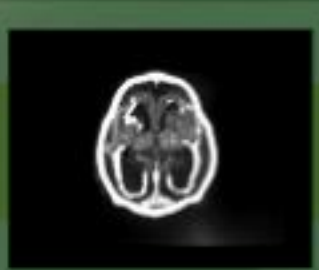
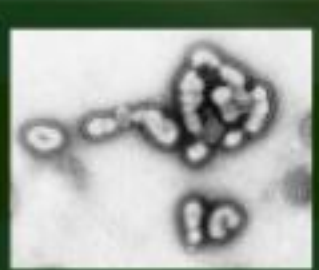
Feigin Cherry Demmler-Harrison Kaplan

FEIGIN & CHERRY'S TEXTBOOK OF PEDIATRIC INFECTIOUS DISEASES

6TH EDITION



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Ralph D. Feigin, M.D.

April 3, 1938–August 14, 2008

With great sadness, we dedicate this sixth edition of the *Textbook of Pediatric Infectious Diseases* to Ralph D. Feigin. As everyone in pediatrics and, in particular, pediatric infectious diseases, knows, Ralph was an extraordinary individual, and his untimely death leaves a void that will never be filled.

Ralph Feigin was born in New York City on April 3, 1938. He graduated from Columbia College in New York City in 1958 and received his M.D. from Boston University School of Medicine in 1962. He married Judith S. Zobel, a childhood friend, in 1960 while in medical school. Ralph completed his first two years of pediatric residency at Boston City Hospital and his third year at the Massachusetts General Hospital. He then fulfilled his military service requirement at the United States Army Research Institute of Infectious Diseases, Ft. Detrick, Frederick, Maryland. While at the United States Army Research Institute, he participated in significant studies relating to circadian periodicity and susceptibility to infections, as well as other studies that resulted in eight publications for which he was the first author. After completing his service commitment, he was Chief Resident at Massachusetts General Hospital during the 1967-68 academic year.

Ralph was recruited to Washington University in St. Louis by Phil Dodge in 1968, and soon thereafter he and one of us (JDC), who was then at St. Louis University, got together and forged an academic and personal friendship that continued until the time of his death. Almost 40 years ago, the two investigators recognized the need for a comprehensive book on pediatric infectious diseases, but because of their busy schedules the plan was put on hold, and in 1973 Jim moved to California. In 1976, the pediatric research meetings were held in St. Louis, and at this time Jim and Ralph met with W. B. Saunders representatives, and the book was conceived. The first edition of the textbook was published 5 years later in the fall of 1981. In comparison with this 6th edition, it was a modest effort, with 44 chapters and 124 contributors.

At Washington University and St. Louis Children's Hospital, Ralph developed one of the finest infectious diseases divisions in the country. His "Feigin Rounds" were an unparalleled learning experience and were legendary among medical students and residents. In 1977, Ralph moved to Houston, Texas, to accept the challenge of being the Chair of Pediatrics for Baylor College of Medicine and the Physician-in-Chief at Texas Children's Hospital. During the ensuing 30 years, the Department grew from 43 faculty members to almost 500. One of us (SLK) came under Ralph's spell in St. Louis and moved to Houston with him. The other one of us (GJD-H), an intern in Houston in 1977, was waiting for Dr. Feigin when he arrived.

In Houston, Ralph served as the Chair of Pediatrics for Baylor College of Medicine and the Physician-in-Chief at Texas Children's Hospital for 31 years. For 7 years of his tenure, he also served as President and CEO of Baylor College of Medicine. In addition to his commitments in Houston, Ralph served in leadership roles on more than 100 local, regional, and national committees and professional societies. His efforts in persuading government officials of all ranks helped children in Texas, the United States, and in all parts of the world. Many consider him to be the foremost pediatrician in the world.

As will be noted in the table of contents of this 6th edition of Feigin and Cherry, Ralph made his usual contributions to the text. In spite of his illness, he contributed to the book and made decisions relating to it until right before his death. Not only was Dr. Feigin a powerhouse of energy, speed, and unsurpassed accomplishments, but he also was a gentleman, full of compassion, warmth, and kindness, and a man who kept people and patients first in his heart and mind. He was a loving husband to his wife, Judy, and a proud father to his three children, Susan, Debra, and Michael; doting grandfather to his six grandchildren, Rebecca, Matthew, Sarah, Rachel, Jacob, and Eli; and a mentor to so many of us in the field of pediatrics and pediatric infectious diseases. Ralph Feigin is missed by everyone who knew him, particularly by Judy Feigin and the family as well as by the three of us.

JDC
SLK
GJD-H

To our spouses—
Judith Feigin, Jeanne Cherry, Neil Harrison, and Marsha Kaplan

our children—
Susan Feigin Harris and Jonathan Harris, Michael and Barbara Feigin,
Debra Feigin Sukin and Steven Sukin;
James Cherry, Jeffrey Cherry and
Kass Hogan, Susan Cherry, Kenneth and Jennifer Cherry;
Emily Demmler Wolfe and Joshua Wolfe, Matthew Demmler, Amy Demmler, Anna Rose Demmler,
Kelly Harrison, and Haley Harrison;
and Lauren Kaplan, Mindy Kaplan Langland and Lance Langland

and our grandchildren—
Rebecca and Sarah Harris, Matthew and Rachel Feigin, Jacob and Eli Sukin; Ferguson,
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Pneumococcal Infections

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Antifungal Agents

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Bacterial Myositis and Pyomyositis; Human Herpesviruses 6, 7, and 8

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Flaviviruses: Yellow Fever

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Whipple Disease

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Parameningeal Infections

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Herpes Simplex Viruses 1 and 2

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Parainfluenza Viruses; Respiratory Syncytial Virus

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Alphaviruses: Chikungunya; Flaviviruses: Dengue and Dengue Hemorrhagic Fever

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Amebiasis

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Cryptococcosis

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Peritonsillar, Retropharyngeal, and Parapharyngeal Abscesses; *Chlamydia trachomatis* Infections in the Neonate; *Chlamydia* Infections

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Chronic Bronchitis; Human Retroviruses: Impact of Human Immunodeficiency Virus and Acquired Immunodeficiency Syndrome

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Nocardia

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Tetanus

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Cervical Lymphadenitis

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Pertussis and Other *Bordetella* Infections

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Campylobacter jejuni

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Cystic Fibrosis; Acute Respiratory Distress Syndrome in Children

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Immunomodulating Agents

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Nonvenereal Treponematoses

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Infections in Pediatric Lung Transplantation

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Coronaviruses and Toroviruses, Including Severe Acute Respiratory Virus Syndrome

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Jean McLean Wallace Professor and Chair, Yale School of Medicine, New Haven, Connecticut
Infectious Disease Considerations in International Adoptees and Refugees

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Distinguished Research Professor and Walter G. Ross Professor and Chair, Department of Microbiology, Immunology, and Tropical Medicine, George Washington University School of Medicine and Health Sciences; President, Sabine Vaccine Institute, Washington, D.C.
Blastocystis hominis Infection; *Entamoeba coli* Infection; *Balantidium coli* Infection; Parasitic Nematode Infections; Drugs for Parasitic Infections

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Pneumocystis Pneumonia

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Staphylococcus aureus Infections (Coagulase-Positive Staphylococci)

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Molecular Determinants of Microbial Pathogenesis

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Reye Syndrome

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Health Care–Associated Infections; Programs to Prevent and Control Health Care–Associated Infections

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Coagulase-Negative Staphylococcal Infections; *Vibrio parahaemolyticus*

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Bacterial Skin Infections

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Pneumococcal Infections

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Horace C. Cabe Professor of Pediatrics and Professor and Chairman, Department of Pediatrics, University of Arkansas for Medical Sciences; President, Arkansas Children's Hospital Research Institute; Attending Physician, Pediatric Infectious Diseases, Arkansas Children's Hospital, Little Rock, Arkansas
Pleural Effusions and Empyema; Lung Abscess; Fungal Meningitis; Other Mycobacteria; *Nocardia; Actinobacillus actinomycetemcomitans;* Actinomycosis

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Active Immunizing Agents

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Hepatitis E Virus

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Smallpox; Monkeypox and Other Poxviruses

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Hepatitis B and D Viruses; Hepatitis C Virus

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Viral and Fungal Skin Infections

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Group A, Group C, and Group G Beta-Hemolytic Streptococcal Infections

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Infectious Pericarditis; Renal Abscess; Prostatitis; Pyogenic Liver Abscess; Bacteremia and Septic Shock; Infections in Pediatric Heart Transplantation; *Staphylococcus aureus* Infections (Coagulase-Positive Staphylococci); Coagulase-Negative Staphylococcal Infections

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Cholangitis and Cholecystitis

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The Pharmacokinetic-Pharmacodynamic Interface: Determinants of Anti-infective Drug Action and Efficacy in Pediatrics

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Passive Immunization

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Cyclosporiasis, Isosporiasis, and Microsporidiosis

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Histoplasmosis

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Otitis Media; Bacterial Pneumonias

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Primary Immunodeficiencies

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Candidiasis

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Classification of Fungi

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Babesiosis

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Chronic Fatigue Syndrome

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Esophagitis; Osteomyelitis; Septic Arthritis; Enteroviruses and Parechoviruses

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Appendicitis and Pelvic Abscess; Pancreatitis; *Kingella* Species

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Lymphocytic Choriomeningitis Virus Infection; Arenaviral Hemorrhagic Fevers; Toscana Virus

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Immunomodulating Agents

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Cholera

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Epstein-Barr Virus

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Other *Campylobacter* Species; Bioterrorism

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Acute Rheumatic Fever

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Fever: Pathogenesis and Treatment

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Filoviral Hemorrhagic Fever: Marburg and Ebola Virus Fevers

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Coronaviruses and Toroviruses, Including Severe Acute Respiratory Syndrome

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Viral and Fungal Skin Infections

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Mastoiditis

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Active Immunizing Agents

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Parainfectious and Postinfectious Disorders of the Nervous System

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Leptospirosis

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Listeriosis

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International Travel Issues for Children

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Public Health Aspects of Infectious Disease Control

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Staphylococcus aureus Infections (Coagulase-Positive Staphylococci)

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Caliciviruses

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Hepatitis C Virus

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Infections Related to Craniofacial Surgical Procedures

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Congenital Toxoplasmosis; Toxoplasmosis

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Antibacterial Therapeutic Agents

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Coronaviruses and Toroviruses, Including Severe Acute Respiratory Syndrome

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La Crosse Encephalitis and Other California Serogroup Viruses

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Hantaviruses

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Toxoplasmosis

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Cholangitis and Cholecystitis

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Paracoccidioidomycosis

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Leprosy and Buruli Ulcer: The Major Cutaneous Mycobacterioses

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Mycoplasma and *Ureaplasma* Infections of the Neonate

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Ocular Infectious Diseases

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Nonvenereal Treponematoses

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Classification and Nomenclature of Viruses; Viral Laboratory Diagnosis

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Hantaviruses

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Cryptococcosis

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Campylobacter jejuni

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Diphtheria; Tularemia

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Bartonella (Cat-Scratch Disease)

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Diarrhea-Causing and Dysentery-Causing *Escherichia coli;* Cholera

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Aeromonas

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Leishmaniasis

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The Common Cold

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Bacillus cereus

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Shigella; Salmonella; Cryptosporidiosis

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Acute Respiratory Distress Syndrome in Children

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Metabolic Response of the Host to Infections

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Plague *(Yersinia pestis);* Clostridial Intoxication and Infection; Antimicrobial Prophylaxis

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Fever without Source and Fever of Unknown Origin

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Group B Streptococcal Infections

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Infections in Burn Patients

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Opportunistic Infections in Hematopoietic Stem Cell Transplantation; Coagulase-Negative Staphylococcal Infections

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Campylobacter jejuni

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Hantaviruses; Other Bunyaviridae: Rift Valley Fever

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Amebiasis

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Pneumococcal Infections

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Approach to Patients with Gastrointestinal Tract Infections and Food Poisoning

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Infections of the Oral Cavity

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Other Bunyaviridae: Oropouche Fever

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Rabies Virus

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Parainfectious and Postinfectious Disorders of the Nervous System

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Retroperitoneal Infections

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Trichomonas Infections

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Mimiviruses

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Toxoplasmosis

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Coagulase-Negative Staphylococcal Infections

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Flaviviruses: West Nile Virus

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Parainfectious and Postinfectious Disorders of the Nervous System

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Trypanosomiasis

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Miscellaneous Mycoses

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Rabies Virus

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PREFACE

Morbidity and mortality rates related to infectious diseases decreased dramatically during the first half of the 20th century in the developed world because of major improvements in public health (e.g., clean water, adequate sanitation, and vector control) and personal health. Further major reduction in morbidity and mortality rates occurred in the second half of that century following the introduction of antimicrobial therapy, as well as active and passive immunization efforts. Despite theses advances in the 20th century, infectious diseases in the developed world remain the leading cause of morbidity in infants and children in the early 21st century. Children continue to experience three to nine respiratory infections and one to three gastrointestinal illnesses annually, requiring visits to physicians that outnumber the visits made for the purpose of well-child care. Infectious diseases also are the most common cause of school absenteeism. In more recent years, the emergence of resistance to multiple antibiotics by a large number of bacterial microorganisms (i.e., community-associated methicillin-resistant *Staphylococcus aureus*) has contributed to the morbidity and mortality related to infectious diseases processes, as have new infectious agents (i.e., bocavirus, SARS coronavirus). The developing world is confronted with the same present-day problems seen in the developed world (antimicrobial resistance and new infectious agents), and these challenges are compounded by malnutrition and lack of adequate public health services and antimicrobial agents.

The first edition of our text was written because we and many of our colleagues were concerned that no single reference text existed that comprehensively covered infectious diseases in children and adolescents. With each subsequent edition, including this one, our goal has been to provide comprehensive coverage of all subjects pertinent to the study of infectious diseases in these populations. Any attempt to summarize our present understanding of infectious diseases for serious students of the subject is a formidable task. In many areas, new information continues to accrue so rapidly that material becomes dated before it can appear in a text of this magnitude. Nonetheless, we have endeavored with the help of many of our colleagues to provide the most comprehensive and up-to-date discussion of this field. The new edition will be available online as well as in print. Purchasers can access the online version by registering their PIN number (found on the inside front cover of the book) at www.expertconsult.com. Online access includes not only fully searchable text, photos, illustrations, and tables but references linked to Pub Med access.

To provide a text as comprehensive and authoritative as possible, we have enlisted contributions from a large number of individuals whose collective expertise is responsible for whatever success we may have had in meeting our objective. We offer our most profound appreciation to the 284 fellow contributors from 108 universities or institutions in 15 countries for their professional expertise and devoted scholarship. Their cooperation and willingness to work with us leave us deeply in their debt.

Once again, infectious diseases are discussed according to organ systems that may be affected, as well as individually by microorganisms. In all sections in which diseases related to specific agents are discussed, emphasis has been placed, to the greatest extent possible, on the specificity of clinical manifestations that may be related to the organism causing the disease. Detailed information regarding the best means to establish a diagnosis and explicit recommendations for therapy are provided.

The entire text has been revised extensively. This edition continues the format that was initiated in the fourth edition in that infections with specific microorganisms have been organized to provide appropriate emphasis on the common features that may relate specific microorganisms to one another. Thus, all gram-positive coccal organisms are presented sequentially and are followed by gram-negative cocci, gram-positive bacilli, enterobacteria, gram-negative coccobacilli, Treponemataceae, anaerobic bacteria, and so forth. In addition, special sections of the text have been devoted to discussions of each of the following: molecular determinants of microbial pathogenesis; immunologic and phagocytic responses to infection; metabolic response of the host to infections; interaction of infection and nutrition; pathogenesis and treatment of fever; indigenous flora; epidemiology of infectious diseases; infections of the compromised host; Kawasaki disease; chronic fatigue syndrome; international travel issues for children; infectious disease problems of international adoptees and refugees; nosocomial infections; prevention and control of infections in hospitalized children; pharmacology and pharmacokinetics of antibacterial, antiviral, antifungal, and antiparasitic agents; immunomodulating agents; active and passive immunizing agents; public health considerations; infections in daycare environments; and use of the bacteriology, mycology, parasitology, virology, and serology laboratories. The section on infections in the compromised host has been divided into nine chapters including opportunistic infections in children with bone marrow transplantation, infections in pediatric heart transplant recipients and lung transplant recipients, opportunistic infections in children with liver and intestinal transplantation, opportunistic infections in children with kidney transplantation, infections related to prosthetics or artificial devices, infections related to craniofacial surgical procedures, and infections related to burns. This reorganization has been necessitated by the large number of individuals, particularly post-transplantation recipients, who now serve as the source of many infectious disease problems and constitute a large part of the consulting practice of many pediatric infectious disease physicians.

With some sadness, we have retained a section on bioterrorism, which is necessitated by the current state of world affairs. The section on immunomodulating agents and their potential use in the treatment of infectious diseases has been expanded because information on this subject has become more extensive since the publication of the last edition. Specific sections also have been

devoted to human and animal bites. The subject of biostatistics as applicable to the subspecialty of infectious diseases also has been included. New chapters on human bocavirus and mimiviruses have been added, and the chapters on human metapneumovirus, coronaviruses, and monkeypox have been expanded.

This project could not have been brought to fruition without the help and assistance of many people whose names do not appear in the text. No words are sufficient to adequately convey our gratitude appropriately; we hope that they know they have our heartfelt thanks.

We would like to single out certain individuals for specific mention. We cannot adequately convey our appreciation for the thousands of hours devoted by Dr. B. Lee Ligon, who edited and also proofread every word of the text that was submitted, as well as the page proofs. We are equally indebted to Mary Campbell, who spent an equivalent amount of time and who was specifically responsible for the coordination of the editorial effort, correspondence with our contributors and with the publishers, and coordination of the manuscript preparation process. She also typed and retyped many sections of the text. We also extremely appreciate the efforts of Laura Wennstrom Sheehan, who oversaw and coordinated all events in Los Angeles. In particular, the work of Tiki Valentino and the students in the UCLA office (Lilit Gevorgyan, Judy Diep, Wendy Monteon, Robin Nguyen, Narges Moaddel, Margarita Santiago, Kelley Shin, and Karla Ortiz) deserves much praise.

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CHAPTER 1

Molecular Determinants of Microbial Pathogenesis

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Despite the availability of antibiotics and expansion of vaccination programs, infectious diseases remain a leading cause of morbidity and mortality worldwide. Numerous factors, including an increase in the prevalence of antimicrobial resistance, an increase in global travel, and an increase in the number of individuals with altered immunity, contribute to the continuing importance of infectious agents. In recent years, several microorganisms have been implicated in diseases previously considered noninfectious, and a variety of new and emerging pathogens have been recognized.

Pathogens are defined as microorganisms that are capable of causing disease. Not all pathogens are equal, however, with respect to their pathogenic potential (i.e., their virulence). Many pathogens are commensal organisms and live in harmony with their host under most conditions, causing disease only when normal immune mechanisms are disrupted or absent. Other pathogens produce disease even in the setting of intact immunity and almost always cause symptoms.

For a given pathogen, pathogenic potential is determined by the specific array of virulence-associated genes. Some species of bacteria are capable of natural transformation and readily acquire fragments of DNA from other organisms, expanding or altering their genetic composition, occasionally with consequences related to virulence. Many microorganisms carry virulence-associated genes on mobile genetic elements, including plasmids, transposons, and bacteriophages. These elements may equip the organism with genetic information that facilitates rapid adaptation to an unfavorable or changing environment. Comparison of genomes from pathogenic and nonpathogenic members of a single genus or species has led to the identification of *pathogenicity islands*—large blocks of chromosomal DNA that are present in pathogens and absent from related nonpathogens. These blocks are flanked by insertion sequences or repeat elements and differ in nucleotide composition relative to the surrounding genome, suggesting acquisition by horizontal exchange. Pathogenicity islands contain clusters of virulence-associated genes that encode a variety of factors, including protein secretion systems, secreted effector molecules, adhesins, and regulatory proteins.

To be successful, a pathogen must enter the host, find an appropriate niche, and multiply. Often the pathogen induces damage to the host and then spreads to other tissues, either nearby the initial site of infection or more distant. Ideally, the pathogen stops short of causing death to the host and produces symptoms, such as cough or diarrhea, that facilitate spread to another host.

This chapter addresses several key steps in the pathogenic process. In each case, we present examples that highlight pathogens and paradigms of relevance to infectious diseases in children. As a reflection of our personal bias, we focus primarily on bacterial pathogens.

COLONIZATION

Most infectious diseases begin with microbial colonization of a host surface—typically the skin, the respiratory tract, the gastrointestinal tract, or the genitourinary tract. Although colonization is insufficient for an organism to produce disease, it is a necessary prerequisite. The process of colonization requires specialized microbial factors, called *adhesins*, which promote adherence to host structures and enable the organism to overcome local defenses, such as mucociliary function, peristalsis, and urinary flow. The cognate receptors for these interactions generally are either carbohydrate or protein structures—in some cases expressed on host cells, and in others present in mucosal secretions or in submucosal tissue.

PILUS ADHESINS

Perhaps most common among bacterial adhesins are hairlike fibers called *pili* (also called *fimbriae*). Pili are polymeric structures containing a major structural subunit that usually ranges in size from 15 to 25 kd. Because of their size and morphology, most pili can be seen by negative-stain transmission electron microscopy.

The prototype example among adhesive pili is the P (or Pap) pilus, which is expressed by uropathogenic *Escherichia coli* (UPEC) and has been strongly associated with pyelonephritis. P pili recognize globoseries glycolipids, host molecules that are characterized by a core structure consisting of Gal-α1,4-Gal. The globoseries glycolipids are especially abundant in renal epithelium,[20] accounting for the predilection of P-piliated *E. coli* to adhere to kidney tissue and cause pyelonephritis. As shown in Figure 1–1, P pili are composite structures and consist of two subassemblies, including a thick rod that emanates from the bacterial surface and a thin tip fibrillum that extends distally.[141,226] The pilus rod is a right-handed helical cylinder and comprises repeating PapA subunits, whereas the tip fibrillum has an open helical configuration and contains mostly repeating PapE subunits. The two subassemblies are joined to each other by the PapK adapter protein. PapG contains the adhesive moiety and is located at the distal end of the tip fibrillum, joined to PapE by the PapF adapter.[123]

P pili are assembled in a process that involves a periplasmic chaperone, called *PapD*, and an outer membrane usher, called *PapC* (see Fig. 1–1).[50,142] The crystal structures of PapD alone and PapD interacting with the PapK pilin subunit have been solved, revealing significant insights into PapD interaction with Pap subunits and the mechanism of P pilus assembly.[225] PapD consists of two immunoglobulin-like folds oriented in an L shape with an intervening cleft. The PapK subunit consists of a single immunoglobulin-like fold but lacks the seventh, C-terminal β-strand (strand G1). The absence of this strand leaves a deep groove along the surface of PapK and exposes its hydrophobic core, predisposing the subunit to aggregation and degradation.

In the PapD-PapK complex, the G1 strand of PapD occupies the groove in PapK and completes the immunoglobulin-like fold, a phenomenon termed *donor strand complementation* (Fig. 1–2A). This interaction shields the hydrophobic core of the subunit and stabilizes the protein. Within the groove, the G1 strand of PapD interacts on one side with the C-terminal, F strand of PapK.[225] Ultimately, PapD delivers PapK and other Pap subunits to the PapC usher, which forms a dimer with twin pores in the outer membrane.[148] As the pilus is assembled on the bacterial surface, the N-terminal strand of a neighboring subunit (the one most

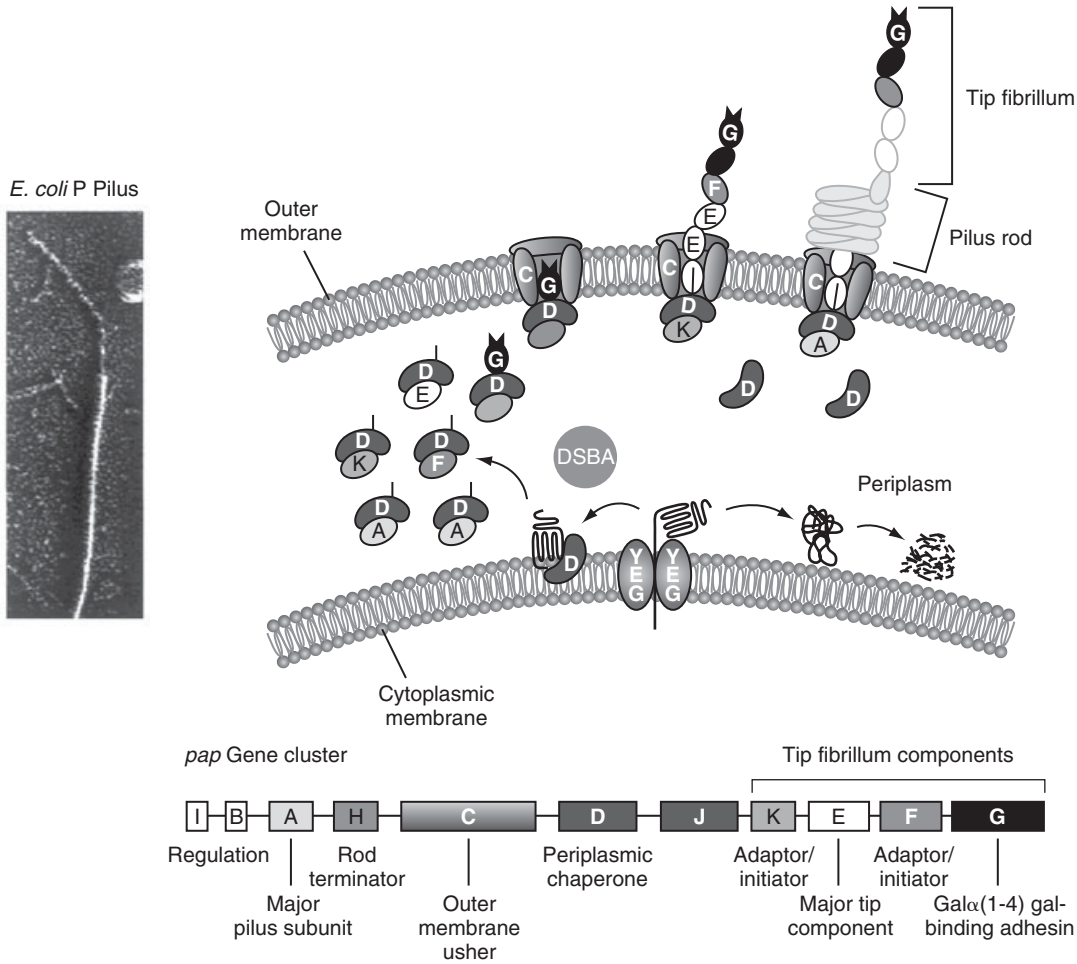


Figure 1–1 Biogenesis and structure of *Escherichia coli* P pili. The *pap* gene cluster and the function of each of the gene products are indicated in the lower portion of the figure. Nascent pilin subunits are complexed with the PapD chaperone and added to the base of the developing pilus via the PapC usher. The mature pilus rod is composed of repeating units of PapA; the tip fibrillum contains the adhesin PapG. The ultrastructure of the pilus is shown in the electron micrograph at the left side of the figure. DSBA, disulfide oxidoreductase that resides in the periplasm; YEG, sec machinery that facilitates protein export from the cytoplasm to the periplasm. *(Courtesy of S. J. Hultgren and F. J. Sauer.)*

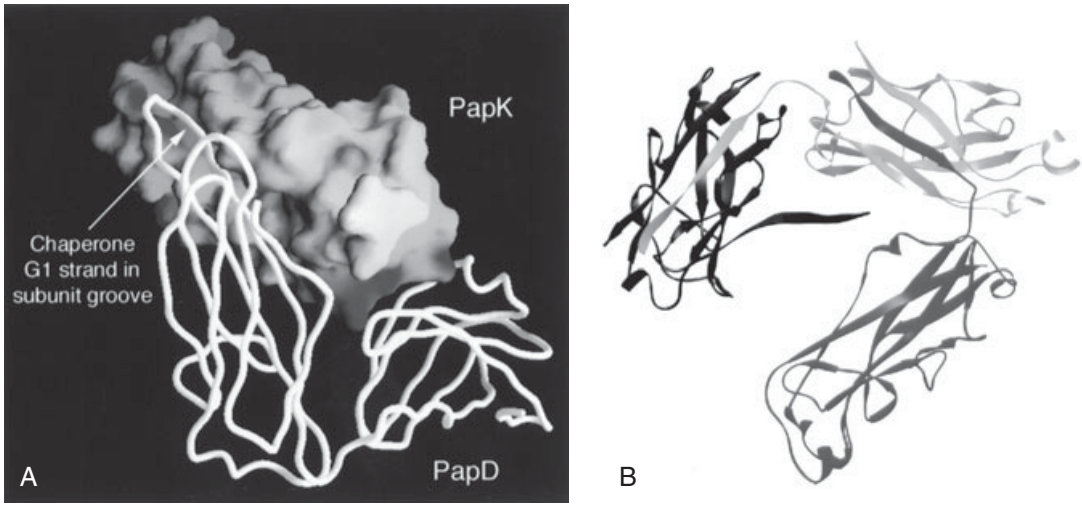


Figure 1–2 **A,** The G1 strand of the PapD chaperone completes the immunoglobulin-like fold of the PapK pilin subunit (donor strand complementation). **B,** The mature P pilus rod comprises a helix (3.28 subunits per turn) of repeating PapA subunits; each subunit is completed by a strand donated by its neighbor. *(Courtesy of S. J. Hultgren and F. J. Sauer.)*

recently added to the pilus base) replaces the G1 strand of PapD in a process called *donor strand exchange*. In the mature pilus, each subunit completes the immunoglobulin-like fold of its neighbor (see Fig. 1–2B).[117] The PapA major subunit employs an intramolecular "hinge" first to exit the usher vertically and then to adopt its final conformation within the helical cylinder that forms the pilus fiber.[176]

More than 30 different bacterial adhesive structures are assembled via the chaperone-usher pathway with a PapD-like chaperone and a PapC-like usher. These PapD-like chaperones can be divided into two distinct subfamilies based on conserved structural differences that occur near the subunit binding site.[118] One subfamily is involved in the assembly of rodlike pili similar to P pili, and the second subfamily participates in the biogenesis of more atypical filamentous structures. The nature of the chaperone is linked directly to the architecture of the adhesive appendage.[239]

Type 4 pili represent a second class of pili, distinguished by a methylated first amino acid (usually phenylalanine), a short positively charged leader sequence, a conserved hydrophobic amino terminal domain, and a tendency to form bundle-like structures. Type 4 pili have been identified in numerous gram-negative bacterial pathogens, including *Neisseria gonorrhoeae*, *Neisseria meningitidis*, enteropathogenic *E. coli* (EPEC), *Vibrio cholerae*, *Pseudomonas aeruginosa*, *Kingella kingae*, *Eikenella corrodens*, *Moraxella* spp., and *Dichelobacter nodosus* (formerly *Bacteroides nodosus*).*

Although the mechanism of assembly of type 4 pili is still being elucidated, existing data suggest that the process is complex. Twenty to 40 gene products are required for the assembly of *P. aeruginosa* type 4 pili, and at least 15 plasmid-encoded proteins are involved in the biogenesis of EPEC type 4 pili.[107,247] Based on studies of *P. aeruginosa*, EPEC, *Neisseria* spp., and *V. cholerae*, the presence of an inner membrane pre-pilin peptidase seems to be a general prerequisite for type 4 pilus biogenesis.[132,144,186] The involvement of at least one protein with a canonical nucleotide binding domain is another common feature. Many of the other proteins required for type 4 pilus assembly resemble proteins from filamentous phage biogenesis systems and DNA uptake and transfer systems, suggesting that similar transport and assembly mechanisms are used.[108,221] More recent evidence indicates that type 4 pili often are glycosylated, with carbohydrate decoration affecting function in at least some cases and perhaps serving to obscure antigenic epitopes.[26,157,234,268]

Despite marked differences in the assembly pathways for type 4 pili and P pili, examination by electron microscopy suggests that, in some cases, significant structural similarities may exist. Gonococcal type 4 pili are composed predominantly of PilE structural subunits polymerized into a helical rod.[195] A minor phase-variable adhesive protein called PilC is displayed at the tip of the pilus and is essential for pilus-mediated binding to epithelial cells.[127,220] These observations suggest that *N. gonorrhoeae* pili may be composite structures with a tip-associated adhesin, analogous to P pili and other pili assembled by the chaperone-usher pathway.

Although adhesive pili are more prevalent in gram-negative bacteria, they also are found in some gram-positive species. One example is *Streptococcus parasanguis*, an oral pathogen and a member of the sanguis streptococcal family. This organism binds to calcium phosphate (the primary mineral component of tooth enamel) and to other oral bacteria, epithelial cells, platelets, and fibronectin. Several adhesins, including pili referred to as *long fimbriae*, mediate these binding functions.

Based on studies of *S. parasanguis* strain FW213, long fimbriae are fashioned primarily from Fap1, a 200-kd protein that includes an unusually long (50 amino acids) signal sequence and a cell wall sorting signal typical of other gram-positive bacterial surface proteins.[283,284] Specific glycosylation of Fap1 seems to be critical to the adhesive function of this fimbrial protein.[246] Similar to gram-negative bacterial pili, long fimbriae seem to have a composite structure with a pilus tip. The tip contains an additional adhesin called FimA, which in purified form is capable of blocking bacterial adherence to saliva-coated hydroxyapatite.[61,191] In work by Burnette-Curley and coworkers,[23] disruption of the *fimA* gene resulted in a 7-fold to 20-fold reduction in the incidence of endocarditis after intravenous inoculation of rats. A second gram-positive organism capable of expressing pili is *Streptococcus agalactiae* (group B streptococcus), a common cause of neonatal pneumonia, sepsis, and meningitis. In *S. agalactiae*, at least two genomic islands encode pilus components, including a major pilin, a pilus-associated anchor, and a pilus-associated adhesin.[55,219]

NON-PILUS ADHESINS

Beyond pili, a variety of non-pilus adhesins exist. In most cases, non-pilus adhesins are proteinaceous and are monomeric or oligomeric surface structures, although isolated examples of carbohydrate and lipid-containing adhesive structures have been identified. Generally, these molecules are difficult to visualize by electron microscopy, even with high-resolution techniques. Similar to pili, for the most part non-pilus adhesins can be classified according to their mechanism of secretion and presentation on the bacterial surface.

Among the best characterized bacterial non-pilus adhesins is filamentous hemagglutinin (FHA), a surface protein expressed by *Bordetella pertussis* and other *Bordetella* species. FHA is synthesized as a large precursor protein with a calculated molecular mass of 367 kd. A cleavage event occurs to eliminate the C-terminal third of the protein and produce the mature 220-kd species.[52] The export of FHA to the surface of the organism occurs via the so-called two-partner secretion pathway, a conserved strategy in which a secreted (TpsA) protein interacts, via specific determinants within its N-terminus, with a cognate outer membrane transporter (TpsB).[109] In *B. pertussis*, the TpsA-type protein FHA is transported by a TpsB-type outer membrane protein called *FhaC*, which has β-barrel pore-forming properties and facilitates translocation of FHA across the outer membrane.[279] Homologous TpsB proteins in other species include those that export the hemolysins of *Serratia marcescens*, *Proteus mirabilis*, and *Haemophilus ducreyi*; the *Haemophilus influenzae* heme:hemopexin binding protein (HxuA); and the *H. influenzae* HMW1 and HMW2 adhesins, among others.[7,36,194,207,264] Although crystallographic data are lacking, the C-terminal portion of FhaC is predicted to form a β-barrel pore in the outer membrane, whereas the FhaC N-terminus may participate in specific recognition of FHA on the periplasmic side.[167]

Examination of purified FHA by transmission electron microscopy and circular dichroism spectroscopy showed that the FHA molecule is 50 nm in length and adopts the shape of a horseshoe nail. It has a globular head, a 37 nm long shaft that averages 4 nm in width but tapers slightly from the head end, and a small flexible tail (Fig. 1–3).[130,156] In the crystal structure of the N-terminus of FHA (the so-called two-partner secretion domain that interacts with FhaC), a series of 19-residue repeat motifs forms a β-helix that is central to the overall structure of full-length FHA (see Fig. 1–3).[30]

Consistent with its large size, FHA contains at least four separate binding domains, three of which have been localized. The region involved in adherence to sulfated saccharides has been mapped to the N-terminus of the FHA molecule.[170] Sulfated saccharides, such as heparin and heparan sulfate, are major components of mucus and extracellular matrix in the respiratory tract

*See references 59, 78, 160, 172, 208, 214, 228, 249, 260, 272.

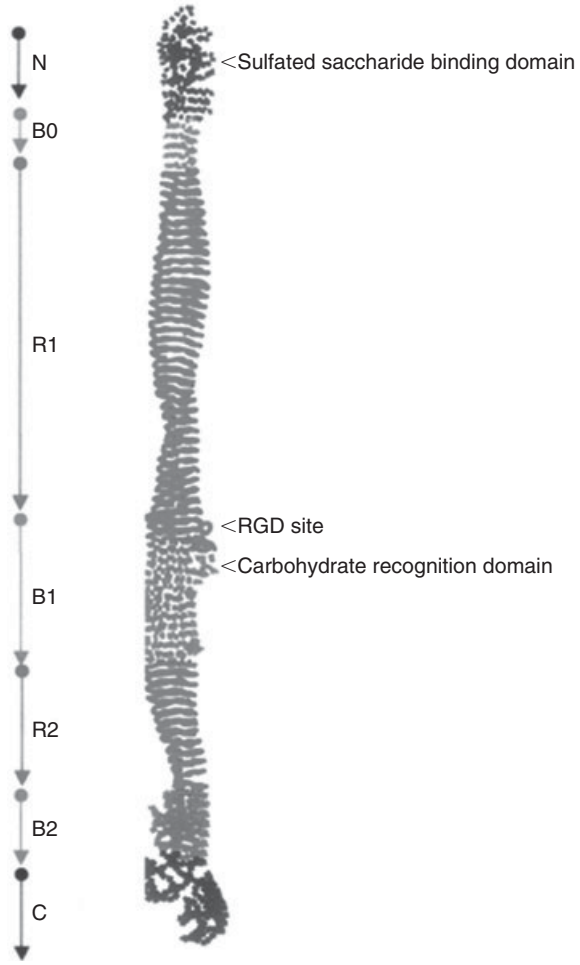


Figure 1–3 Ribbon representation model structure of filamentous hemagglutinin from *Bordetella pertussis*. There are five regions that are assigned β-helical coils, designated B0, R1, B1, R2, and B2. The N-terminus of the protein is designated with "N," and the C-terminus of the protein is designated with "C." The locations of the sulfated saccharide binding domain, the carbohydrate recognition domain, and the arginine-glycine-aspartic acid (RGD) tripeptide are noted. (See companion Expert Consult web site for color version.) *(From Kajava, A. V., Cheng, N., Cleaver, R., et al.: Beta-helix model for the filamentous haemagglutinin adhesin of* Bordetella pertussis *and related bacterial secretory proteins. Mol. Microbiol.* 42*:279-292, 2001.)*

and are found on the surface of epithelial cells.[162,287] The region that recognizes lactosylceramides and promotes adherence to ciliated respiratory epithelial cells and macrophages has been localized to amino acids 1141 to 1279 (the carbohydrate recognition domain).[209] An arginine-glycine-aspartic acid (RGD) tripeptide is located at amino acids 1097 to 1099 and interacts with leukocyte response integrin, a leukocyte integrin that stimulates up-regulation of complement receptor type 3 (CR3).[122] Finally, FHA recognizes CR3 (CD11b/CD18), allowing organisms to be ingested by macrophages without stimulating an oxidative burst.[215,282] The location of the CR3-binding domain is unknown.

A growing number of non-pilus adhesins belong to the so-called autotransporter family. These proteins are synthesized as precursor proteins with three functional domains: an N-terminal canonical signal sequence, an internal passenger domain, and a C-terminal outer membrane domain. The signal sequence directs the protein to the Sec machinery and is cleaved after it facilitates transport of the polypeptide from the cytoplasm to the periplasm. The C-terminal domain inserts into the outer membrane and forms a β-barrel with a central hydrophilic channel. Ultimately, the passenger domain is presented on the surface of the organism and influences interaction with host molecules.[100]

More recent studies have established that autotransporter proteins can be separated into two distinct groups, designated *conventional autotransporters* and *trimeric autotransporters* (Fig. 1–4).[42] In conventional autotransporters, the C-terminal outer membrane domain contains roughly 300 amino acids and is a monomeric β-barrel with a single N-terminal α-helix spanning the pore (Fig. 1–5A).[192,252] In trimeric autotransporters, the C-terminal outer membrane domain contains approximately 70 amino acids and forms heat-resistant, detergent-resistant trimers in the outer membrane. Each trimer forms a β-barrel with four strands from each of the three subunits and with three N-terminal α-helices spanning the pore (see Fig. 1–5B).[168]

An example of a conventional autotransporter adhesin is the *H. influenzae* Hap protein, which was discovered based on its ability to promote adherence and low-level invasion in assays with cultured epithelial cells.[240] Hap also promotes bacterial binding to extracellular matrix proteins and bacterial microcolony formation.[63,101] Examination of chimeric proteins and studies with purified protein have shown that the adhesive activity responsible for Hap-mediated adherence, invasion, binding to extracellular matrix proteins, and microcolony formation localizes to the passenger domain, referred to as Hap_S.[63,101] More detailed characterization of Hap_S has established that the region responsible for interaction with host epithelial cells and microcolony formation resides in the C-terminal 311 amino acids and may have utility as a vaccine antigen.[44,62,151] A prototype member of the trimeric autotransporter subfamily is the *H. influenzae* Hia adhesin. This protein is expressed in a subset of nontypeable *H. influenzae* strains and contains two homologous high-affinity trimeric binding domains, creating the potential for stable multivalent interaction with respiratory epithelial cells.[143,288]

Another group of non-pilus adhesins is typified by intimin, a protein expressed by EPEC, enterohemorrhagic *E. coli*, and the murine pathogen *Citrobacter rodentium*. Intimin contains a flexible N-terminus, a central β-barrel domain that integrates into the outer membrane, and a C-terminal binding domain that interacts with the translocated intimin receptor (Tir).[261] Tir is synthesized by the bacterium, translocated into the host cell cytoplasm via the EPEC type III secretion system,[134,281] and inserted into the host cell membrane, a process that has been modeled in vitro.[212]

The interaction between intimin and Tir triggers a series of host cell events, resulting in receptor clustering, dramatic rearrangement of the actin cytoskeleton, and formation of a distinctive pedestal referred to as an attaching and effacing (A/E) lesion (Fig. 1–6).[134,218] All of the genes essential for formation of A/E lesions are present within a 35-kb region of the EPEC chromosome termed the *locus of enterocyte effacement*, an example of a pathogenicity island.[53,163] This locus is highly conserved in content and organization across all A/E pathogens and contains the genes that encode intimin, Tir, and the EPEC type III secretion system, with a total of 41 open reading frames overall.

In recent years, investigators have identified a large family of non-pilus adhesins involved in adherence to host extracellular matrix proteins, including fibronectin, laminin, vitronectin, collagen, fibrinogen, and a variety of proteoglycans. These adhesins have been classified as *microbial surface components recognizing adhesive matrix molecules* (MSCRAMMs) and are especially prevalent among gram-positive bacteria.[199] In gram-positive organisms, these adhesins are covalently anchored to the cell wall peptidoglycan and have a characteristic primary amino acid sequence. In particular, the carboxy-terminus contains a segment rich in proline and glycine residues, an LPXTG motif (involved in sorting the protein to the cell wall), a hydrophobic

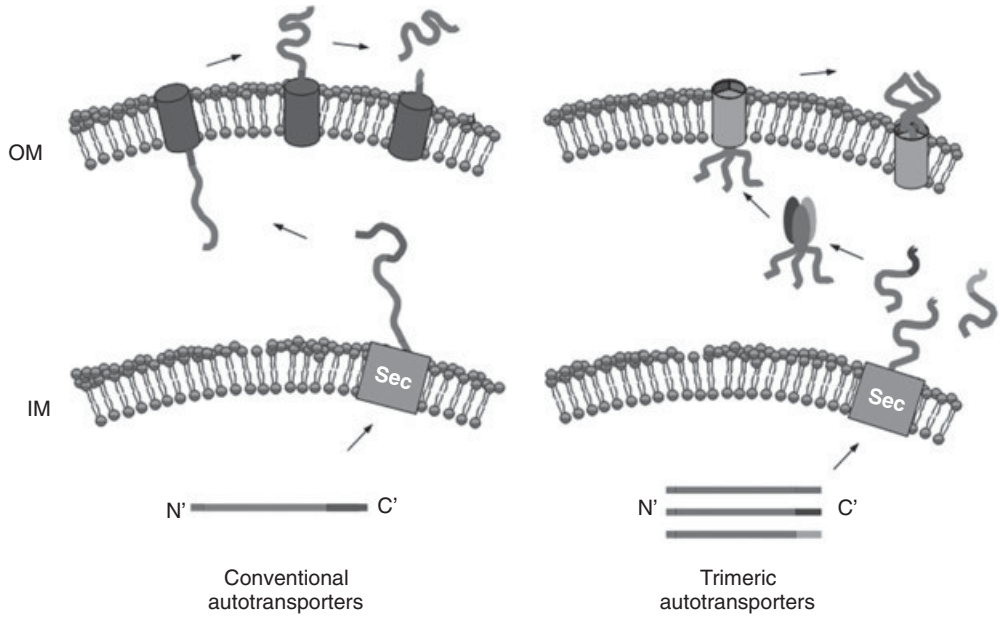
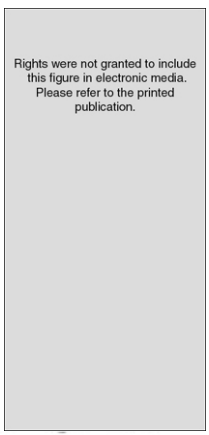


Figure 1–4 Autotransporter protein secretion pathway. Conventional autotransporter secretion is shown on the left, and trimeric autotransporter secretion is shown on the right. Autotransporter proteins are synthesized as preproteins with three functional domains, including an N-terminal signal sequence, an internal passenger domain, and a C-terminal outer membrane β-barrel domain. Protein secretion begins with export of the protein from the cytoplasm via the inner membrane Sec machinery (Sec). Most conventional autotransporters are cleaved on the bacterial surface. IM, inner membrane; OM, outer membrane. (See companion Expert Consult web site for color version.) *(From Cotter, S. E., Surana, N. K., and St. Geme, J. W., III: Trimeric autotransporters: A distinct subfamily of autotransporter proteins. Trends Microbiol.* 13*:199-205, 2005.)*



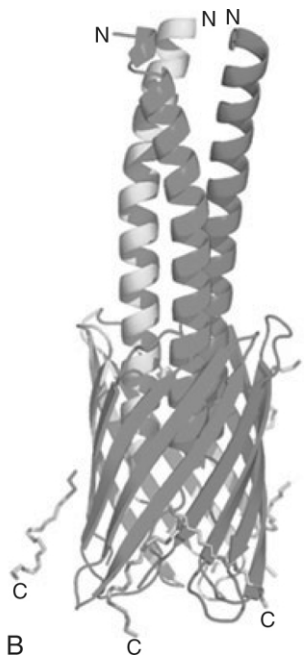


Figure 1–5 Crystal structures of the C-terminal outer membrane β-barrel of autotransporter proteins. **A,** Crystal structure of NalP, a conventional autotransporter. **B,** Crystal structure of Hia, a trimeric autotransporter. (See companion Expert Consult web site for color version.) *(**A** from Surana, N. K., Cotter, S. E., Yeo, H. J., et al.: Structural determinants of* Haemophilus influenzae *adherence to host epithelium: Variations on type V secretion.* In *Waksman, G., Caparon M., and Hultgren, S. [eds]: Structural Basis of Bacterial Pathogenesis. Washington, D.C., American Society for Microbiology, 2005, pp. 129-148. **B** from Meng, G., Surana, N. K., St. Geme, J. W., III, et al.: Structure of the outer membrane translocator domain of the* Haemophilus influenzae *Hia trimeric autotransporter. EMBO J.* 25*:2297-2304, 2006.)*

Figure 1–6 Enteropathogenic *E. coli* are perched on pedestals in the attaching and effacing lesion. *(Courtesy of B. B. Finlay; from Rosenshine, I., Ruschkowski, S., Stein, M., et al.: A pathogenic bacterium triggers epithelial signals to form a functional bacterial receptor that mediates actin pseudopod formation. EMBO J.* 15*:2613-2624, 1996.)*

membrane-spanning domain, and a short, positively charged segment that resides in the cytoplasm and serves as a cell wall retention signal. Adhesive functions typically are located near the N-terminus.[65]

Staphylococcus aureus is a common gram-positive pathogen in children and is capable of producing a variety of MSCRAMMs, including collagen-binding protein (Cna), fibronectin-binding protein A (FnBPA), and clumping factor (ClfA). Strains recovered from patients with septic arthritis commonly express Cna, which mediates binding to cartilage in vitro and seems to play a key role in the pathogenesis of septic arthritis in experimental mice.[198,200,253] The current model of Cna binding suggests that the two major domains (N1 and N2) and an intervening linker polypeptide together form a spherical "hole" through which the bound collagen fiber passes.[294] FnBPA shares homology with *Streptococcus pyogenes* protein F and mediates binding to fibronectin and the gamma chain of fibrinogen.[271] This protein likely is important in *S. aureus* infections of implanted biomaterials, which become coated with fibrinogen and fibrin soon after implantation. ClfA was named based on the observation that it mediates bacterial clumping in the presence of soluble fibrinogen.[164] Similar to FnBPA, ClfA mediates binding to fibrinogen-coated surfaces in vitro and probably contributes to infections of artificial surfaces. The active domain of ClfA is designated region A and contains two amino acid residues (E526 and V527) shown more recently to mediate binding to soluble fibrinogen.[95] The crystal structure of region A shows an immunoglobulin-like fold common to other *S. aureus* MSCRAMMs.[49] Vaccination with ClfA was shown to protect against subsequent development of septic arthritis and death in a mouse model of intravenous *S. aureus* infection.[128]

OTHER MECHANISMS OF ADHERENCE

Candida albicans is a common inhabitant of mucosal surfaces and an important cause of systemic disease, especially in patients with compromised immunity. *Candida* blastospores are capable of efficient adhesion to epithelial cells, leading to budding and division. In addition, germ tube formation occurs, facilitating penetration through the epithelial barrier and dissemination to distant sites.[114] In recent years, several candidate *C. albicans* adhesins have been identified.[8,113,149,290] Of particular interest is a protein called INT1, which shares functional homology with the vertebrate integrin family.

Integrins normally are expressed by cells of the human immune system (neutrophils, monocytes, macrophages) and mediate cellular binding and shape-changing functions. Each integrin is a heterodimer of an alpha chain and a beta chain. Many different alpha and beta chains have been identified, and each combination displays a unique binding specificity. INT1 is an α-integrin–like protein that recognizes the RGD sequence of the C3 fragment iC3b on epithelial cells. In in vitro assays, short peptides encompassing the RGD sequence are capable of inhibiting *C. albicans* adherence by 50 percent, confirming that INT1 plays a significant role as an adhesin and suggesting that other adhesins also exist.[114] Beyond promoting adherence to epithelium, INT1 disguises organisms as leukocytes, allowing evasion of phagocytosis. Introduction of INT1 into *Saccharomyces cerevisiae* confers a capacity for adherence and results in germ tube formation, indicating the importance of this protein in morphogenesis.[73,74]

The adhesive properties of *C. albicans* are tied closely to its morphologic state. Adherence to buccal epithelial cells is greater by organisms bearing germ tubes than by yeast forms.[138] With this information in mind, Staab and coworkers[243] searched a germ tube cDNA library and identified a putative adhesin called *hyphal wall protein 1* (Hwp1), encoded by the *hwp1* gene. Examination of the predicted amino acid sequence of Hwp1 revealed similarity to proteins that are substrates for mammalian transglutaminase enzymes. These enzymes form a cornified envelope on squamous epithelial cells (including buccal epithelial cells) by cross-linking relevant substrates.[244] The interactions of germ tubes with buccal epithelial cells resist stresses (e.g., heating or treatment with sodium dodecylsulfate [SDS]) capable of dissociating most typical microbe-host adhesive pairs, and elimination of expression of Hwp1 results in a marked reduction in adhesion to buccal epithelial cells.[18,242] Hwp1 represents a unique adhesive strategy, employing host transglutaminase enzymes to cross-link Hwp1 (via a glycosyl phosphatidylinositol remnant anchor) directly to surface proteins on buccal epithelial cells.[241]

TISSUE TROPISM

Most microorganisms show restriction in the range of hosts, tissues, and cell types that they colonize. This restriction is referred to as *tropism* and generally reflects the specificity of the interaction between a given microbial adhesin and its cognate receptor. Tropism is determined by the distribution of the relevant host receptor.

P pili of uropathogenic *E. coli* serve as the platform for presentation of one of three different PapG variants, referred to as class I, class II, and class III PapG. All three variants recognize globoseries glycolipids, but each binds with a distinct specificity to the globoseries glycolipid isotypes. Class I PapG preferentially recognizes globotriosylceramide (GbO3, Gal-α1,4-Gal-β1,3-Glc-ceramide); class II PapG preferentially recognizes globoside (GbO4, GalNAc-β1,3-Gal-α1,4-Gal-β1,3-Glc-ceramide); and class III PapG preferentially interacts with Forssman antigen (GbO5, GalNAc-α1,3-GalNAc-β1,3-Gal-α1,4-Gal-β1,3-Glc-ceramide).[250] Globoside is the dominant globoseries glycolipid expressed in human kidney, and most human isolates of *E. coli* associated with pyelonephritis express class II PapG. In contrast, Forssman antigen is the most abundant globoseries glycolipid in dog kidney, and more than 50 percent of canine urinary isolates of *E. coli* express class III PapG.[286] *E. coli* expressing P pili with class II PapG are not found as a cause of urinary tract infection in dogs. The specificity of the PapG variant at the tip of the P pilus influences host range, favoring infection of either human or dog.

The crystal structure of class II PapG bound to Gal-α1,4-Gal was solved by Dodson and coworkers,[51] uncovering the structural basis of PapG binding specificity. The PapG receptor binding site is located on the side of the molecule and must be oriented with its N-terminal to C-terminal axis parallel to the host cell membrane to allow docking to the receptor. This orientation may be facilitated by the flexibility inherent in the tip fibrillum. The PapG binding site consists of two regions. The first forms a β-barrel, and the second is composed of a central antiparallel β-sheet that is flanked on one side by two 2-stranded β-sheets and on the other side by an α-helix. When class II PapG interacts with GbO4, the arginine residue at position 170 in PapG makes contact with the GbO4 side chain. In class I PapG, a histidine residue occupies position 170, interfering with potential contact with the GbO4 side chain. Similarly, class II PapG and class III PapG differ in amino acids required for interaction with the GbO5 side chain.[51]

Group A streptococcus (*S. pyogenes*) is a common cause of infections of skin and soft tissue, including impetigo, cellulitis, and necrotizing fasciitis. Adherence to host cells by *S. pyogenes* is influenced by non-pilus adhesins called *M protein* and *protein F*. M protein forms a fiber and consists of a C-terminal region that anchors the protein in the cell wall, a coiled-coil rod region extending approximately 50 nm from the cell wall, and a short non-helical domain extending more distally.[64] Protein F is a 120-

kd protein that is notable for a tandem repeat element consisting of six repeats of 32 to 44 amino acids adjacent to the C-terminus.[93,193] Based on experiments with a series of isogenic strains that differ in expression of M protein or protein F or both, M protein clearly promotes adherence to human keratinocytes via interaction with the CD46 molecule (also called *membrane cofactor protein*), whereas protein F mediates adherence to epidermal Langerhans cells, which are located in the basal layer of the epidermis.[189,190] M protein and protein F contribute to group A streptococcal adherence to the skin, but each protein directs interaction with a different population of epidermal cells.

Early studies showed that human immunodeficiency virus type 1 (HIV-1) infects $CD4^+$ cells and interacts with the CD4 molecule, but that CD4 alone is insufficient to permit infection. More recent observations have established that numerous host cell chemokine receptors, especially CCR5 and CXCR4, serve as co-receptors for HIV-1 and are required for viral entry into $CD4^+$ target cells. These co-receptors seem to influence the cellular tropism displayed by different HIV-1 variants.[48] All HIV variants are able to replicate in primary T cells, but only some also can replicate in primary macrophages or in immortalized T-cell lines. Asymptomatic HIV-infected individuals carry strains that generally use CCR5 as a co-receptor (termed M5 strains) and are non–syncytium-inducing in vitro. Such strains traditionally have been described as macrophage tropic (M-tropic), but more recent experiments have shown that these M5 strains also can infect $CD4^+$ T cells and peripheral blood mononuclear cells.[205] Rapid viral mutation caused by the error-prone HIV polymerase and HIV reverse transcriptase leads to the production within the host of syncytium-inducing, T cell–tropic (T-tropic) HIV-1 strains, which predominate in the circulation of patients with acquired immunodeficiency syndrome.[48] These variants generally are restricted to CXCR4 (expressed on T cells) as a co-receptor, although some primary syncytium-inducing variants can use CCR5 and CXCR4.[54,60,233] T-tropic, syncytium-inducing strains are characterized by positively charged residues at fixed positions of the V3 loop and changes in charge and length of the V2 region of the viral envelope glycoprotein gp120, which binds to CD4 and co-receptors before viral entry into host cells occurs.[66,67,86] Cellular tropism is closely aligned, but not synonymous, with HIV co-receptor usage.

New HIV-1 infection is selectively established by M-tropic HIV-1 strains, even if the transmitting host harbors more pathogenic non–M-tropic strains as well.[265,292] CCR5 also is expressed on the surface of rectal and vaginal epithelial cells, which may be sites of initial encounter between HIV-1 and the human host.[291] The importance of CCR5 in HIV-1 binding to $CD4^+$ cells is underscored by the observation that individuals homozygous for a 32-bp deletion in CCR5 (the $\Delta 32$ allele) are resistant to infection with HIV-1.[116,152] The $\Delta 32$ heterozygous state does not protect against acquisition of HIV-1, although HIV disease in heterozygous patients may follow an attenuated course.

This allele is found frequently (10 to 14 percent) in white populations, leading to speculation that it provided a survival advantage during one or more historical epidemics of infectious diseases.[185] More recent data suggest, however, that the $\Delta 32$ allele may confer immunodeficiency in the face of challenge with certain viral pathogens, such as West Nile virus.[79,80] CCR5 may have a role in controlling the development of malignancy, including lymphoma, raising some concern about developing anti-HIV pharmacologic agents that target CCR5 function.[146] Finally, co-evolution of viral determinants and host cell receptors may determine the spectrum of tissue and organ involvement within the host. The chemokine receptor CCR8 may facilitate the entry of neurotropic HIV-1 strains into brain cells,[126] and envelopes derived from brain isolates of HIV are adapted to infect cells with low-level CD4/CCR5 expression, such as neuroglia and brain macrophages.[204]

BIOFILMS

After attachment to a particular surface, many pathogens are capable of forming *biofilms*—structured communities of microbial cells enclosed in a self-produced exopolysaccharide matrix.[210] Although most studies of biofilms have involved a single species, it is likely that biofilms relevant to human infection often involve multiple species sharing the advantages of biofilm existence. Human infections associated with biofilms include dental caries, lower airway infection with *P. aeruginosa* in patients with cystic fibrosis, and foreign body infection in patients with prostheses and implanted devices. In addition, formation of biofilms likely occurs during osteomyelitis and endocarditis.[39]

P. aeruginosa is a model organism for the study of biofilms; it forms pillars of stationary (sessile) bacteria held together by an extracellular polysaccharide called *alginate*. Interposed among these pillars are channels that facilitate the flow of nutrients and provide pathways for motile (planktonic) organisms to move about (Fig. 1–7A). In experiments directed at defining the early steps of *P. aeruginosa* biofilm formation, O'Toole and Kolter[187] established that flagella are required for initial bacterial attachment, presumably because these appendages promote movement toward the relevant surface (see Fig. 1–7A). After attachment occurs, type 4 pili and pilus-mediated twitching motility promote formation of microcolonies. In the context of microcolonies, transcription of *algC*, *algD*, and *algU* is activated, resulting in synthesis of alginate.[46] The alternative sigma factor σ^{22} positively regulates the *alg* genes and negatively regulates expression of flagella. Consistent with this observation, pulmonary isolates from patients with cystic fibrosis often are highly mucoid (reflecting expression of alginate) and lack flagella.[71,76]

Development of the complex community present within a biofilm requires intercellular communication. In the case of *P. aeruginosa*, this communication occurs via the LasR-LasI *quorum sensing* system, which involves the acyl-homoserine lactone called *N*-(3-oxododecanoyl)-L-homoserine lactone (3OC12-HSL).[47,196] 3OC12-HSL is synthesized in a reaction catalyzed by LasI and accumulates with increases in population density. Ultimately, 3OC12-HSL reaches a critical concentration and then interacts with LasR, serving to activate transcription of numerous genes. Host inflammatory pathways also are induced directly by accumulated 3OC12-HSL.[238] Organisms with a mutation in *lasI* are capable of attachment and microcolony formation, but the resulting microcolonies remain thin, undifferentiated, and sensitive to dispersion by detergents. Addition of the missing lactone signal to the *lasI* mutant restores development into structured, thick, biocide-resistant biofilms, analogous to biofilms observed with wild-type organisms.[47] Mutation in *lasI* impedes establishment of pulmonary infection in mice.[202,285]

Biofilms constitute a protected mode of growth that allows survival in a hostile environment, such as in the presence of host immune mechanisms or antimicrobial agents.[39] Based on studies of *P. aeruginosa*, sessile bacteria release antigens and stimulate production of antibodies, but these antibodies are ineffective in killing organisms within biofilms.[31] Similarly, sessile *P. aeruginosa* stimulate a diminished oxidative burst and are refractory to phagocytic uptake. In addition, organisms within biofilms are resistant to the effects of many antibiotics—in part because antibiotic agents are unable to diffuse through the biofilm, in part because these bacteria may exist in a slow-growing state, and possibly because these organisms adopt a distinct and protected phenotype.[39] Progress in understanding the mechanisms by which biofilms develop, such as the solution of the

Figure 1–7 In vitro *Pseudomonas* biofilm formation and parallel stages of formation of uropathogenic *Eschericha coli* intracellular bacterial communities [IBCs]. **A,** Dynamics of *Pseudomonas aeruginosa* biofilm formation on an inert surface. Keys to the formation of the biofilm include flagella-mediated attachment, type 4 pilus-based twitching motility, and a quorum sensing system. **B,** Composite representation of the stages of IBC formation and maturation. EPS, exopolysaccharide. (See companion Expert Consult web site for color version.) *(**A** adapted from Kau, A. L., Hunstad, D. A., and Hultgren, S. J.: Interaction of uropathogenic* Escherichia coli *with host uroepithelium. Curr. Opin. Microbiol.* 8:*54-59, 2005. **B** from Costerton, J. W., Stewart, P. S., and Greenberg, E. P.: Bacterial biofilms: A common cause of persistent infections. Science* 284:*1318-1322, 1999. Copyright 1999 American Association for the Advancement of Science.)*

LasI crystal structure,[84] are likely to yield novel approaches to antimicrobial therapy, aimed at disrupting biofilm formation or persistence.

INVASION, INTRACELLULAR SURVIVAL, AND CELL-TO-CELL SPREAD

After adherence to a host surface occurs, many pathogenic organisms are able to invade and survive inside epithelial cells and other non-professional phagocytes (e.g., M cells in intestinal Peyer patches). In addition, some pathogens are able to survive inside professional phagocytes (macrophages and neutrophils). Invasion may represent a mechanism to breach host mucosal barriers and gain access to deeper or more distant tissues. Alternatively, invasion may provide the organism with a special niche, protected from the host's immune mechanisms. Generally, the process of invasion involves a class of molecules called *invasins*, which mediate microbial adherence and entry. Invasion is an active event that relies on underlying host cell functions and is associated with rearrangement of the host cell cytoskeleton. When inside the host cell, the invading or ingested organism usually is localized within a membrane-bound vacuole that contains lysosomal enzymes. In some cases, the pathogen escapes from this vacuole and enters the cytoplasm, a more permissive environment. In others, the pathogen remains in the vacuole and neutralizes lysosomal enzymatic activity. The processes of invasion into cells, survival within cells, cell-to-cell spread, and entry into the circulation define the extent of infection and dissemination.

INVASION

In considering the molecular mechanisms of microbial invasion, perhaps best characterized pathogenic bacteria are the enteropathogenic *Yersinia* species—*Yersinia pseudotuberculosis* and *Yersinia enterocolitica*. These organisms usually are acquired by ingestion of contaminated food or water and typically cause self-limited enteritis or mesenteric adenitis. In infants and other individuals with compromised immunity, they sometimes produce systemic disease. The primary determinant of *Y. pseudotuberculosis* and *Y. enterocolitica* invasion is an adhesive outer membrane protein, invasin, which is encoded by a chromosomal locus called *inv* and binds tightly to a family of β_1-integrins expressed on host cells, including $\alpha_3\beta_1$ integrin on the surface of intestinal M cells.[121] The interaction between invasin and β_1-integrins initiates a cascade of signaling steps in the host cell, resulting in actin rearrangement and formation of large complexes of cytoskeletal elements (e.g., talin, vinculin, α-actinin) called *focal adhesions*.[120] Bacterial entry into the host cell occurs via a "zipper-like" mechanism, with the plasma membrane zippering around the invading organism.

Beyond invasin, two additional proteins called YadA and Ail also influence invasion by enteropathogenic *Yersinia* species. YadA is a 45-kd surface protein that is encoded by the 70-kb *Yersinia* virulence plasmid. It is highly expressed under environmental conditions (e.g., temperature of 37° C) in which invasin is repressed.[57] YadA reaches the bacterial surface via the autotransporter pathway and exists in a trimeric form that is essential for its adhesive activity.[41] Similar to invasin, YadA promotes invasion through binding to β_1-integrins on the host cell surface, but its binding occurs indirectly via extracellular matrix molecules, including collagens, laminin, and fibronectin.[58] Based on studies using a mouse oral infection model, in *Y. enterocolitica*, YadA is essential for survival and multiplication in Peyer patches, whereas in *Y. pseudotuberculosis*, YadA is dispensable for full virulence.[17] Ail is a 17-kd outer membrane protein that also is encoded by a chromosomal locus (*ail*) and mediates high levels of adherence and low levels of invasion in assays with cultured epithelial cells. Ail also mediates resistance to complement-mediated serum killing, independent of an effect on invasion.[14]

Similar to *Y. enterocolitica* and *Y. pseudotuberculosis*, *Listeria monocytogenes* invades epithelial cells via a zipper-like mechanism. Invasion is mediated by proteins called *internalin A* (InlA) and *internalin B* (InlB), which are required for virulence in animal models. InlA interacts with E-cadherin, a host cell transmembrane protein with an intracellular domain that interacts with the cytoskeleton.[169] InlB interacts with C1q on host cells and promotes invasion by activating the PI-3 kinase pathway.[19] Uropathogenic strains of *E. coli* also invade epithelial cells via a zipper-like mechanism, mediated by the FimH adhesin expressed on the tip of type 1 pili. In experiments with cultured bladder epithelial cells, FimH is necessary and sufficient for entry, as shown by examination of a *fimH*$^-$ mutant and of latex beads coated with purified FimH.[161] In vitro experiments suggest further that FimH-mediated bacterial binding to a mannose-coated surface is strengthened by shear forces, such as fluid flow over the surface.[257,258] After FimH-dependent invasion into superficial epithelial cells of the murine bladder occurs, uropathogenic *E. coli* multiply rapidly to form intracellular bacterial communities, which display some features of biofilms (see Fig. 1–7B).[5,129,131] Bacteria derived from this intracellular niche ultimately form a quiescent bacterial reservoir within the epithelium, possibly serving as a seed for recurrent infections.[179]

Salmonella enterica serovar Typhimurium (*S. typhimurium*) is an example of a pathogen that invades cells by a mechanism distinct from zippering. On contact with the epithelial cell surface, *S. typhimurium* triggers a dramatic host cell response characterized by actin rearrangement, calcium and inositol phosphate fluxes, and membrane ruffling. Bacterial entry into the cell occurs rapidly, with organisms appearing in membrane-bound vacuoles within a few minutes of initial contact with the host cell.

The determinants of *S. typhimurium* invasion are encoded by a pathogenicity island called SPI-1, located at centisome 63 on the bacterial chromosome.[72] Especially important is a type III secretion system, which forms a needle-like complex on the bacterial surface and serves to translocate bacterial proteins directly into the host cell, ultimately disrupting the host cell cytoskeleton.[33] Studies of the structure of the needle-like complex have established that the base spans the inner and the outer membranes and is approximately 40 nm in diameter, whereas the needle itself is 8 nm wide and approximately 80 nm long (Fig. 1–8).[35] The base is composed of proteins PrgH, PrgK, and InvG, with InvG playing a key role in pore formation in the bacterial outer membrane. The needle is composed of PrgI, and its length is influenced by a protein called InvJ.[139,140]

The proteins secreted through the *S. typhimurium* needle complex (and other type III secretion systems) and into the host cell are referred to as *effector proteins*. SopE is an effector protein that mediates the initial rearrangement of actin and ruffling of the host cell membrane. It functions as a guanyl-nucleotide exchange factor and activates two host cell Rho guanosine triphosphatase (GTPase) proteins called Rac and Cdc42.[28,92,94] SptP is an effector protein that functions as an antagonist of SopE, mediating reversal of actin rearrangement by converting Rac and Cdc42 to the inactive forms (guanosine diphosphate forms). Consistent with these functions, SopE and SptP directly antagonize each other when co-injected into Ref52 cells.[70] Other effector proteins secreted by the *S. typhimurium* type III secretion system include the inositol phosphate phosphorylase SopB, which disrupts normal host cell signaling mechanisms,[184] and AvrA, which inhibits the nuclear factor (NF)-κB signaling pathway in host cells, down-regulating host inflammatory responses.[34]

Important accessory and regulatory genes also are present in SPI-1. The *sicA* gene is just upstream of the *sipB* and *sipC* genes and encodes an accessory protein with chaperone activity essential for stabilization and translocation of SipB, SipC, and SopE.[263] Other chaperones encoded by SPI-1 are involved in the stabilization and translocation of other effector proteins. The genetic and environmental factors that regulate the expression of type III secretion machinery and secreted proteins also are beginning to be understood.[4]

INTRACELLULAR SURVIVAL

When an organism invades a non-professional phagocyte or is ingested by a professional phagocyte, there are several potential outcomes. Often, the organism is killed. Some pathogens have developed strategies to survive and replicate inside host cells, however, in some cases within a vacuole and in others by escaping from the vacuole.

General agreement is that *S. typhimurium* resides within a membrane-bound vacuole in professional and non-professional phagocytes. The vacuole lacks several lysosomal markers typical of the main endocytic pathway (the mannose-6-phosphate receptor pathway), however, and seems to be distinct from this pathway. Insight into the molecular determinants of intravacuolar survival came in 1996, when two independent groups reported the discovery of a second *Salmonella* pathogenicity island, now called *SPI-2*.[102,188] This island maps to centisome 31 and encodes another type III secretion system, including structural proteins (*ssa* locus),[102] effector proteins (*sse* locus), and accessory proteins (*ssc* locus). In addition, this region encodes a two-component regulatory system consisting of proteins SsrA (formerly SpiR) and SsrB. SsrA is a membrane-located sensor kinase, and SsrB is a transcriptional regulator.[188] Mutations in SPI-2 result in reduced

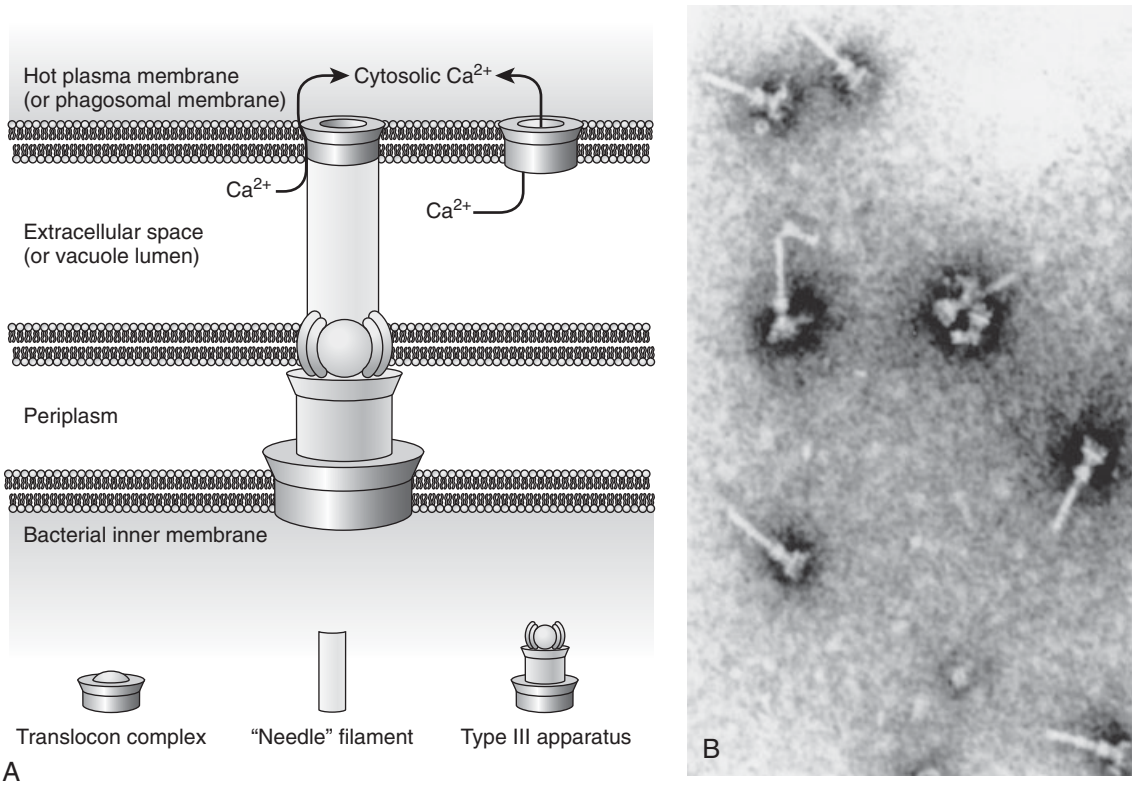


Figure 1–8 **A,** General structure of the gram-negative type III secretion system, represented schematically. **B,** Appearance on electron micrograph of isolated needle complexes from the *Salmonella* type III secretion system. *(**A** from Coombes, B. K., and Finlay, B. B.: Insertion of the bacterial type III translocon: Not your average needle stick. Trends Microbiol.* 13*:92-95, 2005. **B** courtesy of J. E. Galan; from Kubori, T., Matsushima, Y., Nakamura, D., et al.: Supramolecular structure of the* Salmonella typhimurium *type III protein secretion system. Science* 280*:602-605, 1998. Copyright 1998 American Association for the Advancement of Science.)*

survival inside macrophages, with no effect on adherence and invasion in assays with intestinal epithelial cells.[188]

Salmonella SPI-2 mutants show reduced virulence in experimental mice (10^4-fold reduction in 50 percent lethal dose), suggesting that survival inside macrophages is a key factor in the pathogenesis of disease.[229] Expression of SPI-2 genes within the macrophage vacuole depends at least partly on the acidic intravacuolar environment. Inhibition of macrophage vacuolar acidification using bafilomycin A1 (an inhibitor of the vacuolar proton adenosine triphosphatase [ATPase]) results in a sharp attenuation in transcription of SPI-2 genes. This effect is not reproduced by low pH alone outside the vacuole, suggesting that other environmental effects within the vacuole play a role.[29] More recent work indicates that *Salmonella* SPI-2 transcription is activated before invasion occurs, apparently preparing the pathogen for the hostile intracellular environment.[21] As a group, the SPI-2 genes seem to modulate host endocytic and exocytic transport mechanisms.[1]

A third *Salmonella* pathogenicity island called *SPI-3* also influences survival inside macrophages. This island is located at centisome 82 and was discovered by examining the *Salmonella selC* locus, a tRNA gene in which pathogenicity islands reside in some strains of *E. coli*.[10,12] SPI-3 contains the *mgtBC* operon, which permits *S. typhimurium* to grow in environments with low concentrations of Mg^{2+}, including macrophages. In particular, mutation of the *mgtBC* operon abolishes the ability of *S. typhimurium* to replicate in low Mg^{2+} liquid media and in macrophages, and addition of Mg^{2+} to the medium after phagocytosis restores the ability to survive intracellularly. Homologous *mgtBC* genes have been found in other organisms with intracellular lifestyles, such as *Brucella melitensis* and *Yersinia pestis*.[11] In *Salmonella*, the *mgtBC* genes are expressed after internalization into host cells occurs under control of the PhoP-PhoQ two-component regulatory system, a complex that directs expression of many virulence determinants.[158]

The ability to survive within phagocytic cells may provide *Salmonella* with a means to exploit an intrinsic host pathway and disseminate to distant sites. In particular, certain phagocytes express the β_2-integrin CD18, which mediates leukocyte migration in response to various stimuli. During *S. typhimurium* infection, CD18-expressing phagocytes transfer organisms from the intestine to the spleen. Bacterial loads in the liver and spleen are reduced after oral inoculation in CD18-deficient mice, compared with infection in wild-type mice.[266] On the one hand, this function of CD18 facilitates initiation of a systemic immune response and benefits the host. On the other hand, it provides bacteria with a mechanism of transit from the gut to organs of the reticuloendothelial system and elsewhere.

Mycobacterium tuberculosis, another intracellular pathogen, uses an array of mechanisms to ensure intracellular survival. The *M. tuberculosis* vacuole lacks the usual amounts of the vesicular proton ATPase responsible for mediating acidification and fails to acidify to normal levels.[251] In addition, *M. tuberculosis* blocks fusion of the vacuole with acidic lysosomes, further preventing acidification.[222] Similar to intracellular gram-negative bacterial pathogens, *M. tuberculosis* contains an *mgtC* gene, and mutation of this gene results in impaired virulence in cultured human macrophages and in mouse spleen and lung. Low Mg^{2+} concentration and mildly acidic pH inhibit the growth of the *mgtC* mutant, suggesting that the gene is important for survival in the phagosome, where such conditions may exist.[22]

Another factor that influences *M. tuberculosis* survival within macrophages is isocitrate lyase, an enzyme of the glycolytic shunt

that is essential for metabolism of fatty acids. Expression of isocitrate lyase is up-regulated during infection of activated macrophages and is required for full virulence in a murine model of infection, independent of an effect on bacterial growth.[165] The structure of *M. tuberculosis* isocitrate lyase has been solved and may provide a target for new drug therapies against persistent infection because this enzyme is absent from vertebrates.[227,235]

During interaction with macrophages, *M. tuberculosis* (at a low to moderate multiplicity of infection) is capable of stimulating caspase-1 and inducing macrophage apoptosis. This process may be beneficial to the host, preventing systemic spread of infection; less virulent strains of *M. tuberculosis* are more potent inducers of apoptosis.[133] At the same time, *M. tuberculosis* possesses at least two anti-apoptotic mechanisms that influence the outcome of macrophage encounters further. First, *M. tuberculosis* infection enhances host macrophage production of soluble TNFR2, a protein that binds to tumor necrosis factor–α and interferes with apoptosis.[6] Second, *M. tuberculosis* infection activates production of NF-κB, a transcriptional regulator that activates anti-apoptotic pathways within the host cell.[75] Higher multiplicities of infection with virulent strains of *M. tuberculosis* can induce caspase-independent cell death in macrophages, a mechanism proposed to contribute to the formation of necrotic lesions during tuberculosis disease.[147]

Listeria monocytogenes is an example of an organism that escapes from the phagocytic vacuole in macrophages and epithelial cells and moves into the cytoplasm. This organism causes meningitis and focal brain abscesses in humans and has a predilection for the fetoplacental unit. In pregnant women, listeriosis results in fetal loss in 30 percent of cases. Escape from the vacuole is mediated primarily by listeriolysin O, a hemolysin encoded by the *hly* gene. Listeriolysin O interacts with cholesterol in host cell membranes and forms pores, leading to lysis of the phagosome.[69] Mutants defective in *hly* fail to survive intracellularly and are avirulent in mice,[267] although vacuolar escape of *hly* mutants within human cells may be complemented by other bacterial enzymes. Additional factors that contribute to escape from the phagosome include two phospholipase C molecules, one with broad-range specificity (PC-PLC) and another with phosphatidylinositol specificity (PI-PLC). In human epithelial cells, the contributions of PC-PLC and a metalloproteinase called Mpl are most important for vacuolar escape in the absence of listeriolysin O.[87,159,236]

CELL-TO-CELL SPREAD

Movement from one cell to another may help an organism gain a stronger foothold in host tissues. *L. monocytogenes* is one example of a pathogen capable of cell-to-cell spread. When this organism is free in the cytoplasm, actin begins to polymerize on the bacterial surface. Eventually, the condensed actin forms a polar tail or comet, which propels the organism through the cytoplasm and into adjacent cells. The rate of bacterial movement within a cell correlates with actin tail length.[255] Actin accumulation and condensation is mediated by the *L. monocytogenes* ActA protein, which is tightly anchored to the bacterial surface and is expressed asymmetrically over the length of the organism.[237,254] ActA is the sole *Listeria* factor required for actin polymerization because actin tails form in *Xenopus* cytoplasmic extracts containing ActA-coated beads. In these experiments, motility occurs, however, only when ActA is distributed asymmetrically on the beads.[24] ActA seems to interact directly with actin and with a variety of other host cytoskeletal proteins.[69,256] Cytochalasin D is an inhibitor of actin polymerization and inhibits the cell-to-cell spread of *L. monocytogenes* in epithelial monolayers.[45,174]

On reaching the plasma membrane, bacteria protrude from the cell in filipodium-like structures (called listeriopods), which are engulfed by neighboring cells. This engulfment may be part of a normal host process because madin darby canine kidney (MDCK) cells show low-level endocytosis of adjacent cell membrane fragments even in the absence of bacteria.[217] The formation of listeriopods and the engulfment of these structures by neighboring cells are independent of listeriolysin O, PI-PLC, and PC-PLC.[77] When inside a nascently infected cell, *Listeria* escapes from the double-membrane vacuole via the action of PI-PLC, PC-PLC, and Mpl.[69] On arrival in the cytosol, bacteria can enter another cycle of actin-based motility and cell-to-cell spread, although one or two bacterial generations may be necessary to regain motility.[217]

A second pathogen capable of actin-based motility and cell-to-cell spread is *Shigella flexneri*. In *Shigella*, a single protein called IcsA is sufficient to induce formation of an actin tail, similar to that observed in *L. monocytogenes*. IcsA is an outer membrane protein that is encoded on the *Shigella* virulence plasmid and is distributed on the bacterial surface in a polarized fashion, possibly as a result of specialized machinery for autotransporter protein secretion near the poles of some gram-negative bacteria.[125] Initially, IcsA is distributed over the whole bacterial surface, with a predominance at one pole. Over time, a secreted bacterial protease called IcsP cleaves roughly half of the surface IcsA, mostly at the opposite pole, polarizing distribution further.[56,245] IcsP activity is itself tightly regulated by the virulence regulators VirF and VirB.[280]

Elimination of expression of IcsP leads to increased quantities of IcsA and increased actin-based motility, suggesting that IcsA (rather than host factors) is rate-limiting in the motility process.[232] Similar to ActA, IcsA is necessary and sufficient to induce polymerization of the actin tail, and the tail forms at the end where IcsA concentration is highest.[81] Despite the functional similarities between IcsA and ActA, no significant amino acid sequence homology exists between the two proteins. In contrast to ActA, no direct interaction between IcsA and actin has been shown, and IcsA is found throughout the actin tail, not only at the bacterial pole-actin tail junction.

Rickettsia spp. also are capable of actin-based motility and cell-to-cell spread. Based on examination of *Rickettsia rickettsiae*, *Rickettsia* move through the cytoplasm of infected cells 2.5 to 3 times more slowly than do *L. monocytogenes* and *S. flexneri*. In *Rickettsia conorii*, the actin tails seem to be anchored to the bacterial body in a parallel manner, differing from the actin tails in *Listeria* and *Shigella*, which are composed of much shorter actin filaments organized in a random branching network.[83] Details of the bacterial determinants of *Rickettsia* movement remain poorly defined.

DAMAGE TO THE HOST

Damage to host cells and host tissues represents a fundamental mechanism by which a pathogen is able to survive at a given site and then spread within a host. Generally, damage is induced by microbial toxins. Most toxins are released extracellularly and are capable of inducing damage at very low concentrations (exotoxins). Microbial attachment and invasion facilitate toxin delivery to target cells and target tissues and serve to enhance toxicity.

Historically, microbial toxins have been classified according to a variety of criteria, including cellular target of action (e.g., enterotoxins, leukotoxins, neurotoxins), mechanism of action (e.g., adenosine diphosphate [ADP]–ribosylating toxins, adenylate cyclase toxins, pore-forming toxins, proteolytic toxins), and major biologic effect (e.g., hemolytic toxins, edema-producing toxins). In recent years, the term *toxin* has been applied more broadly to include enzymes that mediate damaging effects via phospholipase or hyaluronidase activity.

WHOOPING COUGH AND *BORDETELLA PERTUSSIS* TOXINS

Whooping cough (*B. pertussis* infection) is a classic example of a toxin-mediated disease and involves an interplay of multiple toxins.[135] The pathogenesis of whooping cough begins with *B. pertussis* colonization of the trachea, which is facilitated by a molecule called *tracheal cytotoxin* (TCT). TCT is a naturally occurring disaccharide-tetrapeptide fragment of peptidoglycan and belongs to the family of muramyl peptides.[82] Many gram-negative organisms produce an analogous fragment during normal turnover of cell wall components, but significant extracellular release apparently occurs only in *Bordetella* spp. and gonococci. In most species, an inner membrane protein called AmpG recycles this fragment back into the bacterial cell.[40] More recent crystal structures of TCT in complex with *Drosophila* pathogen pattern recognition receptors offer insight into the specific recognition of TCT via its unique diaminopimelic acid moiety, in contrast to other muramyl peptides.[27,150] TCT is toxic to tracheal epithelial cells in vitro, stimulating nitric oxide synthase and local production of interleukin-1 and causing inhibition of ciliary motility, inhibition of DNA synthesis, and cell death.[97-99,104] During natural infection, TCT likely paralyzes the mucociliary escalator and interferes with clearance of *B. pertussis* and respiratory mucus.

Beyond TCT, *B. pertussis* elaborates a toxin called *adenylate cyclase toxin* (CyaA), which has pore-forming activity and inhibits adenylate cyclase, resulting in accumulation of cyclic adenosine monophosphate (cAMP). In phagocytic cells, the elevated levels of cAMP inhibit oxidative activity and induce apoptosis, disabling this arm of the immune system.[136,137,203] In respiratory epithelial cells, the elevated levels of cAMP may result in increased fluid and mucus secretion, impairing mucociliary function further. Adenylate cyclase toxin has homology to many other bacterial calcium-dependent, pore-forming toxins known as *RTX toxins* (named because of a repeat found in each toxin), with the prototype being the *E. coli* HlyA hemolysin.[40] These toxins create pores in the host cell plasma membrane, ultimately leading to host cell lysis. Among the family of RTX toxins, the general mechanism of pore formation and the predicted amino acid sequences are conserved, but target cell specificities differ.

Pertussis toxin is thought to be a key determinant of the clinical manifestations of whooping cough. This toxin belongs to a family of bacterial ADP-ribosyltransferase enzymes. The target of pertussis toxin is host cell G proteins, resulting in disruption of normal signaling processes. Many biological effects have been ascribed to pertussis toxin and include induction of lymphocytosis, stimulation of insulin release, sensitization to histamine, and disruption of phagocytic cell function; however, the specific relationship between the effects of pertussis toxin and the symptoms of whooping cough remains unclear.[105] *Bordetella parapertussis* is closely related to *B. pertussis* and produces a similar cough illness but fails to produce pertussis toxin because of mutations in the *ptx* promoter region.[180]

HEMOLYTIC-UREMIC SYNDROME AND SHIGA TOXINS

Numerous intestinal pathogens produce Shiga toxins, including *Shigella dysenteriae*, enterohemorrhagic *E. coli* (including *E. coli* O157:H7), and *Citrobacter freundii*, among others. Shiga toxins are classic A-B toxins, consisting of an A subunit that has toxic activity and five B subunits arranged in a pentameric ringlike structure that promotes binding to host cells and delivery of the A subunit. The B subunits interact with host cell globoseries glycolipids, especially the Pk trisaccharide moiety of globotriaosylceramide (GbO3). The A subunit is endocytosed by the host cell and traverses the cytoplasm in membrane-bound vesicles. Some of these vesicles fuse with lysosomes, resulting in degradation of toxin. Others travel in a retrograde fashion to the Golgi apparatus and the endoplasmic reticulum.[224] Within the endoplasmic reticulum, toxin binds to host 28S ribosomal RNA and cleaves a single adenine residue via N-glycosidase activity. Ultimately, this depurination event results in inhibition of protein elongation and cell death.[223]

In humans, *E. coli* O157:H7 is an important cause of hemorrhagic colitis and sometimes produces hemolytic-uremic syndrome. Infection begins with adherence to epithelial cells via intimin and other proteins encoded by the locus of enterocyte effacement, resulting in formation of attaching and effacing lesions analogous to the lesions observed in EPEC infection.[163] After adherence occurs, the organism releases Shiga toxin, which traverses the intestinal epithelial cell and enters the bloodstream.[2] Toxin circulates to distant organs and mediates damage via toxicity to endothelium. Diarrhea likely results from damage to endothelium in small mesenteric vessels, leading to ischemia and sloughing of the intestinal mucosa. The renal effects observed in human hemolytic-uremic syndrome arise from microvascular and glomerular damage with luminal occlusion by fibrin and platelets.[293] Hemolysis and thrombocytopenia likely develop as a consequence of microangiopathy.

Shiga toxins can be divided into two antigenically distinct groups, Stx1 and Stx2, which share 50 to 60 percent homology. Most isolates of *E. coli* O157:H7 recovered from patients with hemolytic-uremic syndrome express Stx2, and some express Stx1 as well.[145] Wadolkowski and colleagues[269] pretreated mice with antibodies against Stx1, Stx2, or both, and challenged these animals with an *E. coli* strain expressing Stx1 and Stx2. Consistent with the importance of Stx2, mice pretreated with antibodies against Stx2 (with or without antibodies against Stx1) were protected from severe disease, whereas 90 percent of mice pretreated with anti-Stx1 alone or anti-cholera toxin died. Pathologic examination of the kidneys showed acute renal tubular necrosis and not glomerular injury, however, suggesting that this model has deficiencies in mimicking human disease.

Understanding the interaction between Shiga toxins and human glycolipid receptors may lead to important therapeutic interventions to prevent hemolytic-uremic syndrome in children with enterohemorrhagic *E. coli* colitis.[96] One initial effort along these lines was a synthetic GbO3 analogue attached to silica particles (Synsorb Pk). Oral administration of this toxin binder did not reduce the severity of hemolytic-uremic syndrome in clinical trials.[262] A more recent strategy has been to express heterologously in nonpathogenic *E. coli* a modified lipopolysaccharide (LPS) containing a sugar moiety that is a mimic for GbO3.[197,206] Oral administration of such bacteria protects mice from subsequent infection with Shiga toxin–producing *E. coli*. Monoclonal antibodies to Shiga toxins have been designed for systemic administration, offering potential activity against toxin already absorbed from the gut; these agents show promising results in animal models of hemolytic-uremic syndrome.[177,230,231] All of these therapeutic strategies would depend on timely recognition of Shiga toxin–producing *E. coli* diarrhea; consequently, additional efforts are being directed at methods to establish this diagnosis early in the course of diarrheal illness.[155]

TISSUE-DEGRADING TOXINS

Numerous toxins have enzymatic activity and are capable of degrading tissue components. One example is hyaluronidase, which degrades hyaluronic acid, a repeating disaccharide glycosaminoglycan involved in cell motility, adhesion, and proliferation in normal hosts. Hyaluronic acid contains alternating *N*-acetylglucosamine and glucuronic acid moieties, connected by β linkages. It is prominent in extracellular matrix when cell turnover and tissue repair are prominent, such as in embryogenesis,

wound healing, and carcinogenesis.[43] The primary host receptor for hyaluronic acid is CD44, which undergoes post-translational modification that varies according to host cell type. Interactions between hyaluronic acid and CD44 are crucial to T-cell and B-cell stimulation, growth of certain lymphoid malignancies, and propagation of certain inflammatory responses.[171]

In *S. pyogenes*, hyaluronidase is a 96-kd protein that is encoded by the *hylA* gene and is released extracellularly. It is proposed to promote invasion through cell layers and tissue planes and is considered one of several *S. pyogenes* spreading factors.[119] *S. pyogenes* also produces a thick "capsule" of hyaluronic acid that can interact with other host cellular and extracellular matrix proteins to contribute to tissue invasion by the organism. Other pathogens that produce a hyaluronidase include *S. agalactiae* (group B streptococcus), *Treponema pallidum*, *Candida*, *Entamoeba histolytica*, and *Ancylostoma braziliense*.[43]

EVASION OF IMMUNITY

To survive and replicate within the host, a pathogen must evade the host immune system. Initially, the organism must circumvent innate immune mechanisms, including mechanical forces, resident phagocytes, and complement activity. Over time, the organism also must overcome adaptive immunity, including the presence of specific antibodies.

ANTIPHAGOCYTIC FACTORS

As described earlier in this chapter, invasin-mediated entry into M cells plays an important role in the early stages of *Yersinia* infection. At the same time, evasion of phagocytosis is crucial to the pathogenesis of *Yersinia* disease. The ability to avoid phagocytosis depends on the *Yersinia* virulence plasmid, which encodes numerous proteins called *Yops*.[38,248] YopE and YopH interfere with ingestion by macrophages and neutrophils via slightly different mechanisms. YopE shares sequence homology with the *S. typhimurium* SptP protein and down-regulates all three of the Rho GTPases (Rho, Cdc42, and Rac), inhibiting actin rearrangement and blocking formation of membrane ruffles (lamellipodia) and spikes (filopodia).[13] YopH is a protein tyrosine phosphorylase that seems to act on a host cell cytosolic protein called Cas, interfering with recruitment of Rho, Cdc42, and Rac, and preventing formation of actin stress fibers, focal complexes, and focal adhesions.[9,13] YopJ is an acetyltransferase that covalently modifies and inactivates intermediate kinases in the mitogen-activated protein kinase and NF-κB signaling pathways, leading to host cell apoptosis.[178,289] Further elucidation of Yop mechanisms may lead to development of Yop inhibitors that abolish the antiphagocytic properties of this pathogen.

Shigella employs another strategy to induce apoptosis in phagocytic cells. This pathogen produces hemorrhagic enterocolitis and is an important etiology of bloody diarrhea in children. Infection begins with ingestion of organisms, which attach to intestinal M cells and cross the intestinal epithelium.[297] On entry into the subepithelial space, organisms are engulfed by resident macrophages and contained in membrane-bound vacuoles. They quickly escape from macrophage vacuoles, however, and move to the cytosol of the cell, where they induce apoptosis.[296] The mechanism of apoptosis involves a protein called IpaB, which is encoded by the *Shigella* virulence plasmid and is injected into host cell membranes via the *Shigella* type III secretion system.[15] Work by Hilbi and coworkers[106] has established that IpaB binds to cytosolic interleukin-1β converting enzyme (caspase-1), a cysteine protease that cleaves interleukin-1β to its active form. Caspase-1 is homologous to many other enzymes involved in cell death pathways, including ced-3 of *Caenorhabditis elegans*. The *S. typhimurium* SipB protein shares homology with IpaB and induces apoptosis by interacting with caspase-1.[103] Insertion of IpaB into host membranes also may facilitate cell-to-cell spread.

EVASION OF COMPLEMENT ACTIVITY

S. pyogenes expresses at least three factors that interfere with host complement activity. Perhaps best known is M protein, which inhibits activation of the alternative complement pathway. This effect is mediated at least in part by the ability of M protein to bind complement factor H, a regulatory protein that inhibits assembly and accelerates decay of C3bBb. More recent studies indicate that serotype M1 and M57 strains express an extracellular protein called Sic (streptococcal inhibitor of complement-mediated lysis), which associates with human plasma proteins called *clusterin* and *histidine-rich glycoprotein* (HRG) and apparently blocks formation of the membrane attack complex (C5b-C9).[3] Studies of epidemic waves of M1 infection show that Sic undergoes significant variation over time, perhaps in response to the selective pressure associated with specific antibodies.[110,111,166] Nonpolar inactivation of *sic* results in reduced mucosal colonization of mice.[153] In addition, *S. pyogenes* produces a serine protease called *C5a peptidase*, which cleaves and inactivates C5a.[278] C5a is a cleavage product of C5 and serves as a powerful chemoattractant for neutrophils. C5a peptidase serves to attenuate the neutrophil response to streptococcal infection.

N. gonorrhoeae is a common cause of cervicitis, urethritis, and pelvic inflammatory disease and is capable of producing disseminated disease. Fresh clinical isolates of *N. gonorrhoeae* typically are resistant to complement-mediated killing, and resistance to complement likely is important in the pathogenesis of disease. Resistance is due in part to sialylation of lipo-oligosaccharide, which involves addition of host-derived cytidine monophospho-*N*-acetylneuraminic acid (CMP-NANA) by a bacterial sialyltransferase. Given the requirement for CMP-NANA, subcultivation in the absence of human serum or human neutrophils is associated with loss of sialylation and loss of resistance. Sialylated lipo-oligosaccharide binds factor H, resulting in down-regulation of activity of the alternative pathway C3 convertase. In addition, sialylated lipo-oligosaccharide interferes with neutrophil phagocytosis and with the normal oxidative burst in neutrophils.[216,277]

A second determinant of resistance to complement-mediated killing is Por1, an outer membrane porin protein that binds factor H and C4b binding protein (C4b BP).[213] C4b BP binds C4b and serves to inhibit assembly and accelerate decay of C4b2a, the classical pathway C3 convertase. Gonococci express a third factor that influences resistance to complement: an outer membrane protein called AniA (for anaerobically induced protein A), which has been shown to be a copper-containing nitrite reductase.[16,25]

EVASION OF HUMORAL IMMUNITY

Numerous pathogens have evolved mechanisms to vary surface-exposed immunogenic molecules, facilitating evasion of a specific antibody response. *Antigenic variation* represents one such mechanism and is characterized by the emergence of modified molecules with novel antigenic properties. *Phase variation* is a second mechanism and is typified by the reversible loss or gain of a given molecule or structure.

N. gonorrhoeae is capable of producing recurrent infection, reflecting the fact that the antibody response to infection fails to provide lasting immunity. In this context, *N. gonorrhoeae* pili are an important target of serum antibody and undergo frequent antigenic variation. Gonococcal pilin expression is controlled by

the *pilE* locus (the expression locus), which contains an intact pilin gene along with promoter sequences. In addition to *pilE*, the gonococcal chromosome contains numerous copies of variant *pil* sequences, called *pilS* loci.[90] These loci are transcriptionally inactive because they lack a promoter and 5′ coding sequence. They can be introduced into the expression locus by RecA-dependent recombination, however, resulting in an altered structural subunit and antigenically variant pili.[115] Because *N. gonorrhoeae* is naturally transformable, horizontal exchange of species-related DNA possibly also contributes to the generation of new *pil* sequences.

The African trypanosomes (including *Trypanosoma brucei*) cause sleeping sickness in sub-Saharan Africa and account for more than 50,000 deaths per year. These organisms avoid humoral immunity by antigenic variation of a large family of proteins called *variable surface glycoproteins* (VSGs), which coat the entire surface of the trypanosome. VSGs are highly immunogenic and stimulate antibodies that lead to efficient and rapid clearing of parasites from the bloodstream. At any given point in time, the organism is able to express a new VSG, however, allowing some organisms to escape the antibody response against the previous VSG.

Each parasite can express more than 100 different VSGs, with variation in expression occurring spontaneously at a rate of 10^{-2} per cell per generation. Overall, the genome of *T. brucei* contains more than 1000 *vsg* genes, including so-called expression sites located near telomeres on mini-chromosomes and silent loci in non-telomeric sites on large chromosomes.[201,259] Generally, VSG antigenic variation occurs by two different mechanisms. The first is termed *in situ activation* and involves the simultaneous activation of a new expression site and inactivation of the old expression site, occurring independent of DNA rearrangement. The second involves DNA recombination, either between the expressed *vsg* and another telomeric expression site (reciprocal recombination) or between the expressed *vsg* and a silent *vsg* locus (gene conversion).[259]

In the case of *H. influenzae*, LPS likely is a key factor in facilitating colonization and is a major target of the antibody response to infection. *H. influenzae* LPS undergoes phase variation. LPS biosynthesis involves multiple enzymatic steps and numerous genes. Among these genes, *lic1A*, *lic2A*, *lic3A*, *lex-2*, *lgtC*, and an *oafA*-like gene contain long stretches of tandem 4-bp repeats within their 5′ coding region. In studies of the *lic* loci, Weiser and coworkers[273] observed that the number of repeats varies spontaneously, generating translational frame shifts with different ATG start codons falling in or out of frame. Such frame shifts result in synthesis of a protein with a different N-terminus or eliminate protein production altogether (when no in-frame start codon exists).

The mechanism of variation in repeat number is presumed to be slipped-strand mispairing, which occurs during DNA replication and involves a single repeat looping out on either the template or the replicating strand. Changes in *lic2A* and *lic3A* influence glycotransferase activity and alter reactivity with monoclonal antibodies directed against specific LPS oligosaccharide epitopes.[88] The *lic2A* gene product is responsible for the addition of a Gal-α1,4-Galβ moiety, which resembles the globoseries glycolipids and protects *H. influenzae* from antibody-mediated killing, possibly by molecular mimicry.[274] *lgtC* may be involved in formation of a Gal-β1,4-Glu moiety.[112] Variation in the *lic1A* gene affects production of a choline kinase responsible for addition of phosphorylcholine (ChoP) to the LPS molecule, a physical change that enhances binding of C-reactive protein and results in susceptibility to serum bactericidal activity.[154,275,276] Expression of *lex2* results in addition of a tetrasaccharide (Gal-α1,4-Gal-β1,4-Glc-β1,4-Glc) to the proximal heptose in LPS and increases resistance to complement-mediated serum killing.[85] Similarly, expression of the *oafA*-like gene results in LPS O-acetylation, which facilitates resistance to serum killing.[68]

ENCAPSULATION

Expression of an extracellular capsule is a common strategy to evade phagocytosis, complement activity, and humoral immunity among pathogenic bacteria, fungi, and parasites. One example is *H. influenzae*, a common cause of childhood bacteremia and meningitis in underdeveloped countries. Among isolates of *H. influenzae*, six structurally and antigenically distinct capsular types are recognized, designated serotypes a to f. Historically, serotype b isolates have accounted for greater than 95 percent of all *H. influenzae* invasive disease, reflecting the distinct virulence properties of the type b capsule, which is a polymer of ribose and ribitol-5-phosphate (PRP) and is encoded by the *cap*b locus.[175] In animal studies comparing derivatives of *H. influenzae* strain Rd expressing type a, b, c, d, e, or f capsule, the strain expressing the type b capsule was associated with the highest incidence of bacteremia after intranasal inoculation of infant rats. Similarly, this strain was associated with the highest magnitude of bacteremia and incidence of meningitis after intraperitoneal inoculation of experimental rats.[295]

In considering the mechanism by which the type b capsule promotes intravascular survival and invasive disease, in vitro studies using mouse peritoneal macrophages and human peripheral blood monocytes provide some insights. Based on work by Noel and colleagues,[183] the type b capsule has been shown to inhibit bacterial binding to macrophages in the absence of complement and a source of C3. In addition, the type b capsule interferes with ingestion by macrophages when anti-PRP antibody is lacking.[182,183] The type b capsule blocks complement deposition on the bacterial surface and resultant complement-mediated bacteriolysis. In almost all isolates of *H. influenzae* type b, the *cap*b locus is a tandem repeat of 18-kb *cap*b gene sequences.[181] As a consequence of this arrangement, the *cap*b locus serves as a template for further amplification of capsule gene sequences in vivo, resulting in increased capsule production. In a study by Corn and associates,[37] 23 of 66 minimally passaged invasive isolates had three to five copies of the 18-kb repeat. Further analysis showed that amplification of the repeat results in augmented resistance to phagocytosis and complement-mediated bacterial killing.[181]

Given the importance of the type b capsule in the pathogenesis of disease, efforts to develop a vaccine effective against *H. influenzae* type b focused on the type b polysaccharide, initially as a plain polysaccharide vaccine and eventually as a conjugate vaccine with the polysaccharide linked to an immunogenic carrier protein. Since routine immunization with conjugate vaccines has been implemented in developed countries, the incidence of invasive *H. influenzae* type b disease in these countries has plummeted.

VIRAL LATENCY

Among viral pathogens, latency represents an important mechanism for viral persistence in the face of host immunity, especially in the case of viruses belonging to the herpes family. Herpes simplex virus is one example, commonly establishing latency after gingivostomatitis and genital tract infection. After infection of a host cell occurs, herpes simplex virus replication commences. Eventually cell death occurs, resulting in cell lysis and release of viral particles, which can infect adjacent cells. This so-called lytic replication is under control of a few *immediate early* (IE) genes, which must be transcribed in moderate amounts to allow expression of the remainder of the viral genome.

IE gene expression is activated by VP16, a viral protein that binds to a sequence common to IE gene promoters.[211] After lysis of the host cell, new virions enter local nerve termini and travel up the long axon to sensory ganglia, where latency is established within days. In the latent state, viral DNA can be detected in the

neuron, but infectious virions cannot be isolated. During latency, IE genes are repressed, and only one small fragment of viral DNA is actively transcribed, yielding the latency-associated transcript (LAT). No protein product has been attributed to the LAT; instead, more recent work has shown the production of a microRNA, transcribed from exon 1 of LAT, which maintains latency by directly inhibiting transforming growth factor–β signaling and promoting survival of infected cells.[89] LAT-deficient mutants are still able to establish initial latency, however, suggesting that IE gene expression may be under multiple controls.[270] The mechanism by which herpes simplex virus is reactivated seems to involve the viral thymidine kinase (TK) gene because TK^- mutants are defective in lytic replication and ordinarily do not reactivate.[32,124] Although a detailed understanding of reactivation is lacking, other viral genes suspected to support reactivation are being studied.[91,173]

CONCLUSION

With the explosion in the development of molecular techniques in recent years, understanding of the specific microbial and host factors involved in the pathogenesis of a variety of infectious diseases has expanded remarkably. As a consequence of this understanding, numerous new vaccines have been developed, including the *H. influenzae* type b and acellular pertussis vaccines. In the coming years, we likely will benefit from novel approaches for treating and preventing human infections. Possible examples might include inhibitors of type III protein secretion systems, antagonists of periplasmic chaperones, and analogues of important host cell receptors. Given the impressive adaptability of pathogenic organisms, however, as new therapeutic agents become available, we must remain vigilant for new microbial strategies allowing evasion of our interventions.

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CHAPTER 2

Normal and Impaired Immunologic Responses to Infection

Michael F. Tosi

This chapter provides an overview of immunologic responses to infection and considers host interactions with different classes of pathogens, normal innate and adaptive immune mechanisms, the developing host responses of neonates, specific primary and secondary immunodeficiencies, and approaches to the evaluation of pediatric patients suspected to have impaired immunity. Human immunodeficiency virus (HIV) and acquired immunodeficiency syndrome (AIDS) are not considered here because they are addressed fully in Chapter 204A. This chapter is intended to supply sufficient information for the development of a basic understanding of mechanisms involved in normal host responses to infection, an appreciation of the underlying basis and clinical presentation of important immunodeficiencies, and a familiarity with general principles of evaluations and management of patients with suspected or documented disorders of immunity. For greater depth and detail regarding specific topics, readers are referred to many excellent reviews.

HOST-PARASITE INTERACTIONS

GENERAL FEATURES OF HOST-PARASITE INTERACTIONS

Humans constantly are exposed to a daunting number and diversity of microorganisms that can cause infection. Many organisms that usually coexist harmoniously with the human host on the skin or on mucous membranes of the oral cavity, upper airways, or lower gastrointestinal tract may invade and become pathogens if the balance of the commensal relationship is disrupted. Other organisms are more virulent, and they overtly attack the host's normal surface barriers and internal defense mechanisms. The human host has evolved a complex array of protective mechanisms designed to defend itself against these continuous microbial challenges.[626] To understand the pathogenesis, pathology, and natural history of infectious diseases, one must be familiar with the features of infectious agents that confer virulence, which are addressed elsewhere in this book. It is equally important to understand the elements of the host's response that contribute to containment, elimination, and protection against subsequent infection with these agents. It is important further to recognize that host responses to infections also may contribute to the pathophysiology of infectious diseases and may injure the host in other ways.

The characteristic features of specific infectious diseases are determined by the interactions of structural components and released products of microbial pathogens with host tissue, cells, and their products. Virulence tactics commonly employed by organisms include adherence to host cell surfaces, internalization within or invasion of host cells, production of toxins, elaboration of surface barriers such as bacterial polysaccharide capsules, usur-

pation of host synthetic mechanisms, and direct inhibition of specific defense mechanisms within host cells. The successful evolution of host strategies to protect against microbial attack has resulted in defenses designed to interfere with or to counteract many of these modes of microbial virulence.[626] In recent years, some of humanity's oldest microbial adversaries (e.g., smallpox, poliomyelitis, measles) systematically have been, or are being, eradicated. In the meantime, previously unrecognized human pathogens, such as HIV-1 and Ebola virus, have emerged as new challenges. Many of our oldest nemeses (e.g., tuberculosis, malaria) continue to elude our efforts to bring them under control, and they remain serious problems world-wide. Continued research at the interface between microbial pathogenesis and immunologic mechanisms is essential for the development of innovative approaches that can support and augment human immune responses to old and new microbial challenges.

MAIN FEATURES OF HOST RESPONSES TO SPECIFIC CLASSES OF INFECTIOUS AGENTS

Viruses

Viruses are obligate intracellular parasites that consist of genetic material in the form of either DNA or RNA that usually is surrounded by a protein coat and may or may not be bound by a lipid envelope.[395] Diseases caused by viruses are remarkably diverse, ranging from mild and merely inconvenient to rapidly fatal and from acute or brief to chronic or lifelong. Certain features are common to the pathogenesis of most viral infections, however. First, viruses must enter host cells to replicate. Viral entry ordinarily is initiated by attachment of a viral surface protein to a specific receptor molecule on the host cell. The specific viral ligands or their corresponding host cell receptors have been identified for numerous viruses. Rhinovirus has evolved a capsid protein that binds to human intercellular adhesion molecule-1 (ICAM-1) on respiratory epithelium[255]; the envelope glycoproteins of HIV-1 interact with CD4 on T lymphocytes and distinct chemokine receptors on lymphocytes or macrophages[163,327,623]; and internalization of adenoviruses depends on interaction between a specific peptide sequence in the penton base complex of the viral capsid and α_V integrins on host cell surfaces.[618]

After the virus has entered the host cell, the cellular synthetic machinery is redirected to the synthesis of viral components. As with many native proteins made by the host cell, a portion of viral protein is processed into peptides and presented on the infected cell surface by major histocompatibility complex (MHC) class I molecules (see later). The host mechanisms most important in defense against most viral pathogens include the production of specific neutralizing antibodies against viral surface proteins, the development of specific $CD8^+$ cytotoxic T-cell responses that eliminate infected cells, and the production of interferons (IFNs) that disrupt viral replication.[367,369,504]

Other host defenses also may exhibit antiviral activity, although the importance of some of these mechanisms in protection against viral infection in humans has not been established as firmly. It is possible that natural killer (NK) cells mediate the destruction of infected host cells[126,542] and that antibody-dependent cellular cytotoxicity (ADCC) may ensue after IgG antibodies bind to viral antigens on the infected cell, permitting subsequent attachment of NK cells or cytotoxic T cells via IgG Fc receptors.[209] IFNs and other cytokines may enhance NK and ADCC activity, and cytokines such as tumor necrosis factor-α (TNF-α) may exert cytotoxic actions on cells infected with certain viruses.[369] Additionally, opsonic complement components bound to viral surfaces can interfere with cell attachment, and the complement-derived membrane attack complex (MAC) can lyse enveloped viruses.[63]

Bacteria

The human host is colonized with a large variety of bacteria at skin and mucous membrane surfaces.[113,482] The integrity of these mechanical barriers ordinarily prevents systemic invasion of local commensal bacteria.[93] The epithelial cells that constitute these barriers, on recognition of an organism as a pathogen, also can release defensins and other microbicidal molecules.[235] In healthy hosts, circulating polymorphonuclear leukocytes (PMNs) help keep the resident flora in check by leaving the bloodstream at the mucosal sites containing the highest bacterial burdens, such as the oral cavity and the lower intestine.[29] This phenomenon helps account for the increased risk of developing local and systemic infection caused by oral and intestinal organisms in patients with severe neutropenia, including patients who receive prolonged chemotherapy for malignancies, and in patients with phagocytic migratory function disorders, such as leukocyte adhesion deficiency syndromes.[29] Important host defenses against most bacteria that invade the human host systemically include the complement system, specific antibodies that promote the opsonic and the bacteriolytic functions of complement, and phagocytes.[2,29,63,279,310]

Fungi

Host mechanisms crucial for defense against fungi are less well understood than are the defense mechanisms directed at bacteria and viruses, but phagocytes and cell-mediated immunity seem to be most important.[197,225] The relative importance of these factors seems to depend on the specific organisms involved, as is shown by clinical observations in patients with isolated defects of one or the other. Severe mucosal infections caused by *Candida* spp. are common occurrences in patients with acquired or primary cell-mediated immune deficits, such as HIV infection, thymic aplasia (see later), chronic mucocutaneous candidiasis, and some forms of severe combined immunodeficiency (SCID), and in patients with disorders of leukocyte migration.[29,197] Disseminated candidiasis more often is attributed to iatrogenic factors, such as prolonged antimicrobial therapy and indwelling vascular catheters. Patients with malignancies, complicated postsurgical courses, and burns also seem to be at increased risk. Although neutrophils from patients with myeloperoxidase (MPO) deficiency kill *Candida* organisms more slowly than do neutrophils from healthy individuals, these patients usually do not develop *Candida* infections, suggesting that this aspect of neutrophil function is not crucial.[29,197]

In contrast to *Candida*, *Aspergillus* infections are not as great a problem for patients with cell-mediated immune defects as they are for patients with defects in phagocytic host defenses, such as chemotherapy-induced neutropenia, or genetic defects in phagocyte killing, such as chronic granulomatous disease (CGD).[61,622] Fungi such as *Histoplasma* and *Cryptococcus*, similar to *Candida*, tend to cause severe infections in patients with defects in cell-mediated immunity, although phagocytes are required for optimal clearance of these organisms.[305,616] The main role of antibodies and complement in protection from fungi probably is to provide opsonic activity to enhance phagocyte function.[181]

Parasites

Parasites, such as protozoa and helminths, comprise such a widely varying group of pathogenic organisms that generalizing about mechanisms of immunity to these organisms as a group is difficult. The importance of specific host mechanisms in defense against certain parasites may be appreciated, however, by consid-

ering the characteristic host responses mobilized by parasitic infection or infestations and examples of special susceptibilities to certain parasites among patients with impairments of different components of immunity. Many helminths induce production by host cells of chemokines that recruit eosinophils and stimulate their production, suggesting a likely role for these cells in antiparasitic defenses; eosinophils have been shown to be important in protection against helminths such as *Strongyloides* and other parasites in this group that can invade tissues. Among the immunoglobulins, IgE seems to play a special role, often in concert with eosinophils, in antihelminthic defenses. IgG also may be important, based on the susceptibility of individuals with hypogammaglobulinemia to hyperinfection with *Strongyloides*. Patients with hypogammaglobulinema or IgA deficiency also are at risk for developing chronic or severe infestations with the flagellate intestinal parasite, *Giardia lamblia*, suggesting a role for some degree of antibody-mediated protection in normal hosts. Patients with primary or acquired disorders of cell-mediated immunity are prone to development of serious central nervous system and ocular manifestations of infection with the protozoan *Toxoplasma gondii*, an obligate intracellular parasite, and hyperinfection with *Strongyloides*.[318,407,454]

FEATURES OF NORMAL IMMUNE FUNCTION

The organization of the immune system often has been viewed as consisting of several separate arms or compartments, such as complement, phagocytes, cell-mediated immunity, and humoral immunity. A more current approach often considers two broader categories—innate immunity and adaptive immunity. The former encompasses the more rapid and phylogenetically primitive, nonspecific responses to infection, such as surface defenses, cytokine elaboration, complement activation, and phagocytic responses, and the latter involves more slowly developing, persistent, and highly evolved antigen-specific responses, such as cell-mediated immunity and antibody production, which exhibit extraordinarily diverse ranges of specificities. Although the individual components of innate and adaptive immunity are addressed separately, the various arms of the immune system engage in a wide range of interactions that may enhance or regulate functions of other components of immunity and add to the already remarkable complexity of the human immune response, and numerous examples of such interactions are provided.

INNATE IMMUNE RESPONSES

Epithelia, Defensins, and Other Antimicrobial Peptides

The epithelium of skin and mucosal tissue functions as a mechanical barrier to the invasion of microbial pathogens. In the last 2 decades, it has become clear that epithelial cells also are a major source of antimicrobial peptides that play important roles in local host defense.[50,233-235,445] Studies of their structure, sources, expression, and actions also have revealed an unexpected range of immunologic activities for these molecules, the functions of which previously were considered mainly antimicrobial in nature.[3,35]

Epithelial cells of mucous membranes of the airways and intestines and keratinocytes express the human β-defensins, HBD-1, HBD-2, HBD-3, and HBD-4. These small cationic peptides are similar to the α-defensins stored in the azurophilic granules of neutrophils, and they display antimicrobial activity against a broad range of bacteria, fungi, chlamydiae, and enveloped viruses.[50,233,235,445] Their production by epithelial cells may be constitutive, as for HBD-1, or inducible, as for HBD-2, HBD-3, and HBD-4. More recent evidence indicates that epithelial cells of the airway or intestine can produce HBD-2 in response to activation by bacterial products via the Toll-like receptors (TLRs), TLR2 or TLR4 (see later), on the epithelial cells.[275,602,608] Stimulation of epithelium by cytokines, including interleukin-1 (IL-1) or TNF-α, also can induce production of defensins.[50,235]

Defensins have been reported to exert their antimicrobial action either by the creation of membrane pores or by membrane disruption resulting from electrostatic interaction with the polar head groups of membrane lipids, with more evidence now favoring the latter mechanism.[50,288] Some microorganisms have evolved mechanisms for evading the action of defensins. Bacterial polysaccharide capsules may limit access of microbial peptides to the cell membrane,[118] and an exoprotein of *Staphylococcus aureus*, staphylokinase, neutralizes the microbicidal action of neutrophil α-defensins.[304]

Several immunoregulatory properties of defensins and related peptides, distinct from their antimicrobial actions, have been documented.[233] Several of these peptides have been shown to facilitate post-translational processing of IL-1β.[464] Some of the β-defensins have been shown to function as chemoattractants for neutrophils, memory T cells, and immature dendritic cells by binding to the chemokine receptor CCR-6.[287,427,445] Separately, HBD-2 has been shown to activate immature dendritic cells via a mechanism that requires TLR4.[72,625] The activation of immature dendritic cells by these mechanisms also promotes their maturation. The β-defensins also act as a chemoattractant for mast cells by an undefined mechanism, and they can induce mast cell degranulation.[426] HBD-2 and several other antimicrobial peptides can interfere with binding between bacterial lipopolysaccharide (LPS) and LPS-binding protein.[522]

Additional antimicrobial peptides of epithelial cells include lysozyme and cathelicidin. Lysozyme, an antimicrobial peptide also found in neutrophil granules, attacks the peptidoglycan cell walls of bacteria and may be released from cells by mechanisms that involve activation of TLR.[456] Cathelicidin, or LL37, similar to lysozyme, is released from neutrophils and epithelial cells. It exhibits broad antimicrobial activity and can inhibit lentiviral replication.[287,556] Cathelicidin also exhibits chemotactic activity for neutrophils, monocytes, and T lymphocytes. This activity is mediated by a formyl peptide receptor-like molecule, FPRL1, rather than the chemokine receptor CCR6 bound by β-defensins.[624]

The release of defensins in response to activation of TLRs and the many actions of these peptides, including their direct antimicrobial activities, their chemoattractant actions for a wide range of immune cells, and their activation of dendritic cell maturation, already suggest a highly complex and regulatory role in the development of host defense and immunity. More recent genomic evidence for the possible existence of 25 additional human defensins that have not yet been characterized suggests that current knowledge describes only a small sample of the overall contribution of these peptides to immune responses.[50,519]

Toll-Like Receptors

Mononuclear phagocytes, including circulating monocytes and tissue macrophages, other phagocytic cells, and many epithelial cells, express a family of receptors that is highly homologous to the *Drosophila* receptor called *Toll*.[99,275,393,602,608] These receptors mediate a phylogenetically primitive, non-clonal mechanism of pathogen recognition based on binding, not to specific antigens, but to structurally conserved pathogen-associated molecular patterns.[9,442,443,629] At least 10 human TLRs bind a range of microbial ligands, such as gram-negative bacterial LPS, bacterial lipoproteins, lipoteichoic acids of gram-positive bacteria, bacterial cell wall peptidoglycans, cell wall components of yeast and mycobacteria, unmethylated CpG dinucleotide motifs in bacterial DNA, and viral RNA.[9,442,443,629]

Gram-positive cell wall components bind mainly to TLR2, and TLR2 also can bind components of herpes simplex virus.[345,568] Gram-negative LPS activates TLR4 indirectly by first binding to LPS-binding protein, which binds to CD14 at the cell surface. The bound CD14 has no transmembrane domain, but it associates directly with an extracellular domain of TLR4.[443,568] TLR5 has been identified as the receptor for bacterial flagellin, TLR9 recognizes CpG motifs of bacterial DNA, and TLR3 has been shown to bind synthetic and viral double-stranded RNA.[59,265,341,345]

Signaling via TLRs occurs via a well-described pathway in which receptor binding generates a signal via an adapter molecule, MyD88, which leads to intracellular association with IL-1 receptor–associated kinase. This association leads to activation of TNF receptor–associated factor-6, which results in nuclear translocation of nuclear factor-κB.[140] NF-κB is an important transcription factor that activates the promoters of the genes for a broad range of cytokines and other proinflammatory products, such as TNF-α, IL-1, IL-6, and IL-8. This signaling pathway, based on studies with TLR4, is similar to, but not identical to, the signaling pathways activated by other TLRs.[140] The activation of cytokine production by TLRs plays an important role in recruiting other components of innate host defense against bacterial pathogens. With large-scale release of cytokines, the deleterious effects of sepsis or other forms of the systemic inflammatory response syndrome show, however, that these pathways have beneficial and potentially harmful effects for the host.[140] Genetic polymorphisms in TLRs may play a role in determining the balance of these effects in certain individuals responding to the challenge of systemic infection.[140,373,374]

In addition to their "first responder" roles in generating an inflammatory response to invading pathogens, TLRs may network with other components of innate and adaptive immunity. TLR4 function is suppressed by activation of cells via the chemokine receptor CXCR4.[326] Activation of some TLRs also can induce expression of the co-stimulatory molecule B7 on antigen-presenting cells (APCs), which is required for activation of naive T cells.[393]

Cytokines

A heterogeneous group of soluble small polypeptide or glycoprotein mediators, often collectively called *cytokines*, form part of a complex network that helps regulate the immune and inflammatory responses. Included in this group of mediators, molecular weights of which range from approximately 8 kd to approximately 45 kd, are the interleukins (ILs), IFNs, growth factors, and chemokines (see separately subsequently). Most cells of the immune system and many other host cell types release cytokines, respond to cytokines via specific cytokine receptors, or both.

New cytokines are being discovered and characterized regularly, and the range of sources and effects of cytokines and their actions and interrelationships are of such complexity that they cannot be addressed here in detail. A list of cytokines and related molecules that play a role in immune function, with selected characteristics, is provided in Table 2–1.[344,445,468] Excellent general reviews are available.[344,369,446,468] Numerous cytokines are considered in subsequent sections because they play important roles in the functions of various immune cells. Two cytokines, IL-1 and TNF-α, are of such fundamental importance in acute host responses to infection that they warrant specific attention here.

IL-1 and TNF-α are small polypeptides, each with a molecular weight of approximately 17 kd, which exhibit a broad range of effects on immunologic responses, inflammation, metabolism, and hematopoiesis.[69,446] IL-1 originally was described as "endogenous pyrogen," referring to its ability to produce fever in experimental animals, and TNF-α, which produces some of the same effects produced by IL-1, originally was named *cachectin* after the wasting syndrome it produced when injected chronically in mice.[69,446] Many of the physiologic changes associated with gram-negative sepsis can be reproduced by injecting experimental animals with these cytokines in the absence of microorganisms. Depending on the doses injected, these effects may include fever, hypotension, and either neutrophilia or leukopenia.[69,446]

In the production of endotoxic shock secondary to gram-negative sepsis, IL-1 and TNF-α are produced by mononuclear phagocytes in response to activation of TLRs by bacterial LPS. They activate the production of other cytokines and chemokines, lipid mediators such as platelet-activating factor and prostaglandins, and reactive oxygen species. They also induce expression of adhesion molecules of endothelial cells and leukocytes, stimulating recruitment of leukocytes by inducing release of the chemokine IL-8 and activating neutrophils for phagocytosis, degranulation, and oxidative burst activity.[69,140]

These all are important, usually beneficial, host responses to infection. At high levels of activation, pathophysiologic effects of this proinflammatory cascade, including vascular instability, decreased myocardial contractility, capillary leak, tissue hypoperfusion, coagulopathy, and multiple organ failure, may occur.[140] For some systemic actions, notably the production of hemodynamic shock, IL-1 and TNF-α are synergistic. IL-1 and TNF-α also induce production of IL-6, a less potent cytokine that exhibits some of the actions of IL-1 and TNF-α.[446] The human host produces several soluble antagonists of IL-1 and TNF-α, including IL-1 receptor antagonist, soluble TNF-α receptor, and anti-inflammatory cytokines, especially IL-10,[140] that can modulate their effects.

The importance of effects mediated by IL-1 and TNF-α in the pathophysiology of septic shock has prompted much active research aimed at blocking their effects to reduce morbidity and mortality. Monoclonal antibodies against TNF-α and other inhibitors of TNF-α or IL-1 have shown early promise in vitro and in animal models of septic shock.[30,45,140,222,446] They have been far more effective at preventing the effects of cytokines than reversing them, however. More recent attempts to address the issue of the timing of intervention have been directed at the intracellular signaling mechanisms activated through the TNF-α receptor or at mediators that appear later than TNF-α. Lipophilic inhibitors of protein tyrosine kinases, enzymes that propagate the cellular signals via TNF-α receptors, have been found to enhance survival in experimental animals, even when administered 2 hours after systemic injection with endotoxin.[594]

Additionally, monoclonal antibodies against a cytokine-like, non-histone nucleoprotein product of macrophages, high mobility group B1 (which appears much later than TNF-α or IL-1 after LPS stimulation) were found to rescue mice from endotoxin shock, when given 2 hours after an otherwise lethal dose of LPS was administered.[606] More recently, clinical trials with activated protein C, a regulatory protein in the coagulation cascade, have shown beneficial effects in selected patients with septic shock by mechanisms that may involve inhibition of activation of NF-κB.[100,482] To date, despite progress, clinical strategies to interfere with the cytokine-induced cascade that leads to endotoxin shock have continued, overall, to meet with limited success.

Chemokines

A specialized group of small cytokine-like polypeptides, chemokines, all of which share the feature of being ligands for G protein–coupled, seven transmembrane–segment receptors, plays an increasingly appreciated and complex role in the immune response as cellular activators that induce directed cell migration, mainly of immune and inflammatory cells.[46,300,324,386,419,500] The chemokines and their receptors have been classified into four families, based on the motif displayed by the first two cysteine

TABLE 2–1 Features of Selected Human Cytokines and Growth Factors

	Main Cellular Sources	Biologic Effects
IL-1	Mo, TL, BL, NK, PMN, others	Broad range of cellular activation in inflammatory and immune responses
IL-2	TL, BL, NK	TL, BL proliferation and activation; enhances TL and NK cytotoxicity
IL-3	TL	General stimulation of hematopoiesis
IL-4	TL, BL, Mast, Mo	TL, BL proliferation; BL isotype switching; stimulates IgE synthesis; enhances MHC class II expression
IL-5	TL	Stimulation of Eo production
IL-6	TL, BL, Mo	Broad inflammatory activity; stimulates BL differentiation and megakaryocyte production
IL-7	Marrow and thymus stromal cells	TL, BL growth and differentiation
IL-8	Mac, Mo, Endo, Epi, PMN, Eo	Activation and chemotaxis of PMN, Eo
IL-9	TL	Mast growth and differentiation; growth of activated TL
IL-10	TL, BL, Mast, Mac	Broad anti-inflammatory actions; inhibits synthesis of several other cytokines (TNF, IL-2, IL-3, IFN-γ)
IL-11	Marrow stromal cells	General stimulation of hematopoiesis; BL growth and differentiation
IL-12	BL, Mo	Stimulation of TL growth; induction of IFN-γ production; enhancement of TL and NK cytotoxicity
IL-13	TL	BL proliferation and isotype switching; enhances MHC class II expression; inhibits production of cytokines by Mac
IL-14	TL, malignant BL	Induces BL growth
IL-15	Epi, Endo, Mo, Mac, marrow stromal cells	Enhances NK growth, development, function; enhances TL growth/migration
IL-17	TL	Enhances TL growth; induces Mac cytokine release
IL-18	Kupffer cells, Epi, spleen, Mac	Promotes TL, BL, NK cytokine release; promotes TL, BL cytotoxicity
IL-21	TL	Promotes BL, TL proliferation; NK cytoxicity
IL-23	Dendritic cells, Mac	Similar to IL-12
IL-25	TL (T_H2), Mast	TL, Mac T_H2 cytokine secretion
IL-27	Dendritic cells, Mac	TL responsiveness to IL-12
IFN-α	Mo, TL	Interference with viral replication; increases MHC class I expression
IFN-β	Epi, Fibro	Similar to IFN-α
IFN-γ	TL, NK	Similar to IFN-α, IFN-β; stimulates Mac inflammatory functions
TNF-α	Mo, Mac, TL, NK	Broad inflammatory effects; fever; cachexia; stimulates catabolism; activation of leukocytes and Endo
GM-CSF	TL, BL, Mo, PMN, Eo, Fibro, Mast, Endo	Growth of PMN, Eo, Mo, and Mac precursors; enhances leukocyte function
G-CSF	Mo, Epi, Fibro	Enhances production and function of granulocytes
M-CSF	Mo, TL, BL, Endo, Fibro	Promotes Mo production; stimulates Mo and Mac function

BL, B lymphocyte; Endo, endothelial cell; Eo, eosinophil; Epi, epithelial cell; Fibro, fibroblast; GM-CSF, granulocyte-macrophage colony-stimulating factor; IFN, interferon; IL, interleukin; Mac, macrophage; Mast, mast cell; MHC, major histocompatibility complex; Mo, monocyte; NK, natural killer cell; PMN, polymorphonuclear leukocyte; TL, T lymphocyte; TNF, tumor necrosis factor.

residues of the respective chemokine peptide sequence. Each of at least 16 CXC chemokines binds to one or more of the CXCRs, CXCR1 through CXCR6. Examples of CXC chemokines include IL-8 and Gro-α. Similarly, at least 28 CC chemokines, such as MIP-1α, RANTES, and eotaxin-1, eotaxin-2, and eotaxin-3, bind to one or more of the CCRs, CCR1 through CCR10. The sole CX3C chemokine, fractalkine, or neurotaxin, binds to CX3CR1, currently the only receptor in its family. The two XC chemokines, including lymphotaxin, bind to the sole receptor in this family, XCR1.

A new nomenclature has been proposed to designate each of the chemokines as a numbered ligand for its respective receptor family. In this system, Gro-α is CXC ligand (L)-1 (or CXCL-1), and IL-8 now becomes CXCL-8. Similarly, RANTES becomes CCL-5, fractalkine is CX3CL-1, and lymphotactin is XCL-1.[300,500] An update of this nomenclature system has been published, tabulating the members of each family with their respective ligands and receptors and the traditional names in human and murine systems.[300]

Virtually every cell type of the immune system expresses receptors for one or more of the chemokines. The cells of virtually any inflamed tissue can release a variety of chemokines, and tissues infected with different bacteria or viruses release chemokines that recruit characteristic sets of immune cells.[244,324] Rhinoviruses induce the release of chemokines that result mainly in recruitment of neutrophils (early in the course of infection), whereas Epstein-Barr virus induces a set of chemokines that result in recruitment of B cells, NK cells, and $CD4^+$ and $CD8^+$ T cells.[244] Almost mutually exclusive sets of chemokines are induced by cytokines associated with T_H1 (IL-12, IFN-γ) versus T_H2 (IL-4, IL-13) immune responses, indicating a tight interplay between cytokines and chemokines in determining the type of immune response to specific infectious challenges generated under different conditions.[75] The specificity of such cellular responses is influenced strongly by the type of chemokines released by specific tissues, the vascular adhesion molecules expressed in those tissues, the chemokine receptors expressed by different populations of leukocytes, and the specific adhesion molecules expressed by leukocytes.[75,244,324]

Modulation of chemokine function may occur by several mechanisms. Chemokines themselves may be potentiated or inactivated by tissue proteases, including tissue peptidases and matrix metalloproteinases.[392,444] Heparan sulfate–related proteoglycans on endothelial cell surfaces tether chemokines locally, where they can activate circulating leukocytes for adhesion most efficiently (see later). Similar proteoglycans free in the extracellular environment may act to bind and sequester chemokines, however, preventing them from interacting with their cellular receptors.[145,346] Finally, in addition to the well-described use of chemokine receptors as co-receptors for viral entry by HIV-1, other viruses, especially members of the herpesvirus family, encode soluble decoy receptors that compete with native host receptors for chemokine binding, disrupting normal host responses.[145,497]

Natural Killer Cells

NK cells are an important cellular feature of innate immunity. They are lymphoid cells that do not express clonally distributed receptors, such as TCRs or surface immunoglobulin, for specific antigens.[411,412,542] They respond in an antigen-independent manner to help contain viral infections before the development of adaptive immune responses, and they aid in the control of malignant tumors.[542,543] NK cells are found in the peripheral circulation and in the spleen and bone marrow. Similar to many other leukocytes, they can be recruited to sites of inflammation by chemokines and other chemoattractants. They seem to be important for the control of tumors in vivo and serve a crucial function in host defense against viral infections, especially infections caused by members of the herpesvirus family.[126,542] Activated NK cells also are an important source of IFN-γ, which limits tumor angiogenesis and promotes the development of specific protective immune responses.[411,412,542,543]

Regulation of NK cell activity involves a balance between activating and inhibitory signals. Several cytokines can activate NK cell proliferation, cytotoxicity, or IFN-γ production, including IL-12, IL-15, IL-18, IL-21, and IFN-αβ.[542] Activating signals via other receptors on NK cells, such as NKG2D, may lead to cytotoxicity or cytokine production, or both, depending on the receptor's association with distinct intracellular adapter proteins that signal via different kinases.[542,598] Other molecules on NK cells may act as co-stimulatory or adhesion receptors, including CD27, CD28, CD154 (CD40 ligand), and LFA-1 (CD11a/CD18).[53,543] Additionally, FcγRIII (CD16), can contribute to NK cell cytotoxic activity by mechanisms that include ADCC.[209,412]

NK cells are able to distinguish normal cells of self origin via receptors that recognize specific MHC class I molecules. Activation of such receptors provides an inhibitory signal that protects healthy host cells from NK cell–mediated lysis. Virus-infected cells and malignant cells may express MHC class I molecules at reduced levels and may be less able to generate inhibitory NK cell signals, rendering them more susceptible to attack by NK cells.[126,542] NK cell inhibitory receptors, which are not well characterized, seem to contain intracytoplasmic tyrosine-based inhibition motifs and to antagonize NK cell activation pathways via protein tyrosine phosphatases.[480,542] The regulation of the phosphorylation state of specific tyrosine residues by activating kinases and inhibitory phosphatases seems to be a pivotal determinant of NK cell activation.

NK cells kill infected or malignant cells by the release of perforin and granzymes from granular storage compartments and by binding of the death receptors Fas and TRAIL-R on target cells via their respective NK cell ligands.[513,542,543] The mechanisms by which perforin and granzymes mediate target cell death are not fully understood. The best available evidence suggests that perforin and one or more of five human granzymes, released along with perforin from cytotoxic granules of NK cells, associate with the cell membranes of target cells, either by binding via the mannose 6-phosphate receptor or by another mechanism that remains to be defined. One or more of the granzymes seems to activate intracellular pathways leading to target apoptosis via pathways that involve the mitochondria or caspases or both.[319,584] Separately, binding of the death receptors also activates caspases, causing target cell apoptosis.[542] Although some tumor cells do not express Fas, NK cells can induce Fas expression on these targets by releasing IFN-γ, then proceed to kill them by binding to the newly expressed Fas.[523]

NK cells engage in several kinds of interactions with other cells of the immune system, including dendritic cells and other APCs. Dendritic cells can influence the proliferation and activation of NK cells by release of cytokines, including IL-12, and by cell surface interactions, including CD40/CD40 ligand (CD40L), LFA-1/ICAM-1, and CD27/CD70.[173] In return, NK cells can provide signals that result in either dendritic cell maturation or apoptosis.[126,542]

Complement System

The complement system consists of more than 30 different free and membrane-bound activation and regulatory proteins. It has multiple key roles in the clearance of invading microbes, including opsonization, recruitment of inflammatory cells, and lytic destruction of pathogens.[62,178,196,198,217,305,308,309,416] Complement and antibody often act synergistically in host defense against infection. Traditionally, they have been known as the heat-labile (complement) and heat-stable (antibody) factors, which contribute to serum opsonic and bactericidal activity. Activation of the complement response to the initial encounter with an organism usually occurs earlier than that of antibody because some components of complement activation are independent of antibodies and can be initiated before specific antibody can be produced. When specific antibody is available, it serves to activate complement more efficiently and to direct complement binding to locations on the microbial surface that support the optimal execution of its effector functions, such as opsonization and killing.

Approximately 90 percent of complement proteins are synthesized in the liver, but some components can be produced locally at sites of infection by tissue mononuclear phagocytes and fibroblasts.[143,463] In healthy individuals, most complement is found in the circulation; less than 10 percent is in mucosal secretions, and little is detectable in cerebrospinal fluid. Circulating complement levels vary over time, particularly in the presence of inflammation. The inflammatory response may lead to increases in levels of complement components such as C3 that are acute-phase reactants or to decreases in individual components and total complement activity as a result of consumption.

The importance of normal complement component levels and activity in host defense has been well established and is based primarily on the increased susceptibility of patients with specific complement component deficiencies to recurrent and severe infections.[176,178,196,305,308] Although the complement response to infection usually is beneficial to the host, it also may be associated with adverse clinical manifestations, such as septic shock and acute respiratory distress syndrome.[212,610]

COMPLEMENT ACTIVATION

Complement proteins are activated in a specific sequence or "cascade" via one of at least three pathways—the classical pathway, the alternative pathway, and the more recently described mannan-binding lectin (MBL) pathway (Fig. 2–1). These pathways converge at C3, and the complement cascade downstream from C3 proceeds identically, regardless of the pathway by which activation occurs. The C3 convertases, C4b2a for the classical and MBL pathways, and C3bBb for the alternative pathway, cleave the C3 molecule at exactly the same location, producing C3b, which binds to the target surface, and C3a, which is released into the fluid phase. Cleavage and activation of C3 lead to a conformational change in C3b that transiently renders its reactive thioester group capable of forming covalent ester or amide bonds with acceptor molecules on the target surface.[291,353]

If the acceptor molecules are situated on the surface of a microorganism, the bound C3b can act as an opsonin to promote phagocytosis, or it can bind with the classical and alternative pathway C3 convertases to form the C5 convertases, C4b2a3b and C3bBb3b. C5 convertases bind and then cleave C5, with release of the C5a fragment into the fluid phase. The bound C5b fragment can initiate formation of the MAC by the sequential incorporation of the remaining terminal components, C6, C7, C8, and multiple molecules of C9. The MAC can insert into the

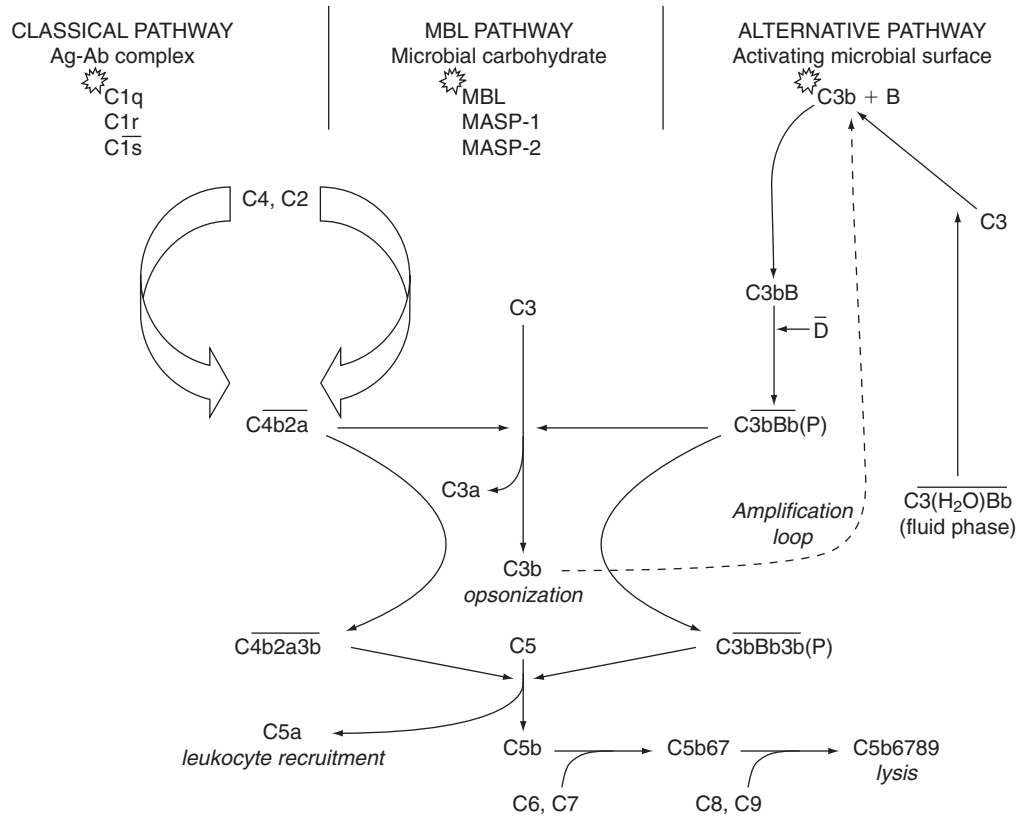

Figure 2–1 The complement cascade. The initial binding events of the classical, mannan-binding lectin (MBL), and alternative pathways are indicated by a *starburst*. These pathways intersect at the conversion of C3 to C3b. This is followed by activation of the terminal components, beginning with the binding and cleavage of C5, releasing C5a and leaving bound C5b to initiate assembly of the remaining components to form the membrane attack complex (C5b, 6, 7, 8, 9). Enzymatically active proteases, or convertases, of the classical and alternative pathways that cleave and activate subsequent components are shown by convention with an *overbar*. The alternative pathway C3 and C5 convertases are shown associated with properdin (P), which increases their stability.

outer membrane of target cells, such as erythrocytes or gram-negative bacteria, and cause cell lysis and death.[309]

Classical Pathway

Ordinarily, the classical pathway is activated by IgM or IgG bound to microbial antigenic targets or by other kinds of antigen-antibody complexes.[178] IgM activates complement more efficiently than does IgG because only one molecule of polymeric IgM is required compared with at least two molecules of IgG.[151] Activation is initiated when C1q binds directly to an immunoglobulin molecule on the surface of an organism or, less often, to a surface molecule of the organism itself. C1r and C1s are activated and bound to C1q sequentially, forming C1qrs. The enzymatic activity of this complex, which resides in the C1s molecule, can cleave multiple molecules of C4 and C2 into two fragments each. The C4a and C2b fragments are released into the environment, whereas C4b and C2a remain bound to each other on the surface of the target to form the classical pathway C3 convertase, C4b2a. C4bC2a can cleave and activate C3 and localize C3b binding to nearby sites on the target surface. As noted previously, some C3b binds with C4b2a to form the classical pathway C5 convertase, C4b2a3b. Activation of the classical pathway ordinarily is not initiated by complexes of antigens with IgG4, IgA, IgD, or IgE.

Alternative Pathway

The classical pathway requires specific antibody and contributes to host defense in immune individuals, whereas the alternative pathway is more important in protection of non-immune hosts, such as premature infants who have low levels of transplacentally acquired antibody and older infants and young children whose maternal antibody has waned, but who have not yet produced their own specific antibodies. The alternative pathway can be initiated by microbial surface macromolecules (e.g., polysaccharide, LPS, teichoic acid), although, as noted earlier, in some circumstances, specific antibody increases alternative pathway efficiency and may direct the location of C3b binding.[309]

A spontaneous low level of hydrolysis of the thioester of C3 in the fluid phase results in an activated form of C3, C3(H_2O). This activated form of C3 can bind factor B, and the latter then is cleaved by factor D to form the fluid-phase C3 convertase C3(H2O)Bb. The constitutive presence of small amounts of this convertase in the fluid phase ensures that a small amount of C3b always is available to bind to microbial surfaces and initiate the alternative pathway.[457] The alternative pathway protein factor B has structural and functional similarities to C2, including the ability to bind to surface-bound C3b. When bound to C3b, factor B undergoes proteolytic cleavage by factor D to release a small soluble fragment, Ba, leaving the larger fragment, Bb, associated with C3b. C3bBb, the alternative pathway C3 convertase, is analogous to the classical pathway C3 convertase, C4b2a. Properdin stabilizes the C3 convertase C3bBb, permitting more efficient activation of C3 to form more C3b, creating the C3 amplification loop (see Fig. 2–1).[211,214] Alternative pathway activation of C3 by this mechanism is several times less efficient than activation via the classical pathway, but it is vital to host defense because it is the principal means by which a non-immune

host can activate C3 until a specific antibody response can be mounted.[175,214]

The most important factor in determining whether or not a specific microbial pathogen would activate the alternative pathway is the biochemical nature of its surface. The surface biochemical features of a microorganism that characterize it as an activator are only partially understood. Microorganisms that bear large amounts of surface sialic acid usually are non-activators, however. The expression of surface sialic acid is one means by which mammalian host cells are protected from complement-mediated lysis in vivo. On surfaces rich in sialic acid, bound C3b is less able to bind factor B because another molecule, factor H, has a strong competitive advantage over factor B under these conditions. When bound by factor H, C3b becomes highly susceptible to further cleavage by factor I (C3b inactivator), resulting in C3bi (or iC3b). Although C3bi can function as an opsonin, it cannot bind factor B. No alternative pathway convertases can be formed, and no amplification loop is established.[62,416]

Organisms whose surfaces do not support activation of the alternative pathway are some of the most successful pathogens in infants and young children, who often lack specific humoral immunity. These organisms include K1 *Escherichia coli*, groups A and B streptococci, *Streptococcus pneumoniae*, *Neisseria meningitidis*, *Haemophilus influenzae* type b, and some salmonellae.[121,309] Development of specific IgG or IgM antibody against these organisms permits some activation of the alternative pathway and efficient activation of the classical pathway of complement at their surfaces and correlates with protection.

Mannan-Binding Lectin Pathway

A third complement activation pathway, the MBL pathway, has been described more recently. It is similar to the classical pathway, but it does not involve antibodies. MBL is a serum protein of the collectin family that has structural and functional similarities to those of C1q. It does not require antigen-antibody complexes to initiate its complement-activating function, however. MBL binds to mannose-containing carbohydrates on microbial surfaces, leading to its association at the microbial surface with activated MBL-associated serine proteases (MASP-1 and MASP-2). These latter proteases seem to have structural and functional similarities to those of C1r and C1s and result in activation of C4, with sequential binding of C4b and C2a and formation of C4b2a, the C3 convertase of the classical pathway. C3 is activated, and the cascade proceeds as described. A more detailed characterization of the MBL pathway and its role in immune responses to infection may be found in an excellent review.[465]

EFFECTOR FUNCTIONS OF COMPLEMENT IN HOST DEFENSE

All complement effector functions in host defense against infection require activation of the complement cascade through at least C3. The cascade needs to be activated through only C3 for effective opsonization, stimulation of leukocytosis, and immune regulation. Activation through C5 is required to produce a normal inflammatory response, including recruitment of phagocytes to sites of inflammation, and activation through C8 is needed for formation of the MAC. Complement may be activated and bound to cell surfaces but may not be able to carry out its effector functions if it is bound at a disadvantageous location.[97,250,290] C3b bound to a pneumococcal cell wall underneath a thick polysaccharide capsule is not accessible to CR1 on phagocytes and does not promote effective opsonophagocytosis. Similarly, complement-mediated killing of some strains of *Salmonella* is prevented when the MAC is bound to long LPS molecules distant from the organism's cell membrane.[309]

Opsonic Activity

Complement opsonic activity is essential for effective removal of organisms from the circulation by macrophages in the liver and spleen and from other sites by neutrophils and tissue macrophages.[82] Opsonins facilitate recognition, binding, ingestion, and killing of microorganisms by phagocytes. Opsonization is particularly important for protection against gram-positive bacteria and fungi because their thick cell walls prevent them from being killed by the MAC.

As noted earlier, activation of C3 leads to a conformational change in C3b that permits its reactive thioester to bind covalently with acceptor molecules on microbial surfaces, where it can serve as an opsonin. C3b also can be cleaved by inactivators such as factor I and CR1 to form iC3b, which may be a more efficient opsonin than C3b.[250,291] Some organisms, including certain serotypes of pneumococci, have surfaces that support degradation of differing amounts of surface-bound C3b to iC3b.[290] Surface-bound C3b and iC3b permit microbes to be recognized by circulating and tissue phagocytes by interacting with the phagocyte surface complement receptors, CR1 (CD35) and CR3 (CD11b/CD18). These interactions lead to binding, ingestion, and intracellular killing of the organisms.[250,291,353]

Antibodies are important opsonins in their own right, but they also facilitate more rapid complement activation and more effective localization of C3b binding to the surface of the organism. In the absence of specific antibody to direct complement binding, complement deposition on the surface of the target would be determined principally by the nature of the surface, and effector functions such as opsonization may not be performed as successfully. As noted earlier, this matter is of particular importance for encapsulated organisms, such as pneumococci, because in the absence of specific anticapsular antibody, C3b would be deposited at the cell wall, where it is inaccessible to phagocyte receptors.[97,290,311]

Inflammation

The cleavage products of several complement proteins contribute to the development of an inflammatory response. C3a stimulates an increase in the number of circulating granulocytes, and C5a serves as a potent stimulus for migration of monocytes, neutrophils, and eosinophils toward the source of C5a gradients being produced at infected tissue sites. C5a also up-regulates phagocytes' expression of CR1 and CR3 and stimulates them to release stored enzymes and other granular contents that also are important mediators of inflammation, aggregation, and production of microbicidal oxidants. C5a-induced neutrophil aggregation and stasis in the pulmonary circulation can be an important feature of the respiratory distress syndrome associated with sepsis.[610]

The anaphylotoxins, C4a, C3a, and especially C5a, induce release of histamine from mast cells and basophils, causing increased vascular dilation and permeability, which permit local influx of other inflammatory mediators.[294] In this way, they help produce the hallmark clinical manifestations of inflammation, swelling, and erythema. When large quantities of anaphylotoxins are released rapidly, they can contribute to septic shock.[212] Carboxypeptidase treatment of C4a, C3a, and C5a destroys their anaphylatoxin activity, but the C5a cleavage product $C5a_{des\ arg}$ maintains some chemoattractant-activating and phagocyte-activating activity.[323]

Microbicidal Activity

Complement can act directly on certain bacteria to kill them. As noted earlier, C5b and the terminal complement proteins C6, C7, C8, and C9 form the MAC, which can kill and lyse target cells such as gram-negative bacteria by penetrating their outer membranes.[309] The C5b-C8 complex serves as a polymerization site for several molecules of C9.[71] Although C9 is not essential

to membrane penetration, its presence as poly-C9 allows it to proceed more efficiently.[569] Electron microscopy of the MAC shows that it is composed of a ringlike structure at the outer surface of the target cell membrane and a perpendicular cylindrical component that penetrates the cell membrane. As has been noted, the MAC cannot penetrate the thick cell walls of gram-positive bacteria and fungi and cannot kill these organisms directly.

The MAC can lyse some virus-infected host cells and some enveloped viruses themselves.[152] Additionally, C1 and C4 can enhance neutralization of virus by antibody.

Immune Regulation

Complement components and fragments can modulate several features of the immune response—directly by binding to CR1, CR2, and CR3 on the surfaces of T cells, B cells, and other cells involved in antigen recognition and indirectly by stimulating the synthesis and release of cytokines.[204] The C3b cleavage product, C3dg, when covalently bound to antigen, brings the antigen close to B cells by binding to B-cell CR2 (CD21).[73,88,119,178,439] Complement decreases the amount of antigen required to induce an immune response and increases the efficiency of the process by facilitating presentation and localization of antigen. C3 influences antigenic localization within germinal centers, and it is involved in anamnestic responses and isotype switching. The importance of complement in the immune response has been shown by the finding that C1-deficient, C2-deficient, C4-deficient, and C3-deficient animals have decreased antibody responses that can be restored by providing the missing protein.[73,88,119,178,439] Deficiency of early complement components, such as C2, in humans also has been associated with antibody deficiencies.[17,119]

Phagocytes

The first recognized cellular mechanism of host defense was the accumulation of phagocytic host cells around a foreign body in starfish observed by Metchnikoff.[397] PMNs, the most abundant circulating phagocytes in the human host, serve as a model here for discussing phagocyte functions. These cells constitute a major line of defense against invading bacteria and fungi. The proliferation of myeloid marrow progenitors and their differentiation into mature progeny are regulated by specific growth factors and cytokines.[47,367,576] The normal half-life of circulating PMNs is approximately 8 to 12 hours.[604] In the absence of active infection, most PMNs leave the circulation via the gingival crevices and the lower gastrointestinal tract, where the resident flora stimulate ongoing local extravasation of PMNs, a process that helps maintain the integrity of these tissues.[30] In response to invasive bacterial infection, circulating PMNs engage in three major functions: (1) migration to the site of infection, (2) recognition and ingestion of invading microorganisms, and (3) killing and digestion of these organisms.

PHAGOCYTE RECRUITMENT TO INFECTED SITES

Activation of endothelial cells that line the microvessels of acutely infected tissue occurs via locally produced cytokines, eicosanoid compounds, and microbial products.[115,534] As a result, the endothelial cells rapidly up-regulate their surface expression of P-selectin and then E-selectin.[70,537] These selectins engage in lectin-like interactions with the fucosylated tetrasaccharide moiety sialyl Lewis X, which is presented on constitutively expressed glycoproteins on PMNs, including L-selectin and P-selectin glycoprotein ligand-1.[350,366,631] These early interactions slow the PMNs in this first adhesive phase of leukocyte recruitment, sometimes described as "slow rolling."[70,115,534]

Within several hours, newly synthesized ICAM-1 is expressed at the endothelial surface.[115,534,536] The slowly rolling PMNs are activated by transient selectin-mediated interactions and locally produced mediators, especially endothelium-derived chemokines, such as IL-8.[365] These chemokines are most effective in activating the PMNs when they are bound by complex proteoglycans at the endothelial cell surface.[346] The activated PMNs produce a conformational activation of their surface β_2 integrins LFA-1 and Mac-1[179,595] and translocate an additional large quantity of Mac-1 from intracellular storage pools to the cell surface.[63,84] The newly translocated Mac-1 also may undergo this activation as the PMN is exposed to increasing concentrations of activating mediators.[179,282]

These activated β_2 integrins interact with the endothelial cell ICAM-1 in this second, firm adhesion phase mediated by integrin-ICAM interactions, which ultimately is necessary for transendothelial migration of the PMNs.[63,115,179,365,525,534,536] Other chemoattractants, such as C5a, N-formyl bacterial oligopeptides, and leukotrienes (e.g., leukotriene B_4), which diffuse from the site of infection, activate PMNs further and provide a chemotactic gradient for migration of PMNs into tissue.[241,419] The receptors for these chemoattractants, similar to the chemokine receptors, are G protein–coupled and have a seven transmembrane–domain structure.[241,419] They constitute important sensory mechanisms of the PMNs for activating adhesion, directional orientation, and the contractile protein-dependent lateral movement of adhesion sites in the PMN membrane necessary for migration.[26,241,419,561]

A scheme for recruitment of PMNs from the microcirculation into infected tissue is presented in Figure 2–2, and a more detailed diagram depicting the surface molecules that mediate PMN-endothelial adhesion is provided in Figure 2–3. Although the specific stimuli and adhesion molecules may vary, this general scheme applies to the local recruitment of virtually all circulating cells of the immune system.[75,244,324]

PHAGOCYTOSIS

After PMNs reach the site of infection, they must recognize and ingest, or phagocytose, the invading bacteria. Opsonization, especially with IgG and fragments of C3, greatly enhances phagocytosis.[290,310] Although non-opsonic phagocytosis may occur, only opsonin-mediated phagocytosis is discussed here. CR1 and CR3, respectively, are the main phagocytic receptors for opsonic C3b and iC3b.[63,213] When PMNs are activated by chemoattractants or other stimuli, CR1 and CR3 are translocated rapidly to the cell surface from intracellular storage compartments, increasing surface expression 10-fold.[63,213] CR3 is identical to the adhesion-mediating integrin Mac-1.[36,63] CR1 and CR3 act synergistically with receptors for the Fc portion of antibodies, especially IgG.[36,310]

Phagocytic cells may express three different types of IgG Fc receptors, or FcγRs, all of which can mediate phagocytosis.[209,587] FcγRI (CD64) is a high-affinity receptor that is expressed mainly on mononuclear phagocytes.[587] The two FcγRs ordinarily expressed on circulating PMNs are FcγRII (CD32) and FcγRIII (CD16).[576,587] FcγRII is conventionally anchored in the cell membrane, exhibits polymorphisms that determine preferences for binding of certain IgG subclasses, and can activate PMN oxidative burst activity directly.[576,587,588] FcγRIII is expressed on PMNs as a glycolipid-anchored protein, although it is anchored conventionally on NK cells and macrophages.[587] Most phagocytes also express IgA receptors. The best-characterized FcαR, CD89, binds monomeric IgA and promotes phagocytosis and killing of IgA-opsonized bacteria.[292,409]

The engagement of phagocyte receptors with microbial opsonins on microbes locally activates cytoskeletal contractile elements, leading to invagination of the phagocyte membrane at

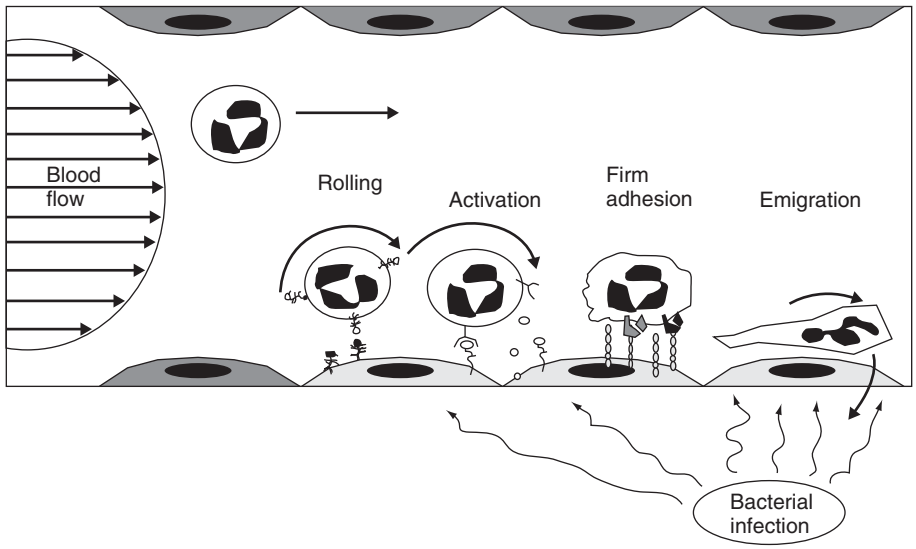

Figure 2–2 Events during polymorphonuclear leukocyte (PMN) recruitment to infected sites. Interactions between microorganisms in infected tissue and host cells and proteins result in elaboration of mediators that diffuse to the local microcirculation and stimulate the endothelial cells. This stimulation induces new surface expression of P-selectin and E-selectin, release of interleukin-8 and other chemokines, and new surface intercellular adhesion molecule 1 (ICAM-1). The endothelial selectins bind to constitutively expressed carbohydrate ligands on circulating PMNs and slow the passage of the PMNs through the microvessels. As the PMNs slow further, they become activated by interaction with chemokines bound to complex glycopeptides on the endothelial surface. This activation of PMNs increases their expression and binding activity of the β_2 (CD11/CD18) integrins, Mac-1 and lymphocyte function-associated antigen 1 (LFA-1). Interactions between these integrins and ICAM-1 (and ICAM-2 in the case of LFA-1) lead to tight adhesion and spreading on the endothelial surface. These latter adhesive interactions also are used for migration between endothelial cells and through the subjacent extracellular matrix in response to the gradient of chemoattractants, such as C5a and bacterial peptides, released at the infected site. Homophilic interactions between platelet–endothelial cell adhesion molecule-1 (PECAM-1) on the PMNs and endothelial cells also seem to contribute to transendothelial migration. *(Drawing courtesy of Dr. Scott Seo.)*

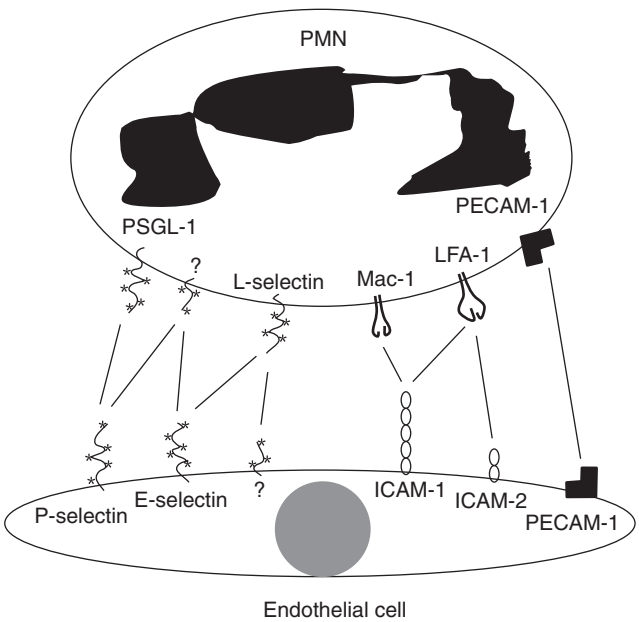

Figure 2–3 Molecular interactions that mediate polymorphonuclear leukocyte (PMN)–endothelial adhesion. The selectins, P-selectin and E-selectin on endothelium and L-selectin on leukocytes, bind to oligosaccharide moieties, including sialyl Lewis X (sLeX), which decorate the selectins and other glycoproteins on the opposite cell, including P-selectin glycoprotein ligand-1 (PSGL-1) on leukocytes. These interactions mediate the early adhesive phase of leukocyte recruitment. The β_2 integrins macrophage antigen 1 (Mac-1) (CD11b/CD18) and lymphocyte function-associated antigen 1 (LFA-1) (CD11a/CD18) on PMNs bind intercellular adhesion molecule 1 (ICAM-1) on endothelial cells. LFA-1 also can bind ICAM-2, which is expressed constitutively on endothelium. Platelet–endothelial cell adhesion molecule-1 (PECAM-1) molecules interact with each other and are expressed on PMNs and endothelial cells.

the site of initial engagement and the extension of pseudopods around the microbe. The ligation of additional opsonin-receptor pairs leads to engulfment of the microbe within a sealed phagosome,[560] as depicted in Figure 2–4. This engulfment is followed by fusion of the phagosome with lysosomal compartments containing the phagocyte's array of microbicidal products.

PHAGOCYTE MICROBICIDAL MECHANISMS

The microbicidal mechanisms of PMNs usually are categorized as either oxygen-dependent or oxygen-independent,[492] and intracellular killing usually occurs after the phagosome fuses with one or more types of lysosomal granules that contain many of the cell's microbicidal weapons. The contents of the three major populations of intracellular granules of PMNs, which contain many of the PMN's microbicidal molecules, either free within the granules or anchored in the granule membrane, are shown in Table 2–2.[84,85,235] Oxygen-dependent microbicidal mechanisms of phagocytes depend on a complex enzyme, nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, which converts molecular oxygen (O_2) into superoxide anion (O_2^-).[42,135] This enzyme is assembled at the activated cell membrane from six or more components that include a cytochrome (*a* and *b* subunits, designated $gp91^{phox}$ and $p22^{phox}$ ["phox" refers to phagocyte oxidase]), a flavoprotein, and a quinone, all of which are associated with the cell membrane, and at least two cytoplasmic proteins, $p47^{phox}$ and $p67^{phox}$, which assemble with the membrane-associated components to form the active enzyme complex.[42,84,135] Each of the main oxidant products derived from these reactions exhibits microbicidal activity, including the earliest products, O_2^- and hydrogen peroxide (H_2O_2), which are less potent than the downstream products hypochlorite (OCl^-) and chloramines (NH_3Cl, RNH_2Cl), chloramines being the most stable.[492] Figure 2–5 shows the sequence of the main oxidative reactions of PMN after the formation of superoxide anion.

TABLE 2–2 Stored Contents of Neutrophil Granules and Vesicles

Primary (Azurophilic) Granules	Secondary (Specific) Granules[a]	Tertiary Granules[b]	Secretory Vesicles[c]
Elastase	Lactoferrin	Gelatinase	Alkaline phosphatase
Cathepsin G	Vitamin B_{12}–binding protein		
Myeloperoxidase	Lysozyme		
Defensins	Gelatinase		
Bactericidal/permeability-increasing protein "p15s"			
Cathelicidin (LL37)			
Lysozyme			

Selected membrane-bound proteins in intracellular granules and vesicles:
f-met-leu-phe receptor[a,b,c]
type 1 complement receptor (CR1)[c]
CD11b/CD18 (CR3, Mac-1)[a,b,c]
cytochrome b_{558}*[a,b,c]*
type III Fcγ receptor (CD16)[?,c]

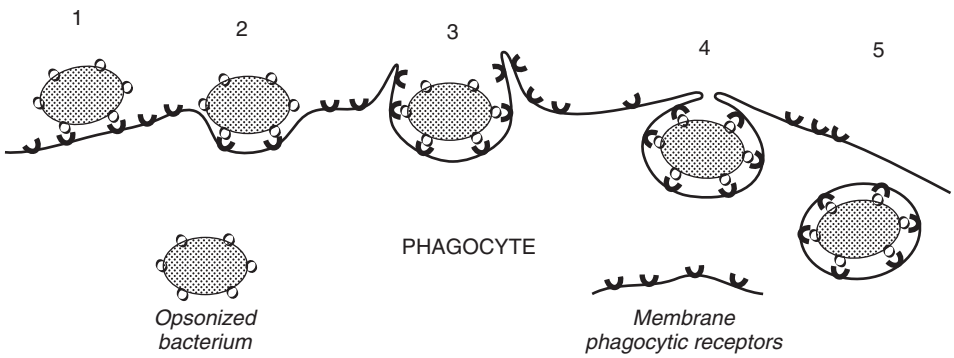

Figure 2–4 Opsonin-receptor-mediated phagocytosis. Phagocytes such as polymorphonuclear leukocytes (PMNs) that reach the site of bacterial infection are activated for enhanced recognition, attachment, and ingestion of bacteria that have been coated with opsonic C3 fragments, IgG, or both via specific receptors on the PMN surface. Binding of opsonized bacteria to opsonin receptors (1) activates contractile elements of the cytoskeleton to produce an invagination at the initial site of attachment (2), with subsequent extension of membrane pseudopods around the organism (3). This process allows engagement of additional opsonin-receptor pairs, as the organism becomes engulfed (4). Finally, the plasma membrane fuses completely around the bacteria to create the phagosome (5), which soon fuses with lysosomal granules, exposing the bacterium to the microbicidal components of the phagocyte.

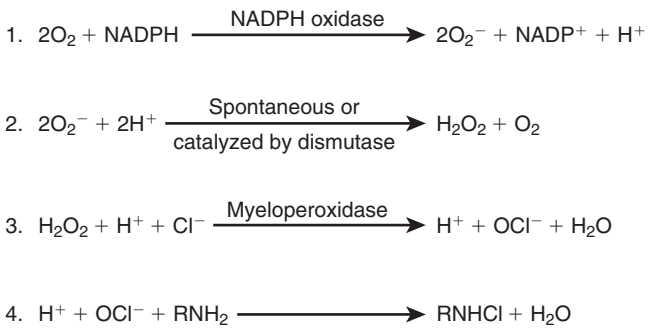

Figure 2–5 Major reactions in the evolution of oxygen-dependent polymorphonuclear leukocyte microbicidal activity. The conversion of molecular oxygen to superoxide anion (O_2^-) by nicotinamide adenine dinucleotide phosphate (NADPH) oxidase is the initial event in the sequence of production of antimicrobial oxidants. Shown in order are the subsequent reactions for production of hydrogen peroxide (H_2O_2), hypochlorite (OCl^-), and chloramines (RNH_2Cl).

Oxygen-independent microbicidal activity of PMNs resides mainly in a group of proteins and peptides stored within primary (azurophilic) granules. Lysozyme is contained in the primary and the secondary (specific) granules of PMN.[551] It cleaves important linkages in the peptidoglycan of bacterial cell walls and is most effective when it can act in concert with the complement MAC.[310] The primary granules contain several cationic proteins with important microbicidal activity. A 59-kd protein, bactericidal/permeability-increasing protein, is active against only gram-negative bacteria.[612] Smaller arginine-rich and cysteine-rich peptides, the α-defensins, similar to the β-defensins of epithelial cells, are active against a range of bacteria, fungi, chlamydiae, and enveloped viruses, and other related molecules include cathelicidin and a group of peptides called p15s.[230,234,235,363] The mechanisms of action of these peptides are not fully understood, although they are addressed in the earlier section on antimicrobial peptides of epithelial cells. Some of these PMN proteins and peptides may interact with each other synergistically to enhance overall antimicrobial activity.[362]

Important Interactions among Innate Immune Mechanisms

A schematic overview of many of the main features of innate immunity discussed previously, along with some of their important interactions, is diagrammed in Figure 2–6. Several levels of interactions are depicted, from initial host-pathogen contact, through a variety of activating signals, to the attack by host effector mechanisms on pathogenic targets.

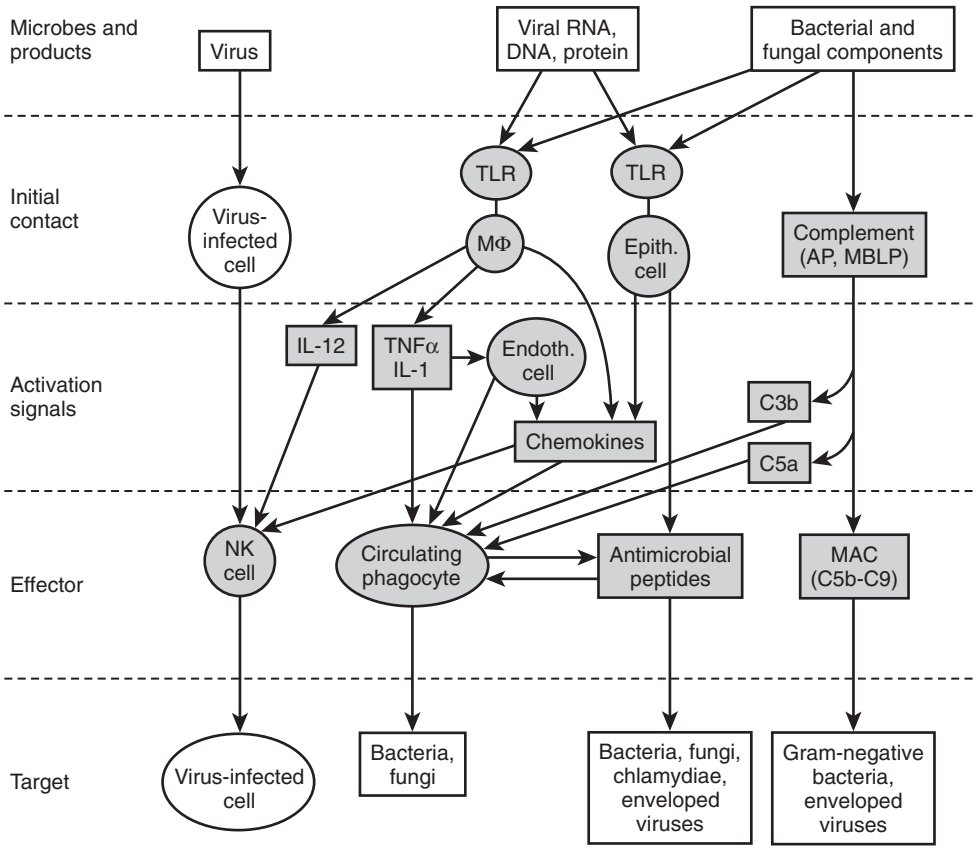

Figure 2–6 Innate immunity: first contact, intermediate signals, and effector mechanisms. Diagrammed are important host responses to infection that are independent of specific cell-mediated immunity or antibodies. Initial contact between the host and microbes or their products may result in viral infection of cells, activation of Toll-like receptors (TLRs) on macrophages (MΦ) and epithelial cells, and activation of the alternative pathway (AP) or mannose-binding lectin pathway (MLBP) of complement. The resulting activation signals, including cytokines (e.g., interleukin-12 [IL-12], tumor necrosis factor–α [TNF-α], IL-1), chemokines, and products of the complement cascade, mobilize cellular (natural killer [NK] cells, phagocytes) and humoral (antimicrobial peptides, membrane attack complex [MAC]) effectors, which attack their respective microbial targets. *(From Tosi, M. F.: Innate immune responses to infection. J. Allergy Clin. Immunol.* 116*:241-249, 2005.)*

ADAPTIVE IMMUNE RESPONSES

Adaptive immunity involves the host's antigen-specific responses to infectious challenges that, once modified, can help clear some infections and can provide specific protection against subsequent challenges. The major steps in the development of adaptive immunity include processing and presentation of specific antigens by APCs, activation of T lymphocytes for specific cytotoxic T-cell activity, T-cell cytokine production, and T-cell help in activating and maturing of antigen-specific B cells for the production of specific antibodies. Innate immune responses often occur in minutes to hours and may activate early cellular responses that are essential for the development of adaptive immunity. The full development of most adaptive immune responses requires days to weeks. When developed, however, adaptive immune responses often can provide durable protection. Figure 2–7 depicts the major events in the adaptive immune response to infection.

Antigen Presentation and Specific Cell-Mediated Immunity

Specific cell-mediated immunity provides T-cell help for production of antibodies by B cells, cytokine production for the stimulation and regulation of a range of immune responses, and cytotoxic T-cell activity against host cells infected with viruses.[184,414,461] The development of cell-mediated immunity requires complex interactions to occur between T cells and APCs via several types of surface molecules on the respective cell surfaces. These interactions include binding of an antigen-specific T-cell receptor (TCR) on the T lymphocyte to a peptide antigen presented on the class I or class II MHC by the APC, with concurrent binding of the class I or class II MHC by CD8 or CD4,[574] as shown in Figure 2–8. Other respective pairs of accessory cell/surface molecules that enhance interactions between T cells and APCs include CD40L/CD40, LFA-1/ICAM-1, and CD28/B7. An additional molecule, cytotoxic T lymphocyte antigen-4, expressed on activated T cells, also can bind to B7 molecules on APCs to generate a suppressive signal that may terminate T-cell activation.[574] The sustained physical interface between T cells and APCs at which these molecular interactions take place has been characterized as the *immunologic synapse*.[96,252]

CLASS I MAJOR HISTOCOMPATIBILITY COMPLEX

Virtually all human cells except neurons express class I MHC.[161] The class I MHC molecule presents antigenic peptides to $CD8^+$ cytotoxic T lymphocytes.[431,565] It consists of a heavy chain that contains the peptide-binding domain and a transmembrane domain and a smaller extracellular subunit, β_2-microglobulin.[77] The three major types of class I MHC heavy chains in humans—HLA-A, HLA-B, and HLA-C—have at least 22, 31, and 12 different alleles, respectively.[630] This polymorphism permits a great diversity in the peptide-binding repertoire in individuals and

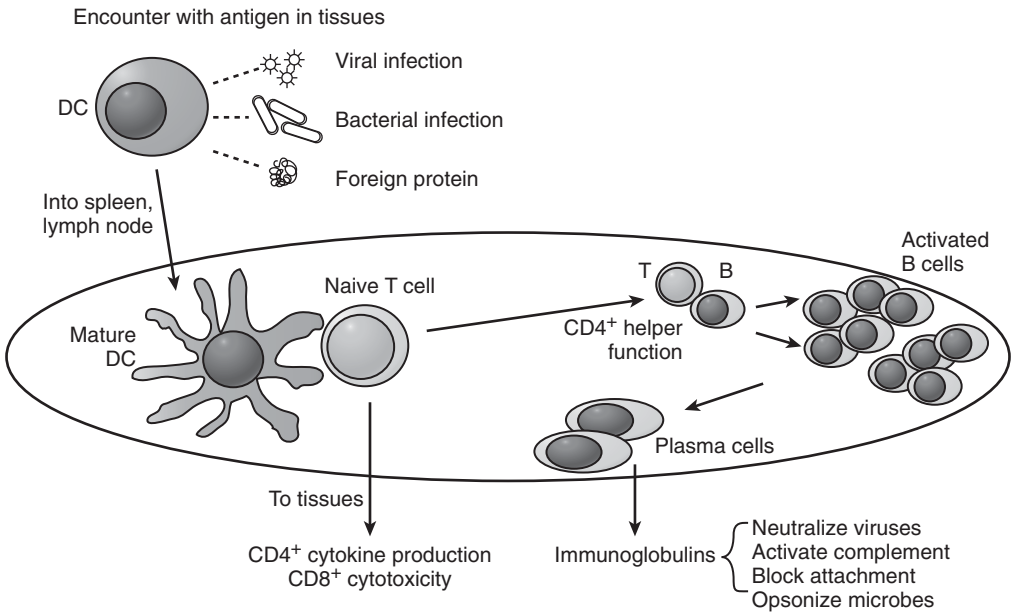

Figure 2–7 A simplified scheme of major events in the development of adaptive immune responses. When antigen-presenting cells, such as dendritic cells (DC), encounter and internalize microbes or their protein antigens in peripheral tissues, they process the microbial proteins and present the resulting antigenic peptides on either class I or class II major histocompatibility complex (MHC) molecules. The activated antigen-presenting cells migrate to lymphoid tissue, where they undergo maturation. When mature DC encounter naive $CD4^+$ or $CD8^+$ T cells expressing T-cell receptors (TCRs) specific for the peptides presented in the appropriate MHC context (CD4/MHC-II; CD8/MHC-I), binding between the cells occurs via TCR/peptide, MHC/$CD4^+$ or MHC/$CD8^+$, and other pairs of accessory molecules, all necessary for stimulating the T cells to become effector cells. Cytotoxic effector $CD8^+$ T cells migrate into the periphery and kill virus-infected cells that present viral peptides via MHC class I. Effector $CD4^+$ cells produce cytokines or provide help for proliferation of antigen-specific B cells in the lymphoid germinal centers and eventual production by plasma cells of specific antibodies that can neutralize viruses, prevent microbial attachment, opsonize microorganisms, or activate complement.

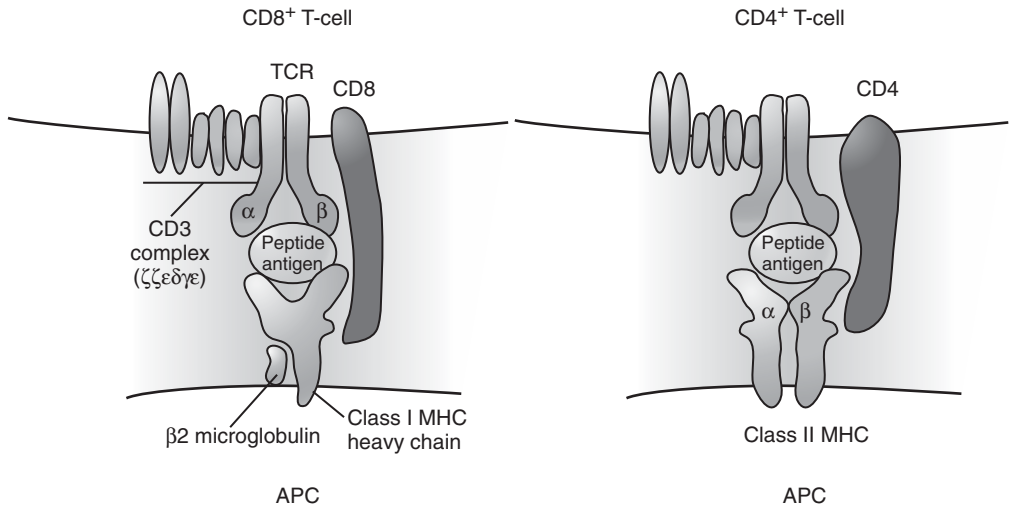

Figure 2–8 Principal cell surface interactions between $CD8^+$ and $CD4^+$ T lymphocytes and peptide antigens complexed with major histocompatibility complex (MHC) class I and class II molecules, respectively. CD3 (composed of six subunits, ζ, ζ, ε, δ, γ, ε) is associated closely with the T-cell receptor (TCR), which recognizes a specific peptide presented on MHC molecules. Class I and class II MHC determinants are recognized by $CD8^+$ and $CD4^+$. Additional or accessory interactions are discussed in the text. APC, antigen-presenting cell. *(Adapted from Lewis, D. B., and Wilson, C. B.: Developmental immunology and role of host defenses in neonatal susceptibility to infection.* In *Remington, J. S., and Klein, J. O. [eds.]: Infectious Diseases of the Fetus and Newborn Infant. 6th ed. Philadelphia, W. B. Saunders, 2006, p. 92.)*

within populations. A restricted degree of MHC genetic polymorphism has been invoked as a possible explanation for the predisposition of certain populations to develop infections.[78]

Class I MHC molecules within the cell ordinarily bind peptides derived from recently synthesized proteins, either of self origin or of infecting viruses.[207,208] A portion of newly synthesized proteins is processed into peptides at a cytoplasmic site termed the *proteasome*.[245] These peptides actively are transported into the endoplasmic reticulum, where they are bound in the peptide-binding cleft of MHC class I. Suitable peptides usually are restricted to 8 to 10 amino acids in length, and they must contain certain amino acids at specific "anchor" positions on the peptide

to bind.[295] Allelic variants of MHC class I may require different amino acids at these anchor positions.[227] The other amino acids of the peptide constitute the antigenic determinant recognized, after trafficking of the MHC-peptide to the cell surface, by a specific TCR on $CD8^+$ cytotoxic T cells, the latter concurrently binding the heavy chain of MHC class I via CD8.[227,295]

CLASS II MAJOR HISTOCOMPATIBILITY COMPLEX

Mononuclear phagocytes, B lymphocytes, and dendritic cells, including specialized tissue-specific dendritic cells such as the Langerhans cells of the skin, all serve the immune system as "professional" APCs.[597] Dendritic cells, the most efficient APCs for primary activation of naive T cells, are macrophage-like cells of a distinct lineage that take up and process antigens in tissues and migrate to local lymph nodes or to the spleen, where they are likely to encounter T cells specific for the presented antigens.[191,263,489,565] A defining feature of these professional APCs is their expression of class II MHC molecules in addition to class I MHC.[161]

Class II MHC molecules are composed of an alpha and a beta chain, which together form a peptide-binding cleft.[98,503] Class II MHC molecules present peptides, 13 to 17 amino acids in length, derived from proteins that are internalized by endocytosis or during phagocytosis of microorganisms.[261,296,503] There are three major types of class II MHC alpha and beta chains—HLA-DR, HLA-DP, and HLA-DQ—each exhibiting a high degree of polymorphism.[383] MHC class II, bound to a separate smaller molecule known as the *invariant chain*, trafficks via the Golgi to endosomal/lysosomal compartments, where it must dissociate from the invariant chain to bind antigenic peptides derived from internalized proteins.[487,572,573] The class II MHC-peptide complexes then move to the cell surface, where the peptides are bound by TCRs of $CD4^+$ T cells, which concurrently bind class II MHC via CD4.[192,372]

Figure 2–9 depicts the essential features of the conventional antigen presentation pathways that involve class I and class II MHC molecules, as described earlier. Alternative mechanisms have been documented by which class I MHC can present peptides derived from internalized exogenous proteins, and class II MHC may present peptides derived from newly synthesized proteins. The importance of these unconventional pathways of antigen presentation in the immune response is not fully understood, but evidence indicates that such "cross-presentation" may be important for generation of $CD8^+$ cytotoxic T-cell response against some viruses or fungi taken up via endocytosis by APCs.[38,313]

CD1 FAMILY OF ANTIGEN-PRESENTING MOLECULES

The CD1 family comprises proteins with significant homology and structural similarity to the MHC class I heavy chain, but

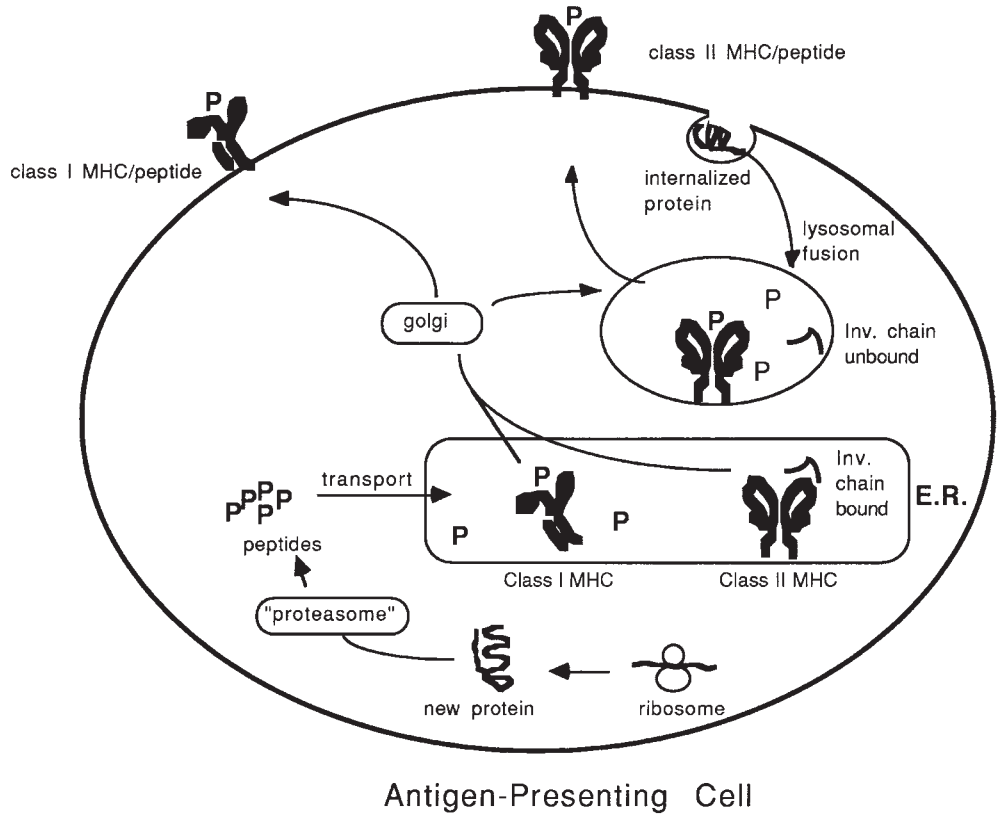

Figure 2–9 Conventional pathways for peptide antigen presentation by class I and class II major histocompatibility complex (MHC) molecules. In the antigen-presenting cell, a proportion of newly synthesized proteins undergoes proteolysis into peptides by enzymes that constitute the "proteasome." The peptides actively are transported into the endoplasmic reticulum (E.R.), where peptides with the appropriate length and sequence bind to MHC class I molecules. MHC class II cannot bind peptides in the E.R. because of interference by the associated "invariant chain." The class I MHC/peptide complex is transported via the Golgi to the cell surface, where it may be recognized by $CD8^+$ lymphocytes. Class II MHC molecules pass via the Golgi to a lysosomal compartment, where conditions favor the release of the invariant chain. This release permits class II MHC to bind peptides derived from internalized proteins that have entered the lysosomal compartment via fusion of endosomes or phagosomes with the lysosome. The lysosome translocates to the cell surface, where the class II MHC/peptide complex may be recognized by $CD4^+$ lymphocytes.

which present lipid and glycolipid antigens. All mammalian species express one or more members of the CD1 family, principally on professional APCs. Four human CD1 proteins—CD1a, CD1b, CD1c, and CD1d—have been identified, each tightly associated with a β_2-microglobulin subunit. Mycolic acid, lipoarabinomannans, and other related components of mycobacteria are the best-documented foreign antigens presented by CD1 molecules, and internalized antigens and antigens synthesized within the APCs by ingested mycobacteria may be presented via distinct trafficking patterns of the CD1-antigen complexes.

Researchers have hypothesized that other antigens, such as the lipoteichoic acids of gram-positive bacteria, the complex capsular polysaccharides of *H. influenzae* and *N. meningitidis*, and the glycosylphosphatidylinositol components of glycosylphosphatidylinositol-linked proteins of malarial parasites and trypanosomes, also could be presented by CD1 molecules. Antigens presented on APC by CD1 molecules are recognized by a specialized subset of CD1-restricted T cells that usually lack CD4 and CD8, known as NK/T cells. These cells share characteristics of NK cells and T cells, exhibiting a limited range of TCR specificity. They represent a sizeable fraction of the T cell compartment, although their function in the immune response is incompletely understood. Greater detail regarding the structure, function, phylogeny, trafficking, expression, and T cell interactions for members of the CD1 family may be found in an extensive review.[470]

T Lymphocytes

The development of T lymphocytes, or T cells, begins when prothymocytes leave the marrow and enter the subcapsular region of the thymus.[190] By mechanisms that are poorly understood, the thymic environment induces the rearrangement of TCR V (variable), D (diversity), and J (joining) gene segments with the eventual expression of mature alpha-beta TCRs complexed with CD3. The T cells, now co-expressing CD4 and CD8, migrate to the thymic cortex, where they undergo screening for TCR specificity to optimize the repertoire for distinguishing self from non-self and to eliminate TCR rearrangements that result in undesirably high self-reactivity. The mechanism by which this screening occurs is the subject of intense investigation and theoretical controversy, and several reviews of the subject are cited here.[249,317,387,396] Thymocytes that do not pass this dual screening procedure receive signals that induce programmed cell death (apoptosis).[396,417,600] Only approximately 5 percent of the original thymocytes pass this screening, after which they express either CD4 or CD8, but not both.[396,417,600]

Mature thymocytes are released into the periphery, where the $CD4^+$ cells serve as the main source of IL-2 and provide help for B-cell antibody production, and the $CD8^+$ cells engage in specific cytotoxic activity. This discussion of T cells and TCRs specifically relates to T cells that express TCRs composed of alpha and beta chains, or alpha-beta T cells. T cells of a distinct type, gamma-delta T cells, are far less numerous in most tissues (intestinal epithelium is a notable exception); exhibit much less TCR diversity than do alpha-beta T cells; may not require an intact thymus for development; and play a role in host responses to certain intracellular bacterial pathogens, including *Listeria* and mycobacteria.[110,264]

Antigen specificity of alpha-beta T cells resides in their TCRs, which are integral membrane proteins that exhibit structural homology with immunoglobulins. TCR diversity results from a rearrangement of V, D, and J segments. There are 100 different V segments, 1 D segment, and 100 different J segments in the complete germline configuration of the TCR genes. Rearrangement of these gene segments into a mature VDJ sequence occurs by the action of a recombinase enzyme complex formed by two proteins, RAG-1 and RAG-2.[440,516] TCR diversity is generated by several factors, including the range of possible combinations of V, D, and J segments; the imprecise action of the recombinase complex; the variability in the number of nucleotides deleted during rearrangement; and the action of another enzyme, *terminal deoxytransferase*, which seems to add nucleotides at random to extend segments during rearrangement.[215,533] The actions of Artemis and DNA ligase IV, two enzymes crucial for the processing and joining of DNA ends, introduce additional sources of variability.[103,200,376] Researchers have estimated that 10^{15} different TCR specificities theoretically could result from the several mechanisms involved in TCR segment rearrangement.[168]

Stimulation of naive $CD4^+$ or $CD8^+$ T cells occurs as they circulate through peripheral lymphoid tissue and encounter dendritic cells and other professional APCs. Localized T-cell migration is highly regulated by specific chemokines and adhesive interactions with local endothelium and involves mechanisms similar to the mechanisms discussed earlier for circulating phagocytes.[194,342] When T cells engage APCs presenting specific peptide antigens on the appropriate MHC molecules, they are activated via their TCR and several costimulatory molecules, especially CD28, to produce IL-2 and to proliferate and differentiate into effector T cells.[134,299] Effector $CD4^+$ T cells may be of the T_H1 or T_H2 type, and this type is influenced by several factors, including the specific cytokines elicited by a particular microbial pathogen. Naive T cells activated in the presence of IL-12 and IFN-γ are likely to develop into T_H1 cells, whereas IL-4 and IL-6 tend to drive development in the direction of T_H2 cells.[413,421] Preferential development of T_H1 effector cells leads mainly to macrophage activation and cell-mediated immunity, whereas T_H2 effector cells, which provide more effective B-cell help, drive the development of humoral immunity.[418]

Activation of naive $CD8^+$ T cells by antigen binding, costimulation by accessory binding molecules on APCs, and exposure to cytokines, including IL-2, leads to clonal proliferation of specific $CD8^+$ cells and their differentiation into cytotoxic effector cells. Effector $CD4^+$ T cells bound in common to an APC may play a role in activating naive $CD8^+$ T cells, either by releasing IL-2 or by activating the APC to provide greater co-stimulation to the $CD8^+$ T cell to make its own IL-2.[33] Antigenically experienced effector $CD8^+$ T cells respond to specific antigen and co-stimulatory molecules on infected host cells by activating cytotoxic mechanisms similar to those described earlier for NK cells, including the release of perforin and granzymes and generation of receptor-mediated signals for target cell apoptosis.[367,504,583]

T-CELL MEMORY

Some proportion of activated $CD4^+$ and $CD8^+$ T cells become endowed with the capacity for long-term antigenic memory and rapidly can become effectors on re-exposure to specific antigen. Whether these cells develop directly from naive T cells or previously have been effector cells, or both, is uncertain, and the mechanisms by which they become memory T cells is poorly understood. Among the features of memory T cells are high levels of expression of CD45RO, the ability to suppress activation of naive T cells of the same specificity, and a homeostatic level of ongoing proliferation in bone marrow and peripheral lymphoid organs.[314,378,509]

T-CELL ACTIVATION BY SUPERANTIGENS

The term *superantigen* describes a class of protein antigens, mainly microbial exotoxins, including most staphylococcal enterotoxins, staphylococcal toxic shock syndrome toxin-1 (TSST-1), and related streptococcal TSST-1-like toxins. These bacterial toxins are potent pyrogens, can induce a potentially lethal toxic shock syndrome, and share binding domains for TCR V regions and MHC class II molecules. Superantigens bypass normal antigen

processing and presentation pathways by binding directly to class II MCH molecules on APCs and to specific variable regions on the beta chain of the T-cell antigen receptor. Through these interactions, superantigens induce a polyclonal activation of T cells at orders of magnitude above levels induced by antigen-specific activation, resulting in massive release of cytokines from T cells and APCs, including TNF-α, TNF-β, IL-1, IL-2, and IFN-γ, which are thought to be responsible for the most severe features of toxic shock syndromes.[16,476]

REGULATORY T CELLS

The existence of T suppressor cells was long a subject of debate among immunologists. Only since the 1990s has solid evidence been developed to support the existence of suppressor T cells, now referred to as *regulatory T cells*. These cells were discovered when thymectomized mice were noted to develop autoimmune disease. Transfer of T cells that expressed CD25, the alpha chain of the IL-2 receptor, from normal adult mice to thymectomized mice prevented autoimmune disease. This population of $CD4^+CD25^+$ regulatory T cells can suppress the activity of other immune cells and has been shown to prevent graft-versus-host disease and allograft rejection. The mechanism of suppression by regulatory T cells is uncertain but may involve direct contact with other cells or secretion of inhibitory cytokines, including IL-10. These inhibitory cytokines can interfere with T-cell proliferation and inhibit the ability of antigen-presenting dendritic cells to promote T-cell activation. The role of regulatory T cells in immunity to infection is only beginning to be studied; some current evidence suggests that the action of regulatory T cells with specificity for microbial antigens may suppress protective immune responses to some infections but also may suppress excessive or injurious host responses.[475]

B Lymphocytes and Immunoglobulins

B LYMPHOCYTES

B lymphocytes (B cells) are the source of humoral immunity in the form of specific immunoglobulin. The earliest recognizable marrow precursors of B cells are pro-B cells, surfaces of which bear the pan-B marker CD19. Further differentiation produces pre-B cells and then mature B cells, the latter expressing cell-surface immunoglobulin by which they recognize and bind antigen. B lymphocytes constitute approximately 20 percent of the lymphocytes in the circulation and peripheral lymphoid tissues, including the lymph nodes, spleen, bone marrow, tonsils, and intestines, and they are identified by the presence of surface immunoglobulin and the pan-B differentiation markers CD19 and CD20.[114,394]

B-cell activation is initiated by recognition and binding of specific antigens to B-cell surface immunoglobulins. Early activation leads to increased expression of receptors that either bind cytokines (e.g., IL-2, IL-4, IL-6) or interact with T cells,[349,459] leading to clonal proliferation and differentiation into memory B cells and plasma cells in the germinal centers of peripheral lymphoid tissue.[321] Some data suggest that B-cell differentiation into memory B cells is favored by exposure to the CD40L on dendritic cells in lymphoid organs, whereas differentiation into plasma cells is favored by exposure to CD23, IL-1a, IL-6, and IL-10.[321,571] The plasma cells, later found in bone marrow and liver and peripheral lymphoid tissue, are responsible for most free production of immunoglobulin.[571]

The B-cell response to protein antigens depends on T-cell help. B cells can process and present antigen to $CD4^+$ T cells they encounter in the lymph nodes and spleen.[320,321,571] B-cell surface immunoglobulin binds to a protein antigen, which is internalized, processed, and presented to the T cell via class II MHC molecules. B cell-mediated activation of T cells during antigen presentation is much more effective for memory T cells, whereas naive T cells are more likely to be turned off or rendered tolerant.[206,229] T-cell help is provided for B-cell proliferation and production of antibody against the specific protein antigen. It is mediated by signaling via CD40L interactions with CD40 on the B cell and by the release of cytokines, which also can induce isotype switching.[429,555] Most B-lymphocyte responses to polysaccharide antigens proceed largely without formal T-cell help, although antibody responses to some such antigens may be enhanced in the presence of T cells.[408]

IMMUNOGLOBULIN

Immunoglobulin molecules may be bound at the surface of B cells or free in the circulation, mucosal secretions, or tissues. Free immunoglobulins function in host defense against infection by binding to microbial surfaces to prevent microbial attachment, activating complement via the classical pathway, neutralizing viruses and toxins, and participating in the formation of immune complexes.[134]

Immunoglobulin molecules are composed of two identical heavy and two identical light chains, as diagrammed in Figure 2–10.[195] The carboxyl terminus of the immunoglobulin molecule is the heavy chain constant, or Fc, region. The amino acid sequence of this region determines the immunoglobulin isotype. The heavy chain is encoded by V, D, J, and constant (C) regions on chromosome 14.[66,605] Each immunoglobulin molecule has a pair of either kappa or lambda light chains, defined by distinct C regions. The variable region of the immunoglobulin molecule contains the antigen binding site. Similar to the TCR, the Fab region consists of two identical heavy and light chain pairs; similarly, broadly diverse antigen specificity results from the variable nature of recombinase-mediated DNA rearrangements of the three hypervariable, or complementarity-determining, regions

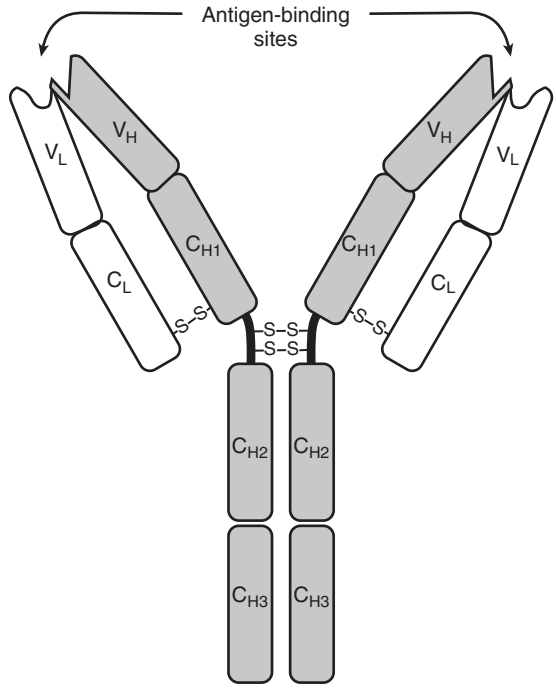

Figure 2–10 Structure of an immunoglobulin molecule. The schematic structure of IgG is shown, depicting the variable (V) and constant (C) regions of the heavy (H) and light (L) chains, the disulfide bonds that link the two heavy chains at the hinge region and the CL region with CH_1, and the antigen-binding sites formed by the complementarity-determining regions of VH and VL.

(CDR1, CDR2, and CDR3) and the four framework regions during development of B cells.[210,381]

The imprecision inherent in this rearrangement, involving mechanisms similar to those described for the TCR, leads to the generation of more than 10^{12} potential antigenic specificities. Somatic hypermutation of variable regions after gene rearrangement occurs adds to the repertoire, and further diversity results from differences in approximation of the three complementarity-determining regions in relation to each other, affecting the three-dimensional structure of the antigen recognition site.[406,458] In contrast to most TCRs, which recognize specific peptide sequences, immunoglobulin molecules can recognize the three-dimensional structures of antigens.[165]

All immunoglobulin is derived from B cells expressing surface IgM. B cells may change immunoglobulin isotype when they differentiate into plasma cells, which produce only one class or subclass of immunoglobulin each. Isotypes other than IgM are the result of isotype switching by replacing a part of the constant region of the immunoglobulin heavy chain with another isotype-specific segment.[555] As already noted, isotype switching primarily depends on specific B-cell interactions with cytokines and T cells. The variable region remains unchanged during isotype switching; no change occurs in antigen specificity. Important features of immunoglobulins, including half-life, localization in tissues, and interactions with cellular IgG receptors, are directly determined by isotype, however.[166]

IMMUNOGLOBULIN ISOTYPES

IgG accounts for approximately 80 percent of circulating immunoglobulin and includes the subclasses IgG1, IgG2, IgG3, and IgG4. The half-life of IgG ordinarily is approximately 21 days (7 days for IgG3).[303] Initial exposure to most microbial protein antigens first induces IgM and then an IgG response consisting of IgG1 and IgG3. IgG2 and IgG4 usually are produced during the secondary immune response. IgG1 usually is made in response to protein antigens.[303] In adults, the main antibody response to polysaccharides is IgG2, whereas in infants, IgG1 predominates.[19] The functions of IgG in host defense include blocking microbial attachment, opsonization, complement activation, toxin and virus neutralization, and promotion of ADCC. IgG1, IgG2, and IgG3, but not IgG4, can trigger complement activation via the classical pathway by binding to C1q.[95]

Free IgM usually exists as an immunoglobulin pentamer that has a molecular weight of approximately 950,000 and is stabilized by a single J chain.[195,336,398,458] Present mainly in the circulation, its half-life is approximately 8 to 10 days. The IgM response is the earliest of the isotype responses, appearing within the first few days of infection, but it is transient. The formation of an IgM response in the absence of an IgG response to infection is not associated with the formation of memory B cells. The main function of IgM in host defense is the activation of complement to promote opsonization and lysis of microorganisms.[166,398]

IgA exists in monomeric circulating and polymeric secretory forms and has a half-life of approximately 7 days.[303] Both forms are produced mainly by plasma cells that have migrated to mucosal sites. Secretory IgA is composed of two or three IgA molecules joined by a stabilizing J segment that is secreted by plasma cells and a secretory component produced by mucosal epithelial cells.[322,336] The secretory component permits delivery of IgA to mucosal surfaces.[430] There are two subclasses, IgA1 and IgA2, which differ in the composition of their heavy chains. Most IgA in the circulation is IgA1, whereas most IgA in secretions is IgA2. IgA1, but not IgA2, may be cleaved at mucosal sites by bacterial proteases.[335] IgA neutralizes viruses at mucosal sites, may block bacterial adhesion, and can act directly as an opsonin to promote phagocytosis and killing of microbes via Fcα receptors.[292,322]

The IgE molecule has a molecular weight of 200,000 and a half-life of only 2.3 days.[303] Most IgE is produced by plasma cells in lymphoid tissue near gastrointestinal and respiratory mucosal surfaces and released into the circulation.[301,564] IgE acts via Fcε receptors to trigger activation of mast cells and basophils, leading to immediate hypersensitivity reactions.[564] Individuals with intestinal metazoan parasites often have elevated serum levels of IgE, and IgE may have a role in protecting against parasitic disease by stimulating mediator release from mast cells, causing intestinal smooth muscle contraction and expulsion of parasites.[564]

IgD has a molecular weight of approximately 180,000 and a half-life of 3 days.[303] It is expressed along with IgM on surfaces of naive B cells but is present in normal adult serum and secretions in very low concentrations. Some antigenic specificity for IgD has been shown, and, although its function in host defense is unclear, it may serve as a secondary antigen receptor on B cells, where it may regulate the development of B-cell antibody responses.[80]

CLINICAL CONDITIONS ASSOCIATED WITH DEFICIENT HOST RESPONSES TO INFECTION

IMMATURE HOST RESPONSES OF THE NEWBORN

A mild febrile respiratory illness in a 10-month-old infant might prompt little more than gentle reassurance over the telephone from the child's pediatrician. If the patient is an infant in the first few weeks of life, however, the physician's response is likely to include an evaluation for systemic bacterial infection and administration of parenteral antibiotics in the hospital until a serious infection can be ruled out. Similarly, in an older infant, the appearance of a few cutaneous perioral vesicles characteristic of herpes simplex virus usually evokes little concern and no specific treatment. The same condition in the first 3 weeks of life is likely to lead to a prolonged hospitalization for antiviral therapy because of the risk of developing serious central nervous system or disseminated infection.[617] It is a well-recognized fact that newborns are much more susceptible to serious disease from many types of organisms than are older children and adults. This predisposition to infection is even more profound in infants born prematurely.[364] The basis for this special vulnerability of neonates is complex and encompasses all arms of the immune system. This vulnerability is of such importance that the clinical approach to possible infections in infants during the first month of life usually is far more aggressive than that in older children.

Cell-Mediated Immunity

Antigen presentation per se, via the mechanisms discussed earlier, seems to be intact in the newborn. Expression of class I and class II MHC molecules has been documented in a broad range of fetal tissues by 12 weeks' gestation,[284,441] and levels of expression are sufficient to mediate normal MHC class II–restricted antigen presentation by neonatal monocytes to maternal or paternal $CD4^+$ T cells and to induce vigorous rejection of allogeneic fetal tissue by $CD8^+$ cytotoxic T cells.[269,283]

By about 20 weeks' gestation, the fetal repertoire of diversity of TCRs has developed fully.[593] At the time of birth, although most basic functions of cell-mediated immunity are present, a high proportion of immature T cells are present in the peripheral circulation and can be identified by their co-expression of CD4 and CD8.[364] This phenotype typifies type II thymocytes, which usually are not found in the periphery in older individuals.

Neonatal T cells seem to be deficient in most of their major functions, including $CD8^+$ T cell–mediated cytotoxicity, delayed hypersensitivity, and T-cell help for B-cell differentiation.

Diminished production of cytokine by neonatal T cells likely accounts for much of this deficiency. The naive status of most neonatal T cells may account for reduced production of cytokine because memory T cells are much more efficient in all of these functions.[364]

B Cells and Antibody

B CELLS

Pre-B cells are found in the fetal liver and omentum by 8 weeks'gestation and in the fetal bone marrow by 13 weeks' gestation.[237,364,545] Pre-B cells with surface IgM have been detected by 10 weeks' gestation. After 30 weeks' gestation and delivery, pre-B cells are seen only in the bone marrow. Mature B cells are present in the circulation by the 11th week of gestation, and have reached adult levels in the bone marrow, blood, and spleen by the 22nd week of gestation.[170,237,545]

Fetal B cells express only IgM, whereas most adult B cells express IgM and IgD. Neonatal B cells may express three immunoglobulin isotypes (e.g., different combinations of IgG, IgA, IgM, and IgD) on their surfaces.[237,260] Data from experiments in mice suggest that exposing B cells with surface IgM, but not IgD, to antigens leads to anergy, or B-cell inactivation. The absence of surface IgD on fetal B cells has been speculated to contribute to the induction of tolerance to self- and, possibly, maternal antigens in utero. In addition, the fetus has a higher proportion of the functionally immature $CD5^+$, or B1, cells than adults do. These cells produce autoantibodies and may play a role in the development of tolerance to self-antigens, maternal antigens, or both.

Although germinal centers are not present in lymphoid tissue at birth, they begin to develop in the first few months of life concomitant with the infant's exposure to antigens.[575] Despite conflicting in vitro data, neonatal T-cell help for B cells probably is comparable to that of adult T cells, as is reflected by the excellent T cell–dependent antibody response of the newborn to immunization with protein antigens such as tetanus toxoid.[174] Neonatal T-cell help is associated with secretion of IgM alone, however, not other isotypes, possibly because neonatal T cells have diminished production of cytokines crucial for promoting isotype switching. Addition of cytokines such as IL-2, IL-4, and IL-6 helps overcome neonatal B-cell dysfunction in vitro.[364] In contrast to adult B cells, neonatal B cells cannot respond to polysaccharides without help from T cells.

ANTIBODY

Maternal IgG accounts for most of the newborn's circulating immunoglobulin because almost none is made by the healthy fetus, and IgG is the only isotype of maternal immunoglobulin that crosses the placenta.[333,384] Maternal transport of IgG can be detected by 8 weeks' gestation, and the newborn's IgG level is directly proportional to gestational age, reaching 100 mg/dL by 17 to 20 weeks' gestation and 50 percent of the maternal level by 30 weeks' gestation (Fig. 2–11).[49,305] By term, the infant has 5 to 10 percent more IgG than the mother does because maternal antibody is transported not only passively but also actively via trophoblast Fc receptors. Trophoblast Fc receptors have higher affinity for IgG1 and IgG3 than for IgG2 and IgG4, and so more of those subclasses are transported from the mother.[199,358] Newborns may have higher proportions of IgG1 and IgG3 than IgG2 and IgG4 compared with adults, and term newborns' IgG1 levels may exceed maternal levels.

By approximately 2 months of chronologic age, the term infant has quantitative IgG that is of approximately half maternal and half infant origin. The physiologic nadir of IgG in all infants is approximately 3 to 4 months of age and ranges from less than 100 mg/dL in very-low-birth-weight preterm infants to about 400 mg/dL in term infants (Table 2–3; see Fig. 2–11).[49,559] Maternal IgG essentially is gone by the time the infant is approximately 12 months old, at which time infant levels are approximately 60

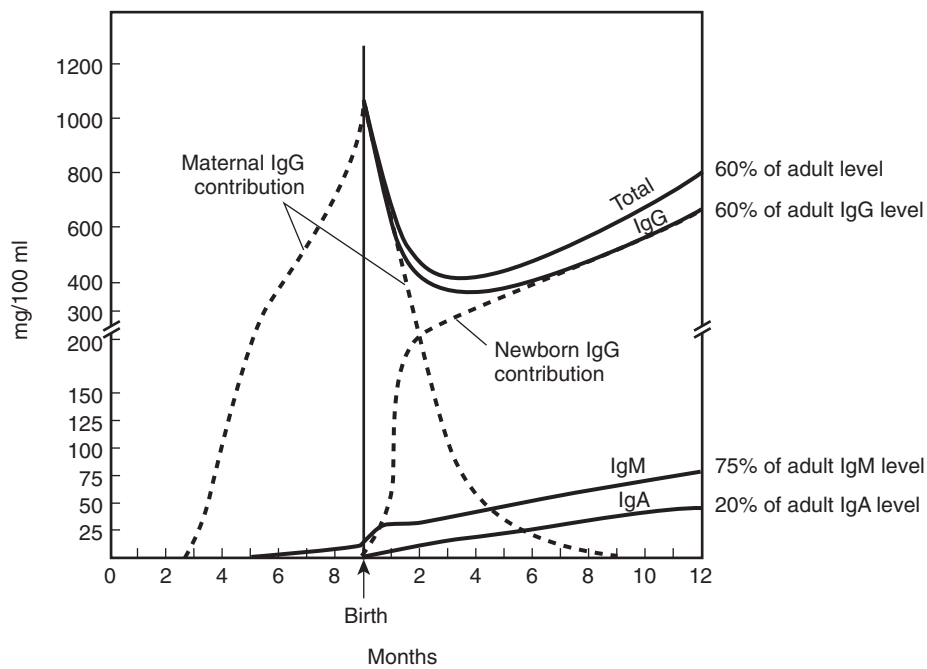

Figure 2–11 Immunoglobulin (IgG, IgM, and IgA) levels in the fetus and infant in the first year of life. The IgG of the fetus and newborn is solely of maternal origin. The maternal IgG disappears by the time the infant is 9 months of age, by which time endogenous synthesis of IgG by the infant is well established. The IgM and IgA of the neonate are synthesized entirely endogenously because maternal IgM and IgA do not cross the placenta. *(After Braun, J., and Stiehm, E. R.: The B-lymphocyte system.* In *Stiehm, E. R. [ed.]: Immunologic Disorders in Infants and Children. 4th ed. Philadelphia, W. B. Saunders, 1996, p. 67.)*

TABLE 2–3 Levels of Immunoglobulins in Sera of Normal Subjects, by Age

Age (mo)	IgG (mg/dL)	IgG (mg/dL)	IgG (mg/dL)	Total Immunoglobulin (mg/dL)
Newborn	1031 ± 200*	11 ± 5	2 ± 3	1044 ± 201
1-3	430 ± 119	30 ± 11	21 ± 13	481 ± 127
4-6	427 ± 186	43 ± 17	28 ± 18	498 ± 204
7-12	661 ± 219	54 ± 23	37 ± 18	752 ± 242
13-14	762 ± 209	58 ± 23	50 ± 24	870 ± 258
25-36	892 ± 183	61 ± 19	71 ± 37	1024 ± 205

**Values were derived from measurements made in 296 children and 30 adults. Levels were determined by the radial diffusion plate method using specific rabbit antisera to human immunoglobulins. Values are ± 1 SD.*

Modified from Stiehm, E. R., and Fudenberg, H. H.: Serum levels of immune globulins in health and disease: A survey. Pediatrics 37:715-727, 1966. Copyright American Academy of Pediatrics, 1966.

percent of adult levels. Production of IgG1 and IgG3 matures more rapidly than that of IgG2 and IgG4, reaching adult levels by the time the child is approximately 8 years old versus 10 and 12 years of age for IgG2 and IgG4.[437]

The fetus normally produces little IgM, IgA, IgE, or IgD, and none is transported from the mother.[285] The presence of IgM levels greater than 20 mg/dL at birth suggests an intrauterine infection. Serum IgA levels at birth in preterm and term infants usually are less than 5 mg/dL and consist of IgA1 and IgA2. Secretory IgA is not detectable until after birth, but it usually is present within the first few weeks of life. IgM and IgA reach approximately 60 percent and 20 percent of adult levels by the time the infant reaches 1 year of age (see Fig. 2–11). Secretory IgA reaches adult levels by the time the child is 6 to 8 years old.[364]

The IgG transferred from the mother to the fetus has been shown to protect the newborn from many infectious agents, including viruses such as varicella, polio, measles, mumps, and rubella, and bacteria such as tetanus, diphtheria, *H. influenzae* type b, and group B streptococcus.[146,147] The infant is not protected by the mother, however, if she does not have specific IgG antibody, even if she is immune to a given organism. The mother may have low or absent levels of circulating IgG antibody but good memory B cells capable of mounting a booster response. In this case, the mother is protected, but she cannot transfer protection to her infant. Similarly, because no IgM is transferred to the fetus, the mother cannot protect her newborn effectively against many gram-negative enteric organisms, even if she is immune.[148,403]

The concept of passive transfer of protective IgG is being used to develop vaccines for maternal immunization before or during pregnancy so that passive transfer of vaccine-induced antibody would result in protection during the neonatal period. Examples of organisms for which such strategies have been investigated include group B streptococcus, *H. influenzae* type b, meningococcus, pneumococcus, rotavirus, and respiratory syncytial virus.[48,203,297]

Researchers have documented that the fetus can respond to antigenic stimulation in the form of maternal immunization with tetanus toxoid vaccine and be primed for a secondary antibody response to repeat immunization after birth.[242,243] Some fetuses near term also can make IgM and IgA antibody to organisms such as toxoplasmosis, rubella, cytomegalovirus, or herpes.[201,422] The amount of fetal antibody produced in response to intrauterine antigenic stimulation is proportional to gestational age.[171,557]

Maternal antibody inhibits the infant's ability to respond to vaccines against certain organisms, such as measles, but it does not prevent the infant from mounting protective immune responses to most normal childhood vaccine antigens, such as tetanus, diphtheria, polio, hepatitis B, and protein-conjugated polysaccharide vaccines.[14] Generally, neonates have protective responses to T cell–dependent antigens, although they may produce less antibody to some antigens than do older infants and adults.[13,164,174,218,540,541]

The newborn's response to T cell–independent type 1 (TI-1) antigens is slightly decreased and to TI-2 antigens is poor.[246] The antibody response to most TI-2 antigens, including the polysaccharide capsules of group B streptococcus, pneumococcus, and *H. influenzae* type b, is not mature until the infant is 18 to 24 months old, although infants 3 months of age can respond to group A meningococcal polysaccharide.[538] In contrast, in the first few weeks of life, infants mount excellent antibody responses to T cell–independent polysaccharide antigens that have been rendered T cell–dependent by covalent conjugation of the polysaccharide to a protein carrier.[7]

A proposal is that immunologic tolerance may be induced in infants by early exposure to some antigens. The subject is controversial, but some data suggest that immunization to pertussis in the newborn period results in lower levels of antibody to subsequent doses of vaccine than does initially immunizing the infant at 1 month of age.[47,52] No evidence indicates, however, that tolerance is induced by administration of tetanus, diphtheria, or oral polio vaccines in the newborn period.[174,518]

The response of premature infants to most routine childhood vaccines, including diphtheria, tetanus, pertussis, and oral and inactivated polio, is comparable to that of 2-month-old term infants.[8,68,122,541] That premature infants do not respond as well to hepatitis B vaccine is well documented; the reasons are unclear.[201,352]

The data are conflicting, but small-for-gestational-age infants may have lower total levels of IgG at birth than those of normal-weight newborns,[199,531,627] possibly because of placental insufficiency. Their response to most routine vaccines is good, but it may be slightly decreased to inactivated polio vaccine.[128]

Complement

Complement proteins do not cross the placenta, but studies of mothers with congenital complement component deficiencies, studies of mother-infant pairs discordant for variants of individual components, and studies of fetal tissues have provided evidence for fetal synthesis of complement beginning as early as 5½ weeks' gestation. Most complement proteins are present by 10 weeks' gestation.[144,333] Levels of complement activity and of individual complement components vary significantly among infants, but generally classical pathway hemolytic activity of term neonates ranges from 50 to 80 percent of maternal values (Table 2–4). Because serum complement levels are elevated during pregnancy, term neonates' levels are approximately 60 to 90 percent of normal adult values.[197,307,628] Alternative pathway hemolytic activity is decreased more consistently than classical pathway activity, and ranges from approximately 50 to 70 percent of normal adult values at term (see Table 2–4).[6,193,434,531] Complement activity usually is lower in premature infants than

TABLE 2–4 Summary of Published Complement Levels in Neonates

	Mean Percentage of Adult Levels	
Complement Component	***Term Neonate***	***Preterm Neonate***
CH_{50}	56-90 (4)*	45-71 (3)
AP_{50}	49-65 (3)	40-51 (2)
C1q	65-90 (3)	27-58 (2)
C4	60-100 (4)	42-91 (3)
C2	76-100 (2)	96 (1)
C3	60-100 (5)	39-78 (4)
C5	75 (1)	—
C6	47 (1)	—
C7	67 (1)	—
C8	20 (1)	—
C9	<20 (2)	—
B	35-59 (8)	36-50 (3)
P	33-71 (5)	13-65 (2)
H	61 (1)	—
C3bi	55 (1)	—

**Number of studies.*

Data from Lewis, D. B., and Wilson, C. B.: Developmental immunology and role of host defenses in neonatal susceptibility to infection. In *Remington, J. S., and Klein, J. O. (eds.): Infectious Diseases of the Fetus and Newborn Infant. 4th ed. Philadelphia, Pa, W. B. Saunders, 1995, p. 65.*

in term infants, with hemolytic activity of both pathways and serum levels of most complement proteins usually corresponding directly with gestational age.[193] Small-for-gestational-age infants have complement levels comparable to levels of infants of the same gestational age who are not small for gestational age.[434,531]

Hemolytic activity of the classical and the alternative pathways increases rapidly and reaches adult levels by the time the infant is 3 to 6 months old (classical pathway) and approximately 6 to 18 months old (alternative pathway). In addition to hemolytic activity, complement-mediated opsonic and bactericidal activity is decreased in newborn sera and generally correlates with C3 and factor B levels.[197] Studies of opsonic and bactericidal activity of newborn sera have been reviewed in detail elsewhere.[197,307,308] Levels of individual complement proteins do not correlate always with their functional activity.[238,291] Zach and Hostetter[628] reported not only that total C3 levels were decreased as measured by enzyme-linked immunosorbent assay, but also that C3 thioester reactivity was decreased, and that it correlated with gestational age. Because the opsonic function of C3 is mediated by its thioester, such a defect may contribute to the newborn's deficiency in functional complement activity. Although complement levels and activity are decreased significantly in most newborns, these abnormalities are mild compared with the abnormalities seen in hereditary complement deficiencies, in which levels of an individual component often are nil. Despite studies in vitro that suggest increased risk of development of infection, the extent to which lower neonatal complement levels predispose the infant to infection in vivo is uncertain.

Phagocytes

The newborn exhibits quantitative and qualitative deficits in phagocytic defenses. Although the number of circulating PMNs usually does not differ greatly from those in older children and adults, under conditions of stress, including systemic infection, the availability of marrow reserves of PMNs is impaired markedly.[131] The ratio of marrow neutrophil reserves to circulating cells in older individuals is nearly 15:1, whereas this ratio in newborns is 2:1 to 3:1.[131,380] Neutropenia is more likely to develop during severe systemic infections in newborns than in older children and adults.[131] The resulting deficiency in PMNs available for delivery to infected sites under conditions of stress is a serious disadvantage for the neonate in containing bacterial and fungal infections. Distinct from this quantitative deficiency in marrow reserves of PMNs are functional impairments of PMNs, which also are important in understanding neonatal phagocytic defenses.

The most important and best-documented functional impairments of neonatal PMNs are related to defective adherence and chemotaxis.[4,5,25-28,339,390,400] Numerous specific structural, functional, and biochemical abnormalities have been documented, any or all of which may contribute to the overall impairment in adhesion and migration of these cells.[280] Impaired adhesion of neonatal PMNs to endothelial cells and other biologic substrates has been linked with deficiencies in the expression or function of the β_2 integrins Mac-1 (CD11b/CD18) and LFA-1 (CD11a/CD18).[4,28,99,312,390] Perhaps the best-documented of these deficiencies is the diminished level of surface expression of Mac-1 on activated neonatal PMNs, although expression on resting PMNs is similar to that of adults.[99,312] The deficiency in stimulated Mac-1 surface expression can be explained sufficiently by the observation that the total cell content of Mac-1 in PMNs from term neonates is only approximately 60 percent of that in adult PMNs.[4] The total PMN content of Mac-1 at the time of birth is related directly to gestational age, and PMNs from very early premature infants (<30 weeks' gestation) may contain less than 20 percent of the Mac-1 content of adult PMNs.[390] The PMN content of LFA-1, which is normal at term, seems to be reduced in infants born before 35 weeks' gestation.[390] In addition to reduced expression of these integrins, reduced adhesive function of the β_2 integrin molecules themselves at the surface of activated PMNs has been documented.[28]

Several other defects of neonatal PMNs that are likely to influence chemotaxis have been documented. They include defective redistribution of surface adhesion sites,[26] impaired uropod formation during stimulated shape change,[27] reduced cell deformability,[310] impaired microtubule assembly,[27] deficient F-actin polymerization,[262,506] reduced lactoferrin content and release,[25] reduced ability to effect membrane depolarization and intracellular calcium ion transients,[507] and impaired uptake of glucose during stimulation by chemoattractants.[5] The importance of each of these individual impairments to the overall deficiency in chemotaxis by neonatal PMNs has not been determined.

Evidence suggests that the number and binding efficiencies of receptors for chemoattractants, including C5a and synthetic bacterial peptides such as the formyl peptide N-f-met-leu-phe, are normal.[26,507,562] Phagocytosis and microbicidal activity of neonatal PMNs have been found in several studies to be similar to that of adult PMNs.[399,400] In some studies in which the assay conditions are designed to expose a potential defect in these functions (e.g., limiting concentrations of opsonins and high bacterial inocula), defects in phagocytosis and killing have been documented, however.[399,400] Whether these potential deficiencies play a role in impaired neonatal phagocyte defenses in vivo is unclear.

PRIMARY OR HERITABLE IMMUNOLOGIC DEFICIENCIES

Physicians who care for children often are faced with the problem of a child who has a predilection for recurrent infections. Most of these children are normal infants or toddlers who have been exposed to a succession of common respiratory infections when they entered daycare or a similar setting for the first time.[117] For most of these children, repeated exposure elicits relative immunity to many or most of the infectious agents. It is an important challenge for the physician to identify infants and children who do not fit into the normal pattern, but who are unusually susceptible to development of infection with respect to frequency, severity, type of causative agent, and response to appropriate

treatment. This challenge usually falls to the pediatrician or family physician because, with few exceptions, the primary immunodeficiencies manifest during infancy and early childhood.[149]

As suggested earlier, an infant or toddler who experiences six to eight presumed viral upper respiratory infections during the course of a winter season, without other complications, ordinarily would not be considered likely to have an immunodeficiency. In contrast, a child who has experienced five episodes of acute otitis media in the previous 4 months, several episodes accompanied by sinusitis or pneumonia, has displayed reasonable cause to suspect a humoral immunodeficiency.[149] For certain organisms, infection in the healthy host is so rare that even a single episode should prompt a high suspicion of impaired host defenses. *Pneumocystis jiroveci* pneumonia strongly suggests a severe defect of T-cell number or function.[149] Similarly, lymphadenitis or osteomyelitis caused by gram-negative enteric bacilli suggests a defect of phagocytic killing, such as CGD.[306,474] The following discussion of specific immunologic defects, their genetic basis, if known, and their infectious consequences focuses on well-characterized prototypic disorders within each class of defects, but addresses other related disorders.

Antibody Deficiencies

Humoral immunity is provided by antibody and plays an important role in host defense against most pathogens, as illustrated by the finding that patients with significant antibody deficiencies develop recurrent and sometimes life-threatening infections.[148,156,439] These patients particularly are prone to otitis media, sinusitis, bronchitis, pneumonia, sepsis, and meningitis. Antibodies participate in complement-dependent and complement-independent opsonization, bactericidal activity, virus and toxin neutralization, and the formation of immune complexes that can be cleared by the reticuloendothelial system.[134,270] The role of immunoglobulins globally and of distinct isotypes and subclasses in humoral immunity is elucidated by the nature of infections found in patients with the specific deficiencies addressed subsequently.

X-LINKED AGAMMAGLOBULINEMIA

X-linked agammaglobulinemia (XLA), first described by Bruton, is a primary immunodeficiency disorder of the B-cell lineage and is the most serious disorder of humoral immunity.[102,357,439,494] XLA is characterized by absent or severely decreased numbers of circulating B lymphocytes and absent or extremely low levels of all classes of circulating immunoglobulins. It is caused by several different mutations in the gene encoding for a B cell–specific tyrosine kinase, *Btk*, which maps to the long arm of the X chromosome at Xq22.[585,596] This abnormality in kinase activity results in an arrest in the development of B cells, usually at the pre-B cell stage, and few B cells or their progeny (e.g., plasma cells) are in the circulation or lymphoid tissues.[248]

Most individuals with XLA develop chronic or recurrent pyogenic bacterial sinopulmonary or gastrointestinal infections, and they may have recurrent skin infections.[102,357,439] Systemic disease, such as sepsis, and serious focal infections resulting from bacteremia, such as meningitis, osteomyelitis, and septic arthritis, do not occur as frequently as do respiratory and gastrointestinal infections, but they occur more commonly and are more severe than in normal hosts. The causative agents of most of these infections are *S. pneumoniae* and *H. influenzae*, but *S. aureus* and *Pseudomonas aeruginosa* and other gram-negative organisms may be implicated. The most troublesome gastrointestinal infections in XLA are caused by *Salmonella*, *Campylobacter*, and chronic infestation with *Giardia lamblia*. Although they have no increased susceptibility to most viruses, these patients have been found to have unusually severe or chronic enterovirus infections, which can be manifested by chronic meningoencephalitis, dermatomyositis, hepatitis, or a combination thereof, and several patients with XLA have developed vaccine-related paralytic poliomyelitis after receiving the live oral polio vaccine.[357]

The only abnormality on physical examination that is not related directly to infections is a paucity of normal B cell–containing lymphoid tissues, such as tonsils, adenoids, and peripheral lymph nodes.[439] Patients who are diagnosed and treated early in life have normal physical examination, growth, and development.

Older individuals with XLA have very low serum levels of IgG, IgM, IgA, and IgE. The diagnosis can be confirmed by studying lymphocyte markers.[439] These patients have a lack of circulating cells that stain for surface immunoglobulin or of B cell–specific monoclonal antibodies against CD19, CD20, or both. The number and function of T lymphocytes are normal in XLA. Establishing the diagnosis in the newborn period may be difficult because maternally derived immunoglobulin bestows normal IgG levels. If there are other reasons to suspect this diagnosis, such as a newborn boy with a documented family history, the diagnosis can be established by documenting a paucity of circulating B cells.

Advances in genetic techniques have enabled maternal carrier detection.[148] In contrast to carriers of some other X-linked genetic diseases, X chromosome inactivation is not random in carriers of XLA. Instead of the two populations of B cells in normal individuals, B lymphocytes of XLA carriers express only one population of B cells—those with the normal allele on the X chromosome—suggesting that B cells with the mutant allele are at a selective disadvantage and do not develop. Prenatal diagnosis is made by genetic studies of amniotic fluid cells or quantitation of fetal circulating B cells.

The prognosis for patients with XLA has improved markedly with earlier diagnosis, high-dose intravenous immunoglobulin (IVIG) therapy, and aggressive use of antibiotics.[439] Before the availability of IVIG, most patients who survived to the third decade of life had chronic lung disease from pulmonary infections and hearing loss from recurrent otitis media.[357]

IgG SUBCLASS DEFICIENCY

Individuals with IgG subclass deficiencies have levels of one or more IgG subclasses that are more than two standard deviations below normal for age, normal to slightly decreased total IgG, normal levels of other immunoglobulin isotypes, and, often, a poor antibody response to certain antigens.* Patients with IgG subclass deficiency who also have IgM and IgA deficiency may have another immunodeficiency disorder, such as common variable immunodeficiency (CVID).

Patients with individual or combined deficiencies of IgG1, IgG2, and IgG3 may be at increased risk for development of infection, particularly if the deficiency is associated with an abnormal antibody response to antigenic stimulation. The most common kinds of infections in patients with IgG subclass deficiency involve the upper respiratory tract. Ordinarily, these patients do not have life-threatening systemic infections.

Because this subclass accounts for approximately 60 percent of total IgG, deficiency of IgG1 is most likely to be associated with subnormal levels of total IgG, and it usually is associated with other subclass deficiencies.[18,19,521,527-529] IgG1-deficient individuals may have recurrent pulmonary infections that can lead to chronic lung disease. IgG2 deficiency usually is associated with normal total serum IgG levels and is more likely to be clinically significant if accompanied by IgG4 or IgA deficiency. Patients with IgG2 deficiency have poor antibody responses to polysac-

*See references 272-274, 410, 437, 450, 451, 520, 521, 526-530.

charide antigens but normal responses to protein antigens. Their infections are localized primarily to the respiratory tract, but some patients have had recurrent meningococcal meningitis or disseminated pneumococcal disease. IgG3 deficiency has been associated with low total levels of serum IgG and recurrent respiratory infections, which also may lead to chronic pulmonary disease.[437] IgG4 deficiency is difficult to diagnose because many normal individuals have low serum levels of IgG4, and most normal infants have no detectable IgG4.[437] IgG4 deficiency seems to be of clinical significance if it is associated with IgG2 and IgA deficiency.

The treatment for children with IgG subclass deficiency usually is individualized according to the frequency and severity of symptoms. Noninvasive infections usually can be treated successfully with appropriate antibiotics. Patients with more severe presentations may benefit from IVIG therapy, but patients who also are IgA-deficient should be followed closely for the formation of anti-IgA antibodies and treated only with IgA-depleted IVIG preparations.

HYPER-IgM SYNDROME

Immunoglobulin deficiency with increased IgM is characterized by low levels of IgG, IgA, and IgE but normal to increased levels of IgM in the circulation and normal numbers of circulating B cells.[433,437] The disorder is caused by an intrinsic T-cell abnormality that alters switching from IgM to other isotypes. The basis for the various genetic forms of this defect is in one of several possible defects that involve interactions between CD40 on B cells and CD40L on T cells. In normal individuals, CD40 on the surface of B cells interacts with CD40L on activated T cells to cause B-cell differentiation into memory B cells and switching from IgM to other isotypes. B cells from patients with hyper-IgM syndrome make only IgM antibody, and their B cells express only surface IgM and IgD. The originally described and most common form of the defect is X-linked recessive and results from a mutation in the gene encoding CD40L, a protein expressed transiently on activated T cells.[14,39] More recently described autosomal recessive forms of this disorder involve mutations either in CD40 itself[216] or in a CD40-activated RNA editing enzyme, activation-induced cytidine deaminase.[481] Other cells have surface CD40, and immunologic abnormalities such as the neutropenia and the increased incidence of infections caused by *Pneumocystis* and of malignancies in patients with the hyper-IgM syndrome also may be the result of impaired cell interactions via CD40.

Clinically, hyper-IgM syndrome is manifested by recurrent bacterial infections, especially of the respiratory tract, beginning after maternal immunoglobulin levels subside during the first few months of life.[433,437] Such individuals are susceptible to the same kinds of recurrent pyogenic infections that are associated with other immunoglobulin deficiencies and to infections with organisms more commonly encountered in patients with T-cell defects (e.g., *P. jiroveci*).[51] Some patients with this syndrome have recurrent diarrhea caused by *G. lamblia* and *Cryptosporidium* that is severe enough to require parenteral nutrition. Life-threatening peritonsillar and peritracheal soft tissue infections also have been observed. Approximately half of patients also have persistent or recurrent neutropenia. Patients with autoantibodies may have thrombocytopenia, hemolytic anemia, nephritis, hypothyroidism, or arthritis, as noted earlier.

Physical examination usually is normal except for the sequelae of infections and lymphoid hyperplasia, which probably is caused by constant antigenic stimulation. Arthritis and arthralgia may be the result of chronic infection or the production of autoantibodies. The diagnosis of X-linked hyper-IgM syndrome may be established by using immunofluoresence to document absent expression of CD40L on activated T cells or absent CD40 expression on B cells, or by showing a mutation in one of the genes encoding CD40, CD40L, or the enzyme activation-induced cytidine deaminase.

IVIG treatment of patients with hyper-IgM syndrome usually results in marked clinical improvement.[51] Patients who have neutropenia with granulocyte colony-stimulating factor (G-CSF) and patients with arthritis or other autoimmune symptoms may need to be treated with steroids. Patients with hyper-IgM syndrome usually do not do as well as patients with XLA because, in addition to their immunoglobulin deficiency, they have an increased incidence of neutropenia, autoimmune disease, and malignancy.

IgA DEFICIENCY

IgA deficiency is the most common immunodeficiency, occurring with a frequency of 1:400. Most of the functions of serum IgA can be performed by IgG and IgM.[156,437] Although deficiencies of secretory IgA may lead to recurrent respiratory or gastrointestinal tract infections, deficiency of serum IgA usually is not associated with increased susceptibility to systemic infections.[24] IgA deficiency has been associated with many other conditions, including recurrent infections, IgG2 deficiency, a family history of immunodeficiency (e.g., relatives with CVID), autoimmune disorders, and malignancy.[23] Recurrent infections are most likely to occur in the subset of IgA-deficient patients who also have IgG2 deficiency.[156,450]

Infections usually are mild and involve the upper respiratory and gastrointestinal tracts. Chronic gastrointestinal disease can be caused by *G. lamblia* infections, nodular lymphoid hyperplasia, lactose intolerance, malabsorption, ulcerative colitis, regional enteritis, or autoimmune disorders (e.g., chronic hepatitis, cirrhosis, pernicious anemia). Approximately 20 percent of IgA-deficient patients have allergies, and many have elevated levels of IgE.[156] Food allergy is a common finding and may be the result of abnormal processing of antigen at mucosal surfaces. Other autoimmune diseases that are associated with IgA deficiency include rheumatoid arthritis, systemic lupus erythematosus, thyroiditis, transfusion reactions, pulmonary hemosiderosis, myasthenia gravis, and vitiligo.[156] IgA deficiency seems to occur sporadically, but familial cases have been described.[24,156]

Serum IgA levels less than 5 mg/dL can be clinically significant because patients with levels this low who receive transfusions may make antibody against donor IgA and have severe reactions when transfused again.[517] Reactions to IVIG also may occur because IVIG preparations contain varying amounts of IgA. IgA-depleted preparations are available and usually are well tolerated.

IgE DEFICIENCY

The clinical significance of selective IgE deficiency is unclear because it has been described in healthy individuals. Some patients with profound antibody and cellular immunodeficiencies lack serum IgE, but several partial cellular immunodeficiency syndromes are characterized by elevated levels of IgE (e.g., DiGeorge syndrome, Wiskott-Aldrich syndrome, Hodgkin disease).[437] The absence of IgE does not correlate with an enhanced susceptibility to infection in developed countries, but it has not been investigated in the developing world, where its role in immunity to parasites may be more important.

TRANSIENT HYPOGAMMAGLOBULINEMIA OF INFANCY

Hypogammaglobulinemia is a normal physiologic phenomenon occurring in all infants beginning about 3 to 4 months of age, when maternal antibody wanes and infant synthesis of immunoglobulin has not compensated yet.[437] The syndrome of transient hypogammaglobulinemia of infancy can be differentiated from physiologic hypogammaglobulinemia by the fact that immuno-

globulin levels of normal infants begin to increase by the time they are approximately 6 months old, whereas the immunoglobulin levels of infants with transient hypogammaglobulinemia of infancy do not begin to increase until they are 18 to 36 months old.[437] Infants suspected to have this syndrome should be evaluated for XLA and CVID (see later) and followed closely until their immunoglobulin levels normalize for age.

ANTIBODY DEFICIENCY WITH NORMAL OR ELEVATED LEVELS OF IMMUNOGLOBULINS

Some individuals with normal levels of all circulating immunoglobulin isotypes are at increased risk of acquiring infection.[18,20,437] The cause of this disorder remains poorly defined, but it may be related to an inability to respond to specific antigens or the induction of tolerance by exposure to certain antigens very early in development. The most common infections in these patients are recurrent sinopulmonary infections, although a few patients have developed pneumococcal sepsis.[20]

Such individuals can be identified by their inability to make antibody in response to stimulation with specific antigens. They do not have abnormal total serum immunoglobulin classes or subclasses or B-cell or T-cell quantity or function. They can respond to some, but not all, antigenic stimulation. It is important to test these individuals with a variety of stimuli. A good way to test for this syndrome is to immunize with protein antigens, such as tetanus and diphtheria toxoids, and with polysaccharide antigens, such as pneumococcal and *H. influenzae* type b capsular polysaccharide vaccines. Patients who can respond to protein but not to polysaccharide antigens usually respond to protein-polysaccharide conjugates. Treatment with IVIG may help prevent recurrent infections in these patients, although their normal overall levels of immunoglobulin could pose difficulties in determining the appropriate doses of IVIG and intervals between infusions.

Defects of Cell-Mediated Immunity: DiGeorge Syndrome

The prototypic pure T-cell defect, DiGeorge syndrome, is characterized clinically by congenital heart disease (usually involving the aortic arch), hypocalcemic tetany, unusual facial features, and recurrent infections.[182] In the classic or complete form of this disorder, there is absence or hypoplasia of the thymus and parathyroid glands, cardiac or aortic arch deformities, and a stereotypic constellation of abnormal facial features.[182,340,567] Although the condition usually is considered to be associated with immunodeficiency because of the thymic hypoplasia, only approximately 25 percent of patients actually exhibit an immunologic defect.[58] Although currently out of favor, the term *partial DiGeorge syndrome* has been used to describe patients with the typical anatomic findings, but without immunodeficiency, or with mild immunologic impairment.[286]

Some sources designate this disorder as an "anomaly" or "sequence," rather than a syndrome, because of confusion about its relationship to 22q11 deletion syndrome (del22q11) or to the more recently defined microdeletion, del22p11.2, a deletion also associated with velocardiofacial syndrome. Robin and Shprintzen[486] hold that the findings that comprise the DiGeorge "sequence," although often associated with del22q11.2, are etiologically heterogeneous and have been associated with other chromosomal deletions, such as del10p and del17p, or del10q13. Numerous individuals with del22p11.2 exhibit abnormalities distinct from those of the DiGeorge sequence.[253,254,286,348,462,486] This genetic and phenotypic heterogeneity should be considered when evaluating patients with the classic facial and cardiac findings that so often have been associated with a serious T-cell defect.

DiGeorge syndrome usually is recognized in the newborn period by the presence of unusual but characteristic facial features, hypocalcemic tetany in the first 2 days of life, or the presence of serious cardiovascular manifestations, most commonly associated with an interrupted aortic arch or truncus arteriosus.[286,371] Because of the serious nature of the cardiovascular defect, many patients with DiGeorge syndrome have not survived long enough for the immune defect to become a clinical problem.[286] However, with improvements in aggressive surgical treatment of the heart defects, more of these infants are surviving long enough to display manifestations of the immunodeficiency that results in an increased frequency and severity of viral and fungal infections and *Pneumocystis* pneumonia. In such patients, management often has included prophylaxis against *Pneumocystis*, periodic immunoglobulin infusions, and avoidance of live virus vaccines.[286] Transplantation of fetal or postnatal thymic tissue has corrected the immunologic problem for the long-term in approximately a third of such attempts.[247,286] More recently, HLA-matched bone marrow transplantation has been successful in some cases.[87,247,286]

Combined Defects of Cellular and Humoral Immunity

SEVERE COMBINED IMMUNODEFICIENCY DISEASE

SCID describes a heterogeneous group of heritable immunodeficiencies that involve serious impairments of cellular and humoral immunity with recurrent severe infections by a wide range of viral, bacterial, and fungal organisms. SCID has many different forms, which have been reviewed in greater detail elsewhere.[104,107,501] At this writing, there are at least 10 genes, abnormalities of which are known to result in SCID. X-linked SCID, the most common form of SCID, is caused by a mutation in the common gamma chain of the receptor for IL-2 and several other cytokines (γ_c).[104,361] The other known forms of SCID are either known or presumed to be autosomal recessive. They include a deficiency of adenosine deaminase (a purine salvage pathway enzyme), a deficiency in Janus kinase 3 (a cytokine receptor signaling molecule), and a defect in the alpha chain of the IL-7 receptor.[104,315] Mutation of one of at least six different genes whose products play a role in TCR or immunoglobulin gene recombination or TCR signaling, including RAG1, RAG2, Artemis, DNA ligase IV, CD3-delta, and CD3-epsilon, also results in SCID.[104,200,315,377] Additionally, SCID is caused by a mutation in CD45, a phosphatase that regulates signaling thresholds in immune cells.[315]

Long-term management of patients with SCID involves modalities employed in T-cell and B-cell disorders, including prophylaxis against pneumocystis pneumonia, avoidance of live viral vaccines, and immunoglobulin replacement therapy.[104] Bone marrow transplantation from HLA-matched siblings has corrected the defect in many cases and is considered the current treatment of choice.[106] Considerable effort has been expended toward development of gene therapy to treat SCID.[361] Adenosine deaminase deficiency is of historical interest in that it is the first heritable disorder for which gene therapy was attempted, although early success was limited.[79,106] More recent advances in retroviral gene therapy for X-linked SCID initially appeared to be successful. However, at least three patients have developed lymphoproliferative disorders similar to lymphocytic leukemia, with malignant cells showing insertion of the vector into the promoter or first intron of a proto-oncogene, LMO2.[83,107,124,220,472]

COMMON VARIABLE IMMUNODEFICIENCY

CVID is a heterogeneous group of combined immunodeficiencies that differ from most other primary immunodeficiencies in that they usually manifest in the second or third decade of life,

although they may manifest at any age.[155,157] Some sources indicate that CVID may be the most common symptomatic primary immunodeficiency. Patients with CVID have normal or only slightly decreased numbers of circulating B cells; low, but not absent, levels of IgG, IgM, and IgA; poor responsiveness to antigens; and abnormal T-lymphocyte function.[159]

Although T-cell and B-cell abnormalities often can be shown, the clinical presentation usually is comparable to that in patients with humoral or B-cell defects (i.e., recurrent bacterial otitis media and sinopulmonary infections).[155,271,494] Occasionally, in addition to having the organisms causing infections in patients with XLA, these patients also have infections with organisms found more commonly in individuals with T-lymphocyte abnormalities, such as *P. jiroveci*, *Mycoplasma pneumoniae*, recurrent herpes simplex virus, and herpes zoster virus infections. Chronic gastrointestinal problems may be caused by *G. lamblia* or other intestinal pathogens. Patients with CVID are prone to development of nodular lymphoid hyperplasia, autoimmune diseases, and malignancies. CVID occasionally has been reported to be familial, and it has been described in families with IgA deficiency. Several gene mutations that have been found more recently in patients with CVID include the genes encoding for CD19, TACI (transmembrane activator and CAML interactor), BAFF-R (receptor for B-cell activating factor of the TNF receptor family), and ICOS (inducible co-stimulatory molecule).[120,514,592]

Patients with CVID usually can benefit from therapy with IVIG, which reduces the incidence of acute infections. Most patients with CVID, even some who undergo long-term treatment with IVIG, develop chronic sinopulmonary disease.[157,449]

Defects of the Interferon-γ and Interleukin-12 Pathways

Macrophages infected by intracellular pathogens, especially mycobacteria or salmonellae, are stimulated via a TLR4-dependent mechanism to release IL-12, along with IL-18, IL-23, and IL-27. These cytokines stimulate T cells and NK cells to produce IFN-γ, via interactions with cellular receptors for the aforementioned cytokines. The released IFN stimulates the macrophage further to release more IL-12 and to activate killing. The mechanism by which this intracellular killing occurs is unknown. This cycle of mutual activation is essential for normal defense against mycobacterial pathogens, and numerous genetic defects of these cytokines, their cellular receptors, or related molecules crucial for receptor-mediated signaling have been associated with increased susceptibility to mycobacterial infections. Deficiencies in this system have resulted from mutations in either of the two receptors for IFN-γ, IFN-γR1 and IFN-γR2, and mutations in STAT1, a molecule crucial for transducing signals from both IFN-γ receptors. Mutations also have been described in the 40-kd subunit of IL-12, IL-12p40, and in the IL-12 receptor, IL-12Rb1. These disorders are uncommon, all involving fewer than 100 known patients.[498]

Mutation of either of the IFN-γ receptors is associated with increased risk of developing mycobacterial infections. Deficiencies of IFN-γR1 may be autosomal recessive or dominant and complete or partial. Most recessive defects are complete and result in absent IFN-γ responsiveness. Dominant IFN-γR1 deficiency is associated with mutations that result in heterozygous truncations of the cytoplasmic domain of the receptor with excessive accumulation of nonfunctional receptor at the cell surface, leading to reduced, but not absent, responsiveness to IFN-γ. Patients with the recessive complete form of this deficiency have a much more severe clinical phenotype than that of patients with the dominant partial form, although the latter have a fivefold greater frequency of nontuberculous mycobacterial osteomyelitis. Defects in IFN-γR2, much less common, also may be recessive or dominant in inheritance and complete or partial. Rare deficiencies in the receptor signaling molecule STAT1 have led to an increased number of mycobacterial infections in a partial deficiency or, in two patients with a recessive complete form, to post-vaccination disseminated bacille Calmette-Guérin disease followed later by death from severe viral infections. The latter probably relates to the additional role of STAT1 in development of IFN-α/β-mediated antiviral activity.[498]

Deficiencies of IL-12p40 or its receptor IL-12Rβ1 are associated with disseminated nontuberculous mycobacterial infections, tuberculosis, and *Salmonella* infections. The receptor deficiency results in unresponsiveness to IL-12. This defect apparently is autosomal recessive with variable clinical penetrance. Deficiency of IL-12p40 also varies in its clinical phenotype and has resulted in deaths caused by severe mycobacterial infections.[452,498,554]

Diagnosis of defects of the IFN-γ pathway usually first involves immunofluorescence flow cytometry to examine cells for deficient or abnormally excessive expression of IFN-γR1. If these studies are normal, studies of STAT1 phosphorylation in response to IFN-γ stimulation may reveal a defect in IFN-γR2 function or expression. For evaluation of the IL-12 pathway, IL-12p40 can be measured in cell supernatants with an enzyme immunoassay, and IL-12Rb1 expression function can be assessed with flow cytometry and, if necessary, by measuring STAT4 phosphorylation after stimulation with IL-12. These studies ordinarily should be done only in a highly specialized reference laboratory.[498]

Complement Deficiencies

Approximately 0.03 percent of the general population have complement deficiencies resulting from acquired or congenital abnormalities of single or multiple complement components or regulatory proteins. Excellent reviews of complement deficiencies are available elsewhere.[175,176,178,185,277,308,499,614]

The most common complement deficiencies are acquired and are transient. They include the relative complement deficiencies in infants (see earlier) and complement deficiencies that result from complement consumption in various inflammatory states, such as connective tissue disorders and acute or chronic infections.[175,176,178,308] Acquired complement deficiencies usually are associated with low levels of more than one complement component.[176] These deficiencies generally are incomplete and are of doubtful clinical significance in host defense against infection.

In contrast, congenital or hereditary deficiencies more often are manifested by abnormality or complete absence of a single complement protein. Deficiencies of individual components may have profound clinical implications because they have been well documented to predispose to acquiring life-threatening infections. Most primary complement abnormalities (C1q dysfunction and C1rs, C4, C2, C3, C5, C6, C7, C8, and C9 deficiencies) are inherited as autosomal co-dominant traits.[176]

Patients with homozygous or heterozygous deficiency of the early classical pathway proteins—C1, C2, and C4—are more prone to develop collagen vascular disease than infections, for reasons that are not fully understood. Approximately 20 percent of patients with homozygous deficiency of early components have problems, however, with recurrent or severe infections that are similar to those seen in C3 deficiency.[176,217,499] Ordinarily, these patients do not have as serious or as frequent problems with infections as do patients with alternative pathway deficiencies because they always can protect themselves via the alternative pathway whether or not they have specific antibody to the infecting organism. Their predilection to development of collagen vascular disease probably is due partly to abnormal solubilization and removal of immune complexes. Although cases of deficiency of one of the isotypes of C4 (C4B) have been reported to be

associated with increased susceptibility to infection caused by encapsulated organisms, subsequent studies have not confirmed this finding.[74,121] C2 deficiency has been associated with antibody deficiencies in individuals with recurrent infections.[17,119]

Deficiencies of alternative pathway proteins predispose to development of serious, often fatal, infections because of the lack of ability to respond promptly to organisms not previously encountered.[176] Although the classical pathway is intact, patients with alternative pathway deficiencies often die before they have the opportunity to make the specific antibody required for its activation. No homozygous factor B–deficient patients have been reported. Properdin deficiency, the only X-linked complement deficiency, has been associated with fulminant, usually fatal, meningococcal infection.[177] Factor D deficiency is rare and seems to predispose to recurrent neisserial infection.[330]

More recently, mutations or variants in the gene for MBL, the initiator of the MBL pathway (see earlier), have been associated with an increased risk of having recurrent infections.[185,277,563] In particular, homozygosity for such mutations or variants was found to be associated with an increased risk for developing systemic meningococcal disease.[223]

Because all three complement activation pathways converge at the activation of C3, patients who are deficient in C3 are unable to mobilize any of the three main effector functions of complement in host defense—opsonization, phagocyte recruitment, or bacteriolysis. One is not surprised that the most serious complement deficiency state is the total absence of C3.[176] Congenital C3 deficiency is rare and results from decreased production of C3.[217,499] Patients with deficiencies of factors H and I have low, but detectable, levels of C3 because absence of either of these regulatory factors allows continuous activation of the alternative pathway and uncontrolled C3 consumption. Patients with C3 deficiency caused by any of these mechanisms have increased susceptibility to infections caused by encapsulated bacteria, such as pneumococci, meningococci, and *H. influenzae* type b. Many of these infections involve the respiratory tract (otitis, sinusitis, bronchitis, and pneumonia), but C3-deficient patients also are predisposed to development of sepsis and meningitis.[176,217,499] In addition, some C3-deficient patients develop one of several different autoimmune diseases.[176,217,499]

Deficiencies of terminal complement proteins C5, C6, C7, and C8 greatly increase the risk of developing systemic infections caused by *N. meningitidis* or *Neisseria gonorrhoeae*.[76] Because C9 is not absolutely required for bacteriolysis—although it renders this process more efficient—C9 deficiency increases the risk of infection to a lesser degree than deficiencies of other terminal components. C9 deficiency was found to be present in approximately 0.1 percent of the population in Japan, and the risk of acquiring meningococcal disease was increased 5000-fold in C7-deficient patients and 700-fold in C9-deficient patients.[420]

The risk of developing infection is higher in patients with C5 deficiency than in patients with deficiencies of other terminal proteins because, in addition to the role of C5 in initiating assembly of the MAC, the free C5 fragment, C5a, is an important chemoattractant and is crucial for leukocyte recruitment to sites of microbial invasion and for bactericidal activity.[175] Although a rare patient with C5 deficiency had recurrent subcutaneous skin infections, as might characterize a disorder of chemotaxis, most patients with this disorder are at increased risk for acquiring meningococcal and disseminated gonococcal infections.

At least one episode of meningococcal disease occurs in approximately 60 percent of individuals who have been identified as having C5, C6, C7, C8, or properdin deficiency, and 75 to 85 percent of documented bacterial infections in complement-deficient patients are meningococcal.[176,217,499] Conversely, approximately 14 percent of patients presenting with sporadic meningococcal diseases may be expected to have a defect in one of the late complement components, and this percentage increases to approximately one third among individuals with two or more meningococcal infections.

Differences in the features of meningococcal disease between individuals with complement component deficiencies and normal hosts have been noted. First, meningococcal disease occurs in older individuals (mean age 17 years versus 3 years), and a higher proportion of disease is caused by groups Y, W135, and X in complement-deficient individuals than in normal individuals.[176] Additionally, the mortality rate in meningococcal infection is much lower than in normal individuals, probably because many patients with one of these deficiencies have developed antibodies to meningococci that can activate the classical pathway leading to normal opsonization and phagocyte activation, functions upstream in the cascade from the MAC. In contrast, individuals with normal complement levels who develop meningococcal infection usually do so because they do not have specific antibodies with which to activate the classical pathway, and meningococci are poor activators of the alternative pathway.

Currently, no specific treatment exists for patients with hereditary complement deficiencies. Replacement of missing complement proteins has been attempted with fresh-frozen plasma, but it is not practical because of the short half-life of most of the components.[55,351,502] Immunization of complement-deficient patients and their close household contacts against encapsulated organisms may reduce the risk of acquiring infections caused by these organisms.

Disorders of Phagocyte Function

GENERAL FEATURES OF PHAGOCYTE DISORDERS

The most frequent reminder of the importance of having an adequate supply of well-functioning phagocytes comes from patients who develop neutropenia after undergoing chemotherapy for malignancies. The high risk of developing bacterial and fungal infections in these patients mainly is the result of a lack of circulating neutrophils available for delivery to infected tissue.[467] The qualitative disorders of phagocyte function discussed in this section result in similar susceptibilities to these infections, either because the circulating cells are unable to migrate to an infected site or because, even having migrated to the infected tissue, they are unable to effect normal microbicidal activity. Some overlap exists among the types of infectious complications associated with disorders of migration versus killing. As a rule, defects of neutrophil migration tend to be associated with infections at skin and mucous membrane sites. In contrast, killing defects are more likely to result in infections of soft tissues and internal organs, although skin infections are common findings.

INTRINSIC DISORDERS OF CELL MIGRATION

Type 1 Leukocyte Adhesion Deficiency

In the late 1970s and the first half of the 1980s, several reports described patients with recurrent bacterial infections, diminished neutrophil motility, and delayed separation of the umbilical cord.[2,29-31,37,89,154,221,268] The neutrophils of these patients were discovered to be markedly deficient in adherence to natural and artificial surfaces, in response to complement-opsonized particles, and in expression of surface glycoproteins in the molecular weight range of 150 to 180 kd. The deficient glycoproteins were found to be members of a family of heterodimeric glycoproteins, LFA-1, Mac-1, and pl50,95, each defined by its own unique alpha subunit, CD11a, CD11b, and CD11c, respectively, but sharing a common 95-kd beta subunit designated CD18.[30,31,36,37,552] A fourth alpha subunit, CD11d, the importance of which remains poorly understood, has been described more recently.

These proteins, also called the β_2 leukocyte integrins, were identified as crucial determinants of adhesion-dependent func-

tions on neutrophils and other phagocytic cells, and their absence seemed to be directly responsible for the striking adherence-dependent defects that characterized the function of leukocytes from patients with this disorder.[29-31,36,37,552] Variously called Mac-1 deficiency, MO1 deficiency, LFA-1 deficiency, CD11/CD18 deficiency, or CR3 deficiency, this disorder, now usually termed *type 1 leukocyte adhesion deficiency* (LAD-1), is an autosomal recessive disorder with one of numerous mutations in the β_2 integrin subunit, CD18, localized to chromosome 21.[31,36,552,553] It has been identified in more than 150 individuals worldwide and has a broad ethnic diversity.[31,36] Patients may exhibit a moderate or severe phenotype, depending on the extent of the defect in protein expression.[30,31] The documented mutations of CD18 that result in LAD-1 have been diverse, leading to abnormalities ranging from complete absence of the protein to extensions of the molecule, truncations of the extracellular portion or of the cytoplasmic domain of the molecule, small deletions, and point mutations.[33,36,282]

Patients with LAD-1 develop recurrent necrotic skin and soft tissue infections with poor or absent formation of pus, and they exhibit poor wound healing.[30,31] They develop a severe generalized form of gingivitis or periodontitis, often losing most or all of their primary and secondary dentition along with some of their alveolar bone.[30,31] They also may develop enterocolitis similar to that seen in neutropenic patients.[30,31] Delayed separation of the umbilical cord, presumably caused by an impaired inflammatory response, is a common feature of the more severe phenotype of this disorder,[30,31] but this finding alone in infants without infectious complications or other characteristic features is of doubtful significance.[619] Pronounced leukocytosis is a common feature of LAD-1, even in the absence of active infection.[30,31] The reason for this feature is not completely understood. The fact that LAD-1 neutrophils are incapable of normal egress from the circulation via the oral cavity or lower intestinal tract was long thought to provide the principal explanation for the high circulating neutrophil counts. More recent studies in CD18-null LAD-1 mice reveal abnormally high circulating G-CSF levels, however, and suggest that a more likely explanation involves the absence of a negative feedback mechanism on production of G-CSF that occurs during normal transendothelial migration of leukocytes and involves IL-17. Absent ongoing transendothelial migration results in failure of this putative feedback mechanism, resulting in elevated G-CSF levels and higher circulating granulocyte counts.[225]

Functional studies of neutrophils from patients with LAD-1 reveal a marked impairment of adherence-dependent functions that require the β_2 integrins, including attachment to various surfaces, orientation in a chemotactic gradient, chemotaxis through nitrocellulose filters or under agarose gels, aggregation, phagocytosis of iC3b-opsonized particles, degranulation or activation of the oxidative metabolic burst in response to such particles, and recruitment of PMNs in vivo to Rebuck skin windows or dermal suction blisters.[29-31,111,232] In contrast, neutrophil functions that are independent of CD11/CD18-mediated interactions, including degranulation or oxidative burst activation in response to soluble stimuli or polarized shape change in suspension in response to chemoattractants, are normal.[29-31] PMNs and NK cells from patients with LAD-1 exhibit impaired ADCC for virus-infected target cells, suggesting that CD11/CD18-mediated cell-cell adhesion is essential for normal killing of virus-infected cells by this mechanism[331] and that the increased severity of viral infections in a few of the most severely affected patients could be related to defective ADCC. Currently, the specific diagnosis of LAD usually is made by showing absent or markedly deficient expression of the CD11/CD18 family of glycoproteins on circulating leukocytes by immunofluorescence flow cytometry, although various other methods have been used.[29-31,37]

Careful attention given to skin and oral hygiene, aggressive management of infections, and meticulous local care of wound sites are important in the care of patients with LAD-1 or any serious disorder of neutrophil migration. Prophylactic antibiotics, usually trimethoprim-sulfamethoxazole, have been used in many of these patients, but their efficacy has not been well established. Granulocyte transfusions have been used with some success to treat severe infections in a few patients with LAD-1.[31,89] Bone marrow transplantation with HLA-matched allogeneic marrow has led to missed results, ranging from complete correction of the phagocytic defect to death from graft-versus-host disease 9 months after transplantation.[31,221] The human CD18 gene has been cloned and sequenced, and human LAD-1 cells have been corrected successfully in vitro with the normal CD18 complementary DNA carried by retrovirus vectors, hinting at the future promise of gene therapy for patients with LAD-1.[31,32] The development of genetic knockout mice deficient in CD18 may provide a useful model for studying gene therapy in vivo and for helping to elucidate features of the underlying deficiency itself.[31,338]

Type 2 Leukocyte Adhesion Deficiency

In 1992, two unrelated patients were reported, both products of consanguineous matings, who exhibited clinical characteristics virtually identical to those described for LAD-1.[228] Expression of the β_2 (CD18) integrins on leukocytes was normal, however. In addition to defects in neutrophil motility, these children exhibited short stature, psychomotor retardation, and the Bombay (hh) erythrocyte phenotype (homozygous for absence of the H antigen). Phagocytosis by PMNs was normal.

This defect has been documented to be caused by one or more mutations of a specific guanosine diphosphate–fucose transporter,[375] resulting in the absence of fucosyl residues on sialyl Lewis X, the tetrasaccharide moiety that serves as an important ligand for members of the selectin family of adhesion molecules.[228,350,600] In vivo and in vitro studies comparing the adhesive functions of PMNs from LAD-1 and this new disorder, now called LAD-2, have provided elegant validation of the distinct roles of selectins and integrins in the recruitment of leukocytes in vivo, with the initial selectin-mediated "rolling" stage (deficient in LAD-2) required first for the second integrin-mediated "firm adhesion and extravasation" stage (deficient in LAD-1) to occur.[600] A deficiency in either mechanism results in defective delivery of PMNs to infected sites and is manifested clinically as a form of LAD. The other somatic and neurologic features of LAD-2 may be related to more widespread consequences of the generalized defect in fucosylation of glycoproteins.[375] Because of the generalized nature of this deficiency, a proposal has been made to designate this disorder *type IIc congenital disorder of glycosylation*.[375]

Type 3 Leukocyte Adhesion Deficiency (Integrin Activation Defect)

In recent years, at least four patients have been reported who have clinical phenotypes that include features of type 1 LAD and Glanzmann thrombasthenia, a bleeding disorder associated with mutations in the $\alpha IIb\beta 3$ integrin on platelets. Laboratory studies of these patients revealed markedly deficient integrin-mediated adhesive functions of leukocytes and platelets despite normal surface expression of leukocyte and platelet integrins. Further studies led to the conclusion that this defect in integrin function was the result of defective "inside-out" signaling pathways that normally lead to integrin activation.[205,325,389] Although the precise molecular defect in this disorder remains uncharacterized, cells from one patient with this disorder have been reported to exhibit deficient activation of a small guanosine triphosphatase, Rap1, an important regulator of inside-out integrin activation.[325] A growing consensus is that this defect of integrin activation be termed *type 3 leukocyte adhesion deficiency* (LAD-3), although this nomencla-

ture does not include reference to the defect in platelet function.[205,325]

Specific Granule Deficiency

Rare patients with hereditary specific granule deficiency have been reported, beginning with the original description by Spitznagel and colleagues in 1972.[90,231,550] These patients exhibited recurrent and severe infections, primarily of the skin and mucous membranes, sometimes involving the lung and, in one patient, the mastoid. Normal human neutrophils contain azurophilic (primary) granules and specific (secondary) granules, the contents of which have been summarized previously. Neutrophils from patients with this disorder exhibit bilobed nuclei and absent specific granules on Wright-stained blood smears. Lactoferrin released from specific granules reduces the negative surface charge of the plasma membrane, contributing to nonspecific adhesiveness of the cell.[231]

The specific granule membrane also contains some of the intracellular store of the important adhesion molecule Mac-1 (CD11b/CD18) that is mobilized to the plasma membrane on stimulation by chemoattractants or other stimuli that induce granule secretion.[63,85] Specific granule deficiency results in marked impairment of adhesion and migration of neutrophils, probably on the basis of diminished intracellular pools of adhesive proteins and the inability to effect the change in surface charge caused by lactoferrin. This impairment leads to the recurrent skin and mucous membrane infections caused by *S. aureus*, gram-negative bacilli, and *Candida* that characterize the natural history of patients with this disorder.[90,231,550] Neutrophils in this disorder also exhibit diminished microbicidal activity, presumably because of diminished amounts of the cytochrome b_{558} component of NADPH oxidase that are stored in the membrane of specific granules.

Although the disorder is probably too rare to make such generalizations, specific granule deficiency likely is autosomal recessive in its mode of inheritance because males and females are represented equally. The documentation of a specific granule deficiency phenotype in mice rendered genetically null for an important myeloid cell transcription factor known as *CCAAT/enhancer binding protein epsilon* (C/EBPε) has led to studies in a few patients with specific granule deficiency that confirmed a deletion in the C/EBPε gene, with absent expression of this transcription factor, although not all patients with this disorder have a mutation of this gene.[183,360] Specific granule deficiency may be diagnosed in a patient with recurrent skin and mucous membrane infections whose neutrophils exhibit the characteristic absence of specific granules on Wright stain and a marked impairment of chemotaxis in vivo and in vitro.

Chédiak-Higashi Syndrome

Chédiak-Higashi syndrome is a complex, rare autosomal recessive disorder characterized by partial oculocutaneous albinism, recurrent pyogenic infections, peripheral neuropathy, and neutropenia.[81] The illness also may involve an accelerated lymphoproliferative phase.[81] Granular cells, including neutrophils, contain giant lysosomal granules that are the apparent result of spontaneous intracellular fusion of azurophilic granules and, to a lesser extent, specific granules.[81] Corresponding disorders of intracellular pigment granules and vesicle trafficking in axons account for the albinism and other manifestations of this disease.[81] Similar disorders have been described in Aleutian mink, beige mice, albino Hereford cattle, and albino whales.[81] The genetic basis of the defect now is known to involve either a nonsense or a frameshift mutation in the gene encoding a large protein called the *lysosomal trafficking regulator*; homologous to the "beige" gene in mice, with all mutations studied so far resulting in a truncated protein.[54,125]

Patients with Chédiak-Higashi syndrome develop recurrent skin and mucosal infections, most often caused by *S. aureus*, which are characteristic of those observed in defects of phagocyte migration.[31,81] They have a consistent defect in cell migration that seems to be related to abnormal regulation of microtubule polymerization on stimulation by chemoattractant agents.[81] The possible role of intracellular levels of cyclic adenosine monophosphate (cAMP) and guanylic acid in this microtubule abnormality has been suggested,[93] but the relationship between cyclic nucleotides and the microtubule dysfunction in Chédiak-Higashi syndrome has not been established. Ascorbic acid has been shown in at least one study to normalize the elevated levels of cAMP and the number of microtubules present within the cell.[93] Studies of two brothers with Chédiak-Higashi syndrome showed abnormally increased tyrosinylation of the alpha subunit of tubulin.[81,423] Phagocytosis is normal, but killing of ingested bacteria is defective or delayed. The reason for this deficient or delayed killing is uncertain but may involve defective phagolysosomal fusion or abnormalities in levels of microbicidal defensins, which also are stored in primary granules.[235]

The diagnosis of Chédiak-Higashi syndrome usually is suspected clinically on the basis of partial oculocutaneous albinism and recurrent pyogenic infections. A Wright stain showing giant lysosomal granules and laboratory studies showing defective cell migration are confirmatory.

Neutrophil Actin Dysfunction

Filamentous actin constitutes the main contractile mechanism of neutrophils for migration and phagocytosis.[560] An extremely rare and apparently heterogeneous disorder, neutrophil actin dysfunction, has been characterized by recurrent skin infections caused by *S. aureus* and *Candida albicans*. Biopsy samples of infected skin lesions in one child showed necrotic tissue with a notable absence of neutrophils. In vivo and in vitro studies revealed severely impaired neutrophil chemotaxis and phagocytosis.[91] The capacity for polymerization of actin from cell extracts also was diminished markedly. PMNs from family members of this patient also were found to be variably deficient in the CD11/CD18 family of glycoproteins that are the basis of LAD-1.[547,548] The nature and significance of this association is uncertain.

Another infant reported to have recurrent skin and mucosal infections and defective neutrophil chemotaxis was found to have abnormally high levels of a 47-kd protein, now identified as *lymphocyte-specific protein–1*, which exhibits actin-binding activity.[138] More recently, a 12-year-old patient with recurrent infections, mental retardation, and abnormal neutrophil chemotaxis was reported to be heterozygous for a substitution of lysine for glutamic acid-364 in non-muscle β-actin.[435] This substitution lies in a region important for binding to profilin and other actin-regulatory molecules. This patient's neutrophils also exhibited reduced production of superoxide, suggesting a possible role for normal actin function in the assembly of the NADPH oxidase complex.

Glycogen Storage Disease Type 1B

Beaudet and colleagues[60] first reported the association of recurrent infection, neutropenia, and impaired neutrophil migration with glycogen storage disease (GSD) type 1B, a metabolic disorder characterized by defective microsomal transport of glucose-6-phosphate. In 1985, Ambruso and coworkers[21] reviewed the features of 21 patients with GSD type 1B, 15 of whom had frequent infections, especially of the skin and subcutaneous tissues. Osteomyelitis, pneumonitis, sinusitis, and septicemia also were reported. Seventeen of these 21 patients were found to have serum inhibitors of myeloid stem cell proliferation, which were presumed to account for their chronic neutropenia. Impaired neutrophil motility was found in 8 of 11 patients in whom this condition was evaluated. Assays of neutrophil microbicidal capacity generally were normal. A specific relationship between the

underlying metabolic defect in GSD type 1B and the mechanism of impaired cell motility has not been established. However, exogenous glucose is an important energy source for chemotaxis,[611] and the uptake of glucose by PMNs in response to chemoattractant stimulation is impaired in patients with GSD type 1B and in neonates, both examples of patients with impaired PMN migration.[5,56]

EXTRINSIC OR SECONDARY DEFECTS OF POLYMORPHONUCLEAR LEUKOCYTE MIGRATION

Defective Neutrophil Chemotaxis Associated with Serum Inhibitors of Cell Function

Many investigators have reported the presence of inhibitors of PMN chemotaxis in the serum of patients with recurrent infection.[337,415,535,546,590,609] In most cases, the pathophysiologic mechanisms of these inhibitors are unknown. In many of the patients described, other associated immunologic disorders could account for at least part of the increased susceptibility to infection. In each case, the patient's neutrophils exhibited diminished chemotaxis in the presence of autologous serum or plasma, whereas identical assays in the presence of control serum or plasma resulted in a normal chemotactic response. Most such inhibitors seem to be immunoglobulins or immunoglobulin-like molecules.

Hyper-IgE Syndrome

In 1966, Davis and colleagues[169] described two young girls with coarse facial features, reddish hair, fair skin, severe eczema, dystrophic nails, staphylococcal skin abscesses, and recurrent sinopulmonary infections. The absence of classic signs of inflammation accompanying the staphylococcal abscesses led to their being characterized as cold abscesses. The term *Job syndrome* was suggested, referring to the similar biblical affliction. Additional patients, including a patient who exhibited a defect in neutrophil chemotaxis reported in 1973 by Clark and associates,[137] were described with a similar disorder, first associated by Buckley and colleagues[109] with very high serum IgE levels.

Subsequent reports of similar patients have shown that certain features are common to all patients with the disease, now called *hyper-IgE syndrome*. These consistent features include a history of staphylococcal infections of the skin and sinopulmonary tract beginning in infancy or early childhood and serum levels of IgE that are greater that 2000 IU/mL.[105,109,188] Other characteristic, but variable, features of this disorder include coarse facies, cold abscesses of the skin and subcutaneous tissues, a chronic eczematoid rash, eosinophilia, and mucocutaneous candidiasis.[105,188] Comprehensive reviews have provided detailed characterizations of the abnormalities of patients with this poorly understood disorder.[105,188] Consistent abnormalities of cell-mediated immune functions in patients with hyper-IgE syndrome suggest that the pathogenic basis involves a defect of T-cell regulation.[239] Documented abnormalities include diminished reactivity to *Candida* and tetanus toxoid in delayed hypersensitivity skin testing, decreased in vitro lymphocyte proliferative responses to these antigens, and reduced numbers of T cells with the CD45RO memory T-cell phenotype.[108,132,188]

A more recent extensive study of 30 patients with hyper-IgE syndrome and 70 of their relatives concluded that this disorder is inherited as a single-locus autosomal dominant trait with variable expressivity.[256] Although the specific gene defect or defects responsible for this disorder remain unknown, a study of 19 kindreds has revealed a genetic locus for hyper-IgE syndrome on chromosome 4, in the proximal 4q region.[257,258] More recently, a novel autosomal recessive form of hyper-IgE syndrome has been described that includes some patients who have developed severe viral infections or a form of autoimmune vasculitis involving the central nervous system.[83,257]

Although hyper-IgE syndrome more properly may belong in discussions of defective T-cell regulation, some patients with hyper-IgE syndrome may have a defect in neutrophil chemotaxis.[188] The defect, if observed at all, usually is intermittent. In several cases, the presence of a serum inhibitor of chemotaxis has been recognized.[188] Donabedian and Gallin[187] showed an inhibitor of granulocyte chemotaxis in supernatants from cultured peripheral blood monocytes from patients with hyper-IgE syndrome. The persistence of infectious complications in this disorder, at times when chemotaxis has been found to be normal and the presence of large purulent collections within cold abscesses, raises questions about the significance of a chemotactic disorder in explaining the markedly increased susceptibility of these patients to recurrent infections.

Impaired Generation of Serum-Derived Chemotaxins

A deficiency in the host's ability to produce chemotaxins derived from serum components may have profound consequences for the recruitment of PMNs to an infected site. The most important serum-derived chemotaxin is the fragment of the fifth component of complement, C5a, and its des-arg form. Several kindreds have been described with either absent or defective C5.[495] The chemoattractant activity measured in activated normal serum virtually is absent in C5-deficient serum.[496] The risk of developing systemic *Neisseria* infections owing to deficient activation of the lytic terminal complement sequence seems far more significant than any phagocytic recruitment defect caused by impaired production of chemotaxins.[62,217] Patients with C3 deficiency also have impaired chemotaxigenesis because C5 cannot be activated. Host impairment usually is more severe because of the importance of C3 in opsonization and its role in the activation of the remainder of the complement cascade.[499]

OTHER SECONDARY OR POORLY DEFINED DISORDERS OF POLYMORPHONUCLEAR LEUKOCYTE MIGRATION

Patients with protein-calorie malnutrition have defective PMN chemotaxis that seems to be based on systemic pre-activation of circulating cells owing to chronic low-level endotoxemia that results from impaired intestinal mucosal integrity.[128,512] Shwachman-Diamond syndrome, is associated with defective PMN migration in addition to pancreatic insufficiency, neutropenia, and growth retardation.[11] Two kindreds with congenital ichthyosis and an associated defect of PMN migration have been described.[402] Patients with severe thermal injuries develop an acquired form of specific granule deficiency with impaired PMN migration beginning approximately 14 days after injury.[231] Children with juvenile periodontitis of various types may exhibit reduced PMN chemotaxis.[437] In some of these patients, this condition has been associated with gingival infections caused by *Capnocytophaga*, an anaerobic gram-negative organism that can elaborate factors that markedly impair PMN migration in vitro.[532] In one such patient, the ultimate diagnosis was LAD-1, a finding that raises some uncertainty about the role of *Capnocytophaga* in such disorders.[282] Several reports have been published of a poorly defined disorder of neutrophil migration termed *lazy leukocyte syndrome*,[10,401] which is characterized by recurrent staphylococcal skin infections, rhinitis, gingivitis, stomatitis, neutropenia despite adequate marrow precursors, and diminished in vivo and in vitro migration of neutrophils.

DEFECTS IN PHAGOCYTE MICROBICIDAL ACTIVITY

As described earlier, the broad array of available phagocyte microbicidal mechanisms may be divided into oxygen-dependent and oxygen-independent mechanisms. To date, no isolated deficiency of a specific oxygen-independent microbicidal mechanism has been described. This section is concerned mainly with the

known deficiencies of oxygen-dependent microbicidal mechanisms of phagocytes, especially CGD, the prototypic defect in this group. PMN migration usually is normal in these killing defects. Monocytes and the fixed phagocytes of the reticuloendothelial system generally share in the deficient microbicidal activity.

Chronic Granulomatous Disease

CGD was one of the earliest syndromes of phagocyte dysfunction to be characterized[474] and probably is the most extensively studied among individual phagocyte defects. It is recognized now to be a family of biochemically and genetically heterogeneous disorders of distinct components of the phagocyte NADPH oxidase complex.[87,128,183] CGD results in the inability of phagocytes to generate superoxide anion and other reactive oxygen species.[183] Organisms that produce catalase pose a special problem for patients with CGD[183,189,306,355] and encompass a broad range of pathogens that includes staphylococci, gram-negative enteric bacteria, *Pseudomonas* spp., yeast, fungi, *Nocardia* spp., and numerous other pathogenic species.[189,306,355,622]

Most microorganisms produce H_2O_2, which might be used, even by the CGD phagocyte, as an effective microbicidal weapon because it feeds into the sequence of oxidant reactions downstream from the defective oxidase enzyme (see Fig. 2–5).[492] Organisms that produce catalase are able to survive within these deficient cells because catalase is an enzyme that degrades H_2O_2 to oxygen and water.[306,492] Infections with catalase-negative bacteria, such as *S. pneumoniae*, *H. influenzae*, and *N. meningitidis*, do not occur with increased frequency in patients with CGD,[622] and these organisms are killed normally in vitro by CGD phagocytes. Phagocyte functions not related directly to oxidative mechanisms of intracellular killing, including adherence, chemotaxis, phagocytosis, and degranulation, usually are normal in patients with CGD.[44,405,474,544]

The genetic defect in CGD may be inherited by X-linked recessive or autosomal recessive mechanisms.[87,160] In the female obligate carriers of X-linked CGD, the proportion of cells that express the defect usually is 35 to 65 percent, depending on the proportion of cells in which random inactivation of the normal versus the affected X chromosome occurs.[376,622] In most of the autosomal recessive forms of CGD, the quantity of the cytochrome in cells is normal, but there is a deficiency in one of two cytosolic proteins, $p47^{phox}$ and $p67^{phox}$, each of which is a crucial component of the fully assembled NADPH oxidase complex.[42,87,136] In the report of a recently created registry of 368 patients with CGD in the United States,[622] greater than two thirds of the patients had the X-linked recessive form with absent $gp91^{phox}$, the larger subunit of the cytochrome b_{558}; approximately 12 percent had an autosomal recessive form with absent $p47^{phox}$; and fewer than 5 percent each had autosomal recessive disease with absence of the $p67^{phox}$ or absence of $p22^{phox}$, the smaller subunit of the cytochrome b_{558}. Approximately 12 percent had an unknown genetic form of the disease.

A more recent review[276] indicates that approximately 5 percent of patients with CGD have normal levels of an abnormal protein that is inactive and that at least 410 different mutations have been reported to result in CGD. These genetically diverse defects all result in defective function of the oxidase and the characteristic CGD phenotype. Included in the above-mentioned registry were two rare adult women whose sons had X-linked disease and who exhibited clinical signs of the CGD phenotype because of dramatically skewed X-chromosome inactivation with 5 percent or less of their phagocytes showing oxidase activity. Overall, patients with X-linked disease have more severe courses and experience higher yearly death rates than do patients with autosomal recessive forms of the disease.[622]

Patients with CGD experience recurrent serious bacterial and fungal infections, usually beginning in the first few months of life. *S. aureus* and gram-negative bacilli, especially *Serratia* and *Burkholderia cepacia*, are the most common causes of infection in patients with CGD, but fungi, especially *Aspergillus* spp., also are prominent etiologic agents.[355,479,622] Infections caused by *Aspergillus* are the most common cause of death in patients with CGD.[622] Lymphadenopathy associated with lymphadenitis and chronic suppuration with poor healing is a common presenting feature of CGD. Granuloma formation at infected sites is a histologic hallmark of this disorder.[306,479] Pulmonary infections and their complications have been the reported cause of death in 50 percent of patients with CGD in some series, and *Aspergillus* predominates.[622]

These infections often are protracted and respond slowly to appropriate antibiotic therapy.[306,479] Progression to lung abscess, empyema, or both occurs in approximately 20 percent of patients with CGD and pneumonia.[306] Liver abscesses occur in approximately half of patients with CGD and may be recurrent.[62,133] The hepatosplenomegaly common in patients with CGD may result from these infections but probably is more likely to result from chronic infections at various sites with systemic lymphoid hyperplasia.[47,306] Osteomyelitis occurs in approximately one third of patients with CGD.[306,479,622] In contrast to normal children, in whom this infection usually involves the metaphyseal area of long bones, patients with CGD more often develop infections of the small bones of the hands and feet. In normal children, *S. aureus* is the most common etiologic agent, and this agent does cause a significant proportion of cases in CGD. However, gram-negative bacilli and *Aspergillus* seem to be the predominant etiologies, and other agents, including *Nocardia*, also may be important etiologic agents in CGD.[462,566,622] Skin infections in patients with CGD may include pyoderma, purulent dermatitis, and cutaneous or subcutaneous abscesses and often are preceded by a chronic eczematoid skin rash[479] but are less of a problem than in patients with leukocyte migration defects.

Although localized infections are the rule in patients with CGD, these patients also may develop septicemia.[306,479,622] The most common cause of septicemia in most series has been *Salmonella*, but other gram-negative enteric bacilli also have been prominent.[306,355] *S. aureus*, the most common etiologic agent of localized infections in CGD, is a proportionally less common cause of septicemia in these patients.[479,622] Other infections sometimes seen in patients with CGD include recurrent urinary tract infections in approximately 6 to 8 percent of patients; ocular infections with conjunctivitis, blepharitis, or both in approximately 20 percent of patients; and, rarely, chorioretinitis.[385,479]

Granuloma formation adjacent to hollow viscera in patients with CGD has been found to produce clinically significant obstruction. Reported examples of this problem include obstruction of the gastric outlet, esophagus, small intestine, and ureters.[22,129,189] This complication usually responds to treatment with corticosteroids.[129]

CGD should be suspected in patients with a history of recurrent indolent infections caused by catalase-positive organisms such as those described earlier, especially if granulomas are found in biopsy specimens of lymph nodes or other tissues. Confirmation of the diagnosis usually rests on the demonstration of an absent or nearly absent oxidative metabolic burst in the patient's phagocytes. This burst can be detected by the slide nitroblue tetrazolium test (Fig. 2–12) or by other measurements of oxidative burst activity, such as cytochrome reduction, lucigenin-enhanced or luminol-enhanced chemiluminescence, oxygen consumption, H_2O_2 production, and flow cytometry of cells loaded with oxidant-sensitive fluorescent dyes.[15,42,57,391,491,579] Prenatal diagnosis has been achieved by the use of the slide nitroblue tetrazolium test with blood from placental vessels obtained at fetoscopy.[425]

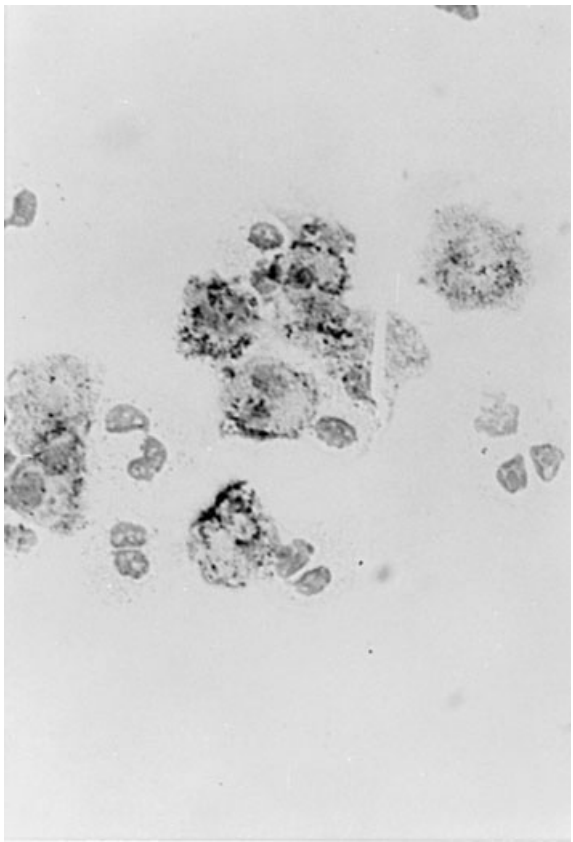

Figure 2–12 Photomicrograph of a slide nitroblue tetrazolium (NBT) test of polymorphonuclear leukocytes (PMNs) isolated from the blood of a maternal carrier of X-linked recessive chronic granulomatous disease. Because of random inactivation of either the normal or the affected X chromosome in maternal carriers of this disorder, approximately half of the PMNs exhibit the granular blue-black staining characteristic of the oxidative reduction of NBT by normal PMNs. In contrast, the remaining PMNs, which express the reduced nicotinamide adenine dinucleotide phosphate (NADPH) oxidase defect of chronic granulomatous disease, are visible only by their nuclear counterstain.

The management of patients with CGD traditionally has relied on antibiotic prophylaxis, usually with trimethoprim-sulfamethoxazole or an oral antistaphylococcal penicillin, and an aggressive approach to the specific diagnosis and treatment of acute infections.[479] The use of antifungal prophylaxis with itraconazole or newer azole derivatives that also have activity against *Apergillus* has become standard in most centers. Granulocyte transfusions have been reported to be at least partially beneficial in a few cases.[112,142] Bone marrow transplantation met with limited success in the late 1970s and early 1980s, with only one successful long-term engraftment reported among four separate attempts in different patients.[251,316,477,615] Allogeneic peripheral blood stem cell transplantation from HLA-identical sibling donors, with prior myeloablative conditioning, has been considerably more promising, as suggested by the European experience from 1985 to 2000—22 of 23 patients "cured," with median follow-up of 12 years.[524] Longer term follow-up is necessary, however, to confirm the promise of this approach to treatment.

One of the most important developments in the treatment of patients with CGD is the administration of IFN-γ. A multicenter study initially showed that daily subcutaneous injections of this agent reduced the requirement for hospitalization of patients with CGD for serious infections by approximately two thirds.[298] IFN-γ does not increase oxidase activity in neutrophils, and the mechanism by which IFN-γ exerts its beneficial effect in CGD has not been determined. Long-term follow-up of patients being treated with IFN-γ has indicated that it continues to reduce the rate of serious infections over the course of many years and generally is well tolerated.[382] This treatment carries some mild systemic side effects, such as fever, fatigue, and myalgia,[298] but it has become part of the standard regimen for managing most patients with CGD.

The definition of the molecular basis for CGD and the cloning of the genes responsible for the various forms of this defect have led to correction of some forms of the defect in cultured cells and the development of animal models of CGD in mice,[183,301,469,599] both of which are crucial steps in developing gene therapy for patients with this disorder in the future. Preclinical studies of gene therapy in mice with CGD have shown promise when bone marrow cells are transduced using retroviral vectors ex vivo and transplanted into irradiated syngeneic recipients.[183] Fifty to eighty percent of circulating neutrophils were oxidase-positive 12 to 14 weeks post-transplantation, and protection from respiratory challenge with *Aspergillus* was achieved.[183] Early human phase I trials with autosomal recessive ($p47^{phox}$) and X-linked CGD, using autologous peripheral blood stem cells retrovirally transduced ex vivo and then re-infused without prior marrow conditioning, have yielded a maximum of 0.2 percent oxidase-corrected neutrophils in the circulation.[379] This accomplishment is still far short of what probably must be achieved to produce a clinically meaningful correction.[183]

Deficiencies of Glucose-6-Phosphate Dehydrogenase and Glutathione Peroxidase

The normal activity of the NADPH oxidase enzyme complex depends on the continued availability of NADPH to reduce molecular oxygen to form superoxide anion.[40,135,491] The primary source of NADPH for this enzyme is the hexose monophosphate shunt. It is provided with the hexose substrate, 6-phosphoglucose, by the enzyme glucose-6-phosphate dehydrogenase, which also generates NADPH in a coupled reaction.[492] The reactions of the hexose monophosphate shunt itself are coupled to two other enzymes, glutathione reductase and glutathione peroxidase, which recycle oxidized and reduced glutathione.[492]

The absence of any of these three enzymes results in a lack of available NADPH to drive the NADPH oxidase. Deficiencies in any of these other three enzymes may result in a phagocyte killing defect similar to that of CGD. Patients with deficiencies of glucose-6-phosphate dehydrogenase or glutathione peroxidase have been described with functional oxidative metabolic defects and presentations that are clinically indistinguishable from CGD caused by defects in the NADPH oxidase itself.[43,505] Glucose-6-phosphate dehydrogenase deficiency usually involves erythrocytes and is associated with hemolytic anemia, especially in conjunction with the administration of sulfonamides.[150] Only when the defect also involves myeloid cells and is severe or complete (<5 percent of normal enzyme levels) is the CGD-like disorder manifested.[43,505] A partial deficiency of glutathione reductase has been reported, with hemolytic anemia and early cataracts, but no increased incidence of infection was noted.[490]

Glutathione Synthetase Deficiency

Glutathione, along with glutathione peroxidase and glutathione reductase, the two enzymes involved in its recycling between oxidized and reduced forms, constitutes a protective mechanism in PMNs against membrane damage mediated by reactive oxygen intermediates formed during PMN activation.[490] The synthesis of an adequate supply of glutathione is crucial to these cells. Two brothers with glutathione synthetase deficiency who presented with neutropenia, hemolytic anemia, acidosis, 5-oxoprolinuria, and recurrent infection were reported by Spielberg and colleagues.[92,549] The PMNs from these patients exhibited elevated

cytosolic H_2O_2 levels, diminished oxidative microbicidal activity, and impaired microtubule assembly. Antioxidant therapy with vitamin E normalized the in vitro abnormalities of the patients' PMNs, and these patients had no further difficulty with recurrent infections.[92] This result suggested that vitamin E protected the cell membranes by scavenging excess H_2O_2 produced during PMN activation and prevented or minimized oxidant-induced membrane damage.

Myeloperoxidase Deficiency

Congenital deficiency of neutrophil MPO, previously thought to be a rare disorder, has come to be recognized as the most common heritable disorder of neutrophil function. Its clinical significance remains in doubt, however. Population surveys made possible with the advent of automated flow cytochemical techniques have indicated an incidence of MPO deficiency of approximately 1 in 2000 individuals.[424,460] Approximately half of these patients have complete absence of this neutrophil enzyme, and the remainder have a partial deficiency. The precise mode of inheritance of this defect has not been established, but MPO is known to be a product of a single gene on chromosome 17.[591] The reaction of MPO with H_2O_2 and chloride, causing the formation of hypochlorite, is one of the most effective microbicidal mechanisms of neutrophils, and cells from some patients with MPO deficiency have been found to exhibit delayed killing of *C. albicans* and *S. aureus*. MPO deficiency rarely has been associated with unusual infectious complications, however.

IMPORTANT EXAMPLES OF SECONDARY IMMUNODEFICIENCY (NOT INCLUDING HUMAN IMMUNODEFICIENCY VIRUS INFECTION)

Asplenia

Fulminant infections can occur in patients who have anatomic or functional asplenia.[512] The mortality rate from these infections in asplenic patients ranges from 40 to 80 percent.[621] The most common pathogens are encapsulated bacteria, including *S. pneumoniae* (50 to 70%), *H. influenzae*, and *N. meningitidis*.[621] Other streptococci, *S. aureus*, *Salmonella*, and other gram-negative bacilli are important but less common pathogens. All can cause fulminant, often fatal, disease characterized by rapid onset of shock. Malaria and babesiosis also are more severe in asplenic individuals. Infections can occur at any time but are most common within the first 2 years after patients undergo splenectomy.

The liver and the spleen are important in phagocytic clearance of bacteria from the circulation, and the spleen is an important site for antibody production. The spleen is more important than the liver in processing antigen in the naive host. The younger the individual is when splenic function is lost, the higher is the risk for developing serious infection. Young children who become asplenic are much more susceptible to fulminant infection than are adults because adults are more likely than are children to have encountered antigens before undergoing splenectomy. Individuals whose indication for splenectomy is thalassemia or Hodgkin disease are at higher risk of dying of overwhelming infection than are individuals who have functional asplenia from sickle-cell disease. Patients who undergo removal of their spleens for spherocytosis or idiopathic thrombocytopenia have a lower risk of developing infection. The lowest risk group consists of adults whose spleens are removed surgically after trauma, who are at little or no increased risk of developing infection.[147]

Congenital asplenia usually is associated with complex congenital cardiac disease and occasionally with structural abnormalities of the gastrointestinal or genitourinary tracts. Asplenia should be suspected in any patient with congenital heart disease and sepsis caused by encapsulated organisms. Anatomic or functional asplenia also should be suspected in patients with erythrocytes that contain Howell-Jolly bodies because these bodies are normally removed from the circulation by the normally functioning spleen. A radionuclide liver spleen scan or other modes of abdominal imaging usually can confirm asplenia or significant hyposplenia.[512]

Elective splenectomy for conditions such as hereditary spherocytosis should be delayed as long as possible, and splenic repair or subtotal splenectomy should be performed whenever possible after trauma. Asplenic patients are managed using prophylactic antibiotics, usually penicillin V or amoxicillin until at least 5 years of age.[147] They also should be immunized against encapsulated organisms at the appropriate ages (e.g., *H. influenzae* type b and pneumococcal conjugate vaccines beginning at 2 months and meningococcal polysaccharide vaccines at 2 years).[147] Patients should be warned about their increased risk of acquiring serious infections caused by malaria and babesiosis.

Sickle-Cell Disease

Immunodeficiency in patients with sickle-cell disease is due largely to their functional asplenia.[512] Part of the risk of developing infection stems from local infarction and tissue necrosis caused by sickling, which causes sludging and resultant tissue hypoxia. The reticuloendothelial system also may be obstructed by having to deal with chronic hemolysis. Patients with sickle-cell disease are protected partially from *Plasmodium falciparum* malaria but have a high incidence of fulminant sepsis and meningitis caused by encapsulated organisms (e.g., *S. pneumoniae*, *H. influenzae* type b, *N. meningitidis*) and *Salmonella*.[224] The relative risk of pneumococcal meningitis developing in children with sickle-cell disease is approximately 500 times that of normal children. *Salmonella* infections often are associated with osteomyelitis or meningitis.[202]

Patients with sickle-cell disease seem to have normal antibody response to most antigens, including age-appropriate responses to vaccines. A deficiency in heat-labile opsonic activity has been reported and may be due to a defect in the alternative pathway of complement. Rare cases of patients with sickle-cell disease who have deficiencies of factor B have been reported, but patients with sickle-cell disease have normal CH_{50} and normal levels of properdin, C3, and factor I.[308] Patients with sickle-cell disease should be managed with prophylactic antibiotics until they are at least 5 years old and should be immunized against *H. influenzae* type b, pneumococci, and meningococci at the appropriate ages.[147]

Cystic Fibrosis

Cystic fibrosis (CF) is an autosomal recessive disorder caused by mutations in both alleles of the gene encoding the protein called *cystic fibrosis transmembrane conductance regulator* (CFTR), a cAMP-regulated chloride channel expressed at the luminal surface of airway epithelial cells.[613] Most patients with CF develop chronic endobronchial infection with *P. aeruginosa* of the mucoid phenotype. This infection is accompanied by an intense chronic airway inflammation with an exuberant influx of neutrophils that leads to bronchiectasis and destruction and fibrosis of lung and airway tissue and early death.[130,334] No systemic disorder of immunity has been documented in CF, but local factors in the airway inflammatory milieu, including neutrophil-derived proteases such as elastase, contribute to secondary impairments in opsonic and phagocytic host defenses by cleaving opsonic antibody and complement fragments and important phagocytic receptors for these opsonins.[581]

Mucociliary clearance of bacteria from the CF airway is impaired because of viscous, dehydrated epithelial lining fluid, although the early pathogenesis of the unique chronic endobron-

chial infection in CF and its relationship to the underlying genetic defect have remained obscure. Several possible explanations have been offered. Researchers have suggested that CFTR on airway epithelial cells may function as a receptor for *Pseudomonas* that the cells normally use to internalize the organisms before being sloughed and cleared by mucociliary action and that this internalization may be deficient in CF epithelial cells.[466] Other evidence suggests that microbicidal peptides of or related to the defensin family, released from airway epithelial cells as a local defense mechanism, may be inactivated by abnormal salt concentrations at the airway epithelial surface, thwarting an important first line of local antibacterial defense.[539]

None of the aforementioned explanations has proved satisfactory. A reduction in cell surface sialic acid on CF epithelial cells unmasks the glycoprotein asialo-GM-1, which seems to function as an epithelial receptor for adhesion by *P. aeruginosa* and may promote early airway colonization.[508] More recently, investigators have proposed that reduced expression of inducible nitric oxide synthase by CF airway epithelial cells may impair airway host defenses against early small inocula of bacteria, but the unresolved question of the relationship between the CF genetic defect and *Pseudomonas* chronic lung infection continues to stimulate active research.[130]

EVALUATION FOR IMMUNODEFICIENCY IN A CHILD WITH RECURRENT OR SEVERE INFECTIONS

Most immunodeficiency disorders can be diagnosed readily by employing a methodical process that begins with careful analysis of the child's presenting history and physical examination.[149,156,178,285,439,512,515] This information serves as the foundation for a rational laboratory evaluation. It is important to bear in mind that it is normal for children to have several infections every year. Normal children who are exposed to other children, particularly older school-aged siblings or classmates, develop approximately one infection per month. Most infections in immunocompetent children are characterized by being mild and localized to the gastrointestinal or upper respiratory tracts, and they either are self-limited or respond rapidly to conventional therapy. Immunocompromised hosts tend to have more frequent, severe, and unusual infections that may not respond readily to appropriate therapy.

HISTORY

A detailed history alone is sufficient to determine whether or not an immunologic evaluation should be pursued in many children who have recurrent or severe infections. If tests for immunity are indicated, the history also serves as a guide to the types of studies that should be performed initially. Table 2–5 provides a list of historical information that is valuable in assessing the likelihood the patient has an immunodeficiency. Whenever possible, the child's complete medical records (including growth charts) should be obtained, particularly if several physicians have provided care, because the history often is complicated, and incomplete or inaccurate information may be misleading.

The age of onset of suspicious infections usually helps define the underlying problem. Children with isolated immunoglobulin deficiencies tend to do well during the first few months of life because they are protected by maternal antibody. They usually start developing serious infections later in the first year of life. Children with cell-mediated or phagocytic disorders may begin developing infections in the newborn period (see earlier). In contrast, healthy children who have been cared for at home by their mothers and who have no siblings often have few infections in the first few years of life but may present for immunologic evaluation when they develop recurrent respiratory infections beginning the first few weeks after entering daycare, preschool, or kindergarten, owing to new exposure to various respiratory viral pathogens.

TABLE 2–5 History in the Evaluation of a Child with Recurrent or Severe Infections

Age at onset of infections
Number, frequency, and periodicity of infections
Nature of infections
Location on body
Organism(s)
Severity
Duration
Nature and duration of therapy
Response to therapy
Hospitalizations
Surgery
Growth pattern
Periodontal disease
Allergies
Immunizations
Exposure history
Contagious diseases in family, school, or community
Number and ages of siblings
Parents' occupations
Babysitting or daycare arrangements
Foreign travel
Parental smoking
Wood furnaces
Family history (especially in males)
Immunodeficiency
Recurrent, severe, or unusual infections
Cause of early deaths
Autoimmune disease
Allergy
Consanguinity
Days of school missed (and why)

The number, nature, and severity of infections help in determining how aggressively to pursue an immunologic evaluation. Certain clinical presentations of disease and causative organisms are associated with a high likelihood of an immunodeficiency. Antibody or complement deficiencies, or functional asplenia, should be suspected in children with recurrent or life-threatening infections, such as sepsis and meningitis caused by encapsulated organisms (e.g., *S. pneumoniae*, *H. influenzae* type b).[102,178,308,357,439,493] Complement deficiencies should be considered in individuals with recurrent or severe neisserial disease.[175] CGD should be suspected in the presence of recurrent or unusual deep tissue infections, such as liver abscess, lymphadenopathy, pneumonia, or osteomyelitis, especially when caused by unusual gram-negative bacteria, such as *Serratia marcescens*, or by *Aspergillus* spp.[622] Pneumonia caused by *Pneumocystis* suggests a T-cell deficiency, either hereditary or due to HIV infection.

Recurrent or even severe infections with group A streptococci have not been associated with immunodeficiency. Recurrent urinary tract infections usually are associated with anatomic abnormalities of the urinary tract and not immunodeficiency.

An essential part of the history is documentation of the child's growth pattern. Children who are thriving, particularly those older than 2 years of age, are much less likely to have serious immune disorders than children with failure to thrive.

The immunization history should be documented carefully because it may prove to be useful in evaluating the child's ability to mount an antibody response to specific vaccine antigens. The

history of recent live viral immunization should be obtained in children who have clinical presentations compatible with polio or measles because infection caused by vaccine strains of these viruses is the first indication of immunodeficiency in some children.

Exposures to contagious diseases may lead to recurrent and occasionally even severe infections in individuals with normal host defenses. Children who never leave the house may have recurrent infections from organisms brought home by older siblings, other relatives, or neighbors. One must assume that children who attend daycare facilities or schools constantly are being exposed to common infections. Familiarity with community patterns of disease, such as prevalent clinical manifestations of enterovirus infection or the beginning of croup, respiratory syncytial virus, influenza, or rotavirus seasons, can be used to reassure families of normal children with frequent mild infections. Environmental pollutants, such as cigarette smoke and wood-burning stoves, also have been associated with an increased risk of acute lower respiratory illnesses developing in children.[485]

Because many immunodeficiencies are hereditary, a detailed family history should be obtained that includes questions about the presence of immunodeficiency, recurrent or severe infections, contributing factors to any early deaths, the gender of affected individuals, and consanguinity. A history of recurrent or severe infections in more than one male relative is highly suspicious of a familial immunodeficiency disorder. Autoimmune diseases may suggest a familial disorder of complement or cell-mediated immunity.[175]

A thorough history of school absenteeism and the reasons the child stays home may be helpful in differentiating medical from psychosocial problems in older children who present for evaluation for recurrent infections, particularly when symptoms are unusual or inconsistent with physical findings. Prolonged absences for vague problems with no physical findings, particularly in the presence of normal growth, are less likely to be caused by infections than are those characterized by well-defined physical or laboratory findings and poor growth. Generally, recurrent severe infections beginning before the child reaches age 1 year, failure to thrive, invasive disease caused by encapsulated or unusual organisms, or family histories of such infections should prompt consideration of an immunologic evaluation.

PHYSICAL EXAMINATION

Physical examination may provide valuable clues as to the nature of the immune disorder. In certain cases, such as some patients with hyper-IgE syndrome[105,281] and DiGeorge syndrome[182] who exhibit the characteristic facies, it may be diagnostic. As noted earlier, one of the most obvious signs that a child may have a serious underlying medical problem is failure to thrive. Every immunologic evaluation *must* include documentation of current growth parameters and a comparison with past growth.

Many immunodeficiency disorders have dermatologic manifestations. Eczematoid rashes are seen in patients with the hyper-IgE syndrome and CGD.[105,281] CGD also is characterized by slow wound healing and the development of hypertrophic scars.[622] Patients with Chédiak-Higashi syndrome have partial albinism.[81] Severe gingival disease and early loss of teeth are prominent clinical features in disorders of neutrophil migration, such as LAD.[30,31]

The chest should be evaluated for physical signs of active disease, such as rales and rhonchi, and evidence of chronic infection, such as an increased anterior-posterior diameter. Pneumonia, bronchitis, bronchiectasis, and scarring can occur with most immunodeficiencies but are associated most frequently with immunoglobulin deficiencies.[102,271,439] Cardiac abnormalities may be associated with primary asplenia or immunodeficiency disorders such as DiGeorge syndrome,[182] and situs inversus should alert the clinician to the possibility of ciliary dyskinesia.[149] Although hepatosplenomegaly may be found in many types of primary immunodeficiency disease, it occurs more frequently in patients with disorders of phagocyte function, especially CGD.[622]

LABORATORY STUDIES

The laboratory evaluation should be guided by the history and physical findings. Simple, inexpensive screening tests often can help narrow the differential diagnosis and streamline the evaluation. One of the first tests that should be performed is a complete blood count with differential and evaluation of the blood smear. This simple test can detect several immunologic abnormalities, including neutropenia, lymphopenia associated with HIV-1 or forms of SCID, the abnormal neutrophil granules associated with the Chédiak-Higashi syndrome, Howell-Jolly bodies found with asplenia, and some malignancies. Chest radiographs should be examined for thymic tissue, mediastinal lymphadenopathy, pneumonia, bronchiectasis, and other evidence of pulmonary infections. Consideration also should be given to evaluating patients with chronic pulmonary disease for CF with a sweat chloride test.

Quantitative immunoglobulin levels provide a useful screening test for evaluating patients with suspected humoral immunodeficiency. IgG2 deficiency often is not reflected in the IgG level, however, because it makes up such a relatively small proportion of total IgG. Patients with suspected humoral immunodeficiency usually should be tested for IgG subclasses and quantitative IgG, IgA, and IgM. Enumeration of B cells and marker studies by immunofluorescence flow cytometry should be considered if more severe forms of humoral deficiency, such as XLA or some forms of CVID, are suspected. Very high IgE levels may be helpful in establishing the diagnosis of hyper-IgE syndrome, although an elevated IgE level is much more common in patients with allergies than immunologic abnormalities. Immunoglobulin levels may be extremely elevated in children with HIV-1 infection.

Children who have normal quantitative immunoglobulin and IgG subclass levels but who continue to have frequent sinopulmonary infections that do not respond well to appropriate medical and surgical management (e.g., ventilation tubes) also can be evaluated by measuring antibody responses to specific antigens, such as tetanus and diphtheria toxoids and *H. influenzae* type b and meningococcal and pneumococcal capsular polysaccharides. Antibody levels can be measured before immunization and approximately 1 to 2 months after immunization is administered to evaluate the child's ability to respond to different kinds of antigens, including T cell–dependent antigens such as diphtheria and tetanus toxoid or T cell–independent antigens such as unconjugated pneumococcal capsular polysaccharide vaccine.

The complement system should be evaluated in individuals with recurrent or life-threatening neisserial disease, including systemic gonococcal infections and sporadic meningococcal disease. The best screening test for hemolytic complement is the CH_{50}. A normal CH_{50} reflects a normal quantity and function of classical pathway proteins (C1, C4, C2), C3, and terminal components through C8, as noted earlier.[143] The alternative pathway proteins can be measured in a similar assay employing rabbit erythrocytes instead of antibody-coated sheep cells. Alternative pathway deficiencies are extremely rare; demonstration of a normal CH_{50} generally is a sufficient indicator of normal complement activity. An abnormal CH_{50} should be repeated immediately, taking care that the specimen is handled correctly. Complement abnormalities may be quantitative or qualitative. If a very low CH_{50} is confirmed, determining the serum levels of

individual complement proteins and their functional activity in consultation with a reference laboratory is important.

Delayed hypersensitivity skin testing with antigens such as *Candida* or mumps antigens may be useful to assess cell-mediated immunity, but results are not always reliable. T-lymphocyte subset quantitation may be helpful in diagnosing such conditions as SCID and HIV, but more sophisticated testing of lymphocyte function, such as mitogen and antigen stimulation, should be done in patients with recurrent or severe fungal infection. These studies ideally should be directed by a clinical immunologist.

Similarly, suspected phagocyte function disorders should be evaluated in consultation with experts in phagocyte function because lack of proper standardization and expertise often leads to misleading results from commercial laboratories. Phagocyte function studies should be directed toward adherence and migration in patients with recurrent skin and mucosal infections, poor or absent formation of pus, and persistent leukocytosis suggestive of a leukocyte adhesion defect. Tests of oxidative metabolic activity and killing should be performed in patients with recurrent staphylococcal or unusual gram-negative or fungal tissue infections suggestive of CGD.

Whenever clinical suspicion of an inherited immunodeficiency is confirmed by appropriate functional laboratory studies, arranging for specific genetic testing to determine the precise nature of a patient's genetic mutation or variation, when such testing is available, may be desirable. This testing may assist greatly in subsequent genetic counseling and would add important information to the clinical database on patients with the specific disorder.

MANAGEMENT OF IMMUNODEFICIENCY DISORDERS

Proper management of immunodeficiency disorders (as described earlier) can enhance markedly the quality of life and life expectancy. Although some children with immunodeficiency disorders have serious problems with autoimmune disease, malignancy, or both, most morbidity and mortalities result from infections. This discussion is limited to the general principles of managing infectious complications of immunodeficiency.

EDUCATION

After the immunologic abnormality has been thoroughly characterized, the first step in management is to educate the family and, when he or she is old enough, the patient regarding environmental risks, how to take medications, and precisely when and where to seek medical care. Families of patients with inherited disorders should receive genetic counseling and be offered the option of prenatal screening if it is available for the disease in question.

EVALUATION FOR INFECTION

Patients with known immunodeficiency disorders should be evaluated promptly and thoroughly for unexplained fevers or any other indication of infection. Immunodeficient individuals are susceptible to a wide variety of pathogens, their responses to appropriate therapy may be slow, and they often require prolonged treatment. Every effort should be made to identify the infecting organism so that treatment can be specific. Unless the pathogen is known, extended courses of empiric broad-spectrum coverage may be required, which may lead to superinfection caused by multidrug-resistant pathogens.

TREATMENT OF INFECTIONS

Many aspects of disease-specific therapy already have been discussed. Generally, patients with immunodeficiency who are susceptible to bacterial infections should be treated empirically and aggressively with antibiotics at the first indication of infection. Antifungal therapy should be added empirically in patients with increased risk of developing fungal infection (e.g., patients with cell-mediated immune and neutrophil disorders) if a prompt response to antibacterial therapy does not occur. When a definitive etiology has been established, treatment should be tailored to the pathogen. The duration of therapy must be individualized, but generally patients with abnormal immune systems should be treated longer than normal hosts who have similar infections.

PREVENTION OF INFECTION

Patients and household members should be immunized with appropriate vaccines as soon as possible after a diagnosis of immunodeficiency has been established.[147] Although many immunodeficient patients, such as those with XLA, cannot respond to immunizations, immunization of household members and other close contacts with vaccines may reduce the patients' likelihood of developing infection. Patients with complement deficiencies, asplenia, and sickle-cell disease should be immunized with vaccines directed against encapsulated organisms, such as meningococci, pneumococci, and *H. influenzae* type b. These patients may not have normal responses to immunization, however, and, if possible, their antibody responses to these vaccines should be measured; if they are low, these patients should receive extra doses of the vaccines. Live viral vaccines should not be administered to patients with primary defects in cell-mediated immunity.

Patients with disorders characterized by recurrent or severe bacterial or fungal infections may require prophylactic antibacterial or antifungal therapy. The benefits of long-term antimicrobial therapy must be weighed carefully against the risks of rapid emergence of multidrug-resistant organisms.

PROSPECTS FOR CORRECTION OF SERIOUS PRIMARY IMMUNODEFICIENCIES

Bone marrow or stem cell transplants have been successful in a few patients with specific immunologic disorders, including SCID,[104,124] Wiskott-Aldrich syndrome,[453] LAD, and CGD.[31,221,615] Gene therapy has raised new possibilities for correcting certain immunologic defects, having shown early promise for some forms of SCID and early success in animal models of CGD.[183,472] The development of malignancies associated with retroviral gene therapy in some cases raises serious concerns about the ultimate feasibility of this approach,[107,472] but the means of overcoming these and other potential obstacles to gene therapy will continue to be sought.

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CHAPTER 3

Metabolic Response of the Host to Infections

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Although infectious microorganisms constitute a continuing threat at all ages of life, they are particularly dangerous in neonates, infants, and young children. Despite the availability of modern sanitation, public health measures, vaccines, and antibiotics, most children have many discrete episodes of acute infection before reaching adulthood. Depending on their severity and duration, infectious illnesses can interrupt normal growth patterns. More importantly, if closely spaced in time, a series of infections can initiate a downhill health spiral, leading to malnutrition, chronic debilitation, immune system dysfunction, and death.[12,14,86,87,129] This possibility is of greatest concern in newborns and weaning children, especially in Third World areas.

The human host normally protects itself against invading microorganisms by maintaining a broad array of general defensive mechanisms and immunologic responses. This array includes the initiation of acute-phase reactions triggered by the release of proinflammatory cytokines from macrophages, monocytes, and other cells.[7,12,14,42] The resulting nonspecific defensive measures typically include fever and anorexia, slow-wave sleep, accelerated production of phagocytic cells, hormonal responses, and participation of many biochemical pathways and molecular mechanisms within body cells.[7,8,10-12,14,170] The acute-phase reaction produces stereotyped patterns of transient metabolic sequelae (diagrammed in Fig. 3–1), which accompany and follow most acute, generalized infectious illnesses and some localized ones.[60] A pediatrician should anticipate these metabolic changes so as to recognize dangerous complications, such as hypoglycemia and electrolyte imbalance, that may occur during an acute infection.

The array of metabolic changes depicted in Figure 3–1 typically is shared as a group of common responses during all generalized acute infectious diseases.[6-9,12,13,141] Similar changes are seen during other types of disease or trauma when they are accompanied by fever or inflammatory reactions.[75] These common acute-phase responses are composed of a hypermetabolic admixture of anabolic and catabolic components. Each 1° C of fever causes basal cellular oxygen consumption to increase approximately 13 percent.[8] The resultant increase in cellular energy expenditure comes at a time when food intake and intestinal absorption are diminished by anorexia and sometimes by vomiting and diarrhea. In the absence of an adequate intake of nutrients, body energy needs are met mostly by the oxidation of metabolic substrates that are derived from nutrient stores already contained within body tissues. The hypermetabolic effects of acute fever are fueled primarily by carbohydrates (some of which are derived from the metabolism of amino acids),[8] but if infectious illnesses become subacute or chronic, body fats become the important sustaining fuel.

Generalized acute-phase metabolic responses may be modified by numerous factors, such as the severity of an infectious process, its duration, and its possible progression to a subacute or chronic disease.[7-12,14] The age and sex of the patient, the presence of genetic resistance (or susceptibility) factors or partial immunity, the adequacy of nonspecific defensive mechanisms and de novo immune responsiveness, the preexisting nutritional status, and the presence or absence of other diseases all combine to modify host metabolic responses through their diverse influences on the infectious process per se.

Superimposed on this general array of common host metabolic responses are additional metabolic changes that occur when an infectious process becomes localized within certain anatomic sites or organ systems. Diarrhea that develops during gastrointestinal infections can lead to depletion of fluids and electrolytes, hepatic infections can lead to derangements of carbohydrate and amino acid metabolism, and infections of the central nervous system that cause neuronal destruction are accompanied by muscle paralysis and atrophy. Other infections localized within the cranial vault often produce an inappropriate secretion of antidiuretic hormone, leading to development of dangerous overhydration in children.[14] The development of shock syndromes during infectious diseases imposes additional metabolic derangements because of progressive stagnant hypoxia.[10,135]

In recent years, the role of genetic variations in host genes important in the immune response to infections has been studied. Wide variations in individual response to several microorganisms, such as *Chlamydia trachomatis*[38] and *Candida albicans*, have been documented.[148]

The total number of discrete metabolic responses known to occur during acute and chronic infectious illnesses continues to expand.[10] The most widely recognized of these multiple responses are grouped for discussion into major categories, including changes in nitrogen, amino acid, carbohydrate, lipid, electrolyte, vitamin, and trace element metabolism. Another important mechanism involved in the host response to infection is oxidative stress, which plays a major role in the systemic inflammatory response in bacterial[153] and viral infections.[147] The important initiating and control mechanisms provided by cytokines, antioxidants, and the endocrine system also are discussed.

Importantly, each of the many individual metabolic changes that occur during the course of an infection must be interpreted in a manner that reflects its longitudinal development and progression over time and its relationship to the evolving phases of the infectious process.[7] Some metabolic changes may be detected during the incubation period, many other responses occur at the onset of fever, and still other phenomena develop during the recovery phase of illness or later during convalescence.

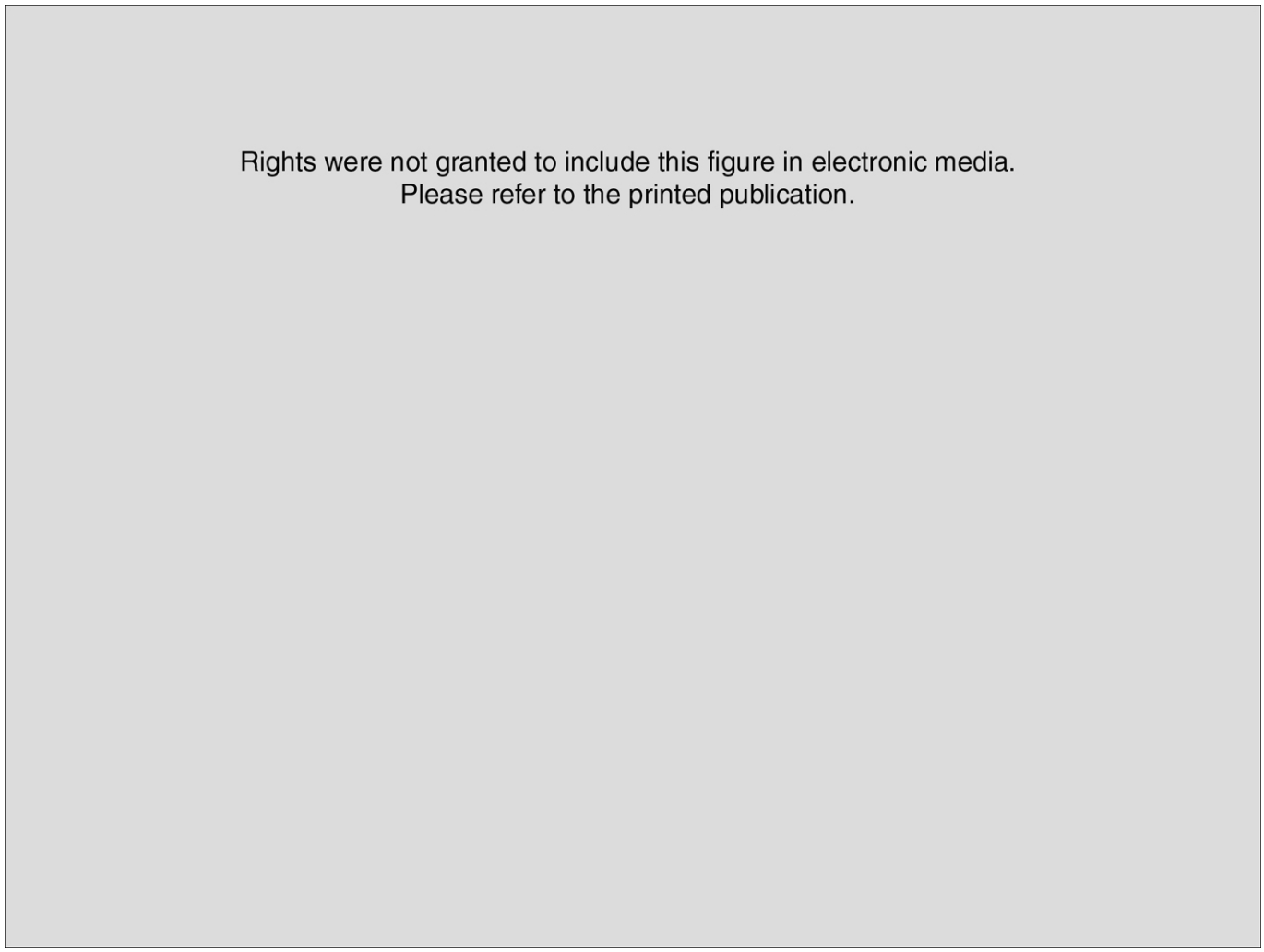

Figure 3–1 Onset time of various host metabolic responses in relation to the sequential phases of a "model" acute, self-limited, generalized infectious illness. *(From McKigney, J. I., and Munro, H. N. [eds.]: Nutrient Requirements in Adolescence. Cambridge, MA, MIT Press, 1976, p. 250.)*

NITROGEN METABOLISM

Fever and its accompanying hypermetabolic state, as induced by acute-phase responses during infectious illnesses, trigger a complex assortment of changes in protein, amino acid, and nitrogen metabolism.[9] Although the catabolic destruction of skeletal muscle protein is most obvious clinically, important anabolic events occur simultaneously. These events involve the synthesis of new proteins and cells that are important in host defense mechanisms. Increases in whole-body protein turnover involve catabolic and anabolic events in chronic infections also, as shown by leucine kinetic studies in patients with human immunodeficiency virus (HIV) infections.[82] Using amino acids isotope tracing techniques to measure muscle protein synthesis, researchers also showed that high plasma HIV RNA interferes with muscle amino acid synthesis and muscle proteolysis.[174]

NITROGEN BALANCE STUDIES

Because the ability of body cells to synthesize new proteins is a fundamental necessity for maintaining all known host defensive mechanisms, including the immunologic ones, understanding the changes that occur in the nitrogen metabolism of an infected individual is important.[110] When the availability of free amino acids within body pools is restricted by diet or disease, the catabolism of certain existing body proteins (chiefly the contractile proteins of skeletal muscle) generates the free amino acids required to synthesize new body proteins with higher priorities in terms of the many new proteins needed for defensive purposes. Information about these metabolic responses has been gained through quantitative analyses of proteins and other nitrogen-containing compounds in tissues, body fluids, and excretions, and through kinetic studies with tagged molecules.[82,99,107,122,158]

One useful approach has been the measurement of nitrogen balance throughout the sequential course of an infectious process.[9,10,110] Daily measurements of nitrogen intake and all nitrogen losses that occur via different routes are obtained to determine whether the body is losing or retaining nitrogen. Other investigative techniques are needed to provide specific information about the molecular mechanisms involved in producing any observed changes in nitrogen balance. Nonetheless, the use of balance techniques has provided information concerning the typical losses of body nitrogen and muscle mass during acute generalized infections (Fig. 3–2). Quantitation of these catabolic losses provides an important framework of reference for more detailed studies of changes in nitrogen metabolism.

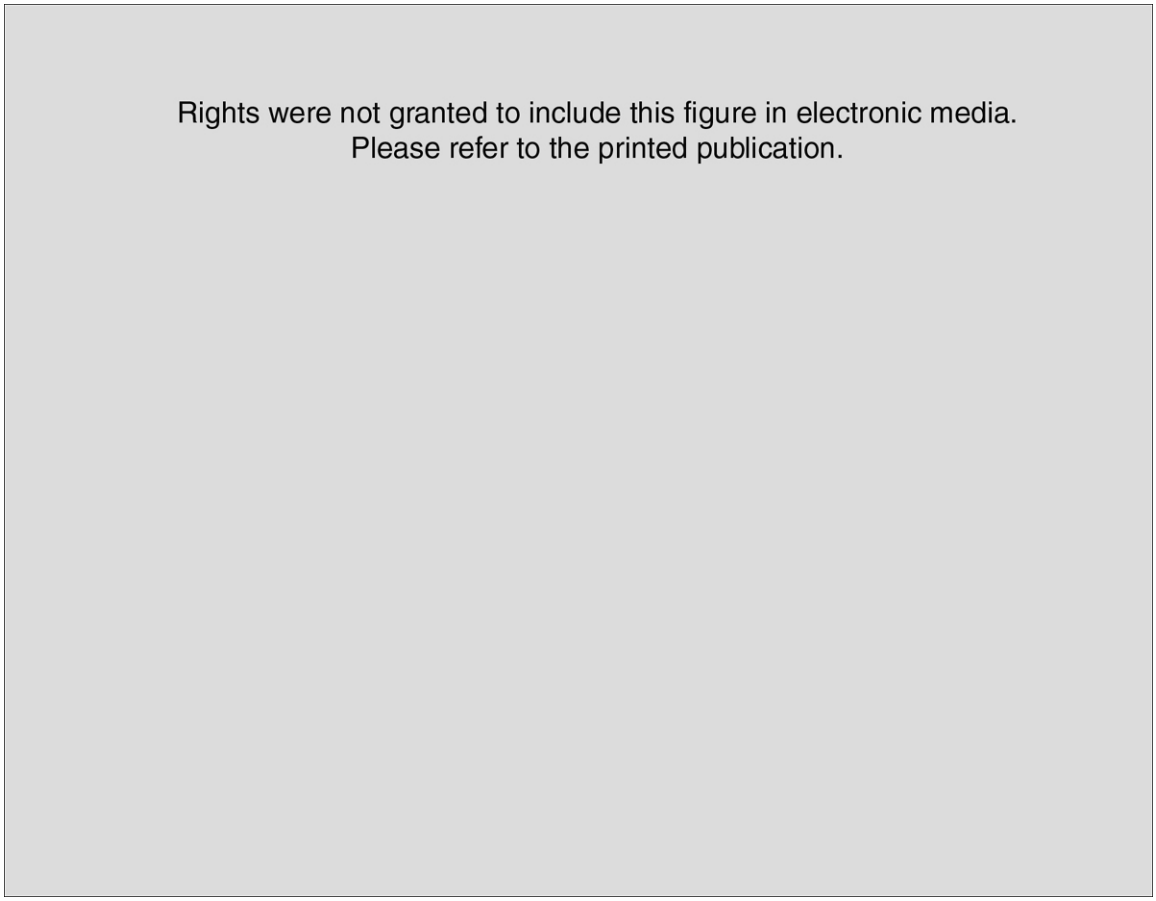

Figure 3–2 Comparisons between the occurrence of fever (top) and changes in daily nitrogen balance (middle) and cumulative nitrogen (bottom) in patients with viral, bacterial, and generalized parasitic infections. Cumulative balance values for pair-fed healthy control subjects show the amounts of body nitrogen loss that can be ascribed to diminished food intake during infection. *(From McKigney, J. I., and Munro, H. N. [eds.]: Nutrient Requirements in Adolescence. Cambridge, MA, MIT Press, 1976, p. 261.)*

A comprehensive series of nitrogen balance studies was obtained in young adult male volunteers subjected to different kinds of experimentally induced infections during the course of studies to test vaccine efficacy.[9] Extensive normal baseline data were obtained on each volunteer before the time of his or her inoculation with or exposure to infectious bacteria, viruses, or rickettsiae. Longitudinal serial measurements made throughout the course of the infectious process allowed for a comprehensive, prospective evaluation of metabolic balance changes in nitrogen and other elements to be obtained.[9]

Nitrogen balances did not change from baseline values during the incubation periods of the infectious diseases studied (see Fig. 3–2). The body began to lose nitrogen only after symptoms and fever had begun. These losses persisted for a period encompassing the acute illness. In convalescence, the subjects began to retain nitrogen so that gradually, over the course of several weeks, body nitrogen losses were regained. Similar nitrogen balance studies were conducted in healthy control volunteers who were not infected.[9,13,15] Instead, these controls were subjected to (1) partial food deprivation to mimic the anorexia-induced reduction in dietary intake measured in the patients with infection, (2) a 24-hour exposure to artificially induced high environmental temperatures to produce an increase in body temperature comparable to that seen in patients with infection, (3) treatment with antibiotic therapy in courses identical to those given to patients with infections, or (4) treatment with oral hydrocortisone using sequentially changing daily doses to mimic the measured adrenal responses of infected subjects.

These balance studies showed that the major losses in body nitrogen resulted from the combined effects of a reduced intake of food plus a continued (or increased) loss of nitrogen via the urine.[9] In contrast, simple starvation produced a prompt decrease in the daily losses of nitrogen via the urine. A patient with an acute infectious illness differed markedly from someone subjected to only simple dietary deprivation. Urinary nitrogen losses did not decline appreciably, or they increased in the presence of fever, whether the fever was caused by an active infection or an artificial increase in environmental temperatures.[15] The control studies also showed that the negative nitrogen balances that occurred during infection were not caused by adrenal glucocorticoid hormones or the antibiotics used in therapy.[7,13] Rather, by terminating infection quickly, antibiotics helped to reverse the loss of body nitrogen, allowing a more rapid restoration of nitrogen stores.

Because the loss of sizable quantities of nitrogen has important nutritional consequences, the same balance data were used to determine patterns of cumulative loss of body nitrogen.[7,9,10] As shown in Figure 3–2, total losses of body nitrogen grew progressively larger as the acute febrile phase of illness continued; the cumulative total loss of body nitrogen persisted throughout early convalescence. When nitrogen balances finally became positive, nitrogen was recovered over the course of weeks as body stores were accumulated slowly.

Studies performed in adult patients with infections such as tuberculosis and malaria suggested that the depletion of body stores of protein nitrogen did not continue unabated during sub-

acute or chronic infection.[67] Rather, a new state of relative nitrogen equilibrium became established as chronically ill patients lapsed into a cachectic state. Total body nitrogen in such chronically ill patients was neither gained nor lost. Vital body processes continued to function for extended periods, despite the presence of cachexia and markedly depleted body nitrogen stores.[8] This state was hazardous at best and was comparable to the threat faced by infants and children with severe protein-energy malnutrition, who constantly face the additional threat of developing dangerous new or superimposed infectious diseases.[86,87,104,129]

Only limited nitrogen balance data have been collected in children with acute febrile infections. These data tend to reflect closely the patterns of change described in adults, with one major exception. Adults normally are in a state of nitrogen equilibrium, with neither a net gain nor a net loss over time, whereas healthy infants and children are consistently in positive balance because they must retain nitrogen to meet their needs for normal growth.

Few prospective measurements are available to document the changes in nitrogen balance throughout an entire infectious process in children. In 1926, Beck[6] in Germany reported a series of metabolic balance studies conducted in healthy infants inoculated with vaccinia virus. One study was begun in a child exposed to varicella 15 days before the onset of pustules; another study was begun in an infant exposed to measles virus 14 days before the onset of clinical illness. The grouped nitrogen balance data from the vaccinated infants are shown in Figure 3–3. Despite the development of a short-lived fever in this mild infection, the infants maintained their usual food intake. The vaccinated infants did not go into negative nitrogen balance, and their rate of growth barely was slowed.[6] In measles or varicella, illness was of greater severity, and a transient period of negative nitrogen balance was recorded.

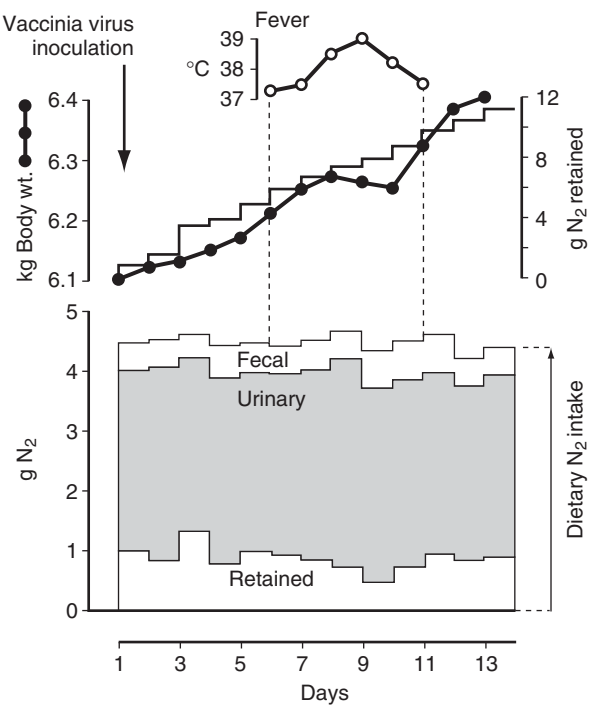

Figure 3–3 Sequential measurements of body weight and cumulative nitrogen balance (top) and daily nitrogen balance (bottom) in a group of five healthy infants inoculated with vaccinia virus. Nitrogen intake values (plotted upward from baseline) and fecal and urinary losses (plotted downward from intake) reveal that the growing infants continued to retain nitrogen despite having brief periods of fever. *(Drawn from numerical data published by Beck.[6])*

Similarly, Viteri and Behar[156] measured nitrogen balance during childhood infections or after administration of live vaccines. Nitrogen loss varied with the severity of the illness. Wilson and colleagues[172] reported changes in nitrogen balances in hospitalized children who were convalescing from kwashiorkor when they developed varicella. A reduction in nitrogen retention occurred, and some children developed negative balances. Despite efforts to maintain a constant dietary intake, the children consumed less food during the course of the infection.

Acute nondiarrheal infections typically do not cause an increased loss of fecal nitrogen; however, if diarrhea is present during an illness, fecal losses of nitrogen and other nutrients can occur.[104,126] Nitrogen losses also can occur through sweat, exudates, blood loss (from illness or from laboratory tests), sputum, gaseous exchanges, or sites of surgical drainage.[9,15]

The absolute loss of body nitrogen, as measured by metabolic balance techniques during infection, also can be used as a guide for estimating the absolute losses from the body of other intracellular elements, such as potassium, magnesium, phosphate, and, to a lesser degree, sulfur and zinc.[9,10] By using the ratios present in normal skeletal muscle of nitrogen to potassium and of nitrogen to magnesium, one can use measured losses of body nitrogen during infection to estimate concomitant losses of potassium and magnesium from the body. Similarly, a ratio of nitrogen to phosphorus in skeletal muscle can be used to estimate inorganic phosphate losses that occur during infection, provided that corrections are made first to account for any phosphate losses originating from demineralized bone. Calculations such as these suggest that the absolute losses of body nitrogen during infection are derived primarily from intracellular sources because they correlate in timing and magnitude with losses of the other principal intracellular elements.[9]

In contrast to infection-induced losses of intracellular elements, the losses of extracellular ions, sodium and chloride, follow an independent course during infection. Calcium losses are minimal in acute infection in the absence of long-term bed rest or paralysis with body immobilization.[9,168]

A newer method to measure total protein metabolism involves the use of a single oral dose of [^{15}N] glycine. This method was used to study children with septic shock as a result of meningococcemia and showed that the protein metabolism of these patients is increased significantly compared with healthy children.[41] A similar method was used previously in children with malaria, HIV, and measles.[41]

URINARY EXCRETION OF NITROGEN-CONTAINING COMPOUNDS

The onset of fever typically is accompanied by an increased urinary loss of creatinine. This loss usually is followed within 1 day by increased losses of urea and ammonia.[9] Although alpha-amino nitrogen losses may increase transiently at the onset of fever in some infections, this excretion tends to be lower than baseline measurements during the remainder of the illness and throughout convalescence. When measured prospectively, excretion of urinary alpha-amino nitrogen or most individual free amino acids did not change appreciably during sandfly fever, a mild virus infection.[159] In contrast, urinary losses of 3-methylhistidine and phenylalanine increased. The increased loss of phenylalanine seemed to reflect its increased concentrations in plasma.[160] Studies using radioactive compounds in rats suggested that most of the excess urea excreted during an acute infection was generated by the deamination of endogenous amino acids derived principally from skeletal muscle.[10]

Increased excretion of one of the amino acids, 3-methylhistidine, serves to indicate the extent of skeletal muscle catabolism.

This amino acid cannot be reused after its release from contractile proteins.[80,178]

Uric acid excretion may increase during febrile periods, but the greatest changes have been reported during infectious mononucleosis.[102] Uric acid losses are ascribed to an accelerated turnover of nucleic acids and enhanced purine degradation.

An increased excretion of urinary diazo reactants commonly occurs during most acute infectious illnesses, especially typhoid fever. These reactants include metabolites, such as kynurenine, 3-hydroxykynurenine, *o*-aminohippuric acid, xanthurenic acid, and anthranilic acid, which are created during the accelerated hepatic metabolism of tryptophan via the kynurenine pathway.[118]

A sudden, dramatic increase in urinary nitrate excretion occurs during acute infections. This increase is a reflection of the cytokine-stimulated production of nitric oxide as a host defensive measure.[44,139,144,157]

METABOLISM OF FREE AMINO ACIDS

The distribution, use, and metabolism of free amino acids undergo profound alterations during the course of acute infectious illness. Measurements in patients show patterns of change that can be explained by studies performed in experimental laboratory animals.[46,110,113,161] As some proteins are produced, others are degraded, and many of the released amino acids are redistributed throughout the body before they are reused.

A diminution in the concentration of most free individual amino acids in plasma may begin before the onset of symptoms. Hypoaminoacidemia generally persists throughout the febrile period of an illness, the largest decreases occurring in the branched-chain amino acids, leucine, isoleucine, and valine.[99] The decline in branched-chain amino acids is caused primarily by an accelerated uptake of free amino acids by the liver. This increased flux from plasma to liver has been shown to occur in several species of animals during a variety of infections.[99] The amino acids that enter the liver are used for a variety of purposes. Amino acids may be metabolized to other compounds, oxidized, or reused for the synthesis of new proteins. Interleukin-6 (IL-6) seems to trigger many of the alterations in hepatic protein and amino acid metabolism that occur during an acute infection.[12,133]

Amino acids become important sources of energy during the hypermetabolic phase of acute infections. Some amino acids are oxidized directly in muscle, whereas others are transformed progressively into glucose and glucagon. Gluconeogenic amino acids, such as alanine, are deaminated; their carbon skeletons are used as the principal substrate for producing glucose; and their nitrogen is used to create urea for excretion.

Serum and the total body pool of certain amino acids are depleted in various infections, presumably to diminish the substrate that some microorganism may use to replicate. Interferon-γ secretion causes a depletion of the body pool of tryptophan to retard the progression of *Chlamydia* infection.[116] A complete spectrum of amino acids is required for the synthesis of new body proteins generated during infection, including hepatic enzymes,[119] such as tryptophan oxygenase and tyrosine transaminase, and the lipoproteins and glycoproteins released into the plasma.[10,110,111] This group of glycoproteins constitutes the "acute-phase reactants," which include alpha$_1$-antitrypsin, amyloid, alpha$_1$-acid glycoprotein, haptoglobin, C-reactive protein, fibrinogen (the third component of complement), and ceruloplasmin.

These acute-phase reactant proteins are produced in excess during infections, even in protein-deficient children who exhibit acute kwashiorkor.[108] Some of the potentially beneficial functions attributed to acute-phase reactants include an amplification of humoral and cell-mediated immunity, antiproteinase effects that could limit or contain harmful enzymes liberated during inflammatory reactions, oxidase activities, and the detoxification of free hemoglobin.[110]

In addition to the accelerated synthesis of proteins within the liver, infection increases the production of phagocytic and lymphoid cells. A need to produce increased quantities of such diverse proteins as the cytokines, complement and kinin components, fibronectin, and the several classes of immunoglobulins also exists. All of these requirements involve the need for free amino acids.

Proinflammatory cytokines and interferon-γ stimulate the production of nitric oxide from arginine, its sole precursor.[44,157] Among the many important biologic effects of nitric oxide are the effects resulting from its being a highly potent microbicidal, parasiticidal, and tumoricidal agent,[139] and from having major immunomodulatory functions.[84] Research also has shown that nitric oxide may act as an antiviral agent against adenoviruses and other similar viruses.[25]

The importance of nitric oxide in these roles may rival those of free oxygen radicals in potency and effectiveness.[144] This newly recognized host defense agent is generated when cytokine-induced nitric oxide synthase initiates the oxygenation of one of arginine's guanido nitrogen groups to produce citrulline and nitric oxide.[139] During sepsis, arginine balance becomes negative, as consumption of arginine exceeds the rate of synthesis. Because arginine is the sole source of nitric oxide, it becomes an essential amino acid in critically ill children.[4] Arginine also plays an important role in wound healing. In patients with infected chronic wounds, total plasma arginine is decreased, and wound healing is impaired secondary to defective usage of arginine.[36]

AMINO ACID AVAILABILITY

In the presence of anorexia and a diminished intake of food nutrients, the body in large measure must use endogenous sources to supply its increased amino acid needs. The principal sources of potentially available endogenous amino acids are the proteins of somatic tissues, including skeletal muscle and skin. The free amino acid pool contains the equivalent of approximately 12.5 g of protein in a normal man, an amount that represents only 0.1 percent of total body protein.[158] Because the normal turnover of protein in a healthy adult is approximately 200 to 250 g each day, the free amino acid pool must be resupplied continually from endogenous and exogenous sources. During infections, the absorption of individual amino acids from the intestine may be depressed and delayed[93] or increased,[33] depending on the infection.

Protein synthesis is not stopped entirely within body somatic tissues during an infection, but it is slowed markedly. At the same time, the rates of degradation of somatic proteins are accelerated so that a net loss of muscle, skin, and other somatic proteins occurs through catabolic wasting. Catabolic degradation of skeletal muscle protein can be initiated by IL-1,[5,8,31,42] which triggers muscular proteases via the intracellular production of prostaglandin E_2. The body apparently sacrifices contractile proteins of skeletal muscle to obtain the free amino acids needed for higher priority requirements.

Some of the amino acids liberated during muscle protein catabolism are lost directly to the plasma. Others, such as the branched-chain group, are used in situ as sources of energy for muscle fibers; after oxidation, their amino groups are used to manufacture new glutamine and alanine, which can be used elsewhere for gluconeogenesis.[80] Because the branched-chain amino acids can be oxidized within skeletal muscle, their rate of release from muscle is decreased, which exaggerates the decline in their concentrations in plasma.[10,99,158] Branched-chain amino acids also play a major role in protein synthesis for lymphocytes and in

immunity.[23] Some of the free amino acids liberated within muscle are retained within contractile cells and reused for the slowed but continuing process of muscle protein anabolism.

This catabolic breakdown of somatic protein to supply amino acids for use in other parts of the body is a highly visible clinical phenomenon. In this regard, the proteins of skin, skeletal muscle, and other somatic structures seem to constitute the principal body pool of "labile" nitrogen, which is called on to maintain protein homeostasis and the physiologic functions of visceral tissues during periods of infectious illness or other stresses. Because infants and small children have very little muscle protein (and a very small "bank" of readily available amino acids), the infection-induced need for free amino acids puts them at a special disadvantage.

To study the complex interrelationships involved in amino acid metabolism, workers have used isotopes to measure the simultaneous rates of anabolism and catabolism in the protein of skeletal muscle and other tissues of experimental animals during the course of infection.[122] In addition, clinically useful estimates of skeletal muscle degradation can be obtained in patients through the quantitative daily measurements of 3-methylhistidine excretion into urine. More than 90 percent of the total body content of 3-methylhistidine is associated with the peptide chains of actin in all muscle and of myosin in white muscle fibers.[178] This unique amino acid is formed in situ through the transmethylation of histidine, but only after the histidine first has been incorporated into the amino acid structure of myofibrillar proteins. When released, 3-methylhistidine cannot be reused by the body and instead is lost via the urine.

The increase in excretion of 3-methylhistidine during acute infections is in keeping with other estimates of skeletal muscle wasting. The extent to which proteolysis occurs during periods of severe surgical sepsis also has been quantified by measuring the differences among concentrations of glucose, lactate, free fatty acids, ketones, and alanine in femoral arterial and venous blood.[106] Neither free fatty acid nor ketone use was increased during occurrences of acute fevers, but an increased oxidation of glucose and of amino acids derived from protein was used to fuel cellular hypermetabolism. Although infection stimulates a sizable increase in the rates of body protein synthesis, the acceleration of protein catabolism is increased even further, and the body experiences a net loss of somatic proteins and even some visceral ones.[158]

Phenylalanine is one of the amino acids released by the accelerated catabolism of somatic proteins. Only limited amounts of phenylalanine can be used, however, for the synthesis of new proteins or for its conversion into tyrosine.[158,160] Phenylalanine concentrations in the circulating free amino acid pool tend to increase rather than decrease during periods of fever. These increases occur coincidentally with the typical declines in plasma values for tyrosine and most other free amino acids. The exaggerated phenylalanine-to-tyrosine ratio can be useful for evaluating these opposing changes during febrile illnesses. An increase in this ratio is a useful clinical index of the severity of infection-induced alterations in amino acid metabolism.[158] During occurrences of infections of great severity and infections with hepatocellular dysfunction, hypoaminoacidemia may be replaced by an excessive accumulation of many other free amino acids in plasma.[10,135,170]

Free tryptophan, also liberated from degrading protein, undergoes an accelerated metabolism via several different metabolic pathways in the liver.[10,96,97,112] In response to the increased hepatic availability of tryptophan or the increased synthesis of tryptophan oxygenase, or both, excess tryptophan enters the kynurenine pathways, where it is converted into different diazoreactant compounds and pyrimidine nucleotides.[112] At the same time, tryptophan enters other pathways, leading possibly to increased synthesis of serotonin or indoleacetic acid.[118]

CARBOHYDRATE METABOLISM

During the febrile phase of infectious illnesses, carbohydrate metabolism provides much of the additional energy needed by body cells. An increased demand for metabolizable energy is met by a resetting of the endocrine control mechanisms to permit a marked acceleration of the synthesis and release of glucose from the liver.[35,54,171] Although some glucose is derived from hepatocellular glycogen, most additional requirements for glucose during acute infections are met by accelerated hepatic gluconeogenic mechanisms. Recycled lactate and amino acids, especially amino acids with gluconeogenic potential, such as alanine, are the principal substrates used by the liver to increase its output of newly synthesized glucose; glycerol (derived from the metabolism of triglyceride lipids) and pyruvate also are used.[80]

Early in acute infectious illnesses, basal blood glucose concentrations tend to become elevated. Diabetic patients who require insulin often develop glycosuria at the onset of febrile infections, and more insulin must be given to maintain their control. The tendency for acute infections to be accompanied by an early modest hyperglycemia also is seen in laboratory animals. Modest hyperglycemia often is noted in animals after they have been injected with bacterial endotoxin.[21,89]

Although an early hyperglycemic response seems to be the initial change in carbohydrate metabolism, the ability of the body to sustain an accelerated production of glucose may be lost. Hypoglycemia may emerge as an important clinical problem. Hypoglycemia generally results from one of two major pathogenic mechanisms: a diminished availability of substrate molecules required for gluconeogenesis or a failure of the metabolic mechanisms needed to produce hepatic glucose. Carbohydrate pathway defects at the molecular level have not been found in overwhelming bacterial infections in laboratory animals.[61,103] Carbohydrate depletion during overwhelming or terminal infections can be ascribed to nonavailability of substrate. If hepatic cells are injured during an infectious disease, such as viral hepatitis, hypoglycemia can result from a failure of gluconeogenic processes.[10,47,126]

The depletion of substrate for adequate gluconeogenesis occurs primarily as a dangerous clinical complication of neonatal sepsis. Infants are born with only minimal amounts of skeletal muscle protein. Because muscle contains the principal "labile nitrogen" pool for supplying amino acids that can be converted into glucose, neonatal infants lack a sufficient quantity of potential substrate molecules for sustaining long-term gluconeogenesis. Newborns especially are prone to develop severe hypoglycemia whenever a septic process becomes established.[176] Hypoglycemia that results from a breakdown of hepatic gluconeogenic mechanisms occurs primarily when liver cells are damaged by an infectious or toxemic process. Hypoglycemia can become an important complicating factor in severe viral hepatitis, yellow fever, or endotoxemia during gram-negative sepsis.[47,89] These conditions can cause hepatocellular damage and a breakdown of enzyme-synthesizing mechanisms within the liver.[89]

A change in glucose tolerance can be detected within hours after the onset of a febrile infection.[121,132] Baseline blood glucose values may be elevated, and disappearance of glucose is slowed moderately as a component aspect of a febrile illness. Under these circumstances, the pancreatic islets produce insulin in excess quantities. Despite an increase in plasma insulin concentrations, glucose disappearance rates are slowed; these combined metabolic changes resemble the changes caused by mild insulin resistance. In low-birth-weight neonates, hyperglycemia could be an ominous sign and suggests the presence of systemic fungal infections.[85]

Bacterial and viral infections also cause increased secretion of pancreatic glucagon,[121,124] which, together with release of catecholamines accompanying infection,[58,60,171] provides hormonal

stimuli for accelerating gluconeogenesis within the liver. These hormonal stimuli initiate their hepatic effects during infection by activating adenylate cyclase.[35] The unusual simultaneous increase in basal concentrations of insulin and glucagon in plasma results from direct cytokine stimulation of pancreatic islet cells.[8,53,175] Because of these hormonal changes and high plasma glucocorticoid values in patients with severe bacterial sepsis, infusions of glucose may fail to suppress hepatic gluconeogenesis.[80,81] The resultant increase in glucose pool size helps to explain the apparent slowing of glucose disappearance and the hyperinsulinemia that occur during infections.

Certain viral infections in children seem prone to initiate acute juvenile-type, insulin-requiring diabetes mellitus. Although the evidence for a viral cause of diabetes has not been established fully in humans, epidemiologic findings indicate that juvenile diabetes may develop several months after onset of certain viral illnesses, especially mumps, coxsackievirus B (type 4) infection, rubella, or cytomegalovirus infection.[101] Additional epidemiologic evidence is based on a tendency for new cases of juvenile-type diabetes to appear in clusters.

Susceptibility to juvenile diabetes also may be influenced by genetic make-up. A high incidence of human leukocyte antigen types B8 and Bw15 has been detected in juvenile diabetics.[127] Studies in laboratory animals suggest that genetically susceptible species may have virus receptor sites on their insulin-producing beta cells. These receptors in species with high-risk haplotypes allow viruses to be absorbed by the pancreatic beta cells, with subsequent destruction of insulin-producing cells.

The cytokine granulocyte colony-stimulating factor influences the uptake of glucose by phagocytic cells and their mobilization during inflammatory reactions.[77] Availability of glucose contributes directly to the respiratory burst that accompanies phagocytosis and to the production of the myeloperoxidase enzymes needed to kill engulfed organisms.

TABLE 3–1 Basic Mechanisms Leading to Observed Effects of Infection on Lipid Metabolism of the Host

Effects Associated with the Presence of Invading Microorganisms
1. Direct effects
   a. Microorganism use of host lipids for replication
   b. Disruption of host cell metabolism by intracellular microorganisms
   c. Localized destruction of fat cells by infectious process
2. Indirect effects
   a. Alterations in host metabolism caused by bacterial exotoxins, endotoxins, or bacterial enzymes
   b. Activation of lipases and other lipid-affecting enzymes within host phagocytes

Effects Secondary to Development of Generalized Illness Due to Infection
1. Decreased dietary fat intake
2. Interference with intestinal digestion and absorption of lipids
3. Alterations in lipid transport
   a. Changing concentrations of lipid transport proteins
   b. Decreased lipoprotein lipase activity (caused by tumor necrosis factor), which allows triglycerides to accumulate in plasma
4. Altered lipid metabolism within host cells caused by proinflammatory cytokines
   a. Activation of cell wall phospholipases by proinflammatory cytokines
   b. Creation, from cell wall polyunsaturated fatty acids, of intracellular arachidonic or eicosapentanoic acids or both
   c. Transformation of arachidonic and eicosapentanoic acids into various eicosanoids (prostaglandins, prostacyclin, thromboxanes, and lipoxins)
5. Other alterations of lipid metabolism within host cells
   a. Altered rates of hormone-mediated lipolysis within fat depots
   b. Accelerated fatty acid synthesis within the liver
   c. Depressed hepatic ketogenesis
   d. Altered rates of lipid uptake and use by peripheral tissues
6. Participation of newly formed eicosanoids in inflammatory and immunologic responses, and coagulation mechanisms
7. Effects related to prior nutritional status of the patient
8. Terminal pathologic hyperlipidemic effects associated with gram-negative sepsis and hypotensive shock

LIPID METABOLISM

As Table 3–1 shows, numerous changes in lipid metabolism have been documented during infection. In contrast to the stereotyped patterns of change in nitrogen and amino acid metabolism and in the accelerated production of glucose, responses involving body lipids vary more from infection to infection. Plasma lipid changes also may be biphasic during the course of a single infection. This complexity results partly from the multiple factors that control the metabolism of lipids during infectious illnesses,[10,135] including the need for hepatic synthesis of serum lipoproteins, variations in the production and use of free fatty acids and ketones as sources of cellular energy, and variable changes in rates of uptake or release of lipids from body fat depots.[10,135]

Lipids have another function that is crucial during acute-phase reactions. This function involves the cytokine-stimulated use of polyunsaturated fatty acids localized within cell walls to serve as precursor molecules for various families of eicosanoids (prostaglandins, prostacyclins, thromboxanes, and lipoxins). These eicosanoid lipids trigger many of the diverse cellular responses seen during infection, inflammation, and immune responses.[73,123]

PLASMA LIPIDS AND LIPOPROTEINS

Because the concentrations of individual lipid moieties in plasma depend on the algebraic summation of input and removal rates, the mechanisms that control the release or uptake of individual lipids must be studied throughout the course of various kinds of infectious illnesses. Concentrations of cholesterol have been reported to increase, to decrease, or to remain unchanged.[10] In severe meningococcal sepsis, high-density lipoprotein and low-density lipoprotein levels are low, and the severity of total hypocholesterolemia correlates with the severity of disease.[152] Other studies have shown that hypocholesterolemia in sepsis may have prognostic value. Decrease of cholesterol parallels increase of C-reactive protein, fibrinogen, and white blood cells in septic patients.[28]

Mild viral infections often are associated with a transient decline in serum cholesterol values. When longitudinal studies were conducted during the course of experimentally induced sandfly fever in young adult male volunteers, plasma values for total and esterified cholesterol, phospholipids, and free fatty acids all declined in conjunction with, or immediately before, the onset of fever.[78] This decline in concentration included the cholesterol and the protein components of the low-density beta-lipoproteins. In contrast, the plasma triglyceride values showed a biphasic pattern of change: An initial decline was followed by an increase above baseline control concentrations during the early convalescent period. Depressions of total serum cholesterol values also have been reported to occur during pneumococcal pneumonia, cholera, tuberculosis, and malaria.

When studied in rhesus monkeys experimentally infected with *Streptococcus pneumoniae* or *Salmonella typhimurium*, rates of [^{3}H] mevalonic acid incorporation into free cholesterol were accelerated,[48] and no evidence of an inhibition of squalene synthesis or its conversion to cholesterol was found. In contrast, the synthesis

of cholesterol was blocked partially when monkeys with diet-induced hypercholesterolemia were subjected to a pneumococcal infection.[48]

Marked increases in the concentrations of total serum lipids and triglycerides are found consistently in patients with infections caused by gram-negative bacilli,[10,69,135] but this response is minimized or fails to occur in patients with viral or gram-positive coccal infections. A similar difference has been noted in monkeys with either *S. pneumoniae* or *S. typhimurium* infection.[70] Tumor necrosis factor, a proinflammatory cytokine with a unique additional function that inhibits the enzyme lipoprotein lipase, seems to be of central importance in accounting for these differences.[69]

Although a release of tumor necrosis factor from activated macrophages may occur in many infections, its release is stimulated markedly by bacterial endotoxins released during gram-negative infections.[10,92] Hypertriglyceridemia occurring during periods of *S. typhimurium* infection in monkeys was generated by tumor necrosis factor–induced mechanisms that reduced the clearance of lipids from plasma and the activity of heparin-sensitive plasma lipoprotein lipase. Hypertriglyceridemia that accompanied pneumococcal infections was far less severe than that observed with gram-negative infections. After endotoxin was administered,[69] hypertriglyceridemia developed chiefly as the result of an impaired lipid disposal mechanism, secondary to release of tumor necrosis factor from macrophages.[8] An acceleration in the rate of hepatic production of triglycerides from free fatty acid precursors also occurred.

LIPIDS AND ENERGY METABOLISM DURING INFECTION

Requirements for cellular energy during periods of chronic infections in laboratory animals seem to be met largely through the catabolism of stored body fat.[82] In contrast, fat wasting does not seem to be as prominent during rapidly lethal bacterial infections. Ketogenesis also seems to be blunted during periods of acute infections.[103] Concentrations of free fatty acid in plasma normally are increased by the actions of catecholamines, growth hormone, and glucagon, which are increased during the course of infection.[10] Concomitantly high concentrations of plasma insulin apparently serve to inhibit lipolysis and ketogenesis.[10,103] The contribution of free fatty acids to energy production during an infection is influenced by the interplay of these hormones, the availability of glucose, and the functional capacity of the liver to take up fatty acids and convert them to triglycerides.[48,49,53,54]

Finally, brown fat is thought to contribute importantly to heat production in newborns via sympathetic nerve activation of this tissue. Because numerous viruses are known to proliferate in brown fat,[136] fever that occurs with neonatal viral infections may involve brown fat thermogenesis.

Acute infectious illnesses produce few effects on the intestinal absorption of fat, although it may be reduced with intestinal infections and parasitemic or enterotoxemic illnesses.[109,126] Lipid precursors needed for the replication of microorganisms within host tissues must be supplied from metabolic pools within the host. Malarial parasites obtain their structural lipids from red blood cell precursors; lipid-containing viruses apparently obtain the lipid components needed for their assembly from lipid-rich membranes already present within host cells.

LIPID METABOLISM AND HOST DEFENSIVE MEASURES

Rapid metabolic responses by cell wall lipids play a major role in initiating a panoply of intracellular events during acute-phase reactions. The attachment of proinflammatory cytokines to cell wall receptors triggers the conversion of polyunsaturated fatty acid in cell wall membranes to a variety of biologically active eicosanoids. Lipids of various eicosanoid families become the messenger molecules that are responsible for many of the acute-phase metabolic and cellular responses[8,42] that occur with infection. These short-lived eicosanoids are biopotent at nanomolar to picomolar concentrations.

In addition to their importance within lymphocytes of the immune system, eicosanoid lipids transmit stimulatory or inhibitory signals between other cells and tissues in health and disease.[73,123] Although they do not initiate disease processes, various eicosanoid lipids (i.e., prostaglandins, leukotrienes, prostacyclin, lipoxins, and thromboxanes) are important components of the pathogenic progression of infectious diseases.[73,123]

Eicosanoids also have important effects on other white blood cells and platelets and on such diverse organs as the brain, lungs, stomach, and kidneys. These eicosanoids seem to be components of complex, often overlapping, regulatory mechanisms that interconnect the immune system, the central nervous system, the endocrine glands, and the functions of many other organs and tissues. These homeostatic (and at times pathogenic) mechanisms are characterized by numerous duplications, amplifications, checks and balances, and feedback control loops.

Biochemical details now are well established concerning the intracellular synthesis of individual eicosanoids from *n*-3 and *n*-6 polyunsaturated fatty acid precursors.[73,123] After cytokines interact with cell wall receptors, they activate cell membrane phospholipase enzymes. These enzymes initiate the release (into the cell interior) of arachidonic acid from cell wall (*n*-6) polyunsaturated fatty acid or eicosapentanoic acid from (*n*-3) polyunsaturated fatty acid. These first steps of eicosanoid synthesis can be blocked by adrenocorticoid hormones. In target cells having cyclooxygenase enzymes, arachidonic acid is oxygenated into 4-series leukotrienes, 2-series prostaglandins, or thromboxanes and prostacyclin. If a cell possesses lipooxygenase enzymes, arachidonic acid is oxygenated into the sometimes less potent 5-series leukotrienes, 3-series prostaglandins, or lipoxins.[123] The highly active eicosanoid molecules are degraded rapidly. Importantly, synthesis of prostaglandins of the 2-series and the 4-series can be blocked by certain nonsteroidal anti-inflammatory drugs, such as aspirin, indomethacin, and ibuprofen.[73,123]

Other fatty acids may participate in defense mechanisms of the host and occasionally even may be involved in pathogenic events of a harmful nature. An increase in the fat content of vital organs is a common occurrence in patients dying of severe bacterial infections; fatty metamorphosis especially is prominent in the liver, kidney, and heart.[66] Hyperlipemia in gram-negative septicemia may be accompanied by fat embolization to the lungs. Other deleterious actions of body lipids involve the liberation of phospholipids from platelets and their subsequent participation in the activation of the blood-clotting cascade leading to disseminated intravascular coagulation. Chronic *Chlamydia* pneumonia infection leads to oxidation of low-density lipoprotein, which promotes atherogenesis.[43]

ELECTROLYTE AND ACID-BASE METABOLISM

Many life-threatening medical emergencies faced by infants and children with acute infectious diseases involve problems in the areas of salt and water or acid-base balance. Some infections cause severe overhydration, and others produce dehydration with hypovolemic shock. Pathogenic mechanisms that come into play during various infections can lead to metabolic alkalosis or acidosis, to respiratory alkalosis or acidosis, or to complex admixtures of these pathophysiologic perturbations.[8,18]

The onset of fever typically is accompanied by tachypnea and accelerated respiratory gas exchange, which leads to an exaggerated loss of dissolved carbon dioxide from blood and a state of

uncompensated respiratory alkalosis.[10] Alkalosis may persist as long as febrile tachypnea lasts, and gas exchange within the alveoli remains unimpeded.

Conversely, infections that produce extensive pulmonary consolidation can impair carbon dioxide exchange and cause respiratory acidosis. Respiratory acidosis also is a complication in patients whose pulmonary musculature no longer can function effectively, such as patients with poliomyelitis, tetanus, botulism, or respiratory distress syndrome.

Metabolic acidosis generally develops whenever an infectious disease process becomes severe. With the hypotension, vascular stasis, and cellular anoxia seen during gram-negative sepsis, the generation of excessive lactic acid and other acidic metabolic products exceeds the capacity of the body's buffering systems.[10,135]

Diarrheal diseases can be accompanied by two other forms of metabolic acid-base derangement.[18,126,163] Toxigenic diarrhea characterized by high-volume stool loss, such as that seen in Asiatic cholera or *Escherichia coli* enterotoxemia, causes an excessive loss of fecal bicarbonate and an alkaline stool, with a resultant decline in blood pH. Bicarbonate is secreted actively in the lower ileum and cannot be reabsorbed completely by the colonic mucosa if high-volume losses of watery stool occur.[18]

In contrast, diarrhea accompanied by only low-volume stool losses, as in rotavirus infections,[126] tends to be associated with an acid stool associated with an exaggerated loss of fecal potassium rather than bicarbonate. If fecal potassium losses persist chronically for a long time or occur rapidly as part of the massive fluid loss of acute secretory diarrhea, such as in pediatric cholera, body potassium can be depleted severely. The loss of cellular potassium can produce metabolic alkalosis, cardiac arrhythmia, paralytic ileus, and weakness in children and the occurrence of hypokalemic vacuolization in cells of the myocardium and renal tubular epithelium. Even if the balance of fluid is restored promptly in these patients, a prolonged state of metabolic alkalosis can persist until body potassium stores are replenished.

The importance of diarrheal diseases in infants and small children is great, with an estimated 2 to 3 million deaths occurring worldwide each year.[104] Life-threatening dehydration can result from the loss of body water and electrolytes during an episode of high-volume diarrhea. Because massive diarrhea produces an iso-osmotic loss of body water and electrolytes, the fluid losses come from the extracellular rather than the intracellular space.[163] The circulating blood becomes thick and viscous because of a relative increase in hematocrit values and a progressive concentration of serum proteins, which can increase to more than twice their normal values. Despite serious dehydration, concentrations of plasma sodium remain normal, when expressed in terms of plasma water.[163] This type of acute, massive diarrhea can lead to the rapid onset of hypovolemic shock, renal failure, and death.

Dehydration usually does not become a problem in infectious diseases that lack protracted vomiting, diarrhea, or prominent sweat loss. Instead, severe generalized infections in children may be accompanied by some retention of body water and salt. Soon after the onset of a febrile illness, the adrenal secretion of aldosterone increases,[9,17] and this mineralocorticoid stimulates the renal retention of sodium and chloride. These electrolytes virtually may disappear from the urine during a severe febrile illness. Retention of salt tends to be accompanied by a retention of body water throughout the illness.[10] Generalized edema is an infection-induced manifestation of kwashiorkor and can be seen during severe infections in well-nourished children. Accumulations of excess water and salt typically are excreted after the acute phase of illness by a transient period of diuresis during the early convalescent period.

Some infections, particularly infections that become localized within the central nervous system, also are complicated by an inappropriate secretion of antidiuretic hormone from the posterior pituitary.[45,169] The ensuing retention of body water may dilute the sodium and chloride in plasma. Redistribution of sodium also may contribute to the development of hyponatremia during infections. Sodium may begin to accumulate within body cells, apparently because the sodium-pumping mechanisms in extracellular membranes may fail to maintain internal electrolyte homeostasis.[10] This form of sodium sequestration is evidence of severe illness. It is not reversed easily and may be a major complication in patients with severe infections, such as meningococcemia or Rocky Mountain spotted fever.

VITAMIN METABOLISM

Although few measurement data are available concerning infection-induced changes in vitamin metabolism, the consensus is that the use or metabolism of most vitamins is accelerated.[16,155] Scattered reports suggest that infectious diseases in humans may be followed by classic scurvy, beriberi, pellagra, or xerophthalmia.[129]

More recent attention has focused on vitamin A, which previously was termed the anti-infection vitamin. Declining concentrations of plasma vitamin A during episodes of childhood infections are accompanied or perhaps caused by a marked urinary loss of this vitamin.[1,142] Not only does the heightened vitamin A deficiency induced by infection contribute to the subsequent development of ocular and conjunctival pathologies, but also subclinical deficiencies of vitamin A and their associated immunologic dysfunctions can heighten the mortality associated with childhood infections,[130] as shown most dramatically in measles.[138] Depressed plasma concentrations of several other vitamins have been reported.[10] In addition to the urinary losses of vitamin A,[1,142] increased excretion of urinary riboflavin and vitamin C may occur in conjunction with negative nitrogen balance.[16]

Vitamins are known to participate in metabolic processes activated during host defensive mechanisms.[8] The rapid synthesis of steroid hormones by the adrenal cortex is accompanied by a decline in the adrenal content of vitamin C. The B group vitamins, vitamin C, and folate all participate in the metabolism of activated phagocytic cells.

Controversy continues to exist over whether massive daily doses of vitamin C can suppress or prevent the common cold and other viral respiratory infections. More than 2 decades ago, the American Academy of Pediatrics Committee on Drugs[2] failed to find sufficient scientific evidence to support Pauling's claim, but new data subsequently were introduced.[63] Concentrations of vitamin C in neutrophils decline during infectious diseases. Vitamin C is recognized for its importance in the locomotive activity of phagocytic cells and its contributions to the immune system.[3]

Intestinal parasites, such as tapeworms, may take up sufficient vitamin B_{12} from the succus entericus to diminish vitamin B_{12} absorption and lead to the development of megaloblastic anemia. The intestinal absorption of fat-soluble vitamins and folate also may be impaired for a time in children with enteric infections or parasitic diseases.[83]

The antioxidant role of several vitamins (A, C, and E) has been recognized more recently. Antioxidants act to reduce oxidative stress within the body by serving as scavengers of singlet oxygen radicals. The antioxidant functions of several other provitamin-A carotenoids are of still greater importance. They include beta carotene, lycopene (the red pigment found in tomatoes and other brightly colored fruits), lutein (from spinach), and zeoxanthin (from kale and other dark green collard greens). Lycopene is more abundant in plasma than is beta carotene and twice as powerful in quenching free oxygen radicals. Lutein and zeoxanthin are retinal pigments, with lutein being found throughout the

retina and zeoxanthin being concentrated in the macula. Antioxidants are thought to protect cell membranes, DNA, and arteries to augment the immune system and the function of natural killer cells and to help prevent heart attacks, macular degeneration, and various cancers, especially prostate cancer. Little is known about the probable role of antioxidants during episodes of infectious diseases of children.

More recent studies on a small sample of adults have suggested that vitamin D is decreased in patients with HIV infection, and that vitamin D levels correlate directly with the CD4 count and inversely with mortality in HIV-infected patients.[154] Vitamin C seems to be decreased in patients with HIV infection, whereas vitamin E concentrations are similar to those in healthy subjects.[143] Oxidative damage is known to play an important role in inflammation, but the exact mechanism by which vitamin C is involved in the antioxidant host defense is unclear. One study that looked at *Klebsiella pneumoniae* infection showed that vitamin C does not prevent lipid and protein oxidation. Another study suggests that vitamin C is protective against oxidative stress, at least partly by decreasing lipid peroxidation.[72] Vitamin C seems to protect against infections in mouse models.[52]

TRACE ELEMENT METABOLISM

As Table 3–2 shows, infectious illnesses often are accompanied by changes in the concentration of several of the trace elements in plasma. The most consistent responses include a decrease in the concentrations of plasma iron and zinc and an increase in plasma copper.[10] This triad of trace-element changes has been reported in bacterial, viral, rickettsial, and parasitic infections. In acute viral hepatitis, serum iron values tend to increase, however, in the second and third weeks of illness. Hepatitis also is associated with an unusual change in the binding of zinc to various ligands.[65] Soon after the acute onset of jaundice, plasma zinc is found to be bound almost entirely as microligands to small molecules, such as the amino acids. This phenomenon contrasts with the normal propensity for approximately 95 percent of plasma zinc to bind predominantly as a macroligand with either albumin or alpha$_2$-macroglobulin. In any event, plasma zinc is sequestered rapidly in the liver, where it becomes tightly bound during acute infections to newly synthesized metallothioneins.[137]

CHANGES IN IRON METABOLISM

An abrupt depression in serum iron concentrations has been observed in virtually all infections in which iron values have been measured except acute viral hepatitis, with pyogenic infections causing the greatest effects. This hypoferremia has been ascribed primarily to an accelerated flux of iron from plasma into the liver, where it becomes localized in reticuloendothelial cells and hepatocytes. There the iron becomes sequestered as intracellular hemosiderin molecules or as a complex with ferritin.[8] Tumor necrosis factor and IL-1 seem to trigger this sequestration of iron by inducing the formation of ferritin,[24] some of which may be found in plasma. Plasma ferritin values provide a valuable clue concerning the quantities of body iron available in storage depots. When iron has become sequestered, it is not released readily until the infection is terminated.

Hepcidin is a peptide involved in major iron metabolic pathways. Hepcidin's secretion in the liver is induced during infections, likely by cytokines such as IL-6. Increased production of hepcidin leads to decreased plasma iron, which can be detrimental to the host but nonetheless acts as a host defense mechanism by reducing the iron available to different microorganisms.[51] Despite a low pool of plasma iron, some microorganisms, such as mycobacteria, have developed survival mechanisms by using the iron stored intracellularly in macrophages.[128]

During acute and chronic infections, the normal mechanisms that allow for the continuing release from tissue stores of the iron needed for use in erythropoiesis seem to be inhibited. If an infection persists for a prolonged period, anemia may develop. Although the "anemia of infection" resembles iron-deficiency anemia in its peripheral manifestations, it develops in the presence of adequate quantities of iron in storage sites and cannot be reversed by the therapeutic administration of iron.[8]

Other factors that influence iron metabolism during episodes of infection include an accelerated destruction of red blood cells and their direct loss in diseases in which hemolysis or bleeding

TABLE 3–2 Infection-Related Changes in Trace Elements

Trace Element	Observed Change during Infection	Suggested Pathophysiologic Mechanisms
Iron	Hyposideremia (common)	Flux of iron into liver and reticuloendothelial system cells
		Increased synthesis of ferritin
		Sequestration of iron in tissue stores
		Diminished iron absorption
	Anemia	Accelerated red blood cell destruction
		Direct blood loss
		Inhibition of erythropoiesis
	Reduced serum iron-binding capacity	Reduced synthesis of transferrin
	Hypersideremia (selective)	Hepatocyte damage during hepatitis
Zinc	Hypozincemia	Accelerated flux of zinc into liver
		Hepatic synthesis of additional metallothioneins
		Negative body balance of zinc
	Hyperzincuria	Hepatitis-induced inhibition of zinc binding to plasma proteins
Copper	Hypercupremia	Accelerated hepatic synthesis and release of ceruloplasmin
Chromium	Hypochromia	Unknown
Manganese	Hypermanganemia	Hepatocyte damage during hepatitis
Cobalt	Hypercobaltemia	Hepatocyte damage during hepatitis
	Macrocytic anemia	Intestinal parasitic competition for available vitamin B_{12}
Gallium	Hypergallemia	Hepatocyte damage during hepatitis
	Accumulation in sites of localized infection	Unknown
Iodine	Accelerated deiodination of thyroid hormones	Increased cellular metabolic rates
		Increased iodine availability for cellular bactericidal functions

is a factor. Excessive laboratory testing of blood samples can occur. In malaria, serum iron values tend to decline, despite the unusually large amounts of hemoglobin released from parasitized red blood cells. The released hemoglobin rapidly complexes with haptoglobin and is taken up by reticuloendothelial cells.[42] As in other forms of hemolytic anemia, a depression of serum haptoglobin values in malaria can serve as an index of the severity of red blood cell destruction.

An inhibition of iron absorption in the intestinal tract also can contribute to an infection-induced depression of serum iron values.[166] Studies using radioactive iron showed that a febrile illness or the febrile response of infants or young children to immunization could depress intestinal iron absorption for several days.[20]

The abrupt decrease in serum iron concentration, which can reach virtually undetectable values, occurs without any appreciable change in total serum iron-binding capacity. Although concentrations of serum transferrin decline along with concentrations of albumin during states of severe protein deficiency, they decline only slowly, if at all, during occurrences of acute infections. The combination of a normal iron-binding capacity and a markedly depressed concentration of serum iron in previously well-nourished children results in an increase in the concentration of serum unsaturated transferrin.[8] Unsaturated transferrin may play an important host defensive role by competing with siderophores of bacteria, which need to acquire iron for their growth and replication.[165] Because of its high affinity constant for iron, unsaturated transferrin is an important potential mechanism for inhibiting bacterial replication.

Increased concentrations of serum iron have been reported during bacillary dysentery, typhoidal infections, and, most commonly, acute viral hepatitis. The increase in concentrations of serum iron is delayed in hepatitis until several weeks after the initial appearance of jaundice; this increase may be large enough virtually to saturate the iron-binding capacity of serum. An impairment of mechanisms accounting for the normal daily hepatic removal of iron from serum has been thought to explain the hyperferremia noted during infections associated with liver cell dysfunction. An alternative hypothesis suggests that hepatitis-induced hyperferremia is caused by an escape of iron from the damaged hepatocytes. The latter explanation also is used to explain the reported increase in concentrations of serum manganese, gallium, and cobalt during acute hepatitis.[10]

CHANGES IN ZINC METABOLISM

Concentrations of zinc decline during episodes of various infectious diseases, as they do with a large variety of other diseases characterized by the presence of an inflammatory process.[10,111] Zinc values rarely decrease, however, as much as iron values do. This difference apparently is because of the large amount of plasma zinc that is bound tightly to alpha$_2$-macroglobulin. This plasma protein remains constant during periods of infection.[10]

Similar to iron, zinc seems to move into the liver at an accelerated rate during episodes of acute infections. This increased flux of zinc from serum to liver apparently is triggered by the action of proinflammatory cytokines released from activated monocytes and macrophages.[8,10] The hepatic uptake and sequestration of zinc are associated with an increased synthesis of zinc-binding metallothioneins within the hepatic cells.[137] The reason for this flux of zinc from serum to liver has not been explained in terms of host defense.

Body balances of zinc are thought to become negative during episodes of infectious diseases because of a combination of factors, including a reduction in dietary zinc intake, a diminished absorption from the intestinal tract, and a concomitant continuation (or increase) of zinc losses via the urine and possibly via the feces and sweat. During periods of acute infectious hepatitis, a marked increase in the urinary loss of zinc occurs. This loss is caused by enhanced glomerular filtration and excretion of zinc microligands.[65] The increased formation of zinc microligands with amino acids, such as histidine and cysteine, permits plasma zinc to be excreted from the body via the urine. Whether an increased binding of zinc to amino acid microligands occurs with infections other than acute hepatitis is unknown; this phenomenon was not detected with sandfly fever. Zinc deficiency is important particularly in developing countries, where zinc deficiency is widespread and plays a significant role in diarrhea, pneumonia, tuberculosis, and malaria.[34]

CHANGES IN COPPER METABOLISM

Concentrations of plasma copper increase in virtually all infections. This increase apparently accompanies the accelerated synthesis and release from the liver of ceruloplasmin, the copper-binding protein of plasma. The increase in plasma ceruloplasmin resembles that of other acute-phase reactant proteins produced by the liver during inflammatory states when triggered by IL-6 and other proinflammatory cytokines.[8] Increases in ceruloplasmin and copper occur later than do the abrupt depressions in serum iron and zinc. The increase in copper values tends to persist longer than the changes in zinc and iron do, apparently as a result of the long in vivo half-disappearance time of ceruloplasmin from plasma. Body balances of copper have not been studied during an episode of an infectious illness.

PROINFLAMMATORY CYTOKINES

The generalized metabolic and physiologic responses to febrile infections are initiated and sustained by a unique control mechanism. This mechanism involves the secretion by various body cells of hormone-like cytokines (mainly monokines and lymphokines).[42] The cytokines include ILs, interferons, colony-stimulating factors, and tumor necrosis factor. Cytokines also function during localized infections.[117]

Complex interactions of cytokines that occur during illness only now are being unraveled.[8,42] Cytokines are not classified as hormones because they are produced by a variety of different cells located throughout the body, rather than by anatomically distinct glands; because cytokines are effective at far lower concentrations than are hormones; and because their interacting array of checks and balances is far more complex than that of hormones. Although cytokines are not components of either the central nervous or the endocrine systems, they interact with and trigger responses by both of these major regulatory control systems.[8,111]

Proinflammatory cytokines (which include IL-1, IL-6, and IL-8, and tumor necrosis factor) initiate and orchestrate the highly complex but stereotyped admixture of concomitant anabolic and catabolic events that make up the generalized host response to febrile infections. Individual components of this generalized response form distinct patterns. Some metabolic responses begin during the incubation period; some, at the onset of fever; some, late in the course of fever; and some, during convalescence.[8,10-12,14,21] No matter which type of microorganism causes an acute, generalized, febrile infection, the onset of most metabolic, biochemical, or physiologic responses seems to occur at consistent, predictable times. These many responses can be categorized temporally by their relationships to the time of onset of clinical symptoms and fever.[8] Virtually every metabolic or biochemical process is influenced in some manner by the body's response to an acute infection. The duration and magnitude of individual components of the generalized response show some

variability from infection to infection. More must be learned about how the cytokines influence this variability in host metabolic responses.

In today's terminology, this overall response is termed an *acute-phase response*, an *acute-phase reaction*, or the *systemic inflammatory response syndrome*. This acute-phase response may accompany other severe medical and surgical problems, in addition to infection.[75] This complex response, which also activates complement and stimulates the immune system, seems to help defend the body.[8,75] On the negative side, acute-phase responses generate important nutritional costs,[8,10,125] costs that can produce severe, life-threatening malnutrition. In addition, acute-phase responses that become excessive or overly prolonged can lead to hypotensive shock, multiorgan dysfunction, and death.[42,79,106,125]

Acute-phase responses are initiated and controlled by the proinflammatory cytokines.[42,62,64,71,79,134,140,167] The cytokine interferon-α also can contribute to the wasting syndrome of acute infections.[32] The acute-phase reaction may be modified by bacterial endotoxins, which uniquely stimulate an exaggerated release of tumor necrosis factor, a cytokine that inhibits lipoprotein lipase enzymes, to account for the high concentrations of plasma triglycerides that develop during gram-negative sepsis.[8,69]

The primary control mechanisms that initiate acute-phase responses normally exist in a standby mode but can be turned on whenever necessary by the activation of macrophages, blood monocytes, or other body cells, and by the subsequent rapid release of proinflammatory cytokines.[8,42,92] The many diverse types of stimuli that can activate these cells include the phagocytosis of microorganisms, tissue debris, or other particulate matter; the effects of polynucleotides, certain drugs and chemicals, antigen-antibody complexes, and bacterial toxins; and the actions of other cytokines. During infectious illnesses, proinflammatory cytokines can be detected in body fluids, body secretions, urine, and stools.[55,88,117,120]

After their release from producing cells, proinflammatory cytokines circulate via the plasma or diffuse through tissue fluids to stimulate cell populations in many locations throughout the body. These cytokines have multiple, often overlapping, actions, and they stimulate the release of companion cytokines. IL-1 previously was termed *endogenous pyrogen*, *leukocytic endogenous mediator*, and *lymphocyte-activating factor*;[42] and tumor necrosis factor previously was termed *cachectin*. Proinflammatory cytokines stimulate the hypothalamic temperature-regulating center to initiate fever,[8,42] endocrine glands to secrete hormones, the liver to take up amino acids and trace elements and to synthesize many different proteins,[8,111,134] the pancreatic islets to release insulin and glucagon,[53,175] and the muscle cells to catabolize contractile proteins.[3,31] These cytokines also stimulate the bone marrow to produce and release neutrophils[111] and synovial cells and fibroblasts to activate collagenase.[42] Proinflammatory cytokines also may affect growth during certain viral infections by exerting a direct effect on bone remodeling.[141] Marrow stimulation involves the effect of IL-1 on progenitor cells[98] and its ability to trigger the release of various colony-stimulating cytokines.[8,59,98] In this regard, IL-1 has been found to be identical with hematopoietin.[98] The proinflammatory cytokines also activate the immune system and cause certain subsets of T lymphocytes to secrete IL-2, IL-3, IL-4, and IL-5.[8,42]

Proinflammatory cytokines are thought to function, after attachment to their specific cell wall receptors, by activating phospholipase A_2 within the cell walls, leading to the intracellular release of arachidonic acid or eicosapentanoic acid, or both, from cell wall polyunsaturated fatty acid phospholipids.[42] Subsequent responses by the cytokine-stimulated cell are determined by the intracellular enzymes that can metabolize these acids to one of many possible eicosanoids.

CONTROL MECHANISMS FOR PROINFLAMMATORY CYTOKINE ACTIONS

Similar to the action of hormones, proinflammatory cytokine actions are regulated by numerous checks and balances. To act on a cell, a cytokine first must attach itself to a protein receptor on an exterior cell wall. Cells produce other similar receptor proteins, however, which are released to float free in plasma.[76,107] Freely circulating receptors can intercept and inactivate their matching cytokine.[50,56] Other unique plasma protein molecules, receptor antagonists, also are produced.[149] They can attach to and block the cellular receptors for specific cytokines.[76,107] Cytokine inhibitor proteins have been identified.[27,150] Other cytokines, such as IL-4 and IL-10, can suppress the cellular production of proinflammatory cytokines.[39,57,76,115,145] Cytokine stimulation of release of cortisol has complex feedback effects because cortisol can block the intracellular formation of eicosanoids stimulated by the cytokines. Cortisol and epinephrine also can stimulate the production of cytokines.[79]

CYTOKINE DETECTION IN BIOLOGIC FLUIDS

Individual components of this complex cytokine system of checks and balances now can be measured in body fluids, and their relationships can be studied throughout the course of an acute-phase reaction.[22,26,42,71,76,140,150] These cytokines and their free receptors can be found in mucosal fluids and in plasma.[105,114,120] Cytokine measurements may have diagnostic and prognostic value.[26,134] Identification of IL-8 in amniotic cord sera was reported to be a specific marker for preterm chorioamnionitis.[134] Raqib and colleagues[120] described longitudinal measurements of cytokines and receptor antagonists in the plasma and stools of patients with acute shigellosis. Concentrations of tumor necrosis factor–α; IL-1β, IL-6, and IL-8; and IL-1 receptor antagonist in stools were quite high when patients with shigellosis first were seen and gradually returned to normal values during the next 2 weeks.[134] IL-6 and IL-8 can be used as markers for neonatal sepsis with coagulase-negative staphylococci and to differentiate between bacterial and enteroviral infections.[151] Proinflammatory cytokines also are responsible for the cachexia associated with acute or chronic inflammatory diseases.[37] The pathogenic or physiologic effects of these interacting molecules in either systemic or localized diseases still are not fully understood.

HORMONAL RESPONSES

Infectious illnesses are accompanied by a variety of endocrine responses, which especially include increased secretion of the hormones that regulate carbohydrate and energy metabolism and the hormones that influence salt and water retention. These hormonal responses are secondary to and often are initiated by the primary release of proinflammatory cytokines from activated cells.

In addition to their participation in physiologic responses, endocrine functions also may be affected by pathologic complications of an infectious process that results in the direct destruction or dysfunction of hormone-producing cells. The adrenal glands may be destroyed if tubercle bacilli localize in these glands or if hemorrhagic necrosis becomes a complication of such infections as acute meningococcemia. Pancreatic islet cells may be destroyed during episodes of viral infections of experimental animals, and the same pathogenic possibility may initiate insulin-requiring diabetes in children. Thyroid function may be impaired after occurrence of a viral infection that triggers an autoimmune thy-

roiditis, and gonadal tissues may be the site of localization and destruction by the mumps virus.

PITUITARY GLAND FUNCTIONS

Secretion of certain of the trophic hormones produced by the anterior pituitary is increased during infection. An increase in adrenocorticotropic hormone production (stimulated by proinflammatory cytokines) triggers an increased secretion of several adrenocorticoids. Concentrations of plasma growth hormone generally are increased in acute infections in which this hormone has been measured,[19,121] but the increases do not seem to correlate directly in timing or magnitude with the presence or severity of fever.[7,10] This increase in plasma growth hormone may be due partly to its production by mononuclear leukocytes.[164] Concentrations of plasma growth hormone increase rapidly in a paradoxical manner if an intravenous infusion of glucose is given to a patient or a laboratory animal with an acute infection.[121] Thyroid-stimulating hormone does not respond during the early phases of an acute febrile illness but may increase during the convalescent period. No increases in the gonadotropic hormones have been documented during an episode of an infection. A release of several anterior pituitary hormones can be stimulated by fever-producing doses of bacterial endotoxin. Such responses have been used in the clinical testing of anterior pituitary function.[74,95]

As described in the earlier discussion of salt and water imbalances, inappropriate secretion of antidiuretic hormone may occur in patients with severe infectious diseases.[45] This phenomenon also seems to be a characteristic response in patients whose infectious process becomes localized within the cranial vault.

ADRENAL FUNCTIONS

Although the adrenocortical hormones are known to influence the ability of a patient to respond to stressful situations, few data define the duration and magnitude of the adrenal response or characterize the spectrum of specific steroid hormones produced in excess.[17,94] Available data suggest that acute generalized infections typically are accompanied by a transient increase in the adrenal output of glucocorticoid hormones that is of short duration. Although these responses generally are limited in magnitude, they serve to maintain a constant concentration of cortisol in plasma throughout the early periods of fever.[17]

The usual diurnal decline in plasma 17-hydroxycorticosteroid values fails to occur during the afternoon and evening hours if fever is present. Cortisol-binding proteins have not been observed to change in plasma during periods of acute infection.[10] The plasma concentrations of unbound, physiologically active cortisol are maintained at or higher than the normal peak early morning values throughout the initial period of a febrile illness.[10,17] The total increase in glucocorticoid secretion rates during early illness ranges from two to five times normal values in infections that have been studied. Lesser degrees of increase have been noted in the adrenocortical output of pregnanetriol and the weak ketosteroid androgens.[17] If an infectious illness becomes chronic, adrenal output generally returns to or even falls below baseline values, and diminished adrenal responsiveness to adrenocorticotropic hormone may occur.

Extremely high concentrations of plasma 17-hydroxycorticosteroids may develop during gram-negative septicemia or before death in patients with other severe acute infections.[91,94] These high terminal values may be ascribed to failures in the hepatic clearance of cortisol from plasma and the metabolic pathways for converting cortisol to water-soluble metabolites; high plasma values are not the result of an extraordinary increase in adrenal secretion rates.[91] In a detailed study of adrenal function in children with meningitis, Migeon and coworkers[94] reported a maximum average increase in cortisol secretion rate of approximately threefold during uncomplicated aseptic or bacterial meningitis. This increase was accompanied by a threefold increase in the excretion of urinary 17-hydroxycorticosteroids during the first few days of illness. On admission to the hospital, these children had concentrations of plasma cortisol that ranged from high normal to twice normal values. Severely ill patients who were dying of adrenal hemorrhage generally had values that were depressed or absent.

Mechanisms by which small increases in adrenal glucocorticoid secretion might protect the host have not been defined. The ability of the liver to produce some of its proteins during periods of infection is known, however, to depend on the permissive presence of glucocorticoid hormones.[17]

The increase in production of aldosterone seems to lag behind the increase in production of cortisol during periods of acute infections and then persists longer. Increases in aldosterone stimulate the intense retention of sodium and chloride by the kidney during episodes of acute infections.[13]

The adrenomedullary secretion of catecholamines may increase in severe infectious diseases.[60,171] High plasma epinephrine and norepinephrine values develop in patients with gram-negative sepsis and bacterial meningitis. The catecholamine response contributes significantly to the acceleration of gluconeogenesis during periods of infection.

CARBOHYDRATE-REGULATING HORMONES

Hormones that serve in the normal regulation of carbohydrate metabolism also participate in the host response to infection. In addition to the heightened secretion of the glucocorticoids, catecholamines, and growth hormone, the major pancreatic hormones insulin and glucagon circulate in increased concentrations in the plasma of patients with acute infectious diseases.[171] The combined effect of these hormonal actions is to accelerate the production of glucose within the liver and to stimulate the release of glucose from stored glycogen. These actions cause the glucose pool size to increase twofold or threefold to provide an important source of metabolizable energy during the early febrile periods of acute infections.

Intravenous glucose tolerance tests performed early in the course of febrile infections in young adult subjects[121,132] led to an exaggerated increase of concentrations of plasma insulin in magnitude and duration, to an appropriate decline in elevated fasting concentrations of plasma glucagon, and to a paradoxical stimulation of release of growth hormone. Extreme hyperinsulinism also was observed after glucose infusions were given to dogs with endotoxic shock.[21]

The modest increase in concentrations of fasting plasma insulin that occurs during periods of infection seems to account for an inhibition of ketogenesis and fat depot lipolysis, two responses that would be expected to occur because of concomitant starvation or semistarvation. The simultaneous combination of high fasting plasma glucagon and insulin values and their effects on the liver and peripheral fat depots and somatic tissues helps to explain the observed differences between the anorexia-induced semistarvation associated with infectious illnesses and the starvation caused by food deprivation.

THYROID HORMONES

Thyroid hormones do not seem to initiate or sustain the hypermetabolic response to fever. Nonetheless, thyroxine (T_4) and triiodothyronine (T_3) are deiodinated at accelerated rates within the body tissues, especially in the liver, during the early phases

of infections studied in humans and experimental animals.[40,162,173] This acceleration in the metabolism of peripheral thyroid hormones accounts for an early decline in serum protein-bound iodine values.[131] Only after an infectious illness has progressed for several days does the thyroid gland hormone output seem to increase. This increase in thyroid secretion persists into early convalescence so that for a time the production of thyroid hormones exceeds apparent body requirements. As a result, protein-bound iodine values in serum are increased in the convalescent period. This sequence of events produces a biphasic response pattern with an initial decrease and a late increase in concentrations of thyroid hormones. Serum concentrations of "reverse T_3" increase during febrile illnesses.[29] Because reverse T_3 has an apparent role in regulating the peripheral cellular actions of T_4 and T_3, its function during acute infectious illness remains to be elucidated.

In studies of patients with falciparum malaria infection, serum T_3 values declined abruptly, whereas serum T_4 values were stable or increased slightly.[162] The decline in serum T_3 concentrations was accompanied by reciprocal increases in reverse T_3. The slowing of T_4 turnover during periods of malaria may be caused by an impaired ability of hepatocytes to metabolize or deiodinate T_4. Hypothalamic suppression in early stages of malaria seems to result in a decreased release of thyroid-stimulating hormone from the anterior pituitary and a secondary decrease in T_4 and T_3 secretion from the thyroid gland. In patients with sepsis and septic shock, thyroid hormones (total and free T_3, T_4, and thyroid-stimulating hormone) are decreased and are associated with poor prognosis.[177]

PROCALCITONIN

Procalcitonin, discovered in the early 1960s, is a prohormone, the precursor of the hormone calcitonin. Multiple studies have shown that procalcitonin is involved in the pathogenesis of infections, and that it can be a useful diagnostic marker for infections such as bacterial pneumonia,[68] bacterial sepsis and septic shock, meningitis, infectious endocarditis, pancreatitis, and urinary tract infections.[30,100,146]

The role of calcitonin is unclear; most likely, it had an evolutionary role, and it currently is nonessential in humans.[30] Serum concentrations of procalcitonin and other precursors of mature calcitonin are elevated significantly during episodes of sepsis and other infectious and noninfectious causes of inflammation. Serum concentration of procalcitonin also is elevated in patients with malaria. The serum concentration of procalcitonin usually correlates with the severity of infection and with mortality.[30]

Secretion of procalcitonin is stimulated by IL-1β and tumor necrosis factor–α in patients with bacterial infections and by interferon-γ in patients with viral infections. The target tissues are lung, liver, kidney, adipose tissue, and muscle.

Several studies have suggested procalcitonin could be used as a marker for bacterial sepsis and could be used to differentiate infectious from noninfectious causes of inflammation. Although procalcitonin has been shown possibly to have greater specificity and sensitivity compared with C-reactive protein as a marker for bacterial infections, it should not be used without evaluation of other clinical and laboratory data in making therapeutic decisions because serum procalcitonin concentration may be normal in patients with sepsis or increased in some individuals with no clinical symptoms.[30]

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CHAPTER 4

Interaction of Infection and Nutrition

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The introduction to the World Health Organization (WHO) report on nutrition and infection, published in 1965, stated the following[343]:

> The concept that malnutrition could make man more susceptible to infectious disease and also alter the course and outcome of the resulting illness has long been current in the history of medicine and public health. Circumstantial evidence is plentiful, principally based on clinical experience. Well controlled observations have been few, and hence clear proof in support of the concept has been slow to accumulate. It has been much easier to demonstrate that infection is often directly responsible for lowering the state of nutrition.

The 2005 WHO report estimated that 1 million children would die before reaching 5 years of age. Infections, including acute respiratory infections (19%), diarrheal diseases (17%), neonatal infections (including neonatal tetanus) (14%), malaria (8%), measles (4%), and human immunodeficiency virus (HIV) (3%),[347] account for most of these deaths. More than half of these deaths are associated with malnutrition.[46] This problem has been called "silent genocide" by WHO officials and is one of the most underreported health problems facing humans.

The association of malnutrition with infection has been documented repeatedly. The devastating effects of long-term semistarvation in infants and small children were described by physicians trapped in the Jewish ghetto of Warsaw by the prolonged Nazi siege.[41,341] They observed gross and microscopic evidence of lymphoid tissue atrophy and the disappearance of delayed hypersensitivity reactions and acute inflammation, as well as the disappearance of clinical allergies, blood eosinophils, and gastric acid in severely malnourished children. More recent data also support the concept that malnutrition renders individuals more susceptible to acquisition of infection. Metabolic, biochemical, and clinical evidence has accumulated that strongly supports the concept that nutrition affects profoundly the progress of infection within the host. The normal host response to infection is described in detail in Chapter 3. Virtually every normal metabolic or endocrine function is altered in some manner by the presence of an infectious illness.

Traditionally, clinical inquiries into the interaction of nutrition and infection have focused on patients with protein-calorie malnutrition. Nutrition is a crucial determinant of immunocompetence and risk for development of illness. Young children with protein-calorie malnutrition exhibit increased morbidity and mortality rates largely secondary to infectious diseases.[59] These children often have isolated deficiencies of single nutrients, and single-nutrient deficiencies and protein-calorie malnutrition are associated with impaired immune responses.

In light of the well-recognized biologic synergism that exists between malnutrition and infection, it is noteworthy that malnutrition has not received comparable attention in child health and survival strategies. This oversight may occur partly because data on mortality gathered from health facilities in developing countries report only the proximate cause of death (usually infectious diseases), such that only the severe cases of nutritional deficiency are recorded as nutritional causes of death.[237]

PROTEIN-CALORIE MALNUTRITION

Protein-calorie malnutrition describes a wide range of clinical conditions resulting from mild to severe undernutrition. The

WHO estimated in 1996[345] that 174 million children younger than 5 years of age in the developing world were malnourished, as indicated by low weight for age, and that 230 million were stunted. Mild protein-calorie malnutrition may be detected primarily by poor growth because when energy consumption is low, amino acids from dietary protein are used for energy, rather than for protein synthesis and growth.

The conditions of kwashiorkor and marasmus represent manifestations of severe protein-calorie malnutrition. Both conditions result from consumption of a diet deficient in protein and calories, but infections also play an important role. Frequently, an acute infection is incriminated as the event precipitating kwashiorkor.[13,117,134,306] Marasmus is characterized by severe wasting, whereas kwashiorkor is manifested by the presence of edema. In marasmus, the prognosis on refeeding is good, whereas treatment of kwashiorkor is more difficult, and the prognosis often is poor.[122] African studies have estimated the mortality rate in kwashiorkor to be 25 to 31 percent.[112,188] In these children, infection was second only to electrolyte abnormalities as a leading cause of death. Inadequate diet, including insufficient intake of energy, protein, and micronutrients (e.g., iron, vitamins, and minerals), leads to weight loss, retarded growth rate, diminished immunity, and mucosal damage. These factors exacerbate the incidence, severity, and duration of infectious diseases, which lead to the loss of nutrients, malabsorption, altered metabolism, and loss of appetite that lead to further inadequate dietary intake.[35]

That various infectious diseases interfere with or influence the responses of host defense is well known. Metabolic responses of the host to infection include increased use of proteins, carbohydrates, lipids, minerals, electrolytes, trace elements, vitamins, and hormones. The normal host responds to infection by initiating an immediate and marked stimulus for protein anabolism. The chemical changes in protein-calorie malnutrition include low serum albumin, low concentrations of essential amino acids in serum, generalized aminoaciduria, lower glycosylated hemoglobin levels, and decreased activity of many enzymes. Reduction in the activity of diphosphopyridine nucleotide, cytochrome *c* reductase, plasma esterase, and leukocyte pyruvate kinase has been documented.

A patient with kwashiorkor, depleted of amino acids and protein, cannot initiate the necessary anabolic response when challenged by infection, which seems to alter the capacity of the host to resist the debilitating effects of infection. Decreased activity of the various enzymes that help to resist infection and the inability to synthesize new enzymes necessary for energy-producing reactions in the body impose an additional burden on an individual with protein-calorie malnutrition. If the diet does not replace calories and protein sufficiently, the individual becomes progressively depleted with each episode of infection. Repeated episodes of infection may be a major factor in precipitating frank kwashiorkor in children on a borderline diet with regard to protein and calories. In many instances, whether the malnutrition or the infection was the event that initiated the ultimate deterioration of the patient cannot be determined.

Postmortem studies have confirmed that acute bacterial infections are the major cause of death in patients with severe protein-calorie malnutrition. Septicemia is the most dreaded infectious complication of protein-calorie malnutrition and has been reported in 31 percent of hospitalized patients. Gram-negative enteric bacilli are the most common cause; less frequent agents include *Haemophilus influenzae*, *Shigella flexneri*, *Pseudomonas aeruginosa*, *Corynebacterium diphtheriae*, *Streptococcus* spp., *Staphylococcus aureus*, and *Neisseria* spp. Tuberculosis occurs more frequently, is more virulent, spreads faster, and reinfects more readily individuals with protein-calorie malnutrition. The rate of urinary tract infections in malnourished children is increased significantly, with gram-negative enteric bacilli encountered most frequently. Diarrhea is a common occurrence in malnourished children, the most common infectious cause being enteric pathogens, such as *Salmonella enteritidis*, enteropathogenic *Escherichia coli*, and nontyphi *Salmonella* spp.[286] The occurrence of *Pneumocystis carinii* infection in malnourished individuals also has been highlighted.[134] Additionally, viral diseases, such as measles, varicella, hepatitis, and herpes simplex, are prevalent in patients with protein-calorie malnutrition.[13,117,306]

Almost all cases of severe protein-calorie malnutrition exhibit biochemical or clinical evidence of micronutrient deficiencies, such as vitamin A deficiency or iron-deficiency anemia. Little evidence exists, however, that any one micronutrient deficiency is the main cause of protein-calorie malnutrition or by itself is responsible for the edema of kwashiorkor.

Although protein is an essential and important nutrient, protein-calorie malnutrition is associated more often with deficient food intake than with protein intake. When commonly consumed cereal-based diets meet caloric needs, they *usually* meet protein needs, especially if the diet also provides modest amounts of legumes and vegetables. Control and prevention of protein-calorie malnutrition should focus on improvements in the quantity and quality of food consumed, immunization, provision of oral rehydration therapy for diarrhea, early treatment of common diseases, regular deworming, and attention given to the underlying causes of protein-calorie malnutrition, such as poverty and inequity.[173]

IN UTERO EFFECTS OF MALNUTRITION

The medical literature reports widespread occurrence of infant low birth weight throughout the nations of the world in which malnutrition is prevalent. Hytten,[137] discussing the relationship of maternal diet to the size of the infant's body, called attention to the fact that dietary status in pregnancy is only part of the broad environmental picture. He emphasized that the mother's nutrition throughout life may be as important as nutrition during pregnancy in ensuring the birth of an infant of normal size. A well-fed mother deprived of food during pregnancy may have been able to lay down a sufficient energy reserve to protect the fetus during intrauterine life despite deficiencies in day-to-day intake. Short maternal stature, which is influenced by biosocial factors, large family size, malnutrition, and chronic disease, frequently becomes intergenerational. Women who were growth retarded as newborns tend to give birth to infants who also are growth retarded, which may be a reflection of poor maternal nutritional status during childhood and adolescence.[183]

Severe nutritional deprivation during pregnancy may affect the size and vitality of the fetus. Abrams and Laros[1] showed that underweight women and women with poor weight gain during pregnancy had an increased risk for delivering an infant weighing less than 2500 g.

Maternal malnutrition results in impaired placental function and chronic vascular insufficiency, which leads to symmetric growth retardation. As nutritional deprivation becomes more severe, first weight, then length, and finally brain mass are affected. Acute placental insufficiency results in asymmetric growth retardation. When the nutritional insult occurs late in pregnancy, when length velocity is declining and weight velocity is increasing, the amount of muscle, fat, and hepatic glycogen is affected adversely. This type of growth retardation is seen in postmature infants; in these infants, placental function no longer is adequate to meet fetal needs, and the fetus must mobilize its own fat and carbohydrate stores.[183]

Maternal malnutrition before or during pregnancy and maternal infection during pregnancy may act alone or in concert to influence the size, gestational age at birth, and vitality of the fetus. Infection during pregnancy occurs commonly in the areas of the world where malnutrition is prevalent. In a prospective study in four Guatemalan villages, 12 percent of village women who were tested serologically throughout pregnancy for cytomegalovirus, herpes simplex, rubella, syphilis, and *Toxoplasma* spp. infection showed seroconversion during pregnancy to one of these agents.[144] The aforementioned infections, varicella, HIV, malaria, and trypanosomiasis are associated with intrauterine growth retardation.[32]

Infant size at birth correlates to a great extent with neonatal survival. Village infants who are small for gestational age at birth have a greater risk for developing subsequent malnutrition and infection than do term infants with an adequate birth weight. Infants with low birth weight (predominantly small for gestational age) have higher rates of infection with *Shigella* spp., *Entamoeba histolytica*, and *Giardia* spp. in the first month of life and exhibit higher occurrences of diarrhea and oral candidiasis in the first 6 months than do term infants.

A case-control study in rural Africa suggests this susceptibility to infectious disease extends far beyond the first 6 months of life.[212] As late as adolescence, increased infectious mortality rates were seen in individuals who had been born during the "hungry season" compared with those born during the "harvest season." These findings suggest that nutritionally mediated growth retardation may impair development of immune function permanently. Animal models highlight these long-term effects of intrauterine malnutrition by showing impaired T-cell–dependent immune responses in the first-generation and second-generation offspring of animals subjected to prenatal nutritional deprivation.[54]

Even if caloric requirements are met during pregnancy, micronutrient deficiencies have been linked to adverse pregnancy outcomes, and the worldwide prevalence of these deficiencies varies greatly.[280] In particular, iodine and folic acid are two micronutrients with proven roles in embryonic/fetal development. Deficiencies are endemic in certain parts of the world and may have devastating consequences for affected infants. More recently, zinc deficiency during pregnancy has been implicated in postnatal immune dysfunction and increased risk for development of infections.[338]

Even in industrialized countries, the consequences of low birth weight can be extensive. Providing enteral nourishment to very-low-birth-weight infants can be difficult because of systemic illnesses, such as respiratory distress syndrome and gastrointestinal tract immaturity. Many of these infants ultimately develop clinical and biochemical signs of malnutrition during the early postnatal period. This malnutrition may compromise an already inadequate immune system and alter host susceptibility to infection. Reductions in spleen, thymus, and body weights were noted in rats after experiencing intrauterine protein-calorie deprivation. After nutritional restoration was achieved in these animals, thymic tissue remained depleted of lymphocytes and significantly reduced in weight.

Newborn infants may develop similar long-term immunologic defects after in utero or early postnatal malnutrition. Low-birth-weight infants born to malnourished mothers have decreased thymus and spleen size and diminished cell-mediated immune responses, reduced transfer of maternal-fetal IgG, and decreased number of T lymphocytes. Infants with fetal growth retardation have shown diminished neutrophil chemotaxis, abnormal nitroblue tetrazolium oxidative reduction, and deficient microbicidal activity.[55,121] After birth, nutritional deprivation may create the environment for frequent episodes of infection. Mechanisms responsible for the increased number and severity of infections in the malnourished host are detailed subsequently.

BREAST-FEEDING

The nutritional, immunologic, psychosocial, and child-spacing benefits of breast-feeding are recognized universally. According to numerous reports, breast-fed infants seem to be less susceptible than are bottle-fed infants to certain infections. The protective effect is most evident for upper respiratory infections, otitis media, and gastroenteritis and has been studied most extensively in developing countries; the impact of breast-feeding on other infections and in more developed countries also is under investigation. Many studies are investigating the specific agents present in human milk that may be important in imparting protection to the infant. In addition to its anti-infective properties, breast milk has been shown to exhibit other advantages over infant formula; however, despite its many benefits, in certain situations, breast-feeding is contraindicated.

The antimicrobial system in human milk constitutes a complex group of biochemical agents that differ widely in structure but have a common effect at the mucosa. These agents include lysozyme, lactoperoxidase, lactoferrin, interferon, complement components, immunoglobulins, leukocytes, lipids, and retinol. These anti-infective factors transmitted by breast milk protect primarily by noninflammatory mechanisms and act to reduce the risk for development of mucosal infection in the gut. Proteins present in breast milk are thought to escape digestion in young infants as a result of low acid, reduced protease activity in gastric and pancreatic secretions, and the presence of protease inhibitors in breast milk.

The component most important to the newborn may be secretory IgA, which is found in high concentrations in colostrum and early milk. Secretory IgA constitutes more than 90 percent of the immunoglobulins in human milk and is formed by intricate processes that may be regulated by cytokines and hormones produced late in pregnancy or during lactation. During this process, B cells producing IgA against respiratory and gastrointestinal infectious agents migrate to the mammary gland in a higher proportion than their percentage of the total IgA-secreting B-cell pool. IgA possesses virus-neutralizing and antibacterial properties and is capable of activating the alternate complement pathway, providing local protection in the gastrointestinal tract.

Lactoferrin is an iron-binding glycoprotein that competes with siderophilic bacteria for ferric iron and interferes with the multiplication of organisms. Lactoferrin also may have a positive influence on cell growth and a negative effect on inflammation. Lysozyme is found in high concentrations in external secretions, including human milk, and lysis-susceptible bacteria by hydrolyzing β-1,4 linkages between *N*-acetylmuramic acid and 2-acetylamino-2-deoxy-D-glucose residues in cell walls. Lysozyme is resistant to digestion by trypsin and denaturation caused by acid. Fibronectin is a high-molecular-weight protein found in breast milk that facilitates the uptake of particulates by phagocytic cells.

Human milk also is rich in carbohydrate moieties that act as receptor homologues and inhibit attachment of pathogenic bacteria, such as *Vibrio cholerae*, *Streptococcus pneumoniae*, *H. influenzae*, and *E. coli*, onto epithelial cells.[72] Certain oligosaccharides in human milk promote the growth of *Bifidobacterium* spp. and lactobacilli in the lower intestinal tract, which produce acetic acid and inhibit the multiplication of bacterial pathogens such as *E. coli* and *Salmonella* and *Shigella* spp. Additionally, lactadherin, a component of human milk, has been found to bind specifically to rotavirus and inhibit its replication in vitro.[222] A study of rotavirus infection in Nicaraguan children supports this finding by showing an association between a longer duration of breast-feeding and decreased symptoms with rotavirus infection.[92]

A plethora of cytokines have been isolated from human milk, many in concentrations exceeding maternal serum, which sug-

gests an active transport mechanism. Viable leukocytes are found in human milk and have been proposed to play a role in protection against infections and immune modulation.[33]

Epidemiologic studies of the association between infections and breast-feeding have come under great scrutiny. Strong evidence has shown that in developing countries, infant morbidity is reduced by breast-feeding. A pooled analysis[348] of studies performed in the Philippines, Brazil, and Pakistan indicates that breast-feeding offers protection against the risks of diarrhea and of mortality from acute respiratory infection. The greatest protection is offered early in infancy and then steadily declines. Multiple small studies from Mexico,[181,330] India,[335] Belarus (diarrhea only; no protective effect against respiratory infections),[163] Haiti,[157] and Nicaragua[92] confirm these results. In a study from Nicaragua, protection against *Giardia* infections depended on the maternal antibody status: breast-fed infants of nonimmune mothers had a higher risk of developing giardiasis and experienced more severe symptoms compared with breast-fed infants of immune mothers.[310]

Many researchers consider that infants in more developed countries have similar benefits.[56,69,233,333,349] In a longitudinal analysis of infants in the United States, the risk for developing otitis media or diarrhea increased as the amount of breast milk received decreased.[265] The effect against otitis media may be the result of transmission of humoral or cellular immune components to the infant or the result of positioning during bottle feeding, which may predispose the infant to otitis media. Data showing general protection against infection are supplemented by studies that investigate the effects of breast milk on specific pathologic organisms. Formula-fed infants are more likely than are breast-fed infants to have colonization[130] or invasive disease[287] caused by *H. influenzae* and a higher risk for acquiring invasive pneumococcal disease.[176] Other studies have shown decreased risk for developing respiratory illnesses,[38,223] meningococcal disease,[210] urinary tract infections,[189] salmonellosis,[255] and human herpesvirus-7[172] infection with breast-feeding.

Despite studies such as these, the evidence in highly developed countries remains controversial. Bauchner and associates[21] presented a meta-analysis of the association between breast-feeding and infections in industrialized countries. Of 20 studies in the meta-analysis, only 6 met strict methodologic standards. These investigators concluded that the evidence supports only a minimally protective effect of breast-feeding. In 1990, a group from Denmark reported their results after following 500 infants prospectively for the first year of life.[258] They were unable to document a protective effect of breast-feeding against infectious illness. A case-control study of 13,224 mother-infant pairs, the largest study published to date, found no measurable association between breast-feeding and risk of neonatal respiratory infections in boys and only a small protective effect in girls.[290] These differences highlight the importance of environmental factors, such as socioeconomic status, parental education, exposure in daycare centers, and parental smoking, to the incidence of infectious disease in infants. Nonetheless, a report of the Department of Health and Human Services Office on Women's Health[83] recommends exclusive breast-feeding except in special situations in which contraindications to breast-feeding exist.

Breast-feeding has distinct advantages over bottle feeding in situations in which infection is not an important factor. Numerous studies in various parts of the world have investigated the possible prevention of atopic or allergic diseases by breast-feeding. Three more recent meta-analyses concluded that there was a lower incidence of asthma[111] and allergic rhinitis[205] in breast-fed infants and a lower incidence of eczema in breast-fed versus formula-fed infants with a family history of atopy.[110] A study analyzing cytokine profiles in formula-fed or breast-fed infants with respiratory syncytial virus (RSV) bronchiolitis suggested that the protective effect of breast milk against infections together with its potential immune-modulatory properties act together to protect infants from more severe RSV disease.[252]

The effect of breast-feeding on the prevention of atopic diseases has not been unchallenged—a cohort study from Brazil found an increased risk for development of asthma associated with being breast-fed for 9 months or longer.[71] The data currently available point toward a protective effect of breast-feeding, however, during the first 6 months of life on allergic or atopic diseases. Although evidence is inconclusive, some studies have implied that breast-feeding provides protection against sudden infant death syndrome, obesity, type 1 and type 2 diabetes, hypercholesterolemia, arteriosclerosis, celiac disease, and other metabolic disorders, and enhances neurodevelopment.[8,149]

Colostrum and early milk contain hormones and growth factors, such as epidermal growth factor, prostaglandins, insulin, and thyroid hormones, that may benefit children whose gut integrity has been compromised by malnutrition and gastrointestinal disease.[242] Whether these components are present in amounts sufficient to have any physiologic effect after the first few months of lactation is unclear, however. Breast milk also contains the digestive enzymes amylase, bile salt–stimulated lipase, and bile salt–stimulated esterase in measurable quantities after 6 months of lactation. Children whose digestive functions are compromised by malnutrition or small bowel overgrowth may benefit from the addition of breast milk enzymes. The combined effects of protection against infections, immune modulation, and maintenance of the integrity and promotion of growth of the intestinal mucosa may work together to explain a much decreased incidence of sepsis and necrotizing enterocolitis in a cohort study of Norwegian extremely-low-birth-weight infants in whom early enteral feeding with human milk was established.[253]

In certain circumstances, breast-feeding is contraindicated. The most common of these situations is maternal infection with HIV, a topic that has been the focus of much recent research. More recent studies estimate that one third to one half of mother-to-child transmissions of HIV occur through breast milk.[164,314] A randomized clinical trial of 401 mother-infant pairs in Kenya[218] showed that the risk for transmission in breast milk was 16.2 percent and that the use of formula feeding prevented 44 percent of infant infections. Risk for transmission is approximately 14 percent in chronic maternal HIV infection and increases to 29 percent in acute infection.

Other factors that may increase transmission of HIV through breast milk include longer duration of breast-feeding, mastitis in the mother, lower maternal CD4 counts, and mixed feedings (i.e., infants fed breast milk and formula).[314] The mechanism by which mixed feedings increase transmission is currently under debate. Some researchers theorize that a subclinical mastitis caused by milk stasis is responsible, whereas others hypothesize that an inflammatory or immunologic response in the infant gut may play a role. Currently, breast-feeding by HIV-infected women is contraindicated in areas where safe alternative sources of infant nutrition exist. Some researchers argue that in areas where environmental contamination precludes the safe use of other infant feeding regimens, exclusive breast-feeding should be promoted. A more recent study conducted in Kenya showed, however, that with access to clean water, formula feeding can decrease HIV transmission rates significantly without substantially increasing morbidity and mortality rates from diarrhea and pneumonia even in resource-poor settings.[194]

Breast-feeding also is contraindicated in mothers who are sputum-positive for *Mycobacterium tuberculosis* or who are carriers of hepatitis B virus. Breast milk has been implicated in the transmission of rubella, cholera, Q fever, human T-cell lymphotropic virus 1, and cytomegalovirus in selected individuals. Human milk also can transmit environmental toxins, such as polychlorinated biphenyl compounds and dichlorodiphenyltrichloroethane. Toxic side effects can occur in nursing infants secondary to passive

excretion of medicines taken by the mother.[88,149] On balance, breast milk is preferable except in unusual circumstances and helps diminish the role of infection during infancy.

IMMUNE SYSTEM AND MALNOURISHED HOST

MUCOSAL IMMUNITY

The first barrier to potential pathogens is the physical integrity of the skin and mucous membranes. This barrier involves single or multiple layers of cells, mechanical clearance mechanisms such as the cilia of the respiratory epithelium, complex carbohydrate structures in the intestinal epithelium, lubricants such as sebum or mucus, and soluble factors such as antibodies and lysozyme.

A "mucosal immune system" has been described in the gut, where tissue resident cells of the monocyte/macrophage lineage and specialized enterocytes act as antigen-presenting cells to lymphocytes. Primed lymphocytes migrate to the Peyer patches, where they proliferate and differentiate. A subset also enters the circulation to reach central immune organs, such as the spleen, bone marrow, and possibly thymus, where the lymphocytes undergo further clonal expansion. Eventually, they become IgA-producing cells that re-enter the circulation and by a selective homing process migrate to the mucosa of the gut and to salivary, lacrimal, bronchial, and lactating mammary glands.[243]

The physical integrity of the cell layers of skin or mucous membranes is affected in numerous nutritional deficiencies. Examples include the metaplastic hyperkeratosis caused by avitaminosis A; the dermatitis, cheilitis, and angular stomatitis from iron, riboflavin, and pyridoxine deficiencies; the mucosal atrophy and dermatosis of pellagra; the acrodermatitis enteropathica associated with zinc deficiency; the spongy gums and subcutaneous hemorrhages of scurvy; and the atrophy of skin and gastrointestinal mucosa of severe protein deficiency.

The carbohydrate composition of intestinal epithelium has been shown to vary according to dietary influences, and a decreased risk for gastrointestinal infections in breast-fed infants is thought to be due partly to decreased carbohydrate receptor sites for intestinal pathogens. Severe protein malnutrition and xerophthalmia have been shown to suppress significantly the secretion of lysozyme in the tears of children.[344] Diminished secretory IgA has been noted in malnutrition, which may increase host susceptibility to infection by permitting increased penetration of infectious agents into the circulation.[292,301] Malnutrition also affects other parts of the "mucosal immune system": Malnourished children have a reduced number of lymphocytes and plasma cells in the interstitial space, and the migration of lymphoblasts from the mesentery is decreased.[63]

HUMORAL IMMUNITY

Numerous studies of B-cell function have been done in patients with protein-calorie malnutrition, albeit with conflicting results. The total number of circulating B cells has been reported to be normal or increased,[312] or diminished.[217,248] Serum immunoglobulin concentrations may be normal or elevated.[293] Cohen and Hansen[65] showed that children with protein-calorie malnutrition and infection synthesized gamma-globulin at three times the rate of uninfected malnourished individuals, documenting the fact that synthesis of gamma-globulin was not rate-limited in malnutrition at the expense of other protein synthesis, such as that of albumin. In protein-calorie malnutrition, antibody affinity is decreased, which may explain the higher frequency of antigen-antibody complexes found in malnourished patients.

In protein-calorie malnutrition, the most dramatic change in humoral immunity concerns IgE. Serum IgE concentrations in healthy, well-nourished children are extremely low. Significant elevations of serum IgE in malnourished children have been reported in the absence of allergy or parasitic infections, possibly caused by an imbalance in T-lymphocyte regulation of IgE-producing B-cell function. The defect in cell-mediated immunity in patients with protein-calorie malnutrition could initiate an exaggerated IgE response during infections with respiratory pathogens, such as RSV or parainfluenza virus, and increase the risk for developing severe bronchiolitis.[153]

Antibody production after immunization with antigen is the best functional measure of humoral immunity. Much of the data on specific serum antibody responses in human malnutrition are conflicting. The serum antibody response to many protein antigens, such as tetanus or diphtheria toxoid, is well preserved. The responses to immunization with viral antigens vary. Responses to yellow fever vaccine, hepatitis, and killed influenza A have been reported to be impaired in protein-calorie malnutrition. Chronic malnutrition also is associated with a poor response to measles vaccine.[331] A diminished antibody response to polysaccharide antigens, such as killed typhoid vaccine (polysaccharide typhoid O antigen), is seen in severe protein-calorie malnutrition. The degree of malnutrition may play a role; mild protein-calorie malnutrition had no impact on the response to a meningococcal group C polysaccharide vaccine.[120]

A diminished antibody response to polysaccharide antigens may be caused by a selective impact on the IgG subclasses that contain antibody to polysaccharide antigens, IgG2 and IgG4.[153] Studies in rats deficient in vitamin A support this hypothesis[156]; however, total serum levels and IgG subclasses in 109 malnourished children from Ghana were found to be normal.[248] Further studies investigating the relationship between IgG subclasses and protein-calorie malnutrition are needed.

CELLULAR IMMUNITY

In patients with malnutrition, the cellular immune system (T-cell system) seems to be the component of the immune system affected most significantly. Impairments have been shown at nearly all levels of T-cell development and function. Histological studies of lymphoid tissues show severe depletion of T-cell areas in malnourished hosts.[197] The total number of circulating T cells, particularly the helper CD4 subset, is decreased.[217] Little change occurs in the number of suppressor T cells; the helper-to-suppressor ratio is decreased significantly.[62,234] The levels of circulating immature ($CD1a^+$, or $CD4^-CD8^-$ "null cell") T cells have been found to be increased in several studies, pointing toward a defect in maturation.[234] Indirect evidence, such as decreased deoxynucleotide transferase activity and increased adenosine deaminase in the serum of malnourished children, also suggests a defect in T-cell maturation.[206]

T-cell function in malnourished hosts seems to be impaired as well. Several studies have shown decreased responses to mitogenic stimuli and decreased production of cytokine, particularly type 1 cytokines (interleukin-2 [IL-2], interferon-γ).[89,251,266] Finally, the level of circulating memory T cells ($CD45RO^+$) has been found to be decreased in malnourished children.[216]

The observed abnormalities in T-cell numbers and function are clinically reflected in decreased responses to skin testing for antigens that normally induce delayed hypersensitivity responses. Chandra[60] reported that malnourished children had a decreased ability to respond to tuberculin antigen after receiving bacille Calmette-Guérin (BCG) immunization, and, in most cases, they could not be sensitized to dinitrochlorobenzene. Smythe and associates[293] reported similar findings in malnourished children and noted lymphocyte depletion in the thymus glands of 118 children with kwashiorkor or marasmus at necropsy. Bhaskaram and coworkers[36] showed that malnutrition did not influence the

ability of BCG-vaccinated children to localize tuberculosis infection; however, malnourished children who did not receive the vaccine had a significantly greater incidence of systemic tuberculosis infection than did BCG-vaccinated malnourished children. Another study of 69 children with protein-calorie malnutrition and 20 healthy controls showed an association between all levels of protein-calorie malnutrition and decreased absolute lymphocyte count, but a decreased response to tuberculin antigen was found only in children with moderate or severe malnutrition.[206]

A few contradicting studies regarding the influence of malnutrition on cellular immunity have been published. McFarlane[195] reported that skin transplants in malnourished rats were rejected, suggesting that all aspects of cell-mediated immunity may not be impaired simultaneously or to the same extent. Good and colleagues[115] have evaluated many aspects of the effect of nutritional status (including chronic protein deficiency) on cellular immune functions, including tumor immunity in animal models. Their results suggest an enhancement of cell-mediated immunity in chronic protein deficiency in the absence of infection. The mechanism for this enhancement and the implications of these findings in humans are unclear.

Most significant is the clinical and epidemiologic evidence for impaired cellular immunity in malnutrition. Numerous studies show that malnourished children are much more susceptible to tuberculosis, measles, disseminated herpes simplex, hepatitis, *P. carinii*, and many other diseases for which prevention requires optimal function of the cellular immune system.

THYMUS

Dourov[85] reviewed the morphologic response of the thymus in malnutrition. In 1845, Simon recognized that the thymus was an "early critical barometer of nutrition." In protein-calorie malnutrition, the severe nutritional defect leads to thymic atrophy and fibrotic changes, with the cortex affected sooner than is the medulla. In contrast to atrophy of liver, kidney, or cardiac muscle, thymic atrophy is characterized more by a loss of cells than by a decrease in cell size. Histologically, corticomedullary differentiation is lost, and fewer lymphoid cells are present. Hassall bodies are enlarged, degenerated, and occasionally calcified. These changes are noted after 5 days of starvation and can be reversed after 6 days of refeeding. In contrast to other organs, thymic tissue does not regain normal size after refeeding. Histopathologic studies on the thymuses of 19 malnourished children at necropsy showed that the degree of lymphocyte depletion correlates with an increase in the extracellular matrix.[186]

In addition to substrate deficiency, stress-related factors may play a role in thymic atrophy. Generally, stress-induced immunosuppression seems to be adrenal-mediated. The stress of malnutrition has been associated with an increase of serum glucocorticoids, a lower binding capacity for steroids, and an increase in their metabolically active forms. Schlesinger and associates[267] found elevated levels of norepinephrine in the thymuses of rats with protein-calorie malnutrition that correlated with decreased immune response.

Functional changes resulting from thymic atrophy also are under investigation. Thymic factors, such as thymosin and thymopoietin, have been incubated with isolated T cells from malnourished children, resulting in a normalization of the maturational characteristics of the lymphocytes.[138,227,234] These observations were confirmed when the results were controlled for infection and zinc content.[140]

The nutritional influence on early thymic development may have lifelong consequences. In rural Gambia, being born during the "hungry" season (July through December) predicts increased infection-related adult mortality. Serial sonographic measurements of thymic indices in children born during the "hungry" season were lower than the measurements in children born during the harvest season, and measurements in all children regardless of birth month were lower when assessed during "hungry" versus "harvest" months. This difference persisted even when adjusted for current weight and infectious markers, pointing toward severe long-term effects of starvation on thymic development and function.[67]

MEDIATORS (CYTOKINES, CHEMOKINES, AND COMPLEMENT SYSTEM)

Data are conflicting with regard to circulating cytokine levels and malnutrition. Normal, decreased, and increased tumor necrosis factor–α (TNF-α) production all have been reported in malnourished children.[17,89] In several studies, a T-helper cell type 1 (T_H1) to T_H2 type shift in cytokine profiles has been reported, with increased levels of IL-4 and IL-10 and decreased levels of IL-2 and interferon-γ.[89,251] These results suggest a diminished ability of malnourished children to mount a T_H1 response important for the elimination of pathogens. The observed skewing of the serum cytokine profile and the reported increased IgE production and increased severity of RSV bronchiolitis in malnourished children also point toward inappropriately hyperactive T_H2 responses and, consequently, more severe tissue damage than in children with intact nutritional status. The available data on cytokine profiles in malnourished children should be interpreted with caution because many confounders, such as chronic infections and living in extremely stressful environments, are likely to coexist in this population and have been shown to influence cytokine profiles.

The proteins of the complement system seem to be sensitive to nutritional stress.[62] Seth and Chandra[278] noted low serum complement in patients with protein-calorie malnutrition. Sirisinha and associates[291] also reported low serum concentrations of all complement components except C4 in malnourished children. Children with kwashiorkor had levels lower than those of children with marasmus. These data are consistent with more recent animal studies that show significantly decreased levels of C1, C2, C3, and C4 in malnourished rats compared with controls.[260]

The cause of the hypocomplementemia is unclear. Electrophoretically distinct C3 breakdown products have been detected in one study, as have increased titers of immunocoagglutinin, an antibody directed to the C423b complex of activated complement. Alternatively, activation of the complement pathway may be a consequence of infection. Complement serum concentrations also may decrease in the malnourished individual as a result of a consumption complementopathy, or a decreased ability to synthesize complement de novo. Complement-derived chemotactic factors and opsonic factors also are depressed. These functional deficits may contribute to an enhanced susceptibility to infection.

Despite these changes, complement may be less susceptible to nutritional stress than other aspects of the immune system. Complement activity seems to be preserved better in patients with severe protein-calorie malnutrition than cell-mediated immunity and it recovers more rapidly after refeeding.[259,261]

PHAGOCYTOSIS

Phagocyte function can be divided into three main phases: (1) adherence and chemotaxis; (2) recognition, opsonization, and engulfment; and (3) postphagocytic events, which include formation of phagocytic vacuoles or phagosomes followed by fusion of lysosomes with phagosomes and degranulation of lysosomal enzymes, microbicidal activity, and associated metabolic changes.[122] Phagocytosis and intracellular microbicidal activity of neutrophils and macrophages depend on various components of the comple-

ment system, antibodies against surface microbial antigens, and possibly acute-phase reactants, such as C-reactive protein. Appropriate phagocytic function also requires adequate pool sizes of neutrophils and mononuclear phagocytes at inflammatory sites and adequate capacity of the bone marrow to produce and mobilize these cells. More recent research suggests that an association exists between low neutrophil count and decreased weight-for-height measurements in children.[321] Protein deprivation in mice results in a marked decrease in phagocyte precursor cell pool sizes as measured colony-forming units in the spleen.[122]

In malnourished individuals, defects in phagocytosis and killing have been identified, but these deficits are subtle. Research has shown that neutrophils from malnourished children have enhanced baseline adhesion; however, after being stimulated with chemotactic factors, the adherence response is decreased, and neutrophil chemotaxis is diminished. These abnormalities reverse with nutritional recovery.[10,122] Schopfer and Douglas[269] investigated the neutrophil function of 46 children with kwashiorkor. Chemotactic response was reduced at early intervals (30, 60, and 120 minutes) and reached values achieved by controls only after 180 minutes, which suggests an early migration defect.

Studies of opsonization and phagocytosis of cells in malnourished animals and humans indicate that neutrophil membrane receptors for Fc-IgG and complement (C3b) are intact. Serum from malnourished patients is deficient in opsonic activity, however, and kinetic studies in malnourished children suggest that the defect in opsonization is related to complement deficiencies.[121] An indirect measure of opsonic activity is fibronectin, and reduced levels of fibronectin have been reported in starvation in rats and in protein-calorie malnutrition in infants. The levels of fibronectin increase to greater than normal values after nutritional rehabilitation.[121]

No evidence has been found for any abnormalities in lysosomal fusion or degranulation in leukocytes of malnourished hosts. Nonetheless, intracellular killing seems to be impaired in malnourished states. Schopfer and Douglas[269] noted that neutrophils from children with kwashiorkor did not kill *Candida albicans* intracellularly as well as did control cells. Seth and Chandra[278] reported impaired intracellular bactericidal killing in patients with kwashiorkor. De la Fuente and Munoz[77] showed a decrease in nitroblue tetrazolium reduction in stimulated and nonstimulated macrophages from mice with protein-calorie malnutrition. Because the nitroblue tetrazolium dye reduction test in nonstimulated phagocytes indirectly measures intracellular hexose monophosphate shunt activity, protein-calorie malnutrition may interfere with this metabolic pathway. Studies from children with kwashiorkor show diminished neutrophil iodination during phagocytosis,[121] suggesting an abnormality in the myeloperoxidase-halide–mediated system.

SINGLE NUTRIENTS

Many barriers exist to analyzing the clinical significance of single nutrients in maintaining normal immune function. First, nourishment is a combination event, and nutrient-nutrient interaction can be of major consequence. Competition for transport may affect absorption or excretion in intracellular and extracellular environments. Second, a hierarchy exists for some nutrient requirements. If an element subserves several functions, as does iron, the prerequisite amount may vary with the function. Third, experimental animal models do not always allow for extrapolation to human medicine. Most species, with the notable exception of the guinea pig, make indigenous vitamin C, and no animal model exists for studying cobalamin deficiency. Finally, infection can affect body stores of essential nutrients. Chandra[58] suggested a framework for evaluation of micronutrients. Alterations in immune responses occur early in the course of reduced intake, and these alterations may predict the risk for developing infection and mortality.[324] In the case of many nutrients, excessive intake also is associated with immune abnormalities.

IRON

Iron deficiency is the most common nutritional deficiency in the world and results in systemic disease involving all cell systems. The role of iron in chronic and acute infection is complex. Experimental and clinical studies have been published that support an impaired immune function in iron deficiency on one hand and a protective role of low iron states against certain infections on the other.[228]

Experimental data suggesting impaired immune function in iron deficiency exist for almost any part of the immune system. Severely iron-deficient rats have an increased number of phagocytes in whole blood, but granulocytic activity as measured by nitroblue tetrazolium dye reduction is decreased significantly.[284] The rate at which granulocytes killed staphylococci was decreased in 819 iron-deficient patients studied by Joynson and coworkers.[147] Retention of iron in the reticuloendothelial system may enable macrophages to detoxify bacterial toxins. Iron in monocytes may enhance antibacterial activity of these cells.[144] Iron also may activate lysosomal hydrolases.[141] Cellular development of lymphoid tissues has been shown to be diminished in iron deficiency; lymphoid tissue from iron-deficient rats shows reduced cellularity, decreased lymphopoiesis, and deranged histology.[284]

In humans with iron deficiency, reduced numbers of circulating T cells and decreased in vitro lymphocyte response to mitogens have been reported.[81] The bactericidal capacity of leukocytes also is reduced, which may be caused by deficient function of iron-dependent myeloperoxidase and cytochrome enzymes.[60] Natural killer cell activity is impaired significantly in moderate and severe iron deficiency compared with controls. In iron-deficient patients studied by Macdougall and coworkers,[184] impaired leukocyte responsiveness, decreased bactericidal capacity, increased IgA, and increased C3 concentrations were observed. Restoration of normal bactericidal function occured before any increase in hemoglobin concentrations was noted, suggesting that tissue iron depletion rather than anemia was an etiologic factor in depressing bactericidal function. Clinically, impairment of delayed cutaneous hypersensitivity responses are found in humans with iron deficiency.[81]

Humoral immunity also may be affected by iron deficiency. In studies in iron-deficient rats, baseline plasma IgG levels were normal, and IgM was low only in severe iron deficiency, but rates of de novo synthesis of IgM and IgG after stimulation were found to be decreased significantly. Although circulating plasma immunoglobulin may be normal in iron-deficiency anemia, iron deficiency may result in diminished antibody production. Cytokine and chemokine production is impaired in individuals with iron deficiency, as has been shown for IL-1 and migration inhibitory factor.[147,269]

Taken together, these studies imply that appropriate iron stores and availability of iron within cells, particularly in selected tissue, is beneficial to the host. Numerous studies suggest, however, that free iron may be detrimental to the host during bacterial infection. Iron may stimulate the growth of the pathogen with which the host is infected, may inhibit bactericidal proteins, and may enhance bacterial metabolism.[337] The percentage of saturation of transferrin with iron in plasma correlates directly with the ability of the sera to support the growth of various microorganisms. Iron in fluids such as plasma, milk, nasal secretions, and saliva is to a greater or lesser extent unavailable to many bacteria and fungi because of the presence of the iron-binding proteins transferrin and lactoferrin.[174,191] Microorganisms produce iron chelators termed *siderophores*. If the supply of

iron in the host is so high that the physiologic processes to withhold it are exceeded, the invading microbes can obtain iron for growth.[284]

The administration of iron to animals by the intravenous, intramuscular, or intraperitoneal route reduces the LD_{50} for *P. aeruginosa*, *Salmonella typhosa*, streptococci, *Klebsiella pneumoniae*, *Salmonella typhimurium*, and *Listeria monocytogenes*.[337] Similarly, the administration of iron intramuscularly to children with kwashiorkor has resulted in overwhelming infection and death. Clinicians in these cases concluded that iron therapy should be deferred until transferrin synthesis was restored by protein nutrition. No evidence exists, however, that gradual oral replenishment of iron predisposes children to more severe infection.

Secondary bacterial infection occurs commonly in patients with bartonellosis and malaria,[175] and bacterial infection, particularly that caused by *S. pneumoniae* and *Salmonella* spp., occurs more frequently in individuals with sickle cell anemia.[19,175] This evidence has been cited by some investigators to support the concept that iron is detrimental to the host during infection. This theory is not justified because patients with these diseases are known to have other deficits that intrinsically increase propensity for infection. The increased incidence of bacterial infection in these individuals cannot be attributed specifically to an increase in free iron.

Malaria frequently is cited as a situation in which iron-deficient states are protective to the host. Field researchers in malaria-endemic countries noted that treatment of iron deficiency, especially with parenteral iron, often was associated with an increase in the incidence of smear-positive malaria. Other studies have manipulated iron availability by using desferrioxamine, which is an iron chelator. In vitro and in vivo, desferrioxamine inhibits the growth of malaria parasites. The effect of desferrioxamine seems to be directly on parasitized erythrocytes, which behave as if they contain a chelation-labile iron pool.[152] In a study of Indian children, no statistical difference was found, however, in the incidence or prevalence of malaria or in the severity of *Plasmodium falciparum* parasitemia at different hemoglobin or nutritional levels.[98]

Several studies[9,185] of iron supplementation have reported fewer episodes of respiratory and gastrointestinal illnesses in iron-supplemented infants, but because of difficulties defining and statistically evaluating infectious episodes, the significance of these findings is not compelling. In a study in Colombia[11] in which iron deficiency was severe, nutritional supplementation and medical care had no impact on the mortality caused by infectious diseases, but supplemented groups experienced an impressive reduction in enteric infections. The relative contributions of iron and other supplements in decreasing morbidity could not be ascertained. Significantly improved iron status and reduced morbidity from upper respiratory tract infections were found in a placebo-controlled trial of iron supplementation in a study on 363 low-income children from Sri Lanka.[78]

Taken together, the depletion of tissue levels of iron as noted in iron deficiency are likely to be detrimental to optimal performance of the inflammatory and immune systems. To date, no studies have identified the point at which iron deprivation interferes with immunologic states. The diverse role of iron in multiple enzyme systems, mammalian and bacterial, may be understood best in a hierarchy of functions in which optimal activity of the different systems may be at different elemental concentrations. The suggestion that iron deficiency protects humans against infection cannot be supported, although caution against rapid parenteral iron repletion in states of acute infection may be advised. Recognizing the importance of maintaining appropriate iron stores, the WHO recommended iron and folic acid supplementation of flour in developing countries as a strategy for breaking the vicious cycle of malnutrition and infection.[327]

SELENIUM

Selenium has three major functions in the tissues and cells of the immune system: reduction of organic and inorganic peroxides, metabolism of hydroperoxides, and modulation of the respiratory burst. Glutathione peroxidase and phospholipid hydroperoxide glutathione peroxidase are selenium-containing enzymes that catalyze the reduction of peroxides formed from general metabolism, drugs, and other initiators of free radical chain reactions. Both of these enzymes also are involved in steps in the synthesis of thromboxane A_2, prostaglandins, leukotrienes, and lipoxins.

Research has shown reduction of eicosanoid biosynthesis in the absence of selenium and glutathione peroxidase. Selenium and the glutathione peroxidases also modulate the production of the oxidizing products of the respiratory burst: O^+_2, H_2O_2, CLO^+, and chloramines, which are used in the phagolysosome to lyse and destroy phagocytized cells. As predicted from these roles, selenium supplementation prevents oxidative stress–induced damage to immune cells. Selenium deficiency in experimental animals is associated with decreased glutathione peroxidase activity in phagocytic cells, release of increased amounts of H_2O_2 by macrophages and peritoneal granulocytes, and increased superoxide formation in macrophages.[58,196,297]

Selenium also apparently boosts cellular immunity by upregulating the expression of the T-cell high-affinity IL-2 receptor, enhancing T-cell response. Splenic leukocytes from selenium-deficient rats proliferated less after mitogenic stimulation than did leukocytes from control rats.[241] Controversy still surrounds the role of selenium in the prevention of infections in humans. Deficiency has been associated with generalized immunosuppression affecting neutrophil function, antibody production, and lymphocyte proliferation. A more recent study investigating selenium levels in 134 patients in intensive care units (ICUs) found that the mean plasma selenium concentration of these patients was 2 standard deviations below that of the general population.[106] This study also showed an inverse correlation between plasma selenium and severity of sepsis in these patients. The treatment of patients in ICUs with supplemental selenium has not been investigated.

In Keshan, China, selenium deficiency has been implicated in the pathogenesis of a dilated cardiomyopathy known as Keshan disease. The seasonal and annual incidences of Keshan disease were characteristic of an infectious disease, and screening of tissues from patients revealed the presence of numerous viruses, including coxsackieviruses. These findings are significant in light of Beck and Levander's[26] studies showing increased virulence of certain coxsackieviruses in selenium-deficient animals.

ZINC

Zinc is a cofactor for more than 300 cellular enzymes, with functions ranging from signal transduction to transcription to replication. Zinc deficiency in humans has been associated with poor growth and development, impaired wound healing, and impaired sensory perception. The low levels of zinc in acrodermatitis enteropathica may be related to the thymic atrophy and high frequency of serious bacterial, viral, and fungal infections seen in these patients because pharmacologic zinc supplementation can reverse all of these symptoms.[219]

Multiple animal models show the association of zinc deficiency with altered nonspecific and specific immune responses.[271,338] Lower zinc levels have been found in serum and hair of otherwise well-nourished children with pneumonia compared with healthy controls in Bangladesh.[281] Patients maintained on parenteral nutrition lacking in zinc showed reduced T-lymphocyte proliferation in response to mitogenic stimulation, which markedly increased after repletion of zinc.[285] A trial in Indian children

showed increased CD3 and CD4 cell counts and an increased CD4/CD8 ratio in children given zinc supplementation compared with controls.[264] Deficiencies in zinc also are associated with reduction in thymulin activity and slower neutrophil chemotaxis; these changes are reversed with the zinc supplementation.[58] Zinc deficiency impairs the function of antigen-presenting cells.[270] Conversely, excessive supplementation with zinc has been shown to cause reduced chemotactic and phagocytic activities and a suppressive effect on lymphocyte proliferation.[70] The inhibition of T-cell proliferation seems to be caused by inhibition of an IL-1 receptor–associated protein kinase by zinc ions at higher concentrations.[339]

Several randomized, double-blind, placebo-controlled trials of zinc supplementation have been done with children in developing countries, most of whom exhibit some manifestations of malnutrition.[231,256,257,263,322] These studies showed the association of zinc supplementation with decreased incidence of and morbidity from respiratory infections and reduced morbidity from diarrhea. Micronutrient deficiencies may be of particular clinical significance in malnourished children with chronic diarrhea or intestinal parasitic infections. Treatment of the infection has been shown to increase serum zinc levels without nutritional intervention, suggesting a vicious cycle comprising malabsorption, immune dysfunction, and inability to clear the infection.[226]

The immunologic consequences of zinc deficiency during pregnancy have received increased attention more recently. Animal studies have shown reduced thymic and spleen size and decreased active and passive immunity in the offspring, which was not fully reversible by postnatal zinc supplementation and partially persisted into the next generation.[338]

OTHER TRACE ELEMENTS

Copper facilitates absorption of iron from the gastrointestinal tract and is essential for the production of red blood cells. It also is crucial for several oxidative enzyme systems. Copper deficiency has been shown to cause anemia of varying degrees and gross ataxia in neonates of many species and defects in formation of connective tissue. Neutropenia has been documented in children with a copper-deficient diet. Copper deficiency is associated with depressed function of the reticuloendothelial system, reduced microbicidal activity of granulocytes, decreased response of splenic lymphocytes to T-cell and B-cell mitogens, and impaired natural killer cell cytotoxicity in animal models. The reduced microbicidal activity of granulocytes is attributed to the role of copper in superoxide dismutase and cytochrome-*c* oxidase enzyme systems.[285] Copper-deficient patients are more susceptible to contracting bronchopneumonia and bacterial sepsis, especially with *E. coli*. Copper-deficient animals show increased mortality rates when exposed to *S. typhimurium*, *L. monocytogenes*, and coxsackievirus B.

The role of chromium in disease control is undefined. Excess amounts adversely affect macrophage and lymphocyte cultures.[109,332] Chromium acts as a cofactor for the potentiation of insulin at the cellular level, and deficiency is characterized by impaired glucose use.[198,236] The role of chromium in impaired glucose homeostasis in kwashiorkor has been documented.[51]

Manganese is another essential trace element necessary for optimal growth. It affects the primary sites of chondroitin sulfate synthesis. In humans, manganese deficiency is characterized by weight loss, transient dermatitis, occasional nausea and vomiting, changes in hair color, hypocholesterolemia, and teratogenicity.

Lymphocyte and neutrophil functions in iodine deficiency are diminished. Iodide interacts with neutrophil peroxidases to form the halide-superoxide system, which is modulated by the amount of thyroid hormone, which supplies the iodide molecule. In hypothyroid patients, the bactericidal activity is decreased and restored after treatment with thyroid hormone.[58]

VITAMIN A

Three of the fat-soluble vitamins—A, D, and E—have recognized effects on immune system function. Before the discovery of antibiotics, researchers noted that urinary tract infections in children responded to vitamin A therapy. In the modern era, vitamin A deficiency is well documented to be a major risk factor for infections in Third World countries. Vitamin A levels in the serum have been used as markers of malnutrition; the independent effects of vitamin A deficiency are significant. Researchers have suggested that improving the vitamin A status of all children who are deficient would prevent approximately 1 to 3 million deaths each year, curtailing the incidence and severity of infectious episodes, especially respiratory and diarrheal infections.[135]

Vitamin A plays an important role in maintaining the integrity of epithelial surfaces. In animals and humans, vitamin A deficiency is associated with keratinizing metaplasia of mucus-secreting epithelial surfaces, particularly of the respiratory, gastrointestinal, and genitourinary tracts, and corneal tissues. This histopathologic alteration is conducive to an overgrowth of bacteria and secondary infections of loculated areas obstructed by keratinized debris. A classic example of this type of alteration is a Bitot spot, a triangular patch of xerotic conjunctiva characteristic of xerophthalmia, which is composed largely of keratin debris and a heavy growth of *Xerosis bacillus*, a saprophytic diphtheroid.

Immune changes in patients with vitamin A deficiency are characterized by disruption of the skin and epithelial barriers, increased bacterial binding to epithelial cells, reduced thymic weight, decreased lymphocyte proliferation, reduced immunoglobulin production, decreased T-helper cell activity, and decreased cytokine production. Vitamin A deficiency also is characterized by reduced phagocytosis, diminished nitroblue tetrazolium dye reduction by neutrophils, and reduced macrophage function.[60,113,275] Lysozyme is a vitamin A–dependent glycoprotein; the activity of this protein is decreased markedly in vitamin A deficiency.[340]

Vitamin A deficiency in children seems to increase their susceptibility to various types of infections. In a malnutrition ward in Bangladesh, 78 percent of children with xerophthalmia had bacteriuria determined by urine culture obtained by bladder tap compared with 17 percent of malnourished peers without xerophthalmia.[44] Studies from South Africa[86] and Israel[66] showed a strong association between poor vitamin A status and increased incidence and severity of acute respiratory infections.

Vitamin A supplementation may reverse some of these susceptibilities. A double-blind, placebo-controlled trial in a malaria-endemic area of Papua New Guinea showed that children supplemented with vitamin A had fewer febrile episodes and decreased parasite counts compared with unsupplemented controls.[282] In contrast, a study from Nigeria failed to establish a relationship between the use of red palm oil, a local oil rich in vitamin A, and decreased disease severity from malaria, whereas such a relationship existed for the overall nutritional status.[68] Finally, a meta-analysis of randomized trials of vitamin A supplementation in developing countries indicated a significant decrease in rates of respiratory and diarrheal disease mortality with vitamin A supplementation compared with placebo in children with no overt deficiency.[113] Vitamin A supplementation may be of particular importance in states of baseline increased susceptibility to infections, such as in children with HIV.[87,100]

Children in Third World countries frequently are caught in a vicious cycle in which infection leads to vitamin A deficiency,

which increases the risk for subsequent development of infection. The impact that vitamin A supplementation would have on childhood morbidity and mortality depends on several factors, including the prevalence and severity of deficiency, aggravating conditions (e.g., protein-calorie malnutrition), associated nutrient defects, virulence of the infectious agents to which they are exposed, and adequacy of supplementation.[296]

The effects of retinoids on the maintenance of the integrity and differentiation of epithelial surfaces on one hand, and their various immunologic effects on the other, raise the interesting question whether retinoid supplementation might help to prevent human papillomavirus–associated cervical dysplasia. In a more recent study, *trans*-leukopene and *cis*-leukopene (but not retinol, tocopherols, vitamin B_{12}, and folic acid) levels correlated with higher rates of clearing of an established infection with oncogenic strains of human papillomavirus.[273]

VITAMIN D

Vitamin D serves as an immunoregulatory hormone and as a lymphocyte-differentiating hormone in addition to its classic role of mineral homeostasis. 1,25-Dihydroxyvitamin D_3 functions in the manner of a steroid hormone in many different cell types. Receptors for 1,25-dihydroxyvitamin D_3 have been identified on the surface of T lymphocytes, monocytes, and macrophages,[326] and an extensive number of RNA polymerase II–transcribed genes that govern lymphokine expression are regulated by this vitamin-hormone.[27] Although vitamin D must be present at a certain concentration for proper immune function, high-dose supplementation also may have detrimental effects. One study has shown suppression of the delayed hypersensitivity response in patients with vitamin D deficiency and in individuals receiving high doses of vitamin D.[145] Activated vitamin D may inhibit T-lymphocyte proliferation and natural killer cell cytotoxicity and decrease concentrations of interferon-γ, IL-2, and IL-12.[180] Additionally, the differentiation of immature dendritic cells into antigen-presenting cells is inhibited in culture by activated vitamin D.[239]

A more recent case-control study from India found subclinical vitamin D deficiency to be a risk factor for pneumonia in children younger than 5 years old.[335] A case-control study from Jordan reported longer hospital stays in children with rickets and pneumonia compared with the nonrachitic controls.[215]

VITAMIN E

Vitamin E enhances immune responses and phagocytosis by acting as an antioxidant to prevent lipid peroxidation of cell membranes. Rapidly proliferating cells of the specific immune and phagocytic systems are prone to peroxidative damage by free radicals, peroxides, and superoxides. In vitro studies of rat alveolar macrophages show that high levels of vitamin E suppress the release of reactive oxygen species after stimulation.[235] These findings suggest that vitamin E may reduce self-inflicted damage to macrophages and surrounding tissue during infection. The antioxidant effect of vitamin E also modulates the biosynthesis and activity of prostaglandins, thromboxane, and leukotrienes.[311]

Several studies have shown that vitamin E deficiency impairs cell-mediated and humoral immunity in different animal models. Some studies suggest that vitamin E deficiency may contribute to the decreased immune function of neonates, especially premature infants.[199] In humans, vitamin E deficiency impairs T-cell–mediated function, which is reversible by vitamin E supplementation. Supplementation in an amount 2 to 10 times greater than the present recommended dose significantly increased humoral and cell-mediated immune responses and phagocytic functions in laboratory animals and humans.[311] Vitamin E seems to interact with other micronutrients in immune modulation. The heightened humoral response noted with vitamin E is synergistic with selenium[124] and copper.[311] In addition, zinc deficiency, even when marginal, can decrease markedly vitamin E serum concentrations.[249]

WATER-SOLUBLE VITAMINS

Among the water-soluble vitamins, B_1, B_6, B_{12}, and C have been implicated in immune function. Vitamin B_6 deficiency impairs immunity by slowing the rate of production of one-carbon units necessary for nucleic acid synthesis in these rapidly proliferating cells. Animal models deficient in vitamin B_6 exhibit impaired antibody production, a delay in IgM-to-IgG class switching, and altered cell-mediated immunity with reduced delayed-type hypersensitivity responses.[315] Humans consuming a diet low in vitamin B_6 or being treated with deoxypyridoxine (vitamin B_6 antagonist) have a decreased number of circulating lymphocytes and reduced antibody production and a mild decrease in the percentage of helper T cells.[200] The thymus is smaller, and thymic hormone activity is decreased in patients with vitamin B_6 deficiency.

Vitamin B_{12} also seems to affect immunity. Patients with vitamin B_{12} deficiency were found to have decreased numbers of lymphocytes, decreased CD8 cells, an altered CD8/CD4 ratio, and suppressed natural killer cell cytotoxicity.[308] All of these parameters improved with supplementation of vitamin B_{12}. Osawa[230] first suggested that thiamine deficiency may predispose to the occurrence of tropical pyomyositis, a hematogenous pyogenic infection characterized by abscess formation in various muscle tissues. Muscle tissue generally is resistant to infection, but lack of thiamine may change the biochemical milieu of the muscle, rendering it more susceptible to infection.

Physiologic quantities of ascorbic acid are necessary for normal metabolism of lipids and iron. One of the major functions of vitamin C is as an antioxidant that protects the $alpha_1$ proteinase inhibitor from inactivation by free radical products of the respiratory burst. $Alpha_1$ proteinase inhibitor is present in plasma, where it reacts with elastase, protecting the extracellular space from escaped proteases from damaged cells. The concentration of intracellular vitamin C is approximately 50 times that found in plasma, which suggests that vitamin C may protect intracellular regions from oxidants that leak into the cytoplasm.

Host susceptibility to infection is increased in scurvy. Vitamin C deficiency is associated with impaired phagocytic activity. Microtubule organization is responsible for phagocytosis and locomotion and depends on the redox state of the cell; vitamin C may modulate it by an antioxidant effect on tubulin tyrosinolation.[126] Macrophages from mice supplemented with vitamin C, vitamin E, or both showed increased random migration, chemotaxis, ingestion, and superoxide anion production compared with controls.[80] Because vitamin C is associated with chemotactic activation of phagocytes, its use has been suggested in patients with disorders of phagocyte function, such as Chédiak-Higashi syndrome.[268]

The possibility that vitamin C may be important in preventing upper respiratory infections has received widespread publicity in the medical and lay presses. Chalmers[53] reported, however, after reviewing 14 clinical trials of ascorbic acid in the prevention and treatment of the common cold, that differences between supplemented and nonsupplemented subjects were minor and insignificant. Nonetheless, in most studies, the severity of symptoms was worse in patients who received placebo. Miller and colleagues[201] performed a double-blind, co-twin controlled study on 44 monozygotic twins of school age. During the 5-month study period, no statistically significant difference in the number or severity of

illness episodes was noted between the recipients of vitamin C and a placebo control group. Several studies have shown a consistent decrease in the duration of the common cold episodes; although most of the results are not statistically significant, all of them point consistently in the same direction.[126] Administration of ascorbic acid is not a panacea for upper respiratory infections. Also, although lack of vitamin C is detrimental, excess intake is of no proven value.

Vitamin deficiencies interfere with host defenses. Infections also may cause or exacerbate certain vitamin deficiencies. Concentrations of vitamins A, B_6, and C have been reported to be lower than normal during acute bacterial and viral infections, and reduced concentrations of folic acid in blood and serum have been found in infants with diarrhea or acute bacterial infection[193] and in adults with tuberculosis or malaria.[250] Severe xerophthalmia in Indian children often is preceded by diarrhea, measles, or a respiratory infection and may have been precipitated by a decline in serum vitamin A and retinol-binding protein concentrations during the course of the infection. A study in children with shigellosis showed increased urinary excretion of vitamin A during infection, which was attributed to impaired renal tubular absorption of low-molecular-weight proteins, such as retinol-binding protein bound to retinol.[207] Infections may alter plasma retinol levels because activation of the acute-phase response may decrease the synthesis of the circulating proteins that transport retinol.[313]

Altered concentrations of circulating vitamins during infection also may be caused by several other mechanisms, including impaired absorption from the gastrointestinal tract, liver cell damage, and altered rates of vitamin excretion. Transient malabsorption of folic acid and vitamin B_{12} has been noted during and after recovery from acute intestinal infections, including cholera and salmonellosis.[178] The urinary excretion of the group B vitamins and vitamin C also has been observed to change during hepatitis and tuberculosis.[125,146]

Taken together, in vitro studies show alterations in immune cell function; animal models and clinical studies show an enhanced susceptibility to infection with deficiencies of macronutrients and micronutrients. More studies are needed, however, to assess particularly mild micronutrient deficiencies, possible synergistic effects, and optimal substitution regimens.

CONSIDERATIONS OF NUTRITION AND INFECTION IN SPECIAL POPULATIONS

Overall, the prevalence of malnutrition in industrialized nations is considerably less than that in developing countries; however, in industrialized nations, certain populations are at increased risk for developing malnutrition. Malnutrition is a common complication in HIV-positive individuals, regardless of their geographic location or economic situation. Critically ill patients have an increased risk for development of malnutrition and infection. Finally, burn patients, premature neonates, cancer patients, and children with cystic fibrosis have special nutritional requirements and metabolic changes that highlight the interactions of infection and nutrition.

HUMAN IMMUNODEFICIENCY VIRUS AND NUTRITION

Acquired immunodeficiency syndrome (AIDS) has become one of the most pressing public health problems, with more than 40 million HIV-infected individuals worldwide (WHO estimate 2005[346]) and more than 1 million in the United States. Infection with HIV has a devastating effect on nutritional status. Malnutrition was one of the earliest recognized complications of AIDS, and unexplained weight loss remains one of the most common initial AIDS-defining diagnoses reported to public health authorities. Patients may lose 30 to 50 percent of their body mass before dying of AIDS. The onset of depletion of body cell mass occurs early in HIV infection and may predate any significant immunodeficiency, suggesting that the virus itself may be responsible.[161]

Most commonly, the malnutrition associated with HIV is multifactorial. Decreased caloric intake, malabsorption of nutrients, and elevated energy expenditure, especially during systemic opportunistic infections, all contribute to malnutrition in these patients. Protein-calorie malnutrition is encountered frequently, as are individual or combined micronutrient deficiencies. All contribute to the growth failure seen in children with HIV, emphasizing the need for early and intensive nutritional intervention and treatment in these patients.

Two major paradigms of weight loss that contribute in patients with HIV infection are starvation and cachexia.[18] Starvation refers to a voluntary or involuntary deprivation of food that leads to increased losses of body fat and extracellular water, with relative sparing of lean body mass. In contrast, cachexia is characterized by a disproportionate loss of lean body mass resulting from specific alterations in metabolism. These alterations during cachexia are caused by shifts in cytokine levels and may include changes in protein metabolism or energy expenditure, appetite changes, or derangement of the sleep-wake cycle.

Investigation of energy metabolism in HIV-infected individuals has produced conflicting results. Some studies report increased resting energy expenditure, and others report that energy expenditure was decreased. A study of 36 HIV-infected children between infancy and 10 years of age showed that cardiac muscle mass and heart rate were correlated inversely with weight and skeletal muscle mass.[204] This finding is inconsistent with the decreased energy expenditure of hypometabolic compensation that occurs in starvation; instead, it suggests that these children have cachexia and intrinsic metabolic changes.

Animal and human studies have found correlations between cytokine levels and anorexia, cachexia, and altered lipid metabolism. Serum levels of interferon-α are increased in some patients with AIDS and are correlated significantly with elevated serum triglyceride levels. Adult patients with AIDS and with wasting were found to have higher plasma concentrations of TNF-α and cholecystokinin octapeptide sulfate, an appetite neuropeptide, and lower levels of β-endorphins compared with well-nourished patients with AIDS and healthy controls.[12] These changes may inhibit appetite pathologically and contribute to weight loss.

Very different metabolic changes have been observed more recently in patients with HIV who are receiving antiretroviral therapy. Lipodystrophy, or fat redistribution syndrome, seems to be associated more strongly with the administration of protease inhibitors than are other antiretroviral agents. Patients with this condition develop a dorsocervical fat pad; abdominal adiposity; and facial, extremity, and buttocks wasting. Increased breast size also is seen in female patients. Other changes include hyperlipidemia with increased levels of total cholesterol and low-density lipoprotein and decreased levels of high-density lipoprotein. These patients also may develop insulin resistance. The mechanism of this syndrome and its long-term consequences are unclear. A study examining the effect of protease inhibitors on growth of children found that despite the protease inhibitor–induced metabolic abnormalities, protease inhibitor therapy had a positive effect on growth parameters, including height, weight, and muscle mass, likely through reducing viral load and improving disease control.[203]

Involvement of the gastrointestinal tract in patients with HIV infections also undoubtedly plays a role in weight loss. Chronic weight loss often is related to gastrointestinal disease and malabsorption. The pathogenesis of malabsorption involves a combination of factors, including primary enterocyte injury with partial

villous atrophy and crypt hyperplasia, ileal dysfunction with bile salt wasting and fat malabsorption, and exudative enteropathy.[162] Small intestine pathology or pancreatic insufficiency may lead to malabsorption of fat, weight loss, and depletion of fat-soluble vitamins.[171] Hypochlorhydria has been found in almost 75 percent of patients with AIDS and can allow for enteric infections and reduced absorption of micronutrients, such as folate and iron. Malabsorption of lactose is a common finding and is more severe in symptomatic than asymptomatic HIV-infected children.[353] Subclinical malabsorption may play a role in early HIV disease, whereas overt malabsorption is found more frequently in advanced HIV infection.

Along with the direct effects of HIV, immunosuppression leads to an increased frequency of gastrointestinal infections, which exacerbate weight loss caused by diarrhea and malabsorption. Protozoal infections can disrupt mucosal architecture in the small intestine, resulting in severe malnutrition. Studies indicate that HIV-infected patients with diarrhea caused by enteric infections experience greater weight loss than do HIV-infected patients with diarrhea for which no pathogen can be identified.[50]

Protein-calorie malnutrition is a common occurrence in patients with AIDS and is considered a predominant cause of morbidity in AIDS. In the United States, 30 to 50 percent of children followed in HIV programs have evidence of protein-calorie malnutrition.[202] The immune effects of protein-calorie malnutrition and HIV infection are similar: Decreased CD4 cells, reversal of the helper-to-suppressor T-cell ratio, impairment of delayed hypersensitivity reactions, and abnormal humoral responses occur in both conditions. A child with protein-calorie malnutrition and HIV infection is likely to have an outcome worse than that of a child with either condition alone.

Low levels of vitamins A, E, B_6, B_{12}, and C and of carotenoids, selenium, and zinc are common findings in many HIV-infected populations. These deficiencies may be caused by decreased dietary intake, diarrhea, malabsorption, impaired storage, or altered metabolism of micronutrients.[277] Some researchers attribute the low levels of zinc in HIV-infected patients to direct use of zinc by the virus for gene expression, multimerization, and integration into the host genome.[23]

More recently, the prognostic significance of the status of selenium has received much attention. Several studies[22,24,49] have shown that selenium deficiency is associated more strongly than other micronutrient deficiencies with mortality in HIV-infected patients. Selenium-deficient, HIV-infected patients had a 10-fold to 20-fold increase in mortality rates compared with HIV-infected patients with adequate selenium levels. Among HIV-infected children who died, children with low selenium levels died at a younger age (mean age at death of 4.3 years versus 8 years), suggesting a more rapid progression of disease in patients with selenium deficiency.[49] The relationship between selenium deficiency and early death in HIV may be associated with the role of selenium in preventing oxidative stress, which may be involved in maintaining viral latency in an infected cell. An in vitro study of HIV-infected monocytes and T lymphocytes emphasized this role by showing that selenium supplementation partially suppresses the TNF-α–mediated replication of the virus.[131]

Controversy currently surrounds the role of maternal micronutrient status, particularly vitamin A, in the perinatal transmission of HIV. Several studies[119,275] show an increased risk for perinatal HIV transmission with maternal vitamin A deficiency; one study[275] showed that the risk for perinatal transmission was correlated inversely with concentration of maternal serum retinol. Other studies showed no association, however, between maternal vitamin A level and risk for transmission of HIV to the infant.[47,48,98,99,303] Further research is required before any conclusions can be drawn. In contrast, administration of vitamin A supplementation in children starting at 6 months of age has been shown to be beneficial in reducing mortality rates and morbidity among HIV-infected children.[100]

In children with HIV, the combination of diarrhea, malabsorption, protein-calorie malnutrition, and micronutrient deficiencies can have devastating effects on growth. HIV-infected infants may exhibit growth failure, failure to thrive, developmental delay, or frank malnutrition by 4 months of age.[101] A retrospective, cross-sectional analysis of 54 children with perinatally acquired HIV showed an early decline in the rate of linear growth (growth failure) with relative preservation of weight for age.[240] A prospective study of HIV-infected infants in Uganda[30] showed an association between mortality and the severity of growth failure. The exact nature of the relationship between growth failure and mortality in HIV infection is unclear. Poor nutritional parameters may represent frequent opportunistic infections and debilitation in infants with already advanced disease; conversely, poor nutritional status may accelerate the progression from asymptomatic HIV infection to symptomatic AIDS.

Nutritional intervention is important for all HIV-infected patients, but it is particularly crucial in the management of infants and children. In 2000, the American Dietetic Association and Dieticians of Canada recommended that children should be referred for a full nutrition evaluation as soon as possible after receiving the diagnosis of HIV infection.[101] Nutrition intervention should begin early in the course of HIV infection in the hopes of stabilizing weight loss and preventing growth failure. Evaluation should address stages of growth and development, anthropometrics, dietary intake assessment, medical data, and any psychosocial or economic issues that may be barriers to establishing adequate nutrition.

Specific dietary interventions include maximizing intake of high-calorie, nutrient-dense foods and vitamin and mineral supplements, especially vitamins A, B_6, B_{12}, E, and C, and riboflavin, zinc, and selenium. In addition, dietary counseling should emphasize the importance of daily ingestion of a full complement of amino acids. If adequate nutritional status cannot be maintained orally, enteral supplementation should be used. To maximize absorption and minimize diarrhea, formulas that have low residue or low lactose and that contain peptides and medium-chain triglycerides should be used. If the patient has intractable diarrhea or impaired function of the gastrointestinal tract, or if nutrient needs are not met by enteral nutrition, home total parenteral nutrition (TPN) may be considered for long-term use; however, TPN therapy may be complicated by metabolic abnormalities (hypertriglyceridemia, hyperglycemia, fluid and electrolyte imbalance) and problems related to catheters, such as infection, hemorrhage, and pneumothorax. Standard nutritional recommendations for patients with AIDS have not been established because of the heterogeneous nature of the complications of this disease.

ROUTE AND COMPOSITION OF NUTRITION IN CRITICALLY ILL PATIENTS

The effects of quality and quantity of nutrients on the immune system have been shown, but the route of administration of nutrition also has been shown to affect host susceptibility to infection. The first experimental evidence that the route of nutrition plays a role in host defense came from Kudsk and associates[166] in the early 1980s. Well-nourished rats were fed the same solution through either gastrostomy or central catheter for 12 days, after which hemoglobin–*E. coli* peritonitis was induced. The 48-hour survival was 60 percent in the enterally fed group compared with 20 percent in the parenterally fed group. Improved survival also was observed in malnourished rats under similar experimental conditions.[165]

Analyses of infection rates in hospitalized patients seem to confirm an association between TPN and susceptibility to infection. In a meta-analysis of data from eight prospective, randomized trials of early enteral versus parenteral feeding of high-risk surgical patients, Moore and colleagues[211] found a 35 percent risk for development of septic complications in patients receiving TPN compared with an 18 percent risk in patients fed enterally. Similar increases in infection rates have been observed in children. Several prospective cohort studies of patients in neonatal and pediatric ICUs have shown that TPN is the most significant risk factor for acquiring a nosocomial infection.[42,114,289]

Numerous hypotheses have been proposed to explain the increased risk for development of infection in parenterally fed patients. Intravenous nutrition may induce immunosuppression directly. In vitro studies of whole blood from infants receiving long-term TPN showed impaired phagocytosis and intracellular killing in response to challenge with coagulase-negative staphylococci, a model for bacteremia.[224,225] The intracellular killing was correlated negatively with duration, but not amount, of parenteral feeding and was found to normalize after the addition of small enteral feeding.[225] Correction of this immune defect with enteral feeding implies the involvement of the gastrointestinal tract in the increased infection risk with parenteral feeding.

Enteral feeding helps to maintain barrier function of the gut through increased gastric acidity, mucus production, and intact peristalsis.[7] Gastrointestinal tract "starvation" in animal models produces mucosal atrophy, bacterial overgrowth, decreased secretory IgA, increased intestinal permeability and translocation of bacteria or toxins, and atrophy of gut-associated lymphoid tissue.[155,177,225] The preliminary evidence suggests that enteral feeding may maintain immunologic integrity in the gut and result in increased resistance to infection.

The composition of enteral diets has been another area of more recent investigation. Certain key nutrients are being recognized for their ability to modulate a variety of inflammatory, metabolic, and immune processes when ingested in increased amounts. Nutritional components such as dietary nucleotides, omega-3 fatty acids, glutamine, and arginine are being called "immune-enhancing agents" because of their potential roles in bolstering and maintaining host defenses against infection.

Nucleotides are thought to have immune-modulating properties because of their role as structural units for DNA, RNA, adenosine triphosphate, and cyclic adenosine monophosphate. The unaltered human gastrointestinal tract contains bacteria, the continuous turnover of which provides an adequate supply of nucleotides, but this flora may be altered in critically ill patients.[20] Periods of rapid growth or certain disease states also may create increased demands for nucleotides. Under these conditions, supplemental dietary nucleotides may spare the energy of de novo synthesis and optimize the function of rapidly proliferating cells of the immune system.[52] Numerous studies have shown the immune-modulating effects of nucleotides in vitro and in animal models.[148,167] More recent studies in children also show these effects. Studies in infants showed that infants receiving nucleotide-supplemented formula had increased natural killer cell cytotoxicity, increased IL-2 production by stimulated mononuclear cells, and higher serum concentrations of IgM and IgA compared with infants fed standard formulas, and they were less likely to experience diarrhea.[52,190,350]

Omega-3 fatty acids may regulate immune function by modulating formation of prostaglandins and regional blood flow. Some inflammatory mediators of shock and sepsis, including prostaglandins, leukotrienes, and platelet-activating factor, are metabolites of omega-6 fatty acids, the primary fat source in many nutritional formulations. Substitution of omega-3 fatty acids for omega-6 fatty acids has been shown to have anti-inflammatory effects.[20] Parenterally fed rats receiving supplemental omega-3 fatty acids had increased splanchnic blood flow in response to endotoxin challenge compared with parenterally fed rats without supplementation.[245] They also had decreased viable bacteria in mesenteric lymph nodes and liver, which may represent decreased bacterial translocation from the gastrointestinal tract or improved bactericidal activity.

Glutamine is the most abundant amino acid in human muscle and plasma and is used as a fuel source for lymphocytes, macrophages, and enterocytes. It also is a precursor of glutathione and nucleotide synthesis. Glutamine is a nonessential amino acid; however, during catabolic illness, glutamine uptake by small intestinal and immune cells may exceed synthesis and release from skeletal muscle, rendering it conditionally essential.[20] Studies of glutamine supplementation to enteral feeding solutions in adult trauma patients have shown significantly less pneumonia, bacteremia, and sepsis in supplemented groups compared with controls.[132] Small individual trials have suggested a beneficial effect of glutamine supplementation in preterm infants.[220] A Cochrane review examining the effect of enteral and parenteral glutamine supplementation on mortality, sepsis, necrotizing enterocolitis, duration of hospitalization, and neurodevelopmental outcome found no clinically significant benefit, however.[317]

Arginine, a nonessential amino acid, has received considerable attention for its immune-enhancing properties. Arginine is a precursor for nitric oxide in vascular endothelial cells, macrophages, and neutrophils, and infusion of arginine stimulates release of growth hormone, glucagon, somatostatin, prolactin, and insulin.[20] A meta-analysis of 15 randomized, controlled trials comparing standard enteral nutrition with commercially available immune-enhancing enteral formulations containing arginine, with or without glutamine, nucleotides, and omega-3 fatty acids, found no effect on mortality, but patients receiving the immune-enhancing formulations showed a decreased risk for developing infection and had shorter hospital stays.[25]

Finally, probiotics such as lactobacillus have received increasing attention as potentially promising supplements for critically ill patients, given their beneficial effects on the gastrointestinal flora in patients with infectious diarrhea or inflammatory bowel disease.[29] Most clinical trials available to date that investigated lactobacillus supplementation in critically ill neonates and adults established safety but were unable to measure any clinical benefit.[74,139]

NUTRITION IN PRETERM INFANTS

Critically ill preterm infants represent a particular nutritional challenge. In addition to all the immunologic abnormalities seen in patients in the ICU, the preterm infant's immune system is immature. Immaturity of the gastrointestinal system renders enteral nutrition particularly difficult and is associated with potentially lethal conditions such as necrotizing enterocolitis, which is not seen in adults. The nutritional requirements of preterm infants exceed the requirements of older patients several-fold in terms of total calories and specific nutrients, calcium, phosphorus, iron, and vitamins. Nutritional management of the critically ill preterm infant is particularly difficult and, at the same time, of utmost importance.

Specialized preterm formulas or human milk fortifiers try to meet the preterm infant's nutritional needs. These formulas are optimized in their caloric content, composition of carbohydrates, type of protein, type of fat, electrolyte and mineral composition, iron content, vitamins, and trace elements. Introduction of enteral feeding often is delayed, however, because of intolerance, immaturity, or an intercurrent episode of infection, hemodynamic decompensation, or necrotizing enterocolitis. Most very-low-birth-weight infants at some point during hospitalization have nutritional deficiencies of vitamin A, vitamin E, or iron, from decreased bone mineralization and growth retardation.

An important strategy in the nutrition of preterm infants is the concept of "trophic feeds." Very small amounts of enteral nutrition, which contribute only minimally to the overall caloric intake, have been shown to avoid many of the devastating consequences of TPN, such as gut atrophy and short gut syndrome. The role of the gut-associated immune system in the overall acquisition of immunocompetence is likely of great importance, and (even trophic) enteral feeds are thought to enhance the development and improve the function of this important part of the immune system. Studies suggest beneficial immunologic effects of early enteral feeds manifesting in decreased incidence of sepsis.[103,250,304] Despite a plethora of studies, the minimal amount at which a clinically significant effect of trophic feeds can be expected is controversial.

BURNS, INFECTION, AND NUTRITION

In the United States, approximately 2.5 million people seek medical care for burns each year. More than 100,000 individuals are hospitalized with burns each year, and 12,000 die. During the past 50 years, great strides have been made in the treatment and management of patients who have sustained thermal injuries. A marked decrease in burn mortality rates, particularly in patients younger than age 35 years, has occurred.[214]

Infection always has been the predominant determinant of wound healing and outcome of burn patients. The incidence of infectious complications in burn patients increases in proportion to the fraction of the body surface injured. The direct effects of heat on skin and underlying tissue render the burn wound particularly susceptible to infection; the denatured protein in burn-injured tissue serves as a rich medium for microbial growth. The thermal thrombosis that renders the eschar avascular precludes delivery of the cellular components of the host defense system and limits delivery of blood-borne antibiotics to the infected wound site. Further microbial proliferation occurs at the interface between viable tissue and the eschar, termed the *subeschar space*. If host defenses are adequate, the eschar is sloughed; however, microbial invasion of the viable tissue occurs if host defenses are deficient.

Pneumonia is the most frequent infection occurring in burn patients. Other infectious complications, such as suppurative thrombophlebitis and septicemia, have been decreasing in incidence as a result of improvements in patient management, wound care, and infection control.[244]

A larger role in the development of sepsis has been attributed more recently to the gastrointestinal tract. The gastrointestinal barrier normally is highly effective in containing its flora. Critically ill patients often experience a breakdown of the barrier, followed by translocation of inert particles and microorganisms across the intestinal wall. This event may play a role in the colonization of burn wounds by gram-negative organisms. Alteration of the indigenous microbial flora, which may be caused by the stress of injury, antibiotics, or composition of enteral feedings, has been shown to influence translocation markedly.

Translocation of bacteria and their toxins from ischemic bowel causes a massive release of cytokines and inflammatory mediators.[79] This "cytokine storm" is associated with depressed cytotoxic activity of T cells, decreased ratio of helper-to-suppressor T lymphocytes, depressed phagocytic activity, reduced intracellular killing, increased superoxide formation, decreased serum concentrations of immunoglobulins, and activation of complement with release of anaphylatoxins.[123]

Major thermal injury is associated with extreme hypermetabolism and catabolism, which occur shortly after successful resuscitation from the shock phase of the burn injury. This hypermetabolic state is characterized by elevated cardiac output, increased energy expenditure, erosion of lean body mass, negative nitrogen balance, and disturbances in glucose metabolism.[316]

The role of nutrition in maintaining immunocompetence and modulating hypermetabolism in burned patients has become increasingly important. The use of early enteral nutrition combined with early excision of nonviable tissue has resulted in reduced energy requirements in burned children.[127] Providing proper nutrition by the enteral route when possible may satisfy caloric needs, regulate microflora, and maintain the integrity of the mucosa of the gut, but it also may blunt the hypermetabolic response after thermal injury.[143]

CANCER AND NUTRITION

Cancer predisposes patients to malnutrition through several mechanisms. Often, patients with cancer have experienced a period of weight loss before being diagnosed with a malignancy. This loss varies for different forms of cancer. Acute lymphoblastic leukemia typically is of rapid onset, and only 6 percent of children have lost a substantial amount of weight at diagnosis. In contrast, 50 percent of patients with stage IV neuroblastoma present with at least some degree of malnutrition.[6]

Malnutrition in patients with cancer originates from a combination of anorexia (i.e., decreased intake) and cachexia (i.e., increased metabolic demand). The latter is particularly evident in children with a very high tumor burden.[238] Anorexia in patients with cancer is due to the malignancy itself and is a common side effect of various cancer treatments. Chemotherapy and radiation often induce substantial nausea, altered smell or taste, vomiting, mucositis, gastric ulcers, or colitis. Certain chemotherapeutic agents, such as vinca alkaloids, can cause severe constipation, as do narcotics, which often are needed to control pain. Pain, malaise, and infections decrease oral intake and are frequent occurrences as a manifestation of the malignancy (bone marrow infiltration leading to immune dysfunction) and as a side effect of therapy. Antibiotics and antifungal agents can cause gastric irritation and diarrhea. Some agents, such as amphotericin B, also may lead to a tubulopathy with urinary losses of electrolytes, amino acids, and trace elements.

Supporting enteral nutrition with nasogastric or gastrostomy tube feeds in patients with cancer is complex. Mucositis and nausea decrease the tolerance for, and compliance with, nasogastric feeds, whereas poor healing and infectious complications affect gastrostomy feedings. Despite these drawbacks, enteral nutrition is much preferred over TPN for its beneficial effect on the gastrointestinal flora and on the structural and functional integrity of the gastrointestinal tract, which helps to prevent translocation of bacteria from the gut into the bloodstream with the associated risk of gram-negative septicemia. In contrast, TPN is associated with a high complication rate, such as central line infections and sepsis, and TPN cholestasis. Nonetheless, both types of nutritional support are used currently in children with cancer.

A variety of clinical studies have highlighted the importance of maintaining adequate nutrition in children with cancer. Malnourished children undergoing cancer chemotherapy have more infectious complications and experience more therapy-related toxicity than do children undergoing therapy with normal nutritional status.[82,151,169,307] In addition to the risks for adverse outcomes from the complications themselves, infections and delayed recovery between cycles of chemotherapy may lead to delays and dose reductions in subsequent cycles. In Brazil, malnutrition has been linked to an increased risk for relapse after treatment for acute lymphoblastic leukemia.[328] Particularly mild nutritional deficits have not been linked conclusively to a decreased rate of overall or event-free survival, however.[232,323] Currently, no national or international standards have been established regarding the nutritional management of children with cancer, and

nutritional support practices vary widely even among centers of the Children's Oncology Group. An urgent need exists for more conclusive studies to be performed on the impact of nutrition on toxicity, infectious complications, and, particularly, survival and on the different types of nutritional support for children undergoing cancer treatment.[168]

CYSTIC FIBROSIS

Malnutrition and growth failure are two common presenting signs of cystic fibrosis with pancreatic insufficiency. The failure to thrive seen in these patients is mostly a consequence of malabsorption; however, chronic infections with repeated exacerbations likely also play a role. Particularly crucial is the absorption of fat-soluble vitamins. The importance of vitamins A, D, and E for the development of the immune system and host defenses against infections has been reviewed earlier in this chapter. Later in life, many children with cystic fibrosis also develop insulin-dependent diabetes mellitus, which decreases further their ability to fight off infections.

Based on the multiple levels of interplay among growth, nutrition, development of the immune system, maintenance of immunologic functions, infections, and pulmonary damage, newborn screening for cystic fibrosis has been proposed. Nutritional deficits have been identified through screening in asymptomatic infants 2 months of age.[247] Early recognition and nutritional intervention not only may prevent failure to thrive but also strengthen host defenses and decrease or delay the severity of pulmonary colonization and infections, and ultimately improve overall survival.

Many studies have addressed this hypothesis and have found conflicting results. Data from the Cystic Fibrosis Foundation Registry suggest that early diagnosis is associated with a survival advantage.[170] Almost all studies confirm that early nutritional optimization through early diagnosis helps to avoid malnutrition and stunting, with benefits measurable in adolescence. Although some studies suggest fewer infectious complications, decreased colonization rate with *P. aeruginosa*, and improved pulmonary function tests in 20-year-old patients who were diagnosed through newborn screens,[2,288,302] other studies have been unable to confirm improved pulmonary function or improved survival through early intervention.[96,288] Malnourished *CFTR* knockout mice showed similar levels of cytokine production, inflammatory cells in bronchoalveolar lavage, and survival after challenge with *P. aeruginosa* as wild-type and nonmalnourished *CFTR* knockout mice.[324]

One also must bear in mind that early intervention is likely to lead to not only early and more targeted nutritional intervention, but also to more aggressive monitoring of pulmonary function tests and colonization with pathogens, with earlier and more aggressive antibiotic intervention. Although most data on newborn screening for cystic fibrosis point toward a beneficial effect of early diagnosis, the exact role of nutritional optimization in preventing colonization and infections needs to be studied in more detail.

OBESITY AND INFECTIONS

In industrialized countries, the burden of morbidity and mortality continues to shift toward chronic diseases. Research has focused on the effects of surfeit nutrition, especially obesity.

Numerous animal studies suggest that obesity increases the susceptibility for, and interferes with the ability to respond appropriately to, infection. When dogs were fed a high-calorie diet, a greater susceptibility to distemper virus and a shorter survival time were noted than in control animals fed a normal diet.[221] Chickens on a high-protein diet experienced greater mortality and morbidity from Newcastle disease agent than did controls that were fed normally.[40] Swiss mice made obese by high-fat diets were less resistant to infection by *S. typhimurium* and *K. pneumoniae* compared with mice fed a standard laboratory diet.[300] High-fat diets result in consistently depressed host resistance to tuberculosis and malaria in rats and to pneumococcal infections in chickens.[187] Erickson and associates[90,91] discovered that high levels of dietary fat, particularly polyunsaturated fat, suppressed the response of lymphocytes to T-cell mitogens. Animal models also have shown the adverse effects of excess cholesterol. Fiser and coworkers[102] found that hypercholesterolemic monkeys developed altered humoral and cellular immune function. Hypercholesterolemia also has been found to increase mortality rates in mice infected with group B coxsackieviruses.[182]

Epidemiologic and clinical data in patients support the concept of obesity-related disturbances in immune function. A cross-sectional study of 1129 preadolescent children suggests that obesity in children may be a predisposing factor to acute respiratory disease.[142] The study showed that children with a body mass index greater than or equal to 20 had twice as high a risk for acquiring acute respiratory infections compared with children with a lower body mass index. Chandra[57] examined the immunocompetence of obese children, adolescents, and adults. Approximately one third of the obese group showed a variable impairment of cell-mediated immune responses and a reduction of intracellular bacterial killing by neutrophils. The obese group had moderately low concentrations of serum zinc and iron, and therapy with these micronutrients for 4 weeks resulted in improvement in immunologic responses.

Obese adolescents and adults experience a greater risk for developing sepsis and wound infections after surgery than do lean control subjects.[97,300] Obese individuals exhibit a slight impairment of delayed cutaneous hypersensitivity responses, reduced helper T-cell populations, decreased lymphocyte response to mitogens, and reduced bactericidal capacity of neutrophils. Humoral immunity also may be affected by obesity. A randomized clinical trial of immunogenicity of hepatitis B vaccine found obesity to be a risk factor for nonresponse to the vaccine.[16] Obese subjects were 2.1 times more likely to be nonresponders than were lean subjects.

Obesity also is implicated in altering cytokine responses. Animal studies have shown that elevated levels of TNF are associated with insulin resistance; Boeck and associates[39] reported that obese patients with significant insulin resistance had little or no detectable plasma TNF compared with control subjects. Genetically obese rodents have attenuated production of TNF and IL-6 in response to lipopolysaccharide compared with their lean littermates.[179] These findings may reflect a refractive state in obesity in which production of TNF is down-regulated. Kolterman and colleagues[158] reported reduced release of migration inhibitory factor by stimulated lymphocytes from moderately obese, nonhyperglycemic subjects to 36 percent of the level of normal-weight controls.

A variety of cell-mediated immune responses have been evaluated in genetically obese animals. Reduced numbers of T lymphocytes in peripheral blood, spleen, and thymus were described in obese rats compared with nonobese littermates. The proliferative response of splenocytes to mitogens and natural killer cell activity also was significantly lower in the obese rats.[309] When obese mice were immunized with lymphoma cells, the cytotoxic response of spleen cells was markedly lower than that of lean controls.[60] When lymphocytes from obese and lean mice were sensitized in vitro rather than in vivo, however, they performed similarly. This observation suggests that the microenvironment of obese animals, which includes hyperlipidemia, hyperglycemia, and altered levels of insulin, glucagon, cortisol, and adrenocorticotropic hormone, may be responsible for impaired cellular responses.

Studies indicate that leptin, a protein known to regulate appetite and energy expenditure, may play a role in the altered immune responses associated with obesity. The molecular structure of leptin is similar to that of IL-6, whereas its receptor is a member of the class I cytokine receptor superfamily. Studies of rodents with genetic abnormalities of leptin or leptin receptor revealed deficits in cell-mediated and humoral specific immunity[192] and in macrophage proliferation and phagocytosis.[108,179]

In humans, genetic leptin deficiency is associated with increased susceptibility to infections. Administration of recombinant leptin to two children with congenital leptin deficiency restored mitogenic responses and cytokine responses of T lymphocytes. This result raises the question as to whether, and if so to what extent, low leptin levels contribute to the immune dysfunction seen in malnutrition. Animal data suggest that malnutrition-induced immunosuppression is reversed, at least partially, through administration of leptin. Weight gain in malnourished children has been shown to lead to an increase in leptin serum concentrations and cytokine production and proliferative responses in T cells. A study conducted in mildly undernourished children in Gambia found that, although leptin serum concentrations were low and correlated well with nutritional status, an association between low leptin levels and depressed immune function as measured by vaccine and delayed hypersensitivity responses could not be established in this population.[213]

The role of leptin in the clinically observed increased susceptibility to infections in obese patients is even less clear. Some researchers have hypothesized that increased serum concentrations of leptin in obesity lead to down-regulation of the leptin receptor and leptin resistance in the target cell. The net effect on a cellular level may be that of decreased leptin signaling in obesity and starvation.[192] Characterizing intracellular signaling pathways through leptin in malnutrition and obesity may help to understand better the influence of different nutritional states on leptin's effect on immune responses. It is hoped that animal and clinical studies will help to define the interrelationship among leptin, infections, and obesity or malnutrition and may aid the discovery of new targets for therapeutic interventions.

EFFECT OF NUTRITION ON RESISTANCE TO SPECIFIC INFECTIONS

When malnutrition diminishes resistance to infection or when infection aggravates malnutrition, the relationship between the two can be described as synergistic. In other situations, malnutrition impedes the multiplication of the agent more than it diminishes resistance of the host. In this case, the interaction between infection and malnutrition can be considered antagonistic. Vitamin A deficient patients have been reported to have a higher incidence of tuberculosis, bronchitis, otitis media, urinary tract infections, and bronchopneumonia. Protein deficiency leaves patients more susceptible to typhus, hepatitis, amebic dysentery, diarrheal disease, and tuberculosis. A reasonable assumption is that most deficiency states decrease host resistance to infection.

EXPERIMENTAL VIRAL INFECTION

The effects of malnutrition on experimental viral infection have been studied, but results have not been uniform. Most studies published before 1965 suggested that starved, fasted, or underfed animals are more resistant to viral infections than are normal animals and that the severity of viral infection is decreased.[28,76,246,298,299,336] Despite some contradicting reports, the concept that healthy animals are more susceptible than are their malnourished counterparts to experimental viral infection gained broad acceptance.[272] This antagonistic effect of malnutrition on viral infection was attributed by some investigators to starvation of the virus at the cellular level, with restriction of viral replication.[34,107]

The first studies to cast doubt on this prevailing concept were done by Woodruff and Kilbourne.[342] Their studies convincingly showed an increased severity of infection with coxsackievirus B3 in male albino mice that were subjected to sustained postweaning undernutrition. Severity of infection was proportional to the magnitude of malnutrition. Virus persisted in the heart, spleen, pancreas, and liver of severely malnourished animals, and mortality rates were highest in these groups. If a quantitatively optimal diet was fed to previously malnourished mice at the time of infection, they were protected from further viremia and death.

Further studies of coxsackievirus B in mice with specific nutrient deficiencies were done by Beck and Levander.[26] In these studies, a normally benign strain of coxsackievirus B behaved more virulently and induced cardiomyopathy in selenium-deficient and vitamin E–deficient mice. The increased virulence was caused by genotypic changes in the virus so that the sequence of the normally benign virus now resembles that of more virulent strains. These viral genotypic changes may be secondary to a decreased immune response in the host, leading to increased viral replication and risk for mutation. Alternatively, the increased oxidative stress in a selenium-deficient or vitamin E–deficient host may result in an increase in free radicals that could damage viral RNA directly and result in mutations. This study is the first example of a host diet having a direct effect on the genetic composition of a pathogen.

Overall, the broad consensus is that malnutrition leads to increased susceptibility, morbidity, and mortality from almost all viral infections. Dengue fever seems to constitute an exception, however: Malnourished children apparently have a lower risk of contracting dengue fever than do children with normal nutritional status.[150] The mechanism underlying this observation is unclear.

MEASLES

Measles may be the most extreme example of a childhood disease that is benign in industrialized populations but associated with high mortality rates in developing nations. This difference can be attributed to many factors, including vaccination patterns, concurrent disease, and available medical care. One of the most important causes of this differential mortality is varying nutritional status. Measles influences the nutritional and immune status by several mechanisms. Similar to any other febrile illness, measles contributes to severe reduction in food intake, vomiting, and increased metabolic losses. Measles also can produce a viral enteritis that results in a protein-losing enteropathy. Measles induces prolonged immunosuppression characterized by a decrease in the number of circulating T cells and impaired proliferation of T lymphocytes that has been shown to last for nearly 6 months.

A study in India revealed a close association between protein-calorie malnutrition and measles, with nearly 25 percent of children hospitalized for severe protein-calorie malnutrition reporting an episode of measles in the preceding 3 to 6 months.[36] In a prospective study done in the urban slums of Hyderabad, measles was associated with a weight loss of 2 to 12 percent of initial body weight. These children also were shown to have retarded growth 6 months after the initial infection, and 4 percent developed clinical signs of kwashiorkor or marasmus. All children with severe protein-calorie malnutrition in the post-measles period were undernourished before contracting the infection, which highlights the importance of nutritional status as a major determinant of the severity of nutritional deficiencies and growth failure that occur in the post-measles period.[36]

In the malnourished host, the epithelial surfaces are affected severely by measles, with eye and mouth involvement, laryngitis,

bronchopneumonia, and gastroenteritis. These children carry and transmit the virus three times longer than do children with normal nutritional status, and they are more susceptible to viral and bacterial superinfection. A study in Nigeria found a mortality rate of 26 percent, with respiratory complications accounting for more than 90 percent of deaths.[94]

Vitamin A deficiency may cause some of the severe manifestations of measles that occur in malnourished hosts. As mentioned previously, vitamin A is essential for maintenance of epithelial surfaces and for the synthesis of the ground substance of the corneal stroma. The striking occurrence of post-measles blindness in approximately 1 percent of all children with measles in developing countries demonstrates the crucial role of vitamin A metabolism in the malnourished host. Vitamin A supplementation in patients with measles in developing countries is associated with significantly decreased morbidity and mortality rates.[113,136] Patients given vitamin A supplementation showed increased serum concentrations of IgG and faster resolution of virus-induced lymphopenia compared with controls, suggesting an increased immunoresponsiveness to the disease. Hyporetinemia is associated with increased severity of measles,[113] but the relationship between cause and effect is unclear. In the case of malnutrition, exhaustion of hepatic stores accounts for decreased retinol and may predispose the patient to severe measles. Alternatively, hepatic stores of retinol may be mobilized inadequately during severe measles.

Vaccination is an extremely important tool in preventing measles and its associated infections. Often, tuberculosis and malnutrition are considered contraindications for administering measles vaccination. In contrast to the natural measles infection, the attenuated virus has no immunosuppressive effect and is unlikely to activate latent tuberculosis.[36] Several studies in undernourished children have shown high rates of seroconversion to the measles vaccine except in cases of severe protein-calorie malnutrition, indicating the efficacy of immunization against measles in undernourished children.[37,75]

BACTERIAL INFECTION

Bacteremia, the most dreaded infective complication of severe malnutrition, varies in incidence among different studies from 2 to 31 percent. Most commonly, bacteremia is caused by gram-negative enteric bacilli, especially *Salmonella* spp. and *E. coli*, and the common organisms that infect normal hosts. Malnourished patients with bacteremia have an increased risk for organ failure and mortality compared with patients with adequate nutrition. Malnutrition also may modify the response of the immune system to bacterial infections and increase the risk for development of long-term sequelae. In a study of Bangladeshi children with antecedent group A beta-hemolytic streptococcal throat infections, poor nutritional status was associated with an increased risk for developing rheumatic fever.[351] This finding is consistent with the steady decline of the incidence of rheumatic fever in the industrialized countries in the first half of the 20th century, before the advent of penicillin, which was due largely to better socioeconomic conditions and consequently better nutrition of large parts of the population.

More recent research has examined the association between nutrition and *Helicobacter pylori* infection. *H. pylori* infection often is acquired in early childhood. The prevalence of *H. pylori* infection has been reported to be higher in breast-fed compared with formula-fed children, and infection rates seemed to correlate with the duration of breast-feeding.[254] A study in Colombian children found an increased incidence of *H. pylori* infection in children with lower consumption of fruits and vegetables, vitamin C, and beta carotene.[116] Because *H. pylori* is associated with depressed gastric acid secretion and loss of the gastric acid barrier, it predisposes hosts to enteric infections and may exacerbate malnutrition. A study in Gambian children reported decreased weight for age in infants with sustained *H. pylori* infections compared with uninfected controls.[73] In contrast, an association between *H. pylori* and decreased adult height could not be shown conclusively in an industrialized setting and may not exist without established baseline malnutrition.[208]

Urinary tract infections usually are caused by bacteria that are physiologically present in the stool. Researchers hypothesized that certain foods, by means of altering the composition of the gastrointestinal flora, may affect the incidence of urinary tract infections. In addition, certain foods may alter the urinary pH and render the bladder milieu more or less conducive to bacterial growth. Based on these theories, dietary modifications have been proposed as preventive and therapeutic interventions. Consumption of berry juices or food items containing probiotic bacteria seems to decrease the incidence of urinary tract infections. Some studies showed a therapeutic effect of cranberries or blueberry products on established urinary tract infections, but a therapeutic effect of probiotic bacteria could not be established.[159] Breast-feeding has been shown to decrease the incidence of urinary tract infections in neonates.[189] Whether this effect is due to transferred immunity, alteration of the infant's immune system, local effects on the bladder milieu, or the bacterial composition of the stool is unclear.

PARASITIC INFECTION

Parasitic disease in humans has been estimated to affect more than 1 billion people, particularly children who are living in developing countries in Africa, Asia, and Latin America, where protein-calorie malnutrition is endemic. The same conditions of poverty, overcrowding, and inadequate sanitation that are associated with parasitic infections also are associated with individuals who are at the highest risk for having malnutrition. A particularly vulnerable time for children seems to be from the time they are 4 months old until they are approximately 5 years old. During this period, the transition from breast-feeding to a home diet occurs, and the exposure to disease in the environment increases. Parasitic diseases may reduce intake, interfere with absorption from the intestine, or cause increased losses of nutrients through gastrointestinal, urinary, or blood loss. Evidence suggests that parasitic infections in humans may reduce voluntary food intake by immunologic mechanisms that cause anorexia. Zwingenberger and associates[354] found elevated levels of TNF and cachectin in humans infected with *Schistosoma mansoni*, which normalized after treatment.

Schistosoma haematobium causes urinary schistosomiasis and is endemic in 52 African and eastern Mediterranean countries. *S. mansoni* and *Schistosoma japonicum* cause intestinal schistosomiasis. *S. mansoni* is endemic in Africa, the Middle East, and a few countries in South America and the Caribbean. Schistosomiasis has been implicated as a major contributor to the two most important forms of malnutrition in the Third World—protein-calorie malnutrition and iron-deficiency anemia. The larval and the adult stages of the infection can alter nutritional status by reducing food and nutrient intake, increasing nutrient excretion (mainly through blood loss, vomiting, and diarrhea), or altering nutrient metabolism within the body.[354]

S. haematobium infection is characterized by hematuria and proteinuria. Infected subjects had mean hemoglobin levels 0.9 to 1.3 g/dL lower than the levels of uninfected controls from their areas. The daily urinary protein losses in urinary schistosomiasis are an average of 1 g/day. *S. haematobium* infection may cause splenomegaly and hepatomegaly. Splenomegaly may be related to increased destruction of erythrocytes and can predispose to anemia, whereas hepatomegaly may alter nutrient metabolism.

Hepatomegaly and splenomegaly are reversible with adequate treatment.[354]

S. mansoni infection is associated with blood loss in the stool. Farid and associates[95] estimated the daily fecal blood loss in seven chronically infected Egyptian patients to be equivalent to 3.3 mg/day of iron. These iron losses are sufficient to produce anemia if persistent and if the daily intake of iron is inadequate. Severe infection with *S. mansoni* has been reported to result in significantly lower height for age and skin-fold thickness than milder or no infection. In particular, hepatosplenic involvement has been associated with lower insulin-like growth factor–I, lower insulin-like growth factor binding protein 3, and lower body mass index in affected children.[229] Increasing evidence indicates that mild and moderate infections also have deleterious effects on childhood nutrition. A double-blind, placebo-controlled trial in Brazilian schoolchildren showed that treatment of mild to moderate intensity *S. mansoni* infections was associated with improvement in height, weight, and body mass index in boys.[15]

The effects of *S. japonicum* are similar to those of *S. mansoni*; however, they tend to be more severe because *S. japonicum* produces 10 times as many eggs per worm pair as does *S. mansoni*. Studies from China report that the presence and intensity of *S. japonicum* infection are related directly to reduced arm circumference, skin-fold thickness, height, weight, and weight-for-height ratios.[352]

Hookworms and *Trichuris* spp. are associated with significant intestinal blood losses. Foo[105] showed that children with hookworm infection were on average 1 kg lighter and 2.4 cm shorter and had hemoglobin levels 1.1 g/dL lower. *Ascaris lumbricoides* infection has been shown to interfere with the absorption of fat and is associated with reduced weight and height and reduced serum concentrations of albumin and vitamin A and C.[128] In addition to reducing fat absorption, *Giardia intestinalis* and *A. lumbricoides* reduce intestinal lactase activity, resulting in lactose intolerance. *Strongyloides stercoralis* infection is associated with a protein-losing enteropathy resulting in significant hypoalbuminemia. To a lesser degree, *Giardia lamblia* has been associated with a protein-losing enteropathy without hypoalbuminemia.[305] Several more recent studies[64,209] have shown an association between infection with *Cryptosporidium parvum* and poor linear growth in children, with more marked and lasting effects seen with younger age at infection.

Malnutrition also may affect reinfection rates after successful eradication of enteral parasites. Follow-up of 585 Brazilian children from a poor neighborhood of São Paulo showed higher reinfection rates in malnourished children; the results maintained borderline statistical significance when corrected for confounding variables, such as income or literacy.[262]

Many parasitic infections are caused by crowded living conditions and poor sanitation. At the same time, parasitic infections place an enormous economical and social burden on societies in which they are widespread. In this context, two more recent studies reported parasitic infections as an independent risk factor for poor cognitive function, even when controlled for socioeconomic or nutritional status.[31,93]

DIARRHEAL DISEASE

Disease of the intestinal tract is the most obvious link between the mutually aggravating conditions of infection and malnutrition.[43] Historically, diarrhea has been a primary cause of childhood morbidity and mortality in developing countries. Poor nutrition increases susceptibility to diarrhea, and diarrhea contributes to deteriorating nutrition. Overcrowding and poor sanitation, conditions that coexist with poverty, also act synergistically with malnutrition to enhance the risk for and morbidity of diarrhea. Steps to improve nutrition and to provide oral rehydration therapy in acute situations can contribute to decreasing diarrheal disease and its effects.

The risk for developing diarrheal disease seems to increase proportionately with the degree of malnutrition. A study of children in the Sudan[160] showed that mild to moderate wasting was associated with a 9 percent increased risk for developing diarrhea, severe wasting was associated with a 34 percent increased risk, and very severe wasting was associated with a 50 percent increased risk compared with normally nourished controls. Gracey[118] has suggested that protein-calorie malnutrition predisposes an individual to having chronic diarrhea by causing changes in the intestinal mucosa. The changes include thinning of the gut wall, flattening of the intestinal villi, inflammatory infiltration of the lamina propria, and alteration of the enterocytes from columnar to cuboidal or squamous.

The gastric mucosa also is abnormal in malnourished states. Chronic gastritis has been noted in Indonesian children in association with a reduction of secretion of gastric acid. This condition may lead to heavy bacterial infestation of the upper gut. The changes seen in the gastrointestinal tracts of children with malnutrition may account for their altered susceptibility to various pathogenic organisms. A cohort study of children in Bangladesh revealed that shigellosis and cholera were common causes of diarrhea in severely malnourished children, whereas rotavirus was seen more often in well-nourished children with diarrhea.[84] Exposure to different pathogens depending on the socioeconomic status also is likely to play a role in this epidemiology.

The mechanisms of nutrient loss in diarrhea that lead to malnutrition include maldigestion resulting in insufficient breakdown of substrates, malabsorption characterized by inefficient uptake, and excessive wastage of nutrients from the body. Bile salt pool depletion and impaired micelle formation in malnutrition cause steatorrhea. Carbohydrate intolerance and malabsorption occur partly because of enterocyte damage and loss of brush border enzymes and partly because of bacterial overgrowth. All of these mechanisms act synergistically with the enteric pathogen to exacerbate the ill effects of malnutrition on the individual.[129,295]

In addition, nonenteral infections commonly coexist and worsen the prognosis. Nutritional restitution is vital and a key factor in survival. It may be initiated through oral feedings or by a modified parenteral route in children who are unable to tolerate oral feedings. Ahmed and coworkers[5] tested a protocol for treating severely malnourished patients with diarrhea that emphasized oral rehydration, immediate refeeding, routine micronutrient supplementation, and broad-spectrum antibiotics. A 47 percent reduction in mortality rates was observed compared with treatment with the conventional protocol, which focused on immediate parenteral rehydration and delayed feeding without routine micronutrient supplementation. Specialized formulas, such as lactose-free formulas or probiotics, may assist further in the treatment of diarrhea, malabsorption, and dehydration, although this approach may be difficult to implement in situations where meeting the most basic needs, such as clean water, is a challenge.[14,294]

Several studies have suggested that breast-feeding and immunization against major pathogens (e.g., measles, rotavirus, cholera) play a protective role in reducing the incidence of morbidity and mortality from diarrhea.[45,133,329] Steps such as improving water quality and availability, ensuring proper food and personal hygiene, controlling zoonotic reservoirs, and improving waste disposal and sanitation would minimize transmission of pathogens to individuals at risk.[129]

RESPIRATORY INFECTION

In the underdeveloped world, acute respiratory infections rank with diarrheal disease as a leading cause of morbidity and mortal-

ity. In developing countries, acute respiratory infections account for approximately 28 percent of childhood deaths and are even more frequent than diarrheal episodes. The annual incidence of pneumonia for children younger than 5 years of age in industrialized countries is 3 to 4 percent compared with 10 to 20 percent in most developing countries. This increased incidence of lower respiratory infection likely relates to the increased incidence of malnutrition in these areas.[283]

Malnourished infants are 10 to 20 times as likely to contract pneumonia as are children of normal weight for age.[66] Severe complications, such as empyema and bronchiectasis, also are more likely to occur in the face of nutritional deficiencies. A community-based study in the Philippines showed that malnutrition was the most important determinant of mortality associated with respiratory disease.[320] Such evidence supports WHO recommendations that in areas of prevalent malnutrition the use of antibiotic therapy be determined on the basis of clinical signs that can be recognized by minimally trained health care workers.[344] The presentation of a child with cough, chest indrawing, inability to drink, or a respiratory rate of more than 50 breaths per minute meets the requirement for antimicrobial therapy.

The World Bank Health Sector Review on Acute Respiratory Infections suggested that the most cost-effective interventions to reduce mortality from respiratory infections are case management, promotion of breast-feeding, vaccination against childhood communicable diseases, reduction of malnutrition, and pneumococcal vaccination. Pneumococcal vaccines such as PCV-7 may not be as efficient in preventing severe infections in Third World countries because the spectrum of the most prevalent strains differs in different parts of the world, and antigens of regionally common strains may not be present in vaccines that are directed toward marketing in industrialized countries. Reduction in malnutrition probably is the most important preventive intervention because mortality correlates directly with nutritional status. Other studies also have stressed the importance of vitamin A supplementation in malnourished children, improved access to health care services, adequate housing, and proper waste management in reducing the transmission and development of acute respiratory diseases in children.[133,318,319,325]

PROPHYLAXIS AND IMMUNIZATION

The link between malnutrition and infection must be considered from biologic and social viewpoints. Strategies for improving the control of disease in the developing world have been reviewed by Keusch and Scrimshaw.[154] Immediate interventions include immunizations, oral rehydration programs, promotion of breast-feeding, adequate weaning foods with increased protein, continued feeding during infection, nutrient fortification, and growth monitoring. Complex measures, such as improved sanitation and general education, are important long-term goals.

The effect of malnutrition on the ability of a child to establish immunity after vaccination varies for different types of vaccines and for different degrees and types of malnutrition. Several investigators have concluded that the immune response to immunization remains unimpaired in balanced mild to moderate malnutrition; however, severe forms of protein-calorie malnutrition, especially when maintained for long periods, may affect negatively the ability to seroconvert after immunization.[3,4,75,276] Response rates to tetanus and diphtheria toxoid are well preserved even in children with severe malnutrition. In contrast, impaired responses to measles, yellow fever vaccine, hepatitis, killed influenza A, and killed typhoid vaccine all have been described in moderate to severe protein-calorie malnutrition. Cell-mediated immune responses after BCG immunization in Indian schoolchildren correlated positively with adequate nutrition and waned more rapidly in malnourished than in normal individuals.[279]

Despite these concerns, most data on vaccine campaigns indicate that virtually every immunization program among populations with malnourished children has been at least partially successful.[312] Adequate studies to define the extent of immunization failure in malnourished populations have not been done. The route of vaccination also may play a role. Flo and associates[104] showed that malnourished rats, after receiving oral immunization with cholera toxin, had diminished levels of total IgA in intestinal fluid and an impaired ability to neutralize cholera toxin in vitro compared with well-nourished controls. This finding suggests that oral immunizations have a diminished capacity to evoke an immune response compared with systemic immunizations in severe malnutrition.

Further research may help to improve the immune response after vaccinations in children with severe protein-calorie malnutrition. Of greater importance, no data are available to answer important questions regarding the possible harmful effects of live viral vaccines in malnourished children.

CONCLUSION

Attempts to reduce the mortality and morbidity referable to infection in a malnourished individual must be predicated on a more comprehensive understanding of this process than that presently available; the need for further research is clear. In the interim, every effort must be expended to control infection and improve nutrition and living conditions throughout the world. Meeting these goals requires education, improvement in sanitation, improvement in prenatal care to reduce the incidence of prematurity, and either greater access to appropriate food supplies or education directed toward improving the use of food of appropriate nutritional quality when it already is available.

Mounting evidence indicates that fine-tuning nutritional interventions in premature neonates, critically ill patients, and children with chronic diseases helps to prevent or decrease the development of infectious complications of these various conditions. It is hoped that further reductions in morbidity and mortality rates can be achieved in these patients as more is learned about the various levels of interplay between nutrition and immune function.

The possible link between cardiovascular disease and the immunologic consequences of obesity, such as increased susceptibility to infections and a chronic inflammatory state, constitutes an interesting platform for new therapeutic intervention in the fight against the number one killer in industrialized countries. It also underscores the importance of fighting obesity early in life. Pediatricians will be asked to play an ever more important role in educating their patients to prevent overweight; helping obese children to stabilize or lose weight; advocating for healthier school lunches and sports; and treating the increasingly more prevalent consequences of obesity, such as hypertension, type 2 diabetes, and obstructive sleep apnea, which were previously rare findings in pediatric patients.

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CHAPTER 5

Fever: Pathogenesis and Treatment

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Fever is defined as a thermoregulated increase in body temperature above normal as the result of a coordinated response to a pathologic insult. Fever has two essential features: abnormal elevation in body temperature and being the end-product of a coordinated physiologic response. The first feature differentiates fever from normal regulated elevations in body temperature (e.g., elevations associated with the circadian rhythm), whereas the latter distinguishes it from conditions in which the regulatory mechanisms are overwhelmed or dysfunctional (e.g., heat stroke). In children, the pathologic insult most likely to result in fever is infection. A variety of other conditions, including malignancies and autoimmune diseases, also may result in this phenomenon, however.

NORMAL BODY TEMPERATURE

Although the general public and physicians alike often refer to "*the*" body temperature, the implication that a single number can represent the thermal state of the entire body is inaccurate. Depending on the site of measurement, body temperature may vary by 1° C or more.[34] These regional variations in temperature do not have a fixed relationship to each other. Although axillary temperatures are consistently lower than rectal temperatures, the absolute difference between the two varies greatly.[4] In addition, even in the healthy state, body temperature is not constant; it varies depending on numerous factors, such as time of day, level of activity, and phase of the menstrual cycle. Generally, clinicians have been most interested in the core body temperature, defined as the temperature of the internal organs of the trunk and head. Under normal circumstances, core temperature is higher than the temperature of more superficial tissues such as skin. Even within these two anatomic regions, temperature gradients exist, however.

The most widely accepted definition of normal body temperature is 37° C (98.6° F).[45] This number is derived from studies performed in the 19th century by Wunderlich.[82] He reportedly arrived at this figure based on the result of several million

measurements conducted in approximately 25,000 individuals. Other, more recent studies have found slightly lower mean temperatures in healthy individuals, despite the fact that these more recent studies are based on oral or rectal temperatures, whereas Wunderlich's studies relied on axillary temperatures.[28] Mackowiak and colleagues[46] determined the mean oral temperature in adults to be 36.8° C (98.2° F), with the upper limit of normal ranging from 37.2° C (98.9° F) at 6:00 A.M. to 37.7° C (99.9° F) at 4:00 P.M. Given the limitations imposed by the technology available at the time, however, perhaps the most surprising aspect of the value reported by Wunderlich is how closely it approximates these more recent determinations.

As noted previously, body temperature fluctuates depending on numerous normal physiologic factors. Core temperature shows a diurnal variation of 1° C, the nadir occurring in the early morning hours and the peak in the late afternoon.[36,46] After exercise and in the postprandial state, body temperature increases.[51,65] In addition, variations in the normal body temperature of women associated with the menstrual cycle are well described, with increases in baseline temperature occurring after ovulation.[15]

THERMOREGULATION

Humans, similar to other mammals, are homeothermic, indicating that they regulate body temperature within a narrow range despite wide variations in the ambient temperature. Regulation of temperature is mediated by a variety of physiologic (e.g., vasoconstriction, sweating) and behavioral (e.g., moving to a warmer environment, putting on additional clothing) responses.

The principal thermoregulatory area is located within the brain in the preoptic area and anterior hypothalamus. Although it frequently is conceptualized as a single center, no single neuronal structure seems to control all aspects of temperature regulation.[10] Rather, a complex interplay occurs among a variety of neural pathways, with the final result being the maintenance of the body's temperature within a narrow range. Regardless of the precise nature of central regulation, body temperature ultimately is a function of the balance between heat gain and heat loss.

Heat energy is a by-product of the inefficiency of the body's normal metabolic processes. It is this "waste" heat that renders the homeothermic state possible. During exercise, the increased metabolic activity in muscle tissue results in increased production of heat, leading to an increase in the body's temperature.[63] Shivering, an involuntary form of muscle activity, is the primary means by which the body generates additional heat under conditions of cold stress. Heat also may be generated by a process known as *non-shivering thermogenesis*. Originally described in rats, non-shivering thermogenesis has been found to occur in a variety of mammals, including humans. It seems to be of greater importance in newborns than in adults. Although this process has been shown to occur in a variety of tissues, brown adipose tissue seems to be the most important site for this phenomenon. Under the control of the adrenergic system, production of free fatty acid is increased, resulting in an uncoupling of oxidative phosphorylation and the production of large amounts of heat.[9,27]

Four mechanisms are responsible for heat transfer: radiation, conduction, convection, and evaporation. Heat loss owing to radiation occurs when heat is transferred directly between two objects not in direct contact. Conduction involves the transfer of heat energy between two objects in contact with each other. Convection is the result of the movement of a fluid or gas across the surface of the body (e.g., as the result of fanning). Evaporative heat loss occurs in association with the energy required to convert liquid to gas form.

Under normal conditions, radiation accounts for most of the body's heat loss. By contrast, conductive losses are smaller under normal circumstances. Conductive losses may become substantial, however, under conditions in which a substantial portion of the individual's body surface is in direct contact with a cooler object (e.g., an unclothed infant in an unheated bassinet). Convective losses are proportional to the amount of air moving over the body surface; these losses are greatest in windy conditions. Conductive and convective heat losses are particularly important in infants and children because of their relatively greater body surface area compared with that of adults. Evaporative losses occur when fluids such as sweat evaporate from the skin's surface. In addition, substantial evaporative losses are associated with respiration.

PATHOGENESIS OF FEVER

In the classic model of fever pathogenesis, exogenous pyrogens stimulate the release of circulating endogenous pyrogens, which act via prostaglandins to increase the set-point of the hypothalamic thermoregulatory center.[17] In this model, exogenous pyrogens are substances extrinsic to the body, primarily various bacterial microorganisms or the products of those microorganisms. Conversely, endogenous pyrogens are a varied group of proteins produced within the human body that share the intrinsic ability to induce fever. The first endogenous pyrogen was described originally more than 60 years ago and was derived from leukocytes, primarily granulocytes, and now is known as interleukin-1.[18] Numerous other cytokines that qualify as endogenous pyrogens, including tumor necrosis factor, interferon-α, interferon-γ, and interleukin-6, have been identified.[44,83]

A variety of alternative and complementary theories of fever pathogenesis have been proposed.[52] The actual mechanisms involved likely are more varied and complex than just described. A variety of intrinsically produced substances (e.g., antigen-antibody complexes) may act as "exogenous" pyrogens.[61] The classic model provides a reasonable framework, however, for understanding most of the observed phenomena associated with the febrile response.

Regardless of the precise pathogenesis, the height of fever seems to be a limit. Retrospective studies of hyperpyrexia in children have found that it is unusual for the body temperature to rise above 41.1° C (106° F), and it rarely rises above 41.7° C (107° F).[56,75] Children with temperatures exceeding this range almost always have an element of heat illness.

EFFECTS OF FEVER

Attempts to treat fever often are predicated on the assumption that fever has harmful effects and that reduction in temperature would abrogate such harm. The evidence with regard to these premises is mixed, however.

ADVERSE EFFECTS

Some animal studies have found that high fever may impair certain immunologic responses, including phagocytosis of staphylococci by polymorphonuclear leukocytes[5,21,22] and lymphocyte transformation in response to mitogens.[60] Whether these isolated in vitro phenomena observed in animal models are relevant to human infection is unknown.

Fever may cause seizures, a phenomenon observed most frequently in young children. The onset generally occurs in infants 6 to 30 months of age. Recurrence is common, occurring in approximately one third of children experiencing an initial febrile seizure. The primary adverse consequences of febrile seizures are the emotional distress experienced by patients and their families and the need for medical evaluation, which may involve invasive

testing and substantial expense. Febrile seizures do not cause brain injury and are not associated with subsequent intellectual or neurologic deficits.[20,77]

BENEFICIAL EFFECTS

Fever may be beneficial—by enhancing the host response to infection and by directly inhibiting the infecting agent. Several studies have illustrated that the immune system responds to mildly elevated body temperature by increasing migration of leukocytes, production of interferon, and lymphocyte transformation and phagocytosis.[2,7,60,62] Studies of bacterial infection in reptiles and fish have shown an increased survival rate in groups maintained at approximately 4° C (reptiles) and 2.5° C (fish) above baseline.[14,37] Kluger and Vaughn[39] showed that rabbits infected with *Pasteurella multocida* had improved survival rates at body temperatures of approximately 4.5° C above normal. Although these data are impressive, their clinical significance with regard to humans has yet to be determined.

Fever also may inhibit the growth and survival of some infectious agents. One potential mechanism for this inhibition is the decrease in serum iron and increase in ferritin that are associated with fever, coupled with the increased iron requirement of many bacteria at higher temperatures.[5,38] Fever therapy was used historically to treat neurosyphilis and gonococcal urethritis, correlating with more recent studies that show that certain gonococci and *Treponema* are eradicated at temperatures of 40° C (104° F) and greater.[12,35] Finally, growth of some pneumococci and viruses seems to be impaired at higher temperatures.[26,71,79]

Several studies have found that the treatment of fever with antipyretics is associated with adverse consequences, providing indirect evidence of a beneficial effect of fever. Ahmady and Samadi[2] reported that the use of aspirin in children with measles prolonged the duration of the illness and was associated with an increase in prevalence of respiratory complications and diarrhea. Other investigators have reported that the length of time to total scabbing in varicella was significantly longer in children treated with acetaminophen compared with children treated with placebo.[19] Several studies in animal models and in humans have found prolongation of viral shedding, depressed neutralizing antibody response, and increased nasal symptoms in association with the use of antipyretics.[24,31,74] Although these studies establish that an association exists between reduction of fever and adverse outcomes, they do not prove a causal relationship. The adverse effects possibly are mediated by some direct physiologic effects of antipyretics, rather than indirectly by their impact on fever.

CLINICAL THERMOMETRY

TYPES OF THERMOMETERS

For many years, glass thermometers containing mercury were the most common type of thermometer used to measure body temperature. This type of thermometer is reasonably accurate for most clinical purposes. Although they are still available, use of mercury-containing thermometers has diminished greatly because of environmental concerns about mercury exposure from broken or discarded thermometers. These thermometers have been replaced largely by digital thermometers or glass thermometers containing liquids other than mercury.

Electronic thermometers (often referred to as *digital thermometers*) previously were used primarily in the hospital and office setting. As their cost has decreased, they are used more frequently in the home as well. Electronic thermometers have the advantage over mercury thermometers of requiring a significantly shorter dwell time, that is, the time they must remain in situ to obtain an accurate reading. Hospital-grade electronic thermometers typically have two modes: monitor and predictive. In the monitor mode, these thermometers function similarly to mercury thermometers in that they must remain in place until equilibration occurs, a process that may require several minutes. In the predictive mode, a complex algorithm is used to estimate the final temperature based on measurements made during the first few seconds. Because the predictive mode produces a temperature reading within seconds, it is the mode used most often in clinical settings. Determinations of temperatures using these two modes have been found to correlate well.[22,53]

Infrared thermometers are a more recent addition to the clinician's armamentarium. Devices that determine the temperature by detecting infrared radiation emitted from the ear drum are used most frequently. Tympanic temperature should provide an accurate estimation of the core temperature because its blood supply is derived from the carotid artery. Additional advantages of this type of thermometer are its speed, acceptance by patients, and decreased risk of cross-contamination compared with oral or rectal thermometers. Studies of the accuracy of tympanic thermometers have yielded mixed results, with numerous studies finding tympanic thermometers inaccurate compared with mercury in glass or electronic thermometers.[13,49] Discrepancies seem to be particularly common in infants who are in the first few months of life.[68] Tympanic thermometers should not be used in young infants because of the importance of fever in making management decisions in these patients.[16]

Even more recently, the temporal artery (TA) thermometer has been introduced. TA thermometers use an infrared sensor to determine skin temperature as the device is passed across the forehead and temporal area. The site of highest measured temperature is assumed to represent that of the temporal artery. An algorithm is applied to the measured temperature to estimate the core temperature. Studies to date suggest that TA thermometer temperatures correlate significantly better with rectal and core temperatures than with temperatures determined by tympanic thermometers. The initial data also suggest that TA thermometers are more sensitive at detecting fever in children than in adults. TA thermometers do not seem to correlate well enough with rectal or core temperature measurements, however, to replace rectal thermometry in clinical situations in which accurate measurement of fever is crucial for making decisions about management. The accuracy of TA thermometer readings is adversely affected by sweating and may be affected by vascular constriction or dilation. In addition, data comparing TA and axillary thermometry are lacking.[25,73]

Several other types of thermometers have been developed. Among them are electronic pacifier thermometers, used for obtaining an oral temperature in infants. Although they are appealing in theory, this type of thermometer has the disadvantage of requiring a prolonged dwell time and has not been found to be sufficiently accurate to recommend its use.[57] Another approach to measuring temperature is the use of liquid crystal thermometers that are applied to the skin of the forehead. Results generally have been disappointing when these thermometers have been compared with more standard techniques.[40,66]

MEASUREMENT SITE

The most common locations for measuring body temperature are the mouth, rectum, axilla, and tympanic membrane. Because of the previously noted regional variations in body temperature, each of these sites has its own range of normal temperatures. The oral cavity historically has been the preferred site for measuring temperature in older children and adults. When taking a temperature orally, one should place the thermometer in the sublingual space because the blood supply for structures in this region

is derived from branches of the carotid arteries and should reflect the core temperature accurately. Younger children usually are unable to cooperate adequately to permit the use of oral thermometers. In addition, the oral temperature may be affected by recent ingestion of hot or cold liquids, and it may be altered by tachypnea.

Rectal temperatures are used frequently in younger children. The rectal temperature correlates well with the core body temperature. The rectal temperature may exceed the core temperature (using the pulmonary artery temperature as the reference standard), however, possibly because of the effects of bacterial activity in the rectum. The use of rectal thermometry has the disadvantage of causing the patient discomfort and is contraindicated in patients with neutropenia because of the risks of causing invasive infection via trauma to the rectal mucosa.

Axillary temperatures are appealing because of the ready accessibility of the axillae. Considerable variability occurs in the readings obtained, however, particularly in younger children. One should not rely on axillary temperatures, particularly in neonates and young children.

TREATMENT

As noted previously, fever may have numerous beneficial effects, convincing evidence of harm owing to fever is lacking, and treatment of fever may be associated with undesirable effects. Routine intervention to reduce fever is not warranted. Rather, physicians should individualize the decision to treat fever and the specific method chosen to do so.

INDICATIONS

Antipyretic therapy often is considered for children who have an increased risk of having febrile seizures, either because of age or because of a history of febrile seizures. Although treatment seems rational in this situation, studies of antipyretic therapy to prevent febrile seizures have failed to show its efficacy.[11,65,76] Even this indication should be considered relative rather than absolute.

Antipyretic therapy also should be considered for children with poorly compensated underlying cardiac or pulmonary disease, significant neurologic impairment, or sepsis, and for children with significant alterations of fluid and electrolyte balance. Definitive controlled trials to support these indications are lacking. Rather, the recommendations are based on the metabolic consequences of fever and their potential adverse impact on the underlying disease.

Perhaps the most frequent indication for the use of antipyretics is to improve the patient's comfort. Despite the absence of definitive studies to support this practice and the potential adverse consequences of using antipyresis, such an approach is reasonable in the absence of definitive evidence to the contrary. In some circumstances, improving the patient's comfort may enhance the ability to assess the seriousness of the patient's illness accurately.[6]

ANTIPYRETICS

A wide variety of antipyretic agents is available. In the United States, the drugs used most frequently for treatment of fever in children are acetaminophen and ibuprofen. Previously, aspirin was the antipyretic used most frequently. Aspirin has fallen into disuse for the management of fever in children, however, primarily because of its association with Reye syndrome, particularly when used in managing children with varicella or influenza.[30] In addition, aspirin has a variety of other adverse effects, including inhibition of platelet function, gastritis and gastrointestinal bleeding, and provocation of asthma exacerbations, although this third complication occurs more frequently in adults.[32,59,67] Aspirin has greater toxicity in situations of overdose than do acetaminophen and ibuprofen.

Each of these agents acts to restore normal body temperature by reducing the set-point of the temperature regulatory center in the hypothalamus. The specific mechanism of action seems to be interference with prostaglandin synthesis in the PAOH. When selecting among the available antipyretic agents, one should consider efficacy and potential toxicity.

Similar to aspirin, ibuprofen inhibits prostaglandin synthesis in a variety of tissues outside the central nervous system. It shares many of the toxicities associated with aspirin. One exception is that ibuprofen lacks the association with Reye syndrome.[58] Ibuprofen inhibits platelet function because of its effect on prostaglandin synthesis, but this effect is reversible with discontinuation of the drug, and platelet dysfunction is short-lived compared with the effect of aspirin.[54] Because prostaglandins are important to the integrity of the gastrointestinal mucosa, inhibition by ibuprofen may result in gastrointestinal upset and bleeding. Although ibuprofen has been associated with exacerbations of asthma in some children, the risk seems to be small and may not be greater than that associated with the use of acetaminophen.[41,42]

Acetaminophen has a lengthy track record of safety. When used in the usual therapeutic doses, it has few adverse effects. Acetaminophen inhibits prostaglandin synthase activity, but this action is inhibited by peroxide. Because peroxide is generated at sites of inflammation, acetaminophen has little anti-inflammatory activity. It also lacks the adverse gastrointestinal and platelet effects of aspirin and ibuprofen.

Recommended dosing of ibuprofen is 5 to 10 mg/kg every 6 hours as needed. Acetaminophen is administered at a dose of 10 to 15 mg/kg every 4 hours, but no more frequently than five times per day. An important note for individuals administering these agents is that a variety of over-the-counter combination medications contain one or the other of these agents. Co-administration may result in inadvertent overdosing.

In addition, recognizing that ibuprofen and acetaminophen come in a variety of formulations is important. Acetaminophen is available as infant drops (concentration 10 mg/mL) and children's liquid (concentration 32 mg/mL). Substitution of children's liquid for infant drops without adjusting the volume administered to reflect the difference in concentration may result in serious toxicity. Acetaminophen also is available in suppository form. Absorption varies, however, and is delayed compared with oral administration. In addition, the medication is not distributed uniformly throughout the suppository, resulting in potential dosing errors if the suppositories are divided before use.[8] The use of acetaminophen in suppository form should be discouraged.

The antipyretic efficacy of acetaminophen and ibuprofen has been the subject of numerous clinical trials.[33,78,80,81] These trials have shown uniformly that both are effective antipyretic agents. Ibuprofen seems to result in a greater decrease in temperature than does acetaminophen, however. In addition, the antipyretic effect of ibuprofen is more prolonged, not surprising in light of ibuprofen's longer half-life compared with acetaminophen. These observations were confirmed in a meta-analysis of trials comparing ibuprofen and acetaminophen.[55]

Acetaminophen and ibuprofen frequently are used in combination.[43] Use of alternating doses often is advocated by practicing physicians.[48] The pathways for metabolism of these drugs are distinct, and, theoretically, metabolism of one should not affect the metabolism of the other. Controlled trials documenting safety and efficacy of combination or alternating use are sparse, however. In the only such study to date, using alternate doses of acetaminophen (12.5 mg per dose) and ibuprofen (5 mg/kg per dose) every 4 hours was found to be associated with a more rapid

reduction in fever, lower mean temperature, and fewer caregiver days absent from work and infant days absent from daycare compared with use of either agent alone.[64] The groups assigned to a single agent received dosing that was either on the low end or infrequent, however, compared with usual practice in the United States.

Another approach to antipyretic treatment frequently employed is the use of a second antipyretic when the initial agent is judged to have resulted in an inadequate response. Theoretically, some individuals may have a better antipyretic response to one agent than another. Although the premise is reasonable, sequential use of acetaminophen and ibuprofen remains unproven in terms of efficacy and safety.

Acetaminophen and ibuprofen have been proven to be remarkably safe when used in the recommended doses. Although ibuprofen may be slightly more efficacious in producing and sustaining fever reduction, acetaminophen remains the antipyretic of choice because of its longer track record and more favorable side-effects profile. Because acetaminophen lacks significant anti-inflammatory activity, ibuprofen or another nonsteroidal anti-inflammatory drug may be preferred in febrile conditions for which anti-inflammatory activity is desired (e.g., juvenile arthritis). Limited data suggest that use of acetaminophen and ibuprofen in combination may be safe and more efficacious than is either agent alone. Prudence suggests, however, that combined therapy seldom is warranted for this generally benign condition. Perhaps most important, patients and their parents should be educated to the benign nature of fever and the lack of evidence to indicate that routine treatment, particularly complete suppression, is either necessary or beneficial.

EXTERNAL COOLING

The use of external cooling in the management of fever has a long history. Compared with standard antipyretics, sponging with tepid water is inferior in fever reduction at 2 to 3 hours, although sponging was found to reduce the temperature more quickly than did antipyretics in one trial.[1,3] External cooling without concomitant administration of antipyretics makes little sense from a physiologic standpoint, however. In a febrile patient whose temperature regulatory center set-point has not been reset by administration of an antipyretic agent, external cooling inevitably results in an increase in the body's heat-production mechanisms.

When used in conjunction with an antipyretic agent, the usual goal of external cooling is to reduce the body's temperature more rapidly or to a greater degree. Several studies have compared the use of external cooling combined with antipyretics with antipyretics alone. Results have been mixed; some studies showed no difference in efficacy of the two approaches, whereas others found combination therapy to be superior.[23,29,47,69,72] Even in the studies in which a difference was found, the superiority of combination therapy was shown primarily in the very early phase of treatment. In addition, the use of external cooling usually is uncomfortable for the patient. When external cooling is to be used, sponging with tepid water is preferred. Alcohol and solutions containing alcohol should not be used for this purpose. Absorption of alcohol vapors via the lungs may occur in sufficient quantities to produce toxicity and even death.[50]

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CHAPTER 6

Indigenous Flora

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The terms *indigenous flora* and *normal flora* describe the microorganisms that colonize the internal and external surfaces of healthy individuals.[113] Colonization is the presence of replicating microorganisms on or within a host, without evidence of the development of disease. The microbial agents that compose the normal flora are called *commensal*, *colonizing*, or *endogenous* organisms. They usually do not cause injury to the host and can be divided into categories of resident or transient flora.[29,159] Resident flora are organisms that are present routinely in a specified anatomic location. *Staphylococcus epidermidis* is a resident organism of the

skin, and *Escherichia coli* is a resident organism of the gastrointestinal tract. Transient flora are organisms that are present only temporarily in a certain anatomic location, such as *Staphylococcus aureus* on the skin or *Pseudomonas aeruginosa* in the gastrointestinal tract. Blood, cerebrospinal fluid, urine, bile, and synovial fluid are considered sterile fluids and have no normal flora.

Environmental factors, host characteristics, and microbial properties all can influence the composition of the indigenous flora. Disruption of the balance among these various factors can have a profound impact on the normal flora and their subsequent potential for pathogenicity. Pathogens are microorganisms that are able to cause disease in the host. Strict pathogens, such as *Neisseria gonorrhoeae* and the rabies virus, always are associated with disease and are not considered part of the normal flora. Organisms such as *P. aeruginosa*, *S. epidermidis*, and *Serratia marcescens* are opportunistic pathogens and do not cause disease except in immunocompromised hosts or in special circumstances that support their dissemination. Central venous catheters can become contaminated with *S. epidermidis*, resulting in a bloodstream infection. Similarly, after a dental procedure, transient bacteremia can cause viridans streptococcal endocarditis in a patient with an abnormal heart valve.

Facultative pathogens fall somewhere between strict and opportunistic pathogens. They have the capacity to cause disease in healthy individuals and compose much of the indigenous flora found in the body (e.g., *Streptococcus pneumoniae*, *E. coli*, and *S. aureus*).[149,159]

It is useful for clinicians to have a general understanding of the normal human microbial flora. First, familiarity with the organisms that compose the normal flora may help with the interpretation of culture reports, such as the isolation of diphtheroids from a peripheral blood culture. Second, when an infectious disease is present, knowledge of the indigenous flora can assist in determining the likely causative agents and provide a basis for determining the initial antimicrobial therapy. This knowledge is especially important because of the increasing number of immunocompromised individuals who are vulnerable to endogenous infections. Third, an understanding of the normal flora may assist in making the decision to withhold antimicrobial therapy when a culture report identifies an organism that is considered to be a pathogen but is not for that patient's particular clinical presentation. The isolation of *S. pneumoniae* from a throat culture of a patient with pharyngitis can be disregarded if one recognizes that this organism is a normal part of the nasopharyngeal microbial environment and an unlikely cause of pharyngitis. Finally, clinicians may prescribe broad-spectrum antimicrobial agents more judiciously if they have an appreciation for the role that indigenous flora play as part of the host's defense system.[138]

ACQUISITION OF INDIGENOUS FLORA BY THE NEWBORN

The fetus normally is in a sterile environment in the uterus. The newborn begins to acquire indigenous microbial flora during delivery. Maternal flora provide the initial source of colonizing organisms. Exposure to organisms from other people and environmental sources contributes to the formation of the neonate's eventual normal flora.[139]

The gestational age, mode of delivery, and type of feeding all can affect the formation of a newborn's indigenous flora. Premature infants requiring prolonged hospitalization have a delay in bacterial colonization and more frequently are colonized with hospital flora, especially *Klebsiella*, *Enterobacter*, and *Citrobacter* spp., compared with healthy term newborns.[49,56,63] Infant-to-infant transmission by the hands of health care workers is an important factor contributing to gram-negative colonization of premature neonates.[56] Infants born by cesarean delivery have delayed intestinal colonization with anaerobic bacteria, and gut colonization can remain altered for 6 months after birth.[21,68,106,144] The intestinal flora also are influenced by the newborn's diet. *Bifidobacterium* spp. and a few other anaerobic bacteria dominate the intestinal flora of breast-fed newborns, whereas infants fed formula have a more complex intestinal microbial flora.[6,13,41,109,156,183] As the infant gets older, the composition of the indigenous flora begins to resemble more closely that of an adult.

MECHANISMS OF COLONIZATION

The normal flora differ substantially among the various anatomic sites because of local barriers to colonization. Environmental conditions, such as moisture, pH, oxygen tension, and nutritional supply, influence the ability of microorganisms to establish residence.[55,113] In certain anatomic regions, mucociliary clearance, epithelial cell turnover, and the flow of secretions restrict colonization to specific species of microbes.[113] Additional barriers include specific and nonspecific immune factors, the production of lysozyme, local attachment-blocking proteins, and microbial competition.[37]

The primary mechanisms of bacterial colonization are adherence to epithelial cells, colonization of mucus, and attachment to other colonizing organisms.[37] Adherence of bacteria to epithelial cells occurs primarily by specific binding between microbial surface antigens (adhesins) and epithelial receptor molecules.[12,72,95] Oral bacteria colonize specific sites within the mouth. *Streptococcus salivarius* binds to the epithelial cells of the tongue and buccal mucosa, whereas *Streptococcus mutans* and *Streptococcus sanguis* bind to tooth enamel.[138,180] Normal flora also can be established by the colonization of the mucous layer without bacterial attachment to host cells, as shown in the intestinal tract.[37] Finally, microbes can attach to colonizing organisms through adhesin-receptor binding. This means is established most clearly in the formation of dental plaque.[180]

EXOGENOUS INFLUENCES ON THE NORMAL FLORA

An individual's normal flora tends to remain stable and consistent but can be affected by a variety of exogenous factors. Antibacterial soaps, topical antiseptics, and deodorants can temporarily suppress the skin flora.[14,74,93] Similarly, brushing teeth with fluoride toothpaste reduces dental microflora. Eating fresh fruits and vegetables may provide a source of transient intestinal colonization with *P. aeruginosa*.[96,134] Cigarette smoke has been found to alter the normal flora and increase the number of potential pathogens in the nasopharynx.[26,27] Viral infections can disrupt the normal flora and predispose the patient to development of bacterial superinfections.[32,50,52,61,70,71,107,132,161] Influenza virus infection increases susceptibility to bacterial pneumonia, otitis media, and bacteremia partly by facilitating the adherence of pathogenic bacteria to respiratory epithelial cells.[61,146,184]

Medications can influence the composition of the normal flora. Acid reduction therapy may permit bacterial overgrowth within the stomach and small intestine, resulting in deconjugation of bile salts and malabsorption.[42,67,92,141,164,166,181] Gastric acid suppression also may reduce the number of ingested pathogens needed to cause enteric disease.[181] Antimicrobial agents can alter significantly the indigenous flora and promote colonization with potential pathogens by eliminating susceptible commensals.[10,40,60,76,82,117,122,123] Such changes can lead to superinfections with overgrowing organisms.[5,23,108,185] Antibiotics can reduce the barrier effect of the normal intestinal flora, permitting *Clostridium difficile* to propagate and produce toxins, resulting in pseudomembranous colitis.[9] Antimicrobial effects on the indigenous

flora depend on the type of antibiotic, route, dose, and duration of administration.

Severe or chronic illnesses alone can cause changes in an individual's indigenous flora.[79,89,90,103] Hospitalized patients with severe illness are much more likely to develop pharyngeal colonization with gram-negative bacilli than are hospitalized patients who are physiologically normal.[89,90] Medical devices may alter the host's normal flora. Thomas and colleagues[165] showed that patients requiring placement of nasogastric tubes had significantly greater nasopharyngeal colonization with aerobic gram-negative bacilli compared with patients in a control group.

BACTERIAL COMPOSITION AT SPECIFIC LOCATIONS

SKIN

Because of the presence of moisture and sebum, most skin flora are associated with sweat glands (Table 6–1). Bacterial concentrations are highest on the face, on the neck, in the finger and toe webs, and in the axillae and groin.[115,116] The organisms occupy the most superficial layers of the epidermis and are found primarily around the hair follicles, although some are located deeper within follicles.[11,115,121] Although soaps and other skin cleansers

TABLE 6–1 Common Indigenous Flora at Various Anatomic Sites

Body Site and Type of Pathogen	Resident Flora	Transient Flora
Skin		
Opportunistic pathogens	*Corynebacterium*, *Staphylococcus epidermidis*, *Micrococcus*, *Peptococcus*, *Brevibacterium*, *Acinetobacter*, *Demodex folliculorum*	Viridans streptococci, *Enterococcus*, *Malassezia*
Facultative pathogens	*Propionibacterium acnes*, *Pityrosporum*	*Staphylococcus aureus*, *Enterobacter*, *Escherichia coli*, *Klebsiella*, *Proteus*, group A streptococci, *Candida*, *Trichophyton*
Eye		
Opportunistic pathogens	Coagulase-negative staphylococci, *Corynebacterium*, *Micrococcus*	*Bacillus*, viridans streptococci, *Propionibacterium*
Facultative pathogens	*Haemophilus*	*S. aureus*, *Streptococcus pneumoniae*
Mouth and Oropharynx		
Opportunistic pathogens	Viridans streptococci, coagulase-negative staphylococci, *Haemophilus*, non–group A beta-hemolytic streptococci, *Treponema*, *Veillonella*, *Porphyromonas*, *Prevotella*, *Lactobacillus*, *Peptostreptococcus*, *Bacteroides*, nonmeningococcal *Neisseria*, *Corynebacterium*, *Gemella*, *Granulicatella*	*Eikenella corrodens*
Facultative pathogens	*Fusobacterium*, *Streptococcus mutans*,* *Actinomyces**	Group A streptococci, *Lactobacillus*,* *Neisseria meningitidis*, *Kingella*, *S. pneumoniae*, *Moraxella*, *Candida*, cytomegalovirus, herpes simplex virus
Nose and Nasopharynx		
Opportunistic pathogens	Coagulase-negative staphylococci, viridans streptococci, *Corynebacterium*	Nonmeningococcal *Neisseria*
Facultative pathogens	—	*S. aureus*, *N. meningitidis*, *S. pneumoniae*, *Moraxella*
Stomach		
Opportunistic pathogens	—	*Streptococcus*, *Lactobacillus*
Facultative pathogens	—	*Helicobacter pylori*
Small Intestine		
Opportunistic pathogens	*Lactobacillus*, *Streptococcus*, *Veillonella*, *Prevotella*, *Porphyromonas*, *Bifidobacterium*	*Candida*, *Entamoeba coli*, *Endolimax nana*, *Iodamoeba bütschlii*, *Trichomonas hominis*, *Chilomastix mesnili*
Facultative pathogens	*Bacteroides*, Enterobacteriaceae, *Clostridium*	*Blastocystis hominis*
Large Intestine		
Opportunistic pathogens	*Bifidobacterium*, *Peptostreptococcus*, *Lactobacillus*, *Veillonella*, *Eubacterium*, *Fusobacterium*, *Prevotella*, *Porphyromonas*, *Enterococcus*, *Peptococcus*, *Ruminococcus*	*Candida*, *Corynebacterium*, *Pseudomonas*, *Mycobacterium avium* complex, *E. coli*, *E. nana*, *I. bütschlii*, *T. hominis*, *C. mesnili*
Facultative pathogens	*Bacteroides*, *Clostridium*, Enterobacteriaceae	*Aeromonas*, *B. hominis*, enterovirus
Anterior Urethra		
Opportunistic pathogens	*Corynebacterium*, coagulase-negative staphylococci, viridans streptococci, *Lactobacillus* (women)	*Mycobacterium smegmatis*, *Bacteroides*, *Fusobacterium*
Facultative pathogens	—	Enterobacteriaceae, *Enterococcus*, *Ureaplasma*, *Mycoplasma*
Vagina		
Opportunistic pathogens	*Lactobacillus*, *Streptococcus*, coagulase-negative staphylococci	*Enterococcus*
Facultative pathogens	*Gardnerella vaginalis*, *Mobiluncus*, *Prevotella*, *Actinomyces*	Group B streptococci, *Candida*, *Trichomonas vaginalis*

**Contribute to dental caries.*

substantially reduce the number of most surface bacteria, organisms within hair follicles and sweat glands quickly re-establish the normal flora.[74,93]

The normal skin flora are primarily of coagulase-negative *Staphylococcus* spp. (especially *S. epidermidis*), *Propionibacterium acnes*, *Corynebacterium* spp. (diphtheroids), and *Micrococcus* spp. *P. acnes* emerges at the onset of puberty, occupies hair follicles and sebaceous glands, and is a major contributing cause of acne. *S. aureus* and group A streptococci are not usual residents of the skin but may cause transient colonization.[152] *Acinetobacter* spp. are a common resident of the toe webs. Other gram-negative organisms, such as *Klebsiella*, *Proteus*, and *Enterobacter* spp. and *E. coli*, are uncommon findings and transient residents of the skin. The fungus *Pityrosporum* (*ovale* and *orbiculare*) normally inhabits the skin,[135,136] but *Candida* spp. do not.[34] Many other microorganisms come in contact with the skin, but because of the skin's dryness and presence of organic fatty acids, they are present only briefly and do not propagate.

Newborns

Within hours, a normal full-term infant's skin becomes colonized with microbes. In addition to typical normal skin flora, potential pathogens (group B streptococci, *E. coli*, *Klebsiella* spp.) frequently are recovered from cultures of the external ear canal.[110,125] Obtaining routine surveillance cultures in an attempt to identify infants at risk for acquiring invasive disease is not recommended, however, because their predictive value is limited.[87,110,125]

CONJUNCTIVAE

The mechanical actions of the eyelids and the washing effect of tears containing antimicrobial substances inhibit microbial colonization of the eye; however, normal flora do exist. The conjunctival flora probably originate from the eyelids and the nasolacrimal ducts. The organisms most commonly isolated are coagulase-negative staphylococci, *Corynebacterium*, *Micrococcus*, and *Propionibacterium* spp. Other isolates include *S. aureus*, *Haemophilus* spp., viridans streptococci, *S. pneumoniae*, and *Bacillus* spp.[102,129,138,177]

RESPIRATORY TRACT

The mouth and oropharynx contain several unique microbial habitats. Viridans (alpha-hemolytic and nonhemolytic) streptococci are the most prominent commensals and include *S. mutans*, *S. sanguis*, *S. salivarius*, *Streptococcus milleri*, and *Streptococcus mitis*.[97,138,159] The flora of the gingival crevice include a diverse collection of facultative and anaerobic organisms. More common isolates include *S. mitis*, *S. mutans*, *Actinomyces*, *Fusobacterium*, *Treponema*, *Veillonella*, and *Peptostreptococcus*. *Bacteroides* spp., *S. sanguis*, and *S. mitis* are the initial colonizers of the tooth surface. Dental plaque forms when *S. mutans* and *Actinomyces*, *Fusobacterium*, *Treponema*, and *Veillonella* spp., along with other organisms, attach to the initial bacterial cell layer and each other.[127,180] The buccal mucosa, tongue, and saliva are colonized most heavily by *S. salivarius*, *S. mitis*, and *Veillonella*, *Gemella*, *Granulicatella*, and *Lactobacillus* spp.[1] Asymptomatic pharyngeal carrier rates of group A streptococci in children range from 3 to 50 percent, with the highest prevalence rates associated with school outbreaks of pharyngitis.[35,45,53,78,130,154,157,178,182]

Compared with flora of the mouth and oropharynx, the microbial flora of the nose and nasopharynx are less diverse. Coagulase-negative staphylococci, viridans streptococci, *Corynebacterium* spp., and *S. aureus* are the most prevalent strains isolated. Nasal carriage rates of *S. aureus* average 20 to 35 percent, and the prevalence of methicillin-resistant *S. aureus* nasal colonization has been increasing, with estimates ranging from 0.2 to 22 percent, depending on demographic characteristics.[2,3,31,77,85,98,150,158,170] Colonization of children with *Neisseria*, *Haemophilus*, *Moraxella* spp., and *S. pneumoniae* is a common occurrence.

Pneumococcal colonization of the nasopharynx generally is transient, averaging in duration from 1 to 4 months.[51,66] Increased colonization is seen with younger age, overcrowding, attendance at a daycare center, winter season, and exposure to tobacco smoke. Nasopharyngeal carriage rates of *S. pneumoniae* average 40 to 50 percent in children and 20 to 30 percent in adults.[59] Nasopharyngeal colonization occurs even with the implementation of pneumococcal protein conjugate vaccine; several studies indicate a change in serotype distribution with an increased presence of drug-resistant strains.[54,83,86] Despite the presence of potential pathogens in the nasopharynx, no convincing evidence supports the use of nasopharyngeal cultures to predict the etiology of acute otitis media, pneumonia, or sinusitis.[58,173]

The sinuses and lower respiratory tract generally are assumed to be sterile. In actuality, bacteria from the mouth and nose probably reach these regions daily but are cleared promptly by local defense mechanisms.[138]

During the first week of life, the newborn's oropharynx usually is colonized by maternal vaginal flora, primarily *Lactobacillus* spp. and *Streptococcus viridans*.[28,119] These bacteria gradually are replaced by mouth flora from the mother and caretakers. *S. salivarius* and *S. mitis* predominate. Anaerobes, *S. mutans*, and *S. sanguis* are uncommon findings until teeth erupt.[97] *Ureaplasma* spp. and *Mycoplasma* spp. from the maternal vagina readily colonize the newborn's respiratory tract.[160] They are a suspected, yet still undetermined, cause of chronic lung disease in premature infants.[75,124,176] Enteric bacilli can be recovered from the throats of more than half of normal infants older than 2 months of age.[7]

GASTROINTESTINAL TRACT

The gastrointestinal tract consists of the esophagus, stomach, small intestine, and colon. The esophagus usually is sterile, and the stomach generally harbors only a few bacteria. The digestive enzymes and acid in the stomach destroy most swallowed organisms, or they pass promptly into the small intestine. Among the microflora of the stomach are acid-tolerant organisms, such as lactobacilli and streptococci,[147] and *Helicobacter pylori*, which survives within the mucous layer overlaying the gastric mucosal epithelium.[22,57,91] The worldwide prevalence of *H. pylori* colonization in children ranges from 10 to 90 percent.[162] Factors associated with colonization include lower socioeconomic status, household crowding, ethnicity, and household contact with carriers.[65,128,168] When the gastric pH is increased, nasopharyngeal and fecal-type flora may colonize the stomach.[181]

Low concentrations of *Streptococcus*, *Lactobacillus*, *Veillonella*, and rare *Bacteroides* spp. can be found in the upper small intestine.[17,30,44] Higher concentrations of the same bacteria plus *Bifidobacterium*, *Clostridium*, and Enterobacteriaceae are present in the lower small intestine.[18,114] Small bowel bacterial overgrowth can occur under conditions of gastric achlorhydria, a blind loop, or dysmotility. This syndrome may result in malabsorption and diarrhea.[94]

The colon contains a large and diverse population of microorganisms.[64,151] More microbes occur in this location than anywhere else in the body. For many years, because of inadequate techniques available for culturing strict anaerobic bacteria, *E. coli* was thought to be the principal resident of the large intestine. It has become apparent that the strict anaerobes outnumber the facultative microbes by 1000 to 1, with hundreds of different anaerobic species being isolated from the colon.[147] Molecular analysis of fecal

flora has expanded understanding of the microbial diversity found in the gastrointestinal tract.[48,175] *Bacteroides* and *Bifidobacterium* spp. comprise the largest percentage of fecal flora. Other major organisms in the colon include *Peptostreptococcus*, *Peptococcus*, *Ruminococcus*, *Enterococcus*, Enterobacteriaceae, *Prevotella*, *Porphyromonas*, *Fusobacterium*, *Clostridium*, *Lactobacillus*, and *Eubacterium* spp.

Newborns

The newborn's intestinal flora usually are derived from organisms within the mother's birth canal and from the newborn's environment. Diet can influence the composition of the infant's intestinal flora.[37,41,106,111] Full-term, breast-fed infants are colonized predominantly with *Bifidobacterium* spp. Formula-fed infants have a more complex intestinal flora in which *Bifidobacterium*, *Bacteroides*, *Enterobacter*, *Clostridia*, and *Enterococcus* spp. are prevalent.[6,13,41,109,156,183] Infants delivered by cesarean section have 6 months' delay in colonization with anaerobic organisms.[21,68,106] At 12 months of age, after the introduction of solid foods, all infants have intestinal flora that more closely resemble those of an adult.[41]

Intestinal colonization of very-low-birth-weight infants is delayed, and the development of anaerobic flora is diminished. Anaerobes, especially *Bifidobacterium* and *Lactobacillus* spp., are thought to protect the host against invasion by pathogens. Paucity of these organisms permits bacterial overgrowth with potential pathogens, especially *E. coli*, *Klebsiella*, *Enterobacter*, and *Citrobacter* spp. Researchers suspect that this aberrant intestinal colonization of premature infants contributes to the pathogenesis of necrotizing enterocolitis.[33,43,101,126]

GENITOURINARY TRACT

The endogenous flora of the genitourinary tract normally are confined to the distal portion of the urethra and the vaginal mucosa. The anterior urethra in men and women commonly is colonized with skin flora (coagulase-negative staphylococci, *Corynebacterium* spp., and streptococci) and may contain *Mycobacterium smegmatis* and *Bacteroides*, *Fusobacterium*, *Enterococcus*, and Enterobacteriaceae. The female anterior urethra also contains *Lactobacillus* spp. Asymptomatic colonization with *Ureaplasma urealyticum*, and to a lesser extent with *Mycoplasma hominis*, is a common event in sexually active individuals.[120,163]

The microbial flora of the vagina is influenced profoundly by hormonal factors.[99,100] During the first few weeks of life, estrogen from the maternal circulation creates in the newborn girl a vaginal environment conducive for the growth of *Lactobacillus* spp. This event is followed by development of a scant vaginal flora, containing mostly coagulase-negative staphylococci, *Corynebacterium*, and occasionally Enterobacteriaceae and *Streptococcus* spp. With the onset of puberty, lactobacilli again become the predominant organisms isolated. Coagulase-negative staphylococci and *Corynebacterium* spp. and various anaerobic streptococci also are common findings.[8,73,133] *Enterococcus*, Enterobacteriaceae, and *Candida* spp. are found less frequently.[8] *U. urealyticum* is present in approximately half of premenopausal women, and *M. hominis* is found in fewer numbers.[120,163] The vaginal colonization rate of group B streptococci in pregnant women is 5 to 35 percent.[4] Heavy colonization with *Gardnerella vaginalis*, *Trichomonas vaginalis*, and *Mobiluncus* and *Prevotella* spp. is associated with bacterial vaginosis.[153]

BENEFICIAL EFFECTS OF INDIGENOUS FLORA

It is generally recognized that pathogens first need to colonize the host before causing disease. The indigenous flora help to protect the host against infections through colonization resistance, also called *bacterial interference*.* Colonization resistance is accomplished by several mechanisms, including competition for nutrients, competition for epithelial cell receptors, production of toxins and bacteriocins, and stimulation of the immune response.[19,24,25,169,179] Suppression of the normal flora with antimicrobial therapy correlates with an increased susceptibility to candidiasis,[148] *C. difficile* colitis,[9] and *Salmonella* infection.[81] Use of probiotic agents, live microbial food supplements (e.g., *Lactobacillus*, *Bifidobacterium* spp.), helps restore the normal intestinal microflora during antibiotic therapy and inhibits the growth of potential pathogens.[36,69,143]

The endogenous intestinal flora seem to stimulate the cellular and humoral mucosal immune system.[16,131,167,174] This relationship influences the development of the newborn's immune system. Germ-free animals have underdeveloped, poorly differentiated lymphoidal tissues and low serum immunoglobulin concentrations. These animals have increased susceptibility to experimental challenges with pathogenic microbes.[47] The intestinal microflora also may affect the development of atopic disease and allergy.[20]

The normal flora provide some nutritional supplement for the host. The intestinal microflora ferment undigested carbohydrates into short-chain fatty acids. The short-chain fatty acids affect colonic epithelial cell transport and serve as an energy source for colonic epithelial cells, the liver, and muscle.[39,137,140,142] The intestinal flora also participate in the enterohepatic recirculation of biliary metabolites and the degradation of toxins and carcinogens.[62,114] In addition, normal flora microbes produce essential vitamins, such as vitamins K and B_{12}. Whether humans use these vitamins in any substantial manner is uncertain.[46]

ADVERSE EFFECTS OF THE INDIGENOUS FLORA

The indigenous flora can have unpleasant or even harmful effects on the host and are the source of intestinal gas and body odor. Oral flora are associated with dental caries and periodontal disease. In special circumstances, bacterial overgrowth of normal flora in the small intestine can result in malabsorption, diarrhea, and weight loss.[84,94] Other clinical conditions can create the potential for bacterial translocation, the invasion of indigenous bowel flora across the intestinal mucosa.[15,104] Intestinal toxins from endogenous bacteria may contribute to the encephalopathy, renal failure, and coagulopathy seen in hepatic failure.[37] Injury to the host from endotoxic shock may be caused to some extent by a hypersensitivity response induced by endotoxin from intestinal flora because germ-free animals are highly resistant to the effects of injected endotoxin.[159] Intestinal flora are speculated to cause cancer by metabolically activating carcinogens or by making carcinogenic products.[112] Finally, the intestinal normal flora may have a causative role in failure to thrive, arthritis, and autoimmune disorders.[37]

SUMMARY

The indigenous flora of humans are unique to specific anatomic locations. They are influenced by intrinsic microbial properties, host characteristics, and exogenous factors. Indigenous flora provide clear benefits to the host and potential adverse affects. Familiarity with the normal flora should help clinicians provide improved care for their patients.

*See references 25, 38, 80, 88, 105, 118, 145, 155, 171, 172.

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CHAPTER 7

Epidemiology of Infectious Diseases

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Epidemiology is concerned primarily with describing and explaining the occurrence of disease in populations. This chapter is a general review of epidemiology as it relates to infectious diseases important in pediatrics. Readers concerned with the epidemiology of a particular disease should consult the appropriate chapter for the relevant information. The principles and methods of epidemiology must be meshed with biostatistics (presented in detail in Chapter 266) and with information from other fields, including, but not limited to, clinical medicine, microbiology, pathophysiology, immunology, demography, and sociology.

The three types of epidemiology are descriptive, analytic or causative, and experimental. All three types are important in infectious diseases.

Descriptive epidemiology provides accounts of the health experiences of populations, including morbidity and mortality. It may or may not be used to support a hypothesis. The data are of two types—incidence and prevalence. *Incidence* is used to denote the numbers of new cases of or deaths from a given disorder that occurs in a defined population during a specified period. Sequential temporal comparisons often are used for assessing trends, and incidence data frequently are useful for setting public health priorities.

Prevalence is used to denote the number of cases of a disorder in the population (or sample) at a single moment in time. Prevalence data are applicable largely to chronic disorders, such as diabetes mellitus, tuberculosis, or human immunodeficiency virus (HIV) infection, and have no utility when applied to acute disorders, such as measles or infection with respiratory syncytial virus. In addition, in studies of infectious diseases, the prevalence of serologic markers may be used to estimate the immune status of a population or the proportion of a group that has had previous exposure to an infectious agent. Examples that have been useful for vaccine development include meningococcal infections[13] and *Haemophilus influenzae* infections.[2] For certain infections such as tuberculosis and diphtheria, skin testing may serve as a substitute for serologic testing to estimate previous exposure to the agent (or to a vaccine).

Analytic, or *causative*, epidemiology searches for clues to the cause of disease. It is based on the principle that disease does not occur at random in the population and classically considers time, person, and place. In other words, differences exist between individuals who acquire a given disorder and individuals who do not. Identification of these differences may lead to ascertainment of causation, inferences about pathogenic mechanisms, or means of control. These differences may be inherent in the individuals themselves and include biologic characteristics (e.g., hereditary, such as race; acquired, such as immunity) and lifestyle. They may be external and consist largely of environmental risk factors for disease, including various factors that influence the likelihood of exposure to an agent, such as geography, weather, contact with vectors (including other humans), and social and economic conditions. In studies of infectious diseases, considerable overlap exists between descriptive and analytic epidemiology because differences in the distribution of disease by person, place, and time often are obvious in descriptive data and provide clues for further study. Analysis of data may lead to the development of useful hypotheses for studies that then generate new data.

Analytic epidemiologic studies of infectious diseases and studies related to other types of conditions generally fall into three categories. The least frequently used, but nonetheless useful, category is the cross-sectional study. Such studies can be conducted in one of two ways. One might examine apparently comparable populations with differing prevalence rates of a given infection for characteristics that might explain these different rates. Alternatively, if a disorder is sufficiently common, one can determine rates of disease in individuals with and individuals without a suspected risk factor. A classic example is the relationship of sickle-cell trait to resistance to *Plasmodium falciparum* malaria, which was examined in both ways.[1] In studies of variations in the prevalence of the sickle-cell gene in Africa, researchers noted that the trait seemed to be more prevalent in areas with a high incidence of malaria. Based on this observation, other studies were conducted and showed that in hyperendemic areas, individuals with the sickle-cell trait might have some resistance to malaria, permitting selective survival in these areas. This observation led to additional studies of the mechanism of this phenomenon, including examinations of the roles of blood groups and other characteristics of erythrocytes that influence the risk of acquiring malaria.

Another type of causative or analytic epidemiologic study is the *longitudinal cohort study*. In this type of study, incidence of infection and routes of transmission may be determined by examining groups of individuals with differences in exposure, which may include timing, duration, intimacy of contact, or disparate sources or mechanisms of potential transmission. The classic studies of the transmission of group A streptococcal infection in military recruits are excellent examples. One particular finding was that transmission of infection from individuals with streptococcal pharyngitis to others in military barracks occurred at rates inversely related to distances between bunks, which established that transmission occurred largely by intimate respiratory contact with droplets containing hundreds or thousands of organisms, rather than by airborne droplet nuclei.[34] Other studies showed that fomites, naturally contaminated with streptococci, did not contribute significantly to the transmission of infection.[26] Studies by Goldschneider and colleagues,[13] by following a cohort of Army recruits at a training camp, showed that recruits without bactericidal antibody against the prevalent strain of meningococcus were susceptible to infection.

Studies of the transmission of staphylococci to newborns are another example of prospective epidemiologic observation. In the 1950s, outbreaks of staphylococcal disease, sometimes severe, occurred in newborn nurseries. In an effort to determine how these organisms were transmitted to infants by personnel or other infants, researchers investigated nurseries with persistently high rates of colonization of infants.[23] Two types of prospective cohort studies were conducted. One consisted of instituting measures that prevented transmission by all but one or two routes, permitting assessment of the importance of those routes in transmission. The other method was the reverse: Some suspected routes of transmission were blocked, and subsequent colonization of infants was monitored. These studies showed that transmission of organisms from personnel who were carriers or from previously colonized infants occurred primarily via the hands of personnel.

Another type of analytic epidemiologic study useful in infectious disease is the *case-control study*. In cohort studies, the investigator compares individuals who are exposed or not exposed to a given agent. The outcome measure of the study is infection or no infection. In contrast, in a case-control study, the samples being studied comprise individuals who have the infection com-

pared with similar individuals who do not have the infection. From individuals in both groups, historical data about previous exposure are obtained. In a case-control study, the investigator starts with diseased individuals and searches for exposure; in a cohort study, the investigator starts with exposure and follows subjects forward in time for development of the outcome. Case-control studies can be used to show a vaccine's effectiveness in actual field conditions after it has been licensed. Rather than having to show efficacy by following many thousands of volunteers, Shapiro and colleagues[31] showed the efficacy of pneumococcal vaccine by comparing 1054 people with invasive pneumococcal infections with 1054 matched controls, looking at the rate of vaccination in each group. Of the case patients, 13 percent had received pneumococcal vaccine, whereas 20 percent of the controls had received the vaccine (effectiveness was 47 percent for all patients and 56 percent for serotypes represented in the vaccine).

Case-control studies are of particular utility when the disease is rare because a cohort study might require an unwieldy number of subjects. A disadvantage of retrospective studies is that they do not provide an estimate of the risk or rate of disease occurring after exposure (i.e., the proportion of exposed or of unexposed individuals in whom the disorder in question actually did develop).

A third type of epidemiologic study useful in infectious diseases is an *experimental* study—*the clinical trial*. Clinical trials are actually a special kind of cohort study in which the investigator determines which subjects are exposed to an intervention. Clinical trials generally are used to determine the efficacy of preventive or therapeutic measures. As such, they often require previous information from analytic epidemiologic studies and from other basic and clinical studies. Sometimes, studies that are, in effect, clinical trials may provide useful information regarding the cause or routes of transmission of an infection. If a vaccine composed of a single purified antigen is associated with elimination of the disease, this information provides strong evidence that antibody directed against that antigen is protective.

To assess the efficacy of either therapeutic or preventive measures, experimental epidemiologic studies require comparison groups of individuals who do not receive the measure in question. Generally, these individuals are given an older agent, an alternative agent, a placebo, or an agent that would have no effect on the infection in question. Having contemporaneous controls is especially important for diseases that have significant year-to-year variation in incidence, to ensure that a decrease in incidence after introducing an intervention was not just a natural event.

An important component of a controlled clinical trial is that treatment and control groups be as similar as possible in characteristics that may affect the outcome. Clinical trials designed to assess the efficacy of either therapeutic or preventive measures must take into consideration many such factors, which usually include age, sex, socioeconomic status, co-morbid diseases, and likelihood of exposure. In any trial, an effort should be made to balance treated and untreated subjects in terms of recognized factors. Not all variables that may lead to bias in the results are recognized in advance, however. To try to ensure as far as possible that these characteristics are distributed approximately equally between the two groups, the process of randomization almost always is necessary. In addition, true randomization must occur; methods involving odd-versus-even record numbers or birth dates, alternate days of the week, and the like are inappropriate. An optimal method is a system of random numbers, as published in most textbooks of biostatistics or available as computer programs. Randomization avoids selection bias, best defined as underlying differences between the treatment and control groups, whether internal (inherent) or external (e.g., the likelihood of exposure). Randomization ensures only lack of bias because one has no assurance in a small clinical trial that unrecognized confounders are distributed equally among groups being compared. If the size of the sample is large, however, unequal distribution, although not impossible, becomes unlikely. Chapter 266 provides a more extensive discussion of types of bias.

Although the principles discussed here and in Chapter 266 apply to all epidemiologic studies, in studies of infectious diseases, three additional factors contribute uniquely to who is and who is not affected: (1) The cause is a specific external agent (the infecting organism); (2) transmission of the organism to the host is required; and (3) certain host factors, such as immunity to infection or disease, may affect the outcome. Recognition of these factors (the infecting agent, transmission, and immunity) evolved gradually over many years.

HISTORICAL PERSPECTIVES

Epidemiology evolved from the study of great epidemic diseases such as plague, cholera, and smallpox. The periodic waves of these diseases, which were associated with high mortality rates, stimulated the first serious efforts to explain the occurrence of disease on the basis of factors other than supernatural or divine forces.

Fundamental to such explanations was the concept of contagion. This factor long had been implicit in attitudes toward victims of leprosy, as exemplified by such early Christian practices as conducting antemortem funerals for lepers, who then were given a bell and cup and forbidden further human contact or, more drastically, were buried alive or burned at the stake.[28] The English physician Thomas Sydenham (1624-1689) introduced laudanum (derived from opium) as a painkiller; recognized the efficacy of Peruvian bark (quinine) in malaria; and revived the Hippocratic idea of "epidemic constitutions" (of atmospheric nature), which by grafting onto existing illness, gave all concurrent illnesses the character reflecting the then prevailing "constitution." These views persisted in colonial America, where they were expounded by such eminent individuals as Noah Webster (of dictionary fame) and Dr. Benjamin Rush of Philadelphia.[37]

Nonetheless, by the mid-18th century, the theory of contagion had gained acceptance for particular diseases, including measles, syphilis, and smallpox. The theory is alleged to have been exploited in an early act of biologic warfare: Massachusetts colonists reportedly presented the blankets of smallpox victims as gifts to the Indians, who then suffered a decimating epidemic.[9a]

The true origin of the concept of immunity is uncertain, but it was applied first in relation to smallpox. Variolation (inoculation of young people with lesion material expected to induce modified, but immunizing, disease) was practiced in China in the 11th or 12th century and in England and the American colonies in the early 18th century. Also popular in rural England at this time was the theory that cowpox, the minor disease acquired from afflicted cattle, induced immunity to smallpox. This theory was verified by Edward Jenner (reported in 1798) and resulted years later in general acceptance of cowpox vaccine (vaccinia) to protect against smallpox.

The germ theory of disease was stated explicitly in 1855 by John Snow, an English anesthesiologist who took up cholera epidemiology as an avocation. Snow argued that the causative agent of cholera was a living cell that multiplied with great rapidity but was too small to be seen under the microscopes then in use.[32] Louis Pasteur (1822-1895) formally validated the germ theory by showing that the microorganisms responsible for fermentation were not generated spontaneously but came from the air.[25] On this basis, Joseph Lord Lister revolutionized surgery by using carbolic acid to combat atmospheric germs and minimize "putrification" in surgical procedures.[30]

In Pasteur's wake, bacteria were cultured with great frequency from ill individuals and often were identified erroneously as causal agents. Robert Koch (1843-1910), who first isolated the bacterial causes of tuberculosis and cholera, also was the first to introduce scientific rigor into the proof of primary causation. His famed "postulates," to be satisfied before a causal relationship between a bacterium and a disease could be accepted, required that (1) the presence of the agent be shown in every case by its recovery in pure culture; (2) the agent not be found in cases of other disease; (3) the agent, when isolated, be capable of reproducing the disease in experimental animals; and (4) the agent be recovered in pure culture from such experimental disease.[12]

Koch's postulates since have been modified, largely to meet problems posed by viruses. As obligate intracellular parasites, viruses cannot be "cultivated in pure culture." In addition, they often are host-specific and do not produce disease in an animal model. Other considerations that were invoked as elements of proof included the significance of recovery of the agent from diseased tissues, the demonstration of an increase in titer of specific antibody in temporal relation to the disease, and, most conclusive, the specific preventive effect of vaccines containing the viral antigen.[16] One further situation not recognized by Koch is that infections with true pathogens do not always cause disease. We now recognize pathogenicity (defined as the proportion of infections that result in disease) as an important characteristic of infectious disease agents.

CAUSE OF DISEASE

GENERAL CONCEPTS

Causation of infectious diseases is defined in terms of the *primary cause* and *contributing factors* (or secondary causes). The former is the specific microorganism (the agent of disease) without which the particular disease cannot occur. Contributing factors affect the likelihood that infection will occur and help determine that disease will result, given infection. Identification of the causative agent may lead to the development of effective means for providing specific protective immunization (e.g., diphtheria and tetanus toxoids, vaccines against polio and measles). Finding the cause of a disease also may lead to other means of control. An example is the discovery of *Legionella pneumophila* as the cause of legionnaires' disease. The discovery of the organism led to the understanding of how the disease is transmitted—via aerosols from cooling towers, which serve as reservoirs for the bacteria.[8,22] Disinfection of these towers has helped to control legionnaires' disease.

Infection and disease are not synonymous, although infection is necessary for disease to occur. *Infection* denotes colonization, multiplication, and completion of the entire pathogenetic process of the organism in the host, usually including induction of an immune response, but without necessarily producing recognizable pathologic and clinical manifestations. *Disease* is present when pathologic and clinical changes occur with infection. There are many examples of infections that may be either asymptomatic or the cause of serious disease in the host, such as poliomyelitis and mumps. When disease occurs, it may vary in severity among infected individuals. Some infections produce full-blown disease in all infected susceptible individuals; measles is an example. Simple *colonization*, in contrast to infection and disease, is a state in which the organism parasitizes the host at an appropriate site, replicates, and may persist, but it fails to proceed further with the processes of infection and disease, including induction of immunity. The *carrier state*, in which the organism persists over time and can be infective for others, may occur after colonization, infection, or disease. Examples of organisms that behave in this way include group A streptococcus and *Neisseria meningitidis*.

Many contributing factors, largely related to the host and to the conditions of exposure, determine whether colonization occurs and whether the subsequent processes of infection and disease occur. These contributing or risk factors are many and varied, and from the standpoint of the host may include, but are not limited to, age, sex, race, immune status, genetic constitution, and general state of health, including underlying diseases. Similarly, contributing factors unrelated to the host may include climate, the presence of vectors, quality of sanitation, intimacy of exposure, and socioeconomic conditions. These contributing factors vary among infectious diseases and are discussed in chapters about specific infectious agents.

AGENT FACTORS

What characteristics of living parasites are significant epidemiologically? Properties directly important to the occurrence of disease are properties that relate to perpetuation of the agent as a species, properties that govern the type of contact required to infect humans, and properties that determine the production of disease. Also important are characteristics useful in classification and specific identification of agents. Some important characteristics are *intrinsic*, in that they can be described after appropriate direct examination of the agent. Others can be described only on the basis of the behavior of the agents in the host; they are *host related*.

Intrinsic Properties

Precise classification and identification of agents are basic to the specific recognition of infections and related disease. Both depend on intrinsic properties, including morphology (which alone provides the basis for identifying most higher parasites), chemical composition (the type of nucleic acid being important in viral classification), and antigenic character. The last is central to specific identification of agent isolates and antibodies induced by infection. Requirements for growth or replication provide keys to the identification of some bacteria (e.g., sugar fermentation) and many viruses that replicate optimally or only in cultures of certain types of cells incubated at specified temperatures. Rhinoviruses replicate best in human diploid cells incubated at 33° C.

In recent years, rapid development of species detection and classification by genome identification has occurred. The use of whole-genome analysis, molecular analysis of gene fragments, and various methods of polymerase chain reaction has resulted in reclassification of some microorganisms and of rapid identification of pathogens, including microorganisms that cannot be cultivated by conventional means. Developments in this field are expected to shift the clinical microbiology laboratory from morphologic and biochemical tests to molecular tests in the coming years.

Several intrinsic properties relate to transmission and long-term survival of infectious agents. Persistence in the free state outside the host depends on requirements for replication (viruses replicate only within the cells of their host, whereas the nutrient requirements of bacteria often exist in food or milk) and on viability under natural conditions of temperature, moisture, and radiation. The ability of agents to persist determines whether transmission requires direct contact, as with influenza viruses, or can involve indirect mechanisms operating over longer periods. Examples include polioviruses, typhoid bacilli, and the bacterial cause of legionnaires' disease.

The spectrum of animals and arthropods that an agent can parasitize (the host range) helps determine the possibilities for successful links in the transmission and reservoir mechanisms. The broader the range, the greater the possibilities. Agents that use arthropod vectors include St. Louis encephalitis virus and

Borrelia burgdorferi, the cause of Lyme disease. The former can infect many avian and mammalian species and a wide range of mosquitoes, whereas the latter is restricted to a few tick species. Among agents requiring no vector, many infect only humans (diphtheria bacillus, the meningococcus, and measles virus), whereas others have multiple natural hosts (rabies virus, most of the *Salmonella* group of bacteria).

Elaboration of exotoxins is an intrinsic attribute of many bacteria and contributes in varying degrees to disease pathogenesis and indirectly to immunity in many infections. Another attribute, which can operate in two opposing ways, is susceptibility to chemotherapeutic agents or antibiotics. Successful treatment may shorten the period of communicability, as in group A streptococcal and *Bordetella pertussis* infections, but it may lead to relaxed precautions against infection; syphilis and gonorrhea are notable examples.

The instability of some intrinsic characteristics as a result of the emergence of genetically different populations because of mutations, selective pressure, gene or plasmid transfer between bacteria, or genetic recombination can be important. One example is the resistance to chemotherapeutic or antibiotic agents that may result from selective pressure (the probable explanation for the rapid acquisition of multiple antibiotic resistance by gonococci and in HIV or plasmid transfer of resistance to antibiotics among enteric bacteria). Antibiotic resistance is of increasing importance, as exemplified by the appearance of multidrug-resistant *Mycobacterium tuberculosis*, penicillin-resistant pneumococci, and methicillin-resistant staphylococci.

Change in antigenic character can diminish the effectiveness of immunity and complicate specific recognition of infection. Influenza A virus is the classic example, with periodic major changes (shift) occurring in either or both crucial surface antigens, hemagglutinin and neuraminidase, associated with pandemic disease and progressive minor changes in hemagglutinin in the interpandemic period. Finally, the emergence of new diseases, such as St. Louis encephalitis, which first affected humans in Paris, Illinois, in 1932, or the appearance of a known disease in a new reservoir, possibly exemplified by the emergence of West Nile virus in North America, can be the result of adaptation of the agent to a new host.[3,4]

Host-Related Properties

Some epidemiologically important properties of infectious agents can be defined only with reference to specific hosts. Such properties include infectivity, pathogenicity, virulence, and immunogenicity.

INFECTIVITY

Infectivity (ability to invade and multiply in a host) is measured conceptually in terms of the minimal number of infective particles required to establish an infection. This number, which can vary from one host to another and within the same host, depending on the portal of entry, host age, and, in some cases, medications, can be determined only experimentally. Except for benign agents such as rhinoviruses or vaccine strains of polioviruses with which challenge of human volunteers is permissible, the infectivity of agents for humans must be inferred from the facility with which they spread in populations or, more directly, from the frequency with which infection develops in exposed susceptible individuals within a reasonable incubation period (*secondary attack rate*). Some researchers have used prisoners to study infectivity of *Salmonella* and *Shigella*, but the ethics of such studies has been questioned. Measles, varicella, and polioviruses are highly infective because they require few infective particles to cause infection; rubella, mumps, and rhinoviruses have intermediate infectivity; and typhoid and tubercle bacilli have low infectivity. Infectivity and pathogenicity may vary among strains of the same organism. The infectivity of group A streptococci is related directly to the amount of M protein in the cell wall, and strains of *Staphylococcus aureus* that appear identical in the laboratory may differ strikingly in infectivity and virulence. Additionally, some evidence indicates that strains of influenza A may vary in infectivity and virulence independent of preexisting immunity in the host. Contemporary studies show that variation in certain genes that are not ordinarily expressed in the clinical diagnostic laboratory are responsible for these variations in infectivity.

PATHOGENICITY

Pathogenicity (ability to induce disease) is measured in terms of the proportion of infections that result in disease. It ordinarily can be determined readily by studies of the incidence and outcome of naturally occurring infections in humans. This proportion may be affected by the size of the infecting dose and numerous host factors, including age. Highly pathogenic agents include typhoid bacilli, rabies, measles, varicella, and rhinoviruses. Agents of intermediate pathogenicity include rubella, mumps, and adenoviruses; polioviruses and the tubercle bacillus have low pathogenicity.

VIRULENCE

Virulence, offered as a synonym for *pathogenicity* in medical dictionaries, is defined more usefully as a measure of the severity of the disease that does occur. Various criteria may be used: days confined to bed, serious sequelae such as persisting paralysis, and death. The measure of virulence is the number of severe cases over the total number of cases, which when death is the criterion becomes the familiar *case-fatality rate*. With this as our measure, the viral agents previously mentioned fall into a different gradient from that based on pathogenicity. Rabies virus (with a case-fatality rate of nearly 100 percent) qualifies as highly virulent, and poliovirus (with a case-fatality rate of 7 to 10 percent for paralytic disease) can be classed as moderately virulent. Measles, with an occasional death from encephalitis or pneumonia, is far down the scale but is still ahead of mumps, varicella, non-fetal rubella, and rhinoviruses, for which the case-fatality rates are very low. Outcomes of severity short of death can be used in a similar fashion.

IMMUNOGENICITY

Immunogenicity (ability to induce specific immunity) is measured best in terms of the degree and duration of resistance conferred by infection. Although agents may differ with respect to the immunogenicity of their intrinsic "protective antigens," more important factors are the sites of primary infection and disease, and the amount of antigen formed during infection to stimulate a host response. Superficial sites, such as the respiratory mucosa, are guarded chiefly by secretory antibody, which is poorly persistent; agents such as rhinoviruses, which replicate only at such sites, are ineffective stimulants of the systemic immune response. The amounts of the respective toxins released during clinical tetanus and diphtheria usually do not induce satisfactory immunity. In contrast, systemic viral infections, as with measles and yellow fever viruses, induce solid and long-lasting immunity.

Agent-Host Relationship

The infected host provides a shelter in which the agent can multiply and from which it may spread. Key questions involve how long the agent can persist in the host and over what period and by what avenues it can escape. The time relationships and descrip-

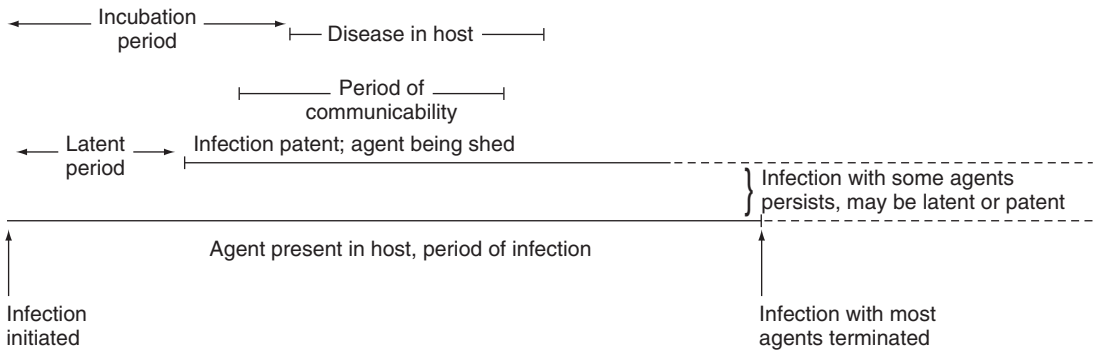

Figure 7–1 Important phases of infection in vertebrate hosts.

tive terms of different phases of infection are suggested schematically in Figure 7–1.

When the agent is not readily recoverable but perhaps is hidden within host cells or at some other site, infection is termed *latent*. Conversely, when the agent is being shed, as in feces or respiratory secretions, or can be recovered from blood or tissues, infection is said to be *patent*. Infections are necessarily latent at first (the *latent period*) and become patent when the agent has multiplied sufficiently for shedding to begin. The *period of communicability* commonly starts soon after initial shedding begins and continues as long as the level of shedding is sufficient for transmission. The rapidity with which disease is spread is related to the length of the latent period, which almost always is shorter (sometimes much shorter) than the better known *incubation period* (time until disease develops). The period of communicability has no consistent relationship to either the occurrence or the duration of disease.

Persistence in the host is important to the agent for as long as escape remains possible. The period of persistence (see Fig. 7–1) varies widely among agents. Infection terminates completely within 2 to 3 weeks with many agents, such as most respiratory viruses, and after a few months with some agents, such as polioviruses or adenoviruses. Truly persistent lifelong infections may become permanently latent as with some herpesvirus group (Epstein-Barr virus, cytomegalovirus) infections. Infection may remain permanently patent (approximately 3 percent of typhoid cases, numerous hepatitis B virus infections), may be intermittently patent (herpes simplex virus infections [HSV]), or after years of latency may recrudesce with patency and associated disease (tuberculosis, Brill disease caused by *Rickettsia prowazekii*, varicella-zoster virus [herpes zoster]).

RESERVOIRS OF INFECTIOUS AGENTS

Reservoir is defined here as the total mechanism responsible for perpetuation of an infectious agent. The reservoir is a continuing chain of transmission from one host to another (including vertebrate and invertebrate species). Chains with long links requiring infrequent transmission are especially favorable to survival of agent species.

Among agents for which humans are the only natural vertebrate host, many contrasting patterns exist. The most common is exemplified by infections with most respiratory viruses, which are characterized by short latent periods (1 to several days) and short periods of communicability (rarely >1 week). The links are short, and frequent transmission is necessary. At the other extreme are long-persisting infections associated with continuous (typhoid carriers, hepatitis B virus, HIV) or intermittent (HSV) *patency* or shedding. The links in this case may be as long as the post-infection life of the host and render generation-to-generation transmission possible. Such transmission also may occur via congenital infection, as in mice infected with lymphocytic choriomeningitis virus. Examples in humans include cytomegalovirus and hepatitis B virus infections. Long links also occur in persistent infections that after many years of latency recrudesce to cause disease and renewed shedding (varicella-zoster virus and *R. prowazekii*).

When infection of invertebrate hosts (vectors) is a link in the chain of transmission, a wide range of reservoir patterns is possible. The simplest involves agents for which humans are the only natural vertebrate hosts (malarial parasites, *R. prowazekii*, dengue virus), with the chain consisting of alternating links of human and vector infection. More commonly, the basic reservoir is a similar alternating chain primarily involving lower vertebrate hosts, with humans being an opportunistic and usually blind-end host. Examples include murine typhus rickettsiae and plague bacillus (both cycling primarily in rats and rat fleas), Lyme disease, and various arboviruses (yellow fever, St. Louis encephalitis). In the case of the latter, the broad vertebrate (numerous avian and mammalian species) and invertebrate (various mosquito species) host range results in very complex patterns that in a given area are defined by the prevalent susceptible host species. The links in these chains are defined temporally by the persistence of patent infection in the vertebrate host and the short life span of the invertebrate host.

Several aspects of infection of the invertebrate (vector) host are important. Typically, infection is acquired in a blood meal and endures for (and does not influence) the life span of the arthropod. Hibernating arthropods may be the long link in the chain by which the agent survives the winter. In at least two instances, infection kills the vector: *R. prowazekii* in the body louse and plague bacilli in the rat flea. As with malaria, infection of the arthropod also may permit completion of an essential stage in the developmental cycle of the agent. Finally, transmission of infection from arthropod to arthropod may be alternate or necessary links in the chain. Transovarial transmission of *Rickettsia tsutsugamushi* (scrub typhus) in mites is essential because the individual mite feeds only once, during the larval stage, on vertebrate hosts. Transovarial transmission also occurs in ticks infected with *Rickettsia rickettsii* (Rocky Mountain spotted fever) and in *Aedes triseriatus* mosquitoes infected with La Crosse virus (California encephalitis), in both cases affording an overwintering mechanism. Venereal transmission of La Crosse virus between mosquitoes also has been shown.[33]

Finally, the inanimate environment can play a role in the reservoir mechanism. Examples are bacteria that can multiply in the free state (salmonellae and staphylococci in food) and agents endowed with unusual survival capacity (tetanus bacillus and *Histoplasma capsulatum*, both of which form highly resistant spores).

It also occurs when a brief sojourn under proper environmental conditions is required for a necessary stage in the life cycle (e.g., hookworm eggs from human feces must hatch into larvae to become infectious).

MECHANISMS OF TRANSMISSION

Transmission, in this context, is defined as the transport of an agent from one vertebrate host to another. It involves escape from the source host and conveyance to and entry into the recipient host. The basic interdependence of these sequential steps is illustrated in Table 7–1, which also presents specific examples of diseases. Although humans are the usual or only source for most agents of human disease, lower vertebrates serve as the major or only (rabies virus) source for some pathogens.

Fomites are intimate personal articles, such as handkerchiefs, playthings, and eating utensils. *Direct contact* includes not only physical contact (shaking hands, kissing, sexual intercourse) but also, in practice, short-range (within 3 ft), airborne transmission by large droplets (>5 μm) containing hundreds or thousands of organisms that descend rapidly to the ground or floor. These heavy droplets are the primary route of transmission of group A streptococcal pharyngitis,[34] pertussis, and influenza virus.

Indirect transmission for respiratory and some other infections includes the acquisition of organisms from dust (e.g., tubercle bacilli), from fomites (inanimate objects in the environment such as bedding), and from airborne droplet nuclei (<5 μm) containing only one or a few organisms that promptly dry, float in the air for long periods, and may be wafted for moderately long distances, such as between rooms or floors in a hospital. Transmission by airborne droplet nuclei is limited to highly infectious agents such as varicella virus. Because respiratory colonization with group A streptococci requires a large inoculum, airborne droplet nuclei play no role in their transmission. Other forms of indirect transmission include inanimate vectors such as food, milk, and water, which are frequent vehicles for spread, particularly of intestinal infections. Another source of indirect transmission is animate vectors, which either may function as a vehicle for transport (as with flies that carry organisms from feces to food) or may be infected. In the latter case, multiplication and transformation in the vector are required for transmission, as with African trypanosomiasis and the tsetse fly.

Conveyance ends when the agent reaches a portal of entry, which to be effective must provide ready access to a tissue in which the pathogen can lodge and multiply. For a given agent, a particular portal (nasal or genital mucosa, oral) often is obligatory or usual, but alternative portals may be possible. Rhinoviruses replicate only in nasal mucosa, whereas typhus rickettsiae typically enter through skin broken by a louse bite but also can infect via ocular or respiratory mucous membranes.

HOST FACTORS

Many biologic and behavioral characteristics of the human host influence the occurrence of infection and subsequent disease. Although the host characteristics to be considered are of widely differing nature, they operate by influencing one or more of the following: degree of exposure, innate susceptibility to infection, and the likelihood of specific immunity. Although much of the following discussion focuses on the individual human host, factors that influence individuals also affect whole human populations.

Infection of the Host

A key principle to be emphasized is the usual existence of a gradient of response to exposure to an infectious agent. Because of this gradient, the occurrence of characteristic overt disease is a notably unreliable measure of the extent of activity of a disease agent. Given a particular exposure, infection may not occur, disease may not result, or the consequences may range from trivial to the fully developed syndrome "characteristic" of the agent. Accumulating data indicate that specific host genes may be responsible for differences in host susceptibility.

An inadequate challenge dose, an unsuitable portal of entry, or specific host immunity also may explain failure of infection to develop. Whether infection causes disease and the extent, nature, and outcome of the resulting disease are determined partly by host-related properties of the agent (pathogenicity and virulence) and partly by host defense mechanisms, a variety of which confront an infectious agent that has reached a site of primary infection. Bacteria and other extracellular parasites stimulate an inflammatory response at the site in an effort to localize the invaders by a retaining fibrin network, and the invaders are destroyed by congregating numerous phagocytic cells. Invader organisms meet sinusoidal passages lined by phagocytic cells in regional (lymph nodes) and bloodstream (bone marrow, spleen, and liver) filters. In addition, clinicians are beginning to understand the importance of so-called innate immunity. This complex network of receptors and responders prevents invader microorganisms from gaining access to tissues. In addition to physical barriers already mentioned, the innate immune system includes a family of receptors known as the Toll-like receptors. These recognition molecules signal the host to activate numerous protective mechanisms. They are discussed in other chapters in this textbook.

Several aspects of the outcome of infection are important, the first being survival of the host. Death is an unsatisfactory outcome not only for the host but also for the many agents for which survival as a species depends on the host. The remaining aspects relate to the surviving host. Was recovery from disease complete,

TABLE 7–1 Typical Modes of Transmission from Host, Conveyance, and Portal of Entry

Agent Shed via	How Conveyed	Portal of Entry	Disease Example
Respiratory secretions	Airborne droplets, fomites, or direct contact	Respiratory	Common cold, influenza, respiratory syncytial virus
Feces	Food, fomites, water, flies	Oral	Poliomyelitis, typhoid, rotavirus
Blood	Arthropod vector, transfusion, needle-stick	Skin—via insect bite, intravenous device	Typhus, dengue, malaria, human immunodeficiency virus, hepatitis B and C
Lesion exudate	Direct contact, sexual intercourse, fomites, flies	Skin, genital or ocular mucous membrane	Syphilis, trachoma, staphylococcal abscess
Aerosols	Droplet nuclei, direct inhalation	Respiratory	Tuberculosis, varicella

or were permanent sequelae present? If the latter, were they stationary (as in paralysis resulting from polioviruses) or potentially progressive (rheumatic heart disease resulting from streptococcal infection, pulmonary tuberculosis)? Another aspect, persistence of the agent, was discussed previously under agent-host relationships. The final aspect is the state of post-infection resistance. If it is incomplete, the recovered host may experience reinfection, with or without disease, and again become a source of infection for others.

Biologic Factors

Biologic factors include characteristics such as age, sex, and race (ethnic group), which are so important and easily ascertained that determining their relationship to occurrence of disease is a usual first step in an epidemiologic description. Biologic factors also include genetic make-up, general health status, and specific immunity.

AGE

The influence of age is illustrated best by patterns of common diseases such as varicella, measles, and mumps before the advent of vaccines. All three diseases occur predominantly in young children, who are affected because of their usual lack of immunity and their high risk of exposure to other children, among whom most infections occur. Older individuals are likely to be immune and, unless they are parents of young children, are unlikely to be exposed to infected individuals. Vaccines that provide only temporary immunity could cause a shift to an older age of presentation in the population.

Age also often is related to the outcome of infections in nonimmune individuals. Demonstration of the influence of age requires that all infections be recognized and classified according to the severity of the resulting disease (if any). When many or most infections are subclinical (as with polioviruses and other enteroviruses), the increase in the case-fatality rate with age is apparent immediately, but special studies to document asymptomatic or mild infections are required to show that the proportion of infections that resulted in clinical disease also increased with age. In contrast, the case-fatality rate for pertussis is highest in young infants. With measles and varicella-zoster virus, initial infection at any age usually results in characteristic disease, but the frequency of serious disease increases with age.

SEX AND RACE OR ETHNIC GROUP

That sex may be a factor is illustrated by the fact that most infectious diseases typically occur more frequently in males than females. The question, with respect to any particular disease, is whether these differences between sexes reflect innate differences in susceptibility to disease or can be attributed to sex-associated differences in behavior, occupation, or stress.

The incidence of many diseases varies greatly between groups defined by race or ethnic group. This variation may be explained by differences in behavior or environment for which socioeconomic status is a marker. Ethnic groups share many genetically determined traits, however, which may include either heightened susceptibility or increased resistance to specific infectious agents. Selective evolutionary pressure may be invoked to explain the greater resistance of whites to tuberculosis and the heightened resistance of blacks to malaria. Differences in host susceptibility based on race, familial clustering, and ethnicity is beginning to be investigated at the genomic level now that the code for the whole human genome has been elucidated. Future investigations likely will be directed at discovering polymorphisms of genes that involve the immune response.

GENERAL HEALTH STATUS

General health status includes the physiologic state, nutritional status, presence of intercurrent disease, and stress. The importance of such factors is commonly accepted in many cases but rarely documented by well-controlled studies. Infancy, during which immune mechanisms are immature, is a period of special vulnerability to many infectious diseases. HSV, enteroviruses, and group B streptococci manifest as disseminated infections in neonates, in marked contrast to their presentation in older individuals. Puberty, associated with rapid growth and change in endocrine balance, is a period of vulnerability to acne and tuberculosis. Pregnancy predisposes to tuberculosis and paralytic poliomyelitis, and varicella is more severe.

Gross protein malnutrition causes definite impairment in the cell-mediated immune response,[17a] a correspondingly increased susceptibility to bacterial and parasitic infections, and increased morbidity and mortality in many viral infections. Viral infections may depress cell-mediated immunity (and increase susceptibility further) to other concurrent infections. In the developing world, where malnutrition may be epidemic, the consequences of diarrhea, measles, and other respiratory virus infections are many times more devastating. Diarrheal diseases also may be the cause and the consequence of malnutrition. Diabetics are especially vulnerable to bacterial infections; measles and pertussis may reactivate quiescent tuberculosis; and, perhaps of greatest importance, otherwise benign respiratory viral infections, notably influenza, may pave the way for development of serious bacterial pneumonia. HIV infection enhances the susceptibility to and severity of tuberculosis, toxoplasmosis, and many other infections.

Finally, stress induced by widely divergent stimuli (including strong emotions, physical exertion, trauma, or excessive heat or cold), according to Selye,[29] may operate through a pituitary-adrenocortical hormonal path to decrease resistance to infections. Widely accepted examples include physical exertion, child-bearing, and rearing of children as factors predisposing to paralytic poliomyelitis and pregnancy and rapid growth during puberty as causes of reactivation of quiescent tuberculosis.

IMMUNITY AND IMMUNE RESPONSE

Immunity and immune response to viruses warrant special attention. Although important in recovery from bacterial infection, antibody response is of questionable significance in viral infections because viruses within cells are inaccessible to antibody, and by the time antibody appears, many or most susceptible cells have been infected already. Nonetheless, the importance of antibody in viral infections is suggested by the great vulnerability of immunodeficient children to vaccine strains of poliovirus, other enteroviruses, and varicella and by the sometimes beneficial effect of passive immunotherapy in progressive vaccinia. The benefits of previous experience with viruses can be seen with the decreased severity of rotavirus infections following the first bout of rotavirus and the protection afforded by varicella-zoster immunoglobulin in highly vulnerable children.

At its maximum (exemplified by post-infection immunity to measles), protection against infection is virtually absolute. At the other extreme (exemplified by many respiratory viral infections), susceptibility to infection persists or wanes as with pertussis, although the severity of related disease may be reduced. In most instances, protection seems to be mediated by antibody, but cell-mediated immunity also may be important. Children with defects in cell-mediated immunity are at particular risk for acquiring infection with varicella, *Pneumocystis carinii*, and otherwise noninvasive fungi.

To the extent that immunity protects the individual against infection or acts to minimize shedding of the agent when infection occurs, an immune host can play little or no part in the

spread of an infectious agent in the population. If a sufficient proportion of a population is immune, an agent transmitted via contact cannot spread, and non-immune members of the population would be spared exposure. The concept of herd immunity assumes that a non-immune individual is protected from a communicable infection by being surrounded by immune individuals. What proportion must be immune to achieve effective *herd immunity* usually is unknown.[10] This concept is valid only in homogeneous, randomly mixing populations in which all possible pairs of individuals have the same probability of making effective contact. Although measles vaccine has been used extensively in the United States, outbreaks of measles continue to occur in segments of the population that failed to accept vaccine, often defined by beliefs, race, economic status, and social behavior.

Human Behavior

Governed largely by habits of the individual and the customs and culture of groups, human behavior greatly influences exposure to and modes of transmission of infectious agents. Cultural factors also underlie attitudes toward preventive and curative practices.

Water is a potential vehicle for many agents. When commonly imbibed without boiling, as in the United States, community water systems constitute potential channels for transmission that usually are well-guarded. Occasional operational failures occur, as exemplified by failure of a water quality–monitoring device in a filtration plant that resulted in an outbreak of *Cryptosporidium* diarrhea affecting an estimated 403,000 individuals in Milwaukee in 1993.[20] Foods and milk, especially items that are consumed raw or after minimal cooking, likewise are excellent vehicles for transmission of disease. Well-known examples include trichinosis from undercooked pork; bacterial enteritis from unpasteurized milk; fish tapeworm from raw fish; and various forms of food poisoning caused by bacterial contamination during handling, poor refrigeration, and inadequate cooking. In recent years, outbreaks of hemorrhagic diarrhea caused by *Escherichia coli* O157:H7, sometimes associated with hemolytic-uremic syndrome, have occurred as a result of improperly prepared products of bovine origin, particularly ground beef.[14]

Closely related to water and foods is the disposal of human excreta. Casual defecation near habitations or in or near running water leads to dissemination of enteric pathogens by filth flies and by water. The use of human feces (night soil) to fertilize crops commonly eaten raw, such as strawberries and lettuce, has an obvious similar potential.

Many more individual types of behavior also are important. Infrequent bathing and laundering of clothes favor infestation with body lice. Inadequate clothing increases exposure to arthropod vectors and, as in young children lightly clad for summer weather, facilitates the exchange of feces. Going barefoot provides exposure to hookworm larvae. Handwashing minimizes the role of hands in indirect transmission of enteric (fecal) and respiratory (nasal secretion) pathogens. Rhinovirus infections result from inserting contaminated fingers into the nose and eyes.[15] Intimate personal contact such as hand shaking, kissing, and play among young children fosters the spread of a wide variety of agents. Even recreation, such as travel, picnics, and camping, may lead to unusual exposure to infectious agents. Sexual behavior is associated with the transmission of numerous infections, including HIV, syphilis, gonorrhea, group B streptococci, and hepatitis B.

ENVIRONMENTAL FACTORS

The environment in concept embraces all that is external to the individual human host. There are three broad environmental areas: *physical*, which includes geologic and climatologic or meteorologic features; *biologic*, which consists of all flora and fauna and all living microbial pathogens; and *socioeconomic*, which encompasses the interrelationships of humans. Environmental factors often act through indirect paths, and some have the potential to affect the agent, the host, and the agent-host relationship. Solar radiation is lethal for many pathogens in the free state, helps humans synthesize vitamin D, and can provoke recrudescence of a latent HSV infection and result in recurrent fever blisters. The capacity of humans to modify adverse environmental conditions beneficially is another important factor.

Geographic and Geologic Factors

Spread of infectious agents on a global scale requires their transport, which is influenced by distance alone and by geographic features—mountain ranges, oceans, rivers—that assist or impede travel. The importance of these factors declines as the extent and speed of travel increase, but it remains substantial, especially in developing countries. The minimal effect of geographic barriers in containing highly infective agents is overcome by modern travel, and rapid spread by airline travel is exemplified by epidemics of severe acute respiratory syndrome, which spread from China throughout the world in a matter of weeks.

The natural paths of travel (including waterways), natural harbors, and the location of mineral deposits help determine where populations concentrate. Water supply, dependent in part on geologic formations, is a factor limiting population size and, together with fossil fuels and mineral deposits, influences the type, extent, and location of industrial development. Soil types vary greatly in their ability to hold and purify water and in their capacity to support vegetation, which influences the type and abundance of animal life. Soil is a determinant of the type and importance of agriculture and a major factor influencing the biologic environment.

Climate

The term *climate* describes the typical annual pattern, along with its seasonal variation, of climatologic conditions in a specified region. Such conditions (climatologic factors) include solar radiation, temperature, humidity, barometric pressure, winds, precipitation (and drought), and lightning. These factors can affect infectious disease agents directly. Many microbial agents in the free state are vulnerable to excessive heat and radiation and uncontrolled drying. The life cycles and reservoir mechanisms of many pathogens, including higher parasites, depend on appropriate temperature and humidity. Maturation and hatching of hookworm larvae from ova deposited in the soil require warmth and reasonable humidity, and the multiplication of malarial parasites and arboviruses in their mosquito vectors and the abundance of the vectors are favored by warm temperatures.

The usual seasonal variation in the incidence of specific infectious diseases suggests important influences of climatologic factors, but how they operate may be hard to determine. Overall, respiratory infections occur more frequently in colder months, but within this period (roughly October through mid-May in the Northern Hemisphere), the prevalence of the many respiratory pathogens varies greatly. Rhinoviruses peak in the early fall and spring, and influenza viruses are most active in midwinter. Parainfluenza virus type 1 and 2 infections usually peak in the fall. Increased congregation of people indoors facilitates transmission, and fluctuations in temperature and humidity not only affect the viability of agents in airborne droplet nuclei or on fingers or fomites but also may affect host susceptibility to infection.

Enterically transmitted infections occur most frequently in the warmer months, presumably largely because of season-related changes in host behavior. The outdoor play of scantily clad children facilitates the spread of skin infection and fecally shed agents

such as enteroviruses. Rotavirus infections are an exception in that they occur most frequently in colder months. More completely understood are the seasonal patterns of infections spread by arthropod vectors; these patterns reflect seasonal variations in the abundance and activity of the vectors and the various lower vertebrate host species, which together constitute the reservoir mechanisms of the specific infectious agents. Climate overall, as a major determinant of the biologic environment, helps determine the abundance and the particular species of flora and fauna in a given area.

Longer term changes in climate have been associated with changes in patterns of infection. The hantavirus outbreak in the southwestern United States in 1993 has been attributed to unusually heavy precipitation in the spring of 1993 after 6 years of drought. This precipitation resulted in marked proliferation of the deer mouse population, the reservoir of the virus.[35]

Socioeconomic Environment

Socioeconomic factors depend on the density and distribution of populations; the available natural resources; the level of social, political, cultural, and scientific development; and, most importantly, the interrelationships of people. These factors typically affect health by indirect means, and, because they often are interrelated closely, evaluating the impact of individual factors is very difficult.

The relationship of population distribution and density to the occurrence of infectious diseases is substantial. Increasing density favors the spread of infectious agents to humans from human and nonhuman sources and the occurrence of related disease and development of immunity. In large and dense populations, agents such as measles virus typically infect in early childhood and persist because a sufficient number of new susceptible individuals are added continuously by birth. In smaller populations, the agents are unable to persist and are reintroduced at unpredictable intervals; as a result, manifestations of "childhood" diseases may be delayed for long periods. Populations of urban and rural areas differ not only in relative density, but also in other important ways. Exposure to zoonotic agents, especially agents prevalent in wildlife and livestock, is greater in rural areas, although rats and stray dogs may abound in city slums. Environmental sanitation (protection of water and milk supplies, safe disposal of sewage) often is a personal problem for rural residents but is handled by cooperative effort in urban populations. In addition, the importance of schools and school buses in facilitating exchange of infectious agents is greater in rural areas, where isolation of farm residents otherwise restricts contact between young children.

The basic population unit is *the household*, membership in which has similar implications for health in rural and urban areas. Family members are similar genetically; share a common diet and economic status; are subject to the same cultural, religious, and educational influences; and are exposed to a common local physical and biologic environment. Most important for contact-transmitted diseases, intrafamilial contact is prolonged and increases in intimacy with household crowding. In the wintertime, many homes are insulated, and the windows are closed so that air exchange is poor and exposure to airborne organisms is great. Prolonged contact is especially important for persisting infections such as HSV and tuberculosis. Family size, regardless of the degree of crowding, is particularly important for acute infections because it determines the number of potential introducers who bring home infections acquired elsewhere. The likelihood of exposure in early childhood increases with family size. Except for early infancy, a period of special vulnerability to some agents (e.g., respiratory syncytial virus, pertussis), early exposure is beneficial because most resulting infections are less apt to have serious consequences.

A population with a highly developed *social and political structure*, through its capacity for cooperative action, enjoys many advantages that directly or indirectly benefit health, including provision of preventive and curative health services, effective environmental sanitation, and well-developed educational facilities. Education closely relates to personal health practices that are based on understanding what individuals should do to minimize disease hazards. Schools, where the educational process begins, have been identified already as important factors in the exchange of infectious agents among children, especially agents spread by contact and airborne droplet nuclei. This matter is offset partly by the benefits derived from school-based immunization programs.

Economic status affects the occurrence of diseases indirectly through its relationship to adequacy of housing, nutrition, level of education, and availability and use of health services. It also is related closely to occupation, which may be associated with exposure to specific infections, such as Rocky Mountain spotted fever (forest workers and hikers in the south Atlantic coast states) and ornithosis (workers in poultry-processing plants).

DISEASE OCCURRENCE IN POPULATIONS

Patterns of occurrence of disease that are not random, but instead reflect the influence of underlying causes (risk factors), not only help predict future occurrence of disease but also provide important clues to understanding causation. Describing the pattern of occurrence of disease begins with definition and classification of disease in the individual so that cases can be identified and counted reliably. The occurrence of disease in defined populations can be expressed quantitatively.

INFECTION AND DISEASE IN THE INDIVIDUAL

Epidemiologic interest focuses on the specific etiologic identification of *infection* and *disease*, terms that are not synonyms. Technically, any deviation from normal function or state constitutes *disease*. Because virtually all infections cause at least some deviation from the normal state (e.g., a change in the white cell pattern in blood and mobilization of such cells at the site of infection), they cause disease. In practice, many infections result in no clinical evidence of disease and are important to the individual only for the reason that they induce immunity. Because subclinical infections help define the overall pattern of occurrence of infection and often play a significant role in its spread, their recognition is important epidemiologically. Subclinical infections go unrecognized except when healthy individuals are observed for infection in longitudinal or case-control studies.

Only infections resulting in disease usually come to the attention of the medical community. To the extent that they are recognized etiologically, they provide the earliest and most available indicator of the pattern of infection in the population.

With few exceptions, such as measles and chickenpox, typical clinical syndromes are not pathognomonic and require the clinician to develop a differential diagnosis. Basically, clinical manifestations depend more on the site or sites of disease than on the infecting agent. Because the number of possible disease targets in the body is small and the number of potential agents is large, reasonably distinct clinical entities may be caused by any of several agents. Notable examples include "common colds," approximately 40 percent of which are caused by rhinoviruses and 60 percent by any of many other viruses, and aseptic meningitis, which may be caused by such agents as mumps virus, many enteroviruses, or *B. burgdorferi*.

Infection with a specific agent may have several possible clinical outcomes. Infection with polioviruses usually is (perhaps

80%) subclinical, but can result in brief febrile illness (approximately 15%), aseptic meningitis (4-5%), or classic paralytic disease (<1 percent). The response to agents with multiple potential targets varies even more widely. Group B coxsackieviruses can cause such disparate entities as acute upper respiratory disease, aseptic meningitis, polio-like paralytic disease, myocarditis, and epidemic pleurodynia (Bornholm disease).

Knowledge of the agents active in the community when a given illness occurs helps narrow the differential diagnosis, but confirming etiologic diagnosis requires laboratory assistance: by culturing the agent, direct visualization in specimens, antigen detection, genome identification using molecular techniques, or demonstration of a specific antibody response in tests of "acute" and "convalescent" serum pairs. The diagnosis is most secure when both approaches suggest infection with the same agent. Although demonstration of the agent in relation to the disease site carries special weight (e.g., in a pharyngeal swab specimen from a respiratory illness), its presence could be the result of preexisting persisting infection unrelated to the current illness. Antibody response indicates a newly acquired infection.

DESCRIBING INFECTION AND DISEASE IN POPULATIONS

Sources of Information

Many sources provide data regarding the incidence of infectious diseases, including U.S. Vital Statistics, which tabulates only fatal cases; the Centers for Disease Control and Prevention (CDC), which receives reports of specific notifiable diseases from state health departments and summarizes them in the *Morbidity and Mortality Weekly Report*; and state and local health departments. In the absence of a focused surveillance project, these data vary in their completeness by source and by disease because of underreporting, subjects who are not seen, and errors in diagnosis.

Among the different states, reporting requirements vary, and adherence to these requirements by providers also varies. Reporting is most complete for uncommon but characteristic disorders of unusual interest, particularly if they are severe or fatal and require hospitalization, such as rabies, anthrax, trichinosis, plague, and diphtheria. Reporting is enhanced by outbreaks, as with measles, mumps, and classic pertussis in recent years. Some notifiable infections, such as leptospirosis and atypical pertussis in partially immune individuals, often are unrecognized and are under-reported. Reporting requirements may change from year to year based on CDC or state interests (neonatal sepsis and West Nile infection) or concerns about bioterrorism. Another source of data, often useful in certain areas, is state health department laboratories, which perform specific microbiologic or serologic tests for providers.

Infectious diseases that are not notifiable by law pose a difficult problem. Although necessarily limited in scale, longitudinal studies of defined populations of families have yielded valuable information. Finally, researchers now can use well-designed serosurveys to make reliable estimates of rates of previous infection with agents that induce long-persisting antibody.

International data on the incidence of infectious diseases are less precise except in well-developed countries. For the developing world, the World Health Organization (WHO) and the United Nations International Children's Emergency Fund provide estimates of the incidence of morbidity and mortality from various infectious diseases in different nations based on local reports, which are not always collected systematically. Continuing collection of such data is important for monitoring the effects of the Expanded Program of Immunization, which is directed at controlling the major vaccine-preventable diseases of childhood. More difficult to develop are definitive data about the incidence and causation of the respiratory and diarrheal diseases, which are estimated to kill 6 million children annually in the developing world (approximately 5% of the yearly birth cohort).

For developing these kinds of statistics, it is important to have a definition of disease that is as useful as possible, which means that the sensitivity and specificity of the definition should be so balanced that as many cases of the disease as possible are identified, while avoiding the confusion that occurs when other disorders with overlapping manifestations meet criteria that are too nonspecific. A well-known example of a useful definition of disease is the Jones criteria for the diagnosis of rheumatic fever, established in 1944 at the request of the National Research Council in an effort to bring order out of chaos at a time when the disease was a major problem in the civilian and military populations.[17] These criteria subsequently have been modified to enhance their specificity by making evidence of a previous group A streptococcal infection a sine qua non for the reason that too many cases of polyarthritis of other causes met the original criteria.[9] Similarly, the criteria for the diagnosis of Kawasaki disease were revised more recently to avoid missing some episodes in infants and missing the opportunity to prevent coronary artery aneurysms by using intravenous immunoglobulin.[24]

Recognizing the importance of standardized diagnostic criteria for surveillance of infectious diseases of public health importance, the CDC published in 1990 case definitions for reportable infections.[36] Optimal use of these criteria and reporting of confirmed and probable cases to the proper authorities are of particular importance currently, when for a variety of reasons, some formerly well-controlled contagious diseases are becoming recrudescent (e.g., mumps, pertussis). All states mandate reporting contagious diseases of major public health importance, particularly diseases affecting children.

Definitions of Terms and Rates

Two commonly used (and often misused) terms, *incidence* and *prevalence*, have significantly distinct meanings. *Incidence* refers to new occurrences of infection or disease, in a population of individuals uninfected or without disease at baseline, during a specified period, commonly a year, whereas *prevalence* refers to the state (infected, ill, immune) of individuals in a population at a specified point in time (point prevalence).

To describe variations in occurrence over limited periods within a single population, such as a city or a state, a simple *numerical incidence* (number of cases or infections) often is used—daily or weekly (during an epidemic), monthly (to reflect seasonal patterns), or annually (to compare successive years). Comparisons among different populations or subgroups within a population or at widely separated times in the same population require use of the *incidence rate*, or *attack rate*, which is defined as follows:

$$\frac{\text{No. new occurrences (cases, infections) within a specified period}}{\text{Population at risk at midperiod}} \times 100, 1000, 10{,}000$$

If 10,000 cases of influenza occurred in a city of 200,000 people in a year, the incidence (or attack) rate would be expressed as 5 per 100 per year. In contrast to *incidence*, which is useful for determining acute and chronic conditions, *prevalence* is applied usefully only in describing conditions of long duration (months or years), such as immunity, persisting infection, and chronic disease. In relation to an acute disease such as influenza, we speak of the incidence of disease and the prevalence of immunity (reflected by antibody). Because most interest is in comparisons of different populations or subgroups, prevalence is

expressed customarily as the *prevalence rate*, which is defined as follows:

$$\frac{\text{No. persons (infected, ill or immune) at a given time}}{\text{Population at that time}} \times 100, 1000, 10{,}000$$

For infections transmitted by contact, the frequency with which infection or disease occurs among exposed susceptible individuals provides a measure of the infectivity of the agent. This frequency, called the *secondary attack rate*, is defined as follows:

$$\frac{\text{No. contacts becoming infected or ill within the maximal incubation period}}{\text{Total number of "susceptibles" exposed}} \times 100$$

The secondary attack rate is applied usefully only to closed groups, households, or classrooms, where exposure safely can be presumed for all members. The first, or primary, case is the presumed source of exposure; other cases that occur within the minimal incubation period are called *co-primary cases*. In calculating the secondary attack rate, primary and co-primary cases are excluded from the numerator and the denominator. Cases that occur subsequently within the maximal incubation period constitute the secondary cases. Cases that develop later are excluded as being derived from outside sources or from tertiary spread. The exclusion of immune individuals from the denominator is feasible only for diseases sufficiently characteristic clinically that the history serves to identify them (e.g., measles, chickenpox). Although immune individuals are not readily identifiable in the case of common respiratory diseases, the secondary attack rate based on all exposed members of the group still remains a useful tool. Its value decreases, however, when the period of communicability of the primary case (as with *Mycoplasma pneumoniae*) is longer than the incubation period because distinguishing between secondary and tertiary cases becomes difficult. Finally, the occurrence of death caused by a specific disease is expressed in two different ways. One, the *cause-specific mortality rate*, is defined as follows:

$$\frac{\text{No. deaths from the disease in a given year in a population}}{\text{Total population at midyear}} \times 100{,}000$$

It is a measure of the effect of the disease on the population. The potential significance of the disease to the affected individual is suggested by the *case-fatality rate*, which is defined as follows:

$$\frac{\text{No. deaths from the disease within a specified period}}{\text{No. cases in the same period}} \times 100$$

RELATING INFECTION AND DISEASE TO PERSONAL CHARACTERISTICS

Multiple characteristics may serve to distinguish one individual from another. Some factors are determined at conception—age, sex, ethnicity, genetic make-up, and birth order. Others, far more numerous, are acquired subsequently. They may be biologic (specific immunity, nutritional state), behavioral (smoking, dietary, recreational habits), or socioeconomic (occupation, educational level, marital status). Many of these characteristics relate to exposure to infectious agents or to susceptibility or resistance to the effects of such agents and to the occurrence and severity of disease.

Relative Usefulness and Importance of Characteristics

Although almost any potentially relevant characteristic of an individual patient can be identified, it is not useful for purposes of description unless we can estimate how many people in the population also possess the characteristic. From census data or other accessible records, numbers of people in groups defined by age, sex, race, occupation, or marital status can be estimated easily. Special surveys would need to be conducted to estimate the prevalence of specific immunity or possibly significant exposure, such as to household pets.

The importance of personal characteristics to the description of a disease varies in two ways. One is in the degree of association that exists between a characteristic and a specific disease. Age is associated strongly with disease caused by prevalent contagious agents, whereas sex usually is not. The second way is in the independence or relative interdependence of characteristics as variables. Inherent characteristics, such as age, sex, and ethnic origin, are independent of one another, whereas acquired characteristics rarely are. The nature of interpersonal contacts, degree of personal hygiene, and usual forms of recreation are associated closely with age or sex or both. The common interdependence of characteristics means that before making an inference from a particular association, one should explore association with other, possibly correlated, characteristics.

Age Patterns

The occurrence of infection and disease generally is related so strongly to age that until possible differences in age distribution are taken into account, differences in occurrence among population subgroups defined by other characteristics cannot be interpreted meaningfully. Age as a characteristic is ascertained easily and reliably for affected individuals and the total membership of the relevant population. Description of the age pattern involves computing a series of *age-specific rates* for sequential age groups, usually defined in intervals of 5 years or multiples thereof (e.g., 0 to 4, 5 to 9, 10 to 19). For conditions of pediatric concern, the use of single-year intervals (<1, 1, 2, 3, and 4 years) to cover early childhood may be more informative. In some cases, especially in the first 2 years of life, a breakdown by months of age would be informative.

Affected individuals in an age group form the numerator, and all individuals in the population in that age group serve as the denominator. Rates so computed describe the age profile of immunity at a specified point in time (age-specific antibody prevalence rates), the age profile of new infections or disease (age-specific incidence rates), or the age profile of deaths caused by a disease (disease-specific and age-specific mortality rates). Age-specific incidence rates for acute infectious diseases indicate the risk of disease occurring in each age group and, depending on the disease agent, more or less accurately reflect the underlying age-specific infection rates.

Age Adjustment of Rates

The need to take age distribution into account when comparing disease in different populations is indicated when (1) the rates vary with age and (2) the distribution of the populations by age differs substantially. From published U.S. mortality data for 1983 and 1984, one can compare pneumonia and influenza mortality rates for Alaska and Florida. During those years, 89 deaths were recorded among 986,000 Alaskans at risk, for an annual mortality rate of 9/100,000. In contrast, in Florida, 4703 deaths occurred from pneumonia and influenza among the 21,792,000 residents

at risk for those 2 years, for a rate of 21.6/100,000, nearly 2.5 times that of Alaska. These rates are called *crude mortality rates.* In this instance, these rates are misleading for the reasons that the likelihood of death occurring from pneumonia and influenza increases with age and the age distributions of the populations of these two states differ markedly. National death rates from these infections are nearly 10-fold greater in people 65 to 74 years of age than in people 55 to 64 years of age. For those years, 17.5 percent and 3 percent of the Florida and Alaska populations were 65 years or older.

To make a valid comparison of the pneumonia and influenza mortality rates for these two states, one must perform an age adjustment; this is a simple process that is not detailed here because the method can be found in available texts of biostatistics and epidemiology. Briefly, one determines mortality rates for specific age groups (usually 5 or 10 years) for the two populations and calculates the deaths that would be expected in a common (or standard) population for the same age groupings by using the age-specific rates of the populations being compared, in this instance those of Alaska and Florida. Summation of these expected deaths permits calculation of the rates that would have occurred in the standard population if the age-specific rates of Alaska applied and if the age-specific rates of Florida applied. In this example, the age-adjusted mortality rate for pneumonia and influenza for Alaska is 35.2/100,000, and that for Florida is 21.8/100,000, nearly the reverse of the crude rates. (The combined population of the two states was used as the standard.)

These age-adjusted rates are not true rates; they are used for comparison. Although often applicable to infectious diseases, age-adjusted rates almost always are required for comparisons of morbidity and mortality from chronic diseases.

Sex Patterns

Because sex is a readily ascertained characteristic of the membership of populations, the occurrence of infections and disease in relation to sex is described easily. Its simplest form is the *sex ratio*, or the ratio of cases in males to cases in females. This ratio is meaningful only when, as in childhood, the population is divided approximately equally by sex. Although males exceed females at birth (106:100), the death rate for males exceeds that for females at all ages (average 1.5:1). From approximately age 20 years on, females outnumber males, the difference increasing with age, which means that when comparing sex-specific rates, age adjustment must be made, or, better yet, the age profiles for the sexes should be compared directly so that important differences in the contour can be seen.

Ethnic or Racial Patterns

A third characteristic by which members of the population can be grouped in describing occurrence of disease is race or ethnic origin, the usefulness of which has decreased with the increasing frequency of mixed marriages. The U.S. census classification is based on information collected regarding race and native origin. Individuals of mixed racial parentage are classified by the race of the nonwhite parent or, if both are nonwhite, by that of the father. People of foreign birth are classified by country. Native-born children of foreign-born parents are identified as "foreign stock" and grouped according to parental origin. Census data provide estimates of population subgroups belonging to several "races" (white, black, Native American, Chinese, Japanese) or "foreign stocks" (including foreign born and first generation).

Although controversial currently, this method yields differences in the occurrence of many infections and other diseases among such population subgroups. Knowledge of such differences is useful in case finding and organizing the application of specific preventive measures. Subgroups defined by ethnicity possess some similarity in genetic constitution that may determine susceptibility or resistance to specific agents. They also may be affected distinctively by environmental factors because of voluntary or involuntary differences in behavior and patterns of living.

Disease Patterns in Kinships

Genetically determined susceptibility and resistance to specific infectious agents have not yet been associated clearly with recognized genetic markers that could serve as a basis for defining population subgroups. Most efforts to look for genetic influences have been studies of occurrence of diseases in individuals of differing degrees of relationship within kinships or in the total memberships of different kinships. With rapid advancement in the database of the human genome, considerable interest has been generated in polymorphisms at loci that may define susceptibility to infectious diseases on a genetic basis. Populations with a high prevalence of certain alleles and known to have an increased risk of certain infections could be studied to determine whether the risk is due to abnormal function of the specified gene.

Family Episodes of Infection and Disease

With respect to contact-transmitted infectious diseases, the family is more important as the basic epidemiologic subgroup of a population than for its shared genes. Observation of family units for episodes of infection and related illnesses has contributed significantly to knowledge of the epidemiology of widely prevalent infectious agents. The situation in all family studies begins with one member's infection, acquired from outside the house. That member then exposes the other family members. The introductory infection and any infections in family members who are exposed constitute a family episode, which is described basically in terms of the times of onset of the related infections and the identities (age, sex, position in the family) of the introducer and the infected and uninfected contacts.

Analysis of cumulated episodes of common respiratory illness, observed in the early studies, identified children as the most frequent introducers (important in community spread). Analysis also yielded estimates of the risk of cross-infection occurring within the family, expressed in terms of secondary attack rates among specified members (e.g., younger children) exposed to specified introducers (e.g., a schoolchild or a parent). Generally, the risk in contacts was related inversely to age overall, as a result of the influence of immunity, and to intimacy of within-family contact (ready exchange between spouses and between children nearest in age). Finally, the relationship of time between the onset of illness in the introducer and onset in family members exposed serves to define the range of incubation periods.

Analysis of the episodes can yield additional information concerning such crucial aspects as mode and duration of the agent's shedding; the spectrum of clinical response to infection, including the proportion that is subclinical; and the significance of previous immunity in the face of close exposure, as measured by the frequency and clinical consequences of the reinfections that result. The results of the analysis of adenovirus episodes in the Virus Watch program are illustrative.[11] Virus appears regularly in the feces and less often (approximately 50%) in the pharynx, and shedding may be abortive (a few days only) or continue intermittently for many months. Overall, 50 percent of infections are subclinical, and illness, typically febrile and respiratory, occurs more commonly with pharyngeal excretion (65%) than with only fecal shedding (31%). Immunity is 85 percent protective against infection; re-infections that do occur usually are subclinical. Young children and especially infants younger than 2 years of age are the usual introducers, and within-family

spread depends more on duration than on the mode of virus excretion by the introducer.

Socioeconomic Patterns

Socioeconomic status covers a complex of characteristics, including levels of education and income and, less tangibly, "social standing." The problem is to discover a useful single indicator. One possibility is area of residence as classified by median income or measures that reflect housing standards, such as type of plumbing and average number of individuals per room. Relevant data are available for census tracts, which have proved useful when the tracts are homogeneous.

Occupation of the head of a household seems to be the one attribute most closely reflecting socioeconomic status. On this basis, the British have defined five broad social classes that directly apply to employed adults and can be extended to cover their dependents. These classes, in descending order, are professional, intermediate, skilled, partly skilled, and unskilled occupations. For use in the United States, based on census-recorded occupations, these terms have been translated as follows:

- Professional workers
- Non-farm technical, administrative, and managerial workers
- Clerical, sales, and skilled workers
- Semiskilled workers
- Non-farm laborers
- Farm workers of whatever level are included in a sixth group as agricultural workers

RELATING INFECTION AND DISEASE TO PLACE

Place is of interest, epidemiologically, when occupied by humans and, unless indicated as relating to work, recreation, or travel, refers here to residence. Place usually is classified geographically (hemisphere, continent, nation) but also can be classified usefully by environmental characteristics such as climate, altitude, stage of economic development, population density, and urban or rural nature. Variations in occurrence of disease with place reflect parallel variations in the operation of causative factors and raise the question of whether these factors are to be found in the characteristics of the physical and biologic environment inherent to the place or in the characteristics of the inhabitants. The former is suggested when the age-adjusted risk of disease increases for immigrants and decreases for emigrants, when risk does not vary among the ethnic groups present, and when similar ethnic groups in other places enjoy a lower risk. One also must consider possible differences in reliability and completeness of recognition and reporting of disease.

Global Variation

On the global scale, the WHO collects and publishes information concerning the occurrences of diseases derived from statistics compiled routinely within nations for morbidity from notifiable infectious diseases and for causes of death. The great variations among nations in the quality and availability of medical care and other health services result in corresponding variations in the reliability and completeness of the data collected by the WHO. Generally, basic demographic data and the quality and availability of health services are equally good in well-developed countries, so specific disease rates can be compared. In less-developed countries, demographic data may be inaccurate and medical services may be inconsistent in quality and concentrated in urban populations, within which their availability varies with economic status. Many illnesses and deaths are unattended medically, especially in rural areas, and births commonly are attended by midwives. Because infant deaths are reported more completely than births are, infant mortality rates may be unreliable.

With respect to infectious and parasitic diseases, knowledge of the frequency of disease is less important than is qualitative knowledge of the distribution and spread of disease. Such knowledge guides the application and enforcement of international control measures and is the basis for advice given by physicians to prospective foreign travelers. Important diseases, such as yellow fever, plague, and cholera, because of their high case-fatality rate and characteristic clinical picture, are almost certain to come to attention when substantial numbers of cases occur. Such knowledge may not be publicized, however, or may not be promptly available. Some countries, in the hope that a new outbreak (perhaps of cholera) will be controlled soon, may withhold information to avoid discouraging economically important tourists.

Two additional considerations are relevant to evaluating the disease hazards of foreign travel. One is the fact that recognized occurrence of disease in the indigenous population may be an inaccurate index of risk to a newcomer. Particular agents, such as polioviruses in the past and hepatitis A virus and Epstein-Barr virus at present, may be so prevalent that infections in natives occur so early in life that they usually are subclinical. The second consideration is the nature of the proposed travel. The usual tourist or business traveler visits chiefly larger population centers and popular tourist attractions, where the most important hazards are pathogens transmitted by food or water. Travelers whose activities bring them into more intimate contact with the people and the biologic environment (Peace Corps workers, military personnel) may encounter additional hazards, such as rabies and the locally prevalent arthropod-transmitted pathogens.

As suggested in considering geographic influences on occurrence of disease, the distribution of many diseases is influenced by relevant environmental factors, rather than political boundaries. In depicting (or predicting) the global distribution of a particular disease, identifying regions defined by the presence of factors thought to be important to disease occurrence is useful.

For most agents pathogenic for humans, the chief environmental requisite is a susceptible human population, and most such agents already exist wherever the size and density of the population are sufficient for them to persist. Concern about global spread is limited to a few important pathogens, such as the cholera vibrio and influenza virus. Cholera is a special case in that its spread also depends on poor sanitation. Neither persistence nor even limited spread should occur after its introduction into highly developed areas. Influenza A virus continues to be a major, and so far unstoppable, threat by virtue of its ability to emerge at irregular intervals in a new antigenic coat, which largely negates the pre-existing widespread immunity.

Local Patterns of Infection and Disease

"Local" units of population for which demographic data are readily available in the United States include "large" units, such as counties, metropolitan areas, and large cities, which contain smaller units (smaller cities and towns [within counties] and census tracts [within metropolitan areas and large cities]). The smaller units, including unincorporated areas within counties, often can be characterized by variables (urban or rural nature, population density, socioeconomic status, racial or ethnic group) that may help explain observed differences in the occurrence of specific infections and related diseases.

Particularly in relation to outbreaks of acute infectious diseases, spot maps commonly are used to show the local distribution of individual cases. Placing new pins (a different color each week) to mark the residences of newly reported cases serves to visualize the outbreak's geographic progression. The final distribution of the pins may help identify a major source of infection.

A classic example is the 1854 outbreak of cholera in the Golden Square district of London, in which the clustering of residences of fatal cases helped Snow incriminate the Broad Street pump as the source.[32] Sometimes, place of work is a better guide to the source of infection than place of residence. In another classic study, that of endemic typhus in Montgomery, Alabama, in the early 1920s, the residences of cases (Fig. 7–2) were scattered widely, whereas the workplaces (Fig. 7–3) were concentrated in relation to feed stores and food-handling businesses, all heavily rat-infested. This finding led Maxcy[21] to perform studies showing the basic role of rats and rat fleas in this disease.

TEMPORAL PATTERNS OF INFECTION AND DISEASE

Definitions

The unit of time used can vary from hours to decades to centuries. In describing acute outbreaks, the units are short—hours for

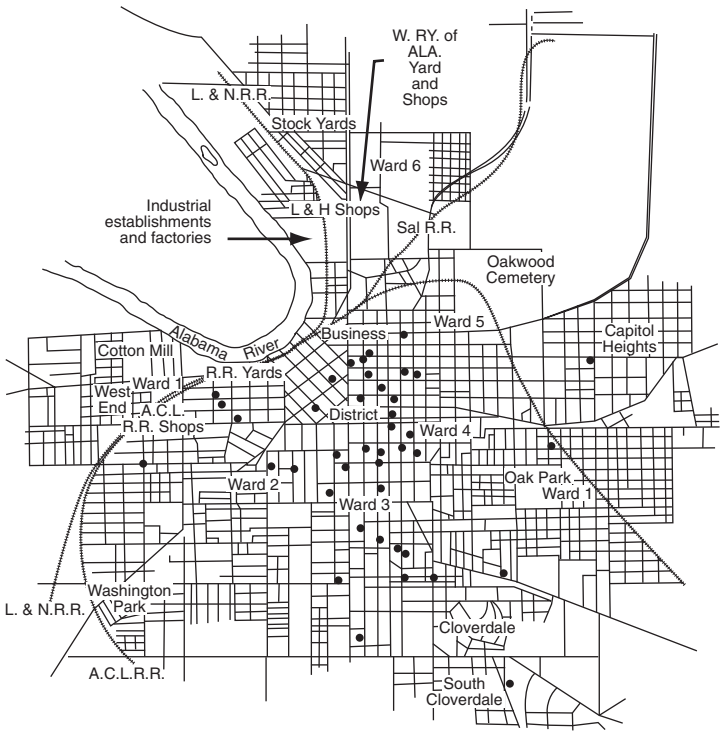

Figure 7–2 Cases of mild typhus (Brill disease) in Montgomery, Alabama, 1922 to 1925, spotted according to residence. *(From Maxcy, K. F.: An epidemiological study of endemic typhus [Brill disease] in the southeastern United States. Public Health Rep.* 41:*2967-2995, 1926.)*

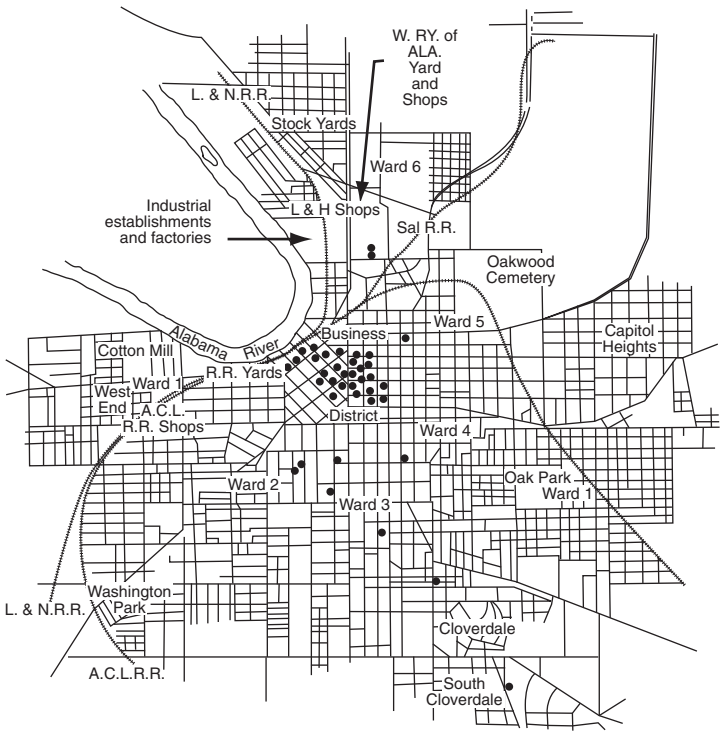

Figure 7–3 Cases of mild typhus (Brill disease) in Montgomery, Alabama, 1922 to 1925, spotted according to place of employment or, if unemployed, according to place of residence. *(From Maxcy, K. F.: An epidemiological study of endemic typhus [Brill disease] in the southeastern United States. Public Health Rep.* 41:*2967-2995, 1926.)*

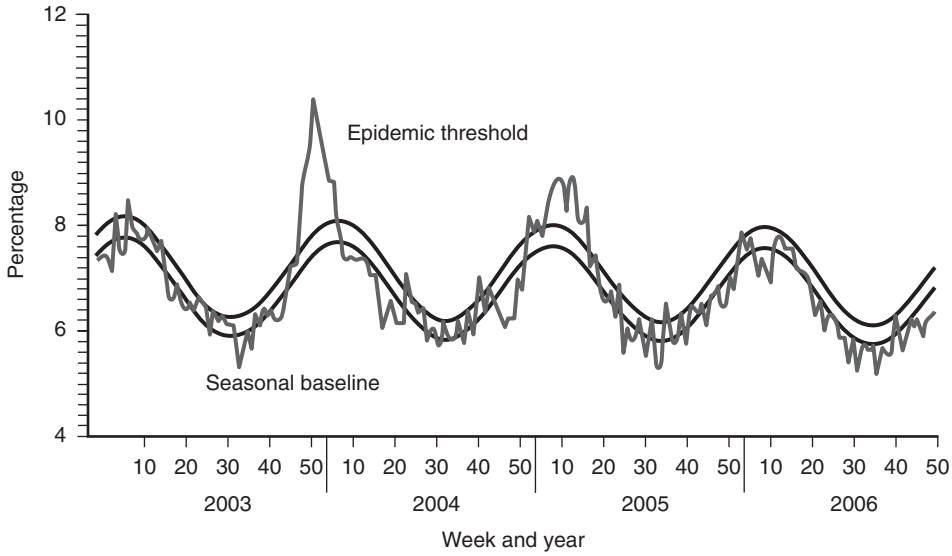

Figure 7–4 Percentage of all deaths attributed to pneumonia and influenza reported by the 122 Cities Mortality Reporting System, by week and year—United States, week ending December 9, 2006. *(From M. M. W. R. Morb. Mortal. Wkly. Rep. 55:1345-1375, 2006.)*

food poisoning, days or weeks for most infectious diseases—whereas long-term time trends are described in longer units of years or decades. Comparisons extending over 1 or more decades may be complicated by changes in diagnostic standards and reporting.

Finally, the meanings of two words commonly used to describe the occurrence of disease over time in a given area should be defined clearly. *Endemic* refers to diseases regularly present. The usual frequency, including expected seasonal variations, is called the *endemic level.* The term *epidemic* applies to any number of cases, small or large, that is a significant increase over the usual, or endemic, level (although just what constitutes a "significant increase" is not clearly defined).

This principle underlies the monitoring of many infectious diseases, deaths, and pneumonia caused by influenza by the CDC. Such monitoring is illustrated in Figure 7–4, in which the weekly ratios of deaths caused by pneumonia and influenza to the total deaths observed from 2003 to 2006 are compared with the ratios expected from use of the time series method[7] and "epidemic threshold," which depicts the upper 95 percent confidence limit. When the observed value exceeds the expected one for 2 successive weeks, an influenza epidemic is indicated. On this basis, epidemics began in week 48 of 2003 and week 9 of 2005.

In practice, especially at the local level, health authorities use the term *outbreak*, rather than *epidemic* (unless the number of cases is very large), to minimize public alarm. The term *pandemic* is used to describe excess disease occurring in many countries, as influenza did in 1957 and 1968.

Time Clusters

Time clusters relate to the recognition and interpretation of events (infections and disease) that occur with some increased frequency within a limited period or clustering in time. An important determinant of clustering of infections is the incubation period. Infection resulting from a known exposure is manifested within a predictable range of time by the initiation of shedding of the agent and the onset of disease (if it occurs). Although the average incubation period of a given disease is remarkably constant, the usual range broadens as the incubation period increases. This range can be estimated by accumulating cases for which the time of exposure is known precisely or approximately and, with this point as day 0 on the time axis, plotting the day of onset of each subsequent case. The width of the resulting cluster provides an estimate of the range of the incubation period. The best estimate is obtained by using only cases with single, clearly timed exposure, such as when contact with the source case occurred only once, as during a playmate's birthday party.

A more readily obtained but less precise estimate is that derived from the cumulative analysis of family episodes of a disease based on the assumption that the initial or primary case is the source of subsequent disease in family contacts. Because the primary case is infectious for several days, on any of which effective exposure may occur, the onset of secondary cases would cluster over a range that theoretically may reflect the true range plus the period of infectivity of the primary case.

Knowledge of the range of incubation periods has several practical implications. For contagious diseases, it can be used to distinguish true secondary cases that occur in families from others arising as a result of extrafamilial sources (cases with onset too soon after that of the primary case are called *co-primary cases*) or representing a second generation of spread within the family (*tertiary cases*). Knowledge of the range of incubation periods also determines the length of time after known exposure that contacts should be observed or possibly held in quarantine for the subsequent development of disease. For poorly contagious diseases, such as typhoid fever, or for noncontagious diseases, such as food poisoning, the distribution of onset in an outbreak can be used to distinguish between "point" epidemics (common time and place of exposure) and outbreaks reflecting exposure to a possibly continuing source. In the former case, onset would fall within the usual range of incubation periods. When the point source is not obvious, investigation can be focused on the interval defined by subtracting the shortest incubation period from the date of onset of the first case and the longest period from the date of onset of the last case.

Short-Term Patterns

EPIDEMICS

"Point" epidemics have a duration limited by the range of the period of incubation of the particular disease because no secondary spread of the agents occurs. Much more commonly, outbreaks or epidemics extend over longer periods. On the basis of the causative agents and the mechanisms of transmission, three distinct types can be recognized.

The first type typically consists of outbreaks of poorly contagious or noncontagious diseases; such outbreaks reflect the new but persisting activity of a source that must be identified and terminated quickly. One example of such a "continuing source" type of outbreak was a community-wide outbreak of salmonellosis in Madeira, California, in 1965, which was traced quickly to the water supply.[27] Unrecognized typhoid carriers working as food handlers and shellfish harvested from sewage-polluted waters contaminated with hepatitis A virus are other examples of outbreaks requiring prompt epidemiologic investigation to identify the source. One of the major causes of point epidemics is food contaminated with *E. coli* O157:H7. More recent outbreaks, such as the contamination of spinach at the growing source, have been reported by the CDC.[6] Often the CDC posts interim reports of investigations on the CDC website (www.cdc.gov) before publication in *Morbidity and Mortality Weekly Reports.*

The element of contagion distinguishes the second type of epidemic, in which disease spreads from person to person. Such epidemics generally are self-limited, and the curve describing them often resembles the bell-shaped curve of a normal distribution. Modification of this curve, as in an abrupt decline from the peak, is the expected result of successful efforts to control the epidemic. Epidemics of contagious diseases occur when the population in which the agent persists or is newly introduced contains a sufficient number of susceptible individuals with contact with one another that is adequate to permit transfer of infection from each new case to, on average, more than one susceptible individual. When this average falls below 1, the curve declines, and when the probability of successful transfer of infection approaches zero, the epidemic stops (typically well before the supply of susceptible individuals has been exhausted).

Reasons that underlie this decline and termination of transmission include seasonal changes in environmental factors that affect viability of the agent, such as temperature and humidity; changes in the behavior of hosts that affect the intimacy of contact, such as indoor school versus outdoor play; and the progressive conversion of individuals from susceptible to immune. Institution of appropriate hygienic precautions likely was an important facet of control and termination of the worldwide outbreak of severe acute respiratory syndrome in 2003.[5] If one assumes constant units on the time axis, the slope of the ascending limb of the epidemic curve is determined by the incubation period (interval between successive cases) and by factors that influence transmission (infectivity of the agent, frequency of adequate contact between susceptible individuals). In the absence of effective control measures, the duration of epidemics of a particular disease depends on the size of the susceptible population and the persistence of favorable environmental factors.

Epidemics of "diseases in nature" constitute the third type. Because susceptible individuals usually are abundant in the human population, these epidemics typically reflect increases in the number of sources of infection in nature. With zoonoses such as arbovirus encephalitis, the initiation, slope of the curve, and duration of the epidemic are determined by the number of susceptible lower vertebrate hosts, the seasonally determined abundance of the vector mosquitoes, and the length of the extrinsic incubation period in the vector. The occurrence of West Nile virus in the United States, which began in August 1999 and has continued with outbreaks in the late summer of each succeeding year in mosquitoes and humans is an example.[3,4]

SEASONAL AND CYCLIC VARIATIONS

Predictable periodic variations in disease take two forms, seasonal and cyclic, neither of which is understood completely in the case of agents infecting only humans. *Seasonal* variations presumably reflect the influence of changes in temperature, precipitation, and length of daylight on the activity of the agent. This variation is readily apparent with respect to diseases, such as Lyme disease (the peak incidence of which typically occurs in the summer months), which depend on lower vertebrate hosts and arthropod vectors, the abundance and activity of which are determined seasonally. Similarly, the increased occurrence of enteric bacterial infections (spread by indirect means) in the warmer months is explainable largely by more rapid bacterial growth in unrefrigerated milk and food, the increase in abundance of flies, and the lack of sanitary precautions associated with summer recreational activities.

The mode of transmission is only a partial determinant of the seasonal pattern. Although the enteroviruses (including polio), the rotaviruses that cause acute gastroenteritis, and hepatitis A virus all are spread chiefly by fecal-oral mechanisms (direct and indirect), the seasonal patterns are distinctly different (late summer and fall for enteroviruses and late winter for rotaviruses and hepatitis A virus). Similarly, although infections with agents present in respiratory secretions and spread by either airborne mechanisms or contaminated fomites occur infrequently in the summer, their seasonal peaks occur at significantly different times. The season of respiratory diseases coincides roughly with the school year. Infections with rhinoviruses occur most frequently in the early fall and late spring, and infections with parainfluenza virus types 1 and 2 occur most frequently in the fall. The annual peaks of RSV vary among fall, winter, and spring. Mumps peaks in late fall, influenza in late winter or early spring, and measles typically in the spring.

Plotting variation over a series of years reveals a roughly regular cyclic variation for some diseases. In larger metropolitan areas, the usual biennial measles epidemic appears as an enlarged annual wave. Before 1950, deaths caused by meningococcal meningitis occurred on a nationwide basis in cycles of 7 to 9 years, a phenomenon presumably terminated by the advent of prophylactic antibiotics. Pandemic influenza A, which provides the most dramatic example of cycling, is explainable by a major change in the antigenic character of the agent.

Long-Term Trends

Except for poliomyelitis during the first half of the 20th century, clearly defined long-term trends in the occurrence of infectious diseases in the United States usually have been downward, and in no case has description or understanding of the mechanisms posed a major difficulty. In the case of poliomyelitis, the increase in the rate of paralytic disease that began about 1880 is attributable to numerous factors, including improved sanitation, which resulted in an expanding proportion of infection occurring among older children and young adults without previous exposure to the viruses. In young infants, most poliovirus infections are benign but produce long-lasting immunity; the older an individual is when infected, the more likely that individual will have paralysis. The frequency of paralytic poliomyelitis is related inversely to the level of sanitation. In the United States, the increase in paralytic disease with epidemics of polio continued unabated (with an apparent sharp increase around 1940 because of changes in reporting criteria) until the widespread use of polio vaccines.

The post-1955 decline in poliomyelitis; the much earlier decline in pertussis and diphtheria; the disappearance of smallpox; and the more recent marked decline in measles, rubella, *H. influenzae* type b, and varicella all are attributable chiefly to widespread use of effective specific vaccines. Similarly, the disappearance of indigenous malaria and the decline of rabies to a negligible level in humans in certain areas reflect the application of a variety of effective control measures.

Emerging Infections

Although in the 1980s one might have said that most infectious disorders had been explored thoroughly epidemiologically and

that little was left to do, this conclusion no longer is the case. Infectious disease epidemiology has become increasingly challenging, and in many ways the problems are more complex and necessitate greater cooperation with additional disciplines, such as microbiology and molecular biology.

In part, this increased complexity is due to so-called *emerging infections*, a term that has come into vogue in recent years and refers to two different groups of disorders. The first is associated with the appearance of previously unrecognized or possibly heretofore nonexistent infections in humans. Well-known examples are legionellosis, hantavirus pulmonary disease, acquired immunodeficiency syndrome (AIDS), Lyme disease, hemorrhagic colitis caused by *E. coli* O157:H7, and Ebola virus infection. The other group includes previously recognized human infections that exhibit changes in epidemiologic behavior or biologic characteristics that enhance their transmission or virulence. These changes usually can be attributed to external influences, such as altered demographics including increasing population and rural-urban migration, international travel, new technology or technologic failure, changes in land use, adaptation of infecting organisms to various influences, and inadequate or underused public health measures.[18] To this list should be added changes in host factors such as immune defenses.

No clear dividing line exists between new infections and old ones that exhibit new behavior. Examples of some with new behavior include multidrug-resistant tuberculosis, penicillin-resistant pneumococcal infections, invasive group A streptococcal (popularly known as flesh-eating bacteria) infections, staphylococcal toxic shock syndrome, community-acquired methicillin-resistant staphylococcal infections, H5N1 influenza ("bird flu"), cryptosporidiosis, and numerous infections that are fostered by immunosuppression or various therapeutic measures such as antibiotics and catheters.

These emerging infections are not minor threats. Although in some instances small populations have been affected to date, the potential for widespread disease of epidemic or even of pandemic proportions exists. This potential can be expected to increase because the demographic and other conditions that have predisposed to the emergence of these infections continue to grow and intensify. Maximal efforts must be expended to reverse this process. To achieve this goal requires worldwide collaborative effort among various disciplines, including epidemiology, microbiology, entomology, immunology, clinical medicine, demography, nutrition, sanitation, and even political science.

The responsibilities of epidemiologists regarding emerging infections may be described in three categories. The first category is surveillance, including recognition of the appearance of a previously unrecognized infection or a new variant of an existing disease. Optimal surveillance requires systematic observations and specific diagnostic criteria to ensure precision. An important part of surveillance is to determine who, when, and where: who is affected (e.g., age and other personal characteristics, contact with others who are ill), when the disorder occurs (e.g., year-to-year variations, season, temporal course of the outbreak), and where it occurs (e.g., geographic locations, urban or rural, local ecology). Second, an important task is to use surveillance and other data to develop an understanding of the epidemiology of the infection, which often provides clues to its etiology and pathogenesis and to approaches to controlling the disease. The third role of epidemiologists is that of monitoring the effects of various control measures, including assessment of the safety and efficacy of new vaccines, such as those for H5N1 influenza, malaria, and AIDS. Chapter 266 contains a description of how the efficacy of vaccines is determined.

The increasing magnitude and speed of international travel enhance the likelihood of global spread of disease and complicate approaches to prevent or contain epidemic infections. Many of the emerging infections are not limited geographically by their ecologic requirements. The nations of the world increasingly depend on each other for surveillance, a task that is not accomplished easily given the logistics; costs (in the face of other needs and priorities, particularly of many developing countries); and the required standardization, collaboration, communication, coordination, and centralized resource for assembly and analysis of surveillance. Although the WHO, the Pan American Health Organization, the U.S. Military and Public Health Service, and various other organizations maintain surveillance systems and laboratories in various parts of the world, these efforts at present are considered to be inadequate except for some infections and in some areas. The development of comprehensive national and worldwide surveillance systems has been urgently recommended.[18]

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Upper Respiratory Tract Infections

CHAPTER 8

The Common Cold

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The common cold is an acute, communicable, viral disease characterized by nasal stuffiness, sneezing, coryza, throat irritation, and no or minimal fever. Although the terms *upper respiratory infection* and *nasopharyngitis* are used frequently as synonyms for the common cold by physicians and other health care workers, the practice should be discouraged; the term *upper respiratory infection* is too broad, and pharyngitis is not present in most colds. To add to the confusion regarding terminology, "a cold" frequently has an even more inclusive connotation to the layperson.

HISTORY

Although the common cold undoubtedly has had an impact on the events of history, the specific symptom complex in ancient times was overshadowed by more severe contagious problems (influenza, plague, smallpox) and by septic diseases (otitis, mastoiditis, pneumonia) that were complications of upper respiratory viral infections. The name *common cold* most certainly arose from the fact that the onset of symptoms included the feeling of chilliness on exposure to cold. This association was perceived as a cause-and-effect relationship. More than 200 years ago, Benjamin Franklin pointed out that colds were caught from other people rather than by exposure to cold.[140]

In 1914, Kruse[114] showed that colds could be transmitted by the nasal instillation in healthy adults of Berkefeld-filtered nasal washings from ill individuals and that the causative agent was smaller than common bacteria. These findings were confirmed clearly in 1930 by Dochez and associates.[51] The way was paved for more extensive study of respiratory viral infections in 1933 when Smith and colleagues[158] reported the isolation and cultivation of an influenza A virus from a human.

The greatest contribution to the present understanding of the common cold has been the use of human volunteers under carefully controlled conditions. The Common Cold Research Unit at Salisbury, England, was established in 1946,[5,179] and volunteer studies at this institution and studies done in the United States* are responsible for the present understanding of colds in adults.

Although studies of respiratory illness in children also have been extensive, controlled virus challenge trials have not been done. Pediatric studies have been most useful in delineating the spectrum of clinical manifestations by age group and seasonal prevalence rates of the different respiratory viruses.

ETIOLOGIC AGENTS

Initial investigations into the etiology of the common cold were based on the hypothesis that only one etiologic agent needed to be discovered. Subsequent studies revealed that many different groups of viruses were involved etiologically and that many types frequently existed within each group. Table 8–1 lists the agents associated with colds. Each of these agents is covered more fully in other chapters; this chapter presents only an overview. As a group, rhinoviruses are the most common cause of colds in children and adults. Also of major importance in the etiology of colds are reinfections with parainfluenza viruses and respiratory syncytial virus (RSV). Although the quantitative contribution of coronaviruses to colds in children is unknown, they probably are significant contributors. More recent studies also indicate that human metapneumovirus causes colds and lower respiratory infections in children.[38,146,185,186]

Enteroviruses and adenoviruses have been implicated frequently in upper respiratory illnesses, but in most instances, the illnesses do not conform to strictly defined colds. Reoviruses cause colds, but their contribution to the overall incidence is unknown. *Mycoplasma pneumoniae*, *Chlamydia pneumoniae*, *Streptococcus pneumoniae*, and *Haemophilus influenzae* infections were identified serologically in one study involving young adults with colds, but in most instances, concomitant viral infections also were noted.[121] The more recently identified human bocavirus also causes coldlike illnesses in some children.[8] Other agents, such as *Coccidioides immitis*, *Histoplasma capsulatum*, *Bordetella pertussis*, *Bordetella parapertussis*, *Bordetella bronchiseptica*, *Chlamydia psittaci*, and *Coxiella burnetii*, also have been associated with illnesses with initial coldlike symptoms.

TABLE 8–1 Infectious Agents Associated with the Common Cold

Category	Agents
Common viruses that usually cause the common cold	Rhinoviruses Parainfluenza viruses Respiratory syncytial virus Coronaviruses Human metapneumovirus
Common infectious agents that occasionally cause illness with common cold symptoms	Adenoviruses Enteroviruses Influenza viruses Reoviruses Human bocavirus
Illnesses with initial symptoms suggestive of the common cold	*Coccidioides immitis* *Histoplasma capsulatum* *Bordetella pertussis*, *Bordetella parapertussis*, *Bordetella bronchiseptica* *Chlamydia psittaci* *Coxiella burnetii*

*See references 1, 25, 41, 44, 46-49, 79-82, 85-88, 101, 102, 108, 124, 128, 172, 173, 175, 177, 178.

TABLE 8–2 Comparative Incidence of Upper Respiratory Infection by Age: London, 1952-1953; Seattle, 1965-1969; and Cleveland, 1948-1950

London		Seattle		Cleveland	
Age (yr)	*Illnesses/Person/Yr*	*Age (Yr)*	*Illnesses/Person/Yr*	*Age (yr)*	*Illnesses/Person/Yr*
0-4	5	<1	3.5	<1	6.9
5-9	3.6	1	3.8	1	8.3
10-16	4.1	2-5	3.7	2-5	8.3
Adult	2.9	6-9	2.7	6-9	6.1
		10-19	1.6	10->11	5.5
		Adult	2.9	Adult	4.8

Data modified from Brimblecombe et al.,[22] Fox et al.,[69] and Badger et al.[10]

EPIDEMIOLOGY

The common cold is an exceedingly frequent illness in childhood. For rhinoviruses alone, more than 100 serologically different viral types can cause this illness. Nonetheless, a general predictability of incidence and seasonal occurrence exists for the common cold. Numerous epidemiologic studies have been conducted on the occurrence of respiratory illnesses, but calculating a precise incidence of the common cold from these studies is difficult because criteria of disease classification have been different. The findings in three carefully performed studies are presented in Table 8–2. Two of these studies were done more than 50 years ago, and the other was done more than 35 years ago. These studies involved a wide range of family life settings, and the average number of colds per year in children varied from three to eight.[10-13,22,69] Adults had on the average approximately one half the number of colds as their children.

In more recent times and in the era of the common use of daycare facilities, studies of the scope and magnitude of those presented in Table 8–2 have not been done. In one study published in the 1980s, the incidence of acute respiratory infections in daycare attendees peaked at 10 per child-year during the second 6 months of life.[46] A study by Wald and associates[181] found that children in home care had 4.7 illnesses per year, whereas the illness rate was 7.1 per year in children in daycare. Of the illnesses, 89 percent in daycare attendees were respiratory infections.

In a study in Arizona, researchers found that 52 percent of 2-year-old children who attended large daycare centers had six to nine colds per year.[15] The conventional spread of colds has its initial focus in the school.[10,13,22] School-age children become infected and introduce secondary infections into the home. Under these conditions, the secondary attack rate was highest in other school-age children and preschool-age children. Generally, the secondary attack rate in adult family members was approximately one half that of the children. The introduction of infection into the family by adults is unusual. In crowded settings, such as a university, infection is common and can be associated with substantial morbidity. In a cohort study of more than 3000 college students, colds and influenza-like illness were common findings and were associated with missed classes, poor performance on class assignments, missed extracurricular activities, and increases in use of medical care.[132]

As noted previously, the present trend toward the use of daycare centers and preschool programs has increased the number of primary infections in younger children and has made them the source from which secondary family infections frequently occur.[130] In a study that involved 606 children who received care at home or in a daycare program during the first 3 years of life, researchers found that children who attended a large daycare facility (six or more unrelated children) had more frequent colds at year 2 and less frequent colds at years 6 through 11 than did the children with home care only.[15]

Close personal contact between children is necessary for the transmission of viruses that cause colds. In the typical pediatric practice office setting, no increased risk for the acquisition of respiratory illnesses by well infants has been shown.[119] Among children, boys tend to have more colds than girls do.[10,22] In the conventional family setting, mothers tend to have at least one more cold a year than their spouses have.[10,22,69,70] The usual incubation period of colds is 1 to 5 days.

In all nonisolated populations, colds occur more frequently during the winter months than during the summer.[50,71,121,179] This seasonal discrepancy in incidence is as apparent in areas of high wintertime mean temperatures as it is in locations with extremely low temperatures. In the tropics, colds are more prevalent during the rainy season. Colds occur throughout the world. In isolated populations in which the number of people is few (e.g., members of Antarctic exploration teams and isolated island communities), colds do not occur unless introduced by a visiting person.[95]

Although colds can be produced regularly in volunteers, the method of transmission of viruses, which results in colds under natural circumstances, is unclear.* In individuals infected with respiratory viruses that cause colds, the greatest concentration of virus is in the nasal secretions. Children tend to have greater concentrations of virus than do adults, and they tend to shed virus for longer periods. Neither secretions related to coughing nor saliva contains appreciable amounts of virus, and both are unlikely sources of contagion. During the process of talking, little virus is disseminated into the air. The greatest amount of virus from an infected individual is contributed to the surrounding environment by sneezing, nose blowing, and the general contamination of external surfaces (including the sufferer's hands) with nasal secretions.

The route of acquisition of virus is by the nose and possibly the conjunctiva. With these facts in mind, one can easily see that a susceptible individual can become infected by the inhalation of virus in droplet nuclei (small particles) resulting from a sneeze, by the direct nasal hit of virus containing large droplets from a sneeze, by nose blowing, or by the inoculation of virus (usually by the fingers of the recipient) from nasal secretions from disseminators that have been transmitted directly or indirectly. In children, most likely the spread either involves close contact with large droplets of nasal secretions containing virus that are applied to the nose from the hands of the future host or occurs by close-range airborne acquisition.

Considerable folklore is related to the catching of a cold. All available evidence to date indicates, however, that cold weather

*See references 23, 26, 41, 44, 45, 49, 79, 81, 85, 93, 101, 106, 124, 150, 168, 179.

per se, chilling, wet feet, and drafts neither cause nor increase the susceptibility of individuals to colds.[52,101] A study of 180 subjects showed that acute cooling of the feet was associated with the onset of common cold symptoms.[107] On day 4, after the cooling of the feet or the control procedure, significantly more subjects in the chilled group thought they were suffering from a cold (14.4%) compared with the control group (5.6%). The subjects who thought they were suffering from a cold had a history of more colds each year compared with subjects who did not develop cold symptoms. In this study, no virologic studies were done, so no evidence exists that the cold symptoms observed were caused by viral infections.

In contrast, in controlled studies in which virologic studies were performed, no increased susceptibility to colds after exposure to chilling conditions was found.[5,52,62] In adults, risk factors for increased susceptibility to colds include stress, smoking, high basal levels of catecholamines, infrequent exercise, low intake of vitamin C, low sleep efficiencies, and lack of diverse social networks.[40,163] Physical stress, as opposed to mental stress, does not seem to be associated with increased susceptibility to colds. In a study of nine healthy men who were competitive cyclists who underwent intensive internal training, the men experienced enhanced performance but had no change in white blood cells, cytokine levels, or the frequency of upper respiratory infections.[56]

PATHOPHYSIOLOGY

The pathophysiology of human infections with viruses that cause colds is presented in the sections of this book covering the individual infectious agents. A general overview is presented here. Few studies on the pathophysiology of respiratory infections have been done in children; the material presented in this section has been derived mainly from studies in adults.* The clinical syndrome of the common cold can occur in association with more than 100 different viral types and in many instances can occur with either a primary infection or a reinfection with a particular viral type.

Although the primary site of virus inoculation in some colds may be on the conjunctival surface, most result from inhalation or self-inoculation of virus onto the nasal mucosa. After acquisition of a virus has occurred, infection of the cells of the local respiratory epithelium develops. This infection varies in the degree of cytopathology on the basis of the viral agent. The infection spreads locally, resulting in an increase in nasal secretions with an increased protein content. Symptoms (nasal stuffiness and throat irritation followed by sneezing) begin on the second or third day and are caused by cellular damage and irritation. Virus shedding is at its maximum in 2 to 7 days, although some shedding may continue for another 2 weeks.

Hilding[94] examined biopsy specimens, scrapings, and smears of nasal secretions and noted that submucosal edema occurred initially, followed by shedding of the ciliated epithelial cells. By the fifth day, the epithelial damage had reached its maximum, with regeneration occurring during the next 10 days. Winther and associates[189] performed similar studies and noted the sloughing of epithelial cells, but they found that the epithelial lining remained continuous with normal cell borders. On the second day of disease, an increase in the number of neutrophils in the epithelium and in the lamina propria occurred. Epithelial mast cells were not involved in the inflammation. The nasal discharge during the second to the seventh day is mucopurulent, owing to its content of desquamated epithelial cells and polymorphonuclear leukocytes.

*See references 9, 36, 53, 109, 133, 136, 148, 155, 168, 178, 179, 188, 193.

In experimental rhinovirus colds in adults, little or no discernible damage to the nasal epithelium has been shown.[80,168,188,190] Arruda and associates[9] noted in adults infected with rhinovirus types 14 and 39 that virus replicates in ciliated and nonciliated cells in the nasopharynx and that only a very small proportion of the cells were infected.

Because damage to the nasal epithelium is not noted in rhinovirus colds, apparently cell death is not the cause of symptoms. Naclerio and colleagues[129] and Proud and associates[147] have found that kinins are generated locally and that their concentrations correlate with the severity of symptoms. The cause-and-effect relationship between kinins and symptoms is questionable, however, because treatment with a bradykinin antagonist failed to lessen symptoms.[176] In addition, steroid therapy was found to reduce the concentration of kinins in nasal wash fluid, but it did not affect clinical symptoms.

More recently, researchers have noted that interleukin-1 (IL-1) may contribute to the pathogenesis of rhinovirus infections.[148] Ongoing molecular and biochemical research has provided information about methods by which rhinoviruses interfere with the host response, induce epithelial expression of IL-6 and IL-8, and mediate infection via activation of kinases and receptor binding activity.[58,111,126,139] Similarly, research into the other viruses of the common cold has helped in the understanding of their similarities and differences, such as the cell-mediated immune response to human metapneumovirus and RSV.[55]

In studies in children with acute upper respiratory infections, IL-1β, IL-8, IL-6, and tumor necrosis factor-α were found to be elevated markedly in nasal lavage fluid.[133] Pacifico and associates[136] found that IL-8 concentrations and white blood cell and neutrophil counts were significantly greater in children with rhinovirus colds than in well children. In an adult volunteer study, Turner and colleagues[178] noted a significant rank correlation between nasal obstruction severity, rhinorrhea severity, and nasal-wash albumin concentrations and the increase in IL-8 concentration from baseline on days 2 to 4 after virus challenge. In a study of 285 children with a parental history of asthma or other respiratory allergy or both, blood specimens were obtained at birth and at 1 year of age.[73] The cytokine responses of mononuclear cells when incubated with phytohemagglutinin, RSV, and a rhinovirus were analyzed. Vigorous IL-13 and interferon-γ responses to phytohemagglutinin and a marked secretion of interferon-γ in response to the viruses were associated with a reduced risk of developing wheezing with viral infections in the first year of life.

Pedersen and colleagues[138] studied nasal mucociliary transport in naturally acquired colds and noted that transport was reduced markedly during the acute illness and that slight impairment remained for approximately 1 month. They point out that because some children have four to six colds during a winter season, these children may have constantly impaired mucociliary transport.

Although viremia has been noted during infections with some of the viruses that cause colds, viremia is not known to occur during the typical common cold. The infection is restricted to the epithelial surfaces of the upper respiratory air passages, including the sinuses and the eustachian tubes. With infection, local interferon is produced and presumably has a major role in controlling the infection.[35] Serum antibody and secretory antibody regularly result from infection. The roles of cell-mediated factors in immunity and disease pathogenesis of colds are unknown.

Levandowski and associates[117] noted in rhinovirus-challenged volunteers that total T cells, and particularly helper T cells, were depressed. The magnitude of this finding correlated with progression of infection and symptoms. In a study in which volunteers received rhinovirus type 39, Skoner and associates[155] found a slight increase in helper ($CD4^+$) and suppressor ($CD8^+$) T cells

during illness. Noting that asthma exacerbations and rhinovirus infections are associated with decreased pH and ammonium levels in exhaled breath condensates, Carraro and colleagues[29] studied whether a direct rhinovirus infection or the host immune response to the infection or both decreased the airway epithelial cell surface pH in vitro. They found that airway epithelial cell pH is affected partly by T-helper type 1 cytokines. This decrease in pH can provide an innate host defense, inhibiting viral replication in the lower airways. By mechanisms not well understood, within already infected cells, low pH is thought to trigger uncoating of rhinoviral RNA from within the endosome, enabling viral replication.[21]

The role of antibody (serum and secretory) in the protection against reinfection and clinical colds is complicated. High levels of secretory and serum antibodies seem to be protective against reinfections.* Clinically abortive colds probably are reinfection colds with early antibody recall. Fleet and colleagues[67] have demonstrated a short-lived heterologous resistance to rhinovirus colds, which probably is not caused by interferon or antibody.

Unexplained constitutional factors also seem to control the clinical manifestations of colds.[152] Although studies have shown genetic disease susceptibility patterns related to tissue types, no studies relating to common respiratory infections have been done.[37] In experimental coronavirus infections in adults, clinical severity correlated with detectable IgE in nasal secretions.[27] This finding suggests that atopy may be related to symptoms in colds caused by coronaviruses. Although clinical symptoms and virologic data suggest that colds are upper respiratory diseases, some studies of pulmonary function also have indicated occult lower respiratory tract involvement.[6,33,142]

CLINICAL PRESENTATION

Because the common cold is caused by more than 100 different viral types, considerable variation in the clinical manifestations occurs. As indicated in the beginning of this chapter, the limits of illness to be considered under the diagnosis of the common cold have been set arbitrarily but rigidly. A disappointing note is that although many comprehensive studies of respiratory viral illnesses of children have been done, little attention has been given to the details of upper respiratory infections.

Illness in children must be considered under two categories—infants and older children. The latter is similar to illness in adults. In studies involving 100 young adults, Jackson and colleagues[99] noted that virtually all patients complained of nasal discharge, nasal obstruction, and sore throat; approximately 80 percent had malaise, postnasal discharge, headache, and cough; slightly fewer than 50 percent reported a feverish feeling and chilliness; and approximately 25 percent noted burning eyes and nasal membranes and muscle aching. In older children, the onset of illness is heralded by dryness and irritation in the nose and a scratchy feeling in the throat.[131] The initial symptoms are followed within a few hours by sneezing and watery nasal discharge; chilly feelings and occasionally muscular aches also are noted. Other complaints include headache, general malaise, anorexia, and low-grade fever.

After a variable period of 1 to 3 days, the illness changes; the nasal secretions become thicker and frequently develop a purulent appearance. Persistent nasal discharge, associated with the trauma of repeated blowing of the nose, leads to excoriation around the nose. Nasal obstruction leads to mouth breathing, which causes drying of the throat, increasing the discomfort in the throat. The usual duration of illness is approximately 7 days, but lingering cough and nasal discharge may persist for 2 weeks or more.[54]

In infants, the manifestations of illness may be more varied. The onset of illness in infants is associated more often with fever (38° C to 39° C [100.4° F to 102.2° F]) than it is in older children. Nasal manifestations in infants are similar to manifestations in older children, but the only other manifestations are irritability and restlessness. Occasionally, coryza is the only symptom. Nasal obstruction may interfere significantly with feeding and sleeping. Vomiting and diarrhea also may occur.

DIFFERENTIAL DIAGNOSIS

Because the clinical entity of the common cold is an arbitrary grouping of signs and symptoms limited to anatomic boundaries and is caused by many different viral types, the approach to the differential diagnosis must consider clinical and etiologic criteria. Many upper respiratory illnesses are caused by numerous infectious agents that should not be confused with colds. A diagnosis of the common cold should not be considered if objective pharyngitis, other enanthema, or evidence of obstructive airway disease is present. Because the common cold is an acute, self-limited disease, the diagnosis should not be considered in a child who has persistent nasal signs or symptoms. Subacute or chronic illness should suggest the possibility of adenoiditis or sinusitis.

The most important differential diagnostic considerations are clinical entities of noninfectious etiology. Allergic rhinitis is a particularly important possibility in a child with "recurrent colds." Careful attention to family history, a search for allergies, the presence or absence of nasal eosinophilia, and the serum IgE value help confirm or exclude this consideration.

Although not reported particularly in pediatric patients, mental stress can lead to vasomotor responses and rhinitis in some susceptible patients. Chemical irritants can cause coldlike symptoms; the clinical response varies greatly among different individuals. Early symptoms of many illnesses, such as pertussis, epiglottitis, measles, and diphtheria, are those of a cold, but in a short time, the more serious nature of the actual illness becomes apparent.

SPECIFIC DIAGNOSIS

The epidemiologic history is the most important aspect of specific diagnosis. In children, if exposure history is requested, a contact usually is uncovered. If strict attention to clinical criteria of the common cold has been adopted, routine laboratory study is unnecessary. Frequently, the physician has an urge to take a throat culture to rule out the possibility of group A streptococcal infection. Usually, one is unnecessary because nasal symptoms are not characteristic of acute streptococcal illness except in infancy, and pharyngitis is not within the limits of the diagnosis of the common cold. The white blood cell count also is of little use.

A specific diagnosis can be made by the isolation of virus from the nasal secretions. It is performed best by using either a nasal-wash technique[84] or a nasopharyngeal swab or aspirate. With laboratory techniques of diagnostic virologic facilities such as those in many university hospitals, parainfluenza viruses, RSV, and most rhinoviruses and influenza viruses are recovered. Direct antigen-detection techniques can be used to identify infections caused by RSV, parainfluenza viruses, adenoviruses, and influenza viruses. Coronaviruses and some rhinoviruses and influenza strains can be recovered only by special laboratory techniques.

*See references 32, 34, 67, 99, 100, 102, 103, 116, 156, 167.

TREATMENT

Although literally hundreds of cold remedies are available, few offer any benefit to the pediatric patient, and many may be harmful.* No clinically available antiviral agents have been shown to be effective in the treatment of colds.

In the approach to a child with a cold, the best assumption is that no therapy is indicated in most cases. Specific symptomatic care can be added in the individual case when it is needed. Many children and adults feel miserable when they have a cold, and therapy with an analgesic often is used. Because aspirin is a risk factor for Reye syndrome in children, the use of acetaminophen rather than aspirin is prudent. The dose per single administration of acetaminophen by year of age is the following: younger than 1 year, 60 mg; 1 to 3 years, 60 to 120 mg; 3 to 6 years, 120 mg; 6 to 12 years, 150 to 300 mg; older than 12 years, 325 to 650 mg. Administration may be repeated three to four times daily in young children and every 4 hours in older children. Acetaminophen rarely should be given to infants younger than 6 months of age.

In adult volunteers with rhinovirus infections, acetaminophen was found to be associated with suppression of the serum neutralizing antibody response, and an increase in nasal symptoms was noted compared with subjects who received a placebo.[77] In another adult volunteer study, administration of naproxen resulted in a reduction in headache, malaise, myalgia, and cough compared with placebo.[161]

Relief of nasal obstruction is the most important therapeutic consideration in young children. Locally applied or orally administered, systemically active decongestants are used frequently, but neither their true efficacy nor their adverse effects have been evaluated carefully. Excessive use of sprays and drops with vasoconstrictive drugs can lead to rebound obstruction, which prolongs the illness. The associated drying effect of vasoconstrictive drugs administered orally can be expected to be deleterious to normal mechanisms of clearance. In young infants, sympathomimetic-antihistamine mixtures in oral drop dosage forms particularly are dangerous because respiratory depression may occur.[75] In addition, in a controlled trial, brompheniramine maleate–phenylpropanolamine hydrochloride was found to be no better than a placebo in relieving cold symptoms in children 6 months to 5 years of age.[39] If vasoactive drugs are used, their use should be restricted to times when maximum benefit would occur (i.e., bedtime), and they should be discontinued within 3 days.

The use of isotonic saline drops and gentle aspiration can be very effective in the temporary relief of nasal obstruction in an infant. Also useful is the general humidification of room air because this moisture tends to dilute tenacious nasal mucus so that its elimination is facilitated.

Antibiotics have no place in the routine therapy of common colds.[76,97,105,112,115,118,135,151,159] Occasionally in children, persistent cough is a problem of such magnitude that it disturbs sleep. For cough, codeine and dextromethorphan often are used.[3] No well-controlled studies support either the efficacy or the safety of codeine or dextromethorphan as an antitussive in children, however. Eccles[61] suggested that the apparent efficacy of liquid cough syrups may be due to the sweetness of the products rather than their active ingredients. The proposed mechanism is that a sweet taste may effect cough at the level of the nucleus tractus solitarius by stimulating the production of endogenous opioids.

In the past, antihistamines often have been given to children with colds, but efficacy had not been shown.[184] In more recent years, first-generation antihistamines, but not second-generation products, have been shown to lessen rhinorrhea in adults with colds.[127,128,168,169,176] Doxylamine succinate, clemastine fumarate, chlorpheniramine maleate, and brompheniramine all have been shown to offer benefit in controlled trials. The effect of these antihistamines is due to their anticholinergenic properties. To date, no studies in children have been reported.

A major controversy relates to the efficacy of vitamin C in the common cold prophylactically and therapeutically. In two carefully controlled volunteer studies, the administration of 3 g of ascorbic acid per day did not prevent or alter the symptoms of experimental colds.[153,183] In addition, several large controlled trials in which vitamin C and placebo preparations have been used to prevent and to treat colds have been conducted.[4,28,42,43,63,108,143] In some of these studies, some benefit was reported, whereas in others, no efficacy was noted. Most probably, the reported benefits are a result of statistical artifacts and placebo effect owing to poor study design, rather than specific pharmacologic drug effects. The antihistaminic action of vitamin C[180,194] probably afforded relief to some patients with allergic rhinitis, who thought that their illnesses were colds. Because of the many toxic effects of ascorbic acid,[16] and because its use in treating respiratory illnesses is questionable at best, giving children vitamin C in excess of normal daily requirements seems unwarranted.

For many years, efforts have been made to develop effective rhinovirus chemotherapy, but showing convincing efficacy in natural infection has been a challenge.[137] Interferon alfa-2b administered intranasally has been shown to have some efficacy in the prevention of rhinovirus colds in controlled clinical trials.[160] The effect is variable, however, and adverse effects of the medication are frequent occurrences.[164] Intranasal interferon alfa-2b was ineffective in the treatment of naturally occurring colds but showed some benefit in experimental coronavirus colds.[89,173] Studies in adults using an antiviral anti-inflammatory combination for the treatment of the common cold showed some benefit compared with either agent used alone.[83] However, the irritation caused by intranasal interferon alfa-2b and the drowsiness associated with first-generation antihistamines render these drug combinations suboptimal.

Zinc lozenges have been used to treat the common cold, and the internet and lay literature are full of claims of efficacy. In well-done controlled studies, efficacy has not been shown, however.[59,60,65,104,120,144,172,181] The use of intranasal zinc preparations also is not well validated and raises some concern because of the possible development of the zinc-induced anosmia syndrome.[2,19] In a double-blind, placebo-controlled trial, the use of intranasal corticosteroid (fluticasone propionate) offered no clinical benefit in young adults with colds and induced prolonged shedding of rhinovirus.[149]

In a controlled trial in adults, a soluble intercellular adhesion molecule-1 product (tremacamra) reduced the severity of rhinovirus colds.[177] The antiviral pleconaril induced an early reduction in severity of symptoms in adults with colds caused by rhinovirus.[90] Pleconaril is an antipicornavirus drug that interacts directly with viral capsid proteins. It blocks attachment of virus to cells through intercellular adhesion molecule-1 and subsequent uncoating and release of viral RNA.

In another randomized, double-blind, placebo-controlled trial, the symptomatic efficacy of pleconaril was linked to its in vivo antiviral effects and to the drug susceptibility of the infecting virus.[140] In March 2002, the Antiviral Drugs Advisory Committee of the U.S. Food and Drug Administration voted against recommending pleconaril for approval of its use in the treatment of the common cold in adults. This decision was based on drug interactions, poor risk-to-benefit ratio, and concerns of development of resistant virus.[137]

Clarithromycin, a macrolide antibiotic, enhances mucosal immunity in mice by increasing levels of IL-12, IgA, and IgG.[110]

*See references 2, 7, 14, 18-20, 24, 30, 31, 47, 57, 59, 60, 72, 74, 75, 83, 92, 96, 98, 113, 122, 123, 134, 137, 141, 145, 157, 162, 165, 170, 181, 192.

In a controlled trial, clarithromycin had no effect on the severity of cold symptoms, however. The intranasal administration of nedocromil sodium has been observed to have a beneficial effect on rhinoviral infections in adult volunteers.[17] Mucolytics have emerged as potential therapeutics for modulating the function of airway epithelial cells and altering the course of viral infections.[8,192] Leukoprotease inhibitors and pulmonary surfactant have been shown to be up-regulated by ambroxol—a mucolytic and antioxidant agent.[7,134]

In one study, adults who took sauna baths once or twice a week were found to have fewer colds than did members of a non–sauna-bathing control group.[64] In another study, volunteers with colds did not benefit from inhaling heated vapor.[68]

The list of available and proposed complementary and alternative medicines thought to be useful for the common cold is long.[20,24,57,191] Blinding subjects in placebo-controlled trials can be difficult, and the placebo effect seems to play a significant role in the popularity of all complementary and alternative medicines.[18,20,31,47,92,170] Fluid extracts of *Echinacea* spp. are popular for the prevention and the treatment of colds.[78,168,175] Because several species of *Echinacea* have been used in prevention and treatment studies, criticism of the negative results in some studies has been expressed by *Echinacea* advocates.[20,30,74,113,154,162,165,170]

In a controlled trial, Taylor and associates[166] evaluated *Echinacea* for the treatment of upper respiratory tract infections in children aged 2 to 11 years old. No benefit was noted, and the *Echinacea*-treated children had an increased occurrence of rash. Preparations of North American ginseng have been reported to decrease the frequency of colds in adults.[14,123,145] No controlled studies have been done in children.

PROGNOSIS

The prognosis of common colds in children is excellent. Secondary complications do occur, however, and frequently they require careful and prolonged therapy. The most common complications are otitis media, sinusitis, bacterial adenoiditis, bacterial pharyngitis, and lower respiratory bacterial infections. The primary viral pathogens identified in hospitalized children with lower respiratory tract infections include RSV, influenza A and B viruses, human matapneumovirus, parainfluenza viruses, adenoviruses, rhinoviruses, and enteroviruses.[112] Infections with multiple agents also occur.

PREVENTION

Studies in isolated populations have shown that when a particular respiratory viral infection has run through the entire group, no further respiratory viral illnesses can occur until a new infected individual enters the population. This type of evidence indicates that quarantine or isolation-type practices could prevent colds. The average urban society of today is so complex, however, that prevention through isolation procedures is impractical. Efforts to control the spread of respiratory virus should be minimal and practical. For children with undue susceptibility to complications, contact with crowds or with infected children and adults should be avoided.

The use of virucidal nasal tissues has been shown to reduce markedly the spread of rhinovirus colds in human volunteers and to reduce modestly colds in the family setting, but as yet no commercial products are available.[48,66] Heikkinen and associates[91] found that the intranasal administration of an immunoglobin preparation by nasal sprays twice a day significantly reduced the occurrence of rhinitis in children attending daycare centers. If confirmed, this form of prophylaxis might be useful for selected children. A study showed that organic acids commonly used in over-the-counter skin care and cosmetic products had substantial virucidal activity against rhinoviruses. The amount of acid applied to the hands correlated directly with the prevention of infection in the deliberate infection model.[171,174]

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CHAPTER 9

Infections of the Oral Cavity

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Although most infections of the oral cavity in children are odontogenic and may be treated simply with local measures, the occasional spread of these infections to adjacent or distant fascial spaces or to the maxilla and mandible may result in life-threatening complications. Consequently, careful attention, including liberal use of the dental consultation, should be given to such infections.[16,53]

MICROBIOLOGIC CONSIDERATIONS IN DENTAL INFECTIONS

NORMAL FLORA

That the oral cavity provides an environment favorable to the growth of microorganisms is substantiated by reports of bacterial counts of 10^8 to 10^{11} organisms/mL of saliva.[3,6] More than 30 species of bacteria normally can be identified in saliva in varying proportions, depending on a dynamic interaction of different microbial ecosystems, including the tongue, the gingival crevice, and the presence of plaque.[50,57] Age, anatomic relationships, eruption of teeth, presence of decayed teeth, diet, oral hygiene, antibiotic therapy, systemic disease, cancer chemotherapy,[51] and hospitalization can modify the microbial population. In the older literature, emphasis was placed on the role of *Streptococcus* and *Staphylococcus* spp. in producing odontogenic infections, to the exclusion of most anaerobic bacteria. This emphasis probably was the result of failure to culture satisfactorily for anaerobic organisms, and it is now well known that the ratio of anaerobic to aerobic organisms ranges from 3 : 1 to 10 : 1.[3,7]

The nomenclature of the oral flora is changing rapidly owing to the improved understanding of the genetic make-up of these bacteria provided by molecular biology techniques. Table 9–1 summarizes changes in nomenclature among selected members

TABLE 9–1 Terminology Changes for Selected Oral Pathogens

Older Terminology	Current Terminology
Streptococcus viridans	*Streptococcus anginosus*
	Streptococcus intermedius
	Streptococcus constellatus
	Streptococcus mutans
	Streptococcus sanguis
	Streptococcus mitis
	Streptococcus salivarius
	Streptococcus vestibularis
Streptococcus milleri	*Streptococcus anginosus*
	Streptococcus intermedius
	Streptococcus constellatus
Bacteroides melaninogenicus	*Prevotella melaninogenica*
	Prevotella intermedia
	Prevotella oralis
	Prevotella buccae
	Prevotella denticola
	Prevotella nigrescens
	Porphyromonas asaccharolytica
	Porphyromonas gingivalis
	Porphyromonas endodontalis
	Porphyromonas salivosa
	Porphyromonas circumdentaria
Streptococcus faecalis	*Enterococcus faecalis*
Streptococcus faecium	*Enterococcus faecium*
Peptococcus species	*Peptostreptococcus* species (main oral pathogen is *P. micros*)

TABLE 9–2 Most Frequent Pathogens Isolated from Orofacial Infections in Two Studies

	Percentage of Cases	
Microorganism	***Lewis et al.****	***Sakamoto et al.**†*
Streptococcus milleri	50	65
Peptostreptococcus species	64	65
Other anaerobic streptococci	8	9
Bacteroides (Prevotella) oralis	40	74
Bacteroides (Prevotella) gingivalis	28	‡
Bacteroides (Porphyromonas) melaninogenicus	24	17
Fusobacterium species	14	52

**Data from Lewis, M. A. O., MacFarlane, T. W., and McGowan, D. A.: Quantitative bacteriology of acute dentoalveolar abscesses. J. Med. Microbiol. 21:101-104, 1986.*
†Data from Sakamoto, H., Kato, H., Sato, T., and Sasaki, J.: Semiquantitative bacteriology of closed odontogenic abscesses. Bull. Tokyo Dent. Coll. 39:103-107, 1998.
‡This organism was not reported in this study.

of the oral flora.[8,61,62] Molecular methods based on the polymerase chain reaction allow direct identification of bacterial species to be made from the oral flora and odontogenic infections by isolation of their DNA or RNA or both. These methods have led to appreciation of the true oral flora, for which 60 percent of species are unculturable. In recent years, many new species and phylotypes have been identified in the normal and pathologic oral flora.

The flora of children is similar to that of adults, with several exceptions. At birth, the oral cavity is sterile, but colonization with *Streptococcus salivarius* occurs rapidly. This organism has been found in 80 percent of cultures taken from 1-day-old infants.[63] The percentage of *Streptococcus* spp. decreases from 98 percent at day 1 to 70 percent at 4 months[48] as other organisms become established. *Staphylococcus* spp., *Neisseria*, *Veillonella*, *Actinomyces*, *Nocardia*, *Fusobacterium*, *Bacteroides*, *Corynebacterium*, *Candida*, and a variety of coliforms gradually become established by the time the child reaches 1 year of age. As the deciduous dentition erupts, anaerobic organisms become well established in the gingival crevice, yet the spirochetes, *Bacteroides* and *Prevotella* spp., and related oral anaerobes, which commonly are associated with the gingival crevice in adults, seem to be present in fewer numbers in patients younger than 13 to 16 years.[6,63] Eruption of deciduous teeth also is associated with the establishment of *Streptococcus mutans* and *Streptococcus sanguis*, which adhere to the enamel surface.

PATHOGENIC ORGANISMS

Not all residents of the oral flora are pathogens. In the odontogenic infections caries and periodontal disease, a progression from initiating infections caused by oral streptococci toward a predominance of oral anaerobes in the more severe and long-standing infections apparently occurs. Caries is initiated primarily by *S. mutans*, a member of the alpha-hemolytic *Streptococcus* viridans group. As tooth decay progresses toward the dental pulp, *Lactobacillus* and *Actinomyces* spp. join the carious milieu. Severe pulpal infections generally are caused by a combination of these same oral facultative streptococci plus obligate anaerobes, such as *Porphyromonas endodontalis*, formerly classified as *Bacteroides melaninogenicus*.[66]

Periodontal infections also are polymicrobial; gram-positive aerobes, primarily streptococci, predominate in gingivitis, and the gram-negative anaerobic rods predominate in bone-destroying periodontitis. Juvenile periodontitis (formerly called *periodontosis*), a particularly aggressive periodontal infection in children and adolescents, shows a predominance of *Actinobacillus actinomycetemcomitans* in its cultivable flora.

Orofacial odontogenic infections that spread beyond the teeth and alveolar processes are polymicrobial, yielding on average four to six isolates per case.[5,34,46] With the use of molecular methods, even greater numbers of species can be identified in these infections, ranging from 5 to 18 species per case.[14] Severe orofacial infections have been associated statistically with *Fusobacterium nucleatum*.[24] The concept of the progression from aerobic streptococci to anaerobic gram-negative rods in orofacial infections is supported further by studies that have found a predominance of streptococci in early infections (in the first 3 days of symptoms) and a predominance of anaerobes in late infections.[34] Table 9–2 lists the frequency with which the major pathogens in orofacial infections were isolated in two studies.[26,36,59]

Infections originating from nonodontogenic causes (facial trauma, surgical manipulation, tonsillitis) are included in most studies of soft tissue and fascial space infections, and contamination from the skin or oropharynx might allow aerobic organisms, such as *Staphylococcus aureus* and aerobic *Streptococcus* spp., to become established.[6] In contrast, infections originating solely from the dental periapical tissues are much more likely to be predominantly anaerobic.

A pitfall in the identification of organisms as described in the older literature was the failure to culture satisfactorily for anaerobic organisms. The more current literature recognizes this fact.[37,49] The preponderance of anaerobic organisms in odontogenic infections mandates the use of anaerobic and aerobic culturing techniques in situations in which cultures are indicated.

ANATOMIC CONSIDERATIONS

Most severe orofacial infections develop consequent to dental infection—periapical, periodontal, or pericoronal. Spread occurs along anatomic pathways of least resistance.[3,7,27,35,65] Periodontal and pericoronal infections rarely have major sequelae because

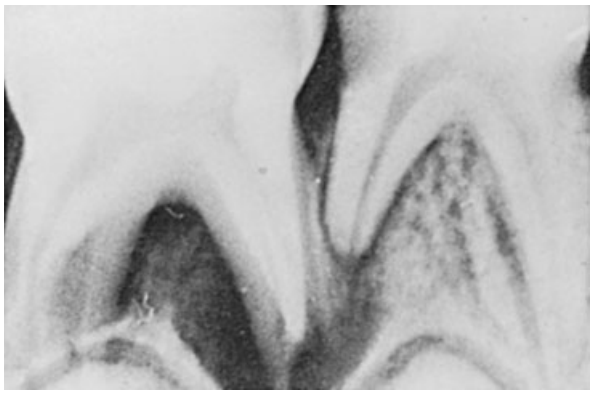

Figure 9–1 Radiolucency representing a chronic periapical abscess involving the mesial root of the deciduous second molar and the distal root of the deciduous first molar. The developing mandibular bicuspids are seen inferior to the deciduous roots. The cause of the abscess is the deep carious lesions in both teeth, which appear to have penetrated the pulp chambers.

they generally drain from the gingival sulcus along the surface of the tooth into the oral cavity. Infections associated with the root apices generally are confined within the bony alveolar process (Fig. 9–1). Should spontaneous intraoral drainage occur through either the periodontium or the pulp chamber, further spread through the marrow spaces is unlikely to occur. If such drainage does not occur, spread through bone (osteomyelitis) or perforation of the cortical plate of the affected jaw may occur. Infections associated with root apices close to the buccal cortical plate generally spread buccally, whereas infections close to the lingual or palatal cortical plate or to the maxillary sinus spread in those directions (Fig. 9–2). When penetration of the cortical plate occurs, infection involves the adjacent soft tissues and may manifest as cellulitis or a soft tissue abscess, which eventually may perforate mucous membrane or skin as a sinus tract (Fig. 9–3).

Perforation of periapical infections through bone follows a typical pattern that results from the position of the root apices in relation to the bony cortex and to muscle attachments (Fig. 9–4). Infections involving maxillary anterior teeth and buccal roots of maxillary posterior teeth generally perforate labially or buccally, whereas infections involving palatal roots of posterior teeth perforate palatally or rarely into the maxillary sinus. The presence of the buccinator muscle attachment superior to the root apices usually confines these infections and fistulas to the oral cavity. In children, maxillary root apices often are superior to the buccinator, however, and infections may spread to the buccal or infraorbital space or to the periorbital tissues. They eventually may drain through the skin.

Infections of the mandibular incisor or canine tooth may spread either labially or lingually because the alveolar process is thin in this area. Labial perforation, which occurs more commonly, may be confined intraorally if the root apices are superior to the origin of the mentalis muscle but may spread extraorally if the apices are inferior to the mentalis attachment (Fig. 9–5). Infections of the mandibular premolar and first molar often perforate buccally, whereas the second and third molars perforate lingually.

When spread of mandibular infections occurs medially, the relationship of the tooth apices to the mylohyoid muscle origin is significant (Fig. 9–6). From the first molar forward, the dental root apices are superior to the mylohyoid, and these infections localize intraorally in the floor of the mouth (sublingual space). The apices of the second and third molars generally are inferior to the mylohyoid, and so the submandibular space is involved, with an extraoral presentation. As in maxillary infections, the relationship of the buccinator muscle to the root apices determines whether the infection spreads intraorally or extraorally.

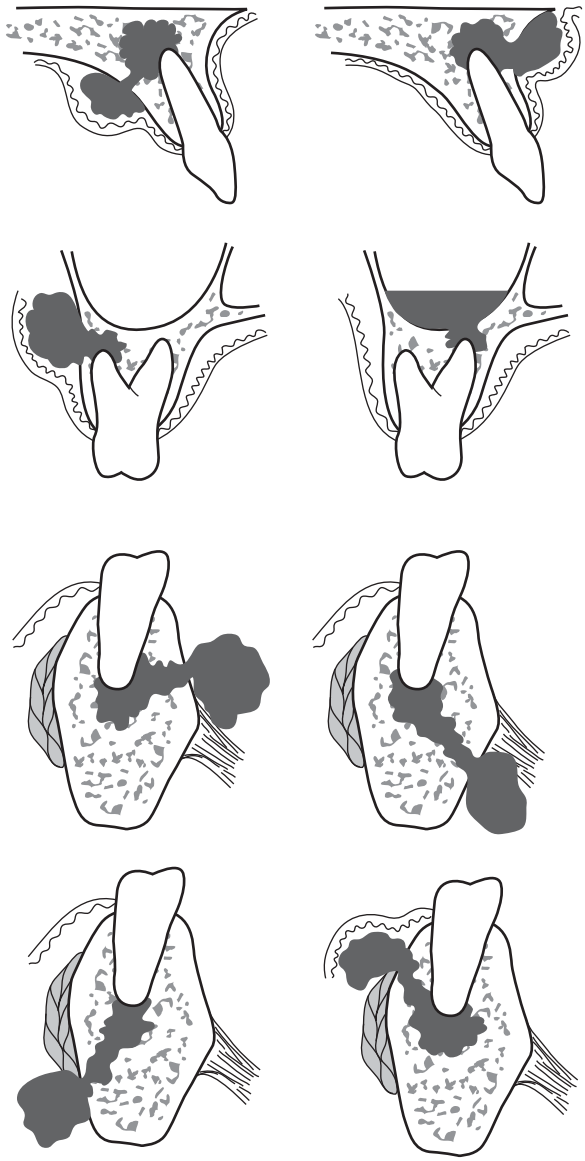

Figure 9–2 Possible pathways of spread of periapical infection. *(From Shafer, W. G., Hine, M. K., and Levy, B. M.: Textbook of Oral Pathology. 2nd ed. Philadelphia, W. B. Saunders, 1963.)*

Two fascial spaces commonly associated with odontogenic infections are the submandibular and masticator spaces.[19,20,35] The submandibular space is formed within the superficial layer of deep cervical fascia inferior to the mylohyoid muscle and inferomedial to the mandible. Anteriorly and posteriorly, it is limited by the bellies of the digastric muscle. Within this space lies the submandibular gland and portions of the facial artery and anterior facial vein. This space is closely approximated to the sublingual and masticator spaces. Infections of the submandibular space may originate in these adjacent spaces and in mandibular posterior teeth.

The masticator space also is formed within the superficial layer of deep cervical fascia. Its name is appropriate because its contents include the masseter, internal and external pterygoid, and temporalis muscles, as well as the mandibular ramus and the inferior alveolar neurovascular bundle. The submandibular, lateral pharyngeal, and retropharyngeal spaces are adjacent.

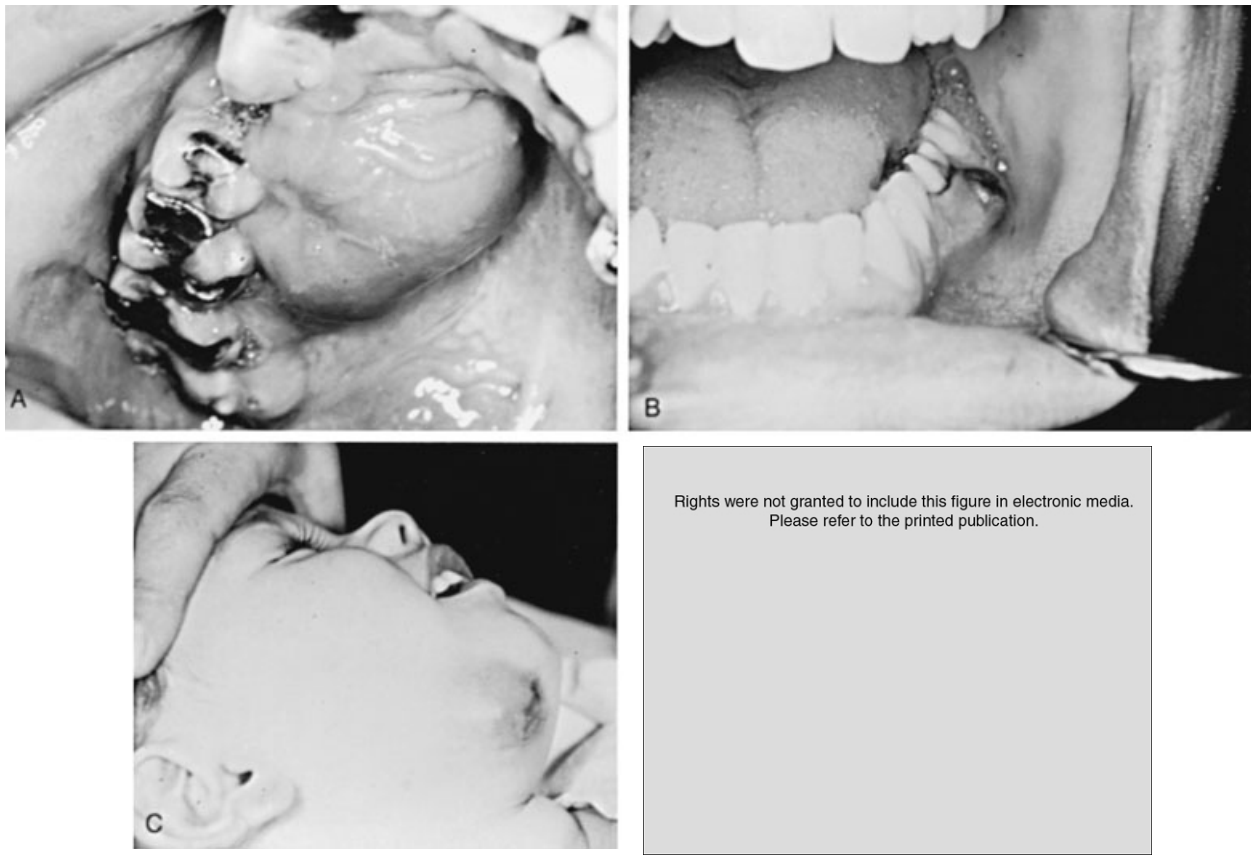

Figure 9–3 Spread of odontogenic infection. **A,** Palatal abscess resulting from infected first premolar. **B,** Intraoral mucosal fistula from periapical abscess of mandibular left first molar. **C,** Soft tissue infection secondary to periapical abscess. **D,** Draining cutaneous sinus tract from a chronically infected lower molar in an adolescent girl. *(**A** from Piecuch, J.: Odontogenic infections. Dent. Clin. North Am. 26:129-145, 1982. **D** from Flynn, T. R., and Topazian, R. G.: Infections of the oral cavity.* In *Waite, D. E. [ed.]: Textbook of Practical Oral and Maxillofacial Surgery. Philadelphia, Lea & Febiger, 1987.)*

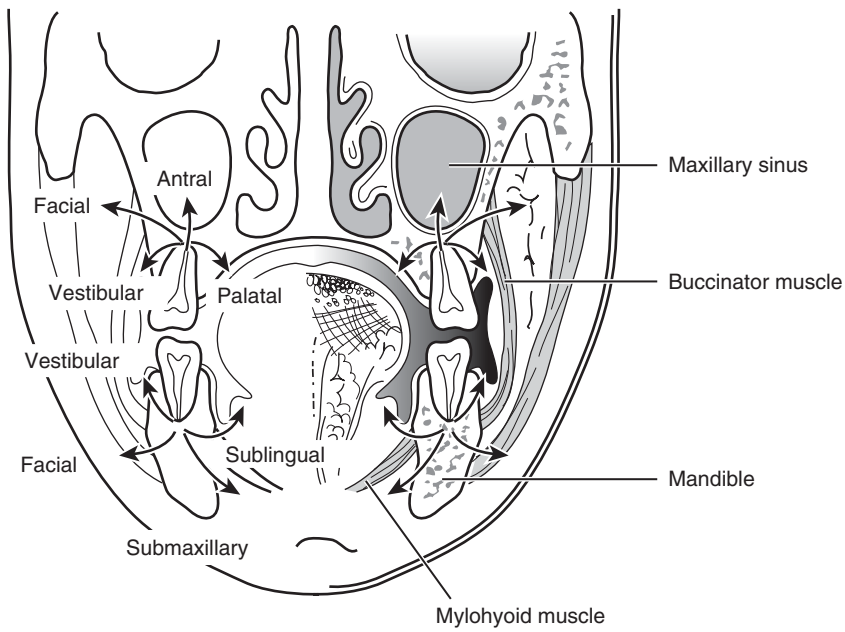

Figure 9–4 Common pathways of spread of periapical infection. *(From Kruger, G.: Textbook of Oral Surgery. 4th ed. St. Louis, C. V. Mosby, 1980.)*

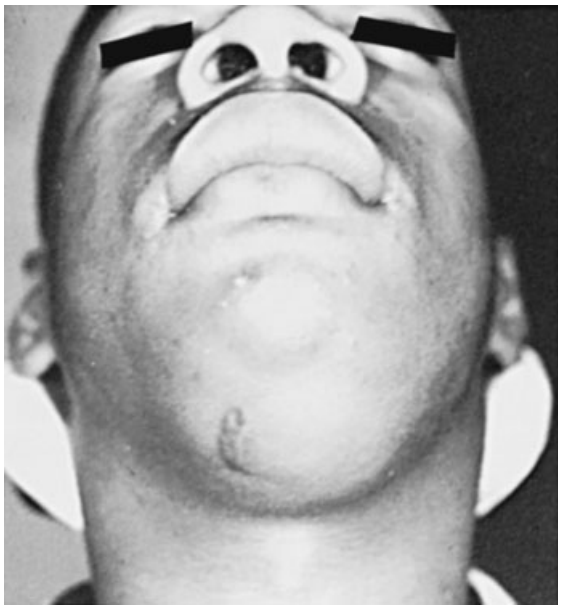

Figure 9–5 Submental space abscess.

Figure 9–6 Relation of tooth apices to the origin of mylohyoid muscle. *(From Waite, D.: Textbook of Practical Oral Surgery. Philadelphia, Lea & Febiger, 1978.)*

Infections of the masticator space may originate in adjacent spaces or spread to it from periapical or pericoronal infections of the mandibular second and third molars and maxillary third molar.

TREATMENT OF ODONTOGENIC INFECTIONS

Patients with odontogenic infections may present with symptoms ranging from minor to life-threatening. Too often, a patient may be given a thorough systemic and extraoral head and neck evaluation while the intraoral search for the etiologic agent is overlooked.

A thorough oral examination begins with an evaluation of the degree of mandibular opening. Interincisal distance on wide opening extends 40 mm or more, even in young children. Painful limitation of the oral opening, or trismus, is associated with inflammation of the muscles of mastication and indicates spread of the infection to the masticator space. In association with a high fever, it can represent a serious turn of events. Teeth are inspected visually for caries by percussion for tenderness and by electric sensitivity or hot and cold stimulation for the pulpal pain response. Gingival tissues are probed for periodontal defects, and salivary glands are palpated for tenderness and milked to observe for purulent discharge from the duct orifices.

GENERAL THERAPEUTIC PRINCIPLES

As with infections elsewhere in the body, the principles of treatment of oral infections involve surgical drainage and antibiotics. Surgical drainage may comprise standard incision and drainage of an orofacial swelling or, in the case of localized periapical infection, endodontic drainage through the pulp or extraction of the offending tooth.

Surgical treatment of odontogenic infections is primary. Dodson and colleagues,[10] in a review of head and neck infections requiring hospitalization of children, found that facial infections of the regions at or above the level of the upper lip and teeth most frequently were upper respiratory or sinus related and that lower face infections primarily were odontogenic. Infections of the upper face resolved without surgery in 65 percent of cases, whereas infections of the lower face resolved without surgery in only 25 percent of cases. Odontogenic infections almost always required some sort of surgical intervention. This finding may be due to the fact that the portal of entry in respiratory infections is through the surface mucosa, whereas the tooth roots carry the invading bacterial pathogens deep into the bone of the jaw, through which the surrounding deep fascial spaces become infected.

Respiratory pathogens frequently are viral, and odontogenic infections almost uniformly are bacterial, which may explain the propensity of odontogenic infections to form abscesses that need to be drained. Odontogenic infections treated with antibiotics only almost always recur in worse form than did their previous manifestation. The indications for treating with antibiotics in addition to appropriate dental surgical therapy are fever, trismus, lymphadenopathy, osteomyelitis, and compromise of the immune system. Minor infections localized to the alveolar processes can be treated by tooth extraction, gingival curettage, or root canal therapy, with or without intraoral incision and drainage, without the use of antibiotics in the non-immunocompromised individual.

Antibiotic selection for odontogenic infections, although ultimately based on Gram stain and aerobic and anaerobic cultures, generally is begun empirically before culture results are available. Penicillin G or penicillin V is the logical first choice for outpatient infections on the basis of lack of toxicity, bactericidal nature, and sensitivity of most streptococci and oral anaerobes to penicillin. Antibiotic sensitivity studies indicate that the oral anaerobes now are largely resistant to erythromycin and most cephalosporins.[52] A study of severe (hospitalized) odontogenic infections found penicillin-resistant organisms in 54 percent of cases and therapeutic failure of empiric penicillin in 21 percent of cases.[13] The duration of previous therapy with β-lactam antibiotics also has been correlated with increased numbers of β-lactamase–producing bacteria in persisting infections. Patients with persisting infection after 3 days of treatment with a β-lactam antibiotic had a 50 percent incidence of β-lactamase–producing bacteria in the infection.[23,31] Clindamycin remains highly effective against the likely oral pathogens, including streptococci and the oral anaerobes; its association with *Clostridium difficile* colitis and its effectiveness in severe orofacial infections indicate that it should be reserved, in this era of increasing microbial resistance to antibiotics, for the most severe cases.

TABLE 9–3 Empiric Antibiotics of Choice for Odontogenic Infections*

Type of Infection	Antibiotic of Choice
Outpatient infections	Penicillin
	Clindamycin
	Azithromycin
Penicillin allergy	Clindamycin
	Azithromycin
Inpatient infections	Clindamycin
	Ampicillin + metronidazole
	Ampicillin + sulbactam
Penicillin allergy	Clindamycin
	Third-generation cephalosporin IV (if the penicillin allergy was not the anaphylactoid type—use caution)

**Empiric antibiotic therapy is used before culture and sensitivity reports are available. Cultures should be taken in severe infections that threaten vital structures.*

The aforementioned considerations suggest that the empiric antibiotics of choice are penicillin in mild odontogenic infections and clindamycin for severe cases or in a patient with penicillin allergy. Table 9–3 lists our recommendations for empiric antibiotic therapy in odontogenic infections.

Second-line antibiotics in odontogenic infections are the cephalosporins, to which effectiveness against the oral anaerobes has been waning, and metronidazole, which is effective against obligate anaerobes only. The safety and effectiveness of metronidazole in children have been established only for treatment of amebiasis.

Other considerations that may be important in the selection of antibiotics for individual cases are as follows: (1) *Eikenella corrodens*, an occasional pathogen in odontogenic infections, is uniformly resistant to clindamycin, which may explain the lack of effectiveness of clindamycin in some cases. (2) Some cephalosporins do not cross the blood-brain barrier in high concentrations, which may be a factor in selection of an antibiotic for an odontogenic infection that is approaching the cranial cavity. Ceftazidime and ceftriaxone cross the blood-brain barrier well. Penicillin is able to cross the blood-brain barrier when the meninges are inflamed. Metronidazole crosses the blood-brain barrier, and its use may be justified in severe odontogenic infections approaching the brain in children. (3) Tetracycline is incorporated permanently into newly formed dentin, causing permanent disfiguring discoloration of the dentition. It should not be used in children until they are at least 9 years old, when all but the third molar teeth would have full crown formation. (4) β-Lactamase inhibitors used in combination with β-lactam antibiotics may improve their effectiveness against resistant anaerobes. A clear clinical advantage of amoxicillin-clavulanate over amoxicillin alone has not been established in adult studies of odontogenic infections, however.[36] (5) Staphylococci are uncommon pathogens in odontogenic infections, and coverage for staphylococci is not indicated in empiric therapy for these infections, although their role in upper respiratory and sinus infections is well known.

NURSING BOTTLE CARIES

Nursing bottle caries is a pattern of tooth decay affecting mainly the primary upper incisors and frequently the upper and lower primary molars in children of bottle-feeding age. It is caused by a practice of putting the child to bed with a nursing bottle filled with a sugar-containing drink, such as milk, fruit juice, or a soft drink. The child sucks on the bottle intermittently during sleep, when salivary secretion is low, and the sugar-containing liquid stays in the mouth for extended periods. This situation provides an excellent environment for the growth of caries-producing organisms, such as *S. mutans*. Nursing-bottle caries can destroy virtually the entire primary dentition of a child as it erupts. Pediatric physicians and dentists should instruct parents to avoid putting their children to bed with nursing bottles or, if they must do so, to use water only in the bedtime drink.

PERIAPICAL ABSCESS

Extension of microorganisms through the root apex leads to the formation of an abscess. Early in this process, the acute abscess is indistinguishable clinically and radiographically from an acute pulpitis, particularly because radiographic evidence of bone destruction may take 7 to 14 days or more to develop. Sensitivity to heat stimulus (relieved by cold), exquisite sensitivity to percussion, and tenderness to finger pressure on the alveolar process are indications that the tooth has become abscessed. Electric pulp testing may be diagnostic if the tooth shows no response to the electric stimulus, but a positive pain response may be equivocal in multirooted teeth. Chronic abscesses are diagnosed more easily by looseness of the tooth, suppuration from draining sinuses or from the gingival crevice (see Fig. 9–3), and radiolucency on the radiographs (see Fig. 9–1). Depending on the path of least resistance, fluctuant areas may be noted in the buccal or lingual mucosa. Spread through the tissues, or cellulitis, may lead to the classic presentation of swollen face, pain, elevated temperature, and malaise.

In 1951, Krogh[31] showed a 3 percent complication rate when 2626 infected teeth were removed at the time of initial presentation. In 1975, Martis and Karakasis[45] published a similar study in which they treated 1376 acute dentoalveolar abscesses by immediate extraction. A 3 percent complication rate was found in this study as well. A complication was defined as further extension of the infection, requiring additional treatment. Hall and associates[22] published a report in 1968 in which 350 patients with odontogenic cellulitis were divided randomly into two groups. The first group had extractions performed on the day of initial presentation, whereas the second group waited (with antibiotics) until the fourth day for surgical treatment to be performed after "localization" had occurred. The investigators' observations showed that extraction did not spread the cellulitis in either group. Patients with earlier extractions recovered more rapidly, whereas patients with delayed treatment had a greater need for incision and drainage, which was twice as likely to be extraoral than intraoral. In 1978, Martis and colleagues[44] showed in a series of more than 2000 patients that extraction without antibiotics in the presence of periapical infection led to the same complication rate as did extraction of noninfected teeth.

Considering the prospect of early relief of symptoms and a 97 percent chance that extraction (or occasionally root canal treatment) will cure the infection, early surgical intervention is mandatory. The use of antibiotics must be determined on an individual basis according to principles outlined previously (Table 9–4).

PERIODONTAL INFECTIONS

Surrounding the teeth is a distinctive, pink keratinized mucosa, the gingiva (Fig. 9–7A). Normal gingiva is attached firmly to the alveolar bone and extends between the teeth as the interdental papilla. A thin cuff of free (nonattached) gingiva surrounds each tooth, and the resulting crevice between the free gingiva and the tooth normally is 1 to 3 mm in depth. It is represented by a thin roll of tissue along each tooth in Figure 9–7A.

Accumulation of food deposits and bacteria in the gingival crevice may result in gingivitis, a localized inflammation of the

free gingiva that manifests as an erythematous, nonpainful swelling of the interdental papillae. In severe cases (see Fig. 9–7B), the gingival architecture may become distorted, and accumulations of plaque are evident. Although gingivitis is prevalent at all ages, affecting more than 50 percent of children[47] and almost all adults to some degree, it often is most severe in compromised hosts, including patients with diabetes and immunosuppressed patients. Poor oral hygiene is the usual precipitating factor for development of gingivitis, and this condition generally responds to dental scaling and improved oral hygiene.

In adolescents and in adults, gingivitis may progress to periodontitis, a progressively severe infection that is characterized by hypertrophied gingivae, tooth mobility caused by irreversible resorption of alveolar bone, and a purulent exudate. This insidious condition usually is painless and may progress for years before being recognized. Localized periodontal treatment and meticulous oral hygiene may arrest the condition.

A rare variant, juvenile periodontitis,[34] usually is localized to the molar and incisor regions of younger, otherwise healthy children. Deep gingival pocketing and severe bone resorption are characteristic of this process and may result in loss of the dentition in these areas. The etiology is thought to involve a gram-negative anaerobe, *A. actinomycetemcomitans*, and localized bacterial inhibition of leukocyte function. Tetracycline in older patients has been useful in combination with periodontal surgery and meticulous home care.

Acute necrotizing ulcerative gingivitis (see Fig. 9–7C) is a specific infection caused by fusiform bacilli and spirochetes. Synonyms include trench mouth and Vincent infection. Erythema at the tips of the interdental papillae soon is supplanted by frank ulceration and foci of spontaneous bleeding. A pseudomembranous necrotic exudate forms along the marginal gingivae and the interdental papillae. The papillae later become blunted. Acute necrotizing ulcerative gingivitis is characterized by pain, foul breath and taste, thick ropy saliva, malaise, and occasionally fever. Theories suggest a concomitant viral etiology. Treatment consists initially of penicillin therapy followed within a few days by localized gingival curettage and oral rinses with 0.5 percent hydrogen peroxide or 0.12 percent chlorhexidine.[40] The safety and effectiveness of chlorhexidine in children have not been established.

TABLE 9–4 Indications for Antibiotic Therapy in Odontogenic Infections

Antibiotic therapy is necessary
Acute-onset facial or oral swelling
Swelling inferior to the mandible
Trismus
Dysphagia
Lymphadenopathy
Fever >38.3°C (>101°F)
Pericoronitis
Osteomyelitis
Antibiotic therapy is *not* necessary*
Asymptomatic periapical abscess
Parulis (draining sinus tract)
Dry socket (alveolar osteitis)
Periodontal disease
Dental extractions
Root canal therapy

**With coexisting immune system compromise, antibiotic therapy may be indicated in some of these conditions.*

PERICORONITIS

Impaction of microorganisms and debris under the soft tissue overlying the crown of a tooth, often a mandibular third molar, or any erupting permanent tooth leads to the development of inflammation. Drainage usually occurs spontaneously from under the flap, localizing the problem. Blockage of natural drainage may lead to spread of infection to adjacent soft tissues and fascial spaces (Fig. 9–8).

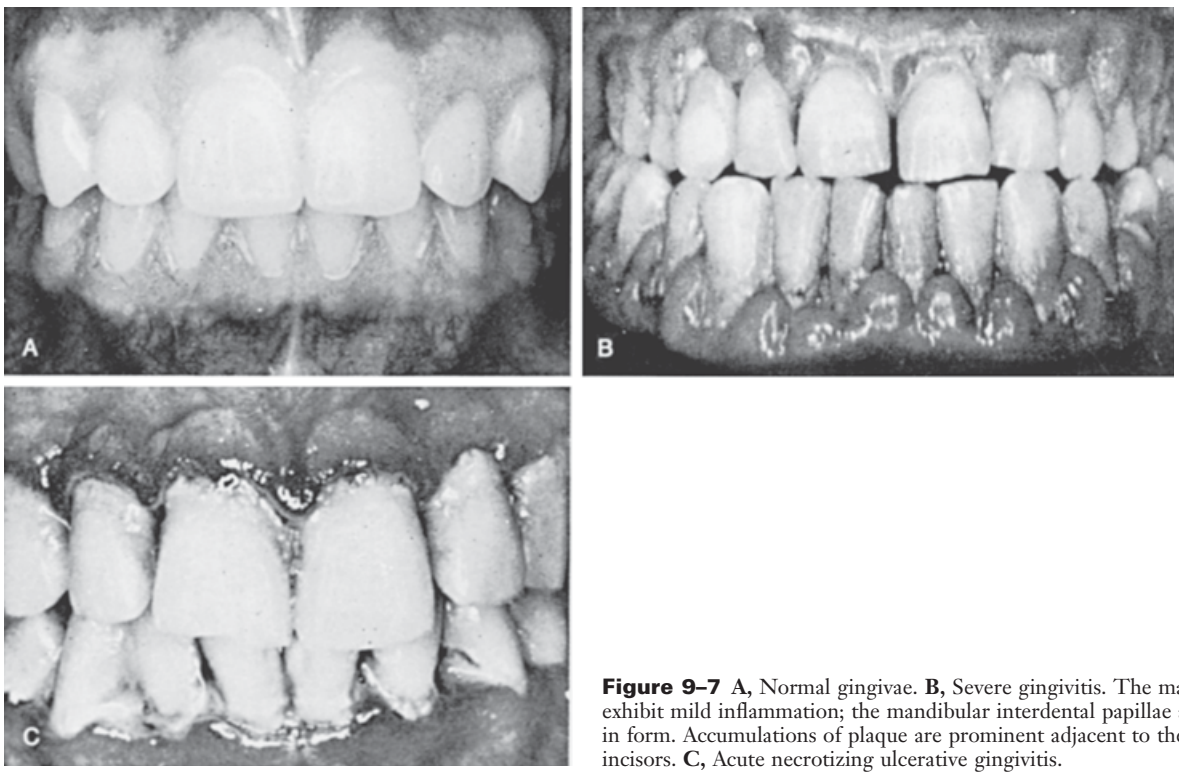

Figure 9–7 **A,** Normal gingivae. **B,** Severe gingivitis. The maxillary gingivae exhibit mild inflammation; the mandibular interdental papillae are distorted grossly in form. Accumulations of plaque are prominent adjacent to the mandibular incisors. **C,** Acute necrotizing ulcerative gingivitis.

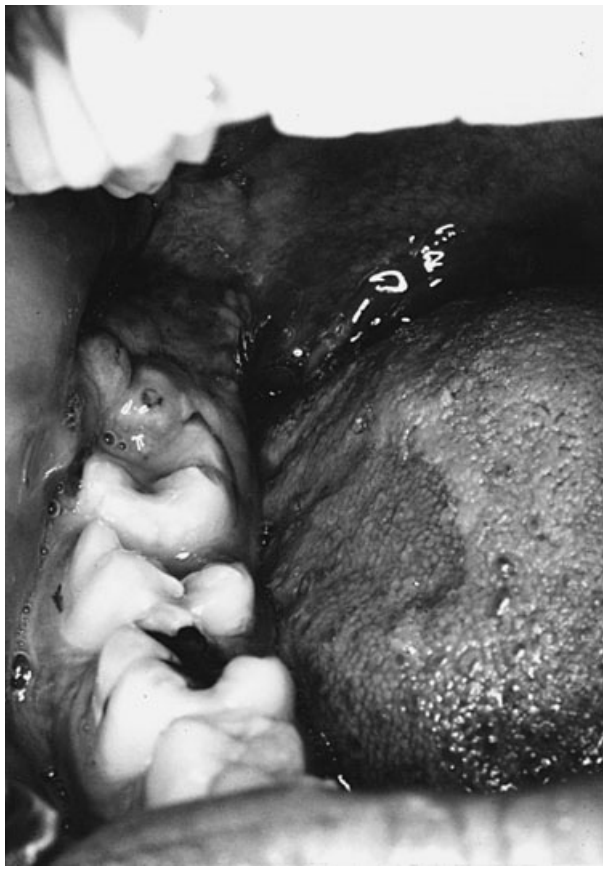

Figure 9–8 Pericoronitis.

Pericoronitis is a polymicrobial infection; the periodontal pathogens *Prevotella* and *Porphyromonas* spp. and oral spirochetes, such as *Treponema denticola*, usually are the causative organisms. The *Streptococcus milleri* group bacteria also have been found to have a significant role in acute pericoronitis.[26] These organisms usually are sensitive to penicillin or penicillin combined with metronidazole. Pericoronitis most frequently occurs around the posterior portion of the crown of the lower third molar because it erupts during adolescence. In most cases, partial eruption of the third molar is caused by insufficient length of the horizontal ramus of the mandible to house all of the teeth. Part of the third molar is trapped under the oral mucosa covering the buccinator muscle and the superior pharyngeal constrictor because they form the most anterior portion of the oropharynx. In cases in which room is insufficient for the eruption of the third molar, the pericoronitis becomes recurrent or chronic, and the impacted third molar should be removed.

Lower third molars lie in proximity to the pterygomandibular space, a portion of the masticator space. When these infections spread to involve this space, trismus results, which obscures the infection to clinical examination. The presence of trismus with a history of pain in the third molar region is an ominous sign of infection involving the masticator space, which, although not manifested by external facial swelling, may begin to involve the deeper parapharyngeal spaces. These infections may become life-threatening. The lower third molar is the most frequent offending tooth in severe odontogenic infections requiring hospitalization, and these infections occur most frequently in adolescents and young adults.[24]

Various treatment modalities, including local incision and drainage and extraction of the tooth, are applicable to pericoronitis.[44,58] Antibiotic therapy is used if fever, trismus, or lymphadenopathy is present (see Table 9–4). Resolution of symptoms should occur in less than 1 week.

ORAL MANIFESTATIONS OF HUMAN IMMUNODEFICIENCY VIRUS INFECTION IN CHILDREN

The most common oral lesions found in children with human immunodeficiency virus (HIV) disease are oral candidiasis, herpes simplex virus infections, linear gingival erythema, parotid salivary gland enlargement, and recurrent aphthous ulcerations. In contradistinction to adult HIV infection, HIV-associated periodontitis and gingivitis are much less common. Neoplastic oral manifestations of HIV infection, such as Kaposi sarcoma, non-Hodgkin lymphoma, and hairy leukoplakia, continue to be rare findings. In contrast to adults, children infected with HIV have a greater susceptibility to bacterial infections, especially with encapsulated organisms, such as *Streptococcus pneumoniae* and *Haemophilus influenzae*. Septicemia from an oral focus of infection can become a life-threatening problem in an HIV-infected child, and optimal oral health must be established and vigorously maintained in these children.[33] Routine use of chlorhexidine gluconate 0.12 percent mouth rinse may be helpful in minimizing gingivitis, candidiasis, and bacterial superinfections of the oral cavity, although its safety and effectiveness have not been shown in children.

Oral candidiasis usually is of the pseudomembranous type, which is seen in the oral cavity as a creamy white plaque that is rubbed off easily, leaving a reddened surface mucosa exposed. Because oral candidiasis is rare in normal children older than 6 months, persistence of oral candidiasis for 2 months or more in a child older than 6 months who has not received antibiotic therapy in the past 2 weeks is suggestive of HIV infection. Persistent oral candidiasis indicates acquired immunodeficiency category P-2D3 (symptomatic infection with secondary infectious diseases) in the Centers for Disease Control and Prevention classification of HIV infection in children younger than 12 years.[30]

Oral candidiasis was associated with a decreased survival time in a study of 99 children with perinatally acquired HIV infection. The median time from birth to the manifestation of the first lesion of oral candidiasis was 2.4 years, and the median time from the appearance of lesions to death was 3.4 years, with a relative hazard rate of 14.2.[29]

Treating oral candidiasis lesions is difficult because of frequent recurrence of oral fungal infection with resistant biotypes of *Candida albicans* or colonization by related but more resistant species, such as *Candida krusei*, *Candida parapsilosis*, and *Candida guilliermondii*. Treatment regimens may progress from nystatin to clotrimazole, fluconazole, and amphotericin B, depending on the extent of disease, clinical response, and culture and sensitivity results.[43] Sudden onset of rampant dental caries has been associated with prolonged oral use of sucrose-containing antifungal antibiotic preparations.[69]

Linear gingival erythema, formerly referred to as HIV gingivitis, is the most common form of periodontal disease seen in children with HIV infection. It is described as a fiery red, 2- to 3-mm-wide linear band of inflammation of the gingiva. Pain is not associated with the lesion, but the gingivae are likely to bleed during tooth brushing or even spontaneously. The microbiology of this lesion is unclear, but *Candida* spp. may be a possible cause.

Parotid salivary gland enlargement, which may be painful and become secondarily infected, has been reported in 14 to 30 percent of HIV-infected children. The enlargement apparently is caused by infiltration of the glands by T8 lymphocytes and has been associated with increased survival time. The median time was 4.6 years from birth to development of parotid enlargement

and 5.4 years from development of lesions to death, with a relative hazard rate of 0.38.[29]

Herpes simplex virus infections, although common occurrences in normal children, seem to be particularly severe and recur more often in HIV-infected children. The lesions appear first as multiple clustered vesicles on the lips or keratinized oral mucosa, which soon rupture to leave painful irregular oral ulcers or crusted labial ulcers. Fever and dysphagia may warrant hospital admission for hydration, nutrition, and therapy with parenteral acyclovir. Less severe cases may be treated with oral acyclovir.

Dental caries is increased in pediatric HIV cohorts. The cause of this finding is unclear. It may be due to xerostomia secondary to parotid enlargement in some cases, prolonged use of sucrose-containing antifungal agents in others, and nursing-bottle caries in still others. The association of nursing bottle caries with pediatric HIV infection may be due to their common increased prevalence in urban dwellers with limited economic resources, although nursing-bottle caries also is found frequently in children with other chronic diseases.[69]

COMPLICATIONS OF ODONTOGENIC INFECTIONS

FASCIAL SPACE INFECTIONS

Spread of infection to the fascial spaces may result in dramatic facial swelling and high fever and, if untreated, respiratory embarrassment. The characteristics of the more common fascial space infections related to odontogenic infection are described here.

Infraorbital space infections generally are related to maxillary anterior teeth and are well localized to the infraorbital fossa by the levator labii superioris and levator anguli oris muscles. Facial swelling lateral to the nose is prominent, as is decreased mobility of the upper lip caused by inflammation of these muscles. If the area is fluctuant, intraoral incision and drainage with placement of a small Penrose drain for 1 to 2 days generally are sufficient treatment. Antibiotics are indicated for all infections of the fascial spaces.

Trismus is the hallmark of infection of the masticator space. Trismus is caused by spasm in the muscles of mastication, which define this large potential space. The resulting inability to open the mouth hinders access to the airway for endotracheal intubation. In addition, abscesses of the masticator space may rupture into the oropharynx, causing aspiration of pus, or they may pass easily around the medial pterygoid muscle to involve the lateral pharyngeal and retropharyngeal spaces. Figure 9–9 shows a 6-year-old boy whose lower primary molar abscesses spread to involve the buccal, pterygomandibular, and lateral pharyngeal spaces. Extraoral and intraoral drainage, prolonged intubation, and extraction of the offending teeth were required.

Infections of the submandibular space (Fig. 9–10) may be localized unilaterally or may involve bilateral structures. Treatment of submandibular space infection is via extraoral incision and drainage.

First described in 1836, Ludwig angina consists of infection of the sublingual and submandibular spaces bilaterally and is characterized by hard, brawny swelling and a minimum of suppuration. The tongue often is edematous and raised to the roof of the mouth, with little mobility (Fig. 9–11). Airway obstruction should be considered imminent; the greatest cause of death in Ludwig angina is blockage of the airway by soft tissue swelling, pus, or blood, which occurred in more than 50 percent of its victims in the pre-antibiotic era.[21] In 1940, Williams[72] published a series of 37 cases of Ludwig angina, which reported a 54 percent mortality rate. The airway management policy at that time was emergency tracheotomy if necessary. Three years later, Williams and Guralnick[73] published a series of 20 cases of Ludwig angina treated with a new policy of immediate establishment of airway security by intubation or tracheotomy and aggressive incision and drainage of all anatomic spaces affected by the infection. By these measures, they were able to reduce the mortality rate of this dreaded infection to 20 percent only 3 years later. Antibiotics were unavailable during the course of these two studies. These studies underscore the importance of airway security and aggressive surgical management in the treatment of severe odontogenic infections.

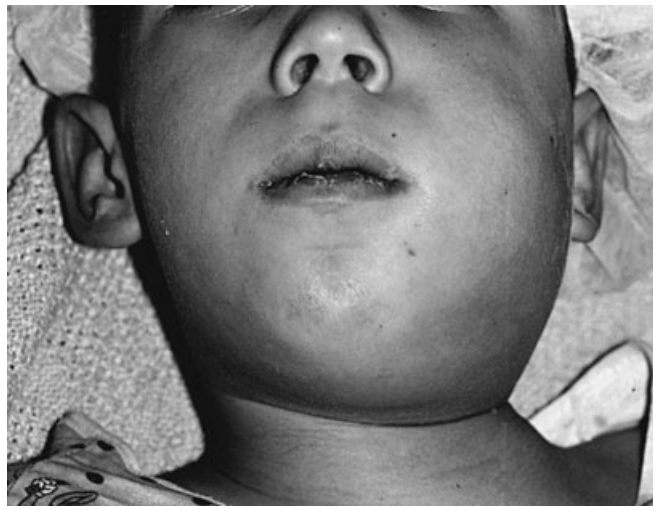

Figure 9–9 Lateral pharyngeal space abscess in a 6-year-old boy. Note the swelling above the hyoid bone and anterior to the sternocleidomastoid muscle. Swelling also occurs in the buccal and submandibular spaces.

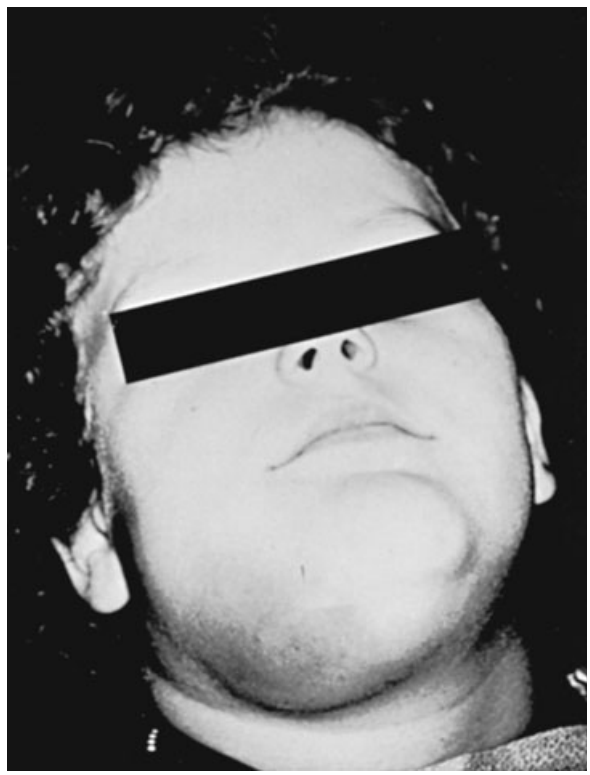

Figure 9–10 Submandibular space abscess.

Today, death rarely occurs from severe odontogenic infections, although the need for tracheotomy or prolonged endotracheal intubation is common. The cause of this infection often is odontogenic infection but may include laceration of the floor of the mouth and mandibular fracture. Usually a disease of middle-aged adults, it is a rare occurrence in children but may occur in greater frequency in immunologically compromised children.[18] Surgical drainage of all four spaces, accompanied by vigorous antibiotic therapy, is indicated.

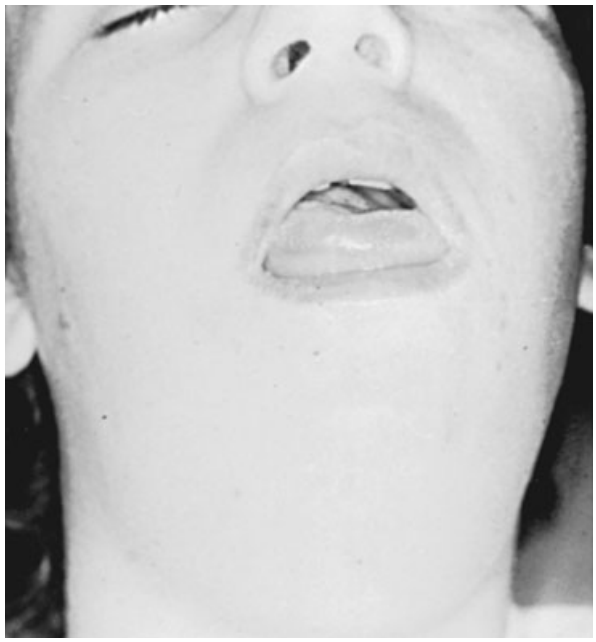

Figure 9–11 Ludwig angina.

NECROTIZING FASCIITIS

Necrotizing fasciitis, which causes a frightening loss of skin and underlying tissues, has received considerable notoriety in the press. Cervicofacial necrotizing fasciitis often is odontogenic and typically causes a superficial spreading of cellulitis that follows the platysma muscle from the cheek down the entire neck to the anterior chest wall (Fig. 9–12A). Figure 9–12B illustrates such a swelling in an 8-year-old boy. The presumptive cause was odontogenic infection of the primary molars, which caused a high fever and a rapidly progressive cellulitis extending from the cheek to the chest. The cause of these infections often is group A streptococci, but a wide variety of microorganisms may be involved.

Wide-spectrum antibiotic therapy is indicated empirically, along with hydration, transfusions if necessary, and support of electrolyte balance, especially with calcium, which may be sequestered by necrotic fat molecules.[1] Timely surgery is important in the management of necrotizing fasciitis. Incision and drainage of the involved spaces, débridement of necrotic tissue, and fasciotomy often are performed as soon as possible after the patient's admission.[71] Hyperbaric oxygen therapy has been advocated as an adjunct in the treatment of necrotizing fasciitis. A significant reduction in the mortality rate and length of hospitalization associated with necrotizing fasciitis has been shown in adults.[71] Necrotizing fasciitis is cited as an indication for hyperbaric oxygen therapy in infants and children and should be considered if available.[70]

ODONTOGENIC SINUSITIS

A significant percentage of cases of sinusitis are odontogenic, especially in adults, because the maxillary sinus follows the erupting permanent tooth roots into the alveolar process. This pneumatization of the alveolar process progresses throughout life and is accelerated by loss of the upper posterior teeth. Dental infections of the periapical upper posterior teeth occasionally rupture through the maxillary sinus floor to involve the paranasal sinuses.

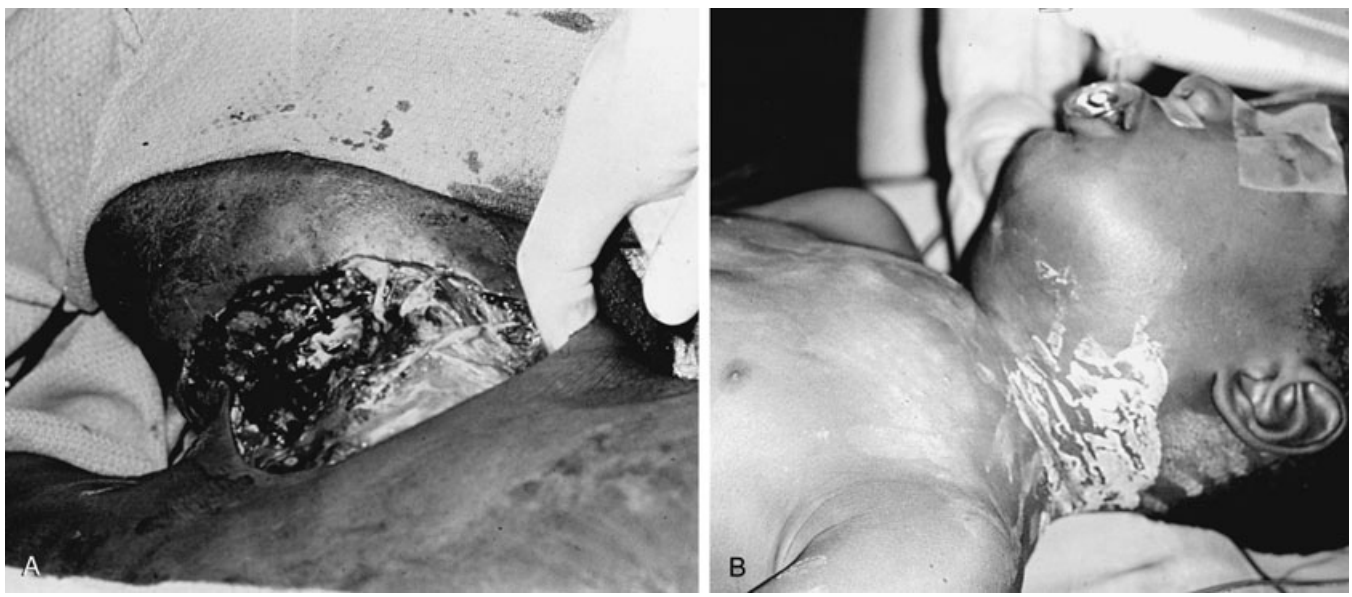

Figure 9–12 **A,** Surgical débridement of necrotic skin and platysma muscle of the left neck of a diabetic female patient with necrotizing fasciitis. Note how easily blunt finger dissection can undermine the skin in the plane of the necrotic platysma muscle. **B,** Necrotizing fasciitis in an 8-year-old boy. Note the swelling extending from the buccal space down the neck and onto the anterior chest wall, following the extent of the platysma muscle. The chalky material on the posterior neck is calamine lotion placed by the patient's mother for vesicles that resembled poison ivy.

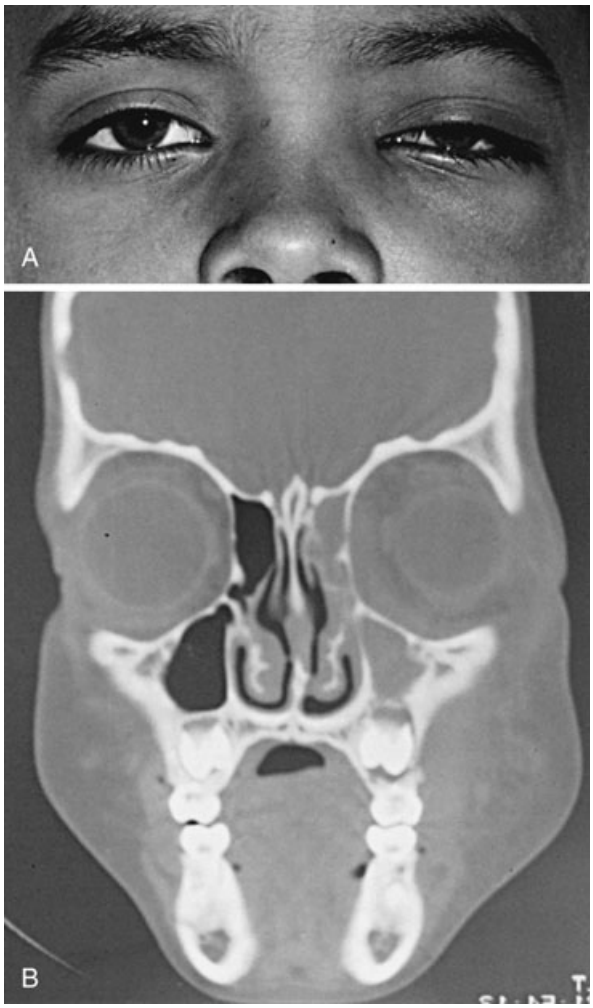

Figure 9–13 **A,** A 9-year-old boy with a left pansinusitis and an abscess of the upper left first primary molar. Note the left periorbital discoloration and swelling, with partial ptosis and displacement of the globe laterally. **B,** Computed tomography scan of the same patient. Note the left maxillary sinus opacification close to the infected tooth, opacification of the ethmoid sinuses, elevation of the periosteum away from the medial orbital wall, and displacement of the globe laterally within the orbit.

Dental infection should be eliminated in the complete treatment of severe recurrent sinusitis in children.

Figure 9–13 illustrates a case in a 9-year-old boy of left pansinusitis, including ethmoiditis and a subperiosteal orbital abscess associated with infected upper primary molars. His treatment involved a team approach of the dental and otolaryngology services for tooth extraction, incision and drainage of the buccal and infraorbital spaces, endoscopic sinus surgery, and external drainage of the orbital abscess.

BUCCAL AND PERIORBITAL CELLULITIS

A child occasionally presents with an acute buccal or periorbital space swelling and cellulitis with no clinically apparent odontogenic cause. These infections tend to occur in young children, usually younger than 36 months. They usually have a history of recent upper respiratory infection or sinusitis. *H. influenzae* type b and *S. pneumoniae* have been implicated as pathogens in these conditions, although the widespread use of conjugated vaccines has reduced drastically the incidence of invasive disease from *H. influenzae*.[17] A possible mechanism for the inoculation of the soft tissues is migration of organisms through emissary veins piercing the thin cortical bone overlying the lateral surface of the maxillary sinus, in cases with existing sinusitis. Unless the infection is severe, incision and drainage usually are unnecessary, and treatment with antibiotics is successful. Blood cultures frequently are positive in more severe cases. The role of lumbar puncture remains controversial in the overall management of infants and young children. The incidence of this infection has declined significantly as a result of the widespread use of *H. influenzae* vaccine.

ORBITAL AND INTRACRANIAL COMPLICATIONS

Odontogenic infections that spread to involve the orbit and the brain are rare. Orbital and intracranial abscesses may have an odontogenic origin, however, and the dental condition of patients with these conditions should be evaluated by a dentist. Probably no more than 5 to 10 percent of orbital cellulitis is odontogenic in origin.[28,74] This infection generally is unilateral and is characterized by proptosis, chemosis, lid edema, and restriction of extraocular motion secondary to edema.[54] No nerve palsies or visual changes are present. Treatment includes surgical drainage, antibiotics, and elimination of the dental infection.

Cavernous sinus thrombosis, which may be difficult to differentiate clinically from orbital cellulitis, is considerably more serious because microorganisms proliferate intracranially. The risk of death is high. Characteristics include bilateral involvement with rapid progression from one eye to the other, proptosis, chemosis, and lid edema. Extraocular movements are limited because of inflammation of the third, fourth, and sixth cranial nerves. Systemic signs of meningeal irritation and ophthalmoscopic evidence of obstruction of the retinal veins also are present.[7,54,65] Treatment includes high doses of parenteral antibiotics, elimination of the causative dental pathosis, and incision and drainage of infected fascial spaces.

Brain abscess and subdural empyema are rare findings today compared with several decades ago. Of the large series of brain abscess cases reported, 0 to 4 percent have been attributed to dental causes.[41,68] All of the studies in which the individual case histories are described disclose a pansinusitis intervening between the dental infection and the brain.[42,60] Odontogenic brain abscesses seem to occur by direct extension through the paranasal sinuses, usually to the frontal lobe through the frontal sinuses. Odontogenic cavernous sinus thrombosis seems to be propagated by an ascending thrombophlebitis.

OSTEOMYELITIS OF THE JAWS IN CHILDREN

Osteomyelitis of the jaws in children usually results from periodontal or, more commonly, periapical infection. Open fracture of the jaws with delayed treatment also is a significant cause of osteomyelitis. Extension from contiguous infections, such as otitis, parotitis, and mastoiditis, occurs much less often.

Osteomyelitis of the jaws occurring in children must be viewed with great concern because it may result in the following problems: (1) loss of primary and permanent teeth; (2) sequestration of segments of the jaws; (3) growth defects, such as mandibular hypoplasia, asymmetry, and ankylosis[12]; (4) disfiguring facial scars and cutaneous fistulas; and (5) lesions suggestive of malignancy, which require open biopsy. For these reasons, osteomyelitis of the jaws in children should be diagnosed rapidly and treated aggressively. Table 9–5 presents a useful classification of this disease.[67]

TABLE 9–5 Osteomyelitis of the Jaws

Suppurative	Nonsuppurative
Acute suppurative	Chronic sclerosing
Chronic suppurative	Facial sclerosing
Primary	Diffuse sclerosing
Secondary	Garré sclerosing
Infantile	Actinomycotic
	Radiation osteomyelitis and necrosis

PREDISPOSING FACTORS

Preexisting systemic disease with accompanying alteration of host resistance plays a major role in the initiation of osteomyelitis of the jaws. It includes such conditions as uncontrolled diabetes, agranulocytosis, leukemia, sickle-cell disease, and febrile illnesses. Conditions that alter the vascularity of bone and the ability to combat infections, including bone tumors, fibrous dysplasia, Paget disease, and radiation to the jaws, also are important predisposing conditions. Major maxillofacial injuries resulting in open fractures of the jaws, especially fractures that are not treated immediately, are an important cause of osteomyelitis.

MICROBIOLOGY

Because the etiology of osteomyelitis of the jaws includes causes other than purely odontogenic infections, the bacterial spectrum is broad. Most instances of osteomyelitis of the jaws are caused by aerobic streptococci (alpha-hemolytic streptococci, *Streptococcus* viridans group), anaerobic streptococci, and other anaerobes, particularly peptostreptococci, fusobacteria, and *Bacteroides* and related genera.[52] Only occasional cases are caused by *S. aureus*, with entry through the skin being the probable route. Other bacteria involved include oral anaerobes, aerobic and microaerophilic cocci, and gram-negative organisms. Specific forms of osteomyelitis are caused by *Actinomyces israelii*, *Treponema pallidum*, and *Mycobacterium tuberculosis*. *Salmonella* organisms have been associated with osteomyelitis of the jaws in patients with sickle-cell anemia.[9]

CLINICAL FINDINGS

Osteomyelitis involves the mandible far more frequently than the maxilla because the poor blood supply to the mandible comes primarily from one major vessel and the periosteal blood supply. Four major forms of the disease, which may be distinguished clinically, are (1) acute suppurative; (2) secondary chronic, which begins as an acute osteomyelitis and becomes chronic; (3) primary chronic, which has no acute phase and always has seemed to be a low-grade infection; and (4) nonsuppurative osteomyelitis. The forms most often seen in children are the acute suppurative, the secondary chronic, and one nonsuppurative form, Garré sclerosing osteomyelitis. These conditions are described in some detail.

SUPPURATIVE OSTEOMYELITIS

Suppurative osteomyelitis usually begins with deep and intense pain in the jaws, high intermittent fever, and an obvious cause, most often a deeply carious or discolored tooth. In the early stages, mental nerve paresthesia occasionally is present. During the course of several days, facial swelling develops, and in 10 to 14 days, teeth begin to loosen, pus exudes around the gingival sulcus, and multiple mucosal or cutaneous sinus tracts form. In addition to the draining sinuses, a firm cellulitis is present in the soft tissues accompanied by trismus and cervical lymphadenopathy. A leukocytosis, ranging typically from 8000 to 15,000 cells/mm^3, is present, although it does not ordinarily reach the levels that are seen in acute osteomyelitis of the long bones. After 10 days to 2 weeks, radiographs may show scattered areas of bone destruction suggestive of a moth-eaten appearance (Fig. 9–14), and periosteal reaction characterized by the laying down of new bone commonly is seen. Smears of specimens and cultures, including cultures of bone sequestra, should be taken whenever possible. Interpretation of cultures must be made with caution because of the possibility of skin and oral contaminants in the specimen.

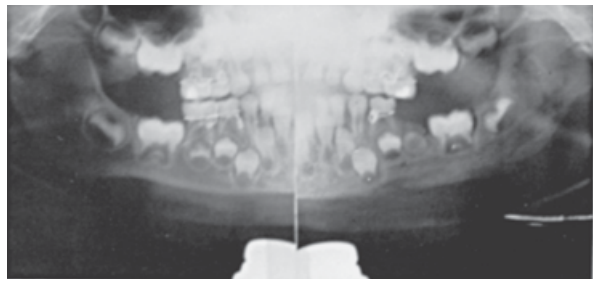

Figure 9–14 Radiograph of the jaws of a 4-year-old girl with suppurative osteomyelitis of the left mandible. The film shows marked destruction of the body and ramus of the mandible.

Initially, antibiotic doses adjusted for age may be given empirically, but the selection of the antibiotic is determined by culture and sensitivity testing of specimens taken directly from the infected site.[15] The antibiotics of choice in mandibular osteomyelitis include amoxicillin-clavulanate, clindamycin, and fluoroquinolones. Fluoroquinolones are excellent choices because of their complete absorption from the gastrointestinal tract and high penetration of bone, but these drugs generally should be avoided in children because they are chondrotoxic during growth. As results from smears and culture are obtained, antibiotics may be changed, unless the infection is responding favorably, in which case no change is made. The involved tooth is removed as early as possible to allow drainage and to provide material for culture.

Antibiotic therapy is continued for at least 2 to 4 weeks after all symptoms subside. If the infection persists, repeated cultures are obtained, and the antibiotic is changed if necessary. The greater vascularity of the jaws may explain their more rapid response to antibiotic therapy and surgery compared with long bones. The duration of intravenous antibiotic therapy in osteomyelitis of the jaws may not need to be as prolonged as in that of the long bones. Consideration should be given to sequestrectomy, saucerization, or the placement of closed-wound irrigation and suction. Saucerization involves the removal of teeth in the immediate area and removal of the overlying buccal plate of bone, allowing access to the medullary portion and sequestra that may be present. Placement of catheters through an extraoral approach occasionally is necessary for closed irrigation and suction. It permits instillation of antibiotics, allowing direct contact with the bone. Hyperbaric oxygen treatment may be considered in chronic cases refractory to antibiotic treatment.[47]

INFANTILE OSTEOMYELITIS

Osteomyelitis of the jaws in a newborn is an uncommon occurrence but warrants special mention because of its serious sequelae. It occurs most often a few weeks after birth and usually involves the maxilla. It is not odontogenic in origin, but is thought to arise from neonatal trauma to oral tissues, hematogenous spread (from

skin, middle ear, mastoid, or tonsils), or an infected nipple.[56] The patient presents with a facial cellulitis centered around the orbit (Fig. 9–15). Irritability and malaise precede development of cellulitis and are followed by marked elevation in temperature, anorexia, and dehydration. Extraorally, inner canthal swelling, palpebral edema with closure of the eye, conjunctivitis, and proptosis may be seen together with a purulent discharge from the nose or from the inner canthus. Oral examination shows swelling of the maxilla on the affected side extending to the buccal and the palatal regions, with fluctuation often present with multiple sinus tracts. *S. aureus* is the organism usually found.

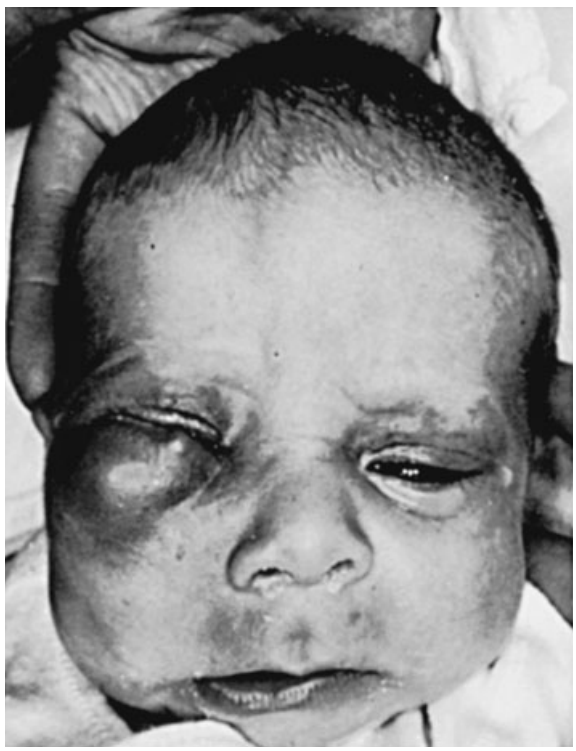

Figure 9–15 Characteristic clinical picture of a 3-week-old child with infantile osteomyelitis. *(Courtesy of Dr. M. Michael Cohen, Sr.)*

Aggressive, prompt treatment must be undertaken to prevent permanent optic damage, neurologic complications, loss of tooth buds and bone, and extension to the dural sinuses. Intravenous penicillin and a penicillinase-resistant penicillin are given simultaneously with surgical drainage of all fluctuant areas, repeated Gram smears, and culture and sensitivity testing. Antibiotics are continued orally for 2 to 4 weeks after all signs of the infection have disappeared. If sequestra form, they should be removed conservatively. Tooth buds may be lost, and surviving teeth may be deformed or discolored after eruption.

GARRÉ SCLEROSING OSTEOMYELITIS

Garré sclerosing osteomyelitis, also known as chronic nonsuppurative sclerosing osteomyelitis and proliferative osteomyelitis of Garré,[4] is notable because of the similarity of some of its characteristics to those of other neoperiostoses. It is characterized by a localized, hard, nontender swelling of the mandible (Fig. 9–16). Lymphadenopathy, hyperpyrexia, and leukocytosis are not present. Garré osteomyelitis is associated commonly with a carious tooth, usually the lower first molar (Fig. 9–17), and a

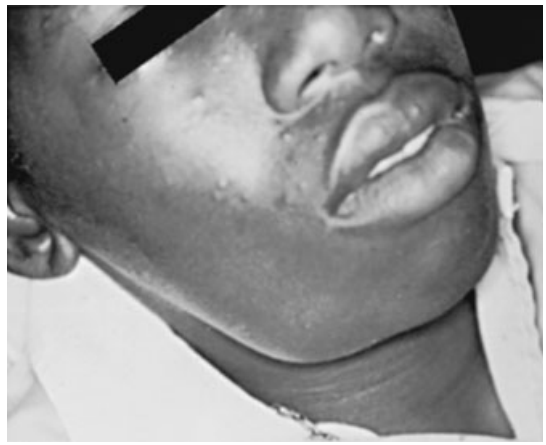

Figure 9–16 Enlargement of the right side of the mandible in a 12-year-old patient with Garré sclerosing osteomyelitis. The swelling is hard and nontender.

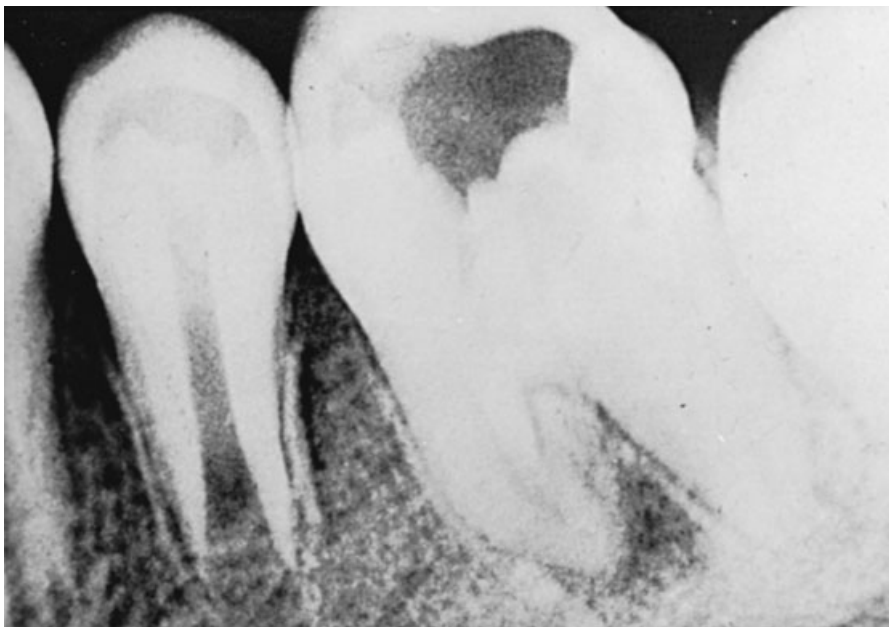

Figure 9–17 Radiograph of a deeply carious lower first molar tooth with periapical spread of infection. It is the usual cause of Garré osteomyelitis.

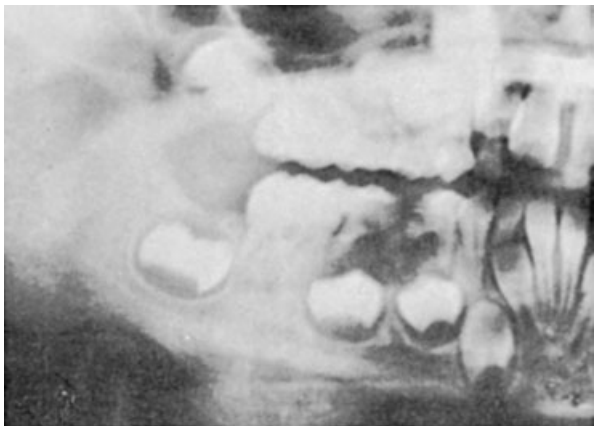

Figure 9–18 Characteristic radiograph of Garré osteomyelitis showing the laminated or onion-peel appearance of the mass. *(Courtesy of Dr. Larry J. Peterson.)*

history of a past toothache. It also may be associated with a recent dental extraction or an infected flap of tissue over an erupting tooth.[39] Radiographs are impressive, showing a focal area of well-calcified bone proliferation that is smooth and that often has a laminated or onion-peel appearance (Fig. 9–18).

Garré osteomyelitis is thought to be a response to a low-grade stimulus, such as a dental infection, which influences the potentially active periosteum of young individuals. Its appearance resembles that of infantile cortical hyperostosis (Caffey disease), osteosarcoma, and Ewing sarcoma, and it must be distinguished from these conditions.[11] Treatment consists of extraction or endodontic treatment of the involved tooth, with continued clinical and radiographic follow-up of the patient to ensure that the new bone formation does not progress. Ordinarily, remodeling occurs over the course of time, but biopsy should be done to rule out neoplasm if the lesion does not regress. No antibiotic therapy is necessary.

HERPES SIMPLEX VIRUS INFECTIONS

Herpes simplex virus type 1 infections[64] commonly are manifested as herpetic gingivostomatitis. Five stages of infection have been identified: (1) primary mucocutaneous infection, (2) acute infection of ganglia, (3) establishment of latency, (4) reactivation, and (5) recurrent infection.

Primary infection is established by direct contact either with individuals who have draining lesions or with an asymptomatic carrier who may continue to shed the virus despite the lack of symptoms. The highest incidence of primary infection seems to be in children 2 to 4 years old. Infants are protected by maternal antibodies, which may explain why this infection is not found in infants younger than 6 months. It appears to have no seasonal variation or male-female difference in incidence.

The incubation period is thought to be approximately 6 days, followed by the development of small vesicles that may coalesce to form larger lesions or ulcers. In severe cases, the lips, gingivae, oral mucosa, and pharynx may be involved. Many patients with primary herpes labialis may be asymptomatic, however, and symptoms may not develop. Healing occurs in 1 to 2 weeks, with gradual crusting of the lesions followed by re-epithelialization.

Latency is thought to continue throughout life, with reactivation occurring at various times, possibly triggered by actinic radiation and emotional or physical stress. Recurrent disease is manifested by vesicles at the mucocutaneous border, which are painful for about 2 days, followed by crusting and complete healing in 7 to 8 days.

Fifty percent of the adult population in industrialized countries and a higher percentage in less-developed countries may have recurrent herpes labialis. Many, if not most, adults who have recurrent "cold sores" are not aware that they can transmit the disease and should be counseled in this regard. Likewise, medical, dental, and nursing personnel should be advised that occurrence of cutaneous lesions (the herpetic whitlow) is possible after direct contact of the practitioner's fingers with lesions during the physical examination.

In treating recurrent herpes labialis in non-immunocompromised adults, acyclovir has been shown to decrease the duration of symptoms by 12 to 24 hours, at a cost of approximately $75.00 per day. Frequent use of acyclovir also may promote the spread of resistant viral strains. The use of acyclovir for treatment of recurrent herpes labialis in non-immunocompromised individuals seems to offer little benefit at significant expense and risk.[55]

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CHAPTER 10

Pharyngitis (Pharyngitis, Tonsillitis, Tonsillopharyngitis, and Nasopharyngitis)

James D. Cherry

Pharyngitis is an inflammatory illness of the mucous membranes and underlying structures of the throat. The clinical diagnostic category includes tonsillitis, tonsillopharyngitis, and nasopharyngitis; inflammation also frequently involves the nasopharynx, uvula, and soft palate. Illness usually is acute but also may be subacute or chronic. Establishing the diagnosis of pharyngitis requires objective evidence of inflammation (erythema, exudate, or ulceration). The symptom of sore throat invariably accompanies pharyngitis, but it should not be used as the sole criterion; sore throat is a common complaint of children with colds in whom no objective evidence of pharyngeal inflammation is present.

Although the clinical finding of pharyngitis suggests an almost exclusive group A streptococcal etiology to many physicians, etio-

logic considerations should include a multitude of viruses, bacteria, and other infectious and noninfectious agents. Etiologically, pharyngitis is subdivided into two categories: illness with nasal symptoms (nasopharyngitis) and illness without nasal involvement (pharyngitis or tonsillopharyngitis). In acute illness, nasopharyngitis nearly always is of viral origin, whereas pharyngitis without nasal signs has diverse etiologic possibilities, including bacteria, viruses, fungi, and other infectious agents. In this chapter, nasopharyngitis and pharyngitis without nasal involvement are considered separately.

HISTORY

Although throat inflammation undoubtedly has been a physical finding of disease throughout human existence, only in more recent years has attention been given to pharyngitis as a primary complaint. The throat findings of diphtheria were mentioned in the 3rd century AD,[164] and Vincent angina was noted in the military before the Christian era,[47] but group A streptococcal infection and pharyngitis were not clearly associated until World War II.[30,141] Although Glover and Griffith[62] mentioned streptococcal tonsillitis in 1931, the major reference to streptococci in the preantibiotic era was in association with scarlet fever, erysipelas, and suppurative processes.[23]

NASOPHARYNGITIS

ETIOLOGIC AGENTS

Etiologic agents of nasopharyngitis, categorized by type of lesion, frequency, season of occurrence, and duration of illness, are listed in Table 10–1. The relative importance of nasal and pharyngeal manifestations also is presented. Although Table 10–1 shows three bacterial agents and one rickettsia, most occurrences of nasopharyngitis are caused by viral infections. The specific infectious agents are discussed fully in their respective sections of this book; only an overview is presented here.

Adenoviruses are the most common cause of nasopharyngitis; types 1, 2, 3, 4, 5, 6, 7, 7a, 9, 14, and 15 account for most illnesses.* Nasopharyngitis also commonly occurs with influenza and parainfluenza viral infections.† Although rhinoviral, respiratory syncytial viral, human metapneumovirus, and human bocavirus infections are common occurrences in children and all always have nasal manifestations (coryza), the occurrence of objective pharyngeal manifestations is less common.[20,51,74,77-80,87,111,130,151] Respiratory symptoms with cough, nasal discharge, and pharyngitis frequently occur in children with rotaviral gastroenteritis.[69,100,143]

EPIDEMIOLOGY

Nasopharyngitis is a common illness of childhood. It tends to be most prevalent in young children, in association with primary infections with respiratory viruses. Nasal symptoms with enteroviruses occur less frequently in school-aged children than in preschool-aged children. In contrast, older children rarely have pharyngitis with respiratory syncytial viral, parainfluenza viral, and rhinoviral infections. Nasopharyngitis caused by adenoviral infection is a particularly frequent occurrence in adolescents and young adults in military training.[33,153,170]

Nasopharyngitis occurs more commonly during the cold weather months (see Table 10–1). No apparent sex predilection has been found. The method of transmission is similar to that of other respiratory viral infections (see Chapter 8).

PATHOPHYSIOLOGY

The pathophysiology of nasopharyngitis is discussed in Chapter 8, in the pharyngitis section of this chapter, and in the chapters discussing the individual viral agents. In nasopharyngitis associated with *Haemophilus influenzae* and *Neisseria meningitidis*, the nasal symptoms may result from a concomitant respiratory viral infection.[23]

CLINICAL PRESENTATION

Because nasopharyngitis is caused by many different etiologic agents, a reasonable expectation is varied clinical manifestations. These differences are highlighted in Table 10–1. Fever occurs in nearly all cases of nasopharyngitis. With adenoviral and influenza viral disease, the pharyngeal findings are most prominent; with the other respiratory viruses, coryza is more notable than are pharyngeal complaints. In adenoviral infections, follicular pharyngitis is the rule, and exudate is a common manifestation. In contrast, patients with the other respiratory viral infections usually present with pharyngeal erythema only. Nasopharyngitis of a viral etiology most often is an acute, self-limited disease lasting 4 to 10 days. Generally, adenoviral illnesses tend to be more prolonged than are illnesses resulting from the other respiratory viruses. Other symptoms in nasopharyngitis are related to the causative virus. Parainfluenza and respiratory syncytial viral infections also might have lower respiratory tract findings (laryngotracheitis, pneumonia, or bronchiolitis), and influenza might be associated with more severe, generalized complaints.

Although respiratory symptoms in association with rotaviral gastroenteritis have been noted frequently, little careful clinical study of the respiratory manifestations has been performed. Lewis and associates,[100] in a careful study, observed a statistically significant occurrence of nasal discharge, cough, and red throat in children with rotaviral diarrhea compared with children with diarrhea caused by other agents.

Nasopharyngitis with *H. influenzae* or *N. meningitidis* infections has been noted mainly in patients with septicemia and meningitis. The nasal symptoms (coryza) usually preceded the pharyngitis and severe systemic disease by a few to several days. In Q fever, the predominant finding is pneumonia. With diphtheria, the exudative pharyngitis and constitutional symptoms are most prominent.

PHARYNGITIS, TONSILLITIS, AND TONSILLOPHARYNGITIS

ETIOLOGIC AGENTS

Etiologic agents of pharyngitis categorized by type of lesion, frequency of occurrence, and duration of illness are presented in Table 10–2. Numerous diverse possibilities exist for the differential diagnosis of pharyngitis. The specific agents or factors are presented in their respective sections of this book, and only an overview is given here.

As with all infectious diseases, etiologic prevalence depends on multiple factors (status of the host, age, season, environment, exposure, and type of lesion) that must be considered in each individual case. In otherwise healthy children, the following infectious agents account for more than 90 percent of acute infections with pharyngeal involvement: *Streptococcus pyogenes*; adenoviruses; influenza viruses A and B; parainfluenza viruses

*See references 11, 21, 33, 35, 87, 116, 133, 153, 157, 170.
†See references 7, 71, 79, 80, 87, 88, 122, 130-132, 139, 140, 147, 160.

TABLE 10–1 Etiologic Agents of Nasopharyngitis

Etiologic Agent	Type of Pharyngeal Lesion*			Relative Importance of Nasal and Pharyngeal Symptoms†		Frequency of Pharyngitis‡	Main Season	Duration of Pharyngitis
	Erythematous	*Follicular*	*Exudative*	*Nasal*	*Pharyngeal*			
Bacteria								
Corynebacterium diphtheriae[181]	+++		++++	+	+++	+	Fall, winter, spring	Acute, subacute
Haemophilus influenzae[174]	++			++	++	+	Fall, winter, spring	Acute, subacute
Neisseria meningitidis[154]	++			+	+++	+	Fall, winter, spring	Acute, subacute
Viruses								
Adenoviruses[11,21,33,35,40,116,133,153,157,170]	++++	++++	++	+	+++	++++	All seasons	Acute
Enteroviruses (polio, coxsackieviruses A and B, echovirus)[24,32,90,91,116,149,150,175]	+++		+	+	+++	+++	Summer, fall	Acute
Influenza A and B[90,131,139,147,160]	+++			+	+++	++	Fall, winter	Acute
Parainfluenza[1-4,7,71,79,80,88,122,140,146,150,152,160]	++		+	+++	+	++	Fall, winter, spring	Acute
Respiratory syncytial[20,77,80,111,130]	++			+++	+	+	Fall, winter, spring	Acute
Rhinoviruses[74,90]	+			+++	+	+	Fall, winter, spring	Acute
Human metapneumovirus[151]	++			+++	+	+	Fall, winter, spring	Acute
Human bocavirus[51]	++			+++	+	+	Fall, winter, spring	Acute
Rotaviruses[70,100,111,143]	++			++	++	++	Fall, winter, spring	Acute
Rickettsia								
Coxiella burnetii[86]	++			++	++	+	All seasons	Acute

**Plus signs indicate the relative degree and severity of the lesion (++++, most marked; +, minimal).*
†Each +, 25%.
‡++++, 76 to 100 percent; +++, 51 to 75 percent; ++, 26 to 50 percent; +, 1 to 25 percent.

1, 2, and 3; Epstein-Barr virus; enteroviruses; and *Mycoplasma pneumoniae*.*

Although group A streptococci are suggested frequently[25] as the only worthy bacterial consideration in the etiology of pharyngitis, the data in Table 10–2 indicate broader possibilities. When streptococci with beta-hemolysis recovered from children and adolescents with pharyngitis are typed, group B, C, and G strains occasionally are found.[5,26,28,58,75,166,167] Turner and associates[166] found that of the group C streptococci, only *Streptococcus equisimilis* caused pharyngitis; *Streptococcus anginosus* (*Streptococcus milleri*) was part of the normal oropharyngeal flora.

Laboratory accidents have provided evidence that *H. influenzae* can cause pharyngitis,[129,173] and children with systemic illnesses caused by *H. influenzae* and *N. meningitidis* frequently have an associated marked pharyngitis.[152,174,178] *Arcanobacterium haemolyticum* and *Corynebacterium ulcerans* occasionally cause an illness mimicking diphtheria.[15,68,89,93,107,134] *A. haemolyticum* also causes an illness that has been confused with streptococcal scarlet fever.[46,89,104,107]

Because anaerobic microorganisms are universal constituents of the normal throat flora, assigning etiologic significance to these agents in throat infections frequently is difficult. Vincent stomatitis and angina seem to result from mixed infections with anaerobes.[48,63,106,169] Brook and Gober[14] noted a significant association between encapsulated organisms of the *Bacteroides melaninogenicus* group (*Prevotella melaninogenica*) and acute tonsillitis in children. My impression is that acute and subacute infections with anaerobes account for numerous pharyngeal infections in adolescents in which cultures do not reveal group A streptococci and infectious mononucleosis test results are negative. Gonococcal and treponemal infections should be considered in sexually active or known exposed teenagers and other children.[17,31,49,84,177]

Adenoviral types 1, 2, 3, 4, 5, 6, 7, 7a, 9, 11, 14, 15, and 16 are the most common causes of pharyngitis in young children, and they are prominent etiologic agents in older children and adolescents.* Pharyngeal involvement frequently is overshadowed by other respiratory symptoms (e.g., cough, coryza) in parainfluenza viral infections and by systemic complaints (e.g., fever, exanthem, meningitis) in enteroviral infections.† An enteroviral etiology should be suspected when small ulcerative lesions are noted that involve the soft palate and uvula and the posterior pharyngeal wall (see Chapter 11). Infection with Epstein-Barr virus causes infectious mononucleosis with pharyngeal involvement similar to that resulting from group A streptococcal infection.[76,171] The clinical manifestations of Epstein-Barr virus are age-related; young children rarely have marked pharyngeal involvement.

*See references 1, 7, 11, 12, 24, 25, 32, 33, 37, 43, 61, 66, 71, 76, 79, 80, 88, 90, 91, 115-117, 122, 130-133, 139, 140, 146-150, 153, 155, 157, 158, 170, 171, 175.

*See references 11, 18, 33, 36, 116, 119, 133, 153, 157, 170.
†See references 1, 7, 24, 32, 71, 79, 80, 88, 91, 116, 122, 130, 132, 140, 146, 149, 150, 155, 160, 175.

TABLE 10–2 Etiologic Agents of Pharyngitis

Etiologic Agent or Factor	Type of Lesion*					Frequency of Occurrence†	Duration of Pharyngitis
	Erythematous	*Follicular*	*Exudative*	*Ulcerative*	*Petechial*		
Bacteria							
Streptococcus pyogenes[12,37,45,61,115,117,148,158]	++++	++	+++		+++	++++	Acute
Other streptococci (groups B, C, and G)[2,26-28,53,75,102,166,167]	+++	+	++			++	Acute
Corynebacterium diphtheriae[112,148,181]	+++		++++			+	Acute
Corynebacterium pyogenes[172]	++++		++++			+	Acute
Corynebacterium ulcerans[104,134,163]	++++		+++			+	Acute
Arcanobacterium haemolyticum[4,15,46,63,89,93,107]	++++	++	+++			+	Acute
Mixed anaerobes (*Prevotella* spp., *Peptostreptococcus*, *Fusobacterium* spp.)[13,14,16,43,63,106,169]	++++		+	++++		++	Subacute
Actinomyces spp.[48]	+			+		+	Chronic
Francisella tularensis[85,168,179]	++++		+++			+	Acute
Haemophilus influenzae[129,159,173,174,178]	++					++	Acute, subacute
Legionella pneumophila[120]	++++					+	Acute
Neisseria meningitidis[152]	++		+			++	Acute
Neisseria gonorrhoeae[84,177]	++		+			+	Acute, subacute, chronic
Leptospira spp.[81,135]	++++					+	Acute
Treponema pallidum[17,31,49,70]	+	+		+		+	Subacute
Borrelia spp.[97]	++++					+	Acute
Streptobacillus moniliformis[138]	+					+	Acute
Yersinia enterocolitica[142,161]	++++		++			+	Acute
Yersinia pseudotuberculosis[145]	+					+	Acute
Streptococcus pneumoniae[109]	+			+		+	Acute
Salmonella typhi[3,121]	+					+	Acute
Rothia dentocariosa[124]	+		+			+	Acute
Mycobacterium tuberculosis[29]				+		+	Chronic
Chlamydia							
Chlamydia pneumoniae[42,67]	++++					++	Acute
Chlamydia trachomatis[123]	++	+	+			+	Acute, recurrent
Viruses							
Adenoviruses[11,35,116,119,133,153,170]	++++	++++	++			++++	Acute
Influenza A and B[131,139,147,160]	+++					+++	Acute
Parainfluenza[1-4,7,71,79,80,122,130,132,140,146,160]	++					+++	Acute
Respiratory syncytial[20,77-80,111,130]	++					+	Acute
Enteroviruses (polio, coxsackieviruses A and B, echovirus)[1,24,82,91,149,150,155,175,176]	+++		+	++		+++	Acute
Epstein-Barr[76,171]	+++	+	++++		++	+++	Acute, subacute
Reoviruses[99,180]	++					+	Acute
Cytomegalovirus[10,95]	+					+	Acute
Herpes simplex[19,23,43,116,176]	++		++	++++		++	Acute
Measles[22]	+++				+	++	Acute
Rubella[22,50]					++	+	Acute
Rhinoviruses[74]	+					+	Acute
HIV[6]	++						Acute
Mycoplasma							
Mycoplasma pneumoniae[25,42,61,66,80]	++	+	+			++	Acute
Mycoplasma hominis[118]	+		+			+	Acute
Rickettsia							
Coxiella burnetii[41,86,126]	++					+	Acute
Fungi							
Candida spp.[98,154]	+		++++			+++	Acute, subacute, chronic
Parasites							
Toxoplasma gondii[96]	+					+	Acute

Continued

TABLE 10–2 Etiologic Agents of Pharyngitis—cont'd

Etiologic Agent or Factor	Type of Lesion*					Frequency of Occurrence†	Duration of Pharyngitis
	Erythematous	*Follicular*	*Exudative*	*Ulcerative*	*Petechial*		
Recognized Illnesses of Uncertain Etiology							
Aphthous stomatitis[64,144]	+			++++		++	Acute, recurrent
PFAPA[45,53,82,85,105,110,125,162]	++		++	++		+	Acute, recurrent
Behçet syndrome[108]	+			++++		+	Chronic, recurrent
Kawasaki disease[73,114]	++	+				+	Acute
Stevens-Johnson syndrome[22]	+		+	++++		+	Acute
Illness in which Host Factors or Therapeutic Agents are Primary Causes							
Neutropenia, other immunodeficiencies, cancer, chemotherapeutic agents, generalized neoplastic disease[22]	+			++++		++	Chronic

**Plus signs indicate the relative degree and severity of the lesion (++++, most marked; +, minimal).*
†++++, 76 to 100 percent; +++, 51 to 75 percent; ++, 26 to 50 percent; +, 1 to 25 percent.
HIV, human immunodeficiency virus; PFAPA, periodic fever, aphthous stomatitis, pharyngitis, and adenitis (syndrome).

Acquired cytomegalovirus infection causes an infectious mononucleosis–like syndrome, but pharyngitis occurs less commonly than it does in Epstein-Barr virus mononucleosis.[95] Primary and recurrent herpes simplex virus infections occasionally have pharyngeal manifestations.[19,23,43,116] Many illnesses that are ascribed clinically to herpes simplex virus actually are misidentified instances of aphthous stomatitis, however. Virtually all instances of herpes simplex virus infections with pharyngitis also reveal lesions in the anterior mouth and externally around the mouth. Although Koplik spots are known universally as the enanthem of measles, many physicians are unaware of the diffuse nature of the associated measles pharyngitis.[22] Primary infection with human immunodeficiency virus may cause the acute retroviral syndrome with fever, nonexudative pharyngitis, lymphadenopathy, exanthem, arthralgia, myalgia, and lethargy.[6]

The role of *Chlamydia* spp. in the cause of pharyngitis is unclear. Grayston and associates[65,67] noted the occurrence of pharyngitis in adolescents and young adults infected with *Chlamydia pneumoniae*. In a study in children, IgM antibody to *Chlamydia trachomatis* was found in children with pharyngitis.[72] Because cross-reactivity between *C. trachomatis* and *C. pneumoniae* exists, the illnesses studied were likely to be caused by *C. pneumoniae*, rather than by *C. trachomatis*. Ogawa and colleagues[123] noted prolonged and recurrent tonsillitis in association with sexually transmitted *C. trachomatis*.

Mild pharyngitis occurs in young children with *M. pneumoniae* infections; in older children, pharyngeal involvement is more pronounced.[25,61,66,80] In young adult volunteers, *Mycoplasma hominis* was noted to cause pharyngitis.[103] Although lower respiratory tract findings and systemic complaints are most marked in Q fever, moderate subjective and mild objective evidence of pharyngitis also is noted.[41,86,126] Exudative pharyngeal involvement with *Candida* spp. commonly occurs.[98,154] *Candida* infection most commonly occurs in children whose normal throat flora has been disrupted and in children who have a compromised immunologic response.

Recurrent aphthous stomatitis usually involves the anterior oral cavity, but occasionally is noted with extensive pharyngeal and soft palate lesions.[64,144] Although L-forms (spheroplasts) of *Streptococcus sanguinis* can be recovered consistently from the lesions of this disease,[64] their role in the etiology is unclear. In 1987, Marshall and colleagues[110] described a new syndrome, periodic fever, aphthous stomatitis, pharyngitis, and adenitis (PFAPA), and it subsequently has been observed by others.[45,53,82,85,105,125,162]

In 1999, Thomas and associates[162] presented an analysis of data in their PFAPA registry of 94 cases. The male-to-female ratio was 1.8:1, and the mean age of PFAPA onset was 2.8 years (95% confidence interval 2.4 to 3.3). The children had an average 11.5 febrile episodes per year, and the average symptom-free interval between episodes was 28.2 days (99% confidence interval 26 to 30.4 days). The average duration of an episode was 4.8 days, with the temperature being greater than 38.3° C for 3.8 days. The average maximum temperature per episode was 40.5° C (95% confidence interval 40.4 to 40.6° C). Along with the periodic fevers, the children also experienced aphthous stomatitis (67%), pharyngitis (65%), cervical lymphadenopathy (77%), chills (80%), headache (65%), abdominal pain (45%), nausea (52%), diarrhea (30%), cough (20%), coryza (18%), and rash (15%). In addition to fever, 97 percent had one or more of the following: aphthous stomatitis, pharyngitis, or adenopathy. The mean leukocyte count was 13,000 with 62 percent polymorphonuclear leukocytes, and the mean erythrocyte sedimentation rate was 41 mm/hr.

In PFAPA, the episodes persist for several years with unchanged findings and periodicity. In the Nashville data, 41 percent of the children had stopped having episodes after a mean of 4.5 years. Before remission, the episodes decreased in frequency over an extended period.[162] Treatment modalities that have offered relief include ibuprofen (85%) and prednisone (90%). Thomas and associates[162] recommend prednisone or prednisolone treatment (1 mg/kg at the beginning of an attack and the same dose on the next morning and 0.5 mg/kg on days 3 and 4). Tonsillectomy, adenoidectomy, and prophylactic cimetidine administration have been associated with remission in some children.

In Behçet syndrome, the ulcerative lesions are subacute or chronic and usually are not associated with surrounding pharyngeal inflammation.[108] In Kawasaki disease, the pharyngeal mucosa is deeply erythematous.[114]

Pharyngeal involvement is a common occurrence in noninfectious illnesses in which host resistance has been altered. The

lesions usually are ulcerative, but secondary bacterial or fungal overgrowth can lead to marked erythematous and exudative findings.

EPIDEMIOLOGY

Pharyngitis is a common occurrence in children and, as noted in Table 10–2, is the result of many different infectious agents. Generally, bacterial pharyngitis occurs more commonly in the cold weather seasons; an enteroviral etiology is most common in the summer and fall. In younger children, viral pharyngitis tends to occur more frequently than does bacterial disease.[36] It has no apparent sex predilection.

Most diseases associated with pharyngitis require close person-to-person contact for spread. Pathogens are transmitted directly by close-range airborne dissemination or indirectly by the hands of the future host. Several food-borne outbreaks of streptococcal pharyngitis have been reported.[44,52] Prechewing of food by adults also may be a cause of streptococcal pharyngitis in infants.[156]

PATHOPHYSIOLOGY

The pathology and pathophysiology of diseases in which pharyngitis is prominent are presented in the chapters of this book on specific infectious agents (in particular, see Chapters 93, 168, and 178).

CLINICAL PRESENTATION

The clinical findings in pharyngitis are highlighted in Table 10–2. Manifestations related to individual pathogens are detailed in the chapters of this book dealing with the specific agents. The onset of pharyngitis usually is sudden and accompanied by fever and the complaint of sore throat. Frequently, parents observe that the child's breath is not normal, and that the throat and particularly the tonsils are red. Other initial complaints include headache, nausea, vomiting, and sometimes abdominal pain. Anorexia is the rule, as is some degree of lessened activity. Parents also report frequently that the child's cervical lymph nodes are enlarged and tender.

Physical examination usually substantiates the parents' observations—the child is febrile with moderate to severe pharyngeal erythema and some degree of cervical adenitis. As noted in Table 10–2, the pharyngeal response varies. With acute common infections, the basic lesion is erythema. Associated with erythema can be follicular, ulcerative, and petechial lesions and generalized or circumscribed exudative areas. Follicular lesions are most characteristic of adenoviral infections, whereas exudative lesions occur most commonly in group A streptococcal infections and in infectious mononucleosis. Meland and associates[113] noted that the absence of cough and the presence of swollen lymph nodes had the highest specificity in predicting a streptococcal cause of pharyngitis. Ulcerative lesions are observed most frequently in enteroviral infections (see Chapter 178). Petechiae on the soft palate frequently are seen in group A streptococcal infections, but also commonly occur in infectious mononucleosis, measles, and rubella.

Occurrences of pharyngitis in children almost always are acute, self-limited diseases. Pharyngitis of viral origin lasts 4 to 10 days, and pharyngitis caused by group A streptococci, if untreated, lasts slightly longer. Subacute and chronic pharyngeal disease is an uncommon occurrence in children, but the etiologic possibilities are numerous (see Table 10–2).

DIFFERENTIAL DIAGNOSIS

As noted in Table 10–2, the differential diagnoses in pharyngitis are numerous, as in nasopharyngitis (see Table 10–1). Although considerable overlap exists in the spectrum of illness in pharyngeal infections, many clues help in ruling in or out certain diagnostic possibilities. The diagnosis of pharyngitis requires carefully eliciting epidemiologic and other historical data (i.e., exposure, season, incubation period, age of patient, associated clinical findings), in addition to observing pharyngeal physical findings.

Most acute instances of nasopharyngitis are of viral origin (see Table 10–1), with adenoviruses accounting for the greatest number of cases. Nasopharyngitis also occurs during epidemic influenza A and B and parainfluenza 1 and 2 infections, and with sporadic parainfluenza 3 infections. In all cases, proper epidemiologic and historical data should be elicited so that early diphtheria and other unusual but treatable illnesses are not diagnosed incorrectly. Retrospective study has indicated that nasopharyngitis occasionally occurs in severe infections from *H. influenzae* and meningococci, so the possible presence of these infections should be considered when the epidemiology suggests it.

Although most cases of pharyngitis without nasal symptoms are caused by viral infections, numerous other etiologic possibilities exist. The approach taken frequently by many physicians is to consider all pharyngitis as being of bacterial origin and to treat it with antibiotics. The initial consideration in a child with pharyngitis should be the duration of illness. Subacute, chronic, and recurrent illnesses (see Table 10–2) generally suggest more unusual problems and require a more deliberate approach. In all instances of acute pharyngitis, ruling out streptococcal disease is mandatory.

SPECIFIC DIAGNOSIS

The epidemiologic history and careful clinical categorization are the most important aspects of specific diagnosis. The most pressing diagnostic need in upper respiratory infections in everyday pediatric practice is distinguishing bacterial from viral disease—who and who not to treat with antibiotics. An approach to the problem is presented in Table 10–3.

A child with the common cold, herpangina, or pharyngoconjunctival fever has a viral disease and does not need therapy with antibiotics. In most instances, when these clinical diagnoses are apparent, performing throat cultures for bacterial pathogens is unnecessary. A child with severe acute pharyngitis with exudate and fever and cervical lymphadenitis also is treated easily because most of these children have disease resulting from group A streptococci.

Only a few children have illnesses at the extremes of those shown in Table 10–3. Most illnesses seen routinely by the physi-

TABLE 10–3 Treatment Considerations in Upper Respiratory Tract Infections

Clinical Entity		Etiology
Common cold, herpangina, pharyngoconjunctival fever		Viral, 100%
↓	Never-Never Land	↓
Marked pharyngitis with exudate and fever and cervical lymphadenitis		Bacterial, 70%

Modified from Cherry, J. D.: Newer respiratory viruses: Their role in respiratory illness of children. In *Schulman, I., et al. (eds.): Advances in Pediatrics. Vol. 20. Chicago, Year Book Medical Publishers, Inc., © 1973. Used by permission.*

cian treating children fall into the large middle area—"never-never land." On clinical grounds, no certain way to make an etiologic diagnosis and specifically to rule out infection caused by *S. pyogenes* exists. Until more recently, the usually recommended approach to management of pharyngitis and nasopharyngitis was to obtain a throat culture to determine whether group A streptococci were present. Because several rapid tests (rapid antigen detection tests [RADT]) for the detection of group A streptococci are available, establishing the immediate diagnosis and providing treatment of streptococcal pharyngitis are possible in the practice setting.* Generally, these rapid tests have high specificity, but sensitivity is suboptimal. If early treatment is desirable today, a reasonable approach to establishing the diagnosis of streptococcal pharyngitis is to do a rapid test. If the test result is positive, specific therapy is instituted. In the case of a negative rapid test result, a routine throat culture is done, and therapy is withheld pending the culture results.[9,39,137]

A question exists regarding whether early treatment of streptococcal pharyngitis is desirable.[40,55,57,136,137] In the public clinic, where following up children with positive throat cultures often is difficult, providing immediate diagnosis and treatment is important. As Pichichero and associates[136,137] and El-Daher and colleagues[40] have shown, early treatment may result in a decreased desirable antibody response, allowing reinfection with type-specific organisms to occur. In settings in which the communication between physicians and parents is satisfactory, an advisable approach in many instances is to withhold the institution of treatment for 1 or 2 days. Gerber and associates[55,57] argue against this approach because providing immediate therapy can reduce the risk of transmission of infection, and they challenge the interpretation of the findings of Pichichero and associates[136,137] and El-Daher and colleagues.[40]

Cultures for other pathogens should be reserved for unusual situations, such as persistent symptoms, indicative epidemiology, or other pertinent historical data. Because *H. influenzae* and *Streptococcus pneumoniae* frequently are part of the normal flora, their isolation is not always etiologically significant. If cultures reveal predominant growths of either *H. influenzae* or *S. pneumoniae*, however, they are likely contributing to the disease process, and antibiotic therapy directed at the specific pathogen would benefit the patient.[23,109,178] When the possibility of disease because of anaerobic agents exists, a Gram-stained smear from an exudative area may be rewarding. When the pharyngeal findings are unique, or when many cases of a similar illness are observed, a viral culture from the throat is indicated.

TREATMENT

The specific treatments of diseases with nasopharyngitis and pharyngitis are discussed in chapters of this book that deal with the individual pathogens. That antimicrobial agents are prescribed inappropriately in children with sore throat and pharyngitis is common knowledge. Based on the etiologic agents noted in Tables 10–1 and 10–2, it is apparent that many infectious agents are associated with nasopharyngitis and pharyngitis, which can be treated effectively with antimicrobial agents. Except in rare specific circumstances, the only common pathogens that require treatment are group A streptococci. The treatment of streptococcal pharyngitis is discussed in detail in Chapter 93. A study noted that physicians prescribed antibiotics to 53 percent of children with sore throat, which is far greater than the 15 to 36 percent likelihood of a group A streptococcal infection.[103] This study also noted that this rate of antimicrobial usage is an improvement from 1995, when the prescribing rate was 66 percent.

Although a multitude of proprietary remedies are available for respiratory infections, and sore throat specifically, none has a place in the care of pediatric patients. Particularly to be condemned are throat lozenges that contain numerous useless ingredients, many potentially harmful. Antibiotic-containing lozenges particularly are to be condemned because they may allow streptococcal disease to go unrecognized. Antiseptic mouthwashes have no value, and decongestants and antihistamines have no proven efficacy and frequently lead to troublesome side effects.

Because children with pharyngitis frequently feel ill, therapy with an analgesic is reasonable. Formerly, aspirin was the analgesic usually recommended. However, because aspirin is an etiologic factor in influenza-associated Reye syndrome and because differentiating influenza viral infections from other respiratory viral infections is difficult clinically, using acetaminophen rather than aspirin is prudent. The dose per single administration of acetaminophen by year of age is as follows: younger than 1 year, 60 mg; 1 to 3 years, 60 to 120 mg; 3 to 6 years, 120 mg; 6 to 12 years, 150 to 300 mg; older than 12 years, 325 to 650 mg. Administration may be repeated three or four times daily in young children and every 4 hours in older children. Acetaminophen rarely should be given to infants younger than 6 months old. In young children, careful attention to adequate hydration is particularly necessary.

PROGNOSIS

Almost all occurrences of nasopharyngitis and pharyngitis are self-limited, and the overall prognosis is excellent. Keeping a constant vigil for streptococcal and other more serious diseases is necessary, however. Failure to diagnose and to treat group A streptococcal infections, syphilis, and other more unusual infections can lead to serious short-term and long-term difficulties.

PREVENTION

Because pharyngitis and nasopharyngitis are caused by infections with many different respiratory pathogens, no practical specific approach to prevention exists. Occasionally, streptococcal disease can be prevented by the judicious use of prophylactic penicillin. For young children or others with undue susceptibility to serious disease with common respiratory pathogens, reducing contact situations (e.g., daycare centers) is prudent. In the 1980s, Paradise and colleagues[127] studied the effect of tonsillectomy on the rate of recurrent throat infections in severely affected children. In this controlled study, children needed to have a history of seven or more episodes of tonsillitis in the preceding year, or five or more episodes in each of 2 preceding years, or three or more episodes in the 3 preceding years. The findings in this study indicated that children in the surgical group had significantly fewer throat infections than occurred in the nonsurgical control group during the ensuing 2 years of follow-up. In a more recent but similar study with less stringent criterion for surgery, Paradise and colleagues[128] found that tonsillectomy or adenotonsillectomy conferred a modest benefit. They concluded, however, that the modest benefit of tonsillectomy or adenotonsillectomy in children with recurrent throat infections does not justify the risks, morbidity, and cost of the operations.

*See references 6, 8, 9, 34, 38, 39, 54, 56, 59, 60, 78, 94, 101, 136, 165.

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CHAPTER 11

HERPANGINA

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Herpangina, a fairly frequent, acute febrile illness that occurs in the summer and fall in temperate climates, is characterized by papular, vesicular, and ulcerative lesions on the anterior tonsillar pillars, soft palate, tonsils, pharynx, and posterior buccal mucosa. It is caused by many different enteroviruses.

HISTORY

Zahorsky[48,49] generally is credited with the identification and characterization of the disease spectrum of herpangina. In his first publication in 1920 entitled "Herpetic Sore Throat," he presented the findings in 82 cases. In 1924, he introduced the term *herpangina* so that the clinical entity would not be confused with other diseases of the mouth and throat. In both articles, Zahorsky noted that Moro previously had referred to a similar illness in 1906. Johnsson and Lindahl[19] also reported that similar syndromes had been observed by Trousseau in 1906 and Marfan in 1924 and Moro in 1906. In 1939, Levine and associates[26] described epidemic herpangina in three summer camps, and in 1941, Breese[4] reported 28 cases that he observed during the summers of 1938 and 1940. In 1951, Huebner and associates[18] and Parrott and colleagues[35] established the etiologic relationship of group A coxsackieviruses to herpangina. In 1965, Cherry and Jahn[10] noted from the literature and from their own observations that herpangina resulted from infection with many echoviruses and group B coxsackieviruses as well as group A coxsackieviruses.

ETIOLOGIC AGENTS

Virologic studies in the early 1950s that used suckling mice inoculation indicated that several group A coxsackieviruses were the cause of epidemic herpangina.[18,20,22,35,39,45] In subsequent years, tissue culture techniques became widely used in diagnostic virology, and many studies revealed additional enteroviral types asso-

TABLE 11–1 Etiologic Agents Found in Association with Sporadic or Epidemic Herpangina Occurrence

Virus	Occurrence		Reference
	Epidemic	*Sporadic*	
Coxsackievirus A			
1	+		20, 44, 45
2	+		20, 39, 44, 45
3	+		20, 39, 44, 45
4	+		20, 39, 44, 45
5	+		9, 20, 44, 45, 47
6	+		20, 39, 44, 45, 47
7		+	20, 37
8	+		20, 39, 44, 45
9		+	10, 20, 25, 37
10	+		20, 39, 44, 45
12		+	47
16		+	7, 10, 20
22	+		20, 44, 45
Coxsackievirus B			
1	+	+	10, 14, 30, 37, 44, 45
2		+	1, 14, 20, 28, 37, 44, 45
3		+	13-15, 20, 24, 33, 37, 44-46
4		+	2, 3, 10, 11, 14, 20, 36, 37, 44, 45
5		+	2, 14, 20, 37, 42, 44, 45
Echovirus			
6		+	20
9		+	10, 20, 23, 37, 42
11		+	31, 43
16	+	+	20, 34
17		+	10
25	+		32
Parechovirus 1		+	31
Enterovirus 71	+		5-8, 16, 17, 21, 27, 40
Herpes simplex		+	12, 29

ciated with herpangina.[10] Except for the initial interest in herpangina in 1950, careful study of the etiology of epidemic disease has not been done. Most herpangina virus-illness associations that have been made during the last 50 years have resulted from secondary findings in other investigations.* Table 11–1 lists viral agents associated with herpangina. In recent years, group B coxsackieviruses and enterovirus 71 have been implicated most frequently. Diagnostic studies using suckling mice inoculation have been performed only rarely during the last 40 years, however. As noted in Table 11–1, herpes simplex virus also can cause a clinical picture suggestive of herpangina.[12,29] Zahorsky[49] suggested that the cause of poliomyelitis also could be etiologic in herpangina because a similar enanthem had been observed in sporadic and epidemic poliomyelitis.

EPIDEMIOLOGY

For a discussion of epidemiology, see sections on coxsackieviruses and echoviruses in Chapter 178.

PATHOPHYSIOLOGY

The pathophysiology of coxsackievirus and the pathophysiology of echovirus infections are discussed in their respective sections in Chapter 178. Simkova and Petrovicova[41] presented specific experimental data related to herpangina. In rhesus monkeys, these investigators found that oropharyngeal lesions typical of herpangina developed 2 to 7 days after oral, intravenous, or subcutaneous administration of coxsackievirus A4. This study indicated that the oropharyngeal lesions were the result of multiplication of virus at the secondary infection site after viremia, rather than a primary manifestation of initial cellular involvement.

CLINICAL MANIFESTATIONS

Although Zahorsky[48,49] and others[18,35] have considered herpangina a specific febrile disease, perhaps a more appropriate approach is to restrict the term *herpangina* to the characteristic oropharyngeal lesions. Herpangina is one of the protean manifestations of enteroviral infections, and it can occur in association with exanthem, aseptic meningitis, encephalitis, acute flaccid paralysis, and other clinical constellations.

The onset of herpangina is typical of most enteroviral infections and is characterized by the sudden awareness of fever.[4,35,48,49] No characteristic prodrome usually is present, but young children may be irritable and occasionally listless and anorexic for a few hours before the febrile state is recognized. The initial temperature can vary, with a range from normal to 41° C (106° F). Generally, the temperature tends to be higher in younger patients. Breese[4] noted that the most common temperature in young children was 39.5° C to 40° C (103° F to 104° F). Older children frequently complain of headache and backache. Vomiting occurs in approximately 25 percent of children younger than 5 years. In one outbreak of illness caused by coxsackievirus A4,[12] initial symptoms were anorexia and drooling (100%); sore throat (50%); coryza (45%); headache (18%); and vomiting, diarrhea, or both (36%).

In most instances of herpangina, the oropharyngeal lesions are present on the first examination at the time of fever or shortly after fever is noted. In the coxsackievirus A4 outbreak described by Forman and Cherry,[12] the enanthem was not observed until 24 to 48 hours after the onset of initial nonspecific symptoms. The characteristic lesions in herpangina are small (1 to 2 mm) vesicles and ulcers. These lesions apparently start as papules, become vesicular, and ulcerate in a short but variable period. In my experience, the lesions most commonly observed are ulcers. Breese[4] noted in many children seen early in the course of the illness that a petechial appearance preceded the appearance of typical vesicular-ulcerative enanthem.

The lesions usually are discrete, with an average of five per patient; some patients have only 1 or 2 lesions, whereas others may have 14 or more. When seen early, the vesicular lesions are observed to enlarge from 1 to 2 mm to 3 to 4 mm during a 2- to 3-day period.[35] Each vesicular and ulcerative lesion is surrounded by an erythematous ring that varies in size up to 10 mm in diameter. The most common site of the lesions is the anterior tonsillar pillars. Lesions also occur on the soft palate, uvula, tonsils, and pharyngeal wall and occasionally on the posterior buccal surfaces. In some cases, additional lesions have been noted on the dorsum or tip of the tongue. By definition, cases in which the primary involvement is on the tongue or anterior mouth and in which the lesions are of a general size greater than 5 mm are not considered to be herpangina.

Aside from the specific lesions, the remainder of the throat appears normal, minimally injected, or mildly erythematous. The usual duration of signs and symptoms is 3 to 6 days. Most cases of herpangina are mild and without complications, but aseptic meningitis and other more severe enteroviral manifestations also occur occasionally. More recently, severe neurologic manifestations have been observed in children with herpangina caused by enterovirus 71.[5,6,8,16,17,21,27,40] During epidemic disease in Taiwan in 1998, children with herpangina and aseptic meningitis, acute

*See references 1-3, 5-8, 10-17, 21, 23-25, 27-32, 34, 36-38.

flaccid paralysis, and rhombencephalitis were described.[16,17,40] In one study, a biphasic course was described, with herpangina occurring first and neurologic manifestations appearing 2 to 5 days later.[17]

Routine laboratory studies are of little value in herpangina. The total white blood cell count may be normal or slightly elevated; the differential count most often is normal.

DIFFERENTIAL DIAGNOSIS

The classic appearance of the oropharynx in herpangina renders establishing the diagnosis easy. Herpangina can be differentiated clearly from bacterial pharyngitis on clinical grounds, so obtaining bacterial cultures seldom is necessary. The follicular lesions of adenoviral infections can be confused with herpangina, but they frequently are exudative, not ulcerative, and associated with a more marked, generalized, erythematous pharyngitis than is seen in herpangina. Additional differential considerations are discussed elsewhere (see Chapters 10 and 178).

SPECIFIC DIAGNOSIS

In most instances, a clinical diagnosis is all that is necessary. Because herpangina is a good indicator of enteroviral disease in a community, however, submission of throat or rectal specimens to a viral diagnostic center can be rewarding.

TREATMENT, PROGNOSIS, AND PREVENTION

No treatment is necessary other than attention to hydration and observation for signs of more severe enteroviral illness. Except in rare instances (associated myocarditis, encephalitis), the prognosis is excellent. No general preventive measures are necessary, but a wise policy is not to expose young children unnecessarily to individuals known to be afflicted.

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CHAPTER 12

Pharyngoconjunctival Fever

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Pharyngoconjunctival fever is an acute, communicable disease syndrome characterized by fever, pharyngitis, and conjunctivitis. It is caused by several serologic types of adenovirus. Illness is epidemic and sporadic.

HISTORY

Shortly after the first isolation of adenoviruses in tissue culture by Rowe and associates[54] in 1953, the clear association of infection with certain adenoviral types and the syndrome of fever, pharyngitis, and conjunctivitis were established.[52] For an approximate 5-year period after the discovery of the adenoviral etiology of pharyngoconjunctival fever, the literature contained numerous confirmatory reports from throughout the world.* In almost all reports, the association between swimming and the contraction of the syndrome was noted. A quick perusal of the reports would suggest that the syndrome and the etiologic agents were new discoveries. Epidemics of pharyngoconjunctival fever–like illness have been noted throughout the 20th century, however. In 1907, Béal[4] in France was perhaps the first to note the syndrome. In the 1920s, epidemics of febrile disease with conjunctivitis associated with swimming in public pools and lakes were noted in Germany[51] and the United States.[2] It is likely that "swimming bath conjunctivitis," as described by Derrick[16] in 1943, was caused by adenoviral infection. An epidemic of conjunctivitis studied by Cockburn and associates[12] in Greeley, Colorado, in 1951 later was proved to have been caused by adenovirus type 3.

In more recent years, reports of pharyngoconjunctival fever have been relatively few.** My experience suggests that this paucity is not because of a decrease in prevalence of the syndrome but because of a general disinterest in the differential diagnosis of viral respiratory disease.

ETIOLOGIC AGENTS

In epidemic pharyngoconjunctival fever, the most likely etiologic agent is adenovirus type 3.† The next most prevalent adenovirus associated with epidemic disease is type 7.[9,17,22,73] One or more epidemics also have been noted with adenoviruses 2, 4, 7a, 11, and 14.[1,7,15,18,19,48,66,67,76] Sporadic occurrences of pharyngoconjunctival fever have been observed in association with infections with adenoviruses 1, 2, 3, 4, 5, 6, 7, 7a, 8, 14, 19, and 13/30 (an intermediate type).‡

EPIDEMIOLOGY

Pharyngoconjunctival fever occurs in large community-wide epidemics, in focal outbreaks, and as sporadic cases. Most major community epidemics have occurred in the summer and have been centered around public swimming facilities. Two community outbreaks involving primarily swimmers have occurred in the winter.[9,23] In swimming-associated outbreaks, infection probably occurs by conjunctival inoculation of adenoviruses from contaminated water. To date, however, the virus has been recovered from the incriminated water in only two outbreaks.[15,46] In one outbreak, adenovirus type 4 was recovered from water samples on two occasions 14 days apart.[15] More recently, adenovirus type 3 was recovered from a pool in which 681 campers had symptoms.[46] In this outbreak, the frequency of swimming and the history of towel sharing increased the risk of acquiring illness. Adenovirus type 3 also was recovered from a sewage outlet area in a lake that was close to a swimming beach.[42]

The incubation period of swimming-associated infections is approximately 5 to 7 days.[6,23,36,67] Secondary cases regularly occur in contacts (most often family members) of swimming-acquired cases. In these instances, the incubation period frequently is slightly longer (9 days).[3,23] This longer period of incubation may be due to a delay in the time of spread of the virus to the contact, rather than an actual prolongation of incubation. Secondary cases probably result from large-droplet respiratory spread to the conjunctiva, the upper respiratory tract, or both. An alternative method would be the contamination of the recipient's hands with eye discharge followed by autoinoculation of the conjunctiva.

In non–swimming-associated outbreaks of adenoviral respiratory illness with appropriate serotypes, conjunctivitis occurs only rarely.[8,21,30,35,41,63] This fact, in conjunction with the finding that pharyngoconjunctival fever occurred after conjunctival administration of virus, but not after nasopharyngeal application in volunteers,[7,58,71] suggests that the conjunctiva must be inoculated directly for the syndrome to occur. After conjunctival inoculation, pharyngeal spread and systemic illness occur. After direct respiratory inoculation, the conjunctiva does not become involved, unless respiratory secretions containing virus are applied to the conjunctiva, presumably by autoinoculation. Hospital outbreaks of pharyngoconjunctival fever–like illnesses have been reported.[20,43] Most instances have occurred in intensive care units. An outbreak of pharyngoconjunctival fever also has been noted in a daycare center.[11]

Although some early epidemic investigations suggested that boys were more susceptible to disease than were girls,[22,66] the incidence of pharyngoconjunctival fever in children does not differ by sex.[6,60] In some cultures, boys had more exposure to swimming and accounted for more cases of illness. In an outbreak that occurred in children hospitalized in Japan for long-term treatment of bronchial asthma, the attack rate was 68.2 percent in boys and only 6.3 percent in girls. This sex difference was attributed to the fact that the boys and girls took separate daily communal baths. Secondary cases in adult family members occur more commonly in mothers than in fathers, presumably because of greater contact with the children.[7,23]

PATHOPHYSIOLOGY

The route of infection with adenoviruses that are capable of causing pharyngoconjunctival fever determines the pathologic manifestations. Conjunctival biopsy specimens in volunteer studies revealed an inflammatory response with lymphocytic infiltration of the submucosal layer.[5,7] Biopsy material from pala-

*See references 1, 3, 5, 7, 8, 12, 17, 19, 21, 22, 25-28, 32-37, 39-42, 47-50, 57, 59-61, 63, 65-67, 69, 71.
**See references 9, 10, 13, 15, 18, 23, 24, 28, 31, 32, 45, 46, 48, 53, 54, 56, 58, 64, 68, 70, 72, 73, 76.
†See references 3, 6, 7, 8, 12, 23, 25, 26, 33, 36-38, 42, 45, 46, 49, 52, 59, 66, 72, 76.
‡See references 5, 7, 10, 24, 26, 30-32, 35, 39-41, 47, 55, 56, 60-65, 68, 70.

TABLE 12–1 Frequency of Symptoms in Epidemic Pharyngoconjunctival Fever

Symptoms	Frequency*
Throat Complaints	++++
Soreness	++++
Cough	++
Foreign body sensation	++
Dry feeling	+
Eye Complaints	+++
Aching or soreness	+++
Burning sensation	++
Lacrimation	+
Photophobia	+
Nasal Complaints	+++
Coryza	++
Stuffiness or blockage or both	++
Sneezing	+
Epistaxis	+
Other Complaints	++++
Headache	++++
Anorexia	+++
Malaise	++
Generalized aches and pains	++
Nausea	++
Vomiting	+
Diarrhea	+
Abdominal pain	+

*++++, *76 to 100 percent;* +++, *51 to 75 percent;* ++, *26 to 50 percent;* +, *1 to 25 percent.*
Data from references 5, 9, 22, 25, 36, 39, 40, 45, 52, 57, 58, 66.

TABLE 12–2 Frequency of Signs in Epidemic Pharyngoconjunctival Fever

Signs	Frequency*
Throat Findings	++++
Erythema and infection	++++
Hypertrophied lymphatic tissue	++++
Particulate exudate	++
Eye Findings	++++
Erythema and infection of palpebral and bulbar conjunctiva	++++
Edema	+++
Granular and follicular involvement	++
Eyes unequally affected	++
Superficial punctate keratitis	+
Lymph Node Enlargement	++++
Cervical	++++
Preauricular	+
Generalized	+
Fever	++++
≥39° C (≥102.2° F)	+++
Other	
Flushed face	+++
Enlarged liver or spleen or both	+

*++++, *76 to 100 percent;* +++, *51 to 75 percent;* ++, *26 to 50 percent;* +, *1 to 25 percent.*
Data from references 5, 9, 22, 25, 29, 40, 52, 57, 58, 66.

tine tonsils of infected volunteers revealed hypertrophy and hyperplasia of the lymphoid tissue, with congestion and edema of the surrounding connective tissue.

CLINICAL PRESENTATION

By definition, pharyngoconjunctival fever is a syndrome characterized by fever, pharyngitis, and conjunctivitis. During epidemics, not all children and adults who have the same infection have the complete syndrome triad. Some patients have only pharyngitis, and some have only conjunctivitis. For purposes of this discussion, all descriptions of frequency of signs and symptoms are calculated from the starting point of 100 percent fever, pharyngitis, and conjunctivitis.

Tables 12–1 and 12–2 summarize the frequencies of specific symptoms and signs.* Although some patients have noted mild prodromal symptoms of headache and malaise, the usual onset of illness is abrupt, with sore throat, generalized aches and pains, eye irritation or pain, and fever. Throat complaints vary from mild to severe. In some patients, only a dry, scratchy feeling is reported; others have noted the feeling of a foreign body. On examination, the tonsils and pharyngeal lymphoid tissue are hypertrophied. The degree of pharyngeal redness and infection varies considerably from patient to patient. Approximately one third of affected patients have follicular exudative lesions that cannot be differentiated from streptococcal disease on clinical grounds. Follicular lesions also have been noted on the soft palate, and the papillae of the tongue may be hypertrophied.

Hypertrophy of the adenoids occurs, which results in nasal blockage. Coryza is a common occurrence. Posterior nasal discharge is a common occurrence and leads to cough in many instances. In some investigations, epistaxis has occurred in 20 percent of the cases.[57,66]

*See references 5, 9, 22, 25, 36, 39, 40, 45, 52, 57, 58, 66.

Generally, complaints related to conjunctivitis are fewer than might be suggested by the usual physical appearance. Most patients note some aching or soreness; photophobia and lacrimation are unusual occurrences. The appearance of the palpebral conjunctiva usually is granular. The lesions may be almost microscopic or 2 to 3 mm in diameter. Hemorrhages occasionally are noted on the bulbar surface. Frequently, involvement starts in one eye and does not involve the other eye until 2 or 3 days later. Occasionally, the involvement is restricted to one eye.

Some degree of anterior and posterior cervical lymphadenopathy occurs in most patients. Preauricular involvement occurs surprisingly infrequently when the degree of eye involvement is taken into consideration. Generalized lymphadenopathy is observed in 10 to 20 percent of affected patients, and liver and spleen enlargement occurs frequently.

Most patients complain of generalized symptoms, but the degree varies considerably among affected patients. Temperatures greater than 39° C (102.2° F) occur in more than 50 percent of the patients, and headache is the rule. General malaise and anorexia are common occurrences; gastrointestinal symptoms occur in approximately 25 percent of cases. Vomiting and diarrhea occur most commonly in younger age groups.

Compared with other respiratory viral infections, the duration of illness with pharyngoconjunctival fever is long. In most patients, the fever is sustained or remittent for 3 to 4 days, then temperature gradually returns to normal within 24 to 48 hours. Approximately 10 percent of patients have fever that lasts longer than 7 days. Throat and eye findings usually are improved considerably by the seventh day of illness, but these findings and nasal complaints, fatigue, and headache may persist for 14 days.

Early in the illness, the total white blood cell count is within normal limits or slightly elevated, with a normal differential count or one with a slight increase in polymorphonuclear leukocytes. During convalescence, many patients have a moderate leukopenia with an equal number of lymphocytes and polymorphonuclear cells. Smears from affected conjunctivae usually do not reveal abnormalities on cytologic examination.

DIFFERENTIAL DIAGNOSIS

Because the symptom triad of fever, pharyngitis, and conjunctivitis is virtually unique to pharyngoconjunctival fever, establishing the diagnosis should be easy. The only difficulty on clinical grounds is in trying to assign a specific type of adenovirus. Generally, major epidemic disease is most likely to be caused by type 3 or 7; sporadic cases can occur with types 1 to 8, 14, 19, and 13/30. Manifestations by different adenoviral types have no known differences.

Of some concern in the differential diagnosis is picornavirus epidemic conjunctivitis (acute hemorrhagic conjunctivitis).[44,74,75] Two enteroviruses (coxsackievirus A24 and enterovirus 70) have been implicated etiologically in several extensive outbreaks of disease. Affected patients have had severe conjunctivitis with preauricular lymphadenitis, but fever and pharyngitis have not been prominent associated signs. In one outbreak, 23 percent of patients studied had upper respiratory tract symptoms.[44]

Generalized diseases that occasionally might be confused with pharyngoconjunctival fever include leptospirosis, psittacosis, *Mycoplasma pneumoniae* infection, Q fever, Newcastle disease virus infection, and prodromal measles. Of these illnesses, all but Newcastle disease virus infection have generalized symptoms that are disproportionately more important than the symptoms of either conjunctivitis or pharyngitis. Human infection with Newcastle disease virus could be confused easily on clinical grounds with pharyngoconjunctival fever. A history of exposure to chickens or other fowl should aid in establishing the diagnosis.

Of more difficulty in making a differential diagnosis are illnesses usually characterized by either pharyngitis or conjunctivitis. Occasionally, infections with influenza viruses, parainfluenza viruses, enteroviruses (other than coxsackievirus A24 and enterovirus 70), and Epstein-Barr virus are confusing. Although eye complaints do occur in these illnesses, severe conjunctivitis usually does not occur.

The differential diagnosis of conjunctivitis includes bacterial infections caused by *Haemophilus influenzae*, *Streptococcus pneumoniae*, *Streptococcus pyogenes*, and *Neisseria gonorrhoeae*. In all of these infections, purulent discharge is greater than that usually observed in pharyngoconjunctival fever. *Chlamydia trachomatis* infections are perhaps the most troublesome in the differential diagnosis. In the past, many cases of swimming pool conjunctivitis were attributed to chlamydial infections. Many such reported cases in reality probably were adenoviral infections. *C. trachomatis* infections can be diagnosed by showing characteristic inclusions in Giemsa-stained scrapings from the palpebral conjunctivae, by direct immunofluorescence, by enzyme immunoassay, or by culture. Epidemic adenoviral keratoconjunctivitis is another differential diagnostic consideration. Other differential diagnostic possibilities that should cause no difficulty include cat-scratch fever, tularemia, *Acanthamoeba* keratitis, and allergic conjunctivitis.

SPECIFIC DIAGNOSIS

In most instances, a clinical diagnosis is all that is necessary. Specific viral diagnosis can be accomplished with ease in any routinely equipped diagnostic virology laboratory. Diagnosis can be made by isolation of virus in tissue culture or by direct antigen detection by indirect immunofluorescence, enzyme-linked immunosorbent assay, or polymerase chain reaction analysis.[11,14,53,68] Cultures from the conjunctivae generally are more diagnostically specific than are cultures from the throat. The recovery of an adenovirus (particularly type 2) from the throat in an isolated case does not indicate an etiologic role for the recovered virus. An adenoviral etiology also can be verified by studying paired serum samples for a titer increase to the adenoviral group antigen.

TREATMENT

Generally, no treatment is necessary or effective in pharyngoconjunctival fever. If conjunctivitis persists and becomes purulent, an investigation for bacterial pathogens and appropriate topical antimicrobial therapy are indicated. Use of steroid-containing ophthalmic ointments should be avoided.

PROGNOSIS

The prognosis generally is excellent. Although superficial keratitis occasionally occurs, permanent scarring is not a problem. Sinusitis, otitis media, and bacterial conjunctivitis are rare secondary complications that, if untreated, can result in long-term difficulties.

PREVENTION

Volunteer studies have clearly indicated that infection and presumable resultant antibody are protective against future disease. Protection theoretically could be achieved through immunization, but priority given to study and to implement an immunization program is low. Because the major cause of pharyngoconjunctival fever caused by adenoviruses is swimming in contaminated water, discretion in bathing locations is advised. Swimming pool water should be chlorinated adequately, and pool filtration systems should be inspected daily. Ill individuals should be excluded from swimming pools during their illness and for at least 2 weeks after recovery.

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CHAPTER 13

UVULITIS

Ellen R. Wald

Infections of the uvula have been reported infrequently in the medical literature. When the uvula is the most inflamed structure in the posterior pharynx of a febrile child, acute infection should be suspected. Other causes of uvulitis include trauma (from instrumentation), inhalant irritation (from cannabis use), vasculitis, and allergy.[3,5,11]

ETIOLOGY

The main bacterial agents that cause uvulitis in children include *Haemophilus influenzae* type b (Hib) and *Streptococcus pyogenes*.[6] Uvulitis caused by *H. influenzae* may occur concurrently with epiglottitis or as an isolated infection.[8,14] Uvulitis caused by *S. pyogenes* seems always to occur in concert with pharyngitis. Brook[2] reported two cases of uvulitis caused by anaerobic bacteria, *Fusobacterium nucleatum* and *Prevotella intermedia*. No search for viral agents has been conducted. Several cases of uvulitis caused by *Candida albicans* have been described in immunocompetent toddlers.[7] In adults, *Streptococcus pneumoniae* and *H. influenzae* have been reported to cause uvulitis.[4,13] In many patients, an associated epiglottitis has been present.[10,13]

EPIDEMIOLOGY

The epidemiology of uvulitis is the epidemiology of its two etiologic agents, *S. pyogenes* and Hib. It occurs in school-age children 5 to 15 years old (the so-called streptococcal age group) in association with pharyngitis. Similarly, it can be seen in the *H. influenzae* age group (3 months to 5 years) if a child has not received the now routine and universally recommended conjugate vaccine to prevent infections caused by Hib. Cases of uvulitis in association with epiglottitis have been reported in the United States and in England.[2,6,12] Infections caused by *S. pyogenes* and *H. influenzae* occur primarily in the winter and spring, but both types can occur throughout the year.

PATHOGENESIS

Uvulitis is an acute cellulitis characterized by dramatic swelling and erythema. Infection of the uvula probably arises from direct invasion by *S. pyogenes* or Hib; both are recognized as normal nasopharyngeal flora. In the latter case, epiglottitis also may arise by direct extension, and the bacteremia may result secondarily from either the uvula or the epiglottis as a primary site of infection.

Uvulitis that is noninfectious may result from injury, chemical irritation, or allergic inflammation. A child ultimately diagnosed to have Kawasaki disease presented with uvulitis.[5]

CLINICAL MANIFESTATIONS

In a review of five patients with streptococcal uvulitis, all had associated pharyngitis.[6] The patients presented with low-grade fever and sore throat. Three of the five patients experienced a choking or gagging sensation in the pharynx that induced coughing and spitting; one of these patients also presented with drooling. Although pharyngitis was noted on physical examination, the swelling and erythema of the uvula were most dramatic (Fig. 13–1). None of the patients had evidence of respiratory distress.

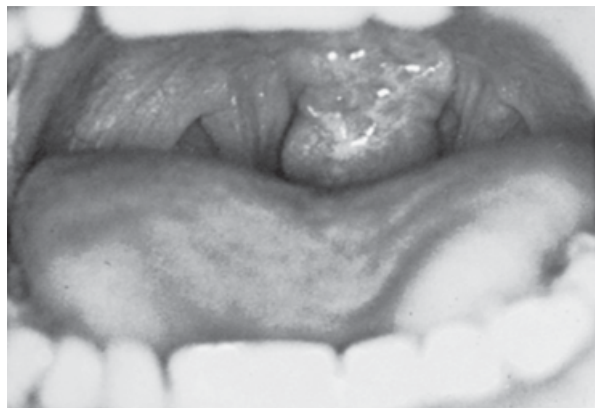

Figure 13–1 Swollen (two to three times normal size) and erythematous uvula in a patient without epiglottitis or pharyngitis.

In most children with uvulitis and epiglottitis, the presentation usually is typical for epiglottitis, with sudden onset of high fever, dysphagia, and increasing respiratory distress. Rapkin[12] reported a case of uvulitis and epiglottitis, however, in which the epiglottitis initially was unsuspected. The same observation has been made in some adults with uvulitis and epiglottitis.[4,10] Lateral neck radiography (performed in one case to evaluate the possibility of a retropharyngeal abscess) belatedly alerted the clinicians to the correct diagnosis.

In patients with uvulitis and no epiglottitis, the presentation may be similar to that of epiglottitis (acute onset of fever, odynophagia, and drooling) or less specific, with fever and irritability or decreased appetite.[8,14] The diagnosis in these cases is provided by physical examination of the oropharynx, which shows a swollen and erythematous uvula (see Fig. 13–1).

DIAGNOSIS

The diagnosis of streptococcal uvulitis is suspected when a school-age child presents with low-grade fever, pharyngitis, and uvulitis. The diagnosis is confirmed by the recovery of *S. pyogenes* from a surface culture of the throat or uvula or both.

The diagnosis of uvulitis caused by *H. influenzae* is suspected in a highly febrile infant or preschool-age child who has uvular inflammation on physical examination. Lateral neck radiography must be performed to evaluate the possibility of epiglottitis, unless obvious signs of upper respiratory obstruction are present, in which case immediate endoscopy is warranted. If epiglottitis is discovered, the airway must be secured, and appropriate parenteral antimicrobials must be initiated after blood and surface culture specimens are obtained. Any surface culture specimen obtained to search for *H. influenzae* must be plated onto chocolate agar. After appropriate culture specimens are obtained, parenteral antimicrobials should be initiated, as in other infections associated with bacteremia caused by *H. influenzae*.

DIFFERENTIAL DIAGNOSIS

The differential diagnosis of a patient with acute onset of fever, dysphagia, and drooling includes herpes simplex gingivostomatitis, uvulitis, epiglottitis, severe pharyngitis, and peritonsillar or retropharyngeal abscess. Although being extremely cautious in examining the pharynx of any patient with suspected epiglottitis is appropriate, some children tolerate attempted visualization of the oral cavity without becoming unduly upset. Instrumentation with a tongue blade should be avoided. If the examination does not show gingivostomatitis or peritonsillar abscess, lateral neck radiography should be performed. If epiglottitis or retropharyngeal abscess is confirmed, management of the airway and administration of antimicrobials are indicated for epiglottitis, and incision and drainage and antimicrobials are indicated for retropharyngeal abscess. If the lateral neck is normal and the uvula is inflamed, uvulitis with or without pharyngitis is confirmed.

TREATMENT

Management of uvulitis is guided primarily by the associated pharyngitis or epiglottitis, if either is present. In the case of streptococcal pharyngitis, penicillin therapy for 10 days is most appropriate. These patients usually can be treated orally with penicillin V, 25 to 50 mg/kg/day, administered in two divided doses.

In the case of uvulitis and epiglottitis, management of the airway is most important and can be accomplished by performing nasotracheal intubation or tracheotomy. Appropriate parenteral antibiotic therapy usually is initiated.

In the case of uvulitis without epiglottitis in an infant or preschool-age child, antimicrobial therapy should be planned for possible Hib bacteremia. Generally, approximately 50 percent of respiratory isolates of *H. influenzae* are β-lactamase producing. Administration of an advanced-generation cephalosporin, such as cefotaxime at 200 mg/kg/day in four divided doses or ceftriaxone at 100 mg/kg/day in one dose or two divided doses, is appropriate. In a patient with serious penicillin hypersensitivity, aztreonam at 100 mg/kg/day in three divided doses also is a satisfactory regimen. After the patient has defervesced and has improved clinically, an oral antimicrobial agent can be substituted. The results of blood and surface cultures now can guide therapy. For an ampicillin-sensitive *H. influenzae* infection, amoxicillin at 45 mg/kg/day in two divided doses should be prescribed to complete a 7- to 10-day course of treatment. For β-lactamase-producing *H. influenzae*, a variety of oral agents, including cefixime at 10 mg/kg in a single daily dose, cefuroxime at 30 mg/kg/day in two divided doses, or amoxicillin-potassium clavulanate at 45 mg/kg/day of amoxicillin in two divided doses, can be prescribed.

Resolution was prompt in the two cases of uvulitis allegedly caused by *C. albicans*. One child was treated with topical nystatin, and the other had spontaneous improvement.[7]

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CHAPTER 14

Peritonsillar, Retropharyngeal, and Parapharyngeal Abscesses

Nira A. Goldstein ✪ Margaret R. Hammerschlag

A deep neck abscess is a collection of pus in a potential space bounded by fascia.[6] These potential spaces are areas of least resistance to the spread of infection. An infection may begin with a minimal area of cellulitis and progress to a deep neck abscess, which may extend to invade adjacent potential spaces; these spaces frequently encompass vital structures in the neck. Destruction and dysfunction of these structures represent the major complications of deep neck infections.[44]

EPIDEMIOLOGY OF HEAD AND NECK SPACE INFECTIONS IN CHILDREN

Data concerning the frequency of head and neck space infections in children are limited. A survey conducted by the American Academy of Otolaryngology found the incidence of peritonsillar abscesses to be approximately 30 per 100,000 person-years, or approximately 45,000 cases annually in the United States and Puerto Rico.[32] A retrospective study from the Children's Hospital of Pittsburgh identified 117 children with head and neck space infections seen between 1986 and 1992.[72] The patients ranged in age from 1 month to 18 years (mean age 7.8 years).[72] Peritonsillar space infections (cellulitis and abscesses) occurred most frequently, accounting for 61 (49%) cases, followed by retropharyngeal space infections, accounting for 27 (22%) cases. Only three (2%) parapharyngeal space infections were seen during the period of the study. Broughton[14] reported seeing 14 pediatric patients, 15 months to 17 years of age, with retropharyngeal and parapharyngeal space infections during a 9-year period at the University of Kentucky Medical Center. Craig and Schunk[18] described 64

cases of retropharyngeal abscesses in children at the Primary Children's Medical Center in Salt Lake City, Utah, from 1993 to 1998, a higher number of cases than previously reported. Kirse and Roberson[40] reported 73 cases of retropharyngeal space infection from 1989 to 1998, with a twofold to fivefold increase in the number of patients treated in the last 4 years of the study compared with the first 6 years.

Peritonsillar abscesses rarely occur in young children. They occur most frequently in patients in their late teens and early 20s. The mean age of the children with peritonsillar infection in Pittsburgh was 11 years, whereas the mean age of the patients with retropharyngeal space infections was 4 years, similar to the mean age of 4.5 years reported by Thompson and associates[71] in their 36-year review of 65 children with retropharyngeal abscesses treated at the Children's Hospital of Los Angeles. Retropharyngeal abscesses have been reported to occur more frequently in young children. In the Salt Lake City series, the median age was 3 years, and 75 percent of the children were younger than 5 years.[18]

All of the peritonsillar infections were associated with tonsillitis, and 15 percent had antecedent infectious mononucleosis shown by positive monospot test results.[72] Results of a meta-analysis of 15 previously reported series of patients with peritonsillar abscess reported by Herzon[32] found prior tonsillar infection rates ranging from 11 to 56 percent, with an overall rate of 36 percent. The high incidence of peritonsillar abscess reported in the American Academy of Otolaryngology study raised the possibility that the decreasing rate of tonsillectomy might increase the risk for development of peritonsillar abscess.[32]

PERITONSILLAR ABSCESS (QUINSY)

A peritonsillar abscess (quinsy) is circumscribed medially by the fibrous wall of the tonsil capsule and laterally by the superior constrictor muscle. Pus may be localized in the superior pole, midpoint, or inferior pole or rarely may be dispersed, with multiple loculations in the peritonsillar space. The superior pole is the most common location, with a frequency range of 41.2 to 70 percent; the remaining inferior locations account for the balance.[7,10,46]

The etiology of peritonsillar abscesses is not constant. They may occur after any "virulent" tonsillitis, with extension through the fibrous tonsil capsule.

CLINICAL MANIFESTATIONS

The recent history may include a sore throat with occasional unilateral ear pain, malaise, low-grade pyrexia, chills, diaphoresis, dysphagia, reduced oral intake, trismus, and a muffled "hot potato voice." Trismus results from irritation and reflex spasm of the internal pterygoid muscle. Sixty-three percent of the children with peritonsillar infection from the Pittsburgh series had trismus.[72] Impaired palatal motion from edema contributes to the muffled voice.

Physical examination reveals minimal to moderate toxicity, dehydration, and drooling. Inspection of the oropharynx may be compromised by trismus. The soft palate is displaced toward the unaffected side, swollen and red, and frequently palpably fluctuant. The edematous uvula is pushed across the midline (Fig. 14–1). The displaced tonsil and its crypts rarely are coated with exudate. The breath is fetid, and ipsilateral, tender, cervical adenopathy is present. The white blood cell count is elevated, with a predominance of polymorphonuclear leukocytes.

Brodsky and associates[11] attempted to identify the clinical signs that might distinguish peritonsillar abscess from peritonsillar cellulitis in a group of 21 children admitted to the Children's Hospital of Buffalo from 1985 through 1987. No significant difference in age, duration of sore throat, fever, or white blood cell count was noted, although a greater degree of pharyngotonsillar bulge and muffled voice was found in the patients with abscess. Patients with peritonsillar cellulitis improved after receiving 24 hours of intravenous antibiotics, whereas patients with peritonsillar abscess had no change or worsening of symptoms. Blotter and colleagues[8] confirmed these findings in 102 patients admitted to Children's Hospital of Columbus, Ohio, between 1995 and 1998.

In uncomplicated cases, computed tomography (CT) scan has not been as useful as clinical assessment and follow-up evaluation in the management of peritonsillar abscess. CT scans are useful in young children with suspected peritonsillar abscess who are

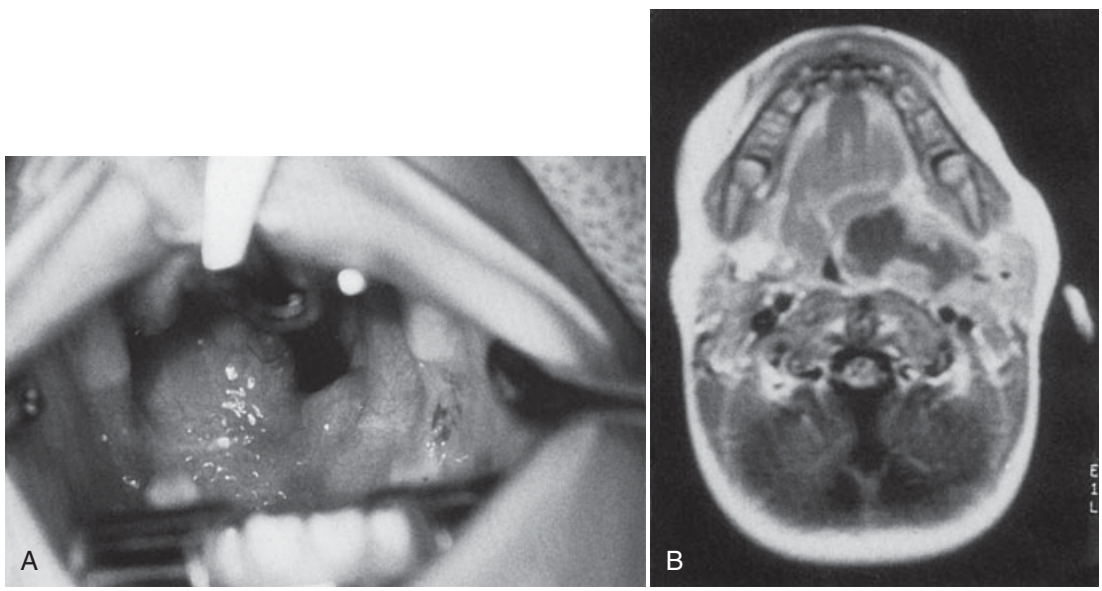

Figure 14–1 Left peritonsillar abscess in an 18-month-old child. *(From Wiatrak, B. J., and Woolley, A. L.: Pharyngitis and adenotonsillar disease. In Cummings, C. W., Flint, P. W., Harker, L. A., et al. [eds.]: Otolaryngology: Head and Neck Surgery. 4th ed. Philadelphia, Elsevier, 2005, p. 4144.)*

not cooperative with examination or children with other suspected deep neck infections.[27]

TREATMENT

Traditionally, management of peritonsillar abscess in children involved hospital admission for intravenous hydration, antibiotic therapy and analgesia, and either intraoral incision and drainage of the abscess or "acute quinsy tonsillectomy" with removal of the medial wall of the abscess. Although older children may tolerate incision and drainage under local anesthetic, the procedure is not well tolerated in young children and carries the risk of potential injury to adjacent vascular structures. The administration of a general anesthetic is required for tonsillectomy in all age groups and often is required for incision and drainage in young children. Acute tonsillectomy often was done to prevent future recurrence of the peritonsillar abscess.

Although it is a commonly accepted clinical observation, a high recurrence rate of peritonsillar abscess has not been well documented. A meta-analysis reported by Herzon[32] of 19 studies from the United States, Europe, and Israel involving 1399 patients found recurrence rates of 10 to 15 percent for peritonsillar abscess. The rates of recurrence seem to be lower in the United States (0 to 17%) than in the series reported from Europe and Israel (3 to 22%). A retrospective analysis of 290 patients treated for peritonsillar abscess found that patients who had a history of recurrent tonsillitis before development of the abscess had a fourfold greater rate of recurrence than patients with no history (40 versus 9.6%).[41] The authors recommended that patients with a history of recurrent tonsillitis before admission be treated with tonsillectomy.[41]

The risks of acute tonsillectomy are not greater than the risks associated with delayed tonsillectomy.[45,51,77] In addition, the morbidity caused by two hospitalizations involving two procedures is reduced by acute tonsillectomy.[4,9,10] An additional benefit of acute tonsillectomy is the ability to evacuate inferior pole abscesses that technically are difficult to drain by needle aspiration or incision and drainage.[46]

Studies have suggested that many peritonsillar abscesses can be managed by simple needle aspiration combined with antibiotic therapy on an outpatient basis.[11,32,58,60,61,66,72,74] An extensive meta-analysis of 10 studies conducted from 1961 through 1994 involving 496 patients with peritonsillar abscesses found that needle aspiration had an overall success rate of 94 percent (range 85 to 100%).[32] This success rate compares favorably with that reported for incision and drainage. Weinberg and associates[74] successfully performed needle aspiration in 41 of 43 children 7 to 18 years old (mean age 13.9 years). All were admitted for intravenous antibiotic therapy, two (5%) required repeated aspiration for resolution, and five (12%) did not respond and required acute tonsillectomy. A pooled analysis of three prospective studies comparing needle aspiration versus incision and drainage showed no significant difference in resolution rates (93.7% for incision and drainage and 91.6% for needle aspiration), but the power was low because of the small sample size.[36] Other studies, which have included adults and children with peritonsillar abscesses, have reported that 0 to 48 percent of patients require hospitalization.[32,51,58,66]

Younger children often require admission to correct dehydration.[27] Younger children also are more likely than are older children to respond to intravenous antibiotics alone and to have negative findings at surgical drainage.[8] The use of conscious sedation has been reported to be a safe and effective approach for the drainage of peritonsillar abscesses in children.[48,67]

In a 10-year retrospective review of 83 children with peritonsillar abscesses by Schraff and colleagues,[61] 65 percent were treated by incision and drainage (51% knife versus 14% needle aspiration), 31 percent had quinsy tonsillectomy, and 4 percent were admitted and treated by intravenous antibiotics alone. Of patients treated surgically, 51 percent of the procedures were done in the emergency department, and 49 percent were done in the operating room. Forty-eight percent of the children required admission, and the average length of stay was 0.9 days (standard deviation 1.37).

A suggested approach to the management of children with peritonsillar abscess is as follows.[33] Cooperative children should undergo needle aspiration of the abscess and treatment with antibiotics. Children who can tolerate liquids orally may be managed as outpatients, and the remainder should be admitted for hydration and administration of intravenous antibiotics. Approximately 4 percent of children require a repeated aspiration for resolution.[32] Children who remain symptomatic after undergoing needle aspiration require incision and drainage or acute quinsy tonsillectomy, depending on the prior history of recurrent tonsillitis. Children who cannot tolerate needle aspiration on initial presentation are admitted for administration of intravenous antibiotics. If no response occurs within 24 hours, incision and drainage or acute tonsillectomy is done, depending on the prior history of recurrent tonsillitis. Delayed tonsillectomy is reserved for children who recover from the peritonsillar abscess without general anesthesia but have a history of recurrent tonsillitis or prior peritonsillar abscess.

Untreated peritonsillar abscess may point, with spontaneous rupture, or extend to the pterygomaxillary space, with potentially fatal complications. Upper airway obstruction, septicemia, and vascular catastrophe may occur. Necrotizing fasciitis also has been reported in adults with peritonsillar abscess.[28,75]

RETROPHARYNGEAL ABSCESS (POSTERIOR VISCERAL SPACE, RETROVISCERAL SPACE, AND RETROESOPHAGEAL SPACE ABSCESSES)

The anterior wall of the retropharyngeal space is the middle layer of the deep cervical fascia, which abuts the posterior esophageal wall (the superior pharyngeal constrictor muscle). The deep layer of the deep cervical fascia circumscribes the posterior wall of this potential space. Inferiorly, these two fasciae fuse to limit the depth of this pocket at a level between the first and second thoracic vertebrae. A retropharyngeal abscess can erode inferiorly through the junction of these fasciae to extend posteriorly into the prevertebral space (Fig. 14–2). Subsequently, pus in the prevertebral space can descend inferiorly below the diaphragm to the psoas muscles.

The retropharyngeal space contains two paramedial chains of lymph nodes that receive drainage from the nasopharynx, adenoids, posterior paranasal sinuses, middle ear, and eustachian tube. These structures are prominent in early childhood and atrophy at puberty.[29] Retropharyngeal abscesses are common occurrences in young children and are thought to be secondary to suppurative adenitis of these retropharyngeal nodes.[3] Other sources of infection are penetrating foreign bodies, endoscopy, trauma, pharyngitis, vertebral body osteomyelitis, petrositis, dental procedures,[72] and branchial cleft anomalies.[35] In one series of 17 cases of retropharyngeal abscesses at the Children's Hospital, Denver, Colorado, 7 children (41%), including 2 neonates (most likely associated with attempts at intubation), had perforations of the hypopharynx or esophagus.[53] In the Pittsburgh series, 63 percent of the children with retropharyngeal abscess had antecedent tonsillitis, pharyngitis, or viral upper respiratory tract infection.[72] Two children had previous trauma; however, no details on the type of trauma were given.

In adults, tuberculosis and syphilis were common causes of retropharyngeal abscesses in the pre-antibiotic era.[56] Four of

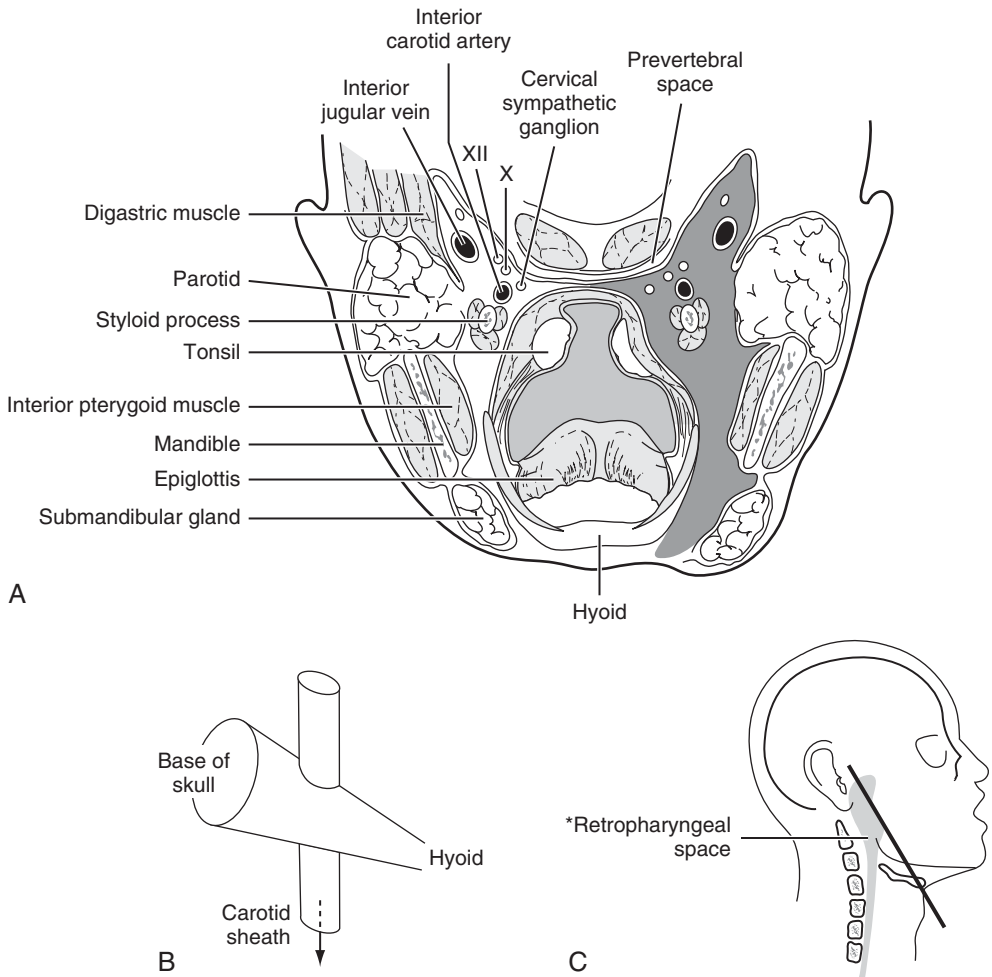

Figure 14–2 A, Oblique transverse section of the oropharynx posterosuperiorly and the hypopharynx anteroinferiorly. Depicted are a peritonsillar abscess on the right and a pterygomaxillary space abscess on the left. The *asterisk* in **C** indicates the location of the retropharyngeal space. **B,** The "cone" of the potential pterygomaxillary space with its carotid sheath. **C,** The vertical dimensions of the retropharyngeal space. The black oblique line represents the level of the drawing in **A.**

seven patients treated for retropharyngeal abscess at Columbia-Presbyterian Medical Center in New York during a 6-year period had serologic evidence of acute primary Epstein-Barr viral infection.[69]

CLINICAL MANIFESTATIONS

The symptoms of retropharyngeal abscess frequently begin insidiously after mild antecedent infection. Signs and symptoms include sore throat, fever, limitation of neck motion, torticollis, neck pain, neck mass, drooling, odynophagia, poor oral intake, and lethargy. Airway stridor or respiratory distress from edema, cellulitis, or an obstructing mass occasionally occurs.[17,18,20,73] Thirty-three percent of the 27 children with retropharyngeal abscess in the Pittsburgh series had torticollis or limitation of neck motion,[72] compared with 53 of the 94 children (83%) in the Las Vegas series.[18] In adults, the symptoms may be milder. Complaints of chest pain by an adult may reflect mediastinal extension.

Early in the course, midline or unilateral swelling of the posterior pharynx occurs. Later, gentle palpation may show a large fluctuant mass in the posterior pharynx. Vigorous palpation should be avoided because it may cause the abscess to rupture into the upper airway. As with other abscesses, the white blood cell count is increased, with a predominance of granulocytes.

Plain films of the neck often are the initial radiologic study, but they must be obtained with the patient in a true lateral position, with the neck in extension, and on inspiration, or the child's retropharyngeal soft tissues may appear abnormally thickened. Widening of the prevertebral soft tissues exceeding the anteroposterior diameter of the contiguous vertebral bodies, or thickening of the retropharyngeal space greater than 7 mm at C2 in children and adults, or 14 mm at C6 in children or 22 mm at C6 in adults suggests retropharyngeal inflammation. Rarely, a prevertebral soft tissue mass, air-fluid level, or gas may be seen. The normal cervical lordosis may be lost or reversed secondary to muscle spasm or local inflammation (Fig. 14–3).

CT scan has rendered the diagnosis and management of deep neck space infections more precise.[12,23,40,43,70,73,76] In contrast to conventional radiologic studies, CT scan distinguishes cellulitis of the neck, which usually does not require surgical treatment, from a deep neck abscess, which does require surgical drainage. With its ability to define differences in tissue density, CT scanning permits an accurate determination of the extent of the abscess and its extension and involvement of adjacent spaces to be made. An abscess is distinguished from cellulitis by a low-attenuation homogeneous area surrounded by a ring enhancement of contrast material (see Fig. 14–3). Kirse and Roberson[40]

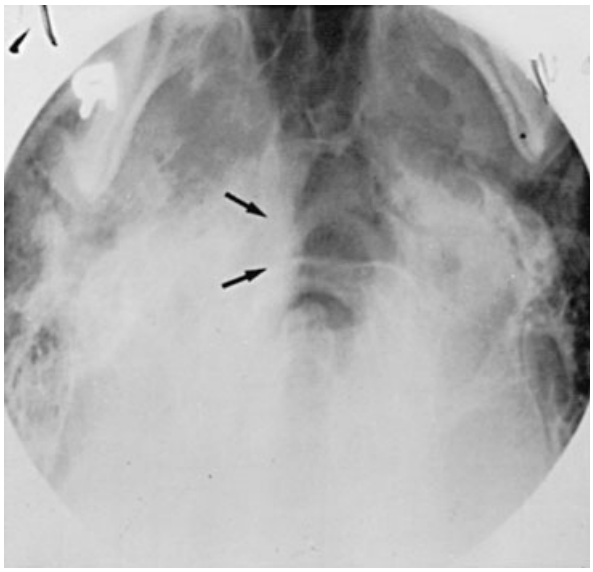

Figure 14–3 Lateral neck radiograph showing a retropharyngeal abscess.

reported scalloping of the abscess wall to be a more useful predictor of the presence of pus than ring enhancement. When more than one space is involved, accurate assessment of these spaces may ensure sufficient surgical drainage. Vascular structures can be identified, as can potential complications such as venous thrombosis. Gas also may be detected by CT.

A 10-year retrospective study from the Massachusetts Eye and Ear Infirmary compared preoperative CT scans with intraoperative findings in 38 patients who underwent surgical exploration of the parapharyngeal or retropharyngeal space within 48 hours after the scans were performed. Overall, the intraoperative findings confirmed the CT scan interpretation in 76.3 percent of the patients.[43] Of the 38 patients, 5 (13.2%) had CT scans indicative of abscesses that were not confirmed at surgery. Exploration of the parapharyngeal or retropharyngeal space revealed cellulitis. The false-negative rate was 10.5 percent. The sensitivity of CT scanning for detection of parapharyngeal or retropharyngeal space abscess was 87.9 percent. Similar findings were reported in the Pittsburgh series; the sensitivity of CT scanning for differentiating an abscess from cellulitis was 91 percent.[72] In three cases, the radiologist's blinded CT scan interpretation did not correlate with the operative findings. Two patients with false-positive interpretations had retropharyngeal infections and underwent needle aspiration. The positive predictive value of CT scans in detection of abscess versus cellulitis was 83 percent. Additional studies have reported that the accuracy of CT in predicting surgical findings was 63 percent, 73 percent, 76 percent, and 92 percent.[23,65,73,76]

TREATMENT

The treatment of choice is administration of intravenous antibiotics and incision and drainage. If the mass is small, a peroral incision with the patient in the Rose position (supine with the neck hyperextended) may provide some drainage, but a slight risk of aspiration exists. If the mass is large and extends lateral to the great vessels, or if fever persists after peroral drainage is performed, an external incision is preferred. A tracheotomy may be required if risk of compromising the airway exists.

Posterior mediastinitis can result from the spread of infection from the retropharyngeal area into the prevertebral space. Other complications may be seen when the abscess extends to the parapharyngeal space and involves the great vessels and cranial nerves.

Reports have documented that patients with small retropharyngeal abscesses may respond to treatment with intravenous antibiotics alone. Broughton[14] described the experience at the University of Kentucky Medical Center, where 8 of 14 patients with deep neck infections seen during a 9-year period were treated successfully by antibiotics alone. All were reported to have small abscesses on CT scan. Possibly some had only cellulitis, however. In the Pittsburgh study, 15 of the children with parapharyngeal or retropharyngeal space infections underwent surgical intervention; 11 (73%) underwent incision and drainage, and 4 (27%) underwent needle aspiration.[72] All had successful outcomes. Twelve (44%) of the children with retropharyngeal infections were treated with intravenous antibiotics alone, and all did well. Ten (37%) of 27 patients in the Las Vegas series with well-defined abscess on CT scan were treated with intravenous antibiotics alone, and no treatment failures occurred.[18]

McClay and colleagues[50] used intravenous antibiotics alone to treat 11 children with deep neck abscesses (retropharyngeal in 8, both retropharyngeal and parapharyngeal in 2) without severe symptoms or airway compromise. Ten children responded and showed clinical improvement within 48 hours, whereas one child required surgical drainage. Close clinical follow-up is mandatory for children treated with intravenous antibiotics alone. Children who do not improve within 24 to 48 hours require surgical drainage.

PARAPHARYNGEAL ABSCESS (PTERYGOMAXILLARY, PHARYNGOMAXILLARY, LATERAL, AND PHARYNGEAL SPACE ABSCESSES)

The potential parapharyngeal or pterygomaxillary space is an inverted conical cavity (see Fig. 14–2B) lying along an oblique axis roughly parallel to the ramus of the mandible (see Fig. 14–2C). The base of the skull at the jugular foramen forms the base of the "cone," and its apex is at the hyoid bone (see Fig. 14–2B). The buccopharyngeal fascia, lateral to the superior pharyngeal constrictor, delineates the medial boundary, and the parotid gland and its partially dehiscent deep layer of the superficial cervical fascia form the lateral wall of this cone. The internal pterygoid muscle and mandible demarcate the cone on its anterolateral aspect. The parapharyngeal space is contiguous with the peritonsillar, submandibular, and retropharyngeal spaces, all of which are potential avenues of egress for an extending parapharyngeal space abscess (see Fig. 14–2A).

The posterior portion of the cone contains the contents of the carotid sheath (carotid artery and internal jugular vein), cranial nerves IX through XII, and the cervical sympathetic chain. The internal pterygoid muscle and fatty connective tissue are anterior.

Involvement of these structures determines the clinical manifestations and complications of the parapharyngeal space abscess. An abscess in the posterior compartment may show medial displacement of the lateral pharyngeal wall and parotid space induration and swelling, with variable overlying facial nerve weakness, carotid artery erosion and hemorrhage,[42] internal jugular vein thrombosis, decreased gag reflex and dysphagia, ipsilateral vocal cord paralysis, weakness of the ipsilateral trapezius muscle, ipsilateral lingual deviation, and Horner syndrome from cervical sympathetic chain involvement.[16]

Extension of the abscess into the anterior compartment causes trismus from irritation of the internal pterygoid muscle. Indura-

tion at the angle of the jaw and medial displacement of the tonsil and pharyngeal wall also occur with an anterior compartment abscess.

By the time a patient with an abscess seeks medical attention, the source of the parapharyngeal space infection may be unclear. Reports indicate variable causes, including incompletely or inadequately treated bacterial pharyngitis, tonsillitis,[3] peritonsillar abscess, dental infections, bacterial parotitis, otitis, mastoiditis (Bezold abscess from a mastoid tip infection traveling along the digastric muscles), petrositis, cervical adenitis with suppuration, cervical vertebral tubercular adenitis in the adult,[5,54] foreign bodies,[16,19] trauma, intravenous drug abuse,[31] branchial cleft anomalies,[68] and cat-scratch disease.[78] Parapharyngeal abscesses seem to be less common than are peritonsillar and retropharyngeal abscesses in children. Only three cases of parapharyngeal abscess and cellulitis were reported in the Pittsburgh series,[72] and only three cases were reported in a 15-year review of pediatric neck abscesses from Rainbow Babies and Children's Hospital in Cleveland, Ohio.[22] Amar and Manoukian[1] reported on 25 children with parapharyngeal abscesses during an 11-year period at Montreal Children's Hospital. Parapharyngeal abscesses occur throughout childhood, with an average age at presentation of 4 to 6 years.[1,63]

CLINICAL MANIFESTATIONS

In addition to the symptoms noted in the preceding description, tender cervical swelling, induration and erythema of the side of the neck, torticollis, sore throat, dysphagia, trismus, hoarseness, malaise, chills, and diaphoresis may occur. Variable low-grade pyrexia with occasional temperature spikes occurs. Examination discloses variable toxicity, respiratory tract distress, laryngeal edema, medial displacement of the lateral pharyngeal wall and inferior tonsil pole, trismus, and, infrequently, drooling. Palpation of the neck reveals a tender, high cervical mass, initially diffuse and later fluctuant. Pharyngeal blood clots may presage erosion of the carotid artery. The complications of parapharyngeal abscesses are related to the structures involved: Involvement of the carotid artery can produce hemiplegia from emboli; internal jugular vein thrombosis with cephalad extension may lead to a cavernous sinus thrombosis, whereas inferior extension leads to internal jugular vein thrombosis. Internal jugular vein thrombosis is characterized by spiking temperature, toxicity with intense diaphoresis, headaches, and increased intracranial pressure. Septic pulmonary emboli occasionally are present.

Extension into the retropharyngeal region by a parapharyngeal abscess may lead to a posterior mediastinitis. Airway obstruction occurring secondary to laryngeal edema and aspiration pneumonia from suppuration of the abscess into the pharynx have been reported. Initially, the parapharyngeal abscess may be difficult to differentiate from a peritonsillar abscess, but the latter usually is less toxic and has a distinct, soft palatal fluctuation.

As described for the diagnosis of retropharyngeal abscess, CT is an extremely useful tool for distinguishing parapharyngeal abscess from cellulitis and for localizing the abscess for surgical planning. In a review of 47 children who presented with deep neck infections to the Children's Hospital of Buffalo during a 5.5-year period, CT scan showed that 34 (77%) of 44 patients who underwent CT scan had involvement of the parapharyngeal and retropharyngeal spaces.[55] The involvement of both spaces had implications for the approach to surgical drainage.

TREATMENT

Intravenous antibiotic therapy with incision and drainage is the primary treatment. An otolaryngologic consultation should be obtained for this potentially complex surgery of the neck. The conventional method of approaching this abscess is an external incision with sufficient exposure to provide immediate access to the common carotid artery for ligation, should erosion of the carotid artery be present.[47]

An intraoral drainage procedure traditionally has been condemned because rapid access to the vital structures of the neck is impossible with this approach. Nagy and associates[55] used an intraoral approach, however, to treat successfully 21 of 22 children with either parapharyngeal or combined parapharyngeal and retropharyngeal abscesses. The authors emphasized that CT with intravenous contrast enhancement showed that all of the abscesses were located medial to the great vessels and were adjacent to the pharyngeal wall. Amar and Manoukian[1] retrospectively compared 15 children with parapharyngeal abscesses who underwent intraoral drainage with 10 patients who underwent external neck drainage and found no complications or recurrences in either group. The children who underwent intraoral drainage had significantly reduced anesthesia time. The duration of intravenous antibiotics and duration of hospital stay also were shorter in the children who underwent intraoral drainage, but the differences between groups were not statistically significant. Cable and coworkers[15] described 12 children presenting to three centers with superior parapharyngeal space abscesses medial to the great vessels who were treated successfully by intraoral drainage using CT-guided systems to assist with localization of the abscess cavity.

The use of CT has made it possible for some patients with parapharyngeal abscesses to be managed with intravenous antibiotics. The number of reported cases is small, however, and usually analyzed with cases of retropharyngeal abscess.[14,55] Nagy and associates[55] used intravenous antibiotics alone to treat 3 (13%) of 24 children with small parapharyngeal abscesses. Sichel and associates[63] used intravenous antibiotics alone to treat successfully 12 children prospectively with infection limited to the parapharyngeal space abscess. Close clinical follow-up is necessary for children with parapharyngeal cellulitis or small parapharyngeal abscesses that are treated conservatively with intravenous antibiotics. Surgical drainage should be performed in children who do not improve within 24 to 48 hours.

In another report, Sichel and colleagues[62] reviewed the CT scans of 29 patients with infections of the parapharyngeal space and divided the patients into two groups: 22 patient with posterior parapharyngeal space infections and 7 patients with anterior parapharyngeal space infections. The patients with posterior infections generally were children who responded well to intravenous antibiotics and did not develop complications. The patients with anterior infections of the parapharyngeal space more commonly were adults who required external drainage in addition to intravenous antibiotics and developed complications including septic shock, respiratory distress, mediastinitis, pericarditis, and lung empyema. The authors suggested that the location of the infection in the fat of the anterior parapharyngeal space resulted in liquefaction of the fat, with formation of pus and rapid spread of the infection to other anatomic spaces.

MICROBIOLOGY OF DEEP NECK ABSCESSES

Group A streptococci (*Streptococcus pyogenes*) and *Staphylococcus aureus* have been considered to be the organisms most frequently associated with pharyngeal space infections. Studies have shown the presence of oral anaerobes in these infections, however; these organisms may be responsible for the gas seen on lateral neck radiographs. This finding is not surprising because the main portals of entry for pharyngeal space infections are the nasopharynx, oropharynx, paranasal sinuses, mastoid, and lower molars, all areas that are colonized with anaerobes.

The most complete microbiologic data available are from studies of peritonsillar abscesses. Flödstrom and Hallander[26] in 1976 reported the results of bacterial cultures on aspirates of pus from 37 patients with peritonsillar abscesses. The ages of the patients were not given. Group A streptococci were isolated from 17 of these patients, whereas 15 had an increase in their antistreptolysin O or anti-DNAase titers. Anaerobes were found in 28 of the cultures, including those of eight patients that also disclosed the presence of streptococci. The most common anaerobic species isolated were fusobacteria (13), peptostreptococci (16), and *Bacteroides* spp. (18). Among the aerobic organisms, *S. aureus* was isolated four times, and *Haemophilus influenzae* was isolated twice. No isolates of aerobic gram-negative enteric organisms were present.

Jokipii and colleagues[37] performed semiquantitative cultures of aspirated pus from 42 peritonsillar abscesses and found similar results. Group A streptococci, the aerobic bacteria isolated most frequently, were isolated in pure culture 4 of 10 times. Anaerobes were more abundant than were aerobes; the most important species, in frequency and quantitatively, were *Bacteroides*, *Peptostreptococcus*, and *Fusobacterium*. Most of the infections were polymicrobial, with two to seven bacteria in 83 percent of the specimens. Subsequent studies from the United States and Finland have reported similar findings.[13,38,60,64]

Kieff and associates[39] found that streptococci were the most frequent isolates; group A streptococci and other non–group A beta-hemolytic streptococci were present in more than 30 percent of patients, and alpha-hemolytic streptococci accounted for another 27.2 percent. The pathogenic role of anaerobic bacteria in peritonsillar abscesses has been reinforced by reports of complications caused by fusobacterial infection in children.[57,59] *Fusobacterium* and *Bacteroides* spp. have been associated with septic thrombophlebitis and pulmonary emboli from the jugular veins.

Data on the microbiology of retropharyngeal and parapharyngeal abscesses in children are more limited. The organisms isolated are similar to those found in peritonsillar abscesses, but with a higher number of anaerobic species. Brook[12] examined aspirated pus from 14 children, 1 to 6 years old (median age 3 years, 2 months), with retropharyngeal abscesses. Anaerobes were isolated from all patients; they were the only organisms isolated in two patients (14%) and were mixed with aerobes in the remainder (86%). The predominant anaerobic species were *Bacteroides*, *Peptostreptococcus*, and *Fusobacterium*. The predominant aerobic species were alpha-hemolytic and gamma-hemolytic streptococci, *S. aureus*, *Haemophilus* spp., and group A beta-hemolytic streptococci. Seventy-one percent of the isolates were β-lactamase–positive and included all isolates of the *S. aureus* group, 6 of 18 of the *Bacteroides melaninogenicus* group (33%), and 2 of 3 of the *Bacteroides oralis* group.

Dodds and Maniglia[22] reported the results of cultures from nine retropharyngeal and three parapharyngeal abscesses from children and adolescents. The organisms isolated were similar to those reported by Brook,[12,13] but the microbiology was not as complete because the study was retrospective and not all specimens may have been processed for anaerobic culture. Streptococcal species were the isolates that occurred most frequently, followed by *S. aureus* and *H. influenzae*. One isolate each of *Fusobacterium necrophorum*, *Escherichia coli*, and *Klebsiella pneumoniae* was found. Asmar[2] performed cultures on material from 17 children with retropharyngeal abscesses; viridans streptococci were isolated from 11 of the abscesses, *S. aureus* from 8, and group A streptococci from 6. The most frequently identified anaerobes were *Peptostreptococcus* spp. Overall, 45 aerobic and 18 anaerobic species were identified.

Rarely, retropharyngeal abscess may result from anterior extension from cervical osteomyelitis. This condition has been described with tuberculosis[56] and atypical mycobacteria causing a retropharyngeal abscess in a similar clinical setting. Barratt and colleagues[3] reported one case of retropharyngeal abscess caused by *Coccidioides immitis* in a 24-year-old woman with Hodgkin disease. The infection also was secondary to cervical vertebral osteomyelitis. Other rare and unusual causes of deep neck abscesses include cat-scratch disease (*Bartonella*),[78] *Streptococcus pneumoniae*,[52] and Kawasaki disease.[34]

Because a large variety of organisms can be found in pharyngeal space infections, obtaining adequate culture specimens is crucial. The optimal material for culture is an aspirate of the pus obtained at operation. Throat swabs or swabs of the abscess obtained after drainage usually are inadequate because of contamination with normal oropharyngeal flora. The pus, when obtained, can be transported in a capped syringe if anaerobic transport media are unavailable. Most pathogenic obligate anaerobes can survive in a purulent exudate, despite extended periods of exposure to air.[25] A Gram stain of the exudate would provide important clues to the bacterial etiology. A Gram stain showing a mixture of organisms suggests a mixed aerobic-anaerobic infection.

Although use of a β-lactamase-resistant antibiotic may be necessary in the treatment of deep neck abscesses because of the presence of β-lactamase-producing bacteria, including *S. aureus* and *Bacteroides* spp.,[12,13,72] results of two studies suggest that penicillin alone was equivalent to broad-spectrum antibiotics for treatment of peritonsillar abscesses.[39,79] Yilmaz and colleagues[79] compared procaine penicillin with intramuscular administration of ampicillin-sulbactam in outpatient treatment of 40 patients with peritonsillar abscesses that were drained perorally. No statistical difference in duration of symptoms and clinical recovery was found between the two groups. Kieff and associates[39] retrospectively evaluated 103 patients with peritonsillar abscesses who were treated with incision and drainage. Fifty-eight patients were treated with broad-spectrum antibiotics including ampicillin-sulbactam, clindamycin, cephalosporins, and metronidazole, alone and in combination; 45 patients were treated with penicillin alone. All patients were hospitalized after drainage, and the clinical outcomes, including duration of hospitalization and fever, did not differ significantly between the two groups. No significant difference in the organisms isolated was found, and failure and complication rates did not differ.

No comparative treatment studies for retropharyngeal or parapharyngeal abscesses have been reported. Treatment in these cases should be based on the results of cultures, as stated before. Drugs that may be effective include penicillin-lactamase inhibitor combinations such as ampicillin-sulbactam, ticarcillin-clavulanic acid, and piperacillin-tazobactam; expanded-spectrum cephalosporins such as ceftriaxone; or penicillinase-resistant penicillins including oxacillin or nafcillin. Cephalosporins, oxacillin, or nafcillin should be used in combination with clindamycin or metronidazole for adequate anaerobic coverage. Erythromycin and other macrolides (azithromycin, clarithromycin) are less satisfactory because of increasing macrolide resistance in group A streptococci and less activity against *Bacteroides fragilis* and *Fusobacterium*.

A study by Hanna and colleagues[30] reported macrolide resistance in 26 percent of group A streptococcal isolates obtained from peritonsillar abscesses seen between August 2001 and July 2002. The routine use of aminoglycoside antibiotics is not indicated because aerobic gram-negative enteric rods rarely are found in these infections. Antibiotic therapy is effective only in conjunction with adequate surgical drainage.

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CHAPTER 15

Cervical Lymphadenitis

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Cervical lymphadenopathy is enlargement of the lymph nodes in the neck. *Cervical lymphadenitis* implies that there is inflammation of one or more nodes. The inflammatory response by the host is triggered by some form of injury or invasion proximal to the involved lymph node or nodes. The nodes become affected secondarily by drainage through connecting afferent lymphatic channels. The injury may be acute or chronic, infectious or noninfectious. Proper anatomic definition of the inflamed node or nodes,[78] combined with knowledge of the structures of the head and neck drained by them, may allow a portal of entry for infectious agents, the most common cause of cervical lymphadenitis in infants and children, to be identified.

Figure 15–1 illustrates the regional lymph nodes commonly affected in infants and children with cervical lymphadenitis. The superficial cervical lymph nodes lie on top of the sternocleidomastoid muscle along the course of the external jugular vein. They receive afferents from the superficial tissues of the neck, mastoid, superficial parotid (preauricular) nodes, and submaxillary glands. Their efferents terminate in the upper deep cervical lymph nodes. The mastoid lymph nodes overlie the mastoid process of the temporal bone and receive drainage from the parietal scalp and inner surface of the pinna. The occipital lymph nodes lie on the upper part of the trapezius and receive afferents from the occipital scalp and superficial portions of the upper posterior neck. Their efferents terminate in the deep cervical glands, as do the efferents from the mastoid nodes.

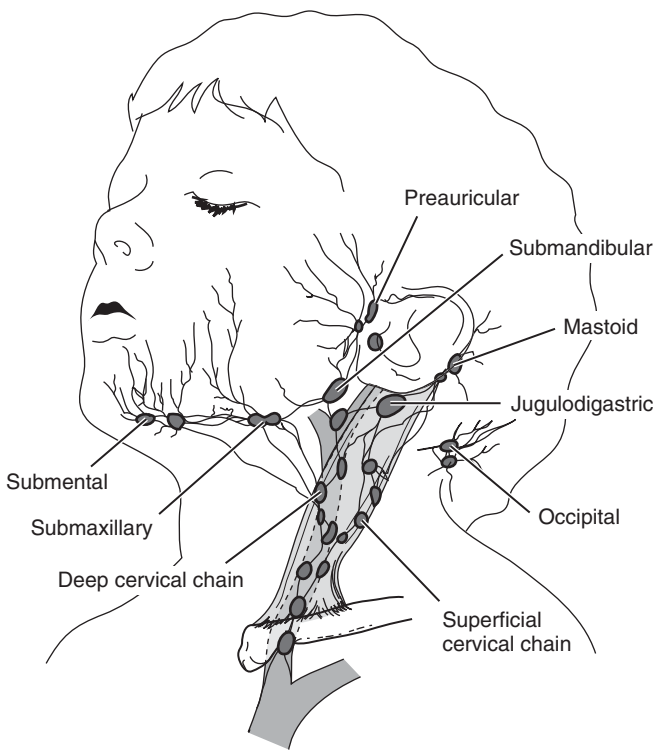

Figure 15–1 The lymphatic drainage and lymph nodes involved in infants and children with cervical lymphadenitis.

The deep cervical lymph nodes lie deep to the sternomastoid muscle along the whole length of the internal jugular vein and are divided into upper and lower groups. The jugulodigastric gland, a member of the upper group, lies at the angle of the jaw below the posterior belly of the digastric muscle. The lymphoid tissue of the palatine tonsil is drained into this gland; it frequently becomes enlarged in patients with "tonsillitis" or with tuberculous infection originating from the tonsils. The larynx, trachea, thyroid gland, and esophagus drain into the lower deep cervical glands. The submental lymph nodes, which lie between the digastric muscles below the myohyoid, receive superficial and deep drainage from the anterior tongue, lower lip, and chin, from both sides of the midline. They send efferents to the submandibular and upper deep cervical glands. The submandibular lymph nodes lie adjacent to the submandibular salivary gland and receive wide, superficial drainage from the lateral aspect of the lower lip, the vestibule of the nose, the cheeks, the medial parts of the eyelids, and the forehead. Deep drainage to these nodes arises from the posterior part of the mouth, gums, teeth, and tongue and from superficial and submental lymph nodes.

Because most of the lymphatic drainage of the head and neck goes to the submaxillary and deep cervical nodes, these glands are involved in more than 80 percent of cases of cervical adenitis in young children. Submental and superficial cervical lymphadenitis is observed less frequently.

EPIDEMIOLOGY

The epidemiology of infectious cervical adenitis is that of its infectious agents. Although cervical lymphadenitis can be a manifestation of focal viral infections of the oropharynx or respiratory tract, often it is part of a more generalized reticuloendothelial response to systemic infection. Viruses commonly associated

with prominent cervical adenitis include Epstein-Barr virus, cytomegalovirus, and human immunodeficiency virus (HIV). In HIV-infected children, cervical adenopathy either may herald or may be a part of the more generalized lymphadenopathy associated with this infection. Although human herpesvirus-6 (HHV-6), the cause of roseola in infants (exanthem subitum), is associated with the development of a mononucleosis syndrome and cervical adenopathy in adults,[3] lymphadenitis is not a prominent feature in children with primary infection.[55] Adenoviral and enteroviral infections are causes of generalized lymphadenopathy rather than of cervical adenitis alone. The epidemiology of cervical adenitis varies with age, geographic location, and socioeconomic status. Generally, lower socioeconomic status is associated with a higher incidence of infection in younger children.

When bacterial in origin, with the exception of group A streptococci and *Mycobacterium tuberculosis*, the agents isolated from these glands are the normal inhabitants of the nose, mouth, pharynx, and skin—*Staphylococcus aureus*, anaerobes, nontuberculous mycobacteria, *Actinomyces* spp.—and person-to-person transmission does not occur. In contrast, group A streptococci and *M. tuberculosis* infection of cervical lymph nodes results from contact with human infection by way of airborne droplets. Except in neonates, for whom male dominance has been reported in cases caused by group B streptococci,[11] infectious lymphadenitis has no gender or seasonal predilection.[13,35]

Any age group may be affected. In neonates, *S. aureus* and group B streptococci are the most common pathogens. Suppurative cervical lymphadenitis caused by *Staphylococcus epidermidis* in an otherwise healthy infant has been reported.[125] Nonetheless, despite the high frequency of nasal colonization by this organism, it remains a rare etiologic agent. There are rare reports of *Streptococcus pneumoniae* causing suppurative adenitis[94] in older children. Some studies[35,141,159] indicate that *S. aureus* has the leading role in infants, whereas in children, either group A streptococci or *S. aureus* is equally likely to be pathogenic. Other reports have varied regarding the relationship between age and probable etiologic agent.[13,24]

Overall, *S. aureus* and *Streptococcus pyogenes* accounted for 65 to 89 percent of consecutive cases in prospectively evaluated series.[13,35,159] Methicillin-resistant *S. aureus* (MRSA) must be strongly considered as a possible etiology because of the increased incidence of nosocomial MRSA and community-associated methicillin-resistant *S. aureus* (CA-MRSA) infections in the United States and elsewhere,[21,42,49,59,61,63,99] and recognition that the highest rates of CA-MRSA colonization and disease are found in children.[42,99] Many studies have shown that CA-MRSA strains predominantly cause skin and soft tissue infections, but a wide spectrum of illness is reported, and invasive CA-MRSA has been associated with a variety of clinical manifestations, including severe illness and death.[59,61,63,101] The impact of the CA-MRSA "epidemic" on pediatric cervical adenitis is less easy to define; however, retrospective and more recent prospective case series report that 6 to 10 percent of CA-MRSA infections manifest as adenitis.[61,63,99] One single-center study found that MRSA was the infectious etiology of no head and neck abscesses from July 1999 through December 2001 but accounted for 34 percent of cases from January 2002 through June 2004.[108]

The epidemiology of bacterial lymphadenitis varies by geographic location. Resurgence of infection with *Yersinia pestis* in the southwestern United States means that, in areas where it is endemic, it also must be considered in the differential diagnosis. Epidemic diphtheria, reported in the Russian Federation in 1990, subsequently spread to several newly independent states in the European region, with the number of reported cases (50,425) peaking in 1995. Although the number of diphtheria cases declined after the implementation of diphtheria control measures, 176 cases from the European region were reported to the World Health Organization (WHO) in 2004, 88 percent from newly independent states. Diphtheria must be added to the list of possible causes of cervical lymphadenitis in that region of the world.[156]

TABLE 15–1 Differentiation of *Mycobacterium tuberculosis* and Nontuberculous Mycobacterial Cervical Adenitis

	NTM MCA	*M. tuberculosis*
Age	1-6 yr	All ages
Ethnicity	White	Black, Asian, Hispanic
Exposure to tuberculosis	Absent	Present
Abnormal chest radiographs	Rare	Often
Residence	Suburban	Urban
TST >15 mm	Uncommon	Often
Bilateral involvement	Rare	Not uncommon

NTM, nontuberculous mycobacteria; TST, tuberculin skin test.

The distinctive epidemiologic features of mycobacterial infection are summarized in Table 15–1. Scrofula caused by *M. tuberculosis* is a rare disease. When it does occur, it usually affects adults and older children. In contrast, children with atypical mycobacterial infection almost always are 1 to 6 years old, live in suburban or rural communities, and have no history of contact with *M. tuberculosis*.* Although *M. tuberculosis* is an infection acquired primarily by inhalation, the gastrointestinal tract or the respiratory tract may serve as the primary portal of entry for nontuberculous mycobacteria.[47,104,114,119] There seems to be an ethnic predilection for nontuberculous mycobacterial infection to occur in whites and for tuberculous infections to occur in blacks, Hispanics, Asians, and the Australian Aboriginal population.[103,139]

The advent of the HIV pandemic had a major impact on the nature and frequency of mycobacterial infections. A marked increase in the incidence of tuberculous infection occurred in HIV-infected adults and children, with annualized case rates for tuberculosis in HIV-infected children increasing from 58 per 100,000 during the years 1981 to 1985 to 478 per 100,000 during the years 1990 to 1992.[51] The availability of highly active antiretroviral therapy (HAART) resulted in significant decreases in the incidence of opportunistic infections, including tuberculous and nontuberculous mycobacterial infections, in HIV-infected children.[48]

The presence of tuberculosis infection in the community means that all children are at risk of exposure to an infectious adult.[20] The annual tuberculosis rate in the United States decreased steadily during the years 1993 through 2005, but the annual decline has decelerated.[31] Of additional concern is the frequency with which drug-resistant tuberculosis is being detected in industrialized and developing countries. During the years 2000 through 2004, 33 percent of tuberculous isolates from industrialized countries were multidrug-resistant (e.g., resistant to isoniazid and rifampin, first-line drugs in the treatment of tuberculosis), and 6 percent were classified as extensively drug-resistant (resistant to isoniazid, rifampin, and three of the six classes of second-line antituberculosis drugs).[32]

The incidence of nontuberculous mycobacterial infections has increased since the 1980s, although some of this apparent increase most likely has resulted from improvements in diagnostic methods. Nontuberculous mycobacteria are ubiquitous and are found in food, water, animals, and soil. An association between cervical adenitis caused by atypical mycobacteria with cold weather is prompted by observations that 68 percent of cases occur in the winter and spring months,[83,112,155] and the disease exhibits a steady incidence throughout the year in cold climates.[47]

*See references 19, 33, 36, 41, 47, 69, 80, 84, 112, 120, 134, 149, 155.

A 10-year Canadian retrospective study reported that 70 percent of cases occurred in girls; however, most reports suggest no gender difference.[112]

Until the 1970s, *Mycobacterium scrofulaceum* was the usual etiologic agent isolated from children, followed by *Mycobacterium avium–intracellulare*.[155] This trend has reversed, with *M. avium–intracellulare* now accounting for 50 to 98 percent of culture-proven cases.[43,58,79,83,102,103,142,155] Previously uncommon species, such as *Mycobacterium kansasii*, *Mycobacterium malmoense*, *Mycobacterium fortuitum*, *Mycobacterium haemophilum*, and *Mycobacterium bohemicum*, also are being detected more frequently.* Some of these uncommon species probably are responsible for many cases of culture-negative mycobacterial lymphadenitis because of their fastidious growth requirements. New diagnostic methods have led to case reports of cervical adenitis secondary to slow-growing species, such as *Mycobacterium lentiflavum* and *Mycobacterium interjectum*.[37,53,57,116,136] Cervical lymphadenitis caused by *Mycobacterium chelonae* is rare, usually involves the submandibular glands, and typically occurs in patients with an antecedent history of dental pathology.[5]

Cervical lymphadenopathy may be the direct result of infection with HIV per se. The development of acute tender adenitis in an HIV-infected child should provoke a search for another etiology, however. Although the typical childhood pathogens remain the most common pathogens in this setting, as in other immunocompromised children, opportunists also should be sought.[51] Patients with HIV infection beginning therapy with potent antiretroviral agents may develop new-onset mycobacterial lymphadenitis (tuberculous and nontuberculous mycobacteria). When this lymphadenitis occurs, it is more localized, is associated with more sinus formation, and is more often caused by nontuberculous mycobacteria (*M. avium–intracellulare*) than by *M. tuberculosis*.[115]

Cat-scratch disease is a common cause of lymphadenitis in children and young adults.[23] In 1988, English and associates[40] first isolated a pleomorphic gram-negative bacillus, later identified as *Afipia felis*, from lymph nodes of patients with cat-scratch disease. *Bartonella henselae* (formerly called *Rochalimaea henselae*), a morphologically similar but genetically distinct pleomorphic gram-negative bacillus, now is recognized as the cause of most cat-scratch disease.[1]

The cervical nodes are the second most common site of cat-scratch disease involvement. Although unusual and severe manifestations of this infection have been described, it remains mostly a mild, self-limited infection in children and adolescents, with no ethnic predilection. Seasonal variation with an increased incidence in fall, winter, and early spring does occur in temperate zones. A history of animal contact with cats usually can be elicited.[26] The importance of bites and scratches by kittens in transmitting this disease has been well defined.[91] However, the absence of a history of traumatic contact with cats in a substantial number of cases has raised the possibility that other modes of transmission exist. Zangwill and associates[161] suggested that fleas might serve as vectors of transmission. Their hypothesis is strengthened by the detection of *Bartonella* DNA by polymerase chain reaction in collections of fleas from cats owned by two infected patients.[161]

PATHOPHYSIOLOGY

Although cervical lymphadenitis is a common entity in pediatric clinical practice, little information exists regarding its pathogenesis. Viral cervical adenitis may be part of either a local response to viruses invading the oropharynx or respiratory tract (e.g., adenoviruses, coxsackieviruses) or a more generalized reticuloendothelial response to systemic viral infection (e.g., Epstein-Barr virus, cytomegalovirus, HHV-6, HIV). Infection attributed to group A streptococci and *S. aureus* is presumed to enter the cervical lymphatics from the oropharynx (group A streptococci) and anterior nares (*S. aureus*). In a patient with group A streptococcal pharyngitis or tonsillitis, whether infection remains localized at the pharyngotonsillar tissues or spreads to cervical lymph nodes and results in suppuration primarily is a function of host response. Although peak attack rates for group A streptococcal pharyngitis are observed among school-age children, suppurative cervical adenitis is an uncommon occurrence. In contrast, infants and children younger than 3 years old rarely have group A streptococci isolated from throat cultures, but this age group more commonly has suppurative cervical lymphadenitis.[118]

In infections attributed to *S. aureus*, colonization of the anterior nares is thought to be a prerequisite for cervical lymphadenitis. Brook and Winter[22,23] arrived at this conclusion because organisms of identical phage types were isolated from the anterior nares and the cervical abscesses of their patients. An investigation of children in St. Louis found no such correlation between isolates from nasal and cervical node cultures.[13] The role of *S. aureus* as a primary pathogen has been the subject of some debate. In most series, 30 percent of aspirates yield mixed cultures of *S. aureus* and group A streptococci, and frequently significant elevations of antistreptolysin O titer are found in the sera of patients whose lymph nodes yielded a pure culture of *S. aureus*.

In a California study, 65 percent of patients had node aspirates yielding a pure culture of *S. aureus*, and 41 percent exhibited an immune response to one or more of the extracellular antigens of group A streptococci.[159] Similarly, the finding that many children improve with penicillin or ampicillin treatment, despite the high prevalence of penicillin resistance among *S. aureus*, suggests that, although streptococci and staphylococci may coexist in these nodes, staphylococci may play a subsidiary role as secondary invaders. Most children with isolates of *S. aureus* from suppurative lymph nodes show no evidence of coexistent streptococcal infection or viral upper respiratory infection. In this more common circumstance, *S. aureus* apparently has the capacity to be a primary invader.

Recovery of anaerobic bacteria from cervical nodes suggests invasion of the lymphatics by mouth flora, often as a result of local tissue destruction by periodontal disease.[24] The delineation of the pathophysiology of cervical lymphadenitis of diverse bacterial etiology requires an understanding of the interaction between a given microorganism (e.g., inoculum size, elaboration of extracellular enzymes, ability to adhere to epithelium) and the host (e.g., humoral and surface immune capacity, degree of trauma).

Tuberculous cervical lymphadenopathy occurs within months of the initial exposure, through pulmonary infection and involvement of the regional and then more distant lymph nodes. It is a rapid process; chest radiographic evidence of active pulmonary disease often is seen. Nontuberculous mycobacteria are ubiquitous in the environment. Oropharyngeal acquisition and local infection lead to lymph node involvement. Most children with nontuberculous mycobacterial cervical lymphadenitis are immunocompetent, although a more recent study suggests that children who develop necrotic nodes may have deficient production of interferon-γ.[102] Despite *M. avium* skin test positivity being linked with pet birds, no clear relationship with lymphadenitis has been shown.[75] The observation that discontinuation of childhood bacille Calmette-Guérin vaccination has been associated with an increase in atypical mycobacterial infection in many countries suggests that this vaccine may have a protective effect.[150] Progressive cervical adenitis developing after bacille Calmette-Guérin vaccination also has been reported.[105]

*See references 10, 43, 58, 79, 83, 102, 103, 111, 112, 142, 155.

CLINICAL PRESENTATION

The clinical manifestations of cervical lymphadenitis vary considerably but are consistent with the diverse etiologies associated with cervical node enlargement in infants and children. To categorize the mode of presentation as either acute or subacute and chronic is useful because, although the boundaries are ill-defined and much overlap exists, common etiologies tend to fall consistently within one or another category. Cervical lymphadenitis of acute onset may be categorized further as either bilateral or unilateral. In most situations, acute, bilateral cervical adenitis is either part of a generalized reticuloendothelial response to a systemic infection or a localized reaction to acute pharyngitis. The presence or absence of associated features (e.g., pharyngitis, enanthems or exanthems, generalized adenopathy, hepatosplenomegaly) aids in making the differentiation.

Acute unilateral cervical lymphadenitis is caused by streptococcal or staphylococcal infection in 53 to 89 percent of cases.[13,35,62,159] In newborns, *S. aureus* is the most common cause, and clinical features are similar to those seen in older children. Group B streptococci have been described as causative in a "cellulitis-adenitis" syndrome in infancy.[11] These infants differ from infants with staphylococcal adenitis in that they are younger; more often are male; and have a greater incidence of systemic symptoms, irritability, and anorexia; 94 percent have associated bacteremia. The typical patient presents with fever, facial or submandibular cellulitis, and ipsilateral otitis media.[11] Isolated cervical adenitis caused by group B streptococci also has been described.[44]

Patients with disease attributed to *S. aureus* or group A streptococci typically are 1 to 4 years old (70-80% of cases), and the male-to-female ratio is equal. Clinically, there is little that helps to differentiate streptococcal from staphylococcal infections. Cervical adenitis can occur as part of the "streptococcosis" syndrome of infancy, with an onset heralded by coryza, an irregular low-grade fever, nasal discharge with excoriation and crusting around the nares, vomiting, and loss of appetite. Lymph node enlargement occurs within a few days of onset and resolves, as do other symptoms, without treatment within 6 to 8 weeks.[118] Suppuration of cervical glands may occur at any time during this interval but seldom does so if antimicrobial therapy is given early in the illness. Group A streptococci also should be suspected as a cause of cervical adenitis in a patient with typical vesiculopustular or crusted lesions of impetigo involving the face or scalp.

Systemic symptoms in children with staphylococcal or streptococcal cervical adenitis usually are minimal or absent unless associated with cellulitis, metastatic foci of infection, or bacteremia. The primary site of lymph node involvement by frequency is submandibular (50-60%), upper cervical (25-30%), submental (5-8%), occipital (3-5%), and lower cervical (2-5%).[13,35,157] Involved nodes generally vary in size from 2 to 6 cm in diameter, and one fourth to one third suppurate. Patients with lymphadenitis caused by *S. aureus* are more likely to have suppuration and a longer duration of symptoms and signs before diagnosis than are patients with disease caused by other bacterial agents (Fig. 15–2).[13,141] Among patients who develop suppurative adenitis, 86 percent do so within 2 weeks of onset.[157]

Approximately one third of patients in one study had concomitant lymphadenopathy at other anatomic sites.[13] A history of recent upper respiratory tract symptoms, including sore throat (40%), earache or coryza (16%), and impetigo (32%), is a frequent finding, as are signs of pharyngitis, tonsillitis, or otitis media.[13,35] These factors do not help to delineate the etiology, however. Hepatomegaly or splenomegaly is a rare occurrence and, if present, should suggest bacteremia or generalized disease processes (e.g., infectious mononucleosis, reticuloendotheliosis, tuberculosis, HIV infection).

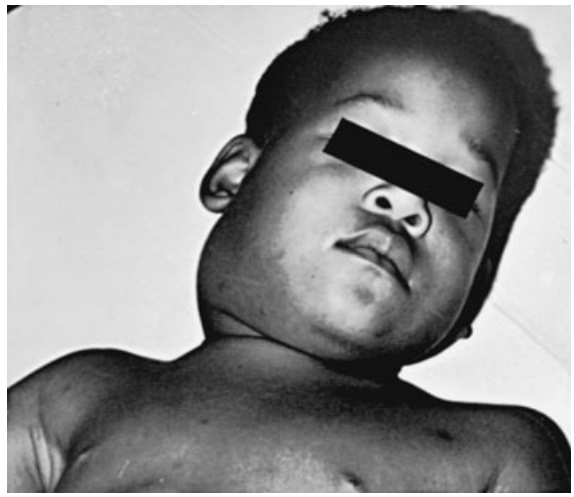

Figure 15–2 A 2-year-old boy with fever and unilateral inflammation of the cervical lymph nodes of 2 days' duration. Needle aspirate culture of this nonfluctuant node grew *Staphylococcus aureus*. Antistaphylococcal therapy resulted in complete resolution of adenitis without surgical drainage.

Kawasaki disease may manifest as a febrile illness associated with bilateral or unilateral cervical lymphadenopathy and may be confused with more common acute pyogenic infections.[152] Other features (e.g., conjunctivitis, oral manifestations, changes in the peripheral extremities, polymorphic erythematous rash) are required criteria for the diagnosis.[95] Although originally termed *mucocutaneous lymph node syndrome*, unilateral lymph node enlargement of at least 1.5 cm is the most inconsistent feature.[15,95] Lymphadenopathy usually subsides when the fever subsides, although in some cases it may follow a more chronic course.

The rapid development of painful lymphadenitis, quickly succeeding the sudden onset of fever, chills, weakness, and headache, is a classic presentation of infection caused by *Y. pestis* (bubonic plague). The groin is the site most often involved. Other locations, including the cervical area, may be affected, however. Establishing the diagnosis and providing treatment quickly are crucial because infection can be fulminant.

In cases of diphtheria, cervical adenopathy develops secondary to infection of the posterior structures of the mouth and proximal pharynx. A whitish gray membrane covers the mucosal surfaces. In severe cases, the cervical adenopathy, which typically is bilateral, can result in a "bull neck" appearance.

Careful physical examination of the head and neck, particularly areas drained by affected lymph nodes, may yield important clues about etiology. The presence of periodontal disease is associated with a higher incidence of anaerobic organisms causing adenitis[24]; the history or presence of tick bites suggests the possibility of tularemia[135]; and the presence of papular or pustular lesions, suggesting an inoculation site, raises the possibility of rarer causes of infection, including *Nocardia*, actinomycosis, sporotrichosis, plague, cutaneous diphtheria, and cat-scratch disease.

Mycobacterial infections, cat-scratch disease, and toxoplasmosis are more common entities presenting as subacute or chronic lymphadenitis. The epidemiologic and clinical features that aid in the differentiation of typical and nontuberculous mycobacterial infections are summarized in Table 15–1. The clinical manifestations virtually are identical (Fig. 15–3).[19,30,97] Typically, a child presents with a history of painless, (so-called cold) cervical node swelling. The submandibular cervical nodes

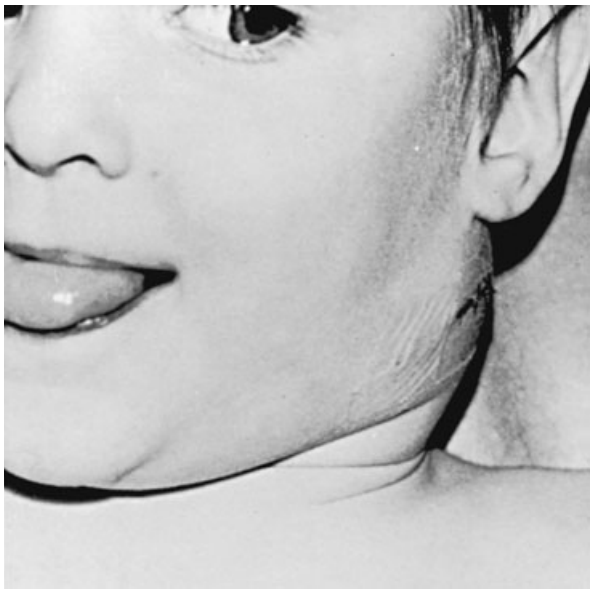

Figure 15–3 A 4-year-old boy who had bilateral nontender enlargement of lymph nodes of 6 weeks' duration without other symptoms. Placement of a TST resulted in 18-mm induration, excisional biopsy acid-fast stain was positive, and cultures grew *Mycobacterium tuberculosis*.

usually are involved in nontuberculous mycobacterial infection,* whereas other cervical nodes are involved more frequently with *M. tuberculosis*.[4,19,65,90,127] As the infection progresses, the skin overlying the node may develop a pinkish or violaceous discoloration caused by increased vascularity, although the skin temperature is not increased. This finding may be followed by adherence of the skin to the underlying mass. If left untreated, fluctuance and spontaneously draining sinus tracts may develop.

A patient with *M. tuberculosis* is more likely than one with atypical mycobacterial disease to be older than 4 years of age and to have generalized lymphadenopathy (10-20% of cases), bilateral node enlargement (10% of cases), a history of exposure to tuberculosis (93% of cases), and an urban residence.[19,90,107] No differences have been noted, however, with regard to duration of adenopathy, fever, and presence or absence of constitutional symptoms. An abnormal chest radiograph has been noted in 28 to 71 percent of cases caused by *M. tuberculosis*,[127,128,138,149] in contrast to the 98 to 100 percent of normal chest radiographs found in patients with nontuberculous mycobacterial adenitis.[47,126,127,142,148]

In a summary of 447 reported childhood cases of nontuberculous mycobacterial infections from 15 countries, Lincoln and Gilbert[74] detected only 6 cases of bilateral cervical node involvement, 4 with abnormal chest radiographs, and none with nodal enlargement other than cervical. Similar findings have been reported by other investigators.[126,148] Intradermal tuberculin skin testing with purified protein derivative uncommonly produces more than 15 mm of induration at 48 hours in a child with nontuberculous mycobacterial infection, but reactions between 5 and 15 mm are common.[76,86] Reactions of 10 to 20 mm can occur with *Mycobacterium marinum* and *M. fortuitum* infection.[114] Tuberculin skin test positivity may persist, even when children are retested many years after infection.[155]

Cat-scratch disease may manifest days to weeks after the initial inoculation. Characteristically, a history of contact with a cat or kitten or a scratch is present. Later, when the primary lesion may have healed, tender regional adenopathy appears. Although axillary nodes most frequently are affected, 25 percent of children have isolated cervical node involvement. Middle cervical and parotid nodes are involved more often than are submandibular ones.[122] Constitutional symptoms, present early in the course of the illness, usually are mild and may have resolved by the time the adenitis appears. Fever is observed in one fourth of patients and, if present, has a mean duration of 5 to 7 days.[85] Nodes suppurate in one tenth to one third of patients.[28,29] Rare manifestations include Parinaud syndrome,[26] encephalopathy,[82] exanthems[28] (usually of the erythema nodosum type), and osteolytic lesions.[27]

Acquired toxoplasmosis may manifest as regional lymphadenopathy, frequently with posterior cervical node involvement.[93,121,146] Most children exhibit few, if any, constitutional symptoms. If present, fatigue and generalized myalgia are prominent. The characteristic location combined with a history of exposure to cats or of eating undercooked meats should raise this diagnostic possibility, and the diagnosis may be confirmed by appropriate serologic testing. It is an uncommon etiology for cervical adenopathy in children living in the United States.

Chronic, recurrent cervical adenitis forms part of the periodic fever, aphthous ulcers, pharyngitis, cervical adenitis (PFAPA) syndrome, a chronic syndrome first described in 1987.[92] It is characterized by periodic episodes of high fever greater than 39° C lasting 3 to 6 days and recurring every 3 to 8 weeks in association with aphthous ulcers, pharyngitis, and cervical adenitis, although symptoms such as abdominal pain, nausea, diarrhea, and headache also are described in 20 to 73 percent of children.[147] In most children, the onset of disease occurs before they reach 5 years of age, the syndrome is self-limited, and recovery without long-term sequelae is the rule. Oral corticosteroids are effective in aborting an attack.[72,109,147]

Kikuchi-Fujimoto disease, also called *subacute necrotizing lymphadenitis*, is an uncommon disorder of uncertain etiology that also may manifest as cervical adenopathy, with or without fever. This disorder was described first in 1972 and seems to have a predilection for Asian women 25 to 30 years old, who generally have a benign course with spontaneous resolution over 3 to 4 months.[46,151] Kikuchi-Fujimoto disease also has been reported in children, typically adolescents, although cases in children 2 years old are documented.[34,71,73,153]

In contrast to the 4:1 female predominance documented in adult series, pediatric Kikuchi-Fujimoto disease occurs more commonly in boys (male-to-female ratio ranges from 1.2:1 to 1.9:1). The most common presentation is unilateral or bilateral lymphadenopathy of multiple nodes. In most cases, posterior cervical chain nodes are involved, and affected nodes may be painful and tender. Fever is an inconsistent symptom. Extranodal manifestations include malaise, nightsweats, weight loss, maculopapular skin rashes, gastrointestinal symptoms, hepatosplenomagaly, arthritis, and aseptic meningitis. Associated laboratory findings include leukopenia (33-100% of cases with prolonged fever), elevated erythrocyte sedimentation rate, mild anemia, elevated C-reactive protein, and elevated liver enzymes.

Diagnosis is established by biopsy of the lymph nodes and not by fine-needle aspiration cytology, showing lymphadenitis with focal proliferation of reticular cells accompanied by histiocytes and extensive nuclear debris. Lymph node biopsy also has been associated with prompt resolution of fever.[34] Treatment generally is supportive, although steroids are reported to provide more rapid resolution of symptoms. The prognosis is favorable; most cases of Kikuchi-Fujimoto disease resolve within 6 months, although a recurrence rate of 3 to 4 percent is recorded. Some children subsequently develop autoimmune disorders, most commonly systemic lupus erythematosus, and regular clinical follow-up looking for signs of evolving autoimmune disorder is

*See references 4, 19, 43, 58, 79, 83, 84, 90, 126, 127, 142, 148, 155.

required.[100,132,153] Whether Kikuchi-Fujimoto disease is infectious or genetic in nature, perhaps the result of infection with a single novel agent or a nonspecific host response to any of a variety of agents, remains to be determined.

Another rare but important cause of cervical lymphadenitis in association with generalized lymphadenopathy or hepatosplenomegaly is hemophagocytic syndrome. This diagnosis should be considered if the aforementioned features occur with prolonged fever, cytopenia in at least two cell lines, low fibrinogen, and high ferritin and triglyceride serum levels.[110] Other features include rash, respiratory distress, hypotension, and coagulopathy. The diagnosis is confirmed by showing hemophagocytosis in bone marrow or lymph node biopsy specimens. Finally, when presented with a history of subacute or chronic lymphadenitis, careful physical examination should be undertaken to exclude obvious local causes (e.g., seborrhea, head lice, tinea capitis, chronic otitis media) before an extensive diagnostic work-up is initiated.

DIFFERENTIAL DIAGNOSIS

Cervical swellings are encountered frequently in pediatric practice, and most of them represent lymph nodes. When considering the diagnostic possibilities in patients with cervical lymphadenitis, one first must ascertain whether or not the pathologic process involves a lymph node, then whether or not its cause is infectious, and, if infectious, the likely etiologic agent. The duration of the cervical swelling aids in the differential diagnosis because most tumors or developmental anomalies have been noted for weeks. Rapid enlargement may occur in the latter, but usually it occurs as a result of secondary infection. Location is a helpful clue because midline masses rarely represent lymph nodes and the most common neck masses of congenital origin (thyroglossal duct cyst, branchial cleft cyst, and cystic hygromas) all have characteristic anatomic locations.

Of the midline masses, thyroglossal duct cysts are the most common.[66,117] These cysts may occur anywhere from the foramen cecum to the thyroid, are midline, and move on protrusion of the tongue. They may have an associated sinus tract, midline or just lateral to it, from which cloudy mucus sometimes can be expressed. They may become infected secondarily, but, in the noninfected state, these cysts are nontender, smooth, and round with well-defined margins. Thyroglossal duct cysts must be differentiated from other midline masses, including epidermoid cysts, lipomas, thyroid tumors, and the rare midline lymph node.

The second most common benign congenital neck mass is the branchial cleft cyst. It usually arises from the second branchial cleft and lies at the anterior border of the sternocleidomastoid muscle. Although such cysts usually manifest as skin dimples, they may become infected secondarily and manifest as inflammatory swellings or draining sinus tracts. A careful examination should detect a sinus tract. Branchial cleft cysts can occur in individuals of any age, but usually occur in school-age children.

Cystic hygromas, considerably less common than thyroglossal duct or branchial cleft cysts, are the third most frequent cause of congenital neck masses. These arise from lymphatics derived from either the jugular vein or the mesenchymal tissue. They may occur elsewhere but usually are found posterior to the sternocleidomastoid muscle in the supraclavicular fossa. Most cystic hygromas appear in the first 2 years of life, many being noted at birth or soon thereafter. They are soft, compressible tumors that transilluminate well, and, although benign in themselves, they may cause symptoms through pressure exerted on surrounding structures. Confusion may arise when cystic hygromas increase in size in association with an upper respiratory tract infection. The latter causes increased lymph flow so that the hygroma persists while other lymph nodes decrease in size after resolution of the infection. In most circumstances, palpation and transillumination readily distinguish these congenital malformations.

These four cervical masses—thyroglossal duct cysts, thyroid tumors, branchial cleft cysts, and cystic hygromas—accounted for 63.7 percent of lesions in children with persistent cervical masses reported by Moussatos and Baffes.[97] Other lesions included neurogenic tumors, parotid tumors, and miscellaneous benign tumors (12.3%). The remainder of masses represented lymph nodes. As a rule, masses located completely anterior to the sternocleidomastoid muscle are benign. The exception is the thyroid tumor.[97] Malignancies that mimic cervical lymph nodes usually are located in the posterior triangle or are multiple masses extending across the anterior and the posterior triangles. In contrast, approximately 50 percent of masses in the posterior triangle represent malignancies, most of which are of lymphoid origin. Although most cysts and tumors manifest as solitary, unilateral, nontender masses, lymph nodes of noninfectious etiology frequently are multiple and bilateral, and they may be mildly tender.

Noninfectious chronic inflammatory involvement of cervical lymph nodes may represent a variety of uncommon, usually benign, but sometimes malignant entities (Table 15–2). Fifty percent of malignant neck masses in children are caused by Hodgkin and non-Hodgkin lymphomas. Neuroblastoma is the second most common malignancy, accounting for 15 percent. The likelihood of a given diagnosis is age-dependent, with neuroblastoma being more common than Hodgkin disease in younger age groups.[62] Thyroid tumors are the third most frequent neck

TABLE 15–2 Noninfectious Etiology of Cervical Adenitis

	Isolated Cervical	Cervical Associated with Generalized Adenopathy
Malignancy		
Hodgkin disease	+	+
Non-Hodgkin lymphomas	+	+
Rhabdomyosarcoma	+	–
Neuroblastoma	+	+
Leukemia	+	+
Metastatic carcinoma	+	–
Thyroid tumors	+	–
Drugs		
Isoniazid	–	+
Phenytoin (Dilantin)	–	+
Serum Sickness	–	+
Collagen Vascular Disease		
Juvenile rheumatoid arthritis	–	+
Systemic lupus erythematosus	–	+
Miscellaneous		
Sarcoidosis	–	+
Reticuloendotheliosis	–	+
Sinus histiocytosis with massive lymphadenopathy	+	+
Histiocytosis X	–	+
Postvaccinial	+	–
Storage disorders	–	+
Kawasaki disease	+	+
Hemophagocytic syndrome	–	+
PFAPA syndrome	+	–
Kikuchi-Fujimoto disease	+	+
Masses Simulating Adenopathy		
Cystic hygroma	+	–
Branchial cleft cyst	+	–
Thyroglossal duct cyst	+	–
Epidermoid cyst	+	–
Sternocleidomastoid tumor	+	–

PFAPA, periodic fever, aphthous ulcers, pharyngitis, cervical adenitis.

TABLE 15–3 Infectious Etiology of Cervical Adenitis

	Isolated Cervical	Cervical Associated with Generalized Adenopathy
Bacterial		
Staphylococcus aureus	+	–
Group A streptococci	+	+
Mycobacterium tuberculosis	+	+
Nontuberculous mycobacteria	+	–
Bartonella henselae	+	–
Gram-negative enterics	+	–
Anaerobes	+	–
Haemophilus influenzae	+	–
Yersinia pestis	–	+
Actinomyces israelii	+	–
Diphtheria	+	–
Tularemia	+	–
Brucellosis	–	+
Syphilis	+	+
Viral		
Measles	+	+
Rubella	+	+
Epstein-Barr virus	+	+
Herpes simplex	+	–
Human herpesvirus 6	+	+
Cytomegalovirus	+	+
Mumps	+	–
Varicella	+	+
HIV	+	+
Fungal		
Histoplasmosis	+	+
Cryptococcus	+	–
Aspergillosis	+	–
Candida	+	–
Sporotrichosis	+	–
Parasitic		
Toxoplasma gondii	+	+

HIV, human immunodeficiency virus.

malignancies. Other entities to be included in the differential diagnosis include leukemia,[25] metastatic carcinoma,[97] phenytoin-induced pseudolymphoma,[25] serum sickness,[25] storage disorders (Gaucher disease, Niemann-Pick disease), collagen vascular disease,[25] sarcoidosis,[64] sinus histiocytosis with massive lymphadenopathy,[12,124] and reticuloendotheliosis or histiocytosis X. Except for malignancies, these disease entities almost always are associated with lymphadenopathy that is not limited to the cervical region and have a variety of clinical and laboratory findings that allow the correct diagnosis to be made.

Numerous infectious agents have been reported in association with cervical adenitis in infants and children (Table 15–3). Among patients evaluated prospectively with needle aspirate cultures of affected lymph nodes, *S. aureus* or group A streptococci are the organisms most frequently isolated.[13,24,35,141,159] No significant difference has been reported that distinguishes between patients with adenitis caused by streptococci or staphylococci with respect to gender, ethnicity, dental problems, symptoms, presence of fever, or site or size of lymph nodes. In patients from whom *S. aureus* is isolated, a longer duration of disease before diagnosis is established, a larger percentage of fluctuant lymph nodes,[13,141] and a tendency toward slower resolution are found.[34] Most patients with bacterial cervical lymphadenitis, including patients with mycobacterial infection, are 1 to 6 years old. Older children are more likely to have negative lymph node aspirate cultures.[13,134,141]

In early studies, anaerobes rarely were associated with cervical adenitis.[13,35] Proper bacteriologic techniques for the isolation of

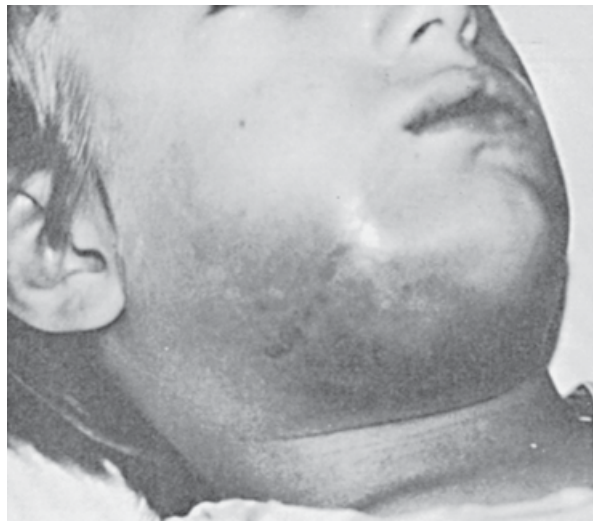

Figure 15–4 A 9-year-old boy who developed high fever and markedly tender submental lymph node inflammation after a tooth extraction. Cultures from this fluctuant mass grew three anaerobes and viridans streptococci.

these fastidious organisms allowed Brook[24] to report anaerobes alone in 18 percent and mixed anaerobic and aerobic bacteria in 20 percent of patients, suggesting that anaerobic organisms may play a more significant role in the etiology of cervical lymphadenitis than recognized previously. An older child with "negative" cultures, especially a child with poor dental hygiene or periodontal disease, may have anaerobic infection, as did the 9-year-old boy in Figure 15–4. Needle aspiration of this cervical mass yielded *Peptococcus*, *Peptostreptococcus*, *Bacteroides fragilis*, and viridans streptococci. Resolution of the lymphadenitis occurred promptly after incision and drainage and penicillin therapy.

Dental disease or manipulation also should suggest the possibility of cervicofacial actinomycosis (lumpy jaw). These patients have a chronic submandibular mass and frequently a fistula from the skin to the oral cavity.[16] Less frequently occurring bacteria,[70] viruses, fungi, and parasites can cause cervical lymphadenitis in children, but these patients usually have less evidence of acute inflammation, with or without adenopathy at additional sites, and historical and physical findings that suggest unusual causes of cervical lymph gland enlargement.

SPECIFIC DIAGNOSIS

A detailed history to ascertain preceding dental problems, presence of skin lesions, animal exposure (including exposure to fleas and ticks), duration of illness, presence of associated symptoms, contact with tuberculosis, presence of risk factors for HIV infection, drug usage (especially phenytoin) or other unusual ingestions (undercooked meat, unpasteurized dairy products), recent travel outside the geographic region of residence, and sites of occult infection drained by the affected node may yield important diagnostic clues in a patient with cervical lymphadenitis. Physical examination should include careful inspection for the presence of dental disease, noncervical lymphadenopathy, hepatosplenomegaly, and oropharyngeal or skin lesions.

Radiologic evaluation of adenitis is unnecessary in most mild to moderate cases. Ultrasonography often is performed when the presenting neck mass is very large, is increasing in size, or has not responded to initial antibiotic therapy. It is useful in diagnosing suppuration and expediting incision and drainage. High-resolution and color Doppler ultrasonography (with or without contrast enhancement) defining longitudinal-to-transverse nodal

ratio and vascularity patterns has had some success in differentiating benign and malignant lymph nodes in adults.[2,96,98,158,160]

In children in whom most adenopathy is infectious or reactive in etiology, ultrasonography is less discriminating. In one study of 35 children, ultrasonography showed significant differences in lymph nodes in 22 children with a diagnosis of Kawasaki disease compared with 8 children with bacterial lymphadenitis, but findings were similar to those of children with lymphadenitis secondary to Epstein-Barr virus infection.[144]

In another study of 146 children, unilateral lymph nodes and cystic necrosis were found only in lymphadenitis caused by cat-scratch disease or bacterial or tuberculous infection.[113] Individual sonographic findings were nonspecific for diagnosis, although these findings combined with clinical signs were helpful. No patients with nontuberculous mycobacterial adenitis were evaluated in this study. Nodal calcifications and spread of nodal masses into the subcutaneous tissues by ultrasonography and characteristic low-density, ring-enhancing lesions with minimal or absent inflammatory stranding of subcutaneous fat seen on computed tomography and magnetic resonance imaging have been reported with nontuberculous mycobacterial adenitis in children.[54,58,77,123] These findings may be helpful in differentiating this etiology from other bacterial causes when the diagnosis is not suspected clinically or early in the course. Differences in clinical presentation between the two entities should obviate the need for such imaging, however, in all but the most complicated of cases.

In the acute stage of cervical lymphadenitis, needle aspiration of the affected node is a valuable diagnostic tool. Sixty to eighty-eight percent of patients with acute cervical lymphadenitis subjected to needle aspiration of the affected node for bacterial and mycobacterial culture have an etiologic agent recovered.[13,24,65,159] Only inflamed nodes should be aspirated, but they need not be fluctuant. No serious complications of this procedure have been recorded.

The largest or most fluctuant node should be selected, and the skin should be cleansed and anesthetized. Skin anesthesia can be induced effectively using a topical anesthetic cream (e.g., lidocaine-prilocaine [EMLA]) placed on the selected aspiration site under an occlusive dressing 30 to 45 minutes before the procedure. An 18- or 20-gauge needle attached to a 20-mL syringe is used. If no material is aspirated, 1 to 2 mL of sterile *nonbacteriostatic* saline is injected into the node, and it is reaspirated. The aspirate should be inoculated directly from the syringe onto aerobic (including chocolate agar) and anaerobic media, onto Sabouraud agar (fungi), and into a broth medium suitable for the early detection of mycobacteria, such as the Bactec radiometric assay. In the last-mentioned system, the release of labeled carbon dioxide in an automated ion chamber system can detect mycobacteria 12 to 17 days after inoculation of the broth.[133] Gram and acid-fast stains are mandatory, and their reading serves as a guide to initial antimicrobial therapy.

Polymerase chain reaction assays used to identify tuberculous and nontuberculous mycobacteria have successfully confirmed their presence in gastric aspirates and in specimens obtained from lymph nodes by aspiration or biopsy.[39,45,52,53,131,136] This technique shows great promise in rapidly providing a specific diagnosis, but it is not yet routinely available.

Thioglycolate broth and anaerobically incubated blood agar plates are incapable of providing optimal conditions for the isolation of many anaerobic bacteria.[13] Optimal methods for cultivation of fastidious anaerobes should be employed because anaerobic organisms may be recovered in 20 percent of cases.[24] Cultures of infected skin lesions and exudates on tonsils also should be done, but not to the exclusion of needle aspiration.

Isolation of group A streptococci from the throat or skin cultures of a patient with lymphadenitis does *not* confirm the etiology of the lymph node inflammation. Patients have been noted to have isolation of group A streptococci from throat and of *S. aureus* from lymph node aspirate cultures.[13,118] Tuberculin skin tests should be performed. Induration of 15 mm or greater suggests infection with *M. tuberculosis*, whereas reactions of 5 to 14 mm may be caused by either a tuberculous or a nontuberculous mycobacterial infection.[86]

In an attempt to develop a rational approach to the use and interpretation of differential skin testing, Huebner and associates,[60] using tuberculin skin tests and nontuberculous mycobacterial antigens, studied 144 children with chronic cervical adenopathy, of whom 123 had mycobacterial culture results available. The low incidence of tuberculosis within the study population (four cases) precluded making an interpretation regarding the utility of these antigens in distinguishing disease caused by *M. tuberculosis* from that caused by other mycobacteria. Children with culture-confirmed mycobacterial lymphadenopathy had significantly larger reactions to nontuberculous mycobacterial antigens than did children with microscopy-negative and culture-negative results. The study was terminated prematurely because of an unacceptably high incidence of a blistering skin reaction to nontuberculous mycobacterial antigens.

Intradermal skin testing, using a crude extract from affected nodes, historically has been used to establish the diagnosis of cat-scratch disease.[28] A diagnosis usually is reached, however, based on the presence of regional adenopathy, a history of cat exposure (particularly if the patient has a history of a scratch or a primary skin lesion), and negative laboratory studies for other causes of lymphadenopathy. *B. henselae*, the more common etiologic agent of this disease, can be isolated from blood if lysis-centrifugation blood cultures or the Bactec blood culture system is used. Isolates may be identified using a commercially available system (Microscan Rapid Anaerobe Panel; Baxter, Sacramento, CA).[1] Serologic methods for the detection of IgG antibodies to *B. henselae* are available[1,161] and should be considered the gold standard diagnostic method. In few cases, a lymph node biopsy should be undertaken to exclude other, more serious, pathologies.

If, after the aforementioned evaluation, the etiology of adenitis remains uncertain, or lymphadenopathy has persisted with no detectable response to antimicrobial therapy, a more intense diagnostic evaluation is indicated. Studies may include a complete blood count; serology for Epstein-Barr virus, cytomegalovirus, HHV-6, HIV, histoplasmosis, coccidioidomycosis, toxoplasmosis, tularemia, *B. henselae*, and *Brucella*; and a radiograph of the chest. If the diagnosis remains in doubt, and the node persists, enlarges, is hard, or is fixed to the adjacent structures, biopsy *should* be performed. Biopsy material should be submitted for the studies outlined earlier for lymph node aspirate cultures and for routine histology; Giemsa, periodic acid–Schiff, and methenamine silver stains; and, in select cases only, viral cultures. If the histology reveals noncaseating granulomas and the child has a history of cat exposure, the most likely diagnosis is cat-scratch disease,[85,87] and serologic testing is indicated. Sarcoidosis involving lymph nodes would have a similar histology but is rare in children and a condition in which isolated cervical node involvement has not been observed.[64,130]

Older children are more likely to have negative cultures of lymph node aspirates[13,141] and to be more frequent candidates for excisional lymph node biopsy. They also are more likely to have lymphomas. It is important that appropriate tissue be excised, especially from adolescents, so that precise diagnostic interpretation can be done. This interpretation can be facilitated by the proper selection of a lymph node to be biopsied; intact removal of the node chosen; and proper fixation, cutting, and staining of the specimen. If only one node or one anatomic group of nodes is enlarged, the largest node should be excised. If several groups of lymph nodes are involved, the site for biopsy should be selected according to the likelihood of diagnostic yield. Biopsy specimens from the lower neck and supraclavicular area have the highest yields.[67] Other areas, including the upper cervical, submandibular,

axillary, and parotid lymph nodes, are much more likely to be affected by reactive hyperplasia, which may or may not be related to the underlying disease process. If lymphoma is suspected, needle biopsies or frozen sections are contraindicated.[18,25]

Even under optimal conditions, many reactive processes, including rheumatoid arthritis, toxoplasmosis, phenytoin-induced adenopathy, dermatopathic adenitis, and infectious mononucleosis, have been noted to simulate lymphoma.[25] Obtaining a thorough history and performing appropriate serologic studies should provide sufficient information for the physician to exclude reactive processes known to simulate lymphoma.

TREATMENT

Optimal management of a child with cervical lymphadenitis depends on an accurate assessment of the underlying etiology. Because almost all cases are associated with infectious agents, every effort should be made to ascertain the etiologic agent so that specific therapy can be initiated. Aspiration of the affected lymph node for Gram and acid-fast stains serves as a guide for initial therapy, and culture and antimicrobial susceptibility form the basis for prescribing specific treatment in patients with bacterial lymphadenitis.[13,35] When the patient presents with typical findings of acute bacterial lymphadenitis, however, empiric therapy may be undertaken without prior needle aspiration. In this situation, close follow-up is essential because failure to show some clinical response after 48 hours of therapy is an indication for this diagnostic procedure to be done.

Acute suppurative cervical lymphadenitis most frequently is caused by infection with *S. aureus* or group A streptococci.[13,22,35,129,141,159] In nodes that progress to abscess formation, *S. aureus* is the most frequent agent isolated,[13,35,129] and drainage is mandatory. Because of the frequency of infection caused by *S. aureus* or group A streptococci, empiric antimicrobial therapy should be directed against these two agents. Penicillinase-resistant penicillins should be used. If the patient requires parenteral therapy and CA-MRSA is not common in the area, oxacillin, nafcillin (150 mg/kg/day), or cefazolin (75 to 100 mg/kg/day) may be used.

When oral therapy is deemed to be adequate, cloxacillin (50 mg/kg/day), dicloxacillin (25 mg/kg/day), or cephalexin (25 to 50 mg/kg/day) is recommended. Augmentin, the fixed combination of amoxicillin and clavulanic acid, provides good activity against methicillin-susceptible staphylococci and streptococci and has an expanded spectrum of activity against the oral anaerobic organisms. These features, combined with its palatability, render it an attractive alternative to the traditional penicillinase-resistant penicillins. Clavulanate-associated diarrhea can be problematic in some children, however. In penicillin-allergic patients, cephalosporins may be used. Once-daily ceftriaxone (50 to 100 mg/kg/day) is an attractive and effective alternative to the parenteral antibiotics that require more frequent administration.

In areas where CA-MRSA is prevalent, clindamycin (30 mg/kg/day) for parenteral or oral use is appropriate for empiric or alternative therapy. In addition to its good activity against anaerobes and methicillin-susceptible *S. aureus*, clindamycin is effective against most CA-MRSA isolates. Because of reports that clindamycin resistance is increasing among CA-MRSA isolates, however, close clinical follow-up to ensure a therapeutic response has been achieved is mandatory when clindamycin therapy is chosen.[42,59,61,63,99] In a severely ill child needing hospitalization or with signs of airway compromise, vancomycin (45 mg/kg/day) is appropriate in combination with another agent until culture results are obtained. Trimethoprim-sulfamethoxazole (10 mg/kg/day of the trimethoprim component) is an alternative choice for oral therapy of CA-MRSA infections[42,59,61,99] but should not be used initially because it is not active against group A streptococci.

Antibiotic therapy may need to be modified if an obvious primary focus of infection suggests a different etiologic agent. In a patient with periodontal or dental disease, adequate anaerobic activity is mandatory, and therapy with penicillin V (50 mg/kg/day), augmentin (40 mg/kg/day), or clindamycin (30 mg/kg/day) should be initiated, pending results of cultures.

Patients with marked lymph node enlargement, moderate to severe systemic symptoms, or concomitant cellulitis frequently require parenteral therapy for the first few days. This therapy allows for a high concentration of the antimicrobial agent within the inflamed tissue and may promote more rapid localization, especially in patients with staphylococcal adenitis. Although the use of parenteral drugs has to be individualized, most infants and children with staphylococcal or streptococcal lymphadenitis respond to orally administered antimicrobials.

Adenitis caused by group A streptococci should be treated with penicillin G (100,000 IU/kg/day) or penicillin V (50 mg/kg/day) for a total of 10 days. In a child with penicillin allergy, erythromycin ethyl succinate (40 mg/kg/day) or cephalexin (25 to 50 mg/kg/day) may be used. Both drugs have been shown to be effective in the treatment of cervical lymphadenitis.[13,22] Treatment should be continued for at least 10 days or approximately 5 days after signs of local inflammation and systemic toxicity have disappeared, whichever is longer. If required, analgesics should be given and not overlooked in infants and children too young to verbalize their discomfort. The average duration of antibiotic therapy is 10 days, unless abscess formation occurs late in the first or early in the second week of treatment.[13] In this situation, incision and drainage are indicated,[13,23,35] and therapy should be continued until resolution of the acute process occurs, usually within another 5 to 7 days.

Some clinical improvement is to be expected within 48 hours after initiation of therapy and is manifested by a decrease in inflammation and tenderness of the lymph node and a decrease in the maximum daily temperature. The size of the lymph node may not show evidence of regression at this stage, and total resolution of fever should not be expected. It is important to record accurate measurements of the node at the time of presentation because a subjective evaluation is an unreliable indicator of lymph node evolution during therapy. If no clinical improvement is noted by 48 hours, needle aspiration is recommended. The history and physical examination should be reassessed, and a more detailed laboratory evaluation should be initiated.

In a study of 284 children admitted to the hospital with acute cervical adenitis, age younger than 1 year and node involvement for more than 48 hours before admission predicted the need for node aspiration.[81] Regression of the size of a lymph node is slow, usually requiring 4 to 6 weeks or more. Persistence of significant enlargement beyond 6 to 8 weeks, even in the face of good initial response to antimicrobial therapy, demands that an underlying disorder be excluded. When signs of acute inflammation have resolved, prolonged antimicrobial therapy is of little value because penetration of antimicrobials through the fibrous capsule of the node is poor.[22] Spontaneous regression occurs in most patients, although it may require several weeks. Uncommonly, reactivation of inflammation may occur, and a meticulous search for an untreated primary source of bacterial infection, such as secondarily infected dermatitis, infestation, foreign body, or dental abscess, should be undertaken. Re-treatment should include specific measures to eliminate the predisposing condition.

If Gram stain of the lymph node aspirate suggests a microorganism other than *S. aureus* or group A streptococci, initial antimicrobial therapy should be directed at the most likely agent until culture results are known. Because attempts to perform careful Gram stains and careful anaerobic cultures of lymph node aspirates in most reported series have been limited, the large number

of infants and children with sterile aspirates may be attributed partly to a failure to isolate fastidious anaerobes indigenous to the mouth. These microorganisms should respond to penicillin G therapy. For penicillin-resistant organisms, clindamycin is a useful alternative drug.

For infants in the first 2 months of life, group B streptococci and *S. aureus* are important pathogens to consider in selecting initial therapy. Penicillinase-resistant penicillins are active against both agents, unless the occurrence of CA-MRSA is frequent, in which case clindamycin or vancomycin should be considered for initial therapy. If group B streptococci are isolated, penicillin G can be substituted. Final bacteriologic identification and antimicrobial susceptibility tests should be the ultimate guide to selecting specific antimicrobial therapy in all patients. Treatment of cervical lymph node infections associated with rarely encountered bacteria, fungi, and parasites listed in Table 15–3 is discussed under those specific disease entities.

Although controversy exists as to whether cervical adenitis associated with *M. tuberculosis* in a child is truly a localized process, only rarely do patients have disseminated infection.[36,51,106] When infection is not localized, pulmonary or hilar lymph node involvement is a common finding.[36,65,107,159] A 2-month regimen of isoniazid (10 mg/kg/day), rifampin (15 to 20 mg/kg/day), pyrazinamide (30 mg/kg/day), and ethambutol (15 mg/kg/day) is recommended for the treatment of uncomplicated pulmonary tuberculosis or isolated cervical lymphadenitis in children. Triple therapy is given daily for the first 2 months of therapy, after which isoniazid and rifampin are administered, either daily or on a twice-weekly basis, for the ensuing 4 months. Directly observed therapy is preferred.[6]

In areas where multiple-drug resistance in *M. tuberculosis* is prevalent, streptomycin (20 to 40 mg/kg/day) or another aminoglycoside (kanamycin, amikacin, or capreomycin) is added for initial treatment until drug susceptibilities are known.[6] The addition of a fifth drug should occur after consultation with an expert in the field because these drugs may have toxic effects, and careful assessment of the risks and benefits is warranted. Detailed discussion of the treatment of tuberculosis in children is provided in Chapter 107. Response to antituberculous therapy is usual, with rapid resolution of symptoms and marked regression of lymph nodes within 3 months. Nodes remain palpable for months, however, because scarring and fibrosis are regular accompaniments to resolution of disease. Draining sinuses, a common complication of lymph node aspiration or incision, and drainage before the advent of effective antituberculous chemotherapy no longer develop.[107]

Cervical lymphadenitis attributed to nontuberculous mycobacteria is much more common in a young child than that caused by *M. tuberculosis.* These microorganisms exhibit in vitro resistance to commonly employed antituberculous drugs. Resistance particularly is common among *M. scrofulaceum* and *M. intracellulare*. Surgical excision is the treatment of choice for nontuberculous mycobacterial lymphadenitis,* and total removal of all the visibly affected nodes is recommended.[4,8,30,56,112,114,120,126,155] Early (within 1 month of onset) removal of affected nodes was associated significantly with better esthetic results in one series.[83] Thorough curettage has been found to be effective,[84,106] but results in higher relapse rates.[43]

Antimicrobial therapy alone is less effective.[90] The new macrolides clarithromycin and azithromycin, rifampin and its analogue rifabutin, ethambutol, amikacin, and cefoxitin show activity against nontuberculous mycobacteria.[38,143] Clarithromycin monotherapy and combination therapy with clarithromycin and ethambutol or rifampin have had some success in the treatment of nontuberculous lymphadenitis.[17,50,58,79,80,84,145] In the largest retrospective case series, 22 children received clarithromycin either alone or in combination with other antimycobacterial agents without surgery, and only one recurrence was documented.[80]

In another study, 10 children (7 of whom had initial incision and drainage or aspiration) were treated for 1 to 14 months with a regimen of clarithromycin (20 to 30 mg/kg/day) in combination with ethambutol (12.5 to 19 mg/kg/day) or rifampin (6 to 20 mg/kg/day). Five children were cured; the remainder needed further surgical procedures.[58] Six of seven patients treated for 6 months in another series had not relapsed after a mean follow-up of 3 years.[79] Rifabutin was used in all children in this study, resulting in side effects in four (neutropenia, yellow skin pigmentation), which disappeared after dose reduction. In HIV-infected children receiving protease inhibitors, rifabutin generally is contraindicated, but it can be given at a much reduced dosage (indinavir or nelfinavir is the preferred protease inhibitor) if deemed necessary.[8]

Despite the lack of prospective studies, macrolide-containing regimens are promising options for the treatment of nontuberculous mycobacterial adenitis for which complete excision of the affected node would endanger the facial nerve or its branches, or if a reduction in size of the swelling would facilitate a complete and esthetic excision at a later stage. The optimal duration of therapy is unknown, but regimens of 4 to 6 months or longer are usual. Expert opinion should be sought, especially as new mycobacterial species are described. Currently, macrolide (clarithromycin or azithromycin) monotherapy or combination therapy should be viewed as a valuable adjunct when surgery is not feasible or is refused.

Cat-scratch disease usually is a benign, self-limited disorder requiring no specific therapy. The use of antimicrobials is controversial, but rifampin, trimethoprim-sulfamethoxazole, azithromycin, ciprofloxacin, and parenteral gentamicin may be useful in promoting fever defervescence and in clinical resolution of systemic cat-scratch disease.[7,9,14,89] If the lymph node progresses to fluctuance, needle aspiration may hasten resolution and relieve discomfort. Surgical excision may be required in a few patients who have persistent problems despite having needle aspiration, or who develop draining sinuses.

PROGNOSIS

With effective antimicrobial therapy, complete resolution of cervical lymphadenitis caused by *S. aureus*, group A streptococci, and *M. tuberculosis* is the rule. Delay in establishing the diagnosis or initiating therapy may prolong the clinical course and may result in complications or sequelae, such as sinus tracts (mycobacteria),[19,33,107] abscess formation,[23] cellulitis or bacteremia (*S. aureus* and *S. pyogenes*),[13] acute glomerulonephritis (group A streptococci),[35] disseminated disease (*M. tuberculosis*),[65] or mycotic carotid artery aneurysm.[154] Except for abscess formation, these complications are rare events. Although lymph node infection caused by *S. aureus* is more likely to result in abscess formation, at least one study has noted a significantly greater duration of infection before treatment in patients in whom *S. aureus* was isolated from the abscess cavity cultures.[13] The extracellular products of this organism (e.g., coagulase, fibrinolysin, hyaluronidase) partly explain its propensity for abscess formation, which may occur in 50 to 70 percent of patients.[23,35,157]

Even in patients whose course is complicated by suppuration, appropriate drainage in conjunction with specific antimicrobial therapy results in prompt resolution of signs and symptoms, and relapse occurs only rarely. Today, surgical excision of affected nodes seldom is recommended except when the disease is caused by nontuberculous mycobacteria, in which case surgical excision remains the treatment of choice. Antimicrobial therapy has been

*See references 8, 38, 43, 83, 88, 112, 114, 120, 137, 142.

responsible for the disappearance of the events commonly associated with cervical adenitis historically, including thrombosis of the internal jugular vein, rupture of the carotid artery, generalized septic embolic phenomena, mediastinal abscess, purulent pericarditis, and even death.[68,140,157]

With the advent of effective antituberculous agents, the prognosis for tuberculous cervical adenitis also is excellent. When surgical excision is performed early in the course of lymphadenitis caused by nontuberculous mycobacterial infection, resolution can be anticipated.[43,83,112,120,126,137] Persistent and recurrent disease is the most frequent complication encountered.[155] Macrolide monotherapy and combination therapy are useful in ameliorating these complications or in cases for which surgery is not feasible.[114,137] Cat-scratch disease usually is a benign, self-limited disorder only rarely requiring therapeutic intervention, such as needle aspiration, to relieve pain.

PREVENTION

Providing appropriate medical and, occasionally, surgical therapy of predisposing conditions (e.g., dental caries, abscess, group A streptococcal pharyngitis or nasopharyngitis, purulent otitis media, impetigo, other infections involving the face and scalp) and minimizing the exposure of infants and children to adults with active tuberculosis should reduce the incidence of cervical lymphadenitis. Some authors suggest that decreased exposure to animals may result in fewer infections,[13] especially for adenitis attributed to toxoplasmosis or *Bartonella*.[29,121]

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CHAPTER 16

Parotitis

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Parotitis, inflammation of the parotid gland, is caused by a variety of infectious agents and noninfectious systemic illnesses. Several terms are used to describe the clinical presentations and etiologic processes that lead to parotid gland swelling and inflammation. *Suppurative parotitis*, first described in the 1800s, is a serious bacterial infection in neonates and postsurgical patients.[49] *Epidemic parotitis*, particularly prevalent in the pre-vaccine era, was caused primarily by mumps virus infection.[56] In the postvaccine era, this form of parotitis also is caused by other viral pathogens and is referred to as *viral parotitis*. Rarely, a more indolent, slowly progressive, granulomatous infection may occur that is referred to as *granulomatous parotitis*. *Recurrent parotitis of childhood* is a unique illness that is characterized by multiple episodes of acute and subacute parotid gland swelling. The histologic findings in

TABLE 16–1 Predisposing Factors for Parotitis

Drug-Induced Xerostomia
Anticholinergics
Antihistamines
Antidepressants
Phenothiazines
Beta blockers
Diuretics
General anesthesia
Disease-Related Xerostomia
Sjögren syndrome
Diabetes mellitus
Chronic liver disease
Cystic fibrosis
Obstruction
Dental appliances
Oral tumors
Radiation therapy
Trauma

this disease include architectural changes in the ducts and chronic inflammation. Many noninfectious systemic illnesses cause persistent or recurrent parotid gland swelling and inflammation, which is referred to as *chronic parotitis*. Bilateral parotid enlargement is a frequent presentation of human immunodeficiency virus (HIV) infection as part of *diffuse infiltrative lymphocytosis syndrome*.

PATHOPHYSIOLOGY

Despite the various agents that cause parotitis, involvement of the gland occurs mainly by three mechanisms. The most common mechanism is a localized infection limited to the gland and surrounding structures. Parotitis may be a manifestation of a systemic infection, as in mumps, or rarely may develop secondary to hematogenous seeding during periods of transient bacteremia. Several common contributing factors and pathophysiologic mechanisms lead to swelling of the gland. The parotid is well encapsulated and consists of superficial and deep lobes that are separated by the facial nerve. The parotid duct (Stensen duct) traverses the buccal soft tissue anteriorly and exits opposite the second upper molar. Thin, watery secretions from the parotid gland cleanse the ductal system and have some bacteriostatic properties, preventing accumulation of bacteria and debris.[36] Factors that predispose individuals to the development of parotitis include side effects of certain drugs and diseases that lead to dehydration, xerostomia, or ductal obstruction (Table 16–1).[36,49] Decreased salivary flow allows retrograde migration of bacteria. Stasis in the ductal system, caused by ductal ectasia, inflammation, calculi, or strictures, allows proliferation of bacteria and inflammation within the gland.

ETIOLOGY

Infectious parotitis may be caused by aerobes, anaerobes, mycobacteria, and viruses (Table 16–2). In all age groups, *Staphylococcus aureus* is the organism most commonly associated with suppurative parotitis.[47,49] Gram-negative pathogens (e.g., *Escherichia coli* and *Klebsiella* and *Pseudomonas* spp.) also may cause suppurative parotitis, particularly in neonates and debilitated or hospitalized patients.[11,31,48,49] The role of anaerobic organisms in this infection has become apparent, especially when poor oral hygiene and oral pathology are associated features.[4,5,39] In cases of recurrent

TABLE 16–2 Reported Infectious Etiologies of Parotitis

Aerobic Bacteria
Staphylococcus aureus
Streptococcus pneumoniae
Streptococcus pyogenes
Viridans streptococci
Francisella tularensis
Haemophilus spp.
Moraxella catarrhalis
Pseudomonas aeruginosa
Escherichia coli
Proteus spp.
Salmonella spp.
Klebsiella spp.
Brucella spp.
Anaerobic Bacteria
Peptostreptococcus spp.
Prevotella spp.
Fusobacterium spp.
Actinomyces spp.
Mycobacteria
Mycobacterium tuberculosis
Mycobacterium avium–intracellulare
Other mycobacteria
Viruses
Mumps
Coxsackieviruses A and B
Echoviruses
Epstein-Barr virus
Influenza A
Parainfluenza viruses 1 and 3
Cytomegalovirus
Herpes simplex virus type 1
Human herpesvirus 6
Lymphocytic choriomeningitis virus
Human immunodeficiency virus

parotitis of childhood, *Streptococcus* spp. are the bacteria most commonly isolated.[21,42,46] Granulomatous parotitis most often is caused by *Mycobacterium tuberculosis* and may occur in the absence of systemic or disseminated tuberculous disease.[36,44,52] Other causes of granulomatous parotitis include *Mycobacterium avium–intracellulare*, *Actinomyces* spp., and gram-negative intracellular organisms (*Francisella tularensis* and *Brucella* spp.).[25,36,59]

In the postvaccine era, the most common viral cause of parotitis still is the paramyxovirus mumps virus. Coxsackieviruses, Epstein-Barr virus, influenza A virus, parainfluenza viruses, adenovirus, human herpes virus–6, herpes simplex virus, cytomegalovirus, and lymphocytic choriomeningitis virus all have been implicated in cases of parotitis.[1,2,16,28,30,32,35,36]

CLINICAL PRESENTATION AND DIAGNOSIS

A detailed history and physical examination are crucial in assisting the clinician in determining the most likely etiology of parotid gland swelling. One should determine the onset and duration of symptoms, their periodicity, and the character of salivary secretions. In addition, the presence of a systemic disease must be excluded. Examination of the parotid gland is achieved best by simultaneous palpation of the intraoral and extraoral salivary structures. Gentle external pressure should be applied to the gland, and the parotid duct should be examined for evidence of purulent secretions or surrounding erythema.

Suppurative parotitis occurs most commonly in neonates or patients with dehydration, poor oral hygiene, malnutrition, immunosuppression, oral trauma, sepsis, or any medication or

disease that decreases salivary secretions.[18] Most often, the disease is unilateral; however, bilateral suppurative parotitis may occur in 17 percent of cases.[42] The disease is characterized by acute onset of pain, swelling, warmth, and induration of the involved gland and purulent discharge from the Stensen duct. Associated physical findings include fever, trismus, malaise, and cervical adenitis. In suppurative parotitis, Gram stain and culture (aerobic and anaerobic) of purulent material from the duct can provide a specific microbiologic diagnosis. In addition, elevation of the white blood cell count with a neutrophil predominance may help differentiate this form of parotitis from viral parotitis and parotid disease of a noninfectious etiology.

Mumps is the most common form of viral parotitis and is characterized by a prodrome of fever, malaise, anorexia, and headache. Usually, the following day, unilateral or bilateral earache and parotid tenderness develop. The gland or glands enlarge during the subsequent 2 to 3 days, and the orifice of the Stensen duct is erythematous and swollen, yet secretions from the duct are clear. At the point of maximal swelling, the angle of the jaw is obliterated, and the earlobe is lifted upward and out. The other salivary glands are involved in 10 percent of cases.[42] Rare systemic manifestations of mumps infection include epididymo-orchitis, meningitis, meningoencephalitis, and oophoritis. More recent outbreaks of confirmed and probable mumps emphasize that this infection may occur in highly immunized populations.[6] In 2006, more than 5000 cases of probable mumps were reported to the Centers for Disease Control and Prevention. Of these, 54 percent were laboratory confirmed or linked epidemiologically to a confirmed case. Although the median age was 22 years, patients ranged in age from 1 month to 96 years. Most cases were from the Midwest (Iowa, Kansas, Wisconsin, Illinois, Nebraska, and South Dakota), and many of the patients had received two doses of measles-mumps-rubella (MMR) vaccine.[6]

Other viral agents may produce similar clinical manifestations and can be differentiated from mumps only by culture and hemagglutination inhibition, complement fixation, or enzyme-linked immunosorbent assay serology.[16] In viral parotitis, the white blood cell count may be normal, slightly elevated, or depressed, with a lymphocytic predominance.

Granulomatous parotitis typically manifests as a painless, slowly enlarging mass without surrounding inflammation. It may be misdiagnosed as a slow-growing tumor until the correct diagnosis often is made by biopsy and culture. *M. tuberculosis* and *M. avium–intracellulare* may cause infection in the parenchyma of the gland or in intraglandular or periglandular lymph nodes.[43,59] Clinical evidence of systemic tuberculous disease usually is absent. Parotitis has been observed as an extension of nontuberculous cervical adenitis.[59] Actinomycosis of the parotid gland causes a slowly enlarging, nodular, nontender gland; associated oral or cervicofacial infection usually is present. Fistulas draining yellow or white material with sulfur granules are common findings.[25]

Recurrent parotitis of childhood is rare, with onset that typically occurs before the child reaches 10 years of age and a peak incidence at approximately 6 years of age.[17,42] Some authors hypothesize that an underlying congenital abnormality, such as sialectasis, is a common predisposing feature.[33,43] Others suggest that selective IgA deficiency may be a contributing variable.[19] Clinically, these children experience repeated episodes of fever, pain, and unilateral swelling of the parotid gland. Purulent material often can be expressed from the Stensen duct and, when cultured, often yields streptococcal organisms. Sialography and ultrasound reveal multiple areas of sialectasis throughout the parotid glands bilaterally, even if only one side is symptomatic. The frequency of attacks varies, and each episode of parotitis may last 2 weeks, when it resolves spontaneously.[10] Several authors have noted that recurrences become less frequent with increasing age and that the disease tends to cease at the onset of puberty or early adulthood.[17,42]

HUMAN IMMUNODEFICIENCY VIRUS AND PAROTID ENLARGEMENT

Salivary gland enlargement has been recognized as a common finding in children infected with HIV since before the era of highly active antiretroviral therapy. The prevalence of this manifestation in HIV-infected individuals is 0 to 58 percent.[46] More recently, in a retrospective report of oral lesions in a cohort of HIV-infected children from Brazil, Miziara and colleagues[40] noted the prevalence of parotid gland enlargement to be 7.6 percent. Although parotid enlargement occurred more commonly in children older than 5 years of age, the prevalence did not differ between children who were receiving antiretroviral therapy without protease inhibitors and children who were receiving highly active antiretroviral therapy.

The exact pathophysiology is unknown, but proposed mechanisms include lymphoepithelial cysts, lymph node enlargement within the gland, cytomegalovirus or Epstein-Barr virus infection, and diffuse infiltrative lymphocytosis syndrome of the gland.[46] This entity in HIV-positive children possibly is associated with the HLA-DR5 and HLA-DR11 phenotype, but the significance of this finding is unclear.[27,46,55] Bilateral parotid enlargement frequently is seen as part of diffuse infiltrative lymphocytosis syndrome, which is characterized by proliferation of $CD8^+$ lymphocytes within the circulation and is of unclear etiology. Growth of the parotid is secondary to infiltration of CD8 lymphocytes into the gland, follicular hyperplasia of intraparotid lymphoid tissue (as occurs in lymph nodes throughout the body in HIV infection), and development of diffuse intraparotid, lymphoepithelial cysts.[8,37] Epstein-Barr virus has been proposed as an impetus for CD8 lymphoproliferation because the virus has been isolated from parotid tissue of some affected patients.[9] The absence of positive serology for the virus renders it an unlikely causative agent, however. HIV, which may be the inciting agent, has been detected in dendritic cells, macrophages, and lymphocytes isolated from the parotid glands of affected patients.[9]

Parotid enlargement may be present in 20 to 50 percent of children with HIV infection and acquired immunodeficiency syndrome (AIDS).[29,33,54,57] The median time from birth to development of parotid enlargement is 4.6 years, and often it is the first manifestation of HIV infection acquired during the perinatal period in an otherwise healthy older child.[27,29] HIV-positive children with enlarged parotid glands tend to have a slower progression to death than do HIV-positive children with oral herpes or candidiasis.[29] Usually, both parotid glands are involved, and an affected patient presents with enlarged, tender parotid glands, xerostomia, and increased serum amylase level.[7] Severity of pain and size of the gland tend to fluctuate without apparent cause. This manifestation of HIV infection and AIDS is still commonly seen in developing countries where access to antiretroviral therapy is limited.

The differential diagnoses for parotid enlargement in an HIV-positive patient include viral and bacterial parotitis. Some patients have preexisting xerostomia that may increase their susceptibility to parotitis. In addition, the immunocompromised state of patients with AIDS may predispose them to development of infection of the parotid gland with other agents, such as cytomegalovirus, Epstein-Barr virus, bacteria, mycobacteria, and fungi.[23,51,58,61] Noninfectious etiologies, such as non-Hodgkin lymphoma or Kaposi sarcoma, also should be included in the differential diagnosis of parotid gland enlargement in an HIV-infected patient, although these manifestations usually are observed in adults.

DIFFERENTIAL DIAGNOSIS

Parotitis most often is diagnosed based on clinical presentation, microbiology, serology, and response to empiric therapy. Ultrasound may be useful as a screening tool to prompt more sensitive modalities if the ultrasound scan is abnormal.[41] Computed tomography is most useful in the presence of anatomic defects, radiolucent calculi, or abscess formation in the parotid gland.[50] X-ray sialography is the gold standard in examining the parotid gland ducts; however, sialography is contraindicated in the setting of acute infection. Magnetic resonance sialography is a promising alternative and has several advantages. In contrast to x-ray sialography, magnetic resonance sialography is not contraindicated during acute parotitis and does not require injection of contrast material or involve manipulation of the Stensen duct.[20] Nonetheless, experience with magnetic resonance sialography is limited, and this mode alone may not be sufficiently sensitive to detect tertiary salivary ductules or calculus disease.[20,61]

Noninfectious causes of parotid swelling and inflammation include collagen vascular diseases (Sjögren syndrome and systemic lupus erythematosus), metabolic disorders (hepatic disease, hyperlipoproteinemia, hyperuricemia), endocrine disorders (diabetes mellitus, hypothyroidism), tumors, leukemic infiltration, drugs (antineoplastic chemotherapy), and poisons (iodine).[36,42,47] Sjögren syndrome, the most common cause of noninfectious parotitis, is caused by lymphocyte-mediated destruction of the exocrine glands.[24,47] Patients with this disease have diminished or absent glandular secretions and mucosal dryness; xerostomia and keratoconjunctivitis sicca are prominent clinical features. In addition, the parotid glands are enlarged bilaterally, are firm, and have an irregular contour. Sialography reveals sialectasia, and saliva from these patients has unique biochemical characteristics. Antibodies to nuclear antigens SS-A and SS-B can be detected in the sera of patients with Sjögren syndrome.[47] Patients with chronic noninfectious parotitis have changes in the ductular architecture or strictures that can predispose them to episodes of infectious parotitis.

TREATMENT

Treatment of parotitis includes rehydration, parotid massage, discontinuation of any medications that diminish salivary flow, and sialalogues (e.g., lemon drops, hard candy, chewing gum), which increase salivary flow.[3,36,47,49] In cases of suspected suppurative parotitis, a broad-spectrum antibiotic regimen that is effective against *S. aureus*, *Streptococcus* spp., gram-negative organisms, and anaerobes should be administered empirically, pending specific culture results. Antibiotic regimens frequently employed include penicillinase-resistant penicillins, first-generation cephalosporins, and clindamycin in combination with an aminoglycoside.[3] Vancomycin should be used if MRSA is the likely pathogen. If the patient has been hospitalized for a prolonged period, or if the predominant organisms on Gram stain of the purulent discharge are gram-negative, ceftazidime should be considered as initial empiric therapy.[47,49]

Surgical incision and drainage of purulent fluid are indicated if there is slow or no response to medical therapy or fluctuant increases.[53] The treatment of viral parotitis consists of antipyretics, analgesia, and hydration. In cases of mycobacterial infection, excision of the gland may be required, in addition to administration of specific antimycobacterial therapy.[44,52] Reports have described successful treatment with clarithromycin and azithromycin of parotitis caused by atypical mycobacteria.[22] In contrast, actinomycosis of the parotid gland is managed medically with penicillin G.[25] Children with recurrent parotitis should be treated with antibiotics during acute episodes, but chronic suppressive antimicrobial therapy is not recommended.

Tympanic neurectomy involves severing the parasympathetic secretomotor fibers of the tympanic plexus to the parotid. This procedure attenuates secretion from the gland and relieves sialectasis and further episodes of parotitis in more than 70 percent of patients.[15,45] Only 10 to 20 percent of these patients require parotidectomy for persistence of symptoms beyond puberty.[13] Although it is the optimal treatment for complete resolution of recurrent parotitis, parotidectomy carries a risk for facial nerve injury. More recently, Nahlieli and colleagues[43] described the use of endoscopy to diagnose and endoscopic irrigation to treat children with recurrent parotitis.

COMPLICATIONS

With improved fluid management of postsurgical patients and the use of broad-spectrum antimicrobial agents, complications secondary to infectious parotitis now are rare events. In neonates or immunocompromised patients, sepsis may be a severe complication of this infection. Abscess formation may result from delayed or ineffective therapy. Compromise of the facial nerve may occur and can resolve with successful treatment of the infected gland.[38] The most serious and rare complication is extension to other structures of the head and neck and along fascial planes to the face, external auditory canal, jugular vein, mandible, and mediastinum.

PREVENTION

Suppurative parotitis can be prevented in postsurgical patients by maintaining adequate hydration and good oral hygiene. The most common form of viral parotitis, mumps, can be prevented by appropriate vaccination. Between 1968 and 1993, a 99 percent reduction in the incidence of new cases of mumps occurred.[60] In the mid-1980s and more recently in 2006, a resurgence of the incidence of mumps in previously vaccinated populations was noted.[6,12,26] The most recent outbreaks occurred among college students and young adults in several states, raising concern of waning immunity in this highly vaccinated population.[6] The current recommendation is to provide two doses of live mumps vaccine for school-age children (i.e., grades kindergarten through 12) with MMR vaccine and to ensure that students in college or other post–high school educational institutions also have received two doses of MMR vaccine.[6,14]

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CHAPTER 17

SINUSITIS

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Sinusitis is an inflammation of the mucosal lining of one or more of the paranasal sinuses. Although this inflammation of sinus mucosa most probably occurs to some degree with every upper respiratory tract infection that produces rhinitis, most of these episodes apparently have a spontaneous resolution.[94] Studies during the last 2 decades estimate that 5 to 10 percent of upper respiratory infections are complicated by acute sinusitis.[2,167,184] This rate range represents a significant increase from earlier reports,[36] possibly because of a greater awareness of the illness and improved imaging techniques. The growing number of

children in daycare has led to an actual increase in the incidence of upper respiratory tract infections.[182,184] In addition, recognition that sinus infection can have a negative effect on the health of children with chronic pulmonary disease has increased the interest in this disease.[108]

When considering a diagnosis of sinusitis in a child, the major problem is to distinguish simple upper respiratory tract infection or allergic inflammation from secondary bacterial infection of the sinuses. Unless complications such as periorbital cellulitis or cavernous sinus thrombosis render the diagnosis obvious, the clinician has no reliable way to establish a diagnosis of acute sinusitis in the office setting. Sometimes, symptoms and signs of sinusitis occur simultaneously with rhinitis, but most often, they occur after an episode of rhinitis. Infection in the sinuses usually persists after the preceding rhinitis has resolved. Sinusitis is classified by the duration of clinical symptoms: acute (≤3 weeks), subacute (3 to 10 weeks), and chronic (>10 weeks). Available data comparing acute and subacute sinusitis are sparse; both entities may have a similar etiology, diagnosis, and prognosis; the distinction seems to be arbitrary and to have no clinical significance.

HISTORY

Purulent sinusitis and its relationship to orbital inflammation have been known for more than 2000 years.[71] Highmore, a 17th-century English physician and anatomist, is given credit for the separation of dental and antral disease.[130] Hunter indicated the importance of surgical drainage in purulent sinusitis and suggested perforating the partition between the maxillary antrum and the nose.[130] During the first half of the 20th century, sinusitis was responsible for considerable morbidity and mortality, and surgical care of sinusitis frequently was lifesaving. Since the advent of antibiotics, sinusitis has had a lower medical profile. Interest in this topic has increased, however. Some factors involved in this increased interest include the advent of the newer surgical techniques of functional endoscopic sinus surgery that yield comparable results to sinus puncture and aspiration,[163] which now have been applied to children; improved diagnostic imaging studies; and the greater social importance of upper respiratory tract infections for parents who must be absent from work to seek treatment for their children.[108]

ANATOMY

All the paranasal sinuses develop as outpouchings of the nasal cavity. Three shelflike structures—the inferior, middle, and superior turbinates—are on the lateral nasal wall. The superior turbinate is not well developed in the first year of life.[194] Beneath each turbinate is the corresponding meatus into which the sinuses open; that is, the frontal, maxillary, and anterior ethmoid sinuses open into the middle meatus. The sphenoid and posterior ethmoid cells open high in the nasal vault into the superior meatus.[175]

The maxillary sinuses develop early in the second trimester of fetal life as lateral outpouchings in the posterior aspect of the middle meatus. They are present at birth,[6,115,146,194] with floors being barely below the attachment of the inferior turbinates.[175] They expand rapidly by the time the child is 4 years of age.[194] Ultimately, at full size, the lateral borders of the maxillary sinuses reach the lateral orbital rims. The position of the floors of the sinuses is determined by the eruption of the dentition.[175] The ostia of the maxillary sinuses are located high on the medial walls of the sinuses, which impedes gravitational drainage of secretions; ciliary activity is required to move secretions from the body of the maxillary sinuses through the ostia into the nose.[175]

The ethmoid sinuses develop in the fourth month of gestation[175] and are present at birth.[115,146,194] They are not a single large cavity but a grouping of cells, 3 to 15 in number, each with its own opening or ostium. They have a honeycombed radiographic appearance and are small anteriorly and large posteriorly. The walls of the ethmoid labyrinth, especially the lateral walls bordering on the orbits, are thin; they are referred to as the *lamina papyracea*.[175]

Development of the frontal sinuses is variable. In adults, 80 percent have bilateral frontal sinuses, 1 to 4 percent have agenesis of the frontal sinuses, and the remainder have unilateral hypoplasia. The position of the frontal sinuses is supraorbital after the child reaches 4 years of age, but they are not distinguished radiographically from the ethmoid sinuses until the child is 6 to 8 years old. The frontal sinuses do not reach adult size for another 8 to 10 years.[175]

The onset of development of the sphenoid sinuses occurs during the child's first 2 years of life, but they remain rudimentary until the child is approximately 6 years of age. They have reached their permanent size, although not their permanent shape, by the time the child is 12 years old.[194]

Although the full development of the sinuses may take 20 years, by the time the child is 12 years old, the nasal cavity and the paranasal sinuses nearly have completed their development and have reached adult proportions.[194] Sinus disease in postpubertal adolescents is similar to that in adults.

The mucosal lining of all the paranasal sinuses is composed of ciliated columnar epithelium and goblet cells.[149] It is continuous and similar to the lining of the nasal cavity except that the mucosa in the nose is thicker and contains more glands. The epithelium of all the paranasal sinuses and nasal cavity is covered in part by a mucus blanket.

PATHOPHYSIOLOGY

The pathogenesis of sinus infection undoubtedly is similar to that of otitis media. The middle ear, with its extension, the eustachian tube, and the paranasal sinuses normally are sterile, but their contiguous areas (nasopharynx and nose) have a dynamic microbial flora. Under normal conditions, ciliary function with mucus flow can be expected to keep the sinuses clear of pathogens. The cilia within the sinuses propel the mucus toward their respective ostia, and from there, nasal ciliary action moves the mucus blanket posteriorly toward the pharynx. Insults that damage the ciliary epithelium and affect the morphology, number, and function of cilia and insults that alter the production or viscosity of the mucus blanket lead to obstruction of the flow of mucus, however, which allows the inoculation of numerous microorganisms into the sinuses that can lead to infection. In a study in adults in whom sneezing, coughing, and nose blowing were stimulated or initiated voluntarily, intranasal pressures were measured, and the deposition of contrast medium (which before the initiation of the event had been inoculated into the nasopharynx) was determined by computed tomography (CT) scan.[66] Results of this study showed that nose blowing introduced viscous fluid into the maxillary sinuses, whereas coughing or sneezing did not generate enough pressure to propel fluid into the sinuses. When instituted, sinus infection is complicated further by inflammatory obstruction of the ostium leading to the nose.

Recurrent chronic sinusitis implies a problem with local mucociliary defense, a defect in systemic immunity, or a fixed anatomic sinus obstruction. Often, the predisposing factors work in tandem, as in a child with a septal deformity and a viral illness.[108] In chronic sinusitis, the mucosa is thickened, and marked edema, vessel dilation, and infiltration of inflammatory cells are present.[166] Goblet cells are decreased in density, and seromucous glands are increased in density compared with their

presence in normal sinuses. The most important factor leading to purulent sinus infection in children and in adults is upper respiratory viral infection.[20,44,108,184]

Wald and colleagues[184] in a large prospective study involving children younger than 3 years of age showed a doubling of the rate of sinusitis (defined as upper respiratory symptoms persisting >15 days) among children in a daycare setting compared with children not in daycare. The differences presumably were due to increased exposure to viral respiratory illnesses. Radiographic studies in children with acute colds regularly indicate abnormalities of the maxillary sinuses, suggesting that the infection involves these areas.[111] These asymptomatic sinus opacifications may persist for 2 weeks after the symptoms of the upper respiratory illness have resolved.[41,92,175] Viral infection that involves the sinuses rarely is differentiated from its primary manifestations, such as the common cold, nasopharyngitis, and influenza, and recovery is the rule. If the effect of the viral infection on the mucosal surface is severe, and is associated with the inoculation of one or more pathogenic bacterial agents and obstruction of an ostium, disease occurs.

The mechanisms by which upper respiratory viral infections set the stage for secondary bacterial infection in the sinuses are complex. Using in situ hybridization, rhinovirus RNA was shown inside epithelial cells of maxillary sinus in 50 percent of a small number of adults with acute sinusitis.[128] This finding is remarkable because in experimental rhinovirus infections, only a small percentage of nasal epithelial cells were noted to contain rhinovirus RNA.[7] These differences may reflect only differences in inoculum between experimental and natural infection, but they may indicate heavier infection in the sinuses than in the nose. Symptoms in upper respiratory viral infections are not caused by extensive damage to ciliated nasal epithelium, but rather to aspects of the host response (see Chapter 8).[7,67,120,122,126,131,132,193]

Other irritants can set the stage for sinus infection. Swimming in ocean, lake, or chlorinated pool water can lead to sinus involvement. Drying of the nasal mucosa, which occurs commonly during the winter in cold climates, may be a precipitating factor. Children with respiratory allergies are prone to sinusitis,[53,101,135-137] and allergy probably is the second most prevalent predisposing factor in childhood sinusitis, acting through mucosa congestion and perhaps depressing local and systemic immune responses.[108,151] The treatment of respiratory allergies may contribute to sinusitis because ciliary damage occurring after the administration of nasal decongestants has been shown in organ culture and animal studies.[41,42,116] Richards and colleagues[141] reported a diagnosis of atopy in 62 percent of a selected cohort of pediatric patients who had documented recurrent sinusitis and were referred to allergy clinics in Los Angeles. Dental infections or extractions also can lead to maxillary sinusitis if the tooth root is adjacent to the maxillary sinus floor.[32]

Sudden change in pressure, as with diving or during descent in an airplane, physically can overcome local mucociliary defense mechanisms and lead to the sudden onset of acute sinusitis.[119] Defects of ciliary function, such as those occurring in the immotile cilia syndrome and Kartagener syndrome, predispose a child to chronic sinusitis.[47,78,89,137,146,155] Refractory sinusitis also occurs commonly in children with primary and acquired immunodeficiency diseases.[28,108,117,147,152,168,179] A growing population of immunocompromised children undergoing treatment for malignancies and organ transplantations and young patients with maternally transmitted and blood-transmitted acquired immunodeficiency syndrome constitute another growing population with a potential for developing sinusitis that is difficult to manage. Finally, anatomic obstruction caused by septal deformities, craniofacial anomalies, foreign bodies, adenoidal hypertrophy, or nasal masses or polyps predisposes children to sinusitis. Nasal polyps in young children usually are not caused by allergies and should be an indication for evaluation for cystic fibrosis.[108]

Immunologic mechanisms are important in the pathogenesis of sinus infections, as indicated by the high prevalence of chronic sinus infections in children with immunodeficiencies.[28,108,117,147,152,168] Sinus and nasal mucus contains IgA, IgG, and IgM and lysozymes.[25,145] Secretory IgA, which is produced locally, is the predominant immunoglobulin in nasal mucus.[69] IgG antibodies in nasal mucus result from passive leakage from plasma cells in the epithelium and submucosa and from the serum.[16] Generally, with the patient's increasing age and as a result of previous exposures, these immunoglobulins develop species-specific and type-specific antibodies that block epithelial colonization by specific microorganisms.

Shapiro and associates[152] studied 61 children with refractory sinusitis and found that 34 had abnormal immunologic studies. Abnormal findings included poor response to pneumococcal type 7 antigen after immunization, IgG3 subclass deficiency, low serum IgA or IgG values, and elevated serum IgE values.

ETIOLOGY

Although most studies on the etiology of sinusitis have involved adults, adequate pediatric data are available. The findings in studies of adults can be applied appropriately to adolescents.

Examining the results of anterior nasal cultures from normal subjects and from subjects with respiratory illnesses is important to clear up confusion regarding the make-up of normal flora. During early investigations of the common cold, Shibley and associates[153] noted that in a group of 13 subjects followed for 4 to 9 months, neither *Haemophilus influenzae* nor hemolytic streptococci were obtained from nasal culture when the subjects were well. When the study subjects were ill with colds, *H. influenzae* was recovered from 9 percent of the cultures, and hemolytic streptococci were recovered from 6 percent. In a study of 500 consecutive medical patients, Jacobson and Dick[83] noted in all but two instances that the recovery of pneumococci and hemolytic streptococci from the nose correlated with nasal or sinus disease. Studies in children have disclosed more varied results. Dunlap and Harvey[44,70] recovered *H. influenzae*, pneumococci, and hemolytic streptococci from the noses of normal children with some consistency. These investigators were interested in carriage and spread of organisms, however, and the state of well-being of their subjects was not delineated clearly.

Orobello and colleagues[123] found that cultures of ipsilateral middle meatus correlated well with maxillary (83%) and ethmoid (80%) sinus cultures. Nasopharyngeal cultures correlated less well, however, with only 45 percent and 40 percent of maxillary and ethmoid sinus cultures being similar.

Yang[195] studied children in a day nursery and noted that neither pneumococci nor *H. influenzae* could be recovered from the noses of well children. Hays and Mullard[73] only rarely could find *Streptococcus pneumoniae*, beta-hemolytic streptococci, or *H. influenzae* in nose cultures from normal children. In an extensive study, Box and associates[15] noted pneumococci in nasal specimens of 38 percent of children without respiratory illness, but in only 3 percent was the growth of great magnitude (>100 colonies per plate). In the same study, *Haemophilus* spp. and beta-hemolytic streptococci were recovered from 14 percent and 1 percent of the cultured specimens from noses. In comparison, in the same study, pneumococci and *Haemophilus* spp. were recovered from 57 percent and 25 percent of the cultures of patients with respiratory illness.

In a more recent study involving 49 children with sinusitis, Ilki and associates[81] found a strong correlation with throat cultures positive for *H. influenzae*, *S. pneumoniae*, or *Moraxella catarrhalis*. Similar organisms were recovered from sinus aspirates.

TABLE 17–1 Etiologic Agents in Sinusitis Analyzed by Patient Age and Type of Illness

	Frequency				Age Group (yr)		
	Overall	*Acute*	*Subacute*	*Chronic*	*≤5*	*6-12*	*>12*
Aerobic Bacteria							
Haemophilus influenzae	++++	++++	++++	++++	++++	++++	++++
Streptococcus pneumoniae	++++	++++	++++	++++	++++	++++	++++
Moraxella catarrhalis	+++	+++	++	+	+++	+	++
Staphylococcus aureus	++	+	+	++	++	++	++
Streptococcus pyogenes	++	++	++	++	+	++	++
Alpha-hemolytic and nonhemolytic streptococci	+		+	+			++
Staphylococcus epidermidis	+		+	+		+	++
Alcaligenes spp.	+			+			++
Escherichia coli	+			+			++
Klebsiella pneumoniae	+			+			++
Pseudomonas aeruginosa	+			+			++
Other*	+			+			++
Anaerobic Bacteria							
Peptostreptococcus spp.	++	+	+	+++		+	++
Prevotella and *Porphyromonas* spp.	++			++		+	++
Fusobacterium spp.	++			++			
Propionibacterium spp.	++			++			
Bifidobacterium spp.	+			+			
Bacteroides fragilis	+	+	+	+			+
Veillonella spp.	+	+	+	+			+
Fungi							
Aspergillus spp.	+	+		+	+	++	++
Alternaria spp.	+	+		+	+	+	+
Penicillium spp.	+			+	+	+	+
Curvularia spp.	+		+	+	+	+	+
Drechslera spp.	+			+	+	+	+
Bipolaris spp.	+			+	+	+	+
Mucor spp. and other Zygomycetes	+	+			+	+	+
Mycoplasma* and *Chlamydia							
Mycoplasma pneumoniae	+	+					+
Chlamydia pneumoniae				+	+	+	+
Other							
L-forms	+			+			++
Mixed aerobes and anaerobes	++	+	+	++			++
Mixed *H. influenzae* with other organisms	++	+	+	++		+	++
Rhinovirus, adenovirus	+	+		+		+	+

**Serratia* spp., diphtheroids, *Enterococcus* spp., *Neisseria* spp., *Haemophilus* spp., *Proteus* spp., *Acinetobacter* spp., *Citrobacter* spp., *Eikenella corrodens*, *Arcanobacterium haemolyticum*.*

Data from references 8, 14, 18-20, 23, 28, 31, 43, 45, 50, 51, 60, 86, 87, 90, 93, 103, 107, 125, 137, 172-174, 181, 185, 186.

In a study performed in the Finnish military, nasal cultures from 183 healthy recruits revealed the following frequencies of specific organisms: *H. influenzae*, 4 percent; *S. pneumoniae*, 1 percent; *M. catarrhalis*, 3 percent; and *Streptococcus pyogenes*, 0 percent.[87] In contrast, in 185 recruits with acute maxillary sinusitis, the percentages of nasal isolates for the same organisms were 61 percent, 25 percent, 7 percent, and 6 percent, respectively. In 91 percent of cases in which a sinus aspirate culture yielded an isolate, the same organism was found in a nasal sample. Similar results have been obtained in other studies done in adults with acute sinusitis, in which nontypeable *H. influenzae* and *S. pneumoniae* account for approximately 74 percent of all bacterial strains recovered in sinus aspirates.[68] In all studies, *Staphylococcus aureus* clearly is part of the normal nasal flora. *S. aureus* is present in well or sick children approximately 50 percent of the time.

Pneumococci, *H. influenzae*, *M. catarrhalis*, and *S. pyogenes* seldom are found in the nose of a healthy child and should suggest a nasal or paranasal infectious illness. The recovery of *S. aureus* cannot be correlated with disease, however.

A review of many reports of children indicates that *S. pneumoniae*, *H. influenzae*, and *M. catarrhalis* are the etiologic agents that occur most commonly in acute and subacute ethmoid and maxillary sinusitis.* In one of the studies[186] of sinus aspirates from 50 children, *S. aureus* was not isolated from the maxillary sinus. Several studies of pediatric patients with chronic sinusitis suggest an increased importance of anaerobes and staphylococcal species.[19,21,39,179] In other studies in children with chronic sinusitis who have undergone surgery, a predominance of coagulase-negative staphylococci, viridans streptococci, and *S. aureus* was noted.[118,123] Several studies[55,71,190] documenting that *S. pneumoniae*, *H. influenzae*, and *M. catarrhalis* were the most common etiologic agents were concerned primarily with orbital involvement; 50 to 85 percent of cases of orbital cellulitis had radiographic evidence of sinusitis. Orbital complications of ethmoiditis primarily affect children. *Staphylococcus* spp., *S. pneumoniae*, and other streptococci have been found in children with orbital involvement from ethmoiditis[3] or frontal sinusitis.[54]

Table 17–1 lists etiologic agents of sinusitis by age of patient and type of illness. In all age groups and in acute, subacute, and chronic disease, *H. influenzae* and *S. pneumoniae* are the principal pathogens in most cases. Also, a large number of different

*See references 55, 71, 73, 77, 78, 107, 115, 172-174, 181, 186, 190.

bacterial species have been recovered from the sinuses of affected patients. In young children, more than 90 percent of all cases of sinusitis are caused by five organisms: *H. influenzae*, *S. pneumoniae*, *M. catarrhalis*, *S. aureus*, and *S. pyogenes*. In adolescents, the same organisms, plus largely penicillin-sensitive anaerobes, account for most cases. As noted in Table 17–1, a variety of gram-negative enteric and other bacilli have been recovered from patients with sinusitis, in most instances from patients who have had various forms of antibiotic therapy before culture. Organisms previously considered to be nonpathogens, such as *Staphylococcus epidermidis*, have been implicated etiologically.

Although clinically recognized sinusitis has occurred rarely in patients with *Mycoplasma pneumoniae* infection, Griffin and Klein[61] noted radiographic evidence of sinusitis in approximately two thirds of a group of Navy recruits with *M. pneumoniae* pneumonia. In adults with chronic suppurative maxillary sinusitis, mycoplasmas have been sought but not recovered.[14,60,161] Bhattacharyya and colleagues[14] noted L-forms in 21 percent of all sinuses in patients with chronic disease.

In a study of 25 adults with chronic rhinosinusitis, *Chlamydia pneumoniae* was recovered from nasopharyngeal samples in 2 patients, but not from any of 10 healthy controls.[45] In addition, the patients were more likely than control subjects (20 percent) to have serum IgG antibody titers to *C. pneumoniae* of 1:64 or greater (72%). IgA antibody titers to *C. pneumoniae* of 1:32 or greater also were more prevalent in the patients (48%) than in the controls (10%). In a study involving 20 children with chronic sinusitis, Cultrara and colleagues[31] cultured material from 13 bilateral endoscopic ethmoidectomies with maxillary antrostomies, 10 adenoidectomies, and 3 bilateral maxillary sinus lavages. They isolated *C. pneumoniae* from a nasopharyngeal swab and adenoid tissue from a 6-year-old child.

Fungal diseases of the sinuses have been well described in adults.[188] Acute fulminate fungal sinusitis is seen in immunosuppressed individuals and is associated with high morbidity and mortality rates.[33,158] *Aspergillus* spp. are the most common fungal causes of sinusitis. Many cases of chronic sinusitis from which a microorganism is not recovered have been thought to be caused by *Aspergillus* spp. infections.[88] The presence of eosinophils, Charcot-Leyden crystals, and hyphae found retrospectively, and not noted on the original examination, in mucus recovered from sinuses suggests that some cases of chronic sinusitis may represent *Aspergillus* hypersensitivity. This allergic aspergillosis in the sinuses is similar to allergic bronchopulmonary aspergillosis. In a series of six patients who were 8 to 16 years of age and had allergic aspergillosis sinusitis, all presented with nasal polyposis and facial deformity, indicating advanced disease.[110] Mucormycosis, an infection caused by Zygomycetes (formerly Phycomycetes), is seen in immunocompromised children and adults.[85] *Drechslera* spp., *Bipolaris* spp., and *Curvalaria lunata* have been added to the list of fungi that can cause sinusitis in children.[13,23,51,157]

Although sinusitis has been reported as a complication of Epstein-Barr virus infection, the sinus infections seem to be a complication of steroid treatment and not specifically the viral infection.[57] *Nocardia* spp. have been reported as a cause of acute sinusitis in an adult transplant recipient[142] and of chronic sinusitis in immunocompetent and immunocompromised individuals.[170]

EPIDEMIOLOGY

Although sinus involvement occurs commonly with respiratory viral infection, sinusitis seldom is identified as a specific illness in previously healthy children. In a survey of all office visits, totaling 2613, Breese and colleagues[17] noted only 6 children (0.23%) in whom the initial diagnosis was sinusitis. The true incidence of sinusitis in childhood is unknown. In 1989, Wald and colleagues[181] estimated that 0.5 to 5 percent of upper respiratory tract infections are complicated by acute sinusitis. More recent estimates by the same authors have been 10 percent.[184] The most recent estimates of greater incidence could be related to a heightened awareness and concern for lost work days by working parents, a possible correlation between pulmonary problems in an increasing number of children with chronic lung disease, better imaging techniques, increased interest in endoscopic sinus surgery,[163] more disease because of more exposure as a result of more children being in daycare,[108] and an increased recognition or perhaps incidence of allergy-related illness.[140,141] Seasonal prevalence has not been studied, but a reasonable assumption is that disease would increase during the cold weather months because it is the time of greatest respiratory viral activity. Cases in older children also can be expected to occur more frequently in association with swimming.

Although it is not well documented, sinusitis seems to be more of a problem in geographic areas where marked temperature changes occur. In children, sinusitis seems to occur more commonly in boys than in girls.[72,115] Ueda and Yoto[167] found abnormal findings in the maxillary sinuses in 135 (6.7%) of 2013 children who presented to an outpatient department with upper respiratory symptoms; of this group, 65 percent were boys, and 35 percent were girls. Manning and associates[109] found similar distribution in a group of 60 children diagnosed by CT or magnetic resonance imaging (MRI). Host factors are important in sinusitis because the illness occurs more commonly in allergic children; in children with chronic ear infections; and in patients with cystic fibrosis, primary humoral immunodeficiencies, and Kartagener syndrome.[22,78,121,146] Although an association between sinus disease and asthma seems to exist, controversy continues regarding whether sinusitis and other upper airway stimuli can induce asthma.[52,141,150,196] A review of hospital admissions of patients with status asthmaticus at the Children's Hospital of Los Angeles showed a marked increase in admissions, and sinusitis was diagnosed in 23 percent.[140] Children with various immunologic defects frequently have sinusitis.

Sinusitis is noncontagious from person to person, but point-source outbreaks are possible from swimming in heavily contaminated water. A cluster of seven cases of invasive nosocomial fungal sinusitis in severely neutropenic patients has been described. It was caused by the release of airborne fungal spores from soil reservoirs that were distributed during hospital construction during a 2-year period.[104]

CLINICAL PRESENTATION

The clinical symptoms of sinusitis vary by age. Older children and adolescents have localized complaints similar to those of adults, whereas in young children, the findings are related less clearly to the sinuses.[176] Table 17–2 presents the overall frequencies of symptoms, signs, and laboratory findings for acute, subacute, and chronic disease.

In young children, disease involves only the ethmoid and maxillary sinuses. In these children, illness frequently has its onset after they have had an upper respiratory viral infection. A period of general improvement may occur, however, between the acute respiratory illness and the onset of symptoms related to sinus infection. The most prominent symptom in all children, and particularly in children younger than 10 years of age, is persistent rhinorrhea. The discharge frequently is purulent, but it occasionally can be serous or watery. Associated with rhinorrhea is cough, which becomes more prominent with increasing duration of disease. The cough particularly is troublesome at night because it is caused by the stimulation of the sinus drainage as it traverses the pharyngeal wall. The posterior drainage also occasionally causes vomiting. Fever is of variable occurrence in

TABLE 17–2 Clinical Findings in Acute, Subacute, and Chronic Sinusitis of Children

	Occurrence (%)	
	Acute and Subacute Sinusitis	*Chronic Sinusitis*
Symptoms		
Fever	50	20
Rhinorrhea	80	80
Cough (persistent and evening)	50	90
Pain/headache	30	30
Sore throat	20	20
Periorbital swelling	30	0
Vomiting	20	10
Allergic history	20	40
Malodorous breath	20	20
Signs		
Rhinorrhea	80	80
Temperature ≥38.3°C (≥101°F)	20	0
Sinus tenderness	20	10
Otitis media	40	60
Posterior pharyngeal pus	0	10
Transillumination positive	30	10
Periorbital swelling	30	0
Malodorous breath	20	20
Laboratory		
Abnormal radiographs	100	100
Maxillary	90	90
Ethmoid	40	40
Frontal and sphenoid	10	10
Unilateral	70	10
Bilateral	30	90
Erythrocyte sedimentation rate elevation	50	10
White blood cell count elevation with an increased percentage of band form neutrophils	40	10

Data from references 3, 10, 72, 75, 84, 89, 91, 115, 121, 141, 146, 159, 178, 187.

sinusitis and generally is related inversely to age and duration of illness. Malodorous breath often is reported by parents. The first evidence of illness in some children is fever and periorbital swelling. In most instances, periorbital cellulitis is a manifestation of ethmoid sinusitis.

Although facial pain and headache are frequent complaints of sinus disease in adults, they have been noted in only approximately one third of the cases in children and are unusual occurrences in young children. The main symptom in older children and adolescents is rhinorrhea. In older patients with more chronic disease, the nasal symptoms may be minimal or absent. Troublesome postnasal drip is a frequent complaint.

Acute isolated sphenoid sinusitis in children is rare but often misdiagnosed because the symptoms are vague and there are no specific physical findings.[112] Findings include fever, headache, and neurologic symptoms. Swimming and diving are possible predisposing factors.

Physical signs in sinusitis also differ by age. Nasal discharge is the most frequent finding in all age groups. Young children are more likely to have a serous or watery discharge, however, than are adolescents. Elevation of temperature occurs more commonly in acute disease and in association with orbital cellulitis. Sinus tenderness, a common finding in older patients, is noted only rarely in children. Particularly significant is tenderness with percussion of the upper molars. Examination of the throat frequently reveals free exudate. Occasionally, the breath is malodorous.

The ears are abnormal in almost half of all patients with sinusitis. In acute disease in young children, it can be acute otitis media, but usually the findings are more suggestive of serous disease. Acute sinusitis frequently is unilateral, whereas chronic disease more often is bilateral.

Children with chronic sinusitis frequently have only minimal complaints. The parent notes that the child does not feel well and frequently reports that the child has had a persistent respiratory infection for months. In a series of children with chronic (>3 months) upper respiratory complaints who were referred to allergy clinics, 60 percent had sinusitis.[121] In this study, the combination of moderate to severe rhinorrhea and cough with minimal sneezing was reported to have a specificity of 95 percent and a sensitivity of 38 percent in predicting the presence of chronic sinusitis. In the referred children in this study, sinusitis was found in 63 percent of atopic children and in 75 percent of non-atopic children.

Laboratory studies other than cultures and radiography are not useful in the evaluation of a child with sinusitis. Herz and Gfeller[75] noted that in their study, erythrocyte sedimentation rates were elevated in only approximately half of the patients, and leukocytosis occurred in only one third. Generally, younger children with orbital cellulitis and ethmoid sinusitis are more likely to have elevated sedimentation rates and white blood cell counts. The American Academy of Pediatrics (AAP) Subcommittee of Sinusitis and Committee on Quality Improvement has recommended that the diagnosis of acute bacterial sinusitis be based on clinical criteria in children who present with upper respiratory symptoms that are either persistent (nasal or postnasal discharge of any quality with or without daytime cough for >10 to 14 days) or severe (temperature >39° C and purulent nasal discharge present concurrently for at least 3 or 4 consecutive days in a child who appears ill).[4]

COMPLICATIONS

Serious complications, including meningitis, osteomyelitis, cavernous sinus thrombosis, and epidural, subdural, brain, and orbital abscesses, occur in untreated sinusitis.* Signs and symptoms of neurologic involvement in sinusitis frequently call for aggressive surgical management of the sinusitis and the intracranial and paracranial lesions. Osteomyelitis of the frontal bone may be a complication of frontal sinusitis[9] and may manifest as Pott puffy tumor if a subperiosteal abscess also is present.

DIFFERENTIAL DIAGNOSIS

Differential considerations in sinusitis are few and are more concerned with whether sinus involvement in a particular child is the primary event or a secondary problem related to a more general host defect. Children with recurrent and chronic sinusitis should be evaluated for respiratory allergy, cystic fibrosis, immunologic deficiency, and Kartagener and other immotile-cilia syndromes.

Foreign bodies in the nose can be mistaken for sinusitis, as can cysts in the maxillary antra. Nasal structural defects (congenital and acquired), such as palatal clefts, unilateral choanal atresia, nasal polyps, and septal deviation, can be confused with sinusitis, but more commonly these problems are predisposing factors in sinus infections.

Dental infections frequently are mistaken for maxillary sinus disease. Dental infections can lead by direct extension to sinus involvement. Primary infections in the region of the eye also

*See references 1, 46, 56, 57, 96, 113, 124, 133, 148, 189, 192.

occur without sinus disease. In young children, a chronic infection of the adenoids can be confused clinically with sinusitis. Infections with *Bordetella pertussis* can be confused with subacute sinusitis.

SPECIFIC DIAGNOSIS

Although persistent nasal symptoms and the presence of other clinical findings as listed in Table 17–2 indicate a diagnosis of sinusitis, the only certain way to make the diagnosis is by obtaining radiographs and cultures reflecting sinus flora. Although some physicians have suggested that maxillary sinus radiographs frequently are abnormal for normal children,[111,154] other data indicate that normal children older than 1 year of age seldom have abnormal radiographs.[92] During infancy, the maxillary sinuses are so small that minimal mucosal edema may "opacify" a sinus on a radiograph. In young children, radiographic examination should consist of two views: lateral and Waters. In older children, Caldwell and basal projections also should be performed. Radiographs in acute upper respiratory viral infections as a rule are abnormal; these radiographs are not false-positive ones, but are the result of viral infections. From a therapeutic point of view, sinus radiographs usually should not be obtained unless nasal symptoms in an upper respiratory illness have not shown signs of improvement after 5 to 7 days.

Plain-film radiographic examination has been supplanted mainly by CT and MRI for the diagnosis of sinusitis. Many endoscopic sinus surgeons consider CT to be a mandatory part of the preoperative evaluation. MRI is useful in cases that may be complicated by orbital or intracranial extension. The high prevalence of incidental sinus opacification in asymptomatic infants and children noted radiographically has been confirmed by CT studies.[35,58,76] Since the advent of MRI, researchers have realized that many incidental sinus abnormalities also occur in adults. These findings in children and adults may be from subclinical or resolving respiratory infections or may be due to unrecognized allergies.[34]

CT has been recognized widely as the standard for the diagnosis of paranasal sinus disease.[12] In particular, coronal thin-section images offer excellent delineation of lesions in the osteomeatal complex.[197] Obtaining axial images is useful for evaluating periorbital and intraorbital complications.[49] In some institutions, a so-called screening CT of the sinuses is performed with a limited number of slices.[64] It can be offered at a cost and radiation exposure that are similar to those associated with plain-film studies, but with much greater accuracy. Some young children and infants require sedation for CT, which limits its suitability.

The AAP Subcommittee of Sinusitis and Committee on Quality Improvement has recommended that imaging studies are not necessary to confirm a diagnosis of clinical sinusitis in children 6 years of age or younger.[4] The need for radiographic evidence as a confirmatory test in children older than 6 years with severe symptoms is controversial. The American College of Radiology considers that the diagnosis of acute uncomplicated sinusitis should be made only on clinical grounds.[114] CT scans of the sinuses should be reserved for patients in whom surgery is being considered as a management strategy.[4] In a meta-analysis of acute uncomplicated sinusitis in children,[82] poor concordance was observed among clinical criteria, plain radiographs, CT scans, ultrasonography, and fluid aspiration. More studies are necessary to determine the optimal set of clinical criteria and other laboratory or radiologic studies for diagnosis of this condition.

Which radiographic technique (plain radiography, CT, or MRI) is selected for evaluation of a child with presumed sinusitis should be determined by availability of techniques and the expertise of the radiologist and by clinical symptoms. Radiographs, in most instances, should not be obtained early in the illness of children with uncomplicated upper respiratory complaints because of the high incidence of transient abnormalities.[5] Radiography is indicated for children with continuing symptoms of sinusitis after extensive medical therapy or for children with possible complications of sinusitis. CT is optimal.[5] A limited CT scan with axial cuts may be obtained. It allows for excellent visualization of sinus anatomy and pathology while limiting the radiation exposure to the child. A limited sinus CT scan has radiation similar to that of a sinus plain-film series. In the absence of low-cost screening CT, plain radiography should be the initial imaging study in most children who have symptoms of sinus disease.[34] Children who have periorbital swelling or proptosis should undergo immediate contrast-enhanced CT studies in axial and coronal planes. If symptoms or CT findings suggest intracranial extension, MRI should be performed.[5,34]

Although ultrasonography would seem to offer an alternative to sinus radiography, some question remains about its dependability unless one normal air-filled maxillary sinus or one opacified maxillary sinus is present for comparison.[174,185] The hallmark of specific diagnosis in sinusitis is similar to that of other infectious diseases: the culture of infected material. Many physicians erroneously considered it an impossible task because of the inability of obtaining material directly from the sinuses of children. As discussed in the section on etiology, nasal cultures that are properly performed reveal the causative organism in most instances. Nasal culture should be taken from the region of the maxillary ostium in the middle meatus. Culture specimens should be obtained from this area and not from the nasopharynx. Wald and associates[185] found no correlation between bacteria isolated directly from the maxillary sinuses and nasopharyngeal and throat culture isolates. Best results are obtained when a vasoconstrictor, such as 0.25 percent phenylephrine hydrochloride, is administered first and the culture specimen is obtained with a wire-cotton swab under direct vision. With this technique, material frequently can be obtained as it comes from the sinus ostium. Bilateral cultures always should be obtained. In serious cases, such as in children with neurologic complications or in treatment failures, performing antral puncture for culture can be lifesaving. Anaerobic and aerobic cultures should be performed on any material recovered by antral puncture.

TREATMENT

ACUTE AND SUBACUTE SINUSITIS

The successful treatment of acute and subacute sinusitis in children depends primarily on the administration of an appropriate antibiotic in adequate dosage for a sufficient period.[4,180] In most instances, therapy should be instituted before obtaining the results of cultures. Antibiotic selection in this situation is not a great problem in children because the etiologic agent is *H. influenzae*, *S. pneumoniae*, *M. catarrhalis*, *S. aureus*, or *S. pyogenes* in more than 90 percent of acute cases.

Initial selection of an antibiotic should be based on the severity of the clinical illness and must take into consideration the antibiotic resistance patterns of the common causative organisms and the cost and ease of administration of the treatment regimen. Today, approximately 50 percent of nontypeable *H. influenzae* and 100 percent of *M. catarrhalis* strains produce β-lactamases and are resistant to amoxicillin.[37,38] In addition, 15 to 38 percent of *S. pneumoniae* strains have either intermediate (7-19%) or complete (7-19%) penicillin resistance.[11,26,40]

The AAP Subcommittee of Sinusitis and Committee on Quality Improvement, in their "Clinical Practice Guideline: Management of Sinusitis," recommended amoxicillin as first-line therapy for children younger than 2 years of age suspected of having acute bacterial sinusitis of mild to moderate severity, who

do not attend daycare, and who have not been treated recently with an antimicrobial agent.[4] They recommend an amoxicillin dose of either 45 mg/kg/day in two divided doses or 90 mg/kg/day in two divided doses. They recommend cefdinir (14 mg/kg/day in one or two doses), cefuroxime (30 mg/kg/day in two divided doses), or cefpodoxime (10 mg/kg/day once daily) for the child who is "allergic" to amoxicillin if the past allergic reaction was not a type 1 hypersensitivity reaction. If the past allergic reaction was of a type 1 nature, they recommend clarithromycin (15 mg/kg/day in two divided doses) or azithromycin (10 mg/kg/day on day 1 followed by 5 mg/kg/day as a single dose for 4 days). They suggest clindamycin (30 to 40 mg/kg/day in three divided doses) as an alternative therapy for the penicillin-allergic patient who is known to be infected with a penicillin-resistant pneumococcal strain. Their recommendation is based on the safety, tolerability, low cost, and narrow spectrum of amoxicillin, and a calculated success rate based on data regarding treatment of acute otitis media.

The AAP committees further recommended that children who did not improve while receiving the lower amoxicillin dose, children with more severe illness, and children who attend daycare should be treated with high-dose amoxicillin-clavulanate (80 to 90 mg/kg/day of amoxicillin component in two divided doses). For children with vomiting, a single dose of ceftriaxone (50 mg/kg/day) given either intravenously or intramuscularly is suggested. After this regimen, the child's therapy is switched to an oral regimen.

The committees' suggestions presented above applied only to children younger than 2 years of age; however, because the spectrum of etiologic agents is similar to that for older children, it should also apply for those older than 2 years of age.

In contrast to the AAP committees' recommendations, we consider that a more appropriate approach is to use only high-dose amoxicillin or amoxicillin-clavulanate (90 mg/kg/day in two divided doses). The duration of treatment in the outpatient setting has not been studied adequately. Wald[178] suggests that therapy should be continued for 7 days after the child becomes symptom-free.

A seriously ill child should be hospitalized, and therapy for β-lactamase–producing staphylococci and highly resistant pneumococci should be implemented, in addition to coverage for amoxicillin-resistant *H. influenzae* and *M. catarrhalis*. This coverage is achieved with vancomycin (40 mg/kg per 24 hours every 6 hours) and cefotaxime (100 to 200 mg/kg per 24 hours every 6 hours) or ceftriaxone (100 mg/kg per 24 hours every 12 hours). Therapy should be adjusted on the basis of clinical response and culture results. The dosage and duration of antimicrobial therapy in sinusitis are crucial considerations. Penicillins penetrate the sinuses poorly.[9,48,65,85,105] The duration of therapy should be a minimum of 10 days.

The relief of obstruction at the sinus ostia and the establishment of drainage are time-honored principles of therapy. To achieve these goals, locally applied and systemically active vasoconstrictive drugs are used. To date, no evidence supports their therapeutic effectiveness, however. The beneficial effects of oral, systemically active, vasoconstrictive drugs are hampered by the fact that their drying effect may be deleterious to the mucus blanket. Topical vasoconstrictor drugs (e.g., phenylephrine hydrochloride and oxymetazoline) are plagued by rebound vasodilation. We consider that these drugs should be used rarely in acute disease; their main use is to relieve pain caused by obstruction, and they should be used only for 2 to 3 days.

CHRONIC AND RECURRENT SINUSITIS

Allergic disorders are common in chronic and recurrent sinusitis.[108,177] Children should be evaluated for allergy, and, when identified, specific treatment should be employed. Specific allergens and irritants should be avoided (e.g., through air filtering, removal of pets, avoidance of tobacco smoke), and pharmacologic management should be implemented.

Nasal saline washes (twice daily in each nostril) are useful because they liquefy secretions and enhance mucociliary transport, which improves sinus drainage and ventilation. Antihistamines may be useful if allergic rhinitis is a contributing factor to the chronic sinus infection. Anti-inflammatory agents also may be useful. In selected cases, use of either topically applied corticosteroids or cromolyn sodium may be beneficial. Corticosteroids should be used carefully because their use occasionally can lead to superinfection in the sinuses with *Pseudomonas* spp., other highly resistant gram-negative bacilli, or fungi. For effective corticosteroid use, Wald[177] suggests using a topical decongestant first so that the steroid preparation can reach the affected areas better.

In chronic or recurrent disease, antimicrobial treatment should be based on culture and sensitivity data. Specific antimicrobial agents are the same as the agents used in acute and subacute disease, but treatment should be prolonged for 3 weeks or more and for 7 days after the resolution of symptoms. *Aspergillus* and *Bipolaris* spp. and other fungal infections require prolonged therapy with an antifungal agent to which the specific agent is susceptible. Itraconazole, ketoconazole, and fluconazole all have been effective in selected cases. Allergic fungal sinusitis can be treated with endoscopic sinus débridement of all fungal and polypoid disease, followed by topical and systemic corticosteroids and close follow-up, including frequent endoscopic cleaning.[30,96,97,134] Allergic aspergillosis of the sinuses can be managed with topical steroids without specific antifungal therapy (see Chapter 28).

In the past, surgical therapy for sinusitis in children was of questionable benefit. Surgical therapy included diagnostic and therapeutic irrigation; permanent drainage procedures in children with complications of sinusitis and in children who had immune defects; and such procedures as adenoidectomy,[99,169] septoplasty, and turbinectomy to relieve anatomic obstructions to improve nasal and sinus ventilation. In one uncontrolled study of children with otitis media with effusion and sinusitis, the sinusitis was improved 6 months after adenoidectomy in 56 percent of children, whereas only 24 percent of similar children who did not undergo surgery had similar improvement.[162]

Historically, creating nasoantral windows was the most common major surgical procedure for treating chronic sinusitis in children.[62,118] Long-term success with this procedure was poor, however, because of the high rate of closure of the windows. A new interest in sinus surgery has resulted from the introduction of endoscopic techniques. Several studies have found endoscopic surgery to be safe and effective.[63,98,106,129,144] Pediatric endoscopic sinus surgery now is recognized as a viable option for children with chronic recurrent sinusitis refractory to medical therapy.[27,102,138] A meta-analysis of 832 children who underwent endoscopic sinus surgery from 1986 to 1996 revealed an overall 88.4 percent positive outcome after surgery.[74]

The goal of functional endoscopic sinus surgery is to remove obstruction at the osteomeatal complex where the mucociliary flow from the frontal, maxillary, and ethmoid sinuses converges.[98,144] This removal results in improved drainage and restoration of normal physiologic function of the frontal, maxillary, and ethmoid sinuses. Surgery involves an anterior ethmoidectomy and enlargement of the natural ostium of the maxillary sinus. Follow-up surgery performed 2 to 3 weeks after the initial surgery sometimes is necessary to remove crusts, blood clots, any stenting material, granulative tissue, and adhesions.[187]

In a study of 210 children with a history of chronic sinusitis for 3 months or longer, functional endoscopic sinus surgery

resulted in successful outcomes in 165 (79%).[98] The follow-up period was 3 to 36 months (mean 18 months), and all of the infections in these children had failed to respond to prior extensive medical management. In this series, no major complications occurred.

Functional endoscopic sinus surgery should be considered for children with chronic or recurrent sinusitis that has failed extensive, prolonged, and adequate medical management.[102] Many pediatric otolaryngologists have resumed performing adenoidectomy as a surgical intervention before proceeding with functional endoscopic sinus surgery.[156] This management includes specific antimicrobial therapy for specific organisms identified by culture; the diagnosis and treatment of allergic and other contributing conditions, such as cystic fibrosis, asthma, or immunologic disorders; and a trial of prophylactic antimicrobial agents.

Orbital and intracranial abscesses and cavernous sinus thrombosis secondary to sinus infection require emergency surgery, which often is lifesaving.[143,148,189,192] Cellulitis, osteomyelitis, and meningitis also frequently require surgery if they do not respond to antimicrobial therapy.[143] Surgery in these cases involves drainage of the sinuses and abscesses. Endoscopic techniques may allow for intranasal drainage and avoidance of development of facial scars.[6] If an intracranial complication involves an intracranial abscess, concomitant craniotomy along with functional endoscopic sinus surgery may be required.[59] Surgical procedures also may be indicated for a child with acute or chronic disease resulting from an identified underlying problem, such as an immunologic deficiency.[191]

PROGNOSIS

The prognosis of identified and adequately treated sinusitis in otherwise normal children is excellent. Frequently, children have subnormal health because sinusitis goes unrecognized; it may be treated only partially because of other clinical impressions, which contributes to the chronicity of the problem. Sinusitis is likely to be recurrent in children with a history of previous chronic disease and in children with repeated adverse exposure, such as swimming in contaminated or irritating water. Children with allergic respiratory disease also are likely to have frequent recurrences. Sinusitis in an immunocompromised child frequently is resistant to cure; long-term continuous therapy can be beneficial in such patients. Signs and symptoms of neurologic involvement in sinusitis frequently call for aggressive surgical management of the sinusitis and the intracranial and paracranial lesions.

Paranasal sinusitis also has been noted occasionally to be associated with bronchial asthma.[127,136] Its successful treatment has resulted in clearing of the asthma.[80,150]

PREVENTION

Sinusitis, as such, is not preventable in most instances. In some individuals, change of lifestyle can do much to improve the situation, however. Sinusitis in some children is related to their swimming habits and can be controlled by elimination of swimming or perhaps by the use of nose plugs. Good allergic management, including intranasal corticosteroid or cromolyn therapy, prevents sinus disease in certain atopic children. Relief of nasal airway obstruction caused by allergic rhinitis, enlarged adenoids, or other anatomic problems also should help to prevent sinusitis. Early attention given to persistent nasal discharge also can be expected to lessen the damage associated with sinus infection.

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CHAPTER 18

Otitis Externa

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Otitis externa constitutes one of the most common otolaryngologic encounters in the emergency department. This condition is addressed commonly as *swimmer's ear* or *tropical ear* because it often occurs after a history of repeated water exposure and it occurs more commonly in warm and humid environments. This condition became of significant interest to the medical field during World War II because of its high incidence among the troops in the South Pacific. During World War II, otitis externa accounted for 50 to 70 percent of the caseload for otolaryngologists in the South Pacific.[22]

Today, otitis externa accounts for 7.5 million annual ototopical prescriptions in the United States and is a significant cause of discomfort because of the associated pain and conductive hearing loss.[36] This chapter discusses knowledge of the basic anatomy of the external canal, different etiologies of otitis externa, and treatment and prevention that are needed by the pediatric practitioner managing this condition.

NORMAL ANATOMY

The external ear is composed of the auricle and external auditory canal (EAC). The auricle of the ear is a skin-covered cartilaginous structure on the temporal region of the head and is an extension of the EAC. The EAC is divided into two regions—a lateral cartilaginous portion and a medial bony portion that ends at the tympanic membrane.

The cartilaginous portion of the canal makes up approximately 40 percent of its 2.5 cm total length and typically is directed slightly upward and backward. This shape and the presence of cerumen help to prevent water and foreign objects from entering the canal.[24] In the anterior portion of the cartilaginous canal are the fissures of Santorini, which provide a route for the spread of infection from the canal into the adjacent parotid and surrounding tissues. The bony canal makes up the remainder of the canal length and is directed slightly downward and anterior. The isthmus, which is the narrowest portion of the canal, corresponds to the bony-cartilaginous junction.

The sensory innervation of the EAC is complex and includes contributions from the fifth, seventh, ninth, and tenth cranial nerves. This sensory innervation is responsible for the exquisite pain associated with otitis externa.[24] The skin of the entire EAC consists of keratinizing stratified squamous epithelium. It is the only keratinizing epithelium that lacks eccrine sweat glands.

Differences exist in the canal skin of the cartilaginous canal and the bony canal. The cartilaginous canal skin is thicker and contains rete pegs, dermis, dermal papillae, hair follicles, and sebaceous and cerumen glands, which are absent in the bony canal. The thinner skin of the bony canal lacks dermal papillae and rete pegs. The lack of subcutaneous tissue in the bony canal allows the tight attachment of the skin to the underlying periosteum, rendering the bony canal wall more vulnerable to trauma.

PROTECTIVE MECHANISMS OF THE EXTERNAL EAR

Three major defense mechanisms protect the EAC and lateral surface of the tympanic membrane: the tragus and antitragus, the skin with its cerumen coat, and the isthmus of the canal. The tragus and antitragus provide a partial barrier to the entrance of the foreign bodies into the EAC.

The skin of the cartilaginous canal contains many hair cells and sebaceous and apocrine glands, such as cerumen glands. Together, these three adnexal structures are termed the *pilosebaceous unit* and provide a protective function in the EAC. Migration of the skin of the EAC also helps to keep the canal free of debris. The pattern of migration is from the tympanic membrane laterally and radially away from the umbo.[1,24] Glandular secretions combine with sloughed squamous epithelium to form an acidic coat of cerumen, one of the primary barriers to infection of the canal. Cerumen is composed of lipids that are hydrophobic, and its major function is to waterproof the canal. Racial and gender differences that exist in the characteristics of cerumen do not seem to have any major clinical significance. Whites and African-Americans produce cerumen with higher levels of lipids compared with Asians, who produce cerumen with higher levels of proteins. Cerumen in males also has been shown to be higher pH than that in females.[18] The acidic nature of cerumen has been shown to inhibit bacterial and fungal growth.[11,18,23]

The canal normally is a self-protecting and self-cleansing structure. The cerumen coat gradually works its way to the lateral part of the canal and sloughs externally. Instrumentation and excessive cleansing of the canal can alter this primary protective barrier and may lead to infection (Table 18–1).

NORMAL BACTERIAL FLORA

The normal bacterial flora of the EAC is a combination of aerobic (80%) and anaerobic (20%) organisms.[9] Aerobic bacteria include *Staphylococcus epidermidis*, alpha-hemolytic streptococci, diphtheroids, and *Pseudomonas aeruginosa*.

In a study by Stroman and colleagues[42] of 291 bacteria isolated from cerumen, 99 percent were gram-positive, whereas of 302 bacteria isolated from the canal, 96 percent were gram-positive. Staphylococci accounted for 63 percent of the cerumen bacteria and the canal bacteria. *Staphylococcus auricularis* is the isolate found most frequently (23% in cerumen and 21% in canal), and *S. epidermidis* is the second most commonly isolated bacteria from cerumen and canal (14% and 17%, respectively). After staphylococci, the coryneform bacteria (diphtheroids) are the organisms most frequently isolated. The third most frequently recovered bacteria belong to the streptococci and enterococci groups. *Alloiococcus otitidis* was isolated with the greatest frequency (>95%) in the cerumen and the canal.[42]

In addition, seven species of *Bacillus* were isolated in the canal and cerumen. From the Micrococcaceae family, *Micrococcus luteus* was isolated most frequently from the canal and cerumen. *Turicella otitidis* was the primary coryneform recovered: 58 percent from cerumen and 65 percent from the canal. *Corynebacterium auris* was the second most frequently isolated coryneform: 12.5 percent from the canal and 12.3 percent from cerumen. Twenty-one other species of coryneforms (including 10 previously undefined species of *Corynebacterium* and 4 of *Microbacterium*) were isolated as well. Coryneforms represented 22 percent of the bacteria in cerumen and 19 percent in the canal. *Propionibacterium acnes* and a variety of *Peptococcus* spp. make up the anaerobic flora.[42]

TABLE 18–1 Predisposing Factors for Otitis Externa

Hot and humid environment
Water exposure
Instrumentation of ear canal
Previous radiation therapy
Eczema
Draining ear
Contact dermatitis

ACUTE OTITIS EXTERNA

Acute otitis externa is an infection of the EAC that often is the end result of a combination of factors. Bacterial and fungal infections of the EAC occur when the natural defenses break down, resulting in a significant reduction in the amount of cerumen, an injury to the skin of the canal, or a shift of the normal canal flora. Humidity, heat, and maceration all produce itching, which often leads to manipulation and instrumentation of the canal, which in turn leads to additional trauma. Otitis externa may be caused by insults that result in the removal of the protective lipid film from the canal, allowing the entrance of organism to the apopilosebaceous unit. Rapid proliferation of bacteria occurs as a result of the warm, dark, and moist canal environment.

Inflammation and infection can cause canal edema or complete obstruction of the canal in severe cases, with purulent discharge. If the infectious process goes untreated, it leads to cellulitis of the auricle and surrounding area.

Symptoms of otitis externa, in addition to pruritus and drainage, include pain and tenderness on palpation or manipulation of the external ear. Pain arises as the soft tissues and skin of the canal distract the periosteal lining of the bony canal. Pain is severe enough to interfere with daily activities and is the major reason for medical consultation.

HISTORY AND PHYSICAL EXAMINATION

Otitis externa is a clinical diagnosis. Interrogation often reveals a history of exposure to water, previous auricular instrumentation, or trauma. The physician should inquire about predisposing factors, such as diabetes, immunosuppression, history of eczema, or previous radiation therapy, all of which may predict a more complicated course.

On physical examination, the pinna may appear swollen or erythematous or may be protruding. Primary care practitioners and emergency department personnel frequently establish the diagnosis of mastoiditis based on auricular protrusion. On manipulation of the auricle, the patient often experiences severe pain. Tragal tenderness is another key feature of this disease. A hand-held otoscope often suffices to establish the diagnosis, but the microscope is recommended for full cleaning of the ear canal. The pinna may appear erythematous with edema and eczematization (Fig. 18–1).The canal appears swollen with various grades of patency, depending on the severity of the infection. Purulent discharge combined with keratin debris usually fills the canal. It is important to attempt to visualize the tympanic membrane if the canal is not completely obstructed. Absence of drainage from the middle ear confirms the diagnosis of otitis externa.

PATHOGENS IN ACUTE OTITIS EXTERNA

Common bacterial pathogens that cause otitis externa include *P. aeruginosa*, *Staphylococcus aureus*, *Escherichia coli*, and *Proteus* spp. (Table 18–2). *Pseudomonas* have been found to be the

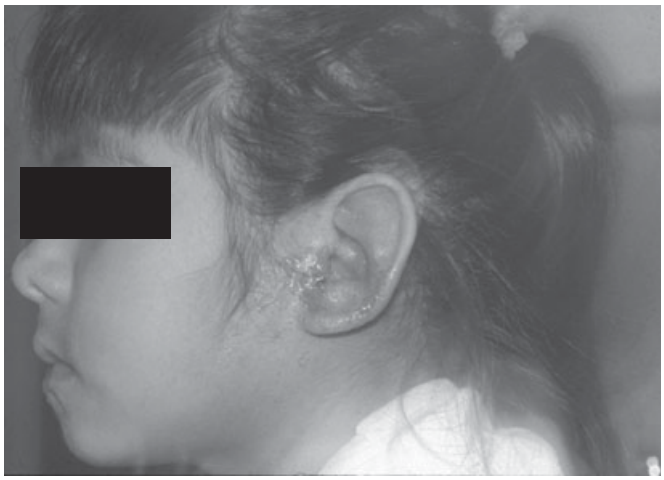

Figure 18–1 Patient with acute otitis externa, with purulent drainage from the ear canal with mild edema and erythema of the pinna. (See companion Expert Consult web site for color version.)

TABLE 18–2 Common Pathogens in Otitis Externa

Gram-Negative Organisms
Pseudomonas aeruginosa
Pseudomonas spp. Nov. "otitidis"
Proteus mirabilis
Serratia marcescens
Gram-Positive Organisms
Staphylococcus aureus
Staphylococcus epidermidis
Corynebacterium auris
Enterococcus faecalis
Fungi and Yeasts
Aspergillus fumigatus
Candida albicans
Candida parapsilosis

predominant organism in various studies.[4,7,35,38] Historically, *Pseudomonas* have accounted for 50 to 80 percent of bacteria isolated from cases of chronic otitis externa.[4,29,31,35,38] In a study by Roland and Stroman,[35] gram-negative bacteria accounted for 53 percent of recovered organisms; 45.3 percent were gram-positive. In this same study, *P. aeruginosa* accounted for 37.7 percent of the total number of isolates, whereas a newly identified species, *Pseudomonas otitidis*, accounted for 2.3 percent of recovered organisms.[35]

Staphylococci are the most common gram-positive organism recovered in cases of otitis externa, accounting for 25 percent of the cases. *S. epidermidis* is the most common staphylococcal species recovered, followed by *S. aureus*. Coryneforms are the second largest group of gram-positive bacteria isolated from cases of otitis externa.[35]

Other organisms that have been identified as pathogens include *Enterobacter*, *Klebsiella*, *Serratia*, *Proteus*, and *E. coli*. Although *Microbacterium* spp. previously were considered normal flora, more recently they have been identified at a 10 times higher rate in infected ears compared with normal controls. *Microbacterium* also have been the single recovered isolate in treatment failures and re-infections.[35]

MANAGEMENT OF ACUTE OTITIS EXTERNA

No consensus exists about the most effective treatment for otitis externa. Treatment usually includes thorough cleaning and suctioning of the purulent debris and application of topical agents. Topical treatment has been shown to be effective and is the mainstay of treatment. A wick usually is placed in the canal to provide adequate delivery of the topical solution. Without the wick and adequate cleaning of the canal, the topical drops may not achieve adequate penetration and are ineffective. When topical therapy is prescribed, various options, including multiple antibiotics, antiseptics, steroids, and combination agents, are available.[9,10,18,34,36] Topical antimicrobial therapy increased absolute clinical cure rates of acute otitis externa by 46 percent and bacteriologic cure rates by 61 percent compared with placebo.[36] Freedman[20] compared topical neomycin/colistin/hydrocortisone with topical placebo and found less severe edema and itching by day 3 and less severe edema, itching, redness, scaling, and weeping by day 7.[36] Without treatment, approximately 15 percent of patients with acute otitis externa have clinical cure within 10 days; however, the cure rate increases to 65 to 85 percent when topical antimicrobial therapy is administered.[20,36]

Rosenfeld and colleagues[36] performed a meta-analysis to assess topical antiseptics and antibiotics and found comparable clinical cures at 7 to 14 days. The most common antiseptics used in the treatment of otitis externa include acetic acid, boric acid/ethyl alcohol and aluminum acetate, and *N*-chlorotaurine.

Topical antibiotic preparations usually are divided into two groups: quinolones and non-quinolone antibiotics. Non-quinolone antibiotics, such as polymyxin, neomycin, gentamicin, tobramycin, and oxytetracycline, have been the mainstay of treatment for otitis externa for several decades. Most of these agents have some degree of ototoxicity, however, which renders them undesirable in the case of a tympanic membrane perforation or a patent pressure equalizing tube.[26,32] The introduction of quinolones in the late 1980s for management of otitis externa and malignant external otitis provided a non-ototoxic alternative treatment. Topical antibiotic therapy allows for the administration of high concentrations of antibiotics, which can overcome organisms with high minimal inhibitory concentrations. Rosenfeld and colleagues[36] reported comparable clinical cure rates for topical quinolone antibiotics compared with non-quinolones at 3 to 4 days, 7 to 10 days, and 14 to 28 days. This same study found no differences in adverse effects between the two antibiotic groups.

More recent studies have reported pseudomonal resistance to fluoroquinolones. Berenholz and colleagues[6] reported 33 percent ciprofloxacin resistance in cases of necrotizing otitis externa.[6] Other studies have shown strains of *S. aureus* and *S. epidermidis* that have developed quinolone resistance.[34] One of the most common mistakes in the treatment of otitis externa is not identifying a tympanic membrane perforation and draining middle ear as the source of the infection, which may have treatment implications because of the ototoxicity of some compounds.

CHRONIC OTITIS EXTERNA

Chronic otitis externa is an inflammatory condition of the ear canal skin. This condition generally is caused by the loss of the protective coating of cerumen and oils secondary to constant mechanical débridement, such as with cotton applicators or with repeated water exposure. Associated pruritus leading to self-cleaning perpetuates the vicious cycle. The ear canal with chronic otitis externa often is more vulnerable to bacterial superinfection.[33] *S. aureus* and *P. aeruginosa* have been shown to secrete proteases that may continue to establish pathologic skin conditions in the EAC in cases of chronic otitis externa.[27] Treatment of chronic otitis externa involves débridement and application of topical anti-inflammatory agents, such as corticosteroids. Cessation of habitual canal manipulation is necessary for achieving a

positive response. For recalcitrant cases, surgical removal of the canal skin and replacement with skin grafts may be required.

OTOMYCOSIS

Otomycosis is a fungal infection of the skin of the external canal. Otomycosis often is the result of superinfection of chronic bacterial infection of the external canal or middle ear. All fungi have three basic growth requirements—moisture, warmth, and darkness—all of which commonly are present in the EAC. The most common fungus is *Aspergillus* spp., although *Candida* spp., *Actinomyces* spp., and Phycomycetes also have been reported.[7] Pruritus is the primary clinical manifestation. Patients also complain of feeling moisture in their ears. Physical examination commonly shows a white, black, or dotted gray membrane in the EAC. Diagnosis often is confirmed with a fungal culture.

Thorough cleaning with removal of the matted fungal debris and topical application of an acidifying solution, such as aluminum sulfate–calcium acetate (Domeboro), or of a drying powder, such as boric acid, often is adequate treatment. Antifungals such as clotrimazole cream or solution (Lotrimin) also may be used. It is important to assess for perforation of the tympanic membrane because antifungals can be ototoxic.[16,26] Gentian violet usually is well tolerated in patients with mastoid cavities, but it permanently stains skin and clothing. Many patients with refractory otomycotic infections have had previous canal wall mastoid surgery and require a hearing aid with a closed mold. Because the patient relies on the hearing aid virtually all day, trauma associated with placing and removing the hearing aid throughout the day can cause a significant problem. Ointments are not recommended for patients with closed hearing aids because they may promote fungal growth secondary to the accumulation of moisture.

NECROTIZING OTITIS EXTERNA

Necrotizing otitis externa is an infection of the EAC that spreads to the surrounding subcutaneous tissues and can lead to osteomyelitis of the skull base.[15] This condition was described first by Chandler[12,14] and was referred to as *malignant otitis externa* because of the aggressive extension of the disease and the poor outcome. Survival rates have improved during the past 2 decades as a result of increased awareness, allowing for earlier diagnosis and treatment.[15,16] Patients often are diabetic, immunocompromised, or undergoing post-radiation therapy.[5,37,39] The infection often begins as any other acute otitis externa involving the bony-cartilaginous junction and often extends through the fissures of Santorini to involve the parotid region. Facial paralysis can occur as a complication of this disease and does so more commonly in children than in adults in cases of necrotizing otitis externa.[30] Facial nerve paralysis in necrotizing otitis externa often is permanent.[13]

P. aeruginosa is the most common pathogen in necrotizing otitis externa. *Proteus mirabilis* also has been reported as a causal agent in some cases.[15,16] Necrotizing otitis externa should be suspected in the presence of a pseudomonal infection that does not resolve and may be associated with facial nerve paralysis. The presence of granulation in the external canal at the bony-cartilaginous junction also should raise suspicion of necrotizing otitis externa.

Diagnostic imaging, including computed tomography or magnetic resonance imaging, of the temporal region often is required.[21] The most common finding of extension beyond the ear canal is retrocondylar fat infiltration.[25] A gallium 67 scan often is diagnostic in the early course of the disease and provides a good way to monitor the efficacy of treatment. A positive technetium 99m scan is diagnostic for acute osteolytic osteomyelitis because it measures osteoclastic activity, but it may remain positive long after the infection has subsided. For this reason, a technetium 99m scan is not ideal for monitoring treatment response.[41]

Treatment of necrotizing otitis externa often involves multiple surgical débridements combined with topical and intravenous antibiotics. Hyperbaric oxygen also has been tried in patients, with some success.[17] In diabetic patients or immunocompromised patients, coadjuvant treatment to control the primary condition is crucial and should be initiated immediately.

DIFFERENTIAL DIAGNOSIS

Not every condition that manifests with pain and purulent discharge from the EAC is otitis externa. Multiple other entities that may manifest with one or more similar symptoms include relapsing polychondritis, suppurative otitis media, and herpetic infections.

Relapsing polychondritis is a progressive inflammatory disorder affecting cartilage. When it involves the ear, it usually involves the pinna and the cartilaginous portion of the EAC, manifesting as swelling and erythema.[3] This condition can affect any cartilaginous structure, including the trachea, the nasal septum, and the larynx. Destruction of the cartilage by inflammatory infiltrates is followed by granulation, fibrosis, and calcifications. It often is accompanied by arthralgias involving one or more joints and rarely affects only one ear.

Herpes simplex and herpes zoster also can affect the EAC. Both conditions manifest with painful vesicles along the EAC (Fig. 18–2) and can be accompanied by facial nerve paralysis (Ramsay Hunt syndrome or herpes zoster oticus).[2,43] Treatment is with antivirals, such as acyclovir or famciclovir.

Dermatologic conditions also can give the appearance of otitis externa. Allergic and contact dermatitis can manifest with erythema, weeping areas, and itching. Allergic dermatitis is a delayed hypersensitivity reaction resulting from substances such as poison

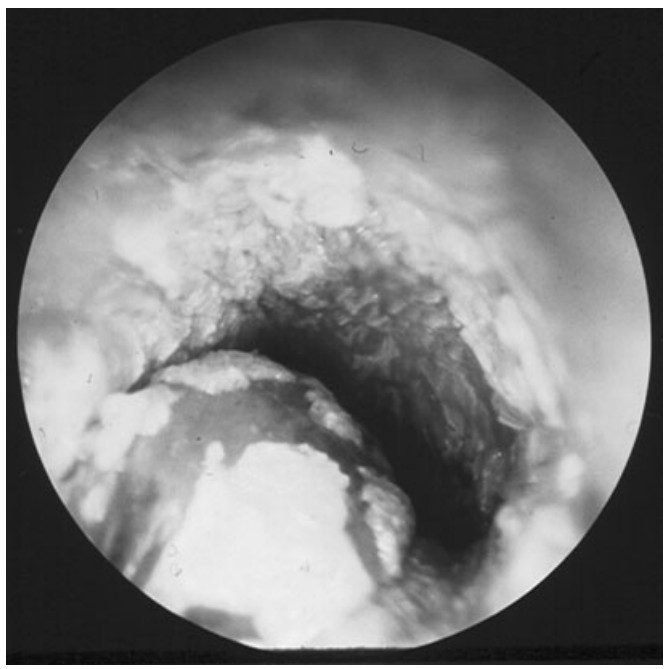

Figure 18–2 Ear canal with multiple vesicles from herpes zoster oticus. (See companion Expert Consult web site for color version.)

ivy, rubber, and nickel compounds.[28,40] Treatment is with administration of topical steroids and removal of the causative agent.

CONCLUSION

Understanding the external ear anatomy and its physiology allows clinicians to comprehend better the natural history of the diseases that affect this region. Despite improvements in diagnostic imaging techniques, no substitute exists for a good history and physical examination to differentiate among various conditions with a similar presentation. Treatment of otitis externa should focus on achieving relief of the acute condition; at the same time, clinicians should try to help prevent recurrences in patients with higher risk factors.

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CHAPTER 19

OTITIS MEDIA

Jerome O. Klein ✪ Charles D. Bluestone

The term *otitis media* denotes inflammation of the mucoperiosteal lining of the middle ear. *Acute otitis media* (*AOM*) is the rapid onset of signs and symptoms of acute infection within the middle ear. *Otitis media with effusion* is an inflammation of the middle ear in which a collection of liquid is present in the middle ear space, and no signs or symptoms of acute infection are present. *Middle ear effusion* denotes liquid in the middle ear. The effusion may be serous, a thin, watery liquid; mucoid, a thick, viscid, mucus-like liquid; purulent; or a combination of these forms. Fluctuating or persisting loss of hearing is present in most patients who have middle ear effusion; hearing impairment is the most frequent complication of AOM or otitis media with effusion. Suppurative complications of otitis media occur when inflammation and infection extend beyond the mucoperiosteal lining of the middle ear (e.g., mastoiditis, epidural abscess). An extended review of otitis media in infants and children was written by the authors and published in 2007.[25]

INCIDENCE AND EPIDEMIOLOGY OF ACUTE OTITIS MEDIA

AOM is one of the most common infectious diseases of childhood. A survey of diagnoses made in office practices in the United States in 1990 identified 24.5 million visits for which the principal diagnosis was otitis media; the number of diagnoses of otitis media had increased from 9.91 million visits recorded in 1975.[171] In Boston, Teele and associates[187] found that about 33 percent of pediatric office visits for illness of any kind were attributable to AOM or otitis media with effusion. The same group of investigators reported that 62 percent of children had had at least one episode of AOM by the time they reached 1 year of age, and 17 percent had experienced three or more episodes.[188] By 3 years of age, more than 80 percent of children had experienced at least one episode of AOM, and 46 percent had had three or more episodes. A similar preponderance of cases of AOM during the first or second year of life with a decline in incidence rate thereafter has been reported by investigators from locations as diverse geographically as Finland[152,175]; Sweden[88]; Cleveland, Ohio[118]; Huntsville, Alabama[86]; and Galveston, Texas.[17] The results of these studies suggest that by the time children reach 3 years of age, they may be categorized into three groups of about equal size relative to acute infections of the middle ear: one group is free of ear infections; a second group may have occasional episodes of otitis media, usually associated with infections of the respiratory tract; and a third group is otitis-prone, subject to repeated (three or more) episodes of acute infection.

A prospective study beginning in 1991 of more than 2000 children in Pittsburgh, Pennsylvania, surveyed during the first 2 years of life provided insight into the prevalence of otitis media, the amount of antibiotic used, and the proportion of children who required surgery for severe and recurrent disease.[142] Middle ear effusion was present 20.4 percent and 16.6 percent of the time during the first and second years of life, respectively; mean number of days of antimicrobial therapy for otitis media was 41.9 and 48.6 in the first and second years of life, respectively (>90% of all antibiotic treatment in the first 2 years of life was for otitis media); and 1.8 percent and 4.2 percent of the children had myringotomy and insertion of tympanostomy tubes in the first and second years of life, respectively.

Three factors are likely to alter the incidence and epidemiology of AOM in the United States in the next few years: (1) the introduction of the seven-valent pneumococcal conjugate vaccine (PCV7) in 2000; (2) the publication of management guidelines by the American Academy of Pediatrics (AAP) and the American Academy of Family Physicians (AAFP) in 2004, which included minimal criteria for establishing the diagnosis of AOM and an option of "watchful waiting," rather than immediate use of antimicrobial drugs; and (3) the educational campaign of the Centers for Disease Control and Prevention (CDC) and other professional groups to influence parents and physicians to avoid inappropriate uses of antimicrobial agents for trivial, usually viral, respiratory tract infections because of concern for development of multidrug resistance. Any one or all of these factors may play a role in decreasing the number of diagnoses of AOM, altering the microbiology of AOM, decreasing the number of surgical procedures for severe and recurrent otitis media, and reducing the volume of antimicrobial agents used in infants and children.

HOST RISK FACTORS

Table 19–1 lists the risk factors for developing severe and recurrent AOM. The peak age-specific attack rate occurs between 6 and 18 months of age. The frequent occurrence of otitis media in otherwise healthy infants is partly a reflection of the fact that the eustachian tube of the young child is shorter, floppier, straighter, and more horizontal than the eustachian tube of the older child. Organisms from the nasopharynx reach the middle ear more readily than they do in older children. By 3 years of age, the incidence of AOM decreases because of changes in the child's anatomy and physiology and maturing immune mechanisms. Children who have had little or no experience with otitis media by the time they reach age 3 years are unlikely to develop problems with middle ear infections, unless some predisposing factor, such as tumor or fracture of the base of the skull or a facial bone or acquired immunodeficiency, occurs.

TABLE 19–1 Risk Factors for Severe and Recurrent Acute Otitis Media

Male gender
Familial aggregation—disease in siblings and parents
Very low birth weight (<1500 g) and gestational age <33 wk
Early onset of disease
Race—Native American, Alaskan Eskimo, Australian Aborigine
Poverty—crowded living conditions, poor sanitation, lack of access to medical care
Prone sleeping position
Use of pacifier
Not breast-fed
Group daycare
Exposure to smoke and environmental antigens
Congenital or acquired immunodeficiency

AOM, similar to most bacterial infections in children, seems to occur more commonly in boys than in girls.[188] A genetic predisposition to AOM has been shown by a study in twins.[37] Histories of severe and recurrent ear infections in siblings and parents are found frequently in families with an otitis-prone child.[61,188] Although prematurity has not been associated previously with predisposition to middle ear infection, a study from the Netherlands suggests that a gestational age of younger than 33 weeks and very low birth weight (<1500 g) are risk factors for developing recurrent otitis media.[51] Age at first episode of AOM is associated significantly with recurrent episodes.[188]

Race and ethnicity as predisposing factors may be difficult to separate from poor social and economic conditions. Particularly high rates of the disease have been observed among Eskimos,[94,153,163] Native Americans,[141] and Australian Aboriginal children.[126] Factors of poverty predisposing children to development of respiratory infections and otitis media include crowded living conditions, poor sanitation, and limited access to medical care.[121]

Although most children with recurrent and severe otitis media have no obvious predisposing factor, a few have altered host defenses, including anatomic changes (e.g., cleft palate or uvula, submucous cleft), alterations of normal physiologic defenses (e.g., patulous eustachian tube, barotrauma), and congenital or acquired immunologic deficiencies (e.g., immunoglobulin deficiency, chronic granulomatous disease, malignancy). Active middle ear disease is a constant event in children with cleft palate.[134,136] Children with acquired immunodeficiency syndrome have a higher age-specific incidence of otitis media beginning at 6 months of age compared with uninfected children.[8] Nasotracheal intubation has been identified as a factor in the development of AOM and otitis media with effusion in neonates and older children.[15,48,145]

ENVIRONMENTAL RISK FACTORS

An increased incidence of respiratory infections, including otitis media, among children in group daycare compared with children

receiving home care has been documented in the United States,[78,128,165] Sweden,[80] and Finland.[3,174] A survey of children in Memphis, Tennessee, found that children in daycare experienced more episodes of otitis media and were more likely to have placement of ventilation tubes.[9] By the second year of life, 21 percent of children in Pittsburgh observed from birth who were in group daycare (7 children or more) had surgical procedures for middle ear disease (almost all were myringotomy and placement of tubes) compared with only 3 percent of children in home care.[198] The increase in incidence of otitis media from 9.91 million office visits in 1975 to 24.5 million office visits in 1990 is associated with increased usage of group daycare for young infants.[171]

Placing infants in daycare usually is necessitated by the professional needs of one or both parents. Paid maternal leave, as is fostered in some European countries, results in an increased proportion of mothers who are breast-feeding and delayed entry of infants into out-of-home daycare. In the Czech Republic, paid maternal leave is available to all women for 9 months after giving birth and is optional to the child's third birthday. Paid parental leave now is reaching a stage of discussion and experimental programs in the United States. The Federal Family and Leave Act guarantees 6 weeks of unpaid leave, but more generous programs are available in Massachusetts, Vermont, Maryland, and Washington.

Infants who are breast-fed have fewer incidents of ear disease than infants who are bottle-fed. In a Boston, Massachusetts, study,[188] breast-feeding for 3 months or more was associated with decreased risk of developing AOM in the first year of life. Although bottle-fed infants are placed in a reclining or horizontal position and breast-fed infants are held in a vertical position, the data suggest that a constituent of breast milk is the important factor and not position during feeding. Of children with cleft palate who were provided breast milk or formula in a similar container, children who received breast milk had fewer cases of middle ear effusion.[139]

Sleep position and use of a pacifier have been identified as risk factors for recurrent episodes of AOM. More episodes of AOM were identified in infants who slept prone (compared with infants who slept supine) in an investigation of 14,000 infants in Bristol, England.[62] Use of a pacifier increased the risk for development of recurrent AOM in Finnish children attending daycare centers.[129] More than three episodes of AOM occurred in 29.5 percent of children younger than 2 years of age using pacifiers compared with 20.6 percent of children not using pacifiers; in children 2 to 3 years old, the incidences of recurrent AOM were 30.6 percent and 13.2 percent, respectively.

Allergy to environmental antigens plays a role in congestion of the mucosa of the eustachian tube. Exposure to smoke can result in goblet cell hyperplasia, mucus hypersecretion, ciliostasis, and decreased mucociliary transport.[192] The availability of a biochemical marker, cotinine, in saliva, serum, or urine has rendered documentation of passive exposure to tobacco smoke more reliable than that provided by history alone. High concentrations of serum cotinine were associated by Etzel and colleagues[53] with increased incidence of AOM and increased duration of middle ear effusion. Kim and colleagues[98] in Houston, Texas, documented the association of invasive pneumococcal infections in children and adults with increased environmental exposures to sulfur dioxide (a marker for air pollution) and higher counts of ragweed pollen.

Studies in the United Kingdom and the United States show seasonal variation in the occurrence of AOM. The pattern within a period of 1 year is sinusoidal, with the peak incidence in December through March and lowest incidence in July through September.[87,123] These findings do not correlate with general climatic conditions because the U.S. studies were performed in Texas and Washington, D.C., and the United Kingdom study was done in northern England. The incidence coincides, however, with the peak incidence of respiratory infections in both countries.

COST ANALYSES

Analyses of cost of management of otitis media have provided additional insights into the epidemiology of otitis media. Direct costs of an episode of otitis media include health care visits, cost of drugs, consultations, surgical procedures, audiometry and remedial speech and language visits, and hospitalizations related to severe AOM or its complications. Indirect costs include transportation, babysitters, and lost time from work. The average total cost of treating an episode of otitis media was estimated to be $116[93] to $131 in 1997 and 2000.[32] In 1996, the combined cost of AOM and its sequelae was estimated to be more than $5 billion every year. Mean costs of surgical procedures for severe and recurrent AOM in a managed care population in northern California for 1997 included myringotomy and placement of tympanostomy tube at $1400, mastoidectomy at $5062, and adenoidectomy at $1500.[32]

ETIOLOGIC AGENTS

The microbiology of otitis media has been documented by cultures of middle ear effusions obtained by needle aspiration (Table 19–2). In 1906, German investigators studied the bacteriology of AOM by aspiration of middle ear fluids. Many microbiologic studies were done, most in European otolaryngologic centers, before the introduction of antimicrobial agents. In the 1960s, investigative studies of the bacteriology of AOM were done in pediatric clinics and offices. Group A streptococcus was the dominant pathogen throughout the early studies but diminished in importance as more cases of mild and moderate disease were studied in pediatric programs, in contrast to the more severe disease referred to otolaryngologic centers. *Streptococcus pneumoniae*, which had been second to group A streptococci in frequency in the studies before 1960, now became the pathogen most frequently identified, followed by *Haemophilus influenzae* and *Moraxella catarrhalis*. More recent studies suggest the extensive use of the conjugate pneumococcal vaccine may have resulted in a decrease in the number of episodes of AOM caused by *S. pneumoniae* and a proportional increase in the incidence of disease caused by *H. influenzae* and *M. catarrhalis*.[21,34]

Bacteria may be isolated from middle ear fluid in approximately two thirds of patients with AOM. The finding that approximately one third of patients have sterile cultures may reflect limitations of bacterial culture methods because antigen detection tests often indicate the presence of pneumococcal capsular polysaccharide in sterile middle ear fluids.[107,113] In addition,

TABLE 19–2 Bacterial Pathogens Isolated from Middle Ear Aspirates in Infants and Children with Acute Otitis Media (Percentage of Children with Pathogen, 1985-1992)*

From Bluestone, C. D., and Klein, J. O.: Otitis Media in Infants and Children. 4th ed. Hamilton, Ontario, B. C. Decker, 2007.

polymerase chain reaction for bacterial genome sequences has identified DNA of *S. pneumoniae*, *H. influenzae*, and *M. catarrhalis* in patients with otitis media with effusion whose cultures were negative for these bacterial species.[149] Concomitant isolation of two or more organisms in the same effusion occurs in 7 percent of cases. Disparate results of cultures in children with bilateral AOM occur in approximately 20 percent of cases.[144] The detection of bacterial biofilms in the middle ear mucosa of children with chronic otitis media raises the possibility of metabolically active bacteria in culture-negative middle ear effusions.[71,149]

Several clinical situations warrant special consideration: (1) The occurrence of purulent conjunctivitis in association with AOM (conjunctivitis-otitis syndrome) usually is attributable to nontypeable *H. influenzae*[27,28]; (2) AOM occurs commonly among children hospitalized in intensive care units, and the bacteriology may be reflective of the hospital environment[48]; (3) renewed signs of AOM within 14 days usually represent relapse, whereas signs occurring 21 or more days after a prior infection are likely to indicate a new infection or recurrence[33]; and (4) children with tympanostomy tubes may develop AOM caused by organisms associated with otitis externa and AOM (e.g., *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Staphylococcus epidermidis*, *S. pneumoniae*, and *H. influenzae*).[164]

The bacteriology of otitis media with effusion mimics that of AOM.[64,65,124,155,170,181] In contrast, the etiologic agents of chronic suppurative otitis media with persistent perforation include *P. aeruginosa*, *S. aureus*, anaerobic bacteria, and enteric gram-negative bacilli.[29,96,133] *Mycobacterium tuberculosis* is a rare but important cause of chronic suppurative otitis media with persistent perforation.[200]

Bacteria found in middle ear aspirates usually are present in the nasopharynx of children with AOM, but multiple pathogens may be present in the nasopharynx that are not present in the middle ear.[54] Although not useful for specific microbiologic diagnosis of AOM, nasopharyngeal cultures are valuable for monitoring antibiotic susceptibility patterns of bacterial pathogens associated with AOM. Several investigators have noted quantitative differences in the nasopharyngeal flora of patients with and without otitis media, and these differences may play a role in the pathogenesis of middle ear disease. Long and colleagues[111] described a significant association between the recovery of abundant *H. influenzae* (at least 50% total colony count) from the nasopharynx and bacteriologically confirmed otitis media. An additional finding was that a semiquantitative nasopharyngeal culture was sensitive and specific in predicting the middle ear pathogen. Similar nasopharyngeal colonization rates for *S. pneumoniae* occur in ill and healthy children.[79,110,111] Gray and coworkers[70] have correlated the occurrence of AOM with nasopharyngeal acquisition of new serotypes of *S. pneumoniae*.

S. pneumoniae is the most frequent cause of severe AOM and suppurative complications. Of the 90 antigenically distinct pneumococcal serotypes, only few serotypes are responsible for most cases of otitis media. In more recent surveys, the most common types responsible for AOM in order of decreasing frequency were types 19F, 23, 14, 6B, and 3 (Table 19–3). All of these types except serotype 3 are included in the conjugate vaccine, PCV7. The serotypes responsible for sequential episodes of AOM were described by Austrian and colleagues.[5] Of interest is the constancy of the four most frequently isolated types in recurrent episodes. Other features of interest in the study are (1)

TABLE 19–3 Distribution of Pneumococcal Serotypes in Children with Acute Otitis Media (%)

Year of Study City Country	1948-1951 Turku Finland	1970-1977 U.S.	1985-1989 Birmingham U.S.	1994-2000 Multinational	1995-1999 Finland	1996-1999 U.S.	2000-2002 Czech Republic and Slovakia
No. Strains	239	958	228	5520	414	500	189
Serotype							
1	10*	2.7	1.8	1.6	—	—	—
3	33.9	6.3	6.1	4	3.1	9.6	9
4	1.7	3.5	0.9	0.7	1	0.4	1.6
5	3.3	0.2	—	1	—	0.4	—
6	5.9	—	14	—			
6A	—	4.9	—	7.3	10.9	9.6	5.8
6B	—	7.3	—	10.1	13.5	10.8	12.7
7	3.8	2.2	0.4	0.8	—	0.4	—
8	2.9	1.9	—	—	—		—
9	—	—	4.4	—	—		—
9N	—	0.8	—	—	1.9		2.1
9V	—	7.1	—	4.6	—	5.2	—
10	—	1.2	—	—	—	—	—
11	1.3	1.2	3.1	1.3	5.8		—
14	1.7	10.2	10.9	13.1	6.3	15.8	11.6
15	1.3	4	—	2.1	5.6		—
18	3.8	5.3	2.2	—	0.2	1.4	3.7
19	10	—	33.2				
19A		5.5		6.6		5.4	1.6
19F		15.7		16.1	14	23.8	22.8
23	5	12.5	14	—			
23A		0.1	—	—	1	0.4	1.1
23F	—	11.4	—	14.9	19.8	10	9.5
27	3.3	0.1	—	—			
35	—	0.1	—	—	2.4		—
Author	Lahikainen, 1953[106]	Austrian et al., 1977[5]	Orange and Gray, 1993[132]	Hausdorff et al., 2002[76]	Eskola et al., 2001[52]	Joloba et al., 2001[89]	Prymula et al., 2006[151]

**Four most frequent serotypes are shaded.*

simultaneous infection of the middle ear fluid by two pneumococcal types, (2) isolation of the same type in consecutive episodes, and (3) isolation of the same type after an intercurrent episode caused by another type. At present, monitoring of pneumococcal serotypes responsible for AOM and invasive pneumococcal diseases is important in determining the durability of efficacy of the conjugate pneumococcal vaccine.

Otitis media caused by *H. influenzae* almost always is associated with nontypeable strains.[84,85,178] The clinical presentation of AOM caused by nontypeable *H. influenzae* usually is less severe than acute infection caused by *S. pneumoniae*, as measured by temperature elevation, ear pain, suppurative complications, and perforation of the tympanic membrane. Before the introduction of the conjugate *H. influenzae* type b vaccine, approximately 10 percent of children with *Haemophilus* spp. otitis had disease caused by type b, and approximately one fourth of these children had concurrent or subsequent bacteremia or meningitis.[74] *H. influenzae* causes AOM in all age groups, including older children and adolescents.[166,168] Of *H. influenzae* strains isolated from middle ear fluids, 30 to 60 percent produce β-lactamase.[105,173,195]

Although *S. pneumoniae* and *H. influenzae* are responsible for most cases of bacterial otitis media, *M. catarrhalis* and group A streptococci are responsible for some cases and should be considered in choosing appropriate antimicrobial agents. The incidence of AOM caused by *M. catarrhalis* in most studies is less than 10 percent but was noted to be 22 percent and 27 percent in 1983 reports from Pittsburgh[105] and Cleveland,[173] respectively, and 18.4 percent in a study of Finnish children observed between 1995 and 1997.[97] Most strains of *M. catarrhalis* isolated from middle ear fluids produce β-lactamase, and some patients fail to improve if they are treated with a β-lactamase-susceptible drug.

During the pre-antibiotic era, otitis media caused by group A streptococci frequently was associated with scarlet fever and often was of a severe and destructive form. In recent years, group A streptococci have been isolated frequently in some studies from Scandinavia but have been found infrequently in most studies from the United States. A survey of Israeli children identified group A streptococci in 350 of 11,311 episodes (3.1%) of AOM during 1999 to 2003. The incidence of mastoiditis was higher for patients with streptococcal AOM than for patients with other pathogens.[169]

Tuberculous otitis was an occasional cause of severe middle ear disease at the turn of the 20th century in the United States and western Europe and still occurs in developing countries. Otitis caused by *M. tuberculosis* is characterized by a painless, watery otorrhea through single or multiple perforations of the tympanic membrane.[176] Other bacteria, including *S. aureus* (which occurs infrequently in the United States but is the etiologic agent in 10% of cases in Japan[6]), gram-negative enteric bacilli (responsible for approximately 20% of otitis media cases in neonates, but a rare finding in older infants), anaerobic bacteria, *Clostridium tetani*, and *Corynebacterium diphtheriae*, are responsible for occasional cases of AOM.

Chlamydia trachomatis[190] has been identified in infants 6 months of age and younger, often associated with pneumonia. The role of *Chlamydia pneumoniae* in AOM is uncertain.[131,183]

The clinical history suggests that viral infection serves as a frequent initiating event of AOM by producing congestion of the mucosa of the upper respiratory tract. Epidemiologic data support an association between viral respiratory infection and the occurrence of AOM.[77] Upper respiratory tract infection with respiratory syncytial virus, influenza viruses, and adenoviruses was associated with a greater risk for developing otitis media than was infection with other viruses. Early studies identified a low viral isolation rate from middle ear fluid in patients with otitis media. A virus was isolated from only 29 of 663 (4.4%) specimens obtained by tympanocentesis and reviewed by Klein and Teele in 1976.[104] A higher virus identification rate in middle ear fluid using culture and antigen detection has been reported.[41,99,162]

Pitkaranta and colleagues[148] used reverse transcriptase polymerase chain reaction to identify viruses in middle ear fluids of children with AOM and otitis media with effusion; evidence of rhinovirus was found in 22 percent and 19 percent, respectively, and respiratory syncytial virus was found in 18 percent and 8 percent, respectively. Ruuskanen and colleagues[159] summarized eight studies published between 1982 and 1990 using immunoassay or isolation; virus was identified in middle ear fluids in 17 percent of the samples—as a single agent in 6 percent and in combination with a bacterial pathogen in 11 percent. Viruses identified in middle ear fluids have included respiratory syncytial virus, influenza viruses, adenoviruses, parainfluenza viruses, enteroviruses, and rhinoviruses.

Concomitant isolation of viral and bacterial pathogens from middle ear fluid seems to be a common finding.[41,162] A survey of the microbiology of new-onset otorrhea in 79 children with tympanostomy tubes identified bacteria in 92 percent, viruses in 70 percent, and bacteria and viruses in 66 percent.[158] In an accompanying commentary, Chonmaitree[40] noted that AOM no longer should be considered a pure bacterial disease and that antibacterial treatment may not result in optimal outcome in some cases because of viral co-infection.

ETIOLOGY IN NEONATES

Clinical investigators have performed needle tympanocentesis to isolate bacterial pathogens causing otitis media in the first 6 weeks of life. Four studies included 169 infants.[14,20,172,189] Bacteria were isolated from middle ear fluid in 68 percent of cases. As in older children, *S. pneumoniae* and *H. influenzae* were the organisms most frequently isolated. Other than the more frequent occurrence of disease caused by gram-negative enteric organisms (approximately 20% cases) and the occasional isolation of other neonatal pathogens (e.g., group B streptococci), the bacteriology of otitis media in this age group was similar to that in older children.

PATHOGENESIS

The pathogenesis of otitis media is likely to follow a similar sequence of events in most children. The patient has an antecedent event (usually caused by an upper respiratory viral infection) that results in congestion of the respiratory mucosa throughout the respiratory tract, including the nose, nasopharynx, eustachian tube, and middle ear; congestion of the mucosa in the eustachian tube results in obstruction of the narrowest portion of the tube, the isthmus. The obstruction results in negative pressure in the middle ear and then development of middle ear effusion. The secretions of the mucosa of the middle ear, which usually drain through the eustachian tube, now have no egress and accumulate in the middle ear. The effusion may be asymptomatic (i.e., lacking the signs and symptoms of acute infection) and is termed *otitis media with effusion*. If pathogenic bacteria or viruses that colonize the nasopharynx are present in the middle ear after obstruction of the eustachian tube has occurred, the organisms multiply, resulting in an acute suppurative infection, with an abscess, and characterized by signs and symptoms of acute infection such as fever and otalgia.[24,25,66,161] For children with recurrent episodes of AOM or otitis media with effusion, anatomic or physiologic abnormalities of the eustachian tube seem to be predisposing factors.

It also is possible that subtle changes in immune response occur that predispose a child to frequent episodes of otitis media. Experimental studies provide evidence that virus-induced

Figure 19–1 Persistence of middle ear effusion after onset of acute otitis media. OME, otitis media with effusions. *(Modified from Teele, D. W., Klein, J. O., and Rosner, B. A.: Epidemiology of otitis media in children. Ann. Otol. Rhinol. Laryngol. 89:5-6, 1980;* in *Bluestone, C. D., and Klein, J. O.: Otitis Media in Infants and Children. 4th ed. Hamilton, Ontario, B. C. Decker, 2007, p. 80.)*

Figure 19–2 The middle ear. *(From Klein, J. O., and Daum, R. S.: The Diagnosis and Management of the Patient with Otitis Media. Copyright Biomedical Information Corporation, New York, 1985.)*

impairments in neutrophil migration and bacterial killing also may be important in the pathogenesis of AOM.[2]

With growth of the skull and change in the position, length, and width of the eustachian tube over the course of time, the predilection to otitis media in accompanying acute infections of the upper respiratory tract in the first 3 years of life diminishes, and the patient has fewer episodes of AOM. Children younger than 3 years of age with similar respiratory infections are predisposed to the complication of acute infection of the middle ear, whereas older children challenged by the same microorganism have signs of upper respiratory infection but need not have a complicating ear infection.

The pathogenesis of persistent middle ear effusion or otitis media with effusion is uncertain. An effective antimicrobial agent sterilizes the acute bacterial infection of AOM. The middle ear effusion, now sterile, may persist for weeks to months. The median duration of middle ear effusion after AOM is approximately 23 days (Fig. 19–1). The type of antibacterial drug used does not seem to alter the duration of fluid in the middle ear after acute infection occurs.

PATHOPHYSIOLOGY

TYMPANIC MEMBRANE

In the presence of otitis media, changes in the tympanic membrane occur rapidly. The presence of congested blood vessels, edema (which obscures normal landmarks), and bulging or sagging of Shrapnell membrane indicate not only a myringitis (inflammation of the tympanic membrane) but also the presence of fluid in the middle ear space. Blebs that appear on the surface epithelium are a consequence of AOM or edema or hydropic degeneration of the membrane.

Inflammation may occur on outer epithelial or inner mucosal sides of the fibrous layer (middle layer) of the drum. In severe cases, infection may involve the fibrous layer itself. The membrane thickens as a result of edema and infiltration of polymorphonuclear leukocytes. All three layers of the drum may undergo dissolution, owing to pressure necrosis resulting from the expanding middle ear abscess or thrombophlebitis of tympanic veins, with resulting perforation. With evacuation of the contents of the middle ear abscess, healing may be rapid, and the perforation usually seals within a few days. In the process of healing, metaplasia of the epithelium, hyaline degeneration, calcium deposition, and scar formation may occur.

Occasionally, when a perforation is close to the margin of the annulus or occurs in Shrapnell membrane, the skin of the external auditory canal (EAC) and the surface squamous epithelium of the tympanic membrane may grow through the aperture and invade the middle ear. This event may lead to formation of a cholesteatoma (epidermal inclusion cyst). Even if the perforation heals, a differential in gas pressure across the tympanic membrane caused by malfunction of the eustachian tube may result in resorption of gas in the middle ear cavity and negative pressure in the middle ear, which causes retraction of Shrapnell membrane or an atrophic scar into the middle ear or mastoid attic.

EUSTACHIAN TUBE

The eustachian tube is approximately 3.8 cm long in an adult. It opens in the fossa of Rosenmüller and extends upward, backward, and laterally to open in the upper anterior wall of the tympanic cavity (protympanum). In a child, the tube is shorter and floppier. The eustachian tube is composed of two portions: the cartilaginous portion extending into the nasopharynx and the bony portion originating in the middle ear. The upper third of the tube is bony; the middle ear opening is the widest; and the medial end (the part joining the cartilaginous eustachian tube), or isthmus, is the narrowest. Pneumatic peritubal air cells arising from the middle ear cavity surround it and can extend to the petrous apex. The internal carotid artery lies anteromedial to this region (Fig. 19–2).

The lower two thirds of the eustachian tube is a narrow, slitlike, fibrocartilaginous passage. It makes a 160-degree angle with the bony portion at its junction. The cross section of the tube

looks like a shepherd's crook, with a cartilaginous superior and medial surface and a fibrous lateral surface.

Three muscles are associated with the eustachian tube. The tensor tympani muscle lies on top of it; the levator palatini muscle lies under it; and the tensor palatini muscle arises on the tube, scaphoid fossa, and spine of sphenoid and then courses around the hook of the hamulus and forms an aponeurosis with its mate (from the opposite side) in the soft palate. This muscle is the only one that acts directly on the eustachian tube.

The eustachian tube area, protympanum, and hypotympanum are lined by ciliated columnar epithelium with goblet cells or secretory cells. The epithelium is continuous with the upper airway system and paranasal sinuses. This area also contains a well-defined subepithelial connective tissue layer, which thins out and may be absent nearing the antrum and mastoid air cell system. The movement of the cilia and mucus blanket always is toward the eustachian tube and nasopharynx. The tube is surrounded by a plexus of lymphoid channels. It has an arterial supply from a branch of the middle meningeal or accessory meningeal artery and from branches of the artery of the pterygoid canal. The nerve supply is from the tympanic plexus (ninth cranial nerve) (sensory) and sphenopalatine ganglion (sympathetics and parasympathetic palatine fiber).

The bony portion is rigid and patulous; the medial two thirds normally is held closed by elastic recoil of the fibrocartilaginous tissue. Contraction of the tensor palatini muscle that inserts in the anterolateral wall opens the tube on swallowing. On average, an adult swallows once per minute while awake and once every 5 minutes while asleep. Suckling infants usually swallow five times per minute.

Mucus and ciliary action flow from the middle ear to the eustachian tube. The eustachian tube acts as a unidirectional valve that favors outflow from the middle ear to the pharynx. Reverse flow can be induced by an increase in pressure in the nasopharynx (Valsalva, barotrauma). During occlusion of the eustachian tube, oxygen and carbon dioxide (and other gases) are absorbed from the middle ear by diffusion into the rich vasculature, and a negative pressure is created. A patent eustachian tube is a crucial prerequisite for subsidence of middle ear disease.

DIAGNOSIS

To establish uniformity of diagnosis, the clinical practice guideline published by the AAP and the AAFP proposed criteria for diagnosis of AOM.[4] The diagnosis required (1) a history of acute onset of signs and symptoms, (2) the presence of middle ear effusion, and (3) signs and symptoms of middle ear inflammation. The presence of middle ear effusion was identified by any of the following: (1) bulging of the tympanic membrane, (2) limited or absent mobility of the tympanic membrane, (3) air-fluid level behind the tympanic membrane, or (4) otorrhea. Signs or symptoms of middle ear inflammation were identified by either erythema of the tympanic membrane or otalgia that results in interference with or precludes normal activity or sleep. The following sections expand these definitions.

CLINICAL PRESENTATION

Children with AOM may have nonspecific signs and symptoms, including fever, irritability, headache, apathy, anorexia, vomiting, and diarrhea. Signs of respiratory viral infection, including cough and coryza, usually are present before the specific signs of ear infection occur. Fever occurs in approximately one third[167] to two thirds[127] of children with otitis media.

Specific signs and symptoms associated with otitis media and its complications and sequelae include the following:

1. Otalgia, or ear pain, is the most common complaint of infants and children with AOM. The symptom is suggested in young infants who are pulling at the ear or excessively irritable. Some infants do not have earache; Hayden and Schwartz[77] identified absence of ear pain in approximately one fifth of 335 consecutively diagnosed episodes of otitis media, usually among children older than 2 years of age.
2. Otorrhea is discharge from the middle ear through a perforation in the tympanic membrane or from the EAC when inflamed. The acute perforation usually is central in the membrane. Relief of the pressure on the tympanic membrane results in immediate pain relief and usually a decrease in temperature. Because the tympanic membrane has a dense network of blood vessels, rapid repair of the membrane occurs, and the perforation usually is unapparent within 24 to 72 hours. If the tympanic membrane seals and mucous membrane infection still is present, fluid may reaccumulate with renewed acute signs of otitis media.
3. Hearing loss occurs whenever fluid fills the middle ear space, whether the fluid is associated with acute infection or with otitis media with effusion. When fluid fills the middle ear space, the median hearing loss is 25 dB (the equivalent of having plugs in the ear canals).[59]
4. Vertigo occurs but is not a common complaint of children with otitis media. Vertigo occurs more commonly in unilateral than bilateral disease and may be caused by labyrinthitis. Older children describe a feeling of spinning, whereas younger children may not be able to verbalize these symptoms but manifest disequilibrium by falling or stumbling.
5. Tinnitus is an uncommon complaint in children, but when it does occur, the symptom often is caused by otitis media and eustachian tube dysfunction.
6. Swelling around the ear, especially in the postauricular area, may be a sign of mastoiditis.
7. Facial paralysis in children occurs as a complication of AOM or chronic otitis media with perforation of the tympanic membrane or as a result of an enlarging cholesteatoma.
8. Conjunctivitis has been associated with AOM caused by nontypeable strains of *H. influenzae*. The conjunctivae are injected, with tearing or purulent discharge.[28]
9. Craniofacial anomalies, such as cleft palate, mandibulofacial dysostosis, and Down syndrome, may predispose to frequent ear disease. Hypernasal speech suggests velopharyngeal insufficiency.

EXAMINATION OF THE EAR

OTOSCOPY

Examination of the ear should begin with observation of the auricle and the external auditory meatus. Palpation of the periauricular areas should be done to indicate presence of periostitis or diffuse external otitis. The ear canal should be examined for inflammation or cerumen that obstructs vision of the tympanic membrane.

For proper assessment of the tympanic membrane and its mobility, a pneumatic otoscope in which the diagnostic head has a secure seal should be used. The speculum should have the largest lumen that can fit comfortably into the child's cartilaginous external auditory meatus. The important landmarks of the tympanic membrane that can be visualized with the otoscope are indicated in Figure 19–3. The otoscopic examination should include observation of the following conditions of the tympanic membrane:

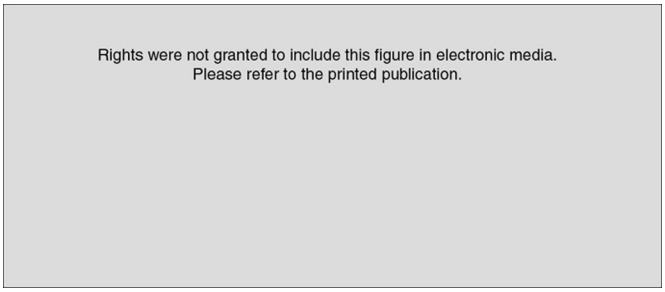

Figure 19–3 Important landmarks of the tympanic membrane that usually can be visualized with the otoscope. *(From Bluestone, C. D., and Klein, J. O.: Otitis Media in Infants and Children. 4th ed. Hamilton, Ontario, B. C. Decker, 2007, p. 156.)*

- *Position*: Normal is slightly convex; bulging indicates increased pressure from positive air pressure or fluid; a retracted drum indicates negative pressure with or without effusion; fullness of the tympanic membrane is apparent initially in the posterosuperior portion of the pars tensa and the pars flaccida because these two areas are the most highly compliant parts of the membrane.
- *Appearance and color*: The normal color is pearly gray and translucent; any congestion of the mucous membrane of the middle ear would be reflected in congestion of the vessels of the tympanic membrane and appear pink; a blue discoloration suggests blood in the middle ear associated sometimes with basal skull fracture; the inflamed middle ear mucosa usually is reflected in a bright red tympanic membrane.
- *Integrity of the membrane*: All four quadrants of the tympanic membrane should be inspected for presence or absence of perforation, retraction pockets, or cholesteatoma.
- *Mobility*: Application of positive and negative pressures by the pneumatic otoscope enables the viewer to determine the presence of an air-filled space (rapid excursion of the membrane on positive and negative pressures) or a fluid-filled space (limited or no excursion of the membrane); a middle ear with negative pressure does not respond to otoscopic negative pressure, and a middle ear with high positive pressure does not respond to otoscopic positive pressure (Fig. 19–4).

TYMPANOMETRY

Tympanometry measures the compliance of the tympanic membrane (Fig. 19–5).[30,45,120,150,154,191] Under normal circumstances, the pressure in the middle ear virtually is the same as the external ambient pressure; the eustachian tube functions to equate middle ear pressure to atmospheric pressure. If for any reason a pressure differential occurs across the tympanic membrane, stress is applied to the drum. Middle ear compliance varies as a function of the pressure differential across the tympanic membrane. When blockage of the eustachian tube with no fluid in the middle ear occurs, the tympanometry curve has a shape similar to that observed on a normal tympanogram, but the point of maximal compliance is shifted to the negative pressure side because maximal compliance is reached when the tympanic membrane reaches a peak of compliance (i.e., when EAC pressure is reduced to the same level as in the middle ear).

If ossicular discontinuity or a flaccid or atrophic tympanic membrane is present, the drum is highly compliant, and a highly peaked tympanogram is obtained. Abnormal tympanograms obtained in the presence of fluid in the middle ear are characterized by the following:

1. Reduced height of the curve (i.e., the middle ear has reduced compliance)
2. Shift to negative pressure sides of the curve and point of maximal compliance (i.e., eustachian tube blockage)
3. A flat curve without definite peak, the most characteristic feature

Although tympanometry with the electroacoustic impedance bridge has proved to be a satisfactory method for detecting the presence of fluid in the middle ear cavity, technical difficulties exist in obtaining accurate readings in infants and children, including the requirement for a secure seal of the probe in the ear canal, the need for a period of quiet to obtain an accurate reading, and decreased accuracy in infants younger than 7 months of age (because of the highly compliant EAC in infants <7 months).[143] In addition, only a few of many instruments currently on the market in the United States have data about sensitivity and specificity based on tympanometric patterns of otoscopic results before myringotomy.[57,130] Less persuasive data are available from studies correlating tympanometry and otoscopy findings.[30,120,150]

ACOUSTIC REFLECTOMETRY

The acoustic otoscope, or reflectometer (Ear Check Pro; Innova Medical, Lenexa, KS), is a handheld instrument that uses a microphone in the probe tip placed in the opening of the child's external ear canal. A professional and a consumer model were developed. The tip measures the level of transmitted and reflected sound from a less than 90 dB sound source that varies from 1800 to 4400 Hz in a 750-msec period. Acoustic energy is reflected back toward the probe tip from the ear canal and eardrum. The operating principle is based on the fact that a sound wave in a closed tube would be reflected when it strikes the end of the tube. Sound reflectivity is measured in units that indicate the status of a fluid-filled or air-filled middle ear.[44] Babonis and associates[7] found that tympanometry and reflectometry had comparable accuracy in predicting middle ear effusion documented by myringotomy. Acoustic reflectometry has some technical advantages compared with tympanometry: Accurate readings can be obtained in crying children, and a secure seal of the probe tip in the ear canal is not required.

AUDIOMETRIC TESTS

Audiometric testing may be employed to measure auditory acuity and evaluate conductive hearing losses, but assessment of hearing is an inaccurate method for identifying middle ear effusion. Hearing loss is the most prevalent complication of otitis media and is present uniformly whenever fluid fills the middle ear. The audiogram usually reveals a mild to moderate conductive hearing loss (median 25 dB).[59] Obstruction of the eustachian tube early in the clinical course of otitis media results in absorption of gases from the middle ear and drum retraction. The reduced compliance of the drum results in increased stiffness in the ossicular chain system. The audiogram reveals a low-frequency conductive hearing loss. As serous effusion appears and the middle ear fills with serum and pus, the ossicular system has an increased mass applied to it, and the audiogram flattens out, resulting in a high-frequency conductive hearing loss.

TYMPANOCENTESIS AND MYRINGOTOMY

Tympanocentesis, a needle aspiration of the middle ear effusion, is used primarily for establishing the presence or absence of an effusion and for microbiologic study. Because cultures of the

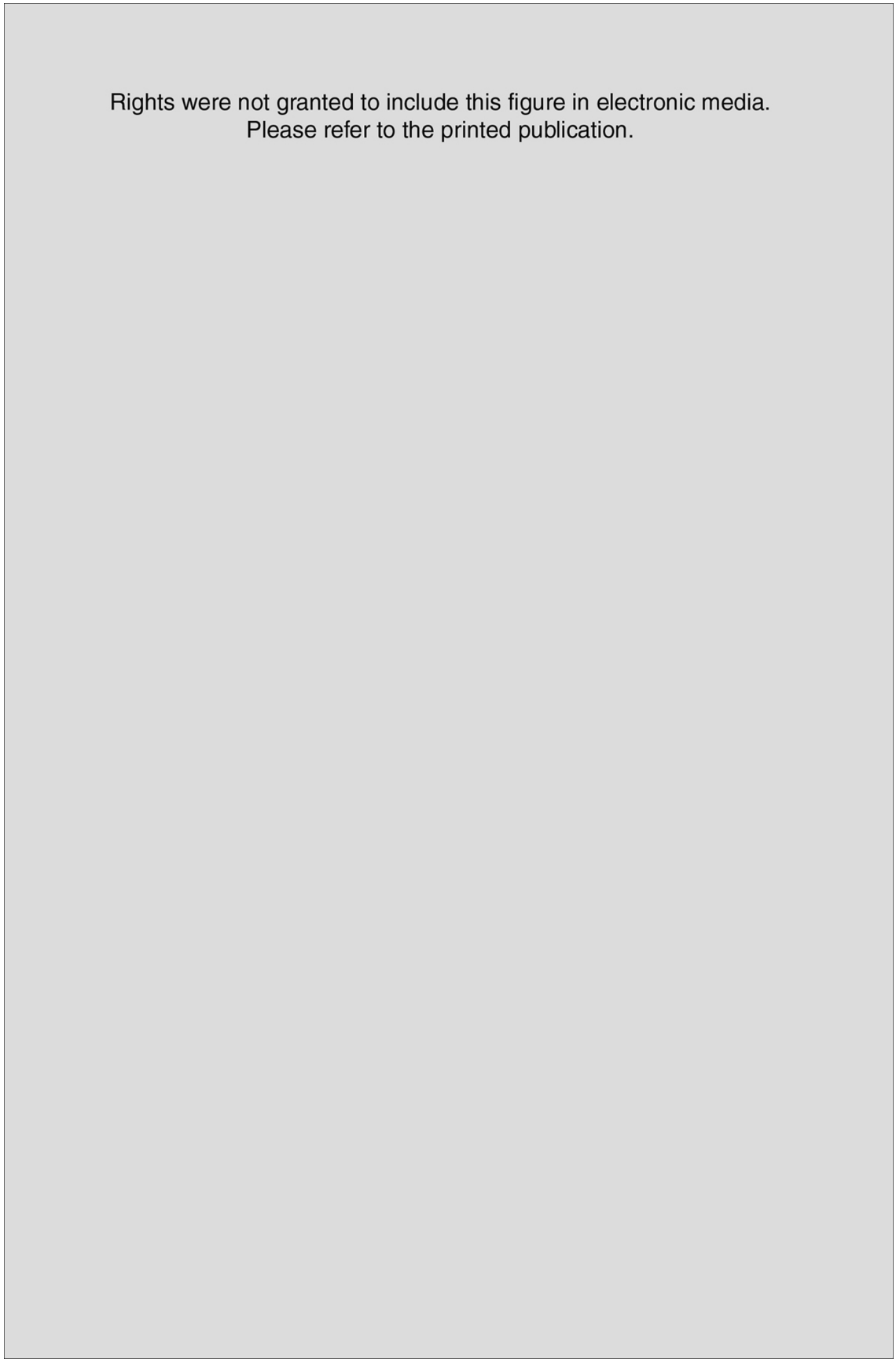

Figure 19–4 Otoscopic views and corresponding lateral sections through the tympanic membranes and middle ear show the various positions of the drum with their respective anatomic landmarks. *(From Bluestone, C. D., and Klein, J. O.: Otitis Media in Infants and Children. 4th ed. Hamilton, Ontario, B. C. Decker, 2007, p. 153.)*

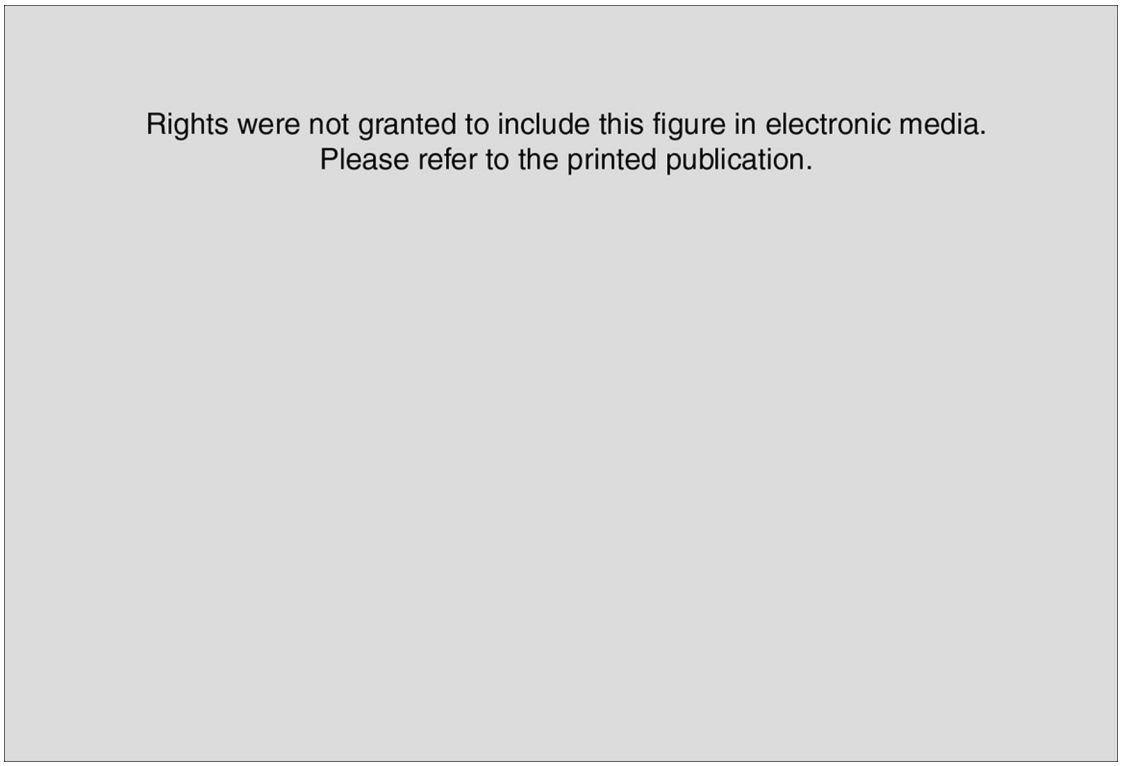

Figure 19–5 Tympanogram types related to presumptive conditions of the middle ear. *(From Bluestone, C. D., and Klein, J. O.: Otitis Media in Infants and Children. 4th ed. Hamilton, Ontario, B. C. Decker, 2007, p. 171.)*

upper respiratory tract are of limited value in providing specific microbiologic diagnosis of otitis media, only materials obtained by aspiration of the middle ear abscess can be considered a true reflection of the etiology of AOM. Myringotomy is an incision in the anterior lower quadrant of the tympanic membrane for therapeutic drainage. Tympanocentesis or myringotomy should be considered in patients who at onset appear toxic or are seriously ill, in patients who are toxic after initiation of antimicrobial therapy, in the presence of suppurative complications (including mastoiditis and meningitis), and in immunologically deficient patients in whom an unusual organism may be present.

RADIOGRAPHY

Radiographic evaluation of the temporal bone is indicated when complications or sequelae of otitis media are suspected or present. Plain radiographs are of limited value in the diagnosis of osteitis of the mastoid or cholesteatoma; computed tomography and magnetic resonance imaging are more precise and should be obtained if a suppurative intratemporal or intracranial complication is suspected.

DIFFERENTIAL DIAGNOSIS

Inflammation or foreign body in the external ear canal may produce ear pain simulating that of AOM. When external otitis or furunculosis of the EAC is present, the patient often has severe itching in the ear canal and pain elicited by manipulation of the pinna. The canal may be narrowed and so tender that performing an otoscopic examination is impossible.

An erythematous tympanic membrane may be caused by an upper respiratory tract infection with congestion of the mucosa lining the entire respiratory tract, including the middle ear. A "red drum" also may be produced by trauma or aggressive examination of the EAC and may appear suddenly in a crying child.

Otalgia may be associated with infections of the tonsils, adenoids, teeth, nasopharynx, hypopharynx, or larynx. Tumors in those regions can refer pain to the ipsilateral ear along the tenth cranial nerve. Lymphomas, leukemias, and rhabdomyosarcomas involving the palate, nasopharynx, or base of the skull eventually occlude one or both eustachian tubes, producing serous effusions and otalgia.

AOM must be differentiated from an acute exacerbation of an unrelated disease in a patient with persistent middle ear effusion. Because fluid persists for weeks to months after each episode of AOM, an intercurrent infection, not associated with middle ear disease, may be misdiagnosed as AOM because of the presence of acute systemic signs of an infectious illness plus a middle ear effusion. This event may be one of the reasons for overdiagnosis of AOM. At present, no technique, other than tympanocentesis, is readily available to distinguish a relapse of AOM from a new and recurrent episode of AOM or from an intercurrent and unrelated infection associated with persistent middle ear effusion.

OTITIS MEDIA WITH EFFUSION

The presence of an asymptomatic middle ear effusion has many synonyms, such as secretory, nonsuppurative, and serous otitis media, but the most acceptable term is *otitis media with effusion*. After every episode of AOM, fluid persists in the middle ear for weeks to months (see Fig. 19–1).[186] In a study of children with AOM in Boston, 70 percent of the patients still had effusion at 2 weeks, 40 percent had effusion at 1 month, 20 percent had effusion at 2 months, and 10 percent had effusion at 3 months. Similar results of persistent middle ear effusion after an episode of AOM have been noted in all other clinical studies of AOM. The incidence or prevalence of otitis media with effusion that is

unrecognized by parents and not brought to medical attention has been studied extensively.[35,56,147] The prevalence of effusion varied with age and the time of year. Incidence of otitis media with effusion peaked during the second year of life and was more prevalent in winter than in summer months.[56,147,182] In some children, the duration of otitis media with effusion may be only 1 or several days.[18] Because hearing loss is present whenever fluid fills the middle ear space, physicians are concerned about the many children with prolonged time spent with effusion; the accompanying hearing loss; and possible adverse effects on speech, language, and cognitive development.

Bacteria can be recovered from one third to one half of specimens obtained at the time of myringotomy or tympanostomy tube insertion.[64,65,124,155,170] The bacteriology in such cases has mimicked closely the bacteriology of AOM, with *S. pneumoniae* and *H. influenzae* being the predominant organisms isolated. The significance of this finding at present is unknown. The bacteria merely may colonize middle ear fluid without producing inflammation, or they may play a role in the production or persistence of middle ear fluid. In addition to live bacteria, nonviable bacteria, pneumococcal capsular polysaccharide, and endotoxin have been found in chronic middle ear effusions.[47,64,107]

Much attention has been given to the nature and composition of the middle ear effusion. The presence of biologic mediators of inflammation in the middle ear fluid has been shown[16,108,177]; they include chemotactic factors, macrophage-inhibiting factors, activated complement, histamine, prostaglandins, leukotrienes,[90,91,125] and immune complexes.[197] Elevated levels of IgA, IgE, IgM, and IgG also have been noted in serous effusions.

Clinical evaluation depends on otologic examination and audiologic and tympanometric testing. Symptoms of this disease include conductive deafness, which usually is fluctuant and may be position-dependent. The patient may have a dull ear ache or a sensation of fullness in the ear. The eardrum usually is dull with a poor light reflex and may be retracted. Color may be pale pink or have a ground-glass appearance.

COMPLICATIONS AND SEQUELAE

Intracranial suppurative complications of otitis media, including meningitis, brain abscess, and lateral sinus thrombosis, are uncommon occurrences today in developed countries (Fig. 19–6). Intratemporal complications that occur within the aural cavity and adjacent structures of the temporal bone are seen more commonly. They include acute and chronic perforation of the tympanic membrane, chronic suppurative otitis media, mastoiditis, cholesteatoma and retraction pocket, adhesive otitis media, tympanosclerosis, and ossicular discontinuity and fixation. The most frequent complication is hearing loss that occurs whenever the middle ear cavity is filled with fluid.

HEARING LOSS

Fluctuating or persisting hearing loss is present in most children who have middle ear effusion; impairment of hearing is the most prevalent complication of otitis media with effusion. Audiograms of children with middle ear effusion usually reveal a mild to moderate conductive loss of 15 to 40 dB.[59] With such deficits, the softer speech sounds and voiceless consonants may be missed. The hearing loss is not influenced by the quality of fluid in the middle ear; ears with thin fluids are impaired to the same degree as are ears with fluids of gluelike consistency.[31,199] The hearing impairment usually is reversed with resolution of the effusion. Uncommonly, permanent conductive hearing loss occurs because of irreversible changes from the inflammatory reaction, resulting in adhesive otitis media or ossicular discontinuity. High negative pressure in the middle ear or atelectasis in the absence of effusion also may cause conductive loss.

Sensorineural hearing loss after a case of AOM may occur as a result of increased tension and stiffness of the round-window membrane and is reversible. A permanent sensorineural loss may occur as a result of spread of infection or products of inflammation through the round-window membrane.[112]

Figure 19–6 Intratemporal complications and sequelae of otitis media include the following: A, Infectious eczematoid dermatitis. B, Cholesteatoma. C, Retraction pocket of tympanic membrane. D, Tympanosclerosis. E, Perforation of tympanic membrane. F, Chronic suppurative otitis media. G, Cholesterol granuloma. H, Ossicular discontinuity. I, Facial paralysis. J, Adhesive otitis media with fixation of the ossicles. K, Hearing loss. L, Petrositis. M, Labyrinthitis. N, Mastoiditis with extension into the neck (Bezold abscess). *(From Bluestone, C. D., and Klein, J. O.: Otitis Media in Infants and Children. 4th ed. Hamilton, Ontario, B. C. Decker, 2007, p. 329.)*

EFFECTS OF OTITIS MEDIA ON DEVELOPMENT OF THE CHILD

Children with severe or recurrent otitis media have prolonged time spent with middle ear effusion. Hearing impairment accompanies the effusion in most children. If the hearing impairment occurs at a time of rapid intellectual growth, the result may be impaired development of speech, language, and cognitive abilities (Fig. 19–7). Because language acquisition is dynamic during infancy, any problems in receiving or interpreting sound signals might have a significant effect on development of speech and language. Softer speech sounds and voiceless consonants, in particular, may be missed or confused when effusion is present in the middle ear.

Although many studies have been done and are reviewed in a clinical practice guideline (*Otitis Media with Effusion in Young Children*) published by the Agency for Health Care Policy and Research of the U.S. Department of Health and Human Services,[182] the limitations of design of many of the studies and inconsistencies of the results limit the ability to make conclusions about the effect of otitis media on development. The interested reader should consult the guideline for a valuable review and extensive bibliography. A study by Paradise and colleagues[140] indicates that early placement of ventilating tubes in children with prolonged time spent with middle ear fluid did not measurably improve developmental outcomes at age 3 years. Nonetheless, some infants and young children with recurrent otitis media and prolonged time spent with middle ear effusion possibly have substantive loss in potential for development of speech, language, and cognitive abilities. Selected references about long-term outcomes of otitis media include studies by Gravel and Wallace,[69] Teele and colleagues,[185] and Friel-Patti and associates[60] and reviews by Berman[13] and Klein.[103]

CHILD BEHAVIOR AND QUALITY-OF-LIFE OUTCOMES

Disturbances in the child's behavior associated with otitis media have been reported to include restlessness, frequent disobedience, impaired task orientation in the classroom, short attention span and distractibility, attention deficits, and restricted social interaction. Only selected children may be most affected. Paradise and colleagues[141] found that stress in the parent-child relationship and behavior problems were highest among children from the most socioeconomically disadvantaged homes.

A working parent who has spent a sleepless night attending to a child who is fretful because of ear pain may have work-related and home-related stress. Chase[39] described the response of parents of 1-year-old children to structured interaction tasks; parents of a child who had one or more episodes of otitis media were less effective in gaining the child's attention, less able to respond effectively when the child was distracted from the task, and less able to help the child understand and perform the task.

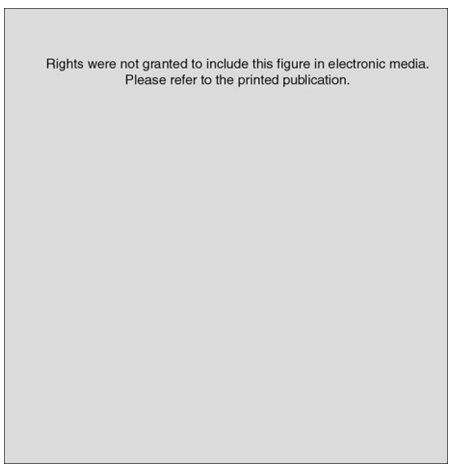

Figure 19–7 Long-term sequelae of middle ear effusion. *(From Bluestone, C. D., and Klein, J. O.: Otitis Media in Infants and Children. 4th ed. Hamilton, Ontario, B. C. Decker, 2007, p. 333.)*

PERFORATION OF THE TYMPANIC MEMBRANE

Acute perforation (not caused by trauma) usually is secondary to AOM but also may occur during the course of otitis media with effusion. The perforation occurs because of pressure of the expanding middle ear contents on the membrane, resulting in local ischemia and tissue damage, usually in the central portion of the membrane. With rupture, the middle ear contents are discharged into the external ear canal, with instant relief of pain and defervescence in acute infection occurring. Because the membrane is highly vascular, the perforation may seal quickly and not be evident within hours to days. If the mucous membrane of the middle ear remains inflamed, fluid may reaccumulate behind the resealed tympanic membrane.

Chronic perforation may occur after an acute episode, spontaneous extrusion, or removal of a tympanostomy tube. If squamous epithelium grows at the edges of the perforation, healing may be prevented, and the perforation persists. The term *chronic suppurative otitis media* is limited to a stage of ear disease in which chronic inflammation of the middle ear and mastoid occurs and in which a nonintact tympanic membrane (caused by perforation or tympanostomy tube) and otorrhea are present. Mastoiditis usually is present, and a cholesteatoma may have formed.

CHOLESTEATOMA

A cholesteatoma usually is a cystic structure lined by squamous epithelium resting on a fibrous strand. The contents of the cyst are the products of desquamation, keratinization, and pus formulation. A cholesteatoma may invade, causing local bone erosion and destruction of the ossicular chain. Aural cholesteatomas can be classified as congenital or acquired.

A congenital cholesteatoma is a congenital rest of epithelial tissue and appears as a white cystlike structure within the middle ear or temporal bone. Acquired cholesteatoma may be secondary to implantation of epithelial tissue or may be a sequela of otitis media or a retraction pocket, or both. Implantation cholesteatoma may develop either from epithelium that has migrated through a perforation of the tympanic membrane or from intraaural epithelium remaining after middle ear or mastoid surgery. Infection caused by such organisms as *S. aureus*, *P. aeruginosa*, *Proteus* spp., nonhemolytic streptococci, and *Aspergillus* spp. may be present. The process of alternating infection and healing causes the advancement of squamous epithelium into the middle ear and antrum.[55,109,160] The persistent infection also stimulates proliferation of the mucoperiosteum of the attic region, creating an accelerated tissue growth (increased production of collagenase) and a destructive and expansive process. It is characterized by a foul smell, pus, squames, and bone destruction.

Management of cholesteatoma is surgical removal of the entire cyst. Antimicrobial therapy may be necessary if secondary infection is present.

ADHESIVE OTITIS MEDIA

Adhesive otitis media is a result of healing after chronic inflammation of the middle ear and mastoid. Fibrous tissue proliferates in the muscosal lining and may impair movement of the ossicles

and result in conductive hearing loss. Adhesive changes may bind the eardrum to the ossicles and surrounding middle ear structures and cause resorption of the ossicles.

TYMPANOSCLEROSIS

Tympanosclerosis, or scarring of the tympanic membrane, may be a sequela of chronic middle ear inflammation or trauma. White plaques are present in the tympanic membrane, with nodular deposits in the submucosal layers. The histopathology is marked by hyaline degeneration resulting from a healing reaction characterized by fibroblastic invasion of the submucosa followed by thickening and fusion of the fibers.

OSSICULAR DISCONTINUITY AND FIXATION

Ossicular chain abnormalities, including osteitis, may be secondary to chronic inflammation in the middle ear or the presence of a retraction pocket or cholesteatoma. The long process of the incus most commonly is involved. Erosion of the blood supply, cholesteatoma, or adhesive otitis media may be the cause of the bone erosion and disarticulation. Conductive hearing loss is present to a varying degree. Diagnosis may be assisted by computed tomography or magnetic resonance imaging.

MASTOIDITIS

Reports from Denmark,[146] Italy,[114] and the United States[95] indicate that mastoiditis continues to be an important complication of AOM in developed countries, although at a low rate. In developing countries, untreated otitis media may lead to persistent perforation of the tympanic membrane, dysarticulation of ossicles, and mastoiditis. Berman[12] estimated that mastoiditis occurred as a complication of otitis media in developing countries in 0.74 percent of cases of otitis, based on community and school surveys, and in 1.7 to 18 percent, based on hospital clinical record reviews. In a survey of eight children's hospitals in the United States between 1993 and 1998 for pneumococcal diseases, Kaplan and colleagues[95] identified 34 children with pneumococcal mastoiditis; serogroup 19 accounted for 57 percent of the isolates. The cases occurred primarily in children younger than 2 years of age.

At birth, the mastoid consists of a single cell, the antrum, connected to the middle ear by a small channel, the aditus ad antrum. Pneumatization of the mastoid bone occurs soon after birth and usually is extensive by the time the child reaches 2 years of age. Likely, whenever AOM occurs, some degree of mastoiditis is present. With healing of the middle ear infection, healing of the mastoid also occurs. In a few cases, mastoid disease progresses with hyperemia and edema of the mucosal lining of the pneumatized cells, accumulation of serous and then purulent exudates in the cells, demineralization of the cellular walls and necrosis of bone, and formation of abscess cavities caused by coalescence of adjacent cells after destruction of the cell walls. Pus may escape into contiguous areas, including the posterior cranial fossa, middle cranial fossa, sigmoid and lateral sinuses, canal of the facial nerve, semicircular canals, and petrous tip of the temporal bone.

Signs of acute mastoiditis with periostitis include fever, otalgia, postauricular erythema, tenderness, and slight swelling. The pinna may be displaced inferiorly and anteriorly.

Initial management of acute mastoiditis includes administration of parenteral antibiotics and myringotomy to provide drainage of the middle ear and mastoid contents. Surgical drainage of the mastoid should be performed if the symptoms of the acute infection, including fever and otalgia, persist. If the infection progresses, causing destruction of the bony trabeculae, a mastoid empyema, mastoidectomy should be performed to prevent spread of the infection to adjacent structures.

PETROSITIS

Petrositis occurs when suppurative infection extends from the middle ear and mastoid into the petrous portion of the temporal bone. Signs of petrositis include pain behind the eye, deep ear pain, persistent ear discharge, and sixth nerve palsy. The triad of pain behind the eye, aural discharge, and sixth nerve palsy is termed *Gradenigo syndrome*. Management is similar to that described earlier for mastoiditis.

LABYRINTHITIS

Spread of AOM into the cochlear and vestibular apparatus through the round (less commonly, the oval) window results in inflammation of the labyrinth. The signs of labyrinthitis include sudden, progressive, or fluctuating sensorineural hearing loss or vertigo in association with otitis media or mastoiditis. Signs of suppurative labyrinthitis (in the absence of meningitis) warrant performing aggressive otologic surgery and administering parenteral antimicrobial therapy.

MENINGITIS

Meningitis may be associated with middle ear infections in three circumstances:

1. *Direct invasion*: A suppurative focus in the middle ear or mastoid spreads through the dura, extends to the pia-arachnoid, and causes generalized meningitis.
2. *Inflammation in an adjacent area*: The meninges may become inflamed if there is suppuration in an adjacent area, such as the mastoid air cells.
3. *Concurrent infection*: Otitis media arises by spread of bacteria from the upper respiratory tract, and meningitis concurrently invades the blood from the upper respiratory focus.

Children with cochlear implants are at risk for development of meningitis. Although the implant has been implicated in the subsequent development of meningitis, some children likely had an underlying congenital inner or middle ear malformation that provided a pathway for the bacterium to enter the brain.[23]

FACIAL PARALYSIS

Facial paralysis may occur as a sequela of AOM because of exposure of the facial nerve in the middle ear cleft caused by a bony dehiscence. The palsy usually is unilateral. The paralysis usually resolves with medical therapy for AOM, but if paralysis of the facial nerve persists, decompression may be necessary.

OTHER SUPPURATIVE COMPLICATIONS

The middle ear and mastoid air cells are adjacent to the dura of the posterior and middle cranial fossa, the sigmoid venous sinus of the brain, and the inner ear (Fig. 19–8). Suppuration in the middle ear or mastoid may spread to these structures, producing suppurative complications, such as meningitis, extradural abscess, subdural empyema, focal encephalitis, brain abscess, and lateral

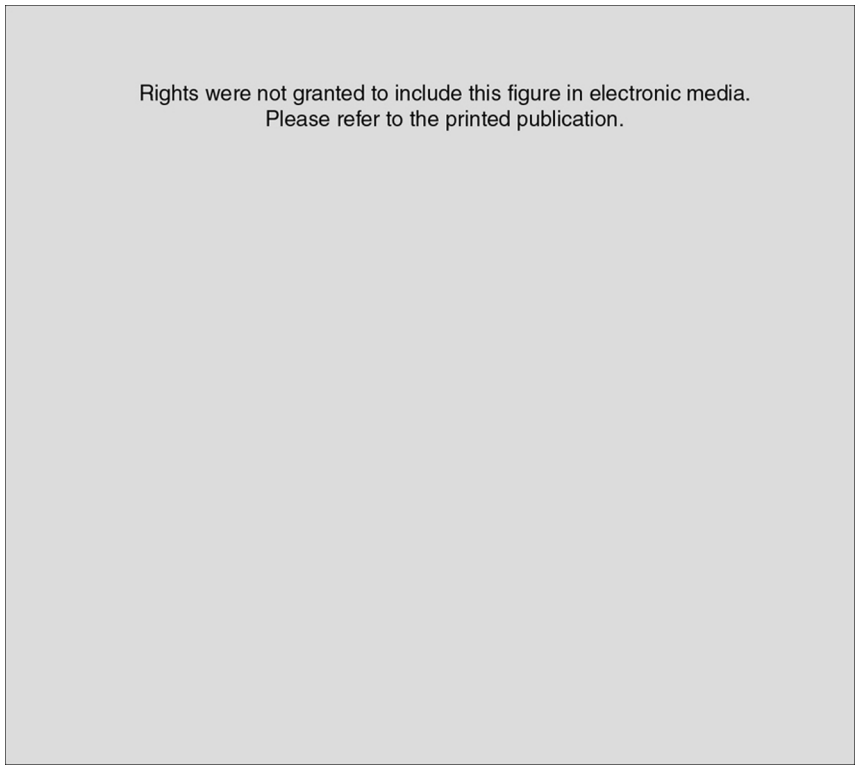

Figure 19–8 Suppurative complications of otitis media and mastoiditis. A, Subperiosteal abscess. B, Extradural abscess. C, Subdural empyema. D, Brain abscess. E, Meningitis. F, Lateral sinus thrombosis. *(From Bluestone, C. D., and Klein, J. O.: Otitis Media in Infants and Children. 4th ed. Hamilton, Ontario, B. C. Decker, 2007, p. 434.)*

sinus thrombosis. Intracranial complications should be suspected when a child with acute or chronic otitis media develops persistent and severe headache, severe otalgia, and change in affect or level of responsiveness. Conversely, children with diagnosed intracranial infection, such as meningitis, should have middle ear or mastoid disease assessed as the origin of the central nervous system disease.

Intracranial extension of infection from the middle ear into the intracranial area may occur because of any of the following:

1. Progressive thrombophlebitis, permitting infection to spread through the intact bone
2. Erosion of the bony walls of the middle ear or mastoid
3. Extension along preformed pathways, such as the round window, dehiscent sutures, skull fractures, and congenital or surgically acquired bony dehiscences.

The microbiology, pathogenesis, diagnosis, and management of intracranial complications of otitis media and mastoiditis are discussed extensively elsewhere (see Chapters 20, 37, 38, and 42).

MANAGEMENT OF ACUTE OTITIS MEDIA

Management of AOM focuses on the choice of an appropriate antimicrobial agent or an option to observe initially rather than treat. Decongestants and antihistamines may provide some comfort for a patient who has congestion of the upper respiratory tract, but they provide no benefit in terms of earlier resolution of the middle ear infection. The antimicrobial agent should have a spectrum of activity that includes *S. pneumoniae* and *H. influenzae* and have documented clinical and microbiologic efficacy, limited side effects, availability in a convenient dosage schedule, palatability when provided in suspension, and reasonable cost. When treated with appropriate antimicrobial therapy, a patient should have substantial resolution of signs and symptoms within 72 hours and absence of signs of relapse, recurrence, or suppurative sequelae.

TABLE 19–4 Daily Dosage Schedule for Antimicrobial Agents Useful in Acute Otitis Media

Agent	24-Hr Dosage
Amoxicillin	40-80 mg/kg in 2-3 doses
Amoxicillin-clavulanate	40-80 mg/kg in 2 doses (90 mg/kg in 2 doses for Augmentin ES-600)
Cefprozil	30 mg/kg in 2 doses
Cefpodoxime	10 mg/kg in 2 doses
Cefaclor	40 mg/kg in 2-3 doses
Cefixime	8 mg/kg in 1 dose
Cefuroxime axetil	30 mg/kg in 2 doses
Loracarbef	30 mg/kg in 2 doses
Ceftriaxone	50 mg/kg in 1 dose* (1-3 days)
Ceftibuten	9 mg/kg in 1 dose
Cefdinir	14 mg/kg in 1-2 doses
Erythromycin-sulfisoxazole	50 mg/kg erythromycin, 150 mg/kg sulfisoxazole in 4 doses
Clarithromycin	15 mg in 2 doses
Azithromycin	30 mg/kg in 1 dose (1 day) 10 mg/kg in 1 dose (3 days) 10 mg/kg in 1 dose (day 1); 5 mg/kg in 1 dose (days 2-5)
Trimethoprim-sulfamethoxazole	8 mg trimethoprim, 40 mg sulfamethoxazole in 2 doses

**Intramuscular route.*

Table 19–4 lists the daily dosage schedules of 15 oral or parenteral antimicrobial agents approved for the indication of therapy of AOM. In addition, ofloxacin otic and ciprofloxacin (combined with dexamethasone) otic have been approved by the

U.S. Food and Drug Administration (FDA) for treatment of AOM in children with tympanostomy tubes in place.

CLINICAL IMPLICATIONS OF ANTIBIOTIC RESISTANCE

Increased resistance of bacterial pathogens to available antimicrobial agents has been a constant concern since the introduction of antimicrobial agents. Multidrug-resistant pneumococci add complexity to the choice of optimal antimicrobial agents for AOM. At present, the incidence of strains of pneumococci that are nonsusceptible to penicillin averages approximately 25 percent in the United States but is more than twice that in southeastern and southwestern states. Reasons for the regional differences are unknown. The major risk factors for presence of multidrug-resistant pneumococci are recent (within 30 days) administration of an antimicrobial agent and attendance in daycare. In addition, the increasing proportion of β-lactamase–producing strains of *H. influenzae* and *M. catarrhalis*, now 30 to 75 percent in the United States, warrants consideration in choice of optimal agents.

DIFFUSION OF ANTIMICROBIAL AGENTS INTO MIDDLE EAR FLUIDS

Most antimicrobial agents of value for treatment of AOM achieve significant concentrations in middle ear fluid. The concentrations are generally parallel to, although lower than, concentrations of drug in serum. Purulent fluids have higher concentrations of drug than do mucoid or serous fluids. Penicillins and cephalosporins achieve concentrations in middle ear fluids that are approximately one fifth to one third the levels present in serum. Sulfonamides and erythromycin achieved middle ear concentrations that were approximately 50 percent of serum concentrations. An extensive review of concentrations achieved in middle ear fluids is provided in the textbook *Pediatric Otolaryngology*, edited by Bluestone, Stool, and Kenna.[26]

STERILIZATION OF MIDDLE EAR FLUIDS BY ANTIMICROBIAL AGENTS

To define the ability of antimicrobial agents to eradicate bacterial pathogens from middle ear fluids of children with AOM, investigators have used serial aspirates of the infected fluids.[46,82,83,119,122] The initial aspirate identifies the bacterial pathogen of the acute middle ear infection; the second aspirate, obtained days after initiation of therapy, defines the ability of the drug to eradicate the infection. The results of these tests generally are consistent with data available from in vitro assays of the drugs against the major bacterial pathogens and the concentrations of drug achieved in the middle ear fluids.[100] Penicillin-susceptible pneumococcal infections were sterilized by most penicillins, cephalosporins, and macrolides; failure rates of 10 percent or more were identified only in infections treated with cefaclor, cefixime, and cefpodoxime; sulfonamides alone were ineffective, but trimethoprim-sulfamethoxazole was effective.

Dagan[46] reviewed sterilization of middle ear fluids by antimicrobial agents for pneumococci that were penicillin-susceptible and penicillin-nonsusceptible. The sterilization of middle ear fluids infected with susceptible strains was consistent with prior data. Nonsusceptible strains were less readily eradicated from the middle ear fluid, and the failure rates were twofold or more than those for penicillin-susceptible strains. The failure rates for penicillin-nonsusceptible versus penicillin-susceptible strains were 20 percent versus 10 percent for amoxicillin, 53 percent versus 0 percent for ceftriaxone administered as one dose, 9 percent versus 0 percent for ceftriaxone administered as three consecutive daily doses, 62 percent versus 10 percent for cefaclor, 92 percent versus 5 percent for azithromycin, and 79 percent versus 0 percent for trimethoprim-sulfamethoxazole.

Sterilization of middle ear fluids infected with *H. influenzae* was influenced by the strain differences—β-lactamase–positive or β-lactamase–negative for β-lactam drugs. Failure rates for amoxicillin were 21 percent when the strain was β-lactamase–negative, but were similar to placebo (60%) when the strain produced β-lactamase. Ceftriaxone eradicated all isolates of *H. influenzae* whether or not they produced β-lactamase. Cefuroxime axetil was more effective (15% failures) compared with cefaclor (40% failures). Failures for azithromycin were comparable to placebo (57% versus 52%). Although similar data based on dual ear aspirates are unavailable for middle ear infections caused by *M. catarrhalis*, the high proportion of β-lactamase–producing strains suggests microbiologic results similar to those of *H. influenzae*.

STERILIZATION OF MIDDLE EAR FLUIDS WITHOUT ANTIBACTERIAL AGENTS

Using the same technique of dual aspirates to identify the microbiologic efficacy of antibacterial drugs, Howie[81] identified sterilization of infected middle ear fluids without drugs. A placebo replaced active therapy in the dual aspirate study: 2 to 7 days after the initial aspirate identified the presence of pneumococci or *H. influenzae*, 19 percent of the pneumococci and 48 percent of the *Haemophilus* strains no longer were present. The differential clearing of the bacteria with persistence of most pneumococci but resolution of one half of the infections caused by nontypeable *H. influenzae* likely is associated with some immune or bacteriostatic factor in the middle ear inflammatory exudate that acts to inhibit growth of these organisms. These data of spontaneous resolution need to be considered in evaluating the efficacy of new and old antibacterial drugs.

INITIAL OBSERVATION RATHER THAN ANTIMICROBIAL AGENT THERAPY

Before the introduction of sulfonamides in 1936, management of AOM included watchful waiting or, when the suppurative process produced severe clinical signs, use of myringotomy to drain the middle ear abscess. Spread of infection to the mastoid, meninges, or other intracranial foci was a feared complication of otitis media. Early therapeutic trials identified the value of using antimicrobial agents for resolution of clinical signs and decreased incidence of suppurative complications. Most children with AOM respond clinically without use of antimicrobial agents. Children who improve without antimicrobial drugs include one third with AOM who have a bacteriologically sterile effusion and are presumed to have a viral infection and children who have bacterial infections that clear without antimicrobial agents (19% of pneumococcal infections, 48% of infections caused by nontypeable strains of *H. influenzae*, and approximately 75% of AOM caused by *M. catarrhalis*).[100] Because of the increased incidence of bacterial pathogens resistant to available antimicrobial agents, and the data associating extensive use of the drugs with development of resistant strains, limiting the use of antimicrobial agents for children with AOM has been suggested. The option of observation and symptomatic treatment rather than initial antibiotic treatment has been advocated in Western Europe based on studies by van Buchem and colleagues.[193,194] In the United States, interest in this management increased with concerns for multidrug resistance resulting from the extensive use of antimicrobial agents, but the recommendations of the CDC and the AAP in 1998 focused on increasing the accuracy of diagnosis, avoiding use of

antimicrobial agents for otitis media with effusion, and continuing use of these drugs for treatment of AOM.[50]

The AAP and the AAFP in 2004 altered their positions and for the first time provided a guideline for an observation option for selected patients.[4] The guideline recommended that observation without use of antimicrobial agents could be considered in a child older than 24 months of age with uncomplicated AOM with nonsevere signs (mild otalgia, temperature <39º C orally in the past 24 hours) when follow-up was ensured, and antibacterial agents could be started if symptoms persisted or worsened. Table 19–5 lists the options considered in the guidelines.

CHOICE OF ANTIMICROBIAL AGENTS

Oral amoxicillin remains the first-line antimicrobial agent for treating AOM because of the expected low failure rates. The drug-resistant *S. pneumoniae* Therapeutic Working Group of the CDC[49] and the 2004 guidelines of the AAP and the AAFP[4] suggested that an increase in dosage used of amoxicillin for empiric treatment from 40 to 45 mg/kg/day to 80 to 90 mg/kg/day would be effective for more nonsusceptible strains of *S. pneumoniae*. The 2004 guidelines for initial treatment and treatment failures are listed in Table 19–6.

The guideline differentiates treatment choice of drug by severity of signs. Children with nonsevere disease may be treated initially with high-dose amoxicillin, but children with severe signs should be treated with amoxicillin-clavulanate. The use of high-dose amoxicillin-clavulanate responds to concern about failure of amoxicillin in cases of AOM caused by β-lactamase–producing *H. influenzae*. If failure occurred because of a high-level resistant strain of *S. pneumoniae*, however, the same dosage of amoxicillin in the combination would not be advantageous. Cefuroxime axetil, cefpodoxime, and cefdinir have equivalent profiles in vitro against *S. pneumoniae* and *H. influenzae*. Because of bitter taste, cefuroxime and cefpodoxime are not well accepted by young patients, and the better taste of cefdinir increased acceptability and compliance. Intramuscular ceftriaxone was most effective in eradicating nonsusceptible pneumococci when provided in three daily doses, but an alternative regimen is to provide a single dose and, if signs do not resolve at 48 hours, to proceed to a second or third dose.[102]

TABLE 19–5 Guidelines for Management of Acute Otitis Media from the American Academy of Pediatrics and American Academy of Family Physicians[4]

Age (mo)	Certain Diagnosis*	Uncertain Diagnosis
<6	Antibiotics	Antibiotics
6-24	Antibiotics	Antibiotics if severe; observe if not severe†
>24	Antibiotics if severe; observe if not severe†	Observe

**Certain diagnosis = middle ear effusion, rapid onset, symptoms of middle ear inflammation.*
†Not severe = mild otalgia; temperature <39ºC orally or <39.5ºC rectally in past 24 hr.

DOSAGE SCHEDULES

Dosage schedules of the antimicrobial agents of value for therapy of AOM have been determined on the basis of studies of the pharmacokinetics and results of clinical trials (see Table 19–4).

DURATION OF THERAPY

Duration of therapy is based on clinical trials and tradition. Most clinical trials and standard pediatric practice include a 10-day course of an antimicrobial agent. The FDA approved a 5-day schedule of once-a-day azithromycin administered orally based on studies comparing the clinical efficacy of 5-day azithromycin with 10-day amoxicillin-clavulanate courses. These data suggest that short courses of therapy may be appropriate for many children with AOM, although some children (likely children with severe and recurrent disease) would require more prolonged schedules.

CLINICAL COURSE AFTER INITIATION OF THERAPY

The clinical course of a child who receives appropriate antimicrobial therapy includes significant resolution of acute signs within 48 to 72 hours. Instructions to the parent should indicate the need to contact the physician if the signs or symptoms worsen at any time or are unimproved at 72 hours. Persistent ear pain or systemic signs, such as fever, signal the need for re-evaluation to examine for other foci of infections, to determine the need for another antimicrobial agent, or to perform tympanocentesis or myringotomy to incise and drain the middle ear abscess and culture the fluid to identify the pathogen. If a new antibiotic is

TABLE 19–6 Recommended Antibacterial Agents for Patients Who Are Being Treated Initially with Antibacterial Agents or Who Have Failed Initial Management with Antibacterial Agents

From American Academy of Pediatrics.[4]

needed, one with β-lactamase stability and activity against penicillin-resistant pneumococci (if such information is available from local surveillance studies) should be chosen.

Follow-up visits should be made to determine that the child has recovered from the acute infection and to diagnose persistent middle ear effusion if it is present. The utility of the traditional 10- to 14-day visit was reassessed by Hathaway and coworkers[75] and Mandel and colleagues[116]; these investigators concluded that the follow-up visit can be extended to 4 to 6 weeks after onset of treatment in children whose parents thought the disease had resolved at 10 to 14 days. For children who still had signs or symptoms of disease (other than persistent middle ear effusion), the 10- to 14-day visit was recommended. Visits at 4 to 6 weeks and repeated at 1-month intervals if effusion is present are valuable in determining the duration of middle ear effusion after the acute episode and identifying children who may be candidates for placement of tympanostomy tubes.

SYMPTOMATIC THERAPY

Administration of antipyretics and analgesics and application of local heat usually are helpful in treating a child with an acute painful and febrile episode. Acetaminophen or ibuprofen is recommended for children with mild to moderate pain. Topical agents such as benzocaine may provide brief benefit in patients older than 5 years of age. Narcotic analgesia with codeine or analogues should be reserved for patients with moderate or severe pain. An oral decongestant, such as pseudoephedrine hydrochloride, may relieve nasal congestion, and antihistamines may help patients with known or suspected nasal allergy. The efficacy of antihistamines and decongestants for resolution of middle ear effusion is unproven.

MANAGEMENT OF ACUTE OTITIS MEDIA IN A CHILD WITH TYMPANOSTOMY TUBES

Children with tympanostomy tubes also experience AOM. Because the tube permits drainage of the middle ear fluid and an abscess does not develop, the major pathology is inflammation of the mucous lining of the middle ear, and the dominant clinical sign is otorrhea. The bacterial pathogens include those responsible for AOM (*S. pneumoniae*, *H. influenzae*, and *M. catarrhalis*) and bacteria that may invade the middle ear from the EAC (*S. aureus* and *P. aeruginosa*). Although amoxicillin or amoxicillin-clavulanate is the usual choice for treatment of AOM in a child with tympanostomy tubes, ofloxacin otic and ciprofloxacin-dexamethasone otic were effective for children who presented with acute otorrhea. The efficacy of the otic suspensions for AOM in children with tympanostomy tubes indicates that high concentrations of drug reach the middle ear mucosa and result in eradication of bacterial pathogens. Additional advantages of otic versus systemic antimicrobial administration include lesser systemic adverse events (e.g., diarrhea) and absence of effect on the upper respiratory flora, and absence of selection of resistant bacteria.[67,157]

MANAGEMENT OF OTITIS MEDIA WITH EFFUSION

The following options have been investigated for management of a child with prolonged middle ear fluid or with otitis media with effusion:

1. Another 10-day course of a broad-spectrum antimicrobial agent that has activity against β-lactamase–producing organisms has been recommended because bacterial pathogens are found in approximately one fourth of patients with otitis media with effusion; a meta-analysis of blinded studies identified resolution of effusion in 14 percent of cases.[182]

2. Myringotomy or myringotomy and tympanostomy tubes drain the middle ear fluid, aerate the middle ear space, and permit the middle ear mucosa to return to normal.[117]

3. Adenoidectomy with or without tonsillectomy for children who have recurrences after an initial placement of tympanostomy tubes is controversial.[63] A Finnish study concluded that adenoidectomy did not reduce the incidence of AOM in otitis-prone children younger than 4 years of age who received tympanostomy tubes.[72]

4. Steroid therapy alone or with an antibiotic has been shown to be effective in some children with otitis media with effusion. Berman[11] recommended a regimen of prednisone, 1 mg/kg/day (given orally in two doses) for 7 days, with an antibiotic for 14 to 21 days. Children without a history of varicella infection who have been exposed to the virus in the month before receiving treatment should not be given prednisone because of the risk for developing disseminated disease. The guidelines published by the Agency for Health Care Policy and Research concluded that the data were insufficient in sample size or duration of observation to recommend use of steroids for otitis media with effusion.[182]

Topical or systemic nasal decongestants, antihistamines, and anti-inflammatory agents,[1,196] alone or in combination, have been found to be of limited or no value in the management of otitis media with effusion.

We favor an initial course of a broad-spectrum antibiotic for children with otitis media with effusion for 3 or more months. If the effusion does not resolve, use of tympanostomy tubes is the most efficient method for managing prolonged middle ear effusion. The use of tympanostomy tubes first was suggested more than 100 years ago by Politzer, but the procedure did not become readily available until it was reintroduced by Armstrong in 1954. Myringotomy and placement of ventilating tubes result in the following immediate benefits to the patient with otitis media with effusion:

- The effusion is drained, and the fluid-filled space is aerated.
- The middle ear secretions, which constantly are being formed by the secretory cells of the mucosa, are drained.
- The chronically diseased mucosa (characterized by hypertrophic secretory cells) returns to normal.
- The hearing impairment caused by the middle ear fluid disappears.
- Concern for effects of hearing loss on development of speech and cognitive abilities is diminished.
- The child who was not responsive or attentive (a condition often unrecognized as hearing impairment by the parent) becomes more social and more involved with siblings, parents, and playmates.

The procedure may be disadvantageous for the following reasons:

- General anesthesia is required.
- The cost is significant.
- Although uncommon, sequelae, such as persistent otorrhea, permanent perforation, scarring of the membrane, and cholesteatoma, may occur.

In addition, developmental outcomes may not be improved after early rather than delayed insertion of tympanostomy tubes.[138]

Current recommendations for pediatric otitis media with effusion include follow-up visits for 1 to 2 months after the acute

episode. When the effusion persists for 3 or more months, the child should receive medical treatment with a course of antibiotics (2 to 3 weeks) or in conjunction with a 7-day regimen of prednisone. If the effusion fails to resolve with medical management, the child should be referred to an otolaryngologist for consideration of placement of tympanostomy tubes with or without adenoidectomy.[117,137]

PREVENTION

ADVISING PARENTS

Parents of children who have severe and recurrent otitis media or risk factors for developing middle ear infections should be advised of measures that may reduce the incidence of infection, such as breast-feeding; enrolling children in small, rather than large, group daycare centers; and reducing exposure to tobacco smoke. In addition, data about the risks for developing recurrent otitis media associated with the prone sleeping position and use of a pacifier, although requiring corroboration, may be added to the discussion with the parent. Physicians also may advise parents that the seasonal incidence of otitis media suggests that their child's condition is expected to improve in late spring and summer and that aggressive measures of management, including chemoprophylaxis and surgery, may be postponed until the course of disease has been determined in the next respiratory season.

PNEUMOCOCCAL VACCINES

A pneumococcal polysaccharide vaccine was introduced in the United States in the 1970s, but it had limited efficacy in infants 2 years of age and younger and only a modest effect in prevention of AOM. In February 2000, the FDA approved a safe and effective seven-valent conjugate pneumococcal vaccine (PCV7) that was immunogenic in infants 2 months of age. By December 2005, more than 80 million doses had been distributed in the United States (Peter Paradiso, personal communication). PCV7 has been effective in decreasing the incidence of invasive disease and, to a lesser extent, the incidence of AOM. The results of two large PCV7 clinical trials have been published: (1) an evaluation of the efficacy of the vaccine for prevention of invasive bacterial disease, pneumonia, and otitis media in approximately 38,000 Northern California infants[19,58] and (2) a clinical and microbiologic study of the efficacy of PCV7 for prevention of AOM in 1662 Finnish infants.[52] A clinical trial of an 11-valent polysaccharide vaccine conjugated with an outer membrane protein of nontypeable *H. influenzae* was found to be effective in infants in the Czech Republic and Slovakia against AOM caused by *S. pneumoniae* and *H. influenzae*.[151] A 13-valent vaccine is currently being studied for safety and immunogenicity. Table 19–7 lists the serotypes included in the various pneumococcal vaccines.

In the Northern California study, the efficacy of PCV7 for prevention of episodes of AOM was based on clinical criteria. The vaccine was 7.8 percent effective in preventing episodes of AOM, antibiotic prescriptions decreased by 5.8 percent, and 24.9 percent fewer surgical procedures (most were placement of ventilating tubes) were performed in children immunized with PCV7 compared with children who received the control vaccine (conjugate polysaccharide meningococcal group C).

The Finnish trial provided microbiologic data based on aspirates of middle ear fluids in children who had AOM. Children who received PCV7 had 6 percent fewer episodes of AOM; culture-confirmed pneumococcal episodes were reduced by 34 percent. Children who received PCV7 experienced a 57 percent reduction in episodes caused by pneumococcal serotypes included in the vaccine and a 51 percent reduction in episodes caused by cross-reactive pneumococcal serotypes. A 33 percent increase in episodes of AOM caused by nonvaccine pneumococcal serotypes and an 11 percent increase in episodes caused by *H. influenzae* occurred (Table 19–8).

An 11-valent pneumococcal polysaccharide vaccine conjugated with an outer membrane protein of nontypeable *H. influenzae* was found to be effective against AOM caused by *S. pneumoniae* and *H. influenzae* (GlaxoSmithKline Biologics), but it has not been approved (as of May 2008) for immunization of infants in the United States or elsewhere.[151] The vaccine prevented 52 percent of episodes of AOM caused by pneumococcal vaccine serotypes and 35.3 percent of episodes caused by nontypeable *H. influenzae*.

TABLE 19–7 Serotypes in Pneumococcal Vaccines—2008

Serotype	Polysaccharide 23 Type (Merck)	Polysaccharide Conjugates		
		7 Type (Wyeth)	13 Type* (Wyeth)	11 Type* (GSK)
1	+		+	+
2	+			
3	+		+	+
4	+	+	+	+
5	+		+	+
6A	–		+	
6B	+	+	+	+
7F	+			+
7V	–		+	
8	+			
9N	+			
9V	+	+	+	+
10A	+			
11A	+			
12F	+			
14	+	+	+	+
15B	+			
17F	+			
18C	+	+	+	+
19A	+		+	
19F	+	+	+	+
20	+			
22F	+			
23F	+	+	+	+
33F	+			

**Not approved by the FDA as of May 2008.*

TABLE 19–8 Efficacy of PCV7 in Finnish Children with Acute Otitis Media (AOM)

End-Point	AOM Episodes		Vaccine Efficacy Point Estimate
	PCV7 (N = 831)	Control (N = 831)*	
Any AOM	1251	1345	6
Pneumococcal AOM	271	414	34
Vaccine serotypes	107	250	57
Cross-reactive serotypes	41	84	51
Nonvaccine serotypes	125	95	–33
Haemophilus influenzae AOM	315	287	–11
Moraxella catarrhalis AOM	379	381	–1

**Hepatitis B vaccine.*

Adapted from data published in Eskola, J., Kilpi, T., Palmu, A., et al.: Efficacy of a pneumococcal conjugate vaccine against acute otitis media. N. Engl. J. Med. 344:403-409, 2001.

The 23-type polysaccharide vaccine currently available produces an independent antibody response in children 2 years of age or older and in adults. Studies of polysaccharide vaccines in Finland and the United States have indicated that the vaccines were effective in preventing type-specific pneumococcal otitis media if an adequate immune response occurred, but the number of types producing an adequate response in children younger than 2 years was limited.[115,179,184] Administration of the polysaccharide vaccine in older children may provide protection against types not included in PCV7 and has been recommended by the AAP and the Advisory Committee on Immunization Practices of the Surgeon General for high-risk children ages 2 years and older who have received PCV7. For children 2 years old and older who continue to have severe and recurrent AOM, use of the polysaccharide vaccine after administration of PCV7 may be valuable for protection against additional pneumococcal serotypes.

INFLUENZA VIRUS VACCINES

Influenza virus vaccine resulted in a reduction in cases of influenza A and a 36 percent decline in otitis media in children attending a daycare center.[42] A similar reduction (30%) in episodes of febrile otitis media also was reported in children after administration of a live, attenuated cold-adapted intranasal vaccine.[10] Annual administration of influenza virus vaccines should be part of the strategy for reducing the incidence of AOM for children with recurrent and severe disease.

CHEMOPROPHYLAXIS

Use of chemoprophylaxis has succeeded in reducing the number of new symptomatic episodes of AOM in children who have a history of recurrent infections. Children at risk for developing severe and recurrent disease should be considered for chemoprophylaxis despite the concern for development of resistant strains of bacterial pathogens after use of the regimen of a modified dosage of an antimicrobial agent. Results of controlled clinical trials of modified courses of antimicrobial agents compared with trials in which placebo or historical controls were used have been reviewed.[101] Most of these studies used a sulfonamide or a broad-spectrum penicillin. Most reports indicated benefit to the enrollees in reduction of new episodes when they were compared with controls: Amoxicillin efficacy was 44 to 67 percent, and sulfonamide efficacy was 40 to 88 percent (although the efficacy of sulfonamides was reported as only 8% in one study).

We recommend the following protocol based on the results of these studies. Criteria for enrollment should include three documented episodes of AOM in 6 months or four episodes in 12 months. Because children who have episodes of acute infection early in life or have siblings with severe and recurrent ear infections are prone to develop otitis media, prophylaxis also should be considered for children who have one episode in the first 6 months of life plus a family history of ear infections or who have two episodes in the first year of life.

A sulfonamide or amoxicillin is the agent used most often and provides the advantages of demonstrated efficacy, safety, and low cost. The drug is administered at half the therapeutic dose (administered once a day). Amoxicillin is given at a dose of 40 mg/kg, and sulfisoxazole is given at a dose of 50 mg/kg. Chemoprophylaxis should be provided during the fall, winter, and early spring months (when respiratory tract infections are most frequent) for 6 months.

Children, when free of signs of acute infection, should be examined at approximately 2-month intervals to determine whether middle ear effusion is present. Management of prolonged middle ear effusion should be considered separately from prevention of recurrences of acute infection.

Acute infections are expected to occur, although at a lower rate, during the course of prophylaxis. The infection should be treated with an alternative regimen. Amoxicillin-clavulanate or intramuscular ceftriaxone would be a suitable alternative, regardless of the prophylactic agent used.

SURGICAL OPTIONS

When nonsurgical methods of prevention fail to prevent recurrent otitis media, surgery is a reasonable option. Among the options that have been shown to be effective is insertion of tympanostomy tubes, with or without adenoidectomy. Casselbrant and colleagues[36] randomly assigned children into treatment groups receiving amoxicillin, tympanostomy tube placement, or placebo and found that amoxicillin and insertion of the tympanostomy tube were effective. With the current concern about the association of low-dose, long-term antimicrobial prophylaxis and emergence of resistant otitis pathogens,[49] myringotomy and placement of tympanostomy tubes may be more desirable than antimicrobial prophylaxis. When tympanostomy tubes extrude and the child continues to have recurrent episodes of otitis media, replacement of the tympanostomy tubes in conjunction with adenoidectomy has been shown to be effective.[137] As shown by Paradise and coworkers,[135] neither adenoidectomy nor adenotonsillectomy is effective in children not treated previously with tympanostomy tubes, when prevention of otitis media is the only indication.

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CHAPTER 20

Mastoiditis

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Mastoiditis, a suppurative infection of the mastoid air cells, is a potential complication of all cases of otitis media caused by the continuity of the mucoperiosteal lining of the mastoid with that of the middle ear.[20] The spectrum of disease in mastoiditis ranges from asymptomatic cases with apparent spontaneous resolution to progressive disease with life-threatening complications.[7] Since the advent of antibiotic therapy, mastoiditis is seen much less frequently, but the complications remain similar.[17,19,21,81] With mastoiditis occurring less commonly, physicians are less apt to consider the diagnosis, especially when the clinical picture has been masked by antibiotic therapy or when the process is chronic and of low grade. Appropriate antibiotic therapy, often accompanied by surgical drainage, can halt and prevent serious complications if mastoiditis is diagnosed early.

HISTORY

Before the advent of antibiotics, mastoiditis was a frequent complication of otitis media that could be treated only by expectant waiting or surgery.[32,36] When surgery was used, many patients with mastoiditis were cured by simple mastoid drainage alone, with a mortality rate quoted at 2 percent.[36] Intracranial complications of mastoiditis carried a very grave prognosis, however. In the pre-antibiotic era, between 1928 and 1933, 25 of every 1000 deaths at Los Angeles County Hospital in California were caused by intracranial complications of otitis media, such as meningitis, venous sinus thrombosis, and brain abscess. In contrast, between 1949 and 1954, only 2.5 per 1000 deaths at the same hospital were caused by complications of otitis or mastoiditis. The use of antibiotics in treating mastoiditis initially led to a marked decrease in the surgical approach to treatment of this illness.[7,81] The realization that infection can persist and that complications of mastoiditis can occur even while the patient is receiving antibiotic therapy has resulted in the present-day approach of combined antibiotics and surgery.*

BACTERIOLOGY

The bacteriology of acute mastoiditis differs from that of acute otitis media (AOM) (Table 20–1). For decades, the predominate bacterial cause of AOM was *Streptococcus pneumoniae*, with *Haemophilus influenzae* being the second most common isolate. Since the introduction of pneumococcal conjugate vaccine, nontypeable *H. influenzae* is the most frequent pathogen isolated (57%) in AOM, with *S. pneumoniae* being the second most common (31%), *Moraxella catarrhalis* the third most common (7-10%), and group A beta-hemolytic streptococcus the fourth most common (3-10%).[59] Studies on acute mastoiditis (defined as symptoms of <1 month's duration) show that *S. pneumoniae* is the most common isolate, with *Streptococcus pyogenes* and *Staphylococcus aureus* as the second and third most common isolates, respectively.[17,19,25,28,49,54,80]

H. influenzae has been isolated from the middle ear of patients with mastoiditis, but less often than one would expect, given its frequent recovery in AOM without mastoiditis. Gram-negative bacteria, enterococci, anaerobes, and *Mycobacterium tuberculosis* also have been isolated occasionally in patients with acute mastoiditis. An increased incidence of penicillin-resistant *S. pneumoniae* infections has occurred, leading to a higher likelihood of mastoiditis being a complication of otitis media.[53,80] The percentage of pneumococcal mastoiditis caused by penicillin-resistant strains increased from 25 to 44 percent between 1994 and 1998, without an increase in the total number of cases of pneumococcal mastoiditis.[30] Although *M. catarrhalis* is a common cause of otitis media, it rarely is noted in association with mastoiditis.[35]

The bacteriologic spectrum of chronic mastoiditis differs from that of acute mastoiditis. Aerobic cultures of chronic mastoiditis and chronic otitis media show predominantly *S. aureus* and gram-negative bacilli, especially *Pseudomonas aeruginosa*.[4,13,61] In addition, a wide variety of anaerobic organisms can be isolated from an infected mastoid and middle ear.[4,13] Brook[4] studied the aerobic and anaerobic bacteriology of chronic otitis media (of ≥3 months' duration) in 24 children. Anaerobic isolates alone were found in 17 percent, aerobic organisms alone were found in 4 percent, and mixed aerobic and anaerobic infections were found in 79 percent. All cases had from two to seven different bacterial isolates. *Peptococcus* spp., *Actinomyces* spp., and *Bacteroides melaninogenicus* (*Prevotella melaninogenica*) were the most commonly isolated anaerobic organisms. Seventeen patients were infected with β-lactamase–producing organisms (i.e., *S. aureus* or *P. melaninogenica*, *Bacteroides fragilis*, or other *Bacteroides* spp. that were resistant to ampicillin).

M. tuberculosis currently is an uncommon cause of mastoiditis in the United States but continues to be a cause of chronically draining ears in lower socioeconomic groups and immigrants from endemic areas.[5,45,47] Case reports of mastoiditis implicate such organisms as nontuberculous mycobacteria,[52] *Aspergillus fumigatus*,[23] *Paragonimus*-like trematodes,[55] *Nocardia asteroides*,[43] *Actinomyces* spp.,[67] *Blastomyces dermatitidis*,[30] and *Histoplasma capsulatum*.[43] *Pneumocystis carinii* otitis media and mastoiditis have occurred as the first manifestation of acquired immunodeficiency syndrome.[18]

ANATOMY AND PATHOPHYSIOLOGY

The mastoid process comprises the posterior part of the temporal bone and, as such, is adjacent to many important structures. Within the mastoid is an interconnecting system of air cells divided by bony septa that drain superiorly into the middle ear via a narrow aditus.[7,20] Only the superior portion of the mastoid airspace, the antrum, is present at birth; pneumatization of the mastoid starts soon after birth and usually is completed by the time the child is 2 years of age.[2,36] Structures lying anteromedial to the mastoid process include the middle ear and ossicles, the facial nerve, the posterior bony wall of the external auditory canal, the jugular vein, and the internal carotid artery. Posteromedially, the mastoid borders the posterior cranial fossa and the sigmoid sinus. Superiorly, the mastoid borders the middle cranial fossa. Medially, the mastoid cortex encases the cochlea and semicircular canals. The soft tissues and muscles of the lateral neck are located inferiorly. Any or all of these adjacent structures can be affected by extension of a suppurative process in the mastoid.

A certain amount of mastoid inflammation accompanies all cases of otitis media because the mastoid airspaces are continuous with the middle ear cavity and both are lined by a continuous mucoperiosteum.[2,20] The first stage of an ear and mastoid

*See references 21, 22, 25, 39, 44, 49, 54, 68, 79.

TABLE 20–1 Summary of Bacterial Isolates from the Middle Ear, Subperiosteal Abscess, or Mastoid of Children with Mastoiditis in Seven Studies

	Acute Mastoiditis						Chronic Mastoiditis
Isolates	***Ginsburg et al., 1955-1979***[19]	***Hoppe et al., 1975-1992***[28]	***Ogle and Lauer, 1973-1984***[54]	***Nadal et al., 1971-1988***[49]	***Ghaffar et al., 1983-1999***[17]	***Zapalac et al., 1993-2000***[80]	***Brook, 1976-1980***[4]
Streptococcus pneumoniae	14	13	5	9	20*	15†	1
Streptococcus pyogenes	8	4	3	4	4	10	2
Staphylococcus aureus	8	2	1	4	5	3	8
Staphylococcus epidermidis	1	2	2	6	7	12	—
Other aerobic gram-positive cocci	3	—	1	2	2	—	4
Haemophilus influenzae	1	—	2	1	—-	1	—
Pseudomonas aeruginosa	2	—	—	3	5	—	7
Other aerobic gram-negative rods	1‡	1	1	1	4§	—	7**
Anaerobic cocci	1	—	—	1	—	—	23
Anaerobic gram-positive bacilli	—	—	—	1	—	—	14
Anaerobic gram-negative bacilli	1	—	1	—	—	—	24
Other	1¶	—	4	—	—	10¶¶	—
Total patients with cultures	49	28	30	54	49	64	24

**Four of 12 tested isolates were penicillin resistant.*
†*Nine of 15 isolates were penicillin resistant.*
‡*Citrobacter* spp.
§*Escherichia coli*, *Klebsiella oxytoca*, *Proteus mirabilis*, *Serratia marcescens.*
¶*Mycobacterium tuberculosis*.
¶¶*Pseudomonas*, *Prevotella*, and *Bacillus* spp.
***Polymicrobial—unspecified.*
***E. coli (5)*, *Klebsiella pneumoniae (2).*

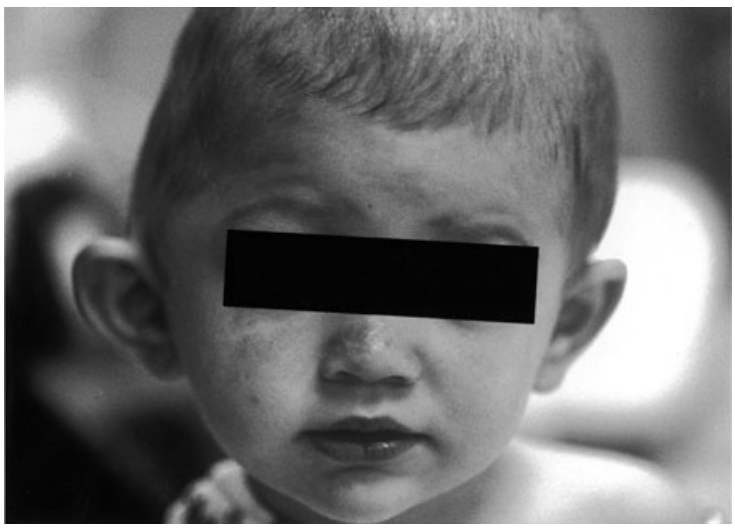

Figure 20–1 An 18-month-old child with right-sided mastoiditis and proptotic right auricle.

infection is associated with hyperemia of the middle ear and the mastoid air cell mucosa. If the infection persists, an exudative stage develops, with serum, fibrin, polymorphonuclear cells, and red blood cells accumulating in the middle ear and mastoid. The accumulation of purulent exudate increases the middle ear pressure, eventually resulting in perforation of the tympanic membrane, followed by drainage of mucopurulent matter from the middle ear and mastoid air cells. Some children also have such marked mucoperiosteal swelling that the drainage of pus from the mastoid is blocked. The pus under pressure creates an environment of local acidosis, hypoxia, and ischemia, causing decalcification and resorption of the bony septa. The term *coalescent mastoiditis* is applied to this process because, with the destruction of the bony septa, the mastoid air cells coalesce into large cavities. Osteomyelitis of the adjacent bone may develop, with subsequent bony erosion and eventual extension of the infection into surrounding structures.[2,20]

Congenital cholesteatomas usually manifest as a "squamous pearl" in the anterosuperior quadrant of the middle ear, abutting the tympanic membrane. They may be associated with recurrent otitis media.[42] Acquired cholesteatomas often are a result of chronic infections and tympanic membrane perforation. The perforation allows for squamous material from the external auditory canal to enter the middle ear space.[57] This tissue contains osteolytic enzymes, leading to bony erosion or mastoid air cell obstruction.[50] Cholesteatomas also may cause slow, insidious erosion of underlying bone, predisposing the patient to extramastoid spread of infection months or years later.[20,61]

CLINICAL PRESENTATION

The classic presentation of acute mastoiditis is a febrile child with ear pain, postauricular swelling, and postauricular tenderness developing days to weeks after the beginning of development of AOM (Fig. 20–1).[17,19,49,54,80] If antibiotics were used to treat AOM, the child may have seemed to improve, only to become ill again while still receiving therapy or after the antibiotics were stopped;

conversely, the child may not have responded to the antibiotics at all. Examination of the tympanic membrane in acute mastoiditis usually shows that it is abnormal.[19,25,49] Early in the course of illness, periosteal inflammation produces swelling and tenderness and sometimes redness over the mastoid process.[2,19] Palpable postauricular fluctuance occurs later, when pus from the mastoid air cells breaks through the underlying bony cortex and forms a subperiosteal abscess.[24] In children older than 1 year of age, the most common area where fluctuance is felt lies behind the ear, where it pushes the earlobe up and out; however, in children younger than 1 year, the fluctuance often may occur above the ear, pushing the pinna down and out.[19]

Chronic mastoiditis is a much more indolent disease process than is acute mastoiditis. It develops when long-standing middle ear disease, usually having a duration of months to years, has been present.[61] Fever and postauricular swelling may or may not be present. Persistent or intermittent drainage of mucopurulent matter from a previously perforated eardrum suggests chronic mastoiditis. Hearing loss and ear pain also may accompany chronic mastoiditis.[61] All these symptoms can be mild enough to be ignored until serious intracranial suppuration occurs. Persistent ear drainage, persistent ear pain, or an otitis media nonresponsive to antibiotics should prompt a search for mastoiditis.

COMPLICATIONS

Complications of mastoiditis include subperiosteal abscess,[24] Bezold abscess,[15,63] facial nerve paralysis,[71] meningitis,[7,19] brain abscess,[7,19] cerebellar abscess,[2] epidural abscess,[2] subdural empyema,[3] labyrinthitis,[20] venous sinus thrombophlebitis,[12,33,56,73] bacteremia,[7,44] benign intracranial hypertension,[3] osteomyelitis of the temporal bone with occasional extension to adjacent bones,[2,20] hearing loss,[58] septic pulmonary emboli,[29] and cerebrospinal fluid otorrhea.[20] Subperiosteal abscesses appear as a postauricular fluctuant mass that obscures the postauricular sulcus. They occur when pus in the mastoid breaks through the bony cortex or extends along vascular channels and dissects under the overlying periosteum.[24]

A Bezold abscess develops when a mastoid infection erodes through the bony cortex of the inferior aspect of the mastoid tip and dissects down the tissue planes to form a deep neck abscess. Fluctuance over the mastoid is not felt. Rather, swelling and tenderness are present below the mastoid process and under the sternocleidomastoid muscle.[15]

The facial nerve runs close to the mastoid and the middle ear, rendering it vulnerable to injury when extension of mastoid or middle ear infection occurs.[20] Pressure on and inflammation of the facial nerve from symptomatic or asymptomatic mastoiditis can lead to transient or permanent facial nerve paralysis that usually is unilateral, although bilateral facial palsy from mastoiditis can occur.[2,71]

Because the temporal bone that houses the mastoid air cells constitutes the floor of the middle and posterior cranial fossae, bony erosion from osteomyelitis, preexisting bony defects, or spread of infection along vascular channels can allow for intracranial spread of mastoid infections into the middle and posterior cranial fossae. The infection may remain confined to the extradural space as an extradural abscess, or it may penetrate the dura and produce a subdural empyema, a brain abscess, a cerebellar abscess, or meningitis.[2,20]

Invasion of infection into the bony labyrinth through the oval or round window triggers a labyrinthitis. Initial tinnitus, hearing loss, nausea, and dizziness progress to severe vertigo, ear pain, vomiting, nystagmus, and difficulties with balance.[20]

Intracranial venous sinus thrombophlebitis is a rare but potentially fatal complication of mastoiditis.[2,20,33,56,69,73] The lateral aspect of the sigmoid sinus is formed by the temporal bone. Venous sinus thrombophlebitis results when an underlying mastoiditis extends through the temporal bone in close proximity to the lateral or sigmoid venous sinus. A perisinus abscess initially is formed, followed by formation of a mural thrombus in the sinus wall. The thrombus eventually may occlude the entire sinus, or it may suppurate and spread along the sinus, resulting in septicemia, increased intracranial pressure, septic emboli, and extension of infection to other intracranial structures.[12,16] The classic findings of a septic thrombosis of the lateral sinus are spiking fevers, shaking chills, and tenderness along the jugular vein associated with acute or chronic otitis. A palpable "cord" at the jugular vein (indicating a jugular vein thrombus) also may be present. When only a perisinus abscess is present, or if the patient is being treated partially with antibiotics, the only symptoms may be a low-grade fever and headache.[22,69]

Benign intracranial hypertension can be seen in association with lateral sinus obstruction secondary to mastoiditis and is termed *otitic hydrocephalus.* Rarely, otitic hydrocephalus can be seen with mastoiditis in the absence of lateral sinus thrombosis. The decreased venous drainage caused by the venous sinus obstruction results in increased intracranial pressure, headache, papilledema, and sixth nerve palsy without enlarged ventricles or a space-occupying lesion.[20,22]

Permanent conductive hearing loss occurs when the middle ear mastoid infection is severe enough to damage or destroy the ossicles. Tuberculous mastoiditis classically manifests with marked conductive hearing loss that often is irreversible.[40]

Osteomyelitis secondary to mastoiditis can spread to adjacent bones. Involvement of the petrous portion of the temporal bone produces a syndrome, described by Gradenigo in 1907, with a triad of abducens paralysis or paresis, severe pain in the distribution of the trigeminal nerve, and suppurative otitis media; additional cranial nerve deficits also may occur.[6,39] Antibiotics may mask the classic signs of petrositis and allow progression to severe intracranial complications, such as meningitis and epidural abscess. Petrositis may be suspected only when antibiotic and surgical management for mastoiditis fails to control chronic ear drainage.[20]

M. tuberculosis mastoiditis is an uncommon finding but should be considered in children who have chronic ear discharge despite having received antibiotic therapy.[40,45] Children can go for months or years with chronic draining ears before the diagnosis of tuberculous mastoiditis is considered.[5,47] Children from lower socioeconomic homes, immigrants from endemic areas, and children with tuberculosis contacts in the family are at risk. The classic presentation in the pre-antibiotic era was an afebrile young child with painless persistent watery ear drainage, an enlarged preauricular lymph node, a history of tuberculosis contact, and often facial nerve paralysis.[64,72]

Tuberculous mastoiditis not always is painless.[76] Sometimes, the diagnosis initially is suspected only when a mastoidectomy wound does not heal.[38,61] Early in the course of the disease, physical examination may reveal small yellow spots (caseating granulomas) on a thickened and hyperemic tympanic membrane. These spots coalesce early and produce tympanic membrane perforations.[38] The discharge through these perforations initially is watery, but later it becomes purulent.[37] Pale, avascular granulation tissue is abundant throughout the middle ear and mastoid and often is seen in the external auditory canal and around the tympanic membrane perforation.[5,64,76] Preauricular and postauricular nontender, enlarged lymph nodes may be present,[37,43] and early and severe hearing loss is characteristic.[76] Often, there is evidence of tuberculosis elsewhere in the body.[76] A 5–tuberculin unit purified protein derivative skin test usually, but not always, is positive.[47]

DIFFERENTIAL DIAGNOSIS

Postauricular swelling, a chronically draining ear, or radiographic evidence of mastoid abnormalities also can appear in other disease entities. Postauricular lymphadenopathy can occur secondary to a scalp infection, causing postauricular swelling. The swelling would be discrete, would not displace the pinna, and would not obliterate the postauricular sulcus.[19] Severe otitis externa may lead to periauricular cellulitis, with postauricular swelling, erythema, and tenderness.[27] Mumps can cause parotid swelling, pushing the earlobe up and out, but the swelling is over the parotid gland, rather than located postauricularly. Histiocytosis,[41] acute lymphocytic leukemia,[48] acute myelogenous leukemia,[70] Burkitt lymphoma,[74] aneurysmal bone cysts,[10] and other benign and malignant tumors of the mastoid bone[8] also can manifest with symptoms clinically suggestive of mastoiditis. Kawasaki disease may mimic acute mastoiditis with postauricular lymph node swelling and ear pain.[62] Children with severe and recurrent ear infections may have an underlying congenital or acquired immunodeficiency.

SPECIFIC DIAGNOSIS

The diagnosis of mastoiditis can be made on clinical grounds alone when a child has an acute episode of fever, otitis media, and posterior auricular tenderness and fluctuance. Temporal bone computed tomography (CT) always is recommended to confirm the diagnosis. Mastoiditis is much less likely when swelling and tenderness over the mastoid process are absent, such as when an infection has been masked by antibiotic treatment or when it has extended to an area other than over the mastoid process. Mastoiditis needs to be considered in all cases of otitis media not responding to antibiotics and in all intracranial suppurative diseases that do not have an apparent focus.

Obtaining an aspirate from the middle ear is an important part of properly diagnosing and managing mastoiditis. Gram stains of aspirates from the middle ear are quite accurate and, as such, can help in the initial selection of antibiotic therapy for chronic mastoiditis. Brook[4] found that in 24 children with tympanocentesis, half of the Gram stains showed a complete correlation with subsequent culture results and the other half showed a partial correlation (one bacterial species was not seen). Leukocytes were seen on all the Gram stains. In addition, cultures from the middle ear accurately reflect mastoid disease.[19,44]

Ginsburg and associates[19] compared the results of cultures of middle ear aspirates and mastoid cultures in 16 patients with acute mastoiditis and found that the same bacterial species was isolated from both sites. A sterile aspiration through an intact tympanic membrane gives the most accurate culture information. If the tympanic membrane is perforated, the purulent drainage may be contaminated by colonizing ear canal flora. An aspirate for culture generally should be obtained from the ear drainage, preferably from as close to the perforation as possible. Aspiration of postauricular fluctuance also is useful in identifying the responsible organisms.[24,54] In addition, specimens should be obtained directly from the mastoid at surgery. All of them should be sent for aerobic and anaerobic cultures with proper anaerobic transport technique. If the child has had a chronic ear infection, or if the child is in a high-risk population for acquiring tuberculosis, mycobacterial stains and cultures also should be obtained and a purified protein derivative should be placed.

A lumbar puncture should be performed if the clinical presentation suggests meningeal irritation. A CT scan should be obtained before performing the lumbar puncture if papilledema or a suggestion of focal intracranial extension is present. Lymphocytosis of the cerebrospinal fluid suggests a parameningeal focus of infection. An immunologic evaluation should be considered if a child has had recurrent episodes of otitis media leading to mastoiditis.

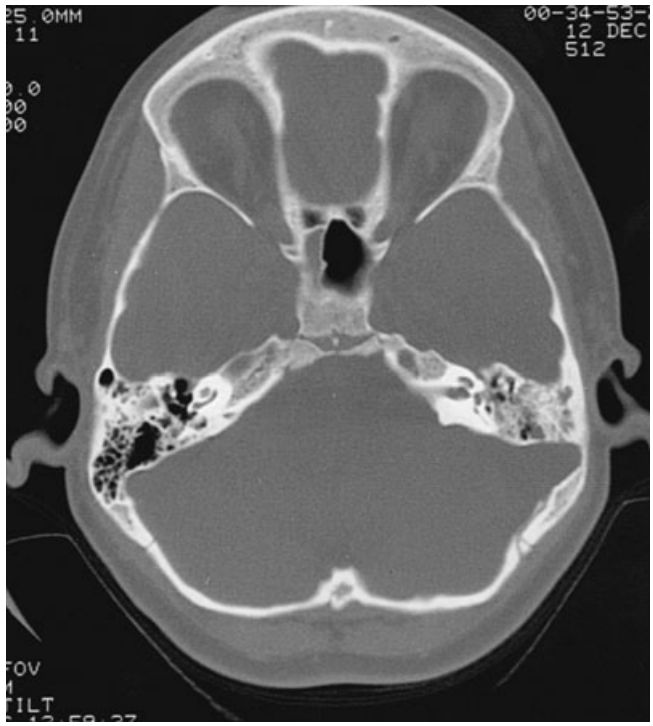

Figure 20–2 Computed tomography scan of the head of a 12-year-old boy with left lateral sinus thrombosis and increased intracranial pressure complicating left mastoiditis. The left mastoid air cells are opacified with marked loss of the fine bony septa, in contrast to the right mastoid air cells.

Peripheral white blood cell counts in mastoiditis may be normal or elevated, often with an increase in band-form neutrophils.[25,49] The erythrocyte sedimentation rate often is elevated in acute mastoiditis, but it usually is normal in chronic mastoiditis.[58]

Mastoiditis often can be diagnosed on clinical findings alone. When a patient is not improving on medical therapy, radiologic imaging of the temporal bone is needed. Changes in the temporal bone are seen best by CT scan.[66] Early in the course of mastoiditis, nonspecific clouding of the middle ear and mastoid is seen. With time, necrosis and coalescence of the bony septa occur. Other CT findings in mastoiditis include hypoaeration of the mastoid and adjacent bony destruction. Figure 20–2 shows a CT scan of a child with unilateral mastoiditis complicated by lateral sinus thrombosis and elevated intracranial pressure. Magnetic resonance imaging is valuable to search for suppurative intracranial complications of mastoiditis.[65,66] In addition, sometimes magnetic resonance angiography or venography (or both) is indicated to diagnose sigmoid sinus thrombosis.[8]

TREATMENT

The pediatrician and the otolaryngologist should work together in the management of a child with suspected or proven mastoiditis because all children with mastoid infections are potential surgical candidates. Some kind of drainage for the middle ear should be provided early in the course of therapy for therapeutic and diagnostic purposes. The drainage could consist of tympa-

nocentesis with subsequent tympanostomy tubes or a myringotomy.[19,25,49,54,80] A specific etiologic diagnosis is more important today than in the past because of the increasing incidence of infections caused by penicillin-resistant pneumococci and methicillin-resistant, coagulase-positive staphylococci.[1,14,26,31,51,60,75]

If the patient has an acute onset of posterior auricular swelling and tenderness with minimal or no posterior auricular fluctuance and no signs of intracranial complications, he or she is likely to respond to antibiotic therapy alone.[25,49,54,79]

Indications for surgical intervention include postauricular fluctuance; a history of chronic ear drainage with postauricular swelling or bony changes on radiographs; facial nerve palsy; nausea, vomiting, and vertigo suggestive of labyrinthitis; meningitis; brain abscess; venous sinus thrombosis with or without intracranial hypertension; epidural or subdural empyema; and petrositis. In addition, if the patient initially is treated medically, a mastoidectomy is indicated if progression of postauricular swelling or fluctuance or persistence of fever, ear pain, or purulent ear drainage occurs while the child is receiving parenteral antibiotics.[17,20,25,49,54,68]

All surgical treatment should include culture of the middle ear or mastoid, either by tympanocentesis or during surgery. If a notable middle ear effusion is present, drainage of the middle ear and equalization of pressure should be done by placement of a tympanostomy tube or a wide circumferential myringotomy.

If postauricular subperiosteal abscess or a Bezold abscess is present, incision and drainage need to be performed. Incision and drainage should be combined with myringotomy and tube placement. In addition, consideration should be given to performing a simple mastoidectomy to remove any diseased mastoid cells or inflammatory mucosa. When a mastoidectomy is done, a biopsy specimen of the inflammatory tissue should be sent to the laboratory for examination to exclude lesions such as eosinophilic granuloma or rhabdomyosarcoma.

For intracranial complications (e.g., subdural or cerebral abscess), a mastoidectomy needs to be performed. Draining the mastoid at the same time as draining the intracranial abscess has been done safely in the same operative setting.[34]

If a patient is found to have a cholesteatoma, a modified radical mastoidectomy, with complete removal of the cholesteatoma, should be performed. A child with a cholesteatoma is likely to need a second-look operation at a later date to monitor for recurrent disease.

Ototopical drops should be included in the postoperative care. Ciprofloxacin ear drops seem to be less toxic than aminoglycoside drops.[11,77,78] All children with mastoiditis should have audiograms performed to help distinguish a purely conductive loss (indicative of middle ear involvement) from a sensorineural loss (a sign of inner ear involvement).

The initial choice of antibiotics for mastoiditis must be made empirically, based on the knowledge of the most likely organisms. Because acute mastoiditis most often is caused by *S. pneumoniae* or *S. pyogenes* and less often by *S. aureus* and *H. influenzae*, oxacillin (150 mg/kg/day divided every 6 hours) and cefotaxime (200 mg/kg/day divided every 6 hours) can be recommended. In cases of severe illness or when possible central nervous system involvement is present, initial administration of vancomycin (60 mg/kg/day intravenously every 6 hours) instead of oxacillin often is desirable because of the possibility that resistant pneumococci or staphylococci are present.

In cases of chronic mastoiditis in which symptoms have been present for longer than 1 month, *S. aureus*, gram-negative bacilli (especially *P. aeruginosa*), and anaerobes are seen more frequently. A broad-spectrum combination of intravenous antibiotics is recommended: an aminoglycoside, such as gentamicin (7.5 mg/kg/day divided every 8 hours), for gram-negative bacillary coverage and a semisynthetic penicillin, such as ticarcillin-clavulanate or piperacillin-tazobactam (200 to 300 mg/kg/day divided every 6 hours). These semisynthetic penicillins are synergistic with gentamicin against *P. aeruginosa*, and they are effective against anaerobes and methicillin-sensitive *S. aureus*. The antibiotics can be adjusted based on the identification and sensitivities of the organisms isolated from the pretherapy cultures. For an intracranial extension of infection caused by *P. aeruginosa*, better penetration of cerebrospinal fluid can be obtained with an antipseudomonal cephalosporin such as cefepime (150 mg/kg/day divided every 8 hours) rather than ticarcillin-clavulanate or piperacillin-tazobactam.

The approach to antibiotic therapy for acute and chronic bacterial mastoiditis should conform to the principles of therapy for osteomyelitis. Treatment is begun with intravenous antibiotics. Intracranial extension of infection or mastoiditis with an organism for which no effective oral antibiotic (e.g., *P. aeruginosa*) exists requires long-term intravenous antibiotic therapy. Otherwise, after the patient has responded well clinically, high-dose oral antibiotic therapy can be considered (e.g., two or three times the normal dose of an oral cephalosporin).[46] Total duration of therapy should be for a minimum of 3 weeks and possibly longer, depending on the severity of illness, the causative organism, and the clinical response. Optimally, the causative organisms and their sensitivities should be known, and an oral agent to which the organisms are sensitive is needed. An oral regimen should not be attempted if vomiting or diarrhea is present because either one would prevent adequate oral absorption. Following the sedimentation rate may be helpful in monitoring treatment. The oral antibiotic should be given strictly every 6 hours rather than four times a day to ensure around-the-clock therapeutic drug levels.

No study has examined the optimal antituberculous chemotherapy for mastoiditis. If *M. tuberculosis* mastoiditis is probable or diagnosed, antituberculous chemotherapy should be started as recommended for tuberculosis of the bone (see Chapter 107).

If fever, purulent ear drainage, or ear pain persists despite antibiotic therapy and surgery, further evaluation is needed to search for either resistant organisms or a persistent site of infection that requires additional surgical drainage. In addition, a slow response to therapy should raise the possibility of an underlying immunodeficiency.

PROGNOSIS

The prognosis of mastoiditis depends on the extent of the infection and the causative organism. Mastoiditis that is treated adequately early in the course of illness before the onset of intracranial extension has a very good prognosis. Facial nerve paralysis is reversible early on,[64,71] and benign intracranial hypertension resolves with treatment of the mastoiditis.[20] Permanent neurologic deficits and death may occur if mastoiditis extends to cause meningitis, brain abscesses, epidural abscess, subdural empyema, or venous sinus thrombophlebitis. Symptoms of mastoiditis may recur if antibiotic therapy is of insufficient duration or if surgical débridement of an infected bone or of an infected cholesteatoma is inadequate. Sensorineural and conductive hearing deficits are reversible early on in mastoiditis; however, chronic infection may produce irreversible hearing loss.

PREVENTION

Early adequate antibiotic treatment of otitis media reduces significantly a child's risk for developing mastoiditis. In addition, rapid treatment of known mastoiditis along with early investigation of persistent ear drainage, persistent ear pain, or an otitis media that is not responding to antibiotic management decreases the risk for developing suppurative complications associated with mastoiditis.

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CHAPTER 21

Epiglottitis (Supraglottitis)

James D. Cherry

Epiglottitis (supraglottitis) is an illness characterized by inflammation and edema of the epiglottis and frequently also of the arytenoepiglottic folds and ventricular bands at the base of the epiglottis.[43] This disorder usually is caused by *Haemophilus influenzae* type b (Hib), and it mainly is a disease of children. The illness is characterized by rapid onset and progression, and without treatment, death caused by obstruction of the airway occurs. Epiglottitis in children is a pediatric otolaryngologic emergency.

HISTORY

The early history of epiglottitis is obscure, probably because of the importance of diphtheritic croup.[44] In 1887, Baron[9] described in detail a 30-year-old woman with epiglottitis who recovered after treatment with hot poultices and steam with tincture of benzoin. In 1900, Theisen[200] described three cases in the United States. Not until the early 1940s did acute epiglottitis become recognized as a definite clinical entity caused by Hib.[3,18,183] In 1948, Rabe,[155] in a study of 347 children with "infectious croup," presented evidence for the division of the clinical illness into three etiologic categories: diphtheritic croup, viral croup, and Hib croup (acute epiglottitis). Since the early 1990s, a dramatic decrease in the number of cases of epiglottitis has occurred in developed countries because of the widespread use of Hib conjugate vaccines.[4,34,64,70,78,80,126,136,138,152,209,215,227]

EPIDEMIOLOGY

In the pre-vaccine era, the epidemiology of invasive Hib disease varied markedly among different population groups.[215,216] Epiglottitis also differed by population group, but this variation was not related necessarily to the rate of overall Hib invasive disease in the population. For example, among Alaskan Eskimos and Navajo Native Americans, whose risk for acquiring invasive Hib disease was 4 to 10 times that of most other American populations, epiglottitis was not recognized among 295 patients with invasive Hib illness.[49,216]

Of Hib invasive disease, the percentage of cases of epiglottitis varied markedly among different localities. For example, in Israel, only 0.3 percent of invasive Hib infections were epiglottitis, whereas in Ireland, Wales, northeast England, Sydney (Australia), and Denmark, the percentage of epiglottitis cases varied between 16 and 32 percent.[50,68,91,109,135,154] In Minnesota and Dallas County, Texas, only 6 and 3 percent, respectively, of invasive Hib infections were epiglottitis.[142] In contrast with these findings in the United States, Europe, and Australia, the most common manifestation of invasive Hib infection in Sweden was epiglottitis.[46,206] In Sweden, the incidence of epiglottitis in children 14 years of age or younger in 1981 to 1983 was 10 per 100,000 per year. The annual incidence in Minnesota and Dallas County, Texas, in children 5 years of age or younger in 1982 to 1984 was 5.4 and 4.4 cases, respectively, per 100,000 per year.[143]

In children in the United States in the pre-vaccine era, the peak occurrence of epiglottitis was during the third year of life, and 72 percent of all cases occurred in children 1 to 5 years of age.[13,16,20,47,65,97] The disease occurred more commonly in boys than in girls; in 8 studies with 611 cases, 58 percent of cases were in boys.[13,16-18,20,47,65,97,141]

In specific geographic areas, marked differences in the yearly percentage of cases of epiglottitis occurred, but no intercity, national, or international cycles of illness were demonstrated.[13,16,47,65,97,141] Seasonal prevalence varied by locality but was not marked. The greatest number of cases in three studies occurred in the winter and spring,[20,97,141] whereas Baxter[16] observed more cases during the summer and in November, and Cohen and Chai[47] found no seasonal pattern.

Epiglottitis is a disease that occurs most commonly in temperate climates.[67,100] In several U.S. military hospitals, a wide geographic variation in incidence was noted; no cases were found among 4625 admissions at Gorgas Hospital in Panama, whereas 1 of 600 admissions to Elmendorf Hospital in Alaska was for epiglottitis.[13] In the past, acute epiglottis had not been reported in either Taiwan or Hong Kong.[96]

Epiglottitis also occurs in adults but less commonly than in children.[71,133,195,207] Annual rates in Rhode Island, Denmark, Finland, and northern California were 1.0, 0.9, 0.2, and 1.8 per 100,000, respectively. In both Ireland and Israel, rates of reported epiglottitis in adults appear to be increasing.[19,84] Whether these increases are true increases or are the result of increased awareness is not apparent.

In the present Hib conjugate vaccine era, the incidence of epiglottitis has fallen dramatically in all countries employing routine immunization, as has the incidence of all invasive disease caused by *H. influenzae*.[4,34,64,73,80,126,136,138,152,209,215,227] In northern Finland, the incidence in children 4 years of age or younger fell from 7.6 per 100,000 before 1988 to 0 per 100,000 after 1988.[4] In Sweden, in the 10-year period from 1986 to 1996, the rate of

epiglottitis in children younger than 14 years fell from 8.2 per 100,000 to less than 1 per 100,000.[74] At the Children's Hospital of Philadelphia, the average annual incidence of epiglottitis declined from 10.9 per 10,000 admissions before 1990 to 1.8 per 10,000 admissions from 1990 through 1992.[80] In this study, investigators also noted that the median age of patients increased from 35.5 months before 1990 to 80.5 months in the post-1989 period.

In recent years, an increase in incidence of invasive Hib disease, including acute epiglottitis, has been reported in England.[136] Many of the cases have occurred in children who had received Hib conjugate vaccine at 2, 3, and 4 months of age but not a booster dose in the second year of life.

ETIOLOGY

Acute supraglottitis in children in the pre-vaccine era almost always was caused by Hib. Lemierre and colleagues[120] and Sinclair[183] first called attention to "Hib laryngitis." This variant of laryngitis was characterized by marked swelling of the epiglottis and arytenoid regions, high fever, and shock. All 10 children described by Sinclair[183] had Hib bacteremia. Rabe[155] recognized a form of "croup" associated with epiglottitis and Hib bacteremia; 25 of 28 blood cultures (89%) yielded this organism. Table 21–1 summarizes 34 pediatric series from the pre-vaccine era with reported blood culture data. In children, 1570 of 2279 (69%) had blood cultures performed; Hib was isolated from 1191 of 1570 (76%).

As the clinical entity gained recognition, the frequency with which blood cultures were obtained and the yield of Hib increased. During 17 years of experience with epiglottitis in Denver, Colorado,[141] researchers found that 40 percent of all blood cultures yielded Hib; however, 70 percent yielded this organism during the last 5 years of the study.

Supraglottitis with bacteremia caused by other organisms in children rarely occurs, but cases have been noted more frequently during the last decade. The following pathogens have been impli-

TABLE 21–1 Etiology of Epiglottitis in Children

Author and Year of Study	Number of Patients	Number of Patients Having Blood Cultures (%)		Patients with Blood Cultures Yielding *Haemophilus influenzae* Type b (%)		Other Bacteria Isolated from Blood (%)*
Sinclair,[183] 1941	10	10	(100)	10	(100)	None
Rabe,[155] 1948	28	28	(100)	25	(89)	None
Berenberg and Kevy,[19] 1958	42	16	(38)	11	(69)	*Streptococcus pneumoniae*
Vetto,[213] 1960	37	2	(5)	2	(100)	None
Margolis et al.,[131] 1972	15	15	(100)	13	(87)	None
Johnson et al.,[97] 1974	55	33	(60)	20	(61)	None
Bass et al.,[13] 1974	97	6	(6)	1	(17)	*Staphylococcus aureus*
Milko et al.,[139] 1974	41	33	(80)	33	(100)	None
Branefors-Helander and Jeppsson,[30] 1975	15	14	(93)	13	(93)	None
Margolis et al.,[130] 1975	32	32	(100)	30	(94)	None
Battaglia and Lockhart,[14] 1975	40	40	(100)	13	(33)	None
Rapkin,[157] 1975	4	4	(100)	4	(100)	None
Smith and Ingram,[189] 1975	8	8	(100)	8	(100)	None
Benjamin and O'Reilly,[18] 1976	61	51	(84)	36	(71)	None
Molteni,[141] 1976	72	29	(40)	10	(34)	None
Breivik and Klaastad,[31] 1978	27	9	(33)	5	(56)	*S. aureus*
Cohen and Chai,[47] 1978	147	49	(33)	28	(57)	*H. influenzae*, not type b
Faden,[65] 1979	48	48	(100)	48	(100)	*S. pneumoniae*†
Bottenfield et al.,[28] 1980	24	22	(92)	18	(82)	None
Briggs and Altenau,[32] 1980	53	44	(83)	30	(68)	*H. influenzae* type a *H. influenzae*, nontypable *Haemophilus parainfluenzae*
Baugh and Baker,[15] 1982	24	22	(92)	18	(82)	None
Broughton and Warren,[35] 1984	24	19	(80)	19	(100)	None
Drake-Lee et al.,[60] 1984	25	19	(76)	19	(100)	None
Sly et al.,[188] 1984	171	89	(52)	71	(80)	None
Claesson et al.,[46] 1984	211	85	(40)	74	(81)	*H. parainfluenzae*
McGregor et al.,[134] 1985	31	31	(100)	19	(61)	None
Vernon and Sarnaik,[212] 1986	60	56	(93)	54	(96)	None
Gerber and Pfenninger,[75] 1986	137	126	(92)	83	(66)	None
Hodge and Ganzel,[90] 1987	25	24	(96)	14	(58)	None
Blackstock et al.,[24] 1987	14	12	(86)	10	(83)	None
Butt et al.,[38] 1988	349	234	(67)	187	(80)	None
Brilli et al.,[33] 1989	41	41	(100)	39	(95)	None
Losek et al.,[127] 1990	169	169	(100)§	131	(78)	*Bacillus* species *S. pneumoniae* *S. aureus*‡
Gorelick and Baker,[80] 1994	142	142	(100)	95	(67)	None
Totals	2,279	1,570	(69)	1,191	(76)	12 (1)

**Each organism represents one patient.*
†*One patient had S. pneumoniae and H. influenzae type b bacteremia.*
‡*One patient had S. aureus and H. influenzae type b bacteremia.*
§*Only patients with blood cultured and no prior antibiotic treatment reported.*

cated in this regard: *Streptococcus pneumoniae*[20,65,127]; *Staphylococcus aureus* (including one case in a 5-day-old baby)[11,31,61,127]; *Haemophilus parainfluenzae*[32,204]; group A,[17,112,186,222] group B,[125,228] group C,[8,176] and group G[92] streptococci; *Pseudomonas aeruginosa*[113] (in a patient with severe combined immunodeficiency syndrome); untypeable and *H. influenzae* type a[32]; and *Bacillus* spp.[127] *Candida tropicalis* was isolated from the blood of a 3½-year-old girl with supraglottitis who had been the recent recipient of an autologous bone marrow transplant.[214]

Candida albicans was noted in a case in a newborn whose mother had vaginal candidiasis and in a 6-year-old boy with chronic mucocutaneous candidiasis.[2] *Candida* spp. epiglottitis was noted in two children infected with human immunodeficiency virus (HIV),[145,181] and *C. albicans* epiglottitis was observed recently in a 2-year-old girl who was immunocompromised as a result of receiving chemotherapy for a primitive neuroectodermal tumor.[132]

The possibility that supraglottitis could be caused by a virus has been noted: a 16-month-old child with type 1 herpes simplex virus stomatitis complicated by stridor and respiratory distress had an epiglottis and arytenoepiglottic folds that were edematous and covered with vesicular lesions resembling those in the oral mucosa.[26] An 18-year-old girl had supraglottitis that also was caused by herpes simplex virus.[138] In addition, parainfluenza type 3 and influenza type B viruses were isolated from the nasopharynx of two children with supraglottic inflammation.[83] *Haemophilus paraphrophilus*[101] was recovered from the epiglottic surface of a single patient, as was *Moraxella catarrhalis* in another patient.[211]

In adults, Hib also has been the major cause of epiglottitis, but other organisms occur more commonly in adults than in children.[9,29,30,43,45,51,66,71,72,76,77,86,88,94,95,99,106,108,133,146,147,162,175,182,186,193,195,205,206] In 1992, Daum and Smith[54] reviewed 474 published cases of epiglottitis in adults, 293 of whom had blood cultures performed; 79 of those cultures (27%) yielded *H. influenzae*. Of these positive cultures, 43 were Hib; 35 isolates were not typed, and one isolate was not Hib. Trollfors and associates[206] in Sweden found that blood cultures were obtained from 185 of 356 (52%) adult patients, and *H. influenzae* was isolated from 53 percent of them. Of these, 53 were Hib, and the type of the remaining 45 was not known.

S. pneumoniae was reported to be isolated from the blood of 18 adults with supraglottitis, 10 of whom were receiving immunosuppressive therapy or were infected with HIV-1,[23,30,93,104,106,118,155,163,164,179] and *H. parainfluenzae* was isolated from the blood of 5 patients.[45,69,128,172,218] In Denmark between 1995 and 2002, *H. influenzae* type f was recovered from 13 cases of epiglottitis in adults.[36] Numerous other infectious agents have been implicated in case reports of adults with supraglottitis.[27,29,56,72,76,77,94,95,98,103,124,129,137,144,146,147,159,160,165,175,185,192,230] These agents include *Pasteurella multocida*, *Kingella kingae*, *Klebsiella pneumoniae*, group A and B streptococci, *Bacteroides* spp., *Fusobacterium necrophorum*, *Vibrio vulnificus*, *Serratia marcescens*, *S. aureus*, *Neisseria meningitidis*, *Aspergillus flavus*, and herpes simplex virus. Epiglottitis has been reported as a complication of infectious mononucleosis caused by Epstein-Barr virus infection.[39]

Epiglottitis also can result from noninfectious causes. Hot foods and water can cause thermal epiglottitis, as can poisoning with corrosive agents including cocaine alkaloid.[16,105,110,115] Hereditary angioedema may manifest with findings typical of epiglottitis.[149]

ANATOMY

The thin, elastic, leaflike epiglottic cartilage is attached to the anterior surface of the thyroid cartilage by the thyroepiglottic ligament (Fig. 21–1). The hyoepiglottic ligament also provides support and anchors the epiglottis to the hyoid bone. The superior aspect of the epiglottis arches slightly posteriorly. Stratified squamous epithelium covers the anterior surface of the epiglottis and the superior third of the posterior portion; respiratory epithelium covers the remaining posterior surface. The stratified squamous epithelium is loosely adherent and creates a large potential space for the accumulation of inflammatory cells and edema fluid.

The arytenoepiglottic folds arise from the epiglottis and terminate posteriorly near the paired arytenoid cartilages. These structures commonly are involved in the supraglottic infection and occasionally are the site of serious disease without epiglottitis per se.[16] Immediately anterior to the epiglottis are the valleculae epiglotticae, where saliva pools before deglutition.

PATHOPHYSIOLOGY

Supraglottic cellulitis with marked edema involving the epiglottis, arytenoepiglottic folds, ventricular bands, and arytenoids is the hallmark of this illness. As the edema increases, the epiglottis curls posteriorly and inferiorly. Inspiration tends to draw the inflamed supraglottic ring into the laryngeal inlet, whereas expiration is unopposed.[177] This "ball-valve" mechanism is thought to produce slight hypoxia without hypercapnia.[167] Diffuse infiltration with polymorphonuclear leukocytes, hemorrhage, edema, and fibrin deposition can be seen microscopically; this infiltration can progress to microabscesses, with Hib occasionally seen in the tissue.[100,162] Frank abscess formation has been documented in adults.[19,124,129,160,165,192,219,225] Infection of the supraglottic larynx

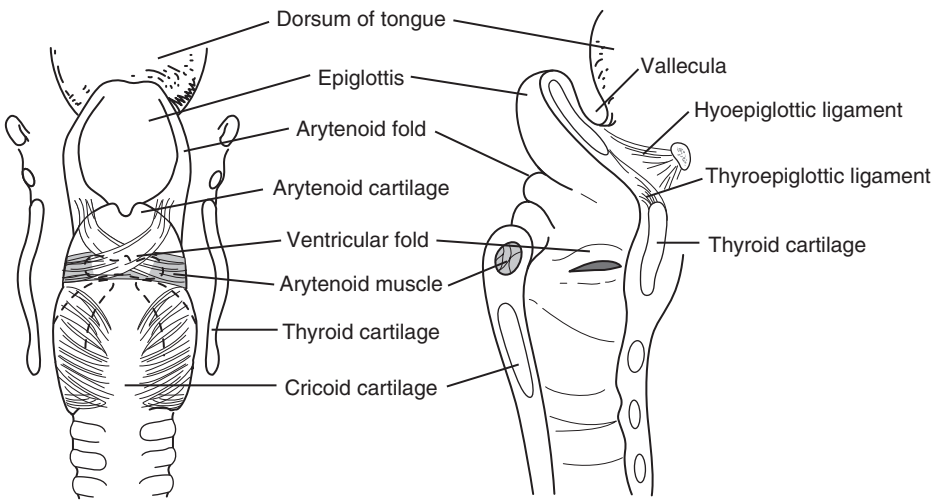

Figure 21–1 Anatomic relationships of the supraglottic larynx. Posterior view *(left)* and sagittal view *(right)*.

may spread inferiorly to involve the paraglottic space,[87] but as a rule, neither upward extension into the laryngeal lymphatics nor downward extension into the subglottic region occurs.[18,100]

Infection of the supraglottic structures probably arises from direct invasion by Hib with subsequent development of bacteremia. The bacteremia appears to be relatively short in duration and of low concentration, as suggested by several observations: (1) the serum concentration of the capsular polysaccharide is directly proportional to the concentration and duration of bacteremia,[168] (2) children with epiglottitis have less Hib capsular polysaccharide in their sera than do patients with meningitis,[217] and (3) the density of Hib in blood is significantly lower in patients with epiglottitis than in patients with meningitis.[117,166,194] In 23 patients with epiglottitis, the geometric mean number of organisms was 123 colony-forming units (CFU)/mL, whereas the geometric mean number in 43 patients with meningitis was 2203 CFU/mL ($p < .001$).[117]

What predisposes the epiglottitis to infection is unknown. Possibly, mild trauma to the epiglottis occurs during food intake. This trauma could result in damage to the mucosal surface, which, in turn, could allow the invasion of organisms that already were present in the upper respiratory tract. Also possible is that a viral infection damages the mucosal surface and thus predisposes the patient to secondary bacterial infection.

Acute-phase sera in most children with epiglottitis lack specific bactericidal and hemagglutinating antibody. Seroconversion regularly occurs after infection.[30,130]

Conflicting evidence exists relating to a possible genetic difference between patients with Hib epiglottitis and other individuals.[5,82,198,225] Whisnant and associates[225] surveyed human leukocyte antigens (HLA) and erythrocytic antigens among 30 children with epiglottitis and 20 patients with meningitis. HLA-A11 was found in 17 and 3 percent of the patients with meningitis and epiglottitis, respectively ($p < .01$). HLA-B5 occurred in 13 and 3 percent of patients with epiglottitis and meningitis, respectively ($p < .05$), whereas HLA-B40 occurred more often (23% versus 10%) in patients with meningitis than in those with epiglottitis ($p < .05$). Moreover, the frequency of HLA-A28 and HLA-B17 was higher among the patients with epiglottitis than in uninfected control subjects.[225] However, the results of another study did not confirm these observations.[82]

The distribution and frequency of MNS erythrocyte antigens in patients with epiglottitis may differ from those observed in others. For example, the NNSS genotype occurred in 6.4 percent of patients with epiglottitis and in 0.5 percent of healthy control subjects ($p < .0002$),[225] but this difference was not confirmed.[82] However, the results of two studies suggest that the MNS genotype occurs less often in patients with Hib meningitis than in patients with epiglottitis.[82,225] In one study, white children with Hib meningitis lacked G2m(n), an allotype antigen of IgG2 subclass heavy chains, more frequently than did control subjects.[5] In another study in a white population in Finland, however, this difference was not noted.[197] The frequency of the Km(1) immunoglobulin allotype in children with epiglottitis did not differ significantly from the prevalence of that marker in blacks or whites. However, in blacks with Hib meningitis, the Km(1) marker occurred less frequently in patients than in control subjects.[82] The identification of one outer-membrane protein subtype of Hib that was associated relatively infrequently with epiglottitis suggests that isotype-specific differences in the propensity of Hib to cause epiglottitis may exist.[196]

CLINICAL MANIFESTATIONS

The classic onset of epiglottitis in children is abrupt, and progression of disease is rapid; careful history on occasion reveals the occurrence of a trivial antecedent upper respiratory tract infection.* The total duration of illness before hospitalization usually is less than 24 hours and occasionally is as short as 2 hours. In one study in which 142 medical records of children with epiglottitis were reviewed, the duration of illness before tracheotomy was performed was found to be 12 hours or less in 73 percent and more than 24 hours in only four patients.[47]

The most common presentation of acute epiglottitis in children includes the sudden onset of fever, severe sore throat, dysphagia, and drooling. Airway obstruction always occurs and is rapidly progressive. It is manifested by distress on inspiration, a choking sensation, irritability, restlessness, and anxiety. The speech is muffled or thick sounding, but hoarseness usually does not occur. The child usually insists on sitting up in a characteristic posture with the arms back, the trunk leaning forward, the neck hyperextended, and the chin pushed forward. This posture increases the diameter of the obstructed airway.

In contrast to acute laryngotracheitis, in which marked inspiratory stridor occurs, the degree of observed stridor in epiglottitis often is not severe. This apparent lack of respiratory distress often leads the unwary physician to underestimate the severity of the child's illness. With progression, the air exchange becomes progressively worse, and hypoxia, hypercapnia, and acidosis develop. These developments cause increased irritability, restlessness, and disorientation, and if an artificial airway is not established, the child will experience sudden cardiorespiratory arrest.

Fever occurs in virtually all children; most temperatures are between 38.8° C and 40.0° C (101.8° F and 104° F). Blood leukocyte counts almost always are elevated; the mean total count was approximately 20,000 cells/mm^3 in five studies.[4,20,47,97,221] The differential cell count reveals an increased percentage of neutrophils and band forms; most patients have absolute band counts that are more than 500 cells/mm^3.

The clinical picture of epiglottitis in adults is more indolent than that in children.[19,86,191,231] Berger and associates[20] reviewed 118 cases of acute epiglottitis seen between 1986 and 2000. Of these patients, 33 percent were admitted to the hospital within 1 day of the onset of symptoms, and 77 percent were admitted within 3 days of onset. Symptoms on admission were as follows: sore throat, 85 percent; odynophagia, 83 percent; temperature elevation, 38 percent; respiratory difficulties, 34 percent; muffled voice, 25 percent; and drooling, 7 percent. In two other studies, an average of 1 to 3 days elapsed before medical aid was sought. The mean temperature was 38.2° C (100.7° F), and some patients were afebrile; the temperature range was 36.6° C to 40.0° C (97.8° F to 104° F). Blood leukocyte counts averaged 17,000/mm^3 (range, 8000 to 32,000/mm^3).[86,191] Sore throat and dysphagia were universal occurrences. Zwahlen and Regamy[231] reviewed the clinical features of 100 reported adult cases of epiglottitis. Of these patients, 78 percent had dyspnea, 49 percent had dysphonia, 41 percent had cyanosis, and 38 percent had stridor. Forty-six percent had edema of the neck. A precordial purring or fluttering sensation was described by one adult patient.[169] In three cases in adults, Ehara[63] noted on physical examination that all had tenderness of the anterior neck over the hyoid bone.

DIFFERENTIAL DIAGNOSIS

The hallmarks of the successful management of acute epiglottitis are an awareness of the condition and an understanding of the rapidity of its progression. A correct early diagnosis frequently is lifesaving. Acute epiglottitis must be differentiated from seven other conditions with symptoms of acute upper airway obstruction. Aspects of the differential diagnosis are presented in the text in Chapter 22 and in Table 22–4.

*See references 13, 14, 18, 20, 30, 33, 47, 65, 86, 97, 99, 122, 127, 130, 131, 141, 150, 155, 184, 213, 224.

In epiglottitis, the important differential points are as follows: the lack of a croupy cough; the presence of a swollen, cherry-red epiglottis; the sitting posture of the child with the chin pushed forward, as well as a reluctance or refusal to lie down; and the relatively greater apprehension and anxiety of the child than the degree of chest retraction suggests. In contrast, the child with acute laryngotracheitis has a normal epiglottis on examination, always has a typical barking cough, is comfortable in a supine position, and frequently appears to have only minimal apprehension in spite of retractions in which the sternum appears to be indenting 2 inches or more.

Acute angioneurotic edema that involves the epiglottis can mimic acute epiglottitis. However, in this condition, the temperature usually is normal, and the patient appears less toxic. This condition usually is brought on by a specific allergic reaction after the ingestion of a food or medication.

Supraglottitis should be considered in children with uvulitis because the clinical features of these disorders may overlap, and both infections may be present. Concomitant uvulitis and epiglottitis were described in association with Hib bacteremia in children[107,158] and with *S. pneumoniae* bacteremia in an adult.[223] Isolated uvulitis has been associated with Hib bacteremia and group A beta-hemolytic streptococcal pharyngitis.[107,123] Uvulitis in the absence of epiglottitis was described in a child with odynophagia, drooling, and Hib bacteremia.[123]

A foreign body lodged in a vallecula or the larynx or in penetrating posterior pharyngeal tissues may cause signs and symptoms that mimic those of acute supraglottitis.[220] A paravertebral collection of pus, from cervical osteomyelitis or parapharyngeal abscess, rarely can spread anteriorly and can produce acute "croup." Congenital anomalies and laryngeal papillomas can be excluded by their chronic course. *C. albicans* has caused neonatal laryngeal obstruction without radiologic epiglottitis.[153]

Infection of supraglottic structures by *Mycobacterium tuberculosis* occurs less commonly than does glottic involvement; tuberculous laryngitis is an exceedingly rare occurrence in children and always is associated with pulmonary lesions.[62] The onset is considerably more insidious than is that of Hib supraglottitis. Nasopharyngeal diphtheria may mimic acute epiglottitis and may be associated with a serosanguineous nasal discharge.

Chronic epiglottic enlargement with edema was observed in two children with cancer who had received radiation therapy to the neck. These clinical features were not confused with those of acute supraglottitis, although one patient had dysphagia and snored.[229] Severe, chronic inflammatory epiglottitis with associated granulomatous lymphangitis was found on histologic examination of tissue obtained from a 19-month-old black child who had epiglottic enlargement without erythema for 3 months.[220] "Tuberculoid" granulomatous lesions were seen at histologic examination of an epiglottic biopsy specimen obtained from a 22-year-old man, HIV status unknown, who presented with weight loss, sore throat, and dysphonia of 1 month's duration.[140] The epiglottitis and arytenoepiglottic folds of this patient were erythematous and edematous.

Lymphangiectasis of the epiglottis produced airway obstruction with stridor and intermittent cyanosis in a 4-month-old white boy. On histologic examination, the epiglottis consisted of multiple dilated lymphatic vessels lined by a single layer of epithelial cells with no discernible wall. The stroma contained scattered lymphocytes and a few neutrophils. This lesion spontaneously regressed, and the child's condition was normal at 1 year of age.[208]

SPECIFIC DIAGNOSIS

The clinical picture of sore throat, dysphagia, drooling, anxiety, and inspiratory distress without significant stridor and the characteristic sitting position should suggest the presumptive diagnosis in most cases. The definitive anatomic diagnosis is made by the visualization of the epiglottis, and the etiologic diagnosis is confirmed by culture of an organism from the blood or the surface of the epiglottis. An Hib origin also can be established by the demonstration of antigenemia or antigenuria.[189,217]

In the typical case, the epiglottis is fiery red and greatly swollen. In children, the epiglottis can be seen by simple depression of the tongue with a tongue blade. In older children and adults, indirect or direct laryngoscopy usually is necessary to confirm the diagnosis. On occasion, the obstruction is caused by swelling of the ventricular bands and the arytenoepiglottic folds so that the epiglottis may appear relatively normal.

Major controversy exists concerning the safety of using a tongue depressor to examine a child with suspected epiglottitis because sudden cardiorespiratory arrest has been noted to occur. However, most instances of cardiorespiratory arrest that I am aware of occurred after the child was forced into a supine position rather than because of the examination itself.[42] In many instances, patients with presumptive epiglottitis can be examined, while they are in an upright position, by using tongue blade or indirect laryngoscopy.

Case management should be individualized. In the child with moderate or advanced disease, the clinical diagnosis should be apparent without having to do an intraoral examination. In this situation, intraoral examination should not be performed, but the child should be prepared for the establishment of an airway. This preparation should be rapid but controlled so that intubation can be performed in an operating room.

The diagnosis of epiglottitis can be established by the classic appearance on a lateral neck radiograph (Fig. 21–2).[156,221]

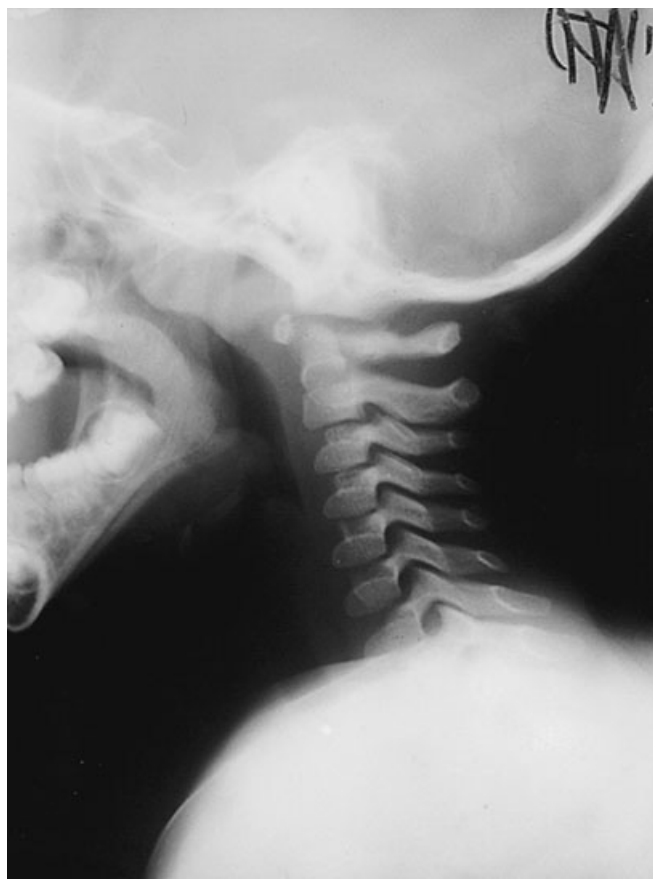

Figure 21–2 Acute epiglottitis. A lateral neck radiograph from a child with acute epiglottitis showing the swollen epiglottis (thumb sign) encroaching on the airway. (*Courtesy of Dr. Ines Bouchat.*)

However, my opinion is that this radiographic procedure rarely is necessary; all too often, it leads to a delay in providing the necessary definitive therapy.[47,100,139] The use of the lateral neck radiograph should be reserved for subacute cases in which the specific diagnosis after completion of a clinical examination is not clear.

The lateral film of the neck, delineating the soft tissues, taken with the patient upright, gives the best view of the upper airway anatomy (see Fig. 21–2). The hypopharynx is dilated; normal cervical lordosis may be replaced by a straight or kyphotic contour. The valleculae are narrowed and may be obliterated. A thickened mass of tissue stretching from the valleculae to the arytenoids emphasizes the appropriateness of the term *supraglottitis*. In adults with epiglottitis, the widths of the epiglottis and arytenoepiglottic folds uniformly exceed 8 and 7 mm, respectively.[174]

When performed, radiography of the neck in the anteroposterior projection usually reveals that tracheal narrowing is absent. However, some children with acute supraglottitis have localized subglottic narrowing indistinguishable from that found in acute laryngotracheitis.[178,187]

All patients with suspected epiglottitis should have a blood culture, and a culture specimen should be obtained from the surface of the epiglottis when an artificial airway is established. Today, cultures are of increasing importance because of the change in epidemiology of Hib infection and the resulting increased likelihood that an organism other than Hib may be causing the illness. A white blood cell count with a differential also may provide useful information. In children who have received antimicrobial treatment before cultures were obtained, performing a direct antigen test for Hib on the blood and urine is worthwhile.

TREATMENT

The treatment of acute epiglottitis should be relatively simple in that it involves only two main aspects of therapy: an airway must be established, and an appropriate antimicrobial agent needs to be administered. However, in the past, the mortality rate from epiglottitis varied from 0 to 32 percent,[47,86] a finding suggesting major differences in the implementation of treatment.

Most deaths occur in transit to the hospital or within the first few hours after arrival. Once the diagnosis is suspected, the patient should be attended constantly by individuals skilled in resuscitation with appropriate equipment for airway stabilization and ventilatory support. Delays of 2 or 3 hours have proved fatal; every effort should be made to reduce the time needed to secure a patient's airway and to initiate antibiotic therapy, before which unnecessary stress should be avoided. In most cases, radiographic confirmation should be omitted. Blood tests, extensive history taking, and delay in transport should be eliminated.

Medical centers and pediatric services that have planned protocols for the diagnostic investigation and treatment of patients with suspected acute epiglottitis generally have better morbidity and mortality statistics than do services that do not have such protocols in place. Pediatricians, radiologists, otolaryngologists, and anesthetists may contribute to the assessment and management of a case; defining roles and responsibilities of each service in advance minimizes confusion and renders the institution of care easier.

SECURING THE AIRWAY

In general, the cornerstone of all management plans is the establishment of an airway in all children in whom the diagnosis of epiglottitis is made.[44] In 1938, Sinclair[183] recognized that tracheostomy was lifesaving. Berenberg and Kevy[19] advocated hospitalization for all patients with epiglottitis but tracheostomy only "if necessary." However, Bass and associates[13] presented a compelling argument for performing routine tracheostomy. Among 83 patients with documented epiglottitis, 11 of whom were adults, these authors noted that 16 (19%) had life-threatening obstruction when they were first seen. An additional 14 (17%) progressed to this point within 6 hours of admission; 9 of the 83 (11%) required emergency tracheostomy while they were hospitalized. Of these 9, 2 died, and 1 suffered anoxic brain damage. All 9 were being monitored carefully, with bedside tracheostomy equipment available and trained personnel nearby. Six of the adults required tracheostomy for survival.[13] Margolis and colleagues[131] noted that elective tracheostomy, performed at the time of diagnosis, eliminated fatalities in 15 consecutive patients. This observation was in contrast to 4 deaths in 20 patients observed until tracheostomy "was required."

A large body of literature attests to the safety and efficacy of nasotracheal intubation as a replacement for tracheostomy,* which has a complication rate of 50 percent.[199,224] Biologically inert tubes, with their decreased risk of complicating subglottic stenosis,[180] and the recollection that endotracheal tube insertion was a universal approach before tracheostomy[139] was an accepted procedure led to the routine use of nasotracheal intubation in this disease. Nasotracheal intubation requires a shorter duration of airway maintenance: 32 patients with epiglottitis managed with tracheostomy had a mean duration of intubation of 7.5 days and a mean hospitalization of 8.8 days. In contrast, a nasotracheal tube was used for a mean of 38 hours with a 6.5-day hospitalization in 5 patients.[139] Diaz and Lockhart[57] managed 104 patients with nasotracheal intubation. The mean intubation time was 53 ± 14.9 hours; 7 patients (6.8%) extubated themselves, and 2 required reintubation. Laryngeal edema occurred in 3 patients (2.9%) who had been intubated. In 2 patients, subglottic granulations required excision.

My position, as well as that of Daum and Smith,[54] is that the argument for performing elective tracheostomy or, preferably, intubation in all children with supraglottitis is compelling, and the procedure should be performed immediately after the diagnosis is established. Whether this "stimulus-response" approach is necessary for adults with epiglottitis is controversial.[12,70,86,151,177,182,191,226] Mayo Smith and associates[133] compared selected clinical features in adults with epiglottitis at the time of diagnosis who died or who received an artificial airway with those of adults who recovered without airway intervention. Few differences emerged. Patients who died or who were managed with airway intervention had respiratory distress and bacteremia more often than did those who recovered without airway intervention. Obviously, the presence of bacteremia was not known on presentation. Moreover, the mortality rate among all adults managed expectantly was 4.6 percent, a figure comparable to the mortality rate (6.1%) of children in a series reported before recommendations were made for routine securing of the airway at diagnosis.[40,133] The preponderance of evidence until very recently has suggested that securing the airway in all adults with supraglottitis by nasotracheal intubation should reduce mortality rates.[7,10,12,13,21,22,51,106,133] However, Frantz and associates[71] reported an analysis of 129 cases of acute epiglottitis in adults in which no deaths occurred in the cohort, and 85 percent of these patients were managed without airway intervention.

In the large series reported by Berger and colleagues[20] involving 118 cases, 19 had airways established on admission (16 endotracheal intubation, 2 tracheotomy, 1 cricothyrotomy), and 6 other patients subsequently required either endotracheal intubation or tracheotomy. The remaining 93 patients were managed successfully without the need for airway intervention. Fifty-eight

*See references 14, 48, 89, 114, 131, 150, 157, 171, 173, 180, 199, 201.

TABLE 21–2 Size of Nasotracheal Tubes Recommended for Children with Acute Supraglottitis

Age	Size (mm)
Birth-6 mo	3.0
6 mo-3 yr	3.5
3-5 yr	4.0
>5 yr	4.5

Data from Rabe, E. F.: Infectious croup: III. Haemophilus influenzae type b croup. Pediatrics 2:559-566, 1948.

of the patients were treated with intravenous corticosteroids in addition to antibiotics, and these patients tended to have more prolonged hospital stays than those who did not receive steroids (5.7 ± 4.1 days versus 4.7 ± 4.3 days; $p = .2$).

To aid in intubation and to reduce long-term sequelae, most investigators have advocated use of a nasotracheal tube 0.5 to 1.0 mm smaller than that predicted by the patient's age.[89,139,171] Recommendations for tube size are shown in Table 21–2. Published criteria for extubation (summarized in reference 14) include those based on duration of therapy and those based on daily examination of the epiglottis and supraglottic structures by direct laryngoscopy[55] or fiberoptic bronchoscopy.[148,210]

Long-term complications of nasotracheal intubation are rare. Thirty-three children with epiglottitis who were managed with nasotracheal intubation (mean duration of intubation, 55 hours) were evaluated 1 to 8 years later. By history and measurement of peak expiratory flow rates, no complications were found.[171] Although additional long-term data are necessary to ensure absence of residua, elective nasotracheal intubation appears to be the procedure of choice.

Several reports documented the recognition of idiopathic pulmonary edema before[119] or, more commonly, after[25,55,59,73,102,190,202] insertion of an endotracheal tube to relieve laryngeal obstruction caused by epiglottitis. One hypothesis to explain this phenomenon is that airway obstruction produces markedly negative intrapleural pressure with increased venous return to the right side of the heart, with decreased left ventricular output and increased pulmonary blood volume.[116] These changes increase the pulmonary microvascular pressure and produce pulmonary hyperemia and edema. Endotoxemia may play a role in altering vascular permeability, but it is not necessary for recognition of this complication of airway obstruction because abrupt onset of pulmonary edema was described when airway obstruction caused by croup, foreign body, and malignant neoplasm was relieved acutely.[37] The frequency of pulmonary edema as a complication of intubation in supraglottitis was approximately 9 percent,[187] and it has occurred in an adult.[161] Continuous positive airway pressure in all intubated patients with epiglottitis probably will provide prophylaxis against this complication,[57,73] but controlled data are lacking.

ANTIBIOTICS

The mainstay of antibiotic therapy for acute epiglottitis in the recent past has been ceftriaxone (50 to 100 mg/kg/day given every 12 hours intravenously) or cefotaxime (100 to 200 mg/kg/day given every 6 hours intravenously) because virtually all cases in both children and adults were caused by Hib. However, in the present conjugate vaccine era, the incidence of all invasive Hib disease has decreased dramatically. Therefore, in children previously vaccinated, the cause is likely to be another organism. Culture results today have added significance because one possible cause that would require a change in therapy is *S. aureus*.

No controlled data exist about the duration of antimicrobial administration, but a course of 7 days seems appropriate. In the event that group A streptococci are isolated from the airway, penicillin is the drug of choice. A first-generation semisynthetic penicillinase-resistant penicillin or vancomycin (if methicillin-resistant *S. aureus* is suspected) should be used for *S. aureus*, whereas erythromycin is indicated for *Corynebacterium diphtheriae*.

OTHER SUPPORTIVE MEASURES

Some authors have advocated corticosteroid therapy on the basis of anecdotal experience in patients with epiglottitis, but no controlled data exist to support its use, and such therapy may be hazardous: among 91 patients with epiglottitis who received corticosteroid therapy, 4 (4%) had evidence of bleeding from the gastrointestinal tract that was sufficient in 2 patients to require transfusion,[58] a phenomenon observed by other investigators.[111] Therapy with racemic epinephrine is without benefit.

Expert respiratory nursing care is essential. Inadvertent extubation must be avoided, particularly in the first 24 hours. Judicious use of sedatives that do not appreciably depress respiration may be appropriate.

COMPLICATIONS

Extraepiglottic complications are not common occurrences in children with acute epiglottitis. In a study involving 72 children with epiglottitis, investigators noted that 25 percent had pneumonia, 25 percent had cervical adenitis, 8 percent had tonsillitis, and 5 percent had otitis media.[141] The other common invasive manifestations of Hib infections (meningitis, arthritis, and cellulitis) rarely are found in conjunction with epiglottitis.[141,170]

PREVENTION

PROPHYLAXIS OF HOUSEHOLD CONTACTS

In the pre–conjugate vaccine era, household contacts of patients with Hib infection were at increased risk for acquiring Hib infection.[81] Whether an increased risk occurred in daycare contacts was unresolved.[53] Although contacts of patients with Hib epiglottitis younger than 5 years are colonized less frequently than are contacts of patients with other Hib invasive infections,[52] secondary disease was described in household contacts when an index patient had epiglottitis.[1,78,79,203] Secondary Hib epiglottitis was described in a child[1] and two adults who were household contacts of a patient with Hib meningitis.[78,79] Hib epiglottitis occurred in two siblings who presented with the condition within 1 day.[85]

Rifampin prophylaxis, 20 mg/kg/day (600 mg/dose maximum) for 4 days, is recommended for all unvaccinated members of a patient's contact group when the index patient has Hib epiglottitis and at least one contact in the group is 4 years of age or younger.[6,41]

The recognition that adults occasionally may acquire secondary infection, particularly epiglottitis, on exposure to children with invasive Hib infection prompted some experts to extend prophylaxis to all of a patient's contact groups, regardless of the presence of one or more contacts who are 4 years of age or younger. All experts, however, recommended that adults and older children be made aware of the signs and symptoms of Hib disease, particularly when the patient's contact group would not receive prophylaxis under current guidelines.

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CHAPTER 22

Croup (Laryngitis, Laryngotracheitis, Spasmodic Croup, Laryngotracheobronchitis, Bacterial Tracheitis, and Laryngotracheobronchopneumonitis)

James D. Cherry

The term *croup* is used to identify several different respiratory illnesses characterized by varying degrees of inspiratory stridor, cough, and hoarseness resulting from obstruction in the region of the larynx. The etiology of croup syndromes is diverse, and the consideration of noninfectious possibilities in the differential diagnosis is of major importance. Table 22–1 classifies etiologic considerations in supraglottic, laryngeal, and infraglottic acute obstructions.

Epiglottitis (see Chapter 21) and diphtheria (see Chapter 101) are mentioned here only for historical perspective and as a consideration in differential diagnosis. Croup is discussed under the subheadings of laryngitis, laryngotracheitis, spasmodic croup, laryngotracheobronchitis, bacterial tracheitis, and laryngotracheobronchopneumonitis.

TABLE 22–1 Clinical Considerations in Acute Supraglottic, Laryngeal, and Infraglottic Obstructions

Infectious
Acute epiglottitis
Laryngitis
Laryngeal diphtheria
Laryngotracheitis
Laryngotracheobronchitis
Laryngotracheobronchopneumonitis
Bacterial tracheitis
Spasmodic croup
Mechanical
Foreign body
Secondary to trauma resulting from intubation
Extrinsic or intrinsic mass
Allergic
Acute angioneurotic edema

Data from references 33, 34, 42, 69, 73, 74, 162, 179, 239.

HISTORICAL ASPECTS

The word *croup* is derived from the Anglo-Saxon word *kropan*, "to cry aloud."[35] Until the 20th century, most crouplike illnesses were thought to be diphtheria. Diphtheritic croup is an ancient disease that has been traced to the time of Homer. The historical trail of diphtheria disappeared in the 5th century and did not reappear until 1100 years later. In the 16th century, epidemics were noted in Europe. Top[217] credits Bretonneau for differentiating diphtheritic croup from spasmodic croup in 1826. In the middle third of the 20th century, the history of croup was marked by three important events: (1) the rapid decline in incidence of diphtheria associated with the use of toxoid; (2) the introduction and widespread use of antibiotics; and (3) the advent of tissue culture techniques, resulting in the establishment of viruses as etiologic agents. After these three events occurred, a prevalent academic view was that all croup was of viral etiology, and bacteria generally were dismissed as causative agents.[36,42,64,180] A careful review of many publications from the first half of the 20th century indicates, however, a causative role for several bacteria, in addition to *Corynebacterium diphtheriae*, in croup.* Bacterial croup (bacterial tracheitis) was rediscovered in 1979.†

In the 1940s, Davison[48] separated spasmodic croup from other, more severe forms of croup. The clinical and pathologic aspects of this entity were poorly defined, and today it often is not separated clinically from more severe forms of croup.

*See references 9, 19, 20, 48, 49, 68, 81, 102, 110, 150, 161, 167, 184, 185.
†See references 33, 34, 39, 53, 58, 59, 63, 65, 67, 85, 89, 91, 98, 111, 116, 136, 137, 148, 153, 160, 162, 201, 208, 209, 213, 227, 236.

TABLE 22–2 Classification and Definition of Infectious Illnesses Involving the Larynx and Infraglottic Region

Category	Other Terms	Definitions
Laryngitis		Inflammation of larynx resulting in hoarseness; usually occurs in older children and adults in association with common upper respiratory viral infection
Laryngeal diphtheria	Membranous croup, true croup, diphtheritic croup	Infection involving larynx and other areas of upper and lower airway due to *Corynebacterium diphtheriae*, resulting in gradually progressive obstruction of airway and associated inspiratory stridor
Laryngotracheitis	False croup, virus croup, acute obstructive subglottic laryngitis	Inflammation of larynx and trachea usually caused by infection with parainfluenza and influenza viruses
Laryngotracheobronchitis and laryngotracheobronchopneumonitis	Membranous laryngotracheobronchitis, pseudomembranous croup	Inflammation of larynx, trachea, and bronchi or lung or both; usually similar in onset to laryngotracheitis, but more severe illness; bacterial infection frequently has causative role
Bacterial croup	Bacterial tracheitis, membranous croup, membranous tracheitis, membranous laryngotracheobronchitis, pseudomembranous croup	Severe form of laryngotracheitis, laryngotracheobronchitis, or laryngotracheobronchopneumonitis due to bacterial infection
Spasmodic croup	Spasmodic laryngitis, catarrhal spasm of the larynx, subglottic allergic edema	Illness characterized by sudden onset at night of inspiratory stridor; associated with mild upper respiratory infection without inflammation or fever but with edema in subglottic region

Data from references 33, 50, 69, 162.
Modified from Cherry, J. D.: Acute epiglottitis, laryngitis, and croup. In *Remington, J. S., and Swartz, M. N. (eds.): Current Clinical Topics in Infectious Diseases. Vol. 2. New York, McGraw-Hill, 1981. Reproduced with permission of The McGraw-Hill Companies.*

TERMINOLOGY

The terminology and classification of infectious illnesses involving the larynx and infraglottic region have evolved over time. Classifications often have mixed etiologic systems with anatomic systems and have led to confusion. Croup often has been presented in articles under the heading of *laryngotracheobronchitis* when the authors actually were discussing laryngotracheitis and spasmodic croup.[112,138,149,176,188,196,214] The term *membranous croup* has been used as the title for articles dealing with bacterial croup.[53,89] This use is confusing because membranous croup historically was diphtheria. Many articles dealing with bacterial croup also have been titled *bacterial tracheitis*.* This term seems inappropriate because most cases of bacterial croup seen today have lower respiratory tract involvement in addition to tracheal findings. Table 22–2 lists the classifications and definitions used in this chapter. In the present era, the physician's knowledge of the clinical symptoms of croup and of the relationship of history and physical findings to the needs of therapy and general prognosis basically has declined.

ETIOLOGY

The etiologic agents in laryngitis, laryngotracheitis, spasmodic croup, laryngotracheobronchitis, and laryngotracheobronchopneumonitis are presented by frequency and severity of illness in Table 22–3. Laryngitis is a common manifestation of infection with many respiratory viruses in older children, adolescents, and adults. Outbreaks of laryngitis in closed population groups (e.g., boarding schools and military training camps) most frequently are caused by adenovirus types 4 and 7, and community outbreaks most often are noted in association with epidemic influenza. Sporadic instances of laryngitis most often are caused by adenoviral infections. Laryngitis also has been reported in association with group A streptococcal infections; the incidence of this association has varied from 2 to 40 percent.[17,155,222]

Generally accepted today is that acute laryngotracheitis and spasmodic croup, which rarely are differentiated clinically, are caused by infection with many different viruses. Although numerous studies of respiratory viral infection exist, almost no attempt has been made to delineate the differences in etiologic spectrum by severity of illness.

Parainfluenza virus type 1 is the most common cause of acute laryngotracheitis and is responsible for frequent and clearly delineated fall and winter epidemics. Croup with parainfluenza type 2 virus seldom is severe but occasionally is related to small outbreaks. Parainfluenza virus type 3 is a frequent cause of sporadic but severe illness.

The most severe laryngotracheitis has been noted in association with influenza A viral infections. Respiratory syncytial virus and several different adenoviruses frequently are isolated in croup. Generally, these illnesses are not severe, but lower respiratory involvement occasionally is a problem. Laryngeal, tracheal, and bronchial involvement commonly occurs in measles.[36] Although rhinoviruses, *Mycoplasma pneumoniae*, enteroviruses, herpes simplex virus, and reoviruses have been associated with croup, they generally cause only minimal distress. More recently, croup has been noted in association with infection with the novel coronavirus NL63, human bocavirus,[8a] and human metapneumovirus.[5a,38,224] Recurrent croup may be due to infection with a human papillomavirus.[239]

Bacteria, other than *Haemophilus influenzae* in epiglottitis and *C. diphtheriae* in membranous croup, generally were dismissed as causative agents in croup until more recently.[42,64,180] Many publications on laryngotracheobronchitis from the first half of the 20th century indicate a role for several common bacterial pathogens.* In 1979, bacterial croup was rediscovered,[111] and numerous reports of this illness have been published since then.†

*See references 39, 58, 59, 63, 65, 67, 98, 111, 116, 136, 137, 160, 201, 227, 236.

*See references 9, 19, 20, 39, 47, 48, 58, 65, 68, 81, 85, 102, 110, 150, 161, 167, 184, 185, 208, 213, 227.
†See references 11, 53, 59, 63, 67, 89, 91, 98, 116, 136, 137, 148, 153, 160, 162, 201, 209, 236.

TABLE 22–3 Etiologic Agents in Laryngitis, Spasmodic Croup, Laryngotracheitis, Laryngotracheobronchitis, and Laryngotracheobronchopneumonitis Presented by Frequency and Severity of Illness

Category	Etiologic Agents	Frequency*	Associated with Outbreaks	Severity†	References
Laryngitis	Adenoviruses				
	Types 4 and 7	++++	Yes	+ to +++	46, 97, 222, 306
	Types 2, 3, 5, 8, 11, 14, and 21	+++	No	+ to +++	
	Influenza viruses	++++	Yes	+ to ++++	6, 97, 169, 222
	Types A and B				
	Parainfluenza viruses				97, 222
	Type 1	++	Yes	+ to +++	
	Types 2 and 3	+	Yes	+ to ++	
	Coronavirus	++	Yes	++	8
	Rhinoviruses and respiratory syncytial virus	++	No	+ to ++	84, 155, 163, 198
	Enteroviruses	+	No	+	97, 222
	Streptococcus pyogenes	+ to +++	Yes	+ to ++	17, 155
Laryngotracheitis and spasmodic croup	Parainfluenza viruses	++++		+ to +++	12, 28-30, 32, 54, 56, 78, 79, 82, 92, 96, 99, 128, 133, 139, 140, 143, 158, 169, 173, 174, 177, 225
	Type 1	++++	Yes		
	Type 2	++	Yes		
	Type 3	++	No		
	Influenza viruses	++			22, 28, 29, 32, 54, 56, 66, 72, 78, 82, 99, 100, 139, 143, 158, 169, 173, 174, 177, 225
	Type A	+++	Yes	+ to ++++	
	Type B	+	Yes	+ to ++	
	Respiratory syncytial virus	++	No	+ to ++	27-30, 32, 54, 56, 77, 78, 82, 99, 128, 140, 154, 173, 174, 176, 218, 226, 232, 237
	Human metapneumovirus	++	Yes	+	5a
	Coronavirus	++	Yes	++	38, 224
	Human bocavirus	+	No	+	8a
	Measles virus	++	Yes	+ to +++	36
	Adenoviruses	++	No	+ to ++	16, 28-30, 32, 54, 82, 96, 99, 128, 133, 139, 140, 158, 173, 174, 176, 204, 218, 225, 226
	Unspecified types and types 1, 2, 3, 5, 6, and 7				
	Rhinoviruses	+	No	+	32, 78, 112, 139, 158
	Mycoplasma pneumoniae	+	No	+	28, 30, 32, 54-56, 82, 99, 140
	Enteroviruses	+	No	+	29, 36, 43, 77, 78, 82, 99, 107, 139, 154, 205, 218, 232
	Coxsackievirus type A9	+	No	+	
	Coxsackievirus types B4 and B5	+	No	+	
	Echoviruses types 4, 11, and 21	+	No	+	
	Herpes simplex viruses	+	No	+	99, 104, 129, 158, 205
	Reoviruses	+	No	+	237
	Human papillomavirus	+	No	+ to +++	239
Laryngotracheobronchitis and laryngotracheobronchopneumonitis	Parainfluenza viruses types 1, 2, and 3	+	No	+++	19, 77, 83, 96, 140, 150, 171, 173
	Influenza viruses types A and B	+	No	++++	66, 72, 100, 174
	Staphylococcus aureus, *S. pyogenes*, *Streptococcus pneumoniae*, *Haemophilus Influenzae*, and *Moraxella catarrhalis*	++	No	++++	9, 11, 19, 20, 47, 48, 53, 59, 63, 67, 68, 81, 89, 91, 98, 102, 110, 111, 116, 136, 137, 148, 149, 161, 162, 167, 184, 185, 201, 236
	Other bacteria	±	No	++++	67, 89, 94, 116, 136, 153, 236
	Cryptosporidium	−	No	++	90

*++++, *most frequent;* +++, *frequent;* ++, *occasional;* +, *rare;* −, questionable.
†++++, most severe; +++, severe; ++, not severe; +, minimal distress.

In the reports from the preantibiotic era, *Streptococcus pyogenes* was the pathogen implicated most frequently. Since 1979, *Staphylococcus aureus* has been the agent implicated most commonly. Other important bacteria are *Streptococcus pneumoniae* and *H. influenzae*. More recently, *Moraxella catarrhalis* has been found to be the causative agent in several cases.[11,67,116,236] In most instances, bacterial croup is likely to be the result of bacterial superinfection in viral disease.* *Cryptosporidium* also has been recovered from the trachea of an infant with a subacute illness.[90]

EPIDEMIOLOGY

Croup accounts for approximately 15 percent of lower respiratory tract disease seen in pediatric practice. In a large 11-year study in a pediatric practice in Chapel Hill, North Carolina, Denny and associates[56] noted the incidence of croup by age and sex. The highest attack rate occurred in children 7 to 36 months of age. Few cases occurred after the sixth birthday. Hoekelman[94] studied the occurrence of illness prospectively in 246 full-term, first-born, well infants during their first year of life. Three infants (1.2%) had croup during the study year. The analysis of a pediatric practice with approximately 3000 active records and approximately 10,000 yearly visits of children younger than 5 years old disclosed five cases of croup in a group of 50 consecutive hospitalized patients.[18]

Although croup occurs occasionally in older children, most cases occur within the first 3 years of life. A review of 211 children hospitalized for croup during a 2-year period at Cardinal Glennon Memorial Hospital for Children in St. Louis showed that 26 percent of the cases were in infants younger than 1 year old, and 73 percent were in children younger than 3 years.[75] Similar data on age have been reported by others.[56,64,69,188,195]

Croup occurs more commonly in boys than in girls.[56] In our studies, two of every three hospitalized children were boys.[75] Berg,[10] Kravitz,[130] and Rosales and Davenport[188] noted similar sex-related illness ratios. Figure 22–1 shows the 3-year seasonal pattern of croup as manifested by emergency department visits at Cardinal Glennon Memorial Hospital for Children. In each of the years, late fall–early winter peaks occurred. In the Chapel Hill studies, an increase was noted in the number of croup cases beginning in September, with a peak in October and November and then a decrease during the next 7-month period.[56] In a 2-year emergency department study in Toronto involving 1700 cases, the peak month of visits and hospital admissions was found to be October.[195] Marx and associates[144] reviewed the National Hospital Discharge Survey data for hospitalizations for croup between 1979 and 1993. They also examined Centers for Disease Control and Prevention laboratory-based surveillance data and published reports with virus isolation studies. Major peaks in hospitalizations for croup occurred in October of odd-number years at the time of peak parainfluenza virus type 1 activity. Minor peaks in hospitalizations for croup occurred each year in February when influenza A, influenza B, and respiratory syncytial viral infections were common occurrences. Epidemic peaks of acute laryngotracheitis reflect community-wide activity with parainfluenza 1 and 2 viruses or influenza A or B outbreaks.[56,83,144]

In the Toronto study, the time of the visit to the emergency department was analyzed.[195] The peak number of visits occurred between 10 P.M. and 4 A.M. During this period, approximately 17 percent of the children seen were admitted to the hospital. In contrast, of children seen between noon and 6 P.M., approximately 50 percent were admitted to the hospital. A study of croup hospitalizations in Ontario over 14 years from 1988 to 2002 noted a biennial midautumn peak and an annual summer trough throughout the entire study period.[194] A striking finding in this study was a marked decrease in hospitalizations for croup after the winter of 1993-1994. This decrease continued in linear fashion for the remainder of the study duration. The authors suggest that the decreasing trend in croup hospitalizations was likely due to the increasing use of corticosteroid treatment in patients who presented to emergency departments with croup.

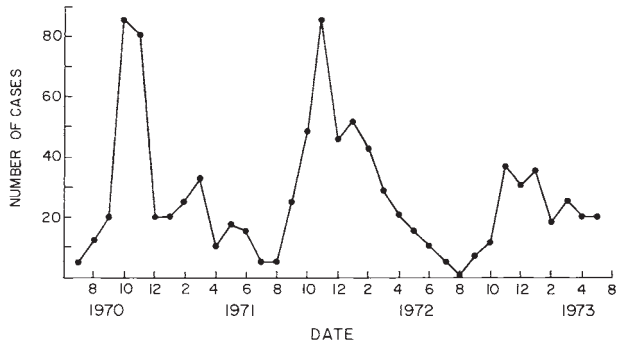

Figure 22–1 Seasonal occurrence of croup, Cardinal Glennon Memorial Hospital for Children emergency department, July 1970 to June 1973.

Because croup is caused by the same viruses that cause other respiratory illnesses, the method of spread probably is similar for all (see discussion of the common cold in Chapter 8). In children, most spread involves close person-to-person contact, with large droplets of virus-containing nasal secretions being applied to the nose from the hands of the future host or by close-range airborne acquisition. Parainfluenza viruses are common causes of colds in adults, so older individuals with trivial illnesses may be the source of more severe childhood croup.

PATHOLOGY

Laryngoscopic studies in acute laryngotracheitis reveal redness and swelling of the lateral walls of the trachea, just below the vocal cords.[48,49,211] Because the subglottic trachea is surrounded by a firm cartilaginous ring, the inflammatory swelling can occur only by encroaching on the patency of the airway; the subglottic space often is reduced to a slit 1 to 2 mm wide. As the disease progresses, the tracheal lumen becomes obstructed further by a fibrinous exudate, and its surface is covered by pseudomembranes composed of the exudative material. The vocal cords frequently are swollen, and their mobility is impaired.

Histologic study of postmortem material from the larynx and trachea reveals marked edema and cellular infiltration in the lamina propria, submucosa, and adventitia. The cellular infiltrate includes histiocytes, lymphocytes, plasma cells, and polymorphonuclear leukocytes.[19,20,157,167,185] The cotton rat model of laryngotracheitis caused by human parainfluenza virus type 3 reveals pathologic findings similar to those noted in human postmortem studies.[168] This model also indicates the time course of events. Early in the course of an infection (first 2 to 4 days), moderate mucosal and submucosal inflammatory infiltrates were noted in the subglottic and proximal tracheal regions. The infiltrates initially contained lymphocytes and neutrophils, and subsequently a mononuclear infiltrate with lymphocytes and macrophages in the submucosa was noted. Cell injury was most marked on days 6 to 8 after infection. The ciliated epithelial cells were blunted, with loss of cilia in large patches. By 12 days after infection, the region reverted to a nearly normal appearance.

*See references 23, 39, 63, 89, 110, 137, 148, 159, 160, 162, 166.

The older literature indicates that classic laryngotracheobronchitis and the same disease with pneumonia represent the extension of disease from the trachea to the bronchi and alveoli. The progressive obstructive disease with exudate and pseudomembrane obstruction at the bronchial and bronchiolar levels usually is the result of secondary bacterial involvement. In bacterial croup, the tracheal wall is infiltrated with inflammatory cells, and ulceration, pseudomembranes, and microabscess formation occur.* In addition to the findings in laryngotracheitis of viral origin, thick pus is present within the lumen of the trachea and lower air passages.[91,116,136,148] Spasmodic croup is an enigma because it occurs in association with respiratory viral infections similar to those that cause more severe laryngotracheitis. Using direct laryngoscopy, Davison[49] noted that the subglottic tissues in spasmodic croup showed noninflammatory edema.

PATHOGENESIS

Although the eventual site of clinically important pathologic change in laryngotracheitis is within the larynx and trachea, the initial acquisition of infection is similar to that of other respiratory viral infections and occurs within the upper air passages, including the nasal and pharyngeal epithelial surfaces. After acquisition of virus, infection of the cells of the local respiratory epithelium develops and spreads locally to involve the larynx and trachea. The initial symptoms of nasal stuffiness and throat irritation reflect the primary sites of involvement. Studies in organ culture systems and in the cotton rat model have shown that several respiratory viruses inhibit tracheal ciliary function and eventually lead to marked destruction of the epithelium and evidence of viral infection in the lamina propria.[126,168,182] In uncomplicated croup, failure of gas exchange within the lung, in addition to hypoxia resulting from subglottic tracheal obstruction, may occur.[163,214]

Parainfluenza viruses types 1, 2, and 3 all are significant causes of respiratory infections in children (see Chapter 191). Infection with parainfluenza virus type 3 occurs in most infants, whereas infections with types 1 and 2 generally occur more frequently in older children. Only a third of children have antibodies against these two viral types by the time they reach age 4 years.

Because parainfluenza viral infections are common occurrences in young children, and because only a few get croup, host factors probably are important factors in the pathogenesis. In contrast with the numerous studies of the pathogenesis of bronchiolitis and respiratory syncytial virus infection, few similar studies relating to parainfluenza viruses and croup have been reported.[37]

The above-presented pathologic data suggest that the findings in laryngotracheitis are directly virus-related (parainfluenza viruses types 1, 2 and 3 and influenza virus types A and B) owing to the direct cytopathic effect of a virus or related to the concomitant host response or perhaps both.[37] In the cotton rat model, the use of topical steroid therapy led to a significant reduction in the degree of inflammatory infiltrates and cell injury, which suggests that the host response contributes to the pathologic findings.[168] The pathogenesis of laryngotracheobronchitis and laryngotracheobronchopneumonitis is similar to that described previously, but with the extension of infection to the lower respiratory tract and, usually, the occurrence of secondary bacterial infection.

Many studies suggest that allergic factors play a role in recurrent croup.[24,93,165,223,229,230,231,238,235] Welliver and associates[230] found that children with croup caused by parainfluenza viral infections had titers of IgE-specific antibody in their nasopharyngeal secretions that were 3.6-fold higher than titers of similarly infected children with only upper respiratory infections. The children with croup had a cell-mediated immune response to parainfluenza virus that was 1.6-fold greater than that of the children with only upper respiratory infections.

In a subsequent review, investigators noted that an atopic disposition might be associated with the development of croup.[229] Children with croup caused by a parainfluenza virus had specific IgE antibodies and released histamine into the airway more frequently than did control patients with parainfluenzal viral upper respiratory infections. They also noted that children with recurrent croup had several atopic features, such as positive skin tests to environmental allergens, and were more likely to develop asthma when they grew older. Also, some children with a history of recurrent croup develop stridor with histamine challenge.

The above-mentioned studies by Welliver and associates[229,230] and other reports make it easy to accept a role of atopy in recurrent croup. What is difficult to explain, however, is why many apparent primary infections result in only spasmodic croup. As I have suggested previously, initial sensitization may be parainfluenza virus group–specific and not type-specific.[37] This proposal suggests that early infection (primary infection) with parainfluenza virus type 3 would set the stage for spasmodic croup with parainfluenza viral types 1 and 2. The primary infection itself could have been mild owing to transplacentally acquired antibody.

CLINICAL PRESENTATION

ACUTE LARYNGITIS

Table 22–4 summarizes clinical characteristics of laryngitis. Laryngitis is mainly a disease of older children, adolescents, and adults that is disturbing but self-limited. The specific clinical manifestation is hoarseness. Other symptoms depend on the causative infectious agent. Adenoviruses and influenza viruses cause the most severe instances of laryngitis. With these viruses, fever usually occurs, and sore throat, headache, muscle aches and pains, and prostration are common symptoms. In contrast, patients with laryngitis resulting from rhinoviral, parainfluenza viral, or respiratory syncytial viral infections have minimal or no fever and few systemic complaints. They usually have pronounced nasal symptoms (coryza and stuffiness), however. Occasionally, hoarseness may persist, which may be a result of secondary bacterial infection of the upper respiratory tract.

ACUTE LARYNGOTRACHEITIS

Although the clinical spectrum of acute laryngotracheitis varies considerably, its manifestations usually are significantly different from the manifestations of the other acute diseases with obstruction in the region of the larynx (see Table 22–4). Onset of illness usually is not alarming and suggests the onset of a cold. Initial symptoms are nasal complaints and include dryness, irritation, and coryza. Ordinary cough and the complaint of sore throat occur frequently. Fever is a usual occurrence within the first 24 hours, which is not true of the common cold. After a period as short as a few hours, but usually after 12 to 48 hours, upper airway obstructive signs and symptoms are seen. The cough first becomes "croupy" (sounding like a barking seal), and then evidence of respiratory stridor (difficulty associated with inspiration) gradually increases. Examination at this time reveals a child with a hoarse voice, coryza, a normal or minimally inflamed pharynx, and a slightly increased respiratory rate with a prolonged inspiratory phase. Temperature nearly always is between 37.8° C and 40.5° C (100° F and 105° F).

*See references 2, 31, 33-35, 39, 53, 58, 59, 63, 67, 85, 91, 98, 111, 116, 136-138, 162, 166, 201, 209.

TABLE 22-4 Differential Diagnosis of Acute Obstruction in the Region of the Larynx

Category	Acute Epiglottitis	Laryngeal Diphtheria	Laryngitis	Acute Laryngotracheitis	Laryngotracheobronchitis and Laryngotracheobronchopneumonitis (Including Bacterial Tracheitis)	Spasmodic Croup	Foreign Body	Acute Angioneurotic Edema
Common age of occurrence	1-8 yr	All ages	Older children and adults	3 mo to 3 yr	3 mo to 3 yr	3 mo to 3 yr	All ages	All ages
Past and family history	Not contributory	No or inadequate immunization	Not contributory	Family history of croup	May be family history of croup	Family history of croup; perhaps previous attack	Occasional history of ingestion	Allergic history; perhaps previous attack
Prodrome	Occasionally coryza	Usually pharyngitis	Usually stuffy nose or coryza	Usually coryza	Usually coryza	Minimal coryza	None	Occasionally cutaneous allergic manifestations
Onset (time to full-blown disease)	Rapid; 4-12 hr	Slowly for 2- to 3-day period	Variable; 12 hr to 4 days	Moderate but variable; 12-48 hr	Usually gradually progressive; 12 hr to 7 days	Sudden; always at night	Usually sudden	Rapid
Symptoms on presentation Fever	Yes; usually 39.5° C (103° F)	Yes; usually 37.8° C to 38.5° C (100° F to 101° F)	Yes; 37.8° C to 39.4° C (100° F to 103° F) with adenoviral and influenza viral infections; usually minimal with other viruses	Yes; variable, 37.8° C to 40.5° C (100° F to 105° F)	Yes; variable, 37.8° C to 40.5° C (100° F to 105° F)	No	No, unless secondary infection	No
Hoarseness and barking cough	No	Yes	Yes	Yes	Yes	Yes	Usually no	No
Dysphagia	Yes; usually severe	Usually yes	No	No	No	No	Frequently yes	Yes
Inspiratory stridor	Yes; moderate to severe	Yes; minimal to severe	No	Yes; minimal to severe	Yes; usually severe	Yes; moderate	Variable	Yes

TABLE 22–4 Differential Diagnosis of Acute Obstruction in the Region of the Larynx—cont'd

Category	Acute Epiglottitis	Laryngeal Diphtheria	Laryngitis	Acute Laryngotracheitis	Laryngotracheobronchitis and Laryngotracheobronchopneumonitis (Including Bacterial Tracheitis)	Spasmodic Croup	Foreign Body	Acute Angioneurotic Edema
Toxic appearance	Severe	Usually no	No	Usually minimal	Usually moderate; may be severe	No	No	No
Signs on presentation								
Oral cavity	Pharyngitis and excessive salivation	Membranous pharyngitis	Normal or mild to moderate pharyngitis	Usually minimal pharyngitis	Usually minimal pharyngitis	Normal	Normal	Pale appearance
Epiglottis	Cherry-red and swollen	Usually normal; may contain membrane	Normal	Normal	Normal	Normal	Normal	Swollen and pale
Radiographs	Swollen epiglottis on lateral film	Not useful	Not useful	Subglottic narrowing on PA film	Subglottic narrowing on PA film; irregular soft tissue densities within trachea on lateral film	Not useful	May reveal foreign body	Swollen epiglottis on lateral film
Laboratory								
Leukocyte count	Usually markedly elevated with increased percentage of band forms	Usually elevated with increased percentage of band forms	Usually normal	Mildly elevated with >70% polymorphonuclear cells	Variable; usually mildly elevated with 70% polymorphonuclear cells; may be increased band count	Normal	Normal, unless secondary infection	Normal; sometimes eosinophilia
Bacteriology	Throat and blood cultures yield *Haemophilus influenzae* type b	Smear and culture from membrane reveal organism	Usually normal flora in throat; occasionally *Streptococcus pyogenes* in throat	Only important if secondary infection suspected	Normal throat flora; tracheal culture often yields *S. pyogenes*, *Staphylococcus aureus*, *Streptococcus pneumoniae*, or *H. influenzae*	Normal flora	Only important if secondary infection suspected	Normal flora
Clinical course	Rapidly progressive; cardiorespiratory arrest occurs within hours if not treated	Slowly progressive obstruction of airway	Hoarseness persists at a constant degree about 4-7 days; occasionally persists 2-3 wk	Variable speed of progression of obstruction; usually does not require surgical intervention	Degree of obstruction usually severe; persists 7-14 days; frequently requires surgical intervention	Symptoms of short duration with treatment; repeated attacks common	Variable depending on size and substance of foreign body	Variable; sometimes leads to rapid asphyxia without therapy

Data from references 48, 49, 53, 63, 70, 76, 89, 91, 111, 136, 137, 152, 215.

The speed of progression and final degree of upper airway obstruction vary. Some children have hoarseness and barking cough but no other evidence of obstruction; in these cases, the symptoms last approximately 3 to 7 days, with a gradual return to normal. In other cases, the obstruction is progressive and leads to severe respiratory distress with supraclavicular and infraclavicular and sternal retractions, cyanosis of varying degrees, and apprehension. With hypoxia, the cardiac rate increases, and the child becomes restless. Without intervention, asphyxial death occurs rapidly in some children. In others, the problem of hypoxia is more prolonged, and respiratory fatigue may lead to the patient's demise. The duration of illness in a severely affected child, regardless of therapy, is rarely less than 7 days and frequently is 14 days.

Laboratory study in acute laryngotracheitis is of only minimal value. The white blood cell count frequently is greater than 10,000 cells/mm^3, and polymorphonuclear cells predominate.[32,164] Very high white blood cell counts (>20,000/mm^3) with numerous band-form neutrophils should suggest bacterial superinfection or the possibility of acute epiglottitis. The posteroanterior chest radiograph reveals the subglottic narrowing (steeple sign), and a lateral neck radiograph indicates the size of the epiglottis.

ACUTE LARYNGOTRACHEOBRONCHITIS AND LARYNGOTRACHEOBRONCHOPNEUMONITIS (BACTERIAL TRACHEITIS)

Laryngotracheobronchitis and laryngotracheobronchopneumonitis are far less common occurrences than are laryngotracheitis and spasmodic croup; however, these illnesses occur more commonly than generally realized.* These entities may be considered an extension of acute laryngotracheitis, as numerous descriptions in the literature suggest.† The severity of the illness is due to secondary bacterial infection. Initial symptoms and signs are similar to those of laryngotracheitis (see Table 22–4). An afflicted child usually has mild to moderately severe illness for 2 to 7 days and then suddenly becomes markedly worse. Occasionally, upper and lower airway obstructions seem to occur simultaneously. In many children, the distress from tracheal obstruction is of such magnitude that the symptoms and signs of lower respiratory involvement go unnoticed. Symptoms and signs associated with extension of disease to the bronchi, bronchioles, and lung substance include rales, air trapping, wheezing, and a further increase in the respiratory rate. Obstruction in these illnesses usually is of such a degree that either intubation or tracheostomy is necessary.

Several instances of laryngotracheobronchopneumonitis with toxic shock syndrome have been observed.[21,31,166,202,209] Generally, children with these staphylococcal infections initially have the onset of croup, then the more severe manifestations of bacterial tracheitis develop, and finally the exanthem and other manifestations of toxic shock syndrome develop. An infant with tracheitis and supraglottitis caused by *M. catarrhalis* has been described.[2] Other findings in laryngotracheobronchitis and laryngotracheobronchopneumonitis are presented in Table 22–4.

SPASMODIC CROUP

In recent years, the clinical entity of spasmodic croup has been incorporated by many physicians into the overall diagnosis of croup. Although distinguishing mild cases of laryngotracheitis from spasmodic croup is difficult at the onset in some instances, the delineation of the two entities is important from prognostic and therapeutic perspectives (see Table 22–4).

Spasmodic croup occurs in children 3 months to 3 years old. The onset always is at night, and the characteristic presentation occurs in a child who previously was thought to be well or to have had a mild cold with coryza as the only symptom. The child awakens at night with sudden dyspnea, croupy cough, and inspiratory stridor. There is no fever. The symptoms apparently are the result of sudden subglottic edema; relief is achieved easily by general reassurance and administration of moist air. The occurrence of spasmodic croup tends to run in families, with repeated attacks occurring in some children. After one attack, the child is likely to have another attack the same evening and on three or four successive evenings. These attacks can be prevented by employing mild sedation at bedtime and ensuring that the bedroom air is adequately humidified.

DIFFERENTIAL DIAGNOSIS

The therapeutic approaches to the various acute obstructions in the region of the larynx vary markedly. Establishing a correct diagnosis is essential and frequently lifesaving. Table 22–4 lists the differential points of eight conditions with symptoms and signs of acute upper airway obstruction.

The most frequent serious differential diagnostic problem is the recognition of acute epiglottitis and its separation from the less fulminant laryngotracheitis. In epiglottitis (a rare disease in children today because of universal immunization with *H. influenzae* type B conjugate vaccines), the important differential points are lack of a croupy cough; the presence of a swollen, cherry-red epiglottis; the sitting posture of the child with the chin pushed forward and a reluctance or refusal to lie down; and the relatively greater apprehension and anxiety of the patient than the degree of chest retraction suggests. In contrast, a child with acute laryngotracheitis has a normal epiglottis on examination, always has a typical barking cough, is comfortable in a supine position, and frequently appears to have only minimal apprehension, despite retractions in which the sternum appears to be indenting 2 inches or more.

Early in the course of epiglottitis, the diagnosis can be confirmed only by the observation of the epiglottis, which can be done without difficulty.[127] Later in the disease, the posture of the child and the history of rapidly progressing disease render the differential from laryngotracheitis readily apparent, so examination of the epiglottis directly (a dangerous procedure if the child is forced to lie down) or indirectly by a lateral neck radiograph rarely is indicated and usually is contraindicated.

Laryngotracheobronchitis and laryngotracheobronchopneumonitis can be recognized by signs of lower respiratory involvement (rales, air trapping, wheezing, and pulmonary infiltrates on the radiograph). Bacterial disease should be suspected in laryngotracheobronchitis and laryngotracheobronchopneumonitis and when symptoms and signs become worse in laryngotracheitis. A lateral radiograph can be useful in the evaluation because it may reveal soft tissue densities within the trachea. Lateral neck and chest radiographs are regarded by many physicians as definitive tests to determine whether to rule out epiglottitis and laryngotracheitis. In a careful study by Stankiewicz and Bowes,[203] the sensitivity and specificity of both radiographs were low, however.

Although a rare occurrence today, laryngeal diphtheria always should be considered and ruled out in croup. Important in this regard are the history of immunization, the pharyngeal evidence of diphtheria, the relative slowness of the disease to progress, and a greater degree of hoarseness caused by direct laryngeal membrane formation.

*See references 11, 34, 39, 53, 58, 59, 63, 67, 85, 89, 91, 98, 111, 116, 136, 137, 148, 153, 160, 209, 236.

†See references 9, 19, 20, 47, 48, 68, 81, 102, 116, 150, 167, 180, 181, 184, 185.

Spasmodic croup rarely should be confused with acute laryngotracheitis, but a perusal of the literature indicates that the two entities most commonly are considered laryngotracheitis, which is unfortunate because prognostic considerations for the two entities are different. Spasmodic croup always is of sudden onset at night, occurs without fever, and is relieved by simple therapeutic modalities.

The possibility of a foreign body and angioneurotic edema always must be considered in upper airway obstructive disease. Differential points are presented in Table 22–4. Rarely, acute upper airway obstruction occurs in adolescents as a result of psychogenic and emotional factors.[80,120,192]

SPECIFIC DIAGNOSIS

The epidemiologic history frequently is an important factor in establishing a specific diagnosis. Obtaining bacterial culture specimens from the throat, laryngeal region, and blood is helpful in diagnosing epiglottitis and important in identifying laryngotracheitis, laryngotracheobronchitis, and laryngotracheobronchopneumonitis when secondary infection is suspected. The white blood cell count should be obtained because it can be helpful when secondary bacterial infection is considered.

A specific etiologic diagnosis can be made by the isolation of virus or its identification by a direct antigen test from a nasopharyngeal specimen. The diagnostic virologic facilities of many medical centers enable rapid identification of parainfluenza viruses, respiratory syncytial viruses, adenoviruses, most rhinoviruses, and influenza viruses to be established.

TREATMENT

During the last 65 years, the treatment of croup has created considerable controversy: tracheostomy versus no tracheostomy, intubation versus tracheostomy, warm versus cold humidification, antibiotics versus no antibiotics, corticosteroids versus no corticosteroids, sedation versus no sedation, and racemic epinephrine therapy versus mist therapy alone. Few of these controversies have been resolved scientifically, but the passage of time has lessened the importance of some of the discrepant opinions.

Of most importance in the evaluation of therapeutic modalities and the specific approach to therapy in croup is making an accurate differential diagnosis. A look at the most important controversy (the use of steroids) indicates that in most instances, cases of spasmodic croup, in which a favorable outcome invariably can be expected, were not separated clinically from cases of laryngotracheitis, in which the outcome is less predictable.

ACUTE LARYNGOTRACHEITIS AND SPASMODIC CROUP

In managing acute laryngotracheitis and spasmodic croup, each case must be treated individually; one child may need minimal therapy, whereas another may require consideration of all modalities. In all children with acute laryngotracheitis, attention should be given to the anxiety and apprehension of the patient and parents. The parents should be reassured immediately, and it is important that the child is not separated from them. Physical examination should be done rapidly by one physician, and all but absolutely necessary procedures should be deferred.

In the past, mist therapy was the cornerstone of management of croup. In more recent years, the value of this therapy has been questioned.[15,135,156,193] It had been my opinion, as expressed in previous editions of this book, that mist therapy in croup was useful. The results of a large randomized controlled trial[193] and the extensive review by Moore and Little[156] indicate, however, that mist treatment offers no benefit in treatment.

Oxygen should be administered to a child who is hypoxemic from respiratory distress. The studies of Newth and associates[163] and Taussig and colleagues[214] indicate that mild hypoxemia occurs more commonly than is realized clinically. The drying effect of oxygen is counterproductive to the removal of tracheal exudate, so it should not be used routinely.

Since 1952, numerous communications in the English medical literature have described the use of corticosteroids in croup. Nine double-blind, controlled studies were conducted before 1989.[61,62,107,127,132,134,199,207,210] In 1964, Eden and Larkin[62] noted no difference between control and methylprednisolone therapy in a study of 50 children with acute croup. In 1967, Eden and colleagues[61] studied another 50 patients and could find no benefit from dexamethasone compared with a control preparation. Sussman and colleagues[210] also could not show any benefit from dexamethasone therapy.

In contrast to these three studies, Skowron and coworkers[199] noted a slight benefit regarding duration of stridor, retractions, fever, and days in the hospital in a group receiving dexamethasone. They suggested, however, that steroids not be used routinely for laryngotracheitis because the overall benefits were minimal, and a potential risk exists in administration of steroids. In 1969, James[107] noted that dexamethasone-treated patients recovered from obstructive symptoms more quickly than did the control group. In another study involving 30 children, Leipzig and associates[134] concluded that dexamethasone in an adequate dose (0.3 mg/kg initially and repeated in 2 hours) given intramuscularly hastens the recovery from uncomplicated croup. This study and its predecessors have major inadequacies in design.[33,220] In a study with 72 children, Koren and colleagues[127] noted that dexamethasone did not offer any benefit to patients with laryngotracheitis but did decrease significantly the respiratory rate in children with spasmodic croup compared with placebo-treated control subjects. Although these findings were statistically significant, the benefits were not clinically significant.

Two additional modest studies of the use of dexamethasone in croup were published in 1988 and 1989, as was a meta-analysis of the evidence from the various randomized trials and a set of related editorials and a review.[4,115,132,198,200,207] Kuusela and Vesikari[132] concluded that dexamethasone was beneficial in acute spasmodic croup, and Super and associates[207] concluded that dexamethasone is beneficial in reducing the overall severity of moderate to severe acute laryngotracheitis during the first day of treatment.

The 1989 meta-analysis suggested that the use of steroids in children hospitalized with croup resulted in a significantly increased number with clinical improvement 12 hours and 24 hours after treatment and a reduced incidence of endotracheal intubation than occurred in the control subjects.[115] In this analysis, improvement at 12 hours was noted to be greater in the children who received higher initial doses of steroid (≥125 mg of cortisone) than in children who received lower doses.

In the early 1990s, nebulized steroids were evaluated in the treatment of croup, which led to another round of testimonials, controlled studies, and treatment articles supporting the efficacy of steroids.* Since the 1989 meta-analysis, more than 15 additional, controlled, steroid treatment trials have been performed, and another meta-analysis was published in 1999.[5] Finally, a large

*See references 5, 45, 84, 86, 101, 106, 108, 114, 122-125, 186, 187, 198, 229.

controlled trial of oral dexamethasone for mild croup was published in 2004, and a comprehensive meta-analysis was published in 2005.[13,190] Also published more recently are many articles comparing doses, types, and methods of administration of steroids and a further round of commentaries.*On the basis of the three meta-analyses and the specific studies analyzed, most reviews on the management of croup recommend the routine use of steroids (administered orally, intramuscularly, or by nebulization) in the treatment of croup.†

In the past, when I reviewed the data on the use of steroids in croup, what concerned me was that the specific clinical entity that was being treated was poorly defined and that no evidence indicated that steroids worked in any illness other than spasmodic croup.[33] Of most concern to me today is that the risks of steroid use have not been evaluated and that, because of small sample sizes in all but two of the available studies, they cannot be evaluated by meta-analysis.

I have seen lower respiratory tract complications develop in three children receiving steroid treatment for croup. In one case, an adenoviral pneumonia worsened, and in the other cases, bacterial tracheitis with pneumonia occurred. In these three cases, they all received corticosteroid treatment over the course of several days and not single-dose treatment. In the study of Super and colleagues,[207] two steroid-treated patients developed pneumonia during therapy; none of the control subjects developed pneumonia. In a more recent trial in which 28 children received nebulized dexamethasone, two children with neutropenia developed bacterial tracheitis.[108] Burton and associates[23] reported the occurrence of *Candida* laryngotracheitis as a complication of corticosteroid and antibiotic treatment in a child with croup, and Myers and colleagues[159] reported an infant who developed multiple pulmonary abscesses caused by *Legionella pneumophila* after receiving prolonged corticosteroid treatment of severe croup.

Today, the standard of care for the initial treatment of laryngotracheitis and spasmodic croup is the use of corticosteroids administered orally, intramuscularly, or by nebulization.[3,14,70] Recommended therapy is a single dose of intramuscular or oral dexamethasone. The limited use of nebulized budesonide also is effective. In the most definitive study, the treatment dose was dexamethasone (0.6 mg/kg) given orally once.[13] In a subsequent review, Bjornson and Johnson[14] noted that the standard dose of dexamethasone is 0.6 mg/kg (oral or intramuscular); they suggested that oral is preferable because absorption is excellent, and peak serum concentrations are achieved as rapidly as with intramuscular administration.

As noted earlier, I and others have observed bacterial and fungal infections as complications in children who have received multiple doses of steroids in croup. It is disturbing to me to note that in pediatric practice many children are receiving 3- to 5-day treatment courses rather than the recommended single-dose schedule. The data from the cotton rat model of human parainfluenza virus type 3 laryngotracheitis provides strong evidence supporting the limited use of steroids in croup.[168] In this model, topical steroid therapy significantly reduced the degree of inflammatory infiltrates and cell damage. The steroid therapy increased the virus load but did not prolong virus shedding.

I believe the most dramatic evidence supporting the use of steroid therapy in laryngotracheitis and spasmodic croup is the marked decrease in the number of hospitalizations for croup in Ontario, which coincided with the 1992 recommendation by the Canadian Pediatrics Society to use dexamethasone for treatment.[103,194] Steroids should not be used in laryngotracheobronchitis, laryngotracheobronchopneumonitis, or epiglottitis.

Because laryngotracheitis is a disease of viral etiology, what seems apparent is that antibiotic therapy would not be indicated and consequently not employed. In our analysis of more than 200 hospitalized children with laryngotracheitis, we noted that antibiotics had been administered to 85 percent.[75] A review of the records in many instances revealed that the physician had given antibiotic therapy because the possibility of epiglottitis had not been ruled out adequately. In several instances, the epiglottis had been observed and thought to be reddened or questionably enlarged.

A second consideration with regard to antibiotic therapy in croup is that the most dramatic reduction in mortality rates coincides with the introduction and widespread use of antibiotics. In my opinion, many of the deaths attributed to croup in the preantibiotic era were caused by secondary bacterial infections. Although use of antibiotics contributed to the reduction in the number of deaths caused by croup, other factors may be important. At about the same time that antibiotics were introduced, disease caused by *S. pyogenes* decreased in incidence and severity. Reasons for the decreased frequency of streptococcal disease were not clear.

Most patients with laryngotracheitis today do not need antibiotic therapy. In severe cases in which bacterial sepsis cannot be ruled out, however, antibiotic therapy should be employed. The pathogens to consider include pneumococci, group A streptococci, *S. aureus*, and *H. influenzae*. In patients with laryngotracheitis in whom fever persists or signs change, secondary infection should be considered. In these instances, appropriate culture specimens should be obtained before therapy is initiated (see the section on laryngotracheobronchitis for specific antibiotic therapy).

The use of nebulized racemic epinephrine, which was introduced by Jordan[112] in 1966 and popularized by Jordan and other members of the Utah group,[1,113] has been adopted widely throughout the United States and elsewhere.[118,131,138,152,189,196] The usual method of nebulization of racemic epinephrine is by intermittent positive-pressure breathing. This form of therapy was associated with a marked reduction in tracheostomies in several series.

In 1973, Gardner and associates[76] performed the first double-blind, controlled study in which racemic epinephrine was nebulized by a compressor without intermittent positive-pressure breathing. They found that saline-treated patients responded as well to therapy as did the racemic epinephrine recipients. In retrospect, this study can be criticized because the investigators failed to differentiate spasmodic croup from laryngotracheitis. In 1975, Taussig and associates[214] reported a small but carefully conducted study with intermittent positive-pressure breathing and racemic epinephrine in which they noted acute improvement in all cases, recurrence of symptoms in 2 hours, and no change in partial pressure of oxygen with clinical improvement; 24 to 36 hours after therapy, treated and untreated children were clinically similar. In another significant study that involved children hospitalized with severe croup without improvement after admission to a high-humidity mist room, Westley and colleagues[233] showed that racemic epinephrine therapy caused definite short-term improvement in children compared with saline treatment. This study is particularly important because a parainfluenza viral etiology was documented in more than 65 percent of the study's subjects, and all cases were clearly laryngotracheitis and not spasmodic croup.

The following points can be made about the use of racemic epinephrine in the treatment of croup: (1) Many children with croup respond to moist air alone; (2) significant rebound occurs after racemic epinephrine therapy, so it frequently needs to be repeated many times; (3) in a hospitalized child with severe acute

*See references 14, 26, 37, 57, 70, 71, 79, 121, 142, 186, 216.
†See references 3, 13, 14, 44, 51, 52, 70, 71, 84, 86, 103, 106, 107, 121-123, 125, 186, 187, 216.

laryngotracheitis, racemic epinephrine should be used. Tracheotomy or endotracheal intubation can be prevented in some cases. The most important issue today regarding the use of racemic epinephrine is whether it can be used safely for outpatient therapy.[40,117,178] In the past, most experts advised against the use of racemic epinephrine in the outpatient setting because of the known rebound that occurs. Some data suggest that with careful observation for a sufficient period (at least 2 hours) after administration, patients can be managed safely, and the number of hospitalizations can be decreased.[40,117,178] In a small study, investigators found that the administration of a helium-oxygen mixture (heliox) resulted in improvement in children with croup similar to that with racemic epinephrine treatment.[228]

The establishment of a mechanical airway seldom is necessary today in patients with laryngotracheitis. The planned procedure has a better outcome than does the procedure performed under emergency conditions. Traditionally, tracheostomy was the preferred method when a mechanical airway was needed.[7,74,240] When careful attention is given to tube size and other aspects of placement and maintenance, however, nasotracheal intubation compares favorably with tracheostomy.[7,240] The management of a child with a mechanical airway requires trained pediatric intensive care physicians and the facilities of an adequately staffed intensive care unit.

Five antiviral drugs have activity against viruses that cause laryngotracheitis.[88,105,146,212,234] Amantadine and rimantadine are approved for use in treating influenza A viral infections, and ribavirin is active against parainfluenza viruses, influenza A and B viruses, and respiratory syncytial virus (see Chapters 251 and 190). The neuraminidase inhibitors, zanamivir and oseltamivir, are active against influenza A and B types.[25,41] At present, consideration of therapy for severe croup that occurs during documented epidemics caused by influenza A or B viruses would seem reasonable. Because of high levels of resistance of influenza A strains to amantadine and rimantadine, treatment with the neuraminidase inhibitors is recommended.[25]

LARYNGOTRACHEOBRONCHITIS AND LARYNGOTRACHEOBRONCHOPNEUMONITIS (BACTERIAL TRACHEITIS)

Generally, all the treatment considerations discussed for laryngotracheitis except corticosteroids and racemic epinephrine by aerosol apply to laryngotracheobronchitis and laryngotracheobronchopneumonitis. Most important, however, because most patients have bacterial disease, antibiotics should be administered to all patients after appropriate culture specimens are obtained. Empiric therapy should be directed against *S. aureus*, *S. pyogenes*, *S. pneumoniae*, and *H. influenzae*. At present, initial treatment with vancomycin (40 mg/kg/day intravenously every 6 hours) and a third-generation cephalosporin, such as cefotaxime (150 mg/kg/day every 6 hours intravenously), is reasonable. If methacillin-sensitive *S. aureus* is isolated, treatment should be changed to oxacillin (150 mg/kg/day every 6 hours intravenously) or a similar agent.

Most children with advanced laryngotracheobronchitis or laryngotracheobronchopneumonitis need the placement of a mechanical airway. Whenever possible, this procedure should be done electively rather than as an emergency.

LARYNGITIS

Patients with laryngitis should rest their voices as much as possible. Increased fluid intake and perhaps the use of a vaporizer may help liquefy secretions and might provide symptomatic relief. Because group A streptococcal infection is a cause of laryngitis, culture should be performed. If it is positive, penicillin or a suitable alternative antimicrobial agent should be administered. In children and adolescents with prolonged hoarseness, sinusitis should be considered. Radiographs of the sinuses and a quantitative culture from the nose should be performed in search of a predominant abnormal bacterial flora. If either is positive, therapy with appropriate antibiotics is indicated. If laryngeal symptoms are persistent, the child should undergo laryngoscopic examination and other appropriate studies to exclude tumor, foreign body, and other chronic diseases.

PROGNOSIS

The prognosis of acute laryngotracheitis has improved markedly during the last 50 years. Today, a child with croup only rarely requires a mechanical airway, and virtually all deaths should be preventable. The child should be observed for the following complications: hypoxemia and cardiorespiratory failure, pulmonary edema, pneumothorax and pneumomediastinum, mechanical problems caused by tracheotomies and nasotracheal tubes, and secondary bacterial infections. Children with a history of croup have a higher prevalence of increased bronchial reactivity than children without such history.[87,141,229]

PREVENTION

At present, acute laryngotracheitis is not preventable. The widespread use of influenza vaccines could reduce the incidence of croup caused by influenza A and B viruses.

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Lower Respiratory Tract Infections

CHAPTER 23

Acute Bronchitis

James D. Cherry

Bronchitis is a common diagnosis in pediatric practice, although little unanimity exists among physicians regarding its exact clinical constellation, and in the true pathologic sense, it probably never occurs as an isolated entity. Acute bronchitis is a febrile illness with cough, rhonchi, and referred breath sounds.[15] Asthmatic bronchitis (infectious asthma), similar to acute bronchitis, but with associated wheezing and expiratory distress, is discussed in Chapter 25. On pathologic examination, the clinical illness of acute bronchitis reflects acute inflammatory disease of the larger air passages, including the trachea and the large and medium-sized bronchi.[25]

ETIOLOGY

Table 23–1 lists various infectious agents associated with acute bronchitis. Infections with adenoviruses, influenza viruses, parainfluenza viruses, respiratory syncytial virus, and *Mycoplasma pneumoniae* account for most cases of acute bronchitis in children. These agents, plus many rhinoviruses, a few enteroviruses, and perhaps human metapneumovirus, human bocavirus, and the newer human coronaviruses, account for virtually all cases in the United States today.

Of the adenoviruses, type 7 has been associated most commonly with acute bronchitis in children. In military recruits, including adolescents, adenovirus types 4 and 7 cause epidemic acute respiratory disease, in which bronchitis is a usual occurrence.[22,64]

Influenza A virus infection is a common cause of severe acute bronchitis, particularly at the time of antigenic shift of influenza A virus subtype and pandemic disease. Acute bronchitis caused by influenza A virus also is a regular occurrence between pandemics in new susceptible individuals (young children) in the population. Influenza B virus also is an important cause of bronchitis, and it was a more common causative agent than was influenza A virus in one large longitudinal study.[13]

All cases of measles involve the bronchi, but measles has been an uncommon occurrence since the advent of widespread use of vaccines. Of the parainfluenza viruses, type 3 is associated most commonly with acute bronchitis. Respiratory syncytial virus is a common cause of acute bronchitis, particularly in very young children. The more recently identified human metapneumovirus is another cause of acute bronchitis.[57]

Of the bacterial agents listed in Table 23–1, only *Haemophilus influenzae* clearly can be incriminated. *Bordetella pertussis* infection involves the trachea and bronchi, but fever is an uncommon event, and the illness is outside the definition of acute bronchitis. When sought, *M. pneumoniae* is a common cause of bronchitis. *Chlamydia pneumoniae* has been found to be the cause of bronchitis in adolescents and young adults.[32]

EPIDEMIOLOGY

The epidemiology of the common viruses that are associated with bronchitis is presented in Section XVII. Chapman and associates[13] published the results of a study of acute bronchitis in a single private group pediatric practice in Chapel Hill, North Carolina. The study occurred during a 104-month period, during which 5489 episodes of lower respiratory illness occurred. Of these illnesses, 40.1 percent were acute bronchitis. The bronchitis attack rate was highest in children in the second year of life (6.71%), and then it decreased gradually to approximately 2 percent in teenagers. In contrast to the age-specific attack rates, the ratio of bronchitis cases to all lower respiratory illness cases increased with age. In the first year of life, the ratio was 0.29; in children 12 years old or older, it was 0.69.

During the first 6 years of life, respiratory syncytial virus and parainfluenza virus type 3 were the most common etiologic agents noted in the Chapel Hill study. During the first 2 years of life, adenoviruses also commonly were associated with bronchitis. In patients older than 6 years, *M. pneumoniae* and influenza A and B viruses were the most common etiologic agents. In a study of cough illnesses of 6 days' duration or longer in university students, investigators found that 15 of 31 students with laboratory evidence of *Bordetella* spp. infection were considered by their primary care providers to have bronchitis.[46]

The incidence of acute bronchitis peaks in the winter months, declines to midsummer, and increases again through the fall. Attack rates generally are higher in boys than in girls.[13,39,65] A sex difference is most pronounced during the first 6 years of life.

PATHOPHYSIOLOGY AND PATHOLOGY

Because acute bronchitis is an illness characterized by clinical features and one not usually associated with death, knowledge of its pathophysiology and pathology is meager. The general pathophysiology of human infections with viruses and *M. pneumoniae* that cause acute bronchitis is presented more completely in the sections of this book related to the individual infectious agents.

In virtually all cases of acute bronchitis, evidence of upper respiratory viral infection (pharyngitis, rhinitis) also is present. Tracheal and bronchial infection apparently is the result of distal spread. In bronchitis, the clinical features result from damage to the ciliated epithelium of the lower trachea and the large and medium-sized bronchi.[25] Although the cytopathologies of the various infectious agents is different,[71] the resulting obstruction of the air passages leads to similar symptoms. The duration of symptoms depends to some extent on the specific initial infectious agent and, in cases of prolonged illness, on secondary bacterial infection.

TABLE 23–1 Infectious Agents Associated with Acute Bronchitis

Agent	Importance in Causation*	References
Viruses		
Adenovirus types 1-7, 12	+++	1, 2, 5, 10-12, 14, 23, 27, 28, 35, 36, 42, 54, 59, 62, 65, 66, 70
Enterovirus	+	30, 35, 36, 59
Coxsackieviruses B	+	12
Echoviruses 8, 12, 14	+	63
Polioviruses	+	27, 63
Herpes simplex	+	27, 59, 63
Influenza virus	+++	1, 6, 8, 10-13, 14, 23, 28, 30, 61, 63
A	++	6, 10, 12, 13, 30, 35, 36, 61, 63
B	++	10, 12, 13, 30, 35
C	+	1
Measles	+	14, 25, 52
Mumps	+	63
Parainfluenza	+++	1, 6, 10-13, 14, 27-30, 33, 35, 42, 59, 61, 63, 66
1	++	10-13, 23, 30, 33
2	++	6, 10-12, 30, 33
3	+++	1, 2, 6, 10-13, 23, 30, 33, 36, 61
4	+	29
Respiratory syncytial virus	+++	1-3, 8, 10-13, 14, 23, 27, 28, 30, 34-36, 38, 45, 55, 59, 60, 62, 63, 66
Human metapneumovirus	+	57
Human bocavirus	+	37
Human coronavirus	+	4, 17, 26
Rhinovirus	++	12, 14, 27, 28, 30, 35, 36, 51, 58, 59
Bacteria		
Bordetella pertussis	+	25, 46
Bordetella parapertussis	–	
Haemophilus influenzae	–	42, 67
Moraxella catarrhalis	–	21, 31
Streptococcus pneumoniae	–	42
Streptococcus pyogenes	–	42, 50, 59
Other		
Chlamydia psittaci	+	10
Chlamydia pneumoniae	+	32
Mycoplasma pneumoniae	+++	10-13, 14, 23, 30, 35, 41, 53, 68

*+++, *very common;* ++, *common;* +, *rare;* –, *of questionable etiologic significance.*

In acute bronchitis, the larynx and subglottic trachea are not involved prominently. Conversely, today, bronchial involvement is seen only occasionally in croup.

CLINICAL PRESENTATION

Initial manifestations of acute bronchitis are upper respiratory in nature and, depending on the etiologic agent, are predominantly nasal, as in the common cold, or show additional objective evidence of pharyngitis, as in nasopharyngitis. Fever usually is present, and temperatures vary from 37.8° C to 39° C (100° F to 103° F) on most occasions. Cough always is present, and its onset can be insidious or abrupt. Initially, the cough is dry and harsh and often brassy in younger children. As the illness progresses, the cough becomes looser. In older children, purulent sputum is raised and expectorated. In younger children, the swallowing of often tenacious sputum frequently leads to gagging and vomiting. Older children may complain of chest pain resulting from coughing.

On initial physical examination, a variable degree of rhinitis usually is present; many patients have diffuse pharyngeal erythema. As the disease progresses, these upper respiratory signs generally decrease. Examination of the chest reveals rhonchi and referred breath sounds. Coarse, changing rales are noted frequently.

In the usual case of acute bronchitis, the illness can be separated into three phases: (1) a 1- to 2-day prodromal period when fever and upper respiratory symptoms predominate, (2) a 4- to 6-day period of marked tracheobronchial symptoms with some fever and general discomfort, and (3) a recovery period that may last 1 or 2 weeks and is characterized by cough and expectoration. Occasionally, the recovery period is particularly distressing and is associated with a low-grade fever, suggesting secondary bacterial infection. Bronchitis caused by *C. pneumoniae* often is insidious in onset and frequently associated with or preceded by pharyngitis.[32,40] Illness persists for several weeks but responds to appropriate antibiotic therapy.

Laboratory study in acute bronchitis is of limited use. Children in whom throat cultures reveal pathogenic bacteria in predominant growth tend to have more severe illness than do children with only viral infections.[16,47] The white blood cell count usually is greater than 10,000 cells/mm^3, and approximately one third of the cases have a predominance of neutrophils.[47] The chest radiograph is normal unless associated pulmonary involvement is present.

DIFFERENTIAL DIAGNOSIS AND SPECIFIC DIAGNOSIS

Because acute bronchitis is a clinical entity caused by multiple etiologic agents, the most difficult differential aspect of diagnosis is the selection of the specific infectious cause. Also important is the separation of acute, self-limited bronchitis from chronic,

more serious problems, such as cystic fibrosis, allergic respiratory disease, and sinusitis.

An epidemiologic history frequently can help in assigning a particular virus, *M. pneumoniae*, *C. pneumoniae*, or *B. pertussis*, as the presumptive etiologic agent. If epidemic bronchiolitis is occurring in the community, respiratory syncytial virus would be a likely cause. Similarly, predictions of causation by influenza virus, parainfluenza virus, adenoviruses, *M. pneumoniae*, *C. pneumoniae*, or *B. pertussis* can be made through clinical epidemiologic observations. Specific etiologic diagnosis can be established through the isolation of an organism or its identification by a direct antigen test from the nasopharyngeal secretions. Serologic study on paired sera may be useful for the diagnosis of *M. pneumoniae*, *C. pneumoniae*, and *B. pertussis*.

Children with protracted illnesses or febrile exacerbations should be examined by culture, radiograph, and perhaps further imaging studies for secondary bacterial infection of the tracheobronchial tree or the lungs or both. Children with chronic recurrent illnesses should be tested for cystic fibrosis, allergic conditions, and anatomic problems, such as gastroesophageal reflux and tracheoesophageal fistula.

TREATMENT

Treatment of acute bronchitis is distinguished more by what *not* to do than by specific modalities. In most mild cases, no specific therapy is indicated.

For children who feel miserable during the initial phases of acute bronchitis, analgesic therapy may be useful. Formerly, aspirin was the recommended analgesic. Because aspirin is an etiologic factor in influenza-associated Reye syndrome and because differentiating influenza viral infections from other respiratory viral infections is difficult, it is prudent to use acetaminophen rather than aspirin. The dose per single administration of acetaminophen by year of age is as follows: younger than 1 year, 60 mg; 1 to 3 years, 60 to 120 mg; 3 to 6 years, 120 mg; 6 to 12 years, 150 to 300 mg; older than 12 years, 325 to 650 mg. Administration may be repeated three or four times daily in young children and every 4 hours in older children. Acetaminophen rarely should be given to infants younger than 6 months of age.

As a result of widespread advertising, a common practice of individuals with acute bronchitis is to use an array of cold remedies, which contain various combinations of antihistamines, decongestants, and antitussives. None has been shown to be useful in acute bronchitis, and in certain stages of illness, they may aggravate the recovery process. Repeated bouts of coughing occasionally result in emesis, exhaustion, or insomnia, and the careful use of antitussive agents (codeine or dextromethorphan) can be helpful.[19] Cough suppressants should be used with caution when a cough is productive.

Intake of fluids should be encouraged to prevent overall dehydration and to decrease the viscosity of new secretions. Use of mist therapy also may help in thinning the exudate-containing respiratory secretions.[24,49]

In severe cases of acute bronchitis, treatment with specific antiviral agents should be considered. When influenza A virus is the likely etiologic agent, amantadine, rimantadine, zanamivir, or oseltamivir therapy may be beneficial.[9]

As noted in Table 23–1, most cases of acute bronchitis are caused by viruses, so antibiotic therapy would not be indicated.[48,69] In cases in which fever returns or no trend toward recovery is seen by the seventh day of illness, the possibility of a secondary bacterial infection should be considered. The association of sinusitis or a throat culture with a predominant growth of a respiratory pathogen (*Streptococcus pneumoniae*, *Streptococcus pyogenes*, *Moraxella catarrhalis*, *H. influenzae*) is an indication for therapy. Infection with *M. pneumoniae* also should be treated, but in contrast to treatment of pneumonia, the therapy usually does not show an impressive response. Bronchitis caused by *C. pneumoniae* should be treated with erythromycin (50 mg/kg/day divided every 6 hours) for 10 to 14 days.[32]

PROGNOSIS

The prognosis in acute bronchitis usually is excellent. Although the duration of cough can be disturbing to the parent and the child, full recovery is the rule. Several studies suggest that lower respiratory illness in the first few years of life may be associated with persistent respiratory symptoms and with abnormalities in lung function in later life.[7,18,43,44] Although none of these studies has followed children with acute bronchitis specifically, the findings in other illnesses (bronchiolitis and croup) indicate a need to observe children with episodes of acute bronchitis carefully as well.

PREVENTION

At present, no practical method of prevention of acute bronchitis in children exists. Because most cases result from infections with common respiratory viruses, the development of vaccines could be expected to be helpful.

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CHAPTER 24

Chronic Bronchitis

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Chronic bronchitis is a serious and costly health problem in adults.[17] In a U.S. review of the epidemiology of chronic bronchitis, affected adult morbidity estimates were greater than 900,000 hospitalizations and 10 to 14 million physician visits each year.[24] The World Health Organization estimates 23.6 million adults have chronic obstructive pulmonary disease, representing more than 15 percent of the adult population, and many of these cases meet the definition for chronic bronchitis.[5] In the adult literature, the accepted definition of chronic bronchitis includes daily excessive production of sputum with manifestation of cough

present on most days for 3 months in a year for not less than 2 successive years.[46]

For most pediatricians, the clinical entity of chronic bronchitis is ill-defined. Chronic cough complex can be associated with other, more common respiratory or cardiac diseases that must be excluded before a diagnosis of chronic bronchitis is considered.[11] Chronic bronchitis usually is described in the literature as chronic productive cough and is synonymous with asthmatic bronchitis.[8,43] The lack of uniform or standardized definitions of pediatric chronic bronchitis leads to wide discrepancies in reported childhood prevalence (Table 24–1). In affected patients, causal relationships and acute exacerbations have been linked to exposure to a noxious inhaled agent (e.g., environmental/industrial pollution, cigarette smoke), specific host factors (e.g., genetic predisposition), and infectious respiratory pathogens. The pathology of the disease entity is unclear. Bronchoscopy of pediatric patients with chronic bronchitis has revealed findings similar to those noted in children with asthma, which reflects the inclusion of asthma in the spectrum of the chronic bronchitis complex.[27] Pediatric bronchoscopic evaluation yields heterogeneous histologic findings (granulocyte and mononuclear cell predominance at lavage and biopsy) that are distinct from findings in adults with chronic bronchitis.[43]

DIFFERENTIAL DIAGNOSIS

Because chronic bronchitis is accepted by most physicians as being a complex of symptoms characterized by persistent cough with or without wheezing, it is imperative that the physician evaluate the patient for diseases that include chronic bronchitis within their spectrum of signs and symptoms. Figure 24–1 includes disorders that have clinical manifestations of chronic cough for longer than 3 months and provides guidelines for diagnostic evaluation of children with chronic cough.[26]

Heading the differential list is asthma,[10] defined as reversible obstructive airways disease with a significant inflammatory component leading to increased edema and production of mucus.

TABLE 24–1 Prevalence of Childhood Bronchitis

Author	Year	Study Subjects	Prevalence (%)	Acute/Chronic Bronchitis
Bland et al.	1974	Kent schoolchildren	5.5	Acute/chronic
Burrows and Lebowitz	1975	Arizona children	7.1	Chronic
Burrows et al.	1977	Arizona retrospective	46.4	Chronic
Kubo et al.	1978	Japanese children	1.4	Chronic
Peat et al.	1980	Sydney schoolchildren	20	Acute/chronic

Modified from Morgan, W. T., and Taussig, L. M.: The chronic bronchitis complex in childhood. Pediatr. Clin. North Am. 31:*851-864, 1984.)*

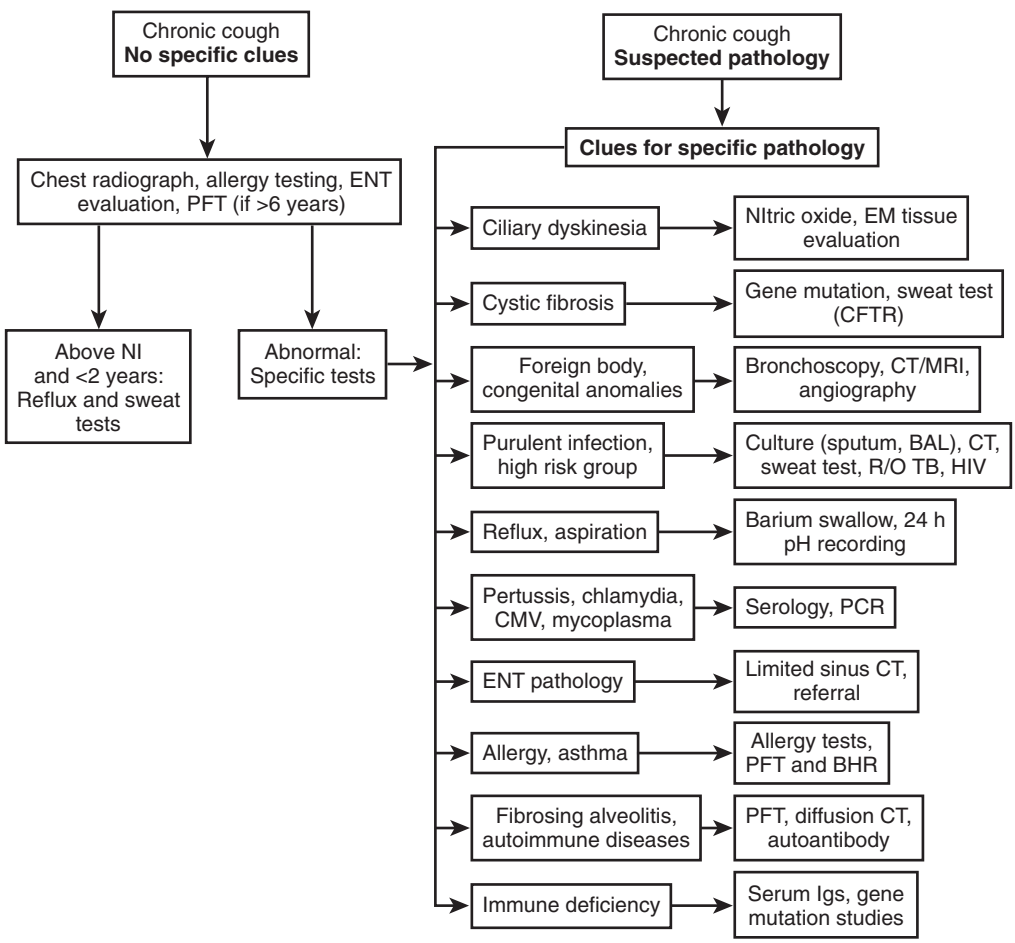

Figure 24–1 Diagnostic evaluation of children with chronic cough. BAL, bronchoalveolar lavage; BHR, bronchial hyperreactivity; CFTR, cystic fibrosis transmembrane conductance regulator; CMV, cytomegalovirus; CT, computed tomography; EM, electron microscopy; ENT, ear, nose, throat; HIV, human immunodeficiency virus; MRI, magnetic resonance imaging; NI, no information; PCR, polymerase chain reaction; PFT, pulmonary function test; TB, tuberculosis. (*Modified from Morice, A. J., Fontana, G. A., Sovijarvi, A. R., et al.: Diagnoses and management of chronic cough. Eur. Respir. J.* 24:*487, 2004.*)

Asthma is a common pediatric lower respiratory disease, affecting more than 6.3 million children younger than 18 years old in the United States.[1] Asthma can be distinguished from chronic bronchitis by pulmonary function evaluation documenting reversal of airway obstruction after delivery of pulmonary bronchodilators. Recurrent episodes of acute bronchitis often can be interpreted as chronic bronchitis, although the intermittent nature of these episodes and absence of a persistent cough usually distinguish this group of patients clinically.[4]

Specific viral infections (e.g., rhinovirus, parainfluenza virus) in children with or without allergic rhinitis may provoke airway hyperreactivity and late asthmatic reactions, which may be confused symptomatically with chronic bronchitis.[6,20] Persistent lower respiratory tract infections (i.e., *Chlamydia* spp., pertussis, *Mycoplasma* spp., and *Mycobacterium* spp.) frequently manifest with the complex of symptoms described and are evaluated best with chest radiographs in a search for enlarged hilar nodes or interstitial lung infiltrates. Respiratory tract secretions for appropriate bacterial and viral culture and serum for determinations of antibacterial antibodies should be obtained. In the case of tuberculosis, a delayed hypersensitivity skin test for *Mycobacterium tuberculosis* antigen should be applied.

Cystic fibrosis is a recessively inherited illness occurring in approximately 1 in 3700 live births, with clinical manifestations of failure to thrive, steatorrhea, nasal polyps, and recurrent lower respiratory tract symptoms. The disorder is associated with mutation of the cystic fibrosis transmembrane conductance regulator (CFTR) gene.[38] Cystic fibrosis can be diagnosed by newborn screening methodologies including identifying typical CFTR gene mutation patterns in DNA extracted from dot blots or other collected blood specimens at or after birth or by detecting abnormally elevated chloride levels (>60 mEq/L) as measured by the sweat iontophoresis test.[40] In clinical practice, one or both modalities may be used for establishing the diagnosis, especially for patients with genetic variations of the CFTR gene not typical of the classic cystic fibrosis phenotype or for patients with uninterpretable sweat test outcomes.

Primary ciliary dyskinesia encompasses the immotile cilia disorders and Kartagener syndrome (rhinosinusitis, bronchitis or bronchiectasis, and situs inversus) and affects 1 in 15,000 white births. Primary ciliary dyskinesia typically is inherited as an autosomal recessive disorder and is associated with defects in mucociliary transport in the respiratory tree. The symptom complex also has been associated with polycystic kidneys, hepatic disease, and central nervous system symptoms. Screening tests for primary ciliary dyskinesia include nasal nitric oxide and in vivo tests of ciliary motility, but these tests are not universally available, and standardization is problematic.[31] More recently, physicians have used tissue biopsy specimens to identify structural defects by electron microscopy, high-speed analysis of cilia beat patterns, and cell culture when establishing the diagnosis has been difficult.[29]

The primary immune disorders associated most frequently with recurrent sinopulmonary infections include selective IgA deficiency, functional antibody deficiencies, hypogammaglobulinemia, and ataxia telangiectasia.[3] Selective IgA deficiency is the primary immune disorder encountered most commonly, with an incidence of 1 in 500. It may be asymptomatic or accompanied by a propensity for atopy and an increase in associated autoimmune disease (most often rheumatoid arthritis and systemic lupus erythematosus). The diagnosis is made readily by evaluation of quantitative serum immunoglobulins defining IgA levels of less than 10 mg/dL. IgA deficiency may be associated with IgG subclass deficiencies (IgG2 deficiency was identified in 85 percent in a series of >150) with or without clinical symptoms. Functional antibody deficiencies have been detected and include inability to respond serologically to vaccination with polysaccharide antigens such as *Streptococcus pneumoniae* or *Haemophilus influenzae*.[13]

Patients with hypogammaglobulinemia represent a diverse group having primary immune disorders that include X-linked agammaglobulinemia, common variable immune deficiency (CVID), and hyper-IgM syndrome. X-linked agammaglobulinemia typically affects boys, with recurrent sinopulmonary infections with onset occurring postnatally as maternal antibody wanes. X-linked agammaglobulinemia is associated with deficiency of a B-cell maturation enzyme, Bruton tyrosine kinase, and is characterized by the absence of circulating mature B lymphocytes. CVID is a disorder of young adults, with immune findings of agammaglobulinemia and variable T-cell defects. Clinical presentation includes lower respiratory and sinus disease. Inherited CVID disorders have been described and include defects in inducible T-cell costimulator gene and transmembrane activator and calcium-modulator and cyclophilin ligand interactor (TACI).[3] Patients with TACI defect and CVID may lack circulating mature B lymphocytes. More often, patients with CVID normally express B-cell markers on circulating lymphocytes. Hyper-IgM syndrome is a rare disorder associated with elevated IgM and low IgG and IgA and recurrent infections including sinopulmonary and hepatic infections. Different gene defects have been identified in patients with hyper-IgM syndrome; patients with defects in nucleotide-modifying enzymes located only in B cells have no associated T-cell defect.

Patients with ataxia telangiectasia have depressed serum IgA concentrations and marked dysfunction of the swallowing mechanism, leading to recurrent lower respiratory tract infections probably secondary to recurrent aspiration. These patients are identified by telangiectasias of the skin and conjunctivae in association with aberrant and progressively deteriorating neurologic symptoms and immunodeficiency (depressed IgA and IgE and aberrant T-cell–mediated immunity). Some patients have depressed IgG subclasses (IgG2, IgG4) as well. The gene mutation in ataxia telangiectasia has been identified as disruption of the ataxia telangiectasia molecules responsible for detection and implementation of DNA repair dependent on phosphatidylinositol-3-kinase signal transduction pathways.

Primary immune disorders with hypogammaglobulinemia can be diagnosed through evaluation of serum immunoglobulin levels. Further characterization includes assessment of antibody production after vaccine or neoantigen challenge, evaluation of circulating lymphocyte cell surface markers, and in vitro proliferative responses to plant lectins or specific antigens. Evaluation of associated gene defects is important for determining corrective interventions (e.g., transplantation, gene therapy) and genetic counseling.

Graft-versus-host disease affecting the lungs has been described in immunocompromised patients after they have undergone bone marrow transplantation. The lesion is caused by chronic pulmonary lymphocytic infiltrates and pulmonary fibrosis mimicking the symptom complex of chronic bronchitis and often is indistinguishable radiographically or clinically from infections that characteristically are pathogenic (i.e., *Pneumocystis jiroveci*, *Candida albicans*, *Aspergillus* spp.).

Secondary immune disorders (prematurity and pediatric human immunodeficiency virus [HIV] infection) also may be associated with significant sinopulmonary infections. Premature infants with attendant severe respiratory distress syndrome requiring positive-pressure ventilation may develop bronchopulmonary dysplasia. Diagnostic criteria include hypoxia requiring oxygen supplementation, characteristic diffuse interstitial markings on chest radiograph, and clinical signs of pulmonary disease (i.e., tachypnea, intercostal retractions). In one series, more than 60 percent of surviving children with bronchopulmonary dysplasia were documented to have significant pulmonary disease and associated morbidity (i.e., increased hospitalizations).[33] Continued improved care for premature infants and resultant decreased

incidence of mortality may increase the population of infants with bronchopulmonary dysplasia.

Pediatric HIV infection is estimated to affect approximately 1.5 percent of infants born to HIV-infected mothers in the United States.[28] This reduced infection rate can be attributed to (1) availability and use of highly active antiretroviral therapy (HAART) to pregnant women and their newborn infants, (2) U.S. practices that minimize exposure to breast milk, and (3) obstetric practices that minimize exposure to infected blood products (cesarean delivery and practices that decrease time from membrane rupture to delivery). For infants in the United States who continue to be infected, with noncompliance to treatment or lack of treatment, HIV is associated with serious lower respiratory tract illness.

In previous years, lymphoid interstitial pneumonitis/hyperplasia, reported in 25 percent of all children with acquired immunodeficiency syndrome, was associated with chronic cough, but this HIV-associated outcome rarely is reported with the advent of improved antiviral medications to suppress replication of HIV. Despite restoration of immunocompetence with HAART, lower respiratory tract infections (viral, bacterial, fungal) are still common occurrences in children infected with HIV.[23] In the face of improved survival for HIV-infected children, the incidence of chronic radiographic lung changes (parenchymal consolidations and nodular disease for >3 months) has been described in 33 percent of children by the time they are 4 years of age.[30] An unexpected consequence of HAART therapy in HIV-infected children may lead to increased risk of developing asthma through an immune reconstitution mechanism.[14] The diagnosis of HIV infection is established by viral diagnostic assays (polymerase chain reaction, p24 antigen) for perinatally exposed infants younger than 18 months of age and by serology (enzyme-linked immunosorbent assay and confirmational assays [Western blot analysis, indirect fluorescent antibody assay]) for perinatally exposed infants 18 months or older.[35]

Anatomic lesions that lead to pulmonary obstructive airway disease can simulate the complex of chronic bronchitis. An infant with chronic cough, poor feeding habits, and failure to thrive should undergo evaluation for gastroesophageal reflux and tracheoesophageal fistula, which are identified most easily by barium swallow or pH probe monitoring. Mediastinal tumors can produce extrinsic obstruction, leading to recurrent cough and wheezing. Congenital heart disease should be considered in this patient group and can be evaluated with chest radiography, electrocardiography, and echocardiography.

Respiratory tract irritants have been implicated as a cause of chronic cough, as documented in adult populations of industrial European nations.[44] An assessment of the public health impact of pollution in Austria, France, and Switzerland concluded that air pollution caused 6 percent of all mortality, or 40,000 attributable cases per year.[19] Nonindustrial, rural communities, such as the forest zone of Nigeria, report virtually no chronic bronchitis, whereas metropolitan New York reports an increased risk for development of upper and lower respiratory tract infections in adults and children who reside in parts of the city with the highest ambient air levels of sulfur dioxide and particulate air pollution.[22] After exposure to urban air particulate matter, animal models with induced chronic bronchitis (exposure to 200 ppm of sulfur dioxide for 6 weeks) have shown pathologic changes consistent with exacerbation of chronic bronchitis—changes in ventilatory capacity and marked pulmonary inflammation.[9]

A correlation between tobacco smoking and reduced ventilatory capacity in adults has been reported by many investigators. Peat and associates[34] described teenagers in Sydney, Australia, with recurrent episodes of bronchitis with worsened lung function when coupled with tobacco smoking. A meta-analysis of 21 relevant publications on relationships between exposure to environmental tobacco smoke and lower respiratory tract infection in infancy and early childhood concluded that exposure to environmental smoke resulted in adverse childhood respiratory outcomes.[21] The outcomes included an increased number of lower respiratory tract infections and an increased number of hospitalizations for these infections. These data suggest that it is imperative for physicians to obtain a smoking history not only for the patient but also for other household members.

Other noxious agents associated with outdoor pollution have been associated with poor control of chronic cough complex diseases such as asthma. In Los Angeles, adults with asthma were more poorly controlled if they resided near areas of heavy traffic.[25] Similar findings of poorly controlled lung disease associated with traffic pollution have been documented in adult and pediatric patients in Lima, Peru.[7] Occupational exposures long have been cited for exacerbating pulmonary diseases. Blanc and colleagues[2] evaluated the risk for developing chronic lung disease from occupational exposure and identified the population attributable risk at 15 percent for chronic bronchitis and chronic obstructive pulmonary disease. Classic examples of occupational exposure to dust leading to increased risk of developing chronic cough are described in coal miners in Great Britain, foundry workers in the Rhine-Ruhr area of Germany, potters in West Virginia, and farmers in Croatia.[47] After the disaster of September 11, 2001, in New York City, firefighters with existing sarcoidosis (reported at higher frequency in firefighters before the disaster) reported increased incidence of bronchial hyperreactivity measured by pulmonary challenge testing.[16]

EPIDEMIOLOGY AND ETIOLOGY

Differentiating the impact of clinical, social, and environmental factors on lower respiratory tract disease, including chronic bronchitis, has been problematic and has led to conflicting outcomes in epidemiologic assessments.[12,42] In addition, the lack of a standardized definition of chronic bronchitis in the pediatric literature leads to confusing interpretation of data when attempting to appreciate the prevalence or etiology of the disease complex.[43] In Table 24–1, the data from Kubo and associates[18] separate acute recurrent bronchitis, asthmatic bronchitis, and chronic bronchitis, leading to a decrease in the prevalence from a proposed 46.4 percent (in the Arizona questionnaire) to 1.4 percent.[27]

Considerable overlap also exists in evaluating etiologic agents for chronic bronchitis. The same viral agents proposed as the exacerbating factors of asthmatic bronchitis (see Chapter 25) are implicated in exacerbations of chronic bronchitis,[1,15] including rhinoviruses, parainfluenza viruses, respiratory syncytial virus, influenza A and B viruses, adenoviruses, and enteroviruses. Persistent adenovirus infection has been implicated as a cause of childhood chronic bronchitis. In a study of 11 children with chronic bronchitis, transbronchial biopsy specimens revealed no evidence of persistent adenovirus infection (culture or polymerase chain reaction),[36] suggesting that viral infections may precipitate cough, but their roles in the pathologic findings associated with chronic bronchitis should be questioned.

Table 24–2 lists predominant bacterial pathogens isolated from washed sputum for a group of 40 pediatric patients with chronic bronchitis and exacerbations of cough and fever; these pathogens include *H. influenzae*, *S. pneumoniae*, and *Staphylococcus aureus*.[18] These bacteria also are implicated as etiologic agents in the triggering of asthmatic bronchitis. Neither *Mycoplasma* nor human metapneumovirus was included in this listing, but this simply may reflect the time-frame (late 1970s) when the Kubo study was conducted.[32,45] Treatment of exacerbations of chronic bronchitis with antibiotic therapy usually is effective in reducing the volume of sputum and purulence during the acute infection but shows no parallel elimination of the cultured microorganisms.

TABLE 24–2 Predominant Pathogens in Washed Sputum of Chronic Bronchitis (40 Cases)

Pathogen	No. Cases
Haemophilus influenzae and *Streptococcus pneumoniae*	21 (52.5%)
H. influenzae	17 (42.5%)
Staphylococcus aureus	2 (5%)
Superinfection with gram-negative rods	
Pseudomonas aeruginosa	4
Klebsiella pneumoniae	2
Escherichia coli	1
Enterobacter cloacae	1

From Kubo, S., Funabashi, S., Uehara, S., et al.: Clinical aspects of "asthmatic bronchitis" and chronic bronchitis in infants and children. J. Asthma Res. 15*:99-132, 1978.*

TREATMENT

When a specific diagnosis is found in association with chronic cough or wheezing, therapy is directed toward the primary disease entity and the clinical presentation of cough. Bronchodilators (beta-adrenergic agents, cromolyn sodium, corticosteroids) and anticholinergic agents are used, when appropriate, for the treatment of chronic cough associated with asthma or acute exacerbations of chronic obstructive pulmonary disease.[41] Appropriate positioning techniques (prone 30 degrees), feeding schedules, and medications (e.g., prokinetics or cholinergics) are indicated in the approach to infants with gastroesophageal reflux. Patients with hypogammaglobulinemia can be helped with supplemental intravenous immunoglobulin preparations currently available commercially (400 to 600 mg/kg per dose every 3 to 4 weeks) in an attempt to decrease the incidence of infections.

It is imperative that patients with chronic pulmonary diseases such as cystic fibrosis, asthma, or ciliary dyskinesias understand the pulmonary irritant effect of tobacco smoking, dust exposure, and air pollution. A change of occupation may be essential for their well-being. It also is imperative to stress the irritant effect of parental smoking on the already compromised pulmonary function of the child.

Antibiotic therapy for chronic bronchitis usually is reserved for patients with severe illness in whom the likelihood of developing a secondary bacterial infection is great. In these instances, antibiotic therapy can be considered and usually consists of amoxicillin (45 to 90 mg/kg per 24 hours), erythromycin (40 mg/kg per 24 hours), or, in adolescents and adults, tetracycline (25 to 50 mg/kg per 24 hours) or quinolones for 5 to 10 days. There do not seem to be significant differences in short-term effectiveness in adults with these outlined treatment regimens.[39] Methylxanthine therapy is used less frequently in chronic bronchitis care. When it is used, certain antibiotics, such as erythromycin, lead to elevated serum concentrations of theophylline, which renders toxicity more likely to occur. Treatment with mucolytic agents in chronic pulmonary disease has not proven to have a significant impact except in adults with already advanced disease with multiple hospitalizations, and no evidence suggests that they are beneficial in children.[37]

As with patients with obstructive lung disease, attention must be aimed at careful and sequential monitoring of pulmonary function. The prognosis for chronic bronchitis varies and is dependent on the specific etiology of this syndrome.

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CHAPTER 25

Bronchiolitis and Infectious Asthma

Robert C. Welliver

Bronchiolitis and infectious asthma, which also is called *asthmatic bronchitis*, *wheezy bronchitis*, or *virus-induced asthma*, are common illnesses of children, characterized by symptoms of upper respiratory tract infection and signs of obstructive airway disease, particularly wheezing. Bronchiolitis and infectious asthma often are considered to be distinct entities, and not all infants who develop bronchiolitis have infectious asthma in later life. Although the two illnesses are similar in terms of clinical presentation and have some mediators in common, the pathologic findings may contrast in some cases. Differences in terms of etiologic agents that precipitate episodes of illness or in terms of response to therapy are more a function of the patient's age (particularly older or younger than 3 years) than of any underlying disease process. *Infectious asthma* is considered as a term defining the occurrence of repeated episodes of bronchiolitis, and the two conditions are discussed together.

DEFINITIONS

Bronchiolitis is an acute communicable disease predominantly manifesting in infancy and characterized by cough, coryza, fever, grunting, tachypnea, retractions, inspiratory crackles, expiratory wheezing, and air trapping. The first (and the most severe) episodes occur most frequently in infants aged 2 to 6 months. *Infectious asthma* is a term that generally refers to infection-induced wheezing occurring beyond infancy. Nonetheless, a patient may experience bronchiolitis during the first months of life and a recurrent episode caused by the same virus in the second year, suggesting an identical underlying nature of the two illnesses. Certain viruses (e.g., respiratory syncytial virus [RSV] and influenza viruses) seem capable of causing bronchiolitis in all children; in contrast, other agents (rhinovirus in particular) seem to induce wheezing only in atopic children.

HISTORY

Although all practicing pediatricians today are familiar with the term *bronchiolitis* and associate it with an acute clinical illness with the signs and symptoms of obstructive emphysema, it has been recognized in the medical literature for only a relatively brief period. In the early years of the 20th century, the term *capillary bronchitis* was used to describe an inflammatory illness of the smallest alveoli.[56] The condition could not be distinguished clinically from pneumonia, however, and whether the pathologic entity (i.e., bronchiolitis) ever occurred separately from pneumonia was doubted. In the 11th edition of *Holt's Diseases of Infancy and Childhood*,[73] published in 1940, capillary bronchitis is discussed only briefly and is considered under pneumococcal pneumonia. Acute bronchiolitis finally was listed as a heading in the sixth edition of the *Textbook of Pediatrics*,[118] published in 1954; even at that time, bronchiolitis was associated with interstitial pneumonitis.

In contrast to the delayed textbook recognition of bronchiolitis, good clinical descriptions were presented in journals. In 1941, Hubble and Osborn[76] published "Acute Bronchiolitis in Children," in which they described an epidemic of bronchiolitis involving 50 hospitalized children. Pratt[131] in 1944 and Nelson and Smith[119] in 1945 published excellent clinical articles. Studies in the late 1950s and early 1960s established the etiologic association of RSV and other viruses with acute bronchiolitis.[6,21,22,24,25,145]

ETIOLOGIC AGENTS

RSV is the major cause of bronchiolitis in infancy and virtually the only important etiologic consideration when major epidemics are occurring. Other agents cause smaller annual outbreaks. Table 25–1 presents the frequency of infectious agents in the overall cause of bronchiolitis. These data were compiled from 12 reports in which respiratory illness in children was observed over an extended period, and in most instances, numerous clinical illness categories were being studied. During major epidemics in the colder months in temperate climates, virologic and serologic studies indicate RSV as the cause in 80 percent or more of the cases, especially in the more severe ones. In nonepidemic situations, more than 50 percent of the isolates or instances of serologic evidence of infection are infectious agents other than RSV.

Sporadic disease rarely is associated with more than a 50 percent association of infectious agent and illness, despite the fact that infections undoubtedly are the cause of sporadic and epidemic bronchiolitis.[107]

Children surviving infantile bronchiolitis often have recurrent episodes of wheezing that, on clinical grounds, seem to be precipitated by upper respiratory infections. Studies designed to determine the infectious agents responsible for these repeated wheezing episodes have yielded variable results, presumably because they involve patients of different ages followed during different seasons of the year.[8,55,98,108-111]

A comprehensive study based on more than a decade of observation of children from birth through 15 years of age[68] revealed that viruses and *Mycoplasma pneumoniae* are the most common etiologic agents (Table 25–2). RSV is the most common cause of recurrent, wheezing-associated respiratory illness occurring in young children, including children who previously experienced bronchiolitis, which strongly suggests the identical nature of infection-related wheezing episodes occurring in children of different ages. With increasing patient age, rhinoviruses and *M. pneumoniae* account for most infection-induced wheezing episodes. The parainfluenza viruses and influenza viruses are important causes of such wheezing episodes throughout childhood, whereas coronaviruses and adenoviruses are less common causes in infancy. Human metapneumovirus and bocaviruses are more recently identified causes of bronchiolitis, and their relative importance as etiologic agents is being determined. RSV remains an important cause of wheezing in adolescents[68] and can cause airway obstruction in middle-aged and elderly individuals.[175]

The role of bacteria as primary agents, synergistically active participants, or secondary invaders in bronchiolitis has interested investigators for many years. No evidence has been found of a primary role for bacteria in bronchiolitis of infants.[6,94] The data on synergistic viral-bacterial infections also are unconvincing. Wood and colleagues[189] in 1954 and Sell[149] in 1960 reported results of studies in which bacteriologic and serologic evidence tended to associate *Haemophilus influenzae* infections with bronchiolitis. Some of the children from these studies later were shown serologically to have had RSV infection.[26] Later studies showed evidence of mixed viral-bacterial infections in bronchiolitis[99,121] and other lower respiratory illnesses.[121]

These serologically defined mixed infections were found no more commonly among patients with bronchiolitis than among patients with mild upper respiratory tract disease, whereas RSV was recovered 10 times as often from patients with lower respiratory tract disease as from patients with upper respiratory tract disease alone.[99] A significant role for mixed viral-bacterial infection in bronchiolitis of infancy is unlikely to exist. Bacterial superinfection is an uncommon occurrence in bronchiolitis (at least in developed countries), as is discussed later in the section on complications and prognosis.

The association of asthma and infectious illnesses was appreciated throughout the 20th century. Initial reports tended to relate bacterial agents to wheezing; in 1909, Carmalt-Jones[19] reported improvement of patients with asthma in association with injections of a bacterial vaccine. Throughout the first half of the 20th century, "bacterial allergy" was the main consideration in infection-related wheezing, and controversy raged about the therapeutic merits of bacterial vaccines. Despite widespread use of bacterial vaccines in the treatment of infectious asthma, no evidence exists that bacterial organisms of the normal flora precipitate asthmatic attacks.[64,74,112,113,173] More recent, large retrospective reviews have shown the rarity with which bacterial infection complicates bronchiolitis, even in severe cases.[133,134] Generally, after a diagnosis of RSV or parainfluenza virus infection has been established, antibiotic courses started empirically can be safely stopped.

TABLE 25–1 Infectious Agents Associated with Acute Bronchiolitis

Infectious Agent	Relative Frequency (%)
Respiratory syncytial virus	50
Parainfluenza viruses	25
Type 1	8
Type 2	2
Type 3	15
Adenoviruses	5
Mycoplasma pneumoniae	5
Rhinoviruses	5
Influenza viruses	5
Type A	3
Type B	2
Metapneumovirus	5-10
Enteroviruses	2
Herpes simplex virus	2
Mumps virus	<1

EPIDEMIOLOGY

Bronchiolitis predominantly is a disease of infancy, although an identical disease occurring in older children arbitrarily may be referred to as another diagnostic entity. In a study involving 1148 children, the peak age of incidence was 2 to 6 months, with more than 80 percent of the cases occurring during the first year of life.[126] In a study of families in or near Houston, Texas, rates of RSV infection were 68.8 per 100 children in the first year of life and 82.6 per 100 children in the second year, including many reinfections. Lower respiratory illness was caused by RSV in 22.4 of 100 children in the first year of life. Of all RSV infections

TABLE 25–2 Principal Agents Recovered from Children of Different Ages with Wheezing Precipitated by Infection

	Frequency of Isolation of Each Age Group†			
Agent*	*0-2 years*	*2-5 years*	*5-9 years*	*9-15 years*
Respiratory syncytial virus	++++	+++	++	++
Adenovirus	++	++	+	0
Parainfluenza viruses	++	++	++	++
Rhinoviruses	+	++ to +++	++ to +++	+++
Metapneumovirus	++	+	+	0
Mycoplasma pneumoniae	+	++	+++	++++

**Other agents that more rarely precipitate wheezing include enteroviruses, herpes simplex virus, cytomegalovirus, coronaviruses, influenza viruses, mumps virus, varicella-zoster virus, Bordetella pertussis, and Coxiella burnetii.*
†++++, *very common;* +++, *common;* ++, *occasional;* +, *uncommon;* 0, *unknown.*
Data from references 7, 40, 45, 51, 68, 74, 94, 99, 107, 109, 115, 149.

TABLE 25–3 Attack Rates of Respiratory Syncytial Virus

Age in months [per Welliver] (*n*)	Infections per 100 Child-Years	LRI per 100 Child-Years	LRI per 100 Infections
0-12 (125)	68.8	22.4	32.6
13-24 (92)	82.6	13	15.8
25-36 (65)	46.2	10.8	23.3
37-48 (39)	33.3	7.7	23.1

LRI, lower respiratory tract infections.
From Glezen, W. P., Taber, L.H., Frank, A. L., et al.: Risk of primary infection and reinfection with respiratory syncytial virus. Am. J. Dis. Child. 140:543-546, 1986.

occurring in children younger than 12 months of age, one third were accompanied by lower respiratory illness. Although the attack rate of RSV decreased with age, the frequency with which lower respiratory disease occurred among individuals infected remained constant (Table 25–3), at least until they reached 4 years of age.[53] In Tucson, Arizona, a study of 1179 children enrolled in a health maintenance organization found that the rate of lower respiratory illness in the first year of life was 32.9 episodes per 100 children; 60 percent of these episodes were diagnosed as bronchiolitis.[192]

Studies in the Washington, D.C., area[84] and in North Carolina[68] estimated the risk of hospitalization for bronchiolitis among infants 0 to 12 months old to be 10 per 1000. A combined study involving 10 centers in Great Britain[28] concluded that the frequency of hospitalization for lower respiratory tract disease (predominantly wheezing) caused by RSV was 1 per 114 infants younger than 1 year of age and 1 per 476 children younger than 5 years. Peak rates of hospitalization were observed for children 1 to 3 months old (1 in 56).

More recent studies suggest that the rate of hospitalization for bronchiolitis may be higher currently than in the above-mentioned older studies. A study of the Tennessee Medicaid population found that the frequency of hospitalization for bronchiolitis was 4.4 and 1.5 per 100 child-years of observation for infants infected in the first and second 6 months of life, respectively.[10] Approximately 123,000 infants younger than 1 year of age are hospitalized annually with bronchiolitis, with RSV infection accounting for 51,000 to 82,000 of these hospitalizations. Including cases diagnosed as pneumonia, RSV infection results in 70,000 to 120,000 hospitalizations.[150]

Between 1979 and 1997, an average of 95 bronchiolitis-associated deaths occurred annually, with 77 percent occurring during the RSV epidemic season. Including fatal cases of bronchiolitis or pneumonia, RSV infection might have resulted in 171 to 510 deaths annually.[151] The yearly incidence of hospitalization for bronchiolitis increased from 12.9 per 1000 infants in 1980 to 31.2 per 1000 in 1996, suggesting an increased severity of illness developing during this interval.[150]

Epidemic bronchiolitis caused by RSV is markedly seasonal in temperate climates, with peak activity occurring from January to April and virtually no activity seen from August to October.[84] Sporadic bronchiolitis cases caused by other agents are seen throughout the year. In their study of 1179 cases, Kim and associates[84] found that 77 percent of the cases of RSV infection occurred between December and June. The lowest incidence occurred in August (2%). Regional differences can be striking. In Miami, RSV activity is year-round, with the maximum number of cases occurring in September and October. Epidemics usually begin in October in other southern states, although still earlier than in the Northeast. Further north (Winnipeg, Canada, and Alaska), the duration of the RSV season again becomes longer, and cases occur essentially year-round. Near the equator, temperature and humidity are positively associated with the number of bronchiolitis cases. In contrast, further from the equator (in either direction), temperature and humidity are inversely associated with bronchiolitis cases. Ultraviolet radiation is negatively associated with bronchiolitis in several regions.[194]

Bronchiolitis occurs more frequently in boys; the male-to-female ratio is approximately 1.5 : 1.[45] In older children, wheezing attributable to viral and mycoplasmal infections also occurs more frequently in boys (male-to-female ratio 1.35 : 1) until they reach 9 years of age, when the incidence becomes equal for the sexes.[68] Crowding may be a major determinant of hospitalization rates for lower respiratory illness caused by RSV, with the incidence of hospitalization for infants 1 to 3 months old residing in rural, urban, and heavily industrialized areas of Great Britain being 1 in 80, 1 in 60, and 1 in 40, respectively. The figures for all children younger than 5 years of age were 1 in 714, 1 in 588, and 1 in 227, respectively.[28] Studies from Tucson, Arizona, indicate that many socioeconomic factors, including absence of breast-feeding, low level of maternal education, and exposure to cigarette smoke, are associated with an increased risk of developing lower respiratory infection at the time of infection with RSV. The greatest risk was conferred by sharing sleeping quarters with two or more individuals.[192]

CLINICAL PRESENTATION

Acute bronchiolitis occurs most commonly in infants 1 to 12 months of age. In most instances, the patient's history reveals exposure to an adult or older child with a common cold or other trivial respiratory infection. Occasionally, as in the daycare setting, the child is exposed to other children with more marked respiratory illness. After exposure, the incubation period is approximately 5 to 7 days. The initial signs include copious nasal discharge (often serous in the early stage), cough, irritability, poor feeding, and vomiting in some cases. Slightly more than 50 percent of infants have fever, with rectal temperatures ranging from normal to 40.6° C (105.4° F) (mean, 39° C [102° F]).[137] Nasal congestion with tenacious secretions and progressive cough and dyspnea dominate the clinical picture.

Symptoms of upper respiratory infection persist for several days, and the onset of lower respiratory infection usually is precipitous, with the time of the onset of wheezing often being recognizable from the caretaker's description of the illness. The maximum severity of illness generally is attained within 24 to 36 hours of the first signs of lower respiratory illness. Apnea may occur and may be sufficiently severe to require mechanical ventilation.[12]

At the time of hospital admission, all patients have cough and evidence of respiratory distress. The pulse is rapid, and the respiratory rate usually is 40 to 80 breaths/min. The breathing is labored, with flaring of the alae nasae; grunting; abdominal breathing; and supraclavicular, subcostal, and intercostal retractions. The degree of retraction of the lower chest wall may be an accurate indicator of the severity of the illness. Wheezing often is audible without the use of a stethoscope, and the chest is full. Hyperresonance may be detected on percussion, and auscultation generally reveals harsh rhonchi, high-pitched or low-pitched expiratory wheezes, or fine inspiratory rales. Occasionally, wheezing is not audible despite other evidence of airway obstruction. A prolonged expiratory phase of breathing may occur, suggesting the presence of a more severe degree of illness. Cyanosis also occurs in severe cases.

Other findings include a mild conjunctivitis in one third of cases, pharyngitis of varied severity in approximately half of affected infants, and otitis media in 5 to 15 percent of cases. The abdomen frequently appears distended, and the liver and spleen usually are palpable; the organs are not enlarged but are pushed down because of emphysema and the flattened diaphragm.

The duration of the hospital course of bronchiolitis varies. Significant improvement occurred in half of the cases within 2 days in one large study.[1] In the same study, approximately one third of the cases had a gradual course without evidence of clear-cut improvement at any one time; 71 percent of the patients were afebrile by the third hospital day. In another study done more than 25 years later,[54] the average duration of hospitalization was 3.4 days. Longer stays were required for infants with initial oxygen saturations of less than 90 percent and infants younger than 6 weeks at the onset of illness. Reasonable criteria for admission in otherwise healthy infants seem to include hypoxia (i.e., oxygen saturation of <90-92%), age younger than 6 weeks, and a degree of respiratory distress sufficient to reduce fluid intake to inadequate levels. Other criteria include apnea, immunodeficiency, premature birth, and the presence of significant underlying heart or lung disease. Most patients can be discharged from the hospital within 2 to 3 days after admission, although mild wheezing still may be present.

In 121 hospitalized patients, the total white blood cell count was less than 12,500/mm^3 in 74 percent, and in 15 determinations had more than 60 percent neutrophils.[1] In another study, a mean leukocyte count of 16,000/mm^3 was determined for children with lower respiratory tract disease, including bronchiolitis, as was an increased percentage of band form neutrophils compared with that of a control group.[137] As in most infections, eosinophil counts in peripheral blood are reduced at the time of acute RSV infection.[48] Nonetheless, some patients maintain detectable eosinophilia in peripheral blood; these infants may be more likely to develop childhood asthma.[37]

Abnormalities of oxygenation frequently are present. Cyanosis may not be evident, even in the presence of markedly reduced oxygenation.[31,152] The respiratory rate is related inversely to the degree of oxygenation except when respiratory failure is imminent. In mild to moderate cases, carbon dioxide retention does not occur because the alveoli that are functioning can compensate for alveoli that are not ventilated. In severe disease, the blood pH is low, and the $Paco_2$ is elevated.[31,152] The technique of pulse oximetry has obviated the need for arterial blood gas sampling except perhaps in severe cases in which hypercarbia is a concern.

In certain patients, clinical findings (e.g., degree of chest wall retractions, wheezing) often are out of proportion to the degree of hypoxia as measured by pulse oximetry.[116,178] Infants with marked dyspnea must be evaluated carefully because respiratory failure may occur precipitously, despite their reassuring oximetry readings. A more meaningful scoring system to assess severity of illness has been developed.[57]

The radiographic appearance of the chest in bronchiolitis varies considerably.[88] Anteroposterior x-rays may be normal in mild cases. In moderate to severe illness, radiographs often appear exceptionally clear because of hyperinflation. The diaphragms often are flattened or depressed. The costophrenic angle is less acute, and the hilar vascular shadows are stretched. Frequently, areas of atelectasis give the appearance of pneumonitis, although true consolidation is a rare occurrence. The heart usually appears small. On the lateral radiograph, the diaphragm is depressed markedly, and reversal of the normal convexity frequently may be seen. The anteroposterior diameter of the chest is increased.

With recurrent wheezing episodes, the prodrome may be considerably shorter in duration with little or no fever and minimal coryza. In these children, differentiation of infection-induced wheezing from more conventional asthma becomes essentially impossible clinically.[186]

PATHOPHYSIOLOGY

The pathophysiology of bronchiolitis deservedly has been the focus of numerous investigations. Several original theories can be discounted reliably, whereas others warrant further study. Pathologic examinations of the lung in bronchiolitis or human airways in organ culture[70] reveal necrosis of the respiratory epithelium with destruction of the ciliated layer; mononuclear cell invasion of the peribronchial tissues; edema of the submucosa and adventitia; and obstruction of small airways with dense plugs consisting of dying epithelial cells, fibrin, and inflammatory cells. In contrast to asthma, mucin (periodic acid-Schiff positive material) usually is absent in fatal bronchiolitis.[184] An associated interstitial pneumonitis often is present, and patchy areas of atelectasis frequently are noted.[188]

The predominant cell types present in the lung of fatal cases are macrophages and neutrophils. Lymphocytes bearing CD4 (helper cells), CD8 (cytotoxic cells), and CD56 (natural killer cells) antigens are almost absent.[184] Even in surviving infants, CD8 lymphocyte counts are less than 1 percent of the total cells in bronchoalveolar lavage fluids,[41] and cytokines characteristically released by CD4 and CD8 cells (interleukin-2 [IL-2], IL-4, IL-5, IL-13, and interferon-γ) are present in low concentrations in nasopharyngeal and tracheal secretions.[184] Adaptive immune responses seem to be poorly developed in infants with severe forms of bronchiolitis. In contrast, staining for markers of apoptosis (caspase 3 and Fas) is positive in infected epithelial cells,[184] suggesting that recovery from primary infection depends on the antiviral activity of phagocytic cells, induction of apoptosis, and perhaps the activity of other innate immune mechanisms.

Recovery from bronchiolitis apparently is complete histologically,[117,187] although plugging of the airway still was prominent in an infant 5 weeks after having an acute episode of bronchiolitis. This infant had experienced an apparent full clinical recovery before eventually dying of acute pneumococcal pneumonia.[117]

Infants may be particularly prone to the development of severe illness as a result of infection of the small airways for many reasons, including the small diameters of their airways. The infant lung is deficient in collateral alveolar ventilation through the pores of Kohn, which develop only in later life.[100] Atelectatic areas cannot be re-expanded readily. Other studies have shown that the small airways in children younger than age 5 years contribute fivefold to sevenfold more to total airway resistance than do small airways of adults.[72] Viral infections involving small airways in young children are more likely to manifest as serious clinical illnesses than are similar infections in adults. Nonetheless, these abnormalities exist in all infants. In contrast, most infants infected with RSV do not develop lower respiratory illness, which suggests that host or environmental factors may be involved in determining the pathogenesis of RSV infection. Table 25–4 lists environmental factors, and potentially important host factors are described later.

TABLE 25–4 Factors Associated with an Increased Risk and Severity of Bronchiolitis and of Postbronchiolitic Morbidity

Factor	Increase in Frequency	Increase in Severity	Increase in Later Morbidity
Crowding	+++	+++	?
Passive smoking	+++	+++	++
Male gender	+	++	++
Absence of breast-feeding	+	+	?
Family history of asthma	±	±	±
Personal atopy	–	–	+++
Congenitally small airways	++	?	–
Airway hyperreactivity	–	+	++
RSV-specific IgE responses	++	++	++

+++, implies strong relationship; ++, implies moderate relationship; +, implies weak relationship; ±, implies controversial relationship; –, implies no relationship; ?, implies unknown relationship.

RSV, respiratory syncytial virus.

Data from references 16, 28, 52, 60, 68, 89, 97, 102-104, 154, 163, 179, 180, 183, 185, 191-193, 195.

One factor that may predispose to the development of lower respiratory illness on RSV infection is the infecting dose of virus. Infants with greater quantities of RSV in their nasopharyngeal secretions are more likely to exhibit severe illness.[15] In fatal cases, abundant virus is evident in epithelial cells plugging the airway lumen by immunohistochemical staining.[184] The fact that crowding is associated with greater risk of developing lower respiratory infection also suggests the importance of the initial inoculum.[187]

Another risk factor for the development of lower respiratory infection may be the relative diameter (or degree of intrinsic constriction) of the airway. When pulmonary function testing is completed on healthy infants before lower respiratory infection occurs, certain infants have lower air flows in smaller airways (and presumably more narrow airways) than do other infants. When followed prospectively, these infants are more likely to develop wheezing early in life than are infants with better air flow.[102] These abnormalities of lung function no longer are associated with an increased risk of wheezing after the child reaches 3 years of age. Instead, evidence of atopy becomes the principal risk factor for recurrent wheezing.[104]

Studies of adolescents reveal reversibility of their airway narrowing after receiving bronchodilator treatment, indicating the airways were constricted, but not stenotic, in early life.[160] Several studies have shown that the airways of infants are intrinsically more reactive to bronchospastic stimuli than are airways of older children,[92,169] particularly children from families with asthma.[193] How long this increased reactivity persists is unknown, and no study has shown that infants with greater degrees of hereditary airway hyperreactivity are more likely to develop bronchiolitis or recurrent virus-induced wheezing. In later childhood, repeated occurrences of viral infections are necessary to sustain this increased reactivity.[177]

In addition to the hereditary airway hyperreactivity, viruses themselves may induce increases in reactivity. Viral infections that clinically appear to be restricted to the upper respiratory tract in adults and children nonetheless result in transient, increased constrictive responses of the airway to a variety of stimuli, including histamine, irritants, and other agents, and small airway dysfunction.[2,3,9,30,39,93] Whether these minor changes contribute to the airway obstruction observed in bronchiolitis is doubtful, in that they are of much lesser magnitude than is the markedly increased reactivity that is observed in asthmatic individuals after exposure to allergens. These virus-induced changes were observed in all infected individuals, including subjects who did not experience wheezing at the time of the virus infection. If airways already are hyperreactive in infants, on a hereditary basis or as a result of a preceding viral infection, the subsequent stimulus of RSV infection may be sufficient to cause airway obstruction.

Immunologic deficits may be expected to lead to more severe forms of virus-induced respiratory illness. Studies of the antibody response to RSV infection in serum and in respiratory secretions show, however, that the nature of responses generally is similar among patients with bronchiolitis or simple upper respiratory illness alone caused by this agent.[82,181] Antibody-directed cellular cytotoxicity expressed against tissue culture cells infected with RSV also is similar in patients with all forms of illness caused by this agent.[80,147] Although investigators have shown that cells infected with RSV can activate complement through classic and alternative pathways,[159] studies suggest that in vivo activation of the complement cascade occurs with equal frequency among patients with all forms of illness caused by the virus.[81]

The concept that serum IgG antibody to RSV, acquired by vaccination or transplacentally, might sensitize the host has been dispelled by results of studies showing that severe bronchiolitis may occur in the absence of circulating antibody[126] and that titers of maternal antibody correlate with protection against infection caused by the virus.[52,123]

Lymphocyte hypersensitivity has been suggested to play a role in the development of severe bronchiolitis. Original field trials showed that a formalin-inactivated RSV (FI-RSV) vaccine induced humoral and cell-mediated immune responses to the virus. Nonetheless, vaccinated subjects manifested more severe forms of illness than did unvaccinated controls when subjects in each group subsequently were infected naturally.[85] Cell-mediated immune responses to viral antigen were greater among recipients of this FI-RSV vaccine compared with control subjects who previously had experienced natural infection.[86] The resulting disease in vaccine recipients was similar in form to bronchiolitis, and the idea that natural RSV bronchiolitis is a consequence of lymphocyte hypersensitivity to the virus has persisted.

Nonetheless, reasons to doubt that the illness that followed FI-RSV vaccination is the same as that following natural bronchiolitis exist. The two FI-RSV vaccine recipients who died after having subsequent natural infection had numerous lymphocytes and eosinophils present in the lung, which is not the case after natural RSV infection.[85,184] Infants surviving RSV infection after receiving the FI-RSV vaccine had high eosinophil counts in peripheral blood, which also is unusual in natural RSV infection.[48] In addition, cytotoxic T-lymphocyte activity is not detected easily in infants after they have had bronchiolitis,[4] and more recent autopsy studies have shown the virtual absence of CD4 and CD8 antigen-positive cells in the lung.[184] Although the development of cell-mediated cytotoxic antiviral responses may be protecting individuals with milder disease, little reason exists to contend that lymphocyte hyperresponsiveness contributes to severe bronchiolitis.

Immediate hypersensitivity to viral antigens has received much consideration as a potential contributing factor in bronchiolitis. The association of a family history of asthma with the development of bronchiolitis in infancy remains controversial.[89,154,161,195] Production of virus-specific IgE and subsequent release of mediators of bronchoconstriction have been documented, however, in infants hospitalized with bronchiolitis caused by RSV and the parainfluenza viruses.[16,179,182,183] In bronchiolitis caused by RSV, the quantities of virus-specific IgE produced and histamine present in respiratory secretions correlate with the severity of illness, as measured by degree of arterial oxygen tension.[183] Leukotriene C_4 is a product of mast cells and eosinophils primarily, but also epithelial cells and macrophages, and is a potent stimulant of airway smooth muscle constriction and mucus secretion. This mediator is released into the airway in acute bronchiolitis,[176] as is histamine,[183] and histamine is found in increased concentrations in plasma of patients with bronchiolitis.[156] In addition, prostaglandins and metabolites can be detected in secretions and blood after bronchiolitis.[156]

A possible role for eosinophils in the pathogenesis of bronchiolitis is supported by the finding of increased concentrations of eosinophil cationic protein in secretions of infants with bronchiolitis and (among high responders) an overall correlation of concentrations of this protein with the degree of hypoxia.[48,49] Peripheral blood eosinophil counts are depressed during the acute phase of most infectious diseases, including RSV infection. Nonetheless, eosinophil counts in peripheral blood are higher in infants with bronchiolitis than in infants with upper respiratory illness only, particularly in boys.[48] This finding is notable because boys generally have more severe forms of bronchiolitis. A contrasting viewpoint is that eosinophils contribute to the clearance of viruses after infection. This view is supported by the fact that eosinophils contain enzymes with ribonuclease activity, which inactivates RSV.[34,35] IgE-dependent and eosinophil-dependent mechanisms may be important in recovery from viral infections, much as they are in parasitic infections.

The airways of asthmatic individuals are infiltrated by T lymphocytes (predominantly T-helper lymphocytes) and eosinophils. T-helper cells can be classified as type 1 (T_H1), which produce primarily interferon-γ and IL-2, or type 2 (T_H2), which produce IL-4, IL-5, IL-13, and others. This finding is important because IL-4, IL-5, and IL-13 are important factors in promoting IgE synthesis and eosinophil migration. Asthma is thought by some researchers to be a result of a T_H2 bias in the airway. An attractive hypothesis is that RSV infection induces airway obstruction and wheezing by inciting the same type of T_H2 responses as observed in asthma. Interferon-γ and IL-4 are found in respiratory secretions of infants with RSV bronchiolitis, but IL-5 and IL-13 rarely are detectable in serum or secretions,[50,174] suggesting that bronchiolitis is not characterized by a T_H2 lymphocyte bias.

Chemokines are proteins released by airway epithelial cells, inflammatory cells, fibroblasts, and other cell types that are chemotactic for leukocytes. Certain chemokines (e.g., IL-8) are active on a broad variety of leukocytes. Others, such as macrophage inflammatory protein 1-alpha (MIP-1α); monocyte chemotactic protein, types 1 through 4; eotaxin; and regulated on activation, normal T-cell expressed and secreted (RANTES), are more selective for eosinophils, basophils, mast cells, and T lymphocytes. These chemokines represent an alternative to T_H2 lymphocytes in terms of initiating the inflammatory response in asthma and bronchiolitis. Studies suggest that MIP-1α, eotaxin, and monocyte chemotactic protein may be particularly important in the pathogenesis of bronchiolitis because they correlate with the degree of hypoxia observed during acute illness.[50] Abnormalities of chemokine responses, rather than T-helper cell responses, may underlie the pathogenesis of bronchiolitis and represent therapeutic targets.

DIAGNOSIS

DIFFERENTIAL DIAGNOSIS

A tendency exists to attribute all infantile respiratory distress occurring after the immediate newborn period to bronchiolitis. The list of causes of infantile dyspnea consists of conditions that are associated with upper and lower airway obstruction, however. Recognition of upper airway obstructive disease should cause little difficulty because the problem is one of distress with inspiration, rather than air trapping. Illnesses that cause lower airway obstructive disease and diseases that suggest this problem are listed in Table 25–5.

The differential diagnosis of allergic disease causes the most difficulty. Generally, the first episode of allergic respiratory disease, when associated with infection, cannot be separated from bronchiolitis by any objective measures. Anatomic defects such as vascular rings can cause obstruction of the airway at many locations; inspiratory or expiratory distress, or a combination, can occur. Frequently, a child with an anatomic defect does not have any detectable difficulty until a trivial respiratory infection occurs, which complicates the diagnostic picture.

Foreign bodies should be considered even in very young infants. Gastroesophageal reflux disease has become recognized as a frequent cause of wheezing in young infants. Because of its obvious therapeutic implications, bacterial pneumonia is the most important differential consideration, although wheezing occurs rarely in association with bacterial pneumonia.[98,99]

TABLE 25–5 Differential Diagnostic Considerations in Acute Bronchiolitis and Infectious Asthma

Allergy
Asthma
Allergic pneumonias (e.g., allergic aspergillosis)
Anatomic cause
Vascular ring, lung cysts, lobar emphysema
Pneumothorax, hydrothorax, chylothorax
Foreign body
Circulatory failure
Congenital and acquired heart disease
Anemia
Nephritis
Infections
Viral, chlamydial, rickettsial, mycoplasmal, bacterial, and fungal pneumonias
Migrating parasites
Irritants
Inhalation of toxic substances (e.g., chlorine gas)
Aspiration pneumonia
Gastroesophageal reflux
Metabolic cause
Poisons (e.g., salicylate)
Acidosis

SPECIFIC DIAGNOSIS

Because bronchiolitis and infectious asthma are clinical diseases with arbitrary boundaries and multiple etiologic agents, outlining a method for establishing specific diagnosis is difficult. When illness is epidemic, RSV usually is the cause. In nonepidemic situations, a careful history and appropriate laboratory studies and radiographs should be considered to exclude the other differential diagnostic possibilities that are listed in Table 25–5.

A specific etiologic diagnosis can be made by the isolation of virus from the nasopharynx. The diagnostic virologic facilities of many medical centers enable the isolation in tissue culture of RSV, parainfluenza viruses, influenza viruses, adenoviruses, rhinoviruses, enteroviruses, and herpesviruses. The use of the shell vial technique has been applied to RSV infection with success.[158] The polymerase chain reaction technique also shows promise in amplification of small quantities of RSV RNA from clinical specimens, although its having superiority to other methods for testing of infants does not seem likely.[128] Rapid detection of viral antigen of RSV directly in nasopharyngeal secretions by commercially available (e.g., enzyme-linked immunosorbent assay) or fluorescent antibody techniques is the method of choice in most laboratories for several reasons.

The accuracy of these techniques often is superior to that of standard cell culture (at least in infants) because antigens remain stable under transport conditions that inactivate live virus. Results also are available several days earlier than by cell culture. Infections with influenza viruses, parainfluenza viruses, and adenoviruses also can be identified by rapid detection techniques, and reliable commercial kits permitting simultaneous testing for each of these agents are the standard in most laboratories.

TREATMENT

The cornerstone of therapy for bronchiolitis is the administration of oxygen because sicker patients usually are hypoxemic.[36,152] Oxygen saturation should be maintained at 92 percent or higher. Usually, an oxygen concentration of 30 percent or 40 percent can achieve this goal, and the patient's respiratory status can be monitored by pulse oximetry; however, blood gas determinations should be obtained when indicated by clinical findings (e.g., cyanosis, agitation). A common practice is to place children with bronchiolitis in tents and administer mist vigorously. Although oxygen should be humidified, the use of mists and aerosols except to deliver specific antiviral therapy is discouraged; mist can act as

an irritant, causing reflex bronchoconstriction, and the water usually does not reach the lower airways.[5]

Although dehydration is a potential problem in children with bronchiolitis because of vomiting and lack of intake, care must be taken not to overhydrate such patients. Because edema is an important part of the pathology of bronchiolitis, excess water may contribute to obstruction of the airway.

Beta-adrenergic bronchodilators have been used frequently in bronchiolitis. Slight improvements in air flow, in oxygenation, and in clinical illness scores have been reported,[63,87,91,143,144] but these effects are not likely to be meaningful clinically; hospitalization is not prevented, and hospital stays are not shortened.[33,43] Improvements observed after the administration of the first dose of these compounds usually are not observed after administration of subsequent doses. In one study, oral and aerosolized beta-adrenergic agents were compared with inactive substances given by the corresponding route. The degree of improvement was the same in all four groups.[47] Combinations of salbutamol and dexamethasone also have no objective effect.[167]

In the past, epinephrine often was administered subcutaneously to older infants in an attempt to differentiate allergic disease from true bronchiolitis. This approach rarely was successful in differentiating one illness from the other or in improving the condition of the patient. Studies have suggested, however, that use of aerosolized, racemic epinephrine produced greater improvement than the use of beta-adrenergic aerosols alone.[144] Other studies of alpha-adrenergic agents have resulted in the same conclusion. Generally, the minor improvements in pulmonary function obtained using alpha-adrenergic agents or racemic epinephrine are not clinically meaningful; the mortality rate is not improved, and the duration of stay in the intensive care unit or in the hospital is not reduced.

Aerosolized sympathomimetic drugs are used frequently in older children with wheezing presumed to be caused by viral infections, with better results than those in infants.[77,91,129,143] Nonetheless, no evidence exists that this form of therapy reduces the need for hospitalization of these patients. Aerosols of ipratropium also have been administered in bronchiolitis without observable benefit.[148] Possible explanations for poor responses to bronchodilators include a paucity of beta-adrenergic receptors in infancy[139]; decreased amounts of smooth muscle capable of responding to bronchodilators surrounding the terminal airways[136]; the nature of the airway obstruction itself (intense plugging with cellular debris)[184,188]; and, presumably, persistence of virus in the airway, stimulating continued inflammation or release of bronchoconstrictive mediators, or both.

Trials of alpha-adrenergic or beta-adrenergic aerosols may be attempted in seriously ill patients,[13,36] although persistence with either of these approaches in the absence of an initial response may prove harmful.[77,124] Most infants hospitalized with bronchiolitis recover quickly with no other intervention than supplemental oxygen and fluid replacement as necessary. A child treated for croup with multiple doses of racemic epinephrine developed ventricular tachycardia and a small myocardial infarction,[18] emphasizing the need to avoid the careless use of these compounds in viral respiratory infections.

Corticosteroids have been employed repeatedly in treating bronchiolitis. Controlled studies have failed to reveal any benefit in terms of prevention of hospitalization, reduced duration of hospitalization, reduced need for intubation, or reduced frequency of recurrent wheezing episodes after bronchiolitis.[8,14,31,32,44,90,138] In a child with nonrespiratory indications for the administration of corticosteroids, these drugs need not be withheld because of fear of complications, although the duration of viral shedding may be extended in individuals treated with steroids.

Early experience with the antiviral substance ribavirin in bronchiolitis suggested that mild subjective benefits and improvement in oxygenation occurred after near-continuous aerosol administration of this compound.[60,61,141,165] The degree of improvement in patients treated with ribavirin was not marked. No study has shown whether the administration of ribavirin could prevent deaths, avoid the need for mechanical ventilation, or shorten the duration of hospital stays.[105,112,157] A meta-analysis of studies using ribavirin showed slight benefits in morbidity and mortality rates that were not statistically significant.[135]

Ribavirin is quite expensive and is difficult to use because of the need for prolonged aerosol administration. Although the drug may precipitate in ventilatory circuits of mechanical ventilators, it can be administered safely if filters are used to prevent plugging of the circuits.[124,125] Because ribavirin is teratogenic in rodents, concern has arisen regarding the safety of health care workers exposed to aerosols of the drug.[65,140] Although absorption may occur in hospital workers, the amount absorbed is negligible.

Other approaches to therapy of RSV infection include the use of interferon-α,[27] vitamin A,[120] DNase,[106] and nitric oxide.[127] The results of these studies were negative. Surfactant[83,171] and leukotriene receptor antagonists[155] have been used in very small studies of bronchiolitis or virus-induced wheezing, with positive effects that require confirmation. Preparations containing a very high titer of neutralizing antibody against RSV have been tested in the prevention and treatment of RSV infection in high-risk populations.[57,66,170] The results of the prophylaxis studies were positive, as discussed later. A significant therapeutic effect of these compounds could not be identified after RSV infection was established, however.[101]

Because bronchiolitis is a viral disease, antibiotics are not useful or necessary. In many instances, the radiographic picture suggests pneumonia, and the blood leukocyte count is elevated; the physician feels compelled to administer antibiotics. Most authorities do not use antibiotics in bronchiolitis or discontinue their use after RSV infection is confirmed because bacterial infection of the lung is rare in bronchiolitis even when infiltrates are identified on chest radiographs. In one large study, secondary bacterial infection occurred in no more than 7 (1.2%) of 565 children with RSV infection.[62] Institution of antibiotics should be considered during the course of therapy when a change in illness suggests the possibility of secondary bacterial infection.

A child with bronchiolitis generally is more comfortable in the supine position with the head end of the crib slightly elevated. Infant seats are used frequently, but are not optimal because the child's head tends to fall to the side or forward, which constricts the upper airway. The sitting position also causes a possibly deleterious upward pressure on the diaphragm. If respiratory failure occurs (virtually absent inspiratory breath sounds, severe inspiratory retractions, inability to maintain an oxygen saturation of >90% in 40% ambient oxygen, cyanosis in 40% oxygen, decreased or absent response to painful stimuli, and a $Paco_2$ of ≥65 mm Hg), ventilatory assistance, such as nasotracheal intubation, neuromuscular blockade, and positive-pressure ventilation,[36,124] is indicated.

PREVENTION

The development of a method to prevent RSV infection is a high priority. The initial adverse experience with formalin-inactivated vaccine (described earlier) has nearly prevented further investigation of inactivated vaccines.[85,172] Later, a live, temperature-sensitive vaccine was developed by adapting RSV to grow at low temperatures in cell culture. This attenuated vaccine was designed to grow at the lower temperatures of the upper respiratory tract but be inactive at the higher temperatures in the lung. In initial field trials, the vaccine strain caused febrile respiratory illnesses in seronegative vaccinees but did not replicate adequately in

seropositive subjects.[23] Further trials of temperature-sensitive mutant RSV strains as vaccines showed improved immunogenicity and some protection, at least against rechallenge with the vaccine strain. Nasal congestion that could cause apnea was common in recipients, however.[78] Numerous other vaccine candidates, including RSV nucleic acid vaccines, bovine RSV strains, parainfluenza virus strains bearing RSV antigens, and human RSV strains with gene deletions or given with immunologically active adjuvants, have been developed. Many of them are in early clinical trials, but development of a successful vaccine is not imminent.

In contrast to the largely negative experience with vaccine development, protection against serious illness caused by RSV infection was achieved using a pooled preparation of human serum obtained from donors with very high titers of neutralizing antibody against RSV.[57] This compound, when administered during the RSV season on a monthly basis to infants and young children with a history of birth at less than 32 weeks' gestation or with bronchopulmonary dysplasia, caused a marked reduction in the severity of illness and rate of hospitalization after RSV infection.

These trials have been repeated successfully using a mouse monoclonal antibody against the RSV fusion protein.[170] The antibody is reconstructed so that it has more than 95 percent of the protein structure of a human antibody. This compound is approximately 50 percent effective in preventing RSV-related hospitalization when administered to high-risk infants, and it has received approval for use in infants born prematurely with or without lung disease of premature birth. Separate trials in infants with hemodynamically significant congenital heart disease have shown a similar reduction in the rate of hospitalization for RSV-related illness, and the compound is now approved for use in these infants as well.[42]

COMPLICATIONS AND PROGNOSIS

Virtually all cases of bronchiolitis in healthy children resolve without acute complications. Secondary bacterial infection in bronchiolitis now is a rare occurrence, at least in developed countries. Scott and colleagues[146] identified minor electrocardiographic abnormalities in 2 percent of 188 children with bronchiolitis. Involvement of other organs apparently does not occur.

The overall mortality rate in bronchiolitis is low. Before the modern era of improved ventilatory support, mortality rates ranged from 2 to 5.5 percent.[36,67,71] A multicenter study in Great Britain published in 1978[28] estimated the mortality rate owing to RSV infection in infancy at 0.5 percent. Deaths should occur rarely except among infants with severe underlying cardiac or pulmonary disease; even in these cases, mortality rate should not exceed 1 percent of cases.[42] Between 1979 and 1997, an average of 95 bronchiolitis-associated deaths occurred annually in the United States, with 20 percent occurring in infants with underlying heart disease, lung disease, or premature birth.[151]

The association of bronchiolitis with the subsequent development of asthma has long been controversial. Fifty percent of patients with bronchiolitis have recurrent episodes of wheezing, although this figure decreases to approximately 10 percent by adolescence and may not be above that of the general population by this time.[96,97,160] Whether this recurrent wheezing is caused by RSV infection or, alternatively, suggests that RSV infection in early life is an indicator of a tendency toward airway obstruction is still being investigated. Recurrent wheezing after having a case of bronchiolitis could reflect an inherited asthmatic trait. Some studies find a strong correlation between atopic family history and postbronchiolitic wheezing,[38,46,142,186,195] whereas others, particularly studies from Great Britain,[132,153,154] do not. Titers of total serum IgE[130,161] and peripheral blood eosinophil counts[37,103,195] have some predictive value for the development of recurrent wheezing after having bronchiolitis. Two studies showed that peripheral blood eosinophilia during viral infections, particularly RSV bronchiolitis, predicts the development of recurrent wheezing in school-aged children.[37,103] Atopy seems to explain a great deal of the recurrent wheezing that occurs after bronchiolitis.

RSV infection in early infancy could damage the developing airway, rendering the airway more prone to obstruction in later life. Pulmonary function tests performed in former patients with bronchiolitis 12 years after an episode of bronchiolitis reveal an increased frequency of airway hyperreactivity in response to challenge with exercise or chemical agents, increased ratios of residual volume to total lung capacity, and reduced expiratory air flow at low lung volumes.[58,79,132,153,162,163,169,190] Although these abnormalities are observed commonly in individuals with asthma, they could not be explained in several of the previous studies simply by the presence of a personal or family history of atopy.[59,132,154] Some of these retrospective studies showed, however, that a single episode of RSV bronchiolitis was not associated with long-term lung dysfunction; abnormal lung function was observed only if at least two episodes of lower respiratory illness had occurred before the patient had reached age 2 years.[69,177]

Prospective studies show that recurrent wheezing in children through age 3 years after bronchiolitis (but not beyond) is related to abnormalities of lung function that existed before RSV infection occurred.[102] Wheezing persisting beyond age 3 years was related, at least partially, to the atopic status of the host.[103,104] No study has shown a relationship between the severity of the initial bronchiolitis episode and the degree of abnormality of long-term lung function.[58,59,132,153] Personal atopy is responsible for many of the long-term manifestations proposed to be sequelae of bronchiolitis. Some factor other than atopy may determine in part the apparent lung abnormalities seen after a case of bronchiolitis,[96,179,191,192] but no direct evidence indicates that this other factor is the initial episode of RSV infection itself.

RSV infection could induce persistent airway hyperreactivity. Retrospective studies have shown that airway reactivity is greater in individuals 1 decade after having an episode of infantile bronchiolitis than in control populations without a history of bronchiolitis.[58,132] Airway reactivity in all children (even children never experiencing bronchiolitis) is greater than that in adults, however, and it is greatest in children of atopic families and in children exposed to cigarette smoke.[193] Airway hyperreactivity occurring after bronchiolitis is not a reflection of the RSV infection itself. One prospective study found no relationship between RSV bronchiolitis and the degree of airway reactivity at age 2 years.[2]

RSV infection possibly can promote sensitization to allergens. In animal models, critically timed RSV infection can enhance temporarily the degree of airway reactivity induced after sensitization to an allergen. Whether this phenomenon occurs in humans is unknown. The absence of T_H2-like cytokine responses at the time of having RSV bronchiolitis[50] suggests, however, that RSV infection in infancy likely does not promote a persistent atopic state in the host.

RSV infection more likely results in the release of mediators of airway obstruction, such as histamine and leukotrienes, through a mechanism other than T_H2 cytokine responses. Chemokines such as MIP-1α, which cause mast cell and basophil degranulation, are possible candidates. Release of these mediators at the time of having RSV infection may result in wheezing in susceptible individuals. These same individuals may develop wheezing again at the time of having allergen exposure in childhood (particularly if they are atopic), but the relationship of bronchiolitis in infancy and such subsequent childhood wheezing would not be causal, but rather would be based on the underlying susceptibility of the airway to obstruction.

The overall outlook for infantile bronchiolitis generally is excellent. In a follow-up study conducted at the author's institution,[179] severe lung disease was not observed in former bronchiolitis patients. All oxygen saturation levels were greater than 95 percent, and at least some of the air flow obstruction present in patients aged 7 to 9 years was reversible with a single bronchodilator treatment, as was confirmed later.[160] Single episodes of infantile bronchiolitis (in the absence of passive smoke exposure and without recurrent wheezing episodes) have not been associated with abnormalities of lung function or airway hyperreactivity in later childhood.[79,163,177]

The natural history of postbronchiolitic wheezing in childhood is for episodes of wheezing to become progressively milder,[58,97,132,153] and the frequency of postbronchiolitic wheezing eventually decreases to essentially the same rate as that of children who did not experience bronchiolitis in infancy.[69,97,160] Nonetheless, the overall prognosis is not entirely benign, and exposure to noxious environmental elements may result in an accelerated deterioration of lung function in later life.[27,186,190] The combination of respiratory tract illness in early life and subsequent cigarette smoking especially may be harmful.[166] Individuals who develop bronchiolitis in infancy should avoid smoking in later life and occupations that are associated with exposure to respiratory irritants.

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CHAPTER 26

Nonbacterial Pneumonia

Kenneth M. Boyer

Nonbacterial pneumonias remain the most frequent pulmonary infections encountered in pediatrics. In recent years, conjugate vaccines against invasive *Haemophilus influenzae* type b (Hib) and *Streptococcus pneumoniae* infections have decreased significantly the importance of these bacterial pathogens.[33,34] Relatively less progress has been made in creating effective vaccines against the most common nonbacterial pathogens that cause pneumonia. Work is progressing on several attenuated viral vaccines,[128] however, and the recommendations for annual influenza vaccination of children have been broadened considerably.[208]

Numerous terms, based on causes, clinical manifestations, or histologic features, are used for these pulmonary infections. Although the connotations differ, viral pneumonia, viral lower respiratory tract infection, atypical pneumonia, infant pneumonitis, and interstitial pneumonia are encountered frequently. The varied causes, excluding bacteria and fungi, cover a broad taxonomic spectrum. With improvements in microbiologic techniques, the number of known causative agents continues to increase. Defining the origin of these infections once was the province of the epidemiologist and virologist, but a sufficient body of knowledge has accumulated to permit informed diagnostic judgment to be made by practicing physicians and rapid specific diagnosis to be confirmed by clinical microbiology laboratories. Although most nonbacterial pneumonias have a good prognosis, occasionally they are life-threatening. Therapy directed against the causative agent may shorten the duration of the course of illness or may avert serious complications. At times, it is lifesaving. This chapter provides an overview of pneumonia syndromes caused by viruses, mycoplasmas, and chlamydiae, as well as by *Coxiella burnetii* (a rickettsia) and *Pneumocystis jiroveci* (an apicomplexan parasite).

HISTORICAL ASPECTS

The development of systematic bacteriology in the late 19th century led to the widespread consensus that pneumonias were bacterial infections with differences in manifestations (bronchial, lobular, lobar) that primarily were the result of differences in anatomic localization. During the 1918 influenza pandemic, most postmortem examinations of patients with pneumonia revealed numerous bacteria in the lungs. Various different species were identified, not exclusively *H. influenzae*, the organism at that time regarded as the cause of influenza. However, in a few cases in

which no bacteria were found, Goodpasture[89] and Winternitz and colleagues[253] found distinctive histopathologic lesions in the lung that they concluded were induced by a nonbacterial agent. Isolation of influenza A virus by Smith and associates[234] in 1933 confirmed their observations, altered the prevailing bacteriologic and anatomic concepts of pneumonia, and ushered in a new era of etiologic diagnosis of respiratory syndromes.

The first isolation of influenza virus and subsequent isolation of *Chlamydia psittaci* (psittacosis) and *C. burnetii* (Q fever) involved transmission of infection to experimental animals such as ferrets and chick embryos. The development of tissue culture techniques in the 1950s enabled researchers to identify numerous other common respiratory viruses: adenoviruses, parainfluenza viruses, respiratory syncytial virus (RSV), enteroviruses, and rhinoviruses. The major etiologic agent of primary atypical pneumonia had been passed to experimental animals in the 1940s, but it was not identified as a mycoplasma definitively until 1962.[42]

In the 1960s and 1970s, the most valuable studies of nonbacterial respiratory infection in pediatrics were comprehensive longitudinal investigations in which epidemiologic and clinical patterns of illness were defined. These studies established that most lower respiratory tract infections in infants and young children are caused by nonbacterial agents, principally respiratory viruses and *Mycoplasma pneumoniae*.

The attention of current investigators is being directed increasingly at developing methods for rapid diagnosis, chemotherapy, and prevention. However, more refined tissue culture and nucleic acid amplification techniques have enabled researchers to identify numerous new agents, including Sin Nombre hantavirus,[111,245] human metapneumovirus,[247] human bocavirus (HBoV),[3] and several strains of coronavirus, including the virus that caused the global epidemic of severe acute respiratory syndrome (SARS).[56,139,247,256]

ETIOLOGY

Three *Mycoplasma* spp., a single *Rickettsia* sp., 3 *Chlamydia* spp., 1 protozoan parasite, and at least 16 different virus groups have been associated with pneumonia syndromes in children. The overall importance of these agents is not measured simply by their incidence. Some agents, although quite common, generally give rise to mild illness; others, less frequently encountered, characteristically cause serious disease. In Table 26–1, the major agents in various age groups are presented by their overall frequency, their typical degree of severity, and their mode of access to the lung. Although the incidence data are representative of numerous major epidemiologic studies,* one should recall that the proportion of pneumonias of unproven cause in most such studies has been approximately 50 percent. Possible explanations for the high percentage of such cases include the following: a bacterial origin[175]; partial treatment with antimicrobial agents[127]; late collection of viral cultures and sera[71]; suboptimal storage, transport, or cultivation of specimens[83,218]; and as yet unidentified agents. The use of antigen tests for bacterial pathogens and of immunoassays and polymerase chain reaction (PCR) assays for viruses accounts for the higher rates of diagnosis in some studies.[110,127,258]

RSV generally is accepted as the agent found most frequently in pediatric pneumonias, particularly those associated with bronchiolitis.† Although infection with this virus is quite common in all age groups, lower respiratory tract involvement is especially prominent in infancy.

The three parainfluenza viruses (types 1, 2, and 3) are second only to RSV as causes of lower respiratory tract disease in infants and younger children. Parainfluenza virus type 3 is the agent most frequently found in pneumonia[85,86,117,198]; infection with parainfluenza virus types 1 and 2 generally produces laryngotracheitis.

Human metapneumovirus, described in children with upper and lower respiratory tract infection in the Netherlands,[246] is thought to account for 10 percent of otherwise unexplained respiratory infections. The clinical symptoms in infected children resemble those caused by RSV.[188,246]

Influenza A and B viruses are not as prevalent overall as are RSV and parainfluenza viruses, but during periods of epidemic spread, they may become predominant isolates in hospitalized children with lower respiratory tract disease[15,26,81,134,193,197,226] The threat of a pandemic of influenza A, secondary to a recombination event resulting in a "humanized" avian virus, keeps influenza in the forefront of concern for global public health agencies.[81,204]

Adenoviruses commonly are isolated in children with pneumonia* and pertussis syndrome.[25,48,186,190] The overall impact of these viruses in the origin of nonbacterial pneumonia in children probably is somewhat less than that of the aforementioned agents; however, many fatal illnesses have been reported. Their common asymptomatic carriage and potential for endogenous activation by unrelated illnesses can render causation difficult to prove.[68,186] Of the 51 known adenoviruses, types 1, 2, 3, 4, 5, 7, 14, 21, and 35 clearly have been associated with pneumonia.[25,135] In certain aboriginal populations such as the Maori, Native Americans, and Inuit, adenoviruses commonly produce severe infection.[114,140] In military recruits, adenoviruses are second to *M. pneumoniae* as a cause of atypical pneumonia.[54]

Rhinoviruses[15,79,199,215,240] have been associated less frequently with pneumonia, although upper respiratory infection with the multiple serotypes of these organisms is common. Some degree of lower respiratory involvement by rhinoviruses also is indicated by the documented role of these viruses in exacerbations of asthma[176] and bronchitis.[171] Among the enteroviruses, primary viral pneumonia has been documented best with coxsackieviruses A9[146] and B1,[58] although coxsackieviruses A16, B4, and B5 and echoviruses 9, 11, 19, 20, and 22 also have been reported.[42,97,230]

Coronaviruses HCo-OC43 and HCo-229E have been implicated as causes of pneumonia since the 1960s, but until recently, this family of viruses was considered a rare cause of human disease.[132,168] The worldwide epidemic of SARS that occurred in 2002 to 2004 focused new interest on these pathogens and led to a new appreciation of their reservoirs in domestic animals and their potential to cause severe pneumonia and respiratory failure.[56,139] Fortunately, the SARS-CoV, no longer circulating, appears to be unique in its pathogenic potential. Two other strains of coronavirus, HCo-NL63[247] and HCo-HKU1,[256] have been discovered more recently. They appear to be among the less common causes of lower respiratory infections, but their clinical manifestations are similar to those caused by the other common respiratory viruses.[61,212]

The respiratory virus most recently described that can cause pneumonia is HBoV.[3] It is a parvovirus closely related to bovine parvovirus and canine parvovirus. (The "bo" in "bocavirus" derives from "bovine," the "ca" from canine.) It was identified using a novel technique based on amplification of nonspecific viral nucleotide sequences, a method that holds promise for identification of other previously uncultivated human viral pathogens. Because only a few population-based studies have been per-

*See references 37, 38, 42, 47, 52, 53, 69, 70, 71, 83, 87, 88, 118, 122, 129, 145, 149, 152, 155, 158, 180, 183, 187, 194, 196, and 244 (major epidemiologic studies).

†See references 18, 20, 24, 27, 112, 123, 133, 165, 179, 235, and 242 (RSV).

*See references 7, 9,19, 25, 28, 39, 83, 113, 115, 116, 126, 185, 202, 234, and 257 (adenoviruses).

TABLE 26–1 Etiologic Agents in Nonbacterial Pneumonia

Etiologic Agents	Frequency*			Usual Degree of Severity†			Mode of Access to Lung
	0-3 mo	4 mo-5 yr	6-16 yr	0-3 mo	4 mo-5 yr	6-16 yr	
Virus							
Respiratory syncytial virus	+++	++++	+	++	++	++	Respiratory
Human metapneumovirus	+	++	?	++	++	?	Respiratory
Parainfluenza viruses							
Type 1	+	++	+	++	++	+	Respiratory
Type 2	+	+	+	++	++	+	Respiratory
Type 3	++	+++	++	++	++	+	Respiratory
Influenza viruses							
Type A	++	+++	+++	++	++	+	Respiratory
Type B	++	++	+	++	++	+	Respiratory
Adenoviruses‡	+	++	++	+++	++	+	Respiratory
Rhinoviruses§	+	±	+	–	++	+	Respiratory
Enteroviruses¶	+	+	+	++	++	+	Respiratory (hematogenous)
Coronaviruses							
Human¶	+	+	+	–	+	+	Respiratory
Severe acute respiratory syndrome	–	+	+	–	++	+++	Respiratory
Human bocavirus	?	±	+	?	?	?	Respiratory
Measles virus	+	++	++	+++	++	++	Respiratory (hematogenous)
Rubella virus	+	–	–	++	–	–	Hematogenous
Human immunodeficiency virus	+	++	+	++	++	++	Hematogenous
Varicella-zoster virus	+	+	+	+++	+++	+++	Hematogenous (respiratory)
Cytomegalovirus	+++	+	+	++	+++	+++	Hematogenous (respiratory)
Epstein-Barr virus	–	+	++	–	++	+	Hematogenous (respiratory)
Herpes simplex virus	++	+	+	++++	+++	+++	Hematogenous (respiratory)
Mycoplasmas							
Mycoplasma pneumoniae	–	+	++++	–	++	+	Respiratory
Mycoplasma hominis	?	–	–	?	–	–	Respiratory
Ureaplasma urealyticum	?	–	–	?	–	–	Respiratory
Chlamydiae							
Chlamydia pneumoniae	–	+	+++	–	+	+	Respiratory
Chlamydia psittaci	+	+	+	–	++	++	Respiratory
Chlamydia trachomatis	++++	–	–	++	–	–	Respiratory
Rickettsiae							
Coxiella burnetii	–	+	+	–	++	++	Respiratory (hematogenous)
Protozoa							
Pneumocystis jiroveci	+	++	+	+++	+++	+++	Respiratory

*++++, *most frequent;* +++, *frequent;* ++, *infrequent;* +, *rare;* –, *no reported cases;* ?, *uncertain.*
†++++, *often fatal;* +++, *severe;* ++, *usually hospitalized;* +, *home management;* –, *no reported cases;* ?, *uncertain.*
‡*Types 1, 2, 3, 4, 5, 7, 14, and 21.*
§*Ninety or more types known.*
¶*Coxsackieviruses A9, A16, B1, B4, and B5; echoviruses 9, 11, 19, 30, and 22.*
¶*Human coronaviruses.*
Data from references 5, 7, 17, 23, 26, 35, 76, 106, 108, 119, 174, 191, 225, and 240.

formed, the contribution of HBoV to the overall epidemiology of pediatric pneumonia remains uncertain. It has been identified in serum and feces, as well as in respiratory specimens[73] and was found to be a relatively common (19%) finding in a Finnish study of children with asthma exacerbations.[2]

Pneumonia is the most frequent serious complication of measles. Kohn and Koiransky[137] demonstrated, by careful radiographic study, that 55 percent of patients with routine measles cases had pulmonary infiltrates early in the illness, a finding suggesting a viral rather than a bacterial cause. Secondary bacterial pneumonia in measles is caused by common respiratory pathogens: *S. pneumoniae*, *H. influenzae*, *Streptococcus pyogenes*, and *Staphylococcus aureus.* Progressive, fatal, primary measles pneumonia (Hecht giant-cell pneumonia) occurs in immunocompromised patients, particularly those with hematologic malignancy or infection with human immunodeficiency virus type 1 (HIV-1).[138,159,228,229] The typical measles rash often is absent. In some persons immunized with killed measles virus vaccine during the 1960s, unusual nodular pneumonia, along with vasculitis of the distal extremities, developed after infection with wild measles virus. Although no longer seen, atypical measles remains important because its pathogenetic mechanism has implications for the development of new vaccines.[74]

Viruses that may attack the lungs by hematogenous spread include varicella-zoster virus (VZV), Epstein-Barr virus (EBV), rubella virus, cytomegalovirus (CMV), herpes simplex virus (HSV), and HIV. Rubella virus, CMV, and HSV may cause interstitial pneumonia in congenitally or perinatally infected infants.[10,106,255,261] CMV and VZV are causes of life-threatening pneumonia in immunocompromised hosts[120,203,252] Pneumonia has been noted in association with primary EBV infections.[8,62] Pulmonary infiltration also is a component of the fatal X-linked lymphoproliferative syndrome caused by EBV.[211] One of the characteristic features of HIV infection in children is lympho-

cytic interstitial pneumonitis, an indolent but progressive process that occurs in approximately one fourth of children in whom acquired immunodeficiency syndrome (AIDS) develops.[221] Both HIV RNA and EBV DNA have been demonstrated in the lung tissue of affected children.[6] The relative contributions of the two agents to the pathogenesis of lymphocytic interstitial pneumonitis are not understood clearly, although EBV is suspected to be the trigger.[131]

Of the 15 known *Mycoplasma* spp. that infect humans, only *M. pneumoniae* is a well-established cause of pneumonia. In children younger than 2 years of age, infection is a common occurrence, but pneumonia is unusual. In children older than 5 years of age, *M. pneumoniae* is the most common cause of nonbacterial pneumonia.[44,71] Studies have associated genital mycoplasmas, in particular *Ureaplasma urealyticum* and *Mycoplasma hominis*, with congenitally and perinatally acquired pneumonia.[32,107]

Three *Chlamydia* spp. have been associated with pneumonia. *C. psittaci* is the well-recognized cause of psittacosis (ornithosis). *Chlamydia trachomatis*, the established agent of inclusion blennorrhea in neonates, causes a characteristic afebrile pneumonitis syndrome in infants aged 4 to 14 weeks.[17] In urban areas in the United States where the condition first was studied carefully (Chicago, Seattle, San Francisco, and Birmingham, Alabama), it was the most frequent cause of pneumonia in that age group.[57,109] With widespread screening and treatment of pregnant women for *Chlamydia*, the incidence of this condition appears to be decreasing. *Chlamydia pneumoniae* was isolated first in 1965 and was recognized as a cause of pneumonia in 1986.[91] It now is considered to be the second most frequent cause of pneumonia in older children and young adults.[92,223]

Of the rickettsiae, only *C. burnetii* is associated with pneumonia, in the form of Q fever. This infection may be severe, but because of its restricted zoonotic niche in domestic farm animals, it is a rare occurrence in children.

P. jiroveci, a protozoan parasite, is an important cause of pneumonia in compromised hosts,[120,203,252] although its incidence in children receiving chemotherapeutic regimens for malignancy has been reduced dramatically with the use of trimethoprim-sulfamethoxazole (TMP-SMX) prophylaxis.[119] *P. jiroveci* is an established cause of pneumonia in premature and debilitated infants,[77] and, with *C. trachomatis*, CMV, and genital mycoplasmas, it has been associated with the afebrile pneumonitis syndrome of infancy.[57,201,237,238] *P. jiroveci* is the most frequent cause of death in infants with HIV infection, although the incidence is reduced markedly in U.S. populations as a result of early diagnosis and treatment of perinatal HIV infection and by the institution of chemoprophylaxis with TMP-SMX.[216,231]

EPIDEMIOLOGY

The major contributors to the overall epidemiology of nonbacterial pneumonia in children are RSV, parainfluenza viruses, *M. pneumoniae*, and, to a lesser extent, influenza viruses A and B.[53] Because of their brief incubation periods and high degree of communicability, these agents often spread through communities in well-defined waves[83,85] (Fig. 26–1). During intervals between epidemics, RSV, parainfluenza viruses types 1 and 2, and influenza viruses A and B rarely are isolated. Between peaks, *M. pneumoniae* and parainfluenza virus type 3 tend to persist endemically. During seasons of respiratory disease in the colder months, an interference phenomenon has been noted whereby peaks of infection by particular agents seldom occur simultaneously[83] (Fig. 26–2).

Annual incidence rates of childhood pneumonia show a rough inverse correlation with age. These rates range from

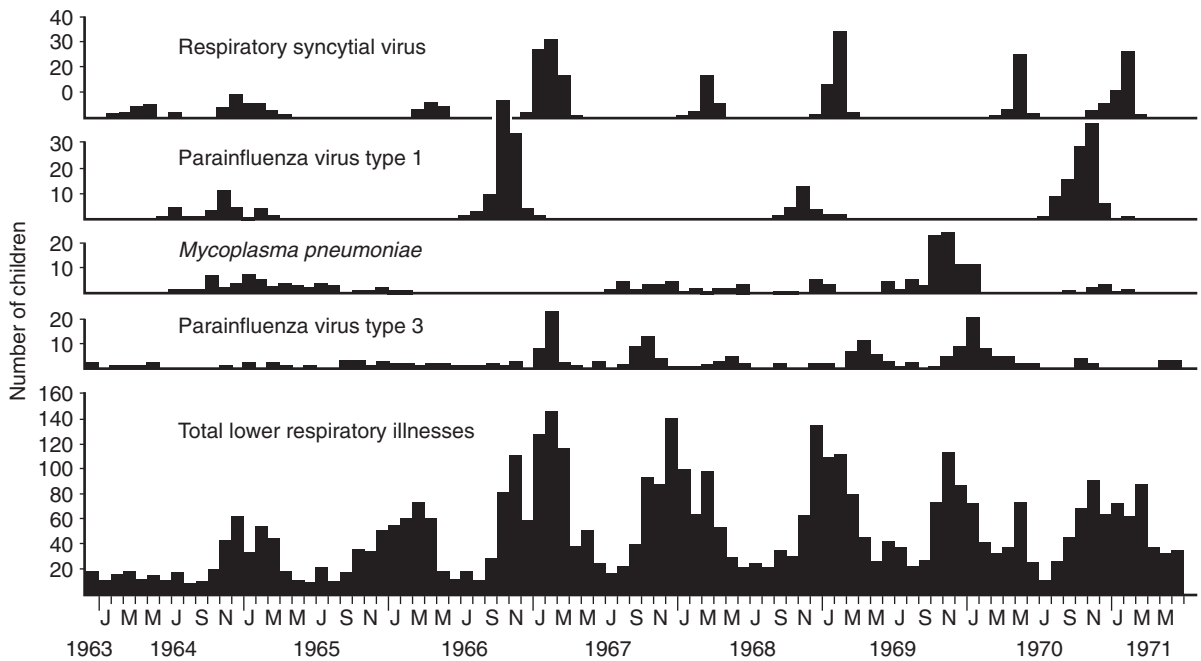

Figure 26-1 Number of isolations, according to month, of four major respiratory pathogens from children with lower respiratory illnesses in Chapel Hill, North Carolina. (*From Glezen, W. P., and Denny, F. W.: Epidemiology of acute lower respiratory disease in children. N. Engl. J. Med.* 288:*500, 1973. Reprinted with permission from the New England Journal of Medicine.*)

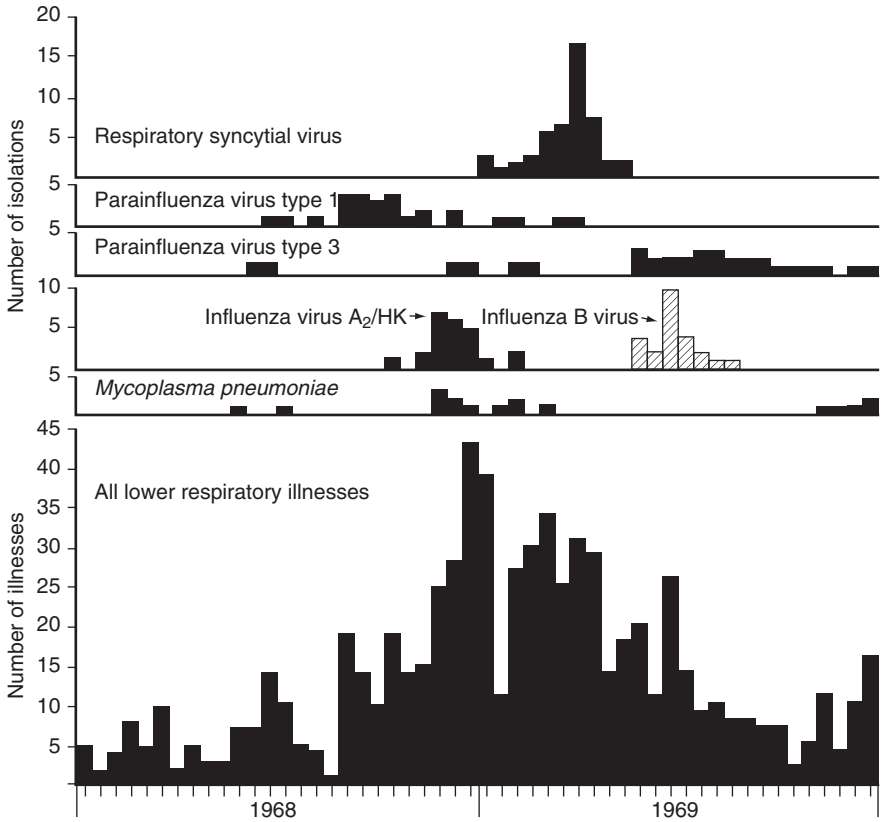

Figure 26-2 Weekly isolations, in 1968 and 1969, of respiratory pathogens from children with lower respiratory illnesses in Chapel Hill, North Carolina. *(From Glezen, W. P., and Denny, F. W.: Epidemiology of acute lower respiratory disease in children. N. Engl. J. Med. 288:500, 1973. Reprinted with permission from the New England Journal of Medicine.)*

40 per 1000 in children younger than 5 years of age to 7 per 1000 in adolescents aged 12 to 15 years.[71,183] RSV is the most common etiologic agent in children younger than 5 years of age; in those older than 5 years, *M. pneumoniae* is the most common.[71] Most studies have shown a male preponderance in pediatric lower respiratory infections on the order of 1.25:1. The increased rates of lower respiratory infection in lower socioeconomic groups correlate best with family size, a reflection of environmental crowding.[83]

Pregnancy, chronic lung disease, valvular heart disease, and neuromuscular conditions in adults predispose them to experiencing greater severity of viral, particularly influenzal, pneumonia.[154] In children, congenital heart disease and bronchopulmonary dysplasia are associated with greater severity of viral pneumonia, especially with RSV.[156] Pulmonary deterioration in patients with cystic fibrosis has been shown to be accelerated by respiratory viral infection.[249] In treated hematologic malignancy, marrow transplantation, and immunosuppressed states, common respiratory viruses have been recognized increasingly as causes of severe pneumonia and respiratory failure.[104,108] Children with these underlying conditions also are prone to developing serious pulmonary infection with such agents as measles virus, VZV, and CMV, which have the capacity for hematogenous dissemination and viral latency. Pediatric HIV infection has added a new category of immunocompromised children susceptible to these pathogens, as well as to the common bacterial agents of pneumonia. Profoundly immunocompromised patients, such as those with severe combined immunodeficiency disease, are prone to acquiring progressive and prolonged pulmonary disease caused by common respiratory viruses.[125]

Transmission of the more common agents of lower respiratory tract disease most often occurs by means of droplet spread from relatively close contact with a source case. Direct inoculation at the alveolar level probably does not occur in most cases because of the extremely small size of aerosolized particles necessary for such transmission to be accomplished. Studies of nosocomially transmitted RSV infection have shown the importance of adults with relatively trivial upper respiratory tract infection as intermediates in transmission to susceptible young infants.[100] School-aged children often introduce respiratory viral agents into households and thus are the source of secondary infections in parents and younger siblings.[83] The increasing use of group daycare by working parents has been associated with enhanced transmission of numerous respiratory pathogens,[151,210] and it certainly has extended the definition of "school age" to increasingly younger children.

PATHOGENESIS AND PATHOLOGY

After the upper respiratory tract has been inoculated, viral agents that cause pneumonia proliferate and spread by contiguity to involve the lower and more distal portions of the respiratory tract. Infected epithelium loses its ciliary appendages, rounds up, and sloughs into the air passages, with subsequent stasis of mucus and accumulation of cellular debris.[1,31,262] When infection extends to the terminal airways, the alveolar lining cells lose their structural integrity. As a result, surfactant production is lost, hyaline membranes form, and pulmonary edema develops. The inflammatory response at the site of tissue damage results in mononuclear infiltration of submucosal and interstitial structures, which further contributes to narrowing of air passages and alveolocapillary block of gas exchange. Relative expiratory obstruction gives rise to hyperinflation and air trapping. Complete obstruction or

stop-valve mechanisms result in atelectasis. Ventilation-perfusion mismatch further exacerbates the hypoxia.

The relative rarity of fatal outcomes in patients with the common causes of nonbacterial pneumonia has resulted in a narrow view of the characteristic pathologic features, limited largely to autopsy studies of overwhelming illness in infants and military recruits. The pathology associated with infection by RSV, adenoviruses, and influenza virus A has been studied most extensively in humans. Studies of the pathology of RSV, parainfluenza virus, and *Mycoplasma* infection have been aided by animal model systems such as mice,[90] the Syrian hamster,[84] and the cotton rat.[95]

Five major pathologic findings have been described in fatal human infections, any or all of which may be present in a given case: acute bronchiolitis, necrotizing bronchiolitis, interstitial pneumonia, alveolar pneumonia, and diffuse alveolar damage. Acute bronchiolitis is characterized by relatively superficial and reversible destruction of ciliated respiratory epithelium, along with accompanying mononuclear infiltration. Necrotizing bronchiolitis extends to the deeper submucosal layers lining the respiratory tract and may not be as readily reversible. It is associated particularly with adenoviral pneumonia.[16] Interstitial pneumonia is a diffuse process in which the inflammatory mononuclear response predominantly involves the peribronchial alveolar septa. In alveolar pneumonia, the alveoli are filled with degenerating lining cells and mononuclear or polymorphonuclear inflammatory cells with or without hyaline membranes. Hyaline membranes consist of fibrin deposits triggered by local release of tissue factor–factor VII complexes, as well as inhibitors of fibrinolysis.[21] When hyaline membranes are present, the process is described as *diffuse alveolar damage*. It is the histopathologic hallmark of the acute phase of the adult respiratory distress syndrome.[219,220] Acute bronchiolitis and interstitial pneumonia are observed in most cases of fatal nonbacterial pneumonia, regardless of cause.[1] Alveolar pneumonia may reflect bacterial superinfection, adult respiratory distress syndrome, or agonal changes associated with intensive ventilator support and oxygen toxicity.

Three important factors that influence the pathologic expression of nonbacterial pneumonia in children are anatomy, preexisting pulmonary disease, and immunity. In young infants, the small caliber of the terminal airways and the absence of interconnections between alveolar spaces (pores of Kohn) contribute to the development of wheezing and lobular atelectasis.[182,254] Preexisting pulmonary disease (e.g., bronchopulmonary dysplasia) is characterized by emphysema, squamous metaplasia of the tracheobronchial tree, hypertrophied goblet cells, and enhanced airway smooth muscle reactivity. An inability to clear the excessive secretions triggered by infection in patients with bronchopulmonary dysplasia often leads to bronchospasm, atelectasis, and respiratory failure.

Immunopathologic mechanisms have been invoked to explain the disparity between infants and older children in the clinical expression of infection by RSV and *M. pneumoniae*.[36,169] Investigators have widely accepted that both viral replication and inappropriately enhanced immune responses contribute to the severity of RSV disease in infants. In a recent study, however, quantities of lymphocyte-derived cytokines were unexpectedly minimal in respiratory secretions, and CD8-positive lymphocytes and natural killer cells were nearly absent in autopsy lung tissue. Thus, it now appears that the severity of RSV in infants relates to inadequate (rather than excessive) adaptive immune responses to robust viral replication and apoptosis of respiratory epithelium.[250] In contrast, cumulative immunity that develops after repeated natural infections with *M. pneumoniae* may account for the more impressive clinical expression of illness in older children and adults.[66] Specific cell-mediated immunity, detectable at low levels in young children but increased in adults, probably contributes to the pathogenesis.

Opportunistic nonbacterial pathogens generally take advantage of the defects in cell-mediated immunity induced by immunosuppressive therapy or HIV infection. Various unique pathologic manifestations of viral pneumonia, including giant-cell pneumonia (leukemia or HIV infection with superimposed measles),[159,228,229] lymphoid interstitial pneumonitis or pulmonary lymphoid hyperplasia (HIV with associated EBV infection),[6,221] and graft-versus-host disease (bone marrow allograft with associated CMV infection), may ensue in these circumstances.[60,241,252]

CLINICAL MANIFESTATIONS

Acute nonbacterial pneumonia in infants and young children generally occurs after 1 or 2 days of coryza, decreased appetite, and low-grade fever. The onset usually is gradual, with increasing fretfulness, respiratory congestion, vomiting, cough, and fever. In very young infants, fever may be minimal, and apneic spells may be the most prominent (and frightening) initial complaint.[29] The most reliable physical findings are those of respiratory distress: tachypnea (respiratory rate =50/min[41]), tachycardia, nasal flaring, and retractions, but without the stridor characteristic of upper airway obstruction. In patients with atelectasis or air trapping, grunting may be present. Cyanosis generally accompanies the apneic spells or coughing attacks, but it may be present at rest in patients with advanced disease or in those with underlying chronic cardiopulmonary conditions.

Other physical findings are quite variable and, in fact, may be normal. Wheezing occurs in infants with associated bronchiolitis or bronchospasm. Hyperresonance may be noted if air trapping is present. Diminished local percussion or breath sounds may indicate lobar consolidation or atelectasis. In interstitial pneumonia, fine crackling rales may be present diffusely or locally. Also important in initial assessment is an evaluation of the young child's state of hydration because increased insensible loss from fever and hyperventilation, coupled with anorexia, can result in significant deficits in fluid.

The afebrile pneumonitis syndrome of young infants, in contrast to the usual acute viral pneumonias affecting this age group, is subacute to chronic in its development and is nonseasonal. Characteristic features include the absence of fever, a "staccato" cough pattern (individual coughs separated by inspirations), and diffuse rales on auscultation.[17] Radiographic findings usually consist of interstitial infiltrates with subsegmental atelectasis. Hypergammaglobulinemia and mild eosinophilia are abnormalities frequently detected in the laboratory.

Most infants with HIV/AIDS and *P. jiroveci* pneumonia have a progressive febrile course leading to respiratory failure over the course of 1 to 3 weeks. Typical upper respiratory symptoms may be absent, and these infants fail to respond to conventional antibiotic therapy. When pneumonia actually is detected by chest radiography, the severity of involvement may not be appreciated because abnormalities often are subtle early in the course. However, even with mild radiographic abnormalities, hypoxia may be obvious and severe. Frequently, the discovery of HIV seropositivity first raises this diagnostic possibility. An important observation is that *P. jiroveci* pneumonia may develop in infants with HIV/AIDS who have CD4 lymphocyte counts in the "normal" range.[231]

Nonbacterial pneumonia in older children and adolescents clinically is more nearly like the pattern seen in adults. Premonitory complaints generally include such systemic symptoms as malaise, myalgia, and anorexia, in addition to upper respiratory symptoms. "Chilliness" may occur, but rigors generally are absent. Cough usually is irritative and nonproductive. A temperature higher than 39° C (102.2° F) is unusual. Although tachypnea, flaring, and retractions typically are present, they may be less apparent than in an infant or young child. Findings on examina-

tion of the chest are more reliable than in infancy and may include local percussion dullness or diminished breath sounds and local or diffuse fine rales. Because apnea rarely occurs in older patients, cyanosis is an ominous sign of advanced disease and respiratory failure. Progression to acute respiratory failure occurred in a disturbingly high proportion of patients with SARS.[143,207]Although mild dehydration often is present, generally it is not evident on examination. Nonspecific rash suggests a viral or mycoplasmal cause.[43]

Radiologic findings in nonbacterial pneumonia are variable and depend on the child's age and the infecting agent.[49,50,160,163,192,217,232] In infants and young children, bilateral air trapping and perihilar infiltrates are the most frequent findings. Patchy areas of consolidation may represent lobular atelectasis or alveolar pneumonia. In older children and adolescents, lobar involvement more frequently is definable, but typically the affected areas are not consolidated completely. Although lobar consolidation may occur in nonbacterial pneumonia, this finding should be distinguished from atelectasis and is more consistent with a bacterial origin. Similarly, although small pleural effusions may be detected on decubitus films in patients with nonbacterial pneumonia,[67] effusions are much more suggestive of a bacterial cause.

Peripheral leukocyte counts are quite variable in nonbacterial pneumonia.[71,187,206] Leukocytosis occurs most frequently in children with influenza and parainfluenza pneumonia,[71] but high counts are more consistent with a bacterial origin.[71,164] Gram staining of sputum or tracheal secretions from patients with nonbacterial pneumonia tends to show epithelial cells as the predominant cell type, with a mixed bacterial population representing the patient's pharyngeal flora. A predominance of neutrophils may be seen, but this finding generally reflects bacterial superinfection or preexisting chronic pulmonary disease.

DIFFERENTIAL DIAGNOSIS

In the differential diagnosis of nonbacterial pneumonia, the following factors need to be considered: status of the host, whether normal or compromised; the environment, whether animate (human and other animal exposure) or inanimate; the age of the patient; and, finally, the season. In certain epidemiologic settings, the diagnosis of nonbacterial pneumonia may be made with relative certainty. Often, however, this category of pulmonary infection is a diagnosis of exclusion. The major conditions to be differentiated include the following: noninfectious pulmonary disease; bacterial pneumonia amenable to conventional antibiotics; and the more unusual bacterial, fungal, or parasitic infections that may require specialized forms of therapy.

Noninfectious conditions that may simulate nonbacterial pneumonia are summarized in Table 26–2. These conditions are particularly relevant to consider in a child with persistent or recurrent pulmonary disease. The line of demarcation between infectious and noninfectious conditions may be blurred. In a child with sickle-cell anemia, for example, pulmonary vascular occlusive crisis is manifested as fever, leukocytosis, and patchy pulmonary infiltrates (the acute chest syndrome).[13,205] Differentiating it from pneumococcal, *Haemophilus*, or mycoplasmal pneumonia, to which a child with sickle-cell anemia has increased susceptibility, may be difficult or impossible. Early recognition of noninfectious conditions either mimicking or underlying pneumonia may prevent recurrence or improve the prognosis. Aspiration resulting from gastroesophageal reflux, for example, is a relatively common correctable cause of recurrent diffuse pneumonia.[45] Early recognition of and initiation of treatment for cystic fibrosis as an underlying condition have clear beneficial effects in reducing irreversible pulmonary damage.[191]

Pneumonias caused by pyogenic bacteria are classically lobar in distribution and exhibit consolidation on radiographs. Atelectasis, conversely, is a common event in viral pneumonia and must be distinguished from true consolidation. Pleural effusions, circular infiltrates, consolidations with convex margins, and pneumatoceles all favor a bacterial cause. In a young child, high fever with significant leukocytosis also suggests a bacterial origin.[166] In contrast to patients with lower counts, children with pneumonia who had leukocyte counts greater than 15,000/mm^3 were found to have a high probability of rapid defervescence with antimicrobial therapy in one emergency department study, presumably on the basis of a bacterial origin.[164]

Other laboratory investigations, such as erythrocyte sedimentation rate, C-reactive protein, and reduction of nitroblue tetrazolium by leukocytes, frequently are positive in children with bacterial respiratory infection.[63,162,189] However, these tests add little to careful initial clinical examination, roentgenographic findings, a differential white count, and, if accessible, Gram stain and culture of tracheal secretions in excluding a bacterial cause.[164] Specific detection techniques for bacterial antigens, used with urine or respiratory secretions, occasionally are helpful. However,

TABLE 26–2 Noninfectious Conditions That May Simulate Nonbacterial Pneumonia in Children

Technical	Damage by Physical Agents	Collagen Disease (SLE, JRA)
Poor inspiratory film	Lipoid pneumonia	Sarcoidosis
Underpenetrated film	Kerosene pneumonia	Neoplasm
Physiologic	Near drowning	Histiocytosis X
Prominent thymus	Smoke inhalation	Bronchogenic cyst
Breast shadows	Iatrogenic pulmonary damage	Vascular ring
Chronic pulmonary disease	Drugs (bleomycin, nitrofurantoin)	Pulmonary sequestration
Asthma	Radiation pneumonitis	Cystic adenomatoid malformation
Bronchiectasis	Graft-versus-host disease	Congenital lobar emphysema
Bronchopulmonary dysplasia	Atelectasis	$Alpha_1$-antitrypsin deficiency
Pulmonary fibrosis	Mucus plug	Allergic alveolitis
Cystic fibrosis	Foreign body	Dust (farmer's lung)
Recurrent aspiration	Congestive heart failure	Mold (allergic aspergillosis)
Gastroesophageal reflux	Pulmonary infarction	Excreta (pigeon breeder's lung)
Tracheoesophageal fistula	Sickle vaso-occlusive crisis	Pulmonary hemosiderosis
Cleft palate	Fat embolism	Desquamative interstitial pneumonitis
Neuromuscular disorders	Pleural effusion	Adult respiratory distress syndrome
Familial dysautonomia	Pleural reaction	

JRA, juvenile rheumatoid arthritis; SLE, systemic lupus erythematosus.

false-negative results occur frequently. Because a bacterial cause cannot be excluded with certainty, these tests seldom are used in clinical laboratories.[122,222]

Positive cultures of blood, pleural fluid, or lung aspirates provide definite evidence of the origin of bacterial pneumonia. Because of the common asymptomatic carriage of potential pulmonary pathogens in children, however, the diagnostic value of upper respiratory tract and tracheal bacterial cultures is debated.[14,86,150] In a child with a suspected nonbacterial pneumonia, the finding of "normal flora" in endotracheal secretions generally is reassuring but may reflect aspiration. In a child who is not doing well, a predominant pathogen in such cultures can be helpful in selecting or altering antimicrobial therapy.[22,42]

Among the less common causes of pneumonia, tuberculosis should never be forgotten. Tuberculin testing should be considered in the initial evaluation and is especially important in children residing in urban areas, in recent immigrants, and in Native Americans. Fungal pneumonia, particularly coccidioidomycosis, histoplasmosis, and blastomycosis, should be considered in children residing in or visiting endemic areas. Often a suggestive history, such as exposure to excavations (backyard swimming pools, geologic or archaeologic "digs"), clean-up chores in old sheds and barns, and exposure to dust storms, may be elicited. Erythema nodosum and eosinophilia are common clinical clues to these entities. Immunodiffusion serologic testing, urine antigen detection, biopsy of skin lesions, and bronchoscopy often are required for establishing the diagnosis. Other fungal pneumonias, such as aspergillosis and cryptococcosis, occur in the setting of immunosuppression. These conditions, coupled with the possibility of *Pneumocystis*, fungal, resistant bacterial, and CMV pneumonia, warrant the use of bronchoalveolar lavage or open lung biopsy as a definitive approach to diagnosis in compromised hosts.[72,255] In an appropriate epidemiologic setting, progressive or recurrent pneumonia should prompt obtaining serologic testing for HIV. At present, pulmonary disease is the most common defining condition in pediatric AIDS.[221] Pulmonary paragonimiasis caused by the lung fluke *Paragonimus westermani* has been recognized as a cause of chronic pneumonia in Indochinese immigrants in the United States.[30]

SPECIFIC DIAGNOSIS

Isolation of virus can be performed in most major medical centers and public health laboratories. With the possible exception of HSV and adenoviruses, respiratory viruses rarely are carried asymptomatically. Thus, identification of an agent in upper respiratory secretions is strong evidence for its causative role in pneumonia. Conventional virologic techniques provide the most sensitive and specific means of identification (see Chapter 264). However, most of the common respiratory viral pathogens, as well as chlamydiae and mycoplasmas, now are identified readily by "rapid" techniques. The available methods and reagents are expanding rapidly and include fluorescent antibody techniques, enzyme-linked immunosorbent assay, direct DNA probes, and PCR.[40,59,78,148,177] Clinical specimens may be tested directly or after pre-incubation in tissue culture systems. In an individual case, serologic diagnosis of acute respiratory viral infection is, in general, less satisfactory than is virologic diagnosis. The difficulty with serology relates to timing of specimens, choice of antigens to test, and variation in the quality and specificity of available reagents.

In contrast, laboratory facilities for the actual isolation of chlamydiae, mycoplasmas, and rickettsiae are less readily available to the clinician. Thus, serologic techniques are important means for establishing a specific diagnosis. Acute-phase reactants (e.g., cold agglutinins in *M. pneumoniae* infection) are of greatest diagnostic help during acute illness but are not invariably present. Serologic tests also can be helpful in diagnosing chlamydial pneumonitis in infants; high levels of immunoglobulin G–specific antibodies are found uniformly at initial evaluation.[17] *Pneumocystis* infection usually is diagnosed by visualizing organisms in specimens obtained by bronchoalveolar lavage during bronchoscopy or by open lung biopsy. Silver impregnation and direct immunofluorescence staining techniques have the greatest sensitivity. Noninvasive diagnosis by serologic and molecular biologic techniques has not proven to be effective.[147,200,238]

Because of the epidemiologic behavior of nonbacterial respiratory infections, a reasonable guess regarding the specific cause often can be based on such factors as age, season, and associated clinical features. If the presence of a particular nonbacterial agent in a community can be established by isolation or serologic means, however, the probability that other patients with similar manifestations will have illness caused by that agent is increased greatly. Regional viral surveillance programs, such as those performed in Rochester, New York,[99] and Houston,[82] can be particularly helpful to practicing pediatricians in this regard.

TREATMENT

Therapy for nonbacterial pneumonia primarily is expectant and supportive. However, specific therapies are available for some of these conditions. Rapid etiologic diagnosis permits appropriate use of these therapies, particularly for hospitalized patients and compromised hosts. Although these treatments shorten the course of illness, they frequently have a less dramatic therapeutic effect than do specific antibiotic therapies for bacterial infections.

The course of uncomplicated viral pneumonia is not influenced by the administration of antibiotics. However, in most cases in which pulmonary involvement is uncovered, antibiotic therapy is administered because bacterial disease cannot be ruled out with certainty, and combined viral-bacterial infection is a common occurrence.[127] In all but the most mild cases, this approach is both reasonable and practical. Of importance, however, is that antibiotic therapy in routine cases be appropriate for the most common bacterial pathogens *(S. pneumoniae* and *H. influenzae).* The increasing prevalence of penicillin-resistant pneumococci now renders treatment with ceftriaxone or cefotaxime a reasonable choice. Concomitant treatment with a macrolide drug such as erythromycin or azithromycin should be considered in children younger than 3 months and older than 6 years because of the high prevalence of chlamydial and mycoplasmal causes.[124,167] In immunocompromised hosts or when secondary infection is a possibility, *S. aureus* (including methicillin-resistant strains) and other hospital-associated and opportunistic pathogens must be considered.[154]

In fulminant viral pneumonia caused by VZV in a compromised host, specific antiviral chemotherapy with acyclovir may be lifesaving, but as many as 50 percent of patients with this condition have complicating bacterial sepsis that requires antibiotic therapy as well.[64] Treatment with zanamivir or oseltamivir should be considered in children with viral pneumonia in the context of a community epidemic of influenza. Treatment of lymphocytic interstitial pneumonia in pediatric patients with AIDS includes antiretroviral therapy with prednisone pulses for increasing hypoxia.[170] Progressive CMV interstitial pneumonia in bone marrow or solid organ transplant recipients is treated best with ganciclovir and intravenous immunoglobulin, ideally with high-titer antibody activity.[60]

TMP-SMX and pentamidine are equally effective for treating *P. jiroveci* pneumonitis, but the former combination is the drug regimen of choice because of its lower toxicity. TMP-SMX may be given orally (20 mg/kg/day of the TMP component divided every 6 hours) or intravenously (15 mg/kg/day divided every 8

hours). Pentamidine should be reserved for patients who are intolerant of TMP-SMX. Atovaquone is a third therapeutic option, pending more complete information on its pharmacokinetic profile in pediatrics. Completion of a course of therapy with any agent should be followed by direct transition to long-term chemoprophylaxis.

Inhalational administration of the antiviral compound ribavirin has been used successfully to treat viral pneumonia caused by RSV and influenza.[80,103,136] Anecdotal experience and in vitro activity suggest that it also may be beneficial in parainfluenza and measles virus infection.[11] Early studies indicated that ribavirin was of particular value for the treatment of RSV infection in nonventilated infants with underlying cardiopulmonary disease,[102] as well as in previously normal infants with RSV pneumonia and respiratory failure.[233] However, several multicenter re-evaluations of the use of ribavirin therapy for RSV-infected infants with and without respiratory failure yielded equivocal results.[142,172,178,251] These observations, coupled with the high cost of the drug,[65] concerns about its possible teratogenicity in medical personnel,[5] and the complexity of its administration during mechanical ventilation,[51] led to reassessment of its use and a change in American Academy of Pediatrics recommendations from "should be used"[5] to "may be considered."[213]

Bronchospasm is present in a substantial proportion of nonbacterial pneumonias. Airway reactivity may be preexisting, or it may arise as part of the pathogenesis of the pneumonia itself. In most hospitals, intervention with inhaled bronchodilators has become part of the routine management of any patient with wheezing, regardless of age or mechanism. Rigorous pulmonary function studies have shown that only approximately 50 percent of previously normal infants with RSV-associated respiratory failure respond to inhaled albuterol.[105] However, in several double-blind controlled trials, inhaled albuterol and racemic epinephrine both showed a statistically significant overall benefit.[173,214,224,227] Other studies of the use of albuterol and ipratropium in mildly ill infants were less convincing.[75,248] None of the available studies of ribavirin therapy for RSV bronchiolitis and pneumonia controlled for the effects of concomitant inhaled bronchodilator therapy.

The use of systemic corticosteroids in nonbacterial pneumonia should be approached with caution. In patients with preexisting asthma or bronchopulmonary dysplasia and acute deterioration triggered by pneumonia, steroids are a noncontroversial element of treatment. In adult patients with AIDS and rapidly progressive *P. jiroveci* pneumonia, short-course steroid therapy clearly is beneficial.[23,76] Comparable data do not exist for pediatric patients with HIV/AIDS. In infants and children without preexisting lung disease, steroid therapy for viral bronchiolitis with or without concomitant pneumonia has shown neither consistent benefit nor harm.[144,236] The value of steroids probably strikes a balance between their anti-inflammatory effects in short-term use and their immunosuppressive effects with more prolonged administration.

Other key elements of supportive therapy include the following: maintenance of adequate hydration, high humidity, and oxygenation and ventilation; mobilization of lower respiratory secretions; and, particularly in young infants, continuous monitoring of respiration. Because of increased insensible loss caused by fever, hyperventilation, and anorexia, mild dehydration frequently is observed initially, and continuing fluid loss is expected during the acute phase of illness. Thus, restoration of deficits and adequate maintenance of fluid intake are desirable. Maintaining nutrition often is difficult when respiratory distress is present; oral feeding is limited or contraindicated. Parenteral nutrition through peripheral veins is adequate during acute self-limited pneumonia. Situations involving more prolonged hospitalization or mechanical ventilation should lead to early consideration of central alimentation.

Mist tents have fallen into disuse because they hinder observation and because mist has little direct therapeutic benefit.[195] However, high humidity is required to prevent the drying effects of supplemental oxygen therapy; slowing evaporation probably also serves to reduce the viscosity of mucous secretions and the magnitude of insensible fluid loss. Mobilization of respiratory secretions by means of vibration and postural drainage is indicated in nonbacterial pneumonias complicated by atelectasis,[153] but it is not helpful in the absence of excessive secretions or mucus plugging.[184] Progression of infiltrates and hypoxia occasionally occurs secondary to pulmonary edema. If it is recognized, diuretic therapy may be beneficial.

Because of the presence of ventilation-perfusion abnormalities and alveolocapillary block, most children with nonbacterial pneumonia have some degree of hypoxemia. In a child with respiratory distress, provision of supplemental oxygen reduces anxiety and ventilation rates. Increases in inspired oxygen to approximately 30 percent are provided easily by nasal cannulas, which are the most convenient means of administration. More severe respiratory distress or cyanosis requires documentation of respiratory status by means of arterial blood gas determination and more exact regulation of inspired oxygen administered by mask or hood. Noninvasive monitoring by means of oximetry can reduce the need for frequent blood gas sampling and arterial lines.[101] Consistent oxygen saturations of 95 percent or more should be the target. In respiratory failure, mechanical ventilation may be required to maintain oxygenation and to control retention of carbon dioxide.[55,161,243] In this instance, management in an intensive care unit setting, with invasive monitoring of gas exchange, is mandated.

Apnea and bradycardia occur commonly in young infants with RSV, parainfluenza, and influenza viral pneumonia.[29,46] These complications are particularly frequent in infants with a history of premature birth. Although the mechanism for these episodes is uncertain, continuous cardiorespiratory monitoring of any young infant with viral pneumonia is prudent.

Acetaminophen or ibuprofen should be used to control high fever and will benefit the patient in terms of comfort as well as reduction in oxygen and nutritional requirements. Expectorants, antihistamines, and cough suppressants, although widely prescribed for upper respiratory tract infections in children and adults, probably have no place in the management of acute nonbacterial pneumonia. In convalescence, persistent irritative coughing that interferes with sleep may be alleviated by the judicious use of antihistamines or dextromethorphan.[4]

PROGNOSIS

In the pre-antibiotic era, pneumonia (of which most cases were "bronchopneumonia") was the most frequent cause of death in children. At present, a fatal outcome rarely occurs but always is a possibility. It is most likely to occur in young infants or compromised hosts.

The incidence of long-term complications of nonbacterial pneumonia is unknown. However, these conditions probably play a role in the development of some cases of bronchiectasis, chronic pulmonary fibrosis, desquamative interstitial pneumonitis, bronchiolitis obliterans, and unilateral hyperlucent lung (Swyer-James syndrome). These complications are well-documented sequelae of measles and adenoviral and influenza viral pneumonia.[130,140,141,157] They occur most frequently today in children who have survived complex and prolonged hospitalization involving aggressive ventilator management of respiratory failure.[219,220]

At a minimum, children with pneumonia should be re-evaluated clinically 2 to 3 weeks after being diagnosed. Provided

the child is asymptomatic, has returned to normal activities, and has a benign physical examination, a follow-up radiograph is not required.[94] Repeated chest radiographs are necessary for children with complicated clinical courses, underlying pulmonary disease, or previous episodes of pneumonia, or if signs or symptoms of respiratory difficulty persist at the time of follow-up. Approximately 20 percent of patients with even uncomplicated pneumonias will show persistent radiographic abnormalities 3 to 4 weeks after the diagnosis has been established, but a selective approach to follow-up films permits early recognition of atelectasis, unresolved infiltrates, or progressive disease.

PREVENTION

Nosocomial spread of respiratory viruses occurs readily in pediatric wards and involves intermediate carriage by medical personnel who have acquired mild upper respiratory tract infections.[98] A reasonable approach to interdicting nosocomial transmission is to group patients with pneumonia and to exclude from ward duties personnel with symptomatic respiratory illness. With the exception of measles, varicella, and SARS, mask or gown isolation has no effect on transmission. Contact precautions, washing hands, and wearing glasses or goggles will minimize the incidence of respiratory infections among personnel and secondary spread. Blood and secretion precautions (universal precautions) are recommended for all hospitalized patients.

Of the common viral causes of pneumonia, vaccines at present are available for only influenza A and B viruses. Annual influenza vaccination with "split-product" vaccines is now recommended for all children aged 6 to 59 months and for children of any age with chronic respiratory, cardiovascular, renal, hepatic, hematologic, or metabolic disorders; with HIV infection; or receiving long-term aspirin therapy. Immunization also is recommended for health care personnel and the household contacts and caregivers of children with predisposing medical conditions.[208] Adenoviral vaccines have been used widely in the military, but their manufacture was discontinued recently. Attenuated, inactivated, and subunit vaccines against RSV, parainfluenza virus type 3, and *M. pneumoniae* have received considerable investigative effort but have not yet proved to be effective.[121,128,181] They present problems in vaccine development because of the possibility of triggering immunopathologic phenomena that could potentiate rather than prevent illness.[169,250]

In compromised hosts, intramuscular passive immunization with pooled immunoglobulin or varicella-zoster immunoglobulin is an established postexposure measure to prevent the acquisition of measles and varicella pneumonia, respectively.[35] Palivizumab is a "humanized" mouse monoclonal antibody specific for the fusion protein of RSV and is administered intramuscularly.[259] It has replaced RSV-specific intravenous immunoglobulin as a means of preventing RSV lower respiratory tract infection in infants with prematurity or other high-risk conditions.[93,209] Other products, such as specific Fab immunoglobulin fragments produced by recombinant DNA technology, are being studied.[12]

P. jiroveci pneumonia can be prevented in pediatric patients with hematologic malignancy or HIV/AIDS by prophylactic administration of TMP-SMX. This medication has become a part of the routine management of these conditions and has reduced the incidence of *P. jiroveci* infection dramatically.[96,119,216] Unless HIV infection can be excluded reasonably by serial PCR studies, seropositive HIV-exposed infants should receive chemoprophylaxis from 4 to 6 weeks of age up to 12 months of age, regardless of their immune status. Infected children aged 1 to 5 years should continue to receive prophylaxis if their CD4 counts are less than 500 cells/μL or the CD4 percentage is less than 15. The criterion for prophylaxis in older children and adults is a CD4 count less than 200 cells/μL or a percentage less than 15. The recommended dosage of TMP-SMX is 150 mg/m^2/day of TMP divided into two doses given on 3 successive days each week.[216]

Opportunistic CMV pneumonia, a major hazard in seronegative high-risk premature infants and recipients of allogeneic bone marrow transplants, can be prevented effectively by the exclusive use of CMV-free blood products.[260,261] In bone marrow transplant recipients who are seropositive and thus are at risk for reactivation of disease, prophylactic acyclovir, ganciclovir, and intravenous immunoglobulin have been shown to reduce rates of infection and interstitial pneumonia.[174,225,241]

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CHAPTER 27

Bacterial Pneumonias

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Bacterial pneumonia is an inflammation of the lung caused by a bacterial pathogen. Pneumonias may be classified in anatomic terms, such as lobar pneumonia, bronchopneumonia, and interstitial pneumonia. The disease usually is categorized by the etiologic agent, however, as in pneumococcal or staphylococcal pneumonia.

HISTORY

Pneumonia has been a frequent and serious human illness throughout recorded history. Histologic examination of Egyptian mummies (1250 to 1000 BCE) revealed hepatization of the lungs compatible with acute pneumococcal pneumonia. The disease was well known to the Greeks and Romans, and the symptoms and management (including a drainage procedure for empyema) were described by Hippocrates. Laennec described the pathologic changes and physical signs of pneumonia and pleurisy in 1819, and Rokitansky distinguished lobar pneumonia from bronchopneumonia in 1842. The early history of pneumonias was reviewed by white.[113a]

In 1881, Pasteur in France and Sternberg in the United States independently isolated, cultured, and described the pneumococcus. Each researcher used inoculation of rabbits with human saliva. Pasteur used saliva from a child who had died of clinical rabies, whereas Sternberg used material from a normal subject. A fatal septicemia resulted in the rabbits, and the organisms were isolated from their blood. In 1882, Friedländer described the pneumococcus in pathologic sections of lung and pleura and in fluid obtained by lung puncture from living patients with pneumonia. In the same laboratory, Gram exposed the sections to a sequence of dyes: aniline-gentian violet, a weak solution of iodine, ethanol, and Bismarck brown. Pairs of elongated cocci retained the dark aniline-gentian violet dye. The organism was referred to as *pneumococcus* by Fraenkel in 1886 because of its role as a cause of pulmonary infection.

Early methods of treating pneumonia included bloodletting; leeching; inhalation of chloroform; subcutaneous injection of gold, silver, and platinum solutions; and oral administration of mercury, quinine, and digitalis. The investigations of the pneumococcus in the late 19th century led to the use in 1891 of small subcutaneous doses of rabbit serum for treatment of patients with pneumonia. These treatments usually failed, but after the many antigenically separable types of the pneumococcus were recognized, specific antisera were prepared. These type-specific sera provided prompt and striking symptomatic improvement and a marked reduction in the fatality rate for pneumococcal pneumonia. Problems arose because of hypersensitivity reactions to the animal sera and the difficulty of making type-specific diagnoses. Responses to these problems included partial elimination of some of the animal protein and adaptation of the Neufeld technique for typing of pneumococci in sputum and body fluids. Use of rabbit antisera resulted in a significant increase in survival rates of patients with pneumonia caused by *Haemophilus influenzae*, and antistreptococcal horse serum or human serum obtained from patients convalescing from scarlet fever was used with success in patients with group A streptococcal pneumonia. Serotherapy was discarded after the introduction of the sulfonamides and penicillin.

Soon after the introduction of the sulfonamides for clinical use in 1935, sulfapyridine was identified as the most potent of the compounds for treatment of pneumococcal disease. By 1943, sulfonamide-resistant strains were reported, however.[101] Abraham and colleagues in 1941[3] and Keefer and colleagues in 1943[56] reported the efficacy of penicillin in treatment of life-threatening infections caused by gram-positive cocci, including *Streptococcus pneumoniae*. Penicillin-resistant pneumococci were identified in epidemic form in South Africa in the 1970s and since have been identified throughout the world.

The approval of a heptavalent conjugate polysaccharide vaccine (Prevnar; Wyeth Lederle Vaccines, Pearl River, NY) by the U.S. Food and Drug Administration (FDA) in February 2000 was the culmination of more than a century of efforts to provide protection for infants, the age group with the highest attack rates for pneumococcal diseases. Whole-cell vaccines had been investigated at the turn of the 20th century and were administered to more than 1 million individuals without serious adverse events, but with uncertain benefit. The importance of capsular polysaccharides to provide serotype-specific antigens was described in the 1930s, and a quadrivalent capsular polysaccharide pneumococcal vaccine was successful in reducing the incidence of serotype-specific pneumonia in military recruits.[63] The results of the military trial led to licensure of a hexavalent capsular polysaccharide vaccine for general use after World War II. The introduction of penicillin and other potent antimicrobials focused physicians' attention on treatment rather than prevention, however, and use of the vaccine lagged. As a result of limited use, the first polysaccharide vaccines were withdrawn in the 1950s.

A 14-valent pneumococcal polysaccharide vaccine was introduced in the United States in 1977, and a 23-valent vaccine was introduced in 1983. Most of the polysaccharides were poor immunogens for children younger than 2 years of age, and the vaccines were used only in children who were 2 years old or older and at risk for contracting invasive pneumococcal infections (e.g., children with sickle-cell disease, functional asplenia, or nephrosis). In October 1990, a conjugate polysaccharide vaccine for *H. influenzae* type b was approved by the FDA. Use of the vaccine has led to a significant decrease in the incidence of invasive disease, including pneumonia caused by *H. influenzae* type b. Similar technology was used to produce a pneumococcal conjugate vaccine. Approval of the pneumococcal conjugate vaccine by the FDA in 2000 resulted in recommendations for universal immunization in infants and selected children aged 2 to 5 years old. By spring 2008, more than 180 million doses of the pneumococcal conjugate vaccine had been distributed worldwide.

Further information about early studies of the pneumococcus and bacterial pneumonias is provided in two reference works of great value that were reprinted in 1979 by the Harvard University Press: *The Biology of the Pneumococcus* by Benjamin White and *Pneumonia* by Roderick Heffron. These works were published first in 1938 (White) and 1939 (Heffron) by the Commonwealth Fund, New York.

Watson and colleagues[112] wrote a brief history of the pneumococcus, highlighting landmarks in infectious disease discovery, including the development of Gram stain, the role of the capsule in resistance to phagocytosis, use of polysaccharides as vaccines, and evidence that DNA encodes genetic information. Podolsky[84] wrote an extensive and insightful review of the therapy of pneumonia before the advent of antimicrobial agents, including a description of early views of treatment of pneumonia by

physiologic support and development of specific pneumococcal serum therapy, concluding with the introduction of the sulfonamides. Of interest are the description of U.S. Public Health Service policy in the 1930s that defined health care as a right and the recognition by states and the federal government that pneumococcal pneumonia was a public health responsibility.[84] Hager[45] wrote a description of the quest for antimicrobials by Domagk and colleagues at the German chemical manufacturer, I. G. Farben, leading to discovery of the sulfonamides, the first successful antimicrobial drug for therapy of bacterial pneumonias. Symposia proceedings have focused on the pneumococcus,[87] polysaccharide pneumococcal vaccines,[55] *H. influenzae*,[30] and lower respiratory tract infections in children in developing countries.[9,35]

MICROBIOLOGY

Because of the difficulty of documenting the microbiology of pneumonia in infants and young children, accurate data concerning the incidence and specific agents of bacterial pneumonia in children are lacking. Austrian[7] estimated that bacteria are responsible for one tenth to one third of all cases of acute pneumonias. Of 540 children with community-acquired pneumonia without empyema diagnosed between 1993 and 1999, only 6.5 percent had a positive blood culture, but 42 percent of children with empyema had a positive blood or pleural fluid culture.[21] A presumptive diagnosis of bacterial or mixed bacterial and viral infection based on antigen detection and antibody assays was made in 45 percent of Finnish children who were hospitalized for lower respiratory tract infections.[75] A reduction of 23 percent of pneumonias in children who received the pneumococcal conjugate vaccine suggests that *S. pneumoniae* is responsible for more than one quarter of pneumonias in infants and children.[64] Bacteriologic findings based on results of lung punctures from 1069 children in developing countries identified *S. pneumoniae*, *H. influenzae*, and *Staphylococcus aureus* as the leading pathogens.[92]

Now, as in the past, *S. pneumoniae* is the leading bacterial cause of pneumonia in all age groups except newborns. *H. influenzae* type b was an important cause of pneumonia in young infants in the United States until the introduction of the heptavalent conjugate polysaccharide vaccine in October 1990. In areas with high rates of immunization, pneumonia and invasive disease caused by *H. influenzae* now are uncommon occurrences. Other species of bacteria are important in special groups: Group B streptococci, *S. aureus*, and some gram-negative enteric bacilli are responsible for pneumonia in newborns; group A streptococci may cause pneumonia in children with viral infections, particularly measles, chickenpox, and influenza; and *S. aureus* and gram-negative enteric bacilli causing pneumonia are a concern in children with malignancy or children who have altered host defense mechanisms. Anaerobic bacteria play significant roles in aspiration pneumonia and lung abscess. Only a few cases of pneumonia caused by *Legionella pneumophila* have been reported in children. Other bacteria responsible for occasional cases of pneumonia include *Neisseria meningitidis*, *Bordetella pertussis*, *Bartonella henselae*,[1] *Bacillus anthracis*, *Salmonella typhi*, *Francisella tularensis*, and *Leptospira* causing leptospirosis (associated with pulmonary hemorrhage).[115]

STREPTOCOCCUS PNEUMONIAE

Although approximately 90 immunologically distinct types of *S. pneumoniae* have been identified on the basis of capsular polysaccharide antigens, relatively few types are responsible for most disease in children. Types 1, 3, 6, 7, 14, 18, 19, and 23 are the types most frequently implicated in pneumonia in children; all types but 1 and 3 are included in the heptavalent pneumococcal conjugate polysaccharide vaccine.

The spectrum of lower respiratory tract illness caused by *S. pneumoniae* ranges from a mild to moderate disease that can be managed without hospitalization to a severe, life-threatening disease that may be complicated by empyema or extrapulmonary manifestations, including meningitis and septic shock. The usual case has a sudden onset, lobar involvement, abrupt termination after appropriate chemotherapy is instituted, and rapid restoration of the involved area of the lung to normal. Although the classic pattern of pneumococcal pneumonia has a lobar distribution, bronchopneumonia and interstitial pneumonia are frequent occurrences.

Multidrug-resistant strains of pneumococci were reported from South Africa[5] in 1977. Some of the strains were highly resistant to penicillin G, requiring more than 4 μg/mL for inhibition, and were resistant to other drugs. The increase in resistance to penicillin has been accompanied by increased resistance to other antimicrobial agents, including other β-lactam drugs, tetracyclines, chloramphenicol, macrolides, clindamycin, trimethoprim-sulfamethoxazole, and other sulfonamides. Resistance of pneumococci to fluoroquinolones is uncommon, and no vancomycin-resistant strains of pneumococci have been reported.[40] The rate of multidrug resistant pneumococci varies in different countries. Incidence is highest in the Far East, with Korea, Taiwan, and Thailand reporting resistance rates of greater than 80 percent. Spain and France have the highest rates of pneumococcal resistance in Europe (30-40%), whereas the Netherlands and Germany have resistance rates of less than 5 percent.

Resistance of *S. pneumoniae* to penicillin is caused by alterations of penicillin-binding proteins. Penicillin resistance is defined by the minimal inhibitory concentrations (MIC) of pneumococci, as follows: less than 0.1 μg/mL = susceptibility; 0.1 μg/mL or higher = nonsusceptibility; among nonsusceptible strains, 0.1 to 1 μg/mL = intermediate status; and 2 μg/mL or greater = high-level resistance. No clinical features distinguish infection caused by a resistant pneumococcal strain, and virulence of infection does not seem to be increased in resistant strains.

Prevalence of multidrug resistance varies by region, with rates highest occurring in the Southeast United States and lowest in the Northeast and Pacific regions. These regional differences are unexplained. Results of surveys of antimicrobial resistance of pneumococcal strains are published frequently; a source of current information is available on the Centers for Disease Control and Prevention (CDC) website (www.cdc.gov). A report[32] of antimicrobial resistance among clinical isolates of *S. pneumoniae* in the United States during 1999 to 2000 revealed 34 percent were nonsusceptible to penicillin, including 21.5 percent with high-level resistance; MICs to all β-lactam antimicrobials increased as penicillin MICs increased. Resistance rates among non–β-lactam agents were 25 percent for macrolides, 9 percent for clindamycin, and 30 percent for trimethoprim-sulfamethoxazole. Resistance to vancomycin was not detected. A trend analysis from the same group extending the surveillance to 2002 to 2003 indicated that the rates of resistance to multiple drugs either have plateaued or have begun to decrease.[33]

The clinical implications of in vitro antibiotic resistance are variable. Daneman and colleagues[29] found high rates of clinical failure among patients with bacteremic pneumococcal disease treated with a macrolide antibiotic to which the organism was resistant; clinical failures were significantly more common among cases of pneumococcal bacteria with isolates having an erythromycin MIC of 1 μg/mL or greater than among isolates exhibiting MICs of less than 0.25 μg/mL. Less certain is the association of penicillin resistance with clinical failure in hospitalized patients treated with a penicillin; results of a study of adults with bacteremic pneumonia caused by penicillin-resistant pneumococci suggested that patients with highly resistant strains (MICs 4-8 μg/mL) failed to respond to a penicillin, whereas patients with

strains with lower MICs did respond.[80] A meta-analysis of 3430 adult patients identified a mortality rate of 19.4 percent in the penicillin-nonsusceptible cases of *S. pneumoniae* pneumonia, in contrast to a 15.7 percent mortality rate in the penicillin-susceptible *S. pneumoniae* group.[102]

HAEMOPHILUS INFLUENZAE

H. influenzae type b accounts for most cases of pneumonia caused by *Haemophilus* spp. Pneumonia caused by *H. influenzae* types a, c, or d is reported rarely, but in a study of serotypes isolated from children with pneumonia in developing countries, types a, c, d, e, or f were responsible for 10 percent of cases.[93] Nontypeable strains are responsible for an uncertain proportion of cases of pneumonia and bronchitis, bronchiectasis, and acute exacerbations of pulmonary disease in patients with cystic fibrosis.

In developing countries, nontypeable strains of *H. influenzae* are important causes of pneumonia. The nontypeable strains gain access to the lung through spread from the upper respiratory tract but are less likely than are type b strains to invade the bloodstream. The diagnosis of nontypeable *H. influenzae* pneumonia in a living child is made only by lung aspiration or blood culture.[93] Of 32 isolates of *H. influenzae* obtained from blood or lung puncture of children with pneumonia in Papua New Guinea, 18 were nontypeable, 8 were types other than b, and 6 were type b; all 6 patients with type b obtained from culture of the lung aspirate also were bacteremic. Only 4 of 18 patients with nontypeable *H. influenzae* in the lung puncture were bacteremic.[94] Of 105 isolates of *Haemophilus* spp. from cultures of blood from children with lower respiratory tract infection in Pakistan, 10 were *Haemophilus parainfluenzae*, 61 were *H. influenzae* type b, and 34 were nontypeable.[113] Similar data were reported in patients with lobar pneumonia in the Gambia.[108]

Nontypeable *H. influenzae* is unlikely to be diagnosed as the cause of pneumonia if microbiologic diagnosis relies on cultures of blood alone. These data also suggest that pneumonia caused by nontypeable *H. influenzae* probably is underdiagnosed in developed and developing countries.

The clinical presentation of pneumonia caused by *H. influenzae* is indistinguishable from that caused by *S. pneumoniae* and includes mild, moderate, and severe disease. In a series of cases in children seen at the Boston City Hospital, 17 children with pneumonia and bacteremia caused by *H. influenzae* type b were identified in a 5-year period; only 4 of the children were judged to be sufficiently ill for admission to a hospital.[68] All 13 children with mild to moderate pneumonia and bacteremia were treated successfully as outpatients. The mild course of pneumonia in these patients contrasts with findings of other investigators who based their reports on the records of children admitted to the hospital.[6,19,53,85] A report of 65 children with *H. influenzae* pneumonia hospitalized at Parkland Memorial Hospital in Dallas during the 14-year period beginning July 1964 included 24 children with pleural effusion, 7 with pneumothorax, and 1 who developed pneumatoceles; 10 children had associated meningitis, and 3 had purulent pericarditis.[42] *H. influenzae* type b was the pathogen cultured most frequently from empyema fluids in children receiving care in Bethesda during the period 1974 to 1987.[19]

Beginning in the 1970s, β-lactamase–producing strains of nontypeable and type b *H. influenzae* were reported throughout the United States.[109] The enzyme cleaves the β-lactam ring of susceptible penicillins, rendering the antibiotic inactive against the enzyme-producing strain. In the United States, 33.5 percent and 41.6 percent of strains of *H. influenzae* in two 1997 surveys were β-lactamase–positive.[52,100] Strains of *H. influenzae* that were β-lactamase–negative but resistant to amoxicillin and amoxicillin-clavulanate also have been identified. A survey in the United States in 1994 and 1995[31] identified 38.9 percent of *H. influenzae* resistant to amoxicillin, including 4.5 percent of strains resistant to amoxicillin-clavulanate (presumably resistant on the basis of a mechanism other than production of β-lactamase).

STAPHYLOCOCCUS AUREUS

Pneumonia and other serious infections caused by *S. aureus* are of particular concern in newborns, patients with altered host defenses, and patients with prior viral respiratory infection (e.g., influenza). In the United States and western Europe, the most severe problem with staphylococcal disease in newborns occurred in the 1950s and ended around 1965. The cyclic appearance and disappearance of virulent strains of *S. aureus* has no satisfactory explanation. The phage type 80/81, which was so devastating in the 1950s, no longer is a major problem in the United States or Europe. Nonetheless, cases of fatal and rapidly progressive staphylococcal pneumonia still occur[78] and are likely to become more frequent occurrences with the widespread increased incidence of community-acquired methicillin-resistant *S. aureus* (CA-MRSA).

The mechanism of β-lactam drug resistance of CA-MRSA is based on the MEC A gene, which confers resistance by encoding a penicillin-binding protein with decreased affinity for β-lactam antibiotics. Although the β-lactam antibiotics act by inhibiting bacterial cell wall synthesis in susceptible organisms, the penicillin-binding protein of CA-MRSA permits cell wall synthesis despite the presence of β-lactam antibiotics. Hospital-acquired MRSA usually is multidrug resistant but susceptible to vancomyin and linezolid. CA-MRSA strains are resistant to β-lactam drugs and macrolides, but they usually are susceptible to clindamycin, trimethoprim-sulfamethoxazole, and some tetracyclines (minocycline and doxycycline). Strains of CA-MRSA (but not hospital-acquired MRSA) carry a gene for a cytolytic toxin, Panton-Valentine leukocidin, which is associated with enhanced inflammatory response and increased necrosis and tissue damage. Although most CA-MRSA infections cause disease of the skin and soft tissues, CA-MRSA was the most frequent cause of pleural empyemas in children in Houston based on a retrospective chart review for the period 1993 to 2002.[91]

In older children, staphylococcal pneumonia may not be differentiated clinically or radiologically from other bacterial pneumonias. In young infants, the course usually is severe; the onset is abrupt, with tachypnea, significant dyspnea, and restlessness. Progression of the disease is rapid, and empyema, abscesses, and pneumatoceles are common findings. Although pneumatoceles are associated with staphylococcal pneumonia, they also may be seen in children with pneumonia caused by *S. pneumoniae*, group A streptococci, and *H. influenzae*. Pneumatoceles may persist for many months but are not a significant cause of morbidity, and they usually require no specific therapy.

GROUP A STREPTOCOCCI

Pneumonia caused by group A streptococci is an uncommon occurrence. Surveys of children hospitalized with pneumonia in Dallas[103] during a 9-year period and in Denver[72] and Chicago[53] during a 5-year period identified only five (Dallas), three (Denver), and two (Chicago) cases caused by group A streptococci. A rare outbreak of group A streptococcal pneumonia occurred among 34 U.S. Marines at a military facility in California.[25] Pneumonia caused by group A streptococci may develop after viral infection, such as influenza, measles, and chickenpox, but it also occurs in children without previous illness. The disease is characterized by necrosis of respiratory tract mucosa and lung tissue with edema and localized hemorrhage. Clinical signs include chills, high and

prolonged fever, dyspnea, and pleuritic chest pain. Patients remain febrile for a mean of 10 days,[103] but patients often remain febrile for 2 to 3 weeks after initiation of therapy with appropriate antibacterial drugs. Bacteremia and pleural effusion are frequent occurrences, and pneumatoceles may occur. The typical pleural effusion begins as a serous fluid, progresses to be serosanguineous, and may become fibrinopurulent.

GROUP B STREPTOCOCCI

Early-onset disease in newborns caused by group B streptococci manifests as a multisystem illness during the first week of life and frequently is characterized by pneumonia, the clinical and radiologic pattern of which simulates respiratory distress syndrome.[2] The pattern of group B streptococci on chest radiograph includes diffuse pulmonary granularity and air bronchograms, similar to the pattern seen in infants with respiratory distress syndrome. Apnea and shock are frequent developments, but pneumonias caused by group B streptococci require lower respiratory pressures on mechanical ventilation than does respiratory distress syndrome. At autopsy, hyaline membranes similar to those seen in infants with respiratory distress syndrome have been observed in the lungs of infants who died of pneumonia caused by group B streptococci, and gram-positive cocci were identified within the hyaline membranes.

ANAEROBIC BACTERIA

Improvements in techniques for isolation and identification of the various genera and species of anaerobic bacteria have provided a better understanding of the anaerobic flora of humans and the roles of these organisms in disease. Anaerobes are present on the skin, in the mouth, in the intestines, and in the genital tract. Anaerobic bacteria may be responsible for pneumonia and lung abscesses in a host who is subject to aspiration. Anaerobic bacteria most commonly responsible for pulmonary infection include *Fusobacterium* spp., *Bacteroides melaninogenicus*, *Bacteroides fragilis*, *Peptococcus*, and *Peptostreptococcus*. The initial lesion of anaerobic infection of the lower respiratory tract is a pneumonitis with a slowly progressive clinical course. Lung abscess and necrotizing pneumonia may be a late consequence of the anaerobic pneumonitis.[12,20]

LEGIONELLA PNEUMOPHILA

In August 1976, 221 cases of respiratory illness caused by an unknown agent occurred among 4500 participants at an American Legion convention in Philadelphia. The disease was marked by high fever, recurrent chills, prominent myalgia, abnormal liver function, and a toxic encephalopathy in addition to respiratory signs. Patients had nonproductive coughs, and their radiologic patterns showed patchy bronchopneumonia, which in some cases progressed to lobar consolidation. Some patients responded promptly to therapy with erythromycin.

Investigators at the CDC isolated small pleomorphic rods from lung tissues taken at autopsy. The rods were stained with silver-impregnation methods and were visualized by direct immunofluorescence; however, they were seen poorly or not at all with Gram stain. The organism was designated *Legionella pneumophila*.

The genus *Legionella* includes aerobic, fastidious, gram-negative rods that require cysteine and some form of iron for growth. Eighteen separable species have been identified. The natural habitat of *L. pneumophila* is aquatic reservoirs, including rivers, lakes, air-conditioning cooling towers, and water distribution systems. Almost all cases of respiratory infection in children have been associated with *L. pneumophila* except for one case caused by *Legionella micdadei* (the Pittsburgh pneumonia agent).[60] Diagnosis is made by culture on buffered yeast extract agar, direct fluorescent antibody staining of respiratory tract secretions, demonstration of antibody by indirect immunofluorescence, and polymerase chain reaction (PCR).

Seroepidemiologic studies suggest that subclinical or minor infections occur in children.[73,88] Prospective studies of children with lower respiratory disease identified few cases caused by *L. pneumophila*, however.[4,79] The development of legionellosis in children with leukemia in relapse,[60] with chronic granulomatous disease,[82] receiving immunosuppressive therapy,[110] or with corticosteroid-induced immunosuppression (a patient with multiple pulmonary abscesses)[74] indicates that the organism should be added to the list of agents that cause pneumonia in immunocompromised children.

NEISSERIA MENINGITIDIS

N. meningitidis usually is associated with asymptomatic carriage in the upper respiratory tract, and pneumonia seldom develops. When pneumonia caused by *N. meningitidis* does occur, it usually is caused by group Y and is accompanied in some cases by bacteremia[51,114] and, rarely, empyema.[43] No distinctive clinical pattern occurs in children, and the diagnosis usually is made by culture of blood.[10,44]

GRAM-NEGATIVE ENTERIC BACILLI

Pneumonia caused by gram-negative enteric bacilli occurs in newborns and children with altered host defense mechanisms but is seen rarely in normal infants and children. Pneumonia caused by *Pseudomonas aeruginosa* and, to a lesser extent, by *Burkholderia cepacia* is a particular problem in children with cystic fibrosis and may occur as a severe, progressive disease leading to a fatal, necrotizing bronchopneumonia. Pneumonia caused by *Klebsiella pneumoniae* is severe, with fever, chills, and a pattern of necrosis and destruction of lung tissue. *Salmonella typhimurium* was cultured by lung aspirate from a specimen of lung in a Malawian child with human immunodeficiency virus (HIV) and lobar pneumonia.[66]

EPIDEMIOLOGY

The respiratory pathogens *S. pneumoniae*, *H. influenzae*, group A streptococci, and *S. aureus* are common inhabitants of the upper respiratory tract. These organisms may be isolated from many healthy children, and it is important to differentiate the many children who are colonized (i.e., multiplication of microorganisms without signs or symptoms of disease and without immune response), children who have asymptomatic or inapparent infection (i.e., multiplication of organisms without signs or symptoms of disease but with immune response), and children with disease (i.e., clinical signs or symptoms that result from multiplication of microorganisms). Colonization may persist for several months. The reason for colonization in some individuals and inapparent infection or disease in others is unknown.

Humans are the only known source for the common bacterial pathogens responsible for respiratory disease. Transmission occurs in most cases by droplet spread—the brief passage of the infectious agent through the air when the source and the patient are near each other (usually within several feet). Spread also occurs during talking or sneezing. The incidence of airborne spread in some cases of staphylococcal infection in children

diminished after introduction of the conjugate pneumococcal vaccine in 2000.[39,98]

Bacterial pneumonia occurs uncommonly in epidemic form in the community, although the incidence of disease increases during periods of epidemic viral infection, as occurs with influenza outbreaks. Legionnaires' disease usually occurs in clusters of cases; most outbreaks have been related to airborne spread from contaminated air-conditioning cooling towers. Hospital-acquired infection may be epidemic (e.g., infections in newborn nurseries during the period of prevalence of virulent strains of *S. aureus*). Common-source outbreaks of pneumonia caused by gram-negative enteric bacilli may result from contaminated aqueous solutions used in humidification equipment.

Pneumonia is of particular concern in developing countries. In 1995, pneumonia caused 2.1 million deaths in children younger than 5 years of age; more children died because of pneumonia than because of acquired immunodeficiency disease (AIDS), malaria, and measles combined.[110] Half of the pneumonias were caused by *S. pneumoniae*.[95] In African countries, rates of pneumonia and invasive pneumococcal disease rates are 10-fold higher than in developed countries.[27] Severe and recurrent pneumonias were associated with development of bronchiectasis in indigenous children living in central Australia.[106]

PATHOGENESIS

The most important factors in development of bacterial pneumonias are the virulence of the pathogen, the absence of specific humoral immunity, and the presence of viral respiratory tract infection. Most bacterial pneumonias are a result of colonization of the nasopharynx, followed by aspiration or inhalation of organisms. The lung is protected from bacterial infection by a variety of mechanisms, including filtration of particles in the nares, prevention of aspiration of infected secretions by the epiglottal reflex, expulsion of aspirated materials by the cough reflex, entrapment and expulsion of organisms by mucus-secreting and ciliated cells, ingestion and killing of bacteria by alveolar macrophages, neutralization of bacteria by local and systemic nonspecific and specific immune substances (i.e., complement, opsonins, and antibodies), and transport of particles from the lung by lymphatic drainage. Pulmonary infection may occur when one or more of these barriers are altered, inhibited, or destroyed. Hematogenous spread to the lung by means of infected emboli arising from a suppurative focus, such as an abscess of the skin or soft tissue caused by *S. aureus*, is an infrequent occurrence.

Animal models suggest that the inflammatory responses in the lung are caused by cell wall components of gram-positive organisms or endotoxins of gram-negative bacteria.[26,104] An increase in cell wall components and endotoxin may occur after antibiotic-caused cell death, with resulting increase in inflammation. The first stage in the healing produced by appropriate antimicrobial drugs may be accompanied by clinical deterioration caused by an early increase in inflammation in the lung.

Pneumonia caused by *S. pneumoniae* begins with acute inflammation and hyperemia of the lower respiratory mucosa, exudation of edema fluid, deposition of fibrin, and infiltration of alveoli by polymorphonuclear leukocytes (i.e., "red hepatization"), followed by predominance of fibrin deposition and macrophage activity (i.e., "white hepatization"). Exudate in the alveoli is digested enzymatically and absorbed or removed by coughing. Resolution then occurs, with return of lung morphology and physiology to normal. In contrast, when the pneumonia is caused by *S. aureus* or *K. pneumoniae*, destruction of tissue and formation of multiple small abscesses frequently occur.

Clinicians have observed that symptoms and signs of minor respiratory infection caused by viruses frequently precede development of bacterial pneumonia. The respiratory virus may act by destruction of respiratory epithelium or up-regulation of bacterial adhesion molecules. Studies in animal models of infection with influenza and reoviruses[58] show a limited period of vulnerability of the lung to bacterial challenge after viral infection. The effects of the viral infection seem to be mediated by alterations in the activity of the alveolar macrophage. A brief period of impaired function of these phagocytic cells results from the viral infection. Staphylococcal[38,69] or pneumococcal pneumonias[36,37] may occur during or shortly after infection caused by influenza virus. Severe pneumococcal pneumonia has been associated with outbreaks of influenza,[77] and increased mortality rates from pneumonia in children occur during epidemics of influenza.[34]

Infections with other respiratory viruses were thought to be uncommon antecedents to bacterial pneumonias.[69] Hall and colleagues[46] found that the risk of secondary bacterial infection developing in infants hospitalized with respiratory syncytial viral infection was low (1.2% of 565 children studied over 9 years). In contrast, the conjugate pneumococcal vaccine was effective in reducing chest x-ray–defined alveolar consolidation associated with respiratory syncytial virus by 12 percent, with parainfluenza types 1 to 3 by 44 percent, and with human metapneumovirus by 40 percent, suggesting that concurrent pneumococcal infection was frequent in virus-associated pneumonias.[64,65] By documenting the decrease of pneumonias in immunized patients, the conjugate pneumococcal vaccine has been valuable in identifying the proportion of pneumonias caused by *S. pneumoniae* and identifying the roles of viral and pneumococcal co-infections.

Anatomic, physiologic, or immune defects predispose patients to single episodes and recurrent lower respiratory tract infection.[83] These defects include congenital anomalies (i.e., cleft palate, tracheoesophageal fistula, or sequestration of lung), congenital or acquired defects in immune function, aspiration (e.g., in children with familial dysautonomia, in a comatose patient, in a child who has a nasogastric feeding tube in place, after seizure, during anesthesia),[67] and alterations in the quality of mucus secretions (e.g., in patients with cystic fibrosis) or impairments in cough or swallowing or mucociliary clearance mechanisms.

Various types of pulmonary infections may develop in children who are being treated with cytotoxic and immunosuppressive drugs for malignancy or for collagen vascular disease or who are recipients of organ transplants. Patients with immune deficits may develop pneumonia caused by aerobic and anaerobic gram-negative bacilli, staphylococci, *Legionella* spp., *Nocardia*, various fungi (including *Aspergillus* and *Candida* spp. and *Pneumocystis carinii*), and viruses such as cytomegalovirus. Some infections in patients with depressed immune response represent reactivation of a latent infection. Newborns can acquire pneumonia by several routes, including transplacental infection, aspiration of organisms present in the birth canal during delivery, and postnatal infection in the nursery or at home from human sources or contaminated equipment or materials.

CLINICAL MANIFESTATIONS

The signs and symptoms of bacterial pneumonia vary with the bacterial pathogen, the age of the patient, and the severity of the disease. Some organisms are associated with a specific pattern of disease, such as the lobar pneumonia of *S. pneumoniae* and the empyema, abscess, and pneumatocele formation caused by *S. aureus*; however, any of these manifestations may result from infection caused by any of the bacterial pathogens. In young infants, signs may be nonspecific, and findings may be sparse on physical examination. Radiologic evidence of pneumonia may be found in infants who appear to have minimal disease or whose signs are more likely to be associated with upper respiratory tract infection. In older children, most cases are mild, and undoubtedly

many cases occur and remain unrecognized because signs of disease do not warrant radiography of the chest. A child with pneumonia who requires hospitalization represents a small but unknown fraction of all children with pneumonia.

Symptoms and signs of pneumonia in children may be classified for convenience into five categories: (1) nonspecific manifestations of infection and toxicity, (2) general signs of lower respiratory tract disease, (3) signs of pneumonia, (4) signs of pleural fluid, and (5) signs of extrapulmonary disease. Nonspecific manifestations of infection and toxicity include fever, headache, malaise, gastrointestinal complaints, restlessness, and apprehension. Rigors may occur and vary from symptoms of chilliness to a sign of teeth-chattering chills.

General signs of lower respiratory tract disease include tachypnea; dyspnea, including shallow or grunting respirations; cough; expectoration of sputum; and flaring of the alae nasae. Because of the importance of tachypnea as a sign of lower respiratory tract disease, reference values for normal patients should be known.[89] Respiratory rates are correlated inversely with age during the first 3 years of life and vary from a median of 47 breaths/min in the first months of life to 38 breaths/min at the end of the first year to 28 breaths/min by 3 years of age. In older children, rates are 15 to 25 breaths/min. Subjects who are asleep have lower respiratory rates than do awake subjects. On the basis of these data, definitions of tachypnea for the purpose of diagnosing lower respiratory tract infection are 50 breaths/min in infants 1 to 11 months old, 40 breaths/min in children 1 to 4 years old, and 30 breaths/min in children 5 years old or older.[59]

Other general signs of pneumonia include a protective position and abdominal findings. The patient may lie on the affected side of the lung with legs drawn up because of chest pain. Abdominal distention may result from gastric dilation because of swallowed air or paralytic ileus. The liver may be displaced downward by the right diaphragm or may be enlarged if congestive heart failure complicates the pneumonia.

Signs of pneumonia may be subtle in young infants. Percussion usually is not valuable in an infant or older child if distribution of the pneumonia is patchy. Dullness to percussion is associated more often in young children with the presence of pleural fluid than with the involvement of the parenchyma of the lung. Auscultatory findings may include rales, but findings are less consistent than in older children. Abnormal findings in older children include dullness to percussion, decreased tactile and vocal fremitus on palpation, and decreased breath sounds and rales over involved areas on auscultation. Intercostal retraction indicates recruitment of accessory muscles, which becomes necessary to assist respiration when significant involvement of the lung is present.

Irritation of the pleura is accompanied by chest pain that may be severe and may limit chest movement. A friction rub may be detected over the involved area of pleura. As the effusion enlarges, dyspnea may increase, but pleuritic pain may diminish and become a dull ache. The pain of pleural irritation may be present at the site of inflammation. If the involved area includes the diaphragm, the pain may be referred to the posterior and lateral neck. Abdominal pain may be so severe as to suggest acute appendicitis. Pleural irritation over the right upper lobe may elicit meningismus, a sign of meningeal irritation without evidence of inflammation. Empyema may extend to involve the mediastinum or pericardium, or it may penetrate the chest wall to manifest as a soft tissue abscess (i.e., empyema necessitatis). Signs of extension of empyema should be sought in a patient who does not respond appropriately to chemotherapy and surgical drainage.

Extrapulmonary infection, including abscesses of the skin and soft tissues, otitis media, sinusitis, and meningitis, may occur concomitantly with bacterial pneumonia. Pericarditis and epiglottitis are particularly likely to be associated with pneumonia caused by *H. influenzae* type b.

DIAGNOSIS

MICROBIOLOGIC DIAGNOSIS

Effective chemotherapy is available to treat all forms of bacterial pneumonia in children. Optimal treatment requires definition of the etiologic agent, however. The physician must differentiate viral or mycoplasmal from bacterial pneumonia; if the agent is bacterial, the probable species must be considered. An effort should be made to obtain adequate materials, including sputum, secretions from the posterior nasopharynx, and blood, for bacteriologic diagnosis. The physician also should consider tracheal aspiration in young children unable to produce sputum, thoracentesis when pleural fluid is present, percutaneous lung aspiration in children who are critically ill, and lung biopsy when tissue diagnosis is important.

Methods for Obtaining Material for Examination and Culture

Sputum usually is not available from children until they are 5 years old; younger children usually swallow their secretions. A Gram-stained smear of sputum is valuable in providing immediate information about the bacterial pathogen; the presence of a significant number of organisms associated with or ingested by polymorphonuclear leukocytes suggests the likely pathogen, whereas the presence of epithelial cells indicates that the material is from the mouth and that further attempts should be made to obtain sputum. The adequacy of the specimen for microbiologic evaluation may be defined by the presence of 10 or more polymorphonuclear cells per low-power field and less than 25 squamous epithelial cells per low-power field.

Secretions from the nasopharynx include organisms that may be responsible for pneumonia, but results of culture of the nasopharynx may be unrevealing or misleading because of the high rate of carriage of bacterial pathogens. Tracheal aspiration through a catheter may be of diagnostic assistance when it is performed with direct laryngoscopy, but it is less valuable when the catheter is passed through the nose or mouth because of contamination with organisms present in the upper respiratory tract. Use of a double-lumen catheter ensures that the specimen remains free of contaminants.

Culture of blood provides specific bacteriologic diagnosis. Bennett and Beeson[14] suggested that most patients with pneumococcal pneumonia have bacteremia at some time during their illness. Reports of pneumococcal pneumonia in adults indicate an incidence of bacteremia of approximately 25 percent.[8,50] Data on the occurrence of bacteremia in children are less well documented. In a study of children in Boston,[99] bacteremia occurred in 8 of 100 consecutive febrile children younger than 2 years of age with radiologic evidence of pneumonia who were seen in a "walk-in" clinic. This and other studies of febrile children seen in clinics for ambulatory children reveal many cases of unsuspected bacteremia in children with pneumonia caused by *S. pneumoniae*,[17] *H. influenzae*,[68] and *N. meningitidis*.[10] The proportion of children with pneumonia who are bacteremic is uncertain, but some data are available from studies of concurrent cultures of blood and lung aspirate. Of 43 Gambian children younger than 10 years of age with pneumonia who had concurrent cultures of blood and lung aspirate, bacterial pathogens were cultured from lung aspirates in 19 children, from blood in 4, and from blood and lung in 10.[108]

The availability of flexible bronchoscopy and bronchoalveolar lavage has added another approach to obtain optimal specimens for microbiologic diagnosis of pneumonia.[13] The technique provides a direct view of bronchial and lung pathology, may provide evidence of endobronchial obstructions, and may identify and remove mucus or mucopurulent plugs. These techniques have

been of particular value in the diagnosis of pulmonary disease in children with AIDS.[15]

Thoracentesis should be considered whenever fluid is present in the pleural space and microbiologic diagnosis is unrevealed by cultures of sputum, blood, or tracheal aspirate. Pleural biopsy should be performed at the time of thoracentesis if tuberculosis or tumor is in the differential diagnosis. The area to be aspirated is defined by physical examination (i.e., the point of maximal dullness), chest radiography, and ultrasonography. Gram or acid-fast stain of the fluid may provide immediate information about the pathogen. Fluid should be sent for culture; cytology; and determination of glucose, protein, and pH.

Aspiration of pulmonary exudate (i.e., lung puncture) can provide direct, specific, and immediate information about the causative agent of pneumonia. The procedure is performed similar to a thoracentesis. Lung puncture should be considered for a child who is critically ill and for whom a specific diagnosis is of immediate importance in guiding antimicrobial therapy, a child whose condition deteriorates after receiving initial therapy and for whom an etiologic agent has not been identified, and a child who has an underlying disease complicating the pneumonia or who is receiving drugs limiting normal host defense mechanisms.[57] A reappraisal of lung puncture in children indicated that a bacteriologic cause was identified in approximately 50 percent of cases and that adverse events were few.[107] Performing open or closed lung biopsy is necessary if tissue diagnosis is important.

Transtracheal aspiration is a safe and useful method of obtaining secretions from the lower respiratory tract of adults with pneumonia who are unable to produce adequate sputum. This method bypasses the mouth flora and permits the investigator to obtain direct culture of tracheal secretions. Transtracheal aspiration has not been used in young children with pneumonia because pediatricians lack experience with the technique and are concerned about the safety of the procedure. Only one report is available; Brook and Finegold[20] used transtracheal aspiration without apparent morbidity to determine the bacteriology of lung abscesses in 10 institutionalized children aged 23 months to 14 years.

Special Methods of Isolation and Identification

Clinical microbiology laboratories have facilities for isolation and identification of aerobic bacterial pathogens associated with pneumonia, but anaerobic bacteria require special techniques. Because many anaerobic bacteria are exquisitely sensitive to oxygen, anaerobic transport media must be provided and special methods must be used to handle materials on arrival in the laboratory.

Identification of bacterial antigens from secretions and body fluids is possible with the use of precipitin reaction, counterimmunoelectrophoresis, latex agglutination, and enzyme-linked immunosorbent assay. Identification of antigen is particularly helpful when prior administration of antimicrobial agents prevents successful isolation of bacteria.

Counterimmunoelectrophoresis of nasopharyngeal secretions may distinguish patients with pneumococcal pneumonia from patients who are carriers of this organism.[24] Detection in urine of polysaccharide antigens of *S. pneumoniae* and *H. influenzae* type b has been used for establishing rapid diagnosis of pneumonia.[18,105] Questions about sensitivity and specificity of the technique need to be answered. Preliminary studies suggest value for PCR for detection of *S. pneumoniae* in whole blood and serum in patients with pneumonia.[90,116] Michelow and colleagues[71] reviewed the diagnosis of lower respiratory infections caused by *S. pneumoniae* by culture, PCR (i.e., whole blood, buffy coat, or plasma), serology, and urinary antigen. A similar study was done by Le Monnier and associates of the microbiologic diagnosis of empyema in children by evaluation of culture, PCR, and pneumococcal antigen detection in pleural fluids.[61]

Laboratory Tests

Elevated white blood cell counts (>15,000 cells/mm^3) frequently, but not invariably, occur in patients with bacterial pneumonia. A white blood cell count less than 5000 cells/mm^3 usually is associated with severe and overwhelming disease. Determinations of erythrocyte sedimentation rate and measurement of C-reactive protein did not distinguish virus from bacterial pneumonia in Finnish children.[78]

The presence of an immune response to infection may be used to document bacterial pneumonia in retrospect. Serologic tests for bacterial pathogens of importance in pneumonia are available only from investigative laboratories.

Chest Radiography

Although the diagnosis of pneumonia may be suggested by clinical signs, pneumonia is defined by chest radiography. In addition to plain radiography, tomography and computed tomography may be used to provide special detail about cavitation, calcification, and patency of central airways.

Radiographic findings may not correlate with clinical signs in young infants. Significant pneumonia may be found by radiography in the absence of clinical signs. Pleural effusion may be identified only with the use of a radiograph taken in the lateral decubitus position. The radiologic pattern may lag behind clinical improvement for weeks to months.

A chest radiograph should be obtained at the conclusion of the illness to determine that the pneumonia has cleared and that no underlying process, such as foreign body, congenital malformation, or residual atelectasis, is present. The precise timing for performing such a study is uncertain, but it should be done when resolution is expected, approximately 4 to 6 weeks after initial signs appear.

To establish uniformity of radiologic diagnosis of pneumonia, the World Health Organization (WHO) created in 2001 the Pneumonia Vaccine Trial Investigator's Group to develop criteria for evaluating chest radiographs. The WHO criteria were used to reinterpret radiographs obtained during the clinical trial of PCV7 in Northern California.[47] The WHO working group established the following criteria:

- *Category 1*. Consolidation/pleural effusion—alveolar consolidation, a dense or fluffy opacity that occupies a portion or whole of a lobe or entire lung and that may or may not contain air bronchograms; or pleural effusion—fluid in the lateral space and not just in the minor or oblique tissue; spatially associated with a pulmonary parenchymal infiltrate or obliterated enough of the hemithorax to obscure an opacity.
- *Category 2*. Interstitial pattern/infiltrate—the presence of linear patchy densities in a lacy pattern involving both lungs and featuring peribronchial thickening and multiple areas of atelectasis.
- *Category 3*. Absence of consolidation/infiltrate/effusion.
- *Category 4*. Radiograph quality insufficient for reading.

Of particular interest in the clinical trial of the heptavalent conjugate pneumococcal vaccine conducted in Northern California (see section on prevention) was the insight obtained in the proportion of radiologically identifiable pneumonias that were caused by the pneumococcus.[16] Children immunized with the conjugate pneumococcal vaccine had approximately one third fewer episodes of radiographically confirmed pneumonia than children who received the control vaccine. Because approximately 80 percent of the pneumococcal types thought to be responsible

for pneumonia in infants were in the vaccine, these data suggest that the pneumococcus was responsible for roughly 40 percent of pneumonias identified by abnormal radiographs. Because lobar pneumonia frequently is caused by the pneumococcus, the 63 percent decrease in radiographically confirmed lobar consolidation in infants who had received the pneumococcal conjugate vaccine was less surprising.

DIFFERENTIAL DIAGNOSIS

The differential diagnosis of bacterial pneumonia includes nonbacterial pneumonias and noninfectious causes of pulmonary disease. During each period of life, certain nonbacterial agents are prominent causes of pneumonia. Pneumonia in neonates may result from congenital infection or infection acquired at the time of delivery because of rubella, toxoplasmosis, herpes simplex infection, cytomegalovirus infection, or syphilis. In infants 2 weeks to 6 months old, *Chlamydia trachomatis* is an important cause of a syndrome of afebrile pneumonia. Throughout childhood, most pneumonias are caused by respiratory viruses, including adenoviruses, influenza viruses, parainfluenza viruses, respiratory syncytial virus, echoviruses, and coxsackieviruses A and B. *Mycoplasma pneumoniae* is an uncommon cause of pneumonia in preschool-aged children, but is an important cause of pneumonia in school-aged children, adolescents, and young adults. Nonbacterial pneumonias that are susceptible to available antimicrobial agents include disease caused by fungi (e.g., histoplasmosis, blastomycosis), *Rickettsia* (i.e., Q fever), and *Chlamydia* (i.e., TWAR agent and psittacosis).

An important consideration is tuberculosis in children with persistent pulmonary disease who do not respond to penicillin or alternatives to penicillin. All children living in areas that have a high risk for tuberculosis should have a tuberculin skin test if admitted to the hospital with a lower respiratory tract infection.

Noninfectious causes of pulmonary lesions include aspiration of gastric contents, aspiration of foreign body, drug reactions, sequestration of lobe, congestive heart failure, atelectasis, sarcoidosis, malignancy or tumor, alveolar proteinosis, pulmonary hemosiderosis, and desquamating interstitial pneumonia.

Bacterial causes of acute empyema include *S. aureus*, *S. pneumoniae*, *H. influenzae*, gram-negative bacilli, and anaerobic bacteria.[19] Bloody exudates (when thoracentesis occurs without trauma) suggest malignancy, infarct of the lung, connective tissue disorder, pancreaticopleural fistula, or tuberculosis.

CLINICAL GUIDELINES IN DEVELOPING COUNTRIES

Acute infections of the lower respiratory tract are the most important cause of death in children younger than 5 years of age in developing countries.[97] The authors of the Programme for the Control of Respiratory Infections of the WHO developed clinical guidelines for case diagnosis of pneumonia for use in developing countries. In these areas, diagnosis and management are provided for children by health care personnel who work in facilities where laboratory and radiologic tests are limited or do not exist.[86] The goals of the program are to simplify diagnosis to the smallest number of readily identifiable signs, to provide a system for classification of the illness, and to define the basis for use of antibacterial agents. The guidelines include assessment of fever, nutrition, lethargy, and color (i.e., presence or absence of cyanosis); measurement of the respiratory rate; observations of chest wall movement to detect retractions; and auscultation for stridor and wheezes.

The guidelines distinguish levels of disease for purposes of management. *Very severe pneumonia* includes central cyanosis and an inability to drink. *Severe pneumonia* includes chest indrawing, without cyanosis, and the ability to drink. *Pneumonia* includes no chest indrawing, but sustained tachypnea (>60 breaths/min for infants <2 months old, >50 breaths/min for children 2 to 12 months old, or >40 breaths/min for children 12 months to 5 years old). *No pneumonia* includes cough in the absence of chest indrawing and tachypnea. Suggested management includes hospitalization and administration of parenteral antibiotics for children with very severe and severe pneumonia, home care and administration of oral antibiotics for children with pneumonia, and no antibiotics but assessment and treatment of other problems for children with cough but without signs of severe respiratory illness.

Simoes and McGrath[96] evaluated the ability of nurses and nursing assistants in Swaziland to recognize pneumonia using the WHO's protocols. Signs of severe disease, including stridor and abnormal sleepiness, often were overlooked, as was audible wheeze, but tachypnea and chest wall retractions were well recognized. In a study of Chinese children, tachypnea (50 cycles/min for infants 2 to 11 months old and 40 cycles/min for children 1 to 5 years old) was a good predictor of radiologically defined pneumonia and was recommended for use by village health care workers in diagnosing pneumonia. Nasal flaring, retractions, stridor, and cyanosis of the tongue had high predictive values but were observed infrequently.[28] A respiratory rate of more than 50 cycles/min or retractions was predictive of pneumonia in children with cough evaluated in Papua New Guinea.[48]

MANAGEMENT

Therapy should be initiated promptly after bacterial pneumonia is diagnosed or strongly suspected. Initial therapy is guided by knowledge of the likely bacterial pathogens in the child's age group because examination of the sputum or tracheal aspirate usually is unavailable in patients younger than school age. The physician must decide whether hospitalization is required for optimal management of the child. Most children with mild to moderate disease can be treated at home. Hospitalization is required for children with severe disease who require hydration, oxygen, or observation; children who are toxic and have a significant degree of pulmonary dysfunction; and children whose families lack the ability to provide therapy and supportive care. Special concern is warranted for infants in the first year of life, when signs of respiratory disease are subtle and disease may progress rapidly.

INITIAL CHOICE OF ANTIMICROBIAL AGENTS BY AGE GROUP

Neonatal Pneumonia

The treatment of neonatal pneumonia is similar to treatment of other severe neonatal infections. Initial therapy must include coverage for gram-positive cocci, particularly group B streptococci, and gram-negative bacilli.

A penicillin is the drug of choice for gram-positive organisms. Penicillin G or ampicillin is used. The latter drug may provide a theoretical advantage because of greater in vitro activity against some enterococci and some gram-negative bacilli, particularly *Escherichia coli* and *Proteus mirabilis*, when used alone or in combination with an aminoglycoside. If the physician has reason to suspect staphylococcal infection, a penicillinase-resistant penicillin is chosen. Infants with pulmonary signs suggestive of severe staphylococcal pneumonia (e.g., abscesses, pneumatoceles, and/or empyema) should be treated with drugs having known efficacy for multidrug-resistant staphylococci, such as vancomycin or linezolid.

Choice of therapy for suspected gram-negative bacillary infection depends on the antibiotic susceptibility pattern for recent

isolates obtained from newborns. An aminoglycoside such as gentamicin has an effective range of in vitro activity. Amikacin and tobramycin have similar activity but may be effective for strains of gram-negative enteric bacilli that are resistant to gentamicin.

Initial therapy is reevaluated when the results of cultures are available. Duration of therapy depends on the causative agent. Pneumonia caused by group B streptococci or gram-negative enteric bacilli is treated for 7 to 10 days; disease caused by *S. aureus* requires 3 to 6 weeks of antimicrobial therapy, according to the severity of the disease.

Pneumonias in Children 1 Month to 10 Years of Age

Most cases of bronchopneumonias in children aged 1 month to 10 years are caused by respiratory viruses. If the initial clinical and radiologic findings are consistent with viral infection, and the child can be observed closely, antimicrobial agents may be withheld pending the results of cultures. *S. pneumoniae* and *H. influenzae* (nontypeable and type b) are the major bacterial agents responsible for bacterial pneumonia in this age group. The rate of disease caused by *H. influenzae* type b is low in children who were immunized previously with the conjugate vaccine. Although multidrug-resistant organisms among pneumococci vary, the concentrations of parenteral ampicillin are sufficient to achieve concentrations in the blood and lung that can inhibit and kill all but the most resistant strains.

If the child is critically ill, vancomycin should be substituted for ampicillin because pneumococci are not resistant to it. Strains of β-lactamase–producing *H. influenzae* resistant to amoxicillin and other susceptible penicillins have been isolated throughout the United States. Nonetheless, amoxicillin still is appropriate initial therapy for a young child with mild disease. If the child is moderately or seriously ill, drugs with efficacy against *S. pneumoniae* and β-lactamase–producing *H. influenzae*, including ampicillin-sulbactam or cephalosporins (i.e., cefuroxime, ceftriaxone, cefotaxime, or ceftazidime), should be administered by parenteral routes.

S. aureus now is an uncommon cause of pneumonia in this age group. If clinical signs compatible with staphylococcal disease are present, however, initial therapy should include a parenteral penicillinase-resistant penicillin. If the disease is severe, drugs of known efficacy for MRSA, such as vancomycin or linezolid, should be used.

Because *M. pneumoniae* and *Chlamydia pneumoniae* are common causes of pneumonia in school-aged children and adolescents, presumptive therapy should include coverage for *S. pneumoniae*, *M. pneumoniae*, and *C. pneumoniae* in children older than 5 years. Erythromycin, azithromycin, and clarithromycin are appropriate drugs for coverage of both pathogens.

Pneumonia in Children 10 Years of Age or Older

S. pneumoniae, *M. pneumoniae*, and *C. pneumoniae* are the major treatable causes of pneumonia in children 10 years of age or older. *H. influenzae* occurs infrequently, and initial therapy need not include coverage for this organism. Therapy outlined earlier for school-aged children is appropriate for children 10 years or older.

CHEMOTHERAPY FOR SPECIFIC PATHOGENS

Pneumococcal Pneumonia

Penicillin G is the drug of choice for children with pneumonia caused by *S. pneumoniae*. For most children with mild to moderately severe disease, an oral penicillin is suitable. Phenoxymethylpenicillin (penicillin V) administered orally provides significant antibacterial activity and approximately twice the peak serum level provided by an equivalent dose of buffered oral penicillin G. Children who appear to be toxic, who have underlying disease, or who have complications (e.g., abscesses, empyema) require the higher serum and tissue antibacterial activity that is provided by aqueous penicillin G administered intravenously or intramuscularly.

Strains of nonsusceptible *S. pneumoniae* have become prevalent in most communities in the United States. Clinical failures have occurred in some patients with meningitis caused by penicillin-resistant pneumococci, but few failures have been identified in cases of sepsis or pneumonia treated with a penicillin or cephalosporin in an adequate dosage schedule. Susceptibility tests should be done on all isolates of *S. pneumoniae* from sputum and body fluids (i.e., blood, cerebrospinal fluid, and pleural fluid). Presumptive therapy for mild to moderate pneumonias need not be altered because of concern for resistant pneumococci, but severe pneumonias should be treated with high-dose parenteral therapy providing high serum and tissue concentrations and stability to β-lactamases, such as intravenous ceftriaxone and cefotaxime. The most appropriate regimen is chosen when results of culture and susceptibility tests are available. Vancomycin is effective uniformly against all pneumococci, including highly resistant strains, and should be considered if susceptibility tests indicate the strain is multidrug-resistant and uniquely susceptible to vancomycin.

The dosage schedule for mild to moderate disease and severe disease is provided in Chapter 235. The duration of therapy depends on the clinical response, but therapy should be continued for at least 3 days after defervescence and significant resolution of radiologic and clinical signs; usually 5 to 7 days is sufficient.

Pneumonia Caused by *Haemophilus influenzae*

Nontypeable and type b strains of *H. influenzae* are susceptible to various antimicrobial agents, including ampicillin or amoxicillin (non–β-lactamase producers), oral or parenteral cephalosporins, sulfonamides, and aminoglycosides. All of these agents have been used with success in systemic infections (including pneumonia) caused by this agent. Amoxicillin is considered the drug of choice for treating young children with mild to moderate pulmonary disease. Because of the concern for β-lactamase–producing strains of *H. influenzae*, a parenteral second-generation (cefuroxime) or third-generation (ceftriaxone, cefotaxime, or ceftazidime) cephalosporin should be used as initial therapy in patients with severe disease when this microorganism is known or strongly suspected to be the pathogen.

A child with mild to moderate disease should be treated for a minimum of 7 days, including a period without fever of at least 3 days. A child with severe disease should be treated for 2 to 3 weeks.

Staphylococcal Pneumonia

The high incidence of staphylococci resistant to penicillin G, in the hospital and in the community, requires the use of a penicillinase-resistant penicillin whenever staphylococcal pneumonia is diagnosed or suspected. Subsequently, if the culture and sensitivity data indicate that the organism is susceptible to penicillin G, the drug should be used because of its greater efficacy and lower cost. Clinical trials indicate that all penicillinase-resistant penicillins are equally effective in treating staphylococcal pneumonia. The increased incidence of CA-MRSA suggests that initial therapy of patients with pulmonary signs suggestive of staphylococcal infection (e.g., abscesses, pneumatoceles, and emypema) should include parenteral drugs of known efficacy, such as vancomycin or linezolid.

The rapid development of empyema, pneumatoceles, and abscesses demands close observation and meticulous nursing care. The antibiotic should be administered parenterally using a

high-dose schedule for 2 to 3 weeks; an oral preparation then may be given for 1 to 3 weeks. The total duration of antibiotic therapy depends on the initial response, the presence of pulmonary and extrapulmonary complications, and the rapidity of resolution of the pneumonia.

Pneumonia Caused by Anaerobic Bacteria

Most anaerobic bacteria that cause pneumonia, including strains of *B. fragilis*, are highly susceptible to penicillin G. Some strains of *B. fragilis* may be resistant to penicillin G and susceptible to chloramphenicol, clindamycin, or cefoxitin. The duration of therapy depends on the extent of the disease; pneumonia without complications clears rapidly with appropriate therapy; 7 days of therapy usually is sufficient.

Pneumonia Caused by Gram-Negative Bacilli

The initial choice of therapy is guided by the following factors: the source of the infection, the disease process present (e.g., burn, cystic fibrosis), the host susceptibility to infection (e.g., deficient immune mechanisms), and the antimicrobial susceptibility pattern for these organisms in the community or hospital. The basis for choice of antibiotic is similar to that outlined for neonatal pneumonia suspected to be caused by gram-negative bacilli. The duration of therapy must be tailored to the clinical course and the response to therapy. Cases of pneumonia with minimal pulmonary lesions and limited symptoms should be treated for at least 3 days after defervescence. Severe cases of pneumonia should be treated for 2 to 3 weeks.

Therapy for Penicillin-Allergic Children

A child who has a significant history of allergic reaction to any of the penicillins must be considered allergic to all of them, and alternative antimicrobial agents must be considered for therapy. If the patient has a history consistent with IgE-mediated reaction to penicillin, cephalosporins also should be avoided. If the patient has a history of a penicillin reaction that was not IgE-mediated and not serious, administration of a cephalosporin usually is safe.[83] Cephalothin, cefazolin, ceftriaxone, and cefotaxime have been used with success in the treatment of pneumococcal pneumonia. Erythromycin, the new macrolides clarithromycin and azithromycin, and clindamycin are active in vitro against gram-positive cocci, but use for pneumococcal and staphylococcal pneumonias should be determined on the basis of susceptibility tests. Because there are no vancomycin-resistant pneumococci and only rare cases of staphylococci that are resistant, vancomycin is the drug of choice for presumptive therapy of a patient who is allergic to penicillin and who has severe pneumonia likely caused by staphylococcal or pneumococcal infections.

Adjuncts to Chemotherapy

Administration of antimicrobial agents is only part of the management of a child with pneumonia. Close observation, nursing care (including suction of excess secretions), and the following supportive measures are crucial:

1. Maintenance of fluid and electrolyte balance
2. Humidification provided by cool mist
3. Oxygen for severe dyspnea
4. Cleansing of the mouth
5. Sparing use of antipyretics because the temperature course provides a guideline for the therapeutic response

More extensive procedures may be required in special circumstances, as follows:

1. Bronchoscopy is important in documenting the presence of a foreign body, tumor, or congenital anomaly.
2. Intubation of the trachea or tracheotomy may be considered when the patient has difficulty clearing secretions and more efficient suction of the lower respiratory tree is required.
3. Drainage of pleural effusions may be necessary when an accumulation of fluid compromises respiration. Thick, tenacious empyema may require intercostal tube drainage or closed-tube thoracostomy. Empyema caused by *S. aureus* may require placement of a tube, whereas the less viscid effusion associated with *S. pneumoniae* and *Streptococcus pyogenes* rarely requires more than frequent thoracentesis. Empyema caused by *H. influenzae* may be thick and viscid, requiring placement of a chest tube, or less viscid, requiring only thoracentesis. Single or multiple thoracenteses are adequate when the volume of fluid is small and the quality of the fluid allows ready drainage, as usually is the case with empyema caused by *S. pneumoniae* or group A streptococci. When large amounts of fluid are present or the fluid is thick and viscid, a closed drainage system with intercostal chest tube under negative pressure is placed; this placement frequently is necessary for empyema caused by *S. aureus*. The tube should be removed as soon as its drainage function is completed because delay might result in local tissue injury, secondary infection, or sinus formation.The use of video-assisted thoracoscopy early in the course of empyema (within 48 hours after admission to hospital) has significantly decreased the duration of fever and the length of hospitalization for patients with pleural empyemas.[91]
4. Intrapleural instillation of antibiotics or fibrinolytics should be considered in cases of empyema when the fluid is loculated because of fibrous adhesions. If a chest tube is in place, antibiotics can be instilled after irrigation through the tube. In susceptible infections, aqueous penicillin G (10,000 to 50,000 U), ampicillin (10 to 50 mg), or a penicillinase-resistant penicillin or cephalosporin (10 to 50 mg) may be inoculated in 10 mL of diluent (i.e., sterile water or normal saline) after the tube is clamped. The clamp is maintained for 1 hour and then released for drainage. The instillations should be repeated three to four times each day that the tube remains in place. Hawkins and colleagues[49] reported successful resolution of 54 of 58 children with empyema who underwent intrapleural placement of catheters and administration of fibrinolytics consisting of tissue plasminogen activator.

PROGNOSIS

In uncomplicated cases of pneumococcal pneumonia in children in the United States, the mortality rate is very low (<1%). A review of mortality from pneumonia in children in the United States, 1939 through 1996, identified reductions in mortality rates that were thought to reflect expanded access to medical care for poor children.[34] In developing countries, pneumonia is a major cause of mortality, accounting for more than one fourth of deaths in children younger than 5 year old. Half of the pneumonia-related mortality occurs in children younger than 1 year old. The WHO estimates that approximately 4 million childhood deaths are caused each year by pneumonia.

Lung morphology and physiology usually return to normal after completion of appropriate antimicrobial therapy. Fibrothorax is a rare occurrence; almost all children resolve thickened pleurae with no effect on lung growth and function. Even after having extensive disease associated with empyema caused by *S. aureus* or *H. influenzae*, children have normal growth and development and normal pulmonary function after recovery.[70] Deaths still result from bacterial pneumonias; however, most deaths of children result from abrupt, overwhelming disease. Asmar and colleagues[6] reported two deaths in 43 children with pneumonia caused by *H. influenzae*; both deaths occurred before antibiotics could be administered.

PREVENTION

PNEUMOCOCCAL VACCINES

The approval of a conjugate polysaccharide pneumococcal vaccine by the FDA in February 2000, followed by the recommendations of various authoritative groups (e.g., American Academy of Pediatrics, American Academy of Family Physicians, and Advisory Committee on Immunization Practices of the Surgeon General) for universal immunization of infants and selective immunization of children at risk who are 2 to 5 years of age, added the most effective mode of prevention of pneumococcal diseases yet available. The available vaccine (Prevnar; Wyeth Lederle Vaccines, Pearl River, NY) is a heptavalent vaccine in which the individual polysaccharides have been purified and directly conjugated to the protein carrier CRM 197, a nontoxic variant of diphtheria toxin. The conjugate vaccine induces type-specific antibodies in infants 2 months of age. The vaccine currently is distributed in 73 countries, and more than 180 million doses have been distributed worldwide (Paradiso P, personal communication May 2008).

The safety and efficacy of the vaccine for prevention of pneumonia were studied in a trial of approximately 38,000 children in Northern California by Black and colleagues at the Kaiser Permanente Vaccine Study Center.[16] Children were randomly assigned to receive the conjugate pneumococcal vaccine or a conjugate polysaccharide meningococcal group C vaccine. Pneumonia was defined clinically and radiologically. Infants and children who had received the pneumococcal vaccine had a 4.3 percent reduction in clinical episodes of pneumonia and a 20 percent decrease in radiographically confirmed pneumonia. Similar results have been reported from developing countries; a nine-valent pneumococcal conjugate vaccine was found to be effective compared with placebo in prevention of 7 percent of clinical pneumonias and 37 percent of first episodes of radiologically diagnosed pneumonias.[27]

The 23-type pneumococcal polysaccharide vaccine produces a satisfactory independent antibody response, but only in children older than 2 years. The polysaccharide vaccine is recommended for children 2 years of age and older who are at risk for developing invasive disease (e.g., sickle-cell disease, HIV infection or other immunodeficiencies, malignancy, nephrosis) after they have received the appropriate number of doses of the conjugate vaccine.

HAEMOPHILUS INFLUENZAE VACCINES

A polysaccharide vaccine for prevention of *H. influenzae* type b disease was introduced in the United States in April 1985. As was true of other polysaccharide vaccines, infants younger than 18 months of age had an inadequate immune response to the capsular polysaccharide. The development of a conjugate vaccine by coupling the capsular saccharide of *H. influenzae* type b and a protein resulted in protective antibodies in infants 2 months of age. The conjugate *H. influenzae* type b vaccine was approved by the FDA in fall 1990 and has resulted in virtual elimination of disease caused by *H. influenzae* type b in infants and children. The conjugate vaccine has had varying efficacy in different regions. The incidence of pneumonia associated with alveolar consolidation or pleural effusion was reduced by 22 percent in Chilean infants, whereas the vaccine had limited efficacy in prevention of clinical or radiologically confirmed pneumonias in Indonesian children.

Because nontypeable strains of *H. influenzae* are important causes of otitis media, sinusitis, and pneumonia, investigators have sought vaccine candidates, including outer membrane proteins, bacterial adherence proteins, and lipo-oligosaccharides. Questions of strain heterogeneity and choice of the number of antigens needed for optimal protection remain unanswered, and no products have moved beyond preclinical testing.[11]

INFLUENZAVIRUS VACCINE

Secondary bacterial pneumonia may complicate primary influenza virus infections. Extensive use of conjugate pneumococcal vaccines in children (and widespread use of influenza vaccines) should limit the incidence and morbidity of the combined infections. Brundage[21] reviewed the epidemiologically and clinically important interactions between influenza and secondary bacterial respiratory pathogens during the 1918 and subsequent influenza pandemics.

CHEMOPROPHYLAXIS

Chemoprophylaxis for prevention of bacterial pneumonias is limited to patients with immunodeficiency. Children who have sickle-cell anemia or who have functional or anatomic asplenia are at risk for developing overwhelming disease caused by *S. pneumoniae*. Daily antimicrobial prophylaxis is recommended for these children, regardless of their immunization status. The chemoprophylactic regimen used most commonly is daily administration of oral penicillin V (125 mg twice daily for children <5 years; 250 mg twice daily for children >5 years). Children with HIV should receive a regimen of trimethoprim-sulfamethoxazole for prophylaxis against infection caused by *P. carinii*.

PREVENTION OF HEALTH CARE–ASSOCIATED PNEUMONIA

Because a hospitalized child is exposed to a variety of pathogens, including bacteria capable of causing pneumonia, and may undergo procedures that compromise the airway, health care providers should be familiar with techniques for limiting exposures. The CDC periodically has provided guidelines for prevention of health care–associated pneumonia, most recently in 2004.[22,23]

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CHAPTER 28

Children's Interstitial Lung Disease and Hypersensitivity Pneumonitis

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Children's interstitial lung disease (ILD) encompasses a large, heterogeneous group of mostly rare, diffuse lung disorders of known and unknown etiology.[43] In many of these disorders, injury to the alveolar wall gives rise to an inflammatory response with subsequent repair that potentially can lead to pulmonary fibrosis. Infections of the lung, either chronic or acute with postinfectious sequelae, form the largest category of children's ILD in the immunocompetent and immunocompromised host. This chapter focuses on the entities that are caused by or associated with infection and the entities that mimic community-acquired pneumonia.

CLASSIFICATION

Table 28–1 presents a general classification of children's ILD. Although a complete description of each disorder is beyond the scope of this chapter, certain conditions warrant brief mention because major differences exist between adult ILD and children's ILD in terms of types and distribution of disease. Usual interstitial pneumonitis, a progressive and fatal disorder, is the most common form of adult idiopathic ILD, but it probably does not occur in children. Also, hypersensitivity pneumonitis (HP) in adults is caused by a variety of occupational exposures but in children usually is caused by exposures to avian antigen. In addition, several entities, such as neuroendocrine cell hyperplasia of infancy and pulmonary interstitial glycogenosis, have been identified more recently and seem to be unique to infants and young children. Finally, the genetic basis for certain types of familial lung diseases has been linked to mutations in genes involved in surfactant metabolism.[97,98,131] With the exception of surfactant protein C deficiency, because of high mortality rates, these disorders have been found mostly in children.

DISORDERS ASSOCIATED WITH INFECTION

The role of infectious agents, such as adenovirus,[146] influenza,[76] *Mycoplasma*,[137] and *Chlamydia*,[56] in the development of chronic lung disease in children has been well documented. Virtually any organism that infects the lower respiratory tract is capable of producing chronic, diffuse lung disease if the injury is severe enough, although most infected children have acute, self-limited disease that resolves completely.

TABLE 28–1 Classification of Pediatric Interstitial Lung Diseases

Pediatric Interstitial Lung Diseases of Known Etiology
Aspiration syndromes
Chronic infection (viral, bacterial, fungal, parasitic)
  Immunocompetent host
  Immunocompromised host
Bronchopulmonary dysplasia
Hypersensitivity pneumonitis (and other environmental exposures)
Lipid storage diseases
Pulmonary alveolar microlithiasis

Pediatric Interstitial Lung Diseases of Unknown Etiology
Primary Pulmonary Disorders
Desquamative interstitial pneumonitis
Lymphocytic interstitial pneumonitis and related disorders
Nonspecific interstitial pneumonitis
Cryptogenic organizing pneumonia
Alveolar hemorrhage syndromes
Pulmonary infiltrates with eosinophilia
Bronchiolitis obliterans
Pulmonary alveolar proteinosis
Pulmonary vascular disorders (proliferative and congenital)
Pulmonary lymphatic disorders

Systemic Disorders with Pulmonary Involvement
Connective tissue disease
Malignancies
Histiocytosis
Sarcoidosis
Neurocutaneous syndromes

Unique Forms of Interstitial Lung Disease in Infancy
Lung growth abnormalities[152]
Neuroendocrine cell hyperplasia of infancy[108]
Follicular bronchitis/bronchiolitis[63]
Cellular interstitial pneumonitis of infancy[123]/pulmonary interstitial glycogenosis[13]
Acute idiopathic pulmonary hemorrhage of infancy[36]
Chronic pneumonitis of infancy[68]/inborn errors of surfactant metabolism[97,98,131]

Bronchiolitis Obliterans

Probably the best example of postinfectious chronic lung disease is found in children who develop bronchiolitis obliterans after having severe adenovirus pneumonia.[146] Bronchiolitis obliterans is characterized by a fibrosing process of the small airways that results in severe, irreversible obstruction of the airways. Clinically, patients present with tachypnea, crackles, wheezing, and a productive cough that persist for more than 6 weeks after the initial illness. Chest radiographs show air trapping and atelectasis; on high-resolution computed tomography (CT) scans, mosaic perfusion, central bronchiectasis, vascular attenuation, and atelectasis are common findings.[24] Occasionally, severe involvement of one lung leads to the development of a unilateral, small, hyperlucent lung, known as Swyer-James syndrome.[146]

Patients with severe adenovirus pneumonia have been shown to have immune complexes containing adenovirus antigen in the lung and increased serum levels of interleukin-6, interleukin-8, and tumor necrosis factor-α.[92,93] These studies suggest that abnormal or excessive host immunologic and inflammatory responses may be important in the development of chronic lung disease from adenovirus in infants and young children. Although lower respiratory tract infection with respiratory syncytial virus, parainfluenza, influenza, measles, varicella,[148] *Mycoplasma*, and pertussis[18] also can result in bronchiolitis obliterans, adenovirus is the most common etiologic agent. The need for mechanical ventilation during the initial illness is a strong independent risk factor for the subsequent development of bronchiolitis obliterans.[24]

Lymphocytic Interstitial Pneumonitis

Well recognized because it is an acquired immunodeficiency syndrome (AIDS)–defining condition in children infected with human immunodeficiency virus (HIV),[15] lymphocytic interstitial pneumonitis (LIP) also occurs in association with autoimmune disorders[81,141] and other immunodeficiencies[22] and in idiopathic and familial forms.[99] LIP is not a true interstitial pneumonitis but rather is a form of pulmonary lymphoproliferative disease. It is characterized histologically by a diffuse infiltrate of mature lymphocytes, along with smaller numbers of plasma cells and histiocytes, in the pulmonary interstitium and alveolar wall.[70,71] The infiltrate also may be found along lymphatic pathways and is evident in the bronchovascular bundle and interlobular septa, but it usually spares the pleura. The lymphocytes are small noncleaved cells that may accumulate as small nodules, sometimes with germinal centers. Generally, the lymphocytes are polyclonal, with B cells and a variety of T cells being identified.[106] Although Epstein-Barr virus has been isolated from the lungs of some AIDS-infected[4,39] and non–AIDS-infected patients with LIP,[85] its role in the development of LIP is unclear. Neither fibrosis nor airspace disease is prominent in this disease.

LIP occurs in 30 percent of children infected perinatally with HIV and typically manifests between the second and third years of life with an insidious onset of cough, tachypnea, dyspnea, and hypoxemia.[65,106,117-119,125] Chest radiographs characteristically reveal a diffuse, symmetric reticulonodular or nodular pattern, occasionally with mediastinal or hilar adenopathy.[86] Among HIV-infected children, the incidence of acute lower respiratory tract infection is higher in children with LIP,[128] and these patients ultimately may develop bronchiectasis.[130]

Other Conditions

Other pediatric chronic lung diseases have been associated with various infectious agents. Perinatal infection or colonization with

Ureaplasma urealyticum has been implicated in inducing pulmonary inflammation and subsequent development of bronchopulmonary dysplasia in premature neonates.[64] A more recent meta-analysis suggested, however, that reporting bias may be partially responsible for this association.[122] Parvovirus has been linked to autoimmune disease associated with ILD and other organ involvement.[9] Finally, attempts have been made to link *Stachybotrys chartarum* and its mycotoxins to acute and recurrent pulmonary hemorrhage in infants, although a causal relationship has not been proven.

In a prospective study of immunocompetent children with chronic diffuse infiltrates, Fan and coworkers[45] found an infectious agent as the underlying cause in 10 (20%) of 51 children with ILD. Identified agents included adenovirus alone in four patients, adenovirus and cytomegalovirus in two, varicella in one, Epstein-Barr virus in one, *Chlamydia* in one, and *Toxocara* in one. Chronic infections or long-term sequelae from acute infections account for many cases of ILD of known etiology in children.

ENTITIES THAT MAY MIMIC COMMUNITY-ACQUIRED PNEUMONIA

Acute Eosinophilic Pneumonia

Acute eosinophilic pneumonia is the most severe form of the idiopathic eosinophilic pneumonias. It is characterized by very large numbers of eosinophils infiltrating the alveoli and interstitium, with resultant acute respiratory failure. Acute eosinophilic pneumonia can be idiopathic or result from inciting triggers such as drugs (minocycline, sertraline) and inhalational exposures (new-onset cigarette smoking). Patients present with acute onset of fever, cough, tachypnea, and dyspnea; pleuritic chest pain; and myalgias. On examination, crackles are present in 80 percent of patients; wheezing is a rare manifestation. Hypoxemia is uniformly present, and patients can progress rapidly to respiratory failure. In contrast to patients with other eosinophilic lung diseases, patients with acute eosinophilic pneumonia generally do not have significant peripheral eosinophilia (>350 cells/mm^3) on presentation as a diagnostic clue. Chest radiographs and CT scans show reticular markings with Kerley B lines and alveolar infiltrates and pleural effusions in 50 percent of cases. Bronchoalveolar lavage (BAL) fluid shows marked eosinophilia (>20%) in most patients. Pleural fluid also has increased eosinophils and a high pH, caused by the basic eosinophil granule contents. Treatment with corticosteroids (methylprednisolone, 2 to 4 mg/kg/day) usually results in a rapid and complete resolution.[3,113]

Pulmonary Vasculitis

In children, the spectrum of pulmonary vasculitides includes Wegener granulomatosis, Churg-Strauss syndrome, microscopic polyangiitis, and pulmonary capillaritis. These disorders can manifest as diffuse alveolar hemorrhage, with fever, dyspnea, hemoptysis (not present in one third of patients), anemia, and patchy alveolar infiltrates on chest radiographs. Other patients may have fever and cavitary or nodular lesions on imaging.[60] Diffuse alveolar hemorrhage can be diagnosed with BAL, when sequentially recovered aliquots of fluid show persistently bloody return. Renal involvement may be present sometimes, with microscopic hematuria and red blood cell casts seen on urinalysis. A positive serum antineutrophil cytoplasmic antibody (ANCA) may be seen with Wegener granulomatosis (especially cytoplasmic ANCA), Churg-Strauss syndrome, and microscopic polyangiitis, and the presence of peripheral and pulmonary eosinophilia would support the diagnosis of Churg-Strauss syndrome.[11] Pulmonary capillaritis is associated with a variety of conditions and requires an open lung biopsy for diagnosis because it may manifest as an ANCA-negative, hematuria-negative diffuse alveolar hemorrhage syndrome.[52] Therapeutic options for pulmonary vasculitis include systemic corticosteroids, cyclophosphamide, and intravenous immunoglobulin; aggressive treatment is required in severe cases.

Connective Tissue Disorders

Pulmonary manifestations can precede other systemic findings in children with systemic lupus erythematosus[23] and juvenile rheumatoid arthritis,[114] and these patients may present with acute respiratory symptoms associated with fever. In acute lupus pneumonitis, signs and symptoms include high fevers, dyspnea, tachypnea, crackles, and cyanosis. Chest radiographs may show areas of consolidation and pleural effusions and interstitial infiltrates and elevation of the hemidiaphragms.[87] Other pulmonary manifestations of systemic lupus erythematosus include pulmonary hemorrhage, pulmonary hypertension, ILD, pneumothorax, and shrinking lung syndrome. Multisystem involvement, including renal and skin, may provide clues to the underlying diagnosis. Treatment usually requires corticosteroids.[23]

Hypersensitivity Pneumonitis

Acute and chronic HP can manifest with fever, cough, dyspnea, and pulmonary infiltrates. HP is discussed in detail later.

CLINICAL PRESENTATION

Most children with ILD have insidious symptoms that may go unrecognized for years. Some children have been misdiagnosed as having asthma and have been treated with bronchodilators.[49] Although a history of wheezing can be elicited in half of patients, it can be documented by physical examination in only approximately 20 percent of cases. Clinical suspicion for children's ILD should arise when patients meet at least three of the four following criteria: (1) presenting symptoms of dyspnea, tachypnea, retractions, cough, exercise intolerance, or respiratory failure; (2) presenting signs of crackles, failure to thrive, clubbing, or respiratory failure; (3) hypoxemia; and (4) diffuse abnormality on chest radiographs or CT not attributable to other known processes.[42]

A careful history should be taken to assess the severity of the disease and to obtain information that may contribute to establishing a diagnosis. A search for precipitating factors should include a history of feeding difficulties that may suggest aspiration; any prior acute or severe respiratory infections; and environmental exposures, especially to birds or molds. Hemoptysis may indicate a pulmonary vascular disorder or hemosiderosis. Joint disease or rash may indicate a systemic process, such as a connective tissue disease. A family history of relatives or siblings with similar respiratory conditions may be clues to genetic or familial lung diseases, such as a defect in surfactant proteins.

On physical examination, tachypnea and retractions often are observed, and crackles commonly are heard, particularly at the bases. In severe cases, cyanosis, clubbing, an accentuated pulmonic component of the second heart sound, and evidence of growth failure are seen. Oxygen saturation usually is normal under all conditions in most patients with mild disease, but desaturation may occur with exercise or during sleep as the disease progresses, and ventilation-perfusion mismatch ensues. Patients with more advanced disease are hypoxemic at rest.

DIAGNOSTIC EVALUATION

A systematic approach to children's ILD is essential to physicians confronted with such a large differential of rare conditions.

TABLE 28–2 Diagnostic Studies for Pediatric Interstitial Lung Disease

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From Fan, L. L.: Pediatric interstitial lung disease. In *Schwarz, M. I., and King, T. E. (eds.): Interstitial Lung Disease, 4th ed. Hamilton, Ontario, B. C. Decker, 2003, pp. 134-151.*

Diagnostic studies can be divided into studies used to assess the extent and severity of disease, to identify disorders that predispose to ILD, and to identify the primary ILD (Table 28–2). In adults with ILD, Raghu[109] suggested a diagnostic process that employs a thorough history and physical examination first, noninvasive tests next, and then invasive studies, including BAL and transbronchial biopsy followed by open lung biopsy if the previous less invasive studies do not provide a specific diagnosis. Based on experience from a retrospective chart review of 48 children with ILD, Fan and colleagues[49] independently developed an algorithm remarkably similar to that of Raghu's and used it prospectively in the evaluation of 51 children presenting with ILD.[45] In that study, a specific diagnosis was established by history and physical examination alone in 1 patient; noninvasive studies alone in 8 others; and invasive studies, including lung biopsy, in another 26. Of the remaining patients, eight had a suggestive diagnosis, and eight had no specific diagnosis. This study suggests that a systematic approach to the diagnosis of children's ILD is useful and that some patients can be diagnosed with noninvasive studies, but most patients require invasive studies, including lung biopsy.

PULMONARY FUNCTION TESTS

In children who are old enough to undergo pulmonary function tests, standard spirometry typically shows a pattern of restrictive lung disease, with reduced forced vital capacity (FVC), forced expiratory volume in 1 second (FEV_1), and a normal or elevated FEV_1/FVC ratio.[49] On measurement of lung volumes, although the total lung capacity (TLC) often is low, the residual volume (RV) may be normal or elevated, resulting in an increased RV/TLC ratio that suggests air trapping and a mixed restrictive/obstructive pattern. These findings also can be documented in infants and young children with the use of infant pulmonary function testing techniques.

HIGH-RESOLUTION COMPUTED TOMOGRAPHY

In the evaluation of children's ILD, high-resolution CT is an important diagnostic modality used to provide precise detail about the extent and distribution of parenchymal disease and to select favorable sites for biopsy. To evaluate the lung parenchyma best with CT scanning, thin sections are necessary to avoid the volume averaging that occurs with 2.5- to 5-mm sections, which obscures the fine parenchymal detail and airway abnormalities. High-resolution CT samples thin sections (usually 1 mm in thickness) at wide intervals (usually 10 mm). With the development of multidetector array or multislice CT, contiguous thin section studies of the entire chest can be performed in a shorter time and with less radiation exposure than with conventional CT.[10]

Inspiratory and expiratory CT scans should be obtained whenever possible. In younger children and infants in whom cooperation is impossible and in whom rapid respiratory rates can cause motion artifact, a controlled-ventilation CT technique using mask ventilation of a sedated patient can be used.[80] Giving several assisted deep breaths to these young children results in a short period of apnea during which the lungs can be inflated to obtain inspiratory images, followed by expiratory images after passive deflation. General anesthesia produces similar results, although frequent large sigh breaths are necessary to prevent dependent atelectasis, which occurs within minutes of intubation.

High-resolution CT may increase the level of diagnostic confidence for the diagnosis of children's ILD, improve diagnostic accuracy, and provide a useful classification system. In a study of 20 children with biopsy-proven ILD, 56 percent of the confident first-choice diagnoses on high-resolution CT were correct.[82] Diseases were classified into five distinct groups based on dominant high-resolution CT features: (1) geographic hyperlucency (bronchiolitis obliterans or bronchocentric granulomatosis), (2) septal thickening (lymphangiomatosis, hemangiomatosis, microlithiasis), (3) ground-glass opacification (desquamative interstitial pneumonitis, LIP, HP), (4) lung cysts and nodules (histiocytosis), and (5) consolidation (aspiration, bronchiolitis obliterans organizing pneumonia). First-choice diagnoses based on high-resolution CT were accurate in 61 percent of cases in a similar study.[25]

BRONCHOALVEOLAR LAVAGE

BAL via flexible bronchoscopy allows for sampling of the cellular and biochemical components of alveolar lining fluid and may be helpful in the evaluation of certain cases of children's ILD. Normal indices for pediatric BAL fluid have been described, against which abnormal results can be compared.[37]

The most common indication for pediatric BAL has been to detect infection in the immunocompromised host, with a diagnostic yield of approximately 50 percent in non–AIDS-infected patients and 75 percent in patients with AIDS.[41] The use of quantitative bacterial cultures may help to differentiate whether recovered organisms represent true infection, colonization, or contamination. Examination of BAL fluid also can identify aspiration or pulmonary hemorrhage by the detection of lipid-laden or hemosiderin-laden macrophages. The presence of lipid-laden or hemosiderin-laden macrophages in BAL may be sensitive but not specific for aspiration or alveolar hemorrhage syndromes.[48] In a murine model, investigators showed that hemosiderin-laden macrophages first appear at 3 days, peak at 1 week, and persist in small numbers for 2 months after a single episode of hemorrhage.[35] BAL also has been used to diagnose pulmonary alveolar proteinosis, lysosomal storage disorders, and histiocytosis.[48]

In immunocompetent children with ILD, Fan and colleagues[48] found that BAL was diagnostic of a primary disorder in only 5 of 29 patients—aspiration was detected in 3, and infection in 2. The differential diagnosis was narrowed in 15 patients by the presence of lymphocytosis, neutrophilia, or eosinophilia. A secondary disorder was uncovered in eight patients. This study suggests that BAL provides some useful information in children with ILD, but that its ability to determine the primary cause is limited.

LUNG BIOPSY

As in adult ILD, lung biopsy is the gold standard for establishing the diagnosis of children's ILD because most diseases are classified in terms of previously defined histopathologic patterns. Although transbronchial or percutaneous needle biopsy may be helpful in certain conditions, a transthoracic approach by either conventional open lung biopsy or video-assisted thoracoscopic surgery (VATS) remains the gold standard for obtaining tissue adequate for diagnosis.

The use of VATS is rapidly becoming the method of choice for lung biopsy in children. Technical modifications have allowed its use even in infants.[116] In a prospective study in a small group of immunocompetent children with ILD, Fan and coworkers[46] found that the diagnostic yield for open lung biopsy (57%) and VATS (54%) was comparable, but the morbidity from VATS was lower in terms of duration of surgery, chest tube insertion, and hospitalization.[46] Although multiple lobe biopsies directed by high-resolution CT have been advocated for the diagnosis of adult and children's ILD, this study did not show a difference in diagnostic yield for single-lobe versus multiple-lobe biopsies. Depending on the handling of the specimen and the expertise of the reviewing pathologist, the diagnostic yield from transthoracic lung biopsy (open lung biopsy and VATS) can be quite high, especially in light of the more recent advances in the understanding of children's ILD.

Lung biopsy material must be handled in a consistent manner to ensure optimal interpretation, and a protocol for such handling was published based on the recommendations of the chILD Pathology Group.[75] A general scheme for division of the biopsy specimen is as follows: (1) microbiology cultures, 35 percent; (2) snap-frozen for polymerase chain reaction or other molecular studies, 10 percent; (3) snap-frozen in cryomatrix for immunofluorescent, laser capture, or other studies requiring frozen sections, 10 percent; (4) fixed in glutaraldehyde for electron microscopy, less than 5 percent; (5) imprints for cytologic examination or rapid identification of organisms, 0 percent; and (6) expanded and fixed in formalin (methods previously described[47]) for light microscopy, 40 percent. It is crucial that the biopsy material be interpreted by a pathologist with considerable expertise in pediatric lung disease because the normal lung of an infant differs greatly from that of an older child or adolescent, and any pathologic finding needs to be interpreted in light of the normal age-dependent variations of lung architecture.

TREATMENT

Supportive care includes providing adequate nutrition, annual influenza vaccination, and aggressive treatment of intercurrent infections; engaging the patient in a carefully supervised fitness and exercise program; having the patient avoid inhalant hazards such as tobacco smoke; and providing selective use of bronchodilators and oxygen for chronic hypoxemia. Patients with underlying systemic disorders need primary treatment for that disorder, such as intravenous gamma globulin for hypogammaglobulinemia. Specific therapy for primary ILD, such as anti-infective therapy for chronic infections, interferon-α for pulmonary hemangiomatosis,[146] and lung lavage for pulmonary alveolar proteinosis, should be used when possible.[84] When environmental agents such as bird antigens are causative, avoiding contact with them is crucial (see the section on HP).

Generally, corticosteroids remain the treatment of choice for most patients with ILD on the presumption that suppression of inflammation may reduce the risk of developing fibrosis.[47] Although controlled clinical studies are lacking, corticosteroids have been used to treat such diverse types of diffuse lung diseases as desquamative interstitial pneumonitis, LIP, and HP.[41] In a retrospective study of pediatric ILD by Fan and coworkers,[49] corticosteroids were judged to be effective in 40 percent (12 of 30) of treated children in terms of improved clinical status, decreased oxygen requirements, and improved pulmonary function. A trial of prednisone or equivalent corticosteroid (1 to 2 mg/kg/day) for at least 6 to 8 weeks probably is warranted.

Alternative but unproven therapy includes pulse steroid therapy, hydroxychloroquine, azathioprine, cyclophosphamide, methotrexate, cyclosporine, and intravenous gamma globulin. Of these, hydroxychloroquine probably has been used most frequently.[5,49,128,133] The precise mechanism of action is unknown, but chloroquine and hydroxychloroquine have shown immunosuppressive effects with the ability to inhibit the functional capabilities of monocytes and the generation of antibody-forming cells. Hydroxychloroquine is preferred over chloroquine because the former has less retinal toxicity. The recommended dose in children for the treatment of ILD is 10 mg/kg/day. A more recent case report described the successful use of infliximab, an anti–tumor necrosis factor-α monoclonal antibody, in reversing bronchiolitis obliterans in a hematopoietic stem cell transplant recipient.[51]

The fact that many alternative pharmacologic approaches are considered for children and adults with ILD implies that conventional therapy often is ineffective. New strategies are being developed based on animal models of pulmonary fibrosis and more recent advances in the cellular and molecular biology of inflammatory reactions. Such therapies would be directed against the action of certain cytokines, oxidants, and growth factors that may be involved in the fibrotic process. The potential to deliver specific inflammatory inhibitors or inhibitors of collagen biosynthesis directly to the lung via aerosolization suggests that disease processes in the lung may be more amenable to novel therapies than are disease processes in other internal organs.

More children are receiving lung transplantation for end-stage ILD. According to the 2006 Registry of the International Society for Heart and Lung Transplantation, between 1991 and 2005, there were 30 reported pediatric lung transplantations worldwide for "idiopathic pulmonary fibrosis," 20 for "interstitial pneumonitis," 14 for "pulmonary fibrosis, other," 8 for "surfactant protein B deficiency," and 7 for "bronchopulmonary dysplasia," together constituting 9 percent of all pediatric lung transplantations.[144] Although the overall 5-year survival after transplantation is still disappointing at approximately 50 percent,[144] outcomes for infants undergoing transplantation for surfactant protein B deficiency seem to be at least similar to the outcomes of infants transplanted for other reasons.[104] A case of recurrent pulmonary alveolar proteinosis in the lung allografts of a child who underwent heart-lung transplantation for lysinuric protein intolerance has been reported.[121]

OUTCOME

The prognosis of children with ILD varies. Infants with neuroendocrine cell hyperplasia of infancy generally do well, although they may be symptomatic and require oxygen for years.[108] At the other end of the spectrum, children with growth failure, pulmonary hypertension, and severe fibrosis do poorly.

The overall mortality rates for children's ILD remain high. In a series of 25 children with fibrosing alveolitis or desquamative interstitial pneumonitis, Sharief and associates[129] reported a poor response to treatment in nine patients, with four deaths. In a review of 28 patients with desquamative interstitial pneumonitis, Stillwell and colleagues[136] reported that only 17 patients survived. In another series of children with a variety of more recently described ILD, Nicholson and coworkers[95] reported four deaths in 17 patients with available follow-up data.

Fan and Kozinetz[44] reviewed the outcome of 99 children with chronic ILD seen in Denver, Colorado, over 15 years (1980 to 1994). As expected, a wide variety of disorders were encountered, and 15 recorded deaths occurred, with a probability that a patient would survive to 24 months, 48 months, and 60 months after onset of symptoms of 83 percent, 72 percent, and 64 percent, respectively. Of the clinical features present at the time of initial evaluation, weight less than fifth percentile, crackles, clubbing, family history of ILD, and symptom duration were not associated with decreased survival rates. A severity of illness score, based on increasing levels of hypoxemia and the presence or absence of pulmonary hypertension, was related significantly to survival, with an increasing score associated with a higher probability of decreased survival rates. A simple scoring system seems to be a useful measure of outcome in children with ILD.

HYPERSENSITIVITY PNEUMONITIS

HP, also known as *extrinsic allergic alveolitis*, is a form of immune-mediated ILD that develops in response to repeated inhalation of finely dispersed organic antigens.[27] HP should be considered in the differential diagnosis of a child who presents with acute or chronic respiratory symptoms associated with fever, and obtaining an environmental exposure history is essential to arrive at the proper diagnosis. A wide variety of organic particles, including mammalian and avian proteins, fungi, thermophilic bacteria, and certain low-molecular-weight volatile and nonvolatile chemical compounds, are known to induce HP in susceptible individuals.[54] Certain systemic medications, such as ciprofloxacin,[134] dapsone,[140] and methotrexate,[28] also have been reported as triggers.

Acute and chronic forms of HP have been described, and repeated exposure can lead to irreversible lung damage. Although exposure to antigens capable of provoking HP occurs commonly in the home and work environment, the overall incidence of the condition in the general population is low. An estimated 5 to 15 percent of individuals exposed to high levels of a specific organic antigen develop clinical disease.

PATHOLOGY AND PATHOGENESIS

Pathologically, HP is characterized by a diffuse, predominantly mononuclear cell inflammation of the small airways and pulmonary parenchyma, often associated with poorly formed, nonnecrotizing granulomas.[126] Foamy macrophages are seen commonly in the airspaces. With advanced disease, interstitial and intra-alveolar fibrosis develops that is indistinguishable from other causes of pulmonary fibrosis.

The mechanisms by which organic dusts induce these characteristic pathologic features of the disease are poorly understood. Evidence supports a type III and a type IV hypersensitivity reaction, as defined by Gell and Coombs.[52b,77] A type III reaction is suggested by the presence of precipitating antibody to the offending antigen, immune complex deposition, and activation of complement. A type IV reaction is suggested by an increased percentage of T lymphocytes in BAL fluid, with a strong predominance of $CD8^+$ subsets and a low CD4/CD8 ratio, and the presence of granulomas on lung biopsy specimen. Considering the small proportion of exposed individuals who develop clinical symptoms, complex interactions among the nature of the antigen, the intensity and duration of the exposure, and the host response in susceptible individuals most likely are involved.

ETIOLOGY

As shown in Table 28–3, HP in adults is caused by a wide variety of occupational and environmental exposures.[126] In contrast, in children, HP is caused mainly by exposure to an array of domestic birds (71%) and fungi (28%), based on a review of 133 reported pediatric cases.* HP also has been reported in a child receiving methotrexate.[28] Familial cases have been identified, with one report describing a mother (who died from the disease) and all of her five children who developed HP from exposure to wild city pigeons.[32]

CLINICAL PRESENTATION

Although a wide variety of antigens can induce HP, the immunologic response and clinical presentation are similar. In the reported pediatric cases referenced previously, the mean age (± standard deviation) was 9.6 (± 3.9) years. The youngest reported patient with HP developed symptoms at 10 weeks of age.[139]

In the acute form, symptoms mimic a flulike illness, with high fevers, chills, dry cough, dyspnea, myalgias, and malaise. These symptoms begin several hours after exposure and diminish during the next 12 to 24 hours, provided that no additional exposure occurs. Physical examination reveals a dyspneic, ill-appearing child, often with bibasilar crackles. Transient hypoxemia and nodular pulmonary infiltrates often are present.

In the subacute form, children have insidious and progressive symptoms. In the reported pediatric cases in which the following specific symptoms were recorded, exercise intolerance was present in 97 percent (93 of 96), cough was present in 96 percent (101 of 105), weight loss was present in 87 percent (46 of 53), and fever was present in 70 percent (51 of 73). On physical examination, crackles were present in 72 percent (66 of 91), and clubbing was present in 35 percent (16 of 46) of cases. The chronic form of HP is an extension of the subacute form resulting from continued antigen exposure, with development of irreversible pulmonary fibrosis.

DIAGNOSIS

A careful and thorough environmental history is a critical component in detecting potential antigens. The presence of the following six predictors can help to establish a clinical diagnosis of HP in adults: (1) exposure to a known offending antigen, (2) positive precipitating antibodies to the offending antigen, (3) recurrent episodes of symptoms, (4) inspiratory crackles on physical examination, (5) symptoms occurring 4 to 8 hours after exposure, and (6) weight loss.[74] Compatible findings on chest radiographs (Fig. 28–1), pulmonary function testing, BAL, and open lung biopsy also aid in establishing the diagnosis.

Detecting precipitating IgG antibodies to the offending antigen can be useful in confirming the diagnosis in a patient with documented exposure and typical clinical features (Fig. 28–2). Fifty percent of individuals who are exposed to a particular antigen develop precipitating antibodies, however, and only a

*See references 1, 2, 6, 7, 12, 14, 16, 17, 20, 21, 29, 32-34, 38, 54, 55, 58, 59, 61, 62, 66, 67, 69, 72, 73, 78, 79, 88, 91, 94, 100, 102, 103, 105, 107, 112, 115, 120, 127, 135, 138, 139, 141, 143, 147, 149-151.

TABLE 28–3 Etiologic Agents of Hypersensitivity Pneumonitis

Rights were not granted to include this table in electronic media.
Please refer to the printed publication.

From Selman, M.: Hypersensitivity pneumonitis. In *Schwarz, M. I., and King, T. E. (eds.): Interstitial Lung Disease, 4th ed. Hamilton, Ontario, B. C. Decker, 2003, pp. 452-484.*

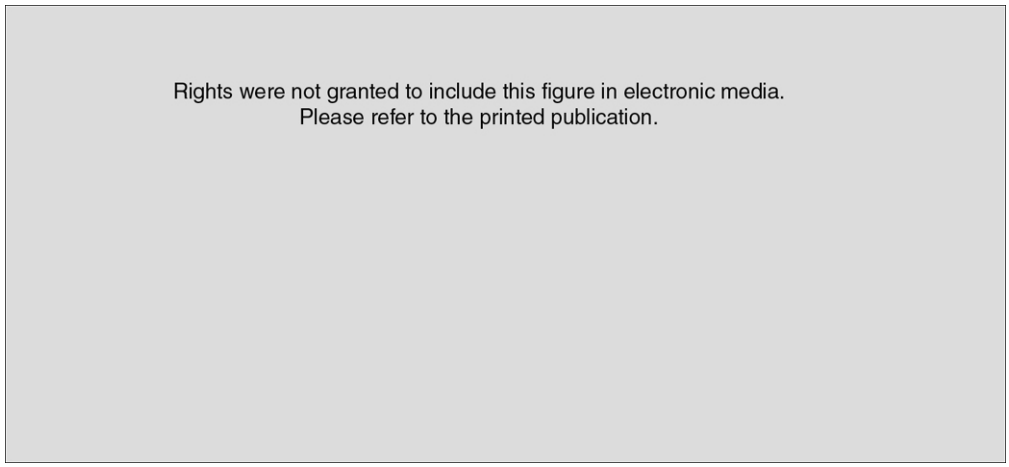

Figure 28–1 Hypersensitivity pneumonitis from cockatiel antigens in an adolescent. Chest radiograph shows bilateral reticulonodular infiltrates. High-resolution computed tomography scan shows diffuse, multiple fine nodules. *(Courtesy of Robin Deterding, M.D., University of Colorado, Denver, CO. From Fan, L. L. Pediatric interstitial lung disease.* In *Schwarz, M. I., and King, T. E. [eds.]: Interstitial Lung Disease, 4th ed. Hamilton, Ontario, B. C. Decker, 2003, pp. 134-151.)*

small percentage of these develop HP.[27] Long-term follow-up has shown that the simple presence of precipitins does not increase the likelihood of developing the condition.[26] Conversely, not all individuals with symptomatic HP have positive precipitins. In reported pediatric cases, positive precipitins were found in 93 percent (120 of 129) of the children tested. Because the quality of antigen material may vary in commercial laboratories, it is preferable to obtain a sample of the suspected antigen directly from the original source and test it against the patient's serum in a reputable laboratory. Researchers have reported using fluorometry to quantify serum IgG antibody against pigeon antigens, with decreasing antibody titers used as a measure of successful antigen avoidance.[89]

To document recurrence of symptoms shortly after exposure to a putative antigen, provocation challenge (inhalation of the antigen in controlled laboratory conditions) has been used in adults.[110] It has not been employed often in children, however, with only 23 of the 133 children reported in the literature having undergone such a challenge, in which 14 (61%) were positive. An in vitro measurement of antigen-induced lymphocyte proliferation compared with unstimulated lymphocyte cultures obtained from BAL or blood also has been described.[101]

Classic features of acute HP on chest radiographs and high-resolution CT scans include poorly defined centrilobular micronodules, with predominance in the upper and middle lung zones (see Fig. 28–1). On high-resolution CT, additional characteristic features are widespread ground-glass attenuation and air trapping, which is especially prominent on expiratory scans.[132] A small percentage of patients also may have lung cysts.[50] In the more chronic phase, diffuse interstitial infiltrates may predominate, with progression to fibrosis and honeycombing that are indistinguishable from usual interstitial pneumonia.[83] Instances of negative CT examinations in patients with biopsy-proven disease have been reported.[57] In the reported pediatric cases of HP, chest radiographs were abnormal in 93 percent (113 of 132).

Pulmonary function tests typically show a restrictive defect, sometimes with an obstructive component. In reported pediatric cases, the mean (± standard deviation) FEV_1 and FVC in the children tested were 54.1 percent (± 19.8) and 52.4 percent (± 20.2) predicted. The pressure-volume curve is shifted down and to the right, consistent with decreased compliance. Low lung volumes and diminished diffusion capacity also are present.[32] Although resting room air oxygen saturation may be normal, desaturation with exercise or sleep may occur. With long-standing disease, resting oxygen desaturation can be seen. In the pediatric cases in which oxygenation was documented, 82 percent (59 of 72) had hypoxemia at rest. Pulmonary hypertension may be present with advanced disease, but in contrast to other forms of pediatric ILD, it may reverse completely with successful treatment.

BAL fluid, obtained by flexible fiberoptic bronchoscopy, typically shows a significant lymphocytosis. In adults, a low CD4/CD8 ratio frequently accompanies the lymphocytosis. The reported normal ratio (mean [± standard error of the mean]) varies from 1.9 (± 0.2) in young normal controls to 7.6 (± 1.5) in elderly controls.[90] Children with HP also have marked lymphocytosis in the BAL compared with healthy pediatric controls (80% versus 12%). Although the CD4/CD8 ratio also may be low compared with that of healthy adults, it is not significantly different from the ratio (0.6) reported in healthy children without lung disease.[111] As with positive precipitins, the presence of BAL lymphocytosis in exposed individuals who are asymptomatic does not predict the development of HP.[26] Analysis of induced sputum shows that it does not reflect accurately the lymphocytosis seen in BAL.[31]

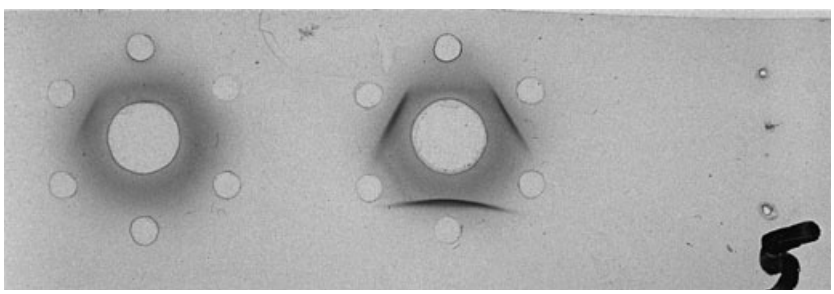

Figure 28–2 Serum-precipitating antibodies against cockatiel antigens in the patient shown in Figure 28-1. *(Courtesy of Robin Deterding, M.D., University of Colorado, Denver, CO.)*

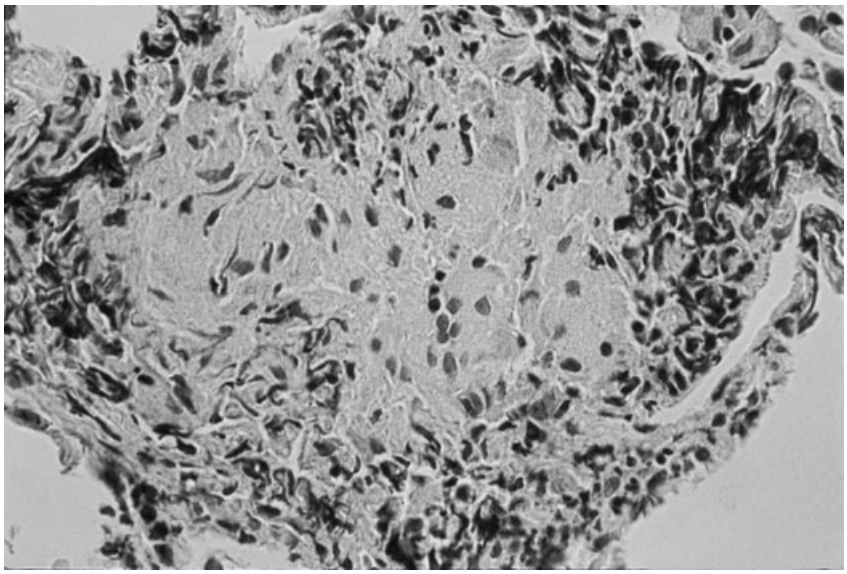

Figure 28–3 Transbronchial biopsy specimen from the patient in Figure 28–1 shows a poorly formed granuloma consistent with hypersensitivity pneumonitis. *(Courtesy of Robin Deterding, M.D., University of Colorado, Denver, CO.)*

Lung tissue obtained by transbronchial (limited to older children and adults) or transthoracic biopsy shows the characteristic histologic features described previously (Fig. 28–3). Of the 83 reported pediatric cases of HP, nine biopsy specimens were obtained, and all showed typical histologic changes.

TREATMENT AND OUTCOME

The mainstay of treatment is eliminating exposure to the offending antigen. In the literature, exposure was eliminated in 97 percent of the pediatric cases (100 of 103), with improvement achieved in all but one fatal case.

The use of corticosteroids often results in rapid improvement of symptoms and reversal of radiographic and lung function abnormalities unless irreversible changes in the lung have occurred. Corticosteroids were used in 70 percent (74 of 106) of the reported pediatric cases, with a positive response occurring in all but the one fatal case. Generally, prednisone was given at 0.5 to 2 mg/kg daily for 2 to 4 weeks, followed by a taper, although specific regimens have not been studied scientifically. Pulsed methylprednisolone also has been used.[19]

The outcome of children with HP, when properly diagnosed and treated, is excellent. In the 106 pediatric cases of HP with reported outcomes, 104 improved or became asymptomatic, 1 patient remained unchanged, and 1 patient died. In this last case, an 11-year-old girl developed classic features after several years of exposure to budgerigars and other birds.[143] Despite removal of the offending antigens and treatment with corticosteroids and penicillamine, she died of respiratory failure 13 months later. The overall prognosis for children with HP is excellent, provided that a prompt diagnosis is made and appropriate treatment consisting of antigen removal and the judicious use of corticosteroids is instituted.

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CHAPTER 29

Pleural Effusions and Empyema

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Collections of fluid in the pleural space have been described in the literature as transudates, pleural effusions, exudates, purulent pleurisy, parapneumonic effusions, empyema, and complicated empyema. A great deal of inexactitude exists in the use of these terms, and comparing methods of diagnosis and management from one study to another is difficult. Standardized definitions are used in this chapter to describe pleural fluid collections.

The term *transudative pleural effusion* refers to fluid in the pleural space that is a nonpurulent effusion and typically nonpneumonic in origin. The term *purulent effusions* refers to effusions that are more cellular (exudative) and typically pneumonic in origin. The term *empyema* describes purulent effusions with chemical or microbial evidence of a severe process requiring drainage. *Complicated empyema* describes the processes associated with loculations or a fibropurulent rind requiring aggressive manipulations for cure. *Parapneumonic effusion* is a general term referring to any pleural exudative process resulting from an inflammatory process in the lung. Although these definitions are arbitrary and the literature is inconsistent in the use of these terms, a standard approach to the classification of these four types of effusions and their management has evolved.

The first description of parapneumonic infection is attributed to Hippocrates, who in the 4th century BCE advocated incision and drainage of empyema 2 weeks after the onset of symptoms. Since then, the physiology and microbiology of effusions have been described, and parapneumonic diseases and the management of fluid collections of the pleural space have been defined. In most cases, the directions of Hippocrates still are relevant: ". . . set him upon a stool, which is not wobbly; someone should hold his hands, then shake him by the shoulders and listen to see on which side a noise is heard. And right at this place—preferably on the left—make an incision, then it produces death more rarely."[58]

This description of open drainage to normal atmospheric pressures was recognized to be associated with significant mortality rates from hemodynamic instability in 1918, when the Empyema Commission of the United States Army recommended that the practice be abandoned[57]; thereafter, closed-tube drainage was introduced, and mortality rates decreased. Developments in radiology, antimicrobials, and surgery resulted in further improvements in care. Today, the major cause of mortality is related to the underlying disease because the management of parapneumonic effusions in children is largely successful and without residual morbidity. The physician must understand the risks for development of pleural effusion and empyema and indications for thoracentesis, implications of the results of pleural fluid studies, and the optimal medical and surgical management of the effusions and empyema.

EPIDEMIOLOGY

Parapneumonic effusions are known and expected complications in children with respiratory tract infections. The frequency of effusions may be 20 percent of patients with viral or mycoplasmal pneumonia[28,34,86] and 75 percent of patients with proven *Staphylococcus aureus* pneumonia.[7] Empyema has been reported to occur in 6.3 to 23 of 1000 admissions of children,[64,89] and as a complication of pneumonia in 1 percent of children in the United States.[19] One of the most extensive studies of empyema in children was from Dallas, Texas; 12 episodes per year during a 19-year period were described.[30] A more recent report from Houston, Texas, suggested an increase in the overall number of hospitalizations per 10,000 admissions for empyema from 1993 to 1998 compared with 1999 to 2002 (8.5 versus 17.8)[89]; investigators elsewhere also have reported increases.[16,83]

In adults, empyema follows a primary pulmonary process in 55 to 60 percent of cases, surgery in 20 percent, trauma in 6 percent, spontaneous pneumothorax in 35 percent, and other causes in 2 percent.[57,84] Hoff and associates[41] found that 11 percent of cases in children had an underlying illness, including hyper-IgE syndrome, hypogammaglobulinemia, acute lymphocytic leukemia, cerebral palsy, Down syndrome, and postsurgical and congenital thrombocytopenia. The remaining cases were related to a primary pulmonary process. Freij and associates[30] found a rate of 8.3 percent with similar risk factors. The mortality rate is highest in the first 2 years of life. After reaching 2 years of age, children have better outcomes than do adults; generally, they have less intrinsic lung disease, have greater elasticity of their chest wall, and heal more quickly than older patients do.[15,35]

PATHOPHYSIOLOGY

The pleurae are mesodermally derived tissues that are approximately 30 to 40 μm thick and permeable to liquid and gas.[9,51] The parietal pleurae, which adhere to the chest wall, are fed from the intrathoracic and superior phrenic arteries and have sensory innervation. The visceral pleurae are splanchnic in origin, with blood flow from the pulmonic and pericardiophrenic arteries and no sensory innervation. The lymphatic structures of the visceral pleurae are microscopic vessels called *lacunae*. They are denser in the lower lobes to accommodate greater venous pressure. Parietal structures called *stomas* are 4- to 10-μm-diameter pores that connect the pleurae to lymphatics. These stomas have valvular function during expiration and inspiration and can filter large structures, such as red blood cells and macrophages.[51] The venous drainage of visceral pleura is into the pulmonic veins and from the parietal pleura into intercostal and bronchial veins. Lymphatic structures are woven below and around the mesothelial cells and ultimately drain into mediastinal, intercostal, and mammary nodes. The structure of the pleura is a surface of mesothelial cells and layers of lymphatic sinuses and pores, elastic fibers, and loose vascular connective tissue, with a fibroelastic layer covering the lungs and chest wall.[51]

The precise flow and distribution of pleural fluid have been debated for some time. Because of the differences in venous pressure in the intercostal and pulmonary veins, fluid is thought to flow from the parietal venous system to the visceral system by drifting from the high-pressure parietal tissues into the negative-pressure pleural space, with reabsorption on the visceral side. The latter is caused by a low venous pressure system on the parietal side and the high oncotic pressure of the pulmonic venous system compared with the pleural space.

The dynamics of this process are described in Starling's equation:

$$\text{Fluid movement} = k \cdot [\{HP_c - HP_{ip}\} - \{COP_c - COP_{ip}\}]$$

in which *k* is the filtration coefficient (a measure of the permeability of capillaries to fluid), *HP* is the hydrostatic pressure, and

TABLE 29–1 Characteristics of Pleural Effusions

	Transudative	Purulent Effusion	Empyema	Complicated Empyema
Appearance	Serous	Thin exudate	Turbid	Thick pus
Mean WBC	1000	5300	25,500	55,000
PMN (%)	50	>90	>95	>95
Protein (fluid/serum ratio)	<0.5	>0.5	>0.5	>0.5
LDH (fluid/serum ratio)	<0.6	>0.6	>0.6	>0.6
LDH (IU/L)		>200	>200	>1000
Glucose (mg/dL)	>60	<60	<60	<40
pH*	7.4-7.5	7.35-7.45	7.2-7.35	<7.2
Imaging	Fluid	Fluid	Fluid	Loculations, thick peel, scoliosis

**Should be examined immediately or stored at 0° C.*
LDH, lactate dehydrogenase; PMN, polymorphonuclear neutrophils; WBC, white blood cell count.

COP is the colloid osmotic pressure of capillaries (*c*) and the intrapleural compartment (*ip*).[14] The consensus is that although the impact of Starling forces on venous flow may play a role in the normal situation, parietal lymphatics absorb most of the excess fluids in pathologic situations and play an important role in normal physiology as well, removing 250 to 500 mL/day in adults.[92] They are the only mechanism for absorbing cells and other debris from the pleura.

A few studies in the past have shown that 20 mL of pleural fluid is found normally in 30 percent of resting adults, 70 percent of exercising adults,[7] and 46 to 67 percent of postpartum women.[41] Some fluid may be transported from the peritoneum to the pleura through small communications.[7] This hypothesis is supported by reports of patients with infected abdominal fluid and pleural effusions in whom the same organisms are recovered in both sites.[13]

The *raison d'être* of the pleural space is unknown. Some mammals, such as elephants, do not have a pleural space.[103] This fact has served as the rationale for using some methods of management of pleural disease that have included chemical obliteration of the pleural space. In the normal situation, pleural fluid in small amounts is a necessary requirement for optimal lubrication of the pleural space and for mechanical coupling of the lung and chest wall.[51] The accumulation of excess fluid (i.e., effusion) occurs in a limited set of circumstances, through excess production or deficient absorption. Increased production occurs when vessels are leaky (e.g., in septic shock) or active secretion of fluid with mesothelial inflammation (e.g., pleural infection) is present. Decreased absorption occurs with decreased oncotic pressure (e.g., nephrosis), increased pulmonary hydrostatic pressure (e.g., congestive heart failure), or lymphatic obstruction (e.g., malignancy).[102]

The mechanisms behind pleural effusions may vary among different infectious diseases. Effusion can be a "sympathetic" pleural response to a bacterial infection in the lung associated with inflammatory cytokines and altered venous or lymphatic drainage because of local edema. Direct or hematogenous extension of a bacterial process can occur in the pleura. *Mycoplasma* particularly is pathogenic in patients with sickle-cell disease, presumably because of pulmonary sludging, which increases pulmonic venous drainage pressures and results in accumulation of effusions. In pneumococcal disease, effusions often develop several days after the acute infection, when bacteria no longer can be recovered. These effusions may be related to immune complex disease.[14] In patients with tuberculosis, the most common cause of pleural effusions is thought to be the rupture of an old granuloma into the pleural space, with a hypersensitivity response similar to the skin test response,[15] which partly explains the low yield in cultures.

After an inflammatory process is initiated, it tends to progress through three classic stages.[3] The first, defined as a *purulent effusion*, is the acute exudative stage, with a thin pleural exudate characterized by normal glucose, lactate dehydrogenase (LDH), and pH. The second transitional fibropurulent stage, categorized as *empyema*, is characterized by turbid fluid, decreased glucose concentration (<60 mg/dL) and pH (7.2 to 7.35), and elevated LDH (>200 U/L). The third chronic organizing stage is notable for a very low pH (<7.2) and glucose level (<40 mg/dL), LDH concentration greater than 1000 U/L, and development of loculations and peel. This fluid is found in patients with *complicated empyema*.

Often, the pleural fluid quality can be predicted without sampling the fluid based on the clinical course of the patient. A patient with anasarca caused by heart failure or nephrosis with bilateral effusions may not need to have an effusion analyzed if otherwise stable. If the same patient has fever, examination of the fluid is necessary to exclude a secondary bacterial infection. Analysis of the pleural fluid is most helpful when the underlying disease is unknown or when a primary pulmonic process is suspected. When patients have effusions caused by hydrostatic imbalance, the effusion is a transudate. Its protein and cell count do not exceed the range of normal pleural fluid (5000 cells/mm^3 and <2 g of protein).[7,92] Patients who have an active inflammatory process may have an exudate (defined by excess protein and cells). In children, the most common cause of exudative pleural processes is pneumonia. In adults, most pleural effusions are related to congestive heart failure or malignancy,[56] but pneumonia is the most common cause of empyema.[71] Table 29–1 summarizes the general differences among pleural effusions.

Table 29–2 lists causes of effusions. Some of these causes, particularly iatrogenic causes, such as invasive procedures and drugs, are important to consider in the differential diagnosis of a difficult case. Others are associated with specific syndromes, such as adult respiratory distress syndrome[94] and yellow nail lymphedema syndrome.[97] Motor vehicle accidents have been identified as a common cause of serosanguineous effusions when disruption of normal mechanical lung function and hematoma occur.[81]

MICROBIOLOGY

Among children with parapneumonic effusions, no prospective study has established firmly the frequency with which effusions occur and how many are associated with particular microbes. Although respiratory viruses infrequently cause symptomatic effusions, the sheer number of cases and the presence of asymptomatic cases likely would implicate viral infection as the most common cause. Definite viral disease has been associated with cytomegalovirus, Epstein-Barr virus, measles, and adenovirus.[30,31,45,63] Other pathogens, such as *Mycoplasma* and *Chlamydia*, are difficult to diagnose but may account for a significant number of pneumonic infections in older children and adolescents that

TABLE 29–2 Causes of Pleural Effusion

Capillary leak
Sepsis syndrome
Vasculitis associated with immune complex disease
Connective tissue diseases
Inflammatory bowel disease
Malignancy (lymphoreticular, sarcoma, neuroblastoma)
Toxins (e.g., TSST-1)
Drugs (phenytoin, isoniazid, nitrofurantoin, amiodarone, methotrexate, bleomycin)
Myxedema
Trauma
Increased hydrostatic pressure
Congestive heart failure
Sickle-cell disease
Pulmonary venous hypertension
Superior vena cava syndrome
Pregnancy
Decreased oncotic pressure
Nephrosis
Cirrhosis
Protein malnutrition
Obstructed lymphatics
Congenital lymphangiectasia
Yellow nail syndrome
Radiation injury
Neoplasia (metastatic disease)
Pneumonia
Pleural inflammation
Pneumonia
Lung abscess with pleural fistula
Pleural infection (e.g., tuberculosis)
Esophageal rupture
Pancreatitis
Iatrogenic
Drugs
Central line misplacement

TSST-1, Toxic shock syndrome toxin-1.

may be associated with effusions in 20 percent of cases.[28,31,86] Viral, mycoplasmal, and chlamydial organisms rarely are isolated in patients with effusions requiring intervention.

The bacteriology of empyema is constantly evolving. Several past articles have established the role of different bacterial pathogens in childhood effusions. A study of 227 children by Freij and colleagues[30] published in 1984 found *Staphylococcus aureus* (29%), *Streptococcus pneumoniae* (22%), and *Haemophilus influenzae* (18%) as the three most frequent causes of parapneumonic effusions (Table 29–3). Subsequent studies show that the frequency of these pathogens has been affected by vaccine[1,16] and antibiotic use. Pathogens are shown in Table 29–4. *H. influenzae* and *S. pneumoniae* vaccines have led to a decreased incidence of these agents as a cause of empyema, whereas *S. aureus* has become the leading cause with antibiotic-resistant strains, accounting for most cases in community-acquired disease.[89] Among cases of *S. pneumoniae* infections, nonvaccine strains are increasing in number and may become a more severe threat.[17,24] Certain groups of children (e.g., neonates,[30] immunocompromised hosts, patients with preexisting chest tubes that become infected with nosocomial pathogens, patients with a ruptured viscus, and patients with foreign body aspiration) are at higher risk for acquiring gram-negative infections.

Administration of antibiotics before the diagnosis of empyema is made influences the recovery of organisms. In one report, the incidence of prethoracentesis antibiotics was 71 percent in culture-negative effusions and only 41 percent in culture-positive effusions.[41] Pretreatment with antibiotics may be associated with a decrease in the number of positive blood cultures and in the number of patients from whom *S. pneumoniae* are recovered.[71] Freij and associates[30] reported the frequency of parapneumonic effusions occurring in children with pneumonia caused by specific pathogens. The rates of effusion by organism were as follows: group A streptococcus, 86 to 91 percent; *S. aureus*, 72 to 76 percent; *S. pneumoniae*, 57 percent; *H. influenzae*, 49 to 75 percent; *Mycoplasma*, 21 percent; and adenoviruses, 11 to 33 percent.

TABLE 29–3 Distribution of Pathogens by Age

	No. Cases					
Pathogen	***0-6 Months***	***7-12 Months***	***13-24 Months***	***25 Months–5 Years***	***6-15 Years***	***Total***
Staphylococcus aureus	27 (41)*	11 (17)	10 (15)	6 (9)	12 (18)	66 (100)
Streptococcus pneumoniae	7 (14)	13 (27)	16 (33)	8 (16)	5 (10)	49 (100)
Haemophilus spp.	4 (10)	15 (38)	18 (45)	3 (7)	0	40 (100)
Sterile	3 (6)	9 (17)	17 (31)	11 (20)	14 (26)	54 (100)
Mixed bacteria	6 (60)	1 (10)	0	1 (10)	2 (20)	10 (100)
Streptococci	1 (20)	0	1 (20)	2 (40)	1 (20)	5 (100)
Gram-negative rods	2 (67)	0	1 (33)	0	0	3 (100)
All cases	50 (22)	49 (21)	63 (28)	31 (14)	34 (15)	227 (100)

**Numbers in parentheses = percentage of cases.*

From Freij, B. J., Kusmiesz, H., Nelson, J. D., et al.: Parapneumonic effusions and empyema in hospitalized children: A retrospective review of 227 cases. Pediatr. Infect. Dis. J. 3:578-591, 1984.

TABLE 29–4 Percentage of Pathogens Recovered in Purulent Effusions from Children

Site/Years (No. Patients)	***Staphylococcus aureus***	***Streptococcus pneumoniae***	***Haemophilus influenzae***	**Other Pathogens**	**Sterile**	**Reference**
Dallas/1964-1982 (227)	29	22	18	8	24	30
Nashville/1977-1989 (61)	11	34	3	11	39	40
Washington, D.C./1973-1985 (33)	15	12	21	52	NR	13
Nigeria/1989-1991 (57)	63	NR	NR	37	NR	58
Israel/1972-1981 (37)	14	41	NR	35	11	63
Dallas/1992-1998 (135)	8	32	1	13	46	23
Houston/2001-2002 (47)	19	9	NR	4	68	89

NR, not reported.

Anaerobes were sought carefully by Brook and Frazier,[13] who found them infrequently in patients younger than 6 years of age. The anaerobes rarely were found in patients with primary pneumonia, occurring most often in patients with lung abscess and aspiration pneumonia.[13] In older patients (7-17 years old), anaerobes were recovered as isolated pathogens in 44 percent of cases.[13] Virtually every bacterial organism has been associated with pleural effusion at one time or another. *Brucella*,[47] *Francisella tularensis*,[82] and *Yersinia enterocolitica*[44] may be associated with the development of pleural effusions. The diagnosis in such cases often is suggested by a unique history in the patient.

Mycobacterial and fungal effusions are rare in children but are well described. In four published reviews, only two patients (from Nigeria) were reported to have *Mycobacterium tuberculosis*.[30,41,59,63] In a series of 303 children younger than 2 years of age with tuberculosis, 3.3 percent had an effusion.[40] In adolescents with tuberculosis, the incidence of effusion likely approximates that of adult disease. In one series of adult patients with primary tuberculous disease, pleural effusion occurred in 29 percent of cases.[20] In another adult series, primarily of reactivation disease, pleural effusion occurred in only 1 percent of the patients.[35] Whether or not co-infection with human immunodeficiency virus (HIV) is increasing the incidence of disease is controversial.[27]

Histoplasmosis has been associated with pleural effusion in 0 to 6 percent of childhood histoplasmosis cases.[73] Blastomycosis has been associated with pleural effusions in 0 to 40 percent of cases.[72,90] Effusions resulting from other fungi (e.g., *Coccidioides*, *Aspergillus*) have been described.[57] Parasitic diseases manifesting with effusions are uncommon but are found in patients with *Entamoeba histolytica* disease, most often from rupture of a hepatic abscess into the pleural space.[57] Echinococcal disease also has been reported.[29]

Pleural effusion associated with adult HIV infection has been reported in 14.6 percent of hospital admissions in one series in which 67 of 160 cases were infectious. Of those cases, 50 were associated with bacterial pneumonia, 10 with tuberculosis, and 5 with *Pneumocystis carinii* pneumonia.[2] Another report on patients infected with HIV suggested that empyema was seen primarily in patients with intravenous drug abuse.[10] We have not seen empyema frequently in our HIV-infected patients, perhaps because of the more recent use of more effective antiretroviral therapy.

Drug resistance in community-acquired pneumonia complicates the management of parapneumonic effusions. Intermediate or fully resistant *S. pneumoniae* were found in 12.8 percent and 10.1 percent of isolates in a study from multiple pediatric centers from 1993 to 2000; 7.5 percent were cephalosporin-resistant.[96] Methicillin-resistant *S. aureus* (MRSA) is a growing concern in empyema. Reports since 2000 have shown that 22 to 78 percent of isolates of *S. aureus* were methicillin-resistant, with many being community acquired.[43]

DIAGNOSIS

CLINICAL PRESENTATION

The clinical presentation of transudative effusions compared with purulent effusions ordinarily is distinctive, but a continuum of symptoms is shared by both. Many of the symptoms associated with pleural processes are caused by the underlying disease that precipitated the effusion, rendering a distinct syndrome difficult to recognize in patients. Disease caused by some pathogens (e.g., anaerobes, fungi, mycobacteria) also may follow a more insidious course, obscuring the symptoms of effusion. A history always should be obtained to identify systemic diseases, such as immunodeficiency diseases, cancer, and rheumatic diseases, or medications that may be associated with effusions.

Symptoms most specific for parapneumonic processes are dyspnea and pleuritic pain. Dyspnea occurs when the volume of the effusion mechanically interferes with breathing, or when pain prevents adequate gas exchange. Pain occurs with irritation of the parietal pleura and on inspiration (i.e., pleurisy). Fever is generated by the inflammatory response and pathogen-specific components (e.g., lipopolysaccharide, toxins). With an acute bacterial process, the fever can be high and hectic, mimicking the fever that occurs with an abscess. Patients in the chronic organizational phase generally have less fever. Cough and malaise are secondary symptoms. Hemoptysis and purulent sputum also may occur. The onset of symptoms of a purulent effusion may be delayed in time and distinct from the symptoms found at the onset of the pneumonia in older children; infants usually have no symptom-free period.[15] In the early phases of effusions, the patient may have no symptoms.

The physical examination usually is revealing. The child is tachypneic in more than 70 percent of cases, but breathing is shallow as a result of the child's attempt to minimize pain. Fever and cough usually occur in more than 90 percent of patients with purulent effusions.[59] The patient may appear toxic, with acute infection. Patients often posture toward the affected side. Classically, auscultation reveals a decrease in breath sounds and occasionally detects a pleural rub, but pleural rubs often are absent in a very young child. Rales from an associated pneumonia may be heard. Depending on the stage of the process, percussion may reveal a level of dullness associated with free-flowing effusion. As the process organizes, it may be less evident. Empyemas can erode through the chest wall into the subcutaneous tissue (i.e., empyema necessitatis) or into a bronchus (i.e., bronchopleural fistula).

IMAGING

The diagnosis most often is made by radiographic examination of the chest. Consolidation of a lobe of the lung is present, with an effusion obscuring the diaphragm (Fig. 29–1). A standard posteroanterior standing view reveals blunting of the costal diaphragmatic gutter. As fluid tracks along the lateral and posterior chest wall, a meniscus configuration is seen. Distinguishing it from pleural thickening may be difficult, and in such cases, a decubitus or cross-table view of the chest allows free-flowing fluid to layer out on the dependent chest wall. In older children and adults, a decubitus layer of fluid of more than 10 mm is considered a sufficient volume of fluid to attempt to extract by thoracentesis.[54] With large volumes of fluid (>1000 mL),[85] compression of the lung and shift of the trachea away from the effusion (Fig. 29–2) may occur. As an empyema develops and organizes, discrete pockets of fluid (i.e., loculations) may form within the pleural cavity (see Fig. 29–2). Occasionally, loculations are confused with lung abscess. Scoliosis also is well defined by the chest radiograph and occasionally is used as an indication for surgery.[41] The observation of an air-fluid level in the pleural space signifies that air has been generated in the pleural space by gas-forming organisms or has entered through a pneumothorax, perforated viscus, or bronchopleural fistula.

Ultrasonography has shown great utility in providing better guidance for thoracentesis of pleural fluid. It is noninvasive and allows empyema to be defined by showing internal echoes and septations (see Fig. 29–2).[90,105] Transudates uniformly are anechoic, although approximately one third of exudates also are anechoic.[105] Ultrasonography is not as precise as is computed tomography (CT) in differentiating a lung abscess from an empyema. CT and magnetic resonance imaging occasionally are required to distinguish parenchymal from pleural disease, identify a non-opaque foreign body, or locate a fistula.[92] CT particularly is useful in a patient whose chest radiograph shows total

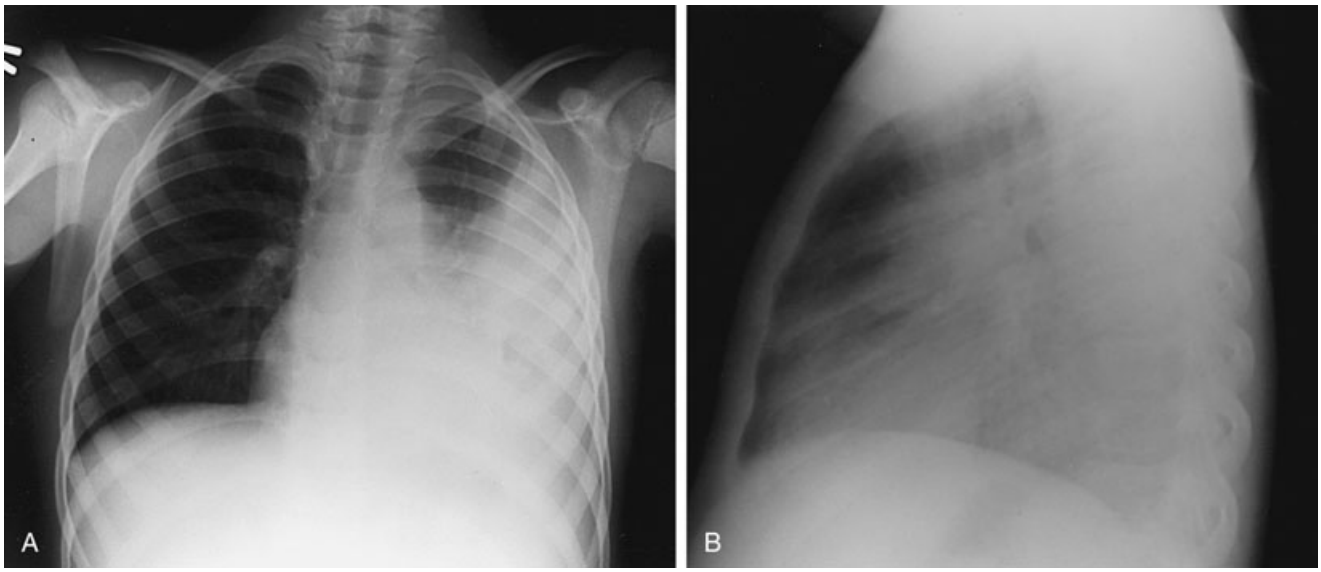

Figure 29–1 Posterior **(A)** and lateral **(B)** chest radiographs show a left pleural effusion in a 9-year-old girl who had symptoms of chest wall pain, fever, and vomiting. After 1 week, chest wall pain and shortness of breath continued, and she was admitted to the hospital and treated with cefuroxime and erythromycin for pneumonia. An ultrasound examination performed 2 days later showed a large pleural effusion that was drained, with a glucose level less than 20 mg/dL, lactate dehydrogenase level greater than 99,000 U/L, protein level 4.5 g/dL, and white blood cell count 51,000 mm^3. Gram stain of the fluid showed gram-positive cocci, but the culture was negative. After failure to respond to intravenous antibiotics alone, the patient was taken to the operating room, where an empyema and several small abscesses were drained, along with decortication and repair of multiple bronchopleural fistulas. The patient was discharged 9 days later and received an additional 10 days of intravenous imipenem-cilastatin. She was well 1 month after hospital discharge.

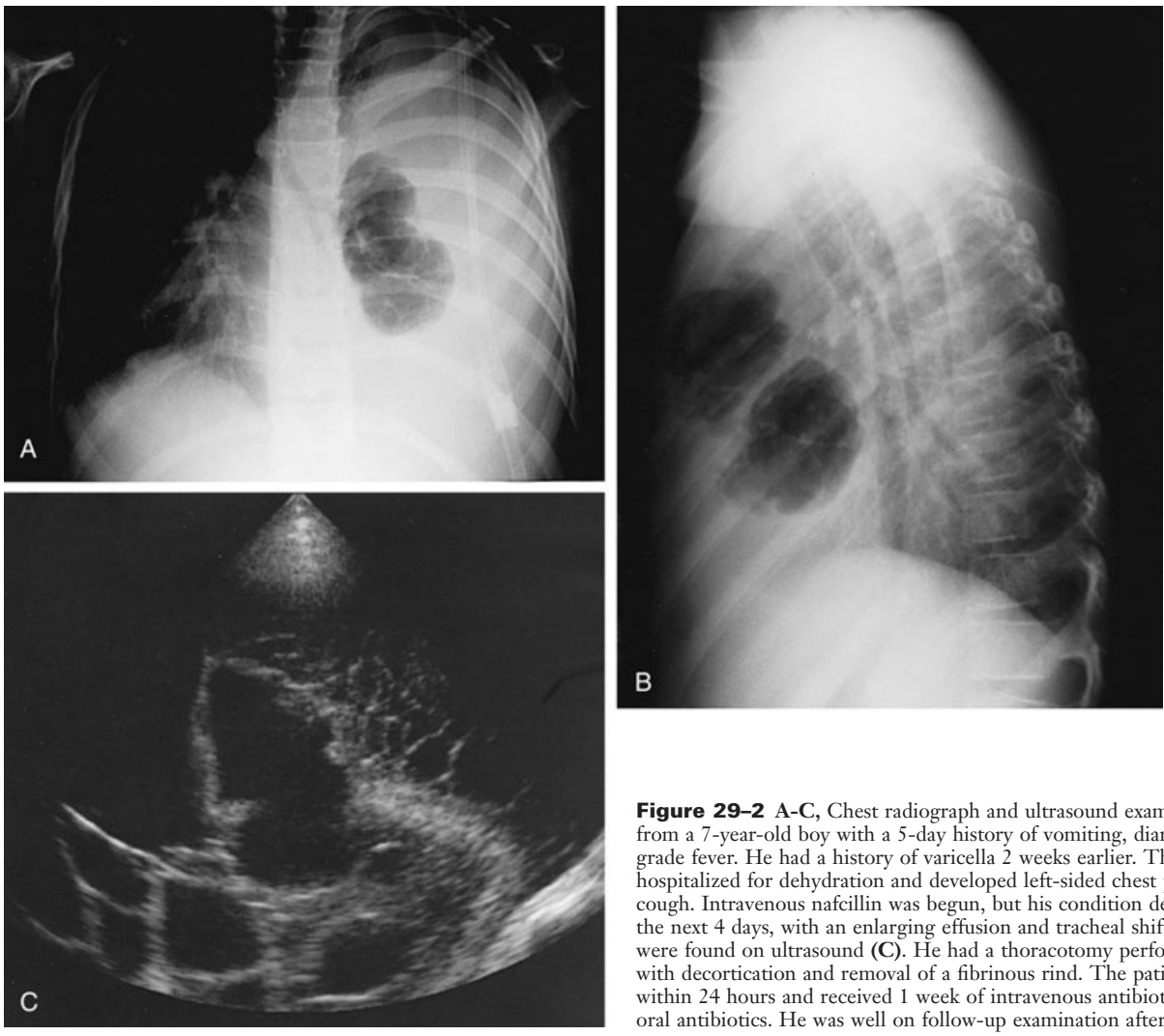

Figure 29–2 A-C, Chest radiograph and ultrasound examination images from a 7-year-old boy with a 5-day history of vomiting, diarrhea, and low-grade fever. He had a history of varicella 2 weeks earlier. The patient was hospitalized for dehydration and developed left-sided chest pain and a mild cough. Intravenous nafcillin was begun, but his condition deteriorated over the next 4 days, with an enlarging effusion and tracheal shift. Loculations were found on ultrasound **(C)**. He had a thoracotomy performed on day 6 with decortication and removal of a fibrinous rind. The patient was afebrile within 24 hours and received 1 week of intravenous antibiotics and 1 week of oral antibiotics. He was well on follow-up examination after discharge.

opacification of the lung, and such tomographic scanning is considered by some physicians to be the study of choice in this situation.[41] Ultrasound can have false-negative results, and CT can have false-positive findings on examination of pleural effusions. One study of adults showed that neither ultrasound nor CT effectively predicted the stage of the effusion or predicted the surgical outcome.[46]

THORACENTESIS

Thoracentesis plays an important role in the management of parapneumonic effusions and in 90 percent of adult cases yields useful information.[22] Some investigators would argue that primary video-assisted thoracic surgery (VATS) should be performed without thoracentesis.[23] Fluid should be obtained from the pleural cavity if fluid is adequate in volume and anatomically accessible and if a microbial diagnosis has not been made or presumed and antibiotic therapy is intended, or if pulmonary function is compromised by the effusion and imaging does not reveal evidence of organization to determine whether further intervention is necessary.

The volume and location of the fluid can be determined precisely by ultrasound examination if the physical examination does not allow localization of the fluid. When a healthy child with apparent or culture-confirmed pneumococcal pneumonia that is community acquired has a small pleural effusion, thoracentesis usually is not required. Small effusions generally can resorb, and a 10 to 50 percent chance of recovering the etiologic organism from blood cultures exists in empyema.[5,30] We suggest, however, that when the clinical presentation is atypical or a moderately sized effusion exists, thoracentesis usually is indicated to define the microbial process. Atypical situations include a history of trauma, foreign body aspiration, prolonged or chronic disease, and underlying systemic diseases (e.g., congestive heart failure, malignancy).

In a classic article published in 1972, Light and associates[56] established the methodology by which transudates could be differentiated from exudates. Such criteria are valuable in determining if antibiotic treatment is indicated, particularly in patients with underlying diseases that predispose them to sterile effusions but who may have a comorbid infectious condition. An exudate was defined by any of the following criteria: a fluid-to-serum protein ratio of greater than 0.5, a LDH fluid-to-serum ratio of greater than 0.6, a glucose concentration less than 50 mg/dL, and a pH less than 7.2. This study was based on the results from 150 adult patients, 103 of whom had exudates.[56]

The criteria of Light and associates have been accepted and are used as guidelines in the management of parapneumonic effusions in adults and children. In 1984, Peterman and Speicher,[69] using adult data, recommended a two-step process to separate transudates from exudates using only the protein and LDH serum-to-fluid ratios in the initial evaluation. If a patient had an apparent exudate, additional studies, including cultures, stains, pH, and glucose, were indicated. Another study of 297 adults compared several criteria for separating transudates and exudates and concluded that the criteria of Light and associates still yielded a high sensitivity (98%) and a specificity of 77 percent. A pleural fluid cholesterol concentration greater than 60 mg/dL also was used and had a sensitivity of 88 percent and specificity of 91 percent for exudates.[79] Although the cholesterol was not recommended for routine use, it was suggested as an extra screening test for patients with congestive heart failure in whom diuretic therapy might lead to increased concentration of pleural fluid protein.[19,38,79]

After an exudate is verified, it must be decided whether chest-tube drainage or other procedures such as VATS are needed. This decision is made by examining several aspects of the pleural fluid. In 1980, Light and associates[55] described pleural fluid findings in adults with exudates in an attempt to determine which patients needed early chest-tube drainage of effusions. Thirty-seven adults with acute pneumonia and parapneumonic effusions were studied. Ten patients were considered to have complicated cases if they required chest tubes or had positive cultures at the time of thoracentesis. No clinical differences were found between the complicated and uncomplicated cases. Patients who required chest tubes had a pleural fluid pH of less than 7.0 and a glucose level less than 40 mg/dL. All patients with uncomplicated effusions had a pH greater than 7.2 and LDH less than 1000 mL. Patients with a pH between 7.0 and 7.2 fell into both categories. Anaerobes were recovered from 6 of 10 complicated cases, and *S. pneumoniae* was recovered from 15 of 27 uncomplicated cases. Cell count and protein analysis were not helpful in separating complicated from uncomplicated cases.

The application of these criteria to children has received limited study. In one series of 61 children, patients who required chest tubes or decortication had a mean pleural fluid pH of 7.24 or 7.10 compared with children who were treated with antibiotics only (pH 7.35). The mean pleural fluid glucose concentration was 74 g/L in the group treated with antibiotics, 10 g/L in the group treated with chest tubes, and 24 g/L in the group treated by decortication.[41] Another, more recent study confirmed that a pH less than 7.2 and low glucose were predictors of re-intervention.[65] These data suggest that the criteria of Light and associates for glucose are appropriate in children, but that the pH at which chest tubes are indicated may be higher for children than for adults. Availability of glucometers and pH meters at the site of thoracentesis is uncommon and might enhance decision-making.

Standard Gram stain and bacterial culture (aerobic and anaerobic) are indicated when thoracentesis is performed in patients in whom diagnosis of infection is considered. The Gram stain usually is positive in patients with bacterial infections; when such infections exist, Gram stain may be used to direct empiric therapy until culture results are known. In one study of children, 12 of 54 sterile effusions from patients with negative blood cultures had a positive Gram stain.[30] Some experts consider that a positive Gram stain indicates a more severe process and that such patients are more likely to require more invasive surgical procedures.[15]

When the total white blood cell count and differential are performed, supportive information may be gained. The results of a peripheral white blood cell count rarely change the clinical management of the patient. The total white blood cell count in empyema fluid can vary from 5000 to 625,000 cells/mm^3, with median values ranging from 5000 to 55,000 cells/mm^3.[30,41] Virtually all cells are neutrophils in bacterial infections. Marked eosinophilia may be seen in parasitic, fungal, tuberculous, or hypersensitivity disease and when blood is found in the pleural space.[15] Numerous small lymphocytes suggest malignancy, tularemia, or tuberculosis.[26,37,69,104] Other studies are required when the history suggests another underlying process. When tuberculosis is suspected by history, specific mycobacterial stains and cultures should be obtained. Identification of mycobacteria by stain and culture may be equivalent to the rate of identification of the disease process by pleural biopsy (approximately 25%).[67] Specific mycobacterial and fungal stains also should be obtained using a Ziehl-Neelsen/auramine stain and potassium hydroxide. Application of newer methods for diagnosis, such as polymerase chain reaction (PCR) and tuberculostearic acid by mass spectroscopy, may be indicated.

When malignancy or metastases are suspected, cytology is necessary.[37] Most effusions in children that prove to be malignant are of lymphoreticular origin. Amylase sometimes is measured and is elevated in cases of esophageal rupture, acute hemorrhagic peritonitis, or pulmonary infarction.[53,85] Countercurrent immunoelectrophoresis and other antigen detection systems

occasionally are used for diagnosis and are useful in pretreated individuals. They are widely available only for disease caused by *S. pneumoniae* and *H. influenzae* type b and not for disease caused by *S. aureus* or anaerobes. Samples for antigen detection require special preparation before analysis because of increased levels of pleural fluid protein, which can create false-positive test results.

Molecular diagnostics is bringing promising advances to the bacteriologic diagnosis of empyema. PCR technology has been compared with routine culture and bacterial antigen and has shown increased sensitivity and good specificity for the diagnosis of *S. pneumoniae* using 16S rDNA or MRSA using the mec-300 gene probe.[24,83] Turnaround times can be quite rapid, exceeding traditional culture methods. The sensitivity of PCR for tuberculous effusions ranges from 20 to 81 percent, and specificity ranges from 78 to 100 percent.[27]

ADDITIONAL DIAGNOSTIC STUDIES

An intradermal test should be applied to any child with a parapneumonic effusion to evaluate tuberculosis as a possible cause. One third of patients with tuberculous effusions have a negative purified protein derivative skin test result.[8] Early morning gastric aspirates are recommended if tuberculosis is suspected.[98] Blood cultures also are indicated because one third of patients can have positive blood cultures and negative pleural fluid Gram stain and culture results.[30] Sputum is a less reliable source from which to determine the microbial cause of an effusion but may be helpful in a patient with purulent sputum and a single predominant organism. It can be diagnostic in older children with reactivation or cavitary tuberculosis and in cases of blastomycosis and histoplasmosis. Cold agglutinins or *Mycoplasma* serology may confirm the cause of a pleural effusion, although the nonspecificity of cold agglutinins and the delay in the increase in antibody titers render these data of marginal use in the acute management of the patient. Viral cultures are useful in only the more unusual cases and generally provide information that is not helpful in the initial management of the patient. Rapid diagnostic antigen assays, such as the rapid tests for influenza A and B, may be useful in defining the primary cause of respiratory disease but do not help exclude secondary bacterial pathogens causing pneumonia and a parapneumonic effusion. When other diseases, such as Wegener granulomatosis[6] and systemic lupus erythematosus, are suspected, disease-specific tests, such as antineutrophil cytoplasmic antibody and antinucleic acid antibody are indicated.

MANAGEMENT

If a patient has an underlying disease process associated with pleural effusion and thoracentesis has excluded bacterial infection (e.g., normal protein and LDH fluid-to-serum ratio, normal glucose level, negative cultures, and Gram stain results), no further treatment is indicated other than treatment of the underlying disease. These patients continue to be at risk for developing infection of the effusion and may require repeat examination of pleural fluid at a later time if infection is suggested clinically.

If a purulent effusion is suggested by imaging, thoracentesis, or surgical findings, empiric antibiotic therapy is indicated. Therapy always should include antimicrobials that are effective against *S. aureus*, *S. pneumoniae*, and *Streptococcus pyogenes*. Empiric therapy choices are becoming more complex as drug resistance patterns continue to change and the ability to make a rapid diagnosis improves (e.g., PCR). In the past, acceptable regimens included nafcillin, first-generation cephalosporins, cefuroxime, and clindamycin. For patients without a confirmed microbial pathogen and sensitivity profile and low incidence of resistant organisms (community-acquired MRSA and penicillin-resistant *S. pneumoniae*), these past regimens based on the level of illness can be used. For mildly ill patients, one can begin with cefazolin or cefuroxime. For moderately ill patients, one can use clindamycin. We use vancomycin therapy for very ill patients and vancomycin and nafcillin for critically ill patients. For moderate to severe disease, cefotaxime or ceftriaxone is added.

In areas with high levels of antibiotic resistance (community-acquired MRSA, penicillin-resistant *S. pneumoniae*), in mild to moderate disease, clindamycin is indicated. For severely ill patients, vancomycin and a third-generation cephalosporin are appropriate. Some experts would add nafcillin, gentamicin, or both to this regimen. In patients who are vaccinated fully against *H. influenzae* (primary series and booster) and for whom the Gram stain is negative, empiric coverage against *H. influenzae* is not required. If *H. influenzae* is suspected, addition of a third-generation cephalosporin (i.e., ceftriaxone or cefotaxime) or single-drug use of cefuroxime or ampicillin-sulbactam would be effective. Ticarcillin-clavulanate, imipenem-cilastatin, meropenem, and piperacillin-tazobactam are useful if anaerobes are considered, particularly if aspiration is thought to play a role, such as in patients with abnormal swallowing. *Streptococcus milleri* has proven exceptionally difficult to manage and can be clindamycin-resistant.

In patients at risk for acquiring gram-negative disease (e.g., neonates, postsurgical patients), addition of an aminoglycoside or an advanced-generation cephalosporin (i.e., cefepime, ceftriaxone, or cefotaxime) is required. Extended-spectrum, semi-synthetic penicillins (e.g., ticarcillin-clavulanate, piperacillin-tazobactam) also are effective, as are carbapenems. Cefepime is helpful in nosocomial pneumonia/empyema because of its current efficacy for *Enterobacter* spp. In patients with renal failure or cephalosporin hypersensitivity, aztreonam is effective therapy for gram-negative infections. Ceftazidime or cefepime, broad-spectrum β-lactams with or without a β-lactamase inhibitor (i.e., ticarcillin–clavulanic acid, imipenem cilastatin, meropenem, or piperacillin-tazobactam), or aminoglycosides are indicated for *Pseudomonas* infection.

Surgical drainage is considered to be a crucial factor in resolving anaerobic infection. Clindamycin and metronidazole are effective, particularly when postsurgical infection or a ruptured gastrointestinal viscus is present. Upper respiratory tract anaerobes may be resistant to penicillins because of β-lactamase–producing oral flora (particularly *Prevotella* and *Porphyromonas* spp.).[13] In these situations, penicillin sensitivity should be documented before a penicillin is used as primary therapy.

For patients who have drug-resistant *S. pneumoniae*, most lung infections without associated central nervous system disease respond to high-dose penicillin (minimal inhibitory concentration >2 μg/mL) or cephalosporins (minimal inhibitory concentration >2 μg/mL).[52,68] In one study, the rates of effusion were similar between penicillin-susceptible and nonsusceptible *S. pneumoniae* isolates.[95] When the pneumococcus is highly resistant to penicillin and cephalosporins and the patient's disease fails to improve, therapy with vancomycin, clindamycin, or both may be required. Recovery of MRSA may require vancomycin therapy. Clindamycin sensitivity should be determined because many MRSA and methicillin-susceptible *S. aureus* isolates are resistant to clindamycin.[88] Susceptibility should be confirmed with performance of a "D" test. Intravenous clindamycin can be switched to oral clindamycin, offering a management advantage over vancomycin. New drugs such as linezolid have limited use at this time because of high cost but may have a role if vancomycin resistance becomes an issue. Daptomycin is specifically avoided in the lung because of its inactivation by surfactant.

With appropriate antibiotic therapy, the duration of fever in uncomplicated cases of purulent effusions usually is less than 48 to 72 hours.[66] When fever persists beyond 72 hours, surgical drainage may be required. The duration of antibiotic therapy is

based on the response of the patient to the medical and surgical therapy provided. In one series of pediatric patients, the total duration of antibiotic therapy for patients with severe pleural infections who did or did not have surgical drainage was comparable (approximately every 12 days).[41] The duration of combined intravenous and oral therapy was 12 to 24 days in the study reported by Freij and associates,[30] with patients with *S. pneumoniae* infection receiving the shortest courses of antibiotic therapy and patients infected with *S. aureus* being treated for longer periods (Table 29–5). A prudent standard is to continue treatment for a minimum of 1 week beyond the last febrile day.

Closed-chest-tube drainage has been the standard treatment of parapneumonic effusions in four classes of patients: (1) patients in whom thick, purulent material is found at thoracentesis; (2) patients with a pleural fluid pH level less than 7.35 and glucose level less than 60 mg/dL; (3) patients for whom antibiotic therapy has not been associated with a timely clinical response (72 hours); and (4) patients in whom pulmonary function is compromised, as shown by severe hypoxemia or hypercapnia. The chest tube is left in place until minimal drainage is noted (usually <50 mL/day).[25]

When closed-chest-tube drainage is not associated with clinical improvement and defervescence of disease, or if the lung parenchyma is trapped by the fibrinopurulent peel or fever persists, decortication often is required. If the pleural involvement is limited, a small incision (minithoracotomy) can be employed.[74] Ultrasonography and CT are required to define these conditions.

Decortication has been advocated as a more expedient way to manage patients. One study reported that patients who had decortication had shorter hospital stays (11.6 days) compared with patients who had thoracentesis or tube thoracostomy (28.3 days).[32] The total number of hospital days also was reduced in another study when patients treated with decortication (16.6 days) were compared with patients treated by chest-tube drainage (21.4 days).[41] In both cases, morbidity from the operative procedure was minimal. Decortication seems to have an advantage in advanced disease in which fibrosis in the pleural cavity has resulted in a large peel. Hoff and associates[41] used an empyema scoring system to assess the need for decortication. Any two of the following are considered indicative of severe disease and need for decortication: anaerobic infection, pH less than 7.2, glucose level less than 40 mg/dL, scoliosis, and lung entrapment.[41]

Other surgical techniques to reduce operative mortality and promote earlier hospital discharge have been proposed.[48,49] VATS is the most popular technique and is gaining support in many centers. Although general anesthesia is required for thoracoscopy, only two small incisions are needed—one through the existing chest-tube tract for a telescope and the second through which operating instruments are passed. This procedure allows adhesiolysis and débridement and should be performed before a thick peel develops.

Proponents of VATS argue that a brief operative procedure and the attendant risks of anesthesia outweigh the child's suffering during thoracentesis (and chest-tube placement) and postoperatively. More importantly, a definitive procedure is done, rather than running the risk of having to perform subsequent chest-tube placements and thoracotomy. The hospital stay is shortened. Doski and colleagues[23] reported a series of 139 children who were studied from 1992 to 1998. By comparing historical cohorts, they showed a shorter length of stay (7 days versus 11 and 12 days) for children who underwent VATS than for groups of children who had thoracentesis, chest-tube drainage, or fibrinolytic therapy and rescue VATS for failure. Twelve of 98 patients who received traditional therapy required thoracotomy, whereas none in the primary VATS group did. A small, randomized study of 20 patients was conducted from 1994 to 1996 and showed that VATS was superior to chest-tube drainage with fibrinolytic therapy. Compared with chest-tube drainage with fibrinolytic therapy, VATS resulted in a higher primary success rate (91% vs 44%), fewer hospital days (8.7 days vs 12.8 days), and lower costs (about $16,000 vs $24,000).[101] Other studies have shown similar results.[33,46,62,76]

The use of radiologist-directed thoracentesis with pigtail catheters (smaller bore than standard chest tubes) for drainage with fibrinolytic therapy has been promoted as a way to reduce the number of operative procedures and achieve shorter hospital stays.[80] This therapy may have value in patients with organizing pleural inflammation and inadequate drainage caused by loculations of pleural fluid without a peel.

TABLE 29–5 Summary of Surgical Management

	No. Cases						
Procedures	***Staphylococcus aureus***	***Streptococcus pneumoniae***	***Haemophilus***	**Sterile**	**Mixed**	***Streptococcus***	**GNR**
DT only	5 (7.5)*	15 (31)	16 (40)	25 (46)	0	1 (20)	0
MT	5 (7.5)	5 (10)	5 (12)	2 (4)	0	0	0
D ± T	56 (85)	29 (59)	19 (48)	27 (50)	10 (100)	4 (80)	3 (100)
Thoracotomy	1 (2)	0	1 (3)	1 (2)	0	1 (20)	0
Open drainage	1 (2)	2 (4)	0	0	1 (10)	1 (20)	0
Decortication	2 (3)	0	0	3 (6)	0	0	0
	Duration of Drainage (days)†						
	n = 39	***n = 24***	***n = 15***	***n = 22***	***n = 7***	***n = 4***	***n = 2***
Range	1-43	2-12	3-41	1-20	4-54	3-6	3
Median	7	4.5	6	4.5	7	5.5	3
Mean	11.8	5.5	9.4	6.4	15.4	5	3
SD	11.1	3.0	9.6	4.7	18.5	1.4	0

**Numbers in parentheses = percentage of cases.*

†Includes only surviving children who required closed-chest-tube drainage only and in whom the exact duration of drainage was known.

DT, diagnostic thoracentesis; D ± T, closed drainage with or without initial thoracentesis; GNR, gram-negative rods; MT, multiple thoracenteses; SD, standard deviation.

From Freij, B. J., Kusmiesz, H., Nelson, J. D., et al.: Parapneumonic effusions and empyema in hospitalized children: A retrospective review of 227 cases. Pediatr. Infect. Dis. J. 3:578-591, 1984.

The use of streptokinase in children with pleural effusion was reported in 1993.[80] The occasional side effects caused by streptokinase reported in adults have generated concern about its safety,[11,78] and its utility has been challenged in adults in a randomized study showing no benefit.[60] Urokinase is less expensive than is streptokinase and has been used in children with minimal adverse effects.[39,50,78,93] In adults and children, these different agents seem to be equivalent,[5,12] although commercial shortages of the products have occurred. Tissue-type plasminogen activator is the fibrinolytic therapy most commonly used at this time. It is less likely to promote allergic reactions.

An early report questioned the benefits of interventional radiologist-placed catheters with fibrinolytics for empyema.[77] A meta-analysis of eight evaluable reports from 2000 to 2004 of operative and nonoperative approaches to empyema suggested that the failure of primary therapy was intermediate for fibrinolytic therapy (9.4%), compared with simple chest-tube drainage (23.6% and either VATS (2.8%) or thoracotomy (3.1%).[4] The differences among these approaches are patient- and center-dependent, and some suggest that with patience even the most severe cases of pleural disease ultimately heal without sequelae.[87] The British Thoracic Society published clinical guidelines for management and concluded that fibrinolytic therapy shortened the hospital stay,[5] as seen in the meta-analysis,[4] but that failure should prompt early consideration of thoracic surgery. The physician must monitor clinical progress carefully and try at the earliest point to identify patients with more complicated disease who would benefit from surgical intervention. The American College of Chest Physicians has suggested that patients requiring surgery include adult patients with large effusions (more than half the hemithorax, loculations, pleural thickening, positive cultures or Gram stains, pH < 7.2, or frank pus).[21] Use of ultrasound also can identify patients with early organization of pleural fluid who have shorter hospitalizations if operative approaches are used.[75] Currently based on limited data, some experts prefer chest-tube and fibrinolytic therapy, whereas other experts choose VATS or minithoracotomy.

Rarely are full thoracotomy and pneumonectomy required for severe pneumonic and parapneumonic disease. An occasional complication of empyema is persistent, organized fluid or air collections in the pleural space, particularly in adults. A high rate of success has been reported using talc pleurodesis in these situations and in patients with noninfectious persistent effusions.[99,103] Long-term complications of this therapy include development of bronchogenic carcinoma and mesothelioma (asbestos-free preparations presumably are not associated with the development of these neoplastic conditions).

One frequent cause of bloody pleural effusions is motor vehicle accidents.[57,81] In one series of 100 children, 56 percent had pleural effusions associated with pulmonary contusions. They were treated with closed-chest-tube drainage; no antibiotics were used, and no infectious complications occurred.[81] Management of pleural hematoma secondary to trauma occasionally is complicated by infection because the bloody pleural fluid is an

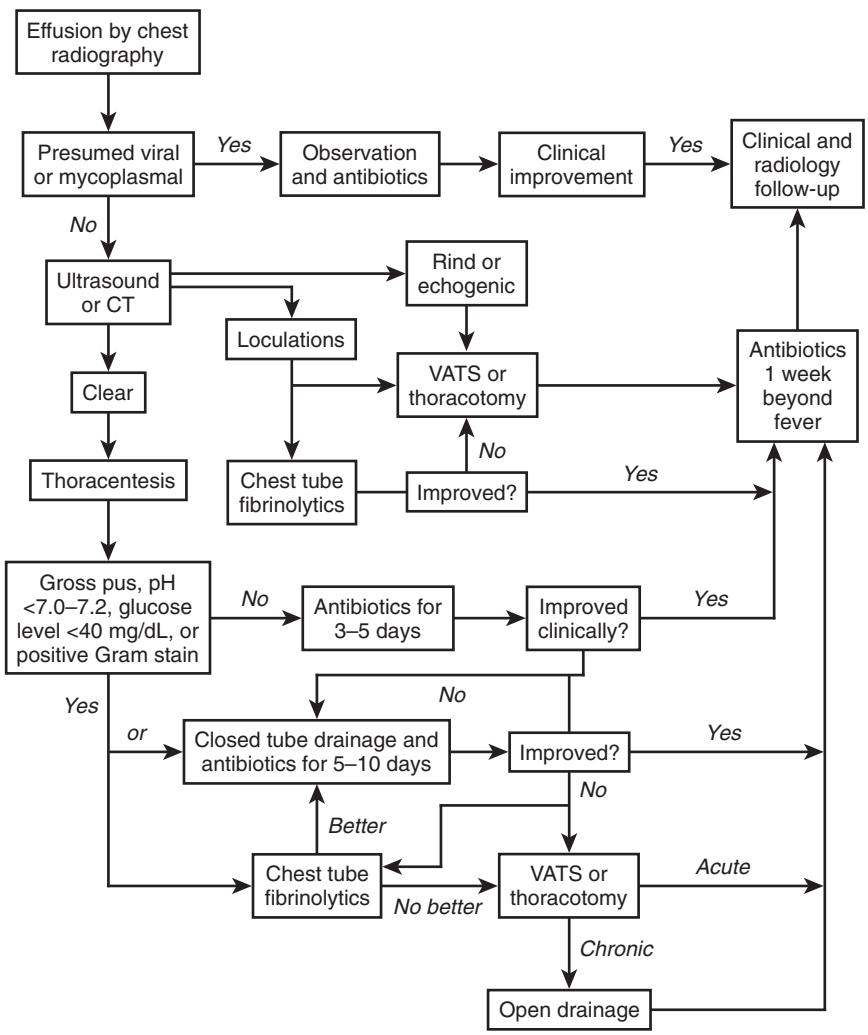

Figure 29–3 Algorithm for the management of pleural fluid collections. CT, computed tomography; VATS, video-assisted thoracic surgery.

excellent growth medium. One study suggested that empyema is less common in post-traumatic effusion with closed-chest-tube drainage than in repeated thoracentesis.[100]

Different biases exist in the management of parapneumonic effusions in children. In suspected empyema based on the clinical history and chest radiograph, we consider that a practical and effective approach is to perform an ultrasound study. If the study suggests complicated empyema (rind or loculations), primary VATS should be performed. If the study result is negative, thoracentesis should be done, and if consistent with empyema, chest-tube placement or VATS should be done. Figure 29–3 is an algorithm incorporating alternatives for the management of pleural collections. Complications from closed-chest-tube drainage include bleeding, infection of the exit wound, bronchopleural fistula, and laceration of the lung. Because of these rare complications, chest-tube placement, performed in the past by the pediatrician, now is delegated more frequently to the surgeon.

In cases of chronic empyema, other approaches are used. A closed tube can be converted into an open drainage tube. This conversion is accomplished safely a minimum of 10 to 14 days into the course of an empyema when the visceral and parietal pleurae fuse, and a pneumothorax can be avoided safely.[57] Other options include open drainage by rib resection and creation of a pleural window. The window ultimately closes with lung expansion and granulation, with disappearance of the pleural space.

PROGNOSIS AND LONG-TERM OUTCOME

The long-term outcome of patients with effusions depends on the underlying cause of the effusion. Patients with empyema who previously were well recover satisfactorily in most cases. Occasional rare complications, such as temporary paralysis of the diaphragm, have been reported.[64] In three retrospective reviews, the percentage of patients who required closed-chest-tube drainage or other surgical procedures ranged from 62 to 80 percent.[30,41,64] The rate of decortication ranged from 4[30] to 43 percent.[41] The relationship of surgical management to the pathogen causing the infection is shown in Table 29–5. The immediate mortality rate for children in recent years has been 0 to 10.8 percent.[30,41,59,64] In one of these studies, the mortality rate was highest for children younger than 1 year old.[30] Studies conducted to evaluate long-term, specific pulmonary disability using pulmonary function tests and lung volumes have shown normalization of these study results over time.[66]

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CHAPTER 30

Lung Abscess

J. Gary Wheeler ❂ Richard F. Jacobs

A lung abscess is an area of necrotic material in the parenchyma of the lung initiated or complicated by infectious organisms. Although possibly originating from a pneumonia, it is distinguished by liquefaction and destruction of parenchymal tissue, organization, and cavitation. Necrotizing pneumonia, which on imaging lacks the "rim" representing the organization of the abscess, has a different clinical course and management.[18] A lung abscess may erupt and form an adjacent empyema. Empyema is defined strictly by involvement of the pleural tissues, however.

In the past, lung abscess was described as a disease of male alcoholics that was managed with surgery.[38] Lung abscess in children was recognized early on as having a different etiology and course than lung abscess in adults.[53] As is true of many infectious diseases, it continues to change as the human host and the microbial environment change. The incidence of lung abscess has declined precipitously in the modern era. Smith[46] reported lung abscesses in 0.33 percent of pediatric admissions in 1934, and Emanuel and Shulman[14] reported a rate of 0.012 percent from 1985 to 1990. In major pediatric referral centers in Chicago, Houston, Dallas, and Montreal, the incidence of lung abscesses has ranged in the last 2 decades from 1.5 to 4.7 cases per year.[4,14,28,48] Many of these cases developed in compromised hosts.

Improvements in pediatric diagnosis and care have resulted in a decrease in the numbers of cases related to underlying diseases. The morbidity and mortality rates also have decreased with use of antibiotic therapy and modern critical care. In 1920, Wessler and Schwarz[53] reported a 33 percent mortality rate, with "invalidism" and hemiplegia occurring in another 27 percent. The mortality rates in the two latest U.S. reports of lung abscess were 11 percent (1982-1993)[14] and 4 percent (1985-1990),[47] and 18.5 percent mortality was reported in Taiwan (1987-2003).[10]

Although lung abscesses occur in children of all ages, two studies have suggested that the trend is away from children younger than 5 years of age to an older population.[14,34] In other studies, the median age ranged from 7 to 9.5 years.[4,7,10,14,18,47] No consistent racial or sexual predisposition to this condition in children has been identified. In the 1950s, one series found boys to be more at risk than girls.[24] In adults, a twofold greater risk exists for men.[32,38] Table 30–1 lists specific risk factors for development of lung abscess in pediatric patients. Most factors are related to some predisposition to aspiration, hematogenous spread, or compromised immunity.

TABLE 30–1 Underlying Risks for 46 Secondary Lung Abscesses in Pediatric Patients

Risk	No.
Neuropsychiatric causes	16
Hematologic/oncologic disorders	11
Primary pulmonary disease	8
Immunodeficiency	4
Congenital heart disease	2
Solvent aspiration	1
Foreign body aspiration	1
Prematurity	1
Chromosomal disorder	1
Endocrinopathy	1

Data from references 13 and 44, and unpublished data from Little Rock, Arkansas, 1989 to 1994.

PATHOPHYSIOLOGY

Two main mechanisms explain formation of a lung abscess. The first mechanism is the introduction of pathogens directly into the airspaces, which typically results in solitary abscesses. The abscess typically follows aspiration, with a resultant neutrophilic reaction and necrosis. Aspiration is thought to be the predominant precipitating factor in adults, particularly in individuals with significant dental disease.[6] Most lung abscesses related to aspiration are polymicrobial and include anaerobes. The prevalence of fluoride and the low incidence of dental disease in children may be additional factors for the reduced incidence of lung abscess in children, although aspiration pneumonia does occur in the absence of dental disease.

The second mechanism in the formation of lung abscess is hematogenous spread. Hematogenous seeding of the lung can lead to an initial pneumonia that develops into an abscess with further organization and cavitation. Primary pneumonia rarely progresses to necrosis and abscess in modern times; this phenomenon is explained largely by the ready accessibility of antibiotics. Emboli from the venous circulation (i.e., septic thrombophlebitis) and right side of the heart (i.e., endocarditis) can cause single or multiple lung abscesses, which often are subpleural. Infection in the head and neck area also is a risk factor for vascular spread to the lung, with resultant lung abscess.[43] This complication occurred commonly in past eras. Lung abscesses complicated tonsillectomies in one third of cases in a 1920 report.[53] The abscesses were theorized to result from aspiration during the operative procedure[46] and characteristically developed 13 to 14 days after the procedure.[53] Great improvements in modern pediatric anesthesia with careful efforts made to prevent aspiration have rendered this complication uncommon today.

A lung abscess tends to have irregular margins, with occasional bullae, and it can dissect into adjacent tissues, such as the mediastinum, bronchi, and pleural space. If the abscess ruptures into a bronchus, air enters and an air-fluid level can be seen radiographically. Dissection into the pleural space creates a purulent effusion, with air noted only if an anaerobic process is present. Dissection into the mediastinum causes a widening of the mediastinum. Air is detected if communication occurs with a bronchus or in the presence of anaerobes. Multiple lung abscesses occur more frequently in hematogenous or embolic disease and are found more often with more sensitive tools, such as computed tomography (CT). Modern reviews of children have suggested that single abscesses are found more frequently than are multiple abscesses.[7,14,47]

Microscopically, a lung abscess is definable by a collection of necrotic material (i.e., highly neutrophilic inflammation); a surrounding irregular, fibrotic wall; and microvascular infarcts. Lymphocytes often are present and seem to play a regulatory role in the formation of the abscess.[44] The infrequency of lung abscesses in patients with human immunodeficiency virus (HIV) infection may be explained by this observation.

The role of preceding viral infection in undermining phagocytic host defenses is supported by the observations of preceding respiratory symptoms in patients with lung abscesses.[1] They manifest primarily in cold weather[4,14] and typically after well-defined viral illnesses, such as varicella, measles, and influenza.[24,26,52] The impact of chemotherapy on phagocytes also may explain the increased numbers of lung abscesses in patients with leukemia and other cancers.

On a macroscopic scale, lung abscesses tend to develop in all parts of the lung. If associated with aspiration, the anatomic site depends on whether the subject was supine or erect at the time of aspiration. Supine patients develop abscesses in the posterior upper and lower lobes, and erect patients develop infection in the middle and basilar lower lobes. Generally, the tendency is for aspiration-related abscesses to develop more on the right side than on the left, presumably because of the more vertical anatomy of the right stem bronchus.[14,23]

Physicians have grouped lung abscesses into primary and secondary categories, presuming that primary and secondary abscesses have different microbiologic factors, management approaches, and outcomes. The arbitrary nature of this distinction is apparent when appreciating how much the microbiology, clinical course, management, and outcome of both conditions overlap.[32] Primary abscesses occur in previously normal hosts without a history of trauma or aspiration of a foreign body. Secondary abscesses occur in the setting of underlying medical illnesses predisposing to infection, airway obstruction, embolization, or aspiration. In an earlier series, secondary abscesses were found more often in children younger than 1 year of age,[26] but this difference was not corroborated in a later study.[14] Primary abscesses were found in 64 percent of patients in Chicago,[14] 33 percent in Houston,[47] 45 percent in Little Rock (1989 to 1994) (unpublished data), 30 percent in Toronto (1956 to 1965),[26] and 30 percent in Taiwan (1987-2003).[10]

In the modern era, lung abscess should trigger a search to exclude underlying factors that may have prognostic value and lead to treatment of the underlying disease. The classic lung abscess syndrome in adults is represented by an alcoholic who aspirates during an alcoholic stupor. In children, a classic presentation would be any child with altered mental status, associated swallowing dysfunction, or both conditions. Foreign bodies can obstruct normal clearance of pathogens and precipitate the development of a lung abscess.[14,27] Obstruction also predisposes to lung abscess in adults who have carcinoma of the lung and rarely in children with metastatic disorders. Ineffective cough in patients with neurodegenerative or myopathic disorders is another risk factor for developing lung abscess, similar to that in an adult alcoholic. Patients with leukemia and patients who are receiving chemotherapy also are at increased risk. Occasionally, a bronchogenic cyst can become infected and mimic a lung abscess. Tricuspid or pulmonary valve endocarditis in children with complicated congenital heart disease places them at risk for developing lung abscesses.

Immunodeficiency is another risk factor for development of a lung abscess. Patients with chronic granulomatous disease and hyper-IgE syndrome typically are found to have lung abscesses. Patients with hypogammaglobulinemia may develop abscesses, although bronchiectasis is the more characteristic finding. The same is true for patients with immotile cilia syndromes and cystic fibrosis, although in the latter group, disease abscesses are uncommon.[10] Among pediatric patients, HIV-1 infection has not been reported as a risk factor in series from Chicago and Houston.[14,47] Additional causes are listed in Table 30–1.

MICROBIOLOGY

The microbiology of lung abscesses seems to be evolving as patients and antibiotics change.[38] In the preantibiotic era, streptococci and *Mycobacterium tuberculosis* were the most commonly reported causes of lung abscesses. After penicillin use began and tuberculosis skin testing, treatment, and control programs became widespread, staphylococci most frequently were recovered from lung abscesses.[39] Development of better culture techniques also increased the identification of anaerobes in lung abscess material; in past eras, these organisms probably were present but not recovered. Anaerobes were suspected by the fetid odor of abscesses, the time course of postoperative aspiration infections, and Gram stains of tissue and pus, which showed fusobacteria and spirochetes.[46,53] Table 30–2 summarizes the microbiology of pediatric lung abscesses reported from 1976 to 2006.

The primary role of anaerobes in lung abscesses has been assumed in aspiration pneumonias. Anaerobes are prominent in the oral cavity and have been recovered from the abscesses of patients with dental disease.[6] In many past studies, lung abscess materials were not transported and cultured for optimal anaerobic growth, however. Successful growth was described when specimens were transported in a closed syringe, with culture inoculation beginning in less than 10 minutes.[7] Later studies, in which optimal culture methods were employed, corroborated the role of anaerobes in lung abscesses in children.[7,47] In one study of mentally retarded children with seizure disorders, poor dental care, and suspected aspiration, transtracheal polymicrobial infections with aerobes were found in 9 of 10 samples.[7] An average of 6.2 isolates were recovered per patient. *Peptostreptococcus* and *Bacteroides* spp. were the anaerobes recovered most frequently.[7] In a larger group of 45 children, 15 of whom had primary abscesses, 14 had polymicrobial infections.[47] Older children with neurologic disorders were the primary patients with anaerobes in both studies. Anaerobes, *Streptococcus pneumoniae*, nontypeable *Haemophilus influenzae*, and *Staphylococcus aureus* have been recovered from normal patients.[47]

The role of tissue lysins and toxins depends on the pathogen and is thought to be crucial for the development of lung abscesses. In mixed infections, synergy probably occurs among the pathogens as proposed by Smith in 1934.[46]

Nosocomial pathogens are becoming more frequent causes of lung abscesses as a result of the increased numbers of patients with extended hospitalizations and use of advanced-generation antibiotics. The widespread use of third-generation cephalosporins has resulted in resistant *Enterobacter* spp. and other gram-negative organisms being recovered in secondary lung abscesses.

TABLE 30–2 Microbiology of Pediatric Lung Abscess

Organism	No.
Staphylococcus aureus	22
Coagulase-negative staphylococci	2
Streptococcus pyogenes	5
Streptococcus pneumoniae	15
Alpha-hemolytic streptococcus	17
Other aerobic streptococci	7
Enterococci	2
Branhamella catarrhalis	3
Escherichia coli	10
Klebsiella	8
Pseudomonas	13
Serratia	1
Eikenella	1
Haemophilus	7
Other gram-negative organisms	2
Bacteroides	19
Peptostreptococcus	12
Other anaerobes	26
Candida	4
Aspergillus	5
Mucor	3
Mycobacterium tuberculosis	1
Total	*185*

These data were reported in the literature from 1976 to 2006. The isolates were recovered by direct aspiration of abscess contents, bronchoscopic aspiration, transtracheal aspiration, blood culture, or culture of surgical specimens. Data from case reports focused on procedures are included, whereas data from case reports focused on the organism recovered are not.[4,7,10,12,14,20,22,24,25,27,28,34,36,45,47,48,51]

In the report by Tan and associates,[47] fungal abscesses always were associated with debilitated, chronically hospitalized patients. Immunosuppression no doubt contributes to the recovery of other unsuspected organisms (e.g., *Legionella*, *Neisseria mucosa*, *S. pneumoniae*, *Citrobacter*)[13,19,40,42] and underscores the value of obtaining specimens in chronically hospitalized patients, atypical cases, and patients not responding to empiric treatment.

Among otherwise normal hosts, tuberculosis can be a cause of single and multiple abscesses. The number of tuberculosis cases manifesting as lung abscess has increased dramatically, and tuberculosis is likely to be associated more frequently with lung abscesses in the future. In patients with an international travel history, unusual pathogens such as parasites (e.g., hydatid cysts)[15] and regional bacteria (e.g., *Pseudomonas pseudomallei*) should be considered.

An important lesson for the physician is the relevance of various respiratory cultures to the microbiology of lung abscesses. Rarely is sputum useful in defining the pathogens in a lung abscess because of three factors: (1) sputum typically is contaminated with abundant mouth flora; (2) if the lung abscess is not ruptured, no direct communication of the pathogens in the abscess occurs with the airway; and (3) sputum is difficult to obtain in preadolescent children. Obtaining cough cultures, performed by gagging a young child and culturing the coughed sputum collected on a swab before it can be swallowed, frequently is unsuccessful. A skilled clinician occasionally can acquire useful information, however.

Bronchoscopy is effective in recovering relevant organisms if the abscess has ruptured and has therapeutic value because it may assist in clearing secretions from the airway. It has been performed infrequently in pediatric practice in the past because of a lack of skilled personnel and pediatric equipment. Rigid bronchoscopy rarely is used to drain the abscess but when performed, highly informative microbiologic information may be obtained. Transtracheal aspirates, likewise performed in few pediatric patients, have similar value. The upper airway frequently is colonized in debilitated patients, and microbiologic information obtained must be interpreted with care. Direct aspiration of the abscess, typically under CT or ultrasound guidance,[49] is an ideal way to provide microbiologic data and plan antimicrobial therapy. Aspiration may have therapeutic value in decompressing the abscess.

CLINICAL FEATURES

Most patients with lung abscesses have had symptoms 1 to 3 weeks before being hospitalized.[14] Fever is reported to be associated with 100 percent of primary abscesses[14,26] and 84 percent of a mixed group of primary and secondary abscesses.[47] All patients with secondary abscesses in a small series had fever.[7] Cough occurs in 53 to 67 percent of cases[26,47] and initially may be nonproductive, becoming purulent when rupture into a bronchus occurs. With necrosis, hemoptysis can occur. Ipsilateral chest or shoulder pain also has been described in some patients, particularly older children.[14,27] Weight loss may be present if the abscess is of more than a few days' duration. Other symptoms are listed in Table 30–3.[47]

Some differences in presentation by age exist. Neonates and young infants typically are febrile, without localizing symptoms. Older children also are febrile but may have more cough or tachypnea and focal pain.

The clinical features of a lung abscess vary with the causative organisms and patient risk factors. Patients with bacterial pneumonia can present with dramatic onset of fever and overwhelming respiratory failure, such as in staphylococcal pneumonia. In these cases, the patient often has a recent history of influenza or varicella infection. Staphylococcal abscesses may not be noticed on chest radiographs until the patient already is on ventilatory support because of the time required for an abscess to organize. Similar presentations are typical of group A beta-hemolytic streptococcus and *S. pneumoniae* infections. Often, a patient has received antibiotics, and a temporary defervescence occurs before the hectic fevers of an abscess re-emerge. In the latter situation, the respiratory symptoms may be less notable but virtually always are present.[14] This biphasic presentation was described more than 70 years ago in postoperative aspiration[46] and continues to be typical of many lung abscesses.

TABLE 30–3 Symptoms and Signs in Patients with Lung Abscess

Symptom	No. Cases	%
Fever	38	84
Cough	24	53
Dyspnea	17	38
Chest pain	11	24
Anorexia	9	20
Purulent sputum	8	18
Rhinorrhea	7	16
Malaise/lethargy	5	11
Hemoptysis	4	9
Diarrhea	4	9
Nausea/vomiting	3	7
Irritability	3	7
Otitis media	2	4
Convulsions	2	4
Weight loss	1	2
Sore throat	1	2
Lymphadenopathy	1	2

From Tan, T. Q., Seilheimer, D. K., and Kaplan, S. L.: Pediatric lung abscess: Clinical management and outcome. Pediatr. Infect. Dis. J. 14:*51-55, 1995.*

Subacute presentations are typical in patients with tuberculosis or fungal abscesses and usually are associated with other chronic systemic symptoms, such as anorexia, weight loss, and malaise. Cough may be prominent. Aspiration pneumonia may take an indolent or acute course, depending primarily on the organisms in the abscess, the volume of aspirated material, and the status of the host.

The physical findings in lung abscess are limited. Children usually have fever,[26,47] whereas adults present with fever less frequently (19%).[38] Tachypnea is a variable finding. Typically, auscultation is unrevealing except in cases of very large abscesses, in which loss of normal breath sounds is perceived. Adults and older children seem to have more discrete physical findings, with rales and decreased breath sounds in approximately one third of adult cases,[38] but they are uncommon findings in young children.[3]

An abscess can rupture into the bronchus, the mediastinum, or the pleura. In all cases, these complications are significant. In children, all organisms seem capable of causing these complications, but polymicrobial and anaerobic infections are suspected most often. Rupture into the bronchus may not be harmful if the volume of the abscess cavity does not overwhelm the host's ability to cough and clear the material. In an immunocompromised host, rupture may lead to disseminated pneumonia and further abscesses or death. Among adult patients who died of lung abscess, 22 percent were found to have died of aspiration of the abscess contents.[16]

In an otherwise healthy individual, rupture into the bronchus can be beneficial because it decompresses the abscess and allows the affected tissues to heal more rapidly. It is associated with the sudden production of foul-smelling, abundant, and sometimes blood-stained sputum. Frank hemoptysis is uncommon. Rupture into the mediastinum can be life-threatening, can be associated with chest pain and cardiac compromise, and requires surgery to

drain the resulting mediastinitis. Rupture into the pleural space results in pleuritic pain, enhancement of symptoms on inspiration, and often a more toxic presentation, and may require drainage.

Routine laboratory information is of limited help. The white blood cell count and erythrocyte sedimentation rate are elevated nonspecifically, and a left shift of the white blood cell differential count typically occurs.[14] Certain laboratory tests, such as the purified protein derivative tuberculosis skin test, HIV serology, or sweat chloride test, may be helpful in revealing an underlying cause. Except for the tuberculosis skin test, such studies should not be performed routinely and should be directed by a family history or other findings, such as chronic diarrhea or lymphadenopathy. Blood cultures are helpful but are positive in fever than 10 percent of cases.[14,47]

DIFFERENTIAL DIAGNOSIS

The major differential diagnoses in the management of a lung abscess are anatomic. A lung abscess must be differentiated from pneumonia, necrotizing pneumonia, pneumatocele, loculated empyema, and a purulent pleural effusion with a bronchopleural fistula. CT or ultrasound may confirm an abscess by documenting central cavitation and differentiating pleural from parenchymal tissues. An abscess may be confused with a congenital cyst, pseudocyst, hydatid cyst, saccular bronchiectasis, pneumatocele, or sequestration. Chest CT allows definition of these entities in many cases by identifying the associated structures, such as the vascular supply and pleural borders.

Apart from the anatomic and infectious causes of lung abscess, the other very rare cause is cancer. Unrecognized metastatic disease from Ewing sarcoma or osteosarcomas with associated central necrosis can mimic an abscess or, by obstructing a bronchus, can promote abscess formation.

DIAGNOSIS

The diagnosis of lung abscess almost always is made by imaging the lung. In most cases, the plain radiograph is adequate to define a lung abscess (Fig. 30–1), showing a thickened cavity with an air-fluid level that can be accentuated by placing the patient in the lateral decubitus or erect position. Atelectasis often occurs as an expanding abscess compressing adjacent tissues. Pleural thickening may occur if the abscess is subpleural. Hilar adenopathy occurs in subacute situations. Visualization can be obtained with bronchoscopy if the abscess ruptures into the bronchus, but it is limited by the location of the abscess and skill of the bronchoscopist. The procedure usually is not performed for anatomic diagnosis but rather to obtain microbiologic specimens or exclude a foreign body.

CT is optimal in its ability to identify smaller or multiple abscesses, document the impact of the abscess on adjacent tissues, identify cystic processes mimicking an abscess, and define an abscess for which an organized pneumonia obscures an air-fluid level on plain film.[20] CT scan of a lung abscess reveals an air-fluid level with an active rim, and the abscess is distinguished from necrotizing pneumonia, which lacks enhancement on contrast studies and lacks a distinct air-fluid level.[18] Nuclear imaging is described[11] but used rarely and adds little to the information obtained by CT.

After a presumed abscess is defined by imaging, needle aspiration of the abscess or bronchoscopic recovery of abscess fluid should allow confirmation and identification of the infectious cause of the process. Based on available pediatric studies, whether either procedure hastens recovery or reveals the microbiologic cause in pretreated individuals cannot be predicted. In a case report in which an infant had abscesses in both lungs, the time to recovery was equal in the abscess that was drained and the other abscesses that were treated medically.[29] An adult study using thin-needle aspiration showed positive cultures in 92 percent of patients not pretreated with antibiotics and in 70 percent of patients pretreated.[37]

Directly aspirating a lung abscess may be difficult or unsuccessful if the abscess is not large or peripheral, and complications such as lung laceration and sterile pleural effusions are real risks. Two groups[18,47] have had satisfactory pediatric experience using direct aspiration under CT guidance (Fig. 30–2). Bronchoscopy particularly is valuable if foreign bodies are suspected or pus can be recovered. When material is obtained, a putrid quality is a clue that anaerobic organisms are present. Typically, the abscess contains pure neutrophils. Occasionally, counterimmune electrophoresis or other antigen detection systems may assist in the microbiologic diagnosis when cultures are negative.[16] Because of

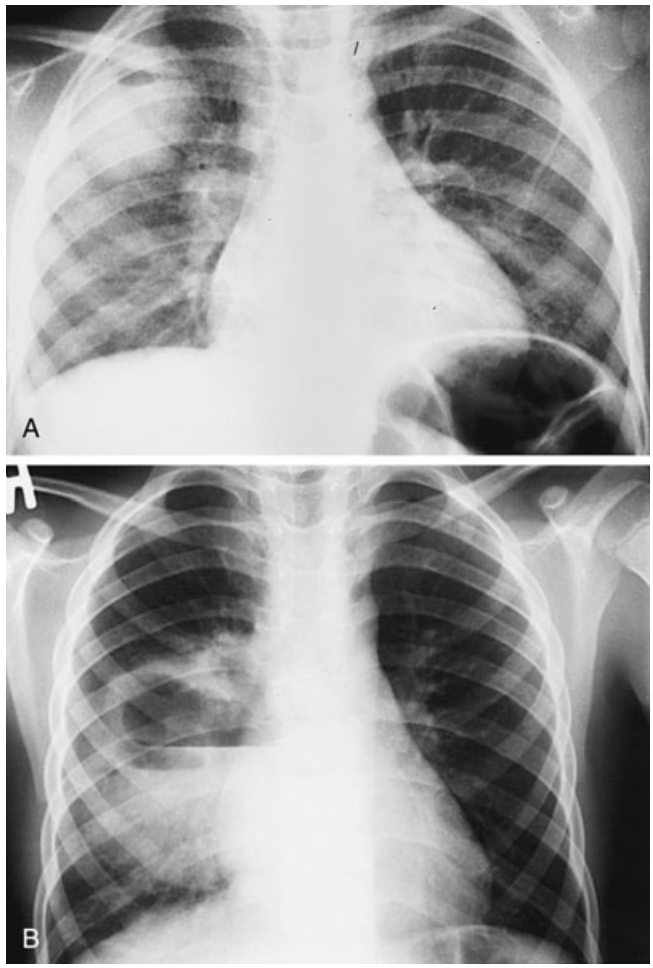

Figure 30–1 **A,** Plain chest film of a 4-year-old African-American boy with symptoms, including cough and fever of 104° F, for 4 days. He was treated initially with oral cefaclor and re-presented with this radiograph showing an air-fluid level in the right upper lobe. On intravenous cefuroxime, he was afebrile in 48 hours and went home on oral cefuroxime axetil after 4 days. **B,** Plain chest film of a 6-year-old African-American boy who presented after 5 days of symptoms with high fever, productive cough, dyspnea, and abdominal pain. The plain chest film reveals multiple air-fluid levels. Nafcillin and cefotaxime were begun. On day 4, an ultrasound-guided diagnostic aspiration recovered thick purulent material, but Gram stain and all cultures, including anaerobic and fungal, were negative. He was afebrile in 7 days and went home on amoxicillin-clavulanic acid at 10 days.

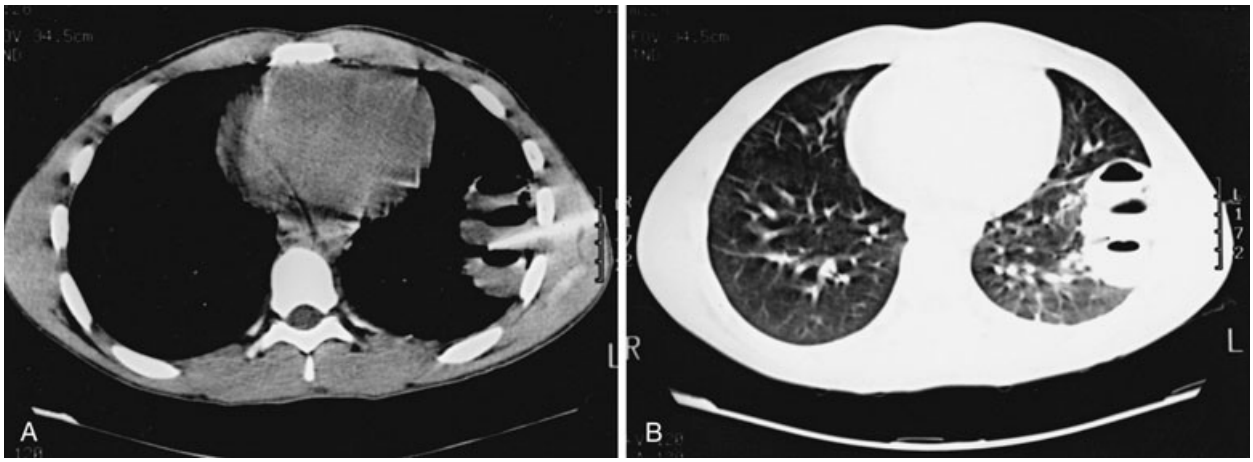

Figure 30–2 **A** and **B**, Computed tomography (CT) scans of a 15-year-old white boy who, 4 days before admission, reported a mild aspiration during fresh-water swimming. He awoke the next day with chest and back pain. He then developed low-grade fever. A plain chest film showed a large, thick-walled abscess in the left lower lobe. CT scans show insertion of a 20-gauge needle into the abscess on the first day of admission **(A)** and detail of the wall thickness and cavitation **(B)**. *Haemophilus influenzae* grew from the aspirate. Ampicillin-sulbactam was initiated and continued for 14 days.

the special handling required, these technologies usually are employed only after standard cultures have failed.

TREATMENT

Although surgery has a role in specific situations, the treatment of lung abscess in children often is successful when antibiotics alone are used.[39] In most cases, the need for surgery is limited to instances of failed antibiotic therapy or to an abscess complicated by rupture into adjacent tissues. Newer techniques, such as hyperbaric oxygen,[8] have been proposed as adjuvant therapy.

The initial choice of antibiotics almost always is presumptive because abscess material may be unavailable. For primary lung abscesses in which no risk factors are identified and in the absence of positive blood cultures, the recommended approach is to begin therapy with a regimen that covers *S. aureus*, *S. pneumoniae*, and the anaerobic microorganisms that normally are found in the upper respiratory tract. Clindamycin, ampicillin plus sulbactam, and ticarcillin plus clavulanate frequently are used in this setting.

For patients at risk for aspiration or who are immunocompromised, gram-negative pathogens also must be considered. This spectrum of pathogens can be addressed with one of several drug regimens: clindamycin and cefotaxime (or an aminoglycoside); ticarcillin plus clavulanate or piperacillin plus tazobactam; or nafcillin (or cefazolin), gentamicin, and metronidazole. The possible emergence of more penicillin-resistant species, such as *Streptococcus milleri* and *Klebsiella*, in community-acquired disease of adults and children supports these broad-spectrum regimens in children.[21,41] Patients with cystic fibrosis are particularly vulnerable to *Pseudomonas* spp. and should receive an aminoglycoside plus an additional antipseudomonal penicillin or cephalosporin. Carbapenem therapy would be an alternative. When endocarditis is present, treatment (e.g., vancomycin plus gentamicin with or without anaerobic coverage) should be provided for staphylococci, streptococci, and enterococci while awaiting results of blood cultures.

S. pneumoniae has been isolated infrequently in lung abscesses (see Table 30–2). Most patients with resistant organisms are still clinically sensitive to achievable doses of penicillins (penicillin G, minimum inhibitory concentrations of 0.12 to 2.0).[35] Drug resistance to *S. aureus* is a much more concerning issue in lung abscess, and empiric therapy with vancomycin is required if methicillin-resistant *S. aureus* is suspected while waiting for confirmation. For a critically ill patient with lung abscess, some experts recommend nafcillin, gentamicin, or both.

With culture information available, therapy is directed specifically at the pathogens isolated. In most patients, oral or intravenous antibiotic therapy has been administered before aspiration of a lung abscess is done, however, and may affect which organisms are recovered.[13] For this reason, coverage should be extended to include organisms that are likely to be present but are not recovered, such as anaerobes, in a setting of aspiration.

All bacterial lung abscesses should be treated with intravenous therapy until the patient is stable and no longer toxic. In approximately two thirds to four fifths of patients, this condition occurs within 3 to 7 days of instituting intravenous therapy.[14,28] After the patient has been afebrile for 48 to 72 hours, initiation of oral therapy may be considered. Certain oral drugs, such as amoxicillin plus clavulanate and clindamycin, achieve therapeutic serum levels and are effective against the spectrum of organisms in lung abscesses.

The length of total therapy for a lung abscess should be 2 to 3 weeks. Complicated infections should be treated intravenously until fever has disappeared and no evidence of continuing inflammation exists. An additional 2 to 3 weeks of oral treatment should follow. Radiographic resolution of a lung abscess that is not drained occurs over the course of weeks. Even with drainage, resolution may not occur more rapidly. Chest radiography should be repeated every 1 to 2 weeks until complete resolution is documented.

Tuberculous lung abscesses may rupture spontaneously into the pleura. Treatment is not directed at the immediate abscess or pleuritis but at preventing spread if it erupts into a bronchus. Preventing reactivation several years later is another goal of therapy.

Clinical failure is defined by persistent fever and toxicity. The length of time treatment with intravenous antibiotics should continue before declaring therapy a failure is not clearly defined. Suggestions in the literature range from 1 to 3 weeks.[2,4,39] When clinical failure occurs, several options are available, including drainage of the abscess, which permits identification of the organisms causing the disease process.

Bronchoscopy may allow direct perforation of the abscess and evacuation of its contents. A risk of fatality occurring from aspiration of abscess contents exists, however.[19]

Needle aspiration (one or more) also is possible under fluoroscopy, ultrasonography, or CT guidance.[18,24,25] Simple CT-guided needle aspiration leads to relief of symptoms in approximately two thirds of cases within 48 hours.[18] The risk of complications seems to be low.[18,25] Percutaneous transthoracic tube drainage has been used in pediatric lung abscesses.[12,27,36,50,51] The risk is lowest if the abscess is peripheral, and adhesion of the opposing pleural surfaces is a consequence of the inflammatory process. When this adhesion occurs, the needle or catheter does not traverse the pleural space. Catheter drainage may be attempted in abscesses larger than 20 cm^3 or 4 cm in diameter[2,18] using 8 to 10 French pigtail catheters. Smaller abscesses are technically difficult to manage.

Chest tube thoracostomy previously was the standard therapy of lung abscesses[31,33]; it is now recommended when conservative therapy fails and may play a role in patients with large abscesses if the abscess abuts the parietal pleura and provides a direct path from the exterior surface to the abscess.[30] A 25-year review of the literature found that significant complications, such as hemothorax, pneumothorax, bronchopleural fistula, empyema, and catheter occlusion, occurred in 9.7 percent of cases, with an overall mortality rate of 4.8 percent.[50]

Surgery for lung abscess is not well described in the pediatric literature. One report that combines surgery for lung abscess and necrotizing pneumonia had no mortality in the postsurgery period and a reversible complication rate of 10 percent.[48] Indications for surgery included associated pyopneumothorax, size of the abscess, and tension pneumatocele. Another possible surgical indication is proximity of a lung abscess to the mediastinum. In the face of antibiotic failure, an open lung procedure and wedge resection may be required, depending on the location of the abscess.[5] The procedure typically is successful and often obviates the need to perform a lobectomy.[22,53] In a complicated case, such as with a gangrenous lung, lobar resection may be necessary. Managing an infant with a lung abscess has required surgery more often than in older children.[45,52]

PROGNOSIS

The outcome for pediatric patients usually is very good when lung abscesses are uncomplicated, and recovery is more rapid than in adults.[32] Most patients have complete symptomatic and radiographic resolution in 3 to 6 weeks, with normal pulmonary function tests at follow-up.[4,14] Complicated disease associated with thoracostomy tubes and empyema more likely leads to residual symptoms (e.g., pleurisy) and persistent effusions or pleural thickening on radiographs. In reports published since 1982, half of patients with lung abscess have required surgical intervention, such as thoracentesis to drain an empyema, lobectomy, or decortication, and 20 percent have required lobectomy or decortication.[4,7,14,47] If patients require lung resection, they may experience immediate surgical complications; long-term exercise tolerance may be limited, and other problems, such as scoliosis, may develop. One study found normal pulmonary functions in patients studied after undergoing lobectomy, however.[34]

Rarely, a residual cavity may develop and become superinfected. The mortality rate is 4 to 11 percent[14,47] for patients with primary and secondary lung abscesses.[14,47] Higher risk of morbidity and mortality occurs in patients with secondary abscesses who have complications caused by either a decrease in host resistance or underlying disease.[14,38,47,48]

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CHAPTER 31

Cystic Fibrosis

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Cystic fibrosis (CF) is the most common inherited lethal disease of whites. It occurs primarily among individuals of Central and Western European origin and affects more than 30,000 Americans and 60,000 people worldwide.[365] The estimated incidence in the United States is 1 : 2000 to 1 : 2600 white,[377] 1 : 19,000 African-American, 1 : 11,500 Hispanic, and 1 : 25,000 Asian-American live births.[226] CF has an autosomal recessive mode of inheritance. Affected individuals are phenotypic homozygotes, and both parents usually are heterozygotes or carriers. The carrier frequency in whites in the United States is approximately 1 in 25, with full siblings of children with CF having a one in four chance of being affected.

Mutations in a single gene located on the long arm of chromosome 7 account for the defective protein in CF.[365] A range of different mutations at the DNA level account for the abnormal protein. The most common mutation (ΔF508) is the absence of three sequential nucleotides, which leads to the deletion (Δ) of phenylalanine (F) at the 508 position on the CF transmembrane conductance regulator protein (CFTR). Approximately 70 percent of individuals with CF have this mutation. To date, more than 1500 mutations in the CFTR gene have been identified,[76] but fewer than 25 occur with a frequency of more than 0.1 percent.[122] Certain populations have higher frequencies of specific mutations, such as W128X in Ashkenazi Jews[226] or G551D in French Canadians.[277]

Five classes of mutations have been proposed to account for reduced CFTR chloride channel function.[141,277,366,391] Class 1 mutations are mutations in which stop codons or frame shift mutations cause early termination of mRNA translation and minimal to no protein production. In class 2 mutations (ΔF508), protein fails to mature, resulting in little expression of CFTR at the cell membrane. Class 3 and class 4 mutations are associated with defective regulation and decreased conductance of chloride at the cell membrane. Protein production and transit to the cell surface occur, but altered chloride conductance is present, or chloride conductance is nonexistent. Class 5 mutations are splice site mutations, which affect the amount of CFTR produced.

Other mutations are associated with unstable CFTR at the cell membrane. The presence of more than 1500 mutations for CFTR has prompted investigators to evaluate the association of genotype with clinical disease. The CF phenotype genotype consortium has shown that certain mutations from classes 3 to 5 are associated with pancreatic sufficiency; however, correlation of CF genotype with the severity of pulmonary disease has been poor. The wide variation in pulmonary disease observed in CF may reflect the effects of environment, patient compliance, polymorphisms in CFTR, and modifier genes that affect the function of the CFTR.

CFTR is a glycoprotein expressed at low levels by surface epithelial cells in the lung, sweat glands, pancreas, liver, large intestine, and testes.[274] Higher levels of expression have been reported in submucosal glands. CFTR protein is a member of a group of membrane transport proteins known as the *ABC-transporter superfamily*.[158] Researchers have confirmed that CFTR functions as an apical chloride channel mediated by cyclic adenosine monophosphate containing 1480 amino acids. It is composed of two membrane-spanning domains, two nucleotide-binding domains that interact with adenosine triphosphate, and a regulatory domain.[6] Activation of the channel is regulated by protein kinase A, which serves as a target for phosphorylation. CFTR in epithelial cell membranes may influence the expression of other proteins important in regulating inflammation, ion transport, and cell signaling, altering the CF phenotype.[286] CFTR regulates the activity of separate calcium-activated chloride channels and down-regulates sodium transport, balancing the rates of chloride secretion and sodium absorption.[116,175,187]

CFTR is responsible for the proper hydration of secretions in the airway, pancreas, and other tissues. An inability to secrete chloride and excessive absorption of sodium and water contribute to altered luminal secretions in patients with CF. In the lung, this alteration leads to decreased airway surface liquid, decreased mucociliary clearance, and a predisposition to chronic bacterial infections.

CLINICAL MANIFESTATIONS

Individuals with CF have exocrine gland dysfunction, which results in progressive suppurative obstructive lung disease, pancreatic insufficiency (85 to 90 percent), elevated sweat electrolytes, male infertility (>95 percent), and female infertility. Less common manifestations include hepatobiliary disease, osteoarthropathy, diabetes mellitus, nasal polyposis, and meconium ileus (Table 31–1).[56,75]

TABLE 31–1 Clinical Features of Cystic Fibrosis at Diagnosis

0-2 years
Meconium ileus
Obstructive jaundice
Hypoproteinemia/anemia
Bleeding diathesis
Heat prostration/hyponatremia
Failure to thrive
Steatorrhea
Rectal prolapse
Bronchitis/bronchiolitis
Staphylococcal pneumonia
2-12 years
Malabsorption
Recurrent pneumonia/bronchitis
Nasal polyps
Intussusception
>13 years
Chronic pulmonary disease
Clubbing
Abnormal glucose tolerance
Diabetes mellitus
Chronic intestinal obstruction
Recurrent pancreatitis
Focal biliary cirrhosis
Portal hypertension
Gallstones
Aspermia

From Maclusky, I, and Levison, H.: Kendig's Disorders of the Respiratory Tract in Children. 5th ed. Philadelphia, W. B. Saunders, 1990, p. 701.

The potential relationship between genotype (the genetic constitution of an individual) and phenotype (the physical expression of that genotype) in CF is an area of active clinical investigation. To date, the correlation of genotype to phenotype has not led to many clear associations between severity or course of pulmonary disease and type of genetic mutations.[43,173] A family-based study of CF involving monozygotic twins, dizygotic twins, and siblings reported that a significant portion of the variability in pulmonary function was attributable to heritability, independent of CFTR genotype.[358] This finding suggests that modifier genes account for a significant amount of the variance observed in CF lung disease. Numerous candidate modifier genes have been investigated to account for the variance in CF pulmonary disease, with more recent reports identifying transforming growth factor-β1 and glutamate-cysteine ligase as candidates.[91,212] In addition to modifier genes, environmental factors, including passive smoke exposure, infection, and socioeconomic status, have been associated with reduced lung function.[68,171,235,287,300] Multiple mutations, incomplete understanding of the physiologic function of CFTR, and the role of modifier genes have delayed understanding of the genotypic influence on phenotype in CF.

A strong association between pancreatic function and genotype has been reported for individuals homozygous for ΔF508.[27] Most subjects homozygous for ΔF508 have pancreatic insufficiency.[72] Obstruction of the pancreatic duct begins in utero, resulting in fibrosis and loss of exocrine pancreatic function. Pancreatic fluid from patients with CF is low in enzyme and bicarbonate concentrations, resulting in maldigestion of fat and protein. Clinically, children commonly present with steatorrhea, protein-calorie malnutrition, muscle wasting, and progressive failure to thrive. A voracious appetite is characteristic, and stools are described as bulky, greasy, and foul-smelling. Approximately 10 to 15 percent of patients have enough preservation of pancreatic function to allow for normal digestion of food (pancreatic-sufficient).[86] At least five mutations from classes 3 to 5 described earlier are associated with pancreatic sufficiency, whereas almost all patients homozygous for ΔF508 have pancreatic insufficiency.[72]

Failure to thrive is a common complication observed at the time of diagnosis. If malnutrition is severe, hypoproteinemia and edema are observed. In addition, malabsorption can lead to vitamin deficiency, especially of fat-soluble vitamins A, D, E, and K. These problems can be reversed with pancreatic enzyme replacement therapy, oral nutritional supplements, and routine vitamin supplements.

Glucose metabolism often becomes impaired with age, as fibrosis of the pancreas occurs in patients with exocrine pancreatic insufficiency. In the 2005 CF registry, 25 percent of adults were reported to have impaired glucose tolerance or CF-related diabetes with and without fasting hyperglycemia. Decreased secretion of insulin and reductions in peripheral glucose use and hepatic insulin sensitivity are observed in patients with impaired glucose tolerance.[220] CF-related diabetes has features of type 1 and 2 diabetes. The prevalence increases with age and is associated with increased morbidity and mortality.[217]

Liver disease in CF is associated with pancreatic insufficiency.[251] Approximately 25 percent of patients with CF develop focal biliary cirrhosis, but less than 5 percent progress to multilobar biliary cirrhosis and portal hypertension. In the absence of CFTR, bile becomes inspissated and associated with periductal inflammation and fibrosis. Liver function tests frequently are abnormal, as is a small, poorly functioning gallbladder. Cholelithiasis has been reported in 12 percent of patients and may be related to loss of bile acids in the stool.[66,107,108] Meconium ileus, the thick inspissated meconium that mechanically obstructs the distal ileum, occurs in 8 to 20 percent of newborns with CF. It also is associated with pancreatic insufficiency.[172] Modifier genes are thought to play an important role in the development of meconium ileus.[24] A similar syndrome (distal intestinal obstructive syndrome) mimicking meconium ileus can occur in older children and young adults with CF.

Absence of the vas deferens with secondary aspermia renders 98 percent of men with CF infertile.[87] Sexual potency is normal, and with microsurgical techniques for sperm aspiration, affected men can become biologic fathers.[211] Men with congenital absence of the vas deferens can have abnormal CF alleles with little clinical expression of disease other than the reproductive system. Infertility in women with CF may be 20 percent related to secondary amenorrhea (malnutrition) and dehydrated cervical mucus.[329]

Pulmonary disease is the primary cause of morbidity and mortality in patients with CF.[378] Expression of CFTR has been localized to the airways and submucosal glands of the lung.[348] Clinical studies in young children with CF have found significant inflammatory changes in the airways in bacterial-positive and bacterial-negative patients.[13,78,140,215,281,347] Imaging studies using high-resolution computed tomography (CT) in infants with CF describe the presence of thickened airway walls and nonhomogeneous air trapping.[80,197,206] The lungs are morphologically normal at birth; within weeks, they begin showing evidence of small airway abnormalities and inflammation. Small and medium-sized airways become obstructed, and neutrophils are the inflammatory cells primarily recovered from bronchoalveolar lavage (BAL) fluid. An intense neutrophilic response leads to the release of proteases that cause chronic injury to the respiratory epithelium and supporting airway structure. The massive numbers of neutrophils subsequently release elastase, which overwhelms the antiproteases in the airway, contributing to enhanced destruction of tissue. Large amounts of neutrophil-derived DNA and cytosol proteins are released into the airway lumen, increasing sputum viscosity and worsening airway obstruction.[78]

Progressive bronchiectasis develops with time, leading to advanced destruction of the airways and parenchyma (Figs. 31–1 and 31–2). Bronchiectatic cysts are prominent, especially in the

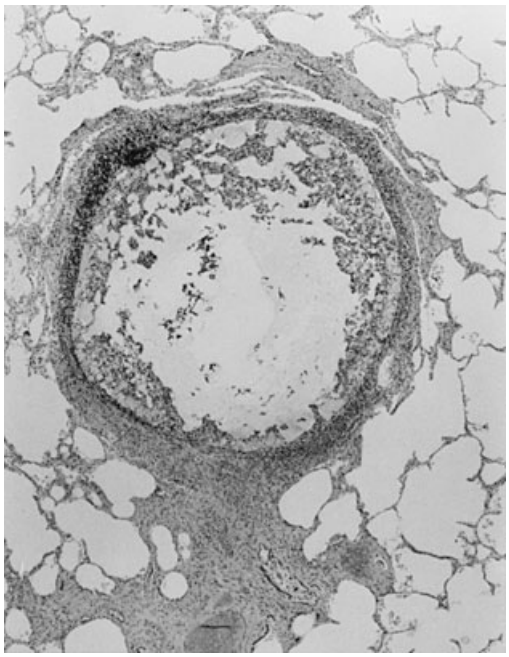


Figure 31–1 Early stages of lung disease in cystic fibrosis are shown in this lung specimen. Airway inflammation and bronchiectasis are present. The surrounding lung parenchyma is normal.

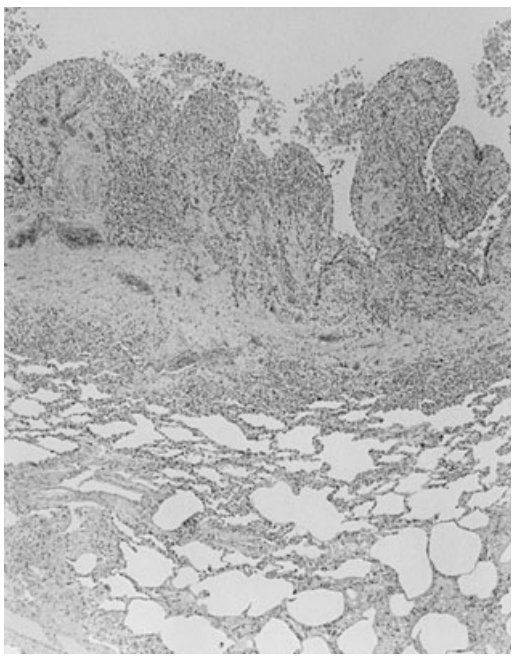


Figure 31–2 Late stages of pulmonary disease. Epithelial ulceration of the airway, loss of smooth muscle from the airway wall, inflammation, and bronchiectasis are present in the large airway at the top of the photomicrograph. Compression of the surrounding lung parenchyma occurs as bronchiectasis increases.

upper lobes. Death eventually occurs from respiratory failure. Progressive deterioration of pulmonary function occurs despite the routine use of anti-inflammatory therapy, mucolytics, airway clearance, and antimicrobial agents. Progressive destruction of the airway and increasing obstruction lead to air trapping, hyperinflation, hemoptysis, and spontaneous pneumothorax.

Many children with CF present during infancy with recurrent wheezing or persistent bronchiolitis. Most infants are asymptomatic at birth; however, many develop tachypnea, wheezing, hypoxia, and hyperinflation after having a respiratory viral infection.[146] These findings often resolve with therapy. As mucopurulent secretions increase, chronic cough develops.[56] Digital clubbing occurs gradually and correlates with severity of lung disease. On examination, there is evidence of crackles and decreased breath sounds secondary to mucopurulent secretions. Acute exacerbations may develop, requiring intravenous antibiotic therapy and frequent hospitalization. Approximately a third of patients with CF in the 2005 U.S. CF registry[75] experienced a pulmonary exacerbation requiring intravenous antibiotics. As lung disease progresses, tolerance for exercise is reduced, dyspnea increases, and respiratory failure develops. There is marked heterogeneity in the rate of progression of pulmonary disease. Some patients live to the sixth decade of life, whereas others die as a result of respiratory failure before their tenth birthday.

In 2005, the median predicted survival for individuals with CF was 36.5 years. Survival for children born in 2000 is expected to increase, with the median age approaching the fourth decade of life. In the United States, 40 percent of people with CF are 18 years old or older, and within 10 years, half are expected to be older than 18 years. As life expectancy has increased, CF has become a disease of children and adults.

DIAGNOSIS

The diagnosis of CF remains a clinical diagnosis in the face of more recent advances in technology. A consensus panel convened by the U.S. Cystic Fibrosis panel recommended a combination of phenotypic features, family history of CF, or positive newborn screen and one or more laboratory tests to diagnose CF.[284] Laboratory tests include identification of known CF mutations, abnormal bioelectric transepithelial membrane properties, and elevated concentrations of sweat chloride. The World Health Organization adopted similar recommendations.[379] Most patients continue to be diagnosed by clinical features and an elevated sweat test. Data from the 2005 U.S. CF registry reported that newborn screening accounted for 17 percent of newly diagnosed patients.[75] In the absence of newborn screening, an in utero diagnosis, or a family history of CF, a strong clinical suspicion is required for early recognition. Most children present with a history of recurrent lower respiratory tract disease and symptoms secondary to malabsorption. Approximately 15 to 20 percent of children have meconium ileus at birth or have a family history of CF or both.

The quantitative pilocarpine iontophoresis sweat test is one acceptable test to establish a diagnosis of CF.[284] A sweat chloride concentration greater than 60 mEq/L is consistent with a diagnosis of CF. Values between 40 and 60 mEq/L are considered borderline, and values less than 40 mEq/L are normal. Some data suggest that sweat chloride concentrations greater than 40 mEq/L in infants younger than 3 months old is highly suggestive of CF. The sweat test should be repeated on two separate occasions. Identification of two CF mutations by genotype is highly specific, but less sensitive. Mutational analysis can be done by several different techniques, with commercial laboratories testing for the most common 30 to 87 mutations. These laboratories identify approximately 90 percent of CF mutations but leave more than 1000 mutations unidentified. Extensive screening for the remaining 10 percent of mutations is expensive and impractical. For rare mutations, gene sequencing is available but expensive.[283,284] The ΔF508 mutation is found in 70 percent of patients with CF in the United States and a similar number of CF patients in the United Kingdom. Because each patient has two chromosomes, however, only 50 percent of patients are homozygous for ΔF508.

Nasal potential difference measurements assess the transepithelial electric potential difference that exists across nasal epithelium. Different patterns of potential difference are found in patients with CF.[284] Abnormalities in chloride transport and sodium absorption alter the transepithelial electric potential difference in CF compared with normal epithelia. The test can be useful in individuals with mild or atypical phenotypic features of CF, but it is not readily available, and it requires experienced personnel to perform.[283,284]

Newborn screening programs have been implemented in some states in the United States and the United Kingdom, Australia, and Europe. These programs identify newborns with CF using low values of trypsinogen in dried blood specimens obtained in routine screening programs for metabolic disorders. Improved nutritional status as a result of newborn screening has been reported in a controlled, randomized trial in Wisconsin.[104] Ninety percent of screened infants diagnosed with CF at birth maintained their weight greater than the 10th percentile compared with only 60 percent of unscreened controls. Children in the screened group were less likely to fall below the 10th percentile for weight and height from early childhood through 16 years of age.[44,105] In addition, cognitive function was improved significantly in the screened group.[182,183] No long-term improvements in pulmonary status were observed in the Wisconsin study; however, several observational studies have reported improved pulmonary outcomes, less colonization with *Pseudomonas aeruginosa*, decreased hospitalization for complications, and improved nutrition in children diagnosed by newborn screening.[5,106,180,188,278,282,386]

In addition to improving nutritional outcomes, newborn screening may result in improved survival rates for children. A systematic literature review of mortality in children with CF reported a survival benefit for patients diagnosed by newborn screening.[67,138] A survival effect also was shown in a study from Wales.[90] Without screening, approximately 60 percent of patients are diagnosed by the time they reach 1 year of age and almost 90 percent by the time they are 5 years. Early diagnosis through neonatal screening seems to improve nutritional outcome, with increasing evidence that these programs are associated with improved pulmonary outcomes and improved long-term survival.

The diagnosis of CF should be based on the presence of one or more clinical features (Table 31–2), a positive newborn screening test, and laboratory evidence of abnormal CFTR function. Laboratory tests include elevated sweat chloride concentration, two identifiable CF mutations, or abnormal in vivo nasal potential difference measurements made across the nasal epithelium.

PATHOGENESIS

The CFTR protein resides in the plasma membrane of epithelial cells and has several functions. In the sweat duct, it acts to absorb chloride; in the lung, it secretes chloride; and in the pancreas, it secretes chloride in exchange for bicarbonate.[342] Organs that are affected by the disease have abnormal chloride and fluid secretion, which impairs fluid movement and leads to ductular obstruction and organ damage. Five classes of CF mutations result in different amounts of CFTR expression and have been observed to produce varying clinical disease. The most common mutation, ΔF508, is a class 2 mutation. The CF protein is misfolded and degraded before reaching the cell membrane.[54,273]

Other classes of mutations produce CFTR that cannot be activated, is decreased in abundance, or has altered conductance. Patients with at least one "mild" allele have some low level of CFTR expression. They retain some degree of pancreatic function, have less severe lung disease, and have sweat chloride concentrations that are borderline normal.[77,79,372] Male patients with congenital bilateral absence of the vas deferens are pancreatic sufficient, exhibit little or no lung disease, can have normal sweat chloride concentrations, and are thought to have CFTR expression that is 10 percent of normal.[9] Carriers for CFTR who are heterozygotes expressing half the expected amount of CFTR are at increased risk for developing pancreatitis, allergic bronchopulmonary aspergillosis (ABPA), and sinusitis.[49,64,93,263]

The range of severity of disease is related to the production of functioning CFTR. The amount of expression of CFTR is important in the pathogenesis of CF. Variation in the severity of disease in individuals with the same genotype indicates other factors, including the environment, genetic modifiers, and medical therapy, are important in long-term outcomes, however.

The exact mechanism by which altered salt and water transport leads to abnormal secretions in the respiratory tract, pancreas, gastrointestinal tract, sweat glands, and other exocrine glands remains under intense investigation. The isotonic, low-volume model has been proposed to account for the pathogenesis of CF.[31,32] In the lung, abnormal respiratory tract secretions leading to decreased airway surface liquid seem to decrease mucociliary clearance and impair defenses to inhaled particulate matter and various microbial pathogens. The airway surface liquid is crucial for lung defense and is composed of a gel and sol layer. The gel or mucous layer is composed of high-molecular-weight mucins with carbohydrate side chains that bind inhaled particulate matter and pathogens. The sol or periciliary liquid layer consists of low-viscosity fluid to hydrate mucins and allows ciliary movement to occur. The periciliary liquid layer is 7 μm in height, allowing cilia to beat freely.

Airway surface liquid volume is regulated by the respiratory epithelium through ion transport processes. Salt concentrations can be changed to regulate the hydration of the airway lining fluid to maintain optimal mucociliary function. The absence of CFTR in the apical cellular membrane decreases the ability of cells to secrete chloride into the periciliary fluid. CFTR inhibits the epithelial sodium channel; in its absence, excessive absorption of sodium occurs. Other channels are available for secretion of chloride (calcium-activated chloride channel) in the respiratory epithelium; however, they cannot compensate for the loss of CFTR. Chloride also can enter the cell through a chloride transporter. The net effect is increased absorption of sodium, chloride, and water, which reduces the periciliary volume, alters the composition of mucins, and decreases mucociliary clearance. The reduction in the periciliary fluid impairs ciliary movement, decreases mucus transport, markedly alters clearance, and sets the stage for airway obstruction, infection, inflammation, and progressive lung destruction.

Altered chloride channel function and fluid secretion help to explain the presence of disease in the sweat gland, intestine, pancreas, and male genital tract. The epithelial cells affected by CFTR mutations in various organs represent different channel and regulatory activities of CFTR but result in deficient secretion

TABLE 31–2 Diagnosis of Cystic Fibrosis

One or More Phenotypic Features
Chronic sinopulmonary disease
Gastrointestinal and nutritional abnormalities
Salt loss syndromes—acute salt depletion
Chronic metabolic alkalosis
Male urogenital abnormalities resulting in obstructive azoospermia
Plus Laboratory Evidence of CFTR Abnormality (One or More)
Elevated sweat chloride concentrations
Identification of two CFTR mutations
In vivo evidence of abnormal ion transport across nasal epithelium

CFTR, cystic fibrosis transmembrane conductance regulator.

of fluids. This deficiency causes accumulation of mucus, obstruction, and various degrees of organ damage. Plugging of pancreatic ducts leads to chronic fibrosis, pancreatic atrophy, and loss of digestive enzymes and islet cells. Similar obstruction in the biliary tract can cause inflammation and focal biliary cirrhosis. Glandular obstruction of the vas deferens causes involution of the wolffian duct and infertility in more than 95 percent of men with CF. Women with CF produce abnormally tenacious cervical mucus, with reported rates of infertility of 20 percent.

Dehydration of airway surfaces and defective mucociliary transport has been termed the *low-volume model*.[32] Obstruction of small terminal airways and of submucosal glands with thickened mucopurulent secretions are the first signs of early disease in infants with CF. Ductular dilation, neutrophil infiltration, glandular hyperplasia, and peribronchiolar inflammation are classic findings of the disease. Mucus adherent to airway surfaces serves as a site for chronic infection. Human cell culture models of normal and CF epithelia have shown the airway surface liquid is decreased in CF,[208,341] and regulation of airway surface liquid volume depends on the regulation of sodium absorption and chloride secretion. Transgenic mouse models overexpressing epithelial sodium channels to mimic CF produce airway obstruction with mucus, neutrophilic inflammation, bacterial infection, and goblet cell hyperplasia.[202]

These findings are similar to observations in humans. Mucociliary clearance is decreased in CF.[31,176,275] Submucosal glands have high expression of CFTR.[100,139] Loss of CFTR function alters the composition of mucins produced by the submucosal glands,[163,176,361] leading to ductular dilation with mucus and obstruction. Mucus is tightly adhered to the respiratory epithelium and increases airway obstruction. Failure to clear mucous plugs, continued mucin secretion, and adherent mucus to the airway surface provides the focus for infection.

The respiratory airways of patients with CF are infected with several bacterial and viral pathogens that occur in an age-dependent order. Chronic infection with *P. aeruginosa* develops in 80 percent of affected individuals by the time they reach 18 years of age.[282] *Pseudomonas* infection is associated with a more rapid decline in lung function and increased rates of mortality[99,115] and becomes the dominant organism colonizing the CF airway. Initial isolates from CF infants and toddlers are unique and suggest an environmental acquisition.[236,321,322] Evidence of person-to-person transmission from chronically colonized CF patients to younger CF children, siblings, and individuals in close contact has been documented.[11,15,53,83,184]

Multiple hypotheses have been proposed to define the mechanism of airway colonization with *P. aeruginosa*. Although several studies have assessed this problem, the exact mechanism of infection and colonization remains debated. The primary hypothesis for airway infection in CF is altered mucociliary clearance secondary to abnormal salt and water transport. As the normal function of CFTR becomes defined, additional hypotheses have been put forward to explain the mechanism of early airway infection and inflammation. They involve altered epithelial cell receptors for bacteria, abnormal mucins that enhance bacterial binding impairing airway clearance, and inactivation of epithelial-derived bactericidal activity.

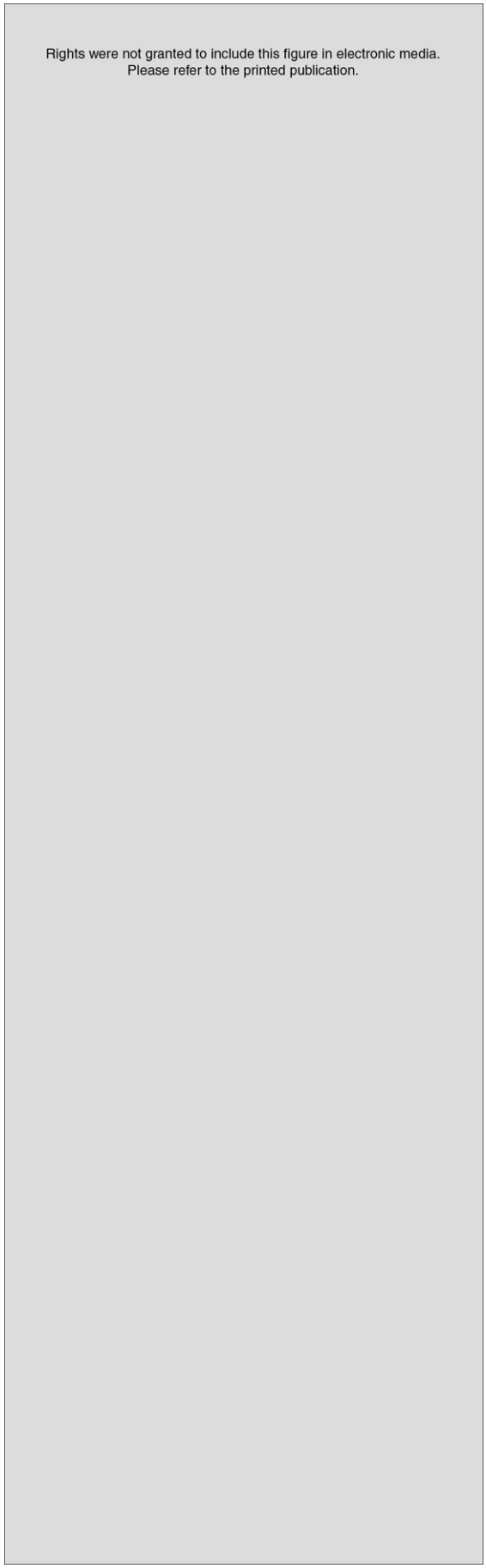
Increasing evidence indicates that the low-airway-surface-liquid-volume hypothesis explains the pathogenic mechanism of lung disease in CF. Dehydration of airway surface liquid and adhesion of mucus to the airway epithelium provide an environment conducive to chronic *Pseudomonas* infection (Fig. 31–3).[380] Depletion of periciliary water decreases ciliary activity, concentrates mucins, and increases adhesion of the mucous layer to the airway surface. Secretion of mucins continues from submucosal glands and goblet cells, producing plugging and airway obstruction. These mucous plaques are poorly cleared from the airway and become the sites of initial infection. Pili extending from the surface of the bacterium are able to bind to mucin.[308] An anaerobic gradient develops within the thickened mucous plugs. *Pseudomonas*, after binding to mucin, is able to penetrate the thickened mucus and grow in an anaerobic environment. *P. aeruginosa* is able to grow in anaerobic conditions because of the production of nitrate reductase, which allows it to cleave oxygen from nitrate.[304] When it is in the anaerobic environment, an alginate polysaccharide is formed. Biofilm-containing macrocolonies of *Pseudomonas* then are established. The established macrocolonies remain within the airway lumen. These macrocolonies are very resistant to antibiotics and host defense and allow chronic infection, inflammation, and airway destruction to occur.[151,250,380]

P. aeruginosa infection of the airway is associated with progressive respiratory impairment and death in most patients with CF. Bacterial colonization and infection of the lower respiratory tract occur early in infants with CF (Fig. 31–4), and airway inflammation often is established at the time the disease becomes clinically manifest.[14] *P. aeruginosa* infection is intermittent before chronic colonization develops.[115,136,281] Initial isolates generally are antibiotic-sensitive and nonmucoid.[37] In contrast, isolates from patients chronically colonized usually are highly antibiotic-resistant, mucoid, and increased in density.[102,324] As lung disease progresses, the phenotype changes and the bacteria adopt a mucoid biofilm mode of growth.[268] As the organism changes from a nonmucoid to mucoid state, differences in the outer membrane proteins are expressed.[143] In addition, hypermutable strains of *Pseudomonas* are isolated and show greater antibiotic resistance.[239] As *P. aeruginosa* converts from a nonmucoid to mucoid phenotype, several bacterial lipoproteins are produced that induce inflammation.[111]

Local production of cytokines and other proteins in response to bacterial infection may play an important role in modulating chronic airway inflammation.[306] Airway inflammation in CF is characterized by marked neutrophilic infiltration, with release of bioactive lipids, oxygen metabolites, myeloperoxidase, and lysozyme.[109] Proteases (e.g., elastase) derived from neutrophils and bacteria directly damage respiratory epithelium and cleave proteins important in host defense.[332,349] Chronic infection, with retention of the by-products of inflammation, ultimately leads to the severe bronchiectatic changes and derangements of gas exchange characteristic of end-stage CF.

Several in vivo studies have assessed the location of bacterial adherence within the lung of CF patients and the attachment of bacteria to CF and non-CF cells in vitro.[20,351] *P. aeruginosa* is found within the lumen of airways of patients with CF obtained from autopsy specimens.[20] These organisms are observed within the inflammatory exudates of the airway and not within epithelial cells lining the lung or in alveolar spaces. In vitro studies have shown adherence of *P. aeruginosa* in areas of epithelial cell destruction.[257,261,351] No adherence to the apical membrane of intact epithelial cells has been noted, however. In contrast, there is evidence in cell culture models that defects in CFTR enhance bacterial binding to immortalized airway epithelial cells.[290] A tetrasaccharide (asialo GM_1) is expressed more on CF than on non-CF cells and promotes *P. aeruginosa* binding to the epithelial membrane. Pseudomonal exoproducts such as neuraminidase increase the amount of asialo GM_1 available for bacterial binding and facilitate bacterial adherence to airway epithelial cells.[42,294]

Mucins also are important in binding bacteria within the airway. Sialylated and neutral forms of mucins bind *P. aeruginosa*.[264] Removal of sialylic acid from mucin by neuraminidase reduces adherence of *P. aeruginosa*. Mucus dehydration can lead to increased concentration of mucins, decreased pH, and a reduction in glutathione, which decreases mucus viscoelasticity.[287] The concentrated mucus impairs neutrophil migration, promotes an anaerobic environment, and reduces mucociliary transport. Mucin, a component of mucus, is decreased in CF, and its decreased content may promote development of infection.[288]



In contrast to the low-airway-surface-volume hypothesis discussed in detail earlier, the "high salt" theory proposes that CFTR maintains low concentration of salt in airway lining fluid. In the absence of CFTR, high concentrations of salt inactivate the secreted antimicrobial substances on airway surfaces.[384] Airway epithelial cells are vital in maintaining a pathogen-free environment within the airway. Smith and colleagues[311] showed that a monolayer of airway epithelial cells was able to kill bacteria when placed on their apical surface. In contrast, CF epithelia were unable to clear the bacterial load. Surface fluid washed from the normal cells showed bactericidal activity; however, fluid from the CF monolayer lacked bacterial killing properties. Goldman and colleagues[130] duplicated the results of this study using a different model. They implicated a human defensin, hBD-1, as the primary agent responsible for bacterial killing. The defensins are low-molecular-weight proteins produced by epithelial cells cytotoxic for bacteria and thought to be salt-sensitive. How deletion of CFTR affects the function of other antimicrobial substances such as lysozyme, which are produced by the respiratory epithelium, remains unclear.

Oxidant stress plays a role in tissue injury observed in CF. Reactive oxygen species are important in killing bacteria and are generated by phagocytic cells and the respiratory epithelium. Oxidants are produced from activated phagocytes, metabolic pathways, inhaled oxidants, and bacteria.[48] Increased oxidant production alters ion transport and increases secretion of mucins.[46,70,167,231,337,339] Excessive oxidant production can cause tissue injury. Increased oxidant production by CF neutrophils has been proposed as a mechanism of airway injury. Activation of reduced nicotinamide adenine dinucleotide phosphate (NADPH) within neutrophils results in production of precursors used by myeloperoxidase to generate chloramines. Chloramines are oxidants with long half-lives and, if produced in excess, potentially can damage the airway epithelium.[34]

One study has examined the activity of NADPH and myeloperoxidase with production of chloramines between CF and non-CF subjects.[374] Significant differences between groups were observed for myeloperoxidase activity and chloramine release. Myeloperoxidase activity and chloramine release were increased

Figure 31–3 Schematic model of the pathogenic events hypothesized to lead to chronic *Pseudomonas aeruginosa* infection in airways of patients with cystic fibrosis (CF). **A,** On normal airway epithelia, a thin mucous layer (*light gray*) resides atop the PCL (*clear*). The presence of the low-viscosity PCL facilitates efficient mucociliary clearance (denoted by *vector*). A normal rate of epithelial oxygen (O_2) consumption (QO_2; *left*) produces no O_2 gradients within this thin ASL. **B-F,** CF airway epithelia. **B,** Excessive CF volume depletion (denoted by v*ertical arrows*) removes the PCL, mucus becomes adherent to epithelial surfaces, and mucus transport slows or stops (*bidirectional vector*). The increased O_2 consumption (*left*) associated with accelerated CF ion transport does not generate gradients in thin films of ASL. **C,** Persistent mucus hypersecretion (denoted as mucus secretory gland/goblet cell units; *dark gray*) with time increases the height of luminal mucus masses and plugs. The increased CF epithelial QO_2 generates steep hypoxic gradients in thickened mucus masses. **D,** *P. aeruginosa* bacteria deposited on mucus surfaces penetrate actively or passively or both (due to mucus turbulence) into hypoxic zones within the mucus masses. **E,** *P. aeruginosa* adapts to hypoxic niches within mucus masses with increased alginate formation and the creation of macrocolonies. **F,** Macrocolonies resist secondary defenses, including neutrophils, setting the stage for chronic infection. The presence of increased macrocolony density and, to a lesser extent, neutrophils renders the now mucopurulent mass hypoxic. (See companion Expert Consult web site for color version.) *(From Worlitzsch, D., Tarran, R., Ulrich, M., et al.: Effects of reduced mucus oxygen concentration in airway Pseudomonas infections of cystic fibrosis patients. J. Clin. Invest.* 109*:317-325, 2002.)*

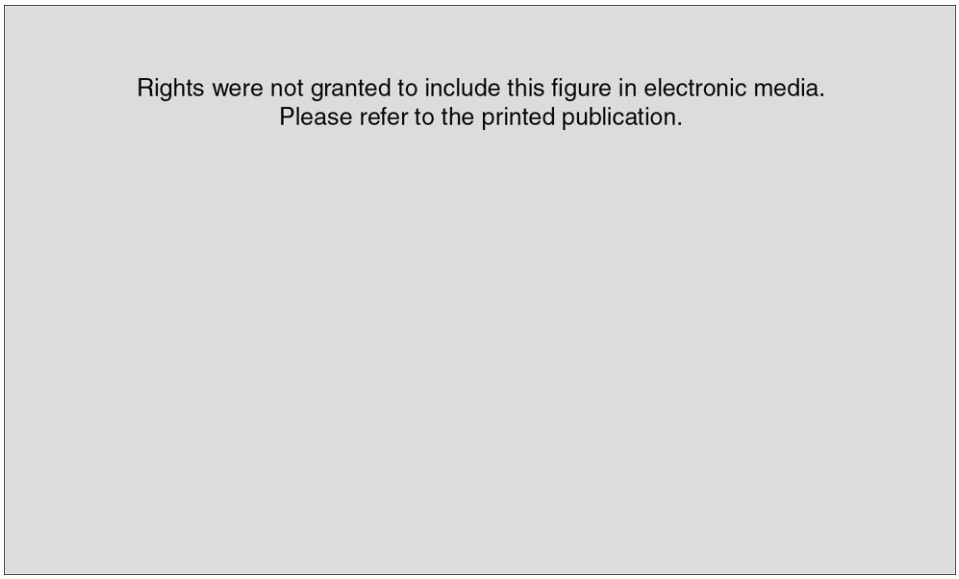


Figure 31–4 Age-specific prevalence of pathogens in patients with cystic fibrosis. *(From CF National Patient Registry, 2000.)*

in subjects with CF. Treatment of CF neutrophils with amiloride reduced oxidant generation, suggesting that altered intracellular ion concentrations or pH may regulate myeloperoxidase activity. Other investigators have suggested alternatively that the circulating neutrophils may be primed to respond to a phagocytic stimulus and that these differences in oxidant generation represent increased neutrophil activity.[135,271]

Antioxidants are produced by enzymatic and nonenzymatic pathways.[48] Mucins, reduced glutathione, alpha-tocopherol, and metal binding proteins function in the airway as antioxidants. The cysteine residues and carbohydrates of mucin account for its antioxidant properties.[71] Secretion of mucins is increased with oxidative stress. In CF, overproduction of mucus with subsequent dehydration may protect bacteria from neutrophil killing, worsening inflammation and causing more harm to the airway. In addition to affecting production of mucins, CFTR also may regulate production of glutathione.[190] Glutathione is an antioxidant found in large amounts in airway lung fluid from normal subjects[47]; however, it is reduced in patients with CF.[285] CFTR may alter glutathione transport, impairing defense to oxidant injury. Whether intracellular glutathione levels are decreased in CF, a factor that could be important in nuclear factor-κB (NF-κB) regulation, remains unclear. Increased oxidant production from neutrophils and impaired antioxidant biosynthesis can be altered by mutant CFTR, increasing the potential for oxidant injury in the CF airway.

Production of cell adherence molecules and inflammatory cytokines is intact in patients with CF.[88,174] Defects in bacterial opsonization, reduction in anti-inflammatory cytokines, and proinflammatory effects of bacterial DNA have been reported, however. Although humoral antibody responses and neutrophil phagocytosis from peripheral blood are normal, increased elastase in the CF airway can alter opsonic receptors, impairing phagocytosis. Large amounts of elastase are present in lavage fluid from airways of patients with CF, overwhelming the normal antiprotease activity. This condition can lead to local impairment in phagocytosis of bacteria within the airway lumen, contributing to bacterial persistence.

Proinflammatory mediators are elevated in BAL fluid from young patients with CF, as are macrophages expressing intracellular cytokines.[170,225,385] Increased numbers of neutrophils and interleukin (IL)-8 in BAL fluid are seen in bacterial culture–negative and bacterial culture–positive infants with CF compared with controls.[155,371] Proinflammatory mediators IL-1, IL-2, tumor necrosis factor-α, and IL-8 are markedly elevated in children with advanced disease.[144,155,371] In contrast, some anti-inflammatory cytokines (IL-10) are found in reduced amounts in BAL from subjects with CF. These observations have prompted some investigators to assess production of IL-10 from bronchial epithelial cells in normal subjects and subjects with CF.[26] Bronchial epithelial cells from normal individuals produce significantly greater amounts of IL-10 compared with cells of subjects with CF. NF-κB is a transcription factor for several proinflammatory mediators and is activated persistently in CF. Inhibitors to NF-κB, including IL-10, are down-regulated.[85,302,360,364]

Enhanced macrophage production of proinflammatory cytokines and decreased production of IL-10 have fostered a hypothesis that the CF gene defect may result in an inability to control airway inflammation after having infections. Alternatively, CFTR could have no effect on production of anti-inflammatory cytokines, and IL-10 could be down-regulated. Although the understanding of this disease pathogenesis is incomplete, airway damage that occurs from excessive inflammation remains a target for new therapeutic modalities.

MICROBIOLOGY OF CYSTIC FIBROSIS

EPIDEMIOLOGY OF PATHOGENS IN CYSTIC FIBROSIS

Most commonly, patients with CF are infected with classic pathogens, including *Staphylococcus aureus*, nontypeable *Haemophilus influenzae*, and *P. aeruginosa*.[8,41,126,214] A few patients may be infected with *Burkholderia cepacia* complex, which can be particularly virulent and, in some patients, associated with increased morbidity and mortality.[68,132,279] More recently, potentially emerging pathogens, such as methicillin-resistant *S. aureus* (MRSA), *Stenotrophomonas maltophilia*, *Achromobacter xylosoxidans*, nontuberculous mycobacteria (NTM), and mold species (primarily *Aspergillus* spp), and, more recently, *Scedosporium* spp., are being reported. Additional potential pathogens include *Ralstonia* and *Pandoraea* spp.[62,193] As described in more detail subsequently, the link between these pathogens and lung disease is not fully elucidated.

The etiologies behind these emerging pathogens are multifactorial. The average life expectancy of patients with CF has increased during the past 4 decades and now is 36.5 years.[75] Patients with CF are treated frequently with prolonged courses

of antimicrobial agents, including oral, intravenous, and aerosolized agents, placing selective pressure on their flora and leading to increased resistance.[38,75] Evidence of health care–associated acquisition of some pathogens, including *B. cepacia* complex, *P. aeruginosa*, and MRSA, is described subsequently. Finally, during the past 2 decades, detection of many potential pathogens has improved as clinical microbiology laboratories processing CF specimens use several selective media and fully speciate non–lactose-fermenting, gram-negative bacilli as described subsequently.[126,145,336,390]

AGE-SPECIFIC PREVALENCE OF PATHOGENS

The U.S. Cystic Fibrosis Foundation has maintained an annual Patient Registry since 1962.[112] The age-specific prevalence for CF pathogens in the United States as reported to this registry in 2005 is shown in Figure 31–4.[75] During the first decade of life, *S. aureus* is the most common pathogen and is harbored by approximately 40 percent of children and adolescents. Nontypeable *H. influenzae* is a less prevalent pathogen and is cultured from the respiratory tracts of 15 percent of children. Over time, *P. aeruginosa* becomes the most prevalent pathogen, but it can be recovered from 30 percent of infants. Approximately 80 percent of American patients with CF are infected with *P. aeruginosa* by the time they reach 18 years old. Overall, *Burkholderia* spp.[191] are recovered from 3 to 4 percent of patients and 10 percent of adults. During the late 1990s, the Cystic Fibrosis Foundation began to collect data on the prevalence of MRSA, *S. maltophilia*, and *A. xylosoxidans*. As shown in Figure 31–4, these organisms are more common than is *B. cepacia* complex and are harbored primarily by adult patients. As noted in patients without CF, MRSA has become increasingly common.[272]

ROLE OF THE CLINICAL MICROBIOLOGY LABORATORY

Ongoing communication between the clinical microbiology laboratory and the CF care team is crucial to ensure that appropriate methodologies are in place to provide appropriate care, to support effective infection control, and to further understanding of the epidemiology of pathogens in CF.[296] Every 5 years, the executive committee of the U.S. Cystic Fibrosis Foundation conducts site visits of accredited CF care centers and meets with the director of the clinical microbiology laboratory in efforts to ensure that standardized practices are in place.[295] In addition, a survey determined that most clinical laboratories processing CF specimens were using selective media and speciating all gram-negative bacilli,[390] which may include molecular identification strategies.

SPECIMEN PROCESSING

Respiratory tract specimens from patients with CF, including specimens obtained after lung transplantation, should be labeled "CF specimens" to ensure proper processing. Timely transport optimizes recovery of pathogens; ideally, specimens should be received by the laboratory within 3 to 4 hours of collection or stored at 4° C and processed within 24 hours. Sputum is the ideal specimen, and at least 1 mL should be collected to ensure an adequate volume is available for plating on selective media. Sputum can be Gram-stained to determine if a specimen contains bacteria and neutrophils rather than epithelial cells. Oropharyngeal cultures often are obtained in children too young to expectorate, but these cultures may not be concordant with specimens obtained from the lower airway.[280] BAL specimens generally are reserved for research protocols, lung transplant recipients, or patients with atypical courses who cannot produce sputum. More recently, induced sputum has been promoted to assess the lower respiratory tract flora of patients with CF who cannot expectorate.[149,244]

SELECTIVE MEDIA

Selective media are needed to isolate specific pathogens, although the use of these media is costly and time-consuming. Current recommendations for selective media are shown in Table 31–3. *S. aureus* strains from patients with CF may be thymidine-deficient and require mannitol salt agar for growth.[295] Detection of *B. cepacia* complex is markedly enhanced by the use of OFBPL (oxidative fermentative bacitracin polymyxin B lactose) agar or *B. cepacia* selective media.[126,145] Multicenter studies conducted by the Centers for Disease Control and Prevention in the 1980s showed that the use of selective media improved the yield of *Burkholderia* spp. from patients with CF.[334] In addition, specimens can be plated on mycosel agar to identify *Aspergillus* spp., although this mold grows well on blood agar. Oropharyngeal swabs from nonexpectorating patients also should be plated on selective media.[307] If NTM are suspected, sputum should be stained for acid-fast bacilli and processed using an additional decontamination step to prevent overgrowth by *P. aeruginosa*.[367] This decontamination step may kill NTM present in low concentrations, however.[21] Quantitative cultures of specimens from sputum are reserved for research purposes.

ANTIMICROBIAL SUSCEPTIBILITY TESTING

The optimal methodology for antimicrobial susceptibility testing for mucoid and nonmucoid strains of *P. aeruginosa* has been elu-

TABLE 31–3 Recommended Media and Processing for Recovery of Cystic Fibrosis Pathogens

Organism	Recommended Media or Processing*†
Staphylococcus aureus	Mannitol salt agar Columbia/colistin–nalidixic acid agar
Haemophilus influenzae	Horse blood or chocolate agar (supplemented or not with 300 mg/L bacitracin) incubated anaerobically
Pseudomonas aeruginosa	MacConkey agar
Burkholderia cepacia complex	OFPBL agar, PC agar, BCSA
Stenotrophomonas maltophilia	MacConkey agar, VIA agar DNase agar confirmatory media or biochemical or molecular identification
Achromobacter xylosoxidans	MacConkey agar Biochemical identification assay
Mycobacterial *spp.*	NALC-NaOH and oxalic acid decontamination step
Yeast	Mycosel agar‡
Other gram-positive organisms	Sheep blood agar supplemented with neomycin and gentamicin (streptococcal selective agar)
Other gram-negative organisms	MacConkey agar

**Detection of some pathogens may be enhanced by prolonging incubation for 4 days to allow slow-growing colonies to become apparent.*

†All media are commercially available.

‡Aspergillus spp. and other molds do not grow well on Mycosel, but do grow well (although not selectively) on many of the media used for cystic fibrosis specimens, especially OFPBL. Standard biochemical and phenotypic methods should be used for confirmation of the identification of molds.

cidated. In studies of 500 multidrug-resistant strains of *P. aeruginosa* from patients with CF, five different antimicrobial susceptibility testing methods were compared: a reference microbroth dilution assay, Kirby Bauer disks, E-tests, and the automated commercial systems Vitek and Microscan. The agar-based diffusion methods (Kirby Bauer disks and the E-test) proved most accurate compared with the reference methods.[39,289] Vitek and Microscan, the commercial systems, had unacceptably high rates of very major (i.e., false-susceptible) and major (i.e., false-resistant) errors.[40] The Clinical Laboratory Standards Institute (formerly the National Committee for Clinical Laboratory Standards) endorses the use of antibiotic-impregnated disks or reference broth microdilution assays to determine the susceptibility of multidrug-resistant strains of *P. aeruginosa* isolated from patients with CF.[229]

An intriguing concept in susceptibility testing is the potential role of susceptibility testing of bacteria grown in biofilms. As described in more detail subsequently, chronic lung infection in CF is thought to reflect a biofilm mode of growth containing bacteria in stationary phase that do not express the antibiotic targets present in log phase bacteria.[69] In vitro studies have shown that the minimal inhibitory concentration (MIC) of log phase bacteria tested in conventional susceptibility testing assays can be very different from the MIC of bacteria growing in stationary phase in biofilms.[147] Log phase–grown *P. aeruginosa* are resistant to macrolide antibiotics (MIC ≥ 128 μg/mL), whereas *P. aeruginosa* grown in biofilms are susceptible to these agents (MIC_{50} 2 μg/mL).[222] Studies are ongoing to compare the outcomes of pulmonary exacerbations treated using the results of conventional susceptibility testing versus the results from biofilm susceptibility testing.

SPECIFIC CYSTIC FIBROSIS PATHOGENS

VIRAL PATHOGENS

Numerous studies have evaluated the role of nonbacterial infections with pulmonary exacerbations in CF. A clear correlation between respiratory viral infection and exacerbations of lung disease has been shown in most of these reports.* Viral lower respiratory tract infections occur more often in younger children and are associated with increased pulmonary disease in 40 percent of cases in CF.[3,355] Respiratory syncytial virus (RSV) has been identified most often with CF pulmonary exacerbations; however, influenza, adenovirus, parainfluenza virus, and rhinoviruses also have been reported.[355] Respiratory viruses are isolated from patients with CF requiring hospitalization, and infection is associated with increased morbidity rates. Infants with CF infected with RSV can experience prolonged hospitalizations, mechanical ventilation, and supplemental oxygen at hospital discharge.[3] Severe viral infections in infants with CF were reported in 31 of 80 children diagnosed by newborn screening. Half of the children hospitalized had a respiratory virus identified at the time of hospitalization, and RSV infection accounted for hospitalization in 7 of the 31 children.[12]

Two studies have reported prolonged bronchiolitis-like syndromes in infants younger than 6 months of age.[119,196] These infants required intensive respiratory therapy, including bronchodilators, chest physiotherapy, and mechanical ventilation. Increased hospitalization was observed for children with CF who were infected with RSV in another study of children younger than 3 years of age.[146] Although prolonged hypoxia and respiratory failure were not observed, pulmonary function was markedly decreased after hospitalization and persisted for several months after infection.[146] Studies in older children with CF have reported reduction in pulmonary function, clinical scores, and radiographic scores and increased hospitalization after viral infection.[65,243,315,362,373] These studies show that infection with respiratory viruses results in clinical deterioration, hospitalization, and decreases in lung function.

Mechanisms to explain the enhanced disease observed with respiratory viral infections are being investigated. Normal airway epithelia use an adenosine-regulated pathway to maintain periciliary fluid volume by regulation of sodium absorption and chloride secretion. In a CF model, this regulation can be normalized by phasic motion simulating movement of the lung. Periciliary volume is restored to normal by release of adenosine triphosphate into the periciliary liquid and activation of alternative chloride channels. Viral infections, such as RSV, up-regulate the extracellular adenosine triphosphatase activity, reducing periciliary fluid volume. Reduction in periciliary liquid volume would promote mucous stasis and plugging.[341] Other investigators have shown impaired innate defense manifested by reduced production of nitric oxide and impaired interferon-γ signaling pathway.[387,388]

The interrelationship between the acquisition of *P. aeruginosa* and viral respiratory infection is unclear. Increased colonization with *Pseudomonas* has been observed during the respiratory viral season,[166] and infection with *Pseudomonas* has been associated with hospitalization for viral respiratory illness. Antipseudomonal antibodies have been reported to increase after having RSV infection, and new infection with *Pseudomonas* has been observed during an acute respiratory illness.[65,256] Other investigators have not observed any change in bacterial infection or change in colonization associated with viral respiratory infection.[238] In cell culture models, RSV acts as a coupling agent between *P. aeruginosa* and enhances attachment to respiratory epithelial cells.[356] Petersen and colleagues[256] and Ong and associates[243] reported more severe pulmonary disease with viral infection in the presence of *P. aeruginosa* colonization, but Przyklenk and coworkers[262] found no correlation. Synergism between bacteria and respiratory virus infection was suggested by Przyklenk and coworkers[262] after an increase in the number of bacterial colony-forming units was found in sputum of subjects with CF. Hordvik and associates[153] and Efthimiou and coworkers[94] noted that patients with CF and severe pulmonary disease recovered slowly from viral infection. The underlying severity of lung disease was crucial in predicting response to viral respiratory infection.

NONTYPEABLE *HAEMOPHILUS INFLUENZAE*

H. influenzae frequently is the earliest pathogen isolated from patients with CF. The advent of the *H. influenzae* vaccine has not had an impact on the prevalence of this organism because patients with CF harbor nontypeable strains. Generally, *H. influenzae* is susceptible to a wide variety of agents and is amenable to treatment. Despite the frequent recovery of this organism from infants and children, the impact of this organism on the clinical course of CF has been difficult to assess because of the frequent occurrence of co-infection with other pathogens.

STAPHYLOCOCCUS AUREUS

Methicillin-susceptible *S. aureus* (MSSA) and MRSA are increasing in prevalence. Patients generally retain the same clone of MSSA for their lifetime.[33,301] Data from the Cystic Fibrosis Patient Registry showed that the overall prevalence of MSSA increased from 37 percent in 1995 to 51.8 percent in 2005.[75] Similarly, MRSA increased from 0.1 percent in 1995 to 17.2 percent in 2005, although the prevalence varied substantially from center to center (range per center, 0 to 24%). The causes

*See references 3, 94, 118, 153, 238, 243, 256, 262, 266, 327, 362, 381.

for this variability are uncertain but could reflect different laboratory practices, different clinical practices, or regional differences in community-acquired MRSA. Although MRSA generally infects older patients, it usually is persistent[272] and can be associated with deterioration in both pediatric and adult patients.[216,345] A more recent report compared the outcomes of patients with MSSA versus MRSA and showed that children (<18 years old) and adults infected with MRSA had worse lung function, more hospitalizations, and more antibiotic use.[272]

Recent hospitalization has been shown to be a risk factor for MRSA, suggesting nosocomial acquisition,[127] but the relative importance of community-acquired MRSA strains (mecA type IV and V) has not been fully elucidated. A more recent case series has suggested that community-acquired MRSA strains expressing Panton-Valentine leukocidin can be associated with increased morbidity, including focal pulmonary infiltrates with cavitary lesions and a greater decline in lung function.[96]

PSEUDOMONAS AERUGINOSA

P. aeruginosa is the most common and important pathogen in patients with CF. Initial strains are nonmucoid, antibiotic-susceptible strains that express pili, flagella, and more highly acylated lipid A component of lipopolysaccharide.[134] *P. aeruginosa* also produces virulence factors, including exotoxin A, exoenzyme S, leukocidin, phospholipase C, elastase, and alkaline protease, which contribute to the pathogenesis of sepsis, acute lung infections, and bacteremia.[50,318,340] These factors may be chemotactic stimuli for neutrophils, and exotoxins may increase the viscosity of secretions and impair ciliary clearance and cause small airway obstruction and ultimately lung destruction.

Over time, *P. aeruginosa* adapts to the CF lung and undergoes genetic and phenotypic alterations. *P. aeruginosa* initially attaches to solid surfaces (e.g., mucin or respiratory epithelial cells), using flagella and type IV pili.[69] Attachment activates genes that synthesize extracellular polysaccharide (alginate),[84] which confers the mucoid phenotype of *P. aeruginosa* unique to chronic infections in CF.[133] Strains associated with chronic infections lack pili, lack flagella, and undergo structural changes in lipopolysaccharide. These changes may render *P. aeruginosa* more resistant to host defenses, including defensins and the innate inflammatory response. The *lasR-lasI* system (quorum-sensing genes) promotes the initial formation of microcolonies, which differentiate into alginate-encased mature biofilms.[69] Bacteria in biofilms avoid ciliary clearance, evade phagocytosis, and are antibiotic-resistant.[309] Biofilms are the proposed mechanism whereby *P. aeruginosa* is able to infect the CF airway chronically and avoid eradication by host defenses and by antimicrobial agents. The CF lung can harbor very high concentrations of *P. aeruginosa*—10^8 to 10^9 organisms per gram of sputum may be present.[376]

Infection with *P. aeruginosa* is associated with increased morbidity and mortality rates caused by recurrent pulmonary exacerbations and a gradual deterioration in lung function.[173,247] Children who are infected with *P. aeruginosa* are more likely to have cough and lower chest radiograph scores than are uninfected children.[156] Investigators have shown that infants younger than 2 years infected with *S. aureus* and *P. aeruginosa* have worse pulmonary function, chest radiograph scores, and 10-year survival rates than do uninfected children.[4] The mucoid phenotype of *P. aeruginosa* is associated with a more rapid decline in lung function.[248]

BURKHOLDERIA CEPACIA COMPLEX

Infection with *B. cepacia* complex and the associated "cepacia syndrome" was reported first in 1979 among adolescent Canadian patients with CF.[161,335] The cepacia syndrome is characterized by a virulent course, high fevers, bacteremia, rapid deterioration in lung function, and early death. Generally, patients with CF do not have bacteremia caused by other pathogens. Different clinical courses can be associated with *B. cepacia* complex, however, including transient colonization, a gradual decline in lung function, or the virulent cepacia syndrome.

TABLE 31–4 Members of the *Burkholderia cepacia* Complex, 2001

Species (Genomovar)	Binomial Designation	Reference
I	*B. cepacia*	Vandamme, 1997
II	*B. multivorans*	Vandamme, 1997
III	Pending	Vandamme, 1997
IV	*B. stabilis*	Vandamme, 1997, 2000
V	*B. vietnamiensis*	Vandamme, 1997; Gillis
VI	Pending	Coenye and LiPuma, 2001
VII	*B. ambifaria*	Coenye and Mahen, 2001
(VIII)	Pending	*
IX	*B. pyrrocinia*	*

**Manuscripts in preparation.*

Through a series of genetic and phenotypic studies, researchers have discovered that *B. cepacia* is actually a complex of several different species, previously called *genomovars*, which are indistinguishable phenotypically but distinguishable genotypically.[63] The genomovars of the *B. cepacia* complex are shown in Table 31–4, but additional genomovars are likely to be described. Several international investigators have described the epidemiology and clinical courses associated with different genomovars, although these studies usually involved a single strain and might not be generalized to all strains of a given genomovar.[192,199,323] Most CF isolates are *Burkholderia cenocepacia* (genomovar III) or *Burkholderia multivorans* (genomovar II). The former may be associated with a more rapidly progressive clinical course,[195] whereas *B. multivorans* is more likely to be associated with transient colonization.[199] Patient-to-patient transmission and clinical deterioration can be associated with *B. multivorans*, however.[192] Most recently, an outbreak of *Burkholderia dolosa* (genomovar VI) occurred, and this newly described genomovar was associated with increased morbidity and mortality rates.[168]

Virulence factors for *Burkholderia* spp. include multidrug resistance, the ability to form biofilms, the ability to reside intracellularly, and the ability to spread from patient to patient potentially via the cable pilus[331] or the *B. cepacia* epidemiologic strain marker.[199] Many outbreak strains do not express these transmission factors, however. Studies are ongoing to unravel potential environmental reservoirs of *Burkholderia* spp.

STENOTROPHOMONAS MALTOPHILIA

S. maltophilia, an intrinsically multidrug-resistant, gram-negative bacillus, is a well-known hospital-acquired pathogen in non-CF patients and is isolated with increasing frequency from the respiratory tract of patients with CF. The overall prevalence of this organism in patients with CF is 12 percent (range 0 to 27% in individual centers).[75,82] In a multicenter, randomized, placebo-controlled study of aerosolized tobramycin conducted in 520 patients 6 years old and older with CF, the baseline prevalence of *S. maltophilia* determined by a core laboratory was 10.7 percent,[41] whereas the national rate among comparably aged patients reported to the Cystic Fibrosis Patient Registry was 2.9 to 3.9 percent, suggesting that many clinical laboratories were failing to identify this potential pathogen. Transient colonization also seems to be a common occurrence; Demko and colleagues[82] reported that 50 percent of patients with CF at their center had

only a single positive culture for this microorganism. Increased use of antibiotics has been shown to be a potential risk factor for acquisition of *S. maltophilia*.[205,338]

The role of this organism as a pathogen in CF is still being investigated. Case-control studies have not shown that *S. maltophilia* has a significant impact on lung function or mortality.[131,205] In contrast, Demko and colleagues[82] found that the 5-year survival of patients with *S. maltophilia* (n = 211) with severe lung function at initial isolation of this organism was 40 percent compared with patients without *S. maltophilia* (n = 471), whose 5-year survival was 72 percent.

ACHROMOBACTER XYLOSOXIDANS

The clinical significance of *A. xylosoxidans* in CF also is unclear. In 1996, the first year that this organism was reported to the Cystic Fibrosis Patient Registry, 2.7 percent of patients harbored this multidrug-resistant, gram-negative bacillus.[74] As described for *S. maltophilia*, however, the nationally reported prevalence most likely is underestimated; 8.7 percent of participants in the aerosolized tobramycin trial had positive cultures for *A. xylosoxidans*.[41] Further complicating the epidemiology of this potentially emerging pathogen are the observations that *A. xylosoxidans* may be misidentified as some other non–lactose-fermenting, gram-negative bacilli, and that *P. aeruginosa*, *B. cepacia* complex, and *S. maltophilia* may be misidentified as *A. xylosoxidans*.[291] In addition, the impact of this organism is difficult to assess fully because *A. xylosoxidans* generally is cultured from patients concomitantly infected with other CF pathogens, but an association has been found between *A. xylosoxidans* and pulmonary exacerbations.[92,103]

ASPERGILLUS SPECIES AND *SCEDOSPORIUM* SPECIES

Patients with CF frequently are colonized with *Aspergillus* spp.; 24.5 percent of participants in the inhaled tobramycin trial indicated they had positive cultures for this mold.[36] Oral and aerosolized antimicrobial agents are risk factors for colonization with *Aspergillus* spp.[22,267]

Of patients with CF, 2 to 10 percent develop APBA, which can be associated with a dramatic loss of lung function.[97,121,207,224] The diagnosis of ABPA can be difficult to establish because of variability in the application of standardized diagnostic criteria, confusion regarding these criteria, and limited recognition by physicians. This immunologically mediated syndrome is marked by a brisk IgE response, specific antibody to *Aspergillus fumigatus*, peripheral eosinophilia, and symptoms of reactive airway disease. Short-lived pulmonary infiltrates may be noted on chest radiograph. Although *Aspergillus* spp. do not normally invade the parenchyma, the airways can become impacted with mucus-containing fibrin, eosinophils, and mononuclear cells, which can cause airway obstruction and bronchiectasis.[97]

A workshop sponsored by the Cystic Fibrosis Foundation sought to further understanding of the diagnostic criteria needed for ABPA in CF.[326] This committee established minimum criteria for the diagnosis, which included (1) new clinical deterioration not attributable to another cause; (2) IgE greater than 500 IU/mL; (3) immediate skin reactivity to *Aspergillus* antigen or IgE antibody to *A. fumigatus*; and (4) precipitants or IgG against *A. fumigatus*, new chest radiograph, or new chest CT findings.

Scedosporium spp. can be isolated from the lungs of patients with CF, but the clinical significance of this microorganism is unknown. In the aerosolized tobramycin trial, 2.4 percent of patients harbored saprophytic fungi,[41] and in a single center, 8.6 percent of 128 patients followed for more than 5 years were colonized or infected with *S. apiospermum*.[58]

NONTUBERCULOUS MYCOBACTERIA

Since the early 1990s, there has been an increasing appreciation that NTM may be pathogens in patients with CF. Olivier and colleagues[241] conducted a natural history study of NTM in patients with CF in 21 CF centers from 1993 to 1997. Using standardized mycobacteriologic methods,[368] these investigators found that the overall prevalence of colonization and infection with NTM was 13 percent. *Mycobacterium avium* complex (MAC) and *Mycobacterium abscessus* were most common, representing 72 percent and 16 percent of isolates, respectively. Patients with NTM were older and had better lung function, a lower prevalence of *P. aeruginosa*, and a higher prevalence of *S. aureus* compared with patients without NTM, suggestive of a "healthy survivor effect." Risk factors for NTM may include geographic proximity to large bodies of water,[241] ABPA, and steroid therapy.[227]

In an individual patient, determining if NTM is colonizing the lung or causing disease can be difficult. Signs and symptoms of mycobacterial disease are nonspecific and may be consistent with CF pulmonary exacerbations. Radiographic signs generally are nonspecific, but small nodules in the lung periphery or progressive "tree and bud" lesions may be noted on high-resolution CT scan.[242] Patients with positive acid-fast bacillus smears and two or more positive cultures are more likely to be infected than colonized with NTM.[7] Patients harboring *M. abscessus* are more likely to fulfill the American Thoracic Society diagnostic criteria for NTM disease than are patients harboring MAC.[137,240] In a study to assess the clinical impact of NTM, 60 incident-case patients with positive NTM cultures were followed for 15 months and did not have increased morbidity compared with control subjects with negative cultures.[240] Because this was a short duration of follow-up, further studies are needed to determine if NTM causes a progressive deterioration in lung function and, if so, the optimal therapy.

TREATMENT OF PATHOGENS IN CYSTIC FIBROSIS PATIENTS

A cornerstone of CF care is the aggressive use of oral, intravenous, and aerosolized antibiotics. The increasing longevity of patients with CF has paralleled the development of effective antibiotics. Antibiotics may be used during several stages of CF lung disease: (1) to prevent acquisition of pathogens, most commonly MSSA; (2) to eradicate initial acquisition of pathogens, most commonly *P. aeruginosa*, in efforts to prevent or delay chronic infection; (3) to treat pulmonary exacerbations caused by classic CF pathogens; (4) to treat patients infected with *P. aeruginosa*, as long-term suppressive therapy; and (5) to treat emerging multidrug-resistant pathogens.

PROPHYLAXIS TO PREVENT ACQUISITION OF *STAPHYLOCOCCUS AUREUS*

Few studies have been conducted on antibiotic prophylaxis in patients with CF, and these studies have focused exclusively on *S. aureus*. The rationale for this strategy is to prevent *S. aureus* infection and to delay acquisition of *P. aeruginosa*.[312,314] From 1985 to 1992, British investigators studied 42 newly diagnosed infants randomly assigned to 12 months of flucloxacillin versus standard care.[23,363] Infants treated with flucloxacillin had fewer infections with *S. aureus* and fewer hospitalizations, but both groups had similar pulmonary function. In a placebo-controlled trial conducted in the United States, 119 patients newly diagnosed with CF (mean age, 16 months) were randomly assigned to receive cephalexin or placebo for 5 to 7 years.[328] No significant differences were found in pulmonary function, the number of

pulmonary exacerbations, nutritional status, or chest radiograph scores in the two groups. In contrast, subjects treated with cephalexin had decreased incidence of infection with *S. aureus* but increased incidence of infection with *P. aeruginosa*. Similarly, an analysis of the German national database showed that patients receiving antistaphylococcal agents had increased acquisition of *P. aeruginosa* and no improvement in lung function.[269]

Antistaphylococcal prophylaxis, although practiced in the United Kingdom in infants, has not been widely endorsed in the United States because of concerns about the emergence of resistance, the increased risk of acquiring *P. aeruginosa*, and the lack of impact on lung function. The different findings in these studies may reflect the different agents studied because cephalexin is broader spectrum than is flucloxacillin or the different durations of therapy, or both. In an era of increasing prevalence of MRSA, strategies to prevent MSSA may prove to be less useful. To date, no studies have been done to assess antibiotic prophylaxis for other pathogens in CF.

EARLY ERADICATION OF *PSEUDOMONAS AERUGINOSA*

An increasingly practiced therapeutic strategy is to use antibiotics to eradicate initial acquisition of *P. aeruginosa* and prevent or delay chronic infection. This strategy was described first in Europe at the Danish Cystic Fibrosis Center.[114,164,333,352,359] The rationale for this approach is that antibiotics may be effective at eradicating initial infection and colonization with *P. aeruginosa* because the organism burden is low, organisms are largely susceptible, and a biofilm has not yet been established.

The optimal regimen for successful eradication is unknown. In some CF centers in Europe, colistin and ciprofloxacin are administered every 3 months after the initial isolation of *P. aeruginosa* has occurred.[114] Compared with historic controls, patients treated with this approach had improved lung function, improved survival rates, decreased prevalence of *P. aeruginosa*, and increased resistance to the therapeutic regimen.[333] In Australia, investigators used intravenous antibiotics followed by ciprofloxacin or aerosolized agents and found that 6 of 24 children no longer had *P. aeruginosa* isolated for 12 months or longer.[13] Compared with children treated with placebo, children treated with inhaled tobramycin within 7 to 12 weeks of initial infection and colonization with *P. aeruginosa* had shorter time to conversion from a positive to negative culture.[370] All 8 subjects treated with inhaled tobramycin compared with 1 of 13 subjects treated with placebo had successful eradication of *P. aeruginosa*.[123]

Despite the concerns about the potential emergence of antimicrobial resistance and lack of long-term studies that show the durability of eradication or an improvement in lung function, the growing consensus has been that early eradication for *P. aeruginosa* has merit. Two large, placebo-controlled studies are ongoing to assess the efficacy of different eradication regimens using different durations of inhaled tobramycin with and without oral ciprofloxacin. Neither of these trials is comparing an active regimen with placebo—evidence that early eradication has been endorsed by most of CF experts.

TREATMENT OF PULMONARY EXACERBATIONS

Pseudomonas aeruginosa

Much effort has been put into standardizing and validating the definition of a pulmonary exacerbation in CF. No currently accepted definition has been adopted universally, however, for use clinically, in quality improvement initiatives, or in clinical research. A combination of clinical signs and symptoms is used to define a pulmonary exacerbation in research trials, and similar factors are used in the clinical setting.

Mild exacerbations often are treated with oral ciprofloxacin[57,150] with or without an inhaled agent such as tobramycin or colistin. Treatment trials supporting the use of inhaled antibiotics for management of an exacerbation are lacking, however.

Several pivotal trials have led to widely accepted principles for intravenous treatment of more severe pulmonary exacerbations. During the 1970s and early 1980s, placebo-controlled trials showed that participants in the placebo group had increased morbidity and mortality rates compared with participants treated with intravenous antibiotics.[128,157,369] Hospitalized participants treated with bronchodilators and chest physiotherapy alone had less improvement in lung function and less reduction in bacterial density compared with patients treated with these interventions plus a β-lactam and an aminoglycoside agent.[270] Treatment with a β-lactam (azlocillin) and an aminoglycoside (tobramycin) agent leads to a significant reduction in bacterial density and a longer time to readmission for a new exacerbation compared with treatment with azlocillin alone.[310]

Studies also have compared various agents, singly or in combination. Most studies enrolled small numbers of patients and concluded that the comparative treatment regimens were equivalent in efficacy, but the studies were insufficiently powered to detect differences.[28,61,129,162,185,213,252,299,305] Many studies showed the emergence of resistance to study drug at the completion of therapy, which did not correlate with clinical response to treatment.[165,246] A larger multicenter trial randomly assigned patients undergoing a pulmonary exacerbation to ceftazidime and tobramycin ($n = 52$) or meropenem and tobramycin ($n = 50$).[25] Participants in the meropenem-treated group had a greater improvement in lung function than did participants in the ceftazidime-treated group (mean increase in forced expiratory volume in 1 second [FEV_1] percent predicted (38.8 percent versus 29.4 percent). Finally, examination of the safety and efficacy of single daily dosing of tobramycin versus multiple daily dosing during a pulmonary exacerbation showed that single daily dosing was associated with the same efficacy and reduced nephrotoxicity; in children, the mean percentage change in creatinine in the single daily dosing group was less than the mean change in the three-times daily dosing group (4.5% vs 3.7%).[313]

Accepted treatment of a pulmonary exacerbation caused by *P. aeruginosa* consists of two parenteral agents from different antibiotic classes to provide synergy and to delay the emergence of resistance.[122,223,265,293] Most commonly, a β-lactam agent with activity against *P. aeruginosa*, such as piperacillin or ceftazidime, is combined with an aminoglycoside agent, generally tobramycin. If a patient is co-infected with another pathogen, such as MSSA, additional agents are added. Treatment trials for pulmonary exacerbations caused by other CF pathogens are unavailable, however.

Antibiotic dosages must be higher or more frequent (or both) in patients with CF because the volume of distribution and clearance are increased in CF.[81] Treatment is administered for 14 to 21 days; treatment outcomes include improved lung function, improved well-being or improved quality of life or both, and a reduction in organism burden. Eradication of pathogens does not occur, however.[245] The Epidemiologic Study of Cystic Fibrosis analyzed the relationship between pulmonary function and treatment strategies for pulmonary exacerbations; centers with patients in the upper quartile of pulmonary function treated pulmonary exacerbations more frequently than did centers in the lower quartile.[221]

Inevitably, *P. aeruginosa* develops increasing resistance to antibiotics. Molecular studies have confirmed that resistance generally develops in the infecting strains, rather than the acquisition of more resistant strains,[237] although superinfection with multidrug-resistant *P. aeruginosa* can occur as described subsequently.[209] Much interest has been generated in optimizing the treatment of pulmonary exacerbations caused by multidrug-resistant *P. aeru-*

ginosa using in vitro synergy testing. Most commonly, checkerboard broth microdilution assays and multiple combination bactericidal testing have been studied.[189,293]

At the Cystic Fibrosis Referral Center for Susceptibility and Synergy Testing at Columbia University, the activity of clinically achievable concentrations of pairs of antimicrobial agents with different mechanisms of action (e.g., a β-lactam agent paired with an aminoglycoside) is tested.[293] To determine if a combination of agents is synergistic, a fractional inhibitory concentration is calculated; synergy is defined as a fourfold reduction in the MIC of agents alone compared with agents in combination.[204] Most multidrug-resistant strains, defined as resistance to all agents in two or more classes of antibiotics,[295] can be inhibited by one or more combinations of agents, but the efficacy of this assay has not been validated in clinical trials. In contrast, a pivotal randomized trial to study the clinical efficacy of multiple combination bactericidal testing synergy studies was performed in Canada and Australia, wherein patients undergoing a pulmonary exacerbation were randomly assigned to treatment guided by multiple combination bactericidal testing versus regimens chosen by individual treating physicians.[2] No significant differences in the time that elapsed until the next pulmonary exacerbation or improvements in lung function between the two treatment strategies were noted.

CF experts have not arrived yet at a consensus regarding the efficacy of inpatient versus outpatient management of pulmonary exacerbations. Outpatient management has advantages; it is less costly, is less disruptive to patients and their families, and involves less risk of acquiring nosocomial pathogens. Patients treated at home were shown, however, to have longer treatment courses and less improvement in lung function.[228] Patients may improve more with hospitalization, as a result of better compliance with antibiotics, bed rest, chest physiotherapy, and bronchodilator treatments.[30]

Staphylococcus aureus and Methicillin-Resistant *Staphylococcus aureus*

The clinical utility of antistaphyloccocal agents is best shown by early studies of infants with CF treated with penicillin before the nearly universal acquisition of β-lactamases by *S. aureus*.[8,214] Infants treated during the antibiotic era had markedly improved survival rates. Experts advocate the use of a first-generation cephalosporin (e.g., cephazolin) or a semisynthetic penicillin (e.g., oxacillin) for treatment of a pulmonary exacerbation associated with MSSA.[122] Treatment trials justifying this approach are lacking, however.

Vancomycin generally is advocated for treatment of MRSA when this organism is considered a pathogen. Although no treatment trials in CF patients using linezolid have been conducted, a case series showed that adults with CF required 600 mg every 8 hours to provide desired pharmacokinetics.[29,298] Linezolid-resistant MRSA did emerge in a child with CF treated for approximately 3 months.[117] Because this agent is available orally and intravenously, the potential for "abuse" exists if clinicians do not use this agent judiciously. Several investigators in Europe have published small case series of various regimens for eradication of MRSA that have included oral agents (e.g., rifampin and fusidic acid) and inhaled agents (e.g., vancomycin).[120,198,201,317] Randomized treatment trials must be performed to establish the safety and efficacy of these strategies.

Burkholderia cepacia Complex

Management of *B. cepacia* complex is more problematic because of higher levels of intrinsic antibiotic resistance and a paucity of clinical trials for this pathogen. Initial isolates may be susceptible to ciprofloxacin, β-lactam antibiotics, chloramphenicol, trimethoprim-sulfamethoxazole, meropenem, and minocycline, but with the exception of *Burkholderia gladioli*, all *Burkholderia* spp. are resistant to aminoglycosides. Temocillin has been used in Europe to treat pulmonary exacerbation caused by *B. cepacia*.[344] Resistance can develop, however, which severely limits therapeutic options. As described for the management of *P. aeruginosa*, combinations of two or three agents are used to treat a pulmonary exacerbation. Even more prolonged courses may be required to improve lung function. Meropenem and minocycline were noted to have the most in vitro activity against 652 multidrug-resistant *Burkholderia* strains isolated from patients with CF and tested at the Cystic Fibrosis Referral Center, and inhibited 28 percent and 33 percent of strains.[197] Treatment may be guided by synergy testing of two or more drug combinations with an evident improvement in pulmonary function.[1,389]

Stenotrophomonas maltophilia and *Achromobacter xylosoxidans*

There are no published treatment trials for patients with CF who are harboring *Saltophilia* or *A. xylosoxidans* undergoing an exacerbation. Although definitive data confirming that these organisms are pathogens in CF are unavailable, clinicians target these multidrug-resistant, gram-negative bacilli if they are consistently recovered from the respiratory tract of an individual patient. At present, the Clinical Laboratory Standards Institute recommends testing ticarcillin-clavulanate, ceftazidime, minocycline, chloramphenicol, trimethoprim-sulfamethoxazole, and levofloxacin against *S. maltophilia*,[60] but CF strains frequently are resistant to these agents.[297]

Nonetheless, similar strategies are used to treat these organisms as described for *P. aeruginosa* or *B. cepacia* complex; two or more parenteral agents are chosen based on susceptibility testing and given for 2 to 3 weeks. In a survey of 263 isolates of *S. maltophilia* from 218 patients with CF isolated from 1995 to 1998, doxycycline, ticarcillin-clavulanate, piperacillin-tazobactam, and trimethoprim-sulfamethoxazole were most active and inhibited 78 percent, 39 percent, 18 percent, and 13 percent of isolates, respectively.[297,389] A survey of 94 *A. xylosoxidans* isolates from 77 patients showed that meropenem and imipenem, or piperacillin with or without tazobactam, were most active.[291]

Nontuberculous Mycobacteria

Treatment of NTM in patients with CF is challenging and guided by the clinical presentation and the mycobacterial species. Before therapy for NTM is initiated, patients should be treated aggressively for the classic CF pathogens they harbor to determine if they clinically improve without the need for specific NTM treatment. If NTM treatment is initiated, a careful history, physical examination, and pulmonary function tests should be done to determine the baseline status of the patient in efforts to monitor response to therapy. Susceptibility testing for NTM is not done routinely for patients who do not have CF. Given the increased antibiotic exposure in CF and the potential for the emergence of resistance, NTM strains isolated from patients with CF should undergo susceptibility testing at a reference laboratory. Treatment can be guided by the initial susceptibility profile.

No treatment trials have been published for NTM in CF, and generally regimens are comparable to the regimens used in patients without CF. Treatment of MAC is straightforward and includes a macrolide agent, rifabutin, and ethambutol. Treatment of *M. abscessus* is more complex. Many experts have a lower threshold for treating *M. abscessus*, given the greater apparent virulence of this species in CF, including after lung transplant.[52] Some experts recommend oral therapy, including a macrolide and linezolid because both have excellent in vitro activity, for stable patients.[35] Some experts add vitamin B_6 to prevent periph-

eral neuropathy, but the pathogenesis of peripheral neuropathy from linezolid is unknown. For more symptomatic patients, intravenous therapy, including cefoxitin or a carbapenem, depending on susceptibilities and amikacin, is recommended. Such patients need to have hearing assessed regularly. Because of the potential for different pharmacokinetic properties in CF and the fact that oral agents may not be well absorbed by patients with CF, serum levels for antimycobacterial agents should be strongly considered.[125,255]

Patients should be followed closely and have monthly sputum testing done for acid-fast bacillus smear and culture. The response to treatment generally is good for patients infected with MAC. Although the optimal duration of treatment has not been studied, experts recommend continuing therapy for 1 year after negative sputum cultures have been obtained. Cure for *M. abscessus* is less likely to occur, and the therapeutic goal may be long-term suppression. After clinical and mycobacterial improvement (i.e., a reduction in organism burden) has been achieved, intravenous antibiotics can be stopped and prolonged oral therapy with a macrolide can be initiated.

Allergic Bronchopulmonary Aspergillosis

Treatment of ABPA also can be very challenging. Although steroids are the treatment of choice because ABPA is immunologically mediated, the response to steroids varies and may have the undesirable consequence of diabetes in this vulnerable patient population. The oral antifungal therapies voriconazole[148] and itraconazole[230] have been used with reported response and toxicity, but no controlled trials have been performed.[223] Monitoring serum levels of itraconazole is desirable because this agent may be malabsorbed if the gastric pH is non-acidic.

Long-Term Suppressive Therapy

INHALED ANTIBIOTICS

Long-term suppressive antibiotic therapy has been used increasingly to treat patients with CF and infected with *P. aeruginosa* to prolong the time between pulmonary exacerbations and to slow the progression of lung deterioration. Numerous oral agents that are not cidal for *P. aeruginosa* (e.g., amoxicillin-clavulanate, trimethoprim-sulfamethoxazole, or tetracycline agents) are used empirically, sometimes in rotation, with anecdotal support coming from patients and physicians. The use of oral antibiotics is widespread; during a 6-month period, 90 percent of patients received at least one course of oral antibiotics.[41] No clinical trials support this practice, however, and it may contribute to antibiotic resistance.

For decades, inhaled agents such as tobramycin, colistin, gentamicin, amikacin, and various β-lactam antibiotics, including carbenicillin and cephaloridine, also have been used for management of chronic infections.[45] Definitive data supporting the use of inhaled agents were derived from a multicenter phase III, double-blind, placebo-controlled, randomized treatment trial of inhaled tobramycin (TOBI) in patients with CF and chronic *P. aeruginosa* infection.[267] Patients received 300 mg of tobramycin twice daily every other month for 6 months. The rationale for administering therapy every other month was to delay the emergence of resistance, to reduce cost, to increase compliance, and potentially to exploit the post-antibiotic effect of aminoglycosides. Subjects randomly assigned to inhaled tobramycin had a 10 percent improvement in FEV_1 compared with subjects who received placebo, who experienced a 2 percent decline in FEV_1. Treated patients also had a reduction in bacterial density, fewer days of hospitalization, and fewer days of intravenous antibiotics. In subset analyses, adolescents had the greatest improvement in pulmonary function. During treatment, patients receiving tobramycin had increased tinnitus that was unassociated with hearing loss. There was minimal systemic absorption of tobramycin because serum levels of tobramycin generally were less than 1 μg/mL.

Although this study confirmed the efficacy of inhaled tobramycin in CF patients with chronic *P. aeruginosa* infection, several questions remain. In routine antimicrobial susceptibility testing, the breakpoint for resistance to tobramycin is MIC of 16 μg/mL or greater. The aerosol route delivers 100-fold higher concentrations of tobramycin without toxicity, however; the median concentration of tobramycin in the phase III trial was 1200 μg/g of sputum.[267] Although the conventional breakpoint for resistance to tobramycin is irrelevant for drug delivered by inhalation, the breakpoint for inhaled tobramycin is unknown. Commercially available susceptibility tests do not measure MICs greater than 8 μg/mL. During the 6-month trial, resistance to tobramycin did occur; a trend was found toward higher MICs in the treatment group (median MIC, 2 μg/mL) compared with the placebo group (median MIC, 1 μg/mL). Only a few subjects had tobramycin MIC of 128 μg/mL or greater, however, and the impact of these higher MICs on clinical efficacy could not be determined because of the small number of such patients.

Investigators also examined the emergence of intrinsically tobramycin-resistant pathogens. Although treatment-emergent *B. cepacia*, *S. maltophilia*, and *A. xylosoxidans* did not increase in the participants randomly assigned to inhaled tobramycin, the incidence of treatment-emergent *Aspergillus* spp., but not ABPA, was increased.

Buoyed by the success of aerosolized tobramycin, other aerosolized agents have been studied. Most recently, aerosolized aztreonam has been developed for long-term suppressive therapy, with a proposed strategy of alternating with aerosolized tobramycin.[124] A phase III trial has been completed, but the results are not yet published.

MACROLIDE ANTIBIOTICS

Much interest has been generated more recently in the use of macrolide agents in CF[154] as long-term suppressive therapy. The rationale for macrolide therapy in CF stems from the successful treatment of diffuse panbronchiolitis with erythromycin, azithromycin, and clarithromycin.[177,186] Diffuse panbronchiolitis is a chronic lung disease, diagnosed primarily in Asian adults, with several clinical features similar to those found in CF, including progressive lung disease caused by mucoid and nonmucoid strains of *P. aeruginosa*.[330] Long-term administration of low-dose macrolide agents to patients with diffuse panbronchiolitis has reduced morbidity and mortality rates.

In vitro studies have provided the scientific rationale for this clinical efficacy. Although macrolides are not cidal for *P. aeruginosa*, subinhibitory concentrations of macrolide agents reduce the production of several virulence factors by *P. aeruginosa*,[218,219] including the formation of biofilm.[160,179] Macrolide antibiotics also may have an anti-inflammatory effect and decrease cytokine production by neutrophils, monocytes, and bronchial epithelial cells.[159,382]

Four azithromycin trials have been conducted in patients with CF so far.[59,101,292,375] Three trials were conducted in patients chronically infected with *P. aeruginosa*[101,292,375] and had similar results; all showed improvement in lung function using similar treatment regimens of azithromycin. Improvements in secondary outcomes, such as decreased hospitalization, decreased antibiotic use, and increased weight gain, also were noted. In the more recent study performed in 82 children and adolescents, most (63 of 82, 77%) with negative cultures for *P. aeruginosa*,[59] participants in the azithromycin-treated group had fewer pulmonary exacerbations and less oral antibiotic use. No differences in lung function or intravenous antibiotics were noted, however, in the

azithromycin-treated group compared with the placebo-treated group. A Cochrane review concluded that short-term (i.e., 3 to 6 months) azithromycin seemed effective in CF, but long-term safety and efficacy remain unknown.[319] Patients treated with azithromycin should be screened for NTM before initiating therapy and annually thereafter because of concern about macrolide resistance in these microorganisms.

LUNG TRANSPLANTATION

Lung transplantation currently is considered a viable therapy for selected patients with end-stage pulmonary disease of CF.[383] Bilateral lung, heart-lung, and living donor lobar lung transplantation all have been performed in patients with CF, but most operations in recent years have used the bilateral lung transplant approach with lungs from deceased donors. By 2006, more than 3000 individuals with CF had undergone lung transplantation worldwide, with nearly 250 procedures performed annually as of 2005.[350] The survival of lung transplant recipients with CF exceeds that of any other diagnostic group for all ages, with projected survival half-life of 6.2 years.[350] Patients with CF nonetheless present special challenges for successful lung transplantation. The life-threatening manifestations of CF generally are limited to the lungs except for patients with advanced liver disease with portal hypertension. Advantages of a lung transplant recipient with CF include relative young age, giving the potential of many years of productive life ahead. In addition, patients with CF have experience with complex medical regimens.

The optimal time to refer potential candidates for evaluation for lung transplant is difficult to determine because the natural history of CF cannot be predicted precisely. In May 2005 in the United States, the United Network for Organ Sharing introduced a new system of lung distribution, which assigns a lung allocation score to each potential candidate. Key parameters used in the lung allocation score include lung function testing, the need for oxygenation, the presence of diabetes mellitus, age, body mass index, the need for ventilatory support, hospitalization in the intensive care unit, and hemodynamic data from cardiac catheterization. Waiting time no longer is considered except in individuals younger than 12 years of age. There are no microbiologic criteria.[95]

Although transplanted lungs do not develop CF, they can become infected with the pretransplant pathogens because the trachea and paranasal sinuses continue to manifest the pathophysiology of CF. Lung transplant recipients with CF are at risk for development of infection via pretransplant microbial flora or newly acquired pathogens.[169,195,316,325] Controversy has ensued about whether microbiologic criteria, specifically pretransplant infection with fungi, NTM, or multidrug-resistant pathogens, should be considered contraindications to performing lung transplantation in patients with CF. Several centers have published their experiences with infections that developed from lung transplantation pathogens; they include invasive aspergillosis; sepsis with *S. maltophilia*, *B. cepacia* complex organisms, and *B. gladioli*; and sternal wound infection.[169] Other centers have reported that morbidity and mortality caused by *P. aeruginosa* infections post-transplant are not higher in patients with CF than in patients who do not have CF.[113,234]

A more recent study indicated that lung transplant recipients with CF and with panresistant, gram-negative organisms other than *Burkholderia* spp. had significantly decreased survival rates after transplantation than a comparable group with antibiotic-sensitive organisms.[142] Patients with CF who are infected with *B. cenocepacia* have increased morbidity and mortality rates from recurrent infection or sepsis or both after lung transplantation. Although Aris and colleagues[10] did not detect patient-to-patient transmission, increased mortality rates were noted with *B. cenocepacia* but not with multidrug-resistant *P. aeruginosa*. Patients infected with genomovar III had increased mortality rates after lung transplantation compared with patients infected with other genomovars before transplantation.[10]

Infection with *M. abscessus* before undergoing transplant can be associated with severe post-transplant complications and death.[55,343] Acquisition of NTM post-transplant can be treated successfully with prolonged combination of antimicrobial therapy.[55,203] Patients with CF are at risk of developing invasive aspergillosis after transplant, but at lower rates than patients without CF.[234,249] Many of these patients with CF were colonized with *Aspergillus* spp. before undergoing transplantations. Treatment with antifungal therapy after surgery should be considered. The U.S. Cystic Fibrosis Foundation held a consensus conference on lung transplantation in 1996.[383] The conferees concluded that microbiologic contraindications to transplantation were limited to active hepatitis B and human immunodeficiency virus. The decision to transplant patients infected with *B. cepacia* complex or multidrug-resistant *P. aeruginosa*, or colonized with *Aspergillus* spp., should be made on a case-by-case basis. Potentially, an understanding of the genomovar infecting a patient may be a useful predictor of mortality.

ANTI-INFLAMMATORY THERAPY

Inflammation associated with chronic bacterial colonization plays a crucial role in CF lung disease. Nonsteroidal agents and glucocorticoids have been used as potential medications for anti-inflammatory therapy. A randomized trial using oral prednisone (1 to 2 mg/kg every other day) showed a reduction in the rate of decline in pulmonary function of patients with CF but was associated with significant steroid-related side effects.[16] Three short-term trials using inhaled steroids have led to mixed results and no clear direction for long-term use.[18,232,357] A systematic review of inhaled steroids for CF was equivocal with respect to efficacy.[17] A multicenter randomized trial of withdrawal of inhaled steroids in CF did not find a difference in pulmonary exacerbations, infection with *P. aeruginosa*, or pulmonary function after inhaled steroids were discontinued.[19] High-dose ibuprofen (20 to 30 mg/kg twice per day) used over a 4-year period showed a decreased rate of decline in pulmonary function with few reported side effects.[181] Macrolide antibiotics decrease inflammation by modulation of inflammatory signaling pathways and decreased cytokine production from inflammatory cells.[98] Several anti-inflammatory agents, including antiproteases, statins, and antioxidants, have been proposed as potential therapies, but they remain under investigation and are not a part of routine clinical practice.

PREVENTION

IMMUNIZATIONS

The use of currently available routine childhood and adolescent vaccines is strongly advocated for patients with CF. Although patients with CF are not at increased risk of developing *Streptococcus pneumoniae* infections, they should receive the pneumococcal vaccine, 7-valent (Prevnar). Annual influenza vaccination is recommended for patients with CF and household members.[104,110] The RSV subunit vaccine, PFP-2 (purified fusion protein vaccine is a fusion glycoprotein that induces serum neutralizing antibodies) has been studied in children with CF for two RSV seasons.[260] Initial studies showed that the RSV subunit vaccine was safe and immunogenic and that it reduced the incidence of lower respiratory tract infections.[259] A large multicenter trial in patients with mild disease failed to show a reduction in the incidence of lower

respiratory tract infection, however.[258] A placebo-controlled trial of RSV monoclonal antibody (Palivizumab) was performed in 186 children.[320] Although Palivizumab was well tolerated, two children were hospitalized (one in each group); the study was not adequately powered for efficacy.

Most recently, the results of a phase III multicenter study of a *P. aeruginosa* vaccine were published.[89] Although this bivalent, antiflagella vaccine was immunogenic and was found to reduce the incidence of *P. aeruginosa* infection and serum antibody titers, it did not prevent chronic *P. aeruginosa* infection, and it did not affect lung function.

INFECTION CONTROL PRECAUTIONS

Although the sources of most pathogens in patients with CF are unknown, it is increasingly recognized that patient-to-patient spread and acquisition from the contaminated health care environment can occur. To date, reports of transmission of bacterial pathogens occurring between individuals with and without CF are rare.[127,152,210] Direct contact, indirect contact, and spread of droplets with infectious secretions all have been implicated as modes of transmission of CF pathogens. Risk factors for transmission were described first for *B. cepacia* complex as shown in Table 31–5.[51,132,194,253,254,346]

Perhaps of most concern have been several reports of clonal spread of *P. aeruginosa* among patients with CF.[209,303,354] These examples have involved obvious phenotypes that triggered an investigation of possible patient-to-patient spread, including an increase in ceftazidime-resistant *P. aeruginosa*[53] or initial colonization of young children with mucoid strains of *P. aeruginosa*.[13,233] These reports have led CF centers in Europe to favor cohorting and segregating patients with *P. aeruginosa*, particularly patients with antibiotic-resistant strains, in hospital and clinic settings.[114,178,209] In the United States, infection control strategies have focused on containment of respiratory tract secretions as described subsequently.[296] Finally, occasional cases of patient-to-patient spread of other potential pathogens, including *S. maltophilia* and *A. xylosoxidans*, have been reported in patients with CF.[276,353]

The U.S. Cystic Fibrosis Foundation convened an expert panel to develop revised recommendations for infection control that addressed inpatient, outpatient (i.e., CF clinic and pulmonary function test laboratories), and non–health care settings, as so much CF care is delivered in the home. These recommendations have implications for treatment, transplantation, and the psychosocial well-being of patients, families, and staff. Several strategies were emphasized (Table 31–6). The guidelines emphasized that all patients with CF could harbor potentially transmissible respiratory tract pathogens, and containing respiratory tract secretions is of paramount importance.

The consensus is that patients harboring *B. cepacia* complex should be cared for apart from other patients with CF and not grouped together because of concern that some strains of *B. cepacia* complex may be more virulent and replace other strains.[200] Patients are hospitalized in single rooms and seen in outpatient settings geographically or temporally apart from other patients with CF. Similarly, patients with CF and MRSA in the respiratory tract should be placed in private rooms without access to common areas.

CONCLUSION

The microbiology of patients with CF is complex and changing. Although the pathogenesis of lung infections is still under active investigation, the current hypothesis suggests multiple etiologies. Appropriate microbiologic processing of respiratory tract specimens is crucial to ensure an accurate understanding of the epidemiology of CF lung disease and to provide appropriate treatment and infection control. Current treatment strategies are directed largely at management of deteriorations of pulmonary function, but increasingly strategies are directed at prevention and preservation of lung function.

TABLE 31–5 Risk Factors Associated with Acquisition of *Burkholderia cepacia* Complex

Risk Factors	Comment
Attendance at CF summer camp[253]	Risk of acquisition (6% incidence) increased with time spent at camp and prevalence of *B. cepacia* at the camp
Slept in same cabin	
Shared a personal item	
Danced with *B. cepacia*–infected camper	
Attendance at summer educational program[194]	3 (20%) of 15 patients acquired same ribotype
Participation in adult CF group	Disband meetings thought to represent extensive social contact
Social contact[132]	
Kissing	
Intimate contact	
Prolonged car rides	
Sibling with *B. cepacia* complex	
Handshaking	2 of 68 cultures positive, 1 from patient and 1 from investigator[254]
	3 patients' hands became contaminated after coughing[132]
Inpatient exposures	Risk of acquisition increased if hospitalized within 3 mo and if hospitalized longer
Recent hospitalization[254]	Interviews with health care workers indicated poor adherence to contact precautions
Use of specific shower	
Sharing hospital room with another patient infected with *B. cepacia*	
Cared for by a medical student	
Respiratory therapy equipment	Reservoirs of large-volume nebulizers grew *B. cepacia*
Sharing equipment	
Hospital nebulizers	
Spirometer[161]	
Mouthpiece filters[132]	

CF, cystic fibrosis.

TABLE 31–6 Infection Control Strategies for Cystic Fibrosis

Guideline	Setting		
	Inpatient	*Outpatient*	*Non–Health Care*
Quarterly cultures of respiratory tract		X	
Appropriate processing of CF respiratory tract cultures	X	X	
Educate patients and families about proper hand hygiene	X	X	X
Implement contact precautions for MDROs, including MRSA	X	X	
Hospitalize patients with MDROs in single-patient room	X	X	
Segregate patients with *B. cepacia* complex from other CF patients	X	X	X
Clean and disinfect respiratory therapy equipment	X	X	X
Avoid socialization among CF patients	X	X	X
Maintain at least 3 ft between CF patients to prevent droplet transmission	X	X	X

CF, cystic fibrosis; MDROs, multidrug-resistant organisms; MRSA, methicillin-resistant Staphylococcus aureus.

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INFECTIONS OF THE HEART

CHAPTER 32

INFECTIVE ENDOCARDITIS

Jeffrey R. Starke

Infective endocarditis results when microorganisms adhere to the endocardial surface of the heart. This process usually occurs on heart valves, although septal defects and mural surfaces can be affected. Most episodes of endocarditis begin on endocardium that has been altered by congenital defects, previous disease, surgery, or trauma. The clinical manifestations depend on the degree of compromise of cardiac function and the occurrence of embolic phenomena. Although bacteria are responsible for most cases, instances of infective endocarditis caused by fungi, chlamydiae, rickettsiae, and perhaps viruses have been described. Advances in the practice of general pediatrics and cardiology during the past 3 decades have contributed to changes in the predisposing conditions and etiologic agents of "modern" infective endocarditis. Before the 1950s, rheumatic fever was the major underlying condition, but its incidence has declined greatly since then.[306] Concurrently, improvements in the medical and surgical management of children with congenital heart disease have increased survival rates. At present, approximately 80 to 90 percent of children with infective endocarditis have congenital heart disease.* Many cases occur after cardiac surgery, especially for replacement of valves and creation of shunts with prosthetic materials.[338] The reported incidence of infective endocarditis in neonates is increasing, probably because of the use of sophisticated and highly invasive techniques in neonatal intensive care nurseries.[93,159,327,397]

Historically, infective endocarditis has been classified as acute or subacute, based on the progression of untreated disease.[432] The acute form has a fulminant course, with high fever, systemic toxicity, and death from sepsis in several days to 6 weeks. The most common etiologic agents are *Staphylococcus aureus*, *Streptococcus pyogenes*, and *Streptococcus pneumoniae*. Children with the acute form often have no underlying cardiac lesion. Subacute disease usually occurs in patients with previous valvular disease or those who have undergone cardiac surgical intervention. It is characterized by a more indolent course (6 weeks to several months) and with low-grade fever, vague systemic complaints, and various embolic phenomena. Viridans streptococci are the most common etiologic agents. This classification ignores the frequent overlap in clinical manifestations caused by various organisms, especially the staphylococci and fungi, which are causes of an increasing number of subacute cases in the postcardiac surgical setting. Classification based on specific etiologic agents is preferable because it has implications for the usual clinical course, predisposing factors, and appropriate medical and surgical management.[217]

EPIDEMIOLOGY

The incidence of infective endocarditis in adults has been difficult to determine because the methods of study and criteria for diagnosis vary among series.[30,118] Accurate figures on the incidence of infective endocarditis in children are difficult to obtain. The most common method of reporting the incidence in pediatric series expresses the number of cases of infective endocarditis as the numerator and the total number of hospital admissions during the analyzed period as the denominator. Zakrzewski and Keith[539] reported an incidence of endocarditis of 1 in 4500 pediatric admissions at the Hospital for Sick Children in Toronto from 1952 to 1962, whereas Van Hare and colleagues[507] at Case Western Reserve in Ohio found an incidence of 1 in 1280 in the period from 1972 to 1982. In a large series from Boston Children's Hospital spanning the period between 1933 and 1972, the incidence before 1963 was 1 in 4500 pediatric admissions, whereas that for 1963 to 1972 was 1 in 1800 admissions.[230] A study from a children's hospital in Australia reported an incidence of 1 in 4500 hospital admissions between 1971 and 1983.[446] More recently, one Japanese center reported an annual incidence of 0.9 cases per 1000 children seen at the cardiology clinic.[156] Although differences in referral patterns at these centers may have introduced bias into these figures, the incidence of infective endocarditis in children appears to be rising. This rise may be explained by the increased survival rate of children with all forms of cardiovascular disease and an increase in the percentage of cases that occur after cardiac surgery and are related to intravascular catheters.[143,238,420] Early surgical correction of many types of congenital heart disease along with effective perioperative antibiotic prophylaxis regimens ultimately may lower the incidence of postoperative infective endocarditis. However, the increasing use of invasive therapeutic modalities, especially intravenous catheters and pacemakers, may lead to an increased incidence of health care–associated endocarditis.[143,312,398]

The average age of children with infective endocarditis is increasing, a phenomenon that may reflect the longer life expectancy created by improved therapy for children at risk.[477] From 1930 to 1950, the mean age for children with infective endocarditis was close to 5 years.[230] Between 1960 and the present, it increased to 8.5 and then to 13 years.[156,167,264,311,420] The number of reports of infective endocarditis in children younger than 2 years had been small but has increased significantly since the late 1980s.[39,167,327,524] The clinical course of infective endocarditis in these young children often is atypical, and some cases are diagnosed at autopsy.[230,375] Before the 1950s, this disease was a rare event in neonates, with only eight autopsy cases reported.[297] Several reports suggest a rapidly increasing rate associated with the development of intensive supportive care in neonates.[46,317,332,362,363] Symchych and colleagues[475] found a 3 percent incidence of bacterial endocarditis among all neonatal autopsies. Endocarditis in neonates frequently occurs on the tricuspid valve when associated with an indwelling central venous catheter.[493] Congenital heart defects also predispose neonates to the development of infectious endocarditis.[93]

Any form of structural cardiac disease may predispose to infective endocarditis, especially with disorders associated with

*See references 25, 73, 89, 260, 264, 278, 285, 350, 358, 420, 477, 489.

TABLE 32–1 Underlying Heart Disease in 266 Children with Infective Endocarditis

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Please refer to the printed publication.

From Kaplan, E. L.: Infective endocarditis in the pediatric age group: An overview. In *Kaplan, E. L., and Taranta, A. V. (eds.): Infective Endocarditis: An American Heart Association Symposium. Dallas, American Heart Association, 1977, pp. 51-54.*

turbulence of blood flow.[163,454] In autopsy and clinical series, children with ventricular septal defect, tetralogy of Fallot, left-sided valvular disease, and systemic-pulmonary arterial communication were at highest risk, whereas those with pulmonary stenosis, coarctation of the aorta, and secundum atrial septal defect were at low risk.[412,420] Hypertrophic obstructive cardiomyopathy rarely is associated with infective endocarditis.[76] Isolated pulmonic or tricuspid valve endocarditis can occur in "otherwise normal" children and adolescents with sepsis or focal bacterial infection,[351] but usually it is associated with congenital heart disease, intravenous catheters, or intravenous drug abuse.[67,348] A bicuspid aortic valve is recognized as an important risk factor for the development of infective endocarditis, especially in elderly men.[318] The underlying heart diseases in 266 pediatric cases of infective endocarditis are listed in Table 32–1.[238]

A cooperative study on the natural history of aortic stenosis, pulmonary stenosis, and ventricular septal defect reported data from a controlled pediatric population collected over a period of 4 to 15 years.[165] In patients not undergoing surgical correction, the risk of acquiring endocarditis by 30 years of age in those with ventricular septal defects was 9.7 percent versus 1.4 percent for aortic stenosis and 0.9 percent for pulmonic stenosis. Aortic valvotomy in children with aortic stenosis actually increases the relative risk, whereas successful repair of ventricular septal defect significantly decreases long-term susceptibility to infective endocarditis.[166] Similarly, endocarditis is an extremely rare occurrence after ligation of patent ductus arteriosus has been performed. At present, palliative systemic-to-pulmonary shunting is the surgical procedure most often complicated by infective endocarditis.[420] In a review of 115 patients with tetralogy of Fallot, Kaplan and colleagues[240] reported an 8 percent incidence of infective endocarditis after placement of a Pott shunt.

The increasing use of prosthetic valves and valved conduit repairs in children with complex heart disease may lead to a larger number of cases of infective endocarditis in the future.[239,245,483] Most medical centers report an incidence of prosthetic-valve endocarditis of 2 to 4 percent after surgery,[57,168,415,454] with the aortic and mitral valves affected most frequently.[219,299]

Older studies arbitrarily divided prosthetic-valve endocarditis into two categories—early and late—based on whether the infection occurred within 60 days of valve placement or later.[34] The rationale for categorizing by time was based on apparent differences in bacteriologic, pathogenetic, and prognostic associations. So-called early cases most often were caused by coagulase-negative staphylococci (CONS), gram-negative bacilli, and fungi, whereas oral and enterococcal streptococci, along with staphylococci, predominated in late cases.[243] These older reports suggested that early cases were acquired by contamination of an intraoperative valve or were secondary to postoperative extracardiac infections, whereas late cases were acquired by the same mechanisms as native-valve endocarditis. Nosocomial bacteremia that develops at any time after the patient has undergone valve placement is a significant risk factor for development of endocarditis.[131,502] Finally, the mortality rate was thought to be higher in early versus late infection.

However, more recent studies have blurred this arbitrary time distinction between early and late prosthetic-valve endocarditis.[57,219] The risk probably is highest in the first 6 to 12 months and decreases to its lowest point beyond 1 year after valve replacement. CONS are the dominant organisms both before and after the 60th postoperative day.[57,242] Clinical and epidemiologic data also suggest that prosthetic-valve infection caused by staphylococci within the first year after placement probably is acquired at the time of surgery.[34] Identified risk factors for the development of prosthetic-valve endocarditis in adults include native-valve endocarditis, black race, male sex, a mechanical (versus biologic) prosthesis, and prolonged cardiopulmonary bypass time[219]; no comparable information is available for children.

Mitral valve endocarditis occurs frequently on an anatomically normal valve in patients with other predisposing factors.[144] An association between mitral valve prolapse and infective endocarditis has been recognized in adults and children. This heart lesion is detected with increasing frequency in adolescent girls and may be only one component of a developmental syndrome.[438] In adults, 40 to 50 percent of cases of infective endocarditis associated with isolated insufficient mitral valves occur in patients with mitral prolapse.[88] In some series of native-valve endocarditis, mitral valve prolapse has been the most common underlying lesion.[318] The reported incidence of infective endocarditis in patients with mitral valve prolapse has varied markedly among studies, from low rates of 14 per 100,000 per year to 5 of 58 patients monitored prospectively for 9 to 22 years.[199] A retrospective epidemiologic analysis involving matched cases and controls yielded an odds ratio of 8.2, indicative of a substantially higher risk for development of endocarditis in patients with mitral valve prolapse than in normal controls.[85]

That the risk of developing infective endocarditis is not uniform for all patients with mitral valve prolapse has become apparent. The risk is increased in patients with a preexisting systolic murmur (but not for those with an isolated click and no murmur), echocardiographically demonstrated regurgitation, and valvular redundancy.[95,298,308] The signs and symptoms of endocarditis associated with mitral valve prolapse may be more subtle than those of other types of left-sided endocarditis.[144,360] However, significant complications are relatively common occurrences and sometimes require valve replacement during the acute illness or during convalescence.[19,461]

Fungal endocarditis is a rare disorder in children but should be suspected in certain clinical and epidemiologic settings. It is more likely to occur after cardiac surgery and rarely occurs on native heart valves. It occurs more commonly in neonates treated in intensive care settings than in older children.[93] Other predisposing factors include (1) the presence of an indwelling vascular catheter, (2) prolonged use of antibiotics, (3) intrinsic (immunodeficiency diseases, malignancy, malnutrition) or extrinsic (corticosteroids, cytotoxic drugs) immunosuppression, (4) bowel surgery resulting in transient fungemia, (5) intravenous drug abuse, and (6) preexisting or concomitant bacterial endocarditis.

Many conditions other than structural heart disease predispose children to the development of infective endocarditis. The most important is the presence of an indwelling central venous catheter, especially in patients who are seriously ill or immunocompromised.[159,167,284,496,523] The catheter acts as a foreign body and presumably causes microscopic damage by abrading endocardial and valve surfaces; such damage results in nonbacterial thrombotic vegetation.[25] Infection of intracardiac pacemaker

wires also can lead to endocarditis.[13] Infection acquired during the placement procedure and infection of the pacemaker pouch are most common. Infective endocarditis, usually of the tricuspid valve, has developed in children with ventriculoatrial shunts placed for the treatment of hydrocephalus.[238] In patients with arteriovenous fistulas created for hemodialysis, bacterial vegetations may develop in the fistula and on heart valves.[276,401] Rarely, penetrating wounds or foreign bodies can initiate endocarditis.[202,307] Piercings of various body parts also have been associated with endocarditis.[2,394] One important group of patients with an increased risk for development of infective endocarditis is intravenous drug abusers.[314,526] In this group of patients, two thirds have no evidence of underlying heart disease. A predilection for involvement of the tricuspid valve, followed by the mitral and aortic valves, has been noted.[155] Roentgenographic evidence of septic pulmonary emboli and signs of tricuspid insufficiency dominate the clinical findings.[432] Within this group of patients, increased rates of infective endocarditis and mortality are associated with infection by human immunodeficiency virus (HIV), particularly as CD4 cell counts fall to less than 200/mm^3.[389]

Although the incidence of infective endocarditis in children may be rising, the prognosis has improved dramatically during the past several decades. Current mortality rates usually are close to 10 percent.[341,420,437] Most survivors remain hemodynamically stable at long-term follow-up.[147,437] However, patients who experience infective endocarditis appear to be at higher risk for developing recurrent endocarditis than are those with similar cardiac abnormalities who have not had previous endocarditis.[454] The patient's functional class before treatment appears to be most predictive of long-term functional status. In one study, 22 percent of children who survived infective endocarditis required surgery related to the infection, including vegetectomy, evacuation of a hematoma, atrioventricular valve replacement, and placement or replacement of a graft or intracardiac shunt.[420]

PATHOPHYSIOLOGY

Clinical observations, autopsy studies, and work with experimental animal models have demonstrated that the occurrence of several independent events is required for the development of subacute infectious endocarditis. The endocardial surface usually is disrupted by stress or injury commonly caused by the turbulence of blood. This surface damage results in the deposition of fibrin and platelets, which form nonbacterial thrombotic vegetations. If bacteria adhere to these deposits, infective endocarditis will result. The surface of the infected vegetation becomes protected by a cover of fibrin and platelets. A tremendous proliferation of organisms (as many as 10^9 colony-forming units per gram) may ensue.[119] The protective sheath isolates the organisms from the action of host neutrophils and antibiotics. The clinical manifestations and complications of infective endocarditis are related to both the hemodynamic changes caused by local infection and the occurrence of embolization and metastatic infection.

In experimental animals, the valvular surface must be damaged, usually by an intravenous catheter, to produce infective endocarditis.[20] The first step in the pathogenesis of subacute infective endocarditis in humans is the development of hemodynamic factors that favor endocardial damage. In an autopsy study of 1024 patients with infective endocarditis, Lepeschkin[277] showed that the location of the endocardial lesions correlated with the impact of pressure; this finding makes a strong argument for the role of mechanical stress as a critical factor in the evolution of the lesions. When associated with valvular insufficiency, infective endocarditis usually occurs on the atrial surface of the mitral valve and the ventricular surface of the aortic valve. Injection of a bacterial aerosol into the air stream passing through a Venturi tube demonstrates how high pressure drives an infected fluid into a low-pressure sink.[407] This process establishes maximal deposition of bacteria in the low-pressure sink immediately beyond the orifice. Mitral insufficiency creates a Venturi effect when blood is driven from the high-pressure left ventricle into a low-pressure atrium; maximal deposition occurs around the mitral annulus on the atrial side. Similarly, with aortic valve insufficiency, the high-pressure source is the aorta and the low-pressure sink is the left ventricle, which leads to deposition on the ventricular surface of the valve.

Lesions also are created more directly by a jet stream causing endocardial damage. For example, in a small, restrictive ventricular septal defect with a left-to-right shunt, a Venturi effect leads to the development of lesions on the right ventricular septal side of the defect, whereas secondary lesions created by the jet effect are located on the right ventricular wall opposite the defect.[518] Heart defects with a surface area sufficiently large to prevent a significant pressure gradient and those in which smaller volumes minimize the gradient do not create the jet and Venturi effects. This difference helps to explain the rarity of endocarditis in patients with atrial septal defects and the increased risk of infection complicating small, but not large, ventricular septal defects.

Once endocardial damage has occurred, collagen is exposed, and platelet and fibrin deposition ensues in a manner analogous to formation of the primary plug of normal hemostasis after vascular injury.[228,519] The sterile platelet-fibrin thrombus that is formed subsequently is referred to as a nonbacterial thrombotic vegetation. In experimental animals, many exogenous stresses, including exposure to cold, high altitude, high cardiac output states, hormonal manipulations, and passage of a sterile catheter across a heart valve, lead to formation of this lesion. Formation of the vegetation reflects two pathogenic mechanisms: hypercoagulability and endothelial damage.[432] To establish experimental infective endocarditis without initial formation of the vegetation is nearly impossible. Microscopic examination demonstrates that this lesion is the one to which microorganisms attach during the early stages of experimental endocarditis. Nonbacterial thrombotic vegetations have been found in both adults and children with malignancy, chronic wasting diseases, uremia, connective tissue diseases, and congenital heart disease and after the placement of intracardiac catheters,[291,367] and they have been associated with embolism and infarction in distant organs.[42]

Once a nonbacterial thrombotic vegetation has been established, transient bacteremia or fungemia may result in colonization of the lesion. Transient bacteremias are common occurrences, especially with traumatization of a mucosal surface. Table 32–2 lists the incidence of bacteremia in adults and children after various procedures.[130,405] The bacteremia usually is of low grade and is proportional to the amount of trauma produced by the procedure and the number of organisms inhabiting the surface. In addition, "silent" bacteremia probably occurs frequently. Many persons have circulating antibodies to their own oral flora, as well as an increase in peripheral T cells sensitized to the flora of their dental plaque.[432] Some children with congenital heart disease may be at increased risk for having gingival colonization and subsequent development of bacteremia with organisms associated with infectious endocarditis, such as the HACEK *(Haemophilus* spp., *Actinobacillus actinomycetemcomitans*, *Cardiobacterium hominis*, *Eikenella corrodens*, *Kingella kingae)* microbes.[457]

The ability of microorganisms to adhere to the platelet-fibrin thrombus is a critical factor in the development of infective endocarditis.[100,188,228] In a canine model, *S. aureus* and the viridans streptococci, which frequently cause infective endocarditis, adhere more readily to normal aortic leaflets than do organisms uncommon in endocarditis.[178] Within isolates of *S. aureus*, strains devoid of microencapsulation are less capable of inducing endocarditis in an experimental model than are encapsulated strains.[21] Specific products released by these organisms, including dextran,

TABLE 32–2 Bacteremia after Various Procedures in Adults and Children

Initiating Event	Percentage of Positive Blood Cultures (%)	Predominant Organisms
Dental extraction (children)	30-65	*Streptococcus*, diphtheroids
Chewing gum, candy, paraffin	0-51	*Streptococcus*, *Staphylococcus epidermidis*
Tooth brushing	0-26	*Streptococcus*
Tonsillectomy	28-38	*Streptococcus*, *Haemophilus*, diphtheroids
Bronchoscopy (rigid scope)	15	*Streptococcus*, *S. epidermidis*
Bronchoscopy (fiberoptic)	0	
Orotracheal intubation	0	
Nasotracheal intubation/suctioning	16	*Streptococcus*, aerobic gram-negative rods
Sigmoidoscopy/colonoscopy	0-9.5	*Enterococcus*, aerobic gram-negative rods
Upper gastrointestinal endoscopy	8-12	*Streptococcus*, *Neisseria*, *S. epidermidis*, diphtheroids, other
Percutaneous liver biopsy	3-14	Pneumococcus, aerobic gram-negative rods, *Staphylococcus aureus*, other
Urethral catheterization	8	Not stated
Manipulation of *S. aureus* suppurative foci	54	

From Everett, E. D., and Hirschmann, J. U.: Transient bacteremia and endocarditis prophylaxis: A review. Medicine (Baltimore) 56:61-77, 1977. © 1977, The Williams & Wilkins Company, Baltimore.

mannan, teichoic acid, and slime, may enhance their ability to colonize the vegetation.[228,254] The amount of dextran produced by various viridans streptococci in broth correlates with both their adherence and their ability to produce endocarditis in the rabbit model.[333,435] *Candida albicans* is readily adherent and produces infective endocarditis in rabbits more easily than does *Candida krusei*, a nonadherent yeast rarely implicated in human infective endocarditis.[433] In addition, endocarditis-producing strains of streptococci and staphylococci are more potent stimulators of platelet aggregation than are other bacteria that do not produce infective endocarditis.[84,197,333] This action may accelerate the formation of an infected vegetation or may increase the removal of organisms from the circulation. The importance of adherence by organisms has been studied by pre-incubating organisms with many classes of antibiotics. After incubation at subinhibitory concentrations, adhesion of streptococcal species to fibrin-platelet matrices and damaged canine valves is decreased.[436] Antibiotics may prevent development of infective endocarditis by both bacterial killing and inhibition of adherence to the vegetation.[172]

Host tissue factors undoubtedly play an important role in adherence of bacteria to the developing thrombus. Once bacteria become adherent to a nonbacterial thrombus, activation of the coagulation system ensues. Some organisms that produce endocarditis may be able to initiate procoagulant activity through microbial enzymes. Activation of the intrinsic coagulation pathway is triggered by exposed connective tissue components and platelet aggregation.[254] However, activation of the extrinsic coagulation pathway probably is the major stimulus for growth of vegetations. Elements of the extracellular matrix, including fibronectin, laminin, and collagen, have been shown to facilitate the adherence of bacteria on fibrin-platelet matrices.[472,495] Fibronectin may be the host receptor for organisms within the nonbacterial thrombotic vegetation.[265,293] Laminin-binding proteins have been found on the cell walls of organisms recovered from patients with endocarditis.[451]

The platelet-organism interaction is complex and not understood completely. *Streptococcus sanguis* produces two cell surface antigens that promote platelet aggregation: a class I antigen promotes adhesion of *S. sanguis* to platelets, whereas coexpression of a class II antigen promotes platelet adhesion or aggregation.[198] The induced platelet aggregation appears to be an important determinant of further development of vegetation and progression of disease in experimental endocarditis. In addition, production of streptococcal exopolysaccharide inversely correlates with platelet adhesion while inhibiting aggregation, thus indicating that surface molecules may enhance endocarditis at only certain pathogenic steps.[469] Platelets also may be involved in host defense within the vegetation. After exposure to thrombin, platelets may release microbicidal proteins with bactericidal activity against some gram-positive cocci; resistance to these proteins may be a virulence factor for *S. aureus* in the development of endocarditis.[357,536]

As bacterial colonization of a nonbacterial thrombotic vegetation progresses, it enlarges by further bacterial proliferation and platelet-fibrin deposition (Fig. 32–1).[336] Kissane [259] described three histologic zones: (1) necrotic endocardium; (2) a broad zone of bacterial colonies, pyknotic nuclear debris, and fibrin; and (3) a thin coating on the surface of fibrin and leukocytes. The location of bacterial colonies below the surface and the minimal infiltration by phagocytic cells create an environment of impaired host resistance that results in extreme bacterial proliferation. The structure of the vegetation diminishes the penetration of antibiotics into the bacterial layer. In addition, the metabolic activity of bacteria within this lesion is slowed, thus rendering antibiotics less effective. The formation of vegetations and erosion of heart valves may cause valvular incompetence and thereby may result in cardiac failure.

Immunopathologic factors may have important roles in both the development and sequelae of infective endocarditis.[32] The susceptibility of a gram-negative bacillus to complement-mediated bactericidal activity is critical to its potential to create endocarditis; only "serum-resistant" organisms produce infective endocarditis in humans and experimental animals.[117] Gram-positive cocci are a more frequent cause of infective endocarditis than are gram-negative bacilli. Gram-positive organisms are resistant to this bactericidal activity; phagocytosis is required for killing.

The frequent presence of hypergammaglobulinemia, splenomegaly, and monocytes in the blood of patients with infective endocarditis indicates stimulation of the humoral and cellular immune systems. Macroglobulins, cryoglobulins, and agglutinating, opsonic, and complement-fixing antibodies have been associated with infective endocarditis.[204,273] Studies in animals pre-immunized with heat-killed streptococci before aortic valve trauma and infection are induced suggest that circulating antibody has a protective role.[434,503] However, antibody to *S. aureus* or *Staphylococcus epidermidis* does not prevent the development of endocarditis in immunized animals, perhaps because this antibody does not enhance opsonophagocytosis.[432] The continuous antigenic challenge created by intravascular organisms leads to increased production of specific antibody (including opsonic,

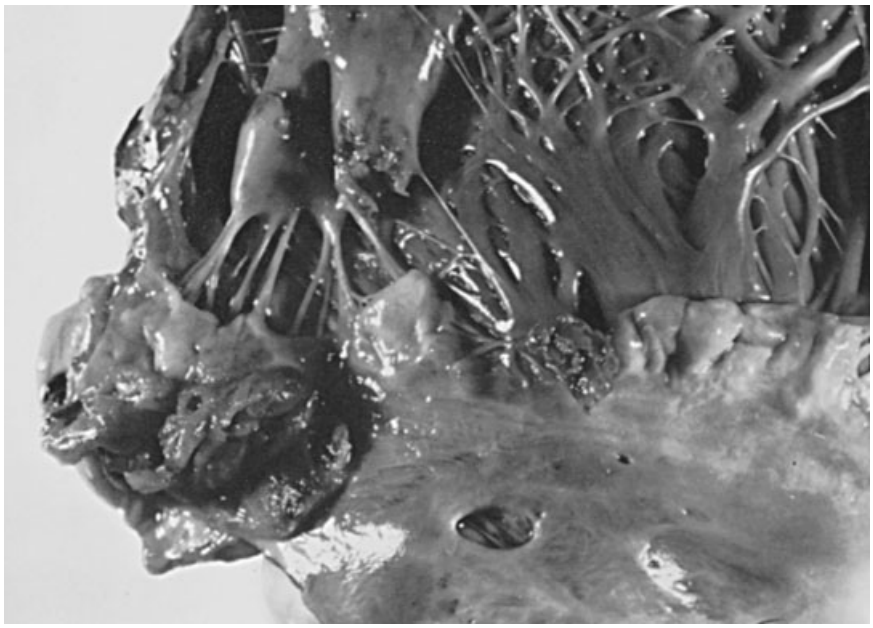

Figure 32–1 Subacute endocarditis of the mitral valve with vegetation and rupture of the papillary muscle caused by *Staphylococcus aureus*. *(Courtesy of Dr. Edith P. Hawkins, Texas Children's Hospital, Houston.)*

agglutinating, and complement-fixing antibodies), cryoglobulins, macroglobulins, and antibodies to bacterial heat shock protein,[391] as well as to the subsequent formation of circulating immune complexes. These complexes are found with increased frequency in patients with a long duration of illness, hypocomplementemia, extravalvular manifestations, and right-sided disease.[36] Quantitative levels of circulating immune complexes may be helpful in distinguishing endocarditic from non-endocarditic sepsis and in monitoring anti-infective therapy. Effective treatment usually leads to a prompt decrease in these levels,[35] whereas relapses may be characterized by rising titers.[246] The diffuse glomerulonephritis occasionally noted with infective endocarditis is caused by subepithelial deposition of immune complexes and complement.[184] Immune complexes can be demonstrated in some diffuse purpuric lesions seen with endocarditis.[292] Bacterial antigens have been found within these complexes.[216]

Further evidence of stimulation of the immune system in infective endocarditis is the development of rheumatoid factor in approximately 50 percent of adults with disease lasting longer than 6 weeks.[525] Titers of rheumatoid factor correlate with hypergammaglobulinemia and, as with immune complex levels, decrease with therapy and increase during relapse. The role of rheumatoid factor in the disease process is unknown, but it may be involved by blocking immunoglobulin G opsonic activity, stimulating phagocytosis, or accelerating microvascular damage.[432] Anti-nuclear, anti-endocardial, anti-sarcolemmal, and anti-myolemmal antibodies also have been identified in patients with infective endocarditis; their role in pathogenesis is unclear.[300]

The pathologic changes that occur in the heart in association with infective endocarditis are secondary to local extension of the infection. The vegetations vary in size from a millimeter to several centimeters; frequently they are singular, but they may be multiple. Valvular stenosis may result from large lesions. Vegetations secondary to certain organisms, especially *Candida*, *Haemophilus*, and *S. aureus* in acute cases, often are large and friable, with a propensity for embolization.[537] Ulcerative lesions may occur and may lead to perforation of the valve and subsequent congestive heart failure. Other local complications include rupture of the chordae tendineae or papillary muscle (see Fig. 32–1), valve ring abscess with subsequent fistula formation and pericardial empyema,[47,62] aneurysms of the sinus of Valsalva or ventricle,[75,160,430] myocarditis, and myocardial infarction.[135] Persistent fever occurring during appropriate medical therapy for infective endocarditis may reflect a persistent vegetation, especially with right-sided disease, or extension of infection into a valve ring and adjacent structures.[113] In such cases, surgery frequently is required.

The pathologic changes in distant organs usually are secondary to embolization with subsequent infarction or metastatic infection. In many cases of infective endocarditis, the causative organism is of low pathogenicity; infections caused by septic emboli often are low-grade because of the reduced propensity of these organisms to invade tissue. However, the emboli in acute *S. aureus* endocarditis frequently cause severe metastatic infections and overwhelming sepsis. Emboli from right-sided heart lesions lodge in the lungs and cause pulmonary infarcts and abscesses, which usually are small and multiple. Left-sided lesions may embolize to any organ but most commonly affect the brain, kidney, spleen, and skin.[260,352] Cerebral emboli have been detected in 30 percent of cases in adults and children and have caused infarction, abscess, mycotic aneurysm, subarachnoid hemorrhage, meningitis, and acute hemiplegia of childhood.[59,180,191,234,313,422] Kidney abscess is a rare occurrence, but infarcts are noted in most patients at autopsy.[330] Amyloidosis involving primarily the kidneys is a rare complication of chronic infective endocarditis.[196] Splenic abscess also is a rare event but can be a fatal complication if undetected.[232] The most common manifestation of embolization to the skin is petechiae. Janeway lesions are septic emboli consisting of bacteria, neutrophils, necrosis, and subcutaneous hemorrhage. Osler nodes are areas of thrombosis and necrosis. They may be related to both immune complex deposition and septic emboli.[5]

CLINICAL MANIFESTATIONS

The signs and symptoms of infective endocarditis are determined by the extent of local cardiac disease, the continuous bacteremia, and the degree of involvement of distant organs as a result of embolization, metastatic infection, and circulating immune complexes.[24] Consequently, the clinical findings are highly variable and mimic those of many other diseases.[418] Unexplained embolic phenomena in any organ should suggest the diagnosis of endocarditis, especially in children with known heart disease.

Patients with acute bacterial endocarditis initially may be seen with florid sepsis; the endocarditis is diagnosed at autopsy. The indolent manifestations of subacute endocarditis may evolve for weeks or months before medical care is sought. Endocarditis frequently occurs in children with preexisting heart disease, so subtle changes in cardiac function may be difficult to detect early in the course. Table 32–3 lists the frequency of the major clinical manifestations of bacterial endocarditis in infants and children.

Fever is the most common symptom of infective endocarditis, but it is absent in 10 percent of cases. It usually is of low grade and has no specific pattern. Chills may accompany the fever, but they rarely are seen in children. Persistent fever during antimicrobial therapy is an uncommon occurrence. Prolonged (>2 weeks) fever is associated with certain etiologic agents (*S. aureus*, gram-negative bacilli, fungi), with culture-negative endocarditis, and with complications such as embolization of major vessels, intracardiac or peripheral abscess, tissue infarction, a need for cardiac surgery, and a higher mortality rate.[48,274] Nonspecific symptoms such as malaise, anorexia, weight loss, and fatigue are common findings. Arthralgia occurs in 24 percent of patients. The arthralgia frequently is multiple and most commonly affects the large joints. Although adults initially may have synovitis,[82] this finding is rare in children. Osteoarticular infection in association with infective endocarditis in adults occurs almost exclusively in intravenous drug users.[429] It is seen very rarely in children. Gastrointestinal complaints are noted in 16 percent of cases and include nausea, vomiting, and abdominal pain. Chest pain occurs in approximately 10 percent of older children and generally is mild and nonspecific. Although chest pain usually is related to diffuse myalgias, it may be secondary to pulmonary complications or cardiac lesions, especially if the tricuspid valve is involved.

Heart murmurs occur in more than 90 percent of children with infective endocarditis, but most patients have underlying heart disease with existing murmurs. The appearance of a new murmur or appreciation of a significant change in a previous one occurs in only 25 percent of cases. Significant blood flow turbulence caused by compromised valvular function must have occurred for a murmur to be detected or to change. The frequent absence of changes in the cardiac examination early in the disease contributes to the long average delay in establishing the diagnosis, especially in children with preexisting heart disease. Congestive heart failure occurs in 30 percent of children with infective endocarditis and is especially common in those in whom a new murmur of valvular insufficiency develops. Endocarditis should be suspected in any child who has rheumatic or congenital heart disease and unexplained deterioration in cardiac function. Although valvular regurgitation is the most common hemodynamic complication of endocarditis, significant obstruction of a valve or shunt requiring rapid surgery rarely occurs.[74]

Neurologic signs and symptoms are reported in approximately 20 percent of children with endocarditis. These signs and symptoms may dominate the clinical findings, especially in patients with endocarditis caused by *S. aureus*.[191,422] The sudden development of cerebral lesions in an infant or child should suggest this diagnosis. The manifestations are those that commonly accompany a cerebral infarct or abscess, namely, acute hemiplegia of childhood, seizures, ataxia, aphasia, sensory loss, focal neurologic deficits, and alterations in mental status.[59] They may be the initial features of endocarditis or may occur years after the infection has been eradicated.[540] Mycotic aneurysms of the cerebral vessels occur rarely in cases of pediatric endocarditis.[63] They usually are single, small, and peripheral but may lead to subarachnoid hemorrhage. Whereas computed tomographic scanning of the brain is useful for delineating central nervous system involvement in patients with infective endocarditis, magnetic resonance imaging may be more sensitive for detecting small infarctions and changes secondary to cerebral edema.[40] Other neurologic manifestations associated with endocarditis include cranial nerve palsies, neuropathy, visual changes, choreoathetosis, seizures, and toxic encephalopathy.

Splenomegaly, a common manifestation of endocarditis in children, occurs in 55 percent of cases. It is found frequently in patients with long-standing disease and other evidence of immune system activation. The spleen generally is nontender and may be associated with mild hepatomegaly. Splenic infarction and abscess are rare events but should be suspected in patients with left upper quadrant abdominal pain that radiates to the left shoulder, a pleural friction rub, or left pleural effusion.

Skin manifestations occur less commonly in children than in adults.[292] Clubbing is found in 10 to 20 percent of children with endocarditis but frequently is related to underlying heart disease. Petechiae are noted in approximately one third of patients, especially those with long-standing disease. These lesions are found most commonly on the extremities, oral mucosa, and conjunctivae. Splinter hemorrhages are linear red or brown streaks seen in the nail beds. They are present in only 5 percent of children with endocarditis and are associated with other conditions.[256] Three other types of lesions are more specific for infective endocarditis but occur in only 5 to 7 percent of patients: Osler nodes, which are small (2 to 10 mm), painful nodular lesions found in the pads of the fingers or toes[5]; Janeway lesions, which usually are painless hemorrhagic macular plaques that frequently occur on the palms and soles[133]; and Roth spots, which are small, pale retinal lesions associated with areas of hemorrhage located near the optic disk.

Other than fever and, perhaps, splenomegaly, no single sign or symptom occurs in more than 50 percent of children with endocarditis. That no classic clinical manifestation exists for this disease is obvious because the chance that even three or more signs will be present is extremely low. The appearance of any one

TABLE 32–3 Clinical Manifestations of Bacterial Endocarditis in Children

Symptom	Average (%)	Range (%)	Physical Finding	Average (%)	Range (%)
Fever	90	56-100	Splenomegaly	55	36-67
Malaise	55	40-79	Petechiae	33	10-50
Anorexia/weight loss	31	8-83	Embolic phenomena	28	14-50
Heart failure	30	9-47	New or change in heart murmur	24	9-44
Arthralgia	24	16-38	Clubbing	14	2-42
Neurologic findings	18	12-21	Osler nodes	7	7-8
Gastrointestinal findings	16	9-36	Roth spots	5	0-6
Chest pain	9	5-20	Janeway lesion	5	0-10
			Splinter hemorrhages	5	0-10

Data from references 49, 89, 91, 156, 230, 264, 311, 358, 418, 446, 453, 477, 484, and 507.

of these clinical features in a child with predisposing heart disease should raise suspicion of infective endocarditis and should lead to an appropriate diagnostic evaluation.[75]

The clinical findings of infective endocarditis in infants and neonates are less specific than are those in older children. The onset more often is acute and related to overwhelming infection.[231,321] In the pre-antibiotic era, these children often had other foci of infection, such as osteomyelitis, meningitis, and pneumonia, that dominated the clinical picture. The widespread use of antibiotics since 1941 has caused a decrease in the number of cases of infective endocarditis secondary to other suppurative infections.[321] Although early studies concluded that congenital heart disease was not an important predisposing factor to endocarditis in infants, more recent reports suggest that it is. At present, infants with heart defects undergo corrective and palliative surgery at a younger age than in the past. Infants in whom postoperative endocarditis does develop probably will have clinical findings more similar to those in older children.

Infective endocarditis is a rare occurrence in neonates and frequently is associated with indwelling vascular catheters.[159,309] It may affect the tricuspid valve and have a fairly "silent" clinical manifestation. Persistent bacteremia or fungemia should lead to a search for a cardiac focus of infection. Deterioration in pulmonary function, coagulopathies, thrombocytopenia, and low-grade murmurs often develop in neonates. Skin abscesses and hepatomegaly also are common findings. The prognosis of infective endocarditis associated with neonatal intensive care usually is favorable, perhaps because the diagnosis often is established relatively early and antibiotics are given rapidly.

Reported series of infective endocarditis in children with prosthetic valves are scarce. In early stages of disease, fever may be the only finding because the other signs of endocarditis are masked by the medical and surgical complications occurring in the immediate postoperative period. Late infections generally produce clinical findings similar to those in native-valve endocarditis. Clinical evidence of systemic embolization occurs in as many as 40 percent of patients.[77] Neurologic complications carry a particularly poor prognosis for survival.[252] A new or changing murmur often indicates valvular insufficiency caused by a paravalvular leak. Florid cardiac failure is the major manifestation if local infection or an abscess creates valve instability and acute, severe regurgitation.

The signs and symptoms of infective endocarditis in intravenous drug abusers may be similar, but these patients have several more distinctive features of their illness. Two thirds of these patients have no predisposing heart disease. The valve most commonly affected is the tricuspid, which leads to a predominance of pulmonary signs and symptoms resulting from pleural effusion, pulmonary infarction, and lung abscesses. Signs of tricuspid insufficiency (gallop rhythm, pulsatile liver, regurgitant murmur) are found in one third of cases.[432] Many patients have extracardiac sites of infection that are helpful in establishing the diagnosis.[482]

LABORATORY FINDINGS

The most important diagnostic procedure is the blood culture. Because many bacteria that usually are not pathogenic cause infective endocarditis, scrupulous aseptic technique must be used to distinguish causative agents from contaminants.[370] The yield of organisms is not increased by obtaining blood from arterial puncture or cardiac catheterization.[37] The bacteremia usually is of low grade and continuous. The first two cultures yield the organism 90 percent of the time; in two thirds of cases, all blood cultures are positive.[522] Therefore, isolated positive cultures generally are not significant. Previous outpatient antibiotic therapy may change the yield significantly.[250] In one study, culture positivity in cases of proven endocarditis was 64 percent in patients who received antibiotics before blood was drawn for culture versus 100 percent in patients without exposure to antibiotics.[371]

When *Candida* endocarditis is suspected, several additional points should be considered. Isolation of *Candida* spp. may require incubation for 1 week or longer. All blood cultures from a patient with *Candida* endocarditis may not be positive, in contrast to the usual situation with bacterial endocarditis; several positive cultures may be interspersed among negative cultures. In patients with fungal endocarditis, *Candida* is isolated commonly from other infected sites, such as urine, sputum, synovial fluid, cerebrospinal fluid, lymph nodes, and bone marrow.[424]

Three to five samples of blood for culture should be obtained from different sites within the first 24 hours in children with suspected endocarditis. Although difficult to obtain in smaller children, 3 to 5 mL of blood per culture is desirable for optimal yield. The samples should be injected into thioglycolate and trypticase soy (or brain-heart infusion) broth and held for at least 3 weeks to detect slow-growing organisms. If gram-positive cocci grow in the broth but fail to grow on subculture, nutritionally variant streptococci should be suspected, and subculture should be performed on media with either L-cysteine or pyridoxal phosphate.[60,459] Poured plates may be used to estimate the degree of bacteremia.

Negative blood cultures are noted in 10 to 15 percent of patients with clinically diagnosed endocarditis.[498] However, when patients have not received antibiotic therapy previously and blood for culture is obtained properly, these cases account for less than 5 percent of the total. Potential reasons for negative cultures include the following: (1) right-sided endocarditis; (2) previous administration of antibiotics; (3) fungal (especially *Aspergillus)* endocarditis; (4) endocarditis caused by *Bartonella* spp., rickettsiae, chlamydiae, or viruses; (5) mural endocarditis; (6) slow growth of organisms *(Candida*, *Haemophilus*, *Brucella*, nutritionally variant streptococci); (7) anaerobic infection; and (8) nonbacterial thrombotic endocarditis or an incorrect diagnosis.[186,190,378,498] In some instances, intraleukocytic organisms may be seen in layered peripheral blood, even when cultures are negative.[386] If surgical resection of vegetations or valve replacement is performed, a cause may be demonstrated by appropriate histologic examination and stains for bacteria and fungi.[336] Organisms also may be isolated from extracardiac sites (bone marrow, urine).

Many nonspecific laboratory findings are abnormal in patients with infective endocarditis (Table 32–4). The total white blood cell count rarely is helpful, but peripheral eosinophilia may be seen with Loeffler endocarditis.[203] The erythrocyte sedimentation rate is elevated in 80 to 90 percent of cases. However, frequently it is normal or low when congestive heart failure or renal failure is present. Serum C-reactive protein levels usually are elevated initially and return to normal during the course of successful therapy.[316] An increase during therapy may result from treatment failure, but can it also be caused by drug allergy or

TABLE 32–4 Selected Laboratory Findings of Bacterial Endocarditis in Children

Laboratory Finding	Average (%)	Range (%)
Positive blood culture	87	68-98
Elevated erythrocyte sedimentation rate	80	71-96
Low hemoglobin (anemia)	44	19-79
Positive rheumatoid factor	38	25-55
Hematuria	35	28-47

Data from references 48, 89, 156, 230, 264, 311, 446, 453, 484, and 507.

intercurrent infection. Rheumatoid factor rarely has been measured in a series of pediatric patients, but when measurements have been made, they have been positive in 25 to 50 percent of children with endocarditis. A positive test may be a diagnostic aid in cases of culture-negative endocarditis when other causes are excluded. Serial measurements may provide evidence of efficacy of therapy, although a fall in the titer of rheumatoid factor may lag behind the clinical and bacteriologic response.[514] Hypocomplementemia is seen in association with glomerulonephritis. Anemia is present in approximately 40 percent of patients, especially in those with long-standing disease. Although hemolysis may occur in the areas of turbulence in the heart, more often it is anemia of chronic disease. Because many patients with cyanotic heart disease normally have a compensatory polycythemia, a serial drop in hematocrit is of more significance than is a single measurement. Leukocytosis occurs in a few patients, but leukopenia is a rare finding in the absence of acute endocarditis with overwhelming sepsis. Hematuria and proteinuria, present in 25 to 50 percent of cases, usually are secondary to microemboli in the kidneys and may be accompanied by "pyuria," casts, and bacteriuria.

Circulating immune complexes are present in most adults with subacute endocarditis, as measured by Raji cell radioimmunoassay [485] or the ^{125}I-Clq binding assay.[541] These immune complexes frequently are absent in acute endocarditis. Low levels of immune complexes have been found in 32 percent of adults with septicemia but not endocarditis, in 10 percent of normal controls, and in 40 percent of noninfected intravenous drug abusers.[36] However, levels higher than 100 μg/mL are correlated highly with the presence of endocarditis. Serial measurement of immune complex levels may aid in monitoring therapeutic efficacy.[35] Systematic investigation of immune complexes has been reported infrequently in children with endocarditis. When immune complexes have been sought, most patients, including two of three children with culture-negative endocarditis, have had significant levels.

When infective endocarditis is suspected but blood cultures remain negative, serologic testing for specific organisms may prove helpful. Several techniques measure antibody to teichoic acids, which are major components of the cell wall of *S. aureus*. These antibodies are present in more than 85 percent of adults with staphylococcal endocarditis, but the false-positive rate is as high as 10 percent.[349] False-negative results correlate with a short (<2 weeks) duration of illness. Specific information about the accuracy of this test in children is lacking, and the tests are not readily available. Serologic testing is available or under investigation for many other organisms that cause infective endocarditis, including *Bartonella*, *Brucella*, *Candida*, *Aspergillus*, *Histoplasma*, *Cryptococcus*, *Chlamydia*, and *Coxiella*.[235,257] In general, the usefulness of these tests in children with endocarditis is unproven. Some patients with non-spirochetal bacterial endocarditis who reside in locales endemic for Lyme disease have significantly elevated levels of antibodies reactive to *Borrelia burgdorferi*.[237] Diagnostic confusion may occur because the signs and symptoms of infective endocarditis and Lyme disease can be quite similar. Other techniques, such as broad-range polymerase chain reaction (PCR), have been used to identify *Bartonella* and other causative agents of endocarditis.[51,512]

Radiographic techniques have not been a great aid in establishing the diagnosis of infective endocarditis. The findings on plain chest roentgenograms are nonspecific, but evidence of complications, such as septic pulmonary emboli or congestive heart failure, may be helpful. Computed tomography may help in establishing the diagnosis of an infected shunt.[499] Immunoscintigraphy using technetium-labeled anti-granulocyte antibodies may yield useful information when the echocardiographic findings are equivocal.[334] Cineangiography is the definitive method to determine the anatomic alterations in the heart resulting from infective endocarditis, but it rarely needs to be performed in children.

The electrocardiogram also is useful in the evaluation of patients with endocarditis because it detects arrhythmias and conduction disturbances that complicate the disease. Ventricular ectopy may be related to myocardial ischemia, myocarditis, or myocardial abscess. New conduction defects imply extension of infection beyond the valve ring into the myocardium. Any degree of atrioventricular block, a new left bundle branch block, or a new right bundle branch block with a left anterior hemiblock may represent extension of infection from the aortic valve into the ventricular septum. Junctional tachycardia, Wenckebach atrioventricular block, or complete heart block may be produced by extension of the infection from the mitral valve annulus into the atrioventricular node or proximal His bundle. In general, an unstable conduction block is more likely to develop in patients with aortic valve endocarditis than in those with mitral infection.[111]

Echocardiography has become a valuable adjunct to the diagnosis and treatment of endocarditis in children.[109,173,266,403,416,417,439] Color Doppler is a sensitive modality for detection of valvular insufficiency, and the results may influence surgical and medical treatment decisions.[145] Echocardiography can be performed by the traditional transthoracic approach or the transesophageal approach.[383] The sensitivity and specificity of transthoracic echocardiography continue to be defined, with positive results obtained in 36 to 100 percent of children in various series of pediatric patients.[52,89,109,247,278,285,420]

In general, two-dimensional echocardiography is more sensitive than is the M-mode technique, especially in cases of right-sided endocarditis,[366] and it is superior in diagnosing complications of the destructive process.[24,328] The smallest vegetation detectable is approximately 2 mm, but the acoustic impedance of the mass relative to the surrounding structures is a more important factor than is size in identifying the vegetation. Echocardiography has identified vegetations in culture-negative cases.[247] Its accuracy in prosthetic-valve endocarditis is diminished by the difficulty in resolution around the prosthetic device.[345,463] Serial evaluation of valvular vegetations generally does not assist in assessing the efficacy of antibiotic therapy because diminution or disappearance of vegetations may take place long after successful medical treatment has been completed.[258,410,511]

The use of transthoracic echocardiography to predict the clinical course and need for operative intervention in patients with endocarditis is controversial.[7,177]A synopsis of many reports that have assessed the role of transthoracic echocardiography in the diagnosis and management of infective endocarditis suggests the following: (1) because of variable sensitivity among studies for detection of vegetations, a negative study does not rule out endocarditis, especially when foreign material is present within the heart; (2) false-positive studies are quite rare (the specificity is high); (3) the reliability of transthoracic echocardiography depends on the experience of the examiner and the technical adequacy of the study; (4) transthoracic echocardiography is valuable in assessing local complications of endocarditis on native valves; and (5) in most but not all studies, patients with a vegetation identified by transthoracic echocardiography have an increased risk for the development of systemic emboli and congestive heart failure.[177,223,295,319,404,427,462]

Although some investigators contend that the presence of a vegetation should hasten early surgery, most suggest that a positive echocardiogram is adjunctive evidence that should be considered along with other clinical parameters when considering surgical intervention. One study suggested that the relative risk for having embolic events associated with echocardiographically visualized lesions is microorganism-dependent, with a significant

attributable risk seen only in patients with viridans streptococcal infection.[455] The absence of a vegetation on transthoracic echocardiography may define a subset of patients at low risk for the development of embolic complications.

Transesophageal echocardiography is a newer technique that has been studied extensively in adults with infective endocarditis.[99] It uses a 5-MHz phased-array transducer with Doppler and color flow encoding capabilities mounted on the tip of a flexible endoscope.[432] Biplane transesophageal echocardiography is considered the standard technique and is superior to transthoracic echocardiography because of improved spatial resolution, lack of acoustic interference from the lungs and chest wall, and closer proximity to posterior structures, such as the mitral valve and left atrium.[98] Multiplane transesophageal echocardiography facilitates and abbreviates the examination procedure and may be more accurate in providing the dimensions of a vegetation associated with infective endocarditis.[98,226]

Transesophageal echocardiography generally is well tolerated by children, even with the use of an adult probe (when the child's weight is more than 7 kg),[439] and rarely is associated with bacteremia.[97,269,382] Transesophageal echocardiography usually is more sensitive than is transthoracic echocardiography in the detection of intracardiac vegetations and is positive in 70 to 95 percent of adults with strongly suspected endocarditis.[342,445] It is significantly more sensitive in the detection of vegetations and complications in infected prosthetic valves.[253,372,409,463,476] Transesophageal echocardiography is particularly useful for detecting an aortic root abscess or involvement of the sinus of Valsalva in adults, and it should be considered in children with aortic valve endocarditis and changing aortic root dimensions on a standard transthoracic echocardiograph.[145,319] It appears to be less helpful for detection of vegetations in right-sided endocarditis.[428] Although a negative transesophageal echocardiographic study does not exclude endocarditis,[450] the procedure should be considered for patients with suspected endocarditis and a negative transthoracic echocardiograph, when the transthoracic echocardiography windows are suboptimal, and when perivalvular extension of infection is suspected.[14,212]

To aid in establishing the diagnosis of infective endocarditis, various sets of clinical criteria have been suggested. The von Reyn criteria were described in 1982, but echocardiographic findings were not included in the case definitions.[510] In addition, isolation of a typical infective endocarditis pathogen from blood cultures was not considered. New case definitions and diagnostic criteria were proposed by investigators from Duke,[121] and they have been modified subsequently[268,283] (Tables 32–5 and 32–6). The inclusion of echocardiographic and blood culture findings in these new criteria has resulted in more flexibility, a higher proportion of "definite" cases, and a more accurate reflection of current clinical practice.[324] These criteria have been validated in large series of infective endocarditis in adults and children.[107,121,200,426] In two pediatric series of clinically defined endocarditis, no cases were rejected by the Duke criteria, whereas 25 and 19 percent were rejected by the von Reyn criteria.[107,464] In one study, three of six pathologically confirmed cases were rated as only probable or rejected by the von Reyn criteria, whereas all were definite by the Duke criteria.[107] However, a more recent study found that 12 percent of pediatric endocarditis cases were not classified as "definite" by the modified Duke criteria.[487]

TABLE 32–5 Definition of Terms Used in the Modified Duke Criteria for Infective Endocarditis

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Please refer to the printed publication.

From Li, J. S., Sexton, D. J., Mick, N., et al: Proposed modifications to the Duke criteria for the diagnosis of infective endocarditis. Clin. Infect. Dis. 30:*633-638, 2000.*

MICROBIOLOGY

Many different microorganisms are capable of causing infective endocarditis in humans. Table 32–7 lists the organisms isolated from patients in major pediatric series. Gram-positive cocci are the etiologic agents in 90 percent of cases in which an organism is isolated. Streptococci remain the bacteria isolated most frequently, although the percentage of cases caused by staphylococci and fungi has been increasing during the past 2 decades.[151,420,507,537] Polymicrobial infective endocarditis, especially in nosocomial settings, also appears to be increasing in incidence.[22] The characteristics of selected organisms and the type of disease that they produce are considered in the following subsections.

STREPTOCOCCI

Several terminologies have been used to classify streptococci. The Lancefield system defines groups (A, B, C, D, E, F, G, H) by serologic reactions. The viridans streptococci are alpha-hemolytic or nonhemolytic, may be Lancefield nontypeable *(Streptococcus milleri*, *Streptococcus mitior*; *Streptococcus salivarius*, most *Streptococcus mutans*, and *S. sanguis*) or typeable *(Streptococcus bovis* group D, some *S. sanguis* group H, some *S. milleri* group F), and display similar characteristics in vivo. They are the most frequent etiologic agents in subacute infective endocarditis and cause 40 percent of cases in children. They may cause rapidly progressive invasive disease.[205,471]

TABLE 32–6 Modified Duke Criteria for the Diagnosis of Infective Endocarditis

Rights were not granted to include this table in electronic media.
Please refer to the printed publication.

From Li, J. S., Sexton, D. J., Mick, N., et al: Proposed modifications to the Duke criteria for the diagnosis of infective endocarditis. Clin. Infect. Dis. 30:633-638, 2000.

TABLE 32–7 Etiologic Agents of Bacterial Endocarditis in Children

Organism	Average (%)	Range (%)
Streptococci		
Viridans	40.3	17-72
Enterococci	4.0	0-12
Pneumococci	3.3	0-21
Beta-hemolytic	2.7	0-8
Other	1.1	0-16
Staphylococci		
Staphylococcus aureus	23.8	5-40
Coagulase-negative	4.7	0-15
Gram-negative aerobic bacilli	4.0	0-15
Fungi	1.1	0-12
Miscellaneous bacteria	2.4	0-10
Culture-negative	12.6	2-32

Data from references 49, 58, 91, 229-231, 264, 321, 420, 453, 484, and 507.

Viridans streptococci are common pathogens in patients with underlying heart disease but are less common in postoperative patients. They are part of the indigenous flora of the human mouth and gastrointestinal tract, and procedures that disrupt mucosal integrity in these areas predispose patients to development of viridans streptococcal bacteremia. In the pediatric population, most blood and cerebrospinal fluid isolates of viridans and nonhemolytic streptococci are not from patients with infective endocarditis.[189] Most strains are exquisitely susceptible to penicillin, although previous administration of antibiotics may promote infection with resistant strains.[262,279] Nutritionally variant viridans streptococci, reclassified as *Abiotrophia defectiva* or *Granulicatella* spp., are recognized as one cause of culture-negative endocarditis in children.[72,136,286,353,393,406] These organisms grow in broth but will not grow on subculture agar-based plates. Bacteriologic failure has occurred in 40 percent of reported cases of endocarditis caused by these organisms despite susceptibility to the antibiotics used.[24,459] Most viridans streptococci have low pathogenicity; however, the *S. milleri* group has a predilection for suppurative complications.[347] The prognosis of endocarditis caused by non-enterococcal streptococci is excellent with good medical and surgical management; the cure rate is more than 90 percent, although complications (emboli, congestive heart failure) occur in as many as 30 percent of cases.

Enterococcal endocarditis occurs much less frequently in children than in adults[322,481] and accounts for only 4 percent of pediatric cases. The organism normally inhabits the gastrointestinal and genitourinary tracts; instrumentation of these areas may cause enterococcal bacteremia. More than 40 percent of adult patients have no underlying heart disease.[402] Endocarditis should be considered in all infants and children with unexplained enterococcal bacteremia. Although the incidence of enterococcal bacteremia appears to be increasing in some neonatal intensive care units, the incidence of associated endocarditis seems to be very low. Factors that may suggest endocarditis in patients with enterococcal bacteremia include (1) preexisting heart disease, (2) community acquisition, (3) a cryptogenic source, and (4) the absence of polymicrobial bacteremia.[301] Differentiation of enterococci from other group D streptococci *(S. bovis)* is important because their respective therapeutic approaches are different.

Endocarditis caused by beta-hemolytic streptococci occurred more commonly in the pre-antibiotic era than today. Most cases are caused by Lancefield group B or G organisms,[1,16,158,517] whereas group C and A streptococci rarely cause endocarditis.[45,114,174,290,395] Group A, B, or C streptococcal infection may lead to large, bulky vegetations, easily seen by echocardiography, and to embolic complications.[17,331,423] Although group B streptococcal bacteremia is a common finding in newborn infants, endocarditis caused by this organism occurs rarely in this age group. Similarly, *S. pneumoniae* accounted for 10 to 15 percent of endocarditis cases in the pre-antibiotic era but currently causes less than 1 percent.[162,222,364] Pneumococcal endocarditis may involve either the aortic or the mitral valve.[126,171,491] In older studies, fewer than 50 percent of affected children had underlying heart disease, but in more recent series, most children have had existing heart disease.[218] The clinical course often is fulminant.[123,275,384] Concurrent meningitis or pneumonia (or both) occurs frequently. Valvular dysfunction and cardiac decompensation are common findings.[48,56,78] Early surgical intervention may be required because the mortality rate is 75 percent when medical management alone is used.[222]

STAPHYLOCOCCI

Staphylococci cause 20 to 30 percent of cases of infective endocarditis in children, but the relative incidence appears to be increasing.[151] *S. aureus* is the etiologic agent in most cases of acute endocarditis and frequently infects normal heart valves.[152,340] The course often is fulminant when the mitral or aortic valve is involved, with frequent suppurative complications occurring both in the heart (myocardial abscess, pericarditis, valve ring abscess) and in other organs.[129,236,329] *S. aureus* is responsible for more than 50 percent of cases of endocarditis in intravenous drug abusers, but the disease tends to be less severe in these patients.[70,71] The origin of the infecting organism is the addict's own nose or skin, not the injection paraphernalia.[497] Endocarditis associated with indwelling vascular catheters or prosthetic valves frequently is caused by *S. aureus*.[227] Endocarditis must be suspected in any patient with *S. aureus* bacteremia, even when a peripheral focus of infection is present. However, most patients with *S. aureus* bacteremia do not have endocarditis. The rise of methicillin-resistant *S. aureus* (MRSA) has rendered treatment more difficult but has had little impact on the rate of local complications.[210]

The incidence of endocarditis resulting from CONS is rising rapidly.[12] CONS is a common etiologic agent of endocarditis

occurring after cardiac surgery,[251,287] and it is occurring more frequently on native valves.[79,80] This organism is the leading agent in prosthetic-valve endocarditis, for which it causes 25 to 67 percent of early cases and 25 to 33 percent of late cases.[168,219,242] CONS endocarditis also has been associated with mitral valve prolapse and the use of intravascular catheters in premature neonates.[23,359] Although metastatic infection rarely occurs, CONS can be locally invasive; the mortality rate of prosthetic-valve endocarditis caused by CONS approaches 75 percent when valve replacement is not performed.

GRAM-NEGATIVE ORGANISMS

Although gram-negative bacteria cause 4 to 5 percent of cases of infective endocarditis in children, the percentage of children with gram-negative enteric bacteremia in whom endocarditis develops is extremely low. Endocarditis should be suspected in patients with gram-negative infection when bacteremia persists despite administration of usually appropriate antibiotic therapy.[65] Burn patients,[214] immunosuppressed hosts, narcotic addicts, and patients with implanted endovascular devices[335] are at an increased risk for development of gram-negative endocarditis. However, in the early postoperative period after cardiac surgery, sustained gram-negative bacillary bacteremia commonly is caused by other foci of infection and does not imply the presence of endocarditis. Many species of gram-negative enteric organisms have caused infective endocarditis in children, but no clear pattern has emerged. Among the gram-negative organisms more commonly reported are *Brucella*, *Escherichia coli*, *Serratia*, *Klebsiella-Enterobacter*, *Salmonella*, and *Pseudomonas*.[108,263,294,335,473] Endocarditis caused by *Salmonella* has been reported in patients with HIV infection.[142] It most often affects previously abnormal heart valves. Endocarditis is a rare complication of tularemia.[478] Cure of left-sided endocarditis caused by the Enterobacteriaceae seldom is achieved with medical therapy alone.[432] Most information about gram-negative enteric endocarditis is limited to case reports and general medicine reviews; discussion of individual organisms is beyond the scope of this review.

Other gram-negative organisms associated with infective endocarditis are the so-called HACEK coccobacilli.[102,137,211] These organisms caused 57 percent of cases of gram-negative endocarditis seen at the Mayo Clinic in Rochester, Minnesota, from 1958 to 1979.[164] Endocarditis caused by *Haemophilus influenzae* has been reported in only four children.[96,305] Cases caused by *Haemophilus parainfluenzae* and *Haemophilus aphrophilus* occur slightly more commonly.[41,81,101,211,225,296] They generally are seen in the setting of preexisting valvular disease and run a subacute course. However, central nervous system complications and emboli to major peripheral arteries are frequent occurrences.[81] Infective endocarditis caused by other organisms of the HACEK group is an extremely rare event in children.[11,138,339,368,392,521] All the bacteria in this group are fastidious, may require 2 to 3 weeks for primary isolation, and need subculturing onto chocolate agar in an atmosphere of 5 to 10 percent carbon dioxide for optimal growth. These procedures should be performed in all cases of culture-negative endocarditis.

Neisseria gonorrhoeae was responsible for 10 percent of cases in the pre-antibiotic era, but fewer episodes have been reported since 1942.[141,220] This pathogen frequently attacks previously normal heart valves and is manifested as an acute illness.[479] Valvular destruction with a need for valve replacement occurs commonly. At present, nonpathogenic *Neisseria* spp. are isolated more frequently in endocarditis than are gonococci, but they usually attack abnormal or prosthetic valves.[53,194,206,215,381,440] Although 1 percent of cases of infective endocarditis in adults are caused by anaerobic bacteria,[139] reports of anaerobic endocarditis in children are exceedingly rare.[86,354,448,467]

GRAM-POSITIVE BACILLI

Infective endocarditis caused by *Corynebacterium* spp. is an unusual finding but may occur on normal or previously abnormal valves.[38,323] Both toxigenic[105] and nontoxigenic[182,449,486] strains of *Corynebacterium diphtheriae* cause endocarditis in children, a finding demonstrating that the toxigenic and invasive properties of the organism are independent. Infection occurs most often on native valves and may be quite aggressive and lead to major vascular complications. *Listeria monocytogenes* endocarditis rarely occurs, has a high mortality rate, and, unlike other forms of listeriosis, usually is not associated with immunocompromised hosts.[33,66] It has not been associated with listeriosis in neonates. Fewer than 40 cases of *Lactobacillus* endocarditis have been reported.[181,213,470] Endocarditis caused by *Erysipelothrix rhusiopathiae* is found predominantly in adults who are farmers or are exposed to farm animals or products.[176,192] Most cases of *Bacillus* endocarditis involve the tricuspid valve in intravenous drug users, but other patients have been affected, including those with prosthetic valves.[458] *Gemella morbillorum*, formerly known as *Streptococcus morbillorum*, is a gram-positive coccus that normally resides in the gastrointestinal tract and is a rare cause of endocarditis.[132,270]

OTHER ORGANISMS

Many different bacteria, including *Acinetobacter*,[179] *Stenotrophomonas*,[408] *Nocardia*,[516] *Actinomyces*,[267] *Streptobacillus*,[414] and *Rothia*,[468] have been associated rarely with endocarditis.[55] Mycobacterial endocarditis is an exceedingly infrequent event.[157]

Infective endocarditis caused by *Coxiella burnetii*, the causative agent of Q fever, is well documented in northern Africa, Europe, and Australia.[4,54,271,282] Most cases are chronic (occurring over a 6- to 12-month period) and involve the aortic valve.[303] Clues to establishing the diagnosis include exposure to parturient cats or rabbits, massive splenomegaly, hypergammaglobulinemia, and thrombocytopenia.[379] The diagnosis usually is confirmed by measurement of antibodies against phase I and phase II antigens, but the organism has been isolated from leukocytes in a shell vial assay and has been demonstrated by immunohistologic techniques.[54,170] At least 20 well-documented cases of infective endocarditis caused by *Chlamydia psittaci* and *Chlamydia pneumoniae* have been reported.[201,235,310,444] Most patients have had preexisting heart disease and a subacute course.[304] *Mycoplasma* endocarditis is exceedingly rare.[112] *Legionella* has been implicated in several cases of prosthetic-valve endocarditis.[492] *Bartonella quintana* and *Bartonella henselae* have been identified as the cause of endocarditis in "culture-negative" cases.[115,224,452] Most described cases have been in immunocompetent individuals.[380] The diagnosis was established by serology, PCR, or special culture techniques.[26,140,149,396,512]

Although culture of bacteria remains the primary method for establishing the microbial cause of infective endocarditis, the number of organisms causing endocarditis that cannot be cultivated by standard culture methods is growing.[148,207] More recently, universal and species-specific primers have been designed to amplify bacterial DNA directly from resected valves. Among the organisms causing endocarditis identified by these methods are *Bartonella*, *Tropheryma whippelii*, *Coxiella*, *Mycoplasma*, *Haemophilus*, *Abiotrophia*, *Gemella*, *Cardiobacterium*, and *Streptococcus*.[161,175,207,289,390]

FUNGI

Most cases of fungal endocarditis in children have been described as occurring after cardiovascular surgery and prolonged intrave-

nous and antibiotic therapy.[125,326,488] More recently, cases have been reported in neonates[315] and after prosthetic-valve placement.[356] The most common causative organism is *C. albicans*, although disease has been attributed to other *Candida* spp., including *C. krusei*, *Candida parapsilosis*, *Candida stellatoidea*, *Candida tropicalis*, and *Candida guilliermondii*.[388,424,441] Among intravenous drug abusers, *Candida* spp. other than *C. albicans* are more common causes of endocarditis.[413] The clinical manifestation usually is indolent and not specific, with symptoms occurring weeks to months before the diagnosis is established. Signs and symptoms caused by emboli to large vessels, especially those supplying the brain, kidney, spleen, and extremities, should alert the physician to the possible presence of fungal endocarditis. Large, friable vegetations occur frequently and can be detected by echocardiography.[465] Cutaneous and ocular manifestations of systemic *Candida* infection may be present.[50] The prognosis of *Candida* endocarditis is poor and is related to the propensity for septic emboli, the tendency for invasion into the myocardium, and the poor penetration of antifungal agents into the bulky vegetation. The diagnosis frequently is delayed by the tendency for negative or intermittently positive blood cultures to occur in this disease.[233] Surgical intervention usually is required.

Aspergillus spp., including *Aspergillus flavus*, *Aspergillus fumigatus*, *Aspergillus terreus*, and *Aspergillus niger*, are the second most frequent causes of fungal endocarditis, and such infections have been reported in 16 children.[28,29] Two thirds of these patients had underlying heart disease. *Aspergillus* endocarditis has been found in immunocompromised hosts with no previous cardiac problems.[534] The most common initial manifestations are fever and embolic phenomena, especially to the central nervous system.[513] Fewer than 15 cases have been diagnosed ante mortem, three by culture of peripheral emboli. In none of the patients was the antemortem blood culture positive. Most cases occur after open heart surgery; the most likely source of the organism is airborne inoculation of the heart during the operation.[28] Surgical removal of all infected material is recommended, although only one child has been treated successfully. Other fungi that rarely cause endocarditis include *Histoplasma capsulatum*, *Coccidioides immitis*,[272] *Cryptococcus neoformans*, *Torulopsis glabrata*, *Trichosporon beigelii*,[249] and *Fusarium* spp.[185,208]

TREATMENT

In the pre-antibiotic era, infective endocarditis was a uniformly fatal disease. With the current improved methods of diagnosis and therapy, 80 to 90 percent of children with this disease can be expected to survive. Mortality rates are higher for acute staphylococcal infection, fungal endocarditis, and prosthetic-valve endocarditis, although the tendency toward earlier surgical intervention for these entities may improve survival rates. The cornerstone of successful therapy is selection of antibiotics with specific activity against the causative organism. Better analysis of pharmacodynamic variables, such as bactericidal activity and the post-antibiotic effects of various drugs, may assist in the selection of optimal therapeutic regimens.[90,255,529] Although persistent infection occasionally complicates treated endocarditis,[400] deterioration in cardiac function is the major cause of morbidity and mortality.

Several general principles provide the basis for the current recommendations for treatment of endocarditis. Parenteral administration of antibiotics is preferred because erratic absorption of oral antibiotics, especially in infants, can lead to therapeutic failure. Although patient selection criteria for the use of outpatient parenteral antibiotic therapy for endocarditis in adults have been suggested, no data have been published about this practice for children.[9] Prolonged treatment, usually 4 to 6 weeks or longer, is necessary to sterilize the vegetations and to prevent relapse. Bacteriostatic antibiotics are not effective and lead to frequent relapses or failure to eradicate the infection, or both. Antibiotic combinations may produce a rapid bactericidal effect through synergistic mechanisms of action. When synergy exists, smaller doses of each drug may be used, thereby reducing toxic side effects. However, certain drug combinations (e.g., penicillin and chloramphenicol) can be antagonistic, and their use should be avoided.

Blood should be drawn for culture for several days to evaluate the effect of the antibiotics. Negative follow-up cultures do not guarantee the success of therapy, but persistent positive cultures usually require that a change or addition to the antibiotic regimen be made. Observation of the patient's clinical course is extremely important. When fever is present initially, the temperature often returns to normal within a few days after therapy is started. However, fever can persist for weeks in patients whose eventual outcome is good. Such patients must be monitored closely for cardiac arrhythmias and congestive heart failure, which may require intensive care observation and electrocardiographic monitoring. Evidence of major embolic phenomena must be sought diligently by physical examination.

Several laboratory tests may aid in monitoring therapy. In all cases of bacterial endocarditis, the minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) ideally is determined for the antibiotics being used because disk susceptibility testing is unreliable and is not quantitative. When combinations of antibiotics are used, tests for bactericidal synergy, such as broth dilution, "checkerboards," or time-kill curves, may give additional information. The role of monitoring the inhibitory and bactericidal activity of the patient's serum is highly controversial. The Schlichter test determines the maximal dilutions of a patient's serum that in vitro inhibit and kill an inoculum of the organism causing the endocarditis.[399] Standardization of this test is poor, with laboratories using variations in inoculum size, in composition of the broth, in timing of samples (at expected peak or trough antibiotic concentrations in serum), in methods of dilution, and in determining the bactericidal end-point. In the rabbit endocarditis model, peak serum bactericidal titers greater than 1:8 correlate with therapeutic success.[64] A retrospective review of 17 reports of serum bactericidal activity in patients with endocarditis failed to show any correlation between titers greater than 1:8 and therapeutic success.[87] A prospective study suggested adjusting antibiotic doses to achieve peak titers of 1:64 or greater and trough titers of 1:32 or greater.[520] At present, no generally accepted recommendation can be made. In general, to attempt to achieve a peak serum bactericidal titer of at least 1:8 or greater seems reasonable if serious drug toxicity is not encountered. However, this level may not be attainable with certain organisms such as enterococci and gram-negative bacilli. Serum bactericidal testing may be particularly useful when synergistic combinations or less well established antibiotic regimens are used or when response to therapy is suboptimal.[533]

Little information is available concerning optimal antibiotic therapy for infective endocarditis in children; most treatment regimens are adapted from studies of adults with endocarditis.[24,530] In general, these regimens have been equally successful (and generally less toxic) in children. Table 32–8 lists recommended doses of the antibiotics commonly used.

After performing the initial evaluation of a patient with suspected infective endocarditis, the physician must make a clinical judgment about when to initiate therapy. If the findings are strongly indicative of the diagnosis or the child is very ill, treatment should be started as soon as blood has been drawn for culture. Initial empiric therapy depends on the clinical setting in which the tentative diagnosis is made. If the infection is subacute, a combination of penicillin G and an aminoglycoside usually is recommended for its activity against viridans streptococci, enterococci, and most gram-negative organisms. If *S. aureus*

TABLE 32–8 Suggested Intravenous Antibiotic Doses and Schedules for Infective Endocarditis in Children

Antibiotic	Daily Dose/kg	Divided Doses Every
Aqueous crystalline penicillin G sodium	300,000 U	4-6 hr
Ampicillin sodium	300 mg	4-6 hr
Ampicillin-sulbactam	300 mg	4-6 hr
Cefazolin	100 mg	6-8 hr
Ceftriaxone	100 mg	12 hr
Ciprofloxacin	20-30 mg	12 hr
Doxycycline	2-4 mg	12 hr
Gentamicin sulfate	3 mg	8 hr or 24 hr
Imipenem/cilastatin	60-100 mg	6 hr
Linezolid	30 mg	8 hr
Nafcillin sodium	200 mg (max., 12 g)	4-6 hr
Oxacillin sodium	200 mg (max., 12 g)	4-6 hr
Rifampin	20 mg	8-12 hr
Vancomycin hydrochloride	40 mg	6-12 hr

endocarditis is a strong consideration (acute manifestation, narcotic addicts), vancomycin and a penicillinase-resistant penicillin should be added to this regimen. Patients who recently have undergone cardiac surgery, especially prosthetic-valve placement, are treated best with an aminoglycoside and vancomycin to "cover" for health care–associated infection caused by MRSA or CONS; some physicians add penicillin G to this regimen to improve activity against streptococci. When culture and susceptibility data are known, antibiotic therapy can be changed as needed.

Most strains of viridans streptococci, *S. pyogenes*, and nonenterococcal group D streptococci are exquisitely susceptible to penicillin, with an MIC of less than 0.2 μg/mL. However, 15 to 20 percent of viridans streptococci have an MIC of 0.2 μg/mL or greater and are defined arbitrarily as relatively resistant.[209] In addition, some strains (particularly *S. mutans* and *S. mitior)* demonstrate tolerance, that is, an MIC to penicillin of less than 0.1 μg/mL but an MBC that is more than 10-fold higher (1.25 to 50 μg/mL). Most strains of nutritionally dependent streptococci are tolerant to penicillin.[24] Clinical failure may occur in endocarditis caused by these tolerant organisms when penicillin alone is used for treatment.[8,209] However, except for nutritionally dependent streptococci, therapy for tolerant viridans streptococci generally should be the same as for susceptible strains.

Although most experts recommend that patients with endocarditis caused by relatively resistant streptococci be treated with high doses of penicillin combined with 2 to 4 weeks of an aminoglycoside, some authorities consider that penicillin alone usually is adequate therapy.[44,110] Synergy in vitro between penicillin or vancomycin and streptomycin, gentamicin, or kanamycin can be demonstrated against virtually all penicillin-susceptible streptococci.[515] This observation correlates with a faster rate of eradication of bacteria from cardiac vegetations in the rabbit endocarditis model when synergistic combinations of antibiotics are used.[119,122] However, streptomycin is not synergistic for strains with high-level streptomycin resistance; gentamicin is the preferred second drug for these rare isolates.[127] In pediatric patients, gentamicin usually is substituted for streptomycin because of its lower toxicity.

Several regimens have been examined in adults with penicillin-susceptible viridans streptococcal native-valve endocarditis (Table 32–9). A 2-week course of penicillin alone leads to an unacceptable relapse rate. However, a 2-week course of intramuscular procaine penicillin and streptomycin cured 99 percent of adults with penicillin-susceptible streptococcal endocarditis in one report.[531] These results are similar to those obtained with β-

TABLE 32–9 Suggested Regimens for Treatment of Native Valve Endocarditis Caused by Highly Penicillin-Susceptible Viridans Streptococci and *Streptococcus bovis*

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From Baddour, L. M., Wilson, W. R., Bayer, A. S., et al.: Infective endocarditis: Diagnosis, antimicrobial therapy, and management of complications. Circulation 111: *e394-e433, 2005.*

TABLE 32–10 Suggested Therapy for Native-Valve Endocarditis Caused by Strains of Viridans Streptococci and *Streptococcus bovis* Relatively Resistant to Penicillin G (Minimum Inhibitory Concentration, 0.12 and 0.5 μg/mL)

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From Baddour, L. M., Wilson, W. R., Bayer, A. S., et al.: Infective endocarditis: Diagnosis, antimicrobial therapy, and management of complications. Circulation 111: *e394-e433, 2005.*

lactams alone for 4 weeks[244] or with penicillin for 4 weeks combined with streptomycin for the first 2 weeks. Gentamicin may be substituted for streptomycin. The 2-week penicillin-gentamicin regimen is the least expensive and is the preferred therapy in uncomplicated cases of penicillin-susceptible streptococcal endocarditis in young adults.[432] In general, the regimen of 4 weeks of penicillin alone is preferred for patients in renal failure or at high risk for developing aminoglycoside-induced ototoxicity. Vancomycin or ceftriaxone administered for 4 weeks can be used in patients with penicillin-susceptible viridans streptococcal endocarditis who have a penicillin allergy.[153,154,442,466] The regimen of 4 weeks of penicillin plus an initial 2 weeks of gentamicin is recommended in adults with a complicated course (symptoms for >3 months)[302] or with infection caused by relatively penicillin-resistant organisms (Table 32–10). Most nutritionally deficient streptococci are tolerant to penicillin. For patients with endocarditis caused by these organisms, a course of 4 to 6 weeks of penicillin with the addition of an aminoglycoside is recommended[24,530] (Table 32–11). In patients with streptococcal

TABLE 32–11 Suggested Therapy for Endocarditis Caused by Enterococcal Strains Susceptible to Penicillin, Gentamicin, and Vancomycin and Other Selected Streptococci*

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From Baddour, L. M., Wilson, W. R., Bayer, A. S., et al.: Infective endocarditis: Diagnosis, antimicrobial therapy, and management of complications. Circulation 111: *e394-e433, 2005.*

TABLE 32–12 Suggested Therapy for Endocarditis of Prosthetic Valves or Other Prosthetic Material Caused by Viridans Group Streptococci and *Streptococcus bovis*

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From Baddour, L. M., Wilson, W. R., Bayer, A. S., et al.: Infective endocarditis: Diagnosis, antimicrobial therapy, and management of complications. Circulation 111: *e394-e433, 2005.*

TABLE 32–13 Suggested Therapy for Native or Prosthetic Valve Enterococcal Endocarditis Caused by Strains Resistant to Penicillin, Aminoglycoside, and Vancomycin

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TABLE 32–14 Suggested Therapy for Endocarditis Caused by Staphylococci in the Absence of Prosthetic Material

Antibiotic(s)	Duration	Comments
Methicillin-Susceptible Staphylococci*		
Nafcillin sodium or oxacillin sodium	6 wk	Benefit of additional aminoglycoside has not been established
optional addition of Gentamicin sulfate	3-5 days	
Cefazolin	6 wk	For patients with non–immediate-type hypersensitivity to penicillin
optional addition of Gentamicin sulfate	3-5 days	
Methicillin-Resistant Staphylococci		
Vancomycin hydrochloride	6 wk	

**If Staphylococcus is penicillin-susceptible (minimal inhibitory concentration ≤0.1 μg/mL), aqueous crystalline penicillin G sodium can be used for 6 weeks instead of nafcillin or oxacillin.*

From Baddour, L. M., Wilson, W. R., Bayer, A. S., et al.: Infective endocarditis: Diagnosis, antimicrobial therapy, and management of complications. Circulation 111: *e394-e433, 2005.*

infection of prosthetic valves or other prosthetic materials, a 6-week regimen of penicillin usually supplemented with an aminoglycoside is recommended. (Table 32–12). None of the regimens discussed has been evaluated specifically in children with endocarditis.

Most strains of enterococci have an MIC to penicillin of 0.4 μg/mL or greater and an MBC of 6.25 μg/mL or greater.[346] All β-lactam antibiotics are bacteriostatic against enterococci and cannot be used alone. However, plasmid-mediated β-lactamase production has been found in rare strains of *Enterococcus faecalis.* Ampicillin-sulbactam overcomes the enzyme production and is effective as therapy.[483] Although therapy with penicillin alone is ineffective, the combination of penicillin and an aminoglycoside is synergistic and produces a bactericidal effect on most enterococcal strains.[323] Unfortunately, 20 to 50 percent of enterococcal strains demonstrate very high resistance (MIC >2000 μg/mL) to streptomycin, and synergy between penicillin and streptomycin does not occur.[195,431] High-level resistance to gentamicin has been found in some isolates, and the incidence is increasing in certain locales.[124,288,369] When these isolates are encountered, all aminoglycosides should be tested because the organism may be susceptible to one while resistant to others.[318] Fortunately, these strains rarely cause endocarditis.[346] Although vancomycin-resistant enterococci have emerged as important nosocomial pathogens, they rarely cause endocarditis in children. Optimal therapy for these strains has not been established, but a combination of high-dose penicillin plus vancomycin and gentamicin may be effective in some cases.[61] Vancomycin-resistant enterococcal endocarditis has been treated successfully with oral linezolid.[10,15] The usual regimens for enterococcal endocarditis are listed in Tables 32–11 and 32–13).

Most isolates of *S. aureus* are resistant to penicillin, but endocarditis caused by penicillin-susceptible (MIC <0.1 μg/mL) isolates can be treated with this agent. In general, a semisynthetic penicillinase-resistant penicillin given for 6 weeks is the drug of choice [374,456] (Table 32–14). The addition of gentamicin to nafcillin produces an enhanced bactericidal effect in vitro and in exper-

imental staphylococcal endocarditis in rabbits.[425] However, the value of this combination in patients has not been proved, and generally it is reserved for children with overwhelming infection. In penicillin-allergic patients or in those with MRSA, vancomycin alone has been recommended, although treatment failures in children with endocarditis have been reported.[134,221,280] The addition of rifampin or the use of β-lactam drugs after desensitization is necessary in some cases.[31,340] Ciprofloxacin has been used, but treatment failures have occurred because of the emergence of resistance.[344,480] The combination of vancomycin and rifampin for at least 6 weeks, plus the addition of gentamicin for the first 2 weeks, is recommended for the treatment of endocarditis caused by MRSA, although clinical trial data are not available. Daptomycin also has been used successfully to treat endocarditis caused by MRSA.[150] Nosocomial infections with *S. epidermidis* usually are treated with vancomycin because of the high incidence of methicillin resistance among these isolates. The addition of rifampin and gentamicin to either nafcillin or vancomycin may increase bactericidal activity and is recommended in cases of prosthetic-valve endocarditis secondary to any staphylococci[24] (see Table 32–13).

Therapy for endocarditis caused by gram-negative organisms must be individualized in accordance with in vitro susceptibility and synergy studies. A regimen of 6 to 8 weeks of combination therapy with two or more drugs may be required, especially with endocarditis caused by *Klebsiella* or *Pseudomonas*.[146,373] Surgical intervention frequently is necessary, especially for infection of the mitral or aortic valves. Endocarditis caused by *Haemophilus* and other fastidious gram-negative organisms usually is responsive to ampicillin, ceftriaxone, or ciprofloxacin alone,[24] but the addition of an aminoglycoside may improve the outcome[24,81] (Table 32–15). Anaerobic bacilli generally are susceptible to penicillin, but infection caused by resistant *Bacteroides fragilis* is treated best by combinations including metronidazole, ticarcillin-clavulanate, or imipenem.

Survival rates of only 10 to 20 percent in patients with fungal endocarditis are related to the poor ability of currently available antifungal agents to sterilize the vegetations. Only rare cures with medical therapy alone have been reported.[424] Most investigators contend that early surgical intervention is mandatory in every patient who has conclusive evidence of intracardiac fungal infection.[460,500,501] Fewer than 50 cases of successful treatment of fungal prosthetic-valve endocarditis have been reported, even when surgery was performed.[169]

Although a prolonged course of antifungal therapy before surgery does not improve the outcome, chemotherapy should be given in conjunction with operative treatment. The drug of choice is amphotericin B at a dose of 0.5 to 1.0 mg/kg/day. This antibiotic may be either fungistatic or fungicidal, depending on the infecting organism. Even though the toxicity of amphotericin B appears to be less severe in children than in adults, side effects that may necessitate alterations in the usual regimen may occur and include fever, chills, phlebitis, anemia, hypocalcemia, renal tubular acidosis, nephrotoxicity, and thrombocytopenia. The optimal dosage of amphotericin B is unknown; total doses of 20 to 50 mg/kg commonly are used. 5-Fluorocytosine[320] and rifampin may act synergistically with amphotericin B against many strains of fungi, but their roles in fungal endocarditis are unproven. Fluconazole is less effective than is amphotericin B for the prophylaxis or treatment of experimental *Candida* endocarditis,[532] but it has been used successfully in a few patients.[92,508]

Treatment of culture-negative endocarditis is problematic.[27] In general, the same criteria used to choose empiric therapy for infective endocarditis can be followed. Antibiotics usually are continued for 6 weeks, and ongoing surveillance for an etiologic agent must be performed. In 52 adults with culture-negative endocarditis, survival correlated with the initial clinical response to antibiotics; most deaths were caused by systemic emboli or congestive heart failure.[378]

Surgery has become a valuable adjunct to medical therapy in the management of infective endocarditis.[30,365,377,385] Several echocardiographic findings suggest a possible need for surgical intervention[30,145] (Table 32–16). Among the generally accepted indications for surgical intervention during active endocarditis are (1) refractory congestive heart failure,[325,490,528] (2) uncontrolled infection,[304] (3) more than one serious embolic episode, (4) fungal endocarditis, (5) most cases of prosthetic-valve endocarditis,[6,104,128,419,538] and (6) local suppurative complications including perivalvular or myocardial abscess with conduction system abnormalities.[43,411,443,474,535] The usual indication for surgical intervention is congestive heart failure in left-sided lesions and persistent infection in right-sided disease.[106,509] Among children with endocarditis after cardiac surgery, repair or takedown of infected graft material commonly is the reason for surgery.[83,361] In general, operative mortality is low even if surgery is performed during the active infection.[343,355] The hemodynamic status of the patient, rather than the activity of the infection, is the critical factor in determining the timing of cardiac surgery or valve replacement.[3,241] The aortic valve is the site most often requiring surgical intervention.[337,387,494] Treatment with recombinant tissue plasminogen activator has been used successfully in some cases of endocarditis when surgery could not be performed safely.[183,281]

TABLE 32–15 Suggested Therapy for Endocarditis Caused by HACEK Organisms in Adults

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From Baddour, L. M., Wilson, W. R., Bayer, A. S., et al.: Infective endocarditis: Diagnosis, antimicrobial therapy, and management of complications. Circulation 111: *e394-e433, 2005.*

TABLE 32–16 Echocardiographic Features Suggesting a Possible Need for Surgical Intervention in Endocarditis

Vegetation
Persistent vegetation after systemic embolization
Anterior mitral valve leaflet vegetation, particularly >10 mm
Embolic event during first 2 weeks of therapy
Increase in vegetation size after 4 weeks of therapy
Valvular Dysfunction
Acute aortic or mitral insufficiency with signs of ventricular failure
Heart failure unresponsive to medical therapy
Valve perforation or rupture
Perivalvular Extension
Valvular dehiscence, rupture, or fistula
New heart block
Large abscess or extension of abscess

Data from Bayer, A., Bolger, A., Taubert, K., et al.: Diagnosis and management of infective endocarditis and its complications. Circulation 98:*2936-2948, 1998; and Ferrieri, P., Gewitz, M. H., Gerber, M. A., et al.: Unique features of infective endocarditis in children. Pediatrics* 109:*931-943, 2002.*

PREVENTION

Accepted medical practice has been to give prophylactic antibiotics to susceptible patients in an attempt to prevent infective endocarditis.[116,248,447] The rationale for such treatment is based on studies indicating that antibiotics can reduce the incidence of bacteremia after various procedures in humans[130] and can prevent experimental endocarditis in animals.[376] However, no controlled trials have documented the efficacy of endocarditis prophylaxis in humans.[505] Prevention of bacterial infection is most likely to be successful and cost-effective when a single antibiotic is directed against a single pathogen and when the disease occurs with high frequency in the absence of prophylaxis. Prevention of endocarditis has not met these ideals because various drugs have been used against numerous organisms, and the disease rarely occurs even if prophylaxis is not given.[116] Fewer than 10 percent of all endocarditis cases can be attributed to bacteremia caused by previous medical, surgical, or dental procedures.[504] Many cases of prophylaxis failure have been reported,[120] but only 12 percent of such patients received antibiotic regimens recommended by the American Heart Association. For reasons that are not clear, mitral valve prolapse was the condition associated most frequently with failure of prophylaxis.

The most common errors in attempted prevention of endocarditis include inadequate medical histories taken by dentists and other health professionals to identify high-risk patients, initiation of prophylactic antibiotics too early, continuation of preventive therapy too long, the use of low-dose antibiotics, lack of prophylaxis for minor dental procedures, and confusion between prevention of rheumatic fever and prevention of infective endocarditis.[187,193] Several studies have shown that adult patients at risk for development of infective endocarditis often have inadequate knowledge of their cardiac lesion, endocarditis, and recommended prophylaxis.[69,261,506] One study demonstrated that the parents of children with heart defects have a low level of knowledge about the importance of good oral health in preventing endocarditis.[103] Another study cast doubt on the cost-effectiveness of endocarditis prophylaxis for urinary catheterization in children.[68]

The American Heart Association published new and radically different guidelines for the prevention of infective endocarditis in 2007.[527] These new guidelines eliminated many of the procedures for which prophylaxis previously was recommended.[94] Now, it is recommended that prophylaxis be undertaken for all dental procedures that involve manipulation of gingival tissue or the periapical region of teeth or perforation of the oral mucosa. Prophylaxis also can be considered for procedures on the respiratory tract or infected skin, skin structures, or musculoskeletal tissue. Prophylaxis no longer is recommended for gastrointestinal or genitourinary tract procedures. In addition, the number of heart lesions for which prophylaxis is recommended has been reduced to only those with the highest risk of endocarditis (Table 32–17).[527] Finally, the specific regimens for prophylaxis have been changed and simplified to encourage more judicious use (Table 32–18). Although immunization against bacteria that commonly cause endocarditis (e.g., viridans streptococci) has been proposed, this approach remains a theoretic possibility.[18]

TABLE 32–17 Heart Conditions with the Highest Risk of Adverse Outcome from Endocarditis for Which Prophylaxis with Dental Procedures Is Recommended

Prosthetic heart valve
Previous infective endocarditis
Congenital heart disease
Unrepaired cyanotic congenital heart disease, including palliative shunts and conduits
Completely repaired congenital heart defect with prosthetic material or device, whether placed by surgery or catheter, during the first 6 months after the procedure
Repaired congenital heart disease with residual defects at or adjacent to the site of a prosthetic patch or device
Cardiac transplantation recipients who develop cardiac valvulopathy

From Wilson, W., Taubert, K. A., Gewitz, M., et al.: Prevention of infective endocarditis: Guidelines from the American Heart Association. Circulation 116: *1736-1754, 2007.*

TABLE 32–18 Endocarditis Prophylaxis Regimens for a Dental Procedure

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CHAPTER 33

Infectious Pericarditis

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Purulent pericarditis generally refers to bacterial infection of the pericardium. Inflammation of the pericardium may result from numerous nonbacterial microorganisms, however, or may occur with a variety of noninfectious illnesses (Table 33–1). Regardless of the cause of pericarditis, the responses of the pericardium are limited to acute inflammation, effusion with or without tamponade, and fibrosis with or without constriction.[18] Because untreated purulent pericarditis is rapidly fatal, suspecting the disease early and approaching the diagnosis aggressively are important.

ANATOMY AND FUNCTION

The pericardium is composed of two loosely approximated layers: visceral and parietal. The visceral pericardium is composed of mesothelial tissue, which closely follows the contour of the heart and extends for a short distance beyond the atria and ventricles to the great vessels. The outer parietal pericardium is a more fibrous structure, composed of layers of collagen interlaced with elastic fibers. The pericardial sac is attached to the diaphragm below; to the sternum in front; and to the thoracic vertebrae, esophagus, and aorta posteriorly. It is surrounded by the lungs on either side and is related closely to the main bronchi and the mediastinal lymph nodes. The phrenic and vagus nerves supply a network of pain fibers to the parietal pericardium.

The dynamics of the pericardial fluid are poorly understood. The pericardial membrane is active in the transfer of water, electrolytes, and small molecules. Molecules of large molecular weight are absorbed poorly from the pericardial space because lymphatic channels are sparse, and drainage must occur primarily through the epicardial capillaries.[64]

Ainger[1] summarized the function of the pericardium as follows: prevention of overdistention of the heart, protection of the heart from infection and adhesions, maintenance of the heart within a fixed geometric position within the chest, and regulation of the interaction between the stroke volumes of the two ventricles.

BACTERIAL PERICARDITIS

POPULATION AND INCIDENCE

Although purulent pericarditis is not a common infection in pediatric patients, it is an important one to recognize because of its life-threatening nature. In an extensive early review of the literature on purulent pericarditis, half of 425 cases occurred in children younger than 13 years of age.[7] In a review of 162 reported children with pericarditis from 1950 to 1977, 67 percent of the children were 48 months old or younger.[27] From 1962 to 1974, 67 cases were recognized at St. Louis Children's Hospital (Table 33–2).[82] During this 12-year period, pericardial disease of all causes occurred in approximately 1 of every 850 hospital admissions.[82] Twelve (18%) of these children had purulent pericarditis.

TABLE 33–1 Causes of Pericarditis

Idiopathic
Benign
Recurrent
Infectious
Purulent
Bacterial—*Staphylococcus aureus*, *Haemophilus influenzae*, streptococci, *Neisseria meningitidis*, *Streptococcus pneumoniae*, anaerobes, *Francisella tularensis*, *Salmonella*, enteric bacilli, *Pseudomonas*, *Listeria*, *Neisseria gonorrhoeae*, *Actinomyces*, *Nocardia*
Tuberculosis
Fungal—*Histoplasma*, *Coccidioides*, *Aspergillus*, *Candida*, *Blastomyces*, *Cryptococcus*
Viral
Coxsackieviruses B
Other—influenza A and B, mumps, echoviruses, adenoviruses, Epstein-Barr virus, hepatitis, measles, HIV, cytomegalovirus
Other
Rickettsial—typhus, Q fever
Mycoplasmal—*Mycoplasma pneumoniae*
Parasitic—*Entamoeba histolytica*, *Echinococcus*
Spirochetal—syphilis, leptospirosis
Chlamydial—psittacosis
Protozoal—toxoplasmosis
Noninfectious
Postpericardiotomy syndrome
Kawasaki disease
Rheumatic fever
Connective tissue disorders—JRA, SLE, dermatomyositis, periarteritis nodosa
Trauma—blunt or penetrating
Metabolic—uremia, myxedema
Hypersensitivity—serum sickness, pulmonary infiltrates with eosinophilia, Stevens-Johnson syndrome, drugs (hydralazine, procainamide, chemotherapy)
Neoplasm—leukemia, metastatic
Postirradiation

HIV, human immunodeficiency virus; JRA, juvenile rheumatoid arthritis; SLE, systemic lupus erythematosus.

TABLE 33–2 Pericarditis in Children, 1962 to 1974 (St. Louis Children's Hospital)*

Etiology	No. Patients
Unknown	28
Purulent	12
Juvenile rheumatoid arthritis	9
Acute rheumatic fever	8
Uremia	5
Viral	2
Blunt chest trauma	2
Dermatomyositis	1

**Patients with postpericardiotomy pericarditis and patients with small effusions at autopsy were excluded from consideration.*

From Strauss, A. W., Santa Maria, M., and Goldring, D.: Constrictive pericarditis in children. Am. J. Dis. Child. 129:822-826, 1975. Copyright 1975, American Medical Association.

Most cases in younger children are infectious. Acute pericarditis was found in 20 children between 1987 and 1997 in a hospital in Iran.[72] The causes of pericarditis were bacterial in eight (40%), collagen vascular disease in six (30%), viral in four (20%), and secondary to mediastinal mass invasion in two (10%). In another series from Turkey, 18 children with purulent pericarditis were encountered from 1990 to 2000.[11] At the Boston Children's Hospital, fewer than 10 patients seen among more than 1700 patients in consultation by the pediatric cardiologists had pericarditis during the period July 1, 2001, to June 30, 2002.[32] Although rare, purulent pericarditis also can occur in neonates.[51] In most series, a marked male predominance has been noted.

ETIOLOGY

Primary purulent pericarditis is a rare disease; it accounted for only 7 of 50 cases of pericarditis reported by Gersony and McCracken.[33] The disease is associated most often with infection from another site, with hematogenous or direct spread to the pericardium. Feldman[27] reviewed all cases of bacterial pericarditis reported in the English language literature from 1950 to 1977. Bacteria were isolated in 146 (90%) of 162 cases. No other infection was found in 10 patients. The most common concomitant site involved was the lung, especially for *Staphylococcus aureus*, *Haemophilus influenzae*, and *Streptococcus pneumoniae*. When septic arthritis, osteomyelitis, or skin infections were found, *S. aureus* usually was the cause of pericarditis. *Neisseria meningitidis* and *H. influenzae* most often were responsible for concomitant meningitis and pericarditis.

Before the introduction of antibiotics, pneumococcal and streptococcal organisms were the most frequent causes of purulent pericarditis in children. Most cases were associated with pulmonary infections. Nearly half of patients with streptococcal pericarditis had associated postinfluenzal pneumonia. Hemolytic streptococci were isolated most often; 10 percent were nonhemolytic streptococci, and 5 percent were viridans streptococci. Kauffman and colleagues[53] reviewed 113 cases of pneumococcal pericarditis reported since 1900. Preceding pneumonia was present in 93 percent, and empyema was present in 66 percent. Pericarditis was thought to be a late event resulting from delay in administering appropriate therapy for pneumonia.

S. aureus is the organism most commonly responsible for purulent pericarditis in children.[6,27,33,45,72] Most cases are the result of hematogenous seeding of the pericardium from staphylococcal pneumonia with empyema, acute osteomyelitis, or soft tissue abscesses. Occasionally, the pericardium is infected during the course of staphylococcal endocarditis. *S. aureus* is the most frequently recovered organism when purulent pericarditis develops within 3 months after the patient has undergone open heart surgery. The clinical course of acute staphylococcal pericarditis is dominated by severe toxemia. In addition to the necrotizing infection produced by *S. aureus*, the organism may release exotoxins, which produce shock and contribute to the high mortality.

S. aureus was isolated from 73 percent of infants who died of purulent pericarditis in the series reported by Gersony and McCracken.[33] It was responsible for 50 percent of cases in children 1 to 4 years old in the review by Feldman.[27] In seven patients younger than 1 month of age, *S. aureus* was isolated from four. This finding is corroborated in literature from other countries.[19,48,72] In one of the latest series, Thebaud and colleagues[86] reported 19 children with purulent pericarditis in a children's hospital in Paris between 1979 and 1994. The mean age of the children was 3 years (range 3 months to 10 years). The organisms isolated were *S. aureus* (three cases), *H. influenzae* (four cases), group A streptococci (three cases), *S. pneumoniae* (three cases), and *N. meningitidis* (one case). Concomitant infections included pneumonia (six cases), osteomyelitis (three cases), cellulitis (one case), and sinusitis (one case). In the series from Turkey, *S. aureus* was isolated from five patients, and *S. pneumoniae* was isolated from one patient.[11] *S. aureus* pericarditis as a complication of varicella has been reported in several children.[9]

In the prevaccine era, the second most frequently encountered organism was *H. influenzae* type b.[16] It was responsible for 22 percent (35 of 163) of the cases in Feldman's review.[27] A single site of coexisting infection, the lung, was identified in 16 of the 35 cases. Meningitis as a single other site of infection was found in 5 of 35 patients, and multiple involvement was found in 7 of 35.[27] Echeverria and colleagues[24] summarized 33 cases from the literature. Pulmonary infiltrates and empyema were seen in 64 percent of patients. Nearly 85 percent had symptoms of an upper respiratory tract infection in the preceding 5 to 12 days. Because *H. influenzae* type b conjugate vaccine has been given routinely to young infants in developed countries, this organism has been eliminated as a cause of invasive infections, including pericarditis.

Pneumococcal, streptococcal, and meningococcal pericarditis have diminished in frequency since the introduction of penicillin.[7] Go and coworkers[35] summarized the 15 reported cases of pneumococcal pericarditis from 1980 to 1998. One was a child. Only four cases did not have an underlying risk factor. In a surveillance study of invasive pneumococcal infections in eight pediatric hospitals, only three cases of pericarditis have been observed in more than 2500 cases of systemic pneumococcal infection during the 6-year period of 1993 through 1999.[52]

Pericardial involvement occurs in approximately 5 percent of young adults with meningococcemia.[21,42] The clinical course generally is milder than that observed with other types of purulent pericarditis. Pericardial involvement rarely is detected at the time of hospital admission. Pericarditis became apparent by the third day in 13 of 17 patients reported by Dixon and Sanford.[21] In some patients, it did not occur until late in the course of therapy. Whether this late-onset pericardial effusion is a part of the meningococcal infection or is related to immune complexes is unclear.[21,67,77] Primary meningococcal pericarditis that occurs without clinical evidence of meningococcemia, meningitis, or any other focal infection has been reported in 16 patients, including 6 children 18 years old or younger (range, 2 to 18 years).[3] Meningococcal serotype C was identified in 11 (79 percent) of 14 cases for which the serotype was known. Cardiac tamponade developed in 88 percent of the patients. Pericarditis also has been reported in two children with W135 meningococcal infection.[4]

Occasionally, other microorganisms cause acute purulent pericarditis. Feldman[27] reported that 11 (8%) of 146 cases of pericarditis in children were caused by *Pseudomonas aeruginosa*. *P. aeruginosa* caused pericarditis in an immunocompetent adult with cystic fibrosis.[2] Pericarditis can occur with pneumonic tularemia, salmonellosis, sepsis from enteric bacilli, listeriosis, and disseminated gonococcal disease.[7] Anaerobic bacteria should be suspected when pericarditis develops in association with lung abscess, intra-abdominal infection including ruptured appendicitis,[84] or a penetrating wound. Callanan and colleagues[12] reported the rapid development of constrictive pericarditis after purulent pericarditis caused by anaerobic streptococcal infection. The child had a history of blunt trauma to the chest with no evidence of a penetrating wound 3 weeks before cardiac tamponade developed. The incidence of anaerobic infection may be underestimated because of improper handling of specimens for culture.[27] Prolonged symptoms related to pericarditis can be associated with *Mycoplasma pneumoniae* infection.[25]

Mycobacterium tuberculosis, previously a common cause of acute pericarditis in the United States,[6] now is responsible more often for chronic pericardial disease. This infection is a complication of miliary tuberculosis and rarely a primary infection. In the series of 2500 children with tuberculosis reported by Lincoln and Savell,[59] pericarditis was diagnosed in 0.4 percent and found at necropsy in 5 percent of patients. A review of 100 cases of tuberculous pericarditis in South African blacks by Desai[20] revealed a marked male predominance (72%). The duration of symptoms, consisting of cough and peripheral edema, in most patients was 0 to 120 days. Most patients were febrile and had congestive heart failure. Generalized lymphadenopathy occurred in nearly 30 percent of patients, pulsus paradoxus occurred in 50 percent, and a friction rub was audible in 25 percent. Of the 52 patients who had pericardiocentesis, 40 percent yielded fluid, but none was positive for acid-fast bacilli. Pericardial effusion was shown in 82 patients, 16 of whom died of tamponade, and another 16 of whom developed constricting pericarditis.

The four stages of tuberculous pericarditis have been described as dry, effusive, absorptive, and constrictive.[69] Granulomata usually are found in the dry stage and heal with no sequelae. The effusive stage occurs commonly with tuberculous lymphadenitis, and 15 to 200 mL of fluid usually accumulates in the pericardial space. The absorptive stage is characterized by thickening of the pericardium with fibrin deposition. Further fibrin deposition and calcification occur during the constrictive phase. The disease may progress through all stages or remain in one stage.

Latent infection in the mediastinal lymph nodes with spread directly into the pericardium is thought to be the mode of involvement with *M. tuberculosis*.[69] The lymph nodes at the tracheal bifurcation often are the source.

Histoplasma pericarditis generally occurs with pulmonary, rather than disseminated, disease.[71] Coccidioidomycosis[15] and, rarely, blastomycosis[41] also may cause pericardial disease. Other pathogenic fungi include *Aspergillus* and *Candida*. These fungi are more serious considerations in patients who are immunosuppressed, have serious burns, or are receiving long-term, broad-spectrum antibiotics after undergoing cardiac surgery.[74]

PATHOLOGY AND PATHOGENESIS

Pericarditis begins with fine deposits of fibrin adjacent to the great vessels; it causes the pericardial membrane to lose its smoothness and translucency. Numerous granulocytes may extend into the myocardium.[37]

Bacterial pericarditis most commonly results from direct extension of infection from involved lung and pleura. Pulmonary infections may spread to the pericardium through the bronchial circulation.[40] Pericarditis also can develop through hematogenous dissemination from infection elsewhere. Pericarditis also may be the result of an immunologically induced response to a primary infection.

As pericardial fluid accumulates, intrapericardial pressure increases. The rate of increase is a function of the speed of accumulation and the compliance of the pericardium. With slow accumulation of fluid, large volumes can be accommodated because of the gradual expansion of the parietal pericardium. As the compliance of the pericardium reaches its maximum, however, further accumulation of even small volumes of fluid results in an abrupt increase in intrapericardial pressure. If pericardial fluid accumulates at a rapid rate, marked elevation in intrapericardial pressure may occur with much smaller volumes of fluid. In a small child, 100 mL can cause severe tamponade, whereas 3 L may accumulate slowly in an older child and not result in tamponade.[1]

The most significant hemodynamic effect of pericardial effusion is restriction of ventricular filling. Ventricular end-diastolic, atrial, and venous pressures increase on the right and left sides of the heart equally. When restriction of ventricular filling becomes more pronounced, the ventricular stroke volume and cardiac output decrease. In an attempt to maintain cardiac output, tachycardia and peripheral vasoconstriction occur. Systemic arterial blood pressure and pulse pressure are reduced markedly. Tamponade occurs when these compensatory mechanisms fail to maintain adequate cardiac output.

CLINICAL MANIFESTATIONS

A diagnosis of purulent pericarditis should be suspected in any patient with septicemia who develops cardiomegaly. The classic signs and symptoms of pericarditis are precordial pain, pericardial friction rub, evidence of cardiac fluid, and muffled heart sounds.[14] Chest pain is not a common symptom, especially in small children; the reported rates vary from 15 to 80 percent.[5,7,33,45,64,68,87] Acute abdominal symptoms may be the presenting complaints of some children.[22]

The most common symptoms and signs of pericarditis are fever, tachypnea, and tachycardia. They also are presenting features of associated systemic infection. If the cardiac shadow is radiographically enlarged, with or without a friction rub, and the tachypnea and tachycardia are out of proportion to the fever, myocardial dysfunction or pericarditis should be suspected.

An evanescent or ubiquitous rub may be detected. The typical sound of a rub is that of a high-frequency murmur,[70] which may have a to-and-fro or triphasic pattern but may not have any correlation with the cardiac cycle.[28] Frequently, the rub is heard better with the patient leaning forward or kneeling.[28] A rub may be differentiated from a murmur by pressing the diaphragm of the stethoscope firmly against the chest wall; this pressure amplifies the rub, and the typical scratchy quality becomes more apparent as the examiner opposes the visceral and parietal pericardium by compression of the chest. Rubs have been known to increase with inspiration.[79] Although a rub is less likely to be heard in the presence of a large effusion, it still may exist.[28] The heart sounds usually are muffled, and the palpable ventricular impulse generally is diminished. Both findings may be present in congestive heart failure, but they may be absent with tamponade.

Cardiac tamponade may be an early complication of pericarditis associated with a systemic infection. Cardiac tamponade means that there is compression of the heart by a tense pericardial sac, usually full of fluid, resulting in a decrease in venous return to the cardiac chambers and a decrease in cardiac output. During inspiration, the intrathoracic pressure decreases, and venous return to the cavae increases. The tense pericardial sac limits the amount of blood that can enter the right atrium because of diastolic compression; a paradoxical increase in jugular venous pressure occurs during inspiration (i.e., Kussmaul sign) (Fig. 33–1).[54]

During inspiration, a small decrease in systolic blood pressure and cardiac output normally occurs and is caused by an increase in pulmonary venous capacitance. It is exaggerated with pericardial tamponade (>10 mm Hg decrease in blood pressure) because of the restricted inflow into the cardiac chambers. This clinical sign has been called *paradoxical pulse*, but it actually is an exaggeration of the normal respiratory cycle (Fig. 33–2).[36]

DIAGNOSIS

The radiographic appearance of a rapidly increasing cardiothoracic ratio without increasing pulmonary vascular markings is more suggestive of pericardial effusion than of congestive heart failure caused by myocardial dysfunction (Fig. 33–3). Fluoroscopy alone generally is of little value; myocardial dysfunction and pericarditis can impair cardiac contractility.

The size of the pericardial shadow does not indicate the severity of hemodynamic effects. It is a function of the rapidity of accumulation and the volume of pericardial fluid. When acute infection results in sudden cardiac tamponade, the heart size may be normal. A large, globular heart shadow with no evidence of increased pulmonary vasculature, particularly in a patient who has signs of right-sided heart failure, is strong evidence for pericardial disease. The lack of pulmonary overcirculation helps to distinguish this condition from myocarditis; however, determining whether pulmonary infiltrates also exist may be difficult.

A plain lateral chest radiograph may show findings consistent with a pericardial effusion.[56] Separation of more than 2 mm between the anterior mediastinal and subepithelial "fat stripes" suggests an effusion. Obliteration of the retrosternal space without evidence of thymic or right ventricular enlargement also indicates pericarditis.

The extent of electrocardiographic abnormalities may be explained by the amount of pericardial effusion and the presence of superficial myocardial injury or myocarditis. Pericardial effusion gives rise to low-voltage QRS complexes as a result of the damping effect of pericardial fluid between the chest wall and the myocardium. Accumulation of fluid and fibrin under pressure also may produce an injury pattern manifested by ST-segment deviation. More than 90 percent of patients have elevation of the ST segment, which occurs most frequently in leads I, II, V_5, and V_6. Widespread T-wave inversion indicative of epicarditis may be seen in the same leads in which ST-segment elevation occurs.

Spodick[80] described four stages of electrocardiographic changes in acute pericarditis. In stage I, ST-segment elevation is pronounced, and the PR segment may be depressed. In stage II, the ST segment begins to return to the isoelectric line, the amplitude of the T wave diminishes, and the PR segment is depressed. By stage III, the ST segment has returned to the isoelectric line, and the T-wave inversion occurs. An incompletely inverted T

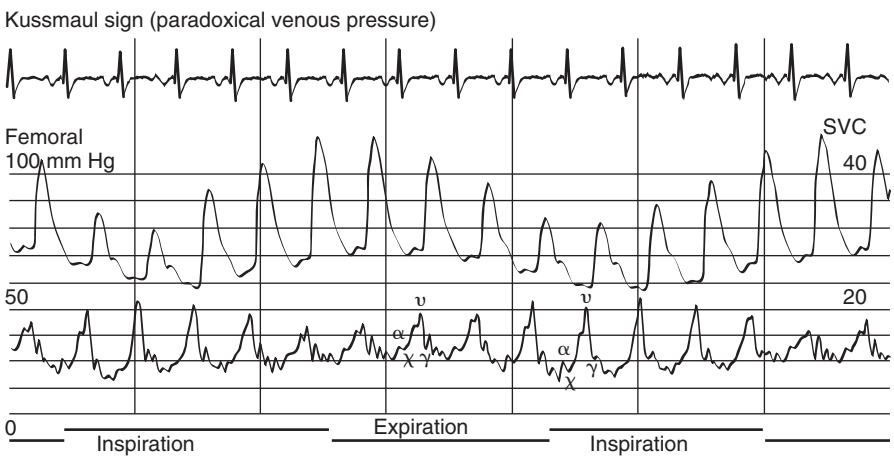

Figure 33–1 Simultaneous recording of right atrial and femoral artery pressures. Notice the increased V wave and exaggerated decrease in the femoral artery pulse with inspiration.

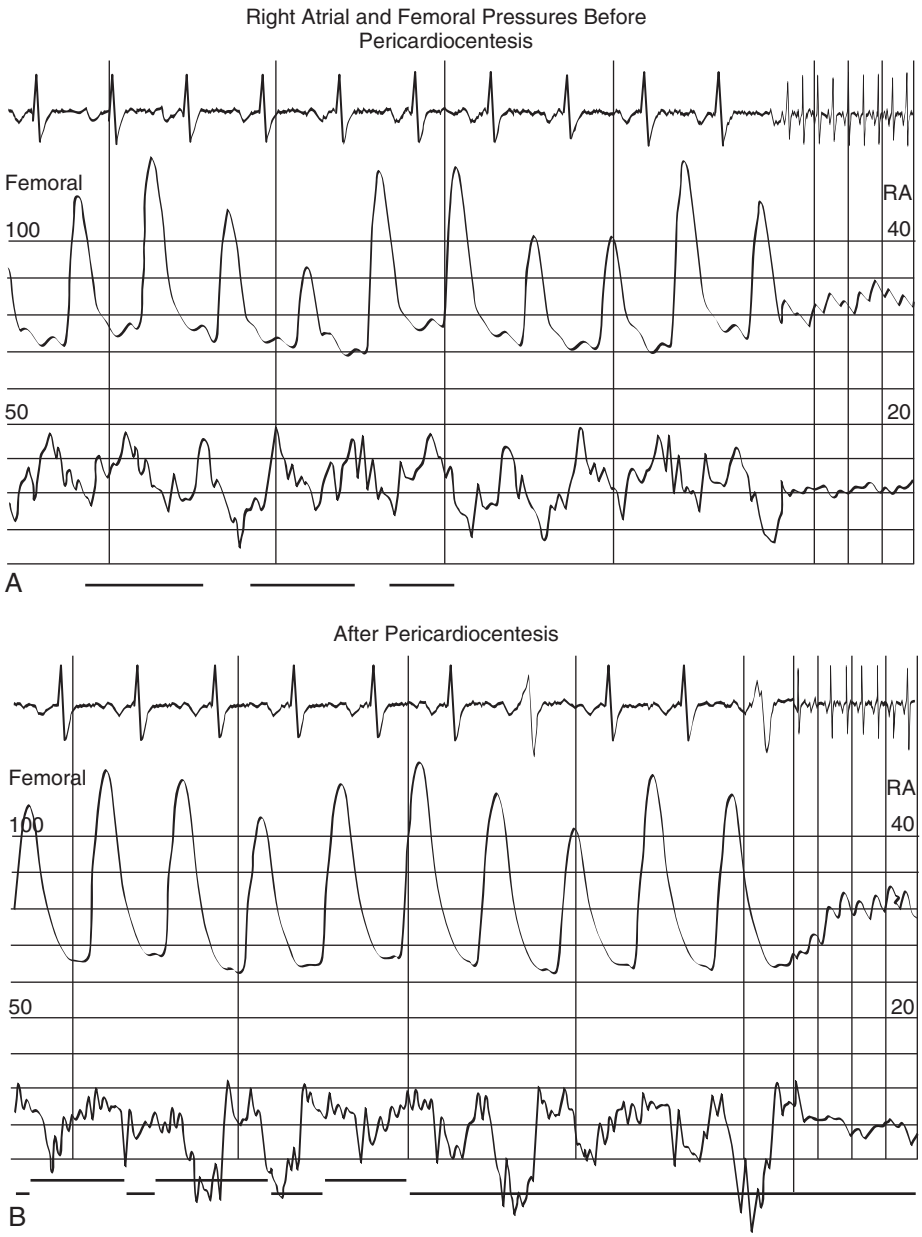

Figure 33–2 **A** and **B,** Recordings of femoral artery and right atrial pressures before **(A)** and after **(B)** pericardiocentesis. **A,** There is an exaggerated decrease in the fall of femoral artery pressure with inspiration and a sustained increase in right atrial pressure. **B,** The recording shows a more normal variation of femoral pressure and a lower right atrial pressure.

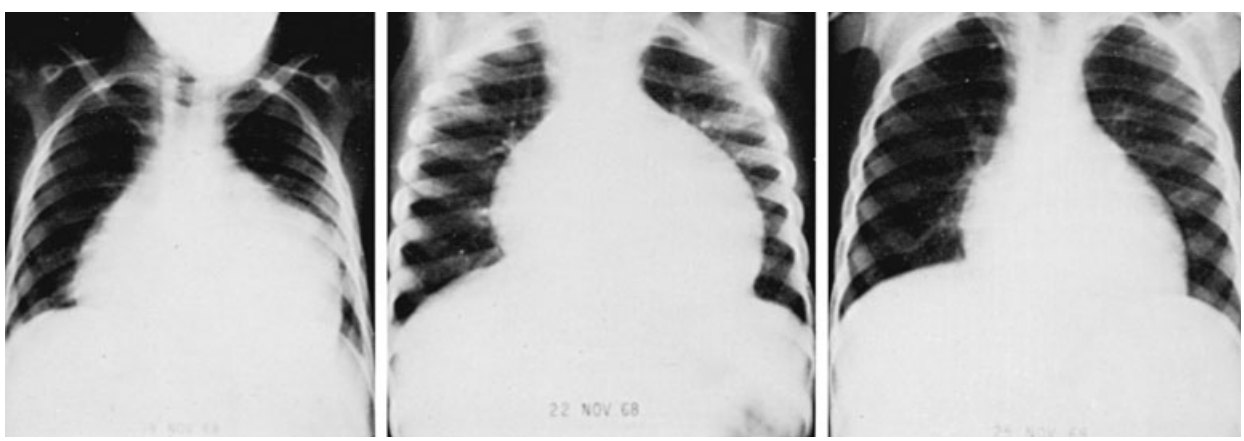

Figure 33–3 In a patient with pericarditis, the first two radiographs show an enlarged cardiac shadow without an increase in pulmonary vascular markings. The third radiograph shows a marked decrease in apparent heart size after pericardiocentesis.

wave (i.e., a diphasic wave or an upright T wave with a notched summit) sometimes is observed. In stage IV, these changes may resolve completely. T-wave abnormalities may persist for life, however, and do not indicate active disease.

Ginzton and Laks[34] compared the electrocardiograms of 19 patients with acute pericarditis with those of 20 healthy patients. By forming a ratio of the amplitude of the ST-segment and T-wave height in all patients, a value of 0.25 or greater in lead V_6 had a positive and negative predictive value of 1.0 for determining the presence of pericarditis. This finding also was true in leads I, V_4, and V_5, although the predictive values were not as high as in lead V_6. Using Spodick's criteria for their patients, Ginzton and Laks[34] were unable to distinguish healthy, normal individuals from patients with acute pericarditis. Their method may prove to be more reliable, although a large study enrolling children has not been performed.

Electrical alternans is seen in a large pericardial effusion. Electrical alternans refers to the alternation in electric amplitude of the T wave and the QRS complex with each cardiac cycle. It is thought to result from the rotational and pendular motion of the heart suspended in pericardial fluid.

Deviations from classic patterns occasionally occur, and single electrocardiographic changes are common findings. All 12 children reported by Okoroma and colleagues[68] had ST-segment elevation, whereas only 3 had concomitant low voltage.

Although many textbooks cite the frequent occurrence of dysrhythmias with pericarditis, these occurrences are unusual in the absence of coexisting heart disease. In one study, 20 of 49 patients with acute pericarditis had no underlying heart disease.[81] Seven patients had no dysrhythmias documented on 24-hour Holter monitoring, and 10 of 20 had infrequent single ectopic beats. Only three patients had supraventricular tachycardia.

M-mode echocardiography is the most sensitive method for diagnosing significant pericardial effusion (Fig. 33–4).[39,46] With a small to moderate effusion, only a "fluid space" is seen posteriorly (see Fig. 33–4B). With a larger effusion, fluid is seen anteriorly and posteriorly, and the septal motion becomes grossly abnormal. The heart may give the appearance of freely swinging (see Fig. 33–4A). Newer echocardiographic techniques, such as two-dimensional sector scanning, are not more useful than the conventional M-mode.

Friedland and colleages[30] prospectively performed transthoracic two-dimensional echocardiography in 36 children with staphylococcal bacteremia; 89 percent had at least one suspected focus of infection, and 19 had community-acquired infections. Vegetations were detected in four children without clinical manifestations of endocarditis. Two other children, including one without suggestive clinical features, had significant pericarditis detected. The researchers concluded that echocardiography should be considered for children with bacteremia caused by *S. aureus*, even when an obvious noncardiac source of infection exists.

Computed tomography and magnetic resonance imaging of the chest are other modalities used to examine the pericardium.[8] They may help to differentiate a bacterial pericarditis from other conditions involving the pericardium. Occult or unsuspected pericarditis has been discerned with radionuclide techniques in immunocompromised patients and in trauma patients.[38,76]

A pericardial effusion may be diagnosed by noticing a discrepancy between the position of a catheter placed adjacent to the lateral wall of the right atrium and the right cardiac border. An injection of radiopaque contrast material into the right atrium may delineate these findings further. Pressure measurements at the time of cardiac catheterization reveal the elevated right atrial pressure and emphasize further the exaggeration of venous, systemic, and left ventricular pressures imposed by inspiration (see Fig. 33–2). Injection of carbon dioxide or air into the pericardium percutaneously may delineate further the pericardial effusion fluoroscopically and differentiate freely moving fluid from loculated areas (Fig. 33–5).

The diagnosis of purulent pericarditis is established definitively only by direct examination of pericardial fluid. Purulent fluid is characterized by a predominance of polymorphonuclear leukocytes; however, it also may occur early in the course of viral and tuberculous pericarditis. Proper handling of pericardial fluid is crucial to recovery and identification of the etiologic agent, as follows:

1. Fluid should be placed directly into broth capable of supporting aerobic and anaerobic microorganisms. The fluid should be plated directly onto agar media, such as blood agar, chocolate agar, or MacConkey agar.
2. Cultures also should be submitted for identification of *M. tuberculosis*, fungi, and viruses.
3. Several slides should be prepared for immediate examination by Gram stain and stain for acid-fast bacilli. Unstained slides should be stored in case of controversy or the need for special histochemical stains.
4. Antigen detection for *S. pneumoniae* or polymerase chain reaction for other microorganisms may be useful in selected

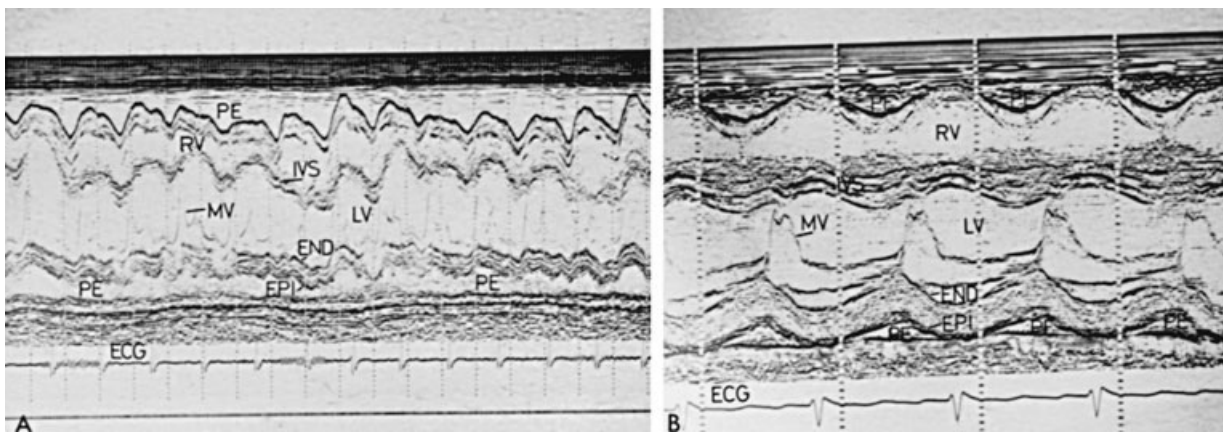

Figure 33–4 **A** and **B**, Serial echocardiograms of a child before pericardiocentesis **(A)** and after pericardiocentesis **(B)**. **A,** Note the large effusion anteriorly and posteriorly with the "swinging" movement of the septum and anterior and posterior walls. **B,** The heart movement is normal, and there remains only a small effusion anteriorly and posteriorly. ECG, electrocardiogram; END, endocardium; EPI, epicardium; IVS, interventricular septum; LV, left ventricle; MV, mitral valve; PE, pericardial effusion; RV, right ventricle.

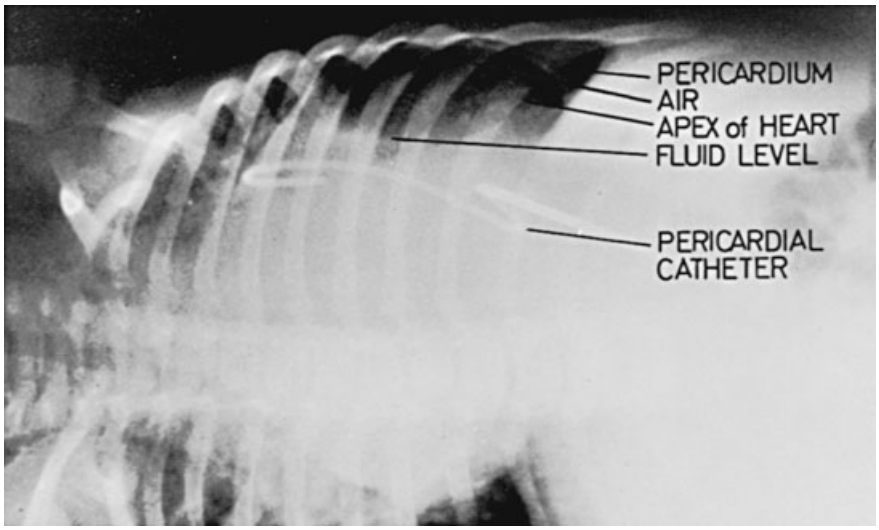

Figure 33–5 Chest radiograph of a patient lying on the right side with a catheter in the pericardium. Air has been injected through the catheter, outlining the pericardium and fluid within the sac.

cases, particularly when the patient has received prior antimicrobial therapy.[58]

The causative microorganism is isolated from blood cultures in many patients. When indicated, cerebrospinal fluid also should be cultured. Because purulent pericarditis often occurs after infections of the lung or pleural space, thoracentesis can reveal the etiologic agent in many cases. Documentation of empyema together with evidence of pericardial disease correlates highly with purulent pericarditis.

Acid-fast bacilli are seen on stained smears of pericardial fluid from 15 to 42 percent of patients with tuberculous pericarditis.[6] Examination of pericardial biopsy specimens by routine methods and with special stains such as the auramine O stain can increase the frequency of identification of *M. tuberculosis*.[66] A negative purified protein derivative skin test does not exclude the diagnosis of tuberculous pericarditis.

Grossly bloody pericardial fluid is observed frequently in patients with *Histoplasma* pericarditis, and an aspirate of the effusion reveals a predominance of mononuclear leukocytes. Growth of *Histoplasma capsulatum* from pericardial fluid rarely is successful. Showing the typical intracellular yeast forms on special stain of pericardial tissue also is helpful. Elevation of the yeast phase of the complement-fixation titer in pericardial fluid allows one to make a more rapid diagnosis.[71] Serum precipitin antibodies to *H. capsulatum* also indicate acute histoplasmosis and are helpful when present. Detecting the polysaccharide of *H. capsulatum* in urine or other body fluids is a rapid and sensitive means by which to establish the diagnosis of histoplasmosis.

DIFFERENTIAL DIAGNOSIS

Any patient with a rapidly increasing heart size in the absence of increasing pulmonary vascular markings should be suspected to have a pericardial effusion. Purulent pericarditis must be differentiated from pericardial effusion caused by collagen diseases, other infectious agents (e.g., viral, tuberculous, rickettsial, protozoan), neoplastic disorders, metabolic disorders, and congestive heart failure.[14] Glycogen storage disease, congenital heart disease, primary myocardial disease, cardiac tumors, and coronary artery aberrations (i.e., anomalous origin from the pulmonary artery, medial wall necrosis, and Kawasaki disease) may be confused with pericardial effusion.[14] Appropriate analysis of pericardial fluid usually permits differentiation of purulent pericarditis from pericarditis caused by other disorders.

TREATMENT

Purulent pericarditis is a potentially life-threatening illness that requires pericardial decompression and open drainage, appropriate antimicrobial therapy, and intense supportive therapy. Ainger[1] stated that more than half of children with purulent pericarditis require early or emergency drainage of the pericardium for relief of critical tamponade. Although bedside needle pericardiocentesis may be lifesaving or necessary for establishing a rapid diagnosis, Fowler and Manitasas[28] reported three deaths related to pericardiocentesis performed by inexperienced physicians. Complications include arrhythmias resulting from myocardial injury, laceration of the coronary arteries leading to hemopericardium and tamponade, and pneumothorax. Ledbetter[57] described a 10-year-old girl with staphylococcal pericarditis who developed an aortic aneurysm after undergoing multiple pericardiocentesis procedures for recurrent tamponade. The subxiphoid approach is recommended, and Hoffman and Stanger[44] have described the proper technique.

Decompression and drainage of the pericardium are safest in a controlled environment, such as in an operating room or under fluoroscopy in the catheterization laboratory. If the patient is awake and agitated, premedication may be required.

If pericardiocentesis does not relieve symptoms successfully, and evidence of tamponade continues, immediate surgical drainage is necessary. Multiple attempts may prove unsuccessful and can lead to serious complications. The pus surrounding the heart may be too thick to be aspirated, as has been seen especially with *H. influenzae* infection.[62] Surgical creation of a pericardial window with a drain occasionally is necessary for complete removal of fluid, which accumulates rapidly. In preparation for evacuation of the pericardial fluid during tamponade, adequate cardiac output can be maintained by stimulating the heart with pharmacologic agents that cause a chronotropic and an inotropic effect. Isoproterenol administered intravenously at a rate of 0.05 to 0.10 μg/kg/min is our drug of choice. It does not replace evacuating the fluid, but it gains time until the aspiration or drainage can be done.

Medications that tend to decrease heart rate and intravascular volume are contraindicated because they compromise the patient further. Wyler and colleagues[89] warn against the use of halothane anesthesia because of its known depressant effect on myocardial function. They described two patients who had reversible cardiac arrest when this agent was used during surgery to relieve tamponade.

Controversy exists regarding the approach and extent of surgery.[26,28,29,55,63,75] A left anterolateral thoracotomy through the fifth intercostal space or a subxiphoid approach with removal of the xiphoid process seems best. Most surgeons favor the creation of a pericardial "window"; however, some favor more extensive removal of pericardial tissue. This decision may be influenced by the severity of pericardial inflammation or the presence of bloody pericardial fluid because these conditions have greater potential for producing acute or chronic constriction. Care must be taken during the procedure not to injure the phrenic nerves.

Morgan and colleagues[62] reviewed 15 children with purulent pericarditis between 1971 and 1981. *H. influenzae* occurred in 7 of 15 patients. Most patients had pericardiocentesis followed by an anterior interphrenic pericardiectomy and recovered completely. In a series of nine children with *H. influenzae* pericarditis, all received a limited left thoracotomy with subxiphoid approach for pericardiostomy, and no deaths occurred.[16] Video-assisted thoracoscopic approaches to managing pericarditis have been described, but further experience is necessary for pediatric patients.[60] For selected patients for whom surgery cannot be performed in a timely manner, the instillation of intrapericardial streptokinase or urokinase or other thrombolytic agents has been successful in draining purulent pericarditis and preventing the need for a more extensive surgical procedure.[50]

Antimicrobial therapy alone is insufficient for the successful treatment of purulent pericarditis. The survival of patients with purulent pericarditis is improved significantly when early pericardial drainage is performed (Table 33–3). In the preantibiotic era, draining the pericardium decreased the mortality rate from nearly 100 percent to 45 percent.[7] Occasionally, patients with meningococcal pericarditis have been managed successfully without pericardial drainage.[18] Fyfe and colleagues[31] described 73 of 79 patients with *H. influenzae* pericarditis seen between 1928 and 1984. The mortality rate before 1960 was 64 percent (7 of 11 patients), although five of seven deaths were reported before the antibiotic era. From 1960 to 1969, the mortality rate was 36 percent, and from 1970 to 1979, it decreased to 11.5 percent. From 1980 to 1984, 25 cases with no mortality were reported.

When the etiologic agent cannot be detected rapidly, the initial antibiotic regimen should consist of two or more drugs. Because *S. aureus* is a major pathogen, a penicillinase-resistant penicillin, such as nafcillin or oxacillin, must be included in a dose of 200 mg/kg/24 hr (maximum 12 g). In regions where infections caused by methicillin-resistant *S. aureus* have occurred in the community, when strains of *S. pneumoniae* resistant to the extended-spectrum cephalosporins are present, or when the infection is nosocomially acquired, vancomycin should be given, 40 to 60 mg/kg/day in four divided doses (maximum 4 g). Cefotaxime (200 to 300 mg/kg/day in three or four divided doses) or ceftriaxone (100 mg/kg/day in one or two doses) should be administered to provide protection against *S. pneumoniae* (including penicillin-resistant strains), *N. meningitidis*, and *H. influenzae* type b for children who may be inadequately immunized.

An aminoglycoside antibiotic should be added to the above-mentioned combined drug therapy when purulent pericarditis occurs after cardiac surgery, in association with genitourinary infections, or in the immunocompromised host. For patients who are allergic to penicillin, vancomycin, clindamycin, or cefazolin is substituted for the treatment of susceptible *S. aureus*; some patients who are allergic to penicillin are sensitive to cephalosporins.

The duration of therapy is empiric and is determined partly by the nature of concomitant infection. Generally, after a pathogen is isolated and the antimicrobial susceptibilities are known, the most specific antimicrobial agent is continued intravenously for 3 to 4 weeks.

Using chemotherapy to treat tuberculous pericarditis has had a major impact on mortality. Before its use, the mortality rate in the acute phase was 80 to 90 percent. The other 10 to 20 percent of patients died of constrictive pericarditis or miliary tuberculosis.[69] The use of three or four drugs, including isoniazid, pyrazinamide, rifampin, and possibly streptomycin, for 9 to 12 months is recommended. The role of corticosteroids in preventing progression to constriction or decreasing mortality is unclear.[23,61,69] In selected cases, pericardiectomy may be indicated to prevent constrictive pericarditis.

Amphotericin B, alone or with other systemic agents, is indicated for treatment of fungal pericarditis. It rarely is required for successful therapy of *Histoplasma* pericarditis, for which nonsteroidal anti-inflammatory drugs are recommended for 2 to 12 weeks, depending on the clinical resolution of symptoms and physical findings of pericarditis.[88] Steroids may be tried for 1 to 2 weeks. As with bacterial pericarditis, open pericardiectomy is crucial for the successful treatment of *Candida* pericarditis.[74]

General supportive therapy in the acute stage of infection may include the administration of oxygen, volume expansion to increase ventricular filling pressure, and isoproterenol to facilitate systolic emptying. Digitalis and diuretics should be used cautiously and only when indicated by decreased myocardial function. Serial electrocardiograms may indicate the presence of occult arrhythmias and alert the physician to the degree of myocardial involvement. The patient must be monitored carefully for signs of reaccumulation of pericardial fluid and for the development of acute constrictive pericarditis. Strauss and colleagues[82] reported this complication in 2 of 12 children with purulent pericarditis. Acute constriction may develop within weeks of the initial pericardial infection[5,10] and has been reported at 8 days.[73] Constrictive pericarditis may be suspected by increasing jugular and central venous pressure, weight gain, enlarging liver, worsening dyspnea, and decreased urinary output. The persistence of heart failure when the cardiac silhouette is becoming smaller also suggests the development of constrictive pericarditis. Complete pericardiectomy should be performed promptly when constriction is suspected.

TABLE 33–3 Influence of Pericardial Drainage on Survival in Purulent Pericarditis in Children

Treatment	Survived	Died
Antibiotics alone	5	28
Antibiotics and pericardial drainage	45	10

Data from references 5, 29, 53, and 65.

PROGNOSIS

The current mortality rate for acute purulent pericarditis ranges from 25 to 75 percent. Accurate statistics are difficult to compute from the literature because the nature and severity of underlying disease have not been considered. Factors that contribute to mortality are delay in recognition, absence of early surgical drainage, presence of cardiac tamponade, degree of myocardial involvement, etiologic agent (particularly *S. aureus*), and age of the patient. Long-term follow-up of children with purulent pericarditis is recommended. They should be followed carefully for the presence of a constrictive component as a sequela to the acute infection. Most children recover fully, however, and return to normal activity.

VIRAL PERICARDITIS

In 1951, Christian[17] suggested that viral infections were responsible for cases of idiopathic or benign pericarditis. A viral cause has not been substantiated in many patients, however.

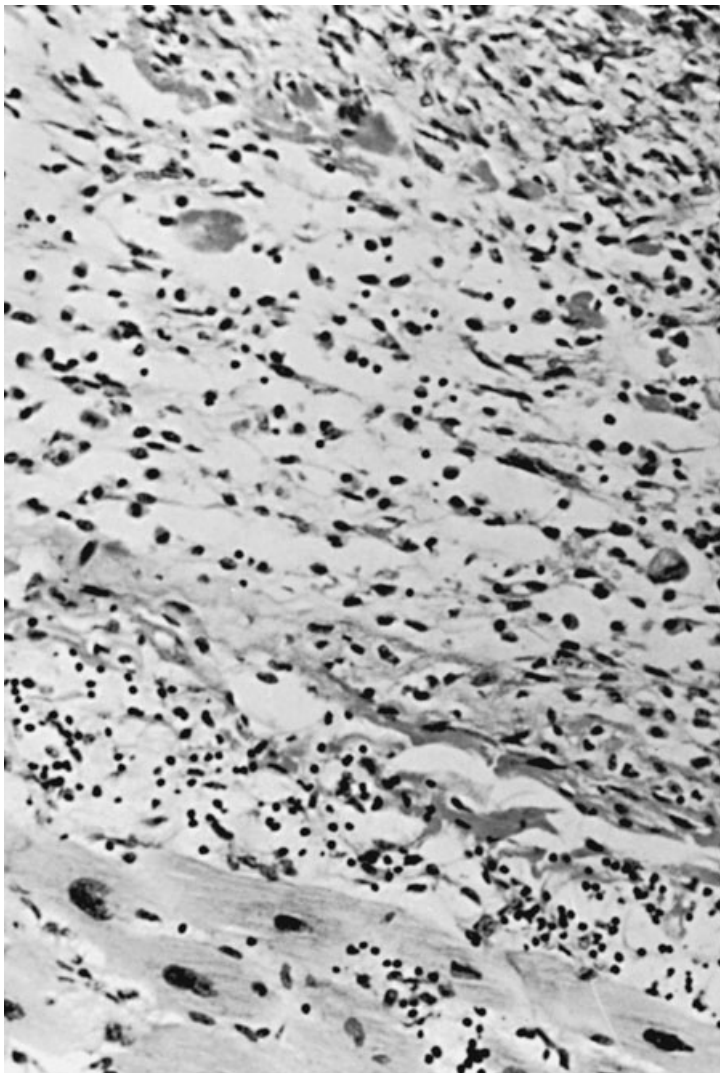

Figure 33–6 In this case of viral pericarditis, there is a layer of fibrin and fibroblasts along the pericardial surface. The mononuclear cell infiltrate in the epicardium extends into the outer myocardium (hematoxylin and eosin staining, ×400). *(Courtesy of Edith P. Hawkins, M.D., Texas Children's Hospital, Houston.)*

ETIOLOGY

The principal viruses implicated in pericarditis are the coxsackieviruses.[65] Adenoviruses have been recovered less frequently.[49] Associations with varicella,[85] cytomegalovirus,[13] smallpox vaccinations, influenza,[43] influenza vaccinations,[83] and infectious mononucleosis[47,78] have been reported.

CLINICAL MANIFESTATIONS

In approximately 40 to 75 percent of cases, the patient has a history of upper respiratory tract infection for 10 days to 2 weeks preceding the onset of symptoms. Fever and chest and abdominal pain are the most common symptoms.[17] A friction rub may be heard in 50 to 80 percent of cases.[87] Children with viral pericarditis generally are less toxic and experience smaller elevations in body temperature than do children with purulent pericarditis. Some appear acutely ill, however. Large amounts of pericardial fluid accumulation and tamponade are rare findings.

INVESTIGATIVE TECHNIQUES

The electrocardiographic, radiographic, echocardiographic, and nuclear scanning findings described for patients with purulent pericarditis also are observed in patients with viral pericarditis. The peripheral leukocyte count may reveal fewer polymorphonuclear leukocytes than in patients with bacterial pericarditis. Mononuclear cell infiltrates in the pericardium with extension into the myocardium may be seen (Fig. 33–6).

If obtained, pericardial fluid should be sent for cell count and viral culture. Nasopharyngeal and rectal samples also should be obtained and cultured for viruses. Acute and convalescent sera should be obtained so that appropriate titers can be measured if a virus is isolated.

COURSE AND PROGNOSIS

Viral pericarditis generally resolves spontaneously over the course of 3 to 4 weeks.[65] Large pericardial effusions and tamponade are rare occurrences.[14] Generally, bed rest for approximately 1 week and analgesics for pain are the only therapy that is required. Constrictive pericarditis is a rare occurrence, but pericarditis may recur.[65]

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CHAPTER 34

MYOCARDITIS

Jesus G. Vallejo

Myocarditis is defined clinically and pathologically as inflammation of the myocardium. The clinical presentation and cause may be quite varied. This entity may go unrecognized in numerous patients whose illness may resolve spontaneously, or it may lead to significant morbidity and mortality.

In the early part of the 20th century, most cases of myocarditis were classified as idiopathic, and a diffuse or focal interstitial inflammation was identified on histologic examination. Rheumatic fever, diphtheria, and other bacterial infections were the only diseases recognized as associated with myocarditis, although some experts suspected that viruses might play a significant etiologic role in many cases.[157] After the discovery in 1947 of the coxsackievirus group and the subsequent isolation and identification of other viruses, the number of cases of myocarditis classified as idiopathic diminished rapidly.[35]

EPIDEMIOLOGY

The diverse clinical manifestations have made the true incidence of myocarditis difficult to determine. The clinical course of acute myocarditis can be insidious, with limited inflammation and cardiac dysfunction, or it can be overwhelming, leading to severe cardiac injury and cardiac failure. As a clinical entity, myocarditis is an uncommon occurrence in children. At Texas Children's Hospital, Houston, Texas, between 1954 and 1977, myocarditis represented 0.3 percent of the 14,322 patients seen by the Cardiology Service.

Because not all cases of myocarditis are recognized clinically, a much higher incidence is recorded in autopsy series. An autopsy incidence of 1.15 percent was found from 4343 studies performed between 1954 and 1977 at Texas Children's Hospital. This rate is considerably lower than the incidence of 6.83 percent reported by Saphir and Simon[157] in 1944 for 1420 autopsies performed on children. In Saphir's series, 32 of 97 cases had or probably had rheumatic carditis,[156] whereas only 2 cases occurred in the Texas Children's Hospital series. The discrepancy is even more pronounced when these observations are compared with those of Burch and colleagues,[26] who showed evidence of interstitial myocarditis in the hearts of 29 of 50 infants and young children undergoing routine postmortem studies.

Some of the discrepancies between the clinical and autopsy series may be explained by the fact that the manifestations of myocarditis are subclinical in many cases and may be recognized only by changes on electrocardiogram (ECG) or perhaps not at all. In many instances, myocarditis is only one component of a generalized illness, and the cardiac dysfunction, if mild, may be overlooked.

ETIOLOGIES

Myocarditis may occur with many common infectious illnesses that affect infants and children (Table 34–1). In most cases of myocarditis, the etiologic agent is never identified, however. In the United States and Western Europe, viruses are the most common causes of acute myocarditis. Myocarditis generally is a sporadic disease, but epidemics have been reported. Most epidemics have been caused by coxsackievirus group B and have affected infants in the newborn period.[46,52] Gear and Measroch[62] were the first to identify coxsackievirus B in association with myocarditis after an epidemic occurred in a nursery in a maternity home in southern Rhodesia.

The association between virus infection and the development of myocardial disease also was made by Grist and Bell,[70] who presented comprehensive serologic data correlating enterovirus infection with acute viral myocarditis. In the World Health Organization record during the 10-year period from 1975 through 1985, the coxsackieviruses B represented the most frequent inflammatory agents in cardiovascular disease (34.6/1000), followed by influenza B virus (17.4/1000), influenza A virus (11.7/1000), coxsackievirus A (9.1 per 1000), and cytomegalovirus (CMV) (8/1000).[27] Karjalainen and colleagues[90] prospectively examined 104 conscripts during the 1978 influenza A virus (H1N1) epidemic in Sweden. The incidence of myocarditis was 9 percent of the 67 verified cases of influenza virus infection.

The development of molecular techniques such as polymerase chain reaction (PCR) has improved the testing of endomyocardial biopsy specimens for potential viral pathogens. A study using PCR identified viral genome in 38 percent of endomyocardial biopsy specimens from patients diagnosed with acute myocarditis.[21] Of the positive PCR samples, 23 percent were positive for adenovirus, 14 percent for enterovirus, and 3 percent for CMV. Parvovirus B19, influenza A virus, Epstein-Barr virus, herpes simplex virus (HSV), and respiratory syncytial virus were detected in less than 1 percent of cases.

Investigators also have speculated for decades on the possibility that acute myocarditis is a common forerunner of idiopathic dilated cardiomyopathy (DCM). Evidence supporting this hypothesis was presented first by Orinius and Pernow,[136] who found cardiac disease in humans years after an apparent uncomplicated coxsackievirus infection. Subsequently, Bowles and colleagues,[22] using a slot-blot hybridization technique, provided conclusive evidence for the presence of enterovirus in endomyocardial biopsy samples from patients with DCM. In another study, Bowles and associates[21] detected viral genomes in 20 percent of 149 patients with the diagnosis of DCM. In these

TABLE 34–1 Causes of Myocarditis

Viruses	Coxsackieviruses A and B
	Echoviruses
	Polioviruses
	Rubella
	Measles
	Adenoviruses
	Vaccinia
	Mumps
	Herpes simplex
	Epstein-Barr
	Cytomegalovirus
	Rhinoviruses
	Hepatitis viruses
	Arboviruses
	Influenza viruses
	Varicella
Rickettsia	*Rickettsia rickettsii*
	Rickettsia tsutsugamushi
Bacteria	*Meningococcus*
	Klebsiella
	Leptospira
	Staphylococcus
	Treponema pallidum
	Haemophilus influenzae
	Hemolytic streptococci
	Mycobacterium tuberculosis
	Salmonella enterica subsp. *enterica* serovar Typhi (typhoid)
Mycoplasma	*Mycoplasma pneumoniae*
	Chlamydia psittaci
Protozoa	*Trypanosoma cruzi*
	African trypanosomiasis
	Toxoplasma
	Amebiasis
Other parasites	*Toxocara canis*
	Trichinella spiralis
Fungi and yeasts	*Actinomyces*
	Coccidioides
	Histoplasma
	Candida
Toxin	Diphtheria
	Scorpion
Drugs	Sulfonamides
	Phenylbutazone
	Cyclophosphamide
	Neo-mercazole
Hypersensitivity/ autoimmunity	Rheumatoid arthritis
	Rheumatic fever
	Ulcerative colitis
	Systemic lupus erythematosus
Other	Sarcoidosis
	Scleroderma
	Idiopathic
	Cornstarch

patients, adenovirus was identified in 12 percent and enterovirus in 8 percent of DCM cases. In all age groups, adenovirus and enterovirus were the viruses most commonly detected in acute myocarditis and DCM.

The discovery that coxsackievirus and adenovirus use the same receptor to infect host cells has provided an explanation for why these unrelated viruses account for most myocarditis cases. Bowles and colleagues[20] proposed that mutations in the coxsackie-adenovirus receptor could represent a host factor for the development of myocarditis or DCM. These investigators screened patients with myocarditis or DCM, including 24 patients in whom a viral etiology was identified by PCR and reverse transcription PCR. In addition, 50 patients who presented with heart failure and had echocardiograms diagnostic for DCM, but in whom no etiology was identified, were screened. Three silent substitutions and an intronic substitution were detected, but no apparent disease-causing mutations were detected. These data suggested that mutations in the coxsackie-adenovirus receptor are unlikely to play an important role in the pathogenesis of viral myocarditis or DCM.

Myocarditis also has been reported as a complication of infection with many other viruses (see Table 34–1). The teratogenicity of the rubella virus in the first 4 months of pregnancy is well known. Ainger and colleagues[3] showed that, because of the persistence of the virus in the fetus, extensive involvement of the myocardium may lead to severe myocarditis. Of 47 infants with congenital rubella, 10 had myocarditis, and 4 died. Morbidity in the survivors was severe. Rubella immunization programs have succeeded in reducing the number of congenital cases, and since 2001, only five infants with congenital rubella syndrome have been reported. Three were born in 2001, one was born in 2003, and one was born in 2004. This epidemiologic evidence strongly suggests that rubella no longer is endemic in the United States.[146]

Osama and colleagues[137] prospectively evaluated 312 cases of varicella over a 1-year period. Eighteen (5.8%) of the 312 cases showed evidence of myocarditis. A statistically significant increase in myocarditis was found in patients who complained of skeletal myalgia.

Currently, neonatal HSV disease in the United States occurs in approximately 1 in 3200 deliveries, resulting in an estimated 1500 cases of neonatal HSV infection annually.[25] Most (85%) infected infants acquire their infections during birth, in the peripartum period. The spectrum of disease ranges from unapparent infection to a fatal encephalopathy. Myocardial involvement has been described, and herpesvirus has been isolated from the myocardium at autopsy.[48,197] Recognition of genital herpes and delivery of the infant by cesarean section reduce the incidence of myocarditis caused by this agent.

In December 2002, the U.S. Department of Defense began mandatory smallpox vaccination for select service members and employees without contraindications to vaccination, and in January 2003, the U.S. Department of Health and Human Services implemented a voluntary civilian smallpox vaccination program. As of June 15, 2003, the Department of Defense identified more than 50 cases of suspected, probable, or confirmed myopericarditis occurring within 30 days of vaccination in these individuals, based on clinical evaluation of symptoms, ECG, cardiac enzyme assays, echocardiography, and the exclusion of ischemic coronary artery disease. Myocarditis occurred in 7.8 per 100,000 primary vaccinees in the U.S. army, an incidence that was 3.6-fold more than that in unvaccinated individuals.[72]

Reports of respiratory diphtheria are rare in the United States in all age groups. The last culture-confirmed case of respiratory diphtheria in a U.S. adolescent was reported in 1996.[179] Acute mortality of this disease is due to toxin-mediated diphtheritic cardiomyopathy, suffocation by the pseudomembrane, disseminated intravascular coagulation, and renal failure. Approximately one third of cases have ECG findings suggesting myocardial involvement, although the rate of cardiac involvement may be 84 percent in severe cases.[15]

PATHOLOGY

Isolated or idiopathic myocarditis is a rare pathologic entity. The pathologic cardiac findings usually are nonspecific; similar gross and microscopic changes occur regardless of the causative agent.[66,132,142,150] Grossly, all four chambers of the heart are enlarged, and the cardiac weight is increased. The heart usually is flabby and pale. In some instances, especially with coxsackievirus B infections, petechial hemorrhages may be seen on the

epicardial surfaces; pericardial fluid may be tinged with blood. On cut section, the ventricular muscle walls may be thinned. Occasionally, the ventricles are hypertrophied or increased in thickness because of edema. The valves are spared. The endocardial surface usually is unaffected but occasionally may be thickened and appear glistening white. This important observation suggested to some investigators that endocardial fibroelastosis, which manifests as congestive cardiomyopathy, represented a progression from acute viral myocarditis.[73,85]

In a study of 64 hearts of children who had myocarditis or endocardial fibroelastosis, Hutchins and Vie[85] found 18 with endocardial fibroelastosis only, five with myocarditis only, and 41 with features of both diseases. When time from onset of illness to death was 2 weeks or less, only myocarditis was evident. When the time interval was 2 weeks to 4 months, a combined picture was seen, whereas only endocardial fibroelastosis with trivial myocarditis was evident when the time from onset of disease to death was more than 4 months.

These findings were supported further by Hastreiter and Miller,[73] who found microscopic evidence of myocarditis after transthoracic needle biopsy of the myocardium in a child who had the classic clinical picture of endocardial fibroelastosis, including left ventricular hypertrophy on ECG. Fruhling and associates[58] extended these observations by showing coxsackievirus B3 in the myocardium of 13 of 28 infants with endocardial fibroelastosis. Ni and associates[130] analyzed 29 myocardial samples from patients with autopsy-proven endocardial fibroelastosis using specific PCR for enterovirus, adenovirus, mumps, CMV, parvovirus, influenza, and HSV. In 90 percent of samples, viral genome was amplified; more than 70 percent of the samples were positive for mumps viral RNA, whereas 28 percent amplified adenovirus. These data suggest that endocardial fibroelastosis also is a sequela of mumps virus infection.

Saphir and Field[156] observed mural thrombi in the left ventricular cavity in some patients with myocarditis and minute emboli in coronary and cerebral vessels. Coronary emboli, although rare, may play a role in the causation of cardiac dysrhythmias, which sometimes accompany myocarditis.

The microscopic picture of acute myocarditis typically shows a focal or diffuse interstitial collection composed predominantly of mononuclear cells, lymphocytes, plasma cells, and eosinophils. Polymorphonuclear leukocytes rarely are seen, unless the cause of the carditis is bacterial. Virus particles and inclusion bodies rarely are recognized.[144,150]

In severe infections caused by any agent, but especially coxsackieviruses and diphtheria, a loss of cross-striation in the muscle fibers, edema, and, sometimes, extensive necrosis of the myocardium occur. The diphtheria exotoxin has a particular affinity for conductive tissue; dysrhythmias, including complete heart block, are common findings in this form of myocarditis. The exotoxin interferes with protein synthesis by inhibiting a translocating enzyme in the delivery of amino acids.

Although the perivascular accumulation of lymphocytes and plasma cells is described in coxsackievirus B myocarditis, it is a minor finding. When myocarditis is caused by rickettsiae,[184] varicella,[123] trypanosomes,[138,141] or other parasites,[181] or when it occurs as a reaction to sulfonamide,[189] this pattern dominates.

Myocarditis seen with bacterial infections usually differs from that of presumed viral origin (Figs. 34–1 to 34–4). Microabscesses and patchy focal suppurative changes may be observed. Frequently, a perimyocarditis may be seen with concomitant bacterial infection of pericardium and myocardium.

Giant cells with or without granulomata are markers for the diagnosis of giant-cell myocarditis.[84] Granulomata have been observed in the myocardium of patients with tuberculosis, syphilis, rheumatoid arthritis, rheumatic heart disease, sarcoidosis, and certain fungal and parasitic infections. Occasionally, giant cells have been seen in interstitial myocarditis (idiopathic or Fiedler). In many cases, giant-cell myocarditis occurs, but no cause is found.

Two types of giant cells are recognized, one of which seems to be myogenic in origin and is thought to represent transitional forms of myocardial fibers. This type of cell has been found without granulomata. The second, more characteristic giant cell probably is derived from interstitial histiocytes. The latter type typically is seen in patients with myocarditis of nonviral cause, whereas the former is a response to viral infection. Hudson[84] described similar cells in an adult who had received neo-mercazole (carbimazole) therapy, and Hodge and Lawrence[79] reported two cases of granulomatous myocarditis associated with phenylbutazone therapy.

PATHOGENESIS

The pathogenesis of myocarditis is poorly understood, insofar as the disease progresses through different phases with distinctly different mechanisms and clinical manifestations. The

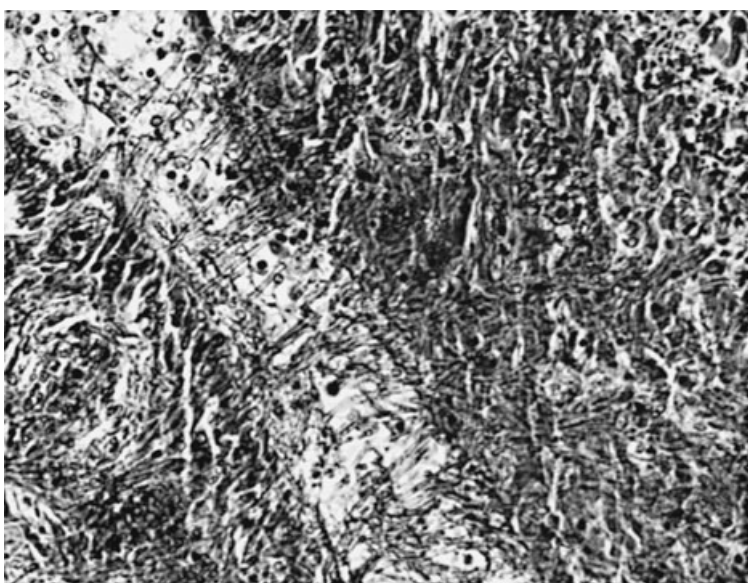

Figure 34–1 *Candida albicans* myocarditis. Notice the focal necrosis of the myocardium with central masses of hyphae and necrotic debris surrounded by mononuclear cell infiltrate (hematoxylin and eosin staining, ×160). *(Courtesy of Edith Hawkins, M.D., Houston, TX.)*

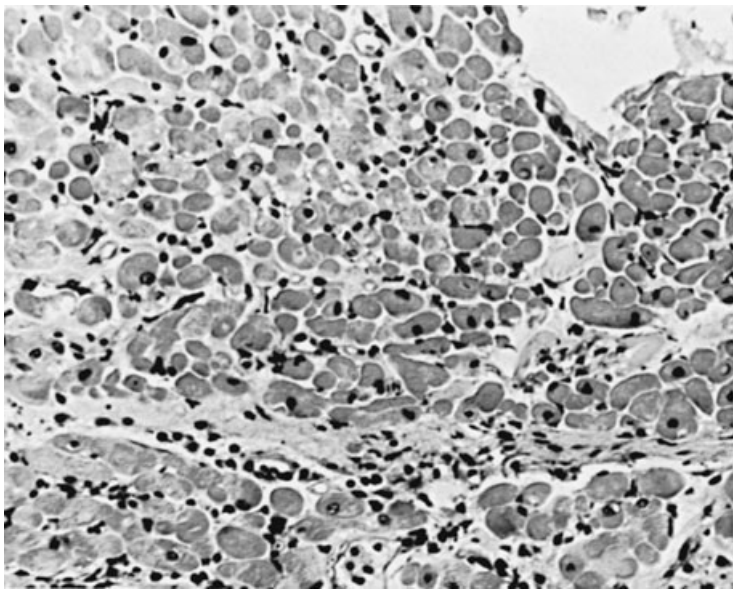

Figure 34–2 Right ventricular biopsy specimen. The presumed viral myocarditis is characterized by focal mononuclear cell infiltrates (hematoxylin and eosin staining, ×160). *(Courtesy of Edith Hawkins, M.D., Houston, TX.)*

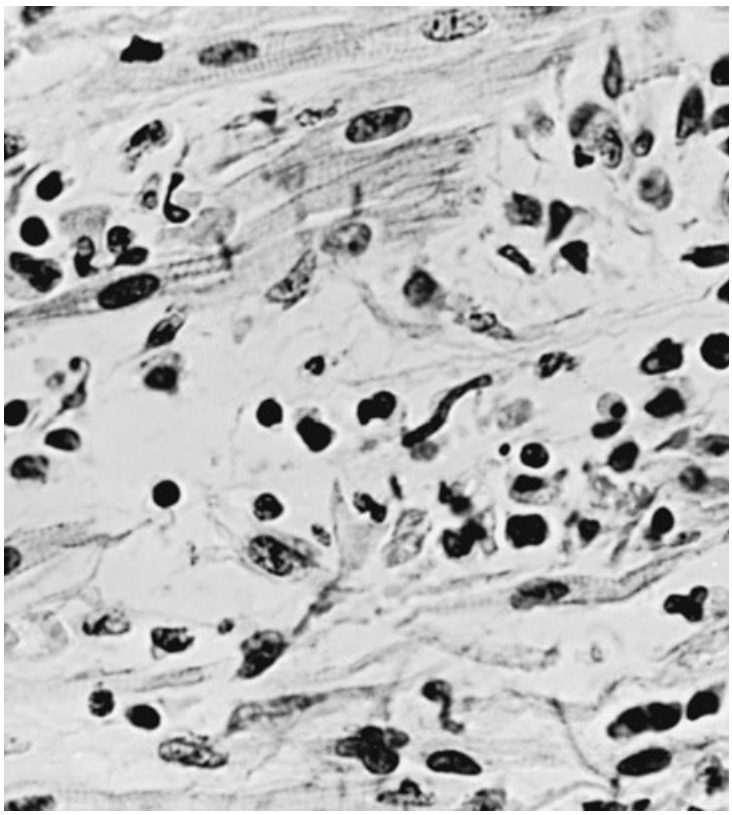

Figure 34–3 Picornavirus myocarditis characterized by interstitial edema, mononuclear cell infiltrates, and focal myofiber disruption (hematoxylin and eosin staining, ×400). *(Courtesy of Edith Hawkins, M.D., Houston, TX.)*

pathophysiology of myocarditis in humans was derived largely from experimental models of coxsackievirus infection. More recently, Liu and Mason[106] have suggested that myocarditis should be viewed as a continuum that comprises three separate phases: acute viral infection (phase I), autoimmunity (phase II), and DCM (phase III).

Phase I of the disease is triggered by the entry and proliferation in the myocardium of the causative virus. Impairment of left ventricular function in mice with histopathologically graded moderate cellular infiltration after coxsackievirus B3 infection supports the important pathophysiologic role of direct viral damage of the myocardium.[173] Phase I concludes with activation of the cellular immune response, which attenuates viral proliferation but also may enhance cardiac injury. Ideally, the immune response should down-regulate to a resting state when viral proliferation is controlled. If immune activation continues unabated despite elimination of the virus, autoimmune disease may result, initiating phase II of the disease. The continuous activation of T cells long after viral clearance occurs is detrimental to the host because cytokine-mediated and direct T-cell–mediated myocyte

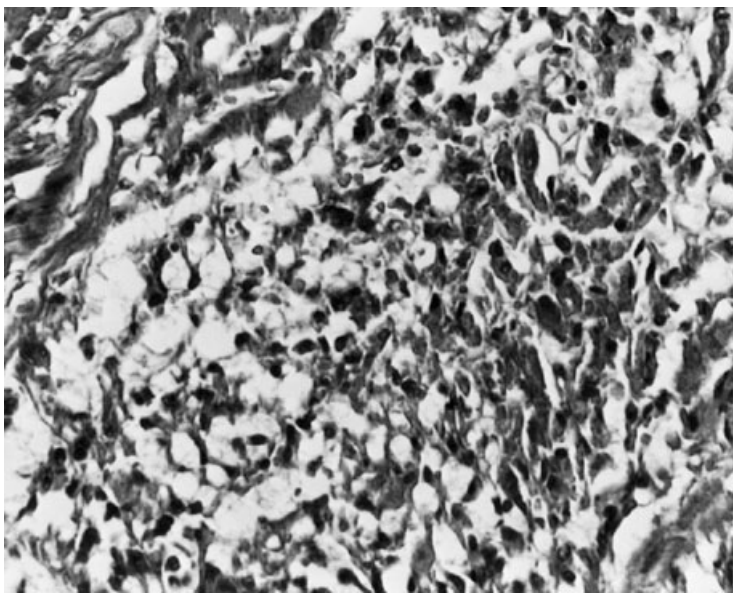

Figure 34–4 Section of myocardium. Vaccinia (smallpox vaccine) myocarditis. Note mononuclear cell infiltrates and fatty degenerative changes (hematoxylin and eosin staining, ×400). *(Courtesy of Edith Hawkins, M.D., Houston, TX.)*

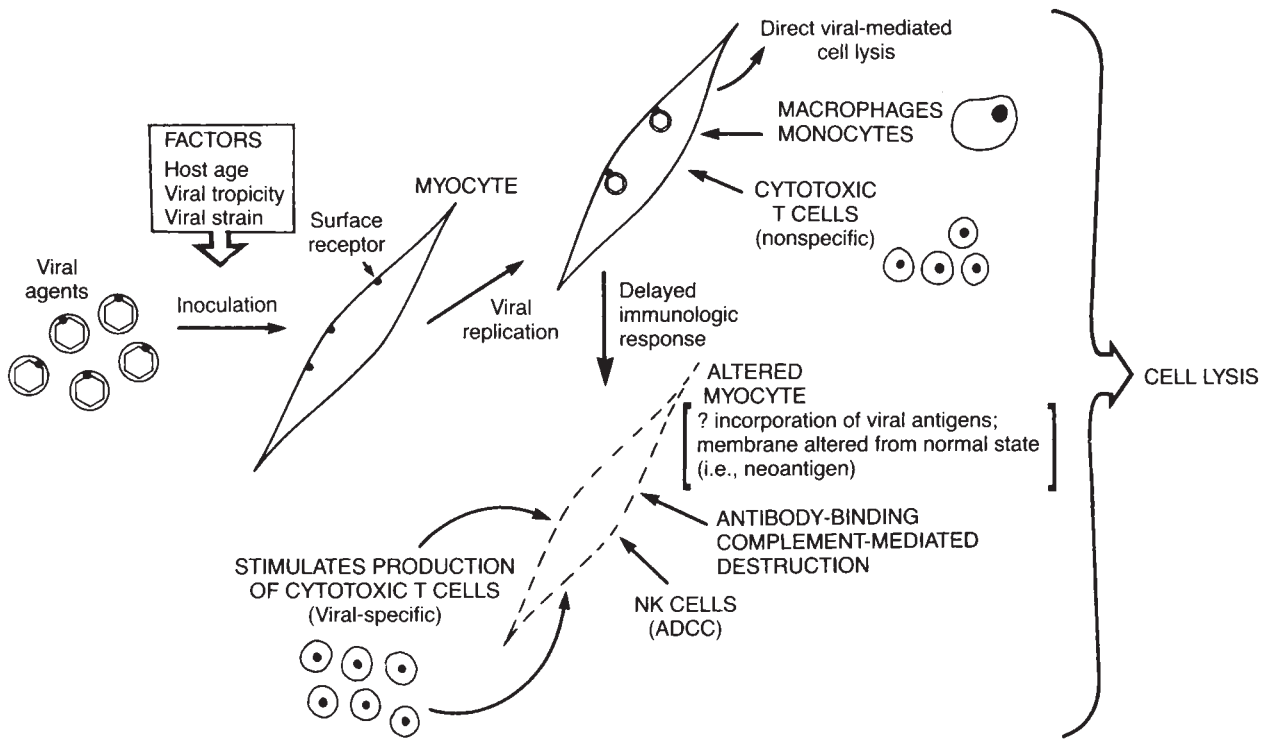

Figure 34–5 Schema for pathogenesis of myocarditis. Viral agents attach to cells by means of surface receptors. After a cell is infected, the cell cycle is changed. Direct virus-mediated cytolysis occurs. Cellular effectors of injury (i.e., macrophages, monocytes, and nonspecific cytotoxic T cells) are involved in the primary reaction. Myocytes that survive are altered in their structure. Cytotoxic T cells specifically targeted against the altered myocyte, natural killer (NK) cells, and complement-activated, antibody-mediated cardiocytolysis or antibody-dependent cellular cytotoxicity (ADCC) participate in the secondary reaction. *(Partially adapted from Maisch, B., Trostel-Soeder, R., Stechemesser, E., et al.: Diagnostic relevance of humoral and cell-mediated immune reactions in patients with acute viral myocarditis. Clin. Exp. Immunol.* 48*:533, 1982.)*

injury leads to impairment of contractile function (Fig. 34–5). Long-term remodeling and progression to DCM characterize phase III of the disease.[82]

The basic function of innate immunity is restriction of the early proliferation of infectious agents. Numerous effector cells and molecules work in concert to restrict this initial spread of an infectious focus. The responding cells include natural killer cells, natural killer/T cells and γδ T cells. Several lines of evidence suggest that mediators of the innate immune system, such as tumor necrosis factor (TNF) and nitric oxide, play an important role in the pathogenesis of viral myocarditis.[115,198] Elevated levels of TNF have been reported in patients with viral myocarditis, and TNF mRNA and protein are consistently up-regulated in the hearts of these patients.[117,158] In mice, the exogenous

administration of TNF aggravates myocarditis, and the neutralization of TNF by antibodies or soluble receptors attenuates the disease.[99,195] These data suggest that TNF production is detrimental in the setting of myocarditis.

More recent studies also have shown that TNF and nitric oxide are beneficial to the host by virtue of their antiviral effects. Mice with defective TNF or nitric oxide expression have increased myocardial injury, a significant increase in viral titers in the heart, and significantly higher mortality rates after infection with encephalomyocarditis virus or coxsackievirus B3.[183,198] These observations highlight the complexity of the innate immune response and suggest that innate immune mechanisms play a dual role in the setting of viral myocarditis. Although the prevailing notion has been that production of cytokine in the heart during viral infection is detrimental, the host-pathogen relationship is changed fundamentally when the host is unable to produce molecules such as TNF or nitric oxide.[183,198]

An important component of the innate immune system uses pattern recognition receptors, such as the Toll-like receptors (TLRs), to recognize pathogen-associated molecular patterns present in microbes.[4] TLRs induce the production of cytokines and interferons and modulate the adaptive immune response. The role of TLRs in the pathogenesis of viral myocarditis is still being explored. More recent studies suggest that cardiac inflammation during viral infection depends on TLRs. Fairweather and colleagues[53] reported that mice with defective TLR4 signaling had decreased coxsackievirus B3 replication and less severe myocarditis 12 days after infection compared with wild-type mice. The presence of TLR4 also was associated with increased production of interleukin-1β and interleukin-18 and increased viral replication in the heart.

In a similar study, Fuse and associates[59] reported that mice deficient in myeloid differentiation factor 88 (MyD88), an adapter protein involved in TLR signaling (except TLR3), also had less myocarditis and attenuated viral replication in the heart after infection with coxsackievirus B3. Coxsackievirus B3–infected, MyD88-deficient mice had significantly higher levels of interferon-β but reduced expression of the coxsackievirus-adenovirus receptor in the heart. The enhanced interferon expression and lower expression of the coxsackie-adenovirus receptor in the heart could explain the attenuation of disease in the MyD88-deficient mice. The final answer on the role of TLRs in the pathogenesis of viral myocarditis awaits further investigation, however.

Although the host genetic factors responsible for the changes observed in the three phases of myocarditis have yet to be defined completely, Woodruff and Woodruff,[192] using a murine model, were the first to show a role for T lymphocytes in the pathogenesis of viral myocarditis. In this study, depletion of T lymphocytes using antithymocyte serum or thymectomy and irradiation led to a decrease in mortality rates and in the inflammatory infiltrate after CVB3 infection. Huber and associates,[83] using BALB/c mice infected with coxsackievirus B3, showed that cytolytic T cells were the agents responsible for the major part of myocardial cell injury. In addition, proinflammatory mediators, such as TNF, released by infiltration cells also adversely affect cardiac function.

Opavsky and colleagues[135] defined the specific contributions of T-cell subsets (CD4 and CD8) and the T-cell receptor beta chain to the pathogenesis of viral myocarditis. When $CD4^{-/-}$ or $CD8^{-/-}$ mice were exposed to CVB3, loss of $CD8^{+/+}$ immune cells did not affect survival significantly, but viral proliferation was attenuated. In contrast, $CD4^{-/-}$ mice showed a trend toward an improvement in survival and a small but significant decrease in the inflammatory infiltrate at 14 days after infection. Mice deficient in CD4/CD8 immune cells and T-cell receptor beta had the best outcome in terms of decreased mortality. A marked decrease in inflammatory infiltrate was noted in CD4/CD8 double knockout mice. Although no significant change occurred in viral titers, a marked decrease in myocardial TNF mRNA 4 days after infection was seen in CD4/CD8 double knockout mice. These same investigators have shown that the T-cell receptor–associated tyrosine kinase $p56^{lck}$ is crucial for coxsackievirus B3 proliferation in the heart and activation of T cells to target the heart.[105] Mice deficient in $p56^{lck}$ were protected against the development of myocarditis, providing further support for the hypothesis that T-cell activation during viral myocarditis contributes to increased inflammation and myocyte destruction in the host.

The ongoing injury that persists may be considered an autoimmune process.[83,191] In murine models, cellular and humoral autoimmunity clearly are involved in the progression to chronic heart disease. Paque and colleagues[140] provide support for this concept. Using CD-1 mice infected with coxsackievirus B3, they found a potassium chloride–extractable antigen in the hearts of mice previously infected with a coxsackievirus B that was specifically immunoreactive with immune mouse peritoneal exudate cells (i.e., stimulated production of a migration inhibitory factor). No viral activity was detected in the animals that had this extractable antigen.

Similar experiments in a primate model confirmed earlier findings in mice and lent further support to similar circumstances in humans.[139] Experimental evidence also has shown that the antigen responsible for cytotoxic T-cell activity is not detectable by antiserum that contains antibodies directed at structural components of the viral capsid. Autoantibodies that have been associated with myocarditis in patients and mice include those directed at cardiac myosin, adrenoreceptor adenine nucleotide translocator, and sarcolemmal and myolemmal proteins. The role played by these autoantibodies in the pathogenesis of myocarditis is unclear; antisarcolemmal antibodies that cross-react with enteroviral epitopes have been shown to damage cardiac myocytes.

More recent studies also have shed light on the mechanisms by which coxsackievirus B3 may contribute directly to the development of myocarditis and DCM. Badorff and colleagues[10-12] reported that the 2A protease encoded by coxsackievirus B3 cleaves dystrophin in cultured myocytes and in infected mouse hearts, leading to disruption of dystrophin and the dystrophin-associated glycoprotein α-sarcoglycan and β-sarcoglycan complex. Dystrophin provides a structural link between the muscle cytoskeleton and extracellular matrix to maintain muscle integrity. Xiong and associates[194] compared the effects of coxsackievirus B3 infection in dystrophin-deficient (*mdx*) and wild-type mice. Coxsackievirus B3 infection significantly enhanced sarcolemmal disruption in the *mdx* mice compared with wild-type mice; the disruption was detectable 2 days postinfection and continued to increase after initial infection. Viral titers were higher in the hearts of *mdx* mice than in the hearts of wild-type mice, indicating greater viral replication in the absence of dystrophin. The observed differences seemed to be a result of more efficient release of coxsackievirus B3 from dystrophin-deficient myocytes. The expression of wild-type dystrophin in cultured cells decreased the cytopathic effect induced by coxsackievirus B3 and the release of virus from the cell. The expression of a cleavage-resistant mutant of the dystrophin protein inhibited coxsackievirus B3–mediated cytopathic effect and viral release further.

PATHOPHYSIOLOGY OF VENTRICULAR DYSFUNCTION IN MYOCARDITIS

With extensive interstitial inflammation, muscle-cell injury, or both, myocardial contractility is reduced. Consequently, the heart enlarges and the end-diastolic volume of the ventricle increases. In the normal heart, an increase in filling volume leads, by the Starling mechanism, to an increased force of contraction, ejection fraction, and cardiac output. In patients with myocarditis, the myocardium is unable to respond in this manner and

cardiac output is reduced. Systemic blood flow may be maintained, however, by use of the cardiac reserve, mediated by the sympathetic nervous system and leading to vasoconstriction of the skin vessels and an increase in heart rate. With progressive disease or any stress (e.g., infection, anemia, fever), the heart may be unable to meet the oxygen demands of the tissues, and the clinical picture of congestive cardiac failure may become evident. Increased end-diastolic volume leads to progressive increase in ventricular end-diastolic pressure, which leads to increased filling pressure, and left atrial and pulmonary venous hydrostatic pressure may be elevated above the colloid osmotic pressure, which normally prevents transudation of fluid across the capillary membranes. Pulmonary congestion and edema and systemic venous engorgement (manifested in infants primarily as hepatic enlargement) are common findings in the more acute forms of myocarditis. In some infants and young children, the presentation is predominantly that of right-sided heart failure.[149]

An appreciation of the disturbance of myocardial function may be gained from the angiographic frames shown in Figure 34–6. The left ventricle is dilated considerably, and the outline is irregular in diastole and systole. The ejection fraction is reduced significantly at 35 percent instead of the normal 60 to 75 percent.

Another means of evaluating left ventricular function is the noninvasive technique of cardiac ultrasound. Gutgesell and colleagues[71] established normal standards for children. An example is shown in Figure 34–7A. The normal shortening fraction (i.e., percentage change in ventricular dimensions between end-diastole and end-systole) is 35 ± 4 percent, regardless of age (range 28–44%). Figure 34–7B illustrates the case of a 4-year-old child with idiopathic myocarditis and shows ventricular dilation with markedly reduced motion of the left ventricular posterior wall and septum, leading to a shortening fraction of only 12 percent. Further assessment of ventricular function can be achieved by measuring systolic time intervals obtained from simultaneous recording of the ECG and the semilunar valve opening and closing points on the echocardiogram.[71]

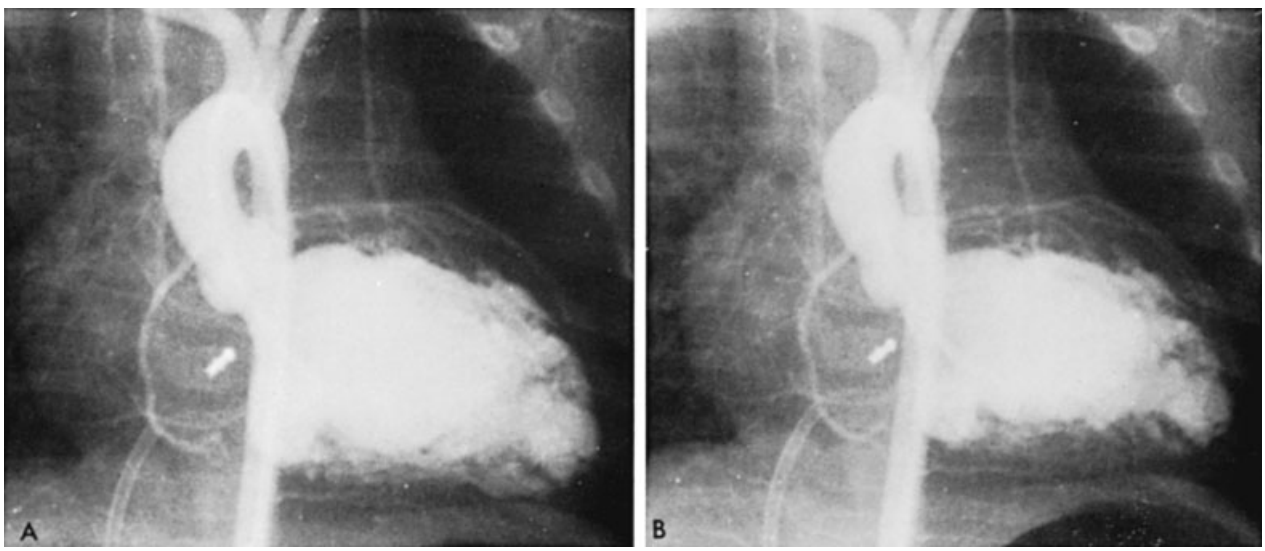

Figure 34–6 **A** and **B,** The end-diastolic **(A)** and end-systolic **(B)** frames from a left ventriculogram of a patient with idiopathic myocarditis show irregularity of the wall and poor contractility.

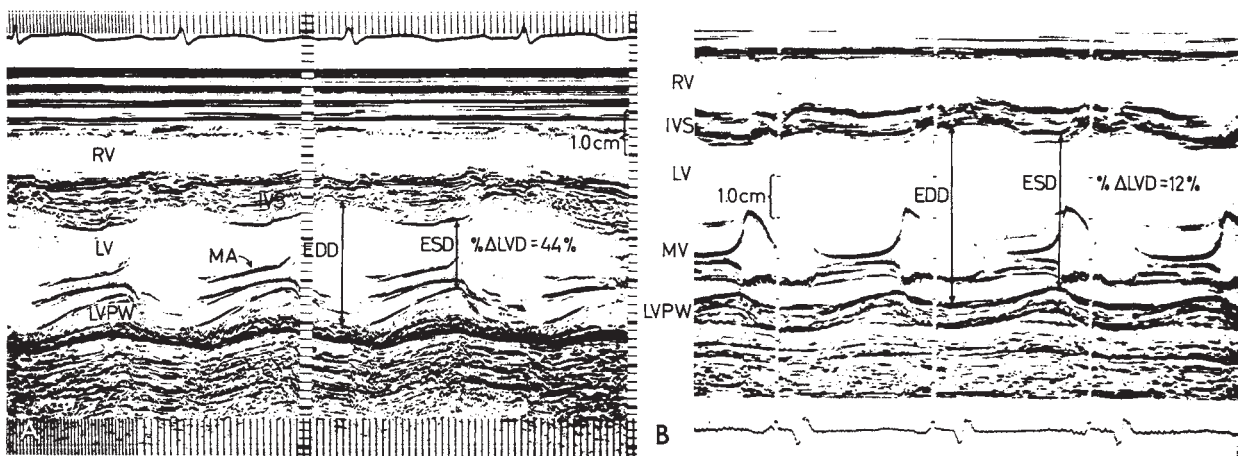

Figure 34–7 **A,** Normal echocardiogram of a 4-year-old child. **B,** Echocardiogram of a 4-year-old child with idiopathic myocarditis shows left ventricular dilation and severely reduced shortening fraction. EDD, end-diastolic dimension; ESD, end-systolic dimension; IVS, interventricular septum; LVPW, left ventricular posterior wall; MA, mitral apparatus; MV, mitral valve, %ΔLVD, percent change in left ventricular dimension (shortening fraction).

CLINICAL PRESENTATION

The clinical presentation of myocarditis varies with the age of the patient and the virulence of the organism. At one end of the spectrum is a fulminant, rapidly fatal illness, and at the other, no apparent clinical disturbance at all. A newborn especially is susceptible to the severe form of myocarditis usually caused by the coxsackieviruses B,[38,92] but it also is recognized with rubella[3] and HSV[193] infections and with toxoplasmosis.[84]

In many of these infections, myocarditis is only one component of a generalized illness, often with severe hepatitis and encephalitis.[17,92] In some instances, however, infections with these organisms may produce only a mild clinical disturbance.[24,86] In the report by Brightman and colleagues,[24] a nursery epidemic of coxsackievirus B5 infection in preterm infants was recognized only by chance because a virologic survey was in progress at the time in the institution. No cases of myocarditis were documented, and all the infants recovered. Findings included lethargy, failure to gain weight, and, in some infants, evidence of aseptic meningitis.

As described in the review by Kibrick and Benirschke[92] of 25 infants with coxsackievirus B myocarditis, vague symptoms such as lethargy and anorexia may herald the onset of the severe disease, emphasizing that close attention should be paid to all symptoms, especially in a newborn, no matter how nonspecific. Four infants had episodes of vomiting, and fever was documented for more than half of the cases; occasionally, the temperature was subnormal. Cyanosis; respiratory distress; and tachycardia, cardiomegaly, or ECG changes occurred in 19 of 23 infants. Tachypnea (a respiratory rate >60/min in a newborn) is an early sign of heart failure in a young infant and should alert the clinician to this diagnosis.

In older infants and children, the manifestations of myocarditis generally are less fulminant than are the manifestations in newborns.[149,154,185,190] An acute and fatal illness has been associated, however, with idiopathic myocarditis[104] and myocarditis caused by enteroviruses,[92] adenoviruses,[75] mumps,[100] chickenpox,[137] diphtheria,[15] CMV,[176] and many of the other causative agents listed in Table 34–1. Some older children have been reported with acute, substernal chest pain consistent with angina and have ECG changes of acute myocardial infarction.[81,119] The usual clinical picture is that of an acute or a subacute illness, which often begins with a mild upper respiratory infection and a low-grade fever.[9] Some infants have only vague, nonspecific suggestions of disease (e.g., irritability, periodic episodes of pallor) before the onset of cardiorespiratory symptoms, which begin a few days to 2 weeks after the onset of the initial symptoms. Abdominal pain may be a prominent complaint in some children.[185]

On examination, these infants and children often are anxious and apprehensive, but some appear apathetic and listless. Pallor may be striking, and mild cyanosis may be present. The skin may be cold and mottled. Respirations are rapid and labored, and grunting may be prominent. The pulse is thready, and blood pressure usually is normal or slightly reduced, unless the infant is in profound shock. The precordium is quiet, without a prominent cardiac impulse. Resting tachycardia invariably is present in children who are critically ill with myocarditis. The heart sounds are muffled, and a prominent gallop rhythm usually is heard. Fine and colleagues[55] found the most sensitive clinical sign of myocarditis to be a soft S_1 at the apex. A prolonged PR interval, which may be a nonspecific finding in many febrile illnesses, also can cause a soft S_1, however, without any other evidence of myocarditis.[162] A high-pitched systolic murmur of mitral insufficiency is heard in some cases. The breath sounds are harsh. Scattered rhonchi and, occasionally, fine crepitations in the lung bases may be detected.

Almost uniformly, the liver is enlarged; edema is a rare finding. Some infants are less distressed and have signs of only mild congestive cardiac failure, without the signs of peripheral circulatory failure. Other infants have no signs of cardiac compromise, and myocarditis is recognized only as part of a generalized illness by a disturbance in the ECG pattern.

DIAGNOSIS

CLINICAL CHARACTERISTICS AND RADIOGRAPHIC EVIDENCE

Myocarditis often is difficult to diagnose, but it should be suspected in any infant or child who presents with congestive heart failure and who has or recently has had a febrile illness. The history should include information regarding travel, exposure to tuberculosis, recent drug ingestion, and illnesses in other family members or schoolmates. A quiet precordium in the presence of a gallop rhythm and decreased intensity or muffling of the heart sounds are findings that strongly suggest the diagnosis. A tachycardia out of proportion to the level of fever also should be viewed with suspicion. A physiologic S_3 is a common finding in normal healthy children and in children with anemia and fever. Sometimes, as with fever and associated tachycardia, the cardiac rhythm may have a gallop cadence. In association with it, however, the precordium is hyperactive, and the heart sounds are crisp and have increased intensity. An unusually prominent S_3 suggests a disturbance of ventricular compliance without other evidence of compromised cardiac function and should be investigated further with an echocardiogram, a chest radiograph, and an ECG.

Chest radiographs of infants and children who have signs of congestive cardiac failure invariably show cardiomegaly, usually of a severe degree (Fig. 34–8). All four chambers may be enlarged, and evidence of pulmonary venous congestion often is found.

Sometimes, especially in newborns, the first sign of illness is acute circulatory collapse, and, in this circumstance, the cardiac size may be normal. The same is true of children who have an arrhythmia rather than congestive heart failure. Other patients may present with Stokes-Adams attacks caused by complete heart block.[103]

The occurrence of an arrhythmia, especially after a febrile illness, should alert the clinician to look for other signs of myocarditis.[33,165] Lind and Hulquist[104] detected significant dysrhythmias in five infants with isolated myocarditis. Four of the five infants died, and three of these infants had paroxysmal atrial tachycardia. Paroxysmal atrial tachycardia has been reported in patients with viral myocarditis[33,164] and has been described in patients with diphtheritic myocarditis.[15] Atrial ectopic tachycar-

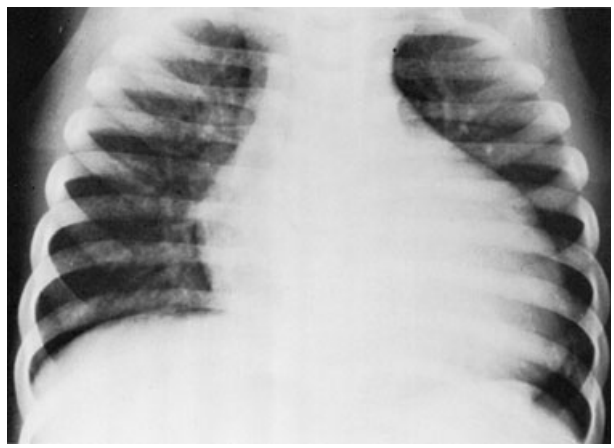

Figure 34–8 Marked cardiomegaly with a mild increase in the pulmonary venous pattern in the upper lobes.

dia may mimic sinus tachycardia and, if not carefully evaluated, may be the primary cause for significant myocardial dysfunction. Complete heart block has been described in children in association with acute idiopathic myocarditis[88,103] and with rubella,[107] coxsackievirus,[159] and respiratory syncytial virus[13,63] infections. In some instances, complete heart block is permanent, and in others, it is temporary.[13,64,88] The ECG is an essential diagnostic tool for all patients with suspected myocarditis.

The classic ECG pattern in myocarditis is one of diffuse low-voltage QRS complexes (<5 mm total amplitude) with low-amplitude or slightly inverted T waves and a small or absent Q wave in leads V_5 and V_6 (Fig. 34–9). The low-voltage signal may be present in the standard leads and the precordial leads. Figure 34–10 shows the ECG of an infant with acute myocarditis and shows a pattern of acute myocardial ischemia. Figure 34–11A shows multifocal extrasystoles and severe intraventricular conduction delay in a patient with diphtheritic myocarditis; the ECG of this child returned to normal over the course of 3 months (see Fig. 34–11B). The ECG from a 5-month-old infant who had mild fever, diarrhea, and vomiting for 3 to 4 days before admis-

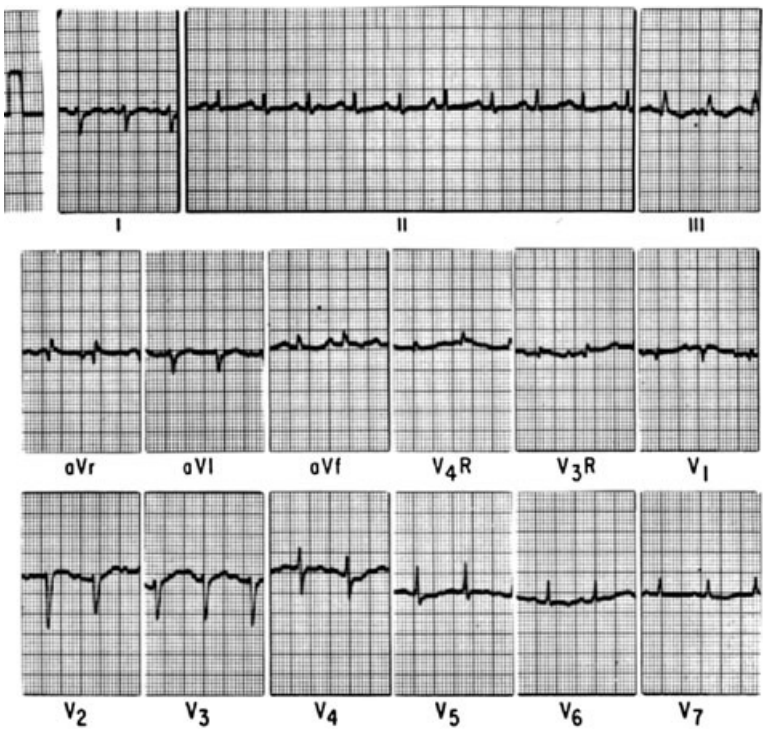

Figure 34–9 Diffuse low-voltage or QRS complexes with T-wave flattening and 1-mm Q waves in the lateral precordial leads represents the classic pattern in myocarditis.

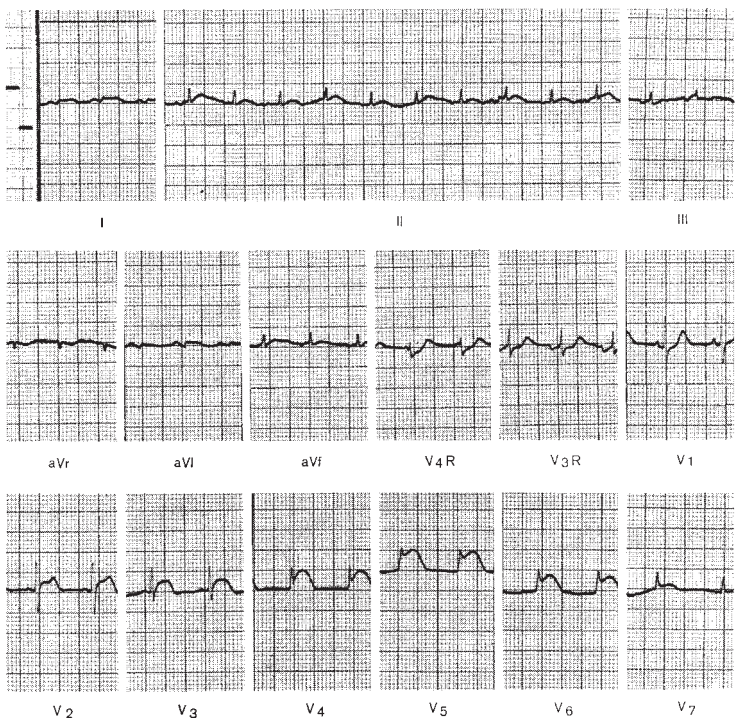

Figure 34–10 In addition to low voltage, there is evidence of acute myocardial ischemia with 4- to 5-mm ST-segment elevation dominantly in the middle and lateral precordial leads.

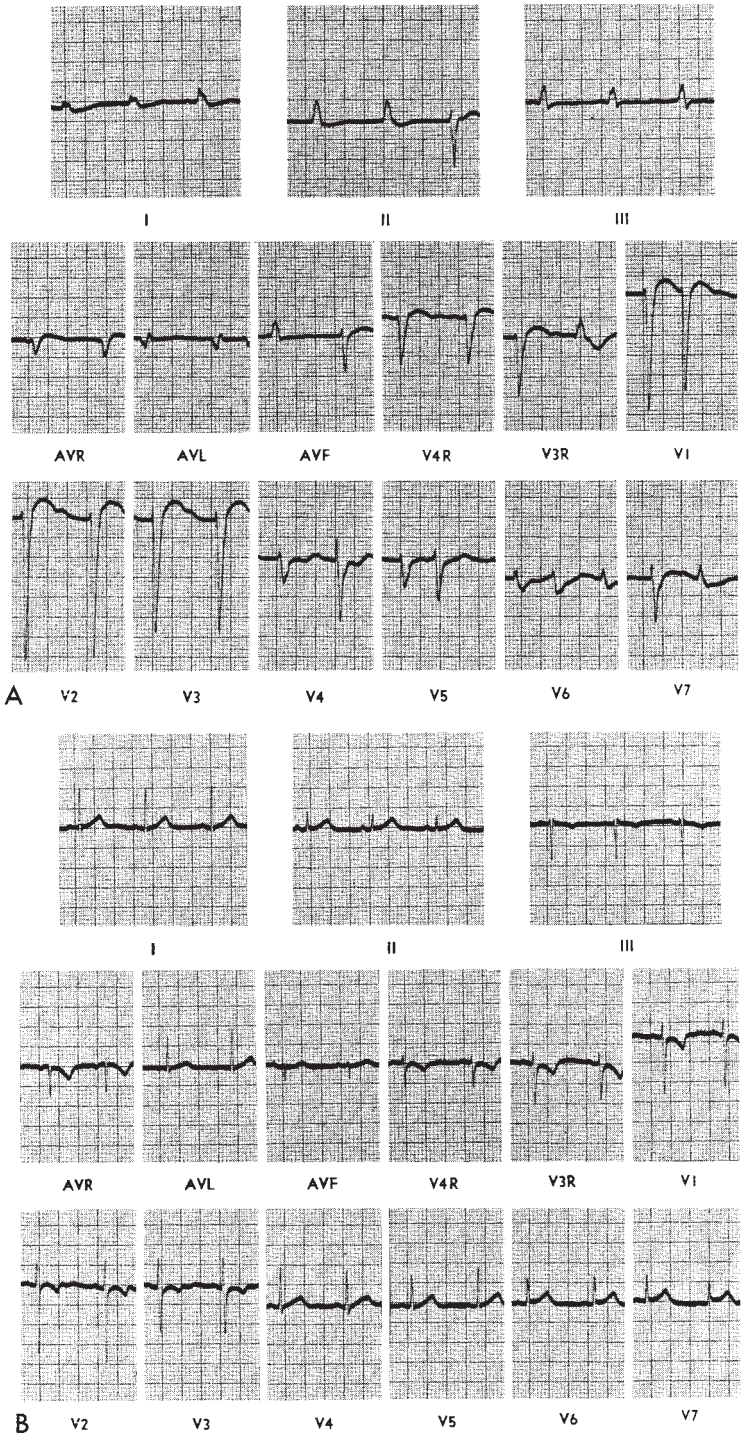

Figure 34–11 **A,** Multifocal premature beats are caused by atrioventricular dissociation and left bundle branch block resulting from diphtheritic myocarditis. **B,** A normal electrocardiogram is shown for the same patient 3 months after an episode of myocarditis.

sion is shown in Figure 34–12. The 2:1 atrioventricular block was associated with normal QRS complexes. This abnormality persisted in the absence of clinical symptoms for 1 year. Figure 34–13 shows a left bundle branch block that was identified in a 10-month-old infant with acute idiopathic myocarditis. Anomalous origin of the left coronary artery from the pulmonary artery was suspected but was excluded by catheterization. This ECG pattern persisted for at least 6 months.

Karjalainen and colleagues[90] studied the ECGs of 87 conscripts 18 and 30 years old, 28 of whom had myocarditis. The most frequent findings were T-wave changes of reduced amplitude or inversion in the left chest leads. Sinus tachycardia followed by premature ventricular depolarizations was the most common dysrhythmia. Take and colleagues[171] examined serial ECGs of 16 patients with confirmed viral myocarditis. They found four patterns: (1) complete normalization in the presence of severe myocardial damage in the acute stage; (2) "pseudoinfarction" patterns with Q waves and poor R-wave progression; (3) permanent conduction disturbances that might require pacemaker support; and (4) chronic dysrhythmias, predominantly ventricular tachycardia and supraventricular tachycardia.

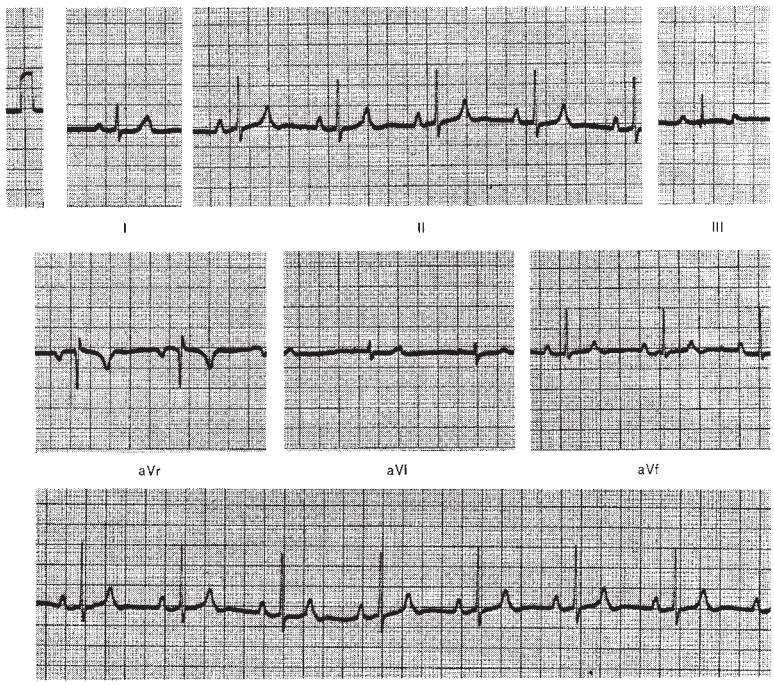

Figure 34–12 Second-degree atrioventricular block with an effective ventricular rate of 60 beats/min. The blocked P wave is placed on top of the T wave in each cycle.

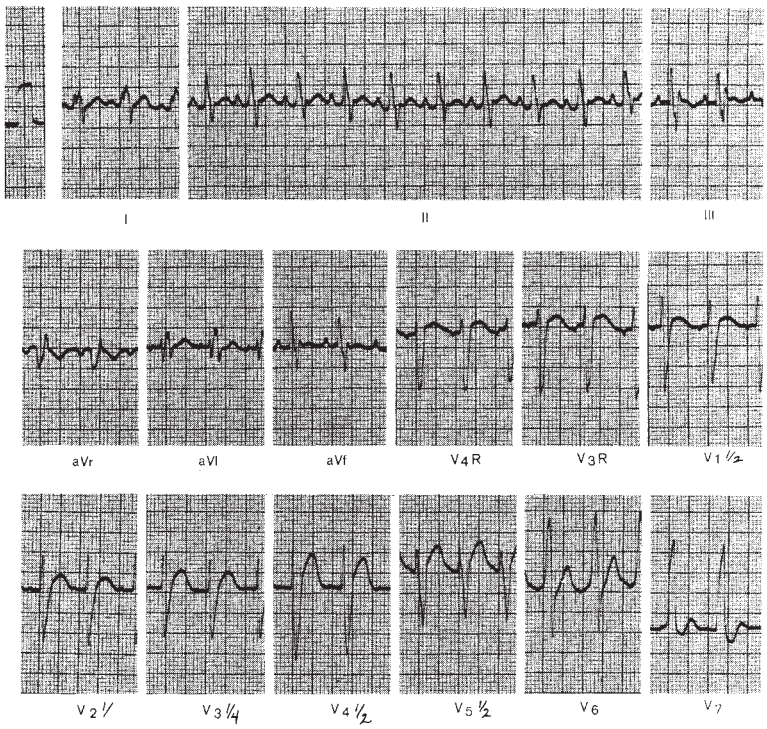

Figure 34–13 Sinus tachycardia (rate 150 beats/min) and left bundle branch block.

Hoshino and coworkers[80] induced coxsackievirus B3 myocarditis in Syrian golden hamsters and found that 80 percent of them had ST-segment or T-wave (or both) changes in the surface ECG. Most of the changes were seen between days 2 and 4, when mortality rates were highest. The endocardial third of the myocardium was most involved histologically, suggesting that the subendocardial myocardial injury corresponded to the observed ST-segment and T-wave changes. Kishimoto and coworkers,[97] using DBA/2 mice, induced myocarditis with encephalomyocarditis virus. Acute changes were correlated with advanced atrioventricular block and with atrial and ventricular premature depolarizations. Sinus tachycardia and low voltage were seen in the late stages in the animals that survived. Over the long-term, the QRS voltages recovered toward normal, possibly reflecting loss of myocardial edema or development of ventricular hypertrophy, or both, as a compensatory mechanism for poor ventricular function.

Although T-wave and ST-segment changes are the most sensitive indices of myocardial ischemia, they also seem to be nonspecific. Prolongation of the PR interval is another nonspecific

ECG finding frequently noted in patients with febrile illnesses. Scott and colleagues[162] showed a 1.49 percent prevalence of these findings in a group of 737 infants and children with respiratory tract infections, but they also found a similar incidence among 108 control children without respiratory infection or other febrile illness. Abt and Vinnecour[1] recorded PR prolongation and T-wave changes in infants and children who had pneumonia without other signs of myocarditis. The QT interval has been prolonged in cases of acute myocarditis, but it also seems to be a nonspecific finding associated with certain infectious diseases, such as measles[151] and poliomyelitis.[89] A diagnosis of myocarditis cannot be established with certainty on the basis of these nonspecific changes.

Noninvasive diagnostic myocardial imaging techniques that are useful in the detection of myocarditis include echocardiography, nuclear imaging with gallium 67–labeled or indium 111–labeled antimyosin antibodies, and magnetic resonance imaging (MRI). Echocardiography is recommended currently in the initial diagnostic evaluation of all patients with suspected myocarditis.[153] The echocardiogram is useful in assessing ventricular function and helps to exclude pericardial effusion as the cause of the cardiomegaly.

Nuclear imaging has been advocated as a potentially helpful laboratory screening test. Some evidence suggests that using gallium 67 to screen patients with idiopathic DCM may select a subgroup of patients who could benefit from endomyocardial biopsy. The biopsy specimen could be used to confirm the presence of active inflammation. O'Connell and colleagues[131] studied 68 patients with DCM who underwent 71 parallel studies with endomyocardial biopsy and gallium scanning. For five of six patients, biopsy samples that showed myocarditis also showed dense gallium uptake, and only 9 of 65 negative biopsy specimens had "equivocally positive" gallium scans. A 36 percent incidence of myocarditis on biopsy was found for positive scans, and only a 1.8 percent incidence of myocarditis on biopsy was found for negative scans. No large studies enrolling children with viral myocarditis have been performed. The use of gallium imaging has diminished over time, mainly because of a lack of specificity.

Indium 111 antimyosin imaging[42] and the presence of major histocompatibility complex class I and II antigens measured by radioimmunoassay using monoclonal antibodies[76] can detect patients with active myocarditis. High titers (1:20) of an IgG antibody (i.e., heart-reactive antibodies) measured by indirect immunofluorescence have been reported in patients with biopsy-confirmed myocarditis.[129] Although none of the methods is yet clinically applicable to all patients, these noninvasive tests offer some hope that the diagnosis of inflammatory heart disease will become safer.

More recent studies suggest that contrast-enhanced cardiac MRI may be a promising technique for diagnosing myocardial inflammation and myocyte injury. Gagliardi and associates[60] reported the first case series on the use of cardiac MRI for the diagnosis of acute myocarditis in 11 infants and children. The authors reported 100 percent specificity and 100 percent sensitivity for T2-weighted spin echo cardiac MRI sequences compared with endocardial biopsy. Cardiac MRI may be a good tool for selecting patients for endomyocardial biopsy.

To undertake a hemodynamic study in patients with a classic picture of acute myocarditis, including an ECG that reveals low voltage, is unnecessary and potentially dangerous. If the clinical or ECG presentation is atypical (i.e., left ventricular hypertrophy with left axis deviation, infarction pattern, or left bundle branch block), the infant should be studied for exclusion of an anomalous origin of the left coronary artery or another unsuspected anomaly.

Endomyocardial biopsy has become a safe and effective means for sampling heart muscle.[5] The technique originally was introduced in 1962 in Japan by Sakakibara and Konno,[155] but it did not gain wide acceptance in the United States for another 10 or more years. Its widest application has been in monitoring the effectiveness of immunosuppressive therapy in heart transplant recipients who may undergo the procedure multiple times.

Endomyocardial biopsy used for establishing the diagnosis of myocarditis and possibly classifying the phase (i.e., active, healing, or healed) of viral infection may have a direct impact on the type of therapy employed. Classification of myocarditis based on histologic evidence found on biopsy specimens has proved to be a difficult and sometimes controversial task. No widespread agreement exists on the criteria for establishing the diagnosis of myocarditis from biopsy samples. Sampling error owing to the small amounts of tissue obtained and the focal nature of sampling and the disease process may lead to misdiagnosis. Samples usually are obtained from the right ventricular septum or apex and should contain at least three and optimally five pieces of tissue. Some investigators[180] have found sampling from other areas of the heart (e.g., left ventricle) to be more sensitive, but these techniques have not been applied widely.

Cases of "borderline myocarditis" (i.e., specimens containing increased numbers of inflammatory cells, but without evidence of myocyte necrosis) may require a repeat biopsy to confirm the diagnosis. Dec and colleagues[40] confirmed the diagnosis of myocarditis in four of six patients with an initial diagnosis of borderline myocarditis. These investigators did not show any significant advantage to sampling the left ventricle during the repeat study. Overinterpretation or misinterpretation has been cited as a major problem in the reading of biopsy specimens. Some studies[168,169,182] used this technique to establish a diagnosis of myocarditis in patients presenting with idiopathic congestive cardiomyopathy and in patients with serious ventricular dysrhythmias with otherwise structurally normal hearts. Edwards and associates[51] looked at 170 endomyocardial biopsy samples and found that more than five lymphocytes per high-power field were consistent with a diagnosis of active lymphocytic myocarditis.

Using endomyocardial biopsy, Fenoglio and coworkers[54] diagnosed myocarditis in 34 patients presenting with congestive heart failure of unknown origin. They classified these patients on the basis of clinical and histologic findings in an attempt to establish subgroups of patients who might benefit from immunosuppressive therapy. Three groups were established: acute, rapidly progressive, and chronic. Immunosuppressive therapy was thought to be significantly beneficial for the last group only in terms of clinical improvement.

Dec and colleagues[41] studied 27 patients referred for endomyocardial biopsy because of congestive heart failure of unknown origin. Two thirds of the patients had biopsy samples read as positive for myocarditis, but in contrast to the study of Fenoglio and coworkers,[54] in which the histologic grouping was slightly different, no correlation was found between histologic classification and outcome. Outcome did not differ between the group receiving immunosuppressives and the group not receiving immunosuppressives. Biopsy results were negative for 30 percent of the patients who already met all the clinical criteria of myocarditis and were positive for two of five patients without any clinical evidence of myocarditis (i.e., viral-like illness, pericarditis, or laboratory evidence of viral infection).

Olsen[134] reviewed 1200 biopsy specimens from patients with a clinical diagnosis of idiopathic DCM and found that slightly more than 25 percent had a diagnosis of myocarditis established on the basis of critical evaluation of their tissue specimens. No large study of pediatric patients has been conducted to confirm this finding in young patients.

Most physicians would agree that many cases of idiopathic DCM probably are the sequelae of unrecognized acute viral myo-

carditis. The role of endomyocardial biopsy in attempting to salvage patients by selecting them for specific therapy has some validity. The hope is that early intervention in some patients guided by this technique would prevent them from progressing to needing transplantation or to death from intractable heart failure.

MOLECULAR DIAGNOSTIC STUDIES

In Situ Hybridization

In 1986, Bowles and colleagues[22] showed the utility of molecular biologic analysis of tissue samples in establishing the diagnosis of myocarditis. Using in situ hybridization, they were able to show enteroviral RNA in the myocardium of patients suspected to have myocarditis. These investigators also showed that enteroviral RNA could be identified in the myocardial tissue samples obtained from patients with end-stage DCM, and the suggestion that some cases of DCM were caused by a previous episode of subclinical myocarditis gained scientific support. The ability to diagnose enteroviral myocarditis and the finding of enteroviral RNA in patients with DCM later were confirmed by other investigators.[9,23] Because of questions of excessive false-positive results, variations among laboratories, and the difficulty in performing the test routinely in hospital laboratories, this method never gained practical popularity.

Polymerase Chain Reaction

Jin and associates[87] first described the usefulness of PCR in identifying the viral genome in myocardial samples obtained from patients with suspected myocarditis. Using reverse transcription PCR, which employs RNA to amplify the corresponding complementary DNA before final DNA amplification, the researchers were able to identify an enteroviral genome from cardiac tissue samples. Patients with DCM were shown to harbor an enteroviral genome within myocardial specimens. Confirmation of the utility of PCR in the etiologic diagnosis of a viral genome in patients with clinical myocarditis and idiopathic DCM quickly followed.[32,50,67,77,127,145,186] Controversy existed, however, because reports of high levels of false-positive results,[50] contamination,[32] and low sensitivity[188] were published. The strength of this rapid (<5 hours) and powerful method of amplification of a specific viral genome is also its weakness. The method depends on the quality and quantity of nucleic acid extraction, but contamination may be commonplace in some laboratories. If any requirements are altered, amplification may not occur, leading to false-negative or false-positive (i.e., contamination) results.

Numerous publications have shown that PCR is a rapid and sensitive method for establishing the diagnosis of enteroviral myocarditis and that it is probably the test of choice in the diagnosis of all virus-induced cardiac disease. Towbin and colleagues[178] used PCR to diagnose adenoviral myocarditis in a fetus with nonimmune hydrops fetalis. In this case, the adenoviral genome was amplified from fetal blood and maternal blood at 29 weeks' gestation and again at delivery at 34 weeks' gestation using blood from the infant and mother and placental specimens. Treatment with digoxin in utero helped the fetus improve clinically, and the infant had normal cardiac function at delivery. Using viral primers designed to amplify enterovirus, adenovirus, CMV, and HSV nucleic acid, Martin and associates[111] reported 34 patients with suspected acute myocarditis, for whom 68 percent of samples analyzed were PCR-positive. In this report, samples from 17 control patients were PCR-negative. Adenovirus was the most common viral genome identified (58%), and enteroviruses were the second most common (29%). A few reported cases were PCR-positive for HSV and CMV.

Lozinski and coworkers[108] confirmed the importance of adenovirus in their study of cases of myocarditis for which no cause had been found previously; in this case, 66 percent of the previously unidentified cases were identified as adenovirus. In a more recent study by Bowles and colleagues,[21] viral genomes were detected in 20 percent of 149 patients with the diagnosis of DCM. In these patients, adenovirus was identified in 12 percent and enterovirus in 8 percent of DCM cases. In all age groups, adenovirus and enterovirus were the viruses most commonly detected in acute myocarditis and DCM.

Schowengerdt and colleagues[161] showed that a variety of viruses might be the inciting cause of rejection in patients after undergoing heart transplantation. Using PCR of endomyocardial biopsy specimens, the investigators showed a direct correlation between histologic rejection and PCR-positive viral study results. Studying patients undergoing serial endomyocardial biopsies, they found that the viral genome could be amplified in transplant-rejecting patients who previously had negative PCR analyses. In these cases, as the rejection grade improved, the PCR results again became negative. The most common viruses correlated with rejection were adenoviruses, CMV, and parvovirus. The researchers postulated that this form of rejection probably is another form of myocarditis, and this hypothesis has been supported by other clinical studies.[187]

VIROLOGIC AND BACTERIOLOGIC STUDIES

For each infant or child with a diagnosis of acute myocarditis, an attempt should be made to identify the offending organism. If the patient is seen early in the illness, isolation of the virus from throat washings, stool, blood, or the myocardium may be possible. Support for an active infection is obtained by showing a fourfold increase in antibody titer to the virus that has been isolated.[19,74,160]

Lerner and colleagues[102] suggested criteria that would help define an etiologic association between a coxsackievirus infection and myocarditis. High-order associations included isolation of the virus from the myocardium, the endocardium, or pericardial fluid and localization of type-specific virus in myocardium, endocardium, or pericardium at sites of pathologic change. Moderate-order associations are determined when virus is isolated from pharynx or feces, and a fourfold increase in type-specific, neutralizing, hemagglutination-inhibiting, or complement-fixing antibodies is shown, or when virus is isolated from pharynx or feces with a concurrent serum titer of 1:32 or greater of type-specific, IgM-neutralizing or hemagglutination-inhibiting antibodies. Schmidt and colleagues[160] stress the usefulness of the IgM-specific antibody titer. Coxsackieviruses B1, B3, B4, B5, and B6 are identifiable with this method. Immunofluorescent methods may be used during histologic analysis to identify specific antigens in the myocardium.[26] In chronic illness, attempts at virologic identification are less fruitful.[69]

Blood for aerobic and anaerobic cultures should be obtained from any infant with fever and signs of compromised cardiovascular function. The erythrocyte sedimentation rate and white blood cell count usually are elevated in acute myocarditis; occasionally, a leukemoid reaction may be identified.[3] A normal value for these tests does not exclude myocarditis. Elevations of serum glutamic oxaloacetic and glutamic pyruvic transaminase levels have been detected, especially in diphtheritic myocarditis, but they may be elevated in any patient with acute myocardial damage. Although a high level of serum transaminase activity usually is an ominous prognostic sign, Tahernia[170] found the ECG to be a more sensitive indicator of the ultimate outcome in children with diphtheritic myocarditis. Creatine phosphokinase and lactate dehydrogenase enzymes also should be measured.

DIFFERENTIAL DIAGNOSIS

Any cause of circulatory failure, especially when acute in onset, may mimic myocarditis. In newborns, heart failure associated with hypoxia, hypoglycemia, and hypocalcemia is well recognized, whereas circulatory collapse may occur with any infection and without direct involvement of the myocardium. A careful history may help to elucidate possible precipitating factors. Biochemical investigations to exclude hypoglycemia and hypocalcemia always should be conducted in any newborn who has signs of heart failure. Blood cultures should be obtained when infection is suspected.

Many infants with structural cardiac defects (e.g., hypoplastic left heart syndrome, aortic valve stenosis) may not have audible murmurs when severely ill. Murmurs usually appear, however, with treatment and improvement in cardiac function. The precordium usually is hyperactive, rather than quiet, and the heart sounds are clear and increased in intensity, rather than muffled. The ECG usually shows severe right ventricular hypertrophy in the former condition and shows right ventricular or left ventricular hypertrophy in the latter; the ECG is useful in the differential diagnosis. The findings on an echocardiogram often are diagnostic.

Beyond the immediate neonatal period, the major disease entities that require differentiation from myocarditis are endocardial fibroelastosis, anomalous left coronary artery arising from the pulmonary artery, Cori type II glycogen storage disease (i.e., Pompe disease), medial necrosis of the coronary arteries, left atrial myxoma,[128] and other congestive cardiomyopathies of undetermined cause. Common to all of these disorders is moderate to severe cardiomegaly, usually associated with congestive cardiac failure, gallop rhythm, and the infrequent occurrence or absence of murmurs. The murmurs are associated primarily with an anomalous left coronary artery and endocardial fibroelastosis. They are not more than grade 3/6 in intensity, are high-pitched, are apically located, and represent some degree of mitral insufficiency. Idiopathic myocarditis occurs primarily in patients older than 6 months of age,[149,150] whereas most of the conditions described earlier manifest before the infant is 6 months old.

Endocardial fibroelastosis, a common cause of congestive cardiac failure in infants, is impossible to differentiate from acute myocarditis on the basis of clinical examination alone. An anomalous origin of the left coronary artery should be identified. The ECG usually shows left axis deviation of the QRS complex in the frontal plane, left ventricular hypertrophy, and a pattern of anterolateral myocardial infarction. It is recognized as a QR pattern with inverted T waves in standard leads I and AVL, a broad Q wave with inverted T waves in precordial leads V_5 and V_6, and loss of anterior forces in the mid-precordial leads. For definitive diagnosis, cardiac catheterization is essential.

Pericarditis, frequently caused by viruses, usually occurs in children rather than in infants. The clinical history may be identical to that of patients with myocarditis; however, considering the degree of cardiomegaly, cardiovascular function is compromised less than in patients with myocarditis, although cardiac tamponade may occur in some cases. Differentiation from myocarditis may be made clinically if the patient has a friction rub, no gallop rhythm, and a typical pattern of chest pain. Further studies may be required, however, to make a conclusive diagnosis. The echocardiogram is invaluable in establishing this diagnosis. It is the most sensitive and least traumatic technique available and easily identifies an effusion. Myocarditis and pericarditis may occur together. This combination is seen most frequently in the pancarditis of rheumatic fever, but it also may occur in coxsackievirus B infections and in many collagen vascular and autoimmune diseases. Myocarditis has been associated with rheumatoid arthritis,[120] systemic lupus erythematosus,[44] and ulcerative colitis.[56,126]

TREATMENT

STANDARD APPROACHES

Intensive medical care is required during the acute stage of the illness. Heart rate, respiratory rate, and blood pressure should be monitored frequently, and a careful assessment of urine output and fluid intake is mandatory. All patients require bed rest. Experimental studies in mice have shown that exercise increases replication of virus in the myocardium and increases the mortality rate from myocarditis by 100 percent.[61] Although extrapolating directly from experimental studies in animals to the human situation always is dangerous, suggesting strict bed rest during the early stages of acute myocarditis seems to be a prudent measure. For infants or children with signs of congestive cardiac failure or shock, oxygen should be administered to maintain a normal arterial blood oxygen tension.

No specific therapeutic modality is known that can reverse the myocardial injury directly, but much can be done to maintain adequate tissue perfusion, prevent metabolic disturbances, and support myocardial function. When congestive cardiac failure is identified, digitalis should be administered. This agent should be used with caution, given the increased expression of proinflammatory cytokine and increased mortality rates observed in murine myocarditis treated with high-dose digitalis.[116] Diuretics also are used frequently to treat cardiac failure. Diuretics have no direct beneficial effect on the myocardium; they should be used cautiously because rapid reduction in extracellular fluid volume may lead to shock, and the loss of potassium associated with vigorous diuresis may precipitate digitalis toxicity. The frequency of administration depends on the clinical state of the patient.

In some instances, especially in newborns, the primary presentation may be shock. The blood pressure usually is maintained close to normal levels until late in the course of disease; it cannot be a reliable index of the severity of the patient's condition. Cold extremities, increasing heart rate, and low urine output are much more sensitive indicators of a reduction in effective circulating blood volume. Although the hearts of these infants and children respond poorly to volume loading, a colloid transfusion may help in selected patients. Albumin as a 5 percent solution in Ringer's lactate or whole blood may be administered. The total amount administered should be guided by the response of the patient in terms of perfusion, urine output, heart rate, and central venous pressure.

These patients may require a high filling pressure (e.g., 12 to 18 mm Hg) to achieve any cardiac output, compared with 5 mm Hg, which would achieve sufficient filling in the normal heart. If the patient remains in shock despite administration of these high filling pressures, a positive inotropic agent is required. Dopamine exerts an inotropic effect on the heart and concomitantly dilates the renal vessels, improving urine output. The usual dose is 2 to 10 μg/kg/min. As the dose increases to 20 μg/kg/min, dopamine has a more dominant alpha-adrenergic effect and may increase systemic peripheral resistance; experts avoid doses greater than 15 μg/kg/min. Dobutamine, which is a sympathomimetic amine that stimulates $beta_1$-adrenergic, $beta_2$-adrenergic, and alpha-adrenergic receptors, may be useful when used in combination with dopamine. It has significant inotropic activity while decreasing left ventricular filling pressure. Dobutamine does not induce the positive chronotropic effect and increased ventricular irritability that are seen with dopamine. Used in combination with low doses of dopamine (<10 μg/kg/min), dobutamine (in doses ≤10 μg/kg/min) may result in significant positive inotropism, while preventing a sinus tachycardia that may compromise cardiac output further.

Isoproterenol, a commonly used inotropic agent, should be avoided because it causes a significant increase in heart rate and may affect cardiac function adversely. When these drugs are used,

ensuring normal acid-base balance is important because action of the agents is decreased significantly by acidosis.

Sodium nitroprusside, phentolamine, and the nitrates have been used in adults. These agents have been used less extensively in children, primarily in the early postoperative period after open heart surgery, to improve cardiac function. They improve cardiac output by indirectly reducing systemic arterial resistance or venous filling pressure, or both. One study showed a marked improvement in the reduction of inflammation, necrosis, and dystrophic calcification in mice infected with coxsackievirus B3 when they were treated with captopril, an angiotensin-converting enzyme inhibitor, early after development of infection.[148] Besides its known afterload-reducing effects, this agent, which contains sulfhydryl groups in its chemical structure, is capable of scavenging oxygen free radicals in vitro. This ability may enable the drug to reduce myocyte damage by free radicals during the acute phase of the infection.

Another study failed to show any significant correlation between improved ventricular function and histologic improvement in patients with myocarditis treated with immunosuppressive therapy. Rather, an increase in the ejection fraction during the first 3 months of therapy seemed predictive of a good outcome.[39] Intravenously administered afterload-reducing agents should not be used, unless facilities are available for constant monitoring of the left ventricular filling pressure (i.e., mean wedge pressure) and unless that pressure is elevated.

Arrhythmias should be recognized and treated vigorously. Digitalis should be used with caution, and intravenous administration of this drug should be used only if the oral route is impossible. Rather than used as inotropic support, digitalis can be used to help control supraventricular arrhythmias. Levels must be monitored, especially when renal function is questionable. Lidocaine should be administered intravenously for acute treatment of complex ventricular arrhythmias, such as couplets and ventricular tachycardia. In cases of intractable ventricular arrhythmias, intravenously administered procainamide can be used. Monitoring the serum level on a daily basis beginning several hours after the drip is started is essential to prevent toxic side effects. Alternatively, amiodarone given intravenously can be used for life-threatening ventricular arrhythmias. Measures of contractility using echocardiographic techniques also are vital because procainamide is a negative inotrope.

A temporary pacing catheter should be inserted when complete heart block occurs. In many instances, the temporary pacing catheter allows time for spontaneous recovery of atrioventricular conduction. A permanent pacemaker may be required; if so, it should be inserted as an elective, rather than emergency, procedure. Only a demand pacemaker should be used because in most instances a return to normal rhythm is expected; use of this type of pacemaker avoids the risk of competition between the patient's inherent rhythm and the pacemaker, as would occur with a fixed-rate device. If infants or children experience Stokes-Adams attacks, a transthoracic pacing wire may have to be inserted as an emergency procedure.

Antibiotics should not be given routinely, unless a bacterial infection is suspected. Appropriate cultures need to be obtained before use of antibiotics.

The use of immunosuppressive agents in the treatment of viral or suspected viral myocarditis is controversial. Immunosuppressant therapy in animal models of viral myocarditis has not been shown to be beneficial. One reason for the lack of efficacy of immunosuppressant therapies may relate to the duality of the effects of the immune system. Corticosteroids enhanced viral titers in the early phase of viral murine myocarditis, whereas cyclosporine caused greater mortality and cardiac insufficiency in encephalomyocarditis virus myocarditis.[123,177] Other studies suggested an exacerbation of virus-induced cytotoxicity when such agents were given in the acute setting and possible interference with the production of interferon.[93,94] A report of 13 children with biopsy-confirmed myocarditis treated with prednisone approximately 3 weeks after symptoms occurred showed a marked reduction in inflammation on the follow-up biopsy specimen.[31] This study was uncontrolled, however, and may not truly represent an accurate account of the role of immunosuppressive therapy.

Mason and colleagues[113] used endomyocardial biopsy as a means of diagnosing and following the effects of immunosuppressive therapy in 10 patients. Eight patients received a combination of prednisone and azathioprine, and two patients received prednisone alone. Four patients improved clinically and histologically while on therapy. Two patients who had medications discontinued had relapses, which were reversed with reinstitution of therapy. Only one patient worsened while on therapy, and that patient died. Although the study by Mason and colleagues[113] was uncontrolled, the reversal of congestive heart failure seen in the two patients who were restarted on therapy suggests the beneficial effect of these agents.

Daly and colleagues[36] treated nine patients using combined immunosuppressive therapy with prednisolone and azathioprine. Seven of the nine patients showed definite hemodynamic and histologic improvement after 2 months of therapy. After 4 months off therapy, only four of the seven patients still showed significant improvement, however. One patient improved with reinstitution of therapy, and two patients deteriorated.

Dec and colleagues[41] treated nine patients in their study with single or combined immunosuppressive therapy. They saw improvement in 4 of 9 patients; however, 6 of 18 patients not receiving immunosuppressive agents also improved, nullifying any statistically significant difference between the two groups. Lymphocytic myocarditis was found in 6 of 12 patients with no obvious cardiac disease, but with high-grade ventricular dysrhythmias, and who had undergone right ventricular endomyocardial biopsy.[182] All six patients received combined immunosuppressive therapy with prednisone and azathioprine. At follow-up, five of six patients had been cured of the dysrhythmia, and active myocardial inflammation had disappeared, as confirmed by repeat biopsy. Although this study was uncontrolled, the fact that none of the patients progressed to a cardiomyopathic state or died of his or her illness suggests a beneficial effect.

Kereiakes and Parmley[91] tabulated most of the studies showing the effects of immunosuppressive therapy in patients with myocarditis. Sixty percent of 82 biopsy-confirmed cases of myocarditis showed improvement with steroids alone or in combination with azathioprine. Patients with lower grade inflammatory changes seem to do better than did patients with higher grade changes. Complications of immunosuppressive therapy, including opportunistic infections and a cushingoid state, have been reported and may limit the amount and type of therapy.[113,168]

Hobbs and associates[78] used combined prednisone and azathioprine to treat 34 adults with biopsy-confirmed myocarditis. Survival was no better for patients with histologic improvement than for patients with persistent infiltrates. Most patients experienced side effects, some of them lethal, from the corticosteroids. The potential benefits of this type of therapy must be weighed against the risks of immunosuppression in each patient with myocarditis. This therapy apparently does not prevent the observed ECG changes seen in untreated patients or the associated neuritis.[175]

Although numerous anecdotal and small case series suggested that patients with viral myocarditis might benefit from early steroid or immunosuppressive therapy, the results of the Myocarditis Treatment Trial suggested that treating patients who received a histopathologic diagnosis of myocarditis with immunosuppressive therapy for 24 weeks (prednisone plus cyclosporine or prednisone plus azathioprine) did not lead to an

improvement in ejection fraction compared with conventional therapy.[114] The major limitation of this study was the unexpectedly low rate of positive biopsy specimens (<10%) and the extension of enrollment to 2 years after the initial clinical presentation. For many patients, the disease possibly already had progressed from ongoing immune-mediated cardiac injury to DCM, and any form of therapy may have been ineffective in this setting.

Various immunomodulatory therapies have been proposed for the autoimmune phase of viral myocarditis. Kishimoto and colleagues[98] found that immunoglobulin therapy suppressed coxsackievirus B3-induced murine myocarditis and increased survival in C3H/He mice after infection with encephalomyocarditis virus. Intravenous immunoglobulin (IVIG) therapy reduced various proinflammatory markers, including TNF, interferon-γ, macrophage inflammatory protein-2, interleukin-6, plasma catecholamines, and soluble intercellular adhesion molecule-1.[98] Exogenous immunoglobulins modulate diverse immune response mechanisms; however, the exact mechanisms by which IVIG modifies myocarditis remains to be determined. Drucker and associates,[47] who investigated the use of IVIG in 21 of 46 children with myocarditis, showed that patients who received this drug had better left ventricular function at follow-up. Survival tended to be higher at 1 year, although the data did not reach statistical significance because of the small number of patients in the study. More recently, in a prospective placebo-controlled trial with IVIG in adult patients with recent-onset DCM or myocarditis, treatment with IVIG did not result in an improvement in left ventricular function compared with placebo-treated patients.[118]

At present, no form of immunosuppressant therapy has proved to be effective in the clinical setting. Routine immunosuppressive therapy no longer is recommended for patients with myocarditis and a stable clinical course. Many clinicians still recommend aggressive immunosuppressive therapy, however, for patients with fulminant myocarditis or a deteriorating clinical course or both.

PROGNOSIS

The prognosis of acute myocarditis caused by coxsackievirus B infection in a newborn is poor. Kibrick and Benirschke[92] reported a 75 percent mortality rate among 25 infants with coxsackievirus B myocarditis. The greatest number of deaths occurred in the first week of the illness. No apparent sequelae occurred in the six infants who survived, although no long-term follow-up data were available. The outlook in other infants and children with clinically recognized myocarditis is better, but mortality rates remain significant (10-25%). Hastreiter and Miller[73] observed complete recovery in 50 percent of patients. Another 25 percent became asymptomatic, but abnormal ECGs or chest radiographs persisted. An abnormality may not be evident on the ECG unless the patients are exercised.[16] Despite lack of symptoms, many adult patients have a reduced working capacity associated with exercise stress testing.[18]

The outcome of myocarditis is related partly to the cause. Patients with diphtheritic myocarditis who have arrhythmias or conduction abnormalities have a very poor prognosis. Tahernia[170] found in his study that all patients with disturbances of conduction died. Begg[15] also reported a 100 percent mortality rate for patients with diphtheritic myocarditis who developed supraventricular tachycardia.

Chronic arrhythmias may persist long after the acute disease has passed. Friedman and colleagues[57] performed a retrospective analysis of 12 patients with biopsy-confirmed myocarditis and complex ventricular arrhythmias at the time of presentation (11 with ventricular tachycardia). Five of the 12 patients still were receiving antiarrhythmic therapy at a median follow-up of 50 months. Complex ventricular arrhythmias still were present in these patients (three with ventricular tachycardia and two with couplets or multiforms), requiring ongoing therapy. The investigators concluded that although the arrhythmias were controlled more easily than at presentation of the patients, ongoing surveillance was essential in ensuring the suppression of these potentially life-threatening arrhythmias. Children who recover from myocarditis, regardless of cause, should be followed indefinitely.

MYOCARDITIS IN CASES OF HUMAN IMMUNODEFICIENCY VIRUS INFECTION

Infection with human immunodeficiency virus (HIV) may affect the heart adversely. Cardiac dysfunction, including congestive heart failure, may occur; many patients who have died and undergone autopsy are shown to have myocarditis. Anderson and associates[6] retrospectively analyzed 71 consecutive necropsy patients who died of acquired immunodeficiency syndrome (AIDS) and found that 52 percent had evidence of myocarditis. Opportunistic agents could account for only a few of the cases, and most were considered idiopathic.

Another study examined autopsy specimens of 26 consecutive cases. Lymphocytic myocarditis was seen in nine patients (35%), and another seven patients had lymphocytic infiltrates without myocytolysis.[14] Acierno[2] correctly pointed out that a distinction must be made between AIDS-associated myocarditis and secondary myocarditis caused by known pathogens or idiopathic myocarditis.

Reilly and colleagues[147] found a 45 percent incidence of myocarditis in 58 consecutive autopsy cases. Congestive heart failure, ventricular tachycardia, and other ECG abnormalities were seen in nearly 60 percent of the patients. Two patients died suddenly, both with myocarditis. In a prospective study of asymptomatic HIV-infected patients, the reported mean annual incidence of progression to DCM was 15.9 cases per 1000 patients. Endomyocardial biopsy specimens revealed myocardial inflammation in 63 of 76 (83%) of these high-risk patients. HIV-infected cardiac myocytes were detected by in situ hybridization in 58 of 76 (76%) patients.

In a prospective multicenter study of 205 vertically HIV-infected children enrolled at a median age of 1.9 years and 600 HIV-exposed children enrolled prenatally or as neonates, the 5-year cumulative incidence of cardiac dysfunction ranged from 18 to 39 percent in HIV-infected children.[167] Myocardial inflammation, with or without cell destruction, is a frequent finding in patients infected with HIV. Whether this response is due to the virus itself or to opportunistic agents or other toxic reactions is unclear. Specific therapy for myocarditis in these patients has not been elucidated, and no recommendations, other than inotropic support with anticongestive and afterload-reducing agents, can be made.

PARASITIC MYOCARDITIS

Parasitic myocarditis is an uncommon form of heart disease in the United States. Chagas disease is caused by infection with *Trypanosoma cruzi*, however, and it is a long-lived infection that affects approximately 17 million individuals in Latin America.[152] Although several countries in Latin America have well-established vector-control programs, contact with contaminated feces or urine from infected triatomine insects remains the main mode of infection.

The major cardiovascular manifestation of Chagas disease is an extensive myocarditis that typically becomes evident years or decades after the initial infection. The disease is transmitted to

humans by various species of blood-sucking reduviid insects. After inoculation, the protozoa multiply and migrate widely throughout the body. Host control of this parasite has been shown to depend on humoral and cell-mediated adaptive responses and elements of the innate immune system.[65]

More recently, a role for TLR signaling in resistance to *T. cruzi* is suggested by the observations that mice deficient in MyD88, an adapter molecule required for signaling events by most TLRs, show enhanced susceptibility to infection with this protozoan parasite.[28] Acute Chagas disease usually is an illness of children, but it can occur at any age.[122] Histologic examination of the heart during the acute phase reveals intracellular parasites with a marked cellular infiltrate, particularly around myocytes that have ruptured and released the parasites.[138] A well-established fact is that intracellular parasites are found in cardiac myocytes only during the acute phase of illness.

Severe myocarditis develops in only a few acute cases, and most deaths are caused by the resultant congestive heart failure and pericardial effusion. Nonspecific ECG changes are seen, but the life-threatening arrhythmias that are frequent occurrences in chronic Chagas disease generally do not occur. In most patients with more acute disease (90% of cases), symptoms resolve gradually over weeks to months.

Chronic progressive Chagas disease develops in 10 to 20 percent of previously asymptomatically infected individuals.[49] It is manifested by a chronic, diffuse, progressive fibrosing myocarditis that involves the myocytes and the atrioventricular conduction system.[124,125] On gross examination, the heart usually is enlarged and flaccid. Thrombus formation frequently occurs, and thrombus may fill much of the apex of the left ventricle in some cases.

Immune-mediated cardiac injury, caused primarily by infiltrating mononuclear cells, probably is the main mechanism responsible for the development of chronic Chagas heart disease. This hypothesis is supported by several observations. Animal models of *T. cruzi* infection have shown lysis of nonparasitized cardiac myocytes by immune effector cells[8]; depletion of $CD4^+$ T-lymphocyte subpopulations abrogates myocardial injury in a murine model of chronic Chagas disease; and myocardial damage can be induced in healthy animals by passive transfer of $CD4^+$ T cells from *T. cruzi*–infected mice.[45] Although $CD4^+$ T cells seem to be crucial in myocardial injury, $CD8^+$ T cells may have a protective role. Mice depleted of $CD8^+$ T cells have a robust parasitemia, but almost no inflammatory infiltrates are seen in the parasite-infected tissues.[172]

Histologic examination reveals focal but widespread areas of cellular infiltrates composed of plasma cells, eosinophils, mast cells, and macrophages.[124] Extensive fibrosis occurs, replacing previously damaged myocardial tissue. In contrast to the situation observed in acute disease, the presence of parasites (a rare finding) in tissue has little correlation with myocardial pathology. Inflammatory changes in the right bundle branch and the anterior fascicle of the left bundle branch explain the frequent occurrence of right bundle branch and left anterior fascicular block.[125]

The clinical manifestations of chronic Chagas disease range from isolated rhythm disturbances to advanced disease characterized by cardiomegaly, chronic congestive heart failure, and arrhythmias. Syncope also is a frequent problem of the disease. In one series of 53 patients with chronic Chagas disease, the most frequent causes of recurrent syncope were ventricular tachycardia (43%) with a poor prognosis and paroxysmal atrioventricular block (21%) with a favorable prognosis.[112] Sudden death caused by ventricular fibrillation is a constant threat and may develop before cardiomegaly or heart failure is diagnosed.[30]

The diagnosis of chronic Chagas disease cannot be made solely on the basis of histologic examination of the heart. Serologic testing is the method of choice for establishing the diagnosis of chronic Chagas disease. Several highly sensitive serologic tests for the detection of anti–*T. cruzi* antibodies, such as indirect hemagglutination, complement fixation, indirect immunofluorescence, and enzyme-linked immunosorbent assay, are available.[95,163]

Benznidazole and nifurtimox are the only available drugs with activity against *T. cruzi*. Although these two drugs have been thought to be ineffective or too toxic, or both, for treating chronic infections, in one report a 60-day course of benznidazole therapy eliminated infection in more than 60 percent of chronically infected children.[7] The current emphasis on the management of Chagas disease is on prevention of infection. In 1991, the Latin American countries of Argentina, Bolivia, Brazil, Chile, Paraguay, and Uruguay initiated a program to decrease transmission of the parasite through vector control and the screening of blood donors. According to more recent studies, transmission has been virtually eliminated in much of the region.[196]

Myocarditis is one of the most serious complications of trichinosis. The disease develops when undercooked meat contaminated with infective larvae of *Trichinella* is eaten. Myocardial invasion by *Trichinella spiralis* has been well described, but encystment within the myocardium has been reported only rarely.[96] At autopsy, the heart may be dilated and a pericardial effusion may be identified. Histologically, a prominent focal infiltrate composed of lymphocytes and eosinophils with interstitial edema and scattered hemorrhages commonly is found.[165]

Myocarditis usually is mild, with few clinical signs and symptoms. This myocarditis may range from chest pain to fatal congestive heart failure, however, and may mimic acute myocardial infarction.[34,68] The frequencies of ECG abnormalities among 154 cases of trichinellosis were determined to be 56 percent.[143] The abnormalities on ECG most frequently observed were a nonspecific ventricular repolarization disturbance (with ST-T wave changes), followed by bundle branch conduction disturbances, and sinus tachycardia. Despite ECG evidence of myocardial involvement, less than 0.1 percent of patients with trichinosis die of this complication.[68]

The definitive diagnosis of trichinosis is based on the presence of the larval forms in tissue biopsy samples, usually from a large tender area such as the gastrocnemius muscle. Serologic testing is available through state laboratories and the Centers for Disease Control and Prevention. Typically, serum antibody titers become positive during or after the third week of illness. The efficacy of mebendazole or albendazole in the treatment of myocarditis caused by *Trichinella* infection has not been evaluated adequately.

Toxocara canis, the principal cause of visceral larva migrans, is a rare cause of myocarditis. Most reported cases have occurred in children younger than 3 years of age.[37,181] Children especially are susceptible to infection with *Toxocara* because of their habit of crawling on the ground and putting objects into their mouths. The myocardial lesions noted on histologic examination have included granulomata and extensive eosinophilic infiltrates with foci of muscle necrosis. The clinical presentation may be acute respiratory distress caused by congestive heart failure, requiring administration of oxygen and diuretic therapy. Asymptomatic infection involving the heart also has been reported.[37]

The definitive diagnosis of *T. canis* infection requires microscopic identification of the larvae in biopsy specimens of the liver or heart, but this finding is infrequent. An enzyme immunoassay for *Toxocara* serum antibodies, which is available at the Centers for Disease Control and Prevention, can provide presumptive evidence of toxocariasis.

Toxoplasma gondii may cause myocarditis as part of disseminated infection or, less frequently, as an isolated cardiac infection. In infants with congenital toxoplasmosis, the clinical manifestations usually are those of meningoencephalitis, but at autopsy, extensive myocardial involvement has been documented.[199] Outside the newborn period, infection with this intracellular

parasite most commonly occurs in immunosuppressed patients with malignant diseases, patients with AIDS,[29] and patients who have undergone cardiac or bone marrow transplantation.[109] Histologic examination of the heart reveals focal interstitial infiltrates consisting of histiocytes, lymphocytes, plasma cells, eosinophils, and very few polymorphonuclear cells.[174] *Toxoplasma* is seen as basophilic masses within a pseudocyst in normal or damaged myocardial fibers.

Clinical manifestations may include arrhythmias (atrial and ventricular), atrioventricular block, atypical chest pain, pericarditis, and heart failure. The diagnosis of toxoplasmic myocarditis requires the exclusion of other specific forms of heart disease and the establishment of evidence of toxoplasmosis with serologic testing. The diagnosis may be aided by endomyocardial biopsy.[109] Treatment with pyrimethamine and sulfonamides (especially sulfadiazine) has been reported in patients with isolated toxoplasmic myocarditis, but the response to therapy has varied. In one series of toxoplasmic myocarditis, relapses occurred in 17 percent of cases after therapy.[101]

Myocardial disease also has been reported after infection with *Echinococcus granulosus* and *Plasmodium falciparum*.[96] Cardiac involvement is estimated to occur in less than 2 percent of cases of echinococcosis.[43] When it does occur, the cysts usually are located in the intramyocardial region and protrude into the adjacent cardiac chambers. The clinical manifestations depend primarily on the location and size of the cyst. Rupture of the cyst is the most dreaded complication because it may lead to pericarditis, anaphylactic shock, or pulmonary emboli. Two-dimensional echocardiography is the preferred imaging study to detect and localize cysts.[133] Myocardial changes also have been documented in fatal malaria, particularly when caused by *P. falciparum*. Histologically, blocking of the coronary arteries and capillaries with parasites, local hemorrhage, and deposit of pigment occurs. Clinical findings suggestive of cardiac involvement are rare, however. In a series of 49 patients with falciparum malaria, no ECG evidence of cardiac involvement was found.[166]

Myocarditis also has occurred in association with primary amebic meningoencephalitis caused by *Naegleria*. In a retrospective study, focal or diffuse myocarditis was documented in more than 40 percent of cases.[110] Myocardial involvement is not a clinically significant manifestation of this uniformly fatal central nervous system infection.

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CHAPTER 35

Acute Rheumatic Fever

Diana R. Lennon

Acute rheumatic fever is an inflammatory disease of the heart, joints, central nervous system, and subcutaneous tissues that develops after a nasopharyngeal infection by one of the group A beta-hemolytic streptococci. The pathogenesis of this disease, a clinical syndrome without a specific diagnostic test, remains an enigma, and specific treatment is unavailable. Prevention of initial and recurrent attacks is possible, however, with penicillin prophylaxis. Rheumatic fever is especially important because of the heart disease that often ensues, and such disease may lead to chronic progressive damage and premature death. As succinctly stated by Lasegue many years ago, "Rheumatic fever licks the joints and bites the heart"[186]—a statement that holds true today.

The unexpected upsurge in this disease in the United States in the late 1980s and 1990s and reports of increasing numbers of invasive group A streptococcal infections have renewed interest in group A streptococci and their abilities. In addition, recognition that rheumatic fever is the leading cause of acquired heart disease in children and young adults worldwide has led to action.[147]

EPIDEMIOLOGY

The overall incidence and severity of acute rheumatic fever have decreased in recent years in developed Western countries and in prosperous countries of Asia.[2] Reliable morbidity data on the occurrence of acute rheumatic fever in total populations are lacking because studies often consider only a segment of a population. The trend seems clear, however. Some of the best long-term data come from Denmark, where rheumatic fever has been a reportable disease for many years[201]; a steady decline has been occurring since 1900, except for a peak during World War II (Fig. 35–1). In the United States, rheumatic fever is not a reportable disease, but mortality rates (Fig. 35–2) and hospital discharge rates (Fig. 35–3 and Table 35–1) have shown a steady decline. Although this decline was already under way, it seems to have been accelerated by the introduction of penicillin.[126]

The dramatic decline in the incidence of rheumatic fever began in the United States in the late 1940s (see Fig. 35–2). During the late 1950s, the 1960s, and the early 1970s, studies in the United States showed annual rates of 13.5 to 62.5 first attacks per 100,000 children 5 to 14 years of age; however, these studies are not strictly comparable in design.[35,52,82,150,170,176] Some of them were conducted in low-income urban areas, locations in which the incidence of rheumatic fever was thought to be higher. Secondary prevention of rheumatic fever with penicillin prophylaxis to protect against recurrent attacks probably became widespread in the 1960s. Denny and colleagues[64] showed the possibility of preventing initial attacks with injectable penicillin in 1950 in military camps. Similar conclusive controlled studies were not repeated in the general or pediatric populations or with oral penicillin.

Figure 35–1 Reported annual incidence of acute rheumatic fever in Denmark, 1862 to 1962. *(Adapted from Public Health Board of Denmark: Reported rheumatic fever incidence in Denmark, 1862-1962.* In *Vendsborg, P., Fauerholdt, L., and Olsen, K. H.: Decreasing incidence of a history of acute rheumatic fever in chronic rheumatic heart disease. Cardiologica 53:332-340, 1968. Used with permission of S. Karger A. G., Basel.)*

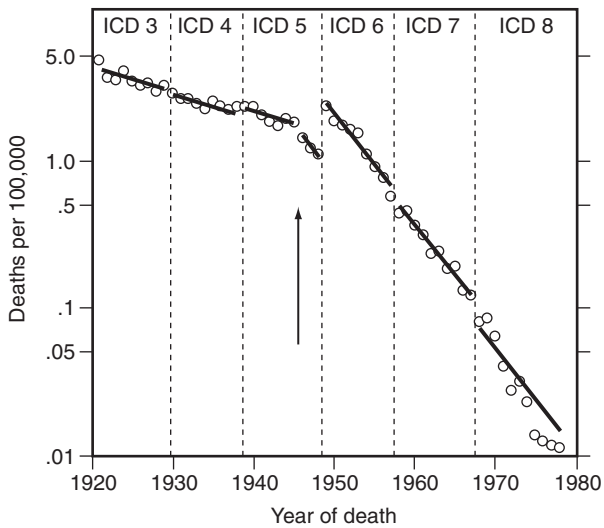

Figure 35–2 U.S. national mortality rates from rheumatic fever in individuals 5 to 19 years old, 1921 through 1978. Values are age-adjusted to the 1950 U.S. population. Trend lines are fitted for each era (1921 through 1945 and 1946 through 1978) separately and take International Classification of Diseases (ICD) revisions into account. The *arrow* separates the two eras. *(From Massell, B. F., Chute, C. G., Walker, A. M., et al.: Penicillin and the marked decrease in morbidity and mortality from rheumatic fever in the United States. N. Engl. J. Med. 318:280-286, 1988.)*

The most compelling evidence that appropriate medical intervention helps reduce the number of initial attacks of rheumatic fever comes from a study by Gordis[82] of health care availability in an inner-city Baltimore population at risk. The rate of acute rheumatic fever was reduced by 60 percent over the course of a decade in only the census tracts receiving a comprehensive care program, with that reduction occurring only in patients with an identifiable preceding clinical respiratory infection. A similar trend with small numbers of patients was seen for the Navajo and Papago Native American populations in school-based intervention programs[14,52] and in an Alaskan program.[33] Other school-based interventions[155] have reduced streptococcal prevalence rates but have not gone the necessary further step to show a reduction in rheumatic fever morbidity in a controlled, carefully demarcated population.

An unexplained high incidence of acute rheumatic fever persists in Hawaii, especially in Polynesian and part-Polynesian children.[46] Similarly, rates are inexplicably higher in larger populations of Polynesian children in New Zealand.[113] In some areas of the continental United States, the rate of endemic rheumatic fever continues to exceed that of the general population in some children traditionally considered to be at risk (urban African-Americans), although not in others (recent Hispanic immigrants).[70] In contrast, in a hospital-based study in another urban area, Hispanic children, reflecting the pediatric population being served, were the most affected.[88]

Since the beginning of 1985, the number of patients with acute rheumatic fever in several centers on the U.S. mainland has increased.* Although these numbers are not large, they represent a definite increase in the outbreak areas (Table 35–2).[193] The resurgence in Utah since 1985 has led to more than 600 cases.[130] Nationally, the number of diagnoses of acute rheumatic fever may have continued to decline gradually from 1984 through 1990.[193] The populations (aside from clusters in military populations)[10,204] generally do not seem to be those considered to have been at risk in the past: Most patients are white and middle class and live in suburban or rural communities with ready access to medical care. In the Utah and Tennessee outbreaks,[200,210] the families were larger than the state average. The military outbreaks[10,205] were the first in 2 decades in U.S. military personnel. Factors other than the widespread use of antibiotics and improved availability of health care may be important. Overcrowded living circumstances long have been considered to be a risk factor,[153] although this hypothesis was not substantiated as an important risk factor by conditional logistic regression in a modern-day study.[202]

The disquieting feature in these more recent outbreaks was the severity of the illness, especially in the Salt Lake City, Utah, outbreak: 72 percent of patients had clinical carditis (Table 35–3), 19 percent had severe carditis with or without congestive heart failure, and three patients required mitral valve replacement. Because this report was from a cardiology center, however, it may reflect a referral bias. Earlier U.S. reports documented clinical carditis in initial attacks of rheumatic fever in 40 to 51 percent

*See references 27, 51, 66, 93, 109, 138, 199, 200, 204, 205, 220.

Figure 35–3 Annual rates (per 100,000) of discharge of patients with rheumatic fever from short-stay nonfederal hospitals in the United States, 1965 to 1983. *(From National Hospital Discharge Survey, NCHS. Adapted from Gordis, L.: The virtual disappearance of rheumatic fever in the United States: Lessons in the rise and fall of disease. Circulation 72:1155-1162, 1985.)*

TABLE 35–1 Reported Incidence of Acute Rheumatic Fever in Studies in the United States, 1970 to 1981

Location	Years of Study	Rate/100,000	Age Range (yr)
Fairfax, VA	1970-1980	1.14	0-18
Rhode Island	1976-1980	0.23	5-17
Memphis, TN	1977-1981	1.88	5-17
Baltimore, MD	1977-1981	0.5	5-19
San Fernando, CA	1971-1980	0.63	5-17

Adapted from Veasy, L. G., Wiedmeier, S. E., Orsmond, G. S., et al.: Resurgence of acute rheumatic fever in the intermountain area of the U.S. N. Engl. J. Med. 316:421-427, 1987; and Markowitz M., and Kaplan, E. L.: Reappearance of rheumatic fever. Adv. Pediatr. 36:44, 1989.

TABLE 35–2 Reported Outbreaks of Acute Rheumatic Fever in the United States: Selected Epidemiologic Features

	Salt Lake City, UT[130,131]	Columbus, OH[60]	Akron, OH[32]	Pittsburgh, PA[132,144]	San Diego, CA[126]	Tennessee[139]*
Time	1985-1992	6/84-9/86	1986	1987-6/90	12/86-7/87	1/87-7/88
No. cases	274	40	23	60	50	26
White (%)	93	80	96	97	50	80
Family income	80% middle	73% middle	$20,000-$40,000	3 of 17 on assistance[132]	NA	$18,000
Suburban-rural residents	Most[130]	85%	Many	75%[137]	NA	20%
History of sore throat (*n*†)	77 (46)	22 (NA)	18 (NA)	4/17 (3/17)[132]; 28/43 (11/43)[144‡]	6 (3)	15 (7)
Family history of rheumatic fever (%)	NA	5	16	64[132]	NA	NA
Recurrences	27	1	0	2	1	0
M types of group A streptococci isolated from						
Patients	1 × M-1; 1 × M-5	NA	M-1, M-5, M-18	NA	§	Mucoid M-18/T-1; mucoid nontypeable
Families	3 × M-3; 1 × M-1; 1 × M-18; 1 × M-78	NA	M-6	NA	NA	NA
Community	9 × M-18 (8/9 mucoid); 1 × M-4, M-5, M-6, M-9, M-11, M-12; 6 nontypeable	Mucoid M-18	NA	NA	NA	Mucoid M-18

NA, not available.
**Family size greater than the state average.*
†*Number who sought treatment.*
‡*Respiratory illness.*
§*San Diego: five of seven available sera positive for antibodies to M-18; three of seven, positive for M-1 antibodies; two of seven, positive for M-5 and M-6 antibodies; and one of seven, positive for M-24 antibodies.*
Adapted from Veasy, L. G., Wiedmeier, S. E., Orsmond, G. S., et al.: Resurgence of acute rheumatic fever in the intermountain area of the U.S. N. Engl. J. Med. 316:421-427, 1987; and Markowitz, M., and Kaplan, E. L.: Reappearance of rheumatic fever. Adv. Pediatr. 36:39-68, 1989.

TABLE 35–3 Clinical Manifestations in Four Outbreaks of Acute Rheumatic Fever

Manifestation	Salt Lake City, UT, 1985-1992 (274 Patients) (%)	Columbus, OH, 1984-1986 (40 Patients) (%)	Northeastern Ohio, 1986 (23 Patients) (%)	Pittsburgh, PA, 1985-6/1990 (60 Patients) (%)	San Diego, CA, Naval Base, 1986-1987 (10 Patients) (%)	Tennessee, 1987-1988 (26 Patients) (%)
Arthritis	36	62	78	43	100	58
Carditis	68*	50	30	52	30	73
Chorea	37	17	9	37	0	31
Erythema marginatum	4	12	1	0	0	4
Subcutaneous nodules	3	0	0	0	10	0

**85 percent with Doppler ultrasound examination.*
Adapted from Markowitz, M., and Kaplan, E. L.: Reappearance of rheumatic fever. Adv. Pediatr. 36:39-68, 1989.

of patients (1951 through 1965).[125] Longitudinal observations at the same institution suggest a decreasing frequency of clinical carditis (73% from 1921 to 1930; 51% from 1951 to 1960).[32,131]

Rheumatic fever in its milder form with arthritis is the sole major manifestation without a serologic search for other causes of polyarthritis, or echocardiography may cause difficulty in making a diagnosis, and the patient may not be admitted to the hospital, which could affect estimates of rates of carditis in studies that are not population-based. With the use of Doppler echocardiography, carditis rates in the Utah patients increased to 91 percent (see Table 35–3). The duration of secondary penicillin prophylaxis and endocarditis prophylaxis is an important consideration for patients with nonclinical carditis. The place of echocardiography in the diagnosis and management of rheumatic fever is evolving (see section on laboratory findings).

A more recent assessment of hospitalized acute rheumatic fever disease in the United States in children younger than 21 years of age found a low rate of 14.8/100,000 hospitalized children (503 cases).[138] The prevalence of rheumatic heart disease, which represents the result of many years of exposure to the risks of acquiring acute rheumatic fever, seems to have declined in the United States over the course of many years.[143]

Many different racial and ethnic groups have been deemed unusually susceptible to rheumatic fever. They usually have been minority groups within a given area who are of lower socioeconomic status than the general population (e.g., Malays in Singapore, Arabs in Israel, Bantus in South Africa, Maori in New Zealand, African-Americans in the United States, aborigines in Australia).[2,39,124] In the United States, when differences in socioeconomic status or degree of crowding were taken into account,[85] the differences in the incidence of rheumatic fever,[84] prevalence of rheumatic heart disease,[143] and mortality from acute rheumatic fever and rheumatic heart disease generally declined or disappeared,[162] at least in the population studied. Rates in African-Americans were slow to decline, however. Rates became very low in the 1970s, at least in some areas, but cases persisted in other areas.[70,108]

Gordis and associates[83] caution that some other socioeconomically determined factor closely paralleling crowding could be the actual determinant of rheumatic fever. Most authorities agree that the reduction in the incidence and severity of rheumatic fever that has been noted in the United States and Western Europe might be due partly to a higher standard of living and less crowding. In a case-control study in the former Yugoslavia, home dampness, change of place of residence during the last 5 years, low maternal education, body weight below normal, frequent sore throats, and a positive family history of rheumatic fever were found to be significant risk factors.[202]

A relationship between group A streptococcal throat infection and rheumatic fever was recognized following observations of the latter occurring after outbreaks of scarlet fever.[150] The attack rate noted after group A streptococcal throat infection varied widely (from 3% at Warren Air Force Base[165] to 0.39% in Chicago children[178]). Further observations in the latter study revealed that exudative pharyngitis, a positive throat culture with persistence of group A streptococci beyond 21 days, and the development of significant antibody (antistreptolysin O [ASO]) responses were associated with a higher attack rate that approached 3 percent in the children studied.

Rheumatic fever, similar to streptococcal infection, occurs most commonly in children 5 to 15 years of age. First attacks of rheumatic fever rarely occur in children younger than 3 years of age or in adults older than 40 years because of the relative infrequency of streptococcal infection at these ages and perhaps other factors.

The incidence of acute rheumatic fever is highest in the spring and winter months in temperate zones and coincides with the seasonal variation in streptococcal pharyngitis. This incidence may be related to the greater tendency for spread of streptococcal infection by closer contact during the colder and damper months,[113,186,202] at least in some climates. In other climates, a seasonal peak for acute rheumatic fever is less pronounced.[31]

PATHOGENESIS

Evidence points toward acute rheumatic fever being preceded by a group A streptococcal upper respiratory tract infection. The events that occur after such infection and that culminate in rheumatic fever are poorly defined, suggesting a complex interaction of numerous factors. With the resurgence of interest in this disease after the outbreaks in the 1980s, laboratory data collected with the use of modern technologies have led to some new insights.[179,180] Pathogenesis involves the host, the environment (see section on epidemiology), and group A streptococci, individually and collectively.

So-called rheumatogenic strains of group A streptococci have been the subject of much discussion, more recently in relation to the latest focal upsurges in rheumatic fever.[25,29,97] Because no factor has been described or isolated, however, such strains remain a hypothesis. To date, rheumatic fever has been shown to occur only after nasopharyngeal infection,[206] although debate and research continue.[55,101] Why the site of infection seems to predispose individuals to the development of rheumatic fever remains an enigma, perhaps related to skin lipids.[100] Acute glomerulonephritis develops after skin or throat infections with a nephritogenic type of group A streptococci (e.g., M-49, M-12).[206]

Certain streptococcal M protein serotypes are implicated strongly and repetitively in epidemics of acute rheumatic fever. Serotypes M-3, M-5, M-14, M-18, and M-24 have been reported more than once in outbreaks, and M-1, M-6, M-19, M-27, and M-29 have been reported once only.[24] Distinct nucleotide sequences that determine different gene subfamilies encoding the M or M-like protein antigenic domain ("emm gene") may be the

cause of these variations in streptococcal rheumatogenic potential, depending on the site of infection.[23] Other equally prevalent M types rarely, if ever, have been associated with epidemics of the disease[24] or have failed to cause recurrences in susceptible patients.[28] The current resurgence lends limited support for this concept: No predominance of a single serotype within a specific geographic zone was identified in any of the published outbreaks (Table 35–4; see Table 35–2). Specific M types and their production of mucoidal colonies (see Table 35–4), considered to be related to the amount of M protein and virulence,[207] may be more relevant to epidemic than to endemic rheumatic fever.

In Auckland, New Zealand, an average of 45 new cases of acute rheumatic fever occur annually in children (annual age-specific rate of 20/100,000/yr). Nine years (1984 to 1992) of surveillance of group A streptococcal isolates from hospitalized pediatric patients (one centralized children's facility for 8 of the 9 years in question) yielded 2410 isolates. Only 3 of 38 throat isolates (32 from well-documented cases of rheumatic fever, 6 from siblings) were strains described as possibly rheumatogenic (one each of M-1, M-3, and M-6).[129] None was described as mucoidal.[129] In that series, M types 6, 53, 55, and 66 (and NZ 1437, now known as M-89, when sibling isolates were included as cases) were statistically more likely to be associated with a case of acute rheumatic fever. Both streptococcal collections[97,129] (see Table 35–4) are limited samples and may not be representative. In addition, as in most series, group A streptococcal isolates are isolated from a few cases and are not supported by streptococcal or type-specific antibody data.

Strain selectivity is a further consideration. No documented evidence has shown that all members of an M type may be equally able to elicit acute rheumatic fever. Some streptococci from a particular serotype may be associated with acute rheumatic fever and acute poststreptococcal glomerulonephritis, although the two sequelae rarely occur simultaneously.[120,128,129] M types have been shown to be composed of genetically diverse streptococci, not all of which may be established within a community.[128] Genetic analysis of serotype M18 from acute rheumatic fever cases in Utah separated by 12 years showed strains nearly genetically identical.[179] The M type denotes possibly nothing more than a shared type-specific marker, with the property of rheumatogenicity as yet remaining elusive. Streptococcal strains that are opacity factor–negative (a lipoprotein lipase) are unlikely to be rheumatogenic, according to some investigators (see Table 35–4).[97,210] This finding does not hold up in all geographic areas.[129] Surveillance of group A streptococci in different geographic zones must be encouraged to guide vaccine development.

TABLE 35–4 Group A Streptococci Isolated from Patients with Rheumatic Fever and from Their Siblings (1986 to May 1988)

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Please refer to the printed publication.

From Kaplan, E. L., Johnson, D. R., and Cleary, P. P.: Group A streptococcal serotypes isolated from patients and sibling contacts during the resurgence of rheumatic fever in the U.S. in the mid-1980s. J. Infect. Dis. 159:*101-103, 1989.*

Although current evidence strongly implicates an immunologic mechanism in the pathophysiology of rheumatic fever, the details of how the disease develops are unclear.[26,53,130,184] Evidence to date strongly suggests an abnormal cell-mediated and humoral immune response to cell membrane streptococcal antigens, which, because of molecular mimicry of human tissues, may result in continued damage to the cardiovascular and nervous systems.[36,184] The findings of circulating immune complexes in most patients[54] and the deposition of C3 and immunoglobulin in the myocardium of patients dying of acute rheumatic fever support an abnormal immune response in rheumatic fever.[102]

M proteins from some rheumatogenic group A streptococcal types share antigenic determinants with myosin, with the sarcolemma of cardiac muscle,[60,61] and with antigens of articular cartilage and synovium.[16] The immune response to streptococci may mistake the host antigens as foreign and result in tissue damage. Other streptococcal antigens, such as the group A carbohydrate component, are candidates for mistaken cross-reaction with a glycoprotein in human heart valves.[81] Group A streptococci have components that can amplify or down-regulate the immune response.[36] The mechanisms for molecular mimicry leading to central nervous system dysfunction are less defined.[130]

The site of the initial streptococcal infection may be important—lymphatic channels have been shown between the tonsils and the heart.[36] Unusual compartmentalization of rheumatic antigen–positive non–T cells has been shown in patients with acute rheumatic fever, with no positive cells detected in rheumatic tonsils but increased numbers in peripheral blood.[86]

Cell-mediated immunity to streptococcal antigens also is enhanced in patients with rheumatic fever.[184] The lymphocytic infiltrate of heart valves was found to be composed predominantly of $CD4^+$ helper cells.[163] Increased expression of HLA-DR on fibroblasts, which can present antigens to $CD4^+$ lymphocytes (cytotoxic/suppressor T cells), has been observed on the heart valves of patients with acute carditis.[66] The cytotoxicity induced in normal human helper and suppressor cells in vitro by purified protein from a type M-5 group A streptococcal organism has been shown to destroy several human cell types, including cultured myocardial cells.[62] T-cell subset study results are conflicting,[141,184] but production of interleukins is reported to be enhanced.[141,142,221] The role of M protein and streptococcal pyrogenic exotoxins as superantigens is being explored and perhaps might explain the exaggeration of the streptococcal immune response.[127,195]

The genetic background of the human host seems to influence susceptibility to rheumatic fever. Aggregation of rheumatic fever cases in families has been recognized for some time.[156] Low concordance for inheritance has been reported in monozygotic twins,[192] although affected siblings have significant concordance for arthritis, residual rheumatic heart disease, and chorea.[181] Patarroyo and associates[149] found that the B lymphocytes of patients with rheumatic fever have a specific marker (883 alloantigen) associated with host rheumatic susceptibility.[218,219] It seems to transcend ethnicity,[90,149] although studies in India were less supportive and may be similar to an immune response gene.[184] This work has been extended with the use of monoclonal antibodies to family members of patients with rheumatic fever.[69,168] Class I HLA molecules have not been associated with acute rheumatic fever. Many studies in different populations have shown an association with HLA-DR, but without a single HLA marker for susceptibility.[184] Genetic factors alone seem highly

unlikely to be responsible for susceptibility to rheumatic fever.[126] No predictive marker for susceptibility to rheumatic fever has been defined.

VACCINE DEVELOPMENT

Immunity to group A streptococci and to rheumatic fever is thought to depend largely on antibodies to M protein, a major virulence determinant; such antibodies can opsonize the bacteria in the presence of neutrophils.[107b] Immunity was thought to be strain-specific and to depend on antibodies to the variable serotype-specific regions of the protein, and earliest vaccine development has followed this pathway.[19] Two early landmark studies showed that vaccines containing purified M protein evoked protective immune response in humans.[20,159] Antibodies against the variable amino-terminal end of the M protein opsonize streptococci in a type-specific manner, but the results of experiments in animals suggest that the conserved carboxyl-terminal end also may be an immune target. Some human evidence suggests that this conserved epitope acts as a subunit vaccine.[160] Complexities in this area include the risk of inducing cross-reacting antibodies that could injure rather than protect.[15,133] Separation of the peptide fragments of M proteins (epitopes) that evoke type-specific and not cross-reacting antibodies is an important step.[34,54]

With more than 120 different defined serotypes of group A streptococci, vaccine development[29,31a] falls into two broad approaches: vaccines based on common protective antigens of group A streptococci and multivalent vaccines based on type-specific N-terminal regions of the M protein. A 26-valent vaccine with components guided by North American surveillance of acute rheumatic fever, invasive infections, and pharyngitis has been shown to be safe and immunogenic in adults.[137] This vaccine includes 80 to 90 percent of important serotypes in North America, and epidemiologic studies indicate that vaccine coverage may be incomplete in other areas such as in Asia[161] or in New Zealand.[129] Information for serotype prevalence in many areas of high endemicity of acute rheumatic fever is urgently needed.

RHEUMATIC FEVER IN DEVELOPING COUNTRIES

Rheumatic heart disease is considered by some physicians to be one of the few preventable chronic diseases.[9] Despite impressive declines in incidence in developed countries (see Figs. 35–1 to 35–3), globally, rheumatic heart disease remains the most common form of acquired heart disease.[105,217] Four fifths of the world's population live in developing countries, where the prevalence of rheumatic heart disease suggests that the incidence of acute rheumatic fever remains at high levels in areas characterized by crowded living quarters and lower socioeconomic conditions. In Soweto, South Africa, the prevalence of rheumatic heart disease in the 1970s was estimated at 7.1 per 1000 schoolchildren.[136] A recent estimate using echocardiography in two developing country settings increased this estimate by nearly tenfold.[122]

Given the difference in medical care delivery, evaluation of the incidence of acute rheumatic fever must be viewed with caution. Estimates suggest, however, an annual incidence of 200 to 400 cases per 100,000 population in Soweto.[105] In India, the prevalence of rheumatic heart disease in school children has been estimated to be 1 to 5.4 cases per 1000.[148] The incidence of rheumatic fever (as judged by hospital admissions for rheumatic heart disease between 1966 and 1980) has remained stable in India during this period of rapid decline in the United States and the West.[2] Community-based secondary penicillin prophylaxis programs in developing countries are considered cost-effective and more achievable than is primary prevention.[105] An estimate of the global burden of group A streptococcal diseases has been undertaken by the World Health Organization (www.who.int/ch-add-health).

PATHOLOGY

The unique pathologic lesion of rheumatic fever is the Aschoff body, which generally is considered to be a granuloma that results from injury to collagen fibers. Classically, Aschoff bodies are found in the heart, usually in the left atrial appendage, but similar foci can be found in the synovia of the joints and in and around joint capsules, tendons, and fascia.[171]

The early pathologic response to rheumatic fever may be an exudative reaction with Aschoff-like bodies as an inflammatory focus. They are cardiac or extracardiac, with a central area of fibrinoid necrosis surrounded mostly by polymorphonuclear leukocytes. Clinically, this condition may be manifested as arthritis and spontaneously subside in 2 to 4 weeks. No residual joint damage results. The proliferative phase of classic Aschoff nodules, which consists of central necrosis surrounded by a rosette of large mononuclear cells, giant multinuclear cells, and other cell types, is confined to the heart and usually causes pancarditis with simultaneous involvement of all three layers (the pericardium, the myocardium, and the endocardium). This event may result in permanent valvular damage in the following order of frequency: the mitral valve, the aortic valve, the tricuspid valve, and, rarely, the pulmonary valve.

The heart disease encountered clinically usually is mitral regurgitation, aortic regurgitation, or both. The scarring that leads to valvular stenosis (mitral or aortic) typically takes decades to develop but may occur much faster in hyperendemic areas. This process is not the full story, however, because although rheumatic mitral valve stenosis occurs more commonly in India[174] and occasionally in other less advantaged populations, it was never a common occurrence in the United States or the United Kingdom at the height of rheumatic fever incidence.[191]

The presence of Aschoff bodies is not evidence of rheumatic activity because these lesions are found in biopsy specimens of the left atrial appendage many years after an acute attack of rheumatic fever. Little is known about the pathology of Sydenham chorea, and the pathologic changes cannot be related to the clinical manifestations. Patients rarely die of this form of rheumatic fever.

CLINICAL COURSE

The stage is set for the development of rheumatic fever in a susceptible host after a pharyngeal infection by one of the types of group A beta-hemolytic streptococci.[115,125,182] If the infection is not treated, most individuals recover from the acute effects of the disease. Acute rheumatic fever develops in approximately 1 to 3 percent of children with known epidemic untreated exudative pharyngitis and a culture positive for group A streptococci. The frequency decreases to less than 1 percent, as shown in the one controlled study involving children,[178] when patients with less severe or less precisely diagnosed streptococcal infections are included.

This finding has been replicated more recently in a large community-based study in an endemic area in New Zealand. The attack rate observed was approximately 0.2 percent[114] with bacteriologic diagnosis and treatment of group A streptococcal throat infection and its effect on rheumatic fever.

The preceding pharyngitis is not recognized as an illness by the patient or parents in approximately 10 to 33 percent of cases of acute rheumatic fever, although 50 to 60 percent of patients

remember having a sore throat.[82] In the New Zealand series, episodes of sore throat (with appropriately increased streptococcal serology) in this carefully monitored series preceded development of acute rheumatic fever cases in 14 of 19 (74%) of the cases enrolled in the program at the time of presentation.[114] In some series, this figure is lower (see Table 35–2). The infection is followed by a latent period that averages 19 days in duration,[164] during which time the patient seems well. The range seems to be between 1 and 5 weeks but has been difficult to establish.[41] In the New Zealand series, the average latent period was 27 days (range, 2 to 49 days) for seven rheumatic cases with proven group A streptococcal pharyngitis.[114] The average latent period is the same for recurrent attacks as for initial episodes.[41]

Acute rheumatic fever then begins. Table 35–3 suggests a clinical profile in the United States, although recurrent cases with their increased risk for carditis are included. In a prospective study in India, 67 percent of initial episodes were associated with migrating arthritis involving one or more of the large joints[173] accompanied by a fever of 38º C to 39º C, malaise, and anorexia. Just as the redness, swelling, and pain in a knee subside, the whole process may start again in the ankle. The elbows and wrists also are likely to be involved. Typically, multiple joints are involved, in tandem with overlap over the course of time, when symptoms are not suppressed by anti-inflammatory therapy. The whole polyarthritic episode usually subsides over 4 weeks, with no residua remaining.

Carditis generally appears early in the, illness (first 2 to 3 weeks) if it is going to occur.[1] The joint inflammation may be low-grade in some individuals, without limitation of motion or outward manifestations of redness and swelling (arthralgia). The literature supporting monarthritis that does not develop into migrating polyarthritis in acute rheumatic fever is unconvincing.[38,89,104,114,157,212] The clinician should act cautiously, however, in an area endemic for rheumatic fever in the early phase of presentation. The development of polyarthritis in a patient with a culture-negative monarthritis (e.g., a hip joint in a patient without prior antibiotic exposure) may be aborted by nonsteroidal anti-inflammatory drugs (NSAIDs). An early echocardiogram may assist in establishing the diagnosis.[3]

At examination, the striking findings are the patient's pallor and discomfort, especially on movement of the affected joints. The pulse is rapid. Examination of the heart may reveal in at least half of patients a grade II/IV apical pansystolic murmur that is transmitted to the axilla (mitral insufficiency) with or without an apical mid-diastolic flow murmur (Carey-Coombs murmur); half of these patients also may experience an early diastolic grade II/IV murmur at the left sternal edge (aortic insufficiency). In addition, less commonly, the child can have congestive heart failure or cardiac enlargement, indicative of active carditis. Carditis is more likely to occur in younger children. Pericarditis may be suspected with muffled heart sounds, a frictional rub, or chest pain. It becomes less common as acute rheumatic fever in a population becomes less severe. Death is a rare, but well-described, sequela of the acute phase of the disease. Murmurs of mitral and aortic stenosis are associated with chronic, but not with acute, rheumatic valve disease.

The distinctive rash, erythema marginatum, is observed in 10 percent of patients (Fig. 35–4). It is neither pruritic nor painful. The pink, slightly raised macules usually seen initially on the trunk and proximal ends of the extremities and never on the face fuse centrally and coalesce to form a serpiginous pattern. The lesions may disappear after a few hours or may reappear intermittently over the course of weeks, especially after a warm shower or bath. Subcutaneous nodules, usually associated with severe carditis, also occur uncommonly (<10% of patients). They are firm and painless and are found over bony surfaces or prominences and over tendons. Acute rheumatic fever is not likely to be diagnosed on the basis of the latter two major criteria without another major criterion.

Sydenham chorea, or St. Vitus' dance, may be the only manifestation of rheumatic fever, or it may be associated with other disease manifestations. It becomes less common as acute rheumatic fever becomes less severe in a population. Chorea is characterized by purposeless (most often bilateral, uncoordinated, involuntary) movements, mostly of the hands, feet, and face, which develop over the course of weeks and are accentuated by excitement and emotional stress. They disappear during sleep. Sensation remains intact. The speech can be explosive and indistinct, and the handwriting can be clumsy. Handwriting is a useful objective means of monitoring the course of the disease. The child has difficulty counting rapidly and holding the protruded tongue still. The fingers and wrists are hyperextended when the fingers are outstretched, and the palms usually are turned outward when the arms are held above the head. Handgrip generally is weak and may consist of spasmodic contractions followed by rapid relaxation.

The patient may be easily irritated and quarrelsome. Chorea typically is a delayed manifestation of rheumatic fever and may develop after other signs of the disease have subsided. Chorea commonly appears 2 to 6 months after the streptococcal infection. Most observers think that residual heart disease occurs less commonly when chorea is the only manifestation of rheumatic fever, but in the echocardographic era this hypothesis may prove not to be the case.[3] The importance of prophylaxis to prevent recurrent attacks and possible subsequent carditis was reaffirmed in Kuwait.[119] Permanent serious residual neurologic deficits have

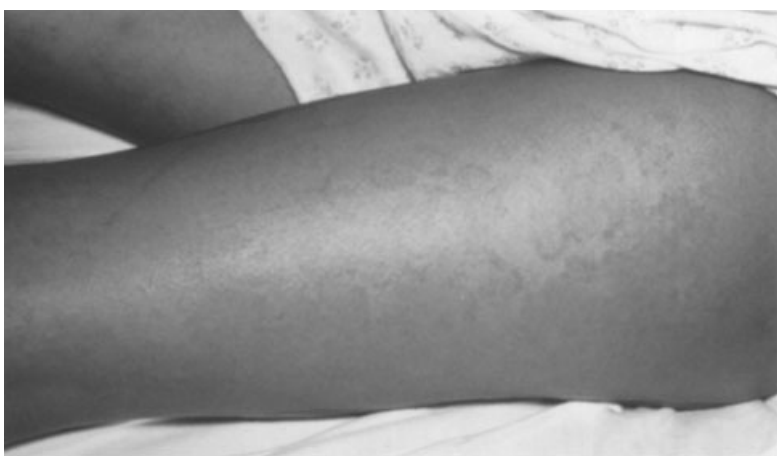

Figure 35–4 Erythema marginatum in an 8-year-old girl with acute rheumatic fever.

not been observed. A 25-year review found the duration of chorea to be 1 to 22 weeks, with a median of 12 weeks.[145] Rare cases may last 2 to 3 years.[4,13,37] Recurrent attacks are common and may occur despite faithful adherence to prophylaxis with intramuscular benzathine penicillin.[22,194] The neuropsychiatric sequelae of chorea were reviewed recently.[139]

Some cases of chorea are mild or atypical and may be confused with motor tics or the involuntary jerks of Tourette syndrome. Confusion may ensue between Sydenham chorea and these conditions. The term *pediatric autoimmune neuropsychiatric disorder associated with streptococcal infection (PANDAS)* refers to a subgroup of children with tic or obsessive-compulsive disorder, whose symptoms may develop or worsen after an episode of group A streptococcal infection. The following five criteria have been used to define the PANDAS subgroup[106,189]:

1. The presence of a tic disorder or obsessive-compulsive disorder or both
2. Prepubertal age of onset (usually between 3 and 12 years of age)
3. Abrupt onset of symptoms or episodic course of symptom severity, or both
4. Temporal association between exacerbations of symptoms and streptococcal infection (approximately 7 to 14 days)
5. Presence of neurologic abnormalities during periods of exacerbation of symptoms (typically adventitious movements or motoric hyperactivity)

The evidence supporting PANDAS as a distinct disease entity has been questioned.[106] In any population with a high prevalence of acute rheumatic fever, clinicians should rarely (if ever) make a diagnosis of PANDAS and rather should err on the side of overdiagnosis of acute rheumatic fever and secondary prophylaxis. If acute rheumatic fever is excluded, secondary prophylaxis is not needed, but such cases should be followed up carefully to ensure that they do not develop carditis in the long-term.

The average duration of an attack of acute rheumatic fever is approximately 3 months when unaltered by anti-inflammatory therapy.[125] Less than 5 percent of cases persist longer than 6 months with active symptoms, so-called chronic rheumatic fever.[191]

LABORATORY FINDINGS

The degree of inflammation in patients with acute rheumatic fever is measured by nonspecific indicators, such as the erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP).[115,125,182] Unless the patient has taken corticosteroids or salicylates, these test results almost always are positive in patients with polyarthritis or acute carditis, whereas they often are normal in patients with chorea. The magnitude of the ESR is proportional to the intensity of the inflammatory reaction but is not site-specific (i.e., it can be high with polyarthritis or carditis). The ESR may be decreased in congestive heart failure, whereas CRP may be elevated in congestive heart failure attributable to any cause. The ESR may remain elevated for 6 weeks to 3 months in an untreated attack of acute rheumatic fever. Anti-inflammatory agents may reduce the ESR, but it rebounds if the drugs are stopped before the rheumatic process has run its course. Although chronic elevation of the ESR (>6 months) is not understood, it is not sufficient reason on its own to limit a patient's activities.[191] The CRP may reflect the patient's rheumatic activity more precisely than may the ESR.[125]

Chest radiographs are useful for detecting cardiomegaly, which may be caused by dilation, preexisting heart disease, or pericardial effusion. The degree of enlargement is helpful in judging severity. The electrocardiogram may show a prolonged atrioventricular conduction time, usually evidenced by a prolonged PR interval or even greater degrees of heart block.[167] Generally, an increase in the PR interval in tracings with comparable rates is considered significant.[125] Upper limits of normal by age are available.[115] Atrioventricular conduction abnormalities per se bear no relation to the ultimate prognosis of patients. Changes of myocarditis and pericarditis also are seen.

The roles of two-dimensional and Doppler echocardiography in establishing the diagnosis and determining the prognosis of acute rheumatic fever are becoming clearer.[1,71,72,115,182,198-200,213] In a prospective blinded study using febrile controls and strict color and pulsed Doppler criteria, pathologic left-sided heart regurgitation could be differentiated from physiologic regurgitation.[1] Several centers using similar strict criteria have observed subclinical carditis in acute rheumatic fever.[73,74,199,200] The status of echocardiographic evidence as a major or minor Jones criterion remains to be settled by the American Heart Association.[71] It has important implications for patients with polyarthritis or chorea as a sole major criterion[3,182] or patients without major criteria and only echocardiographic evidence of mitral or aortic regurgitation. In an area of high endemicity (New Zealand), strict criteria have evolved and are incorporated into common usage.[115] In a prospective study of prevention of acute rheumatic fever, three additional cases (3 of 59 [5%]) met the case definition for acute rheumatic fever using the New Zealand Rheumatic Fever diagnostic criteria, which allows echocardiographic carditis to be a major or minor criterion in the presence of other major or minor criteria and evidence of preceding streptococcal infection.[114]

A positive throat culture for group A beta-hemolytic streptococci as evidence of a recent streptococcal infection seldom is found, and 50 percent of such patients could be carriers of the organism.[99] In the New Zealand prospective study of patients with sore throat episodes (n = 14) and enrolled in the program, 57 percent (8 of 14) had a positive throat culture and appropriately increased serology.[114] A positive culture may be helpful if it can be related to the time of the acute infection.

Corroboration of a previous streptococcal infection may be documented by numerous streptococcal antibody tests.[175,211] Antibody titers may be elevated in the absence of clinical or bacteriologic evidence of streptococcal pharyngitis (5 of 19 acute rheumatic fever cases in the New Zealand program).[114] The ASO titer is the most popular antibody test. It measures the inhibition of rabbit red blood cells by specific antibody to streptolysin O, an extracellular product of beta-hemolytic streptococci that in its reduced form hemolyzes red blood cells. The "normal" level for an ASO titer usually is defined as the highest titer exceeded by only 20 percent of a population, but it is influenced significantly by age, geography, season, and other factors.[98,175] ASO titers of 500 Todd units or greater are rare findings in normal schoolchildren and are good evidence of a recent streptococcal infection. ASO titers less than 250 Todd units could be considered normal; titers of 250 to 320 Todd units should be considered borderline elevated. Approximately 50 percent of patients with acute rheumatic fever have ASO titers in this range, and approximately 60 percent have titers of 500 Todd units or greater.[182] Conversely, ASO titers can be normal in 20 percent of patients with acute rheumatic fever.[185]

A recent streptococcal infection is more likely to be shown if more than one antibody titer is measured (e.g., antistreptokinase and antihyaluronidase).[185] Anti–deoxyribonuclease B is the most favored test because of its better reproducibility.

The Jones criteria (Table 35–5) call for an elevated or increasing titer of an antistreptococcal antibody.[182] The onset of clinical acute rheumatic fever usually coincides with the peak of the streptococcal antibody response. It may stay elevated for many weeks. The absence of an elevated antistreptococcal titer, if three different antibodies are measured, means, however, that the clini-

TABLE 35–5 Guidelines for the Diagnosis of an Initial Attack of Rheumatic Fever (Jones Criteria, 1992 Update)*

Major Manifestations†
Carditis
Polyarthritis
Chorea
Erythema marginatum
Subcutaneous nodules
Minor Manifestations†
Clinical findings
Arthralgia
Fever
Laboratory findings
Elevated acute-phase reactants
Elevated erythrocyte sedimentation rate
Elevated C-reactive protein
Prolonged PR interval
Supporting Evidence of Antecedent Group A Streptococcal Infection
Positive throat culture or rapid streptococcal antigen test
Elevated or rising streptococcal antibody titer

**If supported by evidence of a preceding group A streptococcal infection, the presence of two major manifestations or of one major and two minor manifestations indicates a high probability of acute rheumatic fever.*

†See text for details.

From Guidelines for the diagnosis of rheumatic fever. Jones criteria, 1992 update. Special Writing Group of the Committee on Rheumatic Fever, Endocarditis, and Kawasaki Disease in the Young of the American Heart Association. J. A. M. A. 268:2069-2073, 1992. Copyright 1992, American Medical Association.

cian can be 95 percent certain that the patient has not had a streptococcal infection within the recent past. In patients with pure chorea, antibody levels may have declined to normal because of the length of the latent period between the development of streptococcal infection and the manifestation of this symptom. A slide agglutination test is available (Streptozyme antibody test; Wampole Laboratories, Stamford, CT). This test cannot be recommended at this time because of inconsistencies in results caused by variations in different lots of test material.[216]

The synovial fluid in joints affected by acute rheumatic fever contains 10,000 to 100,000 white blood cells/mm^3, which are mostly neutrophils. The protein concentration is approximately 4 g/dL, glucose levels are normal, and a good mucin clot is present.[92] A more recent report corroborates the cellular findings and highlights similarities with septic arthritis.[89]

DIAGNOSIS

The signs and symptoms of rheumatic fever vary greatly, depending on the stage of the disease, the epidemiology of the rheumatic fever in that place at that time, the severity of the disease, and the sites of involvement. In the absence of a diagnostic test or pathognomonic sign, Jones suggested a series of criteria (major and minor) (see Table 35–5) that have stood the test of time, with ongoing modifications.[71,123]

The keystone on which the Jones criteria (1992 update[182]) rest is the demonstration of a recent streptococcal infection. Because few patients with acute rheumatic fever have positive throat cultures, demonstration of a previous streptococcal infection by a rising titer of one or more of the extracellular streptococcal antibodies is crucial confirmatory evidence to establish a recent streptococcal infection. The mere presence of an elevated titer to one or more of the streptococcal antibodies (see the section on laboratory findings) means only that the subject has had a recent group A beta-hemolytic streptococcal infection.

Clinical manifestations in outbreaks in the 1980s are summarized in Table 35–3. Before this time, during the period of declining incidence in the United States, carditis was found in fewer than half of rheumatic patients and generally was less severe.[131] Joint involvement alone was the most common manifestation, so arriving at a diagnostic certainty was difficult. A common avoidable error is the premature administration of salicylates or corticosteroids before the signs and symptoms become distinct; such therapy leaves in doubt the necessity of administering secondary prophylaxis without a firm diagnosis.

In contrast to the revised Jones criteria (1965), more updated criteria (1992)[182] are designed to establish a diagnosis of the initial attack of acute rheumatic fever; a previous attack of rheumatic fever or rheumatic heart disease no longer is a minor manifestation. Echocardiography currently is not a stand-alone criterion for the diagnosis of acute rheumatic fever in the United States using these criteria (see the section on laboratory findings); however, chorea and indolent carditis are considered stand-alone criteria for establishing the diagnosis of rheumatic fever. Recurrences of rheumatic fever in patients with a reliable past history of rheumatic fever or clear-cut rheumatic heart disease can be diagnosed by the demonstration of a single major or several minor criteria if supporting evidence of a recent group A streptococcal infection is present.

So-called poststreptococcal arthritis has been discussed as a possible entity when the initial symptoms and signs are atypical for acute rheumatic fever, fail to respond to salicylate therapy, or both. In some cases, rheumatic heart disease has ensued.[63a] In all such patients who fulfill the Jones criteria, a diagnosis of rheumatic fever should be considered, particularly for the purpose of administering secondary penicillin prophylaxis.[15]

Criteria have been proposed for establishing the diagnosis of poststreptococcal reactive arthritis that have yet to stand the test of time. A variable response to salicylates in series of this disease has not often been well documented with serum salicylate levels.[80] A shorter latent period is proposed, but the latent period between a group A streptococcal throat infection in original investigations of rheumatic fever varied (mean, 18.6 days), with 10 percent developing disease within 8 days (see the section on clinical course).[164] If this diagnosis is considered and rheumatic fever per se is not supported, vigilant follow-up for cardiac sequelae should ensue, and penicillin prophylaxis would be reconsidered. This diagnosis should be considered with caution in areas endemic for rheumatic fever. An atypical presentation of chorea (e.g., hemichorea) warrants consideration of neuroimaging.[222]

DIFFERENTIAL DIAGNOSIS

Many other diseases might be confused with acute rheumatic fever,[110] including rheumatoid arthritis, suppurative bacterial arthritis (especially gonococcal arthritis in adolescents), reactive arthritis (e.g., after *Yersinia*[107] or *Mycoplasma*[140] infection), infective endocarditis, sickle-cell anemia, leukemia, Lyme disease,[158] and poststreptococcal reactive arthritis (see earlier). In polyarthritis without clinical or echocardiographic evidence of carditis and fulfilling the Jones criteria, rheumatic fever is a diagnosis of exclusion. A follow-up echocardiogram after 2 to 3 weeks may reveal late presenting carditis.[1,203]

With the help of the Jones criteria and time, these diseases usually can be excluded. Heart involvement with rheumatoid arthritis is a rare occurrence. In suppurative arthritis, showing the infecting bacteria by smear and recovery by culture provide the answer. With sickle-cell disease, the bone is affected and not the joint, and a sickle-cell preparation helps establish the diagnosis. A blood smear usually establishes the diagnosis of leukemia.

The diagnosis of isolated Sydenham chorea based on presenting symptoms may be confirmed by an abnormal echocardiogram in the absence of clinical carditis. Other, much rarer

considerations, especially in an area not endemic for rheumatic fever, include systemic lupus erythematosus (SLE), Wilson disease, juvenile Huntington chorea, and various medications.[40,110]

Common errors include diagnosing acute rheumatic fever and prescribing NSAIDs when a single joint is involved (see the section on clinical course)[212]; when an innocent murmur is present; when a nonspecific rash, especially an urticarial or an erythema multiforme rash, erroneously is called erythema marginatum; and when other symptoms similar to chorea (e.g., tics, phenothiazine-induced extrapyramidal syndrome) are misinterpreted.[95] Committing a child to many years of penicillin prophylaxis requires careful decision-making at the time of establishing the diagnosis.

TREATMENT

Therapy for acute rheumatic fever is symptomatic:[41,57,110,136,190] control the inflammation, decrease the fever, and keep cardiac failure in check. Neither salicylates nor corticosteroids are thought to affect severity or outcome.[7] Cardiac drugs (e.g., diuretics, angiotensin-converting enzyme inhibitors, beta blockers) may improve impaired function. The long-term benefits have not been studied systematically. If the physician thinks that a patient has acute rheumatic fever, a trial of salicylates may be useful as symptomatic therapy. If the diagnosis is uncertain, however, pain relief such as acetaminophen should be used as an interim measure to allow migrating polyarthritis to occur. This approach has not been evaluated critically.

Characteristically, the joint inflammation and fever subside in 24 to 48 hours with salicylate treatment if the serum level is 10 to 20 mg/dL, which usually is achieved by a dose of 60 to 100 mg/kg/24 hr (not exceeding 6 g/day in divided doses). This dose may be increased, but the clinician is advised to measure serum salicylate levels and adjust the dose regimen accordingly. A higher dose may result in the undesirable development of salicylism (tinnitus and hyperpnea). Except for occasional patients with rheumatoid arthritis, no other forms of arthritis respond in this dramatic way to aspirin. Salicylate therapy is recommended for 1 to 2 weeks and then can be reduced gradually, keeping in mind that inflammatory markers suggesting ongoing disease activity may persist 3 months or longer. Joint symptoms may recur, obviating a more gradual withdrawal of aspirin.

Most acute rheumatic fever episodes subside within 6 weeks, and 90 percent resolve within 12 weeks. Approximately 5 percent of cases require 6 months or more of salicylate therapy.[186] If lengthy therapy is required, influenza and varicella vaccines are important considerations to reduce the potential for developing Reye syndrome.[50] Newer NSAIDs for the treatment of acute rheumatic fever have been studied in limited fashion.[91,197] Naproxen (10 to 20 mg/kg/day given in two divided doses; maximum dose 1250 mg) had a dramatic effect similar to that of aspirin and is well-tolerated. Advantages are twice-daily administration, the availability of elixir, less hepatotoxicity, and no need to determine serum levels.

No evidence has substantiated that steroid therapy is superior or that treatment with steroid or aspirin decreases the severity or prevents the development of residual heart disease.[6,7,47] Both treatments are palliative and not curative. They are, however, effective anti-inflammatory agents for controlling the acute exudative manifestations of rheumatic fever. Steroids are more likely to reduce acute symptoms promptly and may be indicated for severely ill patients in whom inflammatory edema of the myocardium may be life-threatening during the acute stage of the illness.[8,115] The effect of NSAIDs has not been evaluated adequately.

A randomized controlled trial of intravenous immunoglobulin, a proven immunomodulator, in acute rheumatic fever failed to alter the natural history. No detectable difference was noted in the clinical, laboratory, or echocardiographic parameters found during the subsequent 12 months.[203]

Bed rest has not been studied critically.[125] Restriction of physical activity until the rheumatic process has become quiescent is a time-honored method of treatment. It has been based on the assumption that the workload of the inflamed heart is related to the degree of residual scarring. Suggested guidelines include 6 weeks of bed rest, depending on whether carditis is present, followed by gradual ambulation indoors over the course of a further 6 weeks before outside activity, in modified fashion, occurs.[169] Patients with severe carditis who have congestive heart failure are managed more conservatively.

All patients should receive intramuscular benzathine penicillin, even if the throat culture does not reveal group A beta-hemolytic streptococci. Patients then can be placed on the secondary preventive treatment regimen, which may be either oral penicillin V, 250 mg twice a day, or injections of benzathine penicillin, 1.2 million U every 4 weeks. The parenteral route has been shown to be more effective by the author's group[146] and others (Fig. 35–5).[75,215] In high-risk situations (e.g., after a recurrence in a compliant patient), administration of benzathine penicillin every 3 weeks has been advised (see the section on prevention for more detail).[117]

Rarely, a patient has congestive heart failure.[115] A diuretic or fluid restrictions or both are recommended for mild to moderate failure. Angiotensin-converting enzyme inhibitors should be considered for more severe failure, particularly if aortic regurgitation is present. Experience with beta blockers in acute rheumatic carditis is very limited, and their use is not recommended.

Chorea is benign and self-limited. Mild or moderate chorea does not require any specific treatment aside from rest and a calm environment, perhaps in the hospital. Overstimulation or stress can exacerbate the symptoms. The potential toxicity of recommended medication for severe distressing or limiting chorea should be taken into consideration. Aspirin does not have a significant treatment effect.[125] A randomized trial of prednisone in Sydenham chorea showed efficacy.[151] More studies are awaited to confirm this finding.

Small studies of intravenous immunoglobulin have suggested more rapid recovery from chorea,[188] but they have not shown reduced incidence of long-term valve disease in acute rheumatic fever without chorea.[203] Until more evidence is available, intravenous immunoglobulin is not recommended except for severe chorea refractory to other treatments.

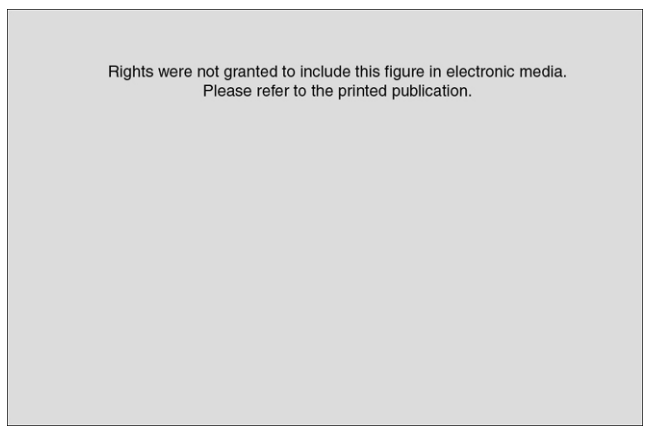

Figure 35–5 Influence of oral and intramuscular penicillin prophylaxis on the recurrence of rheumatic fever. Time is between the last attack and recurrence (years). *(From Newman, J. E., Lennon, D. R., and Wong-Toi, W.: Patients with rheumatic fever recurrences. N. Z. Med. J. 97:678-680, 1984.)*

Carbamazepine and valproic acid now are preferred to haloperidol, which previously was considered the first-line medical treatment for chorea.[63,76] A small prospective comparison of these three agents concluded that valproic acid was the most effective.[152]

Other antichorea medications should be avoided because of potential toxicity. Because of the small potential for liver toxicity with valproic acid, the recommendation is that carbamazepine be used initially for severe chorea requiring treatment and that valproic acid be considered for refractory cases. A response may not be seen for 1 to 2 weeks, and successful medication may reduce, but not eliminate, the symptoms. Medication should be continued for 2 to 4 weeks after chorea has subsided and then withdrawn gradually. Recurrences of chorea usually are mild and can be managed conservatively, but in severe recurrences, the medication can be restarted if necessary.

CARDIAC SURGERY IN RHEUMATIC HEART DISEASE

Aggressive surgical therapy, with increasing acceptance that mitral repair rather than replacement is the treatment of choice for mitral regurgitation,[12] may be indicated in patients with severe active rheumatic heart disease. Extensive published experience in South Africa[17,44] and France[44] with excellent results has challenged the concept that congestive heart failure and death during active carditis are caused exclusively by myocarditis, rather than incompetence of the valve. Studies on left ventricular mechanics support this new evidence.[77] In addition, primary myocardial involvement as evidenced by histologic abnormalities of the myocardium has been well documented during acute rheumatic fever.[144,190] Careful postoperative management, including at least 4 months of physical rest, diuretics, and vasodilation with angiotensin-converting enzyme inhibitors, is thought to improve the long-term outcome by avoiding increased blood pressure and myocardial contractility before the repair has consolidated.

Surgery usually is deferred until active inflammation has subsided. Rarely, valve leaflet or chordae tendinae rupture leads to severe regurgitation; this event requires emergency surgery, which can be performed safely by experienced surgeons, although the risk seems to be slightly higher than when surgery is performed after active inflammation has resolved.[5] Valve replacement, rather than repair, usually is done during the acute episode because of the technical difficulties of repairing friable, inflamed tissue. Nonetheless, very experienced surgeons may achieve good results with repair in this situation.

PROGNOSIS

The prognosis for patients with acute rheumatic fever depends on the initial manifestations, as shown in the 20-year follow-up study from the pre-penicillin era by Bland and Jones.[32] A patient with marked cardiomegaly, congestive heart failure, or pericarditis had about a 70 to 80 percent chance of dying in the 10 years before the advent of secondary prevention programs, open heart surgery, and use of prosthetic valves. The prognosis today is not as poor, although the recurrence rates (with the attendant increased risk of carditis in individual patients) reported after some outbreaks[199,220] suggest a careful look at secondary prevention and its delivery. The risk of having rheumatic heart disease at 1-year[7] and 10-year[49] follow-up is 70 percent for patients with cardiomegaly or heart failure and who survive. Most of these patients have mitral insufficiency, and approximately 50 percent also have aortic insufficiency.

Approximately 50 percent of patients initially are left with residual heart disease after an attack of rheumatic fever. This rate is about the same as it was 25 to 30 years ago. Approximately 25 percent of these patients return to normal cardiac status, however, with a higher chance if the cardiac involvement is mild. Of patients with no or questionable carditis[7] during their attack of rheumatic fever, only 6 percent were found to have heart murmurs when re-examined 10 years later. Heart disease was present at follow-up in 30 percent of the patients initially found to have only apical systolic murmurs and in 40 percent of patients with basal diastolic murmurs during the acute phase. Patients with chorea may have a slightly lower incidence of residual heart disease.[118] More data from the echocardiographic era are required.

PREVENTION

Denny and associates[64,208] made one of the most important research contributions in the last 50 years when they showed that rheumatic fever can be prevented in most susceptible subjects if the preceding pharyngeal infection by one of the group A beta-hemolytic streptococci is treated adequately.[57] These studies used depot penicillin G. The effectiveness of other antimicrobial agents (benzathine penicillin, chlortetracycline, sulfadiazine, oxytetracycline) in the prevention of rheumatic fever also was studied. Eradication of the streptococcus was shown to be essential,[41] and a 10-day course of penicillin treatment was found to be more effective than a 5-day course.[208] From these studies, penicillin, a bactericidal agent with activity against streptococci, became the drug of choice.

Efficacy studies against rheumatic fever per se were performed, mainly in military populations with injectable penicillin. Only one inconclusive study was done in children.[178] These studies more recently have been subjected to meta-analysis,[172] with a relative risk of 0.20 (95% confidence interval 0.11, 0.36; $p < .00001$) favoring the intervention. The ability of oral penicillin to eradicate streptococci in throats is not equal to the ability of injectable penicillin to do so.[18] A school-based, randomized, controlled trial of sore throat clinics using oral penicillin in an area in New Zealand endemic for acute rheumatic fever (43,827 person-years of observation) revealed a clinically useful result, but with uncertainty about the effect size. A meta-analysis of published literature on community-based or school-based controlled studies to prevent first attacks of rheumatic fever revealed a risk of 0.62 (95% confidence interval 0.45, 0.85; $p = .003$), which favors these interventions, suggesting approximately 40 percent efficacy.[111] Our study used supervised oral penicillin treatment[114]; the treatment regimen was unclear in the other studies.[45,52,154] A complete explanation for the decline in rheumatic fever remains unclear.

Streptococcal pharyngeal infection should be identified before treatment is started. Guidelines using clinical parameters have evolved[43,116] and have been tested empirically to guide rational management.[134,135] Streptococcal pharyngeal infection can be diagnosed by throat culture or by using a rapid diagnostic antigen-detection kit.[30,57,79] Most tests have high specificity, so a patient with acute pharyngitis and a positive test result should be treated. Many of the tests have suboptimal sensitivity and should be confirmed by a throat culture. One study found that in one third of individuals with false-negative rapid antigen-detection test results, streptococcal antibody titers increased subsequently, suggesting infection.[78]

Military studies have shown that primary preventive treatment with penicillin is effective even if started 9 days after the infection develops,[42] so physicians can wait 24 to 48 hours for verification of infection by recovery of group A beta-hemolytic streptococci. A dose of 1.2 million U of benzathine penicillin intramuscularly (0.6 million U if weight is ≤27 kg) usually is adequate treatment. Because of the discomfort and a possible, but

small, risk associated with intramuscular penicillin,[94] oral penicillin V (250 mg two or three times a day for children; 500 mg two or three times a day for adolescents and adults) may be preferred in areas in which the incidence of rheumatic fever is low. Erythromycin estolate (20 to 40 mg/kg/day in two to four divided doses; maximum 1 g/day) may be used in patients who are allergic to penicillin. Erythromycin ethyl succinate (40 mg/kg/day two to four times daily; maximum 1 g/day) is an alternative therapy. Accruing evidence supports the use of once-a-day amoxicillin.[48,67,112,177]

All oral treatments should be given for 10 days.[57] Although certain new wider spectrum agents have been administered in shorter courses with the desired streptococcal eradication, on the basis of penicillin's narrow spectrum of antimicrobial activity, the infrequency with which it produces adverse reactions, and its modest cost, it is the drug of choice for nonallergic patients.[30]

Reappearance of acute rheumatic fever in a specific geographic region should draw attention to therapeutic, preventive, and epidemiologic measures for control of the disease. A targeted approach to particularly high-risk population groups in schools may be cost-effective and efficacious.[9,33,52,155,170,196] Because treatment of pharyngitis seems likely to have contributed to the declining incidence of rheumatic fever, obtaining throat cultures (or a rapid antigen-detection test) and administering penicillin treatment, if positive for group A streptococci, still are recommended in low-risk populations, although this recommendation is being challenged by some physicians.[134,135] In addition, a negative culture avoids unnecessarily prescribing antibiotics in the 70 to 80 percent of children with a sore throat attributable to viral pharyngitis, although clinical assessment may obviate having to obtain a throat culture.[135,176]

Prompt administration of antibiotic therapy may shorten the duration of symptoms in patients with group A beta-hemolytic streptococcal pharyngitis.[166] Cultures should be used selectively in age groups in which rheumatic fever rarely occurs (e.g., <4 years of age or >20 years). Signs and symptoms usually not associated with streptococcal infection, such as simple coryza, hoarseness, cough, conjunctivitis, anterior stomatitis, and diarrhea,[58,135] may help target the approach in a low-risk population.

A follow-up throat culture taken after a course of treatment for streptococcal pharyngitis is not recommended routinely, unless the patient remains symptomatic or is from a family with a rheumatic member.[30] Such follow-up cultures probably identify long-term carriers for whom repeated courses of antibiotics generally are not indicated.[30] Streptococcal carriers seem to pose little threat to themselves regarding the development of sequelae from streptococcal infection or dissemination of the organism to people around them. When a symptomatic viral upper respiratory tract infection develops in such a carrier distinguishing whether the group A streptococci isolated indicate current streptococcal infection or identify that individual as a chronic carrier frequently is impossible. In one study, only 43 percent of children with paired sera from whom group A streptococci were recovered showed a significant antibody response to one of two different streptococcal antibodies.[99]

Often, a reasonable approach is to administer a single course of therapy. Indications for obtaining cultures from household contacts vary according to circumstances.[57] Family contacts of high-risk patients should have a culture performed and receive treatment if the culture is positive.

In the pre-penicillin era, 75 percent of individuals developed a recurrence of acute rheumatic fever.[32] Penicillin prophylaxis is well established in its ability to prevent recurrent attacks, with intramuscular long-acting preparations being superior to oral twice-daily delivery.[75,121] Persistent worsening rheumatic heart disease is prevented.[125] Secondary prophylaxis is considered the most cost-effective management of acute rheumatic fever and its sequelae.[187]

Recurrent attacks of rheumatic fever can be prevented by continuous penicillin prophylaxis, administered either orally or parenterally.[121] The parenteral route has been shown to be more effective (1.2 million U of benzathine penicillin intramuscularly at 28-day intervals).[214] In a comprehensive study, children experienced a recurrence rate of only 0.4/100 patient-years of observation (Table 35–6). A Cochrane systematic review found an 87 to 90 percent reduction with parenteral penicillin in four studies.[122] An international study of allergic reactions to long-term benzathine penicillin prophylaxis found that the benefits of preventing recurrence far outweighed the risk of development of a serious allergic reaction.[94] In areas of particularly high risk, administration of benzathine penicillin every 21 days may be more efficacious, although this study was not performed systematically.[118] Serum levels of penicillin toward the end of the time can be unreliable,[96] although this decision should be offset against practicability, cost, and probable compliance.

In a carefully monitored, community-based series with high compliance rates on four weekly injections of penicillin,[87] the penicillin failure rate per se was very low at 0.07/100 patient-years.[183] The program failure rate was 1.4/100 patient-years, with failure primarily resulting from nonadherence. This is in an environment endemic for rheumatic fever, where children had a 1:3 annual chance of having *Streptococcus pyogenes* detected in their throats.[114] A study in Egypt[103] showed that two weekly injections of penicillin resulted in an almost 50 percent reduction in recurrences. This result occurred, however, in an environment in which the rate of recurrences was approximately 45 percent of cases in the four-weekly penicillin comparison arm. A more recent publication supports the use of 1 percent lidocaine hydrochloride as a diluent for benzathine penicillin G to increase tolerability.[6a]

Patients allergic to penicillin may be given erythromycin (250 mg twice a day). Penicillin and sulfadiazine are oral regimens that have been studied for efficacy (see Table 35–6). Sulfadiazine is not readily available in the United States.[11] The lesser efficacy of oral regimens is related at least partly to compliance difficulties.

The risk of rheumatic fever occurring after a group A streptococcal infection increases from an attack rate of 1 to 3 percent with the first attack of streptococcal pharyngitis to 25 to 75 percent with subsequent attacks.[64] Patients who have had carditis are at increased risk for development of further carditis. Patients who have not had clinical carditis have considerably less risk of having cardiac involvement after a recurrence.[68,119] In the echocardiographic era, the dogma that a patient who escapes carditis in the first attack is highly likely to remain free of rheu-

TABLE 35–6 Prophylaxis and Attack Rates of Streptococcal Infection and Rheumatic Fever Recurrence

	Parenteral Benzathine Penicillin	Oral Penicillin	Oral Sulfadiazine
No. years	560	545	576
No./rate of all streptococcal infections, exclusive of carrier state	24/4.3	101/18.5	102/17.7
No./rate of rheumatic recurrences	2/0.4	30/5.5	16/2.8

Adapted from Wood, H. F., Feinstein, A. R., Taranta, A., et al.: Rheumatic fever in children and adolescents: A long-term epidemiologic study of subsequent prophylaxis, streptococcal infections, and clinical sequelae, III: Comparative effectiveness of three prophylaxis regimens in preventing streptococcal infections and rheumatic recurrences. Ann. Intern. Med. 60(Suppl. 5):31-45, 1964.

matic heart disease, even if prophylaxis fails in subsequent attacks, has been challenged.[68,183]

The risk of having a recurrence depends on several other factors, such as the length of time since the most recent attack, and the risk of acquiring streptococcal throat infections according to occupation or living circumstances. If possible, the length of prophylaxis should be individualized. The suggested length ranges from a minimum of 5 years of prophylaxis to a maximum of lifelong prophylaxis.[50] This approach has been validated in a study from Chile.[21]

A more recent publication supports the New Zealand approach.[115,169,183] All patients, regardless of cardiac status, receive at least 10 years of prophylaxis or until they reach 21 years of age (see earlier). Two recurrences in this series occurred in teenagers inadvertently discharged after at least 5 years of prophylaxis. Appropriate discharge of individuals as per the recommended parameters with mild or no carditis apparently was safe. Of equal importance is the prevention of infective endocarditis in patients with rheumatic heart disease or in patients who have had rheumatic fever by the administration of antimicrobial drugs before and after surgical procedures on the eyes, ears, mouth (dental extractions), nose, throat, and gastrointestinal and genitourinary tracts,[59] although opinions are changing on this subject.[213a]

CONCLUSION

Although having effective prophylaxis against a disease[132] for which the pathophysiology is understood incompletely and for which no pharmacologic cure exists is gratifying, rheumatic fever and its sequelae still occur in numerous young people, especially in the developing world and in disadvantaged populations in the developed world.[217] This rate is largely a reflection of complacency about rheumatic fever and rheumatic heart disease by physicians and health care funders, although it also is likely to reflect living conditions and health care access. Renewed educational efforts regarding prevention of rheumatic fever are needed by physicians and the public. The available preventive methods should be applied vigorously.

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CHAPTER 36

Mediastinitis

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The mediastinum is the extrapleural portion of the thoracic cavity situated between the two pleural sacs. The superior and inferior portions are separated arbitrarily by a line extending from the lower manubrium to the fourth thoracic vertebra. The superior mediastinum contains the thymus gland, trachea, esophagus, and aortic arch. The inferior mediastinum is divided into the anterior compartment, containing lymphatic tissue and fat; the middle compartment, containing the heart, pericardium, aorta, bifurcation of the trachea, main bronchi, and numerous lymph nodes; and the posterior compartment, containing the esophagus, thoracic duct, descending aorta, and vagus nerve. *Mediastinitis* refers to inflammation of the tissues located in the mediastinum. Infections of the mediastinum are uncommon occurrences, but often they pose a serious threat to vital structures and can prove extremely difficult to diagnose.

Acute mediastinitis is a fulminant, septic process, and chronic mediastinitis is an indolent infection that produces late symptoms caused by compression of adjacent structures. Acute mediastinitis is subclassified as (1) traumatic, occurring as a consequence of perforation of the esophagus; (2) infection extending to the mediastinum from adjacent structures; and (3) postoperative mediastinitis, usually occurring after thoracic surgery (Table 36–1).

ACUTE MEDIASTINITIS

ACUTE MEDIASTINITIS DUE TO ESOPHAGEAL PERFORATION

The esophagus is a thin-walled organ, and esophageal perforation is the most common cause of acute mediastinitis.[43] Perforation usually occurs at one of the three sites of anatomic narrowing of

TABLE 36–1 Classification of Mediastinitis

I. Acute mediastinitis
  A. Due to traumatic perforation of the esophagus
    1. Spontaneous or post-emetic
    2. Foreign body-associated
    3. Instrumentation or surgery
  B. Due to extension of infection from adjacent structures
    1. Infection of the head and neck
    2. Infections of lungs, pleura, lymph nodes, or pericardium
    3. Subphrenic infection
    4. Vertebral osteomyelitis
    5. Hematogenous dissemination
  C. Postoperative
II. Chronic mediastinitis

the esophagus: (1) the proximal end, located at the level of the cricopharyngeal muscle; (2) the midthoracic segment, where the aortic arch and left main stem bronchus indent the esophagus; or (3) the transdiaphragmatic segment. A correlation exists between the location of the perforation and the injury. Proximal perforations usually are caused by instrumentation or ingestion of a foreign body. The proximal segment is the narrowest, and perforations generally are located in the posterior wall, adjacent to the prevertebral and retrovisceral spaces. Perforations at the aortic arch usually are caused by ingested foreign bodies. Transdiaphragmatic perforations usually are spontaneous. In most such cases, a longitudinal tear occurs on the left posterolateral wall just above the cardia, where the esophagus has little connective tissue support and its intrinsic musculature is weak.

Retching or vomiting can generate sufficient force to cause esophageal perforation, also known as Boerhaave syndrome, but its occurrence is rare.[1] Traumatic perforation can occur after blunt trauma, from ingestion of a foreign body, or as a complication of endoscopic or open surgical procedures. In one large series of acute purulent mediastinitis, ingestion of a foreign body was a major cause of esophageal injury.[7] Children with mediastinitis from erosion of the esophagus caused by a foreign body tend to have small and well-contained perforations. Sharp objects, such as pins and bone fragments, can cause immediate transmural penetration. More commonly, and especially with blunt objects such as coins, teeth, and food particles (e.g., corn chips), the foreign body becomes impacted in the esophagus.[39] Eventually, suppurative necrosis of the wall occurs, with symptoms occurring days, weeks, or months after the ingestion.[22]

Perforations from instrumentation can produce precipitous clinical deterioration during the procedure from transmural laceration.[43] Alternatively, a superficial tear can occur from which infection subsequently extends, and several hours or days can elapse between instrumentation and the onset of symptoms. Repair of esophageal atresia is a common condition associated with a tear of the esophagus in childhood. In one review, 7 of 41 infants had a clinically significant esophageal disruption requiring reoperation 1 to 18 days after repair of esophageal atresia.[6] Mediastinitis complicated repair of esophageal atresia in 3.6 percent of 223 cases in another large series.[41] Postoperative perforations of the esophagus usually represent infectious complications of anastomotic leaks occurring after esophageal resection or of esophageal-pleural fistulas that develop after thoracic surgery. Most of these infections do not become apparent until weeks or months after surgery.

The predominant symptoms of acute mediastinitis after perforation of the esophagus are neck and chest pain, respiratory distress, and dysphagia. Chills, fever of 37.8º C to 39º C (100º F to 102º F), and leukocytosis also are common. Infants can present with tachypnea, tachycardia, stridor, or a supplemental oxygen requirement.[22] Some patients have a staccato breathing pattern characterized by an inspiratory halt with resumption of inspiration after a brief rest.[12] The onset of symptoms usually is abrupt, and the course is fulminant. Approximately 20 to 30 percent of patients are comatose or hypotensive when first seen.[1] Physical examination often reveals cervical tenderness and subcutaneous emphysema in patients with proximal perforations, whereas patients with perforations of the lower esophagus are more likely to have signs suggesting an acute abdominal catastrophe. Examination of the lung fields often shows nonspecific abnormalities. The Hamman sign (a "crunching" sound heard in synchrony with the heartbeat along the left sternal border or cardiac apex) is observed in approximately 50 percent of cases of mediastinal emphysema, but it also occurs with left pneumothorax, dilated esophagus, gastric dilation, bullous emphysema, and pneumoperitoneum.

The principal findings on chest radiographs are a widened mediastinum, subcutaneous and mediastinal emphysema, and pleural effusions. Pleural effusions occur more commonly with perforations of the lower than the upper esophagus and usually involve the left side. Basilar or retrocardiac infiltrates ascribed to chemical pneumonitis can occur in the pulmonary segment adjacent to the site of perforation. Additional changes often include basilar atelectasis, pneumothorax, or a hydropneumothorax. Radiopaque foreign bodies can be detected with plain radiographs, but often they are seen better with mediastinal computed tomography (CT).

Gas in the soft tissues seen on radiography is highly suggestive of perforation of the esophagus if interpreted in the context of a compatible clinical presentation. Gas in the prevertebral tissue or superior mediastinum occurs most commonly with perforation of the upper esophagus. Other conditions, such as chest wall trauma or perforation of the trachea, also can cause mediastinal emphysema.

The diagnosis of acute mediastinitis caused by perforation of the esophagus often can be made on the basis of the clinical setting coupled with routine chest radiograph findings. Contrast-enhanced CT or magnetic resonance imaging (MRI) can provide additional anatomic detail and confirmation of the diagnosis.[47] The findings include esophageal thickening, fluid collections in the mediastinum adjacent to the perforation, and extraluminal air.[2] Other routine laboratory tests are not helpful. Analysis of pleural fluid usually shows a sterile exudate early in the disease course. Pleural fluid amylase levels often are normal within the first 24 hours after perforation. After 24 hours, the pleural fluid amylase level is elevated disproportionately compared with serum levels. Esophagoscopy is unnecessary and is contraindicated except for removing a foreign body.

The standard treatment comprises surgical drainage and repair and antimicrobial therapy and should be undertaken for large perforations, when there is communication with the pleural space or abdomen, when vascular erosion is a concern, and in the setting of underlying esophageal pathology. Nonsurgical management can be considered for children in whom the perforation is a small, well-contained lesion in the upper esophagus in the absence of underlying esophageal pathology.[10,22] Supportive measures include intravenous fluid support, maintenance of an adequate airway, esophageal rest (i.e., no food), and careful monitoring of vital functions.

Selection of antimicrobial treatment optimally is determined by bacteriologic studies. Blood and pleural fluid cultures should be obtained, but they usually are sterile except late in the course. Consequently, decisions with regard to antimicrobials necessarily are empiric and should be directed against oral anaerobic bacteria and streptococci.[5,8]

Mortality rates are 15 to 40 percent and are especially high when recognition of the infection in its early stages is delayed.[1,7,41,43] Mortality rates for children are lower than the rates for adults. An exception is postoperative perforation of the esophagus, which tends to follow a more indolent course.

ACUTE MEDIASTINITIS DUE TO EXTENSION OF INFECTION FROM ADJACENT STRUCTURES

The mediastinum is anatomically well situated for involvement when infection extends downward from the oropharynx. Fascial planes from the supraclavicular region and abdomen traverse the mediastinum, and the lymphatic duct is located in the mediastinum. The lung, situated laterally to the mediastinum, is a frequent locus of potentially serious infection. Despite its position as an anatomic crossroad, however, extension of infection to the mediastinum from adjacent structures occurs infrequently.

In the pre-antibiotic era, retropharyngeal or peritonsillar abscess, Ludwig angina, dental abscess, and other infections of the head and neck were common causes of acute mediastinitis.[8,14,46,51] Since the advent of penicillin, infections of the head and neck usually are contained at the site of origin. The principal spaces that serve as conduits to the mediastinum when these infections do spread are the visceral division of the deep cervical fascia that envelops the esophagus, trachea, larynx, and thyroid gland and the carotid sheath, which extends from the base of the skull, passes through the posterior pharyngomaxillary space along the prevertebral fascia, and enters the chest. Conditions other than pharyngitis and peritonsillar and dental abscesses that have preceded the development of mediastinitis include mastoiditis, laryngectomy, mediastinotomy, tracheostomy, and surgery or trauma of the oropharynx. Mediastinitis can complicate placement of airway stents for management of tracheal or bronchial stenoses in children.[21]

Children have developed mediastinitis after incurring intraoral injuries caused by falling with an object such as a toothbrush in their mouths.[25] Infection spreads to the mediastinum through the retropharyngeal space. Other sharp objects, such as fish bones, can perforate the esophagus, with resultant infection.[36] A penetrating wound to the oropharynx can be caused by falling on a sharp or pointed object such as a pencil. Occasionally, foreign bodies retained in the esophagus for months to years can have life-threatening complications, such as bronchoesophageal fistula or mediastinitis. Reported items causing such morbidity include coins, a heart pendant, a clothespin spring, and a toy soldier.[17] Mediastinitis rarely results in a complication of suppurative pleuropulmonary infection. Extension of infection from vertebrae, ribs, or sternum also is unusual. The main radiographic feature by plain chest radiograph is widening of the mediastinum. Contrast-enhanced cervicothoracic CT scan is crucial for establishing the diagnosis and can reveal heterogeneous infiltration, gas in tissues, abscesses, and fluid collections (Fig. 36–1).

The major bacteria responsible for suppurative infections that originate in the oral cavity and extend to the mediastinum are streptococci and anaerobic oral flora.[8,35] Mixed infection with aerobes and anaerobes should be anticipated when empiric therapy is initiated. Group A streptococcus is a common pathogen when the oropharynx is the original portal of entry. Other streptococci, including *Streptococcus milleri*, *Streptococcus anginosis*, and group F streptococcus, are common isolates. Anaerobic bacteria, principally *Prevotella* spp., *Bacteroides* spp., *Fusobacterium* spp., and peptostreptococci, are the major anaerobic pathogens. Gram-negative aerobic bacteria, including *Pseudomonas aeruginosa*, *Serratia* spp., and *Neisseria* spp., are isolated less often.[28] Clindamycin often is regarded as the agent of choice for streptococci and anaerobes, although some authorities prefer other regimens, including penicillin plus metronidazole, cefoxitin, cefotetan, meropenem, or a β-lactam–β-lactamase inhibitor.[8,13]

Surgical drainage combined with appropriate antibiotic therapy is the cornerstone of treatment. Transcervical incisions usually are employed when spread to the superior mediastinum has occurred. Extension of the infection below the level of the fourth thoracic vertebra requires a parasternal or paravertebral approach, depending on whether the anterior or posterior mediastinum is involved.

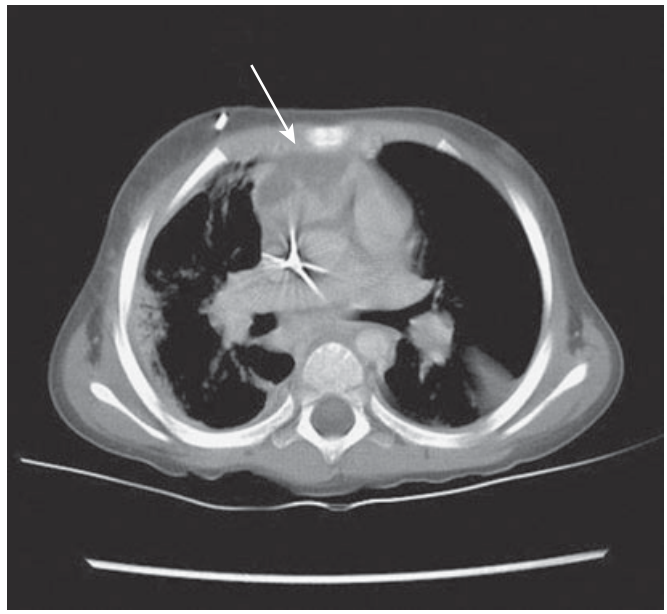

Figure 36–1 Contrast-enhanced computed tomography scan shows a 3 × 3 cm heterogeneous enhancing abscess *(arrow)* in the anterior mediastinum of a child acutely ill with *S. pneumoniae* bacteremia and pneumonia with empyema. Cultures from the mediastinal abscess at the time of surgical drainage were sterile.

TABLE 36–2 Risk Factors for Pediatric Poststernotomy Mediastinitis

Young age
Severe illness (assessed by high ASA score)
Asplenia
Prolonged duration of surgery
Cardiac transplantation
Delayed sternal closure

ASA, American Society of Anesthesiologists.
Data from references 3, 31, 48.

POSTOPERATIVE MEDIASTINITIS

Sternal wound infections are an uncommon complication of median sternotomy for cardiac surgery in children.[3,27,31,38,48] The Centers for Disease Control and Prevention classifies surgical site infections in cardiac patients as superficial or deep wound infection, sternal osteomyelitis, or mediastinitis.[20] Mediastinitis is a serious complication, with potential involvement of contiguous structures, including prosthetic valves, grafts, pericardium, lung, and chest wall. Contemporary incidence data for mediastinitis after median sternotomy in children is 0.2 to 1.4 percent of procedures.[3,27,31,48] The incidence is higher after cardiac transplantation.[27] Postoperative wound infection is more often a complication of double-outlet right ventricle repair, truncus repair, atrial switch, or valvulatory or conduit procedures than of repair of atrial septal defect, ventricular septal defect, or tetralogy of Fallot.[38] Mediastinal infection has developed from an infection of a retained epicardial pacemaker lead.[19]

Some of the well-established risk factors for development of poststernotomy mediastinitis in adults are not applicable to children. Table 36–2 lists factors that are linked to risk for develop-

ment of poststernotomy mediastinitis in children. Obesity and diabetes mellitus, which are risk associations in adults, have not been assessed as potential risk factors in more recent pediatric series. Factors not associated with risk for infection include emergency surgery or prior infection.[31] Intraoperative introduction of organisms is considered the source of most infections. Outbreaks of mediastinitis with *Mycobacterium chelonei* or *Mycobacterium fortuitum* presumably reflect contact with nonsterile water.[23] Epidemics have been traced to operating room personnel who can serve as the source of *Staphylococcus aureus* or other bacteria.[15]

In three contemporary pediatric series, median age at diagnosis of mediastinitis after pediatric cardiac surgery ranged from 6 weeks to 3 years.[3,27,48] Median time to onset of infection after surgery was 10 to 14 days, with a range from 4 to 50 days. The time to infection after initial sternotomy was longer for infections caused by gram-positive bacteria than for infections caused by gram-negative bacteria or fungi in one report.[48] The presenting features include erythema, purulent drainage or tenderness of the sternal incision, wound dehiscence, sternal instability, persistent or recurrent postoperative fever, and leukocytosis. A common clinical sequence is fever and systemic toxicity followed by signs of a sternal wound infection with cellulitis or purulent drainage. The CT abnormalities include mediastinal soft tissue swelling, pleural effusion, and sternal dehiscence or erosion, but CT of the chest does not always reveal abnormalities.[33,50] Consideration should be given to use of alternative testing modalities, such as MRI, when infection is suspected. Lack of compelling radiographic evidence of infection should not delay surgical drainage when clinical signs are evident.

Empiric antibiotic treatment should be initiated based on the expected pathogens and modified according to the results of blood and wound cultures. Approximately two thirds of infections are caused by gram-positive bacteria, one fourth by gram-negative bacteria, and the remainder by yeasts and fungi. The most frequent pathogen is *S. aureus*, which accounts for more than half of infections; coagulase-negative staphylococci and enterococci (including vancomycin-resistant isolates) constitute the remainder of the gram-positive isolates.[3,27,48] In adults, methicillin-resistant *S. aureus* mediastinitis is associated with higher rates of overall mortality, mediastinitis-related mortality, and treatment failure than is infection caused by methicillin-sensitive *S. aureus*, but this association has not been reported for children.[32]

Approximately half of children have associated bacteremia, and infection with *S. aureus* is an independent risk factor for development of bacteremic postoperative mediastinitis.[44] Among gram-negative organisms, *Pseudomonas aeruginosa*, *Serratia* spp., and *Citrobacter* are common isolates. Infection is polymicrobial in one fourth of patients.[3,27,48] *Candida* spp. should be considered in any patient with infection of the mediastinum, particularly when broad-spectrum antimicrobials have been used.[9] Almost any microorganism gaining entry to the mediastinum theoretically can serve as the nidus for infection, and *Mycoplasma hominis*, *Ureaplasma urealyticum*, *Nocardia*, and *Aspergillus* all have been reported, albeit rarely.[16,26,30,49] Heart and lung transplant patients are prone to acquisition of infection with less common and more resistant pathogens, such as *Aspergillus fumigatus* and *Burkholderia cepacia*.[27] *M. fortuitum* can cause apparently culture-negative mediastinitis and has been reported in a child who had undergone a Fontan operation.[45]

Adequate surgical débridement is crucial to successful treatment of postoperative mediastinitis. The sternotomy wound should be reopened, and the sternum and mediastinum should be irrigated and débrided for removal of all devitalized tissue. Some wounds can be managed with direct sternal closure and mediastinal drain; open-wound packing and delayed rectus abdominis flap, pectoralis muscle flap, or omental reconstruction is required in some children.[4,37,48] Pectoralis muscle flap has been an effective treatment even in neonates.[11,48] Early consultation with the hospital plastic surgery and reconstructive team is advisable in patients in whom sternal reconstruction is anticipated.[48] Infection superficial to the sternum can be treated simply with incision, packing, and a short course of antibiotics, usually for 10 to 14 days. Systemic antibiotics must be given for at least 3 to 6 weeks for children with postoperative mediastinitis.

CHRONIC MEDIASTINITIS

Chronic mediastinitis histologically can be fibrosing or granulomatous. Both are rare conditions in which a definite cause often remains elusive. The clinical presentations are identical, and considerable overlap can occur in histologic findings. Fibrosing mediastinitis, also known as *sclerosing mediastinitis*, can be focal or diffuse; calcification within the lesion is observed more often when the process is focal. Some authors propose that common etiologic mechanisms are responsible and that mediastinal fibrosis represents the end stage of mediastinal granuloma, whereas others suggest that the two are distinct entities.[42] Nonetheless, fibrosing mediastinitis stands apart in being associated sometimes with a fibrotic process at another anatomic site, such as the retroperitoneum.

Chronic mediastinitis occurs in virtually any age group. It is rare in very young children, but has been described in toddlers.[40] Many patients are asymptomatic, and the lesion initially is detected by routine chest radiographs showing a widened superior mediastinum near the tracheal bifurcation or the hilum with a lobulated configuration. Concomitant changes in the pulmonary parenchyma that are seen on chest radiograph vary. Symptoms, when manifested, usually reflect compression of adjacent structures, such as the superior vena cava, pulmonary vessels, esophagus, and tracheobronchial tree. The most common symptoms include cough, pleuritic chest pain, dyspnea, and pleuritic chest pain.[42] Low-grade fever, anemia, and weight loss can be present.

When an inciting cause for chronic mediastinitis can be identified, it usually is linked to infection with *Histoplasma capsulatum*. Two types of mediastinal fibrosis caused by histoplasmosis are recognized. Mediastinal granuloma is caused by coalescence of a cluster of mediastinal lymph nodes to form an encapsulated mass that may be large and compress adjacent structures, especially the superior vena cava or esophagus. Surgical resection often is feasible but is advocated only when the obstruction is significant. Fibrosing mediastinitis is thought to result from an exuberant inflammatory response to *H. capsulatum* and to represent a resolved, rather than an active, infectious process. Less common causes of fibrosing mediastinitis include tuberculosis, blastomycosis, cryptococcosis, aspergillosis, zygomycosis, autoimmune disease, nocardiosis, actinomycosis, and lymphoma.[18,24,29,34,40,42]

The diagnostic evaluation of patients with possible chronic mediastinitis should include a chest radiograph and contrast-enhanced CT or MRI. A vascular imaging study may be required if there is evidence of venous or vena caval obstruction or if arterial involvement is present. Tuberculin skin test and histoplasmosis serology should be obtained. Cultures of sputum for *Mycobacterium tuberculosis* and pathogenic fungi rarely are positive. Antigen assay for *H. capsulatum* using blood and urine usually is negative, and skin tests are not helpful. Complement-fixation titers usually exceed 1:8 in patients with chronic histoplasmosis.

Surgical resection of affected tissues can be associated with high morbidity rates, but can be curative. Biopsy of the lesion by mediastinoscopy provides a means to exclude a malignant process and obtain tissue for histopathologic evaluation. Antifungal therapy is not likely to affect resolution of the process when surgically excised tissue shows *H. capsulatum* in the setting

of sterile cultures. Dense fibrosis of mediastinal structures without a granulomatous component does not respond to antifungal treatment. Newer agents used for histoplasmosis, such as fluconazole and itraconazole, also have an uncertain role in therapy.

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CENTRAL NERVOUS SYSTEM INFECTIONS

CHAPTER 37

BACTERIAL MENINGITIS BEYOND THE NEONATAL PERIOD

Ralph D. Feigin ❂ William B. Cutrer

Bacterial meningitis is an inflammation of the meninges caused by bacterial infection. The term *leptomeningitis* denotes inflammation of the arachnoid and pia mater, the usual distribution of meningitis. Infections of neonates, including bacterial meningitis, are presented in Chapter 73, and infections of the central nervous system (CNS) caused by mycobacteria are discussed in Chapter 99.

INCIDENCE AND EPIDEMIOLOGY

Before the discovery and use of antibiotics, bacterial meningitis generally was fatal. Antibiotic therapy has improved dramatically the prognosis in patients with bacterial meningitis, although it continues to be a significant cause of morbidity and mortality in children. The number of deaths attributed to many other infectious diseases in the United States decreased by 10-fold to 200-fold between 1935 and 1968, whereas the number of reported deaths caused by bacterial meningitis decreased by only half during the same period.[379,380] In 1972, the Centers for Disease Control and Prevention (CDC) estimated that in the United States, 29,000 cases of meningitis were caused by *Haemophilus influenzae* type b, 4800 cases were caused by *Streptococcus pneumoniae*, and 4600 cases were caused by *Neisseria meningitidis*.

Population-based studies in South Carolina, Minnesota, Vermont, and New Mexico in the 1970s suggested that the actual incidence of bacterial meningitis ranged from 5.4 to 7.3 cases per 100,000 population.[63,130-133] Studies reported in 1995 suggested that the incidence in children aged 1 to 23 months ranged from 0.7 (for *H. influenzae* type b) to 6.6 (for *S. pneumoniae*) cases per 100,000 population.

Before the widespread use of conjugate *H. influenzae* type b vaccine, *H. influenzae* type b was the most common cause of bacterial meningitis in children in the United States, Canada, and Scandinavia, but this pattern was not universal.[162] Davey and associates[81] reported that between 1968 and 1977, *N. meningitidis* was the most common cause and *H. influenzae* type b was the second most common cause of bacterial meningitis for children and young adults in Great Britain. Data from 2001 showed that meningococcal meningitis accounted for 48 percent of meningitis in children in England.[82] Mortality rates in this group of patients were 3.5 percent for children with meningococcal meningitis, 7.7 percent for children with *H. influenzae* meningitis, and 30 percent for patients with pneumococcal meningitis.

By 1995 in the United States, the most frequent cause of bacterial meningitis in children aged 1 month to 24 months was *S. pneumoniae*, followed by *N. meningitidis*, group B streptococcus, and *H. influenzae* (Table 37–1). In children younger than 1 month, the most common cause of bacterial meningitis was group B streptococcus, followed by *S. pneumoniae*.[333] *Escherichia coli* and *Listeria monocytogenes* are other common causes of meningitis in neonates 2 to 6 weeks of age.[7,35] *N. meningitidis* was the most common cause for individuals 2 to 29 years old, followed by *S. pneumoniae*.

Infants 6 to 12 months old seem to be at greatest risk for acquiring bacterial meningitis; 90 percent of reported cases occur in children between 1 month and 5 years of age.[130] The age distribution of patients with bacterial meningitis has not changed appreciably during the past 40 years.[285,349]

EPIDEMIOLOGY OF *HAEMOPHILUS INFLUENZAE* MENINGITIS

The most dramatic change in the epidemiology of bacterial meningitis since the advent of antibiotics has occurred in the past 2 decades as a result of licensure of conjugate vaccines against *H. influenzae* type b.[330] The first vaccine available was *H. influenzae* type b capsular polysaccharide (polyribosylribitol phosphate [PRP]), which was licensed in April 1985 for use in children 18 to 59 months of age. Newer vaccines with improved immunogenicity for children of younger ages were developed by covalently linking the capsular polysaccharide with protein antigens. In October 1990, the first conjugate, PRP diphtheria CRM_{197} protein conjugate (HbOC), was approved for infant use, and in 1991, the Advisory Committee on Immunization Practices (ACIP) and the American Academy of Pediatrics (AAP) recommended universal infant immunization at 2, 4, and 6 months of age with either HbOC or PRP-meningococcal protein conjugate (PRP-OMP) vaccines.[12,64] Currently, four different conjugate vaccine preparations exist: PRP-T (tetanus toxoid), PRP-OMP, PRP-CRM_{197}, and PRP-D (diphtheria toxoid), which is not licensed in the United States.[395]

The *Haemophilus influenzae* Study Group[5] noted that the number of cases of *H. influenzae* meningitis in children younger than 5 years old reported through the National Bacterial Men-

TABLE 37–1 Age-Specific Incidence in 1995 of Bacterial Meningitis per 100,000 Population

Age	*Haemophilus influenzae*	*Streptococcus pneumoniae*	*Neisseria meningitidis*	Group B Streptococcus
<1 mo	0	15.7	0	125
1-23 mo	0.7	6.6	4.5	2.8
2-29 yr	0.1	0.5	1.1	0.1
30-59 yr	0.2	1	0.3	0.05
>60 yr	0.07	1.9	0.1	0.1

From Schuchat, A., Robinson, K., Wenger, J. D., et al.: Bacterial meningitis in the United States in 1995. N. Engl. J. Med. 337:970-976, 1997.

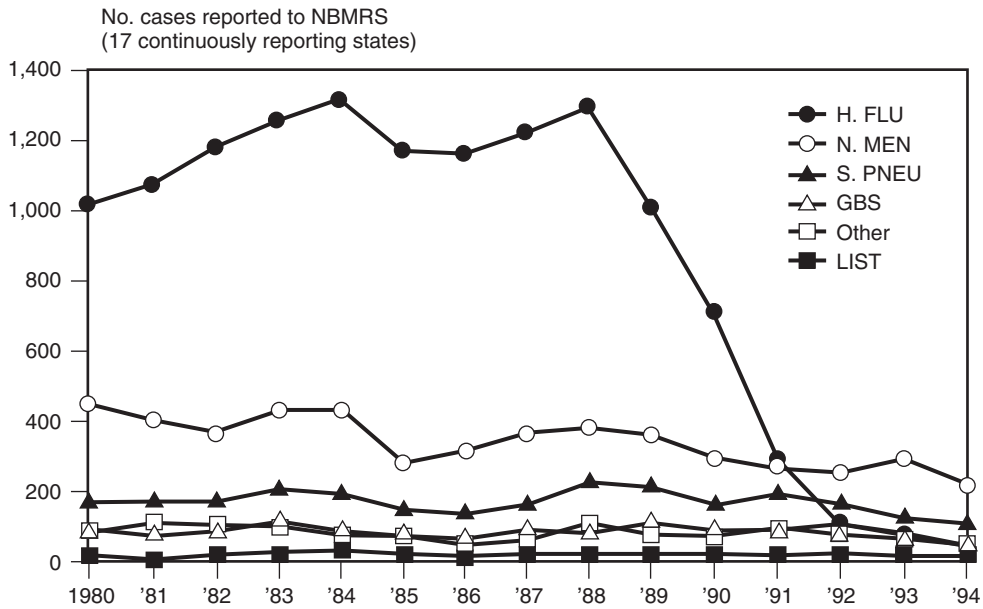

Figure 37–1 Incidence of bacterial meningitis in individuals from birth to 19 years of age. A marked decline in the incidence of *Haemophilus influenzae* type b meningitis is noted during the period between 1988 and 1994. GBS, group B streptococcus; H. FLU, *H. influenzae* type b; LIST, *Listeria monocytogenes*; NBMRS, National Bacterial Meningitis Reporting System; N. MEN, *Neisseria meningitidis*; S. PNEU, *Streptococcus pneumoniae*.

ingitis Reporting System began declining rapidly in 1988 (Fig. 37–1). In another CDC surveillance project conducted from 1989 to 1997, the race-adjusted incidence of invasive *H. influenzae* type b disease among children younger than 5 years declined from 34 to 0.4 per 100,000, a 99 percent reduction.[66] CDC data for 1998 to 2000 show that the incidence of *H. influenzae* infection remains low: 0.4, 0.4, and 0.3 per 100,000 children younger than 5 years in 1998, 1999, and 2000, respectively.[68] The average annual incidence of *H. influenzae* type b infection remains higher in select populations within the United States, including American Indians/Alaskan Natives (14 per 100,000), Hispanics (1 per 100,000), and nonblack Hispanics (0.6 per 100,000).[68]

Schoendorf and colleagues[332] evaluated national trends in mortality from meningitis from 1980 to 1991. From 1980 through 1987, mortality rates from *H. influenzae* meningitis decreased an average of 8.5 percent a year, from 1.72 per 100,000 children in 1980 to 0.94 per 100,000 children in 1987. From 1988 to 1991, mortality rates decreased an average of 48 percent a year, with a death rate of 0.11 per 100,000 children in 1991. The estimated case-fatality rate for *H. influenzae* meningitis was 3.3 percent from 1980 to 1987 and 2.3 percent from 1988 to 1991. In comparison, mortality rates from *S. pneumoniae* decreased 10 percent annually from 1980 to 1987 and 3 percent annually from 1988 to 1991. Similarly, mortality rates from meningococcal meningitis decreased by 13 percent annually from 1980 to 1987 and 12 percent annually from 1988 to 1991.

The dramatic decrease in the incidence of *H. influenzae* meningitis probably has been affected by several factors. The precipitous drop that occurred shortly after universal immunization of infants strongly suggests that this practice has affected the epidemiology of this disease. Conjugate vaccination protects against nasopharyngeal colonization,[268] decreasing the carriage rate of *H. influenzae* type b and diminishing the reservoir for transmission and providing immunity from infection. These factors would lessen the likelihood of development of infection in underimmunized children as well. Other changes in medical practice, such as the widespread use of outpatient antibiotics and improvements in supportive care, may have some effect, as shown by the decrease in case-fatality rates for *H. influenzae* meningitis, the steady decrease in mortality rates before vaccination, and the decrease in instances of meningococcal and pneumococcal disease.

Before universal vaccination was initiated, the incidence of *H. influenzae* meningitis varied worldwide. The incidence of *H. influenzae* meningitis in Scandinavian children younger than 5 years averaged 16 to 28 cases per 100,000 children from 1975 to 1984.[288] The incidence of *H. influenzae* type b meningitis in three Pacific Island countries was 70 to 84 cases per 100,000 children younger than 5 years old in 2003[314] and 67 to 158 cases per 100,000 in Indonesian children younger than 5 years old in 1998 to 2002.[151] The incidence of *H. influenzae* meningitis in the Netherlands was 22 cases per 100,000 children younger than 5 years old.[381] A universal vaccination program in the Netherlands seems to have led to a marked decrease in the incidence, with six reported cases of meningitis caused by *H. influenzae* in a 1-year period after im-plementation of universal vaccination of infants compared with 34 cases per 100,000 children in a 1-year period before vaccination.

The incidence of *H. influenzae* meningitis is markedly higher in nonindustrialized populations. Before the vaccination era, Alaskan Eskimos had an annual incidence of 282 cases per 100,000 children younger than 5 years of age.[393] The Navajo and White Mountain Apache Native Americans had a much higher incidence compared with that of Native Americans in other regions of the United States,[77,242] and the incidence among Australian Aboriginals and among certain African populations, such as those in Gambia and Senegal, was 3 to 10 times higher than that of populations in the United States and Europe.[43,154,176] The incidence of *H. influenzae* meningitis was decreased in Gambia from more than 60 cases per 100,000 children to none per 100,000 in children younger than 5 years old after implementation of *H. influenzae* type b vaccination in May 1997.[6]

Good epidemiologic data concerning the incidence of *H. influenzae* infection are lacking in many other areas of the world. Measuring the extent of *H. influenzae* disease often is difficult, time-consuming, and expensive. In an attempt to decrease complexity, cost, and time required to collect data concerning the incidence of *H. influenzae* infection, the Hib Rapid Assessment Tool was developed. More and more countries are using such tools to estimate the local *H. influenzae* disease burden and to

help determine the need for implementation of a local vaccination program.[395]

In one study, the incidence of *H. influenzae* meningitis was 3.5-fold higher in blacks than in whites, but this distribution of cases seemed to be related more closely to poverty than to race.[130] In whites, no increase in incidence occurred in overcrowded households, but the incidence was higher in rural than in urban areas. Fraser and associates[130] postulated that the increased incidence in rural whites and in blacks was related to lack of access to early medical care.

Ward and colleagues[391] studied prospective data obtained in 19 states to determine the risk of spread of severe *H. influenzae* illness among household contacts of patients with *H. influenzae* meningitis. The risk in children younger than 1 year of age was 6 percent; in children younger than 4 years, 2.1 percent; and in children younger than 6 years, 0.5 percent. The risk of *H. influenzae* disease occurring in household contacts younger than 6 years old is similar to the risk of secondary meningococcal disease occurring in all household contacts, indicating a need for effective antimicrobial prophylaxis.

Spread of *H. influenzae* disease in daycare centers also is well documented.[32,156,252,392] The precise risk of acquiring secondary *H. influenzae* type b infection in daycare centers is unclear.

Recurrent invasive *H. influenzae* type b disease has been reported. Detailed studies suggest that age and high incidence of disease alone are not the only factors contributing to the recurrence of the disease.[55] Patients who have recurrent disease caused by *H. influenzae* type b may represent a subset of a population with unusual disease susceptibility. In addition, children who develop invasive infection caused by *H. influenzae* type b after receiving appropriate conjugate vaccine frequently have subnormal immunoglobulin concentrations and should undergo immune evaluation.[186]

EPIDEMIOLOGY OF MENINGITIS CAUSED BY *STREPTOCOCCUS PNEUMONIAE*

The highest rate of invasive pneumococcal infection occurs in children younger than 2 years old. In a population-based surveillance study of invasive pneumococcal infection in southern California, the age-specific incidence of invasive pneumococcal disease for children 2 years or younger was 145 cases per 100,000. In the pre-vaccine era (before 2000 in the United States), for children 2 to 4 years old and 5 to 14 years old, the age-specific incidence decreased to 25 per 100,000 and 5 per 100,000, respectively.[406] The age-specific incidence for pneumococcal meningitis was 21, 12, 6, 2, and 0.5 cases per 100,000 for the age groups 0 to 6 months, 7 to 12 months, 13 to 24 months, 25 to 60 months, and older than 60 months, respectively. Since the initiation of *S. pneumoniae* vaccination, the incidence of invasive *S. pneumoniae* infections has declined. Data from CDC surveillance shows that from 1998-1999 to 2003, there has been a 94 percent decline in *S. pneumoniae* infection related to *S. pneumoniae* serotypes present in the vaccine (80 cases per 100,000 to 4.6 per 100,000 among children <5 years old).[69]

The risk for development of sepsis or meningitis caused by *S. pneumoniae* depends to some extent on the serotype with which the child is colonized. Although 90 pneumococcal serotypes have been identified, invasive disease, including sepsis and meningitis, in children younger than 6 years old is associated most commonly with serotypes 14, 6B, 19F, 18C, 23F, 4, and 9V.[58] The seven-valent pneumococcal conjugate vaccine (PCV7), added to the national vaccination schedule in 2000, contains each of the above-mentioned most common strains.

The risk of contracting pneumococcal meningitis is 5-fold to 36-fold greater among blacks than among whites and is independent of income or population density.[130] In one study, 11 percent of the black population with pneumococcal pneumonia had sickle-cell disease, a factor known to predispose an individual to development of pneumococcal disease.[130] On the basis of these data, Fraser and associates[130] suggested that 1 of every 24 children with sickle-cell disease may develop pneumococcal meningitis by the time they reach 4 years of age. This incidence is 36-fold greater than the incidence of pneumococcal meningitis in a normal black population and 314-fold greater than that in white children.

Daycare attendance, underlying disease, and lack of breast-feeding were risk factors for acquiring invasive pneumococcal infections in a large case-control study that included children from the United States and Canada.[237] Although cigarette smoking is a major risk factor for the development of invasive pneumococcal disease in adults, whether exposure to cigarette smoke increases the risk of developing systemic pneumococcal infection in children remains unclear.[162,280] The greatest mortality rate occurs in very old and very young patients and has been estimated to be 20 to 60 percent.[123,132,133,146]

Pneumococcal infections generally occur sporadically. Household contacts of a patient with pneumococcal disease are not considered to be at increased risk of acquiring secondary infection. The occurrence of concurrent pneumococcal disease (meningitis and bacteremia) in the household setting has been reported, however.[24,344] Although uncommon in the United States, *S. pneumoniae* has been associated with epidemics in locations such as Northern Ghana.[235]

The incidence of systemic infection with penicillin-resistant *S. pneumoniae* has been increasing steadily worldwide since it first was reported in Australia in the 1960s.[175] Systemic infection with penicillin-resistant pneumococci has become an increasing problem in the United States since the mid-1980s.[359,365,366] In 1995, a CDC multistate surveillance study showed that 35 percent of *S. pneumoniae* cerebrospinal fluid (CSF) isolates were resistant to penicillin.[333]

The first report of meningitis caused by resistant pneumococci was published in 1974,[274] and numerous case reports have appeared subsequently. Tan and colleagues[366] found that patients with systemic infections caused by penicillin-resistant pneumococci were more likely to have received a course of antibiotics within 1 month before acquiring their infection than were matched control subjects who had infections caused by pneumococci but whose isolates were susceptible to penicillin.

More recently, an increase has occurred in the number of cases of systemic infection and meningitis caused by *S. pneumoniae* organisms resistant to penicillin and third-generation cephalosporins. In 1998, CDC surveillance of multiple areas around the United States found that 24 percent of pneumococcal isolates associated with invasive infections were nonsusceptible to penicillin; 14 percent of isolates were nonsusceptible to cefotaxime.[398] As with many studies, rates of resistance were higher among children younger than 5 years old. In a prospective study involving eight children's hospitals nationwide during a 6-year period starting September 1993, a significant increase in the proportion of isolates nonsusceptible to penicillin or ceftriaxone was found. In the sixth year of the study ending August 1998, 37 percent and 11 percent of invasive isolates were nonsusceptible to penicillin and ceftriaxone.[203] Isolates of penicillin-resistant and third-generation cephalosporin–resistant *S. pneumoniae* have been recovered in other regions of the world as well.[136,139,305] Treatment failures in patients with *S. pneumoniae* meningitis resistant to penicillin and third-generation cephalosporins have led to changes in the empiric therapy of suspected bacterial meningitis, as discussed in the treatment section.[22,49,139,212,347]

Data from the Active Bacterial Core Surveillance of the Emerging Infections Program Network after introduction of the pneumococcal conjugate vaccine show an 87 percent decrease in rates of antibiotic-resistant disease by vaccine serotypes.[225] Their

results show that rates of invasive pneumococcal disease with penicillin-nonsusceptible strains and strains nonsusceptible to multiple antibiotics "peaked in 1999 and decreased by 2004."[225] In children younger than 2 years old invasive pneumococcal disease with penicillin-nonsusceptible strains decreased from 70.3 to 13.1 cases per 100,000.[225] The proportion of disease caused by penicillin-nonsusceptible strains varied by location within the United States from 9.4 percent in California to 29.5 percent in Tennessee.[225] The incidence of invasive *S. pneumoniae* disease caused by cefotaxime-resistant *S. pneumoniae* strains has increased in children younger than 2 years of age however, in the post-vaccination period, from approximately 10 percent in 1999 to almost 15 percent in 2003.[225] The decreases in disease caused by vaccine-type *S. pneumoniae* strains were coupled with an increase in non-vaccine serotype *S. pneumoniae* strains, especially with serotype 19A (increased from 2 to 8.3 cases per 100,000).[225]

EPIDEMIOLOGY OF MENINGOCOCCAL MENINGITIS

The carriage rate for *N. meningitidis* in the civilian population has been estimated at various times to range from 1 to 15 percent. Carriage rates in military personnel during epidemic periods have been considerably greater. Meningococcal carriers generally are adults (>21 years old) who harbor the organism for months.

No correlation has been noted between meningococcal meningitis and crowding within households, but disease seems to be more prevalent in urban than in rural areas. In a civilian population, meningococcal meningitis generally is a disease of children and young adults who have been exposed to an adult carrier, usually in the same family, or to individuals with disease or who are carrying the organism in a daycare setting. The estimated likelihood of severe meningococcal disease in family contacts occurring simultaneously with the first case is 1 percent.[231] The rate is 1000-fold greater than the risk in the community. The risk of meningitis being acquired in daycare center contacts of children with meningococcal disease is 1 per 1000.

In a CDC surveillance study of invasive meningococcal disease in the United States, the average annual incidence of disease was 1.1 per 100,000 population for the years 1992 to 1996[310] and remained the same through 2002.[70] The highest age-specific incidence occurred in infants younger than 1 year old (9.2 cases per 100,000),[70] with the peak incidence occurring in infants 4 to 5 months old (15.9 cases per 100,000). The age-specific incidence declined sharply until the 10- to 14-year age range, at which point it began to increase again during the adolescent and early adult years. During the teenage range, the incidence peaked between 15 and 19 years of age (1.5 to 2.2 cases per 100,000). From 1990 to 2002 in the United States, mortality rates for meningococcal disease were 0.1 deaths per 100,000 population per year, with 58 percent of deaths occurring among individuals younger than 25 years old. Infants had a higher mortality rate than that of individuals in different age groups, but they experienced a decline from 1.3 deaths per 100,000 in 1990 to 0.42 deaths per 100,000 population in 2002.[339] Meningococcal disease mortality rates increased each year in individuals between the ages of 10 and 19 years, which was followed by a decline in mortality rates in the years following and throughout most of adulthood.[339]

In the United States, serogroups B, C, and Y account for most meningococcal disease. Meningococcal infections caused by serogroup B caused greater than 50 percent of infection in infants younger than 1 year. In the 1990s, serogroup Y became more common among older patients and caused more pneumonia than did the other serogroups. The overall incidence of serogroup Y infection increased from 2 percent during 1989 to 1991 to 37 percent during 1997 to 2002.[70] A study of the epidemiology of meningococcal disease in New York City from 1989 to 2000 revealed a threefold decrease in rates of serogroup B disease and almost no cases in children younger than 5 years old. The authors noted the coincident increase in the use of *H. influenzae* conjugate vaccine containing serogroup B meningococcal outer membrane protein and postulated the existence of a possible correlation.[263] CDC data reveal that 75 percent of meningococcal infections in individuals older than 11 years are caused by serogroups C, Y, and W-135, all of which are included in the newly available conjugate vaccine. An increased risk of contracting meningococcal infection also exists in college students, especially freshmen who reside on campus in dormitories.[179]

Another change in the epidemiology of meningococcal disease in the United States relates to the increasing number of outbreaks (>10 cases per 100,000 population during 3 months), generally caused by serogroup C. Although these outbreaks account for only 2 to 3 percent of the total number of cases, they cause tremendous public concern and anxiety, which frequently result in misunderstanding of the nature of an outbreak by the media and the public.[189]

An otherwise unprecedented outbreak of meningococcal disease occurred in a sixth-grade elementary school classroom.[106] Five children from a class of 24 developed meningococcal meningitis. In addition, two siblings of one of the index cases also developed meningococcal infection. Detailed epidemiologic investigation suggested that close contact in the classroom (nose-to-nose distances of ≤34 inches) correlated with an increased rate of carriage of *N. meningitidis* and with an increased risk for development of invasive meningococcal disease.

Major outbreaks of meningococcal meningitis have occurred worldwide. In the late 1980s and early 1990s, major epidemics of meningococcal meningitis caused by a specific clone (III-1) of serogroup A *N. meningitidis* occurred throughout sub-Saharan Africa.[2,168,172,292,318] The origins of a pandemic spread of clone III-1 were traced to epidemics in Asia in the early 1980s, with spread through the Near East.[1] An outbreak occurred during the annual pilgrimage to Mecca in 1987,[260,261] with pilgrims carrying clones back to their countries of origin, including the United States and the United Kingdom. Epidemics of closely related strains of clone III-1 serogroup A meningococcal meningitis occurred in the Sudan,[318] Ethiopia,[172] and Chad in 1988,[261] and in Kenya[292] in 1989. A report in 1992 described an epidemic of clone III-1 in the Central African Republic in an area traditionally outside the "meningitis belt."[168] These epidemics generally begin during the dry season and decline at onset of the rainy season. According to the World Health Organization, "in major African epidemics, attack rates range from 100 to 800 per 100,000 population, but individual communities have reported rates as high as 1000 per 100,000."[253] Major outbreaks have occurred in Brazil,[88] in Finland,[288] and at multiple sites in Africa.[399] A clonal outbreak of serogroup W135 (ET-37ST-11 clonal complex) occurred in 2000 among Hajj pilgrims returning to Europe from Saudi Arabia. In 2001, outbreaks with serogroup W135 also occurred in Niger and Burkina Faso.[364]

Shifts within a community or within a country as a whole from one serogroup to another also are associated with an increased incidence of disease for several years after the new serogroup is introduced into the community.[310] The mechanisms underlying the changing patterns of meningococcal serogroups that cause disease are unknown.

Meningococcal infections also occur more frequently in patients with a deficiency of the terminal components (C5-C8) of the complement system.[100,239,319] More recently, an increased risk for contracting meningococcal meningitis has been reported in individuals with an inherited deficiency of C9[273] and with properdin deficiency.[353] Individuals with a complement-depleting underlying illness also are at particular risk for acquiring invasive disease.[290] Screening for complement deficiency in pediatric patients with meningococcal disease is recommended.[232]

PATHOPHYSIOLOGY

ORGANISMS ENCOUNTERED

Any organism may produce meningitis in a susceptible individual. *S. pneumoniae* and *N. meningitidis* are the responsible agents in approximately 95 percent of healthy children older than 2 months. In compromised hosts, infection with other organisms may occur more frequently. The specific organism sometimes may be predicted on the basis of the type of deficit that is present in the host.

ROUTES OF INFECTION

Bacterial infection of the normally sterile leptomeningeal spaces can occur from a distant focus through the bloodstream or by direct invasion from a contiguous focus. Meningitis usually is the result of hematogenous dissemination of organisms from a distant site of infection,[349] often from the respiratory tract. The meninges are seeded with microorganisms during a bacteremic period. Bacterial meningitis in children with otitis media generally follows bacteremia, although direct invasion of the meninges may occur as a complication of otitis media.

The route of infection in bacterial meningitis has been studied by use of a variety of animal models, but the experimental infection was initiated in most cases in a manner that did not mimic human disease. Bacterial meningitis has been induced in rats[264] and monkeys[325] after intranasal inoculation of *H. influenzae* type b. Bacteremia developed hours before meningitis could be detected histologically, a finding that supports the concept that meningitis follows hematogenous dissemination from nasopharyngeal colonization or infection. Marginating bacteria could be detected by fluorescent staining initially in the lateral and dorsal longitudinal (sagittal) sinuses and subsequently spread to the leptomeninges. In the rat model, otitis media seemed to develop by spread of infection from the subarachnoid space to the inner ear and then to the middle ear.

Meningitis may develop after bacterial invasion from a contiguous focus of infection, as in infection of the mastoid or paranasal sinuses, or as a complication of otitis media. Fracture through the paranasal sinuses as a result of head trauma may precede development of meningitis caused by *S. pneumoniae* and *H. influenzae*, which may be recurrent. Direct invasion also may occur in individuals with dermoid sinus tracts or meningomyeloceles, when a direct communication between the skin and the meninges is present. In this setting, infection usually is produced by organisms found on the skin. Recurrent meningitis has been reported in patients with basiethmoidal encephaloceles[362] and a congenital defect in the stapedial footplate.[183] Surgical obliteration of the fistula with temporal muscle and fascia prevented the recurrence of meningitis. Meningitis also may develop subsequent to osteomyelitis of the skull or vertebral column. Rarely, meningitis may develop in the normal host with commensurate microorganisms after having a tooth extraction or getting dental fillings.[74,340]

Neurosurgical procedures, particularly procedures designed for diversion of CSF in children who have hydrocephalus, may lead to development of meningitis. A chemical meningitis also may occur after neurosurgical procedures, especially procedures involving the posterior fossa.[127] In these patients, evidence of inflammation develops rapidly, with elevation of temperature occurring on the first postoperative day.

Infection of the CNS may result from environmental contamination or manipulation. Meningeal infection may be acquired in utero transplacentally or during delivery through contact with the cervix or vaginal canal, which may be colonized with a variety of organisms, particularly group B streptococci and *L. monocytogenes*.[7,25] A newborn, a patient with cystic fibrosis, or a burned child may develop septicemia and meningitis as a result of persistent heavy colonization with *Staphylococcus aureus*. A humidified atmosphere promotes the colonization and growth of such organisms as *Serratia marcescens* and *Pseudomonas aeruginosa*. Placing a patient in this setting leads to an increased frequency of infection with these organisms. Indwelling catheters can predispose a patient to infection by bacterial (and fungal) organisms that generally are of low virulence in the normal host.

FACTORS PREDISPOSING THE HOST TO BACTERIAL MENINGITIS

Factors that predispose the host to the development of infection in other sites also predispose the host to the development of bacterial infection of the CNS. A strong interrelationship exists among factors relating to the host, the organism, and the environment with regard to the pathogenesis and outcome of meningitis. Although presented separately, they must be considered a complex interplay of factors that leads to infection.

An increased incidence of bacterial meningitis is observed in the very young; boys are affected more frequently than girls, and the severity of disease also is increased in these groups. Fraser and associates[133] reported that the greatest morbidity after bacterial meningitis occurred in children affected between birth and 4 years of age. A newborn is predisposed to development of septicemia and meningitis by factors that reflect physiologic deficiencies or immaturity of host defense mechanisms, including (1) decreased phagocytic and bactericidal activity of polymorphonuclear leukocytes, (2) defects in the response of neonatal leukocytes to chemotactic factors, (3) a deficiency in the capacity of leukocytes to support opsonization, and (4) defects in microtubular length and number that decrease the motility of the neonatal leukocytes compared with leukocytes from older children. Deficiencies in serum complement components (C1q, C3, and C5), low levels of serum properdin, and low concentrations of serum IgM and IgA have been documented repeatedly. Despite transplacental acquisition of IgG, antibodies against specific infective agents may be lacking. The precise age at which each of these factors reaches the concentration and functional activity noted in older children and adults is unclear and undoubtedly varies from individual to individual. In part, meningitis in children aged 1 month to 1 year may reflect qualitative or quantitative differences of the inflammatory and immunologic responses in older children compared with infants.

The increased risk for development of meningitis in the normal host with less than completely mature immunologic and inflammatory responses to infection may be attributable to age alone. This factor is exemplified in the report of Cole and associates,[72] who studied the risk of recurrent bacteremia in young children. Within 18 months of having bacteremic illness, none of 42 children older than 24 months had a documented additional episode of bacteremia or systemic infection. Fifteen of 135 children (11%) younger than 24 months at the time of the initial bacteremic disease had at least one additional documented bacteremic illness, however. Of these 15 children, 14 contracted both infections before reaching 2 years of age. Seven of these 15 children had meningitis. Only two patients had documented congenital or hereditary disorders of immunoglobulin or complement concentration or function.

A genetic determination for the predilection of some normal children for the development of bacteremia and meningitis has been suggested.[373] The ability of the host to produce, within the CSF, interleukin-12 (IL-12) and tumor necrosis factor-α (TNF-α)–induced interferon-γ is important in the natural immunity to various microorganisms that may cause meningitis.[223]

Congenital or acquired abnormalities of the immune system may predispose the host to the acquisition of bacterial infections. Congenital deficiency of the three major immunoglobulin classes may predispose the host to the acquisition of severe bacterial infection. Congenital defects of thymic-dependent, small lymphocyte function or combined T and B defects are detrimental to host defense. A deficiency of $CD4^+$ helper-inducer T cells in patients with bacterial meningitis has been reported and may contribute to the impaired antibody synthesis to bacterial capsular polysaccharides in this disease.[303] Multiple studies have shown that deficiencies of various components of the complement system and increased consumption or loss of complement have been associated with increased risk for development of bacterial meningitis caused by encapsulated organisms.[319]

An increased incidence of overwhelming infection, including meningitis, occurs after splenectomy, but the likelihood of development of such infection depends on the age of the child at the time of splenectomy, the time since splenectomy, and the original indication for splenectomy.[101] Congenital asplenia or polysplenia also has been associated with an increased incidence of septicemia and meningitis caused by *S. pneumoniae*,[101] *H. influenzae* type b, and gram-negative enteric microorganisms. Children with sickle-cell disease and other hemoglobinopathies have meningitis caused by *S. pneumoniae*, *H. influenzae*, and *Salmonella* spp. more frequently than do normal children.

Children with malignant neoplasms with or without neutropenia seem to be susceptible to development of meningitis caused by organisms of low virulence that pose a minimal threat to healthy children, presumably because of abnormalities in immunologic function.[355] Decreased production of normal immunoglobulins, delayed and defective antibody responses to antigenic stimuli, production of abnormal immunoglobulins, depression in the clearance mechanisms of the reticuloendothelial system, and depression of cellular immunity have been documented in children with malignant neoplasms involving the reticuloendothelial system. In addition, the use of irradiation or immunosuppressive agents and antimetabolites predisposes the host to the development of infection in the CNS. Attributing the occurrence of bacterial meningitis in this population directly to these agents rather than to the disease for which this therapy has been provided may be difficult. Meningitis occurring after neurosurgical manipulation for tumors of the CNS in non-neutropenic children usually develops within 1 month of the neurosurgery.[355]

Malnutrition also predisposes children and adults to infectious disease. Impaired cellular immune responses, low levels of serum complement, impaired phagocytic activity of neutrophils, and decreased serum concentrations of transferrin have been documented in malnourished children.[114]

Patients with systemic diseases, such as diabetes mellitus, renal insufficiency, adrenal insufficiency, cystic fibrosis, hypoparathyroidism, and exudative enteropathy, have an increased frequency and severity of CNS infections.[47] Children with diabetes mellitus, coma caused by drug overdose, and Cushing syndrome have been shown to be at increased risk for development of bacteremia or meningitis caused by *H. influenzae* type b.[236] Some type of underlying condition was noted for 21 percent (37 of 181) of the children with pneumococcal meningitis in a multicenter surveillance study.[20] The most common of these conditions was some disorder of the CNS, which occurred in 16 children (9%). Defective chemotaxis, phagocytosis, and bactericidal function accompany these disorders and may explain in part the increased susceptibility of these individuals to infection.[114,115]

In the normal host, bacterial infections at sites other than the leptomeninges are associated with an increased incidence of CNS infection. Infection may spread hematogenously to the meninges in children with endocarditis, pneumonia, or thrombophlebitis or by direct extension from sinusitis, mastoiditis, or osteomyelitis of the skull. Development of meningitis after lumbar puncture in children younger than 1 year has been described.[126,371]

An increased risk for development of meningitis also occurs in children after placement of cochlear implants. Reelhuis and colleagues[304] reported the incidence of meningitis caused by *S. pneumoniae* in patients after receiving cochlear implants to be 138.2 cases per 100,000 person-years, which represents a more than 30-fold increase over the age-controlled general population.[304] From 1999 to 2002, some children were implanted with cochlear devices, including a positioner, which were removed from the market subsequently as a result of an associated marked increase in incidence of pneumococcal meningitis. When removing the influence of cochlear devices containing a positioner, the incidence of pneumococcal meningitis among children with cochlear implants was still 16 times higher than that of an age-matched control population.

PATHOLOGY

The most detailed account in English of the pathologic changes occurring with meningitis was written in 1948 by Adams and colleagues.[4] They described the meningeal, cerebral, and vascular changes found post mortem in 14 patients who died of *H. influenzae* infection 14 hours to 76 days after the onset of disease. Although most patients in their series received inadequate treatment (effective antibiotic therapy was unavailable), the pathologic findings they describe differ little from the findings of subsequent reports by Smith and Landing,[350] Rorke and Pitts,[309] and Dodge and Swartz,[96] whose patients died despite administration of antibiotics. These descriptions are summarized later.

A meningeal exudate of variable thickness may be found (Fig. 37–2). Purulent material is distributed widely but may accumulate around the veins and venous sinuses, over the convexity of the brain, in the depths of the sulci, in the sylvian fissures, within the basal cisterns, and around the cerebellum. The spinal cord may be encased in pus. Ventriculitis (purulent material within the ventricles) has been noted repeatedly in children who died of their diseases. Subsequent experience suggests that ventriculitis may be a common finding in children with bacterial meningitis who survive, particularly neonates. Invasion of the ventricular wall with perivascular collections of purulent material has been noted. Loss of ependymal lining and subependymal gliosis may be seen. In some studies, purulent exudate tended to be thicker

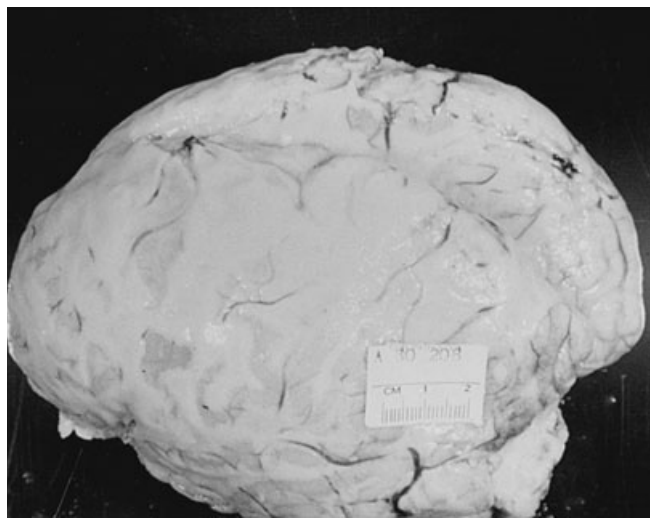

Figure 37–2 Note extensive purulent exudate over entire cerebral cortex in a patient who died as a result of bacterial meningitis.

over the convexity of the brain in patients with pneumococcal meningitis than in patients with other forms of meningitis.[96,309]

Vascular and parenchymatous changes have been shown at necropsy. Polymorphonuclear infiltrates extending to the subintimal region of small arteries and veins have been associated with the exudative meningeal process. Thrombosis of small cortical veins associated with necrosis of the cerebral cortex may be noted. Occlusion of one of the major venous sinuses, subarachnoid hemorrhage secondary to a necrotizing arteritis, and necrosis of the cerebral cortex in the absence of identifiable thrombosis of small vessels rarely may be observed. Reactive microglia and astrocytes may be identified in the cerebral cortex, particularly subadjacent to regions of heavy subarachnoid exudate. Because no bacteria are found in the cerebral cortex, these pathologic changes should be viewed as a noninfectious encephalopathy. "Toxic or circulatory factors" were suggested as possible causes by Adams and associates.[5] Dodge and Swartz[96] suggested systemic hypoxia and fever as additional possible causes. They also noted that an increase in intracranial pressure might interfere with cerebral circulation.

Impaired consciousness, deficits in motor and sensory functions, seizures, and retardation may be observed. Damage to the cerebral cortex, reflecting the effects of vascular occlusion, hypoxia, bacterial invasion, toxic encephalopathy, bacterial factors, inflammatory mediators, small molecule effectors, or some combination of these factors, provides an adequate explanation for these observations.

Hydrocephalus that develops in patients beyond the newborn period is an uncommon complication of meningitis. Most often, hydrocephalus is communicating and is the result of adhesive thickening of the arachnoid around the cisterns at the base of the brain. Less frequently, the aqueduct of Sylvius or the foramina of Magendie and Luschka are obstructed by fibrosis and reactive gliosis. The ensuing ventricular dilation may be coupled with coexistent necrosis of nervous tissue because of the meningitis itself or because of occlusion of cerebral veins and, rarely, arteries. Cerebral necrosis plus increased intraventricular pressure may result in total dissolution of the cerebrum.

Subdural effusions occur frequently during the course of meningitis. The exact pathogenesis is unknown. The high incidence of effusion and the fact that subdural fluid collections may be found early in the course of bacterial meningitis in children suggest, however, that subdural effusions should be considered a concomitant occurrence with meningeal inflammation rather than a complication of the disease. Numerous veins traverse the subdural space, and inflammation of these veins and of the dural capillaries could produce an increase in vascular permeability and loss of albumin-rich fluid into the subdural space. The ratio of albumin to gamma globulin is higher in the subdural fluid of children with meningitis than in serum.[159] When the inflammatory process subsides, formation of fluid generally ceases, but its presence may persist because of a continued transudation through newly formed vessels in the subdural membrane.

Subdural empyema, as opposed to subdural effusion, occurs rarely. It was observed in only 2 of the cases reported by Adams and associates,[5] in 1 of 34 patients examined by Smith and Landing,[350] and in 1 of the patients studied by Dodge and Swartz.[96]

Many factors contribute to the increase in intracranial pressure in patients with meningitis. Endotoxin and fragments of the cell wall of gram-positive organisms are capable of inducing the release of IL-1 and TNF from macrophages and other sources.[93,256] These substances, in addition to other interleukins and arachidonic acid metabolites, affect many systems, including endothelial cells, and profoundly affect the function of the vasculature and its interaction with neutrophils and other inflammatory cells. These substances play an important role in the pathogenesis of increased intracranial pressure and cerebral edema in patients with meningitis by altering cerebral blood flow, intracranial blood volume, and permeability of the cerebral vasculature.[278] Experimentally, the main components of the blood-brain barrier, endothelial cells, astrocyte-end feet, and pericytes, have been found to be sensitive to the effects of such inflammatory mediators.[29] Intercellular junctions, which normally are tight, are open in experimental meningitis, which is associated with an increased permeability to circulating albumin.[298] Pinocytotic vesicles also are noted within the cytoplasm of endothelial cells. Swelling of cellular elements (cytotoxic edema) also has been noted.

Alterations in CSF resorption exacerbate cerebral edema and increased intracranial pressure further. In experimental meningitis, resorption of CSF is diminished as an accumulation of proteins, leukocytes, and other materials interferes with the function of the arachnoid villus.[329]

During the course of meningitis, excess secretion of antidiuretic hormone (ADH) occurs, which induces water retention and exacerbates electrolyte abnormalities already created secondary to the inflammatory processes occurring in the CNS. Cellular electrolyte disturbances may depolarize neuronal membranes, predisposing the host to seizure activity. Increased oxidation of glucose, increased lactate production, and depletion of high-energy compounds such as adenosine 5′-triphosphate and phosphocreatine are observed. Hypoglycorrhachia results primarily from decreased transport of glucose across the inflamed choroid plexus and from increased use of glucose by host tissues. Use of glucose by bacteria and polymorphonuclear leukocytes is of less relative importance.[96,328]

PATHOGENESIS

Most cases of bacterial meningitis progress through four steps: (1) infection or colonization of the upper respiratory tract, (2) invasion of the blood from a respiratory focus, (3) seeding of the meninges by a blood-borne organism, and (4) inflammation of the meninges and brain. Less commonly, infection of the leptomeninges can occur by contiguous spread or hematogenous dissemination from another remote site. The nasopharyngeal mucosa is colonized with *S. pneumoniae*, *N. meningitidis*, or other microorganisms, resulting most commonly in an asymptomatic carrier state or minor upper respiratory tract illness. This attachment is mediated by specific microbial cell surface components. *N. meningitidis* strains possess fimbriae that bind to cell surface receptors on nasopharyngeal mucosal cells[90] and apparently are transported across specialized cells within phagocytic vacuoles.[363]

When in the bloodstream, the common pathogenic organisms (*S. pneumoniae*, *H. influenzae*, *N. meningitidis*, *E. coli* K1, and group B streptococcus) are capable of evading host defense mechanisms through capsular polysaccharides, which inhibit neutrophil phagocytosis and classic complement-mediated bactericidal activity. These bacteria traverse the blood-brain barrier, most likely at the cerebral capillaries and choroid plexus. Fimbriae of *E. coli* have been shown to facilitate attachment in these regions.[286] *N. meningitidis* has been shown to invade via interaction of Opc membrane protein with serum fibronectin and an endothelial surface integrin, which leads to tyrosine kinase activation and subsequent intracellular signaling pathways.[354,378] *S. pneumoniae* has been shown to invade the blood-brain barrier via complex interaction of CbpA protein and platelet-activating factor on activated endothelial cells.[219] When in the CSF, and because of insufficient opsonic and phagocytic activity in the CSF, organisms multiply rapidly, liberating cell wall or membrane components (lipopolysaccharide, lipoteichoic acid, peptidoglycan, bacterial toxins).

Host defenses within the CSF before and after bacterial invasion seem to rely on two important mechanisms available to the

host to clear bacteria.[343] One clearance system requires a type-specific antibody, a functional classic complement system for opsonization, and the presence of competent polymorphonuclear leukocytes for phagocytosis. A second system, which is independent of polymorphonuclear leukocytes, involves activation of the alternative complement cascade and other components of the innate immune system. This sequence begins with the recognition of a "pathogen-associated molecular pattern," such as bacterial lipopolysaccharide, peptidoglycan, or lipoteichoic acid. The pathogen-associated molecular patterns are recognized by host "pathogen recognition receptors," such as C-reactive protein (CRP), mannose-binding lectin, and Toll-like receptors (TLRs). Mannose-binding lectin and CRP can initiate the alternate complement pathway. TLRs (primarily TLR-2 and TLR-4), which can recognize different pathogen-associated molecular patterns, trigger an intracellular signaling pathway that leads to the production of proinflammatory transcription factors such as nuclear factor-κB and the subsequent gene transcription of proinflammatory cytokines such as TNF-α and interferon-β.[245]

Complement and opsonic proteins either are found at very low concentrations or are absent entirely within normal CSF.[128,343] The CSF is devoid of the factors required for bacterial clearance. When bacteria first invade the meninges, the lack of complement and opsonic proteins within the sanctuary of the CNS may permit the bacteria to multiply unrestrained for some time. The slow response of polymorphonuclear leukocytes and the lack of serum-specific antibody available during the initial inflammatory response enhance the probability that bacterial infection will be established.[41,103,152,389,408]

The specific pathophysiologic changes in bacterial meningitis are the result of the bacterial products and the inflammatory response of the host to those products. Initial bactericidal antibiotic therapy results in a rapid release of bacterial products, such as endotoxins, teichoic acid, and peptidoglycans. Augmented permeability of the blood-brain barrier can be induced by bacterial products alone, which cause disruption of the tight junctions between capillary endothelial cells and marked increase in pinocytotic activity within endothelial cells. An influx of serum albumin into the CSF is accompanied by other low-molecular-weight proteins, including components of the complement cascade.[193]

TNF-α and IL-1 seem to be key mediators in initiation of meningeal inflammation. Both proteins stimulate vascular endothelial cells to induce adhesion and passage of neutrophils into the CNS and trigger inflammatory processes. Astrocytes and microglia are capable of producing TNF-α.[374] TNF-α concentrations are elevated (1) in CSF, but not in serum; (2) in animal models of bacterial meningitis; and (3) in patients with bacterial meningitis caused by *H. influenzae*, *N. meningitidis*, *S. pneumoniae*, and *Streptococcus agalactiae*,[269] but not in patients with culture-proven viral meningitis.[301] A complete understanding of TNF-α and the precise role it plays in meningitis has not been achieved. TNF-α levels can be correlated with meningeal inflammation and severity of disease, but lack of TNF-α in the animal model is associated with "increased mortality and stronger deficits in spatial memory."[147]

IL-1 activity can be detected in infants and children with bacterial meningitis, and its presence is correlated significantly with CSF inflammatory abnormalities, TNF-α concentrations, and adverse outcome.[269] TNF-α and IL-1 are capable of inducing phospholipase A_2 activity, which triggers the production of platelet-activating factor and activates the arachidonic acid pathway. This process leads to the generation of prostaglandins, thromboxanes, and leukotrienes from membrane phospholipids of endothelial and polymorphonuclear cells, which modulate multiple aspects of the inflammatory process.

These cytokines activate adhesion-promoting receptors on cerebral vascular endothelial cells, resulting in attraction and attachment of leukocytes to sites of stimuli. These leukocytes release proteolytic compounds that allow intercellular junctions to be traversed. These enzymes in conjunction with platelet-activating factor and the arachidonic acid metabolites injure the vascular endothelium, resulting in increased permeability of the blood-brain barrier and activation of the coagulation cascade.

Superoxide and hydrogen peroxide are secreted by TNF-α-stimulated macrophages, including brain microglia, and leukocytes.[296,303] Hydrogen peroxide induces extensive neuronal damage.[281] In addition, macrophages secrete excitatory amino acids, such as glutamate, which potentially kill *N*-methyl-D-aspartate receptor–positive cells.[227]

For gram-positive bacterial meningitis, lipoteichoic acid peptidoglycans are the bacterial surface elements that induce inflammation.[376] The threshold concentration that triggers inflammation is approximately 10^5 bacterial cell equivalents of cell wall pieces.[376] For gram-negative meningitis, endotoxin is the major inflammatory component, with peptidoglycan serving as an important cofactor.[57] The inflammatory threshold is approximately 2 pg of endotoxin, or approximately 10^5 bacterial cell equivalents.[401]

Cytokines now seem to be the primary drivers of the inflammatory response. The following cytokines are involved in the inflammatory response noted in bacterial meningitis: IL-1, IL-3, IL-4, IL-6, IL-8, IL-10, IL-12, IL-18, interferon-γ, macrophage inflammatory protein, transforming growth factor-β, and TNF-α.[91,134,382,409]

The chemokines are a superfamily of small chemoattractant cytokines that play an important role in the initiation and modulation of inflammation in bacterial meningitis.[357] Complement factors are up-regulated in bacterial meningitis. The activated complement cascade in CSF, acting on up-regulated complement receptors on brain cells, potentially mediates direct brain damage.[53] Complement factors also are chemoattractants that enhance CSF leukocytosis and indirectly produce brain damage in patients with bacterial meningitis.[102]

The most potent final effectors of brain damage in bacterial meningitis seem to be host-derived, low-molecular-weight mediators, such as hydrogen peroxide, hydroxyl radicals, and hypochlorous acid.[299] Nitric oxide also can be induced in brain cells in response to bacterial products.[210] Nitric oxide and superoxide radicals react to form peroxynitrite anion, which decomposes and forms nitrogen dioxide, hydroxyl radicals, and strong oxidant compounds.[36] Peroxynitrite seems to be an important neuronal toxin.[218] Neuronal damage caused by reactive oxygen species and reactive nitrate species occurs via at least two separate pathways. Reactive oxygen species and reactive nitrate species lead to lipid peroxidation and subsequent cell membrane instability and via activation of poly(adenosine diphosphate ribose) polymerase and its subsequent cellular energy depletion.[219]

The various pathways to neuronal cell death are shown in Figure 37–3. Meningitis causes damage in the cortex and the hippocampal region via different mechanisms. Cortical damage is mainly via cellular necrosis surrounded by an area of caspase-3-dependent apoptosis. Damage in the hippocampus is mediated not only via the classic caspase-3-dependent pathway but also by caspase-independent apoptosis mediated by apoptosis-initiating factor.[40,42,257] The caspase-independent apoptotic pathway seems to be important earlier (by 18 hours after infection), whereas the classic caspase-dependent pathway assumes importance later.[257] Braun and Tuomanen[53] have provided a detailed review of the molecular mechanisms of brain damage in bacterial meningitis. In addition, a review by Scheld and coworkers[327] focuses specifically on neuronal injury. The role of inflammatory mediators and oxygen radicals in the pathogenesis of bacterial meningitis is described in detail in a review by Leib and Tauber.[234]

The increasing concentrations of chemotactic factors in the subarachnoid space lead to accumulation of large numbers of neutrophils in the CSF, and the growth of bacteria is not slowed

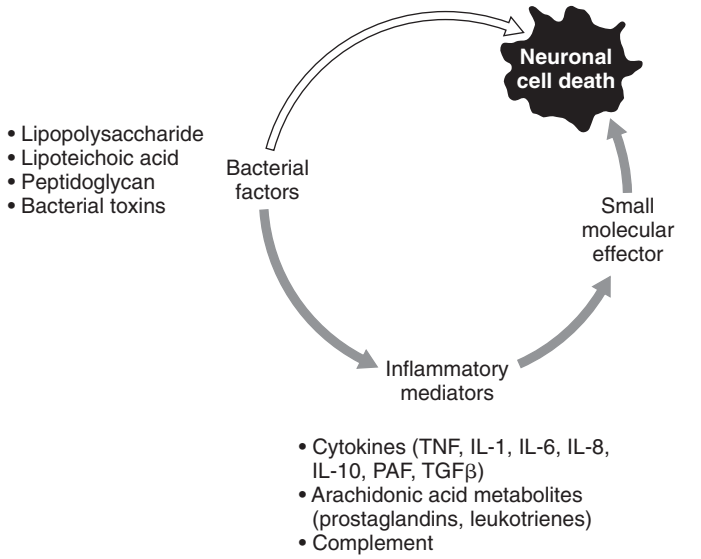

Figure 37–3 Pathways to neuronal cell death. Although bacterial products can induce directly some neuronal toxicity, host-derived inflammatory agents are the primary source of final mediators of injury. IL, interleukin; PAF, platelet-activating factor; TGF, transforming growth factor; TNF, tumor necrosis factor. *(From Braun, J. S., and Tuomanen, E. I.: Molecular mechanisms of brain damage in bacterial meningitis. Adv. Pediatr. Infect. Dis. 14:49-71, 1999.)*

significantly by this response.[102] Impaired phagocytosis by neutrophils in the meningeal spaces may be related to weak activity in fluid medium, lack of complement activity and opsonization, and poor penetration of IgM and IgG through the blood-brain barrier, even during acute *H. influenzae* and *S. pneumoniae* meningitis.[153] Leukocyte entry into the CSF is still important, however. Brandt and colleagues[52] showed that blockage of leukocyte entry into the CSF was associated with poorer prognosis (likely secondary to an increase in bacterial counts in the blood) but did not affect the risk of brain damage.

Bacterial cell wall fragments, endotoxin, or both also contribute to vascular permeability. In experimental *E. coli* meningitis, the CSF endotoxin concentration increased markedly after treatment with β-lactam antibiotics. This increase was associated with an increase in brain water content. This effect could be blocked by polymyxin or a monoclonal antibody, both of which inactivate endotoxin.[368]

The inflammatory and vascular events described earlier act synergistically to produce the clinical symptoms and long-term sequelae that are noted in patients with bacterial meningitis. As described subsequently, altered vascular permeability leads to vasogenic edema, inflammatory and electrolyte changes lead to cytotoxic edema, and alterations in production and absorption of CSF lead to interstitial edema. The cytokines also trigger increased cerebral blood flow and further formation of edema, resulting in increased intracranial pressure.[23] The increased intracranial pressure and vasculitis lead to a subsequent decrease in cerebral blood flow, which does not seem to be caused by loss of autoregulation.[23] Activation of the coagulation cascade predisposes the patient to venous, microvascular, and, rarely, arterial thrombosis. Direct neurotoxic damage by inflammatory cells also may contribute to the neuropathologic changes seen in bacterial meningitis.

CLINICAL MANIFESTATIONS AND PATHOPHYSIOLOGIC RELATIONSHIPS

Inflammation of the meninges generally is associated with nausea, vomiting, irritability, anorexia, headache, confusion, back pain, and nuchal rigidity. In many cases, Kernig and Brudzinski signs are noted. Kernig sign is present when the leg is flexed 90 degrees at the hips and cannot be extended more than 135 degrees. Brudzinski sign is present if the thighs and legs are flexed involuntarily when the neck is flexed. All of these findings suggest irritation of inflamed sensory nerves, which produces a reflex contraction of certain muscles in an attempt to minimize pain. These findings also can be the result of increased intracranial pressure and an associated distortion of nerve roots. These signs can be accompanied by hyperesthesia and photophobia. Currently, no satisfactory pathophysiologic explanation for photophobia exists. Signs of meningeal inflammation may be minimal in the infant, but irritability, restlessness, and poor feeding may be noted. Nuchal rigidity and Kernig and Brudzinski signs may occur late in a young child. Nuchal rigidity may not be elicited in comatose patients or when signs of focal or diffuse neurologic impairment are present. At the time of initial evaluation, 60 to 80 percent of children have a stiff neck.[195] A review of 1064 cases of bacterial meningitis in children beyond the neonatal period revealed that 16 (1.5%) had no meningeal signs during their entire period of hospitalization, despite the presence of CSF pleocytosis.[145] Fever, a hallmark of infection, generally is present; its absence in a patient with signs of meningeal inflammation, although infrequent, is not unusual.

Increased intracranial pressure is the rule; it may be reflected by complaints of headache in older children and by a bulging fontanelle and diastasis of sutures in infants. Papilledema is an uncommon finding in acute meningitis, presumably because of the brief duration of increased pressure at the time of diagnosis. When papilledema is observed, venous sinus occlusion, subdural empyema, or brain abscess should be sought.

Signs of cerebral edema may be present. Vasogenic edema occurs as a consequence of increased permeability of the blood-brain barrier. Interstitial edema may occur secondary to decreased clearance of CSF at the arachnoid villi and subsequent obstructive hydrocephalus. Cytotoxic cerebral edema mediated by the release of toxic factors from neutrophils and bacteria leads to increased concentration of intracellular water and sodium and loss of intracellular potassium. In many cases (88 percent in one prospective study[107]), meningitis is associated with the release of ADH, causing water retention and a relative dumping of sodium by the kidney. If the patient is given excessive free water during therapy, a further increase in intracranial pressure may be noted.

Transient or permanent paralysis of cranial nerves may be noted. Deafness or disturbances in vestibular function are common findings; optic nerve involvement with blindness rarely occurs. Involvement of the eighth cranial nerve may reflect

disease at the level of the cochlear and vestibular end-organs, which may be related to concomitant infection of the inner ear. Paralysis of extraocular and facial nerves may be noted. Torticollis has been reported in two children with partially treated meningitis.[250] Obtundation, stupor, coma, and focal neurologic signs may be seen in children with bacterial meningitis.

The relative frequency with which these findings are noted is shown in Table 37–2, in which data for 235 children with bacterial meningitis who were enrolled prospectively have been analyzed according to the type of organism responsible for meningitis. Overall, 14.9 percent of children were semicomatose or comatose at the time of admission; rates for children with pneumococcal or meningococcal meningitis were higher than rates for children with *H. influenzae* disease. Focal neurologic signs were present at the time of admission in 16.5 percent of the total group (34.3% of children with pneumococcal meningitis). The presence of focal neurologic signs at the time of admission indicated a poor prognosis and could be correlated with persistent abnormal neurologic examinations at 1, 3, and 6 months ($p < .01$) and at 1 year after discharge ($p < .03$). The presence of focal signs at the time of admission also correlated with the presence of retardation ($p < .001$), as determined by detailed psychometric testing after discharge.

Generally, when focal signs are noted in the absence of seizures, cortical necrosis, occlusive vasculitis, or thrombosis of cortical veins has occurred. Thrombosis of meningeal vessels or cortical necrosis may be associated with hemiparesis or quadriparesis and with focal seizures. These signs may appear during the first 3 or 4 days of illness or, less commonly, may be noted after the first or second week of infection. A highly significant association ($p < .001$) between neurologic signs indicative of cerebral injury and late (1 to 15 years after the acute infection) afebrile seizures has been noted.[293] Ataxia has been a presenting sign of meningitis in numerous children and adults. Schwartz[336] described four children who presented with ataxia as an initial symptom. Adolescents with meningitis may present with behavioral abnormalities that may be confused with drug abuse or psychiatric disorders.[28]

Approximately 20 percent of children with bacterial meningitis experience seizures before admissions, and approximately 26 percent have them during the first or second day in the hospital. Green and colleagues[164] retrospectively examined the frequency of seizures before or at the time of presentation in children with meningitis. They found that 111 of 410 (27%) children with bacterial meningitis had seizures at or before the time of diagnosis; 88 of these children had complex seizures (focal, prolonged, or more than one in a 24-hour period). They found that all children with bacterial meningitis who presented with seizures had other signs or symptoms of meningitis, such as altered level of consciousness, nuchal rigidity, or complex seizures and petechial rash. The frequency of seizure activity is similar for children with *H. influenzae* type b or pneumococcal meningitis; seizures occur in children with meningitis caused by these organisms approximately twice as frequently as in children with meningococcal meningitis. In one study of 181 children with pneumo-coccal meningitis, 41 (23%) developed seizures before admission; 75 percent of preadmission seizures were generalized.[20]

Overall, seizures are noted in approximately 30 percent of children with bacterial meningitis. Seizures noted before or during the first several days of hospitalization are of no particular prognostic significance. Specifically, their occurrence does not herald the development of a permanent seizure disorder. Seizures that are difficult to control or that persist beyond the fourth hospital day and seizures that occur for the first time late in the hospital course may be of greater significance and have been associated with permanent sequelae of meningitis. Children with focal seizures have a greater likelihood for development of sequelae of meningitis than do children with generalized seizure activity. Focal or prolonged seizures probably indicate serious cerebral vascular disturbances or cerebral infarction. Seizures that occur before admission have correlated positively with abnormal audiometric studies and permanent hearing handicaps. Seven percent of patients with bacterial meningitis have focal or generalized seizures 3 months to 15 years after recovery from bacterial meningitis.[293]

Collections of fluid in the subdural space can be shown in 50 percent of infants and children during acute illness.[96] In a prospective study of infants 1 to 18 months old with bacterial meningitis, subdural effusions were noted in 43 percent of infants with *H. influenzae* meningitis, 30 percent of infants with pneumococcal meningitis, and 22 percent of infants with meningococcal meningitis. Subdural effusions were found in 24 percent (25 of 103) of children undergoing neuroimaging in the multicenter pediatric surveillance study of pneumococcal meningitis.[20,351] No greater incidence of neurologic sequelae or developmental delay was found on long-term follow-up in patients with effusion compared with patients with bacterial meningitis who did not have effusion.[348,351]

Subdural effusions may cause enlargement in head circumference or may be responsible for abnormal transillumination of the skull. Vomiting, seizures, a full fontanelle, focal neurologic signs, or persistent fever may be noted sometimes, but these signs occur with such frequency in children with bacterial meningitis who do not have subdural effusions that it is difficult to attribute their occurrence to the subdural effusion.[110]

Blindness and optic atrophy may be related to optic arachnoiditis or infarction of the occipital lobe. Spastic paraparesis with sensory loss in the lower extremities may be secondary to meningomyelitis, spinal cord infarction, or both.

Arthralgia and myalgia are noted in many patients with bacterial meningitis, reflecting the systemic nature of the disease. Arthritis also may occur and does so most commonly during the course of meningococcal disease; generally, it is transient. Early

TABLE 37–2 Frequency of Selected Findings in Children with Bacterial Meningitis

	Total Group	*Haemophilus influenzae*	*Streptococcus pneumoniae*	*Neisseria meningitidis*	Others
No. patients	235	151	35	26	23
Level of consciousness (%)					
Irritable or lethargic	184 (78.3)	117 (77.5)	24 (68.6)	21 (80.8)	22 (95.7)
Somnolent	16 (6.8)	13 (8.6)	1 (2.8)	1 (3.8)	1 (4.3)
Obtunded-semicomatose	27 (11.5)	15 (9.9)	8 (22.9)	4 (15.4)	0 (0)
Comatose	8 (3.4)	6 (4)	2 (5.7)	0 (0)	0 (0)
Focal neurologic signs on admission (%)	37 (16.5)	22 (14.6)	12 (34.3)	2 (7.7)	1 (4.3)
Seizures before admission (%)	48 (20.4)	35 (23.2)	8 (23)	3 (11.5)	2 (8.7)
Seizures in hospital (%)	61 (26)	43 (28)	12 (34)	5 (19)	1 (4.5)

findings of arthritis may be related to direct invasion of the joint by the meningococcus. Arthritis that develops late in the course of meningococcal or *H. influenzae* meningitis may be an immune complex–mediated event. Petechial or purpuric lesions may be seen in 50 percent of patients with meningococcal meningitis[96] but also may accompany any infectious or noninfectious disease process in which vasculitis occurs. Purpura, shock, and hypothermia indicate a poor prognosis.

Pericardial effusions may be present; they generally resolve during the course of antibiotic therapy. In some cases, pericardial effusions are the cause of persistent fever, and pericardiocentesis or an open drainage procedure may be required.

Shock may be associated with any form of overwhelming bacteremia, but it occurs most often in patients with fulminant meningococcemia. In a prospective study, 3.8 percent of children with meningococcal meningitis developed profound hypotension. In the same study, shock occurred in 5.5 percent of children with *H. influenzae* meningitis. Endotoxin has been detected by Limulus lysate assay in the blood and CSF of children with meningococcal and *H. influenzae* meningitis.[311] Sixteen percent of the children in the multicenter pneumococcal meningitis study were in shock on admission.[20] Signs of disseminated intravascular coagulation may accompany hypotension in these patients.

Facial cellulitis (including buccal and periorbital cellulitis), pneumonia, epiglottitis, endophthalmitis, and other suppurative manifestations can manifest at the time of admission in any patient with bacterial meningitis. In one study of children with buccal cellulitis, less than 10 percent (7 of 73) had concomitant bacterial meningitis documented by lumbar puncture as part of their initial evaluation.[26] Five of the seven children had no clinical evidence of meningeal irritation. *H. influenzae* and *S. pneumoniae* were cultured from two patients with periorbital cellulitis, and no clinical evidence of meningeal irritation or abnormal CSF cell counts or chemistries was found.[320] In the largest review of facial cellulitis caused by *S. pneumoniae*,[160] 15 of 52 children had a lumbar puncture performed; 2 (13.3%) had a pleocytosis (18 white blood cells [WBCs]—52% polymorphonuclear leukocytes, and 9 WBCs—91% polymorphonuclear leukocytes). The Gram stain and cultures of the CSF specimens were negative. Lumbar puncture should be considered for children with facial cellulitis who are younger than 18 months and possibly bacteremic with *S. pneumoniae*.

DIFFERENTIAL DIAGNOSIS

The signs and symptoms described earlier suggest meningeal or intracranial pathologic processes but are not pathognomonic of acute bacterial infection. Tuberculous meningitis, fungal meningitis, aseptic meningitis, brain abscess, intracranial or spinal epidural abscesses, bacterial endocarditis with embolism, subdural empyema with or without thrombophlebitis, ruptured dermoid cysts, ruptured spinal ependymomas, and brain tumors may show similar signs and symptoms. Differentiation of these disorders depends on careful examination of CSF obtained by lumbar puncture and additional immunologic, radiographic, and isotopic studies as delineated later.

DIAGNOSIS

Early diagnosis and treatment of bacterial meningitis are imperative in reducing mortality rates and morbidity. Physicians must perform a lumbar puncture on any child in whom they suspect the diagnosis after a careful history and physical examination have been performed, unless specific contraindications to this procedure (e.g., clinical signs of increased intracranial pressure in a patient with a closed fontanelle and closed sutures) are present.

An association between performance of a lumbar puncture during bacteremia and the later development of meningitis has been reported.[126] This association was evident only in children younger than 1 year old. Perceptive physicians select children for lumbar puncture in whom clinical signs suggest developing meningitis before the CSF findings are diagnostic. These data suggest a need for careful observation and, if appropriate, hospitalization and antimicrobial therapy for infants younger than 1 year who undergo lumbar puncture and who concomitantly have risk factors (e.g., concurrent high temperature and WBC counts) for the development of bacteremia. Table 37–3 summarizes CSF findings characteristic of various inflammatory diseases of the CNS.

Measurement of pressure, often neglected in infants and young children, is an important component of each CSF examination. When the pressure is very high, only enough fluid to permit a careful examination should be removed. Compression of the jugular vein should be avoided unless compression of the spinal cord is suspected. Xanthochromic CSF derives its color primarily from bilirubin pigment. Hemorrhage, bilirubin staining in icteric patients who have meningitis (i.e., neonates, patients with leptospirosis), or an elevated protein concentration of CSF may be associated with xanthochromia.

CSF should be examined immediately. The total number of WBCs should be counted in a counting chamber, and after cytocentrifugation, a differential cell count should be done on a Wright-stained smear of the sediment. The normal CSF of children 3 months or older contains less than 6 WBCs/mm^3. Ninety-five percent of children older than 3 months have no polymorphonuclear leukocytes in the CSF; the presence of a polymorphonuclear leukocyte in the CSF may be regarded as abnormal. When a lumbar puncture has been performed in a febrile child and a single polymorphonuclear leukocyte has been noted, careful clinical observation is imperative, and treatment should be considered until the results of the culture of the CSF are known.

If the lumbar puncture has been traumatic, a total cell count can be done in a counting chamber. The red blood cells (RBCs) can be lysed by acetic acid, and a cell count can be repeated. If the total number of WBCs compared with the number of RBCs is greater than that in whole blood, one can assume the presence of CSF pleocytosis. One simple way to estimate the WBC count in the presence of RBCs is to allow 1 WBC per 1000 RBCs/mm^3. CSF protein should be measured (usually elevated in bacterial meningitis), and the CSF glucose concentration should be compared with the blood glucose concentration that has been obtained concomitantly. In patients with bacterial meningitis, depression of CSF glucose and of the ratio of CSF to blood glucose (normally approximately 66%) is the rule.

Separate smears should be made, and one smear should be Gram stained for bacteria. A Kinyoun stain for mycobacteria is performed if tuberculous meningitis is suspected. The probability of visualizing bacteria on a Gram stain of CSF depends on the number of organisms present. The percentage of positive smears is 25 percent with less than 10^3 colony-forming units (CFU)/mL, 60 percent in the range of 10^3 to 10^5 CFU/mL, and 97 percent with greater than 10^5 CFU/mL.[226] Quellung and agglutination reactions can provide immediate identification of various organisms if the appropriate type of specific antisera is available.

Treating a child with bacterial meningitis with an antibiotic before performing initial lumbar puncture usually does not alter markedly the morphologic or chemical results obtained (Table 37–4). In patients with *H. influenzae* meningitis (Table 37–5) who were pretreated, CSF cultures frequently grew *H. influenzae*; there is a tendency to pretreat children with pneumococcal or meningococcal disease to render the CSF sterile (see Table 37–4). Even when children received appropriate antibiotics for meningitis intravenously for 44 to 68 hours, the bacterial character

TABLE 37–3 Cerebrospinal Fluid Findings in Suppurative Diseases of the Central Nervous System and Meninges

Condition	Pressure (mm H_2O)	Leukocytes/mm^3	Protein (mg/dL)	Sugar (mg/dL)	Specific Findings
Acute bacterial meningitis	Usually elevated; average 300	Several hundred to >60,000; usually a few thousand; occasionally <100 (especially meningococcal or early in disease); polymorphonuclears predominate	Usually 100-500, occasionally >1000	<40 in more than half the cases	Organism usually seen on smear or recovered on culture in >90% of cases
Subdural empyema	Usually elevated; average 300	<100 to a few thousand; polymorphonuclears predominate	Usually 100-500	Normal	No organisms on smear or by culture unless concurrent meningitis
Brain abscess	Usually elevated	Usually 10-200; fluid rarely is acellular; lymphocytes	Usually 75-400	Normal	No organisms on smear or by culture predominate
Ventricular empyema (rupture of brain abscess)	Considerably elevated	Several thousand to 100,000; usually >90% polymorphonuclears	Usually several hundred	Usually <40	Organisms may be cultured or seen on smear
Cerebral epidural abscess	Slight to modest elevation	Few to several hundred or more cells; lymphocytes predominate	Usually 50-200	Normal	No organisms on smear or by culture
Spinal epidural abscess	Usually reduced with spinal block	Usually 10-100; lymphocytes predominate	Usually several hundred	Normal	No organisms on smear or by culture
Thrombophlebitis (often associated with subdural empyema)	Often elevated	Few to several hundred; polymorphonuclears and lymphocytes	Slightly to moderately elevated	Normal	No organisms on smear or by culture
Bacterial endocarditis (with embolism)	Normal or slightly elevated	Few to <100; lymphocytes and polymorphonuclears	Slightly elevated	Normal	No organisms on smear or by culture
Acute hemorrhagic encephalitis	Usually elevated	Few to >1000; polymorphonuclears predominate	Moderately elevated	Normal	No organisms on smear or by culture
Tuberculous infection	Usually elevated; may be low with dynamic block in advanced stages	Usually 25-100, rarely >500; lymphocytes predominate except in early stages when polymorphonuclears may account for 80% of cells	Nearly always elevated; usually 100-200; may be much higher if dynamic block	Usually reduced; <50 in 75% of cases	Acid-fast organisms may be seen on smear of protein coagulum (pellicle) or recovered from inoculated guinea pig or by culture
Cryptococcal infection	Usually elevated; average 225	Average 50 (0-800); lymphocytes predominate	Average 100; usually 20-500	Reduced in more than half of cases; average 30; often higher in patients with concomitant diabetes mellitus	Organisms may be seen in India ink preparation and on culture (Sabouraud medium); usually grow on blood agar; may produce alcohol in cerebrospinal fluid from fermentation of glucose
Syphilis (acute)	Usually elevated	Average 500; usually lymphocytes; rarely polymorphonuclears	Average, 100; gamma-globulin often high, with abnormal colloidal	Normal (rarely reduced)	Positive reagin test result for syphilis; spirochete not demonstrable by usual techniques of smear or by culture gold curve
Sarcoidosis	Normal to considerably elevated	0 to <100 mononuclear cells	Slight to moderate elevation	Normal	No specific findings

of the chemical and morphologic findings could be discerned in most cases.[46]

The CSF should be cultured on a blood agar plate and a chocolate agar plate. The CSF specimens always should be cultured, even when the fluid appears to be crystal-clear and acellular or nearly so.

In the 1970s, countercurrent immunoelectrophoresis was shown to be a useful technique for rapid diagnosis (within 1 hour) and management of bacterial meningitis caused by *H. influenzae* type b, *S. pneumoniae*, *N. meningitidis* (groups A, C, W135, and D), and group B streptococcus. It may be used to detect antigens from K1 strains of *E. coli* or *L. monocytogenes*.[117,248] The methodol-

TABLE 37–4 Comparison of Cerebrospinal Fluid Findings in Patients with Untreated and Pretreated Meningitis

	Untreated	Pretreated
No. patients	143	91
Total white blood cell count × 10^3		
Mean ± 1 SD	4.9 ± 6.5	4.1 ± 5
Range	0-55	0.006-25.5
Percentage polys		
Mean ± 1 SD	84 ± 21	81 ± 25
Range	0-100	0-100
Glucose (mg/dL)		
Mean ± 1 SD	35 ± 28	32 ± 25
Range	0-109	0-100
CSF/blood glucose (%)		
Mean ± 1 SD	29 ± 21	29 ± 21
Range	0-78	0-94
Protein (mg/dL)		
Mean ± 1 SD	226 ± 228	174 ± 193
Range	13-2290	10-1640
Culture-positive	135	71
Gram stain–positive	114	62

CSF, cerebrospinal fluid; SD, standard deviation.

TABLE 37–5 Cerebrospinal Fluid Findings in Untreated and Pretreated Patients with *Haemophilus influenzae* Meningitis

	Untreated	Pretreated
No. patients	92	57
Total white blood cell count × 10^3		
Mean ± 1 SD	5.6 ± 7.6	4 ± 4.8
Range	0.001-55	0.094-25
Percentage polys		
Mean ± 1 SD	91 ± 15	83 ± 22
Range	0-108	0-99
Glucose (mg/dL)		
Mean ± 1 SD	34 ± 27.5	29 ± 26
Range	0-109	0-99
CSF/blood glucose (%)		
Mean ± 1 SD	29 ± 21	24 ± 20
Range	0-78	0-82
Protein (mg/dL)		
Mean ± 1 SD	214 ± 129	187 ± 227
Range	25-752	29-1640
CIE (μg/mL)		
Mean ± 1 SD	1.83 ± 2.63	2.36 ± 4.73
Range	0-10.24	0-20.48
Culture-positive	91	51
Gram stain–positive	81	45
CIE-positive	77/88	50/55

CIE, counterimmunoelectrophoresis; CSF, cerebrospinal fluid; SD, standard deviation.

ogy is sensitive and can detect nonviable bacteria, permitting the detection of bacterial antigen, even in patients who have been pretreated with appropriate antibiotics. If this technique is employed, it is imperative to use antisera that have the greatest possible sensitivity and specificity.[187,338] Group B meningococcal antiserum, which is available commercially, is unreliable. When pneumococcal antisera are used, material obtained from the State Serum Institute in Copenhagen, Denmark, has proved to be highly efficacious; sensitivity is enhanced by using the various pools of pneumococcal antisera in addition to the omniserum. A negative result of countercurrent immunoelectrophoresis does not exclude the diagnosis of bacterial meningitis.

Latex particle agglutination commercial kits are available for detecting the polysaccharide antigens of *H. influenzae* type b, *S. pneumoniae*, *N. meningitidis*, and group B streptococcus. Latex particle agglutination is superior to countercurrent immunoelectrophoresis in detecting PRP antigen of *H. influenzae* type b in CSF and serum. Nonspecific agglutination of latex particles in serum, urine, and other body fluids may result in an indeterminate test result.[80,188,326,394] A commercial latex particle agglutination kit containing antibody-coated latex particles for *H. influenzae* type b, *S. pneumoniae*, and *N. meningitidis* serogroups is available. The Food and Drug Administration does not recommend latex agglutination testing of urine of infants for group B streptococcus as a method for inferring invasive disease with this organism. Latex agglutination of urine for group B streptococcus or *N. meningitidis* should be avoided because of the high frequency of false-positive results.

The clinical utility of rapid antigen detection in CSF has been questioned in recent years since the decline in frequency of invasive infections caused by *H. influenzae* type b. A positive test response rarely alters the approach to therapy. Maxson and associates[247] examined this issue and suggested that bacterial antigen tests be reserved for patients with suspected bacterial meningitis whose initial CSF Gram stain was negative and whose culture was negative after 48 hours of incubation. Perkins and associates[289] reviewed all latex particle agglutination tests done during a 10-month period at two hospitals and reached the same conclusion. Finlay and colleagues[124] suggested that latex agglutination testing of CSF be used only when the Gram stain is negative when it is consistent with meningococci. In the latter case, clarifying the meningococcal serotype quickly may have implications in large outbreaks for recommending meningococcal vaccine for type A, C, Y, or W135 disease.

The rapidity with which results of bacterial antigen tests are obtained renders them tempting as a means to establish an early diagnosis. The degree to which they affect clinical decisions is unclear.[247] Performing these tests is unnecessary for every patient suspected to have bacterial meningitis, but they could play a role in certain circumstances, such as when a patient's clinical presentation suggests bacterial meningitis and pretreatment with antibiotics or with a traumatic lumbar puncture. Antigen detection is useful in developing countries, where CSF culture yields are lower.[85]

Polymerase chain reaction (PCR) analysis of CSF has been used to detect microbial DNA in patients with bacterial meningitis. Primers are available for the detection of *S. pneumoniae*, *N. meningitidis*, and *H. influenzae* type b simultaneously. Species-specific amplicons have been detected in 89 percent of patients with proven streptococcal, meningococcal, or *H. influenzae* type b meningitis.[300] No false-positive results were noted.

Kotilainen and associates[224] used PCR and DNA sequencing techniques in a prospective study of CSF from patients with suspected bacterial meningitis. The bacterial 23S rRNA gene was amplified from the CSF of 5 of 46 adults with possible infection of the CNS. After sequencing of the 16S or 23S rRNA gene PCR products, 98.3 to 100 percent homology with *N. meningitidis* was observed in all five patients. This PCR method was not timely enough for routine laboratory use because it required 2 to 5 days to obtain results that could be reported to the physician.

Dagan and associates[78] have described the use of PCR for detection of *S. pneumoniae* within CSF. du Plessis and associates[97] have developed a seminested PCR strategy based on the amplification of the pneumococcal penicillin-binding protein 2B gene to detect penicillin-susceptible or penicillin-nonsusceptible *S. pneumoniae* within CSF. PCR detected pneumococci in all of the 18 culture-positive CSF specimens (of 285 total specimens tested). No false-positive results were noted. This test required only a few hours to perform and used only 15 μL of CSF.

Advances in the field have made the use of PCR technology a more viable option in the diagnostic approach to meningitis. The use of real-time PCR has shortened the time needed for completion of specimen analysis from several days to several

hours. PCR technology can be applied in a broad-spectrum manner, such as testing for amplification of bacterial 16s rRNA[334] or in a bacteria-specific manner using specific probes for *Neisseria* and *Streptococcus* spp.[78,262] or other bacterial pathogens. One diagnostic strategy combines the use of broad-range, real-time PCR for detection of bacterial presence in the CSF and is followed by bacterial identification after subsequent DNA sequencing.[89] The use of such PCR techniques is helpful in patients pretreated with antibiotics in whom the culture may be negative.[89,284] The possible addition of gene microarray technology offers the potential to probe for multiple different bacterial species simultaneously.[177]

PCR also is useful in rapidly documenting viral antigens within CSF, reducing the use of antibiotics in selected patients who are treated for presumptive bacterial disease but who may have viral meningitis.[312,331,383] Dicuonzo and associates[92] also documented the great sensitivity of oligoprobes on amplified DNA for the diagnosis of *H. influenzae*, streptococcal, and *Mycobacterium tuberculosis* meningitis. No false-negative results occurred in culture-positive CSF specimens.

Measurement of CRP has been proposed as a test that may be valuable in distinguishing bacterial from viral meningitis. In some studies, overlap in CRP determinations between these groups of patients has been observed. For this reason, we do not consider that one can rely on the CRP result to distinguish bacterial from viral meningitis with sufficient certainty.[112,150,191] Nonetheless, in the best study to date, serum CRP was superior to CSF parameters in distinguishing Gram stain–negative bacterial from viral meningitis.[356] Among 92 patients with viral meningitis, 93 percent had serum CRP levels within the normal range (<20 mg/L). Only one child with Gram stain–negative bacterial meningitis had a serum CRP value within the normal range.

Numerous metabolic changes have been reported to occur in the CSF and blood of patients with meningitis (Table 37–6). CSF lactate has been noted to be elevated significantly in patients with bacterial meningitis. The increase in CSF lactate apparently is related to decreased cerebral blood flow, cerebral hypoxia, and a change to anaerobic metabolism by the brain. Concentration of CSF lactate tends to parallel the CSF cellular response.[315] Although the concentration of CSF lactate in patients with bacterial meningitis generally is greater than that in patients with aseptic meningitis, such is not always the case. In some patients with aseptic meningitis, CSF lactate has been in the range generally observed in patients with bacterial infections. Conversely, in patients who proved to have bacterial meningitis but who had equivocal clinical and CSF findings, measurement of CSF lactate failed to differentiate bacterial from nonbacterial infection.[315] The determination of the concentration of CSF lactate cannot be used reliably to differentiate viral from bacterial meningitis in an individual patient.[220]

Depression of the pH of CSF also has been described in patients with bacterial meningitis. The depression of pH in CSF is more transient than is the elevation of CSF lactic acid, and its measurement is of even less value in the differential diagnosis.[397]

TABLE 37–6 Metabolic Changes Reported in Patients with Bacterial Meningitis

CSF lactate increased
CSF pH decreased
CSF lactate dehydrogenase increased
Creatine phosphokinase increased
Aspartate transaminase increased
CSF and blood elastase-α-proteinase inhibitor increased
CSF vasopressin increased

CSF, cerebrospinal fluid.

Lactate dehydrogenase, creatine phosphokinase, and aspartate transaminase may be elevated in patients with bacterial meningitis. In some cases, total lactate dehydrogenase activity within CSF may be similar in patients with bacterial and aseptic meningitis, but lactate dehydrogenase isoenzymic analysis may permit differentiation of bacterial from nonbacterial infection. This procedure is time-consuming and cumbersome and does not permit establishing a specific etiologic diagnosis in any patient.[198,276]

Despite the application of impeccable clinical judgment, examination of CSF, and use of one or more of the rapid diagnostic techniques, situations arise in which differentiation of bacterial from aseptic meningitis remains problematic. In these cases, a predominance of polymorphonuclear leukocytes generally is found in the CSF, the CSF cell count is less than 100 cells/mm^3, the CSF glucose concentration is normal or nearly so, and the Gram stain result is negative. Most children with aseptic meningitis have a predominance of polymorphonuclear leukocytes on their initial CSF examination.[277] In addition, although patients exhibit signs and symptoms suggestive of meningitis, they do not appear acutely ill. Some investigators have advocated withholding antibiotic therapy in these patients and repeating the lumbar puncture after 6 to 12 hours of close observation.[3,113] Usually, the repeated examination of CSF either substantiates the impression of aseptic meningitis (a shift to a lymphocytic differential is noted) or points more conclusively to a bacterial process. This course of action is not recommended if the patient has been pretreated with antibiotics or is younger than 1 year old. Occasionally, children have a mild CSF pleocytosis, which may have a predominance of polymorphonuclear leukocytes, after they experience seizures.[403,405] Generally, CSF abnormalities should not be attributed to seizures, unless other causes of CNS inflammation have been excluded.

Other approaches to differentiate bacterial from viral meningitis have been suggested. Evaluation of CSF ferritin concentration has been proposed as a test with considerable predictive value. Kim and associates[209] showed in pediatric patients that CSF ferritin concentration is significantly elevated in bacterial meningitis compared with viral meningitis. Using a proposed cutoff of 15.6 ng/mL of CSF ferritin, the test had a sensitivity of 96.2 percent and a specificity of 96.6 percent in differentiating bacterial from viral meningitis.

Additional laboratory data are helpful and should be obtained. Blood cultures should be obtained in every patient suspected to have bacterial meningitis. In one prospective study in which blood was obtained for culture from every patient, the cultures were positive in 80 percent of children with *H. influenzae* meningitis, in 52 percent of children with pneumococcal meningitis, and in 33 percent of children with meningococcal meningitis.[108] Forty-four percent of the entire group had received some form of antibiotic therapy before admission to the hospital and before these blood cultures were performed. If these individuals were excluded, positive blood cultures were obtained from 90 percent, 80 percent, and 91 percent of children with meningitis caused by *H. influenzae*, *S. pneumoniae*, and *N. meningitidis*, respectively.

A thorough search for foci of infection adjacent to or remote from the meninges should be conducted. Repetitive neurologic evaluation also should be performed, and appropriate laboratory studies should be undertaken to define the extent of neurologic dysfunction.

When the concentration of bacteria within the blood is high, a Gram-stained smear of a buffy coat obtained from the blood may reveal the presence of microorganisms. If petechial lesions are present, a smear of the lesions after puncture with a small lancet may reveal microorganisms on Gram stain. A chest radiograph may be helpful in disclosing a focus of infection.

Radioisotope scanning may be helpful in selected patients, such as patients with a leak of CSF. The pattern of distribution of radioactivity recorded by gamma camera coincides with the

accumulation of purulent material. Increased concentration of isotope may relate to the inflammatory response within the meninges or in the periventricular region or to alteration in the blood-brain barrier.[155] Localized concentrations of radionuclide may be seen in children with meningitis, most likely as a result of cerebral vasculitis or infarction.[96] Confirmation of impaired cerebral circulation, including occlusion and narrowing of arteries, sluggish circulation, and retrograde flow, has been provided by the studies of Gado and associates.[140] In these studies, resolution of the arterial lesions was shown in subsequent angiograms in two patients, despite the persistence of neurologic deficits; these findings prompted the authors to suspect vascular spasm at the earlier stage of disease. Hydrocephalus contributed to sluggish circulation through intracerebral vessels in two patients. Tyson and colleagues[377] showed at least transient disturbance in the circulation of CSF in 45 percent of patients with meningitis, but persistent hydrocephalus is a rare complication of purulent meningitis.

Computed tomography (CT) and magnetic resonance imaging (MRI) are noninvasive techniques that permit the prospective and repetitive assessment of children with meningitis. These techniques permit detection of ventricular dilation, subdural effusion, decrease in brain mass, and presence of vascular lesions or of brain infarcts (Fig. 37–4). With these procedures, ventricular dilation may be noted acutely in many children who never develop hydrocephalus after recovery from their disease.[108] Neuroimaging may be indicated in the following situations: (1) focal neurologic signs, (2) persistently positive CSF cultures despite administration of appropriate antibiotic therapy, (3) persistent elevation of CSF polymorphonuclear leukocytes (>30 to 40%) after more than 10 days of therapy, and (4) recurrent meningitis.[215]

Recurrent bacterial meningitis may be the result of a communication between the nasal passage or ear and the meninges. If rhinorrhea or otorrhea is present, a leak may be suspected, but documenting that CSF is present and locating the site of leakage are difficult when the sample is small or contaminated. Sectional (2 mm) coronal cranial CT has been reported to be an easy, noninvasive method for delineating anatomic abnormalities in children with recurrent meningitis.[362]

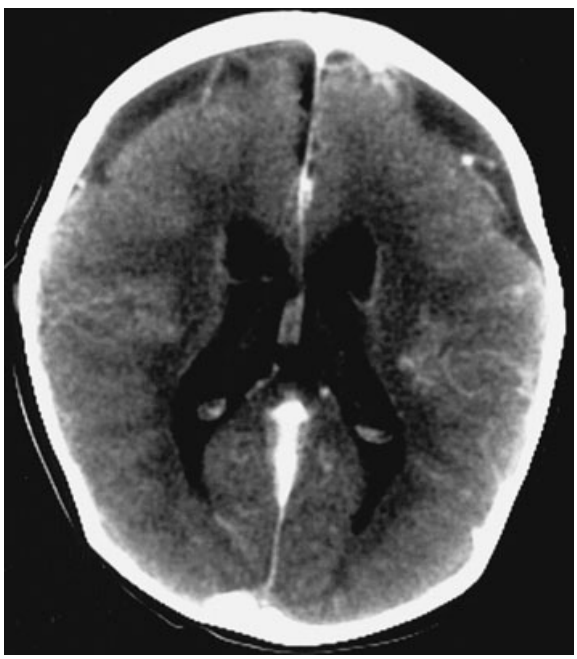

Figure 37–4 Computed tomography scan of a 2-year-old child with bacterial meningitis. Moderately severe ventricular dilation and the presence of bilateral extracerebral fluid collections overlying the convexities of the brain (subdural effusions) are noted. Note the several prominent vessels that run through the subdural space.

Meurman and associates[255] showed that an extra band of transferrin is located in the β_2-fraction after protein electrophoresis of CSF. This extra β_2-transferrin band could not be shown in serum, nasal secretions, saliva, tears, or perilymph and endolymph. The amount of sample required is small (<50 μL). We have applied this immunochemical method successfully in documenting that fluid found draining from the nose or ear was CSF. Differential suction may permit demonstration of the site of the anatomic communication among the nose, the ear, and the meninges. Moderate contamination with other body fluids does not invalidate the method. The method also is noninvasive and safe for the patient.

TREATMENT

ANTIMICROBIAL THERAPY

Prompt treatment of bacterial meningitis with an appropriate antibiotic is essential. Antibiotic selection should include consideration of such factors as the CSF penetration of the antibiotic, the activity of the drug in purulent CSF, the mode of administration of the drug, and the intrinsic pharmacodynamic relationships of CSF drug concentrations to bactericidal activity.[346] The initial selection always should be made before definitive cultures are available and ideally should be based on incidence and susceptibility patterns in the local community.[213]

For many years, ampicillin and chloramphenicol were preferred as the initial empirical therapy for children older than 3 months and thought to have bacterial meningitis. The development of newer cephalosporins and other antibiotics that have excellent bactericidal activity against *H. influenzae* type b, *N. meningitidis*, and *S. pneumoniae* within the CSF led to the current approaches to initial therapy of childhood meningitis. Cefotaxime and ceftriaxone are included in the empiric treatment regimen of choice in most centers.[211]

Cefotaxime is a third-generation cephalosporin that has a broad spectrum of activity against gram-positive and gram-negative organisms. It possesses a high level of resistance to hydrolysis by β-lactamase. Cefotaxime penetrates the blood-brain barrier and provides bactericidal activity in the CSF equivalent to or greater than that of antibiotics that have been used conventionally for treatment of bacterial meningitis in children.[265] It is an excellent choice for inclusion in empiric therapy in children 1 month or older but must be used with ampicillin for initial therapy in children younger than 1 month because *L. monocytogenes* and enterococci cannot be treated with cefotaxime but are sensitive to ampicillin. Vancomycin in a dose of 60 mg/kg/day in four divided doses is recommended in addition to cefotaxime for empiric therapy of children with meningitis because of the frequency with which penicillin-resistant and cephalosporin-resistant pneumococci have been isolated in recent years worldwide. Cefotaxime is given as a daily dose of 225 to 300 mg/kg/day in three or four divided doses intravenously. The higher dosage is preferred by some experts because the higher CSF concentrations achieved by high-dose therapy may be beneficial for patients whose disease may be caused by *S. pneumoniae* when the organisms are of intermediate susceptibility to third-generation cephalosporins.[75]

Ceftriaxone is another third-generation cephalosporin that possesses broad antimicrobial activity against the organisms that cause bacterial meningitis. Ceftriaxone readily penetrates the CSF of patients with inflamed meninges. In patients who receive adjunctive therapy with dexamethasone, meningeal inflammation

may be reduced, possibly decreasing the penetration of antibiotics into the CSF. Gaillard and colleagues[141] found that concentration of ceftriaxone in the CSF of children with bacterial meningitis treated with dexamethasone was similar to that found in children not treated with steroids. The half-life of ceftriaxone in serum is approximately 4 hours; a twice-daily dose regimen provides serum and CSF concentrations far in excess of the minimal bactericidal concentrations of most organisms that cause bacterial meningitis. Several prospective randomized studies have shown that ceftriaxone is comparable to ampicillin plus chloramphenicol for the treatment of bacterial meningitis in children.[21,33,56,76,87,157,361]

Ceftriaxone therapy has been associated with an increased incidence of diarrhea, which is mild and self-limited. An increased incidence of "gallbladder sludge," or precipitation of ceftriaxone salts in the gallbladder, diagnosed by ultrasound, also occurs and generally is asymptomatic but occasionally is associated with clinical symptoms of cholecystitis.[21,324] Ceftriaxone also has a high protein-binding capacity and can displace bilirubin from albumin in vitro[169] and needs to be used cautiously in neonates.

When ceftriaxone is used for the treatment of bacterial meningitis, it can be administered in a dose of 100 mg/kg/24 hours in two divided doses or one daily dose intravenously. Although a once-daily dose has proved to be effective,[49,135,167] is convenient, and lends itself particularly to home therapy for selected patients (after an initial period of hospitalization), we do not advocate single daily dosing; dosing errors, delayed doses, or missed doses undoubtedly occur, and inadequate treatment could result. Although ceftriaxone can be given intramuscularly, a single dose by this route may be impractical.[49] The solution used for intramuscular administration should contain no more than 250 mg/mL. A 15-kg child receiving a daily dose of 80 mg/kg would require 4.8 mL of fluid, a volume too large for injection in a single site in an infant. In addition, for penicillin-nonsusceptible but ceftriaxone-susceptible organisms, administration twice a day may be preferred.[75]

Ampicillin and chloramphenicol have been and continue to be effective as initial treatment of bacterial meningitis when organisms are susceptible to these agents, but they are used infrequently. Ampicillin may be provided intravenously in a dose of 300 mg/kg/24 hours in six divided doses. An initial bolus of 100 mg/kg is given. Chloramphenicol is administered intravenously in a dose of 100 mg/kg/24 hours in four divided doses. No loading dose of chloramphenicol is required.

An increasing number of strains of *S. pneumoniae* that are relatively or completely resistant to penicillin and third-generation cephalosporins have been identified.[54] Tan and coworkers[367] reported a retrospective analysis of five children who had pneumococcal meningitis caused by strains that were penicillin-resistant and that had minimal inhibitory concentrations (MICs) to cefotaxime or ceftriaxone of 0.5 to 2 μg/mL compared with strains that were penicillin-resistant but susceptible to cefotaxime or ceftriaxone (MIC ≥0.25 μg/mL); they found no difference in clinical outcome at the time of discharge. After publication of this report and others with similar findings, the National Committee for Clinical Laboratory Standards established the current guidelines for interpreting the MIC for cefotaxime or ceftriaxone for pneumococci isolated from patients with bacterial meningitis. The guidelines indicate that strains with an MIC of 2 μg/mL or greater are considered resistant, strains with an MIC of 1 μg/mL are intermediate, and strains with an MIC of 0.5 μg/mL or less are considered fully susceptible.[275]

Vancomycin has been used successfully to treat penicillin-resistant pneumococcal meningitis[50,137] and experimental models of cephalosporin-resistant pneumococcal meningitis.[139] In eight reports of treatment failures with third-generation cephalosporins, a variety of treatment regimens were used, all successfully. Vancomycin, alone or in combination with rifampin, chloramphenicol, or both, was used most frequently. The recommendation for empiric therapy of bacterial meningitis advocated by the AAP Committee on Infectious Diseases is to include vancomycin in addition to a third-generation cephalosporin in patients aged 1 month or older.[9] If nonsusceptibility to penicillin (MIC ≥0.1 μg/mL) and cephalosporins (MIC ≥0.1 μg/mL) is documented, treatment is continued with vancomycin plus cefotaxime or ceftriaxone (a synergistic effect is achieved when they are used together) with or without rifampin to complete an appropriate course. Vancomycin should be given at a dose of 60 mg/kg/24 hours in four divided doses. Peak serum concentrations of vancomycin in children whose renal function is normal should be 30 to 40 μg/mL.

Antibiotic tolerance is the ability of an organism to grow in the presence of antibiotics and frequently is a precursor phenotype to resistance. Clinical isolates of *S. pneumoniae* that are tolerant to vancomycin have been reported in the United States.[279] Meningitis caused by a vancomycin-tolerant organism in a patient was treated successfully with cefotaxime (300 mg/kg/day in four divided doses) plus vancomycin (60 mg/kg/day in four divided doses) and rifampin (20 mg/kg/day in two divided doses). Chloramphenicol also may be a suitable alternative for the treatment of these organisms if the pneumococcus proves to be sensitive to this antibiotic. Pneumococcal isolates resistant to penicillin or extended-spectrum cephalosporins frequently are nonsusceptible to chloramphenicol, however.

Cefuroxime is a second-generation cephalosporin that has been shown to be effective in vitro against *H. influenzae* type b, *S. pneumoniae*, and *N. meningitidis*. Initial clinical studies found that cefuroxime had equivalent effectiveness compared with ampicillin plus chloramphenicol. Subsequent studies showed delayed sterilization of the CSF, however; relapse during or after treatment was higher, and more frequent sensorineural hearing loss occurred than with use of ampicillin, chloramphenicol, cefotaxime, and ceftriaxone.[19,86,228,230,323] Cefuroxime *should not be used* to treat bacterial meningitis in children.

Ceftazidime has been efficacious in the treatment of meningitis caused by *P. aeruginosa*.[180,258,306,307] Cefoperazone and cefoxitin within CSF may fail to reach concentrations required to kill all susceptible strains of *H. influenzae* and *S. pneumoniae* and cannot be recommended for the treatment of bacterial meningitis in children.[59,121] Cefpirome concentrations in CSF of patients with bacterial meningitis were found to be significantly higher than the minimum bactericidal concentrations for *N. meningitidis*, *H. influenzae*, and *S. pneumoniae*,[402] but studies documenting its effectiveness in large numbers of children have not been done.

Cefepime is a fourth-generation cephalosporin that has been studied for the treatment of bacterial meningitis. In vitro, cefepime offers no advantage over cefotaxime or ceftriaxone for penicillin-resistant *S. pneumoniae*.[207] In addition, no in vitro data exist for the efficacy of cefepime against cefotaxime-resistant pneumococci. In two clinical trials, the efficacy of cefepime was found to be equivalent to the efficacy of either cefotaxime or ceftriaxone.[316,317] No penicillin-resistant or ceftriaxone-resistant pneumococci were encountered in either study, however. The role of cefepime for the treatment of bacterial meningitis is unclear.

Aztreonam is an antimicrobial agent that belongs to the monobactam family of antibiotics. It is effective against most gram-negative organisms, including *P. aeruginosa*. Limited data suggest its efficacy in the treatment of *Pseudomonas* and *H. influenzae* meningitis, suggesting a potential role for this agent in the treatment of patients who are allergic to penicillin and who are infected with these or other gram-negative organisms.[208,229,375]

If a history of *significant* allergy to penicillin or cephalosporin (anaphylaxis, urticaria, exfoliative dermatitis) is documented, vancomycin plus rifampin or chloramphenicol may be used. A cross-

reactivity of approximately 10 to 15 percent has been noted for cephalosporins in penicillin-allergic patients.

When meningitis is caused by *Streptococcus pyogenes*, ampicillin or penicillin provides effective therapy. If meningitis is caused by a penicillin-resistant strain of *S. aureus*, oxacillin or nafcillin should be used, 200 mg/kg/24 hours intravenously in six divided doses. Vancomycin is effective against *S. aureus* strains resistant to penicillin and to semisynthetic penicillin derivatives[170] or *S. aureus* meningitis in patients who are penicillin allergic. Vancomycin also may be useful in treatment of meningitis caused by *Flavobacterium meningosepticum*.[94] Metronidazole is effective in treatment of anaerobic infection of the CNS when response to conventional therapy has been suboptimal. A dose of 40 mg/kg/24 hours in three or four divided doses results in CSF concentrations of greater than 10 μg/mL.[39]

Imipenem and meropenem have been evaluated for the treatment of meningitis. These carbapenems are active against the bacteria that cause meningitis, but the use of imipenem-cilastatin has been associated with drug-induced seizures.[404]

The safety and efficacy of meropenem and cefotaxime were compared in a prospective randomized trial of 190 children with bacterial meningitis.[216] Seizures occurred within 24 hours before administration of antibiotic therapy in 16 percent of patients randomly assigned to receive meropenem and in 7 percent of patients randomly assigned to receive cefotaxime. Seizures occurred in patients after administration of therapy in 6 percent of children receiving meropenem and in 1 percent of children receiving cefotaxime. None of these seizures could be attributed to drug therapy. All patients responded to therapy with clinical improvement, and bacterial eradication was proved by repeated lumbar puncture in 100 percent of patients in both groups. No significant difference in short-term outcomes occurred between the two groups.

Odio and associates[282] compared the efficacy and safety of meropenem with cefotaxime for the treatment of bacterial meningitis in 258 children who were randomly assigned to the meropenem or cefotaxime group. Clinical cure with or without sequelae was achieved in 97 percent and 96 percent of the meropenem-treated and cefotaxime-treated patients, respectively. At 7 weeks after treatment was concluded, 54 percent of patients treated with meropenem and 58 percent of patients treated with cefotaxime had no sequelae. Seizures were noted in 12 percent of the patients treated with meropenem and in 17 percent of patients treated with cefotaxime; none of the seizures was considered to be drug-related. These data suggest that meropenem is effective in the treatment of bacterial meningitis in children. Few children with pneumococcal meningitis in either study had isolates that were nonsusceptible to cefotaxime. No conclusions can be drawn regarding the usefulness of meropenem in treating pneumococcal meningitis caused by isolates with a cefotaxime or ceftriaxone MIC equal to or greater than 2 μg/mL. Meropenem should be studied further in children who may have meningitis that is caused by organisms resistant to extended-spectrum cephalosporins. For meropenem-susceptible isolates, meropenem alone or in combination with other drugs may provide a satisfactory alternative for patients who do not tolerate vancomycin.[75]

In animal models, the addition of bacterial protein-synthesis inhibitors, such as clindamycin[48] and rifampin,[149] has been associated with a neuroprotective effect, suggesting the possibility of their use as an adjunct therapy. Bottcher and associates[48] showed decreased bacterial cell wall release, a lessened pro-inflammatory response (including decreased leukocyte recruitment), less free radical formation, and decreased apoptosis and cellular damage after the addition of clindamycin to standard antibiotic therapy.

Table 37–7 provides current recommendations for antibiotic treatment of various microorganisms. An appropriate antibiotic should be continued until the patient is afebrile for 5 days, but for at least 7 to 10 days in every patient. Although some data support a shorter course of therapy, we continue to recommend 10 days of treatment for pneumococcal and *H. influenzae* type b meningitis and 7 days for *N. meningitidis* meningitis.[190,240,246] If clinical improvement is noted within 24 hours, a repeated lumbar puncture is unnecessary, in most cases, during the course of treatment or after treatment has been completed. If infection is caused by *S. pneumoniae* that is resistant to penicillin and to third-generation cephalosporins, we recommend a repeated lumbar puncture at 48 to 72 hours to document bacterial sterilization of the CSF. If clinical improvement is slower than anticipated or is not noted, a repeated examination of the CSF is indicated at any time.

In the 1970s, lumbar puncture frequently was performed at the conclusion of therapy. Data from studies performed at that time (Table 37–8) reveal that WBC counts and protein concentrations within the CSF generally had not returned completely to normal and that the CSF-to-blood glucose ratio may have remained depressed. In every case, Gram stain of the CSF should reveal no organisms and cultures should be sterile. If a lumbar puncture is performed at the conclusion of therapy, we consider re-treatment to be mandatory if organisms are seen or grown. It also may be considered if more than 30 percent of the cells are polymorphonuclear leukocytes, or if the CSF glucose concentration is less than 20 mg/dL, and the CSF-to-blood glucose ratio is less than 20 percent.

Bacteriologic relapse after treatment of meningitis (particularly that caused by *H. influenzae* and treated with ampicillin) was highlighted in numerous reports.[27,71,73,173,174] Precise assessment of the frequency of relapse in children who have received an appropriate antibiotic to which the organism is sensitive or an appropriate dose intravenously and for an extended period has been difficult. The relapse rate is currently less than 1 percent.

Some physicians have discharged children with meningitis from the hospital before the conclusion of a course of therapy and prescribed home management. Benefits of home therapy include a decreased risk of acquiring nosocomial infection, a return of the child to his or her normal environment sooner, and a decrease in the total cost of therapy. Financial savings of outpatient, once-daily ceftriaxone for pediatric meningitis have been estimated to be $200 per day.[295]

Bradley and colleagues[49] reported the results of 54 children with bacterial meningitis treated as outpatients for 1 to 8 days (mean 4.6 days) with intramuscular ceftriaxone given once daily. Each dose was given in conjunction with a physician's examination. Each child had to be afebrile for 24 to 48 hours before initiation of home therapy, free of neurologic dysfunction except for auditory or vestibular dysfunction, and without evidence of inappropriate secretion of ADH before being considered for outpatient therapy. No child required readmission or developed neurologic sequelae or relapse. Powell and Mawhorter[295] reported a retrospective review of 26 patients with meningitis or other serious bacterial infections who received some portion of their therapy as an outpatient with ceftriaxone; none of the patients experienced relapse or recurrence.

Waler and Rathore[390] suggest 10 criteria for considering outpatient therapy for children with bacterial meningitis, as follows: (1) the child has received inpatient therapy for at least 6 days, (2) the child is afebrile for at least 24 to 48 hours before initiation of outpatient therapy, (3) the child has no significant neurologic dysfunction or focal findings, (4) the child has no seizure activity, (5) the child is clinically stable, (6) the child is taking all fluids by mouth, (7) the first dose of outpatient antibiotic is received in the hospital, (8) the antibiotic is administered in the office or emergency department setting or by qualified home health nursing, (9) daily examination is performed by a physician, and (10) parents are reliable and have transportation and a telephone. The dose of ceftriaxone of 80 to 100 mg/kg/24 hours intramuscularly may need to be aliquoted to account for the volume of diluent needed to achieve a concentration of no greater than 250 mg/mL. If oral

TABLE 37–7 Recommendations for Antibiotic Therapy

Organism	Antibiotic(s)	Recommended Dosages (IV)
Bacteroides fragilis	Chloramphenicol	100 mg/kg/day in 4 dd
	Metronidazole	30 mg/kg/day in 4 dd
Bacteroides other than *B. fragilis*	Penicillin G	300,000 U/kg/day in 6 dd
Clostridium	Penicillin G	300,000 U/kg/day in 6 dd
Corynebacterium	Penicillin G	300,000 U/kg/day in 6 dd
	Erythromycin	50 mg/kg/day in 4 dd
Enterobacter, *Klebsiella*, *Escherichia coli**	Ampicillin	300 mg/kg/day in 6 dd
	Gentamicin	7.5 mg/kg/day in 3 dd
	Amikacin	15 mg/kg/day in 3 dd
	Cefotaxime	200 mg/kg/day in 4 dd
	Ceftriaxone	100 mg/kg/day in 2 dd
	Ticarcillin	300 mg/kg/day in 4 dd
Haemophilus influenzae	Ampicillin	300 mg/kg/day in 6 dd
	Cefotaxime	200 mg/kg/day in 4 dd
	Ceftriaxone	100 mg/kg/day in 1 or 2 dd
	Chloramphenicol	100 mg/kg/day in 4 dd
Listeria monocytogenes	Ampicillin	300 mg/kg/day in 6 dd
	Gentamicin	7.5 mg/kg/day in 3 dd
	TMP-SMX	20 mg/kg/day in 4 dd (TMP component)
Neisseria meningitidis	Penicillin G	300,000 U/kg/day in 6 dd
Neisseria gonorrhoeae	Penicillin G (if sensitive to penicillin)	300,000 U/kg/day in 6 dd
	Ceftriaxone	100 mg/kg/day in 1 or 2 dd
Proteus mirabilis (indole-negative)	Ampicillin	300 mg/kg/day in 6 dd
P. mirabilis (indole-positive)	Cefotaxime	200 mg/kg/day in 4 dd
	Gentamicin	7.5 mg/kg/day in 3 dd
	Amikacin	22.5 mg/kg/day in 3 dd
	Ticarcillin	300 mg/kg/day in 4-6 dd
Pseudomonas	Gentamicin	7.5 mg/kg/day in 3 dd
	Ticarcillin	450 mg/kg/day in 4 or 6 dd
	Piperacillin	300 mg/kg/day in 4 or 6 dd
	Amikacin	15-20 mg/kg/day in 3 dd
	Ceftazidime	150-200 mg/kg/day in 3 dd
	Meropenem	120 mg/kg/day in 3 dd
Salmonella	Ampicillin	300 mg/kg/day in 6 dd
	Cefotaxime	200 mg/kg/day in 4 dd
	Gentamicin	7.5 mg/kg/day in 3 dd
	Chloramphenicol	100 mg/kg/day in 4 dd
Staphylococcus aureus (penicillinase-negative)†	Penicillin G	300,000 U/kg/day in 6 dd
S. aureus (penicillinase-positive)†	Oxacillin or nafcillin	200 mg/kg/day in 6 dd
S. aureus (resistant to semisynthetic penicillins)	Vancomycin *plus*	60 mg/kg/day in 4 dd
	Rifampin	20 mg/kg/day in 2 dd
Staphylococcus (coagulase-negative)	Vancomycin plus	60 mg/kg/day in 4 dd
	Rifampin	20 mg/kg/day in 2 dd
Streptococcus pneumoniae†	Penicillin G	300,000 U/kg/day in 6 dd
	Chloramphenicol	100 mg/kg/day in 4 dd
	Vancomycin	60 mg/kg/day in 4 dd
	Cefotaxime/ceftriaxone	225-300 mg/kg/day in 3 or 4 dd/100 mg/kg/day in 1 or 2 dd
	Rifampin‡	20 mg/kg/day in 2 dd
Unknown (<1 mo old)	Ampicillin *plus*	300 mg/kg/day in 6 dd
	Cefotaxime *plus*	200 mg/kg/day in 4 dd
	Vancomycin	60 mg/kg/day in 4 dd
Unknown (>1 mo old)	Cefotaxime *or*	225-300 mg/kg/day in 4 dd
	Ceftriaxone *plus*	100 mg/kg/day in 1 or 2 dd
	Vancomycin	60 mg/kg/day in 4 dd
	Nafcillin (if question of staphylococcal infection)	200 mg/kg/day in 6 dd
	Gentamicin (if question of *Pseudomonas*)	7.5 mg/kg/day in 3 dd

**TMP-SMX in a dose of 20 mg (TMP) and 100 mg (SMX) kg/day IV in 4 dd has been used successfully in selected patients with gram-negative enteric meningitis.*
†*Vancomycin may be provided in a dose of 60 mg/kg/day in 4 dd IV if patients are allergic to penicillin or penicillin derivatives or, in the case of Streptococcus pneumoniae, for multidrug-resistant pneumococci or pneumococci that are highly resistant to penicillin. In these cases, addition of rifampin may be considered.*
‡*Should never be used alone.*
dd, divided doses; TMP-SMX, trimethoprim-sulfamethoxazole

chloramphenicol is used, therapeutic serum concentrations of 15 to 25 μg/mL must be documented before discharge and maintained.

H. influenzae type b organisms have been recovered from the throats of patients after completion of a course of treatment for *H. influenzae* type b meningitis. When members of a household to which the patient will return include children 4 years or younger, the patient should be given rifampin, 20 mg/kg once daily for 4 days, to prevent the occurrence of secondary cases. Children with meningococcal meningitis also should receive che-

TABLE 37–8 Cerebrospinal Fluid Findings at Conclusion of Antibiotic Treatment

	Total White Blood Cell Count		Polymorphonuclear Leukocytes (%)		CSF-to-Blood Protein (mg/dL)		Glucose (mg/dL)		Glucose Ratio	
	Mean ± 1 SD	*Range*	*Mean ± 1 SD*	*Range*	*Mean ± 1 SD*	*Range*	*Mean ± 1 SD*	*Range*	*Mean ± 1 SD*	*Range*
Total group	41 ± 80	0-850	5.5 ± 12	0-90	46 ± 72	7-970	47 ± 12.7	21-91	55.7 ± 17	23-156
Haemophilus influenzae	53 ± 98	0-850	5.5 ± 11	0-90	43 ± 37	10-334	47 ± 13	31-100	55 ± 17	31-100
H. influenzae (ampicillin)	56 ± 107	0-850	5.9 ± 12	0-90	44 ± 43	13-334	46 ± 14	21-91	53 ± 17	33-89
H. influenzae (chloramphenicol)	49 ± 83	0-325	5.1 ± 10.7	0-50	41 ± 26	7-127	48 ± 11	27-90	57 ± 13	30-100
Streptococcus pneumoniae	29 ± 30	0-110	5.5 ± 11.5	0-45	42 ± 39	7-211	48 ± 9	22-68	57 ± 13	22-91
Neisseria meningitidis	16 ± 27	0-132	3.4 ± 7.9	0-27	39 ± 46	7-188	47 ± 11	29-77	47 ± 19	23-100
Others	11 ± 18	0-77	7.3 ± 19	0-75	70 ± 197	10-970	48 ± 18	37-73	70 ± 28	44-156

CSF, cerebrospinal fluid; SD, standard deviation.

moprophylaxis to eradicate nasopharyngeal carriage before discharge, unless they were treated with cefotaxime or ceftriaxone.

ADJUNCTIVE THERAPY

As described earlier, the pathogenesis and subsequent sequelae of bacterial meningitis are as much a consequence of the host response to infection as of the bacterial organisms themselves. Anti-inflammatory agents used as adjuncts to antimicrobial therapy may decrease the degree of tissue injury during the course of the disease. Corticosteroids have been suggested as an adjunct to therapy of bacterial meningitis because they may (1) decrease intracranial pressure by decreasing meningeal inflammation and brain water content; (2) modulate the production of cytokines, which lessens the meningeal inflammatory response; and (3) decrease the incidence of sensorineural hearing loss or other neurologic complications of meningitis.[227,281]

Corticosteroids may play a role in acute management of increased intracranial pressure and cerebral herniation, although no data specifically indicate that corticosteroids decrease cerebral edema caused by bacterial meningitis. Odio and colleagues[281] found that dexamethasone therapy in children with bacterial meningitis decreased opening lumbar CSF pressure 12 hours after administration of the first dose, but this effect was lost by 24 hours of treatment. The significance of these findings is unclear because the steroids were not given as specific therapy for increased intracranial pressure, and most of the subjects were not showing signs of impending herniation.

In experimental *H. influenzae* meningitis, administration of dexamethasone 1 hour before, but not 1 hour after, administration of ceftriaxone was associated with significantly reduced TNF-α concentration and indices of inflammation in the CSF.[270] Administration of dexamethasone has been associated with decreased concentration in CSF of prostaglandin E_2 and decreased leakage of some proteins from serum into CSF in rabbits with experimental pneumococcal meningitis.[192] Among patients with bacterial meningitis, steroid-treated patients had significantly lower concentrations of IL-1β, TNF-α, platelet-activating factor, and prostaglandin E_2 in their CSF than those of patients who received antibiotics alone.[254,271,281] Patients tend to become afebrile sooner when they receive dexamethasone, but they have an increased incidence of secondary fevers.[388]

Although the understanding of the pathophysiologic events associated with initiation of the acute inflammatory response has been enhanced by more recent data, the ability of dexamethasone to reduce long-term complications of bacterial meningitis in pediatric patients remains controversial. In a prospective, randomized, double-blinded, placebo-controlled study evaluating adults in Europe with bacterial meningitis, de Gans and colleagues[84] showed a decrease in mortality rates in all patients and in unfavorable outcomes in patients with pneumococcal meningitis. By contrast, no pediatric studies of the use of dexamethasone for treatment of bacterial meningitis have shown an overall change in mortality rates.

In randomized, placebo-controlled trials of dexamethasone as adjunctive therapy in bacterial meningitis[227,281,322,388] and in retrospective studies[158,206] published since 1988, 68 percent (227 of 333) of steroid recipients in the six randomized trials had meningitis caused by *H. influenzae* type b; the remaining 32 percent had meningitis caused by *S. pneumoniae* (50 of 333) or *N. meningitidis* (56 of 333).[297] A meta-analysis of nine controlled trials published before 1991 failed to document a reduced risk of neurologic abnormality at hospital discharge or follow-up examination.[181] A more recent meta-analysis of randomized clinical trials of dexamethasone as adjunctive therapy for bacterial meningitis suggests a beneficial effect for *H. influenzae* meningitis and a possible beneficial effect in preventing severe hearing loss in *S. pneumoniae* meningitis, but only if it is given early.[251]

Most patients with *S. pneumoniae* meningitis in the studies that were reviewed were not treated with vancomycin. This issue is important because the penetrance of vancomycin across the blood-brain barrier is not optimal, and selected studies show that it is reduced further when dexamethasone is used concomitantly.[20,51,138,259] Despite the theoretical clinical concerns, CSF concentrations of vancomycin and ceftriaxone are adequate for treatment of penicillin-susceptible and penicillin-nonsusceptible *S. pneumoniae* when dexamethasone is given at recommended dosages.[104]

Odio and associates[281] found that the administration of dexamethasone immediately before the initiation of cefotaxime therapy was associated with a reduced incidence of neurologic sequelae (14% compared with 38% in patients receiving cefotaxime alone). They found no significant difference in auditory sequelae compared with control subjects. The frequency of neurologic sequelae in placebo-treated patients was significantly higher than that noted in other studies.[322,370,388]

Studies by Lebel and associates[227] and Schaad and colleagues[322] failed to show a significant reduction in the incidence of neurologic sequelae in comparisons of steroid-treated and placebo-treated patients. A prospective, multicenter, placebo-controlled study evaluated 143 children with bacterial meningitis caused by *H. influenzae* type b (58%), *S. pneumoniae* (23%), and *N. meningitidis* (17%).[388] Patients were treated with ceftriaxone and placebo or ceftriaxone and dexamethasone administered within 4 hours of the first dose of antibiotics. No significant difference in neurologic or developmental outcome was found between patients who received steroids or placebo.

Sensorineural hearing loss is a significant sequela of bacterial meningitis. In the two randomized studies by Lebel and associ-

ates,[227] patients treated with dexamethasone were significantly less likely to have moderate or severe bilateral sensorineural hearing loss; however, in one study, patients were treated with cefuroxime, which has been shown to result in delayed sterilization of the CSF and a higher rate of hearing loss compared with ceftriaxone.[323] An additional 100 infants and children with bacterial meningitis were treated with ceftriaxone for 10 days and either dexamethasone or placebo for 4 days. A significant reduction in moderate to severe hearing loss in children with *H. influenzae* type b meningitis ($p < .001$) was reported. No significant differences in other neurologic sequelae were found between the two groups. In addition, two patients receiving dexamethasone developed gastrointestinal bleeding severe enough to require transfusion, and two others developed heme-positive stools.[227] Odio and associates[281] found no significant difference in the incidence of moderate or severe hearing impairment between placebo and steroid groups (16% and 6%) respectively.

The Swiss Meningitis Group[322] found that treatment of children with dexamethasone 10 minutes before administration of ceftriaxone and then for 2 days subsequently resulted in persistent hearing loss in 5 percent (three) of children who had received dexamethasone and in 15 percent (eight) of children who received placebo, a difference that was not significant. One child treated with steroid and five children treated with placebo had unilateral hearing loss only. The group also documented a transient mild to moderate hearing impairment in five children treated with dexamethasone and four children treated with placebo; in six of the nine children, the impairment was caused by a conductive disturbance. Dexamethasone did not alter the incidence or natural history of the transient hearing impairment.

In a multicenter study,[388] audiologic measurements were made early in the course of the disease (within 24 hours of admission) and 6 weeks to 12 months after recovery from disease. The authors found no significant difference in the incidence of persistent moderate or severe hearing loss between children who received dexamethasone within 4 hours of antibiotics and children who received placebo, with the exception of bilateral deafness in children with *H. influenzae* type b meningitis (5 of 72 in the placebo-treated group versus 0 of 67 in the dexamethasone-treated group; $p = .02$). The overall incidence of moderate to severe hearing loss was 14.7 percent (10.3% unilateral and 4.4% bilateral) in the dexamethasone-treated group and 22.9 percent (13.5% unilateral and 9.4% bilateral; $p = .33$ for bilateral loss) in the placebo-treated group. These authors found that 22 children (8 in the dexamethasone-treated group and 14 in the placebo-treated group) had bilateral moderate or severe hearing loss at the initial evaluation. Only one child with *H. influenzae* meningitis and unilateral deafness at initial examination progressed to bilateral deafness. At follow-up, the resolution of hearing loss was nearly identical for each group, with 8 of the 22 children having normal hearing at follow-up, 5 having unilateral deafness, and 9 having bilateral deafness.

These results suggest that hearing loss occurs early in the course of meningitis and that early auditory brain stem response results need to be interpreted cautiously with regard to long-term audiologic sequelae. A strong relationship was noted between hearing loss early in the disease and a low concentration of CSF glucose at manifestation of meningitis, a finding that has been reported previously.[95]

Data from animal studies highlight a potential negative effect of dexamethasone therapy.[233,410] Leib and associates[233] examined infant rats with pneumococcal meningitis and found that the rats treated with dexamethasone had increased hippocampal apoptosis and deficiencies in learned behavior.

The AAP Committee on Infectious Diseases states that dexamethasone therapy should be considered for pneumococcal meningitis in infants and children 6 weeks and older.[9] Dexamethasone also is recommended for treatment of infants and children with *H. influenzae* type b meningitis by the Red Book Committee.[12] The reluctance on the part of some experts to recommend dexamethasone for pneumococcal meningitis is predicated on data that fail to show any diminution in morbidity or mortality rates from meningitis when meningitis is caused by *S. pneumoniae* and dexamethasone is used. The CSF concentrations of vancomycin, ceftriaxone, cefotaxime, and rifampin when they are given in dosages recommended for meningitis in children treated with dexamethasone *generally* are adequate to treat meningitis caused by most nonsusceptible strains of *S. pneumoniae*. Dexamethasone can lead to decreased fever and a misleading impression of clinical improvement, even though sterilization of the CSF has not been achieved.

Dexamethasone should not be used if aseptic or nonbacterial meningitis is suspected; if it is started before the diagnosis of nonbacterial meningitis is made, it should be discontinued immediately. It should not be used in "partially treated" meningitis. No data exist on which to base a recommendation for use of dexamethasone in the treatment of bacterial meningitis in infants younger than 6 weeks or in infants with congenital or acquired abnormalities of the CNS, with or without a prosthetic device. One prospective study in infants concluded that adjunctive dexamethasone therapy does not improve the outcome of neonatal bacterial meningitis.[79] Dexamethasone plus vancomycin may decrease the transport of vancomycin into the CSF of experimental animals with pneumococcal meningitis, but this finding was not observed in nine children who were treated with vancomycin and dexamethasone (0.6 mg/kg/day).[217] Dexamethasone should be used cautiously when vancomycin is used to treat meningitis caused by *S. pneumoniae* that may be resistant to penicillin, third-generation cephalosporins, or both.[51,201]

If dexamethasone is used, it should be used in all patients, regardless of disease severity, and it should be administered as early as possible in the course of treatment in a dose of 0.15 mg/kg/dose intravenously every 6 hours for no more than 4 days. One study[322] found no difference in children treated for 2 days instead of 4 days with 0.4 mg/kg/dose every 12 hours.

Except for hearing loss after *H. influenzae* type b meningitis, no clear evidence establishes that dexamethasone dramatically alters the long-term sequelae of meningitis, and its use is not without risk of causing adverse events. The markedly decreased frequency of meningitis caused by *H. influenzae* type b and the increased frequency of meningitis caused by *S. pneumoniae* nonsusceptible to penicillin (for which therapy with vancomycin may be necessary) suggest that initiation of dexamethasone should be considered carefully and that the clinician caring for the patient should evaluate the risk-to-benefit ratio of such therapy.

Research studies seek additional therapies that could be used to help reduce further mortality, morbidity, or both. Five main areas serve as the targets for therapeutic development: (1) bacterial killing and the release of bacterial products, (2) host recognition of bacteria or its products and the initiation of the inflammatory response, (3) modulation of the inflammatory response with adjuvant dexamethasone, (4) inhibition or interruption of the host inflammatory/neurotoxic mediators, and (5) modulation of the apoptotic pathways.[163] Inhibitors of inflammatory mediators and mediator effector molecules such as TNF-α, matrix metalloproteinases, and nitric oxide and antioxidants, neuroprotective factors (melatonin[148] and brain-derived neurotrophic factor[42]), and other anti-inflammatory therapies (triptans[184]) are being studied in experimental animal models of bacterial meningitis. Although their use is attractive theoretically because of the damage produced by the inflammatory cascade, none has emerged as a realistic potential candidate for general clinical use at present.

SUPPORTIVE CARE

In addition to antibiotic therapy, management of bacterial meningitis includes measures that apply generally to critically ill children.[215] Careful monitoring and attention to detail are essential. Pulse rate, blood pressure, and respiratory rate should be measured carefully every 15 minutes until stable and then every hour while the patient is in the intensive care unit. Temperature should be measured every 4 hours. A thorough neurologic examination should be done at the time of admission and at least daily thereafter. A rapid assessment of neurologic function should be done several times a day for the first several days of treatment. Body weight should be measured daily for at least the first 3 or 4 days. Head circumference should be measured in children younger than 18 months at the time of admission and repeated daily if concerns about increased intracranial pressure persist.

The following laboratory data are suggested if results of lumbar puncture indicate bacterial meningitis: (1) total peripheral WBC count and differential, (2) hemoglobin concentration, (3) hematocrit, (4) platelet count, and (5) serum electrolytes (serum and urine osmolalities may be useful in selected patients). Urine volume and specific gravity should be monitored. A low WBC count may suggest a poor prognosis. Anemia associated with *H. influenzae* type b septicemia has been reported[341,342] and has been attributed to immune hemolysis of RBCs that are coated with soluble bacterial antigens.[341]

Every child with meningitis should be evaluated carefully to identify inappropriate secretion of ADH, recognize seizure activity, and detect the development of subdural effusions. Determinations of body weight, serum electrolytes (serum and urine osmolalities in selected patients), urine volume, and specific gravity should be made at the time of admission and observed closely (every 6 to 12 hours) for the first 24 to 36 hours that the child is in the hospital and daily for several days thereafter. Initially, the child should receive nothing by mouth because of the risk of vomiting and aspiration. In addition, delivery of all fluid intravenously ensures greater accuracy in measurement of intake and output during the critical early days of therapy. Inappropriate secretion of ADH has been documented in 88 percent of children enrolled in a prospective study of bacterial meningitis.[107] Elevated serum concentrations of ADH in the presence of hyponatremia have been documented by direct measurement of ADH concentration in serum obtained from the same children.[111]

An electrolyte solution containing approximately 40 mEq/L of sodium and chloride, 35 mEq/L of potassium, and 20 mEq/L of acetate or lactate should be administered at a rate of 1000 to 1200 mL/m^2/24 hours in a patient without evidence of dehydration or shock. Fluid restriction is continued until it can be documented (frequently within 2 hours), on the basis of objective measures, that ADH secretion is not a factor or has resolved. The best indicators of retention of fluid in excess of solute are body weight and serum sodium concentration. As serum sodium concentration approaches normal (140 mEq/L), fluid administration may be liberalized progressively to normal maintenance levels of 1500 to 1700 mL/m^2/24 hours.

Powell and associates[296] found that elevated concentrations of arginine vasopressin in patients with bacterial meningitis who were clinically dehydrated responded to maintenance fluids plus deficit replacement with 0.9 percent saline. This study confirms that the syndrome of inappropriate secretion of ADH should not be diagnosed in the presence of dehydration. Decreased intravascular volume is a physiologic stimulus for the release of ADH, and its release is not inappropriate. Fluid restriction is not advocated for patients who are dehydrated; rehydration should be performed with careful and frequent assessment of fluid and electrolyte status.

Singhi and colleagues[345] examined the effect of fluid restriction on body water and outcome of 50 consecutive children who had been hospitalized with acute meningitis. These children were divided into two groups—patients with hyponatremia and patients without hyponatremia. Patients in both groups were randomly assigned to receive either normal maintenance or restricted fluids (65 to 70% of the volume of that received by the maintenance subgroup). Eleven to 15 patients were randomly assigned to any of the four subgroups in the study. No significant difference in overall outcome or intact survival was found when comparisons were made between fluid-restricted and non-fluid-restricted groups or within each group between the subgroups that received restricted fluids or maintenance fluids. After combination of the subgroups that received restricted fluids with the subgroups that received maintenance fluids, however, a trend toward higher intact survival and lower mortality rates was noted in the non-fluid-restricted groups. Nonetheless, children who had an extracellular water reduction of 10 mL/kg or greater in 48 hours had a significantly lower intact survival rate (10 of 28, 36%) than that of children with less than 10 mL/kg or no reduction of extracellular water (15 of 22, 64%). The mortality rate also was higher in the former group (7 of 28, 25%) than in the latter group (2 of 22, 9%). The authors concluded that fluid restriction did not improve the outcome of acute meningitis and that a decrease in extracellular water volume at 48 hours may increase the likelihood of having an adverse outcome.

This study is particularly difficult to interpret within the context of previous information in the literature. In studies of large numbers of children with bacterial meningitis, evidence of inappropriate secretion of ADH correlated significantly ($p < .01$) with abnormal neurologic findings, even 3 months after discharge, and with low IQ scores.[116] The original studies were in patients who were *not* fluid restricted.[110] As a result of these findings, coupled with documentation of inappropriate secretion of ADH, a recommendation was made to restrict fluids in patients who are hyponatremic at admission. Fluids are restricted only until evidence of inappropriate secretion of ADH can be excluded (usually within 2 hours).

The average patient in subsequent studies reported by Kaplan and Feigin[197] was fluid-restricted for only 0.75 days. The mortality rate in the largest single study reported by Feigin[105] of individuals who were fluid-restricted was 0.5 percent compared with 9 and 25 percent in the groups reported in the studies by Singhi and associates.[345] Singhi and associates[345] did not assess other important outcome variables, such as the number of patients with hearing loss or patients whose psychometric performance might or might not have been impaired. The total number of patients in any of their study groups was relatively small. Although their data are intriguing, other differences, either in the population studied or in the management of the patients, may have accounted for these differences.

In addition to the increased mortality rates of the patients studied by Singhi and associates[345] noted earlier, an extraordinarily high frequency of hydrocephalus and a very high frequency of seizures and status epilepticus were noted compared with groups of patients who have been studied in the United States. Because cerebral edema and increased intracranial pressure have been noted as major disturbances in seriously ill patients with meningitis and because many of the deaths and some of the sequelae have been related to the effects of cerebral edema and intracranial hypertension, we continue to recommend fluid restriction in patients with hyponatremia who are not dehydrated and liberalization of fluids as soon as the effects of excess ADH secretion have been dissipated (usually <1 day).

Meningitis complicated by shock creates a complex fluid management problem. Shock associated with meningitis is secondary

to septicemia and generally is treated with intravenous infusion of large quantities of fluid to maintain blood pressure and adequate tissue perfusion (see Chapter 69). Patients with meningitis without shock or dehydration benefit from initial fluid restriction to avoid worsening of cerebral edema and severe hyponatremia with subsequent seizures. Children with meningitis and shock should receive sufficient quantities of isotonic fluid to maintain a systolic blood pressure of 80 to 90 mm Hg, a urine output equal to or greater than 500 mL/m^2/24 hours, and adequate cerebral perfusion as indicated by mental status. Central venous pressure monitoring is useful to guide fluid resuscitation and to prevent fluid overload. The addition of albumin (1 g/kg) to intravenously administered fluids may decrease the total volume of fluid needed to maintain adequate perfusion. Vasopressors, such as dopamine, dobutamine, and isoproterenol, also may provide support of blood pressure and perfusion and reduce requirements for intravenously administered fluids.

When increased intracranial pressure is suggested by such signs as progressive lethargy, increased muscle tone, or bulging anterior fontanelle, elevating the head approximately 30 degrees may be helpful. Increased intracranial pressure associated with deterioration in mental status or signs of cerebral herniation (Fig. 37–5) may be treated more vigorously with mannitol administered intravenously (0.5 g/kg) infused during 30 minutes and repeated as necessary. If steroids are used for this purpose, the recommended steroid is dexamethasone in a dose of 10 to 12 mg/m^2/day in four divided doses for no more than 4 or 5 days.[199] Other supportive measures to ensure appropriate delivery of oxygen and nutrients to the brain include a quiet environment, elective intubation, and the use of sedatives.[34]

Head circumference measurement and transillumination permit assessment of the development of subdural effusions or may suggest other causes for an enlarging head. CT may be helpful in detecting large subdural effusions or hydrocephalus. Because effusions can be considered part of the pathophysiologic changes that occur with bacterial meningitis, obtaining CT scans to evaluate effusions does not need to be part of the routine evaluation of a child with meningitis. Neuroimaging (CT or MRI) should be performed in children with focal neurologic signs. In children with hemiparesis or quadriparesis, CT or MRI may document cerebrovascular abnormalities. CT also should be performed in children with papilledema on an emergency basis before proceeding with the initial lumbar puncture. *Administration of antibiotics should not be delayed for diagnostic imaging in patients in whom bacterial meningitis is suspected.*

Subdural effusions should be treated with subdural paracentesis only when one suspects that the effusions are responsible for seizures or for prolonged fever as a result of subdural empyema. Paracentesis also may be useful if the effusion is responsible for symptoms of increased intracranial pressure or is the cause of focal neurologic signs.[107,116] In most cases, subdural taps are not required.

Seizures, when noted, are treated expeditiously. A patent airway must be maintained, and appropriate anticonvulsants must be administered. Sodium phenobarbital (7 mg/kg loading dose) may be administered parenterally followed by a maintenance dose of 5 mg/kg/day in two divided doses. If necessary, diazepam (≤0.2 mg/kg) or lorazepam (0.05 mg/kg/dose, 4 mg maximum) infused intravenously for 1 to 2 minutes may be used. If prolonged seizure control is needed, phenytoin (5 mg/kg/day) in two divided doses may be used. Phenytoin generally does not depress the respiratory center to the same extent as phenobarbital does, and it may benefit the patient by inhibiting the secretion of ADH. If the seizure activity no longer is apparent after the second hospital day and the patient has no focal neurologic signs at the time of discharge from the hospital, anticonvulsants may be discontinued. Phenytoin and phenobarbital can induce hepatic microsomal enzymes; their use may increase the metabolism rate of chloramphenicol and possibly cause a significant decrease in the serum concentration of this antibiotic if it has been used for treatment of meningitis.[294]

An electroencephalogram is indicated in patients with meningitis and seizures when focal seizures are noted, seizures persist more than 72 hours after presentation, seizures occur after the third day of hospitalization, a subdural effusion is noted, or prolonged alteration in sensorium is present. An electroencephalogram may be valuable in distinguishing abnormal intermittent posturing from movements associated with seizure activity.

Persistent fever (>8 or 9 days' duration) has been noted.[27] In the multicenter pneumococcal study, the mean duration of fever was 4.4 ± 3.9 days.[19] Suppurative complications, including subdural or pleural empyema, septic arthritis, and pericarditis, should be sought carefully. The rare occurrence of brain abscess in association with bacterial meningitis also may lead to persistent fever. Nosocomial intercurrent infection, usually viral, may cause prolonged fever in a child with meningitis. Complications of therapy, such as suppurative thrombophlebitis or a urinary tract infection after prolonged catheterization, are additional considerations. Persistent fever may be related to the severity of the infection. Poor therapeutic response (especially in multidrug-resistant organisms) occurs, and repeated lumbar puncture must be considered on an individual basis. Drug fever often is cited, but rarely is the cause of persistent fever and remains a diagnosis of exclusion.

Figure 37–5 Inflammation and hemorrhage of cerebellar tonsils from a child with cerebellar herniation through the foramen magnum due to bacterial meningitis. *(From Kaplan, S.: Current management of common bacterial meningitides. Reproduced with permission of Pediatrics in Review 7:77, copyright 1985.)*

PROGNOSIS AND SEQUELAE

The prognosis in patients with bacterial meningitis depends on many factors, including the following: (1) the age of the patient, (2) the time course or progression of illness before antibiotic therapy is effective, (3) the specific microorganism causing the disease, (4) the number of organisms[119] or the quantity of capsular polysaccharide material present in the meninges and CSF at the time of diagnosis, (5) the rapidity with which CSF is sterilized after initiation of antibiotic therapy, and (6) the presence of disorders that may compromise host response to infection.[230]

The younger the patient and the greater the antigenic load at the time of admission, the worse the prognosis. Bacterial colony counts seem to be a more reliable indication of sequelae than is antigen concentration. Seizures, subdural effusions, bacteremia, and a more prolonged period of fever occur more frequently in children who have more than 10^7 CFU/mL of a particular organism in the CSF at the time of admission.[120] Children with colony counts equal to or greater than 10^7 CFU/mL

also are significantly more likely to experience hearing loss and speech disturbance than children with meningitis, but lower concentrations of bacteria within CSF specimens.[120] The presence of TNF in serum has been associated with a fatal outcome in patients with meningococcal meningitis.[386] Elevated concentrations of IL-1β and TNF within the CSF of patients with bacterial meningitis also have been correlated significantly ($p < .002$) with a higher incidence of neurologic sequelae of disease.[269]

The mortality rate for bacterial meningitis in children who are beyond the neonatal period has been reduced to 1 to 5 percent. Although antibiotic therapy has reduced the mortality rate, 50 percent of the survivors of meningitis have some sequelae of disease.[96,116,144,360] Most studies from which estimates of sequelae have been derived have been retrospective, and the patients were enrolled for many years (1951 to 1968). Although antibiotic treatment of these individuals may have been relatively standardized during this period, ancillary methods employed in their care were not controlled.

The frequency of complications of meningitis can be assessed most appropriately by prospective evaluation. In 1975, Sell[337] began a prospective study of 50 infants and children who recovered from *H. influenzae* meningitis. Fifty percent of this group were entirely normal, 9 percent were normal except for behavioral problems, and 28 percent had significant handicaps. The major handicaps noted included hearing loss (10-11%), language disorders or delayed language development (15%), impaired vision (2-4%), mental retardation (10-11%), motor abnormalities (3-7%), and seizures (2-8%).

Twenty-one postmeningitic children were paired with a sibling and tested by the Wechsler Intelligence Scale for Children. The mean IQ of the postmeningitic children was 86, and the mean IQ of control children was 97 ($p < .05$). Comparison of results for individual pairs revealed that 29 percent of postmeningitic children scored 1 full standard deviation below their siblings; no survivor had a score 1 standard deviation higher than a sibling. Feldman and Michaels[118] reported that children who recovered from meningitis caused by *H. influenzae* and who were evaluated 10 to 12 years later maintained grades and scores comparable with those of their siblings as they progressed to middle school. Their academic success may require more school and family support to compensate for the minor differences in IQs that had been noted.

A study by Taylor and associates[370] attempted to gain additional insight into the sequelae of *H. influenzae* meningitis, with particular emphasis placed on neuropsychologic function. Although the study was retrospective, it permitted a more detailed neuropsychologic assessment made at an earlier age than that reported by investigators in previous studies. In addition, the index patients were compared with their siblings who were closest in age and the same sex, and the study was controlled for occupational and educational status. Only 14 percent of children who had had *H. influenzae* meningitis had any residual neurologic sequelae. Mean full-scale IQ was 102 for the index children and 109 for the control children.

The more recent results of our large prospective study of bacterial meningitis in children revealed that 32.8 percent of children had abnormalities detectable on neurologic examination at the time of discharge, but by 5 years after discharge, specific deficits were noted in only 11.1 percent of the total group.[109] As a result of the onset of late seizures in some of these patients, the frequency of neurologic sequelae 15 years after discharge was 14 percent.[293] Specific complications or sequelae of meningitis in these patients are shown in Table 37–9. Shortly after discharge, hemiparesis or quadriparesis was noted in 30 patients (12.4% of the total group), but at 1 year after discharge, paralysis was noted in only 5 patients. These data reflect the tendency for even major neurologic defects to clear unpredictably with time. This important observation suggests the need to maintain cautious optimism in discussing long-term complications of meningitis with parents.

In 1995, Grimwood and colleagues[166] reported the results of a prospective cohort study of 158 meningitis survivors, 3 months to 14 years old, who were treated in a single center between 1983 and 1986. Between 1991 and 1993, 130 children (82% of the original cohort) were evaluated at a mean age of 8.4 years and a mean of 6.7 years after their meningitis. Blended, audiologic, behavioral, neurologic, neuropsychologic, and sociodemographic assessments were compared with those of sex-matched and grade-matched control children. A systematic increase in the risk of abnormality or for poorer functioning was noted across all categories tested in children with meningitis versus control children. The differences reached statistical significance for tests of fine motor function, intelligence, neuropsychologic function, school behavior, and auditory figure-ground differentiation.

Eleven children who had experienced meningitis (8.5% of the cohort studied) had major deficits (hydrocephalus, persistent seizures, spasticity, blindness, IQ < 70, or profound hearing loss). Twenty-four (18.5%) of the survivors of meningitis and 14 (10.8%) of the control children had minor deficits (IQ of 70 to 80, inability to read, abnormalities in speech discrimination possibly referable to mild to moderate hearing loss, and school behavior problems). Overall, one in four of the children in this study had either a serious disabling sequela or a

TABLE 37–9 Complications or Sequelae of Meningitis

	Total	*Haemophilus influenzae*	*H. influenzae* (Ampicillin)	*H. influenzae* (Chloramphenicol)	*Streptococcus pneumoniae*	*Neisseria meningitidis*	Others
No.	235	151	90	61	35	26	23
Deaths <12 hr in hospital	4	4	3	1	0	0	0
Deaths >12 hr in hospital	1	0	0	0	0	1	0
Shock	8	6	5	1	1	1	0
Paralysis							
Early	30	18	7	11	7	3	2
Persistent	5	4	1	3	4	0	0
Persistent tone	5	4	1	3	1	2	0
Ataxia							
Early	7	5	2	3	2	0	0
Persistent	1	1	0	1	0	0	0
Visual problems	7	4	2	2	3	0	0
Clinically significant hearing deficit	25	17	6	11	5	2	1
Hydrocephalus	1	1	1	0	0	0	0

functionally important behavior disorder or neuropsychologic or auditory dysfunction that adversely affected academic performance.

This prospectively followed cohort was re-evaluated 12 years post-meningitis; impairment, although not severe, still persisted in the post-meningitis patients compared with controls.[18] Children post-meningitis were "more than twice as likely as controls to require special educational assistance" and "took longer to complete tasks, made more errors, were less organized, and struggled within problem-solving situations." Decreased performance in language tasks and executive skills also were seen in children whose age was less than 12 months at onset of meningitis.

Meta-analysis of 19 reports of prospectively enrolled and evaluated cohorts from developed countries published between 1980 and 1990[31] determined the mean probability of mortality to be 3.8 percent for *H. influenzae* type b, 7.5 percent for *N. meningitidis*, and 15.3 percent for *S. pneumoniae*, with an overall probability of 4.8 percent. The mean probabilities of sequelae in the survivors were deafness, 10.5 percent; mental retardation, 4.2 percent; spasticity, paresis, or both, 3.5 percent; and seizure disorder, 4.2 percent. The mean probability of no detectable sequelae was 83.6 percent.

Other specific sequelae or complications of bacterial meningitis that have been observed include cranial nerve involvement, hemiparesis or quadriparesis, muscle hypertonia, ataxia, permanent seizure disorders, and development of obstructive hydrocephalus. Subdural effusions (as noted earlier) are so frequent in young children that they can be considered a part of the general disease process, rather than a persistent or troublesome complication of the meningeal infection. Development of brain abscess after bacterial meningitis is exceedingly rare[122]; when it is found, the possibility that it preceded the development of meningeal infection must be considered, and a careful search for other sites of infections such as endocarditis should be initiated.

Arditi and associates[20] reviewed the outcomes of 180 children with 181 cases of pneumococcal meningitis who were enrolled in a prospective multicenter study between 1993 and 1996. Fourteen (7.7%) of 180 children died. No deaths were related to treatment failure caused by an antibiotic-resistant strain. Of the 166 surviving children, 41 (25%) developed motor defects, and 48 (32%) of 151 children had moderate to severe unilateral or bilateral hearing loss. By CT or MRI of 103 patients, brain infarcts were noted in 39 (38%); subdural effusions, in 25 (24%); hydrocephalus, in 22 (21%); cerebritis, in 12 (12%); and brain edema, in 6 (6%).

So far, the outcome of patients with pneumococcal meningitis caused by penicillin-nonsusceptible or cefotaxime-nonsusceptible isolates has not differed from that caused by susceptible strains.[20,125,205] This finding is explained in part because vancomycin has been administered empirically to most children with suspected bacterial meningitis since the mid-1990s in the United States and in other parts of the world where treatment failures have been reported as a result of antibiotic-resistant *S. pneumoniae*.[283]

Evoked response audiometry was used to detect hearing deficits in the patients described by Feigin and Dodge.[109] Some deficit in auditory nerve function was documented by this sensitive technique in 6 percent of children with *H. influenzae* meningitis, in 31 percent of children with pneumococcal meningitis, and in 10.5 percent of children with meningococcal disease.

Significant hearing loss after bacterial meningitis has been reported frequently. The mechanisms responsible for hearing deficits include spread of infection along the auditory canal and cochlear aqueduct, serous or purulent labyrinthitis, and, with time, replacement of the membranous labyrinth with fibrous tissue and new bone.[38,194,238,241,308] Damage to inner ear structures and subsequent hearing loss also is affected by the immune response of the host. TNF-α has been shown to produce cytolytic effects by the production of oxygen-free radicals. Animal studies have shown a decreased incidence of hearing loss when animals are treated with antibody blockade of TNF-α[16] and scavaging of oxygen-free radical by antioxidant therapy.[214]

Deafness generally is noted early in the course of bacterial meningitis and is independent of the therapy provided.[95,196,202,267,337,385] Ataxia has been reported as a presenting sign of bacterial meningitis in children with hearing loss noted at a later date.[200,336] Presumably, the insult to the vestibular and auditory systems occurred concomitantly in these children. The early loss of hearing noted by several investigators suggests that hearing loss is *not* associated specifically with the use of a particular antimicrobial agent. Early diagnosis and treatment apparently do not prevent the development of deafness in many children who develop hearing loss as a consequence of bacterial meningitis.

Estimates of the frequency of hearing loss in retrospective studies vary from 2.4 to 29 percent.[195,272] In our prospective studies, 7 percent of children have experienced marked to extensive (≥75-dB loss) hearing losses.[196,202] Overall, 48 of 151 (32%) of the children with pneumococcal meningitis in the multicenter study had unilateral or bilateral moderate to severe hearing loss.[20] Occasionally, hearing loss noted early may improve over weeks to months.[308]

In our studies, no correlation has been found between hearing loss and either the age of the patient at the onset of meningitis or the duration of illness before admission.[95,202] A significant correlation was noted between hearing loss and the presence of seizures before admission, the duration of fever in the hospital after therapy had been initiated (which presumably reflects more severe disease), treatment with antibiotics administered orally before a definitive diagnosis of bacterial meningitis was established, and a depressed CSF-to-blood glucose ratio at the time of admission.[95,202,204] Koomen and associates[221] developed a nomogram to predict the probability of development of hearing loss using five factors—duration of symptoms before admission, absence of petechiae, CSF glucose level, infection by *S. pneumoniae*, and ataxia—and were able to identify effectively patients at risk for developing hearing loss with excellent sensitivity (100%) using a cutoff of 0 on the point scale.

Because hearing deficits occur so commonly in patients with bacterial meningitis, hearing evaluation by evoked response audiometry in young, uncooperative children is recommended routinely at the time of or shortly after discharge from the hospital. Repeated audiometric evaluation is recommended after discharge if the results of the initial examination are abnormal. Pure tone audiometry can be used for older, cooperative children. Differentiation of hearing deficits resulting from conductive disturbances from deficits related to damage to the eighth cranial nerve is important. Some children who have repetitive episodes of otitis media may experience conductive loss that is unrelated to the meningitis.

In our studies, the mean IQ (±1 standard deviation) of the entire group of patients (235) after recovery was 94 (±23 standard deviations), with a range of 33 to 150. Twenty-nine children (17.3%) had IQs less than 80, and 22 (11.6%) had IQs less than 70. A comparison of these patients with their siblings and other control children revealed no significant difference in mean IQ. A significantly greater proportion ($p < .01$) of children who recovered from meningitis had IQs less than 80 compared with children from control groups. These results differ from those of Sell,[337] which were noted previously. Tejani and associates[372] also prospectively evaluated children who had recovered from bacterial meningitis using siblings as controls. They reported no significant differences in verbal performances or full-scale IQs between patients with meningitis and the sibling control population.

The prospective nature of these studies has permitted an assessment of factors that herald a poor prognosis and that may be discernible at or near the time of admission. Evidence of inappropriate secretion of ADH was correlated significantly ($p < .01$) with abnormal neurologic examinations at 3 months after discharge and with low IQs. The age of the child correlated inversely with the development of subdural effusion ($p < .01$), the occurrence of hearing deficits ($p < .02$), and low IQ ($p < .05$). The significantly increased impact of the disease on young children could be documented conclusively. The presence of focal neurologic findings in patients who were not postictal at the time of admission correlated significantly ($p < .001$) with abnormal neurologic examination, which was noted previously. Focal deficits indicating cerebral injury noted at admission or during the course of hospitalization were associated significantly ($p < .001$) with the development of late (1 to 15 years after discharge) afebrile seizures.[293] Focal neurologic findings at the time of admission proved to be a reliable predictor of permanent sequelae of bacterial meningitis. Focal deficits at admission also correlated significantly with low IQs ($p < .001$), even at 2 and 3 years after discharge from the hospital. The quantity of antigen in the initial CSF specimen and the number of organisms present also correlated significantly ($p < .01$) with sequelae of meningitis.[116]

Systematic review of neurocognitive impairment in children after CNS infection revealed deficits in cognition and motor function to be very common.[60] Koomen and associates[222] reported one third of Dutch children with non-*H. influenzae* type b meningitis were found on follow-up to have academic or behavioral limitations or both. Childhood meningitis survivors also were found to have a higher incidence of nonspecific symptoms, such as headaches, and significantly more symptoms of inattention, hyperactivity, and impulsiveness compared with nearest-age siblings.[37]

Only one study assessed the value of the Pediatric Risk of Mortality (PRISM) score in predicting outcomes of bacterial meningitis.[244] This study was done in a subgroup of children requiring mechanical ventilation. The best predictor of death and functional states on follow-up evaluation was the PRISM score. When the score was less than 20 within the first 24 hours of admission to the pediatric intensive care unit, a favorable outcome was noted in 82 percent. When the score was 20 or higher, a favorable outcome was noted in only 30 percent ($p < .009$).

CT has revealed evidence of cerebral infarction in children who had a diagnosis of bacterial meningitis established within 1 or 2 days of the onset of symptoms of a febrile illness.[352] In most of these cases, abnormalities in cerebrovascular dynamics (arteritis, thrombosis, thrombophlebitis), ventricular dilation, or both have been observed. In some of these cases, infarction has been associated with profound hypotension related to endotoxemia (personal experience). Brain infarction apparently is *not* related causally to a delay in diagnosis and therapy in many cases.

PREVENTION

HAEMOPHILUS INFLUENZAE MENINGITIS

Since the 1980s, methods of preventing meningitis caused by *H. influenzae* type b have improved. The dramatic decrease in the incidence of *H. influenzae* type b invasive disease and meningitis has been attributed to the introduction of vaccines that initially were found to be effective at 15 months of age[12] and then later found to be effective at 2 months of age.[352] Currently, three conjugate vaccines are approved for infants beginning at 2 months of age: HbOC, PRP-OMP, and PRP conjugated to tetanus toxoid (PRP-T). The ACIP and the AAP recommend universal infant immunization with HbOC or PRP-T at 2, 4, and 6 months of age or PRP-OMP at 2 and 4 months of age.[10,64]

Passive immunization of infants also has been studied with use of bacterial polysaccharide immunoglobulin.[8,321] This preparation given in a single intramuscular dose of 0.5 mL/kg provides significant protection for infants from *H. influenzae* type b disease for 3 or 4 months.

The most comprehensive study concerning the spread of *H. influenzae* type b infection among household contacts was coordinated by the CDC.[391] Data collected from 19 states were analyzed prospectively. *H. influenzae* meningitis was reported in 1403 patients. Eighty-two percent of exposed families were investigated for the occurrence of *H. influenzae* disease within 30 days of its onset in the index patient. Systemic disease caused by *H. influenzae* type b developed in 9 of 1687 contacts (0.5%) who were younger than 6 years. The risk of infection in patients younger than 4 years was 2.1 percent; the risk in children younger than 1 year was 6 percent. The risk of secondary infection of household contacts in the 30 days after onset of meningitis in the index case was 585 times greater than the age-adjusted risk in the general population and was similar to the risk of secondary meningococcal disease in household contacts. This nationwide study provided an important impetus for finding a chemoprophylactic regimen that could prevent secondary infection in household contacts.

A nationwide, collaborative, placebo-controlled trial was conducted subsequently among household (children <6 years old) and daycare center contacts of individuals with invasive *H. influenzae* type b disease.[30] Four of 765 placebo-treated contacts experienced secondary disease versus none of 1112 rifampin-treated contacts ($p = .027$).

The AAP Committee on Infectious Diseases recommends that rifampin be provided orally once each day for 4 days in a dose of 20 mg/kg (maximum dose 600 mg/day) to all household contacts (children and adults) regardless of age in households with at least one unvaccinated contact younger than 4 years.[10] The dose for infants younger than 1 month is not established, but it may be reduced to 10 mg/kg. Rifampin prophylaxis is not required, however, when all of the household contacts younger than 4 years have been fully immunized. All members of households with a fully immunized but immunocompromised child, regardless of age, should receive rifampin because of concern that the immunization may not have been effective.

When two or more cases of invasive disease have occurred within 60 days and unimmunized or incompletely immunized children attend a daycare facility, rifampin should be administered to all personnel and attendees. When a single case has been reported, the use of rifampin is controversial, and many experts recommend *no* prophylaxis.[10] Unimmunized or incompletely immunized children should receive a dose of vaccine and should be scheduled for completion of the recommended age-specific immunization schedule.

No data document the safety of rifampin administered during pregnancy. Prophylaxis with rifampin is not recommended for pregnant women who are contacts of infected infants.

Patients receiving rifampin should be advised routinely that their urine, sweat, and tears will be stained orange. Individuals should be advised to refrain from using contact lenses while receiving rifampin therapy because the lenses may be stained permanently.

PNEUMOCOCCAL INFECTION

In a large randomized trial conducted by Black and colleagues,[45] more than 37,000 infants received either the seven-valent pneumococcal vaccine containing polysaccharides of serotypes 4, 6B, 9V, 14, 18C, 19F, and 23F conjugated to CRM_{197} or meningococcal serogroup C oligosaccharide conjugated to the same protein. Vaccines were administered at 2, 4, 6, and 12 to 15

months of age. The pneumococcal conjugate vaccine was found to be 97.4 percent efficacious in preventing acquisition of invasive pneumococcal infections caused by vaccine serotype isolates. As a result of this study, the seven-valent pneumococcal protein conjugate vaccine is recommended by the ACIP and the AAP Committee on Infectious Diseases for all children younger than 24 months of age to prevent invasive pneumococcal infections.[14,67] Vaccine also is recommended for children 25 to 60 months of age with high risk of acquiring invasive disease and should be considered for otherwise normal children in this age range with other risk features that may increase their risk for development of systemic pneumococcal infection. Antibiotic chemoprophylaxis of children exposed to pneumococcal meningitis is not recommended regardless of immunization status.[9] In asplenic patients (functional and anatomic), daily chemoprophylaxis against pneumococcal infection is recommended with oral penicillin therapy.[9] The reader is referred to the CDC and AAP statements for the details of these recommendations.

MENINGOCOCCAL INFECTION

We advocate the use of chemoprophylaxis in all household members of a patient with meningococcal meningitis and in daycare and nursery school contacts, preferably within 24 hours of the diagnosis of the primary case.[11] Prophylaxis may be provided for individuals who had contact with the patient's oral secretions through kissing or sharing toothbrushes or eating utensils during the 7 days before onset of disease in the index case. Prophylaxis is not recommended routinely for health care personnel unless they have had close exposure through mouth-to-mouth resuscitation, intubation, or suctioning before antibiotic therapy was initiated. Schoolroom classmates and hospital contacts of patients usually are not given prophylactic treatment.

Minocycline and rifampin have proved to be 80 to 90 percent effective in eradicating carriage of meningococci.[171] Both drugs are secreted in the saliva in concentrations greater than the MICs for meningococci. The use of minocycline has been accompanied by frequent and significant vestibular reactions, even after a single dose of 100 mg, and, in our opinion, generally should not be used.[61-63,400]

Rifampin is the drug of choice in most instances and can be used in a dose of 600 mg twice daily for four doses in adults and in doses of 10 mg/kg/dose for four doses in children 1 to 12 years of age. A dose of 5 mg/kg every 12 hours for four doses can be used in children 3 months to 1 year of age.[266] The emergence of rifampin-resistant strains in treated meningococcal carriers has been reported to occur with a frequency of 0 to 27 percent.[83,99,396]

A single intramuscular dose of ceftriaxone has proved to be an effective alternative to rifampin for prophylaxis in meningococcal contacts.[335] This approach to prophylaxis may be particularly useful in circumstances in which compliance with the use of oral rifampin is considered questionable. The efficacy of ceftriaxone has been confirmed for only group A strains, but its effect is likely to be similar for other serogroups. Ceftriaxone has the advantage of easier dosage and administration and safety in pregnancy.[11] Ceftriaxone may be given intramuscularly in a dose of 125 mg for individuals younger than 12 years old and in a dose of 250 mg for individuals 12 years and older.

Ciprofloxacin given to adults in a single oral dose of 500 mg has been effective in eradicating meningococcal carriage.[11] Ciprofloxacin presently is not recommended for individuals younger than 18 years old or for pregnant women.

Because secondary cases may occur several weeks or more after onset of disease in the index case, meningococcal vaccine may be used as an adjunct to chemoprophylaxis when an outbreak is caused by a serogroup contained in the vaccine. The CDC has outlined recommendations for the administration of the meningococcal serogroup C vaccine to control outbreaks.[65]

A serogroup-specific quadrivalent polysaccharide meningococcal vaccine against group A, C, 4, and W135 *N. meningitidis* is approved for use in the United States in children 2 years old and older. The vaccine consists of 50 μg each of the respective purified bacterial polysaccharides. It may be given subcutaneously as a single 0.5-mL dose and can be given concurrently with other vaccines. Immunization with the meningococcal quadrivalent polysaccharide vaccine is recommended for children aged 2 to 10 years in high-risk groups, including children with anatomic or functional asplenia and children with terminal deficiencies of the complement system or with properdin deficiency.

In January 2005, the Food and Drug Administration approved a quadrivalent meningococcal conjugate vaccine, MCV4, which includes serotypes A, C, Y and W-135 for individuals 11 to 55 years old. Because of the conjugate vaccine's ability to induce a T-cell-dependent immune response and subsequently longer immunologic memory compared with polysaccharide vaccines, it is advantageous. The ACIP and the AAP made recommendations that the MCV4 vaccine be given to young adolescents at the 11- to 12-year visit, adolescents at high school entry or 15 years of age (whichever comes first), and students entering college planning to live in a dormitory,[15,44] with the goal of immunizing all adolescents by 2008. In addition to its use in the approved age group, MCV4 has been evaluated for use in the younger pediatric population. Pichichero and associates[291] showed an equivalent safety and tolerability profile of MCV4 compared with the quadrivalent polysaccharide vaccine and "significantly higher and more persistent serum bactericidal antibody responses against meningococcal serogroups A, C, Y, and W-135."

A single dose of serogroup C vaccine seems to be approximately 70 percent effective, for a period of 6 to 9 months, in preventing meningococcal disease in children who are older than 2 years.[369] Single 50-μg injections in children younger than 2 years of age do not produce adequate antibody responses.[369] A serogroup A polysaccharide vaccine has been field tested by the World Health Organization and is effective in children aged 3 months or older.[287,387] Serogroup-specific monovalent vaccines may be used to control outbreaks of disease caused by either type A or type C meningococci and may be valuable for travelers to countries with epidemic disease.[11,105] The vaccine is administered as a single dose parenterally in the volume specified by the package insert. Reactions noted after immunizations previously have been mild and infrequent; localized erythema of 1 or 2 days' duration is not unusual. The safety of the vaccine in pregnant women has not been established.

An effective serogroup B meningococcal vaccine has not been produced to date. The polysaccharide capsule cross-reacts with fetal neural tissues causing autoantibodies. Different strategies have been tried to develop a safe vaccine against serogroup B meningococcus, including use of outer membrane proteins (OMP) and lipopolysaccharide components.[182] Although OMP vaccines possess efficacy in older children and adults, the highest rates of serogroup B disease are in infants and young children, in which the OMP vaccines do not show efficacy.[70] Complete sequencing of the *N. meningitidis* serogroup B genome offers new membrane protein targets for vaccine targeting.[178] Similar to other protein conjugate vaccines, meningococcal C conjugate vaccines are more immunogenic than is the pure polysaccharide vaccine in infants younger than 2 years of age.[243] Because of a high incidence of meningococcal disease in the United Kingdom, a meningococcal serogroup C CRM_{197} was administered in a phased program beginning November 1999 to all children younger than 18 years.[302] Surveillance studies to assess short-term efficacy have shown a 97 percent efficacy for teenagers and a 92

percent efficacy for toddlers in the prevention of meningococcal serogroup C infection.

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CHAPTER 38

Parameningeal Infections

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BRAIN ABSCESS

Brain abscess is an uncommon infection, but it remains a serious and life-threatening disease in children despite advances that have been made in diagnosis and management. Abscess formation may occur in the parenchyma of the central nervous system, in the subdural space, or in the epidural space. It can originate from contiguous site infections (e.g., skull osteomyelitis, chronic otitis), from underlying vascular anomalies (e.g., congenital cyanotic heart disease), after head trauma or neurosurgical procedures, or from cryptogenic sources. These conditions can alter neurologic function by direct destruction of nervous tissue, by infarction after inflammatory occlusion of veins and arteries, or by compression caused by mass effect.

Advances in diagnostic and therapeutic modalities since the 1990s have improved the prognosis of this serious disease. The mortality rate associated with brain abscesses seems to be decreasing. This reduction probably is the result of the advent of head imaging and its use to guide precise location and management, improvement in surgical techniques, and advances in antibiotic development. Goodkin and coworkers[43] identified 54 patients aged 5 days to 34 years of age who were diagnosed with a brain abscess at the Boston Hospital from 1945 to 1980 and 1981 to 2000. They found a reduction in the number of abscesses that occurred in the setting of sinus or middle ear infection (26% versus 11%, respectively) and an increase in the number of children who were treated with antibiotics alone (1% versus 22%). These investigators did not observe, however, any additional decline in the mortality rates in their institution between 1981 and 2000. Fischer and associates[17] found a reduction in mortality rate from 36 percent before 1970 to 14 percent after 1970. Tekkok and Erbengi[45] described a similar decline, from 30 percent in the era before the use of computed tomography (CT) to 6 percent in the last 5 years of their study and zero in the last 3 years of their study. Other case series also have reported a mortality rate of zero.[29]

Although the mortality rate seems to be decreasing, a significant percentage of children continue to have residual neurologic deficits, including epilepsy, permanent motor or sensory dysfunction, visual field defects, and personality changes.[17,20,25] Some children also require placement of a ventriculoperitoneal shunt.[10]

PATHOGENESIS AND PATHOLOGY

Brain abscess is a focal, intracerebral infection that begins as a localized area of cerebritis and eventually ends in a collection of pus surrounded by a well-vascularized capsule. For practical purposes, brain abscesses usually are classified according to the likely entry point of the infection (Table 38–1). Ear and mastoid infections are associated with formation of an abscess at the temporal or cerebellar locations; sinus and dental infections give rise to purulent collections in the frontal lobe; and metastatic spread from distant foci in children with congenital cardiac or pulmonary right-to-left shunts commonly results in involvement of any parenchymal area, including parietal or occipital regions.[17,28,41]

The most common origin of microbial infection in children remains direct or indirect cranial infection arising from the middle ear, paranasal sinuses, or teeth. Seeding of the brain presumably occurs via transit of infecting microbes through the valves and emissary veins that serve these regions. A direct erosion of skull and dura by osteomyelitis-induced sinus or middle ear infection can be another mechanism of bacterial spread.[7] Resulting abscesses tend to be solitary and superficial. Metastatic inoculation of the brain from distant extracranial sources (pulmonary infection, endocarditis) tends to provoke multiple cerebral abscesses, with a distribution that reflects the regional cerebral blood flow of the area affected, usually the middle cerebral artery network.[7]

In children with cyanotic congenital heart disease, bacteria are not filtered out by the pulmonary vascular bed, which allows for systemic spread.[10] This situation rarely occurs in patients younger than age 2 years, and the abscess or abscesses usually are in areas of brain perfused by the middle cerebral arteries. Evidence of associated endocarditis is rare in these cases, although acute bacterial endocarditis may be complicated by septic infarction of the brain and abscess formation.[36]

TABLE 38–1 Primary Source, Usual Location of Lesion, and Associated Neurologic Findings in Children with Brain Abscess

Primary Source	Location of Abscess	Associated Neurologic Findings
Upper respiratory site		
Sinusitis	Frontal lobe	Headache, behavioral changes, motor speech disorders, depressed consciousness, forced grasping and sucking, hemiparesis
Chronic otitis/mastoiditis	Temporal lobe	Dyspraxia and aphasia (dominant hemisphere), ipsilateral third cranial nerve palsy, ipsilateral headache, upper homonymous hemianopsia, motor dysfunction of face and arm
	Cerebellum	Dizziness, vomiting, ipsilateral ataxia and tremor, sixth cranial nerve palsy, nystagmus (toward lesion)
Dental infection	Frontal lobe	
Head trauma	Related to injured site	Variable by region involved
Postoperative	At operative site	Variable by region involved
Metastatic spread	Multiple lesions	Variable by region involved
	If parietal lobe involved	Visual field defects in inferior quadrant, homonymous hemianopsia, dysphasia (dominant hemisphere), dyspraxia and contralateral spatial neglect (no dominant hemisphere)

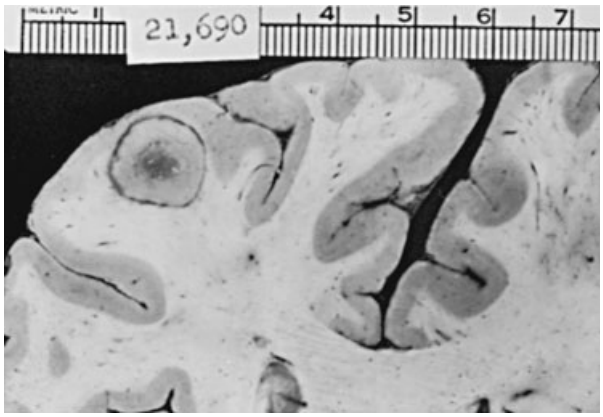

Figure 38–1 Circumscribed cerebral abscess of hematogenous origin.

Formation of an abscess by direct bacterial implantation may complicate compound skull fractures, scalp wounds, anterior cranial fossa or temporal bone fractures, and chronic cerebrospinal fluid (CSF) fistula. Abscesses also rarely may develop during the course of bacterial meningitis.[40] Despite identification of all these potential routes, 20 to 30 percent of cases are classified as cryptic brain abscess for which no obvious predisposing factor can be identified.

The brain is remarkably resistant to microbial infection. Despite the common occurrence of occult bacteremia in infants and children, cerebral abscess is a quite rare disease. This resistance is attributable in part to the abundant blood supply of the brain and the relative impermeable blood-brain barrier.[1] Although certain underlying brain morbidities, such as previous stroke, intracerebral hematoma, or underlying neoplasm, may serve as a nidus of abscess formation in adults, affected children have no apparent predisposing brain lesion. In animal models of infection, induction of abscess usually requires direct inoculation of numerous organisms into the animal's cerebrum.[34]

Experimental animal studies and use of CT scanning techniques have provided evidence of the clinical evolution of a brain abscess.[2] In the early stage of cerebritis (days 1 to 3), a focal area of acute inflammation, vascular dilation, microthrombosis, rupture of small vessels, and edema are present. The center of the lesion then undergoes liquefaction. Expansion of the cerebritis and formation of a necrotic central focus are seen in the late cerebritis stage (days 4 to 9). Establishment of a ring-enhancing dense collagenous capsule of well-vascularized tissue with peripheral gliosis or fibrosis or both occurs at the early capsule stage (days 10 to 14). Finally, during the late capsule stage (>14 days), host defenses act to wall off the abscess, and a well-developed capsule results.

Death can occur if the volume of pus and surrounding edema induce a significant increase of intracranial pressure (ICP) leading to brain herniation. In addition, cerebral abscesses can rupture into the ventricular system or through the cortex into the subarachnoid space, resulting in acute deterioration and a life-threatening event.[10] Figure 38–1 shows the gross appearance of a well-defined abscess of hematogenous origin. Various microscopic features are illustrated in Figures 38–2, 38–3, and 38–4.

The spectrum of microorganisms cultured from brain abscesses has changed with time. This change reflects improved microbiologic isolation techniques, early and aggressive treatment of primary infections, and better neurosurgical procedures. In most more recent series, the predominance of *Staphylococcus aureus* has decreased, and identification of anaerobes has increased.[6,13,47]

Anaerobic bacteria isolated from brain abscesses include species of *Bacteroides*, *Peptostreptococcus*, *Fusobacterium*, *Veillonella*, *Propionibacterium*, *Prevotella*, and *Actinomyces*. Aerobic and microaerophilic streptococci, staphylococci, *Haemophilus* spp., gram-negative enteric bacilli, and *Pseudomonas aeruginosa* also are implicated frequently. In children with impaired host defenses, fungal etiology (*Candida*, *Aspergillus*, *Cryptococcus*, *Histoplasma*, *Coccidioides*, and *Mucor* spp.) or uncommon pathogens, such as *Toxoplasma*, *Nocardia*, *Mycobacterium*, and *Listeria* spp., can be identified.[17] Parasites such as amebae, *Cysticercus*, *Schistosoma*, or *Paragonimus* are very rare causative pathogens.[18] *Citrobacter koseri (diversus)* and *Enterobacter sakazakii* are particularly able to cause brain abscesses in neonates.

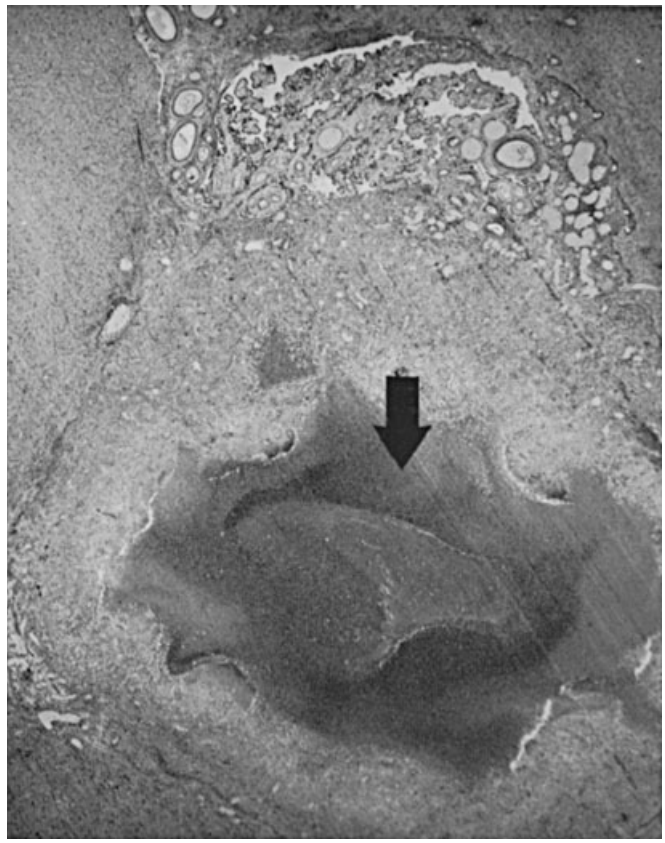

Figure 38–2 Area of necrosis and liquefaction in a cerebral abscess *(arrow)*.

In order of etiologic importance, the predominant organisms causing brain abscess in children are aerobic and anaerobic streptococci (60-70% of cases), gram-negative anaerobic bacilli (20-40%), Enterobacteriaceae (20-30%), *S. aureus* (10-15%), and fungi (1-5%).[3-6,13,17,28,34,35,41,47] Multiple aerobic and anaerobic organisms are isolated in approximately one third of patients, especially in patients with chronic otitis. No growth is reported from 30 percent of properly handled purulent specimens. A reasonable speculation of the likely causative microbes can be made according to the predisposing source of infection (Table 38–2).

CLINICAL MANIFESTATIONS

The clinical presentation of a brain abscess depends on the size of the collection, its location, the multiplicity of lesions, the host's immune status, and the age of the patient. Generally, symptoms and signs can be related to the effect of a space-occupying mass, to the focal neuronal dysfunction of the parenchymal region

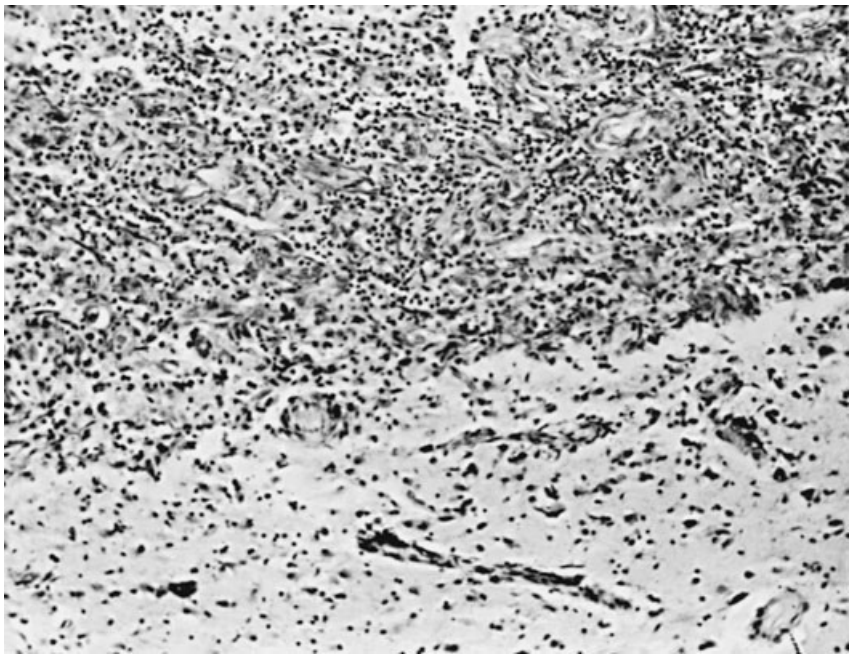

Figure 38–3 Astroglia and fibroblasts forming the capsule of a cerebral abscess.

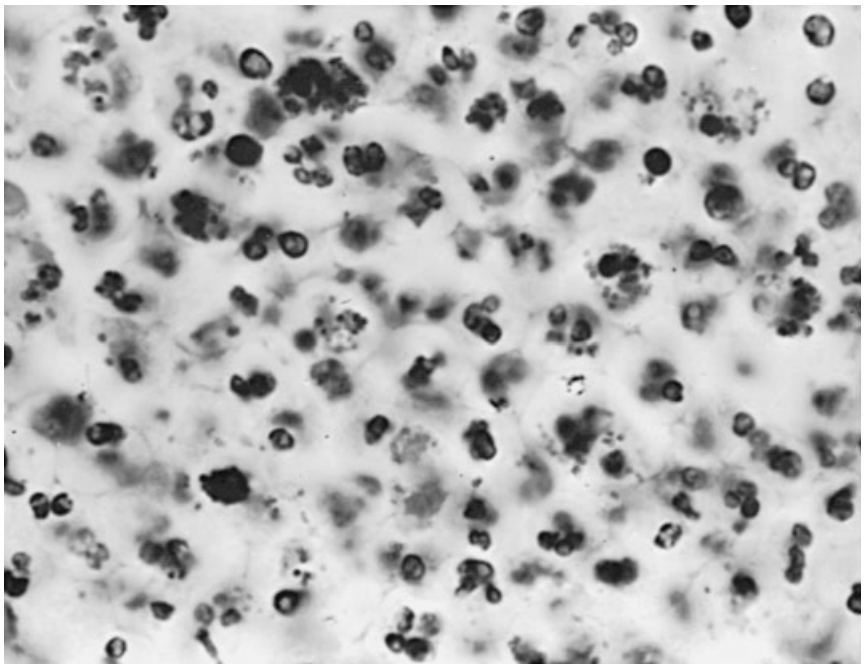

Figure 38–4 Mononuclear and polymorphonuclear leukocytes are in the center of a cerebral abscess.

involved (see Table 38–1), or to accompanying clinical findings of the underlying predisposing infection. Because on early evaluation most children with cerebral abscess present with vague or nonspecific signs and symptoms, the physician must have a high index of suspicion to recognize the condition as early as possible (Table 38–3). When a patient develops significant alterations in mental status, the prognosis is most ominous.[10,41]

In older children and adolescents, headache is the most common initial symptom. Irritability occurs more commonly in infants and small children. The nature of the headache has no particular distinguishing features and usually is poorly localized. A sudden worsening of a preexisting headache can indicate rupture of the brain abscess into the ventricular space, which has a poor prognosis.[8,16]

Absence of fever should not be used to exclude the diagnosis of brain abscess because this sign is detected in only approximately one half of affected children. Drowsiness, confusion, and vomiting occur frequently during the acute phase of disease. Lethargy, stupor, and coma usually are later events and potentially associated with adverse outcome. Papilledema is present in less than one fourth of the cases, but its presence requires immediate neurosurgical assessment and CT scanning.

Focal neurologic disturbances reflect the location of the abscess and can be detected in 30 to 50 percent of cases on presentation (see Tables 38–1 and 38–3).[48] Frontal location is characterized by development of motor speech disorders, memory deficits, personality changes, and depressed consciousness. Hemiparesis occurs with lesions in the postfrontal region or as a con-

TABLE 38–2 Primary Source, Usual Etiologic Pathogens, and Recommended Empiric Antibiotic Therapy in Children with Brain Abscess

Primary Source	Usual Etiologic Microorganisms	Recommended Empiric Antibiotic Combination
Upper respiratory infection		
Sinusitis/dental infection	Viridans and anaerobic streptococci, *Haemophilus* spp., *Fusobacterium* spp., *Bacteroides* spp. (non-*fragilis*)	Penicillin or cefotaxime/ceftriaxone + metronidazole or ampicillin + chloramphenicol or sulbactam
Chronic otitis/mastoiditis	Aerobic and anaerobic streptococci, gram-negative enteric bacilli, *Bacteroides* spp. (including *B. fragilis*), *Pseudomonas aeruginosa*	Penicillin + metronidazole + ceftazidime
Head trauma	*Staphylococcus aureus*, aerobic streptococci, gram-negative enteric bacilli	Oxacillin/nafcillin (vancomycin) + cefotaxime/ceftriaxone
Postoperative	*Staphylococcus epidermidis*, *S. aureus*, gram-negative rods, *P. aeruginosa*	Vancomycin + ceftazidime
Metastatic spread		
Endocarditis	*S. aureus*, viridans streptococci	Oxacillin/nafcillin (vancomycin) + cefotaxime/ceftriaxone + metronidazole
Pulmonary infection	Aerobic streptococci, *Actinomyces*, *Fusobacterium*	Oxacillin/nafcillin (vancomycin) + cefotaxime/ceftriaxone + metronidazole
Congenital heart disease	Viridans streptococci, *Haemophilus* spp., *Haemophilus aphrophilus*	Cefotaxime/ceftriaxone + metronidazole or ampicillin + chloramphenicol or sulbactam
Bacterial meningitis	*S. pneumoniae*, *Haemophilus influenzae* type b, *Salmonella* spp., *Citrobacter* (neonates)	Cefotaxime/ceftriaxone ± vancomycin (resistant pneumococcal strains)
Cryptogenic source and immunosuppression*	Any type of microorganisms	Oxacillin/nafcillin (vancomycin) + ceftazidime + metronidazole
	Nocardia, fungi, *Mycobacterium tuberculosis*	Oxacillin/nafcillin (vancomycin) + ceftazidime + metronidazole

**In areas with significant prevalence of methicillin-resistant staphylococcal strains or penicillin allergy, immunosuppressed children should be treated with broad coverage and with consideration of amphotericin B therapy. Antituberculous therapy should be considered for children with exposure to tuberculosis. Antibiotic regimens are likely to vary by geographic location based on resistant organisms.*

TABLE 38–3 Frequency of Presenting Signs and Symptoms in Children with Brain Abscess

Symptoms	%	Signs	%
Headache	60-70	Focal neurologic deficits	35-50
Fever	50-80	Papilledema	30-40
Vomiting	35-55	Meningeal signs	25-35
Seizures	30-45	Hemiparesis	20-30
Mental changes	30-40	Nerve palsy	10-20
Coma	15-20	Ataxia	5-15

sequence of uncal herniation. Abscess in the temporal lobe is characterized by a contralateral homonymous upper quandrantopia. If the dominant hemisphere is affected, nominal dysphasia and aphasia are characteristic symptoms. Patients with cerebellar abscesses classically exhibit dizziness, nystagmus, defective conjugate eye movements, ataxia, tremor, and hypotonia. Seizures occur in at least 30 to 45 percent of patients, may be focal or generalized, and may occur at any time during the course of the disease. If the parietal region is involved, visual field defects, homonymous hemianopsia, dysphasia, and dyspraxia can be present.

The rapidity with which symptoms develop can vary substantially. Most patients are symptomatic within 1 week of the onset of formation of an abscess. Immunocompromised children can have a more insidious progression of clinical findings. The presentation of brain abscess in infancy can be suspected by bulging fontanelle, vomiting, irritability, and an enlarging head circumference. Seizures occur commonly, particularly in small infants, and at any time during the course of the disease.[14] School-aged children with cyanotic congenital heart disease, notably tetralogy of Fallot or transposition of the great vessels, also can exhibit symptoms and signs related to their chronic cardiac disease.

RUPTURE OF BRAIN ABSCESS INTO THE VENTRICULAR SYSTEM

Rupture of an abscess into the ventricle with consequent ventricular empyema is a dreaded complication because the mortality rate is greater than 50 percent, and residual neurologic deficits, including hydrocephalus, are the rule in patients who survive. Frequently, rupture occurs before the diagnosis of abscess has been established and surgical removal can occur. A sudden worsening in the patient's clinical state heralds this event. High fever, shock, meningismus, and altered consciousness are prominent clinical signs.

Rupture of the abscess into the ventricle is seen more frequently in patients with deep-seated abscesses or in immunocompromised patients.[44] Although a modest pleocytosis and elevated protein concentration in CSF may have been identified earlier, the findings of 50,000 to 100,000 polymorphonuclear leukocytes and markedly reduced glucose concentration in the CSF are usual findings. Organisms may be seen on smear of the CSF and cultured from the fluid. In other words, the patient has developed purulent meningitis, and treatment must include high doses of antibiotics and surgery (see the treatment section).

The concurrence of abscess and meningitis in the past has led to the assumption that brain abscess can be a complication of meningitis; this rarely, if ever, is the case, although meningitis may develop during the incipient stages of abscess formation after intracranial invasion of organisms from a contiguous extracranial source. In such circumstances, the abscess may seem to be a consequence of the leptomeningitis. Given a potential source of infection in the ear or paranasal sinuses, the clinician must be wary and appreciate the possibility of this sequence of events. Abscess has been reported to complicate *Citrobacter* meningitis in infants, but careful pathologic study has shown vasculitis and liquefaction necrosis of the white matter without capsule formation.[47]

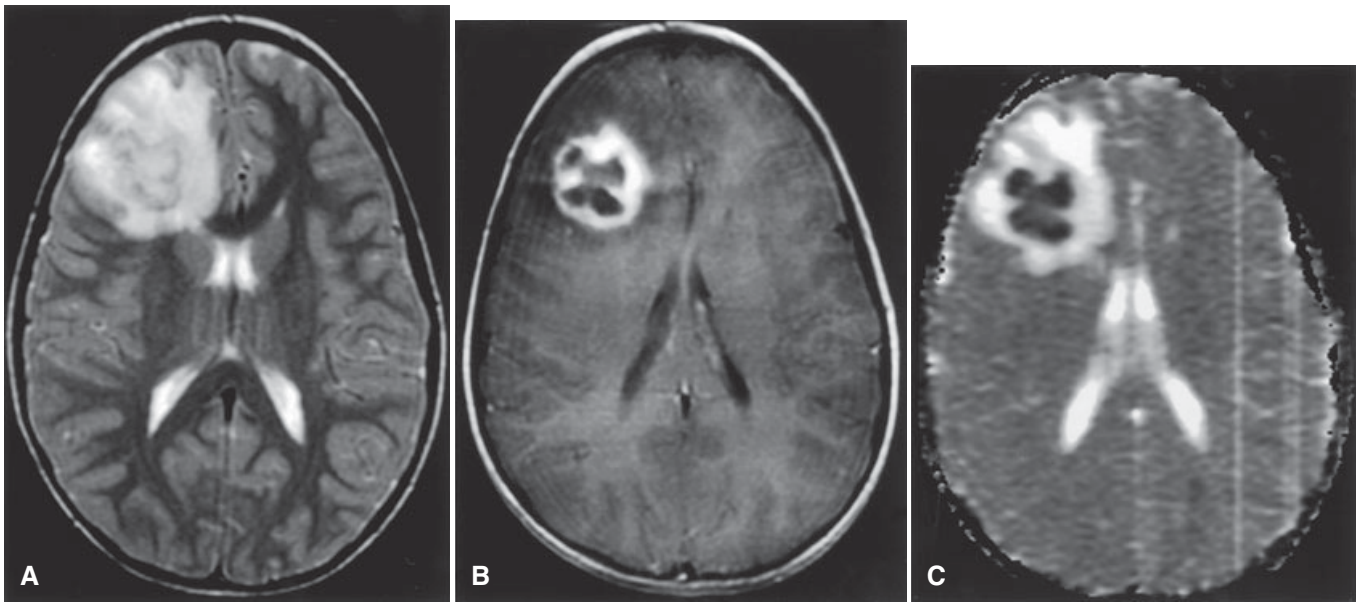

Figure 38–5 Bacterial abscess in a 10-year-old girl. **A,** Axial T2-weighted magnetic resonance (MR) image shows an irregular mass in the right frontal lobe with a hypointense wall *(arrows)* and surrounding vasogenic edema. **B,** Gadolinium-enhanced T1-weighted MR image shows intense, smooth enhancement of the capsule. **C,** Apparent diffusion coefficient map from a line-scan, diffusion-weighted study shows decreased signal within the cavity, compatible with restricted diffusion and suggesting an abscess cavity rather than tumor necrosis. *(Courtesy of F. Kim, M.D., Northwestern University School of Medicine, Chicago.)*

LABORATORY DIAGNOSIS

Laboratory tests frequently are not helpful in supporting the clinical diagnosis of brain abscess.[10,33,41] Children usually have unremarkable leukocyte counts, and the erythrocyte sedimentation rate can be normal. Blood cultures rarely are positive. Performance of a lumbar puncture is potentially dangerous because it can be associated with brain stem herniation.[9] In addition, CSF analysis uncommonly provides useful clinical information. Usual CSF findings include an elevated protein, mild mononuclear pleocytosis, and hypoglycorrhachia. CSF cultures usually are negative, unless the abscess has drained into the ventricular system or has been a complication of meningitis.

Culturing abscess material obtained at the time of surgical drainage provides the best opportunity to make a microbiologic diagnosis. Proper handling and processing, with attention given to optimal anaerobic and aerobic isolation techniques, can identify the causative organism in most cases, especially when antimicrobial therapy has not been instituted previously.[33] When the clinical condition warrants immediate use of empiric antibiotics, Gram stain of the purulent material frequently can help direct selection of the appropriate antibiotic.

DIAGNOSIS

Magnetic resonance imaging (MRI) is the modality of choice for the diagnosis and localization of cerebral abscesses. Similar MRI findings can be observed in infarction, demyelinating disorders, and neoplastic processes. Diffusion-weighted MRI and magnetic resonance spectroscopy have been shown to be effective methods in differentiating cerebral abscesses from tumors.[5,34] Figure 38–5 illustrates the appearance on MRI of a right frontal cerebral abscess in a 10-year-old girl. CT scanning remains an excellent alternative, however, if MRI is unavailable (Fig. 38–6). Intrave-

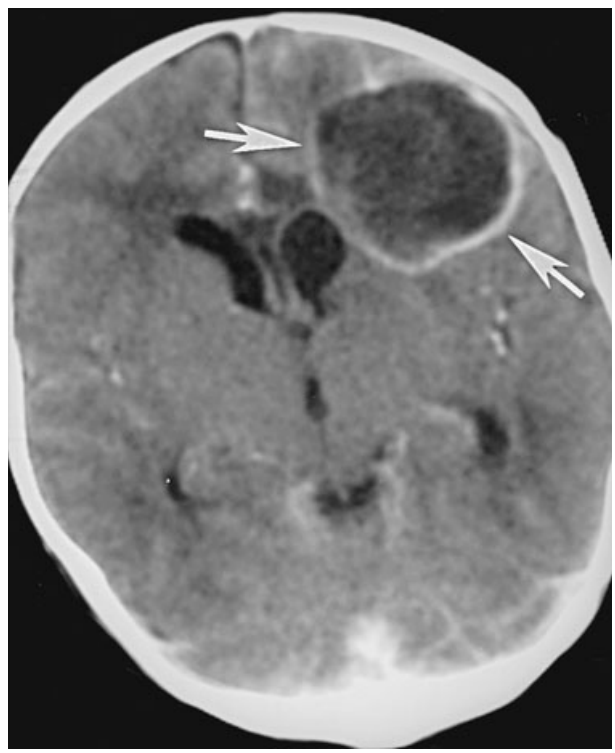

Figure 38–6 Contrast-enhanced computed tomography (CT) scan shows a ring-enhancing left frontal cerebral abscess *(arrows)* in a 3-week-old boy with concurrent *Enterobacter* meningitis. *(Courtesy of C. D. Robson, M.D., Harvard Medical School, Boston, MA, and P. D. Barnes, M.D., Stanford University School of Medicine, Stanford, CA.)*

nous administration of contrast material is advised because the abscess may be missed otherwise.[12,26] Serial CT or MRI scanning, performed weekly or biweekly, provides evaluation of the response to therapy and of the need for repeating the surgical procedure. MRI is more accurate than is CT for establishing the diagnosis of cerebritis, cerebellar, edema formation, and brain stem purulent collections.[19,24] On T1-weighted sequences, brain abscesses appear hypointense and show ring enhancement after administration of intravenous gadolinium. In contrast, on T2-weighted images, the typical mature abscess has a hyperintense central area of pus surrounded by a well-defined hypointense capsule and surrounding edema.

Because specific images allow precise lesion staging, this information provides important clues for the surgeon to suspect the likelihood of encountering purulent material of a well-developed abscess at the moment of drainage. The presence of gas within the abscess cavity on head imaging suggests communication of the abscess with air outside the skull, although gas-forming bacteria in the abscess cavity rarely may be responsible.[49]

Plain skull films generally are normal in children with brain abscess. In small infants, separation of cranial sutures indicates intracranial hypertension. Radionuclide brain scan, arteriography, and ventriculography have been replaced largely by CT and MRI for the diagnosis of brain abscess. Indium-labeled leukocyte scans and thallium-brain single photon emission computed tomography (SPECT) to differentiate brain abscess from tumor in questionable cases have not been studied adequately in the pediatric population, but these methods seem promising in affected adults.[39]

Abnormalities on electroencephalography may be localized and can help in excluding a more generalized, bilateral intracranial disease, such as encephalitis. Unilateral slow waves (delta, 1 to 3/sec) characterize the usual electroencephalographic findings in cases of cerebral abscess.

After the diagnosis of brain abscess has been established, a careful search should be made for a source of infection serving as a site of origin for hematogenous spread or direct inoculation of organisms into the central nervous system. In addition to obtaining data from the history and physical examination, the physician should extend the MRI or CT evaluation to include the mastoids and paranasal sinuses. An echocardiogram should be obtained to assess for concurrent endocarditis. Other testing should be guided by the history and physical examination findings.

TREATMENT

No prospective clinical trials have compared various surgical and medical treatment strategies available to guide the management of cerebral abscesses in children. Most surgical and medical treatment guidelines are based on populations consisting primarily of adult patients. Appropriate management of brain abscesses apparently requires a combined surgical and medical approach,[31] with isolated medical management limited to patients who are neurologically intact and in whom the abscess is in the cerebritis stage[21] or the abscess or abscesses are small,[38] or to patients who are too unstable to undergo a surgical procedure.

The initial treatment of solitary and multiple brain abscesses relies on the aspiration of the cavity contents followed by the initiation of empiric antibiotic coverage while the cultures are processing. CT-guided or MRI-guided stereotactic aspiration is accurate, minimally invasive, and associated with few complications. Mamelak and colleagues[32] suggested performing head imaging biweekly or on any sign of clinical deterioration, with further aspiration if the cavity enlarges or fails to diminish in size after 3 to 4 weeks. Because excisions may be associated with increased risk of developing neurologic sequelae, surgery should be reserved for patients who do not respond to the strategy of repeated aspiration and medical management, and when an abscess results in a significant mass effect.

While results of cultures are pending, the selection of antibiotics should be guided by the primary source of infection, pertinent patient history, and results of microscopic examination of the pus (see Table 38–3). The appropriate duration of antimicrobial therapy for brain abscess is unclear. A 6- to 8-week course of parenteral antibiotics has been recommended traditionally, provided that the etiologic organisms are susceptible and adequate surgical drainage is achieved.[33] In selected cases with uncomplicated infection and complete surgical removal of a well-delineated abscess, shorter courses of treatment (3 to 4 weeks) are likely to be sufficient.[42] Nonetheless, many authorities recommend 2 to 3 months of additional oral antimicrobial therapy to prevent relapses.[10] In fungal, nocardial, and helminthic infection, surgical intervention frequently is required because of failure of medical treatment. Surgical procedures can be used to reduce increased ICP, obtain pus for microbiologic diagnosis, and enhance the antibiotic efficacy. In carefully selected patients (illness duration ≤2 weeks, neurologically intact, no signs of increased ICP, and abscess ≤3 cm in diameter), medical therapy alone can be successful.

With the advent of head imaging and the current methods of treatment employing head imaging, an overall reduction in the mortality rate has occurred; however, a high proportion of children with cerebral abscesses develop neurologic deficits. Eradication of potential sources of infection before the abscess has developed is logical preventive medicine. Early diagnosis and treatment are imperative and can be facilitated by the liberal use of MRI or CT scanning when this diagnosis is even a remote consideration.

ADJUNCTIVE AGENTS

The use of corticosteroids for treatment of brain abscess is controversial. These agents usually are indicated to control life-threatening intracranial hypertension that is associated with risk of herniation or when significant symptoms and signs are presumed to be the result of cerebral edema. Severe brain edema may necessitate the additional administration of intravenous mannitol. Steroids interfere with the inflammatory response, retard the encapsulation process, increase development of necrosis, potentially reduce antibiotic concentrations inside the purulent collection, and alter CT images.[37]

Anticonvulsants are recommended in children who have developed seizures potentially to prevent further episodes. Duration of anticonvulsant therapy should be individualized and guided by an electroencephalographic study in the follow-up phase of disease. Most authors recommend providing at least 3 months of prophylaxis if no more seizures have occurred.[10]

SUBDURAL EMPYEMA

Pyogenic infection in the subdural space is designated as subdural empyema or sometimes, but less correctly, as subdural abscess. The sources of infection and microorganisms responsible are the same as those encountered in brain abscess. It is a rare disease; only 1 case was seen at St. Louis Children's Hospital during a period when 122 patients with bacterial meningitis and 3 patients with cerebral abscess were treated. The primary source of subdural empyema in this single case was not found, which increasingly is true for the pediatric experience. Farmer and Wise[15] and Jacobson and Farmer[27] found associated meningitis in six of eight

infants with subdural empyema (seen over a 16-year period), suggesting that the latter was a complication of the former. We also have encountered this situation, albeit rarely, and the subdural fluid usually is turbid, rather than frankly purulent.

In older children, the infection apparently does not follow leptomeningitis. Although leptomeningitis may complicate subdural empyema, infections of the paranasal sinuses and mastoid region, usually chronic, spread to the subdural space directly because of osteomyelitis or by way of infected veins that penetrate the skull. Extension to cortical veins and to major venous sinuses frequently is associated with subdural empyema, as discussed elsewhere in this section. Why in some cases the infection is restricted to one or another anatomic site or sites (e.g., epidural space, subdural space, parenchyma of the brain or blood vessels) is unknown. Subdural empyema may be hematogenous in origin. It is an infrequent but recognized complication of intracranial surgery. The anatomy of the subdural space is such that the infection often extends widely over one or both cerebral hemispheres, and accumulation of pus in the parafalcine region occurs commonly.

CLINICAL MANIFESTATIONS

The symptoms and signs of the primary source of infection may be prominent, subtle, or absent. Increasingly severe headache, high fever, signs of meningeal irritation, and progressive neurologic deficits referable to the site of the lesion are reported in the typical untreated case. Focal or generalized seizures are prominent, especially in cases of cortical injury from associated vasculitis. Signs of increased ICP become prominent as the mass of pus enlarges. In infants, a fullness of fontanelle, vomiting, and depressed responsiveness are seen. Transillumination of the skull can be positive. Older children also may develop papilledema. As the ICP increases, symptoms and signs progress, ultimately leading to temporal lobe or cerebellar herniation and the characteristic syndromes of these complications.

DIAGNOSIS

The laboratory findings reflect the active infectious process. Peripheral leukocytosis and a predominance of polymorphonuclear leukocytes with immature cells frequently are seen. The CSF in infants reflects the common association with leptomeningitis, and the findings depend on when the fluid is examined. Before the meningitis has been treated adequately, organisms may be cultured; the glucose concentration may be low and the protein concentration high in the CSF. Later, the CSF findings are the same as those for treated meningitis, but viable organisms can be recovered from the subdural collections when the CSF is sterile. Specific antigens may be shown in subdural collections by tests such as countercurrent immunoelectrophoresis in the absence of viable organisms. In older children, the characteristic CSF findings include elevated pressure with a few to a few hundred or more leukocytes, with polymorphonuclear leukocytes predominating. The protein concentration frequently is elevated, the glucose concentration is normal, and the fluid is sterile.

In the past 2 or more decades, subdural empyema in children has become more difficult to diagnose clinically because of antimicrobial therapy. When initiated early, antimicrobial therapy may attenuate the dramatic nature of the disease, especially the symptoms and signs of acute infection. Because the diagnosis of subdural empyema often is confused with that of brain abscess, radiographic studies are necessary to establish the correct diagnosis. CT scanning (Fig. 38–7) and MRI are effective noninvasive techniques, as previously discussed in relation to brain abscess.

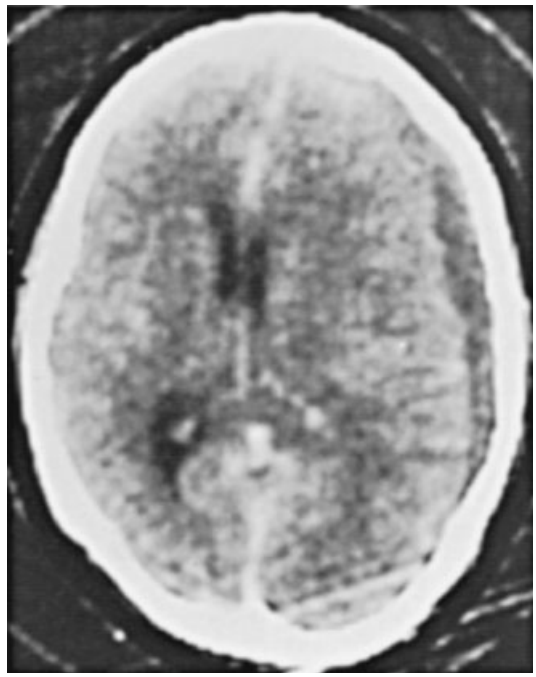

Figure 38–7 CT scan shows a subdural empyema with marked displacement of the ventricular system.

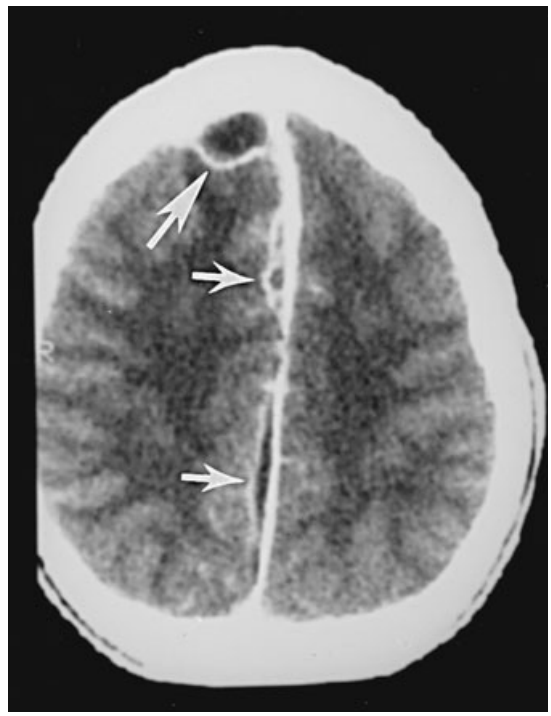

Figure 38–8 Contrast-enhanced CT scan shows a right frontal epidural abscess *(large arrow)* concurrent with an interhemispheric subdural abscess *(small arrows)* in a 13-year-old boy who developed headache, fever, and vomiting after fracture of the right frontal sinus. *(Courtesy of C. D. Robson, M.D., Harvard Medical School, Boston, MA, and P. D. Barnes, M.D., Stanford University School of Medicine, Stanford, CA.)*

Cerebral CT can show a failure of the cerebral vessels to approximate the inner surface of the skull. These studies reveal the extent of subdural disease and may define an epidural abscess or other infectious exudate collection concurrent with subdural empyema (Fig. 38–8).

TREATMENT

Until a causative organism is identified, the patient should receive broad-spectrum antibiotics intravenously in dosages appropriate for bacterial meningitis. After the offending organism is identified, the sensitivities of that organism to specific medications dictate selection of more precise antibiotic therapy. In infants, antibiotic therapy may be sufficient when the fluid is cloudy only, but if thick, purulent material is obtained by subdural paracentesis or, in older children, if the clinical and radiographic evidence indicates a subdural empyema, surgery is requisite. Depending on individual circumstances, craniotomy or multiple burr holes with irrigation of the subdural space should be accomplished.

Mortality and morbidity rates are high (20-40%) in most series but lower in some.[43,50] Early diagnosis and treatment are imperative. The early use of CT scanning seems to be responsible for improved therapeutic results.[50]

EPIDURAL ABSCESS

Because the dura mater is adherent to the inner aspect of the cranium, epidural abscesses rarely attain a large size, and they consequently do not exert significant pressure on the brain. They are important because they can serve as a focus for spread of infection into the subdural space, leptomeninges, or brain. The infection may involve local penetrating vessels and lead to their occlusion, with extension to venous sinuses and other vessels. Infection of the middle ear, mastoid bone, or ancillary air sinuses may lead to epidural abscess, as in the case of subdural empyema and brain abscess. Often, epidural abscess coexists with the other lesions and presumably develops first. Osteomyelitis may be evident, which usually is the case with mastoiditis and often is evident after head injury with comminuted fracture of the skull or after intracranial surgery. A case of an adolescent boy with osteomyelitis and epidural abscess complicating a wrestling injury was reported.[46]

Local pain, tenderness, and fever may be the only signs. Treatment usually includes antibiotic drugs and surgery in the presence of osteomyelitis. Exclusion of associated intracranial infections is mandatory; the epidural infection serves to raise these diagnostic possibilities.

SPINAL EPIDURAL INFECTIONS

Spinal epidural infections may be acute or chronic, and they may be restricted in their extent or extend longitudinally over many segments of the spinal cord because the epidural space offers no resisting structures. Most often, the process affects the posterior region of the spine, with maximal pus or granulomatous tissue found over the dorsal aspect of the cord or spinal roots. In Danner and Hartman's[11] series from the New York Hospital, however, in approximately half of the patients, the pus primarily was anterior to the cord. In the case of spinal osteomyelitis, the pus may be more viscous ventrally. Occasionally, the purulent material may encircle the neural elements. In infants and children, this condition is rare; no case was encountered at the St. Louis Children's Hospital during the 39 months in which 122 cases of bacterial meningitis were seen. In an extensive report by Baker and colleagues[4] covering a 27-year period at the Massachusetts General Hospital, only 6 of 39 patients were younger than 20 years of age, and the youngest was 11 years old. The incidence ranged from 0.2 to 1.2 hospital admissions each year. In approximately half of the patients in this series, acute purulent material was discovered at operation or autopsy, with *S. aureus* incriminated in more than half of the cases. In the remainder, a granulomatous process was found, associated with a wide variety of bacteria. No instance of tuberculous infection occurred, although in many regions of the world, this organism remains an important consideration. Nonbacterial infective agents may include fungi or parasites.

The neural dysfunction probably results from direct compression of the spinal cord, roots, and nerves, but impaired circulation from associated inflammation and occlusion of vessels is at least a contributory factor in some cases. Determining with certainty the extent to which each of the previous mechanisms contributes to neural dysfunction often is difficult. Extensive necrosis of the cord may result in advanced cases in which a prompt diagnosis has not been made.

SOURCES OF INFECTION

In tuberculous and other chronic infections, osteomyelitis and intervertebral disk infection are common occurrences, but adults primarily are affected. Acute spinal epidural infections usually occur after hematogenous spread from furuncles, pharyngitis, dental abscesses, decubitus ulcers, and urinary tract and wound infections.[30] They may complicate spinal surgery, and lumbar puncture rarely has been implicated as a source.[6] Osteomyelitis seldom occurs. The patient may have a history of minor trauma to the back. Presumably, trauma results in local tissue injury or hemorrhage, forming a nidus for the developing infection.

CLINICAL MANIFESTATIONS

Fever is the rule, and the temperature is higher in patients with acute infections. Patients appear septic, and toxic delirium occurs frequently. Heusner,[22] in his classic article dealing primarily with acute epidural abscesses, divided the clinical phases as follows: (1) spinal ache, (2) root pain, (3) weakness, and (4) paralysis. Although certain therapeutic implications render this separation a useful way to consider the disorder, phases 3 and 4 are combined in the following discussion. That the various stages often overlap is axiomatic.

Phase 1: Spinal Ache

Spinal ache was a universal finding in Heusner's experience.[22] In a report from Baker and associates,[4] all 39 patients had backache of various degrees of severity. Local tenderness was absent in only two of their patients and should be searched for by carefully tapping over the spine.

Phase 2: Root Pain

Root pain is characteristic and is an early symptom that may assist in localization of the pathologic process. Root pain especially is prominent with lumbosacral disease in which roots are implicated without involvement of the spinal cord. The diagnosis is suspected infrequently until functional motor and sensory losses occur, which is unfortunate because therapy is most effective during the early stages. Progression to symptoms and signs of spinal cord involvement usually occurs within a few days except when the process is granulomatous, in which case the course tends to be prolonged, extending over several or more weeks.

Phases 3 and 4: Weakness and Paralysis

After weakness and impaired sensation referable to disease of the spinal cord appear, the progression to paralysis can be rapid, and immediate surgical treatment is imperative to maximize the likelihood of reasonable functional recovery. Even appropriate therapy at this stage often is ineffective, however, in restoring

normal neurologic functions. Death occurs in at least 20 percent of cases; this rate has not changed significantly since 1948, despite the availability of a wide range of antibiotic agents.

DIAGNOSIS

The diagnosis of spinal epidural infection is established by MRI of the spine (Fig. 38–9). Imaging also may reveal evidence of concurrent osteomyelitis of the spine, which is found in approximately 20 percent of chronic lesions. After epidural abscess is discovered, immediate neurosurgical intervention is necessary to prevent long-term neurologic sequelae. At the time of surgery, stains and cultures for aerobic and anaerobic bacteria, mycobacteria, and fungi should be obtained. If lumbar puncture is attempted when epidural abscess is suspected, the spinal needle (with stylet) is advanced slowly into the lumbar region, with periodic removal of the stylet and with suction applied gently *before* the thecal sac is entered.

If purulent material is obtained, the diagnosis is established, and the pus must be examined by a Gram-stained smear and cultured on various media under aerobic and anaerobic conditions. The leptomeninges should not be penetrated if purulent material is encountered; otherwise, CSF should be obtained. Characteristically, the CSF is clear or slightly opalescent and yellow if there is a block. Pleocytosis with a few too many hundred cells (with lymphocytes predominating) reflects a contiguous infectious process, but in the absence of meningitis, no organisms can be identified and the CSF glucose concentration should be normal. The protein concentration always is elevated, and the level may be very high (several hundred to 2000 mg/dL) in the case of a partial or total manometric block.

The differential diagnosis includes myelitis caused by bacterial meningitis, syphilis, viruses, and a parainfectious process and by the syndrome of acute transverse myelopathy of unknown cause. Spinal ache is most prominent in acute transverse myelopathy, but as a general rule, the entire illness is compressed in time, with paresis or paralysis evolving over the course of hours or a few days from the onset of disease. Impaired circulation of CSF does not occur in this or the aforementioned disorders. Rarely, a lymphoma may mimic a spinal epidural abscess. Spinal cord tumors, vascular malformations, and arachnoiditis are considerations when the course of disease is prolonged and evidence of sepsis is minimal or absent, as occurs with chronic epidural infections.

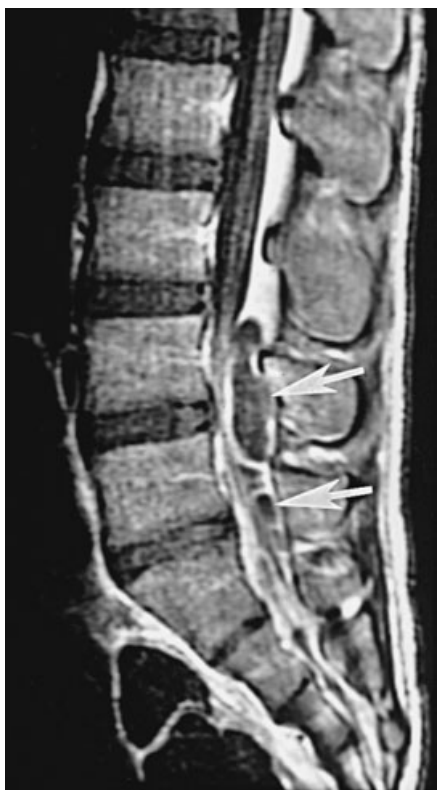

Figure 38–9 Gadolinium-enhanced, sagittal, T1-weighted MR image shows a rim-enhancing lumbar spinal epidural abscess *(arrows)* in a 15-year-old boy with a 3-week history of lower back pain, followed by rapidly progressing left leg numbness and decreased bowel and bladder function. *Staphylococcus aureus* was cultured from purulent fluid removed after L4-L5 laminectomy was performed. *(Courtesy of C. D. Robson, M.D., Harvard Medical School, Boston, MA, and P. D. Barnes, M.D., Stanford University School of Medicine, Stanford, CA.)*

TREATMENT

Prompt surgical removal of purulent or granulomatous material is essential. Surgery should be combined with intravenous administration of an appropriate antibiotic. Initial empiric antibiotic coverage is similar to that for brain abscess, although antifungal therapy need not be included, unless cultures or stains are positive for fungi. Treatment typically should be continued for 3 to 4 weeks, but it should be prolonged for twice this period if the patient has osteomyelitis. Despite advances, the morbidity and mortality rates for spinal epidural abscess remain high. One third of children with the disease die, and another third are left with permanent neurologic sequelae, including weakness, incontinence, and sensory abnormalities. Rapid diagnosis and treatment are essential to ensure a successful outcome.

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CHAPTER 39

Fungal Meningitis

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Fungi are rare causes of meningitis in children. *Candida albicans*, *Cryptococcus neoformans*, and *Coccidioides immitis* are the isolates found most commonly in pediatric patients. Making a diagnosis can be difficult. Fungal meningitis frequently is chronic, and patients may have few symptoms. The lack of obvious meningeal signs and symptoms and the uncommon incidence of a fungal cause often delay establishing the diagnosis. Inherent problems with the diagnosis of fungal infections of the central nervous system (CNS) begin with the basic microbiology of the organisms. Their fastidious growth, the prolonged time needed for culture, and the requirement of special media can render making a diagnosis of meningitis difficult.

Because cultivating many fungi from the cerebrospinal fluid (CSF) frequently is difficult, the use of serologic tests for antibodies and antigens helps define the infection more quickly and with more sensitivity. These tests can be done on CSF, serum, and, in some instances, urine.[177] For most infections, amphotericin B is the drug of choice, although its supremacy is being tested by some of the newer azoles and the introduction of lipid formulations of amphotericin B. Namdar and colleagues[113] compared Abelcet, Amphotec, and AmBisome in neonatal and pediatric patients. CNS disease caused by fungi generally has high morbidity and mortality rates.

EPIDEMIOLOGY

The epidemiology of fungal meningitis depends on many factors. The geographic location of the patient or travel to an endemic area can be an important clue to defining the cause of the infection. The geographic distribution of fungal meningitis varies in the United States and worldwide. Histoplasmosis generally occurs in endemic areas of the Mississippi River valley.[176] Coccidioidomycosis occurs in the San Joaquin Valley and the desert Southwest of the United States and in Mexico.[3,118] Cryptococcosis distribution is worldwide, rather than in discrete endemic areas, but it seems to be associated with pigeon droppings and nesting areas of other birds.[98] Blastomycosis has a sporadic pattern of infectivity but generally occurs in states bordering the Mississippi and Ohio River basins, with occasional outbreaks occurring in the Great Lakes region and Canada.[50,83,143] *Candida* spp., *Aspergillus* spp., *Sporothrix schenckii*, and other fungal pathogens generally are not defined by geographic boundaries but depend more on environmental exposures and the immunocompetence of the individual. Uncommon species of yeasts and fungi, including *Rhodotorula rubra*, *Aureobasidium mansoni*, *Clavispora lusitaniae*, and *Bipolaris spicifera*, have been reported in nosocomial cases of meningitis.[68,89]

Many fungal infections (particularly infections caused by *Candida* spp. and *Histoplasma*) do not usually cause meningitis, unless the host is immunocompromised. The number of cases of cryptococcal meningitis has increased dramatically in association with the acquired immunodeficiency syndrome (AIDS) epidemic. A review from the mid-1980s by the Centers for Disease Control and Prevention showed an incidence rate of 6.8 percent for cryptococcal meningitis in patients with AIDS; however, the rate in pediatric patients with AIDS was 10 times lower (0.6%).[140] In the pre-AIDS era, patients known to be at risk for acquiring

disseminated cryptococcosis included patients with lymphoma, patients on high-dose corticosteroid therapy, and patients with underlying cellular immune dysfunction.[23] Risk factors for developing *Candida* meningitis are similar to those for candidemia and include prolonged antimicrobial therapy, indwelling venous catheters, hyperalimentation, corticosteroid use, recent intra-abdominal surgery, and intravenous drug abuse.[137,157] Pediatric cases occur most commonly in neonates, particularly in very-low-birth-weight newborns.[9,58,70,73,149] In the early 1980s, 3.8 percent of infants weighing less than 1500 g in a large pediatric teaching hospital developed systemic fungal infections, most of which were caused by *Candida* spp. The mean birth weight was 809 g, with 86 percent of the infants weighing less than 1000 g.

Inhalation is the most common means by which many fungi infect the host. Other fungi can be inoculated directly into the skin; this inoculation can occur in the outdoor environment or in the hospital setting (e.g., intravenous catheters), especially in very-low-birth-weight infants.[9]

CLINICAL MANIFESTATIONS

Infections caused by *Candida* spp. in very-low-birth-weight newborns can be particularly difficult to diagnose because of the broad range of symptoms. Most infants with disseminated candidiasis with meningitis present with respiratory distress and a supplemental oxygen requirement, and most progress to require mechanical ventilation.[9] *Candida* usually is identified in endotracheal washings, urine, and blood in patients with *Candida* meningitis, but infants have symptoms on average 11 days before the diagnosis is made. Ophthalmologic examinations may be very important in identifying disseminated *Candida* infections.[28] A careful funduscopic examination also can help determine if increased intracranial pressure is present with papilledema. Marked abdominal distention also occurs commonly in disseminated *Candida* infections in low-birth-weight infants and frequently is associated with guaiac-positive stools. Most patients also have temperature instability, elevated white blood cell counts, and feeding intolerance. Hepatomegaly may indicate the presence of systemic infection.

Physical manifestations that are diagnostic for other causes of fungal meningitis (e.g., *Cryptococcus*, *Blastomyces*, *Histoplasma*) rarely are found because the infections usually are chronic. Looking carefully for other manifestations of fungal infections, especially the presence of skin lesions, is very important, however. All superficial lesions, nodules, and draining abscesses should be investigated because they may give a clue to the cause of subacute and chronic infections (e.g., *Coccidioides*, *Blastomyces*, *Cryptococcus*).[62] Fungal stains, including India ink, should be performed on all biopsy specimens and drainage material, and all specimens should be processed for culture. Bone involvement is a common occurrence with certain fungal infections (e.g., *Cryptococcus*, *Blastomyces*).

The significance of isolating a fungus from CSF cannot be overemphasized. The finding of fungal organisms should be considered a true infection, and appropriate antifungal therapy should be initiated. A single CSF culture for *Candida* or an unlikely meningeal pathogen (*Paecilomyces*) with an otherwise normal CSF should lead the physician to consider the possibility of contamination.[5] Repeat CSF cultures should be sought in such patients.

INFECTION WITH SPECIFIC ORGANISMS

Candidal Meningitis

Candidal meningitis is rare in children. Arisoy and associates[5] reported that 2 percent of all positive CSF cultures were fungal organisms. *Candida* spp. accounted for 94.5 percent of the fungal isolates. Nine of 23 patients were newborns, 8 of whom were very-low-birth-weight infants. Risk factors for positive CSF fungal cultures from neonates included antimicrobial therapy, umbilical catheterization, total parenteral nutrition, intubation, and prematurity.[43,44] Risk factors in children beyond the neonatal period included concurrent bacterial infection, chronic systemic or CNS disease, and the presence of central venous catheters. Histopathology models in animals have revealed hyphal invasion, vasculitis, abscesses, and acute and chronic inflammatory infiltration of meninges and brain parenchyma.[72]

Children with human immunodeficiency virus (HIV) infection are at risk for acquiring disseminated *Candida* infections, including meningitis. In one study, 27 percent of HIV-infected patients with disseminated *Candida* infections had CNS involvement.[95] Nearly all HIV-infected patients who develop *Candida* infection do so as a result of nosocomial infection. Predisposing factors include oral candidiasis, central venous catheters, prolonged antibiotic therapy, and total parenteral nutrition. In HIV-infected patients, neutropenia is not a major risk factor. Simultaneous pulmonary disease, particularly viral, bacterial, or *Pneumocystis carinii* pneumonias, exists in most HIV-infected patients with disseminated *Candida* infection. Most patients are febrile for more than 14 days, with peak temperatures greater than 39º C before the diagnosis is established.

The incidence of infection with *Candida* spp. other than *C. albicans* has increased dramatically in immunocompromised children, particularly children with malignancies. In a case series of children with leukemia, *Candida tropicalis* meningitis was uniformly fatal.[47] In addition to patients with malignancies and HIV infections, other children at risk for developing infection have been reported, including a child with myeloperoxidase deficiency.[97]

Candida spp. have become another concern because of the development of resistance. Intensive efforts to develop standardized, reproducible, and clinically relevant susceptibility testing methods for the fungi have resulted in the development of the NCCLS M27-A methodology for susceptibility testing of yeasts for the azole agents.[131] Reliable breakpoints are unavailable for amphotericin B. Isolates of *Candida glabrata* and *Candida krusei* with increasing resistance to amphotericin B have been described.[175] *Candida lusitaniae* has shown resistance to amphotericin B, and meningitis in an adult patient with a resistant strain has been reported.[138] *C. krusei* has been noted to have azole resistance, particularly in immunocompromised patients receiving suppressive azole therapy. These fungal infections already are difficult to treat, without the added burden of drug resistance. If patients with *Candida* spp. (including *C. albicans*) meningitis are slow to clear infection, or unexpected relapse occurs, susceptibility testing is recommended.

Candida meningitis usually responds to therapy with intravenous amphotericin B alone or in combination with oral flucytosine. The use of flucytosine is controversial; however, most reports support its use in meningitis. Flucytosine has been difficult to use in low-birth-weight infants because of the immaturity of their gastrointestinal tracts and the risks of their developing necrotizing enterocolitis. Amphotericin alone for systemic candidiasis, including meningitis, in neonates has been used successfully in patients who could not tolerate oral medication.[22] The *most* important factor in successful treatment was the initiation of therapy quickly, as soon as systemic *Candida* infection was suspected and frequently before the organism was isolated. Prolonged delays in initiating therapy result in high mortality rates.

Amphotericin B initiated at a dose of 0.25 mg/kg body weight diluted in 5 percent or 10 percent dextrose-water can be infused over 2 to 4 hours. In most neonates, the dose can be increased in 0.25-mg/kg increments at 6- to 12-hour intervals until the

desired 1-mg/kg daily dose is attained. In older children and adolescents, a test dose may be given, with an initial dose of 0.1 mg/kg (1 mg maximum). If this dose is tolerated, the initial dose of therapy (0.25 mg/kg) is given. Many authorities now believe that a test dose is unnecessary and that cautious infusion of the first dose is adequate.[39] In severely ill patients, the dose can be increased rapidly in 6- to 12-hour intervals to 1 mg/kg/dose. Flucytosine generally is recommended in a dose of 150 mg/kg/day, divided every 6 hours. The use of flucytosine often requires monitoring and adjustment of the dosage based on serum determinations.

Large, controlled trials using the azoles and newer agents for *Candida* meningitis are lacking. Huttova and coworkers[67] reported eight neonates with candidal meningitis treated with intravenous fluconazole with and without amphotericin B. Numbers from the study were too small to make conclusive recommendations. Lipid formulations of amphotericin B increasingly are preferred in adults, and liposomal amphotericin B was used successfully in five of six cases of *Candida* meningitis in newborns.[139] Optimal dosing is unknown, but many physicians prescribe liposomal amphotericin B or amphotericin B lipid complex at a dose of 5 mg/kg daily. Marr and associates[104] reported on the use of fluconazole plus flucytosine for the treatment of *Candida* meningitis in a very-low-birth-weight infant. Voriconazole has excellent penetration of the CNS and is active against most *Candida* isolates causing CNS infections; however, the clinical experience with voriconazole is too limited to recommend its use at this time.[74] Caspofungin and the other echinocandins do not achieve adequate CSF concentrations for treatment of *Candida* meningitis.[35,127]

Cryptococcosis

Cryptococcal meningitis was rare in the United States in the pre-HIV era. With the growth of the AIDS epidemic, however, this infection has become a common cause of meningitis in adults in certain areas of the United States and infects 2 to 9 percent of adults with AIDS.[29,38,85,183] It remains an uncommon finding in pediatric patients.[114,132] Cryptococcosis is a systemic fungal infection, and meningitis is its most serious manifestation. Patients with initial pulmonary involvement may have few symptoms but can present with fever, cough, weight loss, and dyspnea on exertion.[105] In adult studies, progressively severe headaches without the presence of fever were common manifestations.[159] Patients with cryptococcal meningitis frequently have few symptoms but can present with nausea, dizziness, and irritability. Nuchal rigidity usually is absent. Careful examination for cranial nerve palsies, which are found in approximately one fifth of adult patients, should be performed. Diplopia, in particular, is one of the most common manifestations of cryptococcal meningitis. Papilledema is seen in nearly one third of patients with cryptococcal meningitis.[37] Patients with coexistent AIDS frequently have very few symptoms.[38]

Pediatric patients with cryptococcal meningitis usually present with signs and symptoms not referable to the CNS.[148] Leggiadro and associates[94] reported on 13 children with AIDS, compiled from 11 institutions, who were diagnosed with extrapulmonary cryptococcosis. Meningitis was diagnosed in 62 percent of these patients and was the most common clinical manifestation of extrapulmonary disease. Patients who initially responded to antifungal therapy did not die of the fungal infection but died of other illnesses related to their immunodeficiency. Death can occur, however, even in an immunocompetent child receiving effective therapy.[135]

Leggiadro and associates[93] reported on eight children with acute lymphoblastic leukemia who developed extrapulmonary cryptococcosis. Of these immunocompromised children, 63 percent had meningitis. Fever was the most common symptom, occurring in 60 percent of the children with meningitis. Headache was present in only 40 percent. A significant number of the children (40%) were completely asymptomatic and had lumbar punctures performed as routine management of acute lymphoblastic leukemia, with the subsequent unexpected growth of *C. neoformans* on culture. One child had cutaneous lesions, and another had ptosis and an unsteady gait. Treatment in this series of patients included amphotericin B (intravenous, intrathecal, or both), alone or combined with oral flucytosine. In these patients with acute lymphoblastic leukemia, relapse was a major complication, occurring in 60 percent of patients thought to have been treated successfully. The relapses occurred within 2 to 6 months of completing therapy. Treatment of the relapses generally included combination therapy of amphotericin B and flucytosine, with the occasional use of intrathecal amphotericin B.

Other illnesses that may lead to cryptococcal meningitis include systemic lupus erythematosus being treated with corticosteroids alone or in combination with azathioprine,[2,93,97,129] chronic mucocutaneous candidiasis,[69,170] and hyper-IgE syndrome.[154] Zoonotic transmission from a pet cockatoo was described in an immunocompromised adult.[115]

Direct examination of the CSF using the India ink test can provide an immediate presumptive diagnosis of cryptococcal meningitis. The sensitivity of this test varies, but in studies of adult patients with AIDS, positive results with the stain approached 75 percent.[29] Other useful stains include silver, periodic acid–Schiff, and mucicarmine. Gram stain of CSF is insensitive and unreliable.

Diagnosis of cryptococcal meningitis is aided by the use of serologic tests. The most common test is the cryptococcal capsular polysaccharide antigen test, which can be performed on serum, CSF, or other sterile body fluids. The sensitivity of this test is nearly 100 percent for the serum of patients who are HIV-positive.[30,85,183] The CSF antigen test in some studies seems to be less sensitive, with a 91 percent sensitivity.[29] False-positive results caused by cross-reactions of antigens in disseminated infections with *Trichosporon beigelii* have been reported.[107] Culture still is the gold standard for establishing the diagnosis and monitoring the success of therapy.

Treatment of cryptococcal meningitis is prolonged and, in immunocompromised patients, frequently requires lifelong maintenance therapy. In adults with HIV infection, multiple regimens have been suggested. Practice guidelines have been published for patients with cryptococcal meningitis who are HIV-negative or HIV-positive.[136] For HIV-negative patients, amphotericin B (0.7 to 1 mg/kg/day) plus flucytosine (100 mg/kg/day in four divided doses) for 2 weeks and then fluconazole (400 mg) for a minimum of 10 weeks, or, alternatively, amphotericin B (0.7 to 1 mg/kg day) plus flucytosine (100 mg/kg/day) for 6 to 10 weeks, is recommended. A repeat lumbar puncture is recommended at 2 weeks. If cultures still are positive, prolonged induction therapy should be continued, and fluconazole when started for therapy should be continued for 6 to 12 months. The investigators who proposed the guidelines do not recommend initiating therapy with fluconazole alone. The use of AmBisome (4 mg/kg) for 6 to 10 weeks is a possible alternative if the patient cannot tolerate amphotericin B or azole therapy.

For HIV-infected patients, the practice guideline study group recommends induction therapy of amphotericin B (0.7 to 1 mg/kg/day) plus flucytosine (100 mg/kg/day in four divided doses) for 2 weeks and then fluconazole (400 mg/day) for a minimum of 10 weeks.[136] When flucytosine cannot be administered, amphotericin B is an acceptable alternative; however, a trial from Thailand evaluated sterilization of the CSF in 64 patients and revealed that the combination of amphotericin B plus flucytosine was the most rapidly fungicidal regimen.[20] The use of lipid formulations of amphotericin B may be helpful in patients who cannot tolerate amphotericin B because of renal toxicity.[33] AmBisome has been effective at doses of 4 mg/kg/day.[92]

In patients with renal compromise associated with amphotericin B, flucytosine levels must be monitored carefully. The recommendation is that the dose of flucytosine be reduced to 75 to 100 mg/kg/day and continuing doses be adjusted to maintain serum flucytosine levels of 25 to 60 μg/mL.[6,48] Serum cryptococcal antigens are not useful in monitoring response to therapy, and the use of CSF cryptococcal antigens to monitor response to therapy also is controversial. Therapy is best judged to be successful by the demonstration of sterility of CSF fungal cultures.

In pediatric patients, a combination of amphotericin B and flucytosine has been used most frequently. In children with AIDS, amphotericin B in a dose of 0.5 to 1 mg/kg/day with or without flucytosine (150 mg/kg/day in four divided doses) is recommended for 4 to 8 weeks, followed by maintenance therapy with fluconazole (3 to 6 mg/kg/day) indefinitely.[174] HIV and AIDS guidelines change frequently, and the physician should consult the most recent guidelines for changes, particularly with regard to need for maintenance therapy.

Histoplasmosis

Infection with *Histoplasma capsulatum*, usually a benign and self-limited disease, is endemic in many parts of the United States. Disseminated disease, including meningitis, is a rare occurrence in children.[80] Case reports of adults generally describe immunocompromised individuals.[54,164] Clinical presentations reported in the literature show a wide variability in the manifestation of meningitis. In these cases, 39 percent of patients presented with meningitis associated with acute dissemination, 25 percent presented with single histoplasmoma that manifested as symptomatic mass lesions alone or with dissemination, 25 percent presented with chronic meningitis without evidence of dissemination, and the remaining patients presented with meningitis as a manifestation of recurrent disease. Rarely, embolization to the brain caused by *Histoplasma* endocarditis has been associated with meningitis.[176]

Meningitis occurring in patients with AIDS has become common in endemic areas. In one report, disseminated histoplasmosis caused 8 percent of the AIDS-defining illnesses in children.[141] The duration of symptoms varies. In patients who do not have AIDS, the symptoms generally last longer than 6 months and can last 7 years.[41,54,122,171] In patients with AIDS, symptoms usually manifest more acutely and in a much shorter timeframe.[176] In this series, neurologic findings occurred in all but 6 percent of the patients.[176] The most common signs and symptoms include depressed consciousness (29%), headaches (24%), confusion (22%), cranial nerve deficits (19%), other focal deficits (16%), seizures (14%), personality changes (12%), and ataxia (11%). Findings more commonly observed with acute bacterial meningitis, such as meningismus, a Babinski sign, or papilledema, were seen in fewer than 8 percent of the cases. In adult patients who do not have AIDS, the death rate is approximately 12 percent, with a relapse rate of 44 percent. In adult patients with AIDS, the death rate is 100 percent in some series.[4,176]

Diagnosis can be aided by serologic testing. High levels of anti-*H. capsulatum* antibodies were detected in the serum of 70 percent of patients tested. CSF serology was helpful in 75 percent of patients who were tested. Culture of the CSF was positive for fewer than half of the cases in one review.[80] In a second series in patients with AIDS, blood cultures were positive for 49 percent, bone marrow cultures were positive for 53 percent, respiratory secretions cultures were positive for 58 percent, and brain or meninges cultures were positive for 75 percent of those tested. Serologic testing can be negative because 10 to 25 percent of patients with disseminated disease lack a positive antibody response. The serology also can be false-positive in patients with other fungal diseases or tuberculosis. The antibody response to acute *Histoplasma* infection may remain elevated for years, without evidence of dissemination or meningeal disease. The use of *Histoplasma* antigen has become widely held as a useful test in immunocompromised patients with disseminated *Histoplasma*. Data from Wheat and associates[176] showed that antigen was found in the urine of six of seven patients with AIDS and meningitis.

Treatment with liposomal amphotericin B (3 to 5 mg/kg/day for a total dose of 100 to 150 mg/kg for 6 to 12 weeks) is considered standard therapy in adults.[178] The liposomal form provides higher CNS levels than does the deoxycholate form. For the deoxycholate form, a total dose of at least 30 mg/kg is considered by many physicians to be necessary to ensure a cure. The use of intrathecal amphotericin B is not recommended for *H. capsulatum* meningitis. A practice guideline for adults written before the previous article recommends that liposomal amphotericin B (3 to 5 mg/kg/day or every other day for 3 to 4 months) be considered for patients who have failed therapy with amphotericin B followed by fluconazole.[179] Johnson and colleagues[75] reported on a trial comparing amphotericin B with liposomal amphotericin B (AmBisome, LAmB) as induction therapy and showed the agents had similar efficacy for treating disseminated histoplasmosis. LeMonte and associates[96] found that amphotericin B combined with fluconazole might have antagonistic effects for treatment of histoplasmosis, but data are needed to determine the efficacy of combination therapy for meningitis.

Patients with AIDS for whom induction therapy was successful must remain on an anti-*Histoplasma* agent indefinitely. A few cases of children with disseminated histoplasmosis and AIDS have been reported, but none with meningitis, and in these children, antifungal therapy must be continued indefinitely.[25,141] The case report by Schutze and associates[141] showed that ketoconazole was ineffective for preventing recurrence of nonmeningeal disseminated disease, and because ketoconazole has poor CNS penetration, it is likely to be ineffective for prophylaxis of meningitis. In patients with AIDS, some success has been achieved with the use of itraconazole for suppressive therapy in disseminated disease. Few data exist, however, on the use of itraconazole for suppressive therapy for meningitis or other CNS lesions caused by *Histoplasma*. For maintenance therapy, fluconazole (high-dose), itraconazole, or intravenous amphotericin B in a weekly dose of 1 mg/kg are available. In one case report, fluconazole was effective in an adult with *Histoplasma* meningitis refractory to amphotericin B therapy.[158]

Coccidioidomycosis

C. immitis (*C. posadasii* in Texas and Central and South America) meningitis is a more common cause of chronic meningitis than is *Histoplasma*.[76] Approximately 1 percent of children with symptomatic pulmonary disease develop disseminated disease.[77] Of patients with disseminated coccidioidomycosis, 15 to 20 percent develop meningitis.[77,82] History of exposure is a crucial factor in diagnosing this disease and relies on careful questioning of the patient about travel to or residence in an endemic area.[155,156] Exposure of wounds to colonized soil has been implicated in at least one pediatric case.[112] An association between facial cutaneous coccidioidomycosis and meningitis has been described.[7]

CSF shows a mononuclear pleocytosis with an elevated protein and decreased glucose concentration. Diagnosing coccidioidal meningitis is made easier by the availability of reliable serologic tests. Smith and associates[150] showed that complement-fixing antibodies appeared in the CSF only in patients with meningitis and provided a sensitivity of 76 percent. Ninety-six percent sensitivity was seen when the complement-fixation test was incubated at 4º C.[119] McGinnis,[106] in his review of the literature based on pooling five studies, showed that *Coccidioides* could be cultured from the CSF in 76 percent of patients with meningitis and was seen on direct examination of the CSF in only 8 percent of the

cases. He concluded that these values were too high and were likely skewed because of reporting bias. Other experts in the field also consider that the rate of positive CSF cultures is much lower (approximately 33%), and that finding the organism in the CSF by direct examination rarely occurs.[153]

Coccidioidomycosis in infancy was described more than 40 years ago and continues to be a problem today.[160] Infants usually have severe disease with high mortality rates and morbidity. In the 1980s, two studies described children with *Coccidioides* spp. meningitis who were treated with oral, intravenous, or intrathecal imidazoles.[64,145] The authors reported promising results with the use of ketoconazole and imidazole therapy compared with standard intravenous or intrathecal amphotericin B.

If untreated, *Coccidioides* meningitis is uniformly fatal. Therapy with intravenous and intrathecal amphotericin B reduced the mortality rate to 30 percent.[119] The use of fluconazole has been found in adult studies to have a success rate of 79 percent.[52] It seems to be safe in clinical use, despite a case report of a pregnant woman with *Coccidioides* meningitis treated with fluconazole who delivered an infant with congenital malformations. The infant was thought to have an autosomal recessive disorder (Antley-Bixler syndrome), however, and not teratogenic malformations caused by fluconazole.[91] Fluconazole may be the superior agent for sustaining remission of meningitis caused by *Coccidioides* because of its oral bioavailability, the toxicity associated with amphotericin B, and the elimination of the need for intrathecal administration.[125]

Galgiani and coworkers[51] have written practice guidelines for the treatment of coccidioidomycosis in adults. Therapy with oral fluconazole is preferred, with most authorities recommending 400 mg/day orally, although some physicians begin with higher doses of 800 to 1000 mg/day. Fluconazole in a dose of 400 to 600 mg/day for 9 to 12 months has been shown to be effective in adults.[52,162] A corresponding dose of 6 mg/kg/day in pediatric patients also has been used, but no controlled studies using this agent in pediatric patients have been published. If treatment is begun with azole therapy, it should be continued for life.[36]

Itraconazole has proven efficacy in nonmeningeal coccidioidomycosis, with 63 percent of adult patients treated showing a complete response.[163] Doses of itraconazole of 400 to 600 mg/day have been reported to be effective in adults. Because itraconazole has variable oral bioavailability, many authorities recommend monitoring for adequate serum drug levels.[169] The use of itraconazole for meningeal involvement led to great hope for the use of an oral agent in this disease.[161] Adult patients with *Coccidioides* meningitis had high relapse rates (40-50%), which rendered them dependent on lifelong therapy with itraconazole.[60] The alternative of continued intrathecal amphotericin B renders this treatment much more appealing.

Well-developed, controlled clinical trials with large numbers of children do not exist for the azoles; some authorities consider intravenous and intrathecal amphotericin B to be the standard therapy for coccidioidal meningitis. It also is recommended for patients who do not respond to fluconazole or itraconazole treatment. Some experts initially use a combination of oral azole with intrathecal amphotericin B with the thought that responses are more prompt with this approach. Amphotericin B can be administered intrathecally into the lumbar area or into the cisterna magna, or by using an Ommaya reservoir.[34,53,87,182] In younger children, intraventricular or intracisternal therapy using the Ommaya reservoir allows ease of administration but has the disadvantage of increasing susceptibility to secondary bacterial infection.

The initial dose of amphotericin B administered into the CSF is 0.025 mg. The dose is increased by doubling until a maintenance dose of 0.1 to 0.5 mg is attained. After this dose has been achieved, therapy can be given every other day, alternating with the intravenous administration of amphotericin B. Therapy is continued until the child's condition has stabilized, at which point intrathecal therapy gradually can be stretched out to every 3 weeks. This program is continued until the CSF indices are normal and culture results have been negative for at least 1 year.

Use of voriconazole has been successful in two case reports, and other similar anecdotal references have been reported at referral centers; some centers use voriconazole, 4 mg/kg every 12 hours orally, for salvage therapy.[32,51,128] Miconazole, an imidazole compound that no longer is commercially available, was used in the treatment of *Coccidioides* meningitis and, in combination with oral ketoconazole, had good results in nine children.[145]

Blastomycosis

Blastomyces dermatitidis is an uncommon cause of chronic meningitis and is difficult to diagnose premortem unless the patient has other signs of systemic blastomycosis. When systemic blastomycosis occurs, it can involve the CNS in 5 percent of cases.[18] Common sites for this organism include bone, genitourinary tract, and skin.[10,19] Although examination of CSF obtained by lumbar puncture usually is negative (9% sensitivity), ventricular fluid (four of four patients tested) seems to have a higher yield.[86] Typically, fungal meningitis is associated with a lymphocytic pleocytosis; however, meningitis caused by *B. dermatitidis* frequently has a neutrophilic predominance.[63] Previously, blastomycosis most often affected patients who were immunocompetent. Patients with AIDS are at a high risk for developing chronic infection.[63] Because of the difficulty in diagnosing meningitis caused by *B. dermatitidis* and its similarities to tuberculous meningitis, patients usually are treated for presumptive tuberculous meningitis.[57,111] Although meningitis is the most common form of CNS blastomycosis, solitary mass lesions also can occur.[133]

Diagnosis relies on the characteristic histopathologic appearance in tissues and occasionally on culture of CSF obtained from the ventricles. If the difficulty in performing these procedures is prohibitive, looking for other sources of blastomycosis, including sputum and urine, is indicated. A study in patients with AIDS showed that examination of sputum was useful for establishing the diagnosis of disseminated blastomycosis.[120]

Treatment of systemic blastomycosis, including meningitis, relies on amphotericin B in a dose of 1 mg/kg/day.[152] The duration of therapy is unknown, but adults with systemic disease usually require a minimum of 2 g to prevent relapse of nonmeningeal disease.[121] In adult patients who have been "cured" of their meningitis, doses of 2400 to 3150 mg were required, which would correspond to a total dose of 35 to 45 mg/kg in a child. The use of lipid formulations of amphotericin B has not been reported for CNS blastomycosis, but this treatment according to current practice guidelines of the Mycoses Study Group may be an alternative for patients unable to tolerate amphotericin B because of toxicity.[27] Azoles should not be considered for primary treatment of CNS blastomycosis. Schutze and colleagues[140] reported that children respond less satisfactorily to oral azole therapy than do adults.

Aspergillosis

CNS infections with *Aspergillus fumigatus* and other *Aspergillus* spp. in immunocompromised patients usually are fatal. Conditions that place the patient at risk include organ transplantation, malignancies, and neutropenia. In most reported cases, patients were receiving high-dose corticosteroid therapy in addition to broad-spectrum antibiotics.[11,88] Many patients had fever, and pulmonary findings preceded neurologic manifestations. *Aspergillus* infection of the CNS usually is acquired by hematogenous spread from the lungs. Other modes of acquisition include extension from a contiguous focus (sinuses) and intravenous drug abuse.[110]

Brain abscesses are the most typical manifestation of *Aspergillus* spp. infection in the CNS, but meningoencephalitis, isolated spinal cord lesions, aqueductal stenosis, and mycotic aneurysms also have been described.[26,45,168,172,181] Magnetic resonance imaging has been suggested to be superior to computed tomography for the delineation of CNS lesions in patients with bone marrow transplantation.[108] The lesions are consistent with acute infarcts.

Some early reports of CNS aspergillosis were in infants who appeared to be normal.[1,101] The diagnosis of meningitis relies on CSF cultures, but the results of a bronchoscopy or biopsy frequently are more helpful and provide a more expedient diagnosis. Use of *Aspergillus* antigen in the CSF has been helpful, and it may be used for serial observations during the course of therapy.[173] Because the disease is uniformly fatal, the importance of treating pulmonary aspergillosis before the development of meningeal involvement cannot be overemphasized. Amphotericin B in doses of 1 to 1.5 mg/kg/day is recommended, with or without the addition of flucytosine, rifampin, or both agents.[58,167] Itraconazole has been used successfully in one case report, as has voriconazole.[109,173]

Sporotrichosis

Sporothrix schenckii, although primarily a lymphocutaneous disease, has been reported to cause meningitis.[42,49,84,123,147] Many of the cases described more recently have occurred in adults with AIDS as an underlying risk factor.[40,134] Meningeal seeding may occur through hematogenous spread from the lungs, as is seen in most other fungal infections.[42] Meningeal involvement with sporotrichosis produces CSF indices and abnormalities similar to those seen with the other fungal meningitides.[41] CSF fungal culture is insensitive for the diagnosis of meningitis caused by sporotrichosis. The use of *S. schenckii* antibody in the CSF has been effective in diagnosing meningitis in patients without other overt signs of this infection.[142]

Treating this infection is very difficult. Amphotericin B alone has been successful occasionally.[81] Some experts recommend the addition of flucytosine, but no studies have evaluated the efficacy of this combination. The azoles, particularly itraconazole, had excellent results in nonmeningeal disease, but successful treatment of meningitis with this agent has not been proved.

Mucormycosis

Meningitis caused by *Mucor* spp. or other Zygomycetes usually occurs as a result of direct extension from paranasal sinus disease. Infection with these organisms most commonly is seen in the immunocompromised host and particularly in patients with diabetes mellitus or patients receiving high doses of corticosteroids. Patients (particularly dialysis patients) undergoing chelation therapy with deferoxamine are at risk for developing infection.[16,17,79,180] The association with deferoxamine therapy is seen with *Cunninghamella*.[130] *Mucor* spp. have been reported as a cause of CNS disease in children but very rare rarely.[61,71] Treatment employs amphotericin B in doses of 1 to 1.5 mg/kg/day, but it usually is unsuccessful. Surgical excision of rhinocerebral infection is recommended along with antifungal therapy.

Other Fungal Infections

Acremonium spp. are common soil fungi that may cause chronic meningitis in humans.[117] *Xylohypha bantiana*, an uncommon dematiaceous fungus, caused a fungal brain abscess in an adolescent girl; this report increases the number of cases reported in the literature to nearly 40.[116] Cerebral chromoblastomycosis also has been reported.[146] Other fungal organisms causing CNS infection include *Paracoccidioides brasiliensis* (i.e., South American blastomycosis),[124] *Prototheca wickerhamii*,[78] *Blastoschizomyces capitatus*,[55] *Rhodotorula* spp.,[103,126] *Pseudallescheria boydii*,[13] *Aureobasidium mansoni*,[68] *Clavispora lusitaniae*,[68] and *Bipolaris spicifera*.[89]

DIAGNOSIS

Specific information about the diagnosis of individual organisms is provided in the previous sections. Table 39–1 provides specific data for some fungal meningitides. Overall, the problem with diagnosing many of these infections is that most patients have nonspecific signs and symptoms without reference to the CNS. Standard culture media may not be useful for these organisms, and it can take weeks before an organism is identified on culture. The use of antigen and antibody testing has proved quite useful for the diagnosis of *Cryptococcus*, *Coccidioides*, and *Histoplasma* infections. The hope is that polymerase chain reaction technology may be helpful in the future, as it has been for bacterial and mycobacterial diagnoses. Neonates may have only a mild CSF pleocytosis. In one series of 16 neonates with definite *Candida* meningitis, the median CSF white blood cell count was 53 cells/mm^3 (range, 0 to 1120 cells/mm^3).[46]

CSF examination may be helpful in some instances. As much CSF as can be removed safely should be obtained, especially at the time of ventriculography or pneumoencephalography.[41] A minimum of 5 mL of spinal fluid has been suggested, based on

TABLE 39–1 Fungal Cerebrospinal Fluid Characteristics

Organism	WBCs	Protein	Glucose	Smears	Serology	Cultures
Blastomyces	Variable up to 15,000 cells/mm^3 with PMNs or lymphocytes	Elevated up to 300 mg/dL	Normal or low	Rare on smear	No good serology	CSF cultures rarely positive; increased yield with ventricular taps
Candida	Mean 600 cells/mm^3 up to 1900 cells/mm^3 with lymphocytes or PMNs	Elevated	Low or normal	40% positive on smears	Serology not helpful	CSF cultures useful
Coccidioides	100-750 WBCs, mostly lymphocytes	150-2000 mg/dL	21-62% serum	Rare on smear	CSF CF antibody positive in 75-95%	CSF cultures positive in 33-60%
Cryptococcus	40-400 WBCs, mostly lymphocytes	High	Low	India ink positive in 25-50%	CSF and serum cryptococcal antigen positive in 85-90%	CSF cultures positive in 75%
Histoplasma	0-300 WBCs, lymphocytes, or PMNs; most 11-101/mm^3	Usually elevated, but can be normal	Usually low (<40 mg/dL) to normal	Rare on smear	Polysaccharide antigen in urine, blood, CSF positive in 61%	CSF cultures positive in 27-65%

CF, complement fixation; CSF, cerebrospinal fluid; PMNs, polymorphonuclear leukocytes; WBCs, white blood cells.
Data from references 57, 64, 106, 175.

experimental work.[99] Repeated cultures of large volumes of CSF may be helpful.[41] The fluid obtained should be centrifuged and the sediment saved for culture and India ink preparation. The supernatant is sent for serologic tests. The India ink test should be interpreted with caution and must be followed with cultures because artifacts frequently can cause misinterpretation.[41] The cumulative efficacy of repeated lumbar punctures for cryptococcal meningitis improved the sensitivity of the India ink smear from 26 percent in one lumbar puncture to 52.6 percent with the second.[106] If large volumes are available, membrane filtration may be used to concentrate the fungal elements. The membrane containing the fungi is placed aseptically on isolation media and incubated at 30° C for 4 weeks. The CSF that passes through the membrane can be used for serology or chemistry determinations.[106] The remaining CSF can be inoculated onto Sabouraud glucose agar, blood agar, and brain-heart infusion agar or into broth media or into both types of media. CSF cultures generally are unhelpful for *Histoplasma*, *Blastomyces*, and other dimorphic fungi.

Serologic techniques are extremely useful for *Cryptococcus* and *Coccidioides*. Approximately 100 percent sensitivity is seen with serum cryptococcal antigen tests in HIV-positive patients.[30,85,183] The CSF antigen test in HIV-positive patients seems to be less sensitive (91%) in some studies.[29] In patients not infected with HIV, the sensitivity of the serologic test in the CSF approaches 90 percent.[65] For coccidioidal meningitis, the sensitivity rate is greater than 76 percent.[119,150]

Candida can be cultured but may require a prolonged incubation period, necessitating institution of empiric therapy while awaiting results of cultures. For other organisms, such as *Histoplasma*, *Blastomyces*, and *Coccidioides*, culturing other body fluids, such as blood, urine, sputum, or draining wounds, can be helpful.

ANTIFUNGAL AGENTS AND TREATMENT GUIDELINES

Amphotericin B remains the treatment of choice for most CNS fungal infections in children. Amphotericin B has less than 5 percent oral bioavailability and is more than 90 percent protein-bound.[14] It has a prolonged terminal elimination half-life of 15 days, with only approximately 3 percent of the parent compound found unchanged in the urine.[8] CSF concentrations are approximately 2 to 4 percent of those found in serum.[53,166] The recommended dose varies depending on the organism and the severity of the illness. In most children and infants, a dose of 1 mg/kg/day seems to be satisfactory, particularly in view of the increased clearance of the drug compared with clearance in adults.[12,151]

Toxicity and adverse effects are universal with the use of amphotericin B. Acetaminophen and diphenhydramine may be helpful in reducing the incidence of fever, nausea, and chills. The effective use of hydrocortisone to reduce febrile reactions has been documented.[165] Great variability in patient responses to this treatment occurs, however. The use of corticosteroids can potentiate water retention and amphotericin B–induced hypokalemia; the dosage of corticosteroids should be kept to a minimum.[53] If chills occur, they can be stopped with the use of meperidine.[21]

Amphotericin B induces reversible impairment of renal function in 80 percent of patients during the first 2 weeks of therapy.[24] In most cases, renal function returns to normal after cessation of administration of the drug. The manifestations of toxicity include renal tubular acidosis, azotemia, oliguria, and potassium-magnesium wasting. Other nephrotoxic drugs worsen the azotemia. Renal failure in adult studies has been shown to be ameliorated by the use of salt loading.[66] The adult studies used 500 mL of a 0.9 percent sodium chloride solution as prehydration and posthydration therapy; in children, 10 mL/kg seems to work as well. Hypokalemia along with hypomagnesemia occurs frequently and may require supplementation. A normocytic, normochromic anemia occurs in many patients who receive amphotericin B, with an 18 to 35 percent decrease in hemoglobin seen after 10 weeks of therapy; this anemia seems to be related partly to changes in erythropoietin levels.[102]

One approach to minimize amphotericin B toxicity while maintaining the drug's pharmacologic spectrum and clinical utility is the use of liposomal and lipid-complexed formulations. The three formulations approved for clinical use include amphotericin B lipid complex (Abelcet; Liposome Company, Princeton, NJ), amphotericin B colloidal dispersion (Amphotec, Amphocil; Sequus Pharmaceuticals, Menlo Park, CA), and liposomal amphotericin B (AmBisome; NeXstar Pharmaceuticals, Boulder, CO; Fujisawa USA, Deerfield, IL). Although each of these lipid delivery systems is unique and has different pharmacokinetic and pharmacodynamic profiles, no clinical comparisons of lipid formulations have been done in children. Their use is discussed briefly in the case reports that exist for the individual infections listed previously. Most of the published pediatric literature describes cases in which AmBisome has been used.

Ketoconazole has not been an effective agent for treating most fungal CNS disease processes. It has been supplanted by the newer azoles, fluconazole and itraconazole, because of their better bioavailability and more favorable results in clinical trials.

Fluconazole is a very effective agent for the suppression of cryptococcal meningitis in patients with AIDS and immunocompetent patients and seems to be superior to amphotericin B for prolonged maintenance therapy in patients with AIDS. Fluconazole absorption by the gastrointestinal tract is not affected by the presence of food or gastric acidity.[15] Fluconazole is highly water soluble and minimally bound to plasma proteins.[90] The terminal elimination half-life of fluconazole is 22 to 31 hours, with 80 percent of the drug excreted unchanged in the urine.[31] Fluconazole readily penetrates into CSF in inflamed or noninflamed meninges, achieving levels that are 60 to 80 percent of serum levels. Rifampin, cyclosporine, and phenytoin have significant interactions with fluconazole. Adverse effects are uncommon, especially compared with the effects associated with amphotericin B. Less than 5 percent of patients have nausea and vomiting. Asymptomatic elevations of plasma aminotransferases occur in less than 1 to 7 percent of patients.[31]

Data for itraconazole use in pediatrics are limited, and itraconazole is not recommended at this time for CNS fungal infections in children. Itraconazole has increased absorption when taken with food.[168] Itraconazole is highly protein-bound (>99 percent); less than 1 percent is excreted unchanged in urine.[59] CSF concentrations are low compared with those of fluconazole; however, itraconazole is much more lipophilic, allowing it to show efficacy in treatment of some fungal meningitides. Additional data on it and the other azoles, particularly for treatment of coccidioidal meningitis in children, should be forthcoming in the next several years,. Promising results from an adult study have been published.[161]

CONCLUSION

With the large increase in the number of immunocompromised children caused by the epidemic of HIV, the advent of new antineoplastic agents, the growth of organ transplantation, and the increased use of corticosteroids, the relative rarity of fungal meningitides has been replaced by a burgeoning upswing in the incidence and prevalence of these infections. New modalities for diagnosis are needed because many of these fungal infections still require weeks for identification. We expect that trials in progress will help determine the role of azoles, amphotericin B, lipid

formulations of amphotericin B, or combinations of these drugs as the optimal treatment of many fungal meningitides.

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CHAPTER 40

Eosinophilic Meningitis

Barbara W. Stechenberg

Eosinophilic meningitis may include any meningitis, infectious or noninfectious, in which one finds a cerebrospinal fluid (CSF) pleocytosis with a significant percentage of eosinophils. The diagnosis of eosinophilic meningitis can be made by the presence of 10 or more eosinophils in the CSF or if at least 10 percent of the CSF leukocytes are eosinophils.[18] Such a finding strongly suggests invasion of the central nervous system (CNS) by a helminthic parasite. In the last 20 years, the term *eosinophilic meningitis* has been applied more specifically to a typical form of meningitis caused by *Angiostrongylus cantonensis*, a rat lungworm found primarily in the Pacific Islands and Southeast Asia. *Gnathostoma spinigerum* and *Baylisascaris procyonis* also may be considered in particular circumstances.

The first documented case of eosinophilic meningitis was reported from Taiwan in 1945.[1] The patient was a 15-year-old boy who developed severe headache and vomiting. Examination of the CSF revealed 528 leukocytes, of which 50 percent were eosinophils. Ten actively moving nematodes also were recovered from the specimen. Since the early 1960s, many new cases have been reported, with particularly large numbers coming from Thailand,[26,27] Tahiti,[32] Taiwan,[47] and Hawaii.[12a,15]

ETIOLOGIC AGENTS

The organism that presumably is involved in most cases is the rodent lungworm. *A. cantonensis*, which as an adult is 17 to 25 mm long and 0.25 to 0.36 mm at its maximum width, has a smooth cuticle, and has three minute lips at the cephalic end. The male has a copulatory bursa supported by bursal rays.

In its life cycle, rodents such as *Rattus rattus* are the principal hosts. These rodents ingest mollusks containing third-stage larvae, which travel from the liver and lung into the general circulation. The larvae selectively leave the circulation to enter the CNS within the first 48 hours. There they develop into young adults in approximately 2 weeks.[44] From the brain, they travel to the pulmonary arteries, where their eggs are laid. The eggs hatch in the pulmonary capillaries; the first-stage forms then travel from the alveolar spaces up the rat trachea to the gastrointestinal tract, from which they are eliminated in the feces. The larvae can survive for approximately 2 weeks under humid conditions. The third-stage larvae develop in the intermediate hosts in approximately 2 weeks.

Another form of eosinophilic meningitis has been attributed to the nematode *G. spinigerum*. The adult *G. spinigerum* is stout and reddish and has a globose cephalic bulb that is separated from the body by a slight constriction. The head and anterior part of the body have spines. Males may be 11 to 25 mm long, and females may be 25 to 54 mm long. The adults lie coiled in lesions along the alimentary canal, from which they release eggs into the feces, where they become embryonated. The eggs hatch on reaching water, releasing a larva that is ingested by a copepod (*Cyclops*) and continues to develop. When the infected copepod is eaten by a fish, frog, snake, or bird, a third-stage larva develops and becomes encapsulated in the intermediate host. When it is ingested by the definitive host (cats, dogs, hogs, mink, humans), the parasite localizes in the stomach wall.[35]

The adult worms of *Baylisascaris procyconis*, the raccoon ascarid, reside in the intestines. They are large, tan-colored roundworms; the female is 20 to 22 cm long, and the male is 9 to 11 cm long. Infection in raccoons usually is subclinical, with the adult female worm producing 115,000 to 179,000 eggs/worm/day, most of which are shed in feces.[12] Young raccoons have a higher prevalence of infection and higher parasitic burden; in their first few months of life, they ingest eggs that are in their environment or sticking to their mothers' fur.

EPIDEMIOLOGY

The geographic distribution of eosinophilic meningitis depends on the distribution of *A. cantonensis*. Many cases have been reported from Taiwan and Thailand. Other areas include Vietnam, the Society Islands (especially Tahiti), Hawaii, the Marshall Islands, and Ponape. Rodents infected with the worm have been documented in other areas of the Pacific Islands and Asia. Disease has been reported from Cuba, Egypt, and many other countries. As infected rodents are carried to other countries on cargo ships, spread of these organisms will continue to increase. Two cases have been diagnosed in the continental United States in travelers from endemic regions.[10,14] An outbreak was described in 12 of 23 young adults who had traveled together to Jamaica.[17,38]

The seasonal incidence of the disease varies in different areas. The months of highest prevalence usually correspond with the more humid periods in each country.

Patients with eosinophilic meningitis have eaten terrestrial snails, slugs, fish, or fresh-water shrimp, all of which can serve as intermediate hosts for the parasites.[41-43] In Thailand, the most common source is the *Pila* snails, which are eaten raw or pickled.[25] They may be served as an appetizer with alcoholic beverages, which may explain partly the increased incidence of the disease in men.[26] The giant African snail *Achatina fulica* is found commonly in Taiwan and may carry thousands of roundworm larvae, explaining the higher incidence of recovery of organisms from the patients in Taiwan.[43] The larvae also may remain infective in water for approximately 60 hours. Cases have been associated with the ingestion of leafy vegetables, presumed to be contaminated by slugs or snails.[15]

The distribution of disease among age groups varies. In Thailand, most of the cases occur in the third to fourth decades of life, but in Taiwan and Hawaii, most cases occur in children younger than 15 years old.[26,47] Although eosinophilic meningitis rarely occurs in very young children, one report documented disease in five children younger than 2 years.[37]

The first confirmed cases of *B. procyonis* neural larva migrans were described in the 1980s in 10-month-old and 18-month-old boys from Pennsylvania and Illinois, respectively.[11,13] Both boys had rapidly progressive, fatal eosinophilic meningoencephalitis. Subsequent reports of fatal and nonfatal disease have been reported from areas with large raccoon populations. They have occurred in rural, urban, and suburban areas. Two risk factors for acquisition of severe infection are contact with raccoons, particularly baby or young animals, or their feces or a contaminated environment, and pica or geophagia. Outdoor contamination of children's play areas by raccoons and proximity of raccoon communal latrines to human habitation may contribute to risk.[33]

PATHOGENESIS

The pathogenesis of this disease in humans has not been studied rigorously but is presumed to parallel the pathogenesis in the rodent host. The larvae are ingested, make their way into the general circulation, and selectively enter the CNS. The number of nematodes found on autopsy specimens has varied. In a 5-year-old Taiwanese girl, 150 nematodes were found on the surface of the cerebrum and cerebellum and in the subarachnoid spaces, and more than 500 were recovered from the normal saline in which the spinal cord and meninges were placed. *A. cantonensis* also were found in the pulmonary arteries.[47]

Pathologic specimens have shown a leptomeningitis in which plasma cells and eosinophils predominate. Tortuous tracks of variable size in the brain and spinal cord parenchyma surrounded by variable reaction and degenerating neurons may be present. Granulomata may form around dead *A. cantonensis*.[24,31]

Hemorrhagic, necrotic tracks caused by the organisms have been associated more commonly with *G. spinigerum* infection.[4] *Angiostrongylus* is capable of causing a vascular reaction, however, including thrombosis and rupture of vessels and arteritis, leading to formation of an aneurysm.[24] The tracks caused by *G. spinigerum* may be larger and more necrotic.

In baylisascariasis, information about the pathology is based on a few fatal cases with massive larvae invasion of the CNS.[12] Swelling of the brain, leptomeningeal congestion, and evidence of herniation have been seen. Necrosis is most marked in the periventricular white matter. Large deposits of eosinophils are present along necrotic migration tracks.

CLINICAL MANIFESTATIONS

On the basis of information obtained from patients with a history of ingestion of the intermediate hosts, the incubation period is 7 to 30 days. Most patients with typical eosinophilic meningitis have an abrupt onset of disease; a more insidious onset may be noted in 20 percent of cases. Headache is the most common and distressing symptom. It usually is intermittent but is frequent and severe. Other common symptoms are nausea, vomiting (often projectile), intermittent somnolence, malaise, anorexia, constipation, and fever with temperatures usually reaching a maximum of 38º C to 39º C in the early phase of the disease, although many patients have no documented fever. Nuchal rigidity is seen more commonly in older patients, often in association with severe headache. Paresthesias occur in a large variety of locations and are expressed as pain, numbness, itching, or a sense of worms crawling on the skin. Some patients also note diplopia with or without strabismus. Convulsions are unusual occurrences.

The findings on physical examination are normal in half the patients. Physical findings may include mild hepatomegaly, mild changes in deep tendon reflexes (usually decreased), nuchal rigidity, absent abdominal wall reflexes, and, less commonly, ophthalmoplegia or facial paralysis. Twelve percent of Thai patients had abnormal ophthalmoscopic examinations.[14]

Examination of the CSF reveals grossly turbid or opalescent fluid in most patients, with leukocyte counts usually 100/mm^3 to 5000/mm^3. All counts usually reach their maximum in the first 3 weeks of disease, decreasing sharply thereafter. Generally, the percentage of eosinophils in the CSF is high, often greater than 50 percent. Fluids with higher cell counts tend to have a higher percentage of eosinophils.[27,47] The proportion of eosinophils may decrease after the first 4 weeks. CSF protein concentration is moderately high (often 50 to 200 mg/dL), but the glucose concentration usually is normal.

Peripheral white blood cell count varies, but the differential cell count often shows striking eosinophilia. Examination of the feces may reveal concurrent infestation with other parasites, such as *Ascaris* or *Trichuris*.

The clinical manifestations of the eosinophilic radiculomyeloencephalitis thought to be associated with *G. spinigerum* overlap to some degree with the manifestations of typical eosinophilic meningitis. Headache is less prominent, however. Many patients report sharp, shooting pains of the trunk or limbs, flaccid paralyses, and impairment of superficial sensation.[28] Impairment of the sensorium may be sudden in association with cerebral hemorrhage. Grossly bloody spinal fluid is a common finding when myeloencephalitis is present, but it is extremely rare in typical eosinophilic meningitis.

Patients with baylisascariasis develop an acute fulminant eosinophilic meningoencephalitis; early features include low-grade fever, increasing lethargy, ataxia, somnolence, and irritability. There is progression to posturing, spasticity, and ocular or cranial nerve involvement. Seizures are common occurrences. Continued deterioration to coma and death, or, with survival, to a persistent vegetative state or severe deficits, may occur.

DIAGNOSIS

The diagnosis of cerebral angiostrongyliasis usually is based on the clinical presentation with associated CSF eosinophilia and an epidemiologic history consistent with possible exposure to the *A. cantonensis* larvae. Rarely, larvae may be recovered from the CSF. Two thirds of patients also have peripheral eosinophilia. Enzyme-linked immunosorbent assay helps to confirm the diagnosis.[39] The absence of focal findings on computed tomography and abnormal enhancement in the globus pallidus may be valuable in establishing a diagnosis.

Gnathostomiasis often is associated with xanthochromic or bloody CSF with an eosinophilic pleocytosis. Peripheral eosinophilia often is pronounced. Computed tomography and magnetic resonance imaging show areas of hemorrhage.[35] Western blot serologies are available,[9] but definitive diagnosis is by recovery of the pathogen, which may be found in subcutaneous tissue.

Baylisascariasis should be suspected in a child with CSF and peripheral eosinophilia, with severe encephalopathy with diffuse white matter disease on neuroimaging,[34] and with or without eye disease in North America or Europe. A history of exposure to raccoons or their feces would strongly suggest the diagnosis. In the absence of brain biopsy, the diagnosis is made serologically.[13]

DIFFERENTIAL DIAGNOSIS

Other helminths may invade the CNS of humans and may be associated with an eosinophilic pleocytosis,[45] including *Taenia solium*, the pork tapeworm causing cerebrospinal cysticercosis; *Schistosoma* spp.; *Paragonimus westermani*; and *Echinococcus*. The diseases produced generally have a chronic and intermittent course. Signs of a space-occupying lesion and convulsions are found frequently. Visceral myiasis with invasion of the CNS by botfly larvae may cause CSF eosinophilia.[3] The larvae of *Trichinella spiralis* can invade the CNS, but pleocytosis seems to be an unusual occurrence.[3] Neurologic involvement in visceral larva migrans caused by *Toxocara canis* rarely occurs; it may occur in association with CSF pleocytosis and eosinophilia.[21,40]

Coccidioidomycosis is the one fungal infection that may be associated with CSF eosinophilia when infection involves the meninges.[30,36] In one series, 30 percent of cases with coccidioidal meningitis had significant CSF eosinophilia.[30] Neurosyphilis and tuberculous meningitis rarely have been associated with eosinophils in the CSF, as have several malignant neoplasms, particu-

larly lymphomas, involving the CNS.[16,22] Other known causes of significant eosinophilic pleocytosis include intrathecal injection of various foreign proteins, rabies vaccination, the insertion of rubber tubing into the CNS during neurosurgery, lymphocytic choriomeningitis,[14] and Rocky Mountain spotted fever.[8] Eosinophilic pleocytosis has been documented in infants with congenital toxoplasmosis and late-onset group B streptococcal meningitis.[20,46]

Eosinophilia in the CSF indicating hypersensitivity has been difficult to document, although one case report described the association of eosinophilic meningitis and ibuprofen therapy.[29] Eosinophilic meningitis developing after implantation of a ventriculostomy catheter impregnated with rifampin and minocycline has been reported.[2] A study documented more than 5 percent eosinophils in the ventricular fluid of patients with shunt-associated pathology, particularly malfunction.[19]

TREATMENT

In a study in Thailand in which the disappearance of headache was used as the criterion for improvement in patients with *A. cantonensis* meningitis, no significant differences were noted among groups when 284 patients were treated with analgesics alone, 96 patients were treated with analgesics and steroids (30 to 60 mg of prednisone daily for 5 days), and 56 patients were treated with analgesics and antibiotics (penicillin or tetracycline).[23] A study that compared adult patients given a 2-week course of prednisolone (60 mg/day) with patients treated with a placebo showed a significant decrease in the number of patients with headache and in the duration of headache in the prednisolone-treated group.[6] Treatment with thiabendazole also has been tried without significant benefit. The combination of albendazole or mebendazole with prednisolone has shown promise.[5,7] Treatment of gnathostomiasis is supportive, although steroids have been used to decrease meningeal inflammation. Baylisascariasis has no effective antihelminthic therapy.

COURSE AND PROGNOSIS

In most patients, eosinophilic meningitis is a self-limited disease characterized by repeated attacks of severe headache, vomiting, intermittent fever, and somnolence. Many patients experience a dramatic improvement soon after undergoing a lumbar puncture, so repeated lumbar punctures may be performed at weekly intervals to relieve the headache. In most patients, most symptoms disappear within 4 weeks of onset, often within a few days after the first lumbar puncture is performed, leaving no sequelae. The mortality rate in large series from Thailand and Taiwan has been less than 5 percent; the incidence of permanent sequelae also has been less than 5 percent for *Angiostrongylus* infection.[27,46] The mortality in gnathostomiasis is higher, ranging from 7.7 to 25 percent.[35] No evidence suggests that immunity develops after recovery from infection; many recurrences have been reported.[27,32,47] In baylisascariasis, the mortality rate is high, and survivors often have severe sequelae.

PREVENTION

Disease can be prevented only by control of rodents and proper cooking of mollusks, shrimp, fish, and other intermediate hosts. Careful washing of fruits and vegetables that may be contaminated by rodent feces also is important. The larvae may remain infective in water for 60 hours, so protection of the water supply should be attempted. Prevention of baylisascariasis depends on eliminating exposure to raccoon feces.

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CHAPTER 41

Aseptic Meningitis and Viral Meningitis

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Aseptic meningitis is an inflammatory process of the meninges. It is relatively common and is caused by many different etiologic factors. The cerebrospinal fluid (CSF) is characterized by pleocytosis, increased protein, and the absence of microorganisms on Gram stain and on routine culture. Usually, the illnesses are self-limited; with some etiologies, however, the resulting diseases may be severe, protracted, recurrent, or progressive, and lead to disability and death.

Serous meningitis, *lymphocytic meningitis*, and *nonparalytic poliomyelitis* are terms that were used in the past to denote aseptic meningitis. Viral meningitis is an inflammation of the leptomeninges caused by infections with many different viruses. Viruses are the causes of most cases of aseptic meningitis.

HISTORY

Aseptic meningitis is a syndrome that first was described by Wallgren in 1925.[289] Wallgren's criteria for this diagnosis included (1) an acute onset with obvious signs and symptoms of meningeal involvement; (2) alteration of CSF typical of meningitis, which may show a small or large number of cells; (3) absence of bacteria in the CSF, as shown by appropriate culture; (4) a relatively short, benign course of illness; (5) absence of local parameningeal infection (e.g., otitis, sinusitis, trauma) or a general disease that might have meningitis as a secondary manifestation; and (6) absence from the community of epidemic disease, of which meningitis is a feature. In 1951, Wallgren[290] redefined aseptic meningitis as a syndrome likely to be encountered in many different infectious diseases.

The clinical occurrence of aseptic meningitis first was recognized in epidemic poliomyelitis and in mumps at the beginning of the 20th century.[93,296] Rivers and Scott[225] reported the recovery of lymphocytic choriomeningitis (LCM) virus from the CSF of several patients with aseptic meningitis in 1935, and in 1934, Johnson and Goodpasture[148] proved that mumps was caused by a virus. The discovery of coxsackieviruses in 1948 by Dalldorf and Sickles[74] and the introduction of tissue culture in 1949 by Enders and colleagues,[94] which resulted in the discovery of echoviruses, paved the way for the widespread investigation into the etiology of aseptic meningitis.

Rasmussen[220] reported on 374 cases evaluated at the Walter Reed Army Institute of Research laboratory between 1941 and 1946 and found the probable or definite etiology in 26 percent of "viral" disease of the central nervous system (CNS). Mumps and LCM viruses were the two etiologic agents identified in his study.

In 1953, Adair and associates[5] reviewed 480 additional cases of aseptic meningitis occurring in military personnel and their dependents from 1947 through 1952 and were able to confirm the etiology in 25 percent of those patients. Herpes simplex virus (HSV) and *Leptospira* spp. were added to the previously identified mumps and LCM viruses as causes of aseptic meningitis. Meyer and associates[188] extended these studies to include 713 more children and adults with acute CNS syndromes of "viral" etiology admitted to military and Veterans Administration hospitals between 1953 and 1958. Of these 713 patients, 430 had the clinical syndrome of aseptic meningitis. Approximately 80 percent of these patients were hospitalized in the United States. An etiologic diagnosis was determined in 71 percent of patients with aseptic meningitis. In addition to the agents identified earlier, poliovirus, coxsackieviruses of groups A and B, echoviruses, and arthropod-borne viruses were identified as causes of aseptic meningitis.

Lepow and colleagues[174,175] reported the probable viral etiology in 54 percent of the 407 patients they studied in Cleveland between 1955 and 1958. In 1958, Lennette and associates[173] determined a viral etiology in 65 percent of 511 children and adults with presumed viral CNS disease in Los Angeles; 368 of these patients were diagnosed as having aseptic meningitis. Sköldenberg[253] analyzed 3117 patients admitted to the Hospital for Infectious Diseases in Stockholm between 1955 and 1964 with the diagnosis of aseptic meningitis, with or without encephalitis or myelitis, and a virologic or clinical diagnosis (or both) of an associated viral infection was established in 72.6 percent. Berlin and associates[25] performed a surveillance study of aseptic meningitis in pediatric ambulatory clinics and emergency departments of three Baltimore hospitals between July 1986 and December 1990. They identified a single viral agent in 169 (62%) of the 274 cases with laboratory study; 168 enteroviruses and 1 adenovirus were identified. Today, with the use of polymerase chain reaction (PCR) and culture and appropriate serologic study,

TABLE 41–1 Etiologic Agents, Factors, and Diseases Associated with Aseptic Meningitis

Viruses
Arboviruses (in the United States: West Nile, St. Louis, California, Colorado tick fever, Eastern equine, Western equine, Venezuelan equine, and Powassan)*
Coronaviruses
Cytomegalovirus
Encephalomyocarditis
Enteroviruses (echoviruses, coxsackieviruses A and B, polioviruses, enteroviruses) and parechoviruses
Epstein-Barr
Hendra and Nipah
Herpes simplex type 1
Herpes simplex type 2
Human herpesvirus-6
Human herpesvirus-7
Human immunodeficiency virus (HIV-1)
Human T-cell lymphotrophic virus (HTLV-1)
Influenza A and B
Lymphocytic choriomeningitis
Measles
Mumps
Parainfluenza
Parvovirus B19
Rhinoviruses
Rotaviruses
Rubella
Varicella-zoster
Variola

Postvaccine
Measles
Mumps
Polio
Rabies
Vaccinia

Bacteria
Atypical mycobacteria
Bartonella henselae
Borrelia spp. (relapsing fever)
Borrelia burgdorferi (Lyme disease)
Brucella spp.
Leptospira spp. (leptospirosis)
Mycobacterium tuberculosis
Nocardia spp. (nocardiosis)
Pyogenic—partially treated
Treponema pallidum (syphilis)

Rickettsia
Coxiella burnetii
Ehrlichia canis
Rickettsia rickettsii (Rocky Mountain spotted fever)
Rickettsia prowazekii (typhus)

Mycoplasma
Mycoplasma hominis
Mycoplasma pneumoniae

Chlamydia
Chlamydia pneumoniae
Chlamydia psittaci

Ureaplasma
Ureaplasma urealyticum

Fungi
Blastomyces dermatitidis
Candida spp.
Coccidioides immitis
Cryptococcus neoformans
Histoplasma capsulatum
Other: *Acremonium* spp., *Alternaria* spp., *Aspergillus* spp., *Blastoschizomyces capitus*, *Cephalosporium* spp., *Cladosporium trichoides*, *Drechslera hawaiiensis*, *Fusarium* spp., *Paecilomyces* spp., *Paracoccidioides brasiliensis*, *Penicillium marneffei*, *Phaeohyphomycosis*, *Pseudallescheria boydii*, *Sporothrix schenckii*, *Trichosporon beigelii*, *Ustilago* spp., *Zygomycetes* spp.

Parasites (Eosinophilic Meningitis)
Flukes: *Paragonimus westermani*, schistosomiasis, fascioliasis
Roundworms: *Angiostrongylus cantonensis*, *Gnathostoma spinigerum*, *Baylisascaris procyonis*, *Strongyloides stercoralis*, *Trichinella spiralis*, *Toxocara canis*
Tapeworms: cysticercosis

Parasites (Noneosinophilic Meningitis)
Acanthamoeba
Naegleria fowleri
Toxoplasma gondii (toxoplasmosis)

Parameningeal Infections

Malignancy
Central nervous system tumor
Leukemia

Immune Diseases
Behçet syndrome
Lupus erythematosus
Sarcoidosis

Miscellaneous
Antimicrobial agents
Epidermoid, dermoid, other cysts
Foreign bodies (shunt, reservoir)
Heavy metal poisoning
Intrathecal injections (e.g., contrast media, antibiotics)
Kawasaki disease
Other drugs

**In other areas of the world, many other arboviruses are important.*

the etiology of most cases of aseptic meningitis can be determined.

ETIOLOGY

Table 41–1 lists etiologic agents and factors in aseptic meningitis. At present, the diagnostic work-up of aseptic meningitis usually is not undertaken vigorously, and the etiologic agent is identified in only approximately 10 percent of all cases. Epidemiologic study and intensive investigations at some centers indicate, however, that most cases result from viral infections. Enteroviruses account for approximately 85 percent of all cases of aseptic meningitis.[44,78-80,195] The following enteroviruses have been associated with aseptic meningitis: polioviruses 1 to 3; coxsackieviruses A 1 to 14, 16 to 18, 21, 22, and 24; coxsackieviruses B 1 to 6; echoviruses 1 to 9, 11 to 21, 24 to 27, and 29 to 33; enterovirus 71; and parechoviruses 1 and 2.

In recent years, multiple outbreaks of aseptic meningitis caused by enteroviruses have been described, including outbreaks caused by echovirus 30 in several countries throughout Eastern and Western Europe, China, Japan, Australia, the Arabian Gulf, the United States, and Brazil.* Echovirus 13 was responsible for reported outbreaks of aseptic meningitis in the United States,

*See references 9, 19, 26, 51, 54, 58, 88, 99, 119, 132, 152, 161, 204, 223, 245, 275, 283, 284, 292, 310.

England, Wales, Germany, Belgium, Spain, France, Lithuania, Israel, Japan, Korea, and Australia.* Enterovirus 71 caused a major epidemic in Taiwan from 1998 to 1999, with multiple cases of hand, foot, and mouth syndrome associated with aseptic meningitis and other neurologic manifestations.[142,177,178,181,249,291,292,304,306] Similar outbreaks of aseptic meningitis caused by enterovirus 71 were reported in Malaysia, Japan, and Australia.[59,106,140,187,248-250] Other enteroviruses involved in more recent outbreaks include echovirus 4 in Italy and Israel/Palestine,[129,217] echovirus 9 in Japan and regions of the United States,[8,57] echovirus 11 among institutionalized children in Israel,[218] echovirus 16 in Cuba,[241] and echovirus 33 in New Zealand.[144] In the United States, the most common specific enteroviral types in the vaccine era are coxsackievirus B5 and echoviruses 4, 6, 9, and 11, with echoviruses 9 and 30 being the most frequently identified etiologies of aseptic meningitis since 2003.[54,56,226]

Sharing seasonality with the enteroviruses, several arboviruses cause CNS disease in North America. Although encephalitis is the most recognizable manifestation of many of these infections, some arboviruses commonly are associated with aseptic meningitis as well.[117,226,230] Since the mid-1990s, outbreaks of West Nile virus meningitis and encephalitis have occurred in Romania, Russia, and Israel.[63,214,278] First detected in the Western Hemisphere in 1999 in New York City, West Nile virus subsequently spread across North America from the Atlantic to the Pacific coasts and into Canada and Mexico.[35,96,145,201,212] Between 1999 and 2005, greater than 18,000 cases were reported in the United States, with more than 700 deaths.[55,134] An estimated 1 in 150 infections results in severe neurologic illness, with meningitis as the primary manifestation in 16 to 40 percent of hospitalized patients.[150] Although the incidence of neuroinvasive disease increases with age, West Nile virus has been responsible for meningitis in young children and adolescents, occurring in at least one quarter of the 150 pediatric cases diagnosed in the United States in 2002.[65,103,133] Even in regions with increased incidence of West Nile virus, episodes of meningitis caused by enterovirus greatly outnumber those caused by West Nile virus.[150]

Before the introduction of West Nile virus, arboviruses accounted for approximately 5 percent of cases of aseptic meningitis in North America, with St. Louis encephalitis virus being the most common.[36,41,46,48] Infection with La Crosse encephalitis virus (a California encephalitis virus subtype) often resembles herpes encephalitis, but it may manifest as aseptic meningitis in children. In a study of 127 patients, mainly children, hospitalized in the southern United States with La Crosse virus infection, headache, fever, vomiting, and seizures were predominant findings. Thirteen percent of these patients had aseptic meningitis.[186] Other California serogroup viruses, such as Jamestown Canyon virus and snowshoe hare virus, and other arboviruses, such as Colorado tick fever, result in aseptic meningitis more frequently than encephalitis.[117,226,262] Tick-borne encephalitis can manifest as aseptic meningitis in endemic areas. Tick-borne encephalitis virus cases were reported more recently in studies conducted in Slovenia and Sweden, and in mild cases, the clinical presentation was that of aseptic meningitis.[125,176,182]

Aseptic meningitis is an occasional manifestation of acute and recurrent genital infections with HSV-2.[15,24,71,87,254,274] In contrast to HSV-1 CNS infections, which without treatment usually are fatal, HSV-2 aseptic meningitis in otherwise immunocompetent patients is a benign, self-limited illness. Herpes family viruses other than HSV-1 and HSV-2 also are potential causes of aseptic meningitis. Although neurologic involvement in primary varicella-zoster viral infections usually is an encephalitis rather than a benign meningitis, pleocytosis is noted occasionally in herpes zoster.[78,218,222,295] Varicella-zoster virus has been identified by PCR in the CSF of patients who had acute aseptic meningitis but no cutaneous lesions.[1,92,147] A variety of neurologic disorders, including aseptic meningitis, are rare complications of Epstein-Barr virus infection.[97,105,121,266,280] Most noncongenital infections with cytomegalovirus in nonimmunocompromised patients are unrecognized; however, occasional instances of aseptic meningitis have been noted.[78-80]

The role of human herpesvirus-6 (HHV-6) in causing meningitis is unclear; although HHV-6 has been found in CSF samples from infants with meningitis, the virus also is detectable in the CSF of asymptomatic individuals.[11,38,305,308] Similarly, PCR identified HHV-7 in the CSF of six children with neurologic diseases, including aseptic meningitis, meningoencephalitis, facial palsy, vestibular neuritis, and febrile seizures.[215] The role of HHV-7 as a causative agent in aseptic meningitis remains to be determined.

Occasionally, meningitis or meningoencephalitis occurs as a manifestation of acute illness with HIV-1 infection.[16,128] Neurologic manifestations develop 3 to 6 weeks after primary infection at the same time as an infectious mononucleosis–like illness.

Although LCM virus was an important historical cause of aseptic meningitis, it rarely is recognized today as a cause of meningitis except in animal-exposure outbreak situations.[17,21,28,81,188] In 1974, eight cases of aseptic meningitis caused by LCM virus were found in New York State.[81] Many sporadic instances of LCM virus infection probably go unrecognized. Physicians should be alert to the possibility in all situations of rodent (pet or wild) exposure. Encephalomyocarditis virus is another rodent virus that rarely is recognized in humans.[293] It is associated with a variety of neurologic manifestations, including aseptic meningitis.[107]

Adenoviral types 1, 2, 3, 5, 6, 7, 12, 14, and 32 have been associated with meningitis and meningoencephalitis.[25,69,78-80,98,159,210,251,257] Although they occur infrequently, adenoviral CNS infections tend to be more severe than are enteroviral infections. Rarely, aseptic meningitis has been noted during illnesses caused by rhinoviruses, influenza A and B viruses, parainfluenza viruses, parvovirus B19 virus, rotaviruses, and coronaviruses.* Most infections with measles, rubella, and variola viruses that involve the CNS are encephalitic.[42,43,45,192]

In the pre-vaccine era, mumps virus was the agent responsible for the greatest number of cases of aseptic meningitis; today in the United States, use of vaccine has rendered mumps rare, although mumps outbreaks with associated cases of aseptic meningitis occur occasionally.[47,57] Aseptic meningitis and encephalitis resulting from administration of mumps vaccine have been noted in Canada, Brazil, Japan, and Europe.† The Leningrad 3, Urabe Am 9, and three Japanese strains of vaccine viruses have been implicated. In the United States, where the Jeryl Lynn vaccine strain has been used exclusively, the rate of encephalitis in vaccinees has been no higher than that of the observed background incidence of similar illness in the population.[47] More recently, a preliminary analysis of the Vaccine Safety Datalink project showed a possible increased risk of developing aseptic meningitis 8 to 14 days after receiving immunization with Jeryl Lynn mumps vaccine strain. A follow-up case-control evaluation of hospitalized cases failed to show an increased risk, however.[30]

Neurologic illness is a rare complication of measles, smallpox, polio, and rabies viral vaccines. In most instances, the illnesses are complex and severe, but occasionally, aseptic meningitis is the only manifestation.[25,42,45,53,191,246,261] A case of aseptic meningitis caused by vaccine-derived poliovirus was reported in the Philippines in 2001.[53] It was in association with two pediatric cases of acute flaccid paralysis that occurred during the same time period.

*See references 52, 61, 62, 85, 151, 158, 162, 198, 200, 258, 275, 277.

*See references 13, 40, 72, 78-80, 141, 157, 203, 205, 209, 224, 285, 301.
†See references 10, 14, 66, 67, 73, 75, 89, 185, 190, 199.

Viral isolates from all three patients revealed type 1 poliovirus derived from the Sabin vaccine strain.

Certain bacteria are important to recognize as etiologic agents in aseptic meningitis because the illnesses are treatable and early initiation of therapy is crucial. Of greatest importance is tuberculous meningitis. Early treatment of this illness nearly always results in complete cure, whereas diagnostic delay or inadequate treatment frequently results in permanent neurologic sequelae. Lyme disease, relapsing fever, brucellosis, leptospirosis, and rickettsial infections are illnesses acquired either directly or indirectly from animals, in which aseptic meningitis may be a part of the disease process.* *Mycoplasma pneumoniae* is an important cause of neurologic illness.[89,124,215,279] Pönkä[216] noted that 8 of 560 hospitalized patients with *M. pneumoniae* infections had aseptic meningitis, and 18 had encephalitis or meningoencephalitis. *Mycoplasma hominis* and *Ureaplasma urealyticum* are rare causes of neonatal meningitis.[108,183,286,287] Meningitis and meningoencephalitis have been associated with *Chlamydia pneumoniae* infections.[12,123,255,267] Partially treated common bacterial meningitides are a common cause of meningitis in which cultures of CSF fail to grow organisms. Antigen detection systems, such as latex agglutination, can be useful in identifying the causative agents in some of these cases.

Numerous fungi and yeasts cause meningitis.[238] Although many fungal meningitides occur almost exclusively in immunocompromised patients, children and adults with normal immune status experience meningitis most commonly caused by the following: *Blastomyces dermatitidis*, *Coccidioides immitis*, *Cryptococcus neoformans*, *Cladosporium* spp., *Histoplasma capsulatum*, and *Paracoccidioides brasiliensis.* In infants who are premature or younger than 1 month of age, *Candida albicans* is an important cause of meningitis, associated with significant morbidity and mortality.[22,120,196]

Parasites occasionally cause aseptic meningitis. Eosinophilic meningitis is caused by *Angiostrongylus cantonensis*, a rat lungworm.[60,128,228,294] Aseptic meningitis caused by *A. cantonensis* has been observed on several islands in the Pacific, and the infection may be acquired by the consumption of freshwater shrimp. *Baylisascaris procyonis*, the intestinal roundworm of raccoons, is a rare cause of neural larva migrans in infants and young children and manifests as acute fulminant eosinophilic meningoencephalitis.[109,110,300] Between 1973 and 2002, 14 probable or confirmed cases were described in eight states throughout the United States, reported approximately once every 1 to 2 years. The infection was fatal in 6 of the 14 children, and all documented survivors were neurologically devastated.[300]

A sterile CSF pleocytosis occurs in 12 to 13 percent of young infants with bacterial urinary tract infections[6,100,269] and in approximately one third of patients with Kawasaki disease who undergo lumbar puncture.[82,116] Numerous drugs and biologicals have been implicated in aseptic meningitis.† Of most importance in pediatrics are trimethoprim-sulfamethoxazole and intravenous immunoglobulin. Other causes of aseptic meningitis are listed in Table 41–1.‡

EPIDEMIOLOGY

Because many different types of organisms cause aseptic meningitis, no unified epidemiologic pattern exists. The epidemiology of the specific individual infectious agents or diseases is presented in detail in the various chapters of this book, and only a brief overview is presented here.

Because approximately 85 percent of all cases of aseptic meningitis are caused by enteroviral infections, the basic epidemiologic pattern of aseptic meningitis reflects these agents. In temperate climates, most cases occur in the summer and fall; infection with enteroviruses is spread directly from person to person, and the incubation period usually is 4 to 6 days. Epidemiologic considerations in aseptic meningitis caused by agents other than enteroviruses depend markedly on season, geography, climatic conditions, animal exposures, and many other factors related to the specific pathogens.

CLINICAL MANIFESTATIONS

Aseptic meningitis has many different causes (see Table 41–1), and clinical manifestations vary with the different diseases. In some instances, the signs and symptoms resulting from meningeal inflammation dominate the clinical illness, whereas in other instances, the main signs and symptoms reflect other organ system involvement. Clinical manifestations in aseptic meningitis, regardless of etiology, also vary markedly by patient age.

ENTEROVIRUSES

Enteroviruses are the most common cause of aseptic meningitis, and they can be considered the prototype for a description of general clinical manifestations of aseptic meningitis.* Even among the enteroviruses, however, significant differences in clinical manifestations exist among the different viral types. Some general aspects of epidemic enteroviral aseptic meningitis are presented by viral type in Chapter 178.

The onset of illness generally is acute, although it may be insidious over the course of a week or so, or may be preceded by a nonspecific acute febrile illness of a few days' duration. Almost all children have fever, and most older children have headache, which most often is retro-orbital or frontal in location. Photophobia is common. Temperature elevation varies, ranging from 38° C to 40.5° C (100.4° F to 105° F), and usually lasts approximately 5 days. Occasionally, fever is biphasic, with the initial elevation occurring before the onset of neurologic signs and symptoms. Anorexia, nausea, and vomiting are common, and abdominal pain and diarrhea also are reported frequently.

Meningeal signs (stiff neck and back, tightness of the hamstring muscles, Brudzinski and Kernig signs) usually are present, but deep tendon reflexes usually are normal or hyperactive. Seizures occur occasionally, usually when concomitant high fever is present. Muscle weakness rarely is reported, but myalgia occasionally is noted. In young children, fever, irritability, and lethargy are the most common findings. Infants may be irritable and show resentment to handling, and the fontanelle may be tense.

Other manifestations of enteroviral infections also occur in children with aseptic meningitis. Most common is pharyngitis, which may occur during infection with all of the neurotropic enteroviral types. Rash occurs commonly, but varies by viral type. With echovirus 9 meningitis, 30 to 50 percent of children have rashes, whereas with echovirus 6, exanthem is rare. Cases of meningitis caused by enterovirus 71 frequently are accompanied by hand, foot, and mouth syndrome. Enanthem, pleurodynia, pericarditis, myocarditis, and conjunctivitis are other findings noted in children with enteroviral aseptic meningitis. Illness often is biphasic with fever, an interlude, then return of fever and neurologic manifestations.

*See references 27, 29, 31, 33, 95, 115, 146, 149, 154, 164, 171, 206, 211, 260, 273, 288, 299, 303, 309.
†See references 17, 37, 83, 101, 118, 127, 184, 187, 197, 219, 302.
‡See references 49, 86, 104, 114, 136, 139, 163, 164, 166, 172, 184, 247, 268.

*See references 3, 8, 9, 32, 50-53, 58, 76, 85, 102, 111, 122, 126, 129, 130, 135, 137, 142, 152, 155, 156, 169, 178, 221, 229, 234, 235, 237, 239, 252, 265, 270, 297.

CSF leukocyte counts vary from a few cells to a few thousand cells; the median is in the range of 100 to 500 cells/mm^4. The percentage of neutrophils also varies greatly. Initially, a predominance of neutrophils commonly occurs, but later, CSF examinations show a decline in the percentage of neutrophils. The CSF protein usually is elevated mildly, and the glucose concentration usually is normal; rarely, hypoglycorrhachia is noted.

The duration of illness varies. Usually, disability because of neurologic involvement lasts 1 to 2 weeks.

ASEPTIC MENINGITIS CAUSED BY OTHER AGENTS

In meningitis caused by arboviruses, involvement of the brain often occurs as well (meningoencephalitis). With St. Louis and California viral infections in children, the illness commonly is benign, without changes in sensorium or other findings indicative of brain involvement. Similarly, meningitis without encephalitis is present in a significant proportion of patients with neuroinvasive West Nile virus infections.[133,212] Seizures occur more commonly in arboviral meningitides than in enteroviral illnesses of otherwise comparable severity.[131] When neurologic disease caused by mumps is recognized, usually evidence of brain involvement is present. Examination of the CSF in mild cases of mumps often reveals pleocytosis.

Tuberculous meningitis usually has a gradual onset over the course of 2 to 3 weeks.[160,193,227] Initially, personality changes, irritability, anorexia, listlessness, and low-grade fever may be present, followed by signs of increased intracranial pressure, such as drowsiness, stiff neck, cranial nerve palsies, inequality of the pupils, vomiting, and convulsions. Finally, coma, irregular pulse and respirations, and high fever occur. In fungal diseases, the course of meningitis is similar to the course of tuberculosis. In tuberculosis and several fungal meningitides, such as those caused by *C. immitis*, *H. capsulatum*, and *C. neoformans*, historical and radiographic evidence of pulmonary disease may be present.

Aseptic meningitis caused by *M. pneumoniae* is unique in that it frequently occurs a few days to 3 weeks after a respiratory illness (pharyngitis, bronchitis, or pneumonia).[124,179,256,279] In nonenteroviral aseptic meningitides, the CSF findings generally are similar to those in enteroviral disease. Generally, the likelihood of a predominance of neutrophils is less in other aseptic meningitides, and low glucose levels are likely in parameningeal bacterial infections, partially treated bacterial meningitides, brain tumors, leukemic infiltration, *M. pneumoniae* infections, fungal infections, and tuberculosis.

RECURRENT ASEPTIC MENINGITIS (MOLLARET MENINGITIS)

In 1944, Mollaret[194] described three patients with recurrent aseptic meningitis whom he had observed over the course of 15 years. Subsequently, many other cases have been reported, and some cases have been noted in children.[34,67,68,138,213,263,272] The illness is characterized by recurrent attacks of fever with meningeal signs and symptoms. The attacks last several days and are separated by symptom-free periods lasting weeks or months. In addition to a lymphocyte predominant pleocytosis, CSF samples obtained from certain patients contain large mononuclear cells (Mollaret cells). The disease remits spontaneously. HSV-2 has been identified by PCR or DNA probes in the CSF of most patients with recurrent meningitis.[18,67,91,167,213,271,272,242,281] Other viruses, such as HSV-1 and Epstein-Barr virus, and noninfectious causes, such as systemic lupus erythematosus, intracranial cysts, and environmental exposures, also have been identified as less frequent etiologies of recurrent meningitis.[121,163,236,240,263]

DIFFERENTIAL DIAGNOSIS

Careful analysis of the history and epidemiologic circumstances may point toward one of the specific causes listed in Table 41–1. During the summer and autumn, the presence of pleurodynia, herpangina, or unexplained febrile eruptions in the community suggests the possibility of enteroviral infections. Acute paralytic disorders in other patients suggests poliomyelitis or West Nile virus. Exposure to mosquitoes and encephalitis in horses implicates certain arboviruses, and exposure to ticks may be suggestive of Lyme disease, relapsing fever, or rickettsial disease, depending on the geographic location and other symptoms of the illness. A history of swimming in waters contaminated by urine from infected animals and exposure to rats in urban slums suggest leptospiral infection. Knowledge of clear-cut exposure to or concurrent evidence of mumps or of one of the common exanthems is helpful in delineating the differential diagnosis. The association of pneumonia or other respiratory illness preceding aseptic meningitis strongly suggests the possibility of *M. pneumoniae* as the etiologic agent.

Most difficult from the diagnostic, therapeutic, and prognostic points of view are instances of incipient or partially treated bacterial (especially when caused by *Haemophilus influenzae*) or mycobacterial meningitis. The clinical findings; the dosage of antibiotic previously used; the spinal fluid smear, latex agglutination, or other rapid antigen identification test; the culture; and the glucose level may be helpful in diagnosing bacterial meningitis. The quantitative determination of C-reactive protein in the CSF also may be useful in differentiating bacterial from viral meningitis.[2,70,77,207,208] Lindquist and associates[180] found that the determination of CSF concentrations of lactate was the most useful test in differentiating bacterial from nonbacterial causes of meningitis. Studies suggest that the presence of tumor necrosis factor-α in the CSF is rare in viral infections, but common in bacterial disease.[7,90,113] When tuberculous meningitis is suspected, a careful evaluation of contacts, a careful examination of an appropriately stained smear from the pellicle of the CSF that was allowed to settle, and a positive tuberculin reaction may confirm the diagnosis. Because combined bacterial and viral infection has occurred, examinations of CSF should be repeated if any doubt exists. The possibility that the observed meningeal reaction is of neither viral nor bacterial origin must be considered. Finally, CNS tumor must be considered in the differential diagnosis, particularly if hypoglycorrhachia and prominent signs of increased intracranial pressure are present.[166]

SPECIFIC DIAGNOSIS

Obtaining a meticulous history is essential. The clinician must evaluate exposure of the patient in the past 2 to 3 weeks to illness in contacts; exposure to mosquitoes, ticks, and animals during recent vacations, picnics, and so on; awareness of illness in animals, especially horses and other Equidae, in the patient's environment; recent travel from the home area; recent injections or medications of any kind; and the possibility of accidental exposure to heavy metals.

The CSF must be examined carefully to exclude disorders that respond to specific therapy. Smears for bacteria, appropriate rapid antigen identification tests, and cultures of the CSF are mandatory; the history and clinical findings may indicate the need for performing acid-fast stain and culture of the sediment for mycobacteria. Other circumstances may indicate the need for excluding fungal or protozoal infection; atypical cells may require cytopathologic study to exclude neural neoplasms, which may manifest acutely.

In any patient suspected to have viral meningitis, spinal fluid, blood, feces, and throat swabs should be collected and sent to a

laboratory offering viral diagnostic services. An additional serum specimen should be collected 10 to 21 days later so that paired sera can be examined for antibody titer increases. This pairing is particularly useful in arboviral, LCM viral, encephalomyocarditis viral, leptospiral, borrelial, rickettsial, mycoplasmal, and toxoplasmal infections. Although these studies may not provide an immediate diagnosis, they may give early warning of a specific epidemic, and they are useful for prognostication, particularly in very young infants.

The introduction of PCR has facilitated the etiologic diagnosis of CNS viral infections, particularly infections caused by enteroviruses and herpesviruses.* PCR detects enterovirus in the CSF more rapidly than does cell culture and has been shown to shorten the duration of hospitalization for children with meningitis, reducing costs.[202,264] PCR is the test of choice for detecting CNS infections caused by HSV, and molecular techniques also have been used to identify in CSF such rare causes of meningitis and meningoencephalitis as varicella-zoster virus, HHV-6, parvovirus B19, and rotavirus.[20,147,165,282] Although such techniques are of limited value for many arboviruses with short periods of viremia and presence in the CSF, real-time PCR analysis of CSF samples used during the 1999 West Nile encephalitis outbreak in New York had 57 percent sensitivity and 100 percent specificity.[64,170]

TREATMENT

Hospitalization usually is necessary because of the possibility of treatable bacterial disease and the frequent need for fluid therapy for dehydration. Treatment is symptomatic. Headache and hyperesthesia are treated with rest; analgesics; and a reduction in room light, noise, and visitors. Antipyretics are recommended for fever. Using acetaminophen rather than aspirin is prudent because of the risk for developing Reye syndrome associated with the latter antipyretic. Codeine, morphine, and the phenothiazine derivatives often are used for pain and vomiting, but they rarely are necessary in children, and they should be avoided because they may induce misleading signs and symptoms. The investigational antiviral drug pleconaril has been shown to be effective in the treatment of enteroviral meningitis; however, this drug presently is unavailable.[84,231,233] Treatment for such illnesses as tuberculous meningitis, fungal meningitides, and other illnesses for which specific therapies are available is covered in specific chapters of this book.

Several weeks after the patient has apparently recovered, a careful neuromuscular assessment should be conducted to ensure that muscular weakness is not a sequela. Bilateral audiometry is recommended, especially when mumps virus was involved.

PROGNOSIS

The prognosis in aseptic meningitis depends on the etiology. Some illnesses have an ominous prognosis (tuberculous meningitis, parameningeal infections, rickettsial infections), but patients usually do well if appropriate specific therapy is instituted early in the course of the illness. In *C. immitis* meningitis, the prognosis for cure is guarded even with early optimal therapy.

In enteroviral and other viral meningitides, children usually recover completely. Some patients complain of fatigue, irritability, decreased ability to concentrate, muscle pain, muscle weakness and spasm, and incoordination for several weeks after an acute illness. Although the outcome of enteroviral meningitis most often is without residual, some infants who have enteroviral meningitis in the first few months of life have an increased risk for altered language development.[23,298] Formally evaluating such children at age 3 to 6 years is important.

PREVENTION

The universal use of polio and mumps vaccines in children clearly is effective in controlling these two diseases. Control of insect vectors by suitable spraying methods and eradication of insect breeding sites is important in the control of many arboviruses. The control of animal vectors such as mice and rats alters the incidence of infections with LCM and encephalomyocarditis viruses.

*See references 4, 8, 64, 112, 129, 143, 153, 161, 167, 232, 243, 244, 276, 307.

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CHAPTER 42

Encephalitis and Meningoencephalitis

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Encephalitis is an inflammation of the brain, and meningoencephalitis is a similar inflammatory illness in which the brain and the meninges are involved. The diagnosis of encephalitis can be established with absolute certainty only by the microscopic examination of brain tissue, and, similarly, the etiology is established only by the recovery from or the demonstration in brain tissue of an infectious agent. In clinical practice, the diagnosis frequently is based on neurologic manifestations, the recovery of infectious agents from other sites in the body, the serologic evidence of a specific infection, and relevant epidemiologic findings.

Encephalitis frequently is classified as primary or as postinfectious or parainfectious. Primary encephalitis is an illness in which encephalitis is the major manifestation. Symptoms are caused by direct invasion and replication of an infectious agent in the central nervous system (CNS), resulting in objective clinical evidence of cerebral or cerebellar dysfunction. Postinfectious or parainfectious encephalitis occurs after or in combination with other illnesses that are not CNS illnesses, or after a vaccine or other product has been administered. Manifestations may be mediated immunologically.

When neurologic clinical findings suggest encephalitis but inflammation of the brain has not occurred (e.g., in Reye syndrome), the condition is identified by the less specific term *encephalopathy*. Frequently, when encephalitis or meningoencephalitis occurs, other areas of the nervous system, such as the spinal cord (myelitis), nerve roots (radiculitis), and nerves (neuritis), also are involved.

HISTORY

Rabies encephalitis was recognized in ancient times in Europe and Asia.[169] In 100 CE, Celsus noted the relationship of animal rabies to human disease. "Sleeping sickness" associated with epidemic influenza was noted early in the 18th century.[345] For the past 100 years, epizootics of encephalitis in equine animals have been observed in the United States, and in 1933, St. Louis encephalitis virus was isolated from the brains of humans dying from epidemic encephalitis.[230,251] Meningoencephalitis was recognized at the beginning of the 20th century as a complication of mumps.[103] Nonpolio enteroviruses have been known for the past 55 years to be a cause of encephalitis; during the same period, more than 400 zoonotic arthropod-borne viruses have been discovered, and of these, 100 or more cause encephalitis in humans.[26]

ETIOLOGY

Table 42–1 presents etiologic agents in acute encephalitis, meningoencephalitis, and acute illnesses with an encephalitic component. All of the infectious agents or diseases are presented more fully and are referenced more completely elsewhere in this book (see Index).

Despite extensive testing and evaluation, the etiology of most cases of encephalitis remained unexplained in more recent prospective studies.[121,193] During the first 2.5 years of the California Encephalitis Project, 334 immunocompetent patients older than 6 months of age were identified who met the case definition of encephalopathy requiring hospitalization and having at least one other sign or symptom, including fever, seizure, focal neurological findings, cerebrospinal fluid (CSF) pleocytosis, or electroencephalography (EEG) or neuroimaging findings consistent with encephalitis.[121] Of these cases, a confirmed or probable viral agent was identified in only 9 percent of cases, and bacterial and parasitic agents were found in only 3 percent and 1 percent, respectively; an etiology was not discovered for 62 percent of the cases. A possible etiology was noted in 12 percent, a noninfectious cause was identified in 10 percent, and a nonencephalitis infection was identified in 3 percent.

VIRUSES

As a group, the herpesviruses are the most frequently identified agents responsible for viral encephalitis. Herpes simplex encephalitis is the most common cause of sporadic fatal encephalitis in the United States, with approximately one third of cases occurring in patients younger than age 20 years but older than 6 months and approximately half occurring in patients older than 50 years.[230,281,335,351] Molecular analyses of paired oral or labial and brain sites have indicated that herpes simplex encephalitis can be the result of a primary infection, a reactivation of latent herpes simplex virus (HSV), or a reinfection by a second HSV.[348] Although in neonates HSV type 2 (HSV-2) is a leading cause of severe and frequently fatal encephalitis,[352] in older children, the usual cause is HSV-1.[71,164,183,219,231,263,273,337] Recurrent genital infection with HSV-2 occasionally is associated with an aseptic meningitis, but this type 2 virus rarely causes encephalitis outside the newborn period except in immunocompromised individuals.

Encephalitis can occur in association with primary infection with varicella-zoster virus (VZV) (chickenpox) and with endogenous recurrent disease (herpes zoster).* In a study in Finland of more than 3000 patients with acute CNS infections of suspected viral origin, VZV constituted 29 percent of all confirmed or probable etiologic agents.[187] In chickenpox, the rate of encephalitis is approximately 0.3/1000 cases,[49] and the case-fatality rate is approximately 17 percent.[265] Of patients with herpes zoster, 0.5 to 5 percent have encephalitis.[168] This complication occurs more commonly in immunocompromised patients.

In infectious mononucleosis, encephalitis occurs in less than 1 percent of cases. Most patients with Epstein-Barr virus encephalitis are adolescents and young adults, and although patients typically present 1 to 3 weeks after the onset of mononucleosis syndrome, encephalitis may be the presenting complaint in Epstein-Barr virus infection.[79,82,96,153,207,335] Caruso and associates[45] reported five children with subacute and chronic neurologic deficits associated with apparent primary Epstein-Barr virus infections. Severe chronic involvement of the brain is a common finding in congenital cytomegalovirus (CMV) infection.[143] Encephalitis caused by acquired CMV infection is uncommon

*See references 1, 42, 74, 84, 101, 148, 167, 188, 189, 271, 305.

TABLE 42–1 Etiologic Agents in Acute Encephalitis and Acute Meningoencephalitis

Etiologic Agents	Frequency*
Viruses	
Spread person-to-person only	
Herpes simplex types 1 and 2	+++
Varicella zoster	++
Epstein-Barr	+
Cytomegalovirus	++
Human herpesvirus type 6	++
Human herpesvirus type 7	+
Enteroviruses	++
Reoviruses	+
Influenza A and B	++
Respiratory syncytial	+
Parainfluenza 1-3	+
Adenovirus	+
Rubella	++
Human Coronavirus	+
Mumps	+++
Measles	++
Variola	+
Hepatitis A, B, C	+
Human parvovirus	+
Rotavirus	+
BK and JC	+
Spread to humans by mosquitoes or ticks	++
Arboviruses†—those that occur in the United States are the following: St. Louis, West Nile, Eastern equine, Western equine, Venezuelan equine, California, Powassan, and Colorado tick fever	
Spread by warm-blooded mammals	
Rabies	+++
Simian herpesvirus (herpesvirus B)	+
Lymphocytic choriomeningitis	++
Encephalomyocarditis	+
Vesicular stomatitis	+
Equine Morbillivirus (Hendra virus)	+
Nipah	+
Monkeypox	+
Bacteria	
Haemophilus influenzae, *Neisseria meningitidis*, *Streptococcus pneumoniae*, *Mycobacterium tuberculosis*, and other bacterial meningitides often have an encephalitic component	+++
Spirochetal infections: *Treponema pallidum*, *Leptospira*, *Borrelia burgdorferi*, and other *Borrelia* spp. infections	+++
Brucella spp.	+
Actinomyces and *Nocardia*	+
Bartonella henselae	+
Listeria monocytogenes	+
Other	
Chlamydia psittaci, *Chlamydia pneumoniae*	+
Rickettsial infections: Rocky Mountain spotted fever, ehrlichiosis, Q fever, and typhus	+++
Mycoplasma infections: *Mycoplasma pneumoniae* and *Mycoplasma hominis*	++
Fungal: *Coccidioides immitis*, *Cryptococcus neoformans*, and other fungal meningitides often have an encephalitic component	++
Protozoal: *Plasmodium* spp., *Trypanosoma* spp., *Naegleria* spp., *Acanthamoeba* spp., *Balamuthia mandrillaris*, and *Toxoplasma gondii*	+++
Helminths: *Trichinella spiralis*, *Schistosoma* spp., *Strongyloides stercoralis*, *Baylisascaris procyonis*	++
Drug: trimethoprim	+

*Frequency refers to the rate of occurrence of encephalitis or encephalitis component in the particular disease cited and not its relative overall occurrence; +++ = frequent, ++ = infrequent; + = rare.

†See Chapters 184, 187, 188, 202 and 203 for viral diseases in other countries transmitted by arthropods.

and usually occurs in immunocompromised children.[135,153,304] Several cases have been described in previously healthy patients, however.

Human herpesvirus type 6 (HHV-6) is an important cause of acute febrile illness and roseola infantum in young children, commonly associated with febrile convulsions.[100] Encephalitis is a rare complication of HHV-6 infection in children with and without roseola.[8,46,164-166,221,247,361] HHV-6 increasingly has become recognized as an important cause of encephalitis in immunocompromised, post-transplantation patients, occasionally manifesting as severe amnesia after they have undergone bone marrow transplantation.[35,70,76,115,213,290,306,338] HHV-7 also they have undergone has been implicated as a causative agent in encephalitis.[263,328,344]

Enteroviruses now are the leading viral cause of neurologic disease in children in the United States, and they are a major cause of encephalitis.* The following viral types have been associated with encephalitis: coxsackieviruses A2, 4 to 7, 9, 10, 16, and B1-5; echoviruses 1 to 9, 11 to 25, 27, 30, and 33; and enterovirus 71. In 1998, an extensive epidemic of enterovirus 71 disease occurred in Taiwan.[64,154,157,208,211,342,357,358] Manifestations of illness in this epidemic varied, and many children had severe neurologic events, including meningitis, meningoencephalitis, encephalitis, cerebellitis, and polio-like syndrome. In particular, numerous children had brain stem encephalitis, with many fatalities occurring. A variety of neurologic illnesses, including encephalitis, have occurred rarely in reoviral infections.[102,173,188,190,364]

Encephalitis occurs with some regularity as a manifestation of influenza viral infection,† more often described in children than in adults. Although particularly notable during 1997 to 2001 influenza A epidemics in Japan, cases of influenza-associated encephalitis and encephalopathy also have been described in North America and Europe, and influenza B has been implicated as well.[237,240,318,326,360] Numerous other clinical CNS manifestations have been shown to occur during the course of influenza infection, including Reye syndrome (see Chapter 56), acute necrotizing encephalopathy, and myelitis.[160,250,289,317] Rarely, encephalitis occurs during the course of respiratory infections with respiratory syncytial virus, human coronavirus, and parainfluenza virus infections.‡

Adenoviruses are uncommon, but not rare, causes of encephalitis and meningoencephalitis.[50,88,89,97,186,244,252] Adenoviral types 1 to 3, 5 to 7, 11, 12, and 32 have been recovered from either the brain or the CSF in affected patients. More recently, a syndrome of transient encephalopathy associated with adenovirus type 3 has been described.[316]

Neurologic involvement is a common manifestation of congenital rubella virus infection,[144] and encephalitis is a rare complication of noncongenital disease.[63,201,232] Some data suggest a rate of encephalitis in rubella between 1/5000 and 1/10,000 cases.[50] In the pre-vaccine era, the encephalitis rate in one epidemic in 1964 was 1/5000 cases, and in another epidemic in 1942, it was 1/6000 cases.[217,301]

Before the use of mumps vaccine became widespread, this virus was the leading cause of meningoencephalitis in the United States. Today, mumps is rare, although extensive mumps outbreaks with associated cases of encephalitis occurred in 2006.[60] The incidence of encephalitis among individuals with mumps is approximately three episodes per 1000 cases, and the case-fatality rate is 1.4 percent.[53] In the pre-vaccine era, measles was an important cause of severe encephalitis in children.[49,51] Measles is a rare occurrence in the United States, and encephalitis occurs uncommonly, at a rate of 0.74/1000 cases of measles; the case-fatality rate is 14 percent. Smallpox (variola virus infection), before its worldwide eradication, was a rare cause of encephalitis.

Hepatitis B virus and potentially hepatitis C virus are rare causes of encephalitis, and encephalitis has been reported as a complication of erythema infectiosum (human parvovirus infection).[16,17,34,141,246,315] Encephalitis and cerebellitis have been noted in association with rotavirus gastroenteritis,[126,212,242] and BK and JC viruses have been detected in the CSF of patients with suspected encephalitis.[23,24,340] A case of encephalitis in a 3-year-old boy caused by vesicular stomatitis virus has been described.[270] This virus can be spread by direct contact with infected animals or by insects.

Arboviruses are the most important worldwide cause of severe encephalitis. The occurrence of specific arboviruses is seasonal and highly geographic. More than 400 different arboviruses exist, and detailed information about illnesses caused by specific types in various areas of the world is provided in Chapters 184, 187, 188, 202 and 203.[26,153,176] In the United States, eight arboviruses (eastern equine, western equine, Venezuelan equine, St. Louis, Powassan, West Nile, California, and Colorado tick fever) that cause encephalitis have been isolated.

First detected in the Western Hemisphere in 1999 in New York City, West Nile virus subsequently spread across North America from the Atlantic to the Pacific coasts, and into Canada and Mexico.[39,55,104,216,162,239,261] Between 1999 and 2005, greater than 18,000 cases were reported in the United States, with more than 700 deaths.[57,150] An estimated 1 in 150 infections results in severe neurologic illness, and, although the incidence of neuroinvasive disease increases with age, West Nile virus has been responsible for encephalitis in young children and adolescents, occurring in approximately one fifth of the 150 pediatric cases diagnosed in the United States in 2002.[73,113,149] Before the introduction of West Nile virus, La Crosse encephalitis virus was the most common cause of pediatric arboviral encephalitis in the United States.[225,294] Of all North American causes of arboviral encephalitis, eastern equine encephalitis virus results in the most severe disease and has the highest case-fatality rate, particularly among infants and children.[280]

Human rabies is uncommon in the United States, but more than 20,000 cases and deaths occur worldwide every year.[356] Since the early 1970s, approximately two cases of rabies have occurred per year in the United States, and approximately 50 percent of the cases have occurred in children and teenagers.[6,52] Although rabies previously was considered to be universally fatal, a 15-year-old girl with clinical rabies survived after induction of coma and administration of antiviral therapy.[353] Encephalitis caused by herpesvirus B is rare; it occurs predominantly in monkey handlers and usually after monkey bites.[163] Except in outbreak situations, lymphocytic choriomeningitis virus rarely is recognized as a cause of encephalitis.[28] Serologic surveys have indicated, however, that neurologic disease caused by this virus is not rare in the United States,[89-91,170,230] and clusters of lymphocytic choriomeningitis virus cases were identified among recipients of solid organ transplants in 2003 and 2005.[108] Rare cases of encephalitis are caused by encephalomyocarditis virus; infection of humans with this virus is common, but most infections go unrecognized.[324]

Since its emergence in Japan in the 1870s, Japanese encephalitis virus, a mosquito-borne flavivirus, has spread across Asia to become the most important cause of epidemic encephalitis worldwide, with an estimated 35,000 to 50,000 cases and 10,000 deaths annually.[309,310,332] Nipah virus, a new paramyxovirus, is the first wide-scale epizoonotic encephalitis with direct animal-to-human, rather than vectorial, transmission.[66] The initial outbreak of Nipah virus encephalitis occurred among pig farmers in Malaysia and Singapore in 1999, and subsequently outbreaks have been identified in Bangladesh and India.[25,62,124,145,156,195,204,321,322] A fatal case of encephalitis caused by Hendra virus (equine morbillivirus), another novel paramyxovirus, was reported in an adult[253];

*See references 22, 50, 58, 59, 71, 88, 89, 91, 97, 136, 153, 175, 179, 186, 198, 230, 234, 236, 244.

†See references 22, 50, 74, 88, 89, 91, 109, 122, 123, 188, 244, 255, 271, 317, 343, 366.

‡See references 16, 29, 49, 88, 91, 188, 241, 319, 341, 359.

previously, this virus had been noted in association with fatal respiratory infections in horses and humans.

In 2003, another emerging pathogen, Chandipura virus, was responsible for a large outbreak of acute encephalitis in 329 children in southern India, with a case-fatality rate of 56 percent.[272] This rhabdovirus transmitted to humans by sandflies was responsible for a second outbreak in western India in 2004, with an even higher case-fatality rate of 78 percent.[61] During the 2003 monkeypox outbreak in the midwestern United States, one child developed severe acute encephalitis and seizures; no additional encephalitis cases were identified.[295]

BACTERIA

Signs and symptoms of acute encephalitis (drowsiness, coma, convulsions, mental confusion) commonly occur in *Haemophilus influenzae*, *Neisseria meningitidis*, and *Streptococcus pneumoniae* bacterial meningitides, but the true etiology usually is established easily by the examination of the CSF. Spirochetal infections are a more common cause of nervous system disease, specifically encephalitis, than generally is appreciated. Encephalitis is a recognized complication of leptospirosis, Lyme disease, and relapsing fever.[29,38,95,257,260,311] *Brucella* spp. are infrequent causes of meningoencephalitis, and *Bartonella henselae* encephalitis is an uncommon complication of cat-scratch disease.[43,120,227,228,245,314,362] Infection with *Listeria monocytogenes* has been shown to mimic herpetic and West Nile virus encephalitis.[81,266]

Neurologic disease is a common complication of pertussis. A 10-year study in the United States indicated a rate of neurologic disease in infants of approximately 9 per 1000 cases.[105] An extensive review by Miller and associates[232] suggested that the neurologic disease occurring with pertussis rarely, if ever, is inflammatory, and it is better classified as an encephalopathy.

OTHER AGENTS

Encephalitis is an uncommon occurrence in psittacosis, occurring in 1 to 3 percent of cases.[44,114] It can be caused by *Chlamydia psittaci* and *Chlamydia pneumoniae*. Neurologic involvement commonly occurs in Rocky Mountain spotted fever.[151,178] In one study, two thirds of the ill children had evidence of encephalitis. Neurologic sequelae are common.[127] Neurologic involvement also occurs in nonspotted fever rickettsial infections.[98,125,191,210,258,273,299] *Coxiella burnetii*, *Ehrlichia canis*, *Rickettsia typhi*, *Rickettsia canada*, and *Rickettsia conorii* all have been implicated.

Mycoplasma pneumoniae is an important cause of encephalitis.* Pönkä[264] noted that 4.8 percent of hospitalized patients with *M. pneumoniae* infections had CNS manifestations, and most of them had encephalitis or meningoencephalitis. *Mycoplasma hominis* is a rare cause of neonatal meningoencephalitis.[215]

Numerous fungi cause neurologic illness.[287] These illnesses occur most commonly in immunocompromised patients, but some infections occur in apparently normal individuals. Meningitis and brain abscess are the most common pathologic events, but encephalitis is associated commonly with meningitis. The following fungal agents are the most common causes of meningoencephalitis in children and adults with normal immunologic status: *Blastomyces dermatitidis*, *Coccidioides immitis*, *Cryptococcus neoformans*, *Cladosporium* spp., *Histoplasma capsulatum*, and *Paracoccidioides brasiliensis*.

Involvement of the brain in parasitic infections is common, and the reader is referred to Section XXII for a complete review. Cerebral malaria is a common complication of *Plasmodium falciparum* infection. Meningoencephalitis and enlarging cerebral mass lesions rarely occur in acute acquired toxoplasmosis.[330] The free-living ameba *Naegleria fowleri* is a rare cause of encephalitis, but infection with this agent usually is fatal.[293,325] Most cases occur in children and young adults and are caused by swimming or playing in contaminated water. Fatal encephalitis also has resulted from infection with *Balamuthia mandrillaris*, a soil ameba formerly thought to be innocuous.[132,339] *Baylisascaris procyonis*, a raccoon roundworm, has been associated with severe and often fatal encephalitis in children.[56,117,118,354] A recurrent encephalitis caused by administration of trimethoprim has been reported.[152]

POSTIMMUNIZATION

Neurologic disease, including encephalitis and meningoencephalitis, has occurred after immunization with a variety of prophylactic and therapeutic preparations. Depending on the type of immunizing agent, the encephalitis can be the result of an immunologic reaction, a CNS infection with the vaccine virus, or a combination of infection and immunologic reaction. Historically, many of the observed neurologic reactions occurred after the administration of antisera prepared in animals in the treatment of specific diseases. Antisera to the following diseases or infectious agents have been noted in association with neurologic illness: tetanus, diphtheria, scarlet fever, tuberculosis, gas gangrene, pneumococcus, gonococcus, meningococcus, and streptococci.[3,161,180,231,279] Of 100 neurologic syndromes complicating administration of serum reviewed by Miller and Stanton,[231] only 10 percent were of a cerebral or meningeal type.

Neurologic disease was a common complication of rabies vaccine derived from animal nervous tissue.[231] The incidence of complication was between 3/1000 and 1/6000 cases.[32,231] Approximately 10 percent of the neurologic disease attributed to this rabies vaccine was meningoencephalitic or encephalomyelitic. Five cases of CNS disease (Guillain-Barré syndrome, demyelination, meningoradiculitis) have been reported in temporal association with the administration of human diploid cell rabies vaccine.[262] This occurrence is so rare that a causal relationship with vaccine is uncertain.

Encephalitis was an important complication of smallpox vaccination.[7,37,110,130,175,199,238,312] The rate of encephalitis varied markedly from one study to another, from 1 in 4000 primary vaccinations in the Netherlands[238] to approximately 1 in 80,000 primary vaccinations in the United States.[200] After reinstatement of the vaccination among military personnel and selected civilian groups in the United States, three cases of suspected encephalitis or myelitis were identified among 665,000 individuals vaccinated from 2002 to 2004.[296]

Neurologic disease, including encephalitis, occurs rarely after the administration of typhoid-paratyphoid vaccine.[231] Neurologic disease also rarely has been attributed to administration of tetanus toxoid and diphtheria toxoid, but the manifestations seldom are central.

Encephalitis and encephalopathy have been observed after the administration of influenza immunization.[119,131,282,355] In the extensive surveillance that occurred in the United States during the period October 1, 1976, to December 16, 1976, when 45,651,113 people received the A/New Jersey/76 influenza vaccine, no epidemiologic evidence of an association between vaccine and encephalitis was noted.[139] Two children who developed acute disseminated encephalomyelitis after receiving Japanese B encephalitis vaccination have been reported.[248]

Neurologic disease developing after administration of a whole-cell pertussis vaccine is a well-known event.[67-69] Pathologic evidence in fatal cases suggests encephalopathy rather than encephalitis.[80] Because neurologic illness similar to that which

*See references 30, 31, 47, 77, 85, 86, 140, 185, 209, 264, 302, 308, 331.

occurs after the administration of pertussis vaccination is a frequent development in infants who have not been vaccinated, establishing a true rate of pertussis vaccine encephalopathy, or that such an entity exists at all, has been difficult. The analysis of studies suggests that encephalopathy caused by pertussis vaccine does not occur.[68,69,134,280]

Neurologic disease, including encephalitis, is a rare complication of measles immunization.[48,51,111,197] The rate in vaccinees in the United States is less than 1/1 million. In contrast, the finding in the National Childhood Encephalopathy Study in England, Scotland, and Wales indicated a rate of 1/87,000 immunizations.[2] This high rate of encephalopathy may be an artifact due to the misclassification of complicated febrile convulsions as encephalopathy. Meningoencephalitis has been shown to be a rare complication of mumps immunization with some vaccine virus strains,[222] although it has not been a problem in the United States. More recent studies in the United States and Finland have failed to show an association between measles-mumps-rubella vaccination and encephalitis or encephalopathy.[214,275] A fatal encephalitis occurred in a 3-year-old child after receiving 17D yellow fever vaccine.[268]

POSTINFECTIOUS ENCEPHALITIS

Postinfectious or parainfectious encephalitis occurs after a demonstrated or presumed viral infection and is thought to be immune-mediated rather than due to a direct effect of the virus in nerve cells.[133,170-172,285] This theory has been studied extensively by Johnson[171,172] and Griffin[133] in encephalitis associated with measles. They have described a periventricular demyelinating disease and have not been able to isolate measles virus or identify measles antigens in nervous tissue. Other investigators, including one of us (J. D. C.), have recovered measles virus, however, from the CSF and brain of affected patients.[111,226,229,269,298] We suggest that immune mechanisms play a role in the pathogenesis of measles and perhaps other postinfectious neurologic illnesses, but the process is stimulated by the direct presence of the antigen in the nervous system. The mechanism of disease is important regarding possible treatment: steroids might be useful in immune-mediated disease but could be detrimental in an acute viral infection.

In contrast to measles, other apparent postinfectious encephalitides that usually have a subacute onset are immune mediated and have multifocal white-matter lesions.* Specifically, acute demyelinating encephalomyelitis (ADEM) usually is subacute in onset and is characterized by optic neuritis, myelitis, ataxia, hemiparesis, cranial nerve palsies, and multifocal white-matter lesions that are easily confused with multiple sclerosis. Subtle features help distinguish between the two disorders, however.[307] If solitary or unilateral lesions are present, it most likely is ADEM, whereas lesions in the corpus callosum are much more common in multiple sclerosis. Lesions greater than 4 cm diameter suggest ADEM, and ADEM lesions generally have indistinct borders compared with multiple sclerosis lesions, which generally have sharp borders. Patients with ADEM may respond dramatically to treatment with steroids and perhaps intravenous immunoglobulin or plasmapheresis.

CHRONIC ENCEPHALITIC OR ENCEPHALOPATHIC ILLNESSES

"Slow infections" that cause encephalitic and encephalopathic illness in humans have been recognized for many years. Many of these illnesses now are recognized as viral infections or caused by prions. Viral illnesses include progressive multifocal leukoencephalopathy (JC, SV40, and BK viruses), subacute sclerosing panencephalitis (measles virus), and acquired immunodeficiency syndrome (AIDS) (human immunodeficiency virus [HIV] type 1 [HIV-1] and HIV-2). Prion diseases, termed *transmissible spongiform encephalopathies*, include kuru, Jakob-Creutzfeldt disease, and Gerstmann-Sträussler-Scheinker disease.[267] Prion diseases are related to scrapie of sheep and bovine spongiform encephalopathy (mad cow disease), which are prion diseases of animals. These chronic illnesses are discussed in Chapter 205.

EPIDEMIOLOGY

Because encephalitis has many different causes, no unified epidemiologic pattern exists. The specific epidemiology of each infectious agent or disease is presented in detail in the respective chapters of this book; only a brief overview is presented here. Most cases occur in the summer and fall, reflecting arboviral and enteroviral etiologies. Encephalitis caused by arboviruses occurs in localized outbreaks and epidemics, with boundaries determined by the range of particular mosquito vectors and the prevalence of natural reservoir animals.

Arboviruses are zoonoses in which humans are infected accidentally by an arthropod vector, humans not being essential in the life cycle of arboviruses. Most commonly, mosquitoes or other insects acquire arboviruses by biting infected birds, which often have prolonged viremia without illness. The insect vectors, although preferring birds, bite other vertebrates, including humans and horses. Encephalitis in horses and mules may be the first indication of incipient trouble in an area; veterinarians often are the first to detect an impending epidemic. Rural exposure is not a sine qua non; urban and suburban outbreaks are frequent.

Although enteroviral disease, including aseptic meningitis, occurs in epidemics, severe encephalitis caused by these agents usually is a sporadic event. Sporadic cases of encephalitis occur in any season; epidemiologic considerations that must be reviewed in a search for the causative agent include geographic area; climatic conditions; animal, water, food, soil, and personal exposures; and host factors.

PATHOGENESIS

Because encephalitis and meningoencephalitis have multiple causes, the lack of a unified pathogenesis is not surprising.* Clinical manifestations of encephalitis can result from a direct or an indirect effect of an infectious agent on the brain. Rabies, arbovirus, herpes simplex, and enteroviral encephalitides are examples in which the viral infections directly involve tissue cells within the brain. In contrast, encephalitic symptoms in bacterial meningitides and in rickettsial infections may be caused by the vasculitis and liberated toxins of the surrounding infection. In addition, in many postinfectious or parainfectious encephalitides, immunologic events clearly are important in the pathogenesis. Measles and *M. pneumoniae* infections are in this category.

Postinfectious or parainfectious encephalitis is an acute demyelinating disease of the brain in which the findings suggest an autoimmune process. Usually, little evidence of an active infectious process is present when symptoms occur. Viral or other agents probably invaded the CNS initially and then were cleared but were a trigger for the subsequent development of disease. An immune (T-cell) response to myelin basic protein occurs.

*See references 4, 11, 15, 36, 83, 138, 181, 202, 224, 227, 238, 323, 359.

*See references 7, 27, 47, 77, 99, 107, 111, 127, 133, 137, 144, 153, 168, 170, 230-232, 235, 349.

In most encephalitides, such as those caused by arboviruses, mumps, and enteroviruses, the CNS infection is secondary to a primary viral infection elsewhere in the body. Generally, the infectious agents, whether from ingestion, as in enteroviral infections, or from the bite of a mosquito, as in an arboviral infection, enter the lymphatic system. In the lymphatics, viral multiplication occurs, which results in seeding of the bloodstream and infection of other organs in the body. Viral multiplication occurs at these secondary infection sites, extensive secondary viremia occurs, and then the CNS becomes infected. Actual involvement of nervous tissue may result from growth across or passive diffusion through brain capillaries or centripetal axonal transport of virus from the olfactory neuroepithelium to the olfactory bulb.[235]

Infection of the brain also may occur through the peripheral nerves. This retrograde spread of virus is important in rabies and HSV encephalitis.

PATHOLOGY

Determining the etiology of encephalitis at autopsy is difficult, although morphologic identification of falciparum malaria, trypanosomiasis, and fungal encephalitis is possible.[80,170,231,232,312] In viral encephalitides, the histopathologist may recognize rabies (Negri bodies) or an agent of the herpesvirus group (intranuclear inclusion bodies).

Tissue sections of the brain generally reveal meningeal congestion and mononuclear infiltration, perivascular cuffs of lymphocytes and plasma cells, some perivascular tissue necrosis with myelin breakdown, neuronal disruption in various stages (including ultimately neuronophagia), and endothelial proliferation or necrosis. A marked degree of demyelination with preservation of neurons and their axons is considered to be predominantly postinfectious or parainfectious (autoimmune) encephalitis. The severity and the extent of observed lesions vary with the infectious agent and with the degree of reaction of the host. The cerebral cortex, especially the temporal lobe, often is affected severely by HSV; the arboviruses tend to affect the entire brain; and rabies has a predilection for the basal structures. Involvement of the spinal cord, nerve roots, and peripheral nerves varies.

CLINICAL MANIFESTATIONS

The clinical findings in encephalitis are determined by the severity of involvement and anatomic localization of the affected portions of the nervous system, the inherent pathogenicity of the offending agent, and the immune and other reactive mechanisms of the patient ("host factors"). A wide range of severity of clinical manifestations exists even with the same etiologic agent. Evidence of brain parenchymal involvement is the hallmark of encephalitis. Children with encephalitis may show evidence of diffuse disease, such as behavioral or personality changes; decreased consciousness; and generalized seizures or localized changes, such as focal seizures, hemiparesis, movement disorders, cranial nerve defects, and ataxia. Some children may seem to be mildly affected initially only to lapse into coma and sudden death. In others, the illness is ushered in by high fever, violent convulsions interspersed with bizarre movements, and hallucinations alternating with brief periods of clarity, and the children emerge with relatively few sequelae.

Most commonly, the initial manifestations resemble an undifferentiated acute systemic illness with fever, headache, or, in infants, screaming spells, abdominal distress, nausea, and vomiting. Signs of an associated mild nasopharyngitis may suggest a respiratory infection. As the temperature increases, new findings direct attention to the nervous system: mental dullness eventuating in stupor; bizarre movements; convulsions; nuchal rigidity, often not as pronounced as in purely meningitic illness; and focal neurologic signs, which may be stationary, progress, or fluctuate. Loss of bowel and bladder control and unprovoked emotional outbursts may occur.

SPECIFIC FORMS OF ENCEPHALITIS

Specific forms of encephalitis or complicating manifestations of encephalitis include Guillain-Barré syndrome and related syndromes, acute transverse myelitis, acute hemiplegia, brain stem encephalitis, and acute cerebellar ataxia. Acute cerebellar ataxia is characterized by an abrupt onset of truncal ataxia resulting in varying degrees of gait disturbance and balance abnormalities. Children with this illness have tremulousness of the head and trunk when in the upright position and of the extremities when attempting to move them against gravity. The duration of illness varies from 3 to 4 days to several weeks. Acute cerebellar ataxia often follows chickenpox or other viral illnesses. In one study, 3 percent were related to immunization.[78] Approximately 90 percent of patients recovered completely from the ataxia, but 20 percent had transient behavioral or intellectual disturbances. Five percent had persistent learning problems.

Brain stem encephalitis (Bickerstaff encephalitis) is a rare disorder, but it is important because clinical signs appear similar to those of a brain stem glioma. The differentiation is important because treatment is radically different. The differentiation is made by the time of onset of symptoms and by the course. Brain stem glioma usually has slowly progressive symptoms developing over the course of several weeks or months. Brain stem encephalitis evolves over 1 to 7 days. Both disorders may be associated with radiographic evidence of brain stem enlargement. Brain stem encephalitis resolves after 1 to 4 weeks, whereas a tumor continues to progress until radiation therapy is given.

Most cases of brain stem encephalitis seem to be postinfectious and are similar to postinfectious cerebellar ataxia, Miller-Fisher syndrome, or Guillain-Barré syndrome. The conditions often overlap.[12] In postinfectious cases, the onset of brain stem encephalitis begins 1 to 3 weeks after a nonspecific viral infection. Brain stem encephalitis has been reported to occur, however, as a result of specific, identifiable, and possibly treatable infectious agents, including HSV,[278,292] VZV,[283,320] CMV,[116,174] and enterovirus 71[157,159] and *M. pneumoniae*,[196,313] *L. monocytogenes*,[5,9,10,13,19] *Propionibacterium acnes*,[41] and *Campylobacter jejuni*.[363]

Some patients with a typical clinical picture of brain stem encephalitis have had anti-GQ1b antibodies in the serum,[65] which may represent a subgroup of postinfectious brain stem encephalitis. Brain stem encephalitis may arise in HIV-infected patients and may be due to a treatable cause, such as herpes simplex encephalitis.[116,143,282] Brain stem encephalitis caused by viral infections may resolve similar to the typical postinfectious type, but some viral infections may lead to permanent neurologic sequelae. In an enterovirus outbreak in Taiwan, the most common neurologic complication was rhombencephalitis, and a 14 percent mortality rate was reported.[157,159] More recent reports suggest that intravenous immunoglobulin may be an effective therapy in some patients.[112] In a Cochrane review, this therapy remains unproven, however.[254]

DIFFERENTIAL DIAGNOSIS

The evaluation of a patient with an acute CNS illness (encephalopathy) must be considered carefully, and the sequence of tests should be dictated by the specific circumstances of the individual patient. Several disease processes may have a presentation similar

to that of encephalitis or meningoencephalitis. The differential diagnosis of acute encephalopathy includes the following:

1. Metabolic diseases, such as hypoglycemia, uremic encephalopathy, hepatic encephalopathy, and rare genetic inborn errors of metabolism, including disorders of glucose or ammonia metabolism
2. Toxic disorders, such as drug ingestion or Reye syndrome
3. Mass lesions, such as tumor or abscess
4. Subarachnoid hemorrhage from arteriovenous malformation or aneurysm
5. Embolic lesions caused by bacterial endocarditis
6. Acute demyelinating disorders, including acute multiple sclerosis, ADEM, and acute hemorrhagic leukoencephalitis
7. Status epilepticus, especially nonconvulsive status epilepticus, such as complex-partial status or absence status
8. Infectious diseases, including viral, bacterial, fungal, chlamydial, mycoplasmal, and parasitic
9. Postinfectious diseases, including Guillain-Barré syndrome, brain stem encephalitis, Miller-Fisher syndrome, and acute cerebellar ataxia
10. Acute confusional migraine

EVALUATION OF A PATIENT WITH ENCEPHALOPATHY OR POSSIBLE ENCEPHALITIS

Obtaining a careful history and performing a physical and neurologic examination are essential in all patients who present with a history consistent with encephalitis. The differential diagnosis previously presented indicates that encephalitis is only one of many disorders that can manifest with an acute or subacute picture of encephalopathy. Although the diagnosis of encephalitis may be determined best with a lumbar puncture and evaluation of the CSF, lumbar puncture may be contraindicated in some disorders and, if performed inappropriately, may lead to serious complications and even death. A child who has a cerebellar tumor with acute obstruction of the fourth ventricle may present with a decreasing level of consciousness caused by the rapidly increasing intracranial pressure (ICP). Nuchal rigidity may be present. The family may not have recognized the more subtle changes in cerebellar functions for the months before the acute obstruction developed and may give a history of acute encephalopathy. In that case, a lumbar puncture could result in herniation through the foramen magnum. It is essential that the patient be assessed for the possibility of increased ICP and the potential for herniation.

The history should be reviewed carefully, questioning specifically for symptoms of neurologic problems that manifested in the days or weeks before the acute disorder occurred. The physical examination must be performed with special attention given to focal neurologic abnormalities, cerebellar signs, and evidence of increased ICP. Conducting a careful funduscopic examination is important but may be impossible in an agitated patient or young child. The presence of papilledema indicates that neuroimaging should be performed before doing the lumbar puncture. If spontaneous venous pulsations are noted on funduscopic examination, ICP is not increased, and the lumbar puncture can be done without imaging the patient.

In addition to lumbar puncture, neuroimaging and EEG can help in determining the cause of the encephalopathy and in determining the most appropriate course of therapy. The history and physical examination are a guide to the most appropriate first test to perform, but generally neuroimaging is the most likely to be helpful. The exception would be a child in nonconvulsive status epilepticus. The history may suggest encephalitis as the most likely diagnosis, but in some patients, nonconvulsive status epilepticus may be clinically indistinguishable from encephalitis.

NEUROIMAGING

Most patients with encephalopathy should undergo neuroimaging to aid diagnosis of treatable conditions, such as HSV encephalitis. Computed tomography (CT) is helpful in the acute setting to identify abnormalities such as tumor or abscess and to decide whether performing a lumbar puncture is safe. CT is not as helpful, however, as is magnetic resonance imaging (MRI) in detecting the subtle changes associated with encephalitis.[40,206,300] In many cases of viral encephalitis, CT and MRI yield normal results or only nonspecific changes, such as swelling[347] or edema.[206] An important exception is herpes simplex encephalitis.

As previously noted, MRI is more sensitive than is CT. In one study, CT initially was positive in only 42 percent of cases, whereas MRI was positive in all cases.[182] MRI in HSV characteristically shows abnormalities in the medial temporal lobes, inferior frontal cortex, and insula.[205,350] The likelihood of finding the abnormalities may be increased by using T2-weighted imaging and fluid attenuated inversion recovery (FLAIR) sequences[14,177] or diffusion-weighted imaging.[297,327] Diffusion-weighted MRI seems to be more sensitive than is FLAIR or T2-weighted sequences in the detection of HSV or other encephalitides.[334] The localization of abnormalities may differ, however, from the classic pattern in young children. In neonatal herpes encephalitis, widespread changes occur in the periventricular white matter, often sparing the medial temporal and inferior frontal lobes. Another pattern has been described in children aged 4 to 13 months in whom the cortex and adjacent white matter of the hemispheres were abnormal.[205]

In addition to herpes, other encephalitides may yield abnormal neuroimaging. CT and MRI results often are abnormal with disorders caused by arbovirus or enterovirus infections. When imaging is abnormal, it usually is nonspecific, showing areas of decreased density (with CT) or increased signal intensity (with MRI) in the gray or white matter. A variety of MRI abnormalities with certain viral encephalitides have been reported. The basal ganglia, brain stem, and thalami have been reported to be abnormal on MRI of patients with eastern equine encephalitis,[93] Japanese encephalitis,[158,192] and enterovirus 71.[157,159] These differences help to distinguish HSV from other, nontreatable causes of viral encephalitis.

Postinfectious disorders most often are associated with selective oligodendrocyte involvement.[21] Imaging shows increased signal in white matter with T2-weighted MRI or low-density white matter with CT.[18,35,147,181,249] Patients with acute hemorrhagic leukoencephalitis, a rare disease that is rapidly progressive and often fatal, may present with a clinical picture similar to that of herpes simplex encephalitis. In contrast to HSV infection, the CT results often are abnormal within the first 1 or 2 days.[284,346] If a patient with suspected herpes simplex encephalitis has abnormal CT results early in the course, acute hemorrhagic leukoencephalitis should be considered.

Another imaging technique that has been reported to be helpful in establishing the diagnosis of encephalitis is single photon emission computed tomography (SPECT). Initial reports suggest that SPECT is more sensitive than is CT. Ackerman and colleagues[2] found that SPECT showed greater sensitivity and more precise localization than did conventional radionuclide scanning and CT. Launes and associates[202] studied 14 encephalitis patients and found that SPECT detected temporal lobe abnormalities in all six of the patients with HSV encephalitis and

yielded normal results in the remaining eight who had other etiologies. A few cases of normal MRI scans but abnormal SPECT in patients with herpes simplex encephalitis have been reported[146,218]; this is less likely to occur with newer MRI sequences such as diffusion weighted imaging. If SPECT is performed, Tc 99 m hexamethylpropyleneamine oxime seems to be superior to Tc 99 m ethyl cysteinate dimer.[87,106] Generally, SPECT should be reserved for cases with normal MRI and a nondiagnostic EEG in which herpes simplex encephalitis is still strongly suspected. Intracranial ultrasonography in neonates has been shown to be helpful in establishing the diagnosis and in follow-up of infants with HSV or CMV infections.[219]

ELECTROENCEPHALOGRAM

Generally, an EEG should be performed in most patients with encephalitis. EEG results of patients with encephalitis generally are normal or nonspecifically abnormal, showing diffuse slowing. Crucial exceptions to the general rule exist, however.

In acute encephalopathy, comatose patients may be in nonconvulsive status epilepticus,[328] which requires immediate and appropriate intervention. The presence of periodic lateralized epileptiform discharges (PLEDs) on EEG strongly suggests the possibility of herpes simplex encephalitis but also may be an indication of seizures.[20] Early in the course of herpes encephalitis, generalized slowing of the background frequencies and focal slowing over the affected temporal lobe may occur. Within a few days, the characteristic PLEDs pattern develops in most cases. Later in the course, the background activity between the bursts of PLEDs gradually may flatten. Occasionally, other areas of the brain seem to be involved, primarily with HSV. PLEDs, although strongly suggestive of HSV encephalitis, are not diagnostic. PLEDs have been reported with stroke and infectious mononucleosis encephalitis,[129] and periodic complexes are characteristic of the slow virus and prion disorders, including Jakob-Creutzfeldt disease and subacute sclerosing panencephalitis.

The EEG abnormalities in neonatal herpes encephalitis are similar. The characteristic EEG results yield periodic or pseudoperiodic complexes, usually triangular or sharp waves, occurring in a multifocal pattern.[233] In one study of 34 infants with herpes encephalitis, 21 underwent EEG; the results of 19 of the 21 were abnormal. The results of three showed only focal slowing, but the other 16 showed the characteristic periodic or pseudoperiodic complexes.[286] The authors reviewed 500 other neonatal EEG records and found 20 with similar complexes; 11 patients had meningoencephalitis of unknown etiology, 3 had hemorrhage, and 2 had asphyxia. Four were placed in a miscellaneous category. Periodic or pseudoperiodic complexes in neonatal EEG results strongly suggest HSV encephalitis, but they are not diagnostic.

SPECIFIC DIAGNOSIS

A meticulous history is essential and must evaluate exposure in the previous 2 to 3 weeks to illness in contacts; exposure to mosquitoes, ticks, and animals during recent vacations or picnics or other outdoor activities; awareness of illness in animals, especially horses and other Equidae, in the patient's environment; recent travel from the home area; recent injections of any kind; and the possibility of accidental exposure to heavy metals, pesticides, or other questionable substances. The CSF must be examined carefully to exclude other disorders that respond to specific therapy. Smears for bacteria, appropriate rapid antigen-identification tests, and cultures of the CSF are mandatory; the history and clinical findings may indicate the need for acid-fast stain and culture of the sediment for mycobacteria. Other circumstances may indicate the need for excluding fungal or protozoal infection; atypical cells may require cytopathologic study to exclude neural neoplasms that may manifest acutely.

The availability of polymerase chain reaction has allowed the definitive and rapid diagnosis of HSV encephalitis to be established, eliminating the need for brain biopsy.[94,194,291,337,352] HSV DNA was detected in the CSF of 53 of 54 patients with biopsy-proven herpes simplex encephalitis.[194] The etiology of encephalitis caused by other herpesviruses also has been determined by polymerase chain reaction assay of CSF.[84,221,335] In addition to its use in herpesvirus infections, polymerase chain reaction is useful in neurologic illnesses caused by enteroviruses and *M. pneumoniae* and in the future is likely to be useful in other encephalitides.[31,184,256,276]

In viral encephalitis, the CSF frequently is clear; the leukocyte count ranges from none to several thousand, often with a significant percentage of polymorphonuclear cells initially, moderate or no elevation of protein, and an initially normal level of glucose relative to the simultaneously determined blood glucose level. In any patient suspected to have viral meningoencephalitis, spinal fluid, blood, feces, and throat swabs should be collected and sent to a laboratory offering viral diagnostic services. An additional serum specimen should be collected 10 to 21 days later. Although these studies may not provide an immediate diagnosis, they may give early warning of a specific epidemic, and the use of specific antiviral chemotherapy may be indicated by the preliminary culture results.

Inquiry regarding recent illness, recent injections, and, especially, recent exposures away from the home environment sometimes is helpful. The incubation periods of some arboviruses are such that mosquito bites acquired at least 1 week earlier or insect bites now healed may give a clue. Occasionally, patients who have traveled to Africa or Asia in preceding weeks present with encephalitis caused by viruses, trypanosomiasis, or falciparum malaria with bizarre systemic and CNS signs and symptoms.

TREATMENT

Acyclovir should be used to treat herpes simplex and VZV encephalitis and perhaps encephalitis caused by Epstein-Barr virus. CMV encephalitis should be treated with ganciclovir. Pleconaril (if it becomes available) should be considered for treatment of enteroviral encephalitis, and oseltamivir should be considered for treatment of encephalitis caused by influenzaviruses A or B. Specific antimicrobial treatment should be used for infections caused by spirochetes, *Chlamydia*, *Mycoplasma*, fungi, and parasites.

General treatment is nonspecific and empiric, aimed at maintaining life and supporting each involved organ system. The effectiveness of various recommended regimens in most instances has not been evaluated objectively. Until a bacterial etiology and, in particular, a brain abscess are excluded substantially, parenteral antibiotic therapy should be administered.

Anticipating and being prepared for convulsions, cerebral edema, hyperpyrexia, inadequate respiratory exchange, disturbed fluid and electrolyte balance, aspiration and asphyxia, abrupt cardiac and respiratory arrest of central origin, cardiac decompensation, and gastrointestinal bleeding are crucial. The syndrome of disseminated intravascular coagulation may be an additional complication.

For these reasons, all patients with severe encephalitis should receive care in intensive care units. Cardiac monitoring should be maintained. Repeat CT and MRI scans are helpful in following comatose patients and often show signs of brain swelling before the patient has the typical clinical indicators of

ICP, such as Cushing triad (systolic hypertension, bradycardia, and slowing of respirations), dilated pupils, and decorticate or decerebrate posturing. The Cushing triad is an unreliable indicator of increased ICP, and, when the other signs of increased ICP occur, they often do so late in the course, when the patient's cerebral perfusion already is at risk.[223] If brain swelling becomes a problem, placing an ICP monitor may be necessary. The ICP should be maintained at less than 15 mm Hg if possible using the standard techniques for reduction of ICP, including hyperventilation, osmotic diuretics, and removal of CSF. As a last resort, inducing a barbiturate coma may be necessary. A related consequence of ICP is the syndrome of inappropriate antidiuretic hormone secretion. Careful monitoring of the fluid and electrolyte balance is essential in all seriously ill patients with encephalitis.

All fluids, electrolytes, and medications initially are given parenterally. In patients with prolonged coma, parenteral hyperalimentation is indicated. Normal blood levels of glucose, magnesium, and calcium must be maintained to minimize the threat of convulsions.

Status epilepticus caused by encephalitis should be treated vigorously using a structured protocol to ensure optimal control.[303,331] The current standard initial therapy is intravenous lorazepam, 0.1 to 0.2 mg/kg, up to 4 mg maximum. Seizures associated with encephalitis may be refractory to the usual therapy, and other anticonvulsants may be required to achieve and maintain control of seizures. In patients who fail initial therapy and are in medically refractory status epilepticus, continuous EEG monitoring usually is recommended to monitor the efficacy of the therapy, especially when the patient is in nonconvulsive status epilepticus.[92,329]

If after a second attempt, lorazepam fails to control the seizures, intravenous phenytoin (preferably phosphenytoin in children) is the next drug of choice. The dose is 18 to 20 mg/kg, maximum 1000 mg, given over 20 minutes. Phosphenytoin is preferred because it can be administered faster and does not cause sclerosis of the veins as does phenytoin and is not as likely to cause cardiac arrhythmias. Virtually all patients who require therapy beyond lorazepam need to be intubated to prevent respiratory embarrassment. If phosphenytoin is unsuccessful, or as an alternative to phosphenytoin, intravenous midazolam has gained favor in recent years.[75,258] The initial dose is 0.1 to 0.2 mg/kg over 5 minutes, with a maintenance infusion starting at 0.05 mg/kg/hr up to a maximum of 0.4 mg/kg/hr. Another alternative therapy is propofol, which generally is administered by an anesthesiologist.

Many methods have been proposed to minimize cerebral edema and to diminish the consequences of cerebral anoxia. The following measures are difficult to evaluate and generally are reserved for patients with severe illness whose condition apparently is desperate.

1. Dexamethasone, 0.1 to 0.2 mg/kg intravenously in an initial dose followed by 0.05 to 0.1 mg/kg intravenously every 4 to 6 hours, is given. This large dose should be reduced gradually after a few days if recovery or improvement is evident. Dexamethasone probably should not be used in acute viral diseases because steroids may potentiate the viral infection.
2. Other substances employed in an effort to reduce elevated ICP include (a) mannitol, given intravenously, as a 20 percent solution in a dose of 0.25 to 1 g/kg over a 30- to 60-minute period (this may be repeated every 8 to 12 hours), and (b) glycerol, by nasogastric tube, using 0.5 to 1 mL/kg diluted with twice that volume of orange juice. This regimen is nontoxic and may be repeated every 6 hours for an extended period.

For more than 40 years, steroids and adrenocorticotropic hormone frequently have been used as empiric therapy for encephalitis. No controlled studies have shown any efficacy, however. In two comparative studies of measles encephalitis, steroids were found to offer no benefit, and in both studies, the steroid recipients seemed to have had worse outcomes.[33,365] More recently, in a carefully controlled study, no benefit of high-dose dexamethasone was found in the treatment of acute encephalitis caused by Japanese encephalitis virus.[155]

In contrast to these investigations are more recent clinical experiences in the treatment of acute disseminated encephalomyelitis, in which MRI studies have indicated multifocal white-matter lesions.[36,138,181,224] Patients treated with steroids often have responded dramatically, with clinical improvement and resolution of the lesions as shown by MRI. Steroids, along with specific antibiotic therapy, also may be beneficial in the treatment of encephalitis caused by *M. pneumoniae* infection.[185,302]

In our opinion, steroids should not be used to treat encephalitis if the patient has an active infection, unless the infection can be treated concomitantly with an effective antimicrobial agent. Plasmapheresis and intravenous immunoglobulin have been used empirically to treat brain stem encephalitis and other encephalitides, but no studies have been done that indicate such therapies are helpful.[243]

Equipment and personnel for handling emergencies such as cardiac and respiratory arrest must be on hand constantly. Early consultation with an anesthesiologist or intensive care specialist is useful in anticipating the need for artificially assisted respiration.

Supportive and rehabilitative efforts are important after the patient recovers. Motor incoordination, convulsive disorders, squint, total or partial deafness, or behavioral disturbances may appear only after some time. Visual disturbances caused by chorioretinopathy and perceptual amblyopia also may make a delayed appearance. Special facilities and sometimes institutional placement may become necessary.

PROGNOSIS

The prognosis in all encephalitides is guarded with respect to immediate outcome and sequelae. Sequelae involving the CNS may be intellectual, motor, psychiatric, epileptic, visual, or auditory. Cardiovascular, intraocular, pulmonary, hepatic, and other systems sometimes are affected permanently. The short-term and long-term prognoses depend to some extent on etiology and age. Young infants usually have severe disease and sequelae. Generally, HSV carries a worse prognosis for survival and residual disability than do the enteroviruses.

Rautonen and associates[274] examined prognostic factors in childhood acute encephalitis at the Children's Hospital, University of Helsinki, during a 20-year period from 1968 to 1987. This study comprised 462 cases with the following etiologies: mumps virus, measles virus, rubella virus, VZV, HSV, enteroviruses, respiratory viruses, *M. pneumoniae*, other agents, and cause undetermined. The investigators found that mortality was fivefold greater in infants compared with older children. Children who were disorientated or unconscious before admission had 4-fold and 25-fold greater risks for death and severe damage than did children whose level of consciousness had been normal. Patients with HSV or *M. pneumoniae* infection had the greatest risks for death or serious residual damage compared with children with encephalitis of other etiologies.

California encephalitis has a low mortality rate but occurs most frequently in the pediatric age group. Of patients who experience seizures in the acute phase of their disease, 25 percent have a permanent seizure disorder.[128] Psychological sequelae were present in 15 percent in one series but were not found to be significant in several series if the children were evaluated several years after their illnesses.[72,220,277]

Prognosis in encephalitis caused by western equine virus is guarded; 56 percent of infants younger than 1 month of age have had recurring seizures with marked motor and behavioral changes. After they reach 1 year of age, the sequelae appear to diminish; only 5 percent of adults have neurologic sequelae. Fifty-seven percent of infants who survived western equine virus infection and who were younger than 1 year of age at the time of infection had major neurologic sequelae requiring either a special school or institutionalization late in life. Severe retardation, paralysis, spasticity, recurrent convulsions, hearing deficits, and speech difficulties all were reported as complications.[100,107]

Eastern equine encephalitis has a high mortality rate. Infants and children younger than 5 years of age who survive usually have severe sequelae consisting of mental retardation, convulsions, and paralysis. These consequences are in contrast to adults older than 40 years of age who survive, who recover completely or have only slight damage.

St. Louis encephalitis has a low mortality rate. Although neurologic sequelae are reported, their incidence is low in the pediatric age group.

PREVENTION

The widespread use of effective attenuated viral vaccines for measles, mumps, and rubella almost has eliminated CNS complications from these diseases in the United States. The control of encephalitis caused by arboviruses has been less successful because specific vaccines for the arbovirus diseases that occur in North America are unavailable. Control of insect vectors by suitable spraying methods and eradication of insect breeding sites is useful.

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CHAPTER 43

Parainfectious and Postinfectious Disorders of the Nervous System

CHAPTER 43a

Parainfectious and Postinfectious Demyelinating Disorders of the Central Nervous System

Timothy Edward Lotze

Acute Disseminated Encephalomyelitis

Acute disseminated encephalomyelitis (ADEM) is a monophasic demyelinating disease of the central nervous system (CNS) that results in acute, polysymptomatic neurologic disability. It also has been termed *postinfectious encephalomyelitis*. It is related to other central inflammatory demyelinating conditions of childhood, including optic neuritis, transverse myelitis, neuromyelitis optica (Devic disease), and multiple sclerosis. Although no single diagnostic test can be used to distinguish among these conditions at the initial presentation, certain clinical features, laboratory results, and imaging findings can be used to ensure a correct diagnosis. Most of these conditions are thought to be caused by autoimmune dysregulation triggered by an infectious agent in a genetically susceptible host.

DIAGNOSTIC CRITERIA

The term *acute disseminated encephalomyelitis* has been used variably in the literature in describing clinical characteristics of this disease. Discrepancies exist among descriptive studies regarding (1) the occurrence of encephalopathy, (2) the association with preceding infection, (3) symptoms that are monofocal or multifocal, and (4) the possibility for recurrence. Lack of standardized diagnostic criteria for ADEM has impeded the ability to understand this distinct disease better as part of the spectrum of demyelinating conditions occurring in childhood.

More recently, the International Pediatric Multiple Sclerosis Study Group developed diagnostic criteria for ADEM.[32] The group created working definitions for monophasic ADEM and for recurrent forms of the disease. Table 43–1 lists the diagnostic criteria. An absolute criterion for a diagnosis of ADEM is the presence of encephalopathy. This criterion is defined to include either behavioral changes, such as lethargy or irritability, or more severe alterations in level of consciousness, such as coma. The onset of the encephalopathy must correspond with the occurrence of the disease state. Premorbid cognitive difficulties, such as learning difficulties or mental retardation, would not be included in this definition. Magnetic resonance imaging (MRI) shows multiple lesions in both hemispheres distributed throughout the white matter (Fig. 43–1). A distinguishing characteristic of ADEM is prominent involvement of the cortical gray matter and deep gray nuclei (basal ganglia and thalamus). Such involvement is atypical for multiple sclerosis and other demyelinating conditions. The lesions of ADEM are asymmetric, showing variable size, shape, and distribution between the hemispheres. MRI showing symmetric and confluent lesions should prompt the clinician to consider other diagnoses, such as leukodystrophies and inborn errors of metabolism. If MRI shows evidence of previous demyelination, the clinician should query the history further for previous attacks, which would suggest either a recurrent form of the disease or a chronic demyelinating condition. Cerebrospinal fluid (CSF) analysis may show a pleocytosis of greater than 50 cells, in contrast to pediatric multiple sclerosis, in which only a slight pleocytosis (<50 cells) is observed.

The International Pediatric Multiple Sclerosis Study Group has defined criteria to distinguish between an evolving pattern for the initial event and for recurrent forms of ADEM. Any new and fluctuating symptoms occurring within 3 months of the initial event are considered to be part of the same inciting event. In addition, symptoms that occur during steroid taper or within 1 month of the patient's completing a steroid taper are considered to be part of the same inciting event. Relapsing ADEM and multiphasic ADEM are the two recurrent forms of the disease. Both forms are defined to occur more than 3 months after the initial event and more than 1 month after completion of steroids. By definition for ADEM, both forms must include a clinical

TABLE 43–1 Diagnostic Criteria of Acute Disseminated Encephalomyelitis

Clinical Features
First clinical attack of demyelinating disease in CNS
Acute or subacute onset
Polysymptomatic presentation
Must include encephalopathy
Acute behavioral change (e.g., irritability, lethargy)
Alteration in consciousness (e.g., somnolence, coma)
Attack should be followed by improvement
Lesion Characteristics on MRI FLAIR and T2-Weighted Images
Multifocal, hyperintense, bilateral, asymmetric lesions in the white matter
At least one or more lesions >1-2 cm
Gray matter, especially basal ganglia and thalamus, may be involved
Spinal cord MRI may show confluent intramedullary lesions
No radiologic evidence of previous destructive white matter changes
Cerebrospinal Fluid
Pleocytosis ≥50 WBCs can be observed
Other
No other etiologies can explain the event
New or fluctuating symptoms and signs occurring within 3 mo of the inciting ADEM event are part of the same acute event

Note: *Symptoms that vary during periods of steroid taper within 3 mo of the inciting event or occur <30 days after discontinuation of all steroids are considered part of the initial inciting event.*

ADEM, acute disseminated encephalomyelitis; CNS, central nervous system; FLAIR, fluid-attenuated inversion recovery; MRI, magnetic resonance imaging; WBCs, white blood cells.

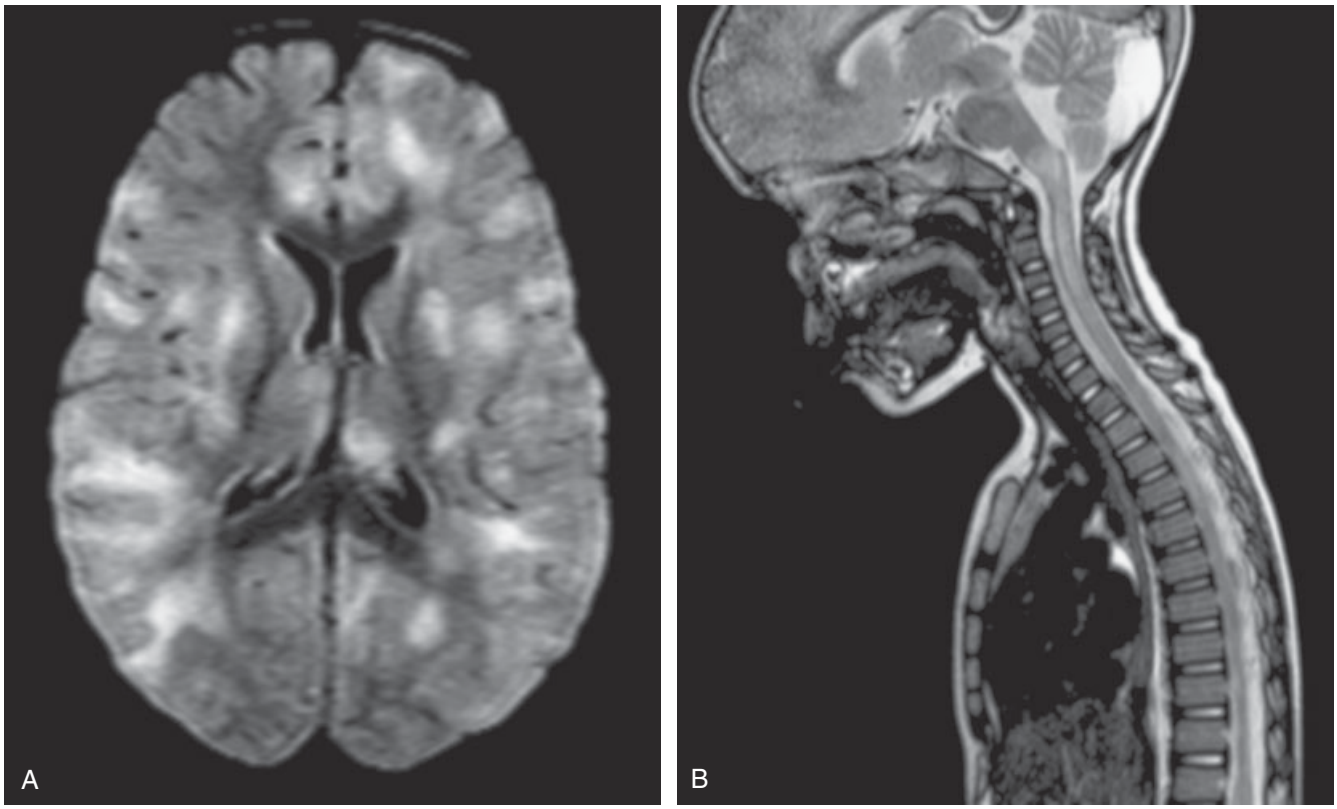

Figure 43–1 **A,** Axial fluid-attenuated inversion recovery (FLAIR) image showing multifocal areas of hyperintensity in both cerebral hemispheres involving cortical gray matter, centrum semiovale, and deep gray nuclei. **B,** Sagittal T2-weighted spine image with increased intrinsic signal consistent with longitudinally extensive transverse myelitis in the same patient. (*Courtesy of Tim Lotze, M.D.*)

presentation with encephalopathy. Relapsing ADEM describes the recurrence of the same symptoms that occurred at the time of the initial presentation. The MRI findings also are similar to the initial event, although they may show enlargement of the previous lesions. Multiphasic ADEM describes children with recurrent disease characterized by symptoms that differ from the initial event. MRI likewise shows development of new lesions different from those seen with the first attack.

CLINICAL MANIFESTATIONS

A febrile illness occurs in 50 to 75 percent of children in the 4 weeks before the onset of typical neurologic symptoms.[38] Preceding vaccinations have been associated with the occurrence of ADEM, but this is less common.[13,47] Fever, headache, vomiting, and meningismus often are present at the time of initial presentation and may persist during the hospitalization.[8,23] Neurologic symptoms typically appear 4 to 13 days after the infection develops or vaccination is administered.[13,23,40,47] New clinical symptoms may continue during hospitalization, and they may alter the treatment for the patient. As per current diagnostic criteria, all children with ADEM have an encephalopathy at the time of presentation.[32] The degree of altered mental status varies, ranging from irritability to somnolence to coma. Encephalopathy may be the initial symptom that brings the child to medical attention. Although alteration in mental state often raises concern for the possibility of seizures, they occur in only one third of patients.[15,47] In addition to having encephalopathy, patients exhibit various other neurologic features. The most common of these are long tract signs, acute hemiparesis, cerebellar ataxia, and cranial neuropathy.[8,15,23,40,47] Aphasia, movement disorders, and sensory deficits occur less commonly.

Demyelination of the optic nerves (optic neuritis) or spinal cord (transverse myelitis) may occur. Symptoms of optic neuritis include vision loss, pain with eye movement, and an afferent papillary defect. Inflammation of the optic disk may be seen on direct funduscopic examination if there is extensive involvement of the optic nerve. Patients with retrobulbar optic neuritis typically have a normal funduscopic examination. Optic neuritis may occur in one or both eyes, with differing degrees of involvement. Symptoms of transverse myelitis include flaccid paralysis of the legs, with a sensory level on examination. The arms can be involved as well if the demyelinating lesion is in the cervical cord. Respiratory failure may occur with high cervical lesions that extend into the brain stem. Bowel and bladder involvement secondary to spinal cord disease results in constipation and urinary retention.

The extent of demyelination in the CNS may not be recognized fully at the time of initial presentation, particularly if the patient has a severe encephalopathy. Imaging of the entire CNS to include the brain, orbits, and spinal cord should be done in all patients meeting diagnostic criteria for ADEM because co-occurrence of transverse myelitis or optic neuritis can have a significant impact on rehabilitation needs and long-term outcome.

CLINICAL VARIANTS

Relapsing ADEM and multiphasic ADEM are the two recurrent forms of the disease. Both forms are defined by reoccurrence of neurologic symptoms more than 3 months after the initial event and more than 1 month after completion of steroids. Most often, recurrence of disease is singular. Repeated events are unusual and should prompt assessment for a different underlying disease

process, including metabolic disorders or primary inflammatory diseases such as multiple sclerosis.

Acute hemorrhagic leukoencephalitis is considered to be a severe variant of ADEM. It accounts for 2 percent of patients with ADEM.[47] Clinical presentation is similar to that of ADEM, including an acute onset of neurologic deficits 1 to 3 weeks after an upper respiratory infection or vaccination. Seizures and coma ensue within hours. The mortality rate is extremely high with fulminant disease. Survivors often have severe residual neurologic deficits. The clinical presentation and imaging features mimic those typically seen in herpes simplex virus encephalitis. Evidence of inflammatory changes and hemorrhage on MRI often is not present for the first few days in herpes simplex virus encephalitis, which may help to distinguish the two conditions.[18] Early recognition with prompt institution of steroids or other immunosuppressive agents can be lifesaving.

Historically, site-restricted forms of demyelination, such as optic neuritis or transverse myelitis, have been included as part of the spectrum of ADEM. Although these conditions share similar underlying pathology, clinical presentations and prognosis are different, and they should be considered to be separate entities. They are considered to be clinically isolated syndromes by the current consensus definitions for demyelinating disease.[32] Clinically isolated syndromes may carry a greater risk for the development of multiple sclerosis or other recurrent forms of demyelinating disease.[49] *Neuromyelitis optica*, also known as *Devic disease*, describes coincident or sequential optic neuritis and longitudinally extensive myelitis. It is not always perinfectious in onset and is associated with antibodies directed against aquaporin-4 water channels in the CNS.[50]

Multiple sclerosis is the second most common cause of acquired neurologic disability in adults. It is an uncommon finding in pediatric patients. Five percent of adults with multiple sclerosis had onset of disease before reaching 18 years of age.[4] Clinical phenotypes consistent with ADEM, relapsing ADEM, and multiphasic ADEM should not be confused with multiple sclerosis. Although they may share some of the same initiating pathologic mechanisms, they are different diseases.[10] The diagnosis of multiple sclerosis requires clinical or radiographic evidence for demyelinating events separated in space and time in the CNS. Patients typically follow a relapsing course of neurologic attacks with resolution or remission of disability between attacks. Evolution into a course of progressive disability occurs in 50 percent of patients who have had the disease for more than 15 years.[7] Treatment consists of medications that modulate the immune response by decreasing migration into the CNS, altering cytokine profiles, and interrupting antigen presentation.

EPIDEMIOLOGY

The estimated incidence of ADEM is 0.8 per 100,000 population per year.[34] ADEM occurs just as frequently in children as it does in adults.[10] It has no specific gender predilection or ethnic distribution.[13,14] In contrast to multiple sclerosis, which generally has a higher incidence at more northern latitudes, ADEM has no appreciable geographic distribution.

PATHOLOGY AND PATHOGENESIS

ROLE OF INFECTION

ADEM is preceded by a viral or bacterial infection in 75 percent of the cases. This infection usually is in the form of a nonspecific upper respiratory infection. Many different pathogens have been identified in association with the illness (Table 43–2). No well-defined latency period has been identified in which an infection can be related to ADEM. Generally, patients present within 1 month of their illness. Correlation between ADEM and an infection occurring more than 30 days prior is more difficult because children are diagnosed with a viral infection four to six times per year, resulting in a positive infectious history in 33 to 50 percent of patients.[38]

TABLE 43–2 Infectious Pathogens Associated with Acute Disseminated Encephalomyelitis

Viral
Coxsackie
Cytomegalovirus
Epstein-Barr
Hepatitis A or B
Herpes simplex
HHV-6
HTLV-1
Human immunodeficiency virus
Influenza A or B
Measles
Mumps
Rocky Mountain spotted fever
Rubella
Vaccinia
Varicella
Bacterial
Borrelia burgdorferi
Campylobacter
Chlamydia
Legionella
Leptospira
Mycoplasma pneumoniae
Rickettsia rickettsii
Streptococcus

HHV-6, human herpesvirus-6; HTLV-1, human T-cell lymphotrophic virus-1.

The phenotypic presentation for ADEM varies depending on the infectious agent. Some organisms have been associated with typical clinical features. Measles virus infection is associated with ADEM in 1 in 1000 cases. The clinical course often is fulminating, with severe neurological sequelae or death in most patients. Introduction of vaccination has reduced markedly the occurrence of measles in developed countries, although vaccine-associated ADEM is described in 2 cases per 1 million.[6]

Post-varicella ADEM is characterized by ataxia in more than 90 percent of patients, occasionally with an explosive onset.[24] Headache and meningismus are typical constitutional signs. Pyramidal symptoms include bilateral symmetric upper motor neuron weakness. Patients also may have mood disturbances with a depressed affect or irritability.

ADEM that develops after a rubella infection is similar to measles, with an explosive onset in most children.[24] There is often profound lethargy and coma and generalized seizures. Most patients with explosive onset of disease have pyramidal signs and myelitis, whereas a milder phenotype is more likely to have ataxia. Both forms also have been associated with brain stem signs.

Group A beta-hemolytic streptococcal infection has been associated with a dystonic extrapyramidal syndrome. Abnormal movements include dystonia, tremor, and parkinsonism. Tics and chorea have not been associated with this condition. Similar to Sydenham chorea and pediatric autoimmune neuropsychiatric disorders associated with streptoccocal infection (PANDAS), behavioral disturbances, such as emotional lability, obsessive-compulsive tendencies, and inappropriate speech, can occur. Changes in mental status can vary from irritability to coma. MRI shows a predilection for demyelination within the basal ganglia, which includes the caudate, putamen, and globus pallidus. Other

deep gray structures, including the thalamus, subthalamus, and substantia nigra, may be affected as well. This finding is distinct from Sydenham chorea and PANDAS, in which imaging is unremarkable. It has been associated with anti–basal ganglia antibodies that cross-react with certain strains of streptococcus.[14]

ROLE OF IMMUNIZATION

Vaccine-associated ADEM has been described with nearly every immunization (Table 43–3). The Collaborating Center for Reference and Research on Viral Hepatitis of the World Health Organization has suggested a maximal period of 3 months to diagnose vaccination-associated ADEM. Post-vaccination ADEM accounts for less than 5 percent of ADEM cases.

Historically, Pasteur rabies vaccination was associated with prototypic ADEM in approximately one in 1000 individuals.[6] The inoculum was derived from rabbit spinal cord injected with fixed rabies virus. The disease was thought to result principally from the neural tissue contaminating the vaccine, rather than the virus itself. This suggestion is supported further by the continued higher incidence of vaccine-associated encephalomyelitis in patients receiving the Semple or duck embryo vaccinations, both of which contain neural tissue. These forms of the vaccination usually are found in developing countries. Experimental allergic encephalomyelitis, the animal model of demyelinating disease, is induced by inoculating myelin or myelin antigens into a suitable experimental animal, further supporting a role of CNS tissue as the causal agent.

Currently, measles, mumps, and rubella vaccination is associated most often with post-vaccination encephalomyelitis. There is a significant difference between the incidence of live-measles vaccination associated ADEM (1 to 2 per 1 million) and the incidence of ADEM previously associated with the measles virus infection (1 in 1000).

Smallpox was eradicated in 1980 following a successful vaccination program that was discontinued in the United States in 1971.[11] The World Health Organization has allowed retention of the variola virus at a designated laboratory in the United States and Russia for the purpose of creating a safer vaccination than the presently available live vaccinia virus. More recent concerns for bioterrorism have resulted in the reinstitution of smallpox vaccination to certain identified groups within the United States that might come into contact with the virus as first responders to a possible smallpox outbreak. The previously reported incidence of ADEM associated with smallpox vaccination was 10 to 300 cases for every 1 million vaccinations. It is estimated, based on these figures, that vaccination of the entire British population would result in approximately 600 cases of vaccine-associated ADEM. Administration of anti-vaccinia gamma-globulin at the time of vaccination can help to prevent this complication.[44]

TABLE 43–3 Vaccines Associated with Acute Disseminated Encephalomyelitis

Diphtheria-tetanus-polio
Hepatitis B
Hog vaccine
Japanese B encephalitis
Measles
Mumps
Polio
Rabies
Smallpox
Tetanus
Tick-borne encephalitis

IMMUNOLOGIC FACTORS

A review of the normal process of immune system regulation and surveillance of the CNS is useful when discussing immune dysfunction in the pathogenesis of ADEM. Similar to other disorders of immune dysfunction, activated $CD4^+$ T cells play a principal role in the disease process. T cells reactive against self-antigens, including myelin components, are present in the normal immune system. Several regulatory mechanisms are thought to prevent activation of these lymphocytes, and failure of such processes results in autoimmune conditions. The thymus plays a crucial role in normal T-cell development by deleting many autoreactive lymphocytes from the immune system. Other peripheral mechanisms also are needed because this thymic depletion often is incomplete. Clonal anergy describes the unresponsive state of T cells that have encountered their antigen without costimulatory factors.[41] A risk for failure of this mechanism exists through release of nonspecific, costimulatory factors from damaged tissue. Another peripheral process is immunologic ignorance, in which no productive reaction exists between the T cell and its corresponding peptide/MHC complex on an antigen-presenting cell. Changes in antigen availability, such as through increased antigen presentation by an invading microorganism, can disrupt this safeguard.[26]

Active regulation is a third process by which regulatory T cells prevent expansion of self-reactive lymphocytes.[31] Regulatory T cells exert their suppressive effects through direct cell-to-cell contact with membrane-bound molecules (CTLA-4) or indirectly through soluble suppressive cytokines, such as interleukin-10 and transforming growth factor-β.[25] Natural killer cells expressing a T-cell receptor also can regulate autoimmune diseases.[37] The presence of such cells within demyelinating regions suggests that some of the inflammation associated with ADEM may have a protective effect.

Although failure of these mechanisms may result in the activation of T cells reactive against myelin antigens, migration of lymphocytes into the CNS is required to produce disease. The CNS has been considered to be a site of limited immunologic surveillance because of the blood-brain barrier and lack of classic lymph vessels. The blood-brain barrier is principally a mechanical diffusion barrier, however, for hydrophilic molecules formed by specialized endothelial cells at the level of the capillaries. Leukocytes readily cross through endothelial cells in post-capillary venules to occupy the perivenular spaces or move on to the neuropil.[21] In addition, antigens and antigen-presenting cells drain from the brain into cervical lymph nodes via the cribriform plate and perineural sheath of the cranial nerves.[12,28] As discussed later, these factors allow for the presentation of antigen in the systemic immune compartment and passage of leukocytes into the CNS for production of disease.

Children with demyelinating disease of the CNS are thought to have a genetic predisposition for such conditions. The strongest data relate to susceptibility genes on chromosome 6p21 in the area of the histocompatibility leukocyte antigen (HLA). Much of the research into these genetic determinants has been related to adult-onset multiple sclerosis.[46] Limited research is available for pediatric demyelinating diseases. Some of the findings show similarities, however, among age groups and various demyelinating diseases. Linkage studies in Russian children found an association between ADEM and HLA-DRB1*01 and HLA-DRB1*017(03) alleles.[24] Korean children with ADEM were found to have higher frequencies of HLA-DRB4*0101 and HLA-DRB1*1501 compared with controls. In adults with multiple sclerosis, the specific genes that confer the highest risk include HLA-DRB1*1501 and HLA-DRB5*0101 among others. In multiple sclerosis, these genes have been linked to earlier disease onset, female gender, a relapsing remitting course, and optic neuritis or spinal involvement as the initial symptom. In addition,

genes associated with this haplotype include transforming growth factor-β family members, CTLA-4, the tumor necrosis factor cluster, interleukin-1 receptor antagonist, interleukin-1, and estrogen receptor. Polymorphisms in these genes also have been associated with increased risk for development of demyelinating disease.[16]

The precise mechanism by which these HLA class II genes confer risk for development of demyelinating disease is unclear. Possibilities include (1) preferential binding of self-antigens by these peptides, (2) preferential linkage of autoreactive T cells to the antigen-presenting cell expressing these peptides, (3) abnormal antigen presentation by certain DR molecules, and (4) engagement of HLA class II molecules leading to intracellular signaling events.[46]

Humoral factors also play a role in the production of demyelinating disease. This finding is based on disease-associated laboratory findings and treatment responses. Analysis of CSF in one third of patients with ADEM shows production of oligoclonal bands, which are not found to be present in the serum.[13] In addition, some patients with post-rabies inoculation ADEM have been found to have positive serum antibodies against myelin basic protein and galactocerebroside.[33]

PATHOGENESIS

ADEM is considered to occur in genetically susceptible individuals prone to immune system dysregulation after an encounter with an appropriate environmental stimulus. Mechanisms of disease production are based principally on two animal models that closely resemble the disease. The first is experimental autoimmune encephalomyelitis.[43] In this model, animals develop monophasic neurologic disease similar to ADEM after receiving immunization with CNS homogenate or encephalitogenic myelin peptides emulsified in Freund complete adjuvant. The second model is Theiler murine encephalomyelitis, in which susceptible mouse strains develop disease after receiving direct injection of Theiler murine encephalomyelitis virus into the cerebrum.[22] In both of these models, increased exposure of the immune system to myelin proteins produces disease.

By comparing these models with ADEM in humans, two pathogenetic concepts have been developed. The inflammatory cascade concept implies a direct CNS infection with a neurotropic pathogen, which results in CNS tissue damage and systemic leakage of CNS-confined autoantigens through a damaged blood-brain barrier into the systemic circulation. Presentation of these antigens within systemic lymphatic organs leads to tolerance breakdown and a self-reactive encephalitogenic T-cell response. The molecular mimicry concept suggests structural amino acid homology between the invading pathogen and myelin basic protein in the host.[17] Although similar to myelin peptides, the amino acid sequence contains subtle differences that fail to prevent immune tolerance and result in activation of myelin-reactive T cells against similar "self" myelin antigens. Epstein-Barr virus provides an example of this. The virus contains a pentapeptide sequence in its nuclear antigen (EBNA) that shares sequence homology with an epitope of myelin basic protein, a major protein of the myelin sheath.[9] Epstein-Barr virus has been implicated in the pathogenesis of multiple sclerosis partly based on this concept of molecular mimicry.[1]

The immunopathologic events leading to ADEM can be divided into two major phases: (1) initial T-cell priming and activation and (2) subsequent recruitment and effector phase.[5] The priming phase occurs in systemic secondary lymphoid organs, where the antigen-presenting cell presents myelin protein antigen and peptides to neuroantigen-reactive T cells. The activated T cells expand and then migrate to the CNS via the postcapillary venules into the perivascular space. In the Virchow-Robin space, the T cells re-encounter their cognate antigen, in the context of HLA class II molecules expressed by dendritic cells.[19] This reactivation allows the T cells to migrate through the glial limitans and enter the brain parenchyma.

Further recruitment occurs through the production of cytokines and chemokines by antigen-presenting cells and activated T cells, promoting migration into the CNS of additional T cells and other leukocytes, such as polymorphonuclear and monomorphonuclear phagocytes.[46] Breakdown of the blood-brain barrier occurs by release of proteases from recruited mast cells, T cells, and monocytes. In addition, production of reactive oxygen radicals occurs, causing further endothelial injury, which leads to the effector phase in which T cells have more of a secondary role to other inflammatory processes that cause demyelination and axonal injury. These inflammatory processes include oxygen and nitrogen radicals, tumor necrosis factor-α, direct and indirect complement activation, antibody-dependent cellular toxicity, myelin phagocytosis, direct axonal injury by $CD8^+$ cytotoxic T lymphocytes, protease secretion, and oligodendrocyte apoptosis.[46] Glutamate-mediated excitotoxic injury of the oligodendrocytes also occurs.[42] The inflammatory process continues for a few days to 2 weeks, resulting in stretches of demyelinated axons, some of which may be transected.

The repair process begins with activation and proliferation of astrocytes. Clearing of debris by macrophages and increased production of anti-inflammatory cytokines and various growth factors by resident cells and T cells occur. Oligodendrocyte precursors become activated and, along with surviving oligodendrocytes, begin the process of remyelination. The clinical and imaging outcome of ADEM most often shows complete recovery. Subtle differences in repaired myelin, including altered thickness and redistribution of sodium channels, may occur, however. In addition, the relative composition of myelin peptides is altered to forms that may have increased vulnerability to further damage[48] and may explain recurrent forms of ADEM.

CLINICAL EVALUATION

ADEM should be considered in a child presenting several days after a febrile illness with subacute onset of encephalopathy and polysymptomatic neurologic deficits. The clinical features require ruling out other possible diagnoses, however, through additional diagnostic tests.

Diagnostic imaging is the most useful tool in establishing the diagnosis. Computed tomography (CT) scans usually are done on an emergent basis for encephalopathic patients. Areas of demyelination may appear as darker areas of hypodensity, with any hemorrhagic component being hyperdense. CT does not adequately show the full burden of disease, however, and may be completely normal. MRI is the most sensitive means for showing the widespread demyelination typical of the disease (see Fig. 43–1). T2-weighted sequences provide the best assessment of the disease, with demyelinating areas being hyperintense and accompanied by surrounding edema. Administration of gadolinium contrast material may show breakdown of the blood-brain barrier with areas of enhancement. This enhancement may appear homogeneous throughout the lesion or show a "broken ring" appearance, with the open edge pointing toward the cortex. Lesions may be large, measuring more than 1 cm in diameter. They typically are rounded with poorly defined margins.

Of most common concern in children presenting with a febrile encephalopathy is the possibility of an underlying CNS infection. A lumbar puncture often is done to investigate this possibility. A lymphocytic pleocytosis may occur (commonly >50 cells). Elevated albumin levels also may be present. Findings of neutrophils and elevated red blood cells alternatively would raise concern for the possibility of herpes encephalitis.

Intrathecal oligoclonal bands may be positive, and IgG synthesis may be increased in 30 percent of patients with ADEM. These abnormalities usually are associated with multiple sclerosis. In ADEM, convalescent testing for both should normalize, whereas tests remain positive in multiple sclerosis. Their relationship to the underlying disease pathophysiology is unclear but is thought to represent a response of B cells within the CNS to the inflammatory process.[3] Serologic studies of the CSF may be useful to uncover the causal agent, although results should be interpreted with caution because many neurotropic viruses have a high prevalence in the general population.

Rarely, patients may present with very large demyelinating lesions, characteristically described as *tumefactive*. In such circumstances, a biopsy may be needed to determine the underlying etiology. Findings typical of demyelination help to rule out a CNS malignancy. Before a biopsy is done, the clinician should investigate for other evidence of demyelinating disease, including imaging of the spinal cord. A comorbid transverse myelitis would provide more evidence of a demyelinating condition and reduce the need for a biopsy. In addition, normal CSF cytology helps to discount an underlying malignancy.

Either a primary or a secondary CNS vasculitis associated with collagen vascular diseases, such as systemic lupus erythematosus or antiphospholipid antibody syndrome, may manifest with a similar clinical and radiologic picture. These diseases are typified by recurrent ischemic strokes. Distinguishing features for antiphospholipid antibody syndrome include a family history of strokes or other thromboses at a young age and fetal loss. Testing for antiphospholipid antibody and lupus anticoagulant is positive. A primary CNS vasculitis with strokes is best investigated through conventional angiography showing an irregular vessel lumen. Leptomeningeal biopsy is considered the gold standard to confirm the diagnosis.

A severe course that principally affects the optic nerves and spinal cord should raise concern for neuromyelitis optica. Serum or CSF testing for the aquaporin-4 water channel antibody that is associated with this disease is clinically available. The test is greater than 90 percent specific for neuromyelitis optica.[50]

Neurophysiologic studies can be useful to evaluate comorbidities and burden of disease. An electroencephalogram should be obtained in patients with seizures and severe encephalopathy to characterize the seizure focus and to investigate for subclinical seizures. Findings often are consistent with a diffuse disturbance in brain function noting slowing of the normal electric rhythms. Visual evoked potentials can evaluate for demyelination of the optic nerves, which may not be appreciated on imaging. The examination measures the time for an electric impulse to travel from the retina to the occipital lobe, with delays suggesting a demyelinating lesion. The patient needs to be attentive to a flashing light stimulus, rendering this test difficult to perform in patients with severe encephalopathy.

TREATMENT

To date, no controlled clinical trials have been conducted regarding the optimal treatment for ADEM. Based on empiric evidence, intravenous high-dose corticosteroids are accepted as first-line treatment.[45] Methylprednisolone is given at 30 mg/kg per dose (maximum 1000 mg) for a total of five doses. Alternatively, dexamethasone, 1 mg/kg per dose for five doses, may be used. A prednisone taper typically is instituted on completion of this regimen with an initial dosing of 1 mg/kg/day and tapering by 5 mg every 5 days. Alternative anti-inflammatory and immunosuppressive therapies may be used depending on the response to the initial steroid treatment. Intravenous immunoglobulin (IVIG) has been found to be beneficial in some patients.[45] IVIG is given 2 g/kg divided over 2 to 5 days.

Plasmapheresis also may be used.[29,30] The patient receives a total of seven treatments consisting of 1.1 to 1.4 plasma volume exchanges over a 14-day course. IVIG and plasmapheresis typically are not considered as first-line treatment for ADEM, partly related to availability of IVIG or pheresis units, cost of treatment compared with steroids, prolonged hospitalization, and need for adequate venous access. Nonetheless, they should be considered as secondary treatment options, especially in the occasional patient who continues to deteriorate during steroid treatment or who fails to show adequate recovery 7 days after completing intravenous steroids. The choice between the two agents is arbitrary. Particular consideration for plasmapheresis might be given in the setting of a comorbid myelopathy because this has been noted to be beneficial in patients presenting with transverse myelitis.[27] Severe cases failing to respond to any of these measures may require immunosuppressive agents, such as cyclophosphamide.[36]

Additional treatment that may be needed for patients depends on the nature of their neurologic deficits. Anticonvulsants should be given for management of seizures. Attention given to bowel and bladder care is important to avoid secondary complications of retention. Impaired swallowing requires adequate nutrition to be provided by gavage feedings. Transverse myelopathy can be associated with autonomic dysfunction, including orthostatic hypotension, necessitating the use of an abdominal binder. Rehabilitation should begin at the time of admission with the assistance of physical medicine and rehabilitation specialists. During the long-term recovery period, modifications to the home and school environment may be needed depending on residual deficits.

OUTCOME AND PROGNOSIS

Historically, ADEM was associated with high morbidity and mortality rates principally related to disease associated with the measles virus. Measles-associated ADEM was associated with death in 25 percent of affected patients, with an additional 30 percent of patients having severe neurologic sequelae.[35] This dismal outcome has improved dramatically with institution of measles vaccination. The introduction of high-dose steroid treatment in modifying the disease course also may have improved the current outcome findings. A 5 percent mortality rate remains associated with this disease.

Currently, 80 to 90 percent of patients show excellent recovery of functional and cognitive deficits.[2,13,24,47] Mild neurologic disabilities, weakness and fine motor difficulties being the most common ones, may be present in some patients. Epilepsy may occur in 6 percent of patients after an episode of ADEM.[13] Neurocognitive testing of children recovering from ADEM has shown mild impairments in attention and executive function and reduced visuospatial and visuomotor skills.[20] The greatest risk for development of neurologic sequelae may be in older patients with sudden onset of severe neurologic symptoms.

Recurrent forms of the disease often raise concern for pediatric multiple sclerosis. The exact relationship between ADEM and multiple sclerosis remains to be better defined. The conditions are distinct, however, based on the clinical presentations, imaging, and clinical courses. Studies investigating risk for disease recurrence or evolution into multiple sclerosis have been limited and do not adequately define ADEM in the context of the current definition, and they do not distinguish between recurrent forms of the disease and multiple sclerosis. Disease recurrence occurs in 30 percent of patients.[38] Most patients have their second attack within the first year after their initial event.[2,39] It often occurs soon after discontinuation of steroid taper, or after an infection or vaccination encountered shortly after the initial event. Treatment and outcome for recurrences are not different from those described for the initial event.

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CHAPTER 43b

INFECTION-ASSOCIATED MYELITIS AND MYELOPATHIES OF THE SPINAL CORD

Mark P. Gorman ● Scott L. Pomeroy

ACUTE TRANSVERSE MYELITIS

The rapid onset of paraplegia and bowel and bladder dysfunction that characterizes acute transverse myelitis (ATM) terrifies affected children and their families. Successful management requires prompt diagnosis and treatment. Because of the association of ATM with numerous infections, specialists in infectious diseases frequently participate in the care of children with ATM. The clinical presentation, radiologic features, differential diagnosis, pathophysiology, treatment, and prognosis of pediatric ATM are reviewed here.

TERMINOLOGY

ATM is a clinical syndrome consisting of progressive symptoms and signs reflecting bilateral sensory, motor, or autonomic dysfunction attributable to the spinal cord. This constellation of symptoms and signs can be caused by a heterogeneous group of disorders, including ATM. Several disorders, including direct infections of the spinal cord, can mimic ATM.

ATM can be associated with more widespread central nervous system (CNS) demyelinating disorders or systemic autoimmune

disorders; in such cases, the term *disease-associated transverse myelitis* has been used. Isolated ATM can be triggered by identifiable preceding infections. Alternatively, ATM can be associated with a nonspecific preceding infection or have no apparent cause; for both of these subgroups, the term *idiopathic ATM* has been used. This last group constitutes the most common category in pediatric ATM. Although the precise pathophysiology of idiopathic ATM is uncertain, the frequent association with preceding infections and vaccinations and accumulating immunologic data suggest an inflammatory cause for the disorder.[12,18,37,56]

CLINICAL PRESENTATION

Idiopathic ATM affects approximately 1.34 persons per 1 million.[12] Six published case series ranging in size from 9 to 50 patients report common symptoms in pediatric patients with ATM. Patients present at a mean age of 8 years, with a slight female predominance.[18,19,25,39,57,59] Patients universally report acute to subacute, bilateral leg weakness, which is symmetric in approximately 67 percent of patients.[18] Involvement of the arms occurs in approximately 40 percent of patients. Approximately 90 percent of patients complain of bowel and bladder dysfunction, ranging from urinary retention and constipation to incontinence. A similar percentage of patients report sensory symptoms, including paresthesias and numbness. Back pain and fever affect nearly 50 percent of patients and may prompt consideration of primary infectious etiologies, such as an epidural abscess. The symptoms of ATM develop rapidly. In one study, the constellation of weakness, sensory changes, bowel and bladder dysfunction, and back pain reached a peak at an average of 5 days (range, 1 to 14 days).[18]

The general examination, although usually unremarkable, may reveal signs suggestive of an underlying systemic infection or autoimmune disorder. Abdominal examination may reveal a distended bladder. A diffuse or dermatomal vesicular rash or its sequelae suggest concurrent or preceding chickenpox or shingles.

On the neurologic examination, the presence of any mental status changes suggests that the myelitis is a component of a more diffuse process (i.e., acute disseminated encephalomyelitis). In the acute stages, muscle tone is flaccid in affected limbs. Detailed muscle strength testing with objective grading serves as a crucial baseline to compare with serial examinations to determine whether the patient is improving. All sensory modalities should be assessed carefully. In one series, preferential involvement of pain and temperature sensation with sparing of vibration sense and proprioception led the authors to speculate that thrombosis of the anterior spinal artery led to most cases of ATM (Fig. 43–2).[59] This series was limited, however, by its retrospective nature and lack of available neuroimaging. In addition, most patients in subsequent series have had involvement of all sensory modalities.[18,25,39,57] A spinal cord sensory level usually is located in the thoracic region (80%) and less commonly in the cervical (10%) or lumbar (10%) area.[12] In the acute phase, deep tendon reflexes are depressed in approximately 70 percent of patients and later become hyperactive. Similarly, Babinski responses may be negative early in the acute phase but soon become positive, indicating upper motor neuron dysfunction.

RADIOLOGIC FEATURES

Every patient with suspected ATM should undergo emergent gadolinium-enhanced magnetic resonance imaging (MRI) of the entire spine to confirm the diagnosis and rule out alternative diagnoses, particularly compressive lesions, such as epidural abscess, epidural hematoma, and extramedullary tumors. T1-weighted and T2-weighted sagittal imaging of the entire spine can serve as an initial screen, followed by axial imaging in areas of suspected pathology.[3]

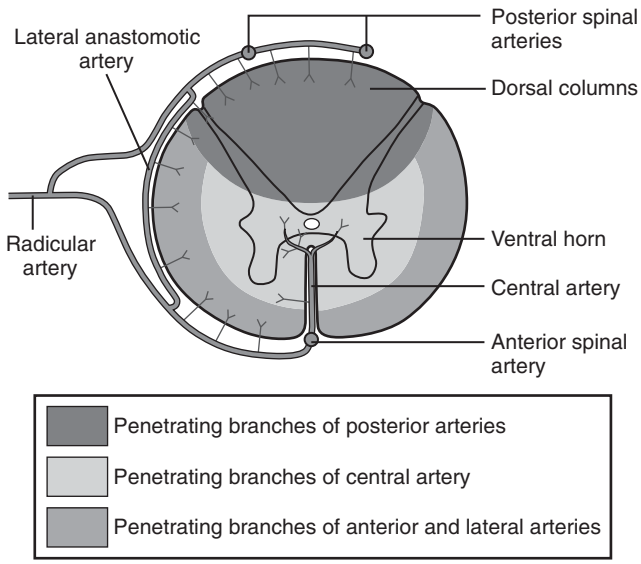

Figure 43–2 The ventral horns, which contain the lower motor neurons, and the lateral spinothalamic tract, which carries pain and temperature sensation, are supplied by the anterior spinal artery. The dorsal columns, which mediate vibration and joint position sense, are supplied by the posterior spinal arteries.

Spinal MRI in ATM typically reveals T1-isointense and T2-hyperintense signals over several contiguous spinal cord segments[18] and may involve the entire spine (Fig. 43–3).[3] This "longitudinally extensive" subtype (extending over three or more vertebral segments) seems to occur more commonly in children than in adults.[3,63] The hyperintense signal can extend into the lower brain stem.[3] Spinal cord swelling with effacement of the surrounding cerebrospinal fluid (CSF) spaces may be present in severe cases (Fig. 43–4). Axial imaging can determine whether gray or white matter or both are affected. Contrast enhancement is variably present and may appear diffuse, patchy, or nodular. In some patients with very suggestive clinical features, initial spine MRI may be normal and should be repeated several days later.[18,39,57] In rare cases, the MRI remains normal but does not rule out the diagnosis.

LUMBAR PUNCTURE

Approximately 50 percent of pediatric patients with ATM have CSF pleocytosis, typically with a lymphocytic predominance. Elevated CSF protein levels, either in isolation or in conjunction with pleocytosis, also are detected in approximately 50 percent of patients. Glucose typically is normal. A normal CSF profile does not rule out ATM because this pattern is seen in approximately 25 percent of patients. CSF cytology should be sent on all patients to assess for the possibility of neoplasm. Additional CSF testing is discussed further subsequently.

DIFFERENTIAL DIAGNOSIS

CONDITIONS THAT MIMIC ACUTE TRANSVERSE MYELITIS

Numerous disorders can affect the spinal cord and produce identical symptoms and signs that mimic idiopathic ATM (Table 43–4). Such conditions must be ruled out through a combination

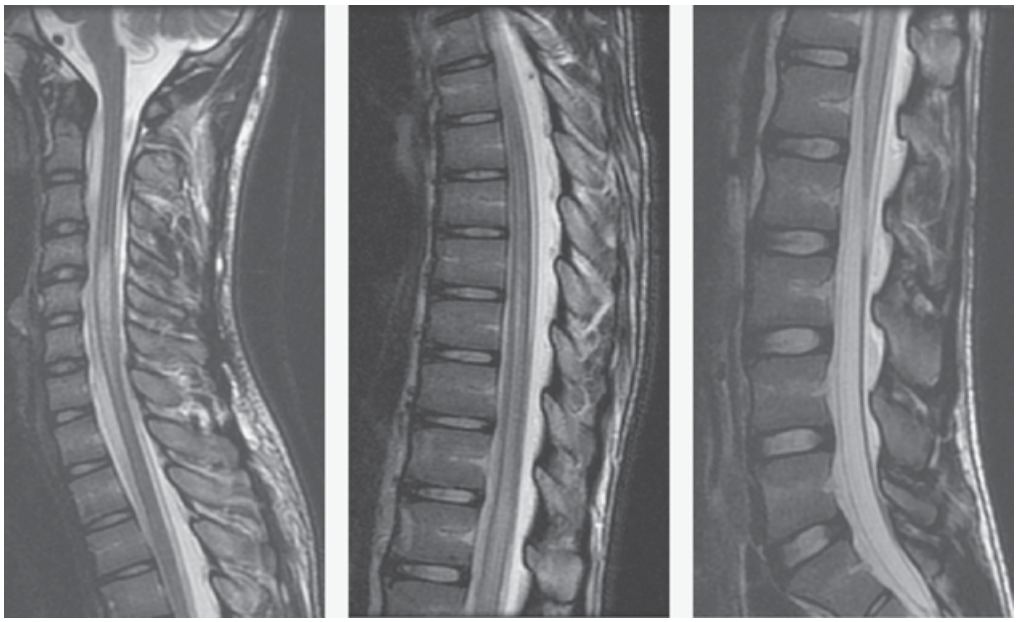

Figure 43–3 Sagittal T2-weighted MR image of the spine of a 16-year-old boy who presented with bilateral leg paresthesias that progressed to paraplegia and urinary and bowel retention. MRI shows diffuse T2-hyperintense signal extending from C4 through the thoracic region to the conus medullaris. The boy's symptoms resolved with administration of high-dose steroids.

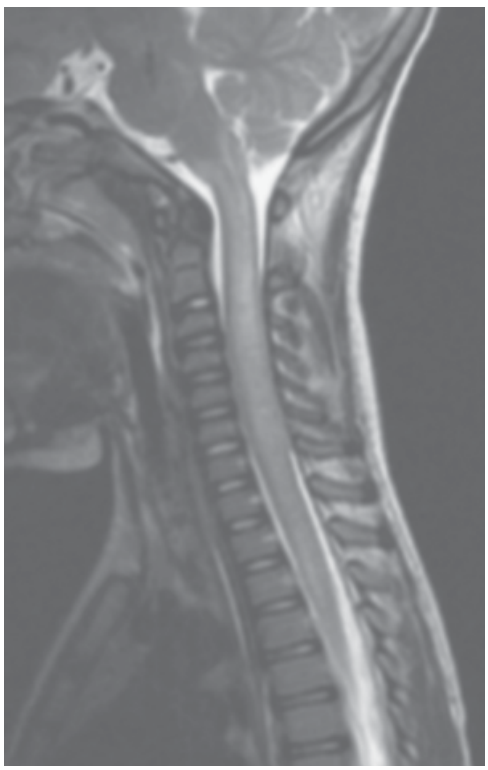

Figure 43–4 Sagittal T2-weighted MR image of a 7-year-old boy with transverse myelitis in the setting of acute disseminated encephalomyelitis. There is T2-hyperintense signal in the cervical cord with associated swelling and effacement of the cerebrospinal fluid spaces. The abnormal signal also extends into the lower brain stem.

TABLE 43–4 Conditions That Mimic Idiopathic Acute Transverse Myelitis

Direct infectious myelopathies
Extramedullary compressive lesions
Epidural abscess
Epidural hematoma
Extramedullary tumors
Guillain-Barré syndrome
Intramedullary spinal cord tumors
Astrocytomas
Ependymomas
Ischemia/infarction
Radiation injury
Traumatic spinal cord injury
Vascular malformations

of history, physical examination, neuroimaging, and laboratory evaluation.

Extramedullary compressive lesions are neurosurgical emergencies that must be diagnosed rapidly for effective treatment. Spinal epidural abscesses are rare in children. In one series of eight children compiled over a 15-year period at a major children's hospital, seven had fever, and all eight had back pain.[8] *Staphylococcus aureus*, some of which were methicillin-resistant, were the most common isolates in this and other series. Spinal epidural hematomas also are rare occurrences in children. They can occur spontaneously or may be associated with anticoagulant use, clotting disorders, trauma, or lumbar puncture.[61] Spinal epidural hematomas usually are located in the lower cervical region and extend over two to three vertebral segments, much fewer than in ATM. In one series of 1147 children with cancer, 70 (6%) developed spinal cord compression at some point during their course.[64] The most common responsible tumor types were primitive neuroectodermal tumor, soft tissue sarcoma, and neuroblastoma. When spinal cord compression is the initial manifestation of cancer, as was the case for 67 percent of the patients in this series, it can mimic ATM. Spinal MRI readily distinguishes each of these disorders from ATM.

The initial clinical presentation of ATM can be quite similar to that of Guillain-Barré syndrome. Both disorders can manifest with back pain, paraparesis, and sensory abnormalities. Although typically absent in both disorders, the presence of deep tendon reflexes would point strongly toward ATM. When deep tendon

reflexes are absent, the presence of a spinal cord sensory level and bowel and bladder involvement suggests ATM. Some patients have albuminocytologic dissociation, which is indistinguishable from Guillain-Barré syndrome. In some cases, ATM and Guillain-Barré syndrome can be distinguished only with MRI. Spinal cord, nerve root, and peripheral nerve pathology can coexist in myeloradiculoneuritis, suggesting that ATM and Guillain-Barré syndrome may share a common pathophysiologic mechanism in some cases.[48]

The most common intramedullary spinal cord tumors in children are astrocytomas (60%), followed by ependymomas (30%).[7,22,35] Although biopsy of the tumors is necessary to establish the exact histologic diagnosis, biopsy of the spinal cord rarely is needed to distinguish tumor from ATM. Despite similarities in presenting symptoms, clinical features can help to distinguish intramedullary tumors from ATM. Intramedullary spinal cord tumors manifest more subacutely or chronically than does ATM, reflecting their low cellular growth rate.[35] In one study, the mean duration of symptoms before presentation of children with intramedullary spinal cord tumors was 254 days.[22] In ATM, the symptoms peak much earlier from onset, with a mean duration of 5 days in one study.[18] On examination, the presence of scoliosis or torticollis suggests the presence of longer standing tumors rather than ATM.[22]

ATM and spinal cord tumors typically are isointense on T1-weighted MR images and hyperintense on T2-weighted images. Although intramedullary tumors display swelling, contrast enhancement, and associated syrinxes more frequently than does ATM, these features also can be seen in the latter (Fig. 43–5; see Fig. 43–4). Other radiographic features may be used to distinguish the two entities. The eccentric location of astrocytomas within the spinal cord[7] can be used to differentiate them from ATM, which usually produces symmetric findings. Although ependymomas can produce symmetric spinal cord expansion because of their origin from the central canal, these tumors usually span three to four vertebral segments,[7] whereas pediatric ATM typically involves longer areas of the spinal cord. In addition, an area of low signal intensity reflecting hemosiderin deposits from chronic hemorrhage at the edge of ependymomas also can serve as a radiographic clue.[7] Because patients with intramedullary spinal cord tumors may be diagnosed on the basis of CSF cytology, this test should be sent on all patients with suspected ATM.

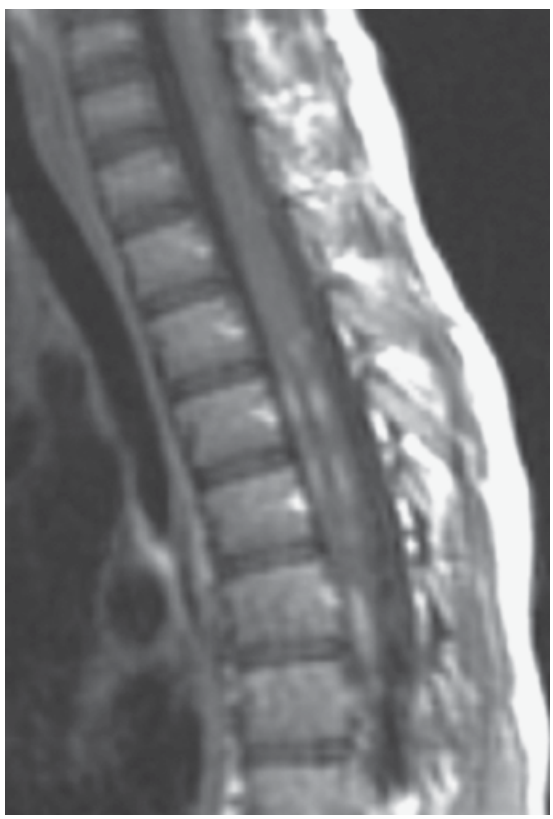

Figure 43–5 Sagittal post-gadolinium T1-weighted MR image of the thoracic spinal cord of a 9-year-old girl who presented with bilateral leg weakness and urinary retention, showing patchy enhancement and a small syrinx. Although these findings raised concern for an intramedullary tumor, her spontaneous improvement and the resolution of enhancement without treatment favored a diagnosis of transverse myelitis.

Clinical features and neuroimaging also can be used to distinguish ATM from spinal cord infarction, which is an exceedingly rare finding in children. The latter diagnosis typically manifests in a hyperacute fashion with dissociated sensory loss (absent temperature and pain sensation with preserved vibration and proprioception) from thrombosis of the anterior spinal artery (see Fig. 43–2). Accompanying MRI signal abnormality in the adjacent vertebral body is highly suggestive of the diagnosis.[60] Diffusion-weighted MRI also may reveal the presence of ischemia and aid in making the differential diagnosis.

The clinical presentation of spinal cord dysfunction in the setting of prior irradiation suggests radiation-induced transverse myelitis as the most likely diagnosis. This entity typically manifests 9 to 18 months after radiotherapy, but it can occur earlier in children.[76]

Although they typically cause slowly progressive symptoms, spinal arteriovenous malformations can manifest acutely as a result of hemorrhage or venous congestion.[71] These lesions usually can be detected as abnormal, dilated vasculature on MRI of the spine; subsequent spinal angiography can confirm the diagnosis.

DISEASE-ASSOCIATED ACUTE TRANSVERSE MYELITIS

ATM with a presumed inflammatory basis can be associated with recurrent CNS demyelinating disorders and systemic autoimmune disorders. In these cases, the term *disease-associated ATM* can be applied (Table 43–5).[5,20]

The presence of changes in mental status and MRI abnormalities of cerebral white matter points toward acute disseminated encephalomyelitis as the correct diagnosis (Fig. 43–6). Spinal cord demyelination in multiple sclerosis tends to be limited to fewer than two vertebral segments and partial in the transverse plane, resulting in mild, asymmetric symptoms compared with the typically severe, symmetric symptoms in ATM.[67] In addition, the recurrence of lesions over the course of time and in different parts of the CNS is required for establishing the diagnosis of

TABLE 43–5 Central Nervous System and Systemic Autoimmune Disorders Associated with Acute Transverse Myelitis

Central Nervous System Disorders
Acute disseminated encephalomyelitis
Multiple sclerosis
Neuromyelitis optica
Systemic Autoimmune Disorders
Antiphospholipid antibody syndrome
Behçet disease
Mixed connective tissue disorder
Neurosarcoidosis
Sjögren syndrome
Systemic lupus erythematosus

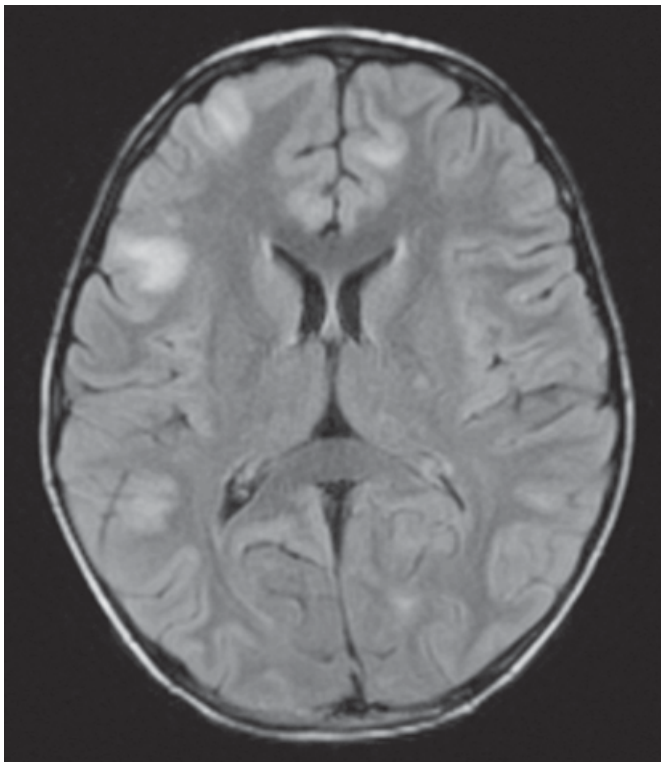

Figure 43–6 Fluid-attenuated inversion recovery (FLAIR) sequence of a brain MR image of the same patient from Figure 43–3, showing multifocal areas of hyperintense signal in the subcortical white matter consistent with acute disseminated encephalomyelitis.

TABLE 43–6 Distinguishing Acute Transverse Myelitis from Other Central Nervous System Demyelinating Disorders

Finding	ATM	ADEM	MS	NMO
Myelitis	+	+/–	+/– (partial)	+
Acute mental status changes	–	+	–	–
Optic neuritis	–	+/–	+/–	+
Abnormal brain MRI	–	+	+	+/–
CSF oligoclonal bands	–	+/–	+	–
Serum NMO-IgG	–	–	–	+/–
Recurrences	+/–	+/–	+	+

+, always present; +/–, variably present; –, usually absent.
ADEM, acute disseminated encephalomyelitis; ATM, acute transverse myelitis; CSF, cerebrospinal fluid; MRI, magnetic resonance imaging; MS, multiple sclerosis; NMO, neuromyelitis optica.

TABLE 43–7 Suggested Diagnostic Work-up for Recurrent Central Nervous System Demyelinating Disorders and Systemic Autoimmune Disorders Associated with Acute Transverse Myelitis

All Patients	Suggestive of Neuromyelitis Optica	Also Consider
Brain MRI with gadolinium	Ophthalmology consultation	Angiotensin-converting enzyme (serum, CSF)
CSF oligoclonal bands	Visual evoked potentials	Other autoantibodies
Antinuclear antibodies	Formal visual field testing	Anti-dsDNA
Antiphospholipid antibodies	Serum NMO-IgG	Anti-La
		Anti-Ro
		Anti-Smith

CSF, cerebrospinal fluid; MRI, magnetic resonance imaging; NMO, neuromyelitis optica.

multiple sclerosis. Previous subtle visual or sensory symptoms suggestive of a recurrent demyelinating disorder may be elicited only with detailed questioning. Oligoclonal bands are present more commonly in multiple sclerosis-related myelitis,[67] but they also have been reported in idiopathic ATM (Table 43–6).[57]

ATM is a necessary feature of neuromyelitis optica (NMO), a severe, relapsing demyelinating disorder restricted mainly to the spinal cord and optic nerves. Specific diagnostic criteria for NMO have been developed.[80] Concurrent or preceding optic neuritis suggests NMO as the correct diagnosis. NMO may manifest initially as ATM, with a future episode of optic neuritis leading to the reclassification of ATM as NMO. Some patients also can have recurrent episodes of idiopathic ATM without optic neuritis; in the literature, such patients have been considered to have a "restricted" or "limited" form of NMO.[63]

A novel biomarker (NMO-IgG) targeting the predominant CNS water channel protein aquaporin-4, which is concentrated in astrocytic foot processes in the blood-brain barrier, has been detected in 73 percent of adult patients with NMO and 52 percent of adult patients with recurrent longitudinally extensive ATM.[44,45] This antibody also has been detected in several pediatric patients with NMO.[52]

ATM also can occur secondary to a variety of systemic autoimmune disorders. Involvement of other organ systems, particularly the skin, lungs, kidneys, and joints, may point to a particular diagnosis, including systemic lupus erythematosus, sarcoidosis, and Sjögren syndrome. A personal or family history of recurrent miscarriages or hypercoagulability is suggestive of antiphospholipid antibody syndrome.

The long list of demyelinating and rheumatologic disorders potentially associated with ATM precludes diagnostic testing for every possible disorder. Table 43–7 presents a schema to guide which tests should be obtained in patients with ATM.

IDIOPATHIC ACUTE TRANSVERSE MYELITIS

Because of the numerous conditions that mimic or are associated with idiopathic ATM, establishing the diagnosis requires a systematic approach. Although all case series have emphasized the presence of bilateral lower extremity motor dysfunction and loss of voluntary bowel/bladder control, definitions of ATM have varied in the literature regarding the duration of symptoms and the necessary diagnostic testing.[18,39] To address this nonuniformity, the criteria proposed by the Transverse Myelitis Consortium Working Group[5] should be used to secure the diagnosis and guide the differential diagnosis and work-up (Table 43–8).

ROLE OF INFECTIONS IN TRANSVERSE MYELITIS

Infectious agents can cause spinal cord dysfunction by directly infecting the spinal cord parenchyma (infectious myelopathy) or by triggering postinfectious, immune-mediated processes (postinfectious ATM). In some cases, such as human T-cell lymphotrophic virus (HTLV)–associated myelitis, damage to the spinal cord may be produced by direct infection and by the immune response to the agent. Several agents, including cytomegalovirus (CMV) and varicella-zoster virus (VZV), are associated with direct CNS infection in certain patients (many of whom are

TABLE 43–8 Transverse Myelitis Consortium Working Group Diagnostic Criteria

Inclusion Criteria	Exclusion Criteria
Development of sensory, motor, or autonomic dysfunction attributable to spinal cord	History of previous radiation to spine within last 10 yr
Bilateral signs/symptoms (not necessarily symmetric)	Clear arterial distribution clinical deficit consistent with thrombosis of anterior spinal artery
Clearly defined sensory level	Abnormal flow voids on surface of the spinal cord consistent with AVM
Exclusion of extra-axial compressive etiology by neuroimaging (MRI or myelography; CT of spine inadequate)	Serologic or clinical evidence of connective tissue disease (sarcoidosis, Behçet disease, Sjögren syndrome, SLE, mixed connective tissue disorder)*
Inflammation within spinal cord shown by CSF pleocytosis or elevated IgG index or gadolinium enhancement. If no inflammatory criteria are met at symptom onset, repeat MRI and lumbar puncture evaluation 2-7 days after symptom onset meet criteria	CNS manifestations of syphilis, Lyme disease, HIV, HTLV-1, *Mycoplasma*, other viral infection (e.g., HSV-1, HSV-2, VZV, EBV, CMV, HHV-6, enteroviruses)*
	Brain MRI abnormalities suggestive of MS*
	History of clinically apparent optic neuritis*
Progression to nadir 4 hr to 21 days after onset of symptoms (if patient awakens with symptoms, symptoms must become more pronounced from point of awakening)	

**Do not exclude disease-associated acute transverse myelitis.*

AVM, arteriovenous malformation; CMV, cytomegalovirus; CNS, central nervous system; CSF, cerebrospinal fluid; CT, computed tomography; EBV, Epstein-Barr virus; HIV, human immunodeficiency virus; HHV-6, human herpesvirus-6; HSV, herpes simplex virus; HTLV-1, human T-cell lymphotrophic virus-1; MRI, magnetic resonance imaging; MS, multiple sclerosis; SLE, systemic lupus erythematosus; VZV, varicella-zoster virus.

Data from Transverse Myelitis Working Group: Proposed diagnostic criteria and nosology of acute myelitis. Neurology 59:499-505, 2002.

immunocompromised) and postinfectious ATM in others. When a specific agent is identified as a trigger of ATM, it should be indicated in the diagnostic terminology (i.e., post-varicella ATM).

Within the postinfectious category of ATM, the preceding infection usually is a nonspecific upper respiratory tract infection without an identifiable, specific microbe.[12,18,59] Approximately 50 percent of patients report having had such an infection with an intervening symptom-free interval of 5 to 10 days.[12,18,59] In instances in which the specific microbe is not identified and in cases in which no associated infection, systemic disorder, or recurrent demyelinating disorder is identified, the term *idiopathic ATM* has been applied.

Clues in the history can suggest the involvement of specific microbes. Immune status should be determined because immunocompromised patients are more susceptible to particular agents, such as CMV.[27] A history of exposure to cats or parakeets may point to *Bartonella henselae* (cats)[66] or *Chlamydia psittaci* (parakeets)[79] as rare causes of ATM. A detailed travel history may lead to the consideration of agents not typically encountered in the patient's primary residence, such as *Schistosoma*.[4]

Vaccination status also should be determined. Some cases of ATM seem to be related to recent vaccination, although causality usually is difficult to prove, given the paucity of reported cases.[18,26,38,57,81] Alternatively, unimmunized children are susceptible to particular agents, such as measles, mumps, and rubella, which have been associated with ATM.[24]

INFECTIOUS MYELOPATHIES

The etiology of an infectious myelopathy usually is established by positive CSF culture or polymerase chain reaction (PCR) results. Biopsy or autopsy results also have provided evidence of direct CNS infection in some patients. Some authors consider elevated CSF titers without positive culture or PCR results to be sufficient evidence of direct CNS infection, but this issue has not been resolved. Viruses constitute the most frequently reported category of infectious myelopathies, with bacteria and parasitic causes being less frequent (Table 43–9).

TABLE 43–9 Partial Listing of Microbes That Cause Infectious Myelopathies

- Cytomegalovirus[49]
- Epstein-Barr virus[48]
- *Echinococcus granulosus*[11]
- Enteroviruses[11]
  - Coxsackieviruses
  - Echoviruses[77]
  - Enterovirus 71[53]
  - Polioviruses (vaccine associated and wild-type)
- Herpes simplex virus-1[54]
- Herpes simplex virus-2[31]
- Human immunodeficiency virus[10,62]
- Human T-cell lymphotrophic virus-1 and -2[10]
- *Mycobacterium tuberculosis*[13]
- *Mycoplasma pneumoniae*[5]
- *Schistosoma haematobium* and *Schistosoma mansoni*[4]
- *Taenia solium* (neurocysticercosis)[11]
- *Treponema pallidum*[13,29]
- Varicella-zoster virus[29]
- West Nile virus[65]

POSTINFECTIOUS ACUTE TRANSVERSE MYELITIS

In postinfectious cases, a specific etiology can be suggested by elevated acute or rising convalescent serum titers, isolation of the agent from systemic sources in the setting of a suggestive clinical picture, or both. In such cases, the presumptive pathophysiology entails an aberrant autoimmune response to the spinal cord via mechanisms such as molecular mimicry, rather than direct CNS invasion by the microbe. A variety of bacterial and viral pathogens and vaccinations have been associated with postinfectious ATM (Table 43–10). Microbes that have been reported to cause infectious and postinfectious myelitis are listed in both Tables 43–9 and 43–10.

TABLE 43–10 Partial Listing of Microbes and Vaccines Associated with Postinfectious or Postvaccinial Acute Transverse Myelitis

Bacteria/Spirochetes	Viruses	Vaccinations
Bartonella henselae[66]	Adenovirus[47]	Hepatitis B[73]
Borrelia burgdorferi[46]	Cytomegalovirus[27]	Influenza[26]
Brucellosis[40]	Coxsackieviruses[56]	Measles, mumps, and rubella[81]
Chlamydia psittaci[79]	Enterovirus 71[33,53]	Poliovirus[38]
Mycoplasma pneumoniae[74]	Epstein-Barr virus[33]	Rabies[26]
	Hepatitis A[15,75]	Smallpox[26]
	Hepatitis B[50]	
	Hepatitis C[32]	
	Herpes simplex virus-1[28]	
	Herpes simplex virus-2[31]	
	Human herpesvirus-6[34]	
	Mumps[58]	
	Rubella[24]	
	Varicella-zoster virus[51]	

TABLE 43–11 Suggested Diagnostic Work-up for Infections Associated with Acute Transverse Myelitis

Blood	Cerebrospinal Fluid	Other
Blood cultures	Bacterial culture	Viral culture of stool and respiratory secretions
Acute and convalescent titers to	Viral culture	Consider stool ova and parasite testing and serum titers if parasitic infection is suspected
Borrelia burgdorferi	PCR testing for	
EBV	CMV	
Mycoplasma pneumoniae	EBV	
	Enterovirus	
	HSV	
	M. pneumoniae	
	VZV	

CMV, cytomegalovirus; EBV, Epstein-Barr virus; HSV, herpes simplex virus; PCR, polymerase chain reaction; VZV, varicella-zoster virus.

TABLE 43–12 Additional Diagnostic Work-up for Infections Associated with Acute Transverse Myelitis

Blood	Cerebrospinal Fluid	Other
Bartonella titers	Cryptococcal antigen	PPD placement
HIV antibody	HTLV antibody	Stool ova and parasite testing
HTLV-1 antibody	Fungal culture	
Parasitic infection titers	VDRL	
RPR		

HIV, human immunodeficiency virus; HTLV, human T-cell lymphotrophic virus; PPD, purified protein derivative; RPR, rapid plasma reagin; VDRL, Venereal Disease Research Laboratory.

The long list of potential pathogens precludes performing diagnostic testing for every possible agent. The work-up instead should focus on the pathogens that are common, treatable, or suggested by particular clues in the history or examination. Table 43–11 lists the infectious disease tests that should be sent for every patient with ATM. Additional infectious disease tests that should be considered depending on the clinical scenario and immune status are listed in Table 43–12. Although a detailed description of every infectious cause of transverse myelitis is beyond the scope of this discussion, several examples of the most commonly reported entities in the literature that highlight general features of the infectious disease aspects of ATM are described subsequently.

CYTOMEGALOVIRUS

Although reported more frequently in immunocompromised patients, CMV myelitis also may affect immunocompetent individuals.[27,75] CMV can infect the spinal cord directly and can produce secondary vasculitis in immunocompromised patients. In such cases, CSF PCR testing frequently is positive.[27] In other cases of reported CMV-associated myelitis, evidence for concurrent or recent infection has included isolation of the virus from blood or urine, detection of anti-CMV IgM in the serum, or significant increases in convalescent CMV serum titers in the absence of viral detection by CSF viral cultures or PCR.[27,75] The inflammatory process in CMV myelitis extends into the brain stem or nerve roots in some cases.[75] Treatment with ganciclovir or foscarnet should be considered.[11] The combined treatment of CMV-associated myelitis with ganciclovir and methylprednisolone was associated with marked recovery in an immunocompetent adult.[27] As illustrated by CMV, myelitis associated with viral infections may be produced by direct infection, accompanying secondary processes such as vasculitis, or postinfectious immune-mediated mechanisms. Whether optimal treatment requires antiviral agents or immunomodulation or both is uncertain.

HUMAN IMMUNODEFICIENCY VIRUS-ASSOCIATED MYELOPATHY

Infections such as HTLV, herpes simplex virus, syphilis, tuberculosis, and VZV can cause myelopathies in patients with acquired immunodeficiency syndrome (AIDS) and seem to be particularly important in developing countries.[13] In addition, human immunodeficiency virus (HIV) itself has been linked to a particular form of myelopathy termed *vacuolar myelopathy*, which is the most common cause of paraparesis in adults with HIV infection in developed countries.[62] Typically occurring in the setting of advanced disease, vacuolar myelopathy has been detected in pathologic series in 22 percent of patients with AIDS.[62] Myelopathy also can occur acutely in conjunction with seroconversion, but this form is less common than is vacuolar myelopathy.[11]

In vacuolar myelopathy, spinal cord pathology reveals noninflammatory myelin loss and spongy degeneration affecting mainly the lateral and posterior columns and producing vacuolization of the white matter, which provides the disorder its name.[62] HIV can be cultured from affected spinal cords, but the exact role of the virus in causing the disorder is unclear. The description of similar vacuolar myelopathies in patients with cancer or other immunocompromising conditions without HIV infection has cast some doubt on the central role of HIV in the disorder. Nutritional or metabolic factors in the vitamin B_{12} pathway may play a central or contributing role.[11]

The clinical presentation is similar to the presentation of other causes of chronic progressive myelopathy, and HIV-associated myelopathy may be underrecognized.[11,23] The diagnosis relies on determining a patient's HIV status and ruling out other bacterial, viral, fungal, and parasitic causes of myelopathy. No specific, effective treatments are available for vacuolar myelopathy.

Children with AIDS encephalopathy frequently develop upper motor neuron symptoms and signs, with resultant delay or regression in motor development.[23] In one series of 15 children with AIDS, only one patient had evidence of vacuolar myelopathy.[23] The most common finding in this series was demyelination with or without axonal loss in the lateral corticospinal tracts in the

spinal cord. The pathologic expression of HIV-associated spinal cord damage seems to be age-dependent.

As illustrated by HIV myelopathy, the existence of co-infections or accompanying systemic illnesses or both may confound the association of particular infectious agents with myelitis. Distinguishing these different causes clinically may be impossible, or they all may contribute to the disorder in some cases; treatment directed at multiple possible mechanisms may be warranted.

MYELOPATHY ASSOCIATED WITH HUMAN T-CELL LYMPHOTROPHIC VIRUS-1 AND HUMAN T-CELL LYMPHOTROPHIC VIRUS-2

The retrovirus HTLV-1 can cause a chronic, progressive myelopathy in immunocompromised and immunocompetent patients. Although reported less frequently, HTLV-2 infection can produce an identical clinical picture.[6] Risk factors for the acquisition of HTLV-1 and HTLV-2 include blood transfusions, intravenous drug use, and multiple sexual partners.[10] HTLV also can be transmitted from mother to infant, usually via breast-feeding. HTLV-1 is endemic to regions closer to the equator, including the Caribbean and southeastern United States.[70] Although the average age of onset is approximately 40 years,[16] rare pediatric cases have been reported.

Approximately 1 of 250 patients with HTLV-1 infection develops myelopathy.[11] Differences in the immune response to HTLV-1 seem to play a role in determining whether an infected individual develops myelopathy. Patients who also are infected with HIV seem to be at higher risk for the development of HTLV-associated myelopathy compared with other patient groups.[10]

The presence of serum and CSF antibodies to HTLV-1/HTLV-2 and of CSF HTLV-1/HTLV-2 DNA (detected by PCR) confirms the diagnosis. Immunomodulation, particularly corticosteroids, may be transiently beneficial early in the course of the disorder, but the progressive nature of the disorder nonetheless ensues.[10,11]

Pathologic specimens of HTLV-associated myelopathy reveal demyelination and degeneration of the long tracts with perivascular T-cell–predominant infiltrates and gliosis.[11] HTLV-infected $CD4^+$ and $CD8^+$ lymphocytes can be detected in the blood, CSF, and white matter of patients with HTLV-associated myelopathy.[9] Although the pathophysiology is uncertain, the evolution of an initially beneficial immune response into a self-destructive autoimmune process has been emphasized in the literature.[9] Possible mechanisms include cytotoxic T-lymphocyte–mediated killing of infected cells, bystander damage from enhanced cytokine production, and cross-reacting autoantibodies directed against CNS autoantigens via molecular mimicry. The example of HTLV-associated myelitis reveals that the boundaries between infectious and postinfectious myelitis may be unclear.

MYCOPLASMA PNEUMONIAE

Antimyelin antibodies reactive against glycolipid components have been detected in patients with *Mycoplasma*-associated inflammatory nervous system disorders, including one patient with ATM.[17,42,74] Such antibodies may be induced via molecular mimicry because of similarities in the glycolipid components of myelin and *Mycoplasma pneumoniae*.[42] Although the specificity of *M. pneumoniae* serologic testing has been questioned,[14] *Mycoplasma*-associated ATM seems to be a true entity based on the available literature, which includes cases of infectious myelopathy with positive CSF *Mycoplasma* PCR results and postinfectious cases.[1,74]

VARICELLA-ZOSTER VIRUS

After producing chickenpox, VZV survives in a latent form in cranial nerve and dorsal root ganglia, particularly in the trigeminal and thoracic regions.[30] Reactivation of the virus increases with immunosuppression and advancing age. Myelitis can occur in immunocompetent patients, often 1 to 2 weeks after the rash of varicella or zoster occurs, but it also can occur without a preceding rash.[30] In such cases, VZV DNA or antibodies can be detected in the CSF, but the virus cannot be cultured. In immunocompromised patients, invasion of the spinal cord by VZV has been described. Treatment with acyclovir or corticosteroids or both in combination may be effective.[21,29]

ROLE OF THE IMMUNE SYSTEM IN IDIOPATHIC ACUTE TRANSVERSE MYELITIS

In cases of idiopathic ATM, infections seem to provoke spinal cord dysfunction via secondary, immune-mediated mechanisms, rather than via CNS invasion. In such cases, a specific triggering agent usually is not identified. Increased peripheral blood lymphocyte responses to myelin basic protein have been shown in the research setting in patients with ATM, three of whom had a preceding viral illness.[2] The patients in this study who did not show increased lymphocyte responses were receiving steroids; reduction of autoimmune cell–mediated responses to CNS myelin antigens may underlie the beneficial effect of these medications in ATM (discussed further subsequently). None of the six patients who were tested in the recovery stage showed significant responses to myelin basic protein, suggesting that the cellular autoimmune reaction is short-lived.

In addition, the production of interleukin-6 by astrocytes seems to lead to nitric oxide–induced injury to spinal cord oligodendrocytes and axons in patients with idiopathic ATM. Interleukin-6 levels are markedly elevated in the CSF of patients with ATM and correlate with long-term disability.[37] The efficacy of corticosteroids in ATM may be due partially to their reduction of interleukin-6 production.[68]

TREATMENT

All patients with ATM should be hospitalized for further monitoring and treatment. Most patients can receive care on the regular ward, although the approximately 5 percent of patients with respiratory involvement from cervical myelitis require intensive care monitoring and may need endotracheal tube placement and mechanical ventilation.

No randomized, controlled treatment trials in ATM have been done. Based on case reports and series that have suggested a beneficial effect,[19,43,68] high-dose corticosteroids have become the standard of care in ATM. In one series of 12 children with severe ATM compared with a historical control group of 17 patients, the use of high-dose intravenous methylprednisolone (IVMP) significantly increased the proportion of children walking independently at 1 month (66% compared with 18%) and with full recovery at 1 year (55% compared with 12%).[19] Although a variety of agents and courses have been used, we use IVMP, 30 mg/kg per dose for 5 maximum 1 g. The need for a prednisone taper is controversial and may be based on whether full or partial recovery is achieved with the intravenous steroids. Most patients improve, often dramatically, with intravenous steroid treatment. Although some patients may improve spontaneously,[59] the collective data suggest that high-dose IVMP should be used in all patients with ATM. For the rare patient who does not improve with IVMP, intravenous immunoglobulins[69] or plasmapheresis can be used.

The use of antimicrobial agents in patients with ATM is controversial. Because most cases are caused by secondary, immune-mediated mechanisms, such treatment may not have significant benefit. Antimicrobial therapy is indicated, however, in cases with highly suspected or proven direct or associated infections, such as doxycycline for *Mycoplasma*,[74] ganciclovir for CMV,[27] and acyclovir for herpes simplex virus and VZV.[29] When antimicrobials are used, agents with good CSF penetration are preferred because direct invasion of the CNS may be present in some cases.

Additional treatment includes pain management, urinary bladder catheterization, bowel regimens, and peptic ulcer and deep venous thrombosis prophylaxis. The early institution of clean intermittent catheterization may improve long-term neuro-urologic outcomes in children with ATM.[72] Physical therapy should be instituted early to maximize the chance for recovery and continued in inpatient rehabilitation or an outpatient setting after the patient has been discharged from the acute care hospital.

Patients with ATM usually are fearful that they will not recover control of their legs or of their bowel or bladder, or that they will have a recurrence. Clinicians caring for patients with ATM must be attuned to their psychosocial needs. Psychiatric consultation may be necessary in some cases. Patients and their families may find the resources of the patient support group Transverse Myelitis Association (www.myelitis.org) helpful.

PROGNOSIS

DISABILITY

Although the variable definitions of recovery reported in the literature preclude a definitive assessment, the prognosis for pediatric patients with ATM generally is favorable and is better than their adult counterparts.[63] Paine and Byers'[59] degree of recovery categories have been the most widely reported outcome scale but are limited by vague terminology. Based on this scale, approximately 80 percent of pediatric patients who receive high-dose IVMP achieve full or good recovery, and 20 percent have a fair or poor outcome.[19,43] Among patients not treated with high-dose IVMP, 60 percent have a full or good recovery, whereas 40 percent have a fair or poor outcome.[59] In one series comprising 33 percent children and 67 percent adults who were not treated with steroids, approximately 33 percent each achieved good, fair, and poor recovery.[12] In a study of adult patients with ATM who were treated with high-dose IVMP and additional immunosuppressive agents if needed, approximately 67 percent were reported to have a good outcome, and 33 percent had a poor outcome. Comparisons with the aforementioned studies are difficult to make because a different outcome scale was used. Future studies in pediatric ATM may be improved by the use of the Extended Disability Status Scale, which has been used widely to assess outcomes in the adult and pediatric multiple sclerosis literature.[41]

During recovery, motor function returns first, with an average time to independent ambulation of 56 days in one study[18] and 25 days in a group of patients treated with high-dose IVMP.[19] Bowel and bladder control recovers more slowly, with an average time to recovery of normal urinary function of 7 months.[18] In one study, 86 percent of children with ATM showed evidence of bladder dysfunction on long-term follow-up.[72] Although these results likely were affected by selection bias for more severely affected patients, this study suggests that urodynamic studies should be considered in all patients after the acute phase has resolved. Based on these results, clean intermittent catheterization and anticholinergic medications may be required.[72]

Patients with hyperreflexia at presentation[57] and independent ambulation at 1 month[18] have a better prognosis. Complete paraplegia, time to maximal deficit of less than 24 hours, and younger age of onset are associated with a poor prognosis.[18,57] Levels of the intracellular neuronal 14-3-3 protein in the CSF of patients with ATM may reflect the extent of neuronal injury and correlate with outcome.[36,37]

RECURRENCES

In contrast to adult patients with idiopathic ATM, who have an approximately 25 percent likelihood of experiencing relapses,[20] most pediatric patients with idiopathic ATM do not have any recurrences. In a series of 24 pediatric patients with a mean follow-up of 7 years, there were no recurrences.[18] In another study of children with a variety of initial acute demyelinating events, only 2 of 29 patients with transverse myelitis had a later demyelinating event.[55,78] In this same study, the presence of myelitis for all patients with acute demyelinating disorders was negatively correlated with recurrence.

The detection of NMO-IgG is predictive of later recurrences and conversion to a diagnosis of NMO.[78] In adult patients with longitudinally extensive ATM, 56 percent of seropositive patients had relapses of either transverse myelitis or optic neuritis within 1 year; none of the seronegative patients had relapses.[78] Some researchers have suggested that NMO-IgG should be checked in all patients with longitudinally extensive ATM to predict more accurately the possibility of relapses.[63] In our anecdotal experience, NMO-IgG usually is not present in the serum of pediatric patients with ATM, even patients with a longitudinally extensive pattern. This finding suggests that NMO-IgG may not play a major role in idiopathic pediatric ATM and may partially explain the differential recurrence rates in pediatric and adult ATM.

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CHAPTER 43c

Parainfectious and Postinfectious Disorders of the Peripheral Nervous System

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Guillain-Barré Syndrome

Guillain-Barré syndrome (GBS) encompasses a spectrum of acute-onset, flaccid paralytic disease involving the peripheral nervous system. The classic form now generally is referred to as *acute inflammatory demyelinating polyradiculoneuropathy* (AIDP). With the decline in the incidence of poliomyelitis, GBS emerged as the most frequent cause of acute, severe generalized human paralytic disease.[195] GBS typically occurs several weeks after an upper respiratory or gastrointestinal tract illness, but it has been associated with other factors, including immunization and surgery. It is characterized by progressive ascending motor weakness, hyporeflexia, and minor sensory disturbances.

No specific diagnostic test for GBS exists; the diagnosis is based on the clinical features supported by other data, including cerebrospinal fluid (CSF) protein elevation, electrophysiologic changes, and pathologic changes of the peripheral nerves. The consensus is that GBS is mediated immunologically; however, the specific immunologic alterations necessary to initiate the events that result in demyelination of human peripheral nerves are unclear. The primary treatment is supportive care, but the efficacy of other therapies, such as plasma exchange transfusion and intravenous immunoglobulin (IVIG) therapy, in shortening the duration of the illness has been shown.[87,96,207,208] Following is an overview of GBS and its variants, including diagnostic criteria, the role of preceding infection and immunization, immunologic aspects, and treatment modalities.

HISTORY

Although bearing the names of Guillain and Barré, GBS was recognized first by Landry in 1859.[114] Landry described 10 patients who presented first with generalized weakness, followed by paresthesia and transitory muscle cramps, and then a rapidly ascending paralysis that involved the respiratory muscles last. He named this disorder *acute ascending paralysis*, postulated that it occurred after another illness, and considered it to be a severe disease because 2 of the 10 patients died. Other reports of this disorder appeared, but in 1916, Guillain, Barré, and Strohl[72] clearly characterized it and were the first to call attention to the "albuminocytologic dissociation," or increased CSF protein with absence of cells. Barré and Guillain favored an infectious cause for the disease.

In 1955, Waksman and Adams[215] pointed out the similarity in the clinical and pathologic pictures between GBS and experimental allergic neuritis in rabbits. In 1963, Melnick[131] found antibodies to nervous tissue in 19 of 38 patients with GBS. In 1960, Osler and Sidell[149] indicated the need for exact diagnostic criteria for GBS and presented 12 diagnostic criteria restricting the definition of the disorder. Most subsequent studies arrived at a broader concept of the disorder, however.[9,13,54] After reports of an increased incidence of GBS in association with the 1976 inoculation program for swine influenza, the National Institute of Neurological and Communicative Disorders and Stroke (NINCDS) proposed a standardized inclusion and exclusion diagnostic criteria.[12,13,14]

DIAGNOSTIC CRITERIA

The diagnosis of GBS is based on the clinical history and examination and supported by clinical laboratory (i.e., CSF) and electrodiagnostic (i.e., nerve conduction velocities) evaluations. The "typical" case of GBS often occurs a few days or weeks after a recognizable, nonspecific infection that is more often viral than bacterial in origin. The paralytic stage usually begins with pain or paresthesia, followed by hypotonia, ascending paralysis without pyramidal tract involvement, and loss of deep tendon reflexes rather than hyperreflexia or pathologic reflexes. Initial weakness usually is noted in the lower extremities and less commonly in the upper extremities or the face. Many patients experience minimal to moderate sensory loss in a glove-stocking distribution. Maximal paralysis is reached in approximately 3 weeks. In 46 to 75 percent of patients, cranial nerves, including VII, IX/X, or both, may be affected, giving rise to facial weakness and difficulties in swallowing. Respiratory weakness occurs in 12 to 20 percent of patients, and some may require mechanical ventilation.

Although not part of any diagnostic criteria, pain has been reported to occur in 55 to 90 percent of patients with GBS, and it may precede weakness in some patients.[176] Approximately 79 percent of children with GBS who are younger than 6 years of age present with pain that may be present for longer than 1 week before the correct diagnosis is made.[140] Multiple pain syndromes that have been described include (1) deep, aching throbbing discomfort in the lower back that radiates to the buttock, thighs, or calves (67%); (2) dysesthesias, described as burning, tingling, or shock-like pain (20%); and (3) myalgias or muscle aches (9%). Severity of pain has not been found to be a predictor of the clinical course.[136]

Routine laboratory tests are unremarkable except for characteristic elevation of CSF protein (typical values of 50 to 200 mg/dL) without appearance of cells. The CSF protein concentration usually peaks between the second and eighth week of illness, with a slow decline occurring thereafter. Although most patients experience "complete" recovery, 20 percent have permanent residual disease (weakness, muscle atrophy), and approximately 1 to 4 percent die of respiratory failure.[19,130]

As Asbury[12] has emphasized, the problem is not with recognition of a typical case but with knowing the boundaries of the disorder. To define those limits, specific criteria were created in 1978 (Table 43–13).[14,15] These criteria consist of specific features required for establishing the diagnosis and additional features that strongly support, cast doubt on, or rule out the diagnosis entirely. The two features required for establishing the diagnosis are progressive motor weakness involving more than one limb and areflexia.

Clinical features supporting the diagnosis include the following: (1) progression of symptoms with signs of motor weakness that cease to progress after 4 weeks; (2) symmetry of symptoms; (3) sensory, cranial nerve, and autonomic dysfunction; (4) recovery; and (5) absence of fever. Laboratory features supporting the diagnosis include CSF protein elevation after the first week of symptoms or an increase in protein on serial lumbar punctures and less than 10 mononuclear leukocytes/mm^3.

Electrodiagnostic studies in GBS show evidence of an axonal, a demyelinating, or a mixed neuropathy. Features suggestive of demyelination include slowing of conduction velocity, prolongation of distal compound motor action potential latencies, prolonged or absent F-wave response, and a partial conduction block

TABLE 43–13 Criteria for Diagnosis of Guillain-Barré Syndrome

Required
Progressive motor weakness in more than one extremity
Areflexia (at least distal with hyporeflexia of the biceps and knee jerks)
Strongly Supportive
Clinical features (in order of importance)
Progression—ceases by 4 wk
Relative symmetry
Mild sensory symptoms or signs
Cranial nerve involvement
Recovery—usually 2-4 wk after progression ceases
Autonomic dysfunction
Absence of fever at onset of neurologic symptoms
CSF Features
Protein—elevated after first week of symptoms, or increasing on serial lumbar punctures
Cells—≤10 mononuclear leukocytes/mm^3
Electrodiagnostic Features
Nerve conduction slowing
Casting Doubt
Marked, persistent asymmetry of weakness
Persistent bladder or bowel dysfunction
Bowel or bladder dysfunction at onset
>50 mononuclear leukocytes/mm^3 in CSF
Presence of polymorphonuclear leukocytes in CSF
Sharp sensory level
Rule Out the Diagnosis
Current history of hexacarbon abuse
Abnormal porphyrin metabolism
Recent diphtheria infection
Evidence of lead neuropathy or intoxication
Purely sensory syndrome
Definite diagnosis of poliomyelitis, botulism, hysterical paralysis, or toxic neuropathy

CSF, cerebrospinal fluid.
Data from references 14 and 15.

TABLE 43–14 New Diagnostic Criteria for Guillain-Barré Syndrome

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Please refer to the printed publication.

From Van der Meche, F. G. A., et al.: Diagnostic and classification criteria for the Guillain-Barré syndrome. Eur. Neurol. 45:133-139, 2001.

or evidence of temporal dispersion (broadening of the compound motor action potential response). Signs of axonal neuropathy include absent or significantly reduced compound motor action potential amplitudes and absence of the features suggestive of demyelination.[87] Early in the course of the disease, many of these findings may not be present, and findings may remain normal in 20 percent of cases.[12,13] The earliest electrodiagnostic signs of GBS are most likely to be decreased or absent F waves, absent h reflex, and the presence of A waves.[5,67,213]

Certain features, including persistent asymmetry of weakness, bowel or bladder dysfunction at onset or a persistence of dysfunction, elevation of cells in the CSF, or a discrete sensory level, cast doubt on the diagnosis. Features that can rule out the diagnosis entirely include (1) a purely sensory syndrome; (2) a current history of hexacarbon abuse; (3) abnormal porphyrin metabolism (increased excretion of porphobilinogen and δ-minolevulinic acid in the urine); (4) evidence of recent diphtheritic infection; (5) lead neuropathy or intoxication; or (6) a definite diagnosis of a condition such as poliomyelitis, botulism, hysterical paralysis, or toxic neuropathy (nitrofurantoin, dapsone, or organophosphorus).[12,13]

A diagnostic process has been suggested.[12] If the clinical features and temporal evolution are typical and without variant features or features that rule out GBS, the diagnosis may be made on clinical grounds alone. Laboratory findings, such as CSF protein elevation, may not appear until after 1 week, and electrodiagnostic findings may never occur. If clinical features are unusual, however, laboratory studies may provide important supportive information, and a diagnosis of GBS may have to be delayed to evaluate these test results fully. Asbury and Cornblath[15] reported that certain variant features are seen occasionally in otherwise typical cases of GBS. These variant features include fever at onset of neuritic symptoms, severe sensory loss with pain, progression beyond 4 weeks, cessation of progression without recovery or with major permanent residual deficit, sphincter dysfunction, central nervous system involvement, no increase in CSF protein 1 to 10 weeks after onset of symptoms, and cell counts of 11 to 50 mononuclear leukocytes/mm^3 in CSF.

Asbury and Cornblath[15] suggested that the presence of one of these symptoms, signs, or laboratory results should raise doubt about the validity of the diagnosis and that the presence of two or more suggest the diagnosis of GBS is incorrect. Manifestations of systemic illness or constitutional symptoms (or both) preceding or coinciding with signs and symptoms of involvement of the peripheral nervous system suggest a diagnosis of a systemic illness or intoxication and not GBS. In patients who have features consistent with GBS and who also have human immunodeficiency virus (HIV) seropositivity, CSF cell counts frequently are elevated.[15,41]

To facilitate use for poliomyelitis surveillance, the diagnostic criteria were simplified by the World Health Organization in 1993 to involve solely clinical features. Cases that fulfilled the aforementioned clinical criteria were diagnosed as GBS. If further electrodiagnostic or pathologic data were obtained, however, the terms *demyelinating* or *axonal* could be applied, and all subtypes were described independently.[219]

With further advancement in diagnostic techniques and identification of additional variant syndromes, a new set of criteria has been proposed to include a systematic approach for further subclassifications and to support future research (Table 43–14).[209] This new classification is based on four main criteria necessary for establishing the clinical diagnosis of GBS: (1) flaccid paralysis that develops subacutely, (2) bilateral onset of weakness (with a tendency for symmetry), (3) loss of reflexes, and (4) other causes

being highly unlikely (ruled out with additional testing as necessary). Electrodiagnostic studies and CSF analysis (as previously discussed) can assist in the diagnosis if the fourth criterion is in question. For all subclassifications, the main criteria first must be met.

Motor-sensory GBS requires the presence of a sensory deficit at some point during the course of the disease. In pure motor GBS, paresthesias may be present, but no sensory deficit at anytime. Miller Fisher syndrome requires weakness of the external ocular muscles and either ataxia or positive anti-G_{Q1b} antibodies in the serum. The bulbar variant requires the onset of weakness to be within the bulbar musculature: facial muscles, tongue, or muscles of deglutition. Lastly, electrodiagnostic or pathologic studies or both can be used to distinguish between the primary axonal and demyelinating forms because this determination cannot be made by clinical signs and symptoms alone.[209]

CLINICAL VARIANTS

GBS typically has an acute onset followed by rapidly ascending weakness. In some patients, the onset is stuttering with periods of progression and plateaus before reaching a nadir of involvement. In other patients, the onset is subacute, with a slow progression that can occur over a few weeks.[123] In addition to weakness and areflexia, other individual features may be observed in patients with GBS, such as total and incomplete external ophthalmoplegia[65,70]; papilledema[42]; and autonomic dysfunction, including hypertension, postural hypotension, and cardiovascular disturbances.[38,42,43,196] Hypertension may be a consequence of sympathetic nervous system hypersensitivity and increased excretion of catecholamines,[42] but it also has been associated with increased renin-angiotensin activity.[183] Cardiovascular disturbances have been reported to be more prevalent in patients with GBS who are severely paralyzed and require mechanical ventilation.[38] Autonomic dysfunction in GBS, including tachycardia and other arrhythmias, may contribute to morbidity.[43]

An axonal form of GBS was described first in 1986. This new variant syndrome seemed to have a more severe disease course with poorer recovery.[58] The pure motor axonal form, termed *acute motor axonal neuropathy* (AMAN), initially was characterized in north China[129] as a summertime epidemic pattern mostly in children and young adults after they experienced an acute diarrheal illness. When sensory symptoms are present, it is called *acute motor-sensory axonal neuropathy*. Although these axonal subtypes tend to have a more severe and rapidly progressive clinical course, the diagnosis can be made only by electrodiagnostic studies or by pathologic examination of peripheral nerves. Despite some initial conflicting reports, recovery can be either rapid or quite prolonged; however, the long-term recovery potential is similar to that of classic demyelinating GBS (AIDP).[83,152,202]

One criticism of the NINCDS Ad Hoc Committee criteria is that they are too restrictive.[12,14] The criteria initially were designed for use during field studies of GBS, and certain variants are allowed: fever at onset of neuritic symptoms; severe sensory loss with pain; occasional progression beyond 4 weeks; major permanent residual deficits; transient bladder paralysis; and, possibly, central nervous system involvement, such as ataxia (cerebellar), dysarthria, extensor plantar responses, and ill-defined sensory levels. These features need not exclude the diagnosis of GBS.[12,13]

Specific variants in which the clinical features are atypical but fall in the spectrum of GBS have been described.[12,13] Findings of ophthalmoplegia, ataxia, and areflexia were described initially by Fisher in 1956, and he postulated that it was a variant of GBS because of the areflexia and CSF findings of cytoalbuminologic dissociation. This clinical syndrome now is referred to as *Miller Fisher syndrome*.[61,172] The rapid onset of these symptoms usually indicates a benign course with fairly complete recovery occurring within weeks to months.[12,13] According to new diagnostic criteria, Miller Fisher syndrome must meet general criteria for GBS and in addition requires onset of weakness in the extraocular muscles and either ataxia or positive anti-G_{Q1b} IgG antibodies (present in 85%).[209]

Similarities with Miller Fisher syndrome occur in a subgroup of patients with Bickerstaff brain stem encephalitis. Bickerstaff and Cloake first described a syndrome of altered mental status, ophthalmoplegia, and ataxia in 1951. This syndrome was termed *Bickerstaff brain stem encephalitis* when Bickerstaff reviewed his collection of cases for the *Handbook of Clinical Neurology* in 1978. Bickerstaff's clinical criteria for Bickerstaff brain stem encephalitis include progressive external ophthalmoplegia and ataxia along with either alteration of consciousness or hyperreflexia. More recent studies have questioned these strict criteria because a subgroup of these patients may have normal or absent reflexes and varying degrees of weakness and cytoalbuminologic dissociation, indicating an overlap with GBS. In addition, 65 percent of patients with a diagnosis of Bickerstaff brain stem encephalitis have positive anti-G_{Q1b} antibodies, which suggests that all of these syndromes may be part of a clinical spectrum of disease.[145]

A rarer form of GBS, pharyngeal-cervical-brachial or bulbar, has been described in adults, and even fewer reports exist in children. This subtype has predominant weakness of the facial and bulbar musculature and weakness in the neck and upper extremities.[134] Sensory loss and areflexia without motor weakness or simultaneous onset of symmetric cranial nerve dysfunction (cranial polyneuritis) also may be accepted as variants of GBS if they are characterized by rapid onset with recovery, elevation of CSF protein, and the typical electrodiagnostic pattern of demyelination.[12,13,147] The presentation of acute sensory neuropathy characterized by symmetric glove-and-stocking sensory loss for pain and temperature (acute numbness) with normal muscle strength and tendon reflexes (acute small fiber sensory neuropathy 1) also is suggested as a variant of GBS.[5,188]

EPIDEMIOLOGY

Worldwide, the incidence of GBS has been reported to range from 0.4 to 4 cases per 100,000, with a median value of 1.3.[110,173,178] GBS can occur at any age; however, peaks occur in older adolescents or young adults and in the elderly. Overall, men tend to be affected more often than are women, at a ratio of approximately 1.25:1. Specific incidence rates do not exist for the various subtypes, although AIDP accounts for approximately 90 percent of cases compared with only 10 percent in Western countries. Approximately two-thirds of patients have had a preceding illness, typically involving the upper respiratory or gastrointestinal tracts, within 6 weeks of diagnosis.[39,100,173,178] No clear seasonal or geographic clustering of GBS has been observed, with the exception of the increased incidence of AMAN related to *Campylobacter jejuni* infection observed in children and young adults in China during the summer months.[129]

Age-related differences in the expression of GBS in children have been observed.[183] In children younger than 5 years old, a greater incidence of bulbar nerve (cranial nerves IX, X, and XII) dysfunction occurs, and muscle weakness is the most frequent initial symptom (72%). In children older than 5 years, limb pain is the most frequent initial symptom (53.4%). The interval between previous illness and onset of GBS is shorter for children older than 5 years and typically is 2 to 14 days. No statistically significant differences occurred in respiratory complications or fatal outcomes between the older and younger children with GBS.

PATHOLOGY AND PATHOGENESIS

GBS has been described as a distinctive neuropathy characterized pathologically by inflammatory lesions scattered throughout the peripheral nervous system.[143,161] Asbury and associates[13,14] studied 19 fatal cases and observed that the pathologic hallmark of this disorder is perivenular mononuclear inflammatory infiltrates, which occurs throughout the peripheral nervous system, even in cases with a short clinical course (1 to 4 days). These authors observed that the lesions were predominantly lymphocytic and that the inflammatory infiltrates tended to cluster about small endoneural and epineural vessels, particularly veins, in a seemingly random, multifocal distribution. All levels, including anterior and posterior roots, ganglia, proximal and distal nerve trunks, terminal twigs, cranial nerves, and sympathetic chains and ganglia, seemed vulnerable to attack. The site of maximal involvement correlated with the degree of premorbid clinical findings. Segmented demyelination was the predominant form of nerve fiber damage, with myelin destruction restricted to the regions of nerve trunks that were infiltrated by inflammatory cells.

Subsequent reports have confirmed the observation that primary demyelination occurs only in tissue infiltrated by inflammatory cells. These studies have shown that the destructive process is affected by macrophages in the presence of lymphocytes and directed only at the part of the Schwann cell plasma membrane forming the myelin sheath.[27,160,161,218,224] Kanda and associates[99] reported the findings of a necropsy of early fulminant GBS in an adult, however. Using semi-thin sections, they observed less extensive mononuclear infiltration than expected and found nerve fibers with myelin splitting, even in regions where inflammatory cell reaction was inconspicuous. Small myelinated fibers were involved preferentially, and no abnormalities of unmyelinated fibers occurred. Sensory roots were involved as severely as motor roots. These authors concluded that the underlying pathologic mechanism of GBS is heterogeneous. They suggested that some cases are cell-mediated, with predominant perivascular infiltration, whereas other cases are humorally mediated, with predominant demyelination without lymphocytic infiltration.

We now know that demyelinating and axonal forms of GBS exist and, as one would expect, that their mechanisms of injury to the nervous system differ. As a result of molecular mimicry, an immune attack is generated against the peripheral nerves and is mediated by immunoglobulins, macrophages, and complement factors. In AIDP, the initial target is the Schwann cell, which is responsible for myelination of the peripheral nervous system. In the axonal forms of GBS, the initial attack is targeted at the nodes of Ranvier, which allows macrophages to penetrate the basal lamina and enter the periaxonal space, causing subsequent axonal degeneration to ensue.[227]

ROLE OF INFECTION

The onset of GBS frequently follows an acute febrile infectious illness. GBS has been reported to occur after childhood illnesses such as mumps,[31] varicella,[23,44,216] measles,[50,116,156] and rubella.[48,180] These childhood illnesses rarely are associated with GBS, however.[48] Epidemiologic studies have indicated the significant occurrence of upper respiratory or gastrointestinal tract illnesses before the onset of GBS and support the concept that both of these categories of illness constitute important risk factors for GBS.[101,132] One case-control study[132] reported a higher incidence of elevated specific complement-fixation antibody titers in GBS cases compared with control cases for infectious mononucleosis and parainfluenza. Although influenza A and B infections have been observed in patients with GBS,[22,141,142,217] this case-control study found no significantly higher incidence of elevated complement-fixation titers in patients with GBS compared with control subjects for influenza A or B.[132] During outbreaks of influenza A2 in 1960 to 1961 and influenza B in 1961 to 1962, the prevalence of GBS was high; however, the greatest number of cases occurred from November 1959 to October 1960, when the prevalence of influenza was comparatively low.[132]

Infectious hepatitis has been reported in association with GBS,[154,158] and four case reports have observed hepatitis B antigenemia in patients with GBS.[60,128,139,140] Acute viral hepatitis rarely is complicated by GBS. GBS has been associated with serologically documented cases of acute A, B, non-A/non-B, and delta hepatitis.[117,199,204] Immune complexes containing hepatitis B surface antigen in the serum and CSF were found in patients with GBS. These complexes were present with acute hepatitis B during the acute phase of GBS and disappeared when the neurologic symptoms resolved.[124,199,204] A causal association has not been established, however, and the fact that many of the populations at risk for hepatitis B and A also are at risk for other acquired viral infections, such as Epstein-Barr virus and cytomegalovirus (CMV), has been emphasized.[199]

Echoviruses and various serotypes of coxsackievirus A and B isolates have been observed in patients with GBS.[17,56,63,94,102,132,153,206] Most isolates were obtained from stool, although some were obtained from CSF in a few cases.[48] Recovery of virus does not prove causation, however; some isolates were recovered when enterovirus was prevalent in the community and in one instance equaled the frequency from control subjects.[48,122] In one series investigating West Nile virus, 1 patient of 224 had a clinical diagnosis of GBS.[126] Direct isolation of coxsackievirus A4 from nerve roots and dorsal root ganglia has been reported.[56] GBS also has been reported to occur after herpes simplex virus type 2 encephalitis,[133,194] in association with HIV infection,[41] and during rabies infection.[210]

Laboratory research has suggested that an important association exists between herpesviruses and GBS. Dowling and Cook[48] reported that 15 percent of patients tested with GBS had high titers of IgM antibodies against CMV antigen in tissue culture cells. Other studies also have observed an association between CMV and GBS.[11,35,45,97,137,157,185] Dowling and Cook[48] reported a wide spectrum of antecedent illness, ranging from asymptomatic infection to typical respiratory tract and gastrointestinal tract symptoms, in CMV-positive patients. A predilection for CMV-positive GBS was found to occur in patients younger than 30 years, corresponding to the age incidence described in heterophil-negative, CMV-induced, mononucleosis-like illness.[48] Time clustering of cases was observed, with CMV antibody–positive cases appearing in 10- to 16-week clusters. Dowling and Cook[48] suggested that in cases associated with preceding surgery, GBS may be consequent to CMV, either acquired during transfusion or caused by activation of latent virus, or occurrence of nontransfusion, non-A/non-B hepatitis.

Dowling and Cook[47] also observed the frequent (8%) occurrence of IgM antibodies to Epstein-Barr virus in GBS. In contrast, less than 2 percent (1 of 75) of GBS patients showed IgM herpes simplex virus–specific antibody.[21] These studies indicate that two herpesviruses are common antecedents of the syndrome, but the precise mechanism by which they initiate destruction of myelin is unknown.

Hart and Kennedy[74] emphasized the difficulty of establishing CMV as a cause of GBS. Using serologic tests, they confirmed active CMV infection in three patients with GBS. They reported that CMV could be isolated in 1 percent of asymptomatic individuals and in 10 percent of pregnant women; hence, isolation may be coincidental. These authors suggested that other viruses may activate latent CMV, and isolation may reflect such a nonspecific phenomenon. GBS also has been observed in association

with HIV infection.[41] Elevated levels of circulating antibody to Epstein-Barr virus, CMV, and other infectious agents are common findings in these patients and may be directly responsible for some cases of GBS occurring in association with HIV infection.

Nonviral infectious agents also may precede occurrence of GBS. A preceding infection with *C. jejuni* associated with a diarrheal illness commonly occurs.[168,169,171] After two case reports,[33,162] a retrospective study was conducted to determine serologic evidence of recent *C. jejuni* infection in GBS.[98] Of 56 GBS patients, 38 percent (20 patients) were found to have serum evidence, CSF serologic evidence (including documentation of increase in titer, two or more elevated antibody titers, or positive CSF titer), or both. Preceding diarrheal illnesses were documented in 20 percent of these patients with GBS. Groups of normal control subjects and subjects with other neurologic disorders had no evidence of having had a recent *C. jejuni* infection. IgA-specific and IgM-specific antibody was found only in the CSF of patients with recent *C. jejuni* infection, suggesting production of specific antibodies by the central nervous system because passive diffusion into the CSF is unlikely.

Patients with GBS who also showed serologic evidence of *C. jejuni* seemed to have a significantly more severe illness; 90 percent of these patients required mechanical ventilation. Electrodiagnostically, GBS preceded by *C. jejuni* selectively elicits the AMAN subtype rather than AIDP.[112] One report noted mild illness in patients with GBS and positive serology and stool culture for *C. jejuni*.[162] Rees and associates,[169] in a prospective, case-controlled study in a cohort of patients with GBS (96 patients) or Miller Fisher syndrome (7 patients) who were admitted to hospitals throughout England and Wales between November 1992 and April 1994, found evidence of recent *C. jejuni* infection in 26 percent of the patients with GBS or Miller Fisher syndrome compared with 16 percent of household controls and 1 percent of age-matched hospital controls. A study in Japan has shown the *C. jejuni* serotype HS:19 to be more common in patients with GBS (67%) than in control patients with enteritis (5%).[200] Seventy percent of the patients with *C. jejuni* infection reported having had a diarrheal illness within 12 weeks before the onset of the neurologic illness. *C. jejuni* was associated with axonal degeneration, slow recovery, and greater disability after 1 year. The median interval from onset of diarrhea to the onset of symptoms for all *C. jejuni*–positive patients was 9 days, suggesting that GBS is a consequence of an immune response to *C. jejuni*, rather than a direct effect of the organism or one of its toxins.[95]

Mycoplasma pneumoniae is the second most prominent nonviral agent reported in association with GBS. One report observed that 5 percent of patients with GBS had serologic evidence of active *Mycoplasma* infection.[66] GBS has been reported rarely after infection with *Haemophilus influenzae*, *Francisella tularensis* (tularemia), *Chlamydia* (psittacosis), *Plasmodium* (malaria), *Mycobacterium tuberculosis*, *Bartonella henselae*, and *Toxoplasma gondii*.[24,69,125,132,135,138,148,184,214]

Patients with GBS frequently give a history of prodromal symptoms. An association between GBS and an infectious agent long has been considered. Isolation of a pathogen in a patient with GBS does not confer a direct correlation, however. McFarlin[127] has suggested that demyelination of peripheral nerves may result from direct infection of Schwann cells by the infectious agent, producing prodromal symptoms, or from immunologic mechanisms triggered by the infection. The second possibility is favored because epidemiologic and virologic surveys of patients with GBS have failed to identify reactivity with a single infectious agent and because the disorder can be triggered by other events, including surgery and immunizations. In addition, the clinical course may be shortened by plasmapheresis.

ROLE OF IMMUNIZATION

GBS occurs infrequently after immunization, including smallpox, diphtheria, tetanus, pertussis, combined mumps-rubella, hepatitis B, rabies, *H. influenzae* type b, and polyvalent pneumococcal vaccines.[40,64,73,92,104] A nationwide GBS surveillance conducted from December 16, 1976, until January 31, 1977, suggested that an excess risk of developing GBS was related to A/New Jersey influenza vaccine for adults 18 years or older.[28] The peak time of onset of GBS was 2 to 3 weeks after the vaccine was given. For the 10 weeks after vaccination with A/New Jersey vaccine, the risk was approximately 13.3 cases per 100,000 for vaccine recipients, which was five to six times higher than that in unvaccinated individuals (2.6 per 100,000).[186,187]

A subsequent survey of adults older than 18 years of age vaccinated in the 1978 to 1979 influenza campaign revealed that the relative risk of developing vaccine-associated GBS (individuals vaccinated within 8 weeks before the onset of GBS) was 1.4, which was significantly below the risk (6.2) associated with A/New Jersey vaccine for the equivalent 8-week period.[28,92,187] The survey of 1979 to 1980 also did not reveal an increased incidence of GBS for vaccinated versus nonvaccinated individuals.[92,100,187] The clustering of onset of GBS in the second and third weeks after the influenza vaccinations were administered in 1976 was not observed after administration of vaccinations in either 1978 to 1979 or 1979 to 1980.[187] These results suggested that A/New Jersey influenza vaccine differed from subsequent influenza vaccines in its ability to trigger GBS.[187] The neurotigenic P2 protein of peripheral nerve myelin has been shown to have been present and biologically active in the 1976 influenza vaccine.[193] P2 may have been a factor in the production of GBS after administration of the A/New Jersey influenza vaccination in susceptible individuals.[192]

Asbury[12] concluded that an "epidemic" of GBS actually occurred, but the precise cause of the illness remains undetermined. Whether a special antigenic site was present on the A/New Jersey viral product remains unknown. Patients with GBS who were vaccinated were spread throughout all 141 lots, and the problem could not be traced to individual lots or a single manufacturer. Surveillance for GBS in the subsequent (1979) influenza programs did not show any excess number of cases of GBS.[12]

GBS has occurred after the administration of rabies vaccine prepared in suckling mouse brain.[26,80] A severe protracted course with involvement of cranial nerves, increased mortality rate (20%), and increased long-term sequelae were observed. GBS occurs less commonly after immunization with sample-type vaccine prepared in brain and spinal cord of mature animals.

The prevalence of GBS cases was increased in children and adults in Finland in 1985 after institution of a mass vaccination program with oral polio vaccine.[103,205] This one-time campaign involved the entire Finnish population, and further study of the association between oral polio vaccine and GBS in Finland was impossible. This potential relationship was examined further during a retrospective epidemiologic survey in southern California.[165] No apparent temporal association between GBS and oral polio vaccine was noted. The frequency of GBS was low in the age groups during which children usually are immunized (before 2 years old and 5 years old). Researchers concluded that the failure to find a correlation between the usual age of oral polio vaccine immunization and the incidence of GBS by age coupled with the failure to find any children with GBS with onset within 1 month of receiving oral polio vaccine immunization provided strong evidence against a causal relationship between administration of oral polio vaccine and development of GBS. They suggested that the differences noted in the outcomes of these studies may be related to the fact that the entire population in the study

in Finland already was vaccinated with inactivated polio vaccine and that different types of oral polio vaccine were used in Finland and California.

In October 2005, five cases of GBS occurring after the administration of the meningococcal polysaccharide diphtheria toxoid conjugate vaccine (Menactra, MCV4) in the United States were reported to the Vaccine Adverse Events Reporting System. Following a heightened awareness, three additional cases were reported. A subsequent investigation determined that the occurrence of eight cases of GBS out of the total number of vaccines given within the time period reported is similar to what might be expected on chance alone, based on an average annual incidence rate of 1.4 per 100,000 per year. The Centers for Disease Control and Prevention and the Global Advisory Committee on Vaccine Safety (World Health Organization) have concluded that no change should be made in vaccine policies, with the exception that individuals with a history of GBS not be vaccinated unless they are thought to have an elevated risk of contracting meningococcal infection.[29]

IMMUNOLOGIC FACTORS

Current opinion strongly favors the hypothesis that GBS is an autoimmune disease. Much evidence suggests that GBS represents an aberrant immune response to peripheral nerve components.[9] McFarlin[127] suggested that the following factors support an autoimmune mechanism in GBS: (1) plasmapheresis shortens the clinical course of GBS; (2) sera from patients with GBS contain IgM antibodies against a component of peripheral nerve myelin; (3) lipid antigen reacts with these antibodies; and (4) at least one lipid, sulfate-3-glucuronyl paragloboside (SGPG), can produce an experimental disease similar to GBS. This hypothesis is supported by the similarities of GBS, experimental allergic neuritis, and Marek disease.[203,215] Serum antibodies or antibody-like factors have been shown in peripheral nervous system tissues in experimental allergic neuritis and in GBS.[28,110,115,137,167,168,190,193,199] P2, a neurotigenic component of peripheral nervous system myelin administered in complete Freund adjuvant, has been shown to induce experimental allergic neuritis,[217] and sensitization to P2 has been reported in GBS.[2-4,192] Sensitized lymphocytes capable of producing demyelination have been found in experimental allergic neuritis and GBS.[10,59,68,222] Evidence of hypersensitization to peripheral nervous system antigens by use of the technique of macrophage migration inhibition factor assay has been reported in experimental allergic neuritis[191] and GBS.[68,106,119,174]

The cause of GBS and the nature of the antigen or antigens against which the immune response is directed are not known precisely. Studies have provided evidence that an intense immunologic response is an invariable accompaniment of the disease.[4,36,93,179,181] Cell-mediated and humoral immunity have been found to be altered in GBS and may contribute to the pathogenesis and pathology of GBS.

Several lines of investigation have implicated a cell-mediated immunologic reaction to the constituent of myelin as being of primary importance in the pathogenesis of GBS.[3,4,9,68,93,106,181] Activated lymphocytes can be identified in early phases of GBS.[46] Secretion of mediators, such as macrophage migration inhibitory factor (a measure of T-cell sensitization), in the presence of P2 protein has been shown during the acute phase of GBS.[20,68,106,174,192] By means of in vitro lymphocytic transformation technique, some researchers found lymphocytes sensitized to P2 in GBS,[4] whereas others have not confirmed these results and suggest that P2 may not be the antigen in GBS.[93] Goust and associates[68] reported finding circulating immune complexes in GBS and an association between increased immune complex and decreased suppressor cell function. Abnormal T-cell subsets have been reported,[120] but other investigators have not reproduced this finding.[86,93] Additional evidence of cell-mediated immunity in GBS is suggested by studies observing demyelinization of rat peripheral nerves in tissue culture by circulating immunocytes from patients with GBS or lymph node cells from animals with experimental allergic neuritis.[10,93,182]

Activation of T cells has been reported in GBS.[4,77,78,201] Alterations in T-cell subsets, including decreased numbers of $CD3^+$ and $CD4^+$ and increased CD19, have been observed.[225] These changes normalized after plasmapheresis occurred in patients who improved. Taylor and Hughes[201] observed an increase in the levels of T cells bearing activator markers (interleukin-2 receptor and transferrin receptor) in the serum of GBS patients compared with normal control subjects. Hartung and associates[77,78] showed T-cell activation in the acute phase of GBS, as evidenced by increased interleukin-2 receptor expression on T cells, increased serum concentrations of interleukin-2 and soluble interleukin-2 receptor, and increased numbers of DR-positive circulating T cells. They reported that increased soluble interleukin-2 receptor concentrations that were found in several samples decreased with clinical improvement. The suggested role of activated T cells in GBS could include cytotoxic effect on Schwann cells, myelinotoxic effects, recruitment of macrophages in a delayed hypersensitivity reaction, or helping B cells to produce antibody against myelin. Activated T cells also may play a role in recovery.[77,78]

Protective and destructive roles have been ascribed to antibodies in GBS.[10,223] One suggestion is that the destructive effects of antibodies are mediated either directly by lysis of peripheral myelin with or without a requirement for complement or indirectly by opsonizing myelin, which then is attacked by macrophages.[93] Serum and CSF immunoglobulins of restricted electrophoretic heterogenicity have been reported to be increased in GBS, and these increased levels return toward normal with clinical improvement.[34,36,118] Autoantibodies to erythrocytes and circulating antigen-antibody complexes have been found in GBS.[34,37,46,49,68] Melnick[131] first showed anti-neural antibody in GBS sera, and anti-neural antibodies also have been reported in the CSF of patients with GBS.[179] The cytotoxic effect of GBS serum in vitro has been reported.[36,38,51] Several studies have suggested the presence of anti-neuronal antibodies in GBS patients. Indirect immunofluorescence has been used to show that sera of patients with GBS have IgG antibody to monkey dorsal root ganglia.[47]

Some findings indicate that sera from patients with GBS react with multiple antigens in peripheral nerve myelin.[49,110,163,226] Complement-fixing antibodies to peripheral nerve myelin have been detected in the sera of patients with GBS.[108,131] Increasing titers of complement-fixing antiperipheral nerve myelin antibodies (IgM) have been found during the acute phase of GBS, and decreasing titers have been observed during convalescence.[108] Koski and associates[108,109] found that some of the antiperipheral nerve myelin antibody in sera from patients with GBS binds a neutral glycolipid of human peripheral nerve myelin and cross-reacts with Forssman antigen, a cross-species antigen found in many infectious diseases. These investigators suggested that IgM antibodies, triggered by multiple infectious agents in patients with GBS, can bind to a glycolipid surface determinant of human peripheral nerve myelin and, after penetration of the damaged blood-nerve barrier, participate in the demyelination of peripheral nerves through activation of complement. Yu and associates[226] found that sera from patients with GBS reacted with antigens in peripheral nerve myelin, including one lipid, SGPG. In rabbits, immunization with SGPG produced weakness and physiologic abnormalities consistent with a demyelinating neuropathy.

PATHOGENESIS

GBS is regarded as an autoimmune disorder involving cellular and humoral immune mechanisms.[209] Immunologic studies have not resulted in a simple concept of the pathogenesis of GBS. The animal model of GBS, experimental allergic neuritis, has allowed researchers to analyze the pathogenetic mechanisms involved in the demyelinating process.[209] In the Lewis rat, GBS can be transferred by $CD4^+$ T cells reactive to neuroautoantigens P2 and P0. In the rabbit, experimental allergic neuritis serum injected intraneurally demyelinates rat nerve largely because of antigalactocerebroside antibody.[159] In humans, no peripheral nerve antigen has been identified as the responsible target.[208] Involvement of the cellular immune system also has been implicated. T and B cells become activated at the onset of GBS, as indicated by an increase of activation markers, including interleukin-2, soluble interleukin-2 receptor, and tumor necrosis factor-α, in serum and CSF.[18,76,189,208] The serum levels of these markers decrease with recovery.[18,189]

Disruption of myelin has been reported secondary to increased serum concentrations of the cytokine tumor necrosis factor-α.[189] Increased serum concentrations of tumor necrosis factor-α are detected in 50 percent of patients with GBS and in 26 percent of patients with unrelated neuropathies and other neurologic disorders. Increased serum concentrations of tumor necrosis factor-α are not specific for GBS, however. Tumor necrosis factor-α serum concentrations correlate with clinical severity, decrease as patients recover,[189] and are similar in patients with GBS whether or not preceding infections were noted. These elevations likely are not merely secondary to the antecedent infection. In contrast, soluble concentrations of interleukin-2 receptor are elevated in healthy relatives of patients with GBS and in patients with GBS. These data suggest that these concentrations may be secondary to environmental or infectious factors, rather than related to the pathogenic mechanism responsible for GBS.[189]

The involvement of complement is suggested by findings that include increased concentrations of the soluble terminal complement in serum and CSF and increases of C3a and C5a in CSF alone.[76,159,208] One study showed a breakdown of the blood-nerve barrier by activated T cells, allowing the development of focal conduction block and demyelinization in the presence of circulating anti-myelin antibodies.[159] Elevated serum concentrations of endothelial leukocyte adhesion molecule-1 have been shown in GBS during the acute phase, with serum concentrations returning to normal by 14 days. Endothelial leukocyte adhesion molecule-1 may be important by virtue of breakdown of the blood-nerve barrier.[148]

The diversity of preceding infectious factors and the observation that GBS may occur after noninfectious factors, such as surgery, trauma, epidural anesthesia, drug administration, and immunizations (Table 43–15),[9,28,57,198] have suggested that infection is not a necessary precondition for the development of GBS. A single factor, such as the release of antigen, may be the common mechanism leading to nerve damage. Also, the peripheral nervous system may possess a limited repertoire of pathologic responses and may react to diverse insults in a restricted fashion.[191] As noted, numerous reports have indicated alterations of cell-mediated immunity and humoral immunity in GBS and have suggested an immunologic basis for the demyelination in GBS. The exact mechanism and precise interaction of a preceding event and the patient's cell-mediated and humoral immune responses in causing demyelination are unknown. Whether GBS is the result of an autoimmune process or represents neural injury from an immune response to viral antigen that might be present in neural tissue is unclear.[36] Proposed mechanisms for the immunopathogenesis of GBS lesions have included antibody or cell-mediated immunity to an infectious agent with secondary neural injury, autoantibody or cell-mediated immunity to peripheral nervous system tissue, and demyelinization caused by deposition of circulatory antigen-antibody complexes in blood vessels of peripheral nerves.[36] One suggestion is that GBS is a syndrome and not a disease and that it may have several different causes.[197]

TABLE 43–15 Antecedent Factors

Strongly Suggestive Evidence
Cytomegalovirus
Epstein-Barr virus
Coxsackieviruses A
Campylobacter jejuni
Mycoplasma pneumoniae
Suggestive Evidence
A/New Jersey 1976 influenza immunization
Survey study or anecdotal reports
Viral Agents
Echoviruses
Coxsackieviruses A and B
Influenza viruses A and B
Measles
Varicella virus
Rubella
Mumps
Hepatitis viruses
Herpes simplex viruses
Rabies
Human immunodeficiency virus
Nonviral Agents
Francisella (Pasteurella) tularensis (tularemia)
Chlamydia
Plasmodium (malaria)
Toxoplasma gondii
Mycobacterium tuberculosis
Noninfectious Agents
Immunizations
Trivalent oral poliovirus
Diphtheria-tetanus-pertussis
Measles-mumps-rubella
Rabies
Influenza, other than A/New Jersey
Polyvalent pneumococcal
Hepatitis
Haemophilus influenzae type b
Surgery
Trauma
Epidural anesthesia
Neoplasm (Hodgkin)
Vasculitides (systemic lupus erythematosus)
Drug hypersensitivities
Heroin addiction
Drug (zimeldine)

Increasingly, researchers have noted that the expression of GBS varies considerably.[52,208] Although the NINCDS Ad Hoc Committee proposed criteria for the research diagnosis of GBS, this committee recognized the occurrence of the many variant subtypes previously discussed. GBS could be viewed as a family of closely related diseases that can be categorized by class of axon (motor, sensory, autonomic, or mixtures), pathologic process (inflammatory-demyelinating, antibody attack), preceding infection, associated antibody, underlying disease mechanism, or response to treatment.[208]

The clinical variability of GBS may reflect different pathogenetic mechanisms. Pathology results suggest the heterogeneity of GBS. Studies by Asbury and coworkers[12,13,15] indicated the early occurrence of inflammatory cells as the primary mechanism

resulting in demyelination. The lesions were found along the entire length of the nerve, but with variable expression among patients corresponding to their clinical deficits. The role of lymphocytic infiltration has been reappraised. Lymphocytic infiltration varies widely among patients with GBS, and severe demyelination can occur without lymphocytic infiltration. Heterogeneity is suggested further by experimental allergic neuritis models.[208] The rat experimental allergic neuritis model is a T-cell–dependent response to the antigen P2. In the rabbit model, demyelination results from a B-cell–dependent response to galactocerebroside. These models suggest that the variability of GBS may depend on the relative contribution of T-cell and B-cell responses.

Serum antibodies against such glycolipids as G_{M1}, G_{M1b}, G_{M2}, GALNAc-GD_{1a}, G_{D1a}, G_{D1b}, G_{D3}, G_{T1a}, G_{T1b}, G_{Q1b}, and L_{M1} have been reported in the acute phase of GBS.[55,75,144,211,227,228] In GBS, the specificity of anti-ganglioside antibodies varies.[31,220] The presence of anti–G_{M1} ganglioside IgG antibodies may be associated with a more severe and predominantly motor form of GBS (AMAN). Among 132 patients with GBS participating in the Dutch GBS trial and for whom suitable pretreatment serum was available, 25 (19%) showed high anti-G_{M1b} antibody titers of the IgG ($n = 15$) or IgM ($n = 14$) class or both ($n = 4$). Patients who were antibody-positive more frequently experienced preceding diarrhea and had serologic evidence of recent *C. jejuni* infection without antecedent evidence of CMV, Epstein-Barr virus, or *M. pneumoniae* infection than did patients who were antibody-negative. In the patients who were antibody-positive, the onset was more rapid, limb weakness was more severe, distal weakness was more prominent, and recovery time was more prolonged. These patients had less frequent sensory deficit or cranial nerve involvement.[229]

Serum anti–G_{Q1b} ganglioside antibodies have been observed in the Miller Fisher (and Bickerstaff brain stem encephalitis) variant of GBS.[31,95,220] Eighty-two percent of patients with the Miller Fisher variant of GBS may have IgG antibodies to G_{Q1b}. The circulating anti-G_{Q1b} IgG antibodies induce presynaptic and postsynaptic blockade. After the patients recovered, this blocking activity was lost, and sera became negative for anti-G_{Q1b} antibodies. These IgG antibodies may play a pathogenic role in the Miller Fisher variant of GBS.[25] In addition, the G_{Q1b} epitope is expressed preferentially on the paranodal regions of the oculomotor, trochlear, and abducens nerves, which correlates with the clinical picture of ophthalmoplegia.[227] Researchers also have hypothesized that the anti-G_{Q1b} antibodies may bind to 1a afferent nerve fibers within the spinocerebellar tract, leading to ataxia.[227]

These findings suggest that some association exists between antigenic specificities of anti-glycolipid antibodies and clinical forms of GBS. The exact role of these antibodies as either neurotoxic and directly responsible for the symptoms or incidental by-products is not clearly established, however.[31,208,220] A decline in anti–G_{D1b} ganglioside titers in relation to clinical recovery has been reported in patients with GBS after receiving plasma exchange.[170] Although studies confirm the activation of the immune system in GBS, whether the increase in activation markers is caused by preceding infection, why they are found only in some and not all patients with GBS, and what their role is in destruction of myelin remain unknown.

Evidence for shared antigenic determinants among certain infective agents associated with GBS and various antigens within peripheral nerve includes the following: (1) shared antigenic determinants between herpes simplex virus ribonucleotide reductase and peripheral nerve P0 glycoprotein have been shown, (2) amino acid sequence homology between CMV and varicella-zoster virus and P0 glycoprotein has been observed, and (3) antibodies to ganglioside have been detected in patients with GBS after they have had an infection caused by *C. jejuni* or *M. pneumoniae*.[7,159] Some studies indicate that molecular mimicry may contribute to the presence of antiganglioside antibodies after infection with *C. jejuni* has occurred.[55,144,228] Humoral immune mechanisms directed at an infectious agent may cross-react with antigens of the peripheral nervous system and cause an immunopathologic disease. The family of glycoconjugates, which includes gangliosides, is one of the myelin antigenic groups with which antibodies have been identified in GBS. The frequency of these antibodies may be 35 percent of patients with GBS. Nonetheless, the presence of high titers of anti-glycoconjugate antibodies is not correlated with the more severe course of this disease.[55,71,98,211]

The association of *C. jejuni* infection preceding presentation of GBS and the occurrence of antiganglioside antibodies may contribute to an understanding of pathogenesis of GBS. *C. jejuni* has a lipopolysaccharide capsule that is rich in glycoconjugates containing sialic acid that resemble human glycoconjugates.[71] A terminal tetrasaccharide immunogenically identical to the ganglioside G_{M1} in human peripheral nerve has been shown on the outer membrane of a *Campylobacter* strain cultured from a patient with GBS.[17] Serologic evidence of an antecedent *C. jejuni* infection has been shown to correlate with the presence of anti-G_{M1} antibodies.[55,95,211,228] Anti-G_{M1} IgG antibodies in GBS sera recognize surface epitopes on whole *Campylobacter* bacteria, and this recognition is strain-specific. *Campylobacter* isolated from patients with GBS and with enteritis have been found to have similar ganglioside-like moieties. This finding indicates that patients who develop GBS respond differently to the ganglioside-like epitopes on *Campylobacter* than do patients with non-GBS diarrhea.[95] The finding also suggests a role for host susceptibility as a determinant for outcome after *Campylobacter* infection.[190]

C. jejuni, but not *Escherichia coli*, bacteria can absorb G_{M1} antibodies of the IgG class.[144] Studies suggest that molecular mimicry might play a role in the pathogenesis of GBS. Molecular mimicry is suggested further by the observation that the G_{Q1b} epitope is present in the lipopolysaccharide fractions of *C. jejuni* isolated from patients with the Miller Fisher variant.[220,228] Direct infection of cells of the peripheral nervous system leading to an immune attack seems unlikely in the case of *C. jejuni* because extraintestinal infection, especially peripheral nervous system infection, probably is irrelevant. *C. jejuni* may generate toxins that directly damage peripheral nerves. Although strain-specific toxins may play a role in post–*C. jejuni* GBS, an immune mechanism remains most likely.[71] Especially appealing is that the association with *C. jejuni* provides indirect support for the shared epitope or molecular mimicry model of the immunopathogenesis of GBS.[190] One hypothesis is that an infectious agent with a specific antigenetic repertoire induces an immune response involving T-cell activation and antibodies that cross-react with gangliosides in peripheral nerves.[55]

In the motor axonal form of GBS, the nodes of Ranvier of the motor fibers are the primary targets. The earliest changes seem to be antibody-mediated and complement-mediated, and T lymphocytes are a rare finding in nerve. The axonal form of GBS has been associated with antiganglioside G_{M1}, G_{M1b}, G_{D1a}, and GalNAc-G_{D1a}, antibodies, and *C. jejuni* infection.[75,227] The epitopes for the aforementioned antibodies have been shown to be located on the nodal axolemma of motor nerves. Most of the Chinese cases are reported to be triggered by recent *C. jejuni* infection in the gut. Surface lipopolysaccharides of *C. jejuni* strains associated with GBS contain epitopes that are similar or identical to G_{M1} ganglioside that is concentrated at the nodes of Ranvier. This finding supports the role of molecular mimicry for inducing GBS.[16]

Conflicting results have been reported concerning the relationship of G_{M1} antibody to axonal degeneration.[113] Antibodies to other gangliosides have been reported to be elevated in sera of patients with GBS during the acute phase. Only a 50 percent

concordance between serologic evidence of *C. jejuni* infection and the presence of anti-G_{M1} antibody was found during large studies. GBS patients with anti-G_{M1} antibodies have had identified infections that included *C. jejuni* (23%), CMV (10%), *M. pneumoniae* (6%), and Epstein-Barr virus (3%).[146] Microorganisms other than *C. jejuni* may trigger an anti-ganglioside response and elicit axonal GBS. Whether the presence of anti-G_{M1} antibodies is associated with extensive axonal loss and poor outcome is controversial. Some patients with GBS and anti-G_{M1} antibodies recover quickly or have conduction abnormalities suggestive of demyelination.[85,113,167,212]

OUTCOME

Most patients with GBS recover spontaneously. Epidemiologic studies have indicated, however, that 10 to 23 percent of patients may require mechanical ventilation, 7 to 22 percent have some residual disability, 3 to 10 percent relapse, and 2 to 5 percent die.[12,30,62,85,105,121,164,221] Patients may be left with residual symptoms, such as facial weakness, weakness in the lower extremities with footdrop, weakness and atrophy of hands, and autonomic dysfunction (impotence and urinary retention).[123] In the North American study of the effect of plasmapheresis,[87] the median time to recovery of independent walking was 85 days for control subjects compared with 169 days for patients who received mechanical ventilation. This same study indicated that children walked at 52 days. Five prognostic factors were identified: age, requirement for respiratory support, rate of progress, abnormal physiologic characteristics of peripheral nerve function, and plasmapheresis. In children, the overall recovery and outcome generally is better than that seen in adults. Much of the literature now uses the Hughes functional disability rating scale in assessing outcome measures. This is a scale from 0 to 6: 0—healthy, 1—minor symptoms and able to run, 2—able to walk 5 m independently, 3—able to walk 5 m with assistance, 4—confined to bed (or chair), 5—requiring assisted ventilation, 6—death.[87]

Complete recovery from motor or sensory deficits after an episode of GBS has been reported in 70 percent of cases after 12 months and in 82 percent after 15 years.[177] Kleyweg and associates[105] compared the outcome of GBS in groups of children and adults. They observed that 22 percent of children compared with 30 percent of adults required mechanical ventilation, with a median duration of 21.5 days in children and 32 days in adults. Mean duration of hospitalization was 84 days for children and 86 days for adults. Two of 18 children died. At 1 year, 77 percent of children had a good outcome, and at 2 years, 83 percent had a good outcome. For adults, good outcomes at 1 and 2 years were observed in 86 percent and 92 percent. Significant slowing of motor and sensory nerve conduction or electromyographic abnormalities may persist for weeks to years after recovery.[164,177] Reported unfavorable signs for complete recovery in children included a period longer than 18 days of plateau between onset of weakness and time of greatest weakness, weakness and maximum motor deficit of greater than 3 weeks, or marked paralysis alone (need for assisted ventilation).[53,177,221]

There are different recovery patterns for demyelinating forms of GBS compared with axonal variants. The axonal varieties overall have a more aggressive and progressive course, reaching their nadir much earlier than do AIDP variants. Demyelinating or AIDP syndromes tend to have a uniform recovery, with most patients having a complete or near-complete recovery over the course of several weeks to months, as previously described. Patients with axonal subtypes (specifically AMAN) seem to have two distinct recovery patterns: a rapid recovery observed within the first few weeks or a prolonged recovery period lasting several months or longer (more often seen with acute motor-sensory axonal neuropathy). Some patients have shown improvements and regained the ability to walk years later,[81,82] which may explain earlier studies that predicted a worse outcome overall for the axonal subtypes because the evaluation period was too brief to measure their long-term recovery potential accurately. The recovery and outcome have been shown to be better and faster in children. One study in children showed similar outcomes at 1 year, regardless of subtype; however, the axonal subtypes had a more protracted course.[202]

TREATMENT

The mainstay of treatment of GBS is supportive care because there is no known cure. Intensive nursing and medical care are essential for proper management. Most deaths are related to respiratory failure, pulmonary embolism, and autonomic dysfunction.[88] Respiratory function must be monitored closely, especially during the acutely progressive phase. The need for endotracheal intubation and mechanical ventilation has been suggested if vital capacity decreases to less than 12 to 15 mL/kg, arterial Po_2 decreases to less than 70 mm Hg, or clinical signs of fatigue develop. Tracheostomy may be indicated if paralysis is prolonged.[175] Physiotherapy and preventive measures for pulmonary embolism are indicated. Because of autonomic dysfunction, electrocardiogram and blood pressure monitoring are essential. Hyponatremia is a reported complication and probably is caused by inappropriate secretion of antidiuretic hormone[155]; fluid and sodium balance and adequate nourishment must be maintained. Reassurance and attention to anxiety, anger, and depression are important during the progressive phase of GBS, especially for patients being ventilated.[88]

Pain is a very common feature of GBS, especially in children, and may be the earliest and most common symptom, occurring in as many as 90 percent of cases. Pain often is either underrecognized or underappreciated and undertreated. Although nonsteroidal anti-inflammatory drugs and opioids can be effective in pain management, they may not be optimal treatment options because of side effects or relative ineffectiveness for neuropathic pain. Gabapentin and carbamazepine have proven efficacy in a variety of neuropathic pain syndromes and in GBS as measured by a reduction in use of intravenous opiates and subjective patient reporting.[136,150,151] Carbamazepine and gabapentin were compared head to head in a randomized, prospective, double-blind, placebo-controlled study. Although both treatments were effective, gabapentin showed earlier and more profound pain relief and less sedation compared with carbamazepine.[151]

A variety of treatment modalities have been used in an attempt to hasten recovery, shorten the duration of time on a ventilator or in an intensive care unit, and decrease the incidence of residual neurologic deficits. These treatments include steroids, plasmapheresis or plasma exchange, CSF filtration, immunoadsorption, and IVIG. The Cochrane Collaboration has published separate systematic reviews of corticosteroids, plasma exchange, and IVIG therapy for GBS.[89,90,166] In 2003, the American Academy of Neurology (AAN) published practice guidelines with regard to immunotherapy in GBS, largely based on the Cochrane systematic reviews of the literature.[91]

The earliest studies investigated the effects of steroids on GBS. Limited evidence suggests that oral steroids slow the recovery process. Intravenous steroids have shown a trend toward a more rapid recovery that is not statistically significant. No effects of intravenous steroids on long-term outcome have been reported, and no significant adverse effects from intravenous steroids have been reported. Currently, corticosteroids are not recommended in the treatment of GBS.[90,91]

Plasma exchange has been shown to be beneficial in the treatment of GBS by shortening recovery time and duration of mechanical ventilation and reducing medical costs. Plasmapher-

esis may be beneficial in patients treated 30 days after the onset of disease, although it is more beneficial when started within 7 days of onset.[166] Studies in children also have shown the efficacy of plasmapheresis for treatment of GBS. In children, the benefits have included decrease in the number of days of mechanical ventilation and time to motor recovery and a decrease in the overall cost of care.[96,207] A further study suggested that two exchanges are better than none for mild GBS, four exchanges are more beneficial than are two for moderate GBS, and six exchanges are no more beneficial than are four in severe GBS.[16]

Although plasmapheresis can be a cumbersome procedure with many technical difficulties, it has been used safely in critically ill children 8 months old. Mild hypotension related to rapid fluid removal has been reported, but no major complications or deaths related to plasmapheresis have been reported in pediatric patients with GBS. Reported complications in adults include hypotension, transfusion reactions, hypocalcemia, arrhythmias, cardiopulmonary arrest, and infection caused by transmitted blood products or sepsis secondary to indwelling catheters. As the only treatment with proven benefit in randomized placebo-controlled trials, plasma exchange is the standard against which all subsequent treatments will be compared. Based on the evidence, current AAN guidelines recommend plasma exchange for nonambulatory patients within 4 weeks of onset of symptoms and for ambulatory patients within 2 weeks of onset.[91,166]

Immunoadsorption has been tried as an alternative to plasma exchange to remove immunoglobulins; however, only one small nonrandomized, unblinded trial and case reports exist suggesting efficacy. Also, CSF filtration has been used in some small studies, but no good large, randomized trials have been done. Currently, the evidence is insufficient to suggest whether or not either of these options is a valid treatment in GBS.[8,79,84,91]

Contraindications to the use of plasmapheresis in some patients with GBS, complications, and lack of universal availability led to the initial studies of IVIG as an alternative therapy to plasmapheresis. Because plasma exchange has proven efficacy in the treatment of GBS, no placebo-controlled trials have been performed of IVIG, only trials comparing IVIG with plasma exchange. IVIG has been shown to have equal efficacy to plasma exchange in speeding recovery in GBS.[89] In addition, some reports indicate that certain GBS subtypes (anti-G_{M1} or anti-G_{M1b}) may respond more favorably to IVIG than to plasma exchange.[111,229] Advantages of IVIG therapy for GBS include its wide availability, ease of administration, safety, and absence of serious complications. Therapy with IVIG generally is less expensive than is plasmapheresis.[207] Current AAN guidelines recommend IVIG for patients with GBS who lose independent ambulation within 2 to 4 weeks of onset of symptoms.[91]

No large trials of IVIG therapy have been performed exclusively in children. Small studies indicate improvement in children similar to that in adults when plasmapheresis or IVIG therapy is used. Plasma exchange and IVIG are treatment options for children with severe GBS (mostly derived from evidence in adult populations). A more recent multicenter, randomized trial[107] investigated the use of IVIG in children and compared IVIG, 2 g/kg (total dose) given over 2 days versus over 5 days, with the primary outcome measure being time required to regain ability to walk unassisted. No statistically significant difference was found between the two groups on the primary outcome measure (19 days versus 13 days). In the 2-day treatment group, early transient deterioration in the disability score before recovery occurred more often. Generally, in centers where it is available, IVIG has emerged as the treatment of choice for moderate to severe GBS in children because of its relative ease of administration, lower incidence of side effects, and overall cost-effectiveness.

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Genitourinary Tract Infections

CHAPTER 44

Urethritis

Ellen R. Wald

Urethritis refers to inflammation of the urethra and periurethral tissues in males and females. It may be associated with a variety of infectious and noninfectious disorders.

EPIDEMIOLOGY

The cause of urethritis varies with the age of the patient, sexual practices, and hygienic standards.[10,45] *Chlamydia* infections and gonorrhea are common occurrences in adolescents; fecal contamination or irritation caused by physical or chemical substances is a more usual occurrence in preschool-aged children. Transmission during sexual activity is the usual means of spread of *Neisseria gonorrhoeae* and *Chlamydia trachomatis* in teenagers and in sexually abused patients; nonvenereal transmission has been described in prepubertal children.[46] Manifestations after nonvenereal spread has occurred may include vaginitis, balanitis, and conjunctivitis in addition to urethritis.[13,46]

The home and social environments of prepubertal children must be examined to identify fully the pattern of spread and infection in the patient and contacts because complex psychosocial diagnoses and therapies often are involved.[38] Children with gonorrhea and chlamydial infections are concentrated in large urban centers, usually in poor socioeconomic environments.

Gonococcal infections in children 2 to 10 years of age should be considered evidence of probable sexual abuse.[1,19,21-23] Household contacts have been found to have positive cultures in 27 to 63 percent of such cases. Prepubertal girls infected with *N. gonorrhoeae* as a result of sexual abuse outnumber boys by a ratio of at least 3 : 1 and in one report 8 : 1.[15]

PATHOPHYSIOLOGY

Infection caused by *N. gonorrhoeae* usually is localized to the urethra in boys and to the vagina in girls; however, rectal carriage sometimes occurs in the absence of urethral colonization. Gonococcal virulence factors include pili, the ability to attach to urethral epithelial cells, and production of extracellular proteases that cleave IgA. Initial attachment of gonococci to the surface of columnar epithelial cells is mediated by pili, which are filamentous outer membrane appendages composed of multiple subunits, the most important of which is pilin.[44] Local invasion involves multiple adhesins interacting with host receptors at the mucosal cell level. After attachment occurs, gonococci become engulfed in a process known as parasite-directed endocytosis.[16] The organisms are able to undergo intracellular replication within phagocytic vacuoles and columnar epithelial cells, which is a successful adaptive response promoting survival.

Chlamydia infections are the most frequent cause of sexually transmitted disease in the United States.[6,9,51] Chlamydiae are structurally complex organisms that are obligate intracellular parasites and contain DNA and RNA. Attachment, which is not understood completely, is the first step in the infectious process of the susceptible host cell. It is followed by phagocytosis and then the failure of cellular lysosomes to fuse with the phagosome containing the elementary body, which may be mediated partly by macromolecules in the chlamydial cell envelope. After these two crucial events occur, the elementary bodies undergo biologic changes, and after approximately 72 hours, they are released from the host cell as new infective elementary bodies (Fig. 44–1).

Urethritis in younger children also may be caused by the introduction of fecal bacteria or pinworms into the urethra during the early years of toilet training, particularly in girls. Inflammation may be related to bubble baths and other chemical and physical irritants. Edema of the mucosa and the presence of inflammation and red blood cells are common histopathologic features of urethritis that lead to dysuria, hematuria, and microscopic pyuria.

CLINICAL PRESENTATION

Gonococcal urethritis is characterized by a 2- to 8-day incubation period after sexual intercourse. The onset often is sudden, with dysuria and copious urethral discharge in boys and leukorrhea in girls. The urethral discharge often is thick, profuse, and yellow. The patient usually has no fever. In prepubertal girls, leukorrhea is more prominent as a sign of gonococcal infection, and urethritis occurs less commonly. This difference may be related to the method of infection and to the different sensitivity of the vaginal epithelial surface to infection in a prepubertal child. Leukorrhea may be minimal in adolescent girls, and dysuria may be absent.[1]

Diagnosis often is made earlier in adolescent boys than in girls, perhaps because of the prominence of urethral discharge in boys and misinterpretation of the significance of leukorrhea in girls. Gonococcal urethritis also may cause asymptomatic pyuria in boys. Occasionally, prepubertal patients have conjunctivitis or balanitis without significant urethritis. Clinical presentations include systemic illness with fever, arthritis, and skin lesions secondary to bacteremia in 3 percent of untreated individuals with mucosal gonorrhea.[1] These lesions often begin on the extremities as small erythematous macules that progress to circular papules with an area of central necrosis.

The clinical presentation of nongonococcal urethritis may be similar to that described for gonorrhea, but it more commonly has a longer incubation period (often 8 to 14 days after sexual intercourse) and a scanty exudate, which may be clear in character and intermittent. This condition also is called nonspecific urethritis and may be present in association with or subsequent to gonococcal urethritis. In the latter case, the scant urethral discharge may persist after the patient has been treated for

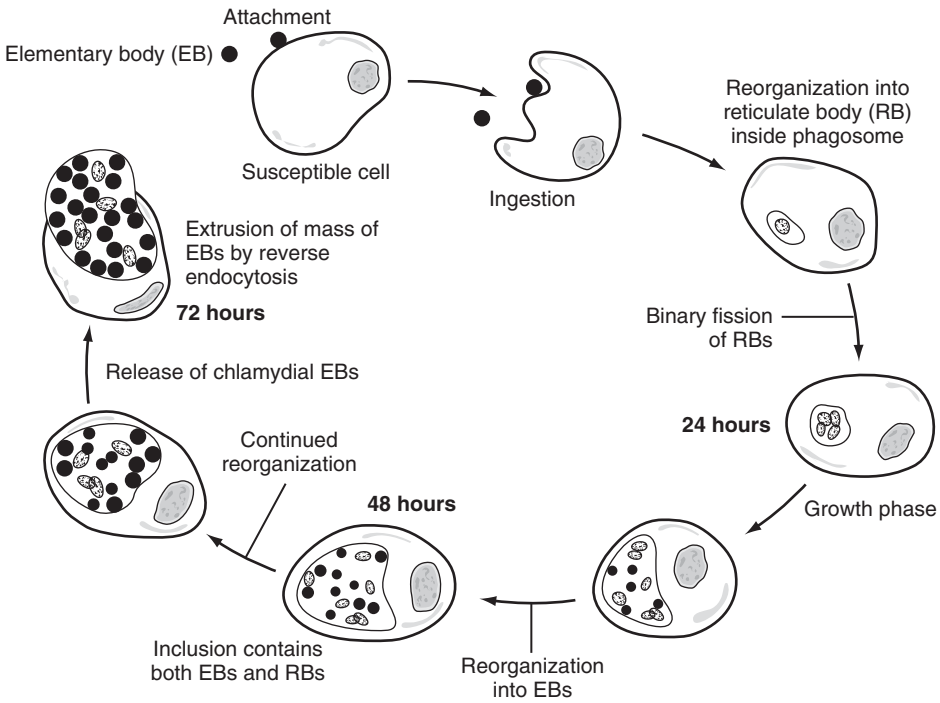

Figure 44–1 Schematic description of the growth cycle of *Chlamydia trachomatis*. *(From Batteiger, B. E., and Jones, R. B.: Chlamydial infections. Infect. Dis. Clin. North Am.* 1*:55-81, 1987.)*

gonorrhea. Asymptomatic urethral colonization with *C. trachomatis* also is reported in males.[49]

An equivalent syndrome, acute urethral syndrome, has been described in sexually active females. The patient experiences an acute onset of dysuria and increased frequency, and pyuria (≥8 white blood cells/mm^3 of midstream urine) is a common finding. Bacterial cultures of the urine often are sterile or show less than 10^5 bacteria/mL; coliform bacteria, *Staphylococcus saprophyticus*, and *C. trachomatis* are the most common causes. Clinical expression of infection with *C. trachomatis* in adolescent girls is characterized by a yellowish, mucopurulent secretion at the cervical os.[7] However, infection with *C. trachomatis* may be asymptomatic in both sexes, an important consideration in designing effective strategies for diagnosis and management of sexual contacts.

A patient with urethritis caused by trauma may have hematuria and dysuria without fever. The trauma may be obvious or related to masturbation or introduction of foreign bodies into the urethra. Patients with urethritis secondary to bubble bath or soap usually have transient dysuria and no systemic signs. Fecal contamination of the urethra may be accompanied by hematuria, dysuria, and pyuria.

DIFFERENTIAL DIAGNOSIS

Table 44–1 lists the differential diagnoses for urethritis.

NONINFECTIOUS

Trauma, bubble bath, detergents found in shampoos, masturbation, radiation, dysfunctional elimination syndrome, and caustic substances may lead to the development of urethritis.[26] Urethritis also may be a component of several systemic syndromes, including erythema multiforme (Stevens-Johnson syndrome), Kawasaki disease, and occasionally other forms of allergy. Reiter syndrome denotes the association of nongonococcal urethritis with conjunctivitis and arthritis.

TABLE 44–1 Etiology of Urethritis

Infectious	Noninfectious
Sexually transmitted infections	**Vasculitides**
Neisseria gonorrhoeae	Reiter syndrome
Chlamydia trachomatis	Erythema multiforme
Trichomonas vaginalis	Kawasaki disease
Herpes simplex virus type 2	**Mechanical**
Mycoplasma spp.	Masturbation
Non–sexually transmitted infections	Foreign body
Staphylococcus saprophyticus	Trauma
Enterobacteriaceae	**Chemical**
Gardnerella vaginalis	Soaps
Streptococcus spp.	Detergents
Enterobius vermicularis	Drugs

INFECTIOUS

The most common forms of urethritis in sexually active adolescents and young adults are gonococcal and so-called nongonococcal urethritis. They may occur together or sequentially. Nongonococcal urethritis has been related causally to infections with *C. trachomatis* in approximately 30 to 50 percent of cases and with *Ureaplasma urealyticum* (T-strain mycoplasma) in approximately 25 percent of cases of nongonococcal, nonchlamydial urethritis in males.[7,50] The remaining cases of infectious urethritis in postpubescent, sexually active patients may be caused by a variety of pathogenic microorganisms, including *Gardnerella vaginalis*, *Mycoplasma hominis*, *Trichomonas vaginalis*, *Candida albicans*, herpes simplex virus type 2, *Treponema pallidum* (syphilis), and other bacteria, such as staphylococci, Enterobacteriaceae, and occasionally streptococci including group B.[18]

Mycoplasma genitalium is a newer species, first isolated from males with urethritis.[8,28] Studies also have implicated a causative role in *Chlamydia*-negative nongonococcal urethritis for anaerobic organisms of the *Bacteroides* spp., in particular *Bacteroides urealyticus*.

In younger children, urethritis usually has noninfectious causes as outlined earlier. Gonorrhea, *Chlamydia*, and fecal bacteria may be important as well.

SPECIFIC DIAGNOSIS

A standard method for diagnosing gonorrhea in a sexually active male is to obtain urethral discharge by manually stripping the urethra or, if that is unproductive, by gently inserting a swab 2 to 3 cm into the distal urethra. The best culture technique for isolating *N. gonorrhoeae*, a fastidious organism, is immediate inoculation of this material onto a selective growth medium, such as regular or modified Thayer-Martin agar. Any delay in inoculation of the plates necessitates the use of a transport method with growth media in a carbon dioxide environment that support the gonococcus at ambient temperatures. These media protect the organism from its marked susceptibility to the effects of drying, cold, and overgrowth by other bacteria. Urethral exudate from a male patient should be Gram-stained at the same time; typical kidney bean–shaped, gram-negative, intracellular diplococci are presumptively diagnostic, with a sensitivity and specificity approaching 100 percent (Fig. 44–2).

Putative gonococcal colonies should be confirmed by oxidase reaction, Gram staining, sugar use tests, rapid enzyme tests, nucleic acid probes, or agglutination reactions with antibodies specific for *N. gonorrhoeae*. The last four tests are especially important in evaluating sites of infection (e.g., the pharynx) or populations of patients with a low prevalence of gonorrhea.

Sexually active females with urethritis also should undergo urethral culture. Although the Gram stain of cervical secretions is only 66 percent sensitive in detecting *N. gonorrhoeae* in adolescent girls, the finding of kidney bean–shaped, intracellular gram-negative diplococci is highly specific and helpful.[52] Vaginal, cervical, and rectal swabs are recommended. Asymptomatic colonization with gonococci seems to occur most commonly in female patients, although it has been described in adolescent boys.[1,25] Pharyngitis, conjunctivitis, balanitis, and other, less common, manifestations of gonorrhea may coexist with urethritis. Samples obtained from these sites should be handled as described earlier. Blood agar and other specialized media may be indicated to identify nongonococcal causes of urethritis.

Nucleic acid amplification (NAA) tests are highly sensitive and specific when used on urethral (males), endocervical swab, and urine specimens.[9] These tests include polymerase chain reaction, transcription-mediated amplification (TMA), and strand displacement assays. Only the TMA assay is approved by the U.S. Food and Drug Administration for testing vaginal swabs from postmenarcheal females. Use of urine specimens increases the feasibility of initial testing and follow-up of hard-to-access populations such as adolescents.[1] These techniques also permit dual testing of urine for *C. trachomatis* and *N. gonorrhoeae*. NAA tests are not recommended for rectal and pharyngeal swabs.[1] Although none of these tests is superior to culture, their ease of use (for urine or self-obtained specimens) renders them extremely attractive.

Gonococcal urethritis in prepubescent boys is diagnosed as described earlier for adolescent boys. Vaginal swabs are most useful in female patients, although vaginal discharge may not be prominent. Endocervical cultures are not recommended for the diagnosis of gonorrhea in prepubescent girls. The yield of vaginal swabs seems to be adequate for most diagnostic purposes; rectal swabs also may be useful in female patients. Showing kidney bean–shaped, gram-negative, intracellular diplococci in a prepubescent boy and girl is useful for establishing a presumptive diagnosis and instituting therapy. Confirmation by culture as described for a sexually active male is necessary.

Other infectious causes of urethritis may be diagnosed by specific techniques, including wet mount for *Trichomonas*, Gram stain and culture on Sabouraud dextrose agar for *C. albicans*, and culture for herpes simplex virus, used in the patients and their contacts. New culture techniques for *Chlamydia*, such as the use of microtiter cell monolayers, have increased the recovery rates, decreased the cost, and shortened the turn-around time for the isolation of *C. trachomatis*.[3] The rigorous transport conditions and the small number of laboratories with cell culture techniques have limited the availability of *Chlamydia* cultures; however, noncultural methods, including direct immunofluorescence staining of smears with use of monoclonal antibodies,[3,20,27,34,47] enzyme-linked immunosorbent assay (ELISA) techniques, and NAA testing, are available.

The use of immunofluorescence and ELISA techniques has been surpassed in sensitivity and ease of use by NAA testing.

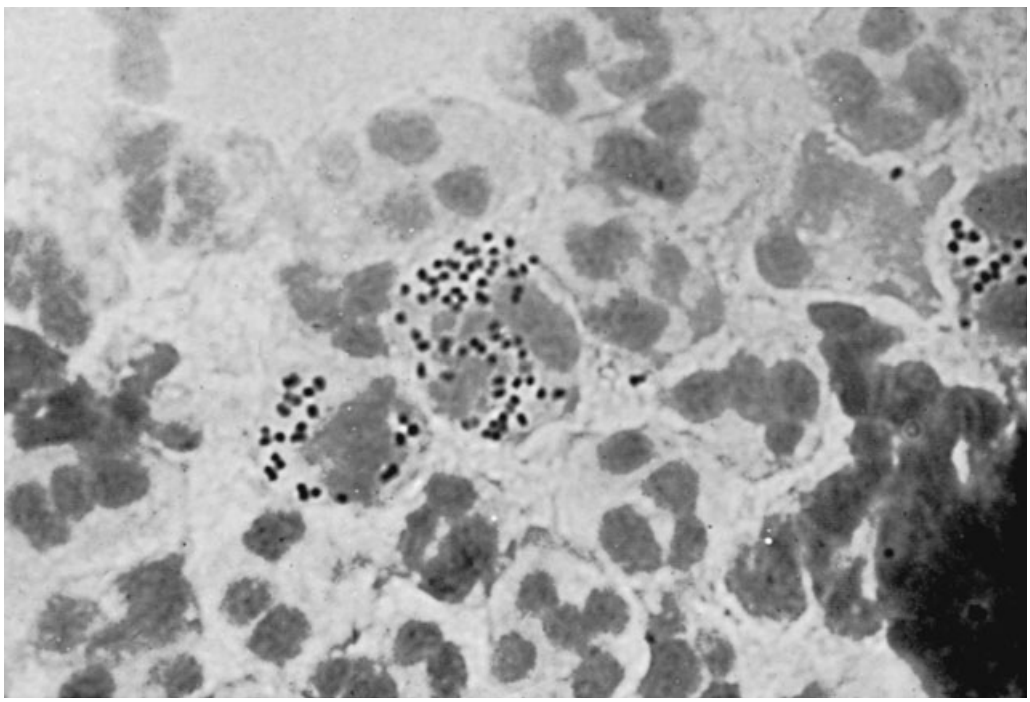

Figure 44–2 Gram-stained smear of urethral discharge from a teenage boy with gonorrhea.

NAA tests such as polymerase chain reaction, TMA, and strand displacement assays are available and are more sensitive than cell culture, DNA probe, direct fluorescent antibody tests, or ELISAs, although specificity varies compared with culture.[1] Polymerase chain reaction, strand displacement assay, and TMA are useful for evaluating urine specimens from either sex. The Food and Drug Administration has approved TMA for testing vaginal swabs from postmenarcheal adolescents. NAA tests are not recommended for specimens obtained from rectal or pharyngeal swabs or for urethral swabs from females.

When documentation of infection is being sought in cases of suspected child abuse, the performance of cultures is strongly preferred and may be the only acceptable test in certain jurisdictions.[1] When culture is unavailable, some experts support using NAA testing if a positive result can be verified by another NAA test. ELISA and fluorescent antibody tests should not be used for testing rectal, vaginal, or urethral specimens from infants and children because of low sensitivity and specificity.[1] Ligase chain reaction seems to be substantially more sensitive in children than is culture for *Chlamydia*.[17,31]

Ureaplasma and other genital *Mycoplasma* spp. can be identified only by culture at this time. This test should be reserved for the evaluation of recurrent cases of urethritis with poor response to treatment.[8,27] Specimens of urethral and vaginal discharge secondary to fecal contamination and foreign bodies can be examined by conventional diagnostic bacterial techniques.

TREATMENT

Table 44–2 presents antibiotic regimens for treatment of urethritis. The treatment of urethritis should include treatment of the sexual partners of the index case to avoid reinfection and further spread of infection. Patients with urethritis frequently have mixed infections with *N. gonorrhoeae* and the pathogens linked with nongonococcal urethritis, such as *C. trachomatis* and *U. urealyticum*. Concurrent infection with *N. gonorrhoeae* and *C. trachomatis* is documented frequently. In a survey of adolescents admitted to juvenile detention centers throughout the United States, screening for sexually transmitted infections was performed on adolescent girls with gonorrhea. Fifty-four percent were coinfected with chlamydia; of adolescent boys infected with gonorrhea, 51 percent were coinfected with *Chlamydia*.[30] A study determining chlamydia and gonorrhea co-occurrence in a high school population showed similar results: of 117 students with gonorrhea, 50 (42.7%) had chlamydia; of 451 with chlamydia, 50 (11.1%) had gonorrhea.[39] Patients with gonococcal urethritis also are at risk for development of early incubating syphilis. One must consider these factors and the possibility of systemic infection when choosing a treatment regimen for urethritis.[36]

Sexual abuse is the most common cause of gonococcal infection among children 2 to 10 years of age. Anorectal and pharyngeal infections with *N. gonorrhoeae* are common and frequently asymptomatic occurrences among these patients.[37]

In 2006, the Centers for Disease Control and Prevention (CDC) published treatment guidelines for sexually transmitted diseases, including urethritis (see Table 44–2). Every attempt should be made to ascertain the specific diagnosis. If doing so is impossible, the treatment regimen chosen always should be appropriate for nongonococcal urethritis and gonococcal infection. The emergence of penicillin-resistant and tetracycline-resistant strains of *N. gonorrhoeae* has led to the abandonment of penicillin and tetracycline for the treatment of gonorrhea.[14]

Single-dose azithromycin for nongonococcal urethritis and either intramuscular or oral single-dose regimens for uncomplicated gonococcal infections are likely to increase compliance.[23,24,32,35,40,41,48,53] Test of cure is not indicated for uncomplicated gonococcal or chlamydial infection when single-dose treatment regimens are used.[9] Emergence of resistance to quinolones initially restricted the use of these drugs in cases of gonorrhea that originated from California, Hawaii, and Asia,[1,4,9] However, in 2007, the CBC recommended that fluoroquinolones no longer be used for treatment of gonorrhea.[9a] If these regimens fail, infections with other pathogens, such as herpes simplex virus or *T. vaginalis*, or bacterial urethritis should be considered. Appropriate testing and specific treatment should be provided when indicated.

TABLE 44–2 Antibiotic Regimen for Urethritis

Nongonococcal Urethritis	
Recommended regimen	Azithromycin 1 g orally in a single dose *or* Doxycycline 100 mg orally bid for 7 days
Alternative regimen	Erythromycin base 500 mg qid for 7 days *or* Erythromycin ethylsuccinate 800 mg qid for 7 days *or* Ofloxacin 300 mg bid for 7 days *or* Levofloxacin 500 mg once daily for 7 days
Chlamydial Infection	
Recommended regimen (adults and adolescents)	Azithromycin 1 g orally in a single dose *or* Doxycycline 100 mg orally bid for 7 days
Alternative regimens	Erythromycin base 500 mg qid for 7 days *or* Erythromycin ethylsuccinate 800 mg qid for 7 days Ofloxacin 300 mg bid for 7 days *or* Levofloxacin 500 mg once daily for 7 days
Children	
<45 kg	Erythromycin 50 mg/kg/day qid for 14 days
≥45 kg and <8 years old	Azithromycin 1 g orally in a single dose
>8 years old	Use same regimen as for adults
Gonococcal Infection	
Recommended regimen (adults and adolescents)	Ceftriaxone 125 mg IM in a single dose *or* Cefixime 400 mg orally in a single dose *plus* Regimen effective for *Chlamydia*
Children	
<45 kg	Ceftriaxone 125 mg IM in a single dose
If bacteremia, arthritis, or meningitis	Ceftriaxone 50-100 mg/kg/day (maximum 2 g/day) IV for 7-14 days
≥45 kg	Use adult regimen

bid, twice a day; IM, intramuscularly; IV, intravenously; qid, four times a day.

PROGNOSIS

Gonococcal urethritis may subside and lead to asymptomatic carriage in female patients. Carriage may last for weeks to months

in adults; this period is undefined in children. Untreated gonococcal urethritis also may lead to prostatitis and epididymitis in male patients and urethral stricture. Asymptomatic genital infections in women can progress to pelvic inflammatory disease with tubal scarring and infertility. Systemic complications of asymptomatic gonorrheal infections include arthritis, endocarditis, and necrotic skin lesions.

Chlamydial infections frequently have been associated with pelvic inflammatory disease in women, resulting sometimes in infertility or ectopic pregnancy. *Chlamydia* can be transmitted to a newborn in the birth canal, which can result in conjunctivitis, pneumonia, or both.

The frequency of *U. urealyticum* is higher in sperm samples from men of infertile couples. It also has been associated in women with premature delivery and postpartum fever. In a newborn, this organism might be linked with the development of bronchopulmonary dysplasia. *Ureaplasma* infection of the central nervous system occasionally is reported in newborns.

PREVENTION

Despite substantial efforts, a specific gonococcal vaccine has not been developed. The mainstays of prevention continue to be education and screening. Prepubescent gonorrhea can be prevented from recurring only by careful family counseling and psychosocial therapy; legal intervention may be necessary.

The availability of diagnostic tests performed on urine (a noninvasive alternative to specimens obtained from the urethra or endocervix) renders extensive screening possible in nontraditional settings such as schools and recreation centers.[22] Large-scale efforts that have implemented screening for all sexually active teens attending school-based clinics have been successful in reducing the prevalence of *Chlamydia* infection in boys.[11]

Prevention of noninfectious causes of urethritis usually depends on education and specific counseling of the family. Physical agents or allergens identified as a cause of urethritis must be removed.

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CHAPTER 45

Cystitis and Pyelonephritis

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Urinary tract infections (UTIs) are the most common serious bacterial infections in children. In several series of children evaluated for fever, UTIs accounted for 5 to 6 percent of infections. They are more common than occult bacteremia, bacterial pneumonia, and bacterial meningitis. UTI is especially common as a cause of infection in white infant girls; UTI may explain febrile episodes in nearly 20 percent of such infants.

EPIDEMIOLOGY

UTIs occur in all age groups and may be symptomatic or asymptomatic. Factors that affect the incidence of UTIs are associated with gender, age, race, circumcision status, and general health.[93] The site of infection may be the bladder (cystitis), ureters (ureteritis), pelvis (pyelitis), and renal parenchyma (pyelonephritis). Infections in neonates and infants are common occurrences. In the first 3 months of life, infections in uncircumcised boys are most common.[116,123] Beyond 6 months, infections in infant girls are substantially more common than are infections in boys; the female predominance of UTIs is maintained throughout the remainder of childhood and adolescence.[31]

RISK OF URINARY TRACT INFECTION

The risk of developing a UTI during childhood seems to have increased since early studies by Winberg and colleagues[159] in 1960. These investigations showed that the risk of developing a UTI during the first 10 years of life was 3 percent in girls and 1.1 percent in boys. In a more recent retrospective study of a cohort of 3556 school entrants, 7.8 percent of girls and 1.6 percent of boys were found to have had symptomatic UTIs as confirmed by significant bacteriuria.[47] In approximately half of these cases, the clinical presentation was consistent with acute pyelonephritis (APN).[42] Another population-based study was performed in Göteborg, Sweden, to describe the incidence rate of first-time symptomatic UTI in children younger than 6 years old. The cumulative incidence rate during the first 6 years of life was 6.6 percent for girls and 1.8 percent for boys.[94] The apparent increase in risk most likely relates to an increased awareness of the diagnosis of UTI as an explanation for fever in children and the more frequent practice of culturing the urine of children who are ill.

Several studies have investigated systematically the prevalence of UTI as the explanation for fever in febrile young children presenting to the emergency department. Although the definition of significant bacteriuria has varied among studies, the overall prevalence of UTI is 3.3 to 5.3 percent.[52,128] White infant girls had significantly more UTIs than did black boy infants. Higher prevalences occurred in uncircumcised boys or boys with abdominal or suprapubic tenderness on examination.[128] White girls with a temperature of 39º C or greater had a prevalence of UTIs of 17 percent.[52] In a large prospective study of febrile (temperature ≥38º C) infants 3 months or younger evaluated in pediatric office settings, 54 percent of infants had urine tested, and 10 percent had UTIs.[99]

RISK FACTORS FOR URINARY TRACT INFECTION

Uncircumcised Boys

The common problem of UTIs in uncircumcised boys, although suspected in the 1970s, was first documented in the 1980s by Ginsburg and McCracken.[35] The strongest evidence of a causal link between an intact foreskin and a UTI comes from several studies conducted by Wiswell and colleagues.[161] In their series, an overall 10-fold increased incidence of UTI was found in uncircumcised compared with circumcised male infants (1.12% versus 0.11%; $p < .001$).[161] Wiswell and other investigators have continued to document this problem.[123,129,160] Where and when rates of circumcision have decreased, the frequency of UTIs in boys has increased. The presence of preputial folds in uncircumcised boys encourages a high density of bacterial growth and contamination of the urethral opening.[14] Circumcision reduces meatal contamination, decreasing the ascent of bacteria into the bladder.[160] The high risk of acquiring UTIs in uncircumcised boys diminishes with age (as the foreskin becomes more retractable) but is still present in the toddler age group.[19]

Dysfunctional Voiding

Dysfunctional voiding is a risk factor for the development of a UTI and an occasional consequence of UTI. Dysfunctional voiding refers to a lack of coordination between the two functions that are essential for normal voiding to occur—relaxation of the urethral sphincter and contraction of the detrusor muscle of the bladder. Ordinarily, the sphincter must relax as the detrusor contracts.[5] The failure of the sphincter to relax causes an obstruction to the outflow of urine. Consequently, voiding pressures and intravesicular pressure are high, the bladder becomes overdistended, dribbling instead of a good flow occurs, and residual urine remains in the bladder after the void. This dyscoordination is termed *dyssynergia*.[5]

Clinical manifestations typically appear after toilet training and include incontinence, enuresis, urinary urgency, and UTI.[29] Constipation is a common occurrence because of the inability to relax the pelvic floor musculature. The presence of dysfunctional voiding also may promote the persistence of vesicoureteral reflux (VUR) and lead to recurrence or contralateral reflux after attempts are made at surgical correction of reflux.[72]

Constipation

The distended rectum in constipated children has been suggested to press on the bladder wall and produce an obstruction to bladder outflow that may cause dysfunctional voiding. Urodynamic studies have shown instability of the detrusor muscle in patients with functional constipation and associated enuresis or UTI.[162] Loening-Baucke[86] studied a group of children referred with encopresis and constipation. The history indicated that many were incontinent of urine, and 11 percent had histories of UTIs. When a vigorous regimen to alleviate the constipation was prescribed, a dramatic improvement occurred in the enuresis and the frequency of recurrent UTI.

Wan and colleagues[151] prospectively evaluated the toilet habits of 77 girls and 24 boys diagnosed with UTIs. Parents were instructed to use a "toilet diary" to record the frequency of voiding and stooling. An abnormal voiding habit was defined as infrequent urination (four times or fewer daily when awake), an abnormal voiding pattern (>4 hours between voidings), or the practice of avoidance maneuvers (repetitive habitual squirming, crossing of legs, or sitting on heels).[149] Constipation was defined as stooling less frequently than every third day. Children with and without abnormal imaging studies were compared. Although abnormal voiding patterns and constipation were identified in both groups, only 10 percent of children with normal images were without constipation or abnormal voiding compared with 60 percent of the group with abnormal images ($p = .0001$). These data strongly suggest that evaluation of children with UTIs should include inquiry into these functional matters, especially when imaging study results are normal.

Sexual Activity

The well-recognized association in women of acute cystitis with sexual intercourse is reflected in the popular, now perhaps outdated, term *honeymoon cystitis*.[101] This phenomenon usually is related to the new onset of sexual activity or a recent change in sexual partners. A novel study of 15 patients with a history of recurrent UTIs involved daily monitoring for the presence of UTI with dipslides and calendars that recorded episodes of intercourse, menses, and the occurrence of symptoms.[101] Eleven patients experienced 16 infections; 12 infections occurred within 24 hours of engaging in intercourse. In 12 control subjects, three infections occurred, all within 24 hours of having intercourse. The authors concluded that in sexually active women, most UTIs are related to intercourse.

These results were reinforced by a large prospective study from Seattle, Washington, which confirmed that the incidence of symptomatic UTI is high in sexually active young women and that a strong and independent association exists between UTI and recent sexual intercourse, recent use of a diaphragm with spermicide, and a history of recurrent UTIs.[59] Most of these same risk factors, including frequency of sexual intercourse and use of spermicide, were documented as risk factors associated with development of APN in healthy women.[124]

Catheters

In the hospital, urinary catheters are major risk factors for acquisition of nosocomial infection. In adults, the risk of developing an infection is approximately 5 percent per day of catheterization.[100] The usual infecting strains include *Escherichia coli*, *Proteus*, *Pseudomonas*, *Klebsiella*, and *Serratia*. Many strains of bacteria that cause infection display antibiotic susceptibilities that are more resistant than usual. The route of infection may be either intraluminal or periurethral. Bacteremia is an unusual complication of nosocomial UTI. In a study of nosocomial infections in a children's hospital, catheter-associated UTI accounted for 48 percent of the UTIs. Secondary bacteremia occurred rarely, with an incidence of 2.9 percent.[21]

PATHOGENESIS

BACTERIOLOGY

Most uncomplicated UTIs are caused by members of a large family of gram-negative bacteria known as Enterobacteriaceae. In most instances, the urinary tract becomes infected by the ascending route. Bacteria derived from the fecal flora colonize the periurethral area and gain access to the urethra. The most common bacterial species in primary and recurrent infections is *E. coli*. Other gram-negative species that commonly cause UTI are *Klebsiella*, *Proteus*, *Enterobacter*, and *Citrobacter*, although virtually any enteric organism can cause UTI. Gram-positive bacterial species account for approximately 5 percent of UTIs and primarily include *Staphylococcus saprophyticus* and enterococcal species. After *E. coli*, *S. saprophyticus* is the most common cause of uncomplicated UTIs in teenagers and young adults of both sexes.

Rarely, the urinary tract may become infected hematogenously in the course of a bacteremic infection. This mechanism is thought to account for at least some cases of neonatal UTI. Increasingly, evidence indicates that even in neonates, most infections occur by the ascending route.

VIRULENCE FACTORS

The key virulence factor for isolates of *E. coli* is the mechanism by which they attach or adhere to the uroepithelial cell.[62] Bacterial adherence is an essential initiating step in all infections. So-called uropathogenic bacteria, derived from the numerous species found in the fecal flora, can attach to specific receptor sites on the uroepithelium and can bind in a nonspecific manner by electrostatic and hydrophobic bonds.[118] A principal means of attachment is through adhesins localized on specialized pili of the *E. coli*. These pili are referred to as *P fimbriae* because they can recognize and agglutinate erythrocytes of the P1 blood group; this P blood group antigen also is present on human uroepithelial cells.

Evidence to support the notion of the increased pathogenicity or virulence of the P fimbriae comes from studies of *E. coli* recovered from children with infection at different levels of the urinary tract. When *E. coli* strains recovered from patients with

pyelonephritis are examined, 76 to 94 percent are P fimbriated; in contrast, strains of *E. coli* recovered from patients with cystitis or asymptomatic bacteriuria are 19 to 23 percent and 14 to 18 percent P fimbriated, respectively.[69,147] Although P fimbriated strains of *E. coli* are common findings in patients with pyelonephritis whose urinary tracts are completely normal, their frequency decreases considerably when strains of *E. coli* are examined from patients with pyelonephritis associated with VUR. Apparently, this virulence characteristic (and others described later) is unnecessary when reflux is present.[89]

The principal adhesin on the tip of the P fimbriae that fosters adherence to the uroepithelial cell is known as the *PapG adhesin*. More recently, 153 *E. coli* organisms recovered from the urinary tracts of infants and children with pyelonephritis were analyzed by polymerase chain reaction for class I, II, and III alleles of the pyelonephritis-associated adhesin gene *papG*. Strains with any class II *papG* alleles were found significantly more often in infants with normal anatomy and function or in infants with clinically insignificant abnormalities than in infants with significant abnormalities (90 of 119 versus 14 of 34 infants; $p < .001$).[63] This virulence factor is more important when the urinary tract is structurally normal than when anatomic features predispose the individual to infection.

Other virulence factors related to the bacterial species causing UTIs are the K antigen, lipopolysaccharides, hemolysins, colicins, resistance to the bactericidal action of serum, and increased iron-binding capacity. In addition to the immunogenic activity of lipopolysaccharides (activating an intense host response), they have been shown to have direct toxic effects on renal cells via their biologically active component lipid A.[144] The K antigen is a capsular polysaccharide that constitutes an outer surface of the *E. coli* organism. The capsule has the capacity to impede phagocytosis and to shield the bacteria from lysis induced by complement.[60] Hemolysins are cytotoxic proteins that can damage renal tubular cells in vitro.

Approximately 50 percent of the types of *E. coli* isolated from the urine of patients with APN produce a pore-forming toxin, alpha-hemolysin. Alpha-hemolysin damages the cell membrane and may activate apoptosis in renal tubular cells.[16] Colicins, elaborated by "uropathogenic" strains of *E. coli*, kill other bacteria that are in their vicinity. In the presence of human serum, many bacteria are killed after activation of complement. Virulent *E. coli* organisms have the capacity to resist this bactericidal effect of serum. Another virulence factor found in bacteria is their ability to acquire and bind iron. Most bacteria require iron for optimal growth and metabolism and have developed mechanisms to acquire iron when the supply is limited. Increased iron-binding capacity, mediated by proteins such as aerobactin, which are made by some *E. coli* strains, provides additional pathogenic potential.

After reaching the bladder, P fimbriated *E. coli* organisms can colonize the ureter even in the absence of VUR.[116] Bacterial colonization of the ureter affects ureteral peristalsis, leading to dilation and a physiologic obstruction. This dilation of the ureter and calyces favors a change in the shape of the renal papillae, which facilitates intrarenal reflux of colonizing bacteria at low pressure. APN develops because the receptors for the P fimbriated *E. coli* are present in the collecting duct and proximal tubules.[102,116]

Experimental studies conducted by Roberts[116] have led to a theory of the chain of events involved in the process that ultimately leads to renal scarring. The initial event is the inoculation of the renal parenchyma with bacteria, which leads to an intense inflammatory response. Liberation of proinflammatory cytokines (interleukin-1, interleukin-6, and interleukin-8) is followed by recruitment of inflammatory cells and a second cytokine burst.[67] This inflammation results in the release of toxic enzymes within the granulocytes and tubular lumen. Superoxide is released simultaneously, generating oxygen radicals that are toxic to the bacteria and to the tubular cells.[117] The resultant death of the tubules intensifies and extends the inflammatory process into the interstitium. At the same time, focal ischemia results from the intravascular aggregation of granulocytes and edema.[66] The tissue damage that results from the toxic enzymes, oxygen radicals, inflammatory response, and ischemia culminates in the creation of renal scars.[62,116]

CLINICAL PRESENTATION

CYSTITIS

Most children with cystitis present with urgency, frequency, or dysuria. Children who have the urge to urinate may have a history of difficulty in initiating the urinary stream. Occasionally, children may complain of abdominal or suprapubic pain. If fever is present, it is low-grade. Suprapubic tenderness may be present on palpation. The urine may be foul-smelling and cloudy in appearance.

PYELONEPHRITIS

Many children who present with APN have impressive chills, spiking fevers, and complaints of back pain. They may have associated gastrointestinal complaints of vomiting and diarrhea, especially vomiting. Lower urinary tract symptoms, such as frequency, urgency, dysuria, and suprapubic discomfort, may or may not be present.

Other findings, such as irritability, poor feeding, vomiting, decreased urinary output, and clinical evidence of dehydration, vary. The youngest children with APN usually present with high fever without other localizing features.[54,93,164]

PHYSICAL EXAMINATION

Features of the physical examination that should be emphasized include (1) an accurate measurement of blood pressure (hypertension may be present in patients who have chronic renal disease), (2) general growth and development (failure to thrive may be a sign of more chronic or recurrent UTI), and (3) a careful abdominal examination (which might reveal tenderness or a mass caused by either an enlarged bladder or an obstructed urinary tract).[142] An effort should be made to elicit the finding of costovertebral angle tenderness in children of all ages. The perineum should be inspected carefully to search for signs of irritation, scars, tears, signs of trauma, labial adhesions, or evidence of vulvovaginitis. In uncircumcised infants, the foreskin may not be retractable, leading to phimosis. A rectal examination should be considered to detect masses or poor sphincter tone, which might be associated with a neurogenic bladder.[142] The lower back should be observed for any lipoma, sinus, pigmentation, or tufts of hair that may be signs of an occult myelodysplasia.

Neurologic examination of the lower extremities and evaluation of the bulbocavernosal reflex often reflect the neurologic integrity of the lower motor neuron reflex arcs. The bulbocavernosus reflex is elicited by squeezing the glans penis or clitoris and observing or feeling a reflex contraction at the external anal sphincter. Absence of this reflex suggests a possible sacral lesion.[5]

ASYMPTOMATIC BACTERIURIA

A large body of work has been produced dealing with the issue of asymptomatic bacteriuria. Data were accumulated during a

long-term study by Kunin[73] of the natural history of recurrent bacteriuria among school-age girls in a well-defined community in central Virginia. Girls were identified in the first grade and were observed prospectively for 10 years. Each year, approximately 0.5 percent of school-aged girls developed asymptomatic bacteriuria. The overall prevalence was 5 percent for the years between entrance to grade school and graduation from high school. Although it was billed as asymptomatic bacteriuria, approximately one third of the girls did have symptoms, and some were known to have had infection or, rarely, abnormalities of the urinary tract before the first screening.

Just a few years after Kunin[73] began his investigations, a similar study was conducted in Göteborg, Sweden. Beginning in 1970, 19,000 girls a year were screened routinely for bacteriuria in Göteborg schools at ages 7, 11, 14, and 16 years.[84] A significant minority had a history of previous infection or symptoms that were referable to the urinary tract.

Savage and colleagues[120] also studied covert bacteriuria in school-age girls. In the three studies, the prevalence of bacteriuria ranged from 0.7 percent[84] to 1.1 percent[73] to 1.6 percent.[120] The risks associated with asymptomatic bacteriuria are difficult to assess from these studies because patients who were truly asymptomatic were difficult to separate from patients with symptoms.

Several prospective studies of infants and school-aged girls from Scandinavia have provided important information regarding the natural history of asymptomatic bacteriuria.[42,154] Most children identified as having asymptomatic bacteriuria among a large cohort of infants (N = 3581) spontaneously cleared the bacteriuria within months. Only 2 of 45 went on to develop symptomatic bacteriuria. In contrast, none of 42 infants who developed symptomatic UTIs had been identified previously as having asymptomatic bacteriuria, suggesting that asymptomatic bacteriuria rarely is a precursor to symptomatic UTI. In addition, prophylactic antibiotics used to treat children with asymptomatic bacteriuria seemed to predispose them to the development of pyelonephritis, usually with microorganisms that had not been present at the outset.[42]

Currently, physicians have little enthusiasm for screening children of any age to discover the presence of asymptomatic bacteriuria. The absence of pyuria in these specimens of urine provides additional evidence that the host is not perturbed by the presence of asymptomatic bacteriuria. The presence of bacteria of low virulence in the urine in asymptomatic patients seems to be protective. These strains apparently prevent invasion by other bacteria and provide a kind of biologic prophylaxis.[42,70]

Rather than screening asymptomatic populations of children, an appropriate approach is vigorous evaluation for the presence of UTIs in febrile children without an obvious focus of infection. In addition, health maintenance examinations should be used as an opportunity to screen for historical information that might suggest the need to collect a urine specimen for culture (Table 45–1). Important items include frequent episodes of unexplained fever, dribbling when urinating, enuresis, encopresis, constipation, urgency, frequency, and dysuria. In addition, it is valuable to know when toilet training was accomplished; frequency of voiding; frequency of stooling; and any apparent difficulties associated with voiding, such as in initiating the urinary stream. The practitioner also should inquire about so-called avoidance maneuvers (e.g., repetitive habitual squirming, crossing of legs, or sitting on heels)[151] and family history of UTI.

TABLE 45–1 Renal-Focused History and Physical Examination

History
Age of toilet training
Characteristics and frequency of voiding (urgency, dysuria, dribbling)
Frequency and characteristics of stooling
Family history of renal disease
Habitual squirming
Color and odor of urine
Unexplained episodes of fever
Physical Examination
Temperature
Blood pressure
Abdominal tenderness
Costovertebral angle tenderness
Suprapubic tenderness
Genital examination (irritation, scars, tears)
Rectal examination (sphincter tone, bulbocavernosus reflex)
Lower back (sinus, pigmentation, lipoma, tufts of hair)

DIFFERENTIAL DIAGNOSIS

INFECTIOUS

E. coli is the most common cause of infection in the urinary tract for primary infections (in which *E. coli* causes 85 to 90% of infections) and recurrent infections (in which *E. coli* causes approximately 75% of infections). Virtually any other gram-negative enteric bacteria may cause infection. Common etiologic agents include *Klebsiella*, *Proteus*, *Enterobacter*, *Serratia*, and *Pseudomonas*. *Proteus mirabilis* is a common cause of UTIs in some series of boys and in nosocomial UTIs associated with catheterization.[71,105] In young women, *S. saprophyticus* is second only to *E. coli* as a cause of cystitis. Rarely, *Staphylococcus epidermidis* has been reported as a cause of pyelonephritis in young boys with anatomic abnormalities of the urinary tract.[40]

In the context of bacteremia or septicemia, occasionally the blood culture and the urine culture are positive for the same bacterial species. In these instances, the kidney has been seeded as part of a hematogenous dissemination. Any organism that is responsible for sepsis, such as *Haemophilus influenzae* type b, *Neisseria meningitidis*, *Neisseria gonorrhoeae*, *Staphylococcus aureus*, *Streptococcus pneumoniae*, or *Streptococcus pyogenes*, may be found in the urine.

Anaerobic infections of the urinary tract are rare occurrences in children despite the high density of gram-positive and gram-negative anaerobes in the fecal flora; this fact relates to the probable lack of adherence of these bacterial species to the uroepithelium. Anaerobic infections of the urinary tract should be suspected when organisms are seen on Gram stain but do not grow in conventional culture, or when the urine of symptomatic children shows no bacterial growth.[13] Another unusual infecting agent that should be suspected in instances in which the Gram stain of the urine shows gram-negative rods but the urine culture is negative is *H. influenzae*.[97]

Fungal infections of the urinary tract usually are caused by *Candida* spp., but they also may be caused by *Cryptococcus neoformans*, *Aspergillus* spp., and the endemic mycoses.[139] Candiduria is an increasingly common form of nosocomial infection that may involve any level of the urinary tract.[50,109] It often occurs in immunosuppressed patients, especially patients who are receiving broad-spectrum antibiotics for treatment of documented or undocumented systemic infections. In many immunosuppressed patients, the infection is complicated by the presence of an indwelling urinary catheter.

Viruses also may cause infection of the urinary tract. For the most part, these infections involve the bladder rather than the kidney, although infection of any part of the urinary tract may occur. The principal etiologic agents are adenoviruses, enteroviruses, coxsackieviruses, and echoviruses. Mumps virus and hepatitis viruses occasionally have been implicated. Type 11 adenovirus has been the most common cause of acute

hemorrhagic cystitis in school-age boys; type 21 also has been documented to be a cause of infection in this age group. In immunosuppressed patients, especially children who have undergone bone marrow transplantation or are recipients of kidney transplants, BK polyomavirus and adenovirus may cause hemorrhagic cystitis.[17,81]

Granulomatous cystitis is the histopathologic description of cystitis caused by *Mycobacterium tuberculosis* and by schistosomiasis and other parasitic infections. Granulomata formed in response to certain parasites, such as *Toxocara* and microfilariae, also may contain numerous eosinophils. *Enterobius vermicularis* infection occasionally leads to signs and symptoms of cystitis and inflammatory changes of the bladder wall.

Xanthogranulomatous pyelonephritis is a rare, chronic, suppurative renal infection. Although it can occur at any age, it typically involves middle-aged women. Cases in children have been reported across all age groups, including infants.[1,114] The patient usually presents with what appears to be an acute UTI, caused most often by *E. coli* or *Proteus* spp. Evaluation of the patient usually reveals a unilateral enlargement of the kidney, often accompanied by urolithiasis and sometimes a staghorn calculus. The differential diagnosis of the mass lesion includes neuroblastoma, Wilms tumor, tuberculosis, and renal carcinoma. The lesion is characterized histologically by granulomata, abscesses, and lipid-laden foam cells. Nephrectomy is the usual means of management.

Infectious urethritis caused by *N. gonorrhoeae* or *Chlamydia trachomatis* is a common cause of symptoms suggestive of UTI. In addition, any etiologic agents of vulvovaginitis may cause inflammation of the distal urethra, with urgency, frequency, or dysuria; they include *Candida* spp., *Gardnerella vaginalis*, *Trichomonas vaginalis*, *S. pyogenes*, *S. pneumoniae*, *H. influenzae*, *C. trachomatis*, *Shigella* spp., and *Yersinia enterocolitica*.

NONINFECTIOUS

Urethral symptoms, such as urgency, frequency, and dysuria, may be caused by any factor or process that gives rise to inflammation in the lower urinary tract. Examples include mechanical irritation (which might result from insertion of foreign bodies, migration of pinworms, or masturbation) and chemical irritation (which might arise from bubble baths or shampoos). Chemical cystitis has been reported from the inadvertent insertion of a vaginal contraceptive suppository (nonoxinol 9) into the bladder. Pharmacologic causes of urethral symptoms include cyclophosphamide and methenamine mandelate, both of which can lead to inflammatory changes in the urinary bladder. Several other agents used in the topical treatment of bladder cancer have been noted to cause cystitis.

SPECIFIC DIAGNOSIS

COLLECTION OF A URINE SPECIMEN

Proper collection of a urine specimen is crucial to facilitate interpretation of the culture. In toilet-trained children, a midstream clean-catch specimen is appropriate for evaluation. When this specimen is used, the definition of significant bacteriuria is 10^5 colony-forming units (CFU)/mL or more. The child is asked to void into the toilet. Straddling the commode in a reverse position creates a natural separation between the urethra and the vulva. Cleansing of the perineum does not result in less contamination of the specimen and is no longer encouraged.[122] The child is asked to begin voiding. A second or two after the void has been initiated, a sterile cup is passed into the stream. The hope is that the initial void succeeds in washing out the distal urethra, the site from which the urine specimen is most likely to be contaminated.

If the child is not toilet-trained, a specimen may be collected by urethral catheterization or suprapubic aspiration. When the urine is collected by urethral catheterization, the perineum is cleaned with 1 percent iodine. A properly sized catheter (10 or 12 French) or a size 5 feeding tube may be used. The catheter is lubricated and inserted into the urethra and threaded a short distance. The first few drops of urine should not be collected in the sterile container. This part of the specimen is the most likely to be contaminated with fecal flora from the distal urethra; these bacteria are not eliminated by the process of perineal cleansing. The remaining urine is collected in a sterile container and sent to the laboratory. When a urine specimen is collected by urethral catheterization, significant bacteriuria is defined as 50,000 CFU/mL or more.[57] This method is preferred when a small volume of urine in the bladder is anticipated and collecting a specimen of urine is necessary so that antibiotics can be initiated.

An alternative to urethral catheterization is suprapubic aspiration. Although some physicians might contend that catheterization is less traumatic than suprapubic aspiration,[142] little evidence supports this notion. The procedure can be done in children of any age; it has been used to obtain specimens of urine in pregnancy.[96] Urine culture specimens obtained by suprapubic aspiration are easy to interpret because the usual source of contamination, the distal urethra, has been bypassed. The presence of any bacteria in a specimen collected by suprapubic aspiration is significant, although most samples contain 10^5 CFU/mL or more.

The patient is in a supine position with the lower extremities flexed (Fig. 45–1 and Table 45–2). The suprapubic area is cleaned with iodine and alcohol. The symphysis pubis is located with the index finger. A 3-mL syringe is attached to a 1½-inch, 22-gauge needle. A spinal needle (21-gauge, 2½- or 3-inch) can be used in older patients. The needle is passed in the midline about 1.5 cm above the symphysis pubis. It is angled about 10 to 20 degrees from the vertical, pointing in a slightly cephalad direction (Fig. 45–2). Negative pressure is applied while the needle is inserted. The procedure is most likely to be successful when the infant can be encouraged to drink and the diaper has been dry for at least 60 minutes before the procedure is done. The success rate of suprapubic aspiration can be improved with the use of a portable ultrasound device.[36] If the suprapubic aspiration is unsuccessful,

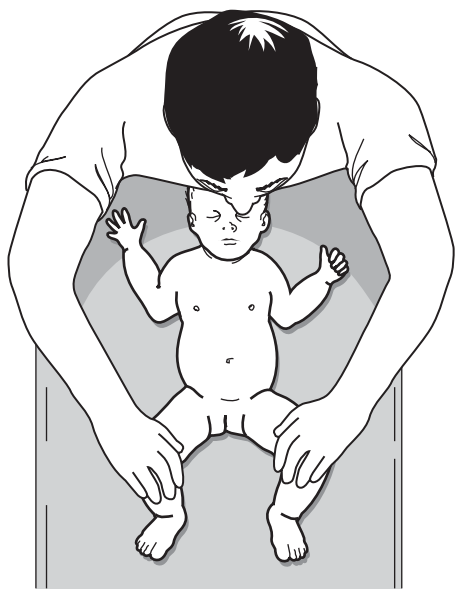

Figure 45–1 Suprapubic aspiration technique, position of patient.

TABLE 45–2 Suprapubic Aspiration Technique

Step 1	Child should not have voided within 1 hr of the procedure
Step 2	Restrain the infant in a supine, frog leg position
Step 3	Clean the suprapubic area with povidone-iodine and alcohol
Step 4	Identify the site of puncture at 1-2 cm above the symphysis pubis in the midline
Step 5	Use a 22-gauge, 1½-inch needle (with 3-mL syringe attached to it) and puncture at 10- to 20-degree angle of the true vertical aiming cephalad; a second attempt can be made at a similar angle aiming caudad
Step 6	Exert suction gently as the needle is advanced until urine enters syringe; aspirate urine with gentle suction. If urine is not obtained, further trials are unlikely to be successful

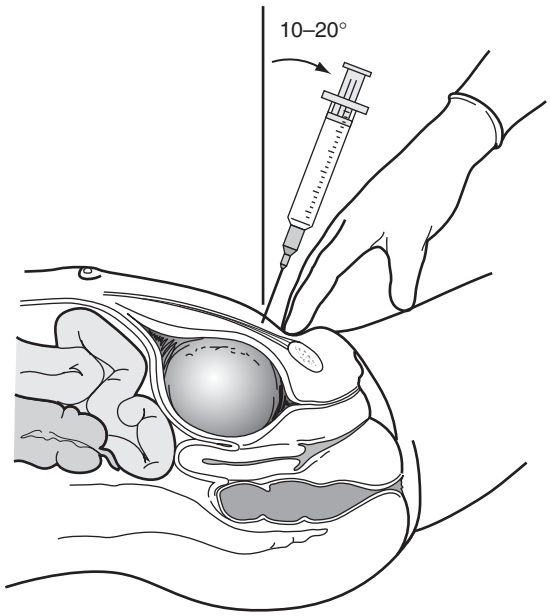

Figure 45–2 Suprapubic aspiration technique, position of needle.

a catheterized specimen should be obtained. Complications of suprapubic aspiration are rare and include formation of a hematoma, perforation of the bowel, and formation of a suprapubic abscess.[111] Suprapubic aspiration is contraindicated if the patient has a bleeding diathesis.

The last method of urine collection is the bag technique. The perineum is washed with soap and water and allowed to dry. A sterile plastic bag is attached. The bag is removed as soon as the patient voids. If the patient has not voided in 20 minutes, the bag is removed, the perineum is cleaned again, and a new bag is attached. If this procedure is followed meticulously, a reasonable specimen can be collected. This specimen is susceptible to contamination from periurethral flora,[121] however, and the technique is not recommended if the patient appears ill and antibiotics need to be started immediately after the specimen of urine is collected. The results of the culture of a bagged specimen are useful only if they are negative. If the culture is positive, a second specimen must be collected by a more reliable method.[3]

DIAGNOSIS OF URINARY TRACT INFECTION

The diagnosis of UTI hinges on the results of culture of a properly collected urine specimen. Disagreement within the literature is substantial regarding the definition of significant bacteriuria.[38] As indicated in the previous section, the definition of UTI varies according to the method by which the urine is collected. This variability in definition acknowledges that although the bladder urine is regarded as a sterile body fluid, contamination of the urine specimen may occur as it passes through the urethra. The distal urethra frequently is colonized with coliforms derived from the gastrointestinal tract. The only urine specimen that bypasses the urethra and is free of contamination is obtained by suprapubic aspiration. When a specimen is collected by this technique, any colony count of coliforms is significant.[164]

TABLE 45–3 Urinary Tract Infection—Definitions

Method of Collection	Colony Count (CFU/mL)
Clean catch	$\geq 10^5$
Catheter	$\geq 5 \times 10^4$
Suprapubic	Any

CFU, colony-forming unit.

In urine that is collected by a midstream clean-catch method, significant bacteriuria is defined, most stringently, by the recovery of 100,000 CFU/mL or more (Table 45–3). For specimens of urine obtained by catheter, significant bacteriuria is defined as 50,000 CFU/mL or more. In each of these instances, physicians recognize that although urine specimens containing lower colony counts rarely may represent true infection, for the most part, lower colony counts usually are the result of contamination of the specimen.[57]

Specimens of urine usually are inoculated onto two different kinds of solid media—one that supports the growth of only gram-negative enteric bacteria (e.g., MacConkey agar) and another that supports gram-positive and gram-negative bacteria (e.g., 5 percent sheep blood agar), with use of a 0.001 calibrated loop. Colonies are counted the next day (18 hours later), and the total is multiplied by 1000 to determine the colony count. The results of the urine culture are unavailable during the practitioner's first encounter with the ill child. Consequently, there is great interest in the development of a method that would predict the results of the urine culture so that appropriate antimicrobial therapy could be initiated presumptively at the time of the initial encounter. Microscopic methods (to evaluate pyuria and bacteriuria) and biochemical tests (which can be evaluated with a dipstick) have been evaluated.

Microscopy

Two surrogate markers for UTI on microscopic assessment are pyuria and bacteriuria. A problem in assessing pyuria and bacteriuria has been the issue of the definition of significant microscopic pyuria and bacteriuria. How many white blood cells (WBCs) in the urine are too many? Should the specimen of urine that is examined be centrifuged or uncentrifuged? How should the WBCs in a specimen of urine be enumerated? Should the number of WBCs on a centrifuged specimen be enumerated as the number per high-power field, or should they be enumerated on a counting chamber as the number of cells per cubic millimeter, as they would be in a sample of cerebrospinal fluid? If the urine is centrifuged, additional variables are introduced: the initial volume of urine, the duration of the spin, and the volume of urine used to resuspend the sediment. All of these variables influence substantially the enumeration of WBCs per high-power field, especially the volume used to resuspend the sediment.

Methods to assess bacteriuria also have raised issues of definition. Should bacteriuria be assessed on a centrifuged specimen or an uncentrifuged specimen, and should the bacteria be evaluated

on a wet mount or a Gram-stained specimen? Should bacteria be enumerated as the number per high-power field?

The standard definition of pyuria in the pediatric literature has been 5 WBCs per high-power field on a centrifuged specimen. Traditionally, microscopic bacteriuria has been expressed as the number of bacteria per high-power field on a Gram stain of a centrifuged specimen of urine. Several investigations undertaken by Hoberman and colleagues[56] have shown, however, that a so-called enhanced urinalysis has greater sensitivity, specificity, and positive predictive value than the standard urinalysis. An enhanced urinalysis is performed on an uncentrifuged sample of urine that has been obtained by catheter. The urine is placed on a counting chamber, and the cells are enumerated as the number per cubic millimeter. A Gram stain is performed in a manner that standardizes the number of drops of urine that are assessed and the number of oil immersion fields that are reviewed.

An enhanced urinalysis is considered to be positive when 10 WBCs/mm^3 or more are present and at least one gram-negative rod in 10 oil immersion fields is present. This definition of significant pyuria is much more sensitive than were previous definitions, and it has performed well for numerous investigators and in neonates and in older infants.[49,82,84,164] A systematic review of the existing literature to assess the performance of rapid diagnostic tests for UTI concluded that use of the traditional definition of pyuria (>5 WBCs per high-power field on a centrifuged specimen) is sufficiently poor that it cannot be recommended for making a presumptive diagnosis of UTI.[38] Huicho and coworkers[61] also performed a meta-analysis of urine screening tests to determine the risk of UTI in children. This more recent study concluded that pyuria of at least 10 WBCs/mm^3 and bacteriuria are best suited for assessing the risk of UTIs in children.

Automated methods to perform urinalysis are being used in many hospitals and laboratories. The most updated automated image-based urinalysis system, the iQ200 (Iris Diagnostics, Chatsworth, CA) received clearance from the Food and Drug Administration more recently. The system uses flow imaging analysis technology and so-called Auto Particle Recognition (APR; Iris Diagnostics) software to classify particles found in an uncentrifuged urine specimen based on multiple parameters. Images are stored and can be viewed later on the workstation screen, eliminating the need for manual microscopy in most cases.[148] The iQ200 provides for a rapid turn-around time, and results correlate well with manual methods, especially for red blood cells, WBCs, and squamous epithelial cells.[74,85]

Pyuria is not specific for UTI. In numerous other conditions, including fever, streptococcal infections, and Kawasaki disease, and after exercise, WBCs are found in the urine. Finding pyuria does not ensure that an infection of the urinary tract is present. Despite reports to the contrary, finding true UTI without pyuria is unusual.[150] Generally, inflammation is expected to accompany infection. The absence of pyuria in children with UTIs is rare; it may occur when a child is being evaluated so early in the clinical course of the infection that the inflammatory response has not yet developed. It also may occur when a child is experiencing an episode of asymptomatic bacteriuria.

The most likely explanation for significant bacteriuria by culture in the absence of pyuria is a contaminated specimen. In most cases when UTI has been reported to occur in the absence of pyuria, the definition of pyuria has been at fault. The requirement for 5 WBCs per high-power field on a centrifuged specimen corresponds to approximately 25 WBCs/mm^3; it is too stringent a requirement, with a low sensitivity for the detection of UTIs in infants and children.

Urine Dipsticks

Urine dipsticks (or reagent strips) have been used to indicate the presence of leukocyte esterase (as a surrogate marker for pyuria) and urinary nitrite (which is converted from dietary nitrates by the presence of gram-negative bacteria in the urine). The conversion of dietary nitrates to nitrites by bacteria takes approximately 4 hours. The test result is most likely to be positive when the urine tested is the first morning void (representing a urine that has incubated in the bladder overnight) or a urine that has been in the bladder for at least 4 hours (e.g., obtained from an older child who may hold urine in the bladder for several hours at a time).

The performance characteristics of leukocyte esterase and nitrites vary according to the definition used for a positive urine culture, the age and symptoms of the population being studied, and the method of urine collection. A nitrite test, although not a sensitive marker in children, is helpful when the result is positive because it is highly specific. A negative nitrite test result has little value in ruling out UTI, however.[25] The leukocyte esterase test has an average sensitivity of 83 percent.[26] It can have a sensitivity of 94 percent in settings in which UTIs are suspected clinically and a sensitivity of 52.9 percent when it is performed on febrile children, most of whom do not have a UTI.[57] The specificity of leukocyte esterase (average 72%; range 64 to 92%) generally is not as good as the sensitivity, reflecting the nonspecificity of pyuria in general. A positive leukocyte esterase test result should be interpreted with caution, depending largely on the population being evaluated.[25]

DETERMINING THE SITE OF INFECTION

Urinalysis is useful for detecting infection but not for determining the location of the infection within the urinary tract (i.e., upper tract versus lower tract). To determine the site of infection (i.e., kidney versus bladder), many investigations of the discriminatory ability of C-reactive protein, erythrocyte sedimentation rate, and total peripheral WBCs have been performed. Several studies have evaluated the accuracy of procalcitonin levels compared with C-reactive protein levels to predict renal involvement among children with febrile UTIs.[8,34,107,112,138] These studies showed that serum procalcitonin concentrations, measured with either an immunoluminetric quantitative test or a rapid semiquantitative test, diagnosed APN with a sensitivity of 70.3 to 94.1 percent and a specificity of 82.6 to 93.6 percent. The specificity of procalcitonin was always higher than that of C-reactive protein, and a highly significant correlation was noted between elevated procalcitonin levels and severity of renal involvement as measured by dimercaptosuccinic acid (DMSA) scintigraphic scores.[107]

IMAGING

The current standard of care is to perform imaging procedures on children with a diagnosis of UTI. The categories of children for whom imaging generally is recommended are (1) any child who experiences an episode of APN, (2) boys of any age with a first UTI, (3) girls younger than 3 years old with a first UTI, (4) girls older than 3 years of age with a second UTI, and (5) girls older than 3 years old with a first UTI if an extenuating circumstance exists (Table 45–4). The extenuating circumstances include a family history of renal disease, recognition of abnormal voiding patterns, poor growth, hypertension, known abnormalities of the urinary tract, and failure to respond promptly to therapy. The imaging studies that usually are considered are renal ultrasonography, contrast voiding cystourethrography (VCUG) to detect VUR, renal cortical scintigraphy, and magnetic resonance imaging (MRI).

TABLE 45–4 Indications for Imaging Procedures in Children with Urinary Tract Infection

Any episode of acute pyelonephritis
Boys with first UTI
Girls <3 years old with first UTI
Girls ≥3 years old with second UTI
Girls ≥3 years old with first UTI if
Positive family history
Abnormal voiding patterns
Poor growth
Hypertension
Abnormalities of urinary tract
Failure to respond promptly to treatment

UTI, urinary tract infection.

RENAL ULTRASONOGRAPHY

The renal ultrasound examination has replaced completely intravenous pyelography as a means to assess the gross anatomy of the urinary tract. Generally, ultrasonography has been performed promptly after diagnosis of the UTI has been made. It is a noninvasive test that can describe the size and shape of the urinary tract, the presence of duplication and dilations of the ureters, the presence of ureteroceles, and the existence of gross anatomic abnormalities such as a horseshoe kidney.[3] It is not sensitive enough, however, to signal consistently the presence of hydronephrosis, hydroureter, VUR, or renal scarring.[30] When ultrasonography was compared with intravenous pyelography for the detection of renal scars, wide interobserver variations were noted, with sensitivity ranging from 40 to 90 percent.[42]

Some investigators have questioned whether routine performance of renal ultrasonography is essential.[54] Given the current frequency with which fetal ultrasound examinations are performed during gestation, the likelihood that ultrasonography would disclose information that is not already known is small. In a study of 306 children younger than 2 years old with UTIs, only 1 child had a clinically important finding discovered by the routine performance of renal ultrasonography.[55] In a more recent study of 255 children younger than 5 years old with a first diagnosed uncomplicated febrile UTI, abnormalities were found on ultrasonography in 14 percent of patients; in none of these patients did the ultrasonography findings influence management.[163]

Most obstructions of the urinary tract are diagnosed in utero. Selective performance of renal ultrasonography is recommended for children with UTIs who do not respond promptly to antibiotic therapy (i.e., have persistent fever or abdominal findings) and in children who did not have a prenatal ultrasound examination performed beyond 30 weeks of gestation at a reliable center.

RENAL SCINTIGRAPHY

In patients with presumed APN, renal scintigraphy with Tc 99m DMSA or Tc 99m glucoheptonate has been shown to be the most practical and reliable method for detecting APN.[126] DMSA and glucoheptonate are amino acids that are cleared by the renal tubules. When these amino acids are labeled with technetium and injected intravenously, they can be used to create an image of the kidney, which reflects vascular flow and tubular function (Fig. 45–3). In experimentally induced APN in piglets, the DMSA scan had a sensitivity of 87 percent and a specificity of 100 percent in showing lesions consistent with APN compared with histology as the gold standard.[119]

In most patients with APN, renal scintigraphy performed during the acute phase of the illness shows a decreased uptake of DMSA (Fig. 45–4). High-resolution pinhole images of the kidney reveal focal, multifocal, or diffuse areas of decreased uptake of isotope in the kidney without loss of volume and with maintenance of the contour of the kidney.[126] DMSA renal scintigraphy can be used to localize the level at which the urinary tract is infected, specifically distinguishing between acute cystitis and

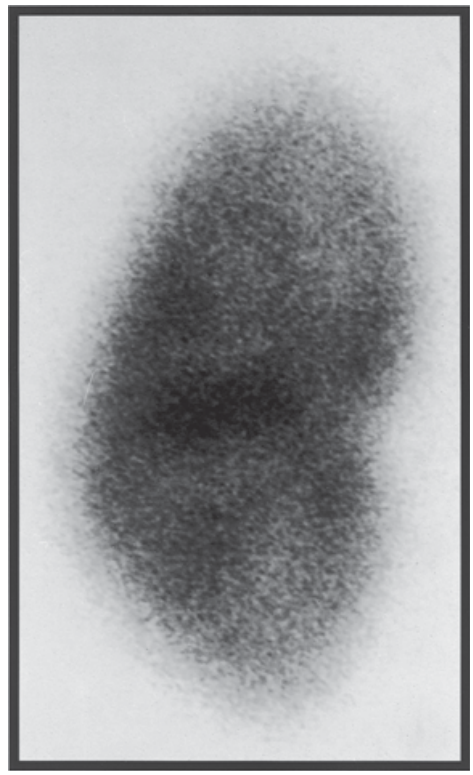

Figure 45–3 Normal renal scintigram.

Figure 45–4 Scintigram showing acute pyelonephritis, manifesting as a photon-deficient area in the upper pole.

Figure 45–5 Scintigram showing a renal scar with loss of the normal contour.

APN with a sensitivity of slightly less than 90 percent. Rarely, a patient may present with classic signs and symptoms of APN in whom renal scintigraphy does not show the usual findings.[32]

The DMSA and glucoheptonate scans also can be used to indicate the presence of renal scars that result from an episode of APN. The scar is indicated by an area in which uptake of the radioisotope is decreased (Fig. 45–5). In this case, the contour of the kidney is not preserved. Scarring also may be indicated by an overall reduction of the size of the kidney. Hoberman and colleagues[53] prospectively evaluated 309 children who were 1 to 24 months old with their first febrile UTI. An initial DMSA scan was performed on all children and showed that 61 percent (190 of 309) had findings compatible with APN, and only 1 child had evidence of previous scars. Repeated scintigraphic scanning was done 6 months after entry in 89 percent to detect renal scarring. A small percentage of renal parenchymal involvement (mean 8.2%) was noted in 9.6 percent of the children. All children whose initial scans were normal had normal scans at follow-up.

DMSA scintigraphy has been suggested as the preferred imaging study to evaluate a child with a first febrile UTI rather than ultrasound or VCUG.[41,46] A strategy to perform VCUG only in patients with an abnormal DMSA scan has been proposed.[41] The rationale is based on the rarity of renal scarring when the initial DMSA is normal. Reflux definitely may be present, however, when DMSA scans are normal (30 of 99 in Hoberman's data).[53] Absence of scarring may be attributable to the effect of prophylactic antibiotics. Renal scintigraphy is the most sensitive study to indicate the presence of small renal scars that may occur after APN.

MAGNETIC RESONANCE IMAGING

MRI can be used to detect the presence of APN. Two studies by Lonergan and colleagues[91,108] showed a sensitivity and a specificity of MRI that are equal to and perhaps even greater than those of renal scintigraphy with DMSA. In both instances, the best pulse sequence to show areas of APN with MRI was gadolinium-enhanced, fast spin-echo inversion recovery. These results have been confirmed by others.[78] Several investigators have urged, however, that MRI not become a screening test for children with UTIs until the detection of minor changes in the renal parenchyma (by either DMSA or MRI) has been proved to be of clinical importance.[78,90] The time and sedation requirements for MRI render it an unattractive choice for routine management.

VOIDING CYSTOURETHROGRAPHY

Two types of cystography are available for the diagnosis of VUR—fluoroscopic contrast VCUG and radionuclide cystography. Contrast VCUG has excellent anatomic resolution and provides detailed images of the bladder and the urethra. It can be used specifically to label the degree of reflux (according to the international classification);[75] to look for trabeculations, diverticula, or ureteroceles of the bladder; and to outline the urethra (which is essential to identify posterior urethral valves causing obstruction). Radionuclide cystography provides less anatomic detail and is most useful when urethral disease is not suspected and when the patient has no history of voiding dysfunction. It is used to observe patients who are known to have reflux (to assess whether reflux is still present) and can be used for screening purposes in siblings of children with reflux and in children of adults known to have had reflux. Compared with conventional contrast VCUG, the amount of radiation exposure is reduced substantially when the radioisotope is used.

The international classification of reflux is a universally used system involving five degrees of reflux.[75] Grade I is reflux into the distal ureter; grade II is reflux into the pelvis; and grades III, IV, and V are reflux with mild, moderate, and severe dilation of the pelvis and calyces (Fig. 45–6).

VCUG is performed on children who have experienced a symptomatic UTI to determine the presence of VUR. Children who have VUR are at risk for developing reflux nephropathy—permanent renal damage secondary to the reflux of infected urine into the kidney. Because reflux often resolves spontaneously over the course of several years, the purpose of identifying a child with VUR is to recommend antibiotic prophylaxis until the reflux either has resolved spontaneously or, in cases of high degrees of reflux, has been corrected surgically.

Although imaging of children with UTI is routine, little evidence exists that diagnostic imaging of children after their first UTI results in prevention of renal scarring, hypertension, or renal failure.[23] In a systematic overview of the literature using the MEDLINE database, no controlled trials or analytic studies evaluating or comparing different management strategies with regard to imaging were discovered.[23] If studies can show with certainty that antibiotic prophylaxis to prevent UTIs in children with VUR is superior to placebo in the prevention of renal scarring, the necessity and importance of performing VCUG would be established. Such a study was funded by the National Institutes of Health to begin in 2007[39a] (see the section on management of VUR).

TREATMENT

ANTIBIOTICS FOR TREATMENT OF ACUTE INFECTION

The treatment of UTI is influenced by the age of the patient, the probable site of infection (cystitis or APN), the degree of toxicity, and the likelihood of adherence to the treatment regimen. Oral antimicrobial therapy is appropriate for children with cystitis and for older children with suspected APN who are neither toxic nor

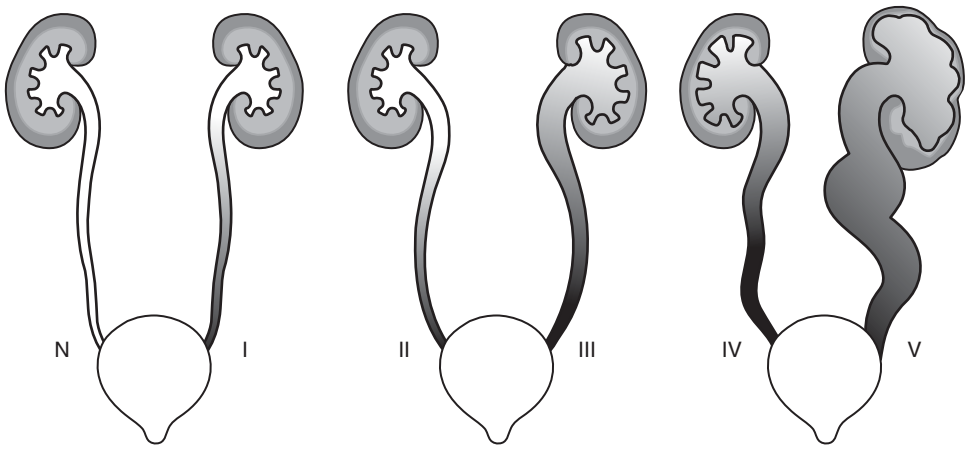

Figure 45–6 Vesicoureteral reflux, international classification.

TABLE 45–5 Antibiotic Treatment

Oral	
Amoxicillin + potassium clavulanate	45 mg/kg/day in 2 divided doses
Cefuroxime	30 mg/kg/day in 2 divided doses
Cefprozil	30 mg/kg/day in 2 divided doses
Cefixime	10 mg/kg/day in 1 dose
Cefpodoxime	9 mg/kg/day in 1 dose
Ceftibuten	10 mg/kg/day in 1 dose
Cefdinir	14 mg/kg/day in 1 dose
Trimethoprim-sulfamethoxazole	10 mg/kg/day (trimethoprim) in 2 divided doses
Parenteral	
Ampicillin + sulbactam	200 mg/kg/day in 4 divided doses
Cefuroxime	150 mg/kg/day in 3 divided doses
Cefotaxime	200 mg/kg/day in 4 divided doses
Ceftriaxone	80 mg/kg/day in 1 dose
Ceftazidime	150 mg/kg/day in 3 divided doses
Cefipime	100 mg/kg/day in 2 divided doses
Gentamicin	7.5 mg/kg/day in 3 divided doses

vomiting. The more complex issue is the management of a young infant with high fever in whom the likely diagnosis is APN. These children traditionally have been admitted to the hospital for parenteral administration of antibiotics.

Numerous choices of oral antimicrobials are available for patients who present with presumed cystitis (Table 45–5). Alternatives include amoxicillin-potassium clavulanate; second-generation cephalosporins, such as cefuroxime and cefprozil; third-generation cephalosporins, such as cefixime, cefpodoxime, ceftibuten, and cefdinir; and the combination agent trimethoprim-sulfamethoxazole. Generally, neither amoxicillin nor first-generation cephalosporins are recommended for first-line therapy because 30 to 50 percent of *E. coli* now are inherently resistant to these agents. Because of some geographic variability in the prevalence of resistance, the practitioner should check with local infectious disease specialists to verify these susceptibility patterns.

In an older patient who appears ill but is not toxic, a repeated visit or telephone follow-up is indicated to ensure that recovery is progressing as predicted. If fever is present, the patient generally becomes afebrile within 1 or 2 days, although occasionally it may take longer. If other indices of recovery, such as general well-being, appetite, and playfulness, are improving appropriately, the persistence of fever is not alarming. If susceptibility test results are available and the organism causing infection is susceptible to the antimicrobial being used, repeating the urine culture after 24 hours is unnecessary.[55] The rapid sterilization of urine is a testimony to the fact that virtually all the antibiotics that are used to treat UTIs are concentrated in the kidney and excreted in the urine.

Duration of treatment for patients who are presumed to have cystitis has been controversial. Conventional recommendations are for 7 to 10 days of antimicrobial therapy. Short courses of therapy, varying from single-dose regimens to 3- or 4-day courses, have been evaluated with mixed results. The potential advantages of short-course therapy include the likelihood of improved adherence to the drug regimen; lower cost of drug; and fewer undesirable side effects, including less alteration of normal flora.[127] A meta-analysis (and the most comprehensive review of the data) was performed by Tran and coworkers[143] after review of 22 published trials and a total of 1279 patients. Amoxicillin in a single dose and trimethoprim-sulfamethoxazole as either a single dose or a 3-day regimen were the short-course regimens most commonly evaluated. The authors found that short-course antimicrobial therapy is less effective than therapy of conventional duration, largely because of the ineffectiveness of single-dose amoxicillin. A 3-day course of trimethoprim-sulfamethoxazole was an effective alternative to the standard course of treatment.

A more recent meta-analysis evaluated 10 trials in 652 children with lower tract UTIs.[58] No significant difference was found in the frequency of positive urine cultures between short-duration (2 to 4 days) and standard-duration (7 to 14 days) oral antibiotic therapy for UTIs in children 1 to 15 months after treatment. In children with clear evidence of cystitis without APN, short-course therapy may be acceptable.[149]

Management of a young infant with presumed APN has been controversial. A study conducted by Hoberman and colleagues[55] compared oral therapy with cefixime (for 14 days) with a combination of intravenous therapy with cefotaxime (for 3 or 4 days) plus oral cefixime for 9 to 10 days. Children were eligible for this study if they were 1 to 24 months of age and presented to the physician with temperature greater than 38.3° C, and were found to have a positive enhanced urinalysis (≥10 WBCs/mm^3 and 1 organism per 10 oil immersion fields). After blood and urine cultures, complete blood count, C-reactive protein determination, and erythrocyte sedimentation rate were performed, the children were randomly assigned to treatment groups. Outcome was evaluated with regard to the short-term measures of sterilization of the urine and time to defervescence and the long-term measures of reinfection and scarring 6 months after the initial infection. No statistically significant differences in any outcome were found.

Bloomfield and colleagues[12] performed a systematic review of the treatment of APN in children. They assessed 18 trials of 2612 children aged 0 to 18 years with proven UTIs and APN. Their results suggested that children with APN could be treated effectively with oral cefixime or with a short course of intravenous therapy followed by oral therapy.[58]

MANAGEMENT OF DYSFUNCTIONAL VOIDING

If a history of dysfunctional voiding is obtained, a behavioral modification approach may be helpful. The key features include frequent volitional voiding every 2 to 3 hours until the voiding pattern has been re-established, increased water intake (1 L/day) in addition to all other fluids consumed, correction of constipation, and adequate perineal hygiene.[28] Referral to a pediatric urologist may be necessary. Children who exhibit persistent inability to relax their sphincters may require training that involves relaxation techniques reinforced with biofeedback. Pharmacologic treatment with anticholinergics may be necessary if uninhibited bladder contractions are shown with urodynamic studies.[28] Special attention must be paid to the management of constipation if anticholinergics are initiated because they would exaggerate the problem.

ANTIBIOTIC PROPHYLAXIS

Although antibiotic prophylaxis commonly is recommended for children with recurrent UTIs,[79] only limited data support this position. In a review of the literature, Le Saux and colleagues[79] found sparse data of low quality to support the use of antibiotic prophylaxis in children with a normal urinary tract. A meta-analysis by Williams and associates[156] revealed five randomized controlled trials assessing the use of prophylactic therapy. Of these, only two trials with 71 children evaluated the effectiveness of long-term, low-dose antibiotics to prevent UTIs.[87,134] The authors concluded that well-designed, randomized, placebo-controlled trials still are required to evaluate this commonly used intervention.

MANAGEMENT OF VESICOURETERAL REFLUX

The clinical significance of VUR as a predisposing factor for UTIs in general or APN in particular and its contribution to the formation of renal scarring have been questioned more recently. As a corollary to the latter, the question also has been framed as to whether long-term antibiotic prophylaxis would prevent renal damage in patients with VUR.[32] The authors concluded that the role of VUR in UTIs needs to be redefined through well-designed, multicenter, prospective, randomized, controlled studies using state-of-the-art renal imaging techniques.

Epidemiology

The incidence of VUR in the general population is thought to be approximately 1 percent.[22] Children usually are tested for the possibility of VUR in one of two clinical situations—during the assessment of prenatally diagnosed hydronephrosis and in the evaluation of a UTI. Reflux is diagnosed in approximately 10 to 20 percent of cases of prenatally identified hydronephrosis; it often is high-grade and occurs more frequently in boys than in girls in this situation.[48,146] The incidence of reflux in girls evaluated after a first UTI is 25 to 40 percent.[26]

An increased incidence of VUR within families suggests a genetic mode of transmission.[68] Rates for prevalence of VUR in identical and fraternal twins were 80 percent and 35 percent, providing evidence that this trait is transmitted in an autosomal dominant fashion. Siblings of children identified to have VUR as part of an evaluation for UTI have a much greater chance of having reflux than the normal population.[4,106] The presence of VUR in these siblings is accompanied by silent renal damage.[15] In addition, a girl who has reflux has a 65 percent risk of having affected offspring.[22] Reflux is much more common among white than black children.

Natural History of Reflux

The natural history of reflux is for spontaneous resolution to occur over time, with a longer period being necessary in more severe types of reflux. Other factors that may affect the rate of resolution include age at diagnosis, gender, unilaterality versus bilaterality, and whether there is dysfunctional voiding. Average figures for spontaneous resolution are 80 to 86 percent for grade II reflux and 40 to 46 percent for grade III reflux during a 5-year period.

In a report from Skoog and associates,[130] spontaneous resolution occurred within 1.65 years of diagnosis in patients with grade II reflux, but 1.97 years was required for resolution in patients with grade III reflux ($p < .04$). Ninety percent of patients with reflux grades between I and III who ultimately had resolution of reflux experienced this result within 5 years. The rate of resolution was approximately 30 to 35 percent per year, although the duration of reflux was shorter for patients in whom the diagnosis was made before they reached 1 year of age compared with patients who were older at the time of diagnosis.[7]

In another study of the natural history of reflux, Schwab and coworkers[125] determined the resolution rate by patient for 179 girls and 35 boys with UTIs and diagnoses of primary VUR between 1981 and 1984. Reflux spontaneously resolved in 68 percent of patients during the study. Grades I to III reflux resolved at a rate of 13 percent per year for the first 5 years of follow-up and then at a rate of 3.5 percent per year during subsequent follow-up. Grades IV and V reflux resolved at a rate of 5 percent per year. Bilateral reflux resolved more slowly than unilateral reflux and more rapidly in boys than in girls. There is no specific age in adolescence at which one can assume reflux will not resolve. Leneghan and colleagues[76] reported cessation of reflux after 14 years of age in 27 percent of patients observed without surgical intervention.

Management of Reflux

The optimal management of children with high grades of reflux (grades III to V) has been the subject of numerous retrospective and prospective studies.[64] Since 1970, two large prospective randomized trials, the Birmingham Reflux study[9-11] and the International Reflux Study in Children,[115] have been performed to compare medical therapy (antibiotic prophylaxis) with surgical therapy (reimplantation of ureters). The International Reflux Study in Children reported results from Europe and the United States. Because entry criteria were different, the results have been reported separately.

In Europe and the United States, the surgical management of VUR is neither superior nor inferior to medical treatment. In both groups, new scars were acquired during the 5-year follow-up period, some in patients who did not show scarring at the time of entry. In other children, scars present at entry worsened.[110] No detectable differences were found between study groups in either renal function or renal growth. The number of episodes of infection also was similar in both groups. The only exception was a greater frequency of episodes of febrile UTIs (presumed pyelonephritis) in children receiving medical therapy.

Wheeler and colleagues[155] conducted a systematic review of randomized trials of the effects of various interventions in patients with VUR on the development of subsequent UTI and renal parenchymal injury. The aim was to evaluate whether any intervention for reflux (surgical) is better than nonsurgical treatment. Eight trials involving 859 evaluable children were reviewed. Seven trials compared antimicrobials alone with surgery plus antimicrobial propylaxis. The risk of a UTI developing by 1 or 2 and 5 years was not significantly different between surgical and medical groups, and the risk of renal scarring or progressive renal damage occurring was similar. The choice of treatment remains a value judgment governed by such local factors as preference of the parents, availability of skilled surgeons, availability of closely supervised medical treatment, and willingness to comply with prolonged periods of prophylaxis.

Does VUR predispose to pyelonephritis? Garin and associates[32] reviewed 10 studies of children with acute UTIs who underwent DMSA scanning and VCUG. Selection of patients in at least one of the studies was biased toward the association between reflux and APN because the VCUG often was performed after the DMSA scan was reported to be abnormal. Six of 10 studies showed statistically significant results indicating that the presence of reflux definitely was associated with the occurrence of APN; all 4 of the remaining studies showed a trend in the same direction.

Antimicrobial Prophylaxis

Although the use of antimicrobial prophylaxis has become routine in the management of children with VUR, substantial controversy currently surrounds this approach, and an increasing literature challenges its benefit. In 2003, a Cochrane Review of the effectiveness of long-term antibiotics for preventing recurrent UTIs in children indicated that most studies published before this date were poorly designed without proper blinding.[157] Although Garin and colleagues[33] contributed a large study suggesting that antibiotic prophylaxis is not an effective intervention compared with no treatment in children with VUR, this study also had many methodologic flaws.[150]

Accepting for the moment that prophylaxis is the most effective medical therapy that can be offered to children with mild to moderate degrees of reflux, the duration of prophylaxis also is an issue. VUR spontaneously remits in most cases during the first 3 to 5 years of life. Conventional wisdom is that prophylaxis should be maintained until reflux ceases, either spontaneously or with surgical intervention. Greenfield and colleagues[39] suggested that two normal VCUGs performed 12 months apart are necessary before prophylactic antibiotics are discontinued. This recommendation is based on the observation that 27 percent of children exhibit VUR again after a single normal study.

Greenfield and colleagues[39] maintained that administration of prophylactic antibiotics should continue until reflux resolves, no matter what the age. They supported this position by noting that older children with reflux may have new scars evolve when they become infected. In contrast, other authors suggest that prophylaxis should be discontinued in older children, even if reflux persists.[2,7,18,141,158] The only two antimicrobial agents that are recommended for prophylaxis of the urinary tract are nitrofurantoin and trimethoprim-sulfamethoxazole. Each agent is used in half the usual therapeutic dose and given before bedtime. These two agents can be used for months to years without the emergence of antibiotic resistance. In contrast, most other agents that are used for treatment of UTIs are not recommended for prophylaxis. Invariably, if agents such as amoxicillin, cephalexin, and second-generation and third-generation cephalosporins are used for prophylaxis, infection with resistant strains emerges within weeks.

Surgery

When antireflux surgery is undertaken, a variety of approaches are available, no one of which seems to be superior. In most cases, the surgical approach is intravesicular. Interest has been gaining in evaluation of an extravesicular approach to reimplantation of the ureters.[95] The advantage of extravesicular approaches seems to be a diminution of the intensity and frequency of bladder spasms and less requirement for postoperative analgesia. This technique is indicated primarily in unilateral VUR.[44] When experienced senior surgeons embark on reimplantation of ureters, the outcome generally is successful.[22] Generally, all surgical techniques have a high success rate of 92 to 98 percent.[44] Repeated operation for persistence or recurrence of reflux seldom is required. Rarely, when children younger than 2 years old undergo surgery, ureteral obstruction may result; its persistence mandates a second operation.[115]

A newer technique for the management of VUR is endoscopic injection therapy using a variety of materials. Developed as a minimally invasive approach to the treatment of VUR in 1984, dextranomer/hyaluronic acid copolymer has emerged as the favored bulking agent since its approval by the Food and Drug Administration.[43,92] Although success is defined differently from after surgery, overall success rates range from 82 to 89 percent with initial injections of dextranomer/hyaluronic acid. The bulking agent is injected beneath the ureteral orifice and effectively closes the distal ureter. The procedure is done in the outpatient setting and is associated with less pain and a quicker recovery compared with traditional reimplantation procedures.[92] The success rates are lower, however, than those of traditional therapy, and the results have questionable durability. Long-term prospective studies are necessary to evaluate endoscopic treatments critically.

PROGNOSIS

Generally, a review of reports suggests that the short-term prognosis for previously normal children who experience an episode of UTI is excellent. In a large cohort of 306 children experiencing their first febrile episode of UTI, 40 percent were found to have reflux.[55] Only five (1.6%) of the children had grade IV reflux; none had grade V reflux. Most of the children with grades I, II, and III reflux can be expected to have spontaneous resolution of the process during the subsequent several years. Although many reports indicate that recurrent UTIs are common occurrences in children who have recovered from UTIs, recurrence during the first 6 months after recovery from the index episode of UTI was an infrequent event in this cohort of children.

Recurrence is seen most commonly in the early months after a symptomatic or asymptomatic UTI has occurred. Symptomatic reinfections (fever, pyuria, and positive urine culture) occurred in 7 children treated orally and in 11 children treated intravenously (for a total of 5.9% of children) during the 6-month follow-up period.[55] Asymptomatic bacteriuria (positive urine culture in the absence of fever and pyuria) occurred in one child treated orally and in two children treated intravenously (1%). Renal scarring occurred in 9.5 percent of all children; the average percentage of renal parenchymal involvement was 8.2 percent. In children who had an abnormal renal scintigram at the time of diagnosis, the frequency of scarring was 15.3 percent. None of the children with a normal renal scintigram at the time of diagnosis developed scars. This experience, reflecting an aggressive approach to the early diagnosis of UTI in infancy, is encouraging with regard to outcome.

When children with a diagnosis of UTI are found to have anomalies of the urinary tract, congenital dysplasias, or massive

degrees of reflux, the prognosis is less optimistic. The most profound degrees of scarring are associated with advanced degrees of reflux. Reflux nephropathy is a major cause of severe hypertension in children and young adults. It occasionally progresses to chronic renal failure,[136] which accounts for approximately 25 percent of the children in the United Kingdom with end-stage renal failure requiring regular dialysis or transplantation.[80] Although surgical correction may relieve the reflux, the overall outcome for children with reflux who have undergone surgical correction has not been shown to be materially better than that for children who have been managed medically.[103,104,137]

Smellie and colleagues[133] undertook a randomized trial of medical management versus surgical correction in 53 children with bilateral severe VUR and bilateral nephropathy. The glomerular filtration rate of the children at enrollment was 20 mL/min/1.73 m^2 body surface area. Children with this degree of severity of renal impairment are rare; recruitment to this study took 5 years. No significant differences were observed in glomerular filtration rate, renal growth, or scarring during a 10-year follow-up period. The failure to find differences may be due to the small sample size and broad heterogeneity of the study group. Nonetheless, no convincing evidence that outcome for renal function is improved by surgical correction of VUR in children with bilateral disease exists.

PREVENTION

Primary prevention of UTI can be accomplished in infant boys by promoting the practice of circumcision. Whether this procedure can be justified simply to prevent UTIs in the context of social rituals is uncertain. Circumcision should be recommended, however, for selected groups of patients, including newborns with prenatal hydronephrosis who are found to have VUR in the neonatal period, boys with high grades of VUR, and boys in whom VUR is associated with unilateral renal agenesis or multicystic kidney.[14]

Other commonly recommended strategies to prevent UTI, such as the avoidance of bathing in tubs or swimming and the instruction in correct wiping techniques, are not accompanied by convincing evidence.[45] The relationship between constipation and UTI is well known. Effective treatment of constipation results in normalization of bladder function and cessation of UTIs.[86]

The role of prophylactic antibiotics for patients with VUR is discussed in the section on treatment. Prophylaxis has not been shown in large, prospective, placebo-controlled, randomized trials to reduce effectively the frequency of UTI and subsequent renal scarring either in patients with reflux or in patients who have experienced an episode of APN.[33,157] Although definite biologic plausibility to this likelihood exists, strong evidence is lacking.

An additional preventive strategy for UTI is immunization. A small pilot study was reported from Turkey.[98] Ten otherwise healthy girls aged 5 to 11 years old with recurrent UTIs were immunized with inactivated uropathogenic bacteria intramuscularly once a week for 3 consecutive weeks. A booster injection was given after 6 months. The frequency of infection was compared with that in a group of 10 age-matched girls with UTIs who were not immunized. Immunization therapy caused a significant reduction in the frequency of infection and an increase in the concentration of the secretory component of IgA in the urine. Other mucosa, such as vaginal and oral surfaces, are alternative targets for vaccine delivery.

A vaginal mucosal vaccine composed of a mixture of heat-killed bacteria from 10 human uropathic strains (6 *E. coli* and 4 other gram-negative enteric bacteria) was developed for women with recurrent UTIs.[145] Fifty-four women completed a double-blind, placebo-controlled, phase 2 trial. Time until reinfection was longest for women who received primary and booster immunization compared with women receiving a placebo. This strategy has potential for further development.

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CHAPTER 46

Renal Abscess

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Although acute pyelonephritis is a common infection in children, the primary development of a renal abscess or progression of pyelonephritis to a renal or perinephric abscess is an uncommon occurrence. In one study conducted over a 10-year period, 8 children with a renal abscess were found among 43,224 discharge diagnoses—approximately 1 case per 5400 pediatric admissions.[5] Six of the eight children were 11 years or older. Although rare, renal abscesses have been reported in neonates.[8] Renal abscess may be a primary problem—that is, one that develops in a kidney without an antecedent infection or underlying anatomic abnormality—or it may occur secondarily in a patient with previously recognized acute pyelonephritis or in a child with congenital urologic abnormalities known to predispose one to the development of pyelonephritis.

A primary renal abscess is thought to develop most often after an episode of bacteremia and frequently occurs in younger children. Hematogenous spread of bacteria to the kidney usually results in a cortical abscess.[5] The most common organisms involved in these abscesses are gram-positive cocci, primarily *Staphylococcus aureus*, and less often a streptococcus. In some cases, a cutaneous infection might have been present before development of the renal abscess, and this infection is thought to be the primary source for the bacteremia.[14] Most children in whom a renal abscess develops hematogenously are normal hosts.[5,10,18,20]

In older children and teenagers, tuberculous abscesses and caseous necrosis of the renal parenchyma also should be included in this primary classification. These infections tend to be indolent, although renal tuberculosis may be complicated by bacterial infection because of associated ureteral strictures and severe tuberculous cystitis. Pediatricians should become familiar with this "adult" malady because of the resurgence of tuberculosis and the generally older age group for which many pediatricians now provide care.

When a renal abscess occurs in association with a recognized urologic disorder, the organism responsible most often is a gram-negative bacillus or an enterococcus, bacteria usually seen in simple urinary tract infections and pyelonephritis.[17] Examples of urologic disorders that one might encounter with these infections include congenital and acquired obstructions (e.g., ureteropelvic and ureterovesical obstruction, retrocaval ureter, ureteral stricture after surgical intervention), calculous disease (obstructing and nonobstructing), infundibular stenosis, and renal dysplasia with cystic changes. Abscesses that occur as a result of infection of the urinary tract generally are found in a corticomedullary location.[5]

Anaerobic organisms also have been implicated as a cause of renal abscess. They often are present simultaneously with the more usual aerobic bacteria, but they can cause infections and abscesses alone. These anaerobic renal infections develop most commonly in association with infections complicating bowel injury or surgery, renal transplantation, malignancy, and orodental infections.[4] The genus of the anaerobic organism may provide a clue to its source. *Bacteroides fragilis* is likely to arise from an intra-abdominal source, whereas an oral site is more common for *Prevotella oralis*.[4]

Children with human immunodeficiency virus infection seem to have an increased risk for development of renal abscesses from the more common traditional organisms[3] and from unusual opportunistic fungal organisms, especially *Aspergillus*.[12] As expected, these children also tend to have a more fulminant course that often requires extensive surgical intervention and drainage.

The presence of a renal abscess implies the destruction and liquefaction of tissue in a confined space. Two other infectious disorders of the kidney, xanthogranulomatous pyelonephritis and acute lobar nephropathy (acute focal bacterial nephritis), frequently are included in this general category, although technically, true abscesses do not always develop in these disorders. Acute lobar nephropathy may progress to renal abscess, however, if not treated appropriately.

Xanthogranulomatous pyelonephritis describes a more chronic form of severe renal parenchymal destruction that often is associated with chronic stone disease. The process may involve the whole kidney or may be focal. In children, the focal form occurs more commonly. The pathognomonic histologic finding is an accumulation of lipid-laden macrophages that coalesce into discrete yellow nodules. Small abscess cavities often are studded throughout the kidney. The organism most frequently recovered from the kidney is *Proteus*, and urinary calculi are common findings. Although these patients often have acute symptoms, the symptoms frequently are superimposed on more chronic manifestations, such as weight loss, failure to thrive, and anemia. Treatment is complete or partial nephrectomy because the renal destruction generally is severe.[19]

Acute lobar nephropathy describes a focal area of intense edema at the site of infection in acute pyelonephritis.[2] It usually is recognized as a mass effect on an initial screening renal ultrasonogram. Severe nephromegaly (renal length >3 standard deviations above the mean for age) is another finding on renal ultrasound suggestive of acute lobar nephropathy.[6] Computed tomography (CT) of the kidney shows poor uptake in the involved segment, but no well-defined liquefaction (Fig. 46–1). Whether this edema is just an exaggerated response to infection or represents a pre-abscess change is unknown. Klar and colleagues[11] described 13 children, 4 months to 8 years of age, with acute lobar nephropathy in a prospective study during a 4-year period. Bacteremia was documented in only one child. Evolution to abscess formation occurred in four (31%). In another prospective study, Cheng and associates[7] found bacteremia occurred in 4 of 80 children (5%) with acute lobar nephropathy. These lesions

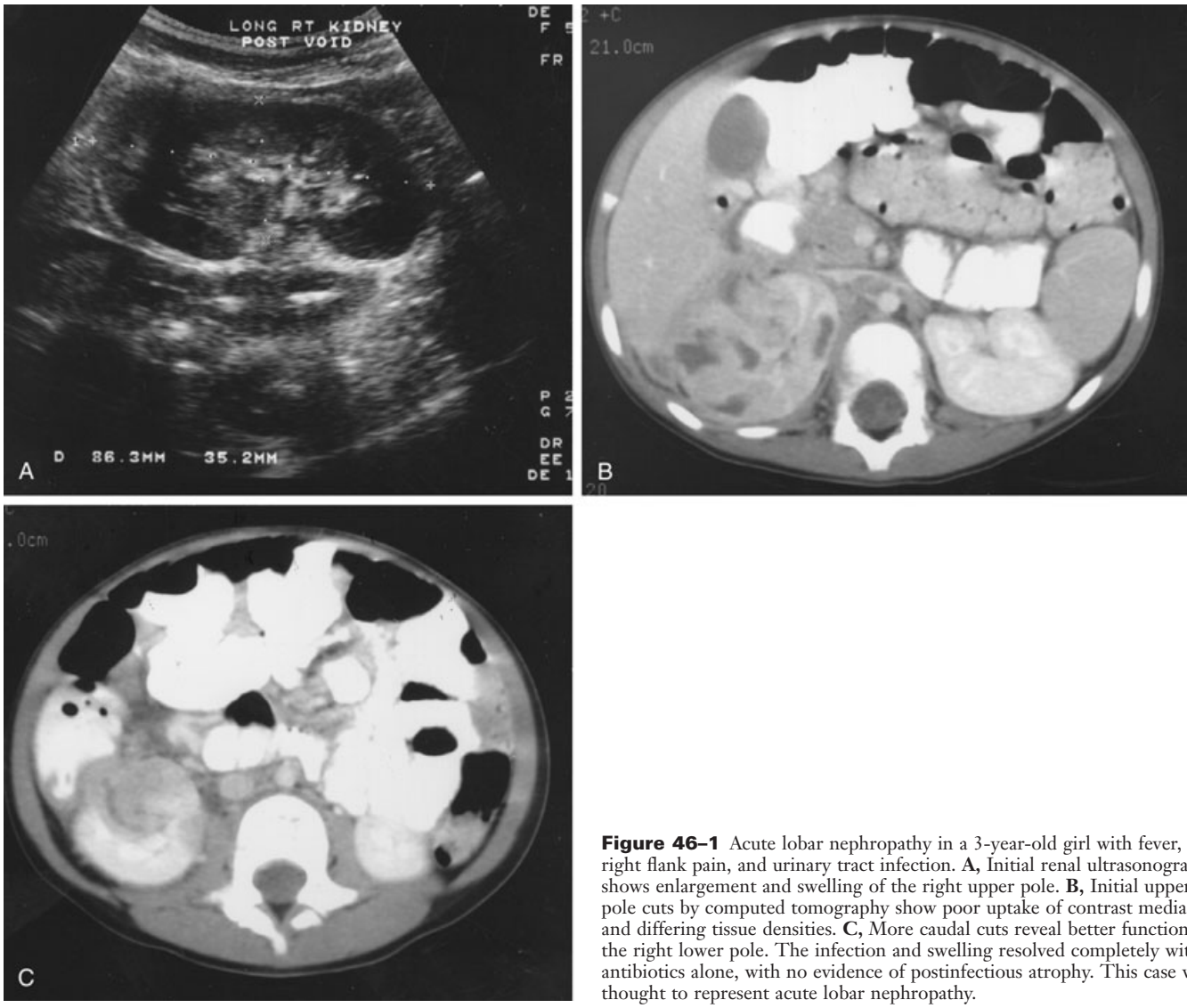

Figure 46–1 Acute lobar nephropathy in a 3-year-old girl with fever, right flank pain, and urinary tract infection. **A,** Initial renal ultrasonogram shows enlargement and swelling of the right upper pole. **B,** Initial upper pole cuts by computed tomography show poor uptake of contrast media and differing tissue densities. **C,** More caudal cuts reveal better function in the right lower pole. The infection and swelling resolved completely with antibiotics alone, with no evidence of postinfectious atrophy. This case was thought to represent acute lobar nephropathy.

generally heal satisfactorily with administration of antibiotics alone.

CLINICAL FINDINGS

Children with acute renal abscess are febrile and have localized pain in the costovertebral location. Prolonged fever is a common finding, especially in older children. Most patients are febrile for longer than 7 days before the diagnosis is established. The initial findings generally do not differentiate, however, between acute pyelonephritis with and without an abscess. If the abscess has spread into the perinephric region, one might be able to recognize psoas muscle irritation in the patient—that is, the patient is more comfortable with the ipsilateral leg in a position of flexion and experiences pain with full extension. A large abscess may be palpable as a flank mass.

Findings on urinalysis can be confusing. With a primary (hematogenous) abscess, the urine may be deceptively benign, and culture generally is negative.[14] Blood cultures may be positive, depending on the duration of the illness when blood is obtained for culture. Abscesses associated with underlying urologic disorders can be expected to contain organisms and pyuria. Generally, severe leukocytosis is present. The erythrocyte sedimentation rate typically is elevated; the same probably is true for C-reactive protein.[20] All these findings are nonspecific, however, and often further studies are needed to establish the diagnosis. If a more indolent process is encountered, one should consider performing appropriate staining and cultures for tuberculosis.

DIAGNOSTIC EVALUATION

All children admitted to the hospital with a febrile urinary tract infection should undergo renal ultrasonography as soon as is reasonable after admission. The findings on this initial study can have a significant effect on the choice of therapeutic options. If both kidneys are normal and unobstructed, organism-specific therapy is satisfactory in most patients. If significant obstruction or stones are present, antibiotic therapy may not be as effective, and interventional drainage may become necessary if the response to antibiotics is inadequate. At the time of this initial screening study, findings may suggest the presence of a renal abscess. Such findings include a mass effect within the margins of the kidney

along with a thickened wall and material of varying sonographic density within the mass (Fig. 46–2). Similar findings can be seen in infections accompanied by severe ureteropelvic junction obstruction or infundibular stenosis with isolated calyceal dilation, in which case purulent material within the dilated collecting system layers out and can mimic a renal abscess (Figs. 46–3 and 46–4).

When the diagnosis of an abscess is suspected on ultrasonography, performing CT of the involved kidney is in order.[9] CT more clearly defines the margins of the abscess, assesses whether loss of function is significant, and can screen the remainder of the kidney for small satellite abscesses.[16] If loss of function in the affected kidney seems to be significant, a dimercaptosuccinic acid renal scan should be done because it is an even more sensitive test to quantitate overall renal function. Currently, gallium 67 scintigraphy is not used frequently to diagnose an obscure inflammatory mass. If CT suggests the diagnosis, percutaneous aspiration can confirm clearly whether an abscess is present, without having to perform additional studies, and, at the same time, provide material for culture.

If a child with a urinary tract infection and a previously normal result on renal ultrasonogram is taking culture-specific antibiotics and a new fever subsequently develops, ultrasonography should be repeated. A previously small, unrecognized abscess

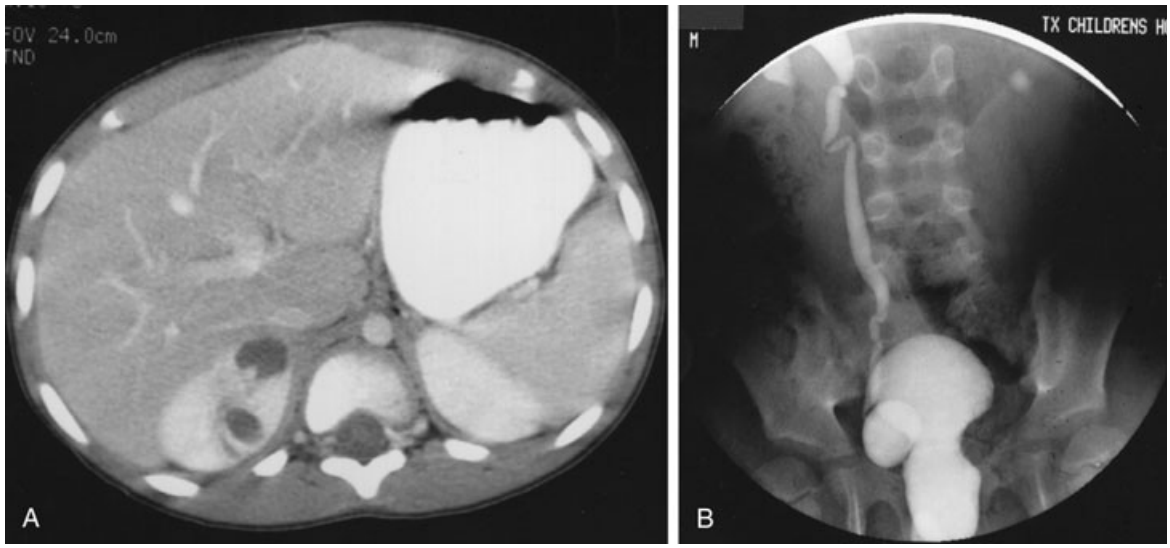

Figure 46–2 Renal abscess (secondary) in a 6-month-old boy with urinary tract infection. A screening renal ultrasonogram showed a small mass in the right kidney. **A,** Computed tomography shows two clearly defined cystic areas consistent with small parenchymal abscesses. Treatment consisted of intravenous antibiotics only. **B,** Voiding cystogram revealed the presence of vesicoureteral reflux.

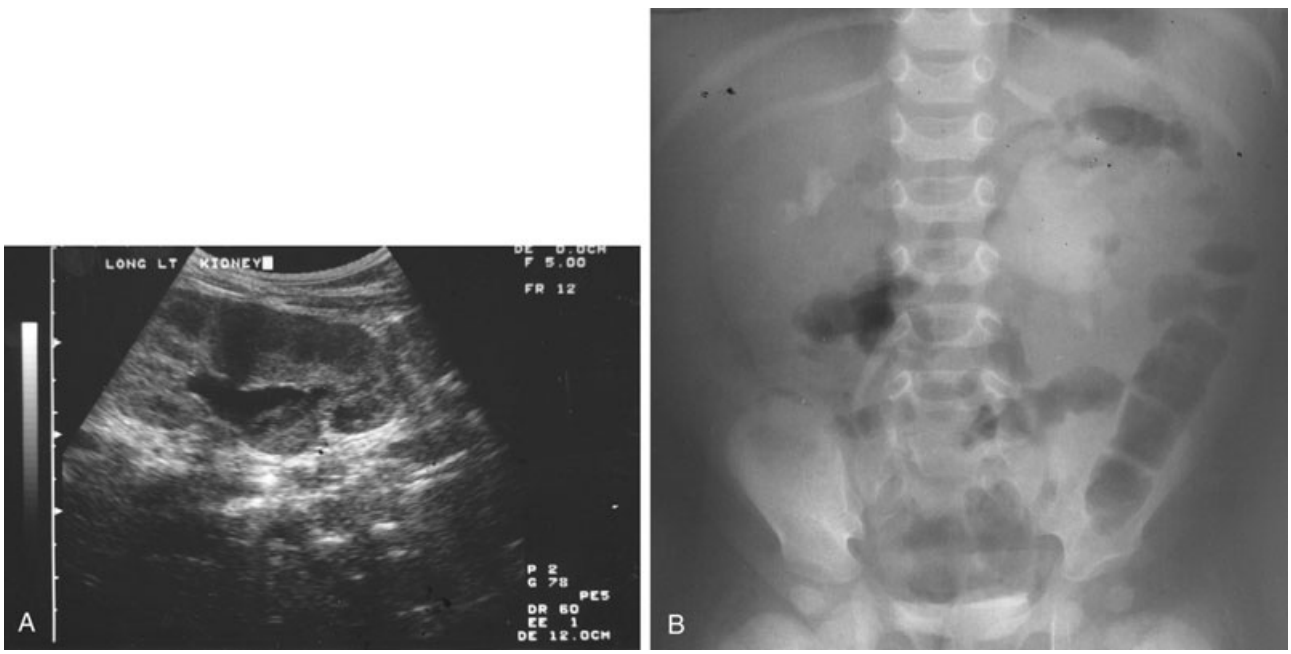

Figure 46–3 Pyonephrosis associated with ureteropelvic junction obstruction in a 3-month-old boy with fever, left abdominal and flank tenderness, and urinary tract infection. **A,** Renal ultrasonogram shows a medially placed cystic mass with layered fluids of different density consistent with purulent material. The position of the mass is most consistent with the renal pelvis. **B,** Intravenous pyelogram confirms ureteropelvic junction obstruction. This infant was treated initially with percutaneous nephrostomy drainage in addition to appropriate antibiotics.

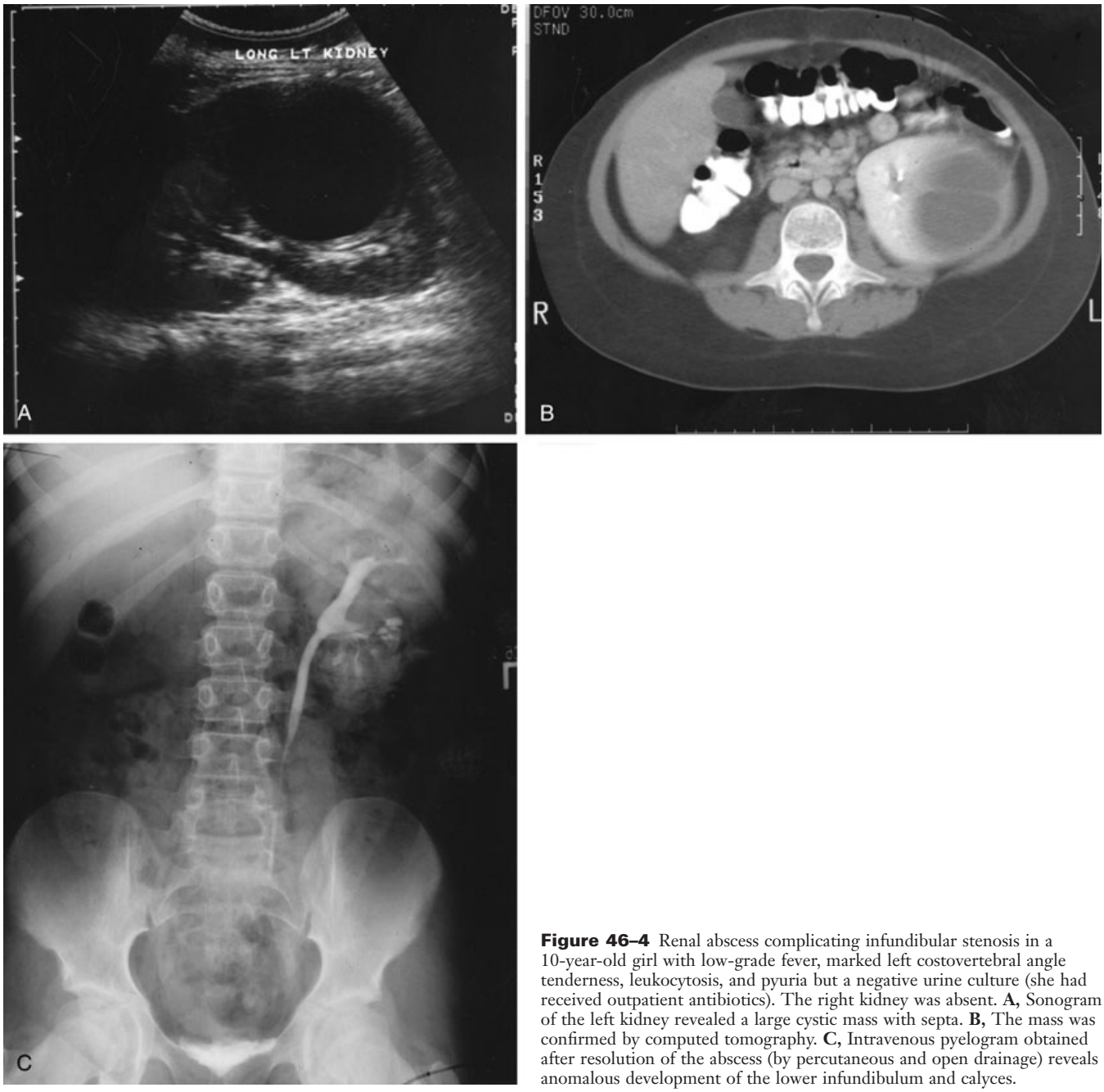

Figure 46–4 Renal abscess complicating infundibular stenosis in a 10-year-old girl with low-grade fever, marked left costovertebral angle tenderness, leukocytosis, and pyuria but a negative urine culture (she had received outpatient antibiotics). The right kidney was absent. **A,** Sonogram of the left kidney revealed a large cystic mass with septa. **B,** The mass was confirmed by computed tomography. **C,** Intravenous pyelogram obtained after resolution of the abscess (by percutaneous and open drainage) reveals anomalous development of the lower infundibulum and calyces.

may have been treated inadequately and now may be more apparent.

Occasionally, imaging studies cannot distinguish between a renal abscess and severe acute lobar nephropathy. The latter condition also may show tissues of differing density by ultrasonography or CT. In these situations, ultrasonography-guided or CT-guided percutaneous aspiration of the lesion may be necessary to determine whether purulent material can be obtained.

THERAPEUTIC CONSIDERATIONS

When the diagnosis of an abscess is confirmed, the choice of therapeutic options is dictated by several factors, including the overall status of the patient, the size and number of abscesses, whether the abscess appears to be unilocular or septate, associated uropathies, and the extent of renal function in the involved kidney. Included in these considerations is some insight into the responsible organism. If it is a solitary abscess in a child with unimpressive urinalysis findings and an otherwise normal kidney, one should suspect a staphylococcal abscess, which can be confirmed by percutaneous aspiration, Gram stain, and culture. If bacteremia caused by *S. aureus* possibly was nosocomial in origin, or if methicillin-resistant *S. aureus* isolates are found commonly in the community, antibiotics such as vancomycin or clindamycin should be included in the initial empiric regimen.

Subsequent therapy is based on the antibiotic susceptibility pattern of the organism isolated. If urinalysis reveals an obvious infection, one can assume that the urine culture reflects the organism responsible for the abscess. In this situation, empiric

therapy is directed against gram-negative enteric organisms. Cefotaxime, ceftriaxone, or ceftazidime plus an aminoglycoside would be suggested. The only caution is if the abscess is complicated by significant stone disease. In this situation, more than one organism may be in the urinary tract, and urine culture alone may be misleading. Percutaneous aspiration can identify accurately the organism responsible for the abscess. The optimal duration of antibiotic therapy for treatment of a renal abscess is not established, but 3 weeks of treatment is superior to 2 weeks for acute lobar nephropathy.[7]

Renal abscesses traditionally have been managed by open surgical drainage. With the introduction of safe percutaneous access, particularly when used with ultrasonography or CT guidance, single, unilocular lesions now can be managed effectively with percutaneous placement of an indwelling catheter that allows not only for primary drainage but also for irrigation of the cavity with appropriate antibiotic solutions.[1,11] Small renal abscesses have been treated successfully with antibiotics alone.[13,18] If the child does not improve with medical therapy alone, some type of drainage procedure is indicated. When function is insufficient to justify renal salvage and ultimate nephrectomy is planned, a short course of specific antibiotics and percutaneous drainage is initiated, if the abscess is large, to decrease inflammation in the perinephric tissues and to reduce the possibility of causing bacteremia at the time of surgery.

When required, surgical repair of associated congenital anomalies of the urinary tract generally is performed at a separate session after the abscess has resolved. Reconstructive surgery in the presence of active infection significantly increases the risk of development of surgical complications and the possibility that additional surgical procedures might be required.

CONCLUSION

Treatment of renal abscess today is multifaceted, and each case must be individualized. In addition to culture-specific antibiotics, treatment may include observation only, percutaneous drainage, or open surgical drainage. Diagnostic evaluation, including renal ultrasonography, CT, and percutaneous aspiration, generally can confirm that an abscess is present and assist in the decision regarding therapeutic options.

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CHAPTER 47

PROSTATITIS

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Prostatitis, a major source of chronic infection and symptoms in men, occurs rarely in prepubertal children. Although prostatitis may develop in postpubertal teenagers, even in this age group the diagnosis is recognized infrequently. The disorder commonly called prostatitis generally is divided into three separate clinical problems: acute prostatitis, chronic prostatitis, and prostatodynia.

Acute prostatitis is a severe infection generally associated with significant toxicity (high fever, elevated white blood cell count, systemic symptoms). Marked urinary symptoms, such as frequency, dysuria, and urinary retention, may be present. The urine usually is infected. On rectal examination, the prostate is enlarged, boggy (edematous), and exquisitely tender. Care must be taken when performing the rectal examination or when inserting a transurethral catheter because bacteremia can result.

The organism responsible usually is one of the gram-negative pathogens that commonly causes urinary tract infection (UTI). The virulence factors of *Escherichia coli* strains recovered from men with acute prostatitis and women with UTIs, such as adhesins and cytotoxins, were similar in one study.[13] Johnson and colleagues[6] found, however, that prostatitis *E. coli* isolates differed significantly from *E. coli* associated with cystitis or pyelonephritis by having a higher prevalence of the genes encoding P fimbriae structural subunits, P fimbriae assembly, fimbriae tip pilins, hemolysin, and cytotoxic necrotizing factor, and a lower prevalence of the gene encoding invasion of brain endothelium virulence factor. Rarely, *Trichomonas vaginalis* can cause prostatitis.

Treatment generally begins with administration of broad-spectrum parenteral antibiotics, such as cefotaxime, and aminoglycosides that are effective against gram-negative enteric organisms, pending the availability of culture-proven sensitivity results and a satisfactory clinical response. In patients older than 17 years of age, a fluoroquinolone also is recommended.

Chronic prostatitis is a more indolent infection associated with intermittent UTI, bladder irritative symptoms, and perineal discomfort. Ejaculation may be painful. Expressed prostatic secretions usually show an increase in the white blood cell count. Chronic prostatitis generally is recognized to occur in two different patterns: bacterial and nonbacterial. The clinical features of the two disorders are remarkably similar, including an increase in the white blood cell count in expressed prostatic secretions, but patients with chronic bacterial prostatitis usually also have recurring episodes of UTI with the same organism, most often one of the gram-negative pathogens.

The diagnosis of chronic prostatitis is related partly to detecting inflammation in expressed prostatic secretions. More precise methods of determining inflammatory parameters, such as counting the white blood cells per cubic millimeter with a hemocytometer, have been proposed.[14] In a large cohort study, the severity of symptoms in men with chronic prostatitis did not correlate with leukocyte or bacterial counts, however.[17] This study suggested that the symptoms associated with chronic pelvic pain syndrome were related to factors other than leukocytes and bacteria.

E. coli isolates associated with chronic prostatitis possess urovirulence profiles similar to those of strains from women with acute uncomplicated pyelonephritis.[1] In most studies, chronic nonbacterial prostatitis occurs more commonly than chronic bacterial prostatitis.

The cause of nonbacterial prostatitis remains enigmatic. Prostatic secretions do not show a common organism consistently, and UTI does not recur. Culture of prostatic secretions is notoriously unreliable, because of the means by which this material is collected; these secretions are contaminated easily as they pass through the urethra.[12] A specific causal relationship in nonbacterial prostatitis has not been found for either *Ureaplasma urealyticum* or *Chlamydia trachomatis*, two organisms commonly implicated in urethritis.[18] Molecular studies using polymerase chain reaction have shown DNA evidence of the presence of bacteria despite negative cultures for the typical bacteria associated with prostatitis.[5] Using these new techniques may lead to greater understanding of the role of infection in the pathogenesis of prostatitis.

Treatment of chronic bacterial prostatitis is frustrating because few patients truly are cured, and relapse occurs commonly after discontinuation of antibiotic therapy. Several studies have confirmed satisfactory concentrations of most common antibiotics in prostatic tissues,[5,8,10] but no study has provided a convincing explanation for the high treatment failure rate. Levofloxacin and other fluoroquinolones penetrate well into seminal and prostatic fluids and reach concentrations equivalent to corresponding plasma levels.[3] Researchers have postulated that antibiotic concentrations may be inadequate within the acini of the prostatic glands and their secretions, but this theory has not been proved.[15]

Normal prostatic secretions exhibit an antibacterial effect that is not present in men with chronic bacterial prostatitis. Several investigators have shown this factor to be free zinc or a zinc-based compound.[16] This antibacterial factor is depressed or absent in men with chronic prostatitis, but whether it causes or results from prostatitis is unknown. The most successful antibiotic therapy has been with trimethoprim-sulfamethoxazole or one of the fluoroquinolones.[9] In many instances, low-dose maintenance chemoprophylaxis with trimethoprim-sulfamethoxazole or nitrofurantoin is the most effective means of controlling symptoms.

A final population of patients have all the symptoms described in patients with chronic (bacterial) prostatitis but no history of UTI and microscopically normal prostatic secretions. To distinguish this group of patients from patients with prostatic infection, the term *prostatodynia* is used. The etiology of prostatodynia is unknown, but most investigators think that the disorder is a form of perineal and urethral muscle dysfunction with sphincter spasm, high voiding pressure within the prostatic urethra, reflux of urine into the prostatic ducts, and, ultimately, the development of chemical prostatitis.[4,11] Treatment generally consists of the use of alpha-adrenergic blocking agents to relax the bladder neck and proximal prostatic urethra and diazepam to relax the striated muscle of the perineum. Although most patients seem to experience some benefit, the results of therapy are difficult to quantitate, and underlying psychosocial issues are thought to be responsible for the symptoms of many patients. Psychological assessment and indicated treatment are essential parts of the total management of these patients.

In the older age group still seen in a pediatric practice, symptoms similar to those of the chronic form of prostatitis also might occur with urethritis. As noted earlier, *Chlamydia* and *Ureaplasma* spp. commonly are found in the urethral discharge in these patients[2] and are thought to be responsible for these specific symptoms. These organisms also have been implicated as a cause of epididymitis in young men. The organisms are transmitted sexually and are treated effectively with a tetracycline (minocycline or doxycycline) or a macrolide (erythromycin or azithromycin).[19] To prescribe these drugs empirically for sexually active young adults with lower tract irritative symptoms and clinical findings consistent with urethritis while awaiting culture results seems reasonable.

Nonspecific urethritis is the term commonly used for the infection in men with irritative voiding symptoms and a clear mucoid discharge and in whom culture of the discharge reveals a mixture of common perineal and gram-negative organisms but without a predominant organism thought to be responsible for the infection. Some of these patients likely have undiagnosed *Chlamydia* or *Ureaplasma* infection. Although specific treatment is empiric, a tetracycline or erythromycin seems to be a prudent first choice in most cases. Finally, one must not overlook the possibility of gonococcal urethritis.

In males, ectopic ureters can drain into the prostatic urethra or the seminal vesicle. With this anomaly, the ipsilateral renal parenchyma generally is dysplastic, the ureter is highly dilated, and the seminal vesicle may be distended markedly and form a large cystlike structure behind the prostate, the bladder neck, and the trigone. If this anomaly becomes infected, pain in the perineum and on rectal examination generally is significant. Occasionally, frankly purulent material is passed at the urethral meatus or expressed from the urethra after rectal examination.

In most cases, the kidney drained by the ectopic ureter is highly dysplastic and shows little or no function. Ultimate management usually consists of nephroureterectomy (ureteroneocystostomy if the kidney works) and, if the seminal vesicle is unusually large, partial excision and decompression of the seminal vesicle. Although these abnormalities are congenital and recognized easily by fetal ultrasonography, they can occur at almost any age if not identified before birth. Unless a palpable flank or lower abdominal mass is felt, infection usually is the initial symptom. Despite the severe degree of abnormality and ureteral and seminal vesicle dilation, the development of infection can be delayed well into the adult years. Infrequently, distortion at the bladder neck can result in obstruction to urinary flow.

SUMMARY

Prostatitis as it is seen and described in adults is a rare event in pediatric patients. When it does occur, it affects pubertal adolescents. Even in these patients, distinguishing true prostatitis from simple urethritis may be difficult.

Appropriate evaluation of an adolescent boy with a lower tract urinary infection generally begins with renal ultrasonography. Special attention should be directed to the region of the bladder and prostate. With this imaging modality, one can assess the presence and normalcy of both kidneys, the degree of thickening of the bladder wall, the ability of the bladder to empty, and whether any cystic masses are located behind the bladder or prostate. Any obvious abnormality justifies obtaining a voiding cystourethrogram. A thickened detrusor or recurrence of infection suggests the possibility of urethral obstruction. Although properly performed voiding cystourethrography does image the entire urethra, other diagnostic tests might include retrograde urethrography or determination of the urinary flow rate, especially if one suspects a urethral stricture. Performing cystoscopy usually is unnecessary if all imaging study results are normal, although cystoscopy may be used to confirm, and sometimes manage, obvious urethral obstruction.

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CHAPTER 48

Genital Infections

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This chapter reviews the clinical aspects of genital tract infections in children and adolescents. Genital infection in boys is limited to balanoposthitis. For details on other genital infections in boys, such as urethritis, epididymitis, and orchitis, refer to the other chapters in this section. Genital tract infections in premenarcheal girls are limited mostly to the vulva and vagina. The clinical presentations and management differ from those of similar infections in older patients. Genital infections in postmenarcheal girls include all the infections found in women; the symptoms accompanying them are similar to those in adults, and their treatment essentially is the same. Genital infections associated with pregnancy are not addressed in this chapter.

GENITAL TRACT INFECTIONS IN BOYS

Genital tract infections in boys are uncommon at any age but can be serious when they occur. Group A streptococcal proctitis and perianal skin infections have been reported.[154,180,261] Perianal pruritus, erythema, and tenderness occur commonly, and abdominal pain and rectal bleeding may be present. Penile involvement occurs rarely if at all.[261]

Balanoposthitis is a condition characterized by inflammation of the prepuce[210] and glans penis.[53] Causes of this condition have been described poorly in the literature; however, researchers have suggested that balanoposthitis results from trauma, irritation, or infection. A few cases of balanitis caused by *Streptococcus pyogenes* and *Staphylococcus aureus* have been reported.[53,60,69,105,160,205,210]

Balanoposthitis occurs more commonly in uncircumcised boys compared with circumcised boys. In one survey, the frequency of balanitis and penile irritation was determined in 272 uncircumcised and 273 circumcised boys.[124] Balanitis was defined as redness or swelling of the entire foreskin or glans, with or without pus, and irritation was defined as redness without swelling or pus. Six percent of uncircumcised and 3 percent of circumcised boys had balanitis. Four percent of the uncircumcised boys were found to have irritation alone compared with 1 percent of the circumcised boys. In another study examining penile problems in boys from birth to 8 years of age, penile inflammation

developed in 8 percent of circumcised and 14 percent of uncircumcised boys.[78]

The diagnosis of balanoposthitis is made on clinical grounds. A boy may complain of soreness, swelling, and discharge around the penis. Examination reveals redness, swelling, and discharge of the prepuce or glans penis, or both. The discharge originates from the area under the foreskin or around the glans penis and not from the urethra. Urethritis may be associated with urethral discharge, but it is not seen commonly. In a case series of 100 boys with balanitis, 100 percent had redness, 91 percent had swelling, and 73 percent had discharge on examination.[75] If an infectious etiology is suspected, Gram stain and culture of the discharge can be performed to determine the cause and guide therapy.

For nonspecific balanoposthitis, local therapy, including sitz baths, gentle cleansing, and application of hydrocortisone cream, is suggested.[244] In cases in which an infectious etiology is suspected, or local therapy has not been effective, a topical or oral antibiotic with coverage against *S. pyogenes* and *S. aureus* can be used.

Circumcision or a dorsal slit procedure may be necessary for recalcitrant or recurrent balanoposthitis. In a study reviewing the reasons for circumcision beyond the neonatal period, 23 percent of 476 boys underwent the procedure for recurrent balanoposthitis.[290] Aside from the need for circumcision, most cases of balanoposthitis seem to be uncomplicated. Cases resulting in necrotizing fasciitis and *Staphylococcus*-induced toxic shock have been reported, however.[53,132]

GENITAL TRACT INFECTIONS IN GIRLS

In this section on genital infections in girls, the normal vaginal microenvironment is reviewed first, followed by specific and nonspecific premenarcheal bacterial and fungal causes for vulvovaginitis. Issues of sexual abuse in children with genital tract infections also are addressed. The section on sexual abuse in children with genital tract infections addresses bacterial and viral sexually transmitted diseases (STDs) in sexually abused children. These sections are followed by postmenarcheal lower genital tract infections of the external genitalia, including clitoris and Bartholin ducts and glands, and bacterial, protozoal, and fungal vulvovaginitis. Vulvovaginal viral infections, granulomatous and ulcerative vulvovaginal disorders, infections of the cervix, and upper genital tract infections are reviewed for premenarcheal and postmenarcheal girls.

These sections provide information regarding infections by specific microbes and syndromes, including trichomoniasis, candidiasis, gonorrhea, syphilis, chlamydial infections, bacterial vaginosis, genital herpes, condyloma acuminatum, molluscum contagiosum, lymphogranuloma venereum, granuloma inguinale, chancroid, tuberculosis, Behçet syndrome, and vulvar vestibulitis, and infections caused by group A streptococci, *Shigella*, and other agents that occasionally cause genital tract disease. Additional information on the microbes can be found in the respective chapters.

Supplemental information concerning the principles of gynecologic infections in children and adolescents can be found in referenced sources.[22,28,29,62,73,129,234] Although the infectious complications of pregnancy are not discussed, pregnancy is a common occurrence in teenage girls. Pregnancy-related infection should be considered when confronted with a septic state in an adolescent girl.

NORMAL VAGINAL FLORA

The lower female genital tract is colonized by nonpathogenic bacteria from birth. Throughout life, this colonization is dynamic and complex. A wide range of aerobic and anaerobic species have been cultured from asymptomatic girls. The results of several modern series are summarized in Table 48–1.

Most girls harbor several organisms in the vagina at any given time. Vaginal specimens obtained for culture during anesthesia for elective surgery from 19 healthy girls aged 3 months to 5.7 years old yielded a mean of 12 bacterial species.[127] Anaerobes predominated, with a mean of 8.7 species versus 3.4 aerobic species. In a series of 25 asymptomatic girls aged 2 months to 15 years, a mean of 8.7 different species (approximately 4 aerobes and 5 anaerobes) per vaginal specimen were detected.[114] Another series of adolescents and young adults aged 13 to 21 years in which only aerobic flora were evaluated noted a mean of approximately three organisms in non-sexually active subjects versus six in sexually active patients.[249] This heterogeneity in vaginal microflora during childhood and adolescence is similar to that found in women.[164]

TABLE 48–1 Vaginal Organisms Isolated from Asymptomatic Girls 2 Months to 16 Years of Age

Organism	%*
Coagulase-negative staphylococci	35-73
Diphtheroids	14-78
Streptococcus viridans	13-39
Enterococci	29-62
Group B streptococcus	5-11
Group D streptococcus	
Staphylococcus epidermidis	
Staphylococcus aureus	
Streptococcus pneumoniae	
Micrococcus spp.	
Gaffkya (Aerococcus) spp.	
Lactobacillus spp.	10-39†
Escherichia coli	12-67
Klebsiella spp.	15-52
Enterobacter spp.	
Proteus spp.	3-5
Pseudomonas aeruginosa	5-6.5
Citrobacter spp.	
Haemophilus influenzae	
Neisseria spp. other than gonococci	
Moraxella (Branhamella) catarrhalis	
Flavobacterium spp.	
Alcaligenes spp.	
Acinetobacter spp.	
Mycoplasma hominis	
Ureaplasma urealyticum	
Gardnerella vaginalis‡	
Peptostreptococcus spp.	29-56
Peptococcus spp.	39-76
Veillonella spp.	
Eubacterium spp.	
Propionibacterium acnes	
Bacteroides fragilis	
Bacteroides melaninogenicus	
Other *Bacteroides* spp.	
Prevotella spp.	
Bifidobacterium spp.	
Clostridium perfringens	
Other *Clostridium* spp.	
Fusobacterium spp.	
Candida spp.	3-18
Other yeasts	
Actinomyces spp.	

**Percentage range when the organism was isolated from patients in at least two studies. If no range is present, the organism was isolated from 3 to 33% of patients in a single study.*

†In one series, 88% of girls were older than 11 years.

‡Isolated in numerous cases without discharge.

Data from references 72, 95, 114, 120, 127, 164, 165, 177, 230.

Different types of bacteria are isolated at various ages. The vagina and its microbial flora form an ecosystem that changes over time from infancy to childhood to adolescence and adulthood.[126] The major forces that influence these changes are fluctuations in estrogen levels and the advent of sexual activity. Hygienic practices and medications, including oral contraceptives and antimicrobial agents, also affect the complex interactions among the various flora present in the vagina in terms of persistence, predominance, and overgrowth. The vaginal flora of most healthy women is dominated by one or more *Lactobacillus* spp., but a substantial minority may harbor other predominant flora, including *Bifidobacterium*, *Gardnerella*, *Prevotella*, *Pseudomonas*, or streptococci, in the absence of lactobacilli.[135]

Gram-negative enteric bacteria and enterococci commonly are encountered in infants and toddlers before completion of toilet training, but less frequently thereafter.[114,127] Younger adolescents have a greater prevalence of anaerobic bacteria than do women. From puberty, aerobic colonization increases with age, onset of sexual activity, and parity.[165] Lactobacilli are the predominant flora in most girls by the end of adolescence and may play a protective role in limiting the overgrowth of other flora.[126] In children, lactobacilli are present more often in girls younger than 2 years than in older prepubertal girls. The increasing presence of yeast and *Gardnerella vaginalis* from puberty to adulthood, although much less common in all ages, parallels that of lactobacilli.[114]

Mycoplasma hominis and *Ureaplasma urealyticum* are present more commonly in sexually active adolescents and in girls of any age who have been sexually abused than in nonsexually active girls. Genital mycoplasmas were found in 17 percent of one series of young girls who were not known to have undergone any sexual abuse.[108,249]

Microbes that usually behave as commensals sometimes are associated with vulvovaginitis. The difference between colonization and disease is at least partially a function of the magnitude of the replication and the quantity of a given bacterial species. In women with bacterial vaginosis in which *G. vaginalis* is a predominant microbe, colony counts generally are greater than 10^7 colony-forming units/g of vaginal fluid. Asymptomatic colonization with *G. vaginalis* usually is associated with colony counts of less than 10^5 colony-forming units/g of vaginal fluid.[165] Alteration in the vaginal microenvironment by factors such as poor hygiene, foreign bodies, or hormonal fluctuations results in loss of environmental constraints on bacterial replication and facilitates the overgrowth of one or more commensals.

When bacterial vaginosis occurs, typically the numbers of lactobacilli, which produce hydrogen peroxide, are decreased. When such a decrease occurs, catalase-negative microbes, such as *G. vaginalis*, *Mobiluncus* spp., and other anaerobes, can increase in number. Lactobacilli probably have mechanisms other than hydrogen peroxide production that may help restrain the growth of other microbes present on the surfaces of the lower genital tract.[165,258]

PREMENARCHEAL VULVOVAGINITIS

Infections and inflammation of the vulva and vagina account for 85 to 90 percent of all genital problems in premenarcheal girls. These conditions are encountered most commonly in children 2 to 7 years old.[213,226] Infections of the vulva and vagina usually occur together and generally are discussed as one entity, vulvovaginitis. The various types of vulvovaginitis are differentiated by determining the presence or absence of specific agents associated with the inflammation in a particular case.

Genital discharge and perineal or vulvar discomfort are the most common symptoms that bring a child with vulvovaginitis to the physician.[133,213] The discomfort may be only minor pain or soreness, or it may be intense perineal burning or pruritus. Genital erythema is the most common sign in girls with vulvovaginitis and is noted in more than 80 percent of cases. Visible discharge is present in one third of cases.[213] The discharge may be scanty serous fluid, bloody, or profuse and purulent. Infections of the vulva and vagina are more likely to be accompanied by moderate or severe inflammation and prominent discharge than is nonspecific vulvovaginitis.[142] Infection can be present at times, however, with neither discomfort nor discharge.

In a series of 80 prepubertal girls aged 2 to 12 years old with vulvovaginitis and no suspicion of sexual abuse, probable bacterial pathogens were found in cultures of 36 percent. Group A streptococci, *S. aureus*, and *Haemophilus influenzae* (non–type b) were the most common isolates.[266] In another series of 74 girls, group A streptococci and *H. influenzae* were isolated in 47 percent and 12 percent. Most of these girls had had symptoms for 7 days or less at the time treatment was sought.[51] These bacteria, especially group A streptococci, causing vulvovaginitis may lead to more severe symptoms that result in care being sought soon after onset, whereas pathogenic bacteria generally are less likely to be found when girls present for care after several weeks of symptoms.

Genital discharge does not always indicate infection or inflammation. Most female infants have a grayish white, mucoid discharge from the vagina during the newborn period. This discharge consists of desquamated vaginal mucosa and cervical epithelium that has undergone hypertrophy because of prenatal stimulation by placental and maternal hormones. Microscopic examination of the material reveals masses of large, superficial vaginal epithelial cells (Fig. 48–1). The condition may last for several weeks, is not pathogenic, and does not require treatment. Urinary leakage from an ectopic ureter opening into the genital tract may mimic a vaginal discharge.

A pubertal girl nearing menarche may have a copious viscous, transparent secretion that fills the vagina, bathes the vulvar tissues, and soils underclothing. The parents of such a girl may be concerned that she has a vaginal infection. In this case, the vulvar and vaginal tissues are thick and moist, a sign of increased estrogen stimulation. Microscopic examination of the vaginal fluid reveals masses of estrogenized superficial vaginal epithelial cells and few leukocytes (Fig. 48–2). Test results for pathogenic bacteria are negative. The parents should be reassured that the girl does not have an infection. Frequent bathing and changes of underclothing are all that is needed.

Poor hygiene with subsequent overgrowth of a mixed aerobic and anaerobic bacterial flora is the most common cause of pre-

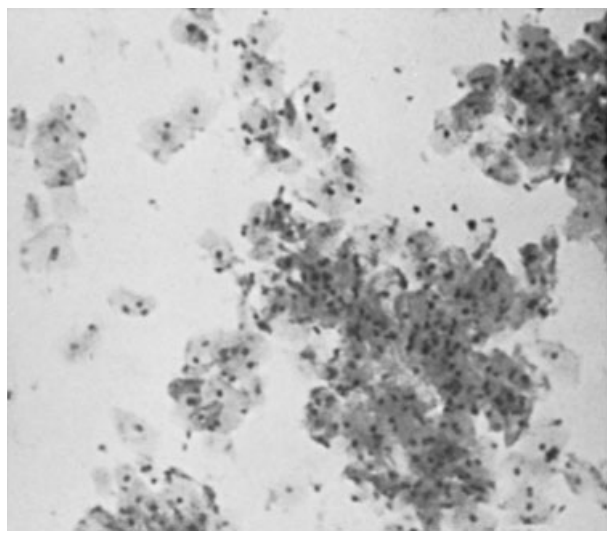

Figure 48–1 Cytosmear from the vaginal discharge of a newborn.

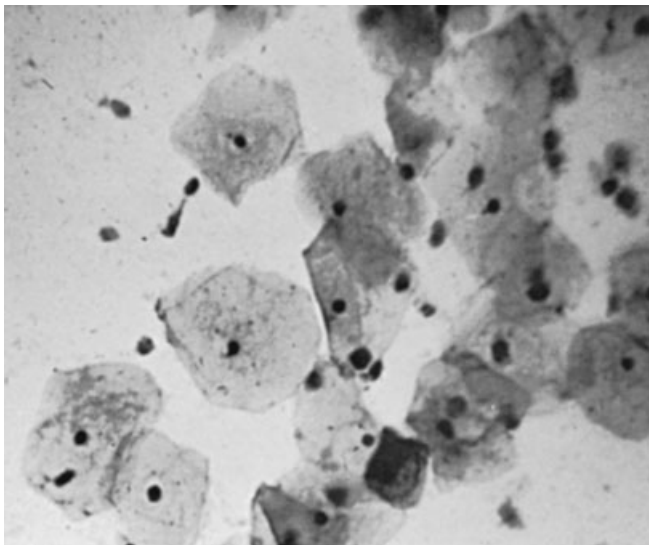

Figure 48–2 Cytosmear from the vaginal secretion of a pubertal girl. Masses of epithelial cells and few bacteria or leukocytes attest to its nonpathologic character.

menarcheal vulvovaginitis.[209] Inflammation of the lower genitourinary tract also may be caused by a variety of specific microorganisms, chemicals, and other physical agents. Contact irritation and allergic reactions induced by soaps, detergents, and medications are frequent causes of vulvovaginitis. Some systemic diseases and focal skin disorders may mimic vulvovaginitis or allow it to develop as a secondary process when these conditions involve the vulvar or perineal tissues.[80,90] Anatomic abnormalities also may be associated with vulvovaginitis. An increased incidence of vulvovaginitis and urinary tract infections has been reported in girls with high posterior commissures.[287] Drainage from an ectopic ureter into the vagina can cause chronic vulvovaginitis and lead to the formation of vaginal calculus. Labial adhesions were present in 7 percent of girls with vulvovaginitis in one series.[213] Table 48–2 outlines the causes of vulvovaginitis in premenarcheal girls.

The history should include information about the manner and circumstances surrounding the onset of the complaint, the characteristics of any discharge, the duration of symptoms, whether the problem is recurrent, and the nature of any recent treatments or medications. Information should be elicited regarding the use of bubble baths, enuresis, history of atopic dermatitis or allergies, anal pruritus (associated with pinworm infection), recent respiratory or skin infections in family members, and hygienic practices. The possibility that the child may have had or has a systemic disorder affecting the genitalia should be explored.

Sexual abuse must be considered when a child has a genital infection, regardless of the nature of the infection or the socioeconomic status of the family. This concern is particularly relevant when the child has a sexually transmissible disease, but any infectious organism can be transmitted to the genitalia by digital contact. Sexual abuse does not require penile penetration of the vagina. The history of vaginal or penile discharge in other family members should be ascertained. Behavioral changes, nightmares, fears, abdominal pain, headaches, and enuresis can be indicators of abuse or other psychosocial stressors.

The characteristics of the discharge frequently do not help identify a specific cause. At the onset of an infection, the discharge likely is thick, purulent, and profuse. It may become scanty and seropurulent in later chronic stages of infection.[133] Discharges that are odorless and bloody or serosanguineous may result from noninfectious conditions, such as vulvar irritation, trauma, precocious puberty, foreign body, urethral prolapse, or tumor. Vulvovaginitis caused by *Shigella* or group A streptococci also can cause bleeding, as can an eroding foreign body. Foul-smelling discharge suggests a foreign body but also may result from a necrotic tumor. Specific diagnoses are made more often when symptoms have been present for less than 1 month.[279]

A general physical examination should precede assessment of the genital tract. The gynecologic examination begins with abdominal inspection and palpation followed by external examination of the perineum and genitalia, including the vulva, urethral meatus, clitoris, hymen, and anus. The examination should be done with the child in a supine, frog leg position. The labia can be retracted gently to visualize the anterior of the vagina. Speculum and bimanual examinations generally are inappropriate in prepubertal children.[214] Note should be made of structural abnormalities, inflammation, sores and ulcerations, and excoriations. If complaints consist only of vulvitis, and findings on external examination are limited to a scanty mucoid discharge and an erythematous introitus, further examination generally is unnecessary.[72]

If the vaginal discharge is purulent, persistent, or recurrent, a thorough gynecologic assessment is warranted. Visualization of the vagina and cervix without instrumentation is possible with the child in the prone knee-chest position.[74] This method is useful in children older than 2 years. In this position, with labial traction, the vaginal muscles relax and stretch the hymenal membrane open. An otoscope head or a magnifying lens with a good wall light is used to visualize the cervix. Because the vagina of a prepubertal child is short, a foreign body or a lesion may be seen.[71]

A rectal examination may be important when persistent discharge, bleeding, or pelvic or abdominal pain is present. The rectal examination may help express discharge from the vagina that previously was not recognized and can permit palpation of hard foreign bodies or abnormal masses.

Visualization with instrumentation (vaginoscopy) is required in some situations. General anesthesia may be required for girls who are small or unable to cooperate, or who may experience significant anxiety or discomfort during the procedure. Conscious sedation for the procedure may be an option in some situations. When a purulent, persistent, or recurrent vaginal discharge is present, samples should be obtained for culture and Gram stain. Gram stain of exudates may provide information rapidly that points toward a specific pathogen and may indicate the need for additional evaluations or interventions. Wet mount preparations may be useful if fungal infection (potassium hydroxide preparations) or pinworms are suspected. Saline wet mount may be indicated for trichomoniasis if sexual abuse is suspected. Urinalysis with microscopic examination should be performed. A complete blood cell count may be useful when pyogenic infection is suspected or bleeding has occurred.

When cultures are indicated, separate vulvar and vaginal specimens may be necessary. The type and duration of discharge and considerations of potential sexual abuse influence this decision. Vulvar specimens alone may be appropriate when etiologies such as group A streptococci are suspected. Exudates emanating from the vagina in prepubertal girls also are adequate specimens for evaluation for gonorrhea, such that vaginal swabs or aspirates are not required. Further information on types of specimens required for specific sexually transmitted infections such as gonorrhea or *Chlamydia trachomatis* is provided in Chapters 100 (gonorrhea) and 206 (*C. trachomatis*).

Options for obtaining vaginal specimens in young girls, when needed, include use of (1) a nasopharyngeal Dacron swab moistened in nonbactericidal saline, inserted carefully through the hymenal opening; (2) a catheter-within-a-catheter technique (Fig. 48–3)[214]; or (3) a sterile newborn suction catheter, with insertion 2 to 3 cm into the vagina.[266] For the catheter-within-a-

TABLE 48–2 Etiologic Factors in Premenarcheal Vulvovaginitis

Bacterial Infections
Nonspecific mixed infections secondary to
- Poor perineal hygiene
- Foreign body in vagina
- Respiratory tract infections
- Skin infections (impetigo)
- Urinary tract infection

Specific nonvenereal infection
- Hemolytic streptococci (groups A, B, F)
- *Escherichia coli*
- *Shigella flexneri*, *Shigella sonnei*
- *Neisseria meningitidis*, *Neisseria sicca*
- *Haemophilus influenzae* type b, nontypeable strains
- *Streptococcus pneumoniae*
- *Corynebacterium diphtheriae*
- *Yersinia enterocolitica*
- *Mycobacterium tuberculosis*
- *Moraxella (Branhamella) catarrhalis*
- *Staphylococcus aureus*

Specific venereal infections
- *Neisseria gonorrhoeae*
- *Treponema pallidum*
- *Chlamydia trachomatis*
- Chancroid *(Haemophilus ducreyi)*
- Granuloma inguinale

Bacterial vaginosis
- *Gardnerella vaginalis*
- *Mobiluncus* species

Fungal Infections
Candida albicans
Other yeasts
Dermatophytes

Protozoan and Parasitic Infections
Trichomoniasis
Amebiasis
Enterobius vermicularis
Hirudiniasis
Schistosomiasis
Other parasitic infections (ascariasis, trichuriasis)

Viral Infections
Venereal
- Herpes simplex
- Condyloma acuminatum (papillomavirus)
- Molluscum contagiosum

Involvement as part of systemic infection
- Measles
- Varicella
- Mononucleosis (Epstein-Barr virus)
- Coxsackievirus
- Smallpox

Infestations
Pediculosis
Scabies

Contact Irritation or Allergic Reactions
Bubble bath preparations
Hair shampoos
Vulvar deodorant sprays
Soaps, laundry detergents
Other medications

Vulvar or Perineal Skin Diseases
Local
- Seborrhea
- Lichen sclerosus et atrophicus
- Lichen planus
- Lichen simplex chronicus
- Premalignant leukoplakia
- Erythrasma (*Corynebacterium minutissimum*)
- Bartholinitis
- Skenitis

Involvement as part of a systemic disorder
- Psoriasis
- Bullous pemphigoid
- Atopic dermatitis
- Drug eruption
- Generalized pruritus with excoriation
- Chronic liver disease
- Chronic renal disease
- Metabolic errors
- Psychosomatic
- Crohn disease
- Sjögren syndrome
- Henoch-Schönlein purpura
- Histiocytosis
- Kawasaki disease
- Stevens-Johnson syndrome
- Typhoid
- Zinc deficiency

Physical Factors
Sand (sandbox)
Chemical or thermal trauma
Physical trauma (accidents, abuse, masturbation)
Nylon, rayon underclothing
Tight garments (maceration in warm climates)
Anatomic abnormalities
- Neoplasms (sarcoma botryoides)
- Polyps
- Labial agglutination, adhesion
- Prolapsed urethra
- Ectopic ureter
- Rectal fistula
- Draining pelvic abscess via fistula

Data from references 11, 12, 72, 80, 81, 134, 163, 183, 250.

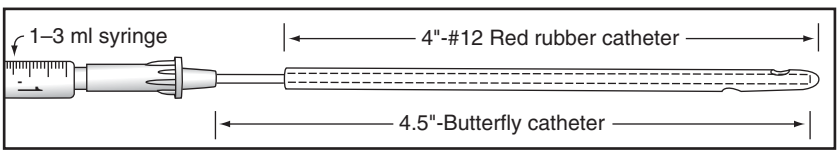

Figure 48–3 Assembled catheter-within-a-catheter for obtaining specimens from a prepubertal child. *(From Pokorny, S. F., and Stormer, J: A traumatic removal of secretions from the prepubertal vagina. Am. J. Obstet. Gynecol. 156:581, 1987.)*

catheter technique, the distal 4 inches of a soft, size 12 bladder catheter and the proximal 4 inches of butterfly needle intravenous tubing are excised from their parent devices by sterile technique. The catheter-within-a-catheter is inserted into the vagina, and fluid is flushed in and out of the upper part of the vagina several times before final aspiration into the syringe and removal of the device.

Nonspecific Vulvovaginitis

Nonspecific vulvovaginitis is identified by vaginal cultures that yield a growth of mixed bacteria not related etiologically to a specific disease. Nonspecific vulvovaginitis is the premenarcheal genital disorder most frequently encountered[133] and is responsible for 25 to 75 percent of cases of vulvovaginitis diagnosed in

this age group in referral centers.[279] In most cases, as noted in Table 48–2, identifiable secondary factors contribute to nonspecific vulvovaginitis.

Several factors other than those listed in Table 48–2 contribute to the occurrence of vulvovaginal infections in young children. The developing immature labia minora and majora flare outward as a young girl squats or sits. As a result, they do not protect the vestibular and vulvar mucosae from contamination, as occurs later in life. The nonestrogenized, prepubertal vulvar and vaginal epithelium, which consists of only a few layers of cells, is traumatized easily and infected readily; however, no evidence suggests that estrogen deficiency is a causative factor in premenarcheal vulvovaginitis. The alkaline vaginal reaction during childhood also is not as resistant to infection as is the acidic vaginal secretion of postmenarcheal girls and women. Because children frequently do not cleanse themselves properly after defecation, the perineum and vulva often are contaminated by fecal material. Nonabsorbent nylon garments or other tight-fitting clothes can lead to maceration, which predisposes to the development of infection.

VULVOVAGINITIS SECONDARY TO POOR PERINEAL HYGIENE

A vulvovaginal infection is considered to be secondary to poor perineal hygiene when bacteria native to the lower gastrointestinal tract are found in properly obtained cultures from the vagina. In a large series of cases of vulvovaginitis subjected to culture, *Escherichia coli* or other coliform organisms were found in 70 percent of patients in the series.[120] Other studies also have found a higher prevalence of *E. coli* in vaginal cultures from girls with vulvovaginitis than in asymptomatic controls.[72] The reappearance and disappearance of premenarcheal vulvovaginitis secondary to poor perineal hygiene are related directly to the appearance and disappearance of coliform organisms in vulvovaginal cultures. The primary role of vulvar and vaginal contamination with fecal material as a result of inadequate cleansing after defecation is supported by the observation that symptoms resolve in most cases when proper perineal hygiene is the only treatment recommended. Fifteen to 20 percent of children have recurrences, usually 1 month or more after resolution of the initial episode. In most instances, recurrences can be attributed to poor perineal hygiene.[120]

Children with nonspecific vulvovaginitis secondary to poor perineal hygiene do not have any uniform historical findings. If asked, the parent may be able to describe the way that the child cleanses herself after defecation. Many children wipe themselves from back to front after defecation. In girls, this practice easily results in fecal contamination of the vulvar area.

On examination, the vulvar mucosa and outer third of the vagina usually are hyperemic and covered with a scant, light gray mucoid discharge. Frequently, a clue to the cause of the infection is fecal soiling around the anus or on the perineum. Inspection of the undergarments may show fecal material in the area that comes in contact with the vulva, unless, as often is the case, the girl has been bathed and dressed in clean clothes before visiting the physician.

Instructing parents regarding perineal and vulvar cleansing when girls are bathed decreases the likelihood that nonspecific vulvovaginitis will develop in their daughters. Young girls should be taught proper hygiene and that they should wash their hands before and after urinating and defecating. Routine inspection of the perineum, vulva, hymen, and clitoris should be a part of routine physical examinations, including well-child checkups.[133]

Proper cleansing of the perineum and anus after defecation and sitz baths leads to the resolution of symptoms and infection in most cases. Sitz baths (warm water with or without Aveeno colloidal oatmeal or baking soda) 10 to 15 minutes in duration should be taken two to six times per day, depending on the severity of the vulvovaginal inflammation.[72] For intense, oozing inflammation, wet compresses with Burow's solution (1:40) or plain water applied every 3 to 4 hours may be used instead of sitz baths.[11] In mild cases, the vulva may be washed twice a day with water or a mild, unscented, nonmedicated soap (e.g., Basis, unscented Dove, Neutrogena). The perineum should be patted dry gently after baths or treatments. Complete drying may be facilitated by sitting for 10 minutes with the legs spread apart. Urinating with the labia and legs spread apart to minimize urinary reflux into the vagina may be helpful. Witch hazel pads (Tucks) may be used for cleansing after defecating and to provide mild analgesia.[252]

White cotton underpants, changed frequently to absorb discharge, and loose-fitting clothing should be worn for several days to a few weeks after the symptoms resolve. Continued wearing of such clothing also may be helpful in preventing recurrences of vulvovaginitis, especially in girls living in warmer climates.

As the inflammation and exudate subside over 1 to 2 days, sitz baths may be reduced in frequency and alternated two to four times a day with the application of either calamine lotion or protective ointments, such as zinc oxide, Desitin, Vaseline, and A and D ointment. If pruritus is a significant symptom, an oral agent such as hydroxyzine hydrochloride (Atarax) or diphenhydramine hydrochloride (Benadryl) may be administered. Topical application of 1 percent hydrocortisone cream or triamcinolone acetonide (Mycolog) cream may be used as the inflammation resolves but should be avoided in the acute phase.[252]

Shampooing the hair while sitting in a bathtub and using harsh soaps, bubble baths, or other preparations that might lead to chemical irritation of the vulvar skin and vaginal mucosa should be avoided throughout the course of vulvovaginitis.[19,32,39] The application of powders should be avoided, at least until the acute symptoms have resolved.

Patients who do not improve after 2 to 3 days of hygienic measures should be re-evaluated. Specimens taken from the vagina should be sent for aerobic and anaerobic bacterial cultures, if these were not done initially. An intravaginal triple-sulfa medication such as Sultrin vaginal cream, which consists of sulfathiazole, sulfacetamide, and sulfabenzamide, may be given.[2,90] Approximately 1 mL is inserted into the vagina with a 5-mL Luer syringe each night for 7 nights (the applicator that comes with the tube of cream is too large to insert into the immature vagina). A 5-cm piece of 12 or 14 French urethral catheter attached to the syringe facilitates application of the cream, if the patient is cooperative (Fig. 48–4). Parents must be warned against and instructed on how to avoid inserting the cream into the child's urethra and bladder.[120] Alternatively, the vagina may be irrigated with a 1 percent povidone-iodine (Betadine) solution with this same method and caveat.[72]

Intractable nonspecific vulvovaginal infections that are not caused by foreign bodies, intestinal parasites, or poor perineal hygiene are encountered occasionally. They are resistant to hygienic measures and topical antibiotics. Reducing vaginal pH from an alkaline or neutral to an acid reaction often helps in these difficult cases and may be achieved either by the local use of estrogen or by daily flushing of a solution of 1 mL of lactic acid (USP) in 250 mL of tap water into the child's vagina with a 10-mL Luer-type syringe and a section of a rubber or plastic urethral catheter, as shown in Figure 48–4.[120]

Estrogens cause thickening of the thin prepubertal vaginal mucosa, which reduces vaginal pH. These events generally are therapeutic. Estrogens are not recommended for the treatment of routine cases of premenarcheal vulvovaginitis for two reasons: the results do not seem to be superior to nonhormonal therapies in these cases,[120] and prolonged administration of topical estrogens may cause isosexual pseudoprecocity. For intractable cases, estrogen should be applied topically and not orally. A globule of estrogen cream (Premarin vaginal cream) measuring not more

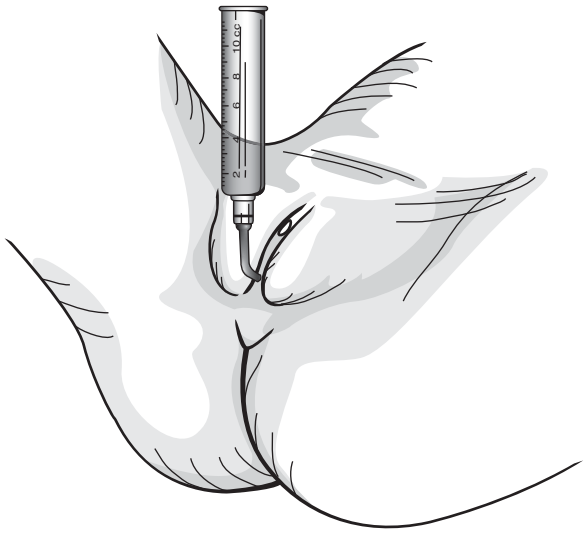

Figure 48–4 The barrel of a Luer-type syringe and attached piece of urethral catheter are used for vaginal lavage in an older child with a vaginal infection.

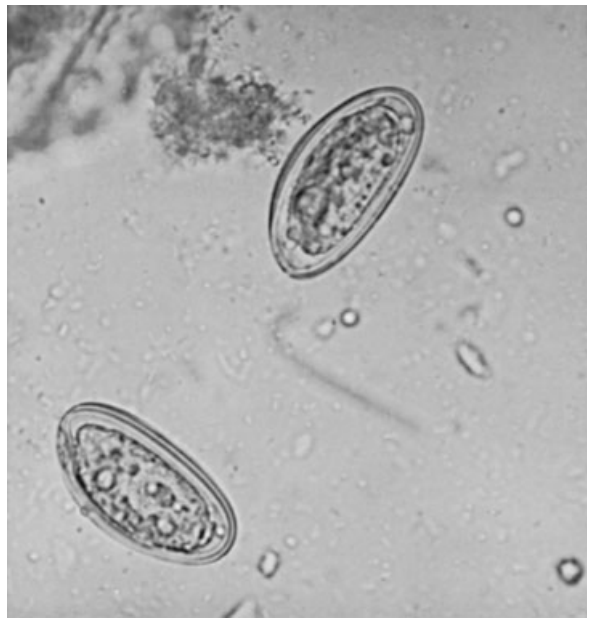

Figure 48–5 Pinworm ova discovered in a vaginal smear from a child with intractable vulvovaginitis.

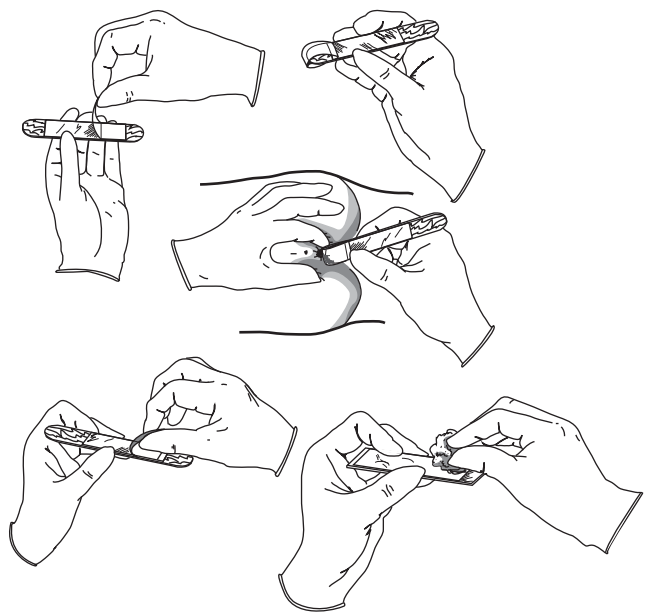

Figure 48–6 Technique for obtaining a perianal smear for detection of pinworm ova, using Scotch tape and a tongue depressor.

than 5 mm in diameter should be rubbed gently onto the inner surfaces of the labia minora and external surface of the hymen. This procedure is repeated daily for 2 to 4 weeks. After 7 to 10 days, the vulvar and vaginal tissues usually thicken, and a mucoid vaginal secretion may appear. The parents must understand clearly that the cream must be used for no longer than instructed. The cream should be discontinued if thelarche occurs.

Oral or parenteral antibiotics may be indicated if symptoms persist for 2 to 3 weeks, or if a specific pathogen that requires antibiotic treatment is isolated. When possible, selection of antibiotic agents and the route of administration should be based on susceptibility testing of organisms isolated from vaginal cultures. Nonspecific vulvovaginal infections are benign, superficial, localized mucosal inflammations that usually respond to less potent chemotherapeutic agents when they are needed. Many antibiotics are absorbed through the vaginal mucosa. The indiscriminate use of vaginal, oral, or parenteral antibiotics may result in the child becoming sensitized to them.

NONSPECIFIC VULVOVAGINITIS SECONDARY TO INTESTINAL PARASITES

Pinworms (*Enterobius vermicularis*) are the causative factor in many cases of recurrent or intractable nonspecific vulvovaginitis in children.[72] Infection occurs when the worms in the lower bowel crawl out of the anus onto the perineum and migrate into the vagina, where they deposit ova. The pinworms may carry *E. coli* and other coliform bacteria into the vagina, which may lead to vulvovaginitis. Other intestinal parasites seldom invade the vagina, although in one case *Ascaris lumbricoides* was discovered in the vagina of a child.[133]

Infestation with pinworms occurs commonly and does not indicate poor hygiene. Pinworm ova may be deposited in playground soil, on toys or books, and on the hands of infected individuals. Infection frequently is asymptomatic.

A child with a pinworm infection and vulvovaginitis usually has a history of a chronic vaginal discharge that has recurred despite repeated attempts to eradicate it. Parents may note worms on the perineum of the child and that she awakens at night because of perineal itching. Other family members or the girl herself may have had pinworms previously.

Examination reveals a low-grade inflammation of the vulva and vagina. Excoriations from scratching may be seen on the perineum. Vaginoscopy (if indicated) shows an inflammatory reaction extending to, but not including, the cervix. Vaginal cultures produce a mixed growth of nonpathogenic bacteria, with *E. coli* and other coliform bacteria generally predominating.

The diagnosis depends on finding pinworm ova on smears from the perineum or in the vaginal discharge or a report from parents that worms are visible on the child's perianal skin. A perineal smear is most likely to show the presence of pinworms, but pinworm ova may be detected in a wet smear of vaginal secretions (Fig. 48–5).

When a perineal smear for the ova of *E. vermicularis* is to be obtained, the parent is given a wooden tongue blade, a piece of Scotch tape, and a glass microscopic slide. The tape is attached to the tongue blade, adhesive side out (Fig. 48–6), and applied

firmly to several areas around the anus. It is removed and applied, adhesive side down, to the glass slide, which is sent to the laboratory for examination.

The type of discharge, the appearance of the vaginal mucosa, or the presence or absence of pruritus does not aid in establishing a diagnosis. A history of a previous pinworm infection in the patient, a member of the family, or a playmate is significant. Pinworms should be suspected when a child has recurrent episodes of intractable nonspecific vulvovaginitis.

Treatment consists of eradicating the pinworms. All members of the family are presumed to be infected and must be treated. Several highly effective drugs are available for this purpose; among them are pyrantel pamoate, given as a single dose of 11 mg/kg, not to exceed 1 g, and mebendazole, 100 mg given orally as a single dose. Either treatment should be repeated after 2 weeks. Three negative perianal smears, taken at weekly intervals, should be obtained before one can assume that the worms have been eradicated. The vulvovaginitis caused by coliform organisms carried on pinworms is treated the same as other cases of nonspecific vulvovaginitis caused by poor perineal hygiene (see earlier).

NONSPECIFIC VULVOVAGINITIS SECONDARY TO VAGINAL FOREIGN BODIES

Foreign bodies account for approximately 4 percent of cases of vaginal discharge in premenarcheal girls.[11] When a foreign body remains in the vagina for some time, it inevitably causes nonspecific vulvovaginitis. Many types of vaginal foreign bodies, including safety pins, glass beads, coins, beans, bits of crayon, and parts of toys, have been reported. The most common objects are bits of toilet paper or shreds of cloth from nightclothes or bedding (Fig. 48–7).[122,134]

The history does not contribute to the diagnosis unless the child has a record of having put objects in her vagina previously. Usually, the parent is unaware that the child has inserted something into her vagina. The child is brought to a physician because of a profuse, foul-smelling, sometimes blood-tinged discharge. The presence of such a discharge is almost pathognomonic for the presence of a foreign body. Even without a profuse discharge or bleeding, a foul odor from the vagina suggests a foreign body.

Examination reveals inflammation of the vulvar and vaginal mucosa. Although foreign material may be seen when the labia are separated, vaginoscopy often must be performed to explore the full length of the vagina. Rarely, a metallic object that has been in the vagina for some time erodes the mucosa and becomes hidden in granulation tissue. When such a condition is suspected, a radiograph should be obtained. Most foreign bodies, such as glass, plastics, paper, or cloth, are not radiopaque, however, and radiographic examination fails to detect the foreign material. Soft foreign bodies, such as toilet paper, can be flushed out of the vaginal canal.

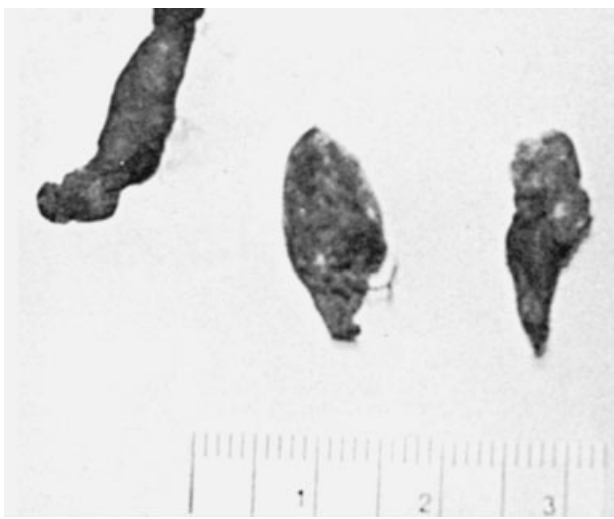

Figure 48–7 Bits of paper or cloth are the vaginal foreign bodies most frequently found in children.

The nonspecific vulvovaginitis caused by a foreign body disappears gradually after removal. Recovery can be hastened by using the hygienic treatments previously described. Repeat episodes are common.

Specific Vulvovaginal Infections

Specific vulvovaginal infections include infections of the premenarcheal vulva and vagina by bacteria that cause specific diseases in other sites.

GONORRHEAL VULVOVAGINITIS

Gonococcal infections of the prepubertal genital tract are manifested as vulvovaginitis and not the endocervicitis seen in postmenarcheal girls and women.[40,72,121,247,277,293] The alkaline environment of the unestrogenized vaginal tissues of young girls apparently limits spread of infection to the upper genital tract. Gonorrhea is found less commonly in children now than in the past but must be considered whenever a girl has vulvovaginitis.

Sexual contact should be suspected strongly and almost always is the source when a child has a gonococcal infection. Whether gonococcal infection is transmitted through nonsexual contact is controversial. Researchers have suggested that transmission may occur from freshly infected bedding, towels, or a toilet seat, or digital transmission from an infected adult. No absolute evidence exists, however, for any of these sources of infection. Gonococcal infections in nurseries have been traced to rectal thermometers, other instruments, fomites, and attendants, but before attributing a gonococcal infection to an environmental source, the possibility of sexual transmission must be considered strongly and investigated, and supportive epidemiologic evidence should be sought (e.g., cultures of potential reservoirs).

Neisseria gonorrhoeae was the most common cause of vulvovaginal discharge among prepubertal girls in Rwanda in the late 1980s. Sexual contact was considered likely in all cases because of a cultural belief that a man with a purulent urethral discharge could be cured by rubbing his penis on the external genitalia of a prepubertal girl.[25] In a prospective study of girls 12 months to 12 years of age in the mid-1990s in Cincinnati, Ohio, who had vaginal discharge and no suspicion of sexual abuse, 4 of 43 had positive cultures for *N. gonorrhoeae*.[250] Such cases should lead to investigation for probable sexual abuse.

The acute stage of gonorrheal vulvovaginitis is characterized by inflammation and a purulent discharge. The child may complain of vulvar discomfort, dysuria, frequent urination, and pain on walking. The child usually is well otherwise. Asymptomatic vaginal infection is rare. On examination, the vulvar tissues are edematous; hyperemic; and covered by a profuse, thick, yellowish discharge that exudes from the vagina. The entire vaginal mucosa is inflamed.

The urethra, paraurethral glands, and the major vestibular (Bartholin) glands rarely are involved in a premenarcheal gonorrheal infection. Vulvovaginal infections, including gonorrhea, in prepubertal children rarely, if ever, affect the upper genitalia (uterus, uterine tubes, ovaries, or pelvic peritoneum). Symptoms suggestive of pelvic peritonitis have been reported in premenarcheal children who had gonorrheal vulvovaginitis; all the patients recovered promptly after the administration of penicillin.[40,88]

The acute phase of infection lasts for a few weeks, after which most of the symptoms disappear except the discharge. The vulvar and vaginal tissues may remain hyperemic and macerated in some cases. The discharge becomes scanty and seropurulent, which may persist chronically.

The diagnosis of gonorrheal vulvovaginitis and its differentiation from other types of vulvovaginitis are established by vaginal smears and cultures. Specimens from the pharynx and rectum to test for *N. gonorrhoeae* also should be obtained. Because of the potential medicolegal use of the test results for *N. gonorrhoeae* among children, standard culture systems should be used for the diagnosis of *N. gonorrhoeae* in children. DNA probes (available for vaginal and urine specimens) and other rapid tests can be used as adjunctive evidence, but they should not be done in place of culture for the diagnosis of gonococcal infection in children. The presence of gram-negative intracellular diplococci in vaginal smears from a child with a history of exposure or with typical clinical findings is sufficient reason to start treatment, but it does not establish a definitive diagnosis. Other related species, particularly *Moraxella (Branhamella) catarrhalis* and *Neisseria sicca*, may be found in association with vulvovaginitis. A definitive diagnosis of gonorrhea is made only when *N. gonorrhoeae* is differentiated from other *Neisseria* spp. on the basis of glucose use. Additional confirmation of *N. gonorrhoeae* is recommended in children. Monoclonal fluorescent antibody tests or DNA probe confirmation tests are available. Isolates should be preserved to permit repeated or additional analyses.

Children weighing less than 45 kg with uncomplicated gonococcal vulvovaginitis and urethritis, pharyngitis, or proctitis are treated with a single dose of 125 mg of intramuscular ceftriaxone. An alternative is a single dose of intramuscular spectinomycin, 50 mg/kg (maximum 2 g), if ceftriaxone is contraindicated. Spectinomycin is not recommended for the treatment of gonococcal pharyngitis. Presently, spectinomycin is unavailable in the United States.[43] Treatment of children who are 8 years or older and weigh more than 45 kg follows the guidelines for postmenarcheal girls. In addition to ceftriaxone, 125 mg intramuscularly, single oral doses of cefixime (400 mg), ciprofloxacin (500 mg), or ofloxacin (400 mg) can be given to these girls. Local treatment is limited to gentle cleansing of the vulva and perineum. For hospitalized children, standard isolation precautions are recommended until 24 hours after effective parenteral therapy has been administered. Empiric therapy with erythromycin, azithromycin, or doxycycline to treat potential concomitant infection with *C. trachomatis* is given routinely to individuals with gonococcal infection.[42]

Follow-up cultures should be obtained from all infected sites 2 weeks after treatment to ensure that it has been effective. If not done as part of the initial evaluation, children confirmed as having gonorrhea should be evaluated for co-infection with *C. trachomatis*, *Treponema pallidum*, *Trichomonas*, human immunodeficiency virus (HIV), and hepatitis B virus.

PREMENARCHEAL CHLAMYDIAL VAGINITIS

Although *C. trachomatis* apparently is an uncommon cause of vaginitis in prepubertal girls, vaginal infection with *C. trachomatis* can occur in children with or without a known history of sexual activity and should be considered in the evaluation of a child with vaginal symptoms. Only a few studies have evaluated young children for *C. trachomatis*. These studies have involved mostly children being evaluated for sexual abuse or vulvovaginal symptoms, or both, and some have included a control group in an attempt to control either for a history of sexual activity or for vaginal symptoms.

In studies evaluating premenarcheal children with vulvovaginitis, recovery of *C. trachomatis* has been low in children with symptoms and healthy control groups. In one study, none of the 35 children with symptoms had *C. trachomatis*, whereas 1 of 35 without symptoms had *C. trachomatis* isolated.[209] In another study conducted in a pediatric gynecology clinic, 4 of 29 (14%) premenarcheal girls were found to have *C. trachomatis*.[37] All four had a homogeneous white discharge, and one had a bloody discharge. Sexual abuse occurred in two of the children and was considered possible in another. In a Cincinnati study evaluating vaginitis in girls younger than 12 years in whom sexual abuse was not suspected, none of the 87 children had a positive culture for *C. trachomatis*.[250] Similarly, in a smaller study of 11 girls with vaginitis, none was found to have *C. trachomatis*.[225]

In studies evaluating children suspected of being sexually abused, rates of recovery tend to be higher than those seen in children with vaginitis. In one study, a history of sexual abuse was discovered in two control children only after *C. trachomatis* was identified.[109] Initially, 2 percent of children with a history of sexual abuse versus 4 percent of healthy controls had positive vaginal cultures for *Chlamydia*. On further questioning, however, the two children in the control group with *C. trachomatis* were siblings who were sexually abused 3 years earlier, changing the rate of *C. trachomatis* vaginal infection in the sexual abuse group to 6 percent and decreasing the rate in the control group to 0 percent. This misclassification, if not discovered, would have changed the conclusion of the study, which suggested that similar rates of *C. trachomatis* infection occur in children with and without a history of sexual contact. This incidence illustrates the importance of thoroughly investigating the possibility of sexual abuse whenever a sexually transmitted pathogen is isolated in a young child. In a Cincinnati study evaluating children for sexual abuse, rates were low in prepubertal girls compared with pubertal girls (0.8% versus 7%),[253] whereas in a study in Raleigh, North Carolina, 6 percent of sexually abused girls younger than 12 years versus 0 percent of control children had *C. trachomatis* isolated from their vagina.[140] Four of the 10 children in the latter study had a vaginal discharge.

The highest rates of *C. trachomatis* infection were seen in a Los Angeles study, in which 47 prepubertal girls were examined for alleged sexual abuse and 17 percent had *C. trachomatis* isolated from vaginal specimens.[89] The variation in rates among studies may reflect differences in the prevalence of *C. trachomatis* in different communities. Although co-infection with *C. trachomatis* has not been studied well in prepubertal children, experience from adult populations has shown that it should be suspected in children with *N. gonorrhoeae* infection. In one study in prepubertal girls, 30 percent with *N. gonorrhoeae* also tested positive for *C. trachomatis*.[225]

One possibility is that a positive chlamydial culture in a young child, especially a child younger than 3 years, may be due to persistence of perinatal infection. Subclinical infections have been reported in 14 percent of infants of mothers with active *C. trachomatis* infection.[238] Many infants infected with *C. trachomatis* at birth remain infected for 372 days in the vagina; 383 days in the rectum; and 866 days in the conjunctiva, nasopharynx, and oropharynx.[21] Persistence of perinatal infection for 18 months and for 6 years has been reported in other studies as well.[172,237,275] Although persistence of perinatal infection can occur, when *C. trachomatis* is identified in vaginal specimens, sexual abuse still must be suspected strongly.

In contrast to premenarcheal gonorrhea infections, chlamydial vaginitis in children often seems to be asymptomatic. Because of study design issues in evaluating children with *C. trachomatis* infection, in which only children with a history of sexual abuse or vaginal symptoms, or both, are included, fully understanding the spectrum of symptoms seen in young children is difficult. In one study evaluating symptomatic children with *C. trachomatis* vaginal infection, 25 percent had a vaginal discharge and 12 percent had vaginal bleeding.[89]

Numerous diagnostic methods for *Chlamydia* are available.[23] Direct tissue culture isolation of *C. trachomatis* remains the gold standard and is the only test that should be used in prepubertal children or in cases in which sexual abuse is under consideration.[42] Culturing for chlamydiae requires isolation of the organism in tissue culture and confirmation of the characteristic intracytoplasmic inclusions by fluorescent monoclonal antibody staining.[42] Although the specificity of tissue culture approaches 100 percent, tissue culture is only 70 to 85 percent sensitive compared with DNA amplification techniques.[23] The low recovery rate in studies of prepubertal children may be due to the low sensitivity of tissue culture or the use of vaginal cultures in young children. Isolation rates from the vagina usually are lower than rates of recovery from the endocervix.[176]

Nonculture chlamydia tests, including enzyme immunoassays, direct fluorescent antibody tests,[1] DNA hybridization, and DNA amplification tests, are available and have been investigated in cervical and urine specimens and approved for use in sexually active postmenarcheal adolescents and adults. These tests have not been field-tested for large numbers of vaginal specimens from premenarcheal girls. In addition, rectal or pharyngeal specimens have not been tested. False-positive results have been reported in vaginal specimens for some of these tests in children, and these tests should not be used in premenarcheal children.[112,215] False-positive results probably are caused by cross-reactivity with common anogenital organisms, such as group A and B streptococci, *Acinetobacter* spp., *N. gonorrhoeae*, *G. vaginalis*, *E. coli*, *Proteus* spp., and *Staphylococcus* spp.[156,228,231,232,274]

The treatment of choice for chlamydial infection, including vaginitis in children weighing less than 45 kg, is erythromycin, 50 mg/kg/day in four divided doses for 10 to 14 days.[42] Because erythromycin base or ethyl succinate is only 80 percent efficacious, a test of cure should be considered 3 weeks after completion of treatment. A second course of therapy is recommended if the repeat culture is positive. Children weighing 45 kg or more but who are younger than 8 years of age should be treated with azithromycin as a single 1-g oral dose. For children 8 years and older, two regimens are recommended: azithromycin as a single 1-g dose or doxycycline at a dose of 100 mg twice a day for 7 days.[42] Because these two regimens are highly efficacious, a test of cure is recommended only if symptoms persist or re-infection is suspected.

PREMENARCHEAL VAGINAL TRICHOMONIASIS

Vaginal trichomoniasis is reported infrequently in prepubertal children. This low prevalence may be accurate or could be due to lack of appropriate testing or limitations of the diagnostic techniques used in studies in prepubertal children. Many diagnostic techniques are available; however, the diagnosis of *Trichomonas vaginalis* infection typically is made by identifying the motile, triflagellated trichomonads in urine or wet preparations of vaginal secretions or through culture of vaginal secretions (see Chapter 226).[42,72] Stained smears of vaginal discharge may reveal trichomonads, but this method is the least sensitive of the available methods. The sensitivity of wet-mount examination of vaginal secretions depends on prompt transport of the specimen to the laboratory before the organisms lyse or lose motility and on the expertise of the individual examining the specimen.[157]

Although culturing of vaginal specimens is more sensitive, this method is not widely available. More sensitive techniques have allowed for the evaluation of culture and wet-mount methods. In a study examining vaginal swab samples using polymerase chain reaction (PCR), culture and wet-mount evaluations were found to have sensitivities of 70 percent and 36 percent.[72] A urine-based PCR enzyme-linked immunosorbent assay had a sensitivity and specificity of 91 percent and 93 percent compared with wet mount or culture.[147] Such techniques may be promising for detection of *T. vaginalis* when vaginal specimens are unavailable and culture of specimens is not feasible. Because of a lower specificity than that of culture, however, these techniques probably never will be recommended for use in prepubertal children in whom sexual abuse would need to be considered.

Trichomoniasis in newborns through the acquisition of *T. vaginalis* from the mother's vagina during delivery, with recovery of *T. vaginalis* from urine, vaginal, and respiratory tract specimens, has been described in several case reports.[5,50,54,169,178,216,260] The prevalence of *T. vaginalis* in healthy, vaginally delivered infants of mothers with *T. vaginalis* is unknown. In one study, *T. vaginalis* could not be identified in any of the 14 female infants of mothers in whom *T. vaginalis* was diagnosed.[34] The overall prevalence of *T. vaginalis* in the 868 mothers was 4 percent, but the denominator of mothers of female infants was not described. In another study of 984 female infants, direct smear or culture (or both) of infant vaginal specimens identified 3 infants with *T. vaginalis*, a prevalence of 0.3 percent.[9] Two of the three infants had a vaginal discharge. The overall prevalence of infection in the mothers of the 984 infants was not reported. Of the three infants with *T. vaginalis*, one mother had a history of vaginal discharge and a negative direct smear for *T. vaginalis*, and the other two mothers had no history of *T. vaginalis*.

Numerous reports in the literature confirm the occurrence of *T. vaginalis* outside the newborn period in prepubertal girls evaluated for vaginitis. The prevalence of *T. vaginalis* in prepubertal girls with vaginitis but no history of sexual abuse ranged from 0 to 4 percent.[102,120,142,209,250] In studies with a healthy control population,[101,120,142] *T. vaginalis* was not identified in any of the children in the asymptomatic control group.

T. vaginalis has been described in varying frequency in sexually abused prepubertal girls. A case report described *Trichomonas* vaginitis in two sexually abused children with vaginal discharge.[143] In two studies conducted in Raleigh, North Carolina, which described isolated pathogens in sexually abused children with a vaginal discharge, 19 percent of 52 children at risk for being sexually abused had positive wet mounts for *T. vaginalis* in one study, whereas only 2 percent of 141 children had *T. vaginalis* in the second study.[140] The variation in detection in this high-risk population cannot be explained easily. In studies systematically evaluating children for sexual abuse regardless of symptoms, *T. vaginalis* has not been recovered. In an Australian study evaluating 160 children younger than 10 years and 95 healthy age-matched controls, none of the children had *T. vaginalis* isolated from vaginal cultures.[93] Similarly, none of the 119 prepubertal girls evaluated for sexual abuse in a Cincinnati, Ohio, study had *T. vaginalis* identified by urinalysis or wet mount of vaginal secretions.[253] Without additional studies using culture techniques and conducted in diverse populations of prepubertal girls, determing the true prevalence of *T. vaginalis* in prepubertal girls who have vaginitis, with or without a history of sexual abuse, is difficult.

A more recent case report suggests that *T. vaginalis* could be transmitted within a family without sexual abuse or contact.[3] In this report, *Trichomonas* vaginitis was diagnosed in the mother, and her three prepubertal daughters were symptomatic with a vaginal discharge. Two of the three girls had *T. vaginalis* identified on wet-mount evaluation of their vaginal specimens. They had no history or evidence of sexual abuse, the father was asymptomatic, and microscopy of an early morning urine specimen was negative for *T. vaginalis*. Although these cases may have resulted from transmission within a family without sexual abuse, identification of *T. vaginalis* in a prepubertal child outside the newborn period should prompt further medical and social evaluation for sexual abuse. Additionally, if *T. vaginalis* is recovered from a child, the child should be evaluated for other sexually transmitted infections, including syphilis, *N. gonorrhoeae*, *C. trachomatis*, hepatitis B, and HIV infection.[72]

Metronidazole (Flagyl) is effective in the treatment of vaginal trichomoniasis in premenarcheal children. The recommended dose for prepubertal girls is 15 mg/kg/day in three divided doses (maximal dose 250 mg three times a day for 7 days), 500 mg twice a day for 7 days, or 40 mg/kg (maximum 2 g) in a single dose.[72] Metronidazole can be made into a suspension for young children and has a low level of toxicity. Possible side effects are described in the discussion of trichomoniasis in adolescent girls. Metronidazole is very effective, with cure rates of 90 to 95 percent; however, if the infection does not respond to treatment, prepubertal children should be re-treated with a 7-day course as described.[4,42] A vaginal preparation of metronidazole is available, but it is not recommended for the treatment of *T. vaginalis* infection.[42]

BACTERIAL VAGINOSIS

Bacterial vaginosis is a cause of vaginal discharge in adolescent girls and women and may be seen in premenarcheal girls. Bacterial vaginosis is caused by a change in the relative proportions of bacteria in the vaginal flora: an overgrowth of anaerobes and *G. vaginalis* and a decrease in hydrogen peroxide-producing lactobacilli.[12] Although overgrowth of *G. vaginalis* often is found in bacterial vaginosis, identification of *G. vaginalis* by culture of the vaginal discharge is not diagnostic because *G. vaginalis* may be present in girls with or without bacterial vaginosis.[42]

Two methods are used to establish the diagnosis of bacterial vaginosis—Gram stain of vaginal discharge and clinical criteria,[42,72] with clinical criteria being used more commonly. The Gram stain method is used to determine the relative concentrations of bacterial morphotypes and evaluate for the overgrowth of anaerobes. This method requires an examiner with expertise in evaluating specimens for bacterial vaginosis and is less practical than is the use of clinical criteria; thus, clinical criteria are used more widely. The clinical criteria used for establishing the diagnosis of bacterial vaginosis are the presence of three of four findings: a grayish homogeneous discharge, the presence of clue cells on a wet-mount evaluation, a pH greater than 4.5, and a positive amine test result (amine or fishy odor when vaginal secretions are mixed with 10% potassium hydroxide). Although these criteria have been used routinely in studies in postmenarcheal women, use of these criteria in premenarcheal girls has been inconsistent, rendering assessment of the prevalence of bacterial vaginosis in this population difficult.

Despite the fact that the presence of *G. vaginalis* is not diagnostic of bacterial vaginosis, studies in premenarcheal girls have examined the presence of *G. vaginalis* in vaginal secretions.[18,93,114,142] In a survey of vaginal flora in children without a vaginal discharge, *G. vaginalis* was isolated from 14 percent.[114] In another study, prepubertal girls with a history of sexual abuse were more likely to have *G. vaginalis* isolated from vaginal specimens (15%) than were girls with no history of sexual abuse and either genitourinary complaints (4%) or no genitourinary complaints (4%).[18] In girls with a vaginal discharge, *G. vaginalis* was isolated from 20 percent of 25 sexually abused girls versus none of 11 girls with no history of sexual abuse. In a study examining the vaginal flora of sexually abused and nonabused 3- to 10-year-old girls, 6 percent of abused versus 1 percent of nonabused girls had *G. vaginalis* in their vaginal secretions.[93] In a study of premenarcheal girls older than 2 years with vulvovaginitis, none of the 50 girls or their age-matched controls had *G. vaginalis* isolated from vaginal specimens.[142] Because *G. vaginalis* has been isolated from symptomatic and asymptomatic girls and from girls with and without a history or evidence of sexual abuse, culture for *G. vaginalis* should not be done in the evaluation of a child with a vaginal discharge or to determine whether sexual contact has occurred.

Bacterial vaginosis is defined poorly in premenarcheal girls. No published studies using the recommended criteria to make the diagnosis exist. Some studies have used some of the criteria, however, in an attempt to examine bacterial vaginosis in premenarcheal girls. In a study examining sexually transmitted infections in girls aged 1 to 12 years who were evaluated for possible sexual abuse, 99 of the 245 girls with a vaginal discharge had an amine test and were examined for clue cells.[139] Seven of the 99 girls (7%) had clue cells or a positive amine test, or both. In a similar study, 22 of 51 girls with a history of sexual contact and a vaginal discharge had an amine test performed and were evaluated for clue cells; all of them were negative for both tests.[140] In the same study, in a second group of girls defined as being at risk for having had undetected previous sexual contact, 30 girls had a vaginal discharge, and 10 had wet-mount preparations performed. One had a positive amine test and clue cells, and one had a positive amine test only. In both of these studies, the full criteria were not used, and only subgroups of girls with vaginal discharge were evaluated.

In another study, 31 abused and 23 nonabused girls aged 2.5 to 13 years had vaginal washes performed along with amine tests and testing for clue cells.[110] The abused girls had an initial visit and a follow-up visit more than 7 days after the abuse occurred. The nonabused girls had only an initial evaluation. One of the 23 nonabused girls had a positive amine test with normal examination results and was asymptomatic. Similarly, 1 of the 31 abused girls had a positive amine test on the initial visit. On follow-up evaluation, clue cells and a positive amine test developed in 4 of the 31 abused girls, and either clue cells or a positive amine test was noted in another 4 of the 31 girls. Either a new vaginal discharge or dysuria developed in five of these eight girls. Three of the eight girls were postmenarcheal. Whether the testing itself could have been responsible for the positive results is unclear because the control group did not undergo the follow-up evaluation.

Until studies using the proper and complete criteria are done in young girls with and without a vaginal discharge, the prevalence of bacterial vaginosis will be unknown and the significance of bacterial vaginosis as a cause of vaginal discharge in premenarcheal girls will remain unclear. At this time, the presence of manifestations of bacterial vaginosis should prompt the health care provider to consider treatment of bacterial vaginosis. In addition, the possibility of sexual abuse should be contemplated because a diagnosis of bacterial vaginosis does not provide evidence of sexual abuse. Because bacterial cultures may be performed in the evaluation of a vaginal discharge in a premenarcheal girl and *G. vaginalis* may be identified, identification of *G. vaginalis* should not be considered evidence of sexual abuse or diagnostic of bacterial vaginosis.

Numerous regimens for the treatment of bacterial vaginosis exist, but no specific recommendations are available for premenarcheal girls. Probably because bacterial vaginosis is diagnosed infrequently, clinical trials evaluating treatment of bacterial vaginosis in premenarcheal girls are lacking. The drugs recommended for use in young girls are those recommended for postmenarcheal women, with dosages based on the child's body weight. In small children (<45 kg), metronidazole can be given at 15 mg/kg/day divided two times a day for 7 days (maximum dose 1 g/day).[42,72] Two intravaginal preparations also are available as alternative regimens for adolescents. Two percent clindamycin given once a day for 7 days or 0.75 percent metronidazole gel given twice a day for 5 days can be used to treat bacterial vaginosis.[42] An alternative regimen for younger girls is oral clindamycin; a dose of 10 to 20 mg/kg/day divided three times a day for 7 days is suggested.[197]

MYCOTIC (FUNGAL) VULVOVAGINITIS

Candida albicans and other fungi can cause vulvovaginitis in infants and children. Although these infections can occur in any child, children with a history of recent antibiotic use, uncontrolled

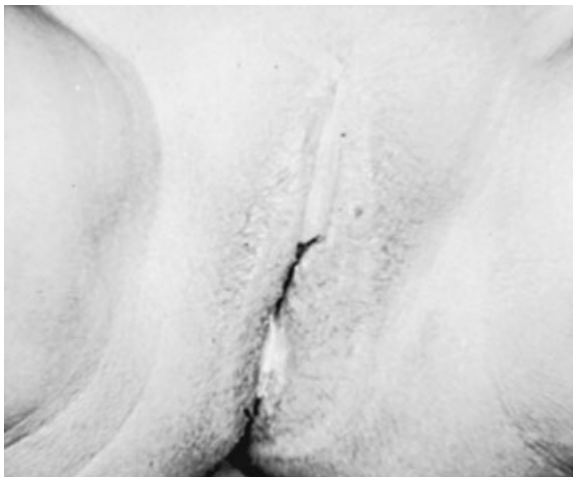

Figure 48–8 Mycotic vulvovaginitis. The child is a diabetic.

diabetes mellitus, or immunosuppression are at an increased risk for development of fungal vulvovaginitis. In children with recurrent or persistent infection, especially with no history of antibiotic use, immunosuppressive conditions, such as diabetes or HIV infection, should be considered. Mycotic infections are not considered sexually transmitted infections, but they are discussed in this section because they can cause genital infections in young children.

Children with fungal vulvovaginitis usually complain of vulvar pruritus and burning. The burning results from urine coming in contact with desquamated or excoriated areas of the vulva. A vaginal discharge also may be present. In diapered children, an erythematous rash in the child's diaper area may be noticed.

Examination reveals findings of diffuse erythema of the vulvar mucosa that may extend onto the perineal area. The involved areas are red and shiny, and whitish plaques and edema may be found. Excoriated areas, caused by scratching, can be seen as well. If the condition has been long-standing, the edema, secondary infection, and repeated scratching may produce thickened and fissured lesions closely resembling chronic eczema or lichen sclerosus et atrophicus (Fig. 48–8). If the vagina is involved, the mucosa is dusky red, and small, whitish plaques on the vaginal surface or a scant white curdled discharge may be noted. The discharge, when present, is odorless.

The diagnosis of fungal vulvovaginitis is made by finding yeast and pseudohyphae on examination of the vaginal discharge or scraped material from the vulvar skin by Gram stain or wet preparation suspended in 10 to 20 percent potassium hydroxide (Fig. 48–9).[72] Further confirmation can be obtained by identifying fungus by culture of material from the vagina or vulva on Sabouraud or Nickerson media.

C. albicans and other fungi have been isolated from the vagina in asymptomatic and symptomatic girls. In a study examining the microbiology of the vagina, yeast was cultured from 48 percent of girls aged 2 months to 2 years, from 12.5 percent of girls aged 3 to 10 years, and from 35 percent of girls aged 11 to 15 years.[122] *C. albicans* (37%) and *Candida tropicalis* (26%) were the species most frequently isolated, but *Candida parapsilosis, Torulopsis glabrata*, non-*Candida* yeast species, and other *Candida* spp. were isolated as well.

In studies evaluating prepubertal girls with vulvovaginitis, fungi have been recovered at varying rates. In one study, *C. albicans* was isolated from 25 percent of 31 symptomatic prepubertal girls with an abnormal vaginal discharge or vulvovaginitis, whereas only 3 percent of asymptomatic girls had *C. albicans* isolated.[95] Another study found different results, with none of the

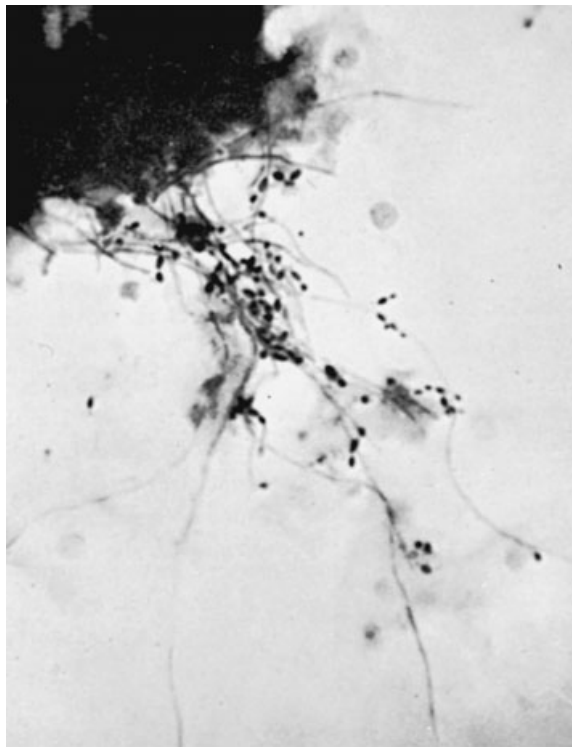

Figure 48–9 Hyphae of *Candida albicans* discovered on a wet smear of vaginal discharge.

50 asymptomatic girls and only 2 percent of the 50 girls with vulvovaginitis having *C. albicans*.[142] Similarly, in a study evaluating girls younger than 12 years with vaginal complaints, only 3 percent of the 74 girls had *C. albicans* isolated.[250] Neither of these groups of girls had a discharge on examination, and both had been treated recently with antibiotics. In a study that examined recovery of microorganisms from girls with vaginitis, 8 percent of the vaginal cultures of the 35 girls evaluated grew yeast.[101] In these latter two studies, assessing the role of yeast in the disease is difficult because there was no control population for comparison.

Several modes of therapy can be used for the management of fungal vulvovaginitis.[72] Numerous antifungal creams, including nystatin, miconazole, clotrimazole, or terconazole, are available. The creams should be applied as prescribed to the affected area after cleansing. In diapered children, the cream should be applied with each diaper change.

Curing fungal infections often is difficult in children who are taking antibiotics or who receive repeated courses of antibiotic therapy. In such cases, a 1-mL dose of nystatin suspension (Mycostatin), 100,000 U/mL, can be injected into the vagina three times a day for 10 days in infants and small children. In addition, the suspension should be administered at the same dose orally four times daily. Fluconazole (Diflucan) also can be administered as a one-time oral dose. A second dose may need to be administered for severe infections.[72] In older girls, vulvovaginal fungal infections can be treated with fungicidal vaginal creams, suppositories, or oral fluconazole as described in the section on vaginal infections in adolescent girls.[42]

NONGONORRHEAL NEISSERIAL VULVOVAGINITIS

Occasionally, other species of *Neisseria* are the causative agents in cases of premenarcheal vulvovaginitis. These gram-negative, intracellular and extracellular diplococci resemble *N. gonorrhoeae*

on stained smears. *M. catarrhalis* also is identical on Gram stain. Gram-negative diplococci should be speciated completely by the microbiology laboratory to avoid misidentifying of nongonococcal diplococci as gonococci and vice versa. Especially for pediatric patients, misidentification can result in serious medicolegal consequences for the family when unwarranted intervention is initiated, or it can result in failure to protect the child's welfare when appropriate measures are deemed unnecessary.[9]

N. sicca, generally considered nonpathogenic, has been isolated from children with vulvovaginal infections that clinically resembled gonorrhea.[283] *Neisseria meningitidis* also has been reported as a cause of vulvovaginitis.[77,102] Nongonococcal *Neisseria* spp. may be considered the cause of vulvovaginal infections when isolated as the predominant flora in the setting of vaginal inflammation and discharge.

Treatment of vulvovaginitis caused by nongonococcal *Neisseria* spp. or *M. catarrhalis* is the same as for *N. gonorrhoeae*. Most nongonococcal *Neisseria* spp. are not resistant to penicillin, but the need for therapy generally is based on the Gram stain finding of gram-negative diplococci, which for therapeutic purposes should be considered gonococci pending culture results. Although data are unavailable, a single dose of ceftriaxone probably should be as effective for these noninvasive *Neisseria* infections as it is for gonorrhea. Chemoprophylaxis, generally with rifampin, should be considered for family members and other contacts in cases of meningococcal vulvovaginitis.

GROUP A STREPTOCOCCAL VULVOVAGINITIS

Group A streptococci are a frequent cause of vulvovaginitis in premenarcheal girls and accounted for 9 to 20 percent of cases in several series.[64,67,120,265,279] Most cases occur in girls aged 2 to 7 years, but cases in infants and teenagers have been reported.[26,64,97,265] A marked seasonal variation in incidence in some geographic regions, with peak rates occurring in late fall and winter, may explain the low number of cases of vulvovaginitis caused by group A streptococci in some series.[79,182] The nasopharynx seems to be the primary reservoir for group A streptococci in these girls. Infection may occur from self-inoculation by hand to nose to the vulvovaginal area.[245] The skin also may serve as the source of group A streptococcal vulvovaginitis.[97,265] Preceding or concurrent symptoms of upper respiratory tract infection are uncommon findings, but many girls with group A streptococcal vulvovaginitis have throat cultures positive for *S. pyogenes*.[97,265] Perineal symptoms have preceded pharyngeal symptoms in some patients.[79] Group A streptococcal vulvovaginitis may occur during the course of scarlet fever.[26,119,120]

The signs and symptoms of group A streptococcal vulvovaginitis often overlap those caused by other bacterial infections, but symptoms usually are abrupt in onset. Most patients seek medical care within 1 week of onset. Vaginal discharge and dysuria are the most common complaints. These girls usually are afebrile. Localized tenderness and an intense, fiery red erythema of the vulvar tissues are frequent findings, but some cases have only mild erythema. Pruritus and excoriation may be present. The discharge usually is seropurulent but may be serosanguineous. The color may be white or green. Petechiae may be present on the vaginal mucosa, and regional papular or scarlatiniform rashes can occur.[79,97,245,265] Acute poststreptococcal glomerulonephritis has been reported in association with group A streptococcal vulvovaginitis.[193] Labial abscesses caused by *S. pyogenes* have been noted rarely in prepubertal girls.[279]

Concomitant streptococcal proctitis and perianal skin infections have been reported.[79,154,261] Perianal pruritus, erythema, and tenderness are common findings. Abdominal pain and rectal bleeding may be present. Penile involvement occurs rarely if at all.[181]

The diagnosis of *S. pyogenes* infection may be missed if vaginal secretions are not plated onto sheep blood agar or other media that readily support the growth of streptococci.[265] The vulvovaginitis caused by group A streptococci usually shows initial response to oral antimicrobial therapy within 24 hours. A 10-day course of oral penicillin, amoxicillin, or erythromycin generally is sufficient. A second course sometimes is necessary when perianal disease is present.[261] Adjunctive use of the hygienic measures discussed for nonspecific vulvovaginitis hastens clinical improvement. Group B and group F streptococci also have been isolated from girls with acute vulvovaginitis.[250]

VULVOVAGINITIS SECONDARY TO BACTERIA THAT COLONIZE THE NASOPHARYNX

Often, a history of an upper respiratory tract infection precedes the onset of vulvovaginitis by a few days. Suspicion that the two conditions are related is strengthened when vaginal cultures yield organisms that commonly colonize the nasopharynx. Vulvovaginitis is assumed to result from autoinoculation of microbes from the nasopharynx to the genitalia. The onset of vulvovaginal symptoms in these infections tends to be more acute, the inflammation and discomfort more marked, and the discharge less profuse and less purulent than in nonspecific vulvovaginitis.[133]

H. influenzae and *S. aureus* are the species isolated most frequently from cultures in this setting. *Streptococcus pneumoniae* is seen occasionally. *H. influenzae* was the organism isolated most frequently in a series of 200 girls with vulvovaginitis.[213] Acute and chronic cases of *H. influenzae* vulvovaginitis do occur, and the discharge usually is mucoid or mucopurulent, yellow, and odorless. Vulvovaginitis can be caused by serotypes a, b, and c and by nontypeable strains.[120,179] Concurrent otitis media or urinary tract infection may be present. All three of these species occasionally are found in vaginal cultures from asymptomatic children. Their isolation in pure culture from symptomatic girls is what leads to the clinical conclusion of cause and effect.

Vulvovaginitis caused by these organisms often responds to the treatment described earlier for nonspecific vulvovaginitis secondary to poor perineal hygiene. Systemic antibiotics may be required for persistent cases or may be helpful early in severely symptomatic cases. The choice of agent depends on the anticipated or known antimicrobial susceptibilities of the specific organism. The vagina is a well-recognized source of *S. aureus* colonization in cases of toxic shock syndrome (TSS) associated with this organism. Such cases generally have occurred in adolescent girls and women in association with the use of tampons. Vulvovaginitis caused by *S. aureus* in prepubertal girls has not been reported in connection with TSS. Labial abscesses caused by *S. aureus* have been described in prepubertal girls.[72]

VULVOVAGINITIS SECONDARY TO SKIN INFECTIONS

Similar to a child with an upper respiratory tract infection, a child with impetigo or an infected superficial wound may transmit bacteria from the wound to the genitalia by hand contamination. Cultures in such cases usually yield hemolytic streptococci or *S. aureus*. Treatment is the same as that described for nonspecific vulvovaginitis secondary to respiratory tract infections.

SHIGELLA VULVOVAGINITIS

Vulvovaginitis may be caused by infection with pathogens from the intestinal tract, especially when the organisms are endemic in a community. *Shigella* spp., mainly *Shigella flexneri* and *Shigella sonnei*, seem to account for most of these cases.[25,28,29,59,189] Vaginal discharge without pain, pruritus, or dysuria is the most frequent manifestation of *Shigella* vulvovaginitis. The course can be acute,

but discharge that persists for 4 weeks to several months before the diagnosis is made is common. Bloody discharge has been observed in approximately half the cases reported from developed countries, but it was not seen in any of 27 girls with *Shigella* vulvovaginitis in Rwanda between 1988 and 1991.[25] The discharge may be purulent and heavy; occasionally, it is absent. The vulvar tissues usually appear inflamed.

In most instances, *Shigella* vulvovaginitis is not associated with current or recent diarrhea.[25,189] The clinical presentation can be very similar to that of a vaginal foreign body. Local application of triple-sulfa cream (Sultrin) may clear the infection in some cases. Refractory cases have been described, however.[59,189] Systemic treatment with an antibiotic to which the *Shigella* isolate is susceptible is recommended. Single-dose therapy with third-generation cephalosporins is ineffective; 5-day courses of oral agents generally are required.[25] Amoxicillin, trimethoprim-sulfamethoxazole, and cefixime are reasonable choices for susceptible isolates; however, most *Shigella* isolates are resistant to ampicillin and trimethoprim-sulfamethoxazole. Ciprofloxacin use has been described in one case.[18] Azithromycin also may be an effective treatment option.[153] As with other causes of vulvovaginitis, adjunctive use of hygienic measures may help resolve the process and prevent recurrence.

OTHER SPECIFIC CAUSES OF PREMENARCHEAL VULVOVAGINITIS

Diphtheritic, amebic, and other types of specific vulvovaginitis in children have been reported in the literature.[84,163,212] Most such cases have had associated primary disease elsewhere.

Diphtheritic vulvovaginitis can be the primary site of infection, but most reported cases have been secondary to nasopharyngeal infection. Although diphtheria seldom occurs today, sporadic cases occasionally appear in areas where immunization coverage is inadequate. The vulva is the most common genital site involved in diphtheria,[212] but diphtheritic lesions may occur in the vagina without vulvar involvement. The diagnosis is suspected when a child has the severe systemic symptoms produced by upper respiratory tract diphtheria and a local ulceration covered by a gray adherent membrane; it is confirmed by finding *Corynebacterium diphtheriae* in discharge from the lesion.[70] Treatment of diphtheria is the same regardless of the site of the infection and is discussed in Chapter 101.

One case of vulvovaginitis with *Yersinia enterocolitica* isolated as the predominant organism was reported in a 4-year-old girl who also had a positive stool culture and associated fever and abdominal pain but no diarrhea.[281] In other members of the community in which she lived, diarrhea developed with cultures positive for *Y. enterocolitica*. The outbreak was linked to contaminated food. Infections with this organism may be missed because special culture techniques are required for isolation.

Rarely, ulcerative vulvovaginitis caused by *Entamoeba histolytica* or related to typhoid fever has been reported. Generally, specific genital infections with pathogenic organisms usually found in the gastrointestinal tract are the result of fecal contamination of the vulva and vagina. Treatment of infections caused by *E. histolytica* is described in Chapter 221.

OTHER LOWER AND UPPER GENITAL TRACT INFECTIONS OF PREMENARCHEAL GIRLS

Other specific and nonspecific infections of the premenarcheal female lower and upper genital tract genitalia, including genital herpes, condylomata acuminata, molluscum contagiosum, granulomatous and ulcerative disorders, cervicitis, and pelvic inflammatory disease (PID), are discussed in the section on genital infections in adolescents.

SEXUALLY TRANSMITTED DISEASES IN SEXUALLY ABUSED CHILDREN

Isolation of an STD in a child (especially a prepubertal child) places a health care provider in an awkward position of having to report the case to a children's protective service agency for investigation of sexual abuse. Management of children who have sexually transmitted infections requires close cooperation among clinicians, laboratories, and child protection agencies. The possibility of nonsexual transmission of these diseases often is raised, particularly when a preliminary investigation cannot elicit a history of sexual abuse. The rate of eliciting evidence of sexual abuse in prepubertal children can be 67 percent, however, in children with STDs.[15] Bacterial sexually transmitted infections are isolated more frequently than are viral infections in sexually abused children. Many challenges are involved in eliciting and validating sexual abuse in children. Verbal communication in children younger than 2 to 3 years old is impossible or difficult to interpret. Fear of disclosure by children or family members because of threats of violence may be a major barrier to validating a case in an older child.[179]

The first step in managing such situations is for a physician to assess critically the type of diagnostic test used to detect the infection. A major problem exists with inappropriate use of nonculture tests for testing for chlamydia.[8,107] The second step is for a physician to perform a genital examination on the child to look for evidence of acute or chronic vulvar and hymeneal trauma. Referral to a physician with expertise in this field is appropriate. A brief interview of the child in a nondirected manner with open-ended questions also should be done. Regardless of whether the child divulges any information, the case should be reported to the local children's protective service agency. The objective is for an agency caseworker to interview the child also in a nonthreatening manner. In addition, the caseworker should request that all household members be tested for STDs. More than one visit and interview by the caseworker may be necessary for such testing to be accomplished. Based on a review of the literature, common STDs in prepubertal children and their potential mode of transmission are summarized in Table 48–3 and discussed in this section.

GONOCOCCAL INFECTION

In infants, perinatal nonsexual transmission is considered the most likely cause of gonococcal infection. Branch and Paxton[27] found that in 1- to 11-month-old infants, all the mothers were found to have gonococcal infection, and no history of sexual contact could be elicited. The authors concluded that transmission of the infection was perinatal, from freshly contaminated hands, or through fomites.

Prepubertal children with gonococcal infection frequently are found to have a history of sexual contact.[139] The rate of eliciting a history of sexual contact in prepubertal children with gonorrhea ranges from 36 to 93 percent.[27,83,136,138] Branch and Paxton[27] reported that 93 percent of children aged 1 to 9 years with gonorrhea had sexual contact with relatives in the household. Ingram and colleagues[136,138] found that 35 percent of 1- to 4-year-old children and 100 percent of children older than 4 years with gonorrhea reported having sexual contact with an older male family member. Folland and coworkers[83] elicited a history of sexual contact from 34 percent of children with gonococcal urethritis and vaginitis.

Other infected adults and children often are found in an infected child's environment.[217] Testing of household members can detect infected adults at a rate of 18 to 29 percent.[8,192] In a retrospective study of 14 Native Alaskan children with

TABLE 48–3 Sexually Transmitted Diseases (STDs) in Prepubertal Children: Modes of Transmission and Implications for Child Sexual Abuse

Infectious Agent	Persistence after Perinatal Acquisition	STD Rate in Alleged Sexual Abuse Cases	Alleged Sexual Abuse Rate in STD Cases	Infection in Household Members of Infected Children	Survival on Fomites	AAP CCAN 2005 Guideline Recommendations for Abuse Reports	Other Comments
Neisseria gonorrhoeae	Up to 1 yr old[27]	5-7%[59]	35%, 1-4 yr old[40] 100%, >4 yr old[136] 93%, 1-9 yr old[27]	18-29% of adults in household[8,192] (abuse was thought unlikely in these series)	24 hr on wet fomites; 2 hr on dry fomites. None from public toilet seats[96,214]	Diagnostic for abuse. Report to Child Protective Services*	Although fomite transmission can occur, sexual transmission must be considered the likely mode, unless strong evidence of an alternative mode is found. Fomite or maternal nonsexual transmission plausible <1 yr old[27]
Chlamydia trachomatis	Up to 3 yr old[238] Rectal and vaginal sites up to 54 wk	6-8%[20,45,137,140] 4% cases vs. 9% controls[111]	75%		No data	Diagnostic for abuse. Report to Child Protective Services*	Perinatal acquisition likely <1 yr old; if >1 yr old, genital infection strongly suggests sexual transmission[137]
Trichomonas vaginalis	Up to 1 yr old	No data	Case report[143]	43% fathers, 72% mothers, with no history of sexual abuse elicited (Poland)[159]	Up to 6 hr on wood surface and in discharge.[149] Isolated from bathing implements[196]	Highly suspicious for abuse. Report to Child Protective Services*	If >1 yr old, genital infection strongly suggests sexual transmission. Bathing in tanks or rivers may increase infection risk in girls (India).[196] Has been isolated from mud baths, warm mineral waters, water from toilets[59,159]
Syphilis	Months to years if untreated	0-5%[59,139]	Case reports		Unlikely, based on difficulty in culturing the microbes, but no data	Diagnostic for abuse. Report to Child Protective Services*	Infection not known to be congenital should be considered sexually transmitted. Clinical manifestations (e.g., late congenital syphilis) may be helpful in discerning mode in some cases[42]
Bacterial vaginosis (*Gardnerella vaginalis*)	No systematic studies; colonization at birth potentially persists for years	7-13%[110]	28%[4]			Inconclusive; conduct medical follow-up	*G. vaginalis* can be found as part of normal flora in nonabused virginal children. Presence of clue cells in a vaginal wet mount, not culture results, is diagnostic.[42]
Haemophilus ducreyi (chancroid)	Asymptomatic persistence unlikely						Should be considered sexually transmitted

TABLE 48–3 Sexually Transmitted Diseases (STDs) in Prepubertal Children: Modes of Transmission and Implications for Child Sexual Abuse—cont'd

Infectious Agent	Persistence after Perinatal Acquisition	STD Rate in Alleged Sexual Abuse Cases	Alleged Sexual Abuse Rate in STD Cases	Infection in Household Members of Infected Children	Survival on Fomites	AAP CCAN 2005 Guideline Recommendations for Abuse Reports	Other Comments
Calymmatobacterium (Klebsiella) granulomatis (granuloma inguinale)							Should be considered sexually transmitted[42]
Herpes simplex virus (genital sites)	Symptomatic congenital infection usually develops within first 6 wk of life		Case reports only	Case report only (mother with an infected finger)[146]	2 hr on latex gloves, toilet seats; 24 hr on speculum; 72 hr on gauze[167]	Suspicious for abuse. Report to Child Protective Services*†	Autoinoculation from oral lesions is possible,[191] but fomite transmission is unlikely because it requires direct contact of viable virus with broken skin or mucous membranes
Human papillomavirus‡ (anogenital warts)	Up to at least 3 yr old and probably years longer[57]		27-90%[47]			Suspicious for abuse. Report to Child Protective Services*§	The possibility of sexual abuse should be considered when oral warts or laryngeal papillomas are found in older children
Human immunodeficiency virus (HIV)	Perinatal acquisition may not become evident for many years in slowly progressive cases	Very low in the U.S.; higher in countries with higher prevalences of HIV infection	26 of 9136 HIV cases in children were associated with sexual abuse by individuals with confirmed or suspected HIV infection[168a]	Common¶	Exposure to infected blood can lead to infection. Casual household contact alone has not been associated with transmission	Diagnostic for abuse if not perinatally or transfusion acquired. Report to Child Protective Services if mother is HIV-negative and child has no transfusion exposure*	History of maternal HIV infection suggests perinatal acquisition unless the child's presentation suggests acute HIV infection or perinatal transmission was reasonably excluded during infancy
Hepatitis B virus	Perinatal transmission can be asymptomatic for years to decades			Household transmission can occur			Nonsexual transmission can occur, so positive HBsAg tests do not necessarily indicate sexual abuse. Vaccination history for hepatitis B virus should be reviewed
Hepatitis C virus	Perinatal transmission can be asymptomatic for years to decades						Sexual transmission has been thought possible, but is currently uncertain. Maternal history may be helpful

**Or other local agency mandated to receive reports of suspected child sexual abuse.*

†Report unless there is a clear history of autoinoculation. Type of herpes simplex virus (1 versus 2) is generally not helpful in determining whether acquisition was by abuse or autoinoculation, although presence of type 1 may increase the likelihood of nonsexual transmission.

‡Laryngeal papillomas in young children are likely perinatally acquired.

§Some experts do not believe reporting is warranted in all cases. In children <3 to 4 years old for whom a thorough evaluation by an experienced child sexual abuse investigator reveals no concerns of abuse, reporting to Child Protective Services may be unnecessary. Close medical follow-up may be appropriate. Physicians must be clear when reporting that anogenital human papillomavirus is not always acquired by sexual abuse even in older children.

¶All siblings should be tested, whether perinatal or postnatal acquisition is suspected, unless perinatal infection is the known route and an older child was born during a time period in which the mother was known to be HIV-negative.

HBsAg, hepatitis B surface antigen.

Modified from Chacko, M., Staat, M. A., and Woods, C. R.: Genital infections in childhood and adolescence. In Feigin, R. D., Cherry, J. D., Demmler, G. J., and Kaplan, S. L. (eds.): Textbook of Pediatric Infectious Diseases. 5th ed. Philadelphia, W. B. Saunders, 2004, p. 577; and Committee on Child Abuse and Neglect.

gonococcal infection, 3 reported having sexual contact. Seven children slept with their parents, one or both of whom had gonorrhea; the authors assumed that these children acquired the infection by nonsexual means.[251]

N. gonorrhoeae has been shown to survive for 20 to 24 hours in infected secretions on towels and handkerchiefs.[263] Although *N. gonorrhoeae* has survived on toilet seats for 2 hours, no gonococci were recovered from toilet seats in public restrooms or in a clinic for STDs.[96,224] Nonsexual transmission to adults is rare.

Nonsexual transmission conceivably occurs in children who sleep and bathe with their parents. Sexual transmission is the more common and most likely mode of transmission, however.[112] The presence of gonorrhea should be considered diagnostic of sexual abuse. An investigation for sexual abuse must be pursued in a child with gonorrhea by reporting the case to the appropriate legal authority.[42]

CHLAMYDIAL INFECTION

C. trachomatis can be transmitted to an infant from an infected mother during the perinatal period. *C. trachomatis* has been isolated from the conjunctiva, nasopharynx, vagina, and rectum of infants born to infected mothers.[238] Perinatally acquired rectal and vaginal chlamydial infection in infants can persist for 372 and 383 days, respectively. Persistent chlamydial infection of the pharynx in infants can persist for 2 years.[21]

Sexual abuse as a potential mode of transmission of *C. trachomatis* infection should be considered in children older than 1 year.[89,111] In a retrospective study, Ingram and associates[137] found that 6 percent of girls who allegedly were sexually abused and no girls who denied sexual abuse had *C. trachomatis* infection. Except for one child, all the children in the control group were determined later to have been sexually abused. In a prospective study, Ingram and associates[139,140] found *C. trachomatis* in 8 percent of girls with a history of sexual abuse compared with 0 percent in girls with no history of sexual abuse. In the former group, three girls also were found to have rectal infection and one to have pharyngeal infection.

Perinatally acquired genital chlamydial infection is a strong possibility in children younger than 1 year. Perinatal transmission is possible in children aged 3 years. After 1 year of age, however, sexual transmission should be considered diagnostic of sexual abuse in children with genital chlamydial infection.[42] An investigation for sexual abuse must be pursued in a child in whom genital chlamydial infection is diagnosed.[42,107] Studies have evaluated the presence of *C. trachomatis* on fomites and its coexistence in family members of infected children.

SYPHILIS

The prevalence of syphilis in children suspected of having been sexually abused is lower than that of gonorrhea or chlamydia. Syphilis infections not found to be acquired congenitally should be considered sexually transmitted. Sexually acquired infection is a strong possibility in all prepubertal children with syphilis. Syphilitic lesions and positive serologic results have been detected in alleged sexual abusers of children with syphilis.[1] No data are available on the survival of *T. pallidum* on fomites. The presence of syphilis should be considered diagnostic of sexual abuse, and an investigation must be pursued.

TRICHOMONAS VAGINALIS INFECTION

T. vaginalis has been found in the nasopharynx and vagina of newborns of infected mothers. Transmission of *T. vaginalis* in infants aged 1 year probably is perinatal. The mode of transmission in children older than 1 year is controversial. Two cases of *T. vaginalis* infection in premenarcheal girls who were sexually abused have been reported.[143] Prevalence studies either do not address the mode of transmission at all or, if they do, do not address the possibility of sexual abuse. In addition, a vaginal wet mount for trichomonads is not performed routinely in children assessed for possible sexual abuse. A survey from Poland found one case of *T. vaginalis* infection in children aged 2 to 7 years and a significantly higher number of cases in 8- to 10-year-old girls. The numbers increased even further for children older than 10 years, suggesting a strong association between *T. vaginalis* and the presence of an estrogenic environment, which promotes glycogen production and decreases vaginal pH.[159]

The Polish survey tested families of women infected with *T. vaginalis* and found that almost a third of their sexual partners and 8 percent of the children (mostly girls) had *T. vaginalis*. When families of men infected with *T. vaginalis* were tested, 91 percent of their sexual partners and 13 percent of the children had *T. vaginalis*. When families of children (mostly girls) infected with *T. vaginalis* were tested, 72 percent of the mothers and 43 percent of the fathers had the infection. The investigators considered that the infection in the latter group originated from mothers and that the primary mode of transmission was nonsexual (beds, sponges, towels, overcrowding). Information regarding sharing of potentially infected fomites, sexual abuse, or physically intimate behavior between parents and children was not gathered in these cases.[159]

Although *T. vaginalis* has been known to survive on fomites in controlled experiments, its ability to spread by these means is unknown. No cases of adults being infected by fomites have been documented. *T. vaginalis* has been found to survive for 6 hours on droplets of discharge and enameled surfaces of wood blocks.[149] It has been isolated from droplets of water splashed from toilets containing the urine of an infected individual.[39] *T. vaginalis* also has been found to survive in mud baths, bathing waters, and warm mineral waters and on moist bathing implements.[159,196] In rural India, a survey found that young girls who bathed in tanks or rivers had a significantly higher risk of acquiring *T. vaginalis* than did girls who used pipe or well water.[46]

T. vaginalis conceivably is transmitted to children nonsexually. The likelihood of perinatal transmission is high in infants younger than 1 year. A child or an infant younger than 1 year with *T. vaginalis* also may have been sexually abused. In a child older than 1 year, the probability of sexual abuse must be considered highly suspicious, and the case must be investigated and reported. Perinatal transmission and transmission of infection through fomites should not be assumed without an investigation for sexual abuse.[42]

BACTERIAL VAGINOSIS

The significance of bacterial vaginosis and *G. vaginalis* and their relationship to sexual abuse in prepubertal girls are unclear and inconclusive. Data on the prevalence of bacterial vaginosis in children with vaginal discharge and suspected sexual abuse are limited. Based on the presence of clue cells and a positive amine test result in vaginal secretions, bacterial vaginosis has been diagnosed in 13 percent of sexually abused children versus 4 percent of girls who denied sexual abuse.[110,139] Similar prevalence rates for *G. vaginalis* have been reported in children who have and have not been sexually abused.[21] No data are available regarding the survival of *G. vaginalis* on fomites.

Although the prevalence of *G. vaginalis* and bacterial vaginosis is higher in sexually abused children than in nonabused children, their significance as a marker for sexual abuse is unclear. *G. vaginalis* is not the sole cause of bacterial vaginosis and is not a

suitable marker of sexual activity. The recommendation is that a child with a vaginal discharge, the presence of clue cells, and a positive amine test be questioned about sexual abuse and be followed medically. An investigation by a children's protective service agency is unnecessary, however.[42]

GENITAL HERPES

Perinatal transmission of herpes simplex virus (HSV) types 1 and 2 in the form of stomatitis occurs in infants. HSV type 2 is an uncommon finding. HSV types 1 and 2 have been isolated in the genital area in children alleging sexual abuse.[94,139] No studies have reported the coexistence of HSV genital infection in household members and infected children, however. Physical contact by a mother's infected finger has been reported.[146] Autoinoculation from the mouth to the genitals as a mode of transmission is possible, especially when oral HSV infection precedes genital herpes lesions.[191] HSV has been known to survive for 2 hours on latex gloves and toilet seats, 24 hours on a speculum, and 72 hours on gauze.[167] Transmission of HSV from fomites requires direct contact of viable virus with either a mucous membrane or a break in the skin, however, rendering fomite transmission unlikely. A child with genital herpes infection should be considered suspicious and evaluated for sexual abuse, and the case should be reported to the authorities.[42]

GENITAL WARTS (CONDYLOMATA ACUMINATA)

Perinatal transmission of human papillomavirus (HPV) from an infected mother to her infant is well-documented. The incubation period after exposure to the virus may range from 1 to 20 months.[57] Data on the presence of HPV DNA in children beyond the neonatal period are inconsistent—1.2 to 27 percent in three different studies.[166,219,282] In addition, the relationship between the presence of HPV DNA and the ultimate development of disease in children is unclear. Because the exact incubation period for the development of genital lesions is unknown, perinatal transmission has been found to be the most likely cause of genital warts in almost 96 percent of patients younger than 3 years.[57] In children 3 years or older, a history of sexual abuse has been elicited in 27 to 90 percent with venereal warts.[47,123]

Condylomata acuminata are acquired by children as a result of close physical contact with an infected individual, by digital infection of the child's genitalia by an infected individual, or by sexual contact with an infected individual.[246] A history of condylomata in other members of the family, particularly the mother or older sisters who care for the child, sometimes may be elicited. Perinatal exposure, poor hygiene, and shared bathing have been suggested sources of infection.[267,268] HPV types 6, 11, 16, and 18 are seen in adults with anogenital warts and are the most common genital types detected in children. This finding has raised questions about warts in children resulting from sexual abuse. In many cases, however, the mode of transmission is unknown.[57] Based on failure to identify sexual abuse, a report from a dermatology clinic concluded that transmission of HPV possibly occurs by fomites.[47] No reports in the literature address survival of the virus on fomites.

Sexual abuse is the most common means of acquiring anogenital HPV infection and should be considered suspicious for sexual abuse and investigated and reported in all prepubertal children older than 3 years who are infected. In children younger than 3 years, although perinatal transmission is likely, sexual abuse should be suspected and investigated.[42]

As recommended by the Centers for Disease Control and Prevention,[42] the possibility of sexual abuse should be considered strongly if no conclusive explanation for nonsexual transmission of a sexually transmitted infection has been identified. When the only evidence of sexual abuse is the isolation of an organism or the detection of antibodies to a sexually transmitted agent, findings should be confirmed, and implications should be considered carefully.

POSTMENARCHEAL LOWER GENITAL TRACT INFECTIONS

Infections of the postmenarcheal female clitoris, urinary tract, vulva, vagina, and cervix produce a variety of overlapping symptoms, including vulvar pruritus, dysuria, and increased or altered vaginal discharge and spotting.[87,175] As a result, distinguishing among various lower genital tract infections based solely on symptoms is difficult. The history, physical examination, and laboratory tests play an important role in assisting the clinician in diagnosing urethritis, vaginitis, or cervicitis.

DISORDERS OF THE CLITORIS

Cellulitis with induration, edema, and erythema, analogous to posthitis in boys, occasionally develops in the clitoral hood. Staphylococci and streptococci are the most common etiologies. Oral antibiotics with efficacy against these organisms usually are effective. Warm soaks or sitz baths also may provide symptomatic relief.[72]

Clitorimegaly with erythema can occur with vulvovaginitis of any etiology but usaully is associated with HSV infections.[67] Edematous enlargement of the clitoris and the labia without erythema has been reported in patients with Crohn disease.[188]

POSTMENARCHEAL URETHRITIS

Urethritis is manifested clinically by dysuria or urinary urgency or both. Attempting to differentiate external from internal dysuria is important. External dysuria is pain from urine flowing over the vulva. Such a history suggests vulvitis and vaginitis. Internal dysuria is pain with initiation of urination and is not associated with urine flowing over the vulva. It indicates urethritis or a urinary tract infection. A careful history and examination assist the physician in differentiating urethritis from vaginitis.

Urethritis occurs commonly, particularly in sexually active postmenarcheal girls. Urethritis with internal dysuria can occur as a result of sexually transmitted infections, such as *T. vaginalis*, *N. gonorrhoeae*, and *C. trachomatis*, and has been implicated as an important cause of dysuria in sexually active girls. It is called *acute urethral syndrome*, and clinical features include dysuria, frequency, and pyuria with significant bacteriuria.[264] Urethral infection by *C. trachomatis* may occur with or without cervical infection; such infection was associated with sterile pyuria in 50 percent of girls with acute-onset dysuria and frequency, and *C. trachomatis* was isolated in 31 percent of these cases.[264]

When a sexually active adolescent girl has internal dysuria, in addition to being tested for conventional uropathogens, she should be screened for common STDs, such as gonorrhea and *Chlamydia* and *Trichomonas* infection.[61] Urinalysis and microscopy for the presence of leukocytes and bacteria should be done in these patients as well. For treatment of urethritis caused by sexually transmitted organisms, the sections in this chapter on trichomoniasis, gonorrhea, and chlamydial infections should be reviewed.

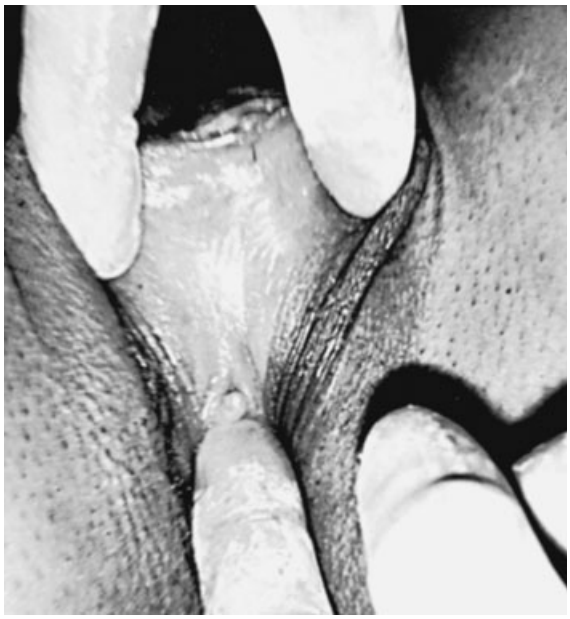

Figure 48–10 Urethral and paraurethral duct discharge is obtained by placing downward and outward digital pressure on the distal urethrovaginal septum.

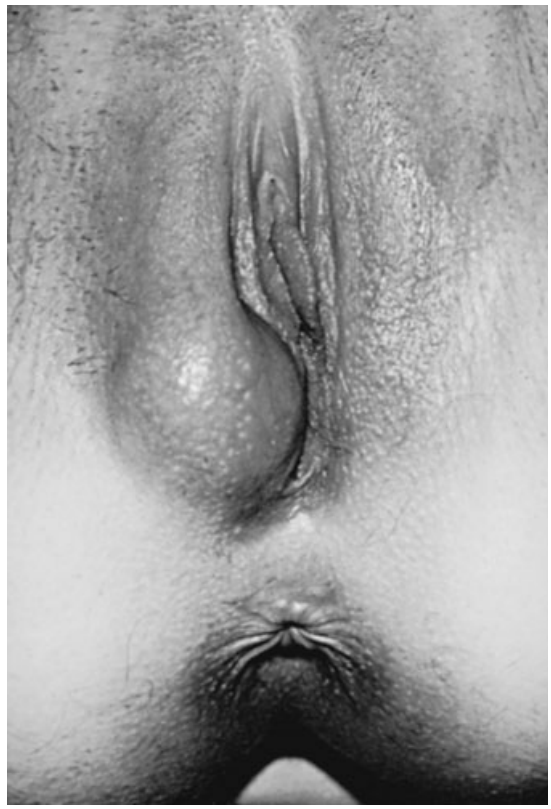

Figure 48–11 Acute Bartholin duct abscess.

PARAURETHRAL DUCT ABSCESS, BARTHOLINITIS, AND BARTHOLIN ABSCESS

The paraurethral ducts lie on each side of the urethral meatus. The Bartholin glands are small, bean-shaped glands that lie on each side of the vaginal opening, behind the hymen. Each gland opens by means of a long single duct immediately external to the hymen. Infections of the paraurethral and Bartholin ducts are found more commonly in women but occasionally occur in adolescent girls.

A paraurethral duct abscess creates a small, exquisitely painful swelling in the urethrovaginal septum. If the abscess is not incised and drained, it may rupture into the urethra and create a urethral diverticulum. Discharge for Gram stain should be obtained from the urethral lumen and the paraurethral ducts by downward and outward pressure on the urethrovaginal septum (Fig. 48–10).

Bartholinitis, or inflammation of the Bartholin ducts, causes pain, tenderness, and a linear rope-shaped swelling, best palpated by holding the vulvar mucosa and labia majora between the fingers. Purulent or mucoid exudate can be expressed occasionally from the Bartholin duct.

N. gonorrhoeae and *C. trachomatis* have been isolated from ductal exudates in women with bartholinitis.[56,223] This condition should be treated with antibiotics that provide coverage for *N. gonorrhoeae*, *C. trachomatis*, and anaerobes. Symptomatic relief may be achieved with sitz baths.

Although a Bartholin abscess is seen most often in women 20 to 29 years old, it does occur in sexually active adolescent girls. It also is the second most common urogenital complication of gonorrhea, after PID, in women. Risk factors for development of Bartholin abscess are similar to those for STDs.[4]

Infection of a Bartholin cyst results in a markedly tender abscess (Fig. 48–11). The abscess can rupture spontaneously and drain foul-smelling, purulent material externally through the skin. Multiple organisms are isolated from Bartholin abscesses. An early study using percutaneous aspirates from abscesses showed predominantly anaerobes and facultative organisms. *N. gonorrhoeae* was isolated in 8 percent of cases, and gram-negative bacilli were isolated in 16 percent. Although genital Mycoplasmataceae organisms were isolated from the duct secretions, they were not isolated directly from abscesses.[168] *Porphyromonas asaccharolytica* (a black-pigmented, gram-negative anaerobe) and *Salmonella panama* (after an attack of *Salmonella* enteritis) have been isolated from a Bartholin abscess.[52,68] Tuberculosis of the Bartholin gland has been reported.[63,133,134] Huffman[133] encountered tuberculosis of the Bartholin gland in a 14-year-old, sexually inactive girl with pulmonary tuberculosis. She had painless, unilateral swelling of the left labium majus with a brown discharge exuding from a small sinus. *Mycobacterium tuberculosis* was isolated in the discharge.

A Bartholin abscess should be incised and drained by applying a surface anesthetic, ethyl chloride, and making a 1- to 1.5-cm, full-thickness incision on the medial aspect of the labium majus. The cavity should be probed with a sterile cotton-tipped swab to break up loculations within the abscess. To allow for further drainage, one should pack the abscess cavity with sterile gauze. Alternatively, a Word catheter, a small rubber catheter with an inflatable balloon tip, can be inserted into the cavity and the balloon inflated with water. Clinical experience indicates that the packing method is far more painful to the patient, and healing takes longer.

Antibiotic coverage for anaerobes, *N. gonorrhoeae*, and *C. trachomatis* should be provided for at least 2 weeks, and a nonsteroidal medication is recommended for inflammation and pain. Frequent sitz baths help further with drainage and healing. Close follow-up during the first week is advised. The packing or the catheter may be removed after 4 days of antibiotics. Although marsupialization frequently was used to treat recurrent Bartholin abscesses, incision plus drainage and primary suture of the abscess cavity along with administration of an antibiotic (clindamycin) has been found to lead to more rapid healing and to decrease significantly the incidence of recurrent abscesses.[13]

POSTMENARCHEAL VULVOVAGINITIS

The most common types of vulvovaginitis in postmenarcheal girls are *T. vaginalis* vaginitis, vulvovaginal candidiasis, and bacterial vaginosis.

Postmenarcheal Vaginal Trichomoniasis

T. vaginalis is a triflagellated protozoan (Fig. 48–12). The organism, which is larger than a polymorphonuclear leukocyte, has a distinctive vibrating or whiplike movement when seen microscopically in fresh wet smears taken from the vagina. It quickly succumbs to reduction of the pH, drying, cooling, and changing the osmotic pressure of the fluid surrounding it. Most infections are encountered in sexually active young women; the incidence increases during the early reproductive years.

Trichomoniasis frequently is asymptomatic. When it is symptomatic, patients complain of a profuse, irritating discharge. The discharge and the pruritus tend to be more severe just before and immediately after a menstrual period. Recurrent exacerbations of the infection occur commonly. Patients occasionally report dysuria and abdominal pain.

Examination reveals diffuse vulvitis with erythema and excoriations and copious leukorrhea that covers the vulvar tissues. The discharge typically is frothy or bubbly, grayish yellow, and watery or mucopurulent. It has a pH of 5 to 7 and an acrid or musty odor. A "strawberry" or punctate vaginal eruption with hemorrhagic spots has been described as being typical of trichomoniasis. Such eruptions frequently are not present, however, even in severe cases. More often, diffuse inflammation causes the vaginal mucosa to be brilliant red.

The diagnosis of trichomoniasis usually is confirmed clinically by finding trichomonads in a wet smear of vaginal fluid. The vaginal specimen is obtained on a cotton-tipped swab and dipped into a small test tube with saline. After the solution has been stirred with the swab, one or two drops of the solution are placed with the swab on a slide. It is important that the slide be viewed under the microscope (dry high power) promptly because the organisms do not remain viable for long outside the vagina and are difficult to detect when they cease to be motile. The observer sees numerous ovoid-shaped, motile organisms. The sensitivity of this test with immediate evaluation is 60 to 70 percent. A vaginal cytosmear including a Papanicolaou (Pap) smear to detect trichomoniasis is not recommended because of the high rate of error in identifying trichomonads in stained smears. Other methods used to diagnose trichomoniasis include isolation by special culture medium, direct fluorescent immunoassay, and nucleic acid amplification test. Culture is the most sensitive, but it is not routinely available. The nucleic acid amplification test is available in some clinical settings (point-of-care test); however, a false-positive result may occur in low-prevalence populations.[42]

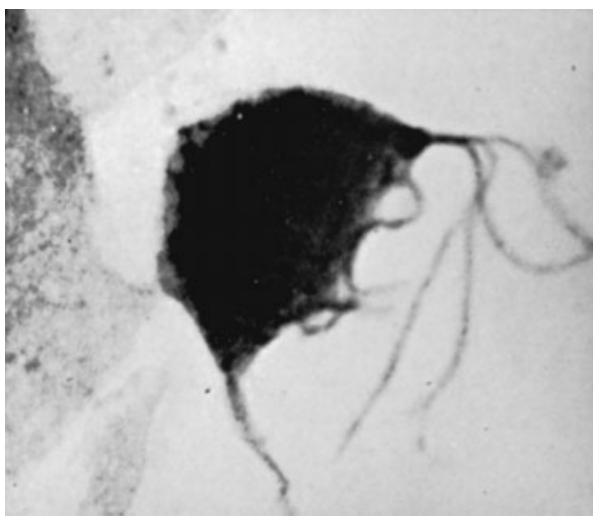

Figure 48–12 *Trichomonas vaginalis* is a triflagellated protozoan that, when motile, is identified easily in wet smears of vaginal discharge.

TREATMENT

A single 2-g oral dose of metronidazole (Flagyl) or tinidazole is the recommended treatment of choice. An alternative regimen is metronidazole, 500 mg orally twice daily for 7 days.[42] The cure rate is 95 percent in girls. If symptoms persist and wet smears from the vagina still show trichomonads, the treatment is repeated. Only in unusually persistent cases is a third course necessary. Strains resistant to metronidazole are rare. The patient should be warned of possible gastrointestinal side effects (nausea, diarrhea). Alcohol can aggravate the side effects of metronidazole therapy and cause a disulfiram-like reaction. The patient should be instructed not to consume alcoholic beverages during treatment. She should be told that trichomoniasis is an STD and that her sexual partner should be treated. Reacquisition of infection should be prevented via sexual abstinence or use of condoms. A follow-up test is unnecessary.

Metronidazole is a pregnancy category B drug, and tinidazole is a pregnancy category C drug; its safety in pregnant females has not been well evaluated. Multiple studies and data analysis subsequently have not shown a consistent association between metronidazole and teratogenic or mutagenic effects in infants.[42] Metronidazole can be given during the first trimester of pregnancy in adolescents.[42] Gentle vaginal douching with vinegar and water may relieve some symptoms but should not be encouraged during pregnancy.[134] Metronidazole gel is less effective in the treatment of trichomoniasis (<50%) and should not be used.[42] During lactation, breast-feeding can be withheld for 12 to 24 hours to reduce the exposure of metronidazole to the infant. Lactation should be withheld for 3 days when using tinidazole.

Postmenarcheal Mycotic Vulvovaginitis

Several factors play roles in causing the increased incidence of vaginal candidiasis after menarche. Menstruation, by altering vaginal pH, may offer a favorable medium for the growth of mycotic organisms. *Candida* spp. are found commonly in the intestinal tract and frequently are detected in stool cultures. Fecal contamination of the vulva during cleansing after defecation at a time when the normal vulvar and vaginal flora are depleted and not able to inhibit fungal growth would permit the development of symptomatic vulvovaginal candidiasis. Wearing tight-fitting underclothes (which keep the perineum warm and moist) is a factor that contributes to the growth of mycotic organisms. Female athletes and ballet dancers seem to be predisposed to development of candidiasis from wearing tight-fitting clothing and from increased sweating. The widespread use of antibiotics that disturb the vaginal flora and the use of oral contraceptives are additional factors that increase the incidence of candidal infection. Other predisposing factors include pregnancy, obesity, uncontrolled diabetes mellitus, immunosuppressive therapy, and heroin or other drug addiction. If a patient has a recurrent unexplainable or resistant infection, a glucose tolerance test should be done to rule out diabetes mellitus.

C. albicans and *Torulopsis glabrata* are the yeastlike fungi found most often in the vagina. *C. albicans* is responsible for 80 to 95 percent and *T. glabrata* for 3 to 16 percent of fungal infections. Other *Candida* spp. are detected less often in the vagina but can be pathogenic. *C. tropicalis*, similar to *C. albicans*, produces systemic infection in immunosuppressed hosts. Mycotic vulvovaginitis causes severe vulvar and vaginal itching; usually, the patient does not have an excessive discharge. The discharge is white,

thick, and curdled and has a yeasty sour odor. The patient may have pain during and after voiding or external dysuria as a result of urine coming in contact with excoriated areas on the urethra and vulva.

Examination in the acute stage reveals intense inflammation of the vulva and vagina that may extend to the perineal skin. Long-standing infection can cause lichenification and hyperpigmentation of the perineal skin. During acute infection, the involved areas are shiny and beefy red with linear excoriations and edema. *C. albicans* typically forms patches of mycelia that create adherent white plaques scattered over the inflamed surfaces. Superficial, red, weeping areas remain after the plaques are pulled away. Other *Candida* spp., notably *C. tropicalis*, do not form adherent plaques but produce a cottage cheese–like discharge similar to that found with *C. albicans.* Vaginal pH usually is normal (<4.5).

The diagnosis is established by wet preparation under microscopy and by finding hyphae and buds of the fungus in vaginal fluid (see Fig. 48–9), in the curdled discharge, in material scraped from the vulvar mucosa, or on the perineal skin. A wet preparation in normal saline may suffice, but debris and cellular material may render identifying the fungus difficult. If so, a smear using 10 percent potassium hydroxide solution is helpful. After one drop of potassium hydroxide has been added to one to two drops of vaginal fluid on a slide, the slide is heated gently until bubbles appear under the coverslip. The potassium hydroxide solution dissolves other extraneous material without affecting the fungus. With an experienced microscopist, this method yields a sensitivity of 86 percent in symptomatic girls; however, the yield may be only 40 percent. The diagnosis frequently is based on clinical findings, and cultural confirmation is unnecessary.

TREATMENT

Several vaginal preparations can be used. The antifungal creams commonly used are imidazoles (clotrimazole and miconazole) and polyenes (nystatin), but the polyenes should not be used because of increasing resistance of fungi to these compounds. A variety of imidazoles are available as over-the-counter vaginal creams, tablets, and coated tampons. Such agents include clotrimazole, miconazole, and terconazole. Intravaginal treatment with one 100-mg vaginal tablet of clotrimazole every night for 7 days produces a cure rate of 90 percent. Comparable cure rates are observed with 100 to 200 mg of clotrimazole or miconazole (2 tablets) for 3 days. The shorter regimen may result in better compliance. Creams and tablets are equally effective.[42]

Oral fluconazole, 150 mg as a single dose, is an effective preparation for the treatment of uncomplicated vulvovaginal candidiasis.[42] Low-dose prophylactic treatment also may be appropriate in these cases. Gentian violet, which has been used for many years, is considered an effective cheap treatment for vulvovaginal candidiasis in countries where the recommended pharmaceutical agents are unavailable.[133] Huffman[133] described painting the cervix and the vaginal and vulvar mucosa with a 1 percent aqueous solution. Care is taken to rotate the speculum so that the anterior and posterior vaginal walls are treated. The creases between the folds of the vulvar mucosa also are covered with the dye. The speculum is reinserted, opened, and left in place for 5 minutes so that all painted surfaces become dry. Treatment with gentian violet is repeated once weekly for 3 weeks and should include one treatment during a menstrual period. According to Huffman, gentian violet can cause herpes-like lesions on the vulva of some patients, and it is extremely messy.

Postmenarcheal Bacterial Vaginosis

Bacterial vaginosis is a noninflammatory polymicrobial condition caused by an ecologic change in the vagina; an overgrowth of anaerobes, especially *Bacteroides* and *Mobiluncus* spp., *G. vaginalis*, and *M. hominis*; and a decrease in the concentration of lactobacilli.[12,90,91,92,207] Bacterial vaginosis is considered an STD based on the occurrence of bacterial vaginosis with other STDs and in male partners of women with this condition. Bacterial vaginosis also has been described in adolescent girls who are not sexually active.[38]

Bacterial vaginosis has been associated with vaginal douching. It also has been noted in connection with postpartum endometritis, premature rupture of membranes, and PID.[131]

The primary complaint is an offensive odor with moderately profuse, gray-colored leukorrhea that stains the underwear. A mild pruritus or dyspareunia may be reported. A clinician often is able to identify this condition simply by the odor of the discharge. Examination shows little or no vulvar or vaginal erythema. The urethral and vulvar glands are not involved. The vagina contains a thick, homogeneous, grayish white discharge. The pH of vaginal secretions in bacterial vaginosis is 5 to 6. *T. vaginalis* tends to be associated with a vaginal pH of 6 to 8.

The diagnosis is made by the presence of any three of the following four criteria: (1) a homogeneous, gray-white malodorous discharge that smoothly coats the vaginal walls[73]; (2) a pH of nonbloody vaginal secretions greater than 4.5; (3) a fishy odor caused by the release of amines when 10 percent potassium hydroxide is added to a nonbloody vaginal specimen (whiff test); and (4) the presence of clue cells in a nonbloody specimen.[12] Microscopic examination of some of the discharge mixed with normal saline solution in a wet-mount preparation shows masses of desquamated vaginal epithelial cells and cellular debris. Clusters of bacteria adhere to the surface of many of the vaginal cells; these "clue cells" (Fig. 48–13) are characteristic of the condition.

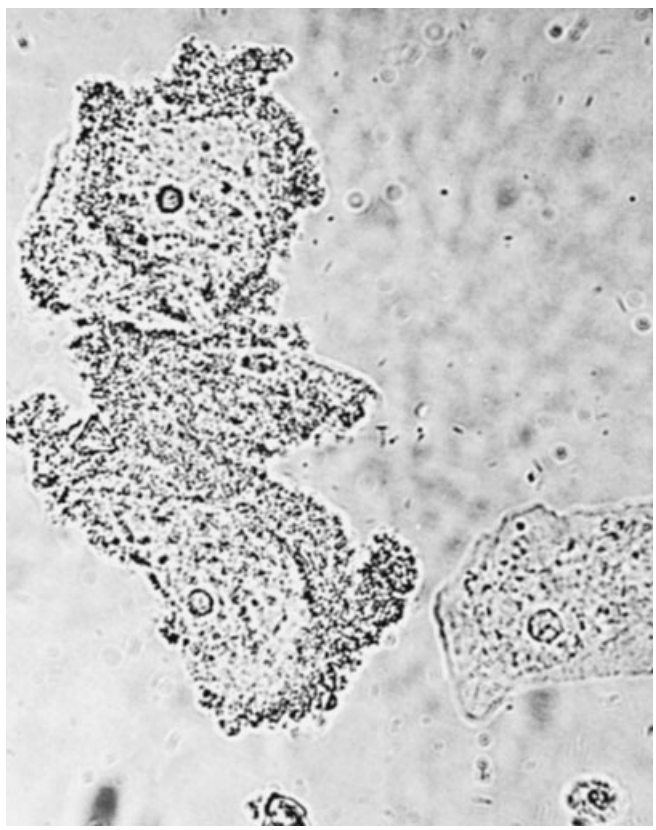

Figure 48–13 The finding of bacteria clinging to the sides of a vaginal epithelial cell ("clue cell") is significant in bacterial vaginosis. *(Courtesy of Dr. Herman L. Gardner.)*

Absence of erythrocytes and leukocytes in the discharge is another characteristic finding.

A Gram stain of vaginal secretions is considered the most reliable diagnostic test for bacterial vaginosis. A predominance of gram-variable cocci and curved rods (anaerobes) and occasional long gram-positive rods (lactobacilli) is seen.[12] A wet mount of vaginal secretions is a rapid and helpful test for clue cells in a busy clinical setting. Cultures for *G. vaginalis* and anaerobes are not useful clinically and are not recommended; Pap tests have no clinical utility for the diagnosis of bacterial vaginosis.

TREATMENT

The goal of therapy for symptomatic bacterial vaginosis is to relieve vaginal symptoms and prevent an ecologic environment that predisposes to the development of PID. Treatment of asymptomatic bacterial vaginosis may be considered before performing a surgical abortion procedure to prevent postpartum PID. In most cases, bacterial vaginosis responds to metronidazole, 500 mg given orally twice daily for 7 days,[42] or 2 percent clindamycin cream intravaginally every night for 7 days or 0.75 percent metronidazole gel intravaginally once a day for 5 days. Alternative regimens include clindamycin, 800 mg orally twice a day for 7 days, or clindamycin ovules, 100 mg intravaginally once a day for 3 days. These regimens provide effective coverage of anaerobes and *G. vaginalis.* Bacterial vaginosis that occurs during pregnancy generally is treated during the first trimester with clindamycin cream. During the second and third trimesters of pregnancy, it can be treated with clindamycin cream or metronidazole gel. Treatment of the sexual partner has not proved to be beneficial.[42]

Postmenarcheal Nonspecific Vulvovaginitis

Nonspecific vulvovaginitis has several contributory factors.[73,133] Vaginal foreign bodies cause discharges that are profuse, extremely malodorous, and sometimes bloody. The most frequent offender is a forgotten menstrual tampon, toilet paper, or a piece of condom. Improper or inadequate cleansing of the perineum after defecation also is responsible for many cases of chronic nonspecific vulvovaginitis, especially in mentally handicapped adolescent girls. If a male partner inserts his penis into the rectum first and then into the vagina or inadvertently touches the anal area before having vaginal intercourse, coliform organisms are carried into the vagina.

A tumor as the cause of a vaginal discharge is a very rare possibility. A discharge, with or without bleeding, may be one of the first symptoms of adenosis or adenocarcinoma of the vagina and cervix. These neoplasms are associated with prenatal exposure to diethylstilbestrol and related synthetic estrogens.

TREATMENT

Treating nonspecific vulvovaginitis in a postmenarcheal girl can be frustrating when no causative agent is found. Often, identifying the causative agent is impossible. In the case of a foreign body, it should be removed. Instructing the patient in proper perineal cleansing after defecation eradicates a major source of nonspecific vaginal infection in teenagers. Providing advice regarding sexual habits is necessary in some cases if coitus-related infections are to be avoided.

According to Huffman,[133] in most cases, nonspecific vulvovaginitis unassociated with chronic cervicitis responds to antimicrobial creams such as Sultrin or AVC vaginal cream inserted at bedtime for 14 days. Generally, creams are preferable because they spread over a larger area of the vaginal mucosa than is treatable with suppositories. Vaginal douching sometimes may be considered helpful in the treatment of nonspecific vulvovaginitis. More recent data suggest, however, that a causative relationship exists between vaginal douching and PID in inner-city populations at high risk for acquiring STDs.[291] Vaginal douching should be discouraged strongly.

Toxic Shock Syndrome

First recognized in the late 1970s, TSS is an uncommon, serious, sometimes fatal acute infection caused by toxin-producing *S. aureus* infection. TSS can occur in males and females of all ages; this entity is discussed fully in Chapter 71. With regard to the genital tract, more than 800 TSS cases occurred in women in 1980 in association with use of a very high absorbency tampon. This type of tampon is no longer marketed. Prolonged retention of currently marketed tampons could be a predisposing factor for TSS or other local infections, but TSS associated with tampon use is now a rare event.

VULVOVAGINAL VIRAL INFECTIONS

GENITAL HERPES

Premenarcheal Patient

HSV infection of the genitalia rarely occurs in children.[116] The mode of acquisition of the infection is not always known, but case reports describe the possibility of autoinoculation from oral lesions, physical contact by a mother's infected finger, and sexual abuse.[146] The presence of genital herpes lesions in a child or any disease that is known to be sexually transmitted in an adult should raise a question about whether the child has been subjected to some type of sexual molestation. The virus can be isolated from the pharynx or vagina of approximately 5 percent of asymptomatic adults. Such individuals are carriers and may transmit the infection to susceptible contacts. That children would have either HSV type 1 or HSV type 2 genital lesions is not surprising.[191]

Herpetic lesions on the vulva begin as small, erythematous spots. Papules quickly develop on the inflamed areas. The papules become serum-filled vesicles that rupture and leave slightly eroded red areas. The latter become covered with crusts, which remain for a few days. The lesions typically cause pruritus and burning. If they do not become secondarily infected, they heal within 2 weeks.

Herpes limited to the genitalia of a healthy child is a painful but relatively benign disease. An infection in a poorly nourished child may spread beyond the vulva and become a serious, life-threatening matter. Treatment of genital herpes is discussed later.

Adolescent Patient

HSV is the most common cause of vesiculoulcerative disease of the adult genitalia.[190] It is sexually transmitted, and its increasing frequency in teenage girls is related to their sexual activity. As noted in Chapter 170, two types of HSV exist and can be antigenically and culturally distinguished from each other. Type 1 is the causative agent in oronasal cold sores. Type 2 is responsible for 70 to 95 percent of genital herpes, but genital infections with type 1 have become more common (5 to 30%), probably related to increasing engagement in genital-oral sex play.[45]

Seroprevalence studies show that type 2 antibodies do not begin to appear until the early teens. The frequency gradually increases through late adulthood and is related to sexual activity, especially with multiple partners. Most individuals with type 2 antibodies are not diagnosed with genital herpes. Type 2 infections are highly contagious and may be transmitted by carriers who are asymptomatic. Because of its frequency and diverse clini-

cal appearance, it should be considered in the differential diagnosis of all vulvar vesiculoulcerative lesions. Because of its acute symptoms, it may mask the presence of other venereal disease acquired concurrently; a patient with genital herpes always should be examined for gonorrhea and syphilis.

Three manifestations of genital disease are recognized: primary initial, nonprimary initial, and recurrent infection. Primary initial genital herpes infection develops with no preexisting herpes antibody. A nonprimary initial infection develops in an individual for the first time with preexisting herpes antibody. Recurrent infection is diagnosed when an individual has a history of previous similar genital infection. Patients with nonprimary initial infection have fewer lesions, less pain, fewer constitutional symptoms, shorter duration of viral shedding, and an overall shorter course of illness.[48]

A primary infection begins with sexual contact with an infected individual 2 to 8 days before the onset of symptoms. The individual probably has a prodromal episode of fever, malaise, and myalgia, often accompanied by vulvar paresthesia and burning, dysuria, and tender nonsuppurative inguinal lymphadenopathy.

Primary lesions, which often involve all the vulvar tissues, the vaginal mucosa, and the cervix, occur during a 2-week period. They first appear as papules surrounded by an erythematous zone and subsequently become vesiculopustular lesions. The vesicles enlarge and rupture, and shallow ulcerations are exposed. During the acute phase of a severe infection, more or less edema and generalized erythema of the vulvar mucosa are present (Fig. 48–14).

The ulcers generally become covered with a firm yellow crust that drops off after a week or so, with a smooth, red area left behind that eventually disappears. The lesions usually are asymptomatic for 10 to 21 days, depending on the severity of the infection. Secondary bacterial infection and a coexisting immunodeficiency state, such as HIV infection, delay healing. Urethral and vesical involvement may cause severe dysuria leading to retention of urine. Proctitis may occur in adolescent girls who engage in anal intercourse, although perianal ulcers also may occur without anal intercourse. The cervical lesions are ulcerations on the exocervix and may range from erythema to severe necrotic cervicitis. Acute cervicitis may be the only manifestation of primary HSV infection.

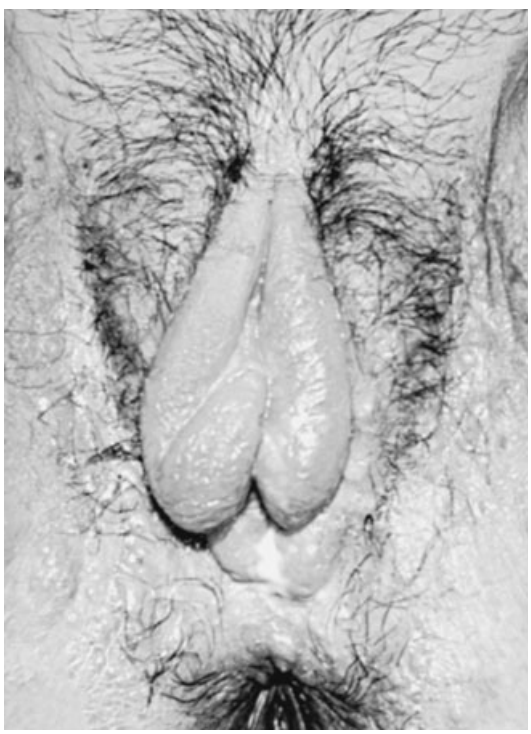

Figure 48–14 Genital herpes in an adolescent patient.

Inguinal lymphadenopathy and moderate lower abdominal pain may be present; if so, they usually occur only with the more severe first eruption. Tender inguinal lymphadenopathy is the last to resolve. If the urethra is involved, the dysuria may be sufficiently severe to cause urinary retention. Insertion of a vaginal speculum may be exquisitely painful. Extension to the perianal area may cause severe discomfort on defecation. Complete healing of lesions at all sites occurs in approximately 3 weeks. When healed, herpetic lesions rarely leave scars.

The diagnosis of genital herpes usually is not difficult to establish. The intense pain and the superficial vesiculoulcerative lesions with their irregular margins and red areolae are sufficiently characteristic to render clinical identification easy in a typical case. Laboratory confirmation should be attempted for all children and adolescents. Direct isolation of HSV by tissue culture is the preferred diagnostic method and is best when the specimen is taken from a lesion within the first 48 hours of the onset of symptoms. The virus can be cultured from the cervix in 90 percent of girls with primary type 2 infections, and the cervix appears abnormal in almost 90 percent of cases with positive cervical cultures. PCR assays for HSV DNA are more sensitive than is culture and are used in settings when available. PCR testing of genital secretions has not been approved by the U.S. Food and Drug Administration (FDA), however.[42]

Type-specific and non–type-specific antibodies to HSV develop during the first several weeks after infection occurs and persist indefinitely. Commonly available serologic tests cannot differentiate type 1 from type 2 virus. Type-specific antisera using glycoprotein G–based assays showing an increase in anti-HSV titer are useful in identifying primary type 2 infections. These tests have limited usefulness for recurrent infection, however. A variety of glycoprotein G–based assays (enzyme-linked immunosorbent assay) are available and have to be specifically requested. Point-of-care HSV tests using glycoprotein G also are available for immediate office-based testing and work best in settings with high prevalence of infection. False-positive results have been reported in individuals with low likelihood of infection.[42]

The presence of multinucleated giant cells in a Pap smear (Fig. 48–15) or in a herpetic fluid smear with a Tzanck preparation is only 40 to 50 percent sensitive compared with culture. These tests may be useful in settings where other diagnostic tests are unavailable but should not be relied on.[42]

For many weeks after the infection has subsided clinically and the patient seems to be cured, type 2 virus can be recovered from the cervix and vagina. The latent virus may infect others or cause recurrent infections in the host. Although the recurrence rate of genital herpes is unknown, recurrent episodes develop in 80 percent of patients with type 2 infection; clinical recurrence of type 1 genital infection is much less common.[48] Factors influencing recurrence rates are the severity of the initial episode and the host immune response to the disease. Emotional stress, heat, moisture, climate change, menstruation, pregnancy, oral contraceptive use, anesthesia, and trauma seem to be triggering factors. The median time from a primary infection to development of a secondary or recurrent infection is approximately 120 days.[48,256,284] Such recurrent episodes, although painful, usually are less severe than are primary infections. Some patients have recurrences immediately preceding or at the time of each menstrual period. Researchers have suggested that genital herpes and squamous cell carcinoma of the cervix are related.[36] Most of the evidence favoring this association originally came from seroepidemiologic studies. A much stronger association now has been observed

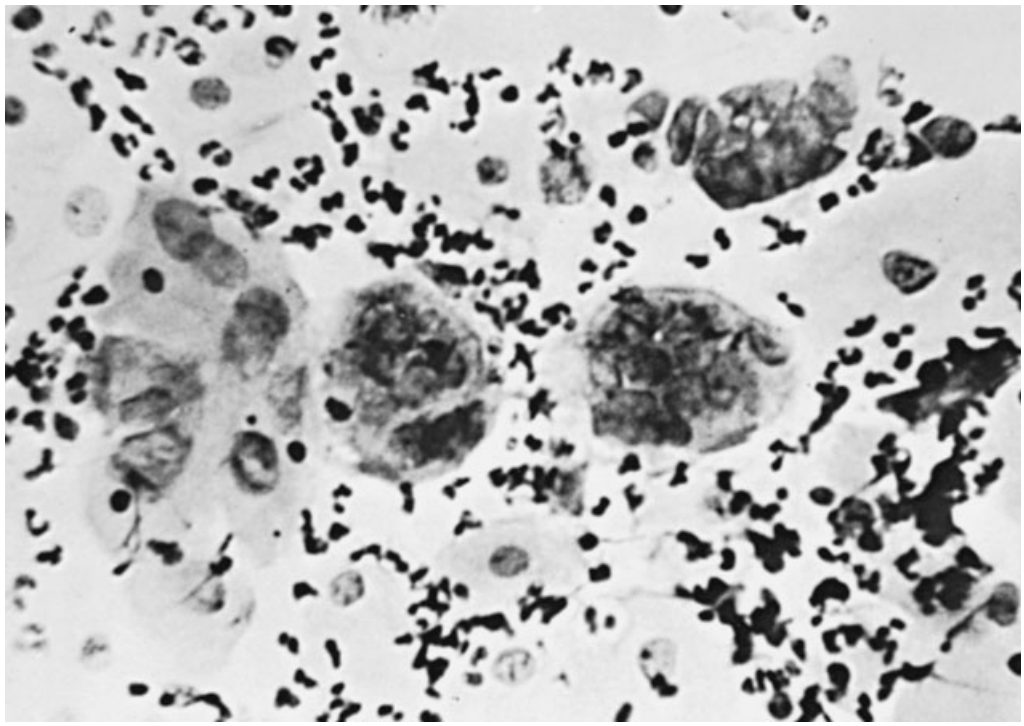

Figure 48–15 Multinucleated giant cells in a vaginal smear are characteristic of genital herpes. *(Courtesy of Dr. Herman L. Gardner.)*

between HPV and cervical cancer. At most, HSV may be causative or may be a cofactor in some cases of cervical cancer.[48]

Treatment

Several medications are available today for the treatment of genital herpes.[42] Acyclovir is the most affordable current drug on the market. For the treatment of initial genital herpes, acyclovir, one 400-mg capsule orally three times a day or one 200-mg capsule five times a day, is administered for 7 to 10 days until clinical resolution. Alternative drugs include famciclovir, one 250-mg capsule three times a day, or valacyclovir, one 1-g capsule twice a day for 7 to 10 days. These drugs are reported to shorten the time that the patient has pain, decrease the number of new lesions, hasten crusting, and reduce the time that the virus can be found in the lesions. The earlier treatment is started after the initial lesions appear, the sooner patients obtain relief.

In severe primary herpes infection with marked systemic symptoms, intravenous acyclovir, 5 to 10 mg/kg/day every 8 hours for 5 to 7 days or until clinical resolution occurs, is recommended. Frequent or severe recurrent disease may benefit from medication if it is started within 2 days of the onset of lesions or at the beginning of the prodrome. Oral acyclovir may be given at 800 mg twice a day, 400 mg three times a day for 5 days, or acyclovir 800 mg three times a day for 2 days. Alternatively, famciclovir, 125 mg twice a day, or valacyclovir, 500 mg twice a day for 3 days, or valacyclovir 1000 mg once a day for 5 days, may be prescribed. Daily suppressive treatment reduces recurrence by at least 75 percent in patients with frequent recurrences. Because of adherence problems, daily suppressive therapy may not be a practical treatment approach for adolescents. The recommended treatment is oral acyclovir, 400 mg twice a day; famciclovir, 250 mg twice a day; or valacyclovir, 500 or 1000 mg once a day for 1 year. Medication should be discontinued after 1 year to reassess the recurrence rate. The dosage regimen of medications for treating prepubertal genital herpes is unknown, and such medications are not used commonly in this age group for mild cases. In moderate to severe cases, clinicians have prescribed approximately 50 percent of the adult dose.[42]

Recurrences can be reduced in frequency in some cases if the patients can avoid stress-inducing situations. Topical anesthetics, cold wet compresses, and sitz baths with 1:40 Burow's solution frequently reduce local discomfort. Severe dysuria may be relieved by urinating when sitting in water. Local therapies used in the past, such as povidone-iodine solutions, photodynamic dye light therapy, and topical surfactant, have not been found to be beneficial and are messy and potentially toxic.

Lesions caused by HSV are common manifestations in patients with HIV infection. Immunocompromised patients benefit from an increased dosage of acyclovir. For severe disease, hospitalization may be required for intravenous acyclovir treatment.[42]

CONDYLOMATA ACUMINATA AND HUMAN PAPILLOMAVIRUS

Condylomata acuminata are encountered in premenarcheal and teenage girls. The agent causing them is HPV, a small, slow-growing virus of the papovavirus group. HPV types 6 and 11 are responsible for low-grade lesions and for 90 percent of genital warts. HPV types 16, 18, and 31 are associated with premalignant and malignant cervical carcinoma in women,[252] with HPV types 16 and 18 causing 70 percent of premalignant and malignant lesions.

Premenarcheal Patient

Warts in premenarcheal children may cover the entire vulva. Most often, however, warts in this population are single or scattered, cauliflower-like lesions (Fig. 48–16). They have a predilection for the smooth, moist mucosa covering the inner surfaces of the labia minora and for the mucosa around the urethral meatus. The vestibular mucosa may be studded with innumerable minute excrescences as well.

Condylomata in children seldom become ulcerative, but sessile condylomata arising within the vagina may become necrotic; produce a bloody vaginal discharge; and resemble, on cursory examination, a mixed mesodermal tumor (sarcoma botryoides) of the vagina. Although not tumors, condylomata acu-

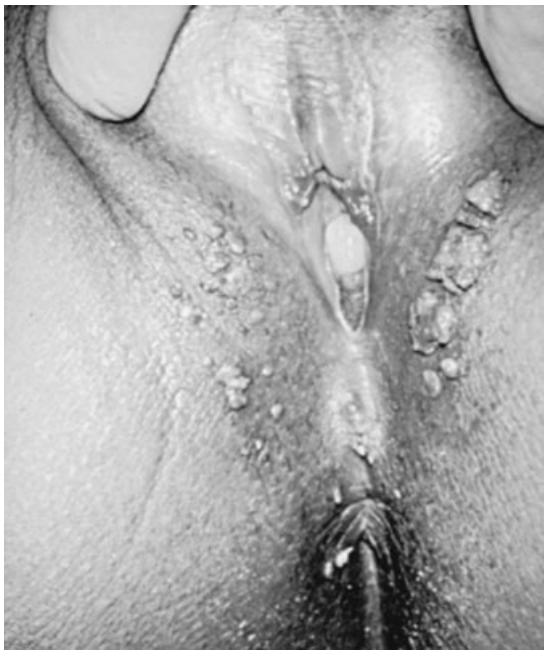

Figure 48–16 Condylomata acuminata usually are single or scattered, small warts in premenarcheal children.

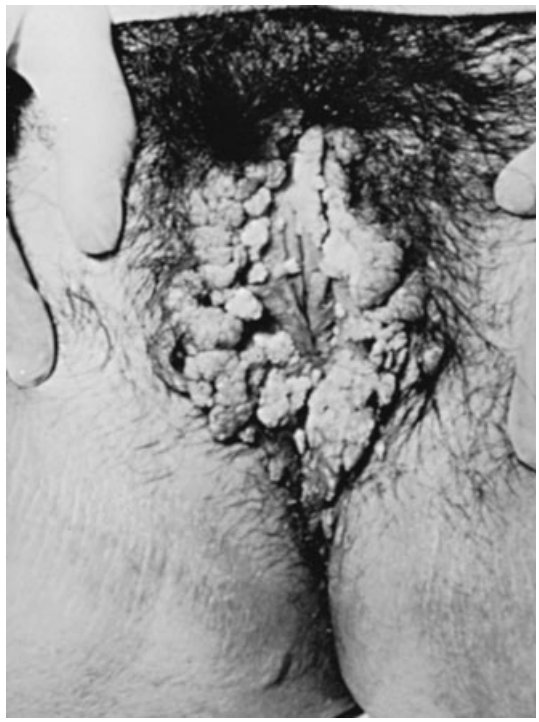

Figure 48–17 Condylomata acuminata may form large masses covering the vulva in postmenarcheal patients.

minata should be considered in the differential diagnosis of vaginal neoplasms. HIV infection should be considered in infants and children with severe, extensive warts.[47,57,100,252]

Adolescent Patient

Condylomata acuminata develop in adolescent girls by sexual transmission. The prevalence of HPV in adolescent girls and young women is 11 to 46 percent, depending on the method of HPV DNA detection used.[229,252] The PCR technique is more sensitive than is the dot-blot hybridization method (ViraPap; Life Technologies, Gaithersburg, MD). The prevalence of HPV in adolescents as detected by DNA isolation techniques is far higher than the prevalence of active disease. The cell-mediated or T-cell immune system seems to play an important role in whether the presence of virus results in clinically manifested disease. Cofactors of HPV infection are herpes, cervicitis, and tobacco use. Immunosuppression from a variety of conditions, including renal transplantation, Hodgkin disease, and HIV infection, is associated with a greater likelihood of development of HPV disease.[229] With regard to the natural history of HPV infection, Moscicki and colleagues[184,185] found that during the course of a 2-year period, 70 percent of cases showed regression, and low-grade squamous intraepithelial lesions (LSIL) did not develop. Daily cigarette smoking was strongly linked to the development of LSIL.[185] Regression was more likely to be seen in young women with LSIL as opposed to high-grade squamous intraepithelial lesions (HSIL). HSIL was more likely to develop in individuals with persistent positive tests showing oncogenic HPV types.

In postmenarcheal girls, condylomata usually form discrete, sessile, vegetative, wartlike growths covered with folded grayish pink epithelium. They are found most frequently on the smooth mucosa of the vulvar vestibule. Usually, they are accompanied by more or less vaginal discharge, which is increased by moisture that exudes from between the leaflike folds of the warty masses. Most often, associated pruritus and secondary infection are present. Warts are more likely to become necrotic in adults than in children. Contact lesions frequently appear on contiguous surfaces. Huge condylomatous masses (Fig. 48–17) that completely hide the introitus are common findings in adolescent patients; if not treated, these huge growths may invade the rectum. Vaginal and cervical lesions commonly accompany the lesions on the vulva.

Treatment

The goal of treatment is removal of warts and amelioration of symptoms, not eradication of HPV. No therapy has been shown to eradicate HPV. When only a few lesions are present, they can be treated in the outpatient setting with 85 percent trichloracetic acid. In infants and children with extensive lesions, Emans and colleagues[74] recommend carbon dioxide laser treatment with general anesthesia. It may be performed in an outpatient surgical setting when few lesions are present. Electrocautery, electrocoagulation, or laser treatment may result in deep scarring and distortion of the vulva. Older children and adolescents usually tolerate cryotherapy without general anesthesia if they know that some tingling or burning sensation is associated with it. Liquid nitrogen may be used in the same manner as solid carbon dioxide.

The therapeutic methods available are 22 to 94 percent effective in clearing exophytic genital warts, but recurrence rates are high, at least 25 percent within 3 months. Treatment seems to be more successful for genital warts that are small and present for less than 1 year.[42] Self-application of medication may be considered in young adults but should be discouraged for adolescents because of the risk of causing dermatologic side effects. When a few lesions are present, they can be treated with 10 to 25 percent podophyllin resin or a solution of 85 percent trichloroacetic acid. The lesions that are treated should be less than 1 cm in diameter and should not be in a confluent mass. Four hours after podophyllin has been applied, the treated area should be washed off with soap and water. The medication is applied to the wart only (not to the surrounding skin), and treatment is

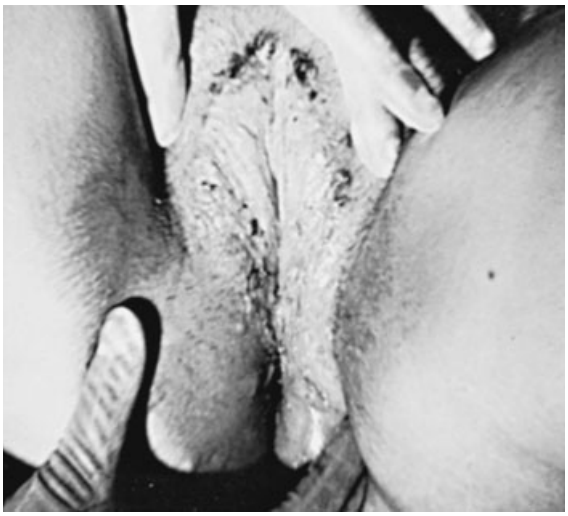

Figure 48–18 The appearance of the vulva 10 days after electrocoagulation and surgical excision of the condylomatous mass shown in Figure 48–17.

repeated once weekly until the lesions have disappeared (usually 3 to 4 weeks).

Urethral, anal, vaginal, and cervical lesions should not be treated with podophyllin. Podophyllin and trichloracetic acid can cause severe irritation of normal skin and can cause ulcerations. Before application, the normal skin around a wart should be covered with K-Y jelly, and a thin or narrow swab should be used for small warts. If excessive medication has been applied, the area should be washed immediately with soap and water, followed by the application of talcum powder or bicarbonate of soda to soothe the area. Imiquimod 5 percent cream also is recommended for the treatment of warts in patients older than 18 years.[41] This medication is self-applied to the lesions, three times a week for 16 weeks. There are reports in the international literature regarding the successful treatment of extensive warts in children using imiquimod 5 percent cream.[171] Laser treatment or cryocautery is recommended if the lesions do not respond to chemical cautery or if they progressively increase in size. Large perianal warts may need to be removed surgically if cryotherapy does not work (Fig. 48–18). Alternative treatments for adolescents are topical 5-fluorouracil in 5 percent creams and intralesional interferon. 5-Fluorouracil is preferable for vaginal and intraurethral warts because it causes erosive dermatitis of normal surrounding skin. The use of 5-fluorouracil seems to be more effective than is the laser in treating exophytic warts in the vagina.[42,57]

An annual Pap smear is recommended in all adolescent girls 3 years after the onset of sexual activity. In an adolescent girl who has not had sex, the first Pap smear is recommended at age 21 years.[294] The quadrivalent HPV vaccine contains HPV types 6, 11, 16, and 18 and has been approved by the FDA to prevent genital warts and cervical cancer. It is recommended that females 9 to 26 years old receive the vaccine at age 11 to 12 years during their routine immunization visit. Catch-up vaccination is recommended for females 13 to 26 years old who have not been vaccinated previously, regardless of their sexual history.[174] FDA approval for a bivalent HPV vaccine containing HPV types 16 and 18, to prevent cervical cancer for a broader age range of women, is in progress.

MOLLUSCUM CONTAGIOSUM

Molluscum contagiosum is a viral infection of the skin characterized by small, discrete, translucent, grayish pink, umbilicated, wartlike papules that sometimes are surrounded by a narrow ring of erythema. The lesions, which are asymptomatic, usually are less than 5 mm in diameter and may be missed by the patient and the examiner. The disease is transmitted by close physical contact and is encountered most often in postmenarcheal patients on the inner surfaces of the thighs and perineum. Although lesions on the lower part of the abdomen and thighs have caused this infection to be classed with venereal diseases, coitus is not necessary for transmission. Pediatricians frequently encounter molluscum contagiosum on the nongenital skin of children. It can be contracted from contaminated towels, bedding, and garments. Lesions of molluscum contagiosum on the lower part of the abdomen, pubis, thighs, or perineum of a child are tacit evidence of sexual contact.[129,170]

The appearance of the lesions usually is sufficient to establish the diagnosis. It can be confirmed by finding large, intracytoplasmic inclusion bodies in a smear or biopsy specimen from a lesion.

Treatment consists of lifting off the roof of each lesion and lightly curetting its base. Cryotherapy and topical imiquimod also give good results.[129,170] A variety of treatment approaches are available for children and adolescents. Curettage is the most efficacious treatment, with a low rate of side effects; however, the procedure requires anesthesia and is time-consuming. The roof of each lesion is lifted off, and the base is lightly curetted. Cryotherapy gives good results. Cantharadin is another useful bloodless alternative, but it can cause blisters. Topical imiquimod holds promise, but an optimal treatment schedule has not been determined.[42]

VULVOVAGINAL GRANULOMATOUS AND ULCERATIVE DISORDERS

Sexually transmitted genital ulcerative diseases occur throughout the world but are found most frequently in tropical countries. Genital ulcers are a common finding on evaluation of adolescent and adult patients with genital symptoms. They are less common findings in young children. Ulcers usually are secondary lesions that result from the breakdown of vesicles, papules, or pustules. By the time that many patients with genital infections seek medical attention for their symptoms, the primary lesion has proceeded to ulceration.[254] Microbial causes vary by geographic region and socioeconomic status: HSV is the most common cause in Western Europe and North America, whereas chancroid is the most common one in the tropics; in the United States, syphilis and chancroid are more common occurrences in urban minority groups, whereas HSV is found more commonly in more affluent groups. Complications of sexually transmitted genital ulcerative diseases also occur more commonly in developing countries and often are the reason for seeking medical care.[187]

HSV infection, syphilis, chancroid, lymphogranuloma venereum, granuloma inguinale, and tuberculosis all may have genital ulcers as a major clinical finding (Table 48–4). Although each of these diseases has a characteristic lesion and course, considerable overlap exists, so a diagnosis based on the history and physical appearance alone often is inaccurate. Herpetic ulcers, contrary to classic manifestations, can be painless, and syphilis chancres can be painful at times. Secondary infection of an ulcerated area may cause pain in lesions that characteristically are painless. In addition, more than one STD may be present in at least 3 to 10 percent of patients with genital ulcers.

Genital ulcers also may result from infestation, fixed drug eruption, mechanical or chemical trauma, autoimmune processes (e.g., Behçet syndrome and Crohn disease), and neoplasia. The presence of ulcers in sites other than the genital regions or oropharynx suggests a noninfectious etiology.

TABLE 48–4 Diagnostic Features of Genital Ulcerations Caused by Sexually Transmitted Diseases

Feature	Primary Syphilis	Genital Herpes	Chancroid	Lymphogranuloma Venereum	Granuloma Inguinale
Incubation period	9-90 days; avg., 2-4 wk	2-7 days	1-35 days; avg., 3-7 days	3 days to 3 wk; avg., 10-14 days	Precise data unavailable; probably a few days to several months
No. lesions	Usually one; may be multiple	Multiple; may coalesce, more with primary episodes than with recurrences	Usually one to three, may be multiple	Usually single	Single or multiple
Description of genital ulcers	Sharply demarcated, round or oval ulcer with slightly elevated edges; may be irregular, symmetric "kissing chancre"	Small, superficial, grouped vesicles, erosions, or both; lesions may coalesce and form bullae or large areas of ulceration; lesions have irregular borders	Superficial, shallow, sharply demarcated ulcer; irregular, ragged, undermined edge; a few millimeters to 2 cm in diameter	Papule, pustule, vesicle, or ulcer discrete and transient; frequently overlooked	Sharply defined, irregular ulcerations or hypertrophic, verrucous, necrotic, or cicatricial granulomata
Base	Red, smooth, and shiny or crusty; oozing serous exudate when squeezed	Bright red and smooth	Rough, uneven, yellow to gray	Variable	Usually friable, rough, beefy granulations; can be necrotic, verrucous, or cicatricial
Induration	Firm; does not change shape with pressure	None	Soft; changes shape with pressure	None	Firm granulation tissue
Pain	Painless; may become tender if secondarily infected	Common; more prominent with initial infection than with recurrences	Common	Variable	Rare
Inguinal lymphadenopathy	Unilateral or bilateral, firm, movable, and nontender; does not suppurate	Usually bilateral, firm, and tender; more common in primary episodes than in recurrences	Unilateral; bilateral rarely occurs; overlying erythema; matted, fixed, and tender; suppuration may occur	Unilateral or bilateral; initially movable, firm, and tender; later indolent; fixed and matted; "sign of groove" may suppurate; fistulas	Pseudobuboes; subcutaneous perilymphatic granulomatous lesions that produce inguinal swellings
Constitutional symptoms	Rare	Common in primary episode; less likely in recurrences	Rare	Frequent	Rare
Course of untreated disease	Slowly (2-6 wk) resolves to latency	Recurrence is the rule	May progress to erosive lesions	Local lesions heal; systemic disease may progress; disfiguring; late complications	Worsens slowly
Diagnostic tests	Darkfield examination, direct immunofluorescence, FTA-ABS, VDRL	Tzanck smear, culture, Pap smear, direct immunofluorescence, electron microscopy, direct immunoperoxidase staining, serology	Culture, biopsy (rarely used), Gram-stained smears have low specificity	Complement fixation, isolation of microorganism by culture	"Donovan bodies" in tissue smears; biopsy

FTA-ABS, fluorescent treponemal antibody absorption test; VDRL, Venereal Disease Research Laboratory.
From Mroczkowski, T. R., and Martin, D. H.: Genital ulcer disease. Dermatol. Clin. North Am. 12:753-764, *1994.*

In the United States, most patients with genital ulcers have genital herpes, syphilis, chancroid, or a combination thereof. Empiric treatment often must be given before diagnostic test results are available, and laboratory confirmation of a specific diagnosis is lacking in at least a quarter of patients with genital ulcer disease. Treatment of syphilis and chancroid should be considered in such circumstances, especially in geographic regions where chancroid morbidity is notable (see later).[41,203] Diagnosis and treatment of the listed infectious etiologies of genital ulcers and Behçet syndrome are discussed in this chapter and elsewhere in this text.

Genital ulcers of any etiology, but especially those caused by herpes, syphilis, chancroid, and granuloma inguinale, are associated with an increased risk of acquiring HIV infection. Serologic testing for HIV infection should be considered in the management of patients with genital ulcers.[41,188] Improved treatment of STDs can affect the rate of HIV seroconversion in a population: HIV seroconversion was reduced by 40 percent over the course of a 2-year follow-up period in rural communities in Tanzania where treatment programs were instituted compared with control communities with no programs.[103]

LYMPHOGRANULOMA VENEREUM

Lymphogranuloma venereum is an STD characterized by chronicity, indolent inflammatory infiltration, granulomatous ulceration, formation of abscesses, and fibrotic cicatrization of the inguinal, perineal, and rectal lymphatics. The infecting agent is *C. trachomatis*, subtypes L1 to L3. It occurs more often in tropical than temperate climates.

Premenarcheal Patient

Lymphogranuloma venereum has been reported in children.[17,98,285] The disease usually is acquired in childhood as a result of sexual contact, but transmission also may occur by accidental inoculation of infected material from family members, such as by handling of garments or towels that have been contaminated by drainage from ulcerative lesions or buboes. Sexual abuse always should be considered, however. Some evidence suggests that transplacental or perinatal transmission of the lymphogranuloma venereum serovars of *C. trachomatis* can occur.[98]

The primary lesion, a small papule or superficial and relatively asymptomatic ulcer, seldom is seen in either children or older patients. A prodromal episode of fever, malaise, and joint pain accompanied by leukocytosis, anemia, and an increased erythrocyte sedimentation rate may precede the local signs. These symptoms often are mild and not diagnostically significant.

The most common manifestations in prepubertal children are inguinal lymphadenopathy and proctitis. The glands may be swollen and tender for a while, and then regress spontaneously. More often, if treatment is not initiated, the lesions progress to formation of an abscess and then rupture, with the development of draining sinuses. Rectal, anal, and deep pelvic tissue infiltration with rectal and colonic strictures is an uncommon finding in children. Arthritis, usually of the knees, and erythema nodosum occasionally occur in children, as they do in older patients.[98]

Adolescent Patient

Adolescents usually acquire lymphogranuloma venereum through sexual activity. The primary lesion—a small papule, vesicle, or shallow ulcer on the vulva (Fig. 48–19), vaginal wall, or cervix—seldom is seen. Cervicitis is a more common finding in primary lymphogranuloma venereum, and the primary lesion often is asymptomatic, heals rapidly, and leaves no scar. The incubation period usually is 2 to 5 days after exposure, but several weeks may elapse before the primary lesion appears.[98,133]

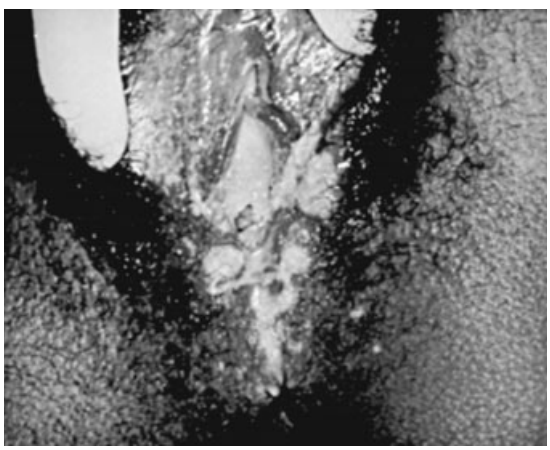

Figure 48–19 Early lesions of lymphogranuloma venereum in the form of herpetic-like ulcers in an adolescent. The diagnosis was confirmed serologically.

Painful enlargement of the inguinal lymph nodes generally occurs after the manifestation of the initial lesions. Inguinal adenitis usually is unilateral in the early stages of infection. The perirectal lymph nodes occasionally are the first to be involved; pain on defecation is an early symptom when this condition occurs. The rectovaginal septum may become involved when the posterior vaginal area is a primary site of infection.

If the disease goes untreated, it may enter a secondary stage called the *inguinal syndrome*, in which the inguinal glands and the surrounding subcutaneous tissues become a brawny mass that adheres to the indurated, purplish red overlying skin. The nodes increase in size and form abscesses (buboes). Unless aspirated, the buboes rupture and create chronically draining sinuses.[133] Enlargement of lymph nodes above and below the inguinal ligament may lead to the "groove" sign.[98] Complaints of lower abdominal pain and backache may indicate concurrent proctitis. The deep iliac nodes may be enlarged in 75 percent of cases; rupture of these nodes may cause large pelvic abscesses.

Healing of draining buboes is slow, and severe scarring occurs. In most cases, healing of buboes indicates the end of the disease, but relapses have been reported in 20 percent of untreated cases. Disseminated disease, hepatitis, pneumonia, arthritis, erythema nodosum, erythema multiforme, and ocular infection can occur in association with the inguinal syndrome.

The anogenitorectal syndrome is a subacute manifestation of lymphogranuloma venereum. It includes proctocolitis and hyperplasia of intestinal and perirectal lymphatic tissue. Perirectal abscesses often develop and lead to ischiorectal and rectovaginal fistulas, anal fistulas, and rectal stricture or stenosis. The clinical findings consist of fever, rectal pain, and abdominal cramping. Progression of the disease causes rectal bleeding and a purulent rectal discharge. Rectal strictures lead to constipation and "pencil stools." Weight loss, bowel perforation, and peritonitis may occur. Genital elephantiasis (esthiomene), a primary infection affecting the lymphatics of the scrotum, penis, or vulva, may occur with chronic infection.

The diagnosis of lymphogranuloma venereum should be considered whenever a patient has a tender, enlarged inguinal gland or an ulcerative or granulomatous lesion of the vulva, perineum, vagina, or cervix. Acutely painful, unilateral inguinal lymphadenitis strongly suggests lymphogranuloma venereum. A definitive diagnosis is made by identification of *C. trachomatis* serotype L1, L2, or L3 in tissue cultures of material from buboes or ulcerative lesions. The sensitivity of culture for lymphogranuloma venereum is only approximately 50 percent, however.[145] Serologic tests may be used when cultures are negative or buboes are not present. Complement fixation has been available since the 1930s and 1940s. In clinical settings suggestive of lymphogranuloma venereum, a complement-fixation antibody titer of 1:64 or higher is considered diagnostic. Most patients with lymphogranuloma venereum have titers of 1:128 or higher. Titers between acute and convalescent specimens may not increase because most patients seek care well after the acute phase of the infection has developed. Cross-reaction with other chlamydial serotypes does occur, but titers higher than 1: 6 rarely are seen with chlamydial urethritis. Microimmunofluorescence is more specific than is complement fixation and can detect IgM and IgG titers. This test is not widely available.[145]

Complement fixation remains the recommended test for diagnosis when the clinical findings lead to a presumptive diagnosis of lymphogranuloma venereum. If complement-fixation titers are negative, repeat testing in a few weeks may be helpful in patients who happen to be evaluated first early in the course of illness. DNA probes or monoclonal antibodies specific for lymphogranuloma venereum serovars may become available in the near future.

Lymphogranuloma venereum must be differentiated from syphilis, with which it may coexist. False-positive Venereal Disease Research Laboratory test results may occur in 20 percent

of patients with lymphogranuloma venereum. When the diagnosis is in doubt, specific tests for antitreponemal antibodies must be used to rule out syphilis. The disease is differentiated from other granulomatous and ulcerative disorders by specific tests for each, by biopsy, and by the clinical appearance of the lesions (see Table 48–3).

Treatment

The earlier the diagnosis is made and treatment is started, the better the response to therapy and the less serious the tissue destruction. Fluctuant buboes are aspirated, not incised, before they rupture. The discharge from the ulcerated areas and buboes is infectious, so precautions against transmission of the disease must be taken. The patient would be more comfortable if kept in bed. Ice-cold compresses may be applied to the inguinal areas. The vulva is cleansed gently twice daily.

The preferred treatment of lymphogranuloma venereum is doxycycline, 100 mg orally twice a day for 21 days.[42] The latter is preferred in pediatric patients younger than 8 years. The alternative regimen is erythromycin base 500 mg orally four times a day for 21 days. Either of these medications also should be given for 21 days. Because of the potential for shorter treatment courses and improved compliance, azithromycin may become a treatment option for lymphogranuloma venereum in the future. It cannot be recommended now because data are insufficient.

After treatment is initiated, patients should be monitored clinically until the signs and symptoms have resolved. Individuals who have had sexual contact with a patient during the 30 days before the onset of symptoms of lymphogranuloma venereum should be examined, tested for chlamydial infection, and treated.[41] Adolescents are likely to be treated before development of the extensive anal and rectal strictures and distorting vulvar cicatrizations occur, for which extensive surgery is sometimes necessary in older patients.

GRANULOMA INGUINALE

Granuloma inguinale is a chronic disease characterized by ulcerative granulomatous lesions of the skin and subcutaneous tissues (Fig. 48–20; see Table 48–4). Common synonyms for the infection include granuloma venereum and donovanosis. The disease most often affects the external genitalia, perineum, and inguinal regions, but the vagina, cervix, and, rarely, distant extragenital sites may be involved. The disease is caused by *Klebsiella granulomatis* (formerly known as *Calymmatobacterium granulomatis*, *Donovania granulomatis*, Donovan bodies), an encapsulated, gram-negative bacillus.[30,65,161,200] Granuloma inguinale occurs commonly in tropical and subtropical regions of the world.[191,201,203] It is relatively rare in the United States and other developed countries.[41,104] The male-to-female ratio seems to be at least 2 : 1. The disease rarely occurs in premenarcheal children, but it should be considered in the differential diagnosis of granulomatous lesions of the genitalia in adolescents.

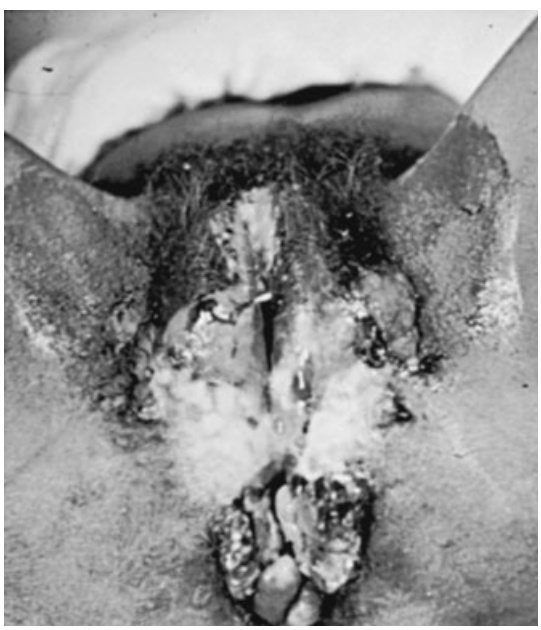

Figure 48–20 Granuloma inguinale.

The disease is transmitted to adolescents and adults during sexual intercourse. The close physical contact of the inguinal, perineal, and genital regions that occurs during sexual activity, and not intercourse per se, probably is more important for transmission of the infection. The organism is not highly contagious; sexual partners of patients with granuloma inguinale often do not become infected. Breaches in the integrity of the skin or mucous membranes, such as with minor trauma, may be required for an inoculum to establish a successful infection.[202] The disease apparently can be transmitted to young children by contaminated clothing or towels. Close physical contact, such as sitting on the lap of an infected parent, also has been reported as a mode of transmission to young children.[295]

The incubation period usually is less than 2 weeks but may be 3 months.[202] The first manifestation of infection usually is single or multiple small, painless hard nodules on the vulva or perineum that erode the skin and form ulcers. Genital tract bleeding is the next most common finding. Ulcers are shallow and have a beefy, granular base that is friable and bleeds easily. The edges are nodular, raised, and undermined. These ulcers usually are painless, unless they become infected secondarily. Regional (inguinal) lymphadenopathy is not associated with granuloma inguinale.[200]

If left untreated, lesions progress slowly outward by eccentric expansion of the leading edge. Over time, this ulcerative stage involves large areas of the perineum, external genitalia, and surrounding skin surfaces. The central areas usually remain ulcerative but may become hypertrophic. Hypertrophic lesions consist of large, vegetating masses with overgrowth of granulation tissue. Less frequently, extensive, destructive necrosis develops in the lesions, or they are dry with sclerotic scarring, which distorts the tissues. Lymphedema of distal tissues occurs commonly during the course of the disease. Lymphatic obstruction or elephantiasis occasionally results in enlargement of the clitoris or labia.[93,149] Subcutaneous granulation develops in approximately 5 percent of cases and may mimic the appearance of bubo formation, termed *pseudobuboes*. When true regional adenopathy occurs in granuloma inguinale, it generally represents a response to a secondary infection or another associated sexually transmitted infection.

Extragenital disease may occur in 6 percent of cases and usually involves the head and neck. Autoinoculation is postulated as the means of transmission of infection of these sites.[86] Occasional involvement of the liver, thorax, and bones remote from the primary genital site suggests that hematogenous spread can occur. Systemic disease is encountered more frequently in girls with cervical lesions and is associated with prolonged spiking fever, anemia, and weight loss.[31,151,220]

Successful isolation of *K. granulomatis* rarely has proved feasible. Reliable culture techniques are unavailable. The diagnosis of granuloma inguinale is based on the presence of Donovan bodies in large, histiocytic cells on crush preparations from lesions (Fig. 48–21). A portion of the granulation tissue is removed and pressed onto a clean slide, which is allowed to air-dry. The slide then is stained with Giemsa, Wright, or Warthin-Starry

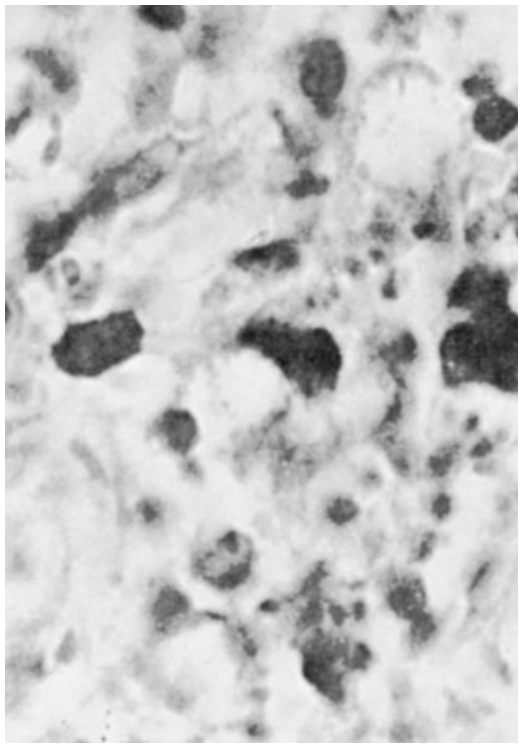

Figure 48–21 Donovan bodies.

stain. Donovan bodies, which are pathognomonic for granuloma inguinale, are vacuolar compartments within the cytoplasm that contain 20 to 30 viable organisms.[65] Histopathologic study of biopsy specimens sometimes is required for establishing a diagnosis. Biopsy specimens should be very early, very sclerotic, or heavily superinfected lesions. Specimens with a scarcity of organisms and smear or crush specimens are likely to be nondiagnostic in such circumstances. Biopsy also should be performed when malignancy is thought possible, or when antibiotic therapy does not lead to improvement.[145]

Complement-fixation and indirect immunofluorescence serologic tests have been developed but have limited specificity.[145] There are no FDA-approved, PCR-based tests for chancroid, but such testing can be performed by commercial laboratories that have developed their own test and conducted a Clinical Laboratory Improvement Act (CLIA) verification study.[41] The differential diagnosis of granuloma inguinale includes carcinoma, syphilis, tuberculosis, chancroid, lymphogranuloma venereum, condyloma acuminatum, blastomycosis, schistosomiasis, and other granulomatous diseases.[20,200,202] STDs such as syphilis may coexist with granuloma inguinale. Darkfield examination of tissue specimens and serologic tests for syphilis should be done. Biopsy may be required to rule out neoplasia or tuberculosis.[86] Chancroid ulcers tend to be deeper and more ragged and often have associated bubo formation. Physical examination alone often is insufficient to distinguish any of these processes from the others (see Table 48–4). Granuloma inguinale also is associated with the development of carcinoma within areas that have been involved in the ulcerative process.

Treatment

Doxycycline, 100 mg orally twice a day for at least 3 weeks and until all lesions have completely healed, is the recommended treatment. Alternatives include azithromycin, 1 g orally once weekly; ciprofloxacin, 750 mg orally twice a day; erythromycin base, 500 mg orally four times a day; or trimethoprim-sulfamethoxazole, one double-strength tablet orally twice a day. Each regimen should be continued until all lesions have healed completely, with a minimum course of 3 weeks.[47] Chloramphenicol and gentamicin also have been used.[227]

Vulvar lesions usually heal within 2 weeks of therapy, but cervical and pelvic lesions may require longer than 3 months. Incomplete treatment can result in recurrence with extensive fibrosis and scarring.[20] The disease recurs in approximately 10 percent of cases. Surgery may be required for complications of granuloma inguinale, such as elephantiasis, strictures, and pelvic abscesses.

Precautions regarding infections should be taken in the home or hospital until the lesions have healed. Sexual contacts of patients with granuloma inguinale within 60 days before onset of the index patient's symptoms should be evaluated and offered therapy, even if no lesions are present. The value of empiric therapy in the absence of overt clinical signs and symptoms has not been determined, however.[41] Adolescent girls who have had granuloma inguinale should be kept under clinical surveillance for many years for the potential development of carcinoma in the perineum and genital tract.

CHANCROID

Chancroid is an acute, ulcerative disease that involves primarily the external genitalia.[7,289] The causative agent, *Haemophilus ducreyi*, is a fastidious, gram-negative coccobacillus. It is a strict human pathogen, and the only known route of transmission is sexual contact. The incubation period usually is 3 to 7 days, but it may be longer. Chancroid rarely is encountered in young children, but occasionally is seen in sexually active adolescents. It occurs far more commonly in boys than in girls for reasons that are unclear, although asymptomatic carriage in girls has been hypothesized.[24,115,206,278]

Chancroid is well established as a cofactor for transmission of HIV infection.[41,42] Ten percent of patients with chancroid may be infected with HIV. Chancroid is found more commonly in tropical and subtropical regions than in developed countries, but its frequency is increasing in many parts of the world.[206] Chancroid is endemic in the United States and occurs primarily in discrete occasional outbreaks. Outbreaks caused by unrelated strains occurred in New Orleans, Louisiana, and Jackson, Mississippi, in the 1990s.[118] The disease probably is underrecognized and underreported.[41]

A recent case series described three girls 5 to 9 years old with chronic skin ulcerations on their lower legs after visiting Samoa. *H. ducreyi* was found in specimens from each child. No evidence of acquisition by sexual contact was found. Lower limb ulcerations were present among household contacts while the girls were in Samoa. Autoinoculation leading to nongenital skin lesions has been described.[278] Although the potential for nongenital transmission of *H. ducreyi* to nongenital sites exists, chancroid lesions in the genital area should be considered the result of sexual contact.

The first sign of chancroid infection of the genital tract is a small, hyperemic macule. The macule becomes a papule and then a pustule before ulceration occurs. The ulcer is painful and usually deep, with irregular borders and undermined edges. The base is gray and covered with purulent exudate laden with the *H. ducreyi* bacillus. Ulcers may occur on the vulva, vaginal mucosa, cervix, or anus but usually are found on the labia minora. Dysuria is a frequent complaint. Tenesmus and rectal bleeding may be associated with anal lesions. Single ulcers are found frequently, but multiple ulcers occur more often. They may become contiguous and form large, eroded areas. Phagedenic destruction of the external genitalia may occur when treatment is not sought early, or a secondary infection develops in the ulcers. In such cases,

scarring persists despite successful eradication of the microbe. Infection, at least experimentally, does not seem to confer protection on re-exposure to the microbe.[6]

Associated, painful inguinal adenitis occurs in 25 to 50 percent of cases. Adenitis occurs more commonly when genital ulcers have been present for more than 10 days. The skin overlying the nodes frequently is erythematous. Discharge from buboes and vulvar lesions is highly infectious. Constitutional symptoms are unusual, and invasiveness beyond regional lymph nodes does not seem to occur even in immunocompromised hosts.

Many patients with chancroid have concurrent infections with other STDs. Patients should be tested for HIV infection and syphilis when chancroid is diagnosed. Testing for both diseases should be repeated in 3 months if the initial results are negative.[42]

In the United States, a probable diagnosis of chancroid is made clinically if (1) one or more painful genital ulcers are present; (2) the individual has no evidence of *T. pallidum* infection by either darkfield examination of ulcer exudate or a serologic test for syphilis performed at least 7 days after the onset of ulcers; (3) the clinical findings, appearance of the genital ulcers, and regional lymphadenopathy, if present, are typical for chancroid; and (4) a test for HSV done on ulcer exudate is negative. When present, the combination of a painful ulcer with tender inguinal adenopathy suggests chancroid. A painful genital ulcer accompanied by suppurative inguinal adenopathy is almost pathognomonic for chancroid.[42]

Definitive diagnosis of chancroid requires isolation of *H. ducreyi* on special culture media. The sensitivity of culture is at best 80 percent.[42] When culture is performed, exudate from the purulent base of the ulcer or material aspirated from buboes should be inoculated onto appropriate media. Gram-negative coccobacilli in a "school-of-fish" grouping on Gram stain suggests *H. ducreyi*, but this pattern often is absent. The diagnosis also can be based on the histologic appearance of tissue obtained by biopsy. There are no FDA-approved, PCR-based tests for chancroid, but such testing can be done by commercial laboratories that have developed their own test and conducted a CLIA verification study.[8,243]

Treatment

Effective agents for the treatment of chancroid in adolescents and adults include azithromycin, 1 g orally in a single dose; ceftriaxone, 250 mg intramuscularly in a single dose; ciprofloxacin, 500 mg orally twice a day for 3 days; and erythromycin base, 500 mg orally three times a day for 7 days.[42] Trimethoprim-sulfamethoxazole no longer is recommended because of the frequency of resistant isolates of *H. ducreyi*. Isolates typically are resistant to penicillin and tetracyclines.

Patients should be re-examined 3 to 7 days after therapy has been initiated. If treatment is effective, ulcers should improve symptomatically within 3 days and show objective improvement within 7 days. If no clinical improvement occurs, one or more of the following should be considered: (1) the diagnosis may be incorrect, (2) co-infection with another sexually transmitted agent may be present, (3) the infecting strain may be resistant to the prescribed antimicrobial agent, (4) compliance with multiple-dose regimens may have been poor, or (5) the patient may have HIV infection. The time required for complete healing of ulcers to occur is related to their size. Large ulcers may require 2 weeks or more.[42]

Resolution of fluctuant lymphadenopathy (buboes) is slower than that of ulcers. Drainage of buboes, when present, usually is required for resolution. Needle aspiration, if adequate drainage can be accomplished, is preferred over surgical incision because it results in less cicatricial scarring. Healed ulcers frequently leave significant scarring.

Individuals who have had sexual contact with an individual who has chancroid during the 10 days before the onset of symptoms should be examined and treated for chancroid, even in the absence of symptoms. Patients co-infected with HIV should be monitored closely. Longer courses of therapy may be required, and healing may be slower. The optimal duration of therapy in these patients is unknown.[42]

TUBERCULOSIS

Even before the advent of chemotherapy for the treatment of tuberculosis, tuberculous disease of the lower genital tract was uncommon.[240,269] The lower genital tract can be affected by primary local infection of the vulvar and vaginal tissues or by scrofulous tuberculosis. Both manifestations of tuberculosis are now exceedingly rare findings. Most such cases occur in young women in developing countries. Tuberculosis of the upper genital tract also is an uncommon occurrence but frequently leads to infertility when it does occur, probably because of scarring of the uterine tubes.[235]

The primary lesions of vulvar tuberculosis may appear as painless, slowly developing, localized, nodular thickening of the skin and subcutaneous tissues or as ulcerative lesions that begin as firm, slightly raised, reddish areas. The ulcers are demarcated sharply and with progression develop undermined edges and granular, grayish brown bases with tubercles and areas of caseation studding their bases.

The scrofulous type is characterized by fistulous tracts and burrowing sinuses that extend from underlying tuberculous infection in the bowel, bladder, or pelvic viscera. Drainage from the ulcers and sinuses keeps the surrounding skin macerated and can lead to extension of the disease.

The diagnosis is straightforward if the patient is known to have visceral tuberculosis, as most often is the case. Isolation of *M. tuberculosis* from the discharge or from lesions or examination of a biopsy specimen from the base of an ulcer is confirmatory. Primary tuberculosis of the vulva must be differentiated from other granulomatous diseases, and the scrofulous type must be distinguished from syphilis, lymphogranuloma inguinale, and Crohn disease.

Treatment

The chemotherapeutic agents used to treat pulmonary and other types of systemic tuberculosis also are effective in treating the disease when it affects the genitalia. Treatment of tuberculosis is described in Chapter 107.

VULVOVAGINAL ULCERATIVE INFECTIONS

BEHÇET DISEASE

In 1937, Behçet described a syndrome characterized by recurrent genital ulcers, aphthous stomatitis, and ocular inflammation.[125,150,181,199,221] Clinical manifestations are not due directly to infection but may result from immunologic events triggered by one or more infections.[204] Arthritis, abnormalities of the central nervous system, a variety of skin lesions including erythema nodosum, and other systemic symptoms also may be associated with the disease. Ocular inflammation frequently is sight-threatening when it occurs. Deep vein thrombosis and arterial occlusion or aneurysms can develop. The onset usually is in patients who are 20 to 30 years old and more frequently in men than in women. Behçet disease is rare in children and may develop more often in girls than in boys.[150] In an international series, 15 percent of children had a family history of Behçet disease.[155]

The cause of Behçet disease is unknown, but an autoimmune basis is suspected. The HLA-B51 phenotype, and especially the B*5101 allele, is associated with Behçet disease in countries with higher prevalences of HLA-B51. Other factors also are involved because a substantial proportion of cases occur in individuals without HLA-B51. Lymphocyte reactivity with peptides derived from human heat shock protein-60 (HSP-60) has been shown. Human HSP-60 is a homologue of streptococcal HSP-65. Aberrant expression of HSP-60 has been found in oral mucosa, skin, and circulating lymphocytes in patients with Behçet disease. In addition to streptococcal species, HSV and *E. coli* antigens have been implicated as potential triggers of Behçet disease.[155,204]

The vulvar lesions take the form of destructive, deep ulcerations that, on recurrence, result in marked scarring and distortion with progressive loss of vulvar tissue (Fig. 48–22). Despite their destructiveness, the lesions are relatively painless. The oral lesions resemble common aphthous ulcers and may precede all other manifestations of Behçet syndrome by 5 to 10 years or longer. Ocular inflammation may be manifested as iridocyclitis, chorioretinitis, or other lesions of the posterior segment. Hypopyon may be seen in some cases.

The vulvar lesions of Behçet syndrome are nonspecific. Diagnosis is based on associated findings and negative test results for diseases that might resemble the vulvar ulcers, notably syphilis, herpes, granulomatous disorders, and Crohn's disease. Histologic study of a biopsy specimen from an ulcer shows only obliterative vasculitis and chronic inflammation.

Many agents have been used to treat Behçet syndrome in the past. Few have been evaluated in controlled trials. Modern treatment regimens usually have used systemic corticosteroids, with or without chlorambucil, colchicine, azathioprine, or thalidomide. More recent studies have suggested that cyclosporine and interferon α-2a may be effective.[10,208] A randomized trial reported in 1998 of the use of colchicine plus benzathine penicillin, with or without interferon α, for the treatment of adults with Behçet disease showed a decrease in the frequency of eye involvement, episodes of arthritis, vascular events, and mucocutaneous lesions in patients treated with all three agents.[155] Infliximab, a humanized monoclonal antibody against tumor necrosis factor-α, may be effective in diminishing new ocular attacks. Etanercept, a tumor necrosis factor receptor analogue, may have an impact on mucocutaneous manifestations.[204] Plastic surgery for correction of vaginal distortions is contraindicated because trauma may be followed by an exacerbation of the disease.

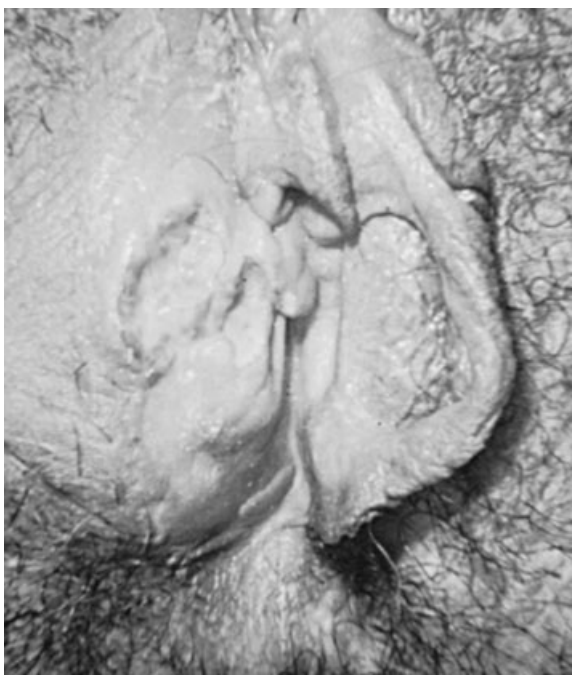

Figure 48–22 Vulvar lesions in Behçet syndrome. *(From Friedrich, E. G., Jr.: Vulvar Disease. Philadelphia, W. B. Saunders, 1976. Reproduced by permission of the author.)*

VULVAR VESTIBULITIS

The vulvar vestibulitis syndrome is the most common type of chronic vulvovaginal pain. Approximately 10 percent of women are affected at some point in their lives. Onset in adolescence can occur, and complaints sometimes may mimic those of sexually transmitted infections. The etiology is unknown and probably multifactorial. An exaggerated inflammatory response of the vestibule (vulvar mucosa) to injury may be involved. Vestibulitis clinically is defined by three signs and symptoms: (1) dyspareunia during intercourse, (2) vestibular tenderness to light touch (e.g., gentle palpation with a cotton swab), and (3) the presence of vestibular erythema. Pain and tenderness are distinctly limited to the vulvar vestibule. Exudates indicate another disease process. Minor ulcerations have been described, but ulceration is not a prominent feature.[27,58,182,220,230]

Debate is ongoing as to the organic versus functional nature of vulvar vestibulitis. Symptoms sometimes respond to biofeedback or cognitive behavioral therapy. Chronic inflammatory changes can be found in the vulvar mucosa of affected women, and surgical resection relieves pain in some patients. Organic and functional components seem to exist, with one or the other predominating in a particular patient.[55,173,296]

Patients with acute vulvar vestibulitis should be evaluated for STDs and treated for any that are found. A history of all medicinal agents or other treatment modalities used by the patient should be obtained. Any agent that might be contributing to the problem should be discontinued if possible. Adolescent girls who have symptoms and signs suggestive of vulvar vestibulitis should be referred for gynecologic evaluation.

CERVICITIS

PREMENARCHEAL

The cervix is not involved when a premenarcheal child has vaginitis because most vaginal infections affect only the distal half of the vagina in children; the exception is gonococcal vaginitis, which in addition usually involves the squamous epithelium over the external cervix. Cervicitis also may occur when a vaginal foreign body is present. Endocervicitis is an unusual finding in premenarcheal girls because the endocervical glands and mucosa are developed poorly before menarche, providing a poor environment for invading organisms.

Erosions of the cervix seldom are seen in children older than 1 year. So-called congenital ectopy is not the result of infection; rather, it is persistence of the fetal paramesonephric (müllerian) glandular epithelium on the outer cervix. This epithelium normally recedes into the endocervical canal as the cervix develops postnatally. Inflammation of the external cervix, which occasionally occurs with vaginitis in a premenarcheal child, need not be treated; it heals as the vaginitis improves.[133]

POSTMENARCHEAL

The structure of the cervix during the reproductive years renders it vulnerable to infections induced by numerous factors. The

long, narrow, deeply pocketed cervical canal, which is lined by open cryptic glands bathed in alkaline secretion and washed periodically by menstrual blood, is an excellent nidus for the growth of bacteria. Pathogenic bacteria in the vagina find ready access to the cervical canal. The use of oral contraceptive pills promotes cervical ectopy, increasing the vulnerability of endocervical cells to chlamydial infection.[130,229]

Early recognition and aggressive treatment of cervicitis in sexually active adolescents are important to prevent serious complications, including PID and tubo-ovarian abscess. Some confusion tends to occur, however, with regard to differentiating normal ectopic columnar epithelium on the exocervix (ectopy) from cervicitis. Ectopy is not an abnormal finding. When the squamocolumnar junction is exposed on the exocervix, it is called *ectopy*. It appears bright shiny red, in contrast to the dull pearly pink appearance of the exocervix. This area does not bleed easily when touched with a swab. During adolescence, the normal cervix may exhibit no ectopy, a small area of ectopy, or sometimes 50 percent ectopy. When the ectopy appears edematous, raised, and friable (often with marked bleeding when touched lightly with a swab), cervicitis should be suspected.[130,229]

Mucopurulent cervicitis primarily is endocervicitis associated with *C. trachomatis*, *M. hominis*, *U. urealyticum*, *N. gonorrhoeae*, *G. vaginalis*, and group B streptococci. In contrast, *T. vaginalis* causes an exocervicitis by extension of vaginitis. Because of the high prevalence of coexisting infections of the cervix, associating any one organism with the clinical signs and symptoms of cervicitis often is difficult. Although studies have shown a strong association between mucopurulent cervicitis and *C. trachomatis*, in most cases no organism is isolated.[130,230,248]

Cervicitis often is asymptomatic, but it should be clinically suspected if an adolescent reports a vaginal discharge and bleeding, especially after having sexual intercourse. Criteria for a clinical diagnosis of mucopurulent cervicitis include the presence of mucopurulent secretion from the endocervix, erythema of the cervix, friable ectopy, and bleeding from the cervix.[34] The mucopurulent secretion in chlamydial cervicitis is tenacious mucoid material mixed with yellow exudate and is difficult to remove from the endocervix. The ectopic area on the cervix may appear erythematous and friable. In chronic chlamydial cervicitis, the ectopic area may be swollen and irregular with a cobblestone appearance, a condition called *hypertrophic cervicitis*. In gonococcal cervicitis, the endocervical mucosa is swollen, intensely inflamed, and often friable as well. In contrast to the mucopurulent discharge present in chlamydial cervicitis, a profuse, yellowish green acrid discharge is present in gonococcal cervicitis.

Cervical erosions occur in sexually active adolescents. Infections other than those listed earlier as causing mucopurulent cervicitis also can involve the cervix. A chancre, the primary lesion of syphilis, may appear on the cervix, where it forms an irregularly shaped ulcer that only remotely resembles a chancre on the vulva. Tuberculosis, HSV, granuloma inguinale, lymphogranuloma venereum, chancroid, schistosomiasis, and actinomycosis also may affect the cervix. The cervical lesions of most of these infections are altered by the warmth and moisture of the vagina and are atypical in appearance. None of these lesions is a common finding, but all of them should be considered in the differential diagnosis when a patient has an unusual ulcerative or fungating lesion of the cervix.[133]

Treatment

The first step is to obtain good specimens from the endocervix. The cervix should be wiped with a large swab to remove vaginal secretions. Endocervical specimens then are obtained for detection of *C. trachomatis* and *N. gonorrhoeae* and a vaginal specimen for a saline wet-mount preparation and potassium hydroxide preparation under microscopy. The types of tests available for chlamydial and gonococcal infection are described in this section under chlamydial and gonococcal cervicitis. If an endocervical Gram stain can be performed, an endocervical specimen should be obtained. This test is useful in a clinical setting. Cervicitis is suspected when an endocervical Gram-stained smear shows more than 5 to 10 polymorphonuclear cells per field under oil immersion. The presence of at least eight pairs of gram-negative intracellular diplococci in at least three polymorphonuclear cells strongly suggests the presence of gonococcal cervicitis. The absence of gram-negative intracellular diplococci, but the presence of more than 5 to 10 polymorphonuclear cells per oil immersion field suggests nongonococcal cervicitis.[34,186] When cervical erosions or ulcers are seen, a specimen for culture of HSV should be obtained. *T. vaginalis* is identified by microscopic examination of vaginal secretions in saline.

The same guidelines for the treatment of gonococcal and chlamydial cervicitis are used for the treatment of cervicitis in nonpregnant adolescent girls or women. Current treatment of gonococcal infection is influenced by the emergence of antibiotic-resistant strains, including penicillinase-producing *N. gonorrhoeae*, fluoroquinolone-resistant strains, tetracycline-resistant strains, and chromosomally resistant strains.[42] The high frequency of chlamydial infection in individuals with gonorrhea (45% in certain populations) and the serious complications resulting from untreated gonorrhea and chlamydial infections also have influenced the treatment approach. Treatment options include intramuscular ceftriaxone, 125 mg in a single dose, cefixime, 400 mg orally in a single dose; plus azithromycin, 1 g orally in a single dose; or doxycycline, 100 mg orally twice a day for 7 days.[42]

In the case of penicillin allergy, a single dose of intramuscular spectinomycin, 2 g, instead of cephalosporins is recommended. Spectinomycin is unavailable in the United States, however.[43] Azithromycin, 2 g, can be used instead of cefixime or ceftriaxone to treat gonococcal cervicitis; however, its cost and severe gastrointestinal side effects need to be considered.[42] Pregnant adolescent girls or women should not be treated with doxycycline. Azithromycin, 1 g orally in a single dose, or erythromycin base, 500 mg orally four times a day for 7 days, is recommended for presumptive *C. trachomatis* cervicitis.[42] If pharyngeal gonorrhea is suspected, the recommended treatment is intramuscular ceftriaxone. When *T. vaginalis* vaginitis is diagnosed, or HSV is suspected strongly, the appropriate treatment for these infections should be given (see sections on trichomoniasis and genital herpes).

Integral to treating the patient is notification, examination, and treatment of the patient's sexual partners if gonococcal or chlamydial infection (or both) is confirmed. Direct notification by the patient should be encouraged, and, based on state laws, a prescription for partner therapy can be given. Written information should be provided with names of diseases and medication side effects to assist with this process. When tests are positive for gonorrhea or chlamydial infection, retesting for these infections is recommended: in 3 weeks if treated with erythromycin or if symptoms persist, and in 3 months if treated with other regimens to detect re-infection from an untreated or new partner.[42]

CHLAMYDIAL

Some of the highest rates of chlamydial cervicitis (8-25%) have been reported in adolescents.[44,82,85,233] It is three to four times more common than gonococcal cervicitis. *C. trachomatis* causes cervicitis by infecting the columnar and transitional epithelium of the cervix. Cervical ectopy seems to be a predisposing factor for acquisition of *C. trachomatis* infection.[16]

C. trachomatis cervicitis is predominantly an asymptomatic disease in adolescent girls.[44] A clinical diagnosis is made when friable ectopy with a mucopurulent cervical discharge (mucopus)

is noted. Chlamydial cervicitis is suspected when a Gram stain of an adequate endocervical smear shows more than 5 to 10 polymorphonuclear cells per oil immersion field in the absence of gram-negative intracellular diplococci.[34,185]

Numerous tests, including culture, direct fluorescent antibody tests, enzyme immunoassay, nucleic acid hybridization tests, enhanced optical immunoassays, and nucleic acid amplification tests, are available today to diagnose *C. trachomatis* infection. Material from the cervix must be obtained directly from the endocervix with a sterile swab. The tissue culture method was considered the gold standard,[236] but because of the high cost of this technique, it is not used widely for adolescents and adults. It continues to be the gold standard, however, for prepubertal children suspected of being infected. Overall, amplification tests are considered the most sensitive and specific tests today, and testing of urine specimens is expected to revolutionize screening and diagnosis. Presently, amplification tests are being used predominantly for research purposes because of their high cost, but gradually they may become available for clinical use.

In large-volume, community-based clinics, the DNA hybridization assay or DNA probe (Gen-Probe) and enzyme immunoassay (Abbot, Wampole, Kodak, Gen-Probe) can be used.[158,242] Both tests perform best in clinic populations with high prevalence rates of *C. trachomatis* infection; however, the DNA probe seems to have greater sensitivity for detection and is simple to perform. The Gen-Probe is approximately 80 percent sensitive and 100 percent specific detecting *C. trachomatis* in girls and women. It can be used as a "mail-out" specimen as well.[242,252] The direct fluorescent antibody test or MicroTrak is considered to be suited best for low-volume laboratories and requires technical expertise. It continues to be used in some hospital-based settings. This test detects *Chlamydia* elementary bodies in cervical secretions and takes 15 to 20 minutes to perform. It has a sensitivity of 75 to 90 percent and specificity of 95 to 97 percent compared with the culture method.[74,242]

The reliability of amplification tests such as PCR, strand displacement amplification, and transcription-mediated amplification in detecting *C. trachomatis* seems to be equivalent for vaginal, cervical, and urine specimens as long as the urine collected is a first-catch specimen. The sensitivity of PCR, ligase chain reaction, strand displacement amplification, and transcription-mediated amplification ranges from 87 to 99 percent, and specificity ranges from 97 to 100 percent.[23,239,252] Treatment of chlamydial cervicitis was discussed in the earlier section on cervicitis.

GONOCOCCAL

Gonococcal cervicitis may be symptomatic or asymptomatic. The risk of acquiring infection varies with the population. Adolescents seen in private practices in the suburbs have a significantly lower rate of gonococcal cervicitis than that noted in adolescents seen in large outpatient hospital clinics or community clinics serving inner-city populations. The rate of asymptomatic endocervical infection in adolescent girls seen in urban settings ranges from 3 to 12 percent. Anal gonorrhea usually is secondary to discharge from gonococcal cervicitis infecting the anus. Primary anal gonorrhea as a result of anal intercourse should be suspected in a sexually active adolescent with anal discomfort. In addition, gonococcal infection should be considered in the differential diagnosis of pharyngitis in sexually active adolescents.[218] Gonococcal endocarditis, arthritis, and dermal abscesses occur, but they are beyond the scope of this chapter.

The initial complaints in gonococcal cervicitis include vaginal discharge, dysuria, urinary frequency, and dyspareunia. Although gonococcal cervicitis is reported to be predominantly an asymptomatic condition in girls and women, many patients, after careful questioning, are found to have symptoms. The discharge is profuse and prevalent. On examination, the vulvar tissues may be inflamed and edematous. Discharge also may be seen exuding out of the urethra and paraurethral ducts in severe cases. On speculum examination, the patient has a normal-appearing cervix, or the cervix appears erythematous and friable, with a foul-smelling purulent discharge draining from the cervical os.

Laboratory diagnosis is made by detecting *N. gonorrhoeae* by culture technique, a DNA hybridization test or DNA probe (Gen-Probe), or an amplification test. Material from the cervix must be obtained directly from the endocervix with a sterile swab. Vaginal swabs that can be obtained by the adolescent also are a recommended method for amplification tests. Although the reliability of detecting gram-negative intracellular diplococci on a Gram stain of cervical secretions is low, this test can be useful in areas where more sophisticated tests are unavailable. The presence of at least eight pairs of gram-negative intracellular diplococci in at least three polymorphonuclear cells strongly suggests gonococcal cervicitis.

Hospital-based settings should continue to use the culture method in children and adolescents for medicolegal cases. The DNA probe (Gen-Probe) can be used in hospital and community-based clinics for the reasons described in the section on chlamydial cervicitis. This test has sensitivities ranging from 90 to 97 percent and a specificity of 99 percent. Amplification tests such as PCR and strand displacement amplification for *N. gonorrhoeae* from vaginal, cervical, and first-catch urine specimens have a sensitivity of 95 to 97 percent and a specificity of 99 to 100 percent.[252,257] The sensitivity of the transcription-mediated amplification test in detecting *N. gonorrhoeae* from urine specimens is poor. Treatment of gonococcal cervicitis was discussed in the earlier section on cervicitis.

UPPER GENITAL TRACT INFECTIONS

PELVIC INFLAMMATORY DISEASE

PID (i.e., infection of the uterine tubes, ovaries, and pelvic peritoneum) is an extremely rare finding in premenarcheal girls and often is undiagnosed until advanced infection occurs.[33,66,133] With the exception of gonorrhea and the inflammation caused by a foreign body, most vulvar and vaginal infections do not involve the upper third of the vagina and do not approach the cervix of premenarcheal patients. The premenarcheal cervix and endometrium apparently are barriers rather than passageways for bacteria causing all types of vulvovaginitis in children.

The causative organisms are diverse. Gonococcal pelvic infection has been reported after sexual abuse has occurred. *S. pneumoniae* serotypes 1 and 2 and *E. coli* also have been reported in pubertal girls with salpingitis and peritonitis and tubo-ovarian abscess.[33]

When intrapelvic infection has been reported in premenarcheal girls, it usually has been part of either generalized primary peritonitis or peritonitis secondary to a ruptured appendix or some other intra-abdominal infection.[72,241] Pelvic infection that is part of an intra-abdominal infection characteristically affects the surfaces of the uterine tubes, uterus, and ovaries and produces perisalpingitis and periovaritis; periappendicitis also is present even if the infection did not begin as appendicitis. An ascending infection from the lower genital tract, such as that caused by gonorrhea, involves the tubal mucosa and produces endosalpingitis and pyosalpingitis. Identical bacteria should be isolated in cultures from the lower genitalia and from the pelvic exudate before an ascending infection is considered to be the cause of a pelvic infection in a specific case in which the patient is a premenarcheal child.

PID in postmenarcheal girls is a common problem. Salpingitis has been reported in virgins, and, although the etiology is

unknown, the organisms are thought to spread hematogenously, lymphatically, or transmurally from intestines or ascending infection from the vagina.[66,74,133] PID occurs primarily in sexually active adolescents at risk for developing ascending infection acquired from STDs.[76,252]

PID is defined as an acute clinical syndrome (unrelated to pregnancy or surgery) attributed to the ascent of microorganisms from the vagina and endocervix to the endometrium, fallopian tubes, or contiguous structures that result in pelvic and generalized peritonitis. The use of terms specifically describing the anatomic sites involved is preferable (e.g., endometritis, salpingitis, salpingo-oophoritis, tubo-ovarian abscess). In most adolescents with acute severe infection, however, differentiating some of these entities is difficult; the term *PID* is used commonly.

Reasons for the development of PID are physiologic and social and include alterations in cervical mucus caused by immature and anovulatory menstrual cycles, onset of sexual activity during early puberty, and multiple sexual partners. Cervical mucus is less viscous during menses and at midcycle, rendering it more permeable to ascending infection. In vitro experiments show that *N. gonorrhoeae*, *C. trachomatis*, *U. urealyticum*, and other aerobic and anaerobic agents can adhere to spermatozoa and migrate with them.[276] Whether this event can occur in vivo is unknown. Most organisms found in nongonococcal and nonchlamydial PID also are found in bacterial vaginosis, suggesting that bacterial vaginosis may be a predisposing or a precipitating factor for the development of PID. In addition, researchers have suggested that *T. vaginalis* can act as a vector for transporting bacteria.[148] Another possible factor is upward transport of bacteria from orgasmic myometrial contractions.[288] Lastly, vaginal douching has been reported to be a risk factor in promoting ascending infection.[198,288,291]

Other factors influencing the risk of acquiring PID are the method of contraception and a history of gonococcal or chlamydial lower genital tract infection, with or without uterine instrumentation, such as the use of an intrauterine device or dilation and curettage. Oral contraceptives are used commonly by adolescents. Their possible protective effect on the development of PID has been reported. Laparoscopic studies also have shown that women with PID who were using oral contraceptive pills have significantly milder degrees of fallopian tube inflammation than those of women who do not use the pills. This finding suggests that the oral contraceptive pill may reduce potential tubal damage. Other studies report, however, that by promoting cervical ectopy, oral contraceptives are a potential risk factor for acquiring chlamydial PID.[248,273,292] In contrast, compared with other methods of contraception, women using depot medroxyprogesterone acetate (Depo-Provera) had a significantly decreased risk of developing PID.[19,292] Risk for acquiring a PID with the intrauterine device seems to be related to the individual's background risk for acquiring sexually transmitted infection.[252]

Women who have had one episode of PID have a 20 to 25 percent chance of having subsequent ones. Reasons for this susceptibility may be re-infection from untreated sexual partners, an inadequately treated first infection, or increased susceptibility of the tubal epithelium to infection.[76,288]

Endometritis

Infections of the endometrium in postmenarcheal girls and women are as rare as previously thought. The gonococcus, on its way upward to the uterine tubes, produces a fleeting asymptomatic infection of the endometrium, or the symptoms produced are overshadowed by those of the vulvar and cervical infections. Evidence also suggests that endometritis occurs in 40 percent of patients with asymptomatic endocervical chlamydial infection.[144] It also is called *subclinical PID*, and repeated episodes have been linked to infertility.[144,286] The role of HIV infection in the development of PID is unclear. An increased prevalence of endometritis in HIV-infected women compared with uninfected women has been noted, and altered immune function might contribute to difficulty clearing endometritis in these cases.[35]

Symptoms specific to endometritis are uncommon. It is commonly an asymptomatic condition. Symptoms include recurrent intermittent or acute suprapubic pain and tenderness with vaginal bleeding or spotting. On bimanual examination, the uterus is tender, and cervical motion tenderness may or may not be present. Endometritis in adolescent girls and young women also has been associated with postabortive and puerperal endometritis. Poor compliance with prophylactic antibiotics places an adolescent at an increased risk for development of endometritis after having an abortion. Young women who engage in high-risk sexual behaviors and use intrauterine devices are likely to have asymptomatic, low-grade, chronic endometritis.

Endometrial tuberculosis is a rare occurrence in the United States and is encountered most often in patients with pulmonary tuberculosis. This entity needs to be considered in HIV-infected girls and women and in geographic areas and regions where tuberculosis is endemic. In a study from a province in Iran, tuberculous endometritis was detected in 72 percent of genital tuberculosis cases in women.[194] The disease may not manifest for years after initial seeding, and the infection is thought to reach the uterus via the gastrointestinal tract through hematogenous seeding and by lymphatic spread. It is not acquired as a result of an ascending infection or through coitus with an infected man. The symptoms of endometrial tuberculosis vary from none to amenorrhea, pelvic pain, dysmenorrhea, abnormal uterine bleeding, and tuberculosis. Often, the patient has a history of healed or active pulmonary disease. Overall, 60 percent of patients with pelvic tuberculosis have tuberculous endometritis.[5,117]

Endometrial tuberculosis is diagnosed by endometrial biopsy and demonstration of acid-fast *M. tuberculosis* in cultures of curettage material or menstrual blood. Menstrual fluid has been reported to be culture-positive more frequently than are biopsy specimens.[117] Endometrial tuberculosis and the management of tuberculosis are described further in Chapter 107.

Salpingitis

The pathogenesis of salpingitis has been studied best with *N. gonorrhoeae* and *C. trachomatis*. In the fallopian tube, gonococci attach to nonciliated epithelial cells and induce sloughing of ciliated epithelial cells into the lumen.[271,288] Gonococci also enter the subepithelial space, where a local inflammatory response, predominantly polymorphonuclear leukocytic infiltrate and anaerobic bacteria, is produced and may reach the bloodstream. A purulent exudate is produced within the tube and may cause pelvic peritonitis. Chlamydial infection produces a similar picture except that it causes a predominantly lymphocytic infiltrate in the submucosa.[211,271,288]

Salpingitis also is caused by several other microbial agents that have been isolated directly from the fallopian tubes. The organisms most commonly recovered from the upper tract in salpingitis are mixed anaerobes (25-84%), followed by *N. gonorrhoeae* (25-40%) and *C. trachomatis* (25-40%). Mixed aerobic and anaerobic infections, including *Bacteroides* and *Peptostreptococcus* spp., account for 25 to 60 percent of cases. These mixed infections occur more frequently in girls and women with severe PID, chronic PID, and recurrent PID. PID develops in approximately 10 to 17 percent of girls and women with endocervical *N. gonorrhoeae* and in 10 to 30 percent with endocervical *C. trachomatis*. At first, researchers thought that *N. gonorrhoeae* initiated the infection and that superinfection with anaerobes followed. They now realize that apparently anaerobes and aerobes can initiate PID without *C. trachomatis* or *N. gonorrhoeae*.[73,74,76,252,288]

Differentiating salpingitis caused by different microbiologic organisms is difficult. *N. gonorrhoeae* has been identified most frequently within the initial 24 hours of development of symptoms. Beyond 48 hours, the most frequent isolates are anaerobes. Gonococcal and chlamydial salpingitis seem to occur most often within 1 week of menstruation.[272] The clinical presentation varies from asymptomatic to severe disease. The severity of infection depends on duration of symptoms and the etiologic agent. Chlamydial PID tends to be less symptomatic than infection from *N. gonorrhoeae*.

Diagnosis

The classic manifestation of acute PID is lower abdominal pain, which usually is bilateral and continuous and may worsen with movement. After the onset of abdominal pain, fever, nausea, and vomiting develop. The temperature may reach 39°C to 39.5°C (102°F-103°F). A chill seldom precedes the fever. Other common initial symptoms include vaginal discharge, irregular vaginal bleeding, and urinary symptoms.

On physical examination, the patient usually looks sick and uncomfortable. Abdominal examination reveals a markedly tender and often tense lower part of the abdomen. Rebound tenderness indicates generalized peritonitis. The genital examination may show a purulent vaginal discharge in the vaginal vault. Even gentle bimanual rectovaginal abdominal palpation causes great distress. Attempted mobilization of the cervix is extremely painful. The uterus is tense and tender. The adnexa may not be outlined because of discomfort produced by the examination. Palpation of the adnexa unilaterally or bilaterally may be exceptionally painful. An adnexal swelling may be palpated, but this finding is unreliable in diagnosing an adnexal mass. In a less acutely tender patient, the examination may reveal a thickened, tender tube.

Common laboratory findings in patients with PID include a peripheral white blood cell count greater than 10,000/mm^3 and an erythrocyte sedimentation rate greater than 15 mm/hr. In addition, endocervical gonorrhea and chlamydial infection may be present simultaneously in 10 to 30 percent of cases. Ultrasonography of the pelvic cavity helps exclude adnexal masses and may detect pelvic abscesses. A study involving the use of pelvic ultrasonography in adolescents with PID showed that the presence of fluid in the cul-de-sac was not helpful in differentiating patients with and without PID.[99] Almost 20 percent of patients with PID had a tubo-ovarian abscess; in the absence of a tubo-ovarian abscess, adnexal volume was significantly larger in adolescents with PID than in adolescents without it.[99] Ultrasonography (transvaginal) can be very useful in ruling out other diagnoses and defining adnexal masses.

The clinical diagnosis of PID is made by having a high index of suspicion of this entity. The clinical criteria for the diagnosis of PID were developed originally by Jacobsen and Westrom.[141] The benefits of using laparoscopy are that an accurate diagnosis can be made rapidly by direct visualization of the fallopian tubes and adjacent structures,[141] tubal exudate can be obtained for culture, and the outcome of therapy can be evaluated.[292] This diagnostic method, however, is not without risks, is not a sensitive diagnostic tool, and adds to the cost of care.[81] For practical reasons, the clinical criteria developed by Jacobsen and Westrom[141] and revised by the Centers for Disease Control and Prevention[42] are recommended to aid in making the diagnosis and subsequently to increase the index of suspicion for PID (Table 48–5). When comparing the reliability of clinical criteria using endometrial sampling as the reference test,[42] the sensitivity of using one criterion—adnexal tenderness—was 95 percent, and the sensitivity of using three criteria—abdominal pain, cervical motion tenderness, and adnexal tenderness—was 83 percent.

The minimum criteria recommended for empiric treatment include abdominal pain with uterine tenderness or cervical motion tenderness or adnexal tenderness.[42] The more criteria that can be met, the more likely that a tubal infection is present. Clinicians who use only the criteria listed in Table 48–5 to treat would miss adolescents with mild disease, however.[42,106,141,280] The differential diagnosis of PID is an acute abdomen. Conditions of the urinary tract that should be considered include cystitis, pyelonephritis, and urethritis. Gastrointestinal tract conditions include appendicitis, constipation, diverticulitis, gastroenteritis, inflammatory bowel disease, and irritable bowel syndrome. Gynecologic conditions include dysmenorrhea, ectopic pregnancy, endometriosis, endometritis, mittelschmerz, torsion or rupture of an ovarian cyst, ruptured follicle, septic abortion, threatened abortion, and pyogenic sacroiliitis. Although pyogenic sacroiliitis is very rare, it was reported in a 13-year-old girl secondary to an infected umbilical ring.[152]

As in PID, establishing an early diagnosis of acute appendicitis and ectopic pregnancy is important. In acute appendicitis, an adolescent is more likely to have nausea, vomiting, a short history of right lower quadrant abdominal pain, lower grade fever, a higher leukocyte count in relation to the fever, and little discomfort during pelvic examination. Occasionally, the appendix hangs over the pelvic brim and causes pelvic tenderness. A ruptured appendix and peritonitis may simulate PID closely. Differentiating PID from ectopic pregnancy is less difficult if the history suggests pregnancy. PID can occur during the first trimester of pregnancy. In ectopic pregnancy, the patient does not have fever or leukocytosis. The symptoms almost always are unilateral, and signs of hemoperitoneum are present if the tube is ruptured.[288] If ectopic pregnancy is suspected, a serum pregnancy test and pelvic ultrasonography (transvaginal) for detection of a gestational sac should be performed immediately.

TABLE 48–5 Clinical Criteria for the Diagnosis of Acute Pelvic Inflammatory Disease

Minimal Criteria
Lower abdominal or pelvic pain with one or more of the following: uterine or adnexal tenderness or cervical motion tenderness
Additional Criteria to Enhance Specificity
Temperature >38.3°C (>101°F)
Abnormal cervical or vaginal mucopurulent discharge
Presence of white blood cells on saline microscopy of vaginal secretions
Elevation of erythrocyte sedimentation rate
Elevated C-reactive protein
Laboratory documentation of cervical infection with *Neisseria gonorrhoeae* or *Chlamydia trachomatis* or both
Specific Criteria
Endometrial biopsy specimen with histopathologic evidence of endometritis
Transvaginal sonography or magnetic resonance imaging technique showing thickened fluid-filled or tubo-ovarian complex
Laparoscopic abnormalities consistent with pelvic inflammatory disease

From Centers for Disease Control and Prevention: Sexually transmitted disease treatment guidelines 2006. M. M. W. R. Recomm. Rep. 55(RR-11):1-93, 2006.

Treatment

The major goals of treatment of PID are preservation of fertility and prevention of other long-term sequelae, including ectopic pregnancy. Although some data suggest that women younger than 25 years old have a better fertility prognosis overall after having PID and ectopic pregnancy, no difference exists among age groups regarding tubal infertility specifically after PID.[288] The earlier the treatment is initiated, the lower the risk of devel-

oping infertility. Girls and women initially evaluated after more than 3 days of abdominal pain are found to have a 2.8-fold increased risk of having impaired fertility (tubal infertility or ectopic pregnancy) compared with those evaluated within 3 days of the onset of abdominal pain.[128] Generally, the more severe the PID, the higher the risk of future infertility occurring. In addition, the more episodes of PID, the higher the risk of future infertility occurring. After one episode, the risk of infertility ranges from 8 to 13 percent; after two episodes, the risk is 20 to 35 percent; and after three or more episodes, it is 40 to 75 percent.[252,270]

To reduce the incidence of sequelae associated with this condition, early recognition of PID, the use of broad-spectrum antibiotics to treat polymicrobial disease, careful clinical re-evaluation 48 hours after initiating antibiotic treatment to assess antibiotic response, and evaluation and treatment of sexual partners are important. Screening the patient for other STDs, such as trichomoniasis, bacterial vaginosis, and syphilis, also is important.

Patients with PID often are treated on an ambulatory basis. The primary reason to hospitalize an adolescent with PID is to ensure compliance with medication when she is unable to follow or tolerate an outpatient oral regimen because of the seriousness of the sequelae and problems with compliance in this age group. Other reasons for hospitalization are an uncertain diagnosis, the presence of a tubo-ovarian abscess, pregnancy, infection with HIV, temperature greater than 38.5°C, nausea and vomiting precluding the use of oral medications, and lack of improvement after 48 hours of oral antibiotic treatment.

The recommendations of the Centers for Disease Control and Prevention for treating PID in ambulatory and hospitalized patients address antibiotic coverage for the polymicrobial etiology of this condition (Tables 48–6 and 48–7).[42] In addition to antibiotic treatment, bed rest is recommended. Intravenous fluids are administered for hydration when necessary. When the diagnosis of PID is certain, oral analgesic agents may be prescribed. Close follow-up is recommended, with a bimanual examination performed after 48 hours of antibiotic treatment to assess the patient's response to treatment.[42] Overall, most studies show that response to antibiotic regimens is similar between HIV-positive and HIV-negative patients. The treatment recommendations for HIV-positive patients are no different.[259] Complications associated with PID are perihepatitis, tubo-ovarian abscess, hydrosalpinx (obstruction of the tube caused by scarring [Fig. 48–23]), chronic abdominal pain from adhesions surrounding the fallopian tubes and ovaries, recurrent PID, ectopic pregnancy, and infertility.[106,252]

TABLE 48–6 Ambulatory Management of Pelvic Inflammatory Disease

Ceftriaxone 250 mg IM in a single dose *or* cefoxitin 2 g IM in a single dose *plus* probenecid 1 g orally in a single dose *or* other parenteral third-generation cephalosporins (e.g., ceftizoxime or cefotaxime)
plus
Doxycycline 100 mg PO two times a day for 14 days
with or without
Metronidazole 500 mg PO twice a day for 14 days

From Centers for Disease Control and Prevention: Sexually transmitted disease treatment guidelines 2006. M. M. W. R. Recomm. Rep. 55(RR-11):1-93, 2006.

TABLE 48–7 Inpatient Treatment of Pelvic Inflammatory Disease

Regimen A
Cefoxitin 2 g IV every 6 hr *or* cefotetan 2 g IV every 12 hr
plus
Doxycycline 100 mg every 12 hr PO or IV
Regimen B
Clindamycin 900 mg IV every 8 hr
plus
Gentamicin loading dose IV or IM (2 mg/kg) followed by a maintenance dose (1.5 mg/kg) every 8 hr

Note: *The above regimens are given for at least 24 hr after the patient improves. After discharge from the hospital, the patient is continued on doxycycline at 100 mg PO two times a day for a total of 14 days.*

From Centers for Disease Control and Prevention: Sexually transmitted disease treatment guidelines 2002. M.M.W.R. Recomm. Rep. 55(RR-11): 1-93, 2006.

PERIHEPATITIS

The classic manifestation of perihepatitis, or Fitz-Hugh–Curtis syndrome,[288] is severe right upper abdominal pain that often radiates to the shoulder. Concurrent left upper abdominal pain also may be present. Lower abdominal pain and evidence of acute or subacute PID are frequent findings. The right upper quadrant pain lasts about 48 hours. Nausea, fever, and leukocytosis are common manifestations. Elevation of the erythrocyte sedimentation rate and liver enzymes may be present. The pathogenesis of perihepatitis is thought to be from direct spread of *N. gonorrhoeae* and *C. trachomatis* from the fallopian tubes into the peritoneal cavity, along the paracolic sulci. From there, they reach the subphrenic space and hepatic surface. Spread from the reproductive tract also is possible via the retroperitoneal lymphatics.[288]

The diagnosis is made by having a high index of suspicion. Perihepatitis frequently mimics cholelithiasis, hepatitis, pleuritis, subphrenic abscess, perforated peptic ulcer, nephrolithiasis, appendicitis, ectopic pregnancy, abdominal trauma, and pancreatitis. Treatment of this condition is similar to treatment of PID.

TUBO-OVARIAN ABSCESS

The formation of a tubo-ovarian abscess is a late manifestation of PID. The incidence of tubo-ovarian abscess ranges from 14 to 38 percent in hospital-based adolescents and adults with salpingitis.[99,106,162,195,255] Although tubo-ovarian abscesses rarely are seen in adolescents and young women who are not sexually active,

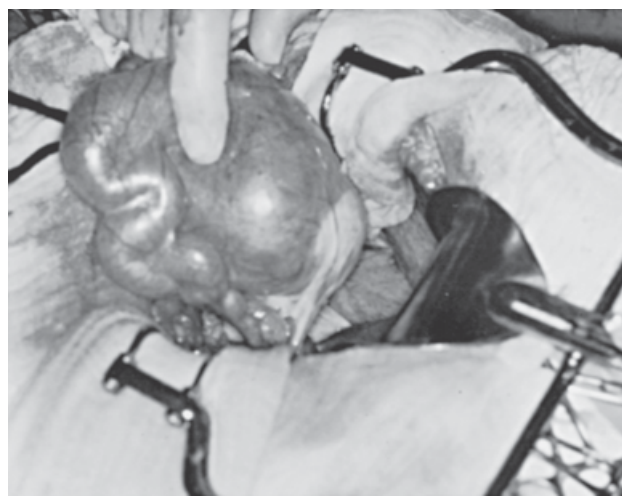

Figure 48–23 Large hydrosalpinx, the result of gonorrheal salpingitis in an older teenage girl who had repeated gonorrheal infections. The other tube also was diseased.

three case reports are in this group.[66] In two cases, *E. coli* and alpha-hemolytic streptococci were isolated, and in one case, *Pasteurella multocida* was isolated. The actual cause for ascending infection in these cases is unknown. Alterations in cervical secretions, which usually serve as a barrier, have been hypothesized. The abscess typically results from a mixture of facultative and anaerobic bacteria, with facultative bacteria dominating the early phase of infection, and bacterial metabolic products producing an environment of low oxygen tension that favors the growth of anaerobic bacteria.[288]

The most common organisms recovered from tubo-ovarian abscesses are *E. coli*, *Bacteroides fragilis*, other *Bacteroides* spp., *Peptostreptococcus*, *Peptococcus*, and aerobic streptococci.[222] Diagnosing the presence of a tubo-ovarian abscess clinically usually is difficult. Adolescents with a tubo-ovarian abscess tend to seek care later in their menstrual cycle (>18 days from the last menstrual period) than do girls without a tubo-ovarian abscess.[255] Bimanual examination frequently does not reveal a pelvic mass.[49] Four potentially useful clinical features that suggest the presence of a pelvic abscess are pain, persistent fever, adnexal tenderness (for >7 days), and an erythrocyte sedimentation rate greater than 30 mm/hr.[49] Ultrasonography of the pelvis is valuable in confirming the presence of an abscess.[99]

The prompt administration of antibiotics has reduced greatly the incidence of pelvic abscess. Most of those encountered today are in adolescents who have not had adequate care and have delayed seeking treatment.[106] Adolescents are far more likely to delay seeking treatment than are young women.[262]

A conservative approach is favored for the treatment of a tubo-ovarian or ovarian abscess (i.e., bed rest, supportive care, intravenous antibiotics).[252] The choice of antibiotics for treating tubo-ovarian abscess should include the following considerations: effectiveness against β-lactamase–producing anaerobes, adequate coverage against resistant *Bacteroides* spp., penetration into the abscess, and ability to remain stable in an abscess environment. The antibiotic regimens for inpatient treatment of PID fulfill these considerations and are appropriate for the treatment of tubo-ovarian abscess (see Table 48–7). As is the case with cefoxitin, cefotetan, and clindamycin, metronidazole provides good activity against anaerobes.[222] Many hospitals prefer the use of triple antibiotics: cefoxitin and gentamicin plus clindamycin or metronidazole.

A clinical response to treatment consisting of a decrease in pain, fever, and total leukocyte count should be noted in 72 hours. Pelvic ultrasonography should be repeated at this time to note any further increase in the size of the abscess. The duration of intravenous and oral antibiotic therapy for a tubo-ovarian abscess should be at least 21 days. The patient may begin oral antibiotics when she is afebrile and asymptomatic and when the size of the abscess has stabilized. The abscess either resolves without drainage or becomes an encapsulated pool of pus. The latter eventually "points" either in the cul-de-sac or anteriorly in the abdominal wall.

Surgical intervention may be needed in 25 percent of cases, either during the initial period or within a year, usually depending on the size of the abscess. An abscess larger than 10 cm has a 60 percent chance, a 7- to 9-cm abscess has a 35 percent chance, and a 4- to 6-cm abscess has a 20 percent chance of requiring surgical intervention. The fertility rate after treatment of tubo-ovarian abscesses may be 20 to 50 percent with conservative medical and surgical approaches.

OVARITIS AND OOPHORITIS

Ovaritis generally refers to inflammation of the ovaries, and *oophoritis* refers to inflammation of the substance of the ovaries, the oocytes in particular. Viral exanthems such as mumps and cytomegalovirus are complicated most frequently by ovaritis or oophoritis. Immunosuppressed children and adolescents are at risk for development of ovaritis and oophoritis. Improved immunization practices have decreased the prevalence of mumps ovaritis. The presence of an enlarged, tender, boggy, smooth mobile ovary in a child with mumps or one of the exanthems is an indication for repeated examinations and watchfulness. The ovary becomes less tender and gradually shrinks. Mumps ovaritis, in particular, may convert one or both ovaries into swellings that are 6 to 8 cm in diameter. The enlargement may persist for several months. Treatment is palliative, with analgesics given for discomfort and fever.[133]

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Gastrointestinal Tract Infections

CHAPTER 49

Esophagitis

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Infectious esophagitis is distinct from other gastrointestinal infections in that it is a rare finding in previously healthy individuals, is predominantly of fungal and viral etiology, and usually is an indicator of a primary or secondary immunodeficiency state. In normal children, infectious esophagitis frequently is associated with conditions that compromise esophageal defense mechanisms.[46]

In view of the numerous predisposing conditions, the incidence of infectious esophagitis in children is difficult to quantify. It has been reported frequently after patients have undergone chemotherapy for hematologic malignancies and after solid organ and hematopoietic stem cell transplantation. Infectious esophagitis has been reported in 3 percent of patients with ataxia-telangiectasia.[29] Before the availability of highly active antiretroviral therapy (HAART), 8 to 10 percent of children infected with human immunodeficiency virus (HIV) developed *Candida* esophagitis.[4,7,37] Despite the availability of HAART, *Candida* esophagitis remains one of the most common opportunistic infections in HIV-infected children and generally is associated with low $CD4^+$ T-cell count (<100/μL), prior episodes of oropharyngeal candidiasis, high plasma HIV-1 load, and neutropenia (<500/μL).[7,8,45]

PATHOPHYSIOLOGY AND CAUSATIVE ORGANISMS

The defense mechanisms of the esophagus against infection include motility (continuous flow of luminal contents, which discourages colonization by microbes), a mucosal lining of stratified squamous epithelium that is resistant to microbial invasion, and local and systemic immune responses. Disruption of any of these protective factors may lead to esophageal infections. Immunosuppression (as seen in patients with acquired immunodeficiency syndrome [AIDS] or after organ transplantation), chronic mucocutaneous candidiasis, malignancy, cancer chemotherapy, and prolonged corticosteroid treatment are the most common underlying etiologic factors for fungal and viral infection of the esophagus.[6-8,19,27,37] In immunocompetent children, dysmotility of the esophagus, in addition to mucosal injury secondary to gastroesophageal reflux, can render the esophagus vulnerable to infections.

Most esophageal infections are caused by fungi and viruses.[3] The esophagus can be infected by local invasion (*Candida*, herpes simplex virus [HSV], bacteria), as a result of systemic infection (cytomegalovirus [CMV], *Candida*, *Pneumocystis*), or by contiguous spread from the mediastinum or neck (tuberculosis, retropharyngeal abscess). *Candida* spp. (especially *Candida albicans*), HSV, and CMV are the major pathogens.[4,37,46] Infections with fungi such as *Pneumocystis*, *Aspergillus*, and *Histoplasma* spp.; viruses such as varicella-zoster virus (VZV), papillomavirus, Epstein-Barr virus, and HIV; and protozoa such as *Cryptosporidium* and *Leishmania donovani* are frequent, well-documented causes of esophagitis.[9,14,15,17,18,23,30,34,41] Bacterial infections of the esophagus probably are under-reported. Of esophageal infections, 10 to 16 percent can have a bacterial etiology, mostly in granulocytopenic patients and as secondary infection in fungal or viral esophagitis. Gram-positive and gram-negative oropharyngeal bacterial flora and *Mycobacterium tuberculosis* are the common pathogens causing esophagitis.[13,44] *Helicobacter pylori* has been isolated from involved tissue in Barrett esophagitis. This "infection" seems, however, to be related to gastroesophageal reflux disease, rather than to direct invasion of the esophagus by *H. pylori*.[31]

CLINICAL FEATURES

Patients with infectious esophagitis can present with esophageal, abdominal, or systemic symptoms (Table 49–1). Odynophagia and dysphagia are the most common symptoms of esophagitis, but they may not be apparent in small children.[7,8,35] In adults, only 59 to 79 percent of patients with documented esophageal infection had these symptoms.[3] Children also may describe a sensation of food "sticking" behind the sternum or a feeling of a food or liquid bolus passing through the chest.

Nearly all patients with AIDS and oral candidiasis and odynophagia have endoscopic evidence of esophageal candidiasis.[5,7,39] Some patients ultimately proven to have esophagitis have no signs or symptoms at presentation, however. Children who develop esophageal candidiasis despite being treated with HAART are less likely to have typical symptoms (e.g., odynophagia and retrosternal pain) or to have concomitant oropharyngeal candidiasis.[8] Similarly, approximately a fourth of adult patients with esophageal candidiasis are asymptomatic.[3]

Drooling is an unusual manifestation in esophagitis per se but may be present with pharyngeal involvement. Fever is caused mostly by systemic or secondary infection, such as disseminated CMV disease or tuberculosis, or complications that occur after esophageal perforation. Fever does not occur commonly in bacterial esophagitis. Cough is characteristic of tuberculosis, tracheobronchial fistulas, or high-grade esophageal obstruction.

Nausea and vomiting are associated more commonly with CMV esophagitis. The abdominal pain in esophagitis may be caused by referred pain from the distal end of the esophagus, associated gastritis (as in CMV infection), or concomitant intra-abdominal infections in immunocompromised hosts. A maculopapular truncal rash and fever may be present in patients with idiopathic esophageal ulceration during acute HIV seroconversion.[34] Diarrhea can be present in CMV infection (diffuse involvement of the gastrointestinal tract) or HIV infection (opportunistic enteral infections, HIV enteropathy). In one report of fungal esophagitis in children, hematemesis was the most common initial symptom.[46] Finally, because physicians seldom seek evi-

TABLE 49–1 Signs and Symptoms of Infectious Esophagitis

Dysphagia
Odynophagia
Oral lesions
Nausea/vomiting
Abdominal pain
Fever
Diarrhea
Cough
Rash
Hematemesis

dence of esophagitis in the absence of dysphagia or odynophagia, infectious esophagitis may be under diagnosed in infants and children.

Oral lesions are seen in patients with *Candida*, HSV, or HIV esophagitis, but they rarely are present with CMV infection and tuberculosis. Oral thrush is a common occurrence, however, in infants and immunocompromised patients. The frequency of *Candida* esophagitis in infants with oral thrush is unknown. The presence of oral thrush and esophageal symptoms does not exclude concomitant esophageal infection with pathogens other than *Candida* or the esophageal ulcerations associated with acute HIV infection. Oropharyngeal lesions are less common findings in HSV esophagitis than in *Candida* esophagitis. Recurrent cold sores, vesicular lesions on the nasolabial folds, and esophageal symptoms sometimes are seen as initial features of HSV esophagitis in immunocompetent adults and children, but their absence does not exclude HSV infection.[3,35]

DIFFERENTIAL DIAGNOSIS

"Pill" esophagitis may be identified by history. Pathologic gastroesophageal reflux is the most common cause of esophagitis in children. In previously healthy children, esophageal symptoms are likely to be caused by reflux esophagitis, whereas in immunocompromised patients, the physician needs to rule out infectious esophagitis. Secondary bacterial or fungal infections can be present in reflux esophagitis and Chagas disease, especially with severe inflammation and obstruction. Absence of reflux symptoms (long-standing heartburn, a water brash taste in the mouth, vomiting, spitting up [in infants], pillow wetting, or coughing) does exclude reflux esophagitis. Achalasia, diffuse esophageal spasm, foreign body impaction, and mediastinal or retropharyngeal abscesses can cause esophageal symptoms and may result in secondary infection.

DIAGNOSIS

Establishing a specific diagnosis is essential for management of infectious esophagitis, particularly because fungal and bacterial superinfections occur commonly in viral esophagitis. Although the clinical profile, barium esophagogram, and endoscopic appearance can provide some clue to the etiology of esophagitis, histopathology, immunohistochemistry, and culture of endoscopic and brush biopsy specimens are essential for confirmation of specific pathogens. Serology for HSV, CMV, or Epstein-Barr virus may help establish the diagnosis in some cases by providing evidence of acute infection. DNA detection methods also may provide evidence of viremia with these herpesviruses. Detection of fungal or viral esophagitis in an ostensibly healthy host should prompt an evaluation for the presence of primary or secondary immunodeficiency states, including HIV infection.

BARIUM ESOPHAGOGRAPHY

The usefulness of an esophagogram in diagnosing infectious esophagitis is limited because it does not provide an etiologic diagnosis; normal or nonspecific findings also are found in some cases of esophagitis. These studies are useful for assessing motility and for excluding obstruction, perforation, and fistulas of the esophagus. Barium studies are not indicated if endoscopy is planned. Double-contrast barium esophagography with air and barium, which details the mucosal lining, is the radiologic investigation of choice. Children may not tolerate this procedure well, however.[25]

Candida esophagitis generally is diffuse, whereas HSV or CMV lesions are found more frequently in the mid to distal portion of the esophagus. Discrete longitudinal plaques, a grossly irregular or "shaggy" appearance, or tiny nodular lesions with a granular appearance are characteristic of *Candida* esophagitis (Fig. 49–1). The presence of discrete superficial stellate ulcers in the mid esophagus with normal-appearing surrounding mucosa is characteristic of HSV esophagitis. CMV lesions may mimic HSV lesions on barium esophagography. Oval or elongated large ulcers are found mostly in CMV infection, however, and idiopathic esophageal ulceration is found in HIV infection.[24,43] Esophagograms of patients with tuberculosis can show intramural pseudodiverticula, extrinsic compression, or esophageal displacement by mediastinal lymph nodes and sinus tracts.[10]

ESOPHAGOSCOPY

The technique for performing endoscopy with videoendoscopes miniaturized to a diameter of 4.8 mm has made the procedure far more tolerable in immunocompromised patients, requiring less sedation and anesthesia and allowing the procedure to be performed even in newborns. These new instruments have biopsy capabilities comparable to older, wider endoscopes.

Characteristic macroscopic lesions are associated with some infectious agents. Macroscopic appearances overlap considerably, however, and histopathologic or immunohistochemical analysis (or both) of endoscopic and brush biopsy specimens is essential for diagnosis. A diffuse esophageal lesion is characteristic of *Candida* infection, whereas CMV and HSV infections mainly involve the distal part of the esophagus. White, longitudinal plaques adhering to the mucosa are characteristic of *Candida* infection.[20] Plaques from oral thrush, common findings in infants and immunocompromised children, can be washed away and reveal a nonulcerated underlying mucosa. Similar-appearing plaques also may be seen in CMV, HSV, bacterial, and "pill" esophagitis, and after sucralfate ingestion.

Small, 1- to 3-mm vesicles are characteristic of HSV esophagitis, but by the time endoscopy is done, these vesicles usually slough off and reveal sharply demarcated ulcers with a raised edge, necrotic base, and normal-appearing surrounding mucosa.[35] In progressive disease, these ulcers may coalesce to resemble *Candida* esophagitis.[1] Multiple superficial ulcers in the distal portion of the esophagus often are seen in CMV esophagitis. Large elongated ulcers also are typical manifestations of CMV infection, but they may occur in idiopathic esophageal ulcerations in HIV infection as well.[24] Complete denudation of the mucosa is an unusual finding with CMV infection.[40] Endoscopy done in patients with VZV esophagitis can show vesicles, discrete ulcers, or necrotizing esophagitis, depending on the stage of the disease. Tubercular ulcers of the esophagus usually are of varying size, distinct, and shallow with a necrotic base.

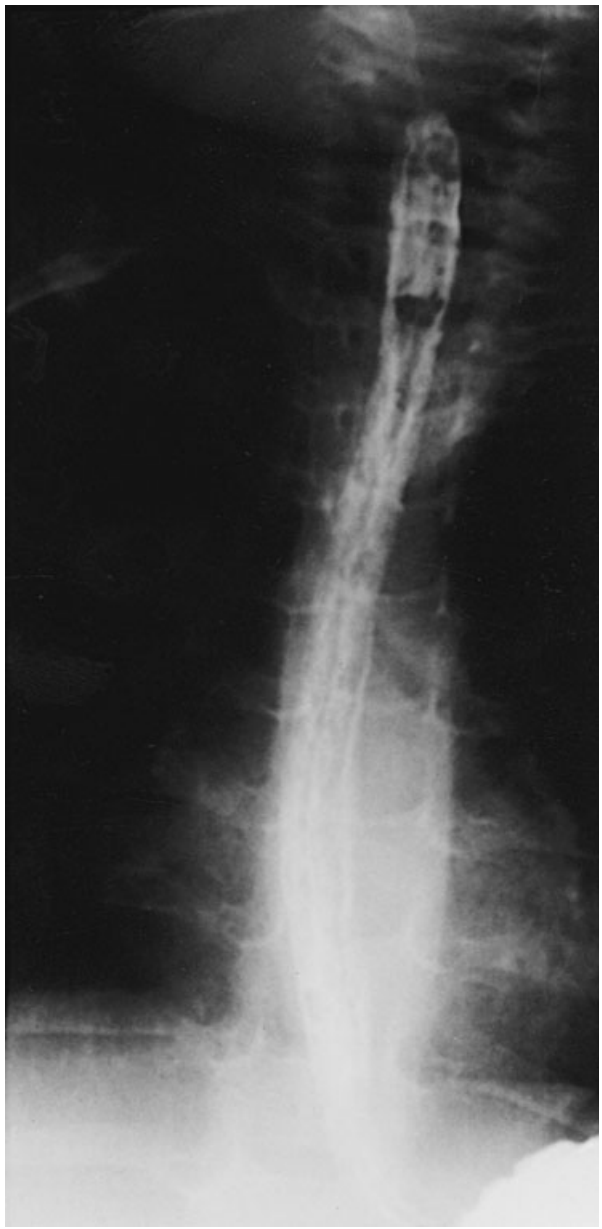

Figure 49–1 *Candida* esophagitis. Barium esophagogram shows diffuse mucosal irregularity suggestive of inflammation and longitudinal filling defects suggestive of plaques. *(Courtesy of Sjirk Westra, M.D., Division of Pediatric Radiology, UCLA Medical Center, Los Angeles.)*

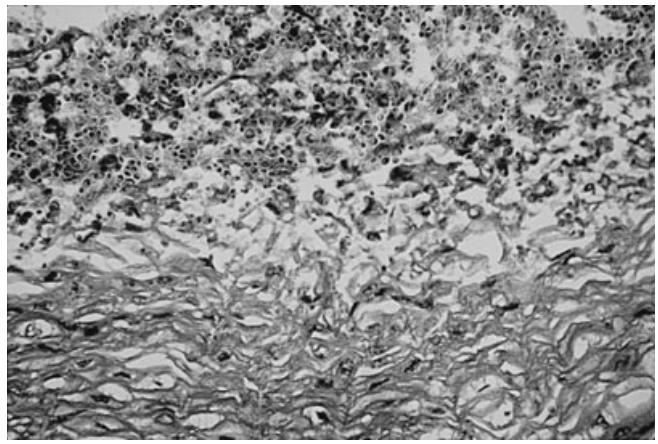

Figure 49–2 *Candida* esophagitis. Photomicrograph shows yeastlike organisms in the esophageal mucosa. Methenamine silver stain. *(Courtesy of Klaus Lewin, M.D., Department of Pathology, UCLA Medical Center, Los Angeles.)*

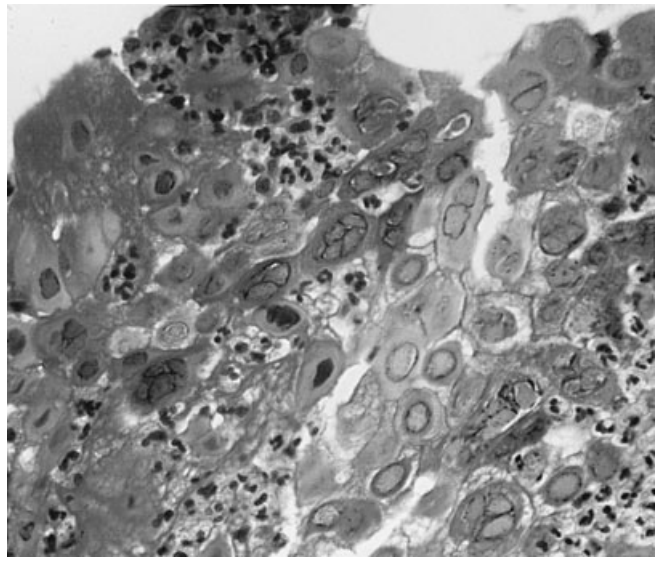

Figure 49–3 Herpes simplex virus esophagitis. Photomicrograph shows viral inclusions in squamous epithelium. Hematoxylin and eosin stain. *(Courtesy of Klaus Lewin, M.D., Department of Pathology, UCLA Medical Center, Los Angeles.)*

BIOPSY AND BRUSHING

Endoscopic biopsy specimens should be obtained from the edge and the base of the lesions. In CMV infection, specimens from the edge do not yield diagnostic information.[40] The pathologist should be alerted to the possibility of fungal, viral, and polymicrobial infection. Appropriate fixatives should be used for routine hematoxylin and eosin stain, Gram stain, and special stains for fungi and bacteria such as *Mycobacterium*. *Candida* and *Aspergillus* can be shown by silver stain, periodic acid–Schiff stain, or Gram stain (Fig. 49–2). Diagnostic histopathologic changes, such as multinucleated giant cells, ballooning degeneration, intranuclear Cowdry type A inclusion bodies, and margination of chromatin in HSV infection (Fig. 49–3), and amphophilic intranuclear inclusions and small multiple cytoplasmic inclusion bodies in CMV infection (Fig. 49–4), can be diagnostic. Immunohistochemical studies and DNA hybridization techniques often are required to establish the diagnosis, however.

Viral culture, with or without immunohistochemical techniques, may aid in confirmation. Material obtained by endoscopy-guided brush biopsy can reveal the features of *Candida* or viral infection described earlier. Blind brushings of the esophagus may be useful for *Candida* infection when endoscopy is impossible or is unavailable.[5] If abdominal pain, fever, or other unusual symptoms are present, the possibility of disseminated or abdominal infection should be excluded by appropriate investigations.

TREATMENT

CANDIDA ESOPHAGITIS

Treatment of esophageal candidiasis requires systemic antifungal therapy. Topical therapy may produce an initial response, but early treatment failures are more common occurrences.[33] Sys-

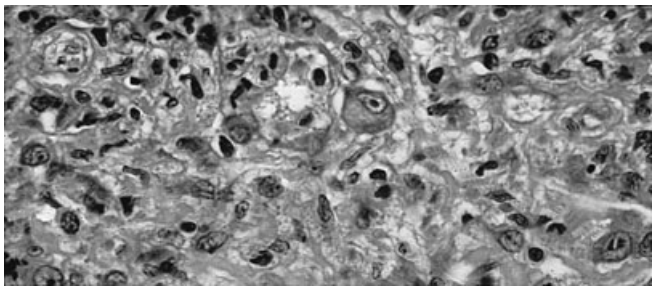

Figure 49–4 Cytomegalovirus esophagitis. Photomicrograph shows intracytoplasmic inclusion bodies in the lamina propria. Hematoxylin and eosin stain. *(Courtesy of Klaus Lewin, M.D., Department of Pathology, UCLA Medical Center, Los Angeles.)*

temic therapy with newer azole medications (fluconazole, itraconazole solution, or voriconazole) is now the preferred approach.[32] Numerous trials have been done comparing the azole agents, most often in adults with AIDS. Patients are less likely to respond and more likely to experience drug-related toxicity when treated with ketoconazole compared with fluconazole.[11] Oral fluconazole and itraconazole suspension seem comparable in efficacy for initial therapy, and some patients whose disease fails to respond to fluconazole may see improvement with subsequent itraconazole therapy.[12,36,44] Regardless of the initial severity of esophagitis, voriconazole and fluconazole seemed comparable in efficacy in one study, although toxicity occurred more commonly with voriconazole.[2,8]

Overall, experience with the azole medications supports recommendations for the initial use of oral fluconazole therapy for *Candida* esophagitis. Therapy generally is given for 14 to 21 days. Intravenous administration of azole medications may be used for patients with severe dysphagia.[26,32]

Infection with azole-resistant variants of *Candida*, including species with intrinsic or acquired resistance to fluconazole (e.g., *Candida krusei*), may occur.[2,8] Although pediatric data currently are limited, large-scale studies in adults have shown that intravenous therapy with echinocandin agents (caspofungin, anidulafungin, or micafungin) is an alternative to azole therapy.[26,32,42]

Intravenous amphotericin B deoxycholate remains an alternative for patients with disease that is refractory to treatment with azole medications.[21,32] Nephrotoxicity is a major toxic effect of amphotericin B, but brief, low-dose therapy (7 to 14 days, 0.3 to 0.7 mg/kg/day) generally is sufficient to treat esophagitis. Lipid formulations of amphotericin B also may be useful for treating refractory disease, but they are not known to be superior in efficacy or lower in toxicity than is the low-dose deoxycholate formulation for the treatment of *Candida* esophagitis.

OTHER CAUSES OF FUNGAL ESOPHAGITIS

Cases of esophagitis caused by *Aspergillus*, *Mucor*, *Cryptococcus*, and *Histoplasma* spp. have been described. Optimal therapy has not been established, but systemic therapy, as for other manifestations of invasive infection with these organisms, has been successful. Currently, this approach would involve the use of intravenous or oral azole agents or an amphotericin B preparation. Echinocandins (micafungin, caspofungin, or anidulafungin) may be useful for *Aspergillus* or *Histoplasma* infections, but they have no activity against cryptococci.

VIRAL ESOPHAGITIS

Although spontaneous resolution of HSV esophagitis may occur in some individuals, antiviral therapy for HSV esophagitis generally is advised. Immunocompromised patients usually are treated with intravenous acyclovir, but in older patients, valacyclovir and famciclovir may be useful alternatives, in view of their clinical efficacy and convenient dosing schedule.[28] Ganciclovir is used for CMV infection.[3,38] Acyclovir is the treatment of choice for VZV, and prophylaxis has reduced effectively the rate of VZV infection in post-transplantation patients. Foscarnet is an alternative treatment for patients with HSV, VZV, or CMV who cannot tolerate or do not respond to initial therapy with acyclovir or ganciclovir therapy. Therapy for 14 to 21 days usually is recommended for esophagitis caused by these herpesviruses. HIV-associated idiopathic ulcers do not respond to empiric antiviral or antifungal therapy, but they may respond to treatment with thalidomide.[16]

BACTERIAL ESOPHAGITIS

Bacterial esophagitis typically occurs in immunocompromised hosts and may involve gram-positive or gram-negative bacteria, or a mixture of both. In immunocompromised hosts, esophagitis may be associated with bacteremia. Consequently, empiric therapy should include broad-spectrum antibacterial agents active against *Staphylococcus aureus*, viridans streptococci, and aerobic gram-negative organisms. Blood cultures always should be performed to exclude bacteremia.[44]

Esophageal infections with *M. tuberculosis* or atypical mycobacteria are treated with regimens appropriate for other systemic infections with these organisms. Lack of response to appropriate therapy may indicate concomitant superinfection by other organisms or resistance to the drugs used. Repeat endoscopy is indicated for documenting eradication of infection.

PROGNOSIS

Candida esophagitis carries a poor prognosis for patients with AIDS. Survival is approximately 1 year after an episode of *Candida* esophagitis in children with untreated HIV infection, and *Candida* esophagitis is an indication for administration of antiretroviral therapy.[4,34] Fungal esophagitis, if not treated successfully, can lead to esophageal strictures, obstruction, perforation, and fistulas.[22,27] Viral esophagitis usually does not have any long-term sequelae.

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CHAPTER 50

Approach to Patients with Gastrointestinal Tract Infections and Food Poisoning

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The approach to treatment of patients with gastrointestinal (GI) tract infections begins with obtaining a thorough medical history including information about epidemiologic factors, a physical examination, and knowledge of the pathophysiologic process and clinical manifestations of various enteropathogens. GI tract infections result in a wide range of symptom complexes and can be produced by a variety of enteropathogens. Most infectious diarrheal illness can be classified into one of several categories based on the causative agent, the pathophysiologic mechanism of the agent, and the clinical response. This information then can be used to determine appropriate laboratory evaluation and therapy. All patients with diarrhea require some degree of fluid and electrolyte therapy; a few need other nonspecific support; and for some, specific antimicrobial therapy is indicated to shorten the duration of the illness and to eradicate fecal excretion of the organism. Practice guidelines for management of infectious diarrhea have been developed by the Infectious Diseases Society of America.[163] Other recommendations include a primer for physicians dealing with diagnosis and management of food-borne illnesses developed jointly by the American Medical Association, Centers for Disease Control and Prevention (CDC), U.S. Food and Drug Administration (FDA), and United States Department of Agriculture[62]; surveillance of food-borne disease outbreaks in the United States by the CDC[245]; guidelines for prevention of

opportunistic infections in patients with human immunodeficiency virus (HIV) developed by the U.S. Public Health Service and the Infectious Diseases Society of America[71]; management of acute gastroenteritis in young children developed by the CDC[66]; and guidelines on acute diarrhea in adults.[111]

EPIDEMIOLOGY AND ETIOLOGY

Establishing the cause of diarrhea often is difficult because of variations in host susceptibility and response to infection, geographic location, season, and complexity of laboratory techniques necessary to identify the wide array of causative agents. In the United States and other parts of the world, acute infectious diarrhea is caused by many enteropathogens; but on the basis of epidemiologic and etiologic considerations, major categories can be differentiated (Table 50–1): diarrhea in childcare centers, hospitals, or other institutional settings and in family members or other close contacts; food- or water-associated diarrhea; antimicrobial-associated diarrhea; diarrhea of travelers; diarrhea in immunosuppressed hosts, including patients with primary and secondary immune deficiencies; and others, which include sporadic episodes in which these categories do not apply. In addition, history of chronic diarrhea or other GI tract diseases should be obtained.

When a patient complains of moderate or severe diarrhea, a decision should be made as to whether he or she should be hospitalized or treated as an outpatient. The following patients with diarrhea should be considered for hospitalization: infants and elderly people who appear toxic, are lethargic, or have high temperatures; patients with excessive loss of fluid and electrolytes through stools, especially in association with vomiting; patients with grossly bloody stools; and patients who are immunosuppressed, including people with acquired immunodeficiency syndrome (AIDS). Once the decision has been made either to hospitalize or to treat on an outpatient basis, appropriate diagnostic procedures and therapy can be instituted.

OUTBREAKS IN CHILDCARE CENTERS

The risk for a person to acquire a GI tract infection varies with age, environment, season, exposure, and immune status. Clinical and epidemiologic data often guide classification of an episode of acute infectious diarrhea into one of the categories mentioned earlier. If predisposing factors are not present, the episode can be classified as sporadic. Most cases of sporadic diarrhea occur in children younger than 5 years old, although all age groups are affected. Infants who are breast-fed are relatively protected from contaminated food and water. Protection also is afforded by factors present in human milk.[310] During and after weaning, the risk for development of diarrhea increases. When an episode of acute infectious diarrhea occurs, an association with other cases should be sought, especially when highly contagious organisms, such as *Shigella* spp., *Escherichia coli* O157:H7, *Giardia lamblia*, *Cryptosporidium* spp., rotavirus, astrovirus, enteric adenovirus, and calicivirus, are isolated and in conditions in which close contact is facilitated, such as institutions for mentally impaired people, childcare centers, hospitals, and other institutional settings.[188,263,289,311,314,412] After respiratory tract illness, diarrhea is the second most common disease among children in childcare facilities,[188] occurring most frequently in children younger than 3 years.

FOOD-BORNE OR WATER-BORNE DIARRHEA

Reporting of food-borne and water-borne diseases in the United States began in 1923 because of concern about typhoid fever and infantile diarrhea.[245] Since 1978, food-borne and water-borne outbreaks have been published in separate annual summaries. The purposes of the outbreak surveillance include disease prevention and control, knowledge of disease causation, and administrative guidance. In 1996, the CDC established the Foodborne Disease Active Surveillance Network (FoodNet) for population-based, active surveillance investigation of food-borne disease in the United States.[362] The active surveillance includes laboratory-confirmed infections for nine pathogens (*Campylobacter* spp., *Listeria* spp., *Salmonella* spp., *Shigella* spp., Shiga toxin–producing *Escherichia coli*, *Vibrio* spp., *Yersinia* spp., *Cryptosporidium* spp., and *Cyclospora* spp.).

An outbreak of food-borne or water-borne disease is defined as an incident in which two or more people experience a similar illness, usually involving the GI tract, after ingesting common food or water.[120,236,245] Surveillance for water-borne disease and outbreaks in the United States is characterized by disease associated with recreational water and disease associated with drinking water and water not intended for drinking.[120,236] Food-borne or water-borne disease can result from ingestion of food or water contaminated with bacteria or a bacterial toxin, a chemical, a virus, or a parasite.[62,236,245] Table 50–2 outlines characteristics of food-borne and water-borne outbreaks, including outbreaks not characterized by diarrhea. Although most enteropathogens can be spread by either food or water, the epidemiology of outbreaks often suggests specific etiologic agents. A determination of the incubation period and the presence or absence of selected clinical findings (especially fever, vomiting, and bloody diarrhea) often leads a physician or other health care professional toward establishing the correct diagnosis in outbreaks of food-borne or water-borne disease.[62] As a general rule, when outbreaks are

TABLE 50–1 Major Categories of Acute Infectious Diarrhea

Category of Diarrhea	Epidemiologic Considerations	Most Commonly Involved Enteropathogens
Outbreaks usually due to person-to-person transmission	Childcare centers, hospitals, and other institutional settings	Enteric viruses, *Cryptosporidium*, *Giardia lamblia*, occasionally bacteria
Food-borne or water-borne	Other people involved after common food or water exposure	See Tables 50–2 and 50–3
Antimicrobial-associated	Recent administration of an antimicrobial agent	*Clostridium difficile*
Travelers	Recent travel to a developing country	Enterotoxigenic *Escherichia coli*, *Campylobacter*, *Shigella*, *Salmonella*, other organisms
Immunosuppressed hosts	Primary or secondary (underlying disease, including HIV infection; recent administration of an immunosuppressive drug or radiation therapy) immune deficiencies	See Table 50–4

divided by an incubation period, illnesses manifesting in less than 1 hour usually are caused by chemical poisoning; illness of 1 to 7 hours, by either *Staphylococcus aureus* or *Bacillus cereus* preformed toxins; illness of 8 to 14 hours, by *Clostridium perfringens* or *B. cereus* enterotoxins; and illness of more than 14 hours, by other infectious or toxic agents (see Table 50–2). Clinical manifestations can involve the central nervous system or be systemic with little or no intestinal tract involvement.

During the period from 1998 through 2002, a total of 6647 outbreaks of food-borne disease, which included 128,370 cases, were reported to the CDC from various regions of the United States.[245] Since 2001, reports of food-borne disease outbreaks have been submitted by state, local, and territorial public health departments through a Web application on the Internet called the electronic Food-borne Outbreak Reporting System.[245] This surveillance system has resulted in an increase in the number of reported outbreaks. Among outbreaks in which the cause was determined (33%), bacterial pathogens caused the largest percentage of outbreaks (55%) and cases (55%), with *Salmonella* serotype Enteritidis accounting for the largest number of outbreaks and outbreak-related cases.[245] *Listeria monocytogenes* accounted for most deaths from any pathogen, followed by *Salmonella* spp. Outbreaks with a known cause accounted for 54 percent of illnesses. Viruses caused 33 percent of outbreaks and 41 percent of cases, predominantly due to noroviruses, with hepatitis A being the second most common viral cause. Parasites caused 1 percent of outbreaks and 1 percent of cases, with anisakis, cryptosporidium, cyclospora, *Giardia*, and trichinella reported as causing outbreaks. Chemical agents caused 10 percent of outbreaks and 2 percent of cases, with ciguatoxin, heavy metals, mushrooms, paralytic shellfish, and scombrotoxin accounting for outbreaks caused by chemical agents (Table 50–3). The etiologic agent was not detected in 67 percent of outbreaks, indicating the need for improved epidemiologic and laboratory investigations. Factors contributing to outbreaks were classified as those that are thought to have led to contamination of a food and those that allowed proliferation of the pathogen in the food. At least one contributing factor was reported in 46 percent of outbreaks reported from 1998 through 2002.[245,438] Major contaminating factors that contributed to outbreaks were bare-handed contact

TABLE 50–2 Characteristics of Food-Borne or Water-Borne Outbreaks of Diarrhea

Usual Incubation Periods	Clinical Illness			Causative Agent	Epidemiologic and Laboratory Diagnosis
	Fever	Diarrhea	Vomiting		
5 minutes-6 hours (usually <3 hours)	Rare	Occasional (see Table 50–3)	Common	Chemical or toxin	Demonstration of toxin or chemical from food or epidemiologic incrimination of food
1-6 hours (usually <1 hour)	Rare	Occasional	Profuse	*Staphylococcus aureus* enterotoxin *Bacillus cereus* emetic toxin	Detection of toxin in food, isolation of organisms in food (>10^5/g) or in vomitus or stool
8-16 hours	Rare	Typical	Occasional	*Clostridium perfringens* enterotoxin *B. cereus* enterotoxin	Isolation of organisms or toxin from food (>10^5g) or stools of ill persons, epidemiologic incrimination of food
16-96 hours	Common	Typical	Occasional	*Shigella* *Salmonella* *Vibrio parahaemolyticus* Enteroinvasive *Escherichia coli* *Yersinia enterocolitica*	Isolation of organisms from food or stools of ill persons
12-72 hours	Clinical syndrome compatible with botulism			*Clostridium botulinum*	Isolation of organism or toxin from food (10^5/g) or stools Demonstration of toxin in serum or food
16-96 hours	Occasional	Typical	Occasional	*E. coli* enterotoxin *V. parahaemolyticus* enterotoxin *Vibrio cholerae* enterotoxin *Y. enterocolitica* enterotoxin *Listeria monocytogenes*	Isolation of organism from food and stools of ill persons Identification of toxin Epidemiologic incrimination of food Blood or cerebrospinal fluid cultures; stool culture usually not helpful
1-11 days	Occasional	Common	Occasional	*Cryptosporidium parvum* *Cyclospora cayetanensis*	Request special stool examination; may need to examine water or food
2 days-weeks	Common	Typical	Frequent	*Bacillus anthracis*	Isolation of organism from blood or contaminated meat
1-7 days	Uncommon	Typical	Frequent	*E. coli* O157:H7 and other Shiga toxin-producing *E. coli*	Isolation of organism from food or stool or identification of toxin in stools of ill persons Epidemiologic incrimination of food
1-3 days	Occasional	Typical	Common	Caliciviruses (noroviruses) Rotavirus	Antigen detection (enzyme immunoassay) in stool Immune electron microscopy of stool Serology
2-5 days	Occasional	Typical	Occasional	*Campylobacter jejuni*	Isolation of organisms from food or stools of ill persons Epidemiologic incrimination of food
7-21 days	Common	Common	Rare	*Brucella abortus*, *Brucella melitensis*, and *Brucella suis*	Blood culture and positive serology
1-4 weeks	Rare	Common	Rare	*Giardia lamblia*	Stool for ova and parasite examination enzyme immunoassay
2 days-8 weeks	Common	Common	Common	*Trichinella spiralis*	Serology, muscle biopsy

TABLE 50–3 Chemical Causes of Food Poisoning

Type of Chemical, Toxin, or Poison	Food	Clinical Symptoms	Onset of Symptoms (hr)
Heavy metals*	Water (through metallic container) and food	Gastrointestinal	1
Scombrotoxin	Fish (tuna, mackerel, marlin, mahi-mahi, bluefish)	*Due to histamine:* flushing, headache, burning of mouth and throat, urticaria, rash, paresthesia	1
Ciguatera	Fish (barracuda, amberjack, snapper, grouper)	*Neurologic:* paresthesia, reversal of cold-hot sensations *Gastrointestinal:* nausea, abdominal pain, vomiting, diarrhea	1-6
Tetrodotoxin	Puffer fish	*Neurologic:* paresthesia, numbness, loss of proprioception, ascending paralysis, death	<1
Paralytic or neurotoxic compounds	Shellfish (clams, oysters, scallops, mussels, other mollusks)	Paresthesia, weakness, respiratory difficulties, dysphasia, dysphonia, gastrointestinal	1-4
Domoic acid	Shellfish (mussels)	Gastrointestinal *Acute CNS:* headache, seizures, hemiparesis, ophthalmoplegia, abnormalities of arousal *Chronic CNS:* memory deficits, motor neuropathy or axonopathy	Within 24
Monosodium glutamate	Chinese food	Burning sensation, heavy feeling in chest, pressure over face, flushing, gastrointestinal	1
Ibotenic acid, muscimol	Mushroom	*CNS:* confusion, delirium, visual disturbances, lethargy	2
Coprine	Mushroom	*Disulfiram-like effect:* nausea, vomiting, headache, hypotension, flushing, paresthesia and tachycardia	2
Muscarine	Mushroom	*Parasympathetic:* sweating, salivation, lacrimation, blurred vision, diarrhea, bradycardia, hypotension	2
Psilocybin, psilocin	Mushroom	*CNS:* hallucinations, anxiety, mood elevation, weakness	2
Diverse, mostly unknown	Mushroom	Gastrointestinal	2
Monomethylhydrazine, gyromitrin	Mushroom	Cellular destruction, gastrointestinal, loss of coordination, convulsion, coma, death	6-12
Amatoxins, phallotoxins	Mushroom	Cellular destruction, gastrointestinal, hepatic and renal necrosis	6-24
Vomitoxin (deoxynivalenol)	Cereals contaminated with *Fusarium* species	Vomiting acutely Altered mucosal immunity with chronic exposure	<3

**Includes antimony, arsenic, cadmium, copper, mercury, thallium, tin, and zinc.*
CNS, central nervous system.

by a food handler or preparer (e.g., ready-to-eat food), inadequate cleaning of processing or preparation equipment or utensils (e.g., cutting board), handling by an infected person or carrier of a pathogen (*Salmonella* spp. or norovirus), and cross-contamination from a raw ingredient of animal origin (e.g., raw poultry on preparatory surface). Factors associated with proliferation of a pathogen in food include allowing foods to remain at room or warm outdoor temperature for several hours, inadequate cold-holding temperatures, slow cooking, insufficient time or temperature during hot holding, and preparation of foods a half day or more before serving. Most of the proliferation factors are associated with outbreaks due to bacteria. The major places where food associated with outbreaks was eaten are restaurants and delicatessens (50%) and private residences (20%); a variety of other or unknown places account for the remainder.[245]

Outbreaks of gastroenteritis caused by caliciviruses have been associated with consumption of contaminated oysters, salads, and bakery products and with transmission of the virus by food handlers.[229,272] The major causes of reported food-borne disease outbreaks due to bacteria are *Salmonella* spp., Shiga toxin–producing *E. coli*, *C. perfringens*, *S. aureus*, *Shigella* spp., and *Campylobacter jejuni*. Bacterial diseases associated with ingestion of unpasteurized milk include salmonellosis and campylobacteriosis and, less frequently, infection with *Brucella* spp., *E. coli*, *L. monocytogenes*, *Mycobacterium* spp., *S. aureus*, *Streptococcus* spp., *Streptobacillus moniliformis*, and *Yersinia enterocolitica*.[245] A nationwide outbreak of *Salmonella enteritidis* gastroenteritis involved 224,000 people in the United States and was associated with ingestion of contaminated ice cream. The origin of the outbreak was pasteurized ice cream premix that was contaminated in tanker trailers that previously had carried nonpasteurized liquid eggs containing *S. enteritidis*.[171] Consumption of raw milk has been associated with a chronic diarrhea syndrome of unknown cause,[294] initially identified in Minnesota and referred to as *Brainerd diarrhea*. It is manifested by acute onset with marked urgency and a lack of systemic symptoms. The duration of illness is at least 9 months and generally has involved adults. Clinical and laboratory data indicate that diarrhea is caused by a secretory mechanism. No evidence of secondary transmission exists. Antimicrobial therapy has not been successful.[294] In addition, an outbreak of chronic diarrhea involving 72 people who ingested untreated drinking water was reported.[297] The cause and pathophysiologic mechanism of the illness remain unknown.

Ingestion of raw fish (sushi or sashimi) has led to infection with *Vibrio parahaemolyticus* and various parasites from infected fish.[87,245] Parasites acquired through ingestion of raw fish include larval nematodes of the family Anisakidae, fish tapeworm of the species *Diphyllobothrium*, the fluke *Nanophyetus salmincola* from salmon, and many other helminths.[363,436] Most worm infections acquired from raw fish in the United States have been acquired from dishes prepared at home and not from sushi restaurants.

Symptoms in patients with chemical poisoning generally begin within 1 to 2 hours after ingestion, although certain mushroom toxins may produce symptoms for up to 24 hours (see Table 50–3). Heavy metals, such as antimony, arsenic, cadmium, copper, mercury, thallium, tin, and zinc, cause irritation of the gastric mucosa, with nausea, vomiting, and abdominal cramps, which

usually resolve 2 to 3 hours after the offending agent has been removed.[62]

The toxic syndromes acquired from fish and shellfish can be grouped clinically into two categories: the histamine-like syndrome of scombroid poisoning and the neurotoxic syndrome, which includes ciguatera, paralytic shellfish poisoning, neurotoxic shellfish poisoning, and puffer fish poisoning caused by tetrodotoxin.[121] The neurologic symptoms produced by fish, shellfish, and the Chinese restaurant syndrome agent include paresthesia, reversal of hot-cold sensations, loss of proprioception, flushing, weakness, and burning sensations. Chinese restaurant syndrome appears to be caused by excessive amounts of monosodium glutamate in foods. Fish poisoning may be caused by scombrotoxin, ciguatoxin, or tetrodotoxin. Scombroid fish poisoning is characterized by symptoms resembling those of histamine release. Shellfish poisoning can be of two types: paralytic, caused by neurotoxins including saxitoxin, and neurotoxic, caused by several poorly characterized neurotoxins. Domoic acid from contaminated mussels causes acute widespread neurologic dysfunction and GI tract manifestations followed by chronic residual memory deficits and motor neuropathy or axonopathy.[303,400]

Infectious diseases associated with consumption of raw and lightly cooked shellfish (mussels, clams, oysters, lobsters, and other mollusks) are caused by bacterial agents that are native to the marine environment and by viral and bacterial agents from sewage effluents and other sources that contaminate environmental waters. As filter-feeding organisms, shellfish amplify public health problems associated with environmental contamination because they accumulate microbial pathogens at densities many times those found in overlying waters.

Public health problems of greatest concern to consumers of molluscan shellfish are associated with viral pathogens. The numbers of cases and outbreaks caused by these pathogens far exceed those of all other infectious causes.[245] The *Vibrio* genus (specifically *Vibrio vulnificus*) presents the most serious problem in terms of the severity of human illness and death, especially in people with liver disease.[180]

Mushrooms produce several clinical syndromes, generally within 2 hours of ingestion (see Table 50–3),[167] except for poisoning caused by amatoxins, phallotoxins, amantin, monomethylhydrazine, and gyromitrin, which may produce symptoms, including death, up to 24 hours after ingestion.[167] Mushrooms also can be contaminated with other agents, including *S. aureus* enterotoxin A.[235]

PulseNet is a national network of public health laboratories that perform pulsed-field gel electrophoresis analysis on bacteria that might be food-borne.[362] This network provides rapid comparison of pulsed-field gel electrophoresis patterns through an electronic database at the CDC. PulseNet has assisted in detecting food-borne outbreaks, especially those that involve multiple states.[362] An annual listing of food-borne disease outbreaks reported to the CDC is available at *www.cdc.gov/foodborneoutbreaks/outbreak_data.htm*.

Since 1971, the number of reported water-borne outbreaks in the United States has averaged 30 per year (range, 13 to 53 years), involving approximately 7600 people annually (range, 1569 to 21,149 people).[120,236] The CDC has published recommendations for management of fecal accidents in disinfected recreational water venues contaminated with formed or liquid stool.[72]

Water-borne disease and outbreaks are reported as those associated with drinking water and water not intended for drinking and those associated with recreational water.[120,236] During 2003 through 2004, 36 water-borne disease outbreaks associated with drinking water were reported; 30 were associated with water intended for drinking, 3 with water not intended for drinking, and 3 with water of unknown source.[236] In the 30 outbreaks associated with drinking water, 4 deaths occurred among the 2760 people who became ill. Etiologic agents were identified in 25, with 68 percent involving pathogens (13 bacterial, 1 parasitic, 1 viral, and 2 mixed organisms) and 32 percent involving chemical or toxin poisonings. The major clinical manifestations in these outbreaks were gastroenteritis (68%), acute respiratory tract illness (26%), and dermatitis (7%). Organisms associated with gastroenteritis were *Campylobacter* spp., *Shigella* spp., *Salmonella typhimurium*, *Giardia*, *Cryptosporidium*, and norovirus. *Legionella* spp. were associated with all of the respiratory tract outbreaks.

Types of water systems involved community, noncommunity, individual, and mixed systems. Types of deficiencies associated with outbreaks included untreated surface water intended for drinking, untreated ground water intended for drinking, water treatment deficiencies, and deficiencies in distribution systems including storage. All water-borne disease outbreaks should be reported to the state health department.

During 2003 through 2004, there were 62 reported water-borne disease outbreaks associated with recreational water that involved 2698 people, resulting in 58 hospitalizations and one death.[120] Of the 62 outbreaks (1945 people), 48 percent were gastroenteritis outbreaks associated with enteric pathogens, chemicals, or toxins (*Cryptosporidium*, *Giardia*, *Shigella* spp., norovirus, *Plesiomonas shigelloides*, and microcystin toxin from blue-green algae); 21 percent were dermatitis; 11 percent were respiratory illness; and 13 percent were other illnesses.

Recreational water–associated outbreaks of gastroenteritis followed exposure to both treated (60%) and untreated (40%) water. In outbreaks that occurred after exposure to treated water, *Cryptosporidium* was associated with 56 percent; in exposure to untreated water, *Cryptosporidium* accounted for 8 percent of outbreaks. There also were 142 *Vibrio* illnesses associated with recreational water exposure.

The main therapeutic modality in most patients with food poisoning is supportive care because most of these illnesses are self-limited. Exceptions include botulism, paralytic shellfish poisoning, tetrodotoxin, long-acting mushroom poisoning, and Shiga toxin–producing *E. coli* (STEC) infection, all of which may be fatal in previously healthy people. Food-borne disease caused by any agent can produce fatalities in infants, elderly people, debilitated people, or people with primary or secondary immune deficiencies. Food irradiation is an important technology that can protect the public against food-borne diseases.[395] Several Web sites that provide information about food-borne disease include *www.ama-assn.org/foodborne*, *www.cdc.gov/foodnet*, *www.cdc.gov/foodsafety*, and *www.fightbac.org*.

ANTIMICROBIAL-ASSOCIATED DIARRHEA

Diarrhea associated with use of an antimicrobial agent is a common occurrence. Changes in bowel flora may result in abnormal stools in the absence of a documented enteropathogen, and discontinuation of the medication may be sufficient to eliminate symptoms. In some patients with antibiotic-induced diarrhea, a severe and potentially fatal form of colitis may develop.[31,44,184,249]

Pseudomembranous or antimicrobial-associated colitis (AAC) refers to the presence of a pseudomembrane or of multiple plaquelike lesions in the colon induced by administration of an antimicrobial agent within the preceding 8 weeks. Pseudomembranous colitis not associated with use of antibiotics also may occur with *Shigella dysenteriae* serotype 1 and STEC.[214] Toxins produced by *Clostridium difficile* are the specific cause of essentially all cases of AAC.[365] Since 2005, an epidemic of *C. difficile* infection that is more serious and more refractory to therapy has been reported.[192,241,256,424] These studies have reported a highly characterized strain designated BI/NAP1, which is responsible for most of these outbreaks. The strain is a hypersecretor of the classic toxins A and B associated with *C. difficile*, possesses an

additional toxin known as binary toxin, manifests deletion of an 18–base pair sequence in the pathogenicity locus responsible for down-regulation of toxin production, and has in vitro resistance to fluoroquinolones.[31,302]

Disease associated with *C. difficile* encompasses a range of severity from colitis, which often manifests as diarrhea, to toxic megacolon, requirement for colectomy, leukemoid reaction, and severe hypoalbuminemia that may result in sepsis, shock, and death.[44,88,134] These complications occur more commonly in older adults. In addition, nosocomial infection with *C. difficile* occurs and can result in asymptomatic carriage or diarrhea.[257] Most antimicrobial agents have been reported to cause this condition; broad-spectrum cephalosporins and clindamycin are implicated most commonly. Fluoroquinolones have emerged as a predominant risk for development of *C. difficile*–associated diarrhea.[302] Cancer chemotherapeutic agents also have been associated with AAC.

The diagnosis of AAC in a patient with diarrhea is supported by the following[29]:

1. A history of ongoing or recent antimicrobial therapy. Twenty to 40 percent of episodes occur after the drug has been discontinued.
2. Finding of leukocytes on a stain of fecal material.
3. Exclusion of other agents known to cause fecal leukocytes.
4. Identification of *C. difficile* toxins in stool specimens.
5. Endoscopy with rectal biopsy to identify pseudomembranes or plaques that contain mucin, fibrin, leukocytes, and sloughed epithelial cells.

Plaque and pseudomembranes not always are present in patients with AAC, but when they are, *C. difficile* usually can be presumed to be the cause of disease. The finding of *C. difficile* in feces suggests but does not confirm that *C. difficile* is the cause of the diarrhea. The finding of fecal toxin is a more useful method of diagnosing *C. difficile*–induced disease than is bacterial culture.[288] The enzyme immunoassay is used now by most laboratories because of ease of processing, cost, and speed of results.[31] This organism and the toxins may be found in the GI tract of some healthy people.[257,265] Approximately 15 to 63 percent of neonates, as many as 50 percent of infants younger than 1 year, and as many as 8 percent of children are asymptomatic carriers.[76,103]

The most important aspect of therapy in patients with AAC is discontinuation of the antimicrobial agent, after which symptoms usually resolve within 1 to 2 weeks; however, if symptoms persist or worsen or if the patient has severe diarrhea, specific therapy with metronidazole or oral vancomycin should be instituted.[169,260,301,399] Rifampin has been used as an adjunct to treatment with metronidazole, but it is not recommended.[230] Teicoplanin, fusidic acid, bacitracin, and nitazoxanide also have been used.[184,277,429] Metronidazole is preferred in guidelines, but vancomycin appears to be more effective in patients with severe disease.[31,276,300,441] After therapy has been initiated, symptoms generally resolve within several days, and fecal toxin disappears. Relapse of colitis after discontinuation of metronidazole or vancomycin has been documented in 10 to 20 percent of patients, generally 1 to 4 weeks after treatment has been discontinued.[32,301,399] Relapses are caused by germination of *C. difficile* spores that persist despite treatment and are not due to development of resistance. Relapses can be treated with a second course of metronidazole. Other modes of therapy include dietary reduction of carbohydrates and administration of probiotics to allow normal intestinal flora to be re-established.[184] Two meta-analyses demonstrated the benefit of probiotics in preventing antibiotic-associated diarrhea and treating *C. difficile* disease.[256,390] One study evaluated a variety of different types of probotics[256]; the other evaluated *Saccharomyces boulardii*.[390] Further research is needed to determine the optimal probiotic, the best dose and duration of treatment, and the effectiveness against specific antimicrobial agents.

TRAVELER'S DIARRHEA

Traveler's diarrhea is the most common illness encountered by people from the United States, northern Europe, Canada, or Australia who travel to Latin America, Asia, the Middle East, or Africa, where diarrhea is hyperendemic.[113,115-117,160] Half of these travelers experience diarrhea within the first few weeks after arrival. Enterotoxigenic *E. coli* (ETEC) is the most commonly identified cause of traveler's diarrhea, but the illness can be caused by a variety of bacteria, viruses, and parasites,[115,116,290,364] including *Shigella* spp., *C. jejuni*, *Aeromonas* spp., *P. shigelloides*, *Salmonella* spp., *V. parahaemolyticus* (in Asia), *G. lamblia*, enteroaggregative *E. coli*, and enteric viruses, specifically norovirus. Information from existing studies concerning pathogens associated with diarrhea among children or elderly people who travel is limited. Studies of diarrhea in travelers visiting the United States and Great Britain from developed and developing countries showed attack rates of 2 and 0.6 percent, respectively.[346]

Clinical illness varies, reflecting the diversity of causative agents. Typically, illness occurs within several weeks of arrival in a foreign country and is defined as passage of at least three unformed stools in a 24-hour period, together with cramps, nausea, fecal urgency, tenesmus, or a combination thereof. Bloody diarrhea, vomiting, and documented elevation of temperature are less frequent signs and symptoms. Approximately 50 percent of people who become ill have mild disease, 15 to 40 percent are forced to alter their scheduled activities, and 15 to 30 percent are confined to bed. Because traveler's diarrhea is caused by a variety of enteropathogens, travelers may experience more than one episode. Traveler's diarrhea is spread by many fecally contaminated vehicles; most studies implicate food, particularly uncooked food,[128,405] and untreated water and ice.[174] Careful attention given to food consumption with avoidance of foods that are not steaming hot, raw vegetables, fruit not peeled by the traveler, tap water, and ice is critical to prevention of disease.[128,174] Predisposing factors and host factors that increase susceptibility to traveler's diarrhea or complicate its course include young age, short duration of travel, eating in restaurants, season, reduced gastric acidity, chronic and active GI tract disease, and immunodeficiency disorders.[128,183]

Several studies have evaluated prevention and treatment of traveler's diarrhea in adults using either antidiarrheal compounds or antimicrobial agents.[117] Compounds that have been shown to be effective chemoprophylactic agents include doxycycline, bismuth subsalicylate, trimethoprim-sulfamethoxazole (TMP-SMX), fluoroquinolones, and nonabsorbable antibiotics including rifaximin.[4,108,113,114,117] These compounds have been shown to offer 50 to 100 percent protection against traveler's diarrhea.[4,117] Potential problems with antibiotic regimens include development of resistance against certain agents, including ETEC, *Salmonella* spp., *Shigella* spp., and *C. jejuni*, and side effects, such as nausea, diarrhea, and photosensitivity reactions. Large doses of bismuth subsalicylate can result in significant concentrations of serum salicylate.[135,316] The bioavailability of salicylate in 1 ounce of Pepto-Bismol is equivalent to that of one 325-mg adult aspirin tablet.[135] Probiotics have been shown to be only minimally effective in prevention of diarrhea.[117]

Because of the uncertain risk of widespread prophylactic administration of these antimicrobial agents, including development of resistance among enteric bacteria, and the mildness of most cases of traveler's diarrhea, prophylactic use of antimicrobial agents is not recommended in children and has limited use

in adults.[117,160] Instructing travelers with regard to appropriate dietary practices as a prophylactic measure is recommended.[128,405] If a person becomes ill in a locale where medical facilities are inadequate, self-treatment may be indicated.[4,117] Antidiarrheal compounds as therapy for traveler's diarrhea have been used in people with nondysenteric disease. Bismuth subsalicylate has been shown to be effective in decreasing the incidence of diarrhea by up to 60 percent.[116] Loperamide should not be used in infants or older children with bloody diarrhea or fever, although it may benefit older children and adults with watery diarrhea, especially when it is given in combination with an antimicrobial agent.[18,107,125] Other nonantimicrobial compounds have been shown to be ineffective or even dangerous or have not been evaluated.[18]

For self-treatment of diarrhea that occurs during travel in areas where medical care may not be available, travelers should take with them medication with expected activity against the prevalent bacteria. A 3- to 5-day antibiotic course may help children who develop diarrhea with three or more loose stools in an 8-hour period, especially if associated symptoms such as nausea, vomiting, abdominal cramps, fever, and blood in stools are present. A fluoroquinolone, azithromycin, or rifaximin for adults and azithromycin for children should be considered.[4,5,106,107,117,125,307] Problems with development of resistance by *C. jejuni* to fluoroquinolones may preclude their use in certain areas.

Approximately 3 percent of people develop persistent diarrhea after an episode of traveler's diarrhea.[109] The differential diagnosis should include persistent infection, particularly with *Giardia*, *Cryptosporidium*, *Cyclospora*, or *Isospora* spp.; antibiotic-associated colitis; small intestinal bacterial overgrowth; transient disaccharidase deficiency; initial manifestation of ulcerative colitis or regional enteritis; postdysenteric colitis; irritable bowel syndrome; and tropical sprue.[109] An underlying immune system defect, such as occurs with AIDS, also should be considered.

HUMAN IMMUNODEFICIENCY VIRUS INFECTION

Diarrheal disease often is a major problem in people with primary and secondary immunologic disorders.[22,356,439] The GI tract must act as the first line of defense against a wide variety of potentially harmful organisms and toxins. Immune deficiencies can result in an increased host susceptibility to intestinal tract infections or an atypical, prolonged, or disseminated course once an organism is established.

Causes of GI tract disease in children with AIDS can be grouped under the major headings of infection, malignant disease, and HIV enteropathy. The location of GI tract involvement by infectious agents determines the clinical symptoms and the ease with which these organisms can be diagnosed and treated (Table 50–4). Organisms and diseases of the GI tract that fulfill the CDC surveillance case definition of AIDS[59] are candidiasis, cryptosporidiosis, cytomegalovirus, herpes simplex, *Mycobacterium avium* complex, *Microsporidium*, isosporiasis, and *Salmonella* spp.[21,225,264] In addition, the AIDS wasting syndrome (HIV infection without superimposed opportunistic enteric infection of known cause) is part of the CDC case definition for AIDS.[59] Infection with any organism requires more attention because of malnutrition and co-morbidity.[203] Patients with AIDS have been shown to have D-xylose malabsorption and steatorrhea, and on jejunal and rectal biopsy specimens, a specific pathologic process can be demonstrated in the lamina propria of the small intestine and colon.[225] Before institution of highly active antiretroviral therapy (HAART), a significant proportion of patients with AIDS and severe small intestinal injury had enterocyte infection,[224] and infections occurred more frequently and were more likely to be severe, recurrent, persistent, or associated with extraintestinal disease.

TABLE 50–4 Organisms That Cause Gastrointestinal Tract Infections in Patients with AIDS

Area	Organisms
Esophagus	*Candida albicans**
	Cytomegalovirus*
	Herpes simplex virus*
Hepatobiliary	Cytomegalovirus
	*Cryptosporidium**
	Hepatotropic viruses
	Mycobacterium avium complex*
Small intestine	*Campylobacter* species
	Cytomegalovirus*
	*Cryptosporidium**
	*Giardia lamblia**
	*Isospora belli**
	Mycobacterium avium complex*
	*Microsporidium** (*Enterocytozoon bieneusi* and *Encephalitozoon intestinalis*)
	Salmonella species*
	Strongyloides stercoralis
Large intestine	*Campylobacter* species
	Clostridium difficile
	Cytomegalovirus*
	Entamoeba histolytica
	Herpes simplex virus*
	Salmonella species*
	Shigella species

**Diseases of the gastrointestinal tract that fulfill the Centers for Disease Control and Prevention surveillance case definition of AIDS.*

The occurrence of opportunistic infections in people infected with HIV should be considered as infections that occurred before and those since the advent of HAART. In 1993, 11,285 HIV-infected children aged 18 years or younger in the United States were hospitalized, compared with an estimated 3419 in 2003 (a 71% decrease).[227] The inpatient fatality rate also decreased from 5 percent in 1994 to 1.8 percent in 2003. In Thailand, the hospitalization rate in children decreased from 31 percent during the first 24-week period to 2 percent during weeks 120 to 144 after initiation of HAART.[327] The mortality rate also decreased from 5.7 percent in the first 24 weeks to 0 to 0.6 percent in the subsequent weeks of HAART therapy.[327] In another study in the United States, the impact of HAART on the overall incidence of opportunistic infection was significant, with a decline in incidence from 14.4 to 1.1 cases per 100 patient-years.[285] A study in adults examined the annual incidence of bacterial diarrhea before and during the HAART era in a large cohort of people with HIV infection.[285] Between 1992 and 2002, the overall rate of bacterial diarrhea in people with clinical AIDS decreased (odds ratio, 0.4; 95% confidence interval, 0.2 to 0.6). *C. difficile* was the most common cause of bacterial diarrhea. HAART can result in complete clinical, microbiologic, and histologic responses in patients with AIDS infected with *Cryptosporidium parvum* and *Enterocytozoon bieneusi*[56] and in patients with HIV enteropathy.[225] Guidelines for prevention of opportunistic infections, including those involving the GI tract, have been published.[64]

ORGANISMS THAT CAUSE ACUTE GASTROENTERITIS

BACTERIA

Many bacterial, viral, and parasitic organisms produce diarrhea in humans. Tables 50–5 and 50–6 show the major bacterial enteric pathogens associated with acute infectious diarrhea.[80] These organisms are discussed here in alphabetical order for ease

TABLE 50–5 Pathogenic Bacteria Associated with Acute Infectious Diarrhea

Agent	Epidemiologic Considerations
Aeromonas hydrophila	Water, food, or animal exposure
Bacillus cereus	Food exposure
Campylobacter jejuni	Animal or food exposure
Clostridium difficile	Exposure to antimicrobial agents
Clostridium perfringens	Food exposure
Listeria monocytogenes	Food exposure
Plesiomonas shigelloides	Water, fish, or animal exposure
Salmonella species	Exposure to carrier; food and reptile exposure
Shigella species	Exposure to an infected person or to contaminated food
Staphylococcus aureus	Food exposure
Vibrio cholerae O1	Food or water exposure
Vibrio cholerae O139	Food or water exposure
Vibrio parahaemolyticus	Seafood exposure
Yersinia enterocolitica	Food or animal exposure

TABLE 50–6 Pathogenic *Escherichia coli* Associated with Acute Infectious Diarrhea

Class of *E. coli*	Abbreviation	Usual Presentation of Disease
Shigatoxigenic	STEC	Bloody diarrhea, water- and food-borne outbreaks, associated with hemorrhagic colitis and hemolytic-uremic syndrome
Enterotoxigenic	ETEC	Watery diarrhea in children living in and travelers visiting developing countries
Enteroinvasive	EIEC	Dysentery in adults and watery diarrhea, occasionally food-borne outbreaks
Enteropathogenic	EPEC	Acute and chronic watery diarrhea in infants in nurseries, diarrhea in children younger than 1 year in developing countries
Enteroaggregative	EAEC	Acute and chronic watery diarrhea in children

of reference. The relative importance of each organism as a cause of diarrhea varies in different populations.

Aeromonas hydrophila

Aeromonas spp. are gram-negative bacteria found in soil and fresh and brackish water worldwide. *Aeromonas* spp. are recognized as colonizers and pathogens of cold-blooded animals, including fish, reptiles, and amphibians.[16] *Aeromonas* spp. have been associated with a wide spectrum of human disease, most frequently gastroenteritis, soft tissue infection, and bacteremia, especially in immunocompromised hosts.[16,196] Of the 14 species in the genus, *Aeromonas hydrophila*, *Aeromonas veronii*, *Aeromonas caviae*, *Aeromonas jandaei*, and *Aeromonas schubertii* have been associated with human disease; the first three are defined as major pathogens and the species associated with gastroenteritis.[196] Because many clinical laboratories cannot perform precise identification, most species isolated are reported as *A. hydrophila*.

The role of *A. hydrophila* in human diarrhea remains uncertain. Despite the association of this organism with acute gastroenteritis and in some studies its more frequent isolation from patients with diarrhea than from healthy controls,[196] volunteers who have been fed the organism have not become ill.[270] *A. hydrophila* has been isolated from stools of less than 3 percent of healthy humans[16]; however, in Thailand, the isolation rate from healthy controls increased with age, up to 27 percent in adults.[16] *A. hydrophila* strains have been shown to produce both cytotoxin and enterotoxin, including heat-labile (cholera-like) enterotoxin and heat-stable enterotoxins.[365] Children with watery diarrhea associated with *Aeromonas* are significantly more likely to have organisms that possess genes for both a heat-labile cytotonic enterotoxin and the heat-stable cytotonic enterotoxin than are control children.[11] Other toxic or invasive properties may be important in production of disease because a fourth of patients have a dysentery-like illness.[196]

Bacillus cereus

Bacillus cereus is an aerobic, spore-forming, gram-positive bacillus that is a rare cause of food poisoning in the United States.[60,244] Although frequently present in food, *B. cereus* should be suspected as the cause of GI tract illness if appropriate symptoms are present and if incriminated food, particularly fried rice, contains 10^5 or more *B. cereus* organisms per gram.[62] Two distinct forms of GI tract illness can be produced by this organism. One is caused by production of a low-molecular-weight (1.2-kd) preformed emetic toxin, cereulide, that survives high temperatures, exposure to trypsin, and pH extremes. The other is caused by a group of three enterotoxins sensitive to high temperatures, proteolytic enzymes, and acids and is formed in vivo.[365] If the strain causing illness produces the emetic toxin, a syndrome occurs that resembles illness produced by staphylococcal enterotoxin, with nausea, vomiting, and abdominal cramps that begin within 1 to 6 hours of ingestion. Rarely, illness associated with the emetic toxin is followed by fulminant liver failure.[97] The diagnosis may be difficult to confirm because heating may kill the organism but leave the toxin intact. If the strain produces enterotoxins, profuse watery diarrhea and abdominal pain begin within 6 to 24 hours, with minimal or no vomiting. Some strains produce both toxins, whereas others produce only one toxin. Symptoms caused by either toxin usually resolve in less than 24 hours, and fever rarely occurs. Spores of *B. cereus* are resistant to heat and, therefore, may withstand a brief period of cooking or boiling. *B. cereus* can grow in temperatures ranging from 25° C to 40° C. *B. cereus* also has been associated with panophthalmitis, endophthalmitis, pneumonia, bacteremia, endocarditis, and meningitis.[105]

Campylobacter

*Campylobacte*r spp. are recognized as one of the most important causes of acute diarrheal disease in humans throughout the world[15,55,202] and are a major bacterial cause of diarrhea in the United States.[15,70] Most diarrheal illness in the United States caused by *Campylobacter jejuni* is food-borne.[245] Currently, 18 *Campylobacter* spp. and subspecies are recognized. *C. jejuni* and *Campylobacter coli* are the two predominant species, although use of selective media and lack of use of stool filtration techniques may preclude isolation of other *Campylobacter* spp.[202,231,411] Many clinical microbiology laboratories do not differentiate *C. jejuni* and *C. coli*. *Campylobacter fetus*, recognized as a cause of fever, bacteremia, and meningitis in immunocompromised hosts and of abortion, rarely causes diarrhea. People infected with *C. jejuni* may develop diarrhea, cramping abdominal pain, chills, and fever. Gross rectal bleeding may occur, and mucus and fecal leukocytes may be present, resembling the illness produced by *Shigella*. The clinical presentation also may mimic that of inflammatory bowel disease. Both a heat-labile enterotoxin and mucosal invasion have been incriminated in the pathogenesis. *C. jejuni* also has been associated with reactive arthritis and Guillain-Barré syndrome.[278,393]

Phylogenetic trees have been established for *Campylobacter* and contain three distinct clades (species groups). The first consists of *C. fetus*, *Campylobacter hyointestinalis*, and *Campylobacter mucosalis*, all generally associated with disease in farm animals, although *C. fetus* and *C. hyointestinalis* produce human disease. The second clade consists of *C. coli*, *C. jejuni*, *Campylobacter helveticus*, *Campylobacter lari*, and *Campylobacter upsaliensis*; all (except *C. helveticus*) have been associated with gastroenteritis in humans. The third clade contains *Campylobacter curvus*, *Campylobacter concisus*, *Campylobacter gracilis*, *Campylobacter rectus*, *Campylobacter showae*, and *Campylobacter sputorum*, organisms generally associated with the periodontal cavity of humans and animals.

Helicobacter pylori (formerly *Campylobacter pylori*) has been isolated from the stomach and duodenum of patients with histologically confirmed type B antral gastritis, peptic ulcer disease, duodenal ulcers, and gastric lymphoma.[46] This organism is not associated with diarrhea. *Helicobacter cinaedi* and *Helicobacter fennelliae* were identified previously as *Campylobacter* spp. but have been reclassified.

Clostridium perfringens

Clostridium perfringens types A, C, and D produce an enterotoxin that is implicated in the pathogenesis of disease caused by this organism.[365] Most food-borne outbreaks are caused by type A strains. *C. perfringens* causes a short-duration food poisoning syndrome.[251] After contaminated meat or poultry products have been ingested, in vivo sporulation occurs in the small intestine, with release of a structural spore protein that has enterotoxic and cytotoxic properties. The heat-labile, 35-kd, single-polypeptide enterotoxin induces fluid accumulation in ileal loops in animals and produces diarrhea in humans. Within 14 hours after ingesting contaminated food, patients experience watery diarrhea and abdominal pain with minimal nausea, vomiting, or fever ensuing. Illness resolves in less than 24 hours. Less than 5 percent of *C. perfringens* isolates contain the chromosomal *cpe* gene encoding this toxin. *C. perfringens* type C also is associated with a rare destructive intestinal disease called *enteritis necroticans* or *pigbel*. These strains produce three toxins (alpha-toxin, beta-toxin, and an enterotoxin) of potential pathogenetic significance.[365] Enteritis necroticans occurs after ingestion of undercooked pig at pork feasts in Papua, New Guinea. It is characterized by vomiting, abdominal pain, bloody diarrhea, and small bowel necrosis, with peritonitis, shock, and death.[275]

Escherichia coli

Several recognized categories of *Escherichia coli* produce diarrhea (see Table 50–6).[279,365] *E. coli* is among the most common cause of bacterial diarrhea in humans worldwide.[315]

Shiga toxin–producing *E. coli* (STEC) produces bloody diarrhea, usually without fever. This hemorrhagic colitis syndrome has been recognized to be caused most often by *E. coli* O157:H7 and other STEC strains, which produce large quantities of a potent cytotoxin[65,210,279,365] similar or identical to the cytotoxin produced by *S. dysenteriae* serotype 1.[210] STEC also is referred to as enterohemorrhagic *E. coli* (EHEC) and verotoxin *E. coli* (VTEC).[323] Several closely related toxins are recognized. The *E. coli* toxin that essentially is identical to Shiga toxin made by *S. dysenteriae* is called Shiga toxin 1 (Stx1, also called verotoxin 1). A structurally and functionally related toxin that is distinct immunologically is called Stx2 (or verotoxin 2). Both toxins are encoded by bacteriophages. Multiple additional variants exist that are more closely related to the second toxin. *E. coli* organisms that produce high levels of cytotoxin are important clinically because, like *S. dysenteriae* 1, they have been incriminated as etiologic agents of hemolytic-uremic syndrome.[317] Most reported outbreaks result after ingestion of contaminated food or water. Healthy cattle harbor the organism as part of their intestinal flora and are the main animal reservoir for STEC. Direct transmission from animals and their environments to humans in public settings where children come in contact with farm animals, such as petting zoos, represents a public health concern.[319] Hemolytic-uremic syndrome is characterized by the triad of hemolytic anemia, thrombocytopenia, and renal insufficiency and is associated most frequently with *E. coli* O157:H7. Approximately 8 percent of people infected with *E. coli* O157:H7 develop hemolytic-uremic syndrome; most reported cases occur in children younger than 5 years old.

Enterotoxigenic *E. coli* (ETEC) disease is caused by heat-stable (ST) and heat-labile (LT) enterotoxins.[279,328,365,398] STa and LT-I are associated with disease in humans and other animals, whereas STb is associated primarily with disease in piglets, and LT-II has been associated only with animal disease. ETEC strains from humans with diarrhea produce STa only, LT-I only, or both together. These toxins often are produced by strains that have colonization factor antigens, which are important in adherence of the organism to the GI tract.[279] ETEC strains belong to many different serogroups and cause disease in patients of all ages, especially infants and children living in developing countries and travelers from developed to developing countries, and outbreaks of food-borne disease in the United States.[328,398]

Enteroinvasive *E. coli* (EIEC) is related antigenically and biochemically to *Shigella* and causes either a dysentery-like illness or watery diarrhea. EIEC possesses 140-Md plasmids that encode invasiveness and contribute to dysenteric illness. This plasmid is related closely to the plasmids that are associated with *Shigella* virulence.[279] The watery diarrhea may be due to an enterotoxin referred to as *EIEC enterotoxin*. Infections generally occur in adults; food-borne outbreaks have been reported.

Enteropathogenic *E. coli* (EPEC) has been incriminated as a cause of both sporadic and epidemic diarrhea in infants, especially in developing countries.[100] Originally, EPEC was a term used to describe all *E. coli* organisms associated with diarrheal syndromes. Currently, the term EPEC is defined more narrowly. Volunteer studies[100] and studies comparing rates of isolation from sick and healthy infants[279] have demonstrated that EPEC organisms are pathogens, although these strains rarely cause diarrhea in older children and adults. The specific mechanisms involved in production of disease may be related to adherence, which can be demonstrated in HEp-2 cells.[101,279] The proposed three stages of EPEC infection include (1) intestinal colonization mediated by the bundle-forming pilus; (2) induction of the attaching and efficacy lesion through intimin and other secreted proteins; and (3) signal transduction, resulting in induction of a net secretory state.[95,100,102,365]

Enteroaggregative *E. coli* (EAEC) is a cause of acute diarrheal illness among many different subpopulations in both developing and industrial regions of the world,[191,192] including the United States. EAEC also has been associated with chronic diarrhea in developing countries[39,156,364] and in adults with traveler's diarrhea.[3,156] Bloody diarrhea has been described in approximately a third of patients. Chronic persistent diarrhea is especially likely to be caused by EAEC.[361,364] Pathogenicity of EAEC in humans has been confirmed in volunteer studies and outbreak investigations.[252,376] EAEC is defined by its aggregative or stacked brick pattern of adherence in HEp-2 cell assays,[279] the "gold standard" for identification. Some strains elaborate an enterotoxin (EAEC ST enterotoxin 1 [EAST1]).[279,359] However, this toxin is not uniquely present in EAEC, nor is it found consistently (only 40-45% of EAEC pathogens are EAST1-positive in some series).[279,361] A plasmid-encoded enterotoxin may be important, but its role is not fully defined.[124,282] Many EAEC pathogens have genes for aggregative adherence fimbriae (*AAF/I* or *AAF/II*).[85,334] A gene probe that appeared in initial studies to be both sensitive and

specific appears to be less useful than is the original HEp-2 cell assay.[131,334]

Listeria monocytogenes

The genus *Listeria* includes six species, of which two are potentially pathogenic, *Listeria monocytogenes* and *Listeria ivanovii*.[410] *L. monocytogenes* is a rare but serious localized and generalized infection in humans. Listeriosis is primarily food-borne. Acute febrile gastroenteritis caused by *L. monocytogenes* contamination of a variety of foods has been described.[28,67,86,161] The foods most frequently implicated are soft cheeses and dairy products; pâtés and sausages; smoked fish; and industrially produced, refrigerated, ready-to-eat products that are eaten without cooking or reheating. *L. monocytogenes* tolerates high concentrations of salt and relatively low pH and is able to multiply at refrigerator temperatures. Bacteremia may complicate diarrheal illness caused by this organism,[28,67,352] especially in immunocompromised hosts, during pregnancy, and in older people. Symptoms include fever (temperature as high as 40.3° C), chills, headache, cramps, myalgia, and diarrhea.[28,291]

Plesiomonas shigelloides

Plesiomonas shigelloides is a gram-negative bacillus that has been associated with opportunistic infections in immunocompromised hosts and with sporadic cases of diarrhea in immunocompetent hosts in a variety of countries.[187,208] In some case-control studies, the organism has been found to be associated with diarrhea, whereas in others, it has not.[12] Whether a subset of *P. shigelloides* has virulence genes that make them pathogens, whereas other members of this species do not have such genes, is unclear. Organisms produce ST and HL enterotoxins, but their associations with disease are unknown.[365] The organism has been isolated from surface water and the intestines of freshwater fish and many animals, including dogs and cats.[404] *Plesiomonas* occurs commonly in tropical and subtropical areas from which most stool isolates have been reported.[187] Patients with *P. shigelloides* infection describe self-limited diarrhea, occasionally characterized by blood and mucus. The organism is a rare cause of extraintestinal illness, such as meningitis or bacteremia.[208] Appropriate antimicrobial therapy appears to shorten the duration of diarrheal illness.[196,208] The organism has failed to produce illness when fed to volunteers, and its role as an enteric pathogen remains unknown.[173]

Salmonella

Identification of *Salmonella* spp. in the laboratory is not difficult, but the various terminologies used to classify *Salmonella* spp. is confusing. Most hospital laboratories biochemically differentiate *Salmonella* ser. Enterica and *Salmonella* ser. Typhi, although other nomenclature schemes may be used.[51] Since 1993, the *Salmonella* isolates most frequently reported have been *S. enterica* serotype Typhimurium and *S. enterica* serotype Enteritidis.[58,73] Several clinical syndromes are caused by *Salmonella*: the carrier state; acute gastroenteritis; bacteremia, enteric fever, or both; and dissemination with localized suppuration, such as abscess, osteomyelitis, or meningitis. Although *S.* ser. Typhi is the prototype of *Salmonella* able to penetrate intestinal mucosa, reach intestinal lymphatic tissue, and disseminate, other *Salmonella* organisms, particularly *Salmonella* Paratyphi A, occasionally behave in this manner.[73,185] Invasion of intestinal epithelium by *S.* ser. Typhi and occasionally by nontyphoidal *Salmonella* strains is a well-known virulence trait of *Salmonella* spp. Most nontyphoidal *Salmonella* serotypes are associated with watery diarrhea. *Salmonella* rarely causes an illness similar to pseudomembranous colitis.[190] *Salmonella* gastroenteritis occurs throughout life but most commonly in the first 5 years of life, decreases during childhood, and remains relatively constant throughout the adult years.[73] Although most episodes of *Salmonella* infection are food-borne, reptiles, including turtles, snakes, lizards, and iguanas, carry certain serotypes of *Salmonella* in their intestinal tracts and have been associated with episodes of salmonellosis.[281,319,387] Numerous outbreaks of disease caused by *Salmonella* after ingestion of contaminated food products, including eggs, milk, ice cream, peanut butter, and fresh produce, have been reported.[48,68,171,245,326] Food-borne outbreaks associated with *Salmonella* have been reported from fairs or festivals, hospitals, nursing homes, and prisons.[73]

Shigella

Four serogroups of *Shigella*, containing more than 40 serotypes and subtypes, exist.[215] *Shigella sonnei* accounts for more than 75 percent of shigellosis in the United States. *Shigella boydii* and *Shigella dysenteriae* are uncommon causes of diarrhea in the United States. In 2005, a strain of *S. sonnei* resistant to ampicillin and TMP-SMX emerged as a cause of prolonged community-wide outbreaks of shigellosis associated with childcare centers in three states.[368] In addition to person-to-person spread, shigellae can be transmitted through contaminated foods, sexual contact, and water used for drinking or recreational purposes.[120,245] Patients with *Shigella* isolated from stool may present with several clinical patterns: asymptomatic excretion,[312] enterotoxin-like watery diarrhea, bacillary dysentery,[385] arthritis similar to that seen in Reiter syndrome,[9] and hemolytic-uremic syndrome occurring after infection with *S. dysenteriae* 1. The arthritis occurs 2 to 5 weeks after the dysenteric illness, characteristically in patients with histocompatibility antigen HLA-B27. Postinfectious arthritis also occurs in patients after they have had *Salmonella*, *Campylobacter*, and *Y. enterocolitica* infections.[119] *S. dysenteriae* 1 produces Shiga toxin in high levels.[385] Infection by *Shigella* spp. rarely occurs in the first few months of life but is a common occurrence in children between the ages of 6 months and 10 years.[73]

Staphylococcus aureus

Two enteric syndromes have been associated with *Staphylococcus aureus*. Although previously it was considered a cause of AAC, whether antibiotic-associated staphylococcal enteritis actually exists as a disease entity is unclear because most antibiotic-associated colitis is associated with *C. difficile*.[33] The existence of the other major staphylococcal enteric syndrome is well established. Staphylococcal food poisoning is caused by ingestion of food contaminated with preformed *S. aureus* ST enterotoxin.[123,235] Illness usually occurs within 2 to 4 hours after ingestion of contaminated food[244] and is manifested by vomiting and diarrhea. Although multiple enterotoxins (A to F) have been described, only enterotoxin types A to E cause enteric disease. Type A has been responsible for more than half of the reported outbreaks of staphylococcal food poisoning in the United States.[186,235] All toxins are antigenically related, low-molecular-weight proteins. Illness caused by these preformed toxins begins within 1 to 6 hours after ingestion and lasts less than 12 hours. Nausea, vomiting, abdominal pain, and diarrhea occur without fever.

Vibrio cholerae

Strains of *Vibrio cholerae* are classified according to somatic or O groups. *V. cholerae* strains are separated further into two main serotypes (Ogawa and Inaba) and two biotypes (classic and El Tor).[209] *V. cholerae* responsible for epidemic cholera belong to serogroups O1 and O139; all other *V. cholerae* strains belong to serogroups other than O1 and O139 and occasionally cause diarrhea or extraintestinal infections.[197] Cholera affects people of all

ages, but children are involved disproportionately. *V. cholerae* O139 infection occurs primarily in Southeast Asia. *V. cholerae* O1 infection occurs primarily in Asia, Africa, and South America, although a focus is present in the Gulf Coast of the United States.[209,243,271] Most clinical isolates of *V. cholerae* O1 in the United States are associated with foreign travel and with ingestion of undercooked seafood,[73,271] and many are resistant to antimicrobial agents.[386] Crabs harvested from the U.S. Gulf coast are a common source of cholera.[73] After Hurricane Katrina in 2005, crabs were the source of illness for certain cases of cholera.[73,74] Epidemic cholera appeared in Peru in January 1991 and subsequently spread throughout the Americas, including the United States.[209] The epidemic strain is biotype El Tor, serotype Inaba. This strain can be differentiated from the strain of *V. cholerae* that is endemic to the U.S. Gulf coast by production of hemolysin and by molecular subtyping techniques.

V. cholerae O1 adheres to and multiplies on small intestinal mucosa. Diarrhea occurs after elaboration of several toxins, the most important of which is cholera toxin, an HL enterotoxin composed of one A and five B subunits.[365] The B subunits bind the toxin to the terminal galactose of the G_{M1} ganglioside receptors present on intestinal mucosal cells. The A subunit adenosine 5′-diphosphate ribosylates the guanosine 5′-triphosphate–binding regulatory protein of adenylate cyclase in gut epithelium.[365] The resulting intracellular increase in cyclic adenosine monophosphate causes inhibition of sodium absorption and causes chloride and fluid secretion in the small intestine. Most non-O1 strains isolated from ill people in the United States lack cholera toxin–like activity[197] and, when tested with gene probes, are found not to possess gene sequences homologous to those of cholera toxin.[209] Two additional toxins are produced by *V. cholerae*, Zot (zonula occludens toxin) and Ace (accessory cholera enterotoxin). Strains of *V. cholerae* belonging to serotypes other than O1 and O139 are much less significant pathogens, although they can cause mild and occasionally profuse, watery diarrhea. Other *Vibrio* spp., including *Vibrio fluvialis*, *Vibrio mimicus*, *Vibrio hollisae*, and *Vibrio furnissii*, have been shown occasionally to cause GI tract disease.[197]

Vibrio parahaemolyticus

Vibrio parahaemolyticus is a gram-negative, halophilic bacterium that inhabits warm estuarine waters worldwide. The organism has been found in water, shellfish, fish, and plankton[180] and has caused outbreaks of gastroenteritis after ingestion of contaminated seafood.[258,271] Although widely distributed in coastal waters, *V. parahaemolyticus* is an uncommon cause of diarrhea where consumption of raw seafood is common. Clinical manifestations of infection with *V. parahaemolyticus* are gastroenteritis in 59 percent of cases and include abdominal cramps, nausea, and, less frequently, vomiting, headache, low-grade fever, and chills; wound infections, including hemorrhagic cellulitis in 34 percent; and septicemia in 5 percent.[87] A dysentery-like syndrome has been described in India and Bangladesh.[193] Preexisting liver disease predisposes infected patients to development of septicemia and death.[180] A selective culture medium is required for isolation of the organism from stool cultures.

Yersinia enterocolitica

Yersinia enterocolitica is a gram-negative bacillus that appears to be a common cause of gastroenteritis among children in Europe and Canada but is a relatively uncommon cause of enteritis in the United States, where *Y. enterocolitica* O8 has been the predominant clinical serotype.[45] The ingestion of contaminated milk or food such as chitterlings[206,232] has been implicated as the mode of transmission in reported outbreaks. The clinical manifestations vary according to the age of the person involved. Illness in children younger than 5 years usually is self-limited gastroenteritis. Stools may contain blood and mucus or be watery. Associated symptoms consist of fever, vomiting, and abdominal pain. Older children may present with abdominal pain associated with mesenteric adenitis that mimics acute appendicitis. Adults develop diarrhea and abdominal pain less frequently than do children but may present with polyarthritis, arthralgia, or erythema nodosum. Patients with beta-thalassemia and iron overload are at an increased risk for development of severe yersiniosis.[6]

VIRUSES

Acute infectious diarrhea of viral origin generally is a self-limited disease characterized by various combinations of diarrhea, nausea, vomiting, abdominal cramps, headaches, myalgias, and low-grade fever.[254,263,289,412] Bowel movements are watery and generally do not contain mucus or blood. Vomiting is the most common manifestation of this condition. Rotavirus, enteric adenovirus, astrovirus, and norovirus are common causes of viral gastroenteritis (Table 50–7). Other viruses, including coronaviruses, Breda virus, parvoviruses, pestiviruses, picobirnaviruses, and toroviruses, have been linked to gastroenteritis in humans with varying degrees of certainty.[34,222,440]

Rotaviruses

Rotavirus is a 70-nm particle that on electron microscopy resembles a wheel with radiating spokes (Fig. 50–1). Rotavirus was associated with diarrhea in children first by Bishop and associates in 1973.[42] Since then, human rotavirus has been established as a

TABLE 50–7 Viruses Associated with Gastroenteritis

Virus	Approximate Size (nm)
Rotavirus	70
Enteric adenovirus (types 40 and 41)	70-80
Astrovirus	20-30
Calicivirus (noroviruses)	35-39
Parvovirus	20-30
Coronavirus	80-180
Pestivirus	40-60
Breda virus	100

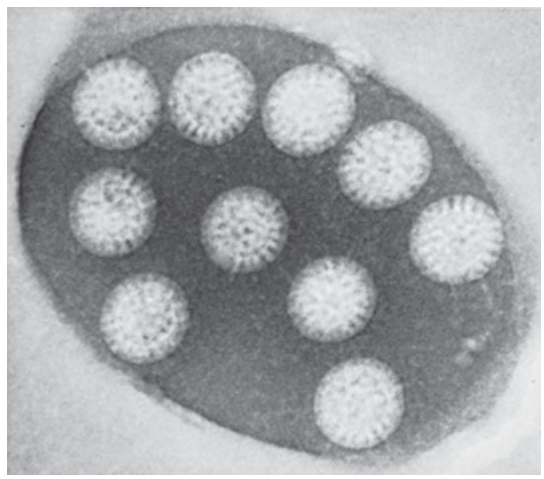

Figure 50–1 Electron micrograph of rotavirus particles in a fecal specimen from an infant with diarrhea. (Phosphotungstic acid, ×238,000.)

major cause of acute gastroenteritis in infants, children, and various animal species worldwide. Six antigenically distinct groups of rotavirus (A through F) have been recognized. Three of these groups (A, B, and C) have been identified in humans. The group A rotaviruses are those associated with infantile diarrhea.[130] Group B rotaviruses have caused epidemics of cholera-like illness in China and sporadic cases elsewhere.[279,366] Group C rotaviruses have caused outbreaks of diarrhea in children in many countries.[286]

Group A rotaviruses have two outer capsid proteins, a hemagglutinin (VP4) and a glycoprotein (VP7), each of which induces neutralizing antibodies.[130] Fourteen different antigenic types of VP7 (which define serotype) and seven human serotypes of VP4 (also designated P serotypes because VP4 is protease sensitive), including two subtypes, are known. This number, combined with the ability of the gene segments to reassort independently, indicates that rotaviruses are complex antigenically.[130] VP7 (also designated G serotype) serotypes 1, 2, 3, 4, and 9 are of epidemiologic importance. Epidemiologic studies indicate that these serotypes are endemic in most regions, that one serotype tends to be predominant at any particular time, that predominant serotypes differ among regions in the same country, and that predictable cycles of change of the predominant serotype may occur.[254]

Rotavirus gastroenteritis affects more than 90 percent of children by the time they are 3 years of age and may cause moderate to severe vomiting that precedes diarrhea. It accounts for 10 to 50 percent of the cases of diarrhea in children and is the most common cause of diarrhea in infants and children during winter months in colder climates. Rotavirus accounts for 35 to 50 percent of infants and young children hospitalized for acute diarrhea. In the United States, rotavirus accounts for 60,000 hospitalizations per year and 37 deaths.[140] Stools usually are watery or soft, and the presence of blood or leukocytes is rare. Asymptomatic rotavirus infections occur frequently,[289,311] and re-infection appears to be a common event.[415,416] The mechanism of spread is fecal-oral; whether respiratory transmission occurs is uncertain. Shedding of virus most frequently occurs from a few days before to 10 days after the onset of illness.[311] Before licensure of the rotavirus vaccine in 2006, outbreaks of rotavirus in childcare centers occurred commonly (usually in the colder months), were caused by a single serotype,[289] and were manifested by the fact that half the infected children were asymptomatic.[30]

The incubation period of human rotavirus infection ranges from 2 to 4 days. Animal models suggest that whereas morphologic changes in the villous tip cells contribute to malabsorption and diarrhea, altered cell function, resulting in enzyme deficiencies, results from enterocyte immaturity and may account for the major part of the disordered physiologic findings. Adults with rotavirus gastroenteritis treated with nitazoxanide showed a significant reduction in time to resolution of symptoms compared with controls (1.5 versus 2.5 days).[341] In hospitalized children, nitazoxanide reduced the duration of rotavirus infection.[338]

The first rotavirus vaccine licensed in the United States was associated with an increased rate of intussusception, resulting in its withdrawal from the market within 14 months of being licensed.[274,332] In February 2006, a bovine-based pentavalent rotavirus vaccine was licensed by the FDA for use in infants in the United States[19,71] as a three-dose vaccine administered orally at 2, 4, and 6 months of age, with all doses administered by the time the child reaches 32 weeks of age. A two-dose orally administered, attenuated monovalent G1 (P8) human rotavirus vaccine is available in many countries throughout the world and is being reviewed by the FDA for licensing in the United States.[343]

Astroviruses

Astroviruses, identified in 1975, are 20 to 30 nm in diameter and have a characteristic five- to six-pointed star. The virus genome is a positive-stranded RNA of about 7500 nucleotides that encodes four structural proteins.[157,422] Eight different antigenic types have been described. Astrovirus gastroenteritis occurs worldwide and has been associated with outbreaks of mild gastroenteritis in schools, childcare centers, pediatric wards, and nursing homes.[94,157,164,263] Illness is restricted primarily to children and elderly people; 80 percent or more of adults have antibodies against the virus. The incubation period is 3 to 4 days.[157] Symptoms include fever and malaise, followed by watery diarrhea that may last approximately 3 days. Vomiting is an uncommon symptom. Short-term intolerance of monosaccharides and more prolonged intolerance of cow's milk protein have been reported after astrovirus infection.[157]

Noroviruses

Members of the genus *Norovirus* in the family *Caliciviridae* have been identified as the most common viral cause of acute gastroenteritis in humans, with an estimated 23 million cases occurring annually.[43,407] The mean incubation period for norovirus is 24 to 48 hours. The primary routes of transmission are fecal-oral, including consumption of fecally contaminated food or water; direct person-to-person contact, especially in schools, childcare centers, restaurants, summer camps, hospitals, nursing homes, and cruise ships[10,69,200,201,221,330]; and through contaminated objects or environments. A low inoculum dose is required for infection, and prolonged, asymptomatic shedding can occur in infected people. Clinical manifestations include acute onset of vomiting, nonbloody diarrhea, or both lasting 12 to 60 hours. Molecular epidemiology techniques have identified substantial diversity in strains, indicating that epidemic strains of norovirus might be more virulent or more environmentally persistent than are nonepidemic strains.[43] The identification of human histo-blood group antigens as norovirus receptors opens a new approach for evaluation of susceptibility and therapy of norovirus infection.[136] The demonstration of an in vitro cell culture infectivity assay for human norovirus may improve understanding of the pathogenesis of human norovirus infections.[389]

Enteric Adenoviruses

Human adenoviruses of subgroups A to F have been identified as etiologic agents in a wide range of human diseases, including conjunctivitis, upper respiratory tract infections, and pneumonia. A subgroup of fastidious adenoviruses (group F) with a distinct set of antigenic determinants and specific tissue culture growth characteristics have been shown to be associated with acute gastroenteritis and are referred to as *enteric adenoviruses*.[50,226,412] These agents fail to propagate in conventional cell lines used to grow adenoviruses but grow readily in 293 cells, an adenovirus type 5 transformed line.

The enteric adenoviruses in group F include serotypes 40 and 41.[226] Types 40 and 41 both appear to be widespread and endemic causes of diarrhea in children. Antibody prevalence to enteric adenovirus increases from 20 percent during the first 6 months of life to 50 percent or greater by the third or fourth year of life.[372] Seasonal shifts in the predominance of types 40 and 41, similar to those described for rotavirus, may occur. A 9-year study in Washington, DC, found that enteric adenovirus types 40 and 41 circulate simultaneously all year.[50] Infection with enteric adenovirus appears to increase in the summer, although with a less marked seasonal variation than that exhibited by rotavirus, which peaks in the winter.[50] Outbreaks of enteric adenovirus diarrhea have been described in childcare centers, where asymptomatic excretion is a common occurrence.[412]

PARASITES

The most important protozoa known to cause diarrhea in various populations in the United States are *Entamoeba histolytica*, *G. lamblia*, and spore-forming protozoa: *Cryptosporidium parvum*, *Isospora belli*, *Microsporidium* spp. (*Encephalitozoon intestinalis* and *Enterocytozoon bieneusi*), and *Cyclospora cayetanensis*. Among helminths, *Strongyloides stercoralis* and *Trichuris trichiura* may produce diarrhea. Data associating *Ascaris* and hookworm with diarrhea are lacking, but both cause abdominal pain. HIV infections stimulated renewed interest in several of these organisms, including various *Microsporidium* spp., *C. parvum*, and *I. belli*.[159] *Balantidium coli* is a cause of bloody diarrhea in humans. The roles of *Blastocystis hominis* and *Dientamoeba fragilis* as causes of diarrhea are not known.

Cryptosporidium

Cryptosporidium organisms are coccidian protozoa that invade and replicate within the microvillous region of epithelial cells lining the digestive and respiratory tracts of vertebrates.[79] *Cryptosporidium* spp. are related taxonomically to *Toxoplasma*, *Sarcocystis*, *Isospora*, and *Plasmodium* spp. Fecal-oral transmission of *Cryptosporidium* oocysts occurs from person to person, through ingestion of contaminated drinking water or recreational water, through consumption of contaminated food, or by contact with infected animals (cattle and sheep).[61,73,120,181,236] Unlike bacterial pathogens, *Cryptosporidium* are resistant to chlorine disinfection and can survive for days in treated recreational water, including swimming pools and recreational water parks.[120] Children aged 4 years old and younger appear to be at a particularly high risk for acquiring this organism.[73] Cryptosporidia have been implicated as a cause of diarrhea in travelers and of epidemics in hospitals, child-care centers, and other institutional settings worldwide. Other groups at risk include animal handlers, travelers to foreign countries with a high prevalence of *Cryptosporidium*, and hospital personnel. The largest water-borne outbreak of diarrhea documented in the United States was caused by *Cryptosporidium*.[246] Volunteer studies in adults showed that 132 oocysts cause disease.[112] The incubation period in humans has been estimated to be 2 to 14 days.

Cryptosporidiosis can be manifested with a wide spectrum of symptoms, including asymptomatic excretion, acute diarrhea, chronic diarrhea, epidemic diarrhea, severe life-threatening watery diarrhea, and biliary tract disease.[79] Watery diarrhea is the hallmark of symptomatic infections, but few if any features distinguish gastroenteritis caused by *Cryptosporidium* in the immunocompetent patient from other enteric infections. Stools do not contain blood or leukocytes. Vomiting, flatulence, abdominal pain, and low-grade fever routinely accompany diarrhea.[168] Symptoms usually subside in an average of 9 days. Patients may have cholera-like illness, transient diarrhea, relapsing episodes, or a protracted clinical course with unremitting, profuse diarrhea lasting for months accompanied by profound malabsorption and weight loss. Before HAART was instituted, enteritis caused by *Cryptosporidium* occurred in 10 to 15 percent of patients with AIDS in the United States and approximately 15 percent of patients with AIDS in the developing world.[248] The frequency with which *Cryptosporidium* and microsporidia are identified in stools of patients with AIDS is a reflection of the CD4 count; identification of the organisms and symptoms is more frequent when the count is less than 100 cells/μL.[141] Antiretroviral therapy is protective against disease.[56,248] Biliary tract infection with *Cryptosporidium* produces two syndromes: sclerosing cholangitis–type lesions, which cause progressive, irregular obstruction and dilation of the intrahepatic and extrahepatic bile ducts,[37] and acalculous cholecystitis, caused by infection of the wall of the gallbladder. Symptoms have been characterized by right upper quadrant pain, nausea, and vomiting.

Entamoeba histolytica

The life cycle of *Entamoeba histolytica* involves encystment of a trophozoite, followed by release of the trophozoite from the cyst under appropriate conditions in the GI tract (Fig. 50–2).[129] Trophozoites vary in size and are found in stools of patients with dysentery or diarrhea. The cyst is more resistant to environmental stresses and is the infective stage. Cysts are found more frequently in formed stools. The minimum period between ingestion of cysts and development of symptoms is 8 days. The incubation period ranges up to 95 days.

The genus *Entamoeba* contains many species, six of which can reside in the human intestinal lumen. *E. histolytica* is the only species definitely pathogenic. *Entamoeba dispar* and *Entamoeba moshkovskii* do not appear to be pathogenic. New approaches to identifying *E. histolytica* are based on detection of *E. histolytica*–specific antigen and DNA in stool and other clinical specimens.[142] Several molecular techniques, including polymerase chain reaction (PCR), have been developed for detection and differentiation of *E. histolytica*, *E. dispar*, and *E. moshkovskii*.

Clinical patterns that occur in patients with amebiasis consist of intestinal amebiasis, with the gradual onset of colicky abdominal pain and frequent bowel movements, tenesmus, and little or no constitutional disturbance; amebic dysentery, characterized by profuse diarrhea containing blood and mucus and the presence of constitutional signs, such as fever, dehydration, and electrolyte alterations; hepatic amebiasis, which usually presents as abscess formation without GI tract symptoms[8]; and asymptomatic

Figure 50–2 **A,** Trophozoite of *Entamoeba histolytica* with ingested material in the cytoplasm. **B,** Cyst of *E. histolytica* seen in a merthiolate-iodine-formalin–stained preparation from a stool specimen. Note two visible nuclei (×1000). *(From DuPont, H. L., and Pickering, L. K.: Infections of the Gastrointestinal Tract. New York, Plenum Publishing, 1980.)*

colonization. Patients may experience tender hepatomegaly, jaundice, weight loss, fever, and anorexia. The frequency of liver abscess in patients with amebiasis is between 1 and 5 percent. The complications of intestinal amebiasis include perforation, ameboma, stricture, hemorrhage secondary to erosion into a blood vessel, intussusception, ischiorectal abscess, fistulas, and rectal prolapse. People in the third through fifth decades of life have the highest incidence of infection and clinical symptoms, although people of all ages are susceptible.

Giardia intestinalis

Giardia intestinalis (formerly *G. lamblia*), a flagellated protozoan, is the most common intestinal parasite identified by public health laboratories in the United States.[63,146] Giardia infection is transmitted by the fecal-oral route and occurs after ingestion of *Giardia* cysts in fecally contaminated food or water or through person-to-person transmission.[120,236,245] The low infectious dose of 10 cysts places people in close contact, including children in childcare, at risk of acquiring infection.[331] The relative contribution of person-to-person, animal-to-person, water-borne, and food-borne transmission to sporadic human disease is unknown. Children appear to be more susceptible to *Giardia* than are adults. Specific conditions that predispose to giardiasis are hypogammaglobulinemia, secretory immunoglobulin A (IgA) deficiency, peptic ulcer disease, biliary tract disease, and pancreatitis. The parasite may exist in two forms: cyst and trophozoite (Fig. 50–3). After being ingested, each cyst divides into two trophozoites, which subsequently mature. The trophozoites usually are seen in duodenal aspirates and loose stools, whereas cysts can be found in formed stools and can remain viable and infectious in water for longer than 3 months. Scanning electron micrographic examination of *Giardia* reveals firm attachment to the intestine (Fig. 50–4).

People vary in their response to infection with *Giardia*, with the following clinical manifestations: asymptomatic; an acute illness with a sudden onset of explosive, watery, foul-smelling stools and flatulence, abdominal distention, nausea, and anorexia, with the absence of blood and mucus; and chronic diarrhea and malabsorption, with exacerbations and remissions of flatulence, abdominal distention, and abdominal pain often lasting for months.[309]

Strongyloides stercoralis

Strongyloides stercoralis is a nematode that infects humans through the intestinal tract or through skin if either comes in contact with soil that contains larvae. About one third of people with strongyloidiasis are asymptomatic, and the remainder may have skin, pulmonary, or, more frequently, GI tract involvement.[373] People at risk include residents and travelers to endemic areas; natives and residents of the Appalachian region in the United States; institutionalized patients; and people treated with corticosteroids, cimetidine, and antacids.[152] Epigastric abdominal pain occurs and is associated with diarrhea that contains mucus and blood. Some patients may complain of nausea, vomiting, and weight loss with

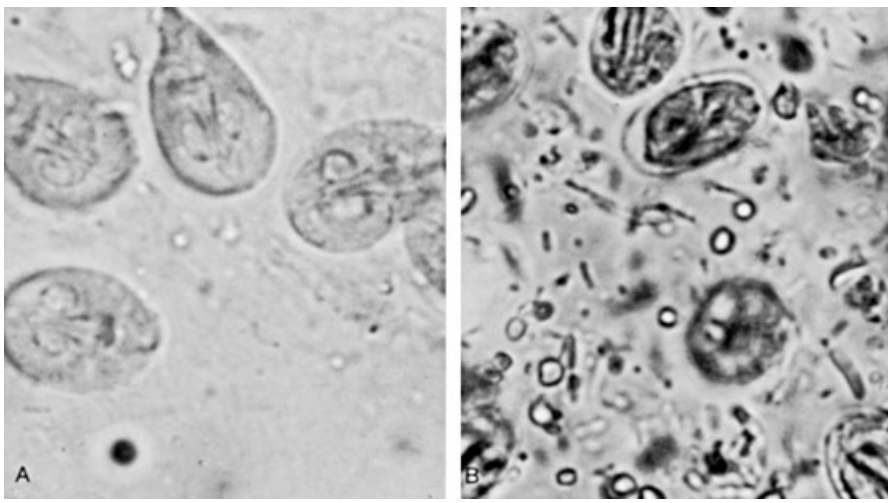

Figure 50–3 **A.** Trophozoites of *Giardia lamblia* seen in a merthiolate-iodine-formalin–stained preparation of stool from a patient with diarrhea (×1000). **B.** Cysts of *G. lamblia* seen in a merthiolate-iodine-formalin–stained preparation of stool from a patient without diarrhea (×1000).

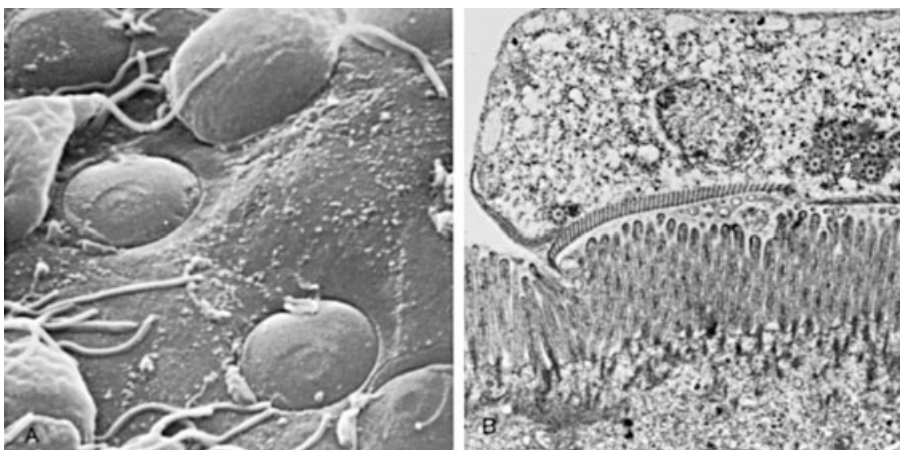

Figure 50–4 **A.** Scanning electron micrograph of an intestinal villus revealing firm attachment of *Giardia muris* trophozoites to the microvillous border. Circular dome-shaped lesions in the microvillous border are produced by attachment of the adhesive disk of trophozoite (×4600). **B.** Transmission electron micrograph of a *G. muris* trophozoite illustrating how lesions in the microvillous border are produced. Note that the edges of the adhesive disk penetrate the microvillous border and compress the microvilli centrally. The microvilli also show some vesiculation under the adhesive disk (×20,000). *(**A**, **B**, Courtesy of Dr. S. L. Erlandsen.)*

evidence of malabsorption. Eosinophilia and an urticarial rash are prominent features of infection. *S. stercoralis* hyperinfection syndrome can present in immunocompromised patients years after exposure and can result in multisystem organ involvement culminating in sepsis.[213] People infected with *S. stercoralis* should be treated with ivermectin or albendazole in an attempt to eradicate the infection.[259]

Isospora belli

Isospora was established as a cause of diarrhea in humans in the early 1900s, when sporadic cases of isosporiasis and a few clinical series were reported in the medical literature.[99] *Isospora belli* gained importance with the advent of AIDS and before HAART was shown to be an important cause of severe and prolonged gastroenteritis.[295] Humans are the only known host for *I. belli*, but the actual prevalence of this parasite is unknown. Infection can occur in adults and children and has been reported in infants with severe diarrhea.[239] This organism also has been implicated as a cause of traveler's diarrhea.[134]

Transmission is fecal-oral from one human to another, but the infective dose in humans is unknown. Oocysts may be present in stools for as long as 120 days after infection occurs. No animal reservoir of *I. belli* has been documented. Transmission is thought to occur by ingestion of oocysts contaminating food, water, or environmental surfaces.[239] *I. belli* oocysts are resistant to commonly used disinfectants and may remain viable for months in a cool, moist environment.

The clinical spectrum of disease caused by *Isospora* is indistinguishable from that described for *Cryptosporidium*. The spectrum includes asymptomatic infection, acute diarrhea in children in developing countries, and chronic diarrhea or severe protracted life-threatening diarrhea in patients with AIDS. Infection of the biliary tract in patients with AIDS by *Isospora* has been associated with acalculous cholecystitis.[36] The incubation period was 8 to 14 days in four subjects who had been exposed to the organism in the laboratory.[170] Fever, malaise, abdominal pain, and headache have been reported. Stools are watery and do not contain blood or leukocytes. Malabsorption, steatorrhea, severe weight loss, and chronic diarrhea lasting months to years are most likely occurrences in immunocompromised hosts.[92]

Microsporidia

Microsporidia are unicellular, spore-forming, obligate intracellular protozoal parasites that cause disease in a wide range of vertebrate and invertebrate hosts.[223,253] Of the more than 100 genera of microsporidia that have been identified, six cause disease in humans. The nontaxonomic term *human microsporidia* can refer to any of the microsporidia known to cause disease in humans: *Enterocytozoon* spp., *Encephalitozoon* spp., *Pleistophora* spp., and *Nosema* spp. Of these, *Enterocytozoon bieneusi* and *Encephalitozoon intestinalis* (formerly *Septata intestinalis)* are the most important in GI tract disease of humans.[26,426] These organisms emerged as important opportunistic pathogens when AIDS became pandemic. Infections with these organisms have been documented in immunocompetent and immunosuppressed people from Africa, Asia, Europe, and North and South America.[367]

In animals, transmission occurs by ingestion of environmentally resistant, infective spores shed into the environment.[253] The person-to-person, fecal-oral route may play a role in transmission, and water-borne disease occurs.[84] The zoonotic potential has been documented for several *Encephalitozoon* species.[253] The clinical spectrum appears to depend on the immune status of the host. *E. bieneusi* and *E. intestinalis* have been detected in intestinal biopsy specimens of patients with AIDS, with a clinical picture of prolonged diarrhea and weight loss.[84] The primary location of all intestinal spore-forming protozoal infection is the small intestine, but colonic infection has been reported with *E. bieneusi*.[159] Infection of the biliary tract with *E. bieneusi* and *E. intestinalis* in patients with AIDS can cause sclerosing cholangitis–type lesions[322,371,434] and acalculous cholecystitis caused by infection of the wall of the gallbladder. *E. intestinalis* can infect lamina propria macrophages, fibroblasts, and endothelial cells and can disseminate to other organs, including liver, respiratory tract, and kidney.[159,434] Extraintestinal infection develops after infection with other species of microsporidia occurs.

Cyclospora

Cyclospora cayetanensis (formerly cyanobacteria or blue-green algae–like bodies) is a coccidian protozoon that first was diagnosed as causing infection in humans in 1977.[293] *Cyclospora* is transmitted by the fecal-oral route; direct person-to-person transmission is unlikely to occur because excreted oocysts require days to weeks under favorable environmental conditions to sporulate and to become infectious. An animal reservoir has not been described. Outbreaks of diarrhea caused by consumption of water and fresh fruits contaminated with *Cyclospora* have been described,[175,245,247] and travelers to developing countries are at increased risk for development of diarrhea caused by *Cyclospora*.[292] Most of the reported cases have occurred during the spring and summer. The mean incubation period appears to be 7 days. Clinical manifestations include asymptomatic excretion, acute watery diarrhea, and diarrhea that may be protracted for days to weeks with frequent, watery stools, which may remit and relapse.

DIAGNOSIS

Determination of the cause of an episode of acute infectious diarrhea depends on epidemiologic information, the clinical syndrome, laboratory tests, and knowledge or assessment of an organism for virulence factors. Because virulence properties determine clinical manifestations of disease, an understanding of pathophysiologic mechanisms guides the laboratory evaluation and empiric therapy. The major virulence properties of enteropathogens include adherence; production of enterotoxin, cytoskeleton-altering toxin, cytotoxin, and toxins with neural activity; and epithelial cell invasion.[365] Certain enteropathogens may produce diarrhea by other mechanisms, and enteric pathogens may possess one or several of these virulence properties. Both host and microbiologic factors ultimately determine clinical expression in the individual patient. Not all of the recognized virulence properties of a given species are obvious clinically in each episode of disease. Virulence properties of individual bacterial, viral, and parasitic enteropathogens are discussed in specific chapters.

LABORATORY EVALUATION

Proper identification of the causative agent of an episode of acute infectious diarrhea will help facilitate initiation of appropriate therapy. A gross examination of the stool specimen should be routine in all patients with diarrhea, even if no laboratory studies are performed. Diarrheal stool that is watery and without mucus or blood usually is caused by an enterotoxin, virus, or protozoan organism, or it may be caused by infection outside the GI tract. The color of stools generally conveys little information if the stool does not contain blood. Infectious causes to be considered when stools contain blood or mucus include a cytotoxin-producing bacterium; an enteroinvasive bacterium causing mucosal inflammation; and an enteric parasite associated with blood in stools, such as *E. histolytica*, *B. coli*, and *T. trichiura.*

TABLE 50–8 Laboratory Tests Used to Detect Enteropathogens

Laboratory Tests	Organisms Suggested or Identified
Microscopic examination of stool	
Fecal leukocytes	Invasive or cytotoxin-producing bacteria
Trophozoites, cysts, oocysts, or spores	*Giardia lamblia*, *Entamoeba histolytica*, *Cryptosporidium*, *Isospora belli*, *Cyclospora*, *Enterocytozoon bieneusi*, *Encephalitozoon intestinalis*
Rhabditiform larva	*Strongyloides*
Spiral or S-shaped gram-negative bacilli	*Campylobacter jejuni/coli*
Stool culture	
Standard	*Escherichia coli*, *Shigella*, *Salmonella*, *Campylobacter jejuni*
Special	*Yersinia enterocolitica*, *Vibrio cholerae*, *Vibrio parahaemolyticus*, *Clostridium difficile*, *E. coli* O157:H7, *Listeria monocytogenes*
Stool cytotoxicity assay	*C. difficile*
Enzyme immunoassay or latex agglutination	Rotavirus, *G. lamblia*, *Cryptosporidium*, enteric adenovirus, *C. difficile*, STEC, *E. histolytica*
Serotyping	*E. coli* O157:H7 and other STEC, enteropathogenic *E. coli*
Latex agglutination after broth enrichment	*Salmonella*, *Shigella*
Tests performed in research or reference laboratories	Toxin-producing bacteria, small round viruses, invasive *E. coli*, EAEC, gene probe or polymerase chain reaction for virulence genes

STEC, Shiga toxin–producing E. coli; EAEC, enteroaggregative E. coli.

When it is present, blood usually is mixed evenly into the stool, except in the case of *E. histolytica* infections, in which blood often is on the surface of the stool, and some STEC infections, in which the stool may be blood streaked. Stools that are particularly foul smelling are consistent with *Salmonella* and other bacteria as well as *Giardia*, *Cryptosporidium*, and *Strongyloides* spp. Stools with little odor suggest an enterotoxin, such as cholera toxin or ETEC, or a viral enteropathogen. Laboratory tests used to detect enteropathogens are listed in Tables 50–8 and 50–9.

MICROSCOPIC EXAMINATION

Microscopic examination of stool specimens for evidence of fecal leukocytes provides information about the cause of diarrhea and helps determine the anatomic location and presence of mucosal inflammation.[313] Fecal leukocytes are produced in response to bacteria that diffusely invade the colonic mucosa and indicate that the patient has colitis. No inflammatory bacterial enteritis exists in which results of the fecal leukocyte examination are uniformly positive.[178] Thus, results of examination are more helpful when they are positive than when negative. When results are positive, the patient probably has an invasive or cytotoxin-producing organism, such as *Shigella* spp., *Salmonella* spp., *Campylobacter* spp., invasive *E. coli*, STEC, *C. difficile*, or *Y. enterocolitica*, although ulcerative colitis and Crohn disease also are associated with fecal leukocytes. Fecal leukocytes generally are not present in stools from patients with diarrhea secondary to viruses, enterotoxin-producing bacteria, or parasites. The leukocytes seen in cytotoxin-associated and invasive bacterial diarrhea syndromes are polymorphonuclear leukocytes. The exception is infection by *S. typhi*, in which the leukocytes are mononuclear. If the fecal leukocyte examination shows evidence of inflammatory enteritis, further laboratory evaluation is indicated. The fecal lactoferrin assay has been shown to be a more accurate test than are fecal leukocytes or occult blood in patients with inflammatory diarrhea.[194]

Normally, examination of stools for ova and parasites is unnecessary unless the patient has a history of recent travel to high-risk areas, stool cultures are negative for other enteropathogens, the patient is involved in an outbreak of diarrhea, diarrhea persists for longer than 1 week, or the patient is immunosuppressed. *G. lamblia* and *S. stercoralis* can be visualized microscopically in stools, duodenal fluid, and small intestinal biopsy material. Both trophozoites and cysts of *G. lamblia* and larvae of *Strongyloides* spp. can be identified on direct smears of stool specimens (see Fig. 50–3); however, the sensitivity of stool examination for most parasites can be improved by use of a concentration technique and by placement of stools in vials containing a stool preservative. Trichrome and iron hematoxylin both are useful as permanent stains for *Giardia* spp. Pooling of preserved fecal samples is an efficient and economical procedure for detecting ova and parasites.[13] Rational use of the stool ova and parasite examination relies on communication between the clinician and laboratory personnel.[49]

For patients in whom giardiasis, cryptosporidiosis, isosporiasis, or strongyloidiasis is considered and in whom stool cultures are negative, aspiration or biopsy of the duodenum or upper jejunum may be indicated. Because these organisms live in the upper intestine, this procedure is more reliable than is examination of stool specimens.[336] Duodenal biopsy is a sensitive and specific method for diagnosing giardiasis, strongyloidiasis, and spore-forming protozoa. Small intestinal biopsy should be considered in patients with characteristic clinical symptoms, negative stool and duodenal fluid specimens, and one of the following: abnormal radiographic findings such as edema and segmentation in the small intestine, abnormal lactose tolerance test results, absent secretory IgA, hypogammaglobulinemia, achlorhydria, AIDS, or severe malabsorptive diarrhea with weight loss. Electron microscopic examination of tissue sections may be useful in identifying fine structures of a parasite (Fig. 50–5).

Medications, including antibiotics, antacids, antidiarrheal compounds, and certain enema and laxative preparations, as well as contrast material for radiographic studies can interfere with identification of an organism by altering morphologic features or causing a temporary disappearance of parasites from stool specimens. Patients should not receive these compounds for 48 to 72 hours before stools are collected for testing. *G. lamblia* and *Cryptosporidium* antigens in feces can be detected by use of one of several rapid and sensitive diagnostic tests.[148,220] *E. histolytica* can be diagnosed by microscopic examination of fresh stool specimens or bowel wall scrapings for cysts or trophozoites (see Fig. 50–2). A concentration technique may be helpful in demonstrating amebic cysts. Examination of several stool samples by an experienced technician may be necessary because excretion of cysts often is intermittent and making an interpretation is difficult. Confusion in differentiating amebic cysts from fecal leukocytes may occur. Microscopy can be used only as presumptive evidence of *E. histolytica* because the nonpathogen *E. dispar* is morphologically identical.[129] Several molecular techniques have been developed for detection and differentiation of *E. histolytica*, *E. dispar*, and *E. moshkovskii*.[142] Numerous serologic tests for amebiasis to detect different types and antibodies are available. Serologic test results for amebae almost always are positive in acute amebic dysentery and hepatic amebiasis. A liver scan may indicate the presence of a liver abscess.

Diagnosis of *Cryptosporidium*, *Isospora*, *Cyclospora*, and microsporidia is based on morphologic appearance and staining of stool or histologic examination of tissue sections.[159] Among the most widely used stains to visualize oocysts of *Cryptosporidium*, *Isospora*, and *Cyclospora* are standard acid-fast or modified acid-fast stains,

TABLE 50–9 Laboratory Evaluation of Patients with Presumed Bacterial Diarrhea

Organism	Tests
Aeromonas hydrophila	Screen colonies grown on MacConkey agar for positive oxidase test result
	Culture on modified blood agar
Bacillus cereus	Culture food (>10^5 organisms/g); demonstrate enterotoxin in food and stool by enzyme immunoassay
Campylobacter jejuni	Stool culture with special media incubated at 42° C with 5% O_2 and 10% CO_2
	Gram stain for "gull wing"–like organisms and fecal leukocytes; darkfield or phase contrast of stool for organisms with darting motility
	Nucleic acid probe
	Serology
Clostridium difficile	Culture feces anaerobically on cycloserine-cefoxitin-fructose agar
	Demonstrate toxins in stool by enzyme immunoassay or tissue culture cytotoxicity with neutralization with antitoxin
Clostridium perfringens	Culture food (>10^5 organisms/g) and feces; stools can be tested for enterotoxin
	Serotype organism
Escherichia coli	Standard stool culture for initial isolation
ETEC	
Stable toxin	Suckling mouse assay
	Gene probe hybridization assay
Labile toxin	Rabbit ileal loop
	Y-1 adrenal or Chinese hamster ovary cell assay
	Gene probe hybridization assay
	G_{M1}, enzyme immunoassay
EPEC	Serogroup, gene probe assay
	Small bowel biopsy for routine microscopy and electron microscopy
EIEC	Gene probe assay
	Biologic assays for invasiveness (Serény or HeLa cell)
STEC	MacConkey sorbitol agar for O157:H7
	Gene probe or polymerase chain reaction to detect toxin gene sequences
	Serotyping
	Toxin enzyme immunoassay
	Free cytotoxin in stool by enzyme immunoassay or tissue culture
	Serologic responses to verotoxins or lipopolysaccharide of *E. coli* O157
EAEC	HEp-2 adherence assay, DNA probes
Listeria monocytogenes	Culture on blood agar
Plesiomonas shigelloides	Culture
Salmonella species	Standard stool culture; blood, bone marrow, and urine cultures if disseminated
	Serotype
Shigella species	Examine stool for fecal leukocytes
	Standard stool culture
Staphylococcus aureus	Culture food and skin lesions of food handlers
	Phage type
	Demonstrate enterotoxin in food, stool, and vomitus
Vibrio cholerae	Culture feces on thiosulfate–citrate–bile salt agar
	Serotype
Vibrio parahaemolyticus	Culture feces on thiosulfate–citrate–bile salt agar
	Test for Kanagawa reaction (beta-hemolysis on Wagatsuma agar), which is a marker for pathogenicity
Yersinia enterocolitica	Standard stool culture with cold enrichment; blood culture if disseminated
	Serology
	Lack of rhamnose fermentation by toxin-producing strains
	Suckling mouse assay for stable toxin

EAEC, enteroaggregative E. coli; STEC, Shiga toxin–producing E. coli; EIEC, enteroinvasive E. coli; EPEC, enteropathogenic E. coli; ETEC, enterotoxigenic E. coli.

which are based on the use of reagents that enhance the penetration of fuchsin into the organism without the need for heating (modified Kinyoun acid-fast stain). In a modified acid-fast stain, *Cryptosporidium* oocysts, which are 4 to 6 μm with four crescentic sporozoites, stain red and can be differentiated readily from yeasts that stain green.[172] Enzyme immunoassays[148,211] and fluorescent monoclonal antibody–based assays[149,345] for detection of *Cryptosporidium* antigen in stool specimens are available. In a study of seven microscopy-based *Cryptosporidium* oocyst detection methods, false-positive results were detected by acid-fast and auramine-rhodamine stains but not by monoclonal antibody–based methods.[23] Oocysts of *I. belli* often are visualized by wet-mount preparations because of their size, which is 20 to 30 μm with four sporozoites in two sporocysts. *Cyclospora* oocysts are 8 to 10 μm in diameter and are nonrefractile spherical organisms containing two sporozoites in two sporocysts that are seen easily on wet-mount preparations and are variably acid fast.

Microsporidia are difficult to differentiate from bacteria and debris because of the small size of the spores, which measure 1 to 2 μm. For detection, formalin-fixed stool or duodenal fluid can be stained with a calcofluor stain, a modified trichrome stain, or a fluorescent stain.[96,425] Gram, acid-fast, periodic acid–Schiff, and Giemsa stains also have been used to stain the organism.[367] A nonspecific fluorescence method or enzyme immunoassay may enhance speed and sensitivity. Small bowel biopsy may be more sensitive than is stool examination for establishing the diagnosis of intestinal microsporidiosis.[35] Spores are gram positive, and parts of the internal structure are positive for acid-fast or periodic acid–Schiff stains. After preliminary identification by these stains has been achieved, further examination by electron microscopy is needed to classify adequately the microsporidia into an appropriate genus. Routine histopathologic studies can provide presumptive identification in infected biopsy tissue; diagnostic confirmation requires electron microscopy. Reliable serologic

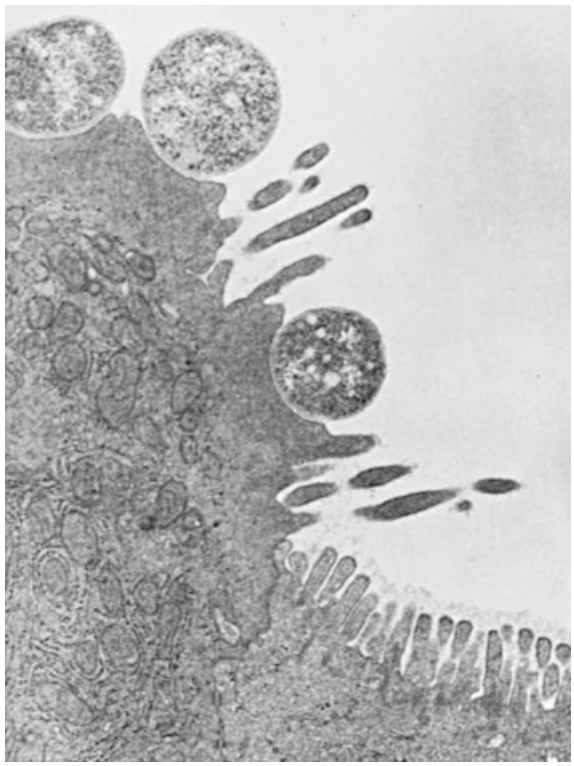

Figure 50–5 Electron micrograph of three *Escherichia coli* organisms adherent to enterocytes that have lost their microvilli and formed pedestals on which the bacteria lie (×25,000). *(Courtesy of Dr. R. J. Rothbaum.)*

tests are not available. Sensitive PCR assays are being evaluated for *E. bieneusi* and *E. intestinalis*.[89,133]

DIAGNOSTIC STOOL CULTURES

Obtaining of stool cultures cannot be justified in all patients with acute diarrhea.[178] Patients with mild, self-limited illness do not need to have stool specimens cultured. When culture is indicated, the specimen should be inoculated onto culture plate media adequate to isolate *E. coli*, *Shigella*, *Salmonella*, and *C. jejuni*. Fecal specimens can be transported to the laboratory in a non-nutrient holding medium, such as Cary-Blair, when culture cannot be performed immediately. This medium prevents drying or overgrowth of specific organisms.

E. coli grown in a hospital microbiology laboratory usually is considered to be normal flora. Proving pathogenicity is difficult because gene probe and PCR assays generally are available only in reference or research laboratories.[279,280] All stool specimens should be evaluated with sorbitol MacConkey medium for *E. coli* O157:H7.[47] *Shigella* organisms are identified in the standard evaluation of stool cultures. However, volunteer studies have shown that these organisms are not always isolated in culture from patients ill with shigellosis.

Salmonella organisms are isolated routinely by clinical microbiology laboratories. Speciation is important in salmonellosis because *S.* ser. *choleraesuis* and *S.* ser. *typhi* cause more severe disease than do other *Salmonella* spp. *S.* ser. *enteritidis* is more variable in severity. Serotyping of *S.* ser. *enteritidis* usually is not helpful in the individual case, although serotyping is crucial in evaluation of an outbreak. Because so many *Salmonella* serotypes exist, isolation of an unusual serotype can be of use in the investigation of a food-borne epidemic or identification of a potential reptile source of infection. As with *Shigella*, isolation of a *Salmonella* spp., even without demonstration of virulence properties, is considered adequate to make an etiologic diagnosis. Serologic studies are of no value in the individual patient.

Some bacterial enteropathogens require modified laboratory procedures for identification.[178] If these agents are suspected, the laboratory should be notified so that appropriate culture methods can be used. *Listeria* is cultured on blood agar plates rather than on the usual enteric media.[410] *Y. enterocolitica* can be isolated from routine media, but a differential selective medium, such as cefsulodin–triclosan (Irgasan)–novobiocin agar, is more effective.[45] If routine enteric media are inoculated, recovery of organisms is optimized by plating them onto MacConkey agar, followed by incubation at 25° C for 48 hours. Cold-enrichment techniques may increase the yield of the organism from contaminated specimens, such as feces. Stool cultures positive for *Y. enterocolitica* only after prolonged cold enrichment may represent environmental strains of low virulence, unrelated to human disease. Biotyping and serotyping for O3, O8, and O9 are helpful in determining the clinical relevance of such isolates.

V. cholerae strains can be isolated from stool with use of thiosulfate–citrate–bile salt–sucrose agar, which is the most convenient and frequently used selective medium. This medium is suitable for most enteropathogenic *Vibrio* spp., except *V. hollisae*. Placement of the specimen into an enrichment broth, such as alkaline peptone water with 1 percent sodium chloride (pH 8.5) for 5 hours before placement on thiosulfate–citrate–bile salt–sucrose agar enhances the isolation of vibrios. Serotyping is necessary to classify organisms into those that cause typical epidemic cholera (i.e., O1 and O139 serotypes) and those that cause less severe disease (i.e., non-O1, or nonagglutinating vibrios).[209]

V. parahaemolyticus, like other vibrios, can be cultured on thiosulfate–citrate–bile salt–sucrose agar. Strains associated with diarrhea are Kanagawa-positive on Wagatsuma agar (i.e., show hemodigestion resembling beta-hemolysis), which is a marker for pathogenicity. *V. parahaemolyticus* can be serotyped on the basis of the O and K antigens.[87]

A. hydrophila can be overlooked easily on standard stool cultures. A specialized blood agar has been suggested for isolation.[16] Oxidase testing of organisms that resemble *E. coli* can select organisms as possible *Aeromonas* spp.[16] If oxidase-positive colonies are found, they can be evaluated biochemically to determine species.

C. difficile can be isolated by anaerobic stool culture on agar containing cycloserine, cefoxitin, and fructose. For establishing a definitive diagnosis, demonstration of the presence of cytotoxin in stool specimens and neutralization with antitoxin or by use of enzyme immunoassay or PCR-based assays are necessary.[14,324,355]

C. perfringens is isolated commonly from feces of well people. Diagnosis of *C. perfringens* food poisoning requires isolation of the organisms from epidemiologically implicated food in a significant quantity (more than 10^5 organisms/g) or demonstration of 10^6 organisms/g of stool from two or more ill people or demonstration of enterotoxin in stools of two or more ill people.[245]

S. aureus may be isolated from food and may not be the cause of illness because not all strains of *Staphylococcus* produce enterotoxin. Conversely, the absence of *S. aureus* from food that has been reheated just before being eaten does not exclude staphylococcal food poisoning because heating may destroy the organism without inactivating the toxin. Confirmation as a cause of food-borne disease requires isolation of the same phage type *S. aureus* from stools or vomitus of two or more ill persons or detection of enterotoxin in epidemiologically implicated food or isolation of 10^5 organisms/g from epidemiologically implicated food, provided the specimen is properly handled.[245]

B. cereus can be diagnosed by demonstration of greater than 10^5 organisms/g in the incriminated food or isolation of the

organism from stool of two or more ill people and not from stool of control patients.[245]

SEROTYPING AND TOXIN DETECTION

Certain serotypes have been associated with ETEC, EIEC, EPEC, and STEC. Studies of somatic *E. coli* antigens are not helpful in establishing the diagnosis of ETEC because more than 50 different serogroups of *E. coli* have been shown to produce ST enterotoxins, HL enterotoxins, or both. Diagnosis of the various pathotypes of diarrheogenic *E. coli* is by assays that identify phenotype directly as well as with genotypic assays. Bacteria initially attach to epithelial cells that respond by forming cuplike pedestals composed of cytoskeletal protein and can be seen on electron micrographic examination in patients infected with EPEC (see Fig. 50–5). Identical lesions can be induced by human STEC strains.

VIRUS DETECTION

Rotavirus has been identified by examination of stool specimens for 70-nm particles by electron microscopy (see Fig. 50–1). Commercially available enzyme immunoassay and latex agglutination kits are available to detect rotavirus antigen in stool specimens. Assay procedures using monoclonal antibodies have improved the sensitivity and specificity to greater than 95 percent.[93,219] Other diagnostic methods less suitable for routine use include gel electrophoresis, PCR, and viral culture. Non–group A rotaviruses are not detected by the commercially available assays.

After the Norwalk virus was cloned and sequenced in 1990,[199] two major types of assays for diagnosing human noroviruses were developed. One type detects the viral antigens or antibodies against the antigens by recombinant enzyme immunoassays, and the other detects the viral RNA by reverse transcriptase–PCR.[269] The report of an in vitro cell culture infectivity assay for human norovirus may improve diagnostic abilities as well as understanding of the pathogenesis of norovirus.[388]

Diagnosis of enteric adenovirus can be established by immune electron microscopy of stool specimens, enzyme immunoassay of stool specimens, or propagation in a line of human embryonic kidney cells transformed by adenovirus type 5 (293 cells).[226] Restriction enzyme analysis is the definitive method for classifying individual enteric adenovirus isolates. Commercially available assays for detection of enteric adenovirus are available.[412]

Astroviruses grow well in human embryo kidney cells in the presence of trypsin. Electron microscopy, immune electron microscopy, immunofluorescence on cell culture, enzyme immunoassay, and PCR can be used as detection methods.[157]

PROCTOSIGMOIDOSCOPY

When symptoms of colitis are severe or the cause of an inflammatory enteritis syndrome remains obscure after laboratory evaluation, proctoscopic examination may help establish the diagnosis. Table 50–10 shows the usual proctoscopic findings in the various enteric syndromes that are characterized by fever, abdominal pain, and diarrhea with mucus and blood. Inflammatory bowel disease enters the differential diagnosis when symptoms of inflammatory enteritis become chronic. Proctitis with or without diarrhea may be related to milk allergy in infants, child abuse, or sexual practices. The etiology of proctitis includes all of these causes as well as *Neisseria gonorrhoeae*, herpes simplex virus, lymphogranuloma venereum, and *Chlamydia trachomatis*.

TREATMENT

Enteric infections generally are self-limited conditions, but nonspecific therapy can provide relief for some patients, and specific therapy may shorten the duration of the illness and eradicate fecal shedding of the organism. In caring for patients with diarrhea and dehydration, the major therapeutic considerations include fluid and electrolyte therapy, dietary manipulation, nonspecific therapy with antidiarrheal compounds, and specific therapy with antimicrobial agents. Increasing numbers of isolates resistant to antimicrobial agents and the risk for worsened illness (e.g., hemolytic-uremic syndrome occurring with Shiga toxin–producing *E. coli*) complicate antimicrobial and antimotility therapy.

TABLE 50–10 Proctoscopic Findings of Persistent Inflammatory Colitis

Organism or Disease	Gross Findings	Microscopic Findings
Shigella species	Diffuse erythema with loss of vascular pattern, mucopurulence, mild friability, occasional aphthoid ulcers	Edema, capillary congestion, focal hemorrhages, crypt hyperplasia, goblet cell depletion, mononuclear and polymorphonuclear leukocyte infiltrate, loss of epithelial cells with microulcerations
Salmonella species	Hyperemic, friable mucosa with petechiae and ulcerations; occasional pseudomembranous changes	Edema, inflammation, microabscesses, ulcerations
Campylobacter jejuni	Diffuse exudative edema	Inflammatory infiltrate with polymorphonuclear leukocytes, eosinophils, mononuclear cells, degeneration, loss of mucus, crypt abscesses, ulcerations
Clostridium difficile	Pseudomembranous colitis with 1- to 5-mm white-yellow nodules or plaques, minimal friability, sometimes nonspecific colitis	Fibrin, mucus, necrotic epithelial cells, leukocytes adherent to the underlying inflamed tissues
Clostridium perfringens	Rarely pseudomembranous colitis	Findings similar to those produced by *C. difficile*
Entamoeba histolytica	Discrete ulcers (millimeters to centimeters in diameter) with undermined edges amid normal mucosa	Trophozoites in flask-shaped ulcers that extend into submucosa, inflammatory cells near periphery but not near trophozoites; wet mount shows motile ameba containing erythrocytes
Ulcerative colitis	Friability, inflammatory polyps on heaped-up granulation tissue, deep linear ulcers	Mucosal ulceration extending to lamina propria, diffuse inflammation, vascular engagement, microabscesses in crypts
Crohn colitis	Hyperemic mucosa with linear ulcers	Inflammation involving all layers of bowel, with lymphocytes, histiocytes, and plasma cells forming granulomas

FLUID AND ELECTROLYTE THERAPY

Patients who develop diarrhea lose fluid and electrolytes through the GI tract by several mechanisms: vomiting, loss of fecal fluid caused by the infecting enteropathogen, and fecal water loss in excess of sodium caused by the intraluminal osmotic effect of unabsorbed nutrients.[179] The composition and amount of lost fluid depend on the rate of stool loss and the causative agent. The higher the rate of stool loss, the greater the sodium loss, probably as a result of rapid passage of intestinal contents through the colon, where sodium-potassium exchange occurs. Stools from patients with cholera or ETEC infection contain sodium in a concentration of 80 to 120 mEq/L, whereas stools from patients with rotavirus infection have sodium concentrations of less than 50 mEq/L.[357] In secretory diarrheal disorders, loss of fluid generally is derived from the small intestine, and colonic reabsorption is overwhelmed. In viral gastroenteritis, small bowel absorptive capacity primarily is impaired, and in dysenteric or invasive diarrhea, reabsorptive capacity of the large intestine is reduced. If vomiting also is a manifestation, this loss is compounded. Continued loss of fluid or electrolytes may lead to dehydration, with potentially severe sequelae. Children, especially infants, are more susceptible to dehydration because they have greater basal fluid and electrolyte requirements per kilogram and because they depend on others to meet these needs.

Important factors to be considered in evaluating patients with diarrhea and possible dehydration include an estimation of deficiency, ongoing daily requirements, continued losses and their replacement, and correction of the underlying cause.[18,179] The clinical signs and symptoms that may help in estimating deficiencies and determining the severity of dehydration include thirst, dryness of the mucous membranes, decrease in urinary output, tachycardia, loss of skin elasticity and turgor, and mottling and coolness of the skin.[18,66] These signs may be misleading in patients who are malnourished or in patients with hypertonic dehydration.

Fluids should not be withheld in treatment of any patient with diarrheal disease. Oral therapy should consist of rapid rehydration with replacement of ongoing losses during the first 6 to 12 hours of therapy with a glucose-electrolyte solution,[18,66,78,357] followed by early initiation of a modified diet.[66,250] If fluid and electrolyte deficits are significant, priority should be given to rehydration as rapidly as possible with oral electrolyte solutions. Ondansetron, an antiemetic, as a single dose reduced vomiting and facilitated oral rehydration in a study of children in an emergency department in the United States.[144]

Intravenous therapy is required only if the patient is in shock, is obtunded, or has ileus; otherwise, fluid and electrolyte therapy should be administered orally. Once the patient is rehydrated, an orally administered maintenance solution containing approximately 50 mEq/L of sodium should be used. Patients with mild diarrhea without clinical dehydration (<3% weight loss) can be managed at home by supplementing their diets with oral electrolyte solutions containing glucose. The glucose in these solutions is necessary to promote intestinal absorption of sodium and water in the small intestine.[370]

Commercial preparations of ready-to-feed glucose-electrolyte solutions are available in the United States and should be used in preference to homemade solutions. The sodium and potassium contents of various commercially available preparations are outlined in Table 50–11. Gatorade and other sports drinks do not supply the quantity of electrolytes necessary to replace those lost with the continued stool losses of severe diarrhea, and they are high in carbohydrate content. High concentrations of sugar are not tolerated well because high osmotic activity may exacerbate diarrhea. The carbohydrate concentration should not exceed the sodium concentration by more than 2:1. If it does, the excess carbohydrate produces osmotic retention of water in the intestine, with subsequent loss in stool.[18] The preparation of glucose and salt solutions at home is not recommended because errors in preparing the solutions may result in hypertonic dehydration in infants.[234] The use of rice-based oral rehydration solutions that contain glucose polymers and amino acids has been shown to increase the absorption of salt, water, and glucose from the intestine and may be more beneficial than are glucose-based oral rehydration solutions.[358,375] In a randomized, controlled trial in an emergency department in the United States, oral rehydration was as effective as intravenous rehydration of moderately dehydrated children.[384]

Other fluids that may be consumed at home include decarbonated soda beverages, fruit juices, and Jell-O. All contain inadequate amounts of sodium and potassium and excessive carbohydrate concentrations, which may exceed the absorptive capacity of the intestine[370] and, therefore, should not be used if rehydration requires more than one or two feedings. Kool-Aid and tea are not recommended because they are low in both sodium and potassium and thus have little advantage over sugar and water. Soon after the child has begun drinking a fluid and electrolyte solution, feeding should be restarted.[18,52,66]

DIETARY MANIPULATION

Of all common childhood illnesses, diarrhea has the most significant adverse nutritional effect. Restoration of feeding is important to reduce the nutritional defects caused by diarrhea.[18,66] However, optimal conditions have not been defined in nutritional management of different diarrheal states.[52] Once rehydration is complete, food may be reintroduced while the oral electrolyte solution is continued to replace ongoing losses from stools and for maintenance. Breast-feeding in infants should be resumed as soon as possible, preferably immediately after rehydration. Some

TABLE 50–11 Content of Representative Solutions Used for Oral Rehydration

Solution	Sodium (mmol/L)	Potassium (mmol/L)	Carbohydrate (mmol/L)	Osmolarity (mOsm/L)
Rehydration				
World Health Organization solution*	90	20	111	310
Reduced osmolar ORS (WHO/UNICEF)	75	20	75	245
Rehydralyte (Ross)	75	20	140	301
Maintenance or Prevention				
Infalyte (Mead Johnson)	50	25	70	200
Naturalyte (Unlimited Beverage)	45	20	140	265
Pedialyte (Ross)	45	20	140	250
Pediatric electrolyte (NutraMax)	45	20	140	250

**Packets of oral rehydration salts are available in the United States from Cera Products (410-997-2334) and Jianas Brothers (816-421-2880).*

infants experience temporary lactose intolerance after having diarrheal illness,[29] although most young children with acute diarrhea can be managed successfully with continued feeding of undiluted nonhuman milk.[18,53,250] Routine dilution of milk and routine use of lactose-free milk formula are not necessary, especially when oral rehydration therapy and early feeding are part of the approach to the clinical management of acute diarrhea.

In some children and infants, the carbohydrate fraction of milk may exacerbate diarrhea because of disaccharidase deficiency acquired as a result of diarrhea.[29] Development of lactase deficiency during a diarrheal illness may require some alteration in diet. In children with moderate or severe acute diarrhea, reduction or elimination of lactose from the diet early in the illness may be necessary to minimize the effects of lactose intolerance. Relative lactose tolerance has been shown to persist for 2 to 6 weeks after some episodes of diarrhea. Other disaccharidases may be reduced during infection, influencing the absorption of other sugars. Soy-based, lactose-free formulas can be used safely during the acute phase of diarrheal illness in infants.[358] If diarrhea persists for more than 3 weeks, conditions that should be considered include not only disaccharidase deficiency but also celiac disease, cystic fibrosis, parasitic disease, allergic gastroenteropathy, bacterial overgrowth syndrome, congenital diarrhea disorders, EPEC or EAEC disease, and chronic nonspecific diarrhea.[41,240]

NONSPECIFIC THERAPY WITH ANTIDIARRHEAL COMPOUNDS

Many compounds are available for symptomatic treatment of patients with diarrhea. These substances are prescribed by physicians, administered by parents, or taken by patients who are eager to relieve the symptoms. Their purpose is to decrease the volume of diarrhea by increasing absorption of water and electrolytes, to decrease intestinal secretion, or to decrease intestinal motility. These over-the-counter and prescription preparations act on the GI tract by one or more of these mechanisms. Table 50–12 lists some commercially available antidiarrheal agents and their mechanisms of action, major value, and toxicity. Most of these compounds are not approved for children younger than 2 to 3 years old.

Drugs that alter intestinal motility can be classified into antimuscarinics and synthetic or natural opium alkaloids.[18,110,321] These compounds usually have a rapid onset of action. They decrease the volume of stool output and relieve abdominal cramps and pain, probably by producing segmental contractions of the intestine, which retard movement of intestinal contents responsible for diarrhea and restrict the intestinal distention that normally causes abdominal pain. Drugs that affect intestinal motility may worsen the symptoms of *Shigella*, STEC, or other invasive and cytotoxin-producing bacteria by inhibiting intestinal transit and allowing the enteropathogen to be in contact with the intestinal mucosa for a longer time.[110,437] These agents may accelerate development of AAC.[287] Drugs that have central opiate–like effects can lead to overdose; fatalities in children have occurred.[152,153,321,344] Two to four doses of these compounds during a 24-hour period may be used in adolescents or adults to treat severe cramps, but prolonged therapy is not advised, and use in children is not recommended. The combination of TMP-SMX plus loperamide and the use of loperamide alone were effective in treating adults with traveler's diarrhea[54,127] and resulted in the shortest mean duration of diarrhea compared with that in patients taking placebo or TMP-SMX alone. In infants, loperamide can cause ileus, emesis, and drowsiness.[273] The practice parameter of the American Academy of Pediatrics does not recommend this class of compounds for treatment of diarrhea in children.[18]

Numerous chemically inert agents are used internally as adsorbents to bind toxins and water to reduce the number and to improve consistency of bowel movements. When these substances are given by mouth, they can adsorb not only bacteria and toxins but also drugs, nutrients, and enzymes. The only agents currently used widely are compounds containing activated attapulgite, which have been shown to be effective in animals by reducing diarrhea and producing formed stools,[139,329] but studies in humans are lacking. Disadvantages include nonspecific changes in adsorption of nutrients, enzymes, and antibiotics, particularly if the absorbent is used for a prolonged time.

TABLE 50–12 Antidiarrheal Compounds Used as Nonspecific Therapy for Patients with Acute Diarrhea

Mechanism of Action	Generic Name	Trade Name	Value	Comments
Alteration of intestinal motility	Loperamide	Imodium advanced, Imodium A-D, Maalox Antidiarrheal, Pepto Diarrhea Control	Decreases diarrhea, rapid onset of action	Numerous side effects and contraindications Not recommended or licensed for use in infants and young children
	Difenoxin and atropine	Motofen*		May potentiate *Shigella*, *Salmonella*, or STEC infections or accelerate the course of antimicrobial-associated colitis
	Diphenoxylate and atropine	Lomotil*		
	Tincture of opium	Paregoric*		
Alteration of secretion	Bismuth subsalicylate	Pepto-Bismol	Decreases diarrhea and cramps of travelers	Potential for salicylate or bismuth overdose, darkens stool
	Octreotide	Sandostatin*	Decreases diarrhea in patients with vasoactive intestinal peptide–secreting and metastatic carcinoid tumors	Used for relief of refractory AIDS-associated diarrhea; not licensed by the Food and Drug Administration for this condition
	Racecadotril	Not available	Decreases diarrhea	Decreases intestinal hypersecretion, enkephalinase inhibitor
Adsorption of toxins and water	Attapulgite	Diasorb, Donnagel, Kaopectate, Rheaban	Increases form of stool	Safe, minimally effective, decreases absorption of nutrients and drugs, causes abdominal fullness
Alteration of intestinal microflora	Probiotics (*Lactobacillus*, *Bifidobacterium*)	Pro-Bionate, Superdophilus	Value unproven	Safe, contraindicated in those with lactose tolerance

**Requires a prescription.*
STEC, Shiga toxin–producing Escherichia coli.

Probiotics are live, nonpathogenic microorganisms that have been studied for prevention and treatment of a variety of disorders including diarrhea, irritable bowel syndrome, and inflammatory bowel disease. When given orally, these organisms recolonize the intestine with saccharolytic flora and alter the intestinal pH as a way of deterring potential pathogens.[17,318] Ingestion of probiotics results in an increased production of short-chain fatty acids and a decrease in pH in the intestine, which may inhibit the growth of *Salmonella* and *Shigella*. Feeding of selected microorganisms, including *Bifidobacterium bifidum*, *Saccharomyces boulardii*, *Lactobacillus acidophilus*, and *Streptococcus thermophilus*, to children and adults has been shown to be effective in prevention against and treatment of intestinal disease. Several meta-analyses have shown moderate clinical benefit of probiotics in treatment and prevention of diarrheal disease in children.[205,360,391,392] There is a need to better define and standardize the optimal probiotic for prevention and treatment of diarrheal disease caused by specific enteropathogens.[318]

Indomethacin, aspirin, chlorpromazine, and imidazole decrease intestinal secretion of fluid and electrolytes. Indomethacin has been shown to be effective in treating radiation-induced diarrhea, and chlorpromazine is effective in managing diarrhea caused by *V. cholerae;* however, the usefulness of these compounds in patients with various forms of acute infectious diarrhea is unknown. Laboratory studies showed that bismuth subsalicylate (Pepto-Bismol) inhibited intestinal secretion caused by *E. coli* and cholera enterotoxins,[126] reduced diarrhea in adult students who became ill in Mexico,[116] and prevented diarrhea among U.S. students traveling to Mexico.[113] Studies supporting its use in children are limited.[382,383] Potential problems with this compound relate to the absorption of salicylate[135,316] and bismuth.[262] Octreotide (Sandostatin) is a long-acting synthetic somatostatin analogue with pharmacologic actions mimicking those of the natural hormone somatostatin. Octreotide has been used in patients with AIDS who have severe refractory secretory diarrhea.[57]

Racecadotril (acetorphan) is an enkephalinase inhibitor that decreases intestinal hypersecretion but not motility in animals and humans by preventing breakdown of endogenous enkephalins in the GI tract. Racecadotril has been shown to decrease 48-hour stool output, median duration of diarrhea, and intake of oral rehydration solution in children with watery diarrhea.[354] The mechanisms and development of effective antisecretory drugs for diarrheal disease have been reviewed.[132] The potential immunomodulatory mechanisms involved in the interrelationships between micronutrients, including zinc and vitamin A, and infectious diseases, including diarrhea, have been reviewed.[7,189,396,419]

SPECIFIC THERAPY WITH ANTIMICROBIAL AGENTS

Antimicrobial therapy is administered to selected patients with gastroenteritis to abbreviate the duration of the clinical course and to decrease excretion of the causative organisms. A stool culture specimen should be obtained when antibiotic treatment is anticipated, and antibiotic susceptibility testing of any suspected pathogen should be performed to ensure optimal therapy. Changing susceptibility patterns renders the initial selection of an antimicrobial agent difficult. Antimicrobial agents should not be used routinely or liberally for gastroenteritis of unknown cause.

Shigella

Several antimicrobial agents have been used successfully in eradicating clinical symptoms and fecal shedding of *Shigella*, but a high prevalence of antimicrobial resistance in the United States limits treatment options.[374] Of the *Shigella* isolates reported in the National Antimicrobial Resistance Monitoring System (NARMS) of the CDC in 2003, 88 percent were *S. sonnei* and 10 percent were *S. flexneri*. Among the 495 shigella isolates, 91 percent were resistant to one or more antimicrobial agents and 23 percent to five or more antimicrobial agents. Resistance to ampicillin occurred in 79 percent of isolates, to TMP-SMX in 38 percent, and to tetracycline in 29 percent. None of the isolates was resistant to ceftriaxone, imipenem, gentamicin, or ciprofloxacin. Susceptibility testing against azithromycin was not performed. Table 50–13 outlines suggested antimicrobial therapy for children and adults who are presumed to have shigellosis or from whom *Shigella* organisms are isolated from stool.

The history of resistance among *Shigella* strains has shown progressive acquisition of multiresistance, first to sulfonamides, shortly after their commercial availability; then to tetracycline, chloramphenicol, and streptomycin less than 10 years after they were introduced; and subsequently to ampicillin, kanamycin, and TMP-SMX.[308,333,394] In children with known ampicillin-susceptible or TMP-SMX–susceptible strains, either drug can be given, but neither should be used as empiric therapy because of increasing resistance. Amoxicillin is not as effective as is ampicillin in the treatment of shigellosis and should not be used.[283] Parenterally and orally administered, extended-spectrum cephalosporins have been used successfully in the treatment of children with shigellosis.[25,122,414] Two-day[122] and 5-day[414] courses of ceftriaxone were effective in eradicating *Shigella* from stool and reducing the duration of diarrhea, but a single parenteral dose of ceftriaxone produced only a moderate reduction in diarrhea and failed to eradicate *Shigella* strains from stools.[207] Cefixime and ceftibuten, orally administered extended-spectrum cephalosporins, have shown good in vitro activity against various enteric pathogens and promising clinical efficacy in patients with shigellosis; cefixime, however, no longer is manufactured in the United States.[25,325]

Ciprofloxacin, norfloxacin, and enoxacin have been used successfully to treat adults and children with shigellosis[217,251,369] and appear to be safe in children.[162] In a study evaluating dosing of ciprofloxacin in adults, 5 days of therapy were effective for patients infected with *S. dysenteriae* type 1. For other *Shigella* spp., a single 1-g dose was sufficient. Ciprofloxacin is approved by the FDA for treatment of GI tract infections caused by *S. sonnei* and *S. flexneri*. In a randomized, double-blind study of 120 children aged 2 to 15 years with shigellosis, ciprofloxacin and pivmecillinam given for 5 days were successful in providing a clinical cure and eradicating the organism from stool.[251] Ciprofloxacin was not associated with development of arthropathy in children in this study. A comparative study of a 5-day course of either azithromycin or ciprofloxacin in adults with shigellosis showed compa-

TABLE 50–13 Antimicrobial Therapy for Patients with Shigellosis

Strain Susceptibility	Antimicrobial Agent
Strain of unknown susceptibility	Ceftriaxone *or* azithromycin *or* ciprofloxacin* *or* ofloxacin*
Trimethoprim-sulfamethoxazole (TMP-SMX)–susceptible strains	TMP-SMX
Ampicillin-susceptible strain	Ampicillin
Suspected or proven multidrug-resistant strain	Ciprofloxacin* *or* ofloxacin*

**Not approved for patients younger than 18 years.*

rable clinical and bacteriologic responses.[217] Patients who are transient asymptomatic carriers may be managed without antimicrobial therapy if they understand and employ excellent standards of personal hygiene. Treatment of these patients, however, reduces fecal shedding of the organism and prevents spread of infection.

Salmonella

Table 50–14 shows antimicrobial therapy for patients with various clinical manifestations of *Salmonella* infection.[260] The type of syndrome produced by *Salmonella* influences the selection and duration of antimicrobial therapy. Antibiotics should not be used in the treatment of patients who are nontyphoid *Salmonella* carriers or in most patients with mild gastroenteritis. Antimicrobial therapy may, on occasion, convert intestinal carriage into systemic disease with bacteremia,[337] prolong excretion of *Salmonella*,[24] produce a bacteriologic or symptomatic relapse,[24] or encourage development or selection of resistant strains. Antimicrobial agents should be considered for patients with enterocolitis if the disease appears to be evolving into one of the systemic syndromes and for patients with a condition that impairs host resistance to infection, including neonates, young infants, and patients with hemoglobinopathies including sickle-cell anemia, AIDS, leukemia, lymphoma, immunosuppression, congenital heart disease, valvular heart disease, prostheses, and uremia.[163] Antibiotic treatment of *Salmonella* infection should be given to all patients with typhoid fever, bacteremia caused by nontyphoidal strains, and dissemination with localized suppuration.

Selection of antimicrobial agents for therapy is complicated by the emergence of *Salmonella* strains that are resistant to multiple antibiotics[2,427] and by increased risk of acquiring *Salmonella* infection associated with prior antimicrobial exposure.[298] Rates of antibiotic resistance among certain serotypes have been increasing, with a substantial proportion of serotypes of Typhimurium and Newport isolates being resistant to multiple drugs.[58] In 2003, 23 percent of non-*typhi Salmonella* isolates tested by NARMS were resistant to one or more antimicrobial agents and 11 percent were resistant to five or more agents.[58] Only 2 percent of isolates were resistant to TMP-SMX, 0.4 percent to ceftriaxone, and 0.2 percent to ciprofloxacin. Azithromycin was not tested.

Several outbreaks of multiresistant *Salmonella* infection in the United States have been traced to animal sources.[91,151,158,266] Resistant strains of *Salmonella* are common findings in retail ground meats, possibly because of use of antibiotics in animals used for food.[432] Human isolates of *Salmonella* should have susceptibility testing performed to guide therapy. Recommended antimicrobial agents include ampicillin, chloramphenicol, TMP-SMX, ceftriaxone, cefotaxime, ceftazidime, and a fluoroquinolone, which is approved only for people older than 17 years.[260,381] Ciprofloxacin, ofloxacin, and azithromycin are active in vitro against *Salmonella*, including *S.* ser. *typhi*, and have been used clinically with success.[27,237,378,418]

Because of the high rate of *S.* ser. *typhi* transmission together with widespread indiscriminate use of antimicrobial agents in some areas of the world, an increase in resistance patterns to ampicillin, chloramphenicol, and TMP-SMX has occurred.[58,342] Of the *Salmonella typhi* isolates reported by NARMS, 26 percent were resistant to one or more antimicrobial agents. One isolate was resistant to ceftriaxone and ciprofloxacin.[58] Because of this high rate of resistance in *S.* ser. *typhi* strains imported by travelers, empiric treatment of *S.* ser. *typhi* should be provided with ceftriaxone or a fluoroquinolone if the patient is older than 17 years. Ampicillin, chloramphenicol, and TMP-SMX should be reserved for patients in whom susceptibility testing of the causative organism has shown susceptibility. Development of resistance of *S.* ser. *typhi* to ceftriaxone and fluoroquinolones needs to be monitored. In areas of the world where *S.* ser. *typhi* strains with reduced susceptibility to fluoroquinolones have been reported, azithromycin was effective for treatment of people with enteric fever,[77,145,155] as was cefixime.[261,306] In a prospective, randomized study of children and adults with confirmed typhoid fever, ceftriaxone administered for 5 days was as effective and safe as a 2- to 3-week course of chloramphenicol,[195] but in another study, short-course therapy failed.[40]

Corticosteroids can be beneficial in treating patients with typhoid fever in whom prompt relief of manifestations of toxemia might be lifesaving,[83] but corticosteroids may increase the relapse rate.[182]

Antibiotics listed in Table 50–14 can be used for treatment of *Salmonella* infections. Patients with defective host defense mechanisms, such as people with AIDS, should be treated with ampicillin or an expanded-spectrum cephalosporin.[380] Ciprofloxacin has been reported to be effective in treating acute diarrhea caused by *Salmonella*,[118,127,128] recurrent *Salmonella* sepsis,[81] and brain abscesses in a neonate.[430] Duration of therapy is influenced by the site of infection and by the host. Patients with bacteremia without a localized infection should be treated for 14 days, whereas those with localized infection, such as osteomyelitis or endocarditis, or patients with AIDS and bacteremia should receive at least 4 to 6 weeks of therapy.[40] In most cases, chronic carriage of *S.* ser. *typhi* is associated with gallbladder disease. The presence of cholelithiasis may significantly affect the efficacy of therapy. When gallbladder disease is present, the failure rate of ampicillin is approximately 75 percent.[204] In patients without gallbladder disease, ampicillin with probenecid or amoxicillin administered for 6 weeks is the treatment of choice for chronic enteric carriers.[305] Ciprofloxacin[137] has been reported to be successful in eradicating *S.* ser. *typhi* in adult chronic carriers. Resistance of clinical isolates and failure of treatment with ciprofloxacin have been noted in patients infected with *S.* ser. *typhi*.[320]

TABLE 50–14 Antimicrobial Therapy for Patients with *Salmonella* Infections

Clinical Manifestation	Antimicrobial Agent
Acute gastroenteritis*	None
Bacteremia or enteric fever†	Ceftriaxone *or* cefotaxime *or* fluoroquinolone† *or* ampicillin‡ *or* chloramphenicol‡ *or* trimethoprim-sulfamethoxazole‡ (TMP-SMX)
Dissemination with localized suppuration (osteomyelitis)† or bacteremia in patients with AIDS	Same as for bacteremia
Meningitis or ampicillin-chloramphenicol–resistant and TMP-SMX–resistant organisms	Cefotaxime or ceftriaxone

**Patients with hyperpyrexia and systemic signs or symptoms should be treated empirically until bacteremia is excluded. Although of unproven efficacy, antimicrobial therapy for children in the first 3 months of life also is recommended by most authorities; a 7- to 10-day course of therapy probably is sufficient.*

†Ciprofloxacin or ofloxacin can be used to treat resistant organisms in patients 18 years of age and older. Both are available for intravenous as well as oral use. Neither is recommended for pregnant women. Azithromycin has been used with success in developing countries.

‡Can be used if the organism is susceptible.

Two typhoid fever vaccines for children older than 2 years, adolescents, and adults are available commercially in the United States for specific situations.[75] A *S. typhi* Vi conjugate vaccine has been shown to be safe, immunogenic, and 90 percent effective in children 2 to 5 years of age,[238] but this vaccine is not available commercially.

Campylobacter

Campylobacter jejuni isolates generally are susceptible to a wide variety of antimicrobial agents, including erythromycin, quinolones, furazolidone, aminoglycosides, tetracycline, chloramphenicol, imipenem, and clindamycin, whereas penicillin, ampicillin, cephalosporins, and TMP-SMX are relatively inactive. Isolation of *Campylobacter* from stool does not imply the need for antimicrobial therapy. In a meta-analysis of eight randomized, controlled trials of antimicrobial therapy versus placebo, antimicrobial therapy shortened the duration of intestinal symptoms by 1.32 days.[402] The decision concerning therapy should be individualized, but therapy should be considered in patients with high temperatures, bloody diarrhea, and severe diarrhea and in immunocompromised hosts. In patients with *Campylobacter* enteritis, erythromycin or azithromycin is the agent of choice when a decision has been made to initiate therapy.[260] Data from NARMS from 1977 to 2003 showed that less than 2 percent of the isolates were resistant to erythromycin and azithromycin.[58,165] In these and other studies, a higher frequency of erythromycin resistance was noted in hog isolates, most of which were *C. coli*.[147,198,285] Strains of *C. jejuni* and *C. coli* that show high-level resistance to erythromycin also appear to be resistant to clarithromycin and azithromycin.[397]

Ciprofloxacin has been licensed by the FDA for treatment of *C. jejuni* enteritis in patients older than 17 years. Development of resistance to ciprofloxacin in *Campylobacter* spp. has been reported.[58,165,347] Data from NARMS show that the rate of *C. jejuni/coli* isolates resistant to ciprofloxacin was 13 percent in 1997 and 18 percent in 2003.[58,165] These high rates of resistance have been related in part to introduction of fluoroquinolones in animal feed.[284,377] In double-blind, placebo-controlled trials of erythromycin for treatment of patients with enteritis caused by *C. jejuni*, erythromycin was shown to eradicate *C. jejuni* promptly from feces but not to alter the clinical course when begun 4 days or more after the onset of symptoms.[20] Studies in which therapy was started early showed that *C. jejuni* was eliminated rapidly from stools, but studies gave conflicting results with regard to resolution of clinical illness.[353,433] The treatment of choice for patients with septicemia caused by *C. jejuni* or *C. fetus* infection is gentamicin or imipenem.[260]

Other Bacterial Agents

Antimicrobial agents have been employed frequently in attempts to treat infantile gastroenteritis caused by EPEC and as a means of controlling the spread of EPEC strains in hospital nurseries (Table 50–15). Although no definitive studies support the effectiveness of these drugs, they may be useful in certain situations, particularly when life-threatening infection occurs or when epidemic spread of the strains continues, despite the use of strict handwashing and appropriate isolation. Agents used in the treatment of EPEC diarrhea include TMP-SMX, but strains may be resistant. If systemic infection is suspected, parenteral therapy should be started and modified according to antimicrobial susceptibility of the organism isolated.

Diarrhea caused by ETEC usually is limited, but studies in adults have shown that antimicrobial agents such as TMP-SMX and ciprofloxacin are effective treatments. The treatment in patients with diarrhea caused by EIEC is similar to that in patients with shigellosis (see Table 50–13). Travelers infected with EAEC have shown response to ciprofloxacin.[156] Antimicrobial therapy of infants and children with hemorrhagic colitis caused by STEC has been reported to increase development of hemolytic-uremic syndrome,[437] but other studies have not supported this observation for STEC[348] or for *S. dysenteriae* type 1.[38]

TABLE 50–15 Antimicrobial Therapy for Bacterial Pathogens Causing Gastroenteritis

Organism	Antibiotic Therapy
Campylobacter jejuni	
Gastroenteritis	None, or if colitis is present, erythromycin or azithromycin or ciprofloxacin
Sepsis	Aminoglycoside
Clostridium difficile (antimicrobial-associated colitis)	Metronidazole or vancomycin
Escherichia coli	
Enteropathogenic	None or TMP-SMX or fluoroquinolone*
Enterotoxigenic	TMP-SMX or fluoroquinolone* or cefixime
Enteroinvasive	Same as for shigellosis (see Table 50–13)
STEC	None
Vibrio cholerae	Doxycycline or tetracycline or TMP-SMX
Vibrio parahaemolyticus	None
Yersinia enterocolitica	
Gastroenteritis	Probably none
Sepsis	TMP-SMX or an aminoglycoside or cefotaxime or ceftizoxime or ciprofloxacin*

**Approved only for patients 18 years of age or older.*
STEC, Shiga toxin–producing E. coli; TMP-SMX, trimethoprim-sulfamethoxazole.

Antimicrobial agents decrease the duration of diarrhea associated with cholera by eradicating the vibrios from the GI tract[420] and thus reducing the volume of fluid loss. Doxycycline is the drug of choice in most instances for O1 and O139 infections, including those in children, in whom the benefits of a 1- to 2-day course outweigh the risk for dental staining.[259,261] TMP-SMX can be used in children younger than 8 years for *V. cholerae* O1, but O139 strains often are resistant. Ampicillin probably is the safest agent for use during pregnancy. Other effective antimicrobial agents are the fluoroquinolones and azithromycin. Single-dose azithromycin has been effective in both children[218] and adults.[350] Single-dose ciprofloxacin has been successful in treatment of adults,[216,349] but resistance was reported in a study from Bangladesh.[350] Diarrhea caused by *V. parahaemolyticus* is self-limited, so antimicrobial therapy shortens neither the clinical course nor the duration of excretion.[87] Antimicrobial therapy has not been proved efficacious in the treatment of uncomplicated enterocolitis caused by *Y. enterocolitica*.[45] Patients with *Y. enterocolitica*–induced septicemia or extraintestinal focal infection or compromised hosts with enterocolitis should receive TMP-SMX, a fluoroquinolone if they are older than 17 years, an aminoglycoside, cefotaxime, or cefotaxime. Table 50–15 outlines the antimicrobial therapy of these bacterial pathogens.

Protozoal Agents

Several drugs have been shown to be effective in the treatment of patients with giardiasis (Table 50–16).[90,150,259,340] Metronidazole may be better tolerated than is quinacrine, is not approved for the treatment of patients with giardiasis in the United States, and is carcinogenic in animals. Quinacrine hydrochloride may produce a yellow discoloration of the skin that disappears after

TABLE 50–16 Antimicrobial Therapy for Patients with Giardiasis

Antimicrobial Agent	Comments
Furazolidone (Furoxone)	Nausea, vomiting, disulfiram-like reaction with alcohol, mild hemolysis in glucose-6-phosphate dehydrogenase–deficient people, hypoglycemia, allergic reactions
Metronidazole (Flagyl)*	Metallic taste, nausea, headache, dry mouth, mutagenic in bacteria, carcinogenic in animals, disulfiram-like reaction with alcohol
Quinacrine HCl	Dizziness, headache, vomiting, diarrhea, yellow-orange skin color; not available commercially but can be obtained†
Nitazoxanide	Abdominal pain, vomiting, and headache uniformly reported; data minimal for children younger than 12 months or older than 11 years and for patients with immune deficiencies
Paromomycin*	Not highly effective, proposed for use in pregnancy
Albendazole*	Anorexia, constipation; clinical trials have shown mixed results
Tinidazole	Given as one dose

**Not a U.S. Food and Drug Administration–licensed indication.*
†Medical Center Pharmacy (203-688-6816) or Panorama Compounding Pharmacy (800-247-9767).

the drug is stopped. Quinacrine is not available commercially but as a service can be compounded (see Table 50–16). Furazolidone is the only one of these three compounds available in liquid form; like quinacrine, it is less expensive than metronidazole. Furazolidone can be used in children if compliance is a problem with quinacrine and metronidazole, both of which have an objectionable taste. Clinical trials using albendazole to treat people with giardiasis have produced mixed results.[150] Treatment of children with albendazole for 5 days,[166] but not 3 days,[166,299] has been effective. Toxicity of albendazole is low, and this compound is effective against many helminths, rendering it useful for treatment when multiple intestinal parasites are identified or suspected. Nitazoxanide has been licensed by the FDA for treatment of children with diarrhea caused by *Giardia* or *Cryptosporidium*. Tinidazole, a nitroimidazole drug similar to metronidazole, is given as a single dose. Paromomycin is not absorbed and is not highly effective but has been proposed for use during pregnancy.[228]

In a review of comparative drug trials for the treatment of giardiasis, Davidson[90] reported that metronidazole cured 92 percent of 219 patients and furazolidone cured 84 percent of 150 patients. Approximately 7 percent of the metronidazole-treated patients and 10 percent of the furazolidone-treated patients had side effects that were serious enough to report. The drugs available in a liquid preparation are furazolidone and nitazoxanide.

In treatment of patients with amebiasis, iodoquinol and paromomycin are the recommended luminal amebicide drugs that are available in the United States.[259] Paromomycin may be used during pregnancy. Diloxanide furoate is an alternative, but it is not available commercially in the United States. These drugs are effective against both cysts and trophozoites in the lumen of the gut, but they are ineffective against tissue forms of the disease. Invasive amebiasis of the intestine, liver, or other organs necessitates the additional use of tissue amebicides, such as metronidazole or tinidazole. Liver abscess or other extraintestinal forms of disease should be treated with metronidazole or tinidazole in the dose recommended for intestinal disease. Table 50–17 lists the recommended drugs for the treatment of children and adults with various forms of amebiasis.

TABLE 50–17 Antimicrobial Therapy for Patients with Amebiasis

Asymptomatic amebic cyst	Iodoquinol (Yodoxin) or paromomycin (Humatin) or diloxanide furoate*
Mild to moderate intestinal disease	Metronidazole (Flagyl) followed by iodoquinol or paromomycin
Severe intestinal disease, liver abscess, or other extraintestinal disease	Metronidazole followed by iodoquinol or paromomycin

**Available in the United States from Panorama Compounding Pharmacy (800-247-9767) or Medical Center Pharmacy (203-688-6816).*

Patients with intestinal strongyloidiasis should receive ivermectin for 1 to 2 days or thiabendazole every 12 hours for four doses. Immunosuppressed patients with disseminated disease may require continued therapy for 2 to 3 weeks, but the mortality rate is high despite administration of therapy. A thorough examination should be performed before immunosuppressive therapy is given to a patient with a past history of infection with *S. stercoralis*.

Therapeutic agents generally have not proved consistently beneficial for treatment of patients with cryptosporidiosis, and only one drug (nitazoxanide) is approved by the FDA for this purpose.[1,143] Infection generally is self-limited in immunocompetent patients, and therapy usually is not warranted.[1,176] In patients with persistent disease, an underlying immunodeficiency should be considered. Spiramycin has been used but is ineffective.[435] Paromomycin may be effective in rapid resolution of chronic diarrhea.[138,421,431] In a prospective, randomized, double-blind, placebo-controlled trial in the treatment of adults with AIDS and symptomatic cryptosporidiosis,[176] paromomycin was not effective. Azithromycin has been shown to be effective in treatment of two children with cancer and four children with HIV infection who had severe diarrhea caused by *Cryptosporidium*.[177,413] Combination therapy with paromomycin and azithromycin has been effective in some patients with AIDS and chronic cryptosporidiosis.[379] In a prospective, randomized, double-blind, placebo-controlled study of 50 children and 50 adults, treatment with nitazoxanide reduced the duration of diarrhea and oocyst shedding.[339] A meta-analysis of immunocompromised people treated with nitazoxanide or paromomycin showed no evidence of effectiveness.[1] Evidence of cyst reduction was not significant for HIV-seropositive people. Nitazoxanide showed a significantly higher rate of achieving parasite clearance.[1] Nitazoxanide is licensed for treatment of children with diarrhea caused by cryptosporidiosis and giardiasis. The best approach for prevention of cryptosporidiosis in HIV-infected patients is maintenance of the immune system function by use of HAART because chronic cryptosporidiosis occurs only in severely immunocompromised individuals.

Hyperimmune bovine colostrum has been used with some success in a child with agammaglobulinemia[408] and in a patient with AIDS.[409] Immune bovine colostrum has been shown to neutralize *C. parvum* sporozoites and partially to protect mice against oral challenge with *C. parvum* oocysts,[304] and oral bovine transfer factor has been used for treatment of cryptosporidiosis.[242] Nonspecific therapy with octreotide may control the severe diarrhea that occurs in patients with AIDS but has no effect on the infection[57] and is not approved for this use by the FDA.

For isosporiasis, TMP-SMX is effective and is the drug of choice.[259] In immunosuppressed patients, TMP-SMX, at a dose of 160 mg TMP and 800 mg SMX four times a day for 10 days, followed by the same dose twice a day for 3 weeks, is recommended for adults. In immunosuppressed children, TMP 5 mg/

kg with SMX 25 mg/kg four times a day for 10 days, followed by the same dose twice a day for 3 weeks, should be used. Ciprofloxacin can be used when TMP-SMX cannot be tolerated, although it is slightly less effective. Other drugs, including pyrimethamine-sulfadoxine (Fansidar), metronidazole, and furazolidone, have been reported to be successful for treating patients with isosporiasis.[296,428] Pyrimethamine has been an effective alternative in patients allergic to sulfa drugs.[296] Problems in the treatment of isosporiasis have been encountered in patients with AIDS, in whom a high incidence of recurrence has been reported after treatment has been stopped. Continuation of therapy indefinitely with either pyrimethamine-sulfadoxine or TMP-SMX in adults has been shown to be effective in preventing recurrence of disease.[296]

On the basis of in vitro studies, several drugs have been used to treat microsporidial infections in humans, but successful treatment with any of them in humans is limited. Therapy with antiparasitic drugs, diet alteration, and antidiarrheal medications often fails to relieve diarrhea and malabsorption associated with microsporidiosis, although octreotide may provide symptomatic relief,[57] and diet modification to include medium-chain triglyceride–based diets has produced clinical improvement.[423] *E. intestinalis* is susceptible to albendazole,[82,212,335] which has been reported to stop diarrhea and weight loss as well as to promote weight gain in patients infected with *E. intestinalis*,[104,267,406] although improvement has not been uniform. Infections caused by *E. bieneusi* are more difficult to treat, but oral fumagillin has been effective.[268] The use of HAART may lead to microbiologic and clinical response in HIV-infected patients with diarrhea due to microsporidia.

Patients with HIV and *Cyclospora* infection respond to TMP-SMX, but relapses are common occurrences.[247,259,417] As few as 7 days of treatment may be adequate in immunocompetent children. HIV-infected patients may need higher doses and long-term maintenance. Although ciprofloxacin is not as effective as is TMP-SMX, it is acceptable for patients who cannot tolerate TMP-SMX.[417]

Patients infected with *S. stercoralis* should be treated with ivermectin or, alternatively, thiabendazole.[260] In disseminated strongyloidiasis, ivermectin or thiabendazole therapy should be continued for at least 5 days. In immunocompromised patients and patients with *Strongyloides* hyperinfection, longer duration of therapy may be necessary[233]; however, the mortality rate is high despite therapy. A thorough examination should be performed before immunosuppressive therapy is given to a patient with a history of infection with *S. stercoralis*.

The clinical significance of *B. hominis* remains unclear, few studies have considered the treatment of large numbers of patients, and case-control studies are lacking.[388] Use of metronidazole or iodoquinol in the same dose as for mild to moderate intestinal disease from *E. histolytica* has been reported to be effective in uncontrolled studies,[388] and they are the recommended drugs of choice. TMP-SMX or paromomycin may be the most appropriate second-choice drug.[259] Treatment should be provided with caution, only after a thorough clinical review of other possible causes of symptoms has been performed.

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CHAPTER 51

Antibiotic-Associated Colitis

George D. Ferry ✪ James Versalovic

Clostridium difficile colonization and infection accounts for 10 to 25 percent of antibiotic-associated diarrhea and is the major cause of antibiotic-associated pseudomembranous colitis.[8] Diarrhea and colitis develop when antibiotics, especially those with a broad spectrum of activity, disturb the bowel microbiota and allow overgrowth of *C. difficile*. Production of toxins then leads to inflammation and secretion of fluids from the colon, resulting in watery diarrhea. If inflammation progresses and pseudomembranous colitis develops, the diarrhea becomes bloody. Colitis induced by *C. difficile* has been reported without prior use of antibiotics, but it is an uncommon occurrence.

HISTORY

Pseudomembranous colitis was recognized as early as 1893[38] and derives its name from the numerous plaquelike lesions in the colon. The plaques are membranes of epithelial debris containing fibrin, mucus, and polymorphonuclear leukocytes overlying necrotic glands.[96] Not until the early 1950s was an association with antibiotics suggested. The organism that initially received the most attention as a possible cause was *Staphylococcus aureus*.[103] Stool cultures frequently were positive for *S. aureus* after antibiotic use, and autopsies showed enterocolitis with ulcers and pseudomembranes in both the small and the large bowel. The association with *C. difficile*, a gram-positive anaerobic bacillus, was shown in 1977 and 1978 with the reports of production of toxins related to pseudomembranous changes in the colon.[9,10,61] *C. difficile* can colonize the intestine without causing diarrhea and was identified as part of the normal microbiota of infants and newborns in 1935.[47] Early studies suggested that *Clostridium sordellii* might be related to pseudomembranous colitis, but subsequent investigation has shown that *C. sordellii* antitoxin neutralizes *C. difficile* cytotoxicity but is not a cause of colitis.[10]

ETIOLOGY AND PATHOGENESIS

C. difficile can be cultured in 7.6 percent of healthy adults,[34,51] but in hospitalized patients, the incidence of colonization and positive cultures reaches 20 percent or more.[74] *C. difficile*–associated diarrhea appears to have significantly increased in incidence in the United States, especially among the elderly,[70] and outbreaks with a more virulent and a more antibiotic resistant strain have been reported in the United States and Canada.[87] The specific *C. difficile* isolates responsible for some of these outbreaks have been characterized as restriction endonuclease analysis group BI, with an increased production of toxins A and B and also positive for the binary toxin CDT, a possible virulence factor.[4,67,71,72]

C. difficile spores are viable for long periods, up to 5 months,[54] and can be cultured from flooring, toilets, and bedding as well as from the stool and hands of carriers (Table 51–1).[34,54] Numerous studies have shown that neonates frequently are colonized with *C. difficile*.[47,49,117] In a London study, 2 to 52 percent of infants in three postnatal wards had positive cultures, but none developed diarrhea or colitis, and no evidence was found that infants were colonized from their mothers.[60] The rate of colonization appears to be related to the length of time infants are hospitalized.[100] In those older than 1 year, colonization decreases significantly.[46] Not only is *C. difficile* a common contaminant in hospitals,[34,54] but person-to-person transmission among children in daycare centers also has been reported.[53] A hospital outbreak of *C. difficile* in children 18 months to 18 years was reported from a pediatric orthopedic ward.[37]

Infection with *C. difficile* occurs commonly and accounts for 10 to 25 percent of cases of uncomplicated antibiotic-associated diarrhea.[8,109] Most infections cause watery diarrhea, but 5 to 10 percent progress to pseudomembranous colitis.[8,55] Pseudomembranous colitis can occur sporadically or in epidemics.[85,91] In daycare centers, the incidence of *C. difficile* infection not related to antibiotics may be as high as 50 percent.[8,53] Community-acquired diarrhea caused by *C. difficile* studied in a health maintenance organization population identified 51 cases, with an incidence of 7.7 cases per 100,000 person-years.[48] Half of these cases occurred after antibiotic use. Increased age and exposure to more than one antibiotic within a 42-day period increased the risk. *C. difficile* diarrhea was an uncommon occurrence in patients younger than 20 years. Risk factors included inflammatory bowel disease, infection with human immunodeficiency virus, and chronic treatment with antibiotics. In a report of 200 Canadian children with *C. difficile*–associated diarrhea, underlying factors such as chemotherapy, Crohn disease, transplantation, immunodeficiency, and Hirschsprung disease were found in 19 percent of patients.[79] A history of antibiotic exposure in the previous 2 months was present in 74.5 and 55.5 percent of children who had been hospitalized. Elemental diets have been reported to increase the growth of *C. difficile*,[50] and both community- and hospital-acquired *C. difficile*–associated diarrhea are associated with the use of proton pump inhibitors.[26,27]

TABLE 51–1 Distribution of *Clostridium difficile* Isolates Taken from the Environment of Two Pediatric Units

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Please refer to the printed publication.

From Kim, K.-H., Fekety, R., Batts, D. H., et al.: Isolation of Clostridium difficile from the environment and contacts of patients with antibiotic-associated colitis. J. Infect. Dis. 143:44-50, 1981.

Virulence may differ among strains of *C. difficile;* some are highly toxigenic, and others have low virulence.[57] This difference may be related to S-layer proteins covering the surface of *C. difficile* serotypes known to cause disease.[94] Production of toxins by the organism and clinical illness are absent in 25 percent of patients with positive cultures.[35] Most toxigenic strains produce both toxin A and toxin B. Toxin B initially was thought to be a cytotoxin with little clinical effect[68,93]; however, more recent studies have shown that it has a significant effect once tissue damage has already occurred.[94] Toxin A produces necrosis and increased cell permeability, and both toxins lead to the production of tumor necrosis factor–α and other cytokines.[94] The increased permeability is responsible for the watery diarrhea.

Studies on the epithelial cell barrier have shown two additional pathways of cell injury.[86,92] *C. difficile* toxins disaggregate actin microfilaments in colonocytes, leading to cell destruction and opening of tight junctions. Toxin A also has a significant chemotactic effect on neutrophils, leading to local inflammation and release of inflammatory mediators.[93] Figure 51–1 illustrates these pathways. In experimental models, injection of toxin A into rabbit ileal loops results in increased fluid secretion, inflammation and necrosis of epithelial cells, and release of prostaglandin E_2 and leukotriene B_4 into the lumen.[113] Another mechanism for diarrhea may relate to decreased anaerobic microbiota and, subsequently, a decreased digestion of carbohydrates, leading to a decreased production of lactic acid and short-chain fatty acids and disturbed function of the colonic mucosa.[12]

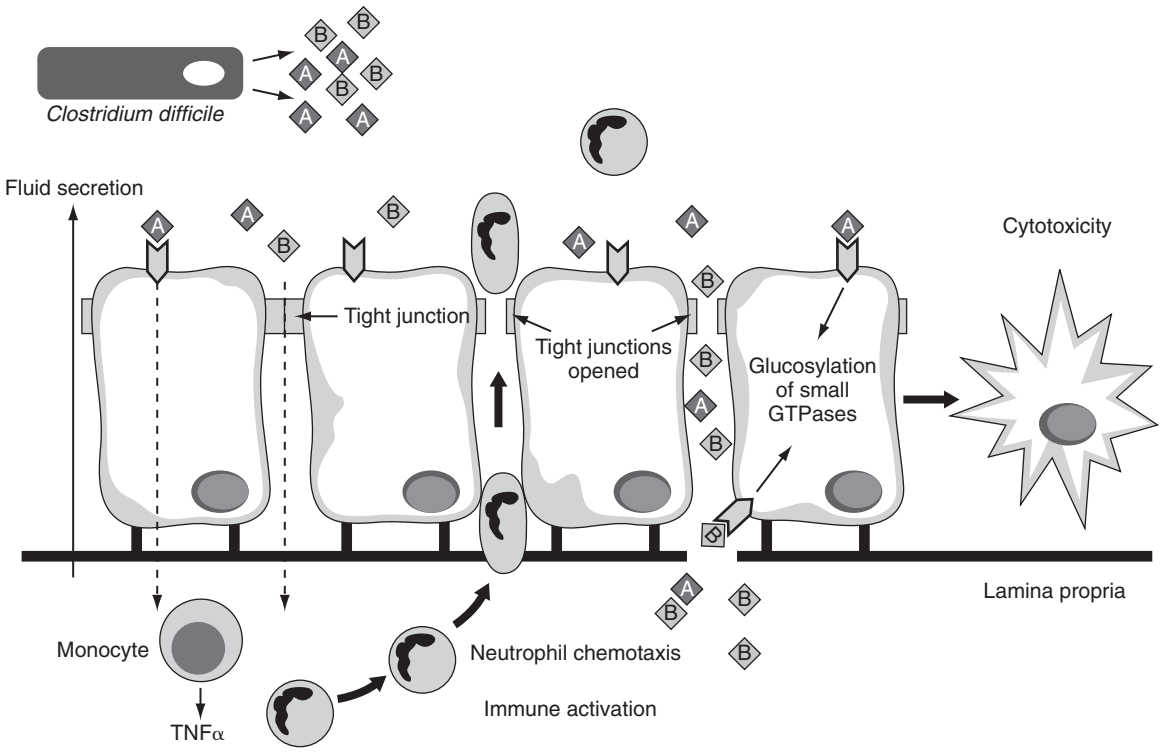

Figure 51–1 Actions of *Clostridium difficile* toxins A and B on intestinal epithelium. *(Courtesy of Ian R. Poxton, Professor of Microbial Infection and Immunity, Medical Microbiology, University of Edinburgh Medical School.)*

Serum antibodies to *C. difficile* toxins A and B occur commonly, being found in 60 to 70 percent of patients older than 3 years.[116] In adults, specific serum IgA and IgG antibodies to toxin A have been detected in 57 to 60 percent of patients.[52] Antibodies also have been found in colonic mucosa and duodenal aspirates in 10 percent of patients. Binding of toxin A was inhibited significantly by colonic aspirates with high-IgA antitoxin-A antibody. These antibodies appear to persist throughout life, and they may have a protective role against recurrence.[58]

Protection against diarrhea and pseudomembranous colitis in newborns may be due to a lack of the intestinal receptor for toxin A[30] or prematurity of the toxin receptor. A study by Enad and colleagues[32] showed that 87 infants colonized with toxin A–positive *C. difficile* had more days of diarrhea than did those who were toxin negative. Although breast milk contains antibody against *C. difficile*, whether it influences disease activity is unclear.[118] *C. difficile* has been suggested to be a causative agent in rare cases of necrotizing enterocolitis, but this suggestion remains controversial.[16,32]

In the normal host, many bacteria, especially lactobacilli, *Bacteroides*, enterococci, and *Escherichia coli*, inhibit growth of *C. difficile*.[99] Treatment with any antibiotic can result in overgrowth of *C. difficile* and lead to pseudomembranous colitis. Oral antibiotics are associated with colitis more often than are parenteral antibiotics, and broad-spectrum antibiotics are responsible for most cases. Multiple antibiotics, including clindamycin, ampicillin, cephalosporin, penicillin, chloramphenicol, gentamicin, trimethoprim-sulfamethoxazole, and rifampicin, have been implicated in antibiotic-associated diarrhea in children.[1,83,111] Fluoroquinolone administration was the most prominent risk factor for *C. difficile*–associated diarrhea (n = 5619 patients) during an epidemic in eastern Canada caused by a hypervirulent strain.[89] Proton pump inhibitors were not associated with disease in this study. Cefixime also has been associated with pediatric pseudomembranous colitis.[45] Long-term antibiotic treatment of acne has been complicated rarely by diarrhea or colitis.[33]

C. difficile infection has been reported in pediatric patients during chemotherapy.[31] The spectrum of illness has been mild to fulminant colitis, just as in antibiotic-associated disease. *C. difficile* also has occurred as an outbreak with associated diarrhea in patients with acquired immunodeficiency syndrome who were admitted to the same hospital ward.[5] In a study in 2005 of 75 pediatric outpatients presenting with diarrhea, positive bacterial isolates were found in 48 percent of patients, and eight children were positive for *C. difficile* toxin.[25]

CLINICAL MANIFESTATIONS

The spectrum of clinical signs and symptoms related to *C. difficile* infection ranges from the asymptomatic carrier state to a fulminant colitis and toxic megacolon.[112] Most patients have watery diarrhea, but 10 to 15 percent have bloody stools.[55] Diarrhea most often begins 3 to 21 days after the use of antibiotics. Most children with diarrhea have a self-limited illness with watery stools, fever, and abdominal pain.[105,111] Patients with pseudomembranous colitis present with cramping abdominal pain and fever, and watery, green, foul stools progress to bloody diarrhea.[29] Colitis often is a mild, self-limited illness once antibiotics are stopped, but it may be severe and require intensive antibiotic and supportive therapy.

Fulminant colitis is an uncommon occurrence but can lead to perforation and colectomy and a high mortality rate (35%).[56] Toxic megacolon, or toxic dilatation of the small bowel, may develop without preceding diarrhea. In these critically ill patients, colitis may lead to perforation and peritonitis. In a series of 201 surgical patients, toxic megacolon developed in five, and four of the five died.[95] Confirmation of the diagnosis may depend on finding the typical pseudomembranous ulcers on colonoscopy. Response to treatment generally is rapid; symptoms clear in 50 percent of patients within 6 to 14 days and in 100 percent by 1 month.

The role of *C. difficile* infection in inflammatory bowel disease has been controversial. Some studies have shown no significant association,[77] whereas others have implicated *C. difficile* in the relapse of Crohn disease and ulcerative colitis.[18,59] The organism has been found in 8 percent of patients in remission,[28] the same as in the general population. The presence of toxin seems to correlate with the degree of bleeding and disease activity.[44] In studies of 59 and 54 patients, toxin was found in 19 and 16 percent of individuals, respectively.[76,114] The majority, 64 and 90 percent of patients, respectively, had received antibiotics previously. These patients responded promptly to treatment with targeted antibiotics, suggesting that the flare of colitis was related to *C. difficile*.

LABORATORY STUDIES

The established standard for diagnosis of *C. difficile* infection is detection of enterotoxins A or B by immunoassays or cell culture–based cytotoxicity tests.[120] Because not all organisms produce toxin, a causative role for *C. difficile* in diarrhea and colitis is based on detection of toxin in the stool rather than a positive anaerobic culture.[42,62] Fresh, liquid stools are the specimens of choice for toxin testing,[24] and all specimens should be tested for both toxins A and B.[69] Production of *C. difficile* toxin can be diagnosed by a variety of techniques. Immunoassays, including laboratory-based enzyme-linked immunosorbent assays and point-of-care immunocard tests, are most commonly used for detection of toxigenic *C. difficile*. Although published sensitivities of these assays exceed 80 percent,[2,35] the reality is that most immunoassays have lower sensitivities when they are used in routine clinical practice. Repeated immunoassay testing usually is recommended when initial testing is negative for *C. difficile* toxin A or toxin B. An alternative and effective approach is the combination of *C. difficile* common antigen testing with toxin detection.[120] This two-pronged approach uses immunoassays to detect a species-specific antigen, glutamate dehydrogenase (GDH or *C. difficile* common antigen), and both enterotoxins A and B. GDH is found in both toxigenic and nontoxigenic strains. Commercial GDH tests are more sensitive than is direct toxin detection, and GDH-positive stools are evaluated subsequently by toxin A or B testing. This approach may detect stools positive for *C. difficile* that may be missed entirely by toxin screening. An enzyme immunosorbent assay test kit combining detection of toxin A and GDH has a negative predictive value for infection with *C. difficile* of 99.6 percent.[24]

Cell culture–based cytotoxicity assays were used widely before the introduction of commercial immunoassays and continue to be a useful approach for detection of *C. difficile* directly in stool specimens. Cell culture testing is tedious and time-consuming for the laboratory, but it may have superior sensitivity for toxin detection if technical expertise is available.[24,35] Fecal material is diluted, and cytopathic effects are evaluated with human fibroblast cell lines to assess the presence of toxin (primarily toxin B). Molecular methods show promise as next-generation methods for direct *C. difficile* enterotoxin gene detection. Polymerase chain reaction amplification, including real-time polymerase chain reaction methods, has demonstrated the ability to detect toxin A (*tcdA*) and toxin B (*tcdB*) genes (DNA) directly in human stool specimens. Published molecular studies described excellent concordance with toxin immunoassays[11,115] while enabling antigen-negative specimens to be detected.[63] Latex agglutination for *C. difficile* antigen is less sensitive than is culture and not as specific as the toxin assay.[42,90] Anaerobic culture of *C. difficile* from human stool specimens, although infrequently practiced in the United States today, may be coupled effectively with immunoassay-based or molecular detection of *C. difficile* enterotoxins. *C. difficile* can be cultured on selective media, such as cycloserine-cefoxitin-fructose agar.[41] Stool culture alone does not determine the presence of toxigenic *C. difficile*, and this approach requires the tedious practice of anaerobic bacteriology for a test that frequently is performed at high volumes.[42]

Peripheral blood leukocytosis of 15,000 cells/mL or more is a common finding in hospitalized patients and may occur with diarrhea or with systemic symptoms of fever and toxicity before the onset of diarrhea.[20] In the appropriate setting, leukocytosis may be an early marker of *C. difficile* infection, with as many as 58 percent of patients being positive for toxins and responding to metronidazole.[119] Stool examination may show evidence of blood and mucus, but fecal leukocytes lack utility for screening because they are present in only 50 percent or less of patients.[35,98]

Endoscopic diagnosis of *C. difficile* is useful when urgent diagnosis is needed before laboratory confirmation of a positive assay for toxin is obtained (Fig. 51–2). Flexible sigmoidoscopy is a rapid diagnostic tool, but in one study of 29 patients with a positive toxin assay, only 55 percent had typical pseudomembranous colitis, 14 percent had nonspecific colitis, and 31 percent were normal.[13] In cases with a typical endoscopic appearance, *C. difficile* toxin is positive in 95 percent.[40] Flexible sigmoidoscopy will detect 90 percent of patients with colitis.[109]

The colon typically appears red with raised, circular, yellow plaques.[40,108] These pseudomembranous plaques vary from 2 to 5 mm in diameter and are scattered throughout the involved area. In the earliest stage, 1- to 2-mm ulcers may be seen but may not have an obvious membrane. Biopsy specimens of these small lesions show the same endothelial degeneration and pseudomembrane as larger lesions do.

Plain radiography of the abdomen in patients with colitis may show a variety of abnormalities, including colonic ileus, small bowel ileus, ascites, and nodular haustral thickening.[17] Colonic findings on computed tomography of the abdomen in adult patients show segmental wall thickening, but this finding is nonspecific.[3] Abdominal ultrasonography also has been used to diagnose cases of *C. difficile* infection.[97] In severely ill patients with pseudo-membranous colitis, leukocyte scintigraphy has demonstrated a constant and diffuse pattern of intense radiotracer.[84] Although not specific, this test may be useful whenever endoscopy cannot be performed.

The presence of *C. difficile* toxin A or toxin B may establish the etiology of diarrhea and colitis; the absence of toxin may not be conclusive. If symptoms are suggestive of antibiotic-associated diarrhea or colitis, studies of stools should be repeated several times before this diagnosis is ruled out.[73]

DIFFERENTIAL DIAGNOSIS

Bloody diarrhea occurring after recent use of antibiotics should suggest the possibility of pseudomembranous colitis. Stool specimens should be obtained for *C. difficile* toxins A and B, along with routine cultures for *Salmonella*, *Shigella*, *Campylobacter*, and enterohemorrhagic *E. coli*. Routine parasitologic testing for *Giardia lamblia* and *Cryptosporidium parvum* by ova and parasite examination or stool immunoassays should be performed. Enteric viruses such as rotaviruses may be an important consideration, especially in children, and may be evaluated with stool antigen immunoassays. The possibility of inflammatory bowel disease, either ulcerative colitis or Crohn disease of the colon, always should be considered, especially if treatment fails to resolve the colitis. A hemorrhagic colitis related to penicillin has been described, but no pseudomembranes were seen on colonoscopy, and studies for *C. difficile* yielded negative results.[80]

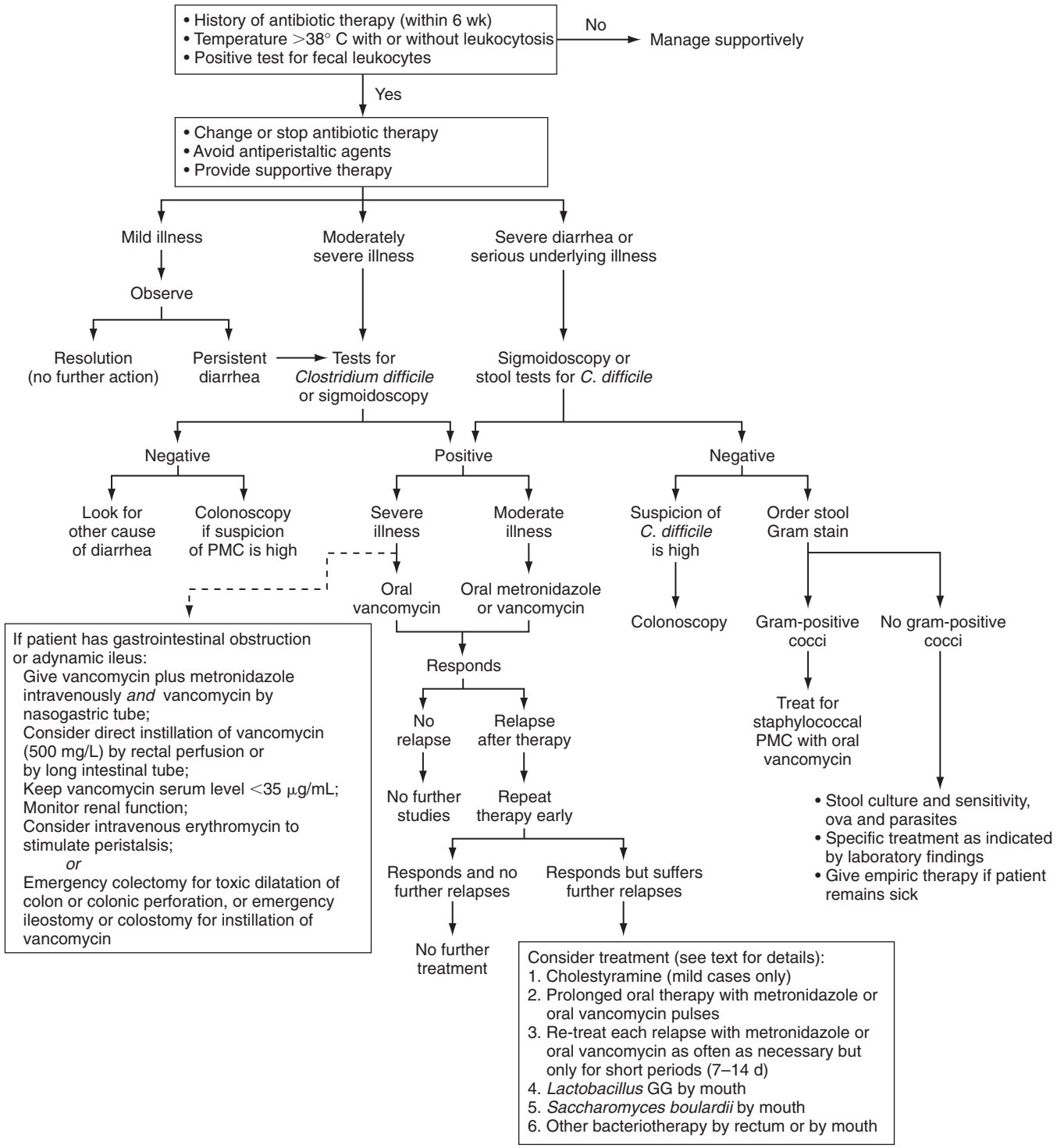

Figure 51–2 Algorithm for management of antibiotic-associated diarrhea and colitis. PMC, pseudomembranous colitis. *(From Fekety, R., and Shah, A. B.: Diagnosis and treatment of Clostridium difficile colitis. J. A. M. A. 269:72, 1993.)*

TREATMENT

In mild cases, discontinuation of antibiotics and implementation of supportive measures alone may lead to gradual resolution of symptoms.[19,108] In a study of 250 hospitalized children aged 5 to 12 years who developed diarrhea while receiving antibiotics, 45 (18%) children had stools positive for toxins A and B.[43] Although bloody diarrhea was a frequent occurrence in these patients, 68.9 percent of cases resolved with discontinuation of antibiotics. Antiperistaltic agents should be avoided because they may cause retention of toxins and complications.[23] Vancomycin has been the accepted treatment of choice for pseudomembranous colitis, but both vancomycin and metronidazole clear 80 to 100 percent of infections (Table 51–2), and metronidazole is considerably less expensive.[6,7,123] The effective oral dose of vancomycin is 40 to 50 mg/kg/day for 7 to 10 days. The adult dose is 125 mg four

TABLE 51–2 Comparison of Results of Treatment with Various Regimens for *Clostridium difficile*–Associated Diarrhea and Colitis

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From Bartlett, J. G.: Clostridium difficile: Clinical considerations. Rev. Infect. Dis. 12: S243-S251, 1990.

times daily for mild cases and up to 500 mg/dose for severe cases.[7,36] Intravenous vancomycin is not effective. Metronidazole is effective in doses of 20 to 40 mg/kg/day; adult doses range from 250 mg four times daily up to 500 mg three times a day. One report of intravenous metronidazole, 500 mg three times daily as initial therapy, showed an excellent clinical response in 10 patients.[39] Oral bacitracin, 25,000 units four times daily, has been used in some adult patients. Systemic signs of infection generally clear within 24 to 48 hours, and diarrhea gradually subsides in 7 to 10 days.

Resistance to metronidazole is being reported, but most of these patients will respond to vancomycin.[15,78,81] Fusidic acid and nitazoxanide both have been shown to be as effective as is metronidazole.[82,121] In a series of 14 adults with severe and recurrent *C. difficile*–associated diarrhea, 64 percent of cases resolved after receiving intravenous immunoglobulin (150-400 mg/kg).[75] Relapse is a major problem and occurs in 5 to 33 percent of patients treated with either vancomycin or metronidazole (see Table 51–2).[106,107] If a relapse produces severe symptoms, retreatment generally is required.[107] In mild relapse, patients may be watched to see whether they clear the infection spontaneously. Other therapeutic regimens to treat relapse have included weaning to the point at which vancomycin or metronidazole is administered every other or every third day.[110] In some patients, administration of cholestyramine has helped clear recurrent diarrhea.[35] One study in children showed an excellent response to immune globulin given intravenously.[64]

Probiotics also have been of interest, especially in patients who relapse. *Saccharomyces boulardii* given daily for 2 weeks produced resolution of symptoms within 1 week in 18 of 19 children with diarrhea caused by *C. difficile*.[21] Relapse occurred in 11 percent of the patients. *Lactobacillus rhamnosus* GG also has been effective in clearing *C. difficile* diarrhea.[14] A combination of oral vancomycin and *S. boulardii* also has been effective in curing relapsed infection.[104] The use of prebiotics has had variable results. Oligofructose has been reported to be beneficial in prevention of relapse,[65] but it is not useful in protecting adult patients while they are receiving antibiotics.[66]

Response to treatment is poor with ileus, toxic megacolon, or colonic perforation. In these cases, instillation of vancomycin by clamped nasogastric tube or direct colonic instillation can be tried.[101] The colonic vancomycin dose for adults is 2000 mg initially, followed by 100 mg every 6 hours.[88]

PREVENTION

To date, no effective means have been implemented to prevent the spread of and colonization with *C. difficile* or to prevent antibiotic-related pseudomembranous colitis. Private rooms and enteric precautions usually are used, but their efficacy is unknown. Bactericidal activity against *C. difficile* spores has been shown with both peracetyl ions and acidified nitrite, both environmentally safe materials.[122] In one study from Turkey of 151 hospitalized patients given antibiotics, prophylactic *S. boulardii* given twice daily reduced *C. difficile*–positive stools from 9 percent in the placebo group to 1.4 percent in the treatment group.[22] A very small number of patients with recurrent *C. difficile*–associated diarrhea have received a vaccine containing toxoid A and B with resolution of diarrhea.[102]

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CHAPTER 52

Whipple Disease

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Whipple disease is a rare, systemic bacterial infection that can be potentially fatal without therapy. In its most common form, Whipple disease affects white, middle-aged men, causing diarrhea, weight loss, abdominal pain, arthralgias, and fever. Although it is extraordinarily rare in children, its recognition may be crucial. Simple treatment with appropriate antibiotics may be curative and lifesaving.[28] Despite successful cultivation of the bacterium *Tropheryma whipplei* (formerly *Tropheryma whippelii*) in 2000,[56] little progress has been made in the development of improved diagnostic tests.

HISTORY

Whipple disease was described in 1907 by George Hoyt Whipple,[77] at that time an Instructor in Pathology at The Johns Hopkins University.[6] Whipple's description probably was not the first; Allchin and Webb apparently described a patient with "Whipple disease" in 1895.[48]

In Whipple's account, a 37-year-old medical missionary was admitted to the Johns Hopkins Hospital with low-grade fever, steatorrhea, and an abdominal mass. The patient had a 5-year history of sporadic migratory polyarthritis. These attacks of arthritis were associated with a gradual loss of weight and strength. His skin was pigmented with a brownish hue. Laboratory evaluation found severe anemia and an enormous number of fatty acid crystals in the stool. Explorative laparotomy revealed large, firm mesenteric lymph nodes, and a diagnosis of either Hodgkin disease or tuberculosis was made. The patient died 1 week later, and autopsy revealed marked fatty deposition within intestinal mucosa and the mesenteric and retroperitoneal lymph nodes. Other findings included polyserositis (peritonitis, pleuritis, and pericarditis) and endocarditis. Histologic examination revealed infiltration of the lamina propria of the small intestine by large,

foamy mononuclear cells that did not stain for fat. Fatty acids and triglycerides were found in dilated lymph channels. Silver stains of the mesenteric lymph nodes showed "great numbers of rod-shaped organisms" that resembled the tubercle bacillus. Whipple suggested that these bacillus-like organisms in the nodes could be the cause.

Whipple[77] reported "a hitherto undescribed disease characterized anatomically by deposits of fat and fatty acids in the intestinal and mesenteric lymphatic tissues." He concluded that the patient had "an obscure disease of fat metabolism," and proposed the term *intestinal lipodystrophy*.[18] Whipple recognized the most important features of this disease except for the involvement of the central nervous system (CNS). In 1949, Black-Schaffer showed that macrophages within the intestinal mucosa of patients with Whipple disease are stained intensely by the periodic acid–Schiff method,[35] proving that the macrophages contained glycoprotein or mucopolysaccharide, not fat, as Whipple had suggested.

In 1992, Relman and colleagues[59] identified a gram-positive bacillus in association with Whipple disease by use of polymerase chain reaction (PCR). They reported a unique 1321-base pair, 16S ribosomal RNA sequence amplified by PCR on intestinal and lymph node tissue from five unrelated patients with Whipple disease. They suggested that the responsible bacillus is a member of the actinomycetes. Relman and colleagues[59] concluded that the phylogenetic relationships of the Whipple disease bacillus, the features of the illness, and its distinct morphologic characteristics provided sufficient grounds to propose a new genus and species name, *Tropheryma whippelii* (from the Greek *trophe*, or "nourishment"; *eryma*, or "barrier," because of the malabsorption it causes; and *whippelii*, in honor of Whipple).

EPIDEMIOLOGY

Whipple disease characteristically occurs in white middle-aged men. Its true incidence and prevalence are unknown because fewer than 1000 cases have been reported worldwide. It is an extremely rare disease in children,[1,2,4,10,34,73] with fewer than 10 cases reported. The youngest patient was a newborn,[15] and the oldest was 83 years old.[42] The peak age at presentation is 40 to 49 years.[24] In a literature review of 114 patients,[42] 88 percent were men and 12 percent were women. Most of these patients were white. Most patients reported as having Whipple disease are from continental Europe or the United States.[13] In an extensive review of 741 cases, Dobbins[20] found that most academic centers in the United States had records of three or four unreported cases. He estimated that for every published report, at least two or three unpublished cases exist, and approximately 1500 to 2000 individuals probably have had Whipple disease.[18]

ETIOLOGY AND PATHOGENESIS

Whipple disease is caused by an organism known as Whipple bacillus or *T. whipplei*.[24,42,56,77] Despite Whipple's account of "great numbers of rod-shaped organisms,"[77] culture of the organism was unsuccessful until more recently. In 2000, Raoult and associates[56] reported that the bacterium *T. whipplei* had been cultured successfully from an aortic valve vegetation in a patient with prolonged endocarditis. The bacteria were isolated after inoculation in a human fibroblast cell line (HEL). Analysis by PCR confirmed that the 16S ribosomal RNA gene of the cultured bacterium was identical to the *T. whipplei* sequence. Subcultures of the bacterium also were obtained, and high-titer polyclonal antibodies against *T. whipplei* were produced. Such antibodies potentially may allow serologic diagnosis to become a reality.

T. whipplei may be a member of the actinomycetes,[59] which are gram-positive bacteria with DNA rich in guanine and cytosine.[78] The genus consists of actinomycetes, streptomycetes, and the nocardioforms.[14] *T. whipplei* seems to be related most closely to the four actinobacteria *Dermatophilus congolensis*, *Arthrobacter globiformis*, *Terrabacter tumescens*, and *Micrococcus luteus*.[59] Using so-called bootstrap analysis, some researchers have argued that the Whipple bacillus is only 67 percent associated with actinobacteria, far from the level needed for scientific conclusion.[67] The Whipple bacillus may represent another, separate, fourth line of descent with the actinomycetes. Amplification, cloning, and sequencing of a 620-base pair fragment of *T. whipplei* heat shock protein led to the conclusion that *T. whipplei* is a member of the actinobacteria.[49]

Scant support exists for a primary humoral immunodeficiency in Whipple disease,[27] but stronger evidence exists for a distinct defect in the cell-mediated immune function. Dobbins[19] reviewed data of 30 patients with HLA-A and HLA-B locus typing and 47 patients with HLA-B27 typing. He found an increased incidence of patients who were positive for HLA-B27 (28%), even with absence of concomitant sacroiliitis. Other reports have failed to confirm the increased association with the HLA-B27 antigen.[3] Marth and associates[44] studied 27 patients with Whipple disease. They found a significantly reduced number of cells expressing the complement receptor 3 L-chain (CD11B), a reduced proliferation to phytohemagglutinin and to sheep red blood cells, and a hypoergic skin reaction. These findings indicated a defect of cell-mediated immunity.

In patients with active disease, the number of $CD8^+$ cells is increased, which results in a reduced CD4/CD8 ratio. Such defects of cellular immunity seem to persist in patients for several years, despite complete remission of the disease. Schoeden and associates[65] were able to culture *T. whipplei* in mononuclear phagocytes deactivated with interleukin-4 (IL-4), IL-10, and dexamethasone. IL-4 was found to be the crucial deactivating signal that rendered monocytes permissive for intracellular multiplication of *T. whipplei*. IL-4 is an immunoregulatory cytokine. Schoeden and associates[65] suggested that host factors, such as an imbalance in the T-helper 1 and T-helper 2 immune response, may contribute to the pathogenesis of Whipple disease.

Desnues and colleagues[17] reported a specific gene expression pattern associated with replication of *T. whipplei* in macrophages. *T. whipplei* organisms are killed by monocytes. The addition of exogenous IL-16 enabled *T. whipplei* to replicate in monocytes and increased bacterial replication in macrophages. *T. whipplei* replication in macrophages was completely prevented after blocking IL-16 activity with the use of anti-IL-16 antibodies. Untreated patients with Whipple disease were noted to have significantly higher circulating IL-16 than that of control subjects and patients treated for Whipple disease. They concluded that response of monocytes and macrophages to IL-16 likely is crucial for replication of *T. whipplei* to occur, in patients with Whipple disease.

Oral acquisition of the Whipple bacillus seems most likely,[24] emphasizing greater involvement of the duodenum and proximal jejunum than the more distal small intestine. Only three reports of siblings with this disease exist; contagious spread of Whipple disease seems unlikely.[32] *T. whipplei* has been identified free in the small intestine next to the glycocalyx of the enterocyte's microvilli, in epithelial cells, and in the lamina propria.[24] Even in patients with extraintestinal Whipple disease, the organism usually is identified in the small bowel.[24] The bacillus seems to spread through the lymphatics and through the systemic circulation[24,36,50] and then can involve several extraintestinal organs.

T. whipplei can be seen faintly by light microscopy. The bacilli are seen best by transmission electron microscopy, which reveals a rod-shaped organism 0.2 μm wide and 1.5 to 2.5 μm long (Fig. 52–1). The ultrastructure of the wall of *T. whipplei* is similar to that of other gram-positive bacteria, with the exception of an

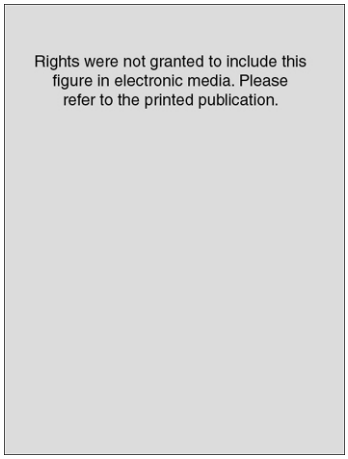

Figure 52–1 Electron micrograph of the invasion of enterocytes by Whipple bacilli. There are numerous bacilli within the lamina propria. (Magnification ×25,000.) *(From Tyor, M. P.: Whipple's disease: The Duke connection. N. C. Med. J. 55:237-240, 1994.)*

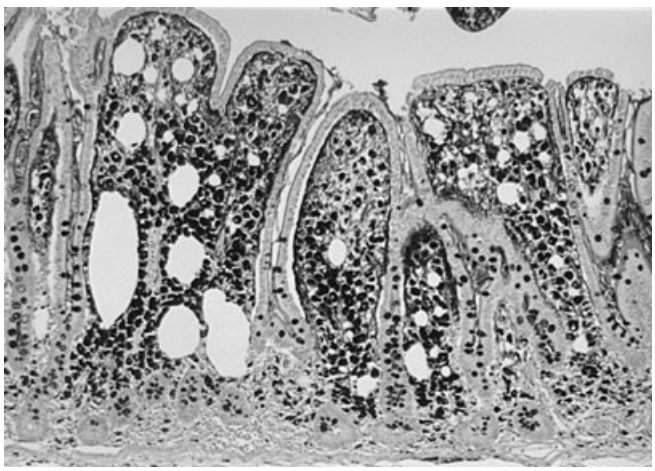

Figure 52–2 Light microscopic photograph of intestinal mucosa from the proximal jejunum of a patient with untreated Whipple disease. The villi appear blunted and swollen with periodic acid–Schiff-positive macrophages stuffed in the lamina propria. *(Courtesy of Kenneth P. Batts, M. D., Mayo Clinic, Rochester, MN; Jeffrey Craver, M. D., DePaul Health Center, Bridgeton, MO; and Milton J. Finegold, M. D., Baylor College of Medicine, Houston, TX.)*

additional surface membrane. This membrane is different from the outer membrane of gram-negative bacteria because it is thinner, has a symmetric profile, and has no periodic acid–Schiff-positive components.[67] After the bacillus has been ingested by the macrophage, the degenerative process that occurs leads to the accumulation of bacterial remnants that are resistant to degradation. The polysaccharide-containing portion of the bacillus wall correlates with these remnants, and its progressive accumulation leads to the typical intramacrophagic inclusions. These inclusions are periodic acid–Schiff-positive and one of the key features in the histologic diagnosis of Whipple disease.[67]

Biopsy specimens from the small intestine in patients with Whipple disease usually show characteristic changes. The intestinal villi are preserved,[24] but distortion of the architecture occurs.[27] A clubbed appearance of the intestinal villi[42] usually is present caused by the accumulation of foamy macrophages in the lamina propria.[24] The enterocytes may appear normal,[21,42] or they may show flattening and vacuolization and occasionally appear cuboidal (Fig. 52–2).[21,42] Lipid accumulation, with large fat droplets within the lamina propria and smaller droplets within and between the absorptive cells, occurs commonly.[42] In some instances, prominent, dilated lacteals are present.[24] Ectors and associates[21] reported reduced and absent lactase and major histocompatibility complex class II (HLA-DR) expression. Lactase and major histocompatibility complex class II expression normalized within 3 to 6 months of starting antibiotic therapy.

Figure 52–3 Electron micrograph illustrating numerous rod-shaped bacilliform bodies within the cytoplasm of a macrophage. (Magnification ×13,750.) *(From Tyor, M. P.: Whipple's disease: The Duke connection. N. C. Med. J. 55:237-240, 1994.)*

The characteristic feature of Whipple disease is the presence of periodic acid–Schiff-positive, diastase-resistant macrophages.[32] These findings are not pathognomonic, however, because intestinal periodic acid–Schiff-positive macrophages can be found in other conditions,[24] such as histiocytosis, melanosis coli, and *Mycobacterium avium-intracellulare* infection, and even within macrophages in healthy individuals. The macrophages in Whipple disease do not stain with Ziehl-Neelsen.[21,32] A sickle-shaped appearance of the periodic acid–Schiff-positive granules often is present in the macrophages of patients with Whipple disease.[42]

Sieracki and Fine[66] observed systemic involvement in the autopsies of five patients with Whipple disease. The sickle-shaped, periodic acid–Schiff-positive macrophages were thought to be specific for Whipple disease, showing involvement of the entire gastrointestinal tract and the pancreas; diffuse involvement of the retroperitoneum and lymph nodes, the adrenals, the liver with sickle-form particles in Kupffer cells and in histiocytes, the brain, the heart, and the visceral pleura of the lungs; and minimal involvement of the genitourinary tract, skeletal muscles, and bone marrow. James and associates[36] examined the vessels of the gastrointestinal system and found abundant bacilli in the arteries of the small intestinal serosa and liver. They noted focal degeneration and fibrosis in the tunica media with arteritis and intimal proliferation. Rickman and coworkers[60] reported a case that confirmed the presence of *T. whipplei* in the vitreous of the eye.

T. whipplei produces a predominantly histiocytic inflammatory reaction, with infiltration by macrophages.[23] Noncaseating, epithelioid cell, sarcoid-like granulomata are located preferentially in peripheral lymph nodes and the liver.[16,23] These granulomata occasionally can be seen in different tissues, including three reports of granulomata in the intestinal tract.[23] Mesenteric lymph nodes often are strikingly enlarged.[13]

Electron microscopy can be useful, often revealing the presence of the rod-shaped bacterium (see Fig. 52–1).[28] Electron microscopy also shows intestinal macrophages containing bacteria with signs of lysis.[67] Silva and associates[67] described steps of a degradative process of the bacillus that starts with disorganization of the surface membrane and the thick outer wall. With the loss of intracellular material, bacterial ghosts composed of the three inner layers of the envelope are present. The two electron-dense layers of the cytoplasmic membrane become disorganized and solubilized, leaving the inner dense layer of the cell wall as the final bacterial remnant (Fig. 52–3).

CLINICAL MANIFESTATIONS

Whipple disease is viewed best as a multisystem illness. It usually manifests with arthralgias and then progresses to involve the gastrointestinal tract.[5] Malabsorption is the key feature of clinical disease, but no specific signs or symptoms for Whipple disease are known. Table 52–1 lists the major symptoms and signs of Whipple disease. In children, failure to thrive, malnutrition, and chronic diarrhea appear most frequently. Abdominal distention, abdominal pain, and generalized lymphadenopathy may be found.[2] Response to antibiotic treatment may be dramatic, with rapid weight gain and resolution of symptoms.[4]

GASTROINTESTINAL TRACT

One of the most common symptoms is weight loss, which is found in 65 to 100 percent of patients.[13,22,24,28,42] Weight loss may be the only symptom.[10] Diarrhea is reported in 60 to 85 percent of patients.[13,24,28,42] The diarrhea usually is watery or fatty in nature.[42] Several mechanisms have been proposed to explain the malabsorption and steatorrhea in Whipple disease.[24] Direct infection and secondary enterocyte dysfunction may prevent the esterification of fatty acids to triglycerides and inhibit the uptake of carbohydrates and amino acids. Blockage of transport of triglyceride-rich chylomicrons into lacteals may result from the deposition of foamy macrophages in the lamina propria. Lymphatic obstruction may occur by involvement of the mesenteric lymph nodes. Malabsorption and diarrhea tend to resolve within a few days after initiation of antibiotic treatment, whereas the lacteal dilation and periodic acid–Schiff-positive macrophages can remain for months to years. Occult gastrointestinal bleeding frequently is found, but melena and gross gastrointestinal bleeding are rare. Hematemesis was reported more recently.[11] Endoscopy revealed diffuse hemorrhagic duodenitis with bleeding on contact.

Abdominal pain is experienced by 60 percent of patients.[13,24,28,42] The pain is nonspecific, is generally epigastric, and may be worse after meals.[42] Abdominal pain and anorexia may lead to reduced calorie intake and further weight loss.[24] Abdominal distention occurs commonly and may be secondary to intra-abdominal lymphadenopathy or to thickening of loops of diseased intestine.[24,42] Ascites occasionally is seen and may be chylous, secondary to lymphatic obstruction.[10,28]

TABLE 52–1 Major Symptoms and Signs in Whipple Disease

Symptoms	Cases (%)
Weight loss	65-100
Chronic diarrhea	60-85
Arthralgia	65-80
Abdominal pain	60
Fever	10-55
Central nervous system–related complaints	10-40
Signs	
Malnutrition	90-95
Hypotension	70
Lymphadenopathy	55
Hyperpigmentation	45-55
Abdominal tenderness	50
Edema	30
Abdominal mass	20
Hepatomegaly	1-14
Splenomegaly	5-10
Ascites	8

Data from references 10, 23, 24, 35, 38.

JOINTS

Arthralgia, the most frequent nongastrointestinal symptom in Whipple disease,[24,42] is present in 65 percent of adult cases.[42] Arthralgia may precede manifestation of gastrointestinal symptoms by many years or decades[24,39] and occurs less frequently in children. Generally, joint symptoms continue unchanged with the onset of gastrointestinal symptoms.[24] Acute migratory arthralgia or arthritis may last days or weeks[28,32,42] and may persist as the disease progresses. The involved joints, in decreasing order of frequency, are knees, ankles, hips, fingers, wrists, elbows, hands, and spine.[24] Examination may reveal joint pain, swelling, limited range of motion, and warmth.[63] Fever sometimes is present.[24] Spondylitis, with or without sacroiliitis, may develop.[4] Permanent joint destruction and deformity are uncommon occurrences, but can be severe.[24,63] Arthrocentesis may reveal an inflammatory arthritis, with cell counts of 6000 to 75,000, often with a polymorphonuclear leukocyte predominance.[24] Synovial biopsy may show periodic acid–Schiff-positive macrophages.[24]

CENTRAL NERVOUS SYSTEM

Whipple disease can be confined to the brain[61] but usually is accompanied by other manifestations.[28] CNS and neurologic manifestations, such as headache, diplopia, meningoencephalitis, depression, confusion, and personality changes, are uncommon manifestations[13,24,42] but may be significant.[13,24,27,42,51,53] Table 52–2 lists the spectrum of potential CNS involvement.

TABLE 52–2 Central Nervous System Symptoms and Signs of Whipple Disease

Ataxia
Confusion
Convulsions
Dementia
Depression
Diplopia
Dizziness
Facial pain
Headache
Hearing loss
Hemiparesis
Hyperphagia
Hyperreflexia (± Babinski sign)
Incoordination
Lethargy, coma
Loss of vibratory and position sense
Meningoencephalitis
Mental and personality changes
Motor weakness
Muscle jerks and twitches
Muscle rigidity
Numbness
Nystagmus
Ophthalmoplegia
Papilledema
Polydipsia
Ptosis
Pupillary abnormalities
Sensory loss
Sleep disorders
Slurred speech
Stiff neck
Tinnitus
Visual difficulties (diplopia, blurring)

EYE

Indirect involvement of the CNS and direct involvement of the eye can produce visual problems.[24] Ophthalmoplegia and diplopia can occur with involvement of cranial nerves III, IV, and VI.[24] Reduced visual acuity and papilledema can occur with compromise of the optic nerve.[24] Numerous ophthalmologic findings, including vasculitis, vitritis, optic atrophy, uveitis, chorioretinitis, vitreous opacities, glaucoma, keratitis, retinal hemorrhages, disk edema, and lacrimal duct obstruction, have been reported.[24,75,76] Rickman and associates[60] reported a case of ocular disease without marked CNS or gastrointestinal disease.

SKIN

Hyperpigmentation of the skin in sun-exposed areas occurs in roughly half of cases.[27,42] The mechanism of hyperpigmentation is uncertain but is not related to adrenal insufficiency.[24] Subcutaneous nodules may be found and can reveal periodic acid–Schiff-positive macrophages and bacilli on electron microscopy.[31]

HEART

The heart frequently is involved in Whipple disease. Endocarditis, myocarditis, pericarditis, pancarditis, and coronary arteritis have been found.[24,46,74] A blood culture–negative endocarditis may be caused by Whipple disease.[24] Chronic aortic regurgitation is the most common clinical finding of endocardial involvement.[24] Pericarditis with polyserositis (pleuritis, peritonitis) has been found.[26] The abnormalities on electrocardiogram are nonspecific but include first-degree atrioventricular block, left ventricular hypertrophy, sinus tachycardia, left bundle branch block, intraventricular conduction delay, old inferior wall infarct, and short PR interval.[27] Sossai and associates[69] reported a case of regression of a right bundle branch block after treatment with antibiotics, but the block spontaneously recurred 2 years later.

SKELETAL MUSCLE

Skeletal muscle may be involved, diagnosed by electromyography or muscle biopsy.[71] Proximal muscle weakness occurs most commonly. Muscle biopsy reveals a nonspecific myopathy.

LYMPH NODES AND SPLEEN

Whipple disease may involve any lymph node in the body.[24] Mesenteric lymph nodes frequently are involved, and splenomegaly is found in 5 to 10 percent of cases.[42]

PULMONARY

Cough, pleuritic chest pain, and dyspnea have been reported.[72] Pleural involvement may appear as a pleural rub. Pleural adhesions and granulomata have been reported at autopsy.[4,72] Chest radiography often reveals pleural thickening, parenchymal shadowing, and elevation of the diaphragm.[72] Lung function tests may show decreased lung volumes.[72]

KIDNEY

Renal involvement in Whipple disease is rare.[24] Granulomata have been found, however, as has focal glomerulonephritis.[38]

HEMATOLOGIC

Anemia occurs commonly and usually is of a hypochromic, microcytic variety.[24,27,28] Macrocytic anemia caused by malabsorption of folate also may be seen. Leukocytosis, thrombocytosis,[52] and bone marrow involvement may be found.[57] Low serum iron concentration and an elevated erythrocyte sedimentation rate frequently are seen.[27] A single case of extraintestinal lymphoma in association with Whipple disease found at autopsy has been reported.[30]

DIAGNOSIS

Despite the successful culture of the organism in 2000,[56] no specific diagnostic tests have been developed. Anemia and low albumin probably are most common, being found in approximately 90 percent of patients. Low serum iron and folate concentrations are seen in approximately 30 percent of cases. Hypokalemia, hypocalcemia, low cholesterol and carotene, prolonged prothrombin time, and increased transaminases are less common findings. Elevated fecal fat is found in more than 90 percent of cases. The D-xylose malabsorption test result frequently is abnormal.

Barium x-ray studies of the small bowel may show marked thickening of the mucosal folds and separation of bowel loops, suggesting a malabsorptive disease. These findings usually resolve completely with successful antibiotic therapy. For CNS Whipple disease, characteristic abnormalities seen on computed tomography scans of the brain include atrophy, focal gray matter lesions, hydrocephalus, and white matter alterations.[73]

A key feature in the histologic diagnosis of Whipple disease is the accumulation of periodic acid–Schiff-positive, diastase-resistant macrophages in the lamina propria of the small intestine.[4,27,43] These findings are not pathognomonic, and infection with *Mycobacterium avium-intracellulare* must be excluded.[43] *T. whipplei* does not stain acid-fast,[43] whereas mycobacteria are identified readily. Small bowel involvement by *M. avium* complex can have endoscopic, histologic, and radiographic findings similar to those of Whipple disease.[55] Periodic acid–Schiff-positive macrophages occasionally can be seen in the mucosa of the large bowel and rectum in unrelated diseases, such as histiocytosis, and in benign conditions, such as melanosis coli and pneumatosis intestinalis.[43] Electron microscopy can confirm the diagnosis by visualization of the characteristic bacillus.[28,67]

Endoscopy of the small bowel may be helpful because characteristic lesions may be seen, and biopsy specimens can be obtained. No clear data suggest where the intestinal specimens should be taken[43] because intestinal involvement usually is patchy. Random biopsy samples of the small bowel are suggested, beginning at the ligament of Treitz.[47] Endoscopic findings include yellow-white plaques and an erythematous, erosive, friable mucosa.[29]

PCR amplification may be used to detect and identify bacterial pathogens. To set up a PCR, the only prerequisite information is the nucleotide sequences flanking each end of the target.[58] PCR has become an important method for establishing the diagnosis of Whipple disease. It may be especially helpful if the histopathologic examination findings are normal or in patients with unusual extraintestinal involvement. Specific identification of *T. whipplei* can be achieved by amplification of the 1321-base pair bacterial, 16S ribosomal RNA gene isolated from infected tissue.[59] Muller and associates[50] applied this PCR technique to show *T. whipplei* in peripheral blood mononuclear cells and cells derived from pleural effusion in a patient with Whipple disease. PCR techniques have been used to detect *T. whipplei* in erythrocytes,[41] cerebrospinal fluid,[6] and resected heart valves with infective endocarditis.[10,12,50]

Sloan and colleagues[68] reported in 2005 another real-time PCR method using a 213-base pair target sequence of the heat shock protein (hsp65) gene of *T. whipplei*. The sensitivity, specificity, and positive and negative values of this LightCycler real-time PCR were compared with the conventional 16S rRNA gene sequence PCR assay and were 98 percent (sensitivity), 99 percent (specificity), 94 percent (positive), and 100 percent (negative). The completion time of the LightCycler assay was approximately 5 hours, which was significantly less than the 2 to 3 days required for the traditional PCR assay.[68]

The diagnostic utility of PCR in Whipple disease is clear, but it cannot be taken as the sole basis for diagnosis. Ehrbar and associates[23] studied the specificity of PCR for *T. whipplei*. They performed elective gastroscopy on patients without known Whipple disease. PCR analysis was positive in 4.8 percent of duodenal biopsy specimens and 11.4 percent of gastric juice samples. Compared with the gold standard of histology and clinical signs, the specificity of PCR for *T. whipplei* was 95.2 percent for duodenal biopsy specimens and 88.6 percent for gastric juice. These findings suggest that *T. whipplei* or a closely related bacterium may be present in some people without known Whipple disease. Street and colleagues[70] detected *T. whipplei* DNA by PCR in the saliva of 35 percent of 40 healthy adults tested, which suggests that this organism can be an oral commensal.

TREATMENT

Antibiotics are the mainstay of therapy for Whipple disease.[13] In vitro antibiotic susceptibility testing using real-time PCR has identified penicillin, doxycycline, macrolides, ketolides, rifampin, aminoglycosides, teicoplanin, chloramphenicol, and trimethoprim-sulfamethoxazole (TMP-SMX) with activity.[7,8,45] Current antibiotic treatment strategies are empiric, based only on the accumulated anecdotal experience, although controlled trials are under way. Generally, response to antibiotics is good and often dramatic. Symptoms such as diarrhea quickly resolve, and weight gain is rapid. Several antibiotics that have been tried alone or in combination include chloramphenicol, penicillin, streptomycin, ampicillin, TMP-SMX, erythromycin, and doxycycline.[13,24,25,28] Keinath and associates[37] analyzed the antibiotic response rate of 88 patients with documented Whipple disease. Thirty-one patients experienced relapse, with a mean time to relapse of 4.2 years after initial diagnosis. CNS relapse occurred in 13 of 88, and all CNS and cardiac relapses were late (Table 52–3). In addition to diagnosis, PCR may prove useful in the monitoring of response to antibiotic therapy.[33,54]

All patients with Whipple disease should receive antibiotics that penetrate the blood-brain barrier.[37,62] Tetracycline and penicillin do not penetrate the blood-brain barrier well, unless the meninges are inflamed.[62] The preferred treatment in adults and children is TMP-SMX given orally twice a day for 1 year.[62] If the small bowel is involved, repeated small bowel biopsy is suggested at least 6 months to 1 year after treatment to document the disappearance of the bacillus. For patients who do not tolerate TMP-SMX, penicillin or ampicillin is recommended. Levy and associates[40] described a case of acquired resistance to TMP-SMX that responded to oral penicillin. Feurle and Marth[25] noted that TMP-SMX was more efficacious than tetracycline in inducing clinical remission of Whipple disease. They observed the development of aqueductal stenosis with hydrocephalus in a patient receiving TMP-SMX treatment, however, and indicated that even TMP-SMX is no safeguard against cerebral recurrence.

CNS relapse has a poor prognosis.[9,24,25,28,37] Feurle and Marth[25] suggested an experimental therapy for CNS recurrence with a highly active bactericidal compound, such as a third-generation cephalosporin, a quinolone, or intrathecal antibiotic therapy, that readily crosses the blood-brain barrier. In adult patients, Keinath and associates[37] recommended treatment with parenteral penicillin (1.2 million U daily) plus streptomycin (1 g daily) for 10 to 14 days, followed by TMP-SMX (1 double-strength tablet twice daily) for 1 year. Because of the extreme rarity of the occurrence of Whipple disease in children, prudent management dictates long-term surveillance after resolution of symptoms.

Because Whipple disease is a malabsorptive disorder, the patient's nutritional needs must be assessed carefully. Specific attention must be paid to replacement of any vitamin or mineral deficiencies. Iron, folate, vitamin D, and calcium typically are given until the steatorrhea resolves.

TABLE 52–3 Treatment of Whipple Disease: Initial Antibiotic Regimen and Relapse

Antibiotics	No. Patients	Total No. Relapses	CNS Relapses
TCN* alone	49	21	9†
PCN + STM + TCN*	15	2	0
PCN/PCN*	8	3	2
PCN + STM	5	2	0
TMP-SMX*	3	0	0
Other	8	3	2
Totals	*88*	*31*	*13*

**Oral therapy.*

†Includes two patients treated with TCN only in whom CNS relapse was the second relapse.

CNS, central nervous system; PCN, penicillin; STM, streptomycin; TCN, tetracycline; TMP-SMX, trimethoprim-sulfamethoxazole.

Data from Keinath, R. D., Merrell, D. E., Vlietstra, R., et al.: Antibiotic treatment and relapse in Whipple's disease: Long-term follow-up of 88 patients. Gastroenterology 88:*1867-1873, 1985.*

CONCLUSION

Whipple disease, despite its rarity, warrants diagnostic consideration in any child with failure to thrive, malnutrition, and chronic diarrhea. Such findings, especially with CNS manifestations or arthralgias, should raise the specter of Whipple disease. Diagnosis of Whipple disease can be made by histology, PCR, and clinical signs. PCR should not be the sole basis of diagnosis because *T. whipplei* DNA has been detected in samples from healthy individuals without Whipple disease.[64,70] Simple antibiotic treatment with oral TMP-SMX can result in dramatic resolution of symptoms, whereas failure to consider this rare disease can lead to catastrophic events.

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SECTION VII

LIVER DISEASES

CHAPTER 53

HEPATITIS

Gail J. Demmler-Harrison

Hepatitis, or inflammation of the liver, may be caused by a variety of infectious agents. Some agents, such as hepatitis A, B, C, D, and E viruses, are primarily hepatotropic and produce disease almost exclusively in the liver, whereas other agents, such as cytomegalovirus (CMV), Epstein-Barr virus (EBV), adenovirus, and the hemorrhagic fever viruses, produce hepatitis as part of a systemic or disseminated illness. With many infectious agents, hepatitis may be subclinical or silent, with very few, if any, signs or symptoms manifested in the patient. Symptomatic or clinical hepatitis may be acute or chronic at initial evaluation, and it may be associated with mild to severe or even fulminant or fatal disease.[64] Children of all ages, from neonates to young adults, both immunocompetent and immunocompromised, may contract infectious hepatitis, and the age and immune status of the host, as well as the history of exposure, may provide clues to identification of the most likely etiologic agent. Noninfectious causes such as autoimmune, genetic, and metabolic disorders, as well as exposure to toxins and idiosyncratic or hypersensitivity reactions to medications or drugs, also are important causes of hepatitis in children. Hepatic steatosis associated with childhood obesity and type 2 diabetes is a growing public health concern for our youth. Severe hypoxia also may damage the liver.

A general approach to an infant or child with hepatitis or hepatic dysfunction, from the perspective of an infectious disease specialist, is presented in this chapter. Detailed information regarding the diagnosis and management of each particular pathogen can be found in the respective chapters in the section of this textbook dedicated to infections with specific microorganisms.

HISTORY

Originally described by Hippocrates in the second century BCE, hepatitis has an ancient historical perspective. The earliest descriptions of outbreaks of hepatitis in the ancient world most likely involved hepatitis A virus (HAV), and it was known as *epidemic jaundice* and *catarrhal jaundice*, or *acute yellow atrophy of the liver* if the disease was severe or fulminant.[21] The earliest recorded outbreak in the United States occurred in Norfolk, Virginia, in 1812.[10] During wartimes, outbreaks of "camp jaundice" or "field jaundice" occurred and probably were caused by HAV, yellow fever, or leptospirosis. Not until 1973 was the cause of "infectious hepatitis" determined to be a 27-nm, nonenveloped viral particle, originally designated enterovirus 72 and now known as HAV.[39] Infection with hepatitis B virus (HBV) has a relatively more recent history. It probably was described originally in the late 1880s in an epidemiologic study published by Luerman in 1885 in Germany, where an outbreak of hepatitis in shipyard and warehouse workers who received smallpox vaccine contaminated with human material was associated with a prolonged form of hepatitis.[74] Other outbreaks of "serum hepatitis" associated with percutaneous therapies, such as gold used for rheumatoid arthritis or contaminated vaccines that were stabilized with human serum have been described.[1,13,58] In the early 1970s, the complete HBV infectious particle identified in serum was called the *Dane particle*, and a specific serologic marker, originally called the *Australian* (*Au*) *antigen* because it was discovered first in the blood of an Australian aborigine and later designated hepatitis B surface antigen (HBsAg), also was characterized and subsequently used to diagnose serum hepatitis caused by HBV.[15,73,109] Not long thereafter, in the mid-1970s, parenterally transmitted "non-A, non-B hepatitis" was described and subsequently identified in 1989 as hepatitis C virus (HCV).[19,59,110,128] The 1970s also marked the discovery of hepatitis delta virus (HDV), a "defective helper virus" that replicates only in the presence of HBV and is associated with chronic or fulminant hepatitis.[114,115] In the 1990s, another enterically transmitted non-A, non-B hepatitis virus was found to be associated with outbreaks of infectious hepatitis in developing parts of the world.[11,52] It was characterized originally as a calicivirus-like particle and subsequently named hepatitis E virus (HEV).[113] No doubt, as our medical knowledge evolves, even more viruses and other infectious agents associated with hepatitis will be discovered and named.

CLINICAL MANIFESTATIONS AND EVALUATION

PATIENT HISTORY

The clinical approach to evaluating and managing a child with hepatitis includes careful attention to the age when first seen; evolution of the initial signs and symptoms; history of exposure to potential pathogens, toxins, or medications; the presence of underlying conditions; and a family history of liver or metabolic disorders. Initial symptoms in older children with acute hepatitis are nonspecific and may include fever, vomiting, poor feeding, anorexia or aversion to specific foods, indigestion, change in taste and smell, lethargy and malaise, mild weight loss, dark urine, pale stool, or jaundice, often with pruritus. Abdominal pain also is a common complaint, and it usually is mild, dull, or aching in quality; located in the right upper quadrant; and unaffected by meals, body position, or bowel movements. Often, an antecedent viral syndrome or "flulike" illness is noted 7 to 14 days before the onset of hepatitis. Rarely, a "serum sickness–like syndrome" may occur at the onset of the illness, before jaundice occurs, and is characterized by fever, rash, and arthritis. In contrast, neonates rarely have signs and symptoms from infection with the hepatotropic viruses and are more likely to have clinical disease with agents that cause congenital or perinatal infection, such as CMV, herpes simplex virus (HSV), rubella virus, parvovirus B19, or *Treponema pallidum*, especially if extrahepatic signs such as splenomegaly, skin lesions, microcephaly, or hearing loss are present. Newborns and young infants may become seriously ill with disseminated HSV, enteroviruses, or adenoviruses and show signs of acute, massive hepatocellular necrosis. A history of administration of blood products at any age suggests hepatitis B, C, or D

or CMV, whereas previous attendance at summer camp or an institutional environment suggests HAV. A history of administration of antimicrobials such as the antifungal azoles, antibiotics, or isoniazid or medications such as acetaminophen suggests drug-induced hepatitis.[133] Exposure of older children to feral kittens is suggestive of infection with *Bartonella henselae* or *Toxoplasma gondii*. Children receiving cancer chemotherapy may have drug-induced hepatitis, and those who have experienced prolonged neutropenia may have fungal disease of the liver. Children with fulminant hepatitis are critically ill and initially may have persistent fever, protracted nausea and vomiting, severe abdominal pain, worsening jaundice, fluid retention with ascites, impaired clotting, and encephalopathy with seizures or coma. Patients with chronic hepatitis, on the other hand, often are clinically asymptomatic unless complications such as cirrhosis, chronic liver failure, or primary hepatocellular carcinoma develop.

PHYSICAL FINDINGS

Physical examination of a child with hepatitis should focus on the abdomen and liver, but it also should include a careful evaluation for extrahepatic manifestations of systemic disease. Tender hepatomegaly, with or without ascites, scleral icterus, and jaundiced skin, often is noted on physical examination of patients with acute hepatitis. Percussion or tapping over the right lower part of the thorax may produce right upper quadrant pain. Fever may or may not be present. Extrahepatic physical findings associated with the hepatotropic viruses, especially HAV, HBV, and HCV, include systemic vasculitis with rash or urticaria, polyarthralgia, and polyarthritis, similar to serum sickness or polyarteritis nodosa. A generalized papular rash, called *infantile papular acrodermatitis* (also known as *Gianotti disease* or *Gianotti-Crosti syndrome*), may accompany HBV infection, especially in young children. Rarely, a patient with acute viral hepatitis may exhibit the Raynaud phenomenon, bullous lesions, or erythema nodosa. Skin excoriations may be present if the jaundice-associated pruritus is severe, and older children and adolescents may have vascular spiders or exacerbation of acne. A child who also has conjunctivitis, pneumonitis, and a maculopapular rash may have a disseminated adenoviral disease. Generalized lymphadenopathy accompanied by pharyngitis and splenomegaly, in contrast, suggests systemic infection with CMV or EBV. Idiosyncratic reactions involving the liver may occur at any time; however, drug-related hypersensitivity hepatitis occurs 1 to 5 weeks after exposure to the agent and usually is accompanied by rash and fever on examination and evidence of nephritis, eosinophilia, and neutropenia. Neonates with acute fulminant hepatitis from disseminated infection with HSV, enteroviruses, or adenoviruses will have fever or hypothermia, respiratory distress, coagulopathy, and a sepsis-like syndrome. Physical examination of a child with fulminant hepatitis and liver failure may actually reveal a small liver or one that is shrinking in size and a child who is confused or has had personality changes. As hepatic failure ensues, the patient may be deeply jaundiced and encephalopathic or comatose, with hyperreflexia, decerebrate posturing, involuntary movements, and asterixis. A distinctive sweetish smell (also called *fetor hepaticus*) from the patient also may be appreciated by an astute observer. Physical examination of a child with chronic hepatitis, on the other hand, may be normal or reveal minimal enlargement of the liver. If the child is obese, fatty infiltration of the liver should be considered as a cause of liver inflammation and dysfunction.

LABORATORY DIAGNOSIS

Laboratory evaluation of a child with acute hepatitis should include a complete blood count and differential, urinalysis, tests of hepatic function, and specific serologic tests, cultures, or detection assays for specific pathogens of interest according to the patient's history or physical findings. In patients with severe or fulminant hepatitis or hepatic failure, serum albumin, electrolyte, and glucose levels also should be determined, in addition to performing a coagulopathy panel with fibrinogen, as well as determination of blood ammonia if encephalopathy is present. In many forms of viral hepatitis, the white blood cell count may be low, usually between 3000 and 4000/mm^3. Atypical lymphocytes also may be seen. Eosinophilia suggests a drug hypersensitivity reaction. If significant leukocytosis is present, sepsis or fulminant hepatitis should be considered. Urinalysis may reveal dark urine and the presence of urobilinogen. In a neonate with jaundice or hepatic dysfunction, an infectious agent such as a gram-negative enteric organism (*Escherichia coli*, for example) may be identified in urine or blood, or evidence of viral infection may be detected in blood, cerebrospinal fluid (CSF), or body secretions. Hematuria with casts or other signs of nephritis in an older child may signify an autoimmune disorder or a drug-induced process as the cause of the hepatitis. Liver enzymes, especially aspartate aminotransferase and alanine aminotransferase, will be elevated in all patients with acute hepatitis, and frequently they are elevated before the onset of clinical symptoms occurs. Most forms of acute hepatitis will be accompanied by elevations of 500 IU/mL or greater. A neonate, however, with transaminase levels higher than 1000 IU/mL is likely to have a potentially life-threatening infection with HSV, enteroviruses, or adenoviruses. Similarly, older children with fulminant hepatitis or hepatic necrosis may have significantly elevated transaminase levels, which gradually fall to less than 500 IU/mL as the disease progresses. Prothrombin levels usually are normal in cases of uncomplicated acute viral hepatitis, but if they become prolonged, severe liver necrosis or fulminant hepatitis should be considered. Levels of alkaline phosphatase and gamma-glutamyltranspeptidase will be elevated in patients with acute hepatitis as well. Serum bilirubin, both conjugated and unconjugated, may be elevated, but it is rarely higher than 4 mg/dL unless fulminant hepatitis with hepatic failure is present. Exceptions to this rule include patients with underlying hemolytic states such as glucose-6-dehydrogenase deficiency or sickle-cell disease; such patients may exhibit marked jaundice and high indirect hyperbilirubinemia, even if the viral hepatitis otherwise is mild. Serum albumin often is normal in acute hepatitis, but it may be low in chronic hepatitis. Some patients will have low levels of nonspecific autoantibodies, such as an elevated homogeneous pattern of antinuclear antibodies, decreased complement levels, or false-positive VDRL (Venereal Disease Research Laboratory) test reactions. The erythrocyte sedimentation rate usually is normal or slightly increased in acute viral hepatitis.

Laboratory investigation to determine the specific etiology of the patient's hepatitis includes detection of HBsAg and anti–hepatitis B core antigen (HBcAg; IgM) in serum, anti-HAV (IgM) in serum and HAV RNA in saliva and serum by polymerase chain reaction (PCR), anti-HCV and HCV in serum by PCR, and anti-HDV antibody (especially if the hepatitis is fulminant) and anti-HEV antibody (if the travel history suggests exposure).[3] Many of the non-hepatitis viruses may be identified by serologic tests that detect virus-specific IgM antibody or by fourfold rises in viral-specific IgG antibody. However, isolation of the specific viral agent in cell culture or detection of viral nucleic acid by PCR in blood, CSF, body fluids such as saliva or stool, or liver tissue provides the most convincing evidence. Bacterial pathogens may be detected by culture or, for agents associated with granulomatous hepatitis, such as *Brucella* and *Mycobacterium*, by culture, serology, or appropriate skin tests. Non-infectious causes such as autoimmune hepatitis may be identified by persistent hypergammaglobulinemia and the presence of autoantibodies, such as positive lupus erythematosus cell tests and anti–liver-

kidney-microsome antibodies (anti-LKM). Metabolic diseases may cause hepatic dysfunction that may mimic acute hepatitis. Laboratory tests that may help differentiate these diseases include sweat chloride or genetic screening for cystic fibrosis, α_1-antitrypsin levels, serum amino acids, and urine-reducing substances and organic acids for metabolic diseases. Serum ceruloplasmin and urine copper levels may help establish the laboratory diagnosis of Wilson disease. Acute hepatotoxicity from overdose of acetaminophen may be predicted from elevated acetaminophen levels. Anatomic causes of hepatic dysfunction, such as biliary atresia in infants and hepatic tumors or steatosis in older children, usually require diagnostic imaging such as ultrasound or liver biopsy for diagnosis.

INFECTIOUS CAUSES

VIRUSES

Hepatitis Viruses

Five hepatotropic viruses are known to cause infectious viral hepatitis in children: hepatitis A, B, C, D, and E. (Table 53–1).

HEPATITIS A VIRUS. HAV is a member of the *Picornaviridae* family and formerly was known as enterovirus 72.[78] A small, nonenveloped RNA virus with icosahedral symmetry (Fig. 53–1), HAV is transmitted by the fecal-oral route, and transplacental transmission has been documented on rare occasion.[27,44] It is transmitted most commonly among young and school-aged children receiving care in group settings such as daycare, summer camp, schools, and institutions.[60] The incubation period generally is 30 days but ranges from 15 to 50 days. HAV infection in young children often is asymptomatic, and usually outbreaks in children are recognized first when symptoms occur in adult caretakers. Older children are more likely to have the classic symptoms of nausea, malaise, jaundice, and tender hepatomegaly. Rarely, HAV causes fulminant hepatitis. Acute infection with HAV is diagnosed serologically by detecting the presence of HAV IgM antibody in serum. No licensed, specific antiviral treatment is available. However, prevention may be accomplished by passive immunization with immune globulin or active immunization with licensed inactivated HAV vaccines.

HEPATITIS B VIRUS. HBV, also known as *Dane particle* and *hepadnavirus type 1*, is a member of the *Hepadnaviridae* family and is a DNA virus that is a 42-nm, nonenveloped spherical particle (Fig. 53–2).[78] It is transmitted through close contact with blood or blood-contaminated secretions, objects, or products.[44] It also has been transmitted by organ transplantation, intravenous drug use, and sexual contact. The incubation period is prolonged, usually 90 days, with ranges of 45 to 160 days reported. Neonates may acquire HBV vertically from mothers who are actively infected with HBV, especially if the mothers also are positive for hepatitis B e antigen (HBeAg). Whereas newborns rarely are symptomatic, children and adults may have mild to moderate symptoms of acute hepatitis or progress to fulminant or fatal disease. HBV also is a common cause of chronic hepatitis in children. Chronic hepatitis will develop in approximately 10 percent of older children and adults infected with HBV; the figure rises to approximately 30 percent if the infection occurs during infancy or early childhood and reaches a striking 90 to 95 percent in infants born to infected mothers who are HBeAg-positive. Acute hepatitis B is diagnosed serologically by the presence of HBsAg or IgM antibody to HBcAg. HBV DNA also may be detected and quantified in serum by PCR during acute hepatitis. Individuals with HBeAg in their serum are highly infectious. Diagnosis of chronic hepatitis B requires a combination of

TABLE 53–1 Causes of Hepatitis in Children

Infectious

Viral

Primary hepatotropic
- Hepatitis A virus
- Hepatitis B virus
- Hepatitis C virus
- Hepatitis D virus
- Hepatitis E virus

DNA viruses
- Adenovirus
- Cytomegalovirus
- Epstein-Barr virus
- Erythrovirus (human parvovirus B-19)
- Herpes B virus
- Herpes simplex viruses 1 and 2
- Human herpesviruses 6, 7, and 8
- Varicella-zoster virus

RNA viruses
- Enteroviruses
- Hemorrhagic fever viruses
- Human immunodeficiency virus
- Measles virus
- Rubella virus
- Syncytial giant-cell hepatitis

Bacterial

Atypical mycobacteria
Bacille Calmette-Guérin (BCG)
Bacillus cereus toxin
Bartonella henselae and *Bartonella quintana*
Brucella species
Listeria monocytogenes
Mycobacterium tuberculosis
Sepsis syndrome with cholestatic jaundice
Urinary tract infection in neonates
Spirochetes
- Leptospira species
- *Treponema pallidum*

Rickettsiae
- *Coxiella burnetii*

Parasites
- *Ascaris lumbricoides*
- *Entamoeba histolytica*
- *Plasmodium* species
- *Toxoplasma gondii*

Fungal

Aspergillus species
Candida species
Cryptococcus neoformans
Histoplasma capsulatum

Non-infectious

Anoxic liver damage
Autoimmune hepatitis
Biliary atresia
Drugs and toxins
Hemophagocytic syndrome
Histiocytosis
Kawasaki disease
Lymphoma
Metabolic and genetic disorders
Obesity with hepatic steatosis (fatty infiltration)
Reye syndrome
Sarcoidosis
Sickle-cell crisis
Toxic shock syndrome
Tumors

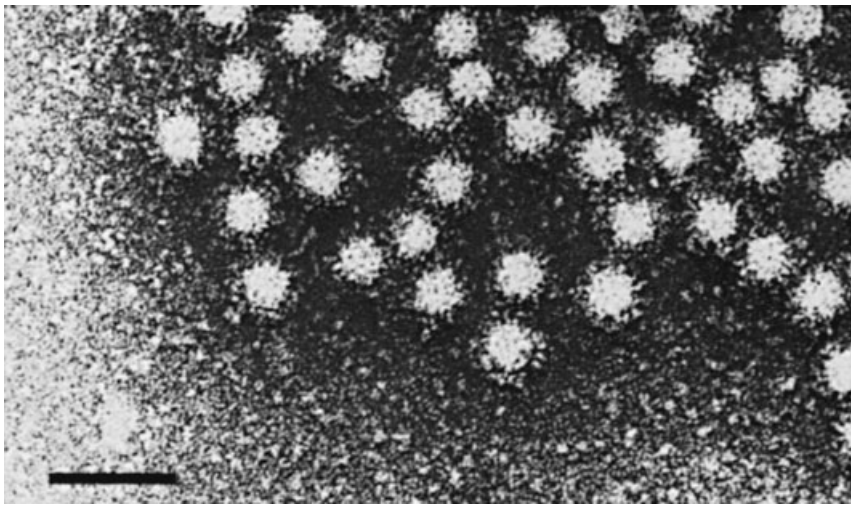

Figure 53–1 Twenty-seven-nanometer hepatitis A virus (HAV) isolated from the stool filtrate of a patient with acute HAV infection. The HAV particles are aggregated by convalescent serum containing anti-HAV antibodies. The *line* represents 100 nm. *(Courtesy of Dr. Jules Dienstag, taken at the Laboratory of Infectious Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Department of Health, Education, and Welfare, Bethesda, MD.)*

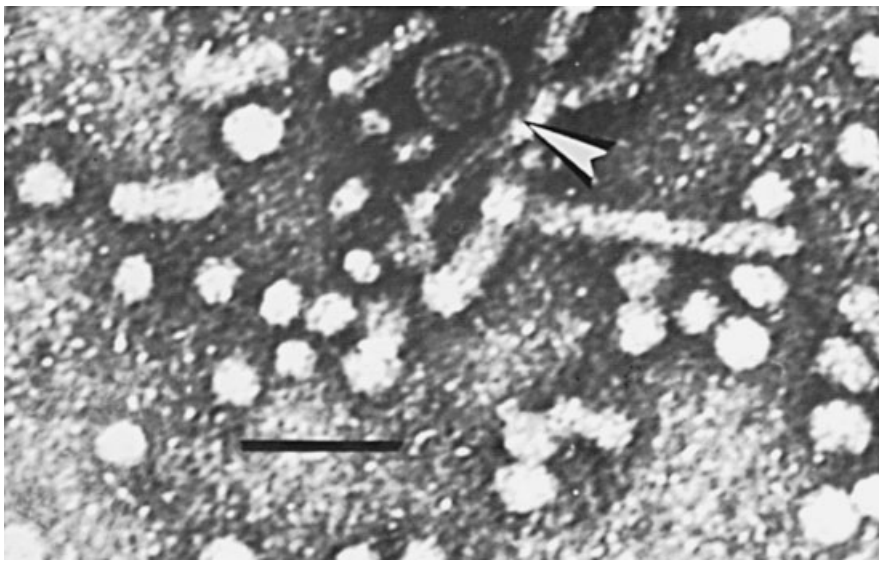

Figure 53–2 Electron micrograph of hepatitis B virus particles. Most particles are 20 to 25 nm in diameter and consist of both spheres and tubules. A larger, 42-nm Dane particle also is present (*arrowhead*). Hepatitis B surface antigen determinants are present on the surface of all three forms.

persistent clinical symptoms and laboratory abnormalities, persistence of specific serologic markers such as HBsAg over a period of at least 6 months, and histopathologic characteristics on liver biopsy. Primary hepatocellular carcinoma also is a long-term complication of infection with HBV. Successful resolution of infection with HBV is marked serologically by the presence of antibody to HBsAg. No licensed specific antiviral therapy is available for acute hepatitis caused by HBV; however, lamivudine and interferon-α may be helpful in treating chronic hepatitis. Famciclovir also has documented activity against HBV and has been used clinically in certain patients. Liver transplantation has been attempted in patients with end-stage HBV-associated cirrhosis, with variable results. Prevention is achieved by passive immunization with hepatitis B immune globulin (HBIG) and active immunization with licensed recombinant vaccines.[57]

HEPATITIS C VIRUS. HCV is a member of the *Flaviviridae* family. It is a small RNA virus with multiple genotypes that appear to have a variable effect on the development of clinical disease and response to antiviral therapy. It is transmitted most commonly to children and adolescents who have been exposed to blood products, clotting factor concentrates before 1987, hemodialysis, organ transplantation, intravenous drug use, cocaine snorting, or tattoos and piercing procedures performed at parlors that do not practice sterile technique. Perinatal transmission from an HCV-seropositive mother to her infant also occurs at an estimated risk of 5 percent, especially if the mother is HCV RNA–positive at delivery.[92,97] Infection with HCV usually does not cause acute hepatitis; however, chronic hepatitis develops in more than 50 percent of children infected with HCV.[24] In adults, cirrhosis, end-stage liver failure, and hepatocellular carcinoma also may develop. Infection with HCV is diagnosed serologically by detecting the presence of HCV IgG antibody and by detecting and quantifying HCV RNA by PCR.[69] Treatment with ribavirin plus interferon may be beneficial in some patients. Liver transplantation may be performed in patients with end-stage liver disease. Currently, no biologic products or vaccines are licensed for prevention of infection with HCV.

HEPATITIS D VIRUS. HDV, also known as the *delta agent*, *delta virus*, *helper virus*, and *defective virus*, is a small, 37-nm RNA virus that requires the presence of HBV, especially HBsAg, to replicate.[107] HDV can co-infect a patient simultaneously or subsequent to infection with HBV. Like HBV, HDV is acquired by exposure to blood products or clotting factors, intravenous drug use, or sexual contact. It also may be transmitted by liver

transplantation, and vertical transmission has been reported. Infection with HDV occurs more commonly in Europe, South America, Africa, and the Middle East and appears to be less common in the United States. The incubation period after superinfection occurs is 1 to 2 months, but it is similar to that for HBV (90 days) if co-infection occurs simultaneously. Co-infection with HDV is associated with more severe disease or progression to fulminant hepatitis in HBV-infected patients.[107] Laboratory diagnosis includes detection of antibody to HDV (anti-HDV) by commercial assays and reference laboratories that can detect IgM-specific HDV antibody, HDV antigen, and RNA by PCR. No specific antiviral therapy is available for HDV. Because HDV cannot be transmitted to or infect humans without HBV, prevention of HBV infection by vaccination prevents the acquisition of HDV infection. HDV co-infection of individuals already infected with HBV, however, cannot be prevented by any licensed biologic product or vaccine.

HEPATITIS E VIRUS. HEV is a small RNA virus that is transmitted by the fecal-oral route.[11,113] HEV infection in U.S. residents is a rare occurrence, but it may be a common cause of acute, self-limited viral hepatitis in developing countries and has been linked to outbreaks associated with contaminated water supplies.[52,101,108] The incubation period is unknown. HEV appears to cause a mild to moderate, acute hepatitis, especially in adults, and may have a high case-fatality in pregnant women. Similar to HAV, HEV does not appear to cause chronic hepatitis. Laboratory diagnosis is established by detection of IgG and IgM antibody to HEV in serum or detection of HEV RNA by PCR in serum or feces performed in reference laboratories or the Centers for Disease Control and Prevention (CDC). No antiviral agent is licensed for HEV, and prevention by immunoprophylaxis is not available. However, the results of clinical trials evaluating the efficacy of a new HEV vaccine may be available soon.

Herpesviruses

Herpesviruses are large DNA viruses with icosahedral symmetry and a glycoprotein envelope, and they share the biologic properties of latency and reactivation. Infections with this family of viruses, the *Herpesviridae*, may be primary or recurrent. All the human herpesviruses (HHVs) and one of the primate herpesviruses (herpes B virus) can cause acute hepatitis during the course of a systemic illness, but primary hepatitis with these viral agents is an unusual event.[42]

HERPES SIMPLEX VIRUS. HSV-1 and HSV-2 most often cause mucocutaneous vesicles or ulcers, and dissemination usually occurs during periods of relative immune compromise, such as pregnancy, the neonatal period, malnutrition, congenital or acquired immunodeficiency syndromes, or organ transplantation. Transient, subclinical hepatitis may occur during acute mucocutaneous HSV disease, but fulminant hepatitis with hepatic necrosis rarely has been documented in a normal host.[38,45,65,83] A special exception to this observation is HSV-associated primary hepatic necrosis and disseminated HSV disease in pregnant women.[45] Most cases occur during the late second or early third trimester, and most, but not all, cases are associated with primary infection with HSV-2.[132,134] Obvious skin lesions may not be present. This disease is associated with high mortality rates in both the mother and infant. Neonates, both term and preterm, are at risk for the development of neonatal HSV hepatitis as part of a disseminated HSV disease that includes viral sepsis–like syndrome, coagulopathy, pneumonitis with respiratory distress, and meningoencephalitis.[62] Skin lesions often are absent in this form of HSV disease. Neonatal HSV hepatitis develops most often in the child's first 2 weeks of life, and transaminase levels may be more than a thousand times higher than normal. Recipients of solid organ, bone marrow, and stem cell transplants may have HSV infection with dissemination within the first 3 weeks after undergoing transplantation, most often as a result of reactivation.[43] Patients with hepatitis secondary to HSV infection may be shedding HSV from a mucocutaneous source or may be viremic, but some may require liver biopsy for confirmation. Acyclovir therapy is recommended for HSV-associated hepatitis, and post-transplant prophylaxis is very effective in preventing post-transplant HSV disease.[132] At least one neonate with HSV-induced fulminant hepatic failure successfully treated by liver transplantation combined with acyclovir therapy has been reported.[31]

VARICELLA-ZOSTER VIRUS. Varicella-zoster virus also causes mucocutaneous vesicles in both immunocompetent and immunocompromised hosts. Primary infection is known as *varicella* (also chickenpox) and is associated with primary and secondary viremias that often seed the visceral organs. Approximately a fourth of healthy children experiencing varicella will have silent hepatitis with transaminase levels at least twice normal.[41,91,105] Fulminant hepatitis with varicella occurs rarely, however, and generally is seen in immunocompromised hosts.[5,89,102,124] Patients have severe abdominal pain with little nausea or vomiting, skin lesions may or may not be present, and transaminases levels may be more than a thousand times higher than normal.[88,119,121] Zoster in a normal host is not associated with fulminant hepatitis, but immunocompromised hosts may experience disseminated zoster with hepatitis and hepatic necrosis.[40] The diagnosis often is based on clinical findings, but viral culture of cutaneous lesions, if present, or blood and liver biopsy also may help establish the diagnosis. Treatment with acyclovir is recommended. Varicella may be prevented or attenuated by passive immunization with varicella-zoster immune globulin (VZIG) or immune globulin intravenous (IGIV) or by active immunization with a licensed live virus vaccine.[17,25]

CYTOMEGALOVIRUS. CMV infection usually is asymptomatic or "silent." However, CMV in a normal host may cause gastroenteritis, pneumonitis, or a mononucleosis-like syndrome that consists of fever, lymphadenopathy, and atypical lymphocytosis. Hepatitis occurs as part of these syndromes but often is silent or mild and rarely is accompanied by jaundice.[67,82] Granulomatous hepatitis also may be associated with CMV.[28] Infants born congenitally infected with CMV frequently have hepatosplenomegaly, elevated transaminase levels, and direct hyperbilirubinemia.[29,129] The hepatitis that newborns and infants experience is self-limited and generally resolves within the first few months of life. If hepatitis with cholestasis persists, other diseases such as extrahepatic biliary atresia should be considered. Solid organ and marrow transplant recipients and patients with acquired immunodeficiency syndrome (AIDS) and other immunodeficiency states may experience persistent fever, malaise, leukopenia, and hepatitis caused by primary or recurrent infection with CMV. Severe liver disease can occur in transplant recipients and may be associated with graft-versus-host disease or graft rejection.[35,36] CMV-associated hepatitis may be diagnosed clinically and supported by virologic evidence of positive cultures, CMV viremia (or a viremia surrogate marker such as CMV antigenemia or CMV DNAemia), or demonstration of involvement of the end-organ by liver biopsy.[34,94] Treatment with ganciclovir, valganciclovir, foscarnet, or cidofovir appears to be beneficial in immunocompromised hosts, and prophylaxis or preemptive therapy with antiviral agents or CMV hyperimmune globulin may prevent the development of severe CMV disease in transplant recipients.[34,37,46]

EPSTEIN-BARR VIRUS. EBV infection also usually is asymptomatic or "silent," but it may cause a mononucleosis-like syndrome with mild hepatitis. However, in rare patients with a

genetic X-linked predisposition, a severe, often fatal lymphoproliferative syndrome with prominent liver involvement may develop. Transplant recipients also may be susceptible to post-transplant lymphoproliferative disease (PTLPD), in which the liver may be involved.[8,22,116] In addition, patients with tumors associated with EBV, such as lymphoma, may have hepatic involvement. The diagnosis of mononucleosis usually is clinical and supported by a positive heterophile or "Monospot" test or by specific EBV serologic tests such as detection of IgM antibody to viral capsid antigen (VCA).[12] The immune response to EBV in immunocompromised hosts may be unusual, and the diagnosis of PTLPD generally is suspected by an increase in EBV DNA genome copies in peripheral blood, generalized adenopathy, or the presence of histopathologic features on biopsy. Treatment involves a reduction in immunosuppression, administration of rituximab (anti-CD20 monoclonal antibody), or adoptive immunotherapy.[26,51,99,120] Antiviral therapy with acyclovir also may be administered, but its effectiveness is controversial.

HUMAN HERPESVIRUSES 6, 7, AND 8. These viruses may involve the liver, especially in immunocompromised patients.[42] Hepatitis associated with HHV-6 may occur in solid organ transplant recipients. In recipients of liver transplants, infection with HHV-6, mostly reactivation, also has been associated with acute rejection, portal lymphocyte infiltration, and impaired function of the grafted liver. However, because HHV-6 is ubiquitous and commonly can be detected in blood and tissue, the role that the virus is playing in the liver disease observed in these patients is not entirely clear.[100] Normal hosts experiencing primary HHV-6 infection may have silent hepatitis with mild elevation of aminotransferase levels. Rarely, severe disseminated disease with fulminant hepatitis has been linked to HHV-6 and HHV-7.[2,9,54,79] HHV-8 (also known as *Kaposi sarcoma virus*) causes a complex neoplasm involving the skin, mucous membranes, and internal organs, most often in patients with severely compromised immune systems. It rarely is seen in children but has been reported in human immunodeficiency virus (HIV)-infected children with advanced AIDS. Recipients of solid organ transplants may be infected with HHV-8. The liver is a common site of visceral disease caused by HHV-8, which most often is associated with tumors rather than hepatitis.[23] Diagnosing infection with HHV-6, HHV-7, or HHV-8 is difficult because of the ubiquity of these viruses and their viral DNA in humans, but the diagnosis is supported by serologic evidence and by detection of viral DNA in blood, in secretions, or more specifically, in tissue.[100] No licensed specific antiviral therapy is available, but these viruses may be inhibited by ganciclovir, foscarnet, and cidofovir. Response also may be noted after withdrawal or reduction of immunosuppression, and with HHV-8, chemotherapy may be indicated.

HERPES B VIRUS. Also known as *herpesvirus simiae* or *cercopithecine herpesvirus 1*, herpes B virus is an alpha-herpesvirus of monkeys that causes severe disease in humans.[131] It can be transmitted from Asian monkeys, such as rhesus and cynomolgus monkeys, to humans through bites or contact with mucous membrane secretions from infected monkeys. The human disease associated with herpes B virus involves skin vesicles at the portal of entry, regional lymphadenitis, and hemorrhagic encephalitis. The virus also may disseminate to the liver and lungs and produce hemorrhagic necrosis, with a high mortality rate. The diagnosis is made by isolation of virus, and the virus is inhibited by acyclovir and ganciclovir.

Adenoviruses

Adenoviruses are small DNA viruses that are members of the viral family *Adenoviridae*. They usually are respiratory or enteric pathogens, but they also can cause disseminated disease with hepatitis and hepatic necrosis in both immunocompetent and immunocompromised hosts and neonates.[56,81,96] Patients often have fever, malaise or lethargy, conjunctivitis, pharyngitis, cough, respiratory distress, vomiting and diarrhea, and a viral sepsis–like syndrome. Hepatitis is marked by hepatomegaly and by elevated aminotransferases and bilirubin. A specific viral diagnosis is made by isolation of adenovirus or detection of adenoviral DNA by PCR from respiratory secretions, blood, stool, or tissue. Adenovirus serotype 5 generally is associated with severe hepatitis, followed in frequency by types 1 and 2.[90] Viral surveillance cultures, blood DNA PCR testing, and virus serotyping and genotyping in immunocompromised patients, such as bone marrow and stem cell transplant recipients and liver transplant recipients at high risk for acquiring severe or fatal adenovirus-associated disease, may allow early intervention before severe, disseminated disease develops.[18,50,81] No licensed antiviral therapy is available, but the virus is inhibited to some extent by antiviral agents such as ribavirin and ganciclovir, and case reports suggest a potential clinical benefit in some patients with severe adenoviral hepatitis treated with cidofovir, an antiviral agent with broad-spectrum activity against many DNA viruses.[16,72]

Erythrovirus—Human Parvovirus B-19

Erythroviruses (i.e., human parvovirus B19) are small DNA viruses. They are members of the family *Parvoviridae* and are responsible for a variety of illnesses, including fifth disease (i.e., erythema infectiosum), arthritis, and anemia. Liver involvement, often severe, may be seen in intrauterine infection with hydrops fetalis.[4,80] Fulminant liver failure with massive hepatic necrosis associated with erythrovirus also has been reported in patients with aplastic anemia.[68] Persistent infection is seen in immunocompromised hosts. Parvovirus infection can be diagnosed by detection of virus-specific IgM antibodies or detection of viral DNA in blood, serum/plasma, bone marrow, secretions, urine, or tissue. No specific antiviral therapy is available, but immunocompromised patients with chronic infection may benefit from receiving IGIV. Severe fetal hydrops usually requires in utero and neonatal blood transfusions for anemia.

Enteroviruses

Enteroviruses are small RNA viruses and members of the *Picornaviridae* family, along with polioviruses and rhinoviruses. The non-polio enteroviruses usually are associated with mild respiratory or gastrointestinal illnesses, myocarditis, or aseptic meningitis, most often occurring in late summer or early fall. Significant hepatitis with hepatic necrosis, however, can occur, especially in neonates with disseminated disease. It often is accompanied by hepatomegaly, thrombocytopenia, viral sepsis syndrome, elevated aminotransferases, and elevated serum bilirubin.[84-86] Although any serotype can cause severe liver disease, echovirus 11 is associated most commonly with severe hepatitis or hepatitic necrosis, especially in neonates.[84] Coxsackie B and echoviruses 9 and 30 also are associated with fatal disease. Enteroviruses may be transmitted to neonates perinatally from the mother or through contact with ill family members. Nosocomial nursery outbreaks with enteroviruses also have been reported.[85] The diagnosis is established by isolation of the virus or detection of viral RNA by PCR in throat, stool, urine, blood, CSF, or tissue samples. Treatment is supportive; however, case reports suggest that IGIV, the investigational drug pleconaril, and even liver transplantation may have some clinical benefit in severely ill neonates.[6,20,63,112]

Measles Virus

Measles virus is an RNA virus that is a member of the *Paramyxoviridae* family, along with parainfluenza viruses, respiratory syn-

cytial virus, metapneumovirus, and mumps virus. Of all the paramyxoviruses, measles virus is associated most often with hepatitis. Approximately 10 to 20 percent of children with measles will have subclinical hepatitis, and severe disease of the liver, lungs, and brain may occur in immunocompromised patients.[47,93,122] Rare reports of severe giant-cell hepatitis, often leading to liver failure, have implicated paramyxoviruses of undetermined type.[106] Measles and other paramyxoviruses may be identified by detection of virus-specific IgM antibody in serum and by isolation of virus in secretions, blood, or tissue. No specific antiviral therapy is licensed for measles virus; however, ribavirin, a broad-spectrum antiviral agent, may have some activity against the virus. Prevention is achieved by vaccination with the live measles vaccine or by postexposure administration of immunoglobulin or IGIV.

Rubella Virus

Rubella virus is a member of the *Togaviridae* family of RNA viruses. Clinical disease associated with infection by rubella virus generally is mild, but as many as 10 percent of children with rubella may have subclinical hepatitis with transient elevation of aminotransferase levels.[126] Congenital rubella syndrome caused by intrauterine infection with rubella virus is, however, associated with significant liver involvement, with hepatomegaly and jaundice being noted at birth.[33,87,125] Congenital rubella syndrome also is associated with intrauterine growth retardation, cataracts, congenital heart disease, thrombocytopenia, purpura, and hearing loss.[66] Because isolating the virus is difficult technically, the diagnosis of rubella most often is made serologically by detection of virus-specific IgM antibody. No specific antiviral therapy is available. Rubella can be prevented, however, by vaccination with the live rubella virus vaccine.

Hemorrhagic Fever Viruses

The hemorrhagic fever viruses are a diverse group of RNA viruses from a variety of different virus families. They include arenaviruses such as Lassa fever virus, bunyaviruses such as hantavirus, filoviruses such as Marburg and Ebola, and flaviviruses such as yellow fever virus and dengue. Hemorrhagic fever is characterized by fever, malaise or lethargy, headache, retroorbital pain, myalgia, conjunctivitis, rash, and intravascular coagulation with hemorrhage. Liver involvement with hepatitis is a very common event, and elevation of aminotransferase levels to 500 IU/mL occurs in almost every patient, with a thousand times the normal range seen in patients who are severely ill.[32] Jaundice is a significant component of yellow fever.[61] The diagnosis is established by serologic means or by detection of the viral agent with electron microscopy or PCR techniques, which in most cases should be attempted only in biosafety level IV reference laboratories. Treatment of most hemorrhagic fevers is supportive; however, intravenous ribavirin reduces the mortality rate associated with Lassa fever and also may be of benefit to patients with hemorrhagic fever caused by other arenaviruses.[74,77]

BACTERIA

Nonviral acute hepatitis can be caused by bacterial illnesses (see Table 53–1).[95] Sepsis with gram-positive organisms, especially pneumococci, or with gram-negative organisms, particularly gram-negative enteric bacteria, can produce hepatic dysfunction, primarily from the cholestatic effects induced by bacterial endotoxins.[95,137] In this form of hepatic dysfunction, the patient will appear jaundiced with mild hepatomegaly, and conjugated bilirubin levels will be elevated out of proportion to the modest elevation in aminotransferase or alkaline phosphatase levels. Neonates also may have jaundice secondary to urinary tract infection with gram-negative enteric organisms. The diagnosis is made by isolating the offending bacteria from blood, urine, or other usually sterile site. Treatment involves specific antimicrobial therapy.

Other bacterial diseases may cause chronic or granulomatous hepatitis, including actinomycosis, brucellosis, listeriosis, nocardiosis, bartonellosis (cat-scratch disease), and tuberculosis.[7,98] Diseases caused by atypical mycobacteria, especially *Mycobacterium avium-intracellulare* complex (MAC complex) or *Mycobacterium mucogenicum*, may be seen, particularly in patients with congenital or acquired immunodeficiency states.[48] Rarely, disseminated disease with hepatitis may occur as a complication of vaccination with bacille Calmette-Guérin (BCG) in children or, in adults, as a complication of bladder irrigation for bladder carcinoma.[7,49,71] A diagnosis of hepatitis caused by these unusual or indolent bacterial pathogens usually is accomplished by isolating the organism from blood or the affected organ. The diagnosis may be supported by positive skin test results in the case of tuberculosis or atypical mycobacterial disease, serologically by elevated titers to *Bartonella quintana* or *Bartonella henselae*, and by positive imaging studies that show hepatic microabscesses, as in the case of hepatic involvement with cat-scratch disease. Antimicrobial therapy is guided by the susceptibility of the offending pathogen.

Bacterial toxins, such as the emetic toxin of *Bacillus cereus*, also have been linked to fulminant hepatic failure in some patients.[75]

Spirochetes

Acute infection with *T. pallidum*, the agent of primary or early secondary syphilis in adolescents or adults, may cause acute or granulomatous hepatitis with serum aminotransferase levels up to 5 to 10 times normal.[70] Jaundice rarely develops, but a chancre of primary disease or a rash of secondary disease often is present. Congenital syphilis also is associated commonly with hepatosplenomegaly in the newborn, along with elevated aminotransferase and bilirubin levels. Other clinical manifestations of congenital syphilis include petechiae or purpura, thrombocytopenia, osteitis, and meningitis. Laboratory diagnosis is confirmed by reactive VDRL and positive fluorescent treponemal antibody tests. Treatment with penicillin is recommended.

Leptospirosis, or infection with the pathogenic bacterium *Leptospira interrogans*, can cause acute hepatitis.[55] Leptospirosis usually is an abrupt, anicteric, flulike illness, but approximately 10 percent of patients will have an icteric or septicemic syndrome with a biphasic clinical course. Patients with the icteric or severe form will exhibit jaundice, hepatomegaly, and characteristic conjunctival injection. Usually, levels of serum bilirubin are elevated out of proportion to the more modest elevations in serum aminotransferases, suggesting a defect in excretion of bilirubin rather than direct hepatic necrosis as the pathogenesis of the jaundice. Meningitis, renal failure, and even liver failure may occur in some patients. The diagnosis should be considered in older children and adolescents with a history of exposure to wild and domestic mammals, especially dogs, rats, and livestock, which may excrete *Leptospira* organisms in their urine, or with a history of exposure to contaminated water in ditches, lakes, or streams. The diagnosis is established by isolating the organisms on special media during the acute phase of illness. Serologic and PCR tests also are available in reference laboratories. Treatment with penicillin or doxycycline is recommended.

Rickettsiae

The rickettsial organism *Coxiella burnetii* causes Q fever, both the acute and chronic forms, in which hepatitis is a prominent feature along with persistent fever, malaise, weight loss, and pneumoni-

tis.[14] Clinical jaundice is a rare finding, and most often the hepatitis is subclinical. A history of exposure to mammals or birds suggests the diagnosis, which can be confirmed serologically by reference clinical laboratories. Treatment with antibiotics, usually doxycycline, is recommended.

PARASITES AND FUNGI

A variety of parasites may invade the liver and occasionally cause hepatic dysfunction or disease.[95,135] Such parasites include *Plasmodium* spp. (malaria), *Entamoeba histolytica* (liver abscess), *Toxoplasma gondii* (toxoplasmosis), and *Toxocara canis* (visceral larval migrans). *Ascaris lumbricoides* (roundworms) may invade the common bile duct and cause acute obstructive jaundice.

Fungi also may invade the liver, usually with only minimal elevation of aminotransferase levels and rarely causing jaundice or elevated levels of bilirubin. Patients with a compromised immune system may have hepatic abscesses with *Candida* spp. or abscesses or necrotic lesions with *Aspergillus* spp., as well as other unusual fungal species. Both immunocompromised and normal hosts may have liver involvement with *Histoplasma capsulatum* or *Cryptococcus neoformans*.[95]

NONINFECTIOUS CAUSES

An important noninfectious cause of acute hepatitis is drug-related hepatitis (see Table 53–1).[133,136] It can range in severity from mild, with subclinical elevation of aminotransferase levels, to severe, with fulminant hepatic failure.[64] A careful history of ingestion of prescription or over-the-counter medications, as well as exposure to toxins or herbal remedies, should be elicited. Although almost any drug can cause acute hepatitis, the most common agents associated with hepatitis in children include acetylsalicylic acid (i.e., aspirin), acetaminophen, isoniazid, rifampin, phenytoin, valproic acid, phenobarbital, β-lactam antibiotics (e.g., oxacillin, nafcillin, and third-generation cephalosporins), sulfa drugs, and antifungal agents such as ketoconazole and fluconazole. The anesthetic drug halothane also can cause acute hepatitis with jaundice.[111] Gold and other metals used to treat arthritis have been associated with hepatitis as well. Carbon tetrachloride is a direct hepatic toxin. The *Amanita* mushroom, if ingested, causes severe liver injury. In addition, chronic hepatitis can develop in children, especially premature infants, who receive prolonged total parenteral nutrition.

Anoxic liver injury also can resemble acute viral hepatitis. It occurs in critically ill children after a period of hypotension, heart failure, or cardiopulmonary arrest. A history of such an inciting event supports the diagnosis. Anoxic liver injury is characterized by an abrupt onset of markedly elevated levels of aminotransferases, often hundreds of times higher than normal, without jaundice, and rapid recovery of enzyme levels to normal or nearly normal after the event has resolved.

Other diseases such as Kawasaki syndrome and toxic shock syndrome, which have been linked to or associated with infectious pathogens, may have hepatitis as part of their manifestation. Similarly, Reye syndrome, characterized by hepatomegaly with fatty infiltration of the liver and often fatal encephalopathy, may occur after a viral syndrome such as varicella or influenza.

Metabolic and genetic disorders may be manifested as hepatitis, especially in infants. Such disorders include cystic fibrosis, α_1-antitrypsin deficiency, galactosemia, glycogen storage disease, urea cycle deficiencies, organic acidemias, tyrosinemia, and lipid storage diseases such as Gaucher and Niemann-Pick disease.[103,127] Of note, cystic fibrosis in neonates may be associated with cholestatic jaundice in the absence of pulmonary disease.[123] Disorders of metal metabolism, such as Wilson disease, may cause acute, chronic, or even fulminant hepatitis in older children.[104,117] In addition, patients with congenital disorders of bilirubin metabolism, such as Gilbert disease, may become jaundiced without significant elevation of aminotransferase levels, especially during an intercurrent viral illness. Sickle-cell crisis also may be manifested as hepatitis.[118]

Tumors of the liver and infiltrative diseases such as lymphoma, histiocytosis, and hemophagocytic syndrome may have hepatitis or hepatic failure as part of the initial manifestation.[53] Another multisystem disorder that may produce chronic granulomatous hepatitis is sarcoidosis.[30]

Anatomic causes of hepatic dysfunction in infants include extrahepatic biliary atresia, which usually develops in the first 2 months of life.[76,129]

Autoimmune hepatitis or other autoimmune disorders with liver involvement also may cause acute or chronic hepatitis, especially in older children and adolescents.[130] In autoimmune hepatitis, hypergammaglobulinemia and autoantibodies usually are present, as are other symptoms such as rash, arthritis, inflammatory bowel disease, thyroiditis, malaise, and persistent fever. Autoimmune hepatitis may respond to steroids or other immunosuppressive agents.

Hepatic steatosis (fatty infiltration) recently has been documented to occur in obese children, especially if they also have type 2 diabetes. These children may have enlarged or tender livers on examination, elevated transaminase levels in serum, abnormal imaging studies showing fatty infiltrates of the liver, and liver biopsies that show inflammation, fibrosis, and even cirrhosis in some cases. Treatment currently includes diet and lifestyle changes with weight loss and exercise.

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CHAPTER 54

CHOLANGITIS AND CHOLECYSTITIS

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CHOLANGITIS

The term *cholangitis* refers to any inflammation of the extrahepatic or intrahepatic biliary system. Clinically, it is seen most often as a consequence of infection in the setting of biliary tract disease or obstruction. The diagnosis of cholangitis implies microbial colonization of the biliary tract, increased biliary pressure, and systemic signs of infection, although all of these findings are not present in all patients. Generally, the etiology and

treatment of infectious cholangitis are similar in adults and children. One specific pediatric population—children with biliary atresia and a hepatic portoenterostomy (HPE)—is particularly susceptible to cholangitis and its sequelae. This population and other patient groups at high risk of developing infectious cholangitis, such as patients with immunodeficiency states, congenital or acquired bile duct abnormalities, or liver transplants, are highlighted in this chapter. Biliary tract obstruction secondary to gallstone disease is discussed in the section on cholecystitis.

ETIOLOGY AND PATHOGENESIS

The central mechanisms leading to biliary tract infection are biliary colonization and stasis. Bile typically is sterile, implying that bactibilia develops either hematogenously via the portal venous system or directly via ascending infection from the gut lumen.[27]

Much is unknown regarding the route of bacterial infection of the biliary tree. Bacterial ascent may occur from the duodenum, most commonly in the presence of abnormal function of the sphincter of Oddi. In addition, bacterial invasion may occur hematogenously via increased gut translocation across the intestine into the portal circulation.[205] Earlier research indicates that infection of bile likely occurs via direct hematogenous spread of bacteria from portal venous flow across the gallbladder wall.[43] Although this mechanism has not been firmly established, the consensus is that transient episodes of bactibilia combined with biliary obstruction may lead to higher concentrations of bacteria in the biliary tract. As biliary pressure increases, bacteria likely migrate from the bile ducts into lymphatic and blood vessels, resulting in bacteremia and clinical signs and symptoms of cholangitis.[27]

In adults, cholangitis typically occurs in the presence of biliary tract obstruction secondary to impaction of gallstones in the common bile duct, leading to bile stasis and secondary infection. In approximately 85 percent of cases of cholangitis in adults, evidence of a common bile duct stone is present.[14] In adults with documented gallstones in the common bile duct (choledocholithiasis), the incidence of positive bile cultures is 30 to 90 percent.[119] Other potential causes of biliary obstruction in adults, possibly leading to a more chronic or recurrent presentation, include neoplasm of the biliary tree or head of the pancreas, other intrinsic or extrinsic strictures, parasitic disease, inflammatory conditions (e.g., primary biliary cirrhosis, primary sclerosing cholangitis [PSC]), and congenital bile duct anomalies. More recently, with improved imaging modalities and a better understanding of the pathogenesis of biliary lithiasis, it has become accepted that biliary sludge or microcalculi may be at the root of nonobstructive cholangitis and recurrent biliary infections.

In children, acute cholangitis occurs most frequently in patients with biliary atresia who have undergone HPE surgery. The HPE, also known as a *Kasai operation*, provides a permissive setup for cholangitis: poor bile flow combined with damaged intrahepatic bile ducts and obligate bacterial colonization with enteric flora in the intestinal conduit.[79,80,103,116,176] In this setting, cholangitis is thought to arise from reflux of jejunal flora through the HPE (Roux-en-Y loop), directly contiguous with the hepatic porta. Cholangitis tends to occur more frequently within 1 year after surgery, usually in patients with evidence of good biliary flow. Decreased biliary and duodenal motility generally are accepted as being associated with a higher incidence of cholangitis,[47,207] but most predictors and exacerbants for the development of cholangitis remain unknown.

Microbiologic evidence of biliary tract infection ideally should involve at least 10^5 organisms per milliliter of bile.[43] Gram-negative rods, primarily *Escherichia coli* and *Klebsiella pneumonia*, are found in infected bile, but *Enterococcus* and *Enterobacter* spp. also often are encountered. Many cases of cholangitis are polymicrobial, lending support for use of broad-spectrum antibiotic treatment regimens. *E. coli* is the organism most commonly isolated, accounting for 20 to 68 percent of infections. Table 54–1 summarizes the frequencies of specific organisms found in adult and pediatric studies.[79,98,141] Anaerobes, such as *Bacteroides fragilis* and *Clostridium perfringens*, also may play a significant role and have been identified in 40 percent of biliary tract infections.[20] Specifically, the latter organism has been implicated in acute emphysematous cholecystitis. Despite these findings in bile, many patients with cholangitis have negative blood cultures, requiring clinicians to have a low index of suspicion for cholangitis in selected circumstances and patient populations.

In addition to bacterial infection, other pathogens—viral, fungal, and parasitic—have been reported in cases of pediatric cholangitis, particularly in immunodeficient patients (primarily patients with human immunodeficiency virus [HIV]; see later) and patients with environmental exposure to parasites in endemic settings. Cholangitis has been reported with viral disease; although most commonly associated with hepatocellular disease, hepatotropic viruses, primarily cytomegalovirus (CMV), hepatitis C, and hepatitis B, have been associated with pathologic findings of bile duct injury with apparent clinical significance.[24,67,110] Depending on the clinical setting and age of the patient, CMV can have a markedly varied presentation, ranging from bile duct paucity seen in congenital CMV infection to hepatomegaly and jaundice in infants and children with primary CMV infection. The role of CMV hepatitis and cholangitis is significant in immunocompromised patients and solid organ transplant recipients (see later).

Fungal cholangitis, specifically with *Cryptococcus neoformans* or *Candida albicans*, has been reported in immunocompromised and immunocompetent patients, although more commonly in the former than the latter population.[53] Although *Cryptosporidium* spp. typically are known to cause cholangitis in the immunocompromised host, one case of a child without a documented immunodeficiency has been reported.[65]

TABLE 54–1 Bacterial Pathogens Isolated from Bile Cultures in Cholangitis*

Organism	Hitch, 1978[80] (*n* = 283)	Keighley, 1975[99] (*n* = 231)	Lewis, 1987[114] (*n* = 23)	Boey, 1980[15] (*n* = 99)
Escherichia coli	230	65	15	46
Klebsiella	193	20	17	28
Enterococcus	179	22	6	14
Pseudomonas	150	1	—	17
Proteus	44	13	—	8
Bacteroides	28	—	2	—
Clostridium	—	11	9	—
Other species	23	—	10	23

**Pediatric and adult data used.*[15,79,98,114]

The biliary tree also is susceptible to parasitic infections in normal hosts. Nematodes—*Ascaris* and, rarely, *Strongyloides*—commonly cause biliary disease in endemic regions. Migrating *Ascaris* larvae may cause either a direct inflammatory response if they pass through the biliary system or a secondary pyogenic cholangitis secondary to obstruction via the worms themselves or eggs. Liver flukes, such as *Clonorchis* (now *Opisthorchis*) *sinensis*, *Opisthorchis viverrini*, and *Opisthorchis felineus*, also may pass through the biliary system through their life cycle. Of these trematodes, *Clonorchis* is found frequently in cases of recurrent pyogenic cholangitis in Asian children.[148] Similar to the worms, the migration of the flukes (*Fasciola hepatica*) through the biliary tree may induce primary inflammation and, later, a secondary bacterial infection. Echinococcal cholangitis has been described in the setting of obstruction secondary to cyst formation from infection with either *Echinococcus granulosus* or *Echinococcus multilocularis*.[27,70,101] A thorough travel history should be obtained in all children presenting with new-onset cholangitis, in particular in patients in whom the most common causes are ruled out rapidly.

Finally, several cases of *Mycobacterium tuberculosis* cholangitis mimicking cholangiocarcinoma in immunocompromised and immunocompetent hosts have been reported in the adult literature.[12,50,104,155,163,219]

CLINICAL PRESENTATION

The classic presentation of cholangitis first described by Charcot in 1877 and known commonly as the *Charcot triad* is reported in 70 percent of patients with cholangitis (Table 54–2). It combines the clinical findings of fever, right upper quadrant pain, and jaundice.[94] The Reynold pentad adds septic shock and altered mental status to the Charcot triad.[115] Studies in children show that fever is the most common presenting symptom, occurring in 100 percent of 105 patients with cholangitis after undergoing HPE.[47] In addition, either acholic stools or an increase in serum bilirubin concentration occurs in 68 percent of patients. Older children and teenagers with cholangitis typically report abdominal pain that may or may not be associated with meals or localize to the right upper quadrant. Patients may report new-onset pruritus as a consequence of retained biliary constituents.

The findings of cholangitis may be markedly attenuated in infants and immunocompromised patients, suggesting that a low index of suspicion be employed in the evaluation of these children at risk. The presentation of cholangitis in an infant with biliary atresia, status post Kasai portoenterostomy, may be as varied as lethargy, increasing jaundice, abdominal tenderness, or fever alone. Finally, patients with ongoing hemolysis (e.g., patients with hemoglobin SS disease) are at high risk for developing cholecystitis and cholangitis from bilirubin stones, yet the symptoms may readily overlap with other causes of abdominal pain.

DIAGNOSTIC EVALUATION

Physical examination may reveal a varied range of toxic appearances, with vital signs suggesting serious systemic infection in more advanced cases (fever, tachycardia, hypotension). Physical examination typically reveals icteric sclera and a distended abdomen, with tenderness localized to the mid or right upper quadrant. Palpation of the liver edge may reveal tenderness. Laboratory studies may reveal an elevated erythrocyte sedimentation rate (81%), leukocytosis or leukopenia (56%), and increased serum levels of conjugated bilirubin.[47] Laboratory evaluations in children should include a standard complete blood count with differential and liver panel (alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, gamma-glutamyltransferase, and fractionated bilirubin [unconjugated and conjugated]). The differential diagnosis of fever, abdominal pain, and jaundice also should include sepsis and hepatitis. No single laboratory test or combination of tests can establish a diagnosis of cholangitis.

TABLE 54–2 Charcot Triad and Reynold Pentad

Charcot Triad	Reynold Pentad
Fever	Fever
Right upper quadrant pain	Right upper quadrant pain
Jaundice	Jaundice
	Septic shock
	Altered mental status

Attempts at identifying a microbiologic agent should be made, and blood cultures should be drawn before antimicrobial therapy is initiated. Most cases are polymicrobial, with a predominance of gut-derived organisms (see Table 54–1). Blood cultures may be positive in 50 percent of pediatric patients with cholangitis, as reported by Ecoffey and colleagues,[47] but this typically is not the case in routine pediatric practice. Other investigators have reported identification of organisms in blood in approximately 10 to 25 percent of patients, which is in keeping with our experience.[227] In patients with a history of hepatobiliary surgery, aerobic and anaerobic cultures should be considered, and in virtually all cases of young children with abdominal pain and jaundice, a urine culture should be considered.

A role for direct testing of hepatic tissue by percutaneous biopsy is controversial, but occasionally this testing may have a place in the setting of negative blood cultures. When used along with blood cultures, hepatic cultures have been shown to increase the diagnostic yield of identifying a microbiologic organism to 75 percent of patients, whereas histologic confirmation of cholangitis may be the only firm evidence of biliary tract infection in some cases.[47]

In practice, liver biopsy rarely is done, and patients are treated empirically. In cases of gallstone-associated biliary tract obstruction with cholangitis that requires endoscopic or surgical decompression, bile should be obtained and cultured at the time of the procedure. In an adult study, 22 of 23 patients with gallstone-associated cholangitis were found to have positive bile cultures.[114]

The initial and principal radiologic evaluation for suspected cholangitis is transabdominal ultrasound, with the primary goal being to search for evidence of biliary tract obstruction, usually associated with dilation of the common bile duct or intrahepatic ducts. Ultrasound examination may reveal anatomic biliary tract abnormalities (choledochal cyst), intrahepatic cysts, and hepatic or other intra-abdominal masses. Other noninvasive imaging modalities include computed tomography (CT) and magnetic resonance cholangiopancreatography (MRCP), which provide increasingly detailed images of the intrahepatic and extrahepatic biliary anatomy.[137,172]

Taken together, these noninvasive imaging techniques should be able to detect the presence of biliary tract obstruction and some congenital anatomic anomalies, although even experienced ultrasonographers still may miss small stones and sludge, especially in the common bile duct. It is important to recognize the technical limitations of the methodology and the possible presence of undetected stones or sludge in the gallbladder or common bile duct (choledocholithiasis) as a cause of biliary tract dilation. Ultrasound misses 40 percent of biliary tract stones.[191] Finally, ultrasound is only 51 to 85 percent sensitive in identifying biliary tract obstruction, which improves to approximately 90 percent with CT, and 95 to 99 percent with endoscopic retrograde cholangiopancreatography (ERCP).[164]

Newer modalities, including endoscopic ultrasound, laparoscopic ultrasound, and helical CT cholangiography, may improve detection in certain patient populations. The 2002 National

Institutes of Health State-of-the-Science Consensus conference regarded endoscopic ultrasound, MRCP, and ERCP to have comparable sensitivity and specificity in detecting common bile duct stones.[146] In the hands of an experienced echoendoscopist, endoscopic ultrasound has been shown in adults to be the procedure of choice to identify small stones and sludge in the common bile duct. This method may prove useful in children as well, and may prevent the unnecessary use of ERCP and the risk of its associated complications.[54,173,191]

After HPE has been performed, imaging may be able to identify lakes of retained bile (bilomas), which may be a source of infection, in a patient with biliary atresia. Because of the inherent risks associated with invasive procedures in these infants, percutaneous drainage of bilomas for culture or other purposes rarely is performed. Similarly, ERCP is not indicated for patients with biliary atresia after HPE, for anatomic reasons.

Nuclear medicine (hepatobiliary iminodiacetic acid [HIDA]) scans have a limited role in the evaluation of cholangitis. HIDA scans may be most helpful in determining if a patient has a complete obstruction of the common bile duct, or if an isolated obstruction of the cystic duct (cholecystitis; see later) is present. Because most pediatric cases of cholangitis occur in patients with biliary atresia after HPE, involvement of the cystic duct rarely is a concern.

Invasive imaging has a role, especially when it may be coupled with therapeutic decompression of an obstructed, and potentially infected, biliary tree. ERCP has proved to be extremely useful in the evaluation and management of biliary tract obstruction and should be considered in the management of a child with evidence of an obstructed common bile duct (see later).[3,26,121,194,195,200,211,237] The role of ERCP in infants and small children is limited by the lack of experience of most pediatric gastroenterologists and should be considered in tertiary care centers that have the requisite expertise. ERCP is the most direct means of obtaining bile for microbial and chemical analysis and provides detailed imaging of the biliary tree.

Practically, ERCP may detect (and remove) small stones and sludge that are missed by ultrasound, CT, or MRCP. ERCP is helpful in establishing the differential diagnosis of biliary tract diseases, such as PSC, which may manifest in a fashion similar to that of infectious cholangitis but have a characteristic radiographic appearance. In the future, as the use of endoscopic ultrasound in pediatrics increases, ERCP may be reserved for those patients in whom stones or sludge have been found by endoscopic ultrasound or in patients with a high pretest probablility of having choledocholithiasis (e.g., patients with sickle-cell conditions).[54,191] One more recent study showed that 34 percent of pediatric patients undergoing ERCP had a normal study.[89] Limiting the indications for ERCP could prevent complications such as pancreatitis and iatrogenic ascending cholangitis. Any child with a first episode of cholangitis warrants a detailed investigation of a possible underlying biliary tract anatomic abnormality.

DIFFERENTIAL DIAGNOSIS

The differential diagnosis for an acutely ill child with fever and clinical evidence of hyperbilirubinemia is broad; a thorough work-up must include consideration of infectious and noninfectious etiologies (Table 54–3). A thorough investigation for hepatic and biliary tract pathology with blood and radiologic studies must be initiated to evaluate for stones or other obstructive processes that may cause symptoms characteristic of the Charcot triad.

Acute viral hepatitis may manifest in numerous ways but often begins with nonspecific signs: fever, headache, anorexia, and jaundice. Laboratory studies typically reveal a greater elevation of serum aminotransferase levels (alanine aminotransferase and aspartate aminotransferase) than the biliary tract enzymes alkaline phosphatase and gamma-glutamyltransferase. In a child with no other evidence of biliary tract obstruction, screening assays for hepatitis infection are warranted.

TABLE 54–3 Differential Diagnosis of Fever, Abdominal Pain, and Jaundice

Cholangitis
Cholecystitis
Cholelithiasis
Sepsis
Hepatitis
Choledochal cyst
Pancreatitis
Urinary tract infection
Leptospirosis and other systemic infections with hepatic involvement
Spontaneous perforation of common bile duct
Biliary cyst
Appendicitis

Pyogenic liver abscesses and amebic abscesses tend to manifest with fever, abdominal pain, hepatomegaly, and focal right upper quadrant tenderness.[148] Laboratory findings vary. CT is considered to be the most sensitive technique for evaluation of these diagnoses.[137] Numerous bacterial, parasitic, and spirochetal infections also should be considered in the differential diagnosis, including, but not limited to, typhoid fever, brucellosis, leptospirosis, borreliosis, amebiasis, and malaria. It is beyond the scope of this chapter to expand on the systemic infections associated with hepatic involvement.[154] Recurrent pyogenic cholangitis is another consideration, although it is rare in the Western Hemisphere and even more so in the pediatric population.[182]

Jaundice may occur with sepsis of any etiology, more commonly in a critically ill infant or child. Clinical evaluation may show a predominantly direct (conjugated) hyperbilirubinemia usually accompanied by a modest elevation of gamma-glutamyltransferase, although serum transaminase levels may not be elevated. These findings are caused by a hepatocellular cholestasis, owing to humoral mediators of sepsis (i.e., endotoxin and proinflammatory cytokines), but they can be exacerbated by biliary sludging that accompanies septicemia. Although sepsis-associated cholestasis historically was linked primarily to gram-negative sepsis, it can be seen in all forms of infection. In some cases, radiologic work-up or biopsy is warranted to rule out ductal obstruction or biliary tract pathology. Jaundice may occur as the sole presentation of sepsis in infants and children, however.[45] Evolving research into sepsis-induced cholestasis points to a complex, multifactorial etiopathogenesis.[30,59,138,215]

Drug-induced cholestasis tends to occur acutely with the onset of jaundice, pruritus, and other symptoms that may mimic cholangitis. It rarely is associated with fever, however. Among the main classes of drugs commonly used in pediatric practice that may lead to significant hepatotoxicity are antimicrobials (mainly trimethoprim-sulfamethoxazole [TMP-SMX], amoxicillin/clavulanate, clindamycin, minocycline, nitrofurantoin, erythromycin, cephalosporins, isoniazid, rifampicin, fluconazole), anticonvulsants (phenytoin, carbamazepine, valproate, felbamate), nonsteroidal anti-inflammatory drugs (aspirin, ibuprofen), antihypertensive agents (propranolol, diltiazem),[21,34] and herbal remedies such as kava kava. Although rarely used in pediatric patients, statins also lead to significant hepatotoxicity.[125] If the offending agent is identified in time, removing it typically leads to rapid improvement. Ceftriaxone warrants special mention because it has been linked directly to cholangitis and cholecystitis, possibly as a result of its biliary concentration and precipitation, leading to sludge and stones.[102,145]

Other systemic illnesses may occur with fever and evidence of biliary tract pathology, and their presentations may overlap with

presentations of infectious cholangitis. PSC is defined as a chronic inflammation of the intrahepatic or extrahepatic ducts leading to a range of biliary tract pathologies, from dilation to obliteration and periductular fibrosis. PSC is seen most commonly in patients with inflammatory bowel disease and affects adolescent boys with inflammatory bowel disease more commonly than girls. Symptoms include systemic findings, such as fatigue, malaise, and weight loss, and evidence of cholangitis, including fever. PSC must be suspected in a jaundiced patient with inflammatory bowel disease or in an adolescent boy presenting for the first time with jaundice. Diagnosis is established best by ERCP or magnetic resonance cholangiography showing irregular narrowing and stricture of the hepatic and common bile ducts and the intrahepatic ducts.[124,169] Patients with PSC are at risk of developing intrahepatic and common bile duct strictures, with subsequent obstruction, sludge or stone formation, cholangitis, and ultimately cholangiocarcinoma.[112]

TREATMENT

Therapeutic goals in the treatment of cholangitis should include general support of the patient, early initiation of appropriate antibiotic therapy, and, in cases of obstruction by stones or stricture, decompression of biliary obstruction via ERCP or surgery. Consulting pediatric surgeons early in the course of evaluating a patient with suspected cholangitis generally is helpful. The importance of decompression and drainage of the bile tract in the face of systemic infection secondary to cholangitis cannot be overemphasized. Other potential etiologies of obstruction, such as choledochal cysts, ultimately require surgical consultation and repair.

Initial and conservative management should include appropriate inpatient monitoring, cessation of oral intake with intravenous fluid support, parenteral antibiotic therapy, and supportive management as warranted by the child's clinical status. Because blood cultures tend to have a low diagnostic yield in cholangitis, antibiotics should be selected empirically, and selection should not rely on culture results.

Selection of a drug or combination of drugs should be based on appropriate coverage of suspected or documented organisms (based on sensitivities) and ability to achieve adequate serum and tissue concentrations. Currently, no single antibiotic or combination is recognized universally as being the definitive therapy for cholangitis in children. Typically, antibiotic therapy is initiated before obtaining culture results. In the past, the goal was to achieve sufficient biliary concentrations, although adequate serum levels are probably a satisfactory surrogate.[43,115] Parenteral antibiotics are indicated in almost all cases of children with suspected biliary tract sepsis. Primary antibiotics should cover gram-negative enteric organisms—*E. coli*, *Klebsiella*, and *Enterococcus*. In the case of a child with clinical sepsis, it is reasonable to add coverage for anaerobic species, particularly *Bacteroides*.[20]

Previously, a combination of ampicillin or penicillin with an aminoglycoside was considered appropriate therapy. Studies in adults suggest, however, that the use of some antipseudomonal penicillins, such as mezlocillin, is more effective alone than is the ampicillin-gentamicin combination.[61] The general recommendation for treatment of a pediatric patient with suspected cholangitis is a semisynthetic penicillin (e.g., piperacillin or mezlocillin) or a third-generation cephalosporin (e.g., cefoperazone) in combination with an aminoglycoside for adequate coverage of *Pseudomonas* spp.[107,227] The addition of metronidazole helps to cover anaerobic gut flora.

Rothenberg and colleagues[176] reported a 62.7 percent success rate after treatment with imipenem-cilastatin or third-generation cephalosporins with or without aminoglycosides and a 58.2 percent success rate using semisynthetic penicillins with aminoglycosides. A prospective trial involving 131 adults with biliary tract infections showed a "cure" in 85 percent of patients treated with ampicillin and tobramycin after a surgical procedure.[141] Although it is limited, a 1987 report of three patients suggests favorable results can be achieved using ciprofloxacin in pediatric patients with cholangitis after they have undergone a Kasai procedure.[84] TMP-SMX, which achieves a high biliary:serum concentration, has been used successfully in similar clinical settings.[80]

As mentioned previously, ceftriaxone probably should be avoided because it has been associated with biliary sludging and cholecystitis.[90] Currently, certain centers have reported the use of broad-spectrum antibiotics, such as meropenem, to cover optimally the enteric organisms most frequently associated with bacterial cholangitis.[201] Meropenem and other broad-spectrum β-lactam antibiotics with a β-lactamase inhibitor may be a good choice because many cholangitis episodes are polymicrobial.[221]

In clinical practice, the choice of antibiotic therapy often is empiric despite attempts to isolate a pathogenic organism. Antibiotic efficacy is determined by clinical and laboratory parameters, such as defervescence and improvement in biliary excretion. Antibiotic resistance is encountered more often in patients with repeated episodes of cholangitis.[47] The duration of antibiotic treatment varies, but a shorter course may result in recurrence.[176] The recommended course of therapy is 14 to 21 days, with longer duration recommended in special cases, such as recurrent or refractory cholangitis, and in the case of intrahepatic abscesses or hepatic surgery. Although oral antibiotic therapy generally has no place in the treatment of cholangitis in children, oral ciprofloxacin has been used in some adult populations.[227]

Ultimately, cholangitis or the risk thereof will not resolve in the presence of ongoing biliary obstruction. Although antibiotic therapy may treat septicemia, timely establishment of biliary drainage is imperative.[48] In 25 percent of adults with cholangitis, medical therapy is insufficient, and decompression via ERCP (or laparotomy) is indicated.[48,98,180] ERCP and percutaneous transhepatic cholangiography generally are recommended as first-line approaches because they pose a lower risk than does open surgical intervention.[157] These procedures can be diagnostic in the case of biliary obstruction and therapeutic, with sphincterotomy or stent placement.

Treatment of parasitic cholangitis should include appropriate treatment of biliary parasites based on regional sensitivities in addition to antibiotic coverage of secondary bacterial infections. Endoscopic or surgical intervention may be required to remove worms or cysts from the biliary tree.[35]

COMPLICATIONS OF CHOLANGITIS

Regardless of the patient's age at the time of presentation, cholangitis can be life-threatening. Other morbidities associated with cholangitis include pancreatitis. Because pediatric patients who present with cholangitis typically have underlying biliary or liver pathology, cholangitis may precipitate a rapid exacerbation of the underlying disease. In diseases such as biliary atresia, cholangitis may hasten the patient's course toward requiring organ replacement.

SPECIFIC POPULATIONS AND CHOLANGITIS

CHOLANGITIS AND BILIARY ATRESIA

In pediatrics, cholangitis is encountered most frequently in the setting of a patient with biliary atresia who has undergone a Kasai procedure. In 1959, Kasai and Suzuki[96] first reported relief of biliary obstruction in children with biliary atresia by HPE. The

procedure, a Roux-en-Y hepatic portoenterostomy, is considered to be standard first-line surgical therapy for infants with biliary atresia, and it is associated with the highest success rates and long-term survival when performed early, usually by 60 days of life. Without this procedure, 90 percent of patients with biliary atresia die before reaching age 3 years, at an average age of 19 months.[95,199]

Cholangitis remains the most frequent complication of the Kasai procedure, occurring in 40 to 60 percent of patients.[152] A subgroup of patients with the pathologic finding of cystic dilation of the intrahepatic bile ducts seems to have the highest risk of developing postoperative cholangitis.[81] The development of cholangitis has been associated with a worsening in long-term prognosis of children with biliary atresia.[85] Complications of cholangitis are severe, ranging from inflammation and scarring, to alterations in biliary flow, and, eventually to biliary cirrhosis. Repeated bouts of cholangitis are associated with worsening liver function, impaired growth, and need for early transplantation. For this reason, early detection, appropriate management, and prompt intervention and treatment of cholangitis are important in this population.

Most cases of post–Kasai procedure cholangitis occur within 1 year postoperatively. In a 1976 review of 49 cases of children who underwent the procedure, 31 achieved good bile flow restoration. Of these, 20 subsequently developed cholangitis, 15 occurring within the first postoperative month and 3 within the second month.[149] Likewise, in a study of 105 cases of cholangitis developing in 101 children after they underwent hepatic portoenterostomies, Ecoffey and colleagues[47] showed that 63 percent of the cases occurred within 3 months after surgery, and 93 percent occurred within 1 year. Rarely, late cholangitis may occur; this has been reported to occur several years after HPE.[66]

The generally accepted mechanism for development of cholangitis after a Kasai procedure is ascending bacterial colonization. This theory is reinforced by reports of a higher incidence of cholangitis developing in patients with good or partial bile flow after the Kasai procedure (78%) than in patients with no obvious bile flow (13%).[47] A 1978 study of 19 patients concluded that all bilioenteric conduits after the procedure were colonized within 1 month, correlating with the high incidence of symptomatic infection at this early stage.[80] The retrograde bacterial colonization may be enhanced by overall changes in intestinal motility after the Roux-en-Y loop.[207] Over time, the intestinal conduit from hepatic porta to jejunum seems to "mature," and the number of episodes of cholangitis diminishes. How this adaptation happens is unknown. Late episodes of cholangitis, occurring 1 to 2 years after a Kasai procedure, likely are related to mechanical obstruction, such as adhesions, and warrant investigation.[116]

Gram-negative enteric organisms constitute most organisms causing ascending cholangitis after HPE. *E. coli* has been found in 50 percent of the first and second cholangitis episodes and in decreasing frequency in subsequent episodes.[47] Anaerobes also are common findings and should be considered when selecting antibiotic treatment.[20] Refractory or recurrent cases occurring after surgery may warrant consideration of fungal disease, principally *Candida*.[33] Attempts to isolate an organism generally are made, but blood cultures typically have a low yield.[176] Bile cultures tend to reflect the multiple enteric organisms that colonize the conduit, but may not specify the true pathogens. Percutaneous liver biopsy has been used successfully to obtain cultures, but it rarely is used for this indication in pediatric patients.[150]

In the intraoperative and postoperative management of children with biliary atresia, attempts have been made to prevent the incidence of cholangitis. Various modifications in the surgical configuration of the intestinal conduit have been suggested to reduce enteric reflux. Initial studies have documented a reduced incidence of ascending cholangitis in the presence of a surgically placed antireflux valve. Despite the reduction of intestinal reflux in these cases, cholangitis continues to occur.[18] Cases of refractory cholangitis may require surgical intervention, especially if obstruction of the conduit or porta hepatis is suspected. The absolute indications for reoperation after HPE are unclear, however.

The role of corticosteroids in improving biliary drainage is controversial and has not been the subject of rigorous controlled trials. Perioperative steroid pulses, typically a 3- to 5-day course of intravenous methylprednisolone, have been shown, however, to be clinically beneficial by decreasing temperature, increasing bile flow, and improving liver function tests.[142,176] A multicenter, randomized, double-blind, placebo-controlled study sponsored by National Institutes of Health has been under way in 10 centers in the United States, to assess the safety and efficacy of using corticosteroids after the Kasai procedure (http://clinicaltrials.gov/ct2/show/NCT00294684?term=basitrank=3). The forthcoming results of this study should yield definitive information regarding the postoperative management of these children. The incidence of cholangitis in the treated and untreated arms is one of the outcomes.

In addition, some centers advocate the use of prophylactic antibiotics, typically TMP-SMX. The benefits of this practice are not well established, however.[32] In adults with recurrent cholangitis secondary to fixed obstruction, as in the case of malignancy, TMP-SMX and ciprofloxacin have been shown to be helpful in preventing episodes.[220] In our practice, TMP-SMX is used as a first-line agent. In selected patients with recurrent episodes of cholangitis on TMP-SMX and a functional HPE, we have used oral ciprofloxacin successfully.

In patients with post–Kasai procedure biliary atresia, each episode of cholangitis is associated with a 1 percent mortality risk. Some studies suggest that no significant clinical difference exists in overall survival rates between patients who have undergone the Kasai procedure and have or have not had cholangitis.[151] Previous studies suggest, however, an 88 percent mortality rate among patients who develop cholangitis within 1 month after undergoing the Kasai procedure, and 16 percent in patients who develop it more than 1 month later. More recent studies suggest that the occurrence of cholangitis is related to early postoperative mortality, and the number of repeated episodes is inversely related to survival.[231] Practically, because cholangitis can be life-threatening, patients with recurrent episodes frequently are considered for liver transplantation.

CHOLANGITIS AFTER LIVER TRANSPLANTATION

Infection remains the most common reason for morbidity and mortality after pediatric liver transplantation, accounting for 20 to 30 percent of postoperative deaths.[178,202,204] Bacterial infection that develops after transplantation tends to occur within the first 2 months postoperatively and generally is of either respiratory tract or intra-abdominal origin. Among patients with severe bacterial infections of intra-abdominal origin, cholangitis and biliary tract infections commonly develop. Gram-negative aerobic bacteria are encountered most commonly in this setting.[183] One adult study showed that 18 percent of 284 patients receiving liver transplantation had confirmed episodes of cholangitis.[222] Pediatric data suggest that the rate of cholangitis after liver transplantation for biliary atresia is 5 to 11 percent.

Biliary tract disease frequently occurs after the post–liver transplantation complication of hepatic artery thrombosis.[122,130] This complication was prevalent in the early days of pediatric liver transplantation. Although it remains a concern, the incidence has decreased owing to the use of microsurgical techniques. Hepatic artery thrombosis eventually leads to damage of the bile duct because the biliary blood supply is exclusively arterial, whereas the hepatic parenchyma is vascularized by the portal

vein and the hepatic artery. Biliary injury leads to altered biliary anatomy and drainage, increasing the risk of development of infection in these patients. Although most pediatric liver transplant recipients have a Roux-en-Y biliary-enteric anastomosis from the donor bile duct to the recipient bowel, ascending cholangitis is rare in this population.

Cholangitis that develops after liver transplantation may manifest with fever, jaundice, elevated liver enzymes, and bacteremia. The variable nature of an immunocompromised patient's response to infection renders the reliability of each of these signs and symptoms suspect, however. Distinguishing these symptoms from the presentation of post-transplant rejection or infection (e.g., with CMV or Epstein-Barr virus) is important. For this reason, liver biopsy often is a necessary part of the evaluation of a post-transplant patient with fever and elevated liver tests.

CHOLANGITIS IN IMMUNOCOMPROMISED PATIENTS

Although biliary disease related to acquired immunodeficiency syndrome (AIDS) may be considered the most common cholangiopathy of immunocompromised hosts, it is not the most frequent one in pediatric practice. Cholangitis also may occur in children with non-AIDS immunodeficiency. Sclerosing cholangitis has been reported in cases of primary immunodeficiency, including familial T-cell deficiency, IgA/IgG deficiency, and X-linked hyper-IgM syndrome.[69,113] Patients with sclerosing cholangitis often present with superimposed bacterial cholangitis because of poor biliary drainage. Hepatobiliary infection also has been reported in children with leukemia.[58,105,189,197]

AIDS-related cholangitis is a well-known complication of AIDS. It tends to occur late in the course of the illness and is more common in adults than in children. With the decline in the number of new AIDS cases in the United States, especially in pediatric patients, this diagnosis has become rare. In 1989, Cello[29] described four distinct patterns of disease in AIDS-related cholangitis as seen on cholangiography—papillary stenosis, sclerosing cholangitis, combined papillary stenosis and sclerosing cholangitis, and long extrahepatic bile duct strictures. The pathogenesis of these changes is unknown and may be related to biliary inflammation secondary to immunodeficiency, infiltration of the mucosa by HIV itself, or opportunistic infection by known gut culprits in AIDS infection.

Opportunistic agents most commonly responsible are CMV, *Cryptosporidium*, *Microsporida*, and, uncommonly, *Mycobacterium avium-intracellulare* and *Isospora*.[144,235] One report of unexplained cholangitis in a small cohort of HIV-positive men identified *Enterocytozoon bieneusi* in the bile of all of the patients.[159] Although AIDS cholangiopathy is extremely rare in children, these pathogens can be found in pediatric patients with primary immunodeficiencies and associated PSC, and with secondary immunosuppression after undergoing solid organ transplantation.[1,40,132,170,228]

Clinical presentation is similar to that in non-HIV patients, with the exception of jaundice, which tends to be less common. In a series of 45 adults with AIDS-related cholangitis, abdominal pain was reported as the most common presenting symptom, occurring in 64 percent of patients, followed by diarrhea (22%), fever (20%), and jaundice (7%). A 1997 review reported that 90 percent of adults present with right upper quadrant or epigastric pain. Twenty percent of patients were asymptomatic and identified by routine blood work alone.[44] Cholangitis has been reported as the initial presentation of HIV infection in a few patients.[17,140]

Diagnostic steps include noninvasive imaging with sonography and CT, and ERCP. Abdominal ultrasound is abnormal in 75 percent of patients and typically shows dilation or wall thickening of the common bile duct. These findings, along with liver function studies, may be suggestive of the disease even in children.[177] ERCP can show further characteristic changes in the biliary tract and has the added advantage of obtaining specimens for biopsy and culture and the possibility of therapeutic intervention.[232] Although AIDS-related cholangitis typically is not directly associated with mortality, most patients with AIDS-related cholangitis die within 1 year of being diagnosed because cholangitis usually occurs in patients with end-stage disease.[120] Therapy generally is symptomatic and should include coverage of bacterial cholangitis, which often is associated because of impaired biliary drainage.

CHOLANGITIS IN ASSOCIATION WITH CONGENITAL ANATOMIC ABNORMALITIES: CHOLEDOCHAL CYSTS AND CAROLI DISEASE

Choledochal cysts occur in 1 in 15,000 births in Western nations and 1 in 1000 live births in Japan.[148] Typically, presenting signs suggest cholestasis and may include jaundice, dark urine, and acholic stools, or patients may present with abdominal masses with or without jaundice. If diagnosed late or untreated, choledochal cysts may result in severe complications secondary to biliary tract obstruction, including cholangitis and pancreatitis. One series of 36 Indian patients reported 13 patients, more frequently children than infants, who presented with cholangitis.[158] Cholangitis is more likely to be the presenting sign for choledochal cyst in adult patients.[31]

Treatment of cholangitis associated with choledochal cysts involves supportive treatment of the patient, including appropriate antibiotic treatment. In the past, cyst-enteric drainage procedures were used as temporizing treatments, and this type of repair was associated with high rates of complications, including recurrent bouts of cholangitis, stones, and cholangiocarcinoma in the remnant duct.[168] The current surgical goal is complete surgical excision; compared with internal drainage procedures, this approach is associated with lower rates of postoperative cholangitis and mortality.[181] Five types of choledochal cysts have been identified, with solitary extrahepatic cysts (type I) being encountered most frequently. After this type of cyst has been excised, reconstruction of the biliary tract may involve a Roux-en-Y choledochojejunostomy or hepatojejunostomy.

Patients who undergo this surgery also are at risk for developing postoperative ascending cholangitis, with an incidence of 8 to 19 percent.[52,185] Although recurrent cholangitis may lead to chronic liver disease in the long-term, antibiotic prophylaxis is not recommended routinely.[92]

Caroli disease describes congenital dilation of the intrahepatic and extrahepatic biliary tree characterized by pure ductal ectasia.[37,38] More commonly, dilation of the intrahepatic ducts is attributed to ductal plate malformation in association with congenital hepatic fibrosis. The association of ductal ectasia with congenital hepatic fibrosis is more common than without and is termed *Caroli syndrome*. Both of these diseases may manifest with clinical signs of liver disease or renal disease secondary to the associated condition of autosomal recessive polycystic kidney disease, or both.[93,118,153] Dilation of the intrahepatic bile ducts results in biliary obstruction and places the patient at increased risk for development of intrahepatic stones and cholangitis, which significantly increases morbidity and mortality rates in Caroli disease and congenital hepatic fibrosis. Diagnosis is suspected in the case of recurrent cholangitis or portal hypertension of unknown etiology, and can be confirmed by ultrasound, cholangiography,[49] or MRCP.

Portosystemic shunting is considered the treatment of choice because this condition typically does not progress to liver failure. Suspicion of cholangitis, whether owing to signs of infection or sepsis or laboratory results suggesting inflammation, should be confirmed via a diagnostic liver biopsy for culture. Treatment of cholangitis in the setting of Caroli disease may be difficult.

Recurrent episodes may occur even after administration of intensive intravenous antibiotic therapy.[224] In some cases, drainage procedures may be used for refractory or recurrent infections. Orthotopic liver transplantation is the treatment of choice in recurrent, life-threatening episodes of cholangitis.[76,224]

Typically, patients with Caroli disease or Caroli syndrome present with cholangitis because the biliary malformation communicates with the extrahepatic biliary tree. Rarely, patients are seen with hepatic cysts associated with autosomal dominant polycystic kidney disease. These lesions are noncommunicating lesions and as such are not prone to infection. In the absence of a history of cholangitis, it is advisable that these rare patients not undergo invasive procedures of the biliary tree because of the risk of microbial seeding and subsequent suppurative cholangitis.[5,100,234]

CHOLANGITIS AFTER ENDOSCOPIC AND OTHER BILIARY PROCEDURES

Although at this point, experience in the use of ERCP in pediatric patients is limited, it is growing, and cholangitis is a known complication of this procedure in adults. In a series of 50 pediatric patients, low-grade fever, abdominal pain, nausea, and vomiting were reported as the most common complaints after undergoing ERCP; one patient was treated for mild cholangitis.[211] Another group reported three cases of sepsis in adults caused by multiresistant *Pseudomonas aeruginosa* after ERCP, ascribed to nosocomial transmission from the endoscope despite negative surveillance cultures.[41] In adults, and rarely in children, biliary stents are used to alleviate a common bile duct obstruction, most frequently malignant.

The major complication of these stents is obstruction and subsequent upstream infection. Clinicians have looked at ways to prolong stent patency. A meta-analysis of randomized or quasi-randomized studies examining the role of antibiotics or ursodeoxycholic acid to maintain stent patency showed no conclusive evidence in favor of either one in the prevention of stent occlusion. In certain children and adolescents with a history of recalcitrant cholangitis, percutaneous transhepatic biliary drainage is used by experienced centers to decompress the biliary tree upstream of the stricture or to dilate the stricture. This procedure is used primarily in patients with PSC and in transplant patients with biliary stenoses secondary to ischemia or rejection.[10,36,56,62,74,209] These patients also benefit from oral antibiotic prophylaxis, frequently ciprofloxacin for its excellent biliary penetration.

CHOLECYSTITIS

Gallstones are the prime initiating factor in cholecystitis. Gallstone disease is a common entity in adults, with more than 700,000 cholecystectomies performed each year in the United States among approximately 20 million adults with gallstones.[109] In children, gallstones and gallstone-related complications occur much less frequently than in adults. In certain pediatric populations, such as patients with hemolytic conditions, gallstone and gallbladder diseases are more common, however, and need to be considered in appropriate clinical settings.[192] In addition, because gallstones are associated with obesity, an increase in pediatric-onset cholelithiasis may occur soon owing to the epidemic in childhood obesity.[193]

The term *cholelithiasis* refers to the presence of gallstones, which may occur silently or in association with clinical symptoms. Biliary colic occurs in the setting of obstruction of either the cystic duct or the common bile duct secondary to gallstones and is associated with characteristic episodic, postprandial right upper quadrant pain. Acute cholecystitis involves inflammation of the gallbladder and can be seen with cholelithiasis (calculous) or in the absence of gallstones (acalculous). In adults, acute cholecystitis almost always is associated with gallstones—90 percent of adult cases occur secondary to gallstones. In contrast, 30 to 50 percent of pediatric cases are acalculous.[216] Acalculous cholecystitis is discussed separately at the end of this section.

ETIOLOGY AND PATHOGENESIS

Gallstones generally are classified as either pigment stones or cholesterol stones. Pigment stones can be divided further into either black or brown pigment stones. Black pigment stones occur in the face of a superabundance of unconjugated bilirubin in the bile and are seen most commonly in hemolytic disease, leading to increased biliary concentration of bilirubin and to calcium-bilirubinate stones. Brown pigment stones are less common and are associated with biliary infection or parasitic infestation, or with the presence of a foreign body (e.g., retained suture material) or biliary obstruction. Cholesterol stones are the end result of biliary cholesterol supersaturation secondary to an imbalance in the cholesterol : phospholipid : bile salt equilibrium or other precipitating factors.[42] These stones tend to occur in the face of either elevated cholesterol production or decreased bile salt pool.

In pediatric practice, pigment stones are seen more frequently than are cholesterol stones, in direct contrast to adult populations, in which cholesterol stones are more prevalent. Friesen and Roberts[55] described 693 pediatric cases of gallstone disease, of which 72 percent involved pigmented stones. The greatest experience with pigmented gallstones in children is in children with hemolytic diseases, primarily sickle-cell anemia and thalassemia, but also in patients with hereditary spherocytosis and other red cell membrane defects, pyruvate kinase deficiency, glucose-6-phosphate dehydrogenase deficiency, and autoimmune hemolytic anemia.[77,167,190] The prevalence of pigment gallstones in hemolytic disease increases with age. In children younger than 10 years old, the frequency is 12 to 14 percent; the frequency increases to 36 to 42 percent in individuals 10 to 20 years old.[16,188]

Total parenteral nutrition predisposes children to develop biliary tract disease, and in particular gallstone formation.[165] This condition occurs more commonly in premature neonates, especially neonates with enteral diseases, in which prolonged fasting, sepsis, immaturity of the enterohepatic circulation of bile acids, small bowel bacterial overgrowth, and prolonged duration of total parenteral nutrition contribute to biliary stasis and increased prevalence of gallstones and sludge.[6,99,106,187,208,226] The occurrence of gallstones secondary to total parenteral nutrition does not lead to increased prevalence of complications, however, because many of these gallstones are clinically silent.

Cholesterol stones are found most frequently in adults and have an increased prevalence in women from puberty to menopause and in obese patients.[184] Some of these characteristics may translate to pediatrics as well. Although boys and girls exhibit an equal incidence in gallstones at young ages, the incidence in girls increases significantly after puberty, and female predominance of gallstone disease continues through menopause.[147] Related risk factors for the presence of cholesterol stones in children and adolescents include obesity, pregnancy, and the use of oral contraceptives. In addition, conditions that are associated with decreased ileal bile salt resorption predispose to stone formation. This association includes patients who have undergone ileal resection or bypass and patients with Crohn disease.[174]

Gallstones are found in 28 percent of adults with cystic fibrosis at the time of autopsy.[196] The presence of gallstones in children with cystic fibrosis is well documented, but the pathogenesis likely is multifactorial.

TABLE 54–4 Patient Populations Predisposed to Gallstone Formation

Pigment Stones	Cholesterol Stones
Hemolytic disorders	Pregnancy
Total parenteral nutrition	Obesity
Biliary tract anomalies (e.g. Caroli disease)	Rapid weight loss
Parasitic disease (*Ascaris lubricoides*)*	Malabsorption (e.g., Crohn disease, ileal resection)
Ceftriaxone use	Genetic predisposition (e.g., *ABCB4* homozygosity)
Solid organ transplantation (heart, lung, kidney)	Cystic fibrosis and other cholestatic liver diseases

**Brown pigment stones.*

Finally, children with chronic liver disease, and in particular cholestasis, are at increased risk for gallstone formation, owing to a deficit of biliary excretion.[229] Table 54–4 summarizes the pediatric patient populations at risk for development of gallstones.[57]

The genetics of biliary stone formation has been the focus of intense research. The consensus is that biliary stone formation, affecting the gallbladder and the common bile duct, is the product of genetic predisposition and environmental risk factors. In adults, a distinction is made between the "common" polygenic predisposition to gallstones and the rarer oligogenic predisposition. Briefly, the first, polygenic group refers to patients with evidence of increased risk of developing biliary stone formation, probably associated with numerous factors, including lithogenic (*LITH*) genes. The oligogenic group refers to a small group of patients with known mutations in genes involved in bile synthesis and export.[72,229] The clearest examples to date are defects in the *ABCB4* transporter in humans[175] and the *Mdr2* homozygous null mutant in mice.[108] Both conditions lead to an increased propensity toward cholelithiasis and choledocholithiasis; however, whether these subjects have a profile of biliary tract infections different from that of the polygenic group is unknown.

The most likely cause of calculous cholecystitis is gallstone obstruction of the cystic duct, which leads to increased intraluminal pressure with gallbladder distention, mucosal damage, and release of inflammatory mediators. The end result is acute inflammation of the gallbladder. Any bacterial infection probably is a secondary occurrence to biliary obstruction.[109]

CLINICAL PRESENTATION

Acute cholecystitis typically manifests with abdominal pain and vomiting. Pain may be localized to the right upper quadrant; however, in pediatric patients, this making distinction can be difficult, and it may be difficult to differentiate from other causes of acute abdominal pain in children. As in the model of appendicitis, initial pain with obstruction of the cystic duct tends to be visceral pain that may be epigastric and poorly localized. With gallbladder inflammation, the pain becomes parietal—more localized to the right upper quadrant and associated with some peritoneal signs, such as pain with movement. With regard to the duration of pain, cholecystitis should produce pain that is more long-standing than uncomplicated biliary colic. Although obstruction of the cystic duct is associated with the acute onset of pain followed by resolution within 3 to 6 hours, true gallbladder inflammation may produce a more persistent pain, lasting 6 to 12 hours or longer.

The presence of jaundice with right upper quadrant pain and emesis in a patient without hemolysis or increasing jaundice in hemolytic patients warrants a thorough investigation for the possible presence of choledocholithiasis and cholecystitis. Fever, although classically described in the presentation of cholecystitis, is not universally present. In a retrospective study of 198 adults who presented to an emergency center with acute cholecystitis, 59 percent were afebrile.[71] Physical examination may show a tender right upper quadrant. The examiner should try to elicit Murphy's sign, for which tenderness on palpation of the right upper quadrant worsens with palpation of the liver edge or gallbladder and may be most apparent with deep inspiration. The patient may exhibit voluntary guarding or peritoneal signs. A palpable mass, representing the inflamed gallbladder with adjacent omentum, may be present.

EVALUATION

Laboratory evaluation may show leukocytosis, although this finding is not universally present in adults or children with cholecystitis.[71,165] Bilirubin levels may be elevated, especially in the setting of hemolysis. A conjugated hyperbilirubinemia should cause one to be concerned about choledocholithiasis, or sepsis-associated cholestasis. Transaminases and amylase and lipase are ordered frequently on initial evaluation to investigate for other causes of epigastric or right upper quadrant pain with vomiting or fever or both, such as pancreatitis and viral hepatitis. As in the evaluation of cholangitis, early consultation with a pediatric surgeon is appropriate.

Abdominal ultrasound is the test of choice in establishing the diagnosis of cholecystitis. Findings on ultrasound include a thickened or irregular, hyperreflexive gallbladder wall with or without the presence of gallstones. Experienced sonographers may be able to elicit tenderness over the gallbladder, the "sonographic Murphy sign." Ultrasound is useful for detecting gallstone disease, showing 90 percent in some cases, although in other studies, ultrasound has a markedly lower sensitivity to detected common duct stones.[54] Without suspicion of gallbladder inflammation, however, the finding of gallstones alone by sonogram does not indicate cholecystitis. Other false-positive studies may occur with the finding of thickened gallbladder walls in patients with hypoalbuminemia, renal failure, or heart failure.[156] The inability to visualize the gallbladder by ultrasound may indicate the finding of a diseased, chronically obstructed gallbladder.[68]

Hepatobiliary scintigraphy (HIDA scan) can be useful in confirming the diagnosis of acute cholecystitis. The study employs a technetium-labeled iminodiacetic acid agent that is excreted into the bile ducts. Subsequent images are taken, which in a normal study should fill the gallbladder, extrahepatic ducts, and duodenum. In the case of cystic duct obstruction, a positive study fails to show the gallbladder filling. The sensitivity and specificity of this study are high, with results approaching 90 to 100 percent and 90 to 95 percent.[143,166] The test may be augmented by attempts to stimulate gallbladder contraction using a cholecystokinin analogue. Plain abdominal radiographs and oral cholecystography are infrequently used in the current clinical environment.

ACALCULOUS CHOLECYSTITIS

Acalculous cholecystitis is a rare but important cause of cholecystitis in pediatric patients. Generally, acalculous gallbladder disease in children occurs in various clinical settings, ranging from congenital gallbladder abnormalities, to idiopathic gallbladder distention without inflammation (acute hydrops of the gallbladder), to acute or chronic cholecystitis. The exact pathogenesis of acute acalculous cholecystitis is unclear; however, stagnation of the bile may be an important factor, as suggested by several predisposing clinical conditions that lead to bile stasis. In addi-

TABLE 54–5 Conditions Associated with Acalculous Gallbladder Disease

Biliary tract anomaly (e.g., choledochal cyst)
Bone marrow transplant
Burns
Chemotherapy in oncology patients
Critical illness in ICU patients
Crohn disease, Kawasaki disease, systemic lupus erythematosus
Infectious agents (atypical microbes)
Postoperative state (e.g., cardiac surgery)
Sepsis
Sludge
Systemic inflammatory states
Total parenteral nutrition
Traumatic spinal cord injury

ICU, intensive care unit.

tion, sphincter of Oddi spasm or dysfunction, as occurs after administration of opiates, could lead to retrograde reflux of inflammatory enzymes or infectious agents. Alternatively, changes in the gallbladder vascular supply may weaken the gallbladder mucosa and allow biliary components to damage the gallbladder wall.[64] Acalculous cholecystitis has been associated with many infectious and noninfectious clinical scenarios. Bacterial, viral, and parasitic agents have been implicated in the setting of acalculous cholecystitis. Frequently, gallstone or biliary microlithiasis missed by standard diagnostic evaluations may lead the clinician to the presumptive diagnosis of acalculous cholecystitis. One adult study revealed that 33 of 35 patients with abdominal pain and a negative transabdominal sonogram had gallbladder sludge and stones, and 21 of those patients also had common bile duct involvement.[136]

Reports of true acalculous disease have been described with systemic infection from *Salmonella* spp., *Mycoplasma pneumoniae*, *Leptospira interrogans*, *Brucella* spp., Rocky Mountain spotted fever, group A streptococci, group B streptococci, and *Staphylococcus aureus*.[8,9,39,78,82,131,198,212,230] Examination and culture of cholecystectomy specimens from patients with acalculous cholecystitis have revealed positive cultures for *Helicobacter* spp. and *Campylobacter jejuni*.* Associations with viral disease include CMV, mumps virus, Epstein-Barr virus, and hepatitis A virus.[2,11,12,13,19,28,60,161] Acalculous cholecystitis has been associated with *Plasmodium falciparum* infections (malaria).[46,126,179,186,233] In immunosuppressed hosts, acalculous cholecystitis also has been described in fungal infections with *Candida* or *Aspergillus*, or parasitic infections with *Giardia* and *Cryptosporidium*.[144]

Noninfectious systemic diseases associated with acalculous cholecystitis or hydrops include neoplastic disease (i.e., leukemia), Henoch-Schönlein purpura, lupus erythematosus, and Kawasaki disease.[25,83,129] Table 54–5 lists several conditions with which acalculous cholecystitis has been reported.[86,88,171,203,213,214]

Clinical presentation is similar to that of patients with calculous disease, with right upper quadrant pain, nausea, vomiting, anorexia, and fever being the most common symptoms. Leukocytosis, jaundice, or a palpable right upper quadrant mass also may be present.[216] The most common setting for acalculous disease is in critically ill or chronically ill patients with concurrent acute or chronic symptoms. A high degree of clinical suspicion is crucial in making the diagnosis in these populations because often the findings suggestive of cholecystitis are obscured by the patient's systemic disease. The most common sonographic finding is a thickened gallbladder wall and a possible sonographic Murphy sign. Gallbladder distention, sludge, or pericholecystic fluid also may be found on transabdominal ultrasound.[87] In addition to ultrasound, the HIDA scan is an important tool in the evaluation of acalculous cholecystitis. Failure to visualize the gallbladder on scintigraphy and a low ejection fraction in response to a cholecystokinin analogue are suggestive of acalculous disease.[127,143,162,236]

MANAGEMENT

Management strategies of gallstone disease in children vary, contingent on individual clinical scenarios. Silent gallstones, which are asymptomatic and often detected incidentally by ultrasound, can be followed conservatively and sonographically without emergent or elective surgery. Although conservative management of adults with asymptomatic stones is described in multiple reports, no such guidelines exist for children. Patients with hemolytic disease in whom gallstones are detected should be considered for early cholecystectomy, although this plan is controversial.[4,73,91,135,225] In patients with sickle-cell anemia, the benefit of timely surgery may outweigh the morbidity of associated abdominal pain crises and the overall morbidity and mortality of surgery in these patients, which increases with age.

In acute calculous cholecystitis, surgery is the mainstay of therapy. Patients should be admitted to the hospital for monitoring, intravenous hydration, and pain control. Antibiotic therapy often is initiated, even without clinical evidence of sepsis or perforation. The choice of antibiotics is similar to that for cholangitis and typically covers gut luminal flora (e.g., a synthetic penicillin plus an aminoglycoside with or without the addition of mezlocillin).[75,227] Surgical treatment may be limited to cholecystotomy, especially in chronically ill children with a high risk of developing complications. Generally, early cholecystectomy should be performed in patients with acute cholecystitis secondary to gallstone disease. Laparoscopic procedures are becoming more widely used in experienced pediatric surgical centers and are associated with positive outcomes in children and adults.

Optimal management of acalculous cholecystitis has not been investigated fully, primarily because of the lack of a diagnostic standard and varied patient population base. This situation leads to an individualized approach that typically starts conservatively with observation but may involve removing oral feeds and placing the patient on intravenous hydration and antibiotics, or it leads to more invasive management with cholecystectomy or biliary manometrics via ERCP.[63,87] Attempts should be made to diagnose and treat any underlying condition in the patient. A more recent treatment protocol supports the close monitoring of these patients with frequent examinations, ultrasonography, and blood tests to determine if the need for cholecystectomy or ERCP seems likely. If ultrasound shows improved findings, conservative management is continued with close follow-up. If biliary dyskinesia is suspected secondary to sphincter of Oddi dysfunction, ERCP offers a diagnostic and therapeutic option at experienced centers.[51,160,218]

COMPLICATIONS

Acute cholecystitis may lead to complications, even if it is recognized in a timely fashion. An inflamed, obstructed gallbladder may wall off to form an intraluminal abscess or empyema. Perforation of the gallbladder is the most frequent complication. A localized perforation may develop into a pericholecystic abscess that is palpable as a tender right upper quadrant mass on examination. Free perforation with peritonitis is associated with a 30 percent mortality rate, but it is found in only a few patients, estimated at 1 to 2 percent of adult patients.[109] In each of these

*See references 7, 22, 23, 53, 97, 111, 117, 128, 133, 134, 210, 223.

situations, prompt surgical intervention and intravenous hydration and antibiotic therapy are crucial in management.

Aside from gallbladder inflammation, complications of gallstone disease may include pancreatitis and choledocholithiasis. Gallstone pancreatitis was a frequent complication of gallstone disease in a series of 50 pediatric patients.[165] Choledocholithiasis with ascending cholangitis, a well-known complication of gallstones, is a rare cause of cholangitis in pediatric patients. Secondary common bile duct stones, usually originating from the gallbladder, can be found in patients with acute cholecystitis by ultrasound, ERCP, MRCP, or intraoperative cholangiography. The possible presence of choledocholithiasis should be considered in any patient with known gallstone disease who presents with increasing jaundice, fever, and right upper quadrant pain, and requires urgent clinical investigation and decompression, typically via ERCP.[206]

BILIARY TRACT INFECTIONS AFTER CARDIOTHORACIC SURGERY

In adults, the association between cardiothoracic surgery and biliary complications is well known and covered extensively in the literature.[123,139,217] These complications frequently manifest as cholangitis or cholecystitis, commonly with stone or sludge formation. The high incidence of biliary complications that develop after cardiothoracic surgery is ascribed to prolonged fasting, metabolic disturbances, vagotomy, and hypoperfusion. The last, in particular, is thought to predispose the biliary tree, which is exquisitely reliant on adequate arterial perfusion, to development of focal ischemia and secondary strictures. In pediatrics, although it is commonly accepted by clinicians as a frequent association, no studies have been published. The clinical implications of the adult studies and unpublished pediatric observations is that patients may present after cardiothoracic surgery with acute biliary disease warranting aggressive empiric management occasionally in immunosuppressed hosts who have undergone thoracic organ transplantation.

Infections of the intrahepatic and extrahepatic biliary tree in children differ from infections in adults by the nature of the populations they affect. First, patients with biliary atresia and portoenterostomy are subject to ascending cholangitis and the risk of developing sepsis and rapidly progressive liver disease. Second, children with hemoglobinopathies are prone to developing cholecystitis because of their increased incidence of cholelithiasis. In these two groups and in other pediatric patients, the same principles of management apply: Biliary obstruction must be sought and alleviated when possible, and aggressive parenteral antimicrobial therapy must be initiated promptly because of the risk of developing sepsis and worsening liver disease.

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CHAPTER 55

Pyogenic Liver Abscess

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Pyogenic liver abscesses are encountered infrequently in healthy children and generally have been reported more commonly in the compromised pediatric host. The rarity of liver abscesses may be explained partly by the rich blood supply, unique architecture, and extensive reticuloendothelial system of the liver, all of which present an effective barrier against bacterial invasion.

The precise incidence of pyogenic liver abscesses in children is unknown. Adult patients with hepatic abscesses constitute approximately 8 to 20 cases per 100,000 admissions; a 0.29 to 0.57 percent incidence of liver abscesses has been found in autopsies of adult patients.[11,48] In an early large series of liver abscesses in children, Dehner and Kissane[10] reported a 0.38 percent

incidence at autopsy in patients younger than 15 years old; 11 of 27 (41%) patients were younger than 2 years, and 18 of 27 (67%) patients were younger than 6 years. In a review of admissions to Milwaukee Children's Hospital, Chusid[9] found five children (four of whom were <6 months old) with at least one hepatic abscess, and estimated an incidence of 3 cases for every 100,000 admissions. Pineiro-Carrero and Andres[43] estimated an incidence of approximately 25 cases per 100,000 admissions in their pediatric population (11 patients >14 years old). Pyogenic liver abscess in children is encountered more frequently in developing countries compared with developed countries.

PATHOGENESIS

Bacteria can establish an inflammatory focus in the liver by four major routes. Direct extension from contiguous structures is the most common mode in adults and precedes up to 60 percent of hepatic abscesses in adults.[7,24,44,48] Biliary tract infection (cholangitis, cholecystitis), pancreatitis, and penetrating gastric or duodenal ulcer are examples of diseases associated with liver abscesses caused by extension from a contiguous focus of infection. In a review of this problem at St. Louis Children's Hospital, 3 of 27 children (11%) were considered to have a liver abscess secondary to inflammation of contiguous organs.[10] Although biliary tract disease occurs infrequently in children, ascending cholangitis is a particularly frequent complication of the hepatic portoenterostomy procedure for congenital biliary atresia and may lead to infections of the liver in such patients.[14] Liver abscesses also may develop as a complication of liver transplantation, especially if technical problems related to vascular supply or biliary drainage develop.[30]

The portal system is the second most common route by which bacteria may reach the liver in adults; 6 to 27 percent of liver abscesses in adults derive from this source.[24,32,48] In newborns, solitary liver abscesses, especially abscesses caused by gram-negative organisms, have complicated the use of umbilical vein catheterization or have been secondary to omphalitis.[6] Prematurity and necrotizing enterocolitis also are important predisposing conditions.[13]

Portal vein inflammation and bacteremia can be associated with infections within the abdominal cavity. Appendicitis, diverticulitis, perirectal abscesses, regional enteritis, ulcerative colitis, and omphalitis are possible sources of portal vein sepsis.[10] A pyogenic liver abscess may be an unusual complication of an ingested foreign body, with subsequent development of portal venous bacteremia.[38] Since antibiotics have been available, portal vein inflammation and pyelophlebitis have become less common sources of hepatic infection in children.

Systemic bacteremia with hematogenous spread of bacteria to the liver through the hepatic artery seems to be the most common source of liver abscess in children, but it is implicated in less than 20 percent of adult patients. In the St. Louis series, the systemic hematogenous route was responsible for 21 of 27 (78%) cases of liver abscesses.[10] In five of the patients examined before 1940, the bacteremia was associated with infection that would be considered manageable today (pneumonia, cellulitis, and osteomyelitis). Seven of 13 patients encountered after 1940 had bacteremia associated with leukemia. Anaerobic bacteremia associated with retropharyngeal or peritonsillar abscesses presumably has preceded development of anaerobic liver abscesses in several children.[8] Likewise, liver abscesses in neonates may be preceded by a systemic bacteremia without evidence of portal or biliary tract involvement.[35]

Liver abscesses occur more frequently in compromised pediatric hosts than in healthy children. Johnston and Baehner[25] reported that hepatic or perihepatic abscesses were present in 41 of 92 (45%) patients with chronic granulomatous disease. In a registry of 368 patients with chronic granulomatous disease from the United States, a liver abscess occurred in 27 percent of patients.[57] Over the course of 10 years, 15 children were diagnosed with pyogenic liver abscess in a large referral center for pediatric liver disease in the United Kingdom.[36] Three children (20%) had chronic granulomatous disease. In addition to functional disorders of phagocytes, chronic neutropenia predisposes to the development of liver abscesses.[42] Wintch and colleagues[58] noted that 5 of 10 children with hepatic abscesses in their institution had an underlying defect in host defense. Primary hemochromatosis predisposes to multiple liver abscesses caused by *Yersinia enterocolitica* in particular.[54] Pyogenic liver abscesses also are associated with Papillon-Lefévre syndrome, a rare autosomal recessive disease characterized by palmoplantar keratoderma and periodontitis.[1]

Penetrating and nonpenetrating trauma to the liver may lead to liver abscesses, presumably caused by bacterial proliferation within small collections of blood and bile that result from the trauma. Hepatic abscess may be a rare complication of ventriculoperitoneal shunts after penetration of a peritoneal catheter into the liver.[39] This mode of infection has been reported in seven children.[22] Liver abscess also is a complication of percutaneous liver biopsy.[16]

Unexplained or cryptogenic hepatic abscesses are encountered in most series and accounted for 40 to 50 percent of cases in many series.[24] Lee and Block[33] have proposed that these cryptogenic liver abscesses "originate from anaerobic bacterial invasion of hepatic infarcts." This theory is supported by reports that describe pyogenic liver abscesses as a complication of hepatic infarction in patients with sickle-cell anemia.[50] Normal gastrointestinal bacterial flora were isolated from 9 of 11 patients with liver abscesses at the Mayo Clinic. This finding suggested to Lazarchick and associates[32] that unrecognized intra-abdominal collections of pus were responsible. Although the reasons are unclear, diabetes mellitus also predisposes to the development of liver abscesses.[21,44] Nematode infection with larvae migrating through the liver is thought to be another predisposing factor for the development of pyogenic abscesses in children. The larvae induce liver granulomata that trap bacteria, leading to formation of an abscess.[41] In one study from Brazil, positive serology for *Toxocara canis* was significantly more frequent for patients with pyogenic liver abscess (10 of 16) than for the 32 age-matched controls (4 of 32).[46]

Biliary tract disease generally predisposes to the development of multiple liver abscesses. In contrast, blunt trauma to the liver or portal system inflammation most commonly predisposes to a single abscess. In neonates, liver abscesses may be solitary or multiple because of systemic bacteria.[35,37] Solitary abscesses are the most common findings in the right lobe of the liver.[32]

Hepatic and splenic abscesses caused by *Candida* spp. are well described in patients with cancer.[52] Multiple abscesses are typical findings. These organs presumably are infected hematogenously, usually when the host is neutropenic.

MICROBIOLOGY

Gram-negative organisms have been the predominant isolates from liver abscesses in adults. *Escherichia coli*, *Klebsiella*, *Aerobacter*, *Pseudomonas*, and *Proteus* spp. have been implicated most frequently. *Klebsiella* spp. were the most common organism isolated from children with pyogenic liver abscess in Taiwan.[53] *Klebsiella* spp. also predominated in adult Asian patients in a report from New York.[45] Anaerobic organisms also are important; anaerobic organisms were recovered from 45 percent of patients with liver abscesses in the UCLA series.[49]

In contrast to the adult experience, Dehner and Kissane[10] reported that 33 percent of liver abscesses in children were caused

by *Staphylococcus aureus*, whereas gram-negative organisms were found in only 32 percent. Two or more organisms were recovered from liver abscesses in 52 percent of children. In a review of 96 children (no neonates) with pyogenic liver abscesses, *S. aureus*, gram-negative enterics, and anaerobes were the organisms isolated most commonly, in that order.[28] *S. aureus* is the most common isolate that causes pyogenic liver abscess in patients with chronic granulomatous disease. In neonates, gram-negative enterics are isolated most commonly. Anaerobes, particularly *Fusobacterium necrophorum*, have been isolated from liver abscesses in children without underlying disease.[15] Human rotavirus–like particles were identified in the material aspirated from a liver abscess, but they were considered a secondary phenomenon and not the primary etiology of the liver abscess.[19] Fungi, particularly *Candida albicans*, have been associated with liver abscesses in children with leukemia and neutropenia who have received parenteral hyperalimentation.[3] Liver or splenic abscesses also may be an unusual complication of brucellosis.[55]

CLINICAL MANIFESTATIONS

The clinical manifestations of pyogenic liver abscesses are nonspecific. A high index of suspicion and an awareness of this illness are necessary to establish the diagnosis. A history of preceding abdominal surgery or trauma is helpful when present, as is the knowledge that the hosts response to infection is compromised.

Fever, nausea, vomiting, anorexia, weakness, and malaise are prominent symptoms that may last several weeks. Abdominal or pleuritic pain, weight loss, and diarrhea are less common manifestations. A history of abdominal pain and fever of unknown origin in an otherwise healthy child suggests the diagnosis of pyogenic liver abscess.[27] In contrast, fever often is not observed in neonates.[13] Patients with a macroscopic or single abscess frequently experience a subacute to chronic course. In contrast, patients with multiple abscesses generally experience a more acute febrile illness.

Hepatomegaly occurs in 40 to 80 percent of patients; abdominal tenderness occurs less frequently. Right upper quadrant tenderness or even a mass may be subtle and not appreciated, unless the physician specifically and carefully examines this region. Other physical findings include jaundice (generally associated with biliary tract disease and not liver abscesses), abdominal distention, and evidence of pleuropulmonary involvement (i.e., elevated or fixed hemidiaphragm, rales, and pleural effusion).

DIAGNOSIS

Routine laboratory studies are of little help in attempting to establish a diagnosis. Anemia, leukocytosis, and an elevation in C-reactive protein are common findings. Liver function tests generally reflect underlying disease of the liver itself and usually are not caused by the abscess. When abscesses occur secondary to biliary tract obstruction, alkaline phosphatase and bilirubin concentrations generally are elevated. Transaminase concentrations usually are normal to mildly elevated in most cases. A rapidly enlarging, tender liver in a patient with normal transaminase concentrations should alert the clinician to the possibility of liver abscess. Lazarchick and colleagues[32] found that the serum albumin concentration was the most important test with regard to prognosis; 14 of 16 patients with a serum albumin level of less than 2 g/dL died.

Blood cultures are positive more commonly in patients with multiple abscesses than in patients with solitary abscesses. Overall, however, blood cultures usually are sterile in children with pyogenic liver abscess.

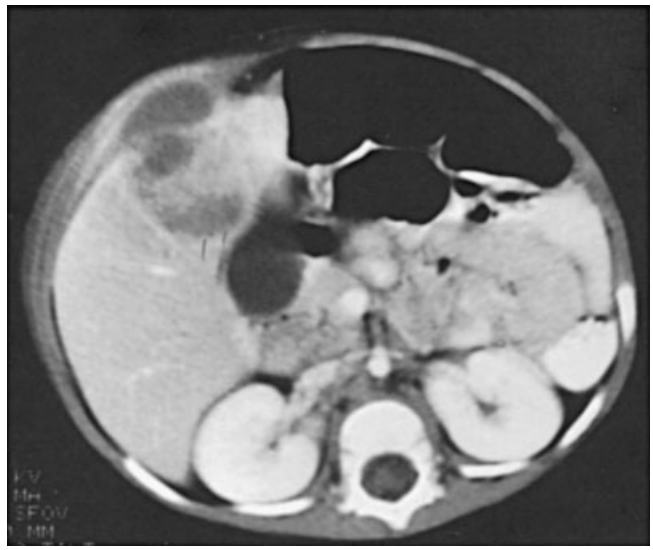

Figure 55–1 Abdominal computed tomography scan showing a 4-cm × 5-cm encapsulated, septate, circular mass within the liver. A low-density soft tissue mass is noted in the abdominal wall from apparent extension from the intrahepatic mass.

More than 50 percent of adult patients have abnormalities on chest radiography. Atelectasis, pulmonary infiltrates, pleural effusion, and elevated or fixed right hemidiaphragm are the most common findings.

Computed tomography (CT) currently provides the most accurate information concerning the size, location, and number of abscesses within the liver parenchyma (Fig. 55–1).[26,31,43] Lesions measuring 1 cm in diameter can be detected by CT. Multiple small abscesses may appear in clusters in a pattern suggesting early coalescence of the abscesses.[23] Liver abscesses appear as areas of low attenuation. The "target" lesions of hepatic candidiasis are not visualized by CT when the patient is neutropenic, and scans may need to be repeated before these characteristic lesions are observed.[52] Structures contiguous with the liver also are shown by CT; this information is important when a surgical approach to drainage is being planned.

Magnetic resonance imaging does not have any major advantages over CT for detecting or characterizing liver abscess but may show characteristic features to distinguish an abscess from other focal liver lesions in selected patients.[2,34] Ultrasonography also is a sensitive technique for detecting liver abscesses, and because it is noninvasive and does not require exposure to radiation, it is recommended for initial evaluation.[29,31] Hepatic angiography defines the vascular anatomy in the area of the liver abscess further and may provide information necessary for surgical management in selected cases. Nuclear medicine techniques rarely are indicated as a diagnostic method if liver abscess is suspected.

TREATMENT

Numerous reports have documented that patients with undiagnosed and untreated liver abscesses generally die and that surgical drainage of the solitary pyogenic liver abscess is the key to successful treatment. The choice of extraserous or transperitoneal open drainage or percutaneous closed aspiration depends on the location and size of the abscess and the experience and preference of the surgeon.[18,24,40,48]

Numerous groups have described percutaneous catheter drainage of liver abscesses in adults.[4,5,17,29] A catheter is placed

into the cavity under CT or ultrasound guidance; material is aspirated, and when an abscess is documented, a draining catheter is placed. The cavity can be irrigated with saline initially. Criteria for the selection of patients for percutaneous drainage have been established.[17] Generally, percutaneous drainage is not indicated for patients with multiple large abscesses or multiloculated abscesses.[24] The optimal route of percutaneous aspiration is directly into the abscess cavity and does not involve any uninfected organs or space. Drainage may proceed for 2 weeks or more, or until drainage from the cavity is decreased, the patient is afebrile and improving, and radiography shows that the cavity is becoming smaller.[20] Surgical backup is mandatory when this drainage technique is used because spillage of abscess material into the peritoneal cavity, hemorrhage, and other complications may occur. Percutaneous drainage of liver abscesses in children has been performed successfully, and this technique should be considered an alternative approach to surgical drainage of such abscesses, especially in the right lobe of the liver.[12,43]

Appropriate antibiotic therapy initially is based on knowledge of the organisms most commonly involved, Gram stain of the purulent material, and culture and susceptibility to antibiotics of the organisms that are recovered. If a hematogenous source of infection is suspected or the host has an immunodeficiency disease, *S. aureus* and streptococci are more likely. Biliary tract disease and blunt trauma are associated more frequently with gram-negative aerobic and anaerobic organisms. A logical antibiotic combination for the initial therapy of children with liver abscesses includes a penicillinase-resistant penicillin, such as nafcillin, plus an aminoglycoside. Vancomycin or clindamycin is selected if strains of *S. aureus* resistant to methicillin are present in the community.

The optimal duration and route of administration of antibiotics for a child with a solitary pyogenic liver abscess that has been drained have not been determined. Generally, 2 to 4 weeks of antibiotic therapy administered parenterally, followed by an appropriate oral antibiotic to complete a minimum 4-week total course, should be adequate. Penicillin, ticarcillin-clavulanate, piperacillin-tazobactam, clindamycin, cefoxitin, or metronidazole is administered for anaerobic isolates, depending on susceptibility. Meropenem is useful for polymicrobial infections, including infections caused by gram-negative aerobic and anaerobic rods.

Multiple liver abscesses are more difficult to treat because achieving complete surgical drainage usually is impossible. Prolonged antibiotic therapy plus treatment of any underlying illnesses is the keystone to effective management. Duration of treatment can be modified, depending on the evidence for resolution of the abscesses as determined by repeated ultrasound examination or CT.

Fungal liver abscesses are difficult to document by culture of the abscess material; histologic evidence of a fungal infection of the liver must be sought.[3] Amphotericin B, with or without flucytosine, is administered in the treatment of fungal liver abscesses.[52] In a neutropenic rabbit model, combination therapy with amphotericin and flucytosine was superior to amphotericin B alone in clearing disseminated candidiasis.[51] The optimal duration of therapy is unknown, but prolonged therapy, guided by repeated CT scans and biopsies, should be provided until the lesions have resolved.[52] Liposomal preparations of amphotericin B or other antifungal agents, such as caspofungin, may be beneficial for selected children with hepatosplenic candidiasis who are intolerant of or failing treatment with traditional amphotericin B.[56]

COMPLICATIONS AND PROGNOSIS

Complications of hepatic abscesses vary and are relatively common. Twenty-eight percent of the patients described by Rubin and associates[48] and 44 percent of the patients studied by Pitt and Zuidema[44] had one or more complications. Possible complications include pleural and pulmonary inflammation, peritonitis, subphrenic or subhepatic abscesses, and hemobilia.[10,48]

Polymicrobial bacteremia, hypoalbuminemia, multiple liver abscesses, or the presence of any complication is associated with increased mortality rates in patients with liver abscesses. Overall mortality rates depend largely on underlying pathologic processes and are difficult to interpret. Mortality figures from more recent reports range from 2.5 to 11 percent in adults.[24,45] In children, the prognosis for pyogenic liver abscess is excellent, generally with a low chance of mortality.[36] An increased awareness and suspicion of liver abscesses, in conjunction with ultrasound or CT of the liver, substantially reduces the mortality rate of this disease.

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CHAPTER 56

Reye Syndrome

Eugene S. Hurwitz

A syndrome involving the acute onset of encephalopathy associated with fatty metamorphosis of the liver and occurring primarily in children was described first by Reye and colleagues[8] in Australia and by Johnson and associates[7] in the United States. The similarities of these two descriptions in separate countries led to the common designation of this clinicopathologic entity as *Reye-Johnson* or *Reye syndrome*. Reye syndrome occurs most frequently after a viral illness and is characterized by the onset of severe vomiting followed by the development of encephalopathy and hepatic dysfunction. The recognition in the early 1980s that the syndrome is associated with the ingestion of aspirin during the antecedent viral illness led to public awareness of this association, a decline in aspirin use for such illnesses in children, and a dramatic decline in the occurrence of this disease in the United States.[1]

EPIDEMIOLOGY

In the United States, national surveillance for Reye syndrome was conducted first during the 1973 to 1974 nationwide outbreak of influenza B and influenza A (H1N1). Such surveillance led to the recognition of outbreaks of Reye syndrome regionally and nationally that were associated with outbreaks of influenza in these and subsequent years.[2] During the first 5 years of surveillance, 250 to 550 cases were reported nationally—an underestimate because it was based on voluntary reporting.[9] Population-based studies conducted in several geographic locations showed that the average annual incidence of the syndrome was one or two cases per 100,000 children younger than 18 years old. Adults rarely were affected. Case-fatality rates reported through national surveillance, initially 40 percent, declined to 20 to 30 percent in later years when the syndrome was prevalent, although this rate undoubtedly was an overestimate because of the tendency to report more severe and fatal cases through this system.

Between 1980 and 1982, four case-control studies reported an association between Reye syndrome and the ingestion of aspirin during an antecedent respiratory or chickenpox illness.[3,11,12] The results of these studies subsequently were confirmed in the Public Health Service Pilot and Main Studies of Reye Syndrome and Medications.[4,5] In these studies, more than 90 percent of patients with Reye syndrome compared with 40 to 70 percent of controls had received aspirin for the antecedent respiratory or chickenpox illness; reported odds ratios were 11.5 to 40. After these studies were reported, publicity and recommendations from various expert panels, including recommendations issued by the Food and Drug Administration in 1985, led to a decline in the use of aspirin and a decline in the incidence of Reye syndrome, particularly in the age group that had been affected most—children 5 to 15 years old.[1]

CLINICAL ILLNESS AND LABORATORY FINDINGS

Reye syndrome is described classically as an illness characterized by the abrupt onset of severe vomiting and progressive encepha-

lopathy in a child who is just recovering from a viral illness, the most common of which are influenza and chickenpox. The onset of these symptoms typically occurs within several days after the onset of the viral illness and commonly during a period when the child seems to be recovering from this illness. In association with severe—often projectile—vomiting, which occurs for a transient period, are progressive encephalopathic changes that may follow stages from delirium through confusion, agitation, and lethargy to coma if untreated.

The definition used by the Centers for Disease Control and Prevention (CDC) and widely adopted for clinical purposes includes (1) evidence of acute encephalopathy manifested by alterations in consciousness and documented, when available, by cerebrospinal fluid with less than 9×10^6/L leukocytes or by biopsy or autopsy evidence of cerebral edema without perivascular or meningeal inflammation in histologic sections of the brain; (2) evidence of liver involvement, including either biopsy or autopsy findings of fatty metamorphosis of the liver if available or, in the absence of such specimens, elevations in liver enzymes (alanine aminotransaminase, aspartate aminotransaminase, or serum ammonia) that typically are more than three times normal levels; and (3) no other more reasonable explanation for the cerebral or hepatic abnormalities. The last requirement emphasizes that Reye syndrome is a diagnosis of exclusion, and that every effort should be undertaken to identify other possible causes for the clinical and laboratory abnormalities.

Liver biopsy or autopsy findings are considered characteristic and include panlobular microvesicular fat and mitochondrial abnormalities on electron microscopic examination showing peroxisome swelling and enlarged pleomorphic mitochondria with loss of dense granules. Additional findings include normal bilirubin levels and absence of jaundice. Most patients also have hypoglycemia and a prolonged prothrombin time. The typically elevated cerebrospinal fluid pressure in patients leads to progressive stages of coma.

Staging criteria for Reye syndrome have been used to define the level of encephalopathy. Patients have been reported with liver involvement, but without evidence of encephalopathy. These patients have been described as having stage 0 encephalopathy and, although they do not meet the Centers for Disease Control and Prevention criteria for Reye syndrome, are considered to have mild disease. Patients with stage I encephalopathy are difficult to arouse and lethargic, whereas patients with stage II are delirious and combative with some movement. Patients with higher stages of encephalopathy (III to V) cannot be aroused and have progressively deeper stages of coma. These patients have a poor prognosis, with a mortality rate approaching 50 percent for patients admitted with stage III or greater and 90 percent for patients admitted with stage V.

Exclusion of other diseases that may resemble Reye syndrome, such as salicylate toxicity, is essential in patients with symptoms resembling this entity. Intensive laboratory investigations should be undertaken to exclude such disorders. In young children, particularly children younger than 3 years of age, inherited metabolic disorders frequently may mimic Reye syndrome and must be excluded. Such metabolic disorders include disorders of fatty acid oxidation, urea cycle disorders, carnitine transport defects, and organic acidemias. Laboratory studies must be performed for the younger age group to exclude these disorders before a diagnosis of Reye syndrome is made, particularly because some of these disorders can be treated effectively. With the declining incidence of Reye syndrome in the typical age group (5 to 15 years) after virtual elimination of the use of aspirin in children, an increasing number of patients with features of Reye syndrome are in this younger age group and ultimately are found, after careful evaluation, to have one of the many metabolic disorders that mimic this syndrome.

TREATMENT AND PREVENTION

The mainstay of treatment of Reye syndrome is early recognition of disease and supportive care focusing on various measures to control intracranial pressure and electrolyte and other abnormalities. Patients should have glucose levels monitored, and early infusion of glucose is considered by many physicians to improve outcome. Comatose patients should be transferred to tertiary care centers that have experience in caring for such patients and can monitor and treat elevated intracranial pressure. When such measures were undertaken before the decline in incidence of this disease, they were associated with improved outcome and decreased mortality. Other therapeutic measures that have been used include efforts to reduce ammonia levels, such as exchange transfusions, peritoneal dialysis, and total-body washout via cardiopulmonary bypass. With advances in supportive care, the mortality rate declined to 10 to 20 percent in later years before Reye syndrome became extremely rare.

Since the association between Reye syndrome and aspirin has been recognized, aspirin no longer is recommended or used for the treatment of febrile illnesses in children. Alternative antipyretics, including nonsteroidal anti-inflammatory drugs and acetaminophen (Tylenol), have replaced aspirin as the primary therapy for such illnesses. These medications have not been associated with an increased risk for development of Reye syndrome. Children with some disorders, including juvenile rheumatoid arthritis and Kawasaki disease, continue to be given aspirin to treat these disorders. Efforts to reduce the risk of development of Reye syndrome in these children have included influenza vaccination annually and vaccination against chickenpox. Careful monitoring of these children also is necessary to ensure early recognition and treatment of Reye syndrome should it occur.

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CHAPTER 57

APPENDICITIS AND PELVIC ABSCESS

Thomas L. Kuhls

The ability to diagnose appendicitis accurately in a child is one of the most fundamental skills that a pediatric surgeon has to master, although establishing the diagnosis often is difficult in young patients. The surgeon ultimately is responsible for deciding whether a child is taken to the operating room for an appendectomy; however, a primary care physician often is the first person to evaluate a patient who complains of abdominal pain. Pediatricians with expertise in infectious diseases frequently are involved in the care of children who present with subtle or atypical manifestations of appendicitis; have unusual microorganisms recovered from their appendices; or have complications as a result of appendiceal rupture, such as the development of wound infections, sepsis, peritonitis, intra-abdominal abscesses, and pelvic abscesses.

HISTORY

In the 16th century, physicians began describing patients with clinical manifestations suggestive of perforated appendicitis. Until the late 1800s, the inflammatory process was called *typhlitis* or *perityphlitis* because the illness was thought to originate from the cecum. In 1886, Fitz[52] recognized that the source of the inflammation was the appendix and suggested that a laparotomy be performed early in the course of the illness. Shortly afterward, McBurney[114] reported that in patients with appendicitis, tenderness was greatest 2 inches from the anterior iliac spine on a line drawn to the umbilicus. Despite intensive research and refinement of understanding of appendicitis during the 20th century, the rates of removing nondiseased appendices or finding already perforated appendices at laparotomy remained at approximately 20 percent until more recently when ultrasound and computed tomography (CT) examinations have been used routinely in children with suspected appendicitis, resulting in a possible decrease in misdiagnosis of the disease.

Despite the continued difficulties in diagnosing appendicitis, the mortality rate from appendicitis has decreased greatly since Fitz reported a 40 percent operative mortality rate. In 1936, Bancroft and Skoluda[16] reported a mortality rate of 8 percent and a complication rate of 11 percent for appendicitis, most likely because of the availability of general anesthesia and better aseptic surgical techniques. The second major improvement in outcome occurred in the 1940s, when sulfonamides and banked blood became widely available.[112] In the 1970s, anaerobes such as *Bacteroides fragilis* were found to cause postoperative infections frequently in patients with appendicitis, and treating these microorganisms was found to reduce further the rate of postoperative complications.[99] Currently, death from appendicitis is a rare occurrence in the United States.

EPIDEMIOLOGY

Reported incidences of acute appendicitis vary widely, depending on where the studies were performed and what methodologies were used. The consensus is that the number of cases of acute appendicitis has been decreasing during the past few decades.[86,115] Investigators have estimated that more than 70,000 children are diagnosed with appendicitis each year in the United States.[71] In 1997, appendicitis-related hospitalizations accounted for 0.6 percent of all hospitalizations in the United States, resulting in approximately 1 million hospital days and $3 billion in hospital charges.[40] Although appendicitis occurs in all age groups, the highest incidence occurs during the second decade of life.[3,107] Appendicitis is an uncommon event in children younger than 5 years of age and occurs extremely rarely in infants younger than 6 months. Researchers have suggested that patients with acquired immunodeficiency syndrome (AIDS) have a higher incidence of appendicitis than the normal population.[95]

Numerous studies have shown that the peak rates of appendicitis occur during the summer months, whereas the lowest rates occur during the winter months.[3,107] A study from Italy found a higher rate of pediatric appendicitis in winter, whereas appendicitis in adults was observed more frequently during the summer.[56] The reasons for these seasonal patterns are unknown, but changes in diet and exposure to allergens have been suggested as explanations.[107] Also, enteric infections occur most frequently during the summer and may play a role in increasing the incidence of appendicitis in summer.

Most studies show a modest increase in incidence of appendicitis in males compared with females.[3,107] An estimated lifetime risk for developing appendicitis is 8.6 percent for males and 6.7 percent for females.[3] In a study of acute appendicitis in California, the rate of appendicitis in whites was twice that of blacks and Asians.[107] More recently, Hispanics were shown to have the highest rate of appendicitis-related hospitalizations in the United States.[40] Whether the reported racial differences are due to errors of measurement, sociodemographic factors, environmental factors, factors related to body constitution, or genetic factors is unknown. Children with appendicitis more frequently have a history of having family members who previously have had appendicitis, suggesting that genetic background plays a role in the susceptibility to appendicitis.[18] Decreased dietary fiber and ingestion of refined carbohydrates also have been suggested to increase the risk for developing appendicitis.

The rates of perforation with appendicitis are significantly higher in Asian, Hispanic, and black children compared with non-Hispanic white children.[64,78,145,179] Similarly, children without health insurance or Medicaid have rates of perforation higher than those of children with private health insurance.[145,178]

Researchers have suggested that the pediatric rupture rate is a good candidate for inclusion in the National Healthcare Disparities Report.[78] Already, studies at local institutions have found no differences in perforation rates based on socioeconomic status, showing improved access to early medical care in their communities.[133,171]

PATHOPHYSIOLOGY

The initial event in the development of most cases of appendicitis is thought to be obstruction of the appendiceal lumen.[174] Microorganisms rarely invade the appendiceal mucosa and initiate the inflammatory process. Appendiceal obstruction can be caused by inspissated feces (fecalith), hypertrophied lymphoid tissue that develops during a systemic viral infection or bacterial enterocolitis, parasitic infestation, appendiceal wall hemorrhage associated with anaphylactic purpura, inspissated barium, or ingested seeds. Continued production of mucus by the appendiceal mucosa distal to the obstruction causes the appendix to distend. Vascular congestion and ischemia occur as the increased intraluminal pressure of the appendix becomes greater than the venous pressure, and edema develops as lymphatic flow becomes obstructed.

Stasis of intestinal flow and intestinal ischemia allow the microorganisms in the appendix to invade the tissues, enhancing the already developing inflammatory response. Bacteria may translocate across the appendiceal wall and reach the peritoneal cavity.[17] If the process is severe and arteriolar blood flow to the appendix is obstructed, transmural infarction occurs and the appendix ruptures. Microorganisms are liberated into the peritoneal cavity, causing generalized peritonitis and formation of an abscess. Animal studies have suggested that synergism occurring between enteric aerobes, such as *Escherichia coli*, and anaerobes, such as *B. fragilis*, is important in the development of intra-abdominal and pelvic abscesses after perforation.[62] An association between the development of an abscess after appendiceal perforation and the presence of *Streptococcus milleri* also apparently exists.[69]

As the appendix distends in the early stages of appendicitis, the visceral afferent autonomic nerves that enter the spinal cord at T8 to T10 are stimulated, referring the pain to the epigastric and periumbilical areas of the abdomen.[174] When the inflammatory response reaches the serosal surface of the appendix, the parietal peritoneum is stimulated, and the pain intensifies in the right lower quadrant. If perforation occurs, the peritoneal inflammatory response causes more generalized abdominal tenderness.

Although appendiceal obstruction may play an important role in the early stages of most cases of appendicitis, not all obstructed appendices become inflamed. Ten percent of normal appendices removed during abdominal surgical procedures contain inspissated fecal material. Also, children may develop recurrent, crampy abdominal pain, possibly from intermittent appendiceal obstruction.

The classic description of the pathophysiology of appendicitis does not explain easily many epidemiologic features of this disease, including its higher incidence in males and in certain races. The amount and reactivity of the lymphoid tissue in the wall of the appendix have been suggested to be key determinants to the development of appendicitis. The amount of lymphoid tissue in the appendix is greatest during adolescence, when the disease process is most prevalent, and the amount most likely is controlled genetically.

In a case-control study from Italy, prolonged breast-feeding during infancy was associated with a decreased risk for developing acute appendicitis later in life.[144] The investigators hypothesized that breast-feeding may have decreased the amount of stimulation to intestinal lymphocytes by microbial and food antigens early in life, so that appendiceal lymphoid tissues were less reactive to antigenic challenge during adolescence and adulthood. Appendicitis may be observed less frequently during infancy and the newborn period because the appendix is more funnel-shaped, the diet is primarily liquid, recumbent posture is maintained for prolonged periods, and gastrointestinal and respiratory infections develop less frequently during this time.[101]

CLINICAL MANIFESTATIONS

In school-aged children and adolescents with appendicitis, the median duration of symptoms before hospital admission is 24 to 28 hours.[151] Pain in the right iliac fossa is the most common sign of appendicitis, occurring in 88 to 99 percent of patients.[151,156] Pain shifts from the periumbilical area to the right lower quadrant of the abdomen in approximately two thirds of children with appendicitis. In three fourths of children, the pain worsens during movement. The characteristics of the abdominal pain do not always predict accurately which children have appendicitis. Of children found to have mesenteric adenitis at laparotomy, 25 percent report a shift in abdominal pain to the right iliac fossa and 33 percent experience worsening of pain during movement.[151]

Nausea and vomiting are found in 86 to 96 percent of children with appendicitis.[151,156] Vomiting usually occurs after the onset of abdominal pain but may precede pain in nearly 20 percent of cases. If only nausea or vomiting is present, it is less likely the child has appendicitis.[151] Anorexia occurs less commonly in children than in adults, occurring in 47 to 91 percent of cases of appendicitis. Because 50 percent of children found to have normal appendices at surgery complain of anorexia, differentiating appendicitis from other causes of right iliac fossa pain is not always helpful. Similarly, complaints of diarrhea (9-16%), constipation (5-28%), and dysuria (7%) occasionally can be elicited from children with appendicitis.[151,160] Fever may be helpful as a clinical sign of appendicitis if it is present (>37.5º C in 68-96% of cases), but absence of fever does not exclude the possibility of acute appendicitis. Very high temperatures (>39º C) suggest that perforation already has occurred or that another intra-abdominal process is present.[174] Rarely, children present with erythema and tenderness of the scrotum as the only manifestation of acute appendicitis.[216]

During the physical examination, the child frequently lies quietly on the examination table with the right hip flexed. Tenderness in the right iliac fossa is the most sensitive sign of appendicitis, occurring in 93 to 100 percent of cases of appendicitis.[151,156] The psoas muscle may become irritated from the inflamed appendix, causing the child to feel increased pain when the right hip is flexed actively. Likewise, if the obturator internus muscle is involved, pain is elicited when the flexed thigh is rotated internally.[174] Guarding is found in 80 to 91 percent of cases of appendicitis compared with 50 percent of cases of mesenteric adenitis and 8 percent of cases of nonspecific abdominal pain.[151] Similarly, rebound tenderness is found in 56 to 83 percent of cases of appendicitis, 33 percent of cases of acute mesenteric adenitis, and 1 percent of cases of nonspecific abdominal pain.[151]

The development of diffuse abdominal tenderness and the absence of bowel sounds usually indicate perforation. Extremely hyperactive bowel sounds suggest that the patient may not have appendicitis. Occasionally, a mass can be palpated in the right lower quadrant of the abdomen in children with appendicitis who are relaxed or well sedated. Rectal tenderness is present more commonly in children with appendicitis (44-68%) than in children with other causes of abdominal pain (12%); however, findings during the rectal examination seldom alter the clinical decision of the surgeon.[151]

In preschool-aged children, the diagnosis of appendicitis is more difficult to establish because of the inability of young children to express their symptoms and because they often do not cooperate during the physical examination.[210,211] Young children with appendicitis often are seen early in the course of their symptoms and are prescribed antibiotics, antihistamines, or antipyretics. By the time one realizes that the child has appendicitis, the appendix usually is perforated (50-90%).[210,211] In contrast to older children, in preschool-aged children vomiting is the initial symptom of appendicitis most frequently observed, and abdominal pain may be absent or may never localize in the right iliac fossa.[151] Sleep disturbances, irritability, restlessness, and crying are common manifestations of appendicitis in this age group. A preschool-aged child is more likely to have a palpable inflammatory mass at presentation.[174]

During the newborn period, appendicitis is an extremely rare occurrence.[101,164] Symptoms of neonatal appendicitis include abdominal distention; vomiting; irritability; diarrhea; erythema, edema, or cellulitis of the abdominal wall; gastrointestinal hemorrhage; abdominal rigidity; lethargy; and jaundice. Usually, the symptoms of neonatal appendicitis are indistinguishable from the symptoms of necrotizing enterocolitis. Underlying conditions, such as total colonic Hirschsprung disease, meconium plugs, or hernias, may predispose a newborn to developing this condition.

In children who are undergoing chemotherapy for leukemia, acute appendicitis may manifest with only vague abdominal pain, abdominal distention, lack of abdominal guarding, fever, dehydration, diarrhea, or unusual symptoms such as gastrointestinal bleeding.[8] Symptoms of appendicitis in immunocompromised patients may be identical to the symptoms of typhlitis.

The differential diagnosis of acute abdominal pain in children is extensive. Table 57–1 outlines conditions that can manifest with symptoms suggestive of acute appendicitis.

TABLE 57–1 Differential Diagnosis of Acute Appendicitis in Children

Cecal and Colonic Diseases
Constipation
Crohn disease
Infectious colitis (bacterial, parasitic)
Intestinal obstruction
Necrotizing enterocolitis (newborns)
Typhlitis (leukemia patients)
Small Intestinal Diseases
Duodenal ulcers (acute and perforated)
Gastroenteritis (including mesenteric adenitis)
Intestinal duplication
Intestinal obstruction
Intussusception
Meckel diverticulitis
Volvulus
Hepatobiliary and Pancreatic Diseases
Cholecystitis
Hepatitis
Hydrops of the gallbladder
Pancreatitis
Other Diseases
Omental torsion
Pneumonia
Psoas abscess
Spontaneous peritonitis
Reproductive Tract Diseases
Intrauterine and ectopic pregnancy
Ovarian torsion
Ovarian cysts
Pelvic inflammatory disease
Testicular torsion
Urinary Tract Diseases
Hydronephrosis
Pyelonephritis
Urachal abscess
Urolithiasis
Wilms tumor
Systemic Illnesses
Anaphylactoid purpura
Cytomegalovirus infection (in patients with AIDS)
Diabetic ketoacidosis
Kawasaki disease
Lymphoma (Burkitt)
Porphyria
Rocky Mountain spotted fever
Sickle-cell disease
Tuberculosis

AIDS, acquired immunodeficiency syndrome.

DIAGNOSIS

The diagnosis of acute appendicitis should be established without laboratory studies when a child complains of abdominal tenderness in the right lower quadrant that initially started in the periumbilical area, develops nausea and vomiting, and has rebound tenderness in the right lower quadrant with guarding during an abdominal examination.[21,174] The child should be taken to the operating room for an appendectomy as soon as possible before perforation occurs.[92] One third of children do not have all of the classic clinical manifestations of appendicitis, however; great emphasis has been placed on using various laboratory tests to help clinicians diagnose the disease accurately. Similarly, patients who have received prior oral antibiotics may have milder symptoms and signs of classic appendicitis, necessitating further diagnostic studies.[49]

For decades, physicians have valued peripheral blood leukocyte counts, neutrophil counts, C-reactive protein concentrations, and erythrocyte sedimentation rates to help them distinguish appendicitis from other causes of abdominal pain. When properly evaluated, these tests have been found, however, to be too insensitive to use as reliable tools for diagnosing appendicitis.[46,77,140,159] Normal results do not rule out the possibility that the child has appendicitis, although these tests help to confirm a physician's suspicions when results are positive. These nonspecific tests usually have elevated findings, however, during advanced disease, such as when perforation occurs.[5,136,142] Certain groups of patients commonly have normal leukocyte counts despite having acute appendicitis. Black patients with acute appendicitis frequently do not develop leukocytosis.[100,106] Patients with AIDS who develop appendicitis also frequently do not have elevated white blood cell counts.[24,124]

Routine radiographic studies for the diagnosis of appendicitis in children no longer are suggested. A chest radiograph often was obtained because right lower lobe pneumonia can cause severe abdominal pain in children. Radiographs of the abdomen are neither sensitive nor specific enough for diagnosing childhood appendicitis. Abnormalities described in association with acute appendicitis include an abnormal bowel gas pattern, a mass, a fecalith, and obliteration of normal fat planes in the right lower quadrant. Gas in the appendiceal lumen is thought to be diagnostic of acute appendicitis, but it may occur rarely in its absence. Most children do not have radiographic findings of appendicitis, or findings are nonspecific.

Graded compression ultrasonography has become the diagnostic procedure of choice in some institutions when evaluating

a patient with possible appendicitis.[57,58] A transducer is used to apply gradual pressure to the abdomen. The technician must ensure that all gas and fluid contents from the loops of bowel are expressed for the examination to be adequate. A noncompressible, enlarged (>6 mm in diameter in adolescents) appendix or a fecalith is the major criterion used for diagnosing appendicitis by ultrasonography. Interruption in the continuity of the echogenic submucosa suggests necrosis of the appendiceal wall and impending perforation. An echogenic periappendiceal mass indicates inflammation of the mesenteric or omental fat. Loculated or generalized fluid collections suggest that perforation already has occurred.

Pooled studies of graded compression ultrasonography have shown that the procedure is 88 percent sensitive and 94 percent specific in diagnosing acute appendicitis in children.[44] False-positive ultrasound results occur in obese patients who have noncompressible appendices because of overlying fat and in children who have inflamed appendices caused by Crohn disease, ulcerative colitis, or adjacent salpingitis. False-negative results occur if retrocecally located appendices are not visualized properly; if the cecum is filled with gas or feces and is not compressed adequately; or if perforation has occurred, allowing the appendix to be compressible. In one study, a noncompressible appendix was identified in only 38 percent of pediatric patients with perforated appendicitis, rendering the other ultrasound findings of appendicitis important in diagnosing the disease.[146] The examination should be directed to diagnose other causes of abdominal pain that can mimic appendicitis when a normal appendix is found during the ultrasound evaluation.

The advantages of using ultrasonography over CT are that it is inexpensive, safe, and widely available. It is especially useful in adolescent girls with abdominal pain because gynecologic causes of the pain can be evaluated easily at the time of appendiceal examination.

Most North American pediatric surgeons prefer CT over ultrasonography as the diagnostic procedure of choice for appendicitis.[122] High-resolution CT has higher sensitivity (94%) and similar specificity (95%) than ultrasonography in diagnosing appendicitis, and it is less operator dependent.[44] Intravenous contrast agents and high-resolution, thin-section scanning techniques must be used to visualize the appendix adequately. An enlarged appendix with a circumferentially and symmetrically thickened bowel wall is the most common CT finding in appendicitis. Periappendiceal inflammatory reaction or fluid collections may be identified. If the appendix is not well visualized, the presence of a fecalith, along with pericecal inflammatory changes, strongly suggests appendicitis. Fecaliths can be visualized in normal appendices by CT, however, and are of no clinical significance unless other inflammatory changes are present.

In recent years, helical CT scanning techniques that are focused only to the right lower quadrant using rectal or no contrast material have been shown to be accurate in diagnosing appendicitis in children.[50,105] By using a focused approach, CT scans may be completed within 5 minutes. Because of the high accuracy rates of CT in diagnosing appendicitis, some physicians have suggested that all children with suspected disease should have a focused CT scan.[141] In most community hospitals and medical centers, waiting for results of a CT scan may delay a surgical consultation and increase the rate of perforation before surgery.[98] Also, CT scanning is expensive and uses significant amounts of ionizing radiation in children, who have greater radiosensitivity of organs and tissues than adults do.

Whether the increasing use of ultrasound and CT scans to diagnose appendicitis in children has decreased the misdiagnosis of the disease and subsequent negative appendectomy rate in hospitals is unclear.[53,82,84,110,111,218] In a single study, the misdiagnosis rate was lower in hospitals that perform frequent pediatric appendectomies.[178] Most children with appendicitis have surgery in nonchildren's hospitals, however.[37] Radiolabeled autologous leukocyte scans also have been used to diagnose appendicitis in children; however, this modality should be reserved for atypical presentations of disease when localizing signs are not present.[72]

MICROBIOLOGY

Numerous microorganisms have been implicated as a cause of acute appendicitis; considerable debate has ensued as to whether simply isolating an organism from the appendiceal lumen is sufficient proof to define causation (Table 57–2).[2]

BACTERIA

In most cases of appendicitis, bacteria do not seem to be involved directly in the initial stages of the inflammatory process. Microorganisms that normally inhabit the appendix are liberated into the peritoneal cavity when appendiceal perforation occurs or when translocation through the inflamed tissues is present, and polymicrobial infections develop as a complication of the disease process.[19,195] In a study of 30 adolescents and adults with nonperforated and perforated appendicitis, 223 different anaerobes and 82 aerobes were recovered from cultures of the appendiceal tissues, peritoneal fluid, and contents of abscesses.[19] An average of 10 different organisms were isolated per specimen collected.

In most microbiologic studies of appendiceal tissues and peritoneal fluid specimens from patients with appendicitis, *B. fragilis* is the strict anaerobe isolated most frequently, occurring in more than 70 percent of patients.[19,153] Other anaerobes that are isolated frequently include *Bacteroides* spp., *Bilophila wadsworthia*, *Peptostreptococcus* spp., *Fusobacterium* spp., and *Clostridium* spp.[201,213] A gram-negative anaerobic rod that develops a pigment in culture and is bile resistant also has been identified frequently.[152,153] Other anaerobes, such as *Turicibacter sanguinis*, continue to be newly described in patients with appendicitis.[23]

E. coli are the aerobic or facultative anaerobic bacteria isolated most frequently from children with appendicitis. *E. coli* are found in more than 75 percent of patients.[19,153] Researchers have suggested that certain *E. coli* strains with type 1C fimbriae may contribute to the development of appendiceal inflammation.[167] Enterohemorrhagic *E. coli* O157:H7 and O111:H have been isolated infrequently from the stools and peritoneal fluid of children with appendicitis.[199,205]

Viridans streptococci of the *S. milleri* group, including *Streptococcus anginosus*, can be found in more than 60 percent of cultures from children with appendicitis.[79,153] Group D streptococci are isolated in approximately 20 to 30 percent of patients with appendicitis, whereas *Pseudomonas* spp. are isolated slightly less frequently.[153] Other aerobes or facultative anaerobes that can be isolated from appendiceal tissues, abscesses, or blood include *Citrobacter* spp.; *Klebsiella* spp.; *Enterobacter* spp.; *Proteus* spp.; *Morganella morganii*; *Providencia rettgeri*; *Eikenella corrodens*; groups C, F, and G beta-hemolytic streptococci; and staphylococci.[45,70,81,148,153,195]

Rarely, encapsulated organisms, such as *Streptococcus pneumoniae*, *Haemophilus influenzae*, and *Haemophilus segnis*, have been isolated from appendiceal tissues or peritoneal fluid of young children with appendicitis, and often these organisms have been isolated in pure culture.[13,117,127] Very rarely, organisms such as *Pasteurella multocida*, *Streptococcus pyogenes*, and *Actinomyces* spp. have been cultured from patients with appendicitis.[149,162,169,198] *Shigella*, *Salmonella*, *Campylobacter*, and *Yersinia* spp. also have been isolated occasionally from appendiceal tissues or peritoneal fluid of patients with nonperforated and perforated appendicitis; whether they played a role in the pathogenesis of disease is

TABLE 57–2 Microorganisms Associated with Acute Appendicitis in Children

Anaerobes
Bacteroides spp.
Bilophilia wadsworthia
Clostridium spp.
Fusobacterium spp.
Peptostreptococcus spp.
Pigmented bile–resistant, gram-negative rod
Turicibacter sanguinis

Enteric Aerobes and Facultative Anaerobes
Campylobacter spp.
Citrobacter spp.
Enterobacter spp.
Enterococcus spp.
Escherichia coli
Klebsiella spp.
Morganella morganii
Proteus spp.
Providencia rettgeri
Salmonella spp.
Shigella spp.
Streptococcus milleri group
Yersinia spp.

Other Bacteria
Actinomyces spp.
Atypical mycobacteria (in patients with AIDS)
Brucella melitensis
Chromobacterium violaceum
Corynebacterium appendicis
Eikenella corrodens
Haemophilus spp.
Human monocytic ehrlichiosis
Kluyvera ascorbata
Pasteurella multocida
Pseudomonas spp.
Staphylococcus spp.
Streptococcus pneumoniae
Streptococcus pyogenes

Parasites
Angiostrongylus costaricensis
Anisakis spp.
Ascaris lumbricoides
Balantidium coli
Cryptosporidium parvum
Entamoeba histolytica
Enterobius vermicularis
Schistosoma spp.
Strongyloides stercoralis
Taenia spp.
Trichuris trichiura

Viruses
Adenoviruses
Coxsackieviruses B
Cytomegalovirus
Epstein-Barr virus
Measles

Fungi
Candida albicans
Histoplasma capsulatum
Mucor spp.

unknown.[20,29,88,96,118] Much more commonly, these organisms cause enterocolitis or mesenteric adenitis, with symptoms mimicking those of appendicitis.[203]

In recent years, appendicitis has occurred during systemic infections caused by *Brucella melitensis* and *Ehrlichia chaffeensis*.[7,173] Rarely, *Kluyvera ascorbata*, *Arcobacter butzleri*, and *Chromobacterium violaceum* have been isolated from patients with appendicitis, as have newly described aerobes and facultative anaerobes, such as *Corynebacterium appendicis*.[31,33,97,215]

Rarely, isolated primary tuberculosis can occur in children.[66,147] The progression of disease usually is rapid. The clinician should be suspicious when caseating granulomas are observed in histopathologic sections of the appendix.

Adults and children with appendicitis usually are not bacteremic at the time they are diagnosed, especially if the appendix is not perforated. Occasionally, *Klebsiella pneumoniae*, *E. coli*, *B. fragilis*, and *B. wadsworthia* are isolated from the blood of patients with nonperforated appendicitis.[22,138,161] In a review of 1000 children and adults with appendicitis, 10 percent of patients with perforation had positive blood cultures, whereas none of the patients without perforation had bacteremia.[100] A higher rate of bacteremia may occur when laparoscopic surgery is done because of the air that is forced into the peritoneum, although the clinical significance of the induced bacteremia is unknown.[132]

In immunocompromised patients who develop appendicitis, the microorganisms that are isolated from appendiceal tissues or peritoneal cultures usually are identical to the microorganisms found in immunocompetent patients.[124] In adults infected with human immunodeficiency virus, appendicitis has been caused by cytomegalovirus infection and neoplastic obstruction of the base of the appendix by Kaposi sarcoma.[34,129] Patients with AIDS who have gastrointestinal *Mycobacterium avium* complex or *Mycobacterium tuberculosis* infections may develop symptoms that mimic appendicitis.[41,204] Atypical mycobacteria have been isolated from an appendiceal abscess from a child with AIDS.[48]

PARASITES

Roundworms, such as *Ascaris lumbricoides*, plausibly may obstruct the appendiceal lumen occasionally and initiate the cascade of inflammatory events leading to perforated appendicitis.[108,134] Parasites such as *Enterobius vermicularis* can be identified in the lumen of 1 to 12 percent of surgically removed appendices obtained from patients living in highly endemic areas.[10,38] Pinworms have been found more frequently, however, in appendices with no evidence of appendiceal inflammation in some studies, suggesting that pinworms probably are a part of the normal appendiceal flora and do not play a role in the pathogenesis of appendicitis.[38,163,217] Whether some parasites may cause abdominal pain that mimics the symptoms of appendicitis necessitating surgical intervention is unclear.

Scattered reports from mostly developing nations describe other worms, including *Taenia* spp., *Anisakis* spp., *Trichuris trichiura*, *Strongyloides stercoralis*, *Schistosoma* spp., and *Angiostrongylus costaricensis*, that have been identified in the lumina of appendices from patients with appendicitis.[11,55,65,91,165] Similarly, protozoa such as *Balantidium coli*, *Entamoeba histolytica*, and *Cryptosporidium parvum* have been found in inflamed appendices of immunocompromised and immunocompetent patients, but whether they play a role in the pathogenesis of disease is unknown.[27,43,67,150]

VIRUSES

The role that viruses play in causing appendicitis also has been debated. One suggestion is that a systemic viral infection may induce hypertrophied lymphoid aggregates that obstruct the appendiceal lumen. In the 1960s, elevated levels of antibodies against coxsackieviruses B and adenoviruses were found in the sera of some children with appendicitis.[196] A later study could not confirm this finding, however.[120] Six adolescents with infectious mononucleosis have developed appendicitis, and cytomegalovirus

has been observed occasionally in immunodeficient and otherwise healthy patients' inflamed appendices.[85,104,129,194] Other children have had histologic evidence of measles virus or adenovirus infection.[137,155] Because of the rarity of documented simultaneous viral infections and appendicitis, whether these viruses play a major role in the pathogenesis of acute appendicitis is doubtful.

FUNGI

Rarely, *Candida albicans* is isolated from inflamed appendices or abscess cultures, but its role in the pathogenesis of disease is unknown. Perforation of the appendix from intestinal mucormycosis has occurred in granulocytopenic patients and premature newborns.[130,193] Also, appendicitis has been described in individuals with histoplasmosis.[94]

TREATMENT

NONPERFORATED APPENDICITIS

In previously healthy children with signs of acute appendicitis and no clinical evidence of perforation, nasogastric suctioning should be established and imbalances in fluid and electrolyte concentrations should be corrected quickly. The child should be taken to the operating room as soon as possible for exploratory laparotomy and appendectomy. Many teaching hospitals now perform appendectomies only during day and evening hours, because of limitations on resident work hours and decreased services available at night.[122] No differences have been noted to date in perforation rates, lengths of stay, or complication rates in children who are diagnosed with appendicitis at night and given analgesics until a scheduled morning surgery.[1,191,214] Morphine can be used to reduce the severity of abdominal pain in children with appendicitis until the child is taken to the operating room and does not impede establishing the diagnosis in children under observation for suggestive symptoms and signs of appendiceal disease.[63]

Prophylactic antibiotics given perioperatively decrease the rate of postoperative wound infection, even in noncomplicated cases of childhood appendicitis.[4,175] No consensus exists concerning the appropriate antimicrobial agent or agents that should be used or the appropriate duration of treatment required after surgery to reduce the complication rate. Although some surgeons continue the antibiotics after surgery, prospective, randomized studies show that a single perioperative dose of appropriate antibiotic with antimicrobial activity against *E. coli* and enteric anaerobes is as effective as continuing the antibiotic for 1 to 5 days after surgery.[125,197]

Few data support the routine intraoperative collection of peritoneal fluid or appendiceal cultures in children with nonperforated appendicitis, although 5 to 20 percent of cultures grow enteric aerobes, anaerobes, or both.[32,60] Immunocompromised patients should undergo intraoperative cultures, including cultures for mycobacteria and cytomegalovirus.

Interest in performing laparoscopic appendectomies in children with nonperforated and perforated appendicitis has been increasing.[30,122,143] Advantages of the procedure are a reduction in wound infection, reduction in scarring, shorter hospital stay, and earlier return to normal activity, although the procedure must be done by a surgeon experienced in laparoscopic techniques.[14] The mean total cost of a laparoscopic appendectomy is similar to that of the more commonly performed open appendectomy, and more recent studies in pediatric patients have determined that no differences exist in postoperative pain, time to self-ambulation, and risk of developing a postoperative abscess.[175] In the future, the use of smaller laparoscopic instruments may improve the speed of recovery of children.[121]

PERFORATED APPENDICITIS

Most surgeons advocate early intervention when perforation has occurred to prevent severe complications, such as fistula formation, abscess rupture, and death, despite the high chance of developing postoperative complications.[122] If a laparotomy is done, debate ensues as to whether the wound should be closed primarily and whether transperitoneal drains should be placed at the time of surgery. Surgeons increasingly are performing primary closures without placing drains in children.[47,116,122] A randomized prospective trial of appendiceal drains in children with perforated appendicitis showed no benefit in using drains compared with primary wound closure.[188] Laparoscopic surgery has been used for perforated appendicitis; nonetheless, caution, good surgical judgment, and a low threshold for conversion to an open procedure are advised.[102]

During the surgical procedure, most surgeons irrigate the peritoneal cavity with copious amounts of saline or antibiotics to reduce the quantity of bacteria in the abdomen.[122] To obtain sterile cultures of the lavage fluid, 6 L of lavage fluid per m^2 surface area of the child is required.[135] It is unclear whether the lavage fluid or the addition of antibiotics to the lavage fluid decreases the rate of postoperative complications in children with perforated appendicitis who are receiving systemic antibiotics.[113]

Controversy remains as to whether immediate appendectomy should be done in a child in whom a palpable mass is associated with the appendicitis or evidence of appendiceal rupture with or without abscess formation exists at the time of presentation.[174,207] Greater than 70 percent of children with palpable masses respond to conservative, nonoperative management consisting of administration of intravenous fluids and antibiotics.[174] If the child does not improve, or a walled-off abscess develops, drainage of the area and appendectomy should be done. If the child responds to conservative management, an interval appendectomy should be done 6 to 8 weeks after resolution of the symptoms. Proponents of initial conservative management consider that the complication rate after interval appendectomy is significantly lower than when a procedure is done during the acute stage of disease.

Antimicrobial agents should be administered routinely to children when perforation or appendiceal abscess is suspected or discovered during surgery. Antibiotics active against aerobes and anaerobes that normally inhabit the intestinal tract have been effective in treating children with perforated appendicitis. Treatment failures occur most commonly when *B. fragilis* or *Pseudomonas* spp. are isolated from intraoperative cultures and antimicrobial agents without activity against these organisms are used.[73] Controversy continues regarding the value of obtaining routine intraoperative peritoneal cultures in cases of perforated appendicitis, although most studies show that culture results seldom change the clinical management of patients.[32,60,87,90,182]

The antimicrobial combination of ampicillin, gentamicin, and clindamycin has been the gold standard of therapy since the 1970s. The importance of including ampicillin in the regimen for adequate enterococcal coverage continues to be controversial. Animal studies and clinical trials using antibiotics with poor enterococcal activity have shown that ampicillin probably is not required in the treatment of perforated appendicitis.[62] Because of the increasing problem of ampicillin resistance in enterococci, ampicillin probably should be reserved for the rare child with enterococcal bacteremia or with persistent intra-abdominal infection in which enterococci have been isolated. Some medical

centers use metronidazole instead of clindamycin because of its broader activity against enteric anaerobes, whereas other institutions substitute cefotaxime or ceftriaxone for gentamicin.[170,186]

Efforts have been made to determine whether single antibiotics are effective in treating perforated appendicitis. The only agents that have been shown to be effective in treating children with perforated appendicitis are cefoxitin, imipenem-cilastatin, ticarcillin-clavulanate, piperacillin-tazobactam, ampicillin-sulbactam, meropenem, and ertapenem.[51,109,126,176,177,192,200,212] In a few medical centers, nearly 50 percent of *B. fragilis* isolates are resistant to cefoxitin, raising the question as to whether cefoxitin should be used routinely as a single agent in these institutions.[54] Generally, the convenience of monotherapy does not outweigh the potential development of resistance to these broad-spectrum agents and their associated increased costs.[87] They may be useful in the treatment of appendicitis in children with renal disease or hearing loss when avoiding the use of gentamicin is prudent.

Most patients with perforated appendicitis are treated with intravenous antibiotics for 5 to 10 days; however, limiting the duration of antibiotic use to 3 days does not lead to higher rates of wound infections or intra-abdominal abscesses.[122,180] Efforts have been made to shorten the hospital stay of children with perforated appendicitis. Some institutions have set criteria for hospital discharge and discontinuation of antibiotics, such as absence of fever for 24 hours, ability to eat well, and less than 3 percent band forms on the white blood cell differential.[74] Many surgeons switch to oral antibiotics at home after 3 to 5 days of intravenous antibiotics in the hospital, although the benefit of adding prolonged oral antibiotics to a short course of intravenous antibiotics has not been shown.[157,190] Providing home single intravenous antimicrobial therapy also can reduce costs and hasten hospital discharge in selected children with perforated appendicitis.[51,87,185]

PROGNOSIS AND EARLY COMPLICATIONS

Currently in the United States, the risk of dying as a result of appendicitis is very low. The estimated mortality rate for nonperforated and perforated appendicitis in California during the 1980s was 0.02 percent.[106] In smaller series of children and adults reported in the 1990s, the mortality rate was 0 percent.[112] It has been stated that the risk of death from appendicitis should be the risk of death from general anesthesia.[112] The mortality rate seems to be higher, however, in the rare newborn or premature infant who develops appendicitis. Also, factors contributing to the death of children rarely may include delay in establishing the diagnosis, inadequate fluid replacement, immunodeficiency, and postoperative vascular or infectious complications.

The most predictive factor of postoperative morbidity occurring from appendicitis is perforation.[154] Age, obesity, duration of the surgical procedure, and nutritional status also are risk factors for the development of complications. Wound infection rates in children who receive perioperative antibiotics should be less than 7 percent; infections generally are caused by the same organisms that are isolated in cultures obtained during appendectomy.[175,197] Occasionally, children develop peritonitis, intra-abdominal abscesses, psoas abscesses, fistulas, pyelophlebitis of the portal vein, scrotal abscesses, or pneumoperitoneum during treatment of appendicitis.[15,68,75,158,216] CT scans can be used successfully to detect postoperative abscesses in the first week after surgery.[9] If complications occur, another surgical procedure often is performed, and antibiotic treatment is prolonged. Abscesses may be treated successfully with antibiotics alone and without surgical drainage in stable patients after appendectomy.[42] The next section focuses on pelvic abscess as an early complication of appendicitis because the topic is not discussed elsewhere in this textbook.

PELVIC ABSCESS

The pelvic area is a common site for development of abscesses because it is the most dependent portion of the peritoneal cavity. Pelvic abscesses most commonly occur in children who have had intestinal perforations after appendicitis, have had penetrating abdominal or retroperitoneal injury, or have undergone an abdominal surgical procedure. Occasionally, adolescents with pelvic inflammatory disease or Crohn disease develop a pelvic abscess.[61]

In children with perforated appendicitis, a coexisting pelvic abscess often is diagnosed at the time of laparotomy. In patients who recently have had penetrating trauma to the abdomen, have had pelvic inflammatory disease, or have undergone gastrointestinal surgery, a pelvic abscess should be suspected when they have continued fever or complain of abdominal pain despite receiving adequate treatment of the initial disease process. Symptoms may not develop until days to months after therapy is ended. No characteristic physical findings are associated with a pelvic abscess, although abdominal palpation or rectal examination may elicit tenderness, or signs of intestinal obstruction may be present.

If a pelvic abscess is suspected, contrast-enhanced CT evaluation of the pelvis should be completed. The bladder should be filled before the procedure so that it can displace bowel loops from the pelvis, act as an anatomic marker, and act as a standard of fluidity against which an abscess cavity can be compared. Walled-off fluid collections in the pelvis can be identified, and sometimes the rectum, sigmoid colon, or bladder is compressed because of mass effect from the abscess cavity. Because most pelvic abscesses develop as complications of intestinal or pelvic infections, enteric aerobes and anaerobes are the organisms most commonly isolated from the abscess cavity. Yeasts only rarely cause pelvic abscesses.[202,209] An *Actinomyces*-related pelvic abscess developed in an adult who had an intrauterine device.[139] Rarely, tuberculous abscesses can develop as a complication of genital tuberculosis.[208]

When a pelvic abscess is identified, antibiotics covering intestinal aerobes and anaerobes, such as clindamycin and gentamicin, should be started, and the abscess contents should be drained. In most situations, reaching the abscess cavity by an anterior approach is difficult. Considerable interest has developed in using CT or ultrasonography to guide percutaneous drainage of pelvic abscesses by transgluteal, transrectal, transparacoccygeal, or transvaginal approaches.[28,59,80,103,131] Although placement of a transgluteal catheter is easiest, the sciatic nerve and gluteal vessels must be avoided. Also, an increased risk for development of a wound infection may occur because microorganisms may track along the outside of the catheter to the skin. Many surgeons prefer the transrectal approach because it often is the most direct route to the abscess. Transvaginal drainage has been used with good results in young women, however. Most often, drainage catheters can be removed after 7 to 10 days of treatment.

Abscesses also may develop within the muscles of the pelvic girdle, including the psoas and internal obturator muscles.[25,168,181,183,184,187] Similar to true pelvic abscesses, they usually cause fever and occasionally abdominal complaints in children. Most children begin to limp, refuse to walk, or complain of pain in the buttocks, thigh, or groin. Often, a suppurative hip infection is suspected initially. A pelvic muscle abscess usually is diagnosed by CT or magnetic resonance imaging. Labeled leukocyte scans sometimes are useful in localizing the infection to within the pelvis, especially when the child has no symptoms other than fever or refusing to walk.

Pelvic muscle abscesses can develop as a complication of Crohn disease or appendicitis; however, they often develop after an episode of bacteremia.[166] *Staphylococcus aureus* is the most common cause of a primary pelvic muscle abscess.[25,168,181,183] *S. pneumoniae*, *H. influenzae* type b, *E. coli*, *Enterococcus faecalis*,

S. milleri group, *Yersinia enterocolitica*, *Salmonella* spp., *Proteus mirabilis*, and *Actinomyces* spp. also have been reported to cause hematogenously acquired abscesses.[25,26,35,39,76,83,89,172] Bacteremia secondary to intravenous drug abuse or the presence of central lines occasionally predisposes patients to developing this type of infection.[93,206] Rarely, tuberculous psoas abscesses have been reported, often as a complication of vertebral osteomyelitis.[189]

Pelvic muscle abscesses usually are drained by a percutaneous or surgical approach, and antibiotic therapy is based on Gram stain and culture results. Successful therapy with antibiotics alone has been reported.[184] Duration of treatment is individualized and depends on the child's response and the drainage techniques that were used.

LATE COMPLICATIONS

Most children who have undergone appendectomy or drainage of a pelvic abscess do not have late complications. Occasionally, patients develop signs of bowel obstruction from peritoneal adhesions later. Some studies have suggested that the future risk for infertility is greater in women who have perforated appendices or pelvic abscesses, presumably because of adhesion formation that impairs the migration of ova in the reproductive tract.[123] Although some retrospective studies have suggested that patients with appendectomies have a higher rate of developing malignancies later in life, prospective and controlled studies have failed to show this association.[36,119] Patients who have had appendicitis have been reported to be three times more likely to develop right inguinal hernias than individuals who have not had the appendix removed.[12] It has been suggested that women have a higher long-term risk of developing Crohn disease if they had nonperforated appendicitis earlier in life, whereas patients with previous perforated appendicitis have a more severe course of inflammatory bowel disease.[6]

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CHAPTER 58

Pancreatitis

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Pancreatitis previously was thought to be an uncommon cause of abdominal pain in children and a disease primarily of adults. Because of better recognition of symptoms in children and the more frequent use of medications that cause pancreatic inflammation, acute pancreatitis currently is being diagnosed more frequently in institutions specializing in pediatric care.[90,169]

Compared with causes of acute pancreatitis in adults—primarily alcoholism, cholelithiasis, and trauma—causes of childhood pancreatitis are more diverse. Microorganisms account for a significant proportion of cases of pancreatitis in children. In addition, antimicrobial agents have been associated with severe and occasionally fatal episodes of pancreatitis, and bacterial infections may complicate the natural history of acute and chronic pancreatitis. Pediatricians who care for children with pancreatitis must have expertise in the diagnosis and treatment of infectious diseases.

CLINICAL MANIFESTATIONS

More than 80 percent of children with acute pancreatitis complain of abdominal pain.[16,169] Only 30 percent of pediatric patients have epigastric pain as usually described by adults, however.[165] In children, other sites of focal tenderness or diffuse pain include the right upper quadrant of the abdomen, the periumbilical area, the entire abdomen, and, less commonly, the right lower quadrant of the abdomen. The onset of pain usually is rapid and increases to a maximal intensity in a few hours, but occasionally

the onset may be slow and gradual. Most often, the pain is sharp and excruciating in nature. Only one third of children complain of pain that radiates to other areas, including the back, lower part of the abdomen, upper abdominal quadrants, and anterior chest wall. In school-aged children, the pain often intensifies after meals.

Two thirds of children with acute pancreatitis have vomiting.[16] Children younger than 5 years old occasionally experience vomiting without abdominal tenderness.[176] Fever is present in only 30 percent of children with pancreatitis, but temperatures greater than 38.5° C are observed occasionally.[165]

On physical examination, children classically are found lying quietly on their sides with their knees flexed. They usually have epigastric tenderness to palpation and decreased or absent bowel sounds. Abdominal distention is found in 30 percent of children with pancreatitis and occurs more commonly in preschool-aged children.[165,176] Occasionally, rebound tenderness, guarding of the epigastrium, jaundice, an abdominal mass, or ascites is detected. Rarely, ecchymoses of the flanks (Turner sign) or the umbilical area (Cullen sign) can be identified, but usually only when life-threatening hemorrhagic pancreatitis is present. In severe pancreatitis, children may present with evidence of shock and multiorgan failure.

Chronic pancreatitis occurs when irreversible damage in the pancreatic architecture causes abnormalities in the function of the pancreas.[91] Children with chronic pancreatitis often have lengthy or recurrent bouts of abdominal pain and vomiting.

LABORATORY DIAGNOSIS

The most common useful laboratory test for the clinical diagnosis of pancreatitis in children is measurement of serum amylase, but the level correlates poorly with the severity of the disease.[58] In most studies of childhood pancreatitis, the diagnosis is confirmed when the serum amylase is greater than three times the normal level for the particular laboratory completing the test. The serum concentration increases quickly within hours after symptoms develop. High serum amylase concentrations can be observed, however, in numerous other illnesses, including acute cholecystitis, intestinal obstruction, perforated abdominal organs, appendicitis, salpingitis, ruptured ectopic pregnancy, and salivary gland disease. The serum amylase concentration can return to normal in 24 to 72 hours after the onset of symptoms, and the diagnosis of pancreatitis can be missed. In this situation, the urine amylase concentration can remain elevated for at least 1 week.

Serum amylase concentrations occasionally are not elevated during the course of pancreatitis in children.[133] In a series of children with pancreatitis, 83 percent of patients had elevated serum amylase levels.[169] In addition, marked hyperlipidemia may interfere with the laboratory measurement of amylase.[17] Serum lipase is useful in these situations; high serum concentrations often are not detected until 24 hours after the beginning of the illness. Because lipase is produced only in the pancreas and intestinal cells, measurement of the serum concentration helps distinguish children with high serum amylase concentrations of pancreatic as opposed to salivary origin. Measurement of serum trypsinogen may be the most sensitive and specific way of detecting acute pancreatitis, but it is unavailable in most clinical laboratories.[165]

Laboratory findings in children with severe acute pancreatitis may include leukocytosis with increased immature polymorphonuclear leukocytes, an elevated erythrocyte sedimentation rate, and elevated C-reactive protein level. In children with fulminate hemorrhagic pancreatitis, anemia develops quickly. Other associated findings include hyperglycemia, hypertriglyceridemia, hypoalbuminemia, and hypocalcemia. A new scoring system for children with pancreatitis has shown that severe pancreatitis is more likely when the child who presents is younger than 7 years of age, weighs less than 23 kg, has a total white blood cell count greater than 18,500 cells/mm^3, has an admission lactate dehydrogenase greater than 2000 U/L, has a 48-hour calcium level less than 8.3 mg/dL, has a 48-hour albumin level less than 2.6 g/dL, has a 48-hour fluid sequestration greater than 75 mL/kg/48 hr, or has a 48-hour increase in blood urea nitrogen greater than 5 mg/dL.[32] Elevated transaminases and alkaline phosphatase generally are observed only when the episode of pancreatitis is caused by biliary obstruction, such as in gallstone-related disease.

The radiographic features of childhood pancreatitis also are nonspecific. Radiographs of the abdomen may show localized ileus of the jejunum in the midepigastric or left upper quadrant region adjacent to the pancreas (sentinel loop), a distended transverse colon without visualization of the descending colon because of adjacent pancreatic inflammation (colon cutoff sign), duodenal distention with air-fluid levels, or loss of the left psoas shadow.[63] Occasionally, chest radiography reveals an elevated left hemidiaphragm or pleural effusion.

In recent years, the ability to diagnose pancreatitis in children has been improved greatly by ultrasonography.[169] The echodensity of the pancreas normally is equal to or greater than that of the left lobe of the liver. During acute pancreatitis, edema causes the gland to enlarge and become less dense than the liver. These two findings can aid in establishing the diagnosis of pancreatitis, and complications such as abscesses and pseudocysts can be identified. Also, ultrasonography may delineate dilations of the pancreatic ducts caused by obstruction or ductal stones. Visualization of the pancreas by ultrasonography may be obscured because of overlying bowel gas. In such cases, computed tomography (CT) is useful to detect pancreatic size and density.

CT of the pancreas should be performed in complicated cases of pancreatitis after a few days of treatment to determine the severity of disease and extent of pancreatic necrosis. It is especially useful when surgery is being considered for drainage of abscesses and pseudocysts.

Endoscopic retrograde cholangiopancreatography (ERCP) is being used increasingly in children with pancreatitis to exclude or treat gallstones, pseudocysts, strictures, or *Ascaris* infection.[126,131] In recent years, magnetic resonance cholangiopancreatography has been used as a noninvasive technique for evaluating children with chronic pancreatitis.[11,99]

NONINFECTIOUS ETIOLOGIES

An etiology for pancreatitis in children can be determined now in more than 90 percent of cases if diagnostic evaluation is thorough, especially in children younger than 6 years of age.[90,176] The frequency of each specific cause depends on the patient population of the particular medical center. At Children's Hospital of Michigan, 33 percent of children with pancreatitis have biliary tract–related disease because of the large patient population with sickle-cell anemia.[176] At Yale–New Haven Hospital, drug-related pancreatitis accounts for 30 percent of the total cases of childhood pancreatitis because of the frequent use of immunosuppressive and cancer chemotherapeutic agents in this hospital.[63] Table 58–1 outlines the most common noninfectious causes of pancreatitis in children.

TRAUMA

In many older series of patients, trauma is the leading cause of acute pancreatitis in children. Because the highly vascular pancreas is immobilized by the stomach, duodenum, and vertebrae, it is susceptible to blunt and penetrating trauma. The less developed abdominal wall musculature in pediatric patients may

TABLE 58–1 Noninfectious Causes of Childhood Pancreatitis

Trauma
Blunt
Brain injury
Penetrating
Postoperative
Drugs
Antimicrobials—pentamidine, antimonials, sulfonamides, tetracycline, macrolides, metronidazole, dapsone, nitrofurantoin, isoniazid, ceftriaxone, gatifloxacin, nucleoside analogue reverse transcriptase inhibitors, interferon-α, liposomal amphotericin B
Atypical antipsychotics
Diuretics—thiazides, furosemide
Ethyl alcohol
Growth hormone
Immunosuppressives—azathioprine, steroids, asparaginase, mercaptopurine, tacrolimus
Metformin
Nonsteroidal anti-inflammatory drugs
Propofol
Proton pump inhibitors
Statins
Sulfasalazine
Valproic acid
Obstructive Diseases
Anatomic abnormalities
Cholelithiasis
Genetic and Metabolic Diseases
Aminoacidurias
Cystic fibrosis
Diabetes mellitus
Glycogen storage disease type I
Hyperlipoproteinemia types I, IV, V
Hyperparathyroidism
Recurrent hereditary pancreatitis
Vasculitic and Autoimmune Diseases
Crohn disease
Henoch-Schönlein purpura
Kawasaki syndrome
Systemic lupus erythematosus
Miscellaneous
Anticholinesterase insecticide intoxication
Chronic fibrosing pancreatitis
Lymphoma
Orthotopic liver transplantation
Reye syndrome
Scorpion stings
Tropical chronic pancreatitis

enhance their susceptibility to pancreatic injury after episodes of blunt trauma. Child abuse has been recognized increasingly as a cause of trauma-related pancreatitis. Postoperative pancreatitis occurs most commonly after abdominal or cardiac surgery.[43] Pancreatitis also may be associated with traumatic brain injury in children.[159]

MEDICATIONS

Medications used in pediatric practice increasingly are causing episodes of pancreatitis in children. In most recent pediatric studies, the anticonvulsant valproic acid is the most common medication associated with the development of pancreatitis.[169] The risk of developing pancreatitis does not depend on the serum level of valproic acid, and it is not related to the length of therapy.[142,168] Azathioprine, steroids, mercaptopurine, and asparaginase have been associated with cases of childhood pancreatitis, and other immunosuppressive agents most likely play a role in post-transplantation pancreatitis.[80,135,143,167,171] Acute pancreatitis has been associated rarely with the use of diuretics, including hydrochlorothiazide and furosemide[96]; growth hormone[33,97]; sulfasalazine[49]; atypical antipsychotics[56,82]; statins[73]; propofol[20]; nonsteroidal anti-inflammatory agents, including ibuprofen[92,93]; metformin[46]; and proton pump inhibitors.[174] Alcohol consumption is an uncommon cause of pancreatitis in younger children; however, it can cause illness occasionally in adolescents.

Physicians with expertise in the management of infectious diseases are becoming more aware of drug-induced pancreatitis because many antimicrobial agents can cause pancreatic inflammation. Pentamidine isethionate is used in the treatment of *Pneumocystis carinii* pneumonia, African trypanosomiasis, and leishmaniasis. It may cause hypoglycemia because of toxicity to pancreatic islet cells and is associated with severe and occasionally fatal episodes of pancreatitis.[104,177] In children and adults, aerosolized pentamidine prophylaxis for *P. carinii* pneumonia also has been associated with severe cases of pancreatitis in patients with acquired immunodeficiency syndrome (AIDS).[59,104] Similarly, pentavalent antimonials, such as sodium stibogluconate and meglumine antimonate, used for the treatment of visceral leishmaniasis can induce pancreatic inflammation.[86,173]

Sulfonamides, including trimethoprim-sulfamethoxazole, have been implicated occasionally as a cause of acute pancreatitis in adults.[6,162] Symptoms have recurred when patients have been re-exposed to the medication. The abdominal pain often is accompanied by a hypersensitivity-type rash. Tetracycline-induced pancreatitis has been described in children with and without overt liver disease.[42,154] In addition, clarithromycin,[51,136] erythromycin,[149] rifampin,[125] roxithromycin,[129] dapsone,[31] nitrofurantoin,[112] isoniazid,[71] and metronidazole[116] have been added to the list of agents that can cause pancreatitis in previously healthy individuals when given in routine doses or when high amounts are consumed. Although uncommonly used in children, quinolone antibiotics, such as gatifloxacin, have been associated with hepatotoxicity and acute pancreatitis.[29] An adolescent who was receiving ceftriaxone also developed pancreatitis secondary to obstruction of the biliary tract from gallstones.[100]

Pancreatitis has been a major dose-limiting toxic effect of the human immunodeficiency virus (HIV)–inhibiting nucleoside analogue reverse transcriptase inhibitor class of medications, especially dideoxyinosine, in adult and pediatric patients.[21,22] Most episodes of pancreatitis occur when the dose is 360 mg/m^2/day or more, and usually the pancreatic inflammation resolves when the medication is discontinued. Concomitant administration of pentamidine or another nucleoside analogue reverse transcriptase inhibitor, such as ribavirin, used in the treatment of hepatitis C infection with dideoxyinosine may increase the risk of developing pancreatitis.[108] In pediatric patients with AIDS, serum amylase concentrations often are elevated in patients without pancreatic symptoms, whereas children with pancreatitis can have normal serum amylase concentrations. The serum lipase concentration is useful in evaluating HIV-infected children for possible pancreatic inflammation.[22,104] Increased liver transaminase or lipase concentrations before the administration of dideoxyinosine may be helpful in predicting the children in whom pancreatitis would develop.[22] In all children with symptoms consistent with pancreatitis, dideoxyinosine should be withheld pending the results of a lipase assay, and it should be discontinued if the concentration is elevated. Similarly, dideoxyinosine should be discontinued for 1 week after treatment with pentamidine for *P. carinii* pneumonia.[47]

Interferon-α, which is used in the treatment of chronic hepatitis and malignancies, also has been associated with the development of pancreatitis.[26] Liposomal amphotericin B treatment rarely causes pancreatic toxicity.[147]

OBSTRUCTIVE DISEASES

Obstruction of the common bile duct, pancreatic duct, or sphincter of Oddi may cause pancreatitis.[7] Gallstones or congenital anatomic malformations, including an annular pancreas, pancreas divisum, choledochal cysts, and intrapancreatic duplication cysts, can cause pancreatitis by obstructing normal pancreatic flow. Pancreatitis observed in children with sickle-cell anemia may be caused by obstruction from stones, biliary sludge, or pancreatic microvascular occlusion and ischemia during crises.[3]

GENETIC AND METABOLIC DISEASES

Metabolic diseases often are associated with chronic recurrent episodes of pancreatitis. Such diseases include hyperlipoproteinemias, cystic fibrosis, diabetes mellitus, hyperparathyroidism, aminoaciduria, and glycogen storage disease type I.[54,63,75,160] Recurrent hereditary pancreatitis usually occurs in an autosomal dominant pattern, with onset occurring between infancy and adolescence.[76] It has been linked to chromosome 7q35. The mutation allows trypsinogen to become activated to trypsin within the pancreas. Other children and adults with chronic pancreatitis have been shown to have mutations in the cystic fibrosis transmembrane regulator (*CFTR*) gene similar to individuals with cystic fibrosis,[26] or to have a mutation causing a deficiency of trypsin-specific inhibitor that inactivates low levels of trypsin within acinar cells.[76]

VASCULITIC AND AUTOIMMUNE DISEASES

Pancreatitis also can occur in syndromes in which vasculitis is a major component of the disease process. It has been associated with common pediatric disorders, including Kawasaki syndrome, Henoch-Schönlein purpura, and systemic lupus erythematosus.[28,124,128,146] Increasing data suggest that numerous children with idiopathic chronic pancreatitis may have inflammation from an autoimmune process.[110] Pancreatitis has been a presenting manifestation of Crohn disease.[84]

MISCELLANEOUS CAUSES

Although no longer common, Reye syndrome has been associated with acute pancreatitis.[50] The venom of the scorpions *Tityus trinitatus*, *Tityus asthenes*, and *Leiurus quinquestriatus* can cause pancreatitis in patients who have been stung; however, these species do not live naturally in the United States.[15,121,144] The gastrointestinal symptoms observed in children with anticholinesterase insecticide poisoning may be caused by pancreatitis.[166] Also, tropical chronic pancreatitis seen in developing countries most likely is related to malnutrition and dietary intake of toxins, such as those from the tuber cassava.[14] Rarely, primary pancreatic lymphoma may manifest in children as acute pancreatitis.[41] Chronic fibrosing pancreatitis in which the pathogenesis of the disease process remains unsolved occurs only rarely in the United States.[57]

Although a much less common occurrence than in adults, acute pancreatitis after orthotopic liver transplantation is severe in children and often results in death.[152,153] The cause of pancreatitis in liver transplant recipients most likely is multifactorial but probably involves traumatic injury, biliary obstruction, and immunosuppressive therapy.

INFECTIOUS ETIOLOGIES

Infections caused by various microorganisms have been shown by culture, histologic examination, or elevation of antibody titer during the course of acute pancreatitis in humans (Table 58–2). A true causal relationship usually is not shown, however. Although not all of the following infectious agents have been shown to be associated with childhood pancreatitis, they must be considered as possible etiologic agents because adults with infectious pancreatitis have been described. Compared with previous decades, infectious agents are being encountered less as a cause of acute pancreatitis, most likely because of mumps vaccination.

VIRAL INFECTIONS

Group B coxsackieviruses and mumps virus are the best documented causes of pancreatitis in children. Group B coxsackieviruses usually cause pancreatitis along with other clinical manifestations, including aseptic meningitis, mild diarrhea, rash, and myocarditis.[23,67] They rarely have caused death in young

TABLE 58–2 Microorganisms Associated with Episodes of Acute Pancreatitis

Viruses
Adenoviruses
Cytomegalovirus
Epstein-Barr virus
Group B coxsackieviruses
Hepatitis A virus
Hepatitis B virus
Hepatitis E virus
Herpes simplex viruses
Human immunodeficiency virus
Measles virus
Mumps virus
Parainfluenza viruses
Rotavirus
Rubella virus
Varicella-zoster virus
Parasites
Ascaris lumbricoides
Clonorchis sinensis
Cryptosporidium parvum
Echinococcus granulosus
Fasciola hepatica
Plasmodium falciparum
Taenia saginata
Toxoplasma gondii
Wuchereria bancrofti
Mycoplasmas and Bacteria
Brucella melitensis
Campylobacter jejuni
Escherichia coli
Legionella spp.
Leptospira spp.
Moraxella catarrhalis
Mycobacterium tuberculosis
Mycoplasma pneumoniae
Salmonella spp.
Yersinia spp.
Fungi
Aspergillus spp.
Candida spp.
Cryptococcus neoformans

infants with myocarditis and pancreatitis.[36] How commonly these enteroviruses cause pancreatic inflammation is unknown. In one epidemiologic study, 31 percent of patients with aseptic meningitis during an epidemic of group B coxsackievirus infection had increased serum amylase concentrations.[111] Numerous studies have shown coxsackievirus-induced damage to pancreatic acinar cells in mouse models of infection.[65] Coxsackievirus B strains have been isolated from pancreatic biopsy samples of patients with chronic pancreatitis.[140]

Usually, mumps pancreatitis occurs in the presence of parotitis; however, abdominal pain and vomiting may occur for days before salivary swelling develops.[164] Rarely, mumps virus can cause pancreatitis without other common clinical manifestations.[109] Because more than 80 percent of children with mumps parotitis have elevated serum amylase concentrations, ultrasonography and serum lipase concentrations should be obtained to aid in establishing the diagnosis of pancreatitis.[55] An estimated 15 percent of children with mumps virus infection have abdominal tenderness and vomiting suggestive of pancreatitis.[63] In only a single report has the pancreatitis been hemorrhagic and severe.[44] Occasionally, chronic or recurring pancreatitis develops after mumps infection.[172]

Researchers previously thought that acute pancreatitis occurred in cases of viral hepatitis only when fulminate liver disease developed. Increasingly, children with mild hepatitis A infection and pancreatitis are being described, however.[2,105] In addition, a 16-year-old patient with acute hepatitis A infection died as a result of severe pancreatitis with multiorgan failure.[79] Individuals with acute hepatitis and pancreatitis also have been found to have hepatitis E viral infection.[69,105] Hepatitis B viral antigens have been detected in the pancreatic glandular cells of patients with severe acute hemorrhagic pancreatitis.[138] The role of hepatitis B virus in the pathogenesis of pancreatic inflammation in these patients is unknown; however, a young adult developed three episodes of acute pancreatitis during acute exacerbations of chronic hepatitis B infection that resolved after lamivudine therapy was given.[27]

Human herpesviruses are uncommon causes of childhood pancreatitis in immunocompetent patients. Occasionally, pancreatitis develops in children and adolescents with infectious mononucleosis.[83,107] Acute pancreatitis and occasionally pseudocyst formation also have been reported in previously healthy individuals with varicella infection.[95,155] In addition, adults have developed pancreatitis during a period in which seroconversion to cytomegalovirus was documented, or when antigens were identified in biopsy specimens obtained during ERCP.[78,119]

Viral pancreatitis also occurs in immunocompromised patients. Cytomegalovirus has been identified in pancreatic specimens from autopsies of patients who had AIDS, transplant recipients, and patients who had undergone cancer chemotherapy.[68,72] The symptoms of pancreatitis have resolved in a few patients with AIDS treated with ganciclovir or foscarnet.[30] Adenovirus has caused hemorrhagic pancreatitis and death in a child who received a bone marrow transplant, whereas an infant with disseminated adenoviral infection and pancreatitis survived with cidofovir therapy.[25,115] Varicella-zoster and herpes simplex viruses have caused pancreatitis and death in patients with various immunodeficient conditions.[45,139] A disseminated parainfluenza virus infection in an infant with severe combined immunodeficiency was associated temporally with the development of pancreatitis; however, no attempt was made to culture the virus from postmortem pancreatic tissue.[48]

Whether HIV directly causes pancreatitis is unclear. Laboratory-diagnosed episodes of pancreatitis in adults and children with AIDS do occur, but whether the pancreatic inflammation is caused by HIV or an unrecognized opportunistic pathogen is unknown.[175] HIV-infected children frequently have elevated amylase and lipase levels with no correlation to antiviral therapy.[24] Also, increasing numbers of adults with primary manifestations of HIV infection have presented with acute pancreatitis, suggesting a role of HIV in the pathogenesis of the disease.[157]

Interstitial pancreatitis occurs commonly in children with congenital rubella syndrome.[106] In addition, severe pancreatitis has been identified in immunocompetent and immunocompromised patients with fatal measles virus infection.[161] An adolescent has been described with measles encephalitis and pancreatitis that responded to steroids.[148] One case of an adolescent with rotavirus gastroenteritis in whom pancreatitis developed has been reported.[35]

PARASITE INFESTATIONS AND INFECTIONS

Ascaris lumbricoides can migrate in the intestines to the ampulla of Vater and subsequently to the pancreatic duct or common bile duct. Obstruction of the biliary or pancreatic duct can cause acute pancreatitis.[1,11] Ascariasis is diagnosed when adult roundworms are identified in the duodenum by radiographs of the upper gastrointestinal tract (Fig. 58–1), or more commonly by ultrasonography or ERCP. Often, a history of seeing worms in the feces can be elicited. The flukes *Clonorchis sinensis* and *Fasciola hepatica* and the cestode *Taenia saginata* also can migrate to the pancreatic and biliary drainage systems and cause pancreatitis.[40,89,141] Rarely, hepatic hydatid cysts caused by *Echinococcus* can obstruct biliary drainage and cause pancreatic inflammation.[122] *Wuchereria bancrofti* occasionally has been found to cause chronic pancreatitis.[70] Parasitic infestations should be considered as a cause of pancreatitis, particularly in immigrant children and patients who have traveled to developing nations.

Figure 58–1 An ascaris close to the ampulla of Vater, the body and tail lying in the second and third parts of the duodenum. The patient is a 9-year-old girl with acute pancreatitis.

The protozoan *Cryptosporidium parvum* has been identified in the bile of a patient with AIDS with elevated serum amylase and right upper quadrant abdominal pain.[52] ERCP showed biliary and pancreatic ductal disease, but no other opportunistic pathogens were isolated. Cryptosporidia also have been observed in the interlobular pancreatic ducts of experimentally infected immunocompromised mice.[158] Whether cryptosporidial infection causes pancreatitis in immunocompetent patients is unknown; however, a previously healthy adolescent developed pancreatitis after having cryptosporidial diarrhea.[60] *Toxoplasma gondii* cysts have been found in the postmortem pancreatic tissue of patients with AIDS.[4,64] Rarely, pancreatitis occurs during acute episodes of falciparum malaria.[74] Other systemic manifestations of malaria that often are present include high fever, hepatitis, intestinal malabsorption, encephalitis, and pulmonary insufficiency.

MYCOPLASMAL AND BACTERIAL INFECTIONS

In older children and adults, moderately severe symptoms of pancreatitis have occurred just before or during the course of atypical pneumonia.[5,62] In these cases, most patients have had cold agglutinins in their sera, and all have had significant changes in *Mycoplasma pneumoniae* antibody titer. Some controversy has ensued over whether *M. pneumoniae* can cause acute pancreatitis without evidence of pneumonia. Although complement-fixing IgM antibodies against *M. pneumoniae* often increase significantly during the course of acute pancreatitis, researchers have argued that pancreatic cellular antigenic components similar to *Mycoplasma* lipid antigens are exposed during the disease process and that the antibodies elicited cross-react in *Mycoplasma* serologic assays.[88]

Along with *M. pneumoniae* infection, legionnaires' disease must be considered when acute pancreatitis develops along with pneumonia.[103,170] Miliary tuberculosis also can cause symptoms of pancreatitis.[132] Occasionally, pancreatitis may be the only manifestation of tuberculosis and usually is diagnosed by fine-needle aspiration of the pancreas.[58,113]

Common pyogenic bacteria usually do not cause acute pancreatitis. Secondary invasion of inflamed pancreatic tissue does occur. Some evidence exists that circulating endotoxin from *Escherichia coli* can cause extrahepatic cholestasis and pancreatitis.[37] Acute pancreatitis also has been seen in children with hemolytic-uremic syndrome.[130,134] Pancreatitis can occur during acute episodes of enteritis. *Salmonella typhimurium*, *Salmonella typhi*, *Campylobacter jejuni*, *Yersinia enterocolitica*, and *Yersinia pseudotuberculosis* all have been reported to cause clinically evident and laboratory-confirmed cases of pancreatitis.[10,34,137,145] A single case of *Moraxella catarrhalis* causing severe pancreatitis and death in a 4-year-old child has been reported.[118]

Pancreatitis has been reported in children with leptospirosis.[81,117] *Brucella melitensis* also has been added to the list of uncommon causes of acute pancreatitis.[123] *Helicobacter pylori* has been suggested to influence the clinical course of pancreatitis in humans, but data are still lacking to imply a role in pancreatic pathology.[98]

FUNGAL INFECTIONS

Fungal infections have not been reported to cause acute pancreatitis in immunocompetent patients. *Aspergillus* has caused fatal hemorrhagic pancreatitis, however, in an adult patient with cancer who was undergoing chemotherapy.[53] *Candida* spp. and *Cryptococcus neoformans* have been isolated from the pancreatic tissue of patients with AIDS, but whether they cause clinical symptoms of pancreatitis is unknown.[175]

PATHOGENESIS

Enzymes for polysaccharides, fats, and proteins are produced and stored in pancreatic acinar cells. They exist intracellularly in an inactive precursor form. After cholecystokinin stimulation, the proenzymes are released into the pancreatic ducts and flow into the duodenum. The enzymes normally do not become enzymatically active until they reach the intestinal lumen.

When trypsinogen is activated prematurely to trypsin within the pancreatic acinar cells, autodigestion occurs within the pancreas.[17] The mechanisms that activate trypsinogen and other proteolytic enzymes during specific pathologic processes have not been well elucidated. Drug-induced and infectious causes of pancreatitis are thought to be caused by direct toxic effects on acinar cells. Gallstones, *Ascaris* infection, and congenital abnormalities are thought to damage acinar cells by obstructing pancreatic flow. Traumatic pancreatitis probably occurs as a result of direct injury to the glandular cells, whereas vasculitis may cause changes in pancreatic blood flow, causing ischemia.

Autodigestion of the pancreas causes edema of pancreatic tissue, and microcirculation may be compromised, leading to ischemia, hemorrhage, or necrosis. An inflammatory response develops, which may be mild, as occurs commonly in episodes of infectious pancreatitis, or may be more severe with hemorrhagic necrosis. Major mediators of the intense immune response include tumor necrosis factor, interleukin-1, interleukin-6, interleukin-8, and platelet activation factor.[94] If an imbalance of the proinflammatory response occurs within the pancreas, a systemic inflammatory response including shock may occur, leading to high morbidity and mortality. Also, sepsis may occur because of extensive necrotic tissue within the pancreas and translocation of microorganisms from the intestines.

TREATMENT

Despite increasing recognition of cases of childhood pancreatitis, no major pharmacologic advances have been made in the treatment of the disease since the mid-1970s. Animal data have shown that medications such as glucagon, aprotinin, gabexate mesilate, 5-fluorouracil, octreotide, and somatostatin may be useful in the treatment of pancreatitis; however, trials in adults have not substantiated their efficacy.[38] The continuing main objectives of treatment are to relieve abdominal pain and treat aggressively systemic manifestations, such as shock, electrolyte abnormalities, and anemia. Meperidine continues to be the medication most commonly used for controlling pain.

In the past, children were kept in the fasted state and nasogastric suction was applied to decrease duodenal acid–stimulated secretin release. Feeding with carbohydrate solutions was not restarted until all symptoms had resolved. More recent studies in adults have shown, however, that feeding with a low-fat elemental diet decreases the complication rate of patients with acute pancreatitis and now is considered the treatment of choice over total parenteral nutrition.[101,151] A study has shown that patients with severe pancreatitis can be fed by slow infusion by the nasogastric route without worsening of pain or changes of nutritional status compared with being fed by a nasojejunal tube.[85] Intravenous fluids and colloids are used during the acute episode to maintain intravascular volume. During the entire course of acute pancreatitis, the hematologic and biochemical parameters of the child must be monitored closely.

If the episode of pancreatitis is drug-induced, use of the medication should be curtailed immediately. Often, the symptoms recur if the medication is restarted. Pancreatitis caused by *M. pneumoniae* or bacteria should be treated with proper antimicrobials. Obstructions to pancreatic flow (e.g., gallstones,

roundworms, congenital abnormalities) may have to be excised or altered either by surgery or by endoscopy.[18,77,126]

COMPLICATIONS

During an acute episode of pancreatitis, the systemic inflammatory response syndrome may develop, leading to renal, hematologic, central nervous system, pulmonary, and cardiovascular complications. In 12 percent of children with pancreatitis, an inflammatory mass develops in the first weeks after the onset of illness; however, these masses more commonly occur after trauma.[163] Continued or increasing abdominal pain, nausea, or vomiting often accompanies the development of a phlegmon, abscess, or pseudocyst. An inflammatory phlegmon usually develops into a thin-walled pseudocyst of the lesser sac but may become secondarily infected and induce the formation of an abscess. Patients in whom an inflammatory mass develops must be monitored closely with frequent physical examinations and serial CT scans. In children with pseudocysts, acute abdominal pain accompanied by hypotension often signifies bleeding into the pseudocyst or rupture of the pseudocyst into the peritoneum. Slowly leaking pseudocysts may cause pancreatic ascites. Pseudocysts are resected surgically, drained externally, or drained by endoscopy when complications occur.[9] Approximately 33 percent of pseudocysts resolve spontaneously within 6 weeks.[127]

The development of fever and leukocytosis during pancreatitis should suggest the presence of an infected pseudocyst, pancreatic abscess, or sepsis. In adults, infectious complications account for 80 percent of deaths associated with acute pancreatitis.[19] Isolates from pancreatic abscesses have yielded intestinal flora, including anaerobes, in more than 90 percent of cases, but *Candida* spp. are being isolated more frequently in many medical centers.[8,39] Rarely, *Streptococcus pneumoniae* can be isolated from infected pancreatic tissues of adults with chronic pancreatitis.[150] Carbapenems, such as imipenem and meropenem, are used commonly to treat adult patients with suppurative complications of pancreatitis because these antibiotics penetrate well into pancreatic tissues and have activity against intestinal flora.

Even with early diagnosis and proper antibiotics and surgical intervention, the death rate from pancreatic abscesses is 22 to 57 percent in adults.[63,66] Performing percutaneous catheter drainage under CT guidance may reduce the mortality rate associated with treating pancreatic abscesses.[13] Rarely, fistulas from pseudocysts or abscesses to other abdominal organs may develop.[61]

The role of prophylactic antibiotics in preventing the suppurative complications of acute pancreatitis remains controversial despite 3 decades of debate.[120] Prophylactic antibiotics should be considered only when pancreatic necrosis is significant; however, antimicrobial trials have yielded conflicting results in adults.[87] A more recent meta-analysis on the subject concluded that prophylactic antibiotics do not prevent pancreatic necrosis from being infected and do not prevent death.[102] Infections, when they do occur after the administration of prophylactic antimicrobials, often are caused by multiresistant bacteria or by fungi.

Osteolytic lesions resembling osteomyelitis may develop weeks to months after an acute episode of pancreatitis.[114] Elevated systemic levels of lipase activity possibly may cause intramedullary fat necrosis in the bone. Usually, the lesions are asymptomatic and resolve spontaneously without therapy.

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CHAPTER 59

Peritonitis and Intra-abdominal Abscess

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Intra-abdominal infection can be a life-threatening condition that occurs spontaneously or as a result of intra-abdominal disease, injury, or surgery. Given the compartmental anatomy and physiology of the abdominal cavity, intra-abdominal infection frequently is categorized as peritonitis, intraperitoneal abscess, retroperitoneal abscess, and visceral abscess.[3] This chapter reviews peritonitis and intra-abdominal abscess; liver abscess and retroperitoneal abscess are reviewed in Chapters 55 (liver abscess) and 60 (retroperitoneal abscess).

PERITONITIS

ANATOMY

Knowledge of the anatomic relationships within the abdomen is important for understanding the source and routes of spread of infection. The peritoneal cavity extends from the undersurface of the diaphragm to the pelvis. In males, it is a closed space, whereas in females, the ends of the fallopian tubes penetrate into the peritoneal cavity. The transverse mesocolon and greater omentum separate the upper and lower peritoneal cavity. Peritoneal reflections divide the intraperitoneal space further into several compartments: the lesser sac, the paracolic gutters, and the subhepatic and subphrenic spaces (Fig. 59–1). The most dependent area of the peritoneal cavity is the pelvis. Exudate can extend to any of the recesses within the peritoneal cavity distant from the original source, however, and cause diffuse inflammation.[3] When inflamed, the anterior parietal peritoneum, which is supplied by somatic afferent nerves, gives the sensation of localized pain. Stimulation of the visceral peritoneum causes dull, poorly localized pain.

PATHOGENESIS

Peritonitis is defined as inflammation of the serosal lining of the abdominal cavity or the peritoneum and may be caused by any chemical or infectious agent that irritates the peritoneal surfaces. Noninfectious peritonitis is caused by extravasation of irritants, such as gastric juice, bile, urine, blood, pancreatic secretions, or the contents of a ruptured cyst, into the peritoneal cavity. Although chemical peritonitis generally is aseptic, it may be an important antecedent event to the development of infectious peritonitis.

After peritoneal contamination by bacteria has occurred, the first mechanism of host defense is lymphatic clearance. In experimental peritonitis, this clearance is so efficient that peritonitis and abscess formation occur only if adjuvant substances, such as hemoglobin or necrotic tissue, are present.[23,24,46] In the first hours after bacterial contamination occurs, local resident macrophages are the predominant phagocytic cells. The macrophages then are cleared by the lymphatic system. After bacterial proliferation occurs, polymorphonuclear leukocytes become more numerous in the peritoneal cavity, and inflammation ensues. These peritoneal defense mechanisms also have adverse effects. Fibrin is deposited, which potentially entraps bacteria into a sequestered environment. An increase in splanchnic blood flow causes exudation of fluid into the peritoneal space, further impairing host defenses by diluting important peritoneal opsonins.[23,24] These host responses serve as a means of containing infection, but they also may contribute to the formation of abscesses.

Infectious peritonitis is subdivided into primary and secondary peritonitis based on the pathophysiology of the infection. Peritonitis that is associated with peritoneal dialysis or the presence of a ventriculoperitoneal shunt is a unique form of peritonitis that also is reviewed in this chapter. The microbial etiologies of peritonitis vary with the underlying cause and are summarized in Table 59–1.

PRIMARY PERITONITIS

Primary, or spontaneous, bacterial peritonitis is a rare infection defined as bacterial peritonitis in the absence of intra-abdominal findings, such as intestinal perforation. The incidence of spontaneous peritonitis in children is unknown; however, in the early 20th century, 8 to 10 percent of abdominal emergencies requiring surgical intervention were due to spontaneous peritonitis.[17,70] Freij and colleagues[29] conducted a 22-year review of children with primary peritonitis in Dallas, Texas. Primary peritonitis was diagnosed in 7 previously healthy children compared with 1840 cases of appendicitis during the same period. Currently, 1 to 2 percent of abdominal emergencies requiring surgical intervention are due to primary peritonitis.[37,40]

Now that this condition frequently is recognized clinically with the assistance of computed tomography (CT), the diagnosis often is made without exploratory laparotomy. The peak incidence of spontaneous peritonitis in children occurs when they are 5 to 9 years of age. In children, the most common predisposing factor is nephrotic syndrome, but this form of peritonitis also occurs in children with postnecrotic cirrhosis.[3,17,31,37,41,45,70,71] Spontaneous peritonitis rarely develops in previously healthy individuals without underlying conditions.[32,47]

The exact pathophysiologic mechanism for primary peritonitis is unknown; however, hematogenous inoculation is thought to be the most likely mechanism because the same organism frequently is recovered from cultures of blood and peritoneal fluid.[17,31,37] Alternative mechanisms include peritoneal seeding via the lymphatics, transmural migration through edematous bowel, and ascending infection from the female genitourinary tract.[31,37] In certain cases, impaired host defenses allow proliferation of bacteria that invade the peritoneal cavity, but a few children with primary peritonitis have no apparent impaired defense. Ascitic fluid from patients with nephrotic syndrome or cirrhosis contains lower levels of complement and immunoglobulin than does

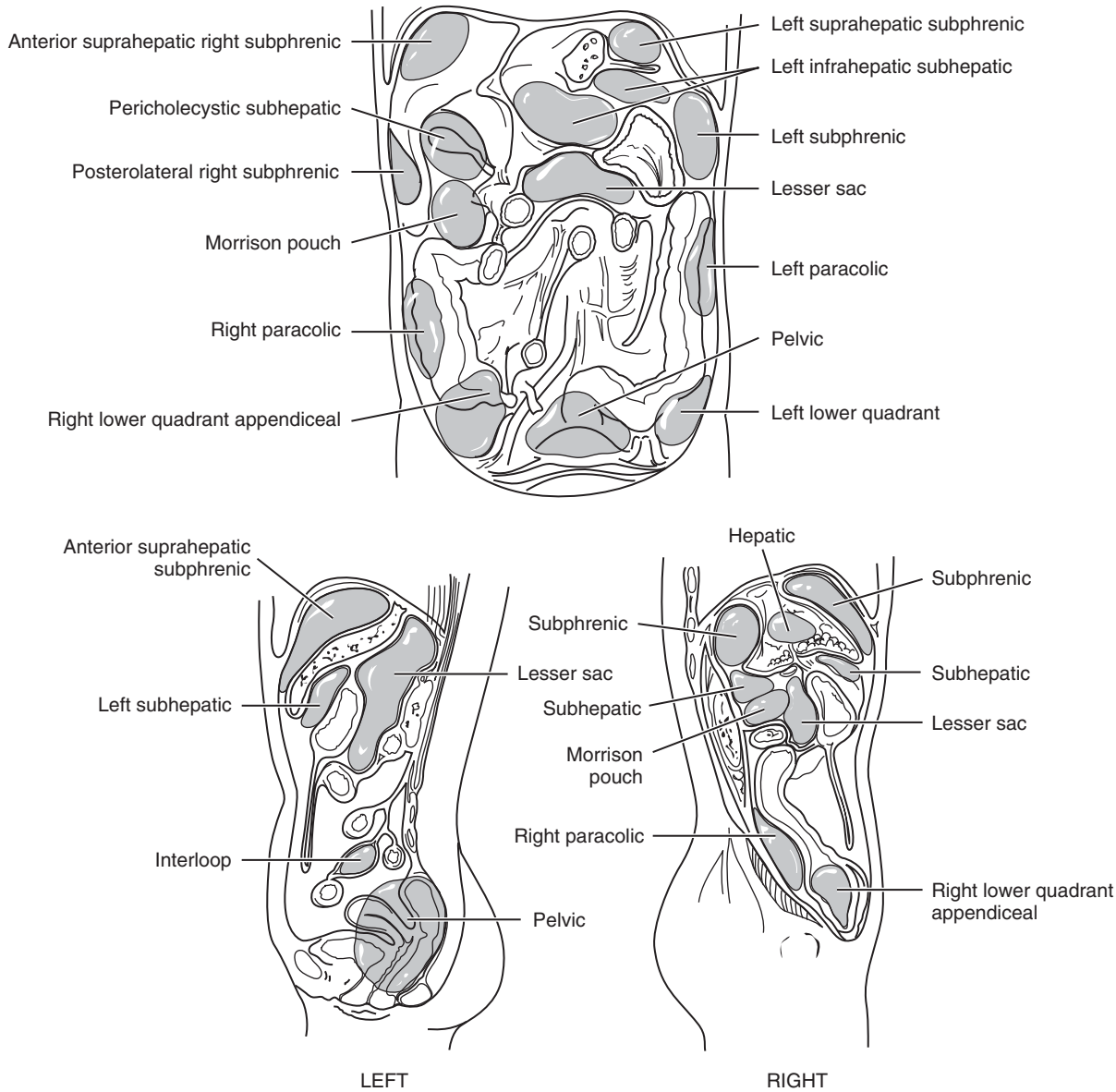

Figure 59–1 Anterior and sagittal views of the peritoneal cavity. *(From Altemeier, W. A., Culbertson, W. R., and Fullen, W. D.: Intra-abdominal sepsis. Adv. Surg. 5:281-333, 1971.)*

peritoneal fluid from a healthy host.[17,70] Deficiency of these important opsonins diminishes the natural clearance of organisms from the peritoneal cavity. Proliferation of organisms triggers the influx of phagocytes, release of inflammatory mediators, and localized or diffuse peritoneal irritation that gives rise to symptoms of abdominal pain and fever.

Since the pre-antibiotic era, researchers have recognized that primary peritonitis frequently is caused by *Streptococcus pneumoniae*,[31] *Streptococcus pyogenes*,[32] and *Staphylococcus aureus*.[17] Rarely, primary peritonitis in prepubescent girls is caused by extension of upper genital tract *S. pneumoniae* infection.[37,66] Since the 1960s, the bacteriology of primary peritonitis has shifted to include an increased proportion of infections caused by gram-negative enteric organisms, such as *Escherichia coli* and *Klebsiella* spp.[17,37,41,71] In some instances, primary *E. coli* peritonitis may occur concurrently with bacteremic urinary tract infection.

Tuberculous peritonitis may be caused by *Mycobacterium tuberculosis* or *Mycobacterium bovis*. It may occur as a complication of primary mycobacteremia or be caused by reactivation of latent intra-abdominal infection within lymphoid tissue, but only rarely does it seem to occur as a function of the ingestion of swallowed organisms from a pulmonary primary focus.[35,65,75] Peritoneal infection with *M. bovis*, which is clinically similar to *M. tuberculosis* peritonitis, is acquired from unpasteurized dairy products and has been reported in children living along the border between the United States and Mexico. These organisms may cause peritonitis from either mycobacteremia or erosion of organisms through the mesenteric lymph nodes or bowel wall into the peritoneal cavity.[20] *Salmonella* spp. rarely cause primary peritonitis and have been reported primarily in patients with underlying conditions.[47]

SECONDARY PERITONITIS

Secondary peritonitis, the most common form of peritonitis, arises as a complication of intra-abdominal injury or disease when microorganisms, secretions, and the particulate material of an

TABLE 59–1 Most Commonly Identified Etiologic Agents

Primary Peritonitis	Secondary Peritonitis
Escherichia coli (25-40%)	Aerobes
Haemophilus influenzae type b	*Enterobacter*
Klebsiella	*Enterococcus*
Mycobacterium bovis	*E. coli*
Mycobacterium tuberculosis	*Klebsiella*
Neisseria meningitidis	*Pseudomonas aeruginosa*
Other enteric gram-negative bacilli	*Serratia*
Other streptococci (alpha-hemolytic and beta-hemolytic)	Anaerobes
Staphylococcus aureus (2-4%)	*Bacteroides fragilis* group
Streptococcus pneumoniae (30-50%)	*Peptostreptococcus*
CAPD-Associated Peritonitis	**VP Shunt–Associated Peritonitis**
Candida	Coagulase-negative staphylococci
Coagulase-negative staphylococci	*Enterobacter*
Enteric gram-negative bacilli	*E. coli*
Mycobacterium	*Klebsiella*
Other fungi	*Pseudomonas*
Pseudomonas	*S. aureus*
S. aureus	
Stenotrophomonas	

CAPD, continuous ambulatory peritoneal dialysis; VP, ventriculoperitoneal.

intra-abdominal organ enter the peritoneal cavity. Congenital or acquired conditions that result in ischemia, inflammation, or perforation of abdominal viscera may be complicated by secondary peritonitis.[46] In premature infants, necrotizing enterocolitis is the most common cause of secondary peritonitis.[51] In infants and children, appendicitis is the most common cause; however, it also may occur with volvulus, intussusception, incarcerated hernia, or rupture of a Meckel diverticulum.[51] Although less common in children than in adults, peritonitis also occurs as a complication of mucosal diseases, such as peptic ulcer, ulcerative colitis, Crohn disease, and pseudomembranous colitis.[51]

Rupture of or injury to an intra-abdominal viscus results in spillage of the luminal contents and contamination of the peritoneal cavity with bacteria, gastrointestinal secretions, and debris. Chemical and infectious sources of inflammation are introduced. The stomach and upper gastrointestinal tract contents contain only 10^3 to 10^4 or fewer organisms per gram because of the low pH of gastric secretions. Gram-negative aerobic organisms colonize the upper gastrointestinal tract. In contrast, the colonic contents have predominantly anaerobes, with 10^{11} anaerobes and 10^8 aerobes per gram.[11,37,64]

Secondary peritonitis usually is a polymicrobial infection, with 5 to 10 different bacterial species of anaerobes and facultative gram-negative bacilli. Synergy among the various bacterial species enhances bacterial proliferation.[11] Members of the *Bacteroides fragilis* group and *Peptostreptococcus* spp. are the anaerobic organisms reported most commonly in secondary peritonitis. Of the aerobic organisms, *E. coli*, *Klebsiella* spp., *Pseudomonas aeruginosa*, and *Enterococcus* spp. are isolated most often. More recently, *P. aeruginosa* has been noted to be present in 20 to 30 percent of children with complicated ruptured appendicitis.[8,38] When secondary peritonitis occurs in patients with a history of prolonged hospitalization, underlying chronic conditions, or recent antibiotic therapy, the etiology may include nosocomial pathogens that have colonized the gastrointestinal tract, such as *P. aeruginosa*, *Enterobacter* spp., *Acinetobacter* spp., or other antibiotic-resistant organisms.

Focal suppurative infection may be present within an intra-abdominal or retroperitoneal solid organ or within intra-abdominal lymphoid tissue. Organisms spread from this purulent focus through the capsule of the organ or lymphoid tissue and enter the peritoneal cavity, with the subsequent development of peritonitis. The intra-abdominal organ or lymphoid tissue may be inoculated via bacteremia (e.g., *S. aureus* and renal infection) or be inoculated as a complication of the normal function of the organ (e.g., *E. coli* and renal infection or *Yersinia* and mesenteric adenitis).[13,36,42]

PERITONITIS AND IMPLANTED DEVICES

Peritonitis is the most significant infectious complication of long-term peritoneal dialysis. Contamination of the dialysis tubing, migration of skin flora from the exit site, or contamination of the dialysate may lead to peritonitis in patients undergoing continuous ambulatory peritoneal dialysis (CAPD). In each instance, a single pathogen usually is isolated.

Gram-positive organisms, coagulase-negative staphylococci, and *S. aureus* account for 30 to 45 percent of peritonitis episodes in children undergoing CAPD. Of CAPD-associated peritonitis episodes, 20 to 30 percent are caused by Enterobacteriaceae.[26] In these instances, contamination of the catheter site with fecal material most often occurs in young children who wear diapers and children with incontinence, an open urogenital sinus, or nephrostomy tubes. The water-borne pathogens *Pseudomonas* and *Acinetobacter* account for 6 percent and 4 percent, respectively, of peritonitis episodes in children receiving CAPD. *Pseudomonas* peritonitis is especially difficult to treat with the dialysis catheter in situ and may recur despite administration of appropriate antimicrobial therapy.[26]

Fungal peritonitis is another complication of CAPD that is difficult to treat successfully without removal of the catheter. Although fungal pathogens have accounted for only 2 percent of peritonitis episodes in children undergoing CAPD, this problem is occurring more commonly.[15,25,26,48,56,76] Most patients with fungal peritonitis have had previous episodes of bacterial peritonitis and antibiotic therapy. The most common fungal pathogens are *Candida* spp.[25,26]; however, rare fungi, such as *Curvularia* spp.,[15] *Fusarium* spp.,[26] *Trichosporon asahii*,[48] and *Aspergillus* spp.,[26] have been reported.[56,76] Other rare causes of CAPD-associated peritonitis include *Mycobacterium fortuitum* and *Mycobacterium chelonae*.[77]

Intra-abdominal infectious complications develop on average in less than 5 percent of infants and children who undergo ventriculoperitoneal shunt placement or revision for hydrocephalus.[43,55,68] Peritonitis, peritoneal pseudocyst, or perforation of the bowel by the abdominal catheter rarely occurs in children with such shunts.[5,30,33,60,61,63,67,72] Cerebrospinal fluid (CSF) in the peritoneal cavity may be seeded during transient bacteremia or a febrile illness or after abdominal trauma. In addition, peritonitis may develop as a complication of infection within the ventricles being drained[68] as organisms descend into the peritoneal cavity via the distal tubing.

A peritoneal pseudocyst containing CSF is the most common manifestation of peritoneal inflammation in patients with ventriculoperitoneal shunts. These patients often have a history of symptoms compatible with a shunt infection before the formation of a pseudocyst and may have signs of peritoneal inflammation and a palpable abdominal mass. The microbial etiology of ventriculoperitoneal shunt–associated peritonitis varies and reflects the pathogenesis of infection. Infections occurring within months of surgery often are caused by skin flora, *Staphylococcus epidermidis*, other coagulase-negative staphylococci, and *S. aureus*.[30,60] The microbiology of late shunt-associated peritonitis is similar to that of spontaneous bacterial peritonitis and may include gram-negative enteric organisms and gram-positive cocci.[60] Peritonitis caused by colonic flora also rarely has been associated with bowel perforation by the distal end of the ventriculoperitoneal shunt.[33,60,67]

CLINICAL MANIFESTATIONS

The initial signs and symptoms of primary bacterial peritonitis include nausea, vomiting, diarrhea, and diffuse abdominal pain.[37,40,41] These signs and symptoms are similar to those of secondary peritonitis caused by a ruptured appendix.

Rupture of the appendix is the most common cause of secondary peritonitis in children; the initial symptoms of anorexia, vomiting, and localized abdominal pain frequently precede the signs and symptoms of diffuse peritoneal inflammation. In primary and secondary peritonitis, patients typically lie very still because any movement exacerbates the abdominal pain. Physical findings include fever, tachycardia, abdominal distention, hypoactive bowel sounds, abdominal tenderness, rebound tenderness, abdominal wall rigidity, and tenderness on rectal or vaginal examination. Peritoneal inflammation is associated with an increase in splanchnic blood flow, capillary permeability, and a shift of fluid into the peritoneal space, which may lead to intravascular hypovolemia and shock, in addition to systemic absorption of endotoxin and bacteria.[46]

Fever and abdominal pain in any child undergoing peritoneal dialysis should be evaluated carefully. Turbid dialysate fluid raises the suspicion of CAPD-associated peritonitis. Similarly, symptomatic children with ventriculoperitoneal shunts should be evaluated for shunt-associated peritonitis.[61,68,80] In a retrospective report of 19 children with ventriculoperitoneal shunts and peritonitis, Reynolds and associates[61] noted that fever and abdominal pain were the most common symptoms in 14 of their patients. Stamos and colleagues[68] found that fever, lethargy, nausea, and vomiting were the most frequently reported symptoms in a review of 23 children with gram-negative infection of ventriculoperitoneal shunts.

Primary tuberculous peritonitis usually is gradual in onset and associated with weight loss, malaise, and nightsweats.[35,37,65] The degree of tenderness is less than that present with acute pyogenic peritonitis and may be nonexistent. Palpation of the abdomen may reveal an extensive, irregular collection of masses, often described as "doughy," caused by widespread granulomatous inflammation.[37]

DIAGNOSIS

Laboratory findings in a child with peritonitis often are nonspecific. The white blood cell count usually is elevated (16,000 to ≥25,000 cells/mm^3), with a predominance of polymorphonuclear leukocytes and an increase in immature forms.[37] The hematocrit may be elevated because of dehydration and hemoconcentration. Mild pyuria is noted occasionally because of irritation of the urinary bladder or ureters.

Diagnostic imaging studies can be useful in evaluating intra-abdominal infections. Upright and lateral decubitus radiographs of the abdomen may show distended adynamic loops of bowel suggestive of ileus and obliteration of the peritoneal fat lines and psoas shadows. Free intraperitoneal air below the diaphragm indicates a ruptured viscus. The presence of a fecalith or right lower quadrant mass may be consistent with appendicitis. Abdominal ultrasound and CT may reveal an underlying cause of the peritonitis.[47,51]

Analysis of peritoneal fluid aspirate or lavage material may be helpful in differentiating primary from secondary peritonitis. Free air, blood, or bile indicates peritonitis secondary to intestinal perforation. In peritonitis, the leukocyte count in peritoneal fluid usually is greater than 250 to 300 white blood cells/mm^3 and sometimes 3000 to 5000 white blood cells/mm^3, with granulocytes predominating in 80 percent of cases.[37,45,70] A total protein content greater than 1 g/dL, a glucose level less than 50 mg/dL, or an elevated lactate dehydrogenase concentration (>25 mg/dL) is consistent with secondary peritonitis.[17,31,37,70]

If a Gram stain of peritoneal fluid shows only gram-positive cocci, primary peritonitis is most likely. The presence of gram-negative bacilli is consistent with primary or secondary peritonitis, but the presence of many different organisms on Gram stain is diagnostic of secondary peritonitis. Bacteremia occurs in 75 percent of patients with primary peritonitis. Specimens of peritoneal fluid and blood should be sent for culture.[37] Similarly, secondary peritonitis also can be associated with bacteremia, suggesting the need for obtaining cultures of blood in addition to peritoneal fluid. Specimens of peritoneal fluid should be processed to optimize the recovery of aerobic and anaerobic organisms, and the use of specific transport tubes or an airless, capped syringe is required.[11,37] The wide variety of pathogens isolated from intra-abdominal infections along with the variable antibiotic susceptibility of these pathogens supports taking an aggressive approach to obtaining samples for microbiologic evaluation.

A child undergoing CAPD who is suspected of having peritonitis should have dialysate sent for cell count, Gram stain, and culture for bacterial, mycobacterial, and fungal pathogens. If a child with a ventriculoperitoneal shunt is suspected of having peritonitis, CSF from the proximal portion of the shunt should be sent for culture, cell count, and determination of glucose and protein levels in addition to Gram stain and culture of peritoneal fluid.[30] Abdominal imaging by ultrasound or CT is useful in identifying a peritoneal pseudocyst and the location of distal tubing.

DIFFERENTIAL DIAGNOSIS

Other infectious diseases that may mimic primary or secondary bacterial peritonitis include mesenteric adenitis, gastroenteritis, hepatitis, streptococcal pharyngitis, lower lobe pneumonia, pyelonephritis, and pelvic inflammatory disease. Noninfectious diseases to be considered in the differential diagnosis are pancreatitis, diabetic ketoacidosis, Henoch-Schönlein purpura, ovarian torsion, sickle-cell pain crisis, and lead poisoning.[51]

TREATMENT

Optimal management of peritonitis involves prompt and aggressive physiologic support, surgical consultation, and antimicrobial therapy. Correction of fluid and electrolyte imbalances and hemodynamic stabilization should be initiated as soon as the diagnosis of peritonitis is suspected. Spontaneous bacterial peritonitis usually is managed medically, unless the diagnosis is uncertain, in which case exploratory laparotomy or laparoscopy is performed. Before resistant strains of *S. pneumoniae* emerged, primary peritonitis in children was treated with aqueous penicillin G.[17,31] Given the increased prevalence of *S. pneumoniae* with reduced susceptibility to penicillin, third-generation cephalosporins such as cefotaxime or ceftriaxone are recommended until susceptibility results are available.[45,71] If primary peritonitis is caused by gram-negative organisms, appropriate empiric therapy includes cefotaxime or ceftriaxone, with or without an aminoglycoside, a carbapenem, ticarcillin-clavulanate, or piperacillin-tazobactam, pending completion of culture and susceptibility testing.

Patients with secondary peritonitis may require either immediate surgery to control the source of contamination and to remove necrotic tissue, blood, and intestinal contents from the peritoneal cavity or a drainage procedure if a limited number of large abscesses can be shown.[43,47,52,79] In cases of phlegmon, or extensive inflammatory edema, surgery usually is not performed acutely because of the child's unstable metabolic state and friable

intra-abdominal tissues. Surgery is delayed for several hours or weeks to allow the inflammation to resolve. Surgery also may be postponed indefinitely.[6,14,74]

Empiric antimicrobial therapy for secondary peritonitis should have activity against anaerobes, especially the *B. fragilis* group, and enteric gram-negative aerobes.[7] Although controversial, some regimens also include an antibiotic effective against enterococci. The gold standard for antimicrobial therapy historically has been clindamycin or metronidazole, gentamicin, and ampicillin.[7,11,27,43,46,52,62] Alternative efficacious regimens, as single or combination therapy, include aztreonam, cefotaxime, cefoxitin, imipenem-cilastatin, meropenem, piperacillin-tazobactam, and ticarcillin-clavulanate.[7,43,46,52,79] Rates of resistance to cefoxitin and clindamycin among the *B. fragilis* group have increased and are reported to be 49 percent; in some institutions, alternative regimens are used routinely.[2,46]

Other studies have examined the use of a single broad-spectrum antibiotic, which allows a portion of the therapy to be delivered less expensively on an outpatient basis. Fishman and coworkers[27] prospectively evaluated the clinical outcomes of 150 children with perforated appendicitis treated postoperatively with a 10-day course of piperacillin-tazobactam. They compared the outcome with that of historical controls treated with a 10-day course of ampicillin, gentamicin, and clindamycin. Rates of postoperative infectious complications were similar in both groups. Bradley and colleagues[8] prospectively identified 87 children with complicated appendicitis in five pediatric centers, also comparing costs and outcomes with historical controls. Although inpatient treatment courses were reduced by an average of 42 percent in meropenem-treated children, outcome measures were equivalent to those of historical controls.

Table 59–2 summarizes randomized trials of monotherapy versus combination therapy for ruptured appendicitis in children. Although no differences in outcome were observed, the potential of emerging resistance to broad-spectrum agents versus the convenience of monotherapy must be considered and balanced against the possible decreased risk of developing nosocomial infection among children who can receive a substantial component of parenteral therapy in the home.[38]

Empiric antibiotic treatment of CAPD-associated peritonitis should be effective against gram-positive and gram-negative organisms until culture results are available. Intraperitoneal antibiotics, with or without concomitant intravenous antibiotics, achieve adequate serum and dialysate concentrations. Vancomycin is used for empiric therapy for gram-positive infections, but if staphylococcal organisms are susceptible to β-lactam agents, treatment with cefazolin is effective.[26] Aminoglycosides (gentamicin or tobramycin) or cephalosporins are used for gram-negative infections; however, because most intraperitoneal antibiotics are absorbed into the systemic circulation, serum aminoglycoside or vancomycin concentrations should be monitored for possible toxicity. Therapy for fungal peritonitis usually is intravenous amphotericin B, although successful use of fluconazole or intraperitoneal amphotericin B has been reported.[26,56] Indications for removal of a dialysis catheter include persistent infection with *S. aureus* or *Pseudomonas*, tunnel infection, or fungal peritonitis.[26]

Treatment of peritonitis associated with ventriculoperitoneal shunts usually requires externalization of the distal end of the catheter in addition to institution of antibiotic therapy.[30] Empiric antibiotic therapy should include an antistaphylococcal agent active against coagulase-positive and coagulase-negative staphylococci. Coagulase-negative staphylococci are a common cause of ventriculoperitoneal shunt infection; vancomycin should be administered pending culture and susceptibility results. If Gram stain of ventricular CSF or peritoneal fluid reveals gram-negative organisms, cefotaxime, ceftriaxone, ceftazidime, or meropenem should be added.[5,61]

The duration of antibiotic therapy for peritonitis should be dictated by the clinical course of the patient because no single regimen or treatment course is accepted universally.[37] Indicators of sufficient therapy include resolution of fever and abdominal pain and return of the leukocyte and differential counts to normal.[37,52,69] Primary peritonitis caused by streptococci is treated successfully with a 10- to 14-day course of antibiotics.[17,37] Primary peritonitis with gram-negative organisms may require 10 days to 3 weeks of antibiotic treatment.[17] The duration of therapy for secondary peritonitis after adequate surgery usually is 5 to 10 days, but it depends on the clinical response to therapy.[37,52,69] Short-course therapy for 5 days has been shown to be efficacious in some patients,[37,52] but longer courses are required if fever persists, or abdominal signs and symptoms are present. Standard therapy for tuberculous peritonitis consists of a minimum of two antituberculous drugs. As with other forms of extrapulmonary tuberculosis in children, empiric therapy with isoniazid, rifampin, and pyrazinamide is advised pending culture and susceptibility results. Although *M. bovis* is resistant to pyrazinamide, most strains are susceptible to isoniazid and rifampin and to ethambutol.

COMPLICATIONS

Acute complications associated with peritonitis include septic shock, adult respiratory distress syndrome, septic thrombophlebitis of the portal vein, acute renal failure, and multiorgan system failure.[46] Postoperative complications include wound infection, adhesions, bowel obstruction, formation of a fistula, and formation of an intra-abdominal or retroperitoneal abscess.

Recurrent peritonitis (tertiary peritonitis) is an entity described as occurring late in the course of therapy for secondary peritoni-

TABLE 59–2 Monotherapy versus Combination Therapy for Ruptured Appendicitis in Children

Study	Monotherapy (A)	Combination Therapy (B)	No. Patients		Complications*	
			A	*B*	*A (%)*	*B (%)*
Meller et al.[49]	Cefoxitin	Clindamycin/gentamicin	29	27	1 (3)	4 (15)
Dougherty et al.[22]	Ticarcillin-clavulanate	Clindamycin/gentamicin ± ampicillin	79	45	14 (18)	5 (11)
Uhari et al.[73]	Imipenem-cilastatin	Metronidazole/tobramycin	9	10	2 (22)	1 (10)
Collins et al.[19]	Ampicillin-sulbactam ± aminoglycoside	Ampicillin/clindamycin ± aminoglycoside	75	39	2 (1)	1 (3)
Fishman et al.,[27] Lund and Murphy[43]	Pipercillin-tazobactam	Ampicillin/gentamicin/clindamycin	150	373	14 (9)	24 (6)
Bradley et al.[8]	Meropenem	Cefotaxime ± amikacin or tobramycin, clindamycin or metronidazole	22	13	2 (9)	1 (8)

**Wound infections, intra-abdominal abscess, or rehospitalization.*
Modified from Kaplan, S. L.: Antibiotic usage in appendicitis in children. Pediatr. Infect. Dis. J. 17:1047-1048, 1998.

tis.[37,44,79] Patients with this condition continue to have symptoms despite receiving appropriate antimicrobial therapy, and peritoneal fluid reveals persistent inflammation. Bacterial cultures often are negative or may yield an organism of low virulence. Multiorgan system failure and a poor outcome frequently are associated with tertiary peritonitis. The mechanism of ongoing peritoneal inflammation is unknown; however, some investigators have proposed that immunoregulatory dysfunction and poor nutrition are contributing factors.

INTRA-ABDOMINAL ABSCESS

Intra-abdominal abscesses often are categorized as intraperitoneal, visceral, or retroperitoneal (see Chapter 60).[4,10] In children, intraperitoneal abscesses are most common. The most common underlying conditions associated with an intra-abdominal abscess in children are appendicitis and trauma.[10,51] Reviews of gangrenous or perforated appendicitis in children indicate that 2 to 20 percent of cases are complicated by the formation of an abscess.[52,62]

Two basic mechanisms exist for the development of an intraperitoneal abscess. In the first mechanism, diffuse peritonitis may cause loculations of purulent material to form in the areas anatomically most dependent—typically the pelvic, subphrenic, and paracolic regions (see Fig. 59–1). The second mode of formation of an abscess involves a localized focus related to contiguous disease or injury in which host defenses and the inflammatory response prevent diffuse spread and peritonitis.[4] The microbiology of intraperitoneal abscesses is polymicrobial and reflects that of the intestinal flora. In a review of intra-abdominal abscess in 36 children, Brook[10,11] noted that the predominant organisms were the *B. fragilis* group, *Peptostreptococcus*, *E. coli*, and other Enterobacteriaceae.

The most common sites of visceral abscess in children are the liver (see Chapter 55), pancreas, and spleen. Underlying conditions that may lead to the development of a pancreatic abscess include pancreatic injury, pancreatitis, and biliary obstruction. Pancreatitis or surgical or accidental injury to the pancreas causes the release of pancreatic enzymes and focal necrosis.[28] Reflux of contaminated bile into the pancreatic duct is hypothesized to be the mechanism by which enteric organisms gain access to the injured pancreas and proliferate. A pancreatic abscess usually is a polymicrobial infection caused by aerobic (*E. coli*, *Klebsiella pneumoniae*, group D streptococci) or anaerobic (peptostreptococci, *B. fragilis* group) organisms that inhabit the gastrointestinal tract. Rare instances of *S. aureus* pancreatic abscess occur as a result of bacteremia.[12,13]

Splenic abscesses are unusual findings in infants and children. Before the 1970s, most reports involved solitary pyogenic abscesses. Since then, the number of reports of multiple splenic abscesses has increased.[16,39,58] Splenic abscess usually is associated with one of five underlying conditions: endocarditis, injury, hemoglobinopathy, immunodeficiency, or adjacent infection. Given the filtering function of the spleen, an abscess can form as a result of any metastatic hematogenous infection, such as endocarditis. Although rare, splenic abscess can be a delayed complication of the nonoperative management of splenic injuries. Splenic infarcts associated with hemoglobinopathies such as sickle-cell disease may become secondarily infected and form an abscess.[16] Immunodeficiency, such as malignancy or acquired immunodeficiency syndrome, is another significant risk factor for the development of multiple splenic abscesses.[39,53,58]

Rarely, infection or disease in a contiguous focus may extend to the spleen. In a review of 56 children with splenic abscesses, 7 (12.5%) were cryptogenic with no apparent cause.[39] In most instances, a single pathogen is isolated, with *S. aureus*, streptococci, *E. coli*, and *Salmonella* spp. being the most common. Fungi, most often *Candida* spp., have been isolated from splenic abscesses primarily in immunocompromised hosts.[39,53,58]

CLINICAL MANIFESTATIONS

The typical clinical features of an intra-abdominal abscess include fever, abdominal pain, and tenderness over the involved area. Subphrenic abscesses also may be manifested as referred pain or pulmonary or pleuritic symptoms. Pancreatic abscess may be associated with a palpable epigastric mass and elevated serum lipase and amylase.[12] Splenomegaly or a splenic mass may be noted in approximately half of patients with splenic abscesses.[39] In postoperative patients, persistence of abdominal symptoms or fever warrants evaluation for an intraperitoneal abscess.[3] Leukocytosis (20,000 to 50,000 cells/mm^3) frequently is present in children with an intra-abdominal abscess.[3,37]

DIAGNOSIS

Imaging studies are helpful in diagnosing an intra-abdominal abscess. Plain radiographs are useful as an initial procedure and may show an extraintestinal air-fluid level, right lower quadrant mass, or localized ileus.[1] Chest radiographs should be obtained because subphrenic abscesses often are associated with a pleural effusion. In a series of 27 children with splenic abscesses, chest radiographs were abnormal in 20 cases, with the most common findings being left pleural effusion and an elevated left hemidiaphragm.[39] Ultrasonography is a useful noninvasive technique that can detect abdominal and pelvic abscesses. The quality of the images depends on the examiner, however. In addition, conditions such as ileus, postoperative drains, or dressings may hinder ultrasound detection of an abscess.[1,47,50] CT is the most sensitive tool for detecting an intra-abdominal abscess, and it provides good anatomic resolution. Disadvantages of CT are the radiation and, if used, exposure to intravenous, oral, or rectal contrast material.[1,47,50]

Although more recent experience with the use of magnetic resonance imaging for detecting an intraperitoneal abscess has been described in the literature,[54] this modality should be considered in children only when an abscess may not be detected more easily by other methods or in patients who should not have exposure to radiation.[54] Gallium scanning is a sensitive technique for diagnosing an abscess, but it is nonspecific, particularly in the abdomen.[1]

TREATMENT

Management of an intra-abdominal abscess includes physiologic and nutritional support, antimicrobial therapy, and drainage. After blood cultures have been obtained, empiric antibiotic therapy should be instituted with agents effective against anaerobes, Enterobacteriaceae, and other enteric flora as discussed earlier for peritonitis. Antibiotic therapy usually is begun before surgery is done to minimize any complications of bacteremia during the procedure. Abscess material should be obtained for culture of aerobic, anaerobic, fungal, and mycobacterial pathogens. Effective surgical management depends on accurate localization of the abscess, discrimination between single and multiple abscesses, and early and adequate drainage.[3,4,46] Traditional therapy for intraperitoneal abscesses has relied on open surgical drainage, although drainage of intraperitoneal abscesses percutaneously under ultrasound or CT guidance now is used often.[1,18,21,46,50,59]

In instances of multiple intraperitoneal abscesses or if the source of peritoneal contamination has not been controlled, laparotomy is indicated.[46] Pancreatic abscesses require intensive surgical and medical therapy. Antimicrobial therapy for mixed aerobic and anaerobic infection is suggested,[12] but splenectomy remains the definitive treatment of bacterial splenic abscesses. In selected patients, percutaneous drainage or splenotomy has the advantage of preserving splenic function, however.[39,53,58] Multiple small splenic abscesses and fungal lesions generally are treated medically.[39] Antibiotic therapy for a pyogenic splenic abscess should be guided by the pathogens associated with the child's underlying condition. Therapy should include antibiotics effective against *S. aureus*, streptococci, and gram-negative enteric bacilli. Specific therapy should be revised after culture and susceptibility results are available.

COMPLICATIONS

Intraperitoneal and visceral abscesses, if not adequately drained, may be associated with significant complications, including ongoing spread of the infectious process and, for splenic or pancreatic abscesses, a high mortality rate. Fistula formation, adhesions, and bowel obstruction may be late complications of intra-abdominal infection.

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CHAPTER 60

Retroperitoneal Infections

Alice Pong ❂ John S. Bradley

Retroperitoneal infections consist primarily of suppurative bacterial infections that originate within the retroperitoneal structures or as an extension from another primary site. In children, these infections are much less common than are intra-abdominal infections; however, they can lead to significant morbidity if missed. Establishing a diagnosis can be difficult because symptoms often are indolent and poorly localized.

The retroperitoneal structures are separated from the intra-abdominal organs by the posterior peritoneal fascia (Fig. 60–1). Structures posterior to this fascia layer, in the anterior retroperitoneal space, include the duodenum, pancreas, and parts of the colon. The kidneys and ureters are encased further by the renal fascia. The iliopsoas and psoas muscles lie at the posterior aspect of the retroperitoneal space and are separated from the other retroperitoneal structures by the transversalis fascia. Pelvic structures, including the bladder, uterus, and rectum, that lie inferior to the pelvic peritoneum constitute the pelvic portion of the retroperitoneal space. The fascial layers limit the spread of retroperitoneal infections. However, the deep location can be difficult to assess by physical examination.

ETIOLOGY AND PATHOGENESIS

Retroperitoneal infections in children arise in numerous anatomic structures. Brook[6] reviewed cases of retroperitoneal infections from five U.S. hospitals from 1974 to 1994. Of 41 children identified, 21 had infections in the anterior retroperitoneal space related to the pancreas (4) and intestines (13), 6 had perinephric abscesses, 7 had iliopsoas abscesses, and 7 had pelvic retroperitoneal abscesses (Fig. 60–2).

Primary infection of the retroperitoneal space can be hematogenous in origin or complicate an ascending urinary tract infection. Secondary infections occur as a direct extension from gastrointestinal perforations, such as ruptured appendices or those related to Crohn disease.[6,19,20] Greenstein and associates[19] reported retroperitoneal abscesses in 12 of 231 patients with Crohn disease. Retroperitoneal infections also can develop secondary to primary infections of the vertebral spine, pelvic bones, and sacroiliac joint.[21,33,36] Suppurative iliac or retroperitoneal lymph nodes are another source of retroperitoneal infections. Prior surgery has been reported as a predisposing factor in perinephric abscesses[7,16] and in vascular grafts in adults.[8] Pancreatic abscesses are seen more frequently in adult patients and are associated with underlying biliary tract disease, alcoholism, surgery, and trauma.[9]

Infections of the perinephric retroperitoneal space include those involving the kidney and adrenal glands. Perinephric abscesses can result from bacteremic inoculation of renal tissue or as a consequence of an ascending urinary tract infection. Nephronia (i.e., focal renal cellulitis) is thought to be an intermediate stage of renal infection between pyelonephritis and renal abscess, resulting from an ascending infection of the urinary tract.[31] Adrenal abscesses are reported more frequently in neonates than in older children and are thought to be related to adrenal hemorrhages that become secondarily infected.[28]

Iliopsoas abscesses may be a consequence of hematogenous seeding of the muscle, with trauma as a predisposing factor (see Fig. 60-2).[20,32] Although primary infection occurs most commonly,[5] the iliopsoas muscle extends from the ribs and lumbar vertebrae to its insertion on the femur and is exposed to the risk of extension of infection from numerous adjacent structures.

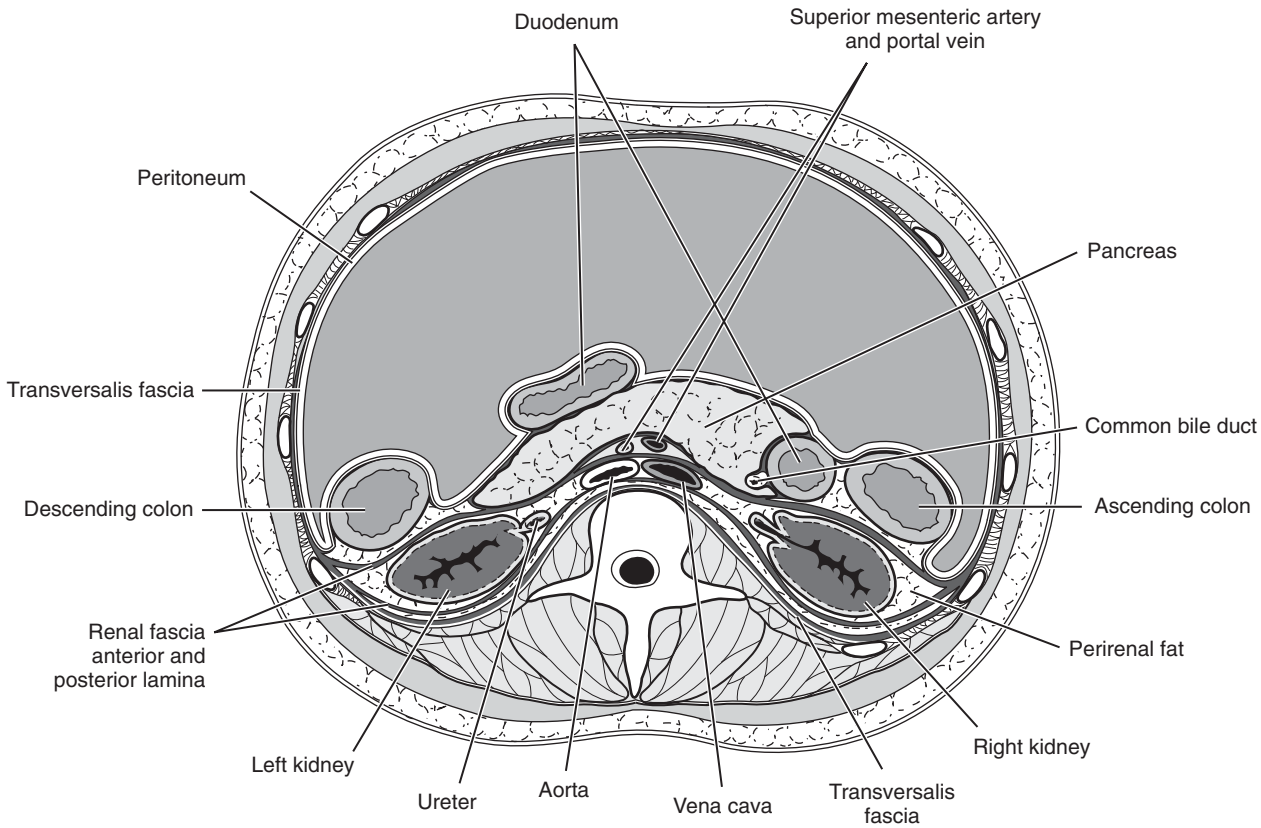

Figure 60–1 A cross section of abdomen at L2 shows the structures within the retroperitoneal space. *(From Altemeier, W. A., and Alexander, J. W.: Retroperitoneal abscess. Arch. Surg. 83:515, 1961, with permission.)*

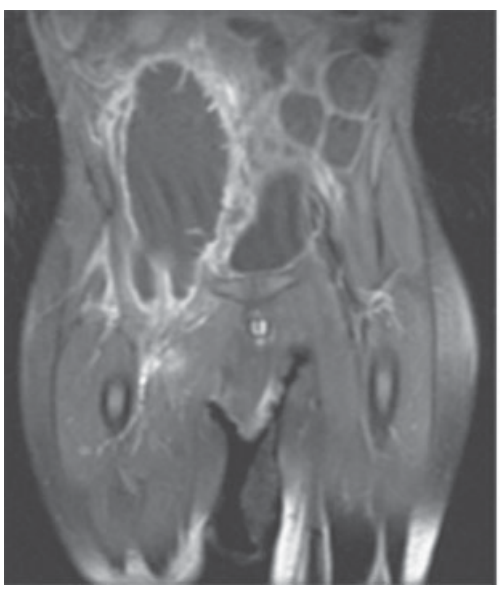

Figure 60–2 Large iliopsoas abscess seen on magnetic resonance imaging. Coronal view with contrast enhancement.

Psoas abscesses have developed as a consequence of vertebral infections, intestinal perforations, and genitourinary sources and as an extension of primary pelvic osteomyelitis.[20,22,31,33] Neonatal iliopsoas abs-cesses also have been reported and present with symptoms similar to those of a septic hip.[14,17,30]

Complications of retroperitoneal abscesses include both rupture into the intraperitoneal space and extension of the infection along fascial planes to adjacent muscles that extend from origins in the pelvis and trunk to insertion sites on the femur. Rupture into the thoracic cavity also has been reported.[1] Other reported complications include pneumonia, recurrent abscess, renal failure, and venous and arterial thrombosis.[12]

MICROBIOLOGY

The microbiology of retroperitoneal infections is determined by the source of the infection and the retroperitoneal compartment involved. Most primary infections thought to result from bacteremia are caused by *Staphylococcus aureus*. Secondary infection related to the gastrointestinal tract is caused by mixed bowel flora including *Escherichia coli*, other gram-negative enteric bacteria, *Pseudomonas* spp., and gastrointestinal anaerobes, particularly *Bacteroides fragilis* and *Peptostreptococcus*.[6] Most infections in the anterior retroperitoneal space are associated with a gastrointestinal source and may be polymicrobial.[4] *Actinomyces* infections, although seen more commonly in the cervicofacial area, also can present as retroperitoneal infections.[3,25]

Ascending infections from the urinary tract usually are caused by *E. coli*. However, perinephric abscesses also are reported as a complication of renal infection caused by *S. aureus*, group B streptococcus, and *Salmonella*.[16,38,39]

S. aureus is the leading pathogen isolated in iliopsoas abscesses unless the infection is secondary to erosion of a primary gastrointestinal focus. In that situation, gram-negative enteric bacteria and anaerobes are more likely to be the causative agents.[5,32] Retroperitoneal necrotizing fasciitis from group A streptococcus also has been reported.[13]

Tuberculosis caused by *Mycobacterium tuberculosis* or *Mycobacterium bovis* may involve the retroperitoneal space as an extension of vertebral tuberculous osteomyelitis.[1,15,22] Abdominal tuberculosis usually is manifested as an intraperitoneal infection but can produce retroperitoneal adenopathy.

CLINICAL PRESENTATION

Children with retroperitoneal infections present clinically in a variety of ways, ranging from nonspecific fever to overwhelming sepsis. The most common clinical symptoms associated with retroperitoneal infections include fever and pain in the hip, back, and abdomen.[12,26] Psoas abscesses often are manifested with the child's limping or refusing to walk.[5] Neonates with a retroperitoneal abscess may present with an abdominal mass.[34] Symptoms often are vague, and pain is not well localized. Patients often have been evaluated previously for fevers and have been treated with antibiotics before a diagnosis has been made.[5,24,33] A delay in establishing the diagnosis is not uncommon.

DIFFERENTIAL DIAGNOSIS

Retroperitoneal infections can be confused with a variety of other infections. Limp and fever caused by pyogenic arthritis of the hip and infection of the sacroiliac joint and pelvic bones occur more commonly than do retroperitoneal infections. Abdominal pain and fever are seen more frequently in patients with intra-abdominal infections, including appendicitis and intra-abdominal abscesses. Trauma and malignant disease are more frequent causes of retroperitoneal masses compared with infectious causes and should be considered.

SPECIFIC DIAGNOSIS

Laboratory tests often are nonspecific and of minimal benefit. Sedimentation rates and leukocyte counts often are elevated.[5,6,32,33] Pyuria often is absent in children with perinephric and renal abscesses, and the urine culture may be negative.[7,16,37,38] However, in patients with nephronia, pyuria and positive urine cultures are more likely.[24,31] Blood cultures may be helpful in identifying a bacterial pathogen. Brook[6] reported that 40 percent of blood cultures were positive for the same organism isolated from abscesses in children with retroperitoneal infections who had blood culture specimens collected. For children with psoas abscesses, Santaella and colleagues[32] reported that 71 percent of blood cultures were positive.

Imaging studies are the most useful diagnostic tools. Ultrasonography can be used to diagnose perinephric infections[7,37] and has been used to diagnose abscesses of the iliopsoas muscle.[20] Computed tomography (CT) with contrast enhancement appears to be the most helpful[11,12,32] because of superior delineation of organ involvement and the extent of infection as well as wide availability and rapid imaging times compared with magnetic resonance imaging (MRI). CT also can provide clues about the primary focus of the infection, thereby helping guide empiric antibiotic therapy. Abscess fluid is seen on CT as areas of low attenuation, often with an enhancing rim.[11,23,38] Percutaneous drainage and biopsy of the lesions also may be accomplished with CT. Although alternative diagnoses also can be evaluated with CT,[35] hematomas and certain tumors may not be distinguished easily radiographically from infection. These noninfectious entities may be identified better by MRI. Another advantage of MRI over CT is superior visualization of bone and inflamed muscle tissue, although calcifications may not be as well identified.[29]

TREATMENT

Percutaneous or open surgical drainage should be considered for all retroperitoneal infections for both diagnostic and treatment purposes. Culture of the aspirated fluid for aerobic and anaerobic bacteria, mycobacteria, and fungi is vital to selection of appropriate antimicrobial therapy. Reports of percutaneous drainage of perinephric abscesses and iliopsoas abscesses are increasing in number.[5,22] These procedures usually are performed with ultrasound or CT guidance. Treatment of patients with antibiotics alone without surgical drainage may not be effective, particularly in cases involving larger abscesses.[1,11]

Initial antimicrobial therapy for retroperitoneal infections should be directed by the presumed source of the infection, with definitive therapy guided by microbiologic culture results. Infections related to gastrointestinal perforation should include coverage directed primarily against enteric gram-negative bacteria and gastrointestinal anaerobes such as β-lactamase–producing *B. fragilis*. Coverage for *Pseudomonas* and *Enterococcus* spp. also should be considered. Historically, antibiotic combinations such as ampicillin for enterococcus, metronidazole or clindamycin for anaerobes, and a third-generation cephalosporin or aminoglycoside for gram-negative bacteria have been used. However, carbapenems such as meropenem, imipenem, and ertapenem as single agents may be more cost effective, particularly if any outpatient antibiotic therapy is being considered. The β-lactam and β-lactamase inhibitor combinations (e.g., ticarcillin-clavulanate, piperacillin-tazobactam), with or without an aminoglycoside, also are likely to be effective.

Infections of renal origin, usually caused by *E. coli* or other gram-negative enteric organisms, can be treated with extended-spectrum cephalosporins such as ceftriaxone or, if abscesses have been drained successfully, with aminoglycosides such as gentamicin and tobramycin. The activity of aminoglycosides may be compromised by the acidic environment of abscess cavities and may lead to clinical failures despite in vitro susceptibility of the organism.[10] Increasingly, resistance to ampicillin is seen in *E. coli*,[2,4] rendering it unreliable for empiric use in severe urinary tract infections. *Pseudomonas* is not an uncommon pathogen in children with recurrent infections caused by anatomic genitourinary abnormalities.[2] *Pseudomonas* spp. usually are resistant to ceftriaxone; extended-spectrum cephalosporins, such as ceftazidime and cefepime, or carbapenems may be needed. Urine culture and susceptibility results help focus the choice of an antibiotic to the narrowest spectrum agent required.

Psoas abscesses and primary perinephric abscesses caused by *S. aureus* should be treated with an antistaphylococcal agent such as nafcillin (or oxacillin or methicillin) or a first-generation cephalosporin such as cefazolin. The prevalence of community-acquired methicillin-resistant *S. aureus* (CA-MRSA) has increased significantly as a soft tissue pathogen, particularly in children.[18,27] Vancomycin or clindamycin should be considered as empiric therapy for serious infections in areas with high rates of CA-MRSA (>5% of all invasive *S. aureus* infections) until culture results are available. These agents also may be effective in treating the patient who is unable to tolerate penicillin or cephalosporin antibiotics.

Infections originating in the vertebrae usually are caused by *S. aureus* or may result from tuberculosis. Empiric antistaphylococcal therapy can be started, but culture and histologic examination of tissue are needed to direct appropriate therapy. For the child with risk factors for acquiring tuberculosis, a positive tuberculin skin test result, and a negative Gram stain result, empiric therapy with three or four antituberculous antibiotics should be considered. A chest radiograph should be obtained to look for evidence of pulmonary tuberculosis.

After adequate drainage is achieved, the duration of antimicrobial therapy depends on several factors, including the organ-

ism, the site and extent of infection, and the clinical improvement. Most drained retroperitoneal bacterial abscesses of renal or muscle origin are treated for 2 to 3 weeks with initial parenteral and follow-up oral antibiotics. Infections involving bone may require 6 to 8 weeks or longer, depending on how quickly the infection responds to treatment. Radiographic studies, erythrocyte sedimentation rate, and C-reactive protein measurements can be helpful to monitor recovery. Tuberculous infections are treated for 6 to 12 months, depending on the presence of bone involvement. *Actinomyces* infection can be difficult to treat because of lack of susceptibility to many antibiotics and the need for prolonged courses of antibiotic therapy, preferentially with penicillin.

PROGNOSIS

Historically, retroperitoneal abscesses are reported to have high morbidity and mortality rates. However, with modern imaging techniques enabling physicians to make more timely diagnoses, the overall prognosis is good, and most children with no underlying disease recover without sequelae.

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SECTION IX

MUSCULOSKELETAL INFECTIONS

CHAPTER 61

OSTEOMYELITIS

Paul Krogstad

The term *osteomyelitis* denotes inflammation of bone and marrow but generally implies the presence of infection. Osteomyelitis is considered acute if diagnosed within 2 weeks of the onset of symptoms, or subacute if symptoms have been present for more than 2 weeks at the time of presentation. Although bacteria are the most common cause, fungi, parasites, and other microorganisms also may cause osteomyelitis.[108] Acute osteomyelitis may evolve into a chronic process, especially if not treated adequately, leading to extensive necrosis of bone.

The incidence of osteomyelitis in normal children has been examined in several populations. Estimates have varied from 1 in 20,000 adolescent girls in New Zealand to 1 in 1000 Australian Aboriginals.[26,45,82] Boys seem to be at greater risk and contract the disease 1.2 to 3.7 times more often than do girls.[82,158,189] Osteomyelitis occurs most often in the first 2 decades of life. Approximately 25 percent of children with osteomyelitis are younger than 2 years old, and 50 percent are younger than 5 years old.[83,121,154,214] The incidence is increased in patients with sickle-cell disease and in some other immunocompromised patients (see the section on special populations).

Microorganisms can be introduced into bone in three ways: (1) by direct inoculation, usually traumatic, but also during surgery; (2) by local invasion from a contiguous focus of infection; and (3) by hematogenous delivery. In children, osteomyelitis generally is of hematogenous origin. Regardless of the route of infection, the common denominator is microscopic bone death. The goal of treatment is to arrest the infection and limit the extent of the injury to bone.

USUAL MICROBIAL ETIOLOGY

Although cultures frequently fail to identify bacterial pathogens in osteomyelitis, most microbiologically confirmed infections are caused by single organisms.[5,154,162] Gram-positive bacteria are responsible for most cases of osteomyelitis in children. *Staphylococcus aureus* is incriminated in 89 percent of cases of osteomyelitis in immunocompetent children.[83,171,207,211] Group A streptococci are next in frequency (Table 61–1). Historically, *Streptococcus pneumoniae* has been a frequent cause of osteomyelitis, but protein conjugate vaccines are likely to diminish markedly the incidence of pneumococcal osteomyelitis. In contrast, *Kingella kingae*, a fastidious gram-negative organism, is being identified increasingly in children as a cause of osteoarticular infection, including osteomyelitis, diskitis, and septic arthritis, and may explain many cases of culture-negative osteoarticular disease.[46,88,134,215,228] Even in more affluent countries, *Salmonella* spp. frequently are a cause of osteomyelitis in immunocompetent patients. *Salmonella* spp. are the most common organism found in cases of osteomyelitis in patients with sickle-cell disease (discussed later). (The microbiologic peculiarities of osteomyelitis in other immunocompromised patients and special populations are discussed in later sections.)

Other aerobic gram-negative bacteria are less common causes of osteomyelitis. Before the development of protein conjugate vaccines, *Haemophilus influenzae* was reported consistently in pediatric case series and caused approximately 5 to 8 percent of cases.[121,154,171,211] As with other invasive *H. influenzae* infections, osteomyelitis caused by this organism usually occurs in children 3 months to 6 years of age.[62,71,89,199] With the advent of effective immunization, cases of *H. influenzae* osteomyelitis are noticeably absent from more recent case series.[5,83,114] Osteomyelitis caused by other gram-negative organisms is an uncommon finding but generally occurs in neonates and young infants. Hematogenous osteomyelitis caused by *Pseudomonas aeruginosa* has been associated with injection of illicit drugs.[102,128,223]

Four distinct clinical entities of infection caused by anaerobic bacteria are recognized: (1) bacteremic seeding of previously normal bones in children and young adults; (2) superinfection of a fracture site already infected with *S. aureus*; (3) indolent (months to years after surgery) infection of a prosthetic device; and (4) contiguous chronic infection,[201] which most often occurs in the skull and the extremities. *Bacteroides* spp. are found most commonly and are associated with paranasal, sinus, or mastoid infection. In most cases, a foul odor is noted when the bone is incised or the focus is opened; trauma often has been an inciting influence.[129]

As noted previously, most cases of osteomyelitis in children develop after an episode of bacteremia and are caused by a single organism. Polymicrobial infections generally reflect the spread of infection from contiguous infectious foci and most often occur in the skull, face, hands, or feet. Distal extremities compromised by vascular insufficiency or immobilized because of peripheral neuropathy also are sites of polymicrobial osteomyelitis (including paraplegia caused by spina bifida). Osteomyelitis caused by fungi and atypical bacterial pathogens occasionally occurs in children and is discussed in greater detail later.

HEMATOGENOUS OSTEOMYELITIS

PATHOGENESIS

In long tubular bones, hematogenous osteomyelitis generally begins in the metaphysis, the broad cancellous end of the bone shaft adjacent to the epiphyseal growth plate. The cartilaginous epiphyseal growth plate (the physis) is nourished by diffusion of nutrients from a narrow plexus of capillaries fed by the metaphyseal branches of the nutrient artery; these capillaries drain into a large sinusoidal plexus that ultimately joins the large sinusoidal veins in the bone marrow (Fig. 61–1).[100] Trauma or emboli lead to occlusion of the slow-flowing sinusoidal vessels, establishing a nidus for infection. Blood-borne bacteria can seed the poorly perfused area and proliferate.[40,111,209]

The high frequency of *S. aureus* in osteomyelitis may reflect specific pathogenic properties of the organism. It has the ability

TABLE 61–1 Etiology of Acute Hematogenous Osteomyelitis in Children: Number of Bacteriologically Confirmed Cases

Organisms	Nelson[154] (*n* = 296) (%)	LaMont et al.[121] (*n* = 90) (%)	Unkila-Kallio et al.[211] (*n* = 44) (%)	Roine et al.[171] (*n* = 38) (%)	Goegens et al.[83] (*n* = 45) (%)
Gram-Positive Bacteria					
Staphylococcus aureus	67	70	89	89	85
Coagulase-negative staphylococci	3	1			
Streptococcus pneumoniae	2	5	2		4
Other streptococci	12	16	2	1	8
Gram-Negative Bacteria					
Haemophilus influenzae	4	8	7	8	0
Pseudomonas aeruginosa	3				
Salmonella spp.	2				
Escherichia coli	<1				
Kingella kingae	<1				
Mixed or unusual organisms	4				

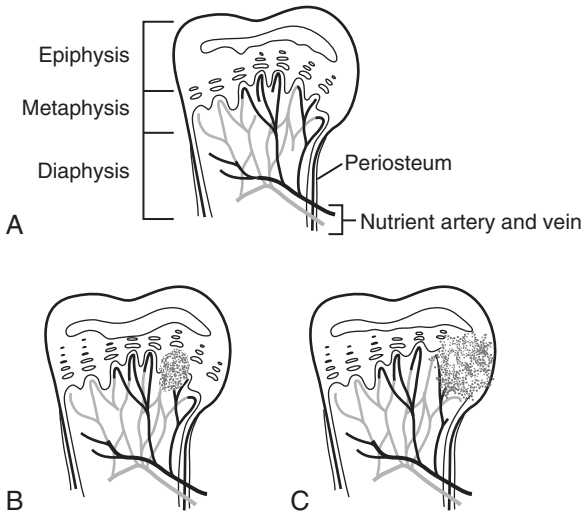

Figure 61–1 **A,** The sluggish blood flow in the sinusoidal venous connections located at the metaphyseal-epiphyseal junction predisposes to the development of traumatic thrombosis and infarction. **B,** Bacteremic seeding of the avascular area initiates the infection, which spreads through the Volkmann canals and the haversian systems and causes septic thrombosis. **C,** Infection tends to spread laterally through the cortex and elevates or ruptures through the periosteum.

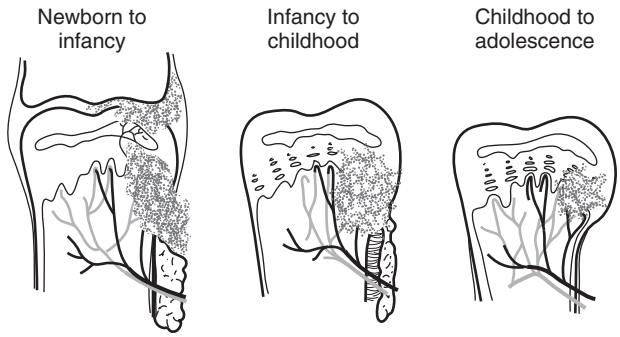

Figure 61–2 In young infants and neonates, particularly in the hip where the epiphyseal growth plate is traversed by nutrient vessels terminating in the distal ossification center, septic thrombophlebitis of the nutrient vessels can lead to growth discrepancies. With the capsule of the joint extending to the metaphysis, rupture of the infection through the cortex leads to the development of septic arthritis. Because of the thin cortex and loose periosteum, the osteomyelitis may come to medical attention as a deep soft tissue abscess. In older infants and young children, the thicker cortex and denser periosteum are a greater barrier to infection. Local tenderness from subperiosteal edema or abscess is the rule. In late childhood and adolescence, the lesion is extremely well localized and rarely penetrates the bony cortex. In this age group, invasive procedures, such as windowing or drilling, are necessary to obtain infected material.

to adhere to type I collagen of bone fibrils.[32] When *S. aureus* binds to collagen, bacterial replication gives rise to microcolonies surrounded by a glycocalyx.[94] Continued injury, elicited by *S. aureus* exoproducts and the host cellular inflammatory response to the injury, causes the accumulation of exudate under pressure. The pressure compresses blood vessels of bone and produces focal bone necrosis. The low ratio of surface area to mass, combined with the blood vessel anatomy described earlier, interferes with reabsorption of necrotic cortical bone[40,111] and the effectiveness of host defense mechanisms.

Since the late 1990s, community-acquired methicillin-resistant *S. aureus* (CA-MRSA) isolates have become a common cause of musculoskeletal infections in the United States and other countries. As described further later, most of these CA-MRSA isolates carry genes encoding the Panton-Valentine leukocidin (pvl).[22] Pvl-positive CA-MRSA has been associated frequently with sepsis, venous thrombosis, and polyostotic disease, suggesting that this or other factors contribute to the development and severity of hematogenous osteomyelitis.[26a,140] The very early stages of osteomyelitis may be aborted by administration of appropriate chemotherapy. In the absence of therapy, necrosis of cortical bone and marrow continues. The exudate under pressure is forced through the haversian systems and Volkmann canals and into the cortex (see Fig. 61–1).

SIGNS AND SYMPTOMS

The bacteremic phase of hematogenous osteomyelitis may be entirely subclinical and associated only with malaise and low-grade fever, or it may be characterized by severe constitutional symptoms with a temperature of 40º C. The subsequent clinical manifestations of osteomyelitis are not related to the severity of initial constitutional signs of infection but are influenced by the age of the child and the etiologic agent (Fig. 61–2).

In newborns, the thin cortex and loosely attached periosteum are poor barriers to the spread of infection. Consequently, the purulence rapidly ruptures through both of these structures into the contiguous muscle bed. With progression of the infection, the purulent material often dissects the muscle bundles, with the

swollen, discolored limb taking the appearance of a sausage. In addition, nutrient metaphyseal capillaries perforate the epiphyseal growth plate in newborns, and the capsule of the diarthrodial joints frequently extends to or is slightly distal to the epiphyseal plate. These anatomic characteristics permit an infection arising in the metaphysis to involve the epiphysis and to extend into the adjacent joint cavity.

In older infants, the cortex (see Fig. 61–2) is thicker and the periosteum is slightly more dense. Consequently, the infection spreads less often to the soft tissues of the extremity. Subperiosteal abscess and contiguous edema readily develop, however. The nutrient metaphyseal capillaries in older infants are atrophic, which, although more recently disputed,[159] is thought to decrease the risk of spread of infection into the adjacent joint space. The subperiosteal purulence almost always is at the metaphysis, the area in which the cortex is the thinnest.[91]

In children and adolescents (4-16 years old), the metaphyseal cortex is considerably thicker, with a dense, fibrous periosteum. The pathogenesis of the infection is the same in this age group, but the infection rarely ruptures and spreads to the outer cortical lamellae. As a result, the signs and symptoms of osteomyelitis in these older children and adolescents usually are more focal.

A newborn with osteomyelitis usually is irritable and displays evidence of pain when the affected extremity is touched or moved. Pseudoparalysis may occur, and if the disease remains untreated, massive swelling of the extremity may be seen. Obtaining a plain radiograph is invaluable in this age group; most of these patients have changes consistent with osteomyelitis on the initial radiograph.[118,147,220] In infants and young children, pain usually is present, as is a limp because osteomyelitis occurs more commonly in the lower extremities. The child refuses to use the affected extremity and displays variable constitutional symptoms.

In older children and adolescents, less restriction of function of the extremity is found compared with infants and young children. The point tenderness is circumscribed more sharply and may be found only as a small area of discomfort at rest. This disease most often affects the lower extremities and produces a mild limp. Most commonly, tubular bones are involved, but osteomyelitis occurs throughout the skeleton (Table 61–2). The hallmark of the disease is the focal nature of symptoms; point tenderness and well-localized pain suggest the diagnosis. Percussion of the long bone away from the area of point tenderness often elicits pain at the site of osteomyelitis in older children and adolescents.

The clinical features of osteomyelitis are influenced by the organisms involved. Osteomyelitis caused by CA-MRSA seems to be more complex and severe than are infections caused by methicillin-susceptible *S. aureus* (MSSA). Life-threatening infections caused by CA-MRSA have been reported in adolescents, and polyostotic disease is more common (15% in one more recent series compared with 2% in a large series that preceded the emergence of CA-MRSA).[22,154] Myositis, pyomyositis, intraosseous and subperiosteal abscesses, and septic thrombophlebitis also seem to occur more frequently with CA-MRSA than with MSSA.[5,86,87,140] In contrast, osteoarticular infections caused by *Kingella* spp. generally have an indolent course, with limb pain often present for longer than a week before initial medical evaluation is made.[134,227] Osteomyelitis caused by *H. influenzae* seems to occur primarily in the upper extremities.[61,62,89,199]

Culture-negative osteomyelitis generally is mild. In one series comparing 45 culture-positive patients with 40 patients with culture-negative osteomyelitis, symptoms were of longer duration, and overlying skin changes were seen less frequently in patients with negative disease. Treatment with β-lactams generally was successful and associated with skeletal sequelae in only one case.[69] Whether these features of culture-negative osteomyelitis suggest a more effective host defense or infection by a less virulent and more fastidious pathogen, such as *K. kingae*, which has been identified in culture-negative disease, is unclear.[215]

TABLE 61–2 Site of Involvement in Acute Hematogenous Osteomyelitis*

Bone Type	%
Tubular	
Femur	25
Tibia	24
Humerus	13
Phalanges	5
Fibula	4
Radius	4
Ulna	2
Metatarsal	2
Clavicle	0.5
Metacarpal	0.5
Cuboidal	
Calcaneus	5
Talus	0.8
Carpals	0.5
Cuneiform	0.5
Cuboid	0.3
Irregular	
Ischium	4
Ilium	2
Vertebra	2
Pubis	0.8
Sacrum	0.8
Flat	
Skull	1
Rib	0.5
Sternum	0.5
Scapula	0.5
Maxilla	0.3
Mandible	0.3

**The bone classification is according to Jaffe.[108]*
Data from Nelson, J. D.: Acute osteomyelitis in children. Infect. Dis. Clin. North. Am. 4:513-522, 1990.

DIFFERENTIAL DIAGNOSIS

Osteomyelitis can be confused with many other conditions associated with fever, pain, and tenderness in an extremity, including fractures, rheumatic fever, septicemia, septic arthritis, cellulitis, Ewing sarcoma, leukemia, reflex neurovascular dystrophy, thrombophlebitis, bone infarction secondary to sickle-cell or Gaucher disease, and toxic synovitis.

DIAGNOSIS

The diagnosis of osteomyelitis generally is suspected based on typical findings, such as fever, focal skeletal pain, warmth, and swelling, and a limp or refusal to use an extremity. The diagnosis is confirmed by an organism shown by culture or Gram stain in an aspirate of bone, or by histopathologic analysis of surgical specimens. In an otherwise healthy individual, the diagnosis is probable when a patient has fever, leukocytosis, elevated acute-phase reactants (elevated C-reactive protein [CRP] or erythrocyte sedimentation rate [ESR]), or a positive blood culture plus one or more of the following: abnormal imaging studies (plain radiograph, magnetic resonance imaging [MRI], or computed tomography [CT]), scintigraphy, or physical findings consistent with osteomyelitis.

Microbiology

The cornerstone of the diagnosis of osteomyelitis is isolation of bacteria or other microbes from bone or from anatomic structures contiguous to bone. Overall, such cultures (bone, subperiosteal exudate, or joint fluid) provide a bacteriologic diagnosis in 66 to 76 percent of cases. Blood cultures yield an organism in about half of cases (36-74% of patients in three series).[154,207,212,215]

In neonates, needle aspiration of soft tissue or incision and drainage of bone may yield the offending organism. In infants and young children, subperiosteal needle aspiration can be done if the point tenderness is localized. In older children and adolescents, noninvasive culturing of the bone is less rewarding. In this age group, windowing or drilling to drain pus from the bone yields valuable material for culture but is controversial; some orthopedic surgeons consider the risk of causing epiphyseal damage and subsequent length discrepancy secondary to the procedure too great. Consequently, blood cultures and imaging methods often represent the extent of the diagnostic evaluation. Nonetheless, it is important to attempt to obtain bacterial cultures early in the evaluation of suspected osteomyelitis because decisions about the type and duration of antimicrobial therapy are greatly facilitated by knowing the susceptibility profile of the etiologic agent.

Radiology

PLAIN RADIOGRAPHS

Conventional radiographs are crucial in establishing the diagnosis of pediatric osteomyelitis and always should be obtained.[26a,39,96] Because bone density must decrease 50 percent to be detected by radiographs,[8] changes in the less ossified bones of neonates are detected more readily than are changes in older children. In contrast, Waldvogel and Papageorgiou[216] found in adults that plain radiographs were of no diagnostic value in 23 percent and were misleading in an additional 16 percent.

Radiographic changes occur in three stages.[34] The first stage, which occurs approximately 3 days after the onset of symptoms, is the formation of a small area of localized, deep soft tissue swelling, usually in the region of the metaphysis (Fig. 61–3). When diagnosis of osteomyelitis is sought early, examination of the radiograph should be directed to the soft tissue rather than the bone. During the second stage, which occurs 3 to 7 days after the onset of symptoms, swelling of the muscles with obliteration of the interposed translucent fat planes can be noted. It is caused by continued spread of edema fluid and can progress, particularly in neonates and young infants, to superficial soft tissue edema; the skin may acquire an orange-peel texture.

Radiographic evidence of bone destruction usually is not detected until 10 to 21 days after the onset of symptoms. The first changes detected include subperiosteal bone resorption, areas of bone destruction, and periosteal new bone formation. The variability depends on the specific bone involved; generally, long tubular bones tend to show bony changes 2 to 3 weeks earlier than membranous or irregular bones.

MAGNETIC RESONANCE IMAGING

MRI is becoming the imaging modality of choice when additional imaging is needed.[39] The major advantages of MRI are that it accurately delineates subperiosteal or soft tissue collections of pus that might require surgical drainage without using ionizing

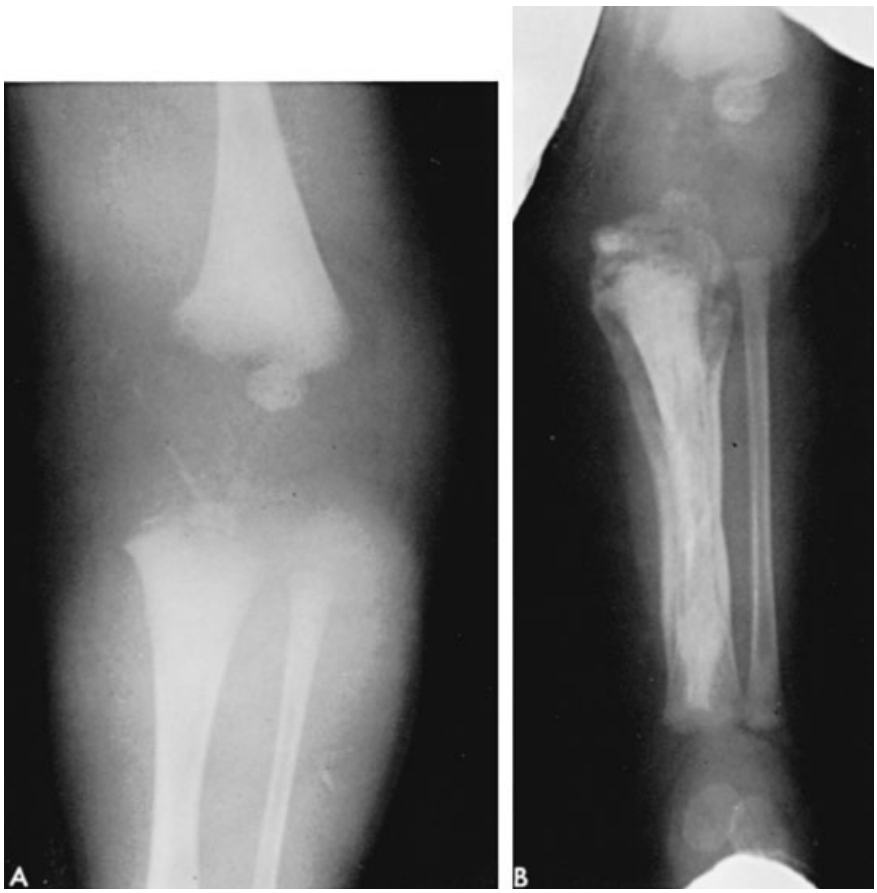

Figure 61–3 **A,** The left knee of an infant shows diffuse soft tissue swelling around the proximal ends of the tibia and fibula. **B,** Six weeks later, the subcutaneous fat lines between the muscles and the skin can be seen, as can an involucrum involving almost all of the tibia.

radiation, and it can identify sinus tracts for removal (Fig. 61–4).[26a,68,164,197] MRI often provides more specific anatomic information than CT or plain radiography.

In acute osteomyelitis, bone marrow edema caused by the accumulation of purulent material leads to decreased signal on T1-weighted images. On T2-weighted images of the same area, increased signal is seen. With commonly used sequences, the sensitivity of MRI for the detection of acute osteomyelitis may approach 100 percent.[141] Fat-suppression sequences, including short-tau inversion recovery, decrease the signal from fat. Inversion recovery sequences allow more sensitive detection of bone marrow edema. MRI may have a particular advantage in establishing the diagnosis of spinal osteomyelitis because a clear distinction can be made between the vertebral body and the adjacent disk. Loss of this border is one of the first abnormalities detected by MRI in spinal osteomyelitis.

The need for sedation in most infants and children and the cost of MRI are the major disadvantages that continue to limit its use. In addition, infarction and other processes can alter the appearance of bone marrow and lower the specificity of MRI and must be considered in the interpretation of the imaging study.

RADIONUCLIDE IMAGING

Radionuclide scanning has been used for decades in the evaluation of suspected osteomyelitis. Despite the fact that bone scanning involves exposure to ionizing radiation, it continues to be used widely because of its ready availability and utility in detecting multifocal disease.[56] Bone imaging employing technetium 99m (Tc 99m) diphosphonate scintigraphy is used most frequently. After being injected, the phosphate adduct is adsorbed to the surface of the hydroxyapatite crystal in bone, and Tc 99m becomes concentrated in the cement line located at the junction of osteoid and mineralized bone.[204]

Most institutions perform a three-phase bone scan for evaluation of infection. Shortly after injection (2-5 seconds), a nuclear angiogram (flow phase) of the area of suspected osteomyelitis is obtained. The second phase (the blood pool phase) consists of a single image obtained 5 to 10 minutes after injection. The third image is obtained 2 to 4 hours after injection. In this later phase, specificity of the diphosphonate compounds for the bone is revealed. Anything increasing local blood flow to the area, particularly if accompanied by inflammation, results in increased general uptake in the first two phases, but osteomyelitis results in focal uptake in the third phase, with the intensity of the signal detected reflecting the level of osteoblastic activity.[36,48,50,56,80,119,180]

Acute osteomyelitis in children is often diagnosed by a Tc 99m scan and treated successfully before bone changes are detected by plain radiographs (see Fig. 61–4).[206] With sensitivity reported to be as high as 95 percent, bone scanning has until recently been a reliable tool in establishing the diagnosis of osteomyelitis.[34] Unfortunately, in one recent report, bone scintigraphy detected only osteomyelitis caused by community acquired-MRSA cases in 53 percent (26/49) of children.[26a] This apparent lack of sensitivity for CA-MRSA may reflect the acuity of disease caused by this pathogen, as changes in radionuclide scans are more characteristic when patients have had an illness of longer duration.[182]

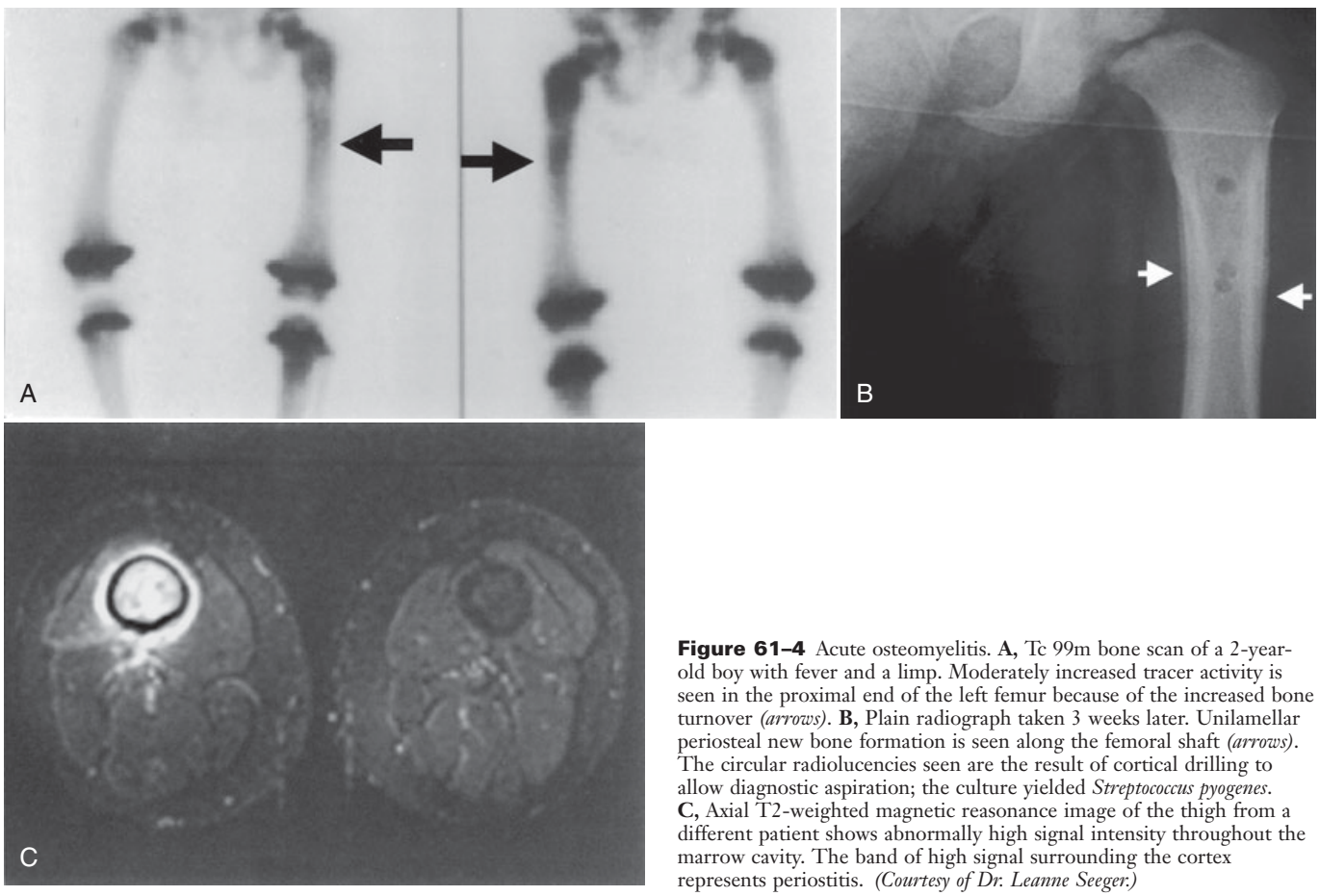

Figure 61–4 Acute osteomyelitis. **A,** Tc 99m bone scan of a 2-year-old boy with fever and a limp. Moderately increased tracer activity is seen in the proximal end of the left femur because of the increased bone turnover *(arrows)*. **B,** Plain radiograph taken 3 weeks later. Unilamellar periosteal new bone formation is seen along the femoral shaft *(arrows)*. The circular radiolucencies seen are the result of cortical drilling to allow diagnostic aspiration; the culture yielded *Streptococcus pyogenes*. **C,** Axial T2-weighted magnetic reasonance image of the thigh from a different patient shows abnormally high signal intensity throughout the marrow cavity. The band of high signal surrounding the cortex represents periostitis. *(Courtesy of Dr. Leanne Seeger.)*

In addition, bone scans that use Tc 99m may be nondiagnostic in neonates.[6,71] Destruction of cortical bone occurs, and periosteal new bone formation often is present on plain-film radiographs of bones with normal Tc 99m uptake. The false-negative result of bone scans probably is due to the paucity of mineralization in neonates' bones. Ischemia of bone, probably caused by infarction, also has been noted on the initial bone scan.[147] Overall, the sensitivity of bone scans in neonates is uncertain, but may be only 30 percent.[71]

Older infants with osteomyelitis and a nondiagnostic Tc 99m bone scan also have been described.[17,95] In such instances, a gallium 67 (Ga 67) scan may be valuable. Ga 67 is a transition metal that, similar to iron, is bound to plasma proteins; the unbound portion (10-25%) is excreted in urine. It localizes in inflammatory foci because of increased capillary permeability (leaking plasma proteins), in vivo leukocyte labeling, binding to lactoferrin in the lesion, and perhaps direct bacterial uptake. Because of the slower elimination of gallium from blood, its uptake in an inflammatory focus depends less on blood flow. Delayed elimination often results in poor contrast of bone to soft tissue, however, and delays making a reliable interpretation for 24 to 72 hours after injection.[101] In one study, 15 of 16 cases of osteomyelitis with a nondiagnostic Tc 99m scan had changes on Ga 67 scan typical of osteomyelitis.[188] Combined evaluation with Ga 67 imaging and Tc 99m bone scanning may lead to greater diagnostic certainty when the studies are not conclusively diagnostic.[185,226] Despite these successes, Ga 67 scans are seldom employed because MRI, repeating plain radiographs, and clinical improvement during therapy usually are sufficient to resolve initial diagnostic uncertainties.

Numerous studies have been conducted to examine the utility of indium 111–labeled leukocyte scans for the diagnosis of osteomyelitis. This method involves removing leukocytes and injecting them back into the patient after in vitro labeling. It has a sensitivity of approximately 86 percent.[177] The sensitivity seems to be best for the detection of lesions in long bones. False-positive scans can result from a variety of processes, including fracture and infarction. Enthusiasm for this modality also is limited by the higher organ absorption of the radiation dose.[73] Numerous other scintigraphic methods for the detection of osteomyelitis, including Tc 99m hexamethylpropyleneamine oxime–labeled leukocytes and monoclonal antibodies, have been examined. At present, these alternative methods do not offer any advantage over MRI and older radionuclide imaging approaches.

COMPUTED TOMOGRAPHY

CT is used occasionally in the diagnosis and management of osteomyelitis because it provides excellent definition of cortical bone and high spatial resolution. CT abnormalities commonly found in osteomyelitis include increased density of bone marrow caused by the accumulation of purulent material and periosteal new bone formation and purulence. CT is particularly useful in detecting sequestra and delineating subperiosteal abscesses. It previously has been used to define infections of the spine; however, MRI has replaced CT for this indication.

TREATMENT OF ACUTE OSTEOMYELITIS

The need for surgical therapy must be considered immediately when osteomyelitis is diagnosed. Reports of soft tissue, subperiosteal, and intramedullary abscesses are becoming more common in the early 21st century, mirroring the increased prevalence of skin, soft tissue, and musculoskeletal infections caused by CA-MRSA.[5,112,140] MRI, ultrasound, and CT imaging may prove useful in assessing these purulent foci.[26a] Drainage of these sites by surgical or interventional radiology techniques also provides the opportunity to obtain cultures to confirm a microbiologic diagnosis. Sequestra, if present at presentation, should be removed. If contiguous infectious foci are present, they should be débrided adequately and treated with effective antimicrobial therapy. Immobilization of the affected extremity or splinting may afford relief from pain and sometimes is used to prevent the development of pathologic fractures when extensive bone involvement is detected by plain radiography.

Although the choice of antimicrobial agents used often is modified during a course of therapy, the therapy initially chosen always should have potent activity against *S. aureus* and group A streptococci because these pathogens represent the primary causes of osteomyelitis. Acute bacterial hematogenous osteomyelitis should be treated initially with parenteral anti-infective agents, in view of the high mortality rate in *S. aureus* osteomyelitis seen in the pre-antibiotic era and more recent reports of fulminant disease with CA-MRSA.[86,87,91,140] The choice of initial therapy has become more complex with the marked increase in prevalence of CA-MRSA in the United States and other countries.

In areas where most (≥90%) *S. aureus* isolates remain methicillin-susceptible, initial therapy may consist of a penicillinase-resistant, semisynthetic penicillin, such as nafcillin or oxacillin, administered parenterally in a dosage of 150 to 200 mg/kg/day in four divided doses.[90,105,171] Cefuroxime, a second-generation cephalosporin, also has been used for empiric therapy, with good results.[154] In areas where CA-MRSA is common, either vancomycin or clindamycin (if >90% of CA-MRSA isolates are susceptible) should be included in the initial empiric therapy for CA-MRSA.[112,139] Currently, most isolates of CA-MRSA remain susceptible to clindamycin, but clinical laboratories should screen for inducible macrolide-lincosamide-streptogramin resistance using the D-test or similar methodology[5,112,127]

Although protein conjugate vaccines have rendered invasive *H. influenzae* infections rare occurrences in the United Sates, antimicrobial coverage for this possibility may be considered for younger children who have not yet completed their immunization series. In these circumstances, addition of a third-generation cephalosporin (ceftriaxone or cefotaxime) to the empiric anti-staphylococcal agent (vancomycin, clindamycin, oxacillin, or nafcillin) might be warranted. Other agents may be administered when epidemiologic factors suggest the possible presence of other pathogens: third-generation cephalosporins or chloramphenical for *Salmonella* osteomyelitis, ceftazidime and aminoglycosides for *P. aeruginosa* or for enteric gram-negative organisms, and clindamycin for suspected anaerobic infections.[63,170]

When an organism is isolated or identified by other means, antimicrobial therapy can be chosen with greater specificity. Staphylococci should be treated with penicillin G if the organisms are susceptible to this antibiotic. In most cases, staphylococci must be treated with a penicillinase-resistant penicillin (oxacillin or nafcillin), clindamycin, or vancomycin. Ceftriaxone also has proved successful in the treatment of most cases of MSSA. Oxazolidinone (linezolid) and streptogramin (quinupristin-dalfopristin) drugs have been used successfully for the treatment of osteomyelitis in some adults with MRSA and MSSA[54] and for the treatment of vancomycin-resistant enterococci. They may prove useful in cases of pediatric osteomyelitis caused by *S. aureus* with decreased susceptibility to vancomycin, but experience with these agents is still limited.

Trimethoprim-sulfamethoxazole and the lipophilic tetracyclines (minocycline and doxycycline) have been used successfully in the treatment of osteomyelitis in children and adults and are readily absorbed by the oral route. Experience with these agents in treating CA-MRSA infections in which pneumonia and bacteremia seem to be common is limited, however, and the potential

for dental staining should be considered with the tetracyclines. Daptomycin, a bactericidal lipopeptide antimicrobial agent, has excellent activity against CA-MRSA, but pediatric pharmacokinetic data and clinical experience also are limited. Daptomycin seems to have poor activity in lung tissue. Reports of pneumonia and sepsis in association with osteomyelitis add to concerns about the utility of daptomycin for the treatment of osteomyelitis.[86,87,112]

Osteomyelitis caused by *S. pneumoniae* strains with decreased susceptibility to penicillin have been managed successfully with a variety of agents, including ceftriaxone, vancomycin, and clindamycin.[24] β-Lactam antibiotics, including oxacillin, nafcillin, and cephalosporins, have been used successfully in the treatment of *K. kingae* infection.[46,88,227,228]

Decades of experience and clinical investigation have led to wide acceptance of sequential use of the intravenous and oral routes of administration of antibiotics to treat pediatric osteomyelitis, which previously was controversial. Completing treatment with oral therapy avoids the cost, pain, inconvenience, and well-known complications of long-term administration of intravenous antibiotics. Central venous catheters have not obviated these concerns. To the contrary, local skin infections, bacteremia, and malfunctions of the catheters seem to be common occurrences, especially among young children.[175]

Oral therapy is most likely to succeed, and oral therapy is an acceptable option when the following criteria are met: An organism has been identified, the patient has the ability to swallow and retain an appropriate medication, the laboratory is able to monitor the degree of antibiotic absorption, and the patient has a clear clinical response to intravenously administered antibiotics.[154,156] In most series in which oral therapy has been evaluated,[29,52,154,203,217] treatment was continued with intravenous antibiotics until the patient was afebrile, until local signs and symptoms of infection were reduced considerably, and until the patient was maintaining caloric and fluid balances by the oral route. In severe or complicated cases, an advisable approach is to delay switching to the oral route until the peripheral leukocyte count has normalized, and the ESR has decreased by 20 percent or more, or until a marked decrease in the concentration of CRP has occurred.[171,212] A review of case series employing sequential therapy affirmed these recommendations and found no evidence that a fixed period of intravenous therapy is beneficial or essential. A transition to oral therapy within 7 days of diagnosis seemed to be equal in outcome to therapy with a fixed initial period of parenteral therapy.[126]

When oral therapy is begun, most antibiotics administered orally for osteomyelitis must be given in doses higher than the doses used for the treatment of other infections.[8,155,203] Specific antibiotics and the recommended starting doses are listed in Table 61–3. Dosages of β-lactams often can be increased to 200 mg/kg/day without having serious adverse side effects. Diarrhea, an infrequent complication of high-dose, oral β-lactam therapy, can be mitigated by a reduction in dose and the administration of probenecid (40 mg/kg/day every 6 hours; maximal dose 2 g/day).[29,154,203] Clindamycin readily achieves high bone levels and does not require higher dose therapy when given orally.[113,139]

TABLE 61–3 Initial Antibiotic Doses for Oral Treatment of Osteomyelitis

Drug	Dose (mg/kg/day)	Interval between Doses (hr)
Amoxicillin	100	6
Cephalexin	150	6
Chloramphenicol	75	8
Clindamycin	40	8
Cloxacillin	125	6
Dicloxacillin	100	6
Penicillin V	125	4

Data from references 29, 76, 154, 203.

The assumption implicit in successful oral therapy is that the antibiotic reaches an effective concentration at the focus of infection. Compliance with the prescribed dose and frequency and absorption into the bloodstream are necessary to fulfill this assumption. Patient (or parent) education and a continuing time commitment by a physician or nurse are essential to maintaining the compliance required for successful treatment. Although eschewed by some physicians,[137,158] therapeutic drug monitoring is recommended by some specialists to show that adequate absorption of orally administered antibiotics is occurring.[155] Rare patients have inadequate serum levels despite receiving high oral dosages.[203] If bacteria have been isolated from the patient, a peak serum bactericidal titer of 1:8 or greater is sought.[165,203]

A disadvantage of assessing serum bactericidal activity is the difficulty of performing the test when other antibiotics are present in the serum sample. Nafcillin administered intravenously would interfere with evaluating the bioactivity of dicloxacillin. Chemical assays of a specific agent circumvent this problem. Dicloxacillin can be administered orally and the dose adjusted based on dicloxacillin measurements before the intravenous administration of another β-lactam has been discontinued. This procedure avoids several days of inadequate therapy should the dosage of the oral agent need to be adjusted.

In most cases, clinical improvement is evident within 3 to 7 days of the initiation of appropriate therapy. To help show that effective therapy is under way, it is helpful to monitor acute-phase reactants. Although CRP and ESR typically increase during the first 3 days of therapy, CRP then begins to decrease rapidly, and typically returns to normal in 7 to 10 days. CRP returns to normal levels in blood more rapidly in children with an uneventful clinical course than in children who ultimately require repeated surgical drainage.[171] A slow decline in serum CRP to normal levels has been associated with more extensive radiographic changes or persistent symptoms 1 to 2 months after discharge from the hospital. Similar to CRP, ESR generally increases during the first several days but declines in the weeks that follow.[212] Failure of ESR to decrease during the second week of treatment may indicate a need for surgical drainage or the development of chronic osteomyelitis.[196]

The outcome of acute osteomyelitis depends heavily on the duration of therapy. In one early report of 45 cases of osteomyelitis, four treatment failures occurred; all had been treated for 10 days or less.[97] Likewise, Dich and colleagues[52] noted a 19 percent failure rate in 37 patients treated for 3 weeks or less; the rate was 2 percent in 48 patients treated for 21 to 50 days. Blockey and Watson[21] provided similar data. The minimal duration of therapy for hematogenous osteomyelitis to minimize the risk of recurrence seems to be more than 3 weeks. A conservative but individualized approach is to administer antibiotics until ESR and CRP are within the normal range, which usually requires 4 to 6 weeks of treatment.[112,154-156,196]

SPECIAL MANIFESTATIONS OF HEMATOGENOUS OSTEOMYELITIS

EPIPHYSEAL OSTEOMYELITIS

Rarely, hematogenous osteomyelitis may arise in the epiphyses of the tubular bones of young children.[78,133,208] Although the pathogenesis is unclear, it may involve delivery of microorganisms to the epiphyses by transphyseal vessels. After the child

reaches 15 to 18 months of age, these vessels are lost, and the physis acts as a physical barrier to the spread of infection from the metaphysis. The vascular anatomy of the epiphysis is very similar to that of the metaphysis, however, and in some cases infection may occur when bacteria are delivered to venous sinusoids by terminal branches of the epiphyseal arteries.

Hematogenous epiphyseal osteomyelitis may be acute or subacute. In the acute manifestation, septic arthritis initially may be diagnosed when joint swelling occurs, and abnormal fluids are removed by diagnostic aspiration. In cases with a more indolent course, pain, limp, or other symptoms prompt an evaluation for the possibility of an osteoarticular infection. The correct diagnosis typically is established when a radionuclide bone scan or plain radiographs taken weeks later show evidence of increased bone turnover or the lytic changes characteristic of osteomyelitis. Administration of appropriate therapy for epiphyseal osteomyelitis has been followed by complete recovery without apparent sequelae 2 to 6 years after diagnosis.

INVOLVEMENT OF NONTUBULAR BONES

Less than 20 percent of all cases of osteomyelitis involve nontubular bones. Infection of the calcaneus seems to be the most common.[154] In patients with hematogenous infection, destruction occurs just under the epiphyseal line in the metaphysis posteriorly and medially, where the blood supply is greatest. It is present in all patients, in addition to destruction of the adjacent epiphysis, particularly in its middle to superior portion. Periosteal new bone formation occurs very late, with 3 to 4 months required for reossification. Osteomyelitis in the other cuboidal bones rarely occurs.

Almost equal in frequency to infection of the calcaneus is infection of the bones of the pelvis.[154] Of the bones of the pelvis, the ischium is involved most commonly. The next most frequently involved bone is the ilium, followed by the sacroiliac joint. The pubis is involved in only 20 percent of cases of pelvic osteomyelitis.[99,152] Pelvic osteomyelitis causes an increase in the ESR in nearly all patients, and two thirds have a peripheral leukocyte count greater than 10,000 cells/mm^3.[152] The most common organism causing pelvic osteomyelitis is *S. aureus*, which is isolated from either blood or an aspirate from the bone lesions in approximately 80 percent of cases.

Establishing the diagnosis of pelvic osteomyelitis often is difficult. Most patients are judged to have disease in the hip at the time that medical attention is sought. Most often, patients with pelvic osteomyelitis have hip pain and a gait abnormality but allow their hips to be put through a passive range of motion. Point tenderness at the site of the lesion can be elicited in approximately 50 percent of these patients. Sacroiliitis frequently is difficult to identify by clinical examination. Pressing down on the pelvis, which stresses the sacroiliac joint, produces local pain. Tenderness in the buttocks or the sciatic notch, if present, is an invaluable diagnostic finding.[1,38,55,148,221] Pelvic osteomyelitis can mimic appendicitis and urinary tract infection[221]; it occurs more frequently in individuals with inflammatory bowel disease. In most patients, plain films of the pelvis are not rewarding, whereas Tc 99m bone scans indicate the diagnosis in approximately 90 percent of cases.[135,152] CT may reveal infection not evident by bone scanning. MRI also is likely to identify pelvic osteomyelitis.[210]

Antibiotic therapy alone is adequate in most cases of pelvic osteomyelitis. Surgery is indicated only when a lack of response to antimicrobial therapy occurs. Osteomyelitis in the pelvic bones has a uniformly good prognosis; chronic infection and sequelae are rare events.

Hematogenous osteomyelitis of the flat bones occurs rarely. It has been described in the skull, ribs, sternum, and scapula.[83,149,154,211]

SPINAL OSTEOMYELITIS

Spinal osteomyelitis can involve either the intervertebral disk or the vertebral bodies per se. Conceptualizing these infections as different entities is worthwhile because of the different pathophysiology and prognosis.[65]

DISKITIS

The intervertebral disk consists of three components: the paired cartilaginous articulation, the fibrous ring (annulus fibrosus), and the nucleus pulposus.[23,65] The axial vessels that parallel the fetal notochord are atrophied by birth, with the avascular, mucilaginous nucleus pulposus remaining. The disk has two arterial supplies: periosteal vessels and vessels descending from the central portion of the vertebral body.[42,64] The vessel from the central portion of the adjacent vertebra begins to atrophy in the first year and is obliterated completely by the time the child is 10 years old. This condition leaves only the capillary network in the annulus fibrosus, which is derived from the terminal radial ramifications of the periosteal vessels. If loss of this vascular supply is precipitous, idiopathic disk necrosis ensues, usually manifested as asymptomatic calcification.[178,194,200] If bacteremia occurs during loss of the blood supply, however, infection of an intervertebral disk may occur.

Infection of an intervertebral disk has no sex preponderance, and most children are younger than 5 years old.[65,184] It occurs almost exclusively in the lumbar region (the L4 and L5 disks are involved most frequently, followed by L3 and L4). The disease comes to medical attention with the patient's refusal to walk. Backache and a progressive limp may be present. Nonambulatory infants often become irritable and refuse to sit. On examination, the most striking feature is percussion tenderness over the contiguous spine; hip pain and stiffness with loss of lordosis of the lower part of the back are observed. Occasionally, compression of the spine produces pain at the infected disk. The mean duration of symptoms before diagnosis is established ranges from 1 day to 18 months. Most patients have had symptoms for several weeks.

Lesions in higher locations (T8 to L1) can mimic gastrointestinal disease with abdominal pain, ileus, and vomiting, but the most important entities in the differential diagnosis are vertebral osteomyelitis and spinal or paraspinal tumors. Most patients have a history of a recent upper respiratory infection.[116,122,169,174,187] Fever generally is absent or low-grade. Peripheral leukocytosis is present in one third of patients, and virtually all have an increased ESR. A few patients undergoing biopsy have cultures that grow microorganisms. Most commonly, *S. aureus* is recovered, along with rare isolates of pneumococci and gram-negative organisms, including *K. kingae*. Evaluation for suspected diskitis usually involves careful elicitation of the history and physical examination, a complete blood count, determination of the ESR, a blood culture, and plain lateral radiographs of the lumbosacral spine.[65]

Typical plain radiographic findings are shown in Figure 61–5. The first finding is narrowing of the disk space, usually not detectable until 2 to 4 weeks after the onset of symptoms. Frequently, this narrowing is overlooked if loss of the normal progressive (from cephalad to caudad) increase in disk width is not appreciated. It is followed by destruction of the adjacent cartilaginous vertebral end-plates and, subsequently, by herniation of the disk into the vertebral body. Rarely, compression or wedging of the vertebral body is noted. In older children, anterior spontaneous fusion is a common finding. In all individuals, reactive bone proliferation is a rare finding, as are paravertebral soft tissue masses.

Because of overlap of this syndrome with noninfectious disk necrosis, investigators in the earlier literature advocated treating

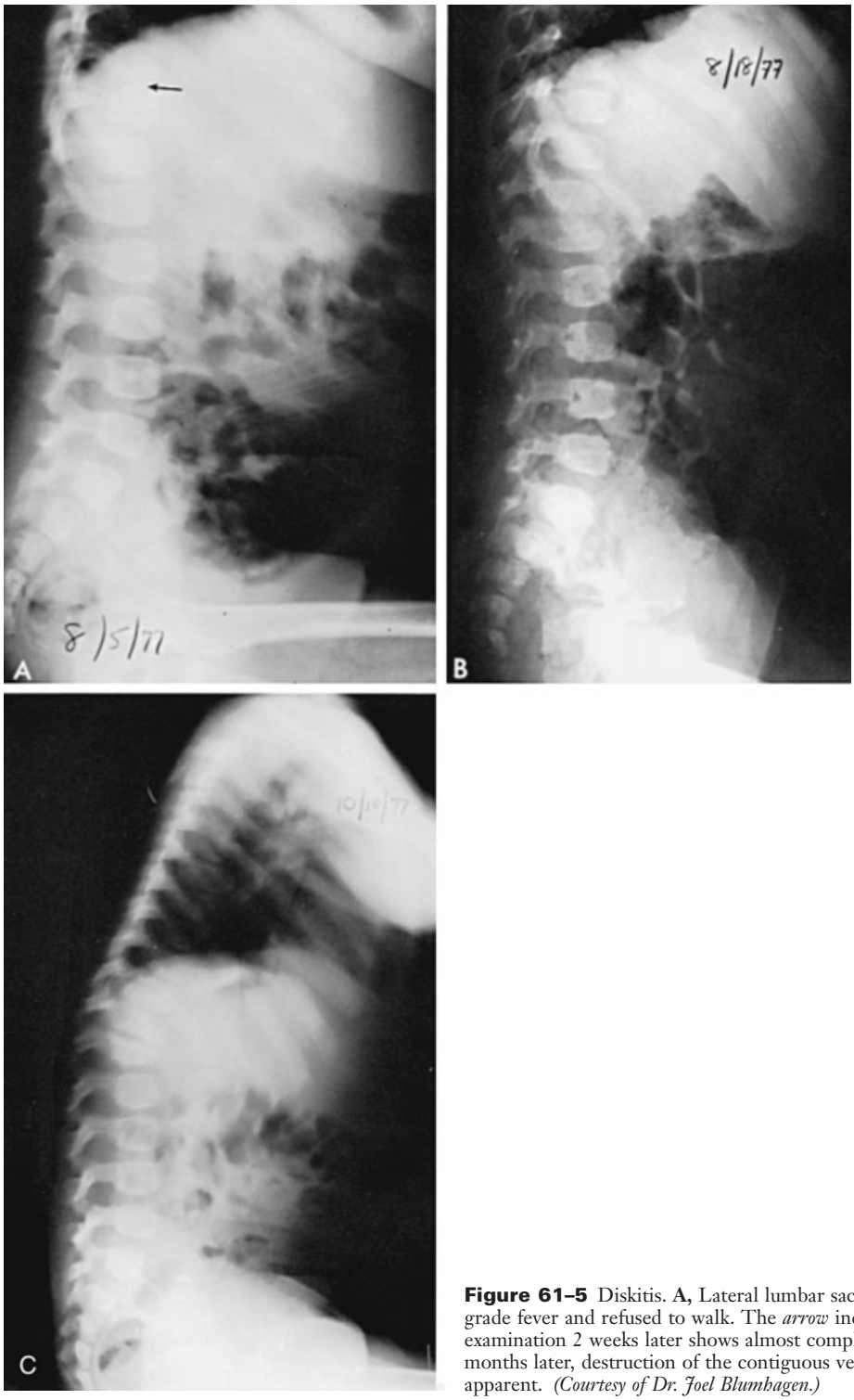

Figure 61–5 Diskitis. **A,** Lateral lumbar sacral spine of a 13-month-old child who had low-grade fever and refused to walk. The *arrow* indicates the narrow disk space. **B,** Follow-up examination 2 weeks later shows almost complete collapse of the intervertebral body. **C,** Two months later, destruction of the contiguous vertebra with marked kyphosis was apparent. *(Courtesy of Dr. Joel Blumhagen.)*

this disease solely by bed rest. In view of the low yield and generally favorable prognosis of diskitis, biopsy or aspiration for culture generally is unnecessary. Although controversial, oral antistaphylococcal therapy often is administered for a prolonged period (5-6 months).[65] Other physicians have suggested giving 5 to 7 days of intravenous antistaphylococcal therapy, followed by 7 to 14 days of a similar oral agent.[44] Very young children generally do well, and the disk space is preserved; spontaneous spinal fusion occurs commonly in older individuals.

VERTEBRAL OSTEOMYELITIS

The intervertebral disk loses its vascular supply with age, a process usually completed by 30 years of age. As a result, bacteremic pyogenic infections of the spine occur most often in the vertebral bodies. The venous drainage of the vertebral bodies is composed of three different freely communicating, but valveless, systems. Intraosseous vertebral veins drain the center of each body and form a large channel that exits through the nutrient

foramen. They anastomose with the anterior and posterior internal plexus (between the dura mater and the vertebral body). The internal venous plexus has anastomotic connections with the external venous plexus through the vertebral ligaments. The external venous plexus communicates freely with the segmental veins on the ventral surface of the body wall (Batson plexus).

Reversal of blood flow or thrombosis in the sluggishly flowing vertebral veins is thought to be the first event in the development of vertebral osteomyelitis. Because of the vascular communication, osteomyelitis usually involves two adjacent vertebral bodies, with the interposed disk initially skipped. The same process of septic thrombophlebitis involving the internal venous plexus can produce an epidural abscess (with cord compression) and infarction of the vertebral body. Spread along the external venous plexus can lead to a paraspinous mass. In the thoracic area, it may produce mediastinitis,[47] and in the cervical area, a retropharyngeal abscess can occur. The lumbar area is involved much more frequently than is the thoracic area, which is involved more frequently than is the cervical region.[2,110,193]

Children with vertebral osteomyelitis usually are older than 8 years of age and seek medical attention because of constant back pain. They may appear toxic and have a low-grade fever (>39º C) after an indolent course (2 weeks to several months).[65] Percussion of the spinal dorsal process frequently elicits exquisite tenderness. Usually, the paraspinous muscles around the involved vertebrae are in spasm, with rigidity of the area. Radiographic examination of a patient with vertebral osteomyelitis shows the earliest change to be localized rarefaction of one vertebral plateau, followed by involvement of adjacent vertebrae. Marked destruction of the bone, usually anteriorly, is followed by abundant anterior osteophytic reactions with bridging and bone sclerosis (Fig. 61–6).

Obtaining an MRI study is useful in establishing the diagnosis of vertebral osteomyelitis[146] because the vertebral disk and the vertebral body are clearly discernible. Most cases of vertebral osteomyelitis begin at the margin between the disk and the anterior part of the vertebral body. In some cases, MRI has detected evidence of osteomyelitis when radionuclide imaging studies were normal.[205] Distinguishing pyogenic osteomyelitis from tuberculous lesions radiologically is impossible, but the latter usually are characterized by less bone destruction, less bone proliferation, and less sclerosis. The osteophytic bridging between adjacent vertebrae is extremely rare in spinal tuberculosis.

Tc 99m and Ga 67 are taken up by the lesion in vertebral osteomyelitis. A possible advantage of scanning with both isotopes is identification of paraspinous abscess with Ga 67,[176] but MRI probably would accomplish the same goal.

Most cases of vertebral osteomyelitis are caused by *S. aureus.* Organisms causing urinary tract infection can cause osteomyelitis, presumably by local spread through Batson plexus.[27,98,124] Urinary tract infection rarely precedes the development of vertebral osteomyelitis, however, and only approximately 2 percent of all cases can be shown to be related to infection of the urinary tract.[77] The best method of establishing the diagnosis is through examination of bone biopsy specimens and cultures.[153] *P. aeruginosa* also has been recognized as a pathogen in vertebral osteomyelitis. All patients were intravenous drug abusers, and the organisms presumably were inoculated along with the illicit drug. Young heroin addicts have been found to have *P. aeruginosa* infection in their intervertebral disks.[28,181] In areas of the world where brucellosis is endemic, spinal osteomyelitis caused by *Brucella* spp. needs to be considered.[130] Fungal pathogens causing vertebral osteomyelitis include *Coccidioides immitis* in endemic areas and *Candida* spp. (often in immunocompromised patients).[144] *Bartonella henselae* also has been found to cause vertebral osteomyelitis.[65]

Therapy for spinal osteomyelitis includes immobilization. Whether immobilization should be accomplished by simple bed rest or with a body cast is controversial. Administration of an antibiotic always is indicated. The average duration of treatment of bacterial vertebral osteomyelitis is 2 months; however, no data are available that can be used for determining the most appropriate duration of therapy. The need for surgical drainage in some cases should not be overlooked because spinal cord compression caused by an epidural or subdural abscess can lead to permanent paraplegia. A paraspinal mass may rupture into the abdominal cavity or erode the aorta; both events are catastrophic complications.[72] The optimal therapy for fungal infection of the vertebra is unclear.

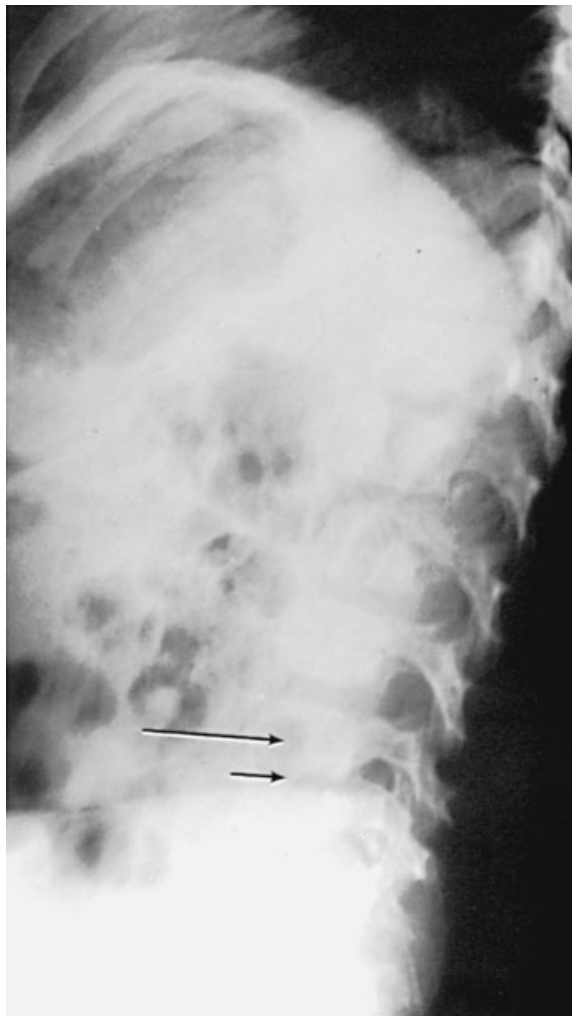

Figure 61–6 Vertebral osteomyelitis. The *large arrow* indicates the lytic lesion with some anterior sclerosis in the lumbar vertebra. The *small arrow* indicates involvement of the adjacent lower vertebra and narrowing of the disk space. *(Courtesy of Dr. Joel Blumhagen.)*

BRODIE ABSCESS

Osteomyelitis occasionally is indolent in presentation. Perhaps the best defined example is subacute osteomyelitis with the development of an intraosseous abscess (Brodie abscess).[191] These patients most often are adolescents with complaints of long bone pain and tenderness. A bony defect with sclerotic margins is detected by plain radiography in most patients. A distinctive "target" lesion has been described in MRI studies of

Brodie abscesses.[138] Concentric layers are seen and reflect a central abscess cavity surrounded by an inner ring of granulation tissue, an outer ring of fibrotic reaction, and a peripheral rim of endosteal reaction that is hypointense on T1-weighted images.

ESR usually is normal. Treatment consists of surgical drainage and curettage followed by antimicrobial therapy, as for other forms of hematogenous osteomyelitis. A variety of organisms, including *H. influenzae*, have been isolated from these lesions,[120] but *S. aureus* and other gram-positive cocci are the pathogens usually involved. The prognosis generally is good, although deformities occur in some cases.

HEMATOGENOUS OSTEOMYELITIS IN SPECIAL POPULATIONS

OSTEOMYELITIS IN NEWBORNS

Infection of the bones of newborns has distinct physiologic and clinical features that warrant emphasis.[7,49,226] Although it is rare, neonatal osteomyelitis may occur in newborns with certain risk factors, such as prematurity, skin infections, and complicated delivery.[72,118] As indicated earlier, the epiphyseal-metaphyseal junction frequently is within the joint capsule, and blood vessels that penetrate the epiphysis are common findings, particularly in the hip, shoulder, and knee of the newborn.

In newborns, osteomyelitis in the long tubular bones frequently (50-70% of cases) is accompanied by contiguous septic arthritis. Fever is present in one third to one half of the newborns; of these, half have a septic or toxic appearance. Half of infants have multiple bones involved. Antecedent infections are present in half of the cases and usually are nosocomial; infections of heel puncture sites, arterial cannulas, lungs, and cut-down sites and cephalhematoma have been described.[7] *S. aureus* is the etiologic agent in more than 90 percent of cases; gram-negative bacilli cause a few cases. Osteomyelitis often is identified when a search is instituted for the source or focus of bacteremia.[226] As noted earlier, plain radiographs often reveal evidence of osteomyelitis at the time of diagnosis, whereas bone scanning may be negative. Bone scanning may be useful, however, in excluding the diagnosis of osteomyelitis; in one study, the negative predictive value was estimated at 82 percent.[226]

Although staphylococci seem to be the most common cause of osteomyelitis in infants, group B streptococci have been found consistently.[58] Infants with group B streptococcal osteomyelitis generally are older (2-4 weeks old), have no recognized preceding infection, and have only a single bone involved, often the right tibia or humerus.[7]

Osteomyelitis in the skull, an uncommon disease, can occur in neonates. Frequently, it is associated with a cephalhematoma, with or without loss of skin integrity,[123] but it can be caused by fetal monitoring without a cephalhematoma.[142] Numerous bacteria have been isolated from such lesions; as one might expect, most of these bacteria have been present as vaginal flora. Radiographic changes (i.e., bony erosion) are a late finding, but CT of the skull seems to assist in establishing the diagnosis. Infection of a cephalhematoma should be considered if it is enlarging, if it is inflamed, or if laboratory evidence of infection is present, such as an increased CRP concentration or leukocyte count. In most cases, the lesion is drained and treated with an antibiotic appropriate for the infecting organism.

In older descriptions of neonatal osteomyelitis, sequelae were common findings. In more recent series,[118,224] approximately three fourths of all cases have had good outcomes, even when the hip has been involved. When seen, sequelae include avascular necrosis of the femoral head, bony deformities, and shortening of the involved limb.[161]

OSTEOMYELITIS IN LONG-TERM HEMODIALYSIS PATIENTS

Patients undergoing long-term hemodialysis, with multiple invasions of the vascular compartment, seem to be at greater risk for development of hematogenous osteomyelitis.[125] Their indwelling intravenous cannulas can be colonized with coagulase-negative staphylococci or *S. aureus*, and osteomyelitis may develop. The thoracic spine and ribs are the bones most commonly involved; other tubular and cuboidal bones that have been traumatized also can become infected.

OSTEOMYELITIS IN CHILDREN WITH HEMOGLOBINOPATHIES

After pneumonia, osteomyelitis is the most common serious infection in children with sickle-cell disease.[11] Children at risk are children with hemoglobin SS, hemoglobin S-Thal, or hemoglobin SO-Arab and certain children with hemoglobin SC disease.[93,186] The clinical manifestations of osteomyelitis are similar to those of other children with osteomyelitis, but there is a propensity for simultaneous involvement of multiple sites, a tendency toward recurrence, and a greater frequency in children 18 to 48 months of age.

As noted earlier, the microbiology of osteomyelitis in children with sickle-cell disease is complex and dominated by *Salmonella* spp.[11,31] Seventy percent of all lesions or blood cultures in children with hemoglobinopathy and presumptive osteomyelitis yield *Salmonella* microorganisms; 10 percent contain *S. aureus*; and aerobic gram-negative rods, including *Shigella sonnei*,[173] *Escherichia coli*,[92] *Serratia* spp.,[70] and *Arizona hinshawii*,[103] are isolated in 7 percent. Although *Salmonella* osteomyelitis occurs in less than 1 percent of normal patients with *Salmonella* bacteremia,[222] the frequency of *Salmonella* osteomyelitis in sickle-cell disease is several hundred times that occurring in the general population, with an incidence estimated at 0.36 percent per annum.[11,53,163]

Many factors probably contribute to the greater incidence of osteomyelitis in patients with sickle-cell hemoglobinopathy. Injuries to the intestinal mucosa from local thrombosis during a thrombotic crisis may facilitate the entrance of *Salmonella* and other enteric organisms into the bloodstream.[31,222] When infection of the bloodstream occurs, the splenic dysfunction in these patients may allow a prolonged period or greater magnitude of bacteremia. Evidence of impaired production of opsonic antibodies also exists. Whether bacteria lodge in infarcted bone, or whether a different pathogenesis exists is unclear. Infarction occurs in the capital femoral epiphysis, hands, feet, and vertebrae, whereas osteomyelitis involves the metaphyseal-diaphyseal junction of long tubular bones.[60,163]

Infants with hand and foot syndrome are not distinguished easily from infants with osteomyelitis of the phalanges of the hands or tarsal bones of the feet.[16,41,219] The changes caused by osteomyelitis that are seen on plain radiographs are more severe than the changes expected in uncomplicated sickle-cell disease. The most common radiographic findings are a longitudinal intracortical diaphyseal fissure and overabundant periosteal new bone formation. The cortical fissures are thought to represent a layer of purulent exudate in and between the periosteal new bone and dead bone.[53]

Radionuclide scans also are used frequently to help differentiate osteomyelitis from bone infarction. In theory, bone infarction should have decreased uptake of Tc 99m in the early "blood pool" phase of the scan; increased uptake would be found only as the lesion healed.[115] In one series with 34 sites of infarction, increased Tc 99m uptake occurred in 10, normal uptake occurred in 9, and decreased uptake surrounded by zones of increased concentration occurred in the remaining 15 sites.[81] Ga 67 or bone marrow scans of lesions with Tc 99m sulfur colloid

also may be valuable in differentiating infarction from infection; increased uptake of the radionuclide is seen more frequently with infection than with infarction.[166] Acute infarction and osteomyelitis cannot always be differentiated by MRI, perhaps because of pre-existing abnormalities in bone marrow. MRI may be useful, however, for presurgical evaluation. Ultrasound imaging also has been used to distinguish osteomyelitis and sickle-cell crisis and, if confirmed, may prove to be a useful adjunct to other imaging modalities.[23]

Generally, patients with bony infarcts have had dactylitis as an infant and multiple episodes; their temperature usually is less than 39º C. Children with hemoglobinopathy and osteomyelitis may lack this history and have a modest leukocytosis of immature granulocytes.[105] If fever, leukocytosis, and local symptoms persist despite provision of hydration and other supportive measures, needle aspiration of the area must be considered. Identification of an infectious pathogen is particularly important because of the large number of possible organisms involved.

OSTEOMYELITIS IN PATIENTS WITH HUMAN IMMUNODEFICIENCY VIRUS INFECTION

Although recurrent invasive bacterial infections often complicate human immunodeficiency virus (HIV) infection in children and adults, few reports of osteomyelitis in these patients exist.[104,143,190] Many of the existing reports involve patients with recent intravenous drug use, which probably acted as a predisposing factor. *S. aureus* was recovered most commonly, although *E. coli*, *Salmonella enteritidis*, *Cryptococcus neoformans*, *Mycobacterium kansasii*, *Histoplasma capsulatum*, and other organisms also were seen in individual cases. To date, no evidence supports the view that the initial signs and symptoms or treatment needed for recovery from osteomyelitis is affected by co-infection with HIV.

OSTEOMYELITIS IN PATIENTS AFTER CLOSED FRACTURES

Acute hematogenous osteomyelitis sometimes develop after closed fractures of tubular bones occur.[33,218] The diagnosis generally is not recognized until fluctuation is apparent at the fracture site. A clue to diagnosis is that after the initial post-fracture pain has subsided, the pain of osteomyelitis occurs. It begins 1 to 6 weeks after the injury occurs. The pain differs from that associated with the fracture by being progressive, and it is not relieved by immobilization. When the cast is removed, local erythema and warmth are apparent and are increased when these findings would seem to be resolving if they were secondary to the fracture. Patients are febrile and may be thought to have another focus of infection before osteomyelitis is discovered. Anaerobic superinfection of staphylococcal osteomyelitis at the fracture site has been recognized.[201] Adequate débridement, administration of appropriate antibiotics, and external fixation have been used in such cases. The outcome varies, however.

OSTEOMYELITIS IN PATIENTS WITH CHRONIC GRANULOMATOUS DISEASE

The phagocytes of patients with chronic granulomatous disease fail to kill intracellular organisms, and infections with catalase-positive bacteria or fungi are frequent complications. Although staphylococci are a common cause of cutaneous infection in chronic granulomatous disease, osteomyelitis is caused most often by *Serratia* and *Aspergillus* spp. Osteomyelitis is attributed less often to staphylococci, *Pseudomonas*, *Burkholderia*, *Nocardia*, and other bacterial and fungal species.[225]

NONHEMATOGENOUS OSTEOMYELITIS

Nonhematogenous infections of the bone arise either through inoculation or from a contiguous focus of infection.

PUNCTURE WOUND OSTEOMYELITIS

Inoculation osteomyelitis most often involves either the patella or the bones of the foot. Soft tissue infections occur after puncture wounds of the foot approximately 15 percent of the time, and osteomyelitis occurs in 1.5 percent of these injuries.[67] Osteomyelitis of the foot that develops after children sustain puncture wounds should be termed *osteochondritis* because it commonly includes infection of the articular cartilage in the metatarsals. Most patients are aged 9 to 18 years. After the initial pain of the puncture wound subsides (in 24-48 hours), the signs of osteochondritis appear after another 48 to 72 hours. Typically, joint tenderness and localized swelling, erythema, and pain over the entrance of the puncture wound are present. Fever is an infrequent finding, and the patient seldom has any other constitutional symptoms. No peripheral leukocytosis is noted, and ESR is increased minimally.

Although the offending organism most commonly is *P. aeruginosa* (90% of the time), staphylococci, streptococci, *Stenotrophomonas maltophilia*,[9] and *Serratia marcescens*[151] also have been isolated.[25,67,106,107,109] In some series,[107] 20 percent of the cases involved infection with *P. aeruginosa* and *S. aureus*. The predominance of *Pseudomonas* can be explained partially on the basis of the mechanism of injury. *Pseudomonas* is not found commonly in surveys of the microbial flora of the skin of the feet,[85,145,198] but often it is found by culture of the sponge liner from children's sneakers.[66] In addition, many patients in whom *P. aeruginosa* osteochondritis develops have received prophylactic treatment with oral antibiotics that have activity against gram-positive bacteria. A semisynthetic penicillinase-resistant penicillin and an aminoglycoside or ceftazidime often are used as initial therapy.[106,107]

Surgical débridement of necrotic cartilage is a key element of treatment because foreign material frequently is found embedded in the soft tissue; débridement also provides the opportunity to identify organisms resistant to the initial antibiotic agents. The local signs and symptoms of infection usually resolve 4 to 5 days after adequate débridement is performed, and the patient is able to bear weight on the foot. In contrast, antibiotic therapy alone after diagnostic aspiration may need to be continued 6 to 8 weeks before weight bearing is possible. Long-term follow-up of *P. aeruginosa* osteochondritis indicates that many patients have asymptomatic radiographic abnormalities.[13] Radiographic abnormalities are found more commonly in patients in whom an adjacent joint was involved initially.[13]

Osteomyelitis also has been described repeatedly after puncture wounds occur from stepping on wooden toothpicks. In these cases, *Eikenella corrodens*, a member of the human oral flora, has been found with other organisms. Surgical débridement to remove toothpick fragments and drain local abscesses is essential.[168]

Osteomyelitis of the patella is a disease of children aged 5 to 15 years old, the time in life during which the patella is vascularized. By adulthood, the vessels are almost completely atrophied. In almost all cases, the diagnosis shows the cause to be by inoculation, such as kneeling on a needle. The most common etiologic agent is *S. aureus*; signs of osteomyelitis appear 1 week after the puncture occurs. No constitutional symptoms occur, but extension of the leg produces pain over the anterior aspect of the patella. The diagnosis is made by isolating the organism from the patella. Radiographic confirmation of the diagnosis often requires 2 to 3 weeks. Because the bone lacks periosteum, no periosteal

elevation is present, but rarefaction and sclerosis may be seen on the profile view or on tomograms. Treatment of this disease is similar to that for other forms of osteomyelitis.

OSTEOMYELITIS CAUSED BY SPREAD OF INFECTION FROM A CONTIGUOUS FOCUS

In children, osteomyelitis related to an infected contiguous focus is a rare finding. Almost all cases of osteomyelitis from a contiguous source in childhood are nosocomial or are caused by an infected burn wound. The probability of postoperative osteomyelitis developing is a function of the surgeon's experience, the technique, the length of time that the wound was open, and whether prophylactic antibiotics were administered.[192] The interval between the precipitating event and pain and the appearance of persistent sinus drainage or ulceration is 2 to 4 weeks. The peripheral white blood cell count often is normal, as is ESR. More than half of cases are caused by multiple organisms. When small draining sinuses are present, correlation between sinus culture and bone biopsy findings is good. With large open areas, organisms obtained by culture of the wound may not be important etiologically, and obtaining a bone biopsy specimen is necessary for making a definitive bacteriologic diagnosis. Staphylococci and streptococci predominate; however, nosocomial gram-negative organisms often are seen.

ORTHOPEDIC FIXATOR DEVICES

Infection involving the wires or pins used for orthopedic stabilization represents a diagnostic and therapeutic challenge. Osteomyelitis is suspected when evidence of inflammation or infection is noted near these materials, but it seldom can be proved.[20] Plain radiographs are essential because they may reveal bone destruction at the site of entry of fixation pins. No controlled studies of treatment are available. Soft tissue débridement and removal of necrotic bone should be done as soon as possible, but fixation devices generally are left in place.[10,160] Prolonged therapy usually is given, guided by the susceptibility pattern of any organisms recovered from local cultures.[160]

UNUSUAL MICROBIAL CAUSES OF OSTEOMYELITIS

ACTINOMYCES

More than half of all actinomycotic infections involve the facial or cervical area. The most common site of actinomycotic bone infection is the jaw, with the mandible being involved more frequently than the maxilla. Local signs and symptoms of fever and discharge from a sinus indicate the presence of the disease. Radiographically, periosteal elevation is followed by lytic changes. Often, several "eggshell" areas of new bone are present.

Forty percent of all actinomycotic infections occur in the vertebral bodies. In this illness, the infection almost always is associated with a focus elsewhere; the condition comes to medical attention because of mild pain, tenderness, and some stiffness. It can be distinguished from tuberculosis radiographically by the diffuse honeycombing of the vertebral bodies and the periosteal reaction; large lytic lesions usually are absent. Although no controlled trials of therapy have been done, long-term (>3 months) penicillin G therapy is indicated at doses of 150,000 U/kg body weight per day (i.e., approximately 100 mg/kg/day). Extensive débridement may be needed and has been linked to successful short-term treatment of mandibular actinomycosis.[12]

BRUCELLA

Although an uncommon cause of osteomyelitis in developed countries, *Brucella* spp. are a well-known cause of skeletal infections, most often following the consumption of unpasteurized milk products. *Brucella* spp. can produce abscesses in the vertebral bodies or long bones, although they are not striking features of the disease. The disease often is subacute in presentation, and malaise, headaches, night sweats, and minimally tender cervical adenopathy with hepatosplenomegaly are common initial findings.

FUNGI

Osteomyelitis may be caused by numerous endemic and opportunistic fungal agents, including *C. immitis*, cryptococci, *Candida* spp., *Blastomyces* spp., and *Aspergillus* spp. Coccidioidomycosis may be characterized by cough, chest pain, night sweats, and anorexia, and it often is associated with erythema nodosum or erythema multiforme. This disease commonly is found in the southwestern United States. Extrapulmonary involvement is suggested by persistent high temperature and toxicity. *C. immitis* occurs primarily in cancellous bone (e.g., vertebral bodies, distal tubular bones, and the skull).[167] These lesions are not radiographically distinct from the lesions seen in osteomyelitis from other causes.[183] Débridement of bone lesions often is needed initially, and years of therapy are required. Oral triazole agents generally are recommended.[74]

Blastomycosis may mimic coccidioidomycosis, but the pulmonary involvement is much more varied, and fusion of the vertebral bodies rarely occurs. A propensity for the development of verrucous, reddened, weeping skin lesions has been noted, and prostate involvement may be seen. Bone involvement occurs frequently, with the skull and vertebral bodies being infected most often. Distinguishing it from other forms of osteomyelitis is impossible by radiographic examination.

Aspergillus osteomyelitis is being recognized with increasing frequency.[35] Most commonly, it is a disease of immunosuppressed patients, with *Aspergillus* pneumonia seen initially followed by disseminated disease. Bone disease occurring by hematogenous spread has developed, however, in normal individuals and after injectable drug use.[43,172] *Aspergillus* osteomyelitis developing after trauma also has been reported.

Other fungi are causes of osteomyelitis. The medical literature contains dozens of reports of osteomyelitis caused by *C. neoformans*, generally in immunocompromised patients and in the setting of disseminated or pulmonary infection. The few reports in pediatric patients generally have involved immunocompromised adolescents. In adolescents and adults, usually only one bone is involved. The lesions generally are slowly destructive, very discrete lesions occurring primarily in the long tubular bones without marginal sclerosis. This radiologic reaction is confused most commonly with tumor and, occasionally, with tuberculosis. Infection of the ribs and skull also has been reported. Although experience with this disease is limited, débridement of the lesions and medical therapy seem to be highly effective.[14,30,37,84,131,132] Cases of *Rhizopus* osteomyelitis have been reported intermittently and apparently are hematogenous in origin.[57]

CHRONIC OSTEOMYELITIS

Chronic osteomyelitis often is the result of bone infection that develops after a surgical procedure or major trauma.[209] Inadequate treatment of acute hematogenous osteomyelitis also can lead to the development of chronic osteomyelitis. The diagnosis

of chronic osteomyelitis usually is straightforward; patients generally have a painful, nonfunctional extremity and may have chronically draining sinuses. Cultures of the purulent exudate or necrotic bone usually reveal *S. aureus.* Gram-negative bacteria, including *H. influenzae*,[120] may be isolated from an intraosseous abscess. Plain radiographs, CT, and MRI all play roles in medical and surgical management by revealing details of the bony and soft tissue involvement, including the formation of abscesses[164] and sequestra (Fig. 61–7).

Treatment of chronic osteomyelitis involves the removal of devitalized bone, management of soft tissue disease, and long-term administration of appropriate antibiotics. Few controlled trials comparing different modes of therapy have been performed. Antimicrobial regimens for chronic staphylococcal osteomyelitis that have been studied include oral cloxacillin plus probenecid for 6 to 12 months; 9 of 19 patients apparently were treated successfully.[15] In another study, the outcome of a 6-week course of nafcillin was compared with that of nafcillin and oral rifampin. No statistically significant differences were observed with the addition of rifampin, but most (10 of 17) of the patients showed no evidence of disease activity 2 years after the cessation of treatment with antibiotics.[157]

The high failure rate in these and other studies has led to investigation of a variety of adjunctive measures to improve the outcome of chronic osteomyelitis. Local irrigation with antibiotic solutions, with[136] and without[3] added detergents, has been examined. The use of surgically implanted polymethyl methacrylate beads impregnated with an antibiotic (usually gentamicin) has been compared with conventional antibiotic therapy and with therapy with both.[19] No differences were seen among the three groups in a preliminary analysis. Hyperbaric oxygen also has been suggested as an aid to therapy, but no comparative studies have been done.[150]

Because perpetuation of chronic infection seems to be caused by the presence of avascular bone and tissue, advances such as laser Doppler flowmetry ultimately may improve the outcome of this disease.[195] Surgical approaches to close open wounds after débridement and improve blood flow with mobilized tissue flaps also seem to bring about prolonged remission in some patients.[4] Meticulous surgical technique is essential to avoid thermal injury and to retain the vascular supply to compromised areas.[202] Complications of chronic osteomyelitis include secondary amyloidosis and local sarcomatosis or carcinomatous changes at the site of infection. The high likelihood of a poor outcome in chronic osteomyelitis must be kept in mind during treatment of acute hematogenous osteomyelitis. Failure to comply with a regimen of oral therapy may result in chronic infection.[196]

CHRONIC RECURRENT MULTIFOCAL OSTEOMYELITIS

Giedion and coworkers[79] first described chronic recurrent multifocal osteomyelitis, an illness characterized by multiple chronic focal, inflammatory lesions in bone with periodic exacerbation and remission, moderate bone pain, and sterile lesions.[213] Initially, this disease is difficult to distinguish from pyogenic osteomyelitis; the only difference seems to be the apparent absence of an infecting agent. Repeated biopsy of lesions that prove to be sterile usually leads to the diagnosis. It occurs more commonly in girls younger than 10 years of age.

At initial evaluation, slightly more than 50 percent of patients have fever, and virtually all have an increased ESR (or increased CRP). The lesions occur primarily in the distal femoral, distal tibial, and proximal tibial regions. Virtually all tubular bones can be involved.[179] Patients may have 1 to 18 lesions at a time; biopsy specimens show a nonspecific chronic inflammatory process. Occasionally, a predominance of plasma cells is present, which erroneously leads to this disease being termed *plasma-cell osteomyelitis*. In the first reported cases, the lesions were symmetric. This feature has not been present consistently, however, as more cases have been described. Many of the cases are in children of northern European origin.[18,117]

Approximately 20 percent of patients have a pustular eruption of the palms and soles at the same time that they come to medical attention with bone lesions; this condition is termed *pustulosis palmaris et plantaris*. Some patients also may have Sweet syndrome, in which painful, indurated, cutaneous plaques are accompanied by fever and leukocytosis. Sweet syndrome and pustulosis palmaris et plantaris may be variations of the same illness. Sweet syndrome and congenital dyserythropoietic anemia also have been associated with chronic recurrent multifocal osteomyelitis.[59]

The long-term outlook in children with this disease generally is good, although numerous relapses may occur.[18] Glucocorti-

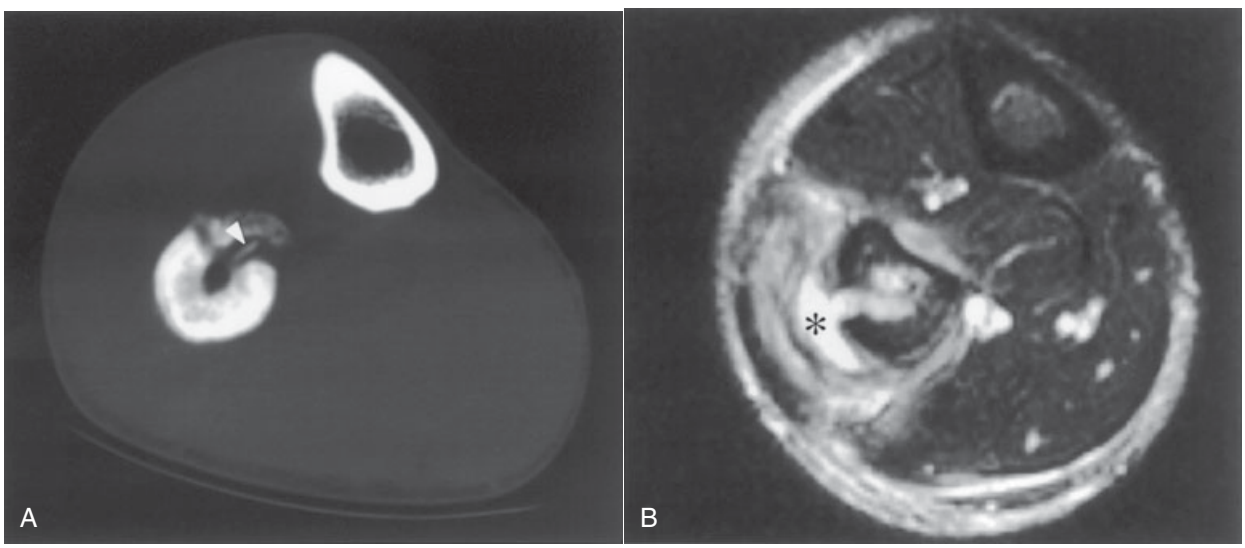

Figure 61–7 Chronic osteomyelitis. **A,** Computed tomography reveals thickening of the fibular cortex and a sinus tract that contains a sequestrum *(arrowhead)*. **B,** T2-weighted axial magnetic resonance imaging from a different level shows a lateral sinus tract through the cortex communicating with a soft tissue abscess *(asterisk)*. Edema is surrounding the entire fibula. *(Courtesy of Dr. Leanne Seeger.)*

coids and nonsteroidal anti-inflammatory drugs have been administered to children with this disease and afford transient relief. Patients usually have a recurrence of symptoms and lesions, however, when these agents are discontinued. Treatment with interferon-γ and tumor necrosis factor–α blocking agents also has been reported to be helpful.[51,75]

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CHAPTER 62

Septic Arthritis

Paul Krogstad

This chapter addresses acute infection of the joints caused by bacteria, fungi, and viruses. Bacterial infections occur most frequently. The terms *septic arthritis*, *acute suppurative pyarthrosis*, and *infectious arthritis* are used interchangeably with regard to bacterial infections; they refer to the presence of organisms in the joint space.

EPIDEMIOLOGY

Septic arthritis occurs most frequently in childhood. The overall incidence of septic arthritis in children has been estimated as 5.5 to 12 cases per 100,000 individuals.[32] Describing the age distribution of septic arthritis is difficult because of the varying age intervals selected by authors of different studies. In a review from the pre-antibiotic era, half of the patients were younger than 20 years old.[45] In a later study, performed after antibiotics became available for systemic use, 31 of 66 patients (47%) were younger than 20 years old.[6] Males are affected more often than are females by ratios of 1.2 to 2.1, and most cases occur in children younger than 10 years old.[10,34,55,59,77,91]

PATHOPHYSIOLOGY

Synovial joints, also termed *diarthrodial joints*, are freely movable articulations containing synovia. Synovia is a transparent viscous fluid that lubricates the joint and nourishes the avascular articular cartilage. The synovium, a connective tissue layer interposed between the fibrous joint capsule and the fluid-filled synovial cavity, is responsible for formation of the joint fluid. The synovium contains a prominent capillary supply embedded in a connective tissue network containing at least two types of cells. One morphologic type (type A) seems to be related to mononuclear phagocytes; fibroblast-like type B cells seem to be responsible for the synthesis of hyaluronic acid. Joint fluid (synovia) is formed by filtration through the capillary network (i.e., the net balance of back-diffusion into the capillary bed and diffusion into the joint space). Diarthrodial joints normally contain small amounts of fluid (e.g., 0.5 to 3 mL in the knee),[81] with glucose and electrolyte concentrations equal to those in plasma. An oxygen partial pressure of 60 to 70 mm Hg, an albumin concentration of 10 to 20 g/L, and an IgG content of 500 mg/L are typical.[22,24,30,81]

Diffusion from the joint space is increased by any mechanism that increases pressure (distention with injected solution, active or passive motion, or external massage). Particulate material is removed from the joint space by synovial membrane macrophages and free monocytes (the latter usually are present in concentrations of $<60 \times 10^6$/L).[75] The viscosity of joint fluid is due to hyaluronic acid; enzymatic depolymerization produces a viscosity approximately equivalent to that of water. With the loss of hyaluronidase from the synovia, the articular cartilage, with continued use, becomes eroded and sclerotic.[9] The vasculature of the synovial membrane is innervated, which has two consequences: Joint pain is localized poorly because it results from stretching of the fibrous joint capsule, and inflammation within the joint cavity elicits an axon reflex leading to vasodilation and warmth over an infected joint. Lymphatic channels drain to the regional lymph nodes and are present in all the joint tissues except cartilage.

Microorganisms can enter the joint space by hematogenous spread, direct inoculation, or extension of a contiguous focus of infection. The synovial membrane has been shown to have highly effective blood flow, approximately equal to that of the brain (if one assumes that 1 g of synovial membrane is in an adult knee joint). Numerous bacteria in blood potentially are delivered to the synovial membrane during transient bacteremia. A history of trauma often is cited as a predisposing factor for development of bacterial arthritis, but the significance of such a history is unclear in view of the great frequency of minor trauma in childhood.

Upper respiratory tract infections frequently precede the development of septic arthritis caused by *Haemophilus influenzae* and *Kingella kingae* and oral ulcers with *Kingella* infections. Similar to traumatic injuries, they are presumed to increase the likelihood of bacteremia occurring.[10,90] Septic arthritis also may develop after joint surgery and joint injections. Gram-negative organisms are the most frequent pathogens when septic arthritis occurs after surgery or instrumentation of the urinary or intestinal tract. *Salmonella* septic arthritis may develop during the course of *Salmonella* bacteremia in a normal host, but it occurs with increased frequency in patients with sickle-cell disease and related hemoglobinopathies. Although septic arthritis occurs in children and adults infected with human immunodeficiency virus (HIV),[58] as yet no data have substantiated that HIV increases the incidence of musculoskeletal infections in children. Septic arthritis has been described during varicella, presumably caused by bacteremia resulting from infection of skin lesions. It must be differentiated

from the apparent ability of varicella-zoster virus to cause joint inflammation on its own.[61,74] Arthritis also occurs during acute infection with other viruses (e.g., variola, Epstein-Barr virus, *Erythrovirus* [parvovirus B19], mumps, measles, and enteroviruses).

Inoculation arthritis occurs after invasion of the joint by a contaminated object. In one series, 5 of 35 cases of septic arthritis were caused by such a mechanism. A predilection for the knee exists; four of five cases cited in this study were secondary to kneeling on sewing needles.[77]

Aside from joint involvement during osteomyelitis, contiguous extension of an infection into the joint space rarely occurs. In one series, 10 of 77 patients with septic arthritis had disease originating from a contiguous focus.[18] None involved the joints of the foot, and eight occurred before the availability of many antibiotics (1951). This high frequency of septic arthritis caused by spread of infection from a contiguous focus has not been seen in more recent studies.

ETIOLOGY

Staphylococcus aureus is the most common agent causing septic arthritis, and infection with community-acquired methicillin-resistant *S. aureus* (MRSA) is now commonly reported.[3,57] Streptococci (especially group A beta-hemolytic organisms and pneumococci) have been responsible for most other gram-positive infections (Table 62–1). *H. influenzae* type b historically has been an important cause of septic arthritis in children younger than 2 years, but now is seen only rarely in areas with widespread immunization.[13,41,55] Arthritis caused by *Streptococcus pneumoniae* also generally occurs in children younger than 2 years old and may diminish in frequency with broader use of protein conjugate vaccines. *K. kingae* has been recognized increasingly as a cause of septic arthritis,[25,39,55,91] perhaps because of improvements in laboratory methods.[92] In one series from Israel, *Kingella* was the most common bacterial isolate (48% of cases), and *S. aureus* was not found.[91] In a few cases, septic arthritis is seen with acute *Neisseria meningitidis* infection. In newborns and sexually active adolescents with suspected septic arthritis, *Neisseria gonorrhoeae* should be considered.[35,51,53]

Salmonella spp. cause approximately 1 percent of the total cases of septic arthritis. Beyond the newborn period, infections with other enteric gram-negative bacteria are rare occurrences in pediatric septic arthritis and often are associated with inoculation, instrumentation, or an immunocompromised state.[35] Infections with *Serratia*, *Aeromonas*, *Enterobacter*, *Bacteroides*, and *Campylobacter* generally occur in patients with malignancy who are immunosuppressed.[1,6,19,35,56,63,67] *Pseudomonas aeruginosa* infections are associated with arthritis in infants, with infection of puncture wounds, or with injectable drug use.[59,63,83] Other rare bacterial causes of septic arthritis include *Propionibacterium acnes*,[93] *Corynebacterium pyogenes*,[67] and *Pasteurella multocida*.[38] *Streptobacillus moniliformis* infection of joints may become evident 2 to 3 days after a rat bite occurs; a macular rash commonly is present at initial evaluation. Discussion of Lyme arthritis is beyond the scope of this chapter, but intermittent, inflammatory arthritis is seen in many patients after *Borrelia burgdorferi* is transmitted by a tick bite.[49,80] *Brucella*, mycobacteria (*Mycobacterium tuberculosis* and atypical species), and *Nocardia asteroides* may cause a chronic monarticular arthritis with a granulomatous reaction.

DIAGNOSIS

CLINICAL FINDINGS

Almost all patients have fever and constitutional symptoms within the first few days of acquiring infection with the most common bacterial pathogens.[31] Table 62–2 presents the frequency of specific joint involvement in hematogenous septic arthritis of childhood. Lower extremity (knee, hip, and ankle) infections consistently account for approximately 80 percent of all cases.[34,45,59,63,77] Focal findings in the joint involved almost always are present.

In infants, in whom the hip is one of the most frequent joints involved, swelling, tenderness, and heat may be absent. Most commonly, the infant lies with the involved leg abducted and externally rotated. Often, dislocation occurs.[65] When the capsule of the joint can be examined, swelling is noted; effusion was present in 22 of 24 cases in one series.[87] Because pain fibers are located in the capsule, any maneuver that increases intracapsular pressure also produces pain. In the hip, this pain can be elicited by compression of the head of the femur into the acetabulum. A portal of entry almost never is apparent, and bilateral hip joint infection occurs in a few cases.[69] Pyogenic sacroiliitis often is accompanied by tenderness detected by pressure applied over the sacrum during a digital rectal examination and by pain experienced during simultaneous flexion, abduction, and external rotation at the hip.[2]

Gonococcal arthritis in newborns has nonspecific prodromal symptoms, including poor feeding, irritability, and fever. The

TABLE 62–1 Bacterial Etiology of Septic Arthritis in Children

	Gram-Positive Bacteria				Gram-Negative Bacteria							
Year of Report	*Staphylococcus aureus*	Streptococci	*Streptococcus pneumoniae*	CNS	*Haemophilus influenzae*	*Kingella kingae*	*Neisseria meningitidis*	*Salmonella*	Non-*Salmonella* Enterobacteriaceae	*Neisseria gonorrhoeae*	Other	Total Cases
1941[45]	50	45	2	10	0	0	0	0	0	14	0	121
1958[77]	18	8	5	2	3	0	0	0	2	0	1	38*
1972[63]	40	20	8	5	37	0	4	2	7	13	11	146*
1975[59]	37	10	0	5	14	0	0	0	2	0	7	75
1987[10]	40	8	5	0	20	0	4	0	2	2	9	90
1995[91]	0	2	3	0	8	19	1	1	1	0	5	40
1999[55]	10	5	2	2	1	3	3	0	4	2	1	33
Total	*195*	*98*	*25*	*24*	*83*	*22*	*12*	*3*	*18*	*31*	*34*	*543*
Percentage of all isolates	36	18	5	4	15	4	2	1	3	6	6	

**Two isolates in one case each.*
CNS, coagulase-negative staphylococci.

TABLE 62–2 Joints Involved in Septic Arthritis of Children

Reference	Knee	Hip	Ankle	Wrist	Elbow	Shoulder	Small Diarthrodial Joints
Heberling[45]	40	50	13	3	8	9	2
Watkins et al.[88]	8	13	2	6	9	2	3
Samilson et al.[77]	8	19	2	0	6	3	0
Nelson[63]	103	48	38	12	35	10	4
Gillespie[31]	37	41	13	2	3	3	0
Yagupsky et al.[91]	16	6	13	1	2	3	1
Goergens et al.[34]	15	15	4	2	4	1	3
Total	*227*	*192*	*85*	*26*	*67*	*31*	*13*
Percentage of all cases	35	30	13	4	10	5	2

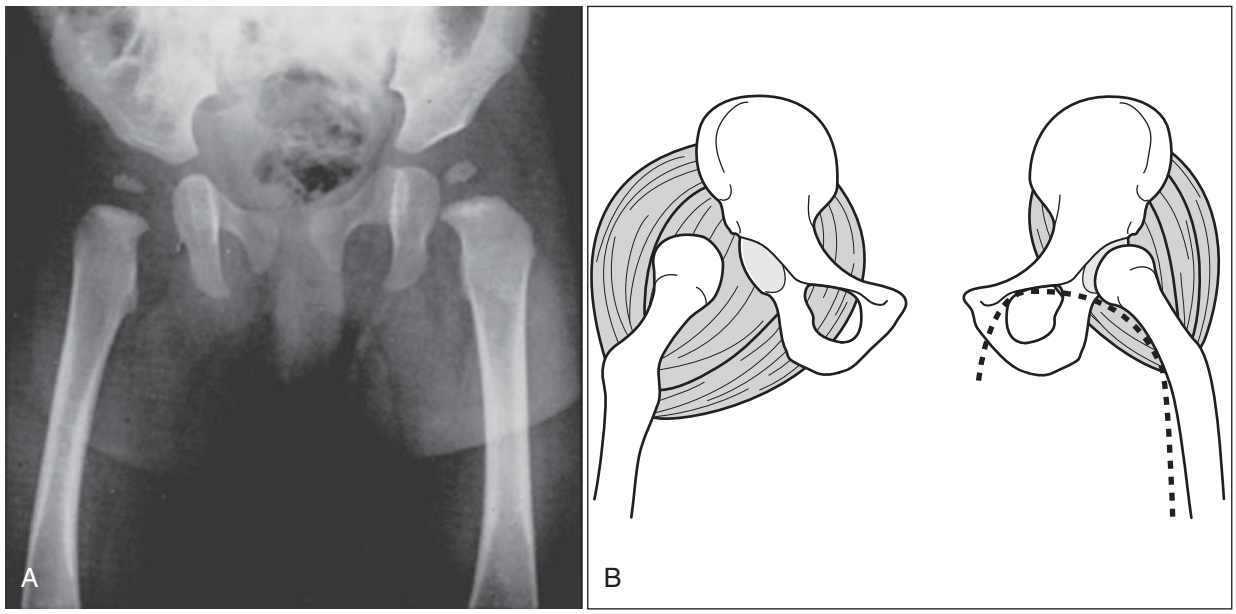

Figure 62–1 **A** and **B**, The obturator sign in the hip is one of the oldest signs of septic arthritis. Another consistent finding is obliteration or lateral displacement of the gluteal fat lines and loss of continuity of the Shenton line. These findings are illustrated radiographically **(A)** and schematically **(B)**.

portal of entry is unknown, and the joints below the hip usually are involved (knee, ankle, and metatarsal). During adolescence, gonococcal arthritis occurs as a manifestation of sepsis with fever, chills, rash, and multiple small joint involvement, often with tenosynovitis.[29] The illness frequently follows the onset of menses by a few days.

RADIOLOGIC FINDINGS

Findings on plain-film radiographs are due to capsular swelling. In the joints readily accessible to physical examination, radiographs add little to the diagnostic evaluation, but when septic arthritis of the hip is suspected in a child, they are a valuable adjunct and may identify other causes of hip pain, such as Legg-Calvé-Perthes disease, slipped capital femoral epiphysis, and fracture. Films of the hip should be made with the child in the frog-leg position and with the legs extended at the knee and slightly internally rotated. The early signs of septic arthritis are caused by swelling of the capsule, which displaces the fat lines. One of the oldest signs is the obturator sign: As the tendon of the obturator internus passes over the capsule of the hip joint, the margins of this muscle are displaced medially into the pelvis (Fig. 62–1).[46] With continued swelling of the hip joint capsule, the femoral head is displaced laterally and upward.[20]

One of the most consistent findings is obliteration or lateral displacement of the gluteal fat lines (see Fig. 62–1).[89] Coincident with filling of the capsule with exudate, the femoral portion of the Shenton line is raised, and its arc is widened.[89] If a technetium bone scan is performed, increased uptake on either side of the joint is seen during the "blood pool" phase of the scan.[86] Ultrasound evaluation has proved useful in evaluation of septic arthritis of the hip.[48,94] In a series of 96 patients, none of the 40 patients with normal ultrasound findings had septic arthritis.[94] Bacterial infections causing pyogenic sacroiliitis may be particularly difficult to diagnose. Bone scans and computed tomography may be negative initially. Magnetic resonance imaging seems to be the diagnostic radiologic method of choice.[2,27]

LABORATORY EVALUATION

Diagnostic evaluation for suspected septic arthritis generally includes determination of the erythrocyte sedimentation rate or C-reactive protein, and the peripheral blood leukocyte count and differential. These may be only mildly elevated in cases of proven infection, however.[26,94] Blood for culture always should be obtained because blood cultures sometimes may yield the pathogen when joint fluid cultures do not.[52]

Joint fluid should be collected in a heparinized syringe so that the large clot that usually forms in fluid obtained from patients with septic arthritis or juvenile rheumatoid arthritis does not preclude enumeration of leukocytes. A cell and differential count should be performed on aspirated joint fluid, and it must be Gram-stained and cultured. Identification of organisms in joint fluid is the primary criterion for diagnosis of septic arthritis. Careful examination of a Gram-stained smear of joint fluid aspirates must be emphasized because joint fluid exerts a bacteriostatic effect on microorganisms and organisms that can be seen but may not grow in culture. Approximately 35 percent of joint aspirates are sterile in patients with other clinical and laboratory findings of a septic joint, including positive blood cultures.[10,63] Joint fluid should be cultured aerobically and under anaerobic conditions. The use of cell lysis culture bottles may enhance the recovery of *K. kingae* and other organisms.[92] *N. gonorrhoeae* is a highly fastidious organism, and oropharyngeal, rectal, and urogenital cultures or detection of gonococcal DNA in urine may be needed to confirm the diagnosis of gonococcal arthritis. Polymerase chain reaction analysis of joint fluid is likely to become a useful adjunct to the diagnosis of culture-negative disease, although it remains investigational.[60,84]

The median synovial fluid leukocyte count in bacterial arthritis in one study was 60.5×10^9 cells/L.[79] In this and other surveys,[75] polymorphonuclear leukocytes accounted for 75 to 90 percent of the white blood cells. Cell density generally is lower in fluid obtained from patients with acute rheumatic fever, juvenile rheumatoid arthritis, and other inflammatory causes of arthritis (Table 62–3).[8,75,79] The glucose concentration often is decreased in septic arthritis, but it may be normal.[79,87] It also may be depressed in rheumatoid arthritis and other conditions. A joint fluid leukocyte density of 5 to 8×10^9 cells/L has been found in joint fluid from patients ultimately proved to have septic arthritis.[75] Minimally turbid fluid with a seemingly low number of cells still should be processed for bacterial culture and Gram stain. Examination of the fluid for uric acid and other types of crystals should be considered in certain children (e.g., children with hyperuricemia).

DIFFERENTIAL DIAGNOSIS

Although bacterial infections are the most common cause of septic arthritis, other microorganisms, including viruses (varicella-zoster, *Erythrovirus* [parvovirus B19], rubella, chikungunya and other togaviruses, variola, vaccinia, certain enteroviruses, others), mycobacteria, and fungi (*Coccidioides immitis*, *Sporothrix schenckii*, *Blastomyces dermatitidis*, *Candida* spp.), may be involved. The differential diagnosis includes obturator internus muscle abscess,[85] epiphyseal osteomyelitis, traumatic arthritis, bacterial endocarditis, villonodular synovitis, leukemia, deep cellulitis, serum sickness, ulcerative colitis, granulomatous colitis, Schönlein-Henoch purpura, traumatic arthritis, fracture, Legg-Calvé-Perthes disease, slipped femoral capital epiphysis, and metabolic diseases affecting joints (e.g., ochronosis in adults with alkaptonuria).

Toxic (or transient) synovitis (also referred to as *irritable hip* and *reactive synovitis*) frequently is seen in children and has milder presenting features than bacterial infections of the hip. The combination of fever, elevated white blood cell count, erythrocyte sedimentation rate (>40 mm/hr), C-reactive protein, and inability to bear weight permits septic arthritis to be distinguished from transient synovitis with high accuracy; greater than 90 percent sensitivity for the detection of septic arthritis has been reported when all of these factors are present.[15,26,52,94] Septic (suppurative) bursitis, although rare in childhood, can be difficult to distinguish from septic arthritis. Children with septic bursitis frequently have a history of recent trauma and fever along with limitation of joint movement.[44,71] Careful physical examination and aspiration of bursal fluid allow the entities to be differentiated.

TREATMENT

ANTIBIOTIC THERAPY

Antibiotic treatment of septic arthritis should target the most common pathogens. In children beyond the neonatal period, *S. aureus* and other gram-positive organisms currently predominate.[13,55,63] Although the risk of acquiring invasive *H. influenzae* infection is low in areas with effective immunization, rare cases continue to be reported. Children younger than 2 years who have not been immunized against *H. influenzae* type b should be treated initially with a regimen that contains an antistaphylococcal agent, such as nafcillin, vancomycin, or clindamycin, and an agent active against this agent (e.g., cefotaxime). Cefuroxime, which is active against *S. aureus* (and other gram-positive pathogens) and *H. influenzae*, is a useful alternative. As with osteomyelitis, vancomycin or clindamycin should be used as the empiric antistaphylococcal agent in areas where community-acquired MRSA is a concern.[3,57] Some authorities advocate that this be done when community-acquired MRSA represents more than 10 percent of *S. aureus* in a geographic area.[50] At present, no regimen is preferred for the treatment of penicillin-nonsusceptible *S. pneumoniae*, but ceftriaxone, cefotaxime, and clindamycin have been used successfully.[14] If β-lactam resistance continues to increase in *S. pneumoniae* and *S. aureus*, other regimens that target highly resistant gram-positive organisms may be needed. Ceftriaxone and cefotaxime are appropriate for the treatment of gonococcal infection.

All antibiotics that have been studied penetrate into joint fluid readily; soon after administration, at the time of peak serum levels, the joint fluid concentration averages 30 percent of the serum value.[5,7,62,73] The efflux of antibiotic from joint fluid back to serum is slow, however. Immediately before the next systemic dose is administered, joint fluid antibiotic concentrations frequently exceed the concentrations present in serum. Antibiotics, whether administered orally or parenterally, can achieve efficacious concentrations in joint fluid.

Injecting antibiotics into the joint space usually is unnecessary because of their excellent penetration. Many antibiotics (e.g., cephalothin) are capable of evoking an intense inflammatory reaction, much as they do if they are infiltrated beneath the skin. Tetzlaff and associates[82] have shown that septic arthritis can be treated for 1 week (or less) parenterally, with the balance of the

TABLE 62–3 Joint Fluid Findings in Childhood Arthritides

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Please refer to the printed publication.

From Ropes, M.: Joint fluid findings in disease. Bull. Rheum. Dis. 7(Suppl.):21, 1957.

drug given orally. These investigators caution that the drug dosage should be adjusted for each patient to ensure a peak serum bactericidal titer of at least 1:8, and that the patient should remain hospitalized so that compliance can be ensured. These investigators considered the minimal duration of therapy to be 3 weeks.

The adequacy of antibiotic therapy can be assessed by serial joint fluid examinations, leukocyte density, and culture results.[87] The time required for resolution of joint symptoms and the time for the synovia to become sterile are proportional to the duration of symptoms before the initiation of appropriate antibiotic therapy.[47] In one study, some patients still had cultures yielding bacteria after undergoing 1 week of therapy. In these patients, cellular density ranged from 25 to 253×10^9 cells/L (mean 109×10^9 cells/L) at the beginning of therapy; 92 percent of the cells were polymorphonuclear leukocytes. By the end of 2 weeks of therapy, all cultures were sterile, and leukocyte density ranged from 4.9 to 23×10^9 cells/L (mean of 12.3×10^9 cells/L).[87] Similar studies of the rate of resolution of indicators of inflammation in joint fluid have suggested that after receiving 9 days of treatment, patients who ultimately recovered completely had a density of 5×10^9 cells/L; however, in patients with recrudescent infection or a poor outcome, the density was 6×10^{10} cells/L.[35]

When an effusion reaccumulates, one should remove it by arthrocentesis, not only to make the patient more comfortable, but also to permit serial assessment of therapy. Generally, disease caused by *S. aureus* and Enterobacteriaceae requires longer treatment than disease caused by *H. influenzae* or meningococci. Radiographs should be obtained during therapy to seek bone changes indicating that osteomyelitis may have been present. Osteomyelitis may require surgical intervention or more prolonged antibiotic therapy.

SURGICAL TREATMENT

In infants, septic arthritis in the hips or shoulders is a surgical emergency; these joints should be drained as soon as the diagnosis is apparent to prevent bony destruction.[69] In a study in adults, the outcome of septic arthritis was better in patients treated by repeated needle aspiration than in patients treated by surgical drainage.[36,37] Eighty percent of the patients treated by needle aspiration were thought to have a good outcome versus 47 percent treated by surgical drainage. All wrist joint infections in this series were treated only by needle aspiration; in treatment of septic arthritis of the knee, however, the outcome was almost equivalent. Similar findings have been reported by others.[11] The investigators suggested that surgical drainage should be performed in any joint whenever the presence of large amounts of fibrin, tissue debris, or loculation prevents adequate drainage by needle aspiration.

PROGNOSIS

Sequelae, including hip dislocation, are more likely to develop in infants and patients with symptoms of longer duration.[87,88] In one study, seven of eight patients in whom "permanent" hip dislocation developed had symptoms for 7 or more days before receiving treatment.[88] Similarly, all patients with spontaneous ankylosis had symptoms longer than 7 days.[88] Comparable data were obtained by Samilson and colleagues.[77] Eight of 10 patients with pathologic dislocation of the hip had symptoms for longer than 7 days.

A detailed analysis of factors affecting satisfactory outcomes in hematogenous septic arthritis of childhood showed no significant difference in the joint when arthrotomy, with or without irrigation, was compared with repeated aspiration.[59] Likewise, the specific antibiotic used had no effect on outcome as long as it was effective against the infecting organism. The literature does suggest that septic arthritis caused by Enterobacteriaceae is associated with more frequent sequelae than when caused by other pathogens.[37,64] *S. aureus* is more likely to cause sequelae than *H. influenzae*.[10] A single randomized, placebo-controlled study has examined the impact of a brief course of dexamethasone on the outcome of bacteriologically confirmed cases of septic arthritis of hematogenous origin in children. Children younger than 3 months were excluded from participation, and *H. influenzae* was responsible for 13 percent of cases among the 100 evaluable patients. After 1 year of follow-up, limping, joint pain, and restriction of movement were found in 26 percent of patients who received a placebo, but in only 1 of 50 patients treated with dexamethasone. If confirmed, this anti-inflammatory therapy may prove useful to limit the sequelae of septic arthritis.[70]

SPECIAL PROBLEMS

NEONATAL SEPTIC ARTHRITIS

Neonatal septic arthritis is a problem that warrants special attention because of its subtle signs and symptoms,[21] its potential for catastrophic consequences of untreated disease,[68] and the unusual organisms occasionally seen. Any newborn who has swelling in the region of the thigh and the buttock and holds that leg flexed with slight abduction and external rotation at the hip should be suspected to have femoral-acetabular septic arthritis. It can occur 1 to 28 days after femoral venipuncture (in most newborns, 5 to 9 days) and should not be confused with femoral vein thrombosis.[4,17]

In most newborns, no toxemia, fever, or leukocytosis is present. More than one joint may be involved when initially evaluated. The progression of disease in newborns can be so indolent that the hip spontaneously drains along the obturator internus, and the condition is manifested as a lower abdominal mass just above the inguinal canal.[28] Problems in recognizing the disease in newborns undoubtedly contribute to the poor outcome. In one series, the delay from onset to diagnosis was an average of 1 week; only two of the nine infants in this series had a normal hip examination at follow-up.[68]

In most series, the causative agents are staphylococci and streptococci,[4,12,17,21,68] but gram-negative organisms often are found. More importantly, arthritis caused by *Candida albicans* has been described,[23] and the gonococcus should not be forgotten. In gonococcal arthritis, the symptoms, which usually become apparent in infants 1 to 5 weeks of age, generally are polyarthritic (more than one joint involved).[33] As previously noted, other symptoms of neonatal gonococcal arthritis are no different from symptoms caused by other pathogens.

Initial antibiotic therapy for neonatal septic arthritis should be directed toward *S. aureus* and the nosocomial gram-negative bacteria that are prevalent in the nursery. Antibiotic therapy can be altered when the susceptibility of the causative bacterium is known. As noted earlier, the usual duration of therapy is 3 to 4 weeks, and radiography should be performed toward the end of treatment. Oral therapy has been used successfully to complete treatment of septic arthritis in newborns,[72] but absorption of antibiotics in this age range is unpredictable.[78] Consequently, either intravenous therapy or measurement of serum bactericidal activity should be used to verify absorption.[82]

FUNGAL ARTHRITIS

Fungal arthritis is rare in children, but may be seen with pathogens of endemic mycoses, such as *Histoplasma capsulatum*, *C. immitis*, and *B. dermatitidis*, and with opportunistic fungal pathogens (e.g., *Candida* spp., *Cryptococcus neoformans*).[55,63] These pathogens usually manifest after an indolent course. The recom-

mended medical treatment generally is that required for other systemic infections caused by these organisms.

JOINT INFECTIONS DURING RHEUMATOID ARTHRITIS

Joint infections that develop during a case of rheumatoid arthritis seem to occur more frequently in adults with rheumatoid arthritis than in children. In one series, only 2 of 17 patients were younger than 10 years.[6] The notable features are that the hips generally are not involved, and infection of multiple joints (17 of 44 patients had more than one joint involved) frequently occurs. Because of the preexisting joint disease, the diagnosis often is delayed, and the outcome usually is poor. Septic arthritis should be considered if an unusual worsening of one joint occurs during a flare-up of rheumatoid arthritis.

REACTIVE ARTHRITIS

After infections with *Shigella* spp.,[16,66] *Chlamydia trachomatis*, *Salmonella* spp.,[43] and *Yersinia* spp., a reactive arthritis can occur.[54] This postinfectious joint inflammation seems to develop with greater frequency in individuals who express the histocompatibility antigen HLA-B*27, perhaps as a result of molecular similarities between bacterial antigens and the human protein. In addition, during the initial infection, bacterial antigens apparently may be deposited in the synovium and may persist for a long time and lead to pathogenic inflammation.[40,54] Generally, the onset of joint symptoms occurs a few days to several weeks after a transient and often mild episode of diarrhea. The arthritis may mimic rheumatic fever and is characterized by daily low-grade fever and an increased erythrocyte sedimentation rate. Reactive arthritis generally can be distinguished from septic arthritis by analysis of synovial fluid; infectious arthritis usually is associated with high white blood cell fluid numbers.[8] Resolution of joint symptoms in reactive cases takes 7 to 10 days.

Reactive arthritis also may occur after acute infections with *N. meningitidis*[42] and *H. influenzae*.[76] Symptoms and signs of joint inflammation often appear 1 week or more after the acute septic episode and resolve without sequelae.

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CHAPTER 63

Bacterial Myositis and Pyomyositis

Charles Grose

Myositis is not a common manifestation of bacterial infection, but when it occurs, the consequences to the patient may be severe or even fatal. *Staphylococcus aureus* and group A streptococci are the most likely causative organisms. Myositis also has been associated with several other infectious agents, including viruses, fungi, and parasites. These pathogens are listed in Table 63–1; they are discussed briefly herein and more thoroughly in the chapters on the specific microorganisms. This chapter focuses on two forms of pyogenic myositis, designated as *acute bacterial myositis* and *tropical pyomyositis*. The former is caused primarily by group A streptococci and the latter by *S. aureus*. Tropical (or staphylococcal) pyomyositis is the more common of the two bacterial diseases and should be considered a distinct nosologic entity.

TABLE 63–1 Infectious Causes of Myositis

Bacterial
Tropical pyomyositis
Acute bacterial myositis
Viral
Influenza myositis
Coxsackievirus myositis
Fungal
Disseminated candidiasis
Parasitic
Trichinosis
Cysticercosis
Toxoplasmosis

PYOMYOSITIS

The pathologic entity termed *spontaneous acute myositis* was recognized by Virchow in the mid-19th century, but the first clinical description of suppurative myositis generally is attributed to the Japanese surgeon Scriba.[20] In 1904, another Japanese surgeon, Miyake,[17] extensively reviewed the subject of skeletal muscle abscesses and added 33 more cases. As the British and French expanded their colonial empires at the turn of the 20th century, the disease was recognized with increasing frequency in the native populations and in the soldiers who lived in the tropical areas of Asia and Africa.[26] It acquired the name by which it now is known widely—*tropical pyomyositis*.[9,10]

The suitability of this designation was confirmed by an epidemiologic study in East Africa, which discovered that the disease was found commonly only in regions with a truly tropical climate (i.e., a fairly constant high temperature and high relative humidity) at an altitude below 4000 ft.[15] Pyomyositis has been described, however, in children from geographic regions of the United States as diverse as New England,[9] northern California,[2] Iowa,[16] and Texas.[11,19,21] Numerous reported cases within the continental United States have occurred in and around San Antonio, Texas.[5] In a 10-year chart review, 1 or 2 cases of pyomyositis per 4000 pediatric admissions occurred annually. In contrast, a review of consultations of pediatric infectious diseases at the University of Iowa Hospital disclosed fewer cases of pyomyositis among children younger than 16 years old.[7] Pyomyositis seems to occur more commonly in children who live in the southernmost regions of the United States (e.g., Texas) than in children who live in the northern regions (e.g., Iowa).

PATHOPHYSIOLOGY

The etiologic agent of the skeletal muscle abscesses in more than 90 percent of cases is *S. aureus*. Phage typing of many isolates in different countries has not identified a particular staphylococcal strain that is more likely to cause pyomyositis.[11] The second most common bacteriologic isolate is *Streptococcus*, including group A and nonhemolytic strains. Whether more virulent streptococcal infections are occurring at the beginning of the 21st century is an issue that remains unresolved.

Miyake[17] studied extensively the experimental conditions under which staphylococci cause muscle abscesses. When healthy rabbits were given boluses of staphylococci intravenously, they occasionally developed small abscesses in the kidney, liver, or spleen but never in the skeletal muscles. When specific muscles were damaged by mechanical pinching or electric current 24 or 48 hours before the intravenous injection of bacteria was administered, small abscesses developed within 2 to 28 days at some of the injured sites in nearly half of the animals. Abscesses were not found in healthy muscle tissue.

The role of trauma was supported further by a study of pyomyositis in the British Army.[3] After physicians found this disease to be a common problem in Gurkha army recruits, they investigated 32 cases and made several observations: Two thirds of the men recalled having experienced trauma at the affected site, the incidence of abscesses increased as the severity of physical training increased, and the abscesses occurred three times more commonly on the dominant (right) side of the body. In an analysis of 78 cases in Uganda, abscesses also were found more commonly on the right side of the body.[15]

From experimental evidence and clinical observations, two conditions commonly are found when pyomyositis occurs: muscle injury and bacteremia, usually staphylococcal. A reported case is illustrative.[11] A 12-year-old girl caught her left foot in the wheel of a moving bicycle and tumbled to the ground. One week later, she developed a furuncle of the foot, and within the next 2 weeks, she developed painful lumps in muscles of the thigh, shoulder, and chest wall (which had been injured during the original accident). Cultures from the furuncle and blood and from the incised muscle abscesses grew *S. aureus*. All isolates were identified as phage type 94. The initial episode of trauma resulted in a staphylococcal skin lesion and, presumably, a bacteremia that seeded sites of previously bruised muscle.

Seven children with pyomyositis are described in Table 63–2. An analysis of all seven cases illustrates the association of pyomyositis with trauma. The sources of muscle trauma have ranged from bicycle accidents to strenuous aerobic exercises. These cases also may explain the predilection of the disease to occur in warmer climates; concomitant skin infections and muscle trauma are more likely to occur in a climate in which children can play or work outside wearing fewer clothes for most of the year.

In tropical countries, pyomyositis is said to occur in individuals who are malnourished and who have multiple parasitic infections. This association has not been confirmed, however, in children with pyomyositis seen in the United States or Australia.[5,7,13] The children have not been malnourished or vitamin-deficient, and they have not had parasitic infestation or marked eosinophilia. Extensive immunologic evaluations also have been normal; the tests included quantitative immunoglobulins, enumeration of T-lymphocyte subpopulations, total hemolytic complement levels, and leukocyte function as tested by reduction of nitroblue tetrazolium.

CLINICAL PRESENTATION

Pyomyositis often is considered a disease of adolescents and young adults, even though it occurs in individuals of all ages, including infants and young children.[2,6,13] Boys are affected more

TABLE 63–2 Pyomyositis and Trauma

Case*	Sex	Age (yr)	Source of Trauma	Circumstances of Trauma	Extent of Disease
1	F	12	Bicycle accident	Thrown from bicycle onto street after foot was caught in the wheel	Right deltoid/right chest wall/left thigh/right groin
2	M	3	Fall while running	Fell while running on street	Left calf/right scapula/right buttock
3	M	11	Hay bale accident	Struck in abdomen by bale of hay thrown from a hay baler	Abdominal wall musculature
4	M	6	Blunt trauma to abdomen	Struck in abdomen during mock fistfight with sibling	Abdominal wall musculature
5	F	17	Aerobic exercises	Injured while instructing others in aerobic exercises	Left thigh
6	M	7	Bicycle accident	Fell from fast-moving bicycle onto street	Left calf
7	F	13	Volleyball accident	Fell several times diving for volleyball during training exercises	Left iliopsoas

**Cases 1 and 2 from reference 11, cases 3 and 4 from reference 5, cases 5 and 6 from reference 7, and case 7 from reference 16.*

often than are girls. As more girls enter competitive sporting activities, however, pyomyositis is being reported in female athletes.[16] Most children with pyomyositis have a solitary lesion, but multiple lesions are common findings. The most common site of abscess formation is the thigh, followed by the calf, buttock, arm, scapula, and chest wall. The muscle lesions are firm or "woody" to palpation, with a well-defined border. The sign of fluctuation may be difficult to elicit. Erythema and warmth often are not apparent because of the deep location of the masses, although diffuse tenderness usually occurs. When a muscle in an extremity is involved, the entire limb may be swollen. Occasionally, pyomyositis also can occur in muscles of the pelvis, in which case pain may be transferred to the hip.[14,19]

In case reports with a clinical history, children with pyomyositis often had similar presenting complaints.[7,11] Many had incurred a recent accidental injury (often involving a leg) that usually was not considered serious. After a few days, the children developed low-grade fever (38.3° C to 39° C), muscle pain, and, occasionally, an impaired gait. These symptoms persisted a few days to a few weeks until a mass appeared. When first examined, many of the patients were considered to have only a contusion or a hematoma; occasionally, a child was diagnosed as having a rhabdomyosarcoma. Although the disease usually occurs in individuals who are otherwise healthy, pyomyositis has been reported in patients with malignancy. Pyomyositis also may develop in children with acquired immunodeficiency syndrome or other immunodeficiency.[13] The pathophysiology of pyogenic muscle abscess may not be the same, however, in immunodeficient individuals with increased susceptibility to bacterial infection. Most children with pyomyositis have no definable immunologic abnormalities.

An unusual clinical presentation is acute abdominal pain. Beck and Grose[5] described two children with pyomyositis whose initial complaints were confined to the abdominal wall. One patient, a 6-year-old child, had been struck in the abdomen in a mock fist-fight with an older sibling. One week later, he developed a low-grade fever and began to walk with a stoop; after another week, his mother detected a "knot" in his right mid-abdominal wall. The second case involved the 11-year-old son of a rancher; the boy was struck in the abdomen by a bale of hay tossed from a hay baler. When he subsequently developed symptoms of abdominal pain, the diagnosis of appendicitis was entertained. When a mass later became palpable in his abdominal wall, rhabdomyosarcoma was suspected. A correct diagnosis was made after the use of scintigraphy and sonography, as described later.[5] A review from Nigeria found muscle abscesses in the anterior abdominal wall to be common.[2]

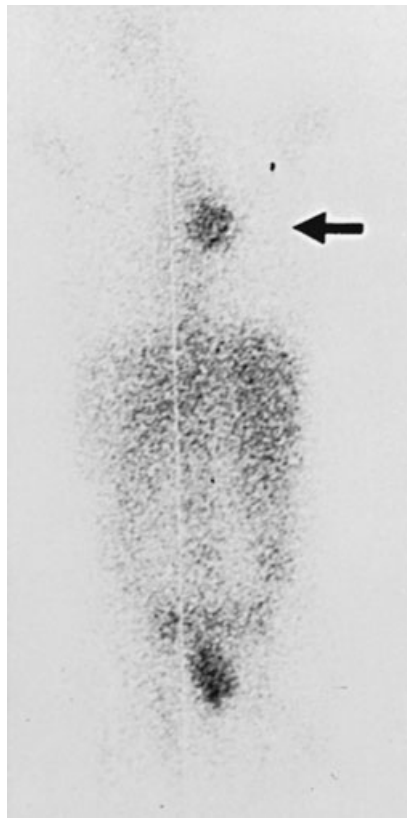

Figure 63–1 Scintigram of a patient with pyomyositis. Posterior gallium 67 citrate scan shows abnormally high uptake over the right scapula (*arrow*), where an abscess cavity was located within the muscle. Increased radioactivity also is observed in the bladder, which is a normal finding.

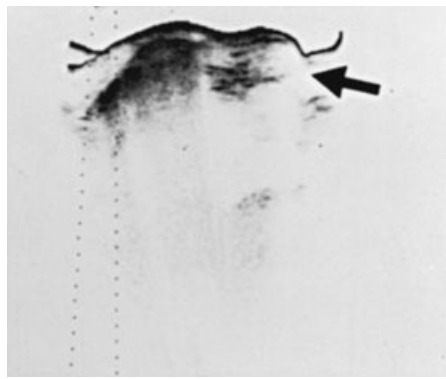

Figure 63–2 Sonogram of the abdominal wall of a patient with pyomyositis. The transverse view of the abdomen shows an abscess cavity in the right belly of the rectus abdominis muscle (*arrow*).

DIAGNOSIS

The diagnosis of pyomyositis should be considered in any child with fever and muscle pain, especially if a recent history of trauma exists. When a child has visible masses at commonly involved sites, such as the thigh, the diagnosis of pyomyositis can be made by needle aspiration of a mass. If a febrile child complains of myalgia in an extremity but has no palpable masses, the differential diagnosis must include more common inflammatory and infectious conditions of the bone or joint. A definitive diagnosis usually depends on one or more radiologic procedures. Plain films may show a soft tissue swelling or even a widened fascial plane suggestive of a mass lesion. A combination of plain radiography and radionuclide (technetium 99m phosphate) bone scintigraphy often can exclude osteomyelitis and pyoarthritis. If the diagnosis still is in question, scanning with gallium or indium can localize a muscle abscess precisely and can visualize other intramuscular abscesses too small to palpate (Fig. 63–1). Alternatively, ultrasonography can detect muscle abscesses and may be preferable as an initial procedure because it avoids the radiation exposure from computed tomography (CT) or scintigrams (Fig. 63–2).[27]

Magnetic resonance imaging (MRI) is very helpful in delineating the extent of a muscle abscess. In many cases, the abscess is much larger than suspected by physical symptoms and signs. The MRI scans of an illustrative case are presented in Figure 63–3. The patient was a 17-year-old girl with a swollen left lower thigh. She worked part-time as an attendant in an athletic club, where she participated in some of the vigorous exercise programs. Radiographs of the knee were normal, whereas a technetium

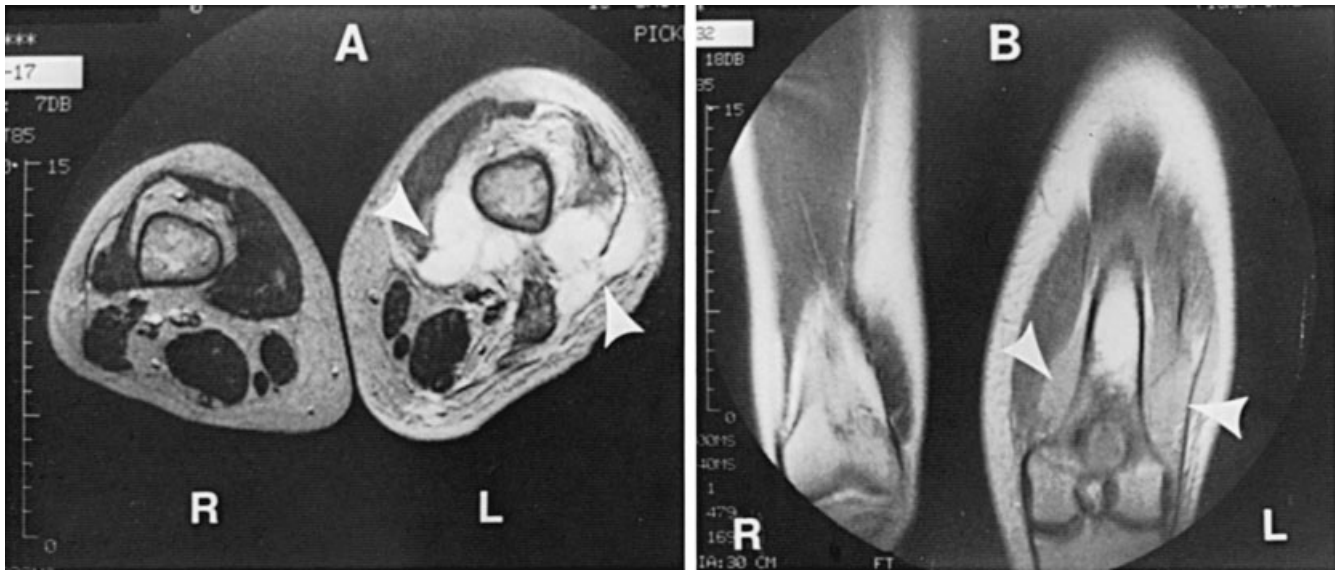

Figure 63–3 Magnetic resonance imaging of the thighs of a patient with pyomyositis. **A,** Axial images of the right (*R*) and left (*L*) thighs. **B,** Coronal images around the distal femoral shafts and femoral condyles. The scans show several well-defined areas of extremely high intensity in the muscle bundles and fascial planes from the middle to distal left femur (*arrows*). The high signal intensity suggests a fluid collection (e.g., an abscess), rather than a neoplastic process. Edema of the subcutaneous tissues also is evident in the lateral aspect of the swollen left thigh. There are no abnormal signals within the bone.

bone scan showed slightly increased radionuclide uptake in the soft tissues around the distal left femur. MRI of the left and right thighs showed a large fluid collection extending from the middle to distal left femur. Surgical exploration identified extensive abscess formation around the posterior aspect of the femur; the culture grew *S. aureus*. The affected muscle groups included the vastus medialis, vastus intermedius, vastus lateralis, and biceps femoris. The bone was not involved. For the reasons described, MRI has become the preferred procedure for establishing the diagnosis of pyomyositis.[14,22,27]

TREATMENT

Because pyomyositis is an abscess of skeletal muscle, the treatment is surgical incision and drainage.[2] Several physicians have seen that smaller lesions resolve spontaneously, even before the antibiotic era. An important role for systemic antibiotics is to prevent the formation of further muscle abscesses, especially in a patient with proven bacteremia. Because *S. aureus* is the most likely agent, a semisynthetic penicillinase-resistant penicillin is the preferred antibiotic in communities with little or no methicillin-resistant *S. aureus* (MRSA). Usually, nafcillin or oxacillin is administered intravenously every 6 hours at a total daily dosage of 150 to 200 mg/kg. When pyomyositis occurs in a patient with penicillin hypersensitivity, clindamycin, 40 mg/kg/day, divided every 8 hours, can be substituted because of its excellent coverage of gram-positive cocci. Parenteral therapy is continued until clinical improvement is evident, usually within a few days after surgical drainage. Thereafter, the antibiotics can be given orally at a reduced dosage for an additional 2 to 3 weeks. A penicillin derivative (dicloxacillin, 25 mg/kg/day, or cefadroxil, 30 mg/kg/day) or clindamycin, 20 to 30 mg/kg/day, is acceptable.

In many communities, MRSA is now a common agent. In a clinical analysis of 182 patients with pyomyositis from Texas Children's Hospital in Houston, Texas, more than 50 percent of the isolated *S. aureus* strains were MRSA.[19] In these communities, initial antibiotic management must include either intravenous clindamycin or vancomycin, 40 mg/kg/day, divided every 6 hours. Clindamycin can be continued as an oral antibiotic. For MRSA isolates resistant to clindamycin, treatment with intravenous vancomycin can be followed by administration of oral linezolid, 30 mg/kg/day, divided every 8 hours, for children younger than 12 years; for children 12 years and older, 20 mg/kg/day, divided every 12 hours, is given. Therapy of MRSA infection may require a longer duration, from 3 to 4 weeks. Before discontinuing antibiotics, the patient should have documented normal levels of C-reactive protein and erythrocyte sedimentation rate.

ACUTE BACTERIAL MYOSITIS

Far less common than tropical pyomyositis is acute bacterial myositis, usually caused by group A streptococci. In this condition, the bacterial infection often is not confined to distinct abscesses within the muscle, but instead it extends diffusely through one or more muscle groups. As with pyomyositis, the disease occurs more commonly in males than in females and usually is associated with prior physical exertion and perhaps minor trauma. In contrast to pyomyositis, most published cases of acute bacterial myositis have occurred in adults and not in children.[4] The disease has been divided by Svane[25] into the following four main types:

1. A malignant form with septicemia and a uniformly fatal outcome
2. An acute form with a more protracted clinical course and other foci of suppuration, such as the bones, joints, or viscera
3. A subacute form with a better clinical prognosis
4. A benign type associated with more distinct muscle abscesses

The benign type of acute bacterial myositis by the Svane classification is the same disease process as described in the preceding section on pyomyositis.

CLINICAL PRESENTATION

The main clinical difference between the acute myositis syndrome and the pyomyositis syndrome is the virulence of the

former disease, especially the malignant type. A case in a San Antonio, Texas, native is illustrative. The patient was a 23-year-old jackhammer operator who was admitted because of high fever, malaise, and pains in his arms. On examination, both upper extremities were tender to palpation, and faint erythema was visible over the same areas. He soon became incoherent, and his general condition quickly deteriorated. Death followed within 24 hours. Blood cultures drawn before death grew group A streptococci, and cultures of the biceps muscles obtained at autopsy grew the same organism. This case is similar to others described in the literature.[4,25] The patient worked as a jackhammer operator, who presumably would incur considerable minor trauma to the muscles of the upper arms and shoulders. Two reported cases of streptococcal myositis in children involved the left thigh and the left paravertebral muscles.[18] The first child developed septic shock and required intensive care for 27 days before a definitive diagnosis was made by CT.

A case of generalized myositis with staphylococcal septicemia has been described in a 15-year-old boy.[1] The patient presented with signs of fever and very diffuse muscle swelling and tenderness. All muscle groups of the extremities appeared to be inflamed and markedly tender; the creatine phosphokinase enzyme levels were markedly elevated. Culture of a muscle biopsy specimen grew *S. aureus*, as did previously drawn blood cultures. The patient experienced a stormy hospital course with severe hypotension and shock syndrome, requiring treatment with intravenous fluids, corticosteroids, dopamine, heparin, and assisted ventilation. He also received a total of 4 weeks of antistaphylococcal antibiotic therapy.

DIAGNOSIS

Blood cultures are positive most often in patients with the most significant clinical symptoms. Cultures of muscle biopsy specimens may yield a profuse growth of bacteria. Rarely, myositis has occurred as a delayed complication of chickenpox, often together with fasciitis.[12,17,23] In this situation, bacteremia may not be documented because the bacterial process usually is the result of contiguous spread from a secondarily infected pock lesion. These streptococcal complications of chickenpox often occur in an extremity, which may become swollen and painful. Under these circumstances, MRI can be an extremely valuable diagnostic tool to gauge the depth and extent of inflammation.[27]

TREATMENT AND THE EAGLE EFFECT

The treatment of acute streptococcal or staphylococcal myositis of the malignant type is a medical emergency. As soon as the diagnosis is suspected, bacteriologic cultures should be obtained, and intravenous therapy should be initiated with a high-dose semisynthetic penicillin (e.g., nafcillin, 50 mg/kg every 6 hours) and vancomycin. If the cultures yield group A streptococci rather than *Staphylococcus*, the antibiotic can be switched to penicillin G at an equivalent high dosage. Surgical consultation is required to evaluate the need for débridement and drainage. Obtaining MRI scans may be advisable to document the extent of the disease and the response to antibiotic therapy. In severe cases of bacterial myositis, the total duration of hospitalization can exceed 4 weeks.[1,18]

The apparent resurgence of serious streptococcal infection has led to an increased interest in what has been called the *Harry Eagle effect*, named after the scientist who first described the failure of penicillin to eradicate group A streptococcal infection in a mouse model.[8] Eagle inoculated mice intramuscularly with group A streptococci and observed a markedly retarded bactericidal action of penicillin on organisms in the older abscesses, even though the bacteria remained highly sensitive to the antibiotic. Even massive doses of penicillin 10,000 times greater than the minimum inhibitory concentration were not effective at eradicating the bacteria. Subsequent studies by other investigators confirmed the existence of the Eagle effect and disclosed that clindamycin showed superior efficacy to penicillin in treatment of streptococcal myositis in the mouse model.[24] In an editorial comment, Stevens[23] observed that penicillin most likely fails to kill stationary-phase streptococci in muscle infections, an explanation for the Eagle effect. He suggested that streptococcal myositis or fasciitis be treated with intravenous clindamycin, 40 mg/kg/day, if the condition fails to respond to penicillin therapy.

MISCELLANEOUS CAUSES OF MYOSITIS

Viral infection occasionally leads to myositis (see Table 63–1). The most common example undoubtedly is myositis associated with influenza virus infection in children. Typically, school-aged children are affected. Muscle groups of the legs often are involved, associated with a marked elevation of serum creatine phosphokinase. Clinical symptoms generally last 2 to 4 days. Another, far less common myositis is caused by coxsackievirus B infection. This disease is called *epidemic pleurodynia* because the myositis frequently involves the chest and upper abdomen. The enteroviral infection is known as *Bornholm disease*, named after the Danish island where it was first described.

Fungal infection (e.g., *Candida albicans*) can cause myositis. Candidal myositis is uncommon, however, and usually occurs in patients who are severely immunocompromised. Frequently, other sites also are involved by the disseminated fungal infection in addition to muscle.

Parasitic infection can cause myositis. The best known is trichinosis, caused by ingestion of the encysted larvae of *Trichinella spiralis*. The larvae typically are found in products containing pork and wild game, such as bear meat. The first signs of illness are fever and abdominal pain. Later, muscle pain is noted in the neck and chest. Eventually, calcifications form in the involved muscles. Treatment of the acute disease with mebendazole or albendazole may be beneficial. Another parasite more common in Central and South America is *Taenia solium*. Ingestion of eggs of this pork tapeworm leads to cysticercosis, with calcified densities in skeletal muscle. Finally, toxoplasmosis infection can lead to symptoms of myositis. During acute infection, *Toxoplasma gondii* can encyst in the skeletal muscle and lead to an inflammatory myopathy closely resembling dermatomyositis.

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SECTION X

SKIN INFECTIONS

CHAPTER 64

CUTANEOUS MANIFESTATIONS OF SYSTEMIC INFECTIONS

James D. Cherry

Many illnesses caused by infectious agents have associated cutaneous manifestations. In some cases, the exanthem may be the hallmark of the disease; in others, it may be only a vague indicator of a more significant underlying process. When an exanthem occurs, it often offers important clues to the etiology of a patient's illness. Although most exanthematous illnesses in children are benign, their differential diagnosis is critical because the early manifestations of potentially fatal bacterial and rickettsial diseases frequently have cutaneous findings.

HISTORY

Exanthematous manifestations of infectious illnesses have been important since medical antiquity. Major epidemics of both measles and smallpox occurred in the Roman Empire and in China at the beginning of the Christian era.[25,130] Scarlet fever was recognized as a distinct entity in the 17th century, and chickenpox and rubella were identified in the 18th and 19th centuries, respectively.[49]

In the writings of the early 20th century, maculopapular exanthematous illnesses of children frequently were referred to by number. Scarlet fever and measles historically were the first two classic maculopapular exanthems of childhood. Which one had the honor of being the "first disease" is unknown today. The "third disease" was rubella, which was recognized by the beginning of the 20th century as a distinct entity.[68,70,87,157,178-180] In 1900, Dukes[68] described an exanthematous illness with the characteristics of both rubella and scarlet fever, which he suggested was a "fourth disease." The general opinion today is that his disease was not a distinct entity. Shaw[180] suggested that Dukes' cases had mild atypical scarlet fever, and Powell[157] raised the possibility that the illness resulted from epidermolytic toxin–producing staphylococci. Most probably, rubella and scarlet fever both were epidemic in the student population under Dr. Dukes' care; combined infections led to the confusion.

Erythema infectiosum (see Chapter 164) commonly is referred to as the *fifth disease*, and roseola infantum (see Chapter 65) qualifies as the *sixth disease*.[179]

During the last 55 years, interest in exanthematous diseases has been renewed because a large number of previously unknown viruses and other infectious agents that cause cutaneous manifestations have been discovered. In addition, the pattern of disease caused by classic exanthem-producing agents has changed; smallpox has been eradicated, the epidemiology of measles and rubella has been altered by immunization, and ecologic changes have resulted in differences in viral and bacterially induced rashes.

ETIOLOGIC AGENTS

Many different types of viruses, chlamydiae, rickettsiae, mycoplasmas, bacteria, fungi, and protozoan and metazoan agents cause illnesses with associated cutaneous manifestations. Although this chapter is devoted to systemic infectious diseases with cutaneous manifestations, the demarcation between exanthematous disease of systemic and local origin is not always readily apparent. For example, the recurrent cold sore caused by herpes simplex virus (HSV) infection frequently is considered a local problem, although its nature and pathogenesis involve central virus latency and host systemic immune functions. Similarly, superficial fungal diseases and other local infections, such as warts, may be quite dependent on more general immunologic functions of the host. The exanthems of enteroviral infections frequently are confused with those caused by insect bites and allergic problems.

Table 64–1 presents viruses that have cutaneous manifestations in humans. Erythema infectiosum is caused by human parvovirus B19.[7,205] This virus also is an important cause of the papular-purpuric gloves and socks syndrome that is an uncommon occurrence and mainly affects young adults.[3,6,50,85,91,184,185] Human parvovirus B19 also has been associated with a vesiculopustular exanthem, erythema multiforme, and other petechial and purpuric rashes. In one study, an erythematous maculopapular rash was noted in 9 percent of children with human bocavirus infections.[8] Adenovirus types 1, 2, 3, 4, 7, and 7a have been isolated from children and young adults with exanthem.[49,110,208,209] The overall clinical expression rate of exanthem in adenovirus infection rarely has been studied. Fukumi and associates[79] noted that rash occurred in 2 percent of adenoviral infections; Hope-Simpson and Higgins[98] indicated a rate of approximately 8 percent.

Eight species in the *Herpesvirus* genus have cutaneous manifestations associated with infection, but clinical expression rates vary greatly. Nearly all primary varicella infections are associated with exanthem, whereas exanthem with acquired cytomegalovirus infection is a rare manifestation.[19,22,49,166,190,208] The incidence of exanthem in Epstein-Barr virus infection varies from 3 percent to nearly 100 percent, depending on whether concomitant ampicillin is administered.[16,28,95,104,111,146,159,197,198] Although firm data are lacking, probably less than 10 percent of primary infections with HSV type 1 are associated with cutaneous manifestations. Erythema multiforme occasionally occurs with recurrent HSV infections.[36,76,107,144] Human herpesvirus–6 (HHV-6) is a major cause of roseola infantum.[11,205,217] HHV-7 also is a cause of roseola infantum[10]; in addition, some evidence suggests that this virus may play a role in pityriasis rosea.[66] HHV-8 infection is necessary

Text continued on p. 762

TABLE 64–1 Clinical Characteristics of Viral Infections with Cutaneous Manifestations

Virus	Disease or Syndrome	Incubation Period (days)	Main Season	Clinical Characteristics	Exanthem		Usual Duration (days)
					Lesions	Distribution	
Human parvovirus B19 (see Figs. 64–6 to 64–8)	Erythema infectiosum; gloves and socks syndrome	7-17	Winter and spring	Biphasic illness with mild prodromal period with headache and malaise for 2-3 days, then 7-day symptom-free period, followed by typical exanthema	Three-stage exanthema: initially, rash on cheeks (slapped-cheek appearance) and then erythematous maculopapular rash on trunk and limbs; finally, rash develops a reticular pattern	Starts on face More prominent on extensor surfaces of extremities	7-21
Human bocavirus			Fall, winter, and spring	Fever, cough, coryza, respiratory distress (bronchitis, bronchiolitis, pneumonia)	Erythematous maculopapular	Mainly face, chest, and trunk	
Human papillomaviruses	Warts		Nonseasonal	Local cutaneous disease	Papular or nodular isolated lesions	Most common on extremities	100+
Adenovirus types 1, 2, 3, 4, 7, and 7a		6-9	Winter and spring	Fever and signs and symptoms of respiratory illness Occasionally, rash occurs after defervescence (roseola-like)	Most commonly erythematous, maculopapular, and discrete (rubelliform), but occasionally confluent (morbilliform) Rarely, erythema multiforme and Stevens-Johnson syndrome	Usually starts on face and spreads downward to trunk and extremities	3-5
Herpes simplex types 1 and 2 (see Fig. 64–5)	Cold sores, genital herpes, neonatal herpes, or other	2-12	Nonseasonal	Primary disease associated with fever and systemic symptoms Recurrent disease caused by exogenous and endogenous infections	Singular or grouped vesicular lesions varying in size from 2 to 10 mm, frequently on a mildly erythematous base Occasionally, erythema multiforme, Stevens-Johnson syndrome, and erythema nodosum	Lesions in primary infection with type 1 virus are mainly in and around the mouth Recurrent type 1 lesions usually perioral Primary and recurrent type 2 lesions usually on genitals	7-14
Human herpesvirus–6 (HHV-6)	Roseola infantum		Nonseasonal	Fever 3-5 days in duration, rapid defervescence, and then the appearance of rash	Erythematous macular or maculopapular	Most prominent on neck and trunk Face and extremities may be affected	1-2
Human herpesvirus–7 (HHV-7)	Roseola infantum		Nonseasonal	Fever 3-5 days in duration, rapid defervescence, and then the appearance of rash	Erythematous macular or maculopapular	Most prominent on neck and trunk Face and extremities may be affected	1-2
Human herpesvirus–8 (HHV-8)	Kaposi sarcoma	Months to years	Nonseasonal	Asymptomatic infection Most commonly noted in AIDS patients but occurs in other immunodeficiency states	Purple to blue nodular, raised lesions	Any epidermal or mucosal surface	Months to years
Varicella-zoster (see Fig. 64–4)	Chickenpox (varicella)	12-20	Late fall, winter, and spring	Malaise and fever of 5-6 days duration	Basic lesion is vesicular, but lesions go through stages: macules, papules, vesicles, and crusts Lesions occur in crops	Lesions more profuse on trunk than on extremities Proximal end of extremities more involved than distal end	8-10
	Herpes zoster		Nonseasonal	Endogenous infection Pain and paresthesia with dermatome distribution	Basic lesion is vesicular, but lesions go through stages: macules, papules, vesicles, and crusts	Lesions localized to area of skin innervated by a single sensory ganglion	10-28

Epstein-Barr	Infectious mononucleosis	28-49	Nonseasonal	Fever, pharyngitis, and lymphadenopathy Exanthem occurs in 3-13% of cases If ampicillin is administered, then exanthema in 50% of cases	Most commonly erythematous, macular, maculopapular, and discrete (rubelliform) In association with ampicillin administration, the rash may be more vivid Erythema multiforme and urticaria may occur	Mainly on trunk and proximal end of extremities	2-7
Cytomegalovirus	Cytomegalovirus mononucleosis		Nonseasonal	Acquired: mild febrile illness with lymphadenopathy Congenital: disseminated disease	Erythematous, maculopapular, and discrete Vesicular or petechial in congenital infection	Located mainly on trunk and proximal end of extremities	2-7
Vaccinia	Roseola vaccinatum, eczema vaccinatum, vaccination "take," or disseminated vaccinia		Nonseasonal	Illness caused by direct exposure via vaccination or exposure to a vaccinee	Vaccination and eczema vaccinatum lesions go through stages: papule, vesicle, pustule, and scab Roseola vaccinatum: erythematous maculopapular lesions Occasionally erythema multiforme Disseminated vaccinia: papular or vesicular lesions	Lesions in roseola vaccinatum, eczema vaccinatum, and disseminated vaccinia are generalized	7-14
Variola	Smallpox	8-17	Seasonal by geographic area	Abrupt onset of high fever, headache, and muscle and joint pain Rash appears 2-4 days after onset	Basic lesion is vesicular, but lesions go through stages: macules, papules, vesicles, pustules, and crusts	Most prominent on exposed body surfaces Starts on extremities and face Spreads centripetally	12-20
Monkeypox				Similar to mild smallpox Exposure to monkeys No human-to-human spread	Similar to mild smallpox	Similar to mild smallpox	
Orf	Ecthyma contagiosum	4-7	Spring	Disease of sheep acquired by humans	Initially erythematous papule Becomes umbilicated, nodular, and then vesicular Occasionally erythema multiforme	Solitary lesion, usually on hands	30-40
Molluscum contagiosum	Molluscum contagiosum			Local cutaneous disease	Umbilicated nodular lesions: singular or clusters	Most common on face, inner aspect of thigh, breasts, and genitalia	100+
Paravaccinia	Milker's nodules	4-7		Human infection acquired from infected calves	Nodular lesion Occasionally erythema multiforme	Solitary lesion, usually on hands	30-40
Tanapox				A virus of monkeys Human infection associated with fever and regional lymphadenopathy	Umbilicated vesicular lesion	Upper part of body Solitary lesion	35-56
Coxsackieviruses A2, A4, A5, A7, A9, A10, and A16; coxsackieviruses B1-B5; echoviruses 1-7, 9, 11-14, 16-19, 22, 24, 25, 30, and 33; enterovirus 71 (see Figs. 64–9 to 64–16)		4-7	Summer and fall	Fever and mild to moderate pharyngitis Occasionally, herpangina, meningitis, and other manifestations of systemic viral infection Exanthem occurs in 5-50% of infections, depending on virus type Rash may occur during fever or after defervescence; hand, foot, and mouth syndrome	Most commonly erythematous, maculopapular, and discrete May have macular, petechial, vesicular, and urticarial components Rarely erythema multiforme	Usually starts on face and spreads downward to trunk and extremities May have peripheral distribution (hand, foot, and mouth syndrome)	3-7

TABLE 64–1 Clinical Characteristics of Viral Infections with Cutaneous Manifestations—cont'd

Virus	Disease or Syndrome	Incubation Period (days)	Main Season	Clinical Characteristics	Exanthem		Usual Duration (days)
					Lesions	Distribution	
Rhinoviruses (many types)		2-4	Fall, winter, and spring	Mild fever and signs and symptoms of respiratory illness Exanthem occurs in about 5% of cases	Erythematous or maculopapular and discrete	Starts on face and spreads downward to trunk and extremities	1-4
Foot and mouth		3-4		Direct animal contact Fever, sore mouth, and lymphadenopathy Vesicles and ulcers within the mouth	Vesicular lesions	Hands and feet	3-6
Colorado tick fever		3-5	Summer	Fever, chills, eye pain, myalgia, and headache Diphasic course Rash in only about 10% of cases	Occasionally maculopapular but usually petechial	Maculopapular rash is generalized Petechial rash most prominent on arms, legs, and trunk	2-7
Reovirus 2 and 3		4-7	Summer	Fever, mild pharyngitis, and cervical adenopathy	Erythematous or maculopapular Discrete or confluent Occasionally vesicular	Starts on face and spreads downward to trunk and extremities	3-9
Rotavirus	Gianotti-Crosti syndrome; infantile acute hemorrhagic edema	2-4	Fall, winter, and spring	Gastroenteritis	Petechial and morbilliform	Generalized	7-14
Chikungunya, o'nyong-nyong, Ross River, Sindbis			During periods of arthropod prevalence	Fever, headache, eye pain, and marked myalgia, arthralgia, and arthritis Geographically localized diseases	Rubelliform and morbilliform Frequently vesicular and petechial	Starts on face and spreads downward to trunk and extremities	
Rubella (see Fig. 64–3)	Rubella (German measles)	15-21	Winter and spring	Mild symptoms with onset 1-5 days before rash Fever usually <38.5° C (101.5° F) Headache, malaise, and suboccipital and postauricular lymphadenopathy	Erythematous, maculopapular, and discrete	Starts on face and spreads downward to trunk and extremities	4-7
West Nile				Sudden onset of fever, chills, and drowsiness Rash may appear during or after fever Geographically localized disease	Erythematous, macular, and maculopapular	Starts on trunk and spreads to extremities	3-6
Dengue and Kunjin		7	During periods of specific arthropod prevalence	Sudden onset of high fever, then severe headache, myalgia, arthralgia, abdominal pain, and marked diaphoresis Fever lasts 5-6 days and ends by crisis Rash appears within 48 hours of onset of fever Geographically localized diseases	Initially, macular, flushed appearance, then erythematous, maculopapular rash May be scarlatiniform Frequently becomes petechial and purpuric Small vesicles occur in Kunjin virus infection	Initial macular rash is more prominent centrally Maculopapular rash may start on hands and feel and spread to trunk	3-10
Influenza A and B		2-5	Fall, winter, and spring	Fever, cough, headache, and muscle aches and pains Usually in young children Rash an occasional occurrence	Erythematous, maculopapular, and discrete (rubelliform) Rarely erythema multiforme	Starts on face and trunk and spreads to extremities	1-3
Respiratory syncytial		2-5	Fall, winter, and spring	Fever, coryza, and respiratory distress (bronchitis, bronchiolitis, or pneumonia) Usually in children <2 years	Erythematous, maculopapular, and discrete (rubelliform)	Starts on face and trunk and spreads to extremities	1-3
Human metapneumovirus			Fall, winter, and spring	Fever, coryza, and respiratory distress (bronchitis, bronchiolitis, or pneumonia)	Erythematous, maculopapular		

Parainfluenza 1-3		2-5	Fall, winter, and spring	Fever, coryza, nasopharyngitis, croup, and bronchitis Usually in young children	Erythematous, maculopapular, and discrete (rubelliform)	Starts on face and trunk and spreads to extremities	1-3
Mumps	Mumps	14-21	Fall, winter, and spring	Fever, headache, and salivary gland swelling	Erythematous, maculopapular, and discrete; also, urticaria and vesicles; rarely, erythema multiforme	Most prominent on trunk	2-5
Measles (see Figs. 64–1 and 64–2)	Measles	8-12	Winter and spring	Onset with fever, cough, coryza, and conjunctivitis About 2 days after onset, appearance of enanthem (Koplik spots); and 2 days later, onset of exanthem	Erythematous, maculopapular, and confluent Develops a brownish appearance, and fine desquamation occurs	Starts behind ears and on forehead Spreads downward over body Confluence most prominent on face, trunk, and proximal end of extremities	5-7
Lassa	Lassa fever			Sudden onset of fever, chills, headache, and sore throat Progresses to pneumonia and renal failure Geographically localized outbreaks	Macular and sometimes petechial	Localized or general	
Hepatitis B	Papular acrodermatitis of childhood	50-180		Insidious onset with arthralgia, arthritis, and rash occurring before jaundice	Maculopapular, macular, or urticarial In young children, papular (Gianotti-Crosti syndrome or papular acrodermatitis of childhood) Rarely, erythema multiforme	Generalized	4-10
Hepatitis C	Mixed cryoglobulinemia (not reported in children)	7-14	Nonseasonal	Acute hepatitis followed by chronic infection Skin findings occur late in disease	Palpable purpura	Mostly buttocks, lower extremities	Variable
Marburg		5-7		Headache, conjunctivitis, photophobia, myalgia, vomiting, diarrhea, and fever (biphasic) Exposure to vervet monkeys	Initially erythematous macular, then discrete maculopapular, and finally confluent maculopapular Exfoliation occurs Occasionally purpura	Generalized	2-14
Ebola	Hemorrhagic fever	5-10	Occurs in outbreaks	Febrile illness that progresses to hemorrhage, shock, and coma	Maculopapular rash that appears toward end of first week of illness	Lateral sides of trunk, groin, and axillae Can become generalized but spares the face	14-60
Hantavirus	Hemorrhagic fever with renal syndrome (nephropathia epidemica)		Spring and summer outbreaks	Febrile illness with hemorrhagic and renal manifestations	Flushing and petechial rash	Face (flushing), skin folds (petechiae)	14-28
HIV		14-60	Nonseasonal	Fever, pharyngitis, myalgia, arthralgias, adenopathy, and rash	Macular	Mainly chest and abdomen	7
Human T-lymphotropic virus	Infective dermatitis		Nonseasonal	Acute onset of eczema	Severe exudative eczema with a crusting, generalized, fine papular rash	Scalp, eyelid margins, perinasal skin, retroauricular areas, axillae, and groin	Months to years

Data from references 1, 2, 4, 6, 7-12, 16, 19, 22, 26, 36, 39, 42, 44, 46, 48-50, 52, 57, 58, 60, 62, 63, 65, 76-79, 85, 91, 95, 97, 98, 102, 104, 106, 111, 114, 116, 117, 120, 121, 126, 135, 137-141, 144, 150, 152, 158, 164, 166, 167, 169-171, 174, 175, 184, 185, 189, 193, 197, 198, 202, 205, 207-209, 216, 217.

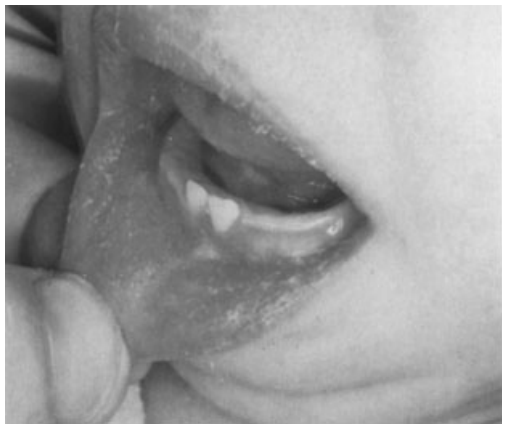

Figure 64–1 Koplik spots with involvement of the buccal and lower labial mucosa. (See companion Expert Consult web site for color version.)

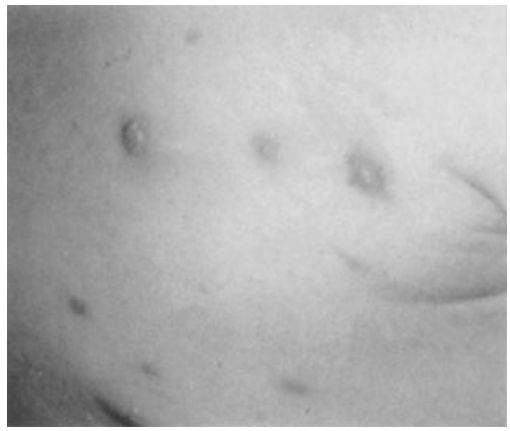

Figure 64–4 Chickenpox exanthem. Typical lesions in all stages: vesicles, papulovesicles, and papules. (See companion Expert Consult web site for color version.) *(From Cherry, J. D.: Newer viral exanthems. Adv. Pediatr.* 16*:233-286, 1969.)*

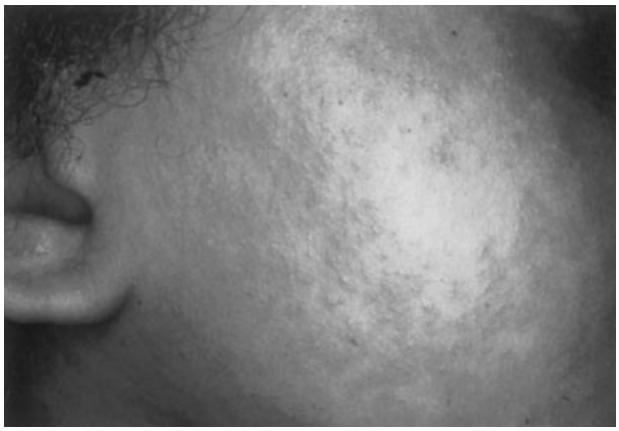

Figure 64–2 Measles exanthem. Note the generalized erythematous confluent base supporting small papular and microvesicular lesions. (See companion Expert Consult web site for color version.)

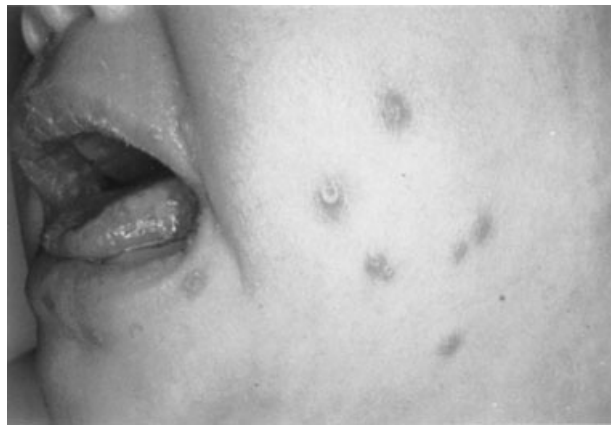

Figure 64–5 Primary herpes simplex virus infection in an infant. Note the severe stomatitis and papulovesicular and vesicular lesions under the lower lip and on the cheek. (See companion Expert Consult web site for color version.)

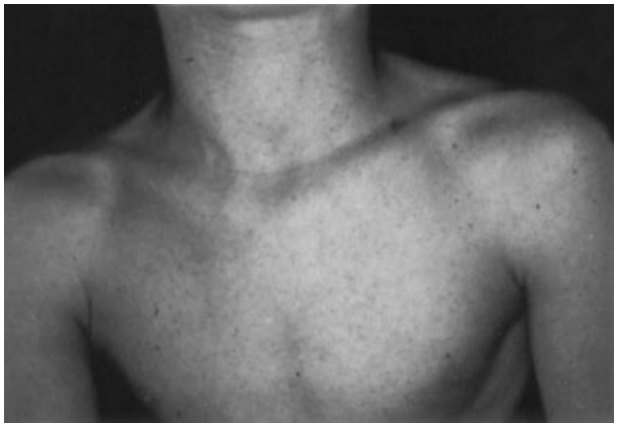

Figure 64–3 Rubella exanthem. The rash is erythematous, maculopapular, and discrete. (See companion Expert Consult web site for color version.) *(From Cherry, J. D.: Newer viral exanthems. Adv. Pediatr.* 16*:233-286, 1969.)*

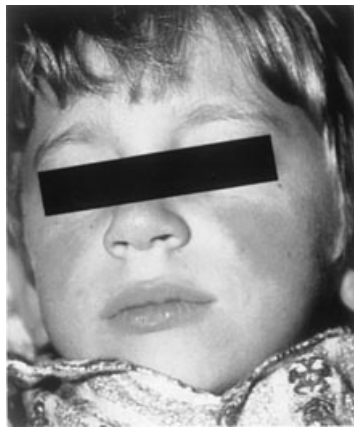

Figure 64–6 Slapped-cheek appearance with a relative circumoral maculopapular rash in erythema infectiosum. (See companion Expert Consult web site for color version.)

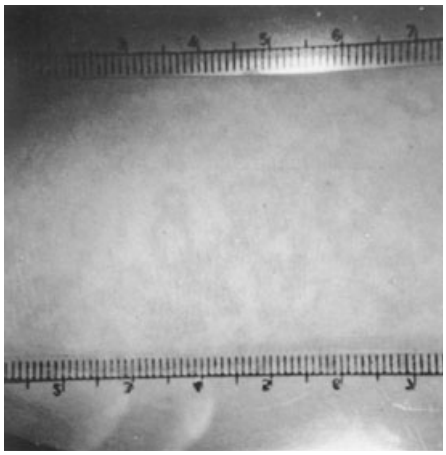

Figure 64–7 Rash with a lacelike, or reticular, pattern in erythema infectiosum. (See companion Expert Consult web site for color version.)

Figure 64–10 Erythematous maculopapular lesions on the buttocks as part of the hand, foot, and mouth syndrome caused by coxsackievirus A16. (See companion Expert Consult web site for color version.) *(From Cherry, J. D., and Jahn, C. L.: Hand, foot, and mouth syndrome: Report of six cases due to coxsackievirus, group A, type 16. Pediatrics 37:637, 1966. Copyright American Academy of Pediatrics 1966.)*

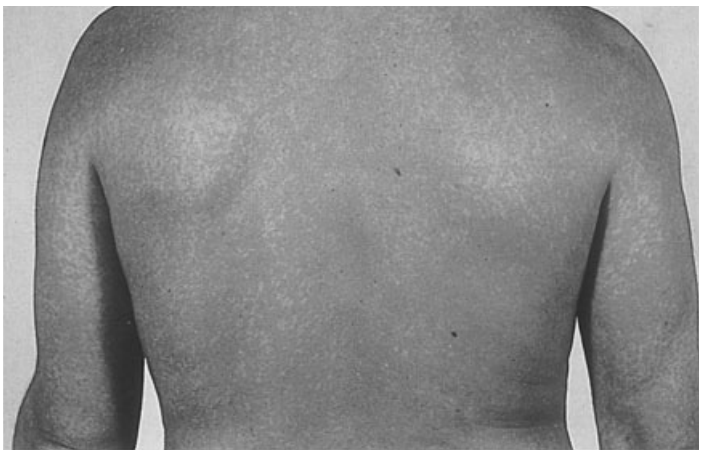

Figure 64–8 Confluent exanthem in a patient with human parvovirus infection. (See companion Expert Consult web site for color version.)

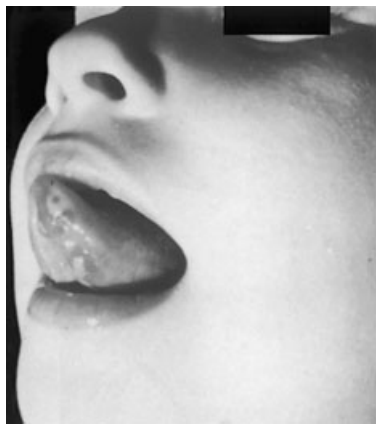

Figure 64–11 Two large ulcerative lesions on the underside of the tongue in a patient with hand, foot, and mouth syndrome caused by coxsackievirus A16. (See companion Expert Consult web site for color version.)

Figure 64–9 Vesicular and maculopapular lesions on the foot and lower part of the leg as part of the hand, foot, and mouth syndrome caused by coxsackievirus A16. (See companion Expert Consult web site for color version.) *(From Cherry, J. D., and Jahn, C. L.: Hand, foot, and mouth syndrome: Report of six cases due to coxsackievirus, group A, type 16. Pediatrics 37:637, 1966. Copyright American Academy of Pediatrics 1966.)*

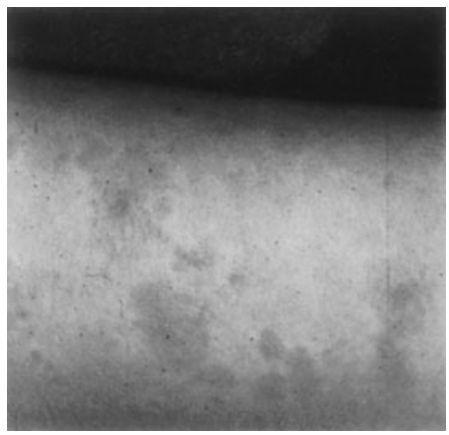

Figure 64–12 Papular-urticarial lesions in coxsackievirus A9 infection. (See companion Expert Consult web site for color version.) *(From Cherry, J. D.: Newer viral exanthems. Adv. Pediatr. 16:233-286, 1969.)*

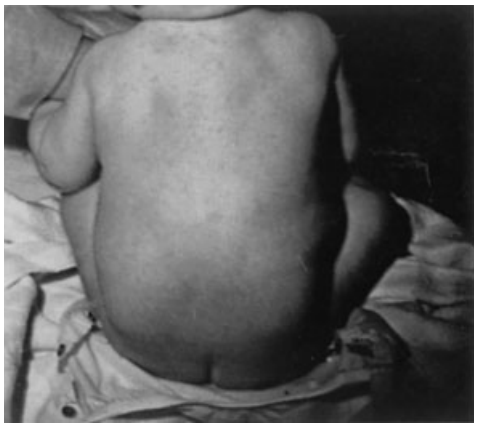

Figure 64–13 Erythematous, discrete, maculopapular, and petechial rash of echovirus 9 infection. (See companion Expert Consult web site for color version.) *(From Cherry, J. D.: Newer viral exanthems. Adv. Pediatr.* 16*:233-286, 1969.)*

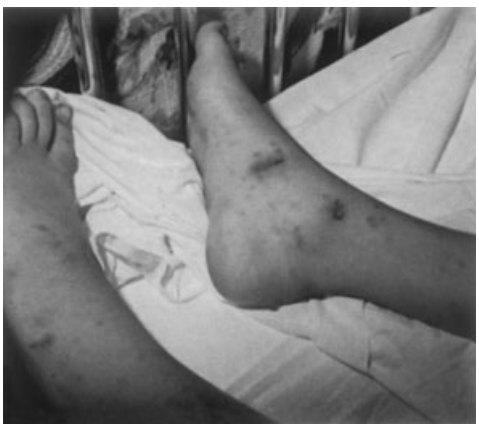

Figure 64–14 Petechial and purpuric rash in a child with coxsackievirus A9 infection. (See companion Expert Consult web site for color version.)

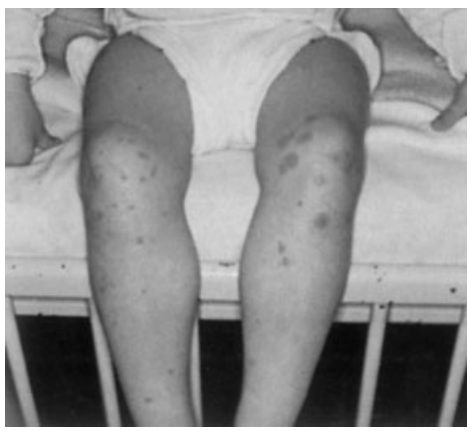

Figure 64–15 Erythematous, papular, papulovesicular, and petechial lesions suggestive of anaphylactic purpura in a child with coxsackievirus A4 infection. (See companion Expert Consult web site for color version.)

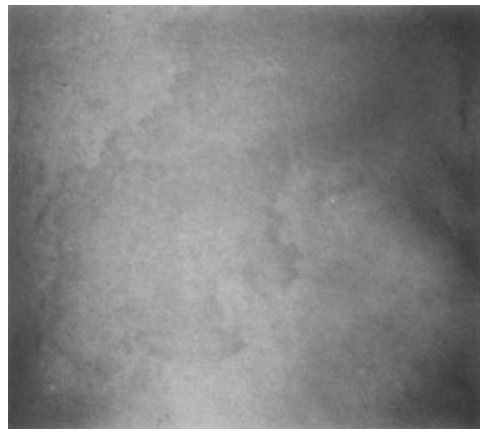

Figure 64–16 Acute urticaria in a child with hand, foot, and mouth syndrome caused by coxsackievirus A16 infection. (See companion Expert Consult web site for color version.)

for the development of Kaposi sarcoma in patients with acquired immunodeficiency syndrome (AIDS) and other immunodeficiency states.[102,114,135]

At present, human illnesses with cutaneous manifestations caused by poxviruses rarely occur. Because smallpox as a disease has ceased to exist, the use of vaccinia virus for immunization has decreased dramatically. However, the terrorist events of 2001 raised concern about the possible use of smallpox virus as a terrorist weapon. Because of this potential danger, smallpox vaccines are being produced and used again. With the increased use of these vaccines, cutaneous complications of vaccinia virus infection can be expected. Monkeypox, orf, and paravaccinia (milker's nodules) continue to occur as isolated events in exposed individuals.[170,208,209] Human infection with tanapox virus is a geographically related illness occurring in limited areas of Kenya.

In the present era, enteroviruses are the leading cause of infection-related exanthematous diseases.[51,55,109,208,209] Thirty-seven types have been associated with rash illnesses. The clinical expression rate varies greatly among the different types; it is as high as 50 percent in children with coxsackievirus A16 and echovirus 9 infections. Only approximately 15 percent of individuals infected with echovirus 4 have exanthem, and rash is a rare occurrence in echovirus 6 infection. Hope-Simpson and Higgins[98] noted exanthem in approximately 5 percent of patients with rhinoviral respiratory illness.

A young adult research worker had an influenza-like illness and a hand, foot, and mouth syndrome–like rash caused by infection with a calicivirus (San Miguel sea lion virus serotype 5) of oceanic origin.[183]

Two percent of patients with Colorado tick fever encephalitis have exanthem.[51] Although infection with reoviruses occurs commonly, exanthem has been noted on only nine occasions.[51,121] A morbilliform rash has been observed in one adult with a rotavirus infection, and a 4-year-old boy was noted to have a petechial rash in association with a rotaviral illness.[60,167] Di Lernia and Ricci[63] described three cases of Gianotti-Crosti syndrome and one child with infantile acute hemorrhagic edema associated with rotavirus infections.

Of the *Togaviridae* family of viruses, rubella virus is the most important as a worldwide cause of exanthematous disease. Several alphaviruses also frequently cause exanthems.[108,141,208,209] Each of these viruses has a marked geographic distribution. Similarly, flaviviruses also have exanthem as part of their clinical expression, and they too have specific geographic boundaries.[208,209] In the New York City area outbreak of West Nile virus infection in 1999, 19 percent of patients had exanthem.[138] The rash was erythematous macular, papular, or morbilliform.

Exanthem generally is not considered to be a manifestation of influenza virus infection, but Hope-Simpson and Higgins[98] noted exanthem in approximately 8 percent of patients from whom influenza B virus was isolated and in 1 or 2 percent of those infected with influenza A virus. Measles virus is the most notable of the *Paramyxoviridae* family with an associated exanthem. However, exanthem occurs rather frequently in young children infected with parainfluenza virus types 1, 2, and 3 and also in those with respiratory syncytial virus (RSV) illnesses.[81,93,94,199,202] Hope-Simpson and Higgins[98] noted a 15 percent incidence of rash in RSV infection and an approximately 15 percent incidence in parainfluenza virus infection. Rash, which was not described further, was observed in four children with respiratory illnesses caused by human metapneumovirus infections.[158] Exanthem also has been noted on rare occasion with mumps virus infection.[49]

Lassa fever virus, Marburg virus, Ebola virus, and hepatitis B virus all have been associated with exanthem on occasion.[42,46,62,75,152,208] Hepatitis B virus is the main cause of papular acrodermatitis (Gianotti-Crosti syndrome) in children.[46,171,175] Chronic hepatitis C virus infection occasionally causes systemic vasculitis and cryoglobulinemia in adults, with purpuric lesions concentrated on the lower extremities.[2,97] Other cutaneous manifestations of chronic hepatitis C virus infection include urticaria, erythema nodosum, lichen planus, and nodular prurigo.[106,216]

Hantaviruses cause two major syndromes throughout the world: hemorrhagic fever with renal syndrome and hantavirus pulmonary syndrome.[4,39,150,174] Exanthem (facial flushing and petechial lesions in skin folds) occurs in approximately 30 percent of patients with hemorrhagic fever with renal syndrome, but rash is not reported in the hantavirus pulmonary syndrome. A macular rash has been noted in association with acute infection with human immunodeficiency virus type 1 (HIV-1).[137,139,140,189] Several reports have associated human T-lymphotropic virus type 1 (HTLV-1) with an atypical form of eczema called infective dermatitis. This exanthem has an acute onset and is somewhat recalcitrant to treatment.[116,117,126]

Chlamydiae, rickettsiae, and mycoplasmas associated with cutaneous manifestations are listed in Table 64–2. Of the chlamydiae, only *Chlamydia psittaci* has been associated with exanthem. In contrast, all rickettsiae that infect humans, with the exception of *Coxiella burnetii*, usually display some cutaneous manifestations as part of their systemic disease.[31,69,84,115,122,143,147,173] Approximately 4 to 7 percent of adults with Q fever have exanthem.[40,187] Of the mycoplasmas that infect humans, only *Mycoplasma pneumoniae* is associated with exanthem.[13,51,53] In epidemics, exanthem occurs in approximately 15 percent of persons with respiratory illness.

In Table 64–3, bacterial agents for which cutaneous manifestations are part of the clinical illness are presented (see Chapter 66). The clinical expression of exanthem varies tremendously among the different etiologic agents, as do the conditions associated with a specific infection. For example, infection with phage group 2 staphylococci usually results in cutaneous disease in young infants, whereas the same organisms rarely cause illness in adults. Symptomatic infection with *Streptococcus pneumoniae* is associated with cutaneous manifestations only occasionally; on the other hand, similar systemic disease with *Neisseria meningitidis* virtually always is associated with the characteristic petechial exanthem. Of the other bacterial agents listed in Table 64–3, exanthem is most important in the following: *Neisseria gonorrhoeae*, *Salmonella typhi*, *Streptobacillus moniliformis*, *Spirillum minus*, *Pseudomonas aeruginosa*, and *Treponema pallidum*.

Fungal, protozoan, and metazoan agents associated with cutaneous manifestations in humans are listed in Tables 64–4, 64–5, and 64–6, respectively. These agents and their diseases, discussed more completely in other chapters, are included here for completeness of the differential diagnosis.

EPIDEMIOLOGY

Tables 64–1 through 64–6 clearly show that exanthematous disease has many possible etiologic agents; hence, no unified epidemiology exists. Epidemiologic events related to specific agents are considered in the appropriate sections throughout this text. Each agent with exanthem as a clinical manifestation has a unique epidemiologic pattern that, if understood, distinguishes it from many of the other agents that cause otherwise identical clinical illnesses. In the evaluation of all patients with rash, exposure, season, and incubation period are important aspects of the diagnostic process.

PATHOPHYSIOLOGY AND PATHOLOGY OF EXANTHEMS

Even though the skin can respond in only a limited number of ways, what is obvious from the extensive number of etiologic agents is that multiple pathogenic mechanisms must occur. In many sections of this book, the pathology and pathophysiology of specific agents are presented in detail. An overview is presented here.

Small vessel vasculitis (leukocytoclastic vasculitis) is a leading event in most exanthematous illnesses caused by infectious agents.[186] The cutaneous manifestations of systemic diseases can be separated into three broad categories. The first category involves dissemination of infectious agents by blood (viremia, bacteremia, and so on), which results in secondary infection at the cutaneous site. The clinical cutaneous findings in this type of infection can be the direct result of infectious agents in the epidermis, dermis, or dermal capillary endothelium or can be the result of an immune response between the organism and antibody or cellular factors in the cutaneous location. The possible events in the skin with this type of infection are presented in Table 64–7. Chickenpox, many enteroviral infections, and meningococcemia are examples of diseases in which infectious agents have reached the skin through the blood and are causing the cutaneous findings without the additional contribution of host immune factors. In illnesses such as measles, rubella, and gonococcemia, the timing, histologic picture, and difficulty of direct recovery of the agent by culture suggest both a direct effect and an immune-mediated response.

The second category of pathogenesis relates to the dissemination of known specific toxins of infectious agents. The infection is in a localized area of the body, but the toxin liberated by the infectious agents reaches the skin by blood-borne dissemination. Three examples of toxin-mediated exanthematous disease are streptococcal scarlet fever, staphylococcal scalded skin syndrome, and toxic shock syndrome.

The third category of pathogenesis in systemic disease with exanthem is poorly understood but appears to have an immunologic basis. Most important in this category are the clinical pictures of erythema multiforme, erythema multiforme exudativum (Stevens-Johnson syndrome), and erythema nodosum. In erythema multiforme associated with *M. pneumoniae* and HSV infection, the respective organisms have been isolated or identified at the skin site. In most instances, however, neither antigen localization nor disseminated toxin has been identified.

Important clinical aspects of exanthematous diseases are the distribution and progression of the lesions, yet little is known of the cause of these aspects. Differences in skin thickness, vascularity, proliferation rate, temperature, and metabolic activity are important in animal diseases with cutaneous manifestations.[51,75,124,134,154] In humans, similar factors must be important but obviously affect the various etiologic agents differently (e.g., the more central exanthem of chickenpox versus that of the hand, foot, and mouth syndrome of coxsackievirus A16 infection).

Text continued on p. 770

TABLE 64–2 Clinical Characteristics of Chlamydial, Rickettsial, and Mycoplasmal Infections with Cutaneous Manifestations

Agent	Disease or Syndrome	Incubation Period (days)	Main Season	Clinical Characteristics	Exanthem		Usual Duration (days)
					Lesions	Distribution	
Chlamydia psittaci	Psittacosis	7-14	Nonseasonal	Fever, chills, headache, and cough Respiratory distress	Erythematous macules Occasionally erythema multiforme or erythema nodosum	Mainly on trunk	2-7
Rickettsia akari	Rickettsialpox	7-14	Nonseasonal	Fever, chills, headache, backache, and malaise 4-7 days after onset of primary lesion at site of mite bite Geographically localized disease	Initial lesion at site of mite bite is papular and then vesicular, and finally an eschar forms Two days after onset of fever, erythematous maculopapular discrete rash occurs Lesions progress to small vesicles and later to scabs	Most prominent on trunk and proximal end of extremities	7-10
Rickettsia typhi	Endemic, murine typhus	7-14	Nonseasonal	Fever and headache Rash appears on 4th-7th day Geographically localized disease	Initially discrete macules and then erythematous maculopapular May become purpuric	Initially upper part of trunk and axilla Progresses to entire body except face, palms, and soles	7-21
Rickettsia prowazekii	Epidemic typhus	10-14	Nonseasonal	Sudden onset of fever, chills, headache, and myalgias Rash appears on 4th-7th day Geographically localized disease	Initially discrete macules and then progresses to maculopapular and petechial lesions Sometimes purpuric	Appears first on trunk and spreads to extremities Spares palms and soles	7-14
Rickettsia tsutsugamushi	Scrub typhus	7-21	Nonseasonal	Sudden onset of chills, fever, and headache	Local lesion at site of chigger bite is present at onset of symptoms; characterized by vesicle, ulcer, and eschar Maculopapular rash occurs 5-8 days after onset of fever	Maculopapular rash first occurs on trunk and then becomes generalized	7-14
Rickettsia rickettsii	Rocky Mountain spotted fever	3-12	Summer	Abrupt onset of fever, chills, and headache Rash appears 2-4 days after onset	Early maculopapular, then petechial, and sometimes purpuric	Rash starts on distal end of extremities Rarely involves the trunk	7-14
Other tick-borne rickettsiae			Tick seasons	Similar to mild Rocky Mountain spotted fever	Similar to Rocky Mountain spotted fever; eschar at site of tick bite	Similar to Rocky Mountain spotted fever	7-14
R. sibirica	North Asian tick-borne rickettsiosis						
R. australis	Queensland tick typhus						
R. conorii	Boutonneuse fever						
R. africae	African tick fever						
Coxiella burnetii	Q fever	20-40	Nonseasonal	Acute febrile illness with chills, headache, and myalgia	Fine discrete macular rash occurring during febrile illness Transient urticarial rash also noted	Mainly on trunk	2-7
Ehrlichia species	Ehrlichiosis	14-28	Tick seasons	Similar to Rocky Mountain spotted fever, but rash usually not on palms and soles	Similar to endemic typhus	Similar to endemic typhus	7-14
Mycoplasma pneumoniae		21	All seasons	Gradual onset of fever, malaise, headache, and cough	Maculopapular rash occurs in 5-15% of cases Vesicular and bullous lesions common (Stevens-Johnson syndrome); more common in males Papular, petechial, and urticarial lesions also noted Erythema multiforme common	Rash most prominent on trunk and proximal end of extremities	7-14

Data from references 13, 14, 18, 31, 35, 40, 51, 53, 69, 84, 92, 115, 122, 129, 136, 143, 147, 160, 173, 187, 210, 215.

TABLE 64–3 Bacteria Associated with Cutaneous Manifestations

			Exanthem	
Agent	**Disease or Syndrome**	**Clinical Characteristics**	**Lesions**	**Distribution**
Gram-positive cocci				
Staphylococcus aureus, exfoliative toxin-producing, mainly phage group 2 (see Figs. 64–17 and 64–18)	Bullous impetigo	Usually occurs in neonates May be epidemic	Rapid progression from vesicles to bullous lesions	Most common in diaper area
	Scalded skin syndrome Toxic epidermal necrolysis (Ritter disease in infants <4 months; Lyell syndrome in older children)	Usually occurs in infants and children 1 month–5 years of age Mucopurulent nasal and eye discharge Fever	Scarlatiniform eruption with exfoliation Nikolsky sign present Crusty appearance around eyes and under nose	Generalized Most marked on trunk
	Staphylococcal scarlet fever or staphylococcal scarlatiniform eruption	Fever and staphylococcal infection in throat, but no evidence of pharyngitis	Scarlet fever–like rash with desquamation Pastia lines present	Generalized
Staphylococcus aureus, non–exfoliative toxin-producing	Septicemic disease	Severe septicemia with osteomyelitis, arthritis, endocarditis, or pneumonia	Diffuse, erythematous, confluent, and macular rash (flush) With endocarditis, may have petechiae and splinter hemorrhages, Osler nodes, Janeway spots	Trunk and proximal end of extremities
Staphylococcus aureus, toxin-1 (TSST-1)–producing	Toxic shock syndrome	Fever, intense myalgias, vomiting, and diarrhea Mental confusion and hypotension	Erythematous, deep red (sunburn-like) rash Desquamation occurs	Generalized
Staphylococcus aureus, non–exfoliative toxin-producing	Folliculitis, furuncles, or carbuncles	See primary skin infections, Chapter 66		
Streptococcus pyogenes	Scarlet fever	Fever, pharyngitis, and cervical lymphadenitis Rash onset within 2 days of first symptoms Incubation period 3-4 days	Diffuse erythematous and fine maculopapular (looks and feels like red sandpaper) Rash darker in skin folds (Pastia lines) Desquamation occurs	Circumoral pallor Generalized rash, with trunk and proximal end of extremities being most involved
	Erysipelas	Fever, headache, and vomiting Localized infection	Circumscribed area that is raised and erythematous Advancing edge is irregular	Anywhere
	Impetigo	Localized superficial pyoderma See primary skin infections, Chapter 66	Discrete and coalescent lesions of a vesicular nature Quickly becomes more pustular and then crusts over with a yellowish brown appearance	Forearms, legs, and face
	Septicemia	Fever and systemic foci of infection	Petechiae	Diffuse
	Miscellaneous skin manifestations of *S. pyogenes* infections		Erythema multiforme, erythema nodosum, and erythema marginatum	
Streptococcus pneumoniae	Septicemia	Fever	Petechiae	Diffuse
Enterococcal and viridans group streptococci	Endocarditis	Endocarditis	Petechiae, splinter hemorrhages, Osier nodes, and Janeway spots	
Gram-negative cocci				
Neisseria gonorrhoeae	Gonococcemia	Fever and polyarthralgias	Papular, petechial purpuric, pustular, or necrotic lesions	Most common on extremities Extensor surfaces over joints
Neisseria meningitidis	Meningococcemia	Fever and pharyngitis Sudden onset of rash	Characteristic rash is petechial or purpuric Early lesions may be erythematous maculopapular, or urticarial	Generalized
Moraxella catarrhalis	Bacteremia	Fever and pharyngitis	Maculopapular and petechial	Generalized

TABLE 64–3 Bacteria Associated with Cutaneous Manifestations—cont'd

Agent	Disease or Syndrome	Clinical Characteristics	Exanthem	
			Lesions	Distribution
Gram-positive bacilli				
Bacillus anthracis	Anthrax	Fever, headache, malaise, and joint pain	Initially, macular, pruritic lesion Later, a papule forms and then vesiculation Vesicles last 2-6 days, and then eschar forms	Usually, single lesion initially at point of exposure, secondary lesions in area develop later
Listeria monocytogenes	Listeriosis	Neonatal meningitis with hepatosplenomegaly	Maculopapular, discrete lesions Pustules	Trunk and legs
Erysipelothrix rhusiopathiae	Crab or fishnet dermatitis	Fever and local pain	Erysipeloid lesion (violet or red)	Hands
Corynebacterium diphtheriae	Cutaneous diphtheria	Secondary infection in cutaneous wounds	Impetigo or ecthyma-like Rarely, erythema multiforme	Exposed surfaces
Arcanobacterium hemolyticum	Scarlet fever–like illness	Fever and pharyngitis	Scarlet fever–like rash Occasionally, rubelliform	Generalized rash with peripheral predominance
Enteric gram-negative bacilli				
Salmonella typhi	Typhoid fever	Malaise, headache, and marked fever Rash onset 10 days after onset of fever	Rose spots, 2- to 4-mm macular lesions	Discrete lesions on abdomen
Other *Salmonella* species	Septicemic salmonellosis	Similar to mild typhoid fever	Similar to typhoid fever	Similar to typhoid fever
Shigella sonnei	Shigellosis	Diarrhea	Urticaria	Diffuse
Campylobacter species		Gastroenteritis	Skin pustules and erythema nodosum	Lower part of legs
Other gram-negative bacilli				
Francisella tularensis	Tularemia	Chills, fever, headache, and localized lymphadenopathy	Initial papule that later ulcerates	Site of inoculation
Haemophilus ducreyi	Chancroid	Local pain and tenderness	Pustular lesions that ulcerate	External genitalia
Haemophilus influenzae	Septicemia	Fever	Petechiae Reddish purple cellulitis	Diffuse Cellulitis mainly on cheeks and extremities
Streptobacillus moniliformis	Rat-bite fever	Fever, chills, malaise, headache, and polyarthritis	Erythematous, maculopapular rash that may become petechial	Most prominent on extremities, including palms and soles
Yersinia pestis	Septicemic plague	Sudden onset of fever	Initial generalized erythema followed by petechiae and purpura	Generalized
Yersinia pseudotuberculosis		Mesenteric lymphadenitis	Erythema nodosum and scarlatiniform eruption	Lower part of legs and generalized
Yersinia enterocolitica	Yersiniosis	Enterocolitis	Erythema nodosum and urticaria	Lower part of legs and generalized
Bartonella bacilliformis	Bartonellosis, Carrión disease, or Oroya fever	Initially intermittent fever, malaise, and myalgias 30-60 days after initial fever, exanthem appears	Erythematous maculopapular Later recurrent nodules	Face and extensor surface of extremities
Bartonella quintana	Trench fever	Usually mild fever, headache, chills, and tibial bone pain	Macular rash	Mainly on trunk
Calymmatobacterium granulomatis	Granuloma inguinale	See *Calymmatobacterium granulomatis*, Chapter 141	Nodular, ulcerovegetative, hypertrophic, or cicatricial lesions	Genitals
Pseudomonas aeruginosa	Ecthyma gangrenosa	Septicemia (usually in immunocompromised patients)	Initially vesicular and then hemorrhagic Become ulcerated with central black necrotic eschar	Anywhere
	Pseudomonas folliculitis (health spa dermatitis)	Headache, malaise, and fatigue	Papular and pustular	Generalized
Burkholderia mallei	Glanders, melioidosis	Fever, malaise, chills, arthralgia, and muscle pains	Nodule or ulcer at site of inoculation and then widespread papules, bullae, and pustules	Generalized
Brucella species	Brucellosis	Acute or subacute febrile illness Exanthem in 8% of cases	Erythematous and maculopapular Occasionally vesicles	Generalized
Legionella pneumophila	Legionnaires' disease	Severe pneumonia	Maculopapular	Anterior of trunk

TABLE 64–3 Bacteria Associated with Cutaneous Manifestations—cont'd

			Exanthem	
Agent	**Disease or Syndrome**	**Clinical Characteristics**	**Lesions**	**Distribution**
Bartonella henselae	Cat-scratch fever	Subacute regional lymphadenitis	Erythematous maculopapular, morbilliform, petechial, erythema nodosum, erythema multiforme, and erythema marginatum May be pruritic	Generalized
Acid-fast bacilli				
Mycobacterium tuberculosis	Lupus vulgaris	Usually associated with other manifestations of tuberculosis	Reddish brown nodular or scaling lesions	Mainly on face and neck
	Papulonecrotic tuberculids	Associated with disseminated tuberculosis	Initially vesicular Become pustules, umbilical, and ulcerated and then form scabs and leave scars	Single or multiple lesions anywhere
Atypical mycobacteria			Granulomatous and ulcerative lesions at site of superficial injury	Usually on hands
Mycobacterium leprae	Erythema nodosum leprosum	General findings of lepromatous leprosy	Erythematous nodular lesions	Disseminated Most prominent on face and extremities
Spirochetes				
Treponema pallidum	Primary syphilis	Chancre	Large ulcers with indurated edges	Genitals
	Secondary syphilis		Erythematous maculopapules that frequently are scaly (psoriasiform)	Generalized, including palms and soles
Treponema pertenue	Yaws		Papular lesions at sites of inoculations Lesions ulcerate, leaving a wart-like appearance	Anywhere
Borrelia burgdorferi	Lyme disease (erythema chronicum migrans)	Skin, cardiac, neurologic, and joint abnormalities	Expanding erythematous, annular lesions	Thighs, buttocks, or axillae
Treponema carateum	Pinta		Initially, erythematous, papular lesions; increase in size during 1-month period and become scaly	Exposed surfaces of body
Spirillum minus	Rat-bite fever	Fever and chills	Discrete, macular rash	Trunk and extremities, including palms and soles
Leptospira species	Leptospirosis	Fever, conjunctivitis, and anorexia Rash rarely noted	Erythematous maculopapular rash	Mainly on trunk
Borrelia species	Relapsing fever	Relapsing fever, headache, myalgia, and photophobia	Morbilliform and petechial Erythema multiforme	Generalized

Data from references 5, 17, 21, 24, 29, 30, 32-34, 37, 38, 41, 54, 59, 64, 67, 69, 71, 74, 86, 88, 90, 99, 100, 101, 103, 105, 112, 113, 118, 119, 123, 125, 128, 132, 142, 145, 149, 151, 153, 155, 156, 161, 162, 168, 172, 177, 188, 190-192, 194-196, 201, 204, 206, 211, 213, 214, 218.

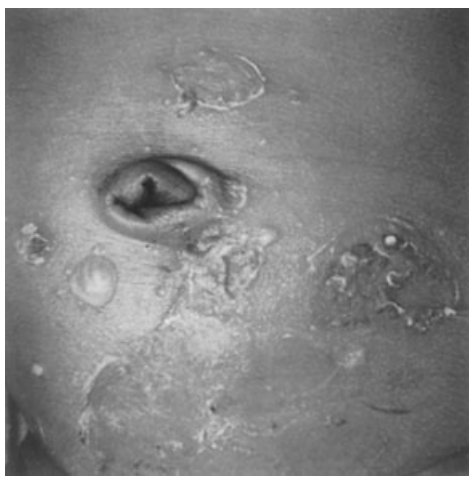

Figure 64–17 Bullous impetigo in a newborn infant caused by exfoliative toxin–producing *Staphylococcus aureus*. (See companion Expert Consult web site for color version.)

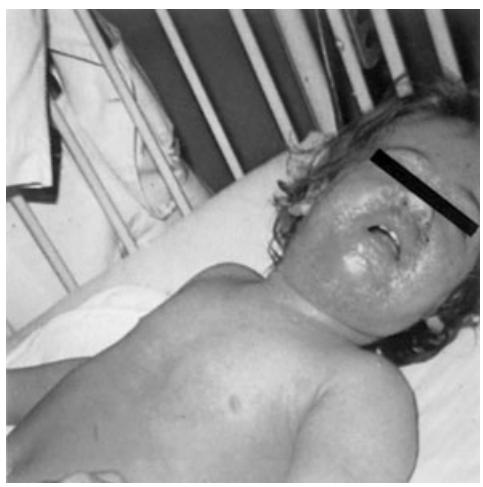

Figure 64–18 Scalded skin syndrome caused by exfoliative toxin–producing *Staphylococcus aureus*. (See companion Expert Consult web site for color version.)

TABLE 64–4 Fungi Associated with Cutaneous Manifestations

			Exanthem	
Agent	**Disease or Syndrome**	**Clinical Characteristics**	**Lesions**	**Distribution**
Dermatophytic fungi	Tinea capitis, tinea cruris, tinea pedis, or tinea circinata		Localized, brownish, maculopapular lesions that are scaly Erythema nodosum	
Candida albicans	Congenital cutaneous candidiasis	Congenital infection	Discrete vesicular lesions	Generalized
	Chronic mucocutaneous candidiasis	Immunodeficiency disease	Confluent, erythematous, and exudative lesions	Generalized, including scalp
	Acquired candidiasis		Confluent, fiery red lesions	Most common in diaper area
Candida spp.	Systemic candidiasis	Severe opportunistic infection	Erythematous nodular lesions	Generalized
Histoplasma capsulatum	Histoplasmosis	Primary respiratory infection	Erythema nodosum, erythema multiforme, and erythematous maculopapular	
Cryptococcus neoformans	Cryptococcosis	Primary respiratory infection	Erythema nodosum and acneiform eruptions	
Coccidioides immitis	Coccidioidomycosis	Primary respiratory infection	Initially, erythematous, maculopapular rash Later, erythema multiforme and erythema nodosum	Generalized maculopapular rash
Sporotrichum schenckii	Sporotrichosis	Cutaneous inoculation	Nodular lesions that ulcerate	Usually, hands, arms, and legs
Blastomyces dermatitidis	Blastomycosis	Primary respiratory infection	Nodular lesions that ulcerate Erythema nodosum	
Scedosporium spp.	No specific syndrome	Severe opportunistic infection	Nodular or necrotic skin lesions	Generalized
Fusarium spp.	No specific syndrome	Severe opportunistic infection	Nodular skin lesions, abscesses	Generalized
Aspergillus spp.	No specific syndrome	Severe opportunistic infection	Nodular and purpuric lesions	Generalized

Data from references 14, 15, 23, 27, 43, 69, 73, 80, 82, 127, 131, 163, 182, 203, 212.

TABLE 64–5 Cutaneous Manifestations of Protozoan and Helminthic Infections

Agent	**Disease or Syndrome**	**Cutaneous Manifestations**
Plasmodium spp.	Malaria	Occasionally generalized urticaria in chronic infection
Toxoplasma gondii	Acquired toxoplasmosis	Occasionally generalized erythematous, maculopapular rash
	Congenital toxoplasmosis	Generalized petechial rash
Giardia lamblia	Giardiasis	Rarely urticaria
Entamoeba histolytica	Amebiasis	Rarely urticaria
Leishmania tropica	Oriental sore	Red nodular lesion that ulcerates; lasts 2-3 months
Leishmania braziliensis and *mexicana*	American cutaneous leishmaniasis	Erythematous papular lesion that vesiculates and ulcerates
Trypanosoma gambiense	African trypanosomiasis	Red nodular lesion at site of bite, followed by generalized, pruritic, erythema multiforme–like rash
Trypanosoma cruzi	American trypanosomiasis or Chagas disease	Nodular lesion at site of bite; generalized recurrent erythematous, maculopapular rash
Trichomonas vaginalis	Vulvovaginalis	Rarely urticaria and erythema multiforme
Ascaris lumbricoides	Roundworm infestation	Erythema nodosum
Enterobius vermicularis	Pinworm infestation	Rarely urticaria
Necator americanus	Hookworm disease	Papules and papulovesicles on exposed surfaces (feet); generalized urticaria
Trichinella spiralis	Trichinosis	Urticaria common; also, generalized maculopapular rash may occur; petechiae frequently develop
Strongyloides stercoralis	Strongyloidiasis; also, creeping eruption (cutaneous larva migrans)	Erythematous, maculopapular lesions on feet; creeping eruption
Ancylostoma braziliense	Creeping eruption (cutaneous larva migrans)	Creeping eruption
Dermatobia hominis	Cutaneous myiasis	Creeping eruption, subacute draining lesions
Schistosoma haematobium, *mansoni*, and *japonicum*	Schistosomiasis	Pruritic papular eruption where exposed; generalized urticaria and granulomatous lesions
Trichobilharzia ocellata, *physellae*, and *stagnicolae*	Swimmer's itch or collector's itch	Initial erythema and urticaria followed by papules and vesiculation; pruritic
Wuchereria bancrofti	Filariasis	Localized erythema urticaria and erythema nodosum
Onchocerca volvulus	Onchocerciasis	Chronic, papular, scaly rash
Echinococcus granulosus and *multilocularis*	Echinococcosis	Frequent urticaria

Data from references 14, 20, 43, 56, 69, 72, 83, 89, 133.

TABLE 64–6 Cutaneous Manifestations of Arthropod Bites and Stings

Agent	Disease or Syndrome	Cutaneous Manifestations
Spiders		
Loxosceles rectus	Recluse spider bite or brown spider bite	Erythema followed by blister and necrosis
Ticks	Tick bite	Initial pruritus at site; becomes ulcerated and granulomatous
Mites		
Sarcoptes scabiei	Scabies	Pruritic burrows in body creases and generalized; become erythematous and then papular urticaria
Trombicula irritans	Chigger bite	Marked pruritus and then papular urticaria
Other mites: food, grain, murine, and fowl		Marked pruritus and then papular urticaria
Lice		
Pediculus humanus	Body lice or pediculosis	Erythematous, maculopapular, pruritic lesions; sometimes urticaria
Phthirus pubis	Crabs	Pruritus and erythema under pubic hair
Bedbugs and kissing bugs		
Cimex lectularius	Bedbug bite	Pruritic papular urticaria
Triatoma sanguisuga	Kissing bug bite	Papular urticaria; occasionally hemorrhagic nodular lesions
Gypsy moth caterpillar		
Lymantria dispar	Gypsy moth rash	Pruritic blotchy erythema and maculopapular
Moths		
Hylesia alinda	Moth-associated dermatitis	Erythema and pruritus; feeling of warmth in area of rash; may have vesicular lesions
Ants		
Solenopsis saevissima	Fire ant bite	Painful papular urticarial lesions that become pustular and then nodular
Fleas		
Pulex irritans (human flea) and fleas of many animals	Flea bite	Papular urticaria
Flies and mosquitoes	Fly and mosquito bite	Papular, nodular, and urticarial lesions in sensitive persons

Data from references 45, 47, 61, 69, 96, 148, 165, 176, 181.

TABLE 64–7 Aspects of Pathogenesis in Exanthems Associated with Blood-borne Dissemination of the Infectious Agent

Anatomic Location	Spread of Agent	Histology	Clinical Expressions	Pathophysiology
Blood	Free in plasma; Associated with leukocytes			
Dermal capillary endothelium	Infection	Damage to vessel, endothelial swelling, perivascular edema, cellular infiltration, hemorrhage	Macule, papule, petechia	Direct effect of agent or immune reaction with pathologic consequences
	To dermis through breaks in basement membrane (secondary to trauma); To dermis by diapedesis; Contiguous spread			
Dermis	Infection; Contiguous spread	Edema, cellular infiltration, hemorrhage, microscopic visualization of organism	Papule, urticaria, purpura, vesicle	Direct effect of agent or immune reaction with pathologic consequences; histamine release
Epidermis	Infection	Cytopathic effects (inclusions, ballooning, vacuolation, necrosis, nuclear disruption), microscopic visualization of organism	Papule, vesicle, ulcer	Direct effect of agent

Modified from Cherry, J. D.: Newer viral exanthems. Adv. Pediatr. 16:*233-286, 1969.*

CLINICAL MANIFESTATIONS

The clinical findings in exanthematous diseases resulting from systemic infections are varied and depend on the inciting pathogens. By examination of skin alone, differentiating an exanthematous disease resulting from systemic infection (e.g., coxsackievirus A9, rubella virus infection) from primary cutaneous diseases of infectious and noninfectious origin (insect bites, acne, and contact with poison ivy) frequently is difficult. In Tables 64–1 through 64–6, the clinical characteristics of viral, chlamydial, rickettsial, bacterial, fungal, parasitic, and arthropod-induced illnesses with primary or secondary cutaneous manifestations are presented. In Tables 64–8 through 64–17, etiologic agents and clinical manifestations are presented on the basis of the more pronounced cutaneous manifestations or syndrome associations. The clinician must keep in mind that other aspects of an illness (e.g., exposure, season, incubation period, geographic location, patient age, associated signs and symptoms) may be more important in determining the underlying etiologic agent. Clinical manifestations of specific exanthematous diseases are presented in greater detail in other chapters of this book.

ERYTHEMATOUS MACULAR EXANTHEMS

When all infectious diseases with exanthems are taken into consideration, the occurrence of illnesses in which the lesions are just macular is rare. However, many important, severe diseases have a transitory erythematous macular rash early in their course, and recognition of this fact can be lifesaving. Infectious agents associated with illnesses in which macular exanthems have been observed are presented in Table 64–8.

The most common rash in infectious mononucleosis is erythematous and maculopapular, but rarely (most often in association with the administration of ampicillin) the exanthem is generalized, confluent, fiery red, and macular. Blotchy or diffuse erythematous macular rashes have been caused specifically by 12 different enterovirus types. Most of these descriptions involve neonates, other very young infants, and adults; children in the peak ages for enteroviral exanthematous diseases do not seem to have solely macular lesions. In neonates, enteroviral disease with a blotchy macular rash in association with fever and lethargy usually is confused with bacterial sepsis.

Patients with dengue, Lassa, and Marburg viral infections frequently have a macular, flushed appearance before other cutaneous manifestations develop. Similarly, in both murine and epidemic typhus, the initial skin manifestations are macular but progress rapidly to more pronounced findings.

Bacterial septicemia with both common and exotic organisms is associated frequently with a generalized flush. In staphylococcal disease, the rash is particularly apparent in endocarditis and osteomyelitis. The most famous disease with a macular rash is typhoid fever. Rose spots occur most commonly on the abdomen, but they also are seen on the chest and back. They are erythematous, macular lesions 2 to 4 mm in size. Lesions likewise have been noted in leptospirosis and psittacosis. In addition, rose spots are seen occasionally in septicemic illnesses caused by other *Salmonella* spp.

The slapped-cheek appearance in erythema infectiosum (Fig. 64–6) is caused by an erythematous macular flush of the cheeks. The full-blown rash in streptococcal scarlet fever is maculopapular, but frequently in mild cases and in those altered by antibiotic therapy, the exanthem is only macular in character (scarlatina).

TABLE 64–8 Infectious Agents Associated with Illness in Which a Macular Exanthem Has Been Observed

Infectious Agent	Illness
Human herpesvirus–6, –7	Roseola infantum
Epstein-Barr virus	Infectious mononucleosis
Coxsackieviruses Bl, B2, B5	—
Echoviruses 2, 4, 5, 14, 17-19, 30	—
Enterovirus 71	—
Dengue virus	Dengue fever
Lassa virus	Lassa fever
Marburg virus	Marburg fever
Parvovirus	Erythema infectiosum
HIV-1	Manifestation of acute infection
Hantavirus	Hemorrhagic fever with renal syndrome
Chlamydia psittaci	Psittacosis
Rickettsia typhi	Murine typhus
Rickettsia prowazekii	Epidemic typhus
Rickettsia quintana	Trench fever
Coxiella burnetii	Q fever
Mycoplasma pneumoniae	—
Staphylococcus aureus	Septicemia and toxic shock syndrome
Streptococcus pyogenes	Scarlatina and septicemia
Bacillus anthracis	Anthrax
Salmonella typhi	Typhoid fever
Salmonella species	Septicemic salmonellosis
Spirillum minus	Rat-bite fever
Leptospira species	Leptospirosis
Yersinia pestis	Plague

ERYTHEMATOUS MACULOPAPULAR EXANTHEMS

An erythematous maculopapular rash is the most common cutaneous manifestation of systemic infection (Figs. 64–2, 64–8, 64–10, and 64–13). It also is an exceedingly common occurrence in allergic conditions. However, all too frequently, the rash of an infectious illness is ascribed to an allergic reaction to an administered drug rather than correctly to the disease process. The converse—an allergic rash illness that is attributed mistakenly to an infectious agent—rarely occurs. Infectious agents associated with illnesses in which maculopapular exanthems occur are presented in Table 64–9.

Both by the number of possible etiologic agents and by total infections, viruses account for the vast majority of illnesses with maculopapular eruptions. Although the distribution and progression of rashes are important aspects relating to the differential diagnosis, the single most important point is whether the lesions are discrete (rubelliform) or confluent (morbilliform). Adenoviruses are not uncommon causes of erythematous maculopapular eruptions. In most instances, signs and symptoms of upper respiratory infection are present. Most commonly, the lesions are discrete, but occasionally, a confluent morbilliform rash is present. A roseola infantum picture—occurrence of rash after the fever falls by crisis—frequently occurs. As a rule, the exanthem in adenoviral infections starts on the head and spreads to the trunk and extremities.

Enteroviruses account for the greatest number of erythematous maculopapular rash illnesses; 36 different serologic types have been implicated. The enteroviral types most commonly associated with maculopapular exanthems are coxsackieviruses A9 and B5 and echoviruses 4, 9, and 16. Echovirus 9 has been the most frequent cause of enteroviral exanthem for the last 35 years (Fig. 64–13). Although morbilliform rashes do occur, the more usual cutaneous manifestation is one suggestive of rubella. The exanthem usually starts on the head and upper part of the trunk and spreads to the extremities.

Although they are not common manifestations of respiratory viruses (rhinoviruses, influenza A and B viruses, RSV, and para-

TABLE 64–9 Infectious Agents Associated with Illnesses in Which Maculopapular Exanthems Occur

Infectious Agent	Illness	Character of Rash	
		Discrete	Confluent
Parvovirus	Erythema infectiosum	+++	+
Human bocavirus		++++	
Adenoviruses 1, 2, 3, 4, 7, 7a		+++	+
Human herpesvirus–6	Roseola infantum	+++	+
Epstein-Barr virus	Infectious mononucleosis	+++	+
Cytomegalovirus		++++	
Vaccinia virus	Roseola vaccinatum	+++	+
Coxsackieviruses A2, A4, A5, A7, A9, A10, A16		+++	+
Coxsackieviruses B1-B5		+++	+
Echoviruses 1-7, 9, 11, 13, 14, 16-19, 22, 25, 30, 33		+++	+
Enterovirus 71		++++	
Rhinoviruses (many types)		++++	
Colorado tick fever virus	Colorado tick fever	++++	
Reoviruses 2, 3		++	++
Rotavirus	Gianotti-Crosti syndrome; infantile acute hemorrhagic edema	++++	
Alphaviruses: chikungunya, Sindbis, o'nyong-nyong fever, Ross River		++	++
Rubella virus	Rubella (German measles)	+++	+
Flavivirus: dengue, Kunjin, West Nile	Dengue, Kunjin fever	++	++
Influenza viruses A, B		++++	
Respiratory syncytial virus		++++	
Parainfluenza viruses 1-4		++++	
Mumps virus	Mumps	++++	
Measles virus	Measles	+	+++
Hepatitis B virus		++++	
Marburg virus	Marburg fever	++	++
Ebola virus	Ebola hemorrhagic fever	+++	+
Rickettsia akari	Rickettsialpox	++++	
Rickettsia typhi	Murine typhus	+++	+
Rickettsia prowazekii	Epidemic typhus	+++	+
Rickettsia tsutsugamushi	Scrub typhus	+++	+
Rickettsia rickettsi	Rocky Mountain spotted fever		++++
Ehrlichia species	Ehrlichiosis	+++	+
Mycoplasma pneumoniae		+++	+
Staphylococcus aureus (exfoliative toxin producing)	Staphylococcal scarlet fever		++++
Streptococcus pyogenes	Scarlet fever		++++
Arcanobacterium hemolyticum		++	++
Neisseria meningitidis	Meningococcemia	++++	
Moraxella catarrhalis		++++	
Listeria monocytogenes	Listeriosis	++++	
Streptobacillus moniliformis	Rat-bite fever	+++	+
Yersinia pseudotuberculosis			++++
Bartonella bacilliformis	Bartonellosis	++++	
Brucella species	Brucellosis	++++	
Legionella pneumophila	Legionnaires' disease	++++	
Bartonella henselae	Cat-scratch fever	+++	+
Treponema pallidum	Secondary syphilis	+++	+
Leptospira species	Leptospirosis	++++	
Borrelia species	Relapsing fever		++++
Coccidioides immitis	Coccidioidomycosis	+++	+
Toxoplasma gondii	Toxoplasmosis	++++	
Strongyloides stercoralis	Strongyloidiasis	++++	

influenza viruses types 1 through 4), exanthems probably occur more often than is generally realized. Because children infected with these agents frequently are given antibiotics, confusion often occurs between an allergic and an infectious etiology. With all the respiratory viruses, the signs and symptoms of respiratory illness (cough, coryza, croup, bronchiolitis, and so on) are prominent. The exanthems virtually are always discrete and rubelliform in character.

In dengue, the exanthem goes through several stages. Initially, it is macular, then erythematous maculopapular, and finally hemorrhagic. Similarly, the exanthems in the rickettsial diseases go through stages that vary in relation to the specific agent (see Table 64–2). In Rocky Mountain spotted fever, the rash starts on the distal ends of extremities. Although the hallmark of meningococcemia is a petechial or purpuric rash, in the initial stages, the exanthem may be erythematous and maculopapular. In addition, maculopapular eruptions are observed in chronic meningococcemia. The most notable cutaneous lesion in coccidioidomycosis is erythema nodosum, but a rubelliform rash early in infection is not an unusual manifestation.

VESICULAR EXANTHEMS

The three main categories of vesicular exanthems are single or localized lesions, generalized lesions in greatest concentration on

TABLE 64–10 Infectious Agents Associated with Illnesses in Which Vesicular Exanthems Occur

Infectious Agent	Illness
Human parvovirus B19	
Herpes simplex virus types 1 and 2	Cold sores, genital herpes, or neonatal herpes
Varicella-zoster virus	Chickenpox (varicella) or herpes zoster
Vaccinia virus	Disseminated vaccinia or eczema vaccinatum
Variola virus	Smallpox
Monkeypox virus	
Orf virus	Ecthyma contagiosum
Tanapox virus	
Coxsackieviruses A4, A5, A8, A10, A16	
Coxsackieviruses Bl-B3	
Echoviruses 6, 9,11, 17	
Enterovirus 71	
Reovirus 2	
Calicivirus of oceanic origin	
Alphaviruses: chikungunya, o'nyong-nyong fever, Ross River, Sindbis	
Kunjin virus	
Mumps virus	Mumps
Measles virus	Atypical measles
Rickettsia akari	Rickettsialpox
Rickettsia tsutsugamushi	
Mycoplasma pneumoniae	
Streptococcus pyogenes	Impetigo
Pseudomonas aeruginosa	
Brucella species	Brucellosis
Bacillus anthracis	Anthrax
Mycobacterium tuberculosis	Papulonecrotic tuberculids
Candida albicans	Congenital cutaneous candidiasis
Leishmania braziliensis	American cutaneous leishmaniasis
Necator americanus	Hookworm disease

TABLE 64–11 Infectious Agents Associated with Illness in Which Petechial and Purpuric Exanthems Occur

Infectious Agent	Illness
Human parvovirus B19	Glove and socks syndrome
Varicella-zoster virus	Hemorrhagic chickenpox
Cytomegalovirus	Congenital cytomegalovirus infection
Variola virus	Hemorrhagic smallpox
Coxsackieviruses A4, A9	
Coxsackieviruses B2-B4	
Echoviruses 4, 7, 9	
Colorado tick fever virus	Colorado tick fever
Rotavirus	
Alphaviruses: chikungunya, o'nyong-nyong fever, Ross River, Sindbis	
Rubella virus	Rubella (German measles) or congenital rubella
Respiratory syncytial virus	
Measles virus	Hemorrhagic (black measles) or atypical measles
Lassa virus	Lassa fever
Marburg virus	
Hepatitis C virus	Mixed cryoglobulinemia
Hantavirus	Hemorrhagic fever with renal syndrome
Rickettsia typhi	Murine typhus
Rickettsia prowazekii	Epidemic typhus
Rickettsia rickettsii and other tick-borne rickettsiae	Rocky Mountain spotted fever
Ehrlichia species	Ehrlichiosis
Mycoplasma pneumoniae	
Streptococcus pyogenes	Scarlet fever or septicemia
Streptococcus pneumoniae	Pneumococcal septicemia
Enterococcal and viridans group streptococci	Endocarditis
Neisseria gonorrhoeae	Gonococcemia
Neisseria meningitidis	Meningococcemia
Moraxella catarrhalis	
Haemophilus influenzae	*H. influenzae* septicemia
Pseudomonas aeruginosa	Ecthyma gangrenosa
Streptobacillus moniliformis	
Yersinia pestis	Septicemic plague (black death)
Bartonella henselae	Cat-scratch fever
Treponema pallidum	Congenital syphilis
Borrelia species	Relapsing fever
Toxoplasma gondii	Congenital toxoplasmosis
Trichinella spiralis	Trichinosis

the trunk and head, and generalized lesions with the greatest concentration on the extremities (Figs. 64–4, 64–5, and 64–9). Infectious agents associated with illnesses in which vesicular rashes develop are presented in Table 64–10. The exanthem in primary or recurrent HSV infection is localized, as it is in recurrent endogenous varicella-zoster infection (herpes zoster), ecthyma contagiosum, tanapox, scrub typhus, anthrax, and papulonecrotic tuberculids (Fig. 64–5).

The vesicular exanthematous disease that occurs most commonly in children today is chickenpox (Fig. 65–4). It should be a readily recognizable disease, but is all too frequently confused with enteroviral infections or insect bites and allergic conditions. Chickenpox has a long incubation period (16 days) and is associated with mild fever and an exanthem that starts on the head and upper part of the trunk and spreads to the extremities. The rash always is more prominent on the trunk than on the extremities. At any time during the first few days of the rash, lesions in all stages (macules, papules, and vesicles) can be seen. Individual lesions in chickenpox form scabs that persist for approximately 7 days.

In contrast to that of chickenpox, the exanthem in enteroviral infections frequently is peripheral in distribution, and the lesions generally heal without scabs. The incubation period (5 days) is much shorter than that of chickenpox. The hand, foot, and mouth syndrome is a common manifestation of enteroviral vesicular rash illnesses (Figs. 64–9 to 64–11). The most frequent etiologic agent in the hand, foot, and mouth syndrome is coxsackievirus A16, but the syndrome also has been attributed to coxsackieviruses A5, A9, A10, B1, and B3 and enterovirus 71.

Enteroviral infections with vesicular exanthems in which the hand, foot, and mouth distribution is not present quite frequently are diagnosed erroneously as insect bites or poison ivy.

PETECHIAL AND PURPURIC EXANTHEMS

A large number of infectious agents are associated with petechial and purpuric skin manifestations (Figs. 64–14 and 64–15). They are listed in Table 64–11. Infectious diseases with hemorrhagic rash can be fulminant fatal events or relatively benign illnesses. On a worldwide basis, meningococcemia is perhaps the most important and feared, although it is not the most prevalent of the petechial and purpuric exanthematous diseases. The relatively sudden onset of fever and a petechial rash must be considered and treated as meningococcemia unless another etiology can be established with absolute certainty. The most important of the

differential diagnostic problems is exanthem caused by enteroviral infection. Many different entero-virus illnesses have a sudden onset with accompanying fever and petechial rash. In addition, the situation frequently is complicated further by the occurrence of meningitis. The most important enterovirus in its ability to mimic meningococcemia is echovirus 9.

Purpuric and petechial lesions in infectious illnesses can result from a direct or indirect (immunologic) effect of the infectious agent at the cutaneous site or from the occurrence of thrombocytopenia. Thrombocytopenia is noted most commonly in acquired rubella virus infections.

URTICARIAL EXANTHEMS

The occurrence of urticaria all too frequently leads the physician to suspect an allergic or dermatologic condition (Figs. 64–12 and 64–16).[199,200] However, what has become quite evident in recent years is that when urticaria develops in association with an acute febrile illness, the cutaneous reaction is a direct effect of an infectious agent, and its mediation does not require an allergic response. Listed in Table 64–12 are infectious agents associated with urticarial exanthems.

Papular urticaria occurs very commonly in children in the summer and fall and most frequently is the result of insect bites (see Table 64–6). However, virtually identical lesions occur in infections with coxsackievirus A as well as with other enteroviruses (Fig. 64–12). The main point for differentiation is that fever regularly develops in the virus-induced exanthems but is not a characteristic associated with insect bites.

Early in the course of meningococcemia, the exanthem can be urticarial, so an illness of sudden onset with fever and this cutaneous manifestation never should be taken lightly.

TABLE 64–12 Infectious Agents Associated with Illness in Which Urticarial Exanthems Occur

Infectious Agent	Illness
Epstein-Barr virus	Infectious mononucleosis
Coxsackieviruses A9, A16, B4, B5	
Echovirus 11	
Mumps virus	Mumps
Hepatitis B virus	
Hepatitis C virus	
Mycoplasma pneumoniae	
Neisseria meningitidis	Meningococcemia
Shigella sonnei	Shigellosis
Yersinia enterocolitica	Yersiniosis
Borrelia burgdorferi	Lyme disease
Plasmodium species	Malaria
Coxiella burnetii	Q fever
Giardia lamblia	Giardiasis
Entamoeba histolytica	Amebiasis
Trichomonas vaginalis	Vulvovaginalis
Enterobius vermicularis	Pinworm infestation
Necator americanus	Hookworm disease
Trichinella spiralis	Trichinosis
Schistosoma species	Schistosomiasis
Trichobilharzia species	Swimmer's itch or collector's itch
Wuchereria bancrofti	Filariasis
Echinococcus species	Echinococcosis
Sarcoptes scabiei	Scabies
Trombicula irritans	Chigger bites
Other mites	Mite bites
Pediculus humanus	Pediculosis
Bedbugs, kissing bugs, ants, fleas, flies, and mosquitoes	Bites and stings

PAPULAR, NODULAR, AND ULCERATIVE LESIONS

In many instances, the lesions in this category occur as single events at the site of primary inoculation. Specific illnesses and etiologic agents are listed in Table 64–13.

DISTINCTIVE CLINICAL FEATURES OR SYNDROMES

(Figs. 64–19 to 64–24)

Erythema Multiforme

Erythema multiforme is a self-limited skin eruption that is erythematous and characterized by distinctive target or iris lesions or both. Small vesicles and urticarial areas also may develop. On occasion, the disease is severe and associated with mucosal involvement and genital lesions. In this latter illness—the Stevens-Johnson syndrome, bullous erythema multiforme, erythema multiforme exudativum major—severe ulcerative, oral, and genital lesions occur; generalized exanthems become bullous, and

TABLE 64–13 Infectious Agents Associated with Papular, Nodular, and Ulcerative Lesions

Agent	Illness
Wart virus	Warts (P and N)
Orf virus	Ecthyma contagiosum (N)
Molluscum contagiosum virus	Molluscum contagiosum (P and N)
Hepatitis B virus	Gianotti-Crosti syndrome (P)
Paravaccinia virus	Milker's nodules (N)
Francisella tularensis	Tularemia (U)
Haemophilus ducreyi	Chancroid (U)
Bartonella bacilliformis	Bartonellosis (N)
Calymmatobacterium granulomatis	Granuloma inguinale (N and U)
Pseudomonas aeruginosa	Ecthyma gangrenosa (U) *Pseudomonas* folliculitis (P)
Burkholderia mallei	Glanders (N and U)
Mycobacterium tuberculosis	Lupus vulgaris (N) Papulonecrotic tuberculids (U)
Atypical mycobacteria	(U)
Mycobacterium leprae	(N)
Treponema pallidum	Chancre (U)
Treponema pertenue	Yaws (P and U)
Sporotrichum schenckii	Sporotrichosis (U)
Blastomyces dermatitidis	Blastomycosis (N and U)
Fusarium species	Opportunistic infection (N)
Scedosporium species	Opportunistic infection (N)
Candida albicans	Systemic candidiasis (N)
Leishmania tropica	Oriental sore (N and U)
Leishmania braziliensis and *mexicana*	American cutaneous leishmaniasis (P and U)
Trypanosoma species	Trypanosomiasis (N)
Necator americanus	Hookworm disease (P)
Schistosoma species	Schistosomiasis (P)
Trichobilharzia species	Swimmer's itch or collector's itch (P)
Onchocerca volvulus	Onchocerciasis (P)
Loxosceles reclusa	Recluse spider bites (U)
Ticks	Tick bites (U)
Sarcoptes scabiei	Scabies (P)
Trombicula irritans	Chigger bites (P)
Other mites	Mite bites (P)
Cimex lectularius	Bedbug bites (P)
Triatoma sanguisuga	Kissing bug bites (P and N)
Solenopsis saevissima	Fire ant bites (P and N)
Fleas	Flea bites (P)
Flies and mosquitoes	Fly and mosquito bites (P)

N, nodular; P, papular U, ulcerative.

Figure 64–19 An erythematous maculopapular discrete and confluent rash on the thigh and arm of a 16-year-old girl with pharyngitis and *Arcanobacterium haemolyticum* isolated from her throat. (See companion Expert Consult web site for color version.) *(From Mackenzie, A., Fuite, L. A., Chan, F. T. H., et al.: Incidence and pathogenicity of Arcanobacterium haemolyticum during a 2-year study in Ottawa. Clin. Infect. Dis. 21:177-181, 1995.)*

Figure 64–20 Numerous flat-topped and dome-shaped, slightly erythematous papules over the skin of the perineum of a young girl with bowenoid papulosis. (See companion Expert Consult web site for color version.)

conjunctivitis is present. The illness is associated with fever and general distress.

Although the pathogenesis of erythema multiforme is unknown, what is clear is that multiple factors, including infectious agents, are responsible for its occurrence. Infectious agents associated with erythema multiforme are listed in Table 64–14. The single most important infectious cause of erythema multiforme and Stevens-Johnson syndrome is *M. pneumoniae*. When *M. pneumoniae* is the instigating agent, the patient nearly always has concomitant pneumonia.

HSV frequently has been recovered from the throats of persons with erythema multiforme, but the cause-and-effect relationship in many cases must be questioned. However, in a recent study, HSV DNA was found in the skin lesions of 11 of 31 patients with erythema multiforme.[58]

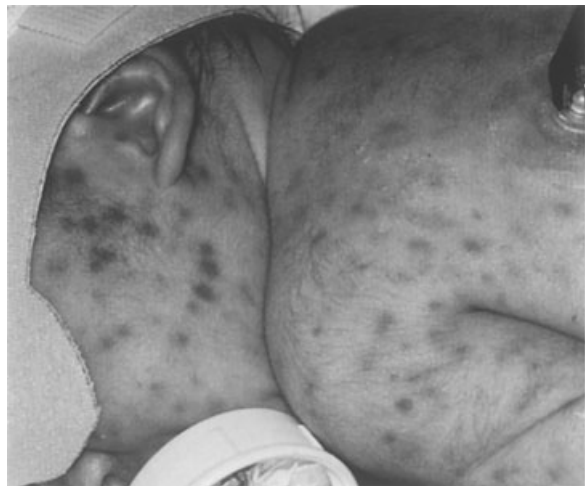

Figure 64–21 Numerous isolated purple papules of dermal erythropoiesis overlying the icteric skin of a neonate with congenital cytomegalovirus infection. (See companion Expert Consult web site for color version.)

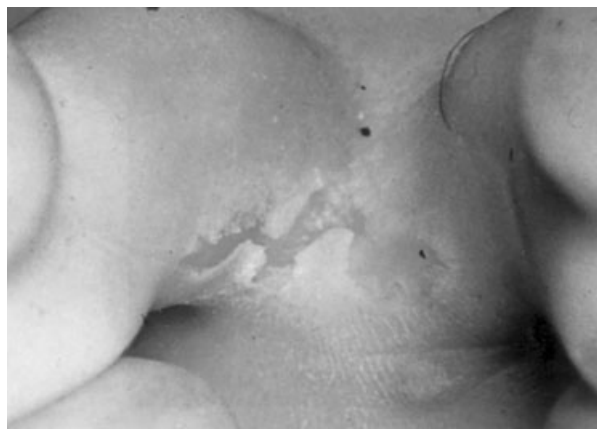

Figure 64–22 Tinea pedis. Peeling, macerations, and fissuring in the fourth interdigital space of the foot are characteristic of dermatophytic infections. (See companion Expert Consult web site for color version.)

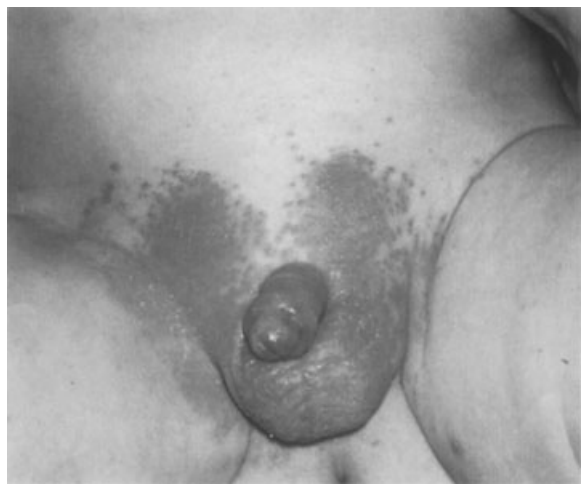

Figure 64–23 Clinical photograph of an infant with *Candida* diaper dermatitis. Confluent and discrete erythematous papules and plaques involving the scrotum, penis, and suprapubic and inguinal area are evident. (See companion Expert Consult web site for color version.)

TABLE 64–14 Infectious Agents Associated with Erythema Multiforme

Agent	Illness
Human parvovirus B19	Erythema infectiosum
Adenovirus 7	Respiratory infection
Herpes simplex virus type 1	Perioral or respiratory infection
Epstein-Barr virus	Infectious mononucleosis
Varicella virus	Chickenpox
Coxsackieviruses A10, A16, B5	Enterovirus syndrome
Echovirus 6	Enterovirus syndrome
Poliomyelitis virus	Poliomyelitis
Vaccinia virus	Smallpox vaccination
Variola virus	Smallpox
Orf virus	Ecthyma contagiosum
Paravaccinia virus	Milker's nodules
Influenza A virus	Influenza
Mumps	Mumps
Hepatitis B virus	Serum hepatitis
Chlamydia psittaci	Psittacosis
Chlamydia trachomatis	Lymphogranuloma venereum
Mycoplasma pneumoniae	Respiratory symptoms
Staphylococcus aureus	Septicemia
Streptococcus pyogenes	Respiratory symptoms
Neisseria gonorrhoeae	Gonorrhea
Corynebacterium diphtheriae	Diphtheria
Pseudomonas aeruginosa	Septicemia
Salmonella species	Gastroenteritis
Francisella tularensis	Tularemia
Yersinia species	Gastrointestinal symptoms
Vibrio parahaemolyticus	Gastroenteritis
Treponema pallidum	Syphilis
Bartonella henselae	Cat-scratch fever
Mycobacterium tuberculosis	Tuberculosis
Mycobacterium leprae	Leprosy
Coccidioides immitis	Coccidioidomycosis
Histoplasma capsulatum	Histoplasmosis
Trichomonas vaginalis	Vulvovaginalis

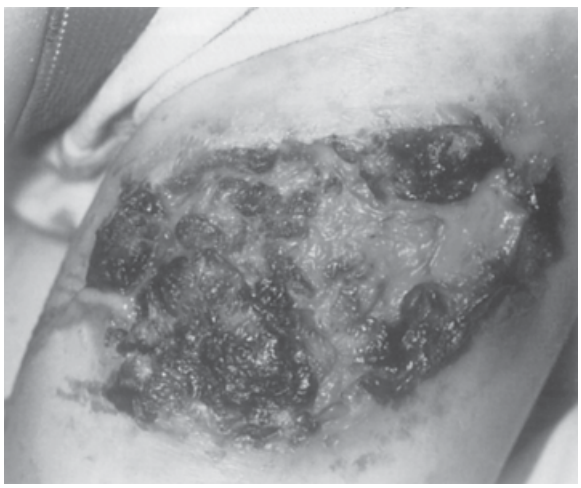

Figure 64–24 An extensive crusted erosion on the left thigh of a child with a cryptococcal skin infection. (See companion Expert Consult web site for color version.)

Erythema Nodosum

Erythema nodosum most commonly occurs on the anterior aspect of the lower part of the legs but may be seen anywhere on the body. The lesions are raised, erythematous, and painful to touch. Their usual size is approximately 2 to 4 cm, with a duration of 2 to 6 weeks.

TABLE 64–15 Infectious Agents Associated with Erythema Nodosum

Agent	Illness
Herpes simplex virus	Perioral or respiratory infection
Epstein-Barr virus	Infectious mononucleosis
Chlamydia psittaci	Psittacosis
Chlamydia trachomatis	Lymphogranuloma venereum
Streptococcus pyogenes	Respiratory infection
Neisseria meningitidis	Meningococcemia
Corynebacterium diphtheriae	Diphtheria
Campylobacter species	Gastroenteritis
Haemophilus ducreyi	Chancroid
Salmonella species	Salmonellosis
Yersinia species	Gastrointestinal symptoms
Brucella species	Brucellosis
Treponema pallidum	Syphilis
Bartonella henselae	Cat-scratch fever
Mycobacterium tuberculosis	Tuberculosis
Mycobacterium leprae	Leprosy
Trichophyton species	Kerion of scalp
Histoplasma capsulatum	Histoplasmosis
Cryptococcus neoformans	Cryptococcosis
Coccidioides immitis	Coccidioidomycosis
Blastomyces dermatitidis	Blastomycosis
Ascaris lumbricoides	Roundworm infestation
Wuchereria bancrofti	Filariasis

Erythema nodosum occurs less commonly today than it did 4 decades ago, and the frequency of specific associated infectious agents also is different. In the past, streptococcal and mycobacterial infections were the agents most commonly related. Now, the exanthem most often is associated with respiratory infection with *Histoplasma capsulatum*, *Cryptococcus neoformans*, and *Coccidioides immitis*. Infectious agents associated with erythema nodosum are listed in Table 64–15.

Hand, Foot, and Mouth Syndrome

The hand, foot, and mouth syndrome is a clearly recognizable viral illness characterized by vesicular lesions in the anterior of the mouth and on the hands and feet in association with fever. Although several enteroviruses (coxsackieviruses A5, A9, A10, A16, B1, and B3 and enterovirus 71) have been implicated, as have HSV and foot and mouth disease virus, most of these cases are caused by coxsackievirus A16.

Roseola-like Illness

Roseola infantum is a classic pediatric illness characterized by fever of 3 to 5 days' duration, rapid defervescence, and then the appearance of an erythematous macular or maculopapular rash that persists for 1 to 2 days. Roseola is an age-related response to infection with many viruses. Recent studies suggest that a leading cause of roseola infantum is primary infection with HHV-6. The following other viruses have been noted in association with roseola: adenoviruses 1, 2, 3, and 14; coxsackieviruses A6, A9, B1, B2, B4, and B5; echoviruses 9, 11, 16, 25, 27, and 30; parainfluenza virus type 1; and measles vaccine virus.

Rocky Mountain Spotted Fever–like Illness

Rocky Mountain spotted fever is a clinical illness characterized by fever and a petechial rash located mainly on the distal ends of extremities. The illness is caused by *Rickettsia rickettsii* and is prevalent in many areas of North America; the infectious agent is transmitted to humans by ticks. In other areas of the world,

other tick-borne rickettsiae *(Rickettsia sibirica*, *Rickettsia australis*, *Rickettsia conorii)* produce similar human illness. Infection with *Ehrlichia canis* also can cause an illness similar to Rocky Mountain spotted fever.

The most important illness confused with Rocky Mountain spotted fever is atypical measles (see Chapter 192). This illness, which has both the constitutional symptoms of Rocky Mountain spotted fever and a rash most prominent on the extremities, occurs almost exclusively after exposure to measles virus in some persons previously immunized with inactivated (killed) measles vaccine.

Rat-bite fever caused by *S. moniliformis* also has been misdiagnosed as Rocky Mountain spotted fever.[156]

Exanthem and Meningitis

Aseptic and also bacterial meningitis frequently are characterized by both exanthem and symptoms and signs of neurologic involvement. Infectious agents associated with exanthem and meningitis are presented in Table 64–16. Of most importance in this category is the differential diagnosis of enteroviral syndromes and meningococcemia.

Exanthem and Pulmonary Involvement

Infectious agents associated with exanthem and pulmonary involvement are listed in Table 64–17. In patients older than 5 years old, the leading cause of exanthem and pneumonia is *M. pneumoniae* infection. In younger children, adenoviruses are the most important etiologic agents. With the exception of enteroviral infections, which are more likely to involve young children, most of the illnesses listed in Table 64–17 occur in older children and young adults.

TABLE 64–16 Infectious Agents Associated with Exanthem and Meningitis

Agent	Illness
Herpes simplex virus type 2	Recurrent genital herpes
Coxsackieviruses A2, A9, B1, B4, B5	Enterovirus syndrome
Echoviruses 4, 6, 9, 11, 14, 17, 25, 33	Enterovirus syndrome
Colorado tick fever virus	Colorado tick fever
Reovirus 2	Respiratory infection
West Nile virus	Meningoencephalitis
Neisseria meningitidis	Meningococcemia
Borrelia burgdorferi	Lyme disease
Listeria monocytogenes	Listeriosis
Toxoplasma gondii	Toxoplasmosis

TABLE 64–17 Infectious Agents Associated with Exanthem and Pulmonary Involvement

Agent	Illness
Adenoviruses 7,7a	Respiratory infection
Herpes simplex virus type 1	Respiratory infection
Varicella-zoster virus	Chickenpox pneumonia
Epstein-Barr virus	Infectious mononucleosis
Coxsackievirus A9	Enterovirus syndrome
Echovirus 11	Enterovirus syndrome
Reovirus 3	Respiratory infection
Measles virus	Measles pneumonia and atypical measles
Chlamydia psittaci	Psittacosis
Mycoplasma pneumoniae	*M. pneumoniae* pneumonia
Neisseria meningitidis	Meningococcal pneumonia
Mycobacterium tuberculosis	Tuberculosis
Histoplasma capsulatum	Histoplasmosis
Cryptococcus neoformans	Cryptococcosis
Coccidioides immitis	Coccidioidomycosis

Gianotti-Crosti Syndrome (Papular Acrodermatitis)

Gianotti-Crosti syndrome is a distinct clinical entity characterized by a papular (lichenoid) exanthem, generalized lymphadenopathy, hepatomegaly, and acute anicteric hepatitis.[46,170,173] In most instances, this illness has been associated with hepatitis B virus infection. The syndrome also has been noted in association with Epstein-Barr virus, cytomegalovirus, coxsackievirus B virus, and RSV infections.[65,111,171,193]

Cutaneous Manifestations Associated with Infections in Immunocompromised Patients

All infectious agents that cause exanthems in immunologically normal children can cause infections in immunocompromised children. However, the clinical manifestations may be different. For example, measles virus infection in a child who is T-cell-deficient may be associated with a severe, progressive pneumonia but not the typical rash. Other viral exanthems that are self-limited in normal children, such as varicella, may be progressive and develop into hemorrhagic skin lesions with disseminated organ involvement in children with T-cell-deficiency.

Of particular concern are bacterial and fungal infections, which are rarely a problem in normal children but are rapidly fatal in granulocytopenic children. These patients have characteristic skin lesions resulting from disseminated infections. Of importance are ecthyma gangrenosa resulting from *Pseudomonas aeruginosa* septicemia and the nodular and purpuric lesions of disseminated fungal infections caused by *Aspergillus*, *Candida*, and other less common agents.

DIAGNOSIS

DIFFERENTIAL DIAGNOSIS

The diagnosis of infectious exanthems frequently is considered an impossible task by many physicians. Other physicians glibly call the first maculopapular exanthem of childhood "roseola" and the first vesicular rash "chickenpox" without consideration of more appropriate choices. The hallmark of diagnosis in exanthematous disease is careful elicitation of historic data. Differential diagnosis requires the consideration of noninfectious etiologies as well as different infectious agents. Listed in Table 64–18 are the major considerations in the diagnosis of diseases with cutaneous manifestations.

A history of exposure is most important in making a differential diagnosis. For example, was the patient exposed to poison ivy, insects, or a person ill with a specific disease? In infectious illnesses with high clinical expression rates (measles, chickenpox, rubella), proper questioning usually reveals a contact case or at least other cases in the community. On the other hand, in ill-

TABLE 64–18 Important Aspects in the Diagnosis of Exanthematous Illness

Exposure	Types of Rash
Season	Distribution of rash
Incubation period	Progression of rash
Age	Exanthem
Previous exanthems	Other associated symptoms
Relationship of rash to fever	Laboratory tests
Adenopathy	

From Cherry, J. D.: Newer viral exanthems. Adv. Pediatr. 16:233-286, 1969.

nesses with low rates of clinical expression of exanthem, such as adenoviral and some enteroviral infections, the source may not be apparent.

Consideration of the seasonal occurrence of different infectious agents, as well as insects, is particularly useful in making a differential diagnosis. In temperate climates, enteroviral and arthropod-mediated diseases occur in the summer and fall. Exanthems with measles, varicella-zoster, and rubella viruses occur most often in the winter and spring. The diagnosis of rubella is important because of fetal consequences. All too frequently, rubella is overdiagnosed and underdiagnosed, both of which can be avoided if its seasonal prevalence is understood.

The incubation period is important in separating the exanthem caused by rubella, varicella-zoster, or measles viruses from rash illnesses caused by enteroviruses or common respiratory viruses. The former have long incubation periods, whereas in the others, the period from exposure to the onset of illness is less than 1 week. Age can be useful. Today in the United States, measles and rubella often are illnesses of adolescents and young adults. Enteroviral exanthem frequency is related inversely to age.

Questioning to obtain a pertinent history of previous exanthems can give useful information if it is done with care. For example, if patients are asked whether they had rubella, the answer is quite unreliable. However, if the past illness is documented by year, season, and symptoms, accurate information often is obtained. The relationship of rash to fever is most significant in the diagnosis of roseola. The presence or absence of fever is important in separating exanthems of infectious and noninfectious etiology. Frequently, insect bites are diagnosed as chickenpox by parents and physicians as well. Chickenpox rarely occurs without fever.

The type and distribution of exanthem obviously are important. They virtually are diagnostic in hand, foot, and mouth syndrome, Rocky Mountain spotted fever, and atypical measles. Enanthem can lead to a specific diagnosis (Koplik spots in measles [Fig. 64–1]) or a category diagnosis (herpangina in enteroviral infections). Other characteristics, such as those listed in Tables 64–8 through 64–17, obviously are useful in delineating a specific illness.

SPECIFIC DIAGNOSIS

As with other infectious diseases, establishing specific diagnosis depends on the acquisition of proper cultures, serologic tests, and microscopic study of secretions or histologic or cytologic preparations. These techniques are discussed in other chapters of this book.

Vesicular lesions always should be scraped for cytologic study or direct antigen identification (varicella, herpes simplex), and, frequently, petechial lesions should be scraped and stained in a search for infectious agents (meningococci). The etiology of viral infections can be established by isolation of virus, direct antigen detection, or serologic methods. In most instances, a virus recovered from the throat indicates acute infection and is the probable cause of a particular illness. Serologic study without culture is useful in diagnosing rickettsial diseases, some viral infections, and a few illnesses of bacterial origin. Serologic study without virus isolation generally is not useful in diagnosing enteroviral illnesses.

TREATMENT, PROGNOSIS, AND PREVENTION

The treatment, prognosis, and prevention of exanthematous diseases are presented in appropriate chapters throughout this text.

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CHAPTER 65

Roseola Infantum (Exanthem Subitum)

James D. Cherry

Roseola infantum (i.e., exanthem subitum, pseudorubella, exanthem criticum, sixth disease, or 3-day fever) is a common, acute illness of young children characterized by a fever of 3 to 5 days' duration, rapid defervescence, and then the appearance of an erythematous macular or maculopapular rash that persists for 1 to 2 days.

HISTORY

Zahorsky[80] generally is given credit for the original description of roseola infantum. In his writings, he pointed out, however, that the syndrome was described in earlier pediatric and dermatology texts.[81-83] Altschuler[1] observed that a British dermatologist, Willan, presented a description of the illness in his 1809 book *On Cutaneous Diseases*. The descriptions in the older literature did not separate the syndrome from the known exanthematous diseases (i.e., measles, rubella, and scarlet fever), an omission that Zahorsky corrected.

In 1921, Veeder and Hempelmann[75] described the syndrome further and noted that leukopenia and relative lymphocytosis occurred. These investigators objected to the name *roseola infantum*, which in the past had been used to describe a large group of diseases with indefinite causes. They suggested the term *exanthem subitum* because it was "descriptive of the most striking clinical symptom, namely, the sudden, unexpected appearance of the eruption on the fourth day." Currently, the term *roseola* is used most commonly to describe the syndrome.

From 1920 through 1940, many excellent clinical descriptions of the syndrome were published.* From 1940 through 1988, articles relating to roseola were concerned with unusual manifestations and complications[8,11-13,22,32,46,48,49,53,56,63] and attempts to recover an etiologic agent.[26,30,40,45,64] In 1988, Yamanishi and associates[78] identified human herpesvirus–6 (HHV-6) in the blood of infants with roseola, and since then, the association between this virus and the disease has been confirmed on many occasions.† HHV-7 also has been found to be the cause of many cases of roseola.[3,14,31,68,71,72,74]

*See references 6, 7, 10, 17, 18, 20, 24, 25, 36, 83, 84.
†See references 2-5, 9, 19, 21, 27, 28, 33-35, 39, 41-44, 54, 58, 60, 66-69, 76, 85.

EPIDEMIOLOGY

In his original article, Zahorsky[80] reported that roseola occurred most commonly in the fall. In his second article, he observed a year-round incidence[81]; in 1925, he pointed out that most cases occurred in the spring, summer, and fall.[82] Breese[10] noted that the greatest number of cases occurred in the summer and early fall. In contrast, 55 percent of Clemens' cases occurred in February, March, and April; 16 percent were seen in October.[17] In a review of 243 cases during a 10-year period, Juretic[38] observed that the peak month was May. Juretic also reviewed the seasonal incidence in 10 other studies and found only minor variations by month. Prevalence was greatest in March, April, and October and least in December. One epidemic of roseola in a maternity hospital occurred in the summer,[36] another epidemic in an infants' home occurred in the fall,[6] and a hospital outbreak occurred in the winter.[18]

Roseola predominantly is an illness of young children. It occurs rarely in infants younger than 3 months old or children older than 4 years. In a review of 1462 cases, the peak age range prevalence was 7 to 13 months of age; 55 percent of the cases occurred within the first year of life, and 90 percent occurred within the first 2 years of life.[38] Occasionally, cases have been seen in older children, adolescents, and young adults and in neonates and other infants younger than 6 months.[25,36]

Although Faber and Dickey[20] found twice as many girls as boys with the syndrome, the sex ratio in most large studies has been equal.[7,10,17,25,45] Although three epidemics have been reported, and cases frequently occur in groups by season, most cases occur sporadically without known exposure. The syndrome, when seen sporadically, generally is considered to be noncontagious, but secondary cases have been reported occasionally.[6,10,18,36] The incubation period range in epidemics is 5 to 15 days.[6,10,18]

The attack rate of roseola has not been well studied. Berenberg and associates[7] stated that roseola is the exanthem most commonly encountered in children younger than 2 years old. Breese[10] found that 16 percent of a group of infants he followed for the first 12 months of life had definite roseola. He estimated that 30 percent of children would have clinical roseola. Juretic[38] looked at the frequency of roseola in 6735 children; the yearly attack rate during a 10-year period ranged from 1 to 10 percent, with a mean of 3.3 percent.

ETIOLOGY

In 1941, Breese[10] reported vigorous attempts to isolate a filterable virus from three children with pre-eruptive roseola. These studies included extensive animal inoculations, but no viral agents were uncovered. In 1950, Kempe and associates[40] reported the passage of the illness to a 6-month-old susceptible infant by the intravenous injection of serum from an 18-month-old child with pre-eruptive roseola. Febrile illnesses without exanthem also were produced in monkeys with serum and throat washings from a child with the syndrome. In similar experiments, Hellström and Vahlquist[30] produced the syndrome in three children aged 6 to 9 days after the intramuscular administration of blood from typical roseola cases.

In electron microscopic studies, Reagan and associates[61] observed uniform virus-like particles (100-110 nm) in the blood of an 18-month-old child with the syndrome. Febrile illness was produced in two monkeys after concentrated virus-containing material was inoculated.

Since the advent of modern diagnostic virology in the early 1950s, numerous viral agents have been recovered from children with roseola. In 1951, Neva and associates[52] studied an epidemic exanthematous illness (i.e., Boston exanthem) caused by echovirus 16, in which many of the illnesses were characteristic of roseola. In 1954, Neva[50] observed additional cases of roseola-like illness associated with echovirus 16 infection. In 1974, Hall and colleagues[27] reported four additional echovirus 16 infections with clinical manifestations of roseola. The reporting of roseola in Rochester, New York, nearly doubled during the time of echovirus 16 activity in the area. Roseola-like illnesses that also have been associated with these enteroviruses are caused by coxsackievirus A6, A9, B1, B2, B4, and B5 and echovirus 9, 11, 25, 27, and 30.[15,16,27,66,77] Outbreaks of roseola that occur in the summer and fall probably are caused by enteroviral infections.

In addition to enteroviruses, adenovirus types 1, 2, 3, and 14 and parainfluenza type 1 virus have been recovered from children with roseola.[23,37,51,77] Saitoh and associates[64] detected rotavirus capsomeres in fecal specimens of nine children with roseola. In contrast to these findings, Gurwith and colleagues[26] studied fecal specimens from five children with roseola, and in none were viral particles identified. One of 13 children in this study did develop antibody to rotavirus around the time of illness, however. In addition to the occurrence of roseola associated with numerous natural viral infections, its pattern (i.e., fever and then rash with defervescence) was observed frequently in recipients of Edmonton B measles vaccine.[15]

In 1988, Yamanishi and associates[78] isolated HHV-6 from four infants with roseola, and all four had significant titer increases for this virus. Shortly after this finding was reported, several other investigators noted similar findings.[2,4,19,33,42,69,73] The implication from these studies, as suggested by the various investigators, is that HHV-6 is the cause of roseola. This viewpoint overlooks or ignores the past experience in which other viral agents have been associated with the clinical syndrome. Subsequent studies indicate that HHV-6 is a major cause of roseola and the cause of acute febrile illness without exanthem in infants.* Since 1993, HHV-7 has been accepted as an additional causative agent in roseola.[3,14,31,68,71,76]

In a study of 1653 infants and young children with acute febrile illnesses, Hall and colleagues[27] found that 160 (9.7%) had primary HHV-6 infections; 27 (17%) of the children who were infected with HHV-6 had roseola. Zerr and associates[85] identified 80 children with primary HHV-6 infections, and of these, 30 percent had roseola. In the same population-based study, 3 of 80 (4%) children without primary HHV-6 infection also had roseola. In a study of clinical roseola, Okada and associates[54] found that 81 percent had serologic evidence of HHV-6 infection, and that 8 percent had an echovirus-18 infection. In a study of roseola in Italy, Braito and Uberti[9] found serologic evidence of HHV-6 infection in only 30 percent of the cases. In 33 percent of the remaining cases, they attributed the illnesses to another infectious agent. In 1994, Hidaka and associates[31] estimated that 73.5 percent, 10.2 percent, and 16.3 percent of their roseola cases were caused by HHV-6, HHV-7, and other viruses. HHV-6 seems to be the major cause of roseola.

PATHOPHYSIOLOGY

The pathophysiology of roseola is unknown. Watson[77] suggested that roseola is not an infection caused by one particular pathogen, but is the result of an immunizing reaction against many different viruses. He also suggested that the rash is caused by the neutralization of virus in the skin at the end of the period of viremia.

Because viremia is common in HHV-6, HHV-7, enteroviral, and adenoviral infections, a reasonable conclusion is that the rash in roseola is related to an immunologic event resulting from the virus that is localized in the skin. Why the pattern of fever and then rash with defervescence is so clearly age-dependent is

*See references 5, 9, 27, 34, 41, 44, 54, 58, 60, 67, 76, 85.

unknown. Most of the viruses that in the past have been associated with roseola cause other exanthematous manifestations in older patients.[15]

CLINICAL PRESENTATION

The basic clinical pattern of roseola is a febrile period of 3 to 5 days, defervescence, and the appearance of a rash that persists for 1 to 2 days. Because the syndrome is caused by many different viruses, the illness apparently may be associated with numerous other symptoms and signs. The major manifestations have been reviewed elsewhere.[7,10,17,25,38,81]

Illness usually occurs with the apparent abrupt onset of fever. Slight irritability and malaise occur frequently, but more commonly, the child's temperature is taken because a parent notices that the child feels warm. The temperature usually is in the range of 38.9°C to 40.6°C (102°F to 105°F). Despite the high fever, the child usually is active, alert, and generally unphased. The fever is constant or intermittent, with its greatest degree occurring in the early evening. Restlessness and irritability occur with higher temperatures. The usual duration of fever is 3 to 5 days, but it has persisted for 9 days. The temperature most often returns to normal by crisis, but in some cases, temperature "lysis" occurs over the course of 24 to 36 hours.

Mild cough and coryza are seen frequently in cases occurring in the winter and spring. Headache and abdominal pain are reported in older children, mainly in the summer and fall. Vomiting and diarrhea occur infrequently.

On initial physical examination during the febrile period, most children appear to be happy, alert, and playful. With high temperatures, some children are irritable; occasionally, a child appears to be sick, which suggests more serious illness, such as meningitis or septicemia. Examination within the oral cavity frequently reveals one or more abnormalities. Mild inflammation of the pharynx and tonsils occurs most commonly. Occasionally, small exudative follicular lesions are noted on the tonsils. In other cases, small ulcerative lesions on the soft palate, uvula, and tonsillar pillars are observed. Usually, the lesions on the soft palate consist of only erythematous macules and maculopapules, presumably because of lymphoid hyperplasia.

Mild injection of the tympanic membranes occurs commonly. Enlargement of the suboccipital, posterior cervical, and postauricular lymph nodes is a common finding, but the degree is not remarkable.

Berliner[8] noticed that children with roseola had palpebral edema. He suggested that the "heavy eyelids" or "droopy" or "sleepy" appearance resulting from this edema was diagnostic of the syndrome before the appearance of the rash. Bulging of the anterior fontanelle also has been observed in roseola.[56]

Appearance of the rash in roseola usually coincides with the subsidence of fever, but it may occur after an afebrile interlude of several hours to 2 days. When defervescence occurs by lysis, onset of the exanthem can occur before the temperature has returned entirely to normal. By definition, it is incorrect, however, to call an illness roseola if the fever and rash are truly concomitant.

Zahorsky[80,81] originally described the rash as morbilliform, but his use of morbilliform was not the same as ours is today (i.e., measles-like, erythematous, maculopapular with confluence). The rash is erythematous and macular or maculopapular, and the lesions are discrete. The lesions are 2 to 5 mm in diameter, and they blanch on pressure. Frequently, individual lesions are surrounded by a whitish ring. The rash is most prominent on the neck and trunk, but the proximal extremities and the face also may be affected. Although they have been reported,[17,24] pruritus and desquamation usually do not occur. The rash usually persists for 24 to 48 hours. In occasional cases, well-documented rashes have been observed to appear and resolve within 2 to 4 hours. Yoshida and associates[79] described a 7-month-old boy with HHV-6 infection and typical roseola initially. On the ninth day of illness, vesicular lesions appeared on the face and limbs, however. These lesions persisted for 12 days.

Except for the white blood cell count, routine laboratory studies are of little use in roseola. The total white blood cell count usually is low. Early in the febrile period, high counts occasionally are found, however. The total count reaches its nadir by the third to sixth day of illness, and then gradually returns to normal over the ensuing 7 to 10 days. During the same time frame, the percentage of lymphocytes increases from a normal value of about 50 percent to 60 to 80 percent on days 3 to 10, and then returns to normal over the next 7 days. Frequently, extreme counts in the range of 3000 cells/mm^3 with 90 percent lymphocytes are found, which raises the consideration of a granulocytic defect.

CLINICAL COMPLICATIONS

The most important complications of roseola are convulsions and other neurologic symptoms.* The incidence of convulsions has varied widely among reports. Juretic[38] did not find one instance of convulsions in the 243 cases in his study. Breese[10] did not report convulsions in any of 100 roseola attacks that he studied. In contrast, Greenthal[25] noted convulsions in 6 percent of his cases, and Faber and Dickey[20] found seizures in 8 of 26 cases of roseola. Möller[48] observed that 8 percent of children admitted to the hospital because of febrile convulsions eventually were diagnosed with roseola infantum.

Möller[48] also reported cerebrospinal fluid evaluations in 29 cases of roseola and febrile convulsions. In six instances, the pressure was elevated; in two, there were 5 white blood cells/mm^3; and in another instance, there were 9 white blood cells/mm^3. In most other cerebrospinal fluid examinations, the findings have been normal, but mild pleocytosis with mononuclear cells has been identified occasionally.[7,32] A surprising number of cases of encephalitis associated with roseola have been reported,[13,22,32,35] and residua have been common. Hemiplegia has occurred after illness,[13,22,59,62] and permanent paresis and mental retardation have occurred in some affected patients. The syndrome of inappropriate secretion of antidiuretic hormone has been reported in roseola associated with HHV-6 infection.[55,65] Facial nerve palsy and Guillain-Barré syndrome also have been noted after HHV-6 induced roseola.[47,57]

Thrombocytopenic purpura was noted in one report in five children with roseola; all of these patients recovered.[53] In a more recent study, Hashimoto and colleagues[29] noted five children with thrombocytopenia during the acute phase of roseola caused by HHV-6 infection. Their data suggested that the thrombocytopenia was due to bone marrow suppression, rather than immune-mediated peripheral consumption. A 14-month-old girl developed a generalized eruptive histiocytoma with rapid progression and then resolution after roseola.[70]

DIAGNOSIS

Although detecting leukopenia with relative lymphocytosis is fortuitous, the only necessity in establishing the diagnosis of roseola is to document the fever, defervescence, and exanthem pattern. Frequently, the first exanthematous illness that a child has is called roseola, regardless of whether the exanthem and the fever are concomitant, or the child has no febrile period at all.

*See references 2, 7, 11-13, 20, 22, 25, 32, 35, 43, 48, 59, 62, 63.

The only problem in the differential diagnosis occurs when a febrile child is receiving antibiotics and a rash follows defervescence. This event occurs frequently, and the child usually is labeled allergic to the antibiotic, rather than suspected of having roseola. In most instances of drug allergy, the exanthem lasts longer than roseola does, and in allergic cases, pruritus and fever may accompany the rash.

TREATMENT AND PROGNOSIS

No specific treatment for roseola exists. When fever is a problem, it may be treated with acetaminophen. Acetaminophen can alter the temperature curve, possibly obscuring the correct diagnosis. Febrile seizures and other neurologic complications should be treated vigorously.

In most cases, the outlook is excellent. When encephalitis occurs, the prognosis must be guarded. Because roseola is the result of infection with multiple different viruses, no practical way to prevent it exists.

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CHAPTER 66

Bacterial Skin Infections

Mary Anne Jackson

NORMAL SKIN

ANATOMY

The epidermal skin layer provides the primary barrier to invasion by microorganisms and an interface between the body and the environment. Hair follicles, sebaceous glands, nails, and sweat glands are considered epidermal appendages and as such may be involved in skin infection. A dermal layer composed of collagen and elastic fibers gives skin its elasticity; however, other cell elements that are present, including mast cells, blood and lymph vessels, and cutaneous nerves, may be involved in the inflammatory process in response to infection. The subcutaneous fat layer is just beneath the dermis and contributes primarily to thermal stability, but it also may be involved when infection extends beyond the epidermal-dermal layer.

FLORA

Colonization is defined as the presence of a microorganism on the skin without either clinical signs or symptoms of infection at the time of isolation. Normal bacterial skin colonization is divided into resident and transient flora. Resident flora predominates and includes typical nonpathogens, such as *Staphylococcus epidermidis* and *Propionibacterium acnes*, in addition to other anaerobic diphtheroids and micrococci. Transient flora include pathogenic organisms, such as *Staphylococcus aureus*, streptococci, gram-negative enterics, and *Candida albicans*; these pathogens usually are present in smaller numbers than the resident flora and may be removed by skin cleansing. Acutely or chronically damaged skin, contact with animate and inanimate environmental sources, and exposure to antimicrobial agents or indwelling devices can modify the skin flora and predispose to infection by resident or acquired transient flora.[80,90]

CUTANEOUS INFECTION AND DERMATOLOGIC MANIFESTATIONS OF SYSTEMIC DISEASE

Dermatologic manifestations of infection can occur when the skin is infected primarily or as a secondary phenomenon. Prompt diagnosis and treatment of certain systemic or disseminated diseases may be accomplished when the secondary dermatologic manifestations are recognized. Empiric treatment of systemic diseases such as endocarditis (septic emboli) or septicemia caused by bacterial pathogens, such as *Neisseria meningitidis* or *Pseudomonas aeruginosa*, is possible when the dermatologic manifestations (purpura fulminans, ecthyma gangrenosum) are noted. Generalized viral infections may be heralded by pathognomonic skin findings, such as occur in varicella or measles. Alternatively, skin manifestations may be mediated by toxin (staphylococcal scalded skin syndrome or toxic shock syndrome [TSS]) or by immunologic mechanisms (gonococcemia).

The list of bacterial infectious agents associated with skin infections is extensive (Table 66–1). This chapter focuses on the bacterial skin infections most frequently encountered by practicing clinicians.

IMPETIGO

NONBULLOUS OR SIMPLE SUPERFICIAL IMPETIGO

The bacterial skin infection most commonly encountered in children is nonbullous impetigo, which accounts for more than 70 percent of impetigo cases in children. This superficial infection is seen predominantly in summer, with insect bites, cutaneous injuries, and primary dermatitis serving as the portal of entry.[46,61]

TABLE 66–1 Bacterial Infectious Agents Associated with Cutaneous Manifestations

Anthrax	*Bacillus anthracis*
Blistering dactylitis	*Streptococcus pyogenes*
	Streptococcus agalactiae
	Staphylococcus aureus
Cellulitis	*S. pyogenes*
	Staphylococcus aureus
	Haemophilus influenzae type b
	Streptococcus pneumoniae
Chancroid	*Haemophilus ducreyi*
Diphtheria	*Corynebacterium diphtheriae*
Ecthyma gangrenosum	*Pseudomonas aeruginosa*
Erysipelas	*S. pyogenes*
	S. agalactiae; group C, G streptococci
	S. pneumoniae
Erysipeloid	*Erysipelothrix rhusiopathiae*
Folliculitis	*S. aureus*
	Coagulase-negative staphylococci
	Klebsiella spp.
	Enterobacter spp.
	Escherichia coli
	P. aeruginosa
	Proteus spp.
Erythrasma	*Corynebacterium minutissimum*
Furunculosis	*S. aureus*
Hidradenitis suppurativa	*S. aureus*
	Streptococcus milleri
	E. coli
	Anaerobic streptococci
Granuloma inguinale	*Calymmatobacterium granulomatis*
Impetigo	
Simple superficial	*S. aureus*
	S. pyogenes
Bullous	*S. aureus*
Lymphogranuloma venereum	*Chlamydia trachomatis*
Melioidosis	*Burkholderia pseudomallei*
Necrotizing fasciitis	*S. pyogenes*
	Polymicrobial
Nocardiosis	*Nocardia brasiliensis*
	Nocardia asteroides
Paronychia	Polymicrobial
Perianal dermatitis	*S. pyogenes*
Pitted keratolysis	Coryneform bacteria
Syphilis	*Treponema pallidum*

Entries in bold are discussed in the text.

Nonbullous impetigo, sometimes called *thick crusted impetigo*, is characterized by the appearance of erythematous maculopapules that rapidly evolve from a vesicular to a pustular stage. Centrally crusted plaques range in size from a few millimeters to 1 cm and are surrounded by a distinct margin of erythema. The honey-colored crust is a classic feature, and removal of the crust results in the reaccumulation of fresh exudate. Regional lymphadenopathy can occur and often is the reason that the patient seeks medical attention. Spread to exposed areas, usually the face, neck, and limbs, occurs frequently. This form of pyoderma often is associated with a 2- to 3-week delay in establishing the diagnosis because the lesions are slow to progress, only mildly tender at the site of the lesion, and generally not associated with systemic signs or symptoms.

Nonbullous impetigo classically has been associated with infection caused by group A beta-hemolytic streptococci (GABHS). More recent data underscore the importance of *S. aureus*, however, which now accounts for most cases of nonbullous impetigo in the United States.[6,50]

Primarily a disease of children, nonbullous impetigo is spread within families and by close physical contact. It is prevalent during warm, humid seasons and is seen year-round in tropical regions. Endemic disease occurs in the southeastern United States and Hawaii.

Epidemics of streptococcal impetigo have been associated with postinfectious glomerulonephritis, and streptococcal strains, including types 2, 31, 49, 53, 55, 56, 57, and 60, have been implicated in such outbreaks.[12,67] Studies published in the 1950s and 1960s from the Red Lake Indian Reservation in Minnesota first confirmed the association of impetigo in school-aged children with a postinfectious nephritis that occurred 18 to 21 days after the onset of impetigo and implicated the so-called Red Lake strain, M-type 49.[5] Further studies in this population performed in the early 1970s found that GABHS was isolated from normal skin in 23 of 31 high-risk children a mean of 10 days before the development of impetigo.[34] Local trauma and other environmental factors seemed to explain the predilection of exposed skin to streptococcal infection, especially the skin of the legs, where 62 percent of the total lesions were noted. Secondary acquisition of streptococcal isolates in other family members occurred a mean of 5 days after the primary case, a time frame that was noted to be significantly shorter than that of secondary respiratory acquisition.[52] Rheumatic fever does not occur as a postinfectious sequela of streptococcal skin infection.

Cutaneous botryomycosis, an indolent infection reminiscent of crusted impetigo, usually is caused by *S. aureus*. Characterized by plaquelike lesions with superficial pustules and crusts, this entity has a predilection for patients with altered immune function.[23] Histologic examination may suggest the diagnosis of actinomycosis if a granulomatous lesion with granules resembling those seen with *Actinomyces* is noted. Successful treatment can be accomplished after the bacterial pathogen has been identified.

BULLOUS IMPETIGO

Bullous impetigo is diagnosed when the primary lesion begins as small vesicles and later appears as flaccid, painless bullae, generally measuring greater than 1 cm (see Fig. 64–17). Initially filled with clear fluid, the lesions eventually may exhibit a purulent fluid level. Rupture of the thin bullae usually reveals a moist, erythematous base that dries to a shiny lacquer-like appearance, sometimes described as a varnished finish (Fig. 66–1). Systemic toxicity is not seen except in neonates, in whom disseminated disease may occur.

In contrast to thick, crusted impetigo, in virtually all cases of bullous impetigo, staphylococci are isolated in pure culture from aspirated bulla fluid. Other bullous dermatitides of childhood, such as pemphigus or Stevens-Johnson syndrome, may be excluded by isolation of the organism. Occasionally, biopsy is done in cases in which extensive bullae or an atypical clinical appearance is noted. Confirmation of a cleavage plane high in the epidermis with gram-positive organisms and polymorphonuclear leukocytes present is a definitive diagnosis of bullous staphylococcal disease.

Infection generally is caused by phage group II strains, particularly phage type 71, but also 3A, 3C, and 55, which are noted to elaborate epidermolytic toxins A and B. Pathologically, these toxins act by disrupting the intercellular attachment of epidermal cells of the stratum granulosum. The toxin is thought to function as a protease in separating the upper layers of the epidermis of adult and infant human skin. Production of antibody to epidermolytic toxin occurs with age; however, it does not protect against the development of new bullous lesions during the localized impetiginous stage of this staphylococcal disease.

Epidemiologically, large outbreaks of bullous impetigo have been traced most notably to hospital nurseries, where identification of infected infants always occurs within the first month of life but after the infant has been sent home. A more severe, generalized form of the epidermolytic toxin-mediated disease (Ritter disease) may be seen in a few infants during one of these out-

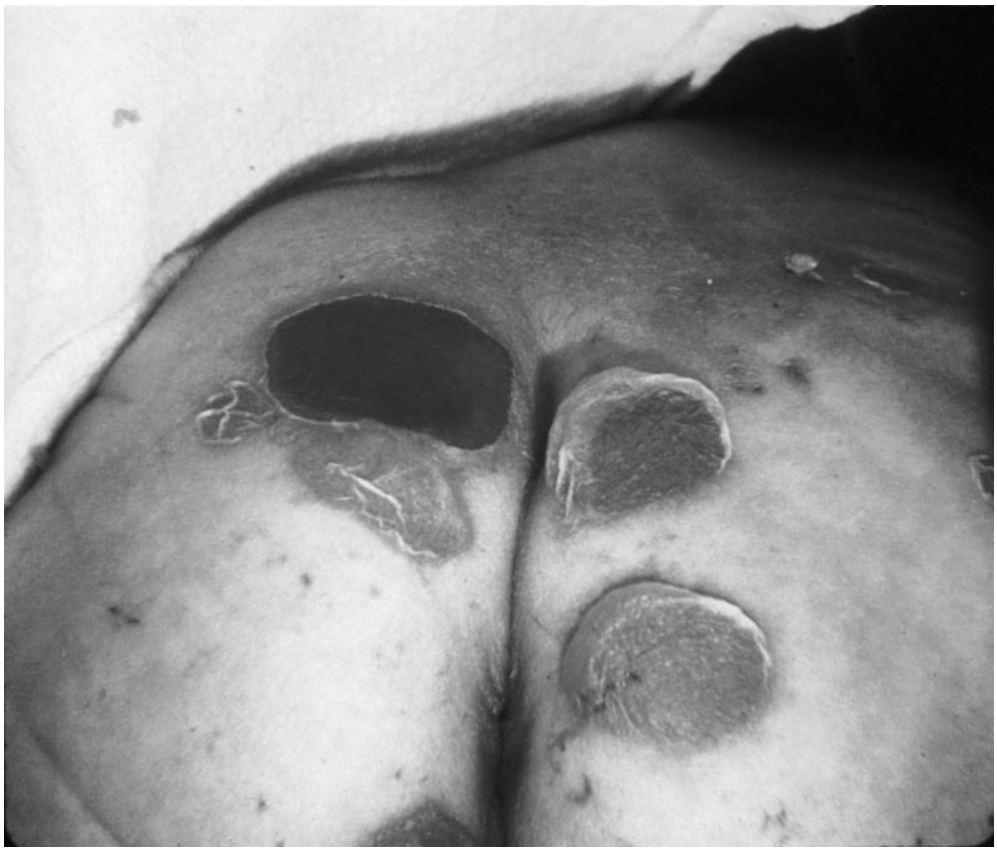

Figure 66–1 Flaccid bullae and shiny lacquer base of staphylococcal impetigo.

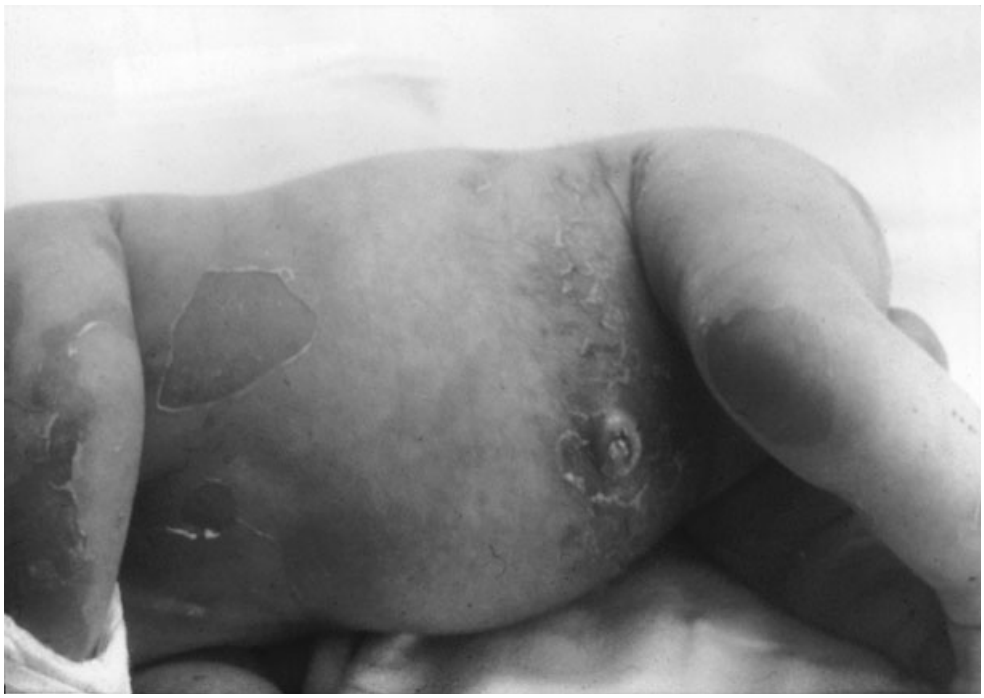

Figure 66–2 Typical appearance of a neonate with Ritter disease.

breaks, underscoring the importance of infection control surveillance practices in recognizing such an outbreak (Fig. 66–2).

TREATMENT OF IMPETIGO

Topical mupirocin may be used in cases of nonbullous impetigo in which adequate coverage of the affected sites can be ensured.[18,33,99] In other cases, systemic treatment with an oral antistaphylococcal antimicrobial agent, such as cephalexin, should be employed.[38,39,45,109] As in other staphylococcal diseases, an increase in community-acquired, methicillin-resistant *S. aureus* (MRSA) cases has been noted in the last decade.[62,63,72,124] In cases for which traditional antistaphylococcal agents are unsuccessful or in patients with recurrent disease, culture should be done to identify the bacterial strain and susceptibility pattern. Currently,

most community-acquired MRSA isolates are susceptible to clindamycin.[17,24]

PERIANAL STREPTOCOCCAL DERMATITIS

Formerly called *perianal cellulitis*, perianal streptococcal dermatitis, a commonly recognized superficial skin infection, is characterized by the presence of marked, well-demarcated, perirectal erythema with associated swelling, pruritus, and tenderness but an absence of systemic symptoms or progressive disease. Approximately half of patients complain of significant rectal pain on defecation, and a third note blood in their stools.[4,29,70,98]

Heavy growth of GABHS is seen on perianal culture, and in one study, isolation of a specific T-type streptococcus (T 28) raised the question of whether certain streptococcal strains have tropism for the perineal region.[104] Asymptomatic patients were evaluated in two studies, and only sparse growth of GABHS was noted in 6 percent of cases.

Perianal streptococcal dermatitis is treated with oral penicillin agents. Topical mupirocin also has been used successfully. Recurrences are noted commonly, however. In one large series of patients, one third had recurrent disease.[87] Intrafamilial spread of disease frequently occurs and may provide a vector for recurrence. For patients with recurrent or persistent disease, clindamycin or a β-lactam agent plus rifampin may be used, and identification and treatment of other affected family members may be necessary.

BLISTERING DISTAL DACTYLITIS

Most commonly identified in school-aged children, blistering distal dactylitis is a distinctive superficial skin infection classically associated with GABHS.[13] Bullae 2 cm in diameter develop over the anterior fat pad of the distal phalanges, sometimes extending to involve the nail folds. Involvement of the proximal phalanges or the palms occasionally is noted. Frankly pustular lesions may occur, but the lesions themselves usually are asymptomatic or only mildly tender. A thin purulent exudate generally is apparent on incision and drainage.[71]

The diagnosis is confirmed by recovery of the etiologic agent on culture, most commonly GABHS, although group B streptococci and *S. aureus* also have been noted.[58] Concurrent recovery of GABHS in the pharynx has been reported in a few cases. Treatment includes a 10-day course of an oral β-lactam agent, usually penicillin or amoxicillin, in addition to incision and drainage of any tense bullae.

ERYSIPELAS

The superficial cellulitis erysipelas, referred to as *St. Anthony's fire* in the Middle Ages, is characterized by the appearance of a bright erythematous plaque with a distinct, elevated border that sharply demarcates affected from unaffected skin. The lesion most often involves the face or lower extremity, although extensive involvement of the trunk has been noted. The involved skin is warm and tender and may have a peau d'orange appearance. Large tension bullae may be seen in the erythematous zone.[65] The patient generally appears toxic and is highly febrile, and rapid extension of the affected skin may occur over the course of hours.[31,66,136]

Histopathologic findings include intense edema with vascular dilation of the dermis and uppermost subcutaneous tissue. Involvement of lymphatic channels and tissue spaces with polymorphonuclear leukocytes is a typical finding.

Surgical wounds, the umbilicus of the neonate, or any break in the skin may serve as the portal of entry; however, the initial lesion may be inapparent. Localized edema, such as occurs from a renal or lymphatic source, is a predisposing factor, and antecedent respiratory infection often is reported. An increased risk for development of erysipelas has been noted in patients with hypogammaglobulinemia, certain malignancies such as lymphoma, or lymphedema complicating radiation therapy.

The diagnosis generally is recognized on clinical grounds, and GABHS traditionally has been isolated by aspiration of the advancing margin of the lesion. A few case reports have identified other streptococci (including group B, C, and G), *Moraxella* spp., *Haemophilus influenzae*, and *Streptococcus pneumoniae* as etiologic agents.[15,30,42,88,102,123,137] A combination of intravenous penicillin and clindamycin should be used until the results of culture are available. Erysipelas has a classic clinical appearance, and appropriate diagnosis and therapy result in a prompt clinical response in most cases. Penicillin prophylaxis may be considered for patients with recurrent erysipelas, particularly patients with underlying risk factors.[16,40]

ECTHYMA

Ecthyma gangrenosa is a deep-seated infectious process that manifests as a necrotic ulcer covered by a black eschar. Usually, the initial lesion, a vesicopustule, sits on an erythematous base; it eventually erodes through the epidermis to the dermis, where it forms a crusted ulcer with heaped-up borders and then becomes frankly necrotic.[64]

This process rarely occurs in an otherwise healthy child, and if it does, an immunodeficiency work-up should ensue. *Chromobacterium violaceum* has been reported to cause ecthyma and similarly should result in an immunologic evaluation focusing on neutrophil defects, including disorders such as chronic granulomatous disease.[22]

Ecthyma can occur as a primary cutaneous infection in an immunocompetent host, and other etiologic agents that have been confirmed include *S. aureus*, *Aeromonas hydrophila*, and GA BHS.[48,57,73,84,96,105,134] A similar-appearing lesion is seen with cutaneous anthrax; however, extensive nonpitting edema of the surrounding soft tissues is an important clue to this diagnosis. Ecthymatous-like lesions have been seen in patients with herpes simplex infection.[85] Additionally, human orf infections result in ulcerative skin lesions that appear similar to lesions of ecthyma; the clue to establishing the diagnosis is contact with an infected animal, usually sheep or goats, or a contaminated fomite.[26]

When an ecthymatous lesion is noted in a febrile neutropenic host, it generally signals disseminated infection. *P. aeruginosa* is the etiologic pathogen identified most commonly in such cases, but other gram-negative pathogens and fungi, including *Enterobacter*, *Escherichia coli*, *Morganella*, *Pseudomonas cepacia*, *Serratia marcescens*, *Stenotrophomonas maltophilia*, *Aspergillus*, *Mucor*, *Fusarium*, and *C. albicans*, have been implicated in ecthyma gangrenosum in compromised hosts.[36,51,53,97,107,112,117,122] Ecthyma has been seen as the heralding manifestation of acute lymphoblastic leukemia in children.[111] Empiric antimicrobial therapy for ecthyma gangrenosum in a neutropenic host should include intravenous therapy with an anti-*Pseudomonas* agent plus an aminoglycoside. Biopsy of the lesion may provide more specific etiologic information and allow for confirmation of antimicrobial susceptibility.

FOLLICULITIS, FURUNCULOSIS, AND CARBUNCLES

Folliculitis, furunculosis, and carbuncles represent a group of infections characterized by their origin in the hair follicles and

the formation of abscesses. Virtually always caused by *S. aureus*, these infections were seen commonly in the 1950s in disease that often involved multiple family members. Outbreaks among athletes likewise have been reported.[130]

By definition, these infections involve sites where body hair is present, including the axilla, breast area, perineum, neck, and extremities. Lesions of folliculitis represent abscesses of a single hair follicle with limited surrounding tissue involvement. When deeper inflammatory nodules are associated with tissue edema, furunculosis is diagnosed. When several interconnecting furuncles are present, the lesion is referred to as a carbuncle. Generally, older children and adolescents are predisposed to the development of follicular infections, and individuals with diabetes mellitus, abnormal neutrophil chemotaxis, and impaired circulation may have recurrent disease.

Although *S. aureus* nearly always is the cause of folliculitis as in the past, outbreaks of so-called hot tub folliculitis have been described and almost always are caused by *P. aeruginosa*; rarely, other gram-negative organisms have been reported.[27,28] Folliculitis in an immunocompromised host often is caused by unusual fungal pathogens.[3,115,127] Although typically associated with hot tubs and whirlpools, a large outbreak of *Pseudomonas* folliculitis reported in 1984 involved 117 individuals after swimming in an indoor pool. An incubation period of 24 to 30 hours could be ascertained, and a typical follicular, pustular eruption was noted. The mean duration of the folliculitis, 15 days, is consistent with other reports, but some patients continued to complain of rash for weeks, and recurrent pustules appearing months later have been reported in other studies.[56]

A more recent report has identified four cases of *Mycobacterium fortuitum* complex furunculosis that developed after pedicures, suggesting this pathogen may be added to the differential diagnosis in such cases when a patient presents with nonhealing furuncles on the lower legs, especially when bacterial cultures have been negative, or the disease is unresponsive to antistaphylococcal therapy (including MRSA).[114] Early recognition and institution of appropriate therapy are essential, and the history of pedicures may be a clue to establishing the diagnosis. Cutaneous myiasis has been reported to manifest as a chronic boil or furuncle, but the diagnosis should be considered only in such cases in which an appropriate travel history is elicited.[32]

Recognition of the typical skin lesion usually is sufficient to establish the diagnosis. Unless the patient has a history of exposure to a hot tub or whirlpool, or recently has had a pedicure, a regimen of antistaphylococcal therapy should be sufficient. In the era of MRSA infection, local susceptibility profiles should be used to confirm appropriate therapy. Isolated boils resolve with drainage alone. Systemic therapy may be considered in cases in which lesions are large or multiple. Trimethoprim-sulfamethoxasole is a good choice for a nontoxic patient older than 2 months. Clindamycin is a good choice for most locales, although resistance may be increasing.[82,91] Because MRSA now accounts for 75 percent of cases in which children present with skin and soft tissue infection, treatment should be individualized, based on type of presentation, patient age, and underlying disease.[81] Systemic agents should be used for 7 to 10 days in patients who are toxic, who have extensive disease, or who have associated cellulitis. Large lesions, specifically larger than 5 cm, should be incised and drained, and culture should be done in all cases to confirm susceptibility testing. In some patients, hematogenous metastatic spread may occur, and a search for foci in the heart, bones, joints, deep tissues, or brain should be done in patients with significant systemic toxicity.

For patients in whom recurrent disease develops, chronic dermatoses, such as eczema, should be identified, and in obese adolescents, the diagnosis of diabetes mellitus should be considered.[7] The patient should be cautioned to refrain from sharing washcloths or towels, and skin trauma and use of irritants such as deodorants should be avoided.

The utility of decolonizing regimens with topical agents, such as mupirocin (nares and perianal area) and chlorhexidine or bleach baths (skin), may be considered in certain cases. *S. aureus* colonization of the nares, rectum, or skin can be detected by culture of these areas, but confirmation of carriage is likely necessary only in specific cases for which decolonization is desirable. The indications for and benefit of decolonization vary depending on host factors, underlying disease, and circumstances related to the health care setting.[126] Outbreaks of MRSA colonization/infection require a multifaceted approach, including attention given to handwashing, cohorting, barrier measures, and decolonization strategies. Examples of special circumstances include the following: (1) patients who are immunosuppressed and colonized, and at risk for development of systemic infections; (2) patients who are more likely to spread the organisms, owing to behavior (e.g., mentally retarded patients); or (3) patients who have repeated infections caused by the MRSA strain that they carry.

Most children with recurrent furunculosis are otherwise healthy, and no specific immunologic evaluation is necessary; however, cases of recurrent furunculosis in patients with hyper-IgE syndrome and common variable immunodeficiency have been reported, and an association with mannose-binding lectin deficiency was confirmed in a family.[78,83,125] Rarely, children with white blood cell defects may have recurrent staphylococcal skin abscesses, and tests of white blood cell function may be considered in specific patients. Data suggest that vitamin C may be beneficial in cases of recurrent folliculitis.[93,94]

HIDRADENITIS SUPPURATIVA

Hidradenitis suppurativa, a chronic, debilitating condition, is a disorder of the apocrine glands that involves primarily skin in the axilla and anogenital region, although scalp, umbilical, and breast involvement has been reported. Seen mainly in adolescents, this androgen-dependent condition is manifested by the development of multiple painful nodules and the formation of deep abscesses in the skin in areas where apocrine glands are present. The formation of fistulas, ulcers, and contracted scars may complicate the course, and recurrent relapses may be noted. Infection usually is polymicrobial, and pathogens to consider include *S. aureus*, gram-negative enterics, and anaerobes. Drainage of abscesses and institution of systemic antimicrobial therapy may be necessary. When fistulas associated with anogenital disease develop, adjacent structures, including the urethra, bladder, and rectum, may be involved. Surgery usually is required for cure. Carbon dioxide laser treatment in some cases may be beneficial.[89]

CELLULITIS

The diagnosis of cellulitis is made when the subcutaneous tissues and dermis are involved in a clinical process manifested as localized edema, erythema, warmth, and tenderness of the tissues. The leading edge of the involved site may be notable, but it is not raised and well demarcated as in erysipelas.

Infection usually is caused by coagulase-positive staphylococci and GABHS; however, infection also is caused by group B streptococci (neonates) and *S. pneumoniae*, and, in the past, *H. influenzae* type b (Hib) cellulitis was described.[131] Streptococcal and staphylococcal cellulitis can involve patients of any age and any site, although the extremity is noted most often. Frequently, the patient has a history of antecedent trauma at the site of involvement, but the injury may not have appeared significant. Some researchers advocate culture of the cellulitic site, but in practice, it rarely is performed. Blood cultures are valuable in individuals

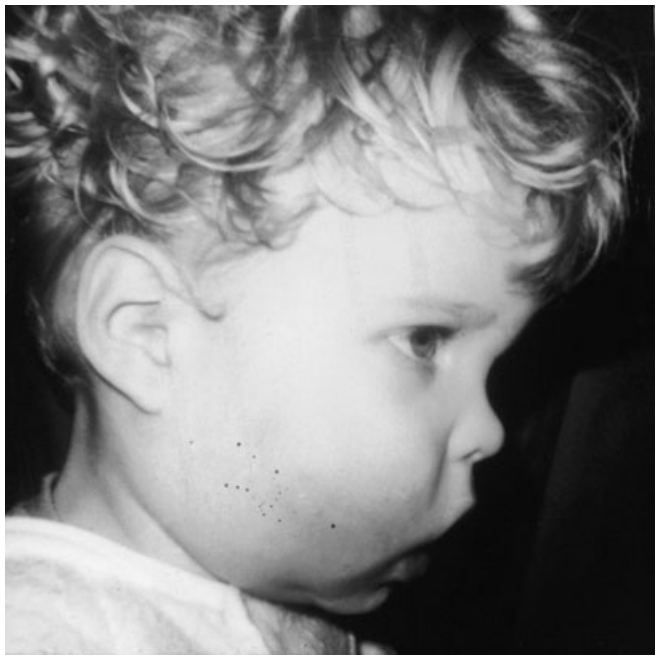

Figure 66–3 Buccal involvement in an infant with invasive *Haemophilus influenzae* type b disease.

with disease caused by group B streptococci, *S. pneumoniae*, and Hib.[75]

Group B streptococcal cellulitis occurs in neonates and generally is seen as part of invasive, late-onset disease. Unilateral involvement of the face or submandibular sites occurs most commonly, but inguinal, scrotal, and prepatellar involvement has been described.[10,69] When cellulitis occurs in an infant younger than 3 months old, group B streptococcal bacteremia should be suspected, even in the absence of other signs of systemic infection.

Pneumococcal soft tissue infections are common findings.[110] Patients with connective tissue disorders, such as systemic lupus erythematosus, seem especially prone, although these infections can occur in healthy infants and children. Sites of involvement include the head, neck, leg, and torso.

Hib cellulitis often involves the face of infants (Fig. 66–3). A violaceous hue of the cellulitic area, which some researchers thought to be pathognomonic, might be observed. This process nearly always was the result of hematogenous seeding by Hib, and meningitis occurred in 15 to 20 percent of such patients.[68,129,140] As has been the case with other forms of Hib disease, almost complete eradication has been achieved in the last decade with the use of conjugate vaccine.

Treatment of simple cellulitis in patients with a clear-cut area of preceding trauma should include an agent that is active against *S. aureus* and GABHS, and in most locales, clindamycin remains a good choice for therapy, although resistance for *S. aureus* (10-15%) and GABHS (2-3%) is noted. The aforementioned caveats for treating staphylococcal skin infections should be followed. In infants with buccal or periorbital involvement or in infants with soft tissue involvement but without a clear-cut focus of infection, a third-generation cephalosporin, such as cefotaxime or ceftriaxone, should be included with clindamycin. Vancomycin always should be included in cases of a patient who is toxic and when metastatic suppurative disease is suspected.

NECROTIZING FASCIITIS

Necrotizing fasciitis is a rapidly progressive bacterial infection of the soft tissues associated with a fulminant course and a high mortality rate. This infection spreads rapidly in the plane between the subcutaneous tissue and superficial muscle fascia and causes widespread necrosis. Prompt and aggressive medical and surgical management is necessary to ensure a good outcome.

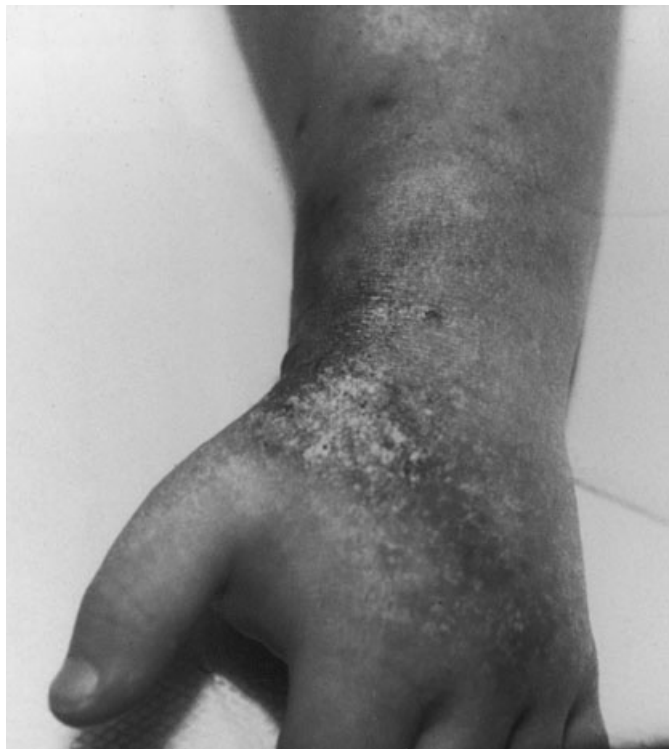

Figure 66–4 Necrotizing fasciitis in a toddler with varicella.

In children, necrotizing fasciitis usually is caused by GABHS; traumatic lesions involving the skin, including varicella, burns, or eczema, may predispose to this aggressive process.[19,47,55,59,76,108,119,120,142] An association has been noted among varicella, ibuprofen use, and invasive GABHS infection, although no data convincingly link this triad to necrotizing infection.[54,92,143] Patients with congenital or acquired immunodeficiencies are at greater risk for the development of necrotizing fasciitis, and in neonates, omphalitis and circumcision are predisposing conditions.

CLINICAL MANIFESTATIONS

The child generally has a high fever and is fussy. In infants, the irritability may be profound and may not appear to be localized to an involved site, unless the clinician is meticulous in conducting the examination. An extremity most commonly is involved, and older infants and children often refuse to bear weight or move the affected extremity. Swelling of soft tissue usually is noted, but the erythema may be subtle. The hallmark tip-off on examination is the finding of intense pain on manipulation of the involved site that is out of proportion to the cutaneous signs. Skin changes that occur during the subsequent 24 to 48 hours include blistering with bleb formation, and a dusky appearance of the involved site is noted as vessels are thrombosed, and cutaneous ischemia develops. Skin necrosis is a late sign and indicates a poor prognosis (Fig. 66–4).[79,135]

Recognition of the manifestations of TSS is crucial because mortality rates of 60 percent have been reported in patients with associated fasciitis. Multisystem complaints, including vomiting, diarrhea, and severe myalgia, are present when GABHS fasciitis is associated with streptococcal TSS. Tachycardia out of propor-

tion to fever and altered mental status may be early signs of TSS. Renal and hepatic dysfunctions occur typically, and symptoms and signs of adult respiratory distress syndrome often are identified.

DIAGNOSIS

The diagnosis cannot be based on the appearance of the involved site because none of the early findings of necrotizing fasciitis are pathognomonic. Plain radiographs usually are normal and of no value in establishing the diagnosis. Magnetic resonance imaging (MRI) is the preferred technique to detect soft tissue involvement. MRI permits visualization of the soft tissue edema infiltrating the fascial planes.[21,113,144] Although MRI may be helpful, it should not delay performing surgical intervention. Waiting for a radiographic procedure to be performed to confirm the diagnosis may serve only to delay implementing definitive surgical therapy and to increase the risk for development of systemic complications, contributing further to the increased morbidity and mortality rates. In the typical clinical scenario in which the index of suspicion for necrotizing fasciitis is high, surgical exploration is appropriate, even in the presence of "normal" MRI findings.

Laboratory manifestations of streptococcal TSS should be sought in any pediatric patient with fasciitis. Typically, the white blood cell count is normal; however, most patients have a significant increase in band forms (>50%) noted on the peripheral blood smear. Thrombocytopenia and evidence of coagulopathy are found commonly, and marked hypoalbuminemia with hypocalcemia is a typical finding. Laboratory findings associated with renal failure, myocardial dysfunction, and adult respiratory distress syndrome may develop during the first 48 to 72 hours.

A microbiologic diagnosis can be made by isolating bacteria from blood, tissue, or wound culture. In some cases, a polymicrobial etiology has been noted, particularly in patients with so-called Fournier gangrene or necrotizing fasciitis of the perineum.[49,74] In these cases, *S. aureus*, GABHS, and one or more anaerobes, including *Peptostreptococcus*, *Prevotella*, *Bacteroides fragilis*, and *Porphyromonas*, have been implicated in infection.[20] Fasciitis caused by *P. aeruginosa* or *Clostridium septicum* has been seen in neutropenic patients. In the last decade, GABHS has been reported widely as a single pathogen and is the etiologic agent in most cases of pediatric fasciitis. As in other cases of invasive disease caused by GABHS, virulence is related to certain structural characteristics of the organism and to its ability to produce biologically active substances, some of which facilitate invasion and spread of the pathogen.

TREATMENT

Surgical débridement of necrotic tissue is the key to managing necrotizing fasciitis, and increased mortality rates have been observed when débridement is delayed more than 24 hours.[106] Mandatory return to the operating room for examination and repeat débridement should occur during the following 24 to 48 hours. Careful management of fluids, attention to pain control, anticipation and management of multisystem organ failure, and administration of appropriate parenteral antimicrobial therapy should be initiated promptly. The use of intravenous immunoglobulin may be considered in cases of TSS-associated fasciitis.[25]

Appropriate therapy includes intravenous penicillin, 150,000 U/kg/day divided into four to six doses; clindamycin, 40 mg/kg/day divided into four doses; and vancomycin, 40 mg/kg/day divided into three to four doses. The use of additional coverage with agents active against *P. aeruginosa* and gram-negative enterics should be considered in neutropenic patients.

Response to therapy is assessed by careful serial examination. Control of pain is crucial in such patients, while keeping in mind that persistent, severe pain suggests ongoing tissue necrosis and may signal the need for further surgical intervention. Careful attention to nutritional support should be maintained throughout the child's hospital stay. Physical therapy is necessary for most patients, especially patients who require amputation, skin grafting, or extensive reconstructive surgery, and providing for the psychosocial needs of the child and the family is imperative.

CONTAMINATED WOUNDS

Although staphylococci and streptococci are the most likely causes of infection after traumatic skin lesions, the list of causes may be extensive, depending on the nature of the injury. Specific pathogens should be considered when infections develop after human or animal bites, soil or water contamination, or various types of injury. Management of such infections depends on recognizing infection patterns and obtaining cultures for careful identification of the specific organism or organisms involved (Table 66–2).

HUMAN BITES

Two types of human bites are described: occlusional and clenched fist (see also Chapter 259). Occlusional bites are related most commonly to child abuse, although in a pediatric patient, a biting toddler may be the culprit. Accidental bites may occur during sporting activities and generally involve the face of a teammate. Clenched-fist injuries are associated with the most prevalent and severe infections that occur after human bites. Rapidly progressive infection may follow despite the patient's receiving early medical attention.[11] When clenched-fist injuries are associated with bite wounds, laceration and puncture wounds commonly occur along the dorsal aspect of the third metacarpophalangeal joint, and bone, joint capsule, or tendon structures may be involved.

Polymicrobial infection is the usual finding, and in such cases, broad-spectrum empiric antimicrobial coverage should be initiated promptly, after adequate drainage has been performed.[118] In a multicenter study of infected human bites, the median number of isolates per wound was four, and aerobes and anaerobes were

TABLE 66–2 Infections Associated with Animal Bites

Nature of Injury	Pathogens Involved
Human bites	Staphylococci Anaerobic and aerobic streptococci *Eikenella corrodens*
Animal bites	
Dog/cat	*Pasteurella* species *Staphylococcus aureus* Streptococci Anaerobes *Capnocytophaga canimorsus*, *Capnocytophaga cynodegmi* *Moraxella* species *Corynebacterium* species *Neisseria* species
Reptile	Enteric gram-negatives Anaerobes
Horse and sheep	*Actinobacillus* species, *Pasteurella* species
Pig	*Flavobacterium* species, *Actinobacillus* species, *Pasteurella aerogenes*
Rat	*Streptobacillus moniliformis*, *S. aureus*

identified. The common pathogens were *Streptococcus anginosus*, *S. aureus*, *Eikenella corrodens*, *Fusobacterium nucleatum*, and *Prevotella melaninogenica*. *Candida* spp. were noted less commonly.[132] Many strains of *Prevotella* and *S. aureus* are β-lactamase producers, and *E. corrodens* is intrinsically resistant to clindamycin and cephalosporins. Ampicillin with clavulanate given orally (simple infections) or ampicillin-sulbactam given intravenously for more complicated infections may be used as a single agent. Unusual pathogens rarely occur. A report of a human bite infection caused by *Mycobacterium ulcerans* is noted; this pathogen is emerging rapidly in West African countries.[35] Antimicrobial prophylaxis should be considered for patients with human bites to the hands, feet, and skin overlying joints and for patients with bites that penetrate deeper than the epidermal layer.[116]

ANIMAL BITES

Animal bite wounds occur commonly, with more than 4 million reported annually in the United States (see also Chapter 260). Children account for more than half of patients who go to emergency departments for care of bite wounds.[2] Although one survey has revealed that rodents and lagomorphs are the biting animals most commonly reported, wounds related to these bites seldom are associated with infection. By contrast, cats and dogs together account for approximately 40 percent of bites, and their bites are associated more frequently with morbidity. The etiology of animal bite infections often is related to the species of animal involved (see Table 66–2).[41,44,77,95]

Approximately 1000 emergency department visits related to dog bites occur each day, at a cost of more than $100 million/yr.[128] The incidence of infection related to dog bites has been estimated at 3 to 17 percent.[9,37] Cat bites are most likely to become infected, probably because of the puncture-like nature of the injury. One study suggests that half of cat bites result in infection, prompting recommendations for prophylaxis of such bites, especially if they involve the face or hands. In the presence of infection, exploration plus débridement of devitalized tissue is necessary, and purulent collections should be drained.

Although *Pasteurella multocida* is implicated most frequently, numerous other pathogens, including *S. aureus*, *Capnocytophaga canimorsus*, and other aerobic and anaerobic bacteria, are associated with dog and cat bites. Depending on the depth of the wound's penetration, underlying structures such as bones, joints, and tendons may be involved in such infections.[60] The wound itself may not appear significant, particularly in the case of puncture wounds, but several clinical features should influence treatment decisions. The presence of tissue edema and point tenderness on palpation over the site should signal that deeper structures may be involved. Soft tissue imaging may be necessary, and appropriate drainage or débridement of involved sites should be pursued.

As with human bites, oral amoxicillin-clavulanate may be used for prophylaxis or treatment of simple infections. Indications for prophylaxis of bite wounds include bites associated with a crush or puncture injury and bites involving the face, hands, feet, and genitalia. Wounds in immunocompromised, especially asplenic, individuals should be considered for prophylaxis. Intravenous ampicillin-sulbactam or ticarcillin-clavulanate can be used for more serious infections. For individuals who have a history of anaphylaxis with penicillin or cephalosporins, a combination of clindamycin plus trimethoprim-sulfamethoxazole or ciprofloxacin can be used.[113,115] Antimicrobial therapy may be modified further depending on the biting animal and suspected pathogen (see Table 66–2).[14]

Appropriate prophylaxis against tetanus should be considered for all bites. Hepatitis B can be transmitted by human bites, and appropriate management should be ensured for susceptible patients. Rabies vaccine should be administered after bat, skunk, or raccoon bites; public health information should be accessed to decide whether rabies vaccine is indicated for other animal bites.

SOIL-CONTAMINATED AND WATER-CONTAMINATED WOUNDS

Four factors that must be considered in the acute care of contaminated wounds include the (1) mechanism of injury, (2) the length of time that transpires from injury to treatment, (3) the type of pathogens that occur in the environment, and (4) the presence of underlying disease in the host.[43] When a traumatic wound becomes infected, a polymicrobial etiology is typical, and common pathogens such as *S. aureus*, gram-negative enterics, and anaerobes characteristically are involved.[1] Unusual and rare organisms, such as nontuberculous mycobacteria, *Nocardia*, *Actinomyces*, fungi including *Aspergillus* spp., and unusual gram-negative organisms, occasionally may be encountered (Table 66–3).[86,100,101,103,138] *A. hydrophila* has been implicated in infections associated with injuries contaminated by fresh water and may produce rapidly progressive wound infection with fascia, tendon, muscle, bone, or joint involvement.[121]

Managing wounds contaminated by soil or water is difficult, especially if the mechanism of injury is a catastrophic event with complex bone and soft tissue injuries. In the acute setting of such an event, complete exploration and thorough débridement with copious irrigation are performed primarily, and signs of infection generally are not present. Days later, the clinician often is faced with the dilemma of a patient who is receiving antimicrobial prophylaxis with broad-spectrum agents and in whom new signs or symptoms develop acutely. Separating infectious from noninfectious complications often is difficult; however, the onset of fever in such a patient, especially in the setting of an open fracture or dural tear, should prompt further evaluation. Careful serial examination of the site of injury is necessary, and more extensive evaluation generally is indicated. Such assessment may include radiographic imaging and specific evaluation of body fluids with appropriate cultures. Because open fractures are associated with an increased risk for development of infection, the utility of antibiotic-impregnated implants, which are biodegradable and osteoconductive, may prove beneficial in such cases.[133,139]

Infections associated with foreign bodies such as wood generally cannot be cured until the foreign body is identified and removed.[141] Puncture wounds should be explored carefully, and

TABLE 66–3 Infections Associated with Soil-Contaminated or Water-Contaminated Wounds

Soil-Contaminated Wounds
Staphylococcus aureus
Group A beta-hemolytic streptococci
Many gram-negative enterics
Enterobacter cancerogenus
Anaerobes
Nocardia asteroides, *Nocardia otitidis-caviarum*
Mycobacterium fortuitum, *Mycobacterium abscessus*
Actinomyces
Aspergillus species
Enterococcus species
Water-Contaminated Wounds
Aeromonas hydrophila
Pseudomonas species
Many gram-negative enterics
Edwardsiella tarda (catfish injury)
Mycobacterium marinum

further débridement of necrotic tissue or drainage of involved sites such as joints may be necessary.[8]

Treatment of simple wound infections associated with soil or water contamination should include an agent such as ciprofloxacin, although deeper tissue infection may develop after a seemingly innocuous injury, and tissue débridement may be necessary. Determining the appropriate therapy for a patient with infection involving extensive soft tissue injury and open fractures is problematic. Administration of an empiric regimen with an agent such as piperacillin-tazobactam, imipenem, or a fluoroquinolone plus vancomycin may be reasonable after evaluation and appropriate culturing have been done.

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CHAPTER 67

Viral and Fungal Skin Infections

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VIRAL INFECTIONS

Cutaneous manifestations of viral infections are common. Exanthems often accompany acute viral infections. The Dukes numbering system of six exanthems is well known to clinicians but is now primarily of historic interest only (Table 67–1). With rare exceptions, skin manifestations that mimic the "typical" features of the six exanthems are seen with multiple infectious agents. Other infections result in lesions such as warts or molluscum contagiosum. This chapter focuses on the common viral illnesses that manifest with cutaneous manifestations. More detailed information on each of these illnesses can be found in the chapters devoted to specific viral agents.

Viruses can invade the skin indirectly as a result of viremia or directly, as with warts or molluscum. Other mechanisms of producing exanthems include interactions between the infecting agent and humoral or cell-mediated factors or a systemic immune response in the absence of viral antigen in the skin.[78]

The type of exanthem encountered depends on the virus, the pathophysiologic mechanisms involved, the location of the eruption, and local and systemic immune factors. A single virus may manifest with a variety of cutaneous reactions within the same host. Clinicians caring for a patient with an exanthem usually are faced with a challenge to determine its cause. Most viral exanthems are benign and self-limited, but understanding the exact cause can be important when evaluating immunocompromised patients or in the setting of a fetus. Similarly, distinguishing a viral exanthem from skin eruptions caused by other infectious agents or drugs is important.

Generally, children presenting with fever and a rash cannot be diagnosed accurately by the presentation of the eruption.[53] The distribution and character of exanthems, such as the often characteristic reticulated erythema after "slapped cheek" exanthem typical of parvovirus B19 infections, are helpful; however, the virus can manifest with several other cutaneous findings.[20,81,86] Typical hand, foot, and mouth syndrome is caused by coxsackievirus, although it has been related more recently to enterovirus 71.[25] The concurrent administration of antibiotics in the setting of Epstein-Barr virus infection is important to know, as is the possible relationship between human herpesvirus infection and certain drugs resulting in drug rash with eosinophilia and systemic symptoms.[35]

Laboratory studies, in addition to specific viral cultures, polymerase chain reaction, and serologies, may be helpful in distinguishing between viral and bacterial diseases or drug eruptions. Coagulation studies may help clarify whether a purpuric eruption is caused by a primary coagulopathy or a viral process. Other laboratory tests, such as streptococcal screens or throat cultures or both, serum antibody titers, viral cultures, and antigen detection methods, are required for evaluating exanthems, depending on the state of the patient being evaluated.

TABLE 67–1 Dukes Classification of Exanthems

First disease*	Measles
Second disease	Scarlet fever
Third disease	Rubella
Fourth disease†	Dukes disease
Fifth disease	Erythema infectiosum
Sixth disease	Roseola infantum

**It is not known definitively if measles or scarlet fever was the first disease.*
†This disorder had characteristics of many infections and today is not believed to represent a distinct entity.
Data from Cherry, J. D.: Cutaneous manifestations of systemic disease. In *Feigin, R. D., and Cherry, J. D. (eds.): Textbook of Pediatric Infectious Diseases. 3rd ed. Philadelphia, W. B. Saunders, 1992, pp. 755-782; Frieden, I. J., and Penneys, N. S.: Viral infections.* In *Schachner, L. A., and Hansen, R. C. (eds.): Pediatric Dermatology. New York, Churchill Livingstone, 1988, pp. 1371-1413; Hurwitz, S.: Clinical Pediatric Dermatology. 2nd ed. Philadelphia, W. B. Saunders, 1993, pp. 318-371.*

WARTS

Although most warts are easily recognized by practitioners and manifest with nothing more than local discomfort or embarrassment for school-aged children, some carry more ominous associations. Human papillomaviruses (HPV), which are DNA viruses, cause this infection. Approximately 120 different serotypes exist, and subclinical infections have been documented.[75] The common wart, or verruca vulgaris, is caused by infection with HPV type 2 or 4. Infection is transmitted by direct contact, and some site specificity exists for each HPV type. Some HPV types, such as 11 and 16, have documented associations with malignancy.[75,124] A vaccine directed against HPV types 6, 11, 16, and 18 has been released in an attempt to address this problem.[24,47] Immunocompromised patients, such as renal transplant recipients, can have persistence of a variety of HPV types, although not always the types having a malignant risk.[9] In healthy hosts, warts can resolve spontaneously within 2 years in most patients.[6] Persistence of infection can be a source of great concern, however, leading to consultation regarding treatment options.

No specific treatments are available for warts. Most treatments are physically destructive in nature, although some incite local immune responses. When discussing treatment options with patients or families, being frank about the lack of predictably useful therapies is important. One of the only therapies subjected to appropriately controlled trials is salicylic acid, which has shown efficacy in approximately 65 percent of the patients so managed. Office therapies using liquid nitrogen, acids such as trichloroacetic acid, or cantharidin have been used with variable success. Over-the-counter cryotherapy units typically do not freeze the lesions as quickly or as thoroughly as does liquid nitrogen. Laser such as carbon dioxide or erbium:YAG lasers are classic destructive lasers. Pulsed-dye lasers also have been useful for some patients through selective destruction of the vasculature. Multiple other modalities, such as duct tape, first suggested in 1978 and more recently by several authors, and antivirals such as cidofovir have been used. The goal of management of this infection must be tailored to the patient and the nature of the lesions.

The finding of condyloma in a young child always raises concern for sexual abuse. When a condyloma is seen in children younger than 2 to 3 years of age, vertical transmission is well recognized to be a means of transmission.[75,124] Caretakers known to have warts also are obvious transmitters of viruses causing such lesions. In these children or in the case of older children, obtaining a careful history regarding the child's behavior and caretakers and examining the child thoroughly for any signs of physical abuse always are important.

MOLLUSCUM CONTAGIOSUM

Molluscum contagiosum represents a common viral infection of the skin (and sometimes mucous membranes) of children and adolescents. It is caused by a poxvirus and has not been grown in culture. The virus replicates within the cytoplasm[38] and is represented by four viral types based on DNA analysis: MCV-1 to MCV-4.[73] MCV-1 accounts for more than 90 percent of infections in the United States.[38,73] The subtypes do not show site specificity. MCV-2 was reported to be seen most commonly in patients infected with human immunodeficiency virus (HIV).[40,73]

EPIDEMIOLOGY

The disease is seen with increasing frequency in sexually active and immunodeficient individuals. Infection occurs at any age, with the highest frequency seen in patients younger than 5 to 10 years old.[17,38] The infection is two to three times more common in school-aged and sexually active males than in females. Transmission is via close contact. Spread is reported to occur via fomites. The incidence of disease is highest in warm climates and in areas of overcrowding.

CLINICAL MANIFESTATIONS

The incubation period for molluscum contagiosum is 2 to 7 weeks, but it has been reported to occur at 1 week of age. Typical lesions are 1 to 5 mm in size, dome-shaped, skin-colored or pink papules with a distinctive central umbilication (Fig. 67–1). Giant lesions 1 to 2 cm can be seen. According to an epidemiologic survey conducted at three pediatric dermatology centers, fewer than 15 lesions usually are seen.[38] In children, molluscum contagiosum is seen most frequently on the face, neck, trunk, and extremities but may be seen on any part of the body, including the mucous membranes.[17,38,72] Periocular lesions may lead to secondary keratoconjunctivitis or trachoma. In some cases, molluscum contagiosum is a sexually transmitted disease, raising the issue of sexual abuse when infection is seen in the genital area. The most common etiology of genital molluscum contagiosum is autoinoculation; however, if the lesions occur solely in the genital area, or a question exists about the patient's social situation, the possibility of abuse should be explored.

Atypical lesions of molluscum contagiosum are seen more commonly, particularly with the improved survival rates of patients with acquired immunodeficiency syndrome (AIDS) and other immunocompromised states. The incidence of molluscum contagiosum in HIV infection is 5 to 18 percent. With improved antiretroviral therapies, molluscum contagiosum has decreased in this population.[21,38]

Molluscum contagiosum lesions in immunocompromised patients often are large, are situated more deeply in the epider-

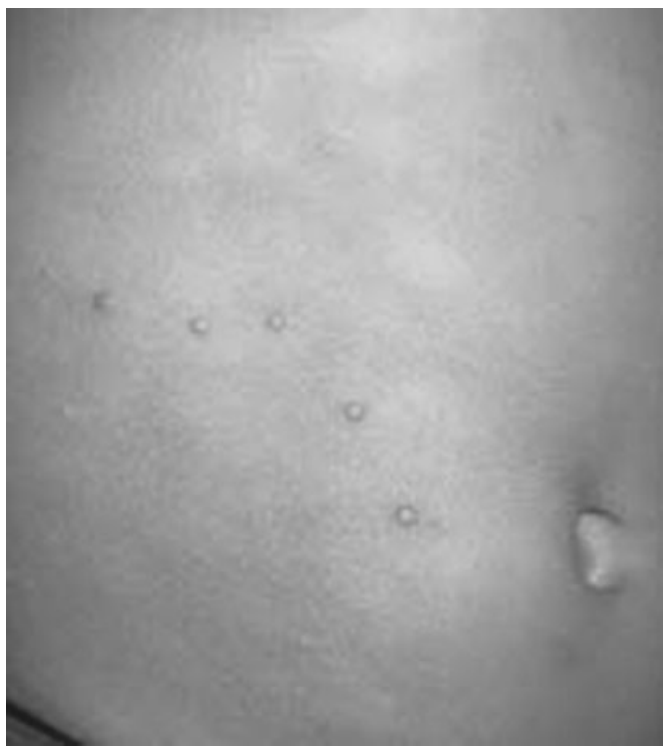

Figure 67–1 Grouped dome-shaped papules of molluscum contagiosum on the skin of the abdomen. (See companion Expert Consult web site for color version.)

mis, and may number in the hundreds. HIV-positive adults usually have molluscum contagiosum on the face, neck, and trunk. In a study of immunocompromised children, molluscum contagiosum was not considered to be common or severe, however. This study included only six patients, and two were disease-free and thought to be immunocompetent at the time of onset of the infection. This and other studies suggest that the presence and degree of cellular immunodeficiency are important in the presentation of molluscum contagiosum.

Patients with atopic dermatitis may be predisposed to develop more severe molluscum contagiosum infection. Available information is insufficient to know if this development is related to the dermatitis itself with its well-described barrier dysfunction or to the use of corticosteroids or other topical agents. The term *molluscum dermatitis* is used to describe an eczematous eruption, more commonly seen in patients with atopic dermatitis, which may occur around molluscum contagiosum lesions and is thought to represent a delayed-type hypersensitivity reaction to viral antigens in the dermis.

Molluscum contagiosum infections in healthy individuals usually resolve spontaneously over the course of several months to 3 to 5 years. For this reason, limited infections can be observed expectantly. More diffuse infections or infections that show continued extension can be considered for treatment. No definitive treatments are available for molluscum contagiosum. Most treatments, similar to those used for warts, are destructive in nature. Immune enhancement for these infections has been tried, but its utility has not been proven. Multiple treatments have been used, although utility is uncertain in all but curettage.

PARVOVIRUS B19 INFECTIONS

The most well-known infection caused by parvovirus B19 is erythema infectiosum or fifth disease.[86] The infection also is termed *slapped cheek disease* because of its initial skin manifestation of bilateral facial erythema (Fig. 67–2). Other manifestations include a papular purpuric glove-and-socks eruption and an eruption with pustules in a bathing-trunk distribution.

The virus is a single-stranded DNA virus that infects erythroid cells. The infection is spread via the respiratory route, with an incubation period of approximately 2 weeks. Most patients are asymptomatic. Serologic assays for IgG and IgM antibodies are used for confirmation of infection. The exanthem of fifth disease coincides with the presence of IgM antibodies. Viral DNA has been found in infected tissues.

Children with fifth disease or erythema infectiosum generally are not ill-appearing. Mild complaints of myalgias or low-grade fevers may be reported. As in adults, arthritis may be seen in older children and adolescents. Outbreaks often spread through schools or families. Most patients present to their physicians with a typical-appearing reticulated blanching erythema. The slapped cheek appearance characteristic of the disorder may be missed or elicited only on questioning.

The papular, purpuric glove-and-socks syndrome is another condition caused by infection with parvovirus B19.[62] When patients present with the eruption, they are infectious based on the finding of viral DNA. The eruption shows a curious distribution of the hands and feet with clear "cutoff" at the wrists and ankles. Changes in oral skin and mucous membrane may be seen, in addition to mild systemic complaints.

Diagnosis of erythema infectiosum or papular purpuric glove-and-socks syndrome is confirmed by serologic testing for antibodies or polymerase chain reaction. A report of loop-mediated isothermal amplification has shown its utility for rapid diagnosis of such infections and has compared favorably with polymerase chain reaction.[134]

GIANOTTI-CROSTI SYNDROME (PAPULAR ACRODERMATITIS OF CHILDHOOD)

Gianotti-Crosti syndrome (papular acrodermatitis of childhood) is a viral exanthem first reported in 1955 by Gianotti,[50] who described a group of children who presented with predominately acrally located, skin-colored or slightly erythematous, lichenoid papules (Fig. 67–3).[120] The flexures usually were spared. Involvement of the buttocks or trunk was seen in some cases. No involvement of mucous membranes occurred. Patients generally were healthy. The initial reports showed prior infection with hepatitis B. The eruption lasted 2 weeks to 2 months.

Since the initial report, similar cases have been associated with other infections, such as Epstein-Barr virus, coxsackievirus, echovirus, other viruses, and streptococcal disease.[85] Most cases are thought to be viral in origin and often caused by Epstein-Barr virus. Topical therapies do not hasten resolution of the exanthem.

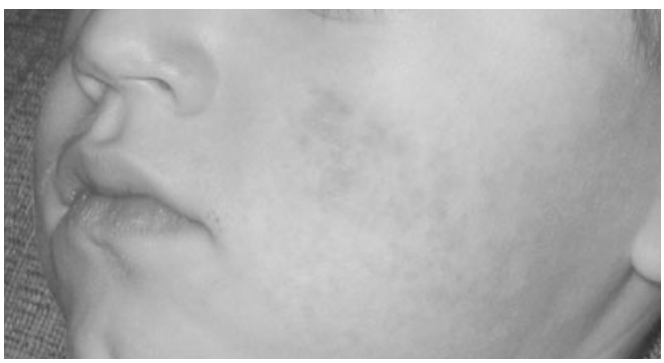

Figure 67–2 Reticulated erythema on the face of a child with erythema infectiosum. (See companion Expert Consult web site for color version.)

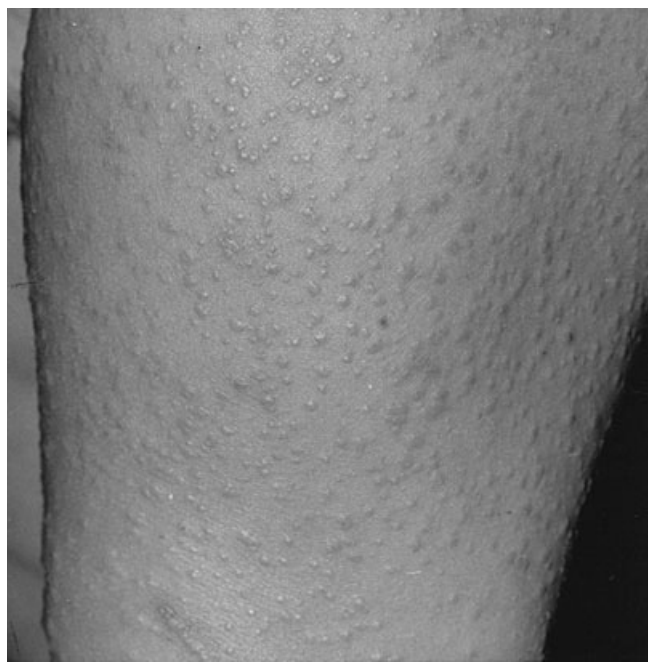

Figure 67–3 Diffusely distributed, isolated, monomorphous skin-colored papules on the thigh of a child typical of Gianotti-Crosti syndrome.

ASYMMETRIC PERIFLEXURAL VIRAL EXANTHEM

Some children have been described with discrete or grouped lichenoid papules or urticarial papules beginning around flexures or the torso (Fig. 67–4).[49] The eruption generalizes over several days and can appear as a more diffuse case of the papular acrodermatitis of childhood. The condition also has been described as the unilateral laterothoracic viral exanthem and seems to be associated with the same infectious agents as described in papular acrodermatitis of childhood.[15] The evolution of this condition is similar to that in the papular acrodermatitis of childhood.

HAND, FOOT, AND MOUTH SYNDROME

Patients presenting with a distinctive eruption of sausage-shaped vesicles on the palms and soles with oral mucosal erosions after a mild viral gastroenteritis represent the classic case of hand, foot, and mouth syndrome. Patients may have erythematous papules found on the skin of the buttocks as well. Cases tend to cluster within homes, daycare centers, or schools. Most cases resolve spontaneously without sequelae over the course of 2 to 3 weeks. Hand, foot, and mouth syndrome traditionally is attributed to infection with coxsackievirus A16; however, more recent data suggest enterovirus 71 as a predominant cause as well.

A more recent publication followed a large outbreak of hand, foot, and mouth syndrome (and herpangina) in Taiwan.[25] As with known epidemiologic data, most cases occurred in younger children (93%, <4 years old; 75%, <1 year old). Enterovirus 71 and coxsackievirus A16 were the viruses predominantly recovered. Enterovirus 71 was seen in 66 percent of these patients. The authors emphasized the potential for mortality from this virus, particularly as a result of pulmonary edema or hemorrhage, although encephalitis also was seen. Enterovirus 71 was seen primarily in inpatients, whereas coxsackievirus A16 was recovered from outpatients.

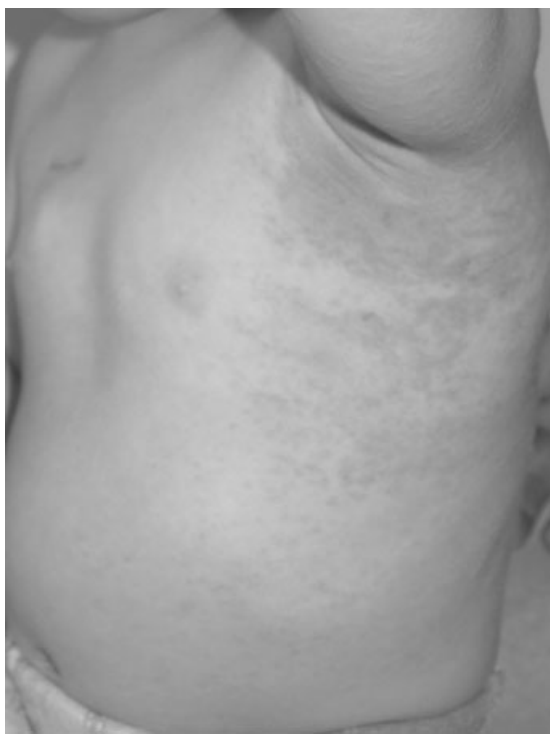

Figure 67–4 Lichenoid papules involving the lateral trunk typical of the unilateral periflexural viral exanthem. (See companion Expert Consult web site for color version.)

HERPES SIMPLEX VIRUS

Herpes simplex virus (HSV) types 1 and 2 are members of the herpesvirus family, which includes varicella-zoster virus, Epstein-Barr virus, cytomegalovirus, and human herpesvirus. HSV-1 and HSV-2 infections may be asymptomatic or associated with classic and unusual clinical manifestations. Although a predilection exists for involvement of the oral mucosa for HSV-1 and the genitalia for HSV-2, either can infect any site. These infections occur worldwide and are transmitted by direct contact. Asymptomatic carrier states exist and are known to be the source of infection in patients. Immunity to HSV-1 generally is acquired during childhood, whereas HSV-2 immunity usually is seen after adolescence.

The virus replicates within the epidermis and travels to regional nerve ganglia. The incubation period ranges from 2 to 20 days. Recurrences, which occur in the same site as the original infection, usually are less severe than is the primary infection. In immunocompetent patients, the infection usually remains localized.

CLINICAL MANIFESTATIONS

Children most often present with oral or perioral disease, such as gingivostomatitis or herpes labialis.[124] Painful vesicles and discrete "punched-out" erosions can be seen on the lips, anterior surface of the tongue, or hard palate. Herpes pharyngitis can be seen in older children and can be caused by HSV-2 infection. Feeding problems and foul-smelling breath can occur in these settings. Grouped vesicles on an erythematous base are a common cutaneous finding (Fig. 67–5).

Disease manifestations can last 10 to 14 days. Herpes gladiatorum is caused by transmission of HSV by direct contact. The name derives from exposure and infection between wrestlers, although the infection also can occur in the setting of close contact between participants of other sports. Eczema herpeticum occurs when cutaneous HSV infection spreads over the skin of individuals with an abnormal skin barrier, such as in atopic dermatitis. Infections can be localized or very diffuse and can be associated with systemic involvement. Herpetic whitlow manifests as painful deep-seated vesicles or pustules on the tips of fingers (Fig. 67–6). This infection results from direct contact with active lesions of HSV. A common presentation is in an infant or

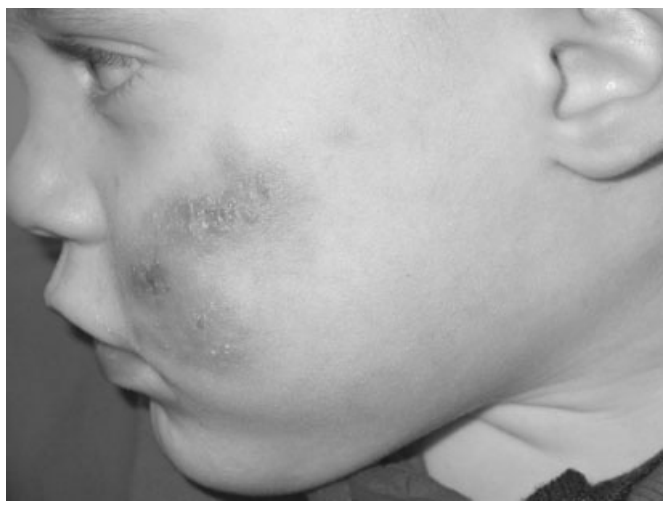

Figure 67–5 Tense vesicles overlying erythematous plaque caused by herpes simplex virus. (See companion Expert Consult web site for color version.)

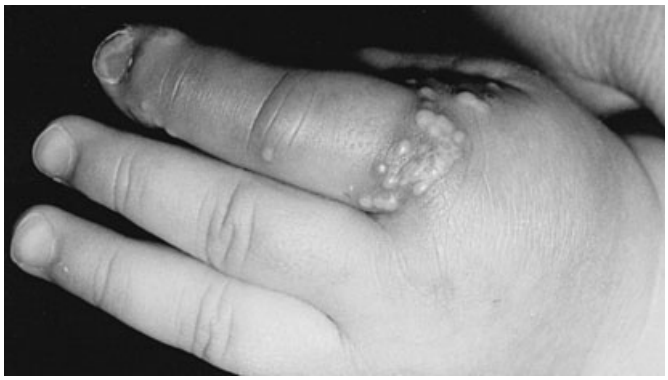

Figure 67–6 Photograph of the left hand of a young child shows grouped tense vesicles typical of a herpetic whitlow.

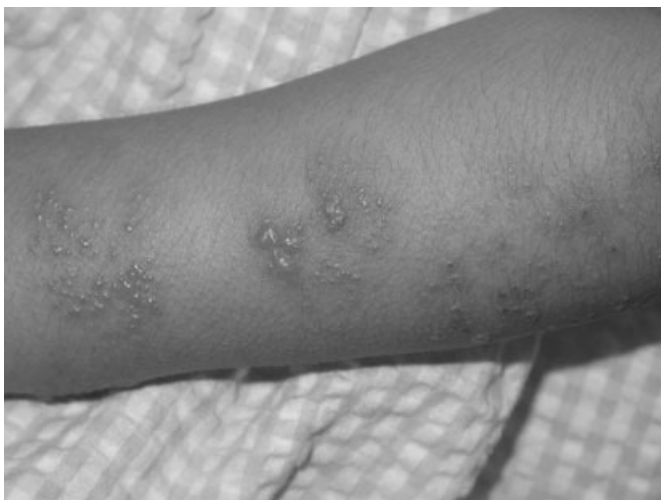

Figure 67–7 Linear array of vesicles caused by herpes zoster. (See companion Expert Consult web site for color version.)

young child who has manipulated a lesion of herpes labialis on a parent. Lastly, HSV infection is perhaps the most common cause of recurrent erythema multiforme.[129] Clinicians should keep this association in mind.

Localized disease and disease without systemic symptoms can be treated symptomatically. Local wound care is appropriate, as indicated. Acyclovir or valacyclovir is useful for cases when antiviral therapy is chosen. Prophylactic antivirals should be considered for cases of recurrent erythema multiforme.

VARICELLA-ZOSTER VIRUS

Chickenpox is the most common presentation of varicella-zoster virus infection in childhood. After a 2-week incubation period, vesicles on erythematous bases ("dewdrops on rose petals") are seen on the head and neck and spread rapidly to the trunk. Involvement of mucous membranes occurs frequently. Lesions are seen in all stages. Constitutional symptoms also may be seen. Unless bacterial infection occurs, scarring seldom occurs. An uncommon and perhaps under-recognized presentation of varicella is photolocalized disease. Several cases of varicella presenting in a photodistributed fashion (i.e., over sun-exposed skin) have been reported.[126]

Shingles or herpes zoster occurs after primary infection with varicella-zoster virus or receipt of vaccination (Fig. 67–7).[112,125] Healthy children who have had a case of chickenpox before reaching 6 to 12 months of age are at risk for developing shingles.[74] Immunocompromised children are at risk for acquiring this infection as well. Ramsay Hunt syndrome is caused by infection of the geniculate ganglion in this setting.[124]

Most cases of chickenpox do not require antiviral therapy. Management of systemic symptoms and local skin care are needed. Antivirals are needed when treating neonates or immunocompromised patients or normal patients with severe skin disease. There is considerable anecdotal experience using corticosteroids and antivirals for the management of Ramsay Hunt syndrome.

FUNGAL INFECTIONS

Superficial fungal infections, which include dermatophytes, yeast, or dematiaceous fungi, are encountered frequently in general pediatric practice. Particularly in an immunocompromised patient, fungal infections also may involve deeper cutaneous structures and other organ systems. This section focuses on the skin manifestations, diagnosis, and treatment of pediatric fungal infections.

SUPERFICIAL FUNGAL INFECTIONS

DERMATOPHYTE INFECTIONS

Dermatophytes invade and grow in hair, nails, and the outer layer of the skin known as the *stratum corneum*. These lesions vary in appearance based on the site of infection and are named accordingly.

Tinea Capitis

Also known as *scalp ringworm*, tinea capitis is seen frequently in prepubertal children. The causative organisms vary according to country; in North America, tinea capitis is caused largely by *Trichophyton tonsurans* and less often by *Microsporum canis*.[101,121,128] *M. canis*, a zoophilic dermatophyte common to suburban and rural areas, can be transmitted to children who handle infected animals such as cats, dogs, and certain rodents; however, it does not spread between humans.[121] Thick, white patches of broken hair that fluoresce under Wood lamp examination characterize these lesions.

Human-to-human transmission of *T. tonsurans* occurs through the shedding of spores from infected skin scales and hair on items such as clothing, bed linen, combs, and hairbrushes. Lesions caused by *T. tonsurans* do not fluoresce under a Wood lamp and vary in appearance from diffuse scaling of the scalp to circumscribed areas of scaling and erythema with or without hair loss, and, in dark-haired individuals, to scaly patches of hair loss that may leave small dark hairs in the follicles (black-dot ringworm) (Fig. 67–8). Tinea capitis lesions may be associated with suboccipital or posterior cervical lymphadenopathy and may produce more inflammatory lesions, including scaly, pustular areas of hair loss and formation of a kerion—a boggy, erythematous mass with follicular pustules that may lead to permanent hair loss and scarring if left untreated (Fig. 67–9).[28,101,128] Another hypersensitivity response to the dermatophyte infection is a dermatophytid or id reaction, which involves papulovesicular inflammatory eruptions adjacent to or distant from the site of the primary infection (Fig. 67–10).[12]

Tinea capitis must be distinguished from alopecia areata, which consists of patches of total hair loss without scalp changes, and trichotillomania, which may be associated with excoriations,

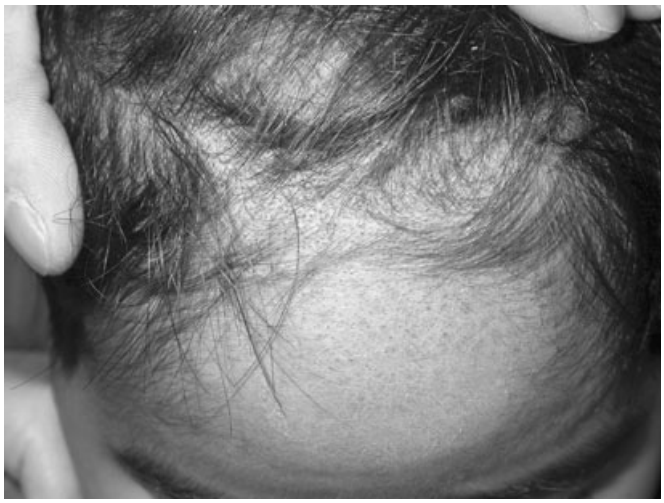

Figure 67–8 Nonscarring alopecia associated with tinea capitis. (See companion Expert Consult web site for color version.)

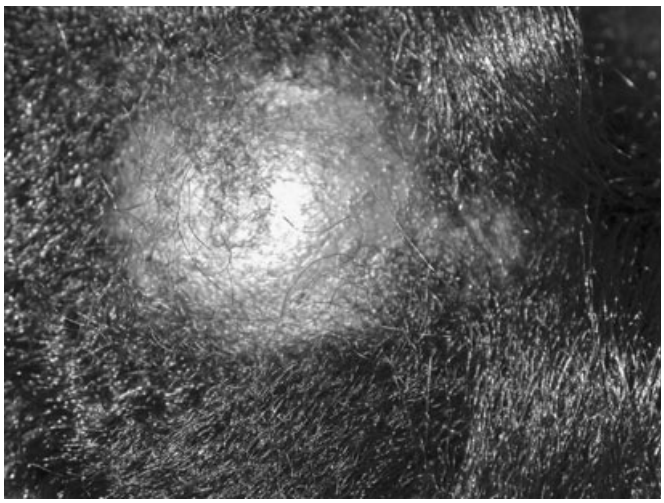

Figure 67–9 Indurated tumor on the scalp typical of kerion. (See companion Expert Consult web site for color version.)

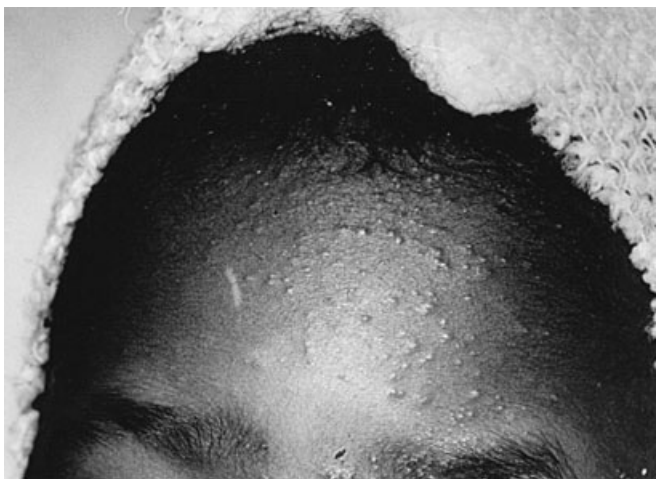

Figure 67–10 Multiple skin-colored papules and pustules on the forehead typical of an id reaction to tinea capitis.

varying lengths of broken hairs, and ill-defined patterns of hair loss. Differential diagnosis also includes traction alopecia, seborrheic dermatitis, psoriasis, and pityriasis amiantacea. In addition, inflammatory lesions of tinea capitis may be confused with bacterial pyodermas of the scalp.[28,121,128]

Tinea Corporis

Tinea corporis refers to dermatophyte infection of the trunk or extremities. Lesions appear as erythematous patches or plaques with well-demarcated, annular borders that may be scaly, pustular, or vesicular.[28,121,128] The application of potent topical corticosteroids may obscure these characteristic borders, resulting in a manifestation termed *tinea incognito*.[121] The differential diagnosis includes seborrheic, atopic, and contact dermatitides; psoriasis; and granuloma annulare.[28,128]

Tinea Faciei

Dermatophyte infections of the face in children are characterized by erythematous, scaly plaques that may be unilateral or occur in a "butterfly" distribution, mimicking cutaneous findings of systemic lupus erythematosus and other collagen vascular diseases (Fig. 67–11). As with tinea corporis, differential diagnosis includes seborrheic, atopic, and contact dermatitides.[28,128]

Tinea Pedis

Tinea pedis, which occurs more commonly in adolescents than in prepubertal children, characterizes dermatophyte infection involving the feet. Findings vary and may be vesicles or erosions over the instep of the feet, fissuring between the toes with surrounding erythema and scaling, or diffuse scaling of the soles in a "moccasin-like" distribution. Differential diagnosis includes juvenile plantar dermatosis, dyshidrotic eczema, atopic dermatitis, contact dermatitis, granuloma annulare, scabies, psoriasis, and erythrasma.[28,121,128]

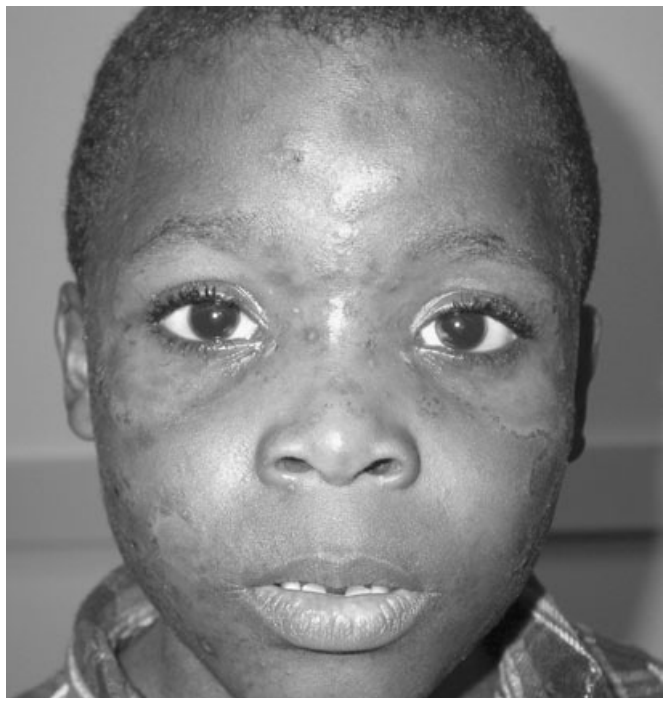

Figure 67–11 Diffuse scaling plaques on the face caused by tinea faciei. (See companion Expert Consult web site for color version.)

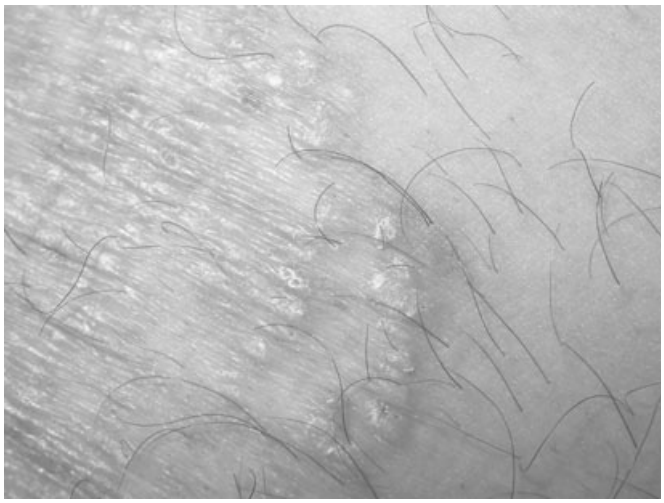

Figure 67–12 Annular scaling plaque on the upper inner thigh caused by tinea cruris. (See companion Expert Consult web site for color version.)

Tinea Cruris

Also predominately seen in adolescents, particularly boys, tinea cruris often occurs in association with tinea pedis and is transmitted through indirect or direct contact with infected skin scales or hair. Risk factors include obesity, friction, moisture, and tight clothing. The characteristic lesions are usually symmetric, well-demarcated, erythematous, scaly eruptions in the inguinal and inner thigh area with potential spread to the buttocks and perianal region. These lesions also may have a raised papular or pustular edge and often are very pruritic, leading to lichenification in chronic disease (Fig. 67–12). The differential diagnosis includes seborrheic dermatitis, *Candida* intertrigo, flexural psoriasis, irritant dermatitis, contact dermatitis, and erythrasma.[28,121,128]

Tinea Unguium

The term *onychomycosis* refers to fungal invasion of the nail plate, which is termed *tinea unguium* when the infection is caused by dermatophytes. The lower incidence of tinea unguium in children compared with adults may be due to faster nail growth, decreased likelihood of nail trauma, and less exposure to tinea pedis, which often is seen concurrently with onychomycosis.[63,106] Clinical findings of infection include superficial white patches on the surface of the nail plate, white spots under the nail, and yellowing and thickening of the nail plate that usually extends from the distal to the proximal end (Figs. 67–13 and 67–14). The differential diagnosis includes psoriasis, lichen planus, hereditary onychodystrophy, and acquired trachyonychia, all of which may be distinguished from tinea unguium by their diffuse nail involvement as opposed to the more limited disease of a dermatophyte infection. Tinea unguium also may mimic *Candida* infection of the nails, but the latter disease differs in that it tends to spread distally from the proximal nail plate.[28,57,63,101,106,128]

DIAGNOSIS

Light microscopy examination with potassium hydroxide should show spores in infected scalp hairs of tinea capitis and branching hyphae in affected nail samples or skin scrapings from advancing margins of lesions on the body (see Fig. 67–14). Fungal culture on Sabouraud dextrose agar treated with chloramphenicol and cycloheximide provides a growth medium selective for dermatophytes and may aid in differentiating various species. Rarely, biopsy may be required for definitive diagnosis.[28,101,121,128]

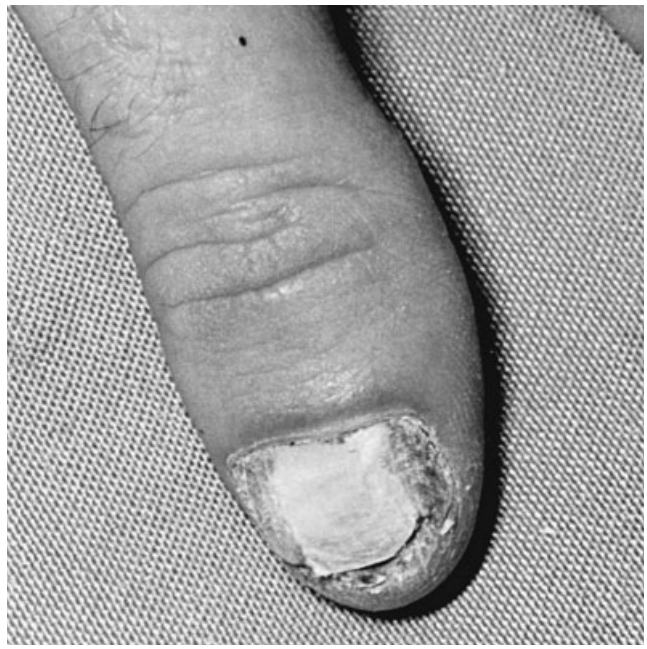

Figure 67–13 Clinical photograph illustrating onychodystrophy and subungual hyperkeratosis involving the left thumb of a child with onychomycosis.

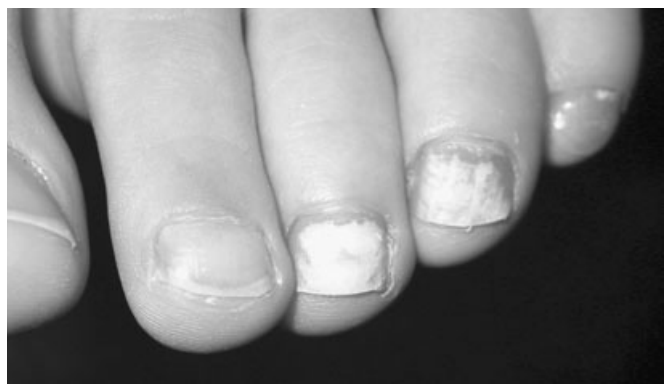

Figure 67–14 White superficial plaques typical of superficial white onychomycosis. (See companion Expert Consult web site for color version.)

TREATMENT

Localized lesions of tinea corporis, faciei, pedis, and cruris generally respond to a 2- to 4-week course of topical antifungals, such as clotrimazole, tolnaftate, ciclopirox olamine, amorolfine, or terbinafine.[101] Severe or refractory disease may require oral antifungal medications. Recurrence of tinea pedis can be prevented by avoiding occlusive footwear and keeping the feet clean and dry.[121]

Griseofulvin is the only oral antifungal medication that is approved by the U.S. Food and Drug Administration (FDA) for use in children. Many agents, including terbinafine, itraconazole, and fluconazole, have been used successfully as off-label alternatives, however, despite limited data regarding their safety and efficacy in children.

Griseofulvin remains the gold standard for the treatment of tinea capitis in the United States. The approved dose of microsized griseofulvin is 11 mg/kg/day for 6 to 8 weeks; however, 20 to 25 mg/kg/day often is required to achieve an adequate response.[114] A 2- to 4-week course of terbinafine also has been shown to be as effective as is griseofulvin, particularly in the treatment of tinea capitis caused by *Trichophyton* spp.[42,45,54,115] For *Microsporum* infections, higher doses or longer duration of therapy with terbinafine may be required.[42,45,54,76,115] Studies have shown that a fluconazole regimen of 6 mg/kg/day for 3 to 6 weeks has a cure rate comparable to that of griseofulvin.[43,54,114] Continuous or pulse itraconazole therapy of 5 mg/kg/day has been noted to result in significant improvement and even cure in *Trichophyton* and *Microsporum* tinea capitis by 6 weeks; however, infection with *Microsporum* may require a longer duration of treatment with itraconazole compared with effective courses for *Trichophyton* infection.[51,54,55] In addition, concomitant topical therapy with selenium sulfide 1 to 2.5 percent shampoo in affected individuals and household contacts reduces the number of viable spores and prevents the spread of infection.[52,105,121]

At present, no antifungal agent is approved by the FDA for treatment of onychomycosis in children, and data are limited regarding efficacy of topical and oral antifungal agents. Topical agents have poor penetration into the nail plate, usually leading to an inadequate response rate. Treating concurrent tinea pedis infections with topical therapy may help prevent recurrences of tinea unguium, however. Although griseofulvin is the drug of choice for treating other dermatophyte infections, it is not recommended for the treatment of onychomycosis because of the lengthy course (≤18 months), inadequate response, and high recurrence rate.

Terbinafine (3 to 6 mg/kg/day for 6 to 12 weeks), continuous or pulse itraconazole therapy (5 mg/kg/day for 3 months), and fluconazole (3 to 6 mg/kg once a week for 12 to 26 weeks) have been reported as safe, effective, and well-tolerated alternatives.[57,59,93,115] Because of their keratinophilic and lipophilic natures, these agents accumulate in the stratum corneum and persist at high concentrations in the nails for months, allowing for shorter courses of therapy.[55,57,59,63,115] Terbinafine may be crushed and taken with or without food. Optimal bioavailability with itraconazole capsules occurs when taken with fatty foods, whereas itraconazole solution should be taken on an empty stomach and may be associated with a higher incidence of gastrointestinal side effects than capsules. Studies regarding use of fluconazole for treatment of onychomycosis in children are limited, but successful therapy has been reported at a pulsed dose of 3 to 6 mg/kg once a week for 3 months.[57,59,115]

CANDIDA

The most common cause of fungal infection in healthy and immunocompromised children is *Candida*, and *Candida albicans* accounts for most cases. These yeast forms have a predilection for moist, warm areas of the body such as mucosal surfaces and intertriginous regions and often colonize these areas, becoming part of the normal flora. Disease is caused by overgrowth and infiltration into the epidermis. Superficial infections may be seen in healthy individuals and individuals with predisposing risk factors, such as prematurity; low birth weight; diabetes mellitus; antibiotic, corticosteroid, or oral contraceptive therapy; and other immunocompromised states. These individuals also are more likely to develop systemic candidiasis.[46,101,128]

Thrush is characterized by superficial, sometimes tender, white plaques on oral mucosa that reveal denuded, erythematous bases when scraped off (Fig. 67–15). These manifestations must be distinguished from other oral cavity lesions, such as aphthous stomatitis, epidermolysis bullosa, herpes simplex, hairy leukoplakia, lichen planus, geographic tongue, erythema multiforme, and burns.[128] Use of pacifiers in infants may increase the risk of development of thrush and colonization of the organism.[11] Oropharyngeal candidiasis may spread to the esophagus in immunocompromised individuals and lead to difficulty with feeding.

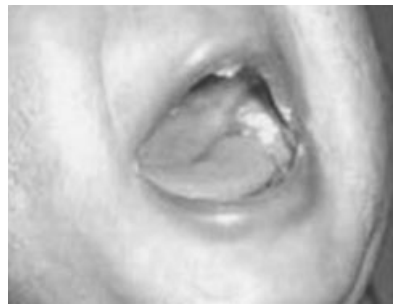

Figure 67–15 White patches on buccal mucosa caused by *Candida albicans*. (See companion Expert Consult web site for color version.)

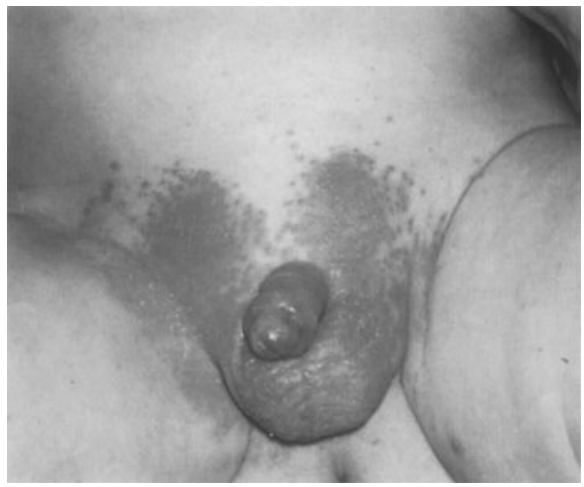

Figure 67–16 Clinical photograph of an infant with *Candida* diaper dermatitis. Confluent and discrete erythematous papules and plaque involving the scrotum, penis, and superpubic and inguinal area.

Diaper candidiasis appears as beefy, erythematous plaques that involve the inguinal creases and perianal region and often are associated with satellite (or surrounding) erythematous papules, plaques, or pustules (Fig. 67–16). These lesions may cause discomfort when the infant urinates onto affected skin. Superinfection with *Candida* should be suspected when irritant diaper dermatitis fails to improve within several days. Other lesions that may be confused with diaper candidiasis include psoriasis, Langerhans cell histiocytosis, and seborrheic dermatitis.[128]

Thumb-sucking or other trauma near nail beds may lead to nontender, erythematous swelling at the base of the nails termed *paronychia* (Fig. 67–17). Some cases are complicated by *Candida* infection. Differential diagnosis of these lesions includes psoriasis, bacterial infection, lichen planus, and pachyonychia congenita. *Candida* also may produce onychomycosis, but its distribution usually involves the proximal nail plate, in contrast to dermatophytosis of the nails, which more commonly affects the distal nail plate.[128]

Adolescent girls may present with vulvovaginal candidiasis, which is characterized by thick, white discharge and white plaques on irritated, erythematous vaginal mucosa. These lesions may cause vaginal itching and dysuria.[128]

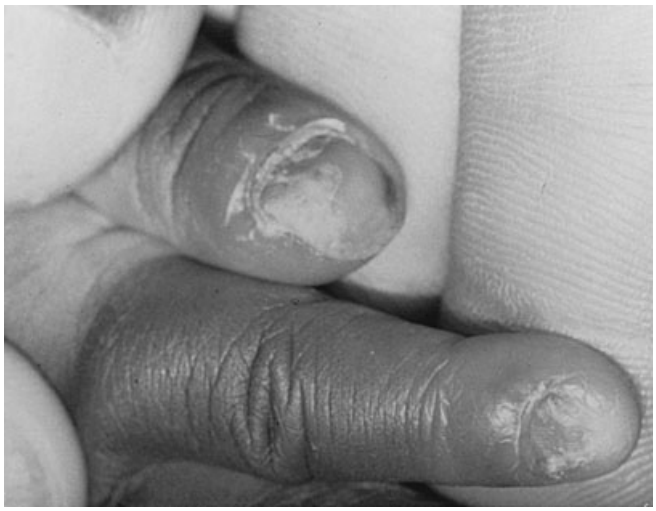

Figure 67–17 Onychodystrophy involving the thumb and index finger, with erythema and edema of the paronychium. These features are typical of *Candida* paronychia.

Figure 67–18 Dense crusting overlying the flank and buttocks of a neonate with *Candida* dermatitis. *(From Rowen, J. L., Atkins, J. T., Levy, M. L., et al.: Invasive fungal dermatitis in the 1000-gram neonate. Pediatrics 95:682-687, 1995.)*

Diagnosis of localized *Candida* infection usually is made clinically. Microscopic examination of potassium hydroxide–prepared scrapings of skin and mucosal lesions reveals pseudohyphae and elongated budding yeast forms. Positive cultures of infected material on Sabouraud dextrose agar or cornmeal agar may signify infection or benign colonization. Biopsy rarely is required for establishing a definitive diagnosis.

Treatment of localized *Candida* infection depends on good hygiene practices and maintenance of a dry environment in the susceptible area. Localized infections usually respond well to topical antifungal therapy. Nystatin solution is the first-line agent used for thrush, but oral fluconazole or itraconazole and intravenous amphotericin B may be required to treat recurrent, refractory, or extensive disease, especially in immunocompromised children.[65] Nystatin or imidazole creams in conjunction with frequent diaper changes, barrier creams, and sometimes low-strength topical steroids are the recommended treatment for diaper candidiasis. Paronychia also is susceptible to nystatin and imidazole creams but may require oral therapy. Vulvovaginal candidiasis often can be treated successfully with topical azole medications, but oral azoles are recommended for refractory or recurrent cases. Adolescents can be treated with a single, 150-mg dose of oral fluconazole.[22,65]

Congenital candidiasis, a rare form of infection acquired in utero as a result of *Candida* chorioamnionitis, can manifest in full-term and premature neonates within the first few days of life as a diffuse, erythematous, papular eruption that progresses to formation of vesicles and pustules, and subsequently crusting and desquamation. Palms and soles also may be affected. Term infants with congenital candidiasis rarely develop systemic manifestations, and the infection usually resolves spontaneously or with topical antifungal agents, such as nystatin, imidazoles, or allylamines. Premature, very-low-birth-weight neonates are at risk for developing ecchymosis and necrosis of the skin with dissemination of the *Candida* infection, requiring treatment with systemic antifungal medication. Congenital candidiasis in these premature infants may manifest as sepsis or pneumonia without cutaneous findings as well.[2,101,119]

Differential diagnosis of congenital candidiasis includes epidermolysis bullosa and infection with *Listeria monocytogenes*, *Staphylococcus*, herpesvirus, and syphilis.[36] Early diagnosis is established by histologic examination of the placenta and umbilical cord, which shows the characteristic pseudohyphae and spores and white microabscesses.[2,36] Microscopic examination and culture of the skin lesions can confirm the diagnosis.

Systemic candidiasis, in which *Candida* spp. can be isolated from the blood, urine, or cerebrospinal fluid, is seen more commonly in immunocompromised individuals, who often present with skin manifestations that include desquamation, abscesses at indwelling catheter sites, and progressive diaper candidiasis. Systemic candidiasis may be associated with signs of sepsis, such as hemodynamic and temperature instability, and respiratory changes.[65,128]

Invasive fungal dermatitis is a rare manifestation of *Candida* infection in premature, very-low-birth-weight infants. *Aspergillus*, *Trichosporon*, and *Curvularia* spp. also have been implicated in this type of infection, but *Candida* is the most common etiologic agent. Besides low birth weight and prematurity, additional risk factors for acquisition of invasive fungal dermatitis include hyperglycemia, steroid use, and vaginal birth. In contrast to congenital candidiasis, invasive fungal dermatitis usually does not manifest until after several days of life. Skin findings include erythematous, macerated plaques and crusted erosions that can spread rapidly over the body and may lead to development of systemic fungemia, with a high mortality rate (Fig. 67–18). Biopsy of these lesions shows invasion through the stratum corneum into the epidermis with occasional extension into the dermis.[107] Survival depends on prompt initiation of systemic antifungal medications.[94,119]

Treatment of disseminated *Candida* infection requires removal of infected indwelling catheters in addition to administration of systemic antifungal therapy. Intravenous amphotericin B is the drug of choice for treating disseminated disease and sometimes is administered in combination with 5-fluorocytosine for synergism. Other options include systemic itraconazole and fluconazole; however, some *Candida* spp. have shown resistance to fluconazole.[2,101,119] Azoles also have been used as prophylaxis for systemic fungal infections in neutropenic patients with hematologic malignancies and bone marrow transplant recipients,[30,66,90] but this practice, especially in preterm neonates, is controversial because of the potential for resistance.[19,23] Newer antifungal agents, such as voriconazole, posaconazole, and caspofungin, have been reported to be effective treatments, particularly in resistant candidemia.[5,85,88,91,114,118] Avoidance of prolonged antibiotic and steroid administration may aid in clearance of *Candida* infection.[65,128]

MALASSEZIA

Malassezia spp., most notably *Malassezia furfur*, are lipophilic yeast forms that are found more readily in humid, tropical environments and are part of the normal skin flora in humans. Colonization begins in the neonatal period and increases with age.[10] *Malassezia* is the organism thought to be responsible for neonatal cephalic pustulosis and tinea (or pityriasis) versicolor in older children, adolescents, and young adults. Invasion of the organism through indwelling catheters, particularly catheters being used for intralipid infusion, in premature, very-low-birth-weight infants also may lead to sepsis and death.[7,103,119] *Malassezia* also has been implicated in the pathogenesis of seborrheic dermatitis, atopic dermatitis, and psoriasis, although this implication is controversial.[7,28,56,84,92,110,123]

Neonatal cephalic pustulosis is a self-limited disease characterized by scattered, erythematous papules and pustules in a head-and-neck distribution (Fig. 67–19). Follicular accentuation and comedones are not observed. Diagnosis usually is made clinically, but may be confirmed by microscopic findings.[10,101,119]

Tinea versicolor is characterized by macules with fine scales that are distributed on the upper body and neck. The macules may appear hypopigmented in dark-skinned individuals or tan or dark brown in lighter skinned individuals (Fig. 67–20). Differential diagnosis includes vitiligo, pityriasis alba, pityriasis rosea, postinflammatory hypopigmentation, melasma, seborrheic dermatitis, contact dermatitis, and tinea corporis. Lesions of tinea versicolor are fluorescent yellow to orange under Wood lamp. Microscopic examination of potassium hydroxide–prepared scrapings of the lesions shows a "spaghetti and meatball" appearance owing to the presence of short, curved hyphae and round spores. Biopsy of the lesions, which usually is unnecessary, reveals the characteristic hyphae and spores invading the stratum corneum.

The recommended treatment is application of 2.5 percent selenium sulfide or ketaconazole shampoo. Other topical agents, such as ciclopirox, terbinafine solutions, sodium hypochlorite, and other azoles, also may be effective. Short 1- to 5-day courses of oral itraconazole, fluconazole, and ketoconazole in single daily doses have been suggested as effective treatments; however, these agents are not approved by the FDA for treating tinea versicolor in children because of their limited safety and efficacy profiles.[12,41,100] It is important to advise patients that skin discolorations may take months to resolve and that recurrences are very common and may require retreatment.[28,100,101,119,128] *Malassezia* sepsis responds well to removal of indwelling catheters and rarely requires systemic antifungal therapy.[119]

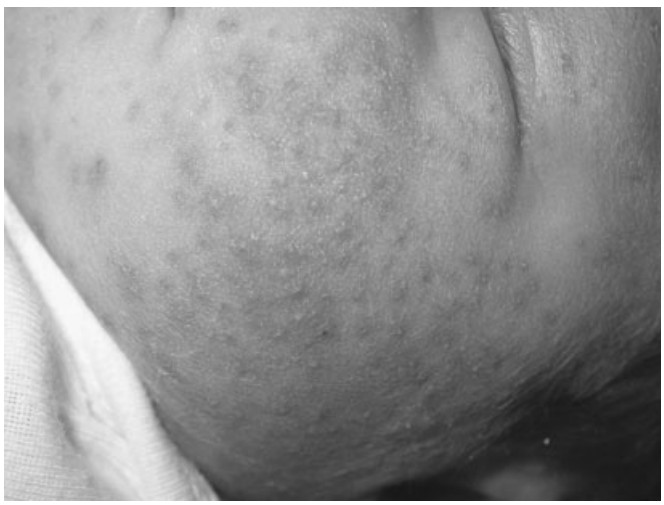

Figure 67–19 Pustules and papules on the face and scalp owing to neonatal cephalic pustulosis. (See companion Expert Consult web site for color version.)

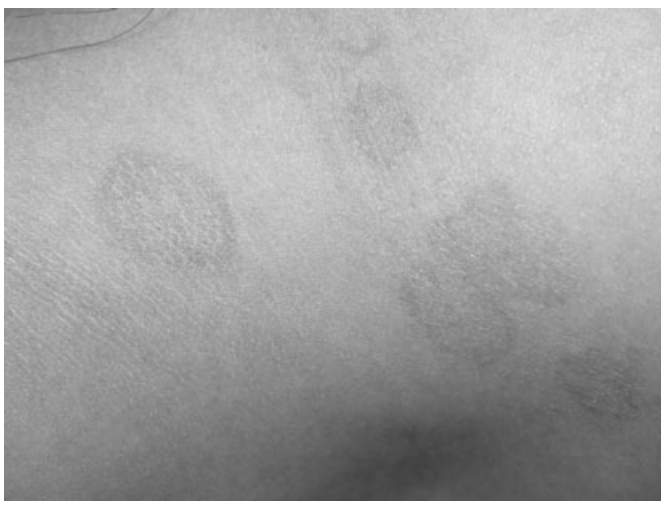

Figure 67–20 Well-circumscribed annular scaling plaques on the neck typical of tinea versicolor. (See companion Expert Consult web site for color version.)

CHROMOBLASTOMYCOSIS

Fonsecaea pedrosoi, and less commonly *Cladophialophora carrionii* and *Phialophora verrucosa*, are found in tropical climates and are organisms responsible for causing chromoblastomycosis, a chronic granulomatous disease affecting skin and subcutaneous tissue. Infection is acquired through traumatic inoculation into the skin. Cutaneous manifestations are described as pink papules that progressively enlarge to become tender, pruritic, hyperkeratotic nodules or verrucous plaques (Fig. 67–21). Scratching may lead to secondary infection or result in autoinoculation of the organism to other areas of the body. Infection may lead to scarring of lymphatic vessels causing obstruction and lymphedema.[16,97,102,107]

Differential diagnosis includes tuberculosis, sporotrichosis, blastomycosis, leishmaniasis, and squamous cell carcinomas. Potassium hydroxide smears of skin scrapings reveal characteristic dark brown, round, sclerotic bodies with horizontal or vertical septa and thick, pigmented hyphae. Sclerotic bodies also are identified easily on hematoxylin and eosin stain. In addition, biopsy of the chromoblastomycosis lesions shows granulomatous inflammation with macrophages and neutrophils and a hyperkeratotic, hyperplastic epidermis with microabscesses.[16,102,107]

Treatment of localized lesions involves surgical excision, cryotherapy, or heat therapy. Chemotherapy with 5-fluorocytosine

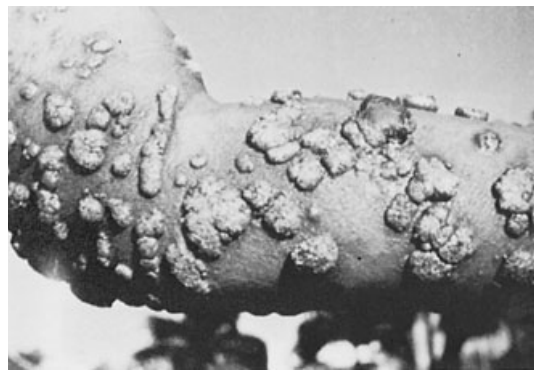

Figure 67–21 Chromoblastomycosis. The warty-like growth of epidermis and dermis is a result of traumatic implantation of the etiologic agent and subsequent autoinoculation from scratching. The agent was *Fonsecaea pedrosoi*.

and antifungal agents such as azoles, amphotericin B, and terbinafine has been used with some success in more widespread disease.[16,107,116]

TINEA NIGRA

Tinea nigra, caused by the organism *Phaeoannellomyces werneckii*, is a superficial fungal infection seen primarily in children and adolescents.[28,80,95] The organism is found in soil, wood, sewage, salted dried fish, and vegetation, mainly in beach environments of Central and South America, Africa, Asia, and, less frequently, in the United States and Europe. Transmission occurs by direct contact.[80,95,96]

Tinea nigra usually is characterized by an asymptomatic, well-demarcated, single brown to black macule or patch primarily on the palm, but may involve other areas of the body as well (Fig. 67–22). Lesions grow slowly and usually are nonscaly.[28,95] Tinea nigra lesions may mimic melanoma, lentigines, junctional nevi, and the hyperpigmented patches of Addison disease.[28,95,96] Under microscopic examination, potassium hydroxide–prepared scrapings of the lesion show yellow to light brown, branched, septate hyphae (Fig. 67–23).[28,96] Diagnosis is confirmed further by culture, which grows oval to spindle-shaped, moist, shiny, black colonies on Sabouraud agar.[95]

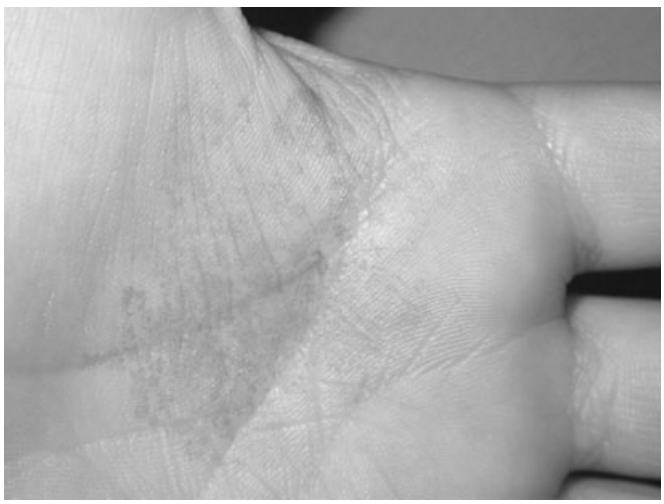

Figure 67–22 Brown patch on the palm of the hand caused by tinea nigra palmaris. (See companion Expert Consult web site for color version.)

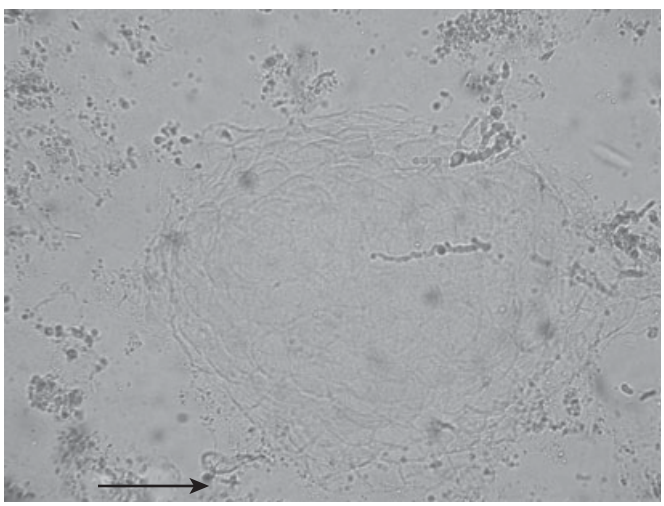

Figure 67–23 Septate hyphae seen by potassium hydroxide examination of scale from the patient in Figure 67–22. (See companion Expert Consult web site for color version.)

Tinea nigra has been treated successfully with keratolytic ointments or solutions, which include salicylic acid and benzoic acid and azole and allylamine creams.[28,95,96] Scraping the lesion with a scalpel also can remove the discoloration.[96]

TRICHOSPORONOSIS

Trichosporon spp., which are found in soil, vegetation, animal feces, and stagnant or fresh water, have a worldwide distribution with a predilection for tropical climates. They also may colonize human skin and digestive, respiratory, and urinary tracts. In immunocompetent individuals, *Trichosporon* organisms produce superficial fungal infections of the hair shaft termed *white piedra* and, less commonly, are one of the causes of onychomycosis. Systemic disease, termed *trichosporonosis*, may develop in immunocompromised patients, particularly neutropenic patients with hematologic malignancies, and in premature, very-low-birth-weight infants with colonization of indwelling catheters.[68,101]

White piedra is described as soft white, tan, or reddish-green nodules encircling the hair shaft that may be associated with scalp hyperkeratosis, brittle hair, and alopecia. These concretions do not fluoresce under Wood lamp examination. Differential diagnosis includes tinea capitis, pediculosis, black piedra, monilethrix, trichomycosis axillaris or pubis, and trichorrhexis nodosa.[70,135] Skin manifestations of disseminated infection include desquamation and serous drainage, purpuric papules or nodules with central ulceration or necrosis, and white plaques.[101,109]

Fungal blood culture reveals germ tube–negative and urease-positive yeast. Potassium hydroxide mounts of affected hair show hyaline septate hyphae, pseudohyphae, rectangular arthroconidia, and spherical blastoconidia that circumscribe the hair shaft.[70,107,109] Culture on Sabouraud dextrose agar shows cream-colored colonies that become wrinkled in a few weeks.[69] Biopsy specimen of skin lesions shows perivascular inflammation with budding yeast cells.[99]

White piedra is a difficult disease to treat because of the high recurrence rate. Although the American Academy of Dermatology Guidelines Committee recommends shaving affected hair to eradicate white piedra,[39] cosmetic and social considerations render this approach an unfavorable option for most patients. Topical antifungal agents that have been tried with varying degrees of success include ciclopirox olamine, imidazoles, chlorhexidine solution, 2.5 percent sulfide shampoo, zinc pyrithione, Castellani paint, mercury bichloride solution, 5 percent sulfur ointment, 5 percent ammoniated mercury ointment, and amphotericin B lotion. Although topical treatments may clear hair shaft concretions, they are not as effective at preventing recurrence. Because of their ability to bind keratin and their excretion in sebum, oral antifungal agents such as itraconazole may achieve higher concentrations in hair follicles and lead to greater success in eliminating colonized *Trichosporon* organisms and preventing relapse.[69,70]

Disseminated trichosporonosis is associated with high morbidity and mortality rates. It has been treated with varying degrees of success using amphotericin B with or without 5-fluorocytosine and the newer azole drugs, such as voriconazole.[4,68]

DEEP FUNGAL INFECTIONS

ASPERGILLOSIS

Aspergillosis, an opportunistic fungal infection caused by *Aspergillus* spp., affects primarily immunocompromised hosts, such as premature infants and children undergoing chemotherapy for

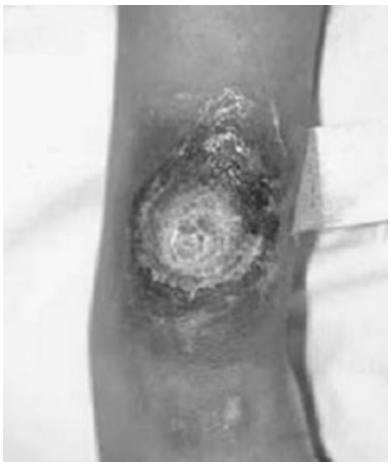

Figure 67–24 Eschar overlying eroded skin on a child with invasive aspergillosis. (See companion Expert Consult web site for color version.)

leukemia. It is acquired most commonly by inhalation of spores into the respiratory tract, with subsequent hematogenous spread to other organ systems including the skin. Cutaneous manifestations of aspergillosis may represent disseminated disease or, less commonly, infection limited to the skin. Premature infants have been reported to develop primary cutaneous aspergillosis at sites of skin trauma caused by adhesive tape, monitor leads, or placement of an intravenous catheter. Lesions appear as erythematous papules, pustules, nodules, or plaques that progressively enlarge and develop necrosis with formation of eschar (Fig. 67–24).[3,14,99,101,119] Differential diagnosis includes ecthyma gangrenosum, cutaneous candidiasis, mucormycosis, vasculitis, and pyoderma gangrenosum.[99,101]

Culture and histopathologic examination, revealing granulomatous inflammation with branched septate hyphae, of the skin biopsy specimen confirms the diagnosis.[14,99,101,119] Treatment of cutaneous aspergillosis includes surgical débridement or excision and administration of systemic antifungals.[101,119] Although amphotericin B remains the drug of choice, itraconazole and newer azoles such as voriconazole and other antifungal agents, including lipid-associated amphotericin B preparations and echinocandins such as caspofungin, also seem to be effective in individuals who cannot tolerate or are refractory to amphotericin B.[44,77,98,111,114] Data regarding safety and efficacy of these alternative therapies in children are limited.

BLASTOMYCOSIS

Blastomyces dermatitidis, a dimorphic fungus that causes North American blastomycosis, is found in the soil in the midwestern United States near the Ohio and Mississippi River valleys and the Great Lakes region, Canada, Central and South America, and tropical areas of Asia and Africa. Human disease is acquired through inhalation of spores, causing lung disease, and, rarely, through direct inoculation into the skin, which may cause primary cutaneous blastomycosis. Infection of the skin usually is due to hematogenous spread from a primary pulmonary focus, however.[13,48,99,113,125,136]

Cutaneous lesions are seen more frequently on sun-exposed areas and may begin as papules or pustules that progressively enlarge; become verrucous or ulcerative; and develop elevated, crusted borders. The central regions may heal, leaving an atrophic, hypopigmented scar, whereas the advancing borders remain active. Subcutaneous nodules with surface pustules and abscess formation may be seen as well. Erythema nodosum has been described as a reactive skin manifestation of pulmonary blastomycosis.[13,48,99,113,136]

Biopsy specimens show noncaseating granulomas with giant cells and microabscesses, and potassium hydroxide smears of purulent material obtained from lesions or biopsy specimens may reveal broad-based budding yeast forms with thick cell walls. Culture on Sabouraud dextrose agar confirms the diagnosis.[13,48,99,113,136]

Amphotericin B is the recommended treatment for blastomycosis with central nervous system involvement and disease in immunocompromised individuals or individuals with severe, disseminated disease. For mild to moderate blastomycosis, itraconazole, sometimes after a course of amphotericin B, is preferred.[13,48,99,113,136] Research on the development of a vaccine against *B. dermatitidis* for immunocompromised individuals in endemic areas or individuals with high exposure is in progress.[34,133]

COCCIDIOIDOMYCOSIS

Coccidioides immitis is a dimorphic fungus endemic to the southwestern United States, Mexico, and Central and South America. Transmission occurs through inhalation of spores from the soil, and the primary site of infection usually is in the lung.[29,99,117] Shortly after the onset of symptoms, primary pulmonary disease may be associated with reactive skin lesions, which do not contain viable organisms. These findings include a generalized, erythematous exanthem, erythema multiforme, Sweet syndrome, or interstitial granulomatous dermatitis.[29,37,99]

Erythema nodosum may be seen 1 to 3 weeks after the onset of infection. The exanthem, which may last several weeks, varies from macular, papular, morbilliform, to urticarial. The exanthem and erythema multiforme lesions may be associated with involvement of the oral cavity, pruritus, target-like lesions, and desquamation of the palms. Sweet syndrome, or acute febrile neutrophilic dermatosis, is characterized by abrupt onset of fever; leukocytosis; and tender, erythematous, well-demarcated papules or plaques. These lesions result from dense, neutrophilic infiltration of the dermis and respond well to topical corticosteroids. Interstitial granulomatous dermatitis, which may persist for 2 months, is a reactive eruption of indurated papules, nodules, and plaques that are caused by interstitial dermal infiltration with macrophages, neutrophils, eosinophils, and leukocytoclastic debris. Erythema nodosum manifests as painful, erythematous, subcutaneous nodules usually found on the lower extremities.[37]

Coccidioidomycosis may spread hematogenously to the skin, a common site of disseminated disease, and produce abscesses, sinus tracts, and granulomatous verrucous nodules or plaques. In contrast to the reactive skin manifestations, these lesions show the characteristic organism under microscopic examination. In extremely rare cases, traumatic inoculation into the skin may result in primary cutaneous coccidioidomycosis characterized by painless, ulcerated, and indurated nodules or plaques associated with regional lymphadenitis. Affected lymph nodes also may ulcerate.[37,99]

Histopathologic examination of infected cerebrospinal fluid and pleural fluid, bronchoalveolar lavage, and biopsy specimens of skin lesions may reveal large spherules containing endospores. Spherules may be seen on culture as well, but specimens should be handled using appropriate safety precautions because the organism may convert to active form during growth on culture plates. Serum antibody titers may be helpful for confirming the disease.[29,37,99,117]

Because uncomplicated primary coccidioidomycosis is self-limited in most patients, administration of antifungal treatment is unnecessary. Individuals with severe or disseminated disease or individuals with risk factors such as immunosuppression should

receive treatment. Amphotericin B is the recommended treatment for severe or progressive disease; otherwise, oral azole antifungals, such as itraconazole and fluconazole, are used most often for initial therapy of nonmeningeal coccidioidomycosis.[29,37,82,99,117] Oral fluconazole with or without administration of intrathecal amphotericin B is recommended for central nervous system involvement.[29,82,117] Additionally, surgical excision of localized infection with or without administration of antifungal therapy may be considered. Immunocompromised patients and patients with meningeal disease may require lifelong suppressive antifungal therapy with an azole to prevent recurrence.[29,37,117] The development of a vaccine against coccidioidomycosis is in progress.[31]

CRYPTOCOCCOSIS

Cryptococcus neoformans, the causative species of cryptococcosis, is an encapsulated yeast found worldwide in soil, fruits, and avian stool. Cryptococcosis affects primarily immunocompromised adults, particularly patients with AIDS, but it also may be seen in children. Infection usually is acquired through inhalation but rarely may spread through direct inoculation. Cutaneous findings, which often mimic other skin diseases, such as molluscum contagiosum and Kaposi sarcoma, vary as papules, nodules, vesicles, purpuric plaques, cellulitis, ulcers, abscesses, and sinus tracts.[27,32,64,99]

Diagnosis of these polymorphic lesions as a manifestation of cryptococcosis is established by visualizing the characteristic encapsulated yeast cells in culture on Sabouraud dextrose agar and on histopathologic examination of a skin sample stained with methenamine silver, periodic acid–Schiff, or mucicarmine. Another diagnostic method includes identification of the organism on smears of infected material prepared with potassium hydroxide and India ink.[27,32,64,99] Although a few cases of isolated cutaneous cryptococcosis have been reported, involvement of the skin usually reflects disseminated disease. Patients whose skin lesions show *C. neoformans* should undergo further evaluation for systemic disease and for cell-mediated immunodeficiencies.[27,32]

Although studies in children are limited, current treatment recommendations for cryptococcosis include a 6- to 12-week course of oral fluconazole or itraconazole for mild to moderate disease in immunocompetent individuals and intravenous amphotericin B in combination with oral fluconazole or flucytosine for more severe disease.[32,64,82,99] Immunocompromised patients continue on lifelong maintenance antifungal therapy because of the high rate of recurrence.[32,82]

FUSARIOSIS

Fusarium spp. are ubiquitous saprophytic fungi that affect primarily immunocompromised hosts, particularly neutropenic patients with hematologic malignancies. Fusariosis may produce localized infection of the nails, cornea, or skin and disseminated disease through hematogenous spread. Rarely, skin disease may result from traumatic inoculation. Lesions are seen most often on the extremities and manifest as tender, erythematous macules or plaques that develop central necrosis, form an eschar, and may become hemorrhagic.[79,87,99,107]

Because the mortality rate of disseminated *Fusarium* infection in neutropenic patients is 80 percent,[79,107] promptly establishing the diagnosis through biopsy and culture is important. Culture of tissue specimens reveals sickle-shaped macroconidia. On microscopic examination, *Fusarium* spp. appear as branched, septate hyphae with a perivascular distribution. Fungal blood cultures also may aid in establishing the diagnosis.[79,87,99,107]

Prognosis of *Fusarium* infection is poor, and treatment options are limited. Combination antifungal therapy with amphotericin B lipid complex and voriconazole and recovery of neutrophil counts have been reported to have some success in resolving the disease.[72,79,87,107,114]

HISTOPLASMOSIS

Histoplasmosis is caused by the organism *Histoplasma capsulatum*, a dimorphic fungus endemic to the Americas, particularly the Ohio, Mississippi, Missouri, and St. Lawrence River valleys. Cases also have been reported in Africa and Asia. The disease is acquired through inhalation of spores in soil contaminated by bird and bat droppings, producing a primary lung infection.[1] Most cases in immunocompetent patients are localized pulmonary infections, producing an influenza-like illness with symptoms that resolve within a few weeks. Disease also may be produced by reactivation of a latent infection.[82] Patients at extremes of age and patients who are immunocompromised, especially patients with AIDS and low CD4 lymphocyte counts, are at risk for developing progressive, disseminated disease.[1,58,60,82,122]

Involvement of the skin, causing chancriform lesions associated with regional lymphadenopathy, rarely has been described as a consequence of direct inoculation. These lesions typically resolve spontaneously in several months. Acute or chronic pulmonary histoplasmosis may produce reactive skin changes, such as erythema nodosum, erythema multiforme, and exfoliative dermatitis. Cutaneous manifestations through hematogenous spread in disseminated histoplasmosis include papules, nodules, ulcerative, or granulomatous lesions often involving oral mucosa. These lesions may progress to thick, verrucous vegetations that cause obstruction of the affected regions.[71,99]

Differential diagnosis includes tuberculosis, *Pneumocystis carinii* pneumonia, and other fungal infections caused by dimorphic fungi.[99] Biopsy specimens of skin lesions show granulomatous inflammation with macrophages, histiocytes, and giant cells and round, narrow-necked, budding yeast cells within phagocytes. Culture of involved tissue, bone marrow, blood, or sputum specimens provides the definitive diagnosis, but *H. capsulatum* may take several weeks to months for growth on culture media to occur. *Histoplasma* antigen may be detected in urine samples of individuals with disseminated histoplasmosis; however, this test is inconsistent in patients with localized infections.[1,99,104,108]

Self-limited disease in immunocompetent individuals does not require treatment. Amphotericin B remains the drug of choice for severe, disseminated histoplasmosis, whereas azole drugs can be effective for treating less advanced disease.[1,130] Itraconazole also is used for lifelong suppressive therapy in immunocompromised patients.[60,82]

MUCORMYCOSIS

Mucormycosis is an opportunistic fungal infection that affects primarily immunosuppressed hosts. Common risk factors include prematurity, diabetes mellitus, malignancies, neutropenia, burns, or malnutrition. Transmission may result from inhalation of spores or, in the case of primary skin disease, from direct inoculation through contaminated occlusive dressings or at sites of invasive procedures, trauma, or burns. Cutaneous manifestations may occur alone or in conjunction with disseminated disease. Mucormycosis has a tendency to invade vascular tissues, leading to inflammation, thrombosis, necrosis, and septic emboli. Infection may spread hematogenously to the skin, causing cellulitis with erythematous papules and pustules that progressively enlarge and develop necrosis with formation of eschar (Fig. 67–25).[89,99,101,132]

Differential diagnosis includes pyoderma gangrenosum, bacterial cellulitis, and necrotizing fasciitis. Tissue biopsy examina-

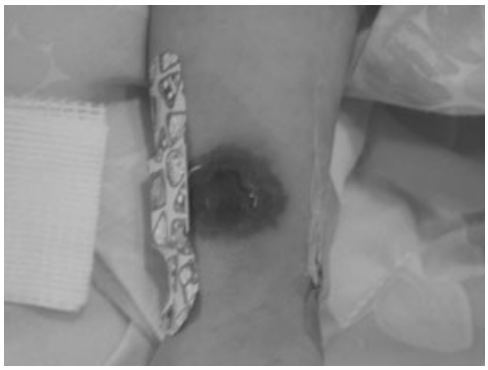

Figure 67–25 Purpura and ulceration in a patient with mucormycosis. (See companion Expert Consult web site for color version.)

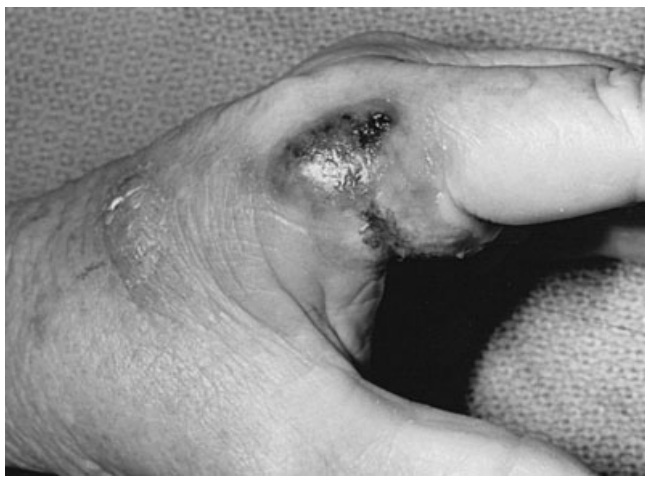

Figure 67–26 Two erythematous nodules on the dorsum of the left hand of a patient with sporotrichosis.

tion of mucormycosis lesions shows characteristic nonseptate hyphae with right angle branches, vascular invasion, necrosis, and inflammatory infiltrate. Administration of intravenous amphotericin B in addition to surgical débridement and wide excision of the skin disease is the recommended treatment.[89,99,101,132]

SPOROTRICHOSIS

Sporothrix schenckii, the organism responsible for sporotrichosis, is found in soil and plants worldwide with a predilection for tropical climates. Sporotrichosis usually is transmitted through inoculation at sites of trauma; however, pulmonary disease may be acquired through inhalation of spores. Infection with *S. schenckii* may produce four forms of disease, with lymphocutaneous being the most common and fixed cutaneous, disseminated skin, and extracutaneous disease being less common.[33,61,83,99,131] Children and individuals with prior exposure to the fungus may present more often with localized skin manifestations.[18] Characteristic lesions include erythematous papules, nodules, and scaly, verrucous plaques that progressively enlarge and ulcerate (Fig. 67–26). Regional lymphadenopathy and spread along the lymphatic drainage may soon follow. Scarring is a common occurrence. Although bone and joints are the most frequent sites of extracutaneous infection, hematogenous spread to almost every other organ system has been described. Disseminated disease should raise the suspicion of immunodeficiency in the affected individual.[33,61,83,99,131]

Definitive diagnosis is made by culture of wound material on Sabouraud agar. Itraconazole is the drug of choice for treating skin disease, whereas intravenous amphotericin B is the recommended treatment for severe, disseminated disease. Saturated solution of potassium iodide at 1 to 2 drops per the child's year of age administered three times daily also has been used as an effective treatment for uncomplicated cutaneous sporotrichosis in pediatric patients.[18,67]

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SECTION XI

Ocular Infections

CHAPTER 68

Ocular Infectious Diseases

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The eye may be affected by a wide spectrum of infections and infestations manifested as primary disease or as part of a larger systemic process. Many infections are vision-threatening, whereas others have important implications affecting the generalized disease process. Findings in the eye, for example, can aid in narrowing the differential diagnosis in some cases of systemic disease, such as congenital viral infections. A helpful approach to delineating the ophthalmic manifestations of infectious diseases is to use an anatomic scheme that considers the primary site of involvement. This chapter proceeds systematically from infections involving the eyelids and ocular surface to those involving intraocular structures and concludes with a discussion of infections involving deeper tissues in the orbit. Some overlap and duplication are unavoidable because disease involvement that truly is isolated to a single anatomic component of the eye or orbit is an unusual occurrence. Most infectious processes involving the eye can be diagnosed accurately with a careful history and detailed ophthalmic examination. Simple tools such as a penlight and a direct ophthalmoscope facilitate establishing a diagnosis in many instances. In some cases, however, special ophthalmologic testing such as slit-lamp examination, indirect ophthalmoscopy, ocular ultrasound, and even biopsy or culture (or both) is required.

INFECTIONS OF THE EYELIDS

Despite being only 2 mm thick, the anatomy of the eyelids is complex and elegant. The eyelids are composed of dense connective tissue, hair follicles, sweat and sebaceous glands, smooth and striated muscles, sensory and motor nerves, and vascular elements. The skin covering the eyelids is the thinnest of the entire body. The internal surface of the eyelid, the part adjacent to the eye, is covered by the palpebral conjunctiva. Bulbar conjunctiva covers the surface of the globe itself. Sebaceous glands, known as the glands of Zeis, are associated with hair follicles. Meibomian glands, located within the tarsal plates of the eyelids, also are sebaceous and drain at the posterior aspect of the lid margin.

Infection of the eyelids may be generalized or focal. Involvement of the skin by an infectious agent is referred to as *dermatoblepharitis*. *Staphylococcus aureus* and *Staphylococcus epidermidis* infections predominate. The exception is angular blepharitis, characterized by inflammation in the lateral canthal region, which frequently is caused by *Moraxella* spp. Impetigo or erysipelas of the eyelids may be caused by *Streptococcus pyogenes*. A variety of infestations, including *Demodex folliculorum*, *Sarcoptes scabiei* (scabies), *Pediculus capitis*, and the pubic louse *Phthirus pubis*, may involve the eyelids.

Though more commonly found in adults, infection of the lid margin does occur in children. Infections of the anterior lid margin most frequently encountered include bacterial blepharitis, molluscum contagiosum, and parasitic diseases. Infections of the posterior lid margin include those associated with chronic meibomian gland dysfunction. Herpes simplex virus (HSV) and herpes zoster virus may involve the lid and lid margin and are discussed elsewhere in this chapter.

ANTERIOR EYELID INFECTION

Staphylococcal Blepharitis

Staphylococcal eyelid infection may be an acute or chronic condition. Typically, the chronic form of the disease is manifested as crusting of the lid margins, especially on awakening. Thickening of the lid margin, seen most easily on slit-lamp examination, is a frequent manifestation. Mild, chronic conjunctival infection often occurs as a spillover phenomenon caused by local reaction to materials secreted by the infecting organism. A child with staphylococcal blepharitis may be completely asymptomatic or may complain of ocular discomfort, burning, a foreign body sensation, or any combination of these symptoms. Lid hygiene usually is all that is required to treat the condition and can be accomplished through eyelid scrubs, with attention given to the lid margin. Tap water can be used, or in refractory cases, the eyelids and lid margins can be cleansed once or twice daily with a 50:50 mixture of baby shampoo and warm water. This mixture is applied gently with a clean washcloth. Lid hygiene is effective in decreasing the concentration of local bacterial flora and reducing or eliminating symptoms from chronic staphylococcal infection. A 2- to 4-week course of erythromycin or bacitracin ophthalmic ointment applied twice daily may hasten resolution and reduce exacerbation and recurrence. Because ophthalmologic ointments can produce temporary blurred vision, they often are used only at night before sleeping.

Molluscum Contagiosum Infection

Molluscum contagiosum is caused by a member of the family *Poxviridae*. Infection of the eyelid may be unilateral or bilateral. Infection may be transmitted by skin-to-skin contact, through fomites, or by auto-inoculation. Widespread development of lesions can occur as a result of auto-inoculation. Epidemics in people who live in closed communities have been reported.[160] Eyelid manifestations typically include an isolated nodule or nodules with mild surrounding inflammation (Fig. 68–1). The nodules vary in size but generally are 1 to 3 mm in diameter. Older lesions frequently become umbilicated and develop a white or waxy-appearing core. Mild conjunctival injection frequently accompanies lesions on or near the margin of the eyelid. The associated conjunctivitis occasionally can be severe and chronic. In chronic cases, corneal epithelial disease may develop.

Molluscum infection is considered to be self-limited, with most lesions resolving spontaneously within a few months.

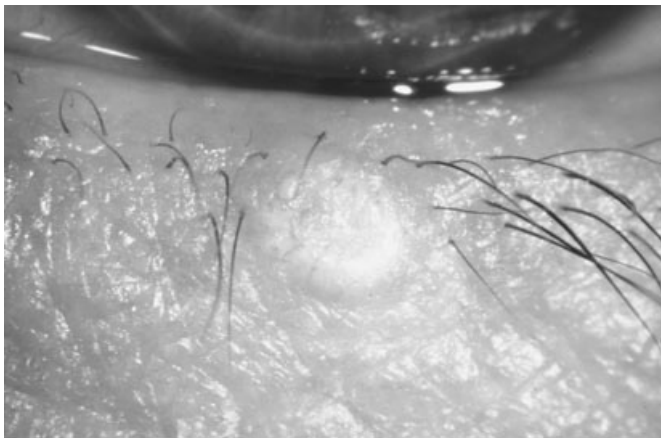

Figure 68–1 *Molluscum contagiosum* typically is manifested as an isolated nodule or nodules approximately 1 to 3 mm in diameter. Older lesions often become umbilicated and develop a white or waxy-appearing core. (See companion Expert Consult web site for color version.)

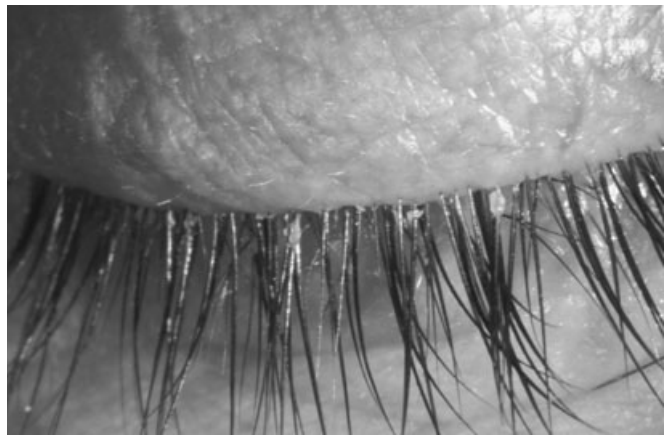

Figure 68–2 Slit-lamp photo of *Phthirus pubis* infestation demonstrating adult lice firmly adherent to the eyelashes and egg cases (i.e., nits) attached to the proximal ends of the hair shafts. (See companion Expert Consult web site for color version.)

However, the infection can be recalcitrant and problematic, especially in immunocompromised patients.[226,234] The presence of recalcitrant or atypical molluscum contagiosum lesions involving the eye have been reported in patients infected with human immunodeficiency virus (HIV).[144,188] Despite the self-limited nature of the disease in immunocompetent individuals, treatment often is advocated to prevent auto-inoculation, which may prolong the course of the disease. Treatment also may be offered to provide symptomatic relief. Mechanical treatments such as cryotherapy, expression or curettage of the central core, excision, and cautery have been effective.[46] Medical treatments that have been effective against molluscum infection remote from the eye include 1 percent imiquimod cream[202] and podophyllotoxin.[203] These agents are not recommended for the treatment of periocular lesions, however, because of the possibility of injury to the eye.

PARASITIC EYELID DISEASE

Phthirus pubis Infestation

The crab louse *Phthirus pubis* is a tiny insect well adapted to living in coarse, widely spaced hair. Infestation most commonly involves pubic, axillary, and body hair. The organism is transmitted by direct person-to-person contact and possibly by fomites.[192] Infestation of the eyelid often is a marker for sexually transmitted diseases, especially in adult patients.[192] Affected patients typically have unilateral, chronic blepharoconjunctivitis, but some patients may be asymptomatic. Although unmagnified ocular examination may reveal signs of the disease, diagnosis is facilitated by slit-lamp examination, which offers a magnified view of the eyelid margins and eyelashes (Fig. 68–2). Slit-lamp examination reveals adult lice firmly adherent to the eyelashes and egg cases (i.e., nits) attached to the proximal ends of the hair shafts. Reddish brown flecks of louse excreta frequently are found at the base of the lashes.[44]

Treatment can be facilitated by simple mechanical removal of the lice and associated nits under magnification offered by the slit lamp or other devices. Medical treatment often is preferred for young children because of their inability to tolerate mechanical removal. The organisms can be smothered by the application of a bland ointment such as petrolatum jelly applied four times each day to the eyelids. Physostigmine ointment administered twice a day frequently is used by ophthalmologists to treat eye lesions.[44] The agent inhibits nerve transmission in the insect and thus is directly toxic to the insect. It should be used with caution in infants and small children. If physostigmine comes into contact with the eye, it has several significant side effects, the most bothersome of which is stimulation of accommodation, which can produce blurred vision that may last for several hours. Medical treatment should be continued for as long as 2 weeks to ensure eradication of lice that emerge from nits during the normal life cycle of the organism.[44]

Patients also should undergo a general physical examination to assess for involvement of other body regions. Lindane shampoo scrubs of the scalp, pubic hair, and body are recommended if infestation is found in these areas. Clothing and bed linen should be laundered, and family members should be examined and treated as necessary. Follow-up at 4 to 6 weeks after treatment is recommended to detect re-infestation.

Demodex Infection

Demodex folliculorum and *Demodex brevis* are mites that frequently infest hair follicles in humans, including the hair follicles of the eyelids.[70] The organisms historically have been considered nonpathogenic parasites,[118] although they have been postulated to cause increased hordeola formation as a result of obstruction of sebaceous glands in the eyelids. *Demodex* is found commonly in patients with rosacea, although a causal relationship is difficult to establish.[172] Rosacea-like eruptions also have been attributed to *Demodex*, and one pathologic report demonstrated a granulomatous dermal inflammation associated with *Demodex* infection.[164] *Demodex* is found more commonly on eyelashes with cylindrical dandruff (Fig. 68–3).[80] Treatment with topical pilocarpine gel was shown in one study to alleviate the ocular itching associated with *Demodex* infection.[79] Because of the frequency of infestation and the paucity of definitive disease caused by the organism, treatment often is considered unnecessary.

POSTERIOR EYELID INFECTION

Hordeolum

A hordeolum (i.e., stye) is an infection of the sebaceous glands in the eyelids. When the glands of Zeis are involved, the term *external hordeolum* is used. The lesion typically points to the skin surface. When the meibomian glands are involved, the term *internal hordeolum* is used. An internal hordeolum may point toward the skin or toward the palpebral conjunctiva. *S. aureus*

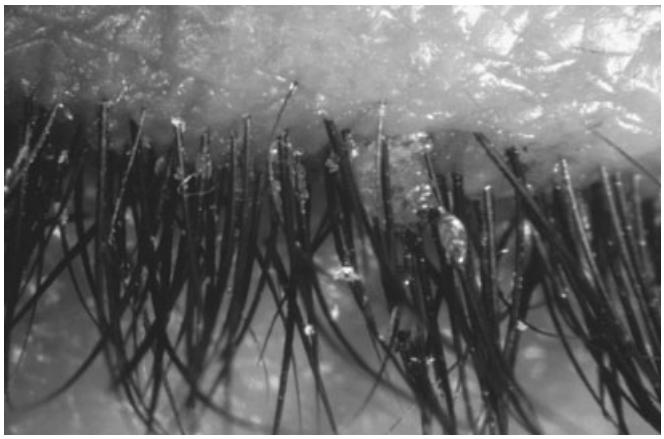

Figure 68–3 *Demodex folliculorum* and *Demodex brevis* are mites that can infect the hair follicles of the eyelids and are found more commonly on eyelashes with cylindrical dandruff. (See companion Expert Consult web site for color version.)

is the most common causal agent of internal and external hordeola.

Patients with disease of the lid margin, such as those with chronic blepharitis, seborrhea, or rosacea, are prone to recurrent hordeola, especially during the first decade of life. Hordeola are manifested as erythematous, elevated, tender nodules. Nodules typically are 5 to 10 mm in diameter and usually solitary, although they may be multiple or bilateral. Patients with a history of recurrent hordeola may have them in various stages of evolution or resolution.

The lesions usually are self-limited and typically resolve within 5 to 7 days with spontaneous drainage of the abscess. Warm compresses may hasten resolution and improve comfort. Parents should be advised to place a clean washcloth soaked in warm tap water on the involved eyelid for 10 to 15 minutes several times each day. Parents should be advised to ensure that the water is not hot enough to result in a burn. For significant coexisting blepharitis, a topical antibiotic such as erythromycin ointment may prove useful by reducing the normal bacterial skin flora of the eyelid and decreasing the risk of recurrence. Systemic antibiotics rarely are indicated for acute hordeola. Children with frequent recurrences, however, may benefit from a short course of systemic antibiotics such as erythromycin (younger than 12 years) or tetracycline (older than 12 years). These agents further decrease the bacterial flora on the eyelid and may reduce the risk of recurrence even more. Lid hygiene efforts, as described for the treatment of staphylococcal blepharitis, should be instituted and maintained for children with recurrent hordeola. Surgical drainage of a hordeolum usually is unnecessary.

Chalazion

Cytologically, a chalazion may represent either a mixed-cell granulomatous inflammation or a suppurating granuloma.[57] The lesion occurs in a meibomian gland as a result of a foreign body reaction to secretions produced by the gland that have been extruded into surrounding tissue. A chalazion may develop after resolution of an internal hordeolum, in which case it is preceded by an acute stage, or it may develop primarily, without a preceding acute inflammatory phase. A typical chalazion appears as a round, nontender nodule within the substance of the eyelid. Multiple and bilateral chalazia may occur in susceptible patients. Recurrent lesions are not uncommon findings. They are typically 2 to 10 mm in diameter.

Spontaneous resolution of smaller chalazia can be anticipated after a period of observation without treatment, sometimes as long as several months. Larger lesions, particularly those greater than 10 mm in diameter, frequently do not resolve without specific treatment. In patients with an acute or chronic inflammatory component, application of warm compresses may be beneficial. Surgical intervention may be warranted to treat medium to large chalazia that are cosmetically objectionable, produce astigmatism by pressing on the cornea, result in mechanical ptosis, or cause other symptoms. The most common surgical treatment offered is incision and curettage through an internal incision on the palpebral conjunctival surface. Surgery is highly effective. It can be performed in the office, but general anesthesia is required for most young children. Because of the need for general anesthesia, we typically recommend deferring surgical treatment until several months has elapsed without spontaneous resolution. Earlier intervention may be recommended if the chalazion is particularly large, if pigmentary changes have developed in the overlying skin, or if astigmatism or ptosis is present in a young child at risk for the development of amblyopia.

Chalazia can be treated by intralesional steroid injection, surgical incision,[58] or both.[72] Dhaliwal and Bhatia[58] reported that incision plus curettage was the procedure of choice for lesions that have been present for 8.5 months or longer and for lesions 11.4 mm or larger in size, based on the results of a prospective study. The major potential drawback of intralesional steroid injection is the risk of complications developing with an otherwise relatively benign condition. Rarely, steroid injection can result in sterile abscess formation or eyelid necrosis. Intralesional injections are done best with a chalazion clamp in place to protect the underlying globe from accidental needle trauma during injection. This device places a metal plate between the chalazion and the eye.

DACRYOADENITIS

Dacryoadenitis is an uncommon ophthalmic condition. Even in a busy ophthalmology practice, dacryoadenitis was diagnosed in approximately 1 in every 10,000 patient visits.[176] The clinical manifestation is variable. Localized tenderness and swelling of the temporal aspect of the upper eyelid usually occur and often produce an S-shaped deformity of the lid margin. Pain is frequently a predominant feature. Associated signs and symptoms include fever, follicular conjunctivitis, mucopurulent discharge, limited extraocular movement, and proptosis.[176] Keratoconjunctivitis sicca has been reported as a consequence of Epstein-Barr virus (EBV)-associated dacryoadenitis in a child.[139]

Before the era of widespread immunization, mumps was a leading cause of dacryoadenitis. Bacteria such as staphylococci, streptococci, and gonococci also have been implicated. Exceedingly rare organisms such as *Brucella* are involved occasionally in bacterial cases.[13] EBV has been implicated as the etiologic agent in a large proportion of nonsuppurative cases. Marked regional lymphadenopathy may be a distinguishing feature of EBV dacryoadenitis.[176]

Appropriate laboratory evaluation includes Gram stain and culture of mucopurulent discharge from the eye. Neuroimaging is indicated when the patient has severe inflammation, proptosis, limitation of extraocular movement, or other orbital signs to rule out a more generalized orbital process. The condition easily can be confused clinically with orbital cellulitis when signs and symptoms are severe. Biopsy of the lacrimal gland may be required to confirm the diagnosis in patients not responding to standard medical treatment or when atypical features are identified on clinical examination or radiographic studies. Blood cultures are indicated for patients with signs of systemic toxicity.

Intravenous nafcillin or vancomycin is a reasonable initial antibiotic choice for severe dacryoadenitis caused by gram-positive organisms. Oral anti-staphylococcal agents may be used

for less severe cases. For gram-negative cases, ceftazidime or other similar agents should be considered. For suppurative cases without Gram-stain guidance, intravenous nafcillin or vancomycin should be considered as initial therapy for severe cases and oral anti-staphylococcal agents for less acute cases.

Therapy in the form of warm compresses and oral analgesics is indicated for nonsuppurative dacryoadenitis. Serum testing for evidence of EBV infection should be considered in patients with regional lymphadenopathy.[176] Noninfectious causes of dacryoadenitis include sarcoidosis, Sjögren syndrome, leukemia, lymphoma, eosinophilic granuloma, and orbital pseudotumor. Noninfectious cases of dacryoadenitis sometimes can be difficult to distinguish from infectious cases. Lacrimal gland biopsy and neuroimaging usually are necessary to establish a diagnosis in noninfectious cases.[129] Intralesional steroid injection has been shown to be effective in the management of acute idiopathic dacryoadenitis; it results in marked improvement of symptoms within 7 days of treatment in most cases.[145]

DACRYOCYSTITIS

Dacryocystitis may result from congenital or acquired lacrimal outflow obstruction. Simple membranous nasolacrimal duct obstruction occurs in as many as 6 percent of the newborn population, and intermittent, mild, self-limited bacterial infection is a commonly associated feature. More severe infection of the lacrimal sac results in dacryocystitis. This condition is manifested as acute erythema, pain, and swelling in the medial canthal region. The swelling typically is located below the medial canthal tendon. Marked epiphora generally is present, and a mucopurulent discharge frequently can be expressed through the lacrimal punctum if the proximal aspect of the lacrimal drainage system is not obstructed. If the infection has resulted in or from obstruction proximal and distal to the lacrimal sac, the overlying skin may become tense as the nasolacrimal sac distends in response to the infectious process. Formation of a fistula to the overlying skin may occur as a result of dacryocystitis, and surgical excision of the fistula tract often is needed after the acute process has resolved. Dacryocystitis is a particularly common finding in neonates with a dacryocele, with dacryocystitis developing in as many as 60 percent of affected neonates.[163]

Aerobic and anaerobic bacteria and fungi may produce dacryocystitis.[29,30] In one study, *S. epidermidis* and *Pseudomonas* spp. were the aerobic organisms identified most frequently, and *Peptostreptococcus* spp. and *Propionibacterium* spp. were the anaerobes isolated most frequently. Less common agents include *Escherichia coli*, *Pseudomonas* spp., *Haemophilus influenzae*, *Pasteurella multocida*, and various anaerobes. Laboratory investigation should include aerobic and anaerobic culture of mucopurulent discharge from the lacrimal sac. Mild massage of the lacrimal sac may be used to facilitate expression of material for Gram stain and culture. A sepsis work-up should be considered for children who are acutely ill and for young infants.

Intravenous nafcillin, vancomycin, or both are good initial therapeutic choices for serious gram-positive infections. Mild cases in older children can be treated with oral antibiotics. Intravenous ceftazidime is a reasonable initial antibiotic choice for gram-negative dacryocystitis. Oral ciprofloxacin is useful for less severe cases in adult patients, although the drug is not approved for use in children. Intravenous nafcillin or vancomycin typically provides good initial empiric therapy in patients when Gram stain guidance is not available.

Ophthalmologic consultation should be requested in all cases of acute dacryocystitis. Decompression of the lacrimal sac by aspiration, incision and drainage, or probing the proximal lacrimal drainage system may be needed to hasten resolution. Probing of the distal lacrimal system often is deferred until the acute infection has subsided, although probing during the acute infection has been reported to be a safe and effective adjunct to treatment.[167] Mucopurulent material obtained during surgical decompression should be sent for appropriate culture.

PRESEPTAL (PERIORBITAL) CELLULITIS

The term *preseptal cellulitis* refers to an infectious process in the eyelids that is isolated to regions anterior to the orbital septum. The orbital septum is a thin layer of fascia that extends vertically from the periosteum of the orbital rim to the tarsal plate within the eyelids. Though penetrated by nerves and vascular structures, the septum provides a barrier that slows the spread of infectious agents into deeper orbital and retro-orbital structures.[198] Typical signs and symptoms of preseptal cellulitis include erythema and edema of the eyelids. Distinctively absent are signs of deeper orbital involvement such as restricted ocular motility, pain with eye movement, and proptosis. Preseptal cellulitis may occur after trauma or be caused by spread of infection from adjacent structures, such as skin and the upper respiratory system.[224]

Post-traumatic Preseptal Cellulitis

Post-traumatic preseptal cellulitis occurs after puncture wounds on the lids, face, or scalp. It also may occur after blunt trauma with no obvious entry wound. The etiologic agents most commonly identified are *S. aureus* and *S. pyogenes*, and polymicrobial infections can occur. Other bacterial causes include non–spore-forming anaerobes such as *Peptococcus*, *Peptostreptococcus*, and *Bacteroides*. Infection by aerobic gram-negative bacilli is an uncommon finding.[10] *P. multocida* is a common organism that produces post-traumatic preseptal cellulitis after dog and cat bites.[124,127] Cellulitis secondary to methicillin-resistant *S. aureus* (MRSA) is a common cause of community-acquired cellulitis in some locations.

Clinical signs and symptoms are determined in large part by the severity of the injury, the interval since injury, and the infecting organisms. The involved lids are edematous, erythematous, and typically quite tender. Fluctuation of subcutaneous tissue may be seen if an abscess has developed. Swelling of the uninvolved contralateral eyelids may occur as a result of lymphedema. As with any form of isolated preseptal cellulitis, vision is unaffected, and proptosis and eye movement disturbances are absent. On rare occasion, eyelid edema may be sufficiently severe to preclude adequate evaluation of the eye. Neuroimaging is required in such cases to assess the globe and rule out involvement of structures posterior to the orbital septum. Ophthalmologic consultation is critical in cases of severe post-traumatic preseptal cellulitis because of the potential for concurrent globe injury.

Laboratory analysis includes Gram stain and aerobic and anaerobic culture of mucopurulent material to aid in therapeutic decisions. Amoxicillin-clavulanate or a related agent is the drug of choice for treating post-traumatic cellulitis caused by a dog bite because of the high prevalence of *P. multocida*. Tetanus prophylaxis should be guided by standard recommendations. Surgical drainage of large abscesses may be required if a rapid response to antimicrobial therapy does not occur.

Nontraumatic Preseptal Cellulitis

The clinical features of nonsuppurative, nontraumatic preseptal cellulitis depend to a large degree on the causative agent. Erythema and swelling of the involved eyelids are typical and often are accompanied by pain. Signs of orbital infection, such as altered vision, proptosis, and eye movement abnormalities, are absent.

Before the advent of *H. influenzae* type b (Hib) vaccine, this organismly frequently was a cause of nonsuppurative preseptal cellulitis in children. Hib is seen infrequently as a cause of preseptal cellulitis today.[62] It was a particularly dangerous agent because of a high risk of spread to the central nervous system (CNS), which occurred in as many as 2 to 3 percent of patients. Though rare today, Hib infections still may be encountered. Like many infecting organisms, Hib gains access to subcutaneous tissue through infected nasal passages.

Streptococcus pneumoniae is now the most common bacterial cause of preseptal cellulitis in the pediatric age group.[62] It occurs in association with upper respiratory tract infection, although constitutional symptoms usually are less pronounced than those associated with Hib infection. A variety of other bacterial agents may cause preseptal cellulitis, but they are seen less commonly. Preseptal cellulitis occasionally has been reported to be due to a variety of other organisms, including *Trichophyton* (ringworm),[218] tuberculosis,[170] and anthrax.[35]

Adenovirus is another particularly common cause of preseptal cellulitis in children. Adenovirus is an important consideration in the differential diagnosis of childhood preseptal cellulitis because, despite being self-limited, the condition can occasionally mimic bacterial infection and prompt unnecessary treatment with antibiotics. Adenovirus can be recognized by its characteristic copious discharge, which may be serous. Swelling of the lid may be prominent, but erythema usually is minimal. Preauricular lymphadenopathy often occurs in older children, and marked conjunctival hyperemia with or without chemosis and subconjunctival hemorrhage may be present. Photophobia also may be noticed in cases of concurrent punctate keratopathy. A history of recent contact with other infected individuals frequently is noted and should be sought. Care should be taken to avoid spreading infection to family members, medical personnel, and others.

Hospital admission should be considered for children younger than 1 year of age with bacterial preseptal cellulitis. Hospitalization also is important for children with signs of systemic toxicity and those with inadequate *H. influenzae* immunization. A sepsis work-up is indicated for children with signs of systemic toxicity and for extremely young children. Ophthalmologic consultation is recommended if orbital involvement is suspected or if clinical examination is inconclusive. Computed tomography (CT) usually is unnecessary for isolated preseptal cellulitis. Cultures of blood obtained from patients with preseptal cellulitis generally are negative but are more likely to be positive in children younger than 2 years. Culture of conjunctival discharge is done often but rarely has significant diagnostic benefit.

A severity index for scoring preseptal cellulitis in children has been reported to help guide treatment decisions.[221] Antibacterial treatment should include intravenous agents for infants and those with signs of serious systemic infection. Intravenous cefuroxime or a combination of nafcillin plus cefotaxime or ceftriaxone frequently is recommended for empiric therapy. Outpatient treatment with intramuscular or oral antibiotics is reasonable for older, less acutely ill children. Intracranial infection has been associated with preseptal cellulitis in pediatric patients. Intracranial involvement should be suspected in any patient 7 years or older with preseptal or orbital cellulitis associated with an orbital subperiosteal abscess, Pott puffy tumor, concurrent sinusitis, complaints of headache, and persistent fever despite intravenous antibiotics.[175]

Systemic antibiotics should be continued for 7 to 10 days. Patients in whom intravenous antibiotics are started initially can switch to an oral antibiotic after they have been afebrile for at least 24 hours and otherwise have improved clinically, unless the possibility of development of sepsis or meningitis remains a concern.

ORBITAL CELLULITIS

Bacterial infection of orbital structures posterior to the orbital septum is the most frequent cause of acute orbital inflammation. Orbital cellulitis occurs more commonly in children and more frequently during cold weather, when sinusitis is more prevalent.

Initial signs and symptoms can vary from mild inflammation to severe and fulminant orbital disease. Cardinal signs and symptoms of infectious orbital cellulitis include proptosis, limited eye movement (including total ophthalmoplegia), pain with eye movement, and an abnormal pupillary response. Decreased vision or even blindness can occur as the most serious ophthalmic complication.[183] Death can result from intracranial extension of the infection if appropriate treatment is not initiated. Elevated intraocular pressure and chemosis of the conjunctiva are common ancillary signs. Preseptal cellulitis often coexists with orbital cellulitis but is not a prerequisite.

Most cases of orbital cellulitis are caused by spread of infection from an adjacent infected sinus.[40,151] Ethmoid sinusitis is the most common predisposing factor. Rare cases are due to penetrating orbital trauma or skin infection involving the face, with spread of organisms into the orbit. Orbital cellulitis may occur infrequently after orbital, ocular, or periocular surgery.[161,230] Orbital cellulitis and cavernous sinus thrombosis have been reported to occur after dental infections and dental surgery.[32]

Comprehensive evaluation of a patient with confirmed or suspected orbital cellulitis includes ophthalmologic and systemic examination. Assessment of visual acuity in both eyes is important for excluding vision loss and establishing a baseline to aid in monitoring progression of the disease or the effects of therapy. Evaluation of optic nerve dysfunction by examining the pupils for an afferent pupillary defect (APD) is important before pupillary dilation is performed to assess the retina. Careful evaluation of extraocular movement should be performed in all extreme positions of gaze to identify restriction of ocular duction and elicit pain on eye movement. The presence or absence of proptosis can be assessed clinically by viewing the eyes from above (bird's-eye view) or below (worm's-eye view) and by comparing their relative positions within the orbits. In severe cases of orbital cellulitis, funduscopic examination may reveal dilation of the retinal venules and signs of compressive optic neuropathy such as optic disk edema. Central retinal artery occlusion has been reported occasionally.[112] Systemic evaluation includes determination of temperature, which usually is in the range of 39° C to 40° C (102° F to 104° F). Sinus examination, a screening neurologic examination, and evaluation for signs and symptoms of sepsis and meningitis should be performed.

In any case of clinically definite or suspected orbital cellulitis, CT of the orbit and brain is indicated. CT can establish or confirm the diagnosis of orbital cellulitis and provides critical information needed to manage the patient. Imaging of the brain is important because orbital cellulitis can evolve into a brain abscess, meningitis, or cavernous sinus thrombosis. However, when a clinical response to treatment is noted, improvement in CT findings frequently is delayed. Therefore, recurrent CT scanning of a child with clinically improving findings is not indicated absolutely unless new signs or symptoms of concern develop.

Microbiologic studies often are acutely unhelpful for the routine patient with orbital cellulitis. Nonetheless, baseline studies remain important because critical information sometimes is acquired. Blood cultures should be obtained at a minimum, and a lumbar puncture with culture of cerebrospinal fluid (CSF) should be considered in infants and those with signs of CNS infection. Culture of the ocular, nasal, and nasopharyngeal mucous membranes is of limited value and can be omitted. However, if surgical drainage is performed, cultures of any material removed should be obtained.

Optimal management of a child with orbital cellulitis requires a multidisciplinary approach. In addition to evaluation and management by an experienced pediatrician, ophthalmologic and otolaryngologic consultation should be obtained. Neurosurgical consultation is required when involvement of the CNS is diagnosed or suspected. In atypical cases and those not responding to treatment, consultation with an infectious disease specialist is warranted.

The most common offending etiologic bacteria are *S. aureus*, *Streptococcus* spp., and *Haemophilus* spp. (other than *H. influenzae*). *S. pneumoniae* also is implicated frequently, and a variety of less common organisms have been reported. *H. influenzae* orbital cellulitis rarely has occurred since widespread use of the Hib vaccine was implemented.[3,62] Fungal infection of the orbit occasionally is encountered, typically in immunocompromised individuals.[137]

The differential diagnosis of orbital inflammation in children includes a broad range of noninfectious conditions, including cavernous sinus thrombosis, idiopathic inflammatory orbital pseudotumor, Wegener granulomatosis, sarcoidosis, leukemic infiltration, lymphoma, rhabdomyosarcoma, necrotic retinoblastoma, metastatic carcinoma, and histiocytosis X. Thyroid ophthalmopathy also can be manifested as an acute orbital inflammatory process, although usually its onset is slow and insidious.

Treatment of all patients with orbital cellulitis requires hospitalization and initiation of intravenous antibiotics as soon as possible after material has been obtained for culture. Infection with penicillin-resistant organisms is an increasingly common occurrence and must be considered during treatment.[37,181] Appropriate initial antibiotic therapy may include nafcillin, metronidazole, and cefotaxime as combination therapy. Given the increasing incidence of MRSA, initial treatment with vancomycin or clindamycin should be considered.[148,181] Other antimicrobial agents may be useful, and local susceptibility patterns should guide the choice of initial antimicrobial therapy. Antibiotic coverage should be modified according to the clinical course and culture results. If the patient fails to respond to antibiotic treatment within 24 to 48 hours, consultation with an infectious disease specialist and a repeat CT scan to look for the development of an orbital abscess should be considered (Fig. 68–4). The mean hospital stay for uncomplicated cases of orbital cellulitis is 10 to 14 days, and oral antibiotics should be prescribed for 7 to 10 days after discharge. A nasal decongestant commonly is prescribed at the time that the diagnosis is established to aid in opening the sinus ostia, promoting drainage of the infected sinus, and speeding resolution. Nasal decongestants should be continued for 7 to 10 days after initiation.

Although treatment of orbital cellulitis with steroids remains controversial, their use in the treatment of acute and chronic sinusitis has become increasingly common. Studies have shown that steroids can reduce levels of inflammatory cytokines in the sinonasal mucosa of individuals with sinusitis.[34,223] In a recent study on the use of steroids in children with orbital cellulitis, Yen and Yen[232] reported no adverse effects of this adjunct treatment with systemic antibiotics. Prospective studies on the use of steroids in orbital cellulitis have been proposed to determine whether they have any clinical benefit.

Children with orbital cellulitis require diligent follow-up while in the hospital. Vision should be assessed at the bedside daily; results may be more accurate if a single examiner routinely assesses vision for a given patient. Pupillary examination for an APD should be performed at each examination. Development of an APD indicates compromise of the optic nerve, warrants escalation of treatment, and usually requires urgent surgical intervention. Reduction of vision, development of an APD, onset of CNS signs, or worsening of systemic status should prompt emergency repeat neuroimaging of the brain and orbit, with further intervention as dictated by results of the scan. The close anatomic relationship of the orbit to the brain, with the orbital venous system freely anastomosing with the facial venous plexus and cranial venous sinus system through a series of valveless veins, seriously increases the potential for spread of infection to contiguous structures, including the brain.

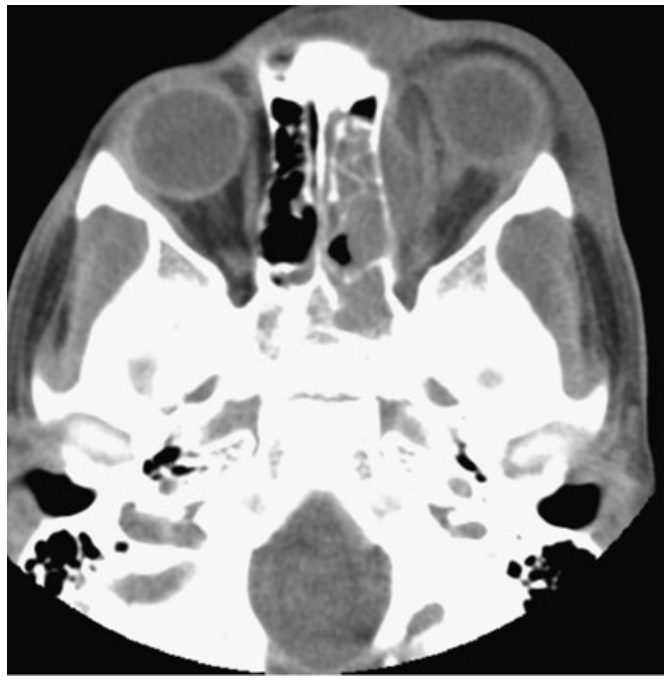

Figure 68–4 Failure of response of orbital cellulitis to antibiotic treatment may be a sign of development of a subperiosteal orbital abscess (seen here in the right medial orbit).

Acute surgical intervention to decompress the orbit or drain an orbital or subperiosteal abscess is indicated if vision loss or an APD is identified at any point during treatment. Immediate neurosurgical consideration is indicated if CNS involvement is documented. Most patients without evidence of vision loss, optic nerve dysfunction, or CNS involvement can be managed successfully medically. The surgical adage that all abscesses should be subjected to incision and drainage does not apply to abscesses associated with orbital cellulitis. Most subperiosteal abscesses located in the medial orbit can be managed successfully with medical therapy alone.[89,180] Formation of an abscess within the substance of the orbit (as opposed to a subperiosteal abscess) seldom occurs. Guidelines for surgical drainage are similar to those for subperiosteal abscess,[180] although such abscesses are more likely to require surgical intervention because of treatment failure or spread of infection to contiguous structures.[89] Provided that clinical improvement continues, no worrisome signs or symptoms of CNS involvement develop, and the patient's overall clinical status does not worsen, repeat neuroimaging of patients with an orbital abscess is unnecessary. Short-term resolution of an orbital abscess often is not obvious on CT. The radiographic appearance of the abscess typically remains unchanged on early follow-up scans, although it may no longer contain viable organisms.

Sinus drainage by an otolaryngologist frequently is required to hasten resolution. Formation of an abscess is not a prerequisite for surgical intervention, and orbital cellulitis without abscess formation may progress to the point of requiring surgery. Despite prompt and appropriate treatment, serious complications such as permanent vision loss and brain abscess can occur.[97,175,183] Most

patients, however, can be treated effectively with no permanent sequelae.

Orbital cellulitis caused by fungal infection has a course and prognosis markedly different from those of bacterial orbital cellulitis, particularly cellulitis caused by mucormycosis and aspergillosis. Fungal orbital cellulitis typically occurs in patients who are immunocompromised or patients with metabolic acidosis, such as those with poorly controlled diabetes. Orbital cellulitis may be manifested as a subacute or chronic process. Orbital apex syndrome is considered to be the most severe form, with loss of function of all cranial nerves traversing the orbital apex into the orbit (i.e., cranial nerves II, III, IV, V, and VI). A black eschar-like lesion may form in the oropharynx or nasopharynx.

Aspergillus orbital infections (most commonly caused by *A. flavus*, *A. fumigatus*, or *A. oryzae*) are rare findings in children and may take a slow, chronic course over a period of months or years. No clear predisposing factors exist, but it has a predilection for humid climates and most cases occur in otherwise healthy individuals. Signs and symptoms of orbital infection caused by *Aspergillus* spp. include loss of vision, constant dull pain, decreased or absent ocular motility, and proptosis with firm resistance to retropulsion. Palate and nasopharyngeal lesions occur but are rare. Biopsy is required for establishing the diagnosis.[88]

Effective treatment of orbital fungal infection requires correction of systemic and metabolic disturbances and administration of intravenous antifungal agents such as amphotericin B. Though still considered experimental, posaconazole has been shown to be effective as an alternative treatment in those who fail to respond to or cannot tolerate treatment with amphotericin B.[182] Surgical débridement of the orbit or adjacent infected sinuses frequently is required. Treatment often is unsuccessful, and fatalities are not uncommon, particularly with mucormycosis.

The larva of *Echinococcus granulosus* can produce a hydatid cyst in the orbit. The dog is the definitive host animal, although the organism also may live in the intestines of sheep, goats, cattle, pigs, and other animals. The disease is endemic in the Middle East, Africa, and Asia. Humans become infected by eating contaminated food, typically meat, and infection may occur in any age group. Affected patients have noninflammatory proptosis, decreased ocular motility, and dull orbital pain. Surgical excision is required for treatment. The cyst may be injected with hypertonic saline to kill the parasite, followed by excision.[86]

CONJUNCTIVAL INFECTIONS

The conjunctiva is the mucous membrane covering the inner surfaces of the eyelids and the anterior surface of the sclera. The mucous membrane lining the inner surface of the eyelids is called the *palpebral conjunctiva*, and that covering the globe is called the *bulbar conjunctiva*. The conjunctiva contains numerous small glands that produce most of the aqueous and mucous components of tears that are responsible for producing a smooth, uniform tear film over the cornea. The conjunctiva may be infected by a wide variety of bacterial and viral agents, as well as by noninfectious allergic and toxic agents.

Patients with conjunctivitis may complain of burning, itching, or a foreign body sensation. Significant itching usually signifies an allergic or viral cause. The conjunctival injection (erythema) in eyes with infectious conjunctivitis is located away from the cornea. Conjunctival injection concentrated adjacent to the cornea (i.e., limbal or ciliary flush) suggests keratitis (i.e., inflammation of the cornea), iritis (inflammation of the iris), or iridocyclitis (i.e., inflammation of the iris and ciliary body). When the eye is severely infected, however, such a differentiating pattern may not be discernible. Conjunctivitis usually is accompanied by a discharge that has some important diagnostic properties. Purulence suggests a bacterial cause, a mucoid discharge is seen most often with viral infections, and a serous discharge usually is seen with viral or allergic causes. Patients with isolated conjunctivitis do not have any significant alteration in vision. Conjunctivitis with poor vision warrants search for another diagnosis.[83,135]

BACTERIAL CONJUNCTIVITIS

Bacterial conjunctivitis is a common form of infectious conjunctivitis in children. It is characterized by a purulent discharge and can be unilateral or bilateral. It is useful clinically to divide bacterial conjunctivitis into mild and severe forms because treatment approaches are different.

Mild Bacterial Conjunctivitis

The agents most commonly causing mild bacterial conjunctivitis in children aged 5 years or older are *S. pneumoniae* and *Moraxella* spp.[83] *H. influenzae* was a prominent causative agent before the availability of Hib vaccine. *S. aureus* conjunctivitis is seen most frequently after trauma or surgical manipulation. Conjunctival stains and cultures usually are not necessary because the disease is self-limited or responds rapidly to topical antibiotics.

Many appropriate topical antimicrobial agents for treatment of bacterial conjunctivitis are readily available and include topical moxifloxacin, gatifloxacin, ciprofloxacin, ofloxacin, norfloxacin, tobramycin, gentamicin, erythromycin, sulfacetamide, and combination antibiotics. Aminoglycoside-containing compounds such as neomycin occasionally can cause a dramatic allergic blepharoconjunctivitis that can be worse than the original problem. The choice of using either drops or ointment is best left to the person who will be instilling the medication because neither has any proven therapeutic advantage. With the emergence of multidrug-resistant organisms in many parts of the country, the choice of antibiotic should be modified accordingly. Newer agents, such as the fourth-generation fluoroquinolones (moxifloxacin and gatifloxacin), have many advantages over older drugs, including broad-spectrum coverage, ease of dosing, and pH neutrality. A typical regimen for treatment of mild conjunctivitis is 1 drop or a 0.25-inch bead of ointment placed into the inferior conjunctival fornix three to six times daily for 5 to 7 days, depending on the actual medication. Persistent infection should prompt a return to the physician for reconsideration of the diagnosis. Mild bacterial conjunctivitis generally resolves spontaneously in 7 to 14 days without treatment.[82,83]

Severe Bacterial Conjunctivitis

Severe bacterial conjunctivitis, characterized by pronounced conjunctival infection and copious purulent discharge, usually is caused by *Neisseria gonorrhoeae*, *Neisseria meningitidis*, *S. aureus*, *S. pneumoniae*, and, in children younger than 5 years, *H. influenzae*. A hyperpurulent state in which the copious purulent discharge reaccumulates in a matter of minutes is characteristic of infection with *N. gonorrhoeae*.[96,194]

Severe conjunctivitis demands a comprehensive microbiologic evaluation consisting of stains and cultures. Samples from both eyes should be cultured separately, even if only one eye is involved, to allow the uninvolved eye to serve as a useful control.[205] Gram stains of conjunctival scrapings should be done at the time of culture. A useful culture technique includes the use of a cotton or calcium alginate swab (Calgiswab, Spectrum, Houston) gently rubbed against the palpebral conjunctiva of the lower lid and inoculated onto blood and chocolate agar. If *N. gonorrhoeae* is suspected, culture for chlamydia also is indicated because concurrent infection with both organisms is a common occurrence.

Treatment of severe bacterial conjunctivitis is based initially on results of the stains and later modified according to culture

and sensitivities. If *Neisseria* is strongly suspected, the patient should be treated as though the disease is present, even if the laboratory results are not confirmatory. Ocular *N. gonorrhoeae* infection is a vision- and life-threatening disease. This organism can penetrate the intact cornea and cause microbial keratitis, corneal perforation, and endophthalmitis, in addition to conjunctivitis. *N. gonorrhoeae* conjunctivitis should be considered in three patient groups: neonates after passage through an infected birth canal, sexually active individuals, and victims of suspected sexual abuse. Pediatric infection with *N. gonorrhoeae* requires hospital admission and administration of a systemic antibiotic. Because of the prevalence of penicillin-resistant strains, a broad-spectrum (third-generation) cephalosporin, such as ceftriaxone, is the most appropriate choice of antibiotic.[92] Adjunctive treatment of *N. gonorrhoeae* conjunctivitis with topical moxifloxacin and simple saline irrigation for 5 days can be helpful, but topical agents should never be used as an isolated treatment.[96] Moxifloxacin is a particularly good antibiotic choice because it is effective against chlamydia as well.

Conjunctivitis caused by gram-positive cocci can be treated with topical moxifloxacin, gatifloxacin, ciprofloxacin, or erythromycin. Systemic nafcillin, a second- or third-generation cephalosporin, or both can be added as needed for more extensive infections. Gram-negative bacterial conjunctivitis can be treated with topical erythromycin ointment or an aminoglycoside drip such as gentamicin or tobramycin. Systemic antibiotics can be added if needed.[205]

VIRAL CONJUNCTIVITIS

Adenoviral Conjunctivitis

Viruses are another common cause of infectious conjunctivitis in children. Many pediatric cases of viral conjunctivitis are caused by adenovirus. Serotypes 1, 2, 3, 4, 7, and 10 produce an acute form of conjunctivitis with prominent conjunctival follicles. Serotypes 3 and 7 also may cause pharyngoconjunctival fever. This entity is characterized by conjunctivitis, fever, and pharyngitis.[21] Serotypes 8, 19, and 37 primarily cause epidemic keratoconjunctivitis.[205] The keratitis is characterized by a combination of a punctate epithelial keratitis and an immune response that results in subepithelial infiltrates. Photophobia is a prominent feature, and vision may be decreased markedly with this entity. Epidemic keratoconjunctivitis commonly is associated with pharyngitis and rhinitis, which may precede or coexist with the conjunctivitis.

Adenoviral conjunctivitis is highly contagious. It is transmitted easily from an infected individual to others at home or school. The incubation period is 5 to 10 days but may be as long as 21 days. The virus is shed from the infected conjunctiva for 7 to 12 days after the onset of infection. Frequently, a prodromal upper respiratory infection consisting of fever, pharyngitis, or otitis media occurs.[205] Ocular signs and symptoms include photophobia (with corneal involvement), foreign body sensation, epiphora, bulbar and palpebral conjunctival injection and chemosis (edema), and subconjunctival hemorrhage.[100] Formation of grayish pink friable membranes on the palpebral conjunctiva is a hallmark of the disease and may result in bleeding when removed. Preauricular lymphadenopathy is a common feature in older children and adults. Keratitis may be prolonged, with mild to moderate reduction of vision lasting weeks to months.[100,136]

The diagnosis of adenoviral conjunctivitis usually is clinical. Rarely is laboratory confirmation necessary. Viral cultures are possible when indicated, and a rapid enzyme immunoassay test is available.[228] Treatment of adenoviral conjunctivitis is supportive, aimed at decreasing symptoms. Cool compresses and acetaminophen are helpful, and removal of conjunctival membranes with a cotton swab may relieve the foreign-body sensation. A small amount of bleeding often occurs after removal of these membranes, and the membranes can recur after several days. If corneal subepithelial infiltrates cause significant discomfort or an unacceptable decrease in vision, treatment with a short course of topical steroids may provide symptomatic relief and speed resolution. The use of steroid preparations requires careful monitoring,[135,205] and they should be prescribed only by an ophthalmologist.

The clinician should wear gloves when examining patients with suspected adenovirus infection. Careful handwashing is essential after direct contact. Instruments or equipment used in examining an infected patient should be cleaned with 10 percent sodium hypochlorite solution or other solutions known to eradicate adenovirus. Mini-epidemics have originated in physicians' offices.[113] At home, families should exercise caution and separate the towels and bed clothes of the patient from those of others in the household. Children of school age should be kept home for approximately 7 days or longer to reduce the risk of transmitting the infection to classmates.[205]

Herpes Simplex Virus Conjunctivitis and Complex Forms

HSV conjunctivitis may occur as a primary or as a secondary infection. Ocular infection usually is caused by HSV-1, except in newborns, in whom HSV-2 predominates.[42] Typical initial signs of HSV conjunctivitis include a serous discharge, scant conjunctival follicle formation on the inferior palpebral conjunctiva, and preauricular lymphadenopathy. Eighty percent of cases are unilateral. Eyelid vesicles often occur in primary infections. Bulbar conjunctival ulceration is an unusual occurrence, but when present, it is virtually pathognomonic of primary HSV-1 infection. Keratitis occurs in as many as 50 percent of primary cases and is characterized by mild epithelial irregularities. Dendrites can occur in primary infections. Reactivated HSV keratitis has the characteristic dendritic pattern of epithelial disease. Lid vesicles usually do not develop in reactivated HSV infection.[117]

Primary HSV dermatoblepharitis is seen most commonly in children younger than 6 years old, but it may occur at any age. The initial episode may be associated with an upper respiratory infection, and recurrences are common. Clinical signs include an eyelid vesicular reaction, mild follicular conjunctivitis, preauricular lymphadenopathy, and, occasionally, atypical epithelial keratitis. Secondary bacterial infection can occur. Systemic acyclovir is a safe and effective treatment, although the disease itself typically is self-limited and does not require treatment.[117]

The diagnosis of HSV keratoconjunctivitis usually is made on the basis of the clinical appearance alone. Antigen detection tests or viral cultures can be used when the diagnosis is in question.[113] Viral cultures, when necessary, require special handling.[205] In primary HSV infection, after the skin surface has been swabbed with an alcohol sponge, a tuberculin syringe with a 30-gauge needle is used to aspirate fluid from an intact vesicle. If no vesicles are present, a Dacron swab is wiped on the palpebral conjunctiva of the lower lid. In either case, the viral transport medium is inoculated and taken to the laboratory for special handling.

Treatment of HSV conjunctivitis alone (in the absence of corneal epithelial disease) is somewhat controversial. Oral acyclovir may be used for severe cases of primary HSV infection.[189,201] Three topical antiviral agents are available: iododeoxyuridine, vidarabine, and trifluridine.

When epithelial HSV keratitis is reactivated and the typical dendritic (branched) epithelial lesions are seen, the cornea usually is hypoesthetic. Iritis commonly is associated with HSV keratitis and is characterized by miosis, photophobia, ocular pain, and a foreign-body sensation. Vision may be decreased if the epithelial disease involves the visual axis.[205] Epithelial HSV may be atypical,

more severe, or more complicated in patients receiving topical steroids and in immunocompromised patients.

Treatment with topical trifluridine or vidarabine is indicated in all cases of HSV keratitis. Topical steroid is effective in decreasing corneal scarring caused by HSV stromal keratitis. When using a topical steroid, coverage with a topical antiviral agent such as vidarabine always should be included to reduce the risk for recurrent active viral proliferation during the course of steroid treatment. Oral acyclovir is not effective for treating active HSV keratitis, but it may be useful in treating HSV uveitis.[201] Ancillary treatments include cycloplegic eyedrops to dilate the pupil and pain medications.

Though not helpful in treating acute epithelial disease, chronic suppression with oral acyclovir is effective in reducing the number of recurrences of HSV epithelial and stromal keratitis, especially in patients with stromal keratitis.[201,205] Interferon also significantly adds to the efficacy of topical antiviral therapy, but the adverse side effects of interferon must be weighed against its potential benefits.[201]

External Ocular Infections with Varicella-Zoster Virus

Childhood varicella infection (chickenpox) commonly is accompanied by conjunctivitis. Vesicles, ulcers, or both occur occasionally on the bulbar or palpebral conjunctiva. Conjunctivitis associated with varicella infection does not result in permanent visual sequelae. Corneal involvement rarely occurs and typically heals without sequelae. Occasionally, it may result in stromal scarring and produce irregular astigmatism and reduced vision.

After primary varicella infection occurs, the virus may persist in latent form in the trigeminal nerve ganglia. Herpes zoster ophthalmicus occurs when the ophthalmic division of the trigeminal nerve is affected by reactivation of the virus. The condition occurs more commonly in immunocompromised patients, and recurrences occur infrequently except in these patients.

The diagnosis of herpes zoster ophthalmicus almost always is made on the basis of the characteristic clinical feature of a painful, tender vesicular eruption in the V1 dermatome. Ocular involvement may include keratitis and uveitis. Corneal epithelial lesions may appear dendritiform, with or without subepithelial infiltrates. Uveitis may occur with or without associated keratitis. Usually it is mild, but occasionally it may be severe, with the formation of a hypopyon or hyphema. Immunofluorescence testing of vesicular base scrapings or viral cultures may be helpful if the manifestation is atypical or the diagnosis is in doubt.[125]

Treatment of herpes zoster ophthalmicus involves the administration of oral or intravenous acyclovir. Treatment is most effective when initiated within 72 hours of the appearance of vesicles. Keratitis and uveitis do not respond to topical antiviral therapy.[201] Sometimes topical steroids can be helpful in treating varicella keratitis and uveitis, but such treatment should be administered under the direction of an ophthalmologist.[135]

Chlamydial Conjunctivitis and Trachoma

Chlamydia spp. can cause conjunctivitis and trachoma. *Chlamydia psittaci* rarely causes ocular disease in humans. *Chlamydia trachomatis*, however, has numerous serotypes that affect the eye. Serotypes A, B, Ba, and C cause trachoma, and serotypes B, C, D, Da, D–, E, F, G, H, I, Ia, J, and K cause inclusion conjunctivitis, including neonatal inclusion conjunctivitis. Neonatal chlamydial conjunctivitis is discussed in the section "Neonatal Conjunctivitis."

Inclusion conjunctivitis in older children and adults is manifested as subacute or chronic inflammation of the conjunctiva. The condition may be unilateral or bilateral. A mucopurulent discharge typically occurs, as do follicles on the bulbar and perilimbal conjunctiva. Preauricular lymphadenopathy is a common finding. Punctate epithelial keratitis with subepithelial infiltrates and a superior micropannus (i.e., vascular growth on the cornea) may develop.[52]

The differential diagnosis of inclusion conjunctivitis is sizable and includes viral and bacterial conjunctivitis, molluscum contagiosum, and toxic keratoconjunctivitis from chronic administration of topical agents. Laboratory testing may be necessary to confirm the diagnosis. Giemsa staining of conjunctival scrapings may demonstrate the classic intracytoplasmic inclusions. A fluorescent antibody detection test or enzyme immunoassay is used to make a rapid diagnosis.[205] Rarely are chlamydial cultures needed except when the diagnosis remains in doubt.

Treatment consists of oral erythromycin or doxycycline if systemic infection is suspected. Topical erythromycin or tetracycline alone four times each day for 7 days is effective treatment of infection limited to the conjunctiva.[52,205] Moxifloxacin recently has been shown to be effective against chlamydia as well but is not yet the recommended treatment of choice.

Trachoma remains a leading cause of blindness worldwide, ranking second or third, depending on the region studied. It is a disease of impoverished populations that is exacerbated by inadequate supplies of water and by poor hygiene. Trachoma seldom is seen in developed countries. In the United States, it is encountered on Native American reservations in the Southwest and in individuals arriving in endemic areas of the United States. Eye-seeking flies play an important role in transmission of *Chlamydia* from one person to another.[205]

Trachoma causes blindness by producing chronic inflammation of the palpebral conjunctiva of the upper eyelid with secondary scar formation. Contracture of the palpebral conjunctiva scar causes entropion of the eyelid and trichiasis. Eventually, this situation leads to corneal opacification and vision loss.[204,205] Less commonly, the cornea may be infected directly. The complete course of the disease from initial infection to blindness generally takes decades.

The diagnosis of trachoma usually is made on the basis of clinical findings alone. The disease passes through characteristic stages, from simple inflammation to scarring, entropion, and trichiasis. Multiple stages may coexist in various parts of the eyelids. The World Health Organization classifies the stages of trachoma as follows: TF, trachomatous follicular response; TI, diffuse trachomatous conjunctival inflammation; TS, trachomatous scarring of the palpebral conjunctiva; TT, trachomatous trichiasis; and TO, trachomatous corneal opacification. In the pediatric population, physicians usually encounter only stages TI and TF.[214] The scars on the palpebral conjunctiva, referred to as *Arlt lines*, are linear and multidirectional. Slit-lamp examination may reveal a superior limbal micropannus and Herbert pits (i.e., hollowed-out areas in the superior limbus), which represent sites of resolved follicles.

Treatment of trachoma consists of topical tetracycline or erythromycin ointment instilled twice daily for 2 months and, more importantly, improved sanitation and hygiene to prevent recurrence.[205] Trachoma can be self-limited if hygiene alone is improved.[204] In patients who do not respond promptly to topical treatment or in those with severe disease, systemic antimicrobial agents should be instituted. For those older than 8 years, doxycycline should be administered orally daily for 40 days. Erythromycin can be substituted in younger children or in patients intolerant of doxycycline.

NEONATAL CONJUNCTIVITIS

Neonatal conjunctivitis, or ophthalmia neonatorum, is a common vision-threatening disorder throughout the world, although it has been relegated to a position of secondary importance in the industrialized world, where screening and prophylactic measures

are widespread. It remains a significant cause of childhood ocular morbidity in developing countries.

Dilute topical silver nitrate solution instilled just after birth for prophylaxis against ophthalmia neonatorum was the first treatment used. It was responsible for reducing the prevalence of gonococcal ophthalmia neonatorum from 10 to 0.17 percent of live births in Europe.[205] A mild, self-limited chemical conjunctivitis is associated with the use of silver nitrate,[154] but it lasts 2 to 4 days and resolves with no treatment. Topical erythromycin largely has replaced silver nitrate in developed countries because it is effective without producing a chemical conjunctivitis.[94] In the developing world, regional conflicts, political instability, and burgeoning refugee populations often result in underuse of these simple prophylactic measures.[78,94]

Causes of neonatal conjunctivitis are highly variable among populations. In areas that use silver nitrate drops for prophylaxis, chemical conjunctivitis is the most common cause of neonatal conjunctivitis.[154] Infectious conjunctivitis occurs in 0.5 to 6.0 percent of live births in the United States. The leading infection is *C. trachomatis*.[15,99,185,205] Neonatal infection is caused by ocular exposure to contaminated maternal discharge during normal birth, although it may occur in infants born by cesarean section, especially if the membranes rupture prematurely. Other important infectious agents that may cause ophthalmia neonatorum are *N. gonorrhoeae* and *S. aureus*.[77,194] Bacteria that normally reside in the vaginal or gastrointestinal tract occasionally are implicated in ophthalmia neonatorum. Such bacteria include *Streptococcus* spp., *Haemophilus* spp., *Pseudomonas aeruginosa*, *Moraxella* spp., *Moraxella catarrhalis*, *N. meningitidis*, *E. coli*, and *Enterobacter cloacae*.[185,200]

Viruses occasionally produce neonatal conjunctivitis. Rarely, HSV may cause keratoconjunctivitis.[78] It is associated with distinctive vesicular skin changes and systemic signs, and the diagnosis seldom is in doubt.[9] Life-threatening HSV meningoencephalitis is a serious potential complication of HSV ophthalmia neonatorum.[152] Other viruses occasionally implicated include adenovirus, coxsackievirus A9, cytomegalovirus (CMV), and echovirus.[199]

Certain clinical features may help establish the specific etiologic diagnosis in cases of ophthalmia neonatorum (Table 68–1), but considerable overlap in findings exists, and physicians cannot rely on the history and physical examination alone to make a definitive diagnosis. Even when all appropriate investigative modalities are used, the cause may remain unknown in some cases.

Variations in the time of onset after birth, severity of inflammation, and character of the ocular discharge are common, and none is considered pathognomonic of a specific infectious process, but general considerations are worth reviewing.[185] Silver nitrate conjunctivitis typically begins during the first 48 hours of life and produces a watery discharge with mild inflammation. It is self-limited and resolves within 48 to 72 hours of its appearance.[154] *C. trachomatis* conjunctivitis generally begins 1 to 21 days after birth and usually is apparent by day 7. A moderately copious mucopurulent discharge may be present but can be variable.[99,149] *S. aureus* conjunctivitis also begins during the first 3 weeks of life and can produce a moderately profuse mucopurulent discharge. Many other bacterial agents produce an overlapping clinical picture. Conjunctivitis caused by *N. gonorrhoeae* ordinarily begins somewhat earlier, usually by the third day of life, but it may appear as late as 3 weeks after birth. The hallmark is a copious, hyperpurulent discharge that can reaccumulate in a matter of minutes after it is removed. *N. gonorrhoeae* can penetrate an initially intact cornea and result in perforation of the eye and endophthalmitis.[96,187,199] Conjunctivitis caused by gastrointestinal flora often begins within a few days of birth and can produce a copious purulent discharge.

TABLE 68–1 Clinical Characteristics of Neonatal Conjunctivitis Caused by Various Agents

Agent	Day of Life at Onset	Discharge
Silver nitrate	1 (0-2)	Serous
Chlamydia trachomatis	7 (1-21)	Mucopurulent
Staphylococcus aureus	5 (1-21)	Mucopurulent
Neisseria gonorrhoeae	3 (0-21)	Purulent
Other bacteria	7 (1-21)	Mucopurulent
Herpes simplex virus	5 (0-21)	Serosanguineous
Other viruses	Not established	Probably serous

Viral neonatal conjunctivitis is a rare occurrence. HSV infection may begin during the first 3 weeks of life and usually produces a serous or serosanguineous discharge.[78,152] Other viruses have highly variable characteristics, but a serous discharge is a common finding.

The differential diagnosis of neonatal conjunctivitis includes congenital glaucoma, dacryostenosis with or without dacryocystitis, keratitis, and uveitis. Dacryostenosis is manifested as epiphora and a watery or purulent discharge. Occasionally, conjunctivitis may develop. Because epiphora caused by congenital dacryostenosis ordinarily is not seen until the second or third week of life, it rarely is a serious consideration. Newborns with congenital glaucoma often are photophobic and irritable.[222] They may exhibit increased tearing, conjunctival injection, and corneal edema. Both keratitis and uveitis most often are accompanied by photophobia and pain. The discharge with keratitis can vary but with bacterial causes is purulent. The discharge with uveitis, if present, is watery.

Laboratory testing is important in establishing a specific diagnosis and should include Gram and Giemsa stains, a chlamydial immunoassay, and cultures for aerobic and anaerobic bacteria. Cotton-tipped swabs should be used to obtain material for culture from the conjunctival fornices. Viral and chlamydial cultures and immunoassays can be considered but usually are unnecessary.[14,207] Gonococcal immunoassay and HSV immunochemical tests are available but not in widespread use.

The initial treatment of neonatal conjunctivitis depends on the suspected infectious agent. Broad-spectrum treatment should be considered if the diagnostic possibilities cannot be narrowed. Silver nitrate–induced conjunctivitis is self-limited and does not require treatment. *C. trachomatis* conjunctivitis is treated with tetracycline (1%) or erythromycin (0.5%) ointment four times daily for 3 weeks and with systemic erythromycin for 2 to 3 weeks to prevent or to treat chlamydial pneumonia. Sulfonamides can be used to treat chlamydial conjunctivitis if erythromycin is not tolerated. Topical antibiotics alone are insufficient for the treatment of neonatal *Chlamydia* infection.[87,95,99] Staphylococcal conjunctivitis can be treated with erythromycin ointment or a variety of other topical antimicrobial agents every 4 to 6 hours for 3 to 7 days. Appropriate systemic antibiotic treatment should be considered if the infection is particularly severe.[205]

N. gonorrhoeae infection requires systemic treatment in all cases. Aqueous penicillin G can be considered if resistant strains are unlikely. However, one dose of intramuscular ceftriaxone is the treatment of choice in the United States because it is effective against all *N. gonorrhoeae* strains.[92,171] Eyes should be irrigated with saline every hour until the discharge clears and then in reduced frequency as necessary to reduce the risk for development of corneal infection. This interval usually is 24 to 48 hours after the initiation of systemic treatment. Penicillin G drops are recommended by some ophthalmologists, but such drops do not appear to improve the overall prognosis or speed of recovery.

HSV neonatal conjunctivitis is treated with systemic acyclovir in appropriate doses for as long as 3 weeks.[78] Treatment should be instituted on an emergency basis because of the potential for

development of serious permanent neurologic sequelae and death if treatment is delayed. Topical ophthalmic vidarabine or trifluridine also should be applied to the eyes four times daily for 2 to 3 weeks.

Prevention of ophthalmia neonatorum is simple and requires only instillation of an antibiotic or antiseptic agent within the first hour after birth. Silver nitrate drops (1%), erythromycin ointment (0.5%), and tetracycline ointment (1%) have been effective treatment against *N. gonorrhoeae* and *C. trachomatis*.[95,205] A 5 percent solution of povidone-iodine also has been effective and economical in prophylaxis against a variety of agents, including gonococcal neonatal conjunctivitis. It may be particularly useful in developing countries.[110] As with other sexually transmitted diseases, neonatal conjunctivitis caused by *N. gonorrhoeae*, *C. trachomatis*, or HSV should be addressed according to standard public health policies, with reporting, investigation, and case identification as required by local law.

KERATITIS: CORNEAL INFECTION

The cornea, with its overlying tear film, is the major refracting component of the human eye. Keratitis means inflammation of the cornea. Any irregularity of the corneal surface or an opacity involving the central visual axis of the cornea may impair vision.

The cornea is composed of five distinct layers: epithelium, Bowman membrane, stroma, Descemet membrane, and endothelium. The epithelium is several layers thick and, when healthy, can regenerate without scarring after an insult. The Bowman membrane lies beneath the epithelium. It does not regenerate and, when injured, heals with a scar. The stroma, approximately 0.5 mm in thickness, is the thickest part of the cornea. It is composed of regularly arranged collagen fibrils embedded in a matrix of mucoproteins and glycoproteins. The arrangement of the fibrils is more regular in the posterior aspect of the stroma. Like the Bowman membrane, the stroma heals with scarring. The Descemet membrane is the basement membrane of the corneal endothelium. It can regenerate after an insult and does not opacify. The innermost layer, the endothelium, is derived from neuroectoderm. It consists of a single layer of cells that do not have significant regenerative capacity. The cornea is an avascular structure that is kept clear by virtue of the regular arrangement of collagen fibers in the stroma and by the pumping action of the endothelium, which keeps the stroma relatively dehydrated. The cornea will become edematous and opacify if the endothelium is significantly damaged or diseased.

The external corneal surface is protected from injury and exposure by the eyelids. Reflex tearing in response to mechanical irritation provides further protection. The blink response to threat and the antimicrobial properties of the normal tear film further protect the cornea from injury and infection.

Keratitis is a potentially vision-threatening condition that refers to inflammatory reactions and infectious processes of the cornea. Persons with keratitis usually have erythema of the perilimbal bulbar conjunctiva and eye pain. Additional complaints may include decreased vision and photophobia. The diagnosis of keratitis depends on the presence of a corneal epithelial or stromal infiltrate. The infiltrate can be extremely difficult to see in uncooperative infants and young children. Ideally, the patient is examined with a slit lamp; sometimes sedation is required for adequate examination of uncooperative children. Stains and cultures often are required to establish the diagnosis, and an ophthalmologist should be consulted promptly. Rarely, biopsy of the cornea is needed to obtain material for culture, and corneal transplantation is required occasionally to halt progression of an infection or to treat corneal perforation. Etiologic diagnosis is based on clinical findings and examination of stains and cultures.

ISOLATED EPITHELIAL KERATITIS

Keratitis can be classified further according to the layer of cornea involved. Most infections involve the epithelium or stroma. Isolated epithelial keratitis usually is caused by one of several viruses: HSV, varicella-zoster virus (VZV), adenovirus, EBV, and measles virus (rubeola). The first three organisms are discussed in an earlier section.

EBV causes HSV-like dendritic corneal epithelial lesions. Stromal disease also may occur. The lesions are self-limited and do not respond to antiviral therapy.[136] When measles involves the cornea, it usually does so in the context of transient epithelial infiltrates, which resolve without permanent sequelae.[117] In malnourished children with vitamin A deficiency, however, measles represents a serious threat to life and vision. Deep corneal ulcers may develop during the first few days of measles infection. Rapid progression culminating in corneal perforation and ultimate loss of the eye may occur.[20,75] Whether the ulcers are caused by direct measles virus infection, by the keratomalacia of vitamin A deficiency, or by some combination of these factors has been debated for some time. Measles immunization programs and vitamin A administration programs can reduce the prevalence of childhood blindness in developing nations significantly.

STROMAL KERATITIS

Syphilis and unique parasitic and viral infections can cause a nonsuppurative keratitis isolated to the corneal stroma. Congenital syphilis produces interstitial (stromal) keratitis in children, although the condition usually does not become apparent until late in the first decade of life. An indolent, peripheral corneal haze develops and slowly progresses centrally. Ghost vessels devoid of blood flow are seen in the corneal stroma on slit-lamp examination.[206] The condition is manifested as a bilateral inactive stromal process[190] in 80 percent of cases and is accompanied by iritis, iridocyclitis, or scleritis at some point in the course of the disease. When ocular syphilis is diagnosed, systemic infection is present by definition. The systemic disease should be treated according to accepted guidelines.

BACTERIAL KERATITIS

Bacterial keratitis (i.e., corneal ulcer) most often occurs after trauma that disrupts the normal integrity of the corneal epithelium. The trauma can be mild and occult, such as that caused by lens wear. *S. aureus*, *S. pneumoniae*, *P. aeruginosa*, and *Moraxella* spp. are the most common bacterial causes of severe necrotizing bacterial keratitis.[8,127,217] Other organisms, such as *S. epidermidis*, *Actinomyces* spp., viridans streptococci, and a variety of others, have been implicated in less severe cases.

Accurate identification of the offending organism facilitates treatment. A specimen is obtained by scraping the edge of the ulcer with a sterile platinum spatula. Smears should be fixed in 70 percent methanol, not by heat, and Gram stain for bacteria and acridine orange stain for fungi and *Acanthamoeba* are recommended. The yield of culture-positive cases is higher in laboratories skilled in handling corneal cultures. Material for culture should be inoculated onto fresh media such as blood and chocolate agar (aerobes and facultative anaerobes), Sabouraud agar (fungi), and thioglycolate broth (anaerobic bacteria). Thayer-Martin agar should be plated if *N. gonorrhoeae* is suspected. Initial treatment is based on the resulting stains (Table 68–2) and modified pending cultures (Table 68–3).[17,126]

Medical treatment of bacterial keratitis includes fourth-generation fluoroquinolones, fortified topical antibiotics, and, occasionally, subconjunctival antibiotic injection (see Table

TABLE 68–2 Treatment of Keratitis Based on Smear Morphology

	Antibiotic	
Organism	**Ocular**	**Systemic***
Gram-positive cocci, gram-positive bacilli	Cefazolin†	Nafcillin, IV
Gram-positive filaments	Amikacin‡	Trimethoprim-sulfamethoxazole, IV
Gram-negative cocci	Ceftriaxone† or ciprofloxacin‡,§	Ceftriaxone, IV or IM
Gram-negative bacilli	Tobramycin†	Tobramycin, IV
Acid-fast bacilli	Amikacin‡	
Hyphal fragments	Natamycin,‡ fluconazole¶	Fluconazole, PO
Yeasts	Amphotericin B,‡ fluconazole¶	Fluconazole, PO
Cysts, trophozoites	Polyhexamethylene, biguanide,‡ paromomycin,‡ and propamidine isethionate‡	Itraconazole, PO

**Standard age-appropriate milligram-per-kilogram dosages.*
†*Topical and periocular use only.*
‡*Topical use only.*
§*Use in children 12 years or older.*
¶*Periocular use only.*
IM, intramuscularly; IV, intravenously; PO, orally.

TABLE 68–3 Treatment Based on Identification of Organisms

	Antibiotic	
Organism	**Ocular**	**Systemic**
Micrococcus, *Staphylococcus* (penicillin-resistant)	Cefazolin*	Nafcillin, IV
Micrococcus, *Staphylococcus* (methicillin-resistant)	Vancomycin*	Vancomycin, IV
Streptococcus	Penicillin G*	Penicillin G, IV
Enterococcus	Vancomycin* and gentamicin*	Vancomycin, IV, and gentamicin, IV
Anaerobic gram-positive coccus	Penicillin G*	Penicillin G, IV
Corynebacterium species	Penicillin G*	Penicillin G, IV
Mycobacterium fortuitum-chelonae	Amikacin†	Amikacin, IV,* or clarithromycin, PO
Nocardia	Amikacin†	Trimethoprim-sulfamethoxazole, IV
Neisseria gonorrhoeae	Ceftriaxone*	Ceftriaxone, IV or IM
Pseudomonas species	Ceftazidime,* Tobramycin*	Ceftazidime, IV or IM
Other aerobic, gram-negative bacilli	Ceftazidime* Tobramycin*	Ceftazidime, IV or IM
Filamentous fungi	Natamycin† Fluconazole‡	Fluconazole, PO
Candida species	Amphotericin B† Fluconazole‡	Fluconazole, PO
Acanthamoeba	Polyhexamethylene biguanide,† paromomycin,† and propamidine isethionate†	Itraconazole, PO

**Topical and periocular use.*
†*Topical use only.*
‡*Periocular use only.*
IM, intramuscularly; IV, intravenously.

68–2).[38,156,205] Antibiotics are administered as often as every 15 to 30 minutes initially. Systemic antibiotics are required only for gonococcal, chlamydial, and onchocercal keratitis, as well as for actual or threatened perforation of the cornea, extension of infection to the sclera, or worsening of the process during topical or periocular treatment (or both). Topical steroids are useful if the resulting inflammation threatens to destroy the mechanical or optical integrity of the cornea, but steroids never are used as isolated treatment.[205]

FUNGAL KERATITIS

The most common causes of fungal keratitis include *Candida*, *Aspergillus*, and *Fusarium*. Mycotic keratitis associated with the filamentous fungi *Aspergillus* and *Fusarium* is thought to occur with trauma, whereas keratitis caused by *Candida* is associated more commonly with a preexisting ocular (e.g., dry eye) or systemic (e.g., diabetes mellitus, immunosuppression) condition.[210] Fungal keratitis tends to be relatively indolent in comparison to bacterial keratitis. Typically, white stromal infiltrates with irregular and indistinct borders are detected. Satellite lesions, or those independent of the main lesion, may be evident. Mild concurrent iritis is a common finding. Severe iritis with hypopyon can occur but is an infrequent manifestation of fungal corneal disease.[157] Acridine orange stain is recommended, and culture on a Sabouraud agar plate is indicated. Hyphal fungi are treated with topical natamycin, topical fluconazole, systemic fluconazole, or some combination of these medications. Yeasts are treated with topical or systemic amphotericin B, fluconazole, or both agents.[76]

PROTOZOAN KERATITIS

Protozoan keratitis is a particularly serious vision-threatening process. It usually is caused by *Acanthamoeba* and occurs more

frequently in wearers of soft contact lenses. Use of homemade saline solution is a significant risk factor and should be discouraged.[31] Amebae are ubiquitous organisms in soil, water, and air. They have been identified in hot tubs and in the feces of domestic animals.[134] People living in rural areas may be at special risk. *Acanthamoeba* keratitis also has been reported in children without a history of contact lens use or trauma.[53]

Acanthamoeba corneal ulcers are pleomorphic.[109] Older lesions may exhibit a ringlike infiltrate around a central ulcer. Iritis or iridocyclitis may be intense, and severe pain is a hallmark of the disease. Acridine orange and calcofluor white stains aid in establishing an early diagnosis of the condition. Tandem scanning confocal microscopy of corneal specimens may increase diagnostic accuracy, and the organism may grow on blood and chocolate agar.[31,131,133,138,166]

Treatment of *Acanthamoeba* keratitis is complex and suboptimal. Topical application of polyhexamethylene biguanide (0.02%),[123] chlorhexidine (0.02%), paromomycin (0.1%), or propamidine isethionate (Brolene) drops may be effective. Oral itraconazole (Sporanox) has been used successfully in adults.[127,131,138] Cycloplegic agents are recommended to improve the patient's comfort, and pain medications often are required. Corneal transplantation is needed if the infection progresses despite treatment, if perforation occurs, or if significant corneal opacification remains after the disease is eradicated.

Onchocerca volvulus is a filarial parasite that causes river blindness (i.e., onchocerciasis). The disease is endemic in sub-Saharan West Africa and in areas of Central and South America. The organism is transmitted by the bite of a blackfly of the family Simuliidae.[179] Larvae migrate to subcutaneous tissue and pass through several molts to become adult worms, which encapsulate in nodules and produce microfilariae that pass into blood, skin, and other organs, including the eyes. The conjunctiva, cornea, aqueous humor, vitreous, retina, uveal tract, sclera, and optic nerve all may be infested. Corneal involvement can lead to vision loss caused by corneal opacification, but most severe vision loss is caused by choroidal, retinal, and optic nerve disease.[93] Although children often are infected, blindness usually does not occur until they reach the third or fourth decade of life.[213,220]

Current treatment of onchocercal infection is administration of ivermectin every 6 to 12 months to all individuals living in endemic areas. Ivermectin kills the microfilariae but not the adult worms, which remain until their death.[209] The adult worm may live 10 or more years. Because of the long life span, ivermectin should be taken every 6 months for at least 10 years to treat microfilariae as they develop.

Microsporidia are intracellular protozoa that represent a new emerging ocular pathogen. Interest in the organism has increased in the last decade because of its association with HIV and acquired immunodeficiency syndrome (AIDS). The ocular manifestation corresponds to both the immune status of the patient and the genus of microsporidia involved.[115] Typically, keratoconjunctivitis occurs in immunocompromised individuals, and stromal keratitis is seen in immunocompetent individuals. Keratoconjunctivitis is manifested as bilateral conjunctival inflammation and epithelial keratopathy, which may lead to decreased vision. Stromal keratitis is manifested as an insidious diskiform keratitis with uveitis similar to that seen in HSV stromal keratitis and may require penetrating keratoplasty because of perforation or scarring.

Potassium hydroxide plus calcofluor white and acid-fast stains are thought to be the most efficient means of establishing the diagnosis of microsporidial keratitis.[114] Transmission electron microscopy provides specificity in identification of the genus and possibly the species, but it may lack sensitivity and is laborious.[115] Knowledge of the genus and species may help guide management. Albendazole, fumagillin, itraconazole, metronidazole, and topical propamidine isethionate have been used as therapeutic agents.[43,115]

INFECTIONS PRIMARILY INVOLVING THE UVEA

The uveal tract is the vascular middle coat of the eye. It is situated between the sclera and the retina. Its major function is to provide nourishment for intraocular tissues, including the retina, lens, and cornea. The uveal tract is composed of the iris, ciliary body, and choroid.

Uveitis is a nonspecific term for inflammation of the uvea. If the inflammatory process primarily affects the iris, it is called *iritis*. If the ciliary body is involved, the process is called *cyclitis*. If these two structures together are involved, the process is called *iridocyclitis* (i.e., anterior uveitis). The term *intermediate uveitis* (i.e., pars planitis) applies to inflammation in the region of the ciliary body and peripheral retina. The term *posterior uveitis* usually applies to combined inflammation of the retina and choroids, also called *chorioretinitis*. If the choroid alone is involved, it is called *choroiditis*; inflammation of the retina alone is called *retinitis*. Because of the thinness and close apposition of these two tissue layers, inflammation in one layer frequently "spills over" into the other. The vitreous body occupies the central area of the eye behind the lens. It is composed of water, mucopolysaccharides, and collagen. Though transparent in a normal, healthy eye, the vitreous is subject to inflammation or vitritis, usually as a result of inflammation in adjacent retinal tissue or in the pars plana.

Causes of uveitis are numerous but can be categorized into two main groups: infectious and noninfectious. Infectious causes are discussed in this chapter. Uveitis in any area of the eye may result in pain, conjunctival or episcleral hyperemia, photophobia, lacrimation, and decreased vision, although these symptoms vary relative to the site and the aggressiveness of the inflammation.

With slit-lamp examination, the hallmark of anterior uveitis is the finding of hazy, proteinaceous aqueous humor, called *flare*, and the presence of leukocytes in the anterior chamber. The cell and flare components are graded independently on a 1+ to 4+ scale. If present in sufficiently great numbers, leukocytes can precipitate in the anterior chamber (with location dependent on gravity) and form a mass called a *hypopyon*. The cells can aggregate on the back of the cornea and create fine, medium, and large lesions known as *keratic precipitates*. The conjunctiva usually is injected or hyperemic. With chronic inflammation, the border of the pupil often adheres to the anterior surface of the lens. Such adhesions are called *posterior synechiae* and may cause the pupil to have an irregular shape and size, as well as poor reactivity to light. Chronic or recurrent anterior segment inflammation may lead to the formation of a cataract. Iris nodules or atrophy may develop with long-standing inflammation. Anterior vitreous cells may occur as a spillover phenomenon in the setting of a prolonged or vigorous anterior chamber reaction. Although inflammatory effects on the ciliary body generally compromise its production of aqueous humor and thereby reduce intraocular pressure, cellular and proteinaceous debris can occlude aqueous humor outflow channels and lead to elevated intraocular pressure.

Inflammation primarily involving the posterior segment of the eye usually leads to decreased vision, which may be the symptom for which treatment initially is sought. Pain may be minimal or absent. Inflammatory lesions of the retina and choroid also may lead to the development of cellular debris in the vitreous and cause the patient to perceive "floaters." Initially, the borders of the retinal or choroidal inflammatory foci often are indistinct and cream-colored. In the healing phase, the borders of these lesions increasingly become distinct, and a defined, partially pigmented scar results. Inflammatory perivascular sheathing of the retinal vessels may occur as well. Involvement of the macula with edema or exudates may result from inflammation of the posterior segment, and long-standing edema may evolve into a cystic configuration and cause loss of central visual acuity. The optic nerve may exhibit an inflammatory response; if the optic disk is so

involved, the response is called *papillitis*. Inflammatory debris also may be found in the vitreous.[4,159,233]

Under normal circumstances, the amount of immune traffic within the eye, especially in the anterior chamber, is held to a minimum by a group of immunomodulatory pathways that together form anterior chamber–associated immune deviation (ACAID). Chief among these deviations is active suppression of delayed hypersensitivity; distinctive ocular antigen-presenting cells migrate to the spleen, where they generate an immune response deficient in $CD4^+$ cells but high in $CD8^+$ cells. These regulatory T cells return to the eye to suppress the $CD4^+$ populace, thereby minimizing the arm of inflammation most likely to impair visual clarity.[74,200]

EPIDEMIOLOGY

In the United States, most uveitis cases have noninfectious causes. Large samples demonstrate a predominance of anterior rather than posterior uveitis. The most common category of anterior uveitis is idiopathic; the most common infectious cause of anterior uveitis is herpetic kerato-uveitis (simplex and zoster). Posterior uveitis is caused most frequently by *Toxoplasma gondii*. In developing nations, infectious uveitis plays a larger role. In West Africa, *T. gondii* probably accounts for most cases of intraocular inflammation.[177,178]

VIRAL UVEITIS

Herpes Simplex Virus

Most of the uveal inflammation associated with HSV (typically iridocyclitis) results from the corneal disease. Occasionally, a patient will have iritis as an isolated finding, but most patients with ocular HSV demonstrate conjunctivitis, keratitis, chorioretinitis, or retinal vasculitis. The iritis may require treatment with topical steroids and topical antivirals as prophylaxis against the development of corneal epithelial disease.[216,233]

Varicella-Zoster Virus

Occasionally, varicella infection (i.e., chickenpox) may be associated with a transient iritis that requires no treatment. Though rare, cases of unifocal choroiditis causing visual loss but responsive to acyclovir in children with primary varicella have been reported.[146]

Herpes zoster virus may cause iridocyclitis during the acute stage of the disease; the anterior chamber reaction can persist or recur long after resolution of the cutaneous component of the condition. Herpes zoster always should be considered in the differential diagnosis of chronic unilateral iridocyclitis. Topical steroids are indicated for iritis, with the addition of oral or intravenous acyclovir for severe cases. Segmental iris atrophy is a characteristic sequela of herpes zoster uveitis. Glaucoma, hyphema, retinitis, vasculitis, and extraocular muscle palsy occasionally occur in patients with herpes zoster ophthalmicus. In a profoundly immunosuppressed patient, VZV can cause a devastating retinitis known as *progressive outer retinal necrosis*.[125]

VZV and HSV-2 have been implicated as causes of acute retinal necrosis syndrome. Patients in whom this syndrome is diagnosed range from 13 to 71 years of age, with an average age of 43 years. The syndrome typically occurs in healthy patients. The virus causes a triad of acute vitritis, retinal vasculitis, and peripheral necrotizing retinitis and is bilateral in 33 percent of patients. Treatment consists of intravenous acyclovir. Prophylactic laser photocoagulation may prevent retinal detachment. The visual prognosis is guarded.[22,125]

Epstein-Barr Virus

Ocular EBV involvement has been reported primarily in patients with infectious mononucleosis. Follicular conjunctivitis may be diagnosed in 2 to 40 percent of patients. Corneal stromal inflammation, iritis, episcleritis, optic neuritis, and chorioretinitis occur less commonly. Systemic corticosteroids and acyclovir may be useful in treating sight-threatening complications of chronic intraocular inflammation from EBV infection.[135] Postinfectious uveitis also has been described and is discussed later.

Enteroviruses

Coxsackievirus A24 and enterovirus 70 may cause a painful follicular conjunctivitis called acute hemorrhagic conjunctivitis. Rarely, chorioretinitis may be diagnosed. Treatment of these infections primarily is supportive.[159]

Rubella Virus

Rubella virus can cause congenital and acquired infections. Ocular manifestations of acquired rubella include conjunctivitis in 70 percent of patients, superficial keratitis, and iritis. Rarely, retinitis has been reported.

Congenital rubella syndrome can be manifested as cataracts, glaucoma, microphthalmos, and retinitis. Cataracts occur in 15 percent of patients and glaucoma in 10 percent. Retinal examination reveals a "salt-and-pepper fundus" caused by the alternating pattern of hypopigmentation and hyperpigmentation of the retinal pigment epithelium. The prognosis for patients with retinitis alone usually is good, with vision between 20/20 and 20/40. Glaucoma resulting from rubella commonly requires surgery, as do the cataracts. Iritis is reported less commonly.[23,135]

Mumps Virus

Ocular manifestations of mumps virus includes dacryoadenitis, conjunctivitis, iritis, optic neuritis, and keratitis. Retinitis also has been reported. The prognosis for visual recovery from the retinitis is good, and sequelae of the iritis are rare occurrences.[76,135]

Measles Virus

Measles virus can cause congenital and acquired infections. In congenital infections, a "salt-and-pepper" retinopathy and cataract formation may occur, similar to that found in rubella. Ocular manifestations in acquired measles include conjunctivitis and, much less commonly, retinitis, retinal vasculitis, and optic nerve edema.[12,159]

Subacute Sclerosing Panencephalitis

Between 30 and 75 percent of patients with subacute sclerosing panencephalitis (SSPE or Dawson inclusion body encephalitis) have ocular findings. SSPE is caused by a variant of the measles virus; it differs from the wild-type virus by alteration or absence of viral M protein. Optic nerve edema, inflammation, and subsequent optic atrophy have been reported. Macular retinitis is a common finding, and the contiguous non-neural tissues (vitreous and choroid) almost never are involved. The visual prognosis of survivors is poor.[155,159]

Creutzfeldt-Jakob Disease

The most common ocular manifestation of Creutzfeldt-Jakob disease is cortical blindness. Optic atrophy may result from degeneration of the neurons of the optic nerve.[159]

Human Immunodeficiency Virus and Acquired Immunodeficiency Syndrome

As many as 75 percent of patients with advanced AIDS have ocular findings. Cotton-wool spots occur in more than 50 percent of patients and are bilateral in more than 80 percent. Cotton-wool patches represent focal infarctions of the neural layer of the retina and are the most common ocular finding in patients with AIDS. They generally produce no symptoms and do not decrease vision. These spots are white and fluffy and occur most commonly in the macular portion of the retina. They resolve in 4 to 6 weeks with no residual scars. Occasionally, flame-shaped hemorrhages are detected as well. The retina and choroid in these patients may become infected with HSV, CMV, VZV, syphilis, tuberculosis, ocular histoplasmosis, *Candida*, toxoplasmosis and *Pneumocystis*, although in the highly active antiretroviral therapy (HAART) era, the incidence of CMV retinitis has declined and its advance in existing cases has been slowed or halted. Symptomatic anterior uveitis in the absence of the aforementioned pathogens occurs rarely in patients with AIDS and may be caused by HIV itself.[111]

Cytomegalovirus Infection

CMV infections may occur in preterm neonates and immunosuppressed patients, especially those with AIDS. In neonates with symptoms of CMV infection (i.e., low birth weight, microcephaly, jaundice, thrombocytopenia, hepatosplenomegaly, or petechial rash), congenital CMV infection[18] (i.e., prenatal transmission) may be manifested in the fundus as chorioretinal scars (21%) and optic atrophy (7%).[41]

CMV retinitis develops in approximately 30 percent of adult patients with AIDS, usually those with $CD4^+$ counts less than $50/mm^3$.[63] In the pediatric AIDS population, $CD4^+$ counts of less than $20/mm^3$ may be required for appearance of the retinitis.[64] The disease is less common in pediatric patients with AIDS. Patients with CMV retinitis have no external ocular signs but may complain of loss of vision. Children may not complain of such loss, and the problem may become apparent only after bilateral severe vision loss has occurred.[64] The retinal lesions of CMV typically are yellow-white and often are associated with hemorrhage. The retinitis generally follows a perivascular distribution. The retina becomes necrotic and eventually atrophies, with a gliotic scar. CMV optic neuritis also may develop. Treatment consists of intravenous or intravitreal ganciclovir, intravenous or intravitreal cidofovir, or intravenous foscarnet[64,233] and can often be discontinued when the $CD4^+$ count rises above $100/mm^3$.[212]

Parvovirus Infection

Bilateral panuveitis has been reported to be associated with parvovirus B19 infection.[130]

Human T-Cell Lymphotrophic Virus Infection

Human T-cell lymphotrophic virus (HTLV) is endemic in several regions of the globe: Japan, the Caribbean islands, and parts of central Africa. It probably is responsible for several cases of self-limited, occasionally recurrent uveitis in these regions.[153]

Lymphocytic Choriomeningitis Virus Infection

Lymphocytic choriomeningitis virus (LCMV) is an arenavirus endemic in mice; it is reported in hamsters as well and occasionally is transmitted to humans by direct contact with the rodent or through aerosolization of its feces or urine. Postnatal exposure results in asymptomatic seroconversion or aseptic meningitis, but intrauterine infection can have devastating consequences. Spontaneous abortion, congenital hydrocephalus, psychomotor retardation, and chorioretinitis have been documented. Diffuse chorioretinal scarring identified postnatally may bode a grim visual prognosis. LCMV may be an underdiagnosed cause of unexplained congenital chorioretinitis. The diagnosis is confirmed by elevated LCMV antibody titers. A survey of severely retarded, visually disabled children revealed immunologic evidence pointing to LCMV as the cause of the visual loss in approximately half of those surveyed.[140,143]

BACTERIAL UVEITIS

Syphilis

Syphilis (*Treponema pallidum*) should be considered as a possible cause in all cases of intraocular inflammation. Any patient with confirmed syphilitic uveitis should undergo a lumbar puncture to rule out asymptomatic neurosyphilis.

Ocular manifestations of congenital syphilis include interstitial keratitis, a mottled "salt-and-pepper" fundus, and chorioretinal scarring. Acute interstitial keratitis occurs as a late manifestation of congenital syphilis (5-25 years of age) and is thought to be a hypersensitivity response to treponemal antigen in the cornea. Patients complain of pain and photophobia and have a diffusely opaque cornea and anterior uveitis. Blood vessels invade the inflamed cornea and eventually are obliterated, with ghost vessels left in the corneal stroma. Glaucoma may occur. A unilateral manifestation of interstitial keratitis suggests postnatally acquired lues.

Secondary syphilis may involve any layer of the eye. Episcleritis, scleritis, iridocyclitis (acute, chronic, or recurrent), iris capillary dilation (i.e., iris roseata), vascular papules of the iris (i.e., iris papulosa), inflammatory nodules (i.e., iris nodosa), choroiditis, chorioretinitis, and retinal vasculitis all have been reported, as have optic neuritis and subsequent atrophy.

Tertiary syphilis may have associated gummata of the iris and an Argyll Robertson pupil (i.e., miotic, irregularly shaped pupil with loss of response to light but preservation of the near response). Intraocular inflammation seldom occurs at this stage.

Ocular inflammation secondary to syphilis should be treated as neurosyphilis. With the appropriate doses of penicillin G, the inflammation typically resolves rapidly. Occasionally, topical regional steroids are required to control local inflammation. Treatment of ocular syphilis may lead, at least initially, to a vigorous local and systemic self-limited, febrile response, presumably caused by liberation of spirochetal antigens or toxins. Topical oral corticosteroids may be required to quell this response, which is also known as a *Jarisch-Herxheimer reaction*.[4,132,159]

Lyme Disease

A mild follicular conjunctivitis occurs in 11 percent of patients with stage 1 Lyme disease, caused by *Borrelia burgdorferi*. During the second and third stages, neuro-ophthalmic manifestations, including cranial neuropathy (most often cranial nerves III, IV, VI, and VII), optic neuritis, bilateral keratitis, bilateral iridocyclitis, diffuse choroiditis, vasculitis, intermediate uveitis, and Parinaud oculoglandular syndrome, may be seen. The most frequent manifestation of late Lyme disease is arthritis. Ocular inflammation occurs in approximately 4 percent of children with Lyme arthritis.[108] Early, localized Lyme disease may be treated with doxycycline (8 years and older) or amoxicillin. More advanced or persistent disease is treated best with intravenous ceftriaxone.[6] Antibiotic treatment early in the course of the disease carries a better prognosis than does therapy initiated at later stages.[19]

Leptospirosis

Leptospirosis may cause an anterior uveitis that occurs months after the acute infection. Leptospirosis is identified as an important cause of epidemic panuveitis in southern India; the most common posterior segment manifestations in this group are vasculitis and vitritis.[39]

Tuberculosis

Any structure of the eye may be affected by tuberculosis. Allergic and infectious processes have been implicated as important causes of tuberculous uveitis. Anterior uveitis with or without keratitis has been attributed to tuberculosis. Choroiditis, optic neuritis, and orbital infections have been detected in cases of miliary tuberculosis. The most frequent manifestations of ocular tuberculosis are choroidal nodules and scars; anterior uveitis is an uncommon occurrence.[26] Treatment should be undertaken with the appropriate antituberculous medications. Corticosteroids often are necessary in conjunction with antimicrobial therapy.

Leprosy

Because *Mycobacterium leprae*, the cause of leprosy, grows best at lower temperatures, corneal infections predominate. Corneal involvement is associated with prominence of the corneal nerves, interstitial keratitis, and corneal hypoesthesia, but corneal opacities are often peripheral and not visually significant.[49] Uveal involvement in leprosy frequently is silent and accounts for a large number of the ocular complications from the disease.[48,49]

Brucella Infection

Ocular manifestations of *Brucella* infection are rare but include iritis, focal nodular choroiditis, and panophthalmitis.[159]

Cat-Scratch Disease

Bartonella henselae is a gram-negative rod transmitted to humans by the bite or scratch of an infected animal, often a young cat or kitten. Regional lymphadenopathy is the predominant nonocular finding. A striking stellate neuroretinitis characterized by swelling of the optic nerves and lipid deposition in the retina is the most easily identifiable complication of ocular infection. Other manifestations include intermediate uveitis, optic disk swelling, multifocal choroiditis, and serous macular detachment. The discrete foci of multifocal choroiditis are the most common findings in the posterior segment. The role of antibiotics in this condition is debated.[119,195]

FUNGAL UVEITIS

Histoplasmosis

The diagnosis of presumed ocular histoplasmosis syndrome is based on the clinical picture of disseminated punched-out retinal "histo spots," atrophic retinal changes around the optic nerve, and a clear vitreous.[168] Later in the course of the disease, subretinal hemorrhage and retinal detachment may occur. Ocular histoplasmosis often is bilateral and can result in legal blindness from the loss of macular vision. Presumed ocular histoplasmosis syndrome may occur after an episode of benign systemic histoplasmosis during childhood. Active inflammation and vitreous cells usually are not seen in this syndrome, although case series have documented active chorioretinal inflammation as new-onset lesions and as reactivation of previously quiescent lesions.[36,116] The hallmark histoplasmosis spots appear as white, punched-out, well-demarcated chorioretinal scars and represent healed fungal lesions. Generally, they first appear during adolescence, do not reduce vision, and do not require treatment. Macular disease, which may reduce vision, does not develop until after the patient reaches the second decade of life. Subretinal neovascularization may develop at the site of a macular histoplasmosis spot, with fluid, blood, and lipid accumulating in the subretinal space. This process plus local scarring can result in a marked reduction in central vision.

Macular neovascularization may be treated suitably with laser photocoagulation in an attempt to salvage the remaining central vision. Antifungal drugs may play a role in the unusual setting of demonstrated active histoplasmosis choroiditis.[184]

Candidiasis

Candida spp., including *C. albicans*, are fungi with yeast and filamentous forms. Candidiasis is encountered in immunocompromised patients and in situations involving indwelling catheters, intravenous therapy, chronic antibiotic use, poorly controlled diabetes, and intravenous drug abuse. Candidal chorioretinitis can develop in approximately 9 percent of patients with blood cultures positive for the fungus,[60] although more recent studies have demonstrated that ocular involvement (either chorioretinitis or endophthalmitis) in children is a rare occurrence.[61]

In the eye, *Candida* infection usually begins in the choroid and eventually causes multifocal white chorioretinal lesions. If the fungus proliferates unchecked, it may break through the retina into the vitreous and produce the classic, white, snowball-like "fungus ball." Candidal infection that progresses to endophthalmitis is exceedingly rare if appropriate intravenous antifungals are started promptly on notice of a positive blood culture.

Intravenous amphotericin B is the drug of choice. Other antifungal agents such as fluconazole, flucytosine, and miconazole also may be effective, but none is dramatically so. Surgical treatment of intraocular *Candida* disease is discussed in the section on endophthalmitis.

Aspergillosis

Aspergillus spp. can infect the choroid, retina, and vitreous of immunocompromised individuals. One group of investigators found a high rate (7%) of these unusual infections on reviewing records of deceased liver transplant recipients.[107]

Coccidioidomycosis

Coccidioides spp. have yeast and filamentous forms. Ocular disease consists of a multifocal chorioretinitis that develops during the course of systemic coccidioidomycosis. The lesions initially appear similar to those seen in histoplasmosis; in severe cases, endophthalmitis results, and vitrectomy may need to be performed. In less severe cases, the lesions may respond to intravenous amphotericin B. Occasionally, an isolated granulomatous iridocyclitis may develop.[147]

Cryptococcosis

Cryptococcus neoformans is a yeast-like fungus that can cause multifocal chorioretinitis and endophthalmitis. Most patients with cryptococcosis are severely immunocompromised; many have AIDS. The CNS and eye are involved commonly, and elevated intracranial pressure may cause papilledema and sixth cranial nerve palsy. Intravenous amphotericin B in combination with oral flucytosine is the treatment regimen of choice.[6,120]

Sporotrichosis

The dimorphic fungus *Sporothrix schenckii* is encountered commonly in rotting vegetable matter, wood, and soil. The fungus gains access to the host by traumatic implantation or inhalation. It has been reported to be responsible for anterior uveitis in the setting of a suggestive lesion on a finger of the dominant hand, with presumed hand-to-eye transmission.[219]

PROTOZOAL UVEITIS

Leishmaniasis

Ocular leishmaniasis has been described. Manifestations include conjunctivitis, blepharitis, and anterior uveitis. This trio of findings responds to systemic treatment with sodium stibogluconate.[68]

HELMINTHIC UVEITIS

Toxocariasis

Toxocara canis causes visceral larva migrans, which is not associated with ocular disease. In the retina, larvae get trapped in small capillaries and burrow into surrounding tissue; the dead larvae incite an intense eosinophilic abscess. Ocular toxocariasis has three classic clinical manifestations, almost always involving one eye only. One form occurs in children 2 to 9 years of age and causes an indolent endophthalmitis and leukokoria (i.e., white pupil). Tractional retinal detachment may occur. The eye typically shows little or no external evidence of inflammation, and the patient experiences no pain.

A second form appears in children between 4 and 14 years of age. These patients have reduced vision but little or no external inflammation and no pain. The reduced vision may cause strabismus, which may be the first sign. An inflammatory granuloma is seen in the macula.

The third form of ocular toxocariasis occurs in patients between 6 and 40 years of age but may not be recognized until years later. Patients with this form of toxocariasis have good vision, but a peripheral retinal granuloma is seen on routine eye examination. Vision may be affected if a traction band from the granuloma distorts the macula.

Inactive *Toxocara* granulomata do not respond to medication. When active intraocular inflammation exists in such a magnitude that it poses a further threat to vision, administration of periocular or systemic steroids may be necessary. Anthelmintic agents eradicate migrating larvae, but their effect on "residing" larvae is questionable. When giving antihelmintics, one should combine them with a short course of corticosteroids because death of the larvae may incite vigorous inflammation. Cryotherapy and laser therapy may be useful when the *Toxocara* granuloma is located away from the macula and optic nerve. Vitrectomy may be helpful if significant traction on the retina occurs. Antihelmintic drugs should not be administered after the patient has undergone posterior segment surgery. Visual prognosis is poor if the macula is involved.[159,186,191,229]

Onchocerciasis

Onchocerciasis often causes a severe choroiditis with an overlying retinitis. The various ocular manifestations of infestation with *O. volvulus* are discussed in the section on keratitis.

Loiasis

The *Loa loa* worm can migrate through the tissues of the eye and cause conjunctivitis, iridocyclitis, vitritis, and chorioretinitis. The disease is transmitted by the bite of a mango fly. Vascular obstruction with intraretinal hemorrhage and retinal exudation may occur as well. Medical treatment with diethylcarbamazine can kill the adult worms and microfilariae. Adult worms also can be removed from the eye surgically.[159]

Cysticercosis

The tapeworm *Taenia solium* causes cysticercosis. When the larva gains access to the eye, cysts form in the vitreous or subretinal space in 13 to 46 percent of patients. The living worm may be seen undulating in these spaces. With death of the organism, severe panuveitis can occur. Orbital and subconjunctival involvement occurs less commonly. Surgical removal of the intraocular cysts may prevent the severe inflammation that occurs on death of the worm. Praziquantel can kill the organism, but the ensuing increase in inflammation may be dramatic.[159]

UVEITIS CAUSED BY INSECT-INDUCED DISEASE

Ophthalmomyiasis is the ocular disorder caused by infestation with fly larvae, most commonly the larval form of the sheep botfly *Oestrus ovis*. Maggots may be seen in the conjunctival fornix (cul-de-sac) or inside the eye. Internal ophthalmomyiasis can be diagnosed by noting a motile larva in the anterior chamber, vitreous, or subretinal space. The maggot may leave trails ("railroad tracks") behind throughout the retina. A mild inflammatory response in the anterior chamber (e.g., iritis, iridocyclitis) or vitreous may occur. Treatment is surgical removal of the larva. Corticosteroids may be used to treat the accompanying intraocular inflammation.[65,159]

POSTINFECTIOUS UVEITIS

Increasingly, attention is being given to the role of bacterial and viral systemic illness in the eventual development of sterile intraocular inflammation. This type of uveitis is thought to be caused by an autoimmune response that occurs between sensitized lymphocytes and host tissues that bear some antigenic similarity to the recently cleared pathogen. Disruption of the ACAID is probably a prerequisite for the development of these postinfectious syndromes. Several reports exist of nongranulomatous anterior uveitis occurring weeks or months after streptococcal infection. These uveitides may be associated with other poststreptococcal findings (e.g., arthritis, glomerulonephritis) or may be the sole manifestation. Treatment with cycloplegics and topical corticosteroids is sufficient for the ocular manifestations.[16,215,231]

Uveitis, predominantly of the anterior type, has been reported to occur after illnesses caused by gram-negative enteric bacteria such as *Klebsiella*, *Salmonella*, and *Yersinia*. These gram-negative–induced uveitides are much more likely to occur in the setting of HLA-B27 positivity. The ocular findings often parallel the development of arthritis, thus suggesting that parallel immunologic processes are occurring in both these mesenchymal cavities (i.e., the joint space and the anterior chamber). An association between recent EBV infection and acute tubulointerstitial nephritis and anterior uveitis has been described, with onset of the renal and ocular inflammation occurring several months after the characteristic acute EBV infection.[90]

INFECTIONS INVOLVING PRIMARILY THE RETINA

EYE MANIFESTATIONS OF INTRAUTERINE INFECTIONS (TORCHS COMPLEX)

The TORCHS complex is a group of congenital and perinatal infections that may cause severe systemic and ophthalmic abnormalities. The effect of infection with one of the TORCHS organisms—*T. gondii*, others (LCMV, EBV), rubella virus, CMV, herpesvirus, and syphilis—may be evident at birth or be manifest later in childhood or adulthood. Congenital EBV infection (infectious mononucleosis) has been reported to be associated, possibly, with congenital cataracts.[85] All these infections commonly cause either mild or no clinically evident disease in the mother.[141] The diagnosis cannot always be established on clinical grounds alone, and neonatal and maternal serologic tests must be performed to confirm the clinical suspicion.

Toxoplasmosis

T. gondii is an obligate intracellular parasite that has an affinity for the CNS and retina. The parasite has three forms: tachyzoite, bradyzoite, and sporozoite or oocyst. Human infection may be congenital or acquired. Acquired infection results from the ingestion of undercooked meat contaminated with oocysts or from exposure to the feces of an infected cat, the definitive host. The oocysts release tachyzoites, which multiply intracellularly and result in cell death. In adults, primary acquired infection usually is asymptomatic. The immune response then transforms the tachyzoite into a bradyzoite, which encysts and remains dormant in tissues for years. These cysts have the propensity to rupture sometime later and cause an inflammatory response resulting in recurrent infection.

Congenital infection is transmitted through the transplacental route. In the United States, the reported incidence of congenital toxoplasmosis is one case per 1000 to 10,000 births.[9,47] Seventy percent of the obstetric population is negative for antibodies and, therefore, at risk for infection and transplacental transmission to the fetus.[141] Congenital infection is most severe when acquired in the first trimester and can result in chorioretinitis, intracranial calcifications, microcephaly, mental retardation, and deafness.[47,71,73] Symptomatic neonates with disseminated disease have hepatosplenomegaly, lymphadenopathy, jaundice, fever, anemia, pneumonitis, and a poor prognosis. Other ocular manifestations include cataracts, strabismus, microcornea, vitritis, retinal detachment, optic atrophy, microphthalmos, nystagmus, and ptosis.[141] In a prospective study, 15 percent of infected newborns had chorioretinal scars, indicative of infection in utero; 4 percent had active chorioretinitis; and, in 10 percent, retinal lesions developed by the time that the children were 1 to 2 years of age. Long-term follow-up studies have found that chorioretinal lesions develop in 82 to 85 percent of children with subclinical *Toxoplasma* infection, some with severe visual loss. Infants with asymptomatic toxoplasmosis should undergo regular ophthalmologic examination because retinal involvement can occur later in childhood or adulthood.[27,71,121] Some researchers have suggested that all neonates with toxoplasmosis should receive drug therapy, even if they are asymptomatic.[71]

Toxoplasmosis is the leading cause of acquired necrotizing retinitis and, in many cases, represents reactivation of congenitally acquired infection. Clinically, an area of active retinochoroiditis is adjacent to the border of a chorioretinal scar.[73,174] Associated choroiditis and vitritis may be present. Primary acquired ocular toxoplasmosis manifested as retinochoroiditis is well-documented also, and reports suggest that this route of infection may be more common than originally thought.[27,105] Recurrent ocular disease with postnatally acquired toxoplasmosis has been reported.[25]

T. gondii causes a focal necrotizing retinitis with secondary choroiditis and vitritis. Patients may have floaters, blurred vision, and photophobia. Those with macular involvement can suffer significant visual loss. After the inflammation has resolved, a flat, pigmented chorioretinal scar develops. Visual loss depends on the location of the retinal lesion, with peripheral lesions resulting in little or no visual disturbance and macular lesions capable of producing profound visual loss.

Toxoplasma retinochoroiditis is an emerging problem in patients with AIDS and may be the initial manifestation of this syndrome.[225] The clinical appearance often is atypical.[193] Chronic suppressive therapy is necessary because infection recurs with discontinuation of treatment. A combination of pyrimethamine and clindamycin has been reported to be most effective as prophylaxis.[225]

The diagnosis of *Toxoplasma* retinochoroiditis usually is presumptive and is based on clinical appearance and serologic testing. Several serologic tests are available. The standard serologic diagnosis is based on the presence of anti-*Toxoplasma* IgM in any sample or demonstration of a significant rise in antibody titer in paired sera taken 4 to 6 weeks apart. However, in reactivated congenital infections, which many cases are, *Toxoplasma* IgM results are not positive. The antibody tests most commonly used are indirect immunofluorescent assay (IFA) and enzyme-linked immunosorbent assay (ELISA) for IgM and IgA.[85] False-positive results can occur with both tests in the presence of rheumatoid factor. If clinical infection is suspected and the initial testing results are negative, repeat testing or an alternative testing technique should be considered.

Standard treatment of *Toxoplasma* retinochoroiditis is triple therapy with sulfadiazine (100 to 200 mg/kg/day in four divided doses [maximum, 1.5 g]), pyrimethamine (2 mg/kg/day for 3 days, then 1 mg/kg/day [maximum, 25 mg/day]), and leucovorin (folinic acid) (10 to 25 mg given orally daily for 6 weeks). Folinic acid prevents the leukopenia and thrombocytopenia that may result from pyrimethamine therapy. Weekly complete blood counts and platelet counts are required to monitor toxicity from therapy. Alternative therapy with clindamycin (40 mg/kg/day in four divided doses [maximum, 2.4 g] for 6 weeks) rather than sulfadiazine also has been effective.[208] This therapy is less toxic than is triple therapy and, therefore, is better tolerated. In patients with severe intraocular inflammation, systemic corticosteroids can be used with concurrent antimicrobial therapy.

Preventive measures are twofold: first, avoidance of raw meat and cat feces during pregnancy, especially during the first trimester, and second, treatment of a mother known to have contracted the disease during pregnancy with spiramycin because it has no known teratogenic effects.[45,55,56] In utero pyrimethamine and sulfadiazine treatment of a fetus known to be infected may be effective.[45,47,102]

Lymphocytic Choriomeningitis Infection

LCMV, an arenavirus discovered in 1933, was recognized as a cause of intrauterine infection in 1955 (also see the earlier section "Viral Uveitis").[142] The common house mouse *Mus musculus* is the natural host and reservoir of the virus, but laboratory mice and pet hamsters also may be infected.[7,101] LCMV may be transmitted to humans by airborne means, rodent bites, or food contaminated by rodent urine, feces, or saliva; the fetus may be infected by transplacental transmission.[85] The diagnosis may be confirmed by LCMV titers.

Systemic manifestations, often devastating, include hydrocephaly, microcephaly, periventricular calcifications, neonatal meningitis, hepatosplenomegaly, cerebral palsy, mental retarda-

tion, and seizures.[85] Ocular findings include chorioretinitis, optic atrophy, nystagmus, microphthalmos, strabismus, and cataracts[142]; the chorioretinitis may mimic congenital toxoplasmosis.[28] The most common ocular findings are peripheral chorioretinal scars, although macular involvement is a common occurrence as well. At present there is no effective treatment. Prevention involves avoidance of mice and hamsters by pregnant women.[85,142]

Rubella Infection

The rubella virion is an RNA virus of the family *Togaviridae* that causes a febrile exanthem. Before the advent of rubella vaccine in 1969, rubella, or "German measles," epidemics occurred every 6 to 9 years. The last major outbreak in the United States took place in 1964.[11] With preschool immunization programs, most primary cases now reported occur in individuals between 15 and 24 years of age. Transmission occurs by inhalation of aerosolized droplets in the nasopharynx. Primary infection in adults results in a mild febrile illness associated with lymphadenopathy and a rash. The proportion of susceptible nonimmunized women of child-bearing age ranges from 10 to 25 percent. Fetal infection occurs transplacentally, and the likelihood of transmission from mother to fetus is highest (90%) in the first trimester; transmission at this stage can produce severe fetal damage and result in spontaneous abortion, stillbirth, or multiple congenital anomalies.

The classic congenital rubella syndrome, first described by Sir Norman McAlister in Australia in 1949,[142] is characterized by cardiac defects, ocular abnormalities, and hearing deficits. The incidence of ocular and cardiac defects is higher with exposure early in the first trimester; hearing deficits appear to be associated with exposure late in the first trimester. Givens and associates[84] reported that 88 percent had multiorgan involvement. Hearing loss is the most frequent nonocular manifestation.[142] The most common cardiac defects are patent ductus arteriosus, pulmonary artery stenosis, and pulmonary valve stenosis. Other features include microcephaly, thrombocytopenia, hepatosplenomegaly, and mental retardation.

Ocular involvement occurs in approximately 50 percent of infected infants.[81] Pigmentary retinopathy (i.e., "salt-and-pepper" retinopathy) affects 22 percent of infected infants. The retina has a mottled appearance, most frequently observed in the posterior pole; the optic nerve and vessels usually are normal unless the patient also has glaucoma.

Nuclear cataracts, which affect between 15 and 27 percent of patients, are the second most frequent ocular complication. Glaucoma is seen in approximately 10 percent of eyes; the combination of cataracts and glaucoma is an uncommon finding. Microphthalmos (which occurs in 10-63% of affected individuals), iris atrophy, and iritis also have been reported.[23] Live virus may persist for years in the lens, so appropriate precautions must be taken during cataract surgery to prevent transmission.[85]

The retinopathy associated with rubella usually is asymptomatic and does not necessitate treatment. In some cases, it can be complicated by subretinal neovascularization, which may require laser or macular surgery. Cataracts cause most visual morbidity. Visual rehabilitation depends on early cataract extraction to prevent deprivation amblyopia, correction of aphakia with spectacles or contact lenses, and careful follow-up. Glaucoma in infants and children can be temporized with topical medications, but most cases require glaucoma surgery.

Congenital rubella has long-term consequences for all organs involved. In nearly two thirds of infants with no manifestations at birth, hearing loss or psychomotor deficits subsequently develop. From an ophthalmic standpoint, individuals with congenital rubella need continued follow-up.[84]

Cytomegalovirus Infection

CMV is an enveloped DNA virus of the family *Herpesviridae*. An estimated 80 percent of adults are infected by the time that they reach 40 years of age.[196] In immunocompetent adults, infection is asymptomatic or can cause a mononucleosis syndrome. Individuals can shed virus in saliva, urine, and other body fluids for months to years after initial infection. Transmission occurs through exposure to body fluids, sexual contact, blood transfusion, or organ transplantation, or it can occur transplacentally. Primary infection acquired in the birth canal is not likely to result in serious disease.

Congenital CMV is the most common congenital infection in humans[197] and can result from exposure to the virus in utero or in the birth canal. In utero infection produces more serious disease. CMV infection occurs in 1 percent of all live births, and only 5 to 10 percent of infants with congenital CMV infection are symptomatic. Maternal infection may be primary or recurrent, but primary infection carries the greatest risk (30-40%) for transmission of symptomatic CMV disease to the newborn.[196] Blood transfusions from CMV antibody–positive donors also can result in severe CMV infection in the newborn. Systemic manifestations include intrauterine growth retardation (IUGR), thrombocytopenic purpura, microcephaly with periventricular calcifications, hepatosplenomegaly, pneumonia, and sensorineural deafness.[54]

In symptomatic congenital CMV infection, the retina is the primary site of ocular involvement. Cytomegalic inclusion bodies are seen in all layers of the retina. Patchy white areas of necrotic retina with hemorrhage and vascular sheathing are seen in the peripheral portion of the retina, although the posterior pole can be affected as well.[128] Nonhemorrhagic retinitis also may be seen, and most affected infants do not have active retinitis at birth, although scars may be visible as evidence of previous disease.[41] Resolution of the retinitis results in an atrophic scar with areas of hyperpigmentation. If retinitis involves the retinal periphery, vision may be normal. However, visual loss may occur if the posterior pole is involved or if optic atrophy or retinal detachment occurs. CMV retinopathy develops in 5 to 30 percent of infants with clinically apparent disease.[66] Microphthalmos, optic nerve hypoplasia, optic atrophy, optic nerve colobomata, anophthalmia, corneal opacities, and anterior segment dysgenesis also have been associated with congenital CMV infection.[103] In addition, cyclopia and anophthalmia have been reported.[33]

The diagnosis of CMV retinitis is based on the clinical appearance and the constellation of systemic symptoms and signs. Recovery of the virus from urine, maternal cervical secretions, saliva, or aqueous humor can confirm the diagnosis. Complement fixation can identify IgM antibodies to CMV that do not cross-react with other herpesviruses. Immunofluorescence techniques are more sensitive but less specific than is complement fixation.[128]

Treatment of neonatal or pediatric CMV retinitis is based on the results of treatment of adults with CMV retinitis. Ganciclovir has been shown to stabilize and prevent spread of the disease in infants. Maintenance therapy is not required, but continued follow-up is necessary to detect recurrence. Granulocytopenia and thrombocytopenia can result. Retinal detachment requires vitrectomy, membrane peel, and silicone oil to tamponade the detached retina.[104]

Herpes Simplex Virus Infection

HSV is a double-stranded enveloped DNA virus. Both subtypes, HSV-1 and HSV-2, can cause a vesicular skin eruption. HSV-1 typically is isolated from oral-facial infections; HSV-2 usually is isolated from genital infections. After primary infection occurs, HSV can maintain latency in neuronal ganglion cells and reactivate. Transmission occurs by exposure to infected body fluids

such as saliva, and the risk of transmission is higher when the individual is symptomatic.[152]

Maternal-fetal transmission occurs through infected genital secretions in the birth canal (HSV-2) or exposure to infected individuals with oral-facial herpetic disease (HSV-1) in the postnatal period. In mothers with active genital disease, the risk of transmission to the neonate is 50 percent with vaginal delivery. Most series report that between 70 and 80 percent of neonatal HSV infection is caused by HSV-2. Neonatal HSV infection is life-threatening, and the mortality rate without treatment is 80 percent.[227] The diagnosis is suspected by the clinical findings: lethargy, respiratory distress, anorexia, vomiting, cyanosis, low birth weight, IUGR, microcephaly, seizures, intracranial calcifications, pneumonia, hepatosplenomegaly, and skin vesicles. The virus may be isolated from skin vesicles or from nasal or conjunctival secretions, CSF, or blood. The mortality rate of untreated neonatal HSV is 49 percent.[85]

Ocular sequelae develop in less than 1 percent of immunocompetent adults with HSV infection. In contrast, 17 to 40 percent of infected neonates have ocular disease.[135,196] Thirteen percent of neonates with HSV have eye involvement. Ocular involvement can range from mild conjunctivitis to severe bilateral necrotizing retinitis and may be unilateral or bilateral. Conjunctivitis is the most common manifestation. Conjunctivitis, keratitis, and, occasionally, retinitis develop 2 to 14 days after birth. HSV retinitis causes punctate, white-yellow lesions in the periphery and the posterior pole accompanied by choroiditis, vascular sheathing, hemorrhage, and vitritis. Chorioretinal atrophic scars with variable amounts of pigmentation around the border result after resolution of acute infection.[106,173] Visual prognosis depends on the severity of disease, macular scarring, optic atrophy, or CNS involvement of the visual pathways. Severe chorioretinitis and cortical blindness are the usual sequelae of HSV infection acquired through transplantation, a rare mode of transmission.

El Azazi and colleagues[67] examined children with serologically proven HSV infection 1 to 15 years after neonatal exposure and found a higher prevalence of chorioretinal scars than noted in previous reports (28% versus 4%). This finding suggests that HSV remains dormant in the retina and reactivates later in childhood or adulthood. Chorioretinitis, cataracts, optic atrophy, and microphthalmos have been reported.[91] Acute retinal necrosis from reactivation of HSV-2 has occurred as well.[211]

Ocular HSV infection can be seen in conjunction with a vesicular rash or disseminated disease. The differential diagnosis of ophthalmic complications from HSV includes infection with any of the TORCHS organisms. Identification of neonatal IgM antibody to HSV confirms the diagnosis of in utero infection. Demonstration of intranuclear inclusions and multinucleated giant cells in skin, conjunctiva, and oral and genital lesions may be diagnostic.

The drug of choice to treat neonatal HSV infection is intravenous acyclovir, 30 to 60 mg/kg/day.[165] Conjunctival and corneal disease also can be treated by débridement and topical antivirals such as vidarabine. In neonatal HSV infection, systemic acyclovir should be given regardless of topical treatment. Early diagnosis and treatment can reduce ocular morbidity.

Varicella-Zoster Virus Infection

VZV is a DNA virus of the family *Herpesviridae*; enveloped virions are the infectious agents. Primary infection results in chickenpox, a highly communicable febrile illness with a vesicular rash that appears after 48 to 72 hours of incubation. VZV can remain dormant in sensory ganglion neurons and reactivate as herpes zoster, a painful rash in the dermatomal distribution of the sensory ganglion.

Congenital varicella syndrome is considered a rare entity. One prospective series reported a 24 percent incidence of congenital varicella with serologic or clinical confirmation of maternal infection during pregnancy. Mortality rates can be high if maternal infection develops 5 days before delivery to 2 days after delivery. Systemic complications of VZV infection include cranial nerve palsy, hemiparesis, cicatricial skin lesions, IUGR, developmental delay, seizures, neurogenic bladder, and learning difficulty.[122,162]

Ocular abnormalities in children with congenital VZV infection include chorioretinitis, cataracts, Horner syndrome, optic atrophy and optic nerve hypoplasia, retinal coloboma, and microphthalmos. The chorioretinal scars of VZV infection have a deeply pigmented center with depigmented borders or a gliotic white center with hyperpigmented edges. The neurotropic nature of VZV infection may explain the association of Horner syndrome. Ocular involvement can be unilateral or bilateral. VZV chorioretinitis affects the macula, periphery, or both.

Serologic testing for IgG and IgM antibodies to VZV, a history of maternal infection during pregnancy, and the constellation of systemic findings help establish the diagnosis. Because active infection may occur early in pregnancy, the neonate may have IgG but no detectable IgM antibodies to VZV by the time of delivery. The persistence of elevated IgG antibodies beyond 6 months, when passive immunity through maternal antibodies has waned, without evidence of primary VZV infection postnatally is a helpful indication of infection in utero.

Syphilis

Syphilis is caused by the spirochete *T. pallidum*. Acquired syphilis is a sexually transmitted chronic disease and has three stages of infection. Primary infection is characterized by painless, indurated chancres of the skin or mucous membranes at the site of inoculation. The secondary stage appears as a maculopapular rash, classically involving the palms and soles. Generalized lymphadenopathy, fever, malaise, sore throat, headache, and arthralgias can accompany the rash. Hypertrophic lesions (i.e., condyloma lata) occur in moist mucous membranes. Approximately a third of untreated cases progress to the tertiary stage, which occurs after a variable latent period that may have occasional recurrences of secondary syphilis. Transmission to the fetus can occur with any stage of maternal syphilis.[206]

Pigmentary retinopathy is the most common early ocular manifestation of congenital syphilis. Diffuse mottling in the periphery (i.e., salt-and-pepper retinopathy) is indicative of chorioretinitis in utero. Pigment clumping in the periphery usually has no effect on vision, but macular involvement can cause decreased vision. Retinal changes can appear later in adulthood, thus suggesting that inflammatory changes caused by congenital infection can occur after birth. Salt-and-pepper retinopathy is evidence of previous inflammation, and no treatment is required. Interstitial keratitis (see the section "Stromal Keratitis") is the hallmark of congenital syphilis and occurs in 75 percent of these patients.[206] It usually is not detected until late in the first decade of life. Other reported ocular manifestations include optic neuritis and iritis.

Testing the serum by nontreponemal and treponemal methods confirms the diagnosis of congenital infection. The American Academy of Pediatrics recommends physical examination, quantitative nontreponemal serologic testing, a CSF Venereal Disease Research Laboratory (VDRL) test, long bone radiographs, and antitreponemal IgM testing, as specified by the Centers for Disease Control and Prevention (CDC). The CDC states that congenital syphilis is presumptively diagnosed with a positive VDRL and at least one of the following: physical examination evidence of congenital syphilis, characteristic radiographic long bone findings, VDRL + CSF, an otherwise unexplained elevated CSF protein or cell count, quantitative nontreponemal serologic titer four times greater than the mother's, or a positive FTA-

ABS-19s-IgM test.[5] Treatment is with intravenous penicillin G for 10 to 14 days.[6,132]

ENDOPHTHALMITIS

Endophthalmitis is an infection within the eye that may arise endogenously during septicemia or exogenously from accidental or surgical trauma.[144,153] Sixty-two percent of cases occur after intraocular surgery, 20 percent after penetrating trauma, and 10 percent after glaucoma surgery; 8 percent result from metastatic infection.[60,169] The patient usually has severe ocular pain and visual loss at initial evaluation. Rarely, patients may be asymptomatic. Signs of endophthalmitis include conjunctival injection, vitritis, uveitis, hypopyon, and intraocular membrane formation.

Endophthalmitis is an ophthalmic emergency and necessitates immediate evaluation of aqueous and vitreous cultures and institution of intravitreal antibiotic therapy. Any significant delay in recognition and treatment of endophthalmitis can result in permanent vision loss.

Postsurgical endophthalmitis occurs in 0.086 percent of cataract operations.[2] The source of postsurgical infection may be from the eyelids, conjunctiva, contaminated instruments and irrigating solutions, or contamination by operating room personnel. The routine use of subconjunctival antibiotics after intraocular surgery does not prevent all cases of postoperative endophthalmitis. Vitreous loss, which may occur occasionally as a complication of cataract surgery, increases the risk of infection developing. Patients who have undergone glaucoma filtering surgery are particularly prone to endophthalmitis, primarily because sclerostomy is performed routinely as part of this procedure and the only remaining protection that the eye has from infective organisms is a thin layer of conjunctiva overlying the sclerostomy. Local antimetabolites, often used in conjunction with glaucoma filtering procedures, predispose the conjunctiva to bleb leaks that allow direct access of microorganisms to the eye.

Endogenous (metastatic) endophthalmitis should be suspected when ocular inflammation occurs in a septicemic patient, particularly if the patient is immunocompromised or has an underlying systemic illness such as diabetes or leukemia.[24,150,158]

Post-traumatic endophthalmitis should be considered in any patient with a history of injury and visual loss. A history of a high-velocity projectile presents great concern for a penetrating injury, and apparently minor accidental ocular trauma may result in perforation of the globe with or without a retained foreign body. The likelihood of post-traumatic endophthalmitis occurring increases directly with the extent of the injury and the degree of intraocular contamination.

After the diagnosis is suspected, immediate referral to an ophthalmologist is necessary. Once the patient has been examined, a vitreous and aqueous aspirate is obtained under general anesthesia and material is evaluated with Gram and Giemsa stains. Staining with calcofluor white or acridine orange is performed if fungal infection is a concern. The material is cultured on blood and chocolate agar, thioglycolate broth, and Sabouraud agar. Vancomycin (1 mg) to cover gram-positive organisms and ceftazidime (2.25 mg) to cover gram-negative organisms are injected into the vitreous cavity. If fungal infection is suspected, intravitreal amphotericin B is given. Occasionally, the ophthalmologist uses intravitreal dexamethasone to control associated, intense intraocular inflammation. Gram stain results often are inconsistent with cultures.

The role of vitrectomy in postoperative endophthalmitis has been addressed in the Endophthalmitis Vitrectomy Study (EVS).[69] In eyes with better than light perception vision at initial evaluation, outcome measurements were equal between the vitrectomy group and the vitreous tap or biopsy group. In eyes with light perception–only vision, the EVS found that patients who underwent immediate pars plana vitrectomy did significantly better than those who underwent vitreous tap or biopsy alone.

The most common infectious agents in endophthalmitis are *S. epidermidis*, *Bacillus* spp., *Streptococcus* spp., *S. aureus*, and various fungi. *Bacillus cereus* is isolated in 30 to 40 percent of cases and can cause severe ocular morbidity.[1,50,51,59,98] *S. epidermidis* is the predominant organism in postoperative cases, and *Streptococcus* spp. with filtering blebs and *B. cereus* are the organisms associated most frequently with penetrating trauma. Approximately 65 percent of cases are culture-positive.

Fungal endophthalmitis has become a relatively common form of endophthalmitis in childhood because of the prolonged hospital care required for severely ill immunocompromised children. The most common organism is *C. albicans*. Children with a central line catheter or receiving prolonged intravenous therapy of any type are particularly prone to infection. In patients with *Candida* endophthalmitis, the vitreous may be hazy, and small, white "snowball" localizations of infected material may appear in the vitreous or on the surface of the retina. Daily careful observation may be required during intravenous amphotericin B therapy. If the endophthalmitis clears, no ocular surgical intervention is indicated. If the infection is not adequately controlled with intravenous therapy, vitrectomy with injection of intravitreal amphotericin may be required.

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SECTION XII

Systemic Infectious Diseases

CHAPTER 69

Bacteremia and Septic Shock

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One of the most serious and potentially life-threatening infectious diseases in childhood is a bacteremic illness. Bacteremia may be caused by a wide variety of gram-positive or gram-negative microorganisms, and it may or may not be associated with a specific focus of infection, such as pneumonia or meningitis. Some bacteremias are transient and self-limited; they are not discussed in this chapter.

The incidence of bacteremia in children has been studied in hospital and ambulatory settings. In otherwise normal children, beyond the newborn age group, *Streptococcus pneumoniae*, *Escherichia coli*, *Staphylococcus aureus*, group A streptococcus, *Salmonella* spp., and *Neisseria meningitidis* are the most common microorganisms causing bacteremia.[99,234] Children with underlying illnesses that depress the host response to infection may develop bacteremia caused by these same microorganisms; however, in this population of children, especially when hospitalized, Enterobacteriaceae, *S. aureus*, coagulase-negative staphylococci, and fungi are the most important organisms commonly isolated from blood cultures.[6,170] Indwelling vascular lines, urinary catheters, endotracheal tubes, and other foreign material further predispose already compromised children to nosocomial infections. The incidence of diagnosed septicemia has increased over the years, partly owing to improved medical technology and the greater numbers of individuals with immunocompromising conditions who previously would not have survived.[160]

Gray and colleagues[91] reported that the incidence of bloodstream infections in a pediatric intensive care unit (ICU) during a 3-year period was 39 cases per 1000 admissions. Of the episodes, 64 percent were acquired in the ICU and 20.6 percent were community-acquired infections. Gram-positive and gram-negative organisms accounted for 62 percent and 31 percent of the isolates. Yeasts were isolated in 5.6 percent of episodes. Children with acquired immunodeficiency syndrome or severe immunosuppression caused by human immunodeficiency virus infection also are at increased risk for developing bacteremias caused by gram-negative bacilli, especially *Pseudomonas aeruginosa*.[189]

Using a seven-state hospital discharge database, Watson and associates[234] estimated that the U.S. age-adjusted and sex-adjusted annual incidence of severe sepsis was 0.56 cases per 1000 children, or more than 42,000 cases per year. The highest age-specific incidence occurred in infants (5.16 cases per 1000), with the incidence declining to 0.20 cases per 1000 for children 10 to 14 years old. Half of the children had underlying comorbidity, with neuromuscular, cardiovascular, and respiratory disorders being the most common.

One potential consequence of bacteremia is septic shock, a state characterized by inadequate tissue perfusion that is associated frequently with endotoxemia. Although most children with septic shock have infections caused by gram-negative enteric bacteria, *P. aeruginosa*, or *N. meningitidis*, organisms with endotoxin or lipopolysaccharide (LPS) within cell walls, septic shock also is associated with disease caused by gram-positive bacteria (especially *S. aureus*, *Streptococcus pyogenes*, and viridans streptococci), viruses, rickettsiae, and fungi. New clones of community-acquired methicillin-resistant *S. aureus* (MRSA) in particular have been associated with severe sepsis and septic shock in young children and adolescents.[87] In adults, the frequency of septic shock continues to increase as the population ages, new technology including more complicated surgery and immunosuppressive agents is developed, and antibiotic resistance grows.[194]

Dupont and Spink[64] reviewed the cases of 172 children, 30 days to 16 years of age, who were hospitalized at the University of Minnesota Medical Center with gram-negative bacteremia. Shock occurred in 25 percent of the children, and 98 percent of children with shock died. In meningococcal infections, 11 to 40 percent of children develop hypotension.[56,116,228] During a 10-month study period, Naqvi and colleagues[157] reported that shock occurred in 5 of 39 (13%) episodes of gram-negative bacillary sepsis, with three deaths. Jacobs and associates[107] reviewed the admissions of previously normal children to a pediatric ICU in a large children's hospital for a 30-month period. Hypotension or evidence of peripheral hypoperfusion occurred in 143 children with confirmed bacterial sepsis, mostly *Haemophilus influenzae* type b (Hib), or apparent meningococcemia. Among 1058 consecutive admissions of 916 children to a pediatric ICU in Canada from July 1, 1991, to July 31, 1992, 25 episodes (2%) of septic shock occurred.[178] During a 12-month period, 140 episodes of septicemia (135 bacterial and 5 fungal) were documented in 100 pediatric hematology-oncology patients.[5] Septic shock occurred in 19 percent.

The organisms and case-fatality rates in the study by Watson and colleagues[234] are outlined in Table 69–1. *N. meningitidis* and fungi were associated with the highest mortality rates. Early-onset group B streptococcal infections in neonates and overwhelming *S. pneumoniae* infections in children with splenic dysfunction or asplenia are associated with shock in a high percentage of cases. *S. aureus* or group A streptococcus may cause hypotension in a child with or without other manifestations of toxic shock syndrome.[202]

Advances in understanding of the pathogenesis and pathophysiology of septic shock with respect to the host response to infection have required that more precise clinical definitions of sepsis and expanded syndromes be developed. Much of the impetus for this effort is related to the ability to identify more readily patients with infections who may benefit from administration of newer (expensive) adjunctive measures. An American College of Chest Physicians and Society of Critical Care Medicine Consensus Conference in 1991 developed new terminology to define sepsis and its sequelae.[28] This terminology has been modified for use in children by an international consensus panel of 20 experts in sepsis and clinical research (Table 69–2).[84]

TABLE 69–1 Occurrence and Case-Fatality Rates of Selected Pathogens among Children with Severe Sepsis Based on Age*

	<1 Year (*N* = 4643)		1-10 Years (*N* = 2724)		11-19 Years (*N* = 2308)	
Organism	***Cases (%)***	***Case-Fatality (%)***	***Cases (%)***	***Case-Fatality (%)***	***Cases (%)***	***Case-Fatality (%)***
Neisseria meningitidis	0.3	20	8	10.4	2.3	15.1
Haemophilus influenzae	1.6	4.2	2.4	1.6	1.9	6.8
Pseudomonas	3.6	14.6	7.7	12.4	6.9	9.4
Staphylococcus aureus	2.3	5.7	2.9	0	3.5	3.8
Group A streptococcus	0.3	0	0.7	5	0.2	0
Group B streptococcus	3.1	7.6	0.1	50	0.8	5.6
Fungus	10	10.8	13.3	16.8	10.4	11.6

**Represents data from a seven-state hospital discharge database in 1995.*
Modified from Watson, R. S., Carcillo, J. A., Linde-Zwirble, W. T., et al.: The epidemiology of severe sepsis in children in the United States. Am. J. Respir. Care Med. 167:695-701, *2003.*

TABLE 69–2 Consensus Definitions of Systemic Inflammatory Response Syndrome (SIRS), Infection, Sepsis, Severe Sepsis, and Septic Shock

SIRS

The presence of ≥2 of the following 4 criteria, one of which must be abnormal temperature or white blood cell count:
- Core temperature (rectal, bladder, oral, or central catheter probe) >38.5°C or <36°C
- Tachycardia defined as >2 standard deviations above normal for age in the absence of external factors or drugs; *or* otherwise unexplained persistent elevation of a 0.5- to 4-hr time period; *or* for children <1 yr old, bradycardia defined as a mean heart rate <10th percentile for age in the absence of external factors or drugs or otherwise unexplained persistent depression over a 0.5-hr period
- Mean respiratory rate >2 standard deviations for age *or* mechanical ventilation for an acute process not related to an underlying neuromuscular disease or to general anesthesia
- Peripheral white blood cell count elevated or depressed for age unrelated to medications or >10% immature neutrophils

Infection

A suspected or proven (by culture, tissue stain, polymerase chain reaction assay) infection caused by any pathogen *or* a clinical syndrome associated with a high probability of infection

Sepsis

SIRS in the presence of or caused by suspected or proven infection

Severe Sepsis

Sepsis plus 1 of the following: cardiovascular organ dysfunction, acute respiratory distress syndrome *or* ≥2 other organ dysfunction as defined in the consensus statement

Septic Shock

Sepsis and cardiovascular organ dysfunction as defined in the consensus statement

Modified from Goldstein, B., Giroir, B., Randoph, A.; and the Members of the International Consensus Conference on Pediatric Sepsis: International pediatric sepsis conference: Definitions for sepsis and organ dysfunction in pediatrics. Pediatr. Crit. Care Med. 6:2-8, *2005.*

PATHOPHYSIOLOGY

The pathophysiology of bacteremia is highly variable and depends on the specific microorganism isolated, the immune status of the host, and other factors such as the locations of indwelling catheters. Highly encapsulated organisms, such as *S. pneumoniae*, *N. meningitidis*, and Hib, normally may reside in the nasopharynx and, for reasons that are poorly understood, are capable of invading beyond mucosal barriers into the bloodstream. A preceding viral upper respiratory tract infection may play some role in alterations in local host defense mechanisms that result in bacteremia.[117,143]

Using human columnar nasopharyngeal tissue in organ cultures, Stephens and colleagues[208] showed that *N. meningitidis* organisms were ingested by the columnar cells, then found within phagocytic vacuoles, and later observed within subepithelial tissues, suggesting that the meningococci had penetrated the epithelial layer. In this same model, Hib organisms attach to nonciliated columnar epithelial cells and subsequently are found in the intercellular spaces in association with a preceding disruption of the tight junctions of epithelial cells.[66] After passing the mucosal barriers, Hib may enter the bloodstream directly through pharyngeal blood vessels.[192] Pneumococci adhere to specific ligands on respiratory cells. The inflammatory mediators generated during viral infections up-regulate platelet-activating factor receptor on respiratory cells to which the pneumococci adhere more avidly and subsequently invade.[223] Pili or adhesins of gram-negative enteric organisms seem to be important in attachment and adherence of these microorganisms to specific receptors expressed on epithelial surfaces. Pili also have been shown to be important in the pathogenesis of some gram-positive infections, such as *S. pyogenes*, group B streptococcus, and *S. pneumoniae*.[18,152]

The placement of an endotracheal tube unmasks a greater number of these receptors, presumably through increased protease activity of secretions and decreased cell-bound fibronectin, and leads to colonization of the upper respiratory tract with gram-negative organisms, which are ubiquitous in the environment of an ICU.[244] Altered host defense mechanisms allow these organisms to move beyond epithelial surfaces and cause bacteremia.

The gastrointestinal and genitourinary tracts are major sources of gram-negative organisms responsible for bacteremia. These organisms first may cause localized abscesses or peritonitis if intestinal perforation occurs, or they may translocate the intestinal mucosa, particularly when the mucosa is affected by antineoplastic agents. Viridans streptococci can cause bacteremia in a neutropenic patient with severe mucositis that develops after the patient undergoes chemotherapy.[204] Microorganisms within the bladder may ascend the genitourinary tract and presumably enter the bloodstream through the kidneys. *S. aureus* and *S. pyogenes* are common inhabitants of the skin and skin structures. Any skin wound or foreign matter within the skin tissue renders the skin more susceptible to bacterial invasion. Staphylococci have a unique capability of adhering to solid surfaces, such as catheters, which may be an important prerequisite to colonization and subsequent catheter-related bacteremia.[173]

The pathophysiology of septic shock is very complex. Septic shock associated with gram-negative organisms has been studied most extensively, especially with respect to endotoxin or bacterial LPS, which has multiple biologic effects. Bacterial LPS has three basic components, as follows:

1. Terminal side chains consist of repeating oligosaccharides that differ from strain to strain and are responsible for the antigenic specificity of the O antigens.

2. A core LPS also consists of oligosaccharides but has less diversity in structure among strains than do the terminal side chains.

3. Lipid A is very similar among the different strains and is responsible for most of the biologic activity of endotoxin.

Endotoxin shock has been the subject of intensive animal research, and much of what is known about the pathogenesis of endotoxin shock has been derived from animal models. Although septic shock in humans is not simulated precisely in these animal models because the animals do not have underlying host defense defects, much of what has been learned about endotoxin shock in animals has been corroborated in the human host.

ENDOTOXIN SHOCK IN ANIMALS

Most animal models of endotoxin shock employ infusions of live gram-negative bacteria, usually *Escherichia coli*, or purified endotoxin, after which observations are made. The effects of purified endotoxin depend partly on the species of animal being studied. The effects of endotoxin in animal models are summarized in Table 69–3.

Numerous mediators induced by endotoxin play pivotal roles in the pathogenesis of endotoxin shock. Tumor necrosis factor (TNF) or cachectin, a polypeptide hormone, is a key cytokine mediating septic shock.[219] The tissue injury induced by TNF largely is a result of other mediators that are induced by TNF, including interleukin-1β (IL-1β), IL-6, eicosanoids, and platelet-activating factor.[62,103,217,220] TNF is synthesized by a wide variety of cells (including monocytes/macrophages, natural killer cells, microglial cells, hepatic Kupffer cells) after stimulation by LPS, C5a, viruses, and enterotoxins, among other agents. TNF initiates a cascade of events that leads to endothelial cell injury, an enhanced inflammatory response, and, ultimately, the characteristic findings of endotoxic shock.

Nitric oxide (i.e., endothelium-derived relaxing factor) is the final pathway by which endogenous vasodilators stimulated by endotoxin result in hypotension from altered control of microcirculation. LPS through the release of cytokines induces a form of the enzyme nitric oxide synthase II, which leads to increased production of nitric oxide.[150] Inhibitors of nitric oxide synthase, such as N^G-monomethyl-l-arginine, can reverse or prevent hypotension in animals challenged with LPS.[142]

The pathophysiology of septic shock caused by gram-positive bacteria is similar to that described for gram-negative organisms.[206] Cell wall components, such as peptidoglycan and teichoic acid, promote proinflammatory activity, but are less potent than endotoxin.

TABLE 69–3 Endotoxin Shock in Animal Models

Effects	Mediators
Cardiovascular Effects	
Decreased peripheral vascular resistance[158]	Histamine, bradykinin,[145] serotonin, complement activation, prostaglandins, anaphylatoxins
Decreased cardiac output[8,13,180]	
Depressed myocardial function[140]	
Decreased systemic blood pressure[48]	
Metabolic Effects[180]	
Hyperglycemia[54]	Hypoinsulinemia
Hypoglycemia[70]	
Increased adrenocorticotropic hormone, growth hormone, and antidiuretic hormone[241]	
Decreased calcium[213]	
Increased triglycerides[97]	
Decreased iron, transferrin, and zinc	
Pulmonary Effects	
Congestive atelectasis[29]	Polymorphonuclear leukocytes
Increased capillary permeability[32,101]	Polymorphonuclear leukocytes
Vasoconstriction	Thromboxane, prostacyclin
Bronchoconstriction[23]	Leukotrienes
Central Nervous System Effects	
Decreased regional and total cerebral blood flow[158]	
Increased cerebral oxygen consumption[179]	

ENDOTOXIN SHOCK IN HUMANS

The pathophysiology of septic shock is highly complex and is related predominantly to actions of endogenous mediators released as part of the systemic inflammatory response to an infection. The cascade of events is intertwining, with production of one cytokine stimulating the synthesis of others; synergistic, in that the activities of certain cytokines act in concert; and sometimes antagonistic, with the production of other molecules to inhibit or compete with various cytokines. This complicated response to an infectious stimulus has been studied best for LPS, but a similar series of events occurs in response to gram-positive infections. Although the best understood system is the one that recognizes bacterial LPS, others exist for sensing the presence of bacterial peptidoglycan, DNA, lipopeptides, flagella, viral double-stranded RNA, and other conserved microbial molecules.

The first host protein involved in the recognition of LPS is LPS-binding protein. LPS-binding protein is an acute-phase protein; its role is to bring LPS to the cell surface by binding to LPS and forming a ternary complex with the LPS receptor molecule, CD14.[235,245] Formation of the complex between LPS and CD14 facilitates the transfer of LPS to the LPS receptor complex, which is composed of toll-like receptor 4 (TLR4) and MD2. Studies over the course of several years led to the discovery of the TLR4/MD2 receptor complex as the signaling entity for LPS (Fig. 69–1).[23,24] MD2 is a secreted glycoprotein that functions as an indispensable extracellular adapter molecule for LPS-initiated signaling events, perhaps by aiding in ligand recognition. The resulting signal promotes mononuclear phagocytes to produce reactive oxygen molecules, cytokines, and arachidonic acid metabolites, including prostaglandin and leukotrienes. A counterregulatory protein is a bactericidal, permeability-increasing protein that is stored in the granules of polymorphonuclear leukocytes and inhibits the effects of LPS.[77]

TNF is largely responsible for the biologic effects, including fever, shock, myocardial suppression, capillary leak (i.e., endothelial damage), coagulation alterations, and metabolic changes,[39,40,144,177,218] of LPS in humans. In children, including neonates, the role of cytokines in sepsis caused by a variety of organisms, but especially *N. meningitidis*, is well-documented.[34,56,81,213,228] LPS and TNF each can induce the synthesis of other proinflammatory cytokines, such as IL-1β and IL-6.[63] IL-6 levels in plasma correlate with mortality. IL-8 plasma concentrations also are increased after infusion of LPS or IL-1β.[63,93]

The anti-inflammatory cytokine IL-10 is produced after LPS is injected and inhibits the production of TNF, IL-1β, and IL-6.[138] Naturally occurring inhibitors of TNF or IL-1β are present in serum samples of patients with the sepsis syndrome.[59,60,83] IL-1 receptor antagonist (IL-1a) binds competitively to the IL-1

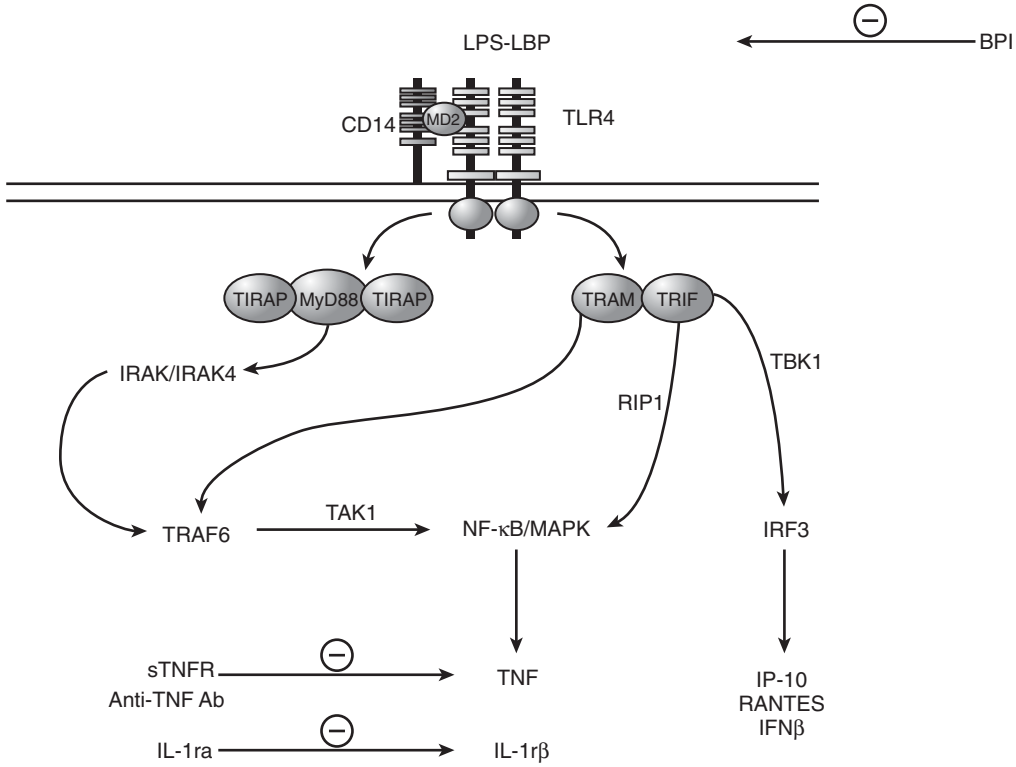

Figure 69–1 Activation of the cytokine network by lipopolysaccharide (LPS). Circulating lipopolysaccharide-binding protein (LBP) recognizes LPS in the plasma and brings it to CD14. This aids the loading of LPS onto the LPS receptor complex, which is composed of dimerized toll-like receptor 4 (TLR4) receptors and two molecules of the extracellular adapter MD-2. Signals activated by TLR4 can be subdivided into signals dependent on MyD88 (and Mal), which occur early (represented by the events illustrated on the left-hand side of the diagram), and signals independent of MyD88, which occur later and use the adapters TRIF and TRAM (depicted on the right). IL-1ra, interleukin-1 receptor antagonist; sTNFr, soluble tumor necrosis factor receptor.

receptor to block the action of IL-1. Soluble TNF receptors bind to circulating TNF, which prevents its proinflammatory actions (see Fig. 69–1).

In humans, gram-negative bacteremia is followed by a decrease in systemic vascular resistance and mean blood pressure and an increase in cardiac output.[24,237] Decreased systemic vascular resistance may be accompanied by activation of the complement and kinin systems.[145] After this early phase, the blood pressure decreases further without change in the central venous pressure. Certain patients, especially children, are able to maintain their cardiac output and cardiac index, and this ability may be associated with increased rates of survival. When peripheral resistance is measured within 12 to 24 hours of the onset of shock, its decrease is significant in patients who survive compared with patients who die.[162] In contrast, cardiac output is reduced significantly in other patients; this decrease is associated with increased concentration of blood lactate, decreased arterial blood pH, and decreased rates of survival.

Depression of myocardial function has been shown in adult and pediatric patients in septic shock.[69] These patients have a reduced ejection fraction, left ventricular dilation, and significantly altered ventricular performance in response to infusion of volume.[164] This depression of myocardial function is transient in survivors, however, reverting to normal within 1 to 4 days.[167] Parker and colleagues[168] found that patients who did not survive septic shock did not have left ventricular dilation or reduction in the ejection fraction. When the systemic vascular resistance index was averaged over the course of time, nonsurvivors had a significantly ($p < 0.05$) lower index than that of survivors of septic shock. This study included three children who were 9 to 17 years of age. Abraham and associates[1] sequentially monitored hemodynamic and oxygen transport measurements in 33 patients with septic shock. In the 24-hour period before the onset of hypotension, the survivors showed significantly greater cardiac index, left cardiac work index, oxygen delivery, and oxygen consumption than the nonsurvivors.

A circulating myocardial depressant factor in patients with septic shock was proposed more than 50 years ago, but myocardial dysfunction was not quantitatively linked to a serum factor until the late 1980s.[140,169,181] Kumar and colleagues[121] later reported that the myocardial depressant activity of sera obtained from patients with septic shock could be eliminated by the immunoprecipitation of TNF and IL-1β. Several investigators also have shown that TNF and IL-1β synergistically depress myocardial function in vitro, and that this effect can be abolished by an inhibitor of nitric oxide.[38,166] Germane to this discussion is the observation that LPS-induced biosynthesis of TNF mRNA and protein is not strictly confined to peripheral mononuclear cells but also may occur within many different tissue compartments. Experimental studies have shown that the cardiac compartment can be a significant source of TNF during septic shock. Kapadia and colleagues[111] showed that administration of LPS leads to intramyocardial production of TNF mRNA and protein in vivo.

These observations raise the possibility that the myocardial depression occurring in sepsis may develop directly in response to the compartmentalized production of TNF and other cytokines within the heart, as opposed to systemic production of these mediators by circulating mononuclear cells. TLR4 is expressed in the heart, and investigators have suggested that it is involved in signaling the cytokine production induced by LPS within the heart.[20]

In children, the most comprehensive investigation of myocardial dysfunction has been in meningococcal septic shock. Thiru and coworkers[214] measured serum concentrations of the cardiac muscle–specific protein cardiac troponin I, which is released from injured cardiac myocytes, in 101 children with meningococcal septicemia. Minimum left ventricular ejection fraction was inversely related to peak cardiac troponin I levels. The degree of myocardial dysfunction, as determined by inotrope measurement, was related directly to peak cardiac troponin I concentrations. Their results suggested that myocardial cell death might contribute, at least in part, to the cardiac dysfunction associated with meningococcal septic shock.

Hematologic changes, such as leukocytosis, leukopenia, and thrombocytopenia, have been observed in human volunteers after receiving an infusion of endotoxin. Thrombocytopenia commonly occurs in association with sepsis of any cause.[50] Septic shock is one of the most common causes of disseminated intravascular coagulation in children. Hageman factor (i.e., factor XII), which initiates the coagulation cascade, can be activated directly by LPS or through endothelial damage induced by bacteria.[134] In septic shock, concentrations of Hageman factor, prekallikrein, high-molecular-weight kininogen, and factor VII are decreased partly as a result of consumption.[53,110] Similarly, levels of inactivators of clotting factors, such as C1 esterase inhibitor, α_2-macroglobulin, and antithrombin III, also are diminished. Corrigan and Jordan[53] diagnosed disseminated intravascular coagulation in 24 of 26 children with septic shock and found that improvement in coagulation parameters seemed to be related most to restoration of blood pressure. Gram-negative bacteremia may be associated with a coagulopathy that is not disseminated intravascular coagulation but is characterized by prolongation of the prothrombin and partial thromboplastin times caused by a reduction in the vitamin K–dependent coagulation factors.[52]

LPS or cell wall components of gram-positive organisms, through cytokine production, activate blood coagulation predominantly through the extrinsic pathway. The procoagulant state is enhanced further by decreased protein C activity, which is an important inhibitor of coagulation factors Va and VIIIa. The fibrinolytic system also is altered by endotoxemia and is mediated by plasminogen activator inhibitor 1–induced suppression. The coagulopathy associated with septic shock is characterized by a procoagulant state and inhibition of fibrinolysis.[125,130] Protein C has anti-inflammatory properties. The antithrombotic, profibrinolytic, and anti-inflammatory actions of activated protein C counteract the effects of cytokine activation, but a deficiency of protein C may be acquired during severe sepsis. Low levels of protein C have been associated with increased morbidity and mortality rates in patients with severe sepsis and septic shock.[73] For meningococcal disease in particular, dysfunction of the protein C activation pathway is a key factor in the development of the thrombosis associated with purpura fulminans. Down-regulation of the endothelial thrombomodulin–endothelial protein C receptor pathway seems to be the mechanism for impaired activation of protein C during severe meningococcal sepsis.[67]

LPS can activate the complement cascade by the classic or the alternate pathway. Significantly depressed concentrations of C3 occur in patients with bacteremia and hypotension compared with normal individuals or with patients with uncomplicated bacteremia, and C3 is activated primarily by the alternate pathway.[68,110,136] In patients with bacteremia and hypotension, C1, C4, and C2 levels were not depressed significantly from values found in normal controls or in normotensive patients with bacteremia. In contrast, C3, C5, C6, C9, properdin, and factor B levels were decreased significantly ($p < 0.05$) in bacteremic patients with shock. In children with meningococcal disease, Tubbs[222] found a mean C3 concentration (as a percentage of normal values) of 132 ± 21 percent for survivors versus 91 ± 21 percent for nonsurvivors. The C3 levels did not correlate with endotoxin levels in sera.

Many metabolic alterations have been documented in the human host during endotoxin shock. Hyperglycemia followed by hypoglycemia can complicate the shock state induced by sepsis.[70,146] Whole-body use of glucose is increased during sepsis, which probably is cytokine-mediated.[147] Glycolysis and gluconeogenesis are increased, but insulin resistance occurs in skeletal muscle. Children with underlying liver disease or with reduced glycogen stores are most likely to develop hypoglycemia during septic shock. Lactic acidosis develops as a result of poor tissue perfusion and cellular hypoxia. Lactic acid concentrations are increased in nonsurvivors and patients with poor or low-flow cardiac output during sepsis.

In clinical studies, Clowes and associates[47] identified a subgroup of patients with low-flow septic shock in whom concentrations of serum insulin were lower than concentrations in a control population. In children with meningococcal sepsis, van Waardenburg and colleagues[225] found higher blood glucose concentrations and significantly lower insulin levels on day 2 or 3 of hospitalization in children with shock compared with children without shock. Levels of plasma insulin and soluble TNF receptor 75 were inversely correlated in these children. Their findings were consistent with the inflammatory response inhibiting insulin secretion.

Hypocalcemia and decreased serum ionized calcium concentrations occur frequently during bacterial sepsis. In one study, 12 (20%) of 60 critically ill adults with bacterial sepsis had hypocalcemia.[247] The mortality rate in the hypocalcemic patients was 50 percent compared with 30 percent in the patients who were normocalcemic. Cardenas-Rivero and associates[42] studied calcium homeostasis in 145 children admitted to an ICU. Of eight children with confirmed sepsis or meningitis (or both) not caused by Hib, seven had hypocalcemia, and six of seven had ionized hypocalcemia. Five of the six children with ionized hypocalcemia had inappropriately normal concentrations of parathyroid hormone, which suggests that transient hypoparathyroidism occurs in some children with sepsis. Hypocalcemia also occurs commonly in patients with toxic shock syndrome.[240] In women with toxic shock syndrome and hypocalcemia, serum concentrations of calcitonin are elevated by unknown mechanisms.[45] Hypocalcemia and elevated calcitonin concentrations also have been documented in children with fulminant meningococcemia.[131]

Procalcitonin levels are elevated in several conditions associated with systemic inflammatory response syndrome, including sepsis, and have been proposed as adjunctive laboratory tests for the early detection of, and indicators for prognosis of, meningococcal disease.[43,96,122] These changes in calcium levels are especially critical because the level of ionized calcium and cardiac output in septic shock can be correlated.[243] Other metabolic changes may occur during septic shock in humans, as follows:

1. Increased concentrations of cortisol and growth hormone (including in neonates[215])[229]
2. Depression of triiodothyronine and thyroxine levels related to poor nutrition[186]
3. Elevations in total concentrations of amino acid in plasma and the preferential use of branched-chain amino acids as an energy source for skeletal muscle[147]
4. Muscle proteolysis, possibly induced by one or more circulating agents in the plasma of patients with serious infections[46]
5. Elevations in concentrations of plasma thromboxane, which are observed in nonsurvivors of septic shock[183]
6. Elevations in concentrations of triglycerides and free fatty acid during gram-negative bacteremia[78,118]

Liver dysfunction is an important aspect of endotoxin shock in adults. Banks and colleagues[16] found that clinical jaundice was apparent in 63 percent of their patients with septic shock, that it was found more commonly in nonsurvivors than in survivors, and that the degree of biochemical liver abnormalities was related to the duration of shock. Postmortem findings included focal liver necrosis, Kupffer cell hyperplasia, portal tract inflammation, venous congestion, and intrahepatic cholestasis.

Adult respiratory distress syndrome (ARDS), or shock lung, is a major complication of septic shock in children.[104,175] The lungs of children with ARDS have characteristic changes consisting of increased lung weight reflecting congestion and atelectasis, alveoli lined with hyaline membranes, microthrombi, hemorrhage, and interstitial edema.[104] Increased capillary permeability and intrapulmonary shunts have been documented in patients with ARDS.[4,55] C5a, a potent chemotactic factor, causes aggregation of polymorphonuclear neutrophils, is elevated in the sera of patients who ultimately develop ARDS, and is found in increased concentrations in bronchoalveolar lavage fluid obtained from patients with ARDS.[94,187] Leukocyte aggregates are thought to be trapped in lung tissue and may cause damage to the endothelium of the pulmonary microvasculature through the release of oxygen radicals, lysosomal enzymes, and products of arachidonic acid metabolism. Although neutrophils play a critical role in the pathogenesis of ARDS, other factors also are important, considering that ARDS can develop in patients who are neutropenic.[163,238] Thromboxane, platelet-activating factor, fibrin, and other substances contribute to the lung injury in ARDS.[190]

The effects of endotoxin shock on the central nervous system have not been studied carefully in humans.[90] The encephalopathy associated with sepsis seems to be caused partly by altered phenylalanine metabolism; concentrations of phenylalanine and its metabolite, phenylacetic acid, are increased in the sera and cerebrospinal fluid of septic adults who are stuporous or comatose.[148]

Endotoxin has been implicated in the pathogenesis of acute renal failure associated with sepsis. Wardle[231,232] showed that 12 of 16 patients with acute tubular necrosis had endotoxemia. Renal arterial blood flow and renal vascular resistance are decreased significantly in baboons 2 to 4 hours after infusion of endotoxin. Inadequate perfusion pressure was associated with renal ischemia and negligible urine output in these animals shortly after administration of endotoxin.[197] Pathologic examination of the kidneys revealed focal necrosis of the proximal tubular epithelium, eosinophilic casts within proximal and distal tubules, and microthrombi in the glomerular capillaries. Endothelin, a potent vasoconstrictor peptide produced by endothelial cells, is elevated in concentration in the plasma of patients with septic shock. Because endothelin contributes to the regulation of regional blood flow, elevated levels suggest that it may relate to renal vasoconstriction and dysfunction.[227]

Endotoxin can be measured in the plasma of patients with gram-negative bacteremia. The presence of circulating endotoxin does not mean that bacteremia is present or ever has occurred because endotoxin presumably may be "absorbed or leak" into the circulation from the gastrointestinal tract.[126,211,221] Endotoxemia may be a valid indicator, however, of impending gram-negative septicemia in febrile patients.[61] Preformed antibody to LPS or lipid A is associated with protection against shock and death caused by gram-negative bacteremia in adults. McCartney and colleagues[138] detected endotoxin in the blood (after chloroform extraction) of patients with gram-negative septic shock; all 18 patients with persistently positive endotoxin assays died. In contrast, nine patients who initially had endotoxemia but subsequently had negative assays survived. Other studies confirm the association between endotoxemia and outcome.[123] Evidence exists that human endotoxin is cleared from the circulation by the liver and can be detoxified by neutrophil enzymes (i.e., acyloxacyl hydrolases).[135,153]

The sequence of events in the evolution of endotoxin shock has been outlined by several investigators.[159] Bacteria, endotoxin, or other bacterial products stimulate the production of TNF and other cytokines, which in concert with endotoxin set off a whole series of events. Potent mediators, including C3a, C5a, eicosanoids, platelet-activating factor, histamine, and myocardial depressant substance, are released. Potent vasodilators cause peripheral vasodilation, and decreased systemic peripheral resistance leads to pooling of blood and decreased venous return to the heart. Mean blood pressure may be low, or it may be normal if cardiac output increases sufficiently to compensate for these alterations despite depression of ventricular function. The central venous pressure, which partially depends on myocardial performance, may be low or in the normal range.

If intravascular volume is increased by the administration of sufficient fluids, shock may be prevented or corrected. Continued hypotension and diminished perfusion pressure may lead, however, to cellular hypoxia and increased production of lactic acid from pyruvate. The microcirculation is altered by local tissue acidosis. Capillary beds become congested, and intravascular fluid may leak into the interstitial spaces. Increased secretion of catecholamine leads to arteriolar and venular constriction and increased peripheral resistance. Pooling of blood is enhanced, which leads to a further diminution in venous return and a reduction of cardiac output. Oliguria, coagulation abnormalities, and additional metabolic alterations indicate multiple organ system failure and presage the death of the patient.

CLINICAL PRESENTATION AND DIAGNOSIS

The signs and symptoms of bacteremia vary, greatly depending on the age and underlying disease of the patient, the duration of illness, and the specific microorganisms. Young, otherwise healthy children aged 3 months to 3 years may present with fever and evidence of an upper or lower respiratory tract infection or no focus of infection and yet have unsuspected bacteremia. Most studies indicated that the risk of developing bacteremia increases as the body temperature increases and that after the temperature exceeds 41°C, almost 25 percent of these children may be bacteremic.[19] In a previously healthy child, the persistence of irritability and the inability to console the child despite optimal environmental conditions have been proposed as key points in the physical examination that should alert the clinician to the possibility of a serious infection, such as bacteremia or meningitis.[137,203]

Underlying illnesses with splenic dysfunction place a child at increased risk for acquisition of infections caused by encapsulated organisms, whereas children with leukemia or other immunosuppressive diseases or children in the ICU are more likely to be infected with gram-negative bacilli or *S. aureus.* A history of diarrhea may suggest *Salmonella* spp. as a possible cause of illness. Preceding skin infections or wounds are important clues to infection caused by *S. aureus* or group A streptococcus. An indwelling vascular catheter may precipitate overlying erythema in a patient with evidence of phlebitis proximally. Gram-positive cocci and gram-negative bacilli can be associated with catheter-related sepsis.[199] Toxic shock syndrome should be considered in a hypotensive girl or woman with a recent menstrual period and history of tampon use, although toxic shock also is associated with *S. aureus* sepsis in males and nonmenstruating females.[87,202] Evidence of osteomyelitis, with or without venous thrombosis, is a very common finding in staphylococcal sepsis.[88] Intra-abdominal sources of infection increase the likelihood of developing anaerobic bacteremia.

Petechiae may be associated with many microorganisms, especially invasive disease caused by *N. meningitidis*.[161] Purpura is an ominous finding and frequently is associated with overwhelming infection caused by *N. meningitidis*, *S. pneumoniae*, and Hib. *P. aeruginosa* is associated specifically with erythema gangrenosum. Almost half of children presenting with erythroderma (diffuse erythema) either had shock at presentation or developed shock in one study.[37] Other skin and soft tissue manifestations of gram-negative sepsis include bullous lesions, cellulitis, fasciitis, thrombophlebitis, and symmetrical peripheral gangrene with disseminated intravascular coagulation.[154] Signs of meningeal irritation or increased intracranial pressure are important because they may modify the approach to management of fluids in a child in shock.

The onset of bacteremia may be heralded by chills, fever, nausea, vomiting, diarrhea, rashes, and petechiae. Initially, the skin feels warm and appears flushed. A change or impairment in mental status may be the first clue to the presence of shock. Hyperventilation also may develop before the onset of clinical shock occurs, which can alert the physician to impending circulatory insufficiency.[25] In time, cold, clammy extremities; a weak pulse; tachycardia; tachypnea; hypotension; and oliguria may occur. The skin over the extremities, the tip of the nose, and the earlobes especially are prone to cyanosis. Auscultation of the lungs may reveal rales, indicating pneumonia or pulmonary edema. Abnormal distention or tenderness to palpation and guarding may be evidence of peritonitis. Costovertebral angle tenderness suggests acute pyelonephritis as a source of bacteremias.

The physician must distinguish among the following three main types of shock in children:

1. *Hypovolemic shock*, such as occurs with blood loss, fluid and electrolyte loss, adrenal insufficiency, or other causes
2. *Cardiogenic shock*, which is associated with drug intoxication, cardiac surgery, arrhythmias, and pericardial tamponade, among other causes
3. *Distributive shock*, which indicates abnormal distribution of blood flow leading to inadequate tissue perfusion (e.g., septic shock, anaphylaxis)

The laboratory evaluation of a child with bacteremia, septic shock, or both conditions should provide information concerning the cause and the data required for optimal supportive management. Several studies showed that a total white blood cell (WBC) count exceeding 15,000 cells/mm^3 in a 3- to 36-month-old child with a temperature greater than 39°C to 40°C and without a focus of infection is an indication that the child is at increased risk for having bacteremia, especially pneumococcal bacteremia.[17] Since the introduction of the pneumococcal conjugate vaccine, pneumococcal bacteremia in this scenario has been greatly reduced.[99,196,210] An erythrocyte sedimentation rate greater than 30 mm/hr and C-reactive protein levels also have been suggested as useful screening tools for detecting serious bacterial infections. A low peripheral WBC count also may suggest septicemia and commonly is observed during episodes of overwhelming bacteremic illnesses.

Procalcitonin levels are elevated in bacteremic children and are related to the severity of illness, such as organ failure and even mortality.[96,122] Commercial kits for the rapid measurement of concentrations of procalcitonin are available in some countries. Hemoglobin and hematocrit results should help differentiate between septic and hemorrhagic shock. Examination of the peripheral smear may disclose evidence of splenic dysfunction (i.e., Howell-Jolly bodies) or fragmented red blood cells, as seen in disseminated intravascular coagulation. Thrombocytopenia, prolongation of prothrombin time and partial prothrombin time, and the presence of fibrin split products are consistent with disseminated intravascular coagulation.[42]

Hyponatremia is a common finding. Concentrations of serum bicarbonate may be depressed, which may signify a state of metabolic acidosis. Elevated lactic acid concentrations result from inadequate tissue perfusion, and in some reports have been significantly greater in nonsurvivors or patients with low-flow states than in survivors or patients with high-flow shock.[47] In pediatric studies, serial lactate levels showing normalization are associated with recovery.[30] Hyperglycemia or hypoglycemia may be encountered. In one study, serum glucose levels greater than 178 mg/dL were associated with a greater risk of death caused by septic shock.[31] Transaminase levels may be elevated and presumably reflect cellular injury. Serum calcium concentrations (preferably ionized calcium levels) should be checked periodically because hypocalcemia may interfere with optimal myocardial function.

A chest radiograph may reveal a pulmonary source of infection or show a secondary pulmonary manifestation of an invasive infection such as pneumonia or septic emboli in children with staphylococcal sepsis.[86] Arterial blood gases obtained early in endotoxin shock usually reveal hypocapnia and normal to elevated pH.[25,26] At this point, the patient has a mixed metabolic acidosis and respiratory alkalosis. If the shock state progresses, the metabolic acidosis becomes so severe that respiratory compensation is ineffective, and the patient becomes acidotic. In some patients, respiratory acidosis accompanies metabolic acidosis. In either case, decompensated metabolic acidosis in a patient with septic shock is associated with a grave prognosis. A major consequence of ARDS is hypoxemia. For patients with ARDS, the chest radiograph characteristically shows bilateral and diffuse hazy infiltrates; opacification of all lung fields usually is seen during the late phases of ARDS.

Concentrations of blood urea nitrogen and serum creatinine may be elevated. Jones and Weil[109] found that the ratio of urine to plasma osmolality was the most valuable indicator of renal impairment in adult patients with shock. When this ratio was greater than 1.5, the likelihood of developing progressive renal failure was remote. A urine osmolality value greater than 400 mOsm/kg also indicated adequate renal function. Many WBCs or WBC casts in the urine may suggest the genitourinary tract as the source of bacteremia. If one or more gram-negative rods are seen on the Gram stain of unspun urine, more than 10^5 colony-forming units/mL of bacteria are likely to be present.

Isolating the organism responsible for bacteremia or septic shock is important for documenting the infection and for providing optimal antimicrobial therapy. With instruments that continuously monitor growth in the blood culture bottles, growth can be detected sooner than if the bottles are inspected just once or twice daily. Before the use of the pneumococcal conjugate vaccine became routine, many authorities recommended that a blood culture be obtained in a 3- to 36-month-old child with temperature greater than 39°C to 40°C and a total WBC count greater than or equal to 15,000 cells/mm^3 and without a specific focus of infection. In this way, instances of "unsuspected" or "outpatient" bacteremia could be identified. In an outpatient setting, almost 90 percent of blood cultures growing true pathogens were positive within 24 hours of incubation using a continuously monitored system.[141] This approach is now less useful in the era of administration of pneumococcal conjugate vaccine to young infants.

In an infant who has received three or more doses of the conjugate pneumococcal vaccine, the likelihood of developing invasive pneumococcal infection is reduced approximately 90 percent. The proportion of children with high fever without localizing findings and a WBC count of 15,000/mm^3 or greater who might have occult pneumococcal bacteremia may be less than 1 percent, a level that no longer justifies this approach. Most organisms isolated from blood cultures in these patients are now more likely to be a contaminant than a true pathogen. Currently, the approach to a febrile child without a source has changed in

many emergency departments such that more selective criteria for obtaining blood cultures are being developed.

When appropriate, cerebrospinal fluid, urine, and other pertinent sites should be cultured before initiating antibiotic therapy, if possible. When an intra-abdominal source of infection is likely, blood and other cultures should be processed anaerobically. Gram stain or acridine orange stain of a buffy coat smear of peripheral blood may reveal evidence of the causative microorganism, especially in an overwhelming infection.[119] Gram stain of material obtained from petechial or purpuric lesions may show gram-negative diplococci suggestive of meningococcus. Polymerase chain reaction for detecting *N. meningitidis* is available in selected laboratories and may be the only method by which infection is documented when cultures are sterile. Bacterial polysaccharide antigens can be detected rapidly in many body fluids by latex agglutination tests. Rapid diagnostic procedures for evaluating outpatients with suspected bacteremia are unreliable. Pneumococcal antigenuria is not detected readily when bacteremia occurs without a specific focus of infection. The value of assays for detecting circulating endotoxin is unclear.

TREATMENT

The initial selection of antibiotics for administration to a child with suspected bacteremia is based on the clinical situation (Table 69–4). If untreated initially, children with occult bacteremia are at risk of developing serious complications, such as meningitis or pneumonia.[133,155] Empiric antibiotic therapy for children who are selected carefully and followed seems reasonable.[17] Two prospective studies of empiric administration of penicillin or amoxicillin in this situation reached different conclusions, however.[44,108] Ceftriaxone has been compared with amoxicillin or amoxicillin-clavulanate in two large studies involving children 3 to 36 months old. Ceftriaxone was marginally superior at best to the oral agents with regard to efficacy.[19,74]

In children who have not received more than two doses of the pneumococcal conjugate vaccine, one approach may be to obtain a blood culture from children aged 3 to 36 months with a temperature of 39° C or greater who have no focal findings and whose peripheral WBC count is greater than or equal to 15,000/mm^3. In such patients, the decision to administer antibiotics expectantly may be based on several factors, especially the ability of the parents to observe the child and communicate this information back to the physician in a timely manner. Ceftriaxone given parenterally, an oral agent such as amoxicillin and amoxicillin-clavulanate, or other oral antibiotics can be administered. If treatment is initiated, a properly collected urine specimen for urinalysis or urine culture also should be obtained so that a urinary tract infection would not be treated inadvertently. Occult bacteremia caused by *S. pneumoniae* intermediate or resistant to penicillin or intermediate to ceftriaxone in an otherwise normal child should resolve with any of the options noted earlier.[115] This approach probably is less useful in an infant or child who has received three or more doses of the conjugate pneumococcal vaccine.

Children who subsequently are determined to have *S. pneumoniae* bacteremia and who have been treated with antibiotics expectantly need to be re-evaluated as soon as the results of the blood cultures are known. If the child appears well and has been afebrile for at least 24 hours and if the parents can observe the child carefully and are able to communicate frequently with the physician, outpatient management can be continued. For children who were not treated initially, who remain febrile, and who are called back to be re-evaluated for a positive blood culture, a parenteral dose of antibiotics is recommended.[15] Close contact with the parents and patient is mandatory no matter how these children are managed initially. Hospitalization for intravenous antibiotics is indicated if the child appears "toxic" or has other signs suggesting a serious infection.

For children with suspected bacteremia who are ill and require admission to the hospital, antibiotics are selected to cover the most serious organisms causing that infection. In normal children aged 3 months or older, a combination of nafcillin (150 to 200 mg/kg/day) or another semisynthetic antistaphylococcal penicillin plus cefotaxime (150 to 200 mg/kg/day) or ceftriaxone (75 to 100 mg/kg/day) covers most of the likely pathogens (e.g., *S. pneumoniae*, *S. aureus*, *S. pyogenes*, *N. meningitidis*, Hib). In areas of the United States where community-acquired MRSA is a problem, an agent effective against MRSA should be included in the initial empiric regimen.[98,113] Vancomycin (45 to 60 mg/kg/day) is the gold standard for treating infections caused by MRSA. Whether adding an aminoglycoside or rifampin to vancomycin is beneficial is unclear. For most community-acquired MRSA isolates, clindamycin (40 mg/kg/day in four divided doses) also is an effective antibiotic. Some experts recommend adding clindamycin to vancomycin or nafcillin theoretically to reduce toxin production. This reduction occurs in vitro, but no clinical data indicate that this approach leads to a decrease in the incidence of morbidity or mortality.[209]

Treatment of bacteremia associated with a genitourinary or gastrointestinal source requires antibiotics to which gram-negative enterics are susceptible. In such cases, initial therapy could consist of an aminoglycoside with an additional antibiotic active against anaerobes, such as clindamycin, metronidazole, ticarcillin-clavulanate, or piperacillin-tazobactam, for a gastrointestinal focus of infection. Extended-spectrum cephalosporins, such as cefotaxime or ceftriaxone, are possible alternative drugs for treating serious gram-negative enteric infections.[112] Optimal management of intra-abdominal or other abscesses usually requires surgical drainage, which should be undertaken as soon as the child's condition allows.

Immunosuppressed children or children with serious illnesses in the ICU require a different initial approach to suspected bacteremia. Gram-negative enterics, *P. aeruginosa*, *S. aureus*, and coagulase-negative staphylococcus are likely to be isolated from these patients.[6] Empiric therapy of nosocomial infection is based on the current antibiotic susceptibility pattern within the hospital.[216] *E. coli*, *Klebsiella* spp., and *Enterobacter* spp. are among the most common organisms causing bacteremia in patients in the pediatric ICU.[185] *E. coli* and *Klebsiella* spp. frequently produce β-lactamases that lead to resistance to ticarcillin or piperacillin. Generally, a combination of an antistaphylococcal semisynthetic

TABLE 69–4 Empiric Antibiotic Regimens for Septic Shock in Infants and Children under Selected Clinical Circumstances

Circumstances	Antibiotics
Normal Child	
Skin findings suggesting meningococcemia or preceding skin trauma or varicella	Cefotaxime or ceftriaxone + vancomycin ± nafcillin
Urinary tract source	Cefotaxime or ceftriaxone + aminoglycoside
Intra-abdominal source	Clindamycin + gentamicin + ampicillin or piperacillin or piperacillin-tazobactam
Immunocompromised Child	
Malignancy or immunodeficiency or neutropenia or central line	Vancomycin + aminoglycoside + ticarcillin-clavulanate piperacillin-tazobactam or ceftazidime
Asplenia or splenic dysfunction	Vancomycin + cefotaxime or ceftriaxone

penicillin or vancomycin (if a central line is present) or, if MRSA is a relatively common nosocomial pathogen, an aminoglycoside and an extended-spectrum penicillin (e.g., ticarcillin-clavulanate, piperacillin-tazobactam) is administered initially until a specific pathogen is isolated.[105] Broad-spectrum penicillins and aminoglycosides frequently exhibit synergy in vitro against gram-negative organisms, especially against *P. aeruginosa*.[124,198] In critically ill patients, a synergistic combination of antibiotics may be beneficial for treating bacteremia caused by *P. aeruginosa* or *Klebsiella* spp., although this subject remains controversial.[100,120]

After a specific organism is identified and antibiotic susceptibilities are known, the most appropriate agent is selected. If *E. coli* or *Klebsiella* spp. are isolated, these organisms can produce extended-spectrum β-lactamases, and the isolates should be tested for this possibility.[172] An extended-spectrum, β-lactamase–producing organism may seem susceptible to the extended-spectrum cephalosporins with minimal inhibitory concentrations of 2 to 8 μg/mL, but treatment failures may occur. If an extended-spectrum, β-lactamase–producing organism is identified, treatment with an extended-spectrum cephalosporin (e.g., cefotaxime, ceftriaxone, ceftazidime) is not recommended.[171] In this case, a carbapenem, such as imipenem-cilastatin or meropenem, is suggested.[171]

Enterobacter spp. may hyperproduce β-lactamase enzymes or be induced to hyperproduce these enzymes and are resistant or can become resistant to the extended-spectrum cephalosporins.[174] In many institutions, more than 30 percent of *Enterobacter cloacae* isolates are resistant to ceftazidime.[35] Cefepime may be more active than is ceftazidime or cefotaxime against *Enterobacter* spp. and other Amp C, β-lactamase enzyme–producing, gram-negative bacilli.[195] A carbapenem with an aminoglycoside is the treatment regimen recommended for treating infections caused by *Enterobacter* spp. resistant to ceftazidime.

In these patients, rapidly achieving therapeutic aminoglycoside levels in the plasma is important.[151] Serum concentrations of the aminoglycoside should be measured within 24 hours of initiation of therapy to ensure that therapeutic concentrations have been reached.

Antibiotics should be administered as soon as the diagnosis of septic shock is suspected. The selection of antibiotics is the same as that for bacteremia. If *S. aureus* is suspected among the potential causes of septic shock, antibiotic therapy is complicated by two factors: MRSA may make up more than 50 percent of community-acquired isolates[114] and nafcillin or oxacillin is superior to vancomycin for the treatment of serious infections caused by MRSA.[89] Nafcillin or oxacillin plus an aminoglycoside may be synergistic against staphylococci, but adding an aminoglycoside increases the risk for nephrotoxicity to develop. Some authorities include nafcillin or oxacillin plus vancomycin plus gentamicin in the initial empiric therapy of patients with life-threatening infections possibly caused by *S. aureus*.[9]

The management of septic shock is directed toward three main objectives: (1) control of the infectious process, (2) restoration of adequate tissue perfusion, and (3) maintenance of efficient respiratory function. Details of management have been the subject of numerous reports.[36,41,194] The reader is referred to these reviews for expert detailed guidance in the fluid management, use of vasoactive agents, and respiratory treatments in children with septic shock.

The benefits of therapy with high doses of corticosteroids in the treatment of endotoxin shock remain unproved. Extensive experimental data document the salutary hemodynamic, metabolic, microcirculatory, and cellular effect of steroids in laboratory models of endotoxin shock.[102] High-dose steroids were never found to be efficacious, however, in randomized controlled trials conducted in adults with severe sepsis or septic shock.[30,205,213]

On the basis of these studies, the routine use of high-dose corticosteroids in patients with severe sepsis or septic shock is not recommended. Later, results of small studies enrolling adults suggested that lower stress doses of hydrocortisone might be beneficial in managing patients with septic shock.[27,33] In one of these trials, placebo was compared with a 100-mg loading dose of hydrocortisone followed by a continuous infusion of 0.18 mg/kg/hr with a reduction of the infusion after shock had been reversed. Twenty patients were randomly assigned to each treatment arm. Infusion of stress doses of hydrocortisone reduced the time to discontinuing the vasopressor therapy and was associated with a trend toward earlier resolution of sepsis-induced organ dysfunction. Overall reversal of shock and mortality rates was unchanged, however. The role of low-dose hydrocortisone in children with septic shock remains controversial.[12,224] Randomized studies are needed to clarify the role of stress doses of hydrocortisone in children with septic shock.

Every effort should be made to ensure an adequate airway, which may require periodic suctioning if the patient is unable to clear pooled secretions. Humidified oxygen in concentrations required to maintain an adequate partial pressure of oxygen should be provided early. The method of oxygen administration (e.g., mask, ventilator) depends on the clinical state of the patient. Intubation and assisted ventilation are indicated if the child shows evidence of impending respiratory failure.

ARDS, usually appearing within 2 days after the onset of shock, may complicate the respiratory and fluid management.[104] Pulmonary edema, atelectasis, decreased pulmonary compliance, and ventilation-perfusion abnormalities are some factors that can lead to inadequate oxygenation. Administration of excessive fluids may contribute to ARDS. The central venous pressure may remain normal, despite the presence of pulmonary edema. Positive end-expiratory pressure, oxygen, and careful attention to cardiovascular parameters are the mainstays of therapy for ARDS.[104,175,191] Steroids are not beneficial after ARDS has been diagnosed.

The general supportive care of a child with septic shock includes attention to nutritional and metabolic requirements.[182] Administration of fluids containing 10 percent glucose may be necessary to prevent development of hypoglycemia. Parenteral alimentation may be the only means by which to provide nutrition, although the optimal amount and composition of elemental nutrients for children with septic shock are unknown. Hypocalcemia should be corrected. Platelet transfusions or fresh-frozen plasma may be necessary to correct coagulopathies. Continuous venovenous hemofiltration with or without dialysis may be instituted for complications of renal failure, such as fluid overload with pulmonary edema or hyperkalemia. It is not surprising that children who have acute renal failure complicating severe septic shock have a significantly higher mortality rate than that of children without acute renal failure.[176]

INVESTIGATIVE THERAPIES

Based on the evolving understanding of the pathophysiology of septic shock, many large, randomized, double-blind, multicenter trials have been conducted to assess the efficacy of agents that neutralize or counteract toxins or cytokines that are important in the evolution of septic shock. These immunomodulating strategies have been used on the basis of experimental data from animal studies. The conditions seen in experimental animal models of sepsis do not reflect the pathophysiologic situation seen in patients with severe sepsis or septic shock, however, which has led to many negative clinical trials (Table 69–5).

Antibody to Lipopolysaccharide

Endotoxemia frequently can be documented in patients with gram-negative sepsis. Endotoxin is released when bacteria are

TABLE 69–5 Clinical Trials of Sepsis Medications in Adults

Trial	Agent Studied	Total Patients (no.)	28-Day Mortality (%)	
			Medications	*Controls*
CHESS	HA-1A human monoclonal antibody[139]	1578	41	37
INTERSEPT	Anti-TNF[49]	564	37.3	39.5
	Steroids[30]	1267	39	35
	IL1-RA[72]	893	31	35
	Soluble TNF receptor[71]	141	40	38
COMPASS	Platelet-activating factor acetylhydrolase[165]	1425	25	24
KyberSept	Antithrombin III[233]	2314	38.9	38.7
OPTIMIST	Tissue factor pathway inhibitor[3]	1754	34.2	33.9
PROWESS	Activated protein C[22]	1690	24.7	30.8
ADDRESS	Activated protein C[2]	2613	17	18.5

TNF, tumor necrosis factor.

killed by bactericidal antibiotics.[200,201] This release may help explain why some patients develop shock after parenteral antibiotics are administered. Because endotoxin is responsible directly or through mediators for many of the adverse effects of gram-negative bacteremia, attempts to neutralize endotoxin have been undertaken. One problem with this approach is that only patients with septic shock caused by endotoxin-producing organisms may benefit from this treatment, and distinguishing a gram-positive from a gram-negative infection is impossible on patient presentation.

Neither E-5[29,92,95] (XMMEN-OEJ; XOMA Corp., Berkeley, CA), a murine monoclonal antibody, nor HA-1A[139,248] (Centoxin; Centocor, Malvern, PA), a different human monoclonal antibody against lipid A, were found to be efficacious in randomized, multicenter trials of gram-negative sepsis in adults. Children were not included in either study, although the pharmacokinetics and safety of HA-1A were established in children.[188] In the one study performed, HA-1A did not reduce the mortality rate in children with meningococcal disease.[57]

Other Potential Adjunctive Therapies

Antibody to TNF, soluble TNF receptors, antiplatelet activating factor, recombinant IL-1 receptor antagonist, antibradykinin, nitric oxide synthase inhibitor, and other therapies have undergone evaluation in large clinical trials of adjunctive therapy for adults with sepsis syndrome. None has proved beneficial.[4,14,72] Some combination of therapies may provide significant benefit in humans with gram-negative septic shock, as has been shown in animals.[193] Using recombinant soluble CD14 is another approach for blocking the action of endotoxin.[97]

Recombinant bactericidal/permeability-increasing protein ($rBPI_{23}$), a human-derived recombinant protein that expresses the amino-terminal half of the whole bactericidal, permeability-increasing protein molecule, has been studied in children with severe meningococcal sepsis.[149] Preliminary evaluation of $rBPI_{23}$ in 26 children with severe meningococcal sepsis in an open-label phase I/II trial was associated with fewer cases of mortality than in historical control patients.[82] A subsequent large, multicenter, randomized, double-blind trial in which 395 children were enrolled was conducted in 22 centers throughout the United States and the United Kingdom.[127] Mortality rates (7.4%) were not diminished significantly in the $rBPI_{23}$ group because the mortality rate in the control group was only 9.9 percent, a rate much less than anticipated. Children receiving $rBPI_{23}$ did have fewer amputations and a better functional outcome than did children receiving placebo. $rBPI_{23}$ is not being developed further.

Based on the complex interaction of the inflammatory and procoagulation responses of the host to infection as outlined in the pathophysiology section, recombinant human activated protein C (drotrecogin alfa activated [rhAPC]) was evaluated for adjunctive treatment of severe sepsis in patients 18 years old and older. In a multicenter, double-blind, placebo-controlled study conducted in several countries, placebo or rhAPC was infused at a rate of 24 μg/kg/hr for 96 hours to patients with severe sepsis.[22] Selected demographic and clinical characteristics of the patients showed that the patients were matched well. The mortality rate at 28 days was 30.8 percent for the 840 patients in the placebo group and 24.7 percent for the patients receiving rhAPC (p = 0.005). The relative risk of mortality was reduced in the rhAPC group by 19.4 percent (95% confidence interval 6.6 to 30.5). The major difference in serious adverse effects was for serious bleeding, which occurred in 3.5 percent and 2 percent of the rhAPC and placebo patients. Recombinant human activated protein C has been approved for use in adults by the U.S. Food and Drug Administration.

Protein C concentrate has been administered to several children with fulminant meningococcemia and thought to be associated with enhanced survival and decreased amputations.[7,239] These findings supported the continued evaluation of rhAPC in the adjunctive treatment of septic shock in children. Phase I and II studies evaluated the pharmacokinetics and safety of rhAPC in children with severe sepsis.[129] An infusion rate of 24 μg/kg/hr resulted in mean serum concentrations of 67 ng/mL in 43 infants and children. (The mean concentration found in adults was 52 ng/mL.) rhAPC did not accumulate during a 96-hour infusion. The estimated half-life was 0.91 hours, and plasma clearance was 0.49 L/hr/kg. In 2005, however, the Food and Drug Administration and the manufacturer announced the discontinuation of a randomized, placebo-controlled trial of rhAPC in pediatric patients with severe sepsis (information available online at www.fda.gov/medwatch/SAFETY/2005/safety05.htm#Xigris2). An interim analysis showed that rhAPC was highly unlikely to show a benefit over placebo in the primary end-point (time to complete organ failure resolution over 14 days). rhAPC was associated with a significantly higher risk for central nervous system bleeding. The risk for intracranial hemorrhage was particularly elevated in patients younger than 60 days. The use of rhAPC is not indicated for use in pediatric severe sepsis.[156]

Intravenous immunoglobulin is recommended for adjunctive therapy for patients with toxic shock syndrome caused by either group A streptococcus or *S. aureus* that is not responding to aggressive therapy after several hours or after appropriate drainage of focal infections.[10] The value of intravenous immunoglobulin in the treatment of septic shock caused by *S. aureus* infection but not associated with toxic shock syndrome has not been established.[80] Tefibazumab is a monoclonal antibody directed against clumping factor A, an adhesin on the surface of *S. aureus*, that is being investigated to determine if it improves the outcome of adults with *S. aureus* bacteremia.[236]

Polymyxin B is an antibiotic that can neutralize endotoxin possibly through a detergent-like action. In experimental models, polymyxin B moderates some of the cardiovascular, metabolic, and lethal consequences of *E. coli* sepsis in rabbits and overwhelming Hib disease in infant rats.[75,230] Clinical studies of polymyxin B have not been conducted in humans with sepsis or septic shock.

Pentoxifylline is a phosphodiesterase inhibitor that has anti-inflammatory properties, including the ability to suppress endotoxin-induced mononuclear cell production of TNF. Pentoxifylline decreases endotoxin or TNF-induced lung injury and increases survival in animals infected with *E. coli* or infused with endotoxin.[128] In a study of human volunteers, a 500-mg dose of pentoxifylline infused 30 minutes before a 100-ng injection of endotoxin from *Salmonella abortus equi* blunted the TNF response but did not affect IL-6 serum levels after administration of endotoxin.[246] Clinical effects, such as fever, myalgia, and headache, were not affected by pentoxifylline. In one small study of 51 patients, a continuous infusion of pentoxifylline was associated with improvement in organ dysfunction scores, Pao_2/Fio_2 ratio, and the pressure-adjusted heart rate compared with placebo-treated patients.[207] Larger clinical studies of pentoxifylline or similar agents are warranted for the adjunctive treatment of sepsis and septic shock.

Plasmapheresis, exchange transfusions, and extracorporeal membrane oxygenation are heroic measures that seem to be beneficial in selected patients not responding to standard management.[21,58,79] These procedures may be considered for such patients when, in the opinion of experienced clinicians, their use is justified and they are the last hope for a successful outcome.[76]

PROGNOSIS

The morbidity and mortality rates for septic shock in children vary with age, the presence or absence of underlying diseases, and the specific microorganisms responsible for the septicemic state. Dupont and Spink[64] reported a 98 percent mortality rate in their series of children with septic shock and gram-negative bacteremia. Jacobs and colleagues[107] reported a 9.8 percent case-fatality rate for otherwise normal children with septic shock. In the pediatric HA-1A study, the overall mortality rate for severe sepsis or septic shock was 31 percent.[188] The overall mortality rate for children with meningococcal infection was 8 percent in a 10-center surveillance study.[116] For the seven-state sepsis cohort, the overall mortality rate was approximately 10 percent.[234] Much progress has been made in the treatment of sepsis and septic shock since the report of Dupont and Spink was published.[64] As with many infections, prevention is more desirable than is treatment. Careful attention given to sterile techniques for insertion and maintenance of intravascular or other lines and other procedures is crucial and may prevent some episodes of bacteremia and septic shock.

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CHAPTER 70

Fever without Source and Fever of Unknown Origin

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Petersdorf and Beeson[76] in 1961 proposed that the term *fever of unknown origin* (FUO) be reserved for persons with an illness persisting for 3 or more weeks and accompanied by temperatures higher than 38.4° C (101.2° F) on at least several occasions. They further specified that the cause of the fever should remain undetermined after at least 1 week of investigation in the hospital. Although this definition was arbitrary, it was useful at that time, when many of the diagnostic tests now in routine use were unknown. The purpose of their precise definition was to explore the cause of fever in this select group of adult patients and to permit comparison of data from different investigations. This exacting definition probably never was applied rigorously in pediatric practice. In children, the term FUO should be reserved for fever of at least 8 days' duration and for which no diagnosis is apparent after the initial work-up in the hospital or as an outpatient.

Many investigators prefer using the term *fever without source* (FWS) for fever of recent onset with no adequate explanation determined by the history or physical examination. The distinction between FUO and FWS is of more than academic interest for several reasons. First, although overlap exists, the differential diagnoses of these clinical conditions are distinct, and the most frequent causes of one are different from the most frequent causes of the other. Second, a child with fever of recent onset generally warrants more immediate evaluation than does a child with FUO. The latter usually does not occur as an emergency and requires timely, but not urgent, diagnostic or therapeutic intervention. Third, although expectant antibiotic treatment of children with FUO generally is not indicated, expectant treatment of infants with FWS is recommended in most cases.

FEVER WITHOUT SOURCE

A convenient definition of FWS *is the occurrence of fever for 1 week or less in a child in whom a careful history and physical*

examination fail to reveal a probable cause of the fever. An estimated 14 to 40 percent of children with fever have no localizing signs or symptoms.[7,12] Stein[89] found that the peak incidence occurs during the second year of life. On the basis of a review of private pediatric practices in upstate New York, Hoekelman and colleagues[43] predicted that every 4 to 5 days a practicing pediatrician would see one child between 1 and 24 months of age with FWS.

Most children with fever of recent onset have acute infectious diseases, the majority of which are self-limited.[100] A few of these patients have serious acute infectious diseases, including meningitis and bacteremia, and a very few have acute noninfectious diseases or chronic disorders. For example, an occasional patient with FWS is discovered to have a disorder such as heat illness, drug poisoning, Kawasaki disease, malignancy, or connective tissue disease. However, these disorders occur infrequently. A physician faced with a child with FWS should consider the possibility of a noninfectious cause or the onset of a chronic disease, but unless a clinical clue suggests one of these entities, investigation in this direction is not warranted.

Many children with FWS are in the prodromal stages of an acute infectious illness, and evidence of a specific infection, such as pharyngitis, otitis media, or pneumonia, develops within hours to days of first being evaluated by a physician. Fever can precede the appearance of specific signs and symptoms by as long as 3 days, as in measles, Rocky Mountain spotted fever, and leptospirosis. In some infections, such as roseola, viral hepatitis, infectious mononucleosis, typhus, and typhoid fever, the interval between the onset of fever and the appearance of specific findings often is more than 3 days.

OCCULT BACTEREMIA

One major concern regarding a young child with FWS is the possibility that the child has occult bacteremia. The patient does not appear ill, is judged clinically well enough to be managed as an outpatient, and does not have an infection commonly associated with bacteremia such as pneumonia, but the blood culture yields pathogenic bacteria such as *Streptococcus pneumoniae*, *Neisseria meningitidis*, *Haemophilus influenzae*, *Escherichia coli*, *Salmonella*, or *Staphylococcus aureus*. Before introduction of the pneumococcal conjugate vaccine, the incidence of occult bacteremia in children with FWS was approximately 3 to 5 percent.[14,25,60,70,93,100] More recent data report an incidence of 0.2 to 3 percent, although this figure can vary depending on vaccine coverage rates in the population studied.[2,3,42,74,85,91] Few prospective, large studies have been performed to further investigate the impact of the pneumococcal conjugate vaccine on the incidence of occult bacteremia in children.[79] Historically, occult bacteremia has been found to occur more commonly in children with FWS than in febrile children of the same age with infections such as pharyngitis, otitis media, or upper respiratory tract infection. In the 1970s, investigators[60] reported the incidence of bacteremia in febrile children without an obvious source of infection to be 9.9 percent, as opposed to 3.3 percent in children with otitis media, upper respiratory tract infection, or a flu-like syndrome. During the same era, Teele and colleagues[93] found a 3.9 percent incidence of bacteremia in children with FWS and a 1.5 percent incidence in comparably febrile children with otitis media or pharyngitis.

The risk of occult bacteremia developing in a child with FWS is age-related, with most cases occurring in children younger than 24 months old. Numerous studies have demonstrated a higher risk for bacteremia or other serious bacterial infections (SBIs) in febrile infants younger than 3 months old.[58,60,84,93] In a group of children with clinically unsuspected meningococcemia, all 12 patients who initially looked well enough to be treated as outpatients were younger than 24 months of age.[31] Bonadio and colleagues[20] found the incidence of positive bacterial cultures to be 12 percent in febrile infants younger than 4 weeks and 6 percent in those between 4 and 8 weeks of age.

The risk of having occult bacteremia and other SBIs increases with the severity of fever. In a prospective study of bacteremia in children seen in the outpatient department, McCarthy and colleagues[61] identified a small, but statistically significant, difference in the incidence of bacteremia between children with temperatures of 40° C (104° F) or higher and those with temperatures of 40.5° C (104.9° F) or higher. In a prospective study in which blood was obtained for culture from all febrile children younger than 2 years old seen in a walk-in clinic, no positive blood cultures were found in 44 children with FWS and rectal temperatures lower than 38.9° C (102° F), whereas five (3.9%) positive blood cultures were found in 129 children with FWS and rectal temperatures of 38.9° C (102° F) or higher.[93] Other studies have reported similar findings.[14,58,85] Several series of children with fevers of 41° C (105.8° F) or higher found a relatively high prevalence of bacteremia and other SBIs, especially meningitis and pneumonia.[19,58,81] However, even in this group of children with very high fever, most of those older than 2 or 3 months of age who looked well did not have an SBI.

The white blood cell (WBC) count has been studied extensively as a potential tool in the diagnosis of occult bacteremia. On the basis of a study of hospitalized children, Todd[94] reported that the absolute number of polymorphonuclear leukocytes and the absolute number of nonsegmented polymorphonuclear leukocytes were more sensitive than was the total WBC count, the percentage of polymorphonuclear leukocytes, or the percentage of nonsegmented polymorphonuclear leukocytes. However, whether information based on hospitalized children—presumably all of whom had serious localized infections or looked ill enough to warrant hospitalization—can be applied to children with FWS who look well enough to be treated on an ambulatory basis is questionable.

Considerable debate continues over the usefulness of the WBC count in febrile children evaluated in the outpatient setting. McCarthy and associates[61] concluded that a WBC count of 15,000/mm^3 or greater was helpful in identifying patients at greatest risk for the development of bacteremia. Dershewitz[32] found a direct relationship between the total leukocyte count and the prevalence of bacteremia and stated that "knowledge of the count was a helpful but limited predictor of patients with positive blood cultures." Other investigators reported that the incidence of bacteremia increased with an increased WBC count[85] and that bacteremia most commonly occurred in patients with counts of 20,000/mm^3 or higher.[66]

Other studies have examined the utility of the WBC count specifically in children with FWS who look well enough to be treated on an outpatient basis. One such study found a sensitivity of 1.0 and a positive predictive value of 0.11 for a total WBC count of 15,000/mm^3.[93] Using a WBC count of 20,000/mm^3 would have decreased the sensitivity to 0.4 while increasing the positive predictive value to only 0.13. In another series, the sensitivity for a WBC count of 15,000/mm^3 was 0.87, with a specificity of 0.73.[14] Kline and coworkers[50] found that a WBC count of 15,000/mm^3 was more sensitive for *S. pneumoniae* bacteremia than for *H. influenzae* bacteremia. Although a total WBC count of 15,000/mm^3 does not accurately predict which child is or is not bacteremic, it is helpful in dividing the population of children with FWS into high- and low-risk groups.

Some investigators have found the erythrocyte sedimentation rate to be no more useful than the WBC count in predicting bacteremia in ambulatory febrile patients.[61] Others have reported that the serum concentration of C-reactive protein (CRP) may be more accurate than is the complete blood count or erythrocyte sedimentation rate in distinguishing bacterial from viral infec-

tions.[59,75] However, recent studies reported conflicting data regarding the utility of the CRP test in screening children aged 3 to 36 months old for occult bacteremia.[44,82] An elevated serum procalcitonin level has been found by some investigators to be at least as sensitive and specific as is CRP in predicting SBI in children with fever and no localizing signs.[51,80]

Other hematologic findings that suggest bacteremia include thrombocytopenia,[29] Döhle inclusion bodies, toxic granulations, and vacuolization of neutrophils. In one study, peripheral blood smears of children younger than 24 months old with acute febrile illnesses were reviewed the following day by a single investigator to determine whether vacuolization and toxic granulations were present; when both abnormalities were present, the positive predictive value for bacteremia was 0.76.[53] The presence of these findings should be considered when estimating the risk for bacteremia.[1,29,69]

Several studies have examined the response to acetaminophen and found no difference in the rate of reduction of temperature or improvement in clinical appearance between bacteremic and nonbacteremic children.[9,95,102] Mazur and associates,[57] however, found that febrile children aged 2 months to 6 years old who did not respond to a dose of acetaminophen by a reduction in temperature of at least 0.8° C in 2 hours had a statistically significant increased risk of developing occult bacteremia in comparison to those who did respond.

The most important aspects of assessment of a febrile child are a careful history and physical examination. Laboratory data are secondary and should be ordered on the basis of the clinical assessment. By definition, a child with FWS has no localizing signs to explain the fever or indicate a site of infection. Many physicians suggest that a general impression can indicate whether the child has occult bacteremia. Some physicians have suggested that careful clinical judgment, based on extensive experience, can identify most, if not all, children with serious illnesses.[18] McCarthy and colleagues,[62-64] in a series of carefully designed studies, elucidated the variables of history and observation that were most useful in assessing febrile children. They found that observation of the variable *playfulness* had the strongest correlation with overall assessment.[63] However, they observed that even an experienced attending pediatrician could identify only 57 percent of seriously ill children by initial impression before performing a full physical examination. Dershewitz[32] found that private pediatricians were no more accurate than were pediatric residents in identifying children with occult bacteremia and that in the private office, pediatricians were no better at predicting bacteremia in familiar patients than in first-time patients.

In a study of 292 consecutive febrile children seen in an emergency department, Waskerwitz and Berkelhammer[97] identified a subgroup of patients who had no localizing signs and who looked so well that they were predicted not to have bacteremia. The physicians were assisted in their assessment by a functional scale that gave 0 to 2 points for the child's eating, drinking, sleeping, and play activities, with a best possible score of 8. The group of patients who had functional scores of 5 or greater, with no localized infection and predicted clinically not to have bacteremia, were free of bacteremia, whereas 14 of 202 patients with functional scores of 4 or less were bacteremic. In this study, the physicians were not able to identify which patients had bacteremia and which did not; rather, they were able to identify one subgroup at high risk for having bacteremia and another at very low risk. Teach and Fleisher[92] found that although Yale Observation Scale scores were higher in patients with bacteremia than in those without, the difference was not clinically useful in detecting bacteremia in well-looking febrile children without a discernible focus of infection. The clinician's overall assessment of the degree of illness of the child appears to be a valuable, but not infallible, tool in estimating the risk of occult bacteremia in children with FWS.

CLINICAL MANAGEMENT OF FEVER WITHOUT SOURCE

As many as 3 percent of children with FWS are bacteremic. Numerous studies (all performed before institution of the routine use of *H. influenzae* vaccine) have shown that if these children were not treated with antibiotics at the time of the initial clinical encounter, 5 to 10 percent would return with bacterial meningitis, 10 percent with localized bacterial infection, and another 30 percent with continued fever and persistent bacteremia.[14,21,43,56,60,61,66,93] In all of these retrospective studies, patients treated initially with antibiotics fared better than those not treated initially, although the decision regarding treatment always was at the discretion of the physician and was not randomized.

In a prospective, randomized investigation, Carroll and associates[25] studied 96 children between the ages of 6 and 24 months who had FWS and a temperature higher than 40° C (104° F). Of 10 patients who were bacteremic, 5 were treated initially with antibiotics on an outpatient basis and 5 were not treated. The difference in outcome between the two groups was statistically significant in favor of the treatment group; four of the five treated patients were improved clinically, as opposed to none of the five untreated patients. Bacterial meningitis did not develop in any of the treated patients, whereas it did develop in two of the five untreated patients. In a prospective, randomized, placebo-controlled study of empiric treatment with amoxicillin in children at risk for developing occult bacteremia, Jaffee and colleagues[46] showed no difference existed between treatment and nontreatment groups. However, the power of this study was low, and a true difference in outcome easily could have been missed.[5] The dosage of amoxicillin used was 125 mg three times daily for children who weighed 10 kg or less and 250 mg three times daily for those weighing more than 10 kg, so some children may have received as little as 37.5 mg/kg/day. Although this dosage is close to the usual recommended dosage of 40 mg/kg/day for infections such as otitis media, Baron and coworkers[15] suggested that considerably higher doses might be required to treat occult bacteremia. In a retrospective study of a private pediatric practice, these investigators found that no complications developed in 11 infants with FWS and bacteremia who initially received 150 mg/kg/day or more of amoxicillin ($p = 0.03$). In contrast, in 5 of 12 such infants not treated or treated with less than 100 mg/kg/day of amoxicillin, complications did develop. A study of the outcome of outpatient management of febrile children who proved to have pneumococcal bacteremia found that those not treated with antibiotics and those still febrile on re-evaluation were most likely to have persistent bacteremia.[6]

One should not be dogmatic about the management of children with FWS. One reasonable approach, based on a careful history, thorough physical examination, and overall clinical impression, is to classify these children as being at low or high risk for the development of occult bacteremia and other SBIs. For the low-risk group, no laboratory investigation is required routinely. For the high-risk group, a complete blood count and blood culture should be obtained. Lumbar puncture, chest radiography, urinalysis, and urine culture are considered on an individual basis. If the patient appears ill, admission to the hospital may be justified, even if all test results are negative. When high-risk children look well enough to be sent home, they are reasonable candidates for expectant antibiotic therapy, pending the outcome of blood culture. For patients clinically considered to be at moderate risk (not clearly high or low risk), the physician has the option of obtaining a WBC count and using the results to decide whether to draw blood for culture and prescribe antibiotics expectantly.

Table 70–1 lists risk factors for the development of occult bacteremia. Current information is not sufficient to warrant the use of scoring systems except as part of investigational series. In the final analysis, the clinician's judgment, taking into account all

TABLE 70–1 Risk Factors for Occult Bacteremia

Factor	High Risk	Low Risk
Age	≤24 mo	>36 mo
Magnitude of fever	≥40°C (104°F)	≤39.4°C (103°F)
White blood cell count	≥15,000/mm^3	<15,000/mm^3
Peripheral blood smear	Toxic granulation or vacuolization of polymorphonuclear leukocytes, thrombocytopenia	Unremarkable
Underlying chronic disorder	Sickle-cell disease, immunodeficiency, malnutrition	None
History of contact with bacterial disease	Contact with *Neisseria meningitidis* or *Haemophilus influenzae*	None
Clinical appearance	Appears ill, "toxic," or unhappy; inconsolable; irritable or lethargic; not eating or drinking enough	Looks well, playful, eating normally, not irritable

available clinical and laboratory data about each patient, is the guide to selecting which children require a diagnostic work-up and expectant therapy with antibiotics.

If the physician elects to prescribe antibiotics while awaiting the results of blood culture, such antibiotic therapy should provide adequate coverage for *S. pneumoniae*, *N. meningitidis*, and *H. influenzae*, although the frequency of *H. influenzae* has decreased dramatically with current immunization practice. A single injection of 50 to 75 mg/kg of ceftriaxone given while awaiting the results of blood culture has been successful in resolving fever, clearing bacteremia, and preventing meningitis and was found to be superior to oral regimens in several series.[13,16,38] Children with a positive blood culture should be recalled for re-evaluation, even if they are afebrile.

Because *S. pneumoniae* is a dominant cause of occult bacteremia, routine use of pneumococcal vaccine is expected to diminish the occurrence of occult bacteremia significantly. Children who have been immunized against both *H. influenzae* and *S. pneumoniae* should be considered to be at relatively low risk for the development of occult bacteremia and may require less work-up, unless they appear ill or have a very high fever.

Infants younger than 90 days old pose a special problem because they have an increased risk for developing SBI, clinical evaluation is more difficult, and a broader spectrum of invading organisms (e.g., group B *Streptococcus*, *E. coli*, *Listeria monocytogenes*) exists. Baker and coworkers[8] showed the safety of managing selected low-risk infants (i.e., normal WBC count, urinalysis, lumbar puncture, and chest roentgenogram and, if diarrhea was present, negative smear for fecal leukocytes) 30 to 90 days of age on an outpatient basis without antibiotics. Jaskiewicz and coworkers[48] found the Rochester criteria (WBC count of 5000 to 15,000/mm^3, band count <1500/mm^3, spun urine specimen <10 WBCs/high-power field, stool specimen [if diarrhea] <5 WBCs/high-power field) to have a 98.9 percent negative predictive value in well-appearing, previously healthy infants younger than 90 days old with no focal infections. One reasonable practice guideline for managing infants with FWS is to hospitalize and treat all who appear toxic and all younger than 28 days. Those between 28 and 90 days of age can be managed as outpatients if they look well and the blood count, urinalysis, and cerebrospinal fluid analysis are within normal limits.[10,11]

FEVER OF UNKNOWN ORIGIN

The exact definition of FUO is a subject of considerable disagreement, and series in the pediatric literature differ in their criteria for inclusion. Brewis[22] defined FUO in children as a temperature of 38.3°C (101°F) or higher for 5 to 7 consecutive days without localizing signs or symptoms. In sharp contrast, McClung[65] and Lohr and Hendley[54] considered children with fever for at least 3 weeks on an outpatient basis or 1 week in the hospital to have FUO. Pizzo and associates,[78] however, required only that the fever be present for 2 weeks, with no distinction made between outpatient or in-hospital status. A reasonable working definition of FUO for clinical purposes is the presence of fever for 8 or more days in a child for whom a careful and thorough history and physical examination and preliminary laboratory data fail to reveal a probable cause of the fever.

Most cases of FUO in children are caused by relatively common diseases. In four series of FUO totaling 418 children, only 5 patients would be considered to have rare disorders (i.e., Behçet syndrome, ichthyosis, variant of "blue diaper" syndrome, diencephalic seizure disorder, and "possible chronic lead and/or arsenic intoxication").[22,54,65,78] The adage that FUO is more likely to be caused by an unusual manifestation of a common disorder than by a common manifestation of a rare disorder certainly is true in pediatrics. The three most common discernible causes of FUO in children, in order of decreasing frequency, are infectious diseases, connective tissue diseases, and neoplasms. In approximately 10 to 20 percent of cases, a definitive diagnosis never is established.

In the United States, the systemic infectious diseases diagnosed most frequently in children with FUO include tuberculosis, brucellosis, tularemia, salmonellosis, and infections caused by rickettsia, spirochetes (e.g., leptospirosis), Epstein-Barr virus, cytomegalic inclusion virus, human immunodeficiency virus, hepatitis viruses, and other viruses. The most common causes of localized infection are upper respiratory tract infections (e.g., sinusitis, otitis, tonsillitis), urinary tract infection, osteomyelitis, and occult abscesses, including hepatic and pelvic abscesses.

The connective tissue disease most commonly manifested as FUO in children is juvenile rheumatoid arthritis, which accounts for more than 90 percent of connective tissue diseases in most series, followed by systemic lupus erythematosus and then by undefined vasculitis.[54,65,68,78] Frequently, a definitive diagnosis of juvenile rheumatoid arthritis can be made only after an extended period of observation because physical examination may yield no findings and the results of specific serologic studies generally are normal or negative.

Malignancy is a less frequent cause of FUO in children than in adults and usually is the third-largest group, after infectious diseases and connective tissue diseases. Malignancy accounted for 7 percent of FUO cases in the series reported by Pizzo and associates[78] and for 13 percent in the Lohr and Hendley[54] series. Leukemia and lymphoma are responsible for most cases of cancer manifested as FUO in children. Other tumors less commonly reported as causing FUO include neuroblastoma, hepatoma, sarcoma, and atrial myxoma.

Although the prognosis for children with FUO is better than that for adults and even though most children with FUO have treatable or self-limited disease, the overall prognosis is far from benign. Mortality rates were 9 percent in the series reported by Pizzo and associates[78] and 6 percent in Lohr and Hendley's series.[54] The prognosis for children in whom a definitive diagnosis is not established during the initial hospitalization is mixed. In most cases, the fever eventually resolves.[36] In some patients, a specific diagnosis is established, whereas other patients continue to have fever without a definitive diagnosis. McClung[65] described 11 such patients, most of whom appeared to do well despite having recurrent episodes of fever.

DIAGNOSTIC APPROACH TO A CHILD WITH FEVER OF UNKNOWN ORIGIN

A child with FUO is admitted to the hospital for more than simply laboratory investigation. Hospitalization provides an opportunity to observe the child, repeat the history and physical examination, analyze all available data, and investigate every potential diagnostic lead. In the Lohr and Hendley[54] series of 54 children with FUO, an incomplete history delayed establishing the diagnosis in 9 cases, and physical findings that were ignored delayed rendering the diagnosis in 4 cases. In McClung's report[65] of 99 pediatric cases of FUO, errors in the history or physical examination obscured the correct diagnosis for at least 10 patients. Failure to use existing laboratory data correctly is another common factor preventing early determination of the diagnosis in children with FUO.[54,78]

Clinical Evaluation

The first and most important step in the diagnostic work-up of a child with FUO is obtaining a complete and detailed history and conducting a physical examination. The clinical evaluation must be thorough and careful, and it must be repeated frequently. Often, a patient or parent eventually recalls information that was omitted or forgotten when the initial history was obtained. Physical findings change, and abnormalities not originally present can appear subsequently. Lohr and Hendley[54] noted that in more than 25 percent of children admitted to the hospital with FUO, significant physical findings developed that were not present at the time of admission.

A detailed history should be obtained regarding contact with infected or otherwise ill persons and any exposure to animals, including pets and wild animals. The number of children with zoonotic infections is increasing each year. Immunization of domestic animals such as the dog against leptospirosis can prevent canine disease, but it does not prevent carriage, excretion, and transmission of this infection. A history of travel extending back to birth must be elicited. Re-emergence of histoplasmosis, coccidioidomycosis, blastomycosis, or malaria years after visiting or living in an endemic area can occur. Inquiring about prophylactic immunizations, precautions taken against the ingestion of contaminated food or water, and malarial prophylaxis is important. Questioning should include the possibility that rocks, soil, or artifacts from geographically distant regions may have been brought into the home, as well as the possibility that contact with persons who have visited distant countries has occurred. Even contact with insects can be important. Tick bites can be a clue to Rocky Mountain spotted fever or tick-borne relapsing fever. North American mosquitoes and some ticks carry a variety of arboviruses.

The physician should determine whether the patient has eaten game meat, raw meat, or raw shellfish. A history of pica should be sought routinely. Ingestion of dirt can suggest a diagnosis of visceral larva migrans, toxoplasmosis, or other infectious diseases. A detailed history regarding all medications, including topical agents and nonprescription items, must be elicited carefully. Any history of surgical procedures should be explored.

Questions designed to determine the genetic or ethnic background of the patient can reveal information that specifically suggests or largely excludes diagnoses such as nephrogenic diabetes insipidus (found in Ulster Scots), familial Mediterranean fever (found in Armenians, Arabs, and Sephardic Jews), familial dysautonomia (found in Jews), and Kikuchi-Fujimoto disease, a benign and self-limited histiocytic necrotizing lymphadenitis (found mostly in young Asian females and characterized by fever, lymphadenopathy, and malaise).

The history should be exacting regarding the duration, height, and pattern of the fever, as well as the circumstances under which temperature elevation occurs, whether the child appears ill or any signs or symptoms develop, and how well the fever responds to antipyretic drugs. A history of "fever" occurring only after exercise or late in the afternoon can indicate parental concern about normal variations in body temperature. A history of high fever occurring in the absence of malaise or other generalized signs can be a clue to factitious fever. The physician also should obtain a careful history regarding how well the fever has been documented. Has a thermometer been used, by whom, and in whose presence? A history of sweating and heat intolerance can indicate hyperthyroidism, whereas a history of heat intolerance with the absence of sweating can be a clue to ectodermal dysplasia.

Several investigators have found that neither the pattern of fever nor its duration was useful in pointing to or establishing a diagnosis in children with FUO.[54,78] However, occasionally the character of the fever can be helpful. *Intermittent fever* is characterized by a return of temperature to normal at least once daily. If the peak of fever is high and the rate of defervescence quick, this pattern often is referred to as *hectic* or *spiking*. Intermittent fevers suggest pyogenic infections but also occur with tuberculosis, lymphoma, and juvenile rheumatoid arthritis. In *remittent fever*, the temperature fluctuates but does not return to normal. A *sustained fever* pattern is characterized by persistent fever with little or no fluctuation and can occur in typhoid fever or typhus. Antipyretic agents can make a remittent or sustained fever appear intermittent. *Relapsing fever* refers to a pattern in which the patient is afebrile for 1 or more days between episodes of fever and can be seen with malaria, rat-bite fever, infection with *Borrelia*, and lymphoma. Recurrent episodes of fever of more than a year's duration can suggest metabolic defects, central nervous system abnormalities in temperature control, and immunodeficient states.

The general activity and appearance of the patient should be observed, vital signs checked, and growth parameters measured. Weight loss is an important, though nonspecific, finding. Impairment of linear growth or short stature can be a clue to inflammatory bowel disease, an intracranial lesion involving the pituitary gland, or a long-standing chronic disease. Examining the patient during an episode of fever to observe the presence or absence of sweating, the effect of the fever on the heart and respiratory rate, the presence or absence of malaise or other symptoms, and the appearance of "toxicity" is helpful. The rash of juvenile rheumatoid arthritis characteristically is evanescent and may be present only during periods of temperature elevation.

Some special aspects of the physical examination merit mention. Hypohidrosis, anomalous dentition, and sparse hair, particularly involving the eyebrows and eyelashes, suggest anhidrotic ectodermal dysplasia. Palpebral conjunctivitis can be a clue to the presence of infectious mononucleosis, Newcastle disease, or lupus erythematosus, whereas predominantly bulbar conjunctivitis can suggest leptospirosis or Kawasaki disease. Phlyctenular conjunctivitis can signal tuberculosis.

Absence of the pupillary constrictor response can be caused by a deficiency of the constrictor sphincter muscle of the eye. This muscle, derived from ectoderm rather than mesoderm, develops embryologically at the same time that hypothalamic structures and function are undergoing differentiation. Absence of this muscle can suggest that the elevation in temperature is the result of hypothalamic or autonomic dysfunction. Careful funduscopic examination can disclose evidence of miliary tuberculosis, vasculitis, or toxoplasmosis. Lack of tears, absence of corneal reflexes, and a smooth tongue with absence of the fungiform papillae suggest familial dysautonomia.

Purulent or persistent nasal discharge can be a sign of sinusitis. The physician should palpate for tenderness over the sinuses.

Hyperemia of the pharynx, even in the absence of exudate or specific symptoms, can be a clue to the diagnosis of infectious mononucleosis, cytomegalic inclusion disease, toxoplasmosis,

tularemia, or leptospirosis. Gingival hypertrophy or inflammation and loosening or loss of teeth can indicate leukemia or Langerhans cell histiocytosis.

The bones and muscles should be palpated carefully. Tenderness over a bone can be found in cases of osteomyelitis or marrow invasion by neoplastic disease. Muscle tenderness can be associated with trichinosis, dermatomyositis, polyarteritis, or various arboviral infections.

The search for skin lesions and rash must be careful, extensive, and repeated. Petechiae can indicate endocarditis or other sources of bacteremia but also can occur with viral and rickettsial infections. A seborrheic rash can be a sign of histiocytosis.

A careful rectal examination is imperative for patients of all ages and can reveal pararectal tenderness or a mass indicative of a pelvic abscess or tumor. A test for occult blood should be performed on any stool found on the examining finger. Examination of the external genitalia should be completed on patients of all ages, and sexually active adolescent females should undergo a pelvic examination.

Laboratory Evaluation

The extent of laboratory investigation depends on the age of the patient, duration of the fever, and history and physical examination findings. Laboratory studies should be directed, as much as possible, toward the most likely diagnostic possibilities. The tempo of the diagnostic evaluation should be adjusted to the severity of the illness. In a critically ill child, speedy evaluation is important. If the patient is less severely ill, however, the evaluation can proceed more slowly; sometimes the fever can disappear without apparent explanation before a definitive diagnosis can be established and any invasive diagnostic procedures have been undertaken.

A complete blood count and careful examination of the peripheral smear are indicated for all patients. Anemia, thrombocytosis, and thrombocytopenia should be noted. Although mild or moderate changes in the total WBC or differential count usually are of no help, in some series, children with more than 10,000 polymorphonuclear leukocytes or 500 nonsegmented neutrophils/mm^3 were found to have a greater likelihood of having SBI.[89,94] Atypical lymphocytes generally indicate viral infection, whereas bizarre or immature forms can suggest leukemia. Although the erythrocyte sedimentation rate is of no specific diagnostic value, it is a general indicator of inflammation and can help in ruling out factitious fever, determining the need for further evaluation, and monitoring the progress of the disease process.

Blood should be obtained from all patients for aerobic and anaerobic culture. In select cases, media appropriate for the isolation of *Francisella* organisms, *Leptospira*, and *Spirillum* also should be used.

Urine analysis and culture should be completed for all patients. In one series of FUO in children, failure to perform urinalysis and failure to investigate pyuria adequately were the most common laboratory errors.[65] Radiographic study of the urinary tract, however, should be performed only when indicated.

All patients should undergo radiographic examination of the chest. Diagnostic imaging of the nasal sinuses, mastoids, and gastrointestinal tract is performed initially only for specific indications but should be done eventually in all children whose fever persists without explanation for a long period. Persistent fever and elevation of the erythrocyte sedimentation rate, with or without anemia, abdominal complaints, anorexia, and weight loss, are sufficient indications for radiographic study to rule out inflammatory bowel disease.

All patients should have an intradermal tuberculin skin test. Control skin tests with antigens such as *Candida* are of limited value because the anergy may be specific for tuberculosis rather than universal for all skin-testing materials.[55,67,71,72] A positive control test result and negative tuberculin test result do not rule out tuberculosis.

Bone marrow examination is most useful in diagnosing cancer (especially leukemia), histiocytic disorders, and hemophagocytic disease. It is less useful in determining infection. Hayani and associates[41] reviewed the results of 414 bone marrow examinations for FUO in children. In only one case was an organism (*Salmonella* group D) recovered from the marrow that also was not recovered from blood or another source. Noninfectious causes of FUO were found in 8 percent of specimens: malignancy (6.7%), hemophagocytic syndrome (HPS, 0.7%), histiocytosis (0.5%), and hypoplastic anemia (0.2%). In most of these cases, the diagnosis had been suspected clinically before the bone marrow was examined.

All patients should undergo a serum test for human immunodeficiency virus infection. Other appropriate serologic tests can help establish a diagnosis of cat-scratch disease, brucellosis, tularemia, Epstein-Barr virus infection, cytomegalic inclusion virus infection, other viral infections, toxoplasmosis, and certain fungal infections.

Hepatic enzymes and serum chemistry, including electrolytes, urea nitrogen, and creatinine, should be determined in all patients. Serum antinuclear antibody should be measured in those older than 5 years. Serum hepatitis antigens, electrocardiography, electroencephalography, echocardiography, and stool culture and examination for ova and parasites generally should be performed in selected cases. Other tests to be considered for individual patients include ophthalmologic examination by slit lamp, radiographic bone survey, technetium bone scan, liver-spleen scan, and abdominal imaging by ultrasonography or computed tomography.[24,77] Computed tomographic scanning, gallium scanning, and indium 111 scanning[37] can detect inflammatory lesions and tumors. Such scanning procedures offer a relatively noninvasive technique for screening patients with FUO for a variety of disorders. Although Steele and associates[88] found that radionucleotide scans seldom led to unsuspected diagnoses in children and suggested that they not be used indiscriminately, gallium scanning has been helpful in diagnosing adult patients with FUO,[40] and it may be a reasonable test for selected children. Lymph node biopsy, liver biopsy, and exploratory laparoscopy are reserved for patients with evidence of involvement of these organs.

In general, antibiotics or other medications should not be administered empirically as a diagnostic measure in children with FUO. Exceptions include the use of nonsteroidal agents in children with presumed juvenile rheumatoid arthritis and the use of antituberculous drugs in critically ill children thought to have disseminated tuberculosis. Empiric trials of broad-spectrum antibiotics generally do more to obscure than illuminate the etiology of FUO and can mask or delay establishing the diagnosis of infections such as meningitis, parameningeal infection, endocarditis, or osteomyelitis.

Examples of disorders that can be manifested as FUO in children are listed in Table 70–2. A few of these disorders are discussed briefly in the following sections.

INFECTIOUS CAUSES OF FEVER OF UNKNOWN ORIGIN

Infectious causes of FUO can be divided into systemic and localized. Immunodeficient states may be considered under the general classification of infections.

Generalized Infections

BRUCELLOSIS

The manifestation of this disease as FUO is explained by the nonspecific symptoms that it engenders and by the chronicity of

TABLE 70–2 Causes of Fever of Unknown Origin in Children

Infectious Diseases

Bacterial
Bacterial endocarditis
Brucellosis
Cat-scratch disease
Leptospirosis
Liver abscess
Mastoiditis (chronic)
Osteomyelitis
Pelvic abscess
Perinephric abscess
Pyelonephritis
Salmonellosis
Sinusitis
Subdiaphragmatic abscess
Tuberculosis
Tularemia

Viral
Cytomegalovirus
Epstein-Barr virus (infectious mononucleosis)
Hepatitis viruses

Chlamydial
Lymphogranuloma venereum
Psittacosis

Rickettsial
Q fever
Rocky Mountain spotted fever

Fungal
Blastomycosis (nonpulmonary)
Histoplasmosis (disseminated)

Parasitic
Malaria
Toxoplasmosis
Visceral larva migrans

Unclassified
Sarcoidosis

Collagen Vascular Diseases
Juvenile rheumatoid arthritis
Polyarteritis nodosa
Systemic lupus erythematosus

Malignancies
Hodgkin disease
Leukemia/lymphoma
Neuroblastoma

Miscellaneous
Central diabetes insipidus
Drug fever
Ectodermal dysplasia
Factitious fever
Familial dysautonomia
Granulomatous colitis
Hemophagocytic syndrome
Infantile cortical hyperostosis
Kikuchi-Fujimoto disease
Nephrogenic diabetes insipidus
Pancreatitis
Periodic fever
Serum sickness
Thyrotoxicosis
Ulcerative colitis

untreated infection. Many physicians, particularly in urban areas, tend to ignore the possibility of this disease and neglect to inquire about a history of exposure to animals or animal products, especially the consumption of unpasteurized goat's milk cheese (see Chapter 139).

CAT-SCRATCH DISEASE

During recent years, many children with FUO have proved to be infected with *Bartonella henselae*. Cat-scratch disease is one of the most common causes of FUO in patients seen at the infectious disease service at Texas Children's Hospital in Houston.[4] Most of the children with this manifestation of cat-scratch disease have hepatosplenic involvement. Jacobs and Schutze[45] reported that *B. henselae* infection was the cause of 4.8 percent of all cases of FUO at the Arkansas Children's Hospital and 10.9 percent of the cases of FUO caused by infection. *B. henselae* infection is best diagnosed by serologic evaluation (i.e., immunofluorescence assay that detects serum antibody to *B. henselae*). Biopsy of lesions (e.g., lymph nodes, liver, bone marrow) may allow visualization of bacilli with the Warthin-Starry silver stain; however, this finding is not specific for *B. henselae*. Management of patients with cat-scratch disease is primarily symptomatic because the disease usually is self-limited. Antimicrobial therapy can be helpful in acutely or severely ill patients, especially those with hepatosplenic disease. Several oral antimicrobial regimens (rifampin, trimethoprim-sulfamethoxazole, azithromycin) and parenteral gentamicin have been used successfully for the treatment of this disease. In particular, rifampin at a dose of 20 mg/kg/day in two divided doses for 14 days has been particularly efficacious.[4] However, the optimal duration of therapy is not known.

LEPTOSPIROSIS

Leptospirosis is caused by a single family of organisms composed of multiple serotypes; it is one of the most widespread zoonoses in the world. Transmission of infection from animal to human can occur by direct contact with the blood, tissue, organs, or urine of infected animals or indirectly by exposure to an environment that has been contaminated by leptospires. The organism also can be acquired from soil or from fresh water after ingestion. Reports indicate that leptospirosis is not a rare disease, that many infections are not associated with occupational exposure, and that urban and suburban cases are becoming more prevalent.[26] Clinical manifestations of leptospirosis usually are not specific. A variety of laboratory aids are available, but specimens must be collected and handled properly. In some cases, establishing a definitive diagnosis may be impossible; negative cultures or failure to demonstrate a rise in antibody titer does not exclude the possibility that the patient has active infection because the organism may not be present in the specimens that have been cultured, the antibody titer may have peaked before an acute-phase specimen was collected, and antibiotic therapy may suppress the development of positive titers or delay their appearance (see Chapter 154).

TOXOPLASMOSIS

Toxoplasmosis should be considered in any child with persistent fever. Cervical or supraclavicular adenopathy is present in most cases, but occasionally fever is the only manifestation. The diagnosis is established by demonstration of a rising serologic titer; antibody to *Toxoplasma gondii* is so prevalent that demonstration of a high titer alone is not diagnostic of acute infection. Demonstration of *Toxoplasma* in tissue sections or body fluid is highly suggestive, although the organism can persist in tissue for years. Isolation of the parasite is not absolutely diagnostic of recent infection (see Chapter 235).

MALARIA

Malaria also should be considered in children with FUO. In addition to fever, splenomegaly usually is present. A history of travel to endemic areas should be sought, although malaria has occurred

in patients who never traveled outside the United States. Disease can occur even in persons who have taken antimalarial drugs when they visited the endemic region. A hiatus of several months can occur between the development of infection and the onset of symptoms. The infection can be transmitted from a person who has visited an endemic area to one who has not when an appropriate mosquito vector is present. Malaria also can be acquired by blood transfusion or by the use of needles and syringes contaminated by the parasite. Demonstration of malarial organisms on appropriately stained thin or thick smears of blood is diagnostic (see Chapter 231).

SALMONELLOSIS

Salmonella organisms are contaminants in many food products. In view of the nonspecific signs and symptoms with which salmonellosis can occur, its association with FUO in children is not surprising. Repetitive blood and stool cultures are most helpful in establishing a diagnosis (see Chapter 121).

TUBERCULOSIS

Tuberculosis is an important cause of FUO in children, as well as in adults. Nonpulmonary tuberculosis is manifested as FUO more frequently than pulmonary tuberculosis is, which usually is evident on routine chest radiographs. FUO occurs most commonly with disseminated tuberculosis or infection of the liver, peritoneum, pericardium, or genitourinary tract. Active disseminated tuberculosis has been well documented in children with negative results on chest radiography and tuberculin skin tests.[72,90] A high index of suspicion and a careful history of possible contacts can be the best diagnostic tools. Funduscopic examination can reveal choroid tubercles. Liver and bone marrow frequently are involved in children with miliary tuberculosis; liver biopsy specimens and bone marrow aspirates should be obtained and processed for morphologic evaluation and culture. If the chest radiograph yields abnormal results, cultures of gastric aspirates, sputum, or both should be obtained. Because nontuberculous mycobacteria (i.e., atypical organisms) are present in the gastric contents of normal individuals, demonstration of acid-fast organisms on smears of gastric secretion does not indicate disease necessarily. Rarely, a patient with tuberculous pericarditis has fever, weight loss, and weakness but no precordial pain or other specific cardiac complaints. Disseminated infection with atypical mycobacteria generally is seen in patients infected with human immunodeficiency virus (see Chapter 107).

TULAREMIA

Generally, failure to consider tularemia in children with FUO may be attributed to a lack of appreciation of the many sources of infection and the various routes of inoculation. The organism can be acquired from contact with a variety of animal species, as well as from ticks, mosquitoes, lice, fleas, flies, and contaminated water. The organism can penetrate mucous membranes and broken or unbroken skin, or it can be inhaled or swallowed. Patients and parents should be questioned about animal contact and the ingestion of rabbit or squirrel meat (see Chapter 144).

VIRAL INFECTIONS

Infection by most viruses produces an illness that is relatively brief. Exceptions to this rule include infections by cytomegalovirus, Epstein-Barr virus, hepatitis viruses, and certain arboviruses. In all of these diseases, symptoms are extremely variable and signs and symptoms frequently are nonspecific. The diagnosis can be established by appropriate cultures and serologic studies (see Section 17 and Chapter 264).

IMMUNODEFICIENCY

A variety of congenital and acquired immunodeficiency states can be manifested as FUO. Patients with immunoglobulin deficiencies (e.g., Bruton agammaglobulinemia) may have a long history of recurrent fever, with or without evident infection, whereas patients with abnormalities in lymphocyte function are more likely to have prolonged fever caused by persistent viral or parasitic infection.

Localized Infections

BACTERIAL ENDOCARDITIS

Infective endocarditis is an infrequent cause of FUO in children. Acute bacterial endocarditis tends to be fulminant in nature, but the subacute form begins insidiously, generally at the site of a preexisting cardiac lesion. Subacute bacterial endocarditis is a rare occurrence in infants and increases in frequency with advancing age. The organisms most commonly encountered are viridans streptococci, enterococci, *S. aureus*, and *Staphylococcus epidermidis*. The absence of a cardiac murmur does not exclude the possibility of endocarditis, especially when the infection is limited to the right side of the heart. Endocarditis also can occur in the absence of positive blood cultures, particularly in association with the following factors: use of antibiotics for an undefined febrile illness, right-sided cardiac lesions, prolonged duration of disease, infection by unusual organisms such as *Brucella* or *Coxiella burnetii*, and inadequate culture methods for the detection of infection with anaerobic organisms. Frequently associated laboratory findings include anemia, leukocytosis, and an elevated erythrocyte sedimentation rate. Several blood cultures (aerobic and anaerobic) should be obtained before starting antibiotics. Echocardiography and gallium scanning can reveal vegetations, but negative results do not rule out endocarditis (see Chapter 32).

BONE AND JOINT INFECTIONS

Infections of bones and joints usually can be diagnosed clinically but occasionally are manifested as FUO. This manifestation occurs commonly in young children who cannot explain where they hurt and is more likely to occur with osteomyelitis than with septic arthritis. Infection of the pelvic bones is implicated most often in this regard. Radioisotopic bone scan and magnetic resonance imaging are more sensitive than are plain radiographs of the bones (see Chapters 61 and 62).

INTRA-ABDOMINAL ABSCESSES

Subphrenic, perinephric, and pelvic abscesses may be manifested as FUO. A history of previous intra-abdominal disease or abdominal surgery or a history of vague abdominal complaints should heighten suspicion of an intra-abdominal collection of pus. The organisms involved most commonly are *S. aureus*, streptococci, *E. coli*, and anaerobic flora. Fever may be the only sign of a pelvic, perinephric, or psoas abscess. Urinalysis generally yields normal results, but the mass can be demonstrated by ultrasound examination, gallium scan, or computed tomography.

LIVER ABSCESS AND OTHER HEPATIC INFECTIONS

Pyogenic liver abscesses are encountered most frequently in immunocompromised pediatric patients but can be seen in otherwise normal children.[49] In some patients, persistent fever is the only finding. Blood cultures usually are sterile, and serum levels of liver enzymes generally are close to or within normal limits. Many patients have hepatomegaly and right upper quadrant abdominal tenderness. The diagnosis can be established by examination of the liver by ultrasonography, radioisotope scanning,

computed tomography, or magnetic resonance imaging; a body gallium scan also can yield positive results. Bacterial hepatitis and bacterial cholangitis can occur in the absence of jaundice and other specific signs of liver dysfunction.[98,101] Granulomatous hepatitis is not a specific disease but rather a syndrome characterized by granuloma formation within the liver. A specific cause cannot be determined in every case. Although most reported cases have been in adults,[86] pediatric cases do occur, particularly with Epstein-Barr virus infection and with cat-scratch disease. The diagnosis can be made by ultrasound or other diagnostic imaging (see Chapter 55).

UPPER RESPIRATORY TRACT INFECTIONS

Frequently, infections of the upper respiratory tract and related organs are manifested as FUO.[54,65,78] Although obvious signs or symptoms would be expected, the complaints often appear trivial and may be ignored. Reported cases of FUO have occurred in children with mastoiditis, sinusitis, chronic or recurrent otitis media, chronic or recurrent pharyngitis, tonsillitis, peritonsillar abscess, and nonspecific upper respiratory tract infection. A parapharyngeal inflammatory pseudotumor manifested as FUO has been reported in a 3-year-old girl in whom anemia and weight loss also developed. The cause never was discerned, but the symptoms resolved after surgical removal of the inflammatory mass.[27]

A syndrome of periodic fever has been associated with recurrent aphthous stomatitis, pharyngitis, and cervical adenitis. Symptoms recur at 4- to 6-week intervals, generally beginning abruptly and resolving spontaneously in 4 to 5 days. The cause of this syndrome remains unknown.[35]

NONINFECTIOUS CAUSES OF FEVER OF UNKNOWN ORIGIN

Central Nervous System Dysfunction

Children with severe brain damage can have dysfunctional thermoregulation, and body temperature in some of these patients can remain elevated for months. Cases of otherwise neurologically normal children who have had fever as a result of central dysfunction also have been reported. Berger[17] discussed a 16-year-old child with recurrent episodes of fever that were thought to represent a form of epilepsy but disappeared when treatment with phenytoin was begun. Wolff and associates[99] reported a 14-year-old child with cyclic episodes of fever, nausea, vomiting, and emotional disturbance caused by a central nervous system lesion.

Diabetes Insipidus

Central and nephrogenic diabetes insipidus can cause FUO in infants and young children. Polyuria and polydipsia may not be appreciated during infancy. Hyperthermia, weight loss, and peripheral vascular collapse can ensue. Signs of dehydration or an increased serum concentration of sodium suggests the diagnosis. The diagnosis is established by simultaneous measurements of urine and serum electrolytes and osmolality during periods of normal hydration and after carefully controlled periods of water deprivation. Serum levels of antidiuretic hormone also can be measured by radioimmunoassay.

Drug Fever

Nearly any medication can be associated with an allergic reaction, including fever. The offending agent may be a prescribed drug, an over-the-counter preparation, or a street drug such as amphetamine or PCP (1-[1-phenylcyclohexyl]piperidine, phencyclidine). Atropine, whether taken systemically or used topically in the form of eyedrops, can cause elevation of temperature. Phenothiazines and anticholinergic drugs can inhibit sweating and impair regulation of temperature. Epinephrine and related compounds can affect thermoregulatory control mechanisms and produce fever. Drug fever can be low-grade or high and spiking. Fever can be continuous or intermittent. Discontinuation of the drug generally is followed by disappearance of the fever within 48 hours, but it sometimes persists for as long as a month as a result of slow excretion of the offending agent.

Factitious Fever

A parent or patient may report the presence of fever that does not exist. The reading of the thermometer can be increased by immersing the bulb in hot liquid or by rinsing the mouth with hot liquid immediately before inserting the thermometer. Clues to factitious fever include absence of tachycardia, malaise, or discomfort despite a markedly elevated temperature; apparent rapid defervescence unaccompanied by diaphoresis; failure of the temperature curve to follow the normal diurnal variation of body temperature; hyperpyrexia; and normal temperature reading when the temperature is obtained rectally by someone who remains in attendance during the procedure. The presence of fever also can be confirmed or excluded by measuring the temperature of a freshly voided urine specimen. The current use of electronic thermometers in most hospitals decreases the possibility of factitious fever in that setting because the nurse or aide usually brings in the thermometer and stays in attendance during the relatively brief period of insertion. In more unusual cases, the patient or parent can induce fever by the injection of infective or foreign materials.

Familial Dysautonomia

Familial dysautonomia (Riley-Day syndrome), an autosomal recessive disorder, is characterized by autonomic and peripheral sensory nerve dysfunction. Eighty percent of patients are children of Jewish parentage, particularly Ashkenazi Jews. Defective regulation of temperature can result in hypothermia or hyperthermia.[30]

A careful history and physical examination can reveal the following: poorly coordinated swallowing movements that lead to recurrent aspiration and pneumonia; recurrent episodes of vomiting; excessive salivation; excessive or diminished sweating; diminished formation of tears; periods of hypotension, hypertension, or both; and erythema or blanching of the skin. The fungiform papillae of the tongue are absent or diminished in number, and the sensation of taste is deficient.[87] Self-mutilation or multiple sites of skin trauma can reflect diminished or absent pain sensation peripherally. Deep tendon reflexes are diminished; corneal reflexes are impaired; and mental deficiency, dysarthria, and emotional lability are common findings.

Excretion of vanillylmandelic acid in urine can be diminished, and excretion of homovanillic acid can be increased. Administration of histamine intradermally can produce a wheal but no flare or pain at the site of injection. Placement of methacholine (2.5%) into the conjunctival sac produces pupillary constriction in children with familial dysautonomia but no response in a normal child. Intravenous infusion of norepinephrine is followed by an exaggerated pressor response, and the hypotensive response to infusion of methacholine is increased.

Hemophagocytic Syndrome

HPS is characterized by prolonged fever, hepatosplenomegaly, cytopenia, and hemophagocytosis in the bone marrow, liver, spleen, or lymph nodes.[47,73] It is a life-threatening and unusual disorder in which uncontrolled proliferation of activated lympho-

cytes and histiocytes results in unregulated hypersecretion of inflammatory cytokines. HPS can be primarily a familial disease or can be manifested as a reactive process triggered by infection, malignancy, immunologic disease, or drugs.

The diagnosis of HPS is suggested by fever, hepatosplenomegaly, cytopenia in at least two cell lines, hypertriglyceridemia or hypofibrinogenemia, and an elevated ferritin level. Because HPS can be manifested initially as FUO and progress to masquerade as overwhelming sepsis, a high index of suspicion is required for establishing the diagnosis. Further investigation should include evaluation of bone marrow, cerebrospinal fluid, or lymph nodes for the presence of hemophagocytosis. Therapy should include treatment of the underlying infection or trigger, if one exists, in addition to appropriate immune modulation therapies. A substantial proportion of HPS cases progress rapidly to death despite administration of appropriate chemotherapy.[47,73]

Inflammatory Bowel Disease

Fever is a prominent feature in many children with inflammatory bowel disease.[28,52,96] A greater percentage of children than adults with regional enteritis have fever. Appropriate contrast-enhanced radiographic studies of the intestines should be undertaken in children with prolonged FUO, even in the absence of findings specifically referable to the gastrointestinal tract, especially if the erythrocyte sedimentation rate is elevated and if the patient has anemia, weight loss, failure of linear growth, or a positive stool guaiac test.

Ulcerative colitis can be manifested as FUO, though less commonly than with regional enteritis. In patients with ulcerative colitis, symptoms referable to the gastrointestinal tract generally are present at the time that the patient is febrile.

Infantile Cortical Hyperostosis

The cause of infantile cortical hyperostosis (i.e., Caffey disease) is unknown. The decreased incidence in recent years suggests an infectious, possibly a viral, cause. Spontaneous hyperplasia of subperiosteal bone begins during infancy and is associated with swelling of the overlying tissues. The skull, mandible, clavicles, scapula, and ribs are affected most frequently, but in some children the long bones and even the metatarsal bones can be involved. Most patients have persistent fever, sometimes as high as 40° C (104° F). Tenderness over the affected regions, irritability, elevated erythrocyte sedimentation rate, and leukocytosis are common findings. The diagnosis is established by the clinical picture in conjunction with radiographically demonstrated periosteal involvement.

Juvenile Rheumatoid Arthritis

Juvenile rheumatoid arthritis is a chronic inflammatory disorder that usually is manifested as one of three distinct syndromes: the systemic form, characterized by high, spiking temperatures (generally once or twice each day), evanescent rash, and lymphadenopathy; a polyarticular form; and a monarticular or pauciarticular form. Fever is associated with all three manifestations but occurs most commonly in the systemic form, in which case it is present in nearly 100 percent of patients. This form also is the one most likely to be manifested as FUO.[23] Arthritis may not develop for months to years after onset of the fever. The diagnosis often needs to be made by exclusion because serologic tests generally are negative.

Periodic Fevers

Familial Mediterranean fever is characterized by episodic fever and abdominal pain.[34] This disease, found in persons of Mediterranean ancestry, is inherited as an autosomal recessive trait. The pattern of recurrent fever, however, is irregular, with varying periods of normality between episodes of fever.

Reimann[83] called attention to a group of patients with recurrent episodes of fever at regular intervals, usually 7 to 21 days. Some of the patients had leukopenia and abdominal or thoracic pain. Reasons for the fever and its periodicity remain unknown. Patients with cyclic neutropenia frequently have fever during acute episodes, but not all the patients reported by Reimann had neutropenia. Cases of children with periodic fever and hyperimmunoglobulinemia D, with or without chills, cervical lymphadenopathy, and occasionally abdominal pain, have been reported,[33,39] primarily from Europe. The cause of the syndrome remains unknown.

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CHAPTER 71

Toxic Shock Syndrome

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Much has been learned about the pathogenesis and pathophysiology of toxic shock syndrome (TSS) since its initial description in 1978 by Dr. James K. Todd and colleagues.[305] The clinical illness is defined by the criteria listed in the case definition formulated for epidemiologic studies (Table 71–1). Though often confused with septic shock, TSS has unique clinical manifestations not generally noted in septic shock, including diffuse erythroderma, delayed desquamation of the palms and soles, conjunctival and pharyngeal hyperemia, muscle injury, rapidly accelerated renal failure, and gastrointestinal symptoms. The capillary leak syndrome, or rapid and massive loss of fluid from capillaries into the interstitial space, loss of peripheral vascular resistance, and subsequent multisystem end-organ failure further characterize this entity. The histopathologic findings are minimal and nonspecific, with extensive interstitial edema of all tissues and minimal perivascular mononuclear cellular infiltrates. TSS can recur after both menstrual and nonmenstrual cases. The highest recurrence rate of 65 percent was reported in a subset of untreated women with menstrual TSS who continued to use tampons during menses.

TABLE 71–1 Clinical Case Definition of Toxic Shock Syndrome

Clinical Findings
Fever: Temperature ≥38.9°C
Rash: Diffuse macular erythroderma
Desquamation: 1-2 wk after onset of illness, particularly on palms, soles, fingers, and toes
Hypotension: Systolic blood pressure ≤90 mm Hg for adults; <5th percentile by age for children <16 yr old; orthostatic drop in diastolic blood pressure ≥15 mm Hg from lying to sitting; orthostatic syncope or orthostatic dizziness
Involvement of three or more of the following organ systems:
Gastrointestinal: Vomiting or diarrhea at onset of illness
Muscular: Severe myalgia or creatinine phosphokinase level greater than twice the upper limit of normal for the laboratory
Mucous membrane: Vaginal, oropharyngeal, or conjunctival hyperemia
Renal: BUN or serum creatinine greater than twice the upper limit of normal or ≥5 white blood cells per high-power field in the absence of a urinary tract infection
Hepatic: Total bilirubin, AST, or ALT greater than twice the upper limit of normal for the laboratory
Hematologic: platelets <100,000/mm^3
Central nervous system: disorientation or alterations in consciousness without focal neurologic signs when fever and hypotension are absent
Negative results on the following tests, if obtained:
Blood, throat, or cerebrospinal fluid cultures; blood culture may be positive for *Staphylococcus aureus*
Serologic tests for Rocky Mountain spotted fever, leptospirosis, or measles
Case Classification
Probable: A case with 5 of the 6 clinical findings described above
Confirmed: A case with all 6 of the clinical findings described above, including desquamation, unless the patient dies before desquamation could occur

ALT, alanine transaminase; AST, aspartate transaminase; BUN, blood urea nitrogen.
From Wharton, M., Chorba, T. L., Vogt, R. L., et al.: Case definitions for public health surveillance. M. M. W. R. Recomm. Rep. 39(RR-13): 1-43, 1990.

When first described, TSS had unique geographic, age, sex, and racial characteristics. It was associated with menses, particularly tampon use, as well as with a phenotypically distinctive type of *Staphylococcus aureus*. In 1994, at least 42 percent of reported cases of TSS were nonmenstrual. *S. aureus* exotoxins now are recognized to be "superantigens," and the endogenous mediators produced by these exotoxins appear to mediate manifestations of the disease.

TSS toxin I (TSST-I) and the staphylococcal enterotoxins are extremely potent stimuli of the in vitro macrophage production of interleukin-1 (IL-1) and tumor necrosis factor-α (TNF-α) and the T-lymphocyte production of IL-2, lymphotoxin (TNF-β), and interferon-γ. These staphylococcal exotoxins are functionally bivalent mitogens that highly selectively bind to major histocompatibility complex (MHC) class II receptors on antigen-processing cells and to selected V_β elements of the T-cell receptor (TCR) specific for each toxin. They now are known as superantigens. Besides *S. aureus*, other bacteria, particularly *Streptococcus pyogenes*, also can produce exotoxins that function as superantigens and produce a toxic shock–like syndrome.

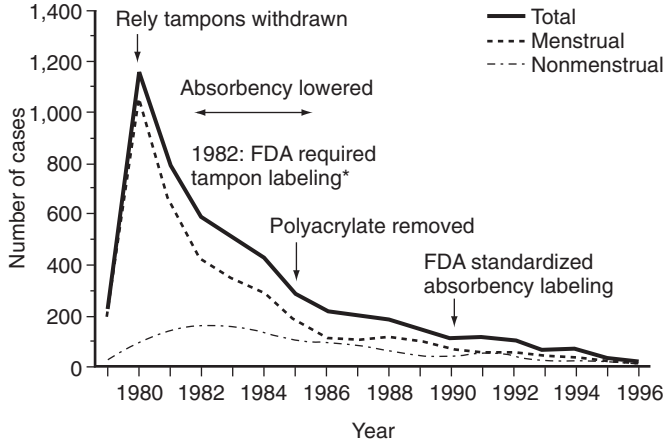
HISTORY

Illnesses resembling TSS and associated with *S. aureus* have been reported since 1927,[7,95,108,293,325] but the initial description of the illness as a disease of children was published in 1978.[305] A Kawasaki-like syndrome described in adults subsequently was recognized to be TSS.[201] The first 12 cases of TSS identified in Wisconsin and Minnesota between July 1979 and January 1980 were reported by state epidemiologists to the Centers for Disease Control and Prevention (CDC) in January 1980.[75,277,315] All 12 cases had occurred in women, and a possible association with

menses was noted. The probable recurrent nature of the illness also was reported.[75,315] In May 1980, the CDC reported findings of the first 55 nationally reported cases,[315] 95 percent of which occurred in women. Of 40 patients for whom a menstrual history was obtained, 38 (95%) had onset during menstruation. Thirteen patients had experienced recurrent episodes of TSS.

By June 1980, case-control studies statistically linking the occurrence of menstrual TSS with tampons had been completed by the Wisconsin Division of Health[75] and the CDC,[277] and similar trends had been noted by the Utah Department of Health.[162] In September 1980, the CDC reported that although TSS had been associated with many tampon brands, women using one particular brand of tampon, Rely (Procter & Gamble), were at greatest risk. This brand was withdrawn immediately and voluntarily from the market by the manufacturer.[24,120,264] Subsequent frequent updates by the CDC documented a decrease in reported cases.[316,317,320]

Microbiologic studies have established that most patients with menses-associated TSS (menstrual TSS) have evidence of vaginal or cervical colonization with *S. aureus*.[22] These strains of *S. aureus* made a characteristic toxin initially called *staphylococcal enterotoxin F*[23] and *pyrogenic exotoxin C*[270] and now known as *TSST-1*.



*FDA, Food and Drug Administration, including definite and probable toxic shock syndrome cases

Figure 71–1 Menstrual and nonmenstrual toxic shock syndrome cases reported to the Centers for Disease Control and Prevention by year, 1979 to 1996. *(From Hajjeh, R. A., Reingold, A., Weil, A., et al.: Toxic-shock syndrome in the United States: Surveillance update, 1979-1996. Emerg. Infect. Dis. 5:807-810, 1999.)*

EPIDEMIOLOGY

SURVEILLANCE AND INCIDENCE

Statewide surveillance for cases of TSS began in Wisconsin[77] and Minnesota[229] in January 1980, in Utah in February 1980,[175] and in other states after the national communications about TSS in spring 1980. In 1982, TSS became a nationally notifiable disease. Between 1983 and 1994, the CDC received reports of 4192 cases of TSS through the National Electronic Telecommunications System for Surveillance (NETSS).[296] The National Center for Infectious Diseases at the CDC maintains a database of TSS cases, including those reported before 1983. In addition, in January 1981, the National Center for Health Statistics recommended use of the International Classification of Diseases (ICD) code 040.89 for TSS, and a study involving reviews of records in 97 percent of Wisconsin general hospitals demonstrated that by 1983 the sensitivity and specificity of using this code in this review of discharge coding was 85 and 95 percent, respectively.[137] Since 1983, a continued downward trend in passively reported cases has been noted (Fig. 71–1).[330]

Of 2509 confirmed cases reported to the CDC through April 1984, 95 percent occurred in females.[249] Among the 2295 women with known menstrual histories, 89 percent had an onset of TSS associated with menstruation. Of 1716 menses-associated cases for which information related to tampon use was available, 99 percent occurred in tampon users and 1 percent in women using napkins or minipads.

The results of an active surveillance study conducted by the CDC in 1986 and 1987 in five states and Los Angeles County confirmed the trends previously noted in the CDC passive surveillance system.[127] The incidence of menstrual TSS was found to be 1 case per 100,000 women aged 15 to 44 years. This rate is a substantial reduction from the reported rates of 2.4 to 12.3 cases per 100,000 women of menstruating age in 1980.[75,175,229,241] Only 55 percent of the cases detected in the 1986-1987 study occurred in women, and 45 percent of all cases were menstrually associated.[38,127] The nationally reported TSS-related mortality rate for menstrual cases decreased from 5.5 percent in 1979 and 1980 to 2.8 percent in 1981 to 1986, then to 1.8 percent in 1987 to 1996 (chi-square for linear trend, $p = 0.0001$).[133]

The striking reduction in the incidence of menstrual TSS was attributed to a decrease in tampon absorbency, changes in composition of tampons and patterns of use, and the impact of publicity on early recognition of symptoms.[77,78,111,228,248,274] Since the 1980s, there has been a paucity of active surveillance data on TSS. Passive reporting during 2000 to 2003 in Minneapolis–St. Paul, Minnesota, suggested an increasing incidence of menstrual TSS cases from 0.8 per 100,000 to 3.4 per 100,000 women of menstrual age.[271] Subsequently, a medical record search for illnesses during 2000 to 2003 that had TSS-based, ICD-9 discharge codes (040.82 or 040.89) and a 20 percent random sample of five other shock or sepsis codes with review of cases was conducted in 25 Minneapolis–St. Paul metropolitan area hospitals. The incidence was not calculated, but the number of menstrual TSS cases (n = 23) was similar to the number of nonmenstrual cases (n = 21), and the gradual increase in total cases from 2000 (n = 9) to 2003 (n = 13) was not significant. Cases associated with methicillin-resistant *S. aureus* (MRSA) and new sites of staphylococcal infection (lung, urinary tract) were noted.[86]

In 1986 and 1987, the incidence of nonmenstrual TSS in women (0.25 case per 100,000 women) and TSS in men (0.16 case per 100,000 men) had changed little since 1980. However, with the declining trend in menstrual TSS, nonmenstrual TSS has accounted for a greater proportion of overall TSS cases. In 1994, only 192 cases of TSS were reported to the CDC through the NETSS (0.1 case per 100,000 population). Among confirmed cases, 42 percent were nonmenstrual.[296] The proportion of nonmenstrual cases reported after surgical procedures increased from 14 percent during 1979 to 1986 to 27 percent during 1987 to 1996.[133] Overall, no significant change in the case-fatality ratio for nonmenstrual cases has occurred: 8.5 percent for 1979 and 1980, 5.3 percent for 1981 to 1986, and 6 percent for 1987 to 1996.[38,133]

Although cases in the United States have been reported in all 50 states and the District of Columbia, significant differences in incidence were noted among the states through 1983.[249] Through mid-1983, the five states of Wisconsin, Minnesota, Colorado, Utah, and California accounted for 44 percent of the total reported cases but represented only 16 percent of the U.S. population.[309] Although intensified surveillance may have been a factor, regional differences in the degree of immunity to TSS-associated *S. aureus* toxins and in the distribution of toxin-producing organisms may have been important factors.[322]

Geographic differences in the occurrence of TSS continued even with the increase in the relative proportion of nonmenstrual cases.[38]

RISK FACTORS FOR MENSTRUAL TOXIC SHOCK SYNDROME

From early 1980 through 1990, most reported cases of TSS occurred in previously healthy, young white menstruating women who were using tampons at the time of onset of the illness. The explanation for this combination of risk factors is complex.

Age

The median age of patients with confirmed menstrual TSS (median age: 21 years, 1979 to 1980; 20 years, 1981 to 1986; 25 years, 1987 to 1996) has varied little since 1980.[127,133,249] Roughly a third of cases occur in adolescents 15 to 19 years of age[249] (Fig. 71–2). The mean age of patients with nonmenstrual TSS (27 years through 1982, 30 years in 1986) is significantly higher than that for menses-associated cases.[127,250] The precise reason for the increased incidence of TSS in adolescent girls is not known, but a lower prevalence of antibody to TSST-I may increase susceptibility in this age group.[324]

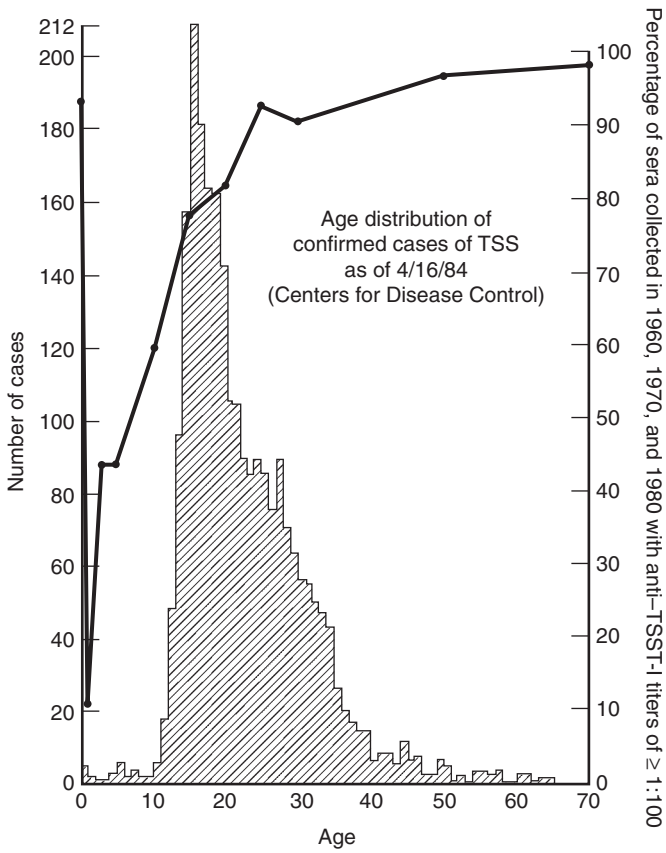


Figure 71–2 Age distribution of patients with confirmed toxic shock syndrome (TSS) reported to the Centers for Disease Control and Prevention before April 16, 1984. The age-specific prevalence of antibodies to toxic shock syndrome toxin I (TSST-I) in a normal population in Wisconsin in the years 1960, 1970, and 1980 is indicated by the *solid connected line*. *(Data from Vergeront, J. M., Stolz, S. J., Crass, B. A., et al.: Prevalence of serum antibody to staphylococcal enterotoxin F among Wisconsin residents: Implications for toxic shock syndrome. J. Infect. Dis.* 148*:692-698, 1983.)*

Race

A striking race-ethnicity distribution is present for menstrual TSS; 97 percent of such cases have occurred in whites, who make up 83 percent of the U.S. population. This distribution is not as striking for nonmenstrual TSS in that 87 percent of nonmenstrual cases have occurred in whites. The reasons for racial differences are not clear and only partially explained by differences in menses-related practices.[111,249,250] Racial differences in antibody to TSST-I may explain to some degree the differences in the distribution of cases.[322]

Menstruation and Tampon Use

The initial observations in 1980 that a high proportion of patients with TSS had an onset during menses is well documented now. The use of tampons as a significant risk factor for the development of TSS was established clearly in six case-control studies conducted in 1980[75,138,162,227,264,277] and one conducted in 1987.[127] Even though these studies varied in design and methodologic technique, all demonstrated that in at least 97 percent of affected individuals, use of tampons during menstrual periods was associated with disease onset. Although 76 to 89 percent of matched control individuals used tampons during temporally comparable menstrual periods, the use of tampons was associated with menstrual TSS in each of these studies, with odds ratios ranging from 11 to 18.[38]

Despite the fact that TSS has occurred and continues to occur with the use of all brands of tampons, the second CDC case-control study demonstrated a greater relative risk for menstrual TSS with the use of one tampon brand, Rely.[120,264] The Tri-State (Minnesota, Wisconsin, and Iowa) TSS Study established that tampons of increasing absorbency were associated with an increasing relative risk for the development of menstrual TSS and that the risk of development of menstrual TSS associated with Rely tampons was greater than that predicted by absorbency alone.[227]

Reasons listed earlier for the striking reduction in incidence of menstrual TSS may be the decrease in absorbency of tampons and changes in composition of tampons.[248] In 1980, products very high in absorbency were used by 42 percent of tampon users.[229] By 1983, this proportion had decreased to 18 percent and, by 1986, to 1 percent.[248] Overall, the absorbency of available tampon brand styles, as measured by the Syngina test,[217] ranged from 10.3 to 20.5 g in 1980 to less than 6 to 15 g in 1990, indicative of an industry-wide decrease in tampon absorbency. The composition of tampons also has changed since 1980. Tampons currently available are composed of cotton or cotton and rayon combinations. In 1980, additives included polyacrylate, polyester foam, cross-linked carboxymethylcellulose, and several surfactants, including Pluronic L-92.[267] Whether these additives enhanced the risk for development of TSS independently or in combination with their increasing absorbency is unclear.[24,227,264]

Two subsequent case-control studies conducted by the CDC and involving patients with an onset of TSS in 1983 and 1984 (CDC III) and 1986 and 1987 (CDC IV) confirmed the Tri-State TSS Study results of a linearly increasing risk for TSS related to increasing tampon absorbency; for each 1-g increase in absorbency, the risk for TSS increased by 34 to 37 percent.[24,116] In addition, the Tri-State TSS Study and the CDC III study both suggested that the effects of absorbency and chemical composition of tampons on TSS risk were independent.[24,38,227]

Continuous use of tampons was associated with a greater risk for the development of TSS than noncontinuous use was in one case-control study[277]; however, this finding was not confirmed in a subsequent investigation.[227] No association between TSS and the frequency of changing tampons has been demonstrated.

As a result of these studies demonstrating the importance of tampons in menses-associated TSS, as well as the voluntary

withdrawal of Rely, several other changes in the manufacture, labeling, and distribution of tampons have occurred. After a request by the Food and Drug Administration (FDA) in November 1980 that tampon manufacturers place warnings on tampon packages, on June 22, 1982, an FDA regulation required that information on TSS and tampon-associated risks appear on tampon packages.[90] In April 1985, two additional tampon manufacturers withdrew their superabsorbent tampons containing polyacrylate from the market on the basis of an in vitro study[208] and a judicial decision.[222] In 1990, the FDA published the in vitro rating system to be used by manufacturers to inform the public about tampon absorbency.[182,217,301]

RISK FACTORS FOR NONMENSTRUAL TOXIC SHOCK SYNDROME

Before 1986, less than 20 percent of confirmed cases of TSS reported to the CDC were nonmenstrual. In the 1986 and 1987 multistate active surveillance study (CDC IV), 54 percent of cases were found to be nonmenstrual.[127] Although the mean age of nonmenstrual cases is higher than that of menstrual cases and the ratio of males to females with nonmenstrual TSS is closer to that in the general population, the clinical findings and complications of nonmenstrual TSS are the same as those occurring in menstrual TSS. The number of confirmed cases of TSS reported in children younger than 10 years of age is surprisingly low, particularly in light of their demonstrated low antibody titers to TSST-I and the increased prevalence of nasal colonization (10%) with TSST-I–positive strains.[38,153,324,334]

Data on risk factors (Table 71–2) were obtained for 559 nonmenstrual TSS cases reported to the CDC through 1986.[38] Associated infections or procedures included nonsurgical cutaneous and subcutaneous infections, 22 percent; childbirth or abortion, 15 percent; infections after a wide variety of surgical procedures, 15 percent; vaginal infections occurring at times other than during menses, 6 percent; vaginal contraceptive sponge use, 5 percent[73]; diaphragm use, 6 percent; and other or unknown sources of infection, 31 percent. The proportion of all nonmenstrual cases reported after surgical procedures increased from 14 percent during 1979 to 1986 to 27 percent during 1987 to 1996. The proportion of female nonmenstrual case patients using barrier contraceptives was significantly less in 1987 to 1996 (6%) than in 1979 to 1986 (14%).[133]

In 1994, nonmenstrual cases accounted for at least 42 percent of all cases of TSS reported to the CDC through the passive surveillance system.[296] The three risk factors necessary for the acquisition of nonmenstrual disease include colonization (or acquisition) of a toxin-producing strain of *S. aureus*, absence of protective antitoxin antibody, and an infected site. TSS has been reported in association with *S. aureus* infections of almost every type, including primary staphylococcal infections and those occurring after surgery, infections associated with disruption of skin or a mucous membrane, and infections occurring after placement of a foreign body[22] (see Table 71–2). Numerous patients with TSS have been reported for whom no obvious focus of infection was found.[38,250] Trauma or surgery in areas of the body frequently colonized with *S. aureus* (nose, skin, vagina) places individuals at enhanced risk for infection and subsequent TSS.

TABLE 71–2 Risk Factors for Nonmenstrual Toxic Shock Syndrome

I.	Colonization with toxin-producing *Staphylococcus aureus*
II.	Absence of protective antitoxin antibody
III.	Infected site
	A. Primary *S. aureus* infection
	Carbuncle
	Cellulitis
	Dental abscess
	Empyema
	Endocarditis
	Folliculitis
	Mastitis
	Osteomyelitis
	Peritonitis
	Peritonsillar abscess
	Pneumonia
	Pyarthrosis
	Pyomyositis
	Sinusitis
	Tracheitis
	B. After surgery: wound infection
	Abdominal
	Breast
	Cesarean section
	Dermatologic
	Ear, nose, and throat
	Genitourinary
	Neurosurgery
	Orthopedic
	C. Skin or mucous membrane disruption
	Burns (chemical, scald, etc.)
	Dermatitis
	Influenza
	Pharyngitis
	Postpartum (vaginal delivery)
	Superficial/penetrating trauma (insect bite, needle-stick)
	Viral infection
	Varicella
	D. After surgical or nonsurgical foreign body placement
	Augmentation mammoplasty
	Catheters
	Diaphragm
	Sponge (contraceptive)
	Surgical prostheses/stents/packing material/sutures
	E. No obvious focus of infection (vaginal or pharyngeal colonization)

HOST RISK FACTORS: GENERAL

Colonization with Exotoxin-Producing *Staphylococcus aureus*

For TSS to develop, an individual must be colonized with or acquire a strain of *S. aureus* that produces TSST-I or one of the staphylococcal enterotoxins. Evidence that staphylococcal enterotoxin B (SEB) alone and, uncommonly, staphylococcal enterotoxin A (SEA) or staphylococcal enterotoxin C (SEC) alone, as well as TSST-I, may be responsible for the manifestations of TSS is compelling. Although many genotypically different strains of *S. aureus* possess the *tst*H gene, one clone has been isolated from 88 percent of menstrual cases and 54 percent of nonmenstrual cases.[214] Colonization with this clone, particularly in menstruating women, clearly provides a significant risk factor. Future work may identify other clones with unique adherence properties to other mucosal or skin sites.

Absence of Protective Antibody Levels

A necessary factor for the development of TSS is the absence of protective antibody levels for the toxin (TSST-I or enterotoxin) produced by the isolate associated with TSS. Thus, more than 90 percent of women with menstrual TSS associated with TSST-I–positive strains have antibody titers of 1 : 10 or lower, whereas a titer of 1 : 100 or higher is considered protective.[22] Because most

adults acquire these antibodies without the development of disease, absent antibody levels may be the result of lack of exposure to a toxin-producing organism or a lacunar inability to respond to the toxin. The failure of most patients, menstrual and nonmenstrual, to form antibody during convalescence may reflect this lacunar nonrecognition of staphylococcal protein antigen, or it may be a reflection of the superantigenic nature of these proteins and failure of the toxin proteins to be presented to the TCR as conventional antigens. In mice and one patient with TSS, in vivo TSST-I–induced proliferation was followed by hyporesponsiveness of TSST-I–responsive $V_\beta 2^+$ T cells.[181]

Interruption of a Mucosal or Skin Surface

Primary deep-tissue staphylococcal infections (e.g., osteomyelitis, pyarthrosis, pyomyositis,[4] endocarditis,[242,331] renal carbuncles, bacteremia) rarely are associated with TSS. Most nonmenstrual cases occur in patients with an altered skin or mucosal surface. Examples of skin disruption associated with TSS include burns, insect bites, needle-sticks, surgical incisions, and varicella. Cases of disruption of mucous membranes can be divided into those associated with respiratory and those associated with genital mucosae. Damage to respiratory mucosa may occur after nasal or other surgery, particularly with placement of stents or packing, in association with a viral respiratory infection such as influenza, or as a result of primary sinusitis, tracheitis, or a parapharyngeal abscess. Heavy colonization of the pharynx also has been associated with TSS. The vaginal mucosa may be damaged in numerous ways, including during placement of tampons or barrier contraceptives,[105,275,313] postpartum,[22,233] or after genital surgery or genital mucosal damage.[106,231,257] Heavy colonization of the vagina without any other apparent risk factor likewise has been associated with TSS.[250] These mucosal infections may provide the right conditions for production of TSST-I, including an aerobic environment, high carbon dioxide concentration, neutral pH, high protein and low glucose concentrations, and low to normal magnesium concentration.[296]

Presence of a Foreign Body

Tampons create an aerobic environment in the vagina, which normally is anaerobic. Tampons of three different types have been shown in humans to change the partial pressure of oxygen of the vaginal wall from an anaerobic environment to that of atmospheric air throughout the 90-minute interval after insertion.[326] Because oxygen is required for the production of TSST-I, researchers have suggested that tampons with enhanced absorbency allow the introduction of increasing concentrations of oxygen to enhance production of toxin.

Several alternative explanations for the role of tampons have been proposed. Tampons may remove vaginal substrates that normally inhibit the growth of *S. aureus.*[22] Another suggested role for tampons is that of inducing cervicovaginal ulceration. Such ulcers have been suggested to enhance bacterial growth or toxin absorption and expose submucosal fibronectin for binding of *S. aureus*.[68,70,329] Tampons can induce chronic cervicovaginal ulcers after long-term continuous use or superficial micro-ulcerations after a brief insertion in otherwise healthy women. However, vaginal ulcerations of the type typically seen in TSS have been found during postmortem examination in women who had never used tampons. This finding suggests that vaginal ulceration, such as that seen in the esophagus and bladder in TSS, may be induced by TSST-I.[174]

Investigations to determine whether individual tampon components can induce or amplify the production of TSST-I in vitro have provided conflicting data, in part because no consensus exists on how best to test tampons and their components to determine their potential to increase risk for the development of TSS. In one setting, although bacterial growth was unaffected, production of TSST-I varied from undetectable levels to 300 μg/mL, depending on the particular brand and style of tampon studied.[22] Other investigators have found that most tampons tested were inhibitory to both bacterial growth and production of TSST-I and that none consistently increased the production of TSST-I.[22]

Two studies clearly demonstrated enhanced production of TSST-I in vitro by the Rely tampon, which is composed of cross-linked carboxymethylcellulose and polyester foam.[238,268] The polyacrylate rayon included in two tampons now removed from the market increased the production of TSST-I under certain conditions,[208] as did the surfactant Pluronic L-92 used in at least one tampon.[268] Neither cotton nor rayon amplifies the production of TSST-I in vitro, nor do cotton tampons adsorb TSST-I or prevent its production.[238,268] Thus, cotton tampons cannot be claimed to be safer than cotton/rayon combinations, although this contention has been disputed.[303] The role of magnesium in controlling the production of TSST-I in the presence of tampons is unclear.[208]

Implanted foreign material such as sutures, central venous lines, and metallic or polymeric implants have been documented repeatedly to enhance the risk of acquiring bacterial infection.[22,155] These infections are characterized by limited spread beyond the tissues in immediate contact with the implants, poor response to antibiotics, and poor healing without removal of the foreign material. Two important differences between TSS and other *S. aureus* infections related to foreign material are the added ability of the organism to produce a toxin that readily disseminates and the limited ability of these strains to produce inflammation.[107] Researchers have suggested that the increased number of surgically associated TSS cases may be related to the number of implanted materials.[133]

The enhanced risk of acquiring infection by *S. aureus* in the presence of foreign material has been defined experimentally in animals. Rats remained asymptomatic after the subcutaneous injection of more than 1×10^6 colony-forming units of *S. aureus*, whereas 3×10^2 colony-forming units invariably led to infection in the presence of a suture. In addition, the ability of sutured tissue to resist infection varies with the kind of material implanted, particularly its physical or chemical configuration. For example, bacterial adherence is eightfold higher for braided sutures of silk, silicone-heated blue polyester, and absorbable polyglycolic acid than for monofilament nylon.[155]

In addition to sutures, other foreign materials associated with TSS-related wound infection are Teflon splints, gauze packing, and mammary implants.[22]

OTHER POTENTIAL HOST RISK FACTORS

Other factors have been examined in an attempt to identify individuals who may be at increased risk for the development of TSS.[80] Some of these factors have included HLA typing,[22] neutrophil function,[140] adherence of *S. aureus* to vaginal epithelial cells,[20] alteration of the cervicovaginal flora,[22] hormonal factors, and personal hygiene practices.[80] Of all these factors, those of potential importance included cervicovaginal flora and hormonal factors.

Vaginal co-colonization with *S. aureus* and one of the Enterobacteriaceae has been postulated to enhance the risk of development of TSS.[55] Prospective examination of 495 healthy women revealed a 7 percent vaginal colonization rate for *S. aureus* TSST-I–positive strains. Women who were colonized with toxin-producing *S. aureus* also were colonized with *Escherichia coli* or other Enterobacteriaceae statistically significantly more often than were women colonized with non–toxin-producing or no *S. aureus*. *E. coli* isolation rates were 54 percent in women with

TSST-I–positive isolates, 15 percent in women with TSST-I–negative isolates, and 11 percent in women with no *S. aureus*. Co-isolation of *E. coli* was the only identified factor associated with vaginal carriage of TSST-I–positive *S. aureus*. Additionally, among the 14 patients with TSS monitored in this study, 9 had *E. coli* as well as TSST-I–positive *S. aureus* co-isolated during the acute TSS episode. The significance of these observations and their relationship to postulated roles for endotoxin in TSS are not clear.

The results of early case-control studies suggested that oral contraceptive steroids may have an effect on vaginal *S. aureus* organisms that may produce or release TSS-associated toxins[75,227] and that this effect was protective. However, one case-control study found no protective effect or enhanced risk associated with oral contraceptive use.[127] Hormonal control is known to be responsible for numerous cyclic changes in vaginal pH and flora, but the role of hormonal factors in the pathogenesis of TSS has not been examined well.

HISTOPATHOLOGY

The histopathologic findings on postmortem examination support the concept that TSS is a toxin-mediated disease.[1,28,36,174,230,333] Striking histopathologic similarities exist between patients with TSS and those with "scarlet fever" reported in 1936.[36] Typically, a total absence of tissue invasion by bacteria and minimal evidence of an inflammatory reaction in most organs are noted. Findings thought to be due to a direct effect of the toxin or mediators (or both) and unrelated to hypoperfusion have included subepidermal ulcerations in the cervix, vagina, esophagus, and bladder; depletion of lymphocytes in lymph nodes; a subepidermal cleavage plane in the skin; and mild inflammatory changes in the kidney, liver, heart, and muscle.

Cervicovaginal ulcerations are the only characteristic lesions noted in the genital tract of patients with fatal menstrual TSS, and such ulcers have been found in a patient with menstrual TSS who had never used tampons.[174,233] The ulcerations are superficial. Capillary vasodilation and thrombosis with inflammation of the mucosa are present, but no deep-tissue bacterial invasion is seen. The layer of vacuolization and separation in the ulcers occurs beneath the basal layer. The same type of ulcer also has been found in the bladder and esophagus, which suggests that these ulcerations may be caused by the toxins or mediators and not by the use of tampons.

Although the myocardium was described as normal in one postmortem series of TSS, in another series of eight fatal cases, all patients had evidence of focal round-cell infiltration with variable degrees of congestion, edema, and hemorrhage.[174,230] Myxoid degeneration was found in all heart valves from four patients in one series. Sections of skeletal muscle have demonstrated only congestion, edema, focal hemorrhage or fiber necrosis, and a mild acute inflammatory infiltrate.

Varying degrees of triaditis or periportal lymphocytic inflammation have been the most consistent findings in the liver; centrilobular congestion with necrosis and mild cellular degeneration also has been described.[150] In the kidney, toxin-mediated mononuclear interstitial nephritis may result from perivasculitis of the adventitia of the renal venules, lesions that probably precede the development of hypotension-induced acute tubular necrosis. The most characteristic findings in the spleen and lymph nodes have been lymphocyte depletion; inactive hypocellular, hypoplastic lymphoid follicles with edema; marked histiocytosis in the interfollicular areas; and hemophagocytosis.

Perivascular lymphocytic infiltrates and bullae that separate at the basement membrane are characteristic of the early skin changes in TSS.[8,10,140] No evidence of vasculitis has been reported.

CLINICAL SPECTRUM

ACUTE PHASE: MODERATE TO SEVERE DISEASE*

Multisystem end-organ damage secondary to loss of peripheral vascular resistance, loss of intravascular volume as a result of endothelial damage and the capillary leak syndrome, and interstitial edema constitute the most important mediator-induced changes of TSS. Prolonged hypotension, interstitial edema, and vascular congestion additionally may result in ischemic organ damage.

The onset of illness in patients with moderate to severe disease is abrupt, with symptoms and signs including fever, chills, malaise, headache, sore throat, myalgia, muscle tenderness, fatigue, vomiting, diarrhea, abdominal pain, and orthostatic dizziness or syncope (Figs. 71–3 and 71–4).

During the first 24 to 48 hours, diffuse erythroderma, severe watery diarrhea (often with incontinence), decreased urine output, cyanosis, and edema of the extremities may be noted. Some patients may have purpura fulminans.[168] Cerebral ischemia and edema rapidly result in somnolence, confusion, irritability, agitation, and occasionally hallucinations, even in individuals without hypotension. Patients with TSS have had signs and symptoms of encephalopathy, cerebral infarction, meningismus,[15,27,135,179,283] and the cauda equina syndrome.[8]

During initial physical examination of a moderately to severely ill patient, fever, tachycardia, tachypnea, a low or unobtainable blood pressure, erythroderma (generally not seen in patients with severe hypotension or in those without T cells)[159] (see Fig. 71–4A), and muscle tenderness are noted in conjunction with peripheral cyanosis and edema, conjunctival hyperemia, subconjunctival hemorrhages (see Fig. 71–4B), beefy red edematous mucous membranes, somnolence, disorientation, and agitation. In menstrual TSS, edema and erythema of the inner aspect of the thighs and the perineum may be noted in conjunction with normal findings on uterine and adnexal examination. In nonmenstrual cases, vaginitis or another focus of infection will be present. In most postoperative cases, the surgical wound is not inflamed. If erythroderma is present, it will be most intense surrounding the infected focus.

Surgical wounds and some abscesses colonized or infected with *S. aureus* and responsible for postoperative or nonmenstrual TSS may have minimal or no signs of inflammation.[6,17,93,250] The production of TNF-α by macrophages in response to TSST-I inhibits neutrophil mobilization in vitro,[107] which may provide an explanation for the absence of signs of inflammation. The incubation period for postoperative or postpartum TSS may be as short as 12 to 48 hours. Relatively few cases have been associated with deep-tissue infection.[22]

Laboratory Changes

Abnormalities in clinical laboratory tests will reflect the endogenous cytokine release, shock, and organ failure associated with TSS. Leukocytosis may not be present, but the total proportion of mature and immature neutrophils generally exceeds 90 percent. The proportion of immature neutrophils usually is 25 to 50 percent of the total number of neutrophils and is associated with a profound and absolute lymphopenia. Thrombocytopenia and anemia are present during the first few days and frequently are accompanied by prolonged prothrombin and partial thromboplastin times. Disseminated intravascular coagulation may be present. Sterile pyuria and cerebrospinal fluid pleocytosis are indicative of generalized involvement of the mucous membranes and serosal surfaces. Elevated blood urea nitrogen and creatinine

*See references 47, 56, 76, 114, 116, 139, 192, 277, 305, 311.

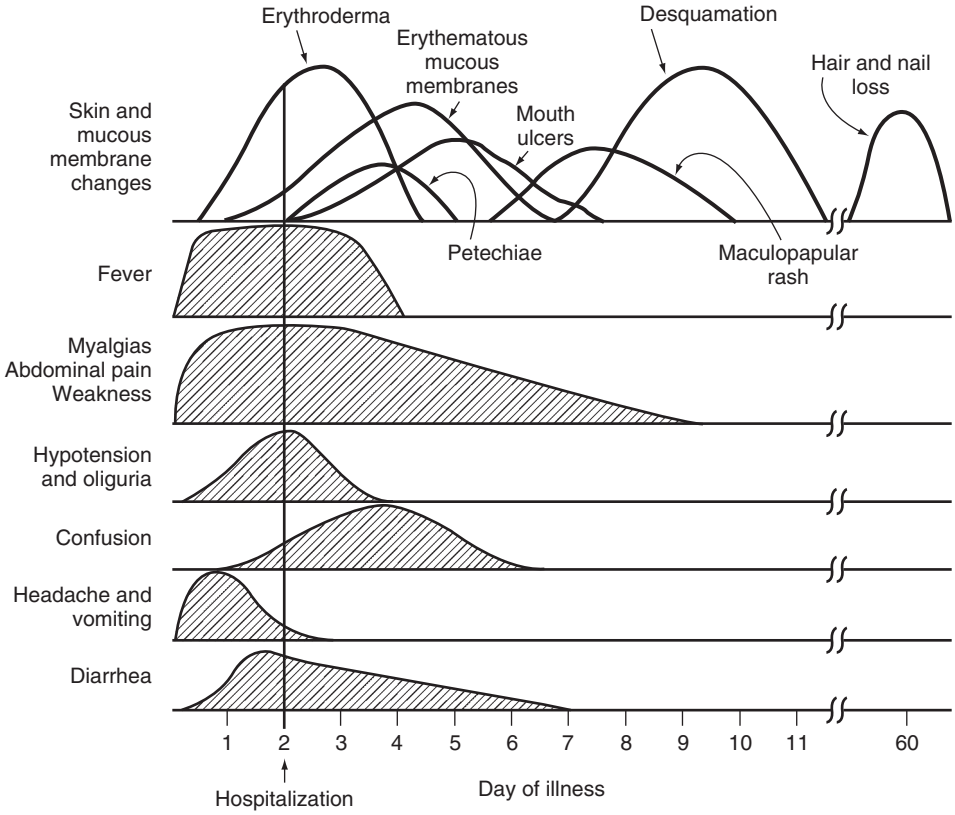


Figure 71–3 Composite drawing of the major systemic skin and mucous membrane manifestations of toxic shock syndrome. *(From Chesney, P. J., Davis, J. P., Purdy, W. K., et al.: The clinical manifestations of toxic shock syndrome. J. A. M. A. 246:741-748, 1981. Copyright 1981, American Medical Association.)*

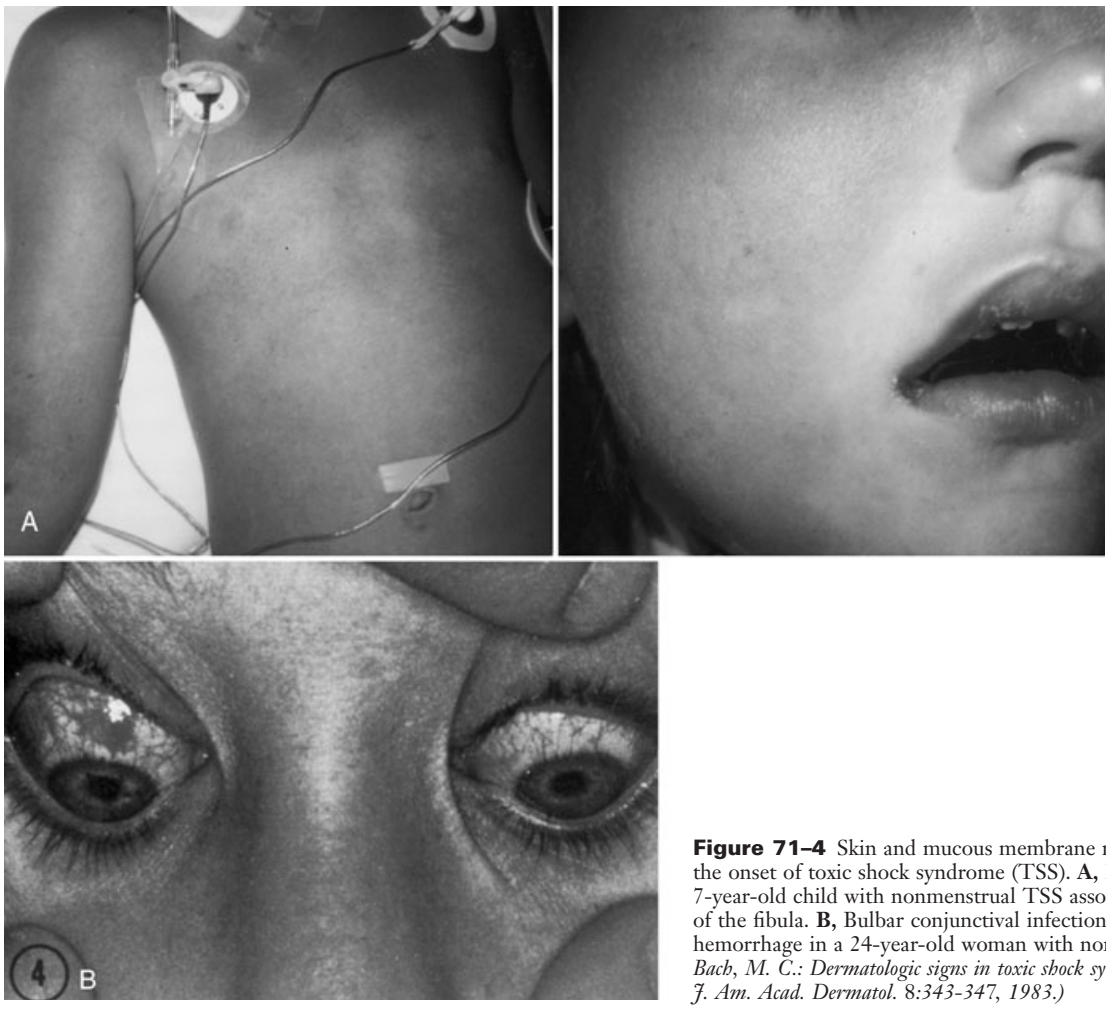


Figure 71–4 Skin and mucous membrane manifestations present at the onset of toxic shock syndrome (TSS). **A,** Diffuse erythroderma in a 7-year-old child with nonmenstrual TSS associated with osteomyelitis of the fibula. **B,** Bulbar conjunctival infection and subconjunctival hemorrhage in a 24-year-old woman with nonmenstrual TSS. ***(B, from Bach, M. C.: Dermatologic signs in toxic shock syndrome: Clues to diagnosis. J. Am. Acad. Dermatol. 8:343-347, 1983.)***

TABLE 71–3 Therapeutic Principles for Management of Toxic Shock Syndrome

1. Identify the focus of infection: débride and irrigate extensively and remove any foreign material
2. Isolate the organism for antimicrobial susceptibility studies
3. Administer parenteral antimicrobial therapy
   Stop enzyme/toxin production with a protein synthesis inhibitor (e.g., clindamycin, erythromycin, gentamicin)
   and
   Eradicate the organism with a bacterial cell wall inhibitor (e.g., β-lactamase–resistant antistaphylococcal antimicrobial agent)
   Consider intravenous immunoglobulin to provide antitoxin antibodies for a subset of patients, including those with the following:
   - Disease refractory to initial fluid replacement and several hours of vasopressor support
   - A focus of infection that cannot be drained
   - Persistent oliguria despite massive fluid replacement and in the presence of pulmonary edema

   Consider additional measures, such as the following:
   - Methylprednisolone to suppress cytokine production and the inflammatory response
   - Fluid therapy to maintain adequate venous return and cardiac filling pressure and to prevent end-organ damage
4. Anticipate the management of multisystem organ failure

levels reflect kidney damage,[50] abnormalities in tests of liver function reflect liver damage and acute cholestasis,[130,150] and the profound hypocalcemia may reflect both hypoproteinemia and high serum levels of a calcitonin-like material.[51,286] Muscle involvement is noted by an elevated creatine phosphokinase level, and the hypophosphatemia that occurs despite impaired renal function is unexplained.[14,51,319] Most of these test results will return to normal within 7 to 10 days of disease onset. *S. aureus* will be cultured from the cervix or vagina in more than 85 percent of patients with menstrual TSS and from the focus of infection in patients with nonmenstrual TSS. Positive blood culture results are rare findings.[69] Antibody to TSST-I or to the staphylococcal enterotoxins will be absent at the onset of disease in more than 85 percent of patients.[23,33,66,259,281,332]

Treatment

The four general principles of treatment of TSS (Table 71–3) are (1) identification and drainage of the focus of toxin production, (2) identification and susceptibility testing of the organism, (3) administration of antimicrobial therapy to block synthesis of the toxin and to kill *S. aureus*, and (4) management of the systemic multiorgan actions of the toxins or mediators.*

LOCATION AND DRAINAGE OF THE INFECTED SITE

The focus of infection should be identified rapidly, any foreign bodies should be removed, and the site should be drained or irrigated completely, even if it does not appear to be inflamed. Performing this procedure is of utmost importance because perpetuation of even a small, undrained focus of infection may result in serious clinical consequences. If TSS occurs in the immediate postoperative period, the wound should be assumed to be the source of infection, regardless of its benign appearance.

IDENTIFICATION AND SUSCEPTIBILITY TESTING OF THE ORGANISM

The incidence of community-acquired MRSA infections has increased dramatically in incidence during the past 10 years.[129] These MRSA strains differ from the long-standing hospital-acquired MRSA strains in that they are acquired in the community by well individuals with no risk factors and are not multiresistant. Most strains are susceptible to clindamycin. Because MRSA strains are fully capable of producing TSST-I,[89,272] community-acquired menstrual and nonmenstrual cases of TSS may be caused by either community or multiresistant hospital-acquired strains of MRSA.[96] To date, vancomycin-resistant strains of *S. aureus* are rare findings.[143,288] Thus, every effort should be made to obtain the organism for susceptibility testing.

ADMINISTRATION OF ANTIMICROBIAL AGENTS

Administration of antistaphylococcal antibiotics is indicated to eradicate organisms and prevent recurrence.[75,76] The infection may be a superficial or deep-tissue infection and may be associated with *S. aureus* bacteremia or bacteriuria. Antistaphylococcal antimicrobial agents should be administered intravenously at maximal doses for age and should be initiated as soon as possible.

Once the patient is stable, no longer vomiting or having diarrhea, and able to take food by mouth, high doses of an oral antimicrobial agent to which the organism is susceptible can be given to complete a total course of 10 to 14 days.

Subinhibitory concentrations of the protein synthesis inhibitors clindamycin, lincomycin, erythromycin, clarithromycin, kanamycin, gentamicin, tetracycline, and linezolid have been shown to suppress TSST-I production in vitro.[88,237,269,291] In one study, clindamycin concentrations $^1/_{64}$ the minimal inhibitory concentration (MIC) were effective in totally blocking TSST-I production.[237] In a mouse model of myositis caused by *S. pyogenes*, another superantigen-producing organism, clindamycin and erythromycin were more efficacious than penicillin.[97,289,292]

Data suggest that subinhibitory concentrations of β-lactam antibiotics actually may *increase* TSST-I production by *S. aureus.* At a concentration of half the MIC, nafcillin can *increase* toxin production 10-fold more than do control conditions.[5] A similar effect has been described for nafcillin and the staphylococcal alpha-toxin.[163] The effect is not seen with vancomycin, another cell-wall-active drug, thus suggesting specificity beyond merely a cell wall effect. Co-administration of a protein synthesis inhibitor with a β-lactam antibiotic blocks the effect.[5]

The choice of initial empiric antimicrobial therapy has become more complex as a result of an increase in the number of community-acquired MRSA infections and the spread of multiresistant MRSA strains in hospitals.[161] The severity of TSS warrants initiating maximally effective therapy. Once the results of susceptibility testing of the organism are available, therapy can be adjusted appropriately. In the past, the most effective initial empiric therapy was a combination of a β-lactamase–resistant penicillin and clindamycin. If there is concern for MRSA strains causing TSS, consideration should be given to initiating therapy with vancomycin and clindamycin. This situation clearly is difficult because most patients with TSS have a degree of renal failure. In addition, if the organism is susceptible to methicillin, the β-lactamase–resistant penicillins generally are more efficacious than is vancomycin. As an option to vancomycin, linezolid has been used successfully for the treatment of staphylococcal TSS.[291] An infectious disease consultation should be considered to help in the management of these patients.

MANAGEMENT OF SYSTEMIC MULTIORGAN ACTIONS OF THE TOXINS OR MEDIATORS

Intravenous Immunoglobulin

Not surprisingly, given the high prevalence of antibodies to TSST-I in adults,[324] high levels of antibody to TSST-I are

*See references 47, 56, 75, 76, 114, 116, 139, 192, 264, 289, 311.

present in IVIG preparations.[46,52,196,233,300] In animal models of TSS, human IVIG given at the time of inoculation of TSST-I–positive *S. aureus* prevented the development of TSS. When IVIG was administered 8 hours later, it decreased the mortality rate from 90 percent in control rabbits to 16 percent. When IVIG was given 29 hours after administration of TSST-I, the increase in survival rates in the IVIG-treated animals still was significant. No adverse reactions were noted in the treated animals, and no evidence was found of disease mediated by the formation of antigen-antibody complexes.[196,197,199,200] Monoclonal antibodies to TSST-I can prevent the development of manifestations of TSS in the rabbit model completely.[34,235,276]

High concentrations of antibodies to TSST-I and the staphylococcal enterotoxins (SEA; SEB; SEC types 1, 2, and 3; SED; and SEE) have been demonstrated in pooled IVIG.[46,52,233,300] These antibodies may inhibit the binding of toxins to MHC class II antigen-processing cells or interfere with presentation of toxin by these cells to the TCR. As a result, production of TNF-α and TNF-β is inhibited by these antibodies in vitro in an apparent toxin-specific manner.[300] Down-regulation of the production of lymphokine induced by streptococcal pyrogenic exotoxin A by IVIG also has been demonstrated in vitro.[215,281]

The results of these in vitro studies have supported the use of IVIG for both streptococcal and staphylococcal TSS.[253] Anecdotal case reports have indicated a beneficial effect on both streptococcal[16,215,338] and staphylococcal[2,52,61,221,233] TSS in humans. The efficacy of IVIG as adjunctive therapy for streptococcal TSS was evaluated in a multicenter, randomized, double-blind, placebo-controlled trial.[72] Although the trial was prematurely terminated because of slow patient recruitment, results from 21 enrolled patients (10 IVIG recipients and 11 placebo recipients) showed 3.6-fold higher mortality in the placebo group. Even though statistical significance was not reached, the trial provides support for IVIG as adjunctive therapy for streptococcal TSS.

Because IVIG is expensive and most patients respond rapidly once standard therapeutic measures are initiated, some authors would reserve IVIG for patients with an inaccessible focus of infection or for those who continue to deteriorate after receiving fluid and vasopressor support for several hours (see Table 71–3). The dose most often used has been 400 mg/kg given as a single dose over the course of several hours. This dose results in a serum antibody titer of greater than 1:100, much higher than that appearing to provide immunity to TSST-I.[233] Some studies have used IVIG doses of up to 2 g/kg.[72] Because early administration of IVIG possibly could blunt the immune response to TSST-I or other toxins and increase the possibility of a recurrent episode, the potential risks and benefits of this form of therapy must be considered for each patient.

The role of endotoxin in the pathogenesis of TSS is unclear. The failure of polymyxin B and anti-J5 antiserum to alter the course of TSST-I–positive TSS in a rabbit model suggests that endotoxin may not be an important mediator of TSS in humans.[198]

Early and sporadic case reports of TSS have suggested therapeutic benefit with naloxone,[59] calcium,[233] and exchange transfusion in severely ill patients unresponsive to the usual forms of therapy.

Corticosteroids

Short courses of methylprednisolone or dexamethasone, if given early in the course of the disease, have been associated with a reduction in the duration of fever and the severity of illness but no reduction in mortality rates.[307] In vitro, dexamethasone has been shown to down-regulate TSST-I–induced cytokine production.[167] Because no controlled prospective study has demonstrated efficacy, the use of steroids probably should be restricted to hypotensive patients unresponsive to fluid resuscitation, antimicrobial agents, and intravenous immunoglobulin (IVIG).

Fluid Replacement

The most important aspect of the nonspecific treatment of symptomatic patients is fluid replacement.[304] Intravascular volume and cardiac filling pressure must be restored rapidly to achieve adequate tissue perfusion. Because of the ongoing capillary leakage, this fluid replacement may far exceed the estimated fluid requirements based on calculated maintenance and fluid deficit volumes. Some adults have required vasopressors and as much as 12 L of fluid during the first 24 hours to stabilize the circulating blood pressure. Pleural, pericardial, and peritoneal effusions and interstitial edema inevitably occur as a result of the continued vascular capillary fluid leak. Close monitoring in an intensive care unit will facilitate determining when the correct intravascular volume has been achieved, as well as detecting and appropriately monitoring and treating myocardial dysfunction, hemodynamic derangements, pulmonary edema, adult respiratory distress syndrome, acute renal failure, encephalopathy, and disseminated intravascular coagulation.

SUBACUTE PHASE: AFTER TREATMENT IS INITIATED

Once treatment is initiated, response usually is rapid. Temperature returns to normal within 48 hours. The hemodynamic changes are observed initially as tachycardia, decreased systemic vascular resistance, decreased central venous pressure, hypovolemia, normal pulmonary artery wedge pressure, and an increased cardiac index.[13,41,67,114,117] Once aggressive fluid therapy has been initiated, myocardial edema and potential failure, along with pulmonary and cerebral edema in the face of renal failure, become the most critical management issues. The reasons for myocardial failure are unclear but probably are related to perivascular inflammation of the coronary vessels, edema, and postulated myocardial depressant factors.[174,230] TSST-I has been shown to inhibit systolic function in isolated rabbit atria, though at higher than usual circulating concentrations.[224] Arrhythmias may result from myocardial damage or electrolyte abnormalities.[193,255] Endomyocardial biopsy in one patient with severe global hypokinesis of the left ventricle revealed no substantial inflammatory infiltrate but a mild to moderate number of T cells scattered diffusely throughout the biopsy specimen.[67]

During the decompensated stage of myocardial dysfunction, the cardiac index falls and pulmonary wedge pressure increases, with both left atrial and ventricular and diastolic diameters being at the upper limits of normal.[114] Reversible electrocardiographic findings include sinus tachycardia, diffuse loss of voltage, flattened T waves, and diffuse nonspecific ST-T wave changes. If fatal arrhythmia does not occur during the decompensated stage, the toxic cardiomyopathy is reversible and rarely results in permanent changes. This process is similar to "stunned myocardium," a transient, postischemic myocardial dysfunctional state.[67]

Pulmonary edema and adult respiratory distress syndrome occur commonly in patients with severe disease when massive fluid replacement is necessary and the capillary leak syndrome continues in the lungs. Pulmonary edema appears rapidly once fluid replacement is initiated and often necessitates intubation and respirator management for several days.[304]

Forms of TSS-associated acute renal failure include prerenal azotemia and both nonoliguric and oliguric renal failure.[50] The type of renal failure manifested may be dependent on the degree of intravascular volume depletion. Unless severe acute tubular necrosis necessitates temporary hemodialysis, repletion of intravascular volume usually results in rapid restoration of renal function and, ultimately, diuresis. Permanent renal damage is an extremely rare event.[46]

The gastrointestinal, musculoskeletal, and hepatic changes resolve rapidly. Sequelae associated with these changes are rare, except for prolonged muscle weakness.[46,79] Joint manifestations generally are self-limited.[22,119]

Management of fluids, electrolytes, and metabolic acidosis in patients with TSS is complex. Although tetany is a rare occurrence, this common severe hypocalcemia may be life-threatening and should be corrected.[51,233,286] Most patients require potassium replacement and management of metabolic acidosis. The use of colloid for fluid replacement and removal of the toxin stimulus for capillary leak syndrome ultimately correct the hypoproteinemia.

The typical dermatologic manifestations follow a predictable sequence (see Fig. 71–3). A dandruff-like flaky desquamation begins on the trunk and extremities 5 to 7 days after the onset of symptoms. From days 10 to 12 and for as long as a month, the characteristic full-thickness desquamation of the fingers, toes, palms, and soles takes place (Fig. 71–5). A variety of atypical dermatologic manifestations, including petechiae and subepidermal bullae, have been described.[10,101,146]

Early in the acute phase, many patients exhibit desquamation of the mucous membranes, which is particularly painful when the oral mucous membranes are involved.[47] In addition, a small number of patients will have reactivated herpes simplex virus type 1 or 2 lesions with the acute illness.[47] A late-onset pruritic maculopapular rash with edema and low-grade fever probably unrelated to antimicrobial therapy occurs in more than 50 percent of menses-associated cases within 7 to 14 days of disease onset.[47,84] The cause of this late-onset rash is unknown.

Telogen effluvium, a common sequela, is a nonspecific response to severe trauma, sepsis, or stress that results in disturbed metabolism and keratinization of the hair follicles and nails. The hair follicles appear to transform prematurely from the growth, or anlagen, phase to the telogen, or resting, phase. Hair and nail loss occurs 4 to 16 weeks after onset of the illness, with restoration taking place in 5 to 6 months.[22,26,47]

The hematologic system seldom is involved with major complications in TSS. Although disseminated intravascular coagulation may be present, gastrointestinal, uterine, or cerebral bleeding rarely occurs. Thrombocytopenia may be present initially in patients with disseminated intravascular coagulation; thrombocytosis is characteristic of the recovery phase. Mild to moderate normocytic, normochromic anemia, which probably is dilutional and a result of suppressed red blood cell synthesis, develops in virtually all moderately to severely ill patients with TSS and resolves during convalescence.[47,49] Hypoferrinemia occurs commonly.[49]

The relatively common toxic or ischemic encephalopathy, rarely complicated by seizures, resolves slowly during the first 4 to 5 days of hospitalization.

Outcome and Sequelae

Death associated with TSS usually takes place within the first few days of hospitalization, but it may occur as late as 15 days after admission. Fatalities have been attributed to refractory cardiac arrhythmias, cardiomyopathy, irreversible respiratory failure, and, rarely, bleeding caused by coagulation defects.[174,230] The duration of circulation of toxins and mediators and the associated hypotension may be the best predictors of the severity of the end-organ damage.

After being discharged, more patients experience prolonged fatigue, muscle weakness, and pain.[46] Sequelae attributed to TSS that appear to be related to a prolonged period of hypotension have included chronic renal failure, gangrene, and telogen effluvium.[79,158,202,258,262] Other sequelae, such as neuropsychological abnormalities, prolonged myalgia and weakness, carpal tunnel syndrome, chronic dermatitis, Raynaud syndrome, new allergies, and recurrences, are explained less easily. Abnormalities such as impaired memory and poorly sustained concentration have been found in patients who did not require any therapy other than intravenous fluids to restore their blood pressure.[258] In one center, patients with nonmenstrual TSS had more serious short- and long-term neuropsychological complications than did patients with menstrual TSS.[158] Sequelae related primarily to the neuromuscular system have resulted in speculation that the TSS-associated toxin may have a direct effect on nerve or muscle tissue. In one patient with TSS, cauda equina syndrome with partial paralysis developed after lumbar laminectomy and staphylococcal meningitis, thus suggesting a neurotoxic effect of the intrathecally produced SEC.[8]

One study compared the sequelae and other long-term effects in 183 (174 menstrual cases) women with TSS and 366 control women hospitalized for appendicitis and appendectomy and matched for age, race, and duration of follow-up.[79] Each subject completed two comprehensive phone interviews. When compared with controls, women with TSS were significantly more likely to report sequelae involving fatigue, the integument (hair loss and nail changes), mental and cognitive skills (problems with concentration, reading difficulty, and memory loss), emotions (menses attitude and emotional changes), and multiple organ

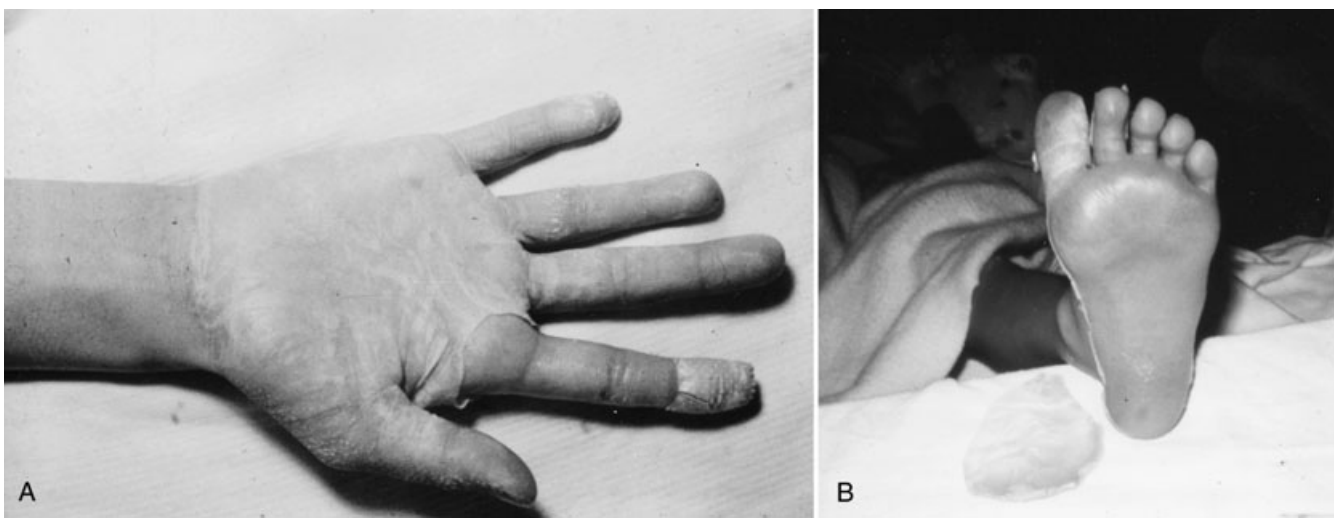


Figure 71–5 Universal full-thickness desquamation of the hands (**A**) and feet (**B**) first noted 7 to 14 days after disease onset and persisting for up to 30 days.

systems (conditions involving the joint, cardiac, muscle, and genitourinary systems). In addition, women with TSS were significantly more likely than controls to report persistence of symptoms. Fertility patterns and pregnancy outcomes were similar in controls and women with TSS, both before and after the index illness.[79]

Recurrences

One of the most puzzling aspects of the pathophysiology of TSS is the high rate of recurrence in patients with inadequately treated menstrual or nonmenstrual disease.[6,47,75,76,191,294] A small number of patients with menstrual disease and repeated tampon exposure have experienced as many as 6 to 12 recurrences. Recurrences associated with menstrual disease have no predictable pattern. In most patients the first episode is the most severe. However, asymptomatic menses may occur between symptomatic episodes, and the most severe episode may occur after one or more milder episodes.[76] The use of antistaphylococcal antimicrobial therapy to which the organism is susceptible in doses recommended for serious infections for 10 to 14 days and discontinuation of tampon use can be expected to reduce the rate of recurrence significantly.[76] Recurrences after nonmenstrual TSS are well described and usually are associated with inadequate initial therapy.[6,158,233] Cisplatin administration resulting in significant hypomagnesemia precipitated several recurrent episodes of TSS in an immunocompromised adult.[25]

The absent or delayed antibody response to TSST-I found in both menstrual and nonmenstrual TSS patients probably accounts for the continued susceptibility to TSS and for the high recurrence rate.[22,33,181,259,281,294,332] The fact that superantigenic toxins are not processed by antigen-processing cells and T lymphocytes as conventional antigens may provide an explanation for the poor convalescent antibody response to these antigens.[166]

Culture-negative, menses-associated, recurrent episodes of TSS continue to occur in a small number of patients despite discontinuation of tampon use and administration of appropriate antimicrobial therapy during the acute episode.[76] Administration of an oral β-lactamase–resistant antistaphylococcal antimicrobial agent during menses has been tried in an attempt to prevent these recurrences but is not always successful. In recurrent cases resistant to this form of prophylaxis, consideration could be given to the untested and empiric use of rifampin, clindamycin, erythromycin, or IVIG (if the patient has no antibody to TSST-I) or to the use of an oral contraceptive.[191]

ATYPICAL MANIFESTATIONS

Mild Disease

Recognition of mild episodes of TSS is particularly important in patients with menstrual TSS because repeated use of tampons and the risk for recurrence are episodic.[48,85,310] Patients with mild or severe menstrual TSS typically do not form antibody to TSST-I during convalescence[22,33,259,281,332] and, without appropriate therapy, may have one or more recurrences. Such recurrences may be mild or severe.[75,76,192] Patients with nonmenstrual TSS also do not form antibodies in convalescence. A mild episode may be recognized only in retrospect, after desquamation or a recurrent episode (or both) develop.[233]

The presence of any combination of fever, headache, sore throat, diarrhea, vomiting, orthostatic dizziness, syncope, and myalgia in a menstruating woman or an individual with a potential *S. aureus* infection, no matter how trivial, should raise suspicion of TSS. A specific laboratory test to confirm the clinical diagnosis is not available. A bacterial culture positive for *S. aureus* may be helpful but is not diagnostic of menstrual cases because *S. aureus* may be cultured from the cervix or vagina in as many as 33 percent of menstruating women.[186,239]

Other laboratory data usually do not reflect multisystem involvement in mild disease, and assays for TSST-I in body fluids are investigational.[195,323] Diagnostic support for the theory that the patient's signs and symptoms represent mild TSS often depends on the constellation of findings present, including subsequent typical desquamation of the palms, soles, toes, or fingers; demonstration that *S. aureus* isolates from the site of infection produce TSST-I or an enterotoxin; absence of antibody to TSST-I or enterotoxins in acute-phase serum; and recurrent disease, if it develops.

Nonmenstrual Disease

The incidence of postoperative cases of TSS after all types of surgery has been estimated to be 3 per 100,000 population.[131] For ear, nose, and throat surgery, the incidence is higher (16.5/100,000 population).[152] A striking feature of most postoperative cases with rapid onset is the absence of any signs of wound infection.[17,131,246,260] The mean time from surgery to the onset of symptoms is 2 to 4 days.[17] Nosocomial acquisition of TSST-I–positive organisms rarely has been documented for postoperative cases.[9,169,212] A wide variety of types of surgery have been associated with TSS.[22,43,211,223,252,278,282]

Burn wounds provide a particularly rich environment for growth of *S. aureus* and the production of toxins.* In burn centers, TSS occurs predominantly in young children with small burns.[98] In one large pediatric burn center, *S. aureus* normally was not cultured from any site on admission.[52] However, it was acquired within a few days of admission and became the most common wound pathogen. Of all wound isolates of *S. aureus*, just 16 percent produced TSST-I. Only 50 percent of the children had antibodies to TSST-I on admission, which reflects the low prevalence of antibodies in this population.[324] Of blood products administered, 76 percent had antibodies to TSST-I, and seroconversion occurred in children receiving these products. A toxic shock–like syndrome developed in 13 percent (7/53) of children, only one of whom had TSST-I–producing *S. aureus* isolated from the wound. SEA and SEB were produced by isolates from three patients. In burn patients, issues regarding the use of prophylactic antibiotics and occlusive dressings and enhancement of TSST-I production by topical antimicrobial agents are unsettled.[99] The mortality rate associated with TSS in children with burns may be as high as 57 percent.[122] Skin disrupted in any way, including disruption by varicella or a tattoo, also may be a focus for TSS.[22,37,65]

Patients whose anterior nares have been colonized by *S. aureus* are at particular risk for the development of TSS when the respiratory mucosa is disrupted by surgery, trauma, or a respiratory infection such as influenza.[22,63,180,312,314] TSS has been reported in association with sinusitis,[22,109] pharyngitis,[22,263] parapharyngeal abscesses,[263] tracheitis,[22,71,92,125,218] pneumonia,[22,74] rubeola,[297] and submandibular space abscesses. TSS occurring after ear, nose, and throat surgery has been associated with the use of nasal splints and packing materials and in part may be the result of interruption of the ciliary blanket.[3,22,121,207,339]

After orthopedic procedures or in association with bone and joint infections, the clinical manifestation of TSS may be confusing as a result of the intense and generalized myalgias associated with TSS, which may be misinterpreted as postoperative musculoskeletal symptoms. The wounds usually appear to be benign.[22,206,240,302,318] Infected abrasions under casts may be focal sites of TSST-I production.[285]

In one center, when cases of nonmenstrual TSS were compared with menstrual cases, patients with nonmenstrual disease

*See references 11, 52, 60, 98, 122, 124, 128, 142, 183, 188, 328.

were found to have a delayed onset of symptoms after the precipitating event, more frequent central nervous system manifestations, less frequent musculoskeletal involvement (myalgia and arthralgia), and a higher degree of anemia.[158] Recurrences occurred in both groups in untreated patients, and mortality rates were not different. Clinical differences between the two categories are suggested to be related to differences in the types of toxins produced. TSST-I was produced with comparable frequency in both groups. SEA was produced less often by nonmenstrual isolates, and menstrual isolates more often produced both TSST-I and SEA. SEB was produced more frequently by nonmenstrual isolates.

Several patients with TSS have been reported to be simultaneously infected with *S. aureus* and *S. pyogenes*.[100] Determining which infection primarily was responsible for the manifestations or whether an amplified effect of exotoxins from both organisms was present was not possible.

Recalcitrant Erythematous Desquamating Disorder

An atypical, subacute variant of TSS has been described in patients with acquired immunodeficiency syndrome (AIDS) and labeled recalcitrant erythematous desquamating disorder. *S. aureus* strains producing TSST-I, SEA, or SEB have been isolated from patients with AIDS and prolonged erythema, extensive cutaneous desquamation, hypotension, tachycardia, and multiple and variable organ involvement.[61,91,164] The illness is recalcitrant, prolonged, and characterized by multiple recurrences. In one patient, elevated levels of TNF and IL-6 were found during severe episodes. When antitoxin antibodies have been measured during recurrence, they have been undetectable. Two patients responded well to IVIG.[61,164] Patients with the combined cellular and humoral immunodeficiencies of AIDS may be at particular risk for the development of severe, frequent, and prolonged recurrent episodes of TSS.[61,91,112,164,284] TSST-I and the enterotoxins may activate human immunodeficiency virus type 1 (HIV-1) gene expression in vivo, as has been observed in vitro.[123]

Neonatal Toxic Shock Syndrome–like Exanthematous Disease

A toxic shock–like illness related to MRSA producing TSST-I, neonatal toxic shock syndrome–like exanthematous disease (NTED), was described initially in neonates in Japan[299] and subsequently recognized in France.[321] These infants are seen in the first week of life with the combination of a generalized erythematous macular rash, thrombocytopenia, elevated acute-phase reactants, and fever. Patients are colonized with MRSA belonging to a single clonal type that produces TSST-I and SEC.[147] Although no focus of infection is present and no exotoxins are detectable in serum, analysis of T cells shows selective expansion of $V_\beta 2$-positive T cells during the acute phase as a result of selective activation of TSST-I–reactive T cells.[298,299] Maternal IgG antibody to TSST-I plays a protective role in preventing NTED.[298] Although complications occur in premature neonates, term infants typically recover within 5 days without active treatment. The limited disease in comparison to TSS has been attributed to the minute amount of exotoxin from colonized sites and to the high susceptibility to induction of anergy in the immature T cells of neonates.[298,299]

Streptococcal and Other Toxic Shock Syndromes

An increase in the incidence and severity of invasive *S. pyogenes* infections was recognized in the 1980s. Manifestations of severe disease have included septicemia with or without a focus of infection; severe, painful cellulitis; necrotizing fasciitis; and in some cases, streptococcal TSS with or without a focus of infection. Streptococcal TSS is similar to staphylococcal TSS in that it appears to be mediated by superantigenic toxins and results in endothelial damage, hypotension, and multisystem organ involvement. In 1993, the Working Group on Severe Streptococcal Infections proposed a consensus definition for streptococcal TSS with clinical criteria, including isolation of group A streptococci from a normally sterile site, hypotension, and two or more of the following: renal impairment, coagulopathy, liver dysfunction, adult respiratory distress syndrome, erythematous macular rash, and soft tissue necrosis.[336]

Streptococcal toxic shock–like syndrome differs from staphylococcal TSS in numerous respects, including a slower onset over the course of several days; usual absence of vomiting, profuse diarrhea, and conjunctival infection; frequent absence of erythroderma or the presence of only a sandpaper-like rash; severe generalized hyperesthesia; extreme pain at the site of skin involvement; and a mortality rate of 40 to 50 percent.[62,290] Both cell-associated and soluble streptococcal virulence factors, including M protein and pyrogenic exotoxins A and B, have been implicated as superantigens mediating the systemic effects of streptococcal TSS.[39,103,141]

Clostridium sordellii has been reported to cause a toxic shock–like syndrome after medical abortion with mifepristone and intravaginal misoprostol.[58,113,279]

UNIQUE MANIFESTATIONS

Work with superantigens suggests that the pathophysiologic events observed in TSS are the result of release of endogenous mediators after monocyte and T-cell activation by TSST-I or the staphylococcal enterotoxins.

The physiologic changes induced by this dysregulation of the immune system are striking in their rapidity of onset and progression and the involvement of almost all body tissues and organs. The consequent functional disorders of many organs appear to be the result of extensive endothelial damage and loss of peripheral vascular resistance. The generalized decrease in vasomotor tone results in pooling of blood in the peripheral vasculature, vascular congestion, and probably relaxation of the microcirculation. Rapid, nonhydrostatic leakage of fluid from the intravascular to the interstitial space, or "second spacing," does occur and is manifested as extensive generalized anasarca-like, nonpitting edema. The universal hypoproteinemia and hypoalbuminemia in patients with TSS suggest that the fluid leaking from the vasculature is high in protein.

Unique clinical manifestations include profuse vomiting and diarrhea, often associated with incontinence; generalized erythroderma; intense erythema of the mucous membranes, including conjunctival injection and subconjunctival hemorrhages; absence of inflammation in surgical wounds and other focal sites of infection; absence of bacteremia; severe hypocalcemia and hypophosphatemia; rapidly accelerated renal dysfunction; universal desquamation of the palms, soles, fingers, and toes; and risk for recurrent disease and long-term cognitive sequelae.

DIAGNOSIS

Application of the case definition for a single episode[330] or recurrent episodes[76] to the patient's illness is currently the only way to confirm the diagnosis (see Table 71–1). Aggressive attempts to find the focus of *S. aureus* replication that always should be undertaken include culture of the cervix and vagina in patients with menses-associated illness and culture of other potentially infected sites that may not appear to be infected obviously in patients with nonmenstrual illness. *S. aureus* isolates could be

examined, when possible, for their ability to produce TSST-I, although such testing seldom is indicated. This test is of limited usefulness for nonmenstrual cases because TSST-I is produced by only 40 to 60 percent of *S. aureus* isolates from such patients.[126] Isolates from these patients could be examined for the presence of the other enterotoxins. Acute and convalescent sera can be tested for the presence of antibodies to TSST-I and the other enterotoxins.[144] Elevated levels of anti–TSST-I in the acute-phase serum of a patient with menstrual-associated TSS would be highly unusual.

In most instances, toxin detection tests are of value only for research or for the rare patient with chronic recurrent or otherwise puzzling disease. Genes for TSST-I and the enterotoxins have been detected in *S. aureus* strains by polymerase chain reaction[19,157,178,194,219,273] and hybridization techniques.[156,216,226] A noncompetitive enzyme-linked immunosorbent assay allows quantitation of TSST-I in clinical samples.[195,209] Reversed passive latex agglutination has been used in hospital laboratories to detect TSST-I and in research laboratories to detect enterotoxins.[83,104] Research laboratories also have developed techniques for detecting the selective expansion of $V_\beta 2$-positive T cells as evidence of host response to superantigenic toxins.[54,187]

DIFFERENTIAL DIAGNOSIS

The differential diagnosis of TSS includes clinical entities in which a rapid onset of fever, erythroderma-like rash, hypotension, and multisystem involvement are observed[42,110,210,233,241,243] (Table 71–4).

PREVENTION AND PROPHYLAXIS

To decrease the risk of development of menstrual TSS, in 1982 the Institute of Medicine Committee on TSS recommended that women, particularly adolescents, minimize their use of high-absorbency tampons.[244] The committee also recommended that women who have had TSS not use tampons because of the increased risk of recurrence and that postpartum women be informed that the use of tampons might increase their risk for the development of TSS.

Although the frequency of changing tampons during a menstrual period has not been associated with risk for the development of TSS, the use of an individual tampon for no more than 12 hours at a time might decrease the risk for menstrual TSS. Women using intravaginal contraceptive devices also should be informed of the potential increase in risk for development of TSS.[275]

Although postoperative TSS is a rare event, it generally occurs in healthy people. As a result of the unexpected and potentially severe consequences of TSS, issues regarding surgical antimicrobial prophylaxis for prevention have been raised. Perioperative systemic antistaphylococcal antibiotics did not prevent TSS in four patients[35,260] and do not eradicate nasal carriage of *S. aureus*.[154] TSS with onset after 48 hours of amoxicillin therapy was reported in two patients after they underwent endonasal sinus surgery.[2] Topical bacitracin ointment on nasal packing does not prevent the development of TSS.[35,87,152,154]

Most authors contend that the rare risk of development of postoperative TSS is comparable to the risk of having a severe antimicrobial reaction and that perioperative antimicrobial prophylaxis is not indicated for clean procedures of short duration.[131,246] Effort should be intensified to recognize postoperative cases early; to open, explore extensively, and irrigate wounds; and to provide immediate antimicrobial and supportive therapy for suspected TSS.

TABLE 71–4 Differential Diagnosis of Toxic Shock Syndrome Based on Clinical Manifestations

Diagnosis	Fever	Exanthem	Shock
Severe invasive *Streptococcus pyogenes* infection	+	+	+
Meningococcemia	+	+	+
Rocky Mountain spotted fever	+	+	±
Ehrlichiosis	+	+	±
Kawasaki disease	+	+	–
Staphylococcal scalded skin syndrome	+	+	–
Toxic epidermal necrolysis	+	+	–
Viral syndromes	+	+	–
Leptospirosis	+	+	–
Systemic lupus erythematosus	+	+	–
Erythema multiforme	+	+	–
Septic shock	+	–	+
Hantavirus pulmonary syndrome	+	–	+
Salmonella infections	+	–	±
Gastroenteritis	+	–	–
Urinary tract infection	+	–	–
Drug reactions			
Phenytoin (Dilantin)	+	+	±
Cocaine	+	+	±
Pseudoephedrine	+	+	±
Inhalational mercury	+	+	±
Quinidine	+	+	–
Sulfonamides	+	+	–
β-Lactam antibiotics	+	+	–
Quinolones	+	+	–

CHARACTERISTICS OF TOXIC SHOCK SYNDROME–ASSOCIATED *STAPHYLOCOCCUS AUREUS* ISOLATES

The ability to produce TSST-I, a previously uncharacterized protein, is the single most distinguishing characteristic of TSS-associated *S. aureus* strains. More than 90 percent of *S. aureus* isolates from patients with menstrual TSS[23,270] and 40 to 60 percent of isolates from patients with nonmenstrual disease[126] produce TSST-I as compared with less than 20 percent of non-TSS strains.

S. aureus strains isolated from patients with TSS are phenotypically different from other strains of *S. aureus*.[22,306] They produce less beta-hemolysis on sheep blood agar and less frequently harbor plasmids than control strains do. An increase in protease production and proteolytic activity by TSS-associated strains has been noted.[306] Next to TSST-I production, production of protease is the most characteristic marker for these strains.

Unlike non-TSS *S. aureus* strains, most TSST-I–positive strains of *S. aureus* require tryptophan for growth and production.[57] In one study, 91 percent of 27 TSST-I–positive vaginal *S. aureus* isolates were tryptophan auxotrophs as compared with 6 percent of 32 TSST-I–negative vaginal *S. aureus* isolates. Eight of 22 TSST-I–producing tryptophan auxotrophs were blocked at the tryptophan synthetase B locus, thus suggesting that the TSST-I gene cluster may have been inserted into this locus and disrupted its function.

Additional differences between TSS and non-TSS strains of *S. aureus* include the resistance of TSS strains to cadmium, arsenate, and penicillin, characteristics that usually are plasmid mediated but, in TSS strains, are chromosomally mediated. As such, these traits must be the result of heterologous insertions. These characteristics are not co-transferred with and therefore presum-

ably not closely linked to the gene segment responsible for TSST-I production.[22,306] TSST-I production is unrelated to methicillin susceptibility or resistance.[272]

ROLE OF STAPHYLOCOCCAL EXOTOXIN TSST-I AND THE ENTEROTOXINS IN TOXIC SHOCK SYNDROME

ROLE OF TSST-I

TSST-I appears to be an important toxin in TSS. Factors supporting this statement include (1) the observation that more than 90 percent of isolates from patients with TSS produce TSST-I, (2) the absence of acute-phase antibody to TSST-I in more than 90 percent of patients with menstrual TSS, (3) the increase in anti–TSST-I antibody during recovery in nonmenstrual cases, (4) absent or low levels of antibody in patients with recurrent menstrual TSS, (5) comparable illness inducible with TSST-I in in vivo animal models, and (6) neutralization of IL-1 stimulation and response to TSST-I by antibody to TSST-I.

Most convincing are experiments in which the ability to cause TSS followed bacteriophage-mediated transfer of the TSST-I–positive chromosomal segment into a recipient *S. aureus* strain without the segment.[82,233] Experiments demonstrating the effectiveness of monoclonal antibodies to TSST-I in reversing the effects of TSST-I provide additional strong support.[171]

ROLE OF ENTEROTOXINS

Because 40 to 60 percent of nonmenstrual TSS *S. aureus* isolates and 5 to 10 percent of menstrual TSS isolates do not produce TSST-I, a role for other staphylococcal exotoxins, including the enterotoxins, has been proposed.[22,66,177,190,234,266,337] Patients with nonmenstrual disease infected with TSST-I–negative strains may have a higher mortality rate.[126] Significant increases in the titer of antibody to the staphylococcal enterotoxins during convalescence strongly suggest a role for these proteins in the pathogenesis of TSS.[12]

In rabbit models, the morbidity and mortality occurring after the injection of TSST-I–negative TSS isolates were comparable to those occurring after infection with TSST-I–positive strains and significantly greater than after inoculation with TSST-I–negative non-TSS isolates.[126] Administration of staphylococcal enterotoxins to animals results in many of the manifestations characteristic of TSS.[21,102]

When large numbers of isolates from patients with TSS have been examined, production of TSST-I alone is the toxin pattern identified most commonly.[22,66,184] Other patterns identified include production of TSST-I in combination with one or more of the identified enterotoxins, production of enterotoxins *only* (one or more), and nonproduction of TSST-I and enterotoxins. Of the TSS strains producing TSST-I, 60.6 percent also produce an enterotoxin.[66,184] SEA is co-expressed with TSST-I frequently, particularly in menstrual isolates. A clone producing both TSST-I and SEA is associated with 88 percent of menstrual TSS cases and may account for as many as 54 percent of isolates from nonmenstrual TSS cases.[44] Seroconversion to SEA occurs more commonly in TSS than in non-TSS *S. aureus* infection, thus further suggesting a role for SEA. TSST-I and SEA have a common MHC class II binding domain and share overlapping domains critical for superantigenic and lethal activities. A monoclonal antibody to TSST-I inhibits these SEA-induced activities.[172]

The third most common pattern of toxin production by TSS isolates is that of TSST-I plus SEC. These isolates have been associated with both menstrual and nonmenstrual cases and particularly with severe respiratory tract TSS-associated infections.[66] NTED also has been associated with MRSA isolates exhibiting a clonal pattern with TSST-1 and SEC production.[147]

The fourth most common pattern of toxin production is that of SEB alone. In one study, strains producing SEB alone accounted for 38 percent of all nonmenstrual TSS isolates.[177,266] This prevalence is significantly higher than that for isolates from non-TSS *S. aureus* infections (15%) or asymptomatic carriers (13%). SEB never is co-expressed with TSST-I. Even though all TSST-I–positive isolates contain the SEB genetic determinant, none produces SEB. SEB is produced only by isolates that have the SEB gene and no TSST-I gene. Given that production of both toxins is mutually exclusive and both genes are located close together on the chromosome, researchers have suggested that the TSST-I genetic determinant may have a preferred site of insertion within the SEB genetic element. Because the TSST-I gene is associated with a variable genetic element and such mobile genetic elements are known to be capable of gene disruption, the gene possibly was inserted within the SEB gene or in a position that interferes with its transcription.[83]

A TSS isolate producing TSST-I along with two other enterotoxins is the fifth most common combination, and an isolate producing no known toxins is the sixth. The identification of new enterotoxins[251,295] capable of producing TSS-like disease in rabbits may account for isolates previously identified as producing no toxins. Isolates producing a non-SEB enterotoxin alone are the least common.

PREVALENCE OF EXOTOXIN-PRODUCING *STAPHYLOCOCCUS AUREUS* STRAINS AND ANTIBODY TO THE EXOTOXINS

PREVALENCE OF EXOTOXIN-PRODUCING ORGANISMS

TSST-I–positive strains of *S. aureus* have been present since at least 1957, and antibody to TSST-I was as prevalent in the general population in 1960 as it was in 1999.[236,324] Thus, the increase in incidence of TSS from 1980 to 1985 is assumed to have been the result of newly introduced cofactors rather than an increase in the prevalence of TSST-I–positive organisms.

At any given time, *S. aureus* is present in 20 to 40 percent of cultures from the anterior nasal vestibule of adults and in as many as 33 percent of nasal cultures from children.[151] A study of 3012 menstruating women between 13 and 40 years of age in North America from 1998 to 1999 showed that 26 percent were colonized with *S. aureus* in at least one of three body sites (nose, vagina, or anus) and 9 percent were colonized vaginally.[236] In women, the prevalence of *S. aureus* in vaginal cultures is higher during menses than at midcycle.[186,225] Vaginal carriage rates vary from 7 percent in premenarcheal and nonmenstruating women to 33 percent in menstruating women.[236,239] TSST-I–positive *S. aureus* is present in 1 to 5 percent of vaginal cultures in women, 7 percent of nasal cultures in hospitalized patients, and 18 percent of nasal cultures in children. In prospective studies of *S. aureus* isolates from healthy individuals and from specimens received for other purposes, 14 to 39 percent of all isolates produced TSST-I and 7 to 14 percent produced SEB.[85,233,236] Overall, between 1 and 7 percent of healthy individuals at any given time are colonized at a mucosal site with TSST-I–positive *S. aureus*.

Of 60 *S. aureus* isolates from blood cultures of patients who did not have TSS, 28 percent produced TSST-I.[46] Presumably, these patients had circulating antibody to TSST-I that prevented the development of TSS despite *S. aureus* infection. Adults and children with persistent nasal carriage of a TSST-I–positive strain have high levels of antibody to TSST-I.[236,254]

Evidence suggests that a single clone of *S. aureus* causes most cases of TSS.[44,214] Multilocus enzyme electrophoresis has demonstrated that the TSST-I gene *tst*H is distributed widely over the whole spectrum of *S. aureus* genotypes. Of 315 *S. aureus* strains collected from around the world, 88 percent of menstrual TSS strains and 53 percent of nonmenstrual strains were indistinguishable by multilocus enzyme electrophoresis. The same clone also was found in 28 percent of vaginal isolates from asymptomatic women. This remarkable phenomenon may reflect the unique ability of this clone to colonize the urogenital epithelium or the unique capability of this clone to cause disease. Although TSS strains are similar in gene content, indicative of a common ancestor, their considerable heterogeneity indicates that the common ancestor was not recent in evolutionary time.[118] Thus, the TSS outbreak was the result of a change in the host environment and not geographic dissemination of a new "hypervirulent" strain.[118]

Even though TSST-I–positive strains may cluster within families, living units, and hospital settings, the occurrence of TSS clusters is a rare event.[169,212] Probable TSS has developed within 24 hours in a husband and wife[115] and in two mother-daughter pairs. Nosocomial acquisition and transmission of TSST-I–positive organisms have been described.[9,169,212]

PREVALENCE OF ANTIBODY TO EXOTOXINS

The prevalence of antibody to TSST-I in 689 Wisconsin residents was found to be 47 percent at 1 year of age, 58 percent at 5 years, 70 percent at 10 years, 88 percent at 20 years, and 96 percent for ages 30 to 50 during the years 1960 to 1983 (see Fig. 71–2).[315] The presence of transplacentally acquired antibody in more than 90 percent of infants also was demonstrated.[324] A study of 3012 menstruating women between 13 and 40 years of age across North America in 1998 and 1999 found similar results, with 85 percent overall and 81 percent of subjects aged 13 to 18 years having positive antibody to TSST-1.[236] Among carriers of toxigenic *S. aureus*, a significantly lower percentage of black women (89%) than white or Hispanic women (98% and 100%, respectively) had positive antibody titers. Mucosal colonization with TSST-I–positive *S. aureus* strains is assumed to result in antibody formation.[254] Coworkers of a nurse in whom recurrent TSS developed were demonstrated to be colonized by TSST-I–positive strains and to form antibody to TSST-I, but not to acquire TSS during a period of several months.[9] Subclinical and mild, unrecognized disease[48] may result in the formation of antibodies as well.

Acquisition of antibodies to the enterotoxins also is age related. By the time that children reach the age of 10 years, the proportion with antibody titers of 1:100 or higher is 15 percent for SEA, 65 percent for SEB, 30 percent for SEC, 5 percent for SED, and 20 percent for SEE. By 22 years of age, the numbers increase to 55 percent for SEA, 77 percent for SEB, and 98 percent for SEC.[22]

Former studies suggested that the prevalence of antibody varies from one area of the country to another. During 1982, of 1017 serum samples obtained from U.S. Air Force recruits between the ages of 17 and 26 years, 35 percent from the Mountain and Pacific states were serosusceptible to TSST-I versus 3 percent from the south Atlantic states and 5 percent from the east south central states. Because low titers appear to indicate susceptibility, the findings in this study suggest that differences in the incidence of TSS among states and regions were explained in part by differences in host susceptibility.[322] However, the 2005 multicenter study of 3012 women from Ohio, New Jersey, Florida, Arizona, and Manitoba, Canada, found no significant regional differences in rates of *S. aureus* colonization or prevalence of antibody.[236]

Sera randomly selected from 87 control women were seronegative more frequently for antibody to TSST-I (24%) than were those from 66 control men (9%), which led to the suggestion that women may be more susceptible than men to TSS.[259] Patients in whom TSS develops have significantly lower levels of antibody to SEB and SEC, as well as to TSST-I, than the general population does.[22,66]

As noted in the discussion on recurrence, acute-phase sera of patients with TSS uniformly demonstrate absent or low levels of antibody to TSST-I. The antibody response to TSST-I is absent or delayed in both menstrual and nonmenstrual TSS.[233,294]

PHYSICOCHEMICAL AND BIOLOGIC CHARACTERISTICS OF TSST-I

PHYSICOCHEMICAL PROPERTIES

The mature secreted TSST-I protein is a single polypeptide chain with a molecular weight of 22,000 daltons and an isoelectric point of 7.2. It is resistant to proteolytic digestion by trypsin but is hydrolyzed by pepsin at pH 4.5. In sterile solution at neutral pH, it is stable for months. When lyophilized, it is a white powder that easily dissolves in distilled water. No loss of serologic activity of the lyophilized powder occurs for at least a year. TSST-I can be heated to 100°C for longer than an hour without loss of biologic activity.[29]

Purification of TSST-I has yielded diffraction-quality crystals that have led to an understanding of its three-dimensional structure.[29] The molecule is folded into two closely associated domains that create two major grooves, the front and backside grooves. The backside groove is larger and more exposed to the external environment. The crystal structures of all the protein superantigens have striking similarity in the two-domain conformational architecture, even though the primary protein segments are different.[189]

REGULATION OF PRODUCTION

S. aureus is highly adaptable and able to live and grow in extremes of temperature, pH, and oxygen concentration. TSST-I production, however, is controlled tightly. TSST-I is not produced in unfavorable conditions, including an anaerobic environment, pH values lower than 6.0 and greater than 8.0, concentrations of clindamycin below the MIC of the organism, glucose concentrations greater than 3 percent, and temperatures lower than 37°C or greater than 40°C.[22,29,335] Conditions and factors that enhance production of TSST-I include uniform aeration of medium through shaking or a roller apparatus, complex medium containing animal protein and low glucose concentrations, a neutral pH, incubation in 5 percent carbon dioxide (under some in vitro conditions), and temperatures of 37°C to 40°C. The role of magnesium is unclear.[88] Twice as much toxin is produced at 40°C as at 37°C. The addition of blood to media does not increase production of TSST-I reliably. Depending on in vitro growth conditions, most strains of TSST-I–negative *S. aureus* produce 3 μg/mL of TSST-I, but some may produce up to 30 μg/mL.

Under optimal in vitro growth conditions, TSST-I production lags behind but parallels bacterial growth. Like other *S. aureus* exotoxins, TSST-I is made primarily during the postexponential phase of growth, when nutrients are scarce and cell density is great.[85] Levels of TSST-I remain stable throughout the stationary phase, even after the organisms begin to die. Under less than optimal conditions, synchronous production of TSST-I and growth of *S. aureus* do not occur. For example, bacterial growth

may be reduced only twofold in an anaerobic environment, whereas TSST-I production is inhibited. Likewise, clindamycin at concentrations below the MIC for the organism will inhibit the production of toxins without altering growth.[269]

Most strains of *S. aureus* do not have the genes for TSST-I or the enterotoxins. TSST-I thus is not necessary for bacterial homeostasis, and its production is a variable genetic trait.

The gene *tst*H is encoded by a transposon-like mobile genetic element.[57,83,148,170] The chromosomal fragment with the structural gene for production of TSST-I is a 10.6-kilobase (kb) unit that has been cloned in *E. coli*. *tst*H has been sequenced and encodes a 234–amino acid protein that is converted by the removal of a signal peptide of 40 amino acids to the mature exotoxin of 194 amino acids.[29,85,170] *tst*H has been inserted in the chromosome to disrupt both the tryptophan synthetase B and SEB genes, thereby preventing the production of both proteins.[57,83]

Production of TSST-I and other post–exponential-phase virulence factors is regulated by three separate genetic loci: the accessory gene regulator (*agr*), the staphylococcal accessory regulator (*sar*), and the extracellular protein regulator (*xpr*).[12,29,85] All three regulators affect gene expression primarily at the level of transcription. During the late log phase of bacterial growth, they activate the expression of TSST-I, alpha- and gamma-hemolysins, serine protease, and nuclease and downregulate the expression of cell wall–associated proteins, fibronectin-binding protein, protein A, and coagulase. Beta-hemolysin, SEB, SEC, and exfoliative toxin A are regulated less tightly by *agr* than TSST-I is. SEA appears to be independent of *agr* in some strains. This complex regulation of exoprotein synthesis probably is the result of a complex interaction between environmental factors and gene products.[29,85] These regulatory functions explain why the production of TSST-I is stimulated under some conditions without SEA or SEC stimulation.[335]

Glyceryl monolaurate is a mild surfactant and emulsifier commonly used in the food and cosmetic industries. It is capable of inhibiting the production of staphylococcal and streptococcal exotoxins at concentrations subinhibitory to growth.[40,245,267] As a lipophilic compound, the mechanism of action appears to be insertion into the cell membrane with resulting interruption of signal transduction. Other surfactants used in the tampon industry have been found to both inhibit and enhance[268] the production of TSST-I in vitro.

KINETICS OF DISTRIBUTION

Detecting TSST-I in body fluids is difficult. In humans, nanogram quantities have been detected by radioimmunoassay in the breast milk of a woman with TSS,[149,323] in serum early during illness in two of four patients,[195] in urine, and in vaginal washings in a small number of patients.[195] By means of the rabbit subcutaneous Wiffle ball abscess model, TSST-I can be detected first in abscess fluid 4 hours after *S. aureus* has been inoculated into the Wiffle ball and in urine 48 hours after inoculation.[195]

For further examination of in vivo target tissues for TSST-I activity, purified TSST-I radiolabeled with iodine 139 has been injected intravenously into rabbits. Measurement of plasma clearance demonstrated a half-life of 1.5 hours. Within 15 minutes of injection, TSST-I was concentrated fourfold in blood cells in comparison to plasma. Toxin persisted in the cellular compartment, and plasma concentrations fell. After 3 hours, most of the radiolabel was found in the spleen.

TSST-I, SEA, and SEB can cross epithelial membranes intact in a fully functional form.[134] In a cellular model of the blood-brain barrier, TSST-1 showed slow bidirectional movement consistent with restricted paracellular diffusion.[18]

CELLULAR INTERACTIONS

TSST-I has been shown to inhibit systolic function in isolated rabbit atria.[224] It also can bind directly to human and porcine endothelial cells and is cytotoxic to porcine endothelial cells because it permits leakage across endothelial cell monolayers.[173,176] However, concentrations of TSST-I needed to induce these and other diverse cytotoxic changes in vitro are higher than the usual tissue and serum levels. Interactions of TSST-I and the enterotoxins with T lymphocytes and antigen-processing cells are described in the next section.

BIOLOGIC FUNCTIONS

Animal Models

Much information regarding pathogenesis has been obtained from both the subcutaneous Wiffle ball and the vaginal tampon rabbit models and a primate model.[81] Rabbits, primates, and other species have been examined for sensitivity to TSST-I or TSS-associated strains of *S. aureus*.[232] Rabbits and baboons exhibit clinical and laboratory changes most consistent with the human syndrome, including pyrogenicity and lethality.* The signs and symptoms observed in these animals after the intravenous injection of purified TSST-I include skin erythema, conjunctivitis, diarrhea, lethargy, tachypnea, respiratory distress, central nervous system changes, and increased capillary permeability, as demonstrated by the skin bluing technique. A variety of laboratory abnormalities consistent with those described in human TSS have been reported for these models.

Immunoregulatory Activities

The absence of in vitro cytotoxicity of TSST-I[22,232] despite potent in vivo biologic effects has suggested an important role for endogenous mediators in TSS. The ability of SEB to induce dramatic T-cell proliferation was recognized a decade ago.[160,166,185] Subsequent work has shown it to be a feature of other bacterial exotoxins, including TSST-I, the staphylococcal enterotoxins, the streptococcal pyrogenic exotoxins A and C, a new streptococcal superantigen, and a streptococcal M surface protein.[166] Since the late 1980s, activation of T cells by these bacterial components or products has become an area of intense interest. The term *superantigen* was coined in 1989 to describe antigens that at concentrations lower than those of conventional antigens (picomolar concentrations) can stimulate proliferation of a large percentage of T cells bearing a TCR beta-chain variable (V_β) sequence or sequences specific for each superantigen. Such activation and proliferation of T cells result in profound alterations in immune system homeostasis by inducing the release of large quantities of monokines (IL-1, TNF-α) and lymphokines (IL-2, TNF-β).[166,280,341]

T cells are activated to produce lymphokines as a result of TCR-antigen binding and activation of signal transduction.[45,94,287] Antigens can bind to the TCR in one of two forms: as conventional antigens or as superantigens. Conventional antigens enter antigen-processing cells by endocytosis. They are broken into small peptides in the lysosomal compartments of the antigen-processing cell and targeted to small vesicles, where they form complexes with one of the allele-restricted MHC class II molecules (HLA-DQ, HLA-DP, and HLA-DR).[280] The complex then is transported to the cell surface, where it is bound in the groove on the heavy chain formed by the alpha and beta chains of the class II molecule. Certain amino acids in residues at critical points

*See references 31, 32, 81, 82, 196, 198, 200, 234, 247, 270, 276.

in the peptide's sequence anchor the peptides in the groove, and this interaction is moderately specific for each different allelic form of the MHC molecule.[280] This complex of peptide and MHC molecule of the same allelic type as the T cell is recognized by the TCR.

The TCR is composed of both alpha and beta glycoprotein chains, each of which is composed of variable (V) and joining (J) segments. The beta chain has an additional diversity (D) segment. As many as 50 V_β and 32 V_α segments are present on human T cells. The $V_{\alpha\beta\alpha\beta}$ segments each are encoded by different genes that undergo gene rearrangement to give more than 10,000 possible combinations to recognize conventional antigenic peptides presented by antigen-processing cells.[16,280] Conventional antigens presented as peptides must be recognized by a specific combination of these elements. Thus, one peptide will activate only 1 in 10^4 to 10^6 T cells, and only $CD4^+$ T cells respond to conventional antigens.

In contrast, superantigens are presented to T lymphocytes in a very different fashion. They bind first as intact proteins (small fragments are inactive) to most allelic forms of MHC class II molecules in an unrestricted fashion at a site outside the peptide-binding groove. They then react with the TCR only through the V_β element or elements specific for that molecule and will react with all T cells carrying that V_β element.[145] Because only 25 to 50 major families of V_β genes exist in humans, each superantigen can interact with 5 to 20 percent of resting T cells, depending on the frequency of T cells expressing that V_β family in each individual's repertoire. Both $CD4^+$ and $CD8^+$ T cells are activated. Features unique to the presentation of superantigens to the TCR V_β receptor thus include the requirement for initial binding of superantigen to the MHC class II molecule (even if unrestricted and not self) outside the antigen-presenting groove and the requirement for an intact superantigen protein.

Once the T cell has recognized the superantigen,[132] both antigen-processing cell and T-cell activation by signal transduction[45] results in release of cytokine by the antigen-processing cell (IL-1, TNF-α) and the lymphocyte (IL-2, TNF-β). In mice and possibly also in humans, the initial T-cell activation occurs in lymph nodes.[205] Within hours of T-cell activation by superantigens, cytokines are detected in vitro and in serum in vivo.

TSST-I binds only to the $V_\beta 2$ element.[256] In humans with TSS caused by TSST-I–secreting *S. aureus*, within 10 to 14 days 30 to 70 percent of the circulating T-cell population will be T cells bearing the $V_\beta 2$ element.[53,54,187] The number of circulating $V_\beta 2$ T cells does not return to normal for several months. In contrast, patients with TSS caused by *S. pyogenes* have a consistent pattern of depletion of certain V_β T-cell types after T-cell activation.[166,220,327] In mice, such depletion is thought to be the result of programmed cell death (apoptosis).[203,204]

Thus, activation of T cells by superantigens may result in massive cytokine release with subsequent selected V_β T-cell expansion, T-cell deletion, or apoptosis, depending on the superantigen and species. The fact that superantigens bind only to selected and specific V_β elements distinguishes them from the nondiscriminating mitogens.

Activation of monocytes and lymphocytes by superantigens involves signal transduction. Activation of both src protein tyrosine kinases and protein kinase C occurs in a manner common to that of other immunoglobulin supergene family members. MHC class II cells also are expressed on beta, endothelial, and dendritic cells. In some instances, superantigens may activate these cells uniquely.

Convincing evidence from the mouse model supports a central role for T-lymphocyte activation in superantigen-mediated disease. Mice that have received cyclosporine to block T-cell activation and lymphokine production or mice with severe combined immunodeficiency disease are protected against SEB-induced lethal shock. Repopulation with T cells results in susceptibility to SEB. The same mice are not protected against endotoxin-induced shock because lipopolysaccharide activates only monocytes.[203-205]

Mononuclear production of IL-1 and TNF-α is central to the shock induced by endotoxin. Production of these two cytokines in addition to production of the lymphokines IL-2, IFN-α, and TNF-β may explain the enhanced severity of superantigen-associated TSS.[30,132,166] These powerful effects on the immune system may account for the tenacity of shock in otherwise healthy individuals. If more than one superantigen is produced by an organism (e.g., TSST-I plus one or more enterotoxins), different V_β specificities would result in an even greater number of T cells being activated, with the potential for development of disease with enhanced severity and mortality.

Once superantigens have resulted in monokine and lymphokine release, the subsequent pathophysiologic events appear to be related to the many and complex interactions of these cytokines,[30,136,340] with the ultimate result being profound endothelial damage. TNF-α and TNF-β and the complex interaction of cytokines, leukotrienes, prostaglandins, adhesion molecules, nitric oxide, platelet-activating factor, complement components, neutrophils, platelets, and endothelium-derived factors appear to be responsible for the extensive endothelial damage and resulting capillary fluid leakage and the decrease in peripheral vascular resistance.[30,136,261,340] In a different setting of antineoplastic therapy, high doses of IL-2 and lymphokine-activated killer cells induce the capillary leak syndrome, which is characterized by rapid weight gain, anasarca, pulmonary edema, hypoalbuminemia, and multiple organ dysfunction in humans.[64,165]

Endotoxin Enhancement

A striking property of TSST-I is its marked ability to enhance the susceptibility of some animals to lethal endotoxic shock.[29,265,270] Intravenous administration of TSST-I to rabbits at less than $^1/_2$ the median lethal dose (LD_{50}) followed 2 hours later by endotoxin at less than $^1/_{500}$ the LD_{50} results in 50 to 100 percent mortality, a 10,000-fold enhancement. In vitro, endotoxin enhances IL-1 production by TSST-I–stimulated monocytes.[20] Whether enhanced endotoxin susceptibility plays any role in human disease is not clear.[55]

STRUCTURE-FUNCTION RELATIONSHIPS

Effort to understand the molecular actions of TSST-I has focused on separating regions that interact with the TCR from those required for lethality.[29,31,32,213] Through the use of TSST-I variants (TSST-ovine and TSST-bovine) and TSST-I molecules with single–amino acid mutations, the areas responsible for binding of TSST-I to both the TCR and the macrophage class II MHC molecule have been defined.[29] In animal models, lethality, induction of fever, and endotoxin susceptibility do not depend on T-cell proliferation as measured by superantigenic activity but must involve interaction of toxin with other host-cell receptors in the body.[29]

Most secreted bacterial superantigens are small, compact globular proteins of 20 to 30 kd.[29] They are protease-, heat-, and acid-resistant and share immunologic, biologic, and functional properties. However, they do not share sequence homology. Although they do not share obvious structural features that would predict their superantigen properties, they may share a common conformational structure.[166] The secreted exotoxins can be divided into two groups based on their amino acid sequence homology. In group 1, SEA, SED, and SEE share 54 to 90 percent homology. In group 2, streptococcal pyrogenic exotoxin A, SEB, and SEC-1, SEC-2, and SEC-3 share 46 to 68 percent homology. In group 3, TSST-I, the exfoliative toxins, and strep-

tococcal pyrogenic exotoxin show no significant homology to each other or to any of the members of group 1 or 2. However, all the secreted superantigens may share a three-dimensional conformation that allows them to interact simultaneously with two different receptors on two different cell types.[29,166]

The structure and binding sites of SEB to the MHC class II molecule have been determined by crystallographic conformation. Two different MHC class II binding sites for SEB exist. The amino-terminal domain forms most of the contact, but residues at the carboxy-terminal domain also contact the MHC class II molecule, as well as the TCR. Although TSST-I is very similar to SEB on the basis of their crystallographic structure, the two proteins do not compete with each other for the same MHC class II HLA-DR sites. Likewise, SEA and SEE have greater than 90 percent amino acid sequence homology but quite distinct patterns of V_β specificity. TSST-I and the exfoliative toxins A and B are unrelated structurally but have the same $V_\beta 2$ specificity.[166,185]

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CHAPTER 72

Acute Respiratory Distress Syndrome in Children

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Ashbaugh and associates[11] first used the term *adult respiratory distress syndrome* in a case series that they reported in 1967. In it, they described a clinical syndrome of diverse etiology that was characterized by the development of fulminant respiratory failure associated with rapidly progressive bilateral pulmonary infiltrates in the absence of cardiac failure. Although the mortality rate in their patients was 67 percent, four individuals treated with continuous positive airway pressure and high positive end-expiratory pressure (PEEP) survived. Postmortem examination of the lungs from nonsurvivors revealed significant pulmonary edema and hyaline membrane formation. They assumed that the presence of hyaline membranes and the favorable response to positive pressure indicated an acquired surfactant deficiency. The term *adult respiratory distress syndrome* was used as an analogy to neonatal respiratory distress syndrome (hyaline membrane disease) caused by insufficient surfactant production and is now referred to as *acute respiratory distress syndrome* (ARDS).

ARDS now is recognized as a relatively common sequela of a variety of local or systemic diseases that result in damage to the vascular endothelium, alveolar epithelium, and alveolar-capillary membrane. Compromise of these structures leads to the final common pathway of ARDS: pulmonary vascular leak, noncardiogenic pulmonary edema, and respiratory failure. The clinical syndrome consists of tachypnea, dyspnea, and marked hypoxemia caused by edema and reduced total lung compliance. Radiographs demonstrate diffuse bilateral opacities. Although considerable research has been conducted in an attempt to identify factors predisposing individuals to ARDS and even more in therapeutic trials, ARDS remains poorly understood and therapy is primarily supportive. Even though mortality rates in adults and children

have decreased during the past 20 years, ARDS remains a life-threatening disease.[77,102,123]

ARDS initially was described in adults, but it has been recognized as a leading cause of mortality in pediatric patients in critical care units. More than 500 cases have been reported in children ranging in age from 2 weeks to 17 years.* The true incidence of pediatric ARDS is unknown but has been estimated to be between 8.5 and 10.4 cases per 100 pediatric intensive care unit (ICU) admissions.[107] The prevalence rate of ARDS in pediatric patients in ICUs has been reported to be 0.6 to 7.2 percent.[23,27] A recent population-based study of pediatric ARDS in Germany reported a prevalence of 5.5×10^{-5} cases per year and an incidence of 3.4×10^{-5} cases per year.[18] As in adults, ARDS in children can result from a variety of injuries that all lead to increased vascular permeability, pulmonary edema, and clinical respiratory failure. Also as in adults, ARDS develops in most children as a result of sepsis, pneumonia, and aspiration.[30,33,44,91,141] Advances in supportive therapy for ARDS have improved outcomes in adults, and the results of several adult clinical trials have been extrapolated for use in children. The overall mortality rates have decreased in both adults and children during the past decade, with recent pediatric studies reporting mortality rates of 22 to 31 percent.[30,33,35,44,141]

DEFINITION

Defining ARDS has been the source of much confusion and, at times, heated debate. Initially, ARDS signified a disease of adults, but the reporting of numerous cases in children prompted the use of *acute* rather than *adult* respiratory distress syndrome. Although both terms occasionally appear, *acute respiratory distress syndrome* currently is the preferred designation. Early attempts at defining ARDS resulted in the use of criteria proposed by the National Heart, Lung, and Blood Institute (NHLBI) that included (1) acute and rapidly progressing pulmonary disease of a noncardiac nature; (2) progressive, diffuse, bilateral pulmonary infiltrates on chest radiographs; and (3) hypoxemia, defined as a ratio of arterial oxygen tension to the fractional concentration of inspired oxygen (Pao_2/Fio_2) of less than 150 without PEEP or less than 200 with PEEP. Continued interest in the systematic study of ARDS from epidemiologic and therapeutic standpoints, combined with continued difficulty in comparing data because of differences in definition, led to the formation of an international discussion group. The European-American Consensus Committee on ARDS then published a unifying definition of ARDS that included (1) impaired oxygenation with a Pao_2/Fio_2 ratio less than 200, regardless of PEEP; (2) bilateral densities demonstrated on chest radiographs; and (3) pulmonary artery wedge pressure less than 18 mm Hg with no evidence of left atrial hypertension.[15] Although this definition of ARDS in pediatric populations is widely accepted, recent evaluation suggests that the Pao_2/Fio_2 ratio used may need to be modified in children.[103]

PATHOPHYSIOLOGY

The pathophysiologic abnormalities associated with ARDS, though studied extensively since the syndrome was originally described in 1967, remain incompletely understood. A host of seemingly unrelated systemic diseases and local insults to the respiratory tract have been reported to result in ARDS. Despite efforts to elucidate the precise chain of events that lead from these initial triggers to massive cellular injury and rapidly progressive respiratory failure, much of the process remains a mystery. Nonetheless, certain cellular and biochemical markers, as well as physiologic characteristics associated with ARDS, have been identified. In addition, the histologic findings seen in ARDS follow a characteristic pattern that has been well described. These pathophysiologic events and findings generally correlate with the clinical findings seen in ARDS.

As mentioned earlier, a wide variety of apparently unrelated disorders and injuries have been associated with the development of ARDS[22,26,33,35,70,91,107,135] and include a broad spectrum of infections (bacterial, viral, and other), inhalation/aspiration injuries, sepsis syndromes, trauma, drug reactions and metabolic disorders, transfusion and stem cell transplantation reactions, and malignancies, among other causes (Tables 72–1 and 72–2). Although the mechanism or mechanisms of injury that unite this group are unknown, underlying damage to the vascular endothelium, alveolar epithelium, or alveolar-capillary membrane (or any combination of such damage) clearly must be present. Several hypotheses have been proposed and are summarized in review articles.[105,111] These hypotheses include activation of the complement cascade, excessive neutrophil and macrophage activity, surfactant dysfunction, and others. A more recent review of ARDS summarizes the importance of disruption of the alveolar-capillary barrier and its constituents—the microvascular endothelium and the alveolar epithelium.[145]

Complement activation occurs after trauma, pancreatic damage, endothelial damage, exposure to endotoxin, and other systemic and respiratory injuries.[99] By-products of complement activation then cause recruitment and activation of neutrophils, which in turn escalate the inflammatory cycle and damage the

TABLE 72–1 Noninfectious Conditions Associated with Acute Respiratory Distress Syndrome

Direct Injury to the Lung	Secondary Injury to the Lung
Inhalation	Anaphylaxis
NO_2	Shock—any cause
Cl_2	Sepsis
SO_2	Trauma
NH_3	Multiple trauma
Phosgene	Fractures
Smoke	Burns
Oxygen toxicity	Head trauma
Aspiration	Blood disorders
Foreign body	Diffuse intravascular coagulation
Gastric fluid (especially with a pH < 2.5)	Massive blood transfusion
Near drowning (fresh or salt water)	Drug overdose
Hydrocarbons	Heroin
Emboli	Methadone
Air	Barbiturates
Fat	Ethchlorvynol
Amniotic fluid	Salicylates
Pulmonary contusion	Propoxyphene
Radiation pneumonitis	Deferoxamine
Asphyxiation/strangulation	Metabolic disorders
	Diabetic ketoacidosis
	Uremia
	Pancreatitis
	Increased intracranial pressure
	Cardiopulmonary bypass
	Hemodialysis
	Cardioversion
	Paraquat ingestion
	Malignancy/lymphoproliferative disorder

From Royall, J. A., and Levin, D. L.: Adult respiratory distress syndrome in pediatric patients. I. Clinical aspects, pathophysiology, pathology, and mechanisms of lung injury. J. Pediatr. 112:*169-180, 1988.*

*See references 13, 27, 33, 35, 53, 59, 70, 84, 90, 107, 109, 111, 112, 136.

TABLE 72–2 Infectious Conditions Associated with Acute Respiratory Distress Syndrome in Children

Author	Year	No. of Patients	Mortality	Viral Isolates	Bacterial Isolates	Fungal	Other
Lyrene and Troug[70]	1981	15	9/15 (60%)	0	*Enterococcus*	0	0
Pfenninger et al.[91]	1982	20	8/20 (40%)	0	Intra-abdominal process, 7/20, NS	0	0
Nussbaum[79]	1983	7	2/7 (29%)	0	*Haemophilus influenzae* type b	0	0
Katz et al.[59]	1984	23	8/23 (35%)	2/23, NS	*Pneumococcus*	0	0
Tamburro et al.[128]	1991	37	19/37 (51%)	Adenovirus, cytomegalovirus, varicella	*Staphylococcus aureus*	0	0
DeBruin et al.*[35]	1992	100	72/100 (72%)	HIV, cytomegalovirus, respiratory syncytial virus	Septic shock syndrome, 64/100, NS; *Bordetella pertussis*	5/100, NS	*Pneumocystis carinii*, 14/100
Davis et al.[33]	1993	60	37/60 (62%)	Respiratory syncytial virus, influenza virus, cytomegalovirus, varicella	Sepsis syndrome, 22/60, NS	4/60, NS	0

**Children with malignancy, compromised immunity, or both.*
HIV, human immunodeficiency virus; NS, organism not specified.

pulmonary parenchyma through the release of oxygen radicals, proteolytic enzymes, and eicosanoids. A strong association has been demonstrated between complement activation and the development of ARDS.[50] However, other investigators have reported that complement activation is nonspecific in predicting the development of ARDS.[40,64,83,150] Additional evidence suggests that the combination of circulating endotoxin and complement activation is potentially more important in the development of ARDS than is complement activation alone.[82] Complement activation further stimulates an inflammatory cascade involving tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), IL-6, IL-8, and other mediators.[14] Additional studies suggest possible roles for other proinflammatory elements, as well as genetic influences related to the inflammatory response.[31,48,89]

Significant, though again not conclusive, evidence suggests a key role for neutrophils in the genesis of ARDS.[14,129] Once activated, neutrophils can damage the lung parenchyma by the release of proteolytic enzymes, generation of toxic oxygen radicals, and initiation of arachidonic acid metabolism. Numerous studies have indicated potential roles for superoxide radicals in the development of ARDS or the presence of elevated concentrations of peroxide in the breath of ARDS patients.[54,124,125] Patients with ARDS similarly have elevated concentrations of elastase and collagenase and increased leukotriene B_4 in bronchoalveolar lavage fluid, findings consistent with neutrophil degranulation.[124,127] Although neutrophils are capable of causing widespread parenchymal lung damage, are characteristically found in ARDS, and probably play some role in the pathogenesis or propagation of ARDS (or both), the occurrence of ARDS in severely neutropenic individuals suggests that they are not essential or singly responsible for its development.[80,115]

Alveolar macrophages may play a critical role in the pathogenesis of ARDS. They are found in abundance in normal airways, and when stimulated by endotoxin or endogenous proinflammatory cytokines, alveolar macrophages synthesize and release TNF and IL-1.[111] Both products promote neutrophil chemotaxis, degranulation, and release of oxygen metabolites, thereby creating a cycle of inflammation within the pulmonary parenchyma. Administration of TNF to animals in experimental models produces pulmonary edema, decreased pulmonary compliance, increased cellularity, and increased lung water, the primary physiologic markers of ARDS.[121,138,139] Conversely, anti-TNF antibody offered protection from ARDS in a baboon septicemia model.[140]

In his review article, Royall[105] also discusses putative roles for oxygen radicals, platelet-derived eicosanoids, proteolytic enzymes, fibrin and its degradation products, and other processes that may be involved in the pathogenesis of ARDS. Likewise, secondary injuries, such as acquired surfactant deficiency, oxygen toxicity, and barotrauma, are thought to contribute to the pulmonary damage. As noted in the same article, a single mediator or mechanism probably is not responsible for all the findings seen in ARDS, and simultaneous activity on several fronts most likely occurs.

The physiologic hallmark of ARDS is damage to the capillary endothelium and alveolar epithelial barriers, leading to disruption of the alveolar-capillary membrane, increased permeability, and noncardiogenic pulmonary edema.[107] Increasing fluid within the alveoli and interstitial spaces leads to decreased total lung compliance, decreased functional residual capacity, increased airway resistance, and increased dead space. The resultant ventilation-perfusion mismatch creates the large intrapulmonary shunt responsible for the profound hypoxemia seen clinically.[63] Additional physiologic derangements seen in ARDS include alterations in peripheral oxygen delivery and consumption, pulmonary hypertension with right ventricular compromise, and end-organ damage (liver, kidney, intestine, bone marrow).[23,111,148]

The histologic findings seen at postmortem examination of patients dying as a result of ARDS are very consistent and have been well described.[12,60,95,137] Historically, three interrelated and overlapping phases are described. They correlate well with clinical progression of the syndrome and include the exudative, proliferative, and fibrotic phases.[14] The early exudative phase typically is recognized from 12 to 96 hours after the onset of respiratory failure.[107,111,137] It is characterized by increased fluid in the alveoli and interstitium and hemorrhagic alveolitis. Grossly, the lungs are rigid, dusky, red-blue, and very heavy.[137] The alveolar fluid is protein-rich, often hemorrhagic, and associated with hyaline membranes.[95] A combined neutrophilic and monocytic infiltrate is observed in the pulmonary capillaries, interstitium, and alveoli (Fig. 72–1A). Although capillaries also contain fibrin plugs and microthrombi, endothelial cells show only subtle abnormalities when compared with the alveolar surfaces, which undergo degeneration and sloughing.[107]

The proliferative phase occurs 3 to 10 days after the onset of ARDS and is characterized by organization of the alveolar and interstitial exudates acquired during the exudative phase.[137] Gross examination reveals the lungs to be solid, pale gray, and slippery because of the generation of connective tissue. The proliferation of type II cuboidal epithelial cells occurs as early as 3 days after onset, with fibrosis visible by 10 days. Type II pneumocytes are metabolically active and responsible for the production of surfactant, and they evolve into the type I epithelial cells that line the alveolar spaces (see Fig. 72–1B). Within the alveolar walls, fibro-

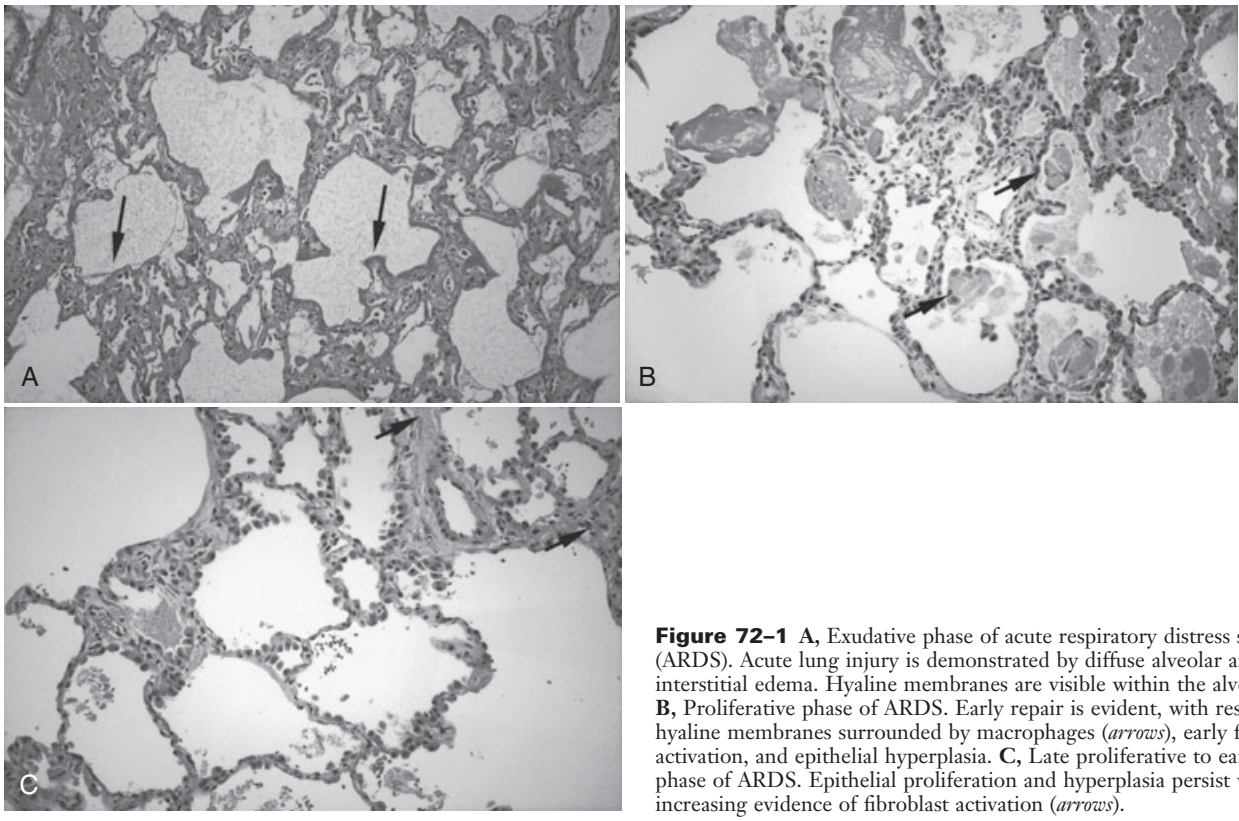

Figure 72–1 **A,** Exudative phase of acute respiratory distress syndrome (ARDS). Acute lung injury is demonstrated by diffuse alveolar and interstitial edema. Hyaline membranes are visible within the alveoli (*arrows*). **B,** Proliferative phase of ARDS. Early repair is evident, with residual hyaline membranes surrounded by macrophages (*arrows*), early fibroblast activation, and epithelial hyperplasia. **C,** Late proliferative to early fibrotic phase of ARDS. Epithelial proliferation and hyperplasia persist with increasing evidence of fibroblast activation (*arrows*).

blasts and myofibroblasts also proliferate and migrate through the basement membrane into the alveolar space. In addition, early resolution of the hyaline membranes and pulmonary edema begins. This phase is considered to be reparative in nature.

Phase III, the fibrotic stage, begins to develop 7 to 10 days after the onset of respiratory failure and is characterized by progressive fibrosis that distorts the primary acinar architecture of the lung. The lungs appear pale and spongy with a cobblestone surface. Cellularity is reduced, alveolar fluid organizes, and fibrosis develops in and around the terminal respiratory units (see Fig. 72–1C). Airspaces are irregularly enlarged and separated by thick bands of collagenous connective tissue. Extensive fibrosis appears to be related to irreversible respiratory failure.[12]

CLINICAL MANIFESTATIONS

The clinical features of ARDS are a reflection of the pathophysiologic changes previously discussed and generally follow a typical course regardless of the initiating event or injury. The syndrome progresses through four stages: the acute injury, a latent period, acute respiratory failure, and a period of severe physiologic derangement.[105,107,111] During the period of acute injury, the physical, laboratory, and radiographic findings associated with the causative disease or injury generally overshadow those associated with ARDS itself. Disorders associated with primary lung injury (e.g., aspiration pneumonitis or inhalation injury) may demonstrate significant physical or radiographic findings, whereas individuals with trauma or sepsis syndromes may have entirely normal auscultative or radiographic examinations of the chest.

The latent period is said to occur from 6 to 48 hours after the onset of ARDS and is characterized by cardiopulmonary stabilization or even improvement in the patient's condition. Subtle physical examination findings (mild tachypnea), radiographic findings (fine reticular infiltrates), and laboratory abnormalities (increased $Paco_2$, increased pulmonary vascular resistance, decreased tissue oxygen delivery, increased serum von Willebrand factor antigen) may suggest the development of ARDS during this phase.[1,108,114]

The diagnosis of ARDS generally is made during the period of acute respiratory failure and is based on appropriate clinical and laboratory findings supportive of the diagnosis combined with a preexisting condition known to be associated with ARDS. The physical examination is remarkable for tachypnea, tachycardia, dyspnea, and cyanosis. The chest is quiet generally, but fine crackles may be present. Hypoxemia refractory to supplemental oxygen therapy is one of the hallmarks of the syndrome. Chest radiographs demonstrate diffuse, bilateral infiltrates suggestive of both interstitial fluid and alveolar filling or atelectasis (Fig. 72–2). Computed tomography may be helpful in characterizing the nature of the pulmonary infiltrates or even distinguishing the cause of the ARDS.[38] Respiratory failure and profound hypoxemia require intubation. The hypoxemia often worsens rapidly despite assisted ventilation, and many patients progress to the phase of severe physiologic abnormalities. During this stage, hypoxemia and hypercapnia refractory to high levels of ventilatory support often denote an irreversible pulmonary process. Multiorgan system failure frequently ensues at this point. The clinical course thereafter depends on the severity and character of the initial illness and the development of complications such as sepsis, disseminated intravascular coagulation, and pulmonary air leak syndromes.

TREATMENT

Although tremendous energy and resources have been invested in research aimed at defining the mechanism of lung injury in

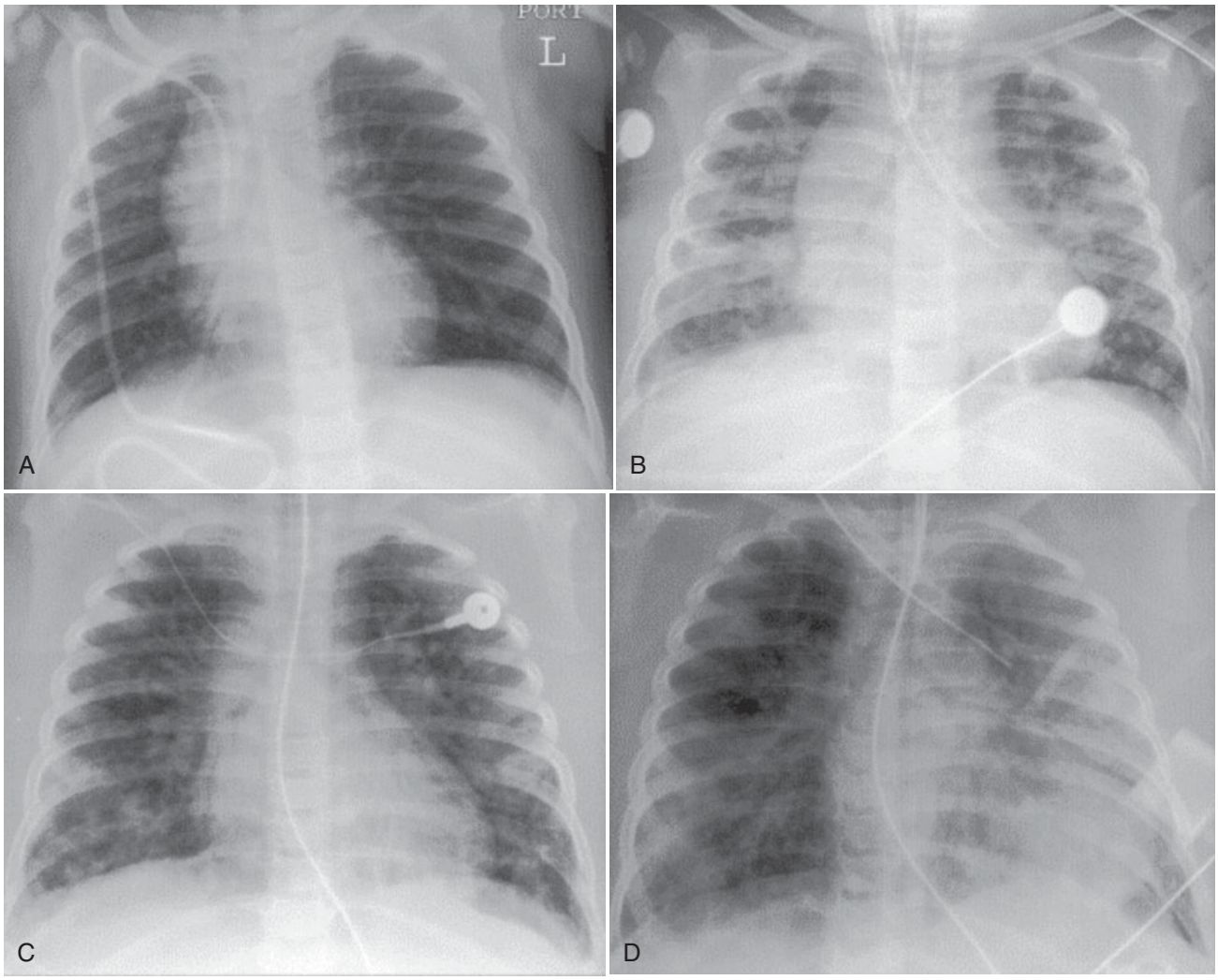

Figure 72–2 Chest radiographs of a 4-year-old child with central line sepsis and acute respiratory distress syndrome. Note the diffuse bilateral infiltrates suggestive of alveolar filling, interstitial fluid, and atelectasis. The time from **A** to **B** was 24 hours, but the intervals from **B** to **C** and from **C** to **D** were several days.

ARDS, a definitive answer and, thus, definitive therapeutic interventions have remained elusive. For this reason, management of ARDS historically has been primarily supportive. The traditional goals of therapy have been to treat the underlying or predisposing condition, maintain adequate end-organ oxygenation through the use of oxygen and supportive ventilation, and minimize complications of therapy or acute/chronic illness (oxygen toxicity, barotrauma, nosocomial infections, negative nitrogen balance, and multisystem organ dysfunction).[106,111] Only in the past decade have significant advances been made in improving supportive care and have attempts been made to treat some of the underlying pathologies encountered in ARDS.

Both Sarnaik and Lieh-Lai[111] and Royall[105] have stressed the importance of adequate monitoring of patients with ARDS. Invasive and noninvasive monitoring modalities allow the physician to assess pulmonary mechanics, the adequacy of peripheral oxygen delivery and consumption, cardiac function, and other critical physiologic parameters. Pulse oximetry, arterial catheters, and central venous pressure monitors are suggested as a minimum, with the use of pulmonary artery catheters as indicated by the patient's clinical status.

Positive-pressure ventilation is a major component of care for patients with ARDS. Several randomized trials have evaluated the usefulness of low–tidal volume ventilation as a method to improve survival and decrease complications. Experimental animal data suggested that high–tidal volume ventilation was associated with inflammation and increased release of inflammatory mediators from the lung into the systemic circulation (e.g., TNF-α, IL-6) when compared with low–tidal volume ventilation strategies.[62,142] Initial human trials with relatively small sample sizes yielded conflicting results.[5,20,21,122] A large multicenter trial sponsored by the NHLBI's ARDS Network (ARMA) enrolled 861 patients at ten sites to compare a ventilatory protocol consisting of tidal volumes of less than 6 mL/kg with conventional ventilation using higher tidal volumes. Plateau pressures were maintained at less than that of 30 cm H_2O.[132] The study reported that the group undergoing low–tidal volume ventilation had a lower rate of mortality than that of controls (31% versus 40%), more mean days free of mechanical ventilation, and more days free of nonpulmonary organ failure. Thus, high tidal volumes and high plateau pressures were associated with worse outcomes in patients treated for ARDS and should be avoided if possible in these patients. Other treatment parameters outlined in the publication suggest maintaining pH between 7.3 to 7.45 by changing the respiratory rate and adjusting the fraction of inspired oxygen and PEEP to achieve adequate oxygenation (Pao_2, 55 to 80 mm Hg, or Spo_2, 88%-95%).

High-frequency oscillation is a mode of ventilation that delivers small tidal volumes and has been proposed as a better method of ventilation for ARDS. Two randomized trials in adults and one in children demonstrated improved oxygenation in patients receiving high-frequency ventilation, but mortality rates were not significantly different from those of patients undergoing conventional ventilation.[10,19,37] These studies had considerably fewer patients than did the ARMA trial. Pediatric case series suggest that this mode of ventilation is safe and can be used as rescue therapy; however, large randomized trials in pediatrics are needed.[9,55,94] The role of high-frequency oscillation versus low–tidal volume ventilation in the routine treatment of ARDS remains to be determined and will require further investigation.

The historical approach to the respiratory management of ARDS has focused on the use of oxygen and PEEP. PEEP is a critical component of mechanical ventilation in the treatment of patients with ARDS. Hypoxemia is profound in patients with ARDS and is refractory to supplemental oxygen given by face mask. The vast majority of patients require intubation and supportive mechanical ventilation. Once the patient is intubated, high concentrations of supplemental oxygen and high levels of PEEP often are needed. PEEP improves pulmonary function in ARDS by reducing ventilation-perfusion mismatch and decreasing intrapulmonary shunting,[32,113] which is accomplished by increasing functional residual capacity by enlarging open alveoli and recruiting atelectatic airspaces. PEEP additionally reduces the repetitive expansion and collapse of terminal airways and redistributes alveolar fluid. The improved lung compliance and decreased ventilation-perfusion mismatch may allow a reduction in F_{IO_2}, thereby decreasing the potential for oxygen toxicity and possibly protecting the lung.[87,151]

Although PEEP is used to treat patients with ARDS, the optimal values have not been established. Several randomized trials have not demonstrated a significant change in mortality rates with increased levels of PEEP.[132,134,144] To determine the effect of high PEEP in the treatment of ARDS, the NHLBI sponsored a multicenter randomized trial comparing patients randomized to high PEEP (12-24 cm H_2O) with a comparable group randomized to low PEEP (5-24 cm H_2O).[134] No significant differences were observed between the two groups in mortality rates, yet the high-PEEP group had improved oxygenation. All patients in the trial were ventilated with low tidal volumes. Mean days of mechanical ventilation and the duration of nonpulmonary organ failure were comparable in both groups. Thus, PEEP should be used to maintain adequate oxygenation, yet the optimal pressure will vary, depending on the severity of the patient's disease.

PEEP significantly improves ventilation-perfusion mismatch, but it can affect cardiac output. Decreased venous return to the heart and a shift in the interventricular cardiac septum can occur with high pressure. These complications are treated by a reduction in left ventricular afterload. PEEP should be used to maximize oxygen delivery at the lowest pressure that achieves this goal. Additional methods of improving mechanical ventilation are prone positioning, inverse-ratio ventilation, partial liquid ventilation, and noninvasive ventilation. Randomized controlled trials of prone positioning have demonstrated that improved oxygenation is achieved with a change in positioning; however, mortality rates in these trials did not significantly decrease with its use.[28,46,49,71,98] For selected patients with an F_{IO_2} requirement of greater than 0.6 or refractory hypoxemia, use of the prone position may be helpful. Because extubation is a potential complication with repositioning, prone positioning should be used with caution at centers unfamiliar with this technique. Studies using perfluorocarbons for partial liquid ventilation have not shown improved outcomes over standard methods of ventilation.[52,56] Noninvasive positive-pressure ventilation is useful in selected patients with acute lung injury, such as pediatric cancer,[92] but it will not provide sufficient ventilatory support for the majority of patients with ARDS.

Many of the predisposing conditions associated with ARDS result in cardiovascular instability. Cardiovascular function should be monitored and maintained with additional therapies (e.g., inotropic agents, fluids) as needed. Patients seen in the early stages of septic shock often require vigorous fluid resuscitation to maintain adequate tissue perfusion.[101] Once the patient has been stabilized for a minimum of 12 hours, a conservative fluid strategy to maintain cardiovascular function and keep the patient's fluid balance stable with no net gain should be considered. A large randomized trial by the Acute Respiratory Distress Network reported more ventilator-free days and ICU-free days with a conservative fluid treatment approach than with a liberal fluid strategy; however, no significant differences were observed in mortality rates.[4] Furthermore, the need for renal dialysis and the prevalence of shock were not significantly different between the conservative and liberal fluid management groups. Patients in shock still should receive fluid resuscitation as needed and would not be candidates for the conservative fluid management strategy. Colloid or crystalloid is used for volume resuscitation.[24,152] A recent large randomized trial reported the equivalence of saline resuscitation and albumin volume resuscitation[43]; however, albumin with furosemide may be useful in patients with hypoproteinemia.[6] Once patients are hemodynamically stable, fluids are restricted to decrease vascular leak and subsequent pulmonary edema. Patients frequently are given diuretics to reduce fluid in the interstitium and alveoli. Positive inotropic support with dopamine or dobutamine may be required.

The overall goal of cardiopulmonary management of ARDS is to maintain good cardiac output and optimize oxygen delivery to the periphery. Other important aspects of the supportive care required for management of ARDS include maintenance of adequate nutrition, avoidance or treatment of complications related to therapy (barotrauma and oxygen toxicity) or prolonged illness (infection), and support of other organ systems.[106] Poor nutrition is a common problem in critically ill children and results in inadequate tissue repair, multiple organ system dysfunction, respiratory muscle weakness, and immune deficiency.[93,120] Hence, nutritional support is of paramount importance in ARDS and must be maintained.[97] The related issues of oxygen toxicity and barotrauma are considered later. Infection is both a common precipitating event in ARDS and an important factor in its course and outcome.[106] Infections typically take the form of bacteremia/sepsis or nosocomial pneumonia. Organisms frequently isolated from the lower respiratory tract are *Klebsiella* and *Pseudomonas*; however, *Escherichia coli*, *Candida albicans*, and *Staphylococcus epidermidis* are not uncommon isolates.[111] A high index of suspicion and a low threshold for initiating therapy should be maintained. Finally, prevention, detection, and treatment of multisystem organ dysfunction or its complications are critical in the overall management of children with ARDS.

As suggested earlier, many of the innovations in the supportive care of ARDS have come in the arena of ventilatory support. Pressure-limited ventilation with a reversed inspiratory-to-expiratory ratio, permissive hypercapnia, high-frequency ventilation (positive pressure, jet, and oscillating), prone positioning, and extracorporeal membrane oxygenation have been reported.* Patients unresponsive to conventional mechanical ventilation may benefit, in terms of improved oxygenation, from any of these methods. Additionally, patients may benefit from lower F_{IO_2} and peak airway pressure to decrease the potential oxygen toxicity and barotrauma. Although many of these newer forms of ventilation

*See references 5, 10, 47, 51, 57, 61, 65, 67, 84, 85.

show promise for children with ARDS, none has been demonstrated conclusively to be superior to another, and all will require more research and clinical experience. At present, many of them are considered "rescue therapy."

Numerous pharmacologic agents have been used for the treatment of ARDS during the past decade. Therapeutic trials with corticosteroids, vasodilators, and prostaglandin E_1 have been reported.[7,16,29,106,149] Improved outcomes have been achieved with short-course corticosteroid therapy for early-phase ARDS and moderate-dose therapy for prolonged ARDS, yet a 7-year multicenter trial did not find a change in mortality rates with moderate-dose methylprednisolone for 14 days in patients with persistent ARDS.[6,25,43,45,72,100,101,133,152] In addition, starting corticosteroid therapy more than 2 weeks after the onset of ARDS was associated with increased mortality rates at 60 and 180 days.[133] Few data exist on the benefits of steroid therapy in pediatrics. Therefore, the role of corticosteroid therapy in the routine treatment of ARDS remains unresolved at the present time. Small trials with a host of other agents have failed to identify universally effective therapies.[61,75] Among the many pharmacologic agents studied, corticosteroid therapy, exogenous surfactant, and inhaled nitric oxide (NO) have been evaluated in large, multicenter trials.

The relative or acquired surfactant deficiency seen in ARDS renders surfactant replacement therapy an attractive area of investigation. In 1993, Lewis and Jobe[66] presented a comprehensive review of the rationale behind surfactant use in ARDS. Theoretically, replacement of endogenous surfactant by exogenous material would increase total lung compliance, decrease atelectasis, and improve pulmonary mechanics, thereby leading to decreased ventilation-perfusion mismatch, improved oxygenation, and less potential oxygen toxicity and barotrauma. The results of several trials have been reported in recent years and appear to offer some hope of improving outcomes in children with ARDS.[41,68,73,117,126,154] Early phase I and II trials in adults were encouraging, but larger randomized trials with synthetic surfactant[8] and natural and recombinant surfactant[118,119] have not demonstrated improvement in the mortality rate. In contrast, a recent randomized trial in children reported a decrease in the mortality rate and improved oxygenation.[153] The randomized trial enrolled 153 infants, children, and adolescents with acute lung injury. Although the mortality rate was greater in the placebo group, no significant differences were observed in the number of ventilator-free days or the duration of respiratory failure. Problems with surfactant therapy include difficulty with delivery and inactivation by lung fluid proteins. As the aforementioned references suggest, solutions to these and other issues related to surfactant therapy for ARDS are being sought aggressively, and researchers hope that surfactant will prove to be a valuable therapy in the future.

Another area of active research for the treatment of ARDS is the use of inhaled NO. NO has been shown to be a potent vasodilator and has been used in numerous settings for infants and children with a variety of cardiopulmonary diseases.[3,58] Numerous clinical trials have suggested a potential role for NO in the treatment of ARDS.[2,34,36,39,76,78,81] However, several randomized trials have not reported a reduction in mortality rates with its use. Improved oxygenation is observed on initiation of therapy, but it often is transient, with the effect lasting just a few days.[36,69,76,104,130,143] NO should be considered rescue therapy pending further investigation.[116,131] Some authors have questioned its use altogether.[74,86] Additional study is required to answer questions regarding the best use of NO therapy for ARDS. Other pharmacologic agents being investigated for the treatment of ARDS include beta-agonists,[88] granulocyte-macrophage colony-stimulating factor (GM-CSF),[96] and activated protein C.[17,110,146,147]

PROGNOSIS

Despite the huge investments of time, money, energy, and talent that have gone into research on the basic pathophysiologic mechanisms involved in the development of ARDS and equal amounts into potential therapies, many questions remain unanswered. Mortality rates have decreased during the past decade since this disease first was described in children. Mortality rates have decreased from 40 to 70 percent (see Table 72–2) to 20 to 31 percent.[30,44,141] New supportive and therapeutic interventions have improved the care of children with ARDS. Most children who survive ARDS eventually have normal radiographic results, and pulmonary function improves gradually with only a mild reduction in forced vital capacity.[42] Long-term physiologic abnormalities show a strong correlation with the severity of the acute illness and the degree of support required in the acute phase of ARDS. The search for innovative therapies to decrease the acuity of illness during the acute phase of the disease and reduce the incidence of iatrogenic complications holds some hope for the future.

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SECTION XIII

INFECTIONS OF THE FETUS AND NEWBORN

CHAPTER 73

VIRAL INFECTIONS OF THE FETUS AND NEONATE

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GENERAL ASPECTS OF VIRAL INFECTIONS OF THE FETUS AND NEWBORN

The fetus and newborn infant are highly susceptible to many different viruses that in most instances cause little or no disease in older age groups. However, relatively few of the hundreds of viruses to which humans constantly are exposed are ever transmitted to the fetus or cause infection in newborn infants. Nonetheless, viral infections are an important cause of neonatal morbidity and mortality. The cumulative frequency of viral infections in the fetus or newborn infant may be as high as 6 to 8 percent of all live births, whereas systemic bacterial disease occurs in only 1 to 2 percent of neonates.[188,229]

Contributing to the frequency of viral infections in this age group is that the infection can be acquired at several different periods during intrauterine and neonatal life: in utero (congenital infection), at the time of birth (natal infection), or after birth but during the neonatal period (postnatal infection). In addition, numerous different outcomes of infection are possible. Congenital infections can result in resorption of the embryo; abortion; stillbirth; congenital malformation; prematurity; intrauterine growth restriction; acute disease apparent in utero, at birth, or shortly thereafter; asymptomatic infection in the neonatal period, but a persistent postnatal infection with neurologic sequelae later in life; or a normal infant with no apparent sequelae. Natal or postnatal infections can cause acute systemic illness leading to death, persistent infection with late sequelae, self-limited disease with no discernible damage, or asymptomatic infection.[188,229]

Recent developments in the fields of diagnostic virology, epidemiology, and viral immunology have expanded our knowledge tremendously and modified our understanding of fetal and neonatal viral infections and their contribution to disease, not only in the neonatal period but also later in life.[188,229] In addition, the development of rubella vaccine and antiviral drugs effective against a few of the agents offers hope for prevention or control of these infections. This chapter provides the physician with an approach to diagnosis and management of, as well as prognostic information about, viral infections that occur in the fetus and newborn infant. Human immunodeficiency virus (HIV), an important perinatal viral pathogen, is covered in another chapter.

PATHOGENESIS

Congenital Viral Infections

Congenital viral infections are secondary to exposure of the fetus during maternal viral infection. Evidence from both humans and experimental animals indicates that most fetal infections are preceded by a systemic viral infection in the mother, with hematogenous spread of the virus to the placenta and subsequently to the fetus (Fig. 73–1). Ascending infection through amniotic membranes also may occur while the fetus is still in utero. Viral infections that occur in the mother during pregnancy and that are limited to the respiratory or gastrointestinal tract may not pose a risk to the fetus but later may be transmitted perinatally to the newborn infant. Even if viremia does occur in the mother, maternal host defense mechanisms and the placenta appear to provide a protective barrier for the fetus. With most viruses known to cause fetal infection—cytomegalovirus (CMV), rubella virus, herpes simplex virus (HSV), varicella-zoster virus (VZV), and vaccinia virus–placental involvement by the virus also has been documented.[74,550] Viruses may reach the fetal circulation by (1) replication through the layers of the placenta, (2) production of virus-induced vascular lesions in the placenta resulting in abnormal communications between the maternal and fetal circulation, or (3) diapedesis of virus-infected maternal leukocytes through the layers of the placenta to the fetal circulation.[74,475,635] Damage to the fetus also may occur in the absence of actual fetal viral infection as a result of severe systemic illness in the mother or alteration of placental function (e.g., abortion or stillbirth in maternal measles, influenza). Viruses demonstrated to have caused congenital infection are listed in Table 73–1. Proof of congenital infection usually consists of the presence of infection or disease caused by the virus or demonstration of the pathogen in the fetus before birth or in the neonate at birth or shortly thereafter.

The effect that congenital infection with various viruses can have on the fetus is shown in Table 73–2.[581-583] Abortion or stillbirth usually occurs when the mother is infected very early in gestation (e.g., rubella) or when the systemic illness in the mother is severe (e.g., measles, influenza). The reasons that premature birth occurs in congenital viral infection are not well understood. Infants with congenital viral infection who are small for gestational age have intrauterine growth restriction, usually the result of decreased numbers of cells in organs.[444,445]

Developmental malformations result from infection of the fetus with the virus (see Table 73–2). Rubella virus is the classic known teratogen; that is, it causes disturbances in organogenesis. VZV has been shown to cause limb hypoplasia and developmental malformations of the eye.[243] Other viruses that result in congenital defects, such as CMV and HSV, cause inflammatory, destructive lesions of already developed organs. Type B coxsackieviruses have been associated with a variety of congenital malformations of the heart,[93-95] and mumps has been linked to endocardial fibroelastosis,[595] but these associations require further substantiation before a causative role can be assigned.

In Table 73–2, congenital disease refers to any manifestation of illness present at birth or shortly thereafter that is secondary to transplacental infection with the virus. Some of the viruses that result in congenital disease cause a chronic persistent infection (CMV, rubella, and hepatitis B virus [HBV]), whereas others cause acute, self-limited, or fatal infection (echovirus, coxsackie-

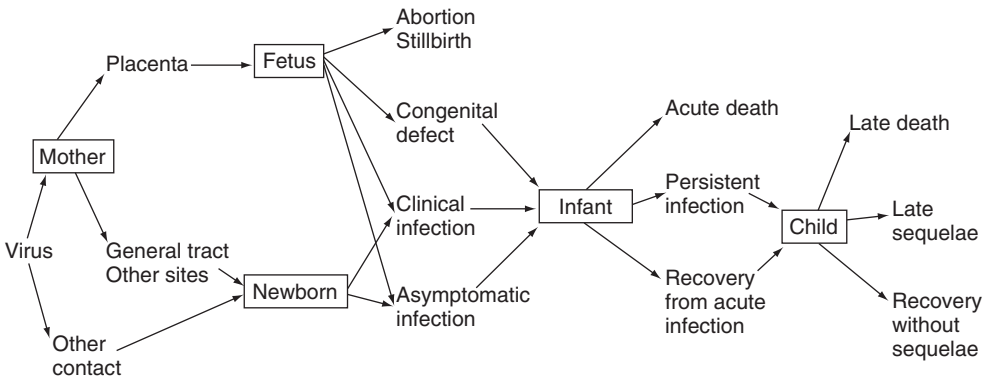

Figure 73–1 Pathogenesis of viral infections in the fetus and newborn.

TABLE 73–1 Period of Transmission of Selected Viruses to the Fetus or Newborn Infant

Viruses	Congenital	Natal	Postnatal
Adenovirus	+	+	+
Cytomegalovirus	++	++	++
Echoviruses	+	+	+
Epstein-Barr	+	–	–
Hepatitis A	–	++	+
Hepatitis B	+	++	+
Hepatitis C	+	++	–
Herpes simplex	+	++	+
Herpesvirus-6	+	–	+
Human immunodeficiency virus	+	++	+
Human parvovirus B19	+	–	–
Influenza	(+)	–	–
Lymphocytic choriomeningitis virus	++	–	–
Measles	+	–	–
Mumps	+	–	–
Parechovirus	–	–	+
Polioviruses	+	–	–
Rubella	++	–	–
Smallpox	+	–	–
St. Louis encephalitis virus	(+)	–	(+)
Type B coxsackieviruses	+	+	+
Vaccinia	+	–	–
Varicella-zoster	++	–	–
West Nile virus	+	–	+
Western equine encephalitis	+	–	–

++, major demonstrated route; +, minor demonstrated route; (+), suggested route, few supporting data; –, route not demonstrated.

virus B, poliovirus, measles, vaccinia, smallpox, western equine encephalitis, and human parvovirus B19). HSV and VZV can cause chronic persistent intrauterine infection, as well as acute transplacental infection at the time of delivery. Because HSV has a relatively short incubation period, ascending amniotic infection with HSV, in association with premature rupture of membranes and maternal genital herpes infection, may cause acute disease that is evident at birth or shortly thereafter. Other viruses, including adenoviruses, Epstein-Barr virus, and respiratory syncytial virus (RSV), also have been detected in the amniotic fluid of both normal fetuses and fetuses with structural abnormalities; however, the direct role these viruses play in fetal infection and disease is not currently known.[418]

Viruses and Congenital Malformation

The role of viruses as etiologic agents in congenital malformations merits special mention. After the recognition by Gregg[198,250] in 1941 that congenital cataracts and other defects occurred in the offspring of mothers with German measles, the concept of an infectious origin for congenital malformations was established firmly. Major malformations occur in 2 to 3 percent of all live births. Although great strides have been made in the diagnosis and management of these defects, their etiologic basis remains largely undefined. Approximately 10 percent are caused by environmental agents such as infections, drugs, or radiation. Another 10 percent have genetic origin and result from familial inheritance or demonstrable chromosomal abnormalities.[475] The remaining 80 percent are of unknown etiology. Most congenital defects may not be caused by environmental or genetic factors acting individually but rather in concert with one another. A genetically predisposed fetus is exposed to the appropriate environmental factor at a particular stage during organogenesis, which then leads to the development of malformations. Because control of the genetic factors contributing to fetal malformation is unlikely to be developed to the point of practical application in the near future, efforts to identify environmental factors that are amenable to control appear warranted.

For several reasons, viruses have been considered to be likely contributors to the 80 percent of malformations that are of unknown etiology. First, the precedent is established that rubella virus and VZV infections during pregnancy do cause congenital defects. Second, most women are expected to have one or more viral infections at some time during pregnancy,[570] so the potential exposure rate is high. Third, a viral infection could be unrecognized in the mother yet produce significant disease in the fetus, which could lead to the occurrence of a "congenital defect of unknown etiology." Fourth, viruses are known to multiply readily in rapidly dividing immature cells, with resultant cell destruction or altered cell function.[426] In the case of more destructive viruses (e.g., measles, vaccinia), fetal death with abortion or stillbirth may occur, whereas with less cytolytic agents (e.g., rubella, CMV), the fetus may survive but defects are produced. Finally, experimental animals provide numerous examples of infection with a virus resulting in little or no disease in the pregnant mother, yet the fetuses are aborted or the newborn offspring are deformed.[208] Therefore, physicians caring for newborn infants should not only be familiar with the patterns of congenital defects currently known to be caused by viruses (CMV, rubella, HSV, and VZV) but also be aware that additional viruses may be added to the list of causative agents in the future.

Natal Viral Infections

Natal viral infections are the result of exposure of the newborn to virus replicating in the genital tract (CMV, HSV, HBV) or to fecal virus contaminating genital secretions (enteroviruses) (see Fig. 73–1 and Table 73–1). Because the incubation periods of HSV and enteroviruses are short, acute postnatal disease may appear in the neonate within 5 to 7 days after natal infection with

TABLE 73–2 Effect of Specific Viruses on the Fetus and Newborn Infant

	Abortion, Stillbirth	Prematurity	Small for Gestational Age	Developmental Malformations	Congenital Disease	Acute Postnatal Disease	Persistent Postnatal Infection
Adenovirus	(+)	–	–	–	(+)	+	–
Cytomegalovirus	+	+	+	+	+(C)	+	+
Echoviruses	–	–	–	–	+(A)	+	–
Epstein-Barr	–	–	+	+	+(C)	–	+
Hepatitis B	–	+	–	–	+(C)	+	+
Herpes simplex	+	+	+	+	+(C,A)	+	+
Influenza	+	–	–	–	–	(+)	–
Lymphocytic choriomeningitis virus	(+)	–	–	+	+(A)	(+)	–
Measles	+	+	–	–	+(A)	–	–
Mumps	+	–	–	(+)	–	–	–
Parechoviruses	(+)	–	–	–	+(A)	+	–
Parvovirus B19	+	–	–	–	+(C,A)	+	+
Polioviruses	+	+	–	–	+(A)	–	–
Rubella	+	+	+	+	+(C)	–	+
Smallpox	+	+	–	–	+(A)	–	–
St. Louis encephalitis	(+)	–	–	(+)	(+)	+	–
Type B coxsackieviruses	+	–	–	(+)	+(A)	–	–
Vaccinia	+	+	–	–	+(A)	–	–
Varicella-zoster	–	+	+	+	+(C,A)	+	+
West Nile	(+)	–	–	(+)	(+)	+	–
Western equine encephalitis	–	–	–	–	+(A)	–	–

+, effect established; –, effect not established; (+), effect suggested, but not proved; (A), acute fatal or self-limited infection; (C), chronic persistent infection.

these agents occurs (see Table 73–2). In contrast, the incubation periods of CMV and HBV are long, and clinically apparent disease, if it occurs, may not be observed for several weeks or even months after birth (see Table 73–2). Persistent postnatal infection can occur after natal infection with CMV, HSV, and HBV.

Postnatal Viral Infections

The source of exposure for postnatal infections of a newborn infant often is the mother, but other sources have been observed and include personnel or other infants in the nursery or newborn intensive care unit and family members (see Fig. 73–1). Neonatal infections, as well as hospital-associated outbreaks of enterovirus[34,526] and parechovirus,[429,654] RSV,[260] rotavirus, adenovirus, rhinovirus, parainfluenza virus, and influenza virus infection, have occurred in nurseries. In addition, health-care-associated HSV infections have occurred in neonates (see Table 73–1).[262,321,487,542,650] Although the maternal respiratory and gastrointestinal tracts are the most common sites from which virus can be transmitted to the neonate postnatally, other viruses, including HIV, human T-cell leukemia virus types 1 and 2 (HTLV I and II), CMV,[277] West Nile virus, rubella virus, hepatitis C virus (HCV), and HBV,[381] have been recovered from breast milk. HSV and VZV lesions on the breast of a mother also may be a source of transmission of virus for the breast-feeding neonate, even though these viruses probably are not present in the breast milk. Finally, HIV, CMV,[697] and HBV[201] have been transmitted to newborn infants by blood transfusion.[199,692] Although most postnatal infections are acute, self-limited processes, severe disease and fatalities have been reported, especially in preterm infants infected with enteroviruses, adenoviruses, or RSV, and persistent postnatal infection may occur with CMV and HBV (see Table 73–2).

EPIDEMIOLOGY

Factors Influencing the Frequency of Infection

Many factors can influence the frequency of infections in the fetus and newborn infant (Table 73–3). Because the mother is the source of the virus causing fetal and neonatal infection in most instances, factors influencing the frequency of maternal infection are of major importance. Except for CMV infection, most congenital infections of the fetus occur after primary viral infection has occurred in the mother. Congenital CMV infection may occur as frequently in mothers known to be seropositive before conception as in seronegative mothers.[603] Congenital rubella, on the other hand, occurs only rarely in immune mothers.[532] Transplacental HBV infection occurs much more often in mothers with acute, primary, symptomatic infection, whereas natal infection is the primary route of transmission in chronic carrier mothers.[470,559] Fetal parvovirus B19 infection and perinatal enteroviral and adenovirus infections acquired from the mother almost always are the result of primary maternal infection. In contrast, natal infections with CMV, HBV, and HSV may result from primary or persistent or recurrent infections in the mother.

The time during pregnancy when the mother is infected (the gestational age of the fetus) is a major factor influencing both the frequency of transmission and the severity of congenital rubella.

TABLE 73–3 Factors Influencing Frequency and Severity of Viral Infections in the Fetus and Newborn Infant

	Time of Fetal or Neonatal Infection		
Factors	Congenital	Natal	Postnatal
Absence of vaccine	++	–	–
Geographic location	++	++	++
Gestational age of fetus	++	+	+
Maternal age	++	+	++
Method of case identification	++	++	++
Presence of epidemic in community	++	+	++
Primary infection in mother	++	++	++
Season of year	++	+	++
Sexual promiscuity	+	++	–
Socioeconomic status	++	++	++

++, major influence; +, minor influence; –, little or no influence.

One hundred percent of infants who have congenital rubella infection during the first 11 weeks of pregnancy have malformations associated with congenital rubella syndrome, 30 percent who have congenital infection from 12 to 20 weeks have malformations, and no malformations develop with infections occurring after 20 weeks.[442] A similar phenomenon occurs with congenital parvovirus B19, CMV, and VZV infections.[282,488,598] In contrast, transplacental HBV and HSV infections appear to develop more frequently when the acute, symptomatic maternal infection occurs in the third trimester rather than the first or second.[704] With viruses such as polio, measles, vaccinia, and smallpox, early-gestation maternal disease results in abortion or stillbirth, whereas late-gestation infection results in congenital disease with acute symptoms in the early neonatal period (see Table 73–2).

Several social and environmental factors may influence the likelihood of development of maternal infection and thereby affect the frequency of infection in the fetus and neonate. The development of rubella vaccine and its licensure in 1969 reduced the incidence of congenital rubella syndrome significantly.[54,473] The successful eradication of smallpox worldwide and discontinuation of the need for smallpox vaccine (vaccinia virus) have eliminated fetal infection with these viruses. Although measles and mumps vaccines have reduced significantly the frequency of occurrence of these diseases in children, whether waning immunity in women of child-bearing age who were vaccinated as infants will predispose to reinfection in pregnant women and subsequent fetal or neonatal disease remains unclear. The presence of epidemics in the community, such as those caused by rubella or enteroviruses, certainly can influence the frequency of maternal and, therefore, congenital, natal, and postnatal infections. The incidence of infection with some viruses (e.g., rubella, primary VZV, enteroviruses, measles, mumps, influenza) clearly is higher during certain months of the year, so the season can influence maternal and neonatal infection rates. In contrast, other viruses causing congenital or natal infection, such as CMV, HBV, or HSV, do not occur in an epidemic or seasonal fashion. Geographic location also can be influential, probably because of differences in the ethnic (and, therefore, genetic) origin of populations in different locations. Rates of chronic carriage of hepatitis B surface antigen (HBsAg) in mothers and the frequency of congenital and natal HBV infection in neonates are much higher in Taiwanese than in U.S. residents.[704] The incidence of numerous maternal viral infections is known to be higher in populations with a lower socioeconomic status, thereby influencing the frequency of congenital, natal, and postnatal infections. Other social factors such as drug abuse, which is associated with higher rates of HBV infection, also can affect the frequency of congenital and natal infections. Because CMV, HBV, and HSV all have been shown to be transmitted venereally, sexual promiscuity in the mother can influence the frequency of these infections in the neonate.

Case identification is a major factor that can have some bearing on the frequency of recognized infection in the neonate. Because most neonates with congenital CMV, rubella virus, or HBV infection are asymptomatic, the use of clinical case-finding methods alone significantly underestimates the true frequency of these infections. Epidemiologic observations (e.g., rubella or enterovirus epidemic in the community), clinical or laboratory information about the mother (e.g., viral illness with a rash or the presence of HBsAg in serum), or screening tests in the newborn (e.g., elevated quantitative IgM in umbilical cord blood) often have been used to select a group of neonates at high risk of contracting congenital infection. The performance of additional laboratory tests in these high-risk neonates to identify potential specific causative agents often has led to the demonstration of infection rates much higher than previously suspected.

Frequency of Infection in the Mother and Neonate

Table 73–4 shows the approximate frequency of the most common viral infections in the mother during pregnancy and in the newborn infant. Although the figures have been obtained by a variety of laboratory methods and some are based on a relatively small or nonrepresentative population sample, they do provide an estimate of the relative frequency of the infections. In many studies, prospective screening of mothers for viral infection during pregnancy or screening of infants for elevated cord blood serum immunoglobulin M (IgM) levels was done to select a population of neonates at high risk of acquiring congenital infection for more detailed virologic investigation.

CMV clearly is the most common cause of viral infection, both in the mother and in the neonate.[105] Surveys in the United States indicate that 30 to 110 per 1000 women excrete virus in urine during pregnancy and that 60 per 1000 are excreters at the time of delivery.[225,284,341,435,452,528] Isolation from cervical swabs is even more common: 80 to 120 per 1000 women during pregnancy and 110 to 130 per 1000 at delivery.[130,341,435,528] Most maternal CMV infections occurring during pregnancy are recurrent rather than primary.[600] Extrapolation from data obtained at the same institution[528,600,605] suggests that two thirds to three quarters of CMV shedding in the cervix or urine during pregnancy is the result of recurrent rather than primary CMV infection in the mother. The frequency of primary CMV infection occurring during pregnancy averages 20 to 25 per 1000 (range, 10 to 40), with higher rates occurring in populations with a larger percentage of susceptible persons (seronegative at the beginning of pregnancy).[251,598] Several factors are known to be associated with increased rates of recurrent urine or cervical shedding: (1) sampling on several occasions rather than a single time; (2) collection of specimens during the third rather than the first trimester because shedding rates are known to increase as gestation progresses; (3) Asian, black, or Native American versus white populations; (4) younger maternal age; (5) lower socioeconomic status; (6) a greater number of lifetime sexual partners; and (7) a history of sexually transmitted diseases.[130,341,452,606] An even more important source for transmission of CMV to the neonate may be breast milk. Postpartum shedding from colostrum or breast milk, the cervix, urine, or saliva was demonstrated in 130 to 280 per 1000 unselected women, and breast milk was the most common site by far.[202,598,605,607]

Congenital CMV infection has been documented in 6 to 24 per 1000 live births, as determined by isolation of virus from the urine of the neonate within the first few days of life.[73,178,530,603] In contrast to rubella, congenital infection with CMV occurs in mothers with either primary or recurrent infections during pregnancy.[603] Congenital CMV infection rates are significantly higher

TABLE 73–4 Approximate Frequency of Infections in the Mother during Pregnancy and in the Newborn Infant

Viruses	Mother (No./1000 Pregnancies)	Neonate (No./1000 Live Births)
Cytomegalovirus		
During pregnancy, congenital	30-120	6-24
At delivery, natal	80-130	20-60
After delivery, postnatal	130-280	140-210
Rubella		
1964 epidemic	20-40	3-7
Interepidemic prevaccine	0.1-2.0	0.1-0.7
Postvaccine	0.15-0.3	0.03
Hepatitis B	1-160	0-61
Enteroviruses	90-600	2-38
Herpes simplex	1-7	0.1-0.6

in lower socioeconomic groups (1.6%) than in middle to upper ones (0.6%).[600] However, among low-income mothers, the status of immunity to CMV is high (82%), and most congenital infections (81%) are associated with recurrent CMV infection during pregnancy. In contrast, seroimmunity to CMV in middle- to upper-income women is lower (55%), and the frequency of congenital infection associated with recurrent maternal CMV also is lower (47%). Although the rate of total congenital CMV infection is lower in mid- to high-income mothers, the proportion of infants born after primary CMV infection is acquired during pregnancy is higher (53% versus 19%). Pooled data from several studies indicate that 30 to 40 percent (range, 20-52%) of mothers with primary CMV infection during pregnancy deliver congenitally infected infants.[12,13,251,364,598,600] Congenitally infected babies born to mothers with primary rather than recurrent CMV infection during pregnancy have a greater frequency of symptoms of CMV disease at birth, higher levels of IgM in cord serum, higher titers of virus in urine, and a greater likelihood of having neurologic sequelae at follow-up.[11,12,598,600] The time during gestation when maternal primary CMV infection occurs does not appear to influence the rate of fetal infection, but fetal damage seems to occur more frequently and to be more severe after the acquisition of maternal infection during the first half than during the second half of pregnancy.[11,251,509,598]

The frequency of natal and postnatal acquisition of CMV by newborn infants is far greater than that of congenital infection. Natally acquired CMV infection occurs at an incidence of 20 per 1000 live births.[528,604] Because approximately one half the infants born to mothers known to be cervical excreters at the time of delivery acquire natal infection[528] and because as many as 130 per 1000 mothers from a low socioeconomic group are excreting CMV at this time, the actual rate of natally acquired CMV infection may be as high as 60 per 1000 live births. A more frequent source for infection of the neonate with CMV is postnatal consumption of virus-infected colostrum or breast milk. In two studies, 58 to 69 percent of infants of nursing mothers who excreted CMV in milk acquired infection.[202,605] Shedding of virus occurred most frequently between 2 and 12 weeks postpartum, and the onset of infant viruria usually occurred when the infants were between 1 and 6 months of age. The aforementioned rates of postnatal infection would project to between 140 and 210 infections per 1000 live births in the United States. These rates, of course, would be affected by all the factors mentioned earlier that influence maternal CMV infection rates, as well as the frequency and duration of breast-feeding.

Yet another source for transmission of CMV to neonates is blood transfusion in infants in neonatal intensive care units. Risk factors include birth weight less than 1250 g, a CMV-seronegative mother, hospitalization for a period longer than 4 weeks, receipt of multiple blood transfusions or a total volume of more than 50 mL, and receipt of blood from a CMV-seropositive donor.[10,50,228,697] The infection rate in high-risk infants may be high: 24 to 31 percent. Morbidity and mortality rates with these infections also are high: clinical disease developed in 88 percent of 34 reported cases, and 24 percent died (CMV was thought to be causal or contributory).

In summary, several factors contribute to the high rates of fetal and neonatal CMV infection: (1) virus can be transmitted from both immune mothers (recurrent infection) and nonimmune mothers (primary infection); (2) virus may be shed or carried in many different sites in the mother's blood (for transplacental infection), urine, cervix, breast milk, and saliva; (3) infection may occur at different times—congenitally, natally, and postnatally; and (4) infection may come from sources other than the mother (e.g., hospital-acquired infection from blood transfusion).

The use of rubella vaccine has modified the epidemiologic patterns of this disease in the United States.[54,184,473] Vaccination has eliminated endemic rubella in the United States. Rates of maternal and congenital rubella during the 1964 epidemic were many times higher than in interepidemic periods. Nonetheless, a comparison of the incidence during the 1964 epidemic with the rates during prevaccine interepidemic periods and what may be happening currently provides useful information. The frequency of serologically proven clinical rubella among 30,000 pregnant women in the Collaborative Perinatal Research Study was approximately 1 per 1000 during the interepidemic years before vaccine licensure but rose to 22 per 1000 during the epidemic. The total figure for rubella during pregnancy is likely to be at least two times higher because as many as one half to two thirds of maternal rubella infections are subclinical or not diagnosed as rubella.[568] An incidence of congenital rubella of 0.7 per 1000 live births was demonstrated during the interepidemic period in a study screening cord blood sera for the presence of rubella-specific IgM antibody.[15] During the 1964 epidemic, an estimated 20,000 babies were born with congenital rubella syndrome in the United States among approximately 4 million live births, an incidence of 5 per 1000 live births.[454] During 1980 to 1982, an average of 11 cases of confirmed congenital rubella syndrome were reported to the Centers for Disease Control and Prevention (CDC) each year.[473] Correcting for under-reporting and missed cases yielded an annual estimate of 110 cases.[473] With an annual national birth rate of 3.5 million per year, this figure projects to 0.03 cases of congenital rubella syndrome per 1000 live births currently in the United States. Assuming a 10 to 20 percent rate of congenital rubella infection during pregnancy, the current estimate is thought to be 0.15 to 0.3 cases of maternal rubella per 1000 pregnancies. Importantly, however, a resurgence in acquired and congenital rubella, particularly in unvaccinated women in correctional institutions and unimmunized communities,[116,412] emphasizes the need for continued surveillance for rubella disease and proper use of the vaccine.

HBV infection during pregnancy may result in acute clinical disease in the mother or, more commonly, may result in an asymptomatic chronic carrier state.[560] The frequency of HBsAg in the serum of pregnant women varies highly and is influenced by geographic location, ethnic origin, socioeconomic status, and other social factors such as illicit drug use and sexual promiscuity. An incidence of 1 to 160 per 1000 pregnancies has been reported in several series.* Infection with HBV in the neonate is even more variable, for several reasons. First, transmission of the virus to the fetus or neonate may occur by several routes: (1) transplacental; (2) natal, from the genital tract; (3) postnatal, by intrafamilial spread through unknown mechanisms; and (4) postnatal, by blood transfusion. Second, infants born to mothers with hepatitis B may follow one of these courses: (1) serum from the infant remains negative for HBsAg, and hepatitis B never develops; (2) umbilical cord blood is positive for HBsAg, but the antigenemia clears and no disease is evident, presumably representing transplacental transmission of antigen only or insufficient virus to cause true infection in the neonate; (3) umbilical cord blood is HBsAg-positive, and clinical or subclinical infection develops with or without persistent hepatitis B antigenemia; or (4) cord blood is antigen-negative, but infection occurs, sometimes not until several months after birth, and persistent antigenemia may or may not develop. Third, because exposed infants may follow one of several courses and because HBV infection may be demonstrable at various times after birth, serial blood specimens must be obtained for evaluation of the true frequency of neonatal infection. Serial blood specimens are difficult to obtain in this age group, and published reports may have based their estimates of infection on one or two blood specimens per infant. Fourth, transplacental infection occurs much more commonly in mothers

*See references 31, 66, 183, 200, 347, 381, 469, 486, 560, 615, 687.

with acute, symptomatic hepatitis, particularly during the second or third trimester of pregnancy, than it does in mothers who are chronic carriers.[560] Finally, even in these mothers, true differences in the rates of transplacental transmission occur: Asians have a much higher incidence than whites do,[704] and mothers who are hepatitis B e antigen (HBeAg)-positive are much more likely to transmit infection than are mothers who have anti-HBe antibody or lack e-antigen markers.[469] Therefore, the incidence of HBV infection in the neonate varies from 0 to 61 per 1000 live births in different reports.*

Data from several studies indicate that perinatal enteroviral infections occur much more frequently than previously realized.[106,307,318,429,436,536,93-95,353,567] Serologic surveys indicate a surprisingly high rate of seroconversion to at least one enterovirus during pregnancy (first serum at the time of enrollment for obstetric care, second serum at delivery): 90 to 600 per 1000 pregnancies.[93-95,353,567] Several factors complicate arriving at an interpretation of the data concerning the frequency of occurrence of maternal enteroviral infections. First, only 2 to 13 of the almost 70 nonpolio enterovirus serotypes were used for antibody testing, thereby resulting in an underestimate of the true frequency for all enteroviruses. Second, the data are reported as the total number of enteroviral infections for a group of pregnant women rather than the percentage of pregnancies complicated by at least one enteroviral infection. Use of these data for calculating the number of pregnancies per 1000 complicated by enteroviral infection would result in an overestimation because more than 25 percent of women may have more than one enteroviral infection during the 9 months of pregnancy.[93]

Data concerning the actual frequency of neonatal enteroviral and parechoviral infection also are difficult to summarize because of differences in the study designs used in the various published results. Modlin and associates[432] reported four echovirus 11 infections among 158 consecutive neonates who had stool samples taken when they were 3 days and 2 weeks of age during the 3-week period of an outbreak of echovirus 11 in Boston. Assuming that live births are distributed evenly throughout the year and that no additional cases of enteroviral infection occurred during the remainder of the year, this incidence translates into two enteroviral infections per 1000 live births. These calculations probably underestimate the true frequency markedly because only echovirus 11 was sought in the diagnostic virology laboratory evaluation of these 158 infants, and, obviously, enteroviral infections occur for more than a 3-week period of the year. A prospective study of all enteroviral infections acquired during the patient's first month of life in Rochester, New York, during the peak enterovirus season (June to October) demonstrated 75 (12.8%) nonpolio enteroviral infections in 586 infants.[307] Fourteen (18.7%) of these 75 infected infants were hospitalized for "suspected sepsis." By using the estimated number of live births per year in the Rochester area and assuming that no additional cases of neonatal enteroviral infection occurred during the remaining 7 months of the year, the authors projected a rate of seven neonatal enteroviral infections serious enough to require hospitalization per 1000 live births. If one considered the total 75 enteroviral infections (14 hospitalized and 61 not hospitalized), the rate would be 38 per 1000 live births. A survey by Kaplan and associates[318] of 77 cases of coxsackievirus B infection occurring during the first 3 months of life in infants hospitalized at the Nassau County Medical Center between 1970 and 1979 yielded an estimated rate of 0.5 per 1000 live births. Because group B coxsackieviruses account for only 45 percent of all enteroviral infections during early infancy[351] and because only 18 to 19 percent of all enterovirus-infected infants may require hospitalization,[307] the actual rate for all enteroviral infections may be 6 per 1000 live births. Despite the variation in estimates, enteroviral infections are clearly a frequent cause of maternal and neonatal infection. Parechovirus infections are being diagnosed and described in the medical literature with increased frequency, but current knowledge of the epidemiology of this infection in neonates is still evolving.[654] The infected genital tract of the mother is the source of virus for most neonatal HSV infections. However, other sources, such as nongenital sites in the mother, family members, and even other infants or caretakers in the nursery, have been implicated. Both genital and neonatal herpes have increased in frequency in recent years.[67,623] Current estimates are that culture-positive genital herpes may occur at a rate of one to seven per 1000[75,77,97-102] during pregnancy and at a rate of one to four per 1000 at the time of delivery.[97,101,450,513,632] However, culture is not the most sensitive method to detect genital HSV-2 infection. Seroprevalence studies have demonstrated HSV-2–specific antibody in 32 percent of women in private obstetric practices,[362] and polymerase chain reaction (PCR) detected HSV DNA in 9 percent of asymptomatic women in labor.[149] Higher rates are associated with lower socioeconomic status, increased numbers of sexual partners, and occurrence of other sexually transmitted diseases. Rates of neonatal herpes have been estimated at 0.1 to 0.6 per 1000 live births.[97,623,669] The greatest risk of acquiring neonatal infection occurs when the mother has an initial genital infection at the time of vaginal delivery.

The frequency of infection with the other viruses listed in Tables 73–1 and 73–2 is so low that numeric estimates per 1000 pregnancies or live births cannot be made.

APPROACH TO DIAGNOSIS

Fetal viral infection may be suspected if the mother is exposed to or experiences an infection with a virus known to transmit to the fetus; or abnormalities detected on routine fetal ultrasound may suggest fetal infection. Fetal abnormalities detected during prenatal evaluation that may be caused by in utero virus infection include intrauterine growth retardation, microcephaly, cerebral ventriculomegaly or hydrocephalus, cataracts, hepatosplenomegaly, hepatic or intracranial calcifications, echogenic bowel, fetal ascites, cardiomegaly, congestive heart failure, fetal hydrops, or poly- or oligohydramnios.[173] Intrauterine cardiac abnormalities, myocarditis, and heart failure may be associated with HIV, parvovirus B19, mumps virus, or adenovirus, and cardiac structural defects may be associated with rubella virus. Limb deformities or dysplasias, especially if they are associated with eye or central nervous system (CNS) abnormalities, may be caused by intrauterine VZV or HSV infection. Consultation with a maternal-fetal medicine specialist is recommended if fetal viral infection is suspected because intervention strategies are now available for many of these infections, which may lessen the morbidity and mortality rates associated with viral infections in the fetus.[1,173,461]

The usual set of circumstances leading one to consider the diagnosis of viral infection in a newborn infant is the presence of clinical or laboratory features in the neonate that suggest this possibility (Table 73–5). Icterus, petechiae, or hepatosplenomegaly at the time of birth or shortly thereafter in a small-for-gestational-age infant suggests a chronic in utero viral infection. On the other hand, acute viral infection in a neonate with suspected sepsis may be present when cultures of blood, spinal fluid, and urine fail to yield a bacterial or fungal agent.

Evaluation of the Mother

Once the suspicion of a viral infection in a newborn infant has been raised, one should proceed with an evaluation of the mother for features that might add further evidence to this possibility.

*See references 31, 66, 183, 200, 347, 381, 469, 486, 560, 576, 615, 687.

TABLE 73–5 Common Manifestations of Viral Infections in the Newborn Infant

Asymptomatic Infection	
Chronic Infection (Early Gestation to Midgestation Congenital)	
General Characteristics	
Manifestations present at birth or shortly thereafter	
Presence of congenital defects	
Specific Features	
General	Small for gestational age
Central nervous system	Microcephaly, seizures, cerebral calcification, hypertonia or hypotonia, cerebrospinal fluid pleocytosis, encephalitis, hydrocephalus, hearing loss
Skin	Icterus, petechiae, purpura, vesicles, hypopigmentation
Eye	Chorioretinitis, cataracts, glaucoma, microphthalmos, optic atrophy
Heart	Patent ductus arteriosus, pulmonary artery stenosis
Abdomen	Hepatosplenomegaly, hepatitis
Lung	Pneumonitis
Musculoskeletal	Bone lesions, limb hypoplasia
Acute Infection (Late Gestation Congenital, Natal, or Postnatal)	
General Characteristics	
Manifestations usually appear several days to weeks after birth	
Absence of congenital defects	
Specific Features	
General	Hyperthermia or hypothermia, irritability, lethargy, jitters, poor feeding, vomiting
Central nervous system	Seizures, hypertonia or hypotonia, full fontanelle, meningitis, encephalitis, hearing loss
Skin	Icterus, petechiae, purpura, vesicles, maculopapular rash
Eye	Conjunctivitis, keratitis
Heart	Myocarditis
Abdomen	Hepatosplenomegaly, hepatitis
Lung	Pneumonitis, respiratory distress, cyanosis

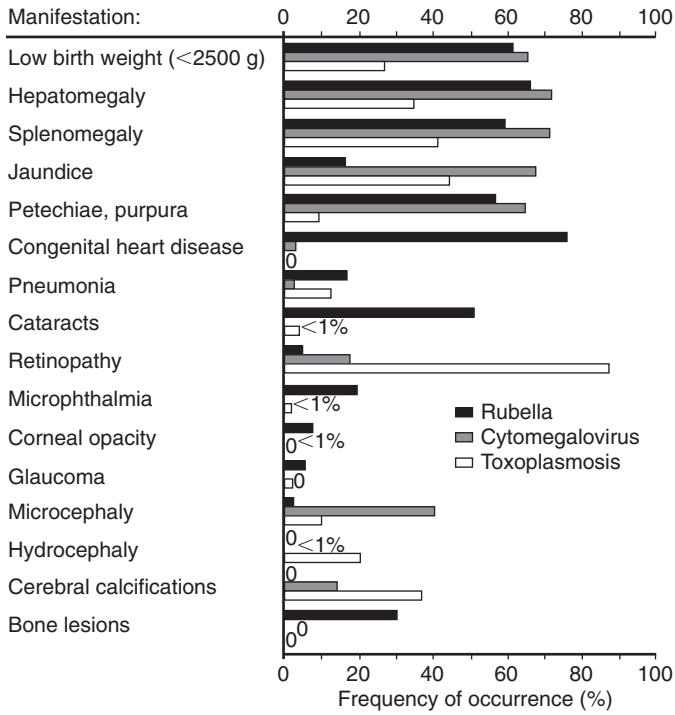

Figure 73–2 Manifestations of symptomatic congenital rubella virus and cytomegalovirus infections and toxoplamosis.

For example, the occurrence of maternal viral illness with an associated maculopapular rash suggests rubella or enterovirus infection in the neonate, whereas ulcerative genital lesions suggest HSV and heterophile-negative infectious mononucleosis is suggestive of CMV. Maternal rash with arthralgias may be caused by parvovirus B19. One should note, however, that most maternal viral infections that lead to fetal or neonatal infection (almost all cases of CMV and HBV infection[612,687] and one half to two thirds of rubella virus and HSV infections[450,568,669,675]) are asymptomatic in the mother. Therefore, the absence of a history of viral infection in the mother certainly does not rule out the possibility of a viral infection in her neonate.

Specimens from the mother for isolation or detection of the viral agent usually are not available. However, the presence of HBsAg- or CMV-specific IgM antibody in maternal serum or isolation of CMV or HSV from the genital tract or an enterovirus from the stool at the time of delivery certainly should lead one to consider these agents in her newborn infant. Serologic documentation of a specific viral illness in the mother during pregnancy requires serum specimens that bracket the illness. Unfortunately, these specimens rarely are available when the pediatrician is considering the possibility of a congenital or neonatal viral illness. Routine IgG antibody determinations on a single specimen obtained from the mother after the birth of a neonate with suspected congenital viral infection are not likely to yield useful information, except if the mother lacks IgG antibody to the pathogen in question. Such a finding in the face of a symptomatic infant would rule out that organism as the cause of the infant's disease. Although the presence of specific IgM antibody against CMV,[253,269,608] rubella virus,[246] parvovirus B19, or HSV[447] in a single maternal serum specimen is highly suggestive of recent infection, IgM tests are known to produce false-positive results and need to be interpreted accordingly. On the other hand, if the maternal viral infection occurs near the time of delivery (e.g., enterovirus meningitis, coxsackievirus B pleurodynia, initial genital herpes), acute and convalescent serum specimens may be obtained from the mother that do demonstrate the diagnostic fourfold or greater rise in antibody titer against a specific agent. Documentation of a particular causative agent in the mother does not constitute proof that the same agent is causing disease in the neonate, but it certainly does provide strong suggestive evidence. Definitive proof, therefore, must come from studies in the newborn infant.

Clinical Features in the Neonate

Certain clinical manifestations of viral disease in the neonate may provide helpful clues to the specific etiologic agent. However, most viral infections are asymptomatic in neonates: more than 95 percent of CMV, two thirds of rubella virus, and most HBV infections. In contrast, less than 1 percent of HSV infections in the neonate are subclinical. To complicate the effort to pinpoint the diagnosis further, the clinical and laboratory manifestations of symptomatic disease caused by numerous agents often have similar patterns (see Table 73–5; Figs. 73–2 and 73–3). However, infants whose congenital viral infections were incurred in early gestation to midgestation have manifestations of disease at birth or shortly thereafter, whereas infants with late-gestation congenital infections or natal or postnatal infections usually do not

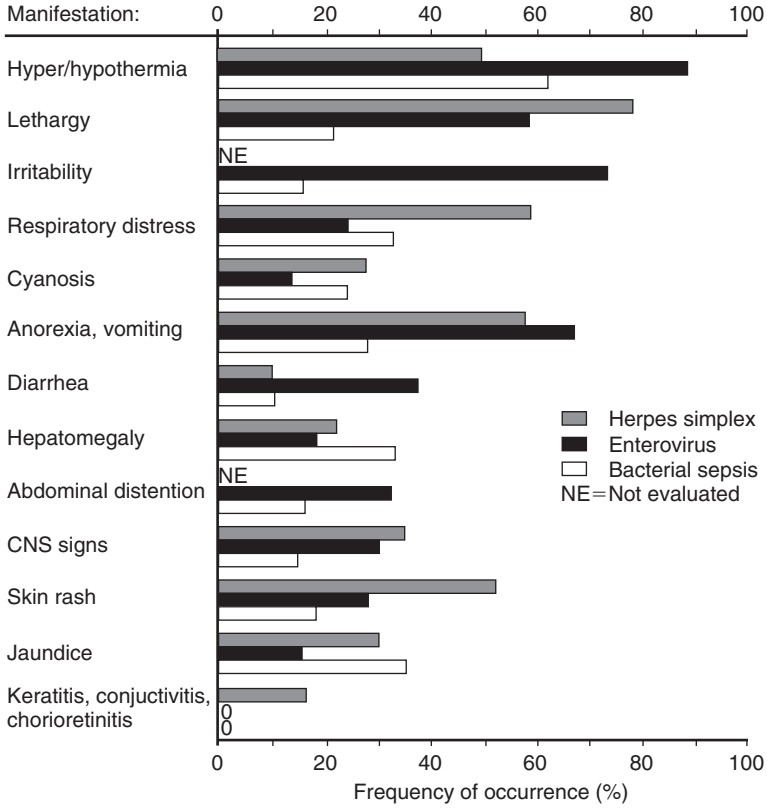

Figure 73–3 Manifestations of herpes simplex virus and enterovirus infections and bacterial sepsis in the neonate.

exhibit signs and symptoms for several days to several weeks after birth (see Table 73–5). In addition, newborn infants who acquire congenital infections in early gestation to midgestation exhibit congenital defects and intrauterine growth retardation, whereas neonates who acquire viral infections near the time of birth have acute disease resembling bacterial sepsis or the viral syndrome typically seen in older children (e.g., enterovirus exanthem, chickenpox) (see Table 73–5). Because the viral agents that commonly cause chronic intrauterine infections are different from those that result in acute neonatal diseases (see Tables 73–1 to 73–3), recognition of these two different patterns helps define a specific etiologic agent.

The most common manifestations of congenital CMV and rubella infection in symptomatic newborn infants are shown in Figure 73–2. Also shown in this figure are the features observed in infants with congenital toxoplasmosis because this infection is important to consider in the differential diagnosis. (Infection with *Toxoplasma gondii* is covered in another chapter.) Note that many nonspecific manifestations, such as low birth weight caused by intrauterine growth retardation, hepatomegaly, splenomegaly, jaundice, and petechiae/purpura, occur with a similar frequency in the three infections. However, certain specific findings may be helpful in the differential diagnosis. The presence of cataracts, congenital heart disease, bone lesions, or microphthalmos is highly suggestive of rubella, whereas chorioretinitis, cerebral calcifications, and hydrocephaly are findings against this diagnosis. In congenital CMV infection, microcephaly, cerebral calcifications, and sensorineural hearing loss are relatively common findings, but congenital heart disease, eye abnormalities, and bone lesions rarely occur. Chorioretinitis, cerebral calcifications, or hydrocephaly should suggest toxoplasmosis or lymphocytic choriomeningitis virus (LCMV). The cerebral calcifications in CMV infection tend to be periventricular, whereas those in toxoplasmosis are scattered through the parietal lobes of the cerebrum. An important note is that some manifestations may not be evident for several months after birth: congenital heart disease, chorioretinitis, microcephaly, hydrocephaly, and cerebral calcifications.

The most frequent findings in the common acute viral infections of the neonate—HSV and enterovirus infections—are shown in Figure 73–3. Because the features in infants with these two kinds of viral infection resemble those associated with bacterial sepsis, the manifestations in neonates with septicemia also are presented. Again, the more common nonspecific features, such as fever or hypothermia, respiratory distress, cyanosis, anorexia or vomiting, and hepatomegaly, occur at a relatively similar frequency in the three infections.[106,107] Lethargy, irritability, and CNS signs are found more commonly with HSV and enterovirus infections, probably because encephalitis and meningitis, respectively, occur more frequently with these two infections. Features that suggest a diagnosis of HSV infection are a vesicular rash and keratitis or conjunctivitis, whereas enterovirus infection usually is associated with diarrhea and abdominal distention. The rash in enterovirus infection usually is erythematous and maculopapular, but petechiae can occur with overwhelming infection. The skin lesions in bacterial sepsis generally are pustules, abscesses, cellulitis, or purpura.

DIFFERENTIAL DIAGNOSIS

The differential diagnosis in a fetus or newborn infant with a suspected viral infection is extensive. In the fetus, noninfectious and genetic etiologies may cause signs and symptoms similar to congenital virus syndromes. Many of the neonatal manifestations shown in Figures 73–2 and 73–3, such as lethargy, irritability, respiratory distress, cyanosis, and anorexia, can be caused by the most common diseases occurring in sick newborn infants—hyaline membrane disease, prematurity, intraventricular hemorrhage, metabolic disturbances, and bacterial sepsis.[106,107,674] Infants

with low birth weight caused by intrauterine growth restriction (small for gestational age) may have congenital malformations, chromosomal abnormalities, placental insufficiency, and inborn errors of metabolism. Hepatosplenomegaly usually is caused by one of the infectious diseases of the neonate. In infants with jaundice, ABO and Rh hemolytic disease and physiologic jaundice should be considered. Noninfectious causes of petechiae/purpura include idiopathic or drug-induced (including transplacental passage) thrombocytopenia, erythroblastosis fetalis, and disseminated intravascular coagulation.

Little firm evidence indicates that infectious agents other than rubella can cause congenital heart disease. However, in utero mumps, adenovirus, CMV, and parvovirus B19 may produce myocarditis and congestive heart failure. Diffuse pulmonary infiltrates early in the neonatal period most commonly are caused by hyaline membrane disease, but in older neonates one should consider bronchopulmonary dysplasia and chlamydial infection. Causes of cataracts other than congenital rubella, HSV, and VZV infection include congenital galactosemia and the oculocerebrorenal (Lowe) syndrome. Chorioretinitis or retinopathy usually is caused by infection with *Taxoplasma gondii* or with viruses such as HSV, CMV, rubella, or LCMV. Microcephaly also can be caused by infection with HSV or VZV; noninfectious causes include Down syndrome, perinatal anoxia, phenylketonuria, and maternal irradiation. Hydrocephalus generally is caused by a congenital malformation of the ventricular aqueductal or subarachnoid space system. In a neonate with manifestations of an acute infection, major diagnostic considerations include serious bacterial infections such as sepsis, meningitis, urinary tract infection, or pneumonia. However, neonatal viral infections also are common causes of fever and sepsis syndrome in the neonate.[92,106-108,236,654,654a] After appropriate samples for bacterial and viral cultures have been obtained from the neonate, systemic antibiotics should be administered until a bacterial infection has been ruled out. Because of the frequency of neonatal HSV disease and its severe morbidity and mortality if untreated, and because results of recent studies have shown viral testing and antiviral treatment to be cost-effective as well as life-saving, some experts recommend administering intravenous acyclovir as well to selected neonates who present with fever or sepsis syndrome and appear to be at high risk for having neonatal HSV infection.[106-108]

LABORATORY DIAGNOSIS

Because the clinical manifestations in the various neonatal viral infections frequently overlap, establishing a specific etiologic diagnosis usually depends on the laboratory. General laboratory tests may show abnormalities suggestive of viral infection. For example, the peripheral white blood cell count may show neutropenia or lymphocytosis, with thrombocytopenia, and liver function tests may be elevated. The approach to the specific laboratory diagnosis of viral infection in a newborn infant is outlined in Table 73–6. Details of the various methods for each virus are covered in the respective chapters for these agents; only general comments relevant to the diagnosis of infection in the fetus or neonate are presented here.

Historically, histopathologic methods were used to attempt to establish a diagnosis of viral infection. For example, examination of stained cells from urine for CMV inclusions or examination of cells scraped from the base of a vesicle or from the conjunctivae for multinucleated giant cells with intranuclear inclusions characteristic of HSV or VZV (Tzanck-stained smear) were performed previously, but they are rarely performed currently. In addition, tissue obtained by biopsy or at postmortem examination may reveal intranuclear inclusions and multinucleated giant cells characteristic of the herpesviruses. Electron microscopy may reveal viral particles in vesicle fluid or tissue. Except for the propensity to involve certain organs (e.g., rubella virus, the heart; HBV, the liver), the pathologic changes induced by viruses other than the herpes group rarely are sufficiently specific to enable one to make an etiologic diagnosis. Even for the herpesviruses, the routine histopathologic methods are only one half to two thirds as sensitive as is isolation of the virus.

TABLE 73–6 Laboratory Diagnosis of Viral Infection in the Newborn Infant

Procedure	Details, Comments
Routine histopathologic methods	Urine cells stained for inclusions Scraping of vesicle base or conjunctiva for multinucleated giant cells Examination of biopsy or autopsy tissue
Isolation of detection of infectious agents	Isolation of infectious agent in cell culture or animals Detection of viral particles by electron microscopy Detection of viral antigen by immunologic methods Detection of viral nucleic acid by DNA probes, often after amplification by PCR
IgG antiviral antibody	Persistence of antibody in serum of infant beyond age of normal decline in maternal transplacental antibody—usually 4-6 mo Variety of methods available (CF, neut, HI, IHA, IFA, ELISA, etc.)—sensitivity of method varies according to specific virus
IgM-specific antiviral antibody	IgM not normally passed transplacentally, presence in cord blood or neonatal serum diagnostic IgM-specific antibodies not always present in neonate False-positive and false-negative results
Quantitative IgM level	Not a specific diagnostic test, suggests intrauterine infection

CF, complement fixation; ELISA, enzyme-linked immunosorbent assay; HI, hemagglutination inhibition; IFA, immunofluorescent assay; IHA, indirect hemagglutination; neut, neutralization; PCR, polymerase chain reaction. Modified from Hanshaw, J. R., Dudgeon, J. A., and Marshall, W. C.: Viral Disease of the Fetus and Newborn. 2nd ed. Philadelphia, W. B. Saunders, 1985.

The most direct, definitive, and preferred method of establishing the diagnosis is isolation of the agent from an appropriate site in the fetus or neonate. Prenatal diagnosis of fetal viral infection may be established by testing amniotic fluid for viral culture and PCR and fetal blood sample or viral-specific IgM and PCR.[310,628] The following sites generally are used in neonates: urine, saliva, and blood for CMV; throat, cataracts, and occasionally spinal fluid or urine for rubella; skin vesicles, buffy coat or whole blood, cerebrospinal fluid (CSF), conjunctivae, throat, stool, and urine for HSV; throat, stool, urine, CSF, or serum/buffy coat for enteroviruses; and skin vesicles for VZV. In addition, isolation of virus can be attempted from biopsy or autopsy specimens. Recovery of a virus from internal body fluids (buffy coat, CSF, urine), vesicle fluid, or tissue from organs is strong evidence of an etiologic association. Electron-microscopic examination of vesicle fluid can demonstrate typical herpesvirus particles in both HSV and VZV infection but cannot distinguish between the two. Immunofluorescence and immunoperoxidase methods are available for many of the viruses and can demonstrate and type viral antigen in cells scraped from lesions or in biopsy material. HBsAg is demonstrable in serum by radioimmunoassay or enzyme-linked immunosorbent assay (ELISA).

Methods for amplifying a particular segment of a viral genome, using methods such as traditional and real-time PCR, are now available in many hospital laboratories and most reference labo-

ratories. Most commonly, testing for CMV, HBV, HSV, VZV, parvovirus B19, adenovirus, and the enteroviruses and parechoviruses is performed in neonates.[479] However, almost any viral pathogen may now be detected using molecular techniques. In general, PCR and other molecular amplification methods are more rapid and sensitive than is virus isolation and are quite specific and may become the diagnostic test of choice for many viral infections. Molecular diagnostic tests also may be performed on any body fluid, but most commonly on blood and CSF. In addition, molecular methods also have been applied recently to diagnose congenital viral infections retrospectively from archived dried blood spots and used prospectively to develop newborn screening programs.[51,659]

Though not as sensitive, immediate, or direct as is isolation or detection of the viral agent using molecular methods, serologic studies are a readily available and traditional means for laboratory diagnosis of viral infection in a fetus or newborn infant. They should be used only when isolation or detection of the organism is not possible. The "TORCH" screen for antibodies *(Toxoplasma*, other, rubella, cytomegalovirus, and herpes simplex) was developed for this purpose. However, proper interpretation of serologic tests in newborn infants requires an understanding of the kinetics of the humoral immune response in the fetus and newborn infant, as well as an appreciation of the transplacental passage of antibodies from mother to fetus. Figure 73–4 illustrates the pattern of immunoglobulin concentrations in the serum of the fetus and newborn infant, as well as older infants and children. Maternal IgG, which contains antibody against viruses to which the mother has been exposed, passes transplacentally, beginning at midgestation. Peak levels are reached in fetal serum at the time of birth (umbilical cord blood); they decline to undetectable levels by the time that the infant reaches 6 to 12 months of age. However, the use of more sensitive assays for IgG antibody detection, such as immunoblotting, has demonstrated that maternal IgG antibody may persist for as long as 15 to 18 months.

In contrast, maternal IgM antibody normally is not passed transplacentally. Because the fetus is in a "protected" environment and usually does not receive an antigenic challenge in utero, fetal immunoglobulin levels remain low and do not begin to rise until after birth, when exposure to a variety of antigens occurs. However, the fetus is capable of mounting a humoral immune response when exposed to an antigen (a virus) in utero. Elevated levels of fetal immunoglobulins, therefore, can be detected at birth in umbilical cord blood. Because maternal IgG is present in such high concentration in cord blood serum, assays for IgM are performed. A fetus challenged in utero with a virus can have specific IgM antibodies against the viral agent, as well as elevated levels of the total IgM fraction. Often, a fetus with detectable virus-specific IgM will have severe in utero disease caused by the virus. Typically, three approaches can be used to make a serologic diagnosis of viral infection in a newborn infant: (1) assay of maternal serum and serum specimens from the infant at birth and at 5 to 6 months of age for antiviral antibody (predominantly IgG activity), (2) assay of neonatal serum for IgM antibody against a specific viral agent, and (3) assay of neonatal serum for quantitative IgM levels, a nonspecific indication of antigenic challenge in utero.

For purposes of illustration, the rubella hemagglutination-inhibition titers of two mother/infant pairs are shown in Table 73–7. Both mothers were exposed to someone with a rubella-like rash during pregnancy. The first mother was susceptible; subclinical rubella developed, and she delivered an infant with congenital rubella, whereas the second mother was immune and delivered an uninfected normal infant. The serum specimen obtained from the first mother at the time of exposure showed no detectable hemagglutination-inhibition titer, whereas the serum at delivery and 6 months postpartum showed high titers indicative of acute rubella virus infection during pregnancy. Serum specimens from her infant at birth and at 6 months of age demonstrated rubella virus hemagglutination-inhibition antibodies at approximately the same level, thus indicating the persistence of antibody formed by the infant and substantiating the occurrence of congenital infection. In the second mother, the rubella hemagglutination-inhibition titers remained unchanged in all three specimens. Her infant had evidence of transplacental maternal antibody in serum obtained at birth but no detectable antibody at 6 months of age. Similar results could be expected from serologic studies with the other viral agents listed in Tables 73–1 and 73–2, not only for congenital infections but also for natal and postnatal infections in which primary viral infection occurred in the mother. Even with natal infections in immune mothers and postnatal infections from nonmaternal sources, the serologic responses in the neonate would be similar to those shown in Table 73–7.

The presence of IgM antibodies in maternal, fetal, or neonatal serum against a specific virus usually is considered to be diagnostic of acute or recent infection with that agent. However, both false-positive and false-negative results can occur. False-positive findings can result from cross-reaction between viruses, espe-

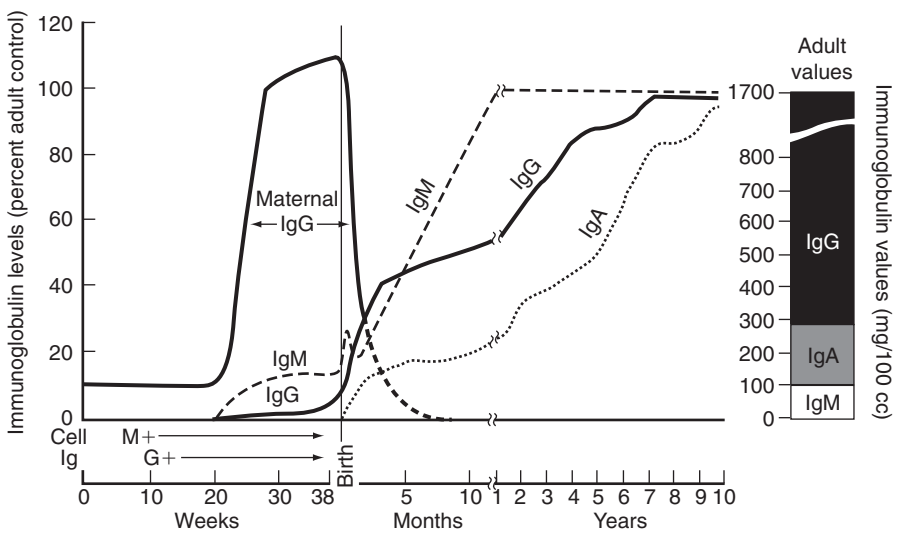

Figure 73–4 Kinetics of fetal and neonatal immunoglobulins. (*From Alford, C. A.: Immunoglobulin determinations in the diagnosis of fetal children. Pediatr. Clin. North Am.* 18:*99-113, 1971.*)

TABLE 73–7 Mother and Infant Serologic Response in Congenital Rubella

	Rubella Hemagglutination-Inhibition Antibody Titers			
Serum Specimens	**Mother A**	**Infant A**	**Mother B**	**Infant B**
At exposure	<8	–	128	–
At birth	1024	1024	128	256
6 mo postpartum	1024	2048	250	<8
Comment	Congenital rubella		Passive transfer of antibody; no congential rubella	

cially the herpesviruses,[458] and the presence of rheumatoid factor (IgM antibody that binds to the Fc portion of IgG) in serum.[129] False-negative results can occur because of a poor or delayed humoral immune response in the fetus/neonate. For example, CMV IgM antibody was present in the serum of only 70 percent of congenitally infected infants, as demonstrated by isolation of virus from urine in the first few days of life.[608]

Determination of a quantitative IgM level in cord-blood serum often has been recommended as a screening test for congenital viral infection. Values above 20 mg/dL are considered to be abnormal, but the normal values in local laboratories should be used as a guide.[18] Unfortunately, contamination of cord-blood serum samples by maternal blood may occur in one half to two thirds of specimens.[15,425] Contamination is determined by demonstrating that the concentration of cord-blood serum IgA, which also is not passed transplacentally, is higher than the IgM level. If one excludes contaminated specimens, the incidence of congenital infection in infants with an elevated IgM level is many times higher than that in infants with normal levels. However, at least 50 percent of infants with a proven congenital infection, particularly those with asymptomatic infection, have a normal IgM level. In two thirds of infants with elevated IgM, infection with a specific etiologic agent could not be diagnosed.[15,18] Therefore, high rates of both false-positive and false-negative results occur. Finally, an elevated IgM level does not enable a specific etiologic diagnosis; it merely provides strong evidence for an intrauterine infection of some type.

The similarities in the clinical signs and physical findings associated with congenital and perinatal infection have led to the recommendation that for establishing appropriate diagnoses of these infections, submission of maternal and neonatal serum specimens for the "TORCH" screen or, in selected instances, a single neonatal specimen for specific IgM antibodies is best. The use of a "TORCH" battery of serologic tests, however, no longer can be recommended routinely because of poor diagnostic return; these tests are difficult to interpret because of transplacental passage of maternal IgG antibodies, and IgM assays may result in false-positive findings.[1] Instead, the laboratory test or tests that should be performed must be dictated by which congenital infection or infections one is trying to diagnose or rule out. As seen in Table 73–6, the variety of tests that are available for establishing the diagnosis of any of the multitude of different potential congenital pathogens renders a "TORCH" assay, and by inference the term "TORCH" itself, obsolete by current standards. Finally, if a congenital infection is suspected in the delivery room, the placenta should be sent for histopathologic studies.

CYTOMEGALOVIRUS

CMV is a ubiquitous agent that usually causes asymptomatic infection in a normal infant, child, or adult. CMV is the most common congenital infection in humans. Overall, the birth prevalence for congenital CMV infection has been estimated at 0.64 percent, with ranges between 0.4 and 1.2 percent reported. Congenital CMV infection occurs after both maternal primary and recurrent infections during pregnancy. It remains a worldwide public health problem for which interventions are critically necessary.[105,323,535] However, patients with immature or impaired host defenses, such as a fetus, newborn infant, or immunosuppressed patient, may exhibit a variety of clinical manifestations during the acute infection and have a greater potential for developing long-term neurologic sequelae.[17,178,228,267,451]

MICROBIOLOGY AND EPIDEMIOLOGY

CMV is a member of the herpesvirus group, which includes HSV, VZV, and Epstein-Barr virus, as well as human herpesvirus 6 (HHV6), HHV7, and HHV8. Several different genotypes or genetic strains of CMV differ, and an individual can have more than one CMV genotype or strain infection.[17,178,228] Different genotypes are associated with congenital CMV infection and disease; gB3 genotype appears to predominate in some studies, but no clear association between genotype and pathogenesis has been identified.[47,691]

Human CMV is limited to the human host and grows only in human cells in tissue culture. Its distribution is worldwide, and no predilection for either sex or a particular season of the year exists.* Studies of antibody prevalence and virus isolation have indicated two peak ages for acquisition of infection: (1) infancy and early childhood and (2) early adulthood (in the sexually active).[228,699] Infection rates are higher and exposure to the virus occurs earlier in life in developing countries, lower socioeconomic groups in industrial nations, and Asian populations.[228] The major source of infection of young infants is the mother, whereas transmission in the daycare setting is an important source for older infants and toddlers.[490,599] Approximately 50 percent of susceptible children between the ages of 1 to 3 years who are in group daycare acquire CMV, mostly by transfer of virus present in saliva on hands and toys. Within 3 to 7 months of their child's acquiring CMV infection in the daycare center, as many as 33 percent of the seronegative mothers become infected.[7] Transmission of CMV from a child in daycare to his mother and her fetus has been confirmed. Thus, CMV infection in young children in daycare has become an important source of maternal and fetal infection.[323,491] In young adults, intimate interpersonal contact appears to be necessary for transmission and an infected sexual partner also may be the source of infection for a pregnant woman and her fetus.

The usual sites for isolation of virus are the urine, cervix, and saliva,[228] but CMV also has been recovered from amniotic fluid,[171] semen,[375,376] breast milk,[202,277] feces,[159] and autopsy and biopsy tissue. Excretion of virus in urine, saliva, or the cervix may be prolonged after primary infection, particularly with congenital disease.[17,178,603,606] Intermittent, recurring shedding also may occur in a significant proportion of seropositive young adults.[528,604,606] Despite this prevalence of virus excretion, CMV seems to be of low communicability.[228] Beyond the neonatal period,

*See references 17, 52, 105, 170, 178, 228, 302, 305, 323, 535, 660.

infection appears to require prolonged or intimate contact and presumably is transmitted through contact with oropharyngeal or genital secretions.[105] Researchers have assumed that CMV may be transmitted sexually because of the frequency of isolation of virus from genital secretions and because the prevalence of antibody is higher in sexually active populations.[17,178] Finally, transmission of CMV by blood transfusion has been documented, particularly in the event of multiple seropositive blood donors.[10,228]

Prenatal transmission of CMV to the fetus presumably is associated with maternal viremia and transplacental passage of the virus, perhaps within virus-infected leukocytes.[17,178] An important observation is that congenital CMV infection occurs in the infants of mothers known to be immune to CMV, thus indicating that transplacental transmission is possible despite circulating antibody in maternal serum.[600,603] In addition, mothers have delivered more than one congenitally infected infant in successive pregnancies.[603,604] Analysis of CMV isolates obtained from the same mothers on repeated occasions are antigenically and genetically identical, whereas strains from different mothers are not.[297] In addition, strains obtained from pairs of mothers and their congenitally infected infant pairs, as well as congenitally infected siblings, usually, but not always, are identical. These results suggest that most CMV transmission from immune mothers to their fetuses or newborns is the result of reactivated latent infection. Recently, however, Boppana and colleagues[82] have documented that acquisition of a new strain of CMV by pregnant immune women can lead to symptomatic congenital CMV infection in their offspring. Fetal CMV infection also may occur in HIV-infected pregnant women.[194,443,558] Symptomatic congenital CMV infection has been documented in a preterm infant who died of disseminated CMV infection and was born to an HIV-infected, CMV-immune mother with acquired immunodeficiency syndrome (AIDS).[558] Among HIV-infected, CMV-immune women without advanced HIV disease, the rate of congenital CMV infection in their infants was similar to that of a comparison group of infants born to CMV-immune, HIV-uninfected mothers.[354,443] In another study, among a cohort of infants born to HIV-infected mothers, infants who were infected with HIV were significantly more likely to have congenital CMV infection than were non–HIV-infected infants, 21 versus 4 percent, respectively.[194] Importantly, HIV-infected infants who are co-infected with CMV appear to have greater immunosuppression and progression of disease than do those infected with HIV alone.[194,354,459]

Natal transmission of CMV results from exposure of the neonate to infected genital secretions at the time of delivery.[17,528,529] If the mother is excreting virus at the time of delivery, 40 to 50 percent of exposed infants become infected.[528] The level of maternal antibody did not influence the frequency or time of onset of infection in the neonate. The usual incubation period for natal infection is 5 to 6 weeks.[529]

The importance of breast milk and blood transfusion as sources for postnatal acquisition of CMV was mentioned earlier in the section "Frequency of Infection in the Mother and Neonate." Although nosocomial transmission of CMV from baby to baby via fomites or health care workers' hands in the neonatal intensive care unit has been documented,[593] it rarely occurs.[8,219]

PATHOGENESIS AND PATHOLOGY

Congenital infection results from transplacental transmission during maternal viremia.[17] However, CMV placentitis may occur without transplacental transmission of virus.[278] If transplacental transmission occurs, the virus spreads through the fetus by the hematogenous route.[400,550] The severity of congenital disease in the neonate usually, but not always, correlates with intrauterine infection at an earlier gestational age.[598] With the exception of blood transfusion-associated infection, natal and postnatal infection with CMV usually is acquired secondary to challenge of the nasopharynx or oropharynx of the infant with virus from infected maternal genital secretions or breast milk.[17,178] Replication of virus in the neonate occurs in the mucosa of the respiratory or gastrointestinal tract, with subsequent viremic spread to target organs. With blood transfusion-associated infection, virus is inoculated directly into the bloodstream. The major target organs are the CNS, eyes, lungs, liver, and kidneys.[17] Of interest is that overt disease in the neonate appears to be associated more commonly with the hematogenous route of inoculation: transplacental infection or infection secondary to blood transfusion.

CMV appears to have a particular affinity for epithelial cells, for ependymal cells lining the ventricles of the brain, and for the organ of Corti and neurons of the eighth nerve.[602] The characteristic pathologic features of CMV infection are cytolysis, focal necrosis and an inflammatory response, the formation of enlarged cells with intranuclear inclusions (cytomegalic cells), and the production of multinucleated giant cells. Healing results in fibrosis and often calcifications, which cause structural damage to the fetal developing organs. Damage may continue after birth because of persistent postnatal viral replication.[17] Replication of CMV in the epithelial cells of blood vessels may result in vascular damage and secondary structural defects. The intrauterine growth restriction associated with symptomatic congenital infection appears to be the result of a reduction in the numbers of cells in various organs rather than a diminution in cell size.[444] Abnormalities resulting from faulty organogenesis secondary to CMV infection are limited primarily to the brain and include microcephaly, optic atrophy, aplasia of various parts of the brain, and microphthalmos.[265] Although a variety of congenital malformations outside the CNS have been observed in infants with congenital CMV infection, they probably are coincidental rather than true teratogenic effects of the virus.[265] Such malformations include a variety of heart lesions, clubfoot deformities, indirect inguinal hernias, high arched palate, dental defects, and hypospadias.[17,265] Other than inguinal hernia in male infants and tooth enamel defects, the extraneural defects in infants with congenital CMV infection have been infrequent, sporadic, and diverse occurrences.

The fetus is capable of mounting a humoral immune response to CMV, as evidenced by the presence of elevated IgM levels and specific CMV IgM antibody in cord serum.[17,530,608] Excessive production of IgM and IgG in the presence of virus replication during the early postnatal course of congenital CMV has resulted in the formation of circulating immune complexes and rheumatoid factor, thereby providing a potential risk for tissue damage to occur by immune complexes.[609] The factors contributing to prolonged replication and excretion of CMV in involved infants are not understood fully, but it does not appear to be a matter of immunologic tolerance because infants do produce specific antibody against CMV.[17]

However, abnormalities in other aspects of the immune response of congenitally infected infants, including decreased lymphocyte blastogenesis and production of immune interferon in response to CMV antigen,[527,613] a decreased percentage of T cells in peripheral blood,[548] and a diminished interferon response in leukocytes challenged with Newcastle disease virus in vitro, have been observed.[210] The degree of suppression of these responses appears to correlate with the presence and amount of virus excretion and the severity of the disease.[529,613] Further investigations are necessary to determine the contribution of these immunologic aberrations to the pathogenesis of congenital CMV infection. The clinical relevance of these immunologic impairments is uncertain. Infants with congenital CMV infection do not have a higher incidence or severity of bacterial infections, although three fatal cases of overwhelming sepsis secondary to

Staphylococcus epidermidis have been described in CMV-infected, low-birth-weight infants.[365] Recently, *Pneumocystis carinii* pneumonia was reported in a 4-month-old infant who had congenital CMV infection; this infant was not infected with HIV but was severely malnourished.[386]

CLINICAL MANIFESTATIONS

From 90 to 95 percent of neonates with congenital CMV infection are asymptomatic in the neonatal period. Babies born to mothers with primary CMV infection during pregnancy are much more likely to be symptomatic as neonates than are newborns of mothers with recurrent infection.[11,17,23,178,510,600] The clinical manifestations present in those with overt disease in the neonatal period are shown in Figure 73–2. These features may occur singly or in combination. The constellation of findings seen in infants with multiorgan disease that primarily affects the reticuloendothelial system and CNS historically has been referred to as *cytomegalic inclusion disease*, a term that rarely, if ever, is used today.

Typical clinical features of symptomatic congenital CMV disease include small size for gestational age, hepatomegaly, splenomegaly, jaundice, petechiae or purpura, pneumonia, microcephaly, chorioretinitis, and cerebral calcifications.[589] The enlargement of the liver and spleen is caused by mild hepatitis, a reticuloendothelial response to chronic infection, and extramedullary hematopoiesis. Hepatitis is associated with direct- and indirect-reacting hyperbilirubinemia and mild elevation of liver enzymes. Liver biopsy specimens have revealed local infiltration and necrosis, multinucleated giant cells, large inclusion-bearing cells, cholangitis, fatty metamorphosis, interstitial fibrosis, and bile stasis.[17,178] Although hepatomegaly and mild alteration in liver function test results may persist for several months after birth, severe chronic liver disease with cirrhosis rarely occurs. Splenomegaly is a common event and may be the only abnormality present at birth. Petechiae and purpura are the result of thrombocytopenia, which usually resolves within a few weeks or months but may persist through the first year of life. Thrombocytopenia may be the only manifestation of CMV infection. "Blueberry muffin spots" are discrete, well-circumscribed lesions often mistaken for purpura; they represent dermal erythropoiesis in the more severely affected infants. A diffuse interstitial pneumonitis occurs in less than 1 percent of newborns with symptomatic disease. Congenital CMV infection also has been associated with defective enamelization of the deciduous teeth.[601]

Involvement of the CNS by CMV results in the most severe sequelae of the disease. CMV infection of the brain causes encephalitis and periependymitis, with resultant ventriculomegaly, gliosis, and calcification. The cerebral calcifications typically are periventricular, a pattern that may be distinguished from the more diffuse pattern observed in congenital toxoplasmosis. In addition, linear calcifications along the lenticulostriate vessels within the basal ganglia and thalamus have been associated with CMV infection. Intracranial calcifications are best visualized by unenhanced computed tomography (CT) scan of the brain; however, cranial ultrasound and brain magnetic resonance imaging (MRI) also have been applied to discern abnormalities.[128] As many as 70 percent of symptomatic infants have neurologic findings, with the most common being microcephaly. Microcephaly may not be present at birth but may become apparent when the child is 1 year of age or older, a period in which differences in growth rates between the brain and somatic tissues are observed. When associated with cerebral calcifications and chorioretinitis, microcephaly carries a high probability of developmental disabilities.[465] On occasion, microcephaly is associated with obstruction of the fourth ventricle, with subsequent development of hydrocephalus. As many as 37 percent of congenitally infected infants with neuroradiologic abnormalities will develop seizures.[623]

The most common ocular abnormalities are chorioretinitis, strabismus, and optic atrophy.[145] Central vision loss may occur in neonates with severe CNS involvement.[145] Although microphthalmos, cataracts, and other eye abnormalities have been observed in congenitally infected infants, they are rare occurrences and most likely are not caused by CMV, but rather represent a coincidental finding with another etiology.[145,265] Permanent vision loss may occur as long-term sequela.

Deafness is the most common sequela of congenital CMV infection. Sensorineural hearing loss occurs in approximately 50 to 75 percent of symptomatic infants and 7 to 15 percent of those with asymptomatic congenital infection. Hearing loss associated with symptomatic infection usually is severe and bilateral, whereas the hearing loss in asymptomatically infected infants usually is unilateral. Both forms of hearing loss are progressive.[230,678] Approximately 20 percent of infants with congenital CMV have late-onset hearing loss that will not manifest until after the child reaches 1 year of age.

Most naturally acquired natal and postnatal CMV infections in full-term newborn infants are asymptomatic and not associated with late neurologic sequelae such as hearing loss and psychomotor retardation. Most of these infections result from reactivation of latent maternal CMV, and infants are born with transplacentally acquired maternal IgG antibody that ameliorates the disease. However, mostly pneumonitis, but also hepatosplenomegaly and lymphadenopathy, has been noted in some infants.[366,597,670,698] Severe disease manifested also by neutropenia and thrombocytopenia has been reported predominantly in preterm infants and is associated with the development of chronic lung disease.[698] In addition, exacerbation of congenital CMV infection has been documented in premature infants receiving corticosteroid therapy for chronic lung disease of prematurity.[648] Similarly, postnatal acquisition of CMV through breast-feeding nearly always is an asymptomatic infection in full-term infants because of the presence of maternal antibodies. CMV infection acquired through breast milk by premature infants with a birth weight of 1000 g or less has been associated with a sepsis-like illness consisting of apnea and bradycardia, hepatosplenomegaly, a distended bowel, pallor, thrombocytopenia, and elevated liver function test results.[263,656]

Transfusion-acquired CMV infection is an important clinical syndrome associated with multiple blood transfusions from seropositive donors to very-low-birth-weight infants in neonatal intensive care units.[10,50,692,697] Eighty-eight percent of babies had hepatosplenomegaly, a "septic" appearance, deterioration in respiratory status, a peculiar gray pallor, and atypical lymphocytosis approximately 4 to 12 weeks after receiving transfusions.[10,50,697] The illness lasted 2 to 3 weeks in most infants, although 24 percent died.

DIAGNOSIS AND DIFFERENTIAL DIAGNOSIS

Primary CMV infection occurring during pregnancy is documented by demonstration of IgG seroconversion with sera obtained before or during early pregnancy and at delivery. Detection of anti-CMV IgM in a single specimen is considered a presumptive primary infection.[12,13,253,364,452,509,608] Caution, however, must be exercised when analyzing the results of CMV IgM tests. Even with the best commercially available assays, at least 5 percent of positive serum IgM tests represent false-positive results. On the other hand, a negative CMV IgG antibody test in the mother of an infant in whom the diagnosis of CMV infection is in question would exclude congenital transmission as a possible source of CMV. CMV avidity tests can be helpful in distinguishing recent primary infections from past recurrent

CMV infections in CMV-seropositive women. The prenatal diagnosis of congenital CMV infection can be accomplished by amniocentesis, with detection of CMV in amniotic fluid by culture and PCR. Fetal blood sampling for CMV IgM determination, complete blood count (CBC) with platelet count, and serial fetal ultrasound examinations can help identify fetuses at high risk for developing disease and sequelae.[41,213,388]

Because the signs and symptoms of symptomatic CMV infection in newborns so often overlap with those in other diseases during this period of life, establishing a definitive diagnosis requires the use of laboratory tests. Isolation of virus from urine or saliva collected within the first 21 days of life is considered proof of congenital infection and is the most sensitive and recommended means of diagnosis.[17,178] The usual specimens for isolation of CMV are urine and saliva,[48] but virus may be recovered from CSF, buffy coat, nasopharynx, tears, and biopsy and postmortem tissue.[465] Congenitally infected infants are known to excrete virus for several years after birth. Isolation of virus in an infant beyond 3 weeks of age does not, by itself, differentiate between natal or postnatal infection and congenital infection, unless negative cultures have been obtained previously. Detection of CMV DNA by PCR in urine, saliva, blood,[235,311] or CSF also may confirm congenital infection, although the sensitivity and specificity of PCR performed on urine may be less than those for viral culture.[179,455] On the other hand, PCR is preferred for detection of CMV in CSF and blood because CMV culture usually is negative.[203,365,643] The presence of CMV DNA by PCR in the CSF of congenitally infected infants has correlated with abnormal neurologic outcome.[643] Similarly, the finding of a positive serum or whole blood CMV PCR in infants with congenital CMV infection has been associated with the development of hearing loss.[86] Quantitative CMV PCR assays also may be used to follow antiviral therapy.

Serologic tests, such as "TORCH" titers performed for specific antibody determination in neonates, rarely are helpful and are not recommended for establishing a diagnosis of congenital infection. If serologic tests that predominantly measure IgG are used, serial serum specimens from birth are required to differentiate congenital from natal or postnatal infection. One observes maintenance of a stable antibody titer during the first 6 months of life in infants with a congenital infection. In contrast, with natal and postnatal infection, a drop in antibody titer occurs during the first 2 to 3 months of life as maternal passive antibody declines, followed by a rise by the time the infant reaches 5 to 6 months of age.[529,607] If the infant is not evaluated until several months of age and serum specimens from earlier life are not available, determining whether the infection is congenital, natal, or postnatal in origin may not be possible.

Detection of CMV antigenemia in blood can be achieved by means of a commercial assay that uses monoclonal antibodies to pp65, a tegument protein of the virus that is present in infected polymorphonuclear leukocytes.[190] Its major use may be for evaluation of a symptomatic infant who has natally or postnatally acquired CMV infection, for which making a differentiation between congenital and acquired CMV infection is difficult at best and the finding of a positive urine CMV culture may be only incidental. The assay is quantitative, so it also may be used to follow antiviral treatment.

Examination for inclusion-bearing cells in urine carries historical significance, but this test yields positive results in only 20 to 50 percent of known virus-positive cases, and it is rarely, if ever, used today.[17,178] Typical intranuclear inclusions also may be seen in biopsy tissue or in megakaryocytes of bone marrow aspirates,[17,178] but virus may be isolated from tissue when these pathologic findings are absent.

The major diseases to consider in the differential diagnosis include congenital syphilis, congenital rubella, congenital toxoplasmosis, erythroblastosis fetalis, disseminated HSV infection, LCMV, neonatal sepsis, and enterovirus infection. Congenital syphilis is suggested by the presence of osteochondritis and periostitis on radiographs of the long bones in an infant born to a mother with reactive serologic tests for syphilis. Darkfield examination of spirochete-laden nasal secretions in infants with rhinitis confirms the diagnosis. The presence of congenital heart disease and cataracts suggests rubella, and chorioretinitis, hydrocephalus, and intracranial calcifications suggest toxoplasmosis, but a definitive diagnosis depends on the laboratory findings. In uncomplicated erythroblastosis, the direct bilirubin and liver function study results remain normal. Vesicular skin lesions suggest HSV or VZV infection. Positive blood cultures confirm the diagnosis of neonatal sepsis. Congenital CMV infection seldom is complicated by bacterial sepsis in a newborn. Neonatal enterovirus infections are seasonal; associated with maternal symptoms of enteroviral disease; and characterized by aseptic meningitis, hepatitis, myocarditis, or gastroenteritis. Biliary atresia, as well as noninfectious and genetic and metabolic disorders, also may mimic congenital CMV disease.

TREATMENT

Although treatment with idoxuridine,[148] floxuridine,[223] 5-fluorodeoxyuridine,[506] cytosine arabinoside,[357,405,506] adenine arabinoside,[58,138] interferon inducers,[506] human interferon,[38,211] and acyclovir[505] has been tried in infants with congenital CMV infection, the only effect was a transient alteration in viral excretion. Unfortunately, little or no effect on the clinical course of the disease was achieved with these early antiviral compounds. Foscarnet, cidofovir, ganciclovir, and valganciclovir are currently licensed for treatment of life- and sight-threatening CMV infections. Of these antivirals, only ganciclovir has been subjected to clinical trials that evaluated the pharmacokinetics and efficacy in treatment of newborns with virologically proven CMV disease.* Neither standard nor CMV hyperimmune intravenous immunoglobulin is recommended for the treatment of congenital CMV infection. However, in nonrandomized studies, maternal administration for in utero fetal treatment with CMV hyperimmune globulin has shown possible benefit in preventing transmission to the fetus and in ameliorating fetal disease.[440,462] Also, the extremely premature infant who develops CMV disease postnatally, after maternal IgG levels have declined and after administration of only CMV-seronegative blood products, may be devoid of virus-specific CMV antibody and, therefore, if CMV IgG levels are nil or negative, may possibly benefit from administration of IGIV or CMV hyperimmune globulin. However, there are no clinical trials published that evaluate the supportive role of immune globulin in the treatment of CMV disease in these special infants.

Clinical studies and experience support the use of ganciclovir in selected neonates. A phase II study of intravenous ganciclovir (4 or 6 mg/kg per dose every 12 hours for 6 weeks) in 47 neonates with congenital CMV infection involving the CNS found that the preferred dose was 12 mg/kg/day.[671] Adverse effects of ganciclovir included neutropenia in 16 (34%) infants, increases in aspartate aminotransferase in 6 (13%), and direct hyperbilirubinemia in 3 (6%). Hearing was improved or stabilized in 16 percent of infants at 6 months or longer of follow-up, thus suggesting efficacy.

Furthermore, a phase III, multicenter randomized study of ganciclovir therapy for infants with congenital CMV infection involving the CNS was performed by the Collaborative Antiviral Study Group (CASG) from 1991 to 1999.[335] This study evaluated

*See references 25, 180, 286, 330, 335, 411, 420, 421, 460, 462, 555, 590, 629, 646, 648.

the safety and efficacy of intravenous ganciclovir (6 mg/kg per dose every 12 hours for 6 weeks) versus no therapy in 100 enrolled CMV-infected neonates with neurologic involvement (≥32 weeks of gestation, birth weight ≥1200 g). Among 47 evaluable infants who completed all protocol follow-up evaluations, those who received ganciclovir were significantly more likely to have either improved or normal hearing at 6 months than were untreated infants, and none of the ganciclovir-treated infants had worse hearing. At 1 year or longer, those who received ganciclovir also were significantly less likely to have worse hearing than were those in the untreated group. Other beneficial effects of ganciclovir therapy included a significant decrease in the median time to normalization of alanine transaminase, as well as improved weight gain and head circumference after 6 weeks of therapy, but no change in mortality rates. Neutropenia developed in 63 percent of ganciclovir recipients, reversible with adjustment or discontinuation of medication. In addition, neurodevelopmental assessments in neonates at 6 to 12 months of age showed that treated neonates had fewer milestone delays and improved growth in head circumference compared with untreated neonates, suggesting another potential benefit of ganciclovir therapy.[335]

Currently, offering ganciclovir therapy to CMV-infected neonates with CNS disease seems reasonable because 90 to 95 percent of these infants will have significant neurologic and sensory sequelae. The decision to treat should be individualized and carefully considered.[25] Because 6 weeks of therapy may not be sufficient for optimal benefit, a phase I/II pharmacokinetic evaluation of oral valganciclovir in neonates with CMV infection of the CNS has been published recently, with the hope that studies evaluating the effects of long-term oral suppressive therapy will be soon conducted.[330,335] Ganciclovir also may be beneficial in treating critically ill neonates, especially preterm infants, with life-threatening CMV disease manifested by viral sepsis syndrome, persistent thrombocytopenia, recurrent or progressive sight threatening retinitis, persistent or severe pneumonitis, persistent hepatitis, or encephalitis.[179]

PROGNOSIS

The major long-term sequelae of neonatal CMV infection are neurodevelopmental, motor, and sensory disabilities.[231,269,367,530,541,602] The most guarded prognosis occurs in neonates born to mothers with primary CMV infection during pregnancy; in infants with symptoms at birth, particularly those of the CNS; in infants with the triad of microcephaly, intracranial calcifications, and chorioretinitis; and in neonates who have elevated quantitative IgM or in whom CMV-specific IgM is present.[17,81,178,231,252,367,465,466,678] The duration of CMV urinary excretion has not been associated with abnormalities in growth or neurodevelopmental deficits.[465] Overall, 25 percent of infants born to mothers with primary CMV infection during pregnancy will have at least one sequela, versus 8 percent of those born to mothers with recurrent infection.[231] Of symptomatic, congenitally infected neonates, approximately 8 percent die in the neonatal period.[82,589] Six percent of symptomatic infants die after the neonatal period, and death often is caused by progressive liver disease, failure to thrive, seizures, or intercurrent illnesses. Death beyond infancy is a result of malnutrition, aspiration pneumonia, or overwhelming infection. Of survivors with symptomatic congenital CMV disease, as many as 90 percent have some evidence of CNS abnormality, such as microcephaly, impaired intellect or development, neuromuscular disorders (seizures, cerebral palsy, spasticity, hemiparesis), sensorineural hearing loss, and ocular abnormalities (usually chorioretinitis).[147,231,492,596,679] Neurologic sequelae of congenital CMV disease, especially sensorineural hearing loss, may progress after the first year of life.[80] For this reason, the recommendation is that CMV-infected newborns have close audiologic and neurodevelopmental follow-up through childhood and adolescence. Cochlear transplantation performed in children as young as 11 months of age, accompanied by intensive speech language therapy, has been used successfully in infants who have profound bilateral hearing loss from congenital CMV disease.

Ninety to 95 percent of infants with congenital CMV infection are asymptomatic in the neonatal period. As many as 25 percent of these infants will have abnormal brain CT scans. Several long-term follow-up studies have indicated that as many as 15 percent will have progressive hearing loss, and at least 10 percent of these infants may have neurodevelopmental and auditory processing differences and disabilities, possibly caused by CMV. The following abnormalities were noted in infected infants when compared with matched control children: IQ less than 90 (32 vs. 16%), significant hearing loss (23 vs. 9%), predicted school failure (36 vs. 14%), and microcephaly (15 vs. 5%).[268,530] Auditory imperception problems also have been associated with congenital CMV infection in children with normal hearing. In addition, approximately 2 percent of infants with asymptomatic congenital infection may have silent, unrecognized chorioretinitis.[145]

As many as one third of neonates who acquire CMV natally from maternal cervical secretions may have acute disease associated with the onset of viruria, regardless of birth weight.[366,698] However, birth weight and age of onset of viral excretion appear to influence the development of neurologic sequelae significantly. Full-term neonates with natally acquired CMV do not have significantly altered behavioral, neurologic, audiologic, speech, and language examinations on long-term follow-up when compared with uninfected controls.[367] In contrast, infants with birth weight less than 2000 g and onset of CMV excretion before they reach 8 weeks of age have associated severe cardiopulmonary disease during the neonatal intensive care unit stay (perhaps because of CMV worsening the pulmonary disease) and a significantly greater percentage of severe handicaps on long-term follow-up than do matched controls.[489] The relationships among low birth weight, early onset of CMV excretion, lower levels of transplacental antibody, and more severe cardiopulmonary disease and their contribution to neurologic sequelae require further evaluation, but natal acquisition of CMV appears to contribute to the development of sequelae in selected situations. CMV disease acquired from blood transfusion in very-low-birth-weight infants also may contribute to neurologic sequelae.[10,17,697]

Infants with congenital CMV infection have many special needs. These infants and children require physical, occupational, and speech therapy, as well as audiologic follow-up to assess their need for hearing aids and cochlear implantation. Ophthalmologic follow-up also is necessary because strabismus, optic atrophy, and, rarely, late onset chorioretinitis may develop and affect visual acuity. Failure to thrive secondary to swallowing dysfunction, such as microaspiration and gastroesophageal reflux, often is present in the more severely affected infants, which renders the need for gastrostomy tube feeding essential for survival. This decision often becomes an ethical issue, as is establishment of resuscitation orders. Severely affected children also may have sleep disorders that may require the use of nighttime sedatives. In less severely affected children, school function must be assessed carefully. Finally, all these issues bring stress to the family unit, and the need for family support cannot be overemphasized.

PREVENTION

Routine serologic screening of pregnant women currently is not recommended routinely by all experts because no prophylactic or

therapeutic interventions, such as vaccines or antivirals, are available during pregnancy. However, many experts recommend that women of child-bearing years be aware of their CMV serostatus and also be aware of the most likely sources of CMV infection.[105] In addition, physicians caring for women who may become or are pregnant should counsel them about hygienic precautions that may reduce the risk of acquiring CMV infection during pregnancy, and educational materials are available to assist in counseling (*www.cdc.gov/cmv* or *www.bcm.edu/pedi/infect/cmv).* During pregnancy, meticulous adherence to standard precautions, especially handwashing after exposure to urine or saliva from young infants and toddlers and avoidance of kissing and sharing utensils with toddlers and young children who are likely to be shedding CMV, currently may be the most effective means of preventing transmission of CMV infection to susceptible pregnant women.[25,105,124,308] CMV often is transmitted within the family settings and in daycare centers caring for infants, toddlers, and young children. The rate of transmission of CMV in the hospital setting appears to be low[181,228] because prolonged or intimate contact seems to be necessary for spread to occur.[17,181,228,693] For this reason and because of the ubiquity of the virus, pregnant women are not excluded routinely from caring for patients infected with CMV.[25] Adherence to universal, standard precautions for all patients is adequate to prevent CMV transmission to patients and health care workers.[181]

Blood transfusion–associated CMV infection in low-birthweight infants in neonatal intensive care units virtually has been eliminated by the use of CMV-seronegative blood donors,[6,88,374,385] frozen deglycerolized red cells,[88,631] removal of the buffy coat by saline washing,[179] and filtration to remove white blood cells. Pasteurization of breast milk effectively kills CMV, although freezing at –20° C significantly reduces but does not eliminate infectivity. If donor breast milk is provided to premature infants or full-term infants born to nonimmune mothers, only that from a seronegative mother should be used.

Passive immunoprophylaxis with CMV hyperimmune globulin has been reported in uncontrolled studies to reduce transmission of CMV and morbidity in the fetus of women experiencing primary CMV infection while pregnant.[462] Randomized, controlled, multicenter trials should be performed, however, to determine if this approach can be beneficial enough to be routinely recommended.

Several vaccine candidates, including two live-attenuated CMV vaccines (AD-169 and Towne strains), have been evaluated in phase I and II clinical trials, and chimeric vaccines combining Towne and Toledo strains have been evaluated.[79,17,90,178] Currently, subunit vaccines using CMV glycoprotein gB, a principal target of the neutralizing antibody response, attached to an adjuvant, is a promising vaccine candidate that has been shown to be safe and immunogenic in phase I trials and currently awaits phase II and III trials. Development of a safe and effective CMV vaccine that can prevent severe congenital CMV disease remains a top priority and is a realistic and attainable goal.[36a,325,551]

RUBELLA

Rubella usually is a mild, often subclinical disease involving school-aged children and young adults. However, rubella virus also can cross the placenta, infect the fetus, and cause fetal death or congenital malformations. Since the original observations were made by the Australian ophthalmologist Norman McAlister were Gregg,[198,250] in which maternal rubella was associated with the birth of offspring with defects of the eye and heart, rubella has been the prototype for congenital viral infection.

MICROBIOLOGY AND EPIDEMIOLOGY

Rubella virus, an enveloped RNA virus in the family *Togaviridae*, has only a single antigenic type but has several newly characterized genotypes.[152,310,518] Genotyping is now used to track the molecular epidemiology of rubella strains associated with outbreaks.[310,518,628] Rubella virus grows in tissue culture from a variety of animal species, but commonly, a viral interference assay with African green monkey kidney cells is used for isolation in the diagnostic virology laboratory.[134] Several serologic tests, including complement fixation, hemagglutination inhibition, immunofluorescence, radioimmunoassay, and enzyme immunoassay (EIA), are available.[134,281] Although hemagglutination inhibition[619] has been the gold standard, many diagnostic virology laboratories now use more sensitive and practical methods such as ELISA, latex agglutination, or fluorescent assay kits.[134] In addition, traditional and real-time PCR assays can now detect and genotype rubella virus RNA in clinical samples.[310,518]

Rubella is worldwide in distribution. Human beings are the only host, and transmission occurs from person to person.[152,681] In the era before rubella vaccine was available, epidemics occurred at 6- to 9-year intervals, and pandemics occurred every 10 to 20 years.[681] Since the vaccine was licensed in 1969, this epidemic pattern has been interrupted; the last major outbreak in the United States was in 1964.[54,473] The peak seasonal incidence is in the spring.[681] In the prevaccine era, the peak age incidence was between 5 and 14 years of age,[681] but in the mid-1970s, the peak age was 15 to 19 years.[473] Currently, no peak age exists. Approximately 5 to 25 percent of women of child-bearing age lack rubella antibody and are susceptible to primary infection.[54,116] The attack rate for rubella in susceptible populations with prolonged intimate exposure is high—95 to 100 percent.[152,681] The frequency of transmission after brief exposure, however, is low.[152,681] The frequency of subclinical infections is much higher in adults than in children, although some serologically diagnosed infections in adults actually may be reinfections rather than primary disease.[293,294,566]

The incubation period for acquired rubella ranges from 14 to 23 days but usually is 16 to 18 days.[566] Virus may be isolated from the throat from 1 week before until 2 weeks after the onset of rash. Rubella virus infection may be subclinical in one third to a half of children and in half to two thirds of adults.[293,566] Rubella hemagglutination-inhibition antibody is detectable in serum within 2 to 3 days after the onset of rash, with peak titers being reached in 3 to 4 weeks.[134] Complement-fixation antibody rises more slowly, with peak titers noted at 4 to 6 weeks after the rash appears. Primary rubella virus infection is associated with an initial response in IgM-specific antibody, followed by an increase in IgG antibody. Reinfection with rubella virus is known to occur, and rates are higher in vaccine-immune than in naturally immune subjects.[293] The vast majority of congenital rubella virus infections occur after primary infection, but a few cases have been reported after reinfection.[131,206,532] If no evidence of rubella-specific IgM can be found in mothers with subclinical reinfection during pregnancy, the fetus is unlikely to be at risk.

PATHOGENESIS AND PATHOLOGY

Transplacental infection of the fetus with rubella virus occurs secondary to maternal viremia during the course of primary infection.[14,16,249] Fetal infection appears to result from embolization of pieces of necrotic placental vascular endothelium.[414] However, involvement of the placenta with rubella virus does not always result in fetal infection, particularly after the first trimester.[16] After maternal immunity, the next most critical factor determining the frequency of fetal infection and the severity of

disease in neonates is the time during gestation that rubella virus infection occurs. Studies of the frequency of fetal infection and congenital defects according to gestational age at the time of maternal infection have used more sensitive methods to detect maternal and neonatal infection and long-term follow-up to detect abnormalities that were not apparent during infancy.[422] These studies indicated higher fetal infection rates than previously realized: 90 percent during the first 11 weeks, 50 percent during weeks 11 to 20, 37 percent from 20 to 35 weeks, and 100 percent during the last month. The congenital defect rate was 100 percent for the first 11 weeks, 30 percent during weeks 11 to 20, and none thereafter. Neonatal purpura and cataracts or glaucoma are observed when maternal rubella occurs during the first 2 months of gestation; congenital heart disease, during the first 3 months; deafness and neurologic deficit, during the first 4 months; and retinopathy, during the first 5 months.[422,646]

Excretion of rubella virus from the throat of congenitally infected infants may continue for several months after birth; approximately 10 to 20 percent of infected infants shed virus in nasopharyngeal secretions at 6 months of age. A small number will continue to shed virus for 1 year or more, and the virus has been recovered from tissues up to several years later.[16,154] Persistence of viral replication after birth may result in continuing damage to involved tissues, especially in the CNS and the eye and lens. In fact, hearing loss and neurologic deficits may appear long after birth in children previously considered well, or the clinical severity of these sequelae actually may worsen as the child's condition is being monitored.[136,154,185]

Rubella virus is a proven teratogenic viral agent that may cause congenital malformations.[475] Knowledge of the mechanisms by which rubella virus causes deformities may lead to a basic understanding of the pathogenesis of these malformations. Rubella virus is noncytolytic in certain tissues in that it does not destroy the cells in which it replicates. This characteristic, if manifested in the fetus, would tend to allow survival but result in disordered function of cells, tissues, and organs. On the other hand, selective cell destruction also may occur in fetal tissues.

In studies of the pathology of therapeutically aborted, rubella-infected fetuses, scattered foci of necrotic cellular damage without inflammatory infiltrates were noted in the endothelial cells of blood vessels and in myocardial cells.[635] Rubella-induced defects could result in defective formation or function of developing tissues by direct cellular destruction or by hypoxic damage secondary to blood vessel obliteration. For example, alteration of the elastic or muscle fibers in the ductus arteriosus could result in failure of postnatal ductus closure. Studies of tissue obtained from infants with rubella syndrome and maintained in culture showed that the cells were infected with rubella virus persistently and had a decreased growth rate and shortened survival time.[521] Naeye and Blanc[445] noted that the growth retardation of infants with rubella syndrome was the result of decreased numbers of cells in organs. This impaired cellular growth, if it occurred during a crucial phase in cardiac development, for example, could result in such cardiac anomalies as septal defects.

Increased numbers of chromosome breaks have been noted in leukocyte cultures of children with congenital rubella.[467] Chromosomal injury possibly results in cell loss during rapid organ development and is, in part, responsible for the congenital anomalies. Persistence of virus possibly results in continuing cell destruction or immunopathologic damage to tissues. In addition, two studies have demonstrated circulating rubella antigen-antibody complexes in 10 infants with congenital rubella and late-onset manifestations such as interstitial pneumonia, hepatosplenomegaly, rash, lymphocytic meningitis, and rapid neurologic deterioration.[78,630] IgG levels were low and IgM levels were elevated, with a diminished number of T cells and an increased proportion of B cells. Delayed maturation of the immune response in congenital rubella was postulated to predispose possibly to persistent antigenemia complexed with IgM and deposition of circulating immune complexes in tissues. Finally, antibodies against thyroid microsomes or thyroglobulin were found in a much larger percentage of children with congenital rubella syndrome than in control subjects.[142] A significant number of the patients with congenital rubella and thyroid antibodies also had thyroid dysfunction. These observations suggest that autoimmunity, induced in some way by persistent infection with rubella virus, plays a role in the late-onset endocrine dysfunction that occurs in children and young adults with congenital rubella syndrome.

Specific pathologic lesions in infants with congenital rubella depend on the gestational age at the time of infection and the particular organs involved. One common finding is necrosis of vascular endothelium, which may be accompanied by damage to organs secondary to vascular obstruction.[416,635] Diffuse intimal changes have been observed in the pulmonary and systemic arteries, as well as the ductus arteriosus.[416] Focal inflammation and necrosis have been seen in the myocardium[352] and structures of the inner ear.

The fetus is capable of an immune response to rubella virus: specific IgM and, occasionally, IgA have been observed in fetal and cord-blood specimens. In fact, hypergammaglobulinemia may be observed during early life in some infants with congenital rubella.[264] On the other hand, hypogammaglobulinemia has been noted in a few infants.[264,504] In 10 to 20 percent of infants with congenital rubella, hemagglutination-inhibition antibody declines to undetectable levels when they reach 1 to 4 years of age.[154,270] Several of these infants failed to respond to immunization with rubella vaccine.[151] In fact, infants with congenital rubella have been reinfected with rubella virus later in life.[414] Several defects in cell-mediated immunity, including diminished responsiveness of peripheral blood leukocytes to phytohemagglutinin and diminished lymphocyte transformation, interferon production, and synthesis of leukocyte migration inhibitory factor in response to challenge with rubella virus antigen, have been observed in infants with congenital rubella.[104,472] Normal responses were observed in healthy, seropositive children and adults. Researchers have presumed, but have not proved, that these abnormalities in cell-mediated immunity play a role in persistent viral excretion in congenitally infected infants.

CLINICAL MANIFESTATIONS

Although the typical clinical features of rubella may occur in pregnant women, as many as one half to two thirds of these infections are subclinical.[568] Typical features that might occur in symptomatic postpubertal women include fever, rash, posterior auricular and postoccipital adenopathy, and arthralgia or arthritis.

As many as two thirds of infants with proven congenital rubella may be asymptomatic in the neonatal period.[549] However, evidence of long-term sequelae was noted in almost three quarters of such infants within the first 5 years of life.[549] The clinical manifestations of congenital rubella syndrome vary highly, not only with regard to specific features but also in relation to the age during which the specific feature occurs. The collected features may be divided into three broad categories: (1) transient—those that are present in the neonatal period and clear after a few months, such as thrombocytopenia and hepatitis; (2) permanent—major malformations that persist and may even worsen as the child grows older, such as congenital heart lesions, cataracts, or hearing loss; and (3) developmental—aspects that do not emerge until childhood or young adulthood, such as behavioral disorders or endocrine dysfunction.[151,569] This categorization of

abnormal features may be useful in determining the prognosis and management of these children.

The manifestations of congenital rubella that are symptomatic in the neonatal period, compiled from several series,[153,292,351,392,540] are illustrated in Figure 73–2. The low birth weight of infants with rubella syndrome results from intrauterine growth restriction; even when born prematurely, the infant often is small for gestational age. The frequency of purpura in most series ranges from 15 to 50 percent. Thrombocytopenia almost always was present in association with the purpura and usually resolved spontaneously in the first month of life. Neonatal thrombocytopenic purpura is a poor prognostic sign because it generally occurs in severely affected infants with multiple manifestations. Thirty-five percent of 58 patients with purpura in one series[153] died during the first year, in contrast to a mortality rate of only 13 percent during the first 18 months for patients from the entire series. Although the thrombocytopenia frequently was profound, death rarely was caused by hemorrhage.

Direct involvement of the liver by rubella virus results in neonatal hepatitis, as evidenced by hepatomegaly, predominantly direct-reacting hyperbilirubinemia, and elevation of liver enzymes.[217] Pathology studies usually demonstrate hepatocellular disease with necrosis, giant-cell formation, bile stasis, and fibrosis, but extrahepatic biliary obstruction also has been demonstrated.[620]

Congenital heart disease occurs frequently and generally is detectable in the neonatal period, although specific lesions may not be defined until later in life. The most common lesions in 87 catheterized patients with congenital rubella were patent ductus arteriosus in 78 percent, right pulmonary artery stenosis in 70 percent, left pulmonary artery stenosis in 56 percent, valvular pulmonic stenosis in 40 percent, mild aortic valvular stenosis in 14 percent, aberrant subclavian artery in 11 percent, and ventricular septal defect in 10 percent.[154] Evidence of active myocardial disease has been noted in some infants.[352]

Interstitial pneumonia with cough, tachypnea, and breathlessness as the major manifestation of congenital rubella has been reported.[498] Most patients with pneumonitis died in the first year of life as a result of their pulmonary disease. Cardiac abnormalities also were present but were thought not to be significant either clinically or from autopsy findings. Microscopic studies revealed acute to subacute chronic interstitial pneumonitis. Rubella virus was isolated from all of the four lung specimens cultured.

Although cataracts are the most characteristic ocular lesion in rubella syndrome, they may not be visualized until after the neonatal period. Worldwide, vision loss from cataracts associated with congenital rubella syndrome remains a global public health problem.[518,650] Retinopathy is described as widespread, with mottled or blotchy, black pigmentary deposits of variable size and location: the "salt and pepper" retinitis.[358] Retinal function usually is not affected adversely. The frequency of retinopathy in the combined data from six series of children monitored for several years was 36 percent.

Bone lesions are another finding in congenital rubella in the neonatal period. Radiographic studies reveal small linear areas of radiolucency and increased bone density in the longitudinal axis of the metaphyseal area of the long bones of the lower and upper extremities.[540] This abnormality results from disturbances in deposition and calcification of osteoid and usually resolves by the time the child is 2 to 3 months of age.

Involvement of the CNS frequently is evident in symptomatic infants. Lethargy, irritability, disturbances in tone, and a bulging fontanelle are common findings. One or more seizures developed in 27 of 100 infants in one series.[185,186] However, they usually occurred after the neonatal period. In most infants with CNS involvement, CSF protein is elevated, but increased cell counts are seen less frequently. Rubella virus may be isolated from the CSF; in one series, 25 percent of 99 CSF specimens obtained during the first 3 months of life from patients with CNS symptoms were positive.[185,186] The extent of impairment at 18 months of age was not readily predictable on the basis of clinical symptoms or isolation of virus in the first few weeks of life. However, severe involvement was found more frequently in infants with seizures and high levels of CSF protein in the first few months of life.[185,186] An important emphasis is the chronic nature of congenital rubella infection, and one should note that although most infants may be asymptomatic in the neonatal period, evidence of disease was seen on follow-up examination in up to 70 percent.

DIAGNOSIS AND DIFFERENTIAL DIAGNOSIS

Because many maternal rubella cases are subclinical and other diseases may mimic symptomatic rubella, establishing a definitive diagnosis in the mother depends on the laboratory. Although rubella virus may be isolated from the throat during the acute phase of the illness, such culture frequently is not a practical means of establishing the diagnosis. The tissue culture cells required for isolation of rubella virus are not always available in routine virology laboratories. Widely used serologic tests include hemagglutination inhibition, ELISA, and fluorescent assay.[127,134] Susceptible individuals lack IgG antibody. Detectable antibody is present in the blood within a few days after onset of the rash, and peak titers are reached within 2 to 3 weeks. If serum specimens are not available until some time after the onset of illness, complement-fixation antibody titers may be useful because peak titers are not reached for 4 to 6 weeks after the rash is manifested. If only a single specimen is available, rubella-specific IgM antibody may be demonstrable by ELISA or immunofluorescence methods, peak 3 to 6 weeks after infection occurs, and persist for several months.[134,281] If maternal rubella infection is diagnosed, then prenatal diagnosis of congenital rubella infection can be made through amniocentesis to obtain amniotic fluid for viral rubella culture and rubella virus RNA detection by PCR and by fetal blood sampling for rubella IgM determination. Laboratory tests ideally should be combined with serial fetal ultrasound evaluations to determine the extent of fetal involvement if the fetus is identified as being infected with rubella.[308,627] Detection of viral RNA by PCR on chorionic villi samples obtained very early in gestation also has been used for prenatal diagnosis.

The most characteristic clinical features of congenital rubella are congenital heart disease, cataracts, microphthalmos, corneal opacity, glaucoma, and radiolucent bone lesions. In infants with these classic features, the clinical diagnosis correlates with laboratory confirmation of congenital rubella 80 percent of the time. However, many of the features, such as low birth weight, hepatosplenomegaly, icterus, and petechiae/purpura, overlap with those found in other infectious diseases of the newborn, and definitive diagnosis requires laboratory confirmation. The diagnosis of congenital rubella is confirmed by isolation of virus from the throat or urine; in addition, virus has been recovered from cataracts, conjunctivae, CSF, feces, bone marrow, and circulating leukocytes. The lens is an excellent site for recovery of virus, especially because cataracts are removed from these infants within the first few weeks of life. The diagnosis of congenital rubella also can be made by serologic means. Because rubella IgG antibody is passed transplacentally, serum levels determined early in the neonatal period mimic those of the mother. Stable or rising serum concentrations of rubella-specific IgG antibody in the serum of infants during their first 4 to 6 months of life can be considered diagnostic of congenital rubella when the clinical picture is compatible. The presence of specific IgM antibody in a single serum specimen obtained from an infant early in life also can be diagnostic, but false-positive test results have been reported. Whenever possible, isolation of virus should be per-

formed for definitive diagnosis. PCR performed on nasopharyngeal secretions ultimately may aid in establishing the diagnosis.[176,658]

The finding during pregnancy that a mother is immune to rubella is not sufficient to exclude the diagnosis because reinfection may have occurred or maternal infection may have developed before the screen was performed. If the diagnosis of congenital rubella is suspected clinically when the mother is thought to be immune, the infant should be screened with rubella-specific IgM ELISA and viral culture or PCR of appropriate clinical specimens. The diagnosis of congenital rubella infection can be eliminated if the mother is nonimmune when the infant manifests clinical signs of possible congenital infection.

The principal diseases to consider in the differential diagnosis include congenital CMV infection, congenital toxoplasmosis, erythroblastosis fetalis, disseminated herpes simplex, neonatal sepsis and congenital syphilis. Infants with congenital CMV infection more commonly have chorioretinitis and microcephaly, whereas congenital heart disease and eye malformations are unusual findings. Infants with symptomatic congenital toxoplasmosis have chorioretinitis, hydrocephaly, and cerebral calcifications but not congenital heart disease, cataracts, or glaucoma. In uncomplicated erythroblastosis, the direct bilirubin and liver function studies remain normal. Disseminated herpes simplex should be differentiated on the basis of the characteristic vesicular skin lesions or the presence of keratoconjunctivitis. Positive blood cultures identify neonatal sepsis. The bone lesions in congenital syphilis are associated with periosteal new bone formation; rhinitis and lesions of the skin and mucous membranes also are seen. Because of overlapping clinical features, however, definitive diagnosis requires laboratory confirmation.

TREATMENT

No specific antiviral therapy exists for congenital rubella. Although amantadine hydrochloride inhibits rubella virus in vitro, treatment with this drug was not shown to alter the clinical or virologic course of the disease.[504] Sensorineural hearing loss associated with congenital rubella syndrome now may be treated with cochlear implantation combined with speech language therapy, and vision loss associated with cataracts can be restored with ophthalmologic surgical procedures that remove the opaque lens and replace it.[317,518,651]

PROGNOSIS

The most common long-term sequelae of congenital rubella are listed in Table 73–8. Deafness is the most frequent finding and may not be apparent for several months to several years after birth. In 15 to 20 percent of children, it may be the only abnormality detectable.[154] Approximately one half of the children may have absent or hyporeactive responses to tests of vestibular function.[424] The hearing loss may be profound and, thus, a major contributor to speech impairment and learning disability. Cochlear implantation and speech language therapy, however, may restore hearing, improve receptive and expressive language abilities, and improve communication skills and school performance.[317] In approximately 50 percent of children with mental retardation, the deficiency is moderate to severe. Although retinopathy is present in a significant proportion of infants, it does not appear to interfere with vision. Cataracts, of course, certainly can interfere with the development of vision, and they should be removed surgically in the first few weeks of life. Most fatalities from congenital rubella occur in the first year of life and are associated with severe congenital heart disease and general debility from multiple defects.[154]

Long-term follow-up evaluation of children with congenital rubella syndrome is available.[136,185,186,414,569] Among 205 children examined at 8 to 9 years of age, 26 percent had severe mental retardation, 18 percent had reactive behavior disorder, 12 percent showed behavior disorder with neurologic damage, and 6 percent displayed autism. Of 29 children with neurologic manifestations of congenital rubella between birth and 18 months of age but with normal intelligence, 93 percent had hearing loss when examined at 9 to 12 years of age; 61 percent, poor balance; 54 percent, muscular weakness; 52 percent, learning deficits; 48 percent, behavioral disturbance; and 41 percent, deficits in tactile perception. Head circumference appears to correlate poorly with intellectual function in patients with congenital rubella.[398] Diabetes mellitus has been observed in 15 to 20 percent of adults with congenital rubella.[415] Onset usually occurs in the second or third decade of life. Other late manifestations[569] of congenital rubella syndrome include hyperIgM syndrome,[481] autoimmune disorders such as chronic lymphocytic thyroiditis,[706] thymic hypoplasia,[238] abnormal dermatoglyphics,[154] chromosomal abnormalities,[33] pancreatic insufficiency,[191] and progressive panencephalitis.[642,663] As one might expect, the severity of long-term sequelae appears to correlate with the number of defects observed in early life.[136,154]

TABLE 73–8 Frequency of Defects in Children with Congenital Rubella

Defect	Percentage of Cases
Deafness	67
Congenital heart disease	48
Psychomotor retardation	45
Retinopathy	39
Cataracts	29
Neonatal purpura	23
No defect	19
Deaths	16
Glaucoma	3

Data from 376 children studied in the New York Rubella Birth Defect Project. Modified from Cooper, L. Z., Ziring, P. R., Ockerse, A. B., et al.: Rubella: Clinical manifestations and management. Am. J. Dis. Child. 118:*18-29, 1969.*

PREVENTION

Active immunization with live, attenuated rubella virus vaccine is the most effective means of preventing congenital rubella syndrome.[539] It has led to the elimination of endemic rubella in the United States and progress toward elimination in Europe and other parts of the world.[117,377,503] The development and use of rubella vaccine in the United States clearly have reduced the frequency of congenital rubella.[54,285,473] Since 2001, fewer than 25 reported cases of congenital rubella syndrome have occurred each year, and genotype analysis showed all cases that have occurred originated in parts of the world other than the United States. However, outbreaks of rubella and congenital rubella syndrome have occurred in the United States, especially in South Texas near the Mexico border. In addition, Hispanic populations and foreign-born individuals from countries that do not have rubella vaccination programs appear to be the highest risk for rubella outbreaks.[377,705] The target population in the United States has been preschool- and school-aged children, whereas in Great Britain, selective vaccination of 11- to 14-year-olds and women immediately after delivery has been the goal. The strategy used in the United States is more effective in decreasing the incidence of congenital rubella syndrome. More recently, immunization programs in the United States have emphasized the need to vac-

cinate susceptible women of child-bearing age.[54,285,473] Moreover, routine prenatal screening for rubella immunity should be performed, and all nonimmune women should be vaccinated during the immediate postpartum period and before being discharged. Breast-feeding is not a contraindication to postpartum immunization. Although the vaccine virus may be transmitted in breast milk, the infection in the neonate is asymptomatic. Immunization with rubella vaccine, however, is contraindicated during pregnancy, and physicians recommend that a woman not conceive during the 3-month period after being immunized.[97,368] Data collected by the CDC from more than 500 pregnancies show that vaccine viruses can cross the placenta and infect the fetus but do not produce the defects associated with congenital rubella syndrome. The rate of isolation of vaccine virus from the products of conception is only 3 percent for the currently used RA 27/3 vaccine. Studies of fetal risk associated with inadvertent administration of rubella vaccine during pregnancy have confirmed rubella vaccine safety in pregnant women and their fetuses.[43] Thus, inadvertent administration of rubella vaccine during the first trimester of pregnancy is not an indication for termination of pregnancy.

Continuing concerns with regard to rubella include reinfection with wild rubella virus in vaccine-immune subjects,[293] failure of rubella herd immunity during an epidemic,[340] arthralgia and arthritis as side effects of the vaccine in children and particularly postpubertal women even though the risk of developing chronic arthropathy is not increased,[328,662] waning immunity that is more profound after immunization than after natural infection,[54,285,473] and, more recently, outbreaks of rubella among unvaccinated pregnant women in custodial institutions, immigration camps in border states, or selected communities, including Hispanic populations and groups of foreign-born individuals from countries with no rubella vaccine program.[16,114,412]

Although administration of gamma-globulin to women during pregnancy may reduce the frequency of symptomatic disease in the mother, it appears to have little effect on the frequency or severity of fetal and neonatal disease.[91,406] Infants with congenital rubella syndrome are considered to be contagious and are maintained with contact precautions until they are at least 1 year of age, unless repeated nasopharyngeal and urine cultures performed after they reach 3 months of age are negative for rubella virus. Health care workers are required to report all suspected and confirmed cases of congenital rubella to their local health department. Therefore, physicians who serve populations at high risk for rubella should be aware of the signs and symptoms of rubella and congenital rubella syndrome so that they may recognize the disease if it occurs in their patients.

MEASLES AND MUMPS

Measles during pregnancy may have severe complications, including maternal pneumonitis, hepatitis, and hemorrhagic sepsis syndrome. On rare instances, maternal measles during pregnancy may be fatal. The adverse fetal effects include spontaneous abortion or premature delivery, and neonates may be born with congenital measles syndrome.[39,136,204]

Mumps rarely if ever causes severe illness in pregnancy. However, mumps RNA has been detected in the myocardium of fetuses and neonates with myocarditis and endocardial fibroelastosis, providing molecular evidence for the long suspected role of mumps virus in these disease processes.[457]

HEPATITIS

Since the late 1970s, a veritable explosion of information concerning infection with hepatitis A virus (HAV), HBV, HCV, hepatitis D virus (HDV), and hepatitis E virus (HEV) has occurred.* HAV has been grown in cell cultures, and two purified viral glycoprotein vaccines have been licensed and recommended for use in high-risk individuals aged 2 years and older.[143,289,383,384] HBV and HCV are discussed later. HDV is a defective virus that infects only persons with acute or chronic HBV infection.[507] The major cause of enterically transmitted non-A, non-B (NANB) hepatitis, a problem confined largely to developing countries,[652] has been shown to be HEV, an RNA virus of the family *Calicivirus*.[355,634] Epidemic NANB hepatitis occurs more frequently during pregnancy and can result in fulminant hepatic failure in these women.

HAV and HDV rarely cause infection of the fetus and newborn.[614,704] Natal transmission of hepatitis A to the newborn can occur if the mother has jaundice or had acute hepatitis within the 2 weeks before and 1 week after delivery. Although most infants are asymptomatic, researchers have recommended that exposed infants receive 0.02 mL/kg of immunoglobulin intramuscularly as soon as possible after delivery. The infant should be maintained with contact precautions for 6 weeks, or for 1 week after the onset of symptoms. Preterm infants may excrete HAV antigen and RNA for several months after acquiring acute infection. Nosocomial transmission of hepatitis A within nurseries has been documented. A report from India indicated a relatively high rate of vertical transmission among eight women with third-trimester HEV infection[326]; more investigations are necessary to determine the true significance of maternal HEV infection for the fetus and newborn infant in the United States. The following section focuses on HBV and HCV.

MICROBIOLOGY AND EPIDEMIOLOGY

HBV, a double-stranded DNA virus with a DNA polymerase, is the prototype member of the family Hepadnaviridae.[296,346] Seroepidemiologic investigation of HBV infection has been enhanced by the identification and development of three major antigen systems, antibody systems, or both: surface antigen (HBsAg and anti-HBs), core antigen (total anti-HBc and IgM anti-HBc), and e antigen (HBeAg and anti-HBe).[296,346] Tests for HBsAg are used widely for establishing the diagnosis of acute or chronic infection with HBV. Anti-HBc is a marker of continuing viral replication in the liver, IgM anti-HBc is present during acute but not chronic HBV infection, and HBeAg is a more specific indicator of infectivity than HBsAg is.[120,288,296,346,572]

HBV accounts for 40 to 50 percent of all cases of hepatitis in the United States and 10 percent of those associated with blood transfusion.[120,572] Between 5 and 10 percent of patients infected with HBV become chronic carriers, and many of them have benign chronic persistent hepatitis or the more serious chronic active hepatitis.[296,346,396,572] A strong association has been noted, particularly in Asian males, between chronic carriage of HBsAg and death from cirrhosis or primary hepatocellular carcinoma.[24,62,409] The age at which HBV infection occurs has a significant effect on the occurrence of clinically overt hepatitis and the development of a chronic carrier state.[241] The vast majority of neonates who acquire HBV from their mothers have subclinical infection, but 60 to 95 percent of infected infants become chronic carriers, particularly when the mother is HBeAg-positive.[60,61,66,183,242,469,614,618,686]

In the United States, the frequency of HBsAg in the general population is 0.2 to 0.9 percent.[115] The highest prevalence of HBV antigenemia in the United States is in Asian immigrants/refugees (13%), Alaskan Natives/Pacific Islanders (5-15%), clients in institutions for the developmentally disabled (10-20%), users of illicit parenteral drugs (7%), sexually active homosexual

*See references 109, 111, 120, 164, 248, 291, 296, 346, 356, 361, 507, 572, 617, 649.

men (6%), household contacts of HBV chronic carriers (3-6%), and patients in hemodialysis units (3-10%).[115] Only 10 to 15 percent of persons reactive for HBsAg have a history of contracting hepatitis. In chronic carriers of HBsAg, antigen has been detected in saliva, feces, urine, wound exudates, vaginal secretions, breast milk, amniotic fluid, and semen.[296,346,572] The prevalence of anti-HBs in the general population is approximately 11 percent; this frequency increases with advancing age and is related inversely to socioeconomic status.[115]

Among children and adults, HBV is transmitted by the parenteral route (blood transfusion, needle-sticks), but infection by nonparenteral routes also occurs.[59,63,572,637] Hepatitis B is not transmitted by the fecal-oral route. In the United States, transmission by blood and blood products now is a rare event because of routine screening of blood donors and viral inactivation of certain blood products.

Clustering of HBV infections in families is known to occur; family members of a known antigen-positive index case have a 10-fold higher prevalence of HBsAg or anti-HBs than do control families of the same ethnic background.[627] Although this clustering within families initially was considered to have a genetic basis,[627] "vertical" transmission of HBV infection from the mother to the infant may be a source.[183,242,469] However, person-to-person spread clearly occurs, but the exact mechanism is not known.[59,637,704] It has been seen in household settings from child to child but not in daycare centers.

Moreover, transmission of HBV has occurred in children born to HBsAg-negative mothers who immigrated to the United States from countries where HBV infection is endemic.[59,637,704] The incubation period for the onset of HBV antigenemia after parenteral inoculation is 2 weeks to 2 months, depending on the dose of virus received. In nonparenteral exposure, the incubation period for antigenemia is 2 to 3 months. The onset of elevated liver enzymes and clinical symptoms follows the onset of antigenemia by 2 weeks to 2 months.[346]

In the fetus and neonate, transmission by the following routes has been suggested: transplacental, either during pregnancy or at the time of delivery secondary to placental leaks; natal, by exposure to HBsAg in amniotic fluid, cervical and vaginal secretions, or maternal blood; and postnatal, by contact with household members who are infected or are chronic carriers or, rarely, by transfusion of blood or blood products. Ninety-five percent of infections occur at the time of delivery, whereas only 5 percent occur in utero. The fetus or newborn infant, therefore, can be infected by hematogenous (transplacental, blood transfusion) or nonparenteral (contamination of the oropharynx or breaks in the skin) routes. Differences in the time of exposure (congenital, natal, postnatal) and in the route of viral inoculation (parenteral, nonparenteral) may account for the wide variation in time of onset of antigenemia in the neonate after birth. The usual age at onset of antigenemia in neonates born to chronic carrier mothers is 2 to 5 months,[31,183,200,381,470,576,615] which is consistent with exposure at the time of birth. Infections secondary to blood transfusion in the neonatal period also are associated with the onset of antigenemia at younger than 2 months of age.[201] Infants with an onset of antigenemia at younger than 2 months of age presumably were exposed to HBV in utero.[200,242,381,470,559,576] In general, infants of mothers with acute hepatitis near the time of birth have antigenemia at an earlier age (1-2 months), thus suggesting transplacental transmission of HBV.[242,559] Infants with an onset of antigenemia after they reach 6 months of age can be assumed to have had postnatal exposure,[559] but the exact source of virus in these infants is unclear.

In summary, evidence is good that infection with HBV can occur transplacentally, at the time of birth, and postnatally by blood transfusion or contamination of the oropharynx with infected secretions.[614] Several studies indicate that transmission by breast milk is a rare occurrence.[65,183,687] Further substantiation for the mother as the primary source of virus for the neonate comes from observations that the HBV serotype almost always is the same in infants and their carrier mothers.[242,470,615]

From 0.1 to 15.6 percent of pregnant women are asymptomatic chronic carriers of HBsAg. The lowest rates occur in the United States and in northern European populations,[183,200,588,687] and the highest rates occur in Chinese populations, regardless of geographic location.[31,66,185,381,615] Intermediate rates are observed in Japanese,[469,576] African,[183,687] South Asian,[183,687] and Mediterranean populations.[486] Rates of transmission of HBsAg from infected mothers to neonates vary highly and are influenced by the sensitivity of the HBsAg assay system used in the study, whether neonatal serum is examined for anti-HBs as well as for HBsAg, the frequency of bleeding and length of follow-up of infants, and the e antigen/antibody status of the mother. In general, HBsAg in a cord-blood specimen is not a reliable indicator of neonatal infection because of the possibility of contamination with antigen-positive maternal blood or vaginal secretions and because of the possibility of transient noninfectious antigenemia from the mother.[183,381,485,687] Demonstration of HBsAg in the serum of the infant during the first several months of life can be considered diagnostic of infection with HBV in the absence of immunization with hepatitis B vaccine. HBsAg may be detected for up to 1 week after the administration of a dose of vaccine. Most infants demonstrate antigenemia by the time they are 6 months of age, with peak acquisition occurring at 3 to 4 months of age.[31,242,381,576]

Factors known to be associated with higher rates of HBV transmission to neonates include the presence of HBeAg and the absence of anti-HBe in maternal serum—attack rates of 80 to 95 percent;[66,183,206,381,469,485,687] an Asian racial origin, particularly Chinese—attack rates of 40 to 70 percent;[31,183,381,576,615] maternal acute hepatitis in the third trimester of pregnancy or the immediate postpartum period—attack rates of 60 to 70 percent;[242,559,560] a higher titer of HBsAg in maternal serum—attack rates parallel the titer;[66,381,615] and the presence of antigenemia in older siblings.[31,469,615] Factors *not* related to transmission include the presence or absence of breast-feeding,[65,183,687] the particular HBV subtype in the mother,[242,470,615] the presence or absence of HBsAg in amniotic fluid,[381] and the presence or absence of anti-HBc in cord blood.[183,200,242] Data conflict regarding the significance of HBsAg in cord blood. Some studies have found no relationship between its presence or absence and subsequent infection of the neonate, whereas others have found a correlation. The reasons for this difference are not understood.[183,381,485,615,687]

From 75 to 100 percent of infants in whom HBsAg develops in serum within the first several months of life have persistent or chronic antigenemia.[31,183,242,469,470,576,615] In some infants, anti-HBs may develop after transient carriage of HBsAg, and in a few (usually those born to asymptomatic carrier mothers), anti-HBs may develop without the infant ever having detectable antigen.[485,576]

Transmission of HBV to infants in successive pregnancies of the same mother has been documented,[192,199] and such transmission has, on occasion, been associated with the development of fatal hepatitis.

PATHOGENESIS AND PATHOLOGY

HBV transmitted to the neonate by the hematogenous route probably seeds the liver directly, whereas nonparenteral exposure requires replication at the portal of entry before spread to the liver through the bloodstream occurs. Electron-microscopic studies of liver biopsy specimens from chronic carrier infants indicate that replication of HBV appears to occur in the nuclei of hepatocytes and that 50 to 100 percent of cells are involved.[197,559] Histopathologic examination demonstrates a diffuse hydropic appearance of the liver cells, with effacement of the normal cord

pattern and the creation of a "cobblestone appearance" in the liver lobule.[559] Only small foci of hepatocytolysis surrounded by macrophages and lymphocytes are present. Infants with symptomatic acute hepatitis have more widespread hepatic necrosis with surrounding inflammatory infiltrates, including giant cells.[199,319] In fulminant disease, massive necrosis with little or no inflammatory response is present.[199,319] In some infants with symptomatic hepatitis, the disease may progress to chronic persistent hepatitis, chronic active hepatitis, cirrhosis, or hepatocellular carcinoma.[184,201,319,399,636,647,690]

CLINICAL MANIFESTATIONS

Although clinically typical acute hepatitis may occur in mothers of infants in whom HBV infection develops, most are asymptomatic chronic carriers of HBsAg. Therefore, routine screening of serum for antigen in all pregnant women is necessary for the identification of infants at risk for development of HBV infection. Maternal HBV infection has not been associated with abortion, stillbirth, congenital malformations, or intrauterine growth retardation.[183,201,576] However, prematurity has been observed, particularly when the mother has acute hepatitis during pregnancy.[559]

A fetus or newborn infant exposed to HBV may follow one of several courses: (1) asymptomatic transient hepatitis B antigenemia, followed by the production of anti-Hbs[200,485]; (2) asymptomatic persistent antigenemia, variably associated with mild and fluctuating elevations in liver enzymes[31,200,242,576,599]; (3) symptomatic hepatitis with recovery and clearance of the antigen[201,222]; (4) symptomatic hepatitis that becomes chronically persistent or chronically active with continued presence of hepatitis B antigenemia[199,201,319,690]; (5) acute fulminant hepatitis with death[201,222]; and (6) asymptomatic neonatal/infant infection with progression to cirrhosis or liver cancer.[84,636,647] Most infants with HBsAg in their serum become asymptomatic chronic carriers. However, liver function studies may be abnormal in some of these infants, and liver biopsy specimens usually show evidence of mild, unresolved hepatitis.[183,201,242,559,576] The long-term significance of these persistent abnormalities in asymptomatic chronic carrier infants is unknown. The factors that determine which pattern of HBV infection occurs in a given newborn infant and the mechanisms responsible for chronic carriage of HBsAg in many infants are not understood.

The source of virus for 29 infants with symptomatic HBV infection in the first few months of life was blood transfusion in 15 (52%), a chronic carrier mother in 9 (31%), and unknown in 5 (17%).[201] The type of clinical disease in these infants was acute, self-limited hepatitis in 16 (55%), severe or fulminant hepatitis in 9 (31%), chronic persistent hepatitis in 2 (7%), chronic active hepatitis in one, and an asymptomatic chronic carrier state in 1. Most patients with fulminant hepatitis died.

DIAGNOSIS AND DIFFERENTIAL DIAGNOSIS

HBV infection in an infant generally is diagnosed by the demonstration of HBsAg in serum. If positive, infants also should be tested for HBeAg and anti-HBc IgM. In addition, PCR is available for detecting HBV DNA in blood. The major diseases to be considered in the differential diagnosis include biliary atresia and acute hepatitis caused by other viruses—HAV, CMV, rubella virus, enteroviruses, adenoviruses, and HSV.

TREATMENT

Treatment with human interferon has resulted in the termination or transient cessation of hepatitis B antigenemia in chronic hepatitis of adults,[249,291,497] but experience in neonates and children is limited.[320,370,453] Lamivudine also is licensed for the treatment of chronic infection in adults. Treatment of established disease in infants with hepatitis B immunoglobulin (HBIG) has not altered the course of illness.[199,222] Therefore, treatment of neonatal HBV infection primarily is supportive.

PROGNOSIS

The long-term outcome of asymptomatic infants with persistent antigenemia is guarded because chronic active and progressive hepatitis occur, as do death from cirrhosis, liver failure, and hepatocellular carcinoma. Death from fulminant HBV infection in neonates or young infants is a rare event but has occurred, particularly in successive children born to the same chronic carrier mother.[192,222]

PREVENTION

Remarkable advances have occurred in the prophylaxis of neonates born to mothers with HBV infection. Initial studies in infants born to HBeAg-positive, chronic carrier mothers demonstrated that administration of HBIG significantly reduced the development of a chronic carrier state.[61,64,192,309,347,523] The most successful regimens were those that began as soon as possible after birth and used multiple doses of HBIG. Anti-HBs developed in most infants who were protected against becoming chronic carriers, thus indicating that passive/active immunization had occurred. However, protection against persistent antigenemia was only 50 to 75 percent effective. Twenty to 30 percent of infants became infected during the second and third years after the protective effects of their passive immunoglobulin subsided.[61,64] This finding suggests that the risk of developing infection continues beyond the time of birth and that active immunization would be necessary to provide long-term protection.

Hepatitis B vaccine (Heptavax B; licensed but no longer produced in the United States) then was demonstrated to be both safe and effective in inducing protective levels of anti-HBs in 95 percent or more of infants receiving three doses of the vaccine.[53,382] Because passive/active immunization (HBIG plus three doses of HBV vaccine) in adults was shown to be safe and as effective in inducing long-term protective titers of anti-HBs as was a dosage of three doses of vaccine alone[702] this regimen was tried in infants. Several investigations have demonstrated 85 to 96 percent efficacy in preventing chronic antigenemia with the combined HBIG/vaccine regimen.[60,396,618,685] Persistent antibody against HBsAg developed in virtually all protected infants. Vaccine failures were thought to occur in babies who already were infected with HBV in utero and, hence, could not be protected by the HBIG/vaccine regimen initiated at birth. Subsequent studies using recombinant DNA vaccines (Recombivax HB, Engerix-B) showed them to be safe and highly immunogenic in neonates (>95%), with an efficacy of 90 to 95 percent.[508,616]

The concentration of HBsAg protein differs in the two vaccines; the pediatric formulation of Recombivax HB contains 10 μg/mL, whereas Engerix-B has 20 mg/mL. The two vaccines are interchangeable, and neither one contains thimerosal.

Because most chronic carrier mothers are asymptomatic, serologic screening during pregnancy would be required to identify neonates needing prophylaxis beginning at birth. Initial recommendations suggested screening during pregnancy in populations with high-risk factors: (1) Asian, Alaskan, or Pacific Island descent; (2) birth in Haiti, sub-Saharan Africa, Eastern Europe, the Middle East, the Caribbean, or Central or South America; (3) acute or chronic liver disease; (4) work or treatment in a dialysis unit; (5)

work or residence in an institution for the mentally retarded; (6) rejection as a blood donor; (7) blood transfusion on repeated occasions; (8) frequent occupational exposures to blood in medical or dental settings; (9) household contact with an HBV carrier or hemodialysis patient; (10) multiple episodes of sexually transmitted disease; or (11) percutaneous use of illicit drugs.[111,115,591] Because routine prenatal history to screen for these risk factors will miss one half to two thirds of asymptomatic chronic carrier mothers,[613,363] the current recommendation is to screen *all* pregnant women for HBsAg early in pregnancy.[35,36,112,119,617] Testing should be repeated late in pregnancy for women who are negative initially and at high risk for acquiring HBV infection or who have had clinical hepatitis since the screening was performed.[123]

Perinatal transmission is prevented in more than 90 percent of cases by the intramuscular administration of 0.5 mL of HBIG and hepatitis B vaccine to both term and preterm infants of HBsAg-positive mothers as soon as possible but within 12 hours of delivery.[115] HBIG efficacy decreases markedly if treatment is delayed beyond 48 hours. HBV vaccine is administered intramuscularly at a separate site at birth (preferably) or within 7 days, and in infants with a birth weight of 2 kg or more, administration is repeated at 1 to 2 months and 6 months after administration of the first dose. In infants with a birth weight less than 2 kg, the initial dose of hepatitis B vaccine does not count toward completion of the hepatitis B vaccine series, and administration of three additional doses of hepatitis B vaccine should commence when the infant is 1 month of age.[122] By the chronologic age of 1 month, all premature infants, regardless of initial birth weight or gestational age, are likely to respond as adequately as do older and larger infants.[329,365,397,494] Testing for HBsAg and anti-HBs is recommended at 1 to 3 months after completion of the vaccine series. The presence of anti-HBs along with the absence of HBsAg indicates successful prophylaxis and immunization. An HBsAg-positive result indicates failure of prophylaxis or in utero infection. Infants who are negative for anti-HBs and HBsAg should receive three additional doses of vaccine at a 0-, 1-, and 6-month schedule, followed by retesting for anti-HBs 1 month after the third dose. Alternatively, additional doses of vaccine (one to three) can be administered and the infant tested for anti-HBs 1 month after each dose has been administered to determine whether subsequent doses are necessary. Household members and sexual contacts of HBsAg-positive mothers should be screened, and if no evidence of previous HBV infection is noted, they also should be immunized.

Infants delivered by HBsAg-positive women are bathed as soon as possible after delivery to remove all maternal blood and secretions. Intramuscular injections should be delayed until bathing is completed; if such bathing is not possible, meticulous cleaning of the site with alcohol is necessary. These infants require standard precautions. Infants who have received both active and passive prophylaxis may be breast-fed.

In 1991, the Advisory Committee on Immunization Practices of the CDC[119] and, in 1992, the Committee on Infectious Diseases of the American Academy of Pediatrics[146] recommended the universal use of hepatitis B vaccine in all infants as the optimal strategy for prevention of HBV infection.[617] Current recommendations continue to support these opinions.[27] For infants with a birth weight of 2 kg or greater and born to mothers whose HBsAG status is negative, the first dose of HBV vaccine should be administered at or soon after birth, the second dose at 1 month or more after the first dose, and the third dose at 6 to 18 months of age. The minimal interval between the second and third doses is 2 months, and the third dose should not be given before 6 months. For infants who receive their first dose after they have reached 2 months of age, the minimal interval between the first and third doses is 4 months. An alternative schedule of the three doses administered at 2, 4, and 6 to 18 months of age concurrently with other routine vaccines may be used for HBsAg-negative infants not vaccinated at birth.

Seroconversion rates in premature infants who have a birth weight of less than 2 kg and are vaccinated shortly after birth are lower than those in larger preterm infants and full-term infants vaccinated at birth.[380] For this reason, for premature infants weighing less than 2 kg at birth and born to HBsAg-negative mothers, initiation of the vaccination series is delayed until they are 1 month of age.[122] The schedule for follow-up doses is the same as for other infants.

Infants with a birth weight of 2 kg or more and born to mothers whose HBsAg status is unknown should receive the first dose of vaccine within 12 hours of birth. Blood should be drawn from the mother at delivery to determine her HBsAg status. If it is positive, HBIG should be given as soon as possible, but no later than when the infant is 1 week of age. If the infant's birth weight is less than 2 kg, HBIG in addition to vaccine should be given within 12 hours of birth. This initial vaccine dose should not be counted as part of the three-dose schedule. The subsequent vaccine schedule is based on the mother's HBsAg status. If it remains unknown, the infant should be treated as though the mother were HBsAg-positive.

Because the rate of perinatal transmission is greatest in mothers who are HBeAg-positive or those who are negative for both HBeAg and anti-HBe, some researchers have suggested administering prophylaxis to neonates born only to this group of mothers.[534,577] However, the occurrence of several cases of hepatitis in babies born to mothers with anti-HBe[174,585] and the demonstration that measurements such as HBV DNA polymerase or HBV DNA by PCR may be more accurate indicators of infectivity than are measurements of HBe antigen or antibody[280,637] indicate that prophylaxis should be given to neonates of all chronic carrier mothers, regardless of their e antigen/antibody status.[27] Because the combined HBIG/vaccine regimen results in effective and long-term protection, breast-feeding is allowed and encouraged in chronic carrier mothers. These significant developments in prophylaxis against perinatal transmission of hepatitis B should reduce the frequency of chronic carriers strikingly and thereby decrease the number of cases of chronic active or progressive hepatitis and death from cirrhosis, liver failure, and hepatocellular carcinoma.

HEPATITIS C VIRUS INFECTION

The remarkable molecular biology effort to isolate and clone the gene of HCV[141] and to express a major nonstructural protein[368] led to the demonstration that HCV is the predominant cause of NANB hepatitis and a major contributor to chronic hepatitis in developed countries.[21-23,121,164,649] Rates of HCV seroprevalence are highest (60-90%) in those with repeated exposure to blood or blood products (e.g., injection drug users, hemophilia patients), intermediate (20%) in those with repeated or inapparent percutaneous exposure (e.g., hemodialysis patients), lower (1-10%) in those with high-risk sexual behavior or household contacts of infected persons, and lowest (0.5-1%) in blood donors.[22,164,649] Overall, an estimated 1.8 percent of the population, including pregnant women, in the United States is infected with HCV.[28]

Among studies of infants born to anti-HCV-positive women, an average of 5 percent (range, 0-25%) of infants were persistently positive for second-generation assay anti-HCV antibody or HCV RNA during a follow-up period of at least 10 months.* The risk of vertical transmission is correlated with higher concentrations of plasma HCV RNA[390,468]; with HIV-1 co-infection, especially in those with advanced stages of AIDS; with the specific genotype of HCV; and, possibly, with the vaginal route of deliv-

*See references 373, 390, 402, 464, 468, 524, 538, 633, 664, 665, 703.

ery. HCV RNA has been detected in the breast milk of infected women, but transmission by breast milk has not been documented, and HCV infection is not a contraindication to breast-feeding.[391]

HCV infection can be diagnosed by antibody titers and molecular methods to detect and quantitate viral RNA. Antigen-detection tests and IgM assays are not available. Antibody titers in infants are confounded by maternal transplacental antibody; this effect presumably is gone by the time that the infant reaches 18 months of age.[27] Antibody testing involves a screening EIA, with repeat positive results confirmed by a recombinant immunoblot assay (RIBA), analogous to what is done for the serologic diagnosis of HIV infection. The second-generation EIA (EIA-2) and RIBA are 95 percent sensitive and 97 percent specific.[254] Infants born to HCV antibody-positive mothers should be screened for anti-HCV with the EIA-2 after they are 18 months of age. RIBA may be used as a supplemental test for specificity; both the EIA-2 and RIBA measure IgG antibody to recombinant HCV antigens. Detection of HCV RNA in infant blood is also available commercially and should be performed when the infant is between 1 and 2 months of age.[29,254] Infants who are infected perinatally should be referred to a hepatologist and have annual screening of liver enzymes, even if they remain asymptomatic.

Controlled trials of interferon therapy in children with chronic hepatitis C are limited.[144] Long-term prognosis for HCV infection in infants and children is not clear, but 85 percent of adults become chronically infected, and HCV is known to be associated with cirrhosis and hepatocellular carcinoma.[22,164,649]

The development of a successful vaccine for hepatitis C must overcome several obstacles, including multiple genotypes of the virus, lack of cross-protective immunity among the genotypes, lack of long-term protection with the same genotype, and lack of successful cultivation of the virus in cell culture.[22,164] In February 1994, the Advisory Committee on Immunization Practices reviewed the available data and concluded that no support was found for the use of immunoglobulin for postexposure prophylaxis. This recommendation is still the current opinion of experts.[28] Immunoglobulin is not protective because blood from anti-HCV-positive donors is excluded from the pool used for preparation and no neutralizing antibody for HCV has been identified as yet. Routine screening of all pregnant women and their newborns for HCV infection cannot be recommended at this time, but screening should be considered in those with known risk factors. For example, infants who received 1 U or more of blood or blood products before 1992 or who received Gammagard/Polygam (Baxter Healthcare Corporation, Glendale, CA) in 1993 or 1994 were screened with liver transaminase tests and for anti-HCV antibody.

HERPES SIMPLEX

HSV, the etiologic agent of cold sores, keratitis, encephalitis, and genital ulcers in older children and adults, causes serious disease in neonates, with high mortality rates and severe neurologic sequelae.

MICROBIOLOGY AND EPIDEMIOLOGY

The two types of HSV, type 1 and type 2, may be distinguished by antigenic, biochemical, molecular, and biologic differences.[156,667] HSV-1 (the "oral strain") usually causes mouth lesions, eye infections, and endemic encephalitis, whereas HSV-2 (the "genital strain") usually is associated with sexually transmitted genital infections.[156,667] However, 8 to 50 percent of genital herpes disease is now caused by HSV-1.[476] Most neonatal disease is caused by HSV-2, but HSV-1 neonatal disease appears in recent studies to be increasing in certain populations.[98]

Antiviral collaborative studies indicate that 4 percent of neonatal herpes cases are acquired in utero, 86 percent natally, and 10 percent postnatally. Three quarters of natal/postnatal cases of neonatal herpes are caused by HSV-2, and the remainder are caused by HSV-1 strains.[480] Virtually all neonatal HSV-2 infections probably are acquired from mothers having an active genital herpes infection at the time of delivery.[477,623,675,695] The majority of neonates with HSV appear to acquire the infection by contact with infected genital secretions at or during delivery from an asymptomatic mother who acquired her first episode of genital herpes infection in the third trimester or near the time of delivery.[96,97,100,102] Because 60 to 80 percent of these mothers have no signs or symptoms of genital herpes at the time of labor and delivery and have a negative past history of genital herpes or sexual contact with a partner who had a genital vesicular rash, some experts recommend routine serologic screening of pregnant women and their partners to identify potential at-risk mothers.[96-98,404,668,674] In a study of 140 pregnant women with cytologically diagnosed genital herpes, only 36 percent had clinically recognized herpetic lesions.[450] Twenty-one percent had nonspecific abnormal findings, and 43 percent were asymptomatic. In addition, 20 to 40 percent of nonpregnant women from whose genital secretions HSV is isolated are asymptomatic.[306,680] Use of more sensitive techniques, such as PCR, detects genital HSV shedding in asymptomatic women even more frequently.[149]

Neonates with HSV-1 infection can acquire the virus from several sources: maternal genital, oral, or breast lesions[97,98,196,389,622,695]; oral herpes in the father or other family members[193,623,695]; or health care–associated transmission from other infected babies or health care workers.[262,393] Although HSV-1 cold sores and asymptomatic oral shedding may be common findings in nursery personnel,[275,276] transmission to a neonate from this source appears to be rare.[414,533,650]

Because most fetal and neonatal HSV infections are transmitted from the mother, a summary of several aspects of herpes infection during pregnancy is appropriate. Fulminant or disseminated primary HSV disease may occur in pregnancy, but whether it develops more often than in nonpregnant women is not clear. A review[495] of seven such cases revealed (1) infection during the third trimester in all; (2) four beginning as a genital HSV infection and three as oral disease; (3) hepatitis in six, encephalitis in two, and pancreatitis in two; (4) maternal death in three (43%)—two from hepatitis and one from encephalitis; and (5) three instances of fetal death—each secondary to severe maternal systemic illness rather than direct infection with HSV. Evidence of visceral disease in a pregnant woman with either genital or oral HSV infection is an indication for providing systemic antiviral therapy.[369]

An increased rate of spontaneous abortion occurs in women with primary genital herpes during early pregnancy, regardless of socioeconomic status.[100,271,274,450,480] Premature delivery does not occur more commonly in prospectively monitored women with recurrent genital herpes,[274,480,657,682] but most reports of neonatal herpes cases find a greater preponderance of premature infants than in the general population.[480,668,675,695] This finding, however, may reflect a higher susceptibility of premature infants to HSV infection secondary to a lack of transplacentally acquired IgG neutralizing antibodies rather than being a cause of the prematurity. Recurrent genital herpes in middle-class pregnant women is no more severe or frequent than in nonpregnant ones,[271,657,682] but older studies demonstrated more frequent and longer episodes in women of lower socioeconomic groups.[450,456] Between 74 and 88 percent of middle-class pregnant women with a history of genital herpes had at least one clinical recurrence during an observed pregnancy.[271,657,682] A mean of 2.7 to 3.0 episodes occurred during gestation, and HSV was isolated from lesions in 56 to 75 percent of the recurrences and in 0.6 to 12

percent of concomitant cervical cultures obtained during the recurrence. Asymptomatic shedding of HSV from the cervix, either between clinical recurrences or in a history-positive woman with no episodes during the observed pregnancy, was detected in 0.5 to 2.3 percent of the cultures obtained. Importantly, the presence of HSV shedding during the latter weeks of pregnancy was not predictive of shedding at the time of delivery.[37,682]

The number of cases of neonatal herpes occurring in the United States each year is far less than one would expect from the probable number of pregnancies complicated by genital herpes. Of the 3.5 million annual pregnancies, 7000 to 200,000 of these women have genital herpes at some time during the pregnancy and 3500 to 14,000 have positive HSV cultures at the time of delivery.[75,255,274,632,657,673,682] The actual number of cases of neonatal herpes per year in Seattle and Atlanta (0.1 and 0.3 per 1000 live births, respectively) would project to a national estimate of 350 to 1050 per year in the United States.[623] Because asymptomatic HSV infection of the neonate rarely occurs, subclinical cases or missed diagnoses probably do not account for the differences between the estimated number of maternal and neonatal infections. More likely, neonatal infection rates in the babies of mothers with genital herpes are far lower than currently assumed, possibly because of the preventive measures used. The estimated infection rates quoted most often currently are: (1) a 33 to 50 percent rate for infants vaginally delivered by mothers with primary genital herpes, (2) a 3 to 5 percent rate for infants vaginally delivered by mothers with recurrent lesions, and (3) less than 3 percent for those delivered by mothers with recurrent asymptomatic shedding at the time of delivery.[75,97,155,480,682] In the early 1970s, Nahmias and associates[450] reported the following neonatal infection rates in babies born to mothers with genital herpes during pregnancy: (1) 33 percent in mothers with primary disease after the 32nd week of gestation, (2) 3 percent in mothers with recurrent episodes after 32 weeks, and (3) 42 percent in mothers with virus-positive lesions at the time of delivery (primary versus recurrent episodes not specified). More recent studies have determined a significant risk of neonatal herpes occurring in babies born to mothers with asymptomatic shedding at the time of vaginal delivery: 33 percent with subclinical first episodes[97] and 0 to 3 percent with recurrent shedding.[97,106-108,481,511] Accurate knowledge of the actual neonatal infection risk is important for making decisions about management (e.g., cesarean section, prophylactic antiviral therapy). However, because researchers have estimated that as many as 75 percent of neonates with HSV disease are born to mothers with no history or clinical findings suggestive of active HSV infection during pregnancy, labor, or delivery, not all neonatal HSV disease is preventable by management decisions made on the basis of current knowledge of known risk factors.[26,101,106-108]

Several factors influence the risk of acquiring neonatal herpes and the severity of neonatal disease once the infection develops. The greater risk of transmitting neonatal infection by mothers with primary, as opposed to recurrent, vesicular or ulcerative lesions at the time of delivery is well established. Also, a woman with virus-positive recurrent lesions at the time of delivery is likely to be a greater risk than is a woman who is asymptomatic and shedding virus identified by HSV surveillance cultures. Other aspects of the anatomic site and the severity of maternal genital herpes also may be associated with a greater risk of development of neonatal disease: (1) cervical as opposed to vulvar or buttock skin involvement—resulting in more virus being shed into vaginal secretions; (2) multiple as opposed to single vesicles or ulcers—again, more virus from multiple lesions; (3) higher titer of virus in vaginal secretions—cultures positive sooner or more intensely positive in the diagnostic virology laboratory; and (4) longer duration of fetal exposure to infected vaginal secretions because of prolonged rupture of membranes, or fetal scalp monitors.[155,478,480]

Investigations by Yeager and associates[695,696] indicate that no anti-HSV antibody or a low titer of such antibody in maternal and neonatal serum is associated with a greater risk of acquisition of neonatal infection and more serious disease and that high-titer antibody is associated with a lower risk. Studies of HSV antibody titers in neonates enrolled in the CASG trial do not show an association between antibody titer and outcome of disease.[676] More investigations are required, therefore, to determine the potential protective role of maternal transplacental anti-HSV antibody.

Premature infants have accounted for 40 to 50 percent of cases of neonatal herpes,[668,676] in contrast to the usual prematurity rates of 6 to 18 percent reported in the general maternal population. Whether the increased frequency of prematurity among neonates with herpes indicates a greater propensity of mothers with genital herpes to deliver prematurely or a greater susceptibility of premature infants to HSV infection is not known. Premature infants appear more likely to have a fatal outcome.[108,668,669]

Instrumentation of the neonate, particularly scalp electrodes for fetal monitoring, is known to increase the risk of acquiring neonatal HSV infection.[205,321,487] Mortality rates appear to be higher in neonates with HSV-2 than in those with HSV-1 infection, probably because of the greater proportion of HSV-2–infected babies with dissemination.[669] In neonatal herpes survivors, neurologic damage occurs much more frequently in infants with HSV-2 than in those with HSV-1 infection.[157,669] Finally, the body sites of involvement in the neonate clearly influence outcome. Babies with disseminated infection (liver, lungs, adrenals), with or without CNS involvement, have the highest mortality rates (70-80%); those with encephalitis have only intermediate rates (30-40%); and those with infection limited to the skin, eye, or mouth have the lowest rates (0%).[676] Babies delivered in a high-risk situation might be given anticipatory antiviral chemotherapy after appropriate cultures have been obtained, whereas those in low-risk situations might be cultured and observed closely for evidence of neonatal herpes.[480,481]

PATHOGENESIS AND PATHOLOGY

As mentioned previously, HSV may be transmitted to the neonate in utero either by a transplacental (congenital infection) or ascending route, at the time of birth (natal infection), or after birth (postnatal infection). Congenital infection probably results from transplacental transmission of virus secondary to leukocyte-associated viremia in a mother with genital herpes, but no direct evidence supports this hypothesis. HSV-2 viremia has been documented in two women with primary genital herpes.[160] A report of 13 neonates with intrauterine HSV infection indicated that primary genital herpes was present during pregnancy in four mothers, recurrent disease was present in one, and no history of genital herpes was elicited in the remaining eight.[301] In the fetus, HSV appears to be transmitted directly from the placenta through the bloodstream to target organs. Because of the known tropism of HSV for the CNS, one is not surprised that most infants with congenital infection have evidence of brain involvement at birth.[301] Intrauterine infection also may occur by an ascending route from an infected maternal genital tract, and such transmission is supported by the development of clinical signs of HSV infection in the first 5 days of life.

The natal infection presumably is acquired secondary to aspiration of infected vaginal secretions into the nares, oropharyngeal cavity, conjunctivae, and upper respiratory tract of the infant. Other portals of entry for natal infection include the scalp, skin, and umbilical cord.[480,668] In postnatal HSV infection, no evidence supports the genital tract as a source of virus. Most postnatally acquired infections appear to result from contact with saliva from persons with oral herpes or with virus carried on the hands of

TABLE 73–9 Congenital Herpes Simplex Virus Infection: Features in 30 Cases*

Feature	Number	Percentage
Culture-positive vesicles or bullae	28/30	93
HSV-2	24/27	89
Low birth weight	22/26	85
Microcephaly, seizures, diffuse brain damage, intracranial calcification	20/30	67
Chorioretinitis, microphthalmos	17/30	57
Small for gestational age	9/25	36

Other features: retinal dysplasia, scars on skin or digits, cataracts, pneumonitis, hepatomegaly.
**Features present at birth or shortly thereafter.*
From Hutto, C., Arvin, A., Jacob, R., et al.: Intrauterine herpes simplex virus infections. J. Pediatr. 110:97-101, *1987.*

personnel.[389,668,695] After natal or postnatal acquisition of HSV occurs, initial replication of the virus occurs at the portal of entry, with subsequent viremic dissemination to viscera. Involvement of the CNS occurs either from hematogenous spread to the brain in infants with disseminated disease, which results in multiple areas of cortical hemorrhagic necrosis, or from retrograde axonal transport of the virus to the CNS from superficial replication in the skin, eye, or mouth.[324,451,668]

CLINICAL MANIFESTATIONS

The clinical features of genital herpes in women have been discussed in detail elsewhere.[99,155,256,449,476] Women with initial or primary infections may have multiple, small, painful, and tender vesicles and ulcers involving the cervix, vagina, vulva, and skin of the perineal region. In addition, patients may complain of inguinal or pelvic pain that is caused by associated lymphadenopathy. Systemic symptoms consisting of fever, malaise, and myalgia usually are present. The total duration of pain is 10 days to 2 weeks, whereas the total duration of lesions may be 2 to 3 weeks. Peak lesion virus titers, from 10^4 to 10^5 plaque-forming units per milliliter, occur during the first week of illness.[99] The total duration of viral shedding lasts from 10 to 14 days.[99,155] Lesions on dry skin progress through the well-defined vesicle, ulcer, crust, and healed stages described for herpes simplex labialis.[594] On moist mucous membranes, vesicles quickly rupture to form shallow ulcers that persist for days and gradually heal from the periphery.[99]

Recurrent genital herpes in women usually is milder and of shorter duration than is primary infection, the vesicles and ulcers are more circumscribed and fewer in number, and the disease appears to be limited largely to the external genitals.[99] Peak virus titers occur during the first few days and are lower, 10^3 to 10^4 plaque-forming units per milliliter. The total duration of pain is 5 to 9 days, duration of vesicles and ulcers is 8 to 11 days, and the duration of viral shedding is 5 to 8 days.[99,155] Women with both primary or initial and recurrent genital herpes may be asymptomatic.[97]

The clinical spectrum in infants with congenital HSV infection is different from that observed in babies with natal or postnatal disease. The most prominent features summarized from 30 cases of congenital infection reported in the literature are shown in Table 73–9.* A vesicular rash, bullae, or cutaneous scars present at birth or within a few days of birth were noted in almost all infants. In some cases, the extent and severity of these lesions resulted in an initial diagnosis of epidermolysis bullosa. At least one case report of limb hypoplasia, similar to congenital varicella syndrome, has been shown to be caused by HSV-2.[312] Two thirds of infants demonstrate extensive involvement of the CNS at birth, either clinically or from autopsy findings: diffuse brain damage, microcephaly, or intracranial calcifications. In some infants, eye findings, including chorioretinitis, microphthalmos, retinal scars and retinal dysplasia, and cataracts, have been noted.[401]

As indicated in Figure 73–3, infants with natal or postnatal herpes commonly have a clinical picture resembling that of bacterial sepsis: alterations in temperature, lethargy, respiratory distress, anorexia or vomiting, and cyanosis.[106-108,266,674,675] The three general patterns of infection are categorized by the extent of disease: (1) disseminated infection with or without CNS involvement, present in 25 percent of cases; (2) infection localized to the CNS in 30 percent of cases; and (3) infection localized to the skin, eye, or mouth in 45 percent of cases.[106-108,334]

The frequency of disseminated disease has decreased after a high of 51 percent reported in the years 1973 to 1981; this decrease probably represents earlier diagnosis and treatment of localized infection before dissemination occurs.[672] The disseminated form of infection in the earlier years of recognition presented in infants between 9 and 11 days of age and usually involved the liver, where it caused fulminant hepatitis with disseminated intravascular coagulation, and the adrenal glands, and it most closely resembled the picture of bacterial sepsis.[279,640] Current reports show disseminated neonatal HSV may present as early as the first week of life, without cutaneous or localizing signs, similar to bacterial sepsis, and confirm that HSV remains an important cause of neonatal morbidity and mortality.[106-108,644] A disseminated infection can affect multiple organs, including the brain, larynx, trachea, lungs, esophagus, stomach, lower gastrointestinal tract, spleen, kidneys, pancreas, and heart. Pneumonia occurs in 37 percent of infants. Markedly elevated transaminase levels, direct hyperbilirubinemia, coagulopathy, and thrombocytopenia are common findings. Approximately 60 to 75 percent of infants with disseminated disease have hematogenously acquired CNS involvement, as manifested by irritability, a bulging fontanelle, localized or generalized seizures, flaccid or spastic paralysis, opisthotonos, decerebrate rigidity, or coma. Importantly, 39 percent of infants with disseminated disease do not have skin vesicles at initial evaluation, and vesicles do not develop during the acute HSV disease; only 56 percent initially have fever, and many are hypothermic on presentation.[106,107,334]

CNS disease from retrograde axonal transmission of virus presents later than does disseminated disease, usually when the infant is 11 to 17 days old but occasionally when the infant is as old as 4 to 6 weeks of age. Typically, these infants have focal seizures that subsequently generalize. Examination of CSF demonstrates mononuclear pleocytosis with a predominance of lymphocytes, a moderately low glucose concentration, and elevated protein levels. A normal cell count, glucose, and protein concentration, however, may be found on the initial lumbar puncture, if performed early in the disease process.[106,107,664] An elevated red blood cell count secondary to hemorrhagic brain involvement may be present but by itself is an unreliable sign. Fever is present at initial evaluation in only 44 percent of infants with HSV encephalitis, and 32 percent of infants do not have skin vesicles either initially or during the course of their illness.

Localized skin, eye, or mouth disease usually presents within the infant's first 7 to 10 days of life. Approximately 83 percent of these neonates have skin lesions: usually single vesicles, occasionally vesicle clusters, and rarely a zoster-like rash.[442] As many as 46 to 80 percent of neonates with skin lesions have 1 to 12 recurrences during the first 6 to 12 months of life.[295,337,669] Approximately 13 to 25 percent of infants have eye involvement; disease is limited to only the eye in one third of these cases. Common

*See references 195, 227, 290, 301, 348, 378, 427, 434, 571, 580, 592, 621.

manifestations include conjunctivitis, keratitis, and chorioretinitis.[258,448] As many as 83 percent of infants are afebrile initially.[106,107] One third of all infants have evidence of herpetic mouth lesions, but involvement of this site alone rarely occurs.

DIAGNOSIS AND DIFFERENTIAL DIAGNOSIS

Up to 80 percent of infants have classic features that would suggest herpes simplex, such as skin vesicles, mouth ulcers, or keratoconjunctivitis. One half of the remaining 15 to 20 percent have either (1) focal or diffuse encephalitis or (2) a sepsis syndrome with pneumonitis, hepatitis, encephalitis, and frequently, disseminated intravascular coagulopathy.[106,107,480] A clinical picture of encephalitis certainly should suggest HSV infection; the combination of pneumonitis, hepatitis, and encephalitis seldom is seen in bacterial sepsis/meningitis and should alert the clinician that a neonatal viral infection is more likely. As many as 32 percent of all HSV-infected neonates do not have vesicular skin lesions that would prompt clinical suspicion and a more rapid diagnosis. Kimberlin and colleagues[334] with the CASG compared the clinical characteristics of neonates with HSV disease in two multicenter studies that they conducted, one from 1981 to 1988 and the other from 1989 to 1997. They found that no progress has been made in decreasing the time interval between the onset of HSV symptoms and the initiation of antiviral therapy; the mean time between the earliest manifestation of an HSV symptom and initiation of acyclovir therapy was between 5 and 7 days. Recent studies have shown neonatal HSV disease presents more commonly than previously appreciated and appears similar in incidence to bacterial meningitis.[106-108] Although acyclovir should not be part of routine antibiotic therapy for all neonates with presumed sepsis, intravenous acyclovir should be provided promptly, similar to the paradigm of bacterial sepsis, pending results of viral studies, if the diagnosis of neonatal HSV is in the differential diagnosis.

The most definitive means of establishing a diagnosis of HSV infection in a neonate is isolation or identification of the virus in clinical or autopsy specimens. Samples for isolation of virus in cell culture should be obtained prior to initiating antiviral therapy and may be obtained from the skin vesicles, nares, throat, nasopharynx, conjunctivae, stool, urine, peripheral blood buffy coat, CSF, brain tissue, and liver biopsy and autopsy material.[187] HSV has been isolated from CSF in up to 25 to 40 percent of cases of encephalitis. HSV also has been isolated from duodenal aspirates of infants with hepatitis. Ideally, specimens should be processed immediately or frozen at –70°C if testing cannot be performed until later. However, HSV is stable for 2 to 3 days at 4°C in commercial viral transport media or media containing trypticase soy broth or brain-heart infusion broth.[694] In cell culture, the typical HSV cytopathic effect often is evident within 1 to 2 days after inoculation. Studies using centrifugation of the specimen onto a cell monolayer at the bottom of a shell vial, followed by staining for HSV antigen the next day, yielded 99 percent sensitivity and 100 percent specificity.[479] Typing of HSV should be performed because recent evidence suggests that neurologic outcomes may be worse with neonatal infection caused by HSV-2 than with infection caused by HSV-1. Moreover, typing might help establish the mode of transmission and provide information useful for future counseling regarding preventive measures.

Direct immunofluorescence assays (DFAs) or immunoperoxidase techniques are available to demonstrate HSV-1 or HSV-2 antigens in cells scraped from the base of vesicles or the conjunctivae or in biopsy or autopsy material.[321,554] Scraping from the base of a vesicle reveals intranuclear inclusions and multinucleated giant cells by the Tzanck test or Wright stain in 60 to 70 percent of cases. Detection of specific HSV antigen from skin and mucocutaneous ulcers by immunofluorescence is preferred and can be accomplished in 70 to 80 percent of cases. Direct detection of HSV antigen by commercial EIA has variable reliability compared with that of traditional cell culture and DFA.[653]

The diagnosis of HSV encephalitis has been improved greatly by the development of PCR for detection of HSV DNA in CSF.[332,333,372,644] PCR has supplanted the need for biopsy testing of brain tissue for HSV diagnostic purposes. It has a sensitivity of 48 to 98 percent and a specificity of 94 to 100 percent. HSV DNA can be detected by PCR in most culture-positive CSF even after 1 week of acyclovir therapy. HSV DNA detection by PCR on infant serum and whole blood is a useful method to document HSV viremia and disseminated HSV infection.[106-108,333,610] Other tests that aid in the detection of CNS abnormalities and help establish CNS involvement with HSV include electroencephalography, enhanced brain CT, and MRI. The characteristic electroencephalographic abnormality is a periodic slow and sharp wave discharge; more commonly, multiple independent foci of periodic activity are present. CT scanning may be normal early in the course of the disease, with characteristic abnormalities appearing 3 to 5 days later. The findings most frequently observed in the acute phase are (1) patchy areas of low attenuation with brain edema in both cerebral hemispheres or (2) hemorrhage or calcification in the thalamus, insular cortex, and periventricular white matter and along the corticomedullary junction. Late findings include multicystic encephalomalacia and ventriculomegaly as a result of brain atrophy and destruction. MRI is sensitive in detecting early abnormalities in the periventricular white matter and in defining the extent of parenchymal lesions.

In most circumstances, serologic assays are not useful for establishing the diagnosis of maternal or neonatal herpes during the acute phase of the disease.[187] Antibody assays may be used for documenting initial or primary genital herpes infection in the mother, as well as for determining whether the mother has a past history of HSV-2 infection. New serologic assays are available that reliably detect serum HSV-2 IgG antibodies.[123] The persistent lack of HSV-2 antibody on repeated samples taken over time in a mother whose infant has clinical evidence of possible HSV infection would render HSV-2 unlikely. However, the possibility of the infection being caused by HSV-1 remains. Immunofluorescent techniques to quantify HSV-specific IgM antibodies have been developed, but these antibodies usually are not detected in serum for 2 or more weeks after onset of the infection in neonates.[447]

The major diseases to be considered in the differential diagnosis of neonatal HSV include bacterial sepsis and meningitis, adenovirus and enterovirus infection, and, to a lesser extent, congenital infection with CMV, rubella virus, and VZV. Inquiry about illness in the mother and other epidemiologic features may be helpful. For example, mothers of neonates with HSV infection may (but often do not) have a history of having had genital lesions or sexual contact with someone who has genital herpes. Infants with sepsis usually have associated factors known to predispose to bacterial infection, such as maternal peripartum infection, premature rupture of membranes, and procedures performed in the intensive care unit or nursery. Enterovirus infection of infants tends to occur in the summer and fall and is associated with signs and symptoms of enterovirus disease in the mother. Adenoviruses, however, occur year-round and may have slight increase in frequency during winter/spring months. Varicella in the neonate usually presents with a history of maternal varicella near the time of delivery or close contact with a caretaker with varicella.

TREATMENT

Studies conducted by the CASG of the National Institute of Allergy and Infectious Diseases (NIAID) have shown that antivi-

TABLE 73–10 Neonatal Herpes: Antiviral Therapy

Outcome	Total (%)	Skin, Eyes, Mouth (%)	Encephalitis (%)	Disseminated (%)
Mortality				
ACV, Ara-A*	17	0	14	54
Historical controls†	49	7	37	76
Developmental Normal				
ACV, Ara-A*	67	94	36	59
Historical controls†	26	73	19	54

**Data from Whitley, R., Arvin, A., Prober, C., et al.: A controlled trial comparing vidarabine with acyclovir in neonatal herpes simplex virus infection. N. Engl. J. Med. 324:444-449, 1991.*

†Data from Nahmias, A. J., Keyserling, H. L, and Kerrick, G. M.: Herpes simplex. In *Remington, J. S., and Klein, J. O. (eds.): Infectious Diseases of the Fetus and Newborn Infant. 2nd ed. Phidelphia, W. B. Saunders, 1983, pp. 636-678.*

ACV, acyclovir; Ara-A, vidarabine (adenine arabinoside).

ral therapy for neonatal HSV disease significantly improves mortality rates and long-term outcomes of infected neonates. Vidarabine was the first commercially available drug for the treatment of neonatal HSV disease.[139] Placebo-controlled trials demonstrated that adenine arabinoside (vidarabine, ara-A) at doses of 15 or 30 mg/kg/day given as a 12-hour infusion significantly reduced the mortality rate from 62 percent in historic cases and placebo controls to 35 percent in treated cases and increased the percentage of normal survivors from 19 to 43 percent.[676] Results were best in infants with disseminated infection and disease localized to the CNS, with the mortality rate being reduced from 70 to 40 percent and the percentage of normal survivors increased from 10 to 30 percent. No difference was found between the 15-mg and the 30-mg dose in side effects, but the higher dose appeared to inhibit more effectively the progression of disease from skin-eye-mouth involvement to disseminated or CNS disease.[674] None of the 49 treated patients had significant adverse clinical reactions or laboratory abnormalities attributable to the drug. On the negative side, 21 percent of infants progressed to more serious disease while receiving therapy, and serious neurologic sequelae developed in 27 percent of the total. Fifteen percent of babies with disseminated or CNS disease continued to excrete HSV in the throat after 10 days of therapy.

The results of a vidarabine-acyclovir comparison trial in the treatment of neonatal herpes did not show any difference in efficacy and safety between the two drugs.[668] When the results with vidarabine and vidarabine-acyclovir were combined, the mortality rate was reduced from 49 percent in untreated historical controls to 17 percent in treated patients (Table 73–10). Normal survivors increased from 26 to 67 percent. Although the results in the total group of patients were encouraging, the outcome in the group of patients with disseminated disease was not: a mortality rate of 54 percent in treated patients versus 76 percent in untreated historical controls and normal development in 59 percent of treated survivors versus 54 percent of controls (see Table 73–10). Factors that predicted mortality and their relative risk were as follows: disseminated disease, 33; CNS disease, 5.8; semicoma or coma, 5.2; disseminated intravascular coagulation, 3.8; prematurity, 3.7; and pneumonitis, 3.6.[669] Factors significantly associated with neurologic sequelae in survivors and their relative risk in these studies were as follows: skin-eye-mouth disease with three or more recurrent skin lesions after completion of acute therapy, 21; skin-eye-mouth disease caused by HSV-2, 14; HSV-2 infection, regardless of disease category, 4.9; CNS disease, 4.4; and seizures, 3.0. In infants with HSV disease limited to the skin, eye, or mouth, neonates with HSV-1 infection were all normal developmentally at 1 year of age as compared with 86 percent of those with HSV-2 infection. Among the latter infants, those with impaired neurologic outcome were significantly more likely to have had three or more skin recurrences in the first 6 months of life.

Because of greater ease of administration, intravenous acyclovir has supplanted vidarabine as the drug of choice for the treatment of neonatal HSV infection. Vidarabine no longer is available commercially. Because mortality and morbidity in neonates with HSV infection remained high using standard (30 mg/kg/day) dosing of acyclovir for 10 days, the CASG evaluated intermediate-dose (45 mg/kg/day) and high-dose (60 mg/kg/day) intravenous acyclovir for 21 days in 79 neonates with neonatal HSV infection.[335] Data were compared with those of a previous CASG study in which all infants received standard doses of acyclovir (30 mg/kg/day) for 10 days. Overall, after stratification for disease category, the survival rate for patients treated with high-dose acyclovir was significantly higher than that for patients treated with the standard dose. Specifically, in neonates who received high-dose acyclovir, those with disseminated disease but not encephalitis had a significantly higher survival rate. The mortality rate at 24 months for patients with disseminated disease who received 21 days of high-dose acyclovir was 31 percent, compared with 57 percent for patients who received an intermediate dose for 21 days and 61 percent for those who received the standard dose for 10 days in the earlier CASG trial. For encephalitis, the mortality rate of infants at 24 months was 6 percent with high-dose acyclovir, 20 percent with the intermediate dose, and 19 percent with the standard dose. In addition, recipients of high-dose acyclovir had less morbidity than did historical control infants who received the standard dose of acyclovir. Patients treated with high-dose acyclovir were 6.6 times as likely to have normal development at 12 months of age. Among infants with encephalitis, however, 31 percent of high-dose acyclovir recipients were developing normally at 12 months as compared with 29 percent of patients treated with the standard dose. With respect to toxicity in the high-dose acyclovir recipients, 21 percent had a transient neutropenia that resolved either during continuation of high-dose acyclovir or after its cessation. Nephrotoxicity occurred in four (6%) infants who had disseminated disease. Because acyclovir is eliminated by the kidneys, the dose should be adjusted for renal disease.[216] High-dose acyclovir did not impede the development of an adequate antibody response to HSV in infected infants.

These studies have led to the current recommendation for the use of intravenous acyclovir at a dose of 60 mg/kg/day for 21 days to treat neonatal CNS and disseminated HSV disease and for 14 days for skin-eye-mouth disease.[26,331] In addition, infants with ocular involvement should receive a topical ophthalmic drug (1-2% trifluridine, 1% idoxuridine, or 3% vidarabine). Consultation with an ophthalmologist is also recommended to assist in the management of ocular HSV in the neonate. All patients with HSV CNS infection should have a repeat lumbar puncture performed at the completion of acyclovir therapy to determine

resolution of abnormal CSF parameters and to document whether the CSF specimen is HSV culture and PCR-negative. A positive CSF HSV PCR at this time is an indication for continuing intravenous acyclovir therapy and for performing repeat neuroimaging studies to evaluate the extent of CNS damage. Repeat samples of blood and CSF should be obtained weekly until HSV studies are negative and provide a guideline for duration of antiviral therapy.

Concern has been raised that some infants may have progressive neurologic injury after acquiring neonatal herpes encephalitis.[257] In addition, infants who have three or more skin recurrences with HSV-2 in the first 6 months of life have been shown to be at increased risk for having neurodevelopmental abnormalities at follow-up, whether as a consequence of previously undetected CNS infection or because of CNS dissemination during skin reactivation.[669] Suppression of HSV skin reactivation by the administration of acyclovir (300 mg/m^2 per dose given orally three times daily) for 6 months is recommended by some experts for selected patients. The infant's blood counts should be monitored while receiving oral acyclovir long term because Kimberlin and coworkers[336] documented suppressive therapy resulted in neutropenia in 50 percent of treated infants and emergence of an acyclovir-resistant HSV isolate in one infant. Acyclovir suppression should be considered after the initial recurrence of skin lesions in the first 2 to 3 months of age or after a recurrent episode of HSV encephalitis. Acyclovir suppression also has been used after development of HSV eye infection in which reactivation might imperil vision.

General supportive measures, such as maintenance of fluid and electrolyte balance, correction of hypoglycemia, management of disseminated intravascular coagulation and shock, control of seizures with anticonvulsants, mechanical support of the respiratory system, nutritional support, and antimicrobial therapy for complicating bacterial infections, also are critical in improving the outcome for neonates with HSV infection.

PROGNOSIS

As indicated in Table 73–10, the overall mortality rate from untreated neonatal HSV infection is 49 percent, and only 26 percent of survivors develop normally. The most guarded prognosis is natal infection that is disseminated, with pneumonitis, or presents as encephalitis localized to the CNS. Between 50 and 60 percent of infants with HSV infection limited to the skin, eye, and mouth who do not receive antiviral therapy progress to having CNS or disseminated disease. As shown in Table 73–10, neurologic sequelae develop in an appreciable proportion of survivors. All infants with neonatal herpes, therefore, require antiviral chemotherapy, regardless of the type or severity of disease at initial evaluation.

The major sequelae in infants surviving neonatal herpes involve the CNS. Diffuse brain damage, seizures, microcephaly, spasticity, paralysis, growth retardation, and chorioretinitis with visual loss all have been observed.[676] Serial CT or MRI of the head may be useful in these infants and can provide prognostic information.[674] Physical therapy and developmental interventions will help optimize the infant's abilities. Most infants with skin involvement have recurrent lesions, which can be managed with oral suppression therapy.

PREVENTION

The major approaches to the prevention of neonatal herpes involve interruption of transmission from sites of HSV infection and disease at the time of delivery and postnatally from a variety of potential sources. Because in utero transplacental congenital infection occurs so infrequently[668] and predicting the occurrence of congenital infection during pregnancy is not possible,[301] no standard recommendations for prophylaxis are available.

Cesarean delivery within 4 hours of rupture of fetal membranes in a mother with active genital herpes at the time of delivery is recommended to reduce the risk of natal infection. Because serial vaginal cultures taken during the latter stages of pregnancy have failed to predict shedding at the time of delivery,[37,71,682] such monitoring is not recommended. Cesarean delivery is recommended only for women with active vesicular or ulcerative lesions at the time of delivery.[45,244,274,485,511,561] Cesarean delivery is not always 100 percent protective, even if the membranes are intact.[274,480,511,561] Between 1.2 and 3 percent and, in one study, up to 33 percent of neonates with HSV infection are delivered by cesarean delivery.[102,673] The cost-effectiveness of performing a cesarean delivery in women with recurrent lesions at delivery has been questioned by some experts, but it remains the current recommendation in the United States.[519] Multiple clinical trials have shown that administration of prophylactic acyclovir and valacyclovir beginning at 36 weeks gestation reduces the risk of clinically apparent HSV genital vesicles and ulcers at delivery, reduces the risk of HSV viral shedding at delivery, and also reduces the incidence of cesarean delivery.[286,562,573,574,673] However, although the hope is that the risk of HSV disease in the neonate also would be reduced, in none of these studies was sufficient power or evidence available to determine the effect of peripartum prophylaxis on the incidence of neonatal HSV. No adverse effects have been seen in infants exposed to maternal acyclovir during pregnancy. Because fetal scalp electrode monitor, forceps, and maneuvers that might cause a break in the infant's skin during delivery appear to increase the risk for acquisition of neonatal HSV, they should be used only with clear indication and a careful assessment of the risk-to-benefit ratio.

The results of antenatal genital HSV cultures from pregnant women with a history of genital herpes do not predict the infant's risk of exposure to HSV at delivery. Because 60 to 80 percent of mothers of babies with neonatal herpes are asymptomatic or have unrecognized infection, the optimal approach may be to perform a screening test to identify women shedding or likely to shed HSV at the time of delivery. Although HSV rapid antigen-detection tests continue to be developed commercially,[479] their sensitivity in *asymptomatic* women still is only 60 to 75 percent. Screening of pregnant women for non–type-specific HSV antibody would be confounded by the presence of HSV-1 antibody, which is present in 60 to 80 percent of the population.[476] The presence of HSV-1 antibody probably represents oral herpes, which poses little risk to the neonate. Although type-specific antibody assays are available to identify pregnant women infected with HSV-2,[123,313] such an approach would miss women with genital HSV-1 infection[155,511] or those with asymptomatic primary HSV-2 infection near the time of delivery before antibody is detectable.[97,511] Its use has been recommended in identifying discordant couples, that is, women with no history of having genital HSV infection but whose sexual partner has had previous infection, so barrier precautions may be used before delivery to decrease the chance of development of a maternal primary infection that poses a greater risk to her infant.[123] Culturing the genital tract of women at the time of vaginal delivery would identify infants exposed to HSV and allow anticipatory management.[478,485,511] However, because genital HSV infection rates are only 0.1 to 0.4 percent,[97,450,513] this approach likely would not be practical or cost-effective for *all* women. Culturing of *selected* women at delivery could focus on (1) positive or partner-discordant HSV-2 serologic tests, (2) those women with a history of genital herpes in themselves or their sexual partners, (3) those women with a history of another sexually transmitted disease or multiple sexual partners, and (4) mothers with a history of maternal fever in labor. However, routine or selective serologic

screening, although recommended by some experts, is not currently a standard recommendation, and further investigation is required to determine whether screening of pregnant women to prevent neonatal herpes is beneficial, practical, and cost-effective.[26,106-108,169]

No studies have been conducted on the optimal management of asymptomatic infants exposed to maternal HSV infection at delivery, although some guidelines on obtaining viral cultures and prophylactic or anticipatory antiviral therapy are available.[26,478-480,511,561] In general, appropriate cultures for HSV should be performed for all infants born to mothers with active genital HSV infection at delivery. In asymptomatic infants, cultures of the conjunctiva, throat, rectum, and possibly urine should be done at 24 to 36 hours of age. Cultures obtained at birth may indicate only contaminating virus, whereas those obtained 1 to 2 days after birth probably represent HSV newly replicating in the mucous membranes. If the infant is delivered by cesarean section within 4 hours of rupture of membranes, no prophylactic antiviral therapy is indicated. If the infant is born vaginally or by cesarean section performed more than 4 hours after rupture of membranes, further management is dependent on the presence of other risk factors. If the mother has primary infection at delivery, a situation in which the neonatal infection rate may be as high as 30 to 50 percent, intravenous acyclovir (60 mg/kg/day) should be initiated. If the mother has recurrent infection, in which the risk of neonatal disease occurring after vaginal delivery is probably less than 3 percent, acyclovir may be withheld pending the results of culture or the development of clinical illness in the neonate that could be caused by HSV. Situations that increase the risk of neonatal transmission despite maternal recurrent disease include prematurity, the use of a scalp electrode monitor, or skin lacerations; prophylactic acyclovir should be considered in these instances if the infant has been delivered vaginally or by cesarean section more than 4 hours after rupture of membranes. In all instances, acyclovir can be discontinued when HSV cultures are negative at 48 to 72 hours and the infant has remained well. All infants should be monitored closely for any clinical evidence of HSV infection. Any positive culture or the occurrence of signs or symptoms suggesting HSV infection in the neonate suggests that a full virologic evaluation, including blood and CSF analysis for HSV by PCR, should be performed and antiviral therapy initiated. Antiviral therapy also should be administered to neonates with positive HSV cultures of mucosal sites, and these neonates should not be assessed as being merely colonized, as all require intravenous acyclovir therapy.[26] The duration of therapy, however, probably can be shortened to 7 or 10 days if no other site of infection is found and the infant remains asymptomatic. Breast-feeding is contraindicated only if the mother has a vesicular lesion on her breast. Physicians also have recommended that circumcision be delayed for 1 month in infants at highest risk of transmission.

Prevention of postnatally acquired neonatal infection needs to take into account the potential sources of HSV: (1) maternal oral herpes or breast lesions; (2) household member, such as father or sibling, or other close contact, such as grandparent or caretaker, with oral herpes or herpetic gingivostomatitis; and (3) health care–associated transmission from other infected babies or health care workers.[193,262,393,622,623,695] Transmission to the neonate from family members with oral herpes lesions can be interrupted by education, avoidance of kissing and nuzzling of the neonate if they have oral herpes ulcers, and avoidance of touching the neonate if they have a herpetic skin lesion or whitlow, as well as common sense personal hygiene, including handwashing. Health care–associated transmission can be reduced by contact isolation and hospital infection control measures, as well as universal precautions and good handwashing techniques.[327,339] Personnel with herpetic whitlow should not have direct patient care responsibilities until the lesion has healed. Infants with HSV infection should be placed in contact isolation for the duration of the illness.[26] The median duration of viral shedding from skin vesicles and mucosal sites in infants receiving acyclovir is approximately 5 to 8 days.[335] Asymptomatic but high-risk infants born to mothers with herpes at delivery also should be in contact isolation; alternatively, they may room-in with the mother in a private room.

ENTEROVIRUS AND PARECHOVIRUS

The enteroviruses and parechoviruses are small RNA viruses that are members of the virus family Picornaviridae. The enteroviruses currently include the polioviruses types 1, 2, and 3; the coxsackieviruses A and B; and the echoviruses. Currently, there are four known parechoviruses: PeV1 (formerly enterovirus 22), PeV2 (formerly enterovirus 23), PeV3, and PeV4.[654] Enteroviruses are common infections in neonates, children, and adults. Coxsackievirus and echovirus infections are fairly common events in young infants and, as with other viral agents, often result in more severe disease in neonates. Neonatal poliomyelitis, however, is a rare occurrence in the United States and is not covered in this section. All four known parechoviruses have caused disease in neonates.[76,654]

MICROBIOLOGY AND EPIDEMIOLOGY

Twenty-three types of type A coxsackieviruses, six types of type B coxsackieviruses, 30 types of echoviruses, three types of polioviruses, five types of other enteroviruses (68 to 72), and four types or parechoviruses are currently recognized.[76,413,653] Most strains grow in tissue culture, but some, particularly type A coxsackieviruses, require inoculation in suckling mice. Because no common or group antigens for the enteroviruses exist, separate antibody titration must be performed for each virus. Therefore, to make a laboratory diagnosis of enteroviral infection by serologic means alone is not recommended. However, antibody titers can be performed after a specific enterovirus has been isolated from the patient or when a concurrent epidemic with a known virus occurs in the community. Detection of enterovirus and parechovirus RNA by reverse transcriptase (RT)-PCR is now a common method of establishing a diagnosis.[158]

The attack rate for enteroviral infections is highest during infancy and early childhood.[109,165-167,359,403,429,436,684] In addition, severe disease occurs much more commonly in neonates than in older children or adults.[429,684] Sixty to 70 percent of neonates infected with enterovirus are males, whereas one study has shown females may predominate in parechovirus infections.[318,371,427,436,517,654] Enteroviruses are worldwide in distribution, and in temperate climates, infections occur predominantly during the summer and fall months, with peaks in July, August, and September.[109,307,413] Parechovirus infection also occurs during summer and fall months.[654] The incubation period for enteroviral infections in children and adults usually is 5 to 8 days, with a range of 2 to 12 days.[543,660] Of the cases reported to the CDC from 1970 to 1979, echoviruses accounted for 57 percent; type B coxsackieviruses, 25 percent; polioviruses, 9 percent; type A coxsackieviruses, 8 percent; and enterovirus types 68 to 71, 0.1 percent.[109] In infants younger than 2 months of age, echoviruses accounted for 51 percent of infections; type B coxsackieviruses, 45 percent; and type A coxsackieviruses, 4 percent.[394,436] In any given year in the United States, usually an epidemic occurs that is caused by a few enterovirus types: echovirus 11 and coxsackieviruses B2 and B4 in 1979, echoviruses 9 and 4 in 1978, echovirus 6 in 1977, coxsackievirus B4 in 1976, and echovirus 9 in 1975.[109]

Outbreaks of enteroviral disease have occurred in normal nurseries and in neonatal intensive care units.*

Acute enteroviral and parechoviral infections may be acquired congenitally, natally, or postnatally.[135,536] However, confusion concerning the incubation period and source of virus for neonatal infection often has ensued. Late-gestation congenital infection is presumed to occur in infants with an onset of illness at birth or within the first few days of life, when their mothers had symptoms of enteroviral disease just before or immediately after delivery.[5,107,133,172,371,429,499] Further evidence of congenital infection is the observation that viremia with echoviruses and coxsackieviruses is known to occur in pregnant women,[135] and virus has been isolated from the placenta of infants with an onset of disease early in life.[89,429] Infants with an onset of enteroviral disease between 3 and 8 to 10 days of age probably acquired the infection from their mothers at the time of birth (natal infection), whereas those with illness appearing after this time probably acquired it postnatally. At least one case of congenital parechovirus infection has been documented in a neonate who presented at 1 day of age.[654] The source of enterovirus for most cases in nurseries appears to be the mother because the age of the infants at the time that the first symptoms appear is usually less than 10 days.[5,107,133,172,371,429,499] However, in some cases and outbreaks, the infection clearly appears to be associated with health care, presumably through spread of virus from other infected neonates by the hands of personnel or from infected personnel themselves.[161,407,429]

Some investigations indicate that enteroviral infections acquired in the community are a common cause of hospitalization for a febrile illness in young infants.[107,165-167,307,359] Enteroviral infections were estimated to account for 20,000 to 40,000 hospitalizations per year in young infants in the United States. Viruses appear more frequently than do bacteria as a cause of fever in the neonate.[107] One study of 182 infants younger than 3 months of age and hospitalized for fever over the course of a year showed viral pathogens were isolated in 41 percent and bacteria in only 15 percent.[359] Enteroviruses accounted for 85 percent of the viral isolates. Among a cohort of 586 newborns prospectively monitored during the enteroviral season in Rochester, New York, 24 (4%) were hospitalized during the first month of life.[307] Two thirds of these 24 were infected with enteroviruses. Risk factors associated with development of infection and more severe enteroviral disease included a particular serotype of virus (e.g., echovirus 22, presumably a more virulent strain); lower socioeconomic status, probably with associated crowding and an increased rate of transmission; bottle feeding (absence of passive antibody in breast milk); and absence of antibody in cord serum.[166,307,429,432]

Evidence suggests that coxsackieviral infections in early pregnancy may cause congenital malformations in the fetus. Brown and colleagues[94,95] demonstrated a significant association between serologic evidence of coxsackievirus A9, B2, B3, or B4 infection in mothers during pregnancy and the birth of infants with anomalies of the cardiovascular, urogenital, and digestive systems. However, specific viral isolation and antibody studies were not performed on the involved infants, and no seasonal distribution was noted in their births. In addition, the observations of Brown and colleagues[94,95] have not been confirmed by others.[207,353,502] The association between maternal infection with coxsackieviruses and congenital malformations, therefore, remains suggestive but not proven. Moreover, no conclusive evidence supports any relationship between maternal infection with type B coxsackieviruses and congenital CNS malformations.[207] More studies are necessary to determine whether or not parechoviral infections are associated with congenital malformations.

*See references 42, 89, 132, 161, 221, 379, 403, 429, 473, 536, 626.

PATHOGENESIS AND PATHOLOGY

Our knowledge of the pathogenesis of congenital enteroviral and parechoviral infections is incomplete because they are rare events. However, studies in gravid mice support the transplacental passage of enteroviruses with resultant fetal infection.[2,311,365,484] By inference from these studies and our understanding of congenital CMV and rubella virus infections, transplacental transmission secondary to maternal viremia probably occurs. Virus then is transmitted through fetal blood to target organs, principally the CNS, liver, heart, lungs, kidneys, and adrenal glands. In congenital infections that are fatal in the early neonatal period, type B coxsackieviruses involve primarily the heart, CNS, liver, and lungs,[239,318,371,684] and echoviruses involve the liver, adrenals, kidneys, CNS, and lungs.[133,299,259,360,428,429,499] Histopathologic findings have consisted of focal myocardial necrosis with type B coxsackieviruses[239,684]; massive hepatic necrosis with echoviruses[299,360,429,499]; and evidence of disseminated intravascular coagulation and adrenal, pulmonary, and renal hemorrhage with both virus groups.[133,239,299,360,371,429,499,684,684] Of interest is that the onset of illness in most neonates with fatal coxsackievirus B and echovirus infection occurred at birth or within the first few days of life, suggesting that transplacental infections carry a worse prognosis.[5,133,299,360,371,429,499] In addition, lack of transplacental transfer of maternal antibody before delivery could play a role.[426,427] Only a few isolated cases of congenital coxsackievirus A infection have been reported.[135]

Natal infection is secondary to aspiration and swallowing of enterovirus-contaminated vaginal secretions at the time of birth. Postnatal acquisition is the result of fecal-oropharyngeal spread of the virus on the hands of the mother, other family members, or hospital personnel.[135,429] Pathogenesis of natal and postnatal infection is similar to that observed in older infants and children. Because most infants with postnatal infection survive, the pathologic features are not well characterized.

CLINICAL MANIFESTATIONS

The clinical features in 134 neonates and very young infants with echoviral and coxsackieviral infections are shown in Figure 73–3. The most common findings, hyperthermia or hypothermia, anorexia or vomiting, and lethargy, are relatively nonspecific findings and are encountered with similar frequency in neonates with other viral infections or bacterial sepsis. Features that appear to be characteristic of enteroviral infection include signs and symptoms of aseptic meningitis and meningoencephalitis (irritability, CNS signs, including seizures); gastroenteritis (anorexia, vomiting, diarrhea, abdominal distention); and an erythematous, maculopapular exanthem. In addition, neonates may manifest a mild, nonspecific febrile illness, a severe sepsis-like disease, respiratory illness, hepatitis that may be fulminant with resultant hepatic necrosis and death, and cardiovascular manifestations secondary to myocarditis. Enteroviral DNA also has been detected by PCR in the respiratory tract of infants with sudden death.[245]

Infants with more serious disease may have a biphasic course that begins as a mild illness with slight elevation of temperature, coryza, anorexia, and diarrhea. After an apparent recovery period lasting 1 to 5 days, infants may have more severe symptoms of aseptic meningitis; myocarditis (tachycardia, tachypnea, respiratory distress, and cyanosis); hepatitis with elevated transaminases; or disseminated infection (abdominal distention, hepatomegaly, petechial rash, disseminated intravascular coagulation).

Milder forms of disease, such as pneumonitis, undifferentiated febrile illness, exanthematous illness, or gastroenteritis lasting only a few days, also occur.[303,371] In a survey of 338 infants younger than 2 months of age with enteroviral infections reported to the

TABLE 73–11 Characteristics Associated with Parechovirus and Enteroviral Infection in Neonates

Seasonal occurrence—summer and fall
Presence of known epidemic in the community
History of maternal viral illness near the time of delivery
Absence of factors predisposing to bacterial sepsis
Nursery outbreak of infectious illness with negative bacterial cultures
Development of culture-negative sepsis or aseptic meningitis in the neonate
Development of myocarditis, hepatitis, or erythematous maculopapular exanthem in the neonate

CDC, 74 percent had severe disease, and 26 percent had mild disease.[436] Five (1%) of these infants died, all in the group with severe disease. Asymptomatic infections in neonates have been detected during outbreaks in nurseries or when routine virologic surveillance was being performed in a nursery.[89] An outbreak of neonatal herpangina caused by coxsackievirus A5 was observed in Thailand.[132] Parechoviruses in neonates also may cause both mild and serious illnesses that cannot be differentiated clinically from enteroviruses and that include fever, seizures, rash, irritability, apnea, tachypnea, diarrhea, meningoencephalitis, hepatitis, and acute myocarditis.[653]

DIAGNOSIS AND DIFFERENTIAL DIAGNOSIS

The major epidemiologic and clinical features that suggest a diagnosis of enteroviral or parechoviral infection in a neonate are listed in Table 73–11. Because the signs and symptoms in neonates with enteroviral and parechoviral disease often mimic those of bacterial sepsis and meningitis, antibiotic therapy generally should be initiated until a bacterial etiology is ruled out. In addition to sepsis and meningitis, the major diseases to be considered in the differential diagnosis include HSV infection, adenovirus, and, to a lesser extent, congenital infection with CMV and rubella virus. HSV infection is nonseasonal, endemic, and may be associated with genital ulcerative lesions in the mother. Symptomatic congenital CMV and rubella infections are associated with intrauterine growth retardation and a chronic rather than an acute infectious clinical picture in the infant.

The most direct means of establishing a diagnosis of enteroviral infection in neonates is isolation of the virus in tissue culture or suckling mice.[479] Virus may be recovered from throat swab, stool, CSF, serum or buffy coat, and biopsy and autopsy tissue. Ideally, specimens should be processed immediately, but enteroviruses may remain stable at 4° C for several days if testing cannot be performed until later. Isolation of virus from CSF, blood, or tissue can be considered diagnostic. A throat, urine, or stool isolate also can be considered etiologic in most neonatal illnesses. RT-PCR performed for detection of enterovirus or parechovirus RNA in CSF or blood samples is more sensitive and rapid than is isolation of virus[526] and is the preferred method for diagnosing meningitis or disseminated infection caused by these viruses in most circumstances.[547,552] Because of genetic differences, parechoviruses cannot be identified by primers used in enterovirus RT-PCR assays. Therefore, RT-PCR for both types of these viruses should be performed for optimal diagnostic sensitivity.[158,654]

TREATMENT

No licensed and effective antiviral chemotherapy exists for enteroviruses. Pleconaril administered orally on a compassionate basis to neonates with severe disseminated enteroviral disease has resulted in clinical recovery.[34,322,537] In adults, pleconaril has decreased the duration of clinical symptomatology in aseptic meningitis significantly and has resulted in cessation of viral shedding in immunocompromised individuals. Case reports and series on administration of pleconaril to neonates with enterovirus infection are published.[34,525,531] Clinical trials evaluating the benefits of pleconaril in neonatal enteroviral sepsis syndrome with liver or cardiac involvement are in progress through the NIAID CASG. Intravenous immunoglobulin often is recommended, especially if myocarditis is present, because it may improve survival.[4,168,261,314]

PROGNOSIS

The outcome is influenced by several factors: the enterovirus serotype causing the infection, the route of transmission of virus to the neonate, the age at acquisition of the infection, prematurity, and severity of the disease in the neonate. Mortality rates are highest for coxsackievirus B infections, intermediate for echovirus infections, and lowest for coxsackievirus A infections.[135,239,318,371] Most of the neonatal deaths caused by enteroviruses reported in recent years have occurred in infants whose onset of disease was at or within a few days of birth and who, therefore, had congenital disease.[133,299,365,371] Infection and mortality rates both appear to be higher for premature infants.[89,429] Death is most likely to occur in infants with acute myocarditis, encephalitis, or severe hepatitis.[135,429] Neurologic sequelae have been observed to occur after meningoencephalitis in the neonatal period,[135,565,677] although most infants who survive enteroviral meningitis appear to have an excellent long-term prognosis.[70] Long-term cardiac sequelae also do not appear to complicate most neonatal enteroviral myocarditis.[239] Long-term follow-up studies of neonates infected with parechovirus have not been performed.

PREVENTION

Polio vaccine has reduced maternal and neonatal poliomyelitis to an extremely rare occurrence, but poliovirus infections still occur in underdeveloped countries without polio vaccination programs. No prospects exist for vaccines for the echoviruses or coxsackieviruses or parechoviruses at this time. With the appearance of a case of enteroviral or parechoviral disease in a newborn nursery or intensive care unit, initiation of vigorous infection control measures involving contact precautions, including such measures for suspected cases and renewed emphasis on handwashing and exclusion of personnel with symptoms of enteroviral disease, is indicated. Of emphasis is that infection control measures applied in the nursery do not prevent congenital or natal transmission of virus from the mother to the infant. The continued appearance of cases in neonates younger than 8 to 10 days of age may reflect a persistent epidemic among pregnant women rather than a failure of nursery infection control measures and spread within the nursery.[626]

VARICELLA-ZOSTER VIRUS

VZV causes both varicella ("chickenpox"), the result of primary infection with the virus, and zoster, caused by reactivation of latent virus.[240,243,476] VZV infection of a pregnant woman results in three separate and distinct syndromes that become apparent in neonates and infants: (1) congenital varicella syndrome with congenital defects secondary to intrauterine VZV infection, (2) neonatal varicella, and (3) zoster in infants.[212,214,240,243,493] Transmission to the fetus occurs as a consequence of maternal viremia

and may result in congenital varicella syndrome, fetal hydrops, or fetal death.[240,243,273] Transmission to the neonate occurs through exposure to virus-infected secretions or lesions present in the mother or close contact and may result in neonatal varicella.

MATERNAL VARICELLA

Knowledge of several features of chickenpox in the mother is necessary to understand the pathogenesis of VZV infection in the fetus and neonate. Because antibody to VZV is present in approximately 90 percent of women of child-bearing age,[243] varicella occurring during pregnancy is a relatively uncommon event. Pregnant women with varicella may develop severe disease, with severe constitutional symptoms, and potentially fatal pneumonia.[272] Maternal chickenpox has been reported to occur in only 0.7 per 1000 pregnancies.[570] The incubation period for chickenpox (from exposure to the onset of rash) usually is between 14 and 16 days, with a range of 10 to 21 days.[240,243]

CONGENITAL VARICELLA SYNDROME

In several large prospective studies, no increase in anomalies was apparent in the offspring of women who had varicella during pregnancy.[87,581-583] However, a prospective study[410] of 43 pregnancies complicated by varicella indicated that (1) 21 percent of the women experienced appreciable morbidity, (2) 24 percent of 33 infants tested had clinical or immunologic evidence of intrauterine VZV infection, and (3) congenital varicella syndrome occurred in 9 percent of 11 women with first-trimester varicella. Pooling of results from several prospective studies indicates an approximate 2 percent risk of fetal malformations caused by maternal varicella during the first 20 weeks of pregnancy.[214,419,493] Of the reported cases of congenital varicella syndrome, 93 percent have occurred after maternal chickenpox and only 7 percent after maternal zoster. Seven percent of reported cases occurred after maternal varicella was acquired at less than the 20th week, with the latest being at 28 weeks of gestation.[19] In another recent prospective multicenter study of 347 pregnant women with varicella, the incidence of congenital varicella syndrome was 0.4 percent, with fetal death and fetal hydrops also observed.[273] One case of congenital varicella syndrome occurring after maternal varicella developed in the third trimester of pregnancy has been reported.[350]

The abnormalities present in 77 infants with congenital varicella syndrome are summarized in Table 73–12. The defects apparently are the result of VZV replication in and destruction of developing fetal ectodermal tissue: skin, peripheral nerves, cervical and lumbosacral spinal cord, brain, and eye.[41,44,233,463,482,545] Diagnosis of the syndrome usually is clinical: a history of varicella in the mother and recognition of the characteristic findings in the fetus or neonate. Prenatal diagnosis may be established by amniocentesis with demonstration of VZV in amniotic fluid by culture or PCR and by cordocentesis and demonstration of VZV-specific IgM in a fetal blood sample. Prenatal ultrasound and fetal MRI imaging may detect abnormalities and document the extent of involvement.[417,655] Virus has not been isolated from these infants, and serologic studies often have been inconclusive.[232,243] However, detection of varicella-zoster DNA by PCR has been used to confirm the syndrome in some cases.[438,461,514,544] Infants with varicella embryopathy do not require isolation. Severely affected neonates may die in infancy.[232,243] However, some children may survive and enjoy productive lives.[556] Children with congenital varicella syndrome also may experience apparent reactivation disease, involving skin and, rarely, brain or other organs.[85,545] Universal vaccination against varicella may decrease the importance of this virus in congenital and neonatal disease.

TABLE 73–12 Abnormalities in 77 Infants with Congenital Varicella Syndrome

Features	Percentage
Skin scars*	61
Eye abnormalities	56
Chorioretinitis	27
Horner syndrome/anisocoria	16
Microphthalmos	19
Cataract	19
Nystagmus	13
Abnormal limb†	47
Hypoplasia	36
Equinovarus	14
Abnormal/absent digits	10
Cortical atrophy/mental retardation	40
Prematurity, low birth weight	36
Early death	26
Dysphagia/aspiration	19
Gastrointestinal tract abnormalities	12
Urinary tract abnormalities	10

**Cicatricial in 37 (79%).*
†Eleven of 28 (39%) with a hypoplastic limb had mental retardation or early death.
Data from Gershon, A. A.: Chickenpox, measles and mumps. In *Remington, J. S., and Klein, J. O. (eds.): Infectious Diseases of the Fetus and Newborn Infant. Philadelphia, W. B. Saunders, 2001, p. 698.*

TABLE 73–13 Congenital Varicella Syndrome: Outcome in Relation to Rash Onset in Mother and Neonate

		Neonatal Deaths	
Day of Rash Onset	Neonatal Cases	Number	Percentage
Mother, antepartum			
5 or more	23	0	0
0 to 4	13	4	31
Neonate, after delivery			
0 to 4	22	0	0
5 to 10	19	4	21

Modified from Gershon, A. A.: Chickenpox, measles and mumps. In *Remington, J. S., and Klein, J. O. (eds.): Infectious Diseases of the Fetus and Newborn Infant. Philadelphia, W. B. Saunders, 1990, pp. 395-445, as modified from Meyers, J. D.: Congenital varicella in term infants: Risk reconsidered. J. Infect. Dis. 729:215-217, 1974, with permission from the University of Chicago.*

NEONATAL VARICELLA

Neonatal varicella refers to the onset of varicella ("chickenpox") within the first 28 days of life.[546] The incubation period, defined as the interval between the onset of rash in the mother and onset in the fetus or neonate, usually is 9 to 15 days, with a range between 1 and 16 days. Neonatal varicella occurs when a pregnant woman experiences varicella during the last 1 to 2 weeks of pregnancy or within the first few days postpartum. The attack rate is approximately 25 to 50 percent. As indicated in Table 73–13, the timing of the onset of disease in the mother and the neonate is a critical factor influencing the outcome in the infant. If disease onset occurs in the mother 5 or more days before delivery or in the neonate during the first 4 days of life, the infection is mild. In contrast, if disease onset in the mother is within 4 days before delivery or in the neonate between 5 and 10 days of age, the infection usually is disseminated and fulminant, and approximately one third of the infants die.[243,423] Other investigations have found that the risk period of the onset of maternal rash extends from 7 days before to 16 days after delivery.[28,512] The generally accepted explanation for this observation is that when

illness in the mother occurs more than 5 days before delivery or when illness in the baby occurs during the first 4 days of life, maternal antibody has time to pass transplacentally and provide passive protection for the infant. However, passive protection does not have time to occur when illness in the mother occurs within 4 days of delivery. Presumably, the immune responses of the neonate are insufficient to retard the growth and dissemination of VZV after intravenous inoculation via the placenta, and disseminated disease results.

The milder form of neonatal varicella resembles the disease in normal, older children, whereas the disseminated variety is similar to that seen in immunosuppressed, leukemic children. In the latter, diffuse pneumonia, severe hepatitis, and meningoencephalitis are the most common clinical manifestations. The diagnosis usually can be established clinically from the characteristic appearance of skin vesicles, and VZV antigen can be demonstrated by immunofluorescence testing of vesicular fluid. Less commonly, VZV may be isolated from vesicular fluid by tissue culture during the first 3 days of the rash, but VZV is difficult to isolate in commercial virology laboratories.[479] VZV DNA can be demonstrated in vesicular fluid by PCR.[300] The major disease to consider in the differential diagnosis is disseminated neonatal HSV infection. With HSV infection, a history of maternal genital herpes may be elicited; characteristic keratoconjunctivitis, mouth lesions, or both may be present in the infant, and growth of the virus in cell culture is markedly different.[476,479] Immunofluorescence testing of vesicular lesions with commercially available specific monoclonal antibodies will rapidly differentiate the two.

Varicella-zoster immunoglobulin (Vari-ZIG) should be administered as soon as possible after birth to a neonate born to a mother with an onset of chickenpox rash between 5 days before delivery and 2 days after delivery.[512] If Vari-ZIG is not available, some experts recommend IGIV, intravenous acyclovir, or both in combination, as an effective alternative.[297] Breakthrough varicella has occurred in neonates properly treated with Var-ZIG, however.[46] Therefore, these infants still must be considered potentially infective and isolated with airborne precautions in a negative-pressure room for 16 days after maternal onset of rash because the incubation period with in utero exposure is decreased. If the newborn is exposed to maternal varicella at delivery, airborne precautions are extended until 21 days after exposure (28 days when Vari-ZIG is given) if hospitalization is required. Exposed infants do not need to be separated from their mothers; on the contrary, rooming-in may be a preferable alternative. If breakthrough neonatal varicella occurs despite Vari-ZIG immunoprophylaxis and the neonatal varicella appears to be becoming severe (extensive skin lesions, high fever and toxicity, hepatitis, pneumonitis), treatment with intravenous acyclovir at 1500 mg/m^2/day or 45 mg/kg/day divided every 8 hours should be administered.[575]

Infants born to mothers with zoster do not require Vari-ZIG immunoprophylaxis because they already have high levels of transplacentally acquired maternal VZV IgG antibody. No special precautions are indicated except that the maternal lesions should be covered and good handwashing when handling the infant must be stressed.

ZOSTER IN INFANCY AND CHILDHOOD

Epidemiologic and serologic evidence indicates zoster is a reactivated infection with VZV.[240,476] Persons with zoster, therefore, have had a previous episode of chickenpox. The vast majority of zoster occurs in older adult patients. The occurrence of zoster in infants and children is somewhat of a paradox because many of these patients have a negative history of having had chickenpox.[103,243,408] However, with several of these cases of childhood zoster, particularly those occurring in infants and young children, the mother had a history of chickenpox during pregnancy.[103,214,240,390] The presumption is that fetal VZV infection occurred, with recovery and no evidence of disease in the neonate at birth. In a few other instances, an infant born to a VZV-immune mother may be exposed to chickenpox or zoster during early life at a time when maternal transplacental antibody still would be present. This passive antibody could provide protection against chickenpox and modify the disease to a subclinical or mild form that was not recognized.[224] Both situations could result in a patient with (1) an unrecognized episode of varicella and (2) the occurrence of zoster as the first overt manifestation of VZV infection.

The major disease in the differential diagnosis of neonatal varicella is HSV infection. Patients with neonatal herpes may have a clinical picture resembling that of zoster,[442] and laboratory evaluation is required to differentiate the two diseases.

HUMAN PARVOVIRUS B19 (*ERYTHROVIRUS*)

Human parvovirus B19 *(Erythrovirus)* is a small, single-stranded DNA virus with two viral capsid proteins, VP1 and VP2.[175] It is the cause of erythema infectiosum, or fifth disease, a mild exanthematous illness in school-aged children.[32] Parvovirus B19 infection in pregnant women is associated with spontaneous abortion, stillbirth, and nonimmune hydrops fetalis secondary to transplacental passage of the virus.[30,338,342,345,578,638,648,661,683] A summary of 22 published case reports of parvovirus fetal infection revealed that (1) only half the mothers had parvovirus-like clinical illness 4 to 13 weeks before fetal death; (2) all fetuses had hydrops fetalis and probable myocarditis; and (3) 19 died in utero at 16 to 26 weeks, and the remaining 3 died within 24 hours of delivery.[26] Laboratory evidence of parvovirus infection includes (1) maternal parvovirus IgM antibody, (2) elevated maternal alpha-fetoprotein, and (3) the presence of parvovirus DNA in amniotic fluid.[32,638,639] Maternal blood also may contain high titers of parvovirus DNA that is detectable by PCR assays. Persistent parvovirus B19 DNA-emia often occurs, and supplementary measurement of VP1 IgG avidity and VP2 IgG epitope-type specificity may be helpful in timing the infection in some cases.[214]

Hydrops fetalis occurs from replication of parvovirus in the fetal bone marrow and resultant profound fetal anemia, with myocarditis playing a potential secondary role.[32,174] Fortunately, not all pregnancies complicated by parvovirus infection result in fetal infection, and hydrops does not develop in all infected fetuses. A British study of 186 pregnant women who had parvovirus IgM antibody and were monitored to term demonstrated that fetal loss occurred in 16 percent (no data on fetal loss rates in a control population) and that 43 percent of 14 fetal tissues tested showed parvovirus DNA.[112,514] Extrapolation from these figures yielded a maximal parvovirus-associated adverse fetal outcome of 7 percent in pregnancies with laboratory-proven parvovirus infection. Extrapolation to the community level yielded the following estimates of fetal death in a pregnant woman exposed to active parvovirus infection: 1.8 percent with home exposure and 1.1 percent or less with school exposure.[112,514] Figures used in this estimate are 7 percent fetal death rate with documented maternal infection cited earlier. Furthermore, only approximately 50 percent of women of child-bearing age are seronegative and, therefore, susceptible, and infection rates after exposure to parvovirus infection are 50 percent in the home and 30 percent with a *widespread* school outbreak. More recent studies have indicated a total fetal infection rate of 25 to 50 percent, but an adverse fetal outcome rate of less than 1 to 2 percent.[247,343,-345,648] Overall, after an acute parvovirus B19 infection during pregnancy, the vertical transmission rate is

approximately 30 to 50 percent, the risk of fetal death is between 2 and 6 percent, and most infected newborns are asymptomatic. In addition, maternal parvovirus B19 infection during pregnancy has not been associated with congenital anomalies. CNS abnormalities, however, have been reported in a few infants with in utero infection.[150] Abnormalities consisted of cerebral atrophy, ventricular enlargement, basal ganglia and periventricular calcifications, and diffuse cortical dysplasia, with hypotonia and later development of cerebral palsy and developmental delay. These findings could be secondary to parvovirus infection of the fetal brain; alternatively, they could be a result of the profound anemia that can occur in the fetus.

Fetal infection is diagnosed best by demonstration of parvovirus B19 DNA in amniotic fluid or determination of parvovirus B19 IgM antibody and DNA levels in fetal blood obtained by cordocentesis. Presence and severity of fetal anemia and thrombocytopenia also can be determined and corrected by intrauterine fetal transfusions into the fetal vein.[162,552] Mild or asymptomatic fetal infections may be managed conservatively with weekly fetal ultrasound examinations because some cases resolve without fetal interventions.[316] Fetal hydrops is readily detectable and monitored by antenatal ultrasound.[661]

In the neonate, detection of virus in blood and CSF can document parvovirus B19 infection. Assays for detection of human parvovirus B19 IgG and IgM antibodies are available commercially. Many infected newborns lack specific parvovirus IgM antibody at birth but will demonstrate persistence of parvovirus B19 IgG antibody beyond infancy, thus documenting infection.

No specific therapy is available. Intravenous immunoglobulin therapy has suppressed or controlled parvovirus-associated chronic anemia in immunosuppressed patients,[233] although no evidence indicates that it would be beneficial in pregnant women with parvovirus infection. Intrauterine transfusions have been used in cases of fetal hydrops associated with severe anemia, although some cases of hydrops resolve spontaneously.

Recent studies on the long-term neurodevelopmental outcome of infants after fetal transfusions for non-immune hydrops associated with parvovirus B19 infection show normal or near normal development in most survivors, supporting the use of intrauterine transfusions for correction of severe anemia and hydrops associated with this intrauterine infection.[177,445] Because some infants have experienced neurodevelopmental disabilities, some experts suggest that parvovirus B19 possibly may invade the fetal and neonatal CNS and produce neurodevelopmental sequelae. However, further studies are necessary to investigate this hypothesis.

Prevention consists largely of the rational use of infection control measures, including handwashing and disposal of used facial tissues. To isolate or quarantine otherwise normal children who have the rash of erythema infectiosum makes no sense because viral shedding has ceased by the time that the rash appears.[32] On the other hand, hemoglobinopathy patients with parvovirus-induced aplastic crisis or immunosuppressed patients with parvovirus-associated chronic anemia are highly infectious,[69] so droplet precautions should be instituted.

LYMPHOCYTIC CHORIOMENINGITIS VIRUS

LCMV is an RNA virus and a member of the Arenaviridae family. It causes a chronic infection with virus excretion in rodents, but it also may infect humans worldwide, especially in Europe, Africa, and the Americas. LCMV most commonly causes a nonspecific febrile illness, aseptic meningitis, or encephalitis. In adults, it also has been associated with nonbacterial orchitis, parotitis, and sudden-onset deafness. On rare occasion, congenital infection and disease have been documented.[55] The incidence of congenital infection with this virus, however, has not been studied systematically, and some experts suggest that it may be under-recognized as a pathogen of the fetus and newborn.[55,79,417,689,700] Congenital infection with LCMV appears to be a common cause of ocular abnormalities such as chorioretinitis, chorioretinal scars, and optic atrophy. Rarely, microphthalmos and cataracts have been observed. Other neurologic abnormalities noted at birth include microcephaly, hydrocephalus, abnormal corpus callosum, cerebellar hypoplasia, porencephalic cysts, and periventricular calcifications. Sequelae such as cognitive delays, major motor disabilities, ataxia, seizures, and vision loss have been seen in these infants. In contrast to other congenital viral infections, signs such as hydrops, hepatosplenomegaly, skin lesions, thrombocytopenia, and hearing loss are uncommon findings in children born with congenital LCMV infection.

The diagnosis of congenital LCMV infection should be suspected in an infant who appears to have a congenital viral infection or congenital toxoplasmosis, but the routine laboratory evaluation for the usual "TORCH" agents does not identify one of these infections. Supporting evidence for congenital LCMV infection includes a mother with prenatal exposure to wild, pet, or laboratory rodents such as mice, hamsters, or gerbils. The laboratory diagnosis of LCMV most commonly is established serologically, and detection of LCMV-specific IgG and IgM antibodies in serum or CSF supports the diagnosis. Detection of the virus by culture or RT-PCR–based methods is also available in reference laboratories. The virus also has been isolated from the CSF of an infant with congenital LCMV.[555] Currently, management is supportive because antiviral therapy has not been evaluated in clinical trials. Prevention of congenital LCMV infection includes minimizing exposure of pregnant women to rodents in the home and workplace.

ADENOVIRUSES

Adenoviruses are DNA viruses and common respiratory and gastrointestinal pathogens of children and adults. Fetal infection with adenovirus has been diagnosed by detection of viral DNA by PCR in amniotic fluid and has been associated with intrauterine myocarditis, fetal tachyarrhythmia, and non-immune hydrops fetalis.[56,140,520,641] Echogenic liver lesions and CNS anomalies also have been described in association with detection of adenovirus DNA in amniotic fluid and fetal tissues. Postnatal adenovirus infections in both term and preterm neonates also occur and may cause keratoconjunctivitis, as well as severe multisystem disease involving the liver, lungs, and brain.[1,72,500] Fatalities are most often associated with prematurity and pneumonitis.[1,92,218,474,625] The virus is transmitted easily in the health care setting and by ophthalmologic instruments, and it can cause outbreaks in neonatal intensive and special care units.[218,226,500]

Diagnosis is established by isolation of adenovirus from blood, CSF, conjunctival swabs, stool or urine specimens, and oropharyngeal or respiratory secretions. Tissue obtained by biopsy or autopsy also may reveal adenoviruses by culture or adenovirus particles by electron microscopy.[474] Histopathology may show smudge cells in lung tissue, and virus-specific immunohistochemical staining may reveal adenovirus antigens in tissue. Direct detection of adenovirus antigen in clinical specimens using rapid immunochromatographic assays or direct immunofluorescence assays can support the diagnosis.[497] Detection and quantification of viral DNA using PCR assays also can provide laboratory diagnosis.[641] Treatment is primarily supportive, and neonates with severe necrotizing pneumonitis may require mechanical ventilation or extracorporeal membrane oxygenation. The antivirals cidofovir and ribavirin have activity against adenoviruses and both have been used to treat severe disseminated adenovirus disease. Neonates with suspected or proven adenovirus infection should be isolated and all ophthalmologic instruments thoroughly

disinfected to prevent health care–associated transmission of the virus.[72,226]

RESPIRATORY VIRUSES—RESPIRATORY SYNCYTIAL VIRUS, INFLUENZA VIRUSES, PARAINFLUENZA VIRUSES, CORONAVIRUSES, HUMAN METAPNEUMOVIRUS

The RNA viruses; influenza A and B viruses (Orthomyxoviridae family), as well as RSV; the parainfluenza viruses types 1, 2, 3, and 4 (Paramyxoviridae family); coronaviruses; and human metapneumovirus are common seasonal winter respiratory pathogens in children and adults. These viruses also may infect neonates, who present with upper or lower respiratory signs and symptoms, or sepsis syndrome. These viruses also may cause health care–associated outbreaks in special care units with cases of serious, even fatal, pneumonia in this special age group.[236,237,315,432,441,583,587,701] Coronavirus, RSV, and influenza A virus appear most frequently, followed by influenza B virus and parainfluenza virus type 3. Neonatal infections with human metapneumovirus and parainfluenza viruses 1, 2, and 4 are less well documented. An outbreak of parainfluenza virus type 3 in an intermediate care nursery was associated with upper respiratory symptoms (nasal discharge and cough), as well as lower respiratory illness, including pneumonia and oxygen requirement. No ventilatory support or fatalities, however, occurred. The outbreak was contained with institution of barrier isolation, reinforcement of hand washing, and temporary closure of the special care unit.[432] Influenza A virus also has been associated with an outbreak in a neonatal intensive care unit.[439] Rapid diagnostic techniques helped identify infected infants, and reinforcement of infection control measures limited the outbreak. Amantadine resistance also was documented in influenza virus isolated from one treated neonate. Term and preterm neonates, especially those infants with chronic lung disease or congenital heart disease, may experience severe or fatal disease with community or health care–acquired RSV infection.[584] More recently, coronaviruses and human metapneumoviruses have been identified as causes of acute illness in neonates, especially premature infants.[236,237,587] Outbreaks of coronavirus infection in neonatal intensive care units also have been documented. Symptoms and signs in infected neonates at presentation included fever, bradycardia, apnea, hypoxemia, and abdominal distension, and chest radiographics showed diffuse pneumonitis.[587] Respiratory viral pathogens should be considered in the differential diagnosis of neonates with fever, apnea, bradycardia, sepsis syndrome, or pneumonitis in whom bacterial and fungal cultures are negative. Rapid diagnostic techniques that detect viral antigens by immunochromatographic assays that provide results within minutes, DFAs that provide same-day diagnosis, and viral culture and viral RNA detection using PCR-based methods are now routinely available and should be used for laboratory diagnosis and confirmation of viral respiratory pathogens in high-risk neonates. Antiviral therapy may be considered in selected neonates with severe respiratory viral disease. The long-term impact on pulmonary function in neonates, both term and preterm, infected with any of these viral pathogens requires further study and investigation.

ARBOVIRUSES

West Nile virus, a single-stranded RNA flavivirus, has caused severe disease in pregnant women. West Nile virus infection in pregnancy has been associated with spontaneous abortion, as well as congenital and neonatal infection.[645] Transmission of West Nile virus appears to occur in less than 5 percent of neonates born to women with West Nile virus infection during pregnancy. Most of these neonatal infections appear asymptomatic. However, congenital abnormalities, including chorioretinitis and CNS abnormalities, have been described in neonates with laboratory evidence of congenital in utero infection with West Nile virus. Possible perinatal transmission of West Nile virus to a neonate through breast milk also has been reported. Neonates with West Nile virus infection may present with fever and encephalitis and may have severe or even fatal illness.[125,126] Neonatal infection with St. Louis encephalitis virus also has been documented in a 19-day-old neonate who presented with fever and seizures.[688] If arbovirus infection, especially West Nile virus infection, is documented during pregnancy, the fetus should be assessed with ultrasound imaging. Fetal infection may be documented by presence of virus-specific IgM in fetal blood obtained by cordocentesis. At delivery, the umbilical cord and placenta may be examined for evidence of virus infection. The infant should be followed for evidence of neurodevelopmental and sensory disabilities, and repeat serologic testing with virus specific IgG and IgM should be performed at 6 months of age, to confirm whether or not infection had occurred. No specific antiviral therapies are available at this time, and studies on the long-term neurodevelopmental outcome of these infants are necessary.

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CHAPTER 74

Chlamydia trachomatis Infections in the Neonate

Margaret R. Hammerschlag

HISTORY

At the beginning of the 20th century, before expectant mothers began being screened for sexually transmitted diseases (STDs), the term *ophthalmia neonatorum* was, for all practical purposes, synonymous with gonococcal conjunctivitis. As neonatal conjunctivitis came under control with silver nitrate prophylaxis, the importance of another form of ophthalmia neonatorum, *inclusion blennorrhea*, was noted. The relationship between maternal genital infection and conjunctivitis of the newborn associated with inclusion bodies within epithelial cells was established by Lindner, Halberstader, Von Prowazek, and others.[10,29] Respiratory infection in infants caused by *Chlamydia trachomatis* probably was reported first in 1941 by Botsztejn,[5] who described an entity that he called *pertussoid eosinophilic pneumonia*.

EPIDEMIOLOGY

C. trachomatis is the most common sexually transmitted pathogen in the United States.[6] The rate of cervical infection with *C. trachomatis* during pregnancy varies from 1 to 37 percent, with the highest rates occurring in women aged 25 years and younger. *C. trachomatis* infection is acquired by the infant from the mother during parturition, as demonstrated in a number of well-controlled prospective studies conducted in the 1970s and 1980s of maternal-infant infection in which infection occurred only in infants born to infected mothers.[11,13,27] No convincing evidence demonstrates horizontal transmission from mother to infant, from other family members to the infant, or from infant to infant after delivery. Infection after cesarean delivery, usually associated with rupture of the membranes, or infection through intact membranes is a rare event but may occur.[3] The overall risk of an infant born to a mother with active chlamydial infection becoming infected at any anatomic site has been reported to be approximately 50 to 75 percent in various studies (Table 74–1).[11,13,27] Infants can be infected at more than one site, including the conjunctiva, nasopharynx, rectum, and vagina. The most frequent clinical manifestation of neonatal chlamydial infection, inclusion conjunctivitis, has been reported to occur in 15 to 37 percent of infants born to mothers with untreated cervical chlamydial infection.[11-13,27] The most frequent site of infection, however, is the nasopharynx, with 78 percent of infected infants having positive nasopharyngeal cultures in one study.[12] Approximately half of infants with inclusion conjunctivitis also will be infected in the nasopharynx. In only a minority of infants with nasopharyngeal

TABLE 74–1 Selected Studies of Perinatal Chlamydial Infection

	Prevalence of Maternal Genital Infection		Proportion of Infants with Chlamydial Infection Born to Infected Mothers				
Author, Year, City	Total	No. Infected (%)	Total	Conjunctivitis (%)	Pneumonia (%)	NP (%)	Rectum/Vagina (%)
Frommell et al., 1979, Denver[11]	340	30 (8.8)	67	39	11	6	NS
Schachter et al., 1986, San Francisco[27]	5531	262 (4.7)	131	17.6	16	11.5	14
Hammerschlag et al., 1989, Brooklyn[13]	4357	341 (7.8)	45	15	1	4	NS

NP, nasopharynx; NS, not studied.

infection does chlamydial pneumonia eventually develop; Hammerschlag and colleagues found that pneumonia subsequently developed in only 4 of 12 (33%) infants with isolated nasopharyngeal infection.[12] The overall risk of pneumonia developing in infants born to chlamydia-positive mothers has been reported to range from 1 to 22 percent.[11,13,27]

Data on the risk of acquiring rectal or vaginal infection are more limited. Bell and colleagues[4] demonstrated that perinatally acquired *C. trachomatis* infection may persist for months to years. Twenty-two infants born to women with culture-documented chlamydial infection were monitored, and positive cultures from the nasopharynx and oropharynx in the infants were detected as late as 28.5 months after birth. Rectal and vaginal infections were asymptomatic and persisted for at least 1 year, which can become an important confounding variable when young children are tested for the presence of *C. trachomatis* during evaluation for suspected sexual abuse.

CONJUNCTIVITIS

C. trachomatis was the most frequent identifiable infectious cause of neonatal conjunctivitis, and conjunctivitis was the major clinical manifestation of neonatal chlamydial infection in the United States in the 1990s.[11,13,27] The introduction of systematic screening and treatment of pregnant women has resulted in a dramatic decrease in the number of perinatal chlamydial infections. However, in countries in which pregnant women are not screened routinely, including many developing countries, *C. trachomatis* remains the most frequent cause of neonatal conjunctivitis.[10-12] The incubation period of *C. trachomatis* conjunctivitis is 5 to 14 days after delivery—or earlier if premature rupture of membranes has occurred. At least 50 percent of infants with chlamydial conjunctivitis also will have nasopharyngeal infection. The manifestation is extremely variable and ranges from mild conjunctivitis with scant mucoid discharge to severe conjunctivitis with copious purulent discharge, chemosis, and pseudomembrane formation. The conjunctiva can be very friable and may bleed when stroked with a swab. Eyelid erythema and edema frequently are present. A Gram-stained conjunctival smear initially may reveal a predominance of polymorphonuclear leukocytes. Chlamydial conjunctivitis needs to be differentiated from gonococcal ophthalmia in some infants, especially those born to mothers who did not receive any prenatal care, had gonorrhea during pregnancy, or abused drugs. An overlap in both incubation periods and clinical findings can occur. Bilateral infections are present in two thirds of cases. A follicular reaction is not seen because infants younger than 3 months old do not have the requisite lymphoid tissue present in the conjunctiva. Though an uncommon finding, chlamydial neonatal conjunctivitis has been noted to induce the long-term sequelae of corneal neovascularization and scarring. However, Hammerschlag and colleagues[12] did not detect micropannus at 1 year of age in seven infants who had culture-documented neonatal *C. trachomatis* conjunctivitis.

PNEUMONIA

As stated previously, approximately 70 percent of infected infants will have positive nasopharyngeal cultures, but the majority of these infections are asymptomatic. Chlamydial pneumonia develops in only approximately 30 percent of infants with nasopharyngeal infection.[12] In infants in whom pneumonia does develop, the manifestations and clinical findings are very characteristic.[1,17,30] The children usually are seen initially when they are between the ages of 4 and 12 weeks. A few cases have been reported in infants as young as 2 weeks of age, but no cases have been seen in infants older than 4 months. The infants frequently have a history of cough and congestion with an absence of fever. On physical examination, the infant is tachypneic, and rales are heard on auscultation of the chest; wheezing is a distinctly uncommon finding.[1,17,20] No specific radiographic findings except hyperinflation are found (Fig. 74–1). Significant laboratory findings include peripheral eosinophilia (>300 cells/cm^3) and elevated serum immunoglobulins. If cultures are performed, infants with *C. trachomatis* pneumonia may remain symptomatic and shed the organism from the nasopharynx for protracted periods.[4,12] Generally, infantile pneumonia caused by *C. trachomatis* appears to be self-limited. Most infants can be managed as outpatients, although a few cases of severe disease requiring hospitalization and assisted ventilation have been reported. *C. trachomatis* pneumonia in infants also appears to be associated with few sequelae, although data are limited. Rarely, infants with *C. trachomatis* pneumonia may have concomitant otitis media.[30]

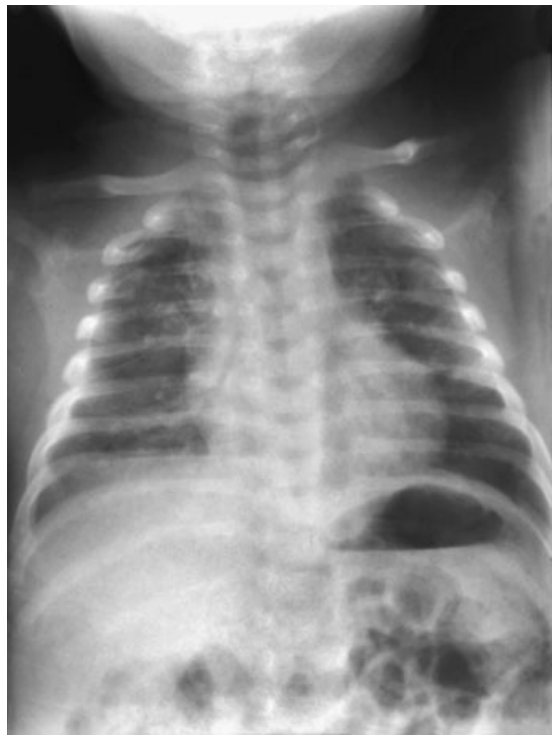

Figure 74–1 Anteroposterior chest radiograph of a severely ill 1-month-old male infant with chlamydial pneumonitis. Diffuse interstitial infiltrates and hyperaeration with a flattened diaphragm are prominent.

DIAGNOSIS

The diagnosis of neonatal conjunctivitis cannot be made on clinical grounds alone. Significant overlap in both incubation period and clinical findings occurs with infections by other organisms, especially *Neisseria gonorrhoeae*. In a high-risk population, particularly infants born to women with no prenatal care, gonococcal ophthalmia must be considered seriously.[13] The incubation period for gonococcal conjunctivitis is usually 3 to 5 days, but it can be longer. The incubation period for chlamydial conjunctivitis is approximately 5 to 14 days. Most cases will be evident by the time that the infant is 2 weeks of age, which is after the infant leaves the hospital. Epidemiologic clues can help the physician decide whether gonococcal ophthalmia needs to be considered. Hammerschlag and colleagues[13] noted that seven of the eight infants with gonococcal conjunctivitis were born to mothers who had not received any prenatal care. Five of these women were

abusers of crack cocaine. Another epidemiologic clue is a history of gonorrhea or other STD during pregnancy.

The clinical manifestation of *C. trachomatis* pneumonitis in infants is fairly characteristic, and one may be able to establish a clinical diagnosis with a degree of certainty.

LABORATORY DIAGNOSIS OF *C. TRACHOMATIS* INFECTION IN INFANTS

The "gold standard" for diagnosis of *C. trachomatis* infection in infants and children remains isolation of *C. trachomatis* by culture from the conjunctiva, nasopharynx, vagina, or rectum. *C. trachomatis* culture has been defined further by the Centers for Disease Control and Prevention (CDC) as isolation of the organism in tissue culture and confirmation by microscopic identification of the characteristic inclusions, preferably by staining with a fluorescein-conjugated, species-specific monoclonal antibody.[6] Enzyme immunoassays (EIAs) have been used by some commercial laboratories as a "screen" for culture confirmation; however, use of EIAs for confirmation of culture results has been associated with a significant number of false-positive results, especially with rectal and vaginal specimens.[7] The CDC has stated strongly that the use of EIAs for this indication is not acceptable.[7] Several nonculture tests, specifically EIAs and direct fluorescent antibody (DFA) tests, have been approved for the diagnosis of chlamydial conjunctivitis in infants. The only EIA and DFA tests still available in the United States are the Pathfinder Chlamydia DFA and EIA Microplate (Bio-Rad Laboratories). These tests appear to perform well with conjunctival specimens, and sensitivities of 90 percent or greater and specificities of 95 percent or greater in comparison to culture have been achieved.[15,24] Unfortunately, their performance with nasopharyngeal specimens has not been as good, with sensitivities ranging from 33 to higher than 90 percent.[15,24] The DNA probe Pace II (GenProbe, San Diego, CA), which is still used in many laboratories, does not have approval for any site in children, including the conjunctiva.

There are currently three Food and Drug Administration (FDA)-approved, commercially available nucleic acid amplification tests (NAATs) for the diagnosis of *C. trachomatis* infection: polymerase chain reaction (PCR) (Amplicor; Roche Molecular Diagnostics, Nutley, NJ), strand displacement amplification (SDA) (ProbeTec; Becton Dickson, Sparks, MD), and transcription-mediated amplification (TMA) (GenProbe). These assays currently have FDA approval for cervical swabs from women, urethral swabs from men, and urine from men and women. Preliminary data suggest that PCR is equivalent to culture for detection of *C. trachomatis* in the conjunctiva and nasopharynx of infants with conjunctivitis.[16] Hammerschlag and associates evaluated Amplicor for the detection of *C. trachomatis* in ocular and nasopharyngeal specimens from 75 infants with suspected chlamydial conjunctivitis.[16] Amplicor was equivalent to culture for eye specimens, with a sensitivity and specificity of 92.3 and 100 percent, respectively. The sensitivity and specificity for nasopharyngeal specimens were 100 and 97.2 percent, respectively. PCR also detected *C. trachomatis* in the urine of 12 of 12 mothers of culture-positive infants.

TREATMENT OF CHLAMYDIAL CONJUNCTIVITIS AND PNEUMONIA IN INFANTS

Oral erythromycin suspension (ethylsuccinate or stearate) (50 mg/kg/day for 14 days) is the therapy of choice for the treatment of chlamydial conjunctivitis and pneumonia in infants.[2,7] It provides better and faster resolution of the conjunctivitis, in addition to treating any concurrent nasopharyngeal infection, which prevents the development of pneumonia. Additional topical therapy is not needed. The efficacy of this regimen has been reported to range from 80 to 90 percent; however, as many as 20 percent of infants may require another course of therapy.[7,12] Erythromycin given at the same dose for 2 weeks is the treatment of choice for pneumonia and does result in clinical improvement, as well as elimination of the organism from the respiratory tract.

Treatment with oral erythromycin has been associated with hypertrophic pyloric stenosis in infants younger than 6 weeks old who were given the drug for prophylaxis after nursery exposure to pertussis.[9,18] Erythromycin is a motilin receptor agonist. Data on the use of other macrolides, including azithromycin or clarithromycin, for the treatment of neonatal chlamydial infection are limited. No studies of clarithromycin have been published; only one small study evaluated azithromycin and found that a short course of azithromycin suspension (20 mg/kg/day orally, one dose daily for 3 days) was as effective as was 2 weeks of erythromycin for eradication of *C. trachomatis* from the conjunctivae and nasopharynx of infants with conjunctivitis.[14]

PREVENTION AND CONTROL STRATEGIES

Because *C. trachomatis* infections are transmitted vertically from mother to infant during delivery, several possible options for intervention exist. The results of several prospective studies of mother-to-infant transmission of *C. trachomatis* demonstrated that neonatal ocular prophylaxis with silver nitrate, erythromycin, and tetracycline ophthalmic ointment does not prevent the development of chlamydial conjunctivitis.[8,13,20] In 1989, Hammerschlag and colleagues[13] compared silver nitrate, erythromycin, and tetracycline as neonatal ocular prophylaxis in a large urban hospital in Brooklyn, New York. The prophylaxis preparations were given within 30 minutes of birth. Chlamydial conjunctivitis developed in 20 percent (15 of 76) of infants born to infected mothers who received silver nitrate drops, 14 percent (13 of 92) of those who received erythromycin, and 11 percent (7 of 62) of those who received tetracycline. There was no effect on the incidence of nasopharyngeal infection and pneumonia. A subsequent study from Taiwan compared silver nitrate, the two antibiotics, and no prophylaxis.[8] This study, in contrast to previous studies, did not specifically monitor infants born to women with culture-documented chlamydial infection but instead monitored all infants delivered during the period of the study—for 4 weeks or until conjunctivitis developed. Again, no difference occurred in the incidence of neonatal chlamydial conjunctivitis among the four groups. The incidence of chlamydial conjunctivitis in the tetracycline, erythromycin, silver nitrate, and no-prophylaxis groups was 1.3, 1.5, 1.7, and 1.6 percent, respectively. Diagnosis of *C. trachomatis* was by DFA rather than culture. No data were given on the prevalence of maternal infection with *C. trachomatis* or *N. gonorrhoeae*. Differences in the prevalence of maternal infection among the four groups could lead to different rates of *C. trachomatis* conjunctivitis in the infants that were unrelated to prophylaxis. Respiratory infection was not assessed. A similar study from a clinic in Kenya compared povidone-iodine, erythromycin ophthalmic ointment, and silver nitrate drops as neonatal ocular prophylaxis.[19] Povidone-iodine was selected because it has a broad antibacterial spectrum in vitro; it also is antiviral and very inexpensive in comparison to the other prophylaxis agents. As with the study from Taiwan, the pregnant women were not screened for *C. trachomatis* prenatally, and chlamydial conjunctivitis in the infants was diagnosed by DFA. Mothers were told to bring their infants back if conjunctivitis developed. Use of povidone-iodine appeared to result in a 50 percent reduction in *C. trachomatis* conjunctivitis in comparison to silver nitrate (5.5% versus 10.5% of infants) and an approximately 30 percent reduction in comparison to erythromycin

(7.4%). There was no difference in the proportions of infants in whom gonococcal ophthalmia developed. As a result of the structure of the study, one cannot be certain whether every infant in whom conjunctivitis developed returned to the clinic. Because the prevalence of chlamydial infection among the pregnant women in the population was unknown, the investigators did not know how many cases of chlamydial ophthalmia to expect. The CDC does not recommend the use of povidone-iodine because of subsequent data that suggest lower efficacy than antibiotics for prevention of gonococcal ophthalmia.[7] Silver nitrate drops no longer are manufactured in the United States. Another approach that has been considered is oral prophylaxis with erythromycin or azithromycin for infants born to mothers with untreated *C. trachomatis* infection.[25] However, several analyses have found this approach to be very expensive in comparison to watching and treating the infants when they become symptomatic. In addition, there is the issue of compliance, and no data exist on the efficacy of either oral erythromycin or azithromycin for prophylaxis.

The most effective method of control of perinatal *C. trachomatis* infection is screening and treatment of pregnant women. In the 1980s, McMillan and colleagues[22] and Schachter and coworkers[28] reported that treatment of pregnant women infected with *C. trachomatis* with erythromycin resulted in a dramatic decrease in chlamydial infection (conjunctivitis and nasopharyngeal infection) in their infants in comparison to infants born to untreated infected women. The introduction of NAATs for diagnosis and the use of well-tolerated antibiotic regimens, including amoxicillin and single-dose azithromycin, have increased the efficacy of prenatal screening and treatment. This approach has been validated by a dramatic decrease in perinatal chlamydial infection in the United States and persistence of these infections in countries in which screening and treatment of pregnant women are not standard practice, such as the Netherlands, India, and China.[21,23,26,31] Rours and colleagues[26] reported that from 1996 through 2001, *C. trachomatis* was responsible for 61 to 64 percent of cases of neonatal conjunctivitis seen in a large university-affiliated hospital in Rotterdam. Even though the rate of *C. trachomatis* infection among pregnant women in Rotterdam exceeded 5 percent, prenatal screening and treatment are not part of routine prenatal care in the Netherlands.

Reasons for failure of maternal treatment to prevent infantile chlamydial infection include poor compliance and reinfection from untreated or new sexual partners. Even with effective screening, some infected women will be missed, depending on the methods used. Additionally, some women do not seek prenatal care.

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CHAPTER 75

Mycoplasma and *Ureaplasma* Infections of the Neonate

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Mycoplasmas are the smallest free-living microorganisms and are characterized by lack of a cell wall. *Mycoplasma hominis*, *Ureaplasma urealyticum*, and *Ureaplasma parvum* are the genital mycoplasmas with clinical significance in neonatal disease.[26,62,164] The two *Ureaplasma* spp. were described previously as the two biovars of *Ureaplasma urealyticum*. Biovar 1 is now *U. parvum*, and biovar 2 is *U. urealyticum*.[70,91,92]

EPIDEMIOLOGY

M. hominis and *Ureaplasma* spp. are sexually transmitted organisms that colonize the urogenital tract of women at rates of 20 to 50 percent and 40 to 80 percent, respectively.[18,105,149] Colonization rates are similar in pregnant and nonpregnant women. High rates of colonization have been associated with younger age, lower socioeconomic status, sexual activity with multiple partners, black ethnicity, and use of oral contraceptives.

In general, cervicovaginal colonization with *Ureaplasma* spp. and *M. hominis* during pregnancy is not predictive of adverse outcomes such as premature delivery, low-birth-weight infants, and spontaneous abortion.[29,49,164] High-density genital ureaplasmal colonization, though, has been associated with chorioamnionitis and preterm delivery.[4] Both *Ureaplasma* spp. and *M. hominis* appear to be capable of invading the upper genital tract in a subpopulation of women, as evidenced by their isolation from the endometrium,[95] placenta,[47,94] and amniotic fluid.[21,25,28,51,65,76,152,173,174] *Ureaplasma* spp. have been associated strongly with histologic chorioamnionitis, postpartum fever, and endometritis.[10-12,23,28,29,143] *M. hominis* is a recognized cause of pelvic inflammatory disease, postpartum septicemia, and endometritis,[26,46,84,106,111] and it has been associated with surgical wound infection after cesarean delivery.[100,127] The role of these organisms in causing spontaneous abortion and premature birth, however, remains controversial and currently unproven.[48,164]

TRANSMISSION

Vertical transmission of *Ureaplasma* ssp. and *M. hominis* from a colonized mother to her newborn occurs in utero or during delivery.[6,8,63,132,164] The relative frequency of occurrence at each time point is not fully known. Acquisition of mycoplasmas by newborns can occur at the time of delivery through contact with a colonized birth canal, but they also have been found on the mucosal surfaces of newborns delivered by cesarean section performed before the onset of labor and rupture of amniotic membranes.[135,147] In utero transmission occurs either transplacentally by the hematogenous route or via an ascending intrauterine infection from a colonized maternal genital tract. Mycoplasmas have been isolated from maternal blood at the time of delivery and from umbilical cord blood, amniotic fluid, endometrium, chorioamnion, placenta, and aborted fetal tissue. Specific IgM antibody responses have been detected in neonatal serum. Postpartum or nosocomial transmission probably occurs, but definitive proof is lacking. The idea was suggested by the finding of ureaplasmal colonization in infants at 3 to 4 weeks of age who were not previously shown to be colonized with *Ureaplasma* spp. while in a neonatal intensive care unit (NICU).[135]

The rate of vertical transmission of *Ureaplasma* spp. ranges from 0.9 to 55 percent in full-term infants and 8.5 to 58 percent in preterm infants, depending on the number and type of mucosal surfaces sampled.[8,31,112,132,134,135,147] *M. hominis* has been isolated from the nasopharyngeal aspirates and gastric secretions of 30 to 42 percent[31,32] and 8 percent,[63] respectively, of infants born to colonized mothers. The rate of vertical transmission of *Ureaplasma* spp. is not affected by the method of delivery or the duration of time after rupture of membranes. Vertical transmission is increased significantly in the presence of chorioamnionitis and intraamniotic infection.[43,140] Colonization of newborn infants increases with decreasing gestational age and birth weight,[7] and it is highest in infants weighing less than 1000 g at birth.[135] Female newborns are more likely than are males to be colonized with *Ureaplasma* because the vagina is a common site of colonization.[52,134]

Colonization with *Ureaplasma* persists through early infancy; 68, 33, and 37 percent of full-term newborns colonized in the throat, eye, and vagina, respectively, are still colonized at 3 months of age.[147] However, most of them lose colonization by the time they reach 2 years of age.[52] Among preterm infants, 65 percent remain colonized at the time of discharge from the NICU or at 28 days of life.[135] Overall, the prevalence of ureaplasmal colonization varies from 2 to 86 percent among infants admitted to NICUs, and as many as 14 to 41 percent of infants have endotracheal aspirate cultures positive for *Ureaplasma* spp.[42,45,71,79,119,132]

CLINICAL MANIFESTATIONS

The roles of *Ureaplasma* spp. and *M. hominis* in neonatal disease continue to be investigated and defined. *M. hominis* and *Ureaplasma* spp. have been recovered from the lungs, brain, heart, and viscera of aborted fetuses and stillborn infants, with histologic findings of bronchopneumonia present in the lungs.[101,148] *Ureaplasma* spp. were isolated from blood in as many as 34 percent of infants younger than 34 weeks' gestational age.[117] Twenty-six percent of preterm infants had positive endotracheal aspirate cultures for *Ureaplasma* spp. in one study.[27] Genital mycoplasmas have been also isolated from the blood, urine, cerebrospinal fluid (CSF), and lung tissue of infants with clinical signs of infection. These organisms frequently colonize the mucosal surfaces of newborns,[88,134] and ascribing disease often is difficult. However, isolation from normally sterile body fluids in symptomatic infants has led to the recognition that these organisms are neonatal pathogens.

NEONATAL PNEUMONIA

Fatal neonatal pneumonia in a term infant was documented by isolation of *Ureaplasma* from lung tissue at autopsy, with demonstrated elevated serum IgG and IgM titers to the organism.[125] Afebrile pneumonitis was reported in infants younger than 3 months old.[144] Pneumonia and persistent pulmonary hypertension were documented in three infants from whom *Ureaplasma* spp. were isolated from blood, endotracheal aspirates, pleural fluid, or lung tissue (or any combination of these sites) at

autopsy.[161] Cultrera and colleagues[40] reported finding an association between colonization of the lower respiratory tract by *Ureaplasma* spp., particularly by *U. parvum* in preterm newborns, and respiratory distress syndrome. The potential role of *Ureaplasma* in neonatal pneumonia[19,54,60,120] has been strengthened by the demonstration of histologic evidence of pneumonia in the lungs of newborn mice and premature baboons; ureaplasmal isolates were obtained from the pleural fluid, lung biopsy specimens, and lung tissue of these experimental pneumonia models.[26,129,167] Crouse and associates[35] demonstrated that pneumonia is produced in newborn mice, but significantly less often in mice older than 14 days, and is potentiated by oxygen therapy. *Ureaplasma* spp. have been shown to induce ciliostasis and mucosal lesions in human fetal tracheal organ cultures.[26] In addition, *Ureaplasma* can induce the production of alveolar macrophage proinflammatory cytokines in vitro,[96] and both *Ureaplasma* spp. and *M. hominis* stimulate the production of tumor necrosis factor-α (TNF-α) and inducible nitric oxide synthase from murine macrophages.[36] Viscardi and colleagues[157] developed the first juvenile mouse model of *Ureaplasma* pneumonia. Their data suggest that *Ureaplasma* alone may cause limited inflammation and minimal tissue injury in the early phase of infection but may promote a mild chronic inflammatory response in the later phase of infection (days 14 to 28), similar to the process that occurs in human newborns. *M. hominis* also has been associated with pneumonia.[153]

CHRONIC LUNG DISEASE

Isolation of *Ureaplasma* spp. from endotracheal secretions, the nasopharynx, the throat, or gastric aspirates (or any combination of these sites) has been associated with chronic lung disease of prematurity. Development of chronic lung disease was described in low-birth-weight infants whose respiratory tracts were colonized with *Ureaplasma* spp. in the first week of life.*

The results from a meta-analysis performed by Wang and colleagues[172] involving 17 publications supported a significant association between ureaplasmal colonization and the subsequent development of chronic lung disease. Crouse and associates[37,38] noted that infants who weighed 1250 g or less at birth, had respiratory disease, and were colonized with *Ureaplasma* spp. in their tracheal secretions were more likely to have radiographic evidence of more severe pulmonary disease than were infants who were not colonized. These findings were not supported by studies of Cordero and colleagues,[33] who did not detect any specific radiographic abnormalities in 183 infants with a birth weight of 1250 g or less and who had endotracheal colonization with *Ureaplasma* spp., gram-negative cocci, or gram-negative bacilli. However, Ollikainen and associates[118] found that preterm infants colonized with *Ureaplasma* spp. had higher leukocyte counts on the first 2 days of life and that they required high-frequency oscillatory ventilation more often than did those not colonized. In addition, the isolation by Walsh and colleagues[168] of *Ureaplasma* spp. from the lungs of infants with chronic lung disease implies an invasive bacterial process as part of the pathogenesis of lung injury. In a retrospective study of 60 ventilated babies born at less than 30 weeks' gestation, 25 of whom were *Ureaplasma* culture–positive, Theilen and associates[151] found that the ventilated babies with *Ureaplasma* in their tracheal secretions had a different clinical and radiologic course than that of infants who were culture-negative. Specifically, they had less acute lung disease but an earlier onset of chronic lung disease.

In contrast to these studies, Heggie,[71] Da Silva,[42] and Couroucli[34] and their colleagues found no association between *Ureaplasma* and the development of bronchopulmonary dysplasia (BPD). Bowman and colleagues[16] used erythromycin to treat extremely-low-birth-weight infants colonized with *Ureaplasma* and found no association between the initial colonization and the development of chronic lung disease. They also called attention to the fact that in the study by Heggie and coworkers,[71] a substantial proportion of the colonized infants were treated with erythromycin, which might explain the lack of an association between colonization and subsequent chronic lung disease. In 1997, Van Waarde and associates[155] noted that preterm infants who were at the highest risk for the development of chronic lung disease (i.e., those with the lowest gestational age and birth weight) also were the ones most likely to be colonized with *Ureaplasma* spp. Castro-Alcaraz and coworkers[30] found that a persistently positive colonization pattern, which accounted for 45 percent of the *Ureaplasma*-positive infants, was associated with a significantly increased risk for the development of chronic lung disease.

One theory suggests that *Ureaplasma* is not the primary cause of BPD but that it might be the cause of undetected pneumonia.[24] This pneumonia results in an increased requirement for supplemental oxygen, and BPD is the result of oxygen toxicity caused by the supplemental oxygen therapy. Viscardi and associates,[156] using the preterm baboon model, suggested that a prolonged proinflammatory response initiated by intrauterine *Ureaplasma* infection contributed to the early development of fibrosis and altered developmental signaling in the immature lung.

The possibility that ureaplasmal colonization of the respiratory tract induces an inflammatory response without direct pulmonary invasion and infection cannot be excluded.[66,98] It has been supported by the finding of elevated levels of TNF-α, interleukin-6 (IL-6), IL-8, and monocyte chemoattractant protein-1 (MCP-1) in the tracheal secretions of colonized infants,[14,26,67,102,146] as well as by elevated white blood cell counts and eosinophilia in infants whose respiratory tract is colonized by *Ureaplasma* spp.[114,118,121] The contribution of *U. urealyticum* and *U. parvum* was evaluated by Heggie and colleagues.[71] Both species colonized preterm infants with a birth weight of less than 1500 g; however, no association was seen between endotracheal colonization with either species and the development of chronic lung disease. In a study performed by Katz and colleagues,[83] *U. parvum* was detected more often than was *U. urealyticum*, but they found no significant difference or trend in the prevalence of either species between infants with or without BPD.

In a meta-analysis, Schelonka and colleagues[137] reviewed 36 articles in which cohorts of neonates were screened for the presence of *Ureaplasma* by culture with or without polymerase chain reaction (PCR) and monitored prospectively for the development of BPD. They concluded that colonization with *Ureaplasma* was associated with higher reported rates of BPD. However, the greatest reported effect was seen in the small studies, and, therefore, reporting bias may be partially responsible for the higher rates of BPD in colonized infants.

NEONATAL CENTRAL NERVOUS SYSTEM INFECTIONS

Both *Ureaplasma* spp. and *M. hominis* have been isolated from the CSF of both full-term and preterm infants.* The repeated isolation of these organisms from the CSF of predominantly preterm infants with suspected meningitis and their association with a CSF pleocytosis consisting of polymorphonuclear or mononuclear cells, hypoglycorrhachia, and elevated protein content support their role in causing neonatal meningitis. Waites and

*See references 2, 7, 8, 14, 22, 26, 55, 69, 75, 78, 81, 82, 93, 120, 122, 123, 133, 142, 168-170.

*See references 9, 56-58, 73, 74, 87, 103, 107, 109, 139, 141, 145, 154, 162, 165.

colleagues[165] noted hemiplegia, hydrocephalus, and developmental delay in survivors. Isolation of *Ureaplasma* spp. from the CSF of preterm infants also has been associated with severe intraventricular hemorrhage.[3,116,165] However, Likitnukul and associates[97] cultured the CSF and blood of 203 infants with suspected sepsis and failed to isolate *Ureaplasma* spp. Primarily in full-term infants, isolation of *Ureaplasma* spp. and *M. hominis* often has been associated with minimal if any CSF abnormalities, and these infants have done well without receiving specific antimicrobial therapy.[72,109,137,154,162] In such instances, the presence of these organisms remains of unclear clinical significance, and their role in producing disease is questionable.

SYSTEMIC INFECTIONS

Nonimmune hydrops fetalis was reported in a newborn at 32 weeks' gestation in whom *U. urealyticum* was isolated from bronchial secretions and lung and brain tissue at autopsy.[115] *M. hominis* has been associated with neonatal septicemia.[26,153]

OTHER INFECTIONS

Osteomyelitis of the femur was associated with isolation of *Ureaplasma* from the blood of a preterm infant.[59] *Ureaplasma* was recovered from a scalp abscess at the site of an internal fetal electrode monitor.[68] *M. hominis* infections have been noted in association with brain and scalp abscesses, ventriculitis, submandibular adenitis, abscesses of subcutaneous tissue, pericarditis, and conjuctivitis.[1,61,80,108,124,131] The clinical significance of isolation of genital mycoplasmas from urine obtained by suprapubic bladder aspiration in infants remains to be determined.[97] In such instances, analysis of urinary sediment has been normal.

DIAGNOSIS

Because of the frequent colonization of newborn infants with mycoplasmas, an etiologic role for these agents cannot be supported by detection of organisms on mucosal surfaces only. The diagnosis of mycoplasmal infection is made by isolating the organism from a normally sterile body fluid or suppurative focus. Genital mycoplasmas may be isolated on special broth and solid media that are available commercially. Shepard 10B broth and A8 agar have been used successfully for cultivation of both *Ureaplasma* spp. and *M. hominis*.[26] Cultures generally become positive within 2 to 5 days.

Cassell and colleagues[22,26] recommended that mucosal specimens be obtained with a Dacron or calcium alginate swab and be placed in a specific mycoplasmal transport medium such as Shepard 10B broth. Specimens should be refrigerated at 4°C until they are transported to the laboratory and be protected from drying. Alternatively, specimens in appropriate transport media can be frozen at –70°C because both *Ureaplasma* spp. and *M. hominis* are stable for long periods of time under these conditions. Specimens should be diluted serially in 10B broth to at least 10^{-3} (preferably to 10^{-5}) to overcome any potential inhibitory substances or metabolites, and an aliquot of the original sample and dilution should be plated directly onto A8 agar. Body fluids (e.g., blood, CSF, pleural fluid) should be inoculated into 10B broth in an approximate 1:10 ratio (usually 0.1 mL of fluid per 0.9 mL of 10B broth). Blood should be collected free of anticoagulants. Broth cultures and agar plates are incubated under 95 percent nitrogen and 5 percent carbon dioxide. The presence of mycoplasmal growth in 10B medium is indicated by a change in color from yellow to pink, which is caused by an alkaline shift in the media as a result of either the urease activity of ureaplasmas or arginine hydrolysis by *M. hominis*. Growth of mycoplasmas in broth culture as indicated by a change in color should be confirmed by inoculation of a broth specimen onto A8 agar. Characteristic colonies of *Ureaplasma* spp. and *M. hominis* can be identified readily on A8 agar after 24 to 72 hours of incubation.

Serologic tests have been used to measure antibody to genital mycoplasmas. Such tests include the metabolic inhibition assay, enzyme-linked immunosorbent assay, mycoplasmacidal test, indirect hemagglutination, indirect immunofluorescence, and IgG and IgM immunoblotting.[26,44,53,126] Use of these tests for establishing the diagnosis of mycoplasmal infection in infants remains problematic and is not well established. None is available commercially, and, therefore, diagnosis of newborns relies on culture results.

PCR assays involving the urease gene, 16S RNA genes, and the multiple-banded antigen genes have been used to detect *Ureaplasma* spp. in neonatal clinical specimens.* On endotracheal secretions, Blanchard and associates[15] reported finding a sensitivity of 100 percent and a specificity of 99 percent in comparison to culture. PCR assays using 16S rRNA and ribosomal DNA targets are available for *M. hominis*.[5,64,99] Ultimately, PCR may aid in identifying colonized or infected infants more readily (results available in 1 day) and reliably, given the fastidious nature of these organisms and the scarcity of microbiology laboratories that routinely perform mycoplasmal culture.

TREATMENT

The decision to treat an infant for possible mycoplasmal infection should be based on the clinical picture and culture results. In general, isolation of mycoplasmas from a normally sterile site in an ill neonate is an indication for consideration of treatment. However, no large randomized clinical trials have determined the efficacy of treatment in neonates. Experience on which to base treatment decisions, the choice of drug, and the duration of therapy is very limited.[171]

In a preterm infant with clinical evidence of sepsis or central nervous system disease in whom routine bacterial and viral cultures are sterile and the infant is not responding to antibacterial or antiviral therapy, the presence of mycoplasmal disease should be suspected and appropriate samples obtained for culture of mycoplasmas. Isolation of mycoplasmas from endotracheal secretions is not diagnostic of pneumonia, and most of these infants do not require any antimycoplasmal therapy. However, if pneumonia is suspected and the infant's clinical condition is deteriorating, a trial of therapy may be indicated despite the unknown efficacy of treatment.

Mycoplasmas are not susceptible to the antimicrobial agents routinely used to treat neonatal infections.[17,159,160,163] Because they lack a cell wall, mycoplasmas are insensitive to penicillins, cephalosporins, polymyxins, sulfonamides, and vancomycin. Although they may have moderate sensitivity to the aminoglycosides, the minimal inhibitory concentrations (MICs) of these agents for genital mycoplasmas usually are too high for therapeutic use. The drugs of choice for the treatment of infection caused by *M. hominis* are chloramphenicol, clindamycin, doxycycline, and tetracycline; for the treatment of ureaplasmal infections, erythromycin or other macrolides, doxycycline, tetracycline, and chloramphenicol are recommended.[89,104,159,166] When possible, antibiotic susceptibility testing should be performed on all clinically significant isolates because of the occurrence of multidrug resistance.

M. hominis is resistant to erythromycin, but in *Ureaplasma* spp., high-level resistance to erythromycin (MIC ≥ 32 mg/mL)

*See references 5, 6, 15, 41, 98, 99, 110, 128, 138, 150.

is found very infrequently. Cardiac toxicity consisting of acute cardiorespiratory deterioration possibly secondary to cardiac arrhythmias has been reported in neonates treated with intravenous erythromycin lactobionate for presumed ureaplasmal pneumonia.[50] Ototoxicity has been seen in adults but not in neonates.[39] Oral erythromycin has been associated with hypertrophic pyloric stenosis in infants younger than 6 weeks.[77] Although the exact duration of therapy is not known, a 10- to 14-day course seems reasonable when clinical improvement and microbiologic eradication are observed during that period.

Azithromycin and clarithromycin are active in vitro against *Ureaplasma* spp. but not *M. hominis*,[90,130,158] but their use in neonates has not been evaluated thoroughly. When given orally to very-low-birth-weight infants colonized with *Ureaplasma* spp., serum levels of clarithromycin at a dose of 7.5 mg/kg every 12 hours were subtherapeutic.[136] For this reason, a dose of 15 mg/kg every 12 hours is recommended.

PREVENTION

Erythromycin administered between 26 and 35 weeks' gestation to pregnant women colonized with *Ureaplasma* spp. was not effective in reducing adverse outcomes such as preterm delivery, low birth weight, or premature rupture of membranes.[49] In the ORACLE I study,[85] in which the use of broad-spectrum antibiotics was evaluated in mothers with premature rupture of membranes, use of erythromycin was associated with prolongation of pregnancy, reduction in neonatal treatment with surfactant, decrease in oxygen dependence at 28 days of age and older, fewer major cerebral abnormalities on ultrasonography before discharge, and fewer positive blood cultures. The ORACLE II study, which consisted of 6295 women with spontaneous preterm labor, intact membranes, and no evidence of clinical infection, showed that none of the trial antibiotics was associated with a lower rate of the composite primary outcome events than occurred in placebo recipients.[86] Because erythromycin therapy does not eliminate *Ureaplasma* spp. from the lower genital tract, it most likely also does not prevent neonatal ureaplasmal colonization.[113] Its effect on prevention of neonatal disease has not been evaluated fully.[13] Administration of erythromycin to preterm infants whose mothers had *Ureaplasma* spp. in their lower genital tract reduced respiratory tract colonization but did not decrease the duration of supplemental oxygen therapy[82] or chronic lung disease.[16,98] Treating very-low-birth-weight infants who have respiratory tract colonization with *Ureaplasma* spp. to prevent chronic lung disease cannot be recommended at present.[20]

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CHAPTER 76

Yeast and Fungal Infections of the Fetus and Neonate

Gail J. Demmler-Harrison

The improved survival of preterm infants since the early 1980s has resulted in the emergence of yeasts and fungi as significant neonatal pathogens.* *Candida albicans* remains the most frequently isolated pathogenic yeast overall, but other *Candida* spp., especially *Candida parapsilosis* and *Candida tropicalis*, and less common pathogenic yeasts, such as *Malassezia*, *Trichosporon*, *Rhodotorula*, and *Pichia/Hansenula*, have increased in frequency. The incidence of invasive fungal disease, often with fatal outcomes, caused by *Aspergillus* and Zygomycetes (*Mucor*, *Rhizopus*, *Rhizomucor*, and *Absidia*) also has increased in neonates, especially infants who were born premature or with an inherited metabolic disorder or primary immunodeficiency.[309]

Reports about premature neonates with serious or fatal infection with rare, darkly pigmented fungi, such as *Phialemonium obovatum*, also have emerged more recently, perhaps heralding an increasing trend for unusual fungal infections in this special age group.[138] In addition, the rare vertical transmission of the endemic pathogenic yeast and fungi *Cryptococcus*, *Coccidioides*, and *Blastomyces* continues to be documented, especially in neonates born to mothers infected with human immunodeficiency virus (HIV).[67] Prompt recognition of pathogenic yeast or invasive fungal disease in neonates and institution of aggressive management that includes appropriate antifungal therapy and, when required, surgical intervention, are important to minimize rates of morbidity and mortality from these serious, often fatal, infections.

NEONATAL CANDIDIASIS

Candida spp. account for approximately 9 percent of all nosocomial infections in the neonatal intensive care unit (NICU). *Candida* spp. infect approximately 1 to 6 percent of infants with a birth weight less than 1500 g (very low birth weight [VLBW]) and 5 to 10 percent of infants with a birth weight less than 1000 g (extremely low birth weight [ELBW]).[177,292,295] The overall case-fatality rate is approximately 30 percent, with a range of 4 to 40 percent reported.[190,211,220,343,411] Neurodevelopmental disabilities also are more common findings in premature infants with invasive candidiasis.[98] In addition to excess mortality and disability rates, neonatal candidiasis is associated with increased length of hospitalizations and excess hospital costs.[343,411]

The species isolated most frequently is *C. albicans*,[27,28,37,41] which represents approximately 40 to 60 percent of isolates from neonates with systemic disease and is the most common *Candida* spp. associated with NICU outbreaks.[18,74,121,313] *Candida* spp. other than *C. albicans* have become increasingly more prevalent, however, and in some NICUs may be isolated more frequently than is *C. albicans*.[63,112,139,306] Among these organisms, *C. parapsilosis* is the most common,* followed by *C. tropicalis;*[124,303] both of them have caused outbreaks in NICUs. *Candida glabrata*,[26,112] *Candida guilliermondii*,[312,314,315] *Candida lusitaniae*,[316] and *Candida krusei*[133,304] also have caused invasive disease in neonates. Most recently, *Candida haemulonii*[193] has emerged as a neonatal pathogen and caused an outbreak in an NICU.[193] Manifestations of neonatal candidiasis range from commonly encountered benign oral and cutaneous candidiasis to systemic infection with candidemia to more severe and often fatal disseminated candidiasis.[27,28,31]

TRANSMISSION, PATHOGENESIS, AND RISK FACTORS

Candida transmitted to the fetus in utero by an ascending route from the colonized vagina of the mother results in

*See references 31, 37, 61, 149, 154, 161, 177, 332, 335, 341.

*See references 41, 85, 96, 113, 115, 166, 211, 218, 319, 373, 395, 405.

congenital candidiasis. Transplacental infection has not been described.[106,170,176,180,215,222] More frequently, vertical transmission to a newborn occurs during birth through the mother's colonized birth canal, which results in the development of oral candidiasis, or thrush, in an otherwise healthy infant.[10,27,65,76,306,382] *Candida* also may be acquired by the infant during breast-feeding if the mother's skin is colonized and from inadequate sterilization of feeding bottles and nipples.[113] Subsequent colonization of the gastrointestinal tract and the presence of *Candida* in stool lead to superficial cutaneous infection involving primarily the perineal area.[27]

Nosocomial transmission occurs and has resulted in nursery outbreaks. Even though *Candida* spp. have been detected on environmental surfaces and from the air samples of neonatal units, person-to-person transmission is the most important mode of transmission.[202,376,382] Horizontal transmission from health care worker to newborn with subsequent colonization of conjunctivae, mucosa, or skin and infant-to-infant transmission via the hands of health care workers have been documented in outbreak investigations that used molecular analysis of *Candida* isolates.* In addition, systemic candidiasis in ELBW infants has been linked to use of topical petrolatum ointment for skin care.[63]

Candidal colonization of the oropharynx of preterm neonates in NICUs is a common occurrence; it occurs in approximately 2.5 to 19 percent of infants in the first few days of life and increases with age and time in the NICU.[121,313] Subsequent colonization of the gastrointestinal tract and endotracheal tube in ventilated neonates seems to predispose them to development of invasive candidiasis.[27,310,314,315] Baley and colleagues[27] studied 146 infants with a birth weight less than 1500 g during an 11-month period. They performed fungal cultures of pharynx, rectum, and endotracheal aspirates within 24 hours of birth, and then weekly while the infants were in the NICU. The overall colonization rate was 27 percent; by 2 weeks of age, 85 percent of the infants were colonized with *Candida* spp. Mucocutaneous disease developed in 28 percent of the colonized infants, and systemic candidiasis was seen in 8 percent.

In high-risk neonates, gastrointestinal colonization also may lead to bloodstream dissemination, particularly if the integrity of the intestinal mucosal lining is disrupted by surgery, ischemia, or enterocolitis.[56,61,108,314,315] In addition, the immature skin of ELBW infants may predispose them to invasive candidal infection.[276,306] Gastrointestinal colonization with *Candida* occurs before the development of candidemia in most preterm infants.[314,315] In addition, Rowen and colleagues[310] showed that VLBW infants with endotracheal colonization by *Candida* had a sixfold increased risk for development of invasive disease.

The relationships among adhesion proteins, lymphocytes, and neutrophils may contribute to yeast pathogenicity in colonized neonates. The ability of *C. albicans* to invade oral and gastrointestinal mucosal tissues and enter the bloodstream may be a major determinant of virulence. In vitro studies of interaction of *C. albicans* with epithelial cells have shown that *C. albicans* invades mucosal tissues by promoting the proteolytic degradation of the junction adherin protein E-cadherin, allowing an increase in cell permeability.[129,160,379] Candidal dissemination in neonates is aided further by abnormal neonatal leukocyte function, as shown by an impaired ability of polymorphonuclear leukocytes to adhere to, ingest, and kill *Candida*, and a reduced capacity of lymphocytes to inhibit candidal growth.[331,357]

In addition, proteomic studies have begun decoding serologic responses to *Candida* cell wall proteins.[285,286] Antibodies made to *C. albicans* cell wall proteins, such as antiwall enolase antibodies, by survivors of systemic candidiasis may provide the basis for novel diagnostic and prognostic indicators and may be candidates for possible future vaccine development. More recently, *Candida* antigens identified by proteomic approaches were shown in murine models to induce protective responses, providing the basis for possible future human clinical vaccine trials.[286]

Risk factors for neonates colonized with *Candida* to progress to systemic infection include prematurity; VLBW and ELBW; prolonged endotracheal intubation with mechanical ventilation; bronchopulmonary dysplasia; indwelling intravenous catheters; prolonged administration of third-generation cephalosporin antibiotics, hyperalimentation solutions, H_2 blockers, and steroids; lack of enteral feedings; abdominal surgery; skin care practices; and early neutropenia.* Several risk factors consistently have been associated with systemic candidiasis, the most common being prematurity and VLBW and ELBW, with attendant impairment of host defense mechanisms; indwelling intravenous catheters; and prolonged use of broad-spectrum antimicrobial therapy, in particular, third-generation cephalosporins, which suppress normal gastrointestinal flora.[314,327,334] Although aminophylline is able to inhibit the activity of human granulocytes in vitro, its use has not been associated clinically with systemic candidiasis in neonates. Studies by Hostetter[37,160] suggested the use of heparin may be another predisposing factor; this in vitro finding also has not been confirmed in the clinical setting, however.

CLINICAL MANIFESTATIONS

ORAL CANDIDIASIS

Oral candidiasis is the most common form of infection with *Candida* spp.[242] Lesions on the mucous membranes of the mouth and oropharynx usually appear on the 7th to 10th day of life as whitish gray plaques that can be scraped easily from the mucosa to expose an erythematous base. Persistent or recurrent infection may be caused by the continued use of bottle nipples and pacifiers that harbor *Candida* on their surface. Primary and acquired immunodeficiency states, including infection with HIV, should be considered if oral thrush fails to clear with appropriate therapy.

CUTANEOUS CANDIDIASIS

Cutaneous candidiasis typically is manifested by erythematous, vesiculopustular lesions found primarily on the skin of the perineum, axilla, and intertriginous areas.[346] The periumbilical area also can be involved.[242] Benign diaper dermatitis with so-called satellite lesions is a frequent occurrence in an otherwise healthy infant, and the peak incidence occurs at 3 to 4 months of age. In VLBW and ELBW premature infants, seemingly benign mucocutaneous lesions, including lesions in the perineum or diaper area, may progress rapidly to invasive dermatitis, producing cutaneous scales, crusting, erosions, ulcerations, or extensive maceration of skin, and result in a potentially lethal systemic candidiasis (Fig. 76–1).[31,72,159,276,306] Scattered, faint, erythematous maculopapular lesions of the skin may be an expression of a serious disseminated candidiasis.[31,306,309] Chronic mucocutaneous candidiasis may be diagnosed in an otherwise well-appearing infant, if *Candida* infections of the skin, mucous membranes, and nails persist or recur, despite administration of antifungal therapy. These neonates have a congenital defect in T-lymphocyte function specific to *Candida* spp.

*See references 18, 119, 124, 163, 165, 167, 218, 295, 303, 304, 373.

*See references 57, 63, 209, 220, 221, 226, 304, 309, 310, 314, 315, 327, 335, 389.

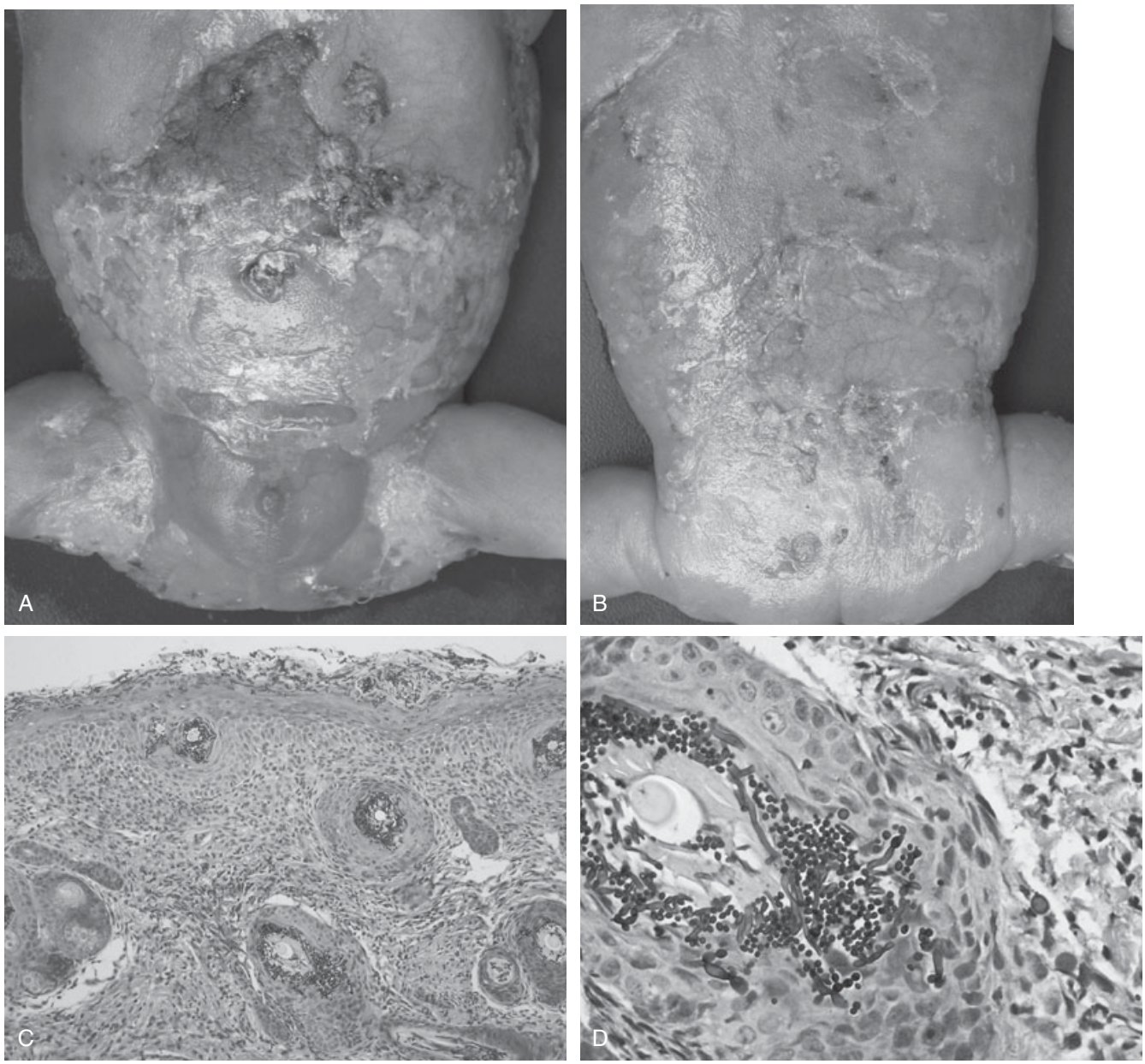

Figure 76–1 A-D, Invasive cutaneous candidiasis. A 3-week-old, former 24-2/7 weeks' gestation premature infant with fatal invasive candidiasis. The skin on the abdomen (**A**) and back (**B**) showed extensive cutaneous ulceration and exudates caused by *Candida* infection. Autopsy examination showed disseminated candidiasis with thromboemboli, infarction, and microabscesses involving the brain, lungs, and kidneys. Skin biopsy specimen showed numerous ovoid yeasts and pseudohyphae infiltrating the stratum corneum and within hair follicles (periodic acid–Schiff stain, 100× original magnification) (**C**) and infiltration of yeasts deep into the dermal connective tissue (periodic acid–Schiff stain, 400× original magnification) (**D**). (See companion Expert Consult web site for color version.) *(Courtesy of Dr. Megan K. Dishop, Department of Pathology, Baylor College of Medicine and Texas Children's Hospital, Houston, TX.)*

CONGENITAL CANDIDIASIS

Congenital candidiasis may cause fetal infection with onset of premature labor or intrauterine demise; it may be evident at birth, or it may appear within the first 24 to 48 hours of life.* Term and preterm neonates may be affected (Fig. 76–2).[348,349] Congenital candidiasis usually is caused by *C. albicans*, but other *Candida* spp., including *C. tropicalis* and *C. glabrata*, also have been reported to cause congenital candidiasis.[230,260]

Congenital cutaneous candidiasis most often manifests as a widespread, mucocutaneous infection, with erythematous maculopapular or vesiculopustular rash.[349,399] Congenital pneumonia also may be a part of the disease, with or without the mucocutaneous or systemic signs.[66,120,185] Congenital systemic candidiasis may manifest as life-threatening, early-onset sepsis syndrome, especially in premature neonates (see Fig. 76–2) with septic shock or meningitis; it also may be accompanied by leukocytosis or neutropenia, or it may mimic congenital leukemia.[66,268] Twins

*See references 32, 59, 60, 66, 106, 170, 173, 176, 180, 203, 237, 305, 306, 328.

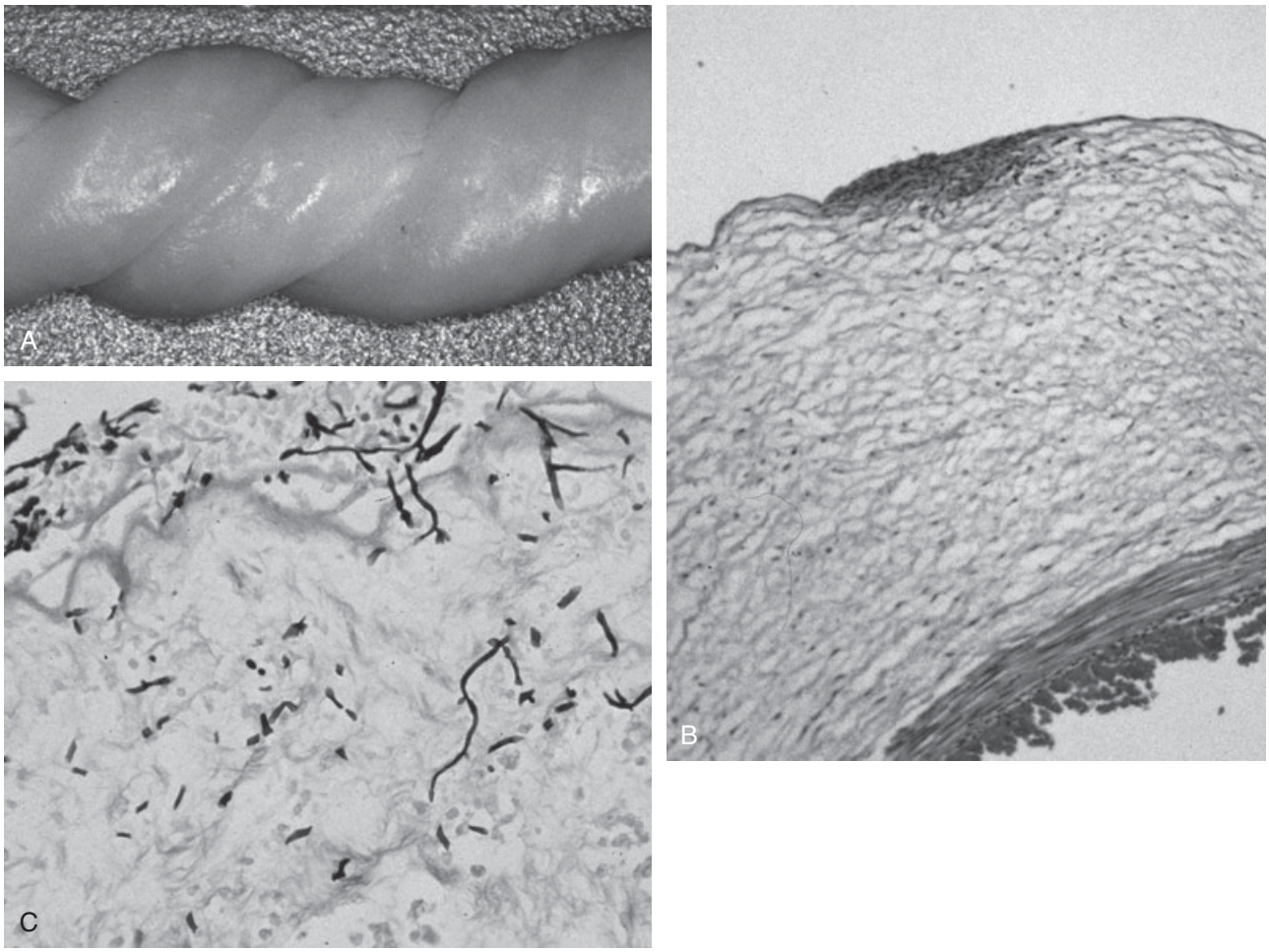

Figure 76–2 A-C, Umbilical cord funisitis in congenital candidiasis. **A,** Umbilical cord from a term infant born with congenital candidiasis (*C. albicans*). Gross examination showed cord edema with pinpoint yellow-white lesions on the surface. **B** and **C,** Umbilical cord from premature infant, born at 24 weeks' gestation with a birth weight of 610 g, to a mother with *Candida* vaginitis at the time of delivery. The infant presented at birth with septic shock associated with disseminated congenital candidiasis. Examination of the umbilical cord showed extensive funisitis with *Candida* microabscesses (hematoxylin and eosin stain, 40× original magnification) (**B**). Microscopic examination showed *Candida* funisitis with budding yeasts and invasive pseudohyphae (methenamine silver nitrate stain, 200× original magnification) (**C**). *(Courtesy of Dr. Edwina J. Popek, Department of Pathology, Baylor College of Medicine and Texas Children's Hospital, Houston, TX.)*

may be affected, sometimes with different *Candida* spp., manifestations, and outcomes for each twin.[203,326,350]

Prenatal diagnosis of intrauterine infection with *Candida* may be determined by amniocentesis.[49,237,305] A presumptive diagnosis in a neonate can be made by identifying budding yeast organisms on potassium hydroxide preparations or Gram-stained smears of the pustular or vesicular skin lesions, blood buffy coat, or gastric fluid aspirate.[294] Growth of *Candida* in culture provides confirmatory evidence. Term newborns with congenital candidiasis usually have infection limited to the skin, mucous membranes, lungs, or gastrointestinal tract; *Candida* rarely is isolated from blood or other sterile body sites. In contrast, premature neonates with congenital candidiasis often have more severe disease, with positive blood cultures and evidence of disseminated disease, including meningitis.[141,348,349] The umbilical cord from a fetus or newborn with congenital candidiasis may show *Candida* funisitis with yellow-white nodules and fungal microabscesses (see Fig. 76–2); the placenta also may have nodules and shows chorioamnionitis on histologic examination.[230,291,325]

Maternal risk factors include a history during pregnancy of *Candida* vaginitis, use of antibiotics, retained intrauterine contraceptive device, cervical cerclage, and prolonged rupture of fetal membranes, although none of these factors may be present in the history.[32,59,76,237,305,328] Poor prognostic factors for neonates include the presence of pneumonia or sepsis, prematurity, and early-onset neutropenia at presentation.[411]

SYSTEMIC CANDIDIASIS

Systemic candidiasis refers to the isolation of *Candida* or its histopathologic demonstration in a normally sterile body site. The most common forms of this clinical syndrome include (1) catheter-associated sepsis, in which *Candida* is isolated from the blood of infants who have central venous catheters, but no evidence of focal infection or disseminated disease; (2) disseminated candidiasis, in which *Candida* is isolated from the bloodstream in association with other foci of infection, such as skin or intestine, regardless of whether the infant has a central venous catheter[61]; and (3) focal disease of an organ or disseminated disease caused by *Candida* spp., without a positive blood culture. No significant differences in presentation of systemic candidiasis caused by different *Candida* spp. have been apparent.[41,43,112,116,163,165,166]

The clinical and laboratory signs of systemic candidiasis usually are nonspecific and resemble signs seen with bacterial sepsis.[37,41,43,220,221] Knowledge of prior colonization with *Candida*

spp. or presence of cutaneous crusting or erosions, dermatitis, or skin maceration provides clinical clues that a sepsis syndrome in a premature neonate may be caused by invasive *Candida* spp. (see Fig. 76–1A and B).[31,61,276,306,309] Signs and symptoms most commonly reported in neonates with systemic candidiasis include respiratory deterioration (74%), apnea and bradycardia (60%), carbohydrate intolerance (56%), skin manifestations (53%),[28,31,37] abdominal distention (49%), temperature instability (35%), guaiac-positive stools (26%), and hypotension (21%).[61,85]

The frequency of end-organ involvement in neonates with systemic candidiasis is difficult to determine precisely. Based on several retrospective, single-center medical literature reviews and one meta-analysis report, the sites most frequently involved in systemic neonatal candidiasis include blood (60-80%), the central nervous system (CNS) (meningoencephalitis, 25-60%),[113,114,123,199] the lungs (pneumonia, 70%),[28,74,185,277] the kidneys (renal candidiasis, 60%),[28,41,107,126,197,272,283,289] the liver and spleen (19%),[42,163,263] and the eyes (endophthalmitis, 30%).[24,25,263] End-organ disease is accompanied most often by positive blood or urine cultures, especially if cultures are persistently positive for more than 5 days.[39,73,263] Not all neonates with end-organ disease with *Candida* spp. have positive blood or urine cultures, however.

The organ involved most frequently in systemic candidiasis seems to be the kidney.[28,39,107,126,197,263] *Candida* may be isolated from the urine of 73 percent of neonates with systemic candidiasis with positive blood cultures, but only 5 to 15 percent of such neonates have documented *Candida* pyelonephritis with renal infiltration, mycetoma, or renal abscess.[7,22,41,153,192,198,206,368] The kidney seems to be particularly vulnerable to the formation of renal cortical abscesses and obstructive masses, which usually occur at the ureteropelvic junction. A neonate with persistently positive urine or blood cultures, hypertension, renal insufficiency or acute renal failure, or oliguria or anuria should undergo ultrasound imaging to determine the presence of a bezoar-associated obstructive uropathy, which requires immediate attention.[22,44,229,289,381,403,404] Urinary catheters or nephrostomy tubes may become colonized with *Candida* and result in secondary infections that may range from asymptomatic to invasive disease with obstruction.[44,229]

CNS involvement occurs in 3 to 23 percent of neonates with systemic candidiasis; percentages of 54 to 64 percent have been reported in a few series.[39,77,79,118,171] In addition to causing meningitis, *Candida* may invade the brain tissue, through hematogenous dissemination, producing focal nodular encephalitis, with minimal inflammation of the meninges (Fig. 76–3). The diagnosis of candidal meningoencephalitis requires a high index of suspicion, followed by thoughtful and careful evaluation.[113,114,123,199]

Candida meningitis may occur in the absence of positive blood cultures; cerebrospinal fluid (CSF) pleocytosis may be minimal or absent, and elevated protein or hypoglycorrhachia is an inconsistent finding. Gram-stained smears of CSF rarely reveal budding yeast, even when CSF cultures are positive for *Candida*.[40,90,123,169] More recent reviews determined that meningitis with *Candida* isolated from the CSF occurred in 8 percent of ELBW infants, but only 37 to 52 percent had *Candida*-positive blood cultures.[40,90,169] CSF findings in neonates diagnosed with *Candida* meningitis also may vary. Of neonates with *Candida* meningitis, 43 percent may have normal CSF.[90] Fernandez and associates,[123] in a 10-year review of candidal meningitis in 23 infants in an NICU, reported that pleocytosis occurred in only 39 percent of infants, and hypoglycorrhachia occurred in 25 percent, despite a positive CSF culture for *Candida* in 74 percent of infants. The remaining neonates are diagnosed when a positive blood culture occurs with an abnormal, but culture-negative, CSF.[98,114,208,254] *Candida* also may colonize and infect external ventricular drains placed for management of hydrocephalus.

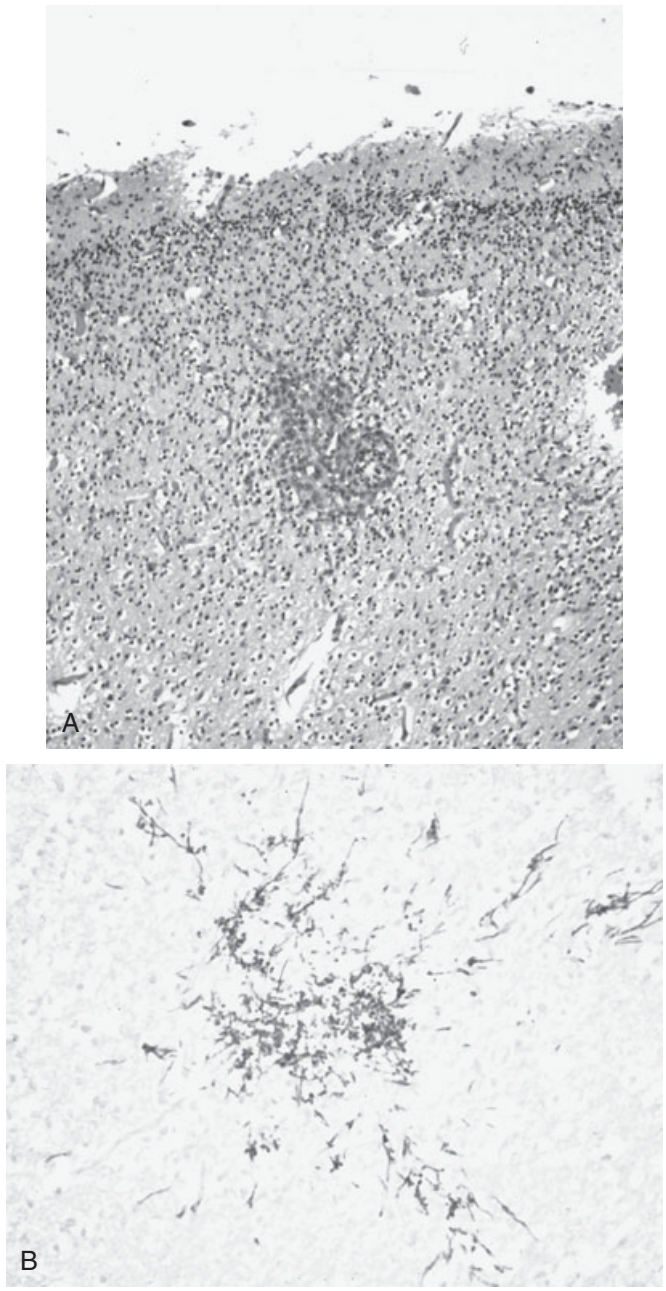

Figure 76–3 **A** and **B,** Invasive candidiasis (*C. albicans*) of the central nervous system. Autopsy examination of a premature infant born at 25 weeks' gestation who died at 1 month of age of disseminated invasive candidiasis. **A,** Central nervous system involvement, with brain microglial nodules, was seen at autopsy examination (hematoxylin and eosin stain, 40× original magnification). **B,** Brain microglial nodules contained *Candida* yeasts and invasive ribbon-like *Candida* pseudohyphae (Gomori methenamine silver stain, 100× original magnification). *(Courtesy of Dr. Edwina J. Popek, Department of Pathology, Baylor College of Medicine and Texas Children's Hospital, Houston, TX.)*

Ventriculitis, brain abscesses, and brain calcifications are unusual complications of *Candida* meningitis that can be diagnosed with brain imaging procedures.[58,141,181,197,198] Mortality or neurodevelopmental disabilities in survivors may occur in 73 percent of neonates with *Candida* meningitis.[40,98,208,254]

Endocarditis is estimated to occur in 5 to 15 percent of neonates with systemic candidiasis.* It is a serious and potentially

*See references 39, 117, 128, 174, 216, 217, 263, 264, 284, 317, 414.

fatal complication of neonatal systemic candidiasis, with reported fatality rates of 25 to 73 percent.[216,217] *C. albicans* has been isolated most often, followed by *C. parapsilosis*. *Candida* endocarditis may occur in neonates with normal hearts and neonates with structural heart disease, and as a postoperative complication of cardiac surgery.[236,246] *Candida* endocarditis in neonates may differ in presentation from *Candida* endocarditis in older children and adults.[115,233] It usually occurs in premature neonates with persistent candidemia associated with an indwelling vascular catheter. Most neonates with *Candida* endocarditis have structurally normal hearts, however, and not all reported cases in neonates have been associated with vascular devices.[104,151,246]

The presentation of *Candida* endocarditis in neonates may be acute and associated with an episode of persistent candidemia, or late onset, often discovered weeks or months after a presumably silent or resolved episode of candidemia.[39,96,179,212,246,301] Cardiac murmurs may be absent. Early literature reports consisted mostly of autopsy diagnoses; however, more recently, echocardiography has proved to be a valuable tool in establishing the diagnosis of *Candida* endocarditis.[236,246,256] Intracardiac vegetations on the mitral, tricuspid, and aortic valves, or the presence of a right atrial mass, in association with persistently positive blood cultures for *Candida*, strongly supports the diagnosis of endocarditis.

Eye disease may occur in 3 to 11 percent of neonates with systemic candidiasis; figures as high as 45 percent have been reported.[77,246,263] Candidal eye disease may complicate systemic candidiasis in term, near-term, and preterm infants.[24-26] More recent reports suggest, however, that the prevalence of ophthalmologic complications in hospitalized infants and children, including premature VLBW infants who are treated aggressively and survive candidemia, may be decreasing.[100,127] Eye disease may occur on the first day of systemic candidiasis but more often is associated with prolonged candidemia.[25,99,263]

Advanced eye disease may be visible on routine examination; however, establishing the diagnosis of endophthalmitis early requires indirect ophthalmoscopy by an ophthalmologist.[77,248,269] Most lesions involve the retina and choroids; characteristic lesions include unilateral or bilateral yellow-white fluffy, retinal, or free-floating vitreal opacities or balls, often accompanied by hemorrhage or inflammatory vitreous haze.[12,24,25,269] The iris and lens of the eye also may be involved.[330,367] Lens abscesses manifesting as lens opacities or cataracts also may occur, and they may be a nidus of ongoing infection poorly reached by systemic antifungal therapy and a source for recurrent disease.[87,103,254,330,367] Untreated, *Candida* endophthalmitis also may progress and produce corneal thinning and perforation of the globe.[240] Rarely, *Candida* endophthalmitis may mimic retinoblastoma.[336] In addition, VLBW infants who experience candidemia may have concomitant endophthalmitis and retinopathy of prematurity, or they may develop more severe retinopathy of prematurity (stage 3 or beyond) or retinal detachment and require laser therapy.[49,136,145,184,245,262] Premature, high-risk, VLBW infants with candidemia should be monitored closely for *Candida* eye disease and for progression of retinopathy of prematurity.[262]

Osteoarthritis is a rare manifestation of systemic candidiasis in neonates.[2,146,162,199,361,387,390,393] It may occur temporarily associated with persistent candidemia or as a late complication, weeks to months later, after apparent resolution of an episode of candidemia.[146,162,390] In addition to premature infants, newborns with underlying inherited disorders of metabolism also may be at increased risk. Clinical features of candidal osteoarthritis in neonates are similar to the features of osteoarthritis caused by bacterial pathogens and include warmth, pain, tenderness, and swelling of the extremity and diminished range of motion. Imaging may show swelling of soft tissue and joint and cortical bone erosion. Culture of aspirated joint fluid or biopsy of bone tissue positive for *Candida* spp. confirms the diagnosis.[387,393]

Intra-abdominal infections, including enteritis, necrotizing enterocolitis (NEC), and peritonitis, and intra-abdominal and hepatosplenic abscesses are estimated to occur in 8 percent of neonates with invasive systemic candidiasis (Fig. 76–4).[39] *Candida* peritonitis and sepsis may complicate surgical procedures performed on the intestine for congenital bowel atresias or bowel perforation associated with NEC.[26,56,99,182,243] Distinct from NEC is idiopathic spontaneous focal intestinal perforation (SFIP), which occurs rarely in VLBW infants and has been associated with systemic candidiasis in half of cases.[1,88,243,290,299,307] SFIP is approximately 12-fold less common than is NEC in preterm neonates and has a distinct combination of clinical and laboratory features that help distinguish it from NEC. Prognosis and management differ, so it is important to understand the difference between these two clinical entities.

Neonates with SFIP are more likely than are neonates with NEC to be smaller at birth, have lower Apgar scores, experience early hypotension or hypothermia, and require more intensive

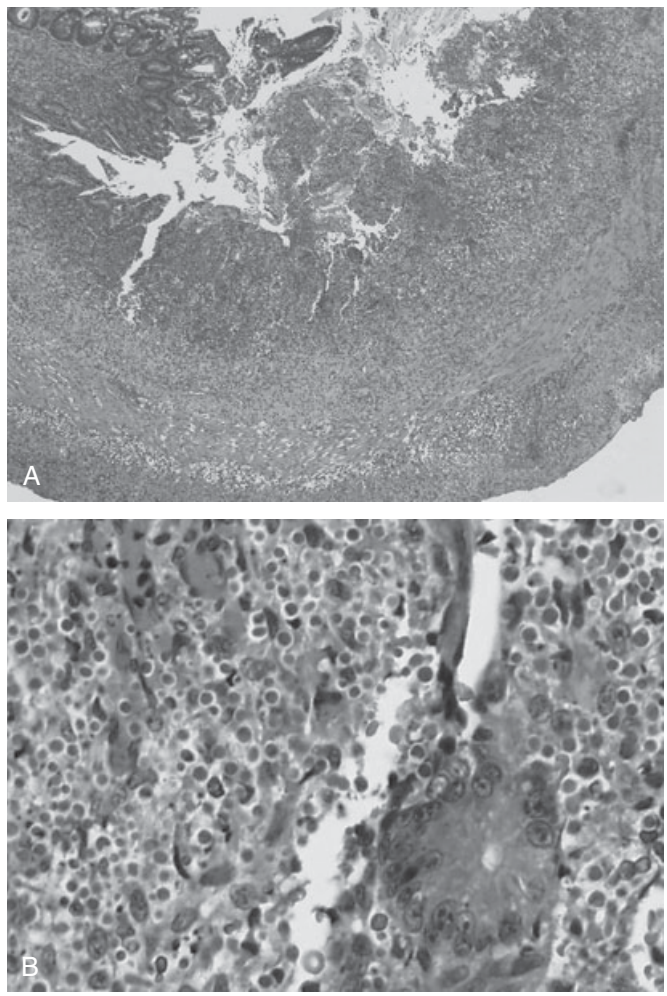

Figure 76–4 **A** and **B,** *Candida* infection complicating necrotizing enterocolitis. A 2-week-old extremely-low-birth-weight premature infant with invasive *Candida* infection associated with necrotizing enterocolitis with perforation. **A,** A large segment of resected bowel showed extensive mucosal erosion and hemorrhage with transmural inflammation (hematoxylin and eosin stain, 40× original magnification). **B,** Numerous yeast organisms infiltrated the eroded mucosa (hematoxylin and eosin stain, 400× original magnification). (See companion Expert Consult web site for color version.) *(Courtesy of Dr. Megan K. Dishop, Department of Pathology, Baylor College of Medicine and Texas Children's Hospital, Houston, TX.)*

neonatal resuscitation.[182,307] Neonates with SFIP present acutely in the second or third week of life (mean, 16.7 days of life), with distention and a bluish discolored abdomen. Radiographs in SFIP reveal a gasless abdomen without pneumatosis intestinalis or portal venous gas commonly associated with NEC. SFIP frequently is an easily resectable focal area of necrosis associated with a single perforation less than 1 cm in size, often located in the terminal ileum, but also may involve the colon or stomach. The remaining bowel may be covered with serosal exudate, but otherwise it appears normal. In contrast, NEC often involves large areas of transmural bowel necrosis that are difficult to resect completely in many patients; *Candida* and other organisms may be seen in multiple sections of resected bowel (see Fig. 76–3B). Histologic findings in SFIP show focal hemorrhagic necrosis, often with *Candida* invading the bowel wall at the site of perforation only, and otherwise healthy bowel. *Candida* spp. and *Staphylococcus epidermidis* or coagulase-negative staphylococci are the predominant pathogens isolated in SFIP, whereas Enterobacteriaceae predominate in peritonitis caused by NEC-associated perforations, with *Candida* spp. isolated in approximately 10 percent of cases of NEC.[1,88,182,243,248,290] A peritoneal culture should be obtained in all neonates with intestinal perforation, regardless of cause, because results of cultures determine the most appropriate antimicrobial therapy for bacteria and *Candida* spp.

Pulmonary candidiasis is seen frequently in congenital candidiasis.[66,185] VLBW infants with endotracheal colonization with *Candida* spp. have an increased risk for development of systemic candidiasis.[132,310] Acquired pulmonary candidiasis with pneumonia may occur in 8 to 10 percent of ventilated premature neonates and must be differentiated clinically and radiographically from asymptomatic *Candida* colonization and pneumonia caused by other pathogens.[132,185,226,277,306,309]

DIAGNOSIS

Congenital or acquired cutaneous candidiasis may be diagnosed by the presence of budding yeast organisms in scrapings of skin lesions, and the species involved is established by culture. Invasive dermatitis caused by *Candida* can be differentiated from benign cutaneous dermatitis by skin biopsy. Histopathologic evidence of organisms beyond the stratum corneum is evidence of more serious invasive dermatitis that leads to potentially fatal systemic candidiasis (see Fig. 76–1C and D).[306,309]

The diagnosis of systemic candidiasis in a fetus or neonate is established by isolation of *Candida* spp. in culture from a normally sterile body fluid, such as amniotic fluid, urine, blood, or CSF, or from an involved site, such as joint or bone. Culture of *Candida* spp. from abscesses and intraoperative tissue specimens also establishes the diagnosis of invasive *Candida* disease. The presence of characteristic yeast or fungal elements in tissue specimens from the placenta, the umbilical cord, or the neonate provides supportive evidence for invasive *Candida* disease, but isolation of the organism from the tissue is required to establish the diagnosis. Methods of detection of *Candida* in blood or CSF by polymerase chain reaction amplification of *C. albicans* DNA or 18S rRNA and detection of *Candida* antigens, such as mannan, in blood or CSF have been developed by a variety of different laboratories; however, they have not been evaluated in neonates and should not replace culture as the definitive test to establish the diagnosis.[285,286,365,366,378] A high index of suspicion is the key to establishing an early and accurate diagnosis, which improves outcome. Systemic candidiasis has been diagnosed first in 20 to 50 percent of infants at autopsy, and a mean delay of 11 days has been reported between the onset of symptomatic disease and the initiation of antifungal therapy.[28]

The diagnosis often is made when a routine blood culture from an infant evaluated for possible bacterial sepsis yields a *Candida* organism. In as little as 4 hours, but usually within 48 to 72 hours, *Candida* grows in routine blood cultures processed in the automated systems used by most clinical laboratories. Yeast also may be detected in smears of blood or buffy coats in high-risk neonates.[287,294] In the evaluation of an infant for possible candidemia, blood for culture should be obtained from a peripheral vein or artery and through all intravascular catheters in place at the time.[311] In addition, at least one blood culture should be obtained after removal of a colonized catheter to determine if candidemia persists after the catheter is removed.

Culture of CSF should be performed if candidemia is documented, even if CSF indices are normal. A CSF specimen that grows *Candida* in a high-risk neonate should not be dismissed as a culture contaminant.

Urine obtained by suprapubic bladder aspiration, often facilitated by ultrasound guidance, is an excellent source for recovery of *Candida*.[283] If the urine grows *Candida*, renal or systemic infection is suggested. Bag specimens of urine are unreliable for establishing the diagnosis because of the high rate of perineal candidal colonization in infants. Catheterized urine specimens, although preferable to specimens obtained by bag, may be contaminated from contact with perineum and penis. Urine obtained from an indwelling urinary catheter may represent colonization of the catheter; a repeat specimen obtained using sterile technique, at least 24 hours after removal of the catheter, is recommended. Fungal culture of urine may yield candidal growth in a premature neonate with disseminated candidiasis, even when other body fluids, such as blood and CSF, do not. Microscopic analysis of urine, Gram-stained smear of urine, or potassium hydroxide preparation of freshly obtained urine may show evidence of yeast or fungi, providing evidence for a presumptive diagnosis and early initiation of antifungal therapy.

In addition to cultures of blood, CSF, and urine, cultures of all involved sites, such as joints, bones, and abscesses, should be performed. The peritoneal fluid from infants with NEC or SFIP who require surgical intervention should be stained and cultured, and resected bowel should be sent for histopathologic examination and culture. Endotracheal cultures positive for *Candida* spp. may represent colonization in an infant at risk for having invasive disease; however, if positive respiratory cultures for *Candida* are associated with pneumonia and respiratory deterioration, invasive pulmonary candidiasis may be present.[132,310]

In addition to culture and histopathology, routine laboratory evaluations may support the diagnosis of systemic or invasive candidiasis.[61] Persistent thrombocytopenia is a frequent early finding in infants with systemic candidiasis. Additional laboratory tests, such as a complete blood cell count, liver function tests, and determination of serum glucose, blood urea nitrogen, and creatinine, may be helpful in assessing the degree of systemic involvement.

Infants whose urine, blood, or CSF cultures yield *Candida*, especially if persistently positive, should have further evaluation done to detect any evidence of dissemination that would influence the prognosis and duration of antifungal therapy.[61,73,254,311] Ultrasound or enhanced computed tomography evaluation of the abdomen, kidneys, and bladder should be done to assess for possible liver or splenic abscesses and evidence of renal infiltration or obstructive lesions.[39,72,197,198,201] Cranial ultrasound may be a useful screen for CNS pathology, such as hydrocephalus. Infants with a positive CSF culture or who are otherwise suspected to have CNS involvement should undergo enhanced computed tomography or magnetic resonance imaging of the brain to detect abscesses, nodules, infarction, or parenchymal calcified granulomata.[141,181] Careful ophthalmologic examination also is recommended, especially if candidemia is persistent, to detect endophthalmitis or lens abscesses. Infants with persistent candidemia, structural or postoperative heart disease, or new-onset

heart murmur should have an echocardiogram done for possible valvular endocarditis or intracardiac mass.[317]

TREATMENT

MANAGEMENT STRATEGIES

Oral candidiasis or thrush in an otherwise healthy infant is treated with oral nystatin suspension or gentian violet. Superficial cutaneous candidiasis in an otherwise healthy neonate is treated with topical nystatin cream or powder. When the diaper area is extensively involved, oral nystatin therapy may be administered as well in an attempt to eliminate the yeast from the gastrointestinal tract. Cutaneous candidiasis that persists or evolves into invasive candidiasis requires systemic antifungal therapy, usually with amphotericin B or fluconazole.

Congenital candidiasis usually requires only topical antifungal therapy in an otherwise healthy newborn; neonates who have pulmonary involvement or who are preterm should receive systemic antifungal therapy because they are at greater risk for dissemination and a poor outcome.[61,66] In these instances, amphotericin B or fluconazole may be used for approximately 10 to 25 days, until clinical signs and symptoms have resolved.

Candidemia is now a common cause of late-onset sepsis in VLBW and ELBW neonates.[255,279] Because it is associated with significant morbidity and mortality rates, and potential risk factors for candidemia among high-risk neonates have been identified, some experts suggest that empiric antifungal therapy with amphotericin B or fluconazole should be administered along with antibiotics at the time that a blood culture is obtained for suspected sepsis in VLBW, extremely premature neonates with gestational ages of less than 28 weeks, especially if they have thrombocytopenia or a history of exposure to third-generation cephalosporin or carbapenem in the previous week.[38,63,220,221,335]

Although many antifungal medications are available, data from well-designed, randomized clinical trials in neonates, especially premature neonates, are insufficient to favor a recommendation of one antifungal agent or combination of agents for invasive or systemic candidiasis.* Most information published is derived from analyses of case series and retrospective reviews.[86,101,253] Published studies on pharmacokinetic profiles, studies evaluating optimal dose or duration of therapy, and studies with safety and toxicity data on neonates are difficult to find.† Recommendations for treatment are based on published experience in adults and older children and opinions of experts in neonatal medicine and pediatric infectious diseases. It is hoped that new data specific to neonates and premature neonates will emerge.

Most experts agree that evaluation for disseminated disease and prompt systemic antifungal therapy, usually with amphotericin B or fluconazole, is indicated if even a single blood culture from a neonate is positive for *Candida* spp.[72,311] Consensus seems to be lacking, however, on which antifungal compound or combination is preferred for treatment of complications such as meningitis, and for how long treatment should be administered.[72,79,123,169,178,253] The most appropriate management for an individual neonate should consider the *Candida* spp. prevalent in the nursery, the *Candida* spp. isolated from the patient, and the usual or known antifungal susceptibility patterns; the site of infection; the availability of pharmacokinetic, efficacy, and safety data; and the ability of the host to metabolize and eliminate the medication.[122,132,140,253,380,410,413]

Isolation of *Candida* from blood obtained through a vascular catheter means the device is colonized with the organism and must be removed promptly to eradicate the infection and reduce the risk of morbidity and mortality.[94,111,352] *Candida* is difficult to eradicate from catheters, and persistence of positive blood cultures from blood obtained through the catheter increases the rates of complications and mortality associated with candidemia.[73,111] In addition to removal of the catheter, systemic antifungal therapy should be administered for 10 to 14 days. If the peripheral blood culture also is positive for *Candida* spp., dissemination via the bloodstream may have occurred, and most experts recommend duration of antifungal therapy to be at least 25 to 30 days.[61,62,311]

Isolation of *Candida* spp. from urine obtained from an indwelling catheter suggests possible colonization of the catheter, requiring removal of the catheter if the infection is to be eradicated. Isolation of *Candida* spp. from a urine specimen obtained by sterile technique denotes invasive renal or systemic disease and requires further evaluation and systemic antifungal therapy. In some patients, medical therapy alone may eradicate the *Candida* infection, even if partial obstruction is present.[7,22,368] Some neonates may require an urgent surgical or endoscopic procedure, however, to relieve the obstruction and restore renal function.[44,51,153,229,289,381,403] Duration of antifungal therapy required for cure may range from 25 to 42 days or longer and should be guided by microbiologic cure and resolution of lesions by imaging studies.[51]

Treatment of meningoencephalitis caused by *Candida* spp. in a neonate usually requires systemic therapy for at least 30 days; longer courses of treatment may be required if ventriculitis or brain abscesses or nodules are present.[79,80,123,169,316] Repeat CSF examination and culture before discontinuing antifungal therapy is recommended to document sterilization and improved CSF parameters of white cells, glucose, and protein.[90] If *Candida* ventriculitis is associated with an indwelling ventricular drain or shunt, prompt removal of the device also is indicated. Repeat CSF examination and culture should be done every 24 to 48 hours until sterile, and treatment should be continued when, and for sometime after, the drain or shunt is replaced.

Treatment of endocarditis or septic thrombophlebitis caused by *Candida* spp. requires prolonged antifungal treatment, at least 30 days and usually 42 to 60 days, depending on clearance of candidemia, clinical progress, and resolution of intracardiac vegetations or lesions as documented by echocardiography.[115,128,233,263] Consultation with a cardiologist or cardiovascular surgeon is recommended.[96,104,246] If septic thrombophlebitis with vascular abscess is present, or an enlarging valvular mass obstructs blood flow, surgical intervention may be considered.[104,115,151,216,241] Reports of resolution of endocarditis with medical therapy alone suggest, however, that it may be a valid option to consider, especially for neonates who are hemodynamically stable, with right-sided, nonvalvular lesions.[115,117,128,151,183,212,317,414]

Treatment of eye disease, such as endophthalmitis, requires systemic antifungal therapy for 25 to 42 days, and consultation with an ophthalmologist is recommended to assess the extent of disease, presence or progression of comorbid retinopathy of prematurity, and response to therapy.[100,136,223,263,356] If an abscess of the lens is present, surgical drainage or lensectomy may be necessary.[25,87,103,330,356,367] Long-term follow-up of neonates with *Candida* endophthalmitis suggests that early medical therapy results in cure for most neonates who survive systemic candidiasis and that invasive procedures usually performed in adults with *Candida* eye disease, such as vitreous taps or intravitreal injections of medications, are unnecessary in most neonates.[12,25,127,136] Neonates with invasive candidiasis should be observed closely, however, for progression of retinopathy of prematurity.[49,145]

Renal disease caused by invasive *Candida* spp. usually responds to systemic antifungal therapy, the duration of which usually is

*See references 8, 29, 52, 62, 68, 86, 101, 102, 131, 140, 145, 172, 253, 265, 274, 321, 354, 410, 413.

†See references 30, 53, 78, 148, 157, 158, 255, 275, 320, 344, 347, 353, 363, 397.

at least 25 days.[7,41,44,153,263] Longer duration of therapy may be required to eradicate renal mycetomas, abscesses, or masses ("fungal balls"). If obstructive uropathy is present, urologic consultation is recommended to determine if nephrostomy or other diverting procedure is indicated to relieve the obstruction temporarily.*

Treatment of osteoarthritis caused by *Candida* spp. usually requires prolonged duration of systemic antifungal therapy (≥42 days).[361,387,393] Aspiration or surgical drainage by an orthopedic surgeon may be necessary if there is an infected joint or bone abscess.[2,146,162,199]

Peritonitis associated with NEC or SFIP usually requires at least 25 days of systemic antifungal therapy, but longer duration may be required if intra-abdominal abscesses develop.[39,88] Surgical management of SFIP also differs from that of NEC; simple surgical procedures, such as suturing or resection with primary anastomosis, may be all that is required for treatment of SFIP, whereas NEC may require extensive bowel resection.†

Duration of systemic antifungal treatment for pulmonary candidiasis is not well established. Treatment should be guided by clinical and radiographic improvement; however, 25 days is likely to be adequate for most patients.[39,132,310]

ANTIFUNGAL AGENTS

The choice of antifungal agents available to treat neonatal candidiasis has expanded beyond the traditional polyene antibiotics, nystatin and amphotericin B deoxycholate, and the pyrimidine analogue 5-flucytosine (5-FC). Lipid formulations of amphotericin B are safe and effective when administered to neonates.[3,51,68] The first antifungal azoles administered to neonates were imidazoles (miconazole and ketoconazole) with unpredictable absorption and toxicities.[83,359,369] First-generation triazoles (fluconazole and itraconazole), available now for almost 2 decades, seem to be effective and safe when administered to most neonates.[44,47,54,375] Rare reports of fetal malformations associated with fluconazole administered prenatally to pregnant women are of potential concern.[5] Newer compounds, including second-generation triazoles (posaconazole, ravuconazole, and voriconazole) and echinocandins (caspofungin, micafungin, and anidulafungin), have emerged as potentially valuable agents to treat resistant yeast and fungal infections in neonates.[8,72,150,158,253,337]

Amphotericin B

Amphotericin B deoxycholate, a polyene macrolide antibiotic, remains the drug of choice for most neonates with systemic candidiasis caused by susceptible *Candida* spp.‡ *Candida* spp. most commonly isolated, including *C. albicans*, usually are susceptible to amphotericin B; however, more recent reports suggest the incidence of amphotericin B resistance may be 20 to 25 percent in *C. albicans* and *C. parapsilosis* isolated from pediatric patients, including neonates.[18,405,410] In addition, resistance is a common finding in *C. lusitaniae* and *C. haemulonii*. Also, the compound seems to be less active in vitro against some species, including *C. parapsilosis*, *C. tropicalis*, and *C. guilliermondii*.[384]

The limited pharmacokinetic data available suggest neonates exhibit considerable interindividual variability in metabolism of the drug, and this variation is speculated to be due partly to genomic factors that affect drug metabolism.[8,353] The usual recommended dose is 1 mg/kg/day infused over the course of 2 to 4 hours; however, dosage ranges of 0.5 to 1.5 mg/kg/day have been used, depending on the diagnosis and organism isolated. Administration of a small initial test dose, slow escalation of doses, and premedication to prevent infusion-related reactions are unnecessary in neonates. The duration of therapy varies with the severity and type of *Candida* infection. For uncomplicated, catheter-associated candidemia in which prompt removal of the intravascular device results in rapid clinical and laboratory improvement, a cumulative dose of 7 to 10 mg/kg usually is sufficient.[62] For disseminated infection, cumulative doses of at least 25 mg/kg often are necessary.[62,311] For meningitis, a 30-mg/kg cumulative dose often is preferred,[79] whereas for endocarditis and osteoarthritis, cumulative doses of 40 to 50 mg/kg have been administered in some cases.[317]

Intrathecal amphotericin B deoxycholate has been used to treat refractory candidal meningitis, but it rarely is needed. The initial intrathecal dose is 0.01 mg, which is increased gradually over 5 to 7 days to 0.1 mg given every other or every third day.

Adverse effects of amphotericin B deoxycholate include nephrotoxicity in 15 percent of neonates, manifested by azotemia, hypokalemia, and hypomagnesemia, often with an elevated serum creatinine concentration and oliguria.[62,78,156,353] Hypokalemia occurs frequently and reflects tubular injury resulting in increased urinary excretion of potassium.[156] Nephrotoxicity may be minimized by avoiding concomitant use of other potentially nephrotoxic drugs during administration of amphotericin B. Animal and adult studies also have suggested higher sodium intake may prevent renal compromise during amphotericin B therapy. More recently, a study of the effects of fluid and electrolyte management on amphotericin B–induced nephrotoxicity in VLBW neonates suggested nephrotoxicity occurred more frequently in neonates with hyponatremia and suggested hydration and higher sodium intake may provide some protection against amphotericin B–induced nephrotoxicity in VLBW neonates.[156]

Hepatic enzyme abnormalities, hyperbilirubinemia,[172,267] anemia, and thrombocytopenia[69] also have been reported, but rarely occur in neonates. Similarly, neonates do not experience the fever, rigors, shaking, chills, or vomiting that are common infusion-related events in older children and adults.

Lipid preparations of amphotericin B, such as liposomal amphotericin B (L-AmB; AmBisome), amphotericin B lipid complex (ABLC; Abelcet), and amphotericin B colloidal dispersion (ABCD; Amphocil, Amphotec), in usual daily doses of 3 to 5 mg/kg (range 2.5 to 7.5 mg/kg), have been used safely and successfully in neonates with invasive candidiasis of many forms caused by a variety of *Candida* spp.* More recent pharmacokinetic and clinical information support their use in neonates, but optimal dosage with standardized duration of therapy has not been established in randomized controlled trials.[86,200,321]

Some reports suggest lipid preparations may eradicate *Candida* infection more rapidly than does amphotericin B deoxycholate when used as first-line therapy for some forms of invasive candidiasis and may benefit neonates who do not respond clinically and microbiologically to amphotericin B deoxycholate therapy.[179,183,200] Although experience with lipid formulations has increased dramatically in recent years, superior efficacy or safety for neonatal candidiasis has not been proven. In addition, lipid formulations are more costly than is amphotericin B deoxycholate, so they are not recommended universally for routine or first-line treatment in neonates.[134,142,152,171,206,386,402] In most centers, lipid preparations are reserved for clinical or microbiologic failure, or when toxicity, primarily renal, occurs during the course of conventional amphotericin B deoxycholate therapy.

Of the lipid preparations, liposomal amphotericin B may have improved CNS penetration; however, some preparations may not penetrate the kidney well.[142] Because these preparations prefer-

*See references 22, 44, 107, 126, 198, 207, 229, 272, 277, 289, 381.
†See references 1, 34, 56, 88, 175, 182, 243, 248, 290, 299, 301.
‡See references 8, 29, 30, 37, 52, 61, 62, 86, 123, 131, 354, 413.

*See references 3, 8, 52, 68, 86, 134, 152, 171, 172, 178, 179, 183, 200, 206, 321, 394, 413.

entially accumulate in organs of the reticuloendothelial system, they may be more useful for the treatment of hepatic or splenic candidal abscesses.[386] Toxicities associated with lipid preparations of amphotericin B are similar to the toxicities seen with amphotericin B deoxycholate and include hypokalemia, hypomagnesemia, renal insufficiency, and hepatic dysfunction; however, occurrence of toxicities may be less frequent or less severe than those of amphotericin B deoxycholate.[68,172,179,394]

5-Flucytosine

The compound 5-FC is a synthetic pyrimidine analogue that is administered orally in a dose of 50 to 150 mg/kg/day divided every 6 hours, in combination with amphotericin B formulations, when meningitis is present.[79,80,311] Some experts also consider using 5-FC if endophthalmitis, endocarditis, persistent candidemia, or osteoarthritis is present, whereas others prefer to use amphotericin B alone and monitor for clinical and microbiologic response.[61,62] Most *Candida* spp. are highly susceptible to 5-FC, and synergy of 5-FC with amphotericin B has been documented.[249] It also is well absorbed from the gastrointestinal tract and diffuses well into the CSF and eye.[53,80]

The dose of 5-FC should be decreased if renal insufficiency is present or suspected, and all neonates should have serum levels monitored frequently to ensure they have adequate levels and to avoid development of toxicity. Recommended therapeutic serum levels range from 25 to 100 mg/L. Some centers recommend steady-state serum levels of 40 to 60 mg/L, whereas others prefer predose and 2-hour postdose serum therapeutic ranges of 30 to 40 mg/L and 70 to 80 mg/L.[275,347] Levels greater than 100 mg/L are associated with toxicity and should be avoided. More recent studies on 5-FC therapeutic monitoring in pediatric patients, including neonates, have shown that the standard recommended daily dose of 100 mg/kg is associated with significantly higher serum concentrations in neonates, prompting recommendations for adjusted dosage schedules in this special age group.[275,336]

Approximately 4 to 8 percent of *C. albicans* isolates, and as many as 22 percent of non-*albicans* isolates, mostly *C. tropicalis* and *C. krusei*, are resistant to 5-FC.[366] The drug should never be used alone to treat candidiasis because resistance is likely to emerge even in susceptible *Candida* spp. if 5-FC is used as monotherapy. Side effects can be significant and include renal toxicity, hepatotoxicity, bone marrow suppression, gastrointestinal intolerance, and hemorrhagic enterocolitis.[53,186,317] These effects usually are associated with elevated serum levels greater than 100 μg/mL; nonetheless, it is prudent to monitor blood counts and liver and renal function tests in all neonates who receive 5-FC. Because of the potential for development of significant toxicity, the need for oral administration in premature neonates who may be unable to tolerate enteral feeds and recommended monitoring of drug levels, and the need for successful clinical experience with amphotericin B monotherapy, routine use of 5-FC requires thoughtful consideration.[62]

Triazoles (Fluconazole, Itraconazole, Voriconazole)

Fluconazole is a first-generation triazole antifungal agent with excellent antifungal activity against most isolates of *C. albicans*.[380] *C. krusei* and the more recently recognized *C. haemulonii* seem to be inherently resistant to fluconazole, and some strains of *C. glabrata*, *C. tropicalis*, *C. parapsilosis*, and *C. guilliermondii* may have reduced susceptibility.[164,193,311,380] Fluconazole resistance also has been detected more recently in *C. parapsilosis*, *C. albicans*, and *C. tropicalis* isolates from neonates.[50,139,319,380,410]

Fluconazole seems to be a safe and effective agent for treatment of neonatal systemic candidiasis and may be first-line therapy in some centers for uncomplicated, catheter-associated candidemia in neonates.* Breakthrough candidemia has been described, however, in neonates receiving fluconazole therapy.[204,246] Because it has excellent penetration of CSF, fluconazole also has been used occasionally to treat neonates with candidal meningitis.[169] Adverse effects associated with use of fluconazole in neonates are minimal and include elevated liver enzymes and skin rashes. Usual recommended doses for term neonates are 3 to 6 mg/kg/day for mucocutaneous disease and up to 6 to 12 mg/kg/day every 24 hours for systemic disease. In premature neonates, the dose interval should be adjusted to every 48 to 72 hours, depending on gestational and chronologic postnatal age. Duration of therapy should be at least 28 days for systemic candidiasis; shorter durations may be acceptable for isolated catheter colonization or mild, noninvasive mucocutaneous candidiasis.

Itraconazole, an oral first-generation triazole with activity against most *Candida* spp. except *C. krusei* is not approved for use in children or neonates. Nonetheless, it has been used rarely to treat systemic candidiasis in neonates.[47,375] Because it does not cross the blood-brain barrier well, itraconazole should not be used to treat neonates in whom *Candida* meningitis is suspected or proven. Clinical experience seems to be limited; similar to fluconazole, emergence of itraconazole resistance has been documented in *C. albicans* and *C. tropicalis* strains isolated from neonates.[140]

The second-generation triazole, voriconazole, has expanded antifungal activity that includes not only *Candida* spp., but also *Cryptococcus neoformans*, *Aspergillus*, *Fusarium*, and *Trichosporon beigelii*. Voriconazole displays nonlinear pharmacokinetics in adults, but it seems to have linear pharmacokinetics in children and neonates.[354] Higher doses in smaller patients may be needed to avoid treatment failures. Experience in neonates is very limited, and the drug is not approved for use in this age group. Nonetheless, isolated case reports of use of the drug in difficult cases appear in the literature. In one case report, the addition of intravenous voriconazole administered in the pediatric dose of 6 mg/kg/dose every 12 hours resulted in subtherapeutic levels; however, 6 mg/kg every 8 hours achieved acceptable serum concentrations and eliminated disseminated fluconazole-resistant *C. albicans* infection in a neonate with persistent infection while receiving liposomal amphotericin B alone.[255] In addition, voriconazole has been administered successfully to neonates with vertically transmitted cryptococcal meningitis and invasive dermatitis caused by *Aspergillus* spp.[130,189,318,333]

Currently, no data on neonates are available on the use of posaconazole, ravuconazole, isavuconazole, or albaconazole, which are antifungal azoles currently in preclinical and clinical development stages.[274]

Echinocandins (Caspofungin, Micafungin)

The echinocandins include caspofungin, micafungin, anidulafungin, and aminocandin and have activity against most *Candida* spp., including *C. krusei*.[64,239,282] Clinical trials showed caspofungin to be comparable to amphotericin B for treatment of candidemia in adult patients.[281] Caspofungin is not approved for use in children, however, and the appropriate dose and safety of this drug have not been established in neonates. Limited published experience using caspofungin to treat neonates with persistent candidemia or progressive invasive candidiasis caused by *C. albicans* or other *Candida* spp., resistant or unresponsive to amphotericin B or fluconazole, suggests that caspofungin may be a safe and effective alternative in selected neonates, alone or when added to amphotericin B.[55,150,224,257,265,334,405]

Caspofungin penetrates the kidneys well, and good levels are achieved in urine. It does not penetrate into CSF or the eye well,

*See references 54, 101, 102, 122, 169, 300, 311, 320, 324, 384, 397.

however, and it should not be used to treat neonates in whom *Candida* meningitis or endophthalmitis is suspected or proven.[137] It also may be less effective for *Candida* endocarditis. Available pharmacokinetic data suggest that caspofungin also requires higher doses in children and neonates, calculated using body surface area rather than body weight (70 mg/m^2 first day, then 50 mg/m^2 once daily).[274]

Micafungin, the newest echinocandin, is not approved for use in this age group. It has been administered to preterm neonates, however, in phase I clinical trials, and case reports of micafungin administered in combination with voriconazole and amphotericin B to treat severe cutaneous aspergillosis in preterm infants have been published.[157,158,318] Limited pharmacokinetic and safety data suggest that VLBW preterm infants tolerate doses up to 3 mg/kg daily, but may require a larger dose (8 to 15 mg/kg) or more frequent dosing compared with older children and adults.[157,363] Neonatal rabbit models suggest that micafungin does not reliably penetrate the CSF; studies administering micafungin to treat human neonates with *Candida* meningitis are unavailable.[157] Currently, no data or published experiences are available on administration of anidulafungin or aminocandin to neonates with invasive candidiasis.

Immunomodulators

Because antibody to fungal proteins is associated with recovery from invasive candidiasis, immunoglobulin and other immunomodulators may be evaluated eventually for treatment or prevention of invasive *Candida* infections. Currently, preclinical assessments of recombinant antibodies (hsp90, Mycograb, Efungumab) show antifungal activity against *Candida* spp. and *C. neoformans*, and synergy with antifungal chemotherapy agents.[155,231,261] No clinical trials evaluating specific antibody preparations for treatment or prevention of invasive yeast or fungal disease in neonates have been performed.

PREVENTION

FLUCONAZOLE PROPHYLAXIS

The high frequency and significant morbidity and mortality rates associated with *Candida* infections in VLBW neonates have been the impetus behind animal models and clinical studies of preventive strategies using antifungal agents.[187,338,377] In early studies evaluating prophylactic regimens, oral nystatin reduced fungal colonization and disease in preterm infants with a birth weight of less than 1250 g, and it has been used to control a nursery outbreak of *Candida* infection.[92,337] Miconazole oral gel decreased rectal fungal colonization, a predisposing factor for the development of systemic candidiasis, compared with placebo in infants with a birth weight of less than 1750 g.[383] Several single-center and cohort studies and a large, multicenter, randomized, double-blind, placebo-controlled trial have shown that fluconazole prophylaxis reduces *Candida* colonization, invasive candidiasis, or mortality rates in high-risk, VLBW premature neonates.*

These published studies presented slightly different methods of selection criteria for neonates for prophylaxis (universal for all infants weighing <1000 or <1500 g versus risk-based selection), doses (3 to 6 mg/kg/day), dosage schedules (daily versus every other day versus twice weekly), routes of administration (intravenous only versus intravenous followed by oral versus oral alone), and duration of prophylaxis (4 weeks versus 6 weeks), and slightly different outcome measures. Fluconazole administration was not associated with significant toxicity in any of the studies. Despite these encouraging findings, however, not all centers have adopted or advocate universal prophylaxis for VLBW infants with birth weight of less than 1500 to 1000 g. Some centers prefer a risk-based strategy, selecting only VLBW neonates with an additional risk factor, such as *Candida* surface colonization, central venous catheter in place, endotracheal intubation, or recent administration of third-generation cephalosporin.[234] Some centers advocate twice-weekly, rather than daily, prophylaxis to reduce health care costs and to minimize neonates' exposure to antifungals, with the speculation that lower and less frequent dosing may delay or prevent emergence of resistant *Candida* spp.[188]

Most studies of fluconazole prophylaxis in neonates did not report changes in patterns of colonization or rates of invasive disease caused by *Candida* spp. other than *C. albican*s, or emergence of resistance to fluconazole.[188,225,228] These studies may not have had sufficient duration of exposure or power, however, to determine a difference during the study period.[228] More recent reports have documented emergence of *Candida* spp. other than *C. albicans* as predominant pathogens[193,273,319] and emergence of resistance to fluconazole in *C. albicans* and other *Candida* spp.[140,380,410] One center with more than 10 years of experience using fluconazole prophylaxis documented emergence of a resistant strain of *C. parapsilosis* that caused invasive candidiasis in neonates.[319]

Similar to well-documented emergence of bacteria that become resistant to frequently used antibiotics, the emergence of *Candida* spp. resistant to antifungal agents is becoming an important consideration in clinical management of invasive candidiasis.[319,410,411] In addition, emergence of invasive yeasts other than *Candida* spp. may occur. An outbreak of *Rhodotorula mucilaginosa*, a fluconazole-resistant yeast, occurred more recently in an NICU that routinely administered prophylactic fluconazole.[280,371]

OTHER PREVENTIVE STRATEGIES

Preventive strategies other than administration of antifungal agents to reduce *Candida* infections in neonates, are important approaches to investigate. Oral supplementation of breast milk feedings with the probiotic *Lactobacillus casei* subspecies *rhamnosus* was shown more recently to reduce significantly the incidence and intensity of enteric colonization with *Candida* spp. in VLBW neonates.[227] Bloodstream infections with *Candida* spp. occurred in neonates enrolled in previous studies evaluating intravenous immunoglobulin for prevention of nosocomial infections in VLBW neonates; however, no studies have evaluated immunoprophylaxis strategies designed specifically for prevention of nosocomial *Candida* infections in VLBW neonates.[391]

The role of patient cohorting or single-room isolation of neonates with *Candida* colonization or infection also was reviewed more recently.[72,247] Published evidence shows that transmission of *Candida* in the NICU occurs by direct or indirect contact, and cross-infection by health care workers has been documented.[18,63,163,165,167,247,303] Guidelines for isolation procedures are unavailable, most likely because currently available information neither supports nor refutes the use of isolation measures to reduce transmission of *Candida* infections in high-risk neonates.[247]

INVASIVE YEASTS OTHER THAN *CANDIDA*

Disseminated candidiasis is the most common fungal infection in neonates. The dimorphic fungi may infect pregnant women and rarely may be transmitted to the fetus or newborn. More recent reports have suggested that unusual yeasts are becoming impor-

*See references 19, 46, 84, 187, 188, 194, 225, 234, 235, 273, 319.

tant causes of invasive disease in neonates and NICU outbreaks, often with high morbidity and mortality rates.[346] It is important to be aware of emerging pathogenic yeasts because a timely diagnosis and effective management may improve neonatal morbidity and mortality from these often challenging and difficult infections.

CRYPTOCOCCUS

Congenital and perinatal infections with the encapsulated yeast, *C. neoformans*, have been reported.[67,110,189,339,342] Fetal demise may occur, especially in pregnant women with untreated or severe disease.[110] Neonates also may aspirate the organism during delivery and present with fever and respiratory symptoms; meningitis, gastrointestinal involvement, and disseminated disease also may occur.[189] Neonates and young infants who present with disseminated *C. neoformans* also may have a congenital immune disorder, such as severe combined immunodeficiency.[342] Systemic antifungal treatment with amphotericin B is preferred, used in combination with 5-FC. Although *C. neoformans* seems to be susceptible to triazole agents, experience using these compounds to treat neonates with cryptococcosis is limited.[333]

MALASSEZIA

Malassezia genus of yeasts includes seven species. The six lipophilic species are *Malassezia furfur*, *Malassezia globosa*, *Malassezia obtuse*, *Malassezia restricta*, *Malassezia slooffiae*, and *Malassezia sympodialis*, and one species, *Malassezia pachydermatis*, is not strictly lipophilic.[17] *Malassezia* spp. frequently colonize the skin of neonates.[16,20,346] By the time they are 1 week old, 5 percent of neonates have positive skin cultures for *Malassezia* spp.; by 2 weeks of age, 37 to 51 percent of VLBW premature neonates have positive skin cultures. By the time they reach 3 to 6 months of age, healthy newborns have been observed to have colonization rates of 48 to 91 percent, as have preterm neonates in the NICU.[16,20,36] *Malassezia* spp. can be introduced into the NICU on the hands of health care workers and be transmitted to neonates; it also seems to persist on environmental surfaces such as incubators.[70,82,396] The species most frequently associated with neonatal infections and NICU outbreaks include *M. furfur*, *M. pachydermatis*, *M. sympodialis*, and *M. globosa*.

Skin infections with *M. furfur* and *M. sympodialis* have been associated with nonfollicular pustulosis skin conditions, such as neonatal cephalic pustulosis and neonatal acne, in neonates.[20,45,259,293] Skin scrapings of the pustules may show neutrophilic cells and fungal or yeast organisms when examined under the microscope, and fungal culture confirms the organism.[20,293] Neonatal cephalic pustulosis usually is a benign, self-limited condition of the skin and responds to topical antifungal therapy. It must be differentiated, however, from more invasive forms of yeast and fungal diseases. Skin infections with *M. globosa* and, to a lesser extent, *M. sympodialis* are associated with pityriasis versicolor.[17]

Intravascular catheters may become colonized with *M. furfur* or *M. pachydermatis* and cause bloodstream infections, often with serious complications in VLBW preterm neonates.[93,392] Risks for development of invasive disease include previous treatment with broad-spectrum antibiotics and prolonged use of intravascular catheters and intravenous fat emulsions or lipid infusions.[82,396] Neonates may present with systemic signs of sepsis, pulmonary decompensation, and thrombocytopenia.[360] Complications of catheter-associated bloodstream infections with *M. furfur* include peripheral thrombophlebitis, thromboembolism, catheter occlusion, adhesion of the vascular catheter to the vein wall, and endocarditis with intracardiac mass and thromboembolic phenomena.[21,95,191,195,323] Neonatal meningitis and urinary tract infections associated with *M. furfur* and *M. pachydermatis* also have been reported.[70]

Cultures of blood, catheter tip, urine, CSF, and involved tissue should be obtained. The organism also has been seen on peripheral blood smear.[48] Diagnosis may be elusive, however, because recovery of *Malassezia* spp. in routine cultures is rare; if the diagnosis is suspected, special fungal cultures on Sabouraud dextrose agar overlaid with sterile olive oil or media containing long-chain fatty acids may enhance recovery of lipophilic *Malassezia* spp. Treatment involves immediate removal of the catheter and discontinuation of lipid infusions. *Malassezia* spp. seem to be susceptible to most triazole agents and amphotericin B.[144,244] Administration of intravenous amphotericin B deoxycholate is recommended for treatment of neonates with invasive disease caused by *Malassezia* spp.

PICHIA

Pichia (*Hansenula*) *anomala* and *Pichia ohmeri* are emerging yeasts that may cause infection in preterm VLBW neonates and infants with severe combined immunodeficiency. In some NICUs, 17 percent of preterm neonates may be colonized, and single cases and NICU outbreaks associated with indwelling catheters, administration of total parenteral nutrition solutions, and abdominal surgery have been described.[13,23,219,256,278,338,364,401] Invasive diseases in preterm neonates include bloodstream infections, sepsis syndrome, abscesses, and meningitis with ventriculitis. Management includes removal of indwelling catheters and systemic antifungal therapy. Although *Pichia* spp. seem to be susceptible to amphotericin B and some antifungal triazoles, no standard treatment recommendations are available. Amphotericin B in combination with 5-FC usually has been administered as treatment for neonates with invasive *Pichia* spp. infections.[23,219,256,364,401]

TRICHOSPORON

Trichosporon spp. colonize the hair, skin, mucous membranes, and gastrointestinal tract. *T. beigelii* has caused invasive dermatitis, late-onset sepsis, pulmonary infiltrates, and urinary tract infections in VLBW preterm neonates with intravascular catheters.* It also may cause endophthalmitis and endocarditis. *Trichosporon asahii* infection in neonates has been reported more recently, suggesting that this species has emerged as a neonatal pathogen.[270,407]

Trichosporon spp. have been isolated from blood, urine, tracheal aspirates, skin lesions, and catheter tip cultures from neonates.[338] The organism may be identified preliminarily as a germ-tube negative yeast and be confused initially with *Candida* spp. Breakthrough *Trichosporon* infections in patients receiving antifungal agents for candidemia have been reported. The clinician may erroneously attribute persistently positive cultures to a resistant *Candida* spp. rather than *Trichosporon* spp.[35,298] *Trichosporon* spp. also share heat-stable antigens with *C. neoformans*, and produce false-positive cryptococcal antigen tests. A real-time polymerase chain reaction assay to detect *T. asahii* DNA in sera has been developed and may help facilitate rapid establishment of the diagnosis of this potentially fatal infection.[238]

*See references 15, 16, 20, 21, 36, 45, 70, 82, 95, 161, 191, 195, 259, 288, 293, 297, 323, 332, 360, 374, 396.

*See references 35, 125, 140, 147, 196, 270, 298, 338, 362, 385, 407, 408.

The organism has unpredictable susceptibility to antifungal agents, and there are no standardized recommendations for management of neonates with invasive *Trichosporon* infection. Most neonates have been treated with amphotericin B deoxycholate or liposomal amphotericin B.[338,407] The organism may be tolerant or resistant to amphotericin B, caspofungin, and posaconazole, and treatment failures with all of these agents may occur.[385] The organism seems to be susceptible to voriconazole, and isolated case reports of successful therapy in older patients using voriconazole, in combination with amphotericin B, have been published, but no published experience in neonates is available.[35,196,298]

RHODOTORULA

Rhodotorula spp. are another emerging opportunistic yeast that has caused catheter-associated bloodstream infections, endocarditis, peritonitis, meningitis, and endophthalmitis in immunocompromised patients and patients in intensive care units.[280,371] *R. mucilaginosa* and, less frequently, *Rhodotorula glutinis* have been documented to cause disease. *Rhodotorula* spp. usually are susceptible to amphotericin B, itraconazole, and voriconazole, but commonly are resistant to fluconazole. More recently, an outbreak of catheter-associated bloodstream infections with *R. mucilaginosa* in preterm neonates in an NICU administering routine fluconazole prophylaxis was reported.[280] Successful management of infected neonates included removal of the catheters and administration of systemic amphotericin B.

INVASIVE FUNGAL DISEASES

CONGENITAL AND PERINATAL TRANSMISSION

Fetal demise, congenital infection, and perinatal transmission associated with the endemic dimorphic fungi, *Coccidioides immitis* and *Blastomyces dermatiditis*, have been described.* Published case reports are very rare, however. Third-trimester acute infection with *C. immitis* seems to carry increased risk of developing severe illness for the mother and fetus. Perinatal transmission for both organisms likely occurs from neonatal aspiration of infected cervicovaginal secretions from a chronically infected maternal genitourinary tract. Cultures of urine, respiratory secretions, and lung tissue are most likely to yield the organisms. If coccidioidomycosis or blastomycosis is diagnosed in a neonate, treatment with systemic amphotericin B is recommended.

ACQUIRED INVASIVE FUNGAL DISEASE

Invasive fungal disease acquired by VLBW or ELBW premature neonates involves primarily the skin, lungs, or gastrointestinal tract. Because many of the invasive fungi that affect neonates also are angioinvasive, sepsis syndrome with hematogenous dissemination to lung, liver, spleen, CNS, and eye may occur rapidly.

ASPERGILLUS

Aspergillus spp. include *Aspergillus flavus*, *Aspergillus fumigatus*, *Aspergillus glaucus*, *Aspergillus nidulans*, *Aspergillus niger*, and *Aspergillus terreus*. *Aspergillus* spp. frequently are found in the environment and the hospital. Infection in neonates and outbreaks in NICUs have been associated with nearby construction or renovation.[9,11,143,271] Diseases associated with *Aspergillus* infection of the neonate include primary cutaneous aspergillosis or invasive dermatitis, pulmonary aspergillosis, and disseminated aspergillosis. Disseminated disease may involve the heart, liver, spleen, lungs, CNS, or eye.*

Neonatal aspergillosis usually manifests in the second week of life.[271,308,400] Risk factors include extreme prematurity and VLBW or ELBW, administration of steroids and broad-spectrum antibiotics, hyperglycemia, and trauma of the extremely vulnerable skin of the premature infant.[143,308] The delicate skin of VLBW infants can be macerated easily by adhesive tape, traumatized at the site of insertion of an indwelling intravascular catheter or by surgical procedures, or damaged by pressure sores, providing a portal of entry for the fungus. Invasive fungal dermatitis then occurs (Fig. 76–5). *Aspergillus* spp. also may gain entry through the lungs and cause invasive pulmonary aspergillosis.[143] Because the fungus has a predilection for invasion of blood vessels, it can disseminate quickly in the bloodstream and occlude vessels, causing thrombosis, infarction, and necrosis of tissue (see Fig. 76–5B and C).

Establishment of the diagnosis requires a high index of suspicion and documentation of the fungus by histopathologic examination and culture of the involved tissue or organ. Suspicious skin lesions may appear early as erythematous plaques, vesicles, scales, or persistent maceration; advanced disease may show dark, necrotic skin lesions; skin biopsy that shows invasion below the stratum corneum establishes the diagnosis of invasive fungal dermatitis (see Fig. 76–5A and B).[400] *Aspergillus* also may be recovered from tracheal aspirate cultures, ascites, and CSF.[135,143] No studies have evaluated the performance of galactomannan antigen tests for the early diagnosis of invasive aspergillosis in neonates.

Early diagnosis followed by prompt and aggressive treatment of a neonate with invasive aspergillosis is important to minimize the usually high morbidity and mortality rates associated with this disease. Traditional treatment has included prolonged, high-dose amphotericin B deoxycholate or lipid complex and surgical excision of accessible tissue, such as the skin and soft tissue, if local disease progresses despite administration of systemic antifungal therapy.[10,271,281] Treatment failures frequently occur.[130,135,143,149] More recently, case reports of premature neonates with primary cutaneous aspergillosis who were treated successfully with voriconazole alone or in combination with amphotericin B lipid complex and micafungin have been published.[130,318]

ZYGOMYCETES (*ABSIDIA, RHIZOPUS, MUCOR, RHIZOMUCOR*)

Zygomycosis, a term preferred now over mucormycosis, usually is caused by the following species of fungi: *Absidia* (*Absidia corymbifera*), *Rhizopus* (*Rhizopus arrhizus*, *Rhizopus microsporus*), *Mucor* (*Mucor amphibiorum*, *Mucor circinelloides*, *Mucor hiemalis*, *Mucor indicus*, *Mucor racemosus*, *Mucor ramsissimus*), or *Rhizomucor* (*Rhizomucor pusillus*, *Rhizomucor miehei*, *Rhizomucor variabilis*). Zygomycosis in a neonate usually manifests during the second and third weeks of life.[296,340,412] Health care–associated outbreaks have been linked to contaminated dressings, bandages, or armboards. Risk factors for acquisition of infection include VLBW, extreme prematurity, indwelling intravascular catheters, exposure to elastic bandages, adhesive dressings and tapes, and armboards or tongue depressors used to split extremities, and host factors such as congenital immunodeficiency or genetic disorders of metabolism that cause metabolic acidosis.[213,252,412]

The Zygomycetes are angiotrophic and aggressively invade blood vessels very early in the disease process (Fig. 76–6C). Invasion of blood vessels leads to thrombosis, infarction, and tissue

*See references 5, 75, 89, 210, 214, 232, 329, 370, 388, 398, 409.

*See references 9, 11, 130, 135, 143, 149, 159, 271, 281, 308, 309, 318, 400.

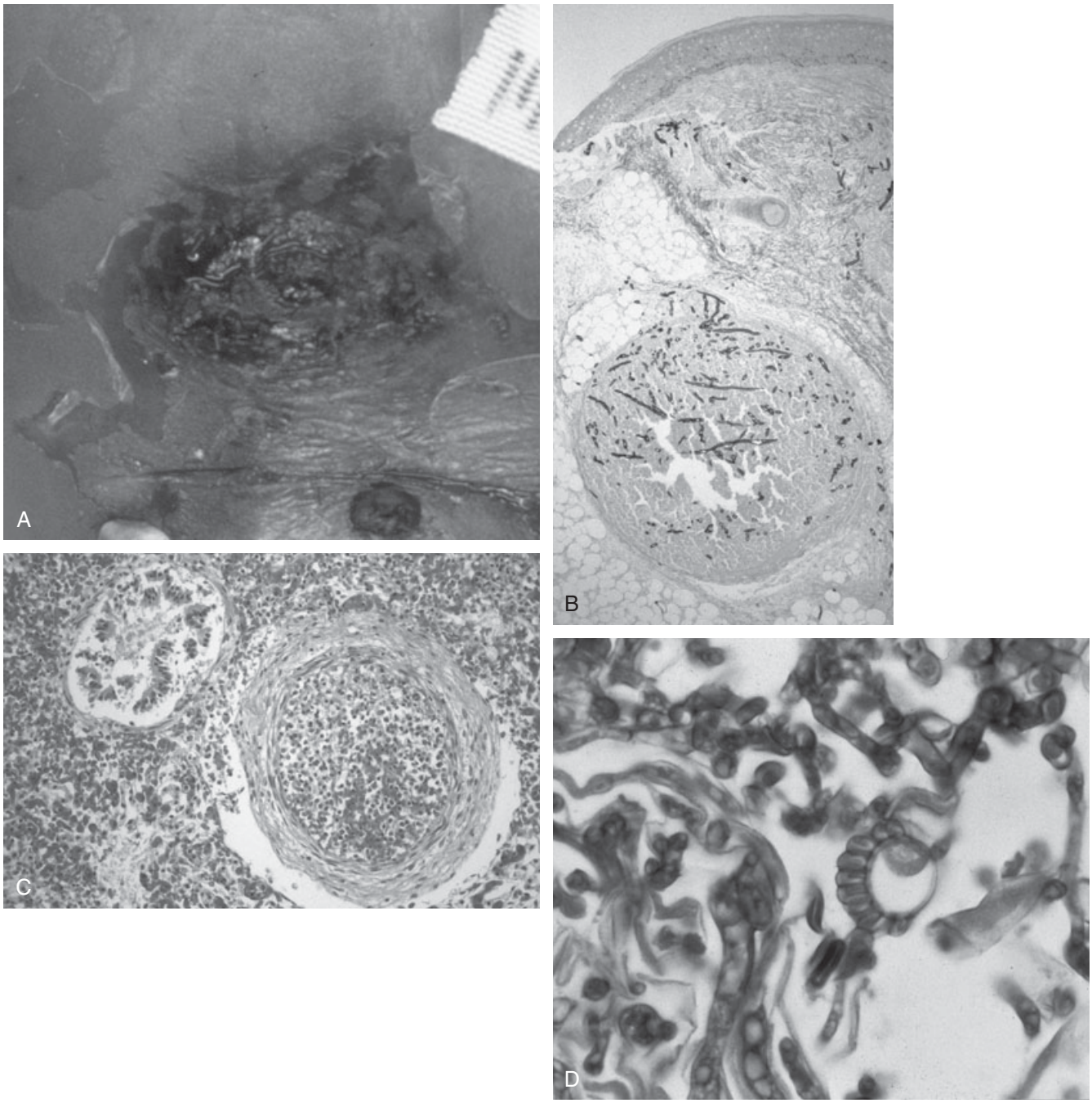

Figure 76–5 A-D, Invasive fungal dermatitis in a premature neonate caused by *Aspergillus flavus*. **A,** Fatal invasive fungal dermatitis with necrotizing fasciitis and disseminated fungal disease in a 23-day-old premature infant born at 26 weeks' gestation. **B-D,** Autopsy examination impressively showed angioinvasive nature of *A. flavus* with infected thrombus in skin (**B**) and lung (**C**) tissue. Uniformly broad, hyaline, septated, right angle branching hyphae with dramatic fungal fruiting body of *A. flavus* were seen in a lung lesion of this infant (**D**) (methenamine silver nitrate stain, 40×, 100×, and 400×, respectively, original magnification). *(Courtesy of Dr. Edwina J. Popek, Department of Pathology, Baylor College of Medicine and Texas Children's Hospital, Houston, TX.)*

necrosis; destruction of local tissue can be extensive, and the fungus may disseminate throughout the body and CNS. The most common presentation of invasive zygomycosis in neonates is primary cutaneous disease. Cutaneous zygomycosis usually begins at an area of minor skin trauma or at the site of intravenous catheter insertion that has come in contact with adhesive tape or bandages, first appearing as a cluster of necrotic vesicles or scales, which then rapidly progresses to extensive necrotizing cellulitis.[14,91,105,168,195,213,252,266,322] It usually involves the skin and soft tissues of the upper extremities, but the lower extremities, abdominal wall, or face also may be presenting sites.

Gastrointestinal zygomycosis in a premature neonate often mimics NEC clinically, but without the characteristic radiographic bowel patterns; rarely, it has presented as Hirschsprung disease.[6,412] Intestinal perforation, of small or large bowel or appendix, with secondary peritonitis, may occur (see Fig. 76–6).[4,6,81,91,97,296,340] Rhinocerebral zygomycosis classically manifests in diabetics with ketoacidosis, but it also may affect neonates with

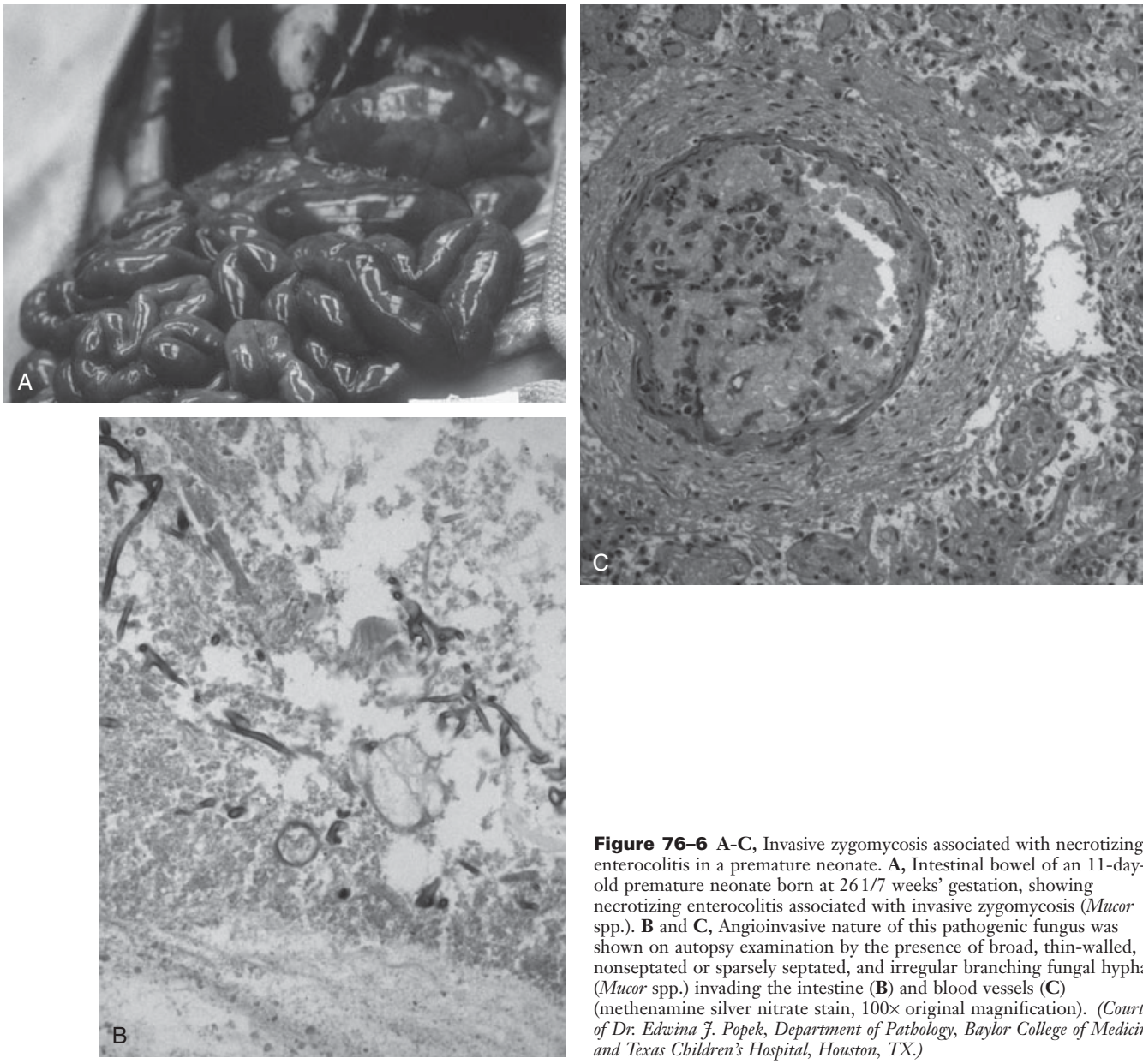

Figure 76–6 **A-C,** Invasive zygomycosis associated with necrotizing enterocolitis in a premature neonate. **A,** Intestinal bowel of an 11-day-old premature neonate born at 26 1/7 weeks' gestation, showing necrotizing enterocolitis associated with invasive zygomycosis (*Mucor* spp.). **B** and **C,** Angioinvasive nature of this pathogenic fungus was shown on autopsy examination by the presence of broad, thin-walled, nonseptated or sparsely septated, and irregular branching fungal hyphae (*Mucor* spp.) invading the intestine (**B**) and blood vessels (**C**) (methenamine silver nitrate stain, 100× original magnification). *(Courtesy of Dr. Edwina J. Popek, Department of Pathology, Baylor College of Medicine and Texas Children's Hospital, Houston, TX.)*

conditions associated with metabolic acidosis and premature infants. The fungus enters via the nasal passages and invades the sinus cavities and orbits, spreading to the CNS by fungal thrombosis of cavernous sinus and internal carotid artery.[413] Pulmonary zygomycosis also may occur, either as a primary pneumonia with consolidation and necrosis or secondary to vascular dissemination from a cutaneous or gastrointestinal source.[413] Disseminated zygomycosis is a fatal fungal sepsis syndrome, usually involving all major organ systems and the CNS.

Diagnosis is established by histopathologic examination of tissue, in which characteristic fungal elements invading the tissue and blood vessels can be seen. Fungal culture confirms the identification of the fungus. Successful management includes early diagnosis and aggressive surgical débridement and resection of involved tissues, to clear margins of resection.[252] Amputation or extensive skin grafts may be necessary in some patients.[205] Aggressive surgery is important because the fungus is resistant to most currently available antifungal agents, including the echinocandins and first-generation and second-generation triazoles. The fungus may be inhibited by amphotericin B given in high doses (1.5 mg/kg/day of amphotericin B deoxycholate or ≥5 mg/kg of lipid formulations), which should be administered as soon as the diagnosis is suspected. The newest triazole compound, posaconazole, seems to have activity against zygomycetes, but experience in administering this new agent to neonates is unavailable.[302]

RARE AND UNUSUAL PATHOGENIC FUNGI

Other unusual pathogenic fungi that have been reported to cause invasive, and often fatal, disease in neonates include *Curvularia* (especially *Curvularia lunata*), which caused invasive fungal dermatitis in a premature infant, caused sternal wound infection complicating cardiac surgery in another neonate, and complicated disseminated neonatal herpes simplex infection in a third neonate (unpublished data) (Fig. 76–7).[309,406] *Phialemonium obova-*

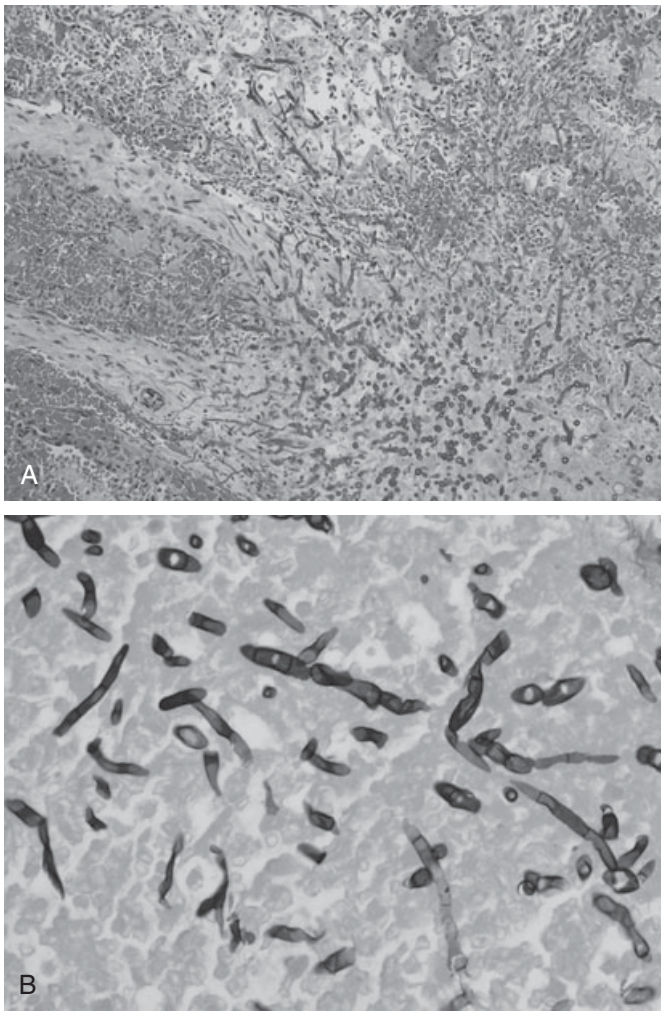

Figure 76–7 **A** and **B,** Disseminated *Curvularia* infection. Fatal invasive fungal infection with *Curvularia* in a 2-week-old term neonate with liver failure because of neonatal herpes simplex virus infection. **A,** The lungs showed angioinvasive fungal hyphae extending from the lumen of a thrombosed vessel into the adjacent alveolated lung parenchyma (hematoxylin and eosin stain, 100× original magnification). **B,** The fungal hyphae of *Curvularia* are narrow, frequently septated, with acute angle branching (methenamine silver nitrate stain, 400× original magnification). (See companion Expert Consult web site for color version.) *(Courtesy of Dr. Megan K. Dishop, Department of Pathology, Baylor College of Medicine and Texas Children's Hospital, Houston, TX.)*

tum reportedly has caused fatal endocarditis in a premature neonate.[138] *Bipolari spicifera* has caused invasive fungal dermatitis with dissemination in a preterm neonate.[250] Several cases of *Trichophyton* (*Trichophyton rubrum* and *Trichophyton violaceum*) causing localized scalp lesions, similar to tinea capitis, in neonates have been reported.[33,71,258,372]

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CHAPTER 77

Congenital Toxoplasmosis

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Toxoplasma gondii is an obligate intracellular protozoan parasite (phylum Apicomplexa, class Sporozoasida, order Eucoccidiida).[126] The first report of human infection occurred in 1908.[18] In 1937, Wolf and Cowen[132] reported a case of congenital granulomatous encephalitis, which Sabin[103] later correctly attributed to *T. gondii* infection. Congenital toxoplasmosis results from placental infection that develops after primary maternal infection and subsequent hematogenous spread to the fetus.[95] *Toxoplasma* is ubiquitous in nature, with the cat family being the definitive host.[35,37]

The organism exists in three forms: (1) *oocyst*, which is excreted in cat feces and is infectious within 2 to 5 days after sporulation; (2) a proliferative stage called a *tachyzoite*; and (3) a tissue cystic stage called a *bradyzoite*. Mammals or birds ingest oocysts from contaminated plants and soil, or, if carnivorous, they may ingest

bradyzoites. When ingested, *Toxoplasma* replicate asexually, and tissue cysts accumulate in the organs and skeletal muscle of these animals. Possible routes of infection for humans are direct ingestion of sporulated oocysts from soil, water, fruits, or vegetables that have been contaminated by cat feces, or ingestion of undercooked meat containing bradyzoites.[6,8,26,95] Humans also have become infected after undergoing organ transplantation, after receiving blood product transfusions, and after having laboratory accidents.[95]

The prevalence of antibody to *T. gondii* in women of childbearing age in the United States varies from approximately 3 to 30 percent, depending on the region of the country.[57,58,111] The lowest seroprevalence rates have been found in the Mountain states, where a dry climate does not favor survival of oocysts in the soil. The highest rates have been noted in the northeastern and southeastern states. In contrast, the seropositivity rate for women in Paris may be 70 percent. These widely disparate seroprevalence rates in different adult populations throughout the world are explained by differences in eating and sanitation practices and conditions that affect survival of oocysts in the environment.[58,95] More recent studies have documented a declining prevalence of infection in the United States.[57] This decline likely is related to decreased rates of contamination of commercial meats with *Toxoplasma* and increased educational efforts aimed at informing the public about avoiding acquisition of infection.[27,58]

The prevalence of congenital infection in the United States has been documented to be 0.08 per 1000 births (1 in 12,000 births) by IgM screening of blood specimens collected on filter paper from newborns in Massachusetts and New Hampshire.[49] This figure compares with a rate of 3 to 10 per 1000 live births in Paris and Vienna. In Massachusetts, a case-control study involving 14 years of newborn screening for congenital toxoplasmosis found that birth of the mother outside the United States, particularly in Cambodia and Laos, and the educational level and higher gravidity of the mother were strongly predictive of congenital infection.[55]

TRANSMISSION

Infection of the fetus occurs during maternal parasitemia, with subsequent infection of the placenta caused by tachyzoites.[36,95] Placental infection represents an important intermediary step between maternal and fetal infection. A delay of 16 weeks between placental infection and subsequent infection of the fetus has been noted and is termed the *prenatal incubation period*.[95] Fetal infection occurs as a consequence of maternal primary infection acquired during pregnancy.[36,83,95] Rare instances of transmission have been reported in women with primary infection shortly before conception.[40,122] Reactivation of latent *Toxoplasma* infection during pregnancy does not lead to fetal infection except rarely in immunocompromised women, such as women infected with human immunodeficiency virus (HIV).[80,87,95,134] In these instances, congenital infection has been documented, although the risk is very low.[30] Maternal re-infection leading to congenital toxoplasmosis also has been reported rarely.[40,95] Congenital toxoplasmosis has occurred in twins and triplets.[13,95] Despite these occasional case reports, most congenital infections are the result of primary maternal infection during pregnancy.

The overall fetal infection rate from untreated maternal infection during pregnancy is approximately 40 percent, although the rate depends on when in pregnancy the mother becomes infected (Table 77–1).[31,95,134] Although the actual rate of fetal infection increases as pregnancy advances, the severity of clinical manifestations is greatest when maternal infection is acquired during the first trimester.[31,95,134] Transmission during breast-feeding in humans has not been shown, although the organism has been detected in human milk.

TABLE 77–1 Vertical Transmission and Severity of Disease for *Toxoplasma gondii* by the Timing of Maternal Infection during Pregnancy

	Trimester of Maternal Infection		
	First	*Second*	*Third*
Overall transmission rate (%)	15	30	60
Proportion of infected children with specific disease severity			
Stillborn/perinatal death (%)	35	7	0
Subclinical (asymptomatic) (%)	18	67	90
Mild disease (%)	6	18	10
Severe disease (%)	41	8	0

CLINICAL MANIFESTATIONS

Acute maternal infection acquired early in pregnancy may lead to fulminant fetal infection and result in stillbirth, nonimmune fetal hydrops, preterm birth, and perinatal death.[1,5,49,95] Chronic *Toxoplasma* infection has been associated only rarely with sporadic abortion.[95] Most infants born with congenital *Toxoplasma* infection are asymptomatic in the neonatal period, with clinical signs and symptoms being present in only approximately 25 percent of infants.[2,55,63,73] Long-term follow-up of these asymptomatically infected infants reveals eye or neurologic disease, or both, in 80 to 90 percent by the time they reach adulthood.[20,31,49,55,116,124] The clinical manifestations of congenital toxoplasmosis in newborns generally are indistinguishable from the manifestations associated with other agents of congenital infection, such as cytomegalovirus and *Treponema pallidum*. The most characteristic clinical findings, frequently referred to as the *classic triad of congenital toxoplasmosis*, are chorioretinitis, intracranial calcifications, and hydrocephalus.[32,95] They are seen in approximately 86 percent (chorioretinitis), 37 percent (intracranial calcifications), and 20 percent (hydrocephalus) of symptomatic infants. These conditions often are accompanied by a combination of signs and symptoms including anemia (59%), jaundice (43%), splenomegaly (41%), seizures (41%), fever (40%), hepatomegaly (34%), lymphadenopathy (32%), microcephaly (9%), and eosinophilia (9%).[32,95]

Involvement of the central nervous system is a hallmark of congenital *Toxoplasma* infection.[12a,13,20,32,72,95,100,111] Hydrocephalus usually is obstructive and often requires ventriculoperitoneal shunting.[95,111] It may be the only manifestation of disease. Abnormalities in cerebrospinal fluid (CSF) occur in approximately 63 percent of infected infants; characteristically, they consist of lymphocytic pleocytosis and an elevated protein level.[32,72,95] The markedly high protein concentrations in ventricular fluid, often exceeding 1 g/dL, and hydrocephalus are explained by periaqueductal and periventricular vasculitis with necrosis, which are associated specifically with toxoplasmosis.[95] Inflammation and necrosis involving the hypothalamus have resulted in hypothermia and hyperthermia. When microcephaly is present, it indicates severe brain damage. Intracranial calcifications may be single or multiple but typically are generalized and located in the caudate nucleus, choroid plexus, meninges, and subependyma.[25,88,95] Periventricular calcifications similar to those associated with cytomegalovirus also have been described. They are visualized best by computed tomography (CT),[72] although ultrasonography has been shown to have excellent correlation with CT findings.[10] The calcifications may resolve with appropriate antimicrobial therapy.[88]

Neurologic sequelae from untreated congenital toxoplasmosis include mental retardation (87%), seizures (82%), spasticity and palsies (71%), and deafness (15%).[32,72,95,111] *Toxoplasma* has been detected in the inner ear and mastoid, with the associated inflam-

mation resulting in deafness. An ascending flaccid paralysis with myelitis also has been reported.[95]

Chorioretinitis caused by *Toxoplasma* occurring at any age is likely to be a result of congenital infection, although acute acquired toxoplasmosis was reported to cause approximately 40 percent of childhood chorioretinitis in one study.[44,79,95] In infants, the most common manifestation is strabismus, whereas defects in visual acuity predominate in older children and adults. The characteristic lesion consists of a focal necrotizing retinitis that often is bilateral.[79] Organisms are present in the retina, and they have a predilection for the macula, with resultant loss of vision. Involvement of the optic nerve also may occur. Other complications include iridocyclitis and cataracts.[79]

Other manifestations of congenital toxoplasmosis include nonspecific maculopapular or petechial rash, myocarditis, pneumonitis, thrombocytopenia, nephrotic syndrome, and abnormalities in immunoglobulin production.[52,95] Bony abnormalities consisting of metaphyseal radiolucencies similar to those seen in congenital syphilis also have been reported. A variety of endocrine abnormalities, including hypothyroidism, diabetes insipidus,[72,95] precocious puberty, and growth hormone deficiency, may occur. All these abnormalities are related to the fact that the organism is capable of widespread dissemination throughout the brain and body, with involvement of virtually all organ systems.

DIAGNOSIS

The diagnosis of congenital toxoplasmosis can be established by isolating the organism from infected body fluids and tissues, such as the placenta, amniotic fluid, fetal blood obtained by cordocentesis, umbilical cord blood, infant blood, and CSF.[22,82,131] Such isolation involves inoculation of the specimen intraperitoneally into laboratory mice and requires approximately 4 to 6 weeks for confirmation. Testing is not widely available but can be done at the Toxoplasma Serology Laboratory, Palo Alto Medical Foundation (860 Bryant Street, Palo Alto, CA 94301; 415-326-8120). Alternatively, a diagnosis can be established by histopathologic examination of the placenta in which tachyzoites are revealed. Polymerase chain reaction (PCR) has been used successfully to detect *Toxoplasma* DNA in amniotic fluid, placenta, CSF, and fetal and infant blood.[22,48,50,82,101,102,131] PCR performed on amniotic fluid obtained by amniocentesis is the preferred method of confirming in utero infection. A negative amniotic fluid PCR result does not always rule out congenital infection, however.[82,131] In addition, interlaboratory variability in performance of PCR assays has been documented.

The most practical and widely used method of establishing the diagnosis is by testing maternal and newborn blood for serologic evidence of *Toxoplasma* infection. Major problems associated with serologic diagnosis include determining the acuity of the maternal infection and differentiating endogenous from transplacentally acquired antibodies in neonatal/fetal infection. In addition, many commercially available immunoglobulin M (IgM) assays have been associated with significant false-positive and false-negative results.[125,131] Table 77–2 is a brief overview of strategies for establishing the serologic diagnosis in maternal and fetal/neonatal sera, including when to send sera for testing in a reference laboratory.

Tests are available that detect *T. gondii*–specific IgG, and they include the Sabin-Feldman dye test,[104] which is considered to be the gold standard but requires live organisms/tachyzoites; the indirect immunofluorescent antibody test; IgG enzyme-linked immunosorbent assay (ELISA); and direct agglutination.[2,82,93,131,133] An AC/HS differential agglutination test has been developed as a confirmatory test to differentiate acute and chronic maternal infection.[16,24,68] This test compares the IgG serologic titer obtained with the use of formalin-fixed tachyzoites (HS antigen) with the titer obtained with acetone-fixed (or methanol-fixed) tachyzoites (AC antigen). The latter preparation contains stage-specific *Toxoplasma* antigens that are recognized by IgG antibodies only during early infection. The enzyme-linked immunofiltration assay compares the immunologic profile of the mother's antibody response and that of her child at delivery and allows discrimination between

TABLE 77–2 Guidelines for Interpretation of Serologic Tests for Toxoplasmosis in Newborns

Newborn Serology		
IgG	***IgM***	**Interpretation**
Positive	Negative	For newborns, 25% have false-negative IgM (competition with high maternal IgG or failure to produce adequate IgM). Repeat testing on second specimen. Consider using alternative assay (IgM ELISA if IFA was used first, IgM IFA if ELISA was used first) If clinically high suspicion (documented acute maternal primary infection in pregnancy *or* compatible illness in infant), specimen should be sent to a reference laboratory for further testing. Clinical evaluation may be initiated (see below) *Reference Laboratory*: 0.5 mL serum to Toxoplasma Serology Laboratory, Palo Alto Medical Foundation, 860 Bryant Street, Palo Alto, CA 94301, 415-326-8120
Positive	Positive	IgM ELISA can be false-positive in the presence of rheumatoid factor, and IgM IFA can be false-positive in the setting of antinuclear antibodies. Further testing needed (IgM ISAGA, IgA EIA, IgE EIA/ISAGA, AC/HS antigen, placental PCR) in reference laboratory (see above) If high likelihood of infection (known maternal primary infection *or* child with clinically compatible illness), begin work-up while reference laboratory tests are pending Initial clinical evaluation of the infant should include ophthalmologic examination, auditory brain stem response to 20 dB of sound, serum alanine aminotransferase, total and direct bilirubin, complete blood count, CSF examination (cell count, glucose, protein, and total IgG), and brain CT for calcifications (more recent data suggest excellent agreement between CT and ultrasound, which can be obtained more quickly and without the need for sedation)
Positive	Equivocal	Repeat testing on second specimen. Consider using alternative assay (IgM ELISA if IFA was used first, IgM IFA if ELISA was used first). If clinically high suspicion (documented acute maternal primary infection in pregnancy *or* compatible illness in infant), specimen should be sent to a reference laboratory (see above)
Negative	Negative	Newborn IgG reflects maternal antibody, so no evidence of maternal infection. Newborn not infected
Negative	Positive	Newborn IgG reflects maternal antibody, so no evidence of maternal infection. Newborn not likely to be infected; consider false-positive IgM. Repeat assay on second specimen (IgG and IgM). Consider using alternative assay (IgM ELISA if IFA was used first, IgM IFA if ELISA was used first)

AC/HS, differential agglutination acetone-fixed/formalin-fixed tachyzoites; CSF, cerebrospinal fluid; CT, computed tomography; EIA, enzyme immunoassay; ELISA, enzyme-linked immunosorbent assay; IFA, immunofluorescent assay; Ig, immunoglobulin; ISAGA, immunosorbent agglutination assay; PCR, polymerase chain reaction.

IgG antibodies of maternal origin and IgG synthesized by the fetus; it has a reported sensitivity of 94 percent and a specificity of 99 percent. The IgG avidity test may be more widely available than is the AC/HS test, which requires working with tachyzoites, and allows timing of the infection from a single maternal specimen.[10,131]

After primary infection occurs, the avidity of IgG antibody for *T. gondii* antigen is low. Urea dissociates low-avidity antibodies, and by determining the percentage of antibodies that resist elution by 6 mol/L urea, one can differentiate infection acquired within the past 12 weeks from an older infection. This test is useful in the first 12 weeks of gestation because the presence of high-avidity antibodies excludes the acquisition of infection in the previous 3 months.

Tests that detect *T. gondii*–specific IgM include (1) the double-sandwich IgM ELISA, which has a sensitivity of 75 percent and a specificity of 100 percent[23,94]; (2) the IgM immunosorbent agglutination assay, which is the most sensitive test but should not be performed on umbilical cord blood because even small quantities of maternal IgM antibodies contaminating the specimen would result in a false-positive test result[84-86]; and (3) the IgM immunofluorescent antibody test, which is not recommended as a first screening test for IgM antibodies because of lower sensitivity than that of either the IgM ELISA or the IgM immunosorbent agglutination assay but may be useful in conjunction with the IgM ELISA.[2,7,23,131] Other tests that can help differentiate acute infection further include a *T. gondii*–specific IgA ELISA and IgA immunofiltration assay; a *T. gondii*–specific IgE immuno-filtration assay; and IgG, IgM, and IgA immunoblotting tests.[19,22,82,92,93,113,131]

Evaluation of a pregnant woman and fetus typically is prompted initially either by detection of seroconversion in the context of a screening program or by serologic evaluation in the context of an acute illness compatible with toxoplasmosis. If the initial IgG is negative, a follow-up test needs to be performed to exclude seroconversion. If the initial test on a pregnant woman reveals IgG antibody, an attempt needs to be made to determine if the primary infection occurred during gestation. An IgM antibody test should be ordered, but the clinician must be aware of the potential for false-positive and false-negative results.[82,125,131] The IgG avidity test can be helpful if the pregnancy is within the first 12 weeks, with a high avidity test virtually excluding infection within the past 12 weeks. If the IgM is determined to be truly positive, and the IgG avidity test does not allow the clinician to exclude acute infection during pregnancy, the clinician needs to move to more sophisticated testing through a reference laboratory (see earlier) because IgM may persist for 12 to 18 months after acute infection.

If recent maternal infection is documented, the fetus should be evaluated and therapy should be started in the mother (see treatment section). The fetus is evaluated by ultrasound, and amniotic fluid should be tested for specific *Toxoplasma* DNA by PCR. PCR has supplanted the need for cordocentesis, and a positive result confirms fetal infection.[95] Postnatally, serologic testing of paired maternal and infant sera should be performed at a reliable laboratory that includes assays for IgG, IgM, IgA, and IgE antibodies. Subinoculation of placental tissue, amniotic fluid, and umbilical cord blood into mice should be considered. If the results of these tests suggest the possible presence of infection, the newborn should be evaluated fully by a complete blood cell count and platelet determination, liver function tests, CSF evaluation (including tests for IgG and IgM antibodies and PCR),[7,72,95] ophthalmologic examination, and CT scan or ultrasound of the head.

The presence of neonatal IgM antibody in serum or CSF or a positive PCR in serum or CSF indicates congenital infection. In addition, at-risk infants should have serologic follow-up to detect increasing serum IgG titers during the first year of life or persistent IgG antibody beyond 12 to 15 months of age, which would be indicative of congenital infection.[7,72] Uninfected infants have a continuous decline in *Toxoplasma* IgG titer, with no detectable IgM or IgA antibodies.

Low IgG titers and an AC/HS differential agglutination test that indicates remote maternal infection do not require further evaluation of the mother or infant, unless the mother is infected with HIV. Because fetal infection has occurred during chronic *Toxoplasma* infection in HIV-infected pregnant women, infants of co-infected mothers should be evaluated serologically at birth for evidence of congenital infection. Whether HIV-infected pregnant women who have low $CD4^+$ counts and who are seropositive for *Toxoplasma* antibody should receive empiric therapy to prevent fetal infection remains unclear. At present, available data are insufficient for routinely recommending such therapy.

TREATMENT

PREGNANT WOMEN

Treatment of an acutely infected woman during pregnancy with spiramycin may prevent transmission of the infection to the fetus.[14,33,127] A meta-analysis including 20 cohorts with 1721 infected mothers and 506 infected children suggested a reduction in transmission (odds ratio 0.48, 95% confidence interval 0.28 to 0.80) if therapy was started within 3 weeks of maternal seroconversion.[120] These results, considered along with the numerous women studied by individual investigators, strongly suggest that intrauterine treatment does reduce transmission of maternal infection to the fetus.[3,15,21,39,47,51] Contrary to the many individual studies, the meta-analysis failed to show that intrauterine treatment ameliorated the manifestations of congenital infection in the children who were born infected.

Spiramycin treatment of pregnant women with recently acquired primary infection should be instituted empirically while further testing is being done, in the hope of preventing spread of infection to the fetus. When fetal infection has occurred, however, maternal treatment with spiramycin does not seem to alter the evolution and severity of disease in the fetus, which is why evaluation of a potentially exposed fetus by PCR amplification of amniotic fluid permits informed decisions to be made concerning termination of pregnancy or treatment of the fetus in utero with pyrimethamine-sulfadiazine.

SEQUENTIAL FETAL AND POSTNATAL TREATMENT

Reports have described improved outcomes in patients treated in utero with continuing treatment during the first year of life compared with historical controls.[39,51] Pregnancies in which fetuses had obvious manifestations on ultrasound examination and most pregnancies with definite first-trimester infection were terminated, however.

Nonetheless, what is remarkable is that when this method of initiating aggressive treatment of fetuses in utero was applied, retinal disease was reported in only 3 of 50 such infants monitored until they reached 2 years of age. This finding contrasts with the presence of retinal or neurologic involvement in 50 percent of asymptomatic newborns detected by serologic screening in Massachusetts[49] and in 75 percent of children whose pediatricians referred them to our National Collaborative Treatment Trial for treatment in the perinatal period.[72] The outcome of pregnancies with infection acquired in the first trimester after in utero treatment also has been reported to be favorable in another study[5] in which only pregnancies in which the fetus had hydrocephalus were terminated.

TABLE 77–3 Treatment Guidelines for Congenital Toxoplasmosis

Condition	Medication	Dosage	Length of Therapy
Congenital infection	Pyrimethamine +	2 mg/kg/day for 2 days, then 1 mg/kg/day for 6 mo, then 3 times weekly (M-W-F) for 6 mo	1 year; monitor weekly complete blood counts and platelets
	Sulfadiazine +	100 mg/kg/day divided twice daily	
	Folinic acid +	5-10 mg 3 times weekly	Dose adjusted to maintain adequate complete blood count
	Prednisone	1 mg/kg/day divided twice daily	Until resolution of elevated CSF protein (<1 g/dL) or sight-threatening chorioretinitis resolves
Pregnant women—acute infection first 21 wk of gestation	Spiramycin—available on request from the Food and Drug Administration (301-827-2127)	1.5 g q12h without food	Until fetal infection documented or excluded (amniotic fluid PCR) at 21 wk gestation. If fetus infected, change to pyrimethamine + sulfadiazine + folinic acid until delivery
Pregnant women—fetal infection confirmed (amniotic fluid PCR positive)	Pyrimethamine +	100 mg/day divided twice daily for 2 days, then 50 mg/day	Until delivery
	Sulfadiazine +	100 mg/kg/day divided twice daily (maximum 3 g/day)	
	Folinic acid	5-20 mg/day	

CSF, cerebrospinal fluid; M-W-F, Monday, Wednesday, Friday; PCR, polymerase chain reaction.

CONGENITAL INFECTION

POSTNATAL TREATMENT

Data regarding the efficacy of postnatal treatment of infants with congenital *Toxoplasma* infection are becoming available.[45,46,49,72] The controlled National Collaborative Treatment Trial is in progress in Chicago.[72] This study seeks to define optimal therapeutic regimens. Physicians treating patients with congenital *Toxoplasma* infection who are younger than 2.5 months may wish to contact this multidisciplinary group regarding potential enrollment of their patients in that study (773-834-4152).

Outcomes to date from the National Collaborative Treatment Trial are substantially better for most, but not all, infants treated from the neonatal period for 12 months with pyrimethamine-sulfadiazine and leucovorin (Table 77–3) than for historical controls receiving no or short-course therapy.[72] Signs of active infection resolve within weeks of initiation of treatment. Overall, 18 of 66 (27%) children in the National Collaborative Treatment Trial have IQ results less than 70 compared to 86 of 101 (85%) in the reported literature.[32,72] No significant diminution in cognitive function occurs over the course of time, and most treated children are functioning well in regular school classrooms. No sensorineural hearing loss has been ascribable to congenital toxoplasmosis in treated children.[72,100,116] A subset of children with significant irreversible neurologic damage already present in the perinatal period have manifested profound developmental delay, motor impairment, and seizures. For the most part, these were children with hydrocephalus, high CSF protein, minimal improvement in brain CT scans after shunting, and often substantial delays in shunt placement or needed revision for shunt failure or other intercurrent medical problems.[116]

Although treatment during the first year of life arrests all signs of active disease, results in normal cognitive and motor outcome for most children and may result in resolution of seizures without recurrence for some treated children, the currently available drugs do not eradicate all cysts containing bradyzoites.[51] In most children, serologic titers of *T. gondii*–specific antibodies rebound 3 to 4 months after treatment is discontinued.[123] To date, new retinal lesions have occurred in 17 of 58 children in the National Collaborative Treatment Trial during 3 to 10 years of follow-up after the 1-year course of treatment.[72] Infected children undergo retinal examination each month for 3 months after discontinuing treatment around their first birthday, then every 3 months until they are old enough to describe visual symptoms accurately, and then every 6 months. In addition, an ophthalmologic evaluation should be performed promptly for any acute visual signs or symptoms that may be related to recrudescence of congenital ocular toxoplasmosis.[79]

Toxoplasmosis and coexistent HIV infection in children is increasingly rare since the advent of highly active antiretroviral therapy for HIV. Most children reported with toxoplasmosis and HIV have had congenital toxoplasmosis and have been symptomatic. For such children, therapy with pyrimethamine-sulfadiazine plus folinic acid is recommended in the doses presented in Table 77–3.

Indications for corticosteroid therapy with prednisone (0.5 mg/kg twice a day) are a CSF protein concentration of 1 g/dL or greater or chorioretinitis that threatens vision. Prednisone is continued until these findings are resolved.

PREVENTION

Routine serologic screening of women during pregnancy has been an effective means of prevention in France and Austria, and it has been advocated in other areas where the incidence of congenital toxoplasmosis remains high.[15] Such strategies have been criticized regarding the cost-effectiveness and feasibility of such strategies and the lack of randomized controlled trials definitively proving benefit.[44,121] Although such studies would be helpful, they are unlikely to be performed, given the current cohort data supporting the interventions. Neonatal screening for IgM antibody also has been advocated so that asymptomatic infants can be detected and treated before neurologic symptoms develop.[45,46,49,61,69,98] This strategy does not detect approximately 25 percent of infected infants who lack anti-*Toxoplasma* IgM antibody, but it allows postnatal treatment, which seems to ameliorate symptoms. The focus of prevention should be on educating women of child-bearing age to avoid ingesting oocysts in cat feces, fruits, or vegetables and encysted bradyzoites in raw meat.[8,11,12,33]

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CHAPTER 78

Perinatal Bacterial Diseases

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In this chapter, we update relevant information on neonatal bacterial infections, with emphasis on epidemiology, pathogenesis, diagnosis, treatment, and prevention strategies. Aspects of the clinical manifestations, laboratory features, and management that are peculiar to the newborn infant are stressed. More complete descriptions of the bacterial pathogens, host-parasite relationships, and spectrum of diseases in older infants and children are presented elsewhere in the text.

ANTIBIOTIC DOSAGE SCHEDULES FOR NEONATES

Much has been written about the irrational use of antimicrobial agents in newborn infants. The "therapeutic misadventures" (i.e., the gray baby syndrome of chloramphenicol, kernicterus associated with sulfisoxazole, enamel hypoplasia after tetracycline therapy, and deafness from streptomycin and kanamycin) of past decades primarily resulted from lack of knowledge about pharmacologic concepts in neonates. Dosage recommendations for babies were calculated from simplified formulas that pared down the usual dosage for adults or from armchair reasoning based on information obtained from healthy men and women. In either case, the amount of antibiotic administered to neonates often was as subtherapeutic as it was toxic.

Physicians have come to realize that many of the physiologic and metabolic processes of the newborn constantly change during the first few days of life and that these alterations profoundly affect the pharmacokinetics of antibiotics. Since 1979, systematic investigations of these drugs have produced a clearer understanding of the factors influencing absorption, distribution, metabolism, and excretion of antimicrobials in newborn infants. As a result, the dosage and intervals of administration for the drugs most commonly used have been defined (Table 78–1).[312,420] These dosage schedules are offered as a guide to the safe and effective use of antibiotics in newborn infants. The suggested regimens must be modified in premature babies weighing less than 1200 g at birth, in patients with reduced renal or hepatic function, and in infants with altered metabolic or physiologic states (e.g., congestive heart failure, shock, hypothyroidism, during exchange transfusions and extracorporeal membrane oxygenation), in whom the volume of drug distribution in the body may be affected profoundly. Under such circumstances, the most effective means of prescribing antibiotics is to monitor serum concentrations and adjust the dosage accordingly.

EPIDEMIOLOGY AND PATHOGENESIS

Throughout pregnancy and until the membranes rupture, the infant's environment usually is sterile. Not until delivery and the immediate neonatal period is the infant exposed to many microorganisms. The human birth canal is host to large numbers of aerobic and anaerobic bacteria, *Mycoplasma*, *Ureaplasma*, *Chlamydia*, fungi, yeast, and viruses. *Staphylococcus epidermidis*, lactobacilli, diphtheroids, and alpha-hemolytic streptococci are found in 50 to 100 percent of vaginal cultures of pregnant women and constitute the predominant aerobic flora.[40,276,485] Significant but less common isolates include *Gardnerella vaginalis*, *Proteus* and *Klebsiella* spp., and group B and D streptococci; miscellaneous organisms, such as *Citrobacter*, *Acinetobacter*, and the *Campylobacter* group, are identified even less commonly.

Certain microorganisms are associated with the occurrence of stillbirths. Among these bacterial agents are *Escherichia coli*, group B streptococci, *Ureaplasma urealyticum*, *Listeria monocytogenes*, and *Treponema pallidum*. In countries with a high prevalence of syphilis, as many as half of all stillbirths are caused by this bacterium.[186,187] In the United States, stillbirths occur in nearly 7 per 1000 of all births, and 10 to 25 percent of them appear to be caused by a maternal/fetal infection. In developing countries, the relative contribution of infection may be even greater.[186]

Obligate anaerobes are present in most vaginal cultures of normal, healthy women.[189] Commonly, multiple anaerobic and aerobic species are present in the same host. Approximately 85

TABLE 78–1 Antibiotic Dosage Schedules in Neonates

		Individual Dose (mg/kg) and Frequency of Administration									
		Weight <1200 g		Weight 1200-2000 g				Weight >2000 g			
Antibiotics	Route	Ages: 0-4 Weeks		0-7 Days		>7 Days		0-7 Days		>7 Days	
Amikacin	IV, IM	7.5	q12h	7.5	q12h	7.5	q8h	10	q12h	10	q8h
Ampicillin*	IV, IM	25	q12h	25	q12h	25	q8h	25	q8h	25	q6h
Cefazolin	IV, IM	20	q12h	20	q12h	20	q12h	20	q12h	20	q8h
Cefotaxime	IV, IM	50	q12h	50	q12h	50	q8h	50	q12h	50	q8h
Ceftazidime	IV, IM	50	q12h	50	q12h	50	q8h	50	q12h	50	q8h
Ceftriaxone‡	IV, IM	50	q24h	50	q24h	50	q24h	50	q24h	75	q24h
Cephalothin	IV	20	q12h	20	q12h	20	q8h	20	q8h	20	q6h
Ciprofloxacin†	IV	—	—	—	—	10-20	q24h	—	—	20-30	q12h
Clindamycin	IV, IM, PO	5	q12h	5	q12h	5	q8h	5	q8h	5	q6h
Erythromycin	PO	10	q12h	10	q12h	10	q8h	10	q12h	10	q8h
Gentamicin	IV, IM	2.5	q18h	2.5	q12h	2.5	q8h	2.5	q12h	2.5	q8h
Imipenem	IV, IM	—	—	20	q12h	20	q12h	20	q12h	20	q8h
Meropenem	IV, IM	—	—	20	q12h	20	q12h	20	q12h	20	q8h
Metronidazole	IV, PO	7.5	q48h	7.5	q24h	7.5	q12h	7.5	q12h	15	q12h
Mezlocillin	IV, IM	75	q12h	75	q12h	75	q8h	75	q12h	75	q8h
Nafcillin*	IV	25	q12h	25	q12h	25	q8h	25	q8h	37.5	q6h
Netilmicin	IV, IM	2.5	q18h	2.5	q12h	2.5	q8h	2.5	q12h	2.5	q8h
Oxacillin*	IV, IM	25	q12h	25	q12h	25	q8h	25	q8h	37.5	q6h
Penicillin G (U/kg)*	IV	25,000	q12h	25,000	q12h	25,000	q8h	25,000	q8h	25,000	q6h
Piperacillin	IV, IM	75	q12h	75	q12h	75	q8h	75	q8h	75	q6h
Piperacillin/tazobactam	IV	50	q12h	50	q12h	100	q8h	100	q12h	100	q8h
Ticarcillin	IV, IM	75	q12h	75	q12h	75	q8h	75	q8h	75	q6h
Tobramycin	IV, IM	2.5	q18h	2	q12h	2	q8h	2	q12h	2	q8h
Vancomycin	IV	15	q24h	10	q12h	10	q12h	10	q8h	10	q8h

**For meningitis, double the recommended dosage.*
†Doses based on anecdotal clinical experience. Not recommended routinely for neonates.
‡Not recommended for premature and/or hyperbilirubinemic neonates.

percent of women with genital colonization by anaerobes harbor *Bacteroides* spp., including *Bacteroides fragilis* in a third of cases. Anaerobic streptococci, *Peptostreptococcus* and *Peptococcus*, are found in approximately 40 percent of women, and *Clostridium* is present in 20 percent. Uncommon anaerobic isolates include *Veillonella*, *Bifidobacterium*, and *Eubacterium*. Vaginal cultures of pregnant women also yield mixed aerobic and anaerobic species, but the number of anaerobes decreases from early pregnancy to delivery.[276]

During the process of delivery, encounters with some of these bacteria initiate colonization of the infant's respiratory and gastrointestinal tracts. In most infants, the microbial flora is established without incident; however, disease caused by one of these organisms develops occasionally in an infant. Factors influencing conversion from colonization to disease are not understood well. Some women have asymptomatic bacterial vaginosis that has been associated with preterm labor and significant vaginal isolation of anaerobic flora.[193]

Worldwide, 1.6 million neonates die every year of infection. In the United States, the infant mortality rate is approximately 6.85 infant deaths per 1000 live births. Neonatal bacterial sepsis corresponds to the eighth leading cause of fatality.[31,233] The incidence of neonatal sepsis ranges from one to eight cases per 1000 live births, with the higher figures corresponding to developing countries. Low birth weight, male sex, and congenital malformation are important risk factors.[61] Extreme prematurity is the greatest risk factor for early-onset sepsis and is associated with an increased risk of having adverse outcomes, including respiratory distress syndrome, bronchopulmonary dysplasia, severe intraventricular hemorrhage, and periventricular leukomalacia.[477] Between 1962 and 1987, the overall incidence of neonatal sepsis in Panorama City, California, was 2.2 cases per 1000 live births; it was 18.6 for infants with birth weights less than 2500 g versus 1.2 for those with birth weights of 2500 g or greater.[263] In the same study, the incidence of meningitis was 0.3 case per 1000 live births and 2.8 and 0.07 for those with birth weights less than and greater than 2500 g, respectively. The highest age-specific incidence of bacterial meningitis occurs during the first month of life.[262]

Socioeconomic factors appear to be important in determining whether infants are at risk for infection. Premature infants and infants with low birth weight are born more frequently to mothers of low socioeconomic class than to those of average or high socioeconomic class.

Although no noticeable sex predilection has been observed for infants with intrauterine infections, a male preponderance is reported in almost all studies. The greater susceptibility of male infants is more evident in cases of sepsis caused by gram-negative enteric bacilli. The reasons behind this male preponderance are unknown but may be related to sex-linked factors in host susceptibility.

The bacterial cause of neonatal sepsis and meningitis varies from one geographic area to another. Although bacterial causes of neonatal sepsis in Western European countries are similar to those in the United States, a different pattern has been identified in Latin America, Asia, and Africa.[36,115] In the latter sites, gram-negative rods are the most frequent pathogens, followed by *Staphylococcus aureus* (Table 78–2).[25,530] Poor sterility standards in preparing intravenous solutions and performing invasive procedures might contribute to these etiologic patterns.[296]

The bacterial pathogens that cause infection in the nursery are different from those encountered when the infant arrives home. In the nursery, besides organisms acquired vertically from mothers, staphylococci (coagulase-positive and coagulase-negative) and gram-negative bacilli constitute the predominant etiologic agents causing nosocomial disease. At home, the infant is exposed to a different environment and to members and pets of the household, which provides opportunity for infection to

TABLE 78–2 Neonatal Pathogens of Sepsis in Hospitals in Different Geographic Areas (1990 to 2004)

	Latin America, Caribbean (%)	Africa	South Asia
All gram-positive	533 (41.7)	606 (38.8)	1857 (31.0)
Staphylococcus aureus	178 (13.9)	224 (14.3)	1206 (20.2)
Coagulase-negative staphylococci	246 (19.2)	122 (7.8)	356 (5.9)
Group B streptococci	53 (4.1)	133 (8.5)	31 (0.5)
Group D streptococci	22 (1.7)	27 (1.7)	132 (2.2)
Listeria spp.	6 (0.5)	7 (0.4)	ND
All gram-negative	709 (55.4)	938 (60.0)	3793 (63.4)
Klebsiella spp	204 (15.9)	441 (28.2)	1450 (24.2)
Escherichia coli	116 (9.1)	155 (9.9)	984 (16.4)
Pseudomonas spp	92 (7.2)	51 (3.3)	576 (9.6)
Acinetobacter spp	26 (2.0)	4 (0.3)	251 (4.2)
Citrobacter spp	11 (1.3)	42 (2.7)	54 (0.9)

Modified from Zaidi, A. K., Huskins, W. C., Thaver, D., et al.: Hospital-acquired neonatal infections in developing countries. Lancet 365:1175-1188, 2005.

develop in the newborn and probably in the household from the newborn.

The current most common bacterial pathogens of the neonatal period in very-low-birth-weight (VLBW) infants (401 to 1500 g) are *E. coli*, group B *Streptococcus* (GBS), and coagulase-negative staphylococci.[409,477] These three organisms account for approximately 65 percent of all bacterial sepsis cases in this population.[477] The bacteria usually are acquired from the mother during the intrapartum period, after the onset of labor or rupture of membranes.[444] The acute septicemic form of group B streptococcal disease can be caused by any of the group B types (B_I to B_{VIII}), and the specific B type causing disease in the infant usually is found in the maternal vaginal tract.[21,22] The gastrointestinal tract is the natural reservoir for GBS and the probable source of vaginal colonization.[79,135,444]

Epidemiologic studies have shown that approximately 10 to 30 percent of pregnant women are colonized vaginally, rectally, or both with GBS, with the highest percentage occurring in developed countries.[79,135,275,400] After routine implementation of maternal cultures and antibiotic prophylaxis guidelines, the incidence of invasive group B streptococcal infection among pregnant women in the United States has decreased dramatically in the past decade.[307]

In a report from Panama,[334] only 5 percent of poor pregnant women seen in a public hospital were colonized by GBS, and approximately 2 percent of documented neonatal sepsis cases were caused by these organisms; in contrast, almost 20 percent of "septic" neonates born to mothers with better socioeconomic status and higher vaginal colonization (seen in a hospital that is only 5 miles away) had group B streptococcal disease.[418] Possibly, better hygienic practices contribute to eradication of many microorganisms from vaginal sites, thereby allowing GBS to colonize the vagina without interference by other microbes. Currently, a sepsis group B streptococcal etiology is relatively common in our country (personal communication).

Vertical transmission from mother to infant occurs in 40 to 70 percent of women colonized with this organism.[1,35,157,528] Infants born to heavily colonized women are more likely to harbor the organism, frequently at multiple sites, than are those born to lightly colonized women.[12,245,370] Some mothers of infants who are infected with GBS are at high risk for having babies in the future who are infected similarly. A low titer of serum antibodies to the type of infecting GBS and persistence of the organism in the mother have been demonstrated.[90]

Although intrapartum mother-to-infant transfer is the initial mode of acquisition of GBS by the newborn, it is not the sole way in which the baby becomes colonized.[1,15,35,369] In a Houston nursery, infant colonization rates increased from 20 to 25 percent at 1 day of age to 60 to 65 percent at 3 to 5 days of age, without a concomitant increase in parturient colonization rates.[369] Nosocomial spread of organisms from the hands of nursery personnel to the infant probably explains the remarkable increase in colonization rates in this nursery. Analysis of the serotype distribution of GBS discloses no significant differences among parturients, 1-day-old infants, nursery personnel, and infants at hospital discharge.

The major sites of colonization in infants are the skin, nasopharynx, and rectum. GBS persists in the nasopharynx for weeks to months, whereas its cutaneous location usually is lost by the time that the infant reaches several weeks of age. For every 100 infants colonized with GBS, disease caused by this organism will develop in an estimated one or two infants.

Group B streptococcal meningitis is caused almost exclusively by the B_{III} organism.[22] These organisms may be acquired from nonmaternal sites. Clusters of three or four cases of group B streptococcal meningitis have occurred in nurseries during short intervals, thus suggesting nosocomial acquisition.[1,27,473]

E. coli is the other important agent implicated in neonatal bacterial disease, with an annual incidence of approximately 6.8 per 1000 live births, and it is the most common gram-negative bacterium causing meningitis during this period.[131,307,477] The *Escherichia* genus is antigenically complex, with at least 177 somatic (O), 103 capsular (K), and 53 flagellar (H) antigens.[56] The epidemiology of this agent in relation to newborn infection was defined more clearly with discovery of the association between the K1 capsular polysaccharide antigen and invasive disease.[403] Strains with K1 antigen are responsible for approximately 80 percent of neonatal meningitis cases caused by *E. coli* and 50 percent of sepsis cases.[20,335,430] K1 strains are associated with more severe disease than non-K1 strains are.[267,313]

Sepsis caused by *E. coli* is associated with greater morbidity and mortality rates than is early-onset group B streptococcal infection. This finding, however, may be confounded by the higher frequency of prematurity among the former infected group.[307]

The explanation for the association between *E. coli* K1 strains and neonatal meningitis is unknown. Animal studies have demonstrated that *E. coli* strains with K1 are highly virulent in mice and that this lethal effect can be prevented completely by pretreatment of mice with minute amounts of specific K1 antibody.[403] The proclivity of K1 strains for the meninges also has been demonstrated in infant rats, in which oral feeding of *E. coli* K1 strains resulted in septicemia and meningitis in approximately 20 percent of experimental animals.[182] Similar feeding experiments with *E. coli* K92 and K100 strains did not cause disease in this animal model. Recently, K1 strains have been categorized into two groups based on their profile for putative virulence factors, lipoproteins, proteases, and outer membrane proteins, which suggests that *E. coli* K1 may use different mechanisms to induce meningitis.[525]

Neonatal colonization with *E. coli* often results from maternal transmission during delivery.[430] Thus, vaginal colonization, observed in 3 to 20 percent of pregnant women, seems to be an important step in neonatal infection. Approximately half of all vaginal strains express the K1 antigen.[11,271] The *E. coli* strains responsible for invasive neonatal infections come from intestinal flora. Evidence suggests, however, that the vagina and amniotic fluid are two barriers that favor selection of a population of highly virulent *E. coli* strains. Most *E. coli* strains that cause neonatal meningitis and septicemia belong to the clone ECOR B2 group.[511]

The highest prevalence rates for rectal colonization with *E. coli* K1 strains are found in pregnant and nonpregnant women aged 16 to 31 years. Approximately 45 to 50 percent of this population have K1 organisms on rectal culture.[430] Studies of pediatric

populations have disclosed colonization rates of 20 to 30 percent for newborns on the second day of life, 40 percent for infants 4 weeks to 1 year of age, and 35 percent for children 1 year to 16 years of age. As expected, the organism is dispersed widely among hospital personnel, who have rectal carriage rates of approximately 40 percent.

Vertical (mother-to-infant) and horizontal (nursery staff–to-infant, infant-to-infant) modes of transmission have been documented for *E. coli* K1 infections.[55,430] Approximately 50 to 70 percent of infants born to culture-positive women acquire *E. coli* K1 strains during the first 48 hours of life; in these instances of vertical transmission, serologic concordance exists for the O and H types of the *E. coli* cultured from the mother and baby.[11] Approximately 10 to 15 percent of infants colonized with K1 strains are born to K1-negative mothers. For this group of babies, *E. coli* is acquired at a later age (3-4 days), presumably from horizontal transmission. Vertical acquisition of K1 organisms has been documented in approximately three fourths of neonates with *E. coli* K1 meningitis. Based on a colonization rate of approximately 200 to 300 infants per 1000 live births and an attack rate of 1 per 1000 live births, the colonization-to-disease ratio for *E. coli* is approximately 200:1 to 300:1.

E. coli containing the K1 antigen was isolated from women throughout their pregnancy and at delivery, and 50 percent of the babies were colonized if their mothers were positive for this organism at the time of delivery.

E. coli infection is highly associated with preterm delivery at less than 34 weeks' gestation and with VLBW neonates and occurs more frequently in infants of women who are heavily colonized.[271] Infants with early-onset *E. coli* sepsis had a poor outcome, with high mortality rates and neurodevelopmental sequelae in 30 percent of survivors. Although amoxicillin resistance is a common finding, a low prevalence of gentamicin resistance exists in coliform isolates.[14] Ampicillin-resistant *E. coli* infections tend to be severe and fatal and appear to be seen more frequently when ampicillin instead of penicillin is used for maternal group B streptococcal prophylaxis.[450] Characterization and comparison of virulence genotypes and phylogenetic analysis will help in understanding the origins and spread of virulence factors within the population of *E. coli* neonatal meningitis isolates.[60,66]

Listeria is a ubiquitous soil organism, and although the animal reservoir for this organism is large, transfer from animals to humans is a rare event and occurs in high-risk persons, such as farmers and veterinarians.[365,399,439] *L. monocytogenes* appears to be a common transient colonizer of the human gastrointestinal tract but with little propensity to cause invasive infection unless host risk factors are present or the gut inoculum is large enough to overwhelm local gastrointestinal preventive barriers. Host factors that increase the risk for *Listeria* infection include pregnancy, acquired immunosuppression associated with organ transplantation, cytotoxic chemotherapy, hemochromatosis, diabetes mellitus, and renal failure.[192,425,439]

The annual incidence of listeriosis in infants younger than 1 year old varies between 1.0 and 11.9 per 100,000, and it is seen more commonly in males. Rates in women of child-bearing age (15-39 years) are between 0.1 and 1.1 per 100,000, with the higher numbers being observed in the Hispanic population.[278,372] Perinatal infection constituted 34 percent (470 of 1378) of cases of listeriosis.[175,461]

Epidemiologic information implicating food as a vehicle for transmission of listeriosis from animals to humans now appears to be established firmly. Food-borne outbreaks have been traced to cabbage, dairy products, and vegetables. Undercooked chicken and hot dogs appear to be frequent sources of infection, as are delicatessen meats and unpasteurized cheese products, especially soft cheese.[367,439] In an outbreak in Canada,[440] *Listeria*-contaminated sheep manure was used to fertilize locally grown cabbage that was stored for the winter in the cold, where the organism is known to survive for long periods. Clinical disease occurred in pregnant women who consumed the processed cabbage months after its original contamination. In 1985, the first well-documented outbreak of listeriosis in humans through contaminated milk products was reported.[163] The milk, which came from a group of farms where listeriosis in dairy cattle was known to have occurred, was well pasteurized, which indicated that pasteurization might not be sufficient to eradicate a large inoculum of *L. monocytogenes*. Linnan and associates[292] reported a large outbreak of perinatal listeriosis in southern California that appeared to be caused by Mexican-style cheese contaminated with raw milk. In a report from Costa Rica, a nosocomial outbreak of listeriosis was associated with the use of contaminated mineral oil for bathing neonates.[448] In 2000, a small outbreak of listeriosis in two previously healthy adults occurred in Ontario, Canada, and was associated with the intake of imitation crab meat. In 2002, 47 cases of human listeriosis were related to ripening solutions used in a cheese-making process.[173,367]

Several large prospective epidemiologic studies have demonstrated that few women are colonized with *Listeria* strains during pregnancy and that the organism is cultured infrequently from healthy premature and term infants or from stillborn fetuses.[229,518] These studies suggest that human carriage of this bacterium does not appear to be of the same magnitude as that for GBS and *E. coli* K1, but asymptomatic colonization with *Listeria* does occur. *Listeria* has been found occasionally in the genitourinary tracts of pregnant women, in the throats of children, and in the noses of men.[197] *L. monocytogenes* rectal carriage rates of 1 to 30 percent of all pregnant or nonpregnant women have been reported.[283] The *Listeria* colonization rate in fecal surveys in the community is approximately 2 to 10 percent.[295,446] A possible venereal nature of listerial colonization has been suggested.[196]

Since the 1980s, epidemic and endemic colonization and disease of the newborn infant with methicillin-resistant *S. aureus* (MRSA) have been reported with increasing frequency in the United States and Europe. Table 78–4 demonstrates the relative frequency of infections caused by *S. aureus* versus those caused by other common neonatal pathogens in nurseries at Parkland Memorial Hospital in Dallas, Texas.

Epidemics of disease caused by MRSA have been reported in the United States and several other countries. Risk factors associated with the development of MRSA infection include lengthy hospitalization, previous antibiotic administration, overcrowding and understaffing, and the presence of predisposing factors such as indwelling central venous catheters, cerebrospinal shunts, mechanical ventilation, and prematurity.[264]

Potential reservoirs of MRSA in the hospital environment include colonized or infected neonates, hospital personnel, and the hospital inanimate environment. MRSA is spread by contact and by air and may circulate among patients, staff, and visitors for several months during an outbreak in hospitals, long-term care facilities, and the community.[64,272,284,327] Although chronic nasal carriage of MRSA by hospital personnel has been implicated in several hospital outbreaks, it generally is an uncommon event and is not necessary for initiation or propagation of hospital outbreaks.[489] Limited data suggest that the hospital inanimate environment may become contaminated with MRSA, thereby possibly sustaining outbreaks of infection.[64,489] Colonized patients without clinical disease contribute substantially to the inpatient reservoir of MRSA. In recent years, an increase in the incidence of community-acquired MRSA infections has occurred in healthy patients without obvious risk factors.[92,224,237]

Coagulase-negative staphylococci also have been found increasingly to be important neonatal pathogens. They are the most common species of the normal flora on the skin, nasal mucosa, and umbilicus of the newborn. With sensitive culture techniques, rates of colonization of the nose, umbilicus, gastrointestinal tract, and cutaneous areas of the neonate with coagu-

lase-negative staphylococci can be as high as 83 percent at 4 days of age.[463] The ubiquitous presence of the organisms and their tolerance to drying and temperature changes contribute to the increased presence of coagulase-negative staphylococci in neonates. Coagulase-negative staphylococci are the most common late-onset organisms isolated in neonatal intensive care units (48% of all infections and 68% of gram-positive infections).[80,239,477,478] In some neonatal intensive care units, disease caused by coagulase-negative staphylococci exceeds that of group B streptococci and *E. coli*.[387]

Prematurity, high rates of colonization, and aggressive treatment of newborn infants in intensive care units (e.g., placement of umbilical or central venous catheters, intravenous parenteral nutrition, and mechanical ventilation) account for coagulase-negative staphylococci becoming important invasive nosocomial pathogens.[478,482] Despite plausible evidence of an increasing prevalence of these organisms as neonatal pathogens, distinguishing between infection and contamination of blood cultures by them often is difficult.[80,208,478]

Most studies have shown that infections with coagulase-negative staphylococci are not associated with significant mortality rates or morbidity in infected infants.[239,251,320] Recently, however, an outbreak of coagulase-negative staphylococcal sepsis characterized by persistent bacteremia and severe thrombocytopenia was reported at the Children's and Women's Health Centre of British Columbia. Some experts suggest that this neonatal infection may not be as benign as has been perceived historically and recommend a large prospective evaluation.[256]

Group D streptococci are normal inhabitants of the gastrointestinal tract and can cause invasive disease. These bacteria are the third most frequent gram-positive organisms associated with late-onset sepsis.[103,422,478] Outbreaks of bacteremia and meningitis related to *Enterococcus faecium* were reported from neonatal intensive care units at the Medical College of Virginia and the Children's Hospital of Denver, Colorado.[119] These organisms have become resistant to ampicillin and vancomycin in many hospitals.[61] Disease caused by these multidrug-resistant enterococci often is difficult to treat. An outbreak of *E. faecium* resistant to vancomycin and teicoplanin was reported in South Korea, where all isolates are shown to have the vanA gene.[281]

Maternal, environmental, and host factors determine the infants in whom invasive bacterial infections will develop when exposed to a potentially pathogenic organism. The presence of any of the following factors can be associated with a 10-fold or greater increased risk for the development of systemic infection: premature onset of labor, prolonged rupture of fetal membranes, chorioamnionitis, and maternal fever. Twin pregnancy remains an independent risk factor for the acquisition of infection with GBS and other organisms after correction for low birth weight. The first-born twin is at a higher risk of contracting ascending intrauterine infection than the second-born is. Infection developed in 3 of 56 twin births, or 54 per 1000 live births, as compared with 7 infections in 603 single births, or 12 per 1000 live births.[371] The basis for the increased risk of acquisition of infection in twins includes the common features of virulent organisms, absence of protective antibody, and similar genetic heritage. Substance abuse by the mother (e.g., heroin) has been shown to alter T-cell activity significantly in the neonate, and such alterations persist through the first year of life.[121] Although numerous microorganisms have been documented to cause maternal bacteremia before delivery, infants born to mothers with bacteremia usually remain well. This phenomenon most likely is explained by a balance among the presence of maternal antibody, the virulence of the organism, and the effectiveness of the placenta in preventing transmission of the organism to the fetus.

Klebsiella pneumoniae is one of the most important neonatal pathogens in developing countries, with an incidence between 4.1 and 6.3 per 1000 live births and fatality rates of 18 to 68 percent. It is the second most frequent gram-negative organism associated with late-onset sepsis in developed nations.[478,530] Although *Klebsiella* strains are part of the normal gastrointestinal and vaginal flora, the resistant nature of the hospital isolates indicates their presence in contaminated environmental reservoirs.[102,296,349]

All arms of the defense system are relatively immature (i.e., lack of previous experience with microorganisms) in a healthy neonate and are impaired further by conditions such as prematurity, hypoxia, acidosis, jaundice, and metabolic derangements. Infants with galactosemia particularly are susceptible to the development of sepsis by gram-negative enteric bacilli.[287] *E. coli* is by far the organism most commonly encountered as a cause of sepsis and meningitis in these infants. The umbilical stump may serve as the portal of entry of microorganisms to the bloodstream. Closure of the umbilical vessels plus subsequent aseptic necrosis of the cord, which begins soon after birth, results in an ideal environment for microorganisms to multiply and invade deeper tissues, with resultant omphalitis. Complications of omphalitis include septic umbilical arteritis, suppurative thrombophlebitis of the umbilical or portal vein, peritonitis, liver abscess, endocarditis, superficial abscess, and necrotizing fasciitis.[10,299]

Of the various microbial virulence factors, the polysaccharide capsule has been studied most thoroughly. Bloodstream infections in infant rats and mice caused by *E. coli* K1 or any of the GBS serotypes can be prevented by pretreatment with type-specific capsular polysaccharide antibody. In infants, mortality and long-term sequelae were increased in cases of meningitis caused by *E. coli* K1 strains versus those caused by non-K1 strains.[217] K1 capsular polysaccharide has been detected in cerebrospinal fluid (CSF) by counterimmunoelectrophoresis in higher concentrations and for longer durations in patients who died or were impaired neurologically than in those who were normal survivors.[217] Concentrations and persistence of K1 capsular polysaccharide in the CSF of neonates with *E. coli* meningitis have been correlated with concentrations and persistence of endotoxin and interleukin-1β in CSF.[311] *E. coli* strains also have been found to resist phagocytosis by normal adult polymorphonuclear leukocytes, thereby resulting in delayed clearance of bacteria from the bloodstream. This delay allows the organism to multiply and achieve the concentration of 1000 colony-forming units per milliliter of blood or more, an inoculum that generally is considered essential for invasion of the meninges. The presence of K1 antigen alone, however, does not appear to account fully for an organism's virulence because nonpathogenic *E. coli* K12 strains that are transformed by plasmid containing the cloned K1 antigen gene do not become virulent on expression of the K1 antigen.[462]

E. coli causing neonatal meningitis usually belongs to the phylogenetic group B2 and to the main serotype O18:K1.[66,244,335] Virulence factors such as fimbrial adhesion S (sfaS), invasion IbeA (ibeA), and cytotoxic necrotizing factor (cnf1), in conjunction with the K1 antigen, facilitate penetration of the brain barrier. These extraintestinal pathogenicity genes of *E. coli* usually are clustered in chromosomal genomic structures known as pathogenicity islands.[66,235,258]

Detailed studies of type III GBS demonstrated that the quantity of sialic acid residues in the capsular polysaccharide and the spatial conformation of the antigenic molecule determine the antiphagocytic properties of this organism. A gene sequence that is specific for type III GBS has been identified and cloned. Strains with multiple copies of the gene sequence repeated within the chromosome have a lower lethal dose required to kill 50 percent of infected animals (LD_{50}) in the infant rat model of disease and are more resistant to opsonophagocytosis than are strains that do not contain the gene structure or have only one or two copies of that sequence.[413]

Studies in children and adults have demonstrated clearly that protection from disease caused by bacteria (i.e., *Haemophilus influenzae* type b, *Neisseria meningitidis*, and *Streptococcus pneumoniae*) possessing polysaccharide capsules is afforded by specific antibody directed against these structures.[331,441] Resistance to bloodstream clearance probably relates in part to relative resistance of the encapsulated organisms to complement. The capsule may protect the deep somatic antigen structures capable of activating the alternative complement pathway. Opsonization is essential for phagocytosis and intracellular killing of these organisms and depends primarily on anticapsular antibody. Studies indicate that levels of B_{III} antibody correlate with in vitro opsonic activity[13] and with in vivo protection in animals experimentally infected with GBS.[502] The lack of type-specific maternal opsonizing antibody is a significant risk factor for the development of systemic disease caused by GBS in the mother and infant.[27,222]

Most pregnant women colonized with group B_{III} organisms have increased concentrations of antibody in their circulation that pass transplacentally to the fetus. Both mother and baby in this instance are protected against disease by that specific B type. Conversely, infants born to mothers with undetectable concentrations of antibody are susceptible to invasion by the group B organisms. In one study, protective B_{III} antibody titers were detected in 73 percent of women whose newborns were well as compared with 17 percent of mothers whose newborn infants contracted group B streptococcal sepsis or meningitis.[26] The same study documented lower concentrations of B_{III} antibody in sick neonates than in healthy infants born to mothers with vaginal colonization. Other studies have shown that premature infants have lower concentrations of B_{Ia}, B_{II}, and B_{III} antibody than do term neonates.[74,107] This finding may explain in part the higher incidence and case-fatality rates of group B streptococcal disease observed in premature infants. Probably, a lack of K1 antibody in the sera of neonates predisposes them to *E. coli* K1 disease as well.[314] Mouse protection studies lend credence to this contention.[403]

Strong parallels exist between the host-parasite relationships found with GBS and with *E. coli*. Both organisms possess immunochemical structures as components of the surface polysaccharide capsule that appear to confer virulence. In both cases, neonatal immunity is mediated, at least in part, by maternally derived serum antibody. For both organisms, asymptomatic infection (i.e., colonization) occurs commonly and clinical disease rarely (i.e., colonization-to-disease ratios of 100:1 to 200:1). Questions concerning the precise role of the complement system in opsonization of these and other bacterial pathogens, the exact concentration of antibody that confers protection, and the feasibility of screening large populations for the absence of antibody need further investigation. The role of local immunity in determining invasion of these bacteria from their sites of colonization (i.e., respiratory and gastrointestinal tracts) needs clarification.

The meninges can be invaded directly from an adjacent infected site, such as skin lesions, meningomyelocele, or a skull fracture. Most cases of meningitis, however, result from bacteremia. After gaining access to the blood, bacteria probably enter the CSF space through the choroid plexus of the lateral ventricle and then spread to the subarachnoid space along normal paths of CSF flow. Because of the absence of antibody and complement in the subarachnoid space, bacteria multiply logarithmically, and as many as 10^8 colony-forming units/mL can be cultured from lumbar CSF. The larger the number of bacteria in CSF, the poorer the prognosis.

As a response to the interaction of bacteria or their cell wall components with central nervous system (CNS) tissues, the local production of inflammatory mediators, such as tumor necrosis factor and interleukin-1β, is an initial step in the cascade of events leading to inflammation and tissue destruction.[340,395,421] Experiments in animals demonstrated that interleukin-1β, tumor necrosis factor, and other mediators can act synergistically in altering the function of the cerebral capillary endothelium (i.e., blood-brain barrier) and in promoting attachment of leukocytes through the expression of adhesion receptors.[421,437] The net result is injury and increased permeability of the usually highly efficient blood-brain barrier that allows transendothelial passage of phagocytic cells and low-molecular-weight serum proteins, including complement. Despite this influx, the opsonic activity of CSF remains low; as a result, phagocytosis is inefficient, thereby allowing continued bacterial growth and meningeal inflammation.

Accumulation of inflammatory exudate and inflammation of the arachnoid villi can alter CSF flow, which coupled with loss of autoregulation of cerebral blood flow, can result in increased intracranial pressure. Hydrocephalus results from aqueductal obstruction by fibrinous debris or from reduced CSF outflow caused by inflammation of the arachnoid villi. The raised intracranial pressure, occlusion of blood vessels traversing the subarachnoid space, and edema of vascular endothelial cells can result in cerebral ischemia and possibly cerebral infarction. Anaerobic glycolysis by poorly perfused cerebral tissue results in increased CSF lactate concentrations and hypoglycorrhachia, which further potentiates swelling of glial and neuronal cells through failure of the adenosine triphosphate–dependent sodium pump; in turn, failure of the sodium pump results in accumulation of intracellular sodium. Inappropriate secretion of antidiuretic hormone also can contribute to cerebral edema.

SEPSIS NEONATORUM

Sepsis neonatorum is a bacterial disease of infants 30 days of age or younger. It involves primarily the bloodstream, although spread to the meninges or other organs occurs in a substantial portion of affected infants.[457] No obvious focus of infection of the bloodstream can be found in most cases. The presence of clinical and laboratory findings distinguishes this condition from the transient bacteremia observed in some healthy neonates. Terminology guidelines to classify infants and children with a systemic inflammatory response syndrome resulting from an infectious process are *sepsis*, *severe sepsis*, *septic shock*, and *multiple organ dysfunction syndrome*.[188,419,423]

Different opinions exist on the appropriate age for differentiating between early- and late-onset sepsis. Although the usual range is 2 to 7 days of age for the early-onset variety, more than 80 to 90 percent of infections in the first week of life have their onset in the infant's first 2 days of life.[476,478] Early-onset sepsis commonly is associated with vertical transmission and late-onset sepsis with the hospital environment or acquisition from human contact.

PREDISPOSING FACTORS

The skin is an important component of innate immunity. Preterm infants are particularly susceptible to the development of infections because the skin lacks the vernix (the naturally protective cutaneous biofilm[527]) and is developmentally immature, easily injured, and functionally compromised, thus becoming an additional risk factor for developing nosocomial sepsis.[116,126] This cutaneous barrier also is affected by malnutrition in developing countries.[125]

In a trial in Egypt consisting of topical application of sunflower seed oil to preterm infants, a substantial improvement in skin condition and a decrease in the risk for late-onset sepsis were observed. Nonetheless, a recent Cochrane review of four trials performed in developed countries using different emollient ointments with mineral oil found an increased risk for the development of coagulase-negative staphylococcal infection and fungal

nosocomial infections, so further clinical studies are warranted before recommending this practice.[116]

Many prepartum and intrapartum obstetric complications are associated with an increased risk for the acquisition of infection in newborn infants. Among these complications are premature onset of labor, prolonged rupture of fetal membranes, uterine inertia with high forceps extraction, and maternal pyrexia.[49,315,363]

Group B streptococcal disease is associated more frequently than are other causes of sepsis with frequent vaginal examinations and intrapartum fever.[450] Preterm delivery is identified consistently as a strong risk factor for the development of group B streptococcal infection. A multicenter study found that approximately 80 percent of group B streptococcal disease occurred in infants born at 37 weeks or fewer and that only 40 percent of cases of early-onset, non–group B streptococcal disease occurred in term infants.[450]

Sophisticated equipment for respiratory and nutritional support combined with invasive techniques provides life support to ill infants. Arterial and venous umbilical catheters, central venous catheters, peripheral arterial and venous cannulas, indwelling urinary catheters, and tracheal intubation provide enormous opportunity for relatively nonvirulent pathogens to establish infection and invade the host.[3,33,78,114,217,242,269,480] The frequency of these infections varies, and they usually are sporadic. Recognizing these opportunistic infections may be difficult because of the severe underlying illnesses requiring intensive therapy and the frequent use of antimicrobial agents in these infants.

CLINICAL MANIFESTATIONS

The newborn infant responds to many varieties of noxious stimuli (e.g., infectious, metabolic, respiratory, traumatic) with a limited repertoire of stereotyped reactions. As a result, many of the manifestations of sepsis have their counterparts in hypoglycemia, hypocalcemia, hypoxemia, hemolytic blood disorders, drug reactions, and surgical events. Most infectious problems in infants cannot be differentiated from other neonatal disorders on the basis of the initial clinical manifestations. The major signs and symptoms of sepsis relate to disturbances in thermoregulation, respiration, and gastrointestinal function.[140,315,354,465]

Abnormalities in temperature regulation frequently are observed as initial complaints. They may take the form of hypothermia (in 40% of cases) or, less commonly, hyperthermia.[61,122,147,503] With the introduction of isolette care of premature infants to maintain an optimal thermic environment, thermoregulatory disturbances commonly become obvious when the nurse reports the need to make frequent changes in the isolette's thermostat to accommodate the infant's loss of regulatory control. Fever can result from a variety of noninfectious causes, such as dehydration, elevation in ambient temperature, and hematomas, or from central origins secondary to neonatal conditions such as anoxia, CNS hemorrhage, and kernicterus.

Another frequent condition seen is respiratory distress manifested as tachypnea, grunting respirations, cyanosis, intercostal and substernal retractions, and apnea. A heart rate persistently in excess of 160 beats per minute can be a sensitive indicator of early-onset neonatal sepsis.[195] Although these findings are indicative of early-onset group B streptococcal disease in particular, they have been associated with infection caused by all of the pathogens commonly encountered in the neonatal period.

Approximately a third of infants have gastrointestinal findings, including poor feeding, regurgitation, vomiting, weak sucking, abdominal distention, diarrhea, and, rarely, gallbladder distention.[376] Although in most cases conditions other than sepsis can explain these findings, bacterial disease always must be considered. In most patients, ruling out sepsis on clinical grounds alone is impossible. Appropriate laboratory studies and therapeutic intervention frequently are necessary in the assessment of these nonspecific clinical manifestations.

Only a small percentage of infants have cutaneous findings (except for jaundice). Such findings include cellulitis, impetiginous lesions, furunculosis, papular lesions (i.e., listeriosis), vascular lesions (i.e., *Pseudomonas*), and exfoliative dermatitis (i.e., phage group II staphylococcal disease). Jaundice develops in approximately a third of infants with sepsis and can occur in infants with urinary tract infection. Occasionally, jaundice is the only sign of infection and occurs in septic infants, regardless of the type of bacterial pathogen.

In utero infection is identified by the presence of bacteria in blood obtained at delivery. Signs of fetal distress may be the first indication of infection in the newborn. Schiano and associates[438] suggested fetal tachycardia in the second stage of labor as a sign of intrauterine infection. Pneumonia or sepsis occurred in 3 of 8 infants with fetal heartbeats more rapid than than 180 per minute, in 7 of 32 infants with a rate of 160 to 179 beats per minute, and in 1 of 167 infants with lower heart rates.

One report suggests that infants with documented sepsis or a sepsis-like illness have abnormal heart rate characteristics for as long as 24 hours preceding their clinical signs. These abnormalities included reduced baseline variability and short-lived decelerations in heart rate. If these findings are confirmed, monitoring of these parameters in neonates at risk for sepsis may lead to earlier suspicion of disease and initiation of more effective and prompt therapy.[200]

ETIOLOGY

Since the middle of the 20th century, a shift has occurred in the microorganisms responsible for neonatal septicemia and meningitis.[140,168,183,315,354] In the 1930s and 1940s, the predominant organism was the group A beta-hemolytic *Streptococcus*. It was replaced in the 1950s by the phage group I *S. aureus* and by coliform organisms. In the last cohort of VLBW infants, gram-negative organisms accounted for 60 percent of infections, with 44 percent caused by *E. coli* and group B beta-hemolytic streptococci accounting for approximately 10 to 11 percent of all infections (Table 78–3).[477] *S. epidermidis* has emerged as an important

TABLE 78–3 Distribution of Pathogens in Early-Onset Neonatal Sepsis in the United States (Neonatal Research Network) 1998 to 2000

Gram-Negative Organisms	
Escherichia coli	37 (44.0)
Haemophilus influenza	7 (8.3)
Citrobacter	2 (2.4)
Other	5 (6.0)
Gram-Positive Organisms	
Group B streptococci	9 (10.7)
Viridans streptococci	3 (3.6)
Other streptococci	4 (4.8)
Listeria monocytogenes	2 (2.4)
Coagulase-negative staphylococci	9 (10.7)
Other	4 (4.8)
Fungi	
Candida albicans	2 (2.4)
Total	84 (100)

Modified from Stoll, B. J., Hansen, N., Fanaroff, A. A., et al.: Changes in pathogens causing early-onset sepsis in very-low-birth-weight infants. N. Engl. J. Med. 347:240-247, 2002.

pathogen in neonates and is responsible for a large proportion of cases of sepsis in newborn intensive care facilities.[39,91,336] A study of VLBW neonates (401 to 1500 g) born between 1998 and 2000 at 15 neonatal centers that belong to the Neonatal Research Network of the National Institute of Child Health and Human Development (NICHD) found a rate of early-onset sepsis of 15.4 per 1000 live births in this group, not a significant decline from the 19.3 per 1000 live births reported between 1991 and 1992. The important differences were a reduction in group B streptococcal sepsis from 5.9 to 1.7 per 1000 live births of VLBW infants and an increase in *E. coli* sepsis from 3.2 to 6.8 when compared with a previous cohort from 1991 to 1993.[477] In a survey of high-risk nurseries participating in the National Nosocomial Infection Surveillance System of the Centers for Disease Control and Prevention (CDC) conducted from October 1986 through September 1994, the pathogen most commonly reported as the cause of nosocomial bacteremia was coagulase-negative staphylococci, which accounted for 51 percent of isolates.[174] The apparent increased incidence of *S. epidermidis* sepsis has been associated with increased survival of very small premature infants and the introduction of invasive procedures.[208] MRSA also has emerged as a nosocomial pathogen of major importance in some nurseries. Prevalence rates for a specific bacterial pathogen vary from nursery to nursery and may change abruptly in any one unit.[38,41,168,183,222] Knowledge of the bacteria most commonly isolated in a nursery or intensive care unit, as well as the antimicrobial susceptibility of these organisms, is invaluable in treating infants with suspected sepsis neonatorum.

In 2004, a total of 308 cases of neonatal group B streptococcal disease, including 146 (47%) early-onset cases and 162 (53%) late-onset cases, were reported to the Emerging Infections Program. Between 1993 (pre-prophylaxis era) and 2003, the absolute difference in the incidence of early-onset disease between blacks and whites had declined by 68 percent. However, racial disparities in the incidence of both early- and late-onset group B streptococcal disease persist. In 2004, rates of early-onset disease per 1000 live births for black infants were 0.73, followed by 0.26 for white infants and 0.15 for those of other races.[95]

During 1999 to 2001, the incidence of early-onset disease in the United States was 0.47 case per 1000 live births; it declined to 0.32 and 0.34 in 2003 and 2004, respectively. In Parkland Memorial Hospital in Dallas, Texas, the incidence of cases has been diminished with the use of obstetric and neonatal chemoprophylaxis since 1995 (Fig. 78–1). Between 1995 and 1999, with implementation of the chemoprophylaxis protocol, no deaths as a result of GBS were reported in Parkland Memorial Hospital, as opposed to 8 percent from 1986 to 1994.[500] During the period from 1996 to 2004, late-onset disease occurred in 0.35 per 1000 live births (range, 0.29 to 0.39 per 1000). The rate of late-onset disease surpassed that of early-onset disease for the first time in 2003, a trend that continued in 2004 (Fig. 78–2).[95]

The etiologic agents of neonatal sepsis and meningitis at Parkland Memorial Hospital in Dallas, Texas, for 1987 through 1999 are shown in Table 78–4.

Coliform bacteria, including *E. coli*, *Klebsiella* spp., and *Enterobacter* spp., were recovered more frequently in Panama and Mal-

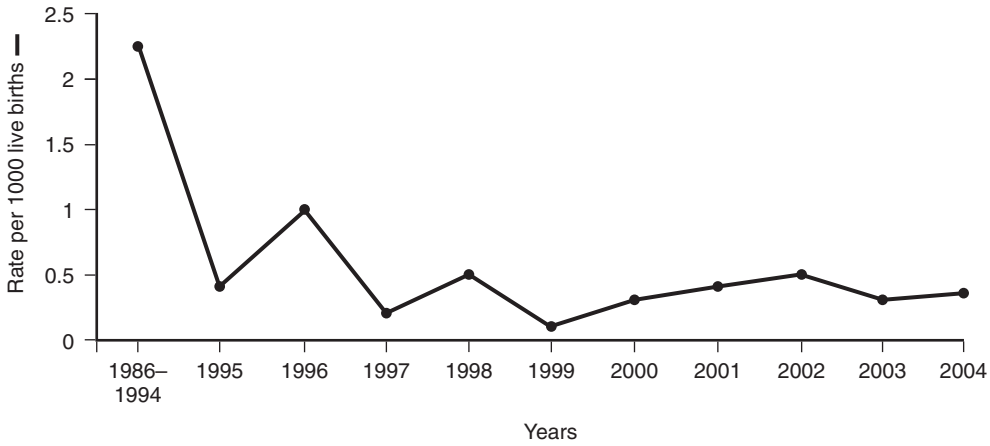

Figure 78–1 Cases of early-onset group B streptococcal infection by year at Parkland Memorial Hospital. *(From Parkland Memorial Hospital, Dallas, Texas [1986-2004]. Data provided by Pablo Sánchez, M.D. Combined obstetric and neonatal chemoprophylaxis was used routinely from 1995 to the present.)*

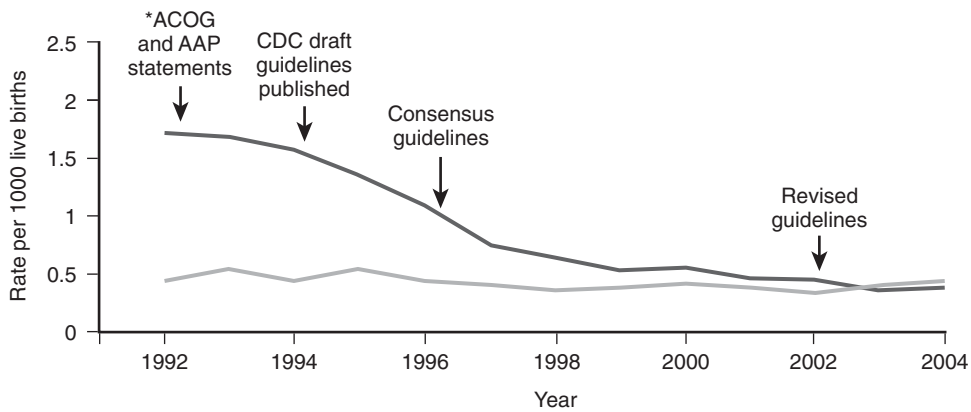

Figure 78–2 Early- and late-onset invasive group B streptococcal disease in infants per 1000 live births from 1992 to 2004, United States. AAP, American Academy of Pediatrics; ACOG, American College of Obstetricians and Gynecologist; CDC, Centers for Disease Control and Prevention. *(Adapted from Centers for Disease Control and Prevention. Early-onset and late-onset neonatal group B streptococcal disease—United States, 1996-2004. M. M. W. R. Morb. Mortal. Wkly. Rep. 54:1205-1208, 2005; and Schrag, S. J., Zywicki, S., Farley, M. M., et al.: Group B streptococcal disease in the era of intrapartum antibiotic prophylaxis. N. Engl. J. Med. 342:15-20, 2000.)*

TABLE 78–4 Main Etiologic Agents Isolated from the Blood and Cerebrospinal Fluid of Neonates with Suspected Sepsis According to Age at Onset and Time Period*

	1987-1994		1995-1999	
Etiologic Agents	**Early Onset**	**Late Onset**	**Early Onset**	**Late Onset**
Gram-positive bacteria	290	426	39	161
Streptococcus agalactiae	233	61	32	5
Enterococci	13	42	1	17
Other streptococci	25	12	2	1
Staphylococcus aureus	8	100	2	22
Coagulase-negative *Staphylococcus*	10	210	0	116
Listeria monocytogenes	1	0	2	0
Gram-negative bacteria	49	80	18	56
Escherichia coli	23	34	13	28
Klebsiella spp.	3	16	1	13
Enterobacter spp.	3	18	1	8
Pseudomonas spp.	1	4	0	2
Acinetobacter spp.	1	2	0	0
Others	18	6	3	5
Total live births	117,478		67,869	
Rate of infection with group B streptococci†				
Early onset (≤3 days)	2		0.5	
Late onset (>3 days)	0.5		0.1	

**Parkland Memorial Hospital, Dallas, Texas (1987 to 1999).*
†*Cases per 1000 live births.*
Data provided by Sithembiso Velaphi, M.D. Intrapartum prophylaxis was used routinely in the 1995-1999 period.

lorca than in Dallas.[225] *S. aureus* was recovered relatively commonly in the three nurseries; a large percentage of the strains isolated in the United States were methicillin-resistant, which underscores the importance of these organisms in some neonatal units. Coagulase-negative staphylococci also were recovered frequently from infants with neonatal sepsis. Anaerobes were responsible for a small percentage of cases of septicemia. Because special techniques for isolation of these relatively fastidious organisms were not used, the actual contribution of these bacteria to the development of sepsis may have been underestimated considerably.

H. influenzae, *S. pneumoniae*, and *N. meningitidis* are occasional causes of neonatal sepsis. Viridans streptococci are being isolated with increased frequency and, in one series, were the major pathogens in neonates with streptococcal septicemia. From 1987 to 1989, viridans streptococci were recovered from the blood of 20 of 273 infants with neonatal sepsis in nurseries at Parkland Memorial Hospital in Dallas, Texas.

The changing distribution of etiologic agents over the course of time has been demonstrated in both developed and developing countries.[294,359,467] In nurseries in the United States, gram-negative enteric organisms were the isolates most frequently recovered 2 decades ago; gram-positive bacteria (mostly GBS and staphylococci) constitute the predominant pathogens today. In the largest public hospital of Panama during an 18-year period (1975 to 1992), the proportional incidence of gram-negative infections also declined with time, whereas that of gram-positive infections increased.[334]

SPECIFIC CLINICAL SYNDROMES

Group B Beta-Hemolytic Streptococcal Infection

Group B streptococcal infection may become evident in a variety of ways, ranging from asymptomatic bacteremia to septicemia, pneumonia, and meningitis. Skin infections, such as impetigo, cellulitis, erythema nodosum–like lesions, adenitis, breast abscess, and scalp abscesses, also may occur.[20,231,345] Group B streptococcal infection may be manifested initially as conjunctivitis, orbital cellulitis, otitis media, or ethmoiditis. These organisms are responsible for an increasing proportion of suppurative arthritis and osteomyelitis cases during the newborn period and have been incriminated in unusual infections such as retropharyngeal cellulitis, pleural empyema, endocarditis, peritonitis, and adrenal abscess.[17,97,231,505,513]

Group B streptococcal colonization can be transient, chronic, or intermittent. Maternal intrapartum colonization is a major risk factor for early-onset disease in infants, and vertical transmission of this organism from mother to fetus occurs primarily after the onset of labor or rupture of membranes. However, colonization early in pregnancy is not predictive of neonatal sepsis.[401]

Culture screening of both the vagina and rectum for GBS late in gestation during prenatal care can detect women who are likely to be colonized at the time of delivery and thus are at higher risk for perinatal transmission of the organism.[72] Almost 21 percent of women are colonized vaginorectally with GBS, and the most frequent capsular serotypes are III and IA.[246]

The most important risk factors for the acquisition of group B streptococcal early-onset disease, in addition to colonization, include gestational age less than 37 weeks, prolonged rupture of membranes (≥18 hours), chorioamnionitis (intrapartum fever ≥38.0°C) , young maternal age, black race, Hispanic ethnicity, and low maternal levels of anticapsular antibody.[26,72,444,447,449,450,477] A multivariable analysis of a large multistate birth cohort in 1998 and 1999 found that intrapartum fever and a previous infant with GBS were associated with greater than a fivefold increase in risk.[444] Six or more vaginal examinations performed during delivery have been associated with a threefold risk for acquisition of group B streptococcal infection, and these factors were not associated with other organisms, probably because of differences in the route of transmission. GBS generally is acquired by vertical transmission, and non-GBS cases usually were seen after the first day of life and may be acquired during hospitalization or through contact.[450]

Universal administration of single-dose intramuscular penicillin to neonates at birth has been considered as an alternative strategy for prevention of early-onset group B streptococcal disease. Randomized trials have shown that this intervention alone[389,458,459] or in combination with a risk-based intrapartum

prophylaxis approach can result in significant prevention of early-onset invasive group B streptococcal disease.[374,500]

Animals studies that explore GBS based on a conserved surface protein that is expressed by all GBS serotypes found that it induced a strong systemic and mucosal antibody response in mice. This finding raises hope that a protein vaccine that would be effective against all serotypes also may prevent maternal colonization with this pathogen and protect neonates against invasive disease.[236] Phase I and II trials of candidates for capsular polysaccharide–protein conjugate group B streptococcal vaccines have been conducted in healthy, nonpregnant adults, and phase I safety and immunogenicity trials were conducted in pregnant women and yielded promising results.[14,31,253]

After implementation of the American College of Obstetricians and Gynecologists and the American Academy of Pediatrics statements in 1992, the CDC chemoprophylaxis draft guidelines in 1994, and the official CDC guidelines in 1996 for prevention of group B streptococcal disease, a reduction in the prevalence of early-onset group B streptococcal sepsis has been documented, from 1.8 per 1000 live births in 1990[253,305,444,531] to 0.5 per 1000 live births in 1999 with an estimate of 4500 early-onset cases and 225 deaths being prevented per year. There was a plateau of tendencies during 1999 to 2002, but then the rates declined 34 percent in 2003 with an overall incidence of 0.32 (Fig. 78–2).[93,94,445] The efficacy of intrapartum prophylaxis adjusted for the presence of fever has been estimated to be 85 percent against early-onset group B streptococcal sepsis and 63 percent against early-onset sepsis attributable to other organisms.[450]

The previous guidelines for intrapartum antibiotic prophylaxis recommended a screen- and risk-based approach, and the modified 2002 guidelines recommend universal screening by vaginal and rectal GBS cultures for all pregnant women at 35 to 37 weeks of gestation.[253,444]

Intrapartum antibiotic prophylaxis is effective in preventing early-onset group B streptococcal sepsis, but the widespread use of antibiotics has led to concern about possible selection for other more virulent organisms and increased antibiotic resistance.[307,477]

Two clinically and epidemiologically distinct forms of illness have been described.[23,37,165,231] The early- or acute-onset form is seen in the first 3 days of life (usually within the first 6 to 12 hours) and is characterized by a high incidence of maternal complications. These infants usually are very ill within hours of delivery and exhibit unexplained apnea or tachypnea, respiratory distress, hypoxemia, and shock. Chest radiographs reveal a diffuse pulmonary infiltrate similar to that seen after aspiration or findings indistinguishable from those of hyaline membrane disease. One of the major diagnostic problems associated with the acute-onset syndrome is clinical differentiation of it from respiratory distress syndrome. Several features of early-onset group B streptococcal disease may be helpful in differentiating it from respiratory distress syndrome. Obstetric complications are encountered commonly in mothers of infants with group B streptococcal disease, whereas they seldom occur in mothers of infants with respiratory distress syndrome.[37,165] Evidence indicates that prenatal complications may protect the infant from respiratory distress syndrome because of increased corticosteroid secretion by the mother.

The proportion of infants with meningitis is higher in those with late-onset infections.[444] Intrauterine infection of the fetus results from ascending spread of GBS from the vagina of a colonized woman, who typically is asymptomatic. Fetal aspiration of infected amniotic fluid can lead to stillbirth, neonatal pneumonia, or sepsis. Infants also can become infected with GBS during passage through the birth canal, although the majority of infants who are exposed to the organism through this route become colonized on skin or mucous membranes but remain asymptomatic.[444]

When compared with infants who have respiratory distress syndrome, infants with streptococcal disease usually are sicker early in the course of illness, and apnea, shock, or both occur within 12 to 24 hours of the onset of infection. Infants with respiratory distress syndrome experience a more gradual evolution of events. In some infants with group B streptococcal disease, rapid progression to respiratory failure and death occurs within 12 hours. This situation is an unusual occurrence in infants with uncomplicated respiratory distress syndrome. The peak inspiratory pressures required to ventilate babies with streptococcal disease are said to be lower than those necessary for infants with respiratory distress syndrome.[2] In preterm infants, leukopenia with increased numbers of band forms occurs commonly in infected cases.[2,300]

The chest radiograph may be helpful in differentiating these two illnesses. Neonatal pneumonia is found in approximately 40 percent of infants with group B streptococcal disease. In the others, a diffuse reticulogranular pattern with air bronchograms is seen and cannot be distinguished from that observed in infants with respiratory distress syndrome. Hyaline membranes are seen pathologically in both illnesses.

The second major form of neonatal group B streptococcal disease is the late-onset syndrome. In contrast to the acute fulminant disease of the first day of life, the late-onset syndrome has an insidious onset after the infant reaches 5 to 7 days of age, although it occasionally may be fulminant.[23,238] The disease almost invariably involves the meninges. Most infants are initially seen in the second through fourth weeks of life, but documented cases have occurred at up to 12 weeks of age. A history of maternal obstetric complications usually is lacking, and the infant almost always has an unremarkable early neonatal history, although late-onset disease does occur occasionally in premature infants.[136] The case-fatality rate is low, on the order of 5 to 15 percent. Some cases of necrotizing fasciitis have been reported, with prematurity probably being the most important risk factor.[274]

GBS can be immunologically subclassified into nine different capsular serotypes (Ia, Ib, and II to VIII); Ia, II, III, and V uniformly are attributed as causes of early-onset disease, and the group B_{III} organism is responsible for approximately 90 percent of all late-onset cases[22,223] (irrespective of clinical manifestation) and cases of meningitis in infants (irrespective of the age at onset). This apparently virulent effect of type III strains, which account for two thirds of group B streptococcal infections in infants, appears to be restricted to young infants. In contrast, type II strains are predominant in isolates from adults with meningitis, but this serotype rarely is isolated from the CSF of infants with meningitis. All the capsular types, especially serotype III, are poorly immunogenic, which helps GBS avoid immune clearance by masking its surface proteins, and the capsular polysaccharide inhibits complement deposition and phagocytosis.[223,268]

The mode of acquisition of B_{III} is uncertain because this organism usually cannot be recovered from maternal sites at onset of the infant's illness. Horizontal transmission of the pathogen from nursery personnel, caregivers at home, and other individuals to the newborn has been proposed as the most reasonable mode of acquisition.[1,22,373]

The clinical features of illness are indistinguishable from those of other forms of purulent meningitis in this age group. An exception to the normal pattern of late-onset infection is the intensive care unit setting, where nosocomially acquired clusters of disease in low-birth-weight infants have been reported.[353,512] In such circumstances, the spectrum of clinical expression is similar to that of early-onset disease, although serotype III still predominates.[353]

The gastrointestinal tract serves as the natural reservoir for GBS and is the probable source of vaginal colonization. Vaginal

colonization is an unusual occurrence in children but becomes a more common finding in older adolescents.[210]

Resistance among group B streptococci has been limited to certain agents, such as macrolides and clindamycin, but not to penicillin. Resistance to erythromycin and clindamycin occurs in as many as 25 and 15 percent of clinical isolates, respectively.[14,221,443]

The impact of intrapartum antibiotic prophylaxis on neonatal infections caused by organisms other than GBS is not clear inasmuch as different studies have shown a range of results, including an increase, no change, and a decrease in frequency.[104,239,317,450,477] The incidence of ampicillin-resistant *E. coli* strains seems to be increasing, but the role of intrapartum prophylaxis in this trend remains unclear. Although the incidence of *E. coli* sepsis increased in VLBW infants in one report (from 3.2 to 6.8 cases per 1000 births), the occurrence of other types of gram-negative sepsis declined in the same report (from 5.1 to 2.6 per 1000 births).[477]

OTHER STREPTOCOCCAL DISEASE

Group A beta-hemolytic streptococcal disease does not occur as frequently now as in previous decades. Disease caused by this organism varies from low-grade chronic omphalitis to fulminant septicemia and meningitis. Because of the explosive nature of this organism in nursery settings, constant surveillance for colonized infants and prompt recognition of illness are mandatory to avert a nursery outbreak of group A streptococcal disease.[178]

Necrotizing fasciitis caused by group A streptococci is a rare event that occurs in approximately 0.018 percent of hospitalized neonates, with a mortality rate of 18 percent but with a long-term morbidity rate of 91 percent.[201] Maternal carriage is considered an important risk factor for neonatal infection, with an approximate rate of 0.03 percent for vaginal and rectal carriage.[319,328]

Group D and G streptococci have been reported to cause an illness indistinguishable from early-onset group B streptococcal sepsis.[141,456] Viridans streptococci are the second most frequent gram-positive organisms in early-onset sepsis, and they usually cause a less severe illness with a lower incidence of respiratory distress, shock, and white blood cell (WBC) count abnormalities.[450,468,477] Dobson and Baker,[138] in a review of 56 neonates with enterococcal septicemia from a single hospital in Houston, Texas, from 1977 through 1986, described two distinct clinical syndromes. Infants older than 7 days were more premature, had lower birth weights, and, in most cases, had infections characterized by a nosocomial origin. When compared with early-onset disease (5 days of age or younger), which was manifested as mild illness with respiratory distress or diarrhea without focal infection, late-onset enterococcal sepsis was heralded by severe apnea, bradycardia, circulatory collapse, and increased ventilation requirements. Focal infections, such as meningitis, pneumonia, scalp abscess, and catheter-related illnesses, were common occurrences.

Staphylococcus Infection

In the mid-1950s, phage group I *S. aureus* was the most common bacterial agent causing serious bacterial disease in newborn infants. Its unique invasive properties caused disseminated disease, including mastitis, furunculosis, suppurative arthritis, osteomyelitis, septicemia, and meningitis, with widespread manifestations. Because bloodstream infection usually occurs after local invasion, the primary focus must be searched for carefully in all septic babies. Changes in the epidemiologic characteristics of the organism, coupled with intensified microbial surveillance and infection control measures, have reduced colonization and disease rates attributable to phage group I *Staphylococcus*.

Coagulase-positive staphylococcal disease in nurseries also has been caused by phage group II organisms.[316] These organisms produce an exotoxin (i.e., exfoliatin) that results in intraepidermal cleavage through the granular cell layer because of disruption of desmosomes.[322] Clinical disease may take one of several forms, including bullous impetigo, toxic epidermal necrolysis (i.e., Ritter disease), and nonstreptococcal scarlatina. The initial finding in Ritter disease is intense, with painful erythema followed by the formation of bullae, which when ruptured leave a tender, weeping erythematous area. A characteristic desquamation of large epidermal sheets occurs approximately 3 to 5 days after onset of the disease. Fine desquamation is observed commonly in the perioral region. Bullous impetigo has been the disease associated most commonly with nursery outbreaks of group II staphylococcal infections.[6]

In premature infants, the risk of acquiring staphylococcal infection increases as a result of poorly developed host defense mechanisms; the presence of central venous, upper gastrointestinal tract, or endotracheal catheters; procedures causing interruption in skin integrity; prolonged total parenteral nutrition; and the use of steroids.[220] By the third day of life, *S. aureus* and coagulase-negative staphylococci colonize the nasopharyngeal compartment of nearly all infants with birth weights lower than 1750 g.[159]

In the 1980s, MRSA emerged as a nosocomial pathogen of considerable importance. MRSA demonstrates resistance to the penicillinase-resistant penicillin class of antibiotics, which includes methicillin, nafcillin, oxacillin, cloxacillin, and dicloxacillin. The mechanism of resistance involves, in part, alteration of penicillin-binding proteins in the periplasm of the bacterium, thereby resulting in a decrease in affinity for these antibiotics. The spectrum of clinical disease caused by MRSA is similar to that caused by methicillin-susceptible *S. aureus*, except that patients with MRSA bacteremia were reported to be less likely to have bone or joint infection.[479] Between 2000 and 2005, the incidence of MRSA infection in the Parkland nursery has varied between 0.12 to 0.45 per 1000 live births. There appears to be a more frequent colonization rate as well (P. Sanchez, personal communication, 2006).[426]

MRSA infection emerged in the late 1990s in healthy children and adults in the community setting. Community-acquired MRSA is associated more frequently with skin and soft tissue infections; however, some cases may progress to invasive infection and death.[169,246] In hospitalized neonates, different clinic characteristics, consisting of omphalitis, preseptal cellulites, otitis externa, pustulosis, mastitis, and bacteremia, can be present.[220]

Staphylococcal infections are a frequent and important cause of morbidity and mortality in nurseries.[19] Outbreaks of skin infections in neonates have been associated with colonized nurses.[54]

Coagulase-negative *Staphylococcus* is the most common organism associated with late-onset sepsis. However, determining which blood culture isolates represent an infection or contamination remains difficult.[330,478] Isolation of these organisms should be considered significant when they grow in aerobic and anaerobic blood culture bottles, when growth occurs within 72 hours, or when they are isolated from two or more sites or from the same site at different times.[39,336,352] *S. epidermidis* disease tends to occur as a late-onset infection (i.e., nosocomial acquisition) and is associated with the usual signs and symptoms of sepsis. WBC count abnormalities are found in approximately half of all infected infants. Major risk factors include prematurity, low birth weight, invasive procedures, central venous catheters, and total parenteral nutrition. Exposure to intravenous lipid emulsions was the major determinant of bacteremia caused by coagulase-negative staphylococci in VLBW infants in one case-control study.[167] These infections frequently are associated with colonization of central venous catheters and involvement of other sites, such as the CNS. Infected patients usually are not very ill and respond well to antimicrobial therapy, but frequently the central venous catheters must be removed to prevent further seeding of the

bloodstream. The mortality rate is low and ranges from 0 to 15 percent in different series.[39,162,336,352] In a cohort of 1313 VLBW infants with late-onset sepsis (Neonatal Research Network), infants with sepsis attributable to coagulase-negative staphylococci were not more likely to die than were infants who were not infected, but the results can be misleading because of the difficulty of determining which patients have true infections.[478]

Escherichia coli Infection

E. coli strains are the most common gram-negative bacteria that cause septicemia in neonates. Unlike illnesses caused by GBS and *L. monocytogenes*, *E. coli* infections do not fit into distinct clinical syndromes of early- and late-onset disease. Approximately 40 percent of *E. coli* strains causing septicemia possess K1 capsular antigen.[430] The clinical features of *E. coli* sepsis generally are similar to those observed in infants with disease caused by other pathogens. Localized *E. coli* infections have included breast abscess, cellulitis, meningitis, pneumonia, lung abscess, empyema, osteomyelitis, septic arthritis, urinary tract infection, ascending cholangitis, and otitis media.

In recent reviews of early-onset sepsis, *E. coli* isolates have shown an increase in resistance to ampicillin, with figures of higher than 80 percent reported in developed and developing countries. Women with ampicillin-resistant *E. coli* infections were more likely to have received intrapartum ampicillin than were those with susceptible strains.[477,530]

Klebsiella pneumoniae Infection

K. pneumoniae is the major pathogen responsible for neonatal sepsis in many developing countries. The incidence of infections varies from 4.1 to 6.3 per 1000 live births, with a fatality rate of 16 to 68 percent. A substantial number of infections occur as early-onset disease. Although species of *Klebsiella* can be normal flora in gastrointestinal and vaginal sites, isolation of resistant strains is more likely related to hospital acquisition in heavily contaminated environmental reservoirs, particularly in developing countries.[58,145,349,530]

Listeria monocytogenes Infection

The pathogenesis and clinical spectrum of diseases caused by *L. monocytogenes* are similar to those caused by GBS. Because the most common foci for neonatal infection are the lung and gut, the fetus probably is infected by the mother swallowing contaminated liquor and through the transplacental route. Infants acquire the infection in two ways, from mothers who are colonized in the gastrointestinal tract after eating contaminated food, with occult sepsis developing and resulting in chorioamnionitis, or from mothers carrying *Listeria* in the gastrointestinal or perianal regions that contaminates the skin and respiratory tract of their babies during birth.[439] Chorioamnionitis diagnosed by transabdominal amniocentesis in pregnant women with intact fetal membranes has been reported,[378] thus favoring the blood-borne route of infection. Early gestational *Listeria* can be associated with abortion or stillbirth. Premature labor in mothers with *Listeria* infection is a common occurrence; in approximately 70 percent of cases, delivery occurs before 35 weeks' gestation.

Evidence of preceding maternal illness often is described in infants with early-onset disease. Symptoms in mothers can be vague (i.e., malaise and myalgia) or distinctive (i.e., fever and chills) and may alert the physician to a risk for *Listeria* infection. Blood cultures often (35%) are positive for *Listeria* in such mothers.

In neonates, two types of illness have been described. "Early-onset" listeriosis can develop after maternal sepsis, and chorioamnionitis can cause abortion, stillbirth, or premature delivery of a severely infected infant. These patients can have pustular skin lesions (granulomatosis infantisepticum) and granulomatous hepatitis, findings often detected at autopsy. The associated mortality rate can reach up to 20 percent.[150,388] The pathogen is acquired transplacentally[499] or by aspiration at the time of vaginal delivery.[42] The infant frequently has hypothermia, is lethargic, and feeds poorly.[451] The organism can be found in the infant's blood, CSF, skin, and placenta; the primary clinical picture is severe sepsis with multiorgan involvement.

A characteristic rash consisting of small, salmon-colored papules scattered primarily on the trunk may be observed in some infants.

Listeria infection should be suspected in premature infants with early passage of meconium. Because meconium is an extremely unusual finding in premature infants younger than 32 weeks' gestational age, its presence should alert the physician to the possibility of *Listeria* infection. Chest radiographs show parenchymal infiltrates suggesting aspiration pneumonitis in most infants. A miliary type of bronchopneumonia also can be seen in some infants. No cases of acute-onset listeriosis mimicking the radiographic picture of hyaline membrane disease have been reported. *Listeria* serotypes Ia, Ib, and IVb produce the early-onset disease, whereas serotype IVb is the predominant type in late-onset meningitic disease.[7]

A delayed form of neonatal listeriosis (late-onset disease) occurs during the second through eighth weeks of life and involves the meninges in almost all cases.[318] Infected infants usually are the term products of an uncomplicated labor and delivery. The onset of symptoms and signs is relatively insidious, and they are indistinguishable from those observed with meningitis caused by other pathogens. Acute *Listeria* encephalitis, which usually is fatal within a few days, is a rare disease in humans. Other clinical forms of disease at this age include *Listeria*-induced colitis with associated diarrhea and sepsis but without meningitis. The bacteriology laboratory should be forewarned of the clinical suspicion of listerial meningitis because these microorganisms frequently are discarded as contaminants because of their tinctorial and morphologic similarities with diphtheroids. Overnight refrigeration of spinal fluid specimens frequently enhances growth of this organism.

The peripheral WBC count usually shows a brisk leukocytosis with a predominance of polymorphonuclear leukocytes in the differential count. A significant elevation in the number of monocytes to 7 to 21 percent of the total WBC count has been documented on admission laboratory evaluation of infected infants.[501] Likewise, a monocytosis of this magnitude can be demonstrated in most of the remaining infants on repetitive determination of the peripheral WBC count. In contrast, monocytes are not found typically in the spinal fluid of infants infected with *L. monocytogenes*. Polymorphonuclear leukocytes predominate in approximately 75 percent of cases, with a relative lymphocytosis detected in the remaining 25 percent. As with other pyogenic meningitides, hypoglycorrhachia and elevated protein concentrations are frequent findings. Examination of stained smears of spinal fluid has not been rewarding in more than 50 percent of cases, a reflection of the relatively low concentration of organisms in this fluid,[155] the atypical morphology, and the variable decoloration resulting from the Gram-staining procedure, which may result in organisms appearing as gram-negative rods or gram-positive cocci.

Pseudomonas aeruginosa Infection

Pseudomonas septicemia may be manifested as characteristic violaceous papular lesions in which central necrosis develops after several days (i.e., ecthyma gangrenosum). Noma (i.e., gangrenous lesions of the nose, lips, and mouth) has been associated with *P. aeruginosa* bacteremia. It is caused by a suppurative vasculitis, and

deep-seated abscess formation is a common finding.[398] A neonate treated with broad-spectrum antimicrobial agents while in an environment potentially contaminated by "water bugs" (e.g., respirators, moist oxygen) particularly is prone to acquisition of disease caused by *Pseudomonas* spp. or other fastidious commensals. The organism usually is a cause of late-onset disease.[282,478] Stevens and colleagues,[475] however, reported nine cases of *Pseudomonas* sepsis, four of which developed in the infants' first 72 hours of life. The clinical and radiologic findings in these four infants were similar to those of hyaline membrane disease.

DIAGNOSIS

The diagnosis of sepsis neonatorum relies heavily on the clinical judgment and diagnostic acumen of the physician. Signs and symptoms may be vague and frequently misleading. Bacterial infection may masquerade as metabolic disease, respiratory distress, environmental stress, and other noninfectious conditions. A physician evaluating an infant with possible sepsis must be guided by a complete perinatal history to elicit factors that place the infant at high risk, by a thorough physical examination with attention to signs suggestive of infection, and by clinical experience. When infection is likely, a laboratory work-up is indicated. When infection is unlikely and not substantiated by the history, physical examination, and clinical judgment, investigating for an infectious process usually is unnecessary. If doubt exists, as frequently is the case, good practice is to proceed with a laboratory work-up.

Recovery of an organism from a meaningful site, such as blood, CSF, urine, abscesses, pleural and peritoneal spaces, joints, bones, and middle ear cavities, substantiates the clinical impression of systemic bacterial disease. Isolation of an organism from mucocutaneous sites, such as the skin, ear canal, nasopharynx, gastric aspirate, and rectum, usually does not reflect the microbiologic status of normally sterile body fluids or tissues.[159] A point of emphasis is that the colonization-to-disease ratio for the major neonatal pathogens is approximately 100:1 to 200:1. For every infant with documented systemic bacterial disease, 100 or more infants are colonized superficially with this organism but are free of systemic bacterial disease.

Several sites to sample blood for culture give reliable results: peripheral vein, umbilical artery, and capillary blood. The preferred site is the peripheral vein. Venipuncture should be performed after the skin has been prepared properly by cleansing with an iodine-containing solution.[146] A two-phase antisepsis procedure using 70 percent isopropyl alcohol followed by chlorhexidine or povidone-iodine has been shown to be superior to one using chlorhexidine or povidone-iodine alone in reducing skin colonization by *S. epidermidis*.[100] The theoretical minimal amount of blood needed for detecting bacteremia is a function of the number of organisms circulating at any given time. Infants with *E. coli* sepsis have 5 to more than 1000 colony-forming units per milliliter of blood.[133] Culturing as little as 0.2 mL of blood should be sufficient for detecting *E. coli* bacteremia in these patients. On the basis of experimental *E. coli* sepsis in rabbits, a cultured volume of blood of 0.2 mL is as sensitive as is 1 mL in detecting bacteremia at a threshold level of five organisms per milliliter of circulating blood.[160] To improve culture sensitivity, a prudent procedure is to obtain at least 0.5 to 1 mL of blood for culture in infants aged birth to 2 months. Optimal results are obtained when the cultured volume of blood is 5 to 10 percent of the total amount of liquid growth medium to be inoculated.

Bacterial growth is seen in most blood cultures within 48 hours. With the use of conventional culture techniques and subcultures at 4 and 14 hours, only 4 percent of cultures that had positive results required more than 48 hours of incubation.[383] With the use of radiometric technique, 98 percent of cultures growing GBS and *E. coli* were identified within 24 hours.[412] We generally recommend obtaining one or two blood cultures before initiating antibiotic therapy. Urine and CSF should be obtained for examination and culture before starting therapy.

Many laboratory tests have been recommended for the evaluation of suspected bacterial disease in neonates. The WBC count is a simple, readily available test that can help in the early detection of sepsis.[46,300,301] Elevated total WBC counts, absolute neutrophils counts, and absolute band counts generally are not helpful singly as indicators of sepsis. Although neutropenia in neonates is caused by infection in most cases, it frequently is associated with other conditions, such as birth asphyxia and pregnancy-induced hypertension.[148]

The usefulness of abnormal WBC counts in the detection of sepsis is enhanced by measurement of the immature-to-total neutrophil ratio; a ratio of 0.2 or greater is a relatively sensitive indicator of neonatal sepsis.[108,379,380] Numerous studies that have evaluated the immature-to-total neutrophil ratio have shown that the ratio is too unreliable to achieve more than limited clinical usefulness. Sensitivities ranging from 90 to 60 percent or less have been reported.[52,53] Elevated ratios caused by a variety of perinatal conditions have been seen in 25 to 50 percent of noninfected, ill infants.[177,260] The ratio's greatest value is thought to be in its good negative predictive value; if the ratio is normal, the likelihood that infection is absent is very high.[177,260] An immature-to-total ratio of 0.8 or greater indicates depletion of bone marrow neutrophil reserves and is a poor prognostic indicator. A combination of all these laboratory findings (i.e., hematologic scoring system), rather than those of any test alone, increases the diagnostic specificity of a bacterial infection.[406] This scoring system is of limited value in late-onset, coagulase-negative staphylococcal infections.[128]

Determining the WBC count and immature-to-total ratio again 6 to 8 hours after the initial evaluation is important because studies in animals and human infants have demonstrated that the WBC count can be normal at the onset of group B streptococcal sepsis and abnormal 4 to 8 hours later. Morphologic changes in neutrophils, such as vacuolization and toxic granulation, suggest the presence of infection. The degree of these degenerative changes in the neutrophils of infected neonates provides no implication of the severity or potential outcome of the illness.[293] Identical morphologic findings can occur as artifacts in citrated blood samples stored for longer than 1 hour before smears are made.

A reduced platelet count associated with placental insufficiency is the most frequent cause of early-onset thrombocytopenia in neonates.[404] The presence of severe thrombocytopenia, or less than 50,000 cells/mL, within 72 hours of birth is an uncommon occurrence and more likely is related to perinatal asphyxia or early bacterial infection (e.g., group B streptococcal sepsis). Late-onset thrombocytopenia almost always is caused by bacterial sepsis or necrotizing enterocolitis. In these cases, platelets fall rapidly, with a nadir at 24 to 48 hours, to counts less than 50,000 cells/mL and tend to persist until the infection is controlled. Slow recovery of platelet counts over a period of 1 to 2 weeks is common.[98,337,404]

The erythrocyte sedimentation rate in infected patients generally is elevated above the normal range of 1 to 2 mm/hr at 12 hours of age to 17 to 20 mm/hr at 14 days of age.[4] Elevated rates usually are not observed until 24 to 48 hours after clinical signs of disease first occur. Other acute-phase reactants, such as C-reactive protein, haptoglobin, prealbumin, orosomucoid, and transferrin, may be useful in establishing the diagnosis of neonatal sepsis and in monitoring the course of the infection.[381] The most extensively studied of these acute-phase reactants is C-reactive protein. Concentrations of this protein increase significantly within a few hours of the onset of infection. In general, other perinatal events have little impact on neonatal C-reactive

protein values.[442] C-reactive protein concentrations decrease rapidly in infected neonates who respond to therapy and, conversely, are elevated persistently in neonates whose infections fail to respond to therapy.[241,429] Reliance on a single C-reactive protein determination as an early indicator of neonatal bacterial infection is not recommended. Serial determinations are helpful, especially in combination with other hematologic tests, when making a decision to stop antimicrobial therapy safely.[65,382]

Fibronectin is a glycoprotein that has been identified on cell surfaces and in extracellular fluids. The concentration of fibronectin in fetal plasma increases with gestational age to values at term of approximately half those found in healthy adults. Plasma concentrations fall significantly during an episode of sepsis[177] but may decrease in noninfectious neonatal conditions such as perinatal asphyxia and respiratory distress syndrome.[526] More data are needed to determine the value of fibronectin concentrations as an indicator of bacterial sepsis.

Detection of interleukin-6 in plasma samples of newborns has been suggested as a reliable early indicator of sepsis.[83,203,218] Interleukin-6 is a pleiotropic cytokine involved in many aspects of the immune system. It is synthesized and released in response to inflammatory stimuli by monocytes, endothelial cells, and fibroblasts after production of tumor necrosis factor and interleukin-1. Interleukin-6 is the major inducer for the synthesis of hepatic proteins, including C-reactive protein, fibrinogen, and other acute-phase reactants. Its sensitivity in the diagnosis of sepsis and necrotizing enterocolitis appears to be high.[218] One study suggests that measuring serum concentration of platelet-activating factor can identify septic infants at risk for necrotizing enterocolitis.[392] An analysis of all studies published indicates that the main diagnostic importance of measuring interleukin-6 in neonates appears to relate to its very high negative predictive value. An initial interleukin-6 value below 20 pg/mL excludes the possible presence of sepsis in more than 90 percent of infants; if it is still below this value when repeated several hours later, its usefulness as a negative predictor of sepsis is even stronger.[158,252,297] More studies are needed, however, to evaluate the precise role of interleukin-6 measurement in guiding physicians to a better diagnostic approach to systemic neonatal infections.

Procalcitonin

Procalcitonin (PCT) has been proposed as a sensitive and specific marker of bacterial infection.[105,176] Several recent studies have found that an elevated serum PCT concentration can be useful in diagnosing antenatal and early-onset infection because of its high positive and negative predictive values. Infants with viral infection, bacterial colonization, and sterile inflammatory stress have normal or slightly raised concentrations.[176] Some studies suggest that this test will be useful in evaluating the severity of the infection and monitoring the clinical course.[105] However, very high serum concentrations have been detected in patients with respiratory distress syndrome, acute lung and inhalation injuries, hemodynamic failure, and severe trauma.[351] Administration of antibiotics during the prenatal, intranatal, and postnatal periods also may alter the relationship between PCT and infection. Although cutoff values usually differ among studies, a concentration of 2 ng/mL is considered reliable in distinguishing between viral and bacterial infection.[498] More extense investigations are needed to determine whether PCT measurement, in a single or repeated sampling, would be of value in differentiating between bacterial infection and colonization and in reducing the number of patients treated unnecessarily with antibiotics.[248]

Other Diagnostic Tests

Detection of bacterial antigens in blood, urine, or CSF confirms the presence of systemic bacterial disease. Diagnostic techniques include countercurrent immunoelectrophoresis, latex particle agglutination, and coagglutination procedures. Countercurrent immunoelectrophoresis is specific but has low sensitivity and can be used to detect infections caused by *E. coli* K1 and GBS.[32,215] Latex particle agglutination and coagglutination tests are more sensitive than is countercurrent immunoelectrophoresis, but they have been associated with a small percentage of false-positive and false-negative reactions.[170,215] They can be used for the detection of disease caused by GBS, *N. meningitidis*, *S. pneumoniae*, and *H. influenzae* type b. The highest yield is achieved by testing concentrated heat-treated urine specimens and CSF. The sensitivity of this test is 90 to 98 percent, with an average false-positive rate of 2 to 6 percent. Perineal contamination may cause false-positive results in healthy colonized infants in the absence of invasive disease when the urine tested is obtained by bag collection.[427] The absence of antigen does not rule out infection. A positive result in the urine antigen test with negative results on blood culture can imply occult infection (e.g., osteomyelitis), partial treatment by intrapartum antibiotic therapy, or a false-positive result. The *E. coli* K1 antigen is identical immunologically to the *N. meningitidis* group B antigen and, therefore, can be detected by *N. meningitidis* group B kits. The usefulness of this test is much decreased, however, by its low sensitivity and the high contamination rate of urine collected by bag with *E. coli* from the gastrointestinal tract.

Direct examination of Gram- or methylene-stained blue buffy coat smears can help in the early detection of neonatal bacteremia if bacteria engulfed by neutrophils are visualized.[152] Some physicians consider only smears with intragranulocytic bacteria to be positive. Bacteria are seen more readily when acridine orange stain is used.[261] This technique requires a smaller volume of blood but cannot distinguish between gram-positive and gram-negative bacteria. Identification of bacteria on Gram-stained smears of tracheal secretions obtained in the first 12 hours of life from infants who require intubation is associated with bacteremia in approximately half of these cases.[452]

Endotoxin elaborated from gram-negative bacteria circulates in blood and is present in urine for considerable periods of time after these fluids have been sterilized. Detection of endotoxin by the *Limulus* amebocyte lysate assay may be helpful in the early identification of infected infants.[254,436] Endotoxin may be present in the blood of septic-appearing infants who have sterile blood cultures. Transient endotoxemia perhaps is responsible for "clinical sepsis" in these infants. The source of endotoxin may be the gram-negative bacterial flora of the bowel. Endotoxin possibly enters the circulation through injured and permeable gastrointestinal mucosa.

Several serologic tests for establishing the diagnosis of listeriosis have been described, but none has become an established routine method. Agglutination reactions, complement fixation, enzyme-linked immunosorbent assay, precipitin, indirect hemagglutination, and antigen fixation tests are available and may help occasionally. Caution is warranted, however, in attempts to use these tests for diagnostic purposes. Genetic studies have shown that an extracellular hemolysin, listeriolysin O, is essential for the intracellular multiplication of *Listeria*. Molecular tests, including DNA hybridization and polymerase chain reaction (PCR), also have been developed for rapid identification of *Listeria* spp. and for differentiating *L. monocytogenes* from other *Listeria* spp.[172]

In one study,[47] French investigators examined whether detection of specific anti-listeriolysin O could be used for the serodiagnosis of human listeriosis. Sera from 28 patients (13 were newborn) infected with *L. monocytogenes* and 101 controls were tested by dot-blot titration with purified listeriolysin O. Twenty-seven patients (96%) with *Listeria* infection produced specific anti-listeriolysin O, which was detected in low titer in 16 percent of healthy controls and in 12 percent of persons who had various bacterial, fungal, and viral infections. Anti-listeriolysin O could

be detected soon after infection and persisted for at least several months. Although this test might be useful for epidemiologic surveys and serodiagnosis of listeriosis, more data are needed before it can be used routinely for the serodiagnosis of human listeriosis.

TREATMENT

After the diagnosis of sepsis is suspected or established and the appropriate samples have been obtained for culture, antibiotic therapy should be instituted. When the infant's condition prompts an evaluation for sepsis, initiation of empiric parenteral antibiotic treatment usually is prudent, despite the fact that only 5 to 10 percent of blood cultures are positive. For practical purposes, we have summarized the recommended empiric antimicrobial treatment of the various bacterial infections occurring during the neonatal period based on the published etiologic organisms identified; therapeutic alternatives also are provided (Table 78–5).

Infants with suspected sepsis should be treated with a combination that includes a penicillin and an aminoglycoside. The choice of antibiotics must be based on the infectious history of the nursery, the antimicrobial susceptibilities of bacteria recently isolated from sick and healthy neonates, the probable etiologic agent, CSF penetration of antibiotics, and the infant's hepatic and renal function. Factors that determine the probable infecting organism include patient age and birth weight; environment (home versus hospital); previous antibiotic therapy; perinatal or nosocomial exposure to pathogens (e.g., MRSA); presence of central lines, drains, or endotracheal tube; and identification of specific infections, such as meningitis, necrotizing enterocolitis, peritonitis, thrombophlebitis, pneumonia, and soft tissue infections.

For treating early-onset sepsis neonatorum, we recommend ampicillin and gentamicin. Ampicillin is effective in vitro and clinically against GBS, *Listeria*, *Proteus*, most enterococci, and 15 to 30 percent of current *E. coli* strains.[450,477] The aminoglycosides have broader antimicrobial activity against many Enterobacteriaceae, including most *E. coli*, *Klebsiella-Enterobacter*, and *Proteus* strains, and, with the exception of kanamycin, against *P. aeruginosa*. Although gentamicin frequently is used, the choice of aminoglycoside (e.g., amikacin, tobramycin, netilmicin) should be based on the antimicrobial susceptibility of nosocomial bacteria within individual nurseries. For infections caused by gentamicin-resistant coliforms, amikacin or third-generation cephalosporins such as cefotaxime and ceftazidime should be used.[232,249,435,496]

Cephalosporins are not active against *Listeria* or enterococci and should not be used without concomitant administration of ampicillin. Moreover, when used with ampicillin, cephalosporins do not offer the advantage of synergism that aminoglycosides do against strains of enterococci. Staphylococci, nosocomial gram-negative organisms, and fungi rarely are encountered in early-onset sepsis neonatorum, and empiric coverage for them generally is not required. When the epidemiologic experience of the nursery suggests *Pseudomonas*, an extended-spectrum penicillin (i.e., ticarcillin or piperacillin) or ceftazidime combined with an aminoglycoside should be administered (see Table 78–1 for dosages). Therapeutic drug monitoring is recommended when aminoglycosides are used, especially in low-birth-weight neonates, and adjustment of the dosage is essential for infants with impaired renal function. Recent interest has focused on evaluating the use of extended intervals for aminoglycoside administration in the neonatal period,[358] and guidelines for drug monitoring in these circumstances need to be evaluated carefully.

Because late-onset sepsis neonatorum is more heterogeneous in its epidemiology than is early-onset disease and may reflect maternal, family, community, or nosocomial sources for the infecting pathogen, the organisms involved cover a broad taxonomic spectrum. As a result, empiric antimicrobial regimens vary. In a previously healthy infant who already has been discharged from the hospital, ampicillin and an aminoglycoside or cefotaxime are recommended unless staphylococcal infection is highly suspected, in which case an antistaphylococcal agent (e.g., oxacillin, nafcillin) should replace ampicillin. Selection of empiric antibiotic regimens can be more difficult for a septic premature infant who has had a prolonged hospitalization, previous antibiotic therapy, possible prolonged tracheal intubation, and placement of a central or peripheral intravascular catheter.

In these patients, major pathogens include coagulase-negative and coagulase-positive staphylococci (including MRSA), aminoglycoside-resistant coliforms, highly resistant opportunistic organisms (e.g., *Pseudomonas*, *Serratia*), fungi, and possibly enterococci. As a result, empiric regimens in this situation should be individualized. Examples include ampicillin, amikacin, and clindamycin for suspected necrotizing enterocolitis, vancomycin and an aminoglycoside or cefotaxime for patients with indwelling central vascular lines, and nafcillin and an aminoglycoside for babies with skin infection. Avoiding empiric vancomycin therapy seems to be a reasonable approach for treating late-onset sepsis because coagulase-negative staphylococci are common contaminants of blood cultures and are associated with a very low frequency of fulminant infection. In institutions with a high

TABLE 78–5 Recommended Empiric Antimicrobial Treatment of Several Neonatal Bacterial Infections on the Basis of Probable Etiologic Microorganisms

Bacterial Infection	Recommendation	Alternatives	Observations
Sepsis			
Early onset (<5 days)	AMPI + GENTA	AMPI + CEFO	
Late onset	AMPI + GENTA	AMPI + CEFO	Readmission of the neonate at term
Nosocomial	VAN ± OXA/NAF + GENTA or AMIK	VAN + CEFTA	Consider AMPHO
Meningitis	AMPI + CEFO	AMPI + GENTA	
Otitis media	AMOX/CLAV	CEFUROXIME	Given orally unless systemic signs present
Urinary infection	AMPI + GENTA	AMPI + CEFO	
Osteoarticular infection	VAN ± OXA/NAF + CEFO	VAN + CEFO	Consider AMPHO
Cellulitis/fasciitis/funisitis/omphalitis	VAN ± OXA/NAF or CLIN + GENTA or AMIK	VAN + CEFTA	Surgery
Pneumonia			
Early onset (<5 days)	AMPI + GENTA	AMPI + CEFO	
Nosocomial	VAN ± OXA/NAF + GENTA or AMIK	VAN + CEFTA	Consider macrolide

AMIK, amikacin; AMOX/CLAV, amoxicillin/clavulanate; AMPHO, amphotericin B; AMPI, ampicillin; CEFO, cefotaxime; CEFTA, ceftazidime; GENTA, gentamicin; NAF, nafcillin; OXA, oxacillin; VAN, vancomycin.

prevalence of MRSA infection, vancomycin generally is used as initial therapy for suspected staphylococcal disease.[251] For infants who fail antimicrobial therapy or have superficial cultures positive for *Candida albicans*, empiric use of amphotericin should be considered. Ceftazidime and cefotaxime should not be used routinely in neonatal units because of the potential for emergence of resistant *Enterobacter* and *Serratia* spp.

After culture and susceptibility studies are available, changes in therapy may be necessary. Ampicillin alone is preferred for enterococcal and *Listeria* infections, whereas ampicillin or penicillin can be used for group B streptococcal disease. Infants infected with these organisms usually receive a combination of ampicillin and an aminoglycoside for the first 3 to 5 days, followed by ampicillin for the balance of 7 to 10 days. The minimal inhibitory concentration (MIC) and minimal bactericidal concentration of ampicillin or penicillin against streptococci and that of nafcillin or oxacillin against *S. aureus* should be considered for the purpose of detecting tolerant strains of these organisms.[259,408,416,460] Tolerant strains are inhibited but not killed by concentrations of these antibiotics, which usually can be achieved in body fluids and are treated best by the addition of an aminoglycoside to ampicillin or nafcillin. The clinical significance of tolerance is uncertain. For *S. epidermidis* infection, vancomycin is the drug of choice unless the isolate demonstrates in vitro susceptibility to nafcillin or oxacillin; most are resistant to these latter drugs. In selected neonates, the addition of rifampin to vancomycin therapy may be beneficial in clearing persistent bacteremia caused by coagulase-negative staphylococci.[484]

Central venous catheters or other foreign bodies frequently must be removed to eliminate the source of these organisms. If a gram-negative enteric isolate is susceptible to ampicillin and aminoglycosides, treatment with either antibiotic alone can be adequate, but we prefer treatment with both drugs for at least a portion of the treatment period. For *Pseudomonas* infections, combined therapy with ticarcillin, piperacillin, or ceftazidime and an aminoglycoside should be used for the duration of therapy. In certain circumstances, antibiotics not approved for infants (i.e., cefepime, imipenem or meropenem, ciprofloxacin) need to be used to treat an infection caused by a multidrug-resistant gram-negative isolate. A few anecdotal reports have been published suggesting successful outcomes of treated neonates.[68,255,270,341]

Although the third-generation cephalosporins have attractive features for the treatment of sepsis neonatorum, such as excellent in vitro activity against GBS and gram-negative enteric bacilli, provision of high serum and CSF concentrations, and no dose-related toxicity, we do not recommend their routine use in the nursery. Clinical studies suggest that they are comparable but not superior to ampicillin and gentamicin and that gram-negative enteric bacilli can become resistant rapidly when third-generation cephalosporins are used for presumptive treatment of neonatal sepsis.

In a 1989 survey of directors of programs in pediatric infectious disease in the United States and Canada, most physicians favored the traditional regimen of ampicillin and gentamicin for initial empiric treatment of sepsis and meningitis.[524] Use of a cephalosporin (cefotaxime in most cases) in conjunction with ampicillin was considered to be appropriate alternative therapy when meningitis was diagnosed. Antibiotics that have the potential to displace bilirubin from albumin-binding sites, such as ceftriaxone and sulfonamides, should be avoided in the newborn period.[338] Currently, no rationale exists for the routine use of chloramphenicol in newborn infants because of individual variations in pharmacokinetics in neonates that are associated with increased risk for the development of toxicity and that necessitate monitoring of the serum drug concentration, its bacteriostatic action against most gram-negative enteric pathogens in vitro, its antagonism with ampicillin against enteric gram-negative rods and GBS, and the availability of equally potent, safer β-lactam antibiotics.

The duration of antimicrobial therapy for neonatal sepsis usually is 7 to 10 days or approximately 5 to 7 days after the clinical signs and symptoms of infection have disappeared. Delayed clinical improvement or persistently positive blood cultures during therapy may indicate that inappropriate antibiotics have been selected or that occult sites of infection (e.g., endocarditis, abscesses, infected foreign bodies) exist.

Blood cultures in bacteremic neonates become positive in 96 percent of infants by 48 hours and in 98 percent by 72 hours.[383] For infants whose initial bacterial cultures are sterile after 48 to 72 hours of incubation, antimicrobial therapy can be discontinued. If no pathogen has been isolated but bacterial sepsis cannot be excluded, a negative C-reactive protein test at 72 hours can help support the decision to discontinue antibiotics.[470] Because postmortem blood cultures can be negative in infants with unequivocal evidence of sepsis (in 18% of patients in one study[469]), blood cultures from septic infants probably are sterile in some at the time of initial evaluation.

A good outcome depends critically on giving careful attention to fluid and electrolyte balance; correcting hypoxia, acidosis, hypoglycemia, and other metabolic abnormalities; and providing nutritional support. The use of fresh-frozen plasma and exchange transfusion as adjunctive therapy in severe neonatal sepsis has not been studied adequately, and no recommendations for their use can be made.[510] Infusion of intravenous immunoglobulin with functional activity against GBS to neonates produces a significant increase in GBS-specific immunoglobulin G that is sustained for several days.[161] Potential therapeutic benefits of intravenous immunoglobulin include enhanced chemotaxis and opsonophagocytosis and improved bactericidal activity of neonatal sera for these organisms. In animal models, a therapeutic effect is achieved only when immunoglobulins are given early in the course of disease.[397]

Experience with human immunoglobulin for intravenous use in septic neonates is limited, but such use appears to be safe. In a double-blind, placebo-controlled study, Weisman and colleagues[516] evaluated the effect of intravenous immunoglobulin (500 mg/kg) on the outcome of 31 premature infants with early-onset sepsis. During the first 7 days after therapy, 5 (29%) of 17 albumin-treated patients and none of 14 patients treated with intravenous immunoglobulin died ($p < .05$). The survival rate at 56 days of age, however, was not improved significantly. Very large doses of intravenous immunoglobulin may induce a blockade of the neutrophil receptors that are necessary for opsonophagocytosis of GBS. Additional studies are required to demonstrate efficacy, safety, and optimal dosage before immunoglobulin therapy can be recommended confidently.

The efficacy of granulocyte transfusions in reducing mortality from severe neonatal sepsis has been reported by several researchers.[88,109,277] Christensen and associates[109] conducted a randomized, prospective, controlled trial of granulocyte transfusions in 16 septic neonates with depleted bone marrow reserves. None of seven infants receiving the transfusions died, whereas only one of nine neonates survived among those not receiving granulocyte transfusions. Cairo and coworkers[88] evaluated the early administration of granulocyte transfusions to neonates with clinical sepsis. Of 23 infants in their study, only 3 had depleted neutrophil storage pools. These researchers also found that the survival rate was improved in neonates with sepsis who received these transfusions as compared with control neonates. Only infants with granulocyte-depleted storage pools are likely to benefit from receiving transfusions. Neither clinical severity nor the degree of neutropenia predicted neutrophil storage pool depletion in septic infants; bone marrow aspiration is required for determining the status of the granulocyte storage pool.[34]

Although these studies are encouraging, they involve a small number of patients, and larger, carefully designed studies are needed. Currently, this strategy is used sporadically in a few

nurseries around the world. The granulocytes normally are obtained from healthy adult volunteers by leukapheresis and then irradiated for prevention of graft-versus-host disease. Approximately 0.5 to 1×10^9 granulocytes per kilogram of recipient body weight are transfused in 20 to 30 minutes.[510] These transfusions usually are tolerated well by neonates, but potential risks include blood group sensitization, graft-versus-host disease, transmission of cytomegalovirus and hepatitis viruses, and volume overload from hydroxyethyl starch.

A more practical and safer approach to reverse sepsis-associated neonatal neutropenia and potentially improve survival is the use of recombinant human granulocyte-macrophage colony-stimulating factor (rhGM-CSF). Several small studies have documented that subcutaneous administration of rhGM-CSF (5 to 10 μg/kg/day for 5 to 7 consecutive days) to septic infants with neutropenia significantly increases the number of various phagocytic cells and decreases mortality rates.[59,77,266,471] Large trials are needed to confirm these benefits before administration of hematopoietic factors can be recommended routinely.

Other nonconventional therapeutic approaches that are being evaluated include extracorporeal membrane oxygenation of neonates with early-onset GBS disease,[227] administration of colony-stimulating factors to neutropenic infants,[87] and immunomodulating strategies (e.g., anticytokine agents, steroids, pentoxifylline, nitric oxide inhibitors).[419] The precise role, if any, of these approaches for the management of newborns with systemic infection needs to be demonstrated in rigorous, carefully designed, double-blind clinical studies.

PREVENTION

Chemoprophylaxis

Studies on the prevention of neonatal infection have focused on those caused by GBS because of its greater prevalence and immunogenicity in comparison to other common neonatal pathogens. Methods proposed for the prevention of neonatal group B streptococcal disease are aimed either at decreasing the likelihood of exposure of the infant to GBS by the use of antibiotic chemoprophylaxis or at decreasing the susceptibility of the exposed infant through improved host defenses by passive or active immunoprophylaxis.

The efficacy of antepartum, intrapartum, or postpartum administration of ampicillin or penicillin has been evaluated in numerous studies. The results have established that selective intrapartum chemoprophylaxis can prevent colonization and disease caused by GBS in the first days of life and prevent postpartum maternal infection with this organism.

Many investigators have attempted to eradicate group B streptococcal colonization from pregnant women during the last trimester. In a prospective, randomized study of women known to be colonized with GBS, Hall and associates[207] demonstrated that treatment with ampicillin (500 mg four times daily for 1 week) briefly reduced maternal colonization, but no difference was found in maternal or infant colonization at the time of delivery. Reinfection from the untreated sexual partner or re-emergence of GBS from an undetectably low population of organisms remaining after antibiotic treatment probably explains failure of antepartum chemoprophylaxis. Gardner and associates[171] treated colonized women in the last trimester of pregnancy and their husbands simultaneously with oral penicillin for 12 to 14 days. Before therapy, 63 percent of husbands also were colonized with GBS in the genital tract, with concordance of isolated serotypes in 88 percent of colonized couples. Such treatment was found to have no effect on the colonization rate of the maternal genital tract at delivery. Other studies also demonstrated that antibiotic therapy had little effect on carriage of GBS.

In contrast, Merenstein and associates[324] demonstrated that treatment of colonized pregnant women with 500 mg of penicillin four times daily at 38 weeks' gestation until delivery resulted in a significant reduction in maternal and infant colonization. Such an approach may eliminate colonization in infants delivered after 38 weeks' gestation; however, because 30 percent of infants with early-onset disease are preterm, the timing of such treatment is inappropriate. The bulk of evidence indicates that antepartum oral antibiotic prophylaxis generally is unacceptable for prevention of early-onset GBS disease.

Parenteral administration of antibiotics during labor has been examined in an attempt to overcome the potential shortcomings of antibiotic administration during pregnancy. In the first published study of intrapartum therapy, 34 women with group B streptococcal genital colonization early in the third trimester were treated at term with intravenously administered ampicillin (500 mg every 6 hours until delivery) at hospital admission.[529] This approach uniformly interrupted vertical transmission of the organism to the infants of treated mothers, which would be expected in approximately 50 percent of infants born to genitally colonized women. Easmon and colleagues[142] conducted a prospective, controlled trial of 87 colonized parturient patients based on vaginal and anorectal cultures obtained at 36 weeks' gestation. Intrapartum prophylaxis with benzylpenicillin during labor significantly reduced the rate of transmission of GBS from mothers to their babies from 45 percent (untreated controls) to 3 percent ($p < .001$). Allerdice and associates[9] prospectively identified 57 women with prenatal group B streptococcal colonization and treated them intrapartum with ampicillin. Seven percent of infants born to treated women were found to have group B streptococcal colonization, and none had invasive disease; 46 percent of infants born to untreated women were colonized, and 7 percent had invasive disease.

Intrapartum chemoprophylaxis given to women with proven GBS reliably prevents colonization of the newborn in the postpartum period. Universal prophylaxis of all pregnant women with group B streptococcal colonization, however, would result in a large number of pregnant women being treated unnecessarily and clearly is unacceptable. Realizing this limitation, researchers started to investigate the feasibility of providing selective, rather than universal, intrapartum chemoprophylaxis.

Boyer and Gotoff[73] were the first to document the efficacy of maternal chemoprophylaxis in high-risk parturients for the prevention of neonatal sepsis. Infants born to women with prenatal cultures positive for GBS and gestation of less than 37 weeks, rupture of amniotic membranes more than 12 hours before delivery, or both, were studied. Eighty women were randomized to receive ampicillin (2 g intravenously and then 1 g every 4 hours until delivery) or no therapy. Infants whose mothers had received ampicillin also were given ampicillin (50 mg/kg body weight every 12 hours intramuscularly for 4 days). Only 1 (2%) of 43 infants born to treated mothers was colonized as compared with 13 (35%) of 37 born to untreated mothers. Boyer and associates,[70,73] in a randomized controlled trial of selective intrapartum chemoprophylaxis using the same selection criteria, demonstrated the efficacy of this approach for prevention of neonatal sepsis and postpartum maternal febrile morbidity.[71] In none of the 85 infants born to mothers in the treatment group versus 5 (6%) of 79 infants born to mothers in the untreated control group did group B streptococcal bacteremia develop ($p = .024$). Additionally, in none of the parturient women did an intrapartum temperature higher than 37.5°C develop in the ampicillin-treated group as compared with four in the control group ($p < .01$). The investigators estimated that their approach had the potential to eliminate more than 50 percent of cases of early-onset group B

streptococcal disease and 75 percent of associated deaths in the United States.

A prospective epidemiologic study of early-onset GBS disease for a 9-year period has provided additional data regarding risk factors for the development of early-onset disease.[70] In this study the relative risk of early-onset disease developing was 7.3 for infants whose birth weight was 2500 g or less (versus those weighing more than 2500 g), 7.2 for infants delivered more than 18 hours after rupture of membranes, and 4.0 for those born to women with intrapartum fever. Overall, 74 percent of the 61 infants had one of these perinatal risk factors at the time that the pregnant women were admitted to the hospital in labor. In Finland, Tupperainen and coworkers,[494] using a rapid latex agglutination test, selected patients solely on the basis of healthy intrapartum colonization. Early-onset group B streptococcal infection developed in 7 (12%) of 58 babies born to mothers who did not receive penicillin, whereas infection developed in only one (3%) of 36 infants whose mothers received penicillin. That infant had intrauterine pneumonia thought to be caused probably by GBS.

Morales and associates[333] selected patients in labor who had positive results on serial coagglutination tests performed on vaginal secretions obtained prenatally. Patients were stratified according to whether their test results were positive after 5 hours' preincubation, which indicated heavy colonization, or after 20 hours' preincubation, which indicated light colonization. None of the infants born to treated, highly colonized mothers was colonized at birth versus 35 percent of the control babies ($p < .001$). In no infant in either group did invasive group B streptococcal disease develop. None of the infants born to treated, heavily colonized mothers was colonized at birth or had early-onset disease; in contrast, 80 percent of the control babies whose mothers were untreated was colonized ($p < .001$), and early-onset group B strepococcal disease developed in three of these control infants ($p = .08$). In another study by the same group,[332] only preterm patients who had premature rupture of membranes were studied, again with use of the results of a rapid coagglutination test on vaginal secretions obtained at the time of hospital admission. Treatment of 36 women with ampicillin resulted in no cases of chorioamnionitis or neonatal sepsis, whereas chorioamnionitis developed in 23 percent of untreated mothers and early-onset group B streptococcal sepsis developed in 27 percent of the babies.

Together, these studies establish the efficacy of selective chemoprophylaxis to prevent early-onset group B streptococcal disease in neonates and postpartum infections in their mothers. Despite endorsement of this approach by the American Academy of Pediatrics and the American College of Obstetricians and Gynecologists, many physicians involved in the management of mothers and their infants are not aware of or do not follow widely published guidelines to prevent acquisition of group B streptococcal disease.[240]

Chemoprophylaxis also has been targeted to neonates at birth. The observation in 1978 that early-onset group B streptococcal disease did not develop in infants born at Mount Sinai Hospital in New York who received intramuscular penicillin at birth for prevention of gonococcal ophthalmia prompted two prospective, randomized studies for evaluation of this regimen. Siegel and colleagues[458,459] studied preterm and term infants and demonstrated the efficacy of a single dose of penicillin administered at birth. The population in this study was characterized by group B streptococcal infection, mostly in term infants who acquired infection at the time of delivery, as evidenced by a delayed onset of symptoms. Because blood for culture was not obtained before penicillin was administered, whether some infections were suppressed inadvertently was unknown. The other investigation of chemoprophylaxis at birth was reported by Pyati and associates.[389] They studied only infants weighing 2000 g or less at birth and found no beneficial effect of penicillin administered at birth. These infants were infected in utero, as determined by the presence of positive blood cultures at the time of delivery in 21 of 24 infants with group B streptococcal disease. Infection had been established before the administration of penicillin at delivery. The population in each of these studies had unique characteristics, and, therefore, the results may not be broadly applicable to all nurseries.

Intrapartum ampicillin prophylaxis coupled with a dose of penicillin to the neonate immediately after birth has been used for years at Parkland Memorial Hospital in Dallas, Texas.[500] This policy has resulted in an approximately 75 percent reduction of group B streptococcal infection rates in neonates (see Table 78–4) without altering the incidence of infections caused by other pathogens. A substantial decline in neonatal early-onset group B streptococcal sepsis related to routine intrapartum prophylaxis also has been documented by the CDC and other U.S. institutions.[44,298] In our Dallas, Texas, series, the incidence of late-onset group B streptococcal sepsis also has declined.

Three special circumstances merit chemoprophylaxis. The first is an asymptomatic twin of an infant with group B streptococcal disease. This twin has an approximately 25-fold increased risk for the development of invasive group B streptococcal disease.[143] Cultures of blood and CSF should be obtained from the twin, and close observation in the hospital with or without treatment is indicated until cultures have been sterile for 72 hours. The second situation is chemoprophylaxis for a pregnant woman who previously has delivered an infant with invasive group B streptococcal disease. Starting at the end of the second trimester, rectal and vaginal cultures are recommended on three occasions at regular intervals for isolation of GBS. If cultures are negative and delivery occurs at term in the absence of maternal risk factors for neonatal infection, prophylaxis can be withheld. If one or more cultures are positive or delivery occurs before 37 weeks' gestation, intrapartum ampicillin should be administered intravenously. The condition of the newborn infant should be assessed; clinical findings and maternal obstetric factors may warrant further laboratory evaluation and antimicrobial therapy. Initiation of chemoprophylaxis in all women colonized with GBS who have two or more risk factors for invasive infection of the neonate seems prudent.[24,179,390]

Several studies[158,286,323,486,493] have indicated that routine administration of prophylactic ampicillin during labor to women with risk factors for GBS-associated infections has facilitated the proliferation of resistant gram-negative bacteria in the vaginal and rectal maternal mucosa and resulted in an increased incidence of neonatal sepsis caused by these organisms, particularly by *E. coli*. Based on these findings, switching to exclusive use of intrapartum penicillin for prevention of early group B streptococcal sepsis in neonates seems reasonable.

Prevention of early sepsis caused by gram-negative bacilli is important in areas of the world where these organisms are prevalent. Unfortunately, no evidence-based medical guidelines on the epidemiology, risk factors, and prophylactic approaches exist for making a clear recommendation. Empiric administration of aminoglycosides during labor frequently is used in Latin American countries, but no published reports have shown its usefulness. We demonstrated that a single, 1-g parenteral injection of ceftriaxone given to high-risk Panamanian pregnant women (i.e., gestation of less than 37 weeks, prolonged rupture of membranes of more than 12 hours, or both) during labor is associated with decreased bacterial colonization and early-onset infection caused by gram-negative enteric bacilli and possibly by GBS.[417] Although this prophylactic strategy seems to be safe and attractive, analysis of cost-effectiveness and careful evaluation for the potential emergence of ceftriaxone-resistant organisms must be done before recommending it for routine use in selected mothers.

Immunoprophylaxis

Effective immunoprophylaxis would be preferable to chemoprophylaxis because of the limitations of antibiotic prophylaxis and because an immunologic approach is more likely to prevent early- and late-onset group B streptococcal disease in neonates and postpartum febrile morbidity in the pregnant woman. The underlying principle is that IgG antibody directed against the type-specific polysaccharide antigen critical to protection against invasive group B streptococcal disease would be provided by passive or active immunization. GBS type-specific polysaccharide vaccines have been developed and found to be associated with low rates of side effects.[28] However, the immune response was unsatisfactory in as many as 40 percent of nonimmune pregnant women who received type III polysaccharide vaccine. These nonresponders did not develop specific antibody, even after repeated vaccine challenge.[28] Response to vaccine possibly is determined genetically, and some of the women in whom antibodies do not respond to vaginal colonization by GBS may be the same women in whom the vaccine will fail. Pregnant women with the highest risk may not benefit from vaccination. Studies of conjugated GBS vaccines using capsular polysaccharide are in progress. Candidate vaccines are immunogenic in adult women, but immune responses are poor even after protein conjugation. Thus, these vaccines are going to be limited for administration to pregnant women. Their use in pregnancy, however, is associated with many medicolegal difficulties.[25,31,181,368]

Maternal immunizations with type III GBS conjugate vaccines have demonstrated 77 percent placental transport of antibodies and persistence of titers in infants up to the age of 2 months. Infant sera uniformly promoted opsonization of type III GBS strains and killing by neutrophils in vitro, thus suggesting that this approach might be an option for the prevention of early- and late-onset disease.[25,31] A recent analysis published by Sinha and coworkers[464] showed that maternal immunization strategies are superior to all other prophylactic strategies, with prevention of 61 to 67 percent of early-onset and 70 to 72 percent of late onset neonatal disease versus a global 55 percent rate of prevention with the current practice of culture-based chemoprophylaxis. Maternal immunization, however, does not prevent disease in neonates who are born before 32 weeks' gestation because not enough antibody would pass transplacentally.

Prevention of late-onset infection, as opposed to early-onset disease, in neonates by the administration of intravenous immunoglobulin to preterm babies has undergone intense clinical scrutiny.[29,30,153,515,517] Premature babies, particularly those born before 32 weeks' gestation, are relatively hypogammaglobulinemic at birth and become more so during the first several weeks of life. Because these same infants are at high risk for the development of infection beyond the first week of life (late onset), intravenous immunoglobulin infusion may provide opsonizing antibody to prevent late-onset infections. Three of the five early clinical trials that examined the efficacy of intravenous immunoglobulin in preventing infection in neonates demonstrated significant favorable responses.[106,112,206,472] Problems with these studies included small sample size, definition of infection, and lack of a blind design.

A well-controlled multicenter study demonstrated the efficacy of intravenous immunoglobulin infusion in reducing late-onset infection rates.[30] Study infants received intravenous immunoglobulin (500 mg/kg) or placebo at 3 to 7 days of age, 1 week later, and every 2 weeks for a total of five infusions or until hospital discharge. Infusions were tolerated well. Although no significant differences in mortality rates or reduction of infections in infants weighing more than 1500 g occurred, the incidence of bacterial infections was reduced significantly in infants weighing less than 1500 g at birth, and the duration of hospitalization was significantly shorter in recipients of intravenous immunoglobulin. Most infections were bacterial in origin, and approximately 70 percent of them were caused by gram-positive organisms, primarily staphylococci. In contrast to the beneficial effect found in this study, two larger, multicenter, well-designed trials showed no significant differences in the rate of nosocomial infection or in the mortality rate between control and treated groups.[153,517] At this time, intravenous immunoglobulin cannot be recommended as routine prophylaxis for low-birth-weight infants. Investigations now are directed at evaluating the usefulness of pathogen-specific hyperimmunoglobulin for the prevention and treatment of sepsis caused by the most common etiologic agents.[515]

PURULENT MENINGITIS

As many as a fourth of neonates with bacterial sepsis have a simultaneous meningeal infection. The incidence of neonatal meningitis varies greatly among institutions in North America. Rates are approximately 0.2 to 0.4 case per 1000 live births but may be as high as 1 case per 1000 live births in some nurseries. In general, group B beta-hemolytic streptococci and *E. coli* strains account for two thirds of all cases of neonatal meningitis in North America. Gram-negative enteric bacilli predominate in many developing areas of the world (Table 78–6).

Information about the bacteria isolated from the CSF cultures of 257 neonates with meningitis treated at Children's Medical Center or Parkland Memorial Hospital in Dallas, Texas, from 1969 to 1989 is presented in Table 78–6. One hundred thirty-six (53%) of these 257 infants had disease caused by GBS. An additional 49 infants (19%) had meningitis caused by *E. coli* strains. *L. monocytogenes* added an additional 7 percent of cases. These three agents accounted for 79 percent of cases seen during the 20-year period. *E. coli* and *Klebsiella-Enterobacter* strains accounted for 77 percent of the gram-negative organisms causing meningitis. For an etiologic comparison, the distribution of meningeal pathogens in a developing setting[329] also is displayed in the same table.

PATHOLOGY

The pathologic findings are similar, regardless of the bacterial cause. Studies of the fulminant, early-onset form of group B streptococcal disease have shown primarily bronchopneumonia with or without hyaline membranes and usually no histologic

TABLE 78–6 Etiologic Agents of Neonatal Meningitis in Nurseries from a Developed and Developing Country

Organism	Isolation Rate in Dallas (1969-1989) *N* = 257	Isolation Rate in Panama (1975-1992) *N* = 105
Gram-negative bacteria	88 (35%)	68 (64%)
Escherichia coli	19%	16%
Klebsiella species	8%	25%
Other gram-negative rods	4%	16%
Pseudomonas aeruginosa	2%	5%
Haemophilus influenzae	1%	1%
Neisseria meningitidis	1%	1%
Gram-positive bacteria	169 (65%)	37 (36%)
Group B *Streptococcus*	53%	5%
Coagulase-negative *Staphylococcus*	<1%	18%
Staphylococcus aureus	2%	10%
Listeria monocytogenes	7%	1%
Enterococci	2%	1%
Streptococcus pneumoniae	1%	<1%

evidence of meningeal involvement. The most consistent finding at necropsy of meningitis cases is a purulent exudate of the meninges and ependymal surfaces of the ventricles.[52] The inflammatory response of neonates is similar to that observed in adults with meningitis, with the exception that babies have a scarcity of plasma cells and lymphocytes during the subacute stage of meningeal reactions. Some patients also have perivascular inflammation. Hydrocephalus and a noninfectious encephalopathy can be demonstrated in approximately 50 percent of infants dying of meningitis.

Subdural effusions occur rarely in neonates. In contrast, effusions are observed commonly (i.e., by computed tomography or magnetic resonance imaging) in infants with meningitis who are aged 3 to 12 months. Various degrees of phlebitis and arteritis of intracranial vessels can be found in all infants. Thrombophlebitis with occlusion of veins may occur in the subependymal zone. K1 antigen has been demonstrated in the brain tissue of infants succumbing to *E. coli* K1 infection.[430] High concentrations of interleukin-1β have been detected in the brain and meningeal tissue of infants succumbing to meningitis.[34]

CLINICAL MANIFESTATIONS

The early signs and symptoms of neonatal meningitis frequently are indistinguishable from those of septicemia and other disorders occurring in the neonatal period. The most frequent signs are temperature instability, respiratory distress, irritability, lethargy, and poor feeding or vomiting. GBS occasionally has been reported to be manifested as hydrocephalus without other signs of infection. Signs suggestive of meningeal involvement, such as a stiff neck, bulging fontanelle, convulsions, and opisthotonos, are the exception in neonates with meningitis. The frequency of these findings as culled from the literature is 17 percent for a bulging fontanelle, 33 percent for opisthotonos, 23 percent for a stiff neck, and 12 percent for convulsions.[52,315,363] The sensitivity of these findings in distinguishing infection of the pia-arachnoid is poor. All newborns being evaluated for sepsis should undergo examination of CSF, especially if antimicrobial therapy is to be instituted.

DIAGNOSIS

Interpretation of CSF values in newborn infants may be difficult. During the first several days of life, the mean WBC count is 15 ± 30 cells/mm^3 (95% confidence limit, 12 to 18 cells/mm^3) in the CSF of healthy or high-risk uninfected babies.[362,431] Approximately 60 percent of these cells are polymorphonuclear leukocytes. During the first week of life, the cell count slowly diminishes in term infants and increases in premature infants. Cell counts in the range of 0 to 10 cells/mm^3 (median, 4 cells/mm^3) are observed in infants at approximately 2 to 4 weeks of age. The WBC count is uncertain when bleeding occurs after the lumbar puncture has been performed. The fixed relationship between the number of WBCs in CSF and peripheral blood has been disputed. A repeat lumbar puncture may need to be performed in 12 to 24 hours to resolve the ambiguity of the traumatic lumbar puncture.

The mean cerebrospinal protein concentration in the first month of life is 64 ± 24 mg/dL, although individual values can be as great as 170 mg/dL, especially in low-birth-weight, premature infants. The ratio of cerebrospinal glucose to blood glucose is 60 to 70 percent and can be greater than 100 percent in term and preterm infants.[431] The upper limits of normal for cellular and chemistry values are higher in uninfected VLBW infants.[67]

These data indicate that CSF values must be interpreted in relation to these normal findings if a diagnosis of neonatal meningitis is to be made early. Comparison of the results of initial CSF evaluations obtained from newborns with proven bacterial meningitis with those from normal or high-risk infants revealed considerable overlap in the findings.[431] For example, approximately 30 percent of infants with group B streptococcal meningitis had normal spinal fluid leukocyte counts (<32 cells/mm^3), whereas only 4 percent of neonates with meningitis caused by gram-negative organisms had normal counts.[432] The ratio of CSF to blood glucose was normal in 45 percent and 15 percent of patients with streptococcal and coliform meningitis, respectively. However, when the total CSF evaluation (including Gram-stained smears) was considered, less than 1 percent of babies with bacteriologically proven meningitis had totally normal CSF on initial lumbar tap. The likelihood of suppurative meningitis is diminished greatly but not impossible if evaluation of CSF discloses no abnormalities. In some patients in whom the diagnosis is obscured, a repeat CSF examination performed 4 to 6 hours after the initial tap (regardless of whether therapy has been instituted in the interim) may help establish the diagnosis. In premature infants with meningitis caused by *S. epidermidis*, the CSF analysis may be only mildly abnormal despite compatible clinical findings.[204]

Careful examination of the stained smears of CSF from every infant with suspected meningitis is important. Grossly clear fluid may contain few WBCs and many bacteria. The stained smears from approximately 20 percent of neonates with proven meningitis are interpreted as showing no bacteria. Because of the low concentrations of organisms, most Gram-stained smears of CSF from infants with *L. monocytogenes* meningitis do not reveal bacteria. The CSF findings in an infant with a brain abscess may include a pleocytosis of up to a few hundred cells, a predominance of mononuclear cells, and an elevated protein concentration. Bacteria may not be seen on a Gram-stained smear of CSF if meningitis is not present. Ventriculitis is diagnosed on the basis of an elevated WBC count (>100 cells/mm^3), identification of an organism by culture, detection of antigen on a Gram-stained smear, increased intraventricular pressure, and dilated ventricles. A cranial computed tomogram with contrast material may show enhancement of the lining tissue of the ventricles.

Several techniques for establishing rapid diagnosis of bacterial meningitis have been described. The first, counterimmunoelectrophoresis, is used to detect bacterial capsular antigens in CSF and other body fluids. Depending on the source of antisera used in this method, meningitis can be diagnosed with counterimmunoelectrophoresis in almost all patients with *H. influenzae* type b and meningococcal groups B and C meningitis if spinal fluid, serum, and urine are tested.[154] Approximately 70 percent of infants with *E. coli* K1 meningitis have detectable K1 antigen in CSF, serum, or both.[301] Group B_{III} streptococcal antigen has been detected in the CSF, serum, and urine of approximately 90 percent of infected infants.[32] Quantitation of antigen is a helpful means of establishing the prognosis of infants with *E. coli* K1 and type III group B streptococcal meningitis.[32,313]

Latex particle agglutination and staphylococcal coagglutination tests have been developed for detection of bacterial antigens in body fluid. The latex particle agglutination method has been found useful in detecting antigen in the CSF of older infants and children with meningitis caused by *H. influenzae* type b; *N. meningitidis* groups A, B, and C; and *S. pneumoniae*.[350,519] This method is more sensitive than is counterimmunoelectrophoresis for measuring the capsular antigen of *H. influenzae*.[508] The staphylococcal coagglutination test has not been used extensively in pediatric patients, but the polyribose phosphate antigen of *H. influenzae* has been detected in body fluids by this method.[481] Both methods can be used for the detection of group B streptococcal infection.

The *Limulus* lysate test detects the presence of endotoxin, a soluble lipopolysaccharide constituent of the cell wall of gram-negative bacteria.[285] Endotoxin can be measured in the CSF of patients with meningitis caused by coliform bacteria, *H. influen-*

zae, and *N. meningitidis*.[304] Endotoxin has been detected in initial CSF specimens obtained from infants with meningitis caused by *E. coli* and other coliforms. Both counterimmunoelectrophoresis and the *Limulus* lysate technique require approximately 1 hour to run and can be performed in most hospital laboratories. If both methods are used, approximately 80 percent of neonates with coliform meningitis can be identified as having disease within an hour of the initial lumbar tap. However, these results are no better than those obtained from a carefully prepared and examined stained smear of CSF, in which bacteria can be identified in approximately 80 percent of patients with documented bacterial meningitis.[431]

TREATMENT

Selection of appropriate antibiotic therapy for meningitis is based in part on achievable CSF concentrations of these drugs in relation to the susceptibility of the pathogens causing disease. The highest concentrations of penicillin or ampicillin in CSF are at least 10 to 100 times greater than the susceptibilities (i.e., MICs) of GBS and *L. monocytogenes.* As a result, sufficient activity remains in CSF for at least 40 to 60 percent of the dosing interval, and most infants with meningitis caused by these two organisms respond promptly to ampicillin or penicillin therapy. CSF cultures usually are sterile within 24 to 36 hours of initiation of therapy. In contrast, concentrations of aminoglycosides (i.e., kanamycin, gentamicin, tobramycin, and amikacin) in CSF usually are equal to or several times greater than the MIC values for coliform organisms and *P. aeruginosa.* Because killing of bacteria by aminoglycosides is concentration-dependent and requires CSF drug concentrations at least fourfold to eightfold the minimal bactericidal concentration, cultures of CSF from infants with meningitis caused by these organisms often remain positive for 2 or 3 days or longer. Documenting bacteriologic cure in patients with meningitis is important because outcome is correlated with the time necessary to eradicate the bacterial pathogen.

Ampicillin and gentamicin or cefotaxime are recommended for initial empiric treatment of neonatal meningitis (for dosages, see Table 78–1). All infants should undergo repeat CSF examination and culture 24 to 36 hours after initiation of therapy. If organisms are observed on methylene blue– or Gram-stained smears of fluid, modification of the therapeutic regimen should be considered. For many years physicians have attempted to increase antibiotic concentrations in CSF by instilling drugs directly into the lumbar intrathecal space. The first Neonatal Meningitis Cooperative Study evaluated 117 prospectively enrolled, randomly treated infants to determine the role of intrathecal gentamicin therapy in the management of neonatal meningitis caused by coliform bacilli.[309] No statistically significant differences were seen in mortality rates, long-term morbidity, or days that CSF cultures remained positive in infants who received lumbar intrathecal gentamicin plus systemic therapy and those who were treated with systemic drugs only.

Data from the Neonatal Meningitis Cooperative Study and from adult neurosurgical patients with meningitis demonstrated that lumbar CSF concentrations of aminoglycoside drugs administered locally usually exceed the MIC values for coliform organisms by 10- to 50-fold.[309,393] However, lumbar instillation does not result consistently in diffusion of these drugs to the level of the cisterna or into ventricular fluid. In contrast, instillation of aminoglycosides into unobstructed ventricles results in rapid and uniform distribution of drug throughout the CSF space.

Data obtained from the second Neonatal Meningitis Cooperative Study demonstrated ventricular fluid gentamicin concentrations were 10 to 130 μg/mL at 1 to 6 hours after administration of a 2.5-mg intraventricular dose and were 1 to 24 μg/mL at 16 to 24 hours later.[308,310] Concentrations in lumbar CSF at comparable intervals after intraventricular administration were 8 to 85 μg/mL and 1.8 to 4.2 μg/mL, respectively. However, results of the intraventricular regimen were inferior to those obtained with systemic therapy alone. The mortality rate was significantly higher in infants who had meningitis and ventriculitis and received intraventricular gentamicin and systemic antibiotics (43%) than in those who were given systemic therapy only (12.5%). The duration of positive CSF cultures was 1 day shorter in those receiving intraventricular therapy. With the use of serial CSF samples from patients enrolled in this study, we demonstrated some years later that intraventricular gentamicin therapy, which resulted in higher gentamicin CSF concentrations, was associated with higher ventricular CSF endotoxin and interleukin-1β concentrations and greater CNS inflammation (i.e., higher CSF leukocyte count, higher protein concentration, and lower glucose concentration), most likely as a result of lysis of organisms.[339] This finding possibly could explain the poor outcomes in these patients versus infants who received parenteral antibiotic therapy only.[339]

On the basis of the findings from the second Neonatal Meningitis Cooperative Study, intraventricular therapy cannot be recommended for the routine management of neonatal meningitis caused by gram-negative enteric bacilli. A controlled study[316] found that ampicillin and moxalactam therapy (a broad-spectrum cephalosporin no longer available) for neonatal coliform meningitis was as effective as is a conventional regimen of ampicillin and amikacin. In that study, moxalactam achieved greater CSF and ventricular fluid concentrations, and bactericidal titers were considerably greater than those achieved with conventional therapy, but this increase did not translate into more rapid sterilization of the CSF, lower case-fatality rates, or improved neurologic outcome in survivors. Although no large controlled trials have evaluated the use of cefotaxime, clinical experience indicates that it can be used safely and effectively for the treatment of gram-negative meningitis in neonates. CSF examination and culture should be repeated 48 to 72 hours after initiation of therapy. If the results still are positive, computed tomography should be performed to rule out the possibility of subdural empyema, brain abscess, or ventriculitis. In all cases caused by *Citrobacter diversus*, cranial computed tomograms should be obtained early because of the frequent association with brain abscess. The duration of antibiotic therapy should be extended, depending on clinical evolution and resolution of the lesion based on repeated tomography. A neurosurgeon should be consulted early for needle aspiration or excision of the abscess. Aminoglycosides probably should not be used because of decreased activity in abscess cavities that have low pH and anaerobic conditions.

Third-generation cephalosporins possess attractive features, including lower MICs for gram-negative enteric bacilli than with aminoglycosides, good penetration into CSF in the presence of inflamed meninges, and a wide therapeutic index, for the treatment of bacterial meningitis in newborn infants. These agents are active against most streptococci but inactive against *L. monocytogenes* and enterococci. Only ceftazidime provides adequate activity against *P. aeruginosa*. Among these agents, cefotaxime is preferred for the treatment of neonatal meningitis because of extensive experience with this drug in the neonatal period and because it does not alter the bowel flora substantially. Cefotaxime can be used singly or in combination with an aminoglycoside. Ceftazidime has been shown to be effective in treating patients with *P. aeruginosa* meningitis. Because of high biliary excretion and marked alteration of the normal intestinal flora and because of potential concern for displacement of bilirubin, we do not recommend routine use of ceftriaxone during the neonatal period. A prospective study of neonatal bacterial meningitis conducted in England indicated that treatment with third-generation cephalosporins has been associated with a decrease in mortality rates from disease seen during the past decade, especially that related to gram-negative bacillary infection.[219]

For premature infants hospitalized in the nursery for prolonged periods, staphylococci, enterococci, and gentamicin-resistant gram-negative organisms are potential pathogens; an alternative antimicrobial regimen should be considered for initial empiric treatment. A combination of nafcillin or oxacillin and amikacin or ceftazidime or cefotaxime could be used as initial empiric therapy. When MRSA or *S. epidermidis* is a potential cause of infection, vancomycin and amikacin or cefotaxime can be used initially. Imipenem, meropenem, cefepime, and ciprofloxacin have been used to treat neonatal meningitis caused by multiresistant gram-negative bacteria. Greater experience is required before they can be recommended for routine use. For neonatal meningitis caused by *Flavobacterium meningosepticum* isolates, combined use of parenteral vancomycin and rifampin has been suggested as the best therapeutic option.[137]

After the pathogen has been identified and susceptibility studies are available, the single drug or combination of drugs that is most effective should be used. In general, penicillin G or ampicillin is preferred for group B streptococcal meningitis, ampicillin for *L. monocytogenes* and enterococci, ampicillin plus an aminoglycoside or cefotaxime for coliforms, and ceftazidime or ticarcillin and an aminoglycoside for *Pseudomonas* infections. The duration of systemic therapy in neonatal meningitis depends on the causative agent and the time necessary to sterilize CSF cultures. As a rule, therapy is given for approximately 2 weeks after bacteriologic cure has been achieved. For meningitis caused by GBS or *Listeria*, approximately 2 weeks of therapy usually is satisfactory. Because delayed sterilization is a common occurrence in infants with gram-negative enteric disease, systemic therapy is given for a minimum of 3 weeks and in some babies for many additional weeks. Final judgment about when to discontinue therapy must be based on the clinical course of the illness and on CSF findings at the time that this decision is to be made.

Despite the beneficial effects of dexamethasone achieved in several prospective, randomized, double-blind studies for the treatment of infants and children with bacterial meningitis,[415] especially that caused by *H. influenzae*,[434] no such data are available for its use in newborns; the use of steroids in neonatal meningitis cannot be recommended. A prospective trial performed in Jordan found lack of effectiveness of dexamethasone for neonatal meningitis in terms of mortality and sequelae rates when compared with the nonsteroid group.[124] Because this study was small (not sufficient statistical power), not blinded, and without a placebo group, additional studies are required to determine whether dexamethasone has a beneficial effect in neonates with meningitis.

PROGNOSIS

Acute complications of bacterial meningitis include communicating and noncommunicating hydrocephalus, subdural effusions (approximately 1% of patients), deafness, and blindness. Ventriculitis occurs in approximately 70 percent of neonates with coliform meningitis and usually is detected at the time of initial diagnosis. Brain abscess is an infrequent complication, except in infants with *C. diversus* meningitis, in whom abscess develops approximately 70 percent of the time.[194,309,310,316] A 30 to 50 percent incidence in neurologic sequelae has been reported in surviving infants.[202] Although gross retardation and neurologic deficits may be obvious in some infants at discharge, most babies appear "well" at this time. Only after prolonged and careful follow-up do the perceptual difficulties, behavioral problems, and other subtle neurologic signs become apparent. Within 4 to 6 weeks of recovering from meningitis, hearing should be evaluated by evoked response audiometry.

Case-fatality rates in neonates with meningitis range from 15 to greater than 30 percent.[131,315,363] A mortality rate of 30 percent was observed in 117 neonates with coliform meningitis enrolled in the first Neonatal Meningitis Cooperative Study.[309] In this project, term infants had a significantly lower rate (18%) than that observed in low-birth-weight (<2500 g) babies (45%) and in infants older than 30 days (48%). Poor outcome after gram-negative enteric meningitis is correlated directly with the presence of ventriculitis, the persistence of positive CSF cultures, the presence and persistence of elevated endotoxin and interleukin-1β concentrations in CSF, a CSF cell count of greater than 10,000/mm^3, and a CSF protein concentration higher than 500 mg/dL.[257] When meningitis is caused by *E. coli* K1, poor outcome is associated with persistence of large quantities of K1 capsular polysaccharide antigen in CSF.[311,313]

Long-term follow-up studies of babies with coliform meningitis enrolled in the Neonatal Meningitis Cooperative Study have revealed that approximately 65 percent of survivors were normal 3 to 7 years after illness. An additional 30 percent were classified as having mild to moderate neurologic sequelae. Many of these latter patients had only slightly abnormal neurologic or psychological evaluations and were considered normal on routine physical examination. About 5 to 10 percent of survivors had severe neurologic or mental impairment requiring custodial care. Approximately 15 to 20 percent of survivors of group B streptococcal meningitis have major sequelae, including spastic quadriplegia, profound mental retardation, hemiparesis, deafness, and blindness.[109] Hydrocephalus develops in 11 percent, and 13 percent have a seizure disorder. The survivors without major sequelae on physical examination, however, appear to function within normal limits and comparable to their siblings.

Edwards and associates[144] reported a 21 percent morbidity rate after group B streptococcal meningitis. Of the survivors, 29 percent had severe neurologic sequelae, 21 percent had minor deficits, and 50 percent were functioning normally. Factors at initial evaluation associated with death or severe disability included coma, decreased perfusion, CSF protein greater than 300 mg/dL, an absolute neutrophil count of less than 1000, and a total leukocyte count of less than 5000/mm^3. A report from Canada noted that duration of seizures longer than 72 hours, presence of coma, use of inotropes, and leukopenia were the most important predictors of an adverse outcome.[265] Our experience with gram-negative enteric bacillary meningitis in 98 identified neonates managed from 1969 through 1989 revealed a case-fatality rate of 17 percent; 61 percent of survivors had long-term sequelae that included seizure disorders, hydrocephalus, physical disability, developmental delay, and hearing loss.[497]

OTITIS MEDIA

Otitis media is diagnosed infrequently in neonates because of the paucity of clinical findings and the difficulty of examining an infant's tympanic membrane. The external canal is narrow and tortuous and often is filled with debris. Because a healthy baby's membrane may appear thickened and dull, mobility of the drum determined by pneumatoscopy should be used as the single most reliable indicator of middle ear abnormalities. The examiner must beware of mistaking movement of the distal interior canal wall for movement of the tympanic membrane. Movement of a normal membrane is seen best in the posterior portion. A reddish or reddish orange color of the tympanic membrane usually indicates infection, provided that the infant is not crying.

The exact incidence of disease is unknown, but a prospective study found that in 34 percent of 70 infants monitored from birth, the first episode of otitis media occurred before they reached 2 months of age.[302] Otitis media develops more commonly in premature than in term infants and occurs almost exclusively in bottle-fed babies. It is a frequent finding in neonates receiving intensive care, especially those with prolonged nasotra-

cheal intubation.[53] The ears of 127 infants with birth weights of less than 2300 g were examined by Warren and Stool[509] three times weekly until discharge from the nursery; in only three patients (2%) did otitis media develop. In contrast, in a study of 125 premature infants in a neonatal intensive care unit, Berman and associates[53] found that 38 infants (30%) had middle ear fluid compatible with the diagnosis of otitis media; this finding was confirmed by tympanocentesis in 13 patients in whom the procedure was performed. Development of otitis media was correlated significantly with nasotracheal intubation for longer than 7 days.

Meconium staining of amniotic fluid and prolonged rupture of membranes are two other risk factors for the subsequent development of middle ear disease.[377] Neonates with cleft palate, Down syndrome, or maxillofacial anomalies are at high risk for the development of chronic middle ear disease. The onset of illness is insidious, and the most common manifestations are rhinorrhea, irritability, and failure to thrive. Fever higher than 38°C is a rare finding. The presence of lethargy, hypotonia, hypothermia, high fever, or jaundice suggests septic complications such as bacteremia or meningitis.

The cause of neonatal otitis media is similar to that observed in older infants and children. *S. pneumoniae*, *H. influenzae*, and *Moraxella catarrhalis* account for more than 50 percent of cases.[62,63,455,487] The important difference from disease in older patients is that 10 to 15 percent of neonates have disease caused by coliforms, GBS, or *S. aureus*. In a review of 137 medical records of infants younger than 2 months with acute otitis media who underwent tympanocentesis in the emergency room of Soroka University Medical Center between January 1995 and May 1999, 20 percent of *S. pneumoniae* isolates were not susceptible to penicillin, thus indicating that antibiotic resistance already may be present at an early age. Pathogens isolated from the middle ear fluid of these infants were *S. pneumoniae* in 46 percent of cases, *H. influenzae* in 34 percent, group A *Streptococcus* in 10 percent, enteric gram-negative bacilli in 7 percent, *M. catarrhalis* in 2 percent, and *Streptococcus faecalis* in 1 percent. Mixed infections were recorded in 20 patients.[495] We occasionally have encountered neonates with otitis media associated with septicemia and pneumonia or meningitis. GBS or coliform organisms were the causative agents in these cases.

The importance of establishing the diagnosis and cause of otitis media in neonates and using appropriate therapy cannot be overemphasized. When otologic examination demonstrates middle ear disease, the infant should be examined carefully for other sites of infection. If none is found, which is the usual case, the infant can be treated on an outpatient basis. Because a small percentage of these cases are caused by coliform bacilli or *S. aureus*, drugs that include those organisms in their spectrum of activity (i.e., second-generation cephalosporin or amoxicillin-clavulanate) are preferable to the aminopenicillins. The patient's condition should be re-evaluated 8 to 72 hours after therapy is initiated to determine whether clinical improvement has occurred and whether the middle ear infection is resolving. In patients demonstrating no improvement, tympanocentesis should be performed for examination of stained smears of middle ear fluid and for culture of the contents. Changes in therapy should be based on the findings of these examinations. If gram-negative organisms or staphylococci are observed, the infant probably is managed best in the hospital by means of parenteral therapy, with administration of an aminoglycoside if coliforms are suspected or an antistaphylococcal penicillin for *S. aureus*. If organisms are not observed in infants with unresolving disease, nafcillin and gentamicin or cefuroxime can be used until the results of culture and susceptibility studies are available. CSF examination should be performed before parenteral therapy is initiated.

All infants with otitis media should be monitored carefully for many months after illness. Misdiagnosis or improper therapy may result in chronic middle ear disease and, occasionally, extension of infection to adjacent structures such as the mastoid or CNS. Infants in whom the first episode of otitis media develops before they reach 2 months of age may require 3 to 4 months to clear the effusions, and recurrent or chronic otitis media is reported to develop in a third of them.[302]

DIARRHEAL DISEASE

Although diarrheal disease during the neonatal period usually is brief and self-limited, it may cause significant morbidity in some infants and represents a potential danger to other infants in the nursery. The advent of modern sterilization practices and increased emphasis on hospital infection control measures has reduced the incidence of nosocomial diarrheal disease significantly.

Numerous factors, including underdevelopment of local and systemic immune responses; lack of fully developed aerobic and anaerobic enteric flora, which protects the gastrointestinal tract of older infants and children; a less effective gastric bactericidal barrier; less intestinal mucus; and less motility, contribute to increased susceptibility to neonatal enteric infections.[357] The infant may have been fed powdered formula that could have been mixed with contaminated water, or a critically ill newborn may have received a broad-spectrum antibiotic in intensive care, in which case highly resistant nosocomial flora pose a special risk.

The importance of breast-feeding in preventing diarrheal disease in infants cannot be overemphasized. The protective effects of breast-feeding have been confirmed in surveys of sporadic gastroenteritis, in community epidemics, and in outbreaks in newborn nurseries. Antibacterial and antiviral factors in breast milk, including lactoferrin, lysozyme, phagocytes, specific secretory immunoglobulins, and lymphocytes sensitive to organisms such as *E. coli*, *Salmonella*, *Shigella*, *Clostridium difficile* toxins A and B, and rotavirus, are well documented.[197,209,357,492] Breast-fed infants are less susceptible to diarrheal diseases than are bottle-fed infants.[205,357]

ETIOLOGY AND PATHOGENESIS

The most common cause of diarrhea in young infants is alteration of diet and feeding practices rather than specific bacterial or viral pathogens. Diarrhea can be a nonspecific symptom of sepsis or urinary tract infection in a newborn infant. Of the infectious causes, rotaviruses are important in infantile diarrheal disease.[326,415] They have been associated with nursery outbreaks of gastroenteritis and necrotizing enterocolitis.[111,410] Studies from France have shown that approximately a third of all neonates shed rotavirus in their stool; of these infants, only 29 percent have associated diarrhea.[101]

Transplacental transfer of rotaviral group-specific or type-specific maternal antibodies to neonates appears to have little effect on the incidence of infection or illness by rotaviruses.[135,136,355] The essential pathogenic feature of rotavirus infection is destruction of the absorptive cells lining the duodenum, jejunum, and possibly the ileum.[325] Lactase exists only in the brush borders of differentiated epithelial cells at these sites and has been suggested to act as a combined receptor and uncoating enzyme for the virus, which may explain why rotavirus infection occurs less commonly in infants of less than 32 weeks' gestation than in more mature infants.[89] In infants between 26 and 34 weeks' gestation, lactase activity is approximately 30 percent of that found in term infants.[280]

Enteropathogenic *E. coli* serotypes once were considered the most common bacterial agents responsible for diarrhea in young infants. Failure to demonstrate on rectal cultures specific sero-

types of *E. coli* designated as enteropathogenic does not rule out coliform disease. Enterotoxigenic strains of *E. coli* possessing nonenteropathogenic serotypes have been identified in nursery outbreaks of diarrheal disease.[75,360] These organisms inhabit the small bowel, where they attach to but do not invade the intestinal mucosa. The enterotoxin produced by these organisms stimulates cyclic adenosine monophosphate, which inhibits sodium and chloride transport across the intestinal wall. As a result, these salts are lost into the lumen of the upper bowel, followed passively by water, which causes a net loss of stools containing high concentrations of electrolytes. *Vibrio cholerae*, some *E. coli* serotypes (almost exclusively nonenteropathogenic strains), *Vibrio parahaemolyticus*, *Aeromonas*, and possibly some *Campylobacter* and *Yersinia* strains are examples of bacteria that cause diarrhea by this mechanism.

The importance of recognizing this form of diarrheal disease was emphasized in a nursery outbreak in the southwestern United States. Severe, watery diarrhea was observed in 59 infants during a 9-month period; in the 7 percent of infants who died, death resulted from altered hepatic function and a hemorrhagic diathesis.[75] An O142/K86/H6 *E. coli* strain was responsible for the outbreak. Because this organism is not a classic enteropathogenic serotype, it was not identified as a pathogen, which thereby delayed establishing the definitive diagnosis and institution of proper infection control techniques. Only by special laboratory techniques was this *E. coli* strain shown to produce labile enterotoxin.

A second mechanism involved in the development of bacterial diarrhea is invasion of the intestinal mucosa. *Shigella* dysentery is the classic example of this disease. Colonic invasion with subsequent destruction of the mucosa causes an outpouring of polymorphonuclear cells and mucus. The resultant diarrhea usually is bloody and contains mucus and pus. *Salmonella* spp. also invade the intestinal mucosa, but destruction is not extensive. The epithelial lining is left intact, and the organisms reach the lamina propria, where an inflammatory response is elicited.[483] *Campylobacter*, *Yersinia*, *Aeromonas* spp., and *C. difficile* also can cause bloody diarrhea.

Although serotyping of *E. coli* to identify the traditional enteropathogenic strains no longer is available routinely, the epidemiologic evidence is strong enough to support a pathogenic role for these strains, even though the mechanism of pathogenicity is unknown. When an index case of diarrhea caused by enteropathogenic *E. coli* or other pathogens is recognized in a nursery, secondary cases are likely to occur. In any nursery infant with diarrhea, a potentially communicable disease should be suspected. For all infants in proximity to the index case, rectal swabs should be tested by culture or by fluorescent antibody technique, which is more sensitive in identifying asymptomatic carriers of enteropathogenic *E. coli.* Ill and healthy colonized infants should be segregated and treated with orally administered neomycin (100 mg/kg/day in three or four divided doses) or with colistin sulfate (15 mg/kg/day in three or four divided doses) for 5 days. Neomycin causes rapid disappearance of the organism and abbreviates the duration diarrhea, but approximately 20 percent of infants revert to an asymptomatic carrier state (J. D. Nelson, personal communication, 1990). Repeated surveillance of infants is necessary until the pathogenic strain has been eliminated from the nursery. A report from Yugoslavia identified a multiresistant strain of enteroaggregative *E. coli* (O4 serogroup) associated with an outbreak of transient, self-limited diarrhea in a neonatal nursery ward.[113]

Campylobacter, a curved, gram-negative bacterium, has been recognized increasingly as a common cause of enteritis. Of the 14 known species, *Campylobacter fetus* and *Campylobacter jejuni* cause human disease most frequently. *C. fetus* causes prenatal and neonatal infections that result in abortion, premature delivery, bacteremia, and meningitis. Infections caused by *C. fetus* appear to be the most common type of *Campylobacter* infection in the first 3 weeks of life and are associated with a high incidence of fetal and neonatal morbidity.[205] In contrast, the most common syndrome produced by a *Campylobacter* species is enteritis caused by *C. jejuni*. Unlike the serious neonatal disease caused by *C. fetus*, infections with *C. jejuni* usually result in mild gastroenteritis, although meningitis occurs rarely. Nursery outbreaks of gastroenteritis caused by *C. jejuni* have been documented definitively.[205]

Although some diarrheal episodes in neonates probably can be caused by *C. difficile*, the diagnostic criteria used in older children and adults are inadequate to establish a definitive diagnosis in this age group. *C. difficile* is a gram-positive anaerobic bacillus that produces an enterotoxin (i.e., toxin A) that causes fluid secretion and a cytotoxin that damages cells (i.e., toxin B).[85,288] *C. difficile* colonic overgrowth and toxin production can result from the selective pressure of antibiotic therapy. A wide variety of antibiotic, antifungal, and antituberculous agents have been associated with *C. difficile* colitis.[205] Although healthy children older than 1 year and healthy adults rarely carry the organisms, as many as two thirds of neonates can be demonstrated to have both *C. difficile* and its cytotoxins in their stool.[205] This high frequency of colonization and the presence of cytotoxin have led to skepticism about the pathogenic potential of this organism in neonates.

CLINICAL MANIFESTATIONS

Although the cause of diarrhea in infants and children may be suspected on clinical grounds, usually this is not possible in newborn infants. As a general rule, diarrhea caused by enteropathogenic strains of *E. coli* is insidious in onset, is associated with 7 to 10 green watery stools daily, and generally is without blood or mucus. These infants do not appear to be acutely ill. Complications rarely occur and primarily are related to dehydration and electrolyte disturbances. *Shigella* infection seldom occurs, usually is episodic in neonates, and generally does not spread within nurseries.[211] Shigellosis in a newborn may occur as a diarrheic or dysenteric syndrome or may be evidenced only by a septic- or toxic-appearing infant. Suppurative complications are rare events, but dehydration and electrolyte disturbances are common and need immediate and constant attention. *C. jejuni* infection typically involves the gastrointestinal tract and produces watery diarrhea or a dysentery-like illness with fever and bloody mucoid stools. Extraintestinal infections related to *C. jejuni*, other than bacteremia, are rare occurrences but include cholecystitis, urinary tract infection, and meningitis.[205] The clinical manifestations in neonates with *C. fetus* infection are similar to those caused by the common neonatal pathogens.[205] *C. jejuni* has been reported to cause bloody diarrhea in otherwise asymptomatic neonates.[84]

A useful procedure for differentiating enteroinvasive from enterotoxigenic diarrhea is examination of fecal material for polymorphonuclear cells. Feces from many patients with dysentery show significant numbers of polymorphonuclear leukocytes, whereas those from patients with enterotoxigenic disease usually show few neutrophils.

TREATMENT

The most important aspect of therapy for diarrheal disease of newborn infants is maintenance of hydration and electrolyte balance. As a rule, parenteral solutions containing appropriate electrolytes should be administered during the time of active diarrhea, and the infant should be examined and weighed frequently to ensure that proper rehydration has been achieved and to prevent the development of complications. Estimates of fluid

loss from diarrhea and vomiting should be recorded carefully and used as a basis for replacement therapy.

Selection of appropriate antimicrobial therapy depends in part on the mechanism of diarrhea. In general, an orally administered, absorbable antibiotic such as ampicillin or trimethoprim-sulfamethoxazole is indicated for disease caused by invasive bacteria (e.g., shigellosis), whereas orally administered, nonabsorbable drugs such as neomycin and colistin sulfate are used for noninvasive organisms that produce enterotoxin (e.g., some *E. coli*).

Antimicrobial therapy for uncomplicated *Salmonella* gastroenteritis is controversial. We do not recommend antibiotics for most infants and children with uncomplicated *Salmonella* disease because such therapy does not shorten the course of illness and can be associated with an increased likelihood of prolonged asymptomatic excretion of the organism. However, neonates and infants younger than 3 or 4 months should be treated with a 7-day course of amoxicillin because of their propensity for the development of a protracted illness or bloodstream invasion with distant foci of infection.[130] Trimethoprim-sulfamethoxazole is a suitable alternative for ampicillin-resistant strains. The asymptomatic carrier state requires no therapy.

Ampicillin formerly was the antibiotic of choice for shigellosis, but in recent years significant resistance to this agent has been observed in many areas of the country. Some strains are susceptible to trimethoprim-sulfamethoxazole in a daily dosage of 10 mg of trimethoprim and 50 mg of sulfamethoxazole per kilogram per day in two divided doses. Sulfa drugs are contraindicated in newborns with jaundice. For multiresistant *Shigella* strains, some authorities recommend third-generation cephalosporins given parenterally (e.g., ceftriaxone) or orally (e.g., cefixime).

Erythromycin is the preferred drug for treating symptomatic *C. jejuni* enteritis. Frequently, if erythromycin therapy is initiated within the first 4 days of illness, excretion of the organism is reduced and symptoms resolve rapidly. An aminoglycoside is the drug of choice for treating *C. fetus* infections; chloramphenicol is an alternative.

Any infant with diarrhea must be isolated from other babies in the nursery. Surveillance of all infants in contact with the index case and institution of infection control measures are mandatory, as discussed earlier.

Probiotics (generally strains of *Bifidobacterium*, *Lactobacillus*, or *Saccharomyces*) have been used for the treatment of acute gastroenteritis with beneficial results. In murine experiments, however, administration of probiotic has shown the potential to causes sepsis in athymic mice, thus suggesting that the presence of immune deficiency in neonates may put them at risk for development of probiotic-induced sepsis. Accordingly, its use is not recommended at present.[76]

URINARY TRACT INFECTIONS

The incidence of bacteriuria in newborn infants ranges from 0.5 to 1 percent for term infants and is approximately 3 percent for premature infants.[348] Urinary tract infections occur more commonly in babies born to bacteriuric mothers and in boys during the neonatal period. The latter observation contrasts with the preponderance of infection in girls beyond the first months of life. The frequency of urinary tract infection and bacteremia is significantly higher in uncircumcised male neonates and young infants.[522] Circumcision reduces the frequency of urinary tract infection by approximately 90 percent. Bacterial colonization of both the prepuce and female perineum may occur because of the presence of maternal urinary tract infection. This prevalence was shown in a study in which 24 percent of infants were bacteriuric when delivered by mothers who had bacteriuria, whereas control infants whose mothers had not been bacteriuric had no bacteriuria. Clinical pyelonephritis occurred in 3 percent of these bacteriuric infants, but only 0.2 percent of 500 control infants of nonbacteriuric mothers had pyelonephritis.[375]

ETIOLOGY

E. coli is the most common etiologic agent of urinary tract infections and accounts for approximately 90 percent of acute infections and 70 to 80 percent of recurrent disease. Approximately 70 percent of *E. coli* strains belong to one of eight common somatic (O) antigen groups, similar to those found in older persons. Several capsular polysaccharide antigens (K1, K2, K12, and K13) are found more often in children with upper tract disease than in those with cystitis.[250,520] The association between K1 antigen and upper tract disease is found significantly more often in newborn and young infants than in older infants and children with *E. coli* urinary tract infection.[520] Fimbriated *E. coli* can attach to specific receptors or uroepithelial cells. Glycolipids of the P blood group constitute a specific receptor that is thought to be associated with pyelonephritis in patients who do not have reflux. When compared with asymptomatic bacteriuric infants, those with febrile urinary tract infections were found to have significantly increased inflammatory signs (e.g., C-reactive protein, microsedimentation rate) and attaching *E. coli*.[304] This finding suggests that bacterial properties determine not only the location of the urinary tract infection but also the severity of inflammation in individual patients.

Proteus, *Klebsiella*, and *Pseudomonas* spp. are encountered in patients with recurrent disease, particularly those receiving prolonged antimicrobial prophylaxis. Gram-positive bacteria, with the exception of enterococci, rarely are encountered as pathogens for the urinary tract. Only a few neonatal cases of renal abscess have been reported in the literature. *S. aureus* and coliforms were the predominant etiologic agents.[466]

CLINICAL MANIFESTATIONS

Most infants with significant bacteriuria are asymptomatic or have nonspecific signs and symptoms. The neonate may appear septic or may have decreased activity, feeding problems, and the other constitutional signs that are seen with infections of other organ systems. Jaundice, hepatomegaly, and thrombocytopenia may be observed in a few infants with urinary tract infection; these findings are associated with septicemia or cholestatic hepatitis in some babies.[50] Localizing signs suggesting urinary tract involvement are unusual findings. When present, they usually consist of a weak urinary stream on voiding or an abdominal tumor from bladder distention, hydronephrosis, or both.

DIAGNOSIS

The diagnosis of urinary tract infection is confirmed by examination and culture of urine. The results of these tests depend largely on the method of urine collection. Most pediatricians obtain urine with a sterile, plastic receptacle applied to the cleansed perineum. However, urine obtained by this method may have an elevated cell count because of recent circumcision, vaginal reflux of urine, or contamination from the perineum. Neonatal asphyxia also may increase the urine cell count. WBCs must be differentiated from round epithelial cells, which appear in the urine in significant numbers during the early days of life. Although pyuria commonly accompanies significant bacteriuria, cells can be few or absent. Direct microscopic examination of uncentrifuged, fresh urine is useful. If bacteria are seen readily in each high-power field, they generally number greater than 10^5 cells/mL.

Glitter cells are thought by many physicians to be diagnostic of urinary tract infection.

Quantitative urine cultures from infants with documented disease usually contain more than 100,000 colonies/mL of a single bacterial species. Any number of bacteria in a urine specimen obtained by percutaneous needle puncture of the bladder should be considered significant. This latter procedure is the single best source of urine for culture and is safe in most newborn infants.[343] The procedure should not be performed in infants who are dehydrated or have bleeding problems. Minor, transient hematuria is an uncommon occurrence, and serious problems from hemorrhage or perforation of the bowel have been rare events. If a "bagged urine specimen" contains fewer than 100,000 colonies/mL of a single species of bacteria or if the culture yields a mixed bacterial population, a repeat urine specimen should be obtained by suprapubic bladder aspiration or catheterization for culture.

Examination of the urinary sediment for antibody-coated bacteria has been found to be useful in differentiating upper tract disease from cystitis in adult patients.[247] Some reports have suggested, however, that this technique is not applicable to infants and children.[520] These studies have demonstrated false-positive and false-negative rates of approximately 30 percent. Because acute pyelonephritis is associated with enlargement of the kidneys secondary to edema and acute inflammatory infiltrate of the medulla and pelvis, ultrasound volume measurements of the kidneys provide a noninvasive method for identifying the probable site of urinary tract infection.[243] Fifteen of 18 children with upper urinary tract infection had increases in volume of 30 percent or greater in at least one kidney, whereas only 4 of 21 children with lower urinary tract infection had increases of greater than 30 percent ($p < .005$).

TREATMENT

Blood and urine should be obtained for culture from all newborn infants with suspected or proven urinary tract infection before antimicrobial therapy is initiated. Antibiotics initially are administered parenterally because sepsis is associated with urinary tract infection in 20 to 30 percent of infants[180] and absorption of antibiotics after oral administration is erratic in some babies. Therapy is initiated with an aminoglycoside and ampicillin to provide antibacterial coverage for the anticipated coliforms, enterococci, and GBS. If the patient has renal impairment, ampicillin and cefotaxime constitute an alternative empiric regimen. Because urine concentrations of these drugs exceed the MIC values of the urinary pathogens manyfold, the usual dosages may be reduced after septicemia has been ruled out.[312] Infants with renal or perirenal abscesses require percutaneous drainage under sonographic guidance or open surgical drainage if the former fails.

A repeat urine culture performed 48 to 72 hours after initiation of appropriate therapy should be sterile or show a substantial reduction in the bacterial count. Infants with persistent bacteriuria should be evaluated for the possibility of inappropriate therapy, obstruction, or perinephric abscess. In uncomplicated disease, therapy usually is continued for a period of 7 to 10 days. Approximately 1 week after discontinuance of therapy, a repeat urine culture is performed.

All infants with documented infection should undergo radiologic evaluation of the urinary tract. An intravenous pyelogram or renal sonograph is obtained during the course of therapy to rule out the possibility of gross congenital abnormalities of the urinary system. Renal sonography is preferred over intravenous pyelography in the acute phase of disease. Congenital malformations are unusual findings in the first week of life but may be present in a significant proportion of infants with urinary tract infections after this age. If obstruction is demonstrated, urologic procedures to ensure proper drainage are mandatory if therapy is to be successful. A voiding cystourethrogram or a radionuclide cystourethrogram should be obtained several weeks after therapy is discontinued. Radiologic abnormalities are found in approximately 45 percent of infants, especially in girls.[69,180]

Physicians have the responsibility of ascertaining that neonates with urinary tract infections do not have congenital abnormalities of the urinary system.[402] In these patients, recurrent urinary tract infections are common events, and physical growth may be retarded until definitive surgery has been performed. Every infant with urinary tract infection should undergo long-term follow-up studies for detection of recurrent infections, many of which are asymptomatic.

Infants found to have anatomic abnormalities (e.g., vesicoureteral reflux) must be protected from reinfection by prophylactic administration of trimethoprim-sulfamethoxazole or nitrofurantoin, and urine cultures or urinary nitrite tests should be performed soon thereafter for children. Although no absolute medical indication exists for routine circumcision of the newborn, cumulative data suggest that circumcision protects against urinary tract infections during early infancy.[523] When compared with the complications of urinary tract infections, short-term complications of circumcision are rare occurrences and mostly minor.[522]

SUPPURATIVE ARTHRITIS AND OSTEOMYELITIS

Osteomyelitis and suppurative arthritis rarely occur in the first 4 weeks of life. The incidence has not changed for many years and is estimated to be one to three cases of bone or joint infections per 1000 nursery admissions. A review of osteoarticular infections from Dallas, Texas, revealed 18 cases (3%) of neonatal arthritis and 18 cases (5%) of osteomyelitis out of 632 arthritis and 365 osteomyelitis cases in infants and children managed from 1959 to 1986.[343] Male infants are affected more often than female infants are (1.6:1), and the incidence is higher in premature than in term infants.

Bone and joint infections can be difficult to detect in neonates and young infants. Early establishment of the diagnosis and appropriate management are vital in preventing orthopedic abnormalities later in life.

ETIOLOGY AND PATHOGENESIS

The infecting organisms in osteomyelitis and septic arthritis vary, but the predominant ones are *S. aureus*, GBS, and gram-negative enteric organisms such as *Klebsiella*, *Proteus*, and *E. coli*. In a study of neonates in Dallas, Texas, *S. aureus* was the etiologic agent in 50 and 44 percent of cases of osteomyelitis and arthritis, respectively, whereas streptococci were responsible for 6 and 22 percent of such cases, respectively. GBS has become the single most common agent associated with arthritis in many areas of the United States.[164] Osteomyelitis and arthritis caused by gram-negative enteric bacilli have remained uncommon events despite the frequency of neonatal bacteremia caused by these organisms. Coliforms caused 11 percent of cases in Dallas and 5 percent in a children's hospital in Stockholm.[45] In Africa and Asia, rates as high as 45 percent have been described.[273] We reported that coliforms and *S. aureus* accounted for two thirds of isolates in neonates with osteoarticular infections in Panama.[424] Gonococcal arthritis and tenosynovitis were common occurrences in previous decades, but they are seen only occasionally today.[117] Other causative agents associated infrequently with newborn infection are *Salmonella*, *Pseudomonas*, *C. albicans*, and *U. urealyticum*.[123,385,386]

Osteomyelitis and arthritis have been reported in newborns after several invasive procedures, including heel puncture, femoral

venipuncture, exchange transfusions, fetal monitoring with electrodes, serial lumbar puncture, and umbilical artery catheterization.[18,48,290,342,364,386,391] Osteomyelitis of cranial bones has complicated infected cephalohematomas. The use of peripheral and central intravascular catheters in neonates has been associated with the development of bacterial and fungal osteomyelitis.[148] Septic embolization from catheter tip thrombi, together with local hypoxia from partial occlusion of the vessel by the catheter, may explain this association.[164,291] A very strong correlation exists between the site of the catheter and localization of infection in the limb; for example, the knees and hips are involved in most cases associated with aortic catheters.[291] Usually, the origin is unknown and presumed to be hematogenous.

During the infant's first month of life, the epiphyseal plate is traversed by multiple small transepiphyseal vessels that provide direct communication between the articular space and the metaphysis of the long bones.[356] As a result, infection of the metaphysis (i.e., osteomyelitis) can spread across the growth plate and penetrate the epiphysis or enter the joint space. Because the perforating vessels disappear when the child is approximately 1 year of age, septic arthritis usually is not associated with osteomyelitis in older infants and children. The two exceptions to this rule are osteomyelitis of the proximal ends of the femur and humerus. The capsule of the hip and shoulder attaches below the proximal metaphysis of the femur and humerus, respectively. Infection of the epiphyseal cartilage may rupture through the periosteum, enter the joint space, and produce purulent arthritis. Because the capsular articulation of the hip and shoulder is permanent, osteomyelitis and septic arthritis may coexist, thus rendering the origin of infection difficult to establish.

The large vascular spaces and thin spongy structure of the metaphyseal cortex in infants permit early decompression of the primary abscess into the subperiosteal space. The abscess then dissects rapidly between the loosely attached periosteum. As pressure increases from the accumulating pus, a subcutaneous abscess can develop that may point and drain spontaneously through the skin and form a sinus tract. Free communication between the original site of osteomyelitis and the subperiosteal space prevents the necrosis and extensive spread of infection within bone that occurs frequently in older children and adults.

CLINICAL MANIFESTATIONS

Two distinct clinical syndromes that may be associated with osteomyelitis in the newborn period have been described.[132,199,490] The first is a benign form in which the earliest sign of bone and joint infection in newborns is failure to move an extremity spontaneously or apparent pain on movement without systemic evidence of infection. Swelling, erythema, and heat localized to the affected part are late findings. Multiple bones or joints can be involved, especially when disease is caused by *S. aureus*. The striking feature of this form is the satisfactory general condition of the infant despite the intensity of the local process. The fatality rate is exceedingly low, and healing is prompt. The second syndrome, a severe form, is characterized by systemic manifestations of sepsis; only later are multiple sites of bone and visceral involvement noted.

Two other clinical entities unique to the newborn are maxillary bone involvement and osteomyelitis caused by GBS. Maxillitis or osteomyelitis of the superior maxilla is an unusual form of bone infection in newborn infants.[289,290] More than 85 percent of all maxillary infections in infants take place in the first 3 months of life, with the peak incidence occurring in the second to fourth weeks.[303] Swelling of the cheek, associated with unilateral nasal discharge of pus, and swelling of the alveolar ridge of the maxilla should alert the physician to this entity. Initially, dacryocystitis or orbital cellulitis may be suspected. The etiologic agent usually is *S. aureus*. Septicemia and death occur commonly in untreated cases.

Group B streptococcal osteomyelitis is manifested during the third and fourth weeks of life (i.e., late onset) and is caused predominantly by type III strains. It affects girls more often than boys, and the humerus is the most common site of involvement. In contrast to the fulminant onset and poor outcome that occur in some infants with late-onset meningitis, group B streptococcal bone and joint infection has an indolent nature and an almost uniformly good outcome. Occasionally, a diagnosis of Erb palsy may be entertained when inflammatory signs are minimal and diminished use of an arm is marked. Manipulations known to predispose neonates to bone and joint infections caused by other organisms have not been reported in infants with group B streptococcal arthritis or osteomyelitis.[299]

DIAGNOSIS

Conventional radiography remains the most useful means of establishing the diagnosis in patients with suspected suppurative arthritis; radiographs may be normal or show enlargement of the joint space. Later in the course of disease, subluxation and destruction of the joint are common findings. In early osteomyelitis, the normal radiographic water markings of tissues adjacent to the affected bone may be obliterated, an indication of deep-tissue inflammation and swelling. Lifting of the periosteum also may be observed, but cortical bone destruction seldom occurs before the second week of illness. A complete skeletal survey should be performed because of the frequent involvement of multiple sites.[164,329] In approximately 10 percent of patients, radiographic abnormalities are not seen during the course of disease. Although radionuclide bone scans are useful in establishing an early diagnosis of osteomyelitis in infants and children, they can be normal in newborn and young infants with confirmed infection. A report indicates a sensitivity of 90 percent for three-phase bone scintigraphy in the diagnosis of neonatal osteomyelitis.[5] Magnetic resonance imaging has been used successfully for establishing the diagnosis of osteomyelitis in the newborn period.

Blood should be obtained for culture from all infants with osteomyelitis or septic arthritis. In view of the varied etiologic bacteria, obtaining specimens of bone or a joint aspirate for culture is imperative, as is percutaneous needle aspiration of intra-articular pus in patients with suspected suppurative arthritis or aspiration of sequestrum in those with suspected osteomyelitis. If pus is obtained, the material should be Gram-stained and cultured.

TREATMENT

Selection of initial antimicrobial therapy is guided by preliminary identification of the pathogen from stained smears of material obtained by needle aspiration. If no microorganisms are seen, initiating treatment with two drugs, a penicillinase-resistant penicillin (e.g., nafcillin, oxacillin) and cefotaxime, is advisable. Vancomycin should be added if MRSA is a possibility. Use of an aminoglycoside with the antistaphylococcal penicillin does not provide adequate coverage for GBS. Definitive treatment is based on culture and susceptibility results. Direct instillation of an antibiotic into the joint space is unnecessary because most drugs penetrate the inflamed synovium and adequate concentrations are achieved in purulent material.[344] The same applies to treatment of osteomyelitis; direct instillation of antibiotics into acutely inflamed bone is unnecessary.[488]

Surgical removal of infected material is an integral part of treatment. Open drainage is essential for managing septic hip disease. The inflammation in osteomyelitis or septic arthritis can

occupy the epiphyseal and metaphyseal sides of the growth plate and result in ischemia and necrosis of the plate and permanent orthopedic damage. For other joints, repeated daily evacuation of fluid with a needle and syringe usually is adequate. In patients with osteomyelitis, the subperiosteal space and the metaphysis should be drained if pus is obtained during diagnostic aspiration. If only a small amount of bloody material is obtained at aspiration, surgery does not need to be performed immediately, and the patient generally can be managed with antibiotic therapy alone as long as the local and systemic signs resolve. If improvement is unsatisfactory, repeat aspiration or open surgical drainage may need to be performed.

Antimicrobial treatment of neonatal musculoskeletal infections caused by staphylococci or coliform organisms is continued for a minimum of 3 to 6 weeks. Group B streptococcal infection is treated with penicillin G or ampicillin for at least 2 weeks. Ten days of therapy usually is adequate for treating gonococcal infection. Use of oral antibiotics as a substitute for parenteral therapy during the second and third weeks of treatment is unwise unless compliance can be ensured and serum bactericidal activity or the antibiotic concentration is satisfactory, which indicates adequate absorption of the orally administered drug. Parenteral antibiotic therapy can be administered at home if the infant can be assessed routinely by a physician.

As a general rule, systemic symptoms disappear within several days of initiation of therapy and adequate surgical drainage, although such local signs as heat, erythema, and swelling may persist for 4 to 7 days. Full range of motion may not return to the involved limb for several months. The erythrocyte sedimentation rate is a useful guide for determining the duration of therapy. The rate usually returns to within the normal range within 2 to 4 weeks; in contrast, C-reactive protein becomes normal sooner. As reported for older infants and children, serial determinations of C-reactive protein may be valuable in assessing the clinical course, but experience is inadequate to use this test in guiding the duration of antimicrobial therapy for neonatal osteoarticular infections.[407] Complete resolution of the radiographic changes may take several months.

Physical therapy to ensure full range of motion should be started as soon as the pain has abated. Assessing residual joint abnormalities and abnormal bone growth patterns in infants frequently is difficult until many months or years have passed. Maintaining good long-term follow-up after treatment has concluded is important.

CONJUNCTIVITIS AND ORBITAL CELLULITIS

Infections of the eye of the newborn can be caused by a variety of microorganisms, including *Neisseria gonorrhoeae*, *Chlamydia trachomatis*, *S. aureus*, and *P. aeruginosa*. From a review of more than 300 eye infections in newborns at Grady Memorial Hospital in Atlanta, Georgia,[16] researchers determined that 29 percent were caused by *Chlamydia*, 14 percent by gonococci, 10 percent by staphylococci, 2 percent by chemical reactions, and 1 percent by mixed gonococcal and chlamydial infections. The remaining 44 percent were of uncertain cause. A prospective, controlled study found that the major microbial causes of neonatal conjunctivitis were *Haemophilus* spp. in 17 percent, *S. aureus* in 17 percent, *C. trachomatis* in 14 percent, *S. pneumoniae* in 11 percent, and enterococci in 8 percent.[428] Other less frequently encountered organisms included *M. catarrhalis*, *Pasteurella multocida*, *N. meningitidis*, and herpes simplex virus.

The incidence of ophthalmia neonatorum has not paralleled the large increase in gonococcal disease rates among adolescent and young adults, almost certainly a result of universal neonatal prophylaxis with 1 percent silver nitrate solution, antibiotic ointment, or systemic penicillin G. Today, we rarely see the invasive, destructive ophthalmitis described so vividly in the early literature.[151]

Numerous agents have been shown to be effective prophylactically against gonococci. The largest series was published by Greenberg and Vandow[198] and involved 250,000 infants treated with 1 percent silver nitrate. A failure rate of 6.6 per 100,000 infants treated with silver nitrate compared favorably with the rate of 22.5 per 100,000 in 86,000 infants who received no prophylaxis. Of the topical antimicrobial agents, tetracycline or erythromycin appears to be comparable in efficacy to silver nitrate and has the advantage of causing fewer cases of chemical conjunctivitis.[361] Penicillin applied topically or given intramuscularly also is effective, although the ointment no longer is available commercially. Bacitracin ointment is ineffective.[110,306] Ceftriaxone as a single dose of 50 mg/kg (for low-birth-weight infants, 25 to 50 mg/kg) can be useful for prophylaxis when the risk is high. Infants born to mothers with active gonorrhea should receive a single 125-mg dose of ceftriaxone intravenously or intramuscularly. Ceftriaxone should not be given to hyperbilirubinemic infants, especially premature ones.

A study by Hammerschlag and associates[211] showed that neonatal ocular prophylaxis with erythromycin or tetracycline ophthalmic ointment does not reduce the incidence of chlamydial conjunctivitis significantly in the offspring of mothers with *Chlamydia* infection when compared with silver nitrate. The investigators concluded that better management of maternal *Chlamydia* infection is required if the incidence of conjunctivitis caused by this organism is to be reduced. They suggested that a small but appreciable incidence of neonatal gonococcal ophthalmia could be prevented by performing better prenatal screening and treating maternal gonococcal infections.

DIAGNOSIS

Any infant with a conjunctival discharge should be evaluated carefully to determine the cause. Three tests should be performed: Gram and methylene blue stain of the exudate, culture of the exudate, and Giemsa stain and culture for *Chlamydia*, if available, of scrapings made from the lower palpebral conjunctiva after the exudate has been removed. The results of the stained smears determine the appropriate therapy. Direct detection of chlamydial antigens in eye scrapings now is possible by means of a commercially available enzyme-linked immunoassay (e.g., Chlamydiazyme). Limited experience in neonates suggests that it is a sensitive and specific test that can provide rapid and reliable results.[213]

DIFFERENTIAL DIAGNOSIS

Conjunctivitis occurring in the first days of life can be chemical or bacterial. Chemical irritants, such as silver nitrate, cause transient conjunctival hyperemia and a watery discharge that rarely turns purulent.

Gonococcal ophthalmia usually becomes apparent within the first 5 days of life and is characterized initially by a clear watery discharge. Conjunctival hyperemia and chemosis are associated with a copious discharge of thick, white purulent material. Both eyes generally are involved, though not necessarily to the same degree. Untreated gonococcal ophthalmia may extend to involve the cornea (i.e., keratitis) and the anterior chamber of the eye. Corneal perforation and blindness can result. Before adequate prophylactic measures were introduced, ophthalmia neonatorum was the most frequent cause of acquired blindness in the United States.

If gram-negative rods are seen in the stained exudate, the greatest concern is *P. aeruginosa* because of the virulent necrotiz-

ing endophthalmitis that can result. In this condition, a relatively mild conjunctivitis can progress to infection of the entire globe within 12 to 24 hours. Invasion of the cornea by small blood vessels (pannus) is characteristic of *Pseudomonas* conjunctivitis. Perforation of the cornea may occur, and blindness from corneal opacity occurs commonly. The ophthalmic disease occasionally can be followed by bacteremia and septic foci in other organs.[86] Prompt diagnosis and immediate institution of appropriate antimicrobial therapy are mandatory.[86]

Conjunctivitis during the second or third week of life can be caused by viral, bacterial, or chlamydial agents. Viral conjunctivitis frequently is associated with other symptoms of respiratory tract disease, such as rhinorrhea, cough, and rash, and several individuals in the family or nursery may have disease simultaneously. The discharge in viral conjunctivitis usually is watery or mucopurulent but rarely purulent. A hemorrhagic discharge can be seen with adenoviral infection. Preauricular adenopathy is a common finding. Staphylococci, streptococci, *Haemophilus*, and occasionally gonococci cause conjunctivitis in this age group. A smear of purulent material helps differentiate these bacterial agents. However, the presence of bacteria on a Gram-stained smear of exudate is not necessarily related etiologically to the conjunctivitis. The exudate may contain normal inhabitants of the skin and mucous membranes, such as staphylococci, diphtheroids, and *Neisseria* spp.

Chlamydial eye infection may begin on the first days of life but usually does not come to the attention of the physician until the second or third week. Conjunctivitis develops in approximately a third of infants exposed to *Chlamydia* during vaginal delivery.[127] Clinical manifestations of chlamydial infection (inclusion blennorrhea) vary from mild conjunctivitis to intense inflammation and swelling of the lids in conjunction with a copious purulent discharge.[190,411] Pseudomembrane formation and a diffuse "matte" injection of the tarsal conjunctiva are common findings. The cornea rarely is affected, and preauricular adenopathy is an unusual occurrence. In the early stages of disease, one eye may appear more swollen and infected than the other, but both eyes are involved almost invariably.

The diagnosis is established by scraping the tarsal conjunctiva and looking for typical cytoplasmic inclusions within epithelial cells (not in the exudate). Exudates are not sufficient for *Chlamydia* testing because specimens must contain epithelial cells that harbor the infecting organism. Newborns with conjunctivitis should have specimens obtained from both the conjunctiva and pharynx.[127]

Specially prepared tissue culture cells for *Chlamydia* are available in some centers, and immunoassays are available commercially for a rapid and specific diagnosis. The conjunctival material obtained is layered carefully onto a microscope slide and stained by the Giemsa method. Without treatment, the acute inflammation continues for several weeks and merges into a subacute phase of slight conjunctival infection with scant purulent material. Occasionally, chronicity develops; some cases persist for longer than a year. The PCR method is used comparably for detection of *C. trachomatis* in conjunctival and nasopharyngeal specimens from infants with conjunctivitis.[214]

TREATMENT

Initial therapy is based on the results of stained smears of exudate and epithelial cells. If gonococci are seen, parenteral penicillin or ceftriaxone therapy is administered. If staphylococci are detected, nafcillin or another penicillinase-resistant penicillin analogue is used. The necessity of using topical antibiotics in these two bacterial infections is dubious. In the presence of acute inflammation, ample antibiotic is present in eye secretions to inhibit bacteria.

Since the beginning of the 1980s, gonococci resistant to penicillin have appeared in the United States and other parts of the world.[139,396] These strains are susceptible to spectinomycin, erythromycin, and the new-generation cephalosporins (e.g., ceftriaxone, cefotaxime). Experience in treating gonococcal ophthalmia with these drugs is limited, as are pharmacologic data for spectinomycin in newborns. Infants with documented gonococcal infections at any site, including the eye, should be examined for disseminated gonococcal infection. This investigation should include a careful physical examination, especially of the joints, and evaluation of blood and CSF cultures. Term, nonhyperbilirubinemic infants with gonococcal ophthalmia should be treated for 4 to 7 days with ceftriaxone (50 mg/kg given intravenously or intramuscularly every 24 hours). Limited data suggest that uncomplicated gonococcal ophthalmia in infants can be cured with a single injection of ceftriaxone (50 mg/kg, up to 125 mg). If the gonococcal isolate is susceptible to penicillin, crystalline penicillin G can be given. The dose is 100,000 U/kg/day given in two doses (or four doses in infants older than 1 week). The eye should be irrigated with buffered saline solution until the eye discharge has cleared. In patients who do not respond satisfactorily, co-infection with *Chlamydia* should be considered. The mother and infant should be tested routinely for *Chlamydia* infection.

Pseudomonas eye infection always should be treated with parenteral therapy consisting of ticarcillin or ceftriaxone and gentamicin. Gentamicin ophthalmic drops are used for simple *Pseudomonas* conjunctivitis, and subtenon injections of gentamicin may be indicated for endophthalmitis.[185]

Orally administered erythromycin (50 mg/kg/day in four divided doses for 14 days) is superior to topically applied tetracycline or sodium sulfacetamide in the treatment of chlamydial conjunctivitis. Topical therapy suppresses chlamydial growth only, whereas erythromycin eradicates the organism in most patients. Topical and oral erythromycin regimens have comparable efficacy, but oral therapy has the advantage of eradicating nasopharyngeal carriage of *Chlamydia*.[373] Although infantile hypertropic pyloric stenosis has been associated with erythromycin administered orally to infants younger than 6 months old, erythromycin remains the antibiotic of choice for the treatment of *C. trachomatis* disease in infants.[228] Eight infants with documented chlamydial conjunctivitis were treated with azithromycin (20 mg/kg once daily for 3 days); failure to eradicate the organism occurred in three patients, but compliance was doubtful in these cases.[212] In infants who do not tolerate erythromycin, oral sulfisoxazole (150 mg/kg/day in four divided doses) can be used after the immediate newborn period. Most cases heal without residua, but if not treated, infection may persist and cause corneal and conjunctival scarring.[127,191] Approximately 25 percent of infants with gonococcal ophthalmia have concomitant infection with *Chlamydia* that requires therapy.

Patients with gonococcal ophthalmia should be segregated, and strict handwashing techniques should be used because the exudate is highly contagious.

CUTANEOUS AND GLANDULAR INFECTIONS

PUSTULAR AND VESICULAR LESIONS

Superficial pustular staphylococcal disease (i.e., impetigo neonatorum) is the most common skin infection of neonates. The lesions tend to concentrate in the periumbilical and diaper areas and rarely become invasive, except when extensive areas are involved or when monitoring devices, catheters, or other invasive devices are used in a gravely ill infant. The lesions respond to simple topical measures; systemic antibiotic treatment usually is not indicated unless extensive involvement of the skin occurs.

The organisms should be phage-typed (they usually belong to group I) so that if additional cases are encountered in the same nursery, the infected infants and their cohorts can be evaluated for the possibility of nosocomial staphylococcal disease. If these infections are caused by the same staphylococcal phage type, prompt measures should be instituted to determine the source and extent of infection to prevent further colonization and disease.

A second form of staphylococcal disease has been recognized with increased frequency in recent years. The disease takes one of several clinical forms, including bullous impetigo, the most common manifestation, and Ritter disease, the eponymic equivalent in newborns of toxic epidermal necrolysis of older infants.[321] These illnesses usually are caused by phage group II staphylococci, which produce an exotoxin (i.e., exfoliatin) that causes intraepidermal cleavage through the granular cell layer as a result of disruption of desmosomes.[322] The initial finding in Ritter disease is intense, painful erythema, not unlike a severe sunburn. During the next hours, bullus formation occurs, and when the bullae rupture, a tender, weeping, erythematous area is left. A characteristic desquamation of large epidermal sheets occurs approximately 3 to 5 days after onset of the illness. A finer desquamation commonly is seen periorally.

Bacteremic complications occur more commonly in neonates than in older infants with the scalded skin syndrome. Treatment consists of systemically administered, penicillinase-resistant penicillin because most phage group II staphylococci are resistant to penicillin. The cleavage plane in this syndrome is very superficial in the epidermis, so little risk for the development of superinfection exists. Steroids are contraindicated. Maceration may occur in intertriginous areas, which should be treated by local soaks with Burow solution.

CELLULITIS AND FASCIITIS

Group A streptococci are the usual cause of diffuse, well-demarcated cellulitis (or erysipelas), although we have seen the same diseases also caused by group B organisms. The involved skin usually is intensely red, hot, and moderately indurated. Occasionally, streptococci can be recovered from material aspirated from the lesion, but blood culture rarely is positive. Parenteral therapy is with penicillin G, and, although the borders continue to advance for the first 12 to 24 hours, stabilization of body temperature and improvement in general appearance of the infant give reassurance that the diagnosis and therapy are correct.

Necrotizing fasciitis is a virulent form of cellulitis that is a relatively rare event in newborns but has a case-fatality rate of up to 50 percent in this age group. Initially, it resembles uncomplicated cellulitis, but the baby rapidly becomes "toxic," the lesion advances progressively, and the central portion becomes discolored and anesthetic. The lesion has borders that generally are indistinct when compared with erysipelas, in which the borders are raised and palpated easily. The disease may be associated with surgical procedures, birth trauma, previous omphalitis, fetal monitoring, or cutaneous infection and has been reported after the infant has undergone circumcision. The abdominal wall is the usual site of involvement, but other areas such as the thorax, back, scalp, and extremities also can be involved.[234] Causative agents include several streptococci, *S. aureus*, *P. aeruginosa*, *E. coli*, and anaerobic bacteria.[394,514,521] In this condition, subcutaneous tissues, including muscle layers, are invaded, and the organism spreads along fascial planes. Extensive surgery involving resection of destroyed tissue is imperative in treating necrotizing fasciitis.[521] Blood and tissue cultures should be performed, and initial antibiotic therapy should consist of a penicillinase-resistant penicillin or clindamycin or vancomycin and an aminoglycoside, pending results of these cultures. Hypocalcemia and hypoproteinemia may complicate the illness. If the infant survives the first days, extensive skin grafting generally is necessary. In a retrospective review, the overall mortality rate for 66 neonatal cases was 59 percent.[234] Having a high index of suspicion, performing prompt aggressive surgery, and providing appropriate antibiotics and optimal supportive care are the mainstays of management.[81,149,234] The role of adjuvant hyperbaric oxygen is controversial, and very limited information is available.

FUNISITIS AND OMPHALITIS

The umbilical cord may be colonized with numerous different potential bacterial pathogens, some of which may have significant epidemiologic importance. Hexachlorophene was used in many institutions in an attempt to reduce or eliminate the staphylococci that were responsible for nursery epidemics in the late 1950s and early 1960s. Although this antiseptic is effective in reducing staphylococcal colony counts, it is not effective in controlling nosocomial staphylococcal disease in nursery units. Widespread use of hexachlorophene occasionally has been associated with CNS spongiform degeneration, particularly in premature infants.[454] A single application of triple dye to the cord results in a significant reduction of all bacteria, particularly staphylococci, streptococci, and coliforms, but its use for care of the umbilical stump at home can delay the time until the cord separates.[99,347,384] Mupirocin ointment applied to the cord or the nasal mucosa also is effective in eradicating staphylococcal carriage.[226]

Group A streptococci may colonize the umbilical cord and be important as a focal point for epidemic streptococcal disease in a nursery. Unlike staphylococci, they tend to cause an inflammatory reaction.[134,347] Streptococcal funisitis (i.e., inflammation of the cord) is mild and is characterized by a wet, malodorous umbilical stump with minimal inflammation. Disseminated disease is an uncommon occurrence, but when present, it results from bloodstream invasion or direct extension to the peritoneal cavity by way of the umbilical vessels. Treatment consists of systemic penicillin G and topical therapy with antibiotic ointment or triple dye. Local therapy is provided for epidemiologic reasons to eliminate surface colonization. Identification of a single infant with group A streptococcal disease in a nursery necessitates immediate institution of infection control measures for identification and segregation of all colonized persons. When a nursery outbreak is suspected, specific M and T typing of the organism is useful in defining the source and spread of infection. A single injection of benzathine penicillin G is satisfactory for treatment of mild superficial infection or for elimination of the organism from colonized persons.[178,347]

Omphalitis, or infection of the umbilicus, has many causes and occurs more frequently in low-birth-weight infants and those with complicated deliveries. The incidence is estimated to be approximately 2 percent, and symptoms start at an average age of 3 days. Culture and susceptibility test results are necessary for the selection of an appropriate antibiotic. *S. aureus* is the most frequent pathogen isolated, followed by gram-negative rods.[433] Some of the complications secondary to omphalitis reported in the literature are spontaneous evisceration of the small bowel through the umbilical cicatrix, necrotizing fasciitis, small bowel obstruction, peritonitis, and superficial, retroperitoneal, or hepatic abscesses.[10,156,166]

BREAST ABSCESS

Breast abscesses are encountered most frequently during the second or third week of life and are more common findings in

girls, particularly those older than 2 weeks.[414] The disease does not occur in premature infants, presumably because of underdevelopment of the mammary glands in these infants. Bilateral disease is a rare event, but a case caused by GBS has been reported.[345]

The major clinical finding is swelling of the affected breast with or without accompanying erythema and warmth. Systemic manifestations are uncommon occurrences, and only a fourth of patients have low-grade fever. The disease sometimes can progress rapidly and involve breast tissue and the entire subcutaneous tissue beyond the breast's anatomic area.[453] This condition is associated with considerable toxicity and systemic signs and symptoms. *S. aureus* is the major pathogen, but coliform bacteria and GBS have become more common causes in the past decade.[414,474] Mixed infection rarely occurs. In 36 infants with mastitis seen in Dallas, Texas, during a 16-year period, 32 cases were caused by *S. aureus*, 1 case by *E. coli*, 2 cases by *Salmonella* spp., and 1 case by both *S. aureus* and *E. coli*.

Breast abscess is diagnosed by examination of stained purulent material obtained from gentle manipulation of the nipple or by needle aspiration of the abscess. If gram-positive cocci are identified, nafcillin or another penicillinase-resistant penicillin is given. For gram-negative bacilli, an aminoglycoside or cefotaxime is appropriate. When no organisms are seen, nafcillin and an aminoglycoside or cefotaxime should be used initially until results of culture are available. Bacteremia is a rare finding in this condition.

If the patient has only mild cellulitis and no discernible fluctuance, antibiotic treatment alone may suffice. We have managed several patients with GBS mastitis successfully in this fashion. In most instances, however, surgical incision and drainage by a skilled surgeon are required. The duration of treatment depends on the rate of response. It generally is rapid, and we have found that healing is complete within 5 to 7 days in most instances. Long-term follow-up studies suggest that some girls will have diminished breast tissue on the affected side.[414]

SUPPURATIVE PAROTITIS

Suppurative parotitis of the newborn usually is easy to recognize, although occasionally it is confused with infection of a preauricular or superior anterior cervical lymph node. We have encountered one instance in which delay in initiating therapy was caused by attributing the swelling to trauma from obstetric forceps. Infection occurs more commonly in low-birth-weight infants than in term infants and boys. Dehydration predisposes to stasis of parotid secretions and subsequent infection. Bilateral infection is a rare event.

Although *S. aureus* accounts for most cases, disease may be caused by coliform bacteria, *Pseudomonas*, pneumococci, and group A streptococci.[129,279] The clinical manifestations include fever, anorexia, irritability, and failure to gain weight. Erythema, swelling, and tenderness over the involved gland may occur. The diagnosis can be confirmed by expressing pus through the parotid duct or by needle aspiration of a fluctuant area. Gram staining of this material helps identify the causative agent; however, one should recognize that material expressed from the duct may be contaminated by mouth microflora.

Selection of antimicrobial therapy should be based on interpretation of the Gram-stained smear of expressed pus. If gram-positive cocci are seen, nafcillin or another penicillinase-resistant penicillin should be used. Vancomycin should be added if MRSA is a possibility. Gram-negative organisms are treated best with an aminoglycoside, cefotaxime, or ceftazidime to cover both coliform bacteria and *Pseudomonas*. If no organisms are seen in the purulent material, a combination of nafcillin and gentamicin or cefotaxime should be used until the results of culture are available. In most cases, antibiotic therapy alone suffices, and surgical incision and drainage are unnecessary. The gland should not be extirpated. Response to therapy generally is rapid. Most patients require only 7 to 10 days of therapy until healing is complete.

SCALP ABSCESS

Scalp abscesses usually are a complication of fetal monitoring with scalp electrodes.[82,504] The number of vaginal examinations, use of more than one electrode, and fetal scalp blood sampling are risk factors for the development of scalp abscesses. Pathogens incriminated include staphylococci, gonococci, and gram-negative enteric bacteria. A polymicrobial flora, including anaerobic organisms, frequently is isolated in scalp abscesses caused by electronic fetal monitoring electrodes. Incision and drainage of the infected site usually is sufficient. If an associated cellulitis occurs, antibiotics are administered and continued for 5 to 7 days.

LOWER RESPIRATORY TRACT INFECTIONS

Neonatal lower respiratory tract infections may be acquired congenitally or postnatally. Perinatal infection results from transplacental transfer of the agent (i.e., congenital infection) or from inhalation of infected amniotic fluid (usually associated with prolonged rupture of membranes) or infected vaginal secretions during delivery. Viruses, bacteria, *Chlamydia*, and spirochetes are the most common causative agents that produce perinatal pneumonia. Common viral agents include cytomegalovirus, rubella, and herpes simplex virus. Common bacterial agents are GBS, *L. monocytogenes*, and coliform bacilli. *Chlamydia* organisms have been implicated as the cause of a chronic interstitial pneumonitis of early infancy (i.e., eosinophilic pertussis-like pneumonia).[43] *Treponema pallidum* produces a severe, sometimes fatal pneumonitis, and mycoplasmas have caused a rare form of fatal congenital pneumonia. We also have seen in Panama in the last 4 years a few cases of congenital pneumonia caused by *Candida* spp. (unpublished observation).

Perinatal lower respiratory tract disease generally becomes apparent clinically in infants from birth to 7 days, occasionally up to 2 weeks of age. Of emphasis is that inhalation of amniotic fluid or maternal vaginal secretions usually is not associated with infection, nor is meconium inhalation, in which case the pneumonitis has a chemical cause. Only a small percentage of inhalation pneumonias are bacterial in origin; the pathogens most commonly encountered in such cases are GBS and coliform organisms. The clinical signs of inhalation pneumonia are caused by obstruction, chemical inflammation, or both.

The second category of pneumonia is acquired postnatally and usually beyond the first week of life. These diseases may be caused by viral or bacterial agents and most frequently are bronchopneumonic in type. Viral disease may be sporadic or occur as part of a nosocomial nursery outbreak. Respiratory syncytial virus is the most important pathogen of lower respiratory tract disease in young infants.[51] This agent causes particularly severe disease in infants with congenital heart disease. The parainfluenza viruses and adenoviruses also cause bronchiolitis and pneumonia during early infancy. An obliterating, necrotizing bronchiolitis may be caused by adenoviruses and result in radiographic hyperlucency of a segment or a lobe in later infancy and childhood.[184]

Documented nursery outbreaks of lower respiratory tract disease have been associated with respiratory syncytial virus,

adenoviruses, echoviruses, influenza A and B viruses, and parainfluenza virus infections. During these outbreaks, many infants are colonized with the epidemic strains, but only a few have clinical disease.

Common bacterial pathogens causing postnatally acquired pneumonia are *S. aureus*, coliform bacilli, and *Pseudomonas*. These infections occur sporadically or epidemically and often are of nosocomial origin. They may be rapidly progressive, necrotic pneumonias that result in pyogenic complications (e.g., empyema, pulmonary abscesses) and metastatic disease in the bones or meninges.

CLINICAL MANIFESTATIONS

The early signs of respiratory disease in neonates and young infants frequently are nonspecific and include change in feeding status, listlessness or irritability, and poor color. More specific findings that may not be present at the onset of illness are tachypnea, dyspnea, cyanosis, alteration of temperature (i.e., hypothermia or fever), coughing, and grunting. Accentuation of the normal irregularity of breathing is a common finding in neonates.

The physical findings of pneumonia are variable. Flaring of the alae nasi, rapid respirations, and sternal and subcostal retractions are common findings. Coughing indicates lower respiratory tract involvement; brassy coughing is found frequently in viral disease. Dullness to percussion is difficult to demonstrate, but when present, it indicates consolidation or effusion. Breath sounds also may be diminished over the affected area. Crackles or wheezes usually can be heard on deep inspiration (or when the baby is crying) but may be absent early in disease. The clinician frequently is surprised by the meager clinical signs in the face of clearly demonstrable and sometimes extensive radiographic findings of pneumonitis.

DIAGNOSIS

The WBC count usually does not help differentiate viral from bacterial pneumonia. An exception is seen in premature infants with acute respiratory distress syndrome caused by GBS. In these patients, the WBC count often reveals leukopenia with an increased proportion of band forms.[308]

Cultures of blood and material from the trachea frequently help in defining the etiologic agent of neonatal pneumonia. Results of cultures of the ear canal, throat, and other external sites typically are unreliable in defining the cause of pneumonia; more often, they are misleading. Lung puncture should be considered for severely ill infants with consolidated pneumonia when the cause is unknown or for an infant who fails to respond to conventional antimicrobial therapy. Material obtained by needle aspiration is Gram-stained for direct visualization and cultured.

A chest radiograph should be obtained for all babies with suspected lower respiratory tract disease. Radiographic evidence of pneumonia may not exist in the absence of specific physical findings. Although the cause of neonatal pneumonia usually can be determined from a radiograph, certain radiologic patterns are associated with specific diseases. With acute-onset group B streptococcal disease, the radiograph may mimic one showing hyaline membrane disease. A consolidating bronchopneumonia with pneumatoceles, with or without empyema, suggests staphylococcal disease. When a lobar infiltrate is associated with expansion of the lobe, *K. pneumoniae* infection should be considered. A miliary type of bronchopneumonia in a septic neonate is characteristic of listeriosis.

SPECIFIC CLINICAL SYNDROMES

Staphylococcal Pneumonia

Primary staphylococcal pneumonia occurs quite frequently in young infants. Epidemics of staphylococcal disease caused by phage group I organisms are rare events today. In the epidemic setting, many infants are colonized with a virulent strain, but only a few have disease. Concomitant viral respiratory tract infections possibly play a role in promoting dissemination of staphylococci among infants and in converting colonization to disease, but such has not been established.

Staphylococci cause a confluent bronchopneumonia consisting of extensive areas of hemorrhagic necrosis and irregular areas of cavitation. The pleural surface usually is covered by a thick layer of fibrinopurulent exudate. Multiple, small abscesses are scattered throughout the affected lung. Rupture of a small subpleural abscess may result in pyopneumothorax. If erosion into a large bronchus occurs, a bronchopleural fistula results.

Most patients with staphylococcal pneumonia have radiographic evidence of bronchopneumonia early in the illness. The infiltrate may be patchy and limited in extent or dense and homogeneous and involve an entire lobe or hemithorax. Bilateral disease occurs in half of affected patients. Pleural effusion or empyema is detected in most infants. Pneumatoceles of various size are common findings. Although no radiographic picture can be considered absolutely diagnostic, progression over the course of a few hours from bronchopneumonia to empyema or pyopneumothorax, with or without pneumatoceles, strongly suggests staphylococcal disease.

Klebsiella pneumoniae Pneumonia

Primary *K. pneumoniae* infection is an unusual event in infants and young children. However, nursery epidemics of *Klebsiella* infection have been reported. During these epidemics, colonization rates are high, but most infants remain asymptomatic. Contaminated fomites are the primary source of nosocomial infection with this organism.

K. pneumoniae pneumonia may be difficult to distinguish clinically from pneumonia attributable to other causes. The disease may have a fulminant course characterized by copious, thick purulent secretions and the formation of pulmonary abscesses and cavitation. The case-fatality rate in sporadic cases is approximately 50 percent but is considerably lower during epidemics.

Pertussis

During 1994 to 2004, the reported pertussis rate per 100,000 inhabitants increased from 1.8 to 8.9. This is the highest figure since 1959. In 2004, 10 percent of the cases occurred in infants 6 months or younger; these infants were too young to have received the first three doses of diphtheria and tetanus toxoids and acellular pertussis (DTaP) vaccine. Adolescents and adults accounted for the majority (67%) of notified cases, which led to a recommendation by the CDC's Advisory Committee on Immunization Practices for the routine use of DTaP vaccine in adolescents aged 11 to 18 years.[96] Although infants formerly acquired disease from siblings, in the past years infection usually has been acquired from one or both parents whose illnesses have not been diagnosed correctly as pertussis. The reason for these epidemiologic changes most likely is better vaccination of schoolchildren. Because immunity starts to wane in adolescence, parents of young infants are susceptible to infection.[346] The infants of these women also are susceptible to the development of infection because of the lack of transplacental immunity.

The onset of disease generally is in the second to sixth weeks of life; the earliest onset reported was at 10 days of age. Most

young infants do not have a characteristic whoop. Pertussis should be suspected when an infant has a paroxysmal cough with excessive mucus. Because apneic spells are common occurrences in these infants, as they also are in those with respiratory syncytial virus, *Chlamydia*, or influenza virus infection, all infants with pertussis are admitted to the hospital for management. Fluorescent antibody testing provides a means of making a rapid diagnosis, but false-negative and false-positive results are common occurrences. Cultures for *Bordetella* should be performed in all infants. PCR-based testing may become the most sensitive means of documenting *Bordetella pertussis* in the future. Mucus for examination is obtained from patients with a flexible nasopharyngeal wire swab or by nasopharyngeal washing or aspiration.

The greatest hazards to an infant with pertussis are asphyxia and secondary bacterial pneumonia. Supportive care is essential. Excessive mucus must be suctioned, and equipment for emergency airway intubation should be at hand. Administration of fluid therapy is necessary, and maintaining adequate nutritional intake may present the greatest challenge. Infants with pertussis should not be placed in mist tents. Mist therapy provides no substantial amelioration of paroxysmal coughing episodes, and it increases the risk for development of secondary infection with *P. aeruginosa* (or with other commensals).

Atelectasis caused by mucus plugs is a common complication of pertussis in small babies. After the infant has passed the stage of severe paroxysms, chest physiotherapy can be initiated. In almost all cases the atelectasis resolves within 2 to 3 weeks.

Chlamydial Pneumonia

Chlamydial pneumonia is manifested between the 4th and 11th weeks of life in most infants.[491] Infants typically are afebrile and tachypneic, and they have a characteristic staccato cough. Only half of the patients have a history of conjunctivitis. Otitis media occurs frequently.[127]

On chest auscultation, rales may be heard, but wheezes are uncommon findings. The chest radiograph reveals hyperexpanded lungs with bilateral interstitial infiltrates. Approximately half of the infants have peripheral eosinophilia (>400 cells/mm^3). A definitive diagnosis made by isolating the organism from the respiratory tract is not possible in many institutions. The role of immunologic techniques for identifying *Chlamydia* in throat swabs is yet to be determined. Serologic testing is available more readily. These infants usually respond to therapy with erythromycin. The clinical course and duration of nasopharyngeal shedding of *Chlamydia* are shortened by treatment with this antibiotic. If left untreated, these infants remain sick for several weeks but do not become acutely ill. Death from this infection seldom occurs.

TREATMENT

Initial antibiotic treatment of suspected bacterial pneumonia should consist of ampicillin or nafcillin and an aminoglycoside or cefotaxime. The most suitable combination of these drugs depends on the clinical features of the illness and the recent experience with bacterial diseases in the local nursery or community. For patients in whom the cause never is defined and staphylococcal disease cannot be ruled out, therapy with nafcillin or vancomycin and an aminoglycoside or cefotaxime is indicated. If the organism is identified, the single most effective drug should be used. Penicillin G or ampicillin is effective against GBS and penicillin-susceptible staphylococci, ampicillin against *Listeria*, and nafcillin or another suitable antistaphylococcal penicillin against penicillinase-producing *S. aureus.* Vancomycin should be used for disease caused by MRSA or coagulase-negative staphylococci. For pneumonia caused by gram-negative bacilli (e.g., *K. pneumoniae* and others), an aminoglycoside or a third-generation cephalosporin should be used. *Pseudomonas* pneumonia is treated best with ticarcillin or ceftazidime in combination with an aminoglycoside. Therapy is continued for 10 to 14 days for disease caused by GBS and *Listeria* and for a minimum of 3 weeks for pneumonia caused by staphylococci or gram-negative bacilli.

Nosocomial pneumonia is a relatively frequent condition of mechanically ventilated premature infants and can be caused by a vast array of microorganisms. Accordingly, treatment with an empiric regimen of broad-spectrum antibiotics is advised until microbial identification is made. Nonetheless, the physician should realize that isolation of bacteria from tracheal aspirates usually represents airway colonization and not necessarily the etiologic agents. For a reliable etiologic approach, a combination of clinical, radiographic, laboratory, and microbiologic findings is recommended. An important matter to recognize is that antimicrobial therapy commonly fails to eradicate microorganisms from the airway of ventilated infants despite clinical and radiographic resolution of pneumonia.[118]

Empyema is managed best by closed drainage and use of chest tubes with the largest possible caliber. Generally, placement of one tube high and anteriorly and a second tube low and posterolaterally is necessary to achieve optimal drainage. Pyopneumothorax is another indication for immediate insertion of a catheter into the pleural space. After the infant has improved clinically and the amount of drainage is minimal, the tubes should be removed. In general, they should not remain in the chest for more than 5 to 7 days. Instillation of antimicrobial agents or enzymes into the pleural space does not help control infection or promote drainage.

Pneumonia may be one manifestation of generalized congenital viral infection. Distinguishing these infections from congenital syphilis and bacterial pneumonia secondary to inhalation is important.

Most infants with inhalation pneumonia do not require antimicrobial therapy. Differentiating infants inhaling sterile fluid from those inhaling infected material frequently is difficult. If doubt exists, therapy with ampicillin and an aminoglycoside should be initiated and continued until the results of cultures are available.

Therapy for pertussis consists of erythromycin administered orally. Antibiotic therapy may lessen the symptoms of pertussis if administered early in the paroxysmal stage and is valuable for rendering the patient noncontagious. Hyperimmune serum probably is not beneficial.

Associations between bronchopulmonary dysplasia and respiratory colonization by *U. urealitycum*, *C. trachomatis*, *M. hominis*, and adenovirus have been shown in different studies.[57,120,216,506]

In a prospective cohort study, isolation of *U. urealyticum* from respiratory tract secretions was associated with radiographic evidence of pneumonia within 7 days of birth, precocious development of bronchopulmonary dysplasia, and a severe pulmonary outcome.[366] A few case reports have suggested that eradication of *Ureaplasma* with erythromycin contributes to faster resolution of symptoms or to a better outcome in neonates with this chronic disease.[8,230,507] Controlled studies are needed to verify these preliminary observations. No experience with the use of newer macrolides in neonates exists.

Recognition of the role of endogenous surfactant inactivation in the pathogenesis of bacterial pneumonia suggests that therapy with exogenous surfactant, in conjunction with antimicrobial drugs, could be beneficial for the management of affected infants. Large, prospective, placebo-controlled randomized trials are needed to explore the effect of surfactant therapy for neonatal bacterial pneumonia.[405]

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SECTION XIV

Infections of the Compromised Host

CHAPTER 79

Primary Immunodeficiencies

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Primary immunodeficiencies include a broad range of congenital and hereditary disorders that manifest with a defect in the development or function of the immune system and, as a consequence, with increased susceptibility to infections and autoimmunity. Although the molecular defects are present from birth, clinical manifestations may not be evident until much later in life. Previously considered of rare occurrence, primary immunodeficiency disorders are estimated to occur in 1 in 10,000 live births, with a wide range of severity depending on the degree of compromise of the immune function, including mild presentation variants that are clinically subtle and difficult to diagnose. This rate is minimal and considered underestimated because it is based on hospital records and does not include two frequent conditions that usually manifest with only minor immunologic abnormalities: DiGeorge syndrome (1 in 3000 live births) and selective IgA deficiency (1 in 500 individuals).

In contrast to primary immunodeficiencies, secondary immunodeficiencies are acquired disorders, with immune dysfunction occurring as a result of exogenous factors or along with some other non-immune primary disease process. Causes of secondary immunodeficiency include infection (e.g., human immunodeficiency virus [HIV]), drugs (e.g., corticosteroids), malnutrition, and neoplastic or metabolic diseases (e.g., Hodgkin disease, diabetes mellitus). Secondary immunodeficiency disorders, such as those resulting from malnutrition or HIV infection, are diagnosed far more frequently than primary immunodeficiencies.

Pediatricians refer children for immunologic evaluation when they present with unusually frequent or severe infections or when they present with infections that are caused by uncommon organisms (Table 79–1). Most of these children are immunocompetent and may have risk factors for increased frequency of infections, such as allergic disorders, anatomic abnormalities, or other clinical conditions that secondarily produce increased susceptibility to development of infections, as discussed earlier. Some evaluated children have a known primary immunodeficiency and benefit from early diagnosis and management.

Understanding of primary immunodeficiencies has increased in recent years with the significant scientific progress that has been made, especially with the description of gene defects responsible for most of the observed immunodeficiency syndromes and leading to the restoration of immune function by gene therapy in two forms of severe combined immunodeficiency (SCID) (see later).

This chapter discusses the elements of the medical history and physical examination that can help to establish the diagnosis of a primary immunodeficiency, in addition to screening laboratory tests that are useful to assess clinically significant immune dysfunction. The most common primary antibody, cellular immunity, complement, and phagocyte deficiencies are described. Also, a few rare but distinctive conditions, including ataxia-telangiectasia and Wiskott-Aldrich Syndrome (WAS), are reviewed.

INITIAL EVALUATION FOR SUSPECTED IMMUNODEFICIENCY

MEDICAL HISTORY

A comprehensive medical history is the key element in identifying children who are likely to have an immunodeficiency. Children with immunodeficiencies often have a history of frequent and severe infections (e.g., pneumonia, meningitis, septicemia, osteomyelitis, abscess of soft tissue or an internal organ). Although infections occur commonly throughout childhood, epidemiologic studies have established a range for the average number of infections that a normal child may have per year. Otherwise healthy children younger than 5 years old average three to eight episodes of upper respiratory infection annually.[14,38] By 1 year of age, 62 percent of children have had at least one episode of acute otitis media, and 17 percent have had three or more episodes.[101] By 3 years of age, more than 80 percent of children have had at least one episode of acute otitis media, and 46 percent have had three or more episodes. Although these studies have not been repeated since routine anti–*Haemophilus influenzae* type b (Hib) and anti-pneumococcal vaccinations were introduced, the frequency of respiratory infections has not changed; however, the causal agents have been replaced by viral and other bacterial pathogens.

Approximately 2 percent of children 1 to 5 years old develop symptomatic urinary tract infection.[92] The incidence of gastroenteritis among children in the United States is approximately two to three episodes per child-year, with rates of five episodes per child-year among children attending daycare centers.[12,44] Occurrence of these infections during infancy or early childhood in normal children is the result of several factors, including immunologic immaturity or naiveté (lack of prior exposure to infectious agents); poor hygiene; mouthing behavior; allergic disease; and frequent exposure to ill contacts in the home, school, or daycare setting.

The courses of individual episodes of infection in an immunodeficient child may be unusually prolonged or associated with unexpected complications (e.g., lung abscess in a child with pneumonia or skull bone osteomyelitis as a complication of sinusitis). Generally, infections that develop over time at multiple body sites are more suggestive of immunodeficiency than are infections occurring at only one site (e.g., recurrent otitis media). In the latter circumstance, a mechanical or anatomic explanation for the infections (e.g., foreign body or occult tracheoesophageal fistula in a child with recurrent pneumonia, congenital fistulous tract to the middle ear in a child with recurrent bacterial meningitis) should be considered.

A history of recurrent infections of defined cause is more meaningful than a history of frequent, self-limited infections of presumed viral cause. Children with primary antibody deficien-

TABLE 79–1 When to Suspect an Immunodeficiency Disorder

Increased number of infections
Increased severity of infectious disease
Poor response to antibiotic therapy
Unusual organisms (opportunistic infections)
Failure to thrive
Poor wound healing
"Cold" *Staphylococcus* species infection (absence of pus)
Recurrent periodontitis
Low granulocyte or lymphocyte count

TABLE 79–2 Common Pathogens in Children with Primary Immunodeficiency

Immunodeficiency	Common Pathogens
Antibody deficiencies	*Streptococcus pneumoniae*, *Haemophilus influenzae*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Mycoplasma* species, *Salmonella* species, *Shigella* species, *Campylobacter* species, rotavirus, enteroviruses, *Giardia* species
Cellular and combined immunodeficiencies	Mycobacteria, *S. pneumoniae*, *P. aeruginosa*, *Candida* species, *Pneumocystis jiroveci*, herpesviruses, adenoviruses
Complement deficiencies	*S. pneumoniae*, *H. influenzae*, *Neisseria* species
Phagocyte deficiencies	*S. aureus*, *Nocardia* species, *P. aeruginosa*, *Serratia* species, enteric gram-negative bacilli, *Candida* species, *Aspergillus* species

cies typically experience infections caused by extracellular bacteria with polysaccharide capsules (e.g., *Streptococcus pneumoniae*, Hib). In contrast, children with primary cellular immunodeficiencies often have infections with unusual or opportunistic viruses, fungi, protozoa, and mycobacteria (Table 79–2). Because of the impairment of T-cell–dependent antibody responses, infections with common bacteria also may be observed. Children with primary deficiencies of complement components typically have recurrent neisserial infections, and children with phagocyte deficiencies may have infections with a variety of catalase-positive bacterial (e.g., *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Serratia marcescens*) and fungal (e.g., *Candida* spp., *Aspergillus* spp.) organisms. Severe infections with atypical mycobacteria often indicate a defect in interleukin-12 (IL-12)–mediated or interferon-γ–mediated responses.

In addition to the microbial cause of infections, several other items of historical information may help to define the risk for and possible nature of a primary immunodeficiency. Because young infants are afforded some protection by the presence of maternal IgG, children with primary antibody deficiencies generally have an initial period of relative well-being, with onset of infections occurring when they are 3 to 18 months of age. Children with severe T-cell, complement, or phagocyte deficiencies may have onset of infections in the first days or weeks of life.

Omphalitis and poor wound healing suggest phagocyte deficiency. Hypocalcemic seizures in the neonatal period and congenital heart disease may be clues to the presence of DiGeorge syndrome. Severe disease associated with administration of live vaccines (e.g., bacille Calmette-Guérin, measles, poliomyelitis, varicella) suggests an immune defect. The absence of potential causes for secondary immunodeficiency should be sought and shown.

Family history can offer important clues to the presence of a primary immunodeficiency. A family history of consanguinity or deaths from infection or from unexplained causes during infancy or early childhood suggests a genetic defect. Many immunodeficiency syndromes have X-linked inheritance patterns (Table 79–3), which explains the male-to-female ratio of 5:1 among children with primary immunodeficiencies seen in the pediatrician's office.

TABLE 79–3 X-Linked Primary Immunodeficiency Disorders

X-linked agammaglobulinemia (Bruton disease)
Immunodeficiency with hyper-IgM (CD40 ligand deficiency)
X-linked ectodermal dysplasia with immunodeficiency (NEMO defect)
Immunodeficiency, polyendocrinopathy, enteropathy, X-linked (IPEX)
X-linked lymphoproliferative syndrome
Severe combined immunodeficiency (common gamma-chain deficiency)
Properdin deficiency
Wiskott-Aldrich syndrome
X-linked chronic granulomatous disease (most cases)

Figure 79–1 Typical facial appearance of a child with DiGeorge anomaly. Notice the microstomia, hypertelorism, upturned nose, and posteriorly rotated and small, low-set ears. (See companion Expert Consult web site for color version.)

PHYSICAL EXAMINATION

Except for patients with DiGeorge syndrome, who have distinctive dysmorphic features, or Omenn syndrome, which is characterized by severe rash during the newborn period, the physical examination in children with primary immunodeficiency may not be revealing, especially when patients are very young and have not been exposed to potential pathogens. Short stature is a feature of some of these congenital disorders (e.g., chronic granulomatous disease [CGD]), with wasting or failure to thrive likely being secondary to recurrent infections or other chronic conditions such as intestinal malabsorption. Oral candidiasis, omphalitis, and multiple skin abscesses may indicate an underlying immune disorder. A paucity of lymphoid tissues (e.g., tonsils, lymph nodes) suggests poor lymphocyte development.

Examples of helpful physical stigmata include the characteristic facial features (Fig. 79–1) and cardiac malformations in chil-

dren with DiGeorge syndrome. Telangiectasia of the bulbar conjunctivae (Fig. 79–2), nasal bridge, ears, and flexor surfaces of the extremities, with or without ataxia, suggests ataxia telangiectasia. Chronic eczema is observed in hyper-IgE and Wiskott-Aldrich syndromes, and severe gingivitis and periodontitis with loss of alveolar bone and dentition can occur in children with LAD (Fig. 79–3).

LABORATORY TESTS

The initial evaluation for immunodeficiency can be done with commonly available, inexpensive laboratory tests to exclude most serious disorders (Table 79–4). This evaluation should be targeted to the type of immunodeficiency (e.g., antibody deficiency versus T-cell defect) suggested by the medical history and physical examination findings. Laboratory test results must be interpreted in the context of the child's age because average normal serum immunoglobulin levels, T-cell counts, and other immune parameters physiologically change with age.[37]

The initial evaluation for immunodeficiency includes a complete blood count and examination of the peripheral blood smear. Because 50 to 70 percent of circulating lymphocytes are T cells, children with severe cellular (e.g., complete DiGeorge syndrome) or combined (e.g., SCID) immunodeficiencies may have lymphopenia. Children with Wiskott-Aldrich syndrome have reduced numbers of platelets, which are small (decreased mean platelet volume), a characteristic that is unique for this syndrome and the related X-linked thrombocytopenia. Large neutrophil cytoplasmic granules are observed in children with Chédiak-Higashi syndrome.

A complete blood count also is useful in excluding congenital neutropenia. Children with LAD often have the opposite, with markedly increased neutrophil and total white blood cell counts. The presence of Howell-Jolly bodies, with or without thrombocytosis, suggests anatomic or functional asplenia. Other tests to rule out HIV infection, malnutrition, or metabolic disorders as causes of secondary immunodeficiency should be considered.

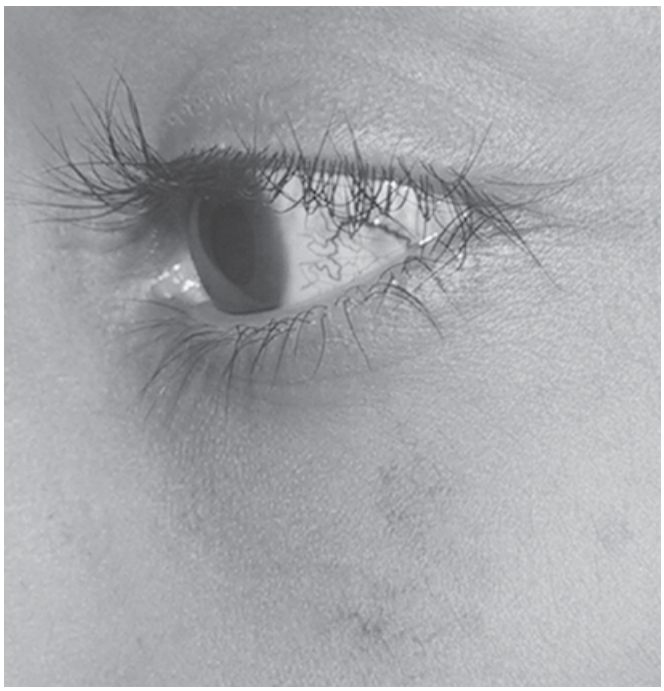

Figure 79–2 Telangiectases of the bulbar conjunctivae in a child with ataxia telangiectasia. (See companion Expert Consult web site for color version.)

TABLE 79–4 Screening Tests for Suspected Primary Immunodeficiency

Immunodeficiency	Screening Tests
All types	Complete blood cell count Peripheral blood smear, for percentage of leukocyte types
Antibody deficiency	Quantitative serum immunoglobulins, IgG, IgA, IgM Post-immunization antibody titers Isohemagglutinins
Cellular immunodeficiency	Delayed hypersensitivity skin tests Chest radiography or chest CT for thymus size Lymphocyte phenotyping for CD3, CD4, CD8, CD19
Complement deficiency	Total hemolytic complement (CH50) assay
Phagocyte deficiency	Nitroblue tetrazolium dye test Dihydrorhodamine (DHR)-1,2,3 Neutrophil CD11a,b,c/CD18 expression

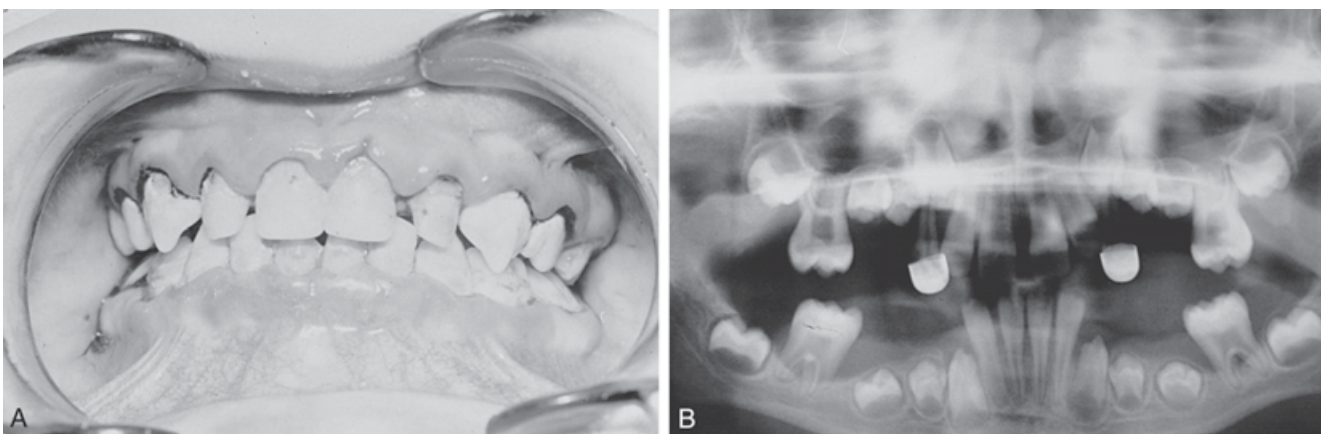

Figure 79–3 **A,** Chronic periodontitis in a boy with leukocyte adhesion deficiency. **B,** Radiograph of the same patient shows extensive alveolar bone loss. *(Courtesy of Dr. Bruce Carter, Texas Children's Hospital, Houston, TX.)*

Evaluation of Humoral Immunity

Screening evaluation of a child with suspected antibody deficiency should include quantitative measurement of serum immunoglobulins and functional assessment of specific antibody responses. Except for the rare deficiency of antibody response to specific antigens, children with primary antibody deficiencies have abnormalities of serum immunoglobulin concentrations. Measurement of serum IgG, IgA, and IgM concentrations identifies children with panhypogammaglobulinemia and children with deficiency of a particular immunoglobulin isotype, such as selective IgA deficiency. Because of marked age-related changes in serum immunoglobulin concentrations, use of age-appropriate normal values for assessment purposes is important.

Functional antibody production usually is evaluated by measuring antibody titers generated in response to immunization with vaccines, such as diphtheria and tetanus toxoids. Antibody responses to polysaccharide antigens do not require cooperation of T cells and are evaluated separately using the pneumococcal polysaccharide vaccine (Pneumovax 23; Merck & Co., West Point, PA) after the child reaches 24 months of age. Younger infants are thought to have a functional immaturity in the ability to respond to this class of antigens. Alternatively, because ABO blood group antigens are polysaccharides and cross-reacting environmental antigen epitopes are ubiquitous, production of anti-polysaccharide antibodies can be assessed by measuring serum isohemagglutinin titers, taking into consideration that children with blood type AB do not form isohemagglutinins. The conjugated pneumococcal (Prevnar; Wyeth-Ayerst, Philadelphia, PA) and Hib (several brand names) vaccines are not suitable for the assessment of anti-polysaccharide antibody responses because they are not pure polysaccharides. Patients vaccinated with the conjugated pneumococcal vaccine still can be evaluated for production of anti-polysaccharide antibodies because the pneumococcal polysaccharide vaccine contains serotype antigens against which the conjugated vaccine does not protect.

Measurement of serum IgG subclasses may be indicated for children with apparent abnormalities in functional antibody production. Further evaluation of children whose screening tests indicate significant quantitative and functional antibody abnormalities should include enumeration of T and B cells in the peripheral blood and in vitro studies of mitogen-induced or antigen-induced B-cell proliferation. The antibody response to neoantigens (e.g., bacterial phages) may be useful for the evaluation of patients receiving immunoglobulin therapy.

Evaluation of Cellular Immunity

The screening evaluation for cellular immunodeficiency starts with delayed hypersensitivity skin tests and, in young infants, posteroanterior and lateral chest radiographs for the assessment of the thymus size (Fig. 79–4). A chest computed tomography or ultrasound scan also may be helpful if available.

Delayed hypersensitivity skin tests are performed using vaccine or microbial antigens to which the child has had prior exposure. Commonly used antigens include tetanus toxoid and *Candida albicans*. Standard initial dilutions are 1:100 for both antigens, but a *C. albicans* dilution of 1:10 may be more appropriate for children aged 5 years or younger because they are less likely to have been exposed to this antigen. The diluted antigen is administered intradermally, and the skin reaction is read at 24 and 48 hours for the presence of wheal formation.

Delayed hypersensitivity skin test responses have low specificity because they depend on previous exposure to the antigen tested and often are absent in children younger than 2 years old. Enumeration of peripheral blood T cells, T-cell subset phenotyping (i.e., $CD4^+$ or $CD8^+$ lymphocyte counts), and mitogen-induced and antigen-induced lymphocyte proliferation studies may be needed to make a reliable assessment of cellular immune function.[37]

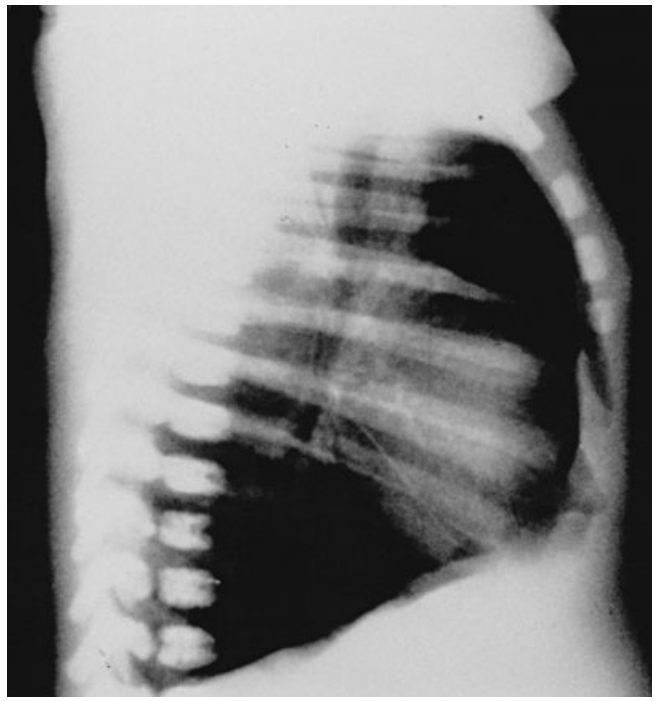

Figure 79–4 Lateral chest radiograph of an infant with severe combined immunodeficiency. Notice absence of the normal thymic shadow.

Evaluation of the Complement System

Deficiencies of components of the classic complement pathway can be detected by performing the total serum hemolytic complement (CH_{50}) assay. This test measures the ability of proteins contained in the fresh patient serum to lyse antibody-coated sheep erythrocytes and reflects the activity of all numbered components of the classic complement pathway from C1 through C9. Complete deficiency of any of these components results in very low levels of CH_{50}. Often, mildly abnormal CH_{50} values are obtained because of inadequate sample handling. Single complement component levels and function should be investigated only when CH_{50} value is zero or near zero, taking into consideration that primary complement deficiencies are rare occurrences. Additionally, measuring the levels of complement components C3 and C4 can be done in most clinical laboratories and may be ordered when complement deficiency is suspected.

Evaluation of Phagocyte Function

A variety of assays are available for assessment of phagocyte function. The nitroblue tetrazolium (NBT) dye test uses the reduction of NBT to formazan by activated phagocytes to measure the oxidative metabolic responses that accompany phagocytosis. Children with CGD show reduced dye reduction (<10% of cells are formazan positive); carriers of X-linked CGD typically have 20 to 90 percent formazan-positive cells. The specificity and sensitivity of this test depend largely on the expertise of the performing laboratory. Flow cytometry is being used increasingly to detect production of hydrogen peroxide in phagocytes, using dihydrorhodamine (DHR)-1,2,3 as the fluorescent indicator, and has proven to be more reliable and sensitive.[50] More sophisticated tests, including assays of chemotaxis, phagocytosis, and bactericidal activity, may be done when the NBT and DHR test results

TABLE 79–5 Gene Defects in Primary Immunodeficiencies

Immunodeficiency	Gene Defect	Chromosome
Antibody Deficiency		
Agammaglobulinemia	Immunoglobulin heavy chain	14q23
	Bruton tyrosine kinase	Xq22
Hyper-IgM syndrome	Activation-induced deaminase	12p13
	CD40 ligand (CD154)	Xq26
Cellular Immunodeficiency		
DiGeorge syndrome	Unknown, TBX1?	22q11.2, 10p13
Severe combined immunodeficiency	RAG1, RAG2	11p13
	JAK3	19p13.1
	Adenosine deaminase	20q13.11
	Common gamma chain	Xq13.1
	IL-7 receptor	5p13
Ataxia telangiectasia	ATM, DNA kinase	11q22.3
Wiskott-Aldrich syndrome	WASP, cytoskeleton protein	Xp11.23
Phagocyte Deficiency		
Chronic granulomatous disease	gp67phox	1q25
	gp47phox	7q11.23
	gp22phox	16q24
	gp91phox	Xp21.1
LAD type I	CD18	21q22

Note: More than 120 gene defects have been shown to cause immunodeficiency syndromes. A more comprehensive list has been compiled by Notarangelo and associates[73] and Geha and colleagues.[41a]

are abnormal, or when high clinical suspicion of a phagocyte disorder exists.[51] Enumeration of CD11a,b,c$^+$/CD18$^+$ white blood cells is indicated when a diagnosis of LAD is suspected.

Genetic Testing

More than 120 gene defects responsible for immunodeficiency disorders have been identified (Table 79–5).[73] Currently, testing for these gene defects is available only in a few specialized laboratories, but testing should be pursued when a primary immunodeficiency with a known genetic defect is suspected. Genetic testing is strongly recommended for an affected patient who is ill, for prenatal diagnosis of an unborn child with an affected sibling, or for an individual who may have inherited or is a carrier of a known immunodeficiency gene defect. For some conditions, knowing the specific gene defect may help when one is assessing the prognosis and therapeutic options. Bone marrow transplantation for X-linked SCID (*IL2RG* gene defect) has been reported to be more successful than for other forms of SCID.[8] Knowledge of the specific gene defect also is useful for genetic counseling, most importantly to answer a common concern of the parents regarding the probability of having another child with a similar condition. Genetic studies for immunodeficiencies usually are used to confirm the diagnosis of DiGeorge syndrome, in which a deletion in chromosome 22 is identified by fluorescence in situ hybridization in approximately 95 percent of patients affected.[98]

MANAGEMENT

Management of an immunodeficient patient requires specialized care that may dictate recommendations according to the specific diagnosis. Hematopoietic cell transplantation is indicated for the most severe immunodeficiencies and necessitates careful individualized assessment of risks and benefits before proceeding with this treatment. One common therapeutic measure is replacement therapy with intravenous or subcutaneous immunoglobulins for patients with proven deficiency of IgG response,

TABLE 79–6 Key Concepts in the Management of Primary Immunodeficiencies

Immune function	T- and B-cell number and function should be assessed periodically
IgG replacement therapy	For patients with low IgG levels and poor antibody function
Immunizations	Live vaccines should not be administered to patients with immunodeficiency except for patients with complement deficiency and selective IgA deficiency
	Patients with complement deficiency may benefit from receiving pneumococcal and meningococcal vaccines because of their increased susceptibility to encapsulated organisms
	Household contacts of children with immunodeficiency should not receive oral poliovirus vaccines because of the risk of transmission to the immunodeficient child
	Other live vaccines (BCG, MMR, and varicella vaccine) may be administered to the household contact
	If the vaccine recipient develops a rash, contact with the immunodeficient child should be avoided[7]
Blood products	When needed, patients with immunodeficiencies should receive only irradiated, cytomegalovirus-negative, leukocyte-depleted blood products
Antibiotic prophylaxis	T-cell–deficient patients should take antibiotic prophylaxis for *Pneumocystis jiroveci*
	Antibiotic prophylaxis is recommended for dental and surgical procedures
	Patients with recurrent infections may benefit from antibiotic prophylaxis
Infectious diseases	Infections should be recognized promptly, and unusual pathogens should be considered
	Antibiotic therapy should be started early and discontinued cautiously
Diet and activity	Patients with immunodeficiency should have a regular diet and lifestyle but should be instructed to avoid eating raw food and playing in environments potentially highly contaminated with pathogens, including daycare centers
	Strict handwashing and reverse isolation may be indicated for patients with poor T-cell function

when presenting alone or in combination with cellular immunity defects. Other recommendations that apply to patients with immunodeficiency include the avoidance of contact with sick individuals and avoidance of live vaccines[6]; the use of irradiated, leukocyte-depleted, cytomegalovirus-negative blood products when needed; use of antibiotic prophylaxis; and prompt establishment of the diagnosis and provision of treatment of infections. Isolation in sterile environments is not recommended, except for patients with severe T-cell deficiency who are awaiting hematopoietic cell transplant, because of the severe impact on psychosocial development and the advances in the treatment and prevention of community-acquired infections (Table 79–6).

SELECTED PRIMARY ANTIBODY DEFICIENCIES

X-LINKED AGAMMAGLOBULINEMIA

Clinical Features

Boys with X-linked agammaglobulinemia (XLA), or Bruton disease, often are healthy during the first months of life because

of the protective presence of transplacentally acquired maternal IgG. As maternal immunoglobulin disappears from the infant, chronic or recurrent infections develop.[25,82,112] Frequent episodes of otitis media, sinusitis, pneumonia, and diarrhea are most common, but infections are not limited to mucosal surfaces; bacteremia, meningitis, and osteomyelitis also may occur. The mean age at establishment of the diagnosis in a retrospective study of 96 patients with XLA was 2.5 years when there was a family history of the disease and 3.5 years when there was not.[112]

S. pneumoniae, Hib, *S. aureus*, and *P. aeruginosa* are the bacterial pathogens observed most frequently in the setting of XLA. *Mycoplasma* spp. infections also occur with increased frequency and have been implicated as a cause of a subacute, destructive arthritis.[107] Gastrointestinal infections may be caused by *Salmonella*, *Shigella*, *Campylobacter*, or rotavirus.[82,112] Chronic giardiasis with intestinal malabsorption has been reported.[75]

Increased incidence of vaccine-associated paralytic poliomyelitis also has been reported.[112] Children with XLA are susceptible to chronic enteroviral meningoencephalitis,[66] which is a severe complication, even with treatment using intrathecal and intravenous immunoglobulin therapy. Most reported cases have a fatal outcome.

Pathogenesis

The defective gene (*BTK*) maps to the midportion of the long arm of the X chromosome. The gene encodes a cytoplasmic protein-tyrosine kinase, the normal function of which is necessary for expansion of B-cell populations during their maturation.[105] Deleterious mutations of the gene have been found in all affected individuals studied. XLA results from developmental arrest of B-cell maturation because lack of the Bruton tyrosine kinase (BTK)–mediated signal. Consequently, blood, lymph nodes, and bone marrow contain markedly diminished numbers of B cells and plasma cells, resulting in hypogammaglobulinemia. Other components of the immune system are not affected.

Diagnosis

Because of the presence of transplacentally acquired maternal IgG, the determination of serum immunoglobulin for establishing the diagnosis of XLA in the child's first 6 months of life is unreliable. Infants with XLA also have low concentrations of other immunoglobulin isotypes (e.g., IgA, IgM), but defining values that clearly differentiate between infants with the disease and normal infants has been difficult. Establishing a definitive diagnosis during early infancy generally relies on immunophenotyping by flow cytometry to show the absence of B cells in peripheral blood. After an infant with XLA reaches 6 months of age, serum IgG concentrations usually are less than 100 mg/dL, and concentrations of other immunoglobulin isotypes are low or undetectable. Isohemagglutinins are absent, and specific antibodies are not produced in response to immunization or natural infection. Recurrent infections in male family members may suggest an X-linked condition. Definitive diagnosis is obtained by finding inactivating mutations in the *BTK* gene sequence.

Treatment and Prognosis

Lifetime immunoglobulin replacement therapy is indicated for all patients with XLA.[61,78] It decreases the frequency of serious infections, reduces the need for hospitalization, and may help to prevent the development of chronic lung disease with progressive decrease of lung function. The dose and frequency of administration of immunoglobulins are adjusted to produce serum IgG trough concentrations of at least 500 mg/dL. For most patients, intravenous immunoglobulins are required at doses of 400 to 600 mg/kg, given every 3 or 4 weeks.

Acute infections in patients with XLA should be treated promptly. Minor middle ear, sinus, and skin infections usually respond to oral antibiotics. Pneumonia and other serious focal or systemic infections should be treated initially with intravenous antibiotics. Empiric antibiotic therapy is directed against common bacterial pathogens, including *S. pneumoniae*, *H. influenzae*, and *S. aureus*. If possible, etiologic diagnosis should be obtained, particularly in cases of severe or chronic infection. Because serum IgG concentrations often decrease during acute infection, the serum IgG concentration should be measured, and additional doses of immunoglobulins may be indicated. Chronic pulmonary disease with bronchiectasis is an important cause of death of patients with XLA. Long-term antibiotic therapy, similar to that used in patients with cystic fibrosis, may be helpful in individual cases. Long-term survivors of XLA are at increased risk for developing lymphoid malignancies.[82]

IMMUNOGLOBULIN DEFICIENCY WITH INCREASED IgM

Clinical Features

Approximately 70 percent of cases of immunoglobulin deficiency with increased IgM (i.e., hyper-IgM syndrome) are associated with X-linked inheritance, due to *CD40L* gene defects, but autosomal recessive and dominant inheritance patterns also have been reported.[57] Patients with this disorder develop recurrent pyogenic infections during infancy as transplacentally acquired IgG wanes. Respiratory tract infections and chronic diarrhea with failure to thrive are common occurrences; septicemia, meningitis, and other serious systemic infections also occur.[60] Opportunistic infections, such as *Pneumocystis jiroveci* pneumonia, also are common occurrences.[113]

Half of patients with hyper-IgM syndrome have neutropenia. It can be intermittent, but it lacks the precise periodicity of cyclic neutropenia. Aphthous ulcers occur commonly. Perirectal ulcers and abscesses also have been reported. Hyperplasia of superficial and deep lymph nodes occur commonly, and intestinal nodular lymphoid hyperplasia may lead to malabsorption and protein-losing enteropathy. Autoimmune conditions, including arthritis and nephritis, have been described. The incidence of lymphoreticular malignancies is increased in these patients.[60,113]

Pathogenesis

Males with hyper-IgM syndrome may have mutations of a gene on the X chromosome that encodes the T-cell ligand (CD154, CD40L) for the B-cell surface molecule CD40.[4,9] Several patients with normal expression of CD40L but defects in the B-cell CD40 signaling pathway also have been described.[26] Interaction of CD40 with CD40L is essential to B-cell proliferation, isotype switching, and terminal differentiation of B cells to antibody-secreting plasma cells. Mutations in the activation-induced cytidine deaminase (*AICD*) and the uracil DNA glycosylase (*UNG*) genes have been found in patients with the autosomal recessive form of hyper-IgM syndrome.[68]

Diagnosis

Patients with hyper-IgM syndrome have a characteristic increase in serum IgM concentrations in association with low to absent concentrations of serum IgA and IgG. Serum IgM concentrations may exceed 1000 mg/dL; however, they can be within normal range. Circulating B cells expressing surface IgM are found in normal numbers, but only a few cells bear surface IgA or IgG. Antibody responses to immunization often are present but consist predominantly or exclusively of IgM because isotype switching does not occur. More than half of patients are diagnosed before

they reach 1 year of age, and 90 percent of patients are diagnosed by the time they are 4 years old. Evaluation of boys with suspected immunoglobulin deficiency with increased IgM should include a determination of CD40L expression in activated T cells, which is done by flow cytometry.[9]

Treatment and Prognosis

Immunoglobulin replacement therapy and antibiotic prophylaxis are indicated.[78] Reports of patients treated successfully with HLA-identical bone marrow transplantation have been published.[102] Patients with hyper-IgM syndrome usually have a clinical course worse than that of patients with XLA. In addition, X-linked forms of hyper-IgM syndrome have increased morbidity and mortality compared with autosomal forms, possibly reflecting that the X-linked condition results from a T-cell defect, rather than a B-cell defect. Causes of mortality include infections other than respiratory, sclerosing cholangitis with liver disease often secondary to *Cryptosporidium parvum* infection, and malignancy.[113]

COMMON VARIABLE IMMUNODEFICIENCY

Clinical Features

Common variable immunodeficiency (CVID) includes a heterogeneous group of disorders with similar clinical manifestations, characterized by hypogammaglobulinemia with low or normal numbers of B cells, abnormal production of antibodies, recurrent infections, and a propensity for autoimmune conditions. Onset can occur at any time from infancy to old age,[27] but symptoms frequently begin during the second or third decades of life.[28]

The clinical manifestations of CVID are similar to those of XLA, with recurrent infections of the respiratory tract, bacteremia, meningitis, osteomyelitis, and septic arthritis. Patients frequently develop bronchiectasis and chronic lung disease. The most common infectious organisms are *S. pneumoniae*, *H. influenzae*, and *S. aureus*.[28] Many individuals with CVID have chronic diarrhea and intestinal malabsorption that have an infectious or autoimmune basis.[28] The inflammation induced by these conditions may result in protein-losing enteropathy and may exacerbate the underlying hypogammaglobulinemia. Organisms implicated in these gastrointestinal infections include *Giardia*, *Campylobacter*, *Salmonella*, and *Cryptosporidium*. Bacterial overgrowth syndrome commonly occurs. Patients with CVID present with increased frequency of ulcerative colitis, Crohn disease, atrophic gastritis with achlorhydria, viral or autoimmune chronic active hepatitis, and cholelithiasis.

Approximately 22 percent of patients with CVID develop autoimmune conditions, which include a chronic arthritis resembling juvenile rheumatoid arthritis; scleroderma, a lupus-like syndrome; hypothyroidism; and autoimmune neutropenia, anemia, and thrombocytopenia.[27,28] Malignancies, including lymphomas and gastric carcinoma, occur commonly among adults with CVID. A pseudolymphoma syndrome with lymphoid hyperplasia of the lung (i.e., lymphoid interstitial pneumonia) and intestine (i.e., nodular lymphoid hyperplasia), massive splenomegaly, and mediastinal adenopathy may be seen in patients with CVID.

Pathogenesis

The pathogenesis of CVID is poorly defined and may correspond to several different genetic defects that result in impaired antibody secretion. Bone marrow B-cell maturation is intact, and normal or low numbers of B cells are present in peripheral blood and lymph nodes, but production of antibody is impaired. Decreased or absent memory B cells is associated with severity of disease and development of autoimmune disorders and malignancies.[2,20] Many patients with CVID seem to have an intrinsic B-cell defect that impairs the ability of these cells to differentiate into immunoglobulin-secreting plasma cells, but a variety of T-cell functional abnormalities, including decreased proliferation of lymphocytes to mitogens and antigens and reduced expression of cytokines, also have been described.[49,96] Most recently, inactivating mutations in the *TACI*, *ICOS*, and *CD19* genes have been reported individually in a small proportion of families with several members affected with CVID and IgA deficiency, providing a link between these two clinical syndromes and supporting the concept of a multiple etiology of CVID.[21]

Diagnosis

Quantitative immunoglobulin determination generally reveals a serum IgG concentration persistently less than 250 mg/dL, with comparable decreases in other immunoglobulin isotypes. Immunophenotyping of peripheral blood lymphocytes shows the presence of mature B cells expressing surface immunoglobulins, with a subset of patients lacking switched memory B cells. Antibody responses to immunizations are subnormal or absent.

Treatment and Prognosis

Treatment of patients with CVID is similar to treatment of patients with XLA. Immunoglobulin replacement therapy is indicated and has been shown to decrease the frequency of respiratory infections.[19] Antibiotic prophylaxis may be indicated as an adjunctive measure in patients who continue to develop infections despite having adequate serum IgG levels. Because of enteric protein loss, patients with CVID and enteropathy may require unusually large doses of immunoglobulin to maintain protective serum IgG concentrations. Chronic lung disease with bronchiectasis resulting from multiple pneumonias is the most frequent cause of morbidity and mortality.[28] Patients with absence of switched memory B cells are at risk of developing lymphoma, splenomegaly, and autoimmune complications.

IgA DEFICIENCY

Clinical Features

IgA deficiency is estimated to occur with a frequency of 1 in 500 individuals and is considered the most common immunodeficiency. Most individuals with IgA deficiency are clinically asymptomatic, however.[33,88] Some patients have frequent, noninvasive viral and bacterial infections of the respiratory tract, although most of these patients also have concomitant IgG2 subclass deficiency. Chronic diarrhea occurs with increased frequency, with *Giardia* being a pathogen commonly implicated. Infections in patients with IgA deficiency usually are less severe than infections observed in patients with XLA or CVID. Individuals with more severe or chronic infections often have another associated immunodeficiency, such as IgG subclass deficiency[80] or, rarely, ataxia telangiectasia.

Patients with IgA deficiency have a higher incidence of atopy than the general population. Autoimmune disorders, including systemic lupus erythematosus, rheumatoid arthritis, and pernicious anemia, and lymphoid and gastrointestinal malignancies are more prevalent in this population.

Pathogenesis

The pathogenesis of IgA deficiency has been compared with that of CVID. The fundamental defect in both disorders is a failure of B cells to differentiate into immunoglobulin-secreting plasma cells. Rare alleles of complement genes within major histocom-

patibility complex III on chromosome 6 have been associated strongly with the development of selective IgA deficiency and CVID, suggesting that these two disorders may be related.[109] Some individuals with selective IgA deficiency subsequently develop CVID. Inactivating mutations in *TACI* and *ICOS* genes have been found in families with members presenting with either of those conditions.[21]

Diagnosis

Selective IgA deficiency is diagnosed by a quantitative serum immunoglobulin determination showing subnormal concentration or absence of circulating IgA, in the presence of normal amounts of the other immunoglobulins. In interpreting test results, an important consideration is that serum IgA concentrations can be undetectable in normal infants younger than 6 to 9 months because of a possible maturational delay. This diagnosis is reliable only after the child reaches 4 years of age.

Treatment and Prognosis

Immunoglobulin replacement generally is not recommended in the management of patients with selective IgA deficiency. Although rarely reported, IgA-deficient patients are at risk for developing IgE-mediated anaphylactic reactions if the patient is sensitized to IgA and receives blood products containing IgA.[17]

Patients deficient in IgA respond adequately to vaccines unless they have concomitant IgG deficiency. Sinopulmonary infections in individuals with IgA deficiency often require unusually long courses of antibiotic therapy. Antibiotic prophylaxis for respiratory infections may be recommended if infections are recurrent. Parenteral antibiotic therapy sometimes is necessary for refractory cases of sinusitis or pneumonia.

IgG SUBCLASS DEFICIENCY

Clinical Features

The most common IgG subclass deficiency reported is IgG2, sometimes occurring in association with deficiency of IgG4 or IgA. The second most common is IgG3 deficiency, which may occur in association with IgG1 deficiency.[46,91] Many individuals with IgG subclass deficiencies are asymptomatic; others have recurrent or chronic bacterial infections, usually of the respiratory tract. Commonly implicated pathogens include *S. pneumoniae*, Hib, and other encapsulated bacteria. Children with selective IgG subclass deficiency usually do not have problems with intestinal malabsorption or autoimmunity.

Pathogenesis

The pathogenesis of IgG subclass deficiency is unknown. Genetic factors have been implicated by studies showing linkage to certain immunoglobulin allotypes.[46] T-cell immunity has been reported to be intact in patients with IgG subclass deficiency.

Diagnosis

A diagnosis of IgG subclass deficiency is supported by finding a marked decrease from age-adjusted normal values in the serum concentrations of one or more IgG subclasses, together with evidence of a functional impairment in antibody responses to immunizations. The total serum IgG concentration may be normal, decreased, or elevated as a result of a compensatory increase in production of unaffected IgG subclasses.

Children with low IgG2 subclass levels may respond poorly to polysaccharide vaccines.[106] Responses to protein antigens (e.g., tetanus toxoid) and protein-conjugated polysaccharide vaccines (e.g., Hib conjugate vaccines) also may be abnormal. In contrast, patients with IgG3 subclass deficiency may respond poorly to protein antigens, but responses to polysaccharides generally are normal. Assessment of the patient's antibody responses to vaccination with polysaccharide and protein antigens can help to establish the functional significance of an IgG subclass deficiency. Because of the age-dependent development of anti-polysaccharide antibody responses, such testing is unreliable in children younger than 2 years.

Treatment and Prognosis

Antibiotic prophylaxis of recurrent sinopulmonary infections and early and prompt treatment of infections form the cornerstone of management for symptomatic IgG subclass deficiency. Immunoglobulin replacement therapy is reserved for patients with abnormal antibody responses and a demonstrated propensity for frequent or chronic infections.[78] Because of the anecdotal evidence of the clinical benefit of this measure, the effectiveness and continued need for immunoglobulins should be reassessed periodically.

TRANSIENT HYPOGAMMAGLOBULINEMIA OF INFANCY

Clinical Features

Many infants come to medical attention because they have recurrent respiratory tract infections (e.g., otitis media, sinusitis) and serum immunoglobulin levels that are lower than laboratory normal ranges. Septicemia, meningitis, and other serious systemic infections are rare findings.[31,65]

Pathogenesis

Transient hypogammaglobulinemia of infancy is a developmental disorder with a delay in the physiologic maturation of immunoglobulin synthesis, resulting in prolongation of the relative hypogammaglobulinemia observed in most normal infants at 4 or 5 months of age, when most maternal immunoglobulins have cleared from the infant's circulation. Patients with transient hypogammaglobulinemia of infancy do not seem to have any inherent defects of B-cell maturation or function, or defects of specific antibody responses.

Diagnosis

Transient hypogammaglobulinemia of infancy is a diagnosis that can be made with certainty only in retrospect. Serum immunoglobulin concentrations may remain low in children until they are several years of age in some cases.[65] Circulating mature B-cell numbers are normal and generate normal antibody responses to diphtheria, tetanus, and pertussis vaccines.

Treatment and Prognosis

Infants with suspected transient hypogammaglobulinemia should be followed clinically, and serial serum immunoglobulin concentration measurements should be performed. Some of these children eventually are diagnosed with CVID, selective IgA deficiency, or another secondary immunodeficiency disorder. Because the condition is self-limited, and production of specific antibodies usually is normal, immunoglobulin replacement therapy is not indicated. Antibiotic prophylaxis for respiratory infections is indicated in cases with recurrent episodes of infection.

SELECTED PRIMARY CELLULAR AND COMBINED IMMUNODEFICIENCIES

DIGEORGE SYNDOME

Clinical Features

DiGeorge syndrome is a common genetic disorder that includes facial, cardiac, parathyroid, and thymic abnormalities.[98] Patients with DiGeorge syndrome may present early in infancy with findings unrelated to immunodeficiency. Congenital heart disease, particularly truncus arteriosus and interrupted aortic arch, usually is detected in the first few weeks of life. Neonatal hypoparathyroidism occurs in almost all infants with DiGeorge syndrome, and hypocalcemic seizures or tetany is a common presenting feature. Characteristic facial features include microstomia and micrognathia; hypertelorism; upturned nose; arched palate; posteriorly rotated and small, low-set ears with notched pinnae; and anti-mongoloid slant of the eyes (see Fig. 79–1). Hypothyroidism, esophageal atresia, tracheoesophageal fistula, and bifid uvula have been described.

Based on the degree of immunodeficiency, clinical manifestations may include predisposition to a wide variety of common and opportunistic infectious diseases. Common findings are recurrent or chronic pneumonias; chronic diarrhea; and candidiasis of the skin, mouth, or esophagus. Recurrent or severe herpesvirus infections (e.g., herpes simplex virus, cytomegalovirus); *P. jiroveci* pneumonia; and other opportunistic viral, fungal, protozoal, and mycobacterial infections occasionally are observed. Fatal graft-versus-host disease may occur if infants receive blood products containing viable lymphocytes during surgical correction of heart defects. Although DiGeorge syndrome is the most common of the cellular immunodeficiencies, with an incidence of 1 in 3000 live births, only a few patients with DiGeorge syndrome have clinically significant immunodeficiency (complete DiGeorge syndrome). Most patients develop adequate T-cell numbers and function by the time they reach 1 year of age and do not have an increased incidence of opportunistic infections (partial DiGeorge syndrome).[23,99] At Texas Children's Hospital, 20 percent of patients with DiGeorge syndrome and mild T-cell deficiency present with an increased incidence of respiratory infections.[23]

Pathogenesis

DiGeorge syndrome is classified as a developmental defect resulting from faulty embryologic development of the third and fourth branchial arches and their derivatives, including parathyroid glands, aortic arch structures, and thymus gland.[98] Partial deletions of chromosomes 22 and 10 are found in more than 90 percent of patients. Three groups of investigators simultaneously reported that they reproduced all the characteristic features of DiGeorge syndrome, working with mice models bearing the deletion of *Tbx-1*, a gene within the chromosome 22 critical region.[116] Whether the absence of this gene in humans is responsible for this syndrome is still not definitive.

Diagnosis

Hypocalcemia, congenital heart disease, and characteristic facies may lead to suspicion of DiGeorge syndrome in the newborn period. A deletion in chromosome 22q is detected by fluorescence in situ hybridization analysis. Lymphopenia varies, and severe T-cell deficiency is rare. Determination of T-cell number and a proliferative response to mitogens allows the clinician to make a better assessment of the degree of immunodeficiency. Quantitative serum immunoglobulin determinations often are normal, but antibody responses after immunization usually are poor. Chest x-ray may show absence of the thymic shadow. A significant group of patients with DiGeorge syndrome are totally or partially thymectomized while heart surgery is performed for repair of characteristic conotruncal cardiac malformations.

Treatment and Prognosis

Initial management should focus on the treatment of hypocalcemia and the surgical correction of congenital heart disease. Immunoglobulin replacement therapy and antibiotic prophylaxis should be considered for patients with recurrent infections. Children with $CD4^+$ lymphopenia or evidence of significant cellular immune dysfunction should receive *P. jiroveci* pneumonia prophylaxis. Infections should be recognized early and treated promptly. Only irradiated, cytomegalovirus-negative blood products and inactivated vaccines should be administered. Several small retrospective studies have suggested that children with partial DiGeorge syndrome and normal or only mildly decreased $CD4^+$ T cell counts may receive vaccines without experiencing serious adverse events.[10,71,81] The safety of live vaccination has not been well established, however, and currently is not recommended. Therapeutic options being investigated for patients with DiGeorge syndrome and severe immunodeficiency include bone marrow transplantation and, experimentally, thymus transplantation.[64]

WISKOTT-ALDRICH SYNDROME

Clinical Features

Wiskott-Aldrich syndrome is an X-linked disorder classically characterized by recurrent infection, bleeding, and eczema. Only a few patients have this classic triad of features, however, and some patients have infectious manifestations alone.[76] Patients commonly present with recurrent otitis media or pneumonia caused by encapsulated bacteria (e.g., *S. pneumoniae*, *H. influenzae*). Septicemia, meningitis, and other serious systemic bacterial infections also may occur. Common opportunistic pathogens include *Candida*, cytomegalovirus, other herpesviruses, and *P. jiroveci*.

Individuals with Wiskott-Aldrich syndrome often have depressed platelet counts. A unique feature is the presence of small platelets (low mean platelet volume) in most cases. Consequently, bruises combined with gastrointestinal bleeding in the first months of life is are common presenting feature of Wiskott-Aldrich syndrome. Life-threatening gastrointestinal or intracranial hemorrhage also may occur. Eczematous lesions are generalized and prone to superinfection.

Patients with Wiskott-Aldrich syndrome have a high incidence of autoimmune disorders.[32] Hemolytic anemia, a juvenile rheumatoid arthritis–like condition, and large or small vessel vasculitis have been reported. Some patients develop autoimmune thrombocytopenia as disease progresses. Lymphoid malignancies, especially non-Hodgkin lymphomas involving the brain, have been reported.

Pathogenesis

The gene (*WASP*) that is mutated in patients with Wiskott-Aldrich syndrome[29] maps to the X chromosome. This gene encodes a protein that is expressed in lymphocytes and platelets and is involved in cytoskeletal organization and formation of pseudopodia. This defect results in failure to form an adequate immunologic synapse, resulting in poor cognate interaction between T cells and B cells.[18] T-cell morphologic and membrane abnormalities and signal transduction defects have been described.[69] X-linked thrombocytopenia without or with only

mild immunodeficiency and eczema has been found to be caused by *WASP* mutations that allow partial protein expression.[108]

Diagnosis

Wiskott-Aldrich syndrome should be suspected in a boy with thrombocytopenia and small platelets. The presence of eczema supports the diagnosis. Serum immunoglobulin concentrations vary, with the most typical pattern showing normal IgG, increased IgA and IgE, and decreased IgM. Antibody responses to protein antigens (e.g., tetanus) usually are normal, but responses to polysaccharides (e.g., *S. pneumoniae*, Hib, isohemagglutinins) are absent.

Patients with Wiskott-Aldrich syndrome are anergic on delayed hypersensitivity skin testing. They have near-normal numbers of circulating T cells, and in vitro proliferative responses to mitogens could be normal. Responses to specific antigens are decreased, however. Monocytes exhibit abnormal chemotaxis and poor antibody-dependent cellular cytotoxicity. The determination of WAS protein expression and the sequencing of the *WASP* gene are available in specialized research laboratories.

Treatment and Prognosis

Bone marrow transplantation results in normalization of cellular immunity, specific antibody responses, and platelet count. It is the definitive treatment of choice; however, mortality is high if the donor is not a matched HLA sibling, or if multiple infections and organ damage have occurred. Splenectomy is indicated for severe thrombocytopenia and may improve the patient's quality of life. Daily antibiotic prophylaxis directed against *S. pneumoniae* and *H. influenzae* should be indicated for patients who have undergone splenectomy. Aspirin is contraindicated because it increases the risk of bleeding. Acute infections should be treated aggressively. Immunoglobulin replacement therapy is indicated for patients with recurrent bacterial infections.

The prognosis of Wiskott-Aldrich syndrome has improved in recent years, with some patients surviving to adulthood.[76] Infection, malignancy, and hemorrhage are the leading causes of death.

ATAXIA TELANGIECTASIA

Clinical Features

Ataxia telangiectasia is characterized by cerebellar ataxia, oculocutaneous telangiectases, variable immunodeficiency with frequent infections, and a high incidence of malignancy.[114] Neurologic signs and symptoms dominate the clinical picture. Ataxia usually becomes evident when the child is approximately 1 year old. Progressive choreoathetosis, myoclonic jerking movements, and oculomotor abnormalities develop subsequently, resulting in severe disability. Telangiectases appear on the bulbar conjunctivae, usually in patients 2 to 5 years old (see Fig. 79–2). They subsequently appear on the nasal bridge, ears, and other areas of sun exposure or trauma. Other cutaneous manifestations include café-au-lait spots, vitiligo, and prematurely gray hair.

Recurrent infections are a major feature of ataxia telangiectasia in most patients. Sinopulmonary infections predominate, but systemic infections are rare despite the immunologic abnormalities.[74] Organisms commonly implicated include S. *pneumoniae* and *H. influenzae*. Fifteen percent of patients with ataxia telangiectasia may develop neoplasias. Non-Hodgkin lymphomas and leiomyomas occur most frequently. Carcinomas (especially of the stomach) occur commonly among adults with ataxia telangiectasia.[114]

Pathogenesis

Ataxia telangiectasia is inherited in an autosomal recessive manner. The defective gene (*ATM*) responsible for the disorder has been identified and maps to chromosome 11.[86] One domain of the ATM protein seems to be important in numerous cellular responses, including cytokine signaling. Another region of the protein is involved in DNA repair. Disease manifestations result from a major defect in one or more DNA repair mechanisms. Breakage and rearrangements of chromosomes, including the T-cell receptor genes and immunoglobulin heavy-chain genes on chromosomes 7 and 14, may explain the observed immunodeficiency.

Diagnosis

A clinical diagnosis of ataxia telangiectasia is possible when the disease is fully manifested. Laboratory studies usually are needed for early diagnosis, however. Increased serum alpha-fetoprotein concentrations are observed in essentially all patients older than 6 months. Most patients have deficiencies in serum IgA and IgE. Specific antibody responses usually decline as the patient ages. Most patients have serum IgM in a monomeric 7S form, rather than the usual pentameric 19S molecule. Common manifestations of immunodeficiency include delayed hypersensitivity skin test anergy and decreased lymphocyte proliferative responses to mitogens and antigens. The sequencing of the *ATM* gene is available in specialized laboratories.

Treatment and Prognosis

No specific treatment is curative for ataxia telangiectasia because of the pathology involving multiple body systems. Infections should be treated promptly with oral or parenteral antibiotics. Continuous prophylactic antibiotic therapy may be beneficial for individual patients. Immunoglobulin replacement therapy is reserved for patients with recurrent infections and abnormal specific antibody responses.

The clinical course and prognosis of ataxia telangiectasia vary. Death from chronic pulmonary disease or malignancy occurs commonly.

SEVERE COMBINED IMMUNODEFICIENCY

Clinical Features

Infants with SCID generally present during the first few months of life with recurrent infections.[16,97] Newborns may present with a rash produced by a graft-versus-host reaction induced by maternal lymphocytes. Recurrent pneumonias, other respiratory tract infections, and persistent oral and cutaneous candidiasis are common, as are chronic diarrhea and failure to thrive. Septicemia and other serious systemic bacterial infections also occur. Causative organisms include routine pathogens (e.g., *S. pneumoniae*, Hib) and more unusual organisms. Life-threatening opportunistic infections, including *P. jiroveci* pneumonia, frequently occur early in infancy. Fatal Epstein-Barr virus–associated lymphocyte proliferative disease has been observed in bone marrow transplant recipients and untreated patients with SCID.[36]

Pathogenesis

SCID represents a heterogeneous group of genetic disorders having in common a profound immunodeficiency with failure of cellular and humoral immune function. Various types of SCID have been defined on the basis of enzymatic, genetic, and immunologic criteria (Table 79–7), including reticular dysgenesis, with

TABLE 79–7 Types of Severe Combined Immunodeficiency (SCID)

Type	Defect
Reticular dysgenesis	Stem cell
RAG1, RAG2 deficiency, Omenn syndrome	Rearrangement of B- and T-cell receptor genes
Artemis deficiency	Rearrangement of B- and T-cell receptor genes
DNA ligase IV	Rearrangement of B- and T-cell receptor genes
X-linked SCID (common gamma-chain deficiency)	Signaling for IL-2, IL-4, IL-7, IL-15, IL-21
JAK3 deficiency	Cytokine signaling
IL-7 receptor deficiency	Signaling for IL-7
CD3 δ or ε deficiency	CD3 signaling
CD45 deficiency	Signaling for CD45, cell activation
Adenosine deaminase deficiency	Metabolite (dATP) toxicity to lymphocytes
MHC class I deficiency	Defect in transporter proteins TAP-1, TAP-2
MHC class II deficiency	Defects in transcription factors RFXAP, RFX5, RFXANK, and transactivator CIITA

impaired lymphoid, myeloid, and erythroid differentiation; absence of T-cell and B-cell differentiation; selective defects of T-cell differentiation; and purine metabolism defects (e.g., adenosine deaminase deficiency [ADA]). Autosomal and X-linked recessive inheritance patterns have been recognized. Individuals with X-linked SCID have a defect in the gamma chain of the IL-2 receptor, and it is the genetic defect identified in approximately half of SCID patients.[16] This protein is a functional component of the receptors for IL-4, IL-7, IL-9, IL-15, and IL-21 receptors.[58,72,85] These functions help to explain why this defect has such a profound effect on lymphoid development and function.

Diagnosis

Infants with SCID typically lack palpable lymph nodes, visible tonsils, and radiographic evidence of a thymus gland. Lymphopenia often is identified.[97] Some patients have panhypogammaglobulinemia, whereas others have depressed concentrations of only one or two immunoglobulin isotypes. Antibody responses almost always are profoundly impaired or absent. Lymphocyte monoclonal phenotyping may reveal the presence of circulating mature B cells. Particularly in boys with X-linked SCID and JAK3-deficient SCID, B cells may account for all circulating lymphocytes. Circulating T cell counts are diminished (<10% of normal range) in most patients with SCID. Delayed hypersensitivity skin test anergy is found, and lymphocyte proliferative responses to mitogens and antigens are severely depressed. Testing for specific genetic defect should be pursued, and a genetic evaluation of immediate relatives for carrier status is recommended for genetic counseling purposes. Biochemical tests for ADA and purine nucleoside phosphorylase levels and enzymatic activities are available in specialized laboratories.

Treatment and Prognosis

Hematopoietic stem cell transplantation (HSCT) is the treatment of choice for most patients with SCID. Prognosis is poor for patients without therapy. HSCT performed with HLA-matched related donors has a survival rate of greater than 95 percent.[8] Most patients do not have such a donor available, however, and receive an HLA-haploidentical transplant. Survival rates with these donors vary from 50 to 75 percent at 5-year follow-up, according to the different transplant centers. This survival rate can increase to greater than 95 percent if the SCID is detected early and HSCT is performed before the infant reaches 3.5 months of age, likely because infections and organ damage are yet to occur at this early age. HLA-matched unrelated donors and HLA-matched cord blood have been used increasingly for different forms of SCID, accompanied with different chemotherapy regimens for myeloablation. Success rates are not uniform; they approximate 75 percent in the best transplant centers.

As an alternative because of the high mortality and morbidity associated with HSCT, French and British investigators have reported success in experimental gene therapy trials for X-linked SCID. Using autologous $CD34^+$ stem cells carrying the correct version of the gene, the investigators were able to restore humoral and cellular immunity in 12 of 14 affected patients.[41,45] Four patients in the French trial developed leukemia that was caused partly by the gene therapy procedure. One of the patients died, and the other three responded to anti-leukemic chemotherapy and are alive and well.

Enzyme replacement[47] and gene therapy[13] have been used for treatment of SCID resulting from ADA deficiency. Polyethylene glycol–adenosine deaminase has shown efficacy to increase lymphocyte counts and provide adequate immunity in most affected patients. Some patients ultimately develop "resistance" to the drug in the form of eliciting neutralizing anti-ADA antibodies or simply showing a decrease of lymphocyte counts. Italian researchers have reported positive results of an experimental gene therapy trial for SCID caused by ADA deficiency with the restoration of cellular and humoral immunity in seven patients, in a treatment protocol that included withdrawal of exogenous ADA and reduced myeloablation.

MISCELLANEOUS CELLULAR IMMUNODEFICIENCIES

Many other disorders of cellular immunity have been described. Particularly noteworthy are disorders characterized for dysregulation of the immune system with autoimmune inflammation. X-linked lymphoproliferative syndrome represents a defect in control of Epstein-Barr virus infection. Mutations in the SLAM-associated protein (SAP), a factor involved in the signal transduction of T cells, have been identified to cause X-linked lymphoproliferative syndrome. The absence of this protein affects the interaction of T cells and B cells, leading to an inability to control B cell proliferation caused by Epstein-Barr virus infection.[42,87] Patients may present with severe and often fatal infectious mononucleosis or with B-cell lymphoma.

Chronic mucocutaneous candidiasis is a condition characterized by chronic, severe *Candida* spp. infection of mucous membranes, skin, and nails, often in association with autoimmune polyendocrinopathy, in which case it is termed *autoimmune polyendocrinopathy ectodermal dystrophy*.[1] T-cell numbers and function usually are normal, but most patients do not manifest delayed hypersensitivity skin test responses to *Candida*, and their lymphocytes fail to proliferate in response to *Candida* antigen in vitro. In cases with autoimmune polyendocrinopathy ectodermal dystrophy, deleterious mutations have been found in the Auto-Immune REgulator (*AIRE*) gene. This gene promotes the expression of many self-antigens in the thymus, facilitating the development of immune tolerance of the newly formed lymphocytes. IPEX (immunodeficiency, polyendocrinopathy, enteropathy, X-linked) is another autoimmune endocrinopathy with immunodeficiency. IPEX results from mutations in the *FoxP3* gene and is characterized by lack of T-regulatory cells and clinically by eczema, chronic diarrhea, diabetes mellitus, and other hormone deficiencies.[56]

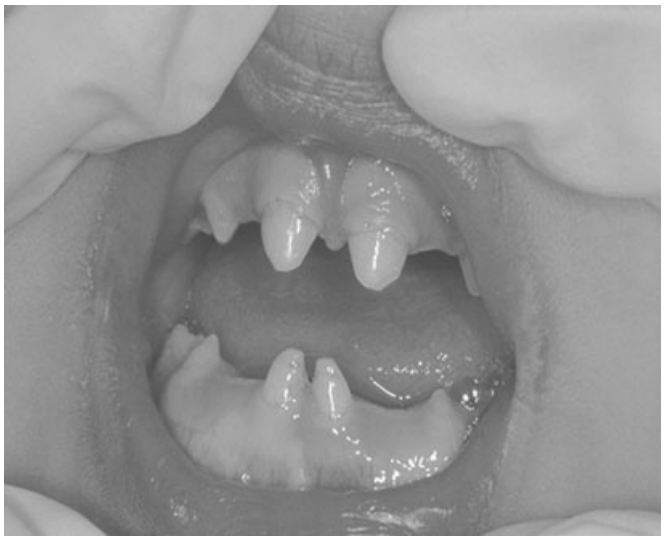

Figure 79–5 Conical teeth characteristic of incontinentia pigmentosa with immunodeficiency, resulting from defects in the *IKKb* gene (NEMO defect). (See companion Expert Consult web site for color version.)

Mutations in the interferon-γ receptor genes have been shown in patients with severe infections caused by atypical mycobacteria.[30,53,83] Patients with a mutation in the IL-12 receptor β2-chain gene or in the IL-12 gene also showed increased susceptibility to mycobacterial disease and disseminated nontuberculous mycobacterial infection.[5]

Deficiency in the nuclear factor-κB enhancing modulator (NEMO) is a newly recognized primary immunodeficiency caused by specific missense mutations in the *IKKG* (also known as *NEMO*) gene, located on chromosome Xq28. NEMO participates in signal transduction mediated by nuclear factor-κB. Most mutations in the NEMO gene are lethal for male fetuses, and female carriers of these mutations may present with incontinentia pigmenti. The surviving male patients with specific missense mutations present with anhidrotic ectodermal dysplasia, conical teeth (Fig. 79–5), low IgG, low IgA, normal or increased IgM levels, and poor T-cell proliferative responses to mitogen and antigen stimuli.[77,93,117]

PRIMARY COMPLEMENT DEFICIENCIES

CLINICAL FEATURES

Three types of complement function deficiencies can cause increased susceptibility to infections: deficiency of opsonic activity of complement proteins, deficiency of the complement lytic proteins, and deficiency of the mannose-binding lectin pathway.[110] Patients with deficiencies of early-acting complement components (e.g., C3, C1, C4, C2) are particularly susceptible to infection with encapsulated bacteria, including *S. pneumoniae* and *H. influenzae*; in addition, they have an increased risk of developing autoimmune disorders, such as systemic lupus erythematosus.[52] *Neisseria meningitidis* is the most important bacterial pathogen observed in patients with deficiencies of terminal complement components (C5 through C9). Recurrent episodes of septicemia and meningitis are especially common.[35,55,84] Although patients with deficiencies of late-acting complement components are at increased risk for developing meningococcal septicemia and meningitis, they seem to suffer lower rates of morbidity and mortality than immunologically normal individuals with systemic meningococcal infection. The frequency of primary complement deficiencies in patients with invasive meningococcal disease is approximately 5 to 10 percent[7,67]; however, the likelihood of having complement deficiency increases dramatically (31%) among individuals who have had more than one episode of invasive disease. A deficiency of opsonization in children with recurrent pyogenic infections and failure to thrive led to the recognition of the mannose-binding lectin pathway deficiencies. The clinical characteristic of these patients is the increased frequency of pyogenic infections.[100]

PATHOGENESIS

Various gene defects are responsible for the many distinct primary complement deficiencies. The particular bacteria that cause infection may indicate the missing component in host defense. C3b, the major cleavage product of C3, is an important opsonic ligand that promotes ingestion and killing of bacteria. Consequently, patients with C3 deficiency have increased susceptibility to bacterial infections (e.g., *S. pneumoniae*, *H. influenzae*), for which opsonization is the primary mechanism of host defense.[84,110] Individuals with deficiencies of C1, C4, or C2 also have increased susceptibility to these encapsulated bacteria because these components are necessary for activation of C3 through the classic pathway. Activation of terminal complement components C5, C6, C7, C8, and C9 results in assembly of the membrane attack complex C5b-9, a multicomponent macromolecule capable of bactericidal activity. Only gram-negative bacteria are susceptible to this bactericidal effect. Infections in patients with deficiencies of terminal complement components are limited to gram-negative bacteria, such as *N. meningitidis*.

DIAGNOSIS

When the history of infections is suggestive, the screening test used for complement deficiencies is the CH_{50} assay, which depends on the activity of all numbered components of the classic complement pathway from C1 through C9. When CH_{50} test results suggest a complement component deficiency, specific immunochemical and functional testing can identify the deficient component.[111]

TREATMENT AND PROGNOSIS

No specific therapy exists for primary complement deficiencies. Meningococcal vaccine is recommended for children with terminal complement component deficiencies.[7] Antibiotic prophylaxis and vaccines for other encapsulated organisms may be beneficial.

PRIMARY PHAGOCYTE DEFICIENCIES

The primary phagocyte deficiencies are a heterogeneous group of disorders having in common a susceptibility for frequent infections, resulting from a decreased number of phagocytic cells (e.g., neutropenia) or because of impaired adhesion, chemotaxis, opsonization and phagocytosis, or intracellular killing (Table 79–8). Infections resulting from quantitative or qualitative phagocyte deficiencies tend to be prolonged and recurrent, with a response to antibiotic therapy that is slower than expected. Common pathogens include *S. aureus*, *P. aeruginosa*, enteric gram-negative bacteria, and certain fungi (e.g., *Candida* spp., *Aspergillus* spp.).

TABLE 79–8 Primary Phagocyte Deficiencies

Quantitative Defects
Infantile agranulocytosis
Familial granulocytopenia
Cyclic neutropenia
Qualitative Defects
Adhesion Defects
Leukocyte adhesion deficiency
Type I, β-integrin deficiency
Type II, fucose-transporter deficiency
Intracellular Killing Defects
Chronic granulomatous disease
X-linked
Autosomal recessive
Glucose-6-phosphate dehydrogenase deficiency
Myeloperoxidase deficiency
Chédiak-Higashi syndrome
Griscelli syndrome

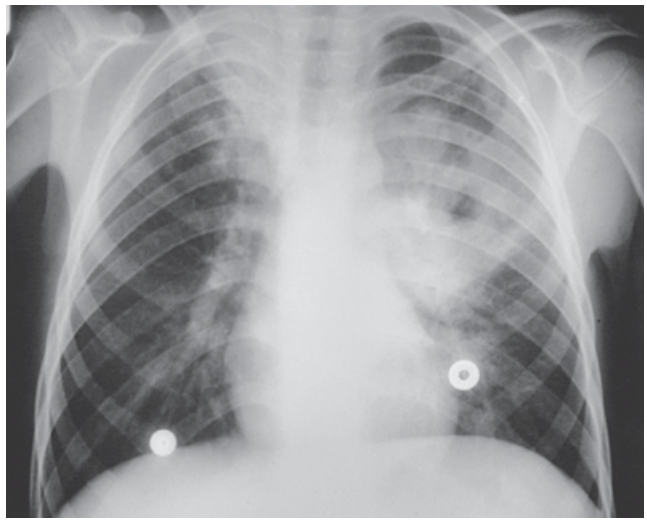

Figure 79–6 Chest radiograph of a 10-year-old boy with chronic granulomatous disease shows a left-sided pulmonary infiltrate and cavitary lung lesion. Biopsy revealed an *Aspergillus fumigatus* infection.

QUANTITATIVE PHAGOCYTE ABNORMALITIES

Primary quantitative phagocyte deficiencies can be observed as solitary defects or in association with other disorders (e.g., Shwachman syndrome). Infantile agranulocytosis (i.e., Kostmann syndrome) is an autosomal recessive disorder characterized by arrest of granulocyte maturation, markedly decreased numbers of circulating granulocytes, and severe infection. The more benign familial granulocytopenia syndrome manifests at various ages from infancy to adulthood, usually with indolent infections of the skin and soft tissues.

Cyclic neutropenia is an autosomal dominant defect of myelopoiesis, resulting from mutation in the leukocyte elastase (*ELA2*) gene, in which periodic disappearance of granulocytes from the circulation occurs.[11] Early granulocyte precursors are present in the bone marrow during periods of granulocytopenia, suggesting transient maturation arrest. Periods of granulocytopenia usually last 5 to 7 days and occur at 14- to 35-day intervals. The length of the cycle generally is constant for any given individual. Fever, malaise, aphthous stomatitis, and skin and soft tissue infections often are observed during periods of granulocytopenia.[94]

Some primary quantitative phagocyte deficiencies, particularly familial granulocytopenia and cyclic neutropenia, respond to therapy with recombinant granulocyte colony-stimulating factor.[39] Alternate-day corticosteroid therapy also has been used with some success in cyclic neutropenia.[115]

CHRONIC GRANULOMATOUS DISEASE

Clinical Features

Patients with CGD present with infections that begin during infancy and recur throughout the patient's life. Occasionally, patients have a mild course of disease and come to medical attention only during adolescence or adulthood.[70] History of suppurative lymphadenitis, soft tissue abscesses, pneumonia, lung abscess, hepatic abscess, and osteomyelitis suggest the presence of CGD. Perirectal abscesses may occur during early infancy. The physical findings of patients with CGD are nonspecific. Lymphadenopathy and hepatomegaly are common, and patients may have aphthous stomatitis. Infections in patients with CGD may progress with few symptoms, and the erythrocyte sedimentation rate and C-reactive protein may be useful to detect active infection.

Patients with CGD generally have infections caused by catalase-positive bacteria and fungi. *S. aureus* accounts for almost one third of all infections of determined cause. *Nocardia*, *S. marcescens*, *Pseudomonas* spp., *Burkholderia cepacia*,[89] and certain enteric gram-negative bacilli also are common findings. *S. marcescens* osteomyelitis particularly is suggestive of the diagnosis of CGD. Fungi, especially *Aspergillus* spp., account for nearly one fifth of all defined infections in patients with CGD (Fig. 79–6).

Patients with CGD often have poor wound healing. Granulomatous obstructive lesions of the urinary[3] and gastrointestinal[62] tracts have been reported. Granulomatous bowel involvement resembles Crohn disease. Corticosteroid therapy may be effective in relieving these sometimes life-threatening lesions.[22] Boys with X-linked CGD may present with the McLeod blood phenotype, which results in difficulty crossmatching for blood transfusion and the potential for hemolytic transfusion reactions.[15]

Pathogenesis

X-linked and autosomal recessive forms of CGD have been described. The various gene defects result in abnormalities of membrane or cytosolic components of the nicotinamide adenine dinucleotide phosphate oxidase complex system.[89] Despite the genetic heterogeneity, all individuals with CGD have in common the failure of the cellular respiratory burst, which ordinarily accompanies phagocytosis and various soluble stimuli. Consequently, oxygen-derived microbicidal factors are not formed, and intracellular killing of phagocytized microorganisms is severely impaired. Catalase-negative microorganisms are killed normally because hydrogen peroxide can accumulate within the phagocytic vacuole.[24,95]

Diagnosis

The diagnosis of CGD historically has been made with the NBT dye test.[51] Neutrophils are stimulated in the presence of NBT, a soluble yellow dye that is reduced by cellular superoxide to formazan, an insoluble blue precipitate. Neutrophils from normal individuals show 100 percent reduction of NBT, asymptomatic carriers show 20 to 90 percent reduction, and patients with CGD show essentially no dye reduction. In patients with mild forms of the disease, the NBT test result may be normal. A more sensitive

method to measure production of hydrogen peroxide is based on chemiluminescence of DHR on activation, detected by flow cytometry; this increasingly is used as a confirmatory test of the disease, replacing the NBT dye test.[50]

Treatment and Prognosis

Specific microbiologic diagnosis of infections should be established whenever possible. Empiric therapy is directed against the organisms known to be common causes of infection: *S. aureus*, *Pseudomonas* spp., and enteric gram-negative bacilli. Surgical drainage or débridement of sites of infection often is required. Anecdotal reports suggest beneficial effects of granulocyte transfusions in patients with CGD and serious bacterial or fungal infections. HSCT has been used successfully with HLA-matched related donors,[59,90] and gene therapy trials for X-linked CGD in humans resulted in gene-corrected, autologous granulocytes detectable for more than 30 months post-treatment, with resolution of chronic infections.[79] The oxidase activity of the gene-corrected cells had decreased progressively, however, by mechanisms of "gene silencing."[79]

Antibiotic prophylaxis with trimethoprim-sulfamethoxazole reduces the frequency of bacterial infections in patients with CGD.[63] Itraconazole and other antifungals given as prophylaxis may be effective for the prevention of *Aspergillus* infections, which usually are severe and difficult to eradicate.[40] The administration of interferon-γ, given by subcutaneous injection three times weekly, reduces the incidence of serious infections in CGD by two thirds without causing major deleterious side effects.[48] Patients should be educated about not performing activities that expose them to environments containing mold spores, such as mulching or raking leaves. The prognosis of CGD has improved remarkably during the past several decades, and now most patients survive to adulthood.

LEUKOCYTE ADHESION DEFICIENCY

Clinical Features

Patients with LAD have severe and recurrent bacterial infections of the skin and soft tissues, mucosal surfaces, and gastrointestinal tract, often beginning during early infancy.[104] Common pathogenic microorganisms include staphylococci and *Pseudomonas* spp. Infants with the severe phenotype may have delayed separation of the umbilical cord secondary to omphalitis. Cutaneous infections may become necrotic, resembling ecthyma gangrenosum or pyoderma gangrenosum. Poor wound healing also is observed. Individuals surviving infancy typically develop severe gingivitis and periodontitis with progressive alveolar bone loss (see Fig. 79–3).

Marked granulocytosis is a hallmark of LAD. Circulating granulocyte counts may range from 15,000/mm^3 to 75,000/mm^3, even in the absence of infection, and counts of 100,000/mm^3 or greater are common during episodes of infection. Despite the presence of granulocytosis, formation of pus is poor because of impaired migration to tissues.

Pathogenesis

LAD type I is an autosomal recessive disorder that maps to chromosome $21_f22.3$. Neutrophils from individuals with LAD are defective in their expression of several surface glycoproteins known as the leukocyte integrin (CD11/CD18) complex. These molecules are crucial for adhesion-dependent functions, and their absence is responsible for defects in leukocyte adherence, chemotaxis, and phagocytosis. The severity of infectious complications of LAD is related directly to the degree of CD18 gene expression. Patients with the severe clinical phenotype have undetectable expression of CD11/CD18 complexes on their phagocytes, whereas individuals with the moderate phenotype generally have 2 to 8 percent expression.

A second form of LAD has been described in two patients with craniofacial dysmorphism, neurologic deficits, recurrent respiratory infections, and marked granulocytosis.[34] Both individuals manifested the Bombay (hh) blood phenotype. In contrast to the neutrophils of patients with LAD type I, neutrophils from patients with LAD type II had normal surface expression of CD18. The molecular basis for the phagocyte dysfunction is a defect in the fucose transporter influencing the glycosylation of sialyl–Lewis X, a carbohydrate ligand for the endothelial adhesion molecules E-selectin and P-selectin. A third form of LAD has been described, characterized by a deficiency of all β integrins, resulting from a deficiency of Rap1 activation, a signal transduction necessary for integrin stabilization.[54]

Diagnosis

The diagnosis of LAD type I is made by flow cytometry using fluorescence-labeled monoclonal anti-CD11/CD18 antibody. In vitro studies reveal abnormalities of phagocyte adherence, chemotaxis, and phagocytosis. Individuals with LAD type II have similar phagocyte function abnormalities, with normal expression of CD18 and down-regulation of selectins. LAD type III is characterized by altered expression of all β integrins.

Treatment and Prognosis

Intercurrent bacterial infections in patients with LAD must be treated aggressively with prolonged courses of parenteral antibiotics. Antibiotic prophylaxis and universal hygiene measures are recommended. Although survival to adulthood is described, almost half of affected individuals die before reaching 2 years of age. Administration of fucose normalizes leukocyte function in LAD type II. Bone marrow transplantation offers the best hope for long-term survival.[103]

OTHER PRIMARY PHAGOCYTE DEFICIENCIES

A variety of other defects of phagocyte chemotaxis, phagocytosis, and intracellular killing have been described. Chédiak-Higashi syndrome is a rare autosomal recessive disorder characterized by partial oculocutaneous albinism, rotatory nystagmus, and peripheral neuropathy. Affected individuals develop recurrent serious or life-threatening infections caused by a wide variety of bacteria. In the accelerated phase of the illness, hepatosplenomegaly, lymphadenopathy, lymphocytic infiltration of multiple body organs, and unexplained febrile illnesses are common. Giant granules are found in numerous different cell types, including leukocytes, in the body. Neutrophils exhibit defective chemotaxis and abnormal bactericidal activity.

Hyperimmunoglobulinemia E (also known as hyper-IgE syndrome, Buckley syndrome, and Job syndrome) is characterized by markedly elevated serum IgE levels, eczema, recurrent staphylococcal abscesses, sinusitis, and otitis media. Coarse facial features are common. In addition to staphylococcal abscesses, recurrent pneumonia with bronchiectasis and pneumatoceles and mucocutaneous candidiasis are common. Other observed features include delay of shedding of primary dentition, recurrent bone fractures, scoliosis, and joint hyperextensibility. Hyper-IgE syndrome manifests with autosomal dominant inheritance and can be diagnosed with certainty only when the patient or family members present with the skeletal manifestations.[43] Missense mutations in STAT 3 gene have been found to cause this disease.[47a]

CONCLUSIONS

Primary immunodeficiencies are rare diseases, with an estimated minimal frequency of 1 in 10,000 live births for most conditions, although the actual incidence is unknown. Mutations in more than 120 genes have been identified to be responsible for primary immunodeficiencies, and several advances have clarified the immunopathogenesis of these conditions.

These diseases should be suspected and investigated in children who present with infections of unusual severity, frequency, or etiologic organisms and unexplained general symptoms, such as fever or failure to thrive. The initial immunologic screening consists of simple laboratory tests, and prompt referral to the clinical immunologist is warranted when a primary immunodeficiency is suspected. Management for these disorders has advanced during recent years to remarkably improved care, reflected in most immunodeficient patients surviving to adulthood. Curative treatment is available for severe immunodeficiencies in the form of allogeneic HSCT; however, it is not without considerable risk of mortality, and cure is not always optimal. Although still experimental, gene therapy has reached the stage of clinical trials for X-linked SCID, ADA-deficient SCID, and CGD, with successful and promising results.

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CHAPTER 80

Opportunistic Infections in Hematopoietic Stem Cell Transplantation

Christian C. Patrick

Hematopoietic stem cell transplantation (HSCT) involves the infusion of stem cells from a donor into a recipient who has received a special conditioning regimen for the procedure. In recent years, the term HSCT has replaced the traditional term bone marrow transplantation (BMT) because hematopoietic stem cells can be obtained from bone marrow, umbilical cord blood, and peripheral blood.[86,117] HSCT has become the standard treatment for many patients with hematologic malignancies and solid tumors, as well as nonmalignant conditions such as primary immunodeficiencies, hemoglobinopathies, bone marrow failure syndromes, and a variety of genetic conditions, including inherited metabolic disorders.*

HSCT can be classified as syngeneic, autologous, or allogeneic, depending on the genetic match of the donor to the recipient of the stem cells. Syngeneic HSCT is transplantation of stem cells from an identical twin. In autologous HSCT, the patient's own stem cells harvested before ablative chemotherapy are transplanted. Autologous HSCT is used for patients who require marrow ablative chemotherapy to treat the underlying malignancy but have healthy bone marrow.[4] Allogeneic HSCT involves the transplantation of stem cells from a human leukocyte antigen (HLA)-matched sibling, an HLA-partially matched family member, or an HLA-matched unrelated donor.[4] In general, engraftment is faster for syngeneic and autologous transplantation when a total HLA match between the recipient and the donor exists. Allogeneic HSCT has a higher chance of success when completely HLA-matched sibling donors are used.

The three main sources of stem cells are bone marrow, peripheral blood, and cord blood. The different cell populations harvested from these sites can be correlated with complications. Peripheral blood progenitor cells from an allogeneic donor can increase the incidence of graft-versus-host disease (GHVD) secondary to an increase in T cells from the transplant.[30] Peripheral blood stem cells contain a high amount of $CD34^+$ cells and an increased amount of T cells, which equates to quicker engraftment but an increased rate of GVHD.[30] Cord blood transplants allow less stringent matching of HLA haplotypes because mismatched cord blood stem cells are less likely to cause GVHD, but they have fewer $CD34^+$ cells, which leads to slower engraftment.[159]

*See references 4, 18, 27, 60, 74, 76, 89, 136, 152, 163.

Infectious complications occurring after HSCT are somewhat predictable based on the underlying primary disease and the acquired immunodeficiencies that occur after transplantation.[96,134,142] Patients undergoing HSCT are at increased risk for infectious complications secondary to the acquired immunodeficiencies that occur after the procedure or secondary to medications (e.g., cyclosporine, steroids) used to treat complications of transplantation.[4] Infections are a major cause of morbidity and mortality and represent the most significant barrier to both immediate and long-term survival after HSCT.

Recovery of immune function after undergoing HSCT is a gradual process that takes place over the course of several months to years. Immune reconstitution after HSCT follows the normal pattern of immune ontogeny, with development from immature to mature immune functions. The general early immune mechanisms, such as phagocytic and cytotoxic functions, recover first, usually by post-transplant day 100, but the specialized functions of T and B lymphocytes may remain impaired for a year or even longer.[36,121]

These immune defects and the gradual recovery of host defenses cause infections to occur in three predictable time periods, as shown in Table 80–1. The first or pre-engraftment period begins with onset of the conditioning regimen and continues until engraftment, about 30 days after transplantation. This phase is characterized by profound neutropenia that lasts 3 to 4 weeks. Treatment with colony-stimulating factors can shorten the duration of this phase.[85,143] The middle or early engraftment period starts after resolution of the neutropenia with transplant engraftment and lasts until approximately 100 days after HSCT. The last or late engraftment period starts about 100 days after transplantation and is associated with selected deficits in both cellular and humoral immune responses and reticuloendothelial function.[134]

EPIDEMIOLOGY

Several risk factors place HSCT recipients at increased risk for acquiring infectious complications; however, there are significant differences in the relative risk for infection after HSCT, depending on the type of transplantation. The degree of immunosuppression is less with autologous HSCT because no risk for

TABLE 80–1 Temporal Association of Infections and Predominant Etiologic Agents after Hematopoietic Stem Cell Transplantation

Phase	Predominant Host Defect	Bacterial Infections	Fungal Infections	Viral Infections
Pre-engraftment (0-30 days)	Neutropenia	*Staphylococcus aureus* *Staphylococcus epidermidis* Viridans streptococci *Pseudomonas aeruginosa* Enterobacteriaceae *Escherichia coli* *Klebsiella pneumoniae* *Enterobacter* species	*Candida* species *Aspergillus* species	Herpes simplex virus
Early engraftment (31-100 days)	Cytotoxic and phagocytic functions	*S. aureus* *S. epidermidis* Viridans streptococci *P. aeruginosa* Enterobacteriaceae	*Candida* species *Aspergillus* species *Pneumocystis jiroveci*	Cytomegalovirus Adenovirus Respiratory viruses
Late engraftment (>100 days)	Cellular and humoral immunity	*Haemophilus influenzae* *Streptococcus pneumoniae*	*Candida* species	Varicella-zoster virus

TABLE 80–2 Diagnostic Evaluation of Patients Undergoing Hematopoietic Stem Cell Transplantation

Complete blood count with differential
Serology for Epstein-Barr virus, cytomegalovirus, human immunodeficiency virus, hepatitis B virus surface antigen, hepatitis C virus, rapid plasma reagin, and toxoplasmosis
Liver function tests
Renal function tests
Stool for ova and parasites
Skin testing with Mantoux purified protein derivative
Posteroanterior and lateral chest radiographs

GVHD exists. Preventive therapy against GVHD, which can include cyclosporine, methotrexate, steroids, and purging of T cells from the graft, increases the risk for development of infection. All these agents enhance the infection rate by depressing the cell-mediated immune response and, in the case of methotrexate, by disrupting mucosal barriers. The presence of GVHD increases the infection rate because it is associated with a delay in the return of normal immune function, prolonged immunodeficiency, and ulceration of the gastrointestinal tract.[4,49] The conditioning regimens for most allogeneic HSCT patients include concomitant total-body irradiation, which compromises the immune system and can cause severe mucositis. Diarrhea also is a very frequent complication in the first week after undergoing irradiation.

The serologic status of the donor and recipient is important as well because many infections in transplant recipients are caused by reactivation of previous infections. Serologic evaluation for cytomegalovirus (CMV), Epstein-Barr virus (EBV), hepatitis B virus, hepatitis C virus, human immunodeficiency virus, and toxoplasmosis should be performed on both donor and recipient before transplantation (Table 80–2).[96,147]

All HSCT recipients have a central venous catheter in place for administration of blood products, nutritional supplements, and medications, thus adding a potential site for development of infection.[115,123] The presence of other indwelling medical devices, such as a Foley catheter or a cerebrospinal fluid shunt, is associated with increased risk for the development of infection.

Knowledge of the epidemiology of the hospital and the transplantation unit allows the risk for acquiring environmental organisms to be assessed. Infection rates can be reduced by preventive strategies that inhibit aerosolization of organisms, such as the use of laminar air flow rooms or high-efficiency particulate air (HEPA)-filtered rooms.

CLINICAL MANIFESTATIONS AND APPROACH

The clinical approach to infections in a patient after HSCT is based on understanding the type of transplantation and the natural history of infections that can occur during each of the different at-risk periods based on immune reconstitution after transplantation. Such knowledge provides the framework for matching possible etiologic agents with the clinical syndromes noted during evaluation.[38,134]

INFECTIONS DURING THE PRE-ENGRAFTMENT PHASE

The pre-engraftment phase begins with the conditioning regimen, usually 7 to 10 days before the time of stem cell infusion, and continues to stem cell engraftment, usually 3 to 4 weeks after transplantation. Profound neutropenia is the major immune defect present during this stage of transplantation.[125,129,134] Evaluation of patients in this phase is similar to that of patients with neutropenia after chemotherapy.

Bacterial Infections

During the neutropenic period, bacterial infections are the most frequent infectious complication in HSCT recipients. These patients are at high risk for acquiring bacterial infection, similar to patients with cancer in whom chemotherapy-induced neutropenia develops.[25] Most infections result from invasion and colonization of the oral mucosa, gastrointestinal tract, or skin by the patient's endogenous flora.[109]

Catheter-related infections are common occurrences, and the spectrum of etiologic agents is similar to that in other chemotherapy-induced neutropenic patients. Among gram-positive organisms, *Staphylococcus aureus* and *Staphylococcus epidermidis* are isolated most frequently, with other gram-positive bacteria such as *Stomatococcus mucilaginosus* also being implicated.[70] Infection with *S. epidermidis* and other coagulase-negative staphylococci continues to occur as long as the central venous catheter remains in place.[32,42,115,123] Infection with viridans streptococci is associated with mucositis after chemotherapy and antibiotic prophylaxis with quinolones, and in some series these bacteria have replaced *Staphylococcus* spp. as the most common cause of bacteremia.[10,25,132,145,155]

Gram-negative bacterial infections develop after mucosal damage occurs as a result of translocation of bacteria from the intestinal lumen into the bloodstream. The organisms most frequently involved from this group are *Pseudomonas aeruginosa* and

the Enterobacteriaceae, including *Escherichia coli*, *Klebsiella* spp., and *Enterobacter* spp., although other gram-negative organisms have been identified. *Enterobacter* spp. are especially worrisome because they display a high rate of an inducible β-lactamase, which limits the effect of front-line drugs such as the cephalosporins.

Fungal Infections

Fungal infections, predominantly with *Candida* spp., occur frequently in this phase.[83] *Aspergillus* spp. represent the second most common fungal infection during this time.[77] Less frequently, infections occur with the agents of mucormycosis (*Mucor*, *Rhizopus*, and *Absidia* spp.), *Trichosporon* spp., *Fusarium* spp., and other saprophytic fungi.[100,157] Fungal infections generally occur after a period of antibiotic therapy and correlate with the degree and duration of neutropenia.[59,106,107,158]

Candida spp. colonizing the gastrointestinal tract disseminate after mucosal injury, usually between 2 and 4 weeks after transplantation. Seeding of the portal circulation leads to hepatosplenic candidiasis manifested as fever and hepatosplenomegaly. Ultrasound or computed tomography reveals multiple round defects in the liver and spleen ("bull's eye" lesions). Candidal infections also can involve the lungs, kidneys, and central nervous system, where they lead to meningitis or brain abscess.[83] Although *Candida albicans* is the most frequent *Candida* spp. causing candidemia, *Candida tropicalis* may be more aggressive.[59,158] Other *Candida* spp., including *Candida krusei*, have emerged as pathogens because of their resistance to the antifungal agent fluconazole, which is used as prophylaxis.[75,167]

Aspergillus spp., *Fusarium* spp., and the agents of mucormycosis use the respiratory tract as their portal of entry and generally are associated with sinopulmonary disease and dissemination to other sites, including the central nervous system.[1,15,37,52,106,107,113,176]

Viral Infections

Herpes simplex virus (HSV) infection usually is the result of reactivation in seropositive patients undergoing HSCT and is one of the most common viral infections in patients after HSCT.[97,164] Reactivation generally occurs in the form of oral and genital lesions. Oral lesions can be difficult to diagnose because lip involvement rarely occurs and the mucosal ulcers are similar to the mucositis caused by the conditioning regimen. HSV reactivation can be complicated by esophagitis, pneumonitis, and bacterial superinfection of skin lesions. Mortality is a rare event, and rapid diagnosis allows timely administration of therapy. The use of acyclovir prophylaxis in HSV-seropositive patients has decreased the incidence of this complication.[47]

Diarrhea after transplantation is related mostly to noninfectious causes, especially total-body irradiation, GVHD, and chemotherapy-related toxicity.[29,118] Viral gastroenteritis can occur throughout the transplant period. Etiologic agents to consider are rotavirus, coxsackievirus, and enteric adenovirus.[6,9,22,29,50,56,64,78] Generally, the enteric viruses are seasonal in their appearance. The course of these enteric infections can be very prolonged, especially with the adenoviruses. In approximately 5 percent of HSCT recipients, adenovirus infection develops and can become latent in lymphoid and renal tissue.[173] A recent study in adult transplant patients has shown that human metapneumovirus can occur during the pre-engraftment phase and cause upper and lower respiratory disease with high mortality rates.[44]

Hemorrhagic cystitis can occur throughout the transplantation period. This condition has several infectious and noninfectious causes (Table 80–3).[133] Chemotherapy-induced cystitis (e.g., high-dose cyclophosphamide) occurs soon after the conditioning regimen. Later in the transplantation period, GVHD can be a contributing cause. The most common infectious causes of hemorrhagic cystitis are adenovirus and the polyomaviruses BK or JC.[2,6,24,48,73] Adenoviral infections can be systemic with associated pneumonitis, hepatitis, and renal insufficiency.[64] Allogeneic transplantation and the use of total-body irradiation are risk factors for acquisition of adenovirus infection.[64] BK viremia has been shown to correlate with hemorrhagic cystitis by polymerase chain reaction (PCR) analysis.[48] Of note, shedding of polyomaviruses in urine may occur in as many as 44 percent of HSCT patients without any clinical symptoms. HSV and CMV also have been associated with hemorrhagic cystitis.

TABLE 80–3 Differential Diagnosis of Hemorrhagic Cystitis after Hematopoietic Stem Cell Transplantation

Infectious Causes
Bacterial
Mainly gram-negative enteric rods associated with urinary tract infection
Fungal
Urinary tract infection
Fungus ball
Viral
Adenovirus
Polyomaviruses BK and JC
Herpesviruses: cytomegalovirus, herpes simplex virus
Noninfectious Causes
Chemotherapy-induced (e.g., cyclophosphamide)
Graft-versus-host disease
Mechanical trauma from a Foley catheter

INFECTIONS DURING THE EARLY ENGRAFTMENT PHASE

The risk for infection decreases after stem cell engraftment occurs. However, severe abnormalities in host defense still exist. The general early immune functions, such as phagocytic and cytotoxic cells, recover first, followed by the cellular and humoral arms of the immune system.[121,134] Another factor that influences immunologic recovery is the presence of acute GVHD.

Bacterial Infections

Bacterial infections continue to occur in this phase as a complication of indwelling central venous catheters or as a complication of acute GVHD and are accompanied by skin and gastrointestinal tract breakdown. The same organisms that cause illness during the neutropenic phase can produce illness in this phase.[10,42]

Fungal Infections

Fungal infections still occur during this phase, with *Candida* and *Aspergillus* spp. being the prominent pathogens. Systemic candidal infections usually occur earlier than aspergillosis.[37,59,158] Fungal pathogens other than *C. albicans*, *Aspergillus fumigatus*, and *Aspergillus flavus* are increasing in prevalence.[125]

Development of brain abscess after HSCT usually occurs during this phase and, in contrast to immunocompetent hosts, is caused mainly by fungal pathogens. The most common etiologic agents of brain abscess in transplant recipients are *Aspergillus* and *Candida* spp. Despite initiation of aggressive antifungal and surgical therapy, the outcome of this complication after HSCT is very poor.[1,63]

In the past, *Pneumocystis jiroveci* pneumonia occurred in 5 to 10 percent of allogeneic HSCT recipients during this phase. However, the routine use of trimethoprim-sulfamethoxazole

prophylaxis has decreased its incidence significantly. Patients with *P. jiroveci* pneumonia usually have hypoxemia, dyspnea, cough, fever, and bilateral infiltrates.[154] The treatment of choice is trimethoprim-sulfamethoxazole.

Viral Infections

In the past, CMV infections predominated during this phase, but their incidence has decreased since the routine use of prophylactic ganciclovir was initiated. CMV infection generally occurs between 40 and 50 days after the transplantation. Most commonly, it results from reactivation in seropositive patients, but it also can occur in seronegative patients who receive their stem cells from a seropositive donor. The immunomodulatory effects of CMV infection, especially in a CMV-seronegative recipient of a CMV-seropositive donor, place the patient at high risk for developing bacterial and fungal infection.[112] The clinical manifestations of CMV infection vary from asymptomatic infection to the constellation of fever, hepatitis, and leukopenia to life-threatening diseases such as esophagitis, interstitial pneumonitis, and encephalitis.[46,175]

Pneumonia continues to be a common problem after HSCT, and the outcome still is poor.[40] Pulmonary infiltrates in an HSCT recipient must be distinguished from infectious processes or noninfectious pulmonary complications after HSCT (Table 80–4). Diffuse alveolar hemorrhage is manifested as gradually worsening dyspnea and repeated bloody aspirates from bronchoscopic examination.[69] Idiopathic interstitial pneumonitis is a process of widespread alveolar damage characterized clinically by varying degrees of respiratory failure and diffuse interstitial infiltrates in the absence of infection.[91] Idiopathic interstitial pneumonitis occurs in two peaks, one in the first few weeks and the other near the end of the early engraftment period. It is thought to be related to the chemotherapy and total-body irradiation used in the conditioning regimen and has a high mortality rate.

Viral respiratory infections may occur throughout the transplantation period but are more severe during the early engraftment period. Etiologic agents include respiratory syncytial virus, human parainfluenza viruses 1 to 4, influenza viruses A and B, adenovirus, rhinoviruses, and nonpolio enteroviruses.[64,66,165,166] Distinctive features of respiratory viral infections in immunocompromised hosts include a high frequency of nosocomial acquisition, prolonged persistence of infection, a higher rate of progression to pneumonia, and high mortality rate in association with the infection.[95]

Respiratory viral infections occur throughout the year in HSCT recipients, although seasonal variations in the type of respiratory viral infection are similar to those seen in the general pediatric population, with respiratory syncytial virus and influenza viruses predominating during the winter and human parainfluenza viruses during the spring and summer.[95]

The differential diagnosis of jaundice and increase in liver enzymes after HSCT is very broad (Table 80–5) and presents a challenge in distinguishing infectious from noninfectious causes of hepatic disease. Two major noninfectious causes of liver disease after HSCT are GVHD of the liver and hepatic veno-occlusive disease.[49,104] The latter is a complication related to the conditioning regimen and is characterized by weight gain, hepatomegaly, and direct hyperbilirubinemia without elevation of liver enzymes.[104] The differential diagnosis of infectious causes includes viral hepatitis with any of the hepatitis viruses from A through C, as well as other hepatotropic viruses such as CMV, EBV, adenovirus, HSV, and varicella-zoster virus (VZV).[51,64,80,84,147]

EBV can reactivate after HSCT and cause EBV-associated lymphoproliferative disease. This disease is manifested as fever, hepatosplenomegaly, and lymphadenopathy and can progress to lymphoma. These EBV lymphomas are of donor origin and can occur for as long as 6 months after HSCT.[94]

Protozoan Infections

Toxoplasmosis is an infrequent, but almost always fatal, infection after HSCT. It usually occurs 2 to 6 months after transplantation and in most cases is the result of reactivation of a previous infection. The brain is the organ most frequently affected. Cerebral toxoplasmosis is manifested as neurologic focal signs, fever, seizures, headache, and altered mental status. Imaging of the central nervous system typically shows multiple lesions in both hemi-

TABLE 80–4 Differential Diagnosis of Pneumonitis after Hematopoietic Stem Cell Transplantation

Infectious Causes
Bacterial
Enterobacteriaceae
Legionella pneumophila
Staphylococcus aureus
Chlamydia trachomatis
Mycoplasma hominis
Fungal
Aspergillus spp.
Candida spp.
Pneumocystis jiroveci
Mucormycosis
Viral
Cytomegalovirus
Human parainfluenza viruses 1 to 4
Respiratory syncytial virus
Influenza viruses A and B
Adenovirus
Rhinovirus
Nonpolio enteroviruses
Human herpesvirus 6
Noninfectious Causes
Radiation
Chemotherapy (e.g., bleomycin)
Bronchiolitis obliterans organizing pneumonia
Underlying malignancy
Pulmonary edema
Diffuse alveolar hemorrhage
Idiopathic interstitial pneumonitis
Pulmonary vascular disease

See references 6, 9, 52, 54, 64, 89, 93, 158, 159, 160.

TABLE 80–5 Differential Diagnosis of Hepatitis after Hematopoietic Stem Cell Transplantation

Infectious Causes
Bacterial
Cholestatic liver injury secondary to septicemia
Viral
Hepatitis viruses A to D
Herpes simplex virus
Epstein-Barr virus
Cytomegalovirus
Adenovirus
Varicella-zoster virus
Echovirus
Human herpesvirus 6
Noninfectious Causes
Acute graft-versus-host disease
Veno-occlusive disease secondary to sepsis chemotherapy (e.g., cyclosporine)
Drug-induced (e.g., acetaminophen)
Parenteral hyperalimentation

spheres and basal ganglia with peripheral enhancement after infusion of contrast medium.[102,103,141]

Dermatologic Manifestations

Rash is a common symptom after HSCT and can occur in any phase after transplantation.[99] The differential diagnosis is broad and includes both infectious and noninfectious causes (Table 80–6). Noninfectious causes include acute GVHD, chemotherapy-induced toxicity, and drug eruptions, most commonly caused by the β-lactam antibiotics used for empiric treatment of febrile neutropenia. Because the etiology of these lesions generally is indistinguishable on a clinical basis, skin biopsy is helpful in determining the diagnosis.

Infectious skin disorders after HSCT may be secondary to bacterial, fungal, or viral causes (see Table 80–6). Embolic lesions can be a manifestation of systemic bacterial or fungal infection, especially with *C. tropicalis*, *Aspergillus* spp., and *Fusarium* spp.[15,137] Lesions are tender and usually papular but can be purpuric, nodular, or necrotic and scattered on the trunk and extremities.

Primary infection with human herpesvirus type 6 after HSCT generally is associated with self-limited clinical symptoms, including a diffuse maculopapular rash. Asymptomatic reactivation with human herpesvirus type 6 appears to be a common occurrence after allogeneic HSCT. Human herpesvirus type 6 might have a possible role in pneumonitis, meningoencephalitis, and bone marrow dysfunction after HSCT.[23,26,81] Human parvovirus B19 is another cause of erythroderma after HSCT, as well as a rare cause of anemia.[138]

INFECTIONS DURING THE LATE ENGRAFTMENT PHASE

The late engraftment period is characterized by decreased risk for the development of infection, especially in autologous transplant recipients because they have more rapid recovery of immune function. During this phase, the central venous catheter usually is removed and immunosuppressive therapy to prevent GVHD is discontinued. Most recipients are outpatients at this point, and educating recipients and their families to avoid environmental exposure to opportunistic pathogens is very important.

Serious infections still can occur, particularly in patients with chronic GVHD or inadequate stem cell engraftment. Chronic GVHD predisposes patients to infection by delaying recovery of the immune system and its effects on target organs; in addition, the steroid therapy that patients receive likewise predisposes them to infection. Moreover, the cellular and humoral arms of the immune system are not recovered fully at this stage, thus placing the patient at risk for the development of infection. Most defects in immune function resolve by 1 year after transplantation.[114,131]

TABLE 80–6 Differential Diagnosis of Rash after Hematopoietic Stem Cell Transplantation

Infectious Causes
Bacterial
Embolic lesions of systemic gram-negative bacteremia
Cellulitis of central venous catheter exit site or tunnel infections
Fungal
Focal dermatitis (*Candida* species and superficial dermatophytes)
Embolic lesions of systemic fungal disease (*Aspergillus* and *Fusarium* species)
Viral
Varicella-zoster virus
Herpes simplex virus
Human herpesvirus type 6
Human parvovirus B19
Noninfectious Causes
Graft-versus-host disease
Chemotherapy
Drug-induced (e.g., β-lactam antibiotics)
Radiation for conditioning therapy

Bacterial Infections

Encapsulated bacteria, including *Haemophilus influenzae* type b, *Neisseria* spp., and *Streptococcus pneumoniae*, are the predominant causes of bacterial infection not related to catheters during this phase. These infections are secondary to deficient opsonization activity and decreased function of the reticuloendothelial system.[114]

Viral Infections

The predominant viral infection contracted during this period is caused by VZV, which occurs in 25 to 40 percent of pediatric HSCT recipients. Most infections represent reactivation, and risk factors include both acute and chronic GVHD, as well as allogeneic transplantation. VZV infection usually is characterized by localized vesicles in a dermatomal distribution. Disseminated infection occurs more frequently with VZV than with HSV, especially in patients with GVHD.[65,88,97]

DIAGNOSIS AND LABORATORY FINDINGS

The most important diagnostic evaluation is a complete physical examination because it can identify a site of infection that may be missed by laboratory tests. Sites requiring special attention include the skin, the oral mucosa to look for ulcers and thrush, the nares to look for necrotic lesions suggestive of invasive mold infection, the sinuses to look for tenderness, the chest, the abdomen, and the perianal area. All suspicious lesions should be cultured. Signs of infection may be subtle in patients who are neutropenic because inflammation is minimal.

The diagnostic approach should be guided by knowledge of which organisms are frequent pathogens during the specific phase after the transplantation (see Table 80–1). Except for microbiologic evaluations, laboratory tests are of limited value. Blood cultures for bacteria, fungi, and viruses should be obtained for any febrile episode. Use of the Wampole isolator blood culture system (Wampole Laboratories, Cranbury, NJ) may increase the yield of fungal pathogens and can help in establishing the diagnosis of central line infections by the use of quantitative cultures. Urine cultures are indicated in the presence of hemorrhagic cystitis or if the patient has a Foley catheter in place. Evaluation of cerebrospinal fluid should be reserved for patients with neurologic signs and symptoms.

Diagnosis of mold infections depends on tissue histology and culture of samples obtained by bronchoscopy, bronchoalveolar lavage, paranasal sinus washings, lung biopsy, or skin biopsy because these organisms are not recovered routinely from blood cultures. Recent improvements in antigen-based tests, including galactomannan and 1-3-β-D-glucan, allow establishing the diagnosis and noninvasive monitoring of antifungal therapy, although the false-positive rate appears to be higher in infected children than in adults.[144,149,174] Fungal surveillance cultures are not indicated for asymptomatic HSCT patients.[128] Additionally, Gram stain, acid-fast stains, and other special stains can be useful in identifying microorganisms early in the infectious process.

Nasopharyngeal swabs or washes for viral culture and direct fluorescent antibody testing are helpful for identifying respiratory viruses in patients with upper respiratory tract symptoms. Shell vial assay is a rapid viral diagnostic technique used for a

variety of viruses, including CMV, adenovirus, and respiratory viruses. The base of vesicular skin lesions should be scraped and the cells examined by direct fluorescent antibody stain with specific monoclonal antibodies to confirm the diagnosis of either HSV or VZV infection.

Nucleic acid detection, such as with PCR, has shown usefulness in the rapid diagnosis of certain pathogens, including adenovirus, CMV, EBV, human herpesvirus type 6, and parvovirus B19.[12,23,26,87] Additionally, quantification of viruses has allowed the response to therapy to be evaluated. Blood assays to detect viral antigens such as pp65 also have been used for establishing the diagnosis of CMV infection.[11,13]

Serologic testing is useful for diagnosing toxoplasmosis, *Bartonella henselae*, and fungal infections such as histoplasmosis and blastomycosis. However, serologic assays have limited value because patients are immunosuppressed and immunoglobulin therapy is administered to most patients.

Other laboratory tests are of limited value. Complete blood counts are useful to determine engraftment status. Liver and renal function tests can provide evidence of disease from an infectious agent or can indicate noninfectious causes of hepatic or renal disease such as chemotherapy or GVHD.

Performance of radiology studies should be based on assessment of the patient. A chest roentgenogram should be part of the work-up of a febrile, neutropenic patient and any patient with respiratory symptoms. Computed tomography scans of the paranasal sinuses, chest, and abdomen are helpful in evaluating and monitoring patients with invasive fungal disease.[37] Ultrasound examination can be used to diagnose abdominal problems such as typhlitis. Neuroimaging studies should be reserved for patients with neurologic signs and symptoms.

MANAGEMENT AND THERAPY

The initial management of HSCT recipients with fever and neutropenia is similar to that of cancer patients with chemotherapy-induced fever and neutropenia.[79,124,129] A standardized antibiotic regimen should be developed to ensure adequate coverage of organisms identified by the hospital's environment and to allow the hospital's staff to compare outcomes of patients. Empiric antibiotic therapy directed against the predominant pathogens should be started after obtaining appropriate samples for culture.[79] One approach consists of an empiric antibiotic regimen of ceftazidime with or without vancomycin, a combination that provides adequate therapy for gram-positive cocci and gram-negative bacteria. If *P. aeruginosa* is suspected, an aminoglycoside should be added. Other approaches using cefepime or quinolones have shown equal efficacy.[16,17]

The choice of any specific antimicrobial therapy should take into account the transplantation center's spectrum of organisms, the patient's surveillance isolates, and the antibiotic susceptibility pattern within the community and hospital. If fever persists after 3 or more days, re-evaluation of the patient may lead to modification of the antibiotic regimen.[79]

THERAPY FOR FUNGAL INFECTIONS

Empiric amphotericin B is recommended for the treatment of occult fungal disease in patients who remain persistently febrile after 5 to 7 days of antibiotic therapy, without identification of a bacterial cause.[79] The introduction of expanded-spectrum triazoles and cell wall–active agents such as echinocandins has changed the antifungal armamentarium.

Amphotericin B has been the traditional drug of choice for *Candida* infection, but recent data support the use of liposomal amphotericin B and itraconazole, which have similar clinical efficacy with reduced toxicity.[119,146,160,172] Voriconazole has become the agent of choice for the treatment of invasive aspergillosis in immunocompromised hosts.[33,71] Posaconazole, a triazole, will probably be the best option for the treatment of Zygomycetes. Caspofungin, an echinocandin active against *Candida* and *Aspergillus*, is available only in an intravenous formulation and probably is relegated to a second-line agent.[161] However, interaction with other drugs may impair its use in HSCT recipients. Combination therapy may be potentially antagonistic, but the efficacy of any of these combinations has not been established.[105] Fluconazole has activity against most *Candida* spp. and exhibits good penetration of the central nervous system.[40]

Surgery plays a prominent role in the treatment of mold infections, especially in neutropenic patients. However, the outcome of invasive mold infections in HSCT patients remains dismal.

THERAPY FOR VIRAL INFECTIONS

Treatment of viral infections is started once the diagnosis is established, if therapy is available. Acyclovir is the recommended therapy for both HSV and VZV infections. Famciclovir and valacyclovir also can be used to treat HSV infections, but they are available only for oral administration. CMV pneumonitis is treated with a combination of intravenous gamma-globulin and ganciclovir. In the case of acyclovir-resistant HSV or ganciclovir-resistant CMV infection, foscarnet is the drug of choice.[111] Cidofovir also has been used for CMV infection.[93]

Respiratory syncytial virus infections have been treated with aerosolized ribavirin and more recently with respiratory syncytial virus immunoglobulin.[34,35,45] Aerosolized ribavirin also has been used for the treatment of human parainfluenza virus infection.[41,45]

Intravenous ribavirin has been used for the treatment of adenovirus hemorrhagic cystitis, with anecdotal success.[20,53] More recently, cidofovir treatment of adenoviral infections has been associated with clinical improvement, as well as viral clearance in cultures and PCR analysis.[14,87,110,173]

The recommended therapy for infection with influenza A virus includes oral amantadine or rimantadine. Their efficacy, particularly in the treatment of severe disease, has not been established in immunocompromised hosts. Both drugs also are useful as prophylaxis for influenza A virus infection.[68] Zanamivir and oseltamivir, both neuraminidase inhibitors, appear promising for the treatment of both influenza A and B virus infection, but data are lacking regarding their use in pediatric immunocompromised patients.[68]

Adoptive immunotherapy by transferring virus-specific cytotoxic T lymphocytes to patients who have undergone allogeneic HSCT has proved to be useful in treating CMV and EBV infection and in reconstituting immune dysfunction selectively against these viruses.[72,130,162]

PREVENTION OF INFECTIONS AFTER TRANSPLANTATION

Measures to prevent infectious complications are a high priority for all HSCT recipients. Regular hand hygiene remains the best strategy for prevention of infections in HSCT recipients. Every HSCT recipient should undergo a dental evaluation before conditioning to assess oral health and any needed dental procedures should be performed to decrease the risk for the development of oral complications and infections after transplantation.[31,132] Strong effort should be made to enforce published guidelines for preventing indwelling catheter–related infections.[115,123]

The Centers for Disease Control and Prevention recently published extensive guidelines for the prevention of opportunistic infections in HSCT recipients that go beyond the scope of this chapter.[21] A brief review of preventive strategies, including

targeted antimicrobial prophylaxis, prevention of exposure, enhancement of immune reconstitution with colony-stimulating factors, and active and passive immunization, is presented here.

ANTIMICROBIAL PROPHYLAXIS

Antimicrobial prophylaxis must be weighed against the toxicity and the drug interactions of the prophylactic agent. Prophylaxis against *P. jiroveci* infection with trimethoprim-sulfamethoxazole is started after engraftment and is taken orally three times a week for 6 months after transplantation. For patients with intolerance to sulfa-containing medications, other options for prophylaxis include atovaquone, dapsone, or inhaled pentamidine.[90,98] Quinolone prophylaxis has been the major antibiotic class used to prevent bacterial infections, but breakthrough infections are problematic.[108]

Administration of fluconazole or itraconazole for 75 to 100 days after HSCT is associated with persistent protection against disseminated candidal infections and candidiasis-related death; in a randomized, placebo-controlled trial, these drugs resulted in an overall survival benefit in allogeneic HSCT recipients.[101,169] However, emergence of resistant pathogens such as *C. krusei* and some strains of *C. albicans* has complicated the routine use of fluconazole.[18] Low-dose amphotericin B is similar in efficacy to fluconazole in preventing fungal infections, but the latter is tolerated better.[171] The use of aerosolized amphotericin B to prevent *Aspergillus* infection needs further study.[74,140] In some centers voriconazole is used for prophylaxis.

Antiviral prophylaxis is indicated to prevent infections with herpesviruses during high-risk periods. Acyclovir has been used to prevent reactivation of HSV in seropositive transplant recipients during the neutropenic phase after transplantation. This strategy can reduce the incidence of reactivation of HSV in seropositive recipients from 80 to 5 percent during the first month after transplantation.

Strategies used to prevent CMV infection include prophylactic and preemptive therapy. Prophylactic therapy is administered to all recipients at risk for acquiring CMV infection and is used routinely for allogeneic transplant recipients when the donor is CMV-seropositive.[57,139] Ganciclovir is the agent of choice for prophylaxis of CMV infection.[57,58] Ganciclovir usually is given for 3 to 4 months after engraftment; however, its use has been associated with neutropenia.[57,135] Preemptive therapy is administered only to recipients who have evidence of CMV replication based on plasma PCR or pp65 antigenemia.[11,13] Primary CMV infection also can be prevented in CMV-seronegative HSCT recipients by transfusing blood products that are leukocyte filtered and seronegative for CMV.[148]

ISOLATION MEASURES

The use of laminar air flow protection for HSCT patients is controversial, and, although some studies have proved its effectiveness, it is very expensive.[39] HEPA filtration is associated with similar efficacy as laminar air flow in preventing airborne pathogens such as *Aspergillus* spp. Major indications for strict isolation of patients include colonization with resistant bacteria, viral respiratory infections, and disseminated VZV infection.[13,126]

COLONY-STIMULATING FACTORS

The use of granulocyte and granulocyte-macrophage colony-stimulating factors in stem cell transplant recipients is safe and effective in reducing the period of neutropenia after HSCT.[85,143,153] A meta-analysis of children receiving chemotherapy has shown a significant reduction in febrile neutropenic episodes, documented infections, and length of hospitalizations.[151] However, there has been a report of an increase in myeloid leukemia or myelodysplastic syndrome in patients with acute lymphocytic leukemia receiving granulocyte colony-stimulating factor.[127] These growth factors also appear to be safe and effective in transplant recipients who do not show engraftment 3 to 4 weeks after transplantation.

ACTIVE IMMUNIZATION

Immunizations should be part of the routine follow-up care of HSCT recipients. Most allogeneic and a large proportion of autologous and syngeneic transplant recipients lose their immunity to vaccine-preventable diseases. The degree that immunity is lost depends on multiple factors, including the donor's immunity, the type of transplant, the interval since the transplant, the presence of GVHD, and the use of immunosuppressive medication.[3] Moreover, transplant recipients are at increased risk for acquiring infections with encapsulated bacteria, such as *H. influenzae* type b and *S. pneumoniae*, for which vaccines are available.[7,55] For all these reasons, reimmunization of HSCT recipients between the first and second year after transplantation is very important.[92,142] When immunizing transplant recipients, recent administration of immunoglobulin must be kept in mind because it may interfere with the response to vaccinations.

An optimal schedule for administering vaccinations to pediatric patients after HSCT has not been established. Some experts will reimmunize HSCT recipients without performing serologic titers for specific antigens, such as diphtheria and tetanus, whereas others obtain titers 1 year after transplantation and provide vaccinations accordingly.[170] A schedule for immunization of HSCT recipients based on the recommendations of the Centers for Disease Control and Prevention Advisory Committee on Immunization Practices and the Infectious Diseases Committee of the American Academy of Pediatrics is presented in Table 80–7.[3,21] Specific recommendations probably will change as further data become available.

Immunizations can be started 12 months after HSCT as long as the patient has no persistent complications such as chronic GVHD and is not receiving immunosuppressive therapy. If the patient is younger than 7 years old, diphtheria and tetanus toxoids (DT) or diphtheria and tetanus toxoids with acellular pertussis vaccine (DTaP) can be used for diphtheria and tetanus.[120,156] For patients older than 7 years of age, immunization with DT at 12, 14, and 24 months after transplantation elicits an immune response.

H. influenzae type b vaccine is included in the first round of immunizations 12 months after HSCT. Boosters of these vaccines are given 14 and 24 months after transplantation.[7,62,122,156]

TABLE 80–7 Immunizations in Patients after Hematopoietic Stem Cell Transplantation

Recommended Time after HSCT	Immunizations
12 mo	Td,* IPV, Hib, pneumococcal, meningococcal hepatitis B
14 mo	Td, IPV, Hib, pneumococcal, hepatitis B
24 mo	Measles-mumps-rubella,† varicella, Td, IPV, Hib pneumococcal and hepatitis B

**DTaP or DT if the patient is younger than 7 years; Tdap if >11 years of age and indicated.*

†Do not use live virus vaccines for patients who have active chronic graft-versus-host disease or who are receiving immunosuppressive therapy.

Td and DT, diphtheria and tetanus toxoids; DTaP, diphtheria and tetanus toxoids and acellular pertussis vaccine; Hib, Haemophilus influenzae type b vaccine; IPV, inactivated polio vaccine.

Immunization with the 23-valent pneumococcal vaccine is recommended 12 and 24 months after transplantation.[3,5,21] The second dose is not a booster dose but provides a second opportunity for immunization in patients who failed to respond to the first dose.[3]

The measles-mumps-rubella vaccine (MMR) should be given 24 months after transplantation to both autologous and allogeneic HSCT patients. It should not be given to patients with chronic GVHD or to those receiving steroids or any other form of immunosuppressive therapy.[82] The live attenuated varicella vaccine should be avoided in patients less than 24 months after HSCT.[3] Of note, a report using an inactivated varicella vaccine has shown efficacy in autologous transplant recipients.[67]

Only the inactivated polio vaccine should be given to HSCT patients. This vaccine should be administered 12, 14, and 24 months after transplantation.

The inactivated influenza vaccine (subvirion or purified surface antigen vaccines) should be given 6 months after HSCT and yearly thereafter in early autumn. Children younger than 9 years old who are receiving influenza vaccine for the first time require two doses given 1 month apart. Children younger than 12 years old should receive only split-virus influenza vaccine.[3,21,43,68] Live attenuated influenza vaccine should not be given.

Vaccination for hepatitis A is not recommended routinely but may be considered 12 months or more after HSCT for patients with chronic liver disease or chronic GVHD, for those living in areas endemic for hepatitis A, and for those in areas experiencing outbreaks. Hepatitis A immunization is given in two doses 6 to 12 months apart.[3,21]

Use of hepatitis B vaccine is unclear, but administration 12, 14, and 24 months after HSCT is reasonable with serologic follow-up.

Health care workers and household contacts of HSCT recipients should have immunity to or be immunized against hepatitis A, influenza, polio, measles, mumps, rubella, and varicella.[3,21,142]

PASSIVE IMMUNIZATION

Intravenous immunoglobulin commonly has been administered in the first months after allogeneic HSCT to prevent infections and acute GVHD and is approved by the Food and Drug Administration.[8,19,116] The use of intravenous immunoglobulin in conjunction with ganciclovir after transplantation has been shown to decrease the incidence of CMV pneumonia, as well as bacterial infections and interstitial pneumonia.[150,168] The optimal dose is not known. However, two meta-analyses have drawn divergent conclusions regarding the efficacy of intravenous immunoglobulin.[8,28,61]

The indications for passive immunization with specific immunoglobulin preparations, such as hepatitis B, rabies, and tetanus vaccines, in transplant recipients are similar to those in otherwise healthy individuals.[3,21] Patients with tetanus-prone wounds sustained during the first year after transplantation should be given tetanus immunoglobulin, regardless of their tetanus immunization status.[3] Passive immunization with varicella-zoster immunoglobulin is recommended for susceptible patients with known exposure to varicella.[3]

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CHAPTER 81

Infections in Pediatric Heart Transplantation

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Each year in the United States, more than 350 children undergo heart transplantation,[66] and infection is an important cause of morbidity and some mortality in these patients. The Registry of the International Society for Heart and Lung Transplantation found that for children who underwent heart transplantation between 1992 and 2004, non-cytomegalovirus (CMV) infection was the cause of mortality in 14 percent of patients in the first 30 days after receiving transplants, peaked at 16.4 percent for 30 days to 1 year after transplantation, and thereafter accounted for 4.3 to 8.6 percent of deaths until more than 5 years after transplantation.[17] The Pediatric Heart Transplant Study Group prospectively collected data from 22 pediatric centers in the United States from January 1993 to December 1994 on 332 children younger than 18 years (mean age, 5.5 years) who had undergone heart transplantation.[123] One or more infections (276 total) occurred in 41 percent of the patients (mean follow-up time, 11.8 months) for an average of 0.84 infections per patient; 22 percent had one infection, 8 percent had two infections, and 11 percent had three or more infections during the study period (Table 81–1). In a similar multicenter study in adults who had undergone heart transplantation between January 1990 and June 1991, infec-

TABLE 81–1 Types of Infections Encountered in 332 Children after Heart Transplantation in a Multi-Institutional Study*

Type	No.
Bacterial (total)	**164**
Coagulase-negative staphylococci	25
Enterobacter species	21
Pseudomonas aeruginosa	16
Viral	
Cytomegalovirus	51
Varicella-zoster virus	11
Respiratory syncytial virus	10
Herpes simplex	6
Other viruses	8
Fungal	**19**
Candida species	12
Pneumocystis carinii	7

**276 infections in 136 patients.*
Data from Schowengerdt, K. O., Naftel, D., Seib, P. M., et al.: Infection after pediatric heart transplantation: Results of a multiinstitutional study. J. Heart Lung Transplant. 16:1207-1216, 1997.

tions developed in 31 percent of 814 patients, with 22 percent having one infection and 9 percent having two or more infections.[91] Bacterial infections were the most common type of infection occurring after transplantation in pediatric and adult patients.

Immunosuppressive therapy for children who have undergone heart transplantation usually consists of some combination of cyclosporine or tacrolimus, mycophenolate mofetil or azathioprine, and corticosteroids. Induction immunosuppression may include interleukin-2 receptor antibodies; OKT3 is now being used less frequently.[29,152] Cyclosporine and tacrolimus predominantly block the effect of interleukin-2 on T cells, an action resulting in a diminished T-cell response to mitogen stimulation.[53] The infections seen in heart transplant patients outside the postoperative period generally are a result of this block in T-cell function. Because the types of infections seen in these patients vary with the time elapsed since transplantation, this chapter is organized in such a manner.

PRETRANSPLANTATION EVALUATION

Several infectious agents may be transmitted to the patient via the transplanted organ or can become "reactivated" after transplantation. Determining the antibody status of the recipient and the donor against selected microorganisms (CMV, Epstein-Barr virus [EBV], *Toxoplasma gondii*) helps physicians anticipate or diagnose infections that develop after transplantation. A reasonable pretransplant evaluation for children is outlined in Table 81–2. The child's immunization status is documented, and vaccinations are completed when possible (i.e., hepatitis B vaccine or *Streptococcus pneumoniae*). The Committee on Infectious Diseases of the American Academy of Pediatrics recommends that for immunized children older than 12 months who are scheduled to undergo solid organ transplantation, serologic tests be performed for rubeola, mumps, rubella, and varicella to determine whether protective titers are present.[4] If possible, appropriate vaccines should be administered at least 1 month before the patient undergoes transplantation. Evidence of selected active infections is a contraindication for transplantation. Chemoprophylaxis should be considered strongly for children with a positive tuberculin skin test (purified protein derivative). Dental status also is assessed.

In addition to having the routine pretransplant evaluation as outlined in Table 81–2, each patient should be screened carefully for selected infections appropriate to the individual circumstances. If surgery is planned during the respiratory disease season and the patient has respiratory symptoms just before having surgery, screening for influenza virus or respiratory syncytial virus (RSV) by rapid techniques may facilitate prescribing antiviral therapy postoperatively. In areas where community-acquired methicillin-resistant *Staphylococcus aureus* (MRSA) is an important cause of infection, performing surface cultures to detect MRSA colonization may modify the choice of antibiotics used for surgical prophylaxis.[64] Applying mupirocin intranasally may influence the rate of postoperative MRSA infections.[98] Children from resource-poor countries may harbor *Salmonella* or intestinal parasites asymptomatically, and these organisms can cause serious infection after the child undergoes transplantation. Preoperative stool cultures for enteropathogens and examination of the stool for parasites may alert the clinician that these pathogens are present and could be the etiology of postoperative infections.

If the patient is being mechanically ventilated before undergoing transplantation, review of recent tracheal aspirate cultures may help in the selection of empiric antibiotics for initial treatment of suspected nosocomial sepsis or pneumonia. Pretransplant infections associated with procedures such as implantation of ventricular assist devices may require prolonged antibiotic therapy after transplantation is performed.[85,131] These infections typically are caused by common nosocomial pathogens and are not a contraindication to undergoing heart transplantation.

TABLE 81–2 Evaluation of Children before Heart Transplantation

Serology
Cytomegalovirus
Epstein-Barr virus
Toxoplasma gondii
Human immunodeficiency virus
Hepatitis A, B, and C
Rubeola, mumps, rubella, varicella*
Cultures
Nasopharyngeal, stool, or tracheal aspirates†
Skin tests
Purified protein derivative
Freezing of an extra aliquot of serum
Review of the child's immunization status

**For children who are >12 mo old and previously immunized.*
†See text for an explanation.

SURGICAL PROPHYLACTIC ANTIBIOTICS

Prophylactic antibiotics typically are administered to patients undergoing heart transplantation surgery. For each institution, selection of prophylactic antibiotics should be based partly on the organisms isolated from postoperative wound infections in that center and the antimicrobial susceptibility of these organisms. Cefazolin generally is a reasonable choice for prophylaxis, unless MRSA is a nosocomial pathogen of concern, in which case vancomycin is suggested.[20] In areas where MRSA is a common community pathogen, vancomycin or some other antibiotic active against MRSA might be considered instead of cefazolin, as determined by preoperative surveillance cultures. Routine use of extended-spectrum cephalosporins is discouraged because it may lead to colonization of the patient by antibiotic-resistant, gram-negative organisms that hyperproduce β-lactamase, such as *Enterobacter cloacae*. Recommendations for the duration of prophylactic antibiotic treatment in these patients are not definite, but some centers continue prophylactic antibiotics for 2 to 5 days or more postoperatively, or until all lines and chest tubes have been removed.

IMMEDIATE POSTOPERATIVE INFECTIONS

COMMON INFECTIONS

During the month after the patient undergoes heart transplantation, the types of infections encountered are the same as those complicating major thoracic surgery. Pneumonia and bacteremia are the most common postoperative infections. The frequency of bacteremia and the distribution of organisms are similar in children and adults after undergoing heart transplantation.[91,123] In a multi-institutional pediatric study, the risk of development of any infection was 25 percent 1 month after transplantation. Overall, 60 episodes of bacteremia occurred in the 136 patients who became infected, and the bloodstream was the most common site of bacterial infection.[123] Lung abscesses and mediastinitis are seen less frequently. Familiarity with the organisms and the antimicrobial susceptibility of isolates recovered from other children in the intensive care units (ICUs) in which these patients receive care helps direct the initial selection of empiric antibiotics.

Postoperative bacteremia is related predominantly to the indwelling lines required for monitoring and infusion of medica-

tion. *S. aureus*; coagulase-negative staphylococci; *Enterococcus* spp.; and gram-negative enteric organisms such as *Enterobacter* spp., *Pseudomonas aeruginosa*, *Klebsiella* spp., and *Escherichia coli* are the most common causes of nosocomial bacteremia in the pediatric ICU.[113] Other foci of infection, such as pneumonia or mediastinitis, also may result in bacteremia.[11,49] Vancomycin plus an aminoglycoside is a typical empiric antibiotic combination for suspected bacteremia in patients with central lines in place and without focal evidence of infection. Vancomycin therapy should be discontinued as soon as possible if an organism requiring the administration of vancomycin is not isolated.[110] A bacterial line infection may be eradicated successfully without removing the line, but the line should be removed if blood cultures remain positive or the patient's clinical condition deteriorates.[121] Fungemia, generally with *Candida albicans* or other *Candida* spp., also may be associated with line-related infections. Central lines complicated by fungemia should be removed immediately.[39] Centrally placed lines must be removed as soon as practical so that catheter-associated infections can be prevented.

As in other critically ill children, pneumonia is a particularly common occurrence in heart transplant patients because of the operative site and requirements for intubation and mechanical ventilation. During the first postoperative week, definite bacterial pneumonia developed in 3 of 22 children (14%) in an early study from Pittsburgh.[57] In the pediatric multi-institutional study, 56 bacterial lung infections were identified, 24 of which developed in patients maintained on a ventilator at the time of transplantation.[123] Nosocomial pneumonia caused by gram-negative bacilli such as *Pseudomonas* and *Enterobacter* or *S. aureus* is especially common in this setting.[113] Daily chest radiographs taken until the patient is released from the ICU may identify pneumonitis before it is clinically suspected. Gram stain and culture of a tracheal aspirate can help guide therapy for lobar pneumonia.

A broad-spectrum combination of antibiotics, such as an extended-spectrum penicillin with a β-lactamase inhibitor (i.e., piperacillin-tazobactam or ticarcillin-clavulanate) plus an aminoglycoside, usually is initiated until a pathogen or pathogens are identified. Empiric therapy should be based on the antibiotic susceptibility patterns of the common nosocomial pathogens in the specific ICU in which the patient is receiving care. Vancomycin also should be considered if MRSA is part of the resident flora in the ICU. Computed tomography (CT) of the chest may detect basilar and retrocardiac pneumonia, which may not be visualized readily by conventional chest radiographs. CT or ultrasound generally is helpful in assessing the size or characteristics of pleural effusions that may require drainage.

If interstitial pneumonitis is encountered, a more aggressive approach to determining an etiology is warranted. Bronchoscopy with bronchoalveolar lavage should be considered strongly. In children who have pulmonary infiltrates after undergoing heart transplantation, flexible bronchoscopy is more useful for establishing a fungal or viral etiology as opposed to a bacterial cause of pneumonia because most patients have received broad-spectrum antibiotics before undergoing the procedure.[140] Lavage fluid is pooled and processed for bacteria including mycobacteria, viruses, fungi, and protozoa by using culture techniques and special stains. *Legionella* may be an important consideration in some centers.[19,111] Noninfectious causes of pulmonary infiltrates in these children include pulmonary edema, atelectasis, hemorrhage, and adult respiratory distress syndrome.

Urinary tract infections (UTIs) also are common occurrences in the month after undergoing heart transplantation. Urinary catheterization and the immunosuppressive agents contribute to the risk for developing a UTI. Gram-negative enteric organisms (*E. coli*, *K. pneumoniae*, *P. aeruginosa*, and *Enterobacter* spp.), enterococci, and *Candida* spp. are isolated most commonly. Removal of the catheter as soon as possible minimizes the potential for development of a UTI, which has occurred in approximately 10 percent of adults. In the multicenter pediatric study, the urinary tract was the site of 16 bacterial infections.[123] In addition, UTIs developed in three children (14%) in Pittsburgh during the 2 to 3 weeks after undergoing transplantation.[57]

The broad-spectrum antibiotics used to treat the bacterial complications of transplantation promote *Candida* infection of the urinary tract. Along with removal of the urinary catheter, short-course intravenous amphotericin B for 10 days or less or fluconazole with careful dosing because of drug interactions with cyclosporine and tacrolimus is an option for treating candidal cystitis.[27,43,74,103] In some patients, a urine culture positive for *Candida* is a clue that a disseminated *Candida* infection is present and that further investigation is necessary to exclude the involvement of other organs, especially the kidneys.

Risk factors for early infection in the pediatric multi-institutional study were younger recipient age (particularly <6 months), mechanical ventilation at the time of transplantation, positive donor CMV serology with a CMV-negative recipient, and longer donor ischemic time.[123]

STERNAL WOUNDS AND MEDIASTINITIS

Sternal wound infections and mediastinitis occur in less than 5 percent of adult patients receiving modern immunosuppressive therapy for heart transplantation.[70,91,92] Most of these infections occur during the first postoperative month, usually within the first 2 weeks, and are superficial. Almost all are caused by bacteria. Staphylococci and other gram-positive bacteria generally are responsible for 50 percent of cases, and the remainder are caused by a variety of gram-negative bacilli.[94] Surgical wound infections developed in eight children in the pediatric multi-institution study, although the site of the infection was not noted.[123] Over a 15-year period, 15 (0.2%) children at Texas Children's Hospital, Houston, Texas, developed mediastinitis after undergoing cardiac surgery; 2 children had undergone heart transplantation.[144] At another children's hospital, between 1995 and 2003, 3 percent (5 of 165) of children undergoing heart and lung transplantation developed mediastinitis.[81]

Postoperative bleeding requiring re-exploration is a risk factor for development of mediastinitis. Fever, incisional pain, and an unstable sternum suggest mediastinitis; however, patients may have no specific evidence of infection, including fever. The white blood cell count may be elevated. A pericardial effusion frequently is detected with the development of mediastinitis, and pericardiocentesis may yield purulent material. CT of the chest may show a fluid collection or abscess within the mediastinum and can detect sternal osteomyelitis. Most cases of mediastinitis are caused by *S. aureus*, coagulase-negative staphylococci, or gram-negative bacteria. Median sternotomy wound infections after repair of a congenital heart lesion occur in less than 1 percent of children in large centers. Mediastinitis caused by gram-negative bacilli in association with pneumonia and bacteremia developed in 3 of the 22 children in the Pittsburgh series; each occurred within 2 weeks postoperatively.[57] Two of the three patients died. *Candida* spp. caused both cases of mediastinitis in the two children after heart transplantation. Long and colleagues[81] reported that the mediastinitis that developed in the five patients who had undergone heart and lung transplantation was caused by *E. coli*, *Torulopsis glabrata*, *Aspergillus fumigatus*, *Burkholderia cepacia*, vancomycin-resistant enterococcus.

A superficial median sternotomy wound infection not associated with an unstable sternum can be treated by local drainage of the infected subcutaneous tissue and administration of appropriate antibiotics.[34] Vacuum-assisted closure may be an important aid in addition to antibiotics.[1] A more aggressive approach is required for more serious infections associated with an unstable sternum, mediastinitis, or osteomyelitis of the sternum.[23,34,70]

Adequate drainage and débridement of the area are crucial, and any involved wires should be removed. Mediastinal drains usually are kept in place for several days. Some authorities recommend irrigating the drains with povidone-iodine, but the duration of irrigation is uncertain. A reoperation after the initial drainage procedure may be necessary. Pending culture results, antibiotic therapy is directed against *S. aureus* and gram-negative bacilli. A 4- to 6-week course of antibiotics usually is recommended. Antifungal therapy is initiated if a yeast or fungus is noted on Gram or special stains or isolated from cultures. Careful attention given to surgical technique to minimize postoperative bleeding and early withdrawal of chest and mediastinal tubes placed intraoperatively decrease the incidence of these potentially fatal infections.

OTHER INFECTIONS ENCOUNTERED DURING THE FIRST POSTOPERATIVE MONTH

Herpes Simplex

Herpes simplex infections of the oral mucosa and other superficial surfaces are common after heart transplantation. Oral herpes simplex was observed in 21 percent (11 of 53) of children undergoing transplantation at Stanford.[14] In the pediatric multiinstitutional study, only six episodes of herpes simplex infection were noted. Visceral involvement is unusual, although it may develop.[78] Herpes simplex infections typically occur approximately 13 days (range 0 to 4 months) after transplantation and are reactivation processes, not newly acquired infections.[107] A decrease in lymphocyte transformation in response to viral antigen in vitro may explain the increased rate of infection that occurs in the first 12 weeks post-transplantation. In a large randomized study comparing azathioprine with mycophenolate mofetil in adult heart transplant recipients who also were receiving cyclosporine and steroids, herpes simplex infection occurred more commonly in the group treated with mycophenolate mofetil (23% versus 16%, $p < .05$).[36] Institution of antiviral therapy for herpes simplex infection is warranted in these immunocompromised patients. The most common antiviral agent for herpes simplex is acyclovir, which can be administered orally or intravenously. Valacyclovir dosing in children has not been established, and a suspension is unavailable.

Some authorities recommend providing prophylactic acyclovir for heart transplant recipients who are seropositive for herpes simplex.[52] Acyclovir is given intravenously during the perioperative period and then orally for 30 days. Other physicians suggest that because labial or oral herpes simplex is treated so easily, a prophylactic approach is not warranted. In view of concern related to toxicity, drug interactions, and the possibility of resistance developing, I favor the approach that targets treatment after a mucocutaneous lesion is noted.

Legionella pneumophila

Legionella pneumophila infection should be included in the differential diagnosis for fever, respiratory symptoms, and pulmonary infiltrates that develop after heart transplantation.[25] *Legionella* pneumonia can develop during the first postoperative month, but the frequency with which this infection occurs varies among transplant centers. Although legionnaires' disease is an uncommon occurrence in children, nosocomial infections have been documented in a children's hospital, and immunosuppression is a risk factor.[19,22] Appropriate cultures and direct fluorescent antibody stains for *Legionella* should be performed on sputum, other respiratory secretions (obtained by invasive techniques), pleural fluid, or lung tissue to detect this pathogen in a timely fashion. A *Legionella* urinary antigen test is available for serogroup 1 antigens and is quite sensitive.[141]

Macrolides should be considered for empiric therapy if *Legionella* is a serious consideration in children with nosocomial pneumonia. Erythromycin or azithromycin is provided for 2 weeks to complete therapy. Macrolides interact with many of the immunosuppressive agents administered to these patients, however. Quinolones also are quite active against *Legionella* and avoid many of these interactions; in adult transplant patients, they have become the agents of choice.[141] The use of quinolones should be considered for pediatric organ transplant recipients in whom infection with *L. pneumophila* is suspected.[26] In hospitals caring for patients with transplants, routine surveillance culture of the hospital water supply is recommended.

Respiratory Syncytial Virus

Ten episodes of RSV infection were noted in the pediatric multiinstitutional study.[123] RSV can be acquired in the hospital soon after undergoing transplantation or can be acquired in the community before undergoing surgery or after discharge. Too few patients in whom RSV infection developed after they underwent heart transplantation have been described to comment on the clinical features. Two children in the early Pittsburgh study were noted to be infected with RSV, both infections occurring on postoperative day 10. Rapidly progressive patchy infiltrates on chest radiographs developed in one child after undergoing heart-lung transplantation, and the second child had only mild upper respiratory symptoms.[49] Tachypnea, cough, fever, wheezing, and the use of accessory muscles occurred commonly in the 18 pediatric liver transplant recipients from Pittsburgh with RSV infection.[106] Radiographic changes included interstitial and lobar infiltrates, atelectasis, and pleural effusion in 12 patients. Two patients required mechanical ventilation after the onset of symptoms related to RSV infection occurred; three others were intubated before acquiring RSV infection and subsequently had complicated courses.

Morbidity and mortality rates related to RSV infection are increased in otherwise immunocompetent children with congenital heart disease, especially when associated with pulmonary hypertension.[84] Because RSV can be acquired in the hospital, RSV infection should be considered in a young transplant patient with respiratory symptoms and fever, especially during the colder months.[59] RSV infection is documented by culture or by rapid detection of RSV infection by enzyme-linked immunosorbent assay or fluorescent antibody testing of respiratory secretions.

The decision to administer ribavirin to these patients is based primarily on the severity of the illness. In a small group of children with underlying bronchopulmonary dysplasia or congenital heart disease, aerosolized ribavirin seemed to be associated with more rapid improvement than that in patients given placebo.[60] Administration of aerosolized ribavirin to a heart transplant recipient with proved or suspected moderate to severe RSV infection is reasonable. As is the case with children requiring mechanical ventilation because of severe RSV lower respiratory tract infection, however, the efficacy of ribavirin in this situation is unknown.[137] The 2006 *Red Book* states, "A decision about ribavirin administration should be made on the basis of the particular clinical circumstances and the experience of the physician."[7,109]

The monoclonal antibody palivizumab was found to be safe, well tolerated, and effective in preventing serious RSV infections in young children with hemodynamically significant congenital heart disease in a large multicenter randomized, double-blind, placebo-controlled trial.[41] Although no specific recommendations have been made regarding the use of palivizumab in children who have undergone heart or other organ transplantation, it would be reasonable to administer it to children 24 months or younger after they undergo heart transplantation and especially postoperatively when the patient is stable.[102,108] The combination of ribavirin and RSV immunoglobulin has been administered to

pediatric bone marrow transplant recipients with RSV lower respiratory tract infection.[31] The outcome in these patients was improved over that of historical controls, but no randomized trials have been conducted. RSV immunoglobulin was not efficacious in treating RSV lower respiratory tract infection in children with congenital heart disease who were younger than 2 years.[114] Although palivizumab reduced the concentration of RSV in the tracheal aspirates of children with respiratory failure caused by RSV, its efficacy in treatment is unknown.[86]

INFECTIONS BETWEEN THE FIRST AND SIXTH POSTOPERATIVE MONTHS

CYTOMEGALOVIRUS

CMV is the virus that most frequently infects immunosuppressed cardiac transplant patients. Asymptomatic or symptomatic infections are noted most commonly between the first and sixth months after undergoing transplantation and rarely after the seventh month. In adult series, 12 to 90 percent of patients had evidence of CMV infection postoperatively.[33,55,91] In the multiinstitutional pediatric study, 51 episodes of CMV infection occurred in 332 patients and accounted for 60 percent of the viral infections, with a peak occurrence in the second month after transplantation.[123] CMV infection occurred more frequently in older children than in infants. In another study, infants younger than 120 days had CMV infection and disease less commonly than did infants older than 120 days after undergoing heart transplantation.[46] Maternal antibody to CMV may have been protective in the younger infants. In the Pittsburgh series, seven children (32%) had CMV infections, with onset occurring at a mean of 33 days post-transplant (range 23 to 43 days).

CMV infection in transplant recipients occurs in three or four possible settings. In a seronegative recipient, primary CMV infection is acquired through the transplanted heart, through blood transfusions from seropositive donors, or from the community. Seropositive recipients can have reactivation of latent CMV infection or be re-infected with a second strain of CMV from the heart or from blood products derived from seropositive donors.[24] The exact site within the donor heart where CMV may reside in a latent form is unknown, but it may be either cardiac cells or leukocytes that remain within the donor heart. When primary CMV infection is acquired from the donor organ, CMV disease tends to be more severe than if CMV infection is acquired from blood or blood products.[155]

Several risk factors for development of CMV infection after undergoing organ transplantation are recognized. Donor and recipient serologic status and the immunosuppressive regimen are the most significant risk factors for acquiring CMV infection after undergoing heart transplantation. Gorensek and colleagues[55] found that positive recipient CMV serology before transplantation and a larger than average dose of corticosteroids were significant risk factors for acquiring CMV infection. Among the group of patients with CMV infection, positive recipient serology was associated with asymptomatic infection, and excessive steroid dosing was a risk factor for acquiring symptomatic CMV infection. CMV tissue invasion occurred more commonly in patients receiving mycophenolate mofetil compared with azathioprine.

The clinical manifestations of CMV infection vary. Patients may seroconvert, or a latent infection may be reactivated, as determined by positive cultures, but these patients have no symptoms attributable to the CMV infection. Fever, leukopenia, and thrombocytopenia are common postoperative manifestations of systemic CMV infection. Patients may complain of arthralgias, myalgias, and nonspecific abdominal pain. Atypical lymphocytes are noted more commonly in adult than pediatric patients. CMV infection can cause hepatitis, pneumonitis, retinitis, myocarditis, and gastrointestinal disease, including colitis.[33,40,42,54,55,69,130] The retinitis may be asymptomatic or associated with complaints such as floaters or scotomata. Ophthalmologic screening for CMV retinitis is recommended for all patients 3 to 4 months after cardiac transplantation.[42]

Of the tissues invaded by CMV, involvement of the lung leads to the greatest mortality rate—13 percent in one study.[73] CMV pneumonitis is characterized by fever, hypoxemia, and, usually, diffuse interstitial infiltrates, although lobar consolidation may occur.[129] Pulmonary infections with other viruses or with bacteria or *Pneumocystis carinii* and other pathologic processes (infarction) may coexist with CMV pneumonitis. Gastritis, gastric ulceration, duodenitis, esophagitis, pyloric perforation, and colonic hemorrhage can be documented by endoscopy. In the multiinstitutional pediatric study, the lung or gastrointestinal tract was the site of CMV infection in 13 (lung) and 6 (gastrointestinal tract) episodes.[123] Death related to CMV in the pediatric study occurred in 6 percent. In the 2005 Heart Transplant Registry report, CMV accounted for 2.5 percent of deaths in the 30 to 365 days after transplant, 0.5 percent of deaths 1 to 3 years after transplant, and zero deaths after 3 years.[17]

The diagnosis of CMV infection can be based on changes in antibody titer to CMV in paired sera run in parallel with the use of established tests or on changes in CMV IgM results. Isolation of CMV or detection of CMV DNA from a variety of sources, such as urine, blood, bronchial washings, or tissues, also establishes that CMV infection is present. In a seronegative recipient, the possibility of active CMV infection can be anticipated by periodic monitoring of CMV serology and cultures. Whether the CMV infection is causing a symptomatic or invasive illness is more difficult to establish. Histopathologic evidence of CMV infection, such as typical viral inclusions or detection of antigen in tissue by special stains, is required to confirm organ involvement by CMV, although this criterion often is not considered a requirement for clinical trials of preventive measures for CMV disease. Cultures of the buffy coat are positive more frequently in patients with symptomatic than in patients with asymptomatic CMV infection; in patients with primary CMV infection and lung involvement, CMV cultures tend to be positive earlier in the postoperative period than in patients who do not have lung involvement.[55] CMV antigen-positive leukocytes detected by monoclonal antibodies to the early antigen of CMV were found 10 to 28 days before increases in CMV antibody occurred in five patients with active CMV infection.[148] Polymerase chain reaction (PCR) can detect DNA from CMV in blood and other tissues readily and with great sensitivity.[45] The antigen and PCR techniques have been used for early detection of CMV infection so that preemptive antiviral therapy can be initiated.[35]

In addition to the CMV infection syndromes, CMV infection itself seems to affect the transplant recipient adversely in other ways. Symptomatic or asymptomatic CMV infection is associated with a higher rate of graft rejection or graft loss, a greater risk of development of fungal infection, more frequent and earlier graft atherosclerosis, and a significantly lower survival rate than occur in patients who do not have CMV infection.[45,116] In the multiinstitutional study, CMV-positive donor serology in conjunction with CMV-negative recipient serology was a risk factor for the acquisition of earlier infection with any organism.[123]

CMV infection of the wide variety of cells that it invades leads to the activation of protein synthesis and the production of multiple immunologically active molecules, including cytokines, especially tumor necrosis factor-α, which adds to the immune deficits induced by the immunosuppressive agents.[45,75] Allograft injury or rejection may be associated with CMV infection of the transplanted organ itself.[45]

Successful treatment or suppression of visceral CMV disease by ganciclovir, a nucleoside analogue active in vitro against CMV, requires a timely diagnosis. Ganciclovir has been shown to alter

CMV disease favorably in heart transplant patients, along with allowing a reduction in immunosuppressive therapy, when possible.[71] The standard approach for treating symptomatic CMV infection is administration of 2 to 3 weeks of intravenous ganciclovir, although the optimal duration of this therapy and the need for maintenance oral doses are unclear.[117] The dose is 5 mg/kg every 12 hours with careful monitoring of hematologic parameters and renal function if renal function initially is normal. Modification of the dose is necessary if renal function is impaired. Viremia should be cleared before discontinuing therapy.[45] The most common adverse reactions to ganciclovir are neutropenia, thrombocytopenia, impaired renal function, seizures, and other central nervous system (CNS) abnormalities.

The role of CMV hyperimmunoglobulin in treating CMV infection in these patients requires further study. After bone marrow transplantation, the addition of CMV immunoglobulin to ganciclovir may be superior to ganciclovir alone in treating CMV pneumonia.[38,112]

In one pediatric study, symptomatic CMV disease developed in five children after they had undergone heart transplantation; each had blood cultures and PCR positive for CMV.[48] Four were treated with ganciclovir for 14 days; one received ganciclovir for 30 days. All received CMV-IgG (150 mg/kg) weekly for 3 weeks. Symptomatic CMV disease was treated successfully in each case.

If possible, prevention of CMV infection in heart transplant recipients is optimal. Only seronegative blood should be used for transfusions when the recipient and the donor are seronegative. Careful control of immunosuppressive therapy, especially with corticosteroids, may help avoid the acquisition of some infections. In the situation of a seronegative recipient of a heart from a seropositive donor, prophylactic administration of CMV immunoglobulin may be useful in some patients. In one study involving the prevention of CMV disease, ganciclovir was compared with CMV immunoglobulin (misoprostol [Cytotec]) in 31 CMV-seropositive heart transplant recipients in whom OKT3 monoclonal antibody was used for early immunoprophylaxis.[2] CMV disease and visceral involvement occurred more frequently in the group given CMV immunoglobulin (40%) than in the group given ganciclovir (6%; $p = .03$). CMV-IgG would be expected to be more beneficial in recipients who are CMV-seronegative.

In one large randomized double-blind, placebo-controlled trial of CMV-seropositive transplant recipients, ganciclovir significantly reduced the incidence of CMV illness during the first 120 days after heart transplantation (9% versus 46% in controls, $p < .001$).[89] No differences were noted between the study groups for seronegative recipients. Combining ganciclovir and CMV-IgG for prophylaxis of high-risk seronegative recipients of hearts from seropositive donors has resulted in mixed findings. Avery[11] found that the combination was not particularly effective; symptomatic CMV syndrome developed in 50 percent of patients. Garjarski and associates[48] provided CMV-IgG (150 mg/kg intravenously at weeks 0, 2, 4, 6, and 8 and 100 mg/kg intravenously at weeks 12 and 16) plus ganciclovir (5 mg/kg every 12 hours intravenously for weeks 1 and 2 and 6 mg/kg/day intravenously at weeks 3 and 4) to 19 children who were recipients of heart transplants from CMV-seropositive donors. CMV disease occurred in 3 of the 10 children who were CMV-seronegative and in 1 of the 10 recipients who were seropositive. Adverse effects of these agents were not reported.

In the study from Stanford, high-risk recipients received CMV-IgG immediately after undergoing transplantation (150 mg/kg administered within 72 hours after transplantation, followed by 100 mg/kg at weeks 2, 4, 6, and 8 and 50 mg/kg at weeks 12 and 16).[146,147] In addition, ganciclovir was administered intravenously immediately after transplantation at a dose of 5 mg/kg every 12 hours for 14 days, followed by 6 mg/kg/day for the next 2 weeks. These patients were compared with a historical control group at the same institution that received ganciclovir in the 2 to 3 years before CMV-IgG was used. The 27 recipients treated prophylactically with ganciclovir and CMV-IgG had a higher disease-free incidence of CMV, a lower incidence of rejection, and a higher survival rate than those of the historical cohort treated with ganciclovir alone. The combination looks promising for prevention of CMV disease in high-risk heart transplant recipients but requires a randomized trial before the combination can be recommended routinely.

CMV resistant to ganciclovir may emerge as a result of ganciclovir prophylaxis or treatment.[13] Foscarnet or cidofovir is an alternative agent in this situation.

In a meta-analysis of CMV prevention strategies for post-transplantation CMV disease, Small and associates[136] concluded that universal prophylaxis and preemptive approaches are equally effective in reducing the incidence of CMV disease.

EPSTEIN-BARR VIRUS

EBV may cause a spectrum of diseases, including a mononucleosis-like syndrome, polyclonal lymphoproliferation, or monoclonal lymphoproliferation, usually of B cells, in pediatric heart transplant recipients. The transplanted organ is thought to be the most frequent source of EBV. Post-transplantation lymphoproliferative disorders (PTLDs) refer to B-cell expansion that may be localized, nodal, extranodal, or widely disseminated. The largest series of children who have undergone heart transplantation is from Pittsburgh; in this series, PTLD developed in 7.7 percent (6 of 78) of pediatric heart transplant recipients.[18] A major risk factor for the subsequent development of PTLD was being seronegative for EBV before undergoing transplantation. PTLD developed in one third of seronegative recipients of thoracic organs who acquired primary EBV infection (10 of 30). PTLD developed in none of the children who were seropositive before transplantation. Almost all these cases occurred within 1 year of transplantation.

In another series, 19 children were EBV-seropositive and 31 were EBV-seronegative before undergoing heart transplantation. PTLD developed in 1 of 19 patients who were seropositive before undergoing transplantation and in 12 of 19 who became seropositive after transplantation.[157] It did not develop in any of the 12 recipients who remained EBV-seronegative. In contrast to the Pittsburgh experience, the mean time to confirmation of PTLD was 29 months (range 3 to 72 months).

Webber and associates[153] reviewed the experience with PTLD after heart transplantation among 1184 primary organ recipients in 19 centers from 1993 to 2002. Fifty-six patients (5%) developed PTLD a mean of 23.8 months post-transplantation.

Symptoms of PTLD may include fever, malaise, sore throat, and lymphadenopathy. Some children may have splenomegaly, CNS symptoms such as lethargy or seizures, or gastrointestinal complaints.[15,18] Concurrent opportunistic infections are common. Nodules in the lung may be noted on chest radiographs.

The diagnosis of PTLD requires biopsy of involved tissue showing lymphoid proliferation with an immunoblastic component. Molecular techniques typically detect EBV nucleic acids in tissue. EBV serology also is helpful. Quantitative measurement of EBV DNA and RNA in peripheral blood is used to detect primary infection or reactivation at a very early time point and to monitor viral loads serially over time to detect PTLD in the most timely manner.[115] When reported as EBV copies per 10^5 peripheral blood lymphocytes, patients with PTLD typically have viral loads between 500 and 5000, which are much greater than the loads detected in normal latency.

Management generally involves decreasing the immunosuppressive regimen or discontinuing it temporarily. Anti-CD20

antibody (rituximab) is a commercially available monoclonal antibody that specifically binds to the CD20 antigen of normal and malignant B cells and results in antibody-dependent and complement-dependent cytotoxicity. Rituximab has been administered to children with PTLD after they have undergone solid organ transplantation, with some success.[126] Antiviral therapy with acyclovir or ganciclovir also is administered. Some centers administer ganciclovir, acyclovir, or intravenous gamma-globulin for prevention of PTLD, although no prospective studies have confirmed that such an approach is efficacious.[56]

TOXOPLASMA GONDII

An increased incidence of toxoplasmosis is apparent in recipients of heart transplants compared with other organ transplants, although it remains an uncommon infection after heart transplantation.[138] In the pediatric multi-institutional study, toxoplasmosis was not mentioned. *T. gondii* has a predilection for muscle and can be transmitted to the recipient from the heart of a seropositive donor. Active *T. gondii* infections may occur in donor hearts.[118] Less commonly, reactivation of old infection occurs in the recipient. The greatest risk for acquisition of toxoplasmosis occurs in a seronegative recipient of a heart from a seropositive donor. Clinical symptoms usually develop after the first postoperative month and generally within 3 months of transplantation.[61,83,90] Fever alone may be the only clinical manifestation. Dissemination of the parasite to the CNS may lead to signs and symptoms of meningoencephalitis, such as lethargy, seizures, coma, and hemiparesis. Chorioretinitis may result in diminished visual acuity. A sepsis-like picture, pneumonia, and cutaneous lesions are unusual manifestations.[10]

CT or magnetic resonance imaging of the brain may detect ring-enhancing mass lesions, which typically are multiple and in periventricular locations. Definitive diagnosis of CNS toxoplasmosis requires the demonstration of tachyzoites or cysts in tissue by biopsy or at necropsy. Serologic tests help monitor seronegative patients for seroconversion and allow a more aggressive approach to be taken to the early diagnosis of toxoplasmosis.[135] *T. gondii* has been seen on endomyocardial biopsy specimens routinely obtained to monitor for rejection.[82]

Therapy for toxoplasmosis with pyrimethamine and sulfadiazine may lead to recovery; these drugs also should be administered if the patient seroconverts.[47] Prophylactic administration of pyrimethamine to seronegative recipients of hearts from seropositive donors is recommended.[156] Prophylactic trimethoprim-sulfamethoxazole also seems to be protective in this high-risk situation.[12,94] Spiramycin is not a useful prophylactic agent.[135]

ASPERGILLUS FUMIGATUS

A. fumigatus is the non-*Candida* fungal infection most commonly reported outside the immediate postoperative period in most series and may be noted first at necropsy.[104] In an early Stanford series, *Aspergillus* infections (four pulmonary, four disseminated) occurred in 8 of 72 (11%) cyclosporine-treated patients 12 to 45 days postoperatively.[65] In a follow-up report, 54 *Aspergillus* infections developed in 620 consecutive heart transplant recipients between 1980 and 1996.[94] Most commonly, *Aspergillus* infections were in the lung (n = 31) or were disseminated (n = 17). The median time to onset was 52 days. Disseminated aspergillosis was the most common infectious episode responsible for the highest mortality rates in this series. One child in Pittsburgh had disseminated aspergillosis.[57] In the multi-institutional study, seven non-*Candida* fungal infections occurred: *Aspergillus* spp., two; *Cryptococcus*, *Rhizopus*, and *Rhizomucor*; one each; and unspecified, two.[123] All patients with disseminated infection died.

CNS invasion occurs in many patients with pulmonary aspergillosis, and aspergillosis is the most common cause of brain abscess in organ transplant recipients.[61,93,133] Alterations in mental status occur most frequently, and seizures may occur in 40 percent of cases. On CT scans of the head, multifocal lesions are seen commonly and show a predilection for the junction of the gray and white matter. Mediastinitis and endocarditis are other manifestations of invasive aspergillosis.[79,127] Although isolation of *Aspergillus* from respiratory secretions in a patient with pneumonitis does not establish a diagnosis, aspergillosis is so difficult to establish firmly and is so frequently fatal that treatment should be considered seriously based on this culture alone.

Voriconazole is the agent of choice for invasive aspergillosis.[63] The prospective administration of the combination of voriconazole and caspofungin has been found to be superior to liposomal amphotericin B in historic control solid organ transplant patients with invasive aspergillosis.[134] Itraconazole should not be used for aspergillosis in heart transplant recipients now that superior agents are available.[96] Caspofungin alone also may be beneficial in some patients.[9] Performing surgical drainage and débridement is important for managing most infections.[24] Some type of drainage procedure is indicated if CNS aspergillosis is documented or suspected, although the response to therapy with amphotericin B generally is dismal. In some centers, inhalation of aerosolized amphotericin B (20 mg in sterile water three times per day) has been used prophylactically throughout the hospital stay to prevent the acquisition of invasive aspergillosis.[81] Liposomal amphotericin B may provide an alternative therapy for patients failing treatment with or intolerant of conventional amphotericin B for other mycoses.[37,150]

INFECTIONS AFTER THE SIXTH POSTOPERATIVE MONTH

NOCARDIA ASTEROIDES

A dry cough, fever, and the presence of a solitary pulmonary nodule or abscess on a chest radiograph are characteristic of infection with *N. asteroides*.[77,132] Although pulmonary nodules are characteristic of *Nocardia*, *Aspergillus* and CMV can be associated with nodules as well.[95] Some patients are asymptomatic despite having an abnormal chest radiograph. Infection with *Nocardia* was noted in only 3 percent of cyclosporine-treated patients in the early Stanford series.[65] The median time to the onset of infection was 225 days. A similar incidence of lung nodules or masses secondary to *Nocardia* was noted in a series from New York.[62] In the follow-up Stanford series, 23 episodes of *Nocardia* infection (3.7%) occurred, 19 in the lung.[94] The median onset of infection in the second series was 147 days. *Nocardia* infections are rare in pediatric heart transplant recipients.

Nocardia is isolated best from direct lung tissue specimens, but it may be cultured from bone, skin, or other sites of involvement as well. If a cutaneous skin lesion of *Nocardia* is recognized, other sites should be evaluated promptly for involvement.[51] The formation of single or multiple abscesses in the CNS may result from hematogenous dissemination.[61] Seizures may develop after invasion of brain parenchyma.

The drug of choice for treating *N. asteroides* infection is a sulfonamide. Prolonged administration (average 10 months) generally leads to clearing of the pulmonary lesions. Minocycline is an alternative therapy.[105] The incidence of *Nocardia* infection has declined with the introduction of routine prophylaxis with trimethoprim-sulfamethoxazole.

PNEUMOCYSTIS CARINII

P. carinii pneumonia has been reported in approximately 3 to 4 percent of heart transplantation recipients during the post-cyclosporine era.[44,58,65] In the multi-institutional study, *P. carinii* was noted in seven children (2.1%).[123] Fever, a nonproductive cough, and tachypnea are typical symptoms; hypoxemia is characteristic. The chest radiograph classically shows a diffuse interstitial infiltrate that can progress rapidly. The most expeditious method of documenting *P. carinii* pneumonia in children is methenamine silver or specific antibody staining of fluid or tissue obtained by bronchoalveolar lavage or lung biopsy. Co-infection with CMV or other pathogens occurs commonly.

Treatment of *Pneumocystis* pneumonia in a pediatric heart transplant recipient is identical to that for other immunocompromised children. Use of trimethoprim-sulfamethoxazole prophylaxis for at least 4 months after transplantation has decreased markedly the incidence of this infection in recipients of heart transplants.[100]

STREPTOCOCCUS PNEUMONIAE

S. pneumoniae is an important community-acquired pathogen in heart transplant recipients, who are at increased risk for acquiring this organism.[3] In the multi-institutional pediatric study, two episodes of pneumococcal infection were recorded.[123] During a 10-year period, 9 (11%) of 80 cardiac transplant patients in Little Rock, Arkansas, had 12 episodes of pneumococcal bacteremia.[142] Over an 11-year period, 4 of 105 children undergoing heart transplantation in Toronto had systemic pneumococcal infections.[145] In the follow-up series from Stanford, seven pulmonary infections with *S. pneumoniae* were reported.[94] In an eight-center pediatric surveillance study spanning 5 years, pneumococcal infection developed in 10 patients after a median time from transplantation of 17 months (range, 5-76 months).[125] Three of the 10 patients had two episodes, and one patient had three episodes of pneumococcal infection. The median age of the 10 patients at the time of the first pneumococcal infection was 26 months (range, 15-89 months). The pneumococcal serotypes of the isolates from these patients were the same as noted in healthy children and generally would be covered by the 23-valent pneumococcal vaccine or the 7-valent pneumococcal conjugate vaccine.

OTHER VIRUSES

Influenza and parainfluenza viruses can cause serious infections at any time after transplantation, but especially in the immediate postoperative period.[8,87] Additional risk factors for development of severe disease leading to death are young age and augmentation of immunosuppression. Fever, cough, rhinorrhea, and pharyngitis are typical symptoms of upper respiratory infections. More serious manifestations include adult respiratory distress syndrome; a requirement for intubation and mechanical ventilation; a sepsis-like picture; or CNS symptoms such as headache, photophobia, and lethargy. Viral infection may enhance the likelihood of allograft rejection occurring.

For influenza A infection, oseltamivir, amantadine, or rimantadine should be considered in younger children older than 12 months of age with a heart transplant because of their enhanced predisposition for severe or complicated influenza infection.[5] In older children, zanamivir or oseltamivir is indicated. Experience with these agents in patients with solid organ transplants is limited, however.[119] Early antiviral treatment of influenza in bone marrow transplant recipients was associated with decreased progression to pneumonia and shortened viral shedding in one study.[97]

Parvovirus B19 infection is recognized to cause severe anemia associated with low or no reticulocytes in recipients of heart transplants, similar to the red blood cell suppression in other immunosuppressed patients.[99] In one child, severe pneumonia developed in association with fever and a blanching maculopapular rash involving the face, trunk, and extremities.[68] The parvovirus B19 genome also has been detected in myocardial biopsy specimens of children experiencing cardiac allograft rejection. Of six children described in one report, one had a diffuse rash and two had persistent rejection despite receiving aggressive therapy.[124] In a series of children from Loma Linda who underwent myocardial biopsy for possible rejection, parvovirus genome was detected in 5 of 553 biopsy samples taken from 149 children.[128] Intravenous immunoglobulin is beneficial for the treatment of anemia related to parvovirus B19, but its efficacy in treating pneumonia or possible allograft rejection is unknown.

Varicella virus infection remains a common childhood illness that can cause life-threatening disease in immunocompromised children. In a group of 28 children younger than 10 years old at the time of undergoing heart transplantation who had been monitored for at least 1 year between 1986 and 1999, 14 cases of primary infection with varicella-zoster virus were identified.[32] The mean time post-transplant was 3 years (range, 9 months to 7.5 years). All children were seronegative at the time of transplantation. These children were treated successfully with either parenteral followed by oral acyclovir or oral valacyclovir (mean dose 77 mg/kg/day) for 7 days. Only one child had recurrent varicella, and none had zoster. Routine administration of varicella vaccine to young children helps to decrease most concerns about varicella developing after organ transplantation because of herd protection. Some patients do lose serologically measured immunity to varicella after undergoing transplantation, although the significance of this loss is unclear.[151]

Adenovirus has been associated with serious infections after solid organ transplantation. Among 28 children with solid organ transplants (9 heart) and adenoviral infections, the most common symptoms were fever, diarrhea, vomiting, and abdominal pain.[28] Infection occurred a median of 1.6 months after transplantation. Only two of these patients received antiviral therapy, and all survived the infection. Cidofovir may be beneficial in some patients with disseminated infection, especially of the lungs, or increasing viral loads determined by PCR.[66]

IMMUNOSUPPRESSIVE AGENTS AND ANTIBIOTICS

Cyclosporine and tacrolimus have improved the success of organ transplantation considerably. Generally, the incidence of infection seems to be less since the introduction of cyclosporine than with the earlier immunosuppressive regimens, although morbidity and morality rates remain high in heart transplant recipients.[62] Cyclosporine serum levels are monitored carefully to ensure that concentrations associated with optimal immunosuppression and minimal adverse effects are maintained. Some antibiotics interfere with the pharmacokinetics of cyclosporine or tacrolimus, which may lead to an increase or decrease in their levels.[21,76,101,120,122,139,143] Table 81–3 outlines these interactions. Because cyclosporine and tacrolimus are nephrotoxic, the antimicrobial agents (amphotericin B, aminoglycosides, acyclovir, ceftazidime) administered to heart transplant recipients may be additive; renal function must be monitored carefully.

TABLE 81–3 Effect of Various Antibiotics on Cyclosporine/Tacrolimus Levels

Increase Cyclosporine/Tacrolimus Level	Decrease Cyclosporine/Tacrolimus Level
Clarithromycin	Sulfadiazine
Azithromycin	Rifampin
Erythromycin	Trimethoprim-sulfamethoxazole
Ketoconazole	? Nafcillin
Fluconazole	? Isoniazid
Voriconazole	? Ciprofloxacin (counteracts immunosuppression)
Itraconazole	
Caspofungin	

IMMUNIZATIONS

No specific guidelines exist for the immunization of children after they have received a heart transplant, although studies examining the immunogenicity of selected vaccines in this population are being conducted. A prudent approach is to follow the recommendations that the Committee on Infectious Diseases of the American Academy of Pediatrics has developed for immunizing immunosuppressed children.[4] Ideally, the patient will have received the recommended routine vaccines before undergoing transplantation.

Children who have received transplants before reaching 2 to 3 years of age do not respond to the pneumococcal polysaccharide vaccine as well as do children who are older when they receive a transplanted heart, even though the vaccine is administered several years after they receive the transplant.[50] An impairment in immunoglobulin isotype switching from IgM to IgG and especially IgG2 seems to result from the immunosuppressive therapy that these children are receiving.[49] In one study, the antibody response of children aged 2 to 18 months old following solid organ transplantation was compared with age-matched children to the 7-valent pneumococcal conjugate vaccine (PCV7) followed by the 23-valent polysaccharide vaccine (PV23) 2 months later.[80] The antibody levels achieved were significantly higher in the controls, and a second dose of PCV7 given 2 months after the administration of the first PCV7 dose did not lead to higher antibody levels.

For previously unimmunized transplant recipients aged 2 to 18 years old, conjugate pneumococcal vaccine is recommended, followed by the 23-valent pneumococcal vaccine at least 2 to 12 months later. Ideally, children will have completed their PCV7 series before undergoing transplantation.[6] Ongoing immunosuppression seems to prevent maturation of the response to polysaccharide antigens in younger children, which it is hoped that the conjugate vaccine can overcome. The 23-valent pneumococcal vaccine is less immunogenic in adults after they have undergone heart transplantation than it is in healthy controls.

Antibodies to the influenza vaccine develop in most children, but previous exposure predicts a better response.[88] Annual administration of influenza vaccine seems to be safe and immunogenic in children after they have undergone solid organ transplantation. In one study, low-level histologic rejection occurred after the administration of influenza vaccine.[16] In a later study, the administration of inactivated influenza vaccine was not associated with an increased risk of rejection.[154] Depending on the time of year and the approximate date of transplantation, influenza vaccination is appropriate for the patient and all household contacts and health care workers to whom the patient might be exposed. The live-attenuated influenza vaccine is not recommended for individuals receiving immunosuppressive therapy or their close contacts.

After transplantation has been performed and after immunosuppressive therapy has been initiated, administration of live viral vaccines is contraindicated. The enhanced inactivated polio vaccine should be given to the child and to normal siblings. Measles, mumps, rubella, and varicella vaccines can be given to the siblings. Diphtheria, tetanus, and acellular pertussis inactivated vaccines should be given at the routine booster schedule, although antibody responses may not be equivalent to those observed in normal children. Varicella vaccine should be administered before transplantation is performed if varicella antibody is not detected at the pretransplant evaluation in a child aged 12 months or older. Whether the antibody response data generated in children with leukemia during maintenance chemotherapy can be applied to heart transplant recipients who must continue daily immunosuppressive therapy is unclear.

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CHAPTER 82

Infections in Pediatric Lung Transplantation

Jill A. Hoffman

The first pediatric lung transplant was reported to the International Society for Heart and Lung Transplantation (ISHLT) in 1986, and by the end of 2005, a total of 963 transplants had been reported to the registry.[446] In addition, living lobar transplantation (LLT) is being performed at some centers as a way to address the shortage of available organs,[395,408,461] but it still accounts for less than 10 percent of all pediatric lung transplants.[446] At Children's Hospital Los Angeles (CHLA), the first pediatric LLT was performed in 1993 on a 13-year-old boy with cystic fibrosis (CF). He received lobes from each of his parents and survived for 5 years, unfortunately succumbing to renal failure while waiting for a kidney transplant.[461] Fifty-three LLT procedures (34% of total lung transplants) have been performed on pediatric patients at CHLA.[40]

The primary indication for lung transplantation in the pediatric population is CF. Most pediatric transplants, 56 percent regardless of age and almost 70 percent of transplants in 12- to 17-year-old patients, were performed for this diagnosis. By age, most pediatric transplant recipients are adolescents (>60%).[446] The most common diagnoses were complex congenital heart disease in infants younger than 1 year of age undergoing lung transplantation and primary pulmonary hypertension in children aged 1 to 5 years. Since 2000, approximately 66 pediatric lung transplants have been performed annually at 27 centers.[446]

Infectious morbidity and mortality rates remain high for lung transplant recipients (LTRs) and are probably greater than for most other solid organ transplant recipients (SOTRs).[8] This high incidence results from constant communication of the lungs and fresh bronchial anastomosis (BA) with the environment and from the high preoperative microbial burden of patients who constitute the majority of recipients of pediatric lung transplants. Denervation of the transplanted lung, interruption of the bronchial and lymphatic circulation, abnormal cough reflexes, and impaired mucociliary clearance also probably play roles.[8] In addition, these patients receive relatively high-dose, long-term immunosuppression; at 5 years, most patients still are maintained on tacrolimus (60% of patients) and corticosteroids (98% of patients).[446] In the first year after lung transplantation at CHLA, the overall incidence of infection was 0.24 episode per patient per month, or 2.88 infections per patient per year.[237] Fifty-five percent of all infections occurred in the first month after transplantation, with infections becoming much less frequent thereafter (Tables 82–1 and 82–2).[237] A summary of the causes of infection in this cohort during the first year after transplantation is presented in Tables 82–3 and 82–4.[237]

The additional indirect impact of infections on the outcome of LTRs also is recognized. Cytomegalovirus (CMV) and probably other viruses, as well as bacterial infections, have been implicated in immunologically mediated processes leading to bronchiolitis obliterans syndrome (BOS) or chronic graft dysfunction, acute rejection, graft loss, and death.[8]

The overall survival of pediatric LTRs as reported to the ISHLT is 74, 59, 49.5, and 30 percent at 1, 3, 5, and 10 years, respectively, similar to that of adult recipients.[446] There is no difference in survival according to donor lung source, living versus deceased.[33,408,446] Infection is the single most common cause of death from 1 month to 1 year after transplantation (44%), and it remains a significant cause of death in all periods through 5 years after transplantation.[446] BOS causes approximately 40 percent of deaths after the first year. Graft failure accounts for a third of deaths in the first month and 8 to 20 percent thereafter.[446] In the series of 75 pediatric transplants at CHLA (12 heart-lung, 18 cadaveric, 45 LLTs), the mortality rate

TABLE 82–1 Timetable of Infections for Pediatric Lung Transplant Recipients

Time of Initial Evaluation	Infection/Pathogen	Comments
0-1 Month	Wound, respiratory tract, line/bloodstream, and urinary tract infections	Related to the surgical procedure: *Candida*, *Staphylococcus* species, *Pseudomonas* species
	HSV stomatitis	Reactivation
	CMV infection	
	HHV-6?	
	Bronchial anastomosis (*Aspergillus*)	
	Other: rabies, West Nile virus, lymphocytic choriomeningitis virus, endemic mycoses	Unusual pathogens preexisting in the recipient or in the donor graft
1-6 Months	CMV disease	Onset of opportunistic infections from immunosuppression
	EBV	
	Aspergillus	
	Mycobacterium tuberculosis	
	Pneumocystis jiroveci	
	Endemic mycoses	
	HHV-6	
	Respiratory tract, line/bloodstream, and urinary tract infections	Continued bacterial (*Pseudomonas*) and candidal infections common through 3-4 months
	Respiratory viruses	Community-acquired infections
>6 Months	Respiratory viruses	Community-acquired infections
	Persistent *Pseudomonas*, *Burkholderia cepacia*, and *Aspergillus* infections	Persistence of organisms from cystic fibrosis in the proximal airways and sinuses
	Opportunistic infections:	Patients receiving continuous high-level immunosuppression or therapy for steroid-resistant rejection, CMV infection
	Pneumocystis jiroveci	
	Cryptococcosis	
	Nontuberculous mycobacteria	
	EBV/PTLD	
	Herpes zoster	

CMV, cytomegalovirus; EBV, Epstein-Barr virus; HHV, human herpesvirus; HSV, herpes simplex virus; PTLD, post-transplant lymphoproliferative disease.
Adapted from Rubin, R. H., Ikonen, T., Gummert, J. F., and Morris, R. E.: The therapeutic prescription for the organ transplant recipient: The linkage of immunosuppression and antimicrobial strategies. Transpl. Infect. Dis. 1:*39, 1999.*

TABLE 82–2 Timing of Infections in 75 Pediatric Lung Transplant Recipients at Childrens Hospital Los Angeles

Months after Transplantation	% of Episodes
0-1	55
1-3	18
3-6	13
6-9	8
9-12	7

TABLE 82–3 Etiology of Infections in 75 Pediatric Lung Transplant Recipients in the First Postoperative Month at Childrens Hospital Los Angeles

Etiology	Number of Episodes
Pseudomonas, respiratory tract	22
Candida, respiratory tract	13
Herpes stomatitis	12
Cytomegalovirus infection	10
Aspergillus infection of the respiratory tract, bronchial anastomosis	4

TABLE 82–4 Etiology of Infections in 75 Pediatric Lung Transplant Recipients in the First Year at Childrens Hospital Los Angeles

Etiology	% of Total Infections
Bacterial infections	55
Pseudomonas spp.	18 (35% of bacterial infections)
Viral	27
Cytomegalovirus	12 (42% of viral infections)
Fungal	16
Candida	10
Aspergillus	4

was 18.6 percent at 1 year, 50 percent of which was attributed to infection.[237]

IMMUNOSUPPRESSION AND TIMING OF INFECTION

An understanding of the mechanisms and effects of immunosuppressive therapy is fundamental to predicting the timing and types of infections that occur after transplantation, and several excellent reviews are available.[349,352] This predictability of certain infections has facilitated establishing more timely diagnosis and determining strategies for preventive antimicrobial therapy.[127] Historically, the mainstay of therapy has included corticosteroids and combinations of the following medications: azathioprine, the calcineurin inhibitors (CNIs) cyclosporine and tacrolimus, sirolimus, mycophenolate mofetil (MMF), and polyclonal and monoclonal antibodies. In recent years, substitution of MMF and tacrolimus for azathioprine and cyclosporine, respectively, has become standard, and "steroid-sparing therapy" also is gaining favor. These regimens may provide a superior cost-benefit ratio in terms of prevention of rejection and risk of infection. A general time line of the occurrence of specific infectious agents in pediatric LTRs is presented in Table 82–1.

OVERVIEW OF INFECTIONS AND ANTIBIOTIC USE IN SOLID ORGAN TRANSPLANTATION

The "therapeutic prescription," as described by Rubin and colleagues,[352] for successful organ transplantation requires a careful balance between immunosuppression and the use of antimicrobials therapeutically, prophylactically, or preemptively to manage the risk of rejection versus infection. A transplant recipient's risk for developing infection is determined by multiple factors, including (1) the technical skill of the surgeon; (2) the quality of perioperative care; (3) the net state of immunosuppression; (4) the patient's infectious exposure, including that from the community, hospital, and donor grafts; (5) the underlying condition of the patient (e.g., colonization in CF); and (6) the virulence of the organisms. Preventive/prophylactic strategies can alter these patterns, most notably for CMV and fungal infections, and delay their appearance past the expected time periods.[350] In sum, these considerations indicate that although a standard set of antimicrobials can be prescribed, they must be tailored closely to the risks, exposure, and immunosuppression of each individual.[353]

General principles of infection and therapy have been proposed by Rubin and Marty [353] and include the recognition that a transplant recipient is likely to have a greater microbial load and more advanced infection at the time of diagnosis, thereby requiring longer therapy with greater potential for drug toxicity. Early establishment of the diagnosis and provision of therapy are paramount to a successful outcome. Furthermore, a broader range of organisms must be considered. Although a common practice in medicine is to assume a single diagnosis for a given manifestation, a recognized caveat is that transplant recipients may have "simultaneous and sequential" infections.[353] In fact, many infectious processes are influenced directly by proceeding or concurrent events. The prototype of this interaction is the immunomodulating effect of CMV infection such that alterations in the cytokine/chemokine milieu, T-lymphocyte subsets and function, neutrophil activity, and activation of endothelial cells emerge. The result of these derangements is further immunosuppression, enhanced production of virus, and subsequent increased susceptibility to additional infections. Other putative immunomodulating viruses include human herpesvirus-6 (HHV-6), Epstein-Barr virus (EBV), and the hepatitis viruses.[353,373]

Length of therapy depends on consideration of the degree of current immunosuppression and location of the infection. In general, experts suggest treating until all signs and symptoms of infection are gone, followed by a "buffer period" of weeks to months, depending on the infection.[353] Finally, drug interactions and toxicities, which may be synergistic, must be taken into consideration when prescribing medications in these complex patients.[265,352]

SITES OF INFECTION

THORACIC CAVITY: RESPIRATORY TRACT INFECTIONS, INCLUDING PNEUMONIA AND ANASTOMOTIC SITE INFECTIONS

The vast majority (80%) of infections in LTRs occur in the thorax—lung, mediastinum, pleural space[132]—with 35 to 66 percent of all infections being manifested as pneumonia.[8] In the CHLA cohort of patients during the first 12 months after transplantation, 62 percent of all infections were pneumonia.[237] The onset of pneumonia in LTRs appears in a bimodal pattern, with most cases occurring in the first postoperative month. A second, albeit smaller group has late (<6 to 12 months) recurrent episodes of gram-negative pneumonia associated with a poor outcome. The immunologic consequences of these infections in some patients may include bronchiolitis obliterans with organizing pneumonia (BOOP), which is characterized by inflammation and fibrotic granulation tissue of the small airways extending into the alveoli. The mortality rate of patients with BOOP is high (50%), and those who survive appear to be at an increased risk for developing BOS (41%).[8,69,132]

Unique to lung transplantation are BA infections, most of which are fungal and caused by *Aspergillus* and sometimes *Candida* species.[162,176,217,298,318] The BA is susceptible to such infections for a variety of reasons. Anatomically, the post-transplant bronchus is relatively devascularized, with the blood supply flowing retrograde from the pulmonary arterial circulation; neovascularization from collaterals takes as long as 1 month to occur. In the interim, the bronchus may experience ischemia and subsequent necrosis and sloughing of bronchial epithelium. Evolving surgical techniques have diminished this risk of ischemia in recent years. This environment is optimal for developing infection caused by saprophytic flora, such as *Aspergillus*, present in ambient air to which the airways are in direct contact. In addition, local reaction to suture material, postoperative corticosteroid use, and immunosuppressive therapy have a negative impact on healing and increase the patient's susceptibility to infection. As a result of this early tenuous anatomy, infections at this site are most likely to occur within the first 2 to 3 months after surgery.[76,162,176,298,300] BA infections rarely can lead to early catastrophic complications, such as dehiscence and hemorrhage. They are linked to substantial late bronchial complications, including dehiscence, bronchial stenosis, malacia, and retransplantation.[294,298]

Some studies have demonstrated that pretransplant isolation of *Aspergillus* spp. has not been found to be a risk factor for the development of *Aspergillus* infection after transplantation.[199,300,323] Others suggest that patients with CF and perioperative *Aspergillus* colonization may be at heightened risk for the development of early BA infection. Although the numbers are small, the authors speculate that the presence of *Aspergillus* at the site of the fresh, devascularized anastomosis renders it more vulnerable.[300] Postoperative isolation of *Aspergillus* from airways appears to identify patients at risk for the later development of BA abnormalities.[76,176,294,298] The overall incidence of airway infections, complications, or both ranges from 5 to 25 percent.[76,162,176,216,298] The mortality rate associated with BA infection and its complications is reported to be 2 to 7 percent.[176,298] Finally, one small study evaluating the effect of early sirolimus-based immunosuppression on the rate of rejection described poor wound healing and anastomotic complications in an unexpectedly high number of patients treated with sirolimus, which resulted in changes in immunosuppression strategies at that institution.[158]

BLOODSTREAM INFECTIONS

The second most common site of infection is the bloodstream. Two recent studies have looked specifically at the epidemiology of bloodstream infections (BSIs) in pediatric and adult LTRs.[91,315] Both studies found that BSI occurred in approximately 25 percent of transplant recipients, with staphylococci, *Pseudomonas aeruginosa*, and *Candida* spp. being the most common organisms identified. Late infections in both groups consisted of a wide range of gram-negative organisms. Both studies demonstrated that post-transplant BSI, especially early (<30 days) BSI in children and candidal BSI in both populations, was associated significantly with mortality. In the pediatric population, most BSI was related to central venous catheters and was not secondary to other sites of infection (5% with pneumonia, sternal wound, ruptured subglottic cysts). These infections were most likely to occur in the first 7 days after transplantation. In adult transplant recipients, BSI largely was associated with pulmonary infections. Specifically, *P. aeruginosa* bacteremia was more likely to develop in adult

patients with CF (20% of the transplant cohort) than in patients with other indications. *Burkholderia cepacia* and *Staphylococcus aureus* BSI was seen in similar frequency in non-CF patients. In addition, BSI in patients with CF was associated with a decreased risk for death when compared with other LTRs with similar infections, possibly because antimicrobials active against resistant gram-negative organisms were being administered at the time that the infection developed. In children, *Pseudomonas* BSI was not associated with the diagnosis of CF (45% of the transplant cohort), nor with any outcome measures in this study.

Empiric therapy in patients with suspected BSI should include agents active against staphylococcal and pseudomonal spp., with a low threshold for adding antifungal therapy. Furthermore, the need for indwelling central catheters should be assessed routinely, and catheters should be removed as soon as clinically feasible.

SELECTED PATHOGENS

BACTERIA

Pseudomonas aeruginosa and *Burkholderia cepacia* Complex

P. aeruginosa and *B. cepacia* play prominent roles in postoperative infections in patients with CF, the majority of pediatric LTRs. Despite the removal of colonized lung tissue with the procedure, the proximal airways and sinuses remain colonized and a source of infection postoperatively in the immunosuppressed transplant recipients. In patients with multidrug-resistant (MDR) organisms, this concern is heightened. MDR, gram-negative organisms have been defined as those resistant to all agents in two or more classes of effective drugs[357] (e.g., resistance to all aminoglycosides, β-lactams, fluoroquinolones). *B. cepacia* complex organisms are resistant to aminoglycosides intrinsically, having high rates of resistance to β-lactams, inducible resistance to fluoroquinolones, and resistance to colistin and polymyxin.[53]

As lung transplantation was emerging as a treatment option, patients with MDR organisms generally were excluded.[299] Such exclusion was based on data suggesting that pretransplant infection with MDR *P. aeruginosa* and *B. cepacia* complex diminished the chance of survival and, therefore, was considered a (relative) contraindication to transplantation.[213,262,388]

More recent data have documented that the presence of preoperative MDR and even pan-resistant *P. aeruginosa*, but not *Burkholderia cenocepacia*, may not have an impact on survival; these patients may be more likely to acquire postoperative infections, but they generally respond to treatment without increases in mortality rates.[22,107,128,199,299] The most recent ISHLT guidelines for selection of LTRs list "colonization with highly resistant or highly virulent bacteria, fungi, or mycobacteria" as a relative contraindication.[309] Additionally, it acknowledges that although "certain resistant pathogens may increase risk of poor outcome . . . it is not possible currently to identify absolute contraindications based on either the type of organisms or the pattern of antibiotic resistance." Despite the status of the immunocompromised host, the relatively low virulence of some of these organisms, the new healthy lung epithelium and improved airway function of the transplanted lungs, the aggressive use of antimicrobials during and after the procedure, and discordance between in vitro susceptibilities and in vivo efficacy may account for the diminished influence that MDR organisms have on the outcomes of transplant recipients.[29]

Recent analysis has shown that *B. cepacia* complex represents a group of nine related species that possess variable pathogenic potential in abnormal hosts.[240,241] Misidentification of *B. cepacia* complex organisms at the genus (*Alcaligenes*, *Stenotrophomonas*, and *Pseudomonas* most commonly) and species levels is a common occurrence.[240,271] This information is critical to evaluating patients considering transplantation and, in postoperative care, to assessing their risk for developing invasive disease.[240] There are species-specific differences in antimicrobial resistance and virulence. Several putative virulence/transmissibility factors that have been identified for *B. cenocepacia* (formerly genomavar III, ET-12 strain)[240] include quorum sensing,[389] cable pili (*cbl*A) for enhanced binding to respiratory epithelium,[359] increased neutrophil recruitment with suboptimal activation of polymorphonuclear neutrophils and macrophages,[358] and production of exopolysaccharides that inhibit neutrophil chemotaxis and scavenge reactive oxygen species.[56]

In the United States, *B. cepacia* complex is present in 3.2 percent of the CF population, with *B. cenocepacia* accounting for 50 percent of cases. In Canada, 15 percent harbor *B. cepacia* complex,[70] and as many as 80 percent of cases involve *B. cenocepacia*.[99] Some controversy continues regarding performing transplantation on patients who have *B. cenocepacia*. Data demonstrate increased morbidity and mortality rates associated with this organism in nontransplanted patients with CF[195,228,229,255] and in LTRs,[23,70,100,240] although successful transplantation has been accomplished in several centers.[70,101,237]

One of the largest and best characterized reports of *B. cepacia* complex in LTRs comes from the Toronto Lung Transplant Program, which documented that from 1988 to 1995, 15 of 28 (54%) patients with preoperative sputum positive for *B. cepacia* (genomavar III, ET-12) died after undergoing transplantation of causes related to this infection, whereas 4 of 25 (16%) *B. cepacia*–negative patients died. The 1-year survival rate for colonized patients was 67 percent versus 92 percent for those without colonization. Of note, 60 percent of *B. cepacia*–related deaths took place in the first 3 months after transplantation, with the additional deaths occurring between 14 and 48 months post-transplantation as a result of infection associated with BOS. Of the 13 surviving patients, 4 had complications related to *B. cepacia*, including abscess and empyema, and were successfully treated medically and surgically. In all surviving patients (34 total), the rate of BOS in the *B. cepacia* group (46%) was comparable to that in the *B. cepacia*–negative group (48%).[70]

More recently, the Toronto center has had improved mortality statistics with the use of 2 to 3 weeks of aggressive antimicrobial therapy, including tobramycin (intravenous, inhaled), ceftazidime, chloramphenicol, and trimethoprim-sulfamethoxazole (TMP-SMX), and a reduction in immunosuppressive therapy. Only one of five transplant patients with MDR *B. cepacia* has died of *B. cepacia*/BOS since this regimen was implemented. One other patient survived a postoperative *B. cepacia* abscess, and three additional patients remained *B. cepacia*–negative and clinically stable.[70]

In vitro synergy or multiple combination bactericidal testing (MCBT) for MDR or pan-resistant gram-negative organisms has been suggested to facilitate the use of effective multidrug regimens.[1,2,53,224,357] In vitro testing can identify combinations of antimicrobials that inhibit bacterial growth. How these data translate to patient care, however, has yet to be fully demonstrated. Recent published data did not reveal better outcomes, specifically the time to the next exacerbation, when using combination therapy to treat CF patients infected with MDR bacteria.[2] In this report, study patients received two-drug combinations based on MCBT, but these patients did not necessarily have MDR/pan-resistant organisms, nor had they failed conventional therapy. Extrapolating these results to LTRs with MDR organisms is difficult. The CHLA has been using MCBT from the University of Ottawa since 2001; our experience suggests that this approach, with as many three or four drugs used in combination, is helpful for managing preoperative and postoperative patients with MDR/pan-resistant organisms, including *B. cepacia* complex.[237] Clearly,

additional data are needed to assess this approach for LTRs with MDR organisms.

Optimal therapy for infections caused by these gram-negative organisms is not known. Therapy should be tailored to susceptibility studies. The duration of therapy requires individualization but generally is no less than 2 weeks, and treatment should be continued for a significant period after the signs and symptoms of infection have resolved. Combination therapy (two or three drugs) usually is recommended. For *P. aeruginosa*, an extended-spectrum, antipseudomonal β-lactam and an aminoglycoside, if renal status allows, with or without aerosolized tobramycin, can be used. Aerosolized tobramycin can deliver drug levels above the minimal inhibitory concentrations (MICs) of resistant organisms directly to the lungs and thereby reduce the bacterial burden with minimal systemic toxicity.[141] Aerosolized tobramycin should not be used as single therapy in transplant recipients at risk for dissemination. Colistin in either an aerosol or intravenous formulation may be helpful for pan-resistant organisms, but renal toxicity may preclude its systemic use. For *B. cepacia*, combinations that include ceftazidime, meropenem, TMP-SMX, tetracyclines, quinolones, and chloramphenicol may be used.[185] A protocol using aerosolized aztreonam has shown some benefit at CHLA.[124,145,462] Therapy is initiated in patients identified with *B. cepacia* complex, preoperatively if possible, and continued through the first year after transplantation. Preliminary data suggest that outcomes are similar to those of patients who are colonized with susceptible *Pseudomonas* organisms. Further investigation is needed in this promising area of therapy.

Other Gram-Negative Organisms

Infections with other gram-negative organisms, including extended-spectrum, β-lactamase–producing organisms (*Escherichia coli* and *Klebsiella pneumoniae*), have been described in other transplant populations, such as pediatric liver or intestinal transplantation, and notably in outbreak situations.[152,337] No data exist on rates in pediatric LTRs. Some increased risk related to antimicrobial exposure, infection control practices, stays in intensive care units, and hospitalization is likely.[289] Carbapenems (meropenem, imipenem) generally are considered the drugs of choice for extended-spectrum, β-lactamase–producing organisms, especially for empiric therapy.

Mycobacterium tuberculosis

M. tuberculosis (MTB) remains an important pathogen in SOTRs. As noted by many authors, it behaves as an opportunistic infection in these patients in that its frequency is greatly increased in comparison to the normal population (up to 74-fold in some U.S. studies), it has diverse manifestations with high rates of extrapulmonary disease, and mortality rates are high (20-40%).[290,351,383] Most cases of MTB arise from reactivation of latent infection, although acquired cases are seen, especially in pediatrics.[269,287,425] Multiple cases of donor-derived MTB infection, including pulmonary MTB in LTRs[340] and extrapulmonary MTB in renal and liver transplants, have been documented.[150] In addition, nosocomial transmission to transplant recipients has occurred.[193]

Important differences appear to exist in the epidemiology of MTB infection in different transplant populations and for adults versus pediatric patients. Most data are available for the former, who are more likely to have reactivation of latent disease and higher rates of liver toxicity with therapy. In the United States and Europe, MTB infections occur most frequently in LTRs (2-6.5%), followed by liver (0.9-2.3%) and heart (1-1.4%) recipients.[383] Studies of MTB infection in SOTRs, including small numbers of children, suggest that most cases occur within the first post-transplant year, with a median time of 9 months for all SOTRs and 3.5 months for LTRs. Risk factors for early MTB infection include allograft rejection; receipt of OKT3 or anti–T-cell antibodies, which also predict dissemination; and previous exposure to MTB, with radiographic evidence of old infection or a history of a positive tuberculin skin test (TST). Co-infection with CMV, mycoses, *Pneumocystis jiroveci*, and *Nocardia* has been associated with the development of disease.[287,290] As would be expected, most manifestations include pulmonary disease (71%), with interstitial infiltrates, nodules, effusion, and cavities seen on imaging studies. Extrapulmonary disease (16%) includes a wide variety of gastrointestinal (GI) disease and skin, musculoskeletal, genitourinary, lymph node, and central nervous system (CNS) involvement. In one study, a third of patients had disseminated disease, 91 percent of whom were febrile as compared with 64 percent of those with localized disease.[383] Significant predictors of mortality, which was 17 percent for LTRs, were disseminated disease, previous rejection, and receipt of OKT3 or anti–T-cell antibodies.[383]

Several studies have investigated mycobacterial infections in LTRs.[46,202,257,287] Two of the studies were conducted in countries with a low endemicity of MTB, Australia and North America (rates of MTB, 5 to 15/100,000), and two in Spain (rates of MTB, 25 to 49/100,000). Rates of MTB in LTRs in the two former studies were less than 1 percent,[202,257] but they were 2.6 percent and 6.4 percent (500-fold higher than the national average) in the Spanish studies.[46,287] Indeed, nontuberculous mycobacterial (NTM) infections were more common than were MTB infections in the first two reports, whereas NTM infection was encountered only once in the Spanish study. No deaths from mycobacterial disease were reported in three studies,[46,202,257] but the mortality rate was 43 percent in the final report.[287]

Few data exist on MTB in pediatric SOTRs and none in LTRs in particular. Two small series of MTB in pediatric liver transplants have been published.[269,425] Initial symptoms included fever in most patients. Pulmonary findings were most common. Pediatric patients may be seen later than adults after transplantation (median of 8 months versus 4 months) as a result of differences in acquisition of disease.[425] TST results were negative or minimal in all patients. A 5-mm induration (two patients) should be considered positive in an immunocomprised patient with compatible symptoms.[18] Furthermore, screening of family members was very useful in both series of patients, thus suggesting that as for immunocompetent children, the index case of exposure is in the home. Invasive diagnostic procedures were needed to confirm the diagnosis in most patients. No deaths were directly attributable to MTB. An additional case report of MTB complex disease in a pediatric renal transplant recipient highlights another potential difference between pediatric and adult cases.[73] *Mycobacterium bovis* disease, which can be indistinguishable from MTB but often is manifested as abdominal disease, developed in this child. It usually is acquired from the ingestion of unpasteurized milk products from infected cows. It appears to be distinctly more common in children and, in the United States, is seen most often in children who have immigrated from or visited countries where *M. bovis* is endemic in cattle, such as Mexico.[88] The importance of recognizing this infection is its inherent resistance to pyrazinamide and the implications that this resistance has for therapy.

Diagnosis of MTB in the transplant setting can be challenging because symptoms may be nonspecific, such as fever of unknown origin, or unusual, such as abdominal manifestations; co-infections also may modify the signs and symptoms. Delayed-type hypersensitivity often is suppressed in transplant candidates and recipients, thus rendering TST unreliable. Moreover, just a minority of patients undergo skin testing before transplantation. Consequently, a high index of suspicion is required, and frequently, aggressive diagnostic techniques, such as bronchoscopy, laparoscopy, and tissue biopsy, are needed.[269,299,425]

Several new methods are available for culture-based diagnosis. Broth-based culture systems coupled with DNA probes allows rapid (2 or 3 weeks for positive and negative smears, respectively) establishment of a diagnosis.[314,363] In addition, culture-based diagnostics are the only widely available methods by which to assess susceptibility. Technologies for the rapid detection of MDR organisms are still experimental.[24,363,412] Two commercially available kits are available in the United States for diagnosis based on nucleic acid amplification (NAA) assays.[314] They are approved for smear-positive (MTD, GenProbe; Amplicor, Roche) and smear-negative samples (MTD), but there is still some debate on the optimal use of these tests. The American Thoracic Society and the Centers for Disease Control and Prevention have published recommendations,[19,65,363] although they have not been studied in transplant recipients or pediatric populations specifically. Newer modalities such as the microscopic-observation drug susceptibility (MODS) assay may allow rapid, inexpensive diagnosis and susceptibility testing in countries with limited resources.[190,286,314] Finally, new tests exist for the detection of latent MTB: the "QuantiFERON-TB Gold," a whole-blood enzyme-linked immunosorbent assay (ELISA), and "T-SPOT. TB," an ex-vivo enzyme-linked immunospot (ELISpot) assay.[267,339,363] Both assays rely on interferon production from peripheral blood mononuclear cells in response to MTB antigens. The advantage of these tests is they may allow MTB to be distinguished from NTM infection and bacille Calmette-Guérin (BCG) vaccine response. In adults, these tests have higher specificity than TST does, especially with regard to BCG, and may be most useful in low-endemic, high-income settings.[267,313,394] However, their use in young children and immunocompromised patients has not been fully evaluated.[313,394]

Specific guidelines exist for the treatment of MTB in children infected with human immunodeficiency virus (HIV).[15] None is available for pediatric SOTRs, and the optimal therapy has not been defined.[41] Clearly complicating factors include liver toxicity from single- and multiple-drug regimens and drug interactions, most notably between rifampin and CNIs. Isoniazid (INH) alone and in combination with other first-line drugs, such as rifampin and pyrazinamide, can lead to toxicity requiring discontinuation of all or some of these drugs and the need to institute new regimens.[290,383] Drug toxicity is most notable in adult liver transplant recipients. Liver biopsy may be necessary to distinguish drug toxicity from rejection or hepatic granuloma from MTB infection.[290,351,383] Pediatric patients generally tolerate first-line therapy, with occasional need for reduced dosage and rarely discontinuation.[73,269,425]

The use of rifampin in SOTRs remains controversial. Significant morbidity of MTB from graft dysfunction and loss can be due to the simultaneous use of rifampin with CNIs. Rifampin lowers serum levels of cyclosporine and tacrolimus considerably, as well as decreases steroid levels somewhat, via induction of cytochrome P-450. Some authors argue that rifampin can be used successfully with increased CNI dosing and careful monitoring of CNI serum levels.[257,425] Others suggest that a longer duration of therapy (up to 50%) with rifampin-free/INH-containing regimens has a comparable outcome without the risk of rejection and potential graft loss, and they do not recommend the use of rifampin.[46,290] Most authors agree that regimens should contain INH if at all possible.[46,287,290,383] If rifampin-free regimens are used, therapy should be prolonged to a minimum of 12 to 18 months.[46,383] Ethambutol and second-line therapies (aminoglycosides and quinolones) may be considered if a first-line drug cannot be used.[290,351] If both INH and rifampin are not used, therapy should be continued for 2 to 3 years.[350]

Recommendations for chemoprophylaxis of MTB infection are similarly without consensus. Some authors suggest that all SOTRs with a positive TST of 5-mm or greater induration be treated with INH. They reason that the morbidity and mortality from MTB and the potential risk for nosocomial transmission from unidentified patients are high enough to warrant the risk of hepatotoxicity.[383] Others use a staged approach in which INH therapy is initiated in LTRs and other non–liver transplant recipients as soon as possible but deferred in liver recipients until liver function is stable.[290] A third strategy is active surveillance, with initiation of INH only if additional risk factors are present, such as recent conversion, exposure to active MTB, recipient or donor with a history of MTB without adequate therapy, or the existence of significant abnormalities on chest radiographs.[351]

Nontuberculous Mycobacterial Infections

NTM infections are more common occurrences in LTRs in areas where MTB is not endemic, including the United States.[110] NTM organisms are divided into slowly growing species, *Mycobacterium avium* complex (MAC), *Mycobacterium kansasii*, *Mycobacterium marinum*, and *Mycobacterium ulcerans*, and rapid growers, *Mycobacterium fortuitum* group, *Mycobacterium abscessus*, and *Mycobacterium chelonae*.[16] Patients with chronic lung disease and CF in particular have a high incidence of NTM colonization. As many as 24 percent of patients with CF are colonized with NTM organisms, most commonly *M. abscessus* or MAC.[121,305,308,330] The contribution of these organisms to progression of lung disease in pretransplant patients still is being defined.[87,121,156,166,305-307] In one center, 20 percent of patients with end-stage CF referred for transplantation were colonized with NTM organisms, which were isolated from 14 percent of CF LTRs. The prevalence of post-transplant invasive disease was approximately 3 percent.[66] Most authors do not consider that the presence of NTM infection before transplantation should serve as an exclusion to transplantation, but these organisms clearly are responsible for serious and fatal post-transplant infections.[66,74,110,202,257,360,410]

Clinical manifestations of NTM infection in lung transplantation vary by organism and include pulmonary, cutaneous, and disseminated disease.[110] In a survey of transplant centers regarding *M. abscessus* infection in LTRs, the majority had pulmonary infections, followed by skin involvement; several patients had both. Disease occurred at a median of 18.5 months after transplantation (1-110 months). Pulmonary disease may be manifested as chronic cough, sputum production, and fatigue. Skin infections may involve surgical sites (sternal wounds) and the extremities.[66,257] Painful, erythematous cutaneous or subcutaneous nodules may develop into abscesses and ulcerate.[74]

Diagnosis requires a high level of suspicion. Cutaneous lesions should be subjected to biopsy for histology, special stains, and culture. The differential diagnosis includes fungal and nocardial infections. These organisms are ubiquitous, and differentiating respiratory colonization from infection may be difficult. The diagnosis of NTM infection should be entertained, with a low threshold for performing bronchoalveolar lavage (BAL) or biopsy for any unexplained or unresolving pleuropulmonary disease.[110,157] The American Thoracic Society has published diagnostic criteria that include signs and symptoms present after the treatment of other possible diseases, such as radiologic evidence of progressive pulmonary disease, including infiltrates, cavities, nodules or bronchiectasis, and bacteriologic evidence of NTM infection, generally on multiple respiratory specimens or on biopsy.[157]

Providing therapy for NTM infection may be difficult and depends on the species isolated. Most authors recommend combination therapy that includes a macrolide. For MAC, therapy with a macrolide, ethambutol, and rifampin, with or without an aminoglycoside, depending on the severity of disease, is recommended.[157] Therapy for *M. abscessus* is undefined because this organism is notoriously resistant to many antimicrobials. Even regimens based on in vitro susceptibilities may not produce a cure. Combinations that include a macrolide, amikacin, and cefoxitin or a carbapenem, based on susceptibility testing, are

general recommendations. Acquired resistance mutations to clarithromycin and amikacin can occur.[157] The duration of therapy is prolonged and depends on the patient's clinical, radiologic, and microbiologic response, but the suggested minimum duration is 12 to 18 months. Reduction of immunosuppression, surgical excision or débridement, and treatment of co-infections may improve the outcome.[74] Relapses are not uncommon. Consideration of lifelong suppression is recommended by some experts for patients with a high burden of disease and persistently high levels of immunosuppression.[110] Most patients who can complete long-term therapy improve (32% cleared their infection, 36% improved); minimal response to therapy was seen in 23 percent of LTRs.[116] Fatal infections occur rarely.[74,360,410] Drug interactions between macrolides and rifampin and CNIs and sirolimus occur. Both can lower serum levels of immunosuppressive drugs and trigger rejection.

The optimal management of patients infected or colonized with NTM organisms before transplantation is debated. A report of 146 patients with CF who underwent lung transplantation found a highly significant association between pretransplant isolation of *M. abscessus* and post-transplant disease, thus suggesting that these patients remain colonized in respiratory tree sites above the anastomosis. The hilar lymph nodes in two patients were positive for *M. abscessus*, thus suggesting other potential reservoirs.[66] Given the concern for emergence of resistance and toxicity related to long-term therapy, prolonged antimycobacterial therapy cannot be recommended for all patients. Some centers opt for pretransplant or peritransplant prophylaxis, whereas others observe for evidence of disease.[110]

FUNGAL INFECTIONS

Aspergillus

Aspergillus infections remain a major challenge to the success of lung transplantation. Manifestations range from semi-invasive tracheobronchitis and BA infections (up to 60% of infections) to invasive pulmonary disease (30%) and, rarely, dissemination to extrapulmonary sites, including the CNS (10%).[139,376] The published incidence of invasive *Aspergillus* (IA) infections varies considerably and probably reflects differences in dates of publication, patient populations that included adults and children, underlying disease such as CF, net state of immunosuppression, use of prophylactic measures, local issues of exposure (e.g., construction), and rates of rejection and viral infection, among others. In addition, distinguishing between isolation of *Aspergillus* from airway cultures versus true invasive disease may be difficult. Despite these caveats, rates of IA infection in LTRs are the highest among SOTRs. Studies suggest that invasive disease occurs in 3 to 33 percent of LTRs, although rates of colonization/isolation of *Aspergillus* may be considerably higher. In most cases, colonization appears to be transient and does not lead to invasive disease.[57,139,148,173,273,283,285,300,376]

Infections usually develop 1 to 6 months after transplantation and are a major cause of death during this time period.[217,273,283,376] Early infections, those that develop less than 3 months after transplantation, are more likely to be tracheobronchitis or BA site infections, whereas invasive pulmonary or disseminated aspergillosis occurs more often in late-onset infections.[376,390] Patients who require retransplantation or have BA infection may present in the first month.[76,162,176,283]

Recent studies have identified different risk factors for the bimodal pattern of the development of IA.[139,375] Risk factors for early disease in LTRs include *Aspergillus* colonization in the 6 months before transplantation or early post-transplant colonization, a complicated postoperative period, and CMV disease.[57,139,173,189,285,300] Corroboration of the importance of CMV is the observation that a significant decrease in IA was associated with the use of prophylactic ganciclovir in heart transplant recipients. This decrease no doubt reflects the immunomodulating effects of the virus on host immune status.[347,437] Patients in whom IA infection developed later than 3 months after transplantation were older (<50 years); "overimmunosuppressed" with the use of sirolimus, tacrolimus, or both for chronic or refractory graft rejection or dysfunction; or both older and overimmunosuppressed.[139,378] In both the early and late groups, IA was associated with renal failure and repeated bacterial infections.

Aspergillus fumigatus causes the majority of disease, although non-*fumigatus* species are becoming more common.[57,273] Such species include *Aspergillus flavus*, *Aspergillus niger*, *Aspergillus nodularis*, *Aspergillus ustus*, and *Aspergillus terreus*. This change may be important because of the recognized potential for differences in antifungal susceptibility of some non-*fumigatus* species. For example, *A. terreus* is innately resistant to amphotericin B, and *A. ustus* has diminished susceptibility to the azoles.[32]

Clinical findings in patients with pulmonary disease are largely nonspecific and may include cough, shortness of breath, and hypoxia, although chest pain and occasionally hemoptysis may raise clinical suspicion of IA.[84,272] Symptoms are seen more commonly in patients with diffuse disease than in those with focal or nodular disease.[95] Although fever is a common early manifestation of IA in neutropenic hematology patients,[95] it occurred in only 15 percent of LTRs with *Aspergillus* infection and usually more often in patients with disseminated (50%) or invasive pulmonary disease (20%) than in those with tracheobronchial or BA infections (0%).[376] Lack of fever also has been documented in other studies of non-neutropenic patients with IA.[84,272] Patients with disseminated disease may have involvement of the sinuses, orbits, musculoskeletal system (osteomyelitis), ophthalmologic structures, cutaneous sites, and CNS. Symptoms of CNS disease may include headache, sinus pain, cranial nerve abnormalities, seizures, changes in mental status, or other focal neurologic findings.[32,95,148]

The radiologic manifestation of IA in LTRs is highly variable.[95,104,369,414] Chest radiographs are highly insensitive and nonspecific for IA. Computed tomography (CT) of the chest has become an integral part of early diagnostic strategies and has been incorporated into recent definitions of invasive fungal infections in immunocompromised hosts.[25] On chest CT scans of neutropenic adults, large nodules and cavitary lesions are common findings.[25,95,155] In addition, much has been made of the early "halo sign," a macronodule surrounded by a perimeter of ground-glass opacity (edema and hemorrhage), and the later "air crescent sign," a crescent-shaped air interface between viable lung and infarcted tissue at the periphery of a nodule, seen with recovery and return of neutrophils. These findings are considered highly suggestive, though not pathognomonic, of IA.[25,59,155] Unfortunately, these findings are seen less commonly in LTRs and in children in general. Multiple ill-defined pulmonary nodules were the most common findings in adult LTRs, with approximately half demonstrating halo signs. Cavitation was rarely seen, and no air crescent signs were observed.[84,104] In a review of immunocompromised children with IA, radiographic findings were highly variable and included consolidations and small nodular masses and effusions, with little evidence of cavitation and no halo or crescent signs.[414]

The gold standard for diagnosis of IA is the demonstration of small hyaline, septate, dichotomously branched hyphae in tissue with evidence of associated tissue damage or positive cultures from a normally sterile body site in the face of signs and symptoms of invasive disease in an immunocompromised host.[25,32,95,179] Blood cultures rarely are positive and, therefore, are not helpful. Unfortunately, obtaining adequate tissue specimens in a timely manner frequently is not possible. An immunocompromised patient with neutropenia or taking steroids may not exhibit

obvious signs or symptoms of invasive disease until late in the course of infection.[6] Additionally, these patients often are deemed too ill to undergo the invasive procedures necessary to obtain tissue for culture.[384] Therefore, the diagnosis is established late in the course of the disease or at autopsy. Indeed, frequently it is not established but is based on radiology and host factors.

Early diagnosis is thought to be critical to improving the outcome of patients with IA, especially now with the availability of more active therapeutic agents and the possibility of combination therapy. In addition to early and frequent CT scanning in high-risk patients, especially those who do not respond to broad-spectrum antibiotics, several recent advances have been achieved in molecular/non–culture-based approaches to establishing a diagnosis. The most widely used and well characterized of these modalities is the Platelia *Aspergillus* serum galactomannan antigen, detected by double-sandwich ELISA.[276,277,403,452,454] This test detects very small amounts of a polysaccharide cell wall component of *Aspergillus* and other fungal species that is released into blood with invasive growth in tissues. A positive test (<0.5 ng/mL) requires confirmation by a second sample for diagnosis. False-positive tests have been documented, notably in patients receiving piperacillin-tazobactam,[3,26,246,250,382,406,435,444] which may be related to cross reactions to *Penicillium* spp.–derived semisynthetic penicillins,[404] although this theory has not been proved conclusively. In general, false-positive tests result in transient and low-positive findings.[276,452] Pediatric patients appear to have higher rates of false positivity,[175,342,405] but the mechanism for this observation remains unclear. Previous antifungal therapy has been shown to decrease the sensitivity of the assay.[258,261,342]

In controlled trials, twice-a-week galactomannan screening of very-high-risk adults with hematologic malignancies and hematopoietic stem cell transplant (HSCT) recipients had high sensitivity and specificity and allowed the diagnosis of IA to be established, often many days before the development of fever and CT findings, with the potential for initiating early therapy.[252-254,258] The addition of galactomannan testing to early CT scans also was found to be helpful in establishing the diagnosis of IA.[55,251,342] The galactomannan assay has been incorporated into recent diagnostic criteria for the diagnosis of IA in immunocompromised patients with cancer and in HSCT recipients.[25] To date, no studies have documented a survival advantage in patients with early diagnosis by galactomannan testing.

In SOTRs and non-neutropenic patients, this test appears to be less sensitive.[129,183,223,327,426] For example, twice-a-week screening of 70 consecutive LTRs, with IA documented in 17.1 percent, revealed a sensitivity of 30 percent and a specificity of 95 percent.[183] False-negative results occurred most commonly in patients with tracheobronchitis, followed by pulmonary disease, versus patients with disseminated disease. Two of five patients with pulmonary *Aspergillus* and negative tests had received antifungals previously. False-positive results occurred in 36 tests on 14 patients, with a median value of 0.868; consecutive positive tests were seen in 5 patients. The majority of these results occurred in the first 2 weeks after transplantation. None of these patients was receiving piperacillin-tazobactam.

Finally, use of the galactomannan assay for specimens other than serum is being evaluated for cerebrospinal fluid (CSF) and BAL fluid.[35,208,247,292,361,436] Assays on these fluids appear to be more sensitive and to offer some advantage in certain patient populations, such as non-neutropenic SOTRs, although controlled clinical trials have not been published. False-positive galactomannan results have been linked to the use of Plasmalyte (Baxter) for lavage fluid instead of normal saline.[163] The product contains gluconate, which is produced by fermentation in *A. niger* and results from contamination of small amounts of galactomannan by this process.

Several other molecular-based methods for detection that are being investigated include the Fungitell beta-glucan assay, which measures a cell wall component found in many fungi, including *Aspergillus* and *Candida* spp., and, like the galactomannan assay, does not detect zygomycetes.[201,284,301,310,325] It is approved by the Food and Drug Administration (FDA) and is available in kit form. Cross-reacting glucan-containing material occurs commonly in the environment, and false-positive tests have been demonstrated in patients maintained on dialysis or exposed to gauze and with gram-positive bacteremia. In addition, false-positive results are associated with the use of some antimicrobials and with sample manipulation, thus suggesting that laboratory contamination is possible.[106,179,264,325,328,454]

The *Aspergillus* polymerase chain reaction (PCR) remains nonstandardized, but most assays rely on detection of fungal sequences of 18S or 28S rRNA or mitochondrial genes in whole blood, plasma, serum, or BAL fluid.* More recent protocols have used real-time PCR, which allows rapid, automated, quantitative, and reliable results, with less chance of contamination.† Clinical information is limited on both these methods. No studies have been published on the use of these tests in SOTRs, but some show promise in febrile neutropenic cancer patients and HSCT recipients. The optimal clinical specimen and PCR protocol have yet to be established.

Several studies have compared the use of these three methods.[48,52,201,292,325,361] No reproducible results suggest that one test is convincingly or consistently better than the others. Several authors suggest that combining two methodologies may improve diagnostic reliability.[48,85,109,281,292,325,361]

Despite historically poor outcomes of IA in immunocompromised patients, new mold-active therapies appear to be improving mortality figures.[40] The Infectious Diseases Society of America (IDSA) practice guidelines for *Aspergillus* infections, published in 2008, recommends therapy with voriconazole as primary therapy for most patients,[445a] as it has demonstrated superiority.[27,97,174] Additionally, voriconazole has shown promise for treatment of CNS disease, which historically has been uniformly fatal.[174,364,442] Lipid formulations of amphotericin B can be recommended as alternative therapy for patients unable to receive voriconazole. For patients refractory to or intolerant of primary therapy, lipid formulations of amphotericin B, itraconazole, and caspofungin are FDA approved for treatment of IA. Other antimicrobials that have activity against *Aspergillus* include posaconazole and other echinocandins, although these are not approved for this indication.

Most data on the use of voriconazole for IA involved adult HSCT recipients and patients with leukemia, although some studies contained small numbers of pediatric patients and SOTRs. The paucity of data in these groups is important for several reasons. The optimal dosing for pediatric patients remains unclear, and much higher doses than currently recommended probably may be tolerated and beneficial.[396,441] This dosing information is currently under investigation.[396] In addition, several important drug interactions occur with medications routinely used in SOTRs. The most important of these interactions involve tacrolimus and sirolimus, in which concomitant use with voriconazole can lead to decreased metabolism, via cytochrome P-450 3A4/5 (CYP3A4/5), and result in greatly increased serum levels of the immunosuppressant.[354] The use of tacrolimus can be controlled safely by reducing the dose by 50 percent generally and monitoring daily levels.[354,424] The effect of voriconazole on sirolimus levels is even more pronounced, and it is labeled as an absolute contraindication by the manufacturer.[354,356] Several case reports/small series describe the use of these two drugs together, with significant dose reduction of sirolimus (up to 90%) and close follow-up of serum sirolimus levels.[263,266,356] The second azole to be approved for use by the FDA recently is posaconazole, which

*See references 51, 118, 165, 168, 196, 226, 244, 386, 420, 460.
†See references 47, 67, 86, 197, 198, 201, 207, 292, 361, 392, 457, 458.

is available only in an oral formulation. Currently, based on several randomized multicenter studies, it is licensed for prophylaxis of invasive fungal disease in patients with fever and neutropenia and those with chronic graft-versus-host disease.[82,418] Posaconazole also has been used for the treatment of *Aspergillus* in patients refractory or intolerant of other therapy, most of these patients having received an amphotericin product or itraconazole.[215,365,443] The use of posaconazole as primary therapy for IA currently is being investigated. Azoles are generally well tolerated. Unique events associated with voriconazole include transient visual disturbances (photopsia) and cutaneous photosensitivity. Both drugs have been associated with hepatic toxicity, which can be fatal.[108,174]

The polyenes AmBD and its lipid formulation (LFAB) are active against *Aspergillus* spp., except for *A. terreus*, and exert their activity on the cell membrane. AmBD was the "gold standard for serious fungal infections for almost 40 years" but now has been supplanted by newer, more effective and less toxic agents, including the azoles, echinocandins, and LFAB. The recent consensus suggests that LFAB may be more effective than AmBD is in certain settings and is clearly less toxic. LFAB and posaconazole are the most broad-spectrum antifungals and are the only agents active against zygomycetes.[108] One of these drugs should be used singly or in combination if the diagnosis of a zygomycelial infection, which can be clinically indistinguishable from IA and equally fatal, is being considered.

Echinocandins have a unique mode of activity; they inhibit the biosynthesis of 1,3-β-glucan in the fungal cell wall, which is absent in mammalian cells, thus offering a high therapeutic index.[108] Moreover, fewer notable drug interactions have occurred. Initial reports suggested that concomitant use of caspofungin, the first licensed echinocandin, with cyclosporine caused increased hepatotoxicity, although subsequent studies did not substantiate this interaction.[108,260] Caspofungin has been demonstrated to be useful in and has been licensed for the treatment of IA in patients refractory to or intolerant of other therapies[249] and for empiric antifungal therapy in patients with fever and neutropenia.[445] Several studies have addressed the safety, efficacy, and dosing of caspofungin in pediatric patients.[130,159,278,302,440] All studies have found caspofungin to be safe and well tolerated in children, even neonates. As was seen with voriconazole, extrapolation of pediatric dosing from adult dosing led to underdosing of caspofungin, which has been revised on the basis of pediatric pharmacokinetic data.[440] No prospective data on the efficacy of caspofungin for IA in immunocompromised pediatric patients have been published, although two small retrospective studies (64 and 25 patients), consisting mostly of children with leukemia, showed promising results with caspofungin, either alone for less aggressive disease or in combination with voriconazole for first-line therapy.[159,278] A second echinocandin, micafungin, also has been licensed recently for prophylaxis of *Candida* infections in HSCT recipients and for treatment of esophageal candidiasis.[103,421] Randomized, controlled data on the use of micafungin for primary treatment of IA are lacking. Several reports of high-risk patients (HSCT recipients, hematologic malignancy) suggest that it is useful, either alone or in combination, in the setting of refractory IA.[68,96,209] Some studies included small numbers of children, who appeared to have similar success rates. In an assessment of its safety, tolerability, and pharmacokinetics in febrile, neutropenic children, dose-limiting toxicity did not occur with doses of 4 mg/kg/day or lower, and young children cleared the drug up to 1.5 times faster than older children and adults did.[367] Optimal dosing regimens are still under investigation. A final echinocandin, anidulafungin, has been approved for the treatment of candidal infection.[423] It also has potent in vitro activity against *Aspergillus*. A unique property of anidulafungin is that its pharmacokinetics is similar across a variety of patient populations, including children, which suggests that dose adjustments are not necessary. The drug appears to have an excellent safety profile, with minimal drug-drug interactions. Its use in the treatment of IA has yet to be defined, but most likely it will be similar to other echinocandins.

Combination therapy remains controversial. Advantages of combination therapy include possible synergistic interaction of two drugs with different targets, lower doses leading to less toxicity, greater spectrum of activity, and decreased potential for the development of resistance.[234] Potential disadvantages include antagonistic antifungal activity, increased overall drug interactions, enhanced toxicity when adding new agents, and great increases in cost.[194,288,434]

Preclinical data from in vitro and animal studies investigating the use of combinations of antifungal agents against *Aspergillus* are generally promising.[194,288,397,399,434] Complexities and differences in methodologies used to determine in vitro efficacy, experimental design, and animal models confound interpretation of the data and render extrapolating to patients difficult.[288,397,399,453]

The comparator to combination therapy in most clinical data to date is primary therapy with LFAB, not voriconazole, which is now considered the drug of choice for IA. Combination treatment also has been studied for salvage therapy in patients who have already failed therapy; however, most studies are retrospective.[96,211,212,248,259,297,320,377,399] Preferred combinations include azoles (voriconazole or posaconazole) with echinocandins (caspofungin or micafungin).

A single prospective multicenter observational study has evaluated combination therapy in SOTRs.[377] Forty transplant recipients (13 lung transplants, or 33%) received voriconazole and caspofungin as primary therapy for IA (2003 to 2005); 47 patients (9 lung transplants, or 19%) received LFAB (1999 to 2002). A trend toward decreased mortality rates at 90 days in the combination group was noted, and subset analysis demonstrated that in transplant recipients with renal failure or *A. fumigatus* infection, combination therapy was associated with improved 90-day survival ($p = 0.04$ and $p = 0.03$, respectively). No patient required discontinuation because of side effects or intolerance. These authors note that currently, "combination therapy constitutes our standard for the therapy of invasive aspergillosis."[291] Data from prospective, randomized trials in which voriconazole is compared with combinations that include voriconazole still are needed to evaluate first-line therapy. Many authors recommend caution in using combination therapy before the results of controlled, prospective investigations are available, even though they acknowledge that widespread use of combination therapy already is evident.[30,194,288,397,399,434]

No definitive data are available on how long to treat IA, although the natural history of disease, assessment of risk factors, and the state and length of immunosuppression, along with investigations on response to therapy and animal studies, can inform us on regimens most likely to be effective. Clearly, one must balance the risk of incomplete therapy and recurrent disease with the dangers of increased toxicity and expense from prolonged therapy. One thoughtful approach, based on these many variables, suggests use of the "most effective therapy first for 10 to 12 weeks, or for at least 4 to 6 weeks beyond resolution of all clinical and radiographic abnormalities, whichever is longer."[372] Length of therapy should be individualized, and factors such as recovery from neutropenia or other immunocompromised state, extent of disease, presence of graft-versus-host disease, and coinfections (e.g., CMV) predict poorer outcome and slower response and probably will prolong duration of therapy for patients with these comorbid findings.

In terms of adjunctive therapy, surgery may be recommended for cutaneous and soft tissue infections,[171] for lesions on the great vessels or major airways to prevent massive hemoptysis[37,58,60,312] in patients with endophthalmitis[447] and osteomyelitis,[205] and for removal of remove of residual lesions before hematopoietic stem

cell transplantation is performed. Immunomodulatory strategies that improve host immune response, in theory, are attractive adjuvant therapies. Alveolar macrophages, polymorphonuclear neutrophils, and pulmonary dendritic cells are critical first-line defenses against IA.[344] Therefore, modalities that improve the number or function of these cells may improve outcome. Decreasing the use of immunosuppression and steroids as much as possible should be the goal in transplant recipients with IA. Additional strategies, including enhancement of T_H1 immune responses, exogenous administration of colony-stimulating factors (granulocyte colony-stimulating factor [G-CSF], granulocyte-macrophage colony-stimulating factor [GM-CSF]) or cytokines (interferon-γ) to activate or recruit phagocytes (or both), G-CSF donor-primed white blood cell transfusions, and augmentation of innate pathogen recognition pathways (toll-like receptors, pentraxins), all still are investigational.[137,140,343,366,398,400] Finally, vaccine strategies and adoptive immunotherapy have shown promise in animal models of IA and await further refinement and clinical trials.[43-46,64,366]

When *Aspergillus* causes invasive disease, mortality rates are high (30% to 100%) and notably so for invasive and disseminated forms of the infection.[57,139,273,390] In a review of English language articles on *Aspergillus* infections in LTRs, the overall mortality rate was 53 percent (59/112), 24 percent (9/38) for tracheobronchial or BA infections, 82 percent (18/22) in patients with invasive pulmonary disease, and 67 percent (2/3) for disseminated invasive infection.[376] These published data are on adult patients (19-64 years old) and reflect the limited therapeutic options previously available (before the advent of extended-spectrum azoles and echinocandins). Somewhat surprisingly, several studies in which the mortality of IA in neutropenic patients was compared with that of non-neutropenic patients found a comparable or higher incidence of mortality in the latter group.[84,272] Authors speculate that a lower index of suspicion may delay establishing the diagnosis and that suboptimal monitoring or lack of the use of *Aspergillus* active fungal prophylaxis may lead to high mortality rates.

Despite the lack of controlled trials, two surveys document that most lung transplant centers in the United States and worldwide use prophylactic or preemptive systemic antifungals (itraconazole or amphotericin formulations), aerosolized amphotericin formulations, or both in patients with preoperative or postoperative isolation of airway *Aspergillus*.[113,188] Because *Aspergillus* infections are acquired through the lungs and most infections are pulmonary, attaining high pulmonary drug levels via aerosolization and limiting systemic toxicity are attractive options for prevention.[384] Several nonrandomized, non–placebo-controlled trials have suggested a reduction in IA infections with the use of aerosolized amphotericin formulations.[61,111,112,176,283,285,316,338] A single retrospective study incorporating the use of voriconazole prophylaxis in LTRs has been published.[184] This study compared targeted itraconazole prophylaxis, with or without inhaled amphotericin, in patients colonized by *Aspergillus* preoperatively or postoperatively with universal voriconazole for a minimum of 4 months. The rate of IA at 1 year was 1.5 percent in patients receiving voriconazole versus 23.5 percent in the other group. In addition, there was a significant delay in *Aspergillus* colonization in patients receiving voriconazole, thereby permitting healing of the BA, and a considerable decrement in immunosuppressive therapy in most patients by that time. Abnormalities in liver enzymes were common findings; twice as many patients had to discontinue antifungal therapy in the voriconazole group because of side effects. All liver enzymes returned to normal within 2 months of terminating therapy. Finally, interactions with CNIs need to be managed by decreasing doses and monitoring serum levels of tacrolimus. Based on two phase III studies, posaconazole appears to be the drug of choice for prophylaxis of invasive fungal infections in certain high-risk patient populations.[82,418] The generalizability of these findings to SOTRs has not been examined. Further randomized, multicenter studies are needed to clarify which agents and prophylactic strategies are most effective.

Prevention of IA through environmental controls also is important.[319,324,336] Cases of IA in transplant recipients can be nosocomial or community acquired. Most germane to prevention is air control inasmuch as both epidemic IA and sporadic IA correlate with concentrations of *Aspergillus* in the air.[5] A protective hospital environment should consist of high-efficiency particulate air (HEPA) filtration, positive air pressure, high air exchange rate, properly sealed rooms, and removal of carpets, plants, and water-damaged ceiling and floor tiles.[83,116,164,324,413] In addition, the environment and air should be monitored frequently for changes in particulates and fungal spores. Patients should be wearing "fit-tested" masks (N95) when leaving protected environments, effort should be made to have construction barriers in place, and patient transport routes should be adjusted accordingly if construction is occurring in the hospital and clinics.[319] When patients are discharged home, they should be counseled to avoid certain high-risk exposure to agents such as dust and mold if possible.[324]

Candida

Candida infections in LTRs often occur early, within the first month, in the form of BSI; urinary tract infections, especially if indwelling catheters remain in place; respiratory tract infections (tracheitis); pneumonia; and, rarely, BA site infections. Although colonization of the respiratory tract is a common occurrence, primary invasive candidal pneumonia is not. Invasion usually occurs when comorbid conditions are present.[217] Rarely, disseminated disease of abdominal organs and the CNS occurs.[321] The source of infection usually is endogenous, although contributions from donor organs also are possible. Risk factors for candidal infections in transplant recipients include surgical complications, use of broad-spectrum antibiotics, CMV infection, and indwelling foreign bodies.[217,321]

The diagnosis of candidal infection can be challenging and requires differentiating colonization from infection because *Candida* spp. are ubiquitous, especially in the upper GI and respiratory tracts. Although the diagnosis of most candidal infections is made by culture, these techniques are insensitive and, except for blood culture, nonspecific. If recovery of *Candida* spp. from BAL fluid is associated with respiratory symptoms or deterioration of lung function (e.g., increased sputum production), treatment should be considered, although other causes such as rejection and co-infections should be taken into account. *Candida* in the blood should be treated, and indwelling catheters should be removed as soon as feasible. Organisms should be identified to the species level because of species-specific susceptibility profiles. Early germ tube determination can confirm the presence of *Candida albicans*, and a negative test can alert the physician to the presence of non-*albicans* species, which may have an impact on the choice of antifungal agent.[321] Newer molecular probes, such as PNA FISH, can identify *C. albicans* rapidly from positive blood cultures.[7]

The Fungitell beta-glucan assay measures a cell wall component of many fungi, including *Candida* spp., and, therefore, is not specific for *Candida*. It has been FDA-approved as an adjunct for the diagnosis of invasive *Candida*, although its role in the early diagnosis of these infections is under investigation.[321] Use of the serum galactomannan antigen assay and PCR techniques shows some promise, but these techniques are still experimental, and the ubiquity of *Candida* increases concern about contamination with PCR.[133,321] The usefulness of these tests in LTRs with candidal infections is currently unknown.

The choice of agent for the treatment of *Candida* infections has become more complicated; non-*albicans Candida* spp. are becoming more prevalent, as are azole-resistant *C. albicans* isolates.[321,326] *Candida krusei* is innately resistant to fluconazole and itraconazole, whereas *Candida glabrata* has variable susceptibility

to these drugs. Voriconazole and posaconazole are likely to be effective. Treatment failures have been noted with the use of echinocandin therapy for *Candida parapsilosis* infections, and some *Candida lusitaniae* strains may be resistant to polyene antifungals.[108] Putative risk factors for infections with these organisms include widespread use of fluconazole, prolonged pretransplant hospitalization, and long-term use of broad-spectrum antibiotics. The choice of initial antifungal therapy should be based on azole exposure, susceptibility profiles, dominant species at the institution caring for the patient, comorbid conditions, sites of involvement, and the use of other medications that might have significant drug-drug interactions.[321] Fluconazole is appropriate for *C. albicans* infections in moderately ill patients. For severely ill or hemodynamically unstable patients or for those with suspected or proven *C. glabrata* or *C. krusei* infection, the use of LFAB or an echinocandin is preferred. Alternative agents include the newer azoles voriconazole and posaconazole. Duration of therapy, as with other infections, should be individualized, but treatment should be continued for at least several weeks after the last positive culture has been obtained and the signs and symptoms have resolved.[322] As noted earlier for *Aspergillus* infections, issues of dosing in pediatrics with the newer antifungals and drug-drug interactions should be considered. Several excellent resources are available for detailed treatment algorithms.[321,322,391] Updated IDSA guidelines for the treatment of candidal infections are expected in 2007. Antifungal susceptibility testing should be considered on non-*albicans Candida* spp. and for *C. albicans* infections that are refractory to conventional therapy. Interpretive breakpoints are available for most common antifungals, although those for the echinocandins still are being determined.[6,13,321]

Cryptococcus

Though relatively rare, cryptococcosis is the third most common fungal infection seen in solid organ transplantation, after *Aspergillus* and *Candida*.[430] Disease can be newly acquired, reactivated from latent disease, or derived from the donor organ.[147,187,464] Although meningitis generally is thought to be the most common manifestation of cryptococcal disease,[187,464] two recent studies suggest that in SOTRs, CNS disease and pulmonary disease are equally common occurrences.[374,430,433] Disseminated disease also is a common finding (61% of cases) and is most likely to be seen in liver transplant recipients and least likely in LTRs.[374] Infection occurs less frequently in pediatric patients than in adults[385] and more commonly in the northeastern United States than in other locations.[430] It is most common in heart and liver transplant recipients and least common in LTRs.[430,433,464] Additional unique properties of cryptococcal infections appear to be seen in the SOTR population, notably an intriguing relationship between the immunosuppressive regimen and clinical findings. A review of published reports of cryptococcal infections in transplant recipients and a prospective multicenter study suggest that patients receiving CNI-based immunosuppressive therapy were less likely to acquire CNS disease and had lower mortality rates.[187,374] A proposed mechanism for protection of this site is the temperature-dependent antifungal activity of CNIs, which suppresses fungal growth at 37° C but not at 24° C, coupled with the high level of CNS penetration by tacrolimus.[187] Additionally, these authors suggest that cutaneous infections may appear more like bacterial cellulitis than the typical molluscum contagiosum–like lesions seen in HIV-infected patients.[187,385] Finally, an immune reconstitution syndrome that correlates with the evolving host response to infection also has been described in SOTRs with cryptococcosis,[381] and it may be associated with allograft loss, as described in renal transplant recipients.[380]

The diagnosis can be made by culture, visualization of encapsulated yeast with India ink or other special stains, and cryptococcal capsular polysaccharide antigen in serum or CSF.[72] Serum cryptococcal antigen is most reliable for CNS and disseminated disease but not for pulmonary infection.[430,433]

The mainstay of therapy for severe, disseminated disease with CNS involvement remains combination induction therapy with an amphotericin-containing regimen plus flucytosine.[355,379] Therapy should continue for an extended period because relapses are well documented, especially in HIV-infected patients. The risk of relapse in SOTRs and the optimal length of therapy are not known. Suppressive therapy with fluconazole for 6 to 12 months has been suggested after induction therapy.[355] Duration of treatment less than 6 months has been associated with an increased rate of relapse.[379] Management of elevated intracranial pressure also should be considered.[72] For less severe disease localized to the lungs, fluconazole alone may be adequate.[355,379] Mortality rates vary by site of infection and type of transplant and possibly by immunosuppressive regimen. The overall mortality rate for cryptococcal disease in SOTRs is 14 to 30 percent. The highest rates are seen in liver and kidney transplant recipients with disseminated disease or fungemia, or both (20-70%). The lowest mortality rates (3-13%) are seen in localized pulmonary disease.[374,433,464]

Pneumocystis jiroveci (Formerly *Pneumocystis carinii*)

Pneumocystis pneumoniae (PCP) infections are caused by the renamed fungal pathogen *Pneumocystis jiroveci*.[402] PCP has been associated notably with defects in T-cell immunity, as in HIV infection, in patients with leukemia, and with the immunosuppression used in SOTRs. It also is described in patients with other immune defects, including those associated with steroid use and radiation therapy, neutropenia, and congenital immunodeficiency.[341] Serologic data suggest universal, albeit asymptomatic, infection occurs by the time the patient is 4 years of age. In most cases, symptomatic PCP in an immunocompromised host is thought to be reactivation of latent infection, although person-to-person transmission also may occur.[93]

Heart-lung recipients and LTRs are at increased risk for developing infection, with reported attack rates of 6.5 to 43 percent without prophylaxis, up to a 10-fold increase from heart transplants alone.[341] Higher rates of infection in LTRs have been associated with CMV co-infection, use of cyclosporine and steroids, and receipt of therapy for rejection. In addition, cases can occur many years after transplantation. Therefore, many experts suggest the benefit of long-term and perhaps lifelong prophylaxis, especially if additional risk factors are ongoing.[332,341] The drug of choice for prophylaxis remains TMP-SMX, and its use is associated with dramatic decreases in the incidence of PCP infection. Additional benefits of this drug include prevention of many community-acquired respiratory, GI, and urinary tract pathogens and protection against most *Toxoplasma gondii* and *Nocardia* infections. Multiple regimens exist, and 3-day/wk dosing appears to be as effective as is daily dosing in all populations studied. Toxicity remains an issue for some patients and includes bone marrow suppression, decreased renal function, hepatitis, and rash/Stevens-Johnson syndrome, which may be severe. Alternative regimens exist for patients intolerant of TMP-SMX, but they should be considered second line because of breakthrough PCP infection or diminished coverage of non-PCP pathogens mentioned earlier. Such alternatives include pentamidine, dapsone, atovaquone, and clindamycin/pyrimethamine. Details of these regimens have been reviewed recently.[341]

PCP infection is manifested as progressive dyspnea, cough, tachypnea, chest pain, and cyanosis over the course of days to weeks. In addition, fevers, sweats, and flulike symptoms may be prominent. Hypoxia and shortness of breath, in the context of normal or minimal findings on chest radiographs, are common findings. Steroids and CNIs may alter the signs and symptoms and render early diagnosis more difficult to establish. The diag-

nosis is confirmed by identification of *P. jiroveci* in respiratory samples or lung tissue. Immunofluorescent staining of organisms with monoclonal antibodies is the technique of choice; Gomori methenamine silver nitrate, which stains cyst forms only (5-10% of the total organisms), is the most reliable staining method.[332] BAL fluid and tissue obtained by open lung biopsy are preferred specimens because they have the highest diagnostic yield.[341] Newer molecular techniques, including PCR, are promising but are nonstandardized and not commercially available.[114,238]

The mainstay of therapy for severe PCP infection remains high-dose TMP-SMX for 14 to 21 days.[126] Therapy can be continued through mild adverse events, such as rash and minimal perturbations in transaminases and complete blood counts. Dose reduction, desensitization, or both may be helpful, and renal dosing should be implemented if renal dysfunction is present. Steroids may be added for severe disease.[332] For patients intolerant of TMP-SMX or those less seriously ill, alternative therapies can be used. Second-line drugs are dapsone with trimethoprim, atovaquone, and intravenous pentamidine. Third-line therapies include trimetrexate, clindamycin/primaquine, and pyrimethamine/sulfadiazine, as well as several others under study.[126] Relapse in patients without acquired immunodeficiency syndrome (AIDS) is an uncommon occurrence with TMP-SMX therapy if a reduction in immunosuppression can be accomplished and co-infections such as CMV do not occur.[126]

Endemic Mycoses

Cases of endemic mycoses—histoplasmosis, blastomycosis, and coccidioidomycosis—causing disease in SOTRs have been reported. Infection may be caused by latent reactivated disease or by donor-derived and newly acquired infections. Because of the nonspecific findings, latency of these organisms, increased worldwide travel, and organ procurement from different parts of the country, infections in transplant recipients may be difficult to recognize, especially if they occur in nonendemic areas.

Coccidioidomycosis, caused by *Coccidioides immitis* and *Coccidioides posadasii*, is the endemic mycosis most commonly seen after transplantation. It occurs in as many as 9 percent of SOTRs in endemic areas of the southwestern United States (especially California and Arizona), northern Mexico, and Central America.[40] Unlike histoplasmosis and blastomycosis, pretransplant infection poses an increased risk for development of post-transplant disease. In addition, if secondary prophylaxis is not given, the mortality rate is very high (70%).[40] Pulmonary infections may be accompanied by an acute onset of respiratory symptoms and fever and progress rapidly to respiratory failure. Nonspecific symptoms of anorexia, weight loss, and fatigue may herald extrapulmonary disease. Dissemination to bones, skin, and the CNS occurs commonly. The time of highest risk appears to be in the first 3 months after transplantation, with the majority of infections occurring by 1 year. Donor-derived coccidioidomycosis has been established in LTRs and liver and renal organ recipients.[280,415,463] None of the patients had evidence of pretransplant coccidioidomycosis, and none lived or traveled to endemic areas. Several of the donors had documented epidemiologic risk factors. These patients all had fulminant pulmonary or disseminated infection within the first 2 weeks after undergoing transplantation. Three of the four infections were fatal.

The diagnosis of coccidioidomycosis can be made definitively by culture, usually from respiratory samples or urine and rarely from blood or CSF.[20] Histopathologic evaluation may reveal large spherules with the presence of endospores. Serologic tests also are available and include tube-precipitin antigen (IgM), which can be detected early in disease, and complementation fixation (IgG), which arises later in the course of infection and is helpful in monitoring response to therapy.[20,40] Immunocompromised patients may have negative serologic findings. Skin testing for delayed hypersensitivity no longer is available in the United States. The optimal therapy for SOTRs with coccidioidomycosis is unknown. Most experts suggest that patients with rapidly progressive, nonmeningeal disease should receive amphotericin B–based therapy until stabilized, followed by a prolonged course of azole therapy (fluconazole, itraconazole, possibly voriconazole or posaconazole). For meningitis, high-dose azoles are indicated, probably lifelong. Patients with evidence of previous infection should receive secondary prophylaxis with azole therapy for a minimum of 6 to 12 months after undergoing transplantation.[20,40,135]

Histoplasmosis capsulatum infection is a rare occurrence after transplantation, even in patients with presumed pretransplant infection. Cases have been observed in conjunction with outbreaks in areas hyperendemic for histoplasmosis, the Mississippi and Ohio River valleys and Central America.[419] Two cases of histoplasmosis in renal transplant recipients were linked by molecular typing to donor-derived disease.[236] Manifestations include pulmonary, mediastinal, inflammatory, and disseminated syndromes with or without CNS involvement.[455] The diagnosis can be made by culture, histopathology, serology, or antigen detection.[455] In immunosuppressed patients with disseminated disease, urine antigen detection and culture appear to be the most useful (sensitivity of 80%).[451] Long-term therapy (3 to 4 months) with LFAB generally is recommended for immunocompromised patients with disseminated or CNS involvement, followed by 6 to 12 months of itraconazole or posaconazole.[450,455] Monitoring antigen levels is beneficial to assess response to therapy and relapse.[451]

Blastomycosis dermatitidis, the causative agent of blastomycosis, appears to be even more uncommon in the post-transplant setting. Indeed, it is a rare event in series of fungal infections in immunocompromised patients, including patients with AIDS, even in endemic areas of the south central and north central United States. Several case reports of post-transplant disease have been described. Manifestations can involve pulmonary, cutaneous, and disseminated disease with or without CNS involvement.[200,368] Most immunocompromised patients should receive an amphotericin-containing regimen until stabilized, followed by a prolonged course (6-12 months) of an azole. Most data are available for itraconazole, although voriconazole and posaconazole also may be effective.[71,200]

Emerging non-*Aspergillus* Mycelial Fungi

The impact of non-*Aspergillus* mycelial fungi is currently unknown for LTRs. Published reports suggest that these mold infections are becoming increasingly common. These organisms include non-*Aspergillus* hyalohyphomycetes, or hyaline hyphae without pigment (*Scedosporium apiospermum*, *Fusarium* spp.); phaeohyphomycetes, or dematiaceous pigmented molds (*Cladophialophora bantaina*, *Scedosporium prolificans*, *Exophiala jeanselmei*, *Pyrenochaeta romeroi*, *Cladosporium* spp.); and zygomycetes, or nonseptated hyphae (*Rhizopus* spp., *Mucor* spp.). All have been described in SOTRs.[182] Many of these organisms have unique susceptibility profiles and thus are less amenable to conventional antifungal agents. The newer azoles, voriconazole and posaconazole, show some promise for therapy.[108] Indeed, these infections may be more likely to be disseminated and fatal than those caused by *Aspergillus* spp.[182]

VIRAL INFECTIONS

Cytomegalovirus

The epidemiology of CMV in SOTRs has changed in recent years. Advances in diagnosis and in preemptive and prophylactic

strategies and therapy have decreased the impact of early CMV in transplant populations. However, it remains one of the most important pathogens in SOTRs, with wide-ranging effects on morbidity and mortality. The source of infection can be endogenous reactivation, or it can be carried by the donor graft or leukocyte-containing blood products and cause primary infection or re-infection. Rarely, primary infection may be acquired from the community.[346]

The publication of definitions has helped standardize studies on rates of CMV and the impact of therapy.[242] The effects of the virus have both profound direct and indirect consequences.[242,346,466] Direct effects of the virus range from CMV infection, which can be detected by viremia (culture positive), antigenemia (pp65 in leukocytes), and DNAemia or RNAemia (generally by PCR), to true end-organ disease. The diagnosis of end-organ disease, such as pneumonitis, GI disease, hepatitis, and CNS disease, requires signs or symptoms of disease at that site with detection of CMV by culture, histopathology, immunohistochemical staining, or in situ hybridization. Except for CNS disease, detection by PCR is not sufficient for establishing the diagnosis because it is probably too sensitive and may signify transient viremia. In addition, the identification of co-pathogens is important and may confound the diagnosis. CMV syndrome with fever, neutropenia, or thrombocytopenia (or any combination) and detection of CMV in blood also have been defined in SOTRs.[242]

The indirect, immunomodulatory effects of CMV appear to have broad-reaching consequences, as mentioned previously. Epidemiologically, CMV has been associated with dysfunction and rejection of the graft, accelerated atherosclerosis (heart transplant), secondary infections, and BOS.[242,467]

The incidence of CMV infection in LTRs is higher than that in other SOTRs. The reasons are multifactorial but include intense immunosuppression, high levels of CMV latency, viral load, and recurrence associated with the lung and its transplantation.[31,468] The incidences of infection and disease are reported to be 54 to 92 percent without prophylaxis[468] and 30 to 86 percent with various different strategies, thus pointing to the difficulty in identifying the optimal regimen.[467] The greatest risk factor for developing CMV infection is donor/recipient (D/R) mismatch, with D^+/R^- having the highest rate of infection, although infection does occur in D^+/R^+ and rarely in D^-/R^- matches.[89] Other risks include the use of antilymphocyte antibody and blood-product transfusion.[468] A study of pediatric LTRs reported that CMV viremia developed in 23 percent of 194 patients.[89] CMV prophylaxis was given to all D^+ or R^+ patients and consisted of 42 days of intravenous ganciclovir. The median time at onset was 80 days. A first episode of viremia was associated with retransplantation or death between days 90 and 365. Early viremia, before 42 days, was not associated with mortality. This study did not find an association between viremia and BOS, but patients with viremia were statistically more likely to experience two or more episodes of acute rejection.

A combination of tools can be used to reduce the incidence of CMV infection. The use of CMV-negative or leukocyte-free blood products for CMV-negative recipients is standard practice in most transplant centers and has been shown to reduce infection rates. Matching of D/R serologic status probably would reduce infection rates but also would limit the donor pool for R^- recipients significantly and is not considered to be indicated. The final strategies use different regimens of antivirals to suppress replication and immunoglobulin products for enhanced passive immunity.[467] These regimens can be prophylactic, in which all patients at risk are treated for a certain period, or preemptive, in which highly sensitive screening techniques, antigenemia (pp65) or DNAemia, are used to identify patients needing therapy. To complicate matters, regimens can include multiple antivirals such as intravenous ganciclovir or acyclovir, and/or oral ganciclovir, acyclovir, or valganciclovir for various periods. A recent international survey of pediatric lung transplant centers reported variable approaches to reducing the incidence of CMV infection.[90] All centers used prophylactic ganciclovir or valganciclovir, generally based on serostatus stratification; the duration of therapy ranged from 3.5 weeks to indefinitely, but most used 12 weeks. In addition, 50 percent of responding centers used CMV intravenous immunoglobulin (IVIG) with variable schedules. One center used a combination of prophylaxis followed by preemption using antigenemia. Although most centers used active surveillance of antigenemia for detection of CMV, PCR also was used, and one center used viral culture. The monitoring regimen also varied considerably.

A consensus of evidence-based recommendations by an expert advisory group has been published recently.[468] The recommendations are as follows: 1. All D^+ or R^+ LTRs should be considered for prophylaxis. 2a. Prophylaxis with valganciclovir for at least 100 days significantly reduces CMV infection. Some limited data suggest that longer duration (up to 180 days) may reduce infection rates further.[142,469] 2b. Combination prophylaxis with CMV IVIG should be considered, if available, because evidence suggests that its use may reduce infection rates further.[466,469] 3. Monitoring should be performed every 2 weeks for the first 6 months after transplantation to assess for breakthrough viremia and disease. Whole-blood quantitative PCR is the method of choice and should be validated at each center. If a preemptive approach is being used, monitoring should be performed more frequently. 4. Breakthrough disease should be treated with ganciclovir, 5 mg/kg every 12 hours for up to 21 days, until the viral load is below detection. Immunosuppression should be reduced if possible. 5. Infection that occurs after the initial prophylaxis has been stopped should be treated with ganciclovir or valganciclovir until the viral load is below detection. 6. Resistance should be considered in patients with breakthrough viremia, recurrent infections, or poor response to therapy. Genotypic analysis should be performed on isolates recovered from these patients. Foscarnet is the drug of choice, with or without ganciclovir, for possible resistant strains. Of note is that valganciclovir is not FDA-approved for prophylaxis in LTRs, although it is for other SOTRs. Available data suggest that it is safe and effective in LTRs as well.[181,469] Data in pediatric patients are lacking. These authors believe that the data are stronger for the safety and efficacy of prophylaxis than for preemptive strategies, although they acknowledge that some centers have used the latter approach effectively.

Epstein-Barr Virus/Post-transplant Lymphoproliferative Disorder

The disease manifestations of primary EBV infection in SOTRs range from uncomplicated mononucleosis to conditions indistinguishable from malignant lymphoma. Early lesions of EBV-driven lymphoproliferation, such as the plasmacytic hyperplasia, are seen in mononucleosis-like syndromes, within the context of normal tissue architecture. The term post-transplantation lymphoproliferative disease (PTLD) generally is reserved for proliferation of EBV-positive immunoblasts and atypical lymphocytes associated with effacement or destruction of normal tissue architecture. These disorders can be classified further into polymorphic or monomorphic PTLD and finally malignant lymphomas that may contain clonal chromosomal abnormalities. These tumors are characterized by rapid and progressive growth despite a reduction in immunosuppression. Fatal non-PTLD viral syndromes can occur in SOTRs as well.[154] The reader is referred to several excellent, in-depth reviews on these topics.[154,335,409]

The manifestation of EBV/PTLD is varied and depends on the site of involvement, which may be intrathoracic, extrathoracic

(abdominal, head and neck, CNS), or disseminated and intranodal or extranodal.[235] Often, PTLD is seen in the allograft, as with lung, liver, and intestinal transplantation.[154] Signs and symptoms of PTLD can be nonspecific, such as fever of unknown origin, malaise, weight loss, and sore throat.[42] Abdominal pain, GI bleeding, intestinal obstruction and perforation, allograft dysfunction such as changes in respiratory status in LTRs, diffuse lymphadenopathy on physical examination or CT, or hepatosplenomegaly should raise the index of suspicion for PTLD. Focal neurologic findings also can be seen with CNS disease. An additional challenge is to differentiate allograft rejection from PTLD; both entities in LTRs may be characterized by allograft dysfunction with diffuse consolidation on imaging, without obvious lymphadenopathy or a mass. Because the therapies for these two conditions are diametrically opposed, increasing or decreasing immunosuppression, this distinction obviously is critical.[154,335]

Risk factors for the development of PTLD have been identified.[42,79] Pretransplant EBV seronegativity (as a surrogate marker for the risk of development of post-transplant primary EBV infection) is probably the most important predisposing factor for the development of PTLD. Because primary EBV infection occurs almost universally in children, older individuals are already immune and young children undergoing transplantation are, therefore, at greatest risk. In addition, concomitant CMV infection or CMV mismatch before transplantation increases the risk for developing PTLD. Patients at highest risk by type of transplant include recipients of lung and intestinal organs. This risk is probably multifactorial and includes more intense immunosuppressive regimens and transplantation of large amounts of lymphoid tissue with the graft, thereby increasing recipients' potential exposure to donor-derived EBV. The published incidence of PTLD in pediatric LTRs ranges from 7.7 to 26.3 percent.[42,79,329] Certain immunosuppressive regimens, such as the use of OKT3 and polyclonal antilymphocyte antibodies and possibly tacrolimus in pediatric patients, are linked to the development of PTLD. Many experts acknowledge that the overall intensity of immunosuppression, not a specific agent, is most important in defining the risk for development of PTLD.

PTLD most often occurs within 12 months after transplantation.[42] Late PTLD has been documented and appears to have specific properties, including an increased incidence in older recipients, long duration of immunosuppression, EBV-negative disease, and poorer prognosis.[79,120,227]

The diagnosis of PTLD requires a high index of suspicion. The gold standard is histopathologic examination of tissue from either excisional or fine-needle biopsy. Specimens should be processed by pathologists familiar with the morphologic classifications, and ancillary tests that may be helpful include staining for EBV-encoded RNA 2 by in situ hybridization (EBERS) and the presence of CD20, which has implications for therapy.[335] Imaging also can be used for presumptive diagnosis, to guide biopsy, and for follow-up.[329,370] In LTRs, CT of the chest may reveal discrete nodules, airspace consolidation, or mediastinal lymphadenopathy. Extrathoracic involvement is less common but may include abdominal lymphadenopathy, liver/spleen lesions, and a thickened bowel wall. On head and neck imaging, the cervical lymph nodes, pharynx, orbit, sinus, and rarely the brain are found to be involved occasionally.

Patients at the highest risk for mortality are those in whom primary EBV infection develops early after transplantation is performed; therefore, every effort should be made to identify these patients. Pretransplant serologic assessment of donors and recipients for both EBV and CMV can alert physicians to most of these high-risk patients, as donor/recipient mismatch of either virus appears to play an important role.[42,79] Once identified, these patients should be monitored carefully by PCR for evidence of primary infection, and careful examination and use of CT scanning for early diagnosis of disease should be included. Several strategies have been used for the prevention of EBV/PTLD in high-risk patients.[154,335] The use of EBV-negative donors, though probably effective, would reduce the donor pool substantially and generally is not advocated. The use of prophylactic antiviral therapy has not been proved to prevent the development of PTLD. Although these agents are active against the lytic viral infection that proceeds PTLD, they are not active in the latent viral phase that characterizes PTLD. Acyclovir and ganciclovir often are used for prevention of CMV, and historical comparisons of the incidence of PTLD in certain high-risk patients receiving them suggest some benefit[92]; however, PTLD has developed in some patients while receiving these antiviral agents. The use of IVIG is similarly a potential, but unproven strategy and is currently undergoing evaluation.[154]

Preemptive strategies aimed at identifying primary infection by blood PCR monitoring have received significant attention.[29,36,151,154,335,345,401,417] In these protocols, high-risk patients undergo EBV quantitative viral load monitoring at frequent intervals, usually weekly, to identify increases associated with infection, a prerequisite for the development of EBV/PTLD. Many issues remain regarding the use of EBV viral load.[151,153,335] For example, these assays are not standardized and therefore are difficult to compare among institutions. Many questions are raised: Which compartment is most revealing and should be sampled—peripheral blood lymphocytes, whole blood, or serum? Is there a threshold level at which therapy should be instituted? How does serostatus affect the sensitivity and specificity of viral loads? Additionally, not all patients with EBV/PTLD have elevated viral loads, and viral loads may be elevated without evidence of disease; therefore, these assays lack specificity. Although these assays are now widely used at transplant centers, further controlled studies are necessary to optimize their use in immunocompromised patients.

When patients with high viral loads are identified, several therapeutic options are available; however, few controlled trials of these interventions have been performed, and the optimal timing and use of each are unknown at this time.[151,336] All experts agree on immediate reduction or cessation, if possible, of immunosuppressive regimens. Most patients with nonmalignant lesions will respond to this maneuver alone. The use of antiviral agents has become routine, though without proven benefit. Patients with a persistently high viral load should be evaluated aggressively for the presence of PTLD on examination and imaging, and suspicious lesions should undergo biopsy for the diagnosis of EBV/PTLD. When the diagnosis of PTLD is made, ideally, immunosuppression should be discontinued, the optimal duration of which is uncertain. Re-initiation of immunosuppressive agents usually is prompted by rejection or clear evidence of clinical and virologic response. Surgical resection and local radiation therapy may be beneficial for localized disease and in the event of GI involvement.

Monoclonal B-cell antibody therapy is now an attractive option for $CD20^+$ lesions not responsive to a reduction in immunosuppression.[335] The currently available product is an anti-CD20 antibody (rituximab). Initial retrospective data reported complete remission rates of 62.5 percent,[282] and these data have been corroborated in several prospective trials.[39,119,303] This therapy generally is well tolerated, although long-term hypogammaglobulinemia is a common occurrence in recipients. In addition, severe CMV and hepatitis B and C infections, as well as parvovirus B19–induced aplasia and enteroviral meningoencephalitis, have been associated with its use.[334] Detailed recommendations for the initial approach to pediatric transplant recipients with EBV/PTLD have been published.[151] For refractory disease, cytotoxic chemotherapy may be indicated. Other investigational therapies include anticytokine therapy (anti–interleukin-6), adoptive immu-

notherapy (EBV-specific cytotoxic T lymphocytes, lymphokine-activated killer cells), and boosting of the host immune response (interferon-α). Vaccine technology is in progress.[151,334]

Other Herpesviruses: Herpes Simplex Viruses 1 and 2, Varicella-Zoster Virus, Human Herpesviruses 6, 7, and 8

Infections with herpes simplex virus (HSV) 1 and 2 are common occurrences in SOTRs, and in older children and adults, HSV usually is a reactivation disease manifested as orolabial or genital disease.[311] Primary disease occurs in young children who contract the infection from viral shedding of close contacts. In LTRs, serious or disseminated disease such as esophagitis, hepatitis, and pneumonitis can occur.[387] Infections tend to occur early, within the first month after transplantation, unless prophylaxis is used. As with other herpesviruses, concomitant infection with CMV is not an uncommon event. The diagnosis can be made clinically when typical lesions are present. Culture and PCR technology can be helpful for unusual manifestations. Prophylactic strategies with ganciclovir or acyclovir for CMV can prevent HSV. Intravenous acyclovir and oral valacyclovir or famciclovir are effective therapies for localized mucocutaneous disease. More severe disease should be treated with intravenous formulations only. Resistance of HSV in SOTRs is rare, but when it occurs, it should be treated with foscarnet.[311]

Primary varicella-zoster virus (VZV) infection can occur in pediatric LTRs, as can reactivation of previous disease leading to herpes zoster. No data on VZV infections in SOTRs have been published since the widespread use of varicella vaccine (Varivax), but, presumably, rates of infection after transplantation are low. Patients should be screened for evidence of immunity, and seronegative transplant candidates should receive varicella vaccine, optimally at least 6 weeks before transplantation.[311] If the vaccine does not prevent disease, it may mitigate development of severe disease. In addition, susceptible family members also should receive vaccine. Exposure in postoperative patients should be treated with a high-titer varicella immunoglobulin preparation if available or IVIG within 96 hours of exposure. Additionally, some experts recommend the use of prophylactic acyclovir if exposure such as household or intimate contact is likely to lead to development of disease or is revealed past the window for immunoglobulin prophylaxis to be effective. Varicella vaccine, which is an attenuated live viral product, generally is not recommended in post-transplant recipients at this time. Limited data suggest that it is safe and may ameliorate disease in some immunocompromised patient populations, such as those with leukemia, HIV infection, or renal transplants.[54] Detailed recommendations for prophylaxis are given in the American Academy of Pediatrics *Red Book*.[17]

Patients taking steroids, especially during the incubation period, are at increased risk for the development of severe disease. Hemorrhagic and disseminated disease, including encephalitis, hepatitis, and pneumonitis, can occur in immunocompromised patients.

Herpes zoster is a common event in adult transplant recipients; rates in children have not been published for SOTRs. A recent study of SOTRs aged 16 to 74 years found a 15 percent incidence of herpes zoster in LTRs, with a mean time to onset of 14 months and a median of 9 months (9 days-5.8 years).[149] The diagnosis is made clinically and with the use of viral culture or PCR techniques if necessary. Infections should be treated with intravenous acyclovir and pain control. Steroids should be diminished if possible.

HHV-6 is an emerging opportunistic viral pathogen. Seropositivity reaches nearly 100 percent in early childhood, and HHV-6 is responsible for febrile illnesses with or without GI or upper respiratory symptoms, febrile seizures, roseola (exanthema subitum, sixth disease), and asymptomatic infection in normal infants and young children.[9] Therefore, disease in all but the very youngest transplant recipients probably represents reactivation. Several studies have documented HHV-6 by PCR or culture (or both) in a majority (66-90%) of apparently asymptomatic adult LTRs.[192,231] Reactivation of HHV-6 can occur in the setting of CMV antiviral prophylaxis. These infections occur early after transplantation (median of 6 and 18 days), and most are without obvious clinical manifestations that could be ascribed to HHV-6 alone. In immunocompromised patients, HHV-6 appears to exert direct and indirect effects on the host. It probably is responsible for rare cases of encephalitis, hepatitis, pneumonitis, febrile illnesses, and bone marrow suppression.[231] The presence of HHV-6 alone or as a cofactor to CMV may augment the immunomodulatory effects and increase the risk associated with fungal and other infections, as well as allograft rejection, BOS, and mortality.[192,275,296]

Many questions regarding HHV-6 in this population remain unanswered. PCR of peripheral blood lymphocytes is the most sensitive method for detection of virus, but it cannot differentiate between latent and active virus. Routine monitoring of asymptomatic patients is not recommended. Data are insufficient to recommend prophylactic or preemptive therapies, although the virus is susceptible to achievable levels of ganciclovir, foscarnet, and cidofovir. Finally, which patients should be treated remains unclear. Treatment with ganciclovir, foscarnet, or cidofovir may be considered in patients with compatible syndromes if other causes are eliminated. Whether asymptomatic patients with documented HHV-6 infection would benefit from therapy with regard to the indirect effects of the virus remains unknown.[5]

The role of HHV-7 and HHV-8 in SOTRs is undefined. HHV-7 is similar to HHV-6 in normal hosts, although it appears to be less prevalent and occurs later in life. Symptomatic disease is less well characterized, but it may be responsible for similar syndromes seen with HHV-6 in normal children and transplant recipients.[311] Evidence of HHV-8 infection in normal children is rare. It is associated with Kaposi sarcoma in immunocompromised patients, as well as body cavity lymphomas, PTLD, and Castleman disease.[10] It also may be associated with fever and bone marrow suppressive syndromes of donor origin in transplant recipients.[245]

Community-Acquired Respiratory Viruses: Respiratory Syncytial Virus, Parainfluenza Virus, Human Metapneumovirus, Influenza, and Adenovirus

The paramyxoviruses respiratory syncytial virus (RSV), parainfluenza virus (PIV), and human metapneumovirus (hMPV) are common causes of upper and lower respiratory tract disease in normal children; symptoms range from congestion and rhinorrhea to laryngeal tracheobronchitis (croup), bronchiolitis, and pneumonia.[12-14] Disease can be severe and persistent in immunocompromised patients, although the exact impact of these viruses on LTRs is not well studied. Co-infection and sequential infection with other respiratory viruses, including CMV, probably occurs.[143] Importantly, transmission occurs from person to person, from exposure to nasopharyngeal secretions, from infected individuals, and from fomites. Consideration should be given to screen recipients for incubating respiratory viruses before they undergo transplantation, especially if high levels of circulating virus are present in the community or illness is present in family members, or both. Strict adherence to hospital infection control practices is most important in reducing nosocomially acquired disease from visitors and health care workers. Patients and their families should be counseled on avoidance of exposure and on good handwashing practices when discharged from the hospital.[81]

RSV can cause severe lower respiratory tract disease in LTRs. Although most infections resolve, with or without therapy, fatal

cases have been reported, as have long-term declines in pulmonary function.[38,143,268,448] The diagnosis can be made by rapid antigen-detection kits, standard viral culture, or more rapid shell vial culture methods, and some centers offer PCR detection; the preferred specimen is nasal washings, although in adults with lower tract disease, BAL fluid is recommended,[14,81] Risk factors for poor outcome in immunocompromised patients include neutropenia, lymphopenia, age younger than 1 year, underlying lung disease, and augmented immunosuppression.[81] High mortality rates in HSCT recipients have led to prevention and treatment strategies that can serve as models for care in SOTRs,[102,144,456] though they are not without controversy because no controlled data exist.[38,146] Supportive care remains the mainstay of therapy. For children with upper tract disease and risk factors or lower tract disease, aerosolized ribavirin should be considered. In addition, because outcomes, even in treated HSCT recipients, are poor with ribavirin alone, some experts suggest combination therapy with RSV IVIG or monoclonal antibody (palivizumab) for immunocompromised patients with significant infections.[38,81,407] Effective prophylaxis with palivizumab and RSV IVIG has been achieved in young infants with lung disease.[160,274] Care must be taken when extrapolating to other patient populations, given the unexpected results of some studies in infants with complex congenital heart disease who are administered RSV IVIG[371] but not pavilizumab.[123] Some experts would consider the use of immunoprophylaxis in young infants undergoing organ transplantation during the RSV season.[81]

hMPV was described in healthy young children in 2001. It appears to have signs and symptoms similar to those of RSV, and almost universal infection probably occurs by 5 years of age.[422,459] The impact of hMPV on immunocompromised patients is as yet uncharacterized, in part because it has been identified relatively recently and diagnostic tests are not widely available. The virus can be cultured, but reverse transcriptase PCR appears to be the test of choice. Antigen assays are not yet available.[12] When found in adult LTRs with upper and lower respiratory tract symptoms, it usually is a single pathogen or co-pathogen.[143,225] In addition, it has been associated with episodes of allograft rejection and mortality.[225] Therapy is supportive, with treatment of co-pathogens if identified.

PIV (types 1 to 4) is isolated frequently as a cause of upper respiratory infections and croup in normal children.[13] PIV can cause severe lower tract infection in immunocompromised patients, including respiratory failure in LTRs, most notably caused by serotype 3.[21,431,449] Though rarely fatal in adults, persistent declines in pulmonary function and acute allograft rejection are important consequences of PIV infection.[268,431,449] A retrospective study on PIV in pediatric SOTRs (liver, small bowel, lung, heart, kidney) found significant age-related morbidity and mortality.[21] Although 44 percent of all patients with PIV had upper respiratory infections, only 11 percent of children younger than 1 year old had limited disease. A 16 percent mortality rate was associated with PIV, and predictors of mortality included age younger than 6 months, infection occurring less than 3 months after transplantation, and augmented immunosuppression. Infection in these children occurred in the context of community outbreaks. The diagnosis can be made most rapidly by antigen detection; culture isolation can take weeks. Respiratory secretions obtained from nasal washings or BAL fluid can be used.[38] No proven therapy for PIV in LTRs is available. Intravenous and aerosolized ribavirin has been used in immunocompromised hosts, but without randomized controlled data or clear evidence of efficacy.[38,268]

Despite available immunoprophylaxis and chemoprophylaxis, influenza viruses A and B remain an important cause of morbidity in the normal population and lead to a considerable rate of mortality in the very young and the elderly.[11] Transplant recipients are at risk for acquiring community-acquired infection during the yearly epidemics, as well as nosocomial infection from visitors, hospital workers, and other transplant recipients as a result of outbreaks occurring in the inpatient setting.[256,348,429] Influenza infection is relatively rare in SOTRs—4.2 percent of adult LTRs over the course of a 10-year-period and 2.6 percent of pediatric SOTRs over a similar period[21,38]—but LTRs appear to be at a uniquely high risk of acquiring infection. In the adult study, the incidence was 41.8 cases per 1000 person-years for adult LTRs versus 2.8 and 4.3 per 1000 in liver and kidney transplant recipients, respectively.[432] The authors speculate that optimal protection from influenza infection afforded by serum and secretory antibodies in the respiratory tract are altered in LTRs, thereby leading to higher attack rates.

In addition to the usual symptoms of acute onset of malaise, fever, myalgias, and respiratory symptoms, GI symptoms also may be prominent.[136] Most pediatric SOTRs with influenza had minimal disease consisting of upper respiratory symptoms, although 3 of 13 patients (23%) died.[21] In these children, steroid bolus and OKT3 therapy given within a week of diagnosis of infection were associated with mortality. Transplant recipients may be at increased risk for acquiring secondary bacterial infections and nonpulmonary complications such as hepatitis, myocarditis, and aseptic meningitis.[38,432]

Influenza is unique among the respiratory viruses in that both vaccine and chemoprophylatic strategies are available, as are several effective therapies. Unfortunately, immunosuppressed patients may not respond well to influenza vaccine, and, if disease is not recognized promptly, the effectiveness of therapy for established disease may not be optimal. Studies on the immunogenicity of influenza vaccine in pediatric SOTRs are more encouraging than in adult reports, which have documented poor response, especially when associated with MMF or sirolimus, and occasional association with acute rejection.[54,167] In pediatric renal transplant recipients, response was similar to that of normal controls, without evidence of rejection,[117,134] although in pediatric heart transplant recipients protective antibody responses were not achieved until three doses of vaccine were administered.[4] The current recommendation is that influenza vaccine be administered yearly to all SOTRs and those awaiting transplantation, as well as to household contacts and caretakers.[11,54,81] Chemoprophylaxis can be administered to patients who have not been vaccinated or who have not had sufficient time to respond to vaccination, especially if influenza is circulating in the community. Agents that are approved for prophylaxis of influenza A in children older than 1 year include amantadine and rimantadine, although resistance commonly is reported to these antivirals, and oseltamivir, which is additionally active against influenza B.[11,429]

The diagnosis of influenza can be made by rapid antigen testing, which is highly sensitive in ill children, though less so in adults with a lower burden of disease. PCR techniques, including multiplex assays for detection of multiple respiratory viruses in one sample, are currently under development but are not yet widely available.[80,131,222,411]

Most experts would recommend therapy with antiviral agents in an LTR with suspected (community epidemic with compatible symptoms) or proven influenza, although their utility in such patient populations has yet to be defined.[38] The choice of agent depends on the type of virus (A or B) and information about resistance. The neuraminidase inhibitors oseltamivir and zanamivir are currently favored because of their broad spectrum of activity and less reported resistance.[81,429] Some would consider the addition of aerosolized ribavirin for severe lower tract disease.[81] The implications of pandemic influenza, including higher disease burdens and mortality and longer duration of viral shedding with considerable potential for spread of disease, for those who already have undergone transplantation and those awaiting transplants probably would be significant. These authors speculate that transplantation under these circumstance probably would be curtailed.[220]

Despite recent interest, published data on adenovirus (AdV) infections in pediatric SOTRs are limited, and most describe infection in liver transplant recipients. The majority of information on AdV in pediatric immunocompromised hosts comes from those undergoing hematopoietic stem cell transplantation. AdV infections in SOTRs appear to be more common occurrences in young pediatric than adult transplant recipients[94,279,304] because young transplant recipients may remain naïve to many of the 51 recognized serotypes. AdV infections have multiple putative sources, including the donor organ, reactivation from latency in host tissues, and infection acquired from the community or nosocomially.[62,210,270,279,331]

Cases of acute, disseminated, and fatal disease have been described in cardiothoracic transplant recipients. In LTRs, infections occur in the early post-transplant period and often cause disease in the graft in the form of necrotizing pneumonia.[49,304] AdV has been associated with graft dysfunction, BOS, retransplantation, and death.[50]

Several methods that are used for the identification of AdV include culture, direct identification of antigens, and serology, as well as histologic examination of tissues for the presence of AdV inclusions and immunohistochemical staining.[75,81] Viral isolation by culture can be expedited by centrifugation in shell vial assays and immunofluorescent staining with adenoviral monoclonal antibodies, although viral serotyping cannot be done with this method. Direct identification of AdV antigens, usually performed on respiratory specimens, can be achieved by radioimmunoassay, immunofluorescence, or ELISA techniques, which are rapid and specific but less sensitive than culture.

Detection of virus with culture techniques and direct antigen detection are insensitive in identifying patients at risk for the development of disseminated disease. In addition, cultures may take a week or longer, and neither method may detect low levels of circulating virus. PCR is emerging as a powerful tool for detection of AdV in body fluids and tissues and for serotyping.[115,161,170,232,239,362] The virus's ability to establish latency can render interpreting the presence of virus or viral DNA in clinical specimens a challenge.[138,233] A single determination of the presence of AdV by PCR in immunocompromised patients may be nonspecific and perhaps misleading. As with other important viruses that establish latency, quantitative viral load patterns appear to be more informative with regard to the pathogenic role of AdV in these patient populations. Studies in pediatric HSCT recipients support the use of blood PCR surveillance as a method to identify patients at risk for disseminated disease.[78,115,161,170,232,239,362,439] These methods have not been validated in LTRs.

The optimal therapy for AdV has not been determined. Supportive care and reduction of immunosuppression are the mainstays of therapy currently recommended.[81] Recent data suggest that administration of cidofovir may be helpful in severely ill patients with disseminated disease and rising viral loads.[63,178,230,243,438,465] Use of this agent may be associated with significant renal toxicity,[169,230,333] and use of modified dosing regimens has been investigated with some success.[63,178,293,438] Strategies involving adoptive immunotherapy also may be feasible and efficacious in the future. Data on these therapeutic modalities have been reviewed recently.[177]

Although a detailed discussion is beyond the scope of this chapter, the link between respiratory viruses and BOS or chronic allograft dysfunction should be addressed briefly. BOS is a progressive condition, without good treatment options, and the greatest impediment to long-term survival for LTRs. It affects 50 percent of patients alive 5 years after transplantation and is the leading cause of death in adult and pediatric LTRs 1 year after transplantation.[416,446] Although the exact mechanisms are still being determined, the final common pathway is epithelial injury and intraluminal proliferation of fibroblasts leading to air flow obstruction.[186,203,427] Stimulation of T cells and release of cytokines and chemokines by viruses are leading candidates for the initiation of these pathways. Many studies have linked viral infections, such as CMV, AdV, RSV, PIV, and others, often working in concert, to the development of BOS.[122,136,219,317,428,431] The role of these agents has yet to be proved, although it is biologically plausible.[186] Reduction of BOS remains an incentive for the continued development of preventive strategies against these viral infections.

ZOONOSES: RABIES, WEST NILE VIRUS, LYMPHOCYTIC CHORIOMENINGITIS VIRUS, AND *BORDETELLA*

Isolation of unusual pathogens often is a sign of an unexpected environmental exposure.[28,352] This statement is epitomized by the recent reports of SOTRs with uncommon zoonotic infections.[214] In the case of donor-derived rabies, lymphocytic choriomeningitis virus, and West Nile Virus infections, multiple cases were traced to a donor or donors with retrospectively assessed exposures or risk factors (or both).[125,172,191,393] West Nile virus in SOTRs also has been community acquired,[206,221] and cases of *Bordetella bronchiseptica* in transplant recipients have been traced to sick pet dogs.[34,77,295] These cases are humbling in many respects. Many of these infections have no established therapy and, as expected, are associated with increased morbidity and mortality in SOTRs.[98,125,206,221,295] Therefore, prevention is of utmost importance. These cases advise us to counsel our post-transplant recipients on avoidance of potentially infectious exposures, such as sick pets and arthropod vectors of diseases.[214,218,295] Undeniably, they remind us of the almost limitless pathogens that may befall SOTRs, including those not yet described. These cases have newly informed the policies for reporting suspected donortransmitted conditions and opened a new dialogue on how screening procedures of organ and blood donors are conducted.[105,125,180,204]

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CHAPTER 83

Opportunistic Infections in Liver and Intestinal Transplantation

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For more than 20 years, liver transplantation has been established as an effective treatment of end-stage liver disease in children. Improved surgical techniques and the availability of new and more potent immunosuppressive regimens have led to enhanced short- and long-term survival rates that now approach or exceed 80 percent. In response to these excellent results, more children are being referred for liver transplantation, and increasing numbers of pediatric centers routinely perform this procedure. More recently, intestinal transplantation, performed as an isolated procedure or in combination with the liver or other organs, also is gaining expanded acceptance as treatment of refractory intestinal failure in children, particularly those who experience

progressive liver failure as a result of hyperalimentation-induced liver disease. Although this newer procedure has been performed routinely at only a limited number of centers, an increasing number of transplant programs are beginning to offer intestinal transplantation.

Infectious complications have been a significant cause of morbidity and mortality in children undergoing liver transplantation since this procedure gained initial acceptance in the 1980s. However, improvements in immune suppression management along with the increasing availability of new antimicrobial agents and diagnostic tools have resulted in improved treatment regimens that have reduced the impact of infectious complications on these children. The introduction of intestinal transplantation brought with it many of the same infectious complications seen after liver transplantations, as well as a number of infectious issues that appear to be unique to this procedure. The experience garnered from the treatment of patients undergoing liver and other organ transplant procedures has been translated effectively to the management of infections occurring after intestinal transplantation. Although development of increasingly effective treatment strategies is of great benefit to the children undergoing these procedures, emphasis also focuses on the development of strategies aimed at prevention of infectious complications in children undergoing abdominal transplantation. This chapter provides a general overview of the problem of infections that develop after liver transplantation and intestinal transplantation in children.

PREDISPOSING FACTORS

Liver transplantation and intestinal transplantation are associated with a set of technical and medical conditions that predispose one to a unique set of infectious complications. The abdomen is a common site of infection in patients undergoing both these procedures,[29,77] almost certainly as a result of local ischemic injury and bleeding as well as potential soilage with contaminated material.[59] Additional factors predisposing recipients to infection can be divided into those that exist before transplantation and those secondary to intraoperative and post-transplant activities.

PRETRANSPLANT FACTORS

The underlying illnesses leading to transplantation may be associated with intrinsic risk factors for the development of infection. Some disorders may have required palliative surgery that increases the technical difficulty of the transplant procedure and may be associated with an enhanced risk for the development of post-transplant infection.[25] For example, in children undergoing liver transplantation for biliary atresia, a Kasai procedure (choledochojejunostomy) may have been performed previously and predisposed them to recurrent episodes of bacterial cholangitis before transplantation, thereby increasing the likelihood of colonization with multiple antibiotic–resistant bacteria, which can cause infection after transplantation. Similarly, children undergoing liver transplantation for cystic fibrosis may have an increased risk for the development of invasive aspergillosis if they were colonized with this pathogen before receiving the transplant. Complications of end-stage liver disease (as part of a primary liver disease or as a consequence of hyperalimentation in patients with intestinal insufficiency) also may predispose the patient to the development of infection after undergoing transplantation. A history of one or more episodes of spontaneous bacterial peritonitis before transplantation in patients with ascites has been associated with an increased rate of bacterial infection developing after liver transplantation[120] and could be expected in patients with liver disease associated with intestinal insufficiency. Finally, children undergoing intestinal transplantation experience frequent episodes of gastrointestinal-associated bloodstream infections. Recurrent exposure to antimicrobials to treat these episodes of bacteremia increases the likelihood that colonization and disease with multiply resistant bacterial and fungal pathogens will occur after transplantation. Risks for the occurrence of bacterial translocation after intestinal transplantation, with resultant bloodstream infection, include the presence of a colon graft,[21] hospitalization before transplantation, and treatment with mycophenolate mofetil.[77]

Age, another important pretransplant factor, is a major determinant of susceptibility to certain agents, severity of expression of infection, and immune maturation. Young children undergoing abdominal transplantation may experience moderate to severe infection with certain viral (e.g., respiratory syncytial virus [RSV]) or bacterial (coagulase-negative staphylococci) pathogens, as opposed to the milder illness experienced by adult recipients infected with these pathogens. In contrast, infections caused by certain pathogens, such as *Cryptococcus neoformans*, are seldom manifested before young adulthood.[124] Age also is an important factor governing clinical expression of infection with cytomegalovirus (CMV) and Epstein-Barr virus (EBV). When transplants are performed in young patients, a high likelihood exists that they will be seronegative for CMV and EBV and, therefore, susceptible to primary infection, which is more severe than that caused by reactivation.[11,60,96]

Donor-related issues represent another set of pretransplant factors. Transplant recipients are at risk for acquiring infections that may be active or latent within the donor at the time of organ harvesting. The best described examples of donor-associated infections are CMV and EBV.[10,12,19,96] Infections caused by CMV and EBV have been more severe after intestinal transplantation than after transplantation of other organs. This difference may be due to the fact that the intestine is an organ rich in lymphoid tissue, which may result in transmission of a larger viral load from the donor than with other graft types. Similarly, adenovirus has been isolated more commonly from pediatric recipients of intestinal transplants than from other organs, which also may be related to donor transmission in the accompanying lymphoid tissue.[83,92] Although the frequencies of some donor-associated pathogens (e.g., human immunodeficiency virus [HIV],[30] hepatitis B, hepatitis C) have decreased substantially with better diagnostic screening tests,[5] recent evidence now demonstrates donor-associated transmission of West Nile virus and rabies.[67,112] Because organs from a single donor often go to disparate sites, it is important for the recipient center to report back to United Network for Organ Sharing (UNOS) any unusual infections that possibly could have come from the donor.

INTRAOPERATIVE FACTORS

Operative factors unique to liver transplantation may predispose the recipient to infectious complications. For example, liver transplant recipients undergoing Roux-en-Y choledochoduodenostomy experience more infectious episodes than do those who undergo choledochocholedochostomy with T-tube drainage.[70,102] However, usually only the former option is performed in children undergoing liver transplantation because of the small size of their bile ducts. For combined liver-intestinal transplantation, evolution to en bloc replacement of the liver and intestine avoids the need for performing an additional biliary anastomosis and minimizes the risk of development of infection related to biliary complications. Prolonged operative time (>12 hours) during the initial transplant procedure has been associated with an increased risk for developing infection after transplantation[36,70] and probably is a surrogate marker for the technical difficulty of the surgery. Intraoperative events, such as contamination of the operative field, also predispose the recipient to postoperative infections.

Finally, inability to close the abdomen after the transplant has been performed, as a result of size discrepancy or intraoperative complications, appears to increase the risk for development of postoperative infections.

POST-TRANSPLANT FACTORS

Technical problems, immunosuppression, the presence of indwelling cannulas, and nosocomial exposure are major postoperative risk factors for the development of infectious complications. Thrombosis of the hepatic artery is the most serious technical problem after liver transplantation and predisposes the recipient to the development of areas of necrotic liver, hepatic abscesses, and bacteremia.[99,102] Bile duct strictures, developing as a sequela of a thrombosed hepatic artery and ischemia or as a result of technical difficulties, may predispose the recipient to the development of cholangitis.[102] Retransplantation represents a high risk for the development of intra-abdominal infection after intestinal transplantation.[77]

Immunosuppression is the critical postoperative factor predisposing to the development of infection in all transplant recipients. Immunosuppressive regimens have evolved in an attempt to achieve more specific control of rejection with the least impairment of immunity. Thus, this evolution is aimed not only at improved control of rejection but also at a decreased rate of morbidity and mortality from infections. The use of cyclosporine-based regimens has resulted in a decreased incidence of infections in renal and cardiac transplant recipients.[29,61,89] The introduction of tacrolimus has allowed many patients to be managed without steroids.[49,118] Although reported rates of infection have been similar in liver transplant recipients treated with either tacrolimus or cyclosporine, an apparent decrease in morbidity and mortality rates, especially from viral pathogens, has been noted with tacrolimus.[2,49] In contrast to these results, some centers have reported an increased rate of EBV-associated post-transplant lymphoproliferative disease (PTLD) in patients receiving tacrolimus.[24] However, data from the University of Pittsburgh suggest that the short- and long-term incidence of EBV-associated PTLD appears to be similar in pediatric liver transplant recipients treated with either cyclosporine or tacrolimus.[15]

Children undergoing intestinal transplantation require a higher baseline level of immunosuppression than do patients undergoing most other solid organ transplant procedures.[96] With this increased level of immunosuppression has come an increased risk for the development of infection. In an effort to overcome this risk, numerous alternative immunosuppressive strategies have been explored. Looking at one such alternative strategy, Loinaz and colleagues[77] found an increased risk for the development of bacterial infection after intestinal transplantation with both mycophenolate mofetil and daclizumab.

Treatment of rejection with additional or higher doses of immunosuppressants increases the risk for development of invasive and potentially fatal infection. Of particular concern is the use of antilymphocyte preparations, especially OKT3, which often is indispensable in the management of rejection refractory to steroids.[10,36,70] Newer antilymphocyte antibodies (e.g., thymoglobulin) also are likely to be associated with an increased risk for the development of infection.

The prolonged use of indwelling cannulas at any site is an important cause of infection throughout the postoperative course. The presence of central venous catheters is a cause of bacteremia after transplantation. This is a particularly important consideration for children undergoing intestinal transplantation, for which maintenance of long-term central venous access has been required for prolonged periods after transplantation. Urinary tract infections and bacterial pneumonia are associated with the use of urethral catheters and prolonged nasotracheal or endotracheal intubation, respectively.[36,70]

Nosocomial exposure constitutes the final group of postoperative risk factors. Transplant recipients, especially children, may be exposed to many common viral pathogens (e.g., rotavirus, RSV, or influenza) while hospitalized. In addition, all transplant recipients are at risk for exposure to transfusion-associated pathogens (e.g., hepatitis B, hepatitis C, HIV). Finally, the presence in the hospital of heavy areas of contamination with pathogenic fungi, such as *Aspergillus*, may increase the risk for acquisition of invasive fungal disease in these patients. The rate of fungal colonization increases during times of hospital reconstruction. Implementation of infection control policies aimed at the local epidemiology of circulating infections is paramount.

TIMING OF INFECTIONS

The time of onset of infection with various pathogens after transplantation tends to be predictable. Most clinically important infections occur within the first 180 days after transplantation.[46,70] The timing of infections can be divided into three intervals: early (0-30 days after transplantation), intermediate (30-180 days after transplantation), and late (>180 days after transplantation). In addition, some infections may occur throughout the postoperative course. These divisions, though arbitrary, generally are useful in approaching a patient with fever after undergoing transplantation and can be used as a guide to the differential diagnosis. An overview of the infectious complications occurring during each of these time periods is provided in Tables 83–1 to 83–3 and is summarized in the following sections.

EARLY INFECTIONS (0 TO 30 DAYS)

Early infections tend to be associated with preexisting conditions and surgical manipulation (Table 83–1). In general, they are caused by either bacteria or yeast. Bacterial infections are particularly common developments after intestinal transplantation and have been reported in up to 90 percent of recipients.[62,77,116] As many as half of these early infectious complications may develop in the first 2 weeks after the patient has undergone abdominal transplantation.[9] Cholangitis or spontaneous bacterial peritonitis occurring at or near the time of liver transplantation may lead to the development of intra-abdominal infection after the procedure. Herpes simplex infection also can reactivate and cause early symptomatic disease,[70] although reactivation is an uncommon occurrence in children. Technical difficulties (e.g., thrombosis of the hepatic artery or portal vein, biliary strictures) predispose recipients to early development of bacterial infections. Likewise, the development of bile leaks and bowel perforations is associated with polymicrobial intra-abdominal infections, primarily consisting of enteric bacteria and *Candida* species, in the first month after transplantation.[38] Bacteremia may be seen in intestinal transplant recipients in association with the presence of central venous catheters, intestinal rejection, or PTLD involving the intestine.[41,107] Finally, re-exploration of the abdomen has been linked to increased rates of fungal infection.[70]

INTERMEDIATE PERIOD (31 TO 180 DAYS)

The intermediate period (Table 83–2) is the typical time of onset of infections associated with donor transmission (either organ or blood products), reactivated viruses, and opportunistic infections. CMV infection peaks in incidence during this time.[10,70] However,

TABLE 83–1 Differential Diagnosis of Infectious Complications during the Early Period (0 to 30 Days) after Pediatric Liver and Intestinal Transplantation

Clinical Syndrome	Associated Pathogens
Wound infection	*Staphylococcus aureus*
Superficial	Enterococci
Deep	Enterobacteriaceae
	Candida species
Intra-abdominal infection	Enterobacteriaceae
Peritonitis	Enterococci
Intra-abdominal abscess	*Candida* species
Intrahepatic abscess (isolated liver and liver-intestine transplants) with or without bacteremia	
Bloodstream infection associated with	
Central venous catheters	Coagulase-negative staphylococci
	Enterococci
	S. aureus
	Candida species
Hepatic artery thrombosis (isolated liver transplant only)	Enterobacteriaceae
	Enterococci
	Candida species
Intestinal rejection (intestine only)	Enterobacteriaceae
	Enterococci
	Candida species
Bacterial cholangitis (isolated liver transplant only)	Enterobacteriaceae
	Enterococci
	Candida species
Urinary tract infection	Enterobacteriaceae
	Enterococci
	Candida species
Ventilator-associated pneumonia	Enterobacteriaceae
	Enterococci
	S. aureus
	Candida species
Nosocomial acquisition of common community pathogens	Respiratory syncytial virus
	Parainfluenza virus
	Influenza virus
	Rotavirus
Noninfectious causes	Rejection
	Drug fever

TABLE 83–2 Differential Diagnosis of Infectious Complications during the Intermediate Period (31 to 180 Days) after Pediatric Liver and Intestinal Transplantation

Clinical Syndrome	Associated Pathogens
Viral syndrome	CMV
Fever, leukopenia, thrombocytopenia ±Atypical lymphocytosis	EBV
Hepatitis	CMV
	EBV
	Adenovirus
	Hepatitis B
	Hepatitis C
Enteritis	CMV
	EBV
	Rotavirus
	Adenovirus
	Clostridium difficile
PTLD	EBV
Bacterial cholangitis*	Enterobacteriaceae
	Enterococci
	Candida species
Pneumonia	*Streptococcus pneumoniae*
	CMV
	Adenovirus
	RSV
	Parainfluenza
	Influenza
	Pneumocystis jiroveci
	Aspergillus fumigatus
Bacteremia†	Enterobacteriaceae
	Enterococcus
	Candida species
Adenopathy	EBV/PTLD
Pulmonary nodules	EBV/PTLD
	A. fumigatus

**Typically only seen in isolated liver transplant recipients and usually associated with the presence of technical complications (e.g., biliary stricture).*
†Seen in intestinal transplant recipients in association with the presence of central venous catheters, intestinal rejection, or PTLD involving the intestine.
CMV, cytomegalovirus; EBV, Epstein-Barr virus; PTLD, post-transplant lymphoproliferative disease.

TABLE 83–3 Differential Diagnosis of Infectious Complications during the Late Period (>180 Days) after Pediatric Liver and Intestinal Transplantation

Clinical Syndrome	Associated Pathogens
Bacterial cholangitis*	Enterobacteriaceae
	Enterococci
	Candida species
PTLD	EBV
Bacteremia†	Enterobacteriaceae
	Enterococcus
	Candida species
Varicella/zoster	Varicella-zoster virus

**Usually associated with the presence of technical complications (e.g., biliary stricture).*
†Seen in intestinal transplant recipients in association with the presence of central venous catheters, intestinal rejection, or PTLD involving the intestine.
EBV, Epstein-Barr virus; PTLD, post-transplant lymphoproliferative disease.

if CMV prophylaxis is instituted, disease from this virus may occur after 180 days. The intermediate period also is when many patients begin to have EBV-associated PTLD[60] and *Pneumocystis jiroveci* (formerly *P. carinii*) pneumonia (PCP).[70]

LATE INFECTIONS (>180 DAYS)

Late infections developing after abdominal transplantation (Table 83–3) are less well characterized than those of other periods because patients usually have been discharged from the transplant center to their respective homes, which often are quite far away. This renders accumulating accurate data on these late infections difficult. Nonetheless, problems such as recurrent episodes of bacterial cholangitis in liver transplant recipients (typically associated with underlying problems of the biliary tree), bacteremia associated with intestinal graft rejection, and PTLD (in both groups of patients)[78] occur in this period. In addition, children who are at high risk for the development of CMV disease and have been maintained on prophylaxis may have the onset of CMV disease delayed to the late period.[17]

INFECTIONS OCCURRING THROUGHOUT THE POSTOPERATIVE COURSE

Iatrogenic factors are important causes of bacterial and fungal infection at all times but predominately in the early transplant period. Central venous lines are maintained for a variable time; the risk of infection developing persists for the entire period that

the catheter remains in place. This problem is particularly important for recipients of intestinal transplants, in whom central line access is maintained until the graft is fully functional. Similarly, the presence of urethral catheters and endotracheal tubes also increases the risk for the development of infections whenever they are in use.

Nosocomial acquisition of community viruses, such as RSV, rotavirus, and influenza A or B, can occur at any time after transplantation. These viruses spread easily in hospital environments from personnel or other hospitalized patients to transplant recipients. It is, therefore, important to modify diagnostic considerations according to local epidemiologic considerations.

BACTERIAL AND FUNGAL INFECTIONS

Bacterial and fungal pathogens are important causes of morbidity and occasionally mortality in children undergoing liver or intestinal transplantation (or both). With the exception of infections related to the use of indwelling catheters, sites of bacterial infection tend to occur at or near the transplanted organ. Accordingly, the intra-abdominal space is an important site of infection after any of the abdominal transplant procedures. Adding to the complexity of management of bacterial infections in children undergoing abdominal transplantation is the fact that recovery of multiply antibiotic-resistant bacteria is occurring increasingly frequently. Outbreaks of colonization and disease caused by vancomycin-resistant *Enterococcus faecium* and extended-spectrum β-lactamase (ESBL)-producing *Klebsiella pneumoniae* have been reported in pediatric liver and intestinal transplant recipients.[40,42] These multiply resistant bacteria have been transmitted from patient to patient, thus prompting the imposition of strict infection control procedures. Reports of multiply resistant strains of *Enterobacter cloacae* associated with de-repression of a chromosomally located, broad-spectrum, β-lactamase enzyme have identified this very resistant organism as an important pathogen after liver transplantation.[20] More recently, isolates of *E. cloacae* identified to also be carrying ESBL enzymes have been recovered from our intestinal transplant candidates and recipients and, as a result, have limited our antimicrobial alternatives even further. The increasing prevalence of these multiply antibiotic-resistant organisms limits the therapeutic options available for the treatment of bacterial infections that occur after abdominal transplantation; in some cases, effective antimicrobials may be unavailable to treat these complications. Knowledge of the results of previous cultures and local antimicrobial resistance patterns is critical in choosing initial empiric antibiotic therapy in these patients to maximize their outcomes.

LIVER TRANSPLANTATION

Bacterial and fungal infections are common early problems after liver transplantation.[22,23,57,62,122,130] Rates of bacterial infection of 40 to 70 percent have been reported from multiple series.[49,70,102] Bacteremia often occurs in association with intra-abdominal infection or with the use of central venous catheters, but it can develop without an obvious source. Enteric gram-negative organisms account for more than half of these episodes. Bacterial infections involving the abdomen or surgical wound are common developments in most series. Infectious complications of the transplanted liver also occur. The most important complication is hepatic abscess associated with hepatic artery or portal vein thrombosis, often accompanied by persistent bacteremia. However, the introduction of frequent surveillance Doppler studies early after transplantation to monitor for the development of thrombosis, coupled with the use of operative thrombectomy and thrombolysis, has essentially eliminated the development of hepatic abscesses in this population.

Ascending cholangitis is a common occurrence after liver transplantation and usually is associated with biliary abnormalities. This diagnosis typically is made on clinical grounds in a patient with fever and biochemical evidence of biliary inflammation. Enteric gram-negative bacteria and enterococcal species predominate. However, because this clinical picture can be identical to that of acute graft rejection, liver biopsy should be performed to differentiate these processes. A cholangiogram is performed to assess the status of the biliary tract in patients with proven cholangitis.

As many as 40 percent of children undergoing liver transplantation may contract a fungal infection during the first year after the procedure.[122] *Candida* spp. are the most common fungal pathogens, and infection usually is associated with an intra-abdominal focus or indwelling catheter. Infections caused by *Candida* spp. usually are recognized in the first month after transplantation, with *Candida* peritonitis most likely occurring in the first 2 weeks after liver transplantation in association with a bile leak or bowel perforation. Other risk factors associated with the development of *Candida* infection include a prolonged duration of intubation after transplantation, hepatic artery thrombosis, volume of blood transfused, and exposure to steroids within the 3 months before the transplant procedure. Recovery of *Candida* from a Jackson-Pratt drain in the early postoperative period may be the first indication of either of these two technical complications and may occur before the onset of clinical symptoms of intra-abdominal infection.[40] Accordingly, recovery of *Candida*, alone or in combination with enteric bacteria, should prompt initiation of antimicrobial therapy and aggressive evaluation for the presence of these complications. Early initiation of treatment is particularly important, given an attributable mortality rate of up to 33 percent for candidal infections in pediatric liver transplant recipients.[40] The availability of fluconazole and the newer echinocandin antifungal agents (e.g., caspofungin and micafungin) has increased the number of therapeutic options for the treatment of *Candida* infection. However, acquired or inherent resistance to the azoles is an increasing concern, as are drug-drug interactions between azoles and echinocandins with both cyclosporine and tacrolimus.

Episodes of invasive aspergillosis are uncommon occurrences but can be fatal.[50] Children undergoing liver transplantation for cystic fibrosis are at particular risk for infection with *Aspergillus*.[121] We have observed early disseminated disease in children with cystic fibrosis undergoing liver transplantation, which has prompted the use of perioperative antifungal prophylaxis in liver recipients with cystic fibrosis and a history of recovery of *Aspergillus* before transplantation. Because data defining the precise duration of prophylaxis necessary to protect against this complication are not available, prophylactic treatment has ranged from 1 month of intravenous amphotericin to prolonged use of an oral azole agent (e.g., itraconazole or voriconazole). The availability of newer antifungal agents, including the advanced-generation azoles (voriconazole and posaconazole), as well as the echinocandins, has increased the number and complexity of therapeutic options for the treatment of aspergillosis in children undergoing liver transplantation. A summary of a suggested approach to the diagnosis and management of fungal infections that occur after liver transplantation in children is provided in Table 83–4.

INTESTINAL TRANSPLANTATION

A relatively small number of children have undergone intestinal transplantation. Many have received combined liver and intestine or multivisceral transplants. Bacterial infection occurs frequently in these patients.[41,106] One recent series reported that as many as

TABLE 83–4 Overview of Diagnosis and Management of Fungal Infections after Liver and Intestinal Transplantation in Children

	***Candida*—Noninvasive (Mucositis, Dermatitis and Cystitis)**	***Candida*—Invasive**	***Aspergillus***	***Cryptococcus***	**Others (*Histoplasma, Mucor, Fusarium, Blastomycetes, Alternaria,* etc.)**
Frequency*	Common	Common	Uncommon	Rare	Rare
Diagnostic Tests	Clinical examination Culture Gram stain	Culture Gram stain Histology	Culture Gram stain Histology Radiographic staging[¶]	Culture Antigen test India ink stain Histology CSF examination	Culture Histology Antigen testing (when appropriate)
Treatment					
Primary	Nystatin	Amphotericin B[†]	Amphotericin B[†]	Amphotericin B[†]	Amphotericin B[†]
Secondary	Clotrimazole Topical amphotericin B[‡]	Fluconazole[§,¶] Echinocandin therapy** 5-Flucytosine[††]	Echinocandin therapy** Itraconazole[§,‡‡,§§] Voriconazole[§,¶¶] 5-Flucytosine[††]	Fluconazole[§,¶] 5-Flucytosine[††]	Azole therapy (for susceptible organisms)[§]
Adjunctive	Fluconazole[§,||]	Removal of central lines	Surgical resection		Surgical débridement
Duration of therapy	Dependent on the rate of clearance	Dependent on the rate of clearance: minimum of 14 days	Dependent on the rate of clearance: minimum of 4 weeks, usually 8-12 weeks	Minimum of 6-8 weeks Many would continue with fluconazole indefinitely	Dependent on the rate of clearance
Follow-up	Clinical examination Repeat urine analysis/cultures	Dependent on the clinical scenario	Dependent on the clinical scenario	Clinical examination Antigen testing Repeat culture of the appropriate source (sputum, CSF, urine) Radiographs if relevant	Clinical examination Antigen testing Repeat culture of the appropriate source (sputum, CSF, urine) Radiographs if relevant

**Common, greater than 5 percent; uncommon, 1 to 5 percent; rare, less than 1 percent.*
†*Amphotericin B is dosed at 0.75 to 1.0 mg/kg/day; lipid formulations are used if renal failure is present.*
‡*Topical amphotericin B for bladder wash for noninvasive candiduria. Ultrasound of the kidneys is recommended to determine that no invasive disease is present.*
§*Azole use must be accompanied by close follow-up of levels of cyclosporine or tacrolimus. In general, tacrolimus dosing should be cut in half when using a standard dose of fluconazole.*
¶*Radiographic staging includes computed tomography of head, chest, and abdomen.*
||*Fluconazole is the alternative first-line drug for invasive disease if the species is known to be sensitive to fluconazole and the patient is clinically stable. Fluconazole is dosed at 6 to 12 mg/kg/day based on the severity of infection.*
***The use of either of the approved echinocandins (caspofungin or micafungin) may be an appropriate alternative for the treatment of invasive candidiasis, candidemia, and aspergillosis. Dose adjustments may be necessary in the face of impaired liver function. Monitoring of tacrolimus levels is indicated because these agents may decrease tacrolimus levels.*
††*5-Flucytosine should not be used alone but is synergistic when used in conjunction with amphotericin B. Flucytosine is dosed at 100 to 150 mg/kg/day divided every 6 hours.*
‡‡*Itraconazole can be used long-term for patients who have been treated for invasive Aspergillus, but in general it is not recommended as first-line therapy.*
§§*Itraconazole absorption can be erratic. Accordingly, monitoring of itraconazole levels is recommended. Itraconazole is dosed at 3 to 5 mg/kg/day as a single dose. Dosing adjustment based on monitoring of levels is recommended. Adjustment of cyclosporine or tacrolimus dosing should be individualized.*
¶¶*Voriconazole levels should be checked in patients treated with oral therapy.*
CSF, cerebrospinal fluid.

92 percent of children undergoing intestinal transplantation experienced an average of 2.9 episodes of bacterial infection per patient.[77] In more than 80 percent of these patients, the first bacterial infection occurred during the first 2 months after receiving the transplant. Bacteremia, which can be explained in part by disruption of the mucosal barrier associated with harvest injury or intestinal allograft rejection, is a common finding.[41,106] Coagulase-negative staphylococci and enterococci and gram-negative enteric bacilli account for most episodes. As noted earlier, antibiotic resistance is seen commonly in recovered pathogens. In our experience, episodes of gut-associated bacteremia frequently are responsible for secondary infection of central venous catheters, and persistent positive cultures are produced even after resolution of clinical symptoms. Accordingly, treatment strategies aimed at preserving the catheter (e.g., antibiotic lock therapy) may need to be implemented in conjunction with systemic antibiotics to achieve a sustained clinical cure.

Intra-abdominal and wound infections also are seen commonly in this population; they occur in more than a third of patients and typically are detected during the first month after transplantation. Gram-negative enteric pathogens, which frequently demonstrate multiple-antibiotic resistance, as well as enterococci (often exhibiting vancomycin resistance), are the most common organisms associated with these complications. Recurrent laparotomy has been identified as a risk factor for intra-abdominal infection. One unique aspect of intestinal transplantation is the potential inability to achieve abdominal wall closure. Although failure to close the abdominal wall is an obvious risk for the development of intra-abdominal infection, the use of abdominal mesh as part of an effort to resolve difficult abdominal wall closure also has been associated with the development of superficial and deep wound infection.[28] Successful treatment of mesh-related infections may require removal of the mesh to obtain a sustained clinical cure.

Another important site of infection is the intestine itself. *Clostridium difficile* enteritis can be accompanied by a pattern of fever, abdominal pain, and diarrhea that easily can be mistaken for graft rejection or viral infection caused by CMV, EBV, and adenovirus. Accordingly, the diagnosis of *C. difficile* enteritis must be considered in any child in whom fever and changes in stool output are noted. In one small series, *C. difficile* enteritis was diagnosed in nearly 10 percent of children undergoing intestinal transplantation.[129] The frequent exposure to antibiotics and prolonged hospital stays that children undergoing intestinal transplantation experience are major risk factors for the development of this complication. Standard treatment with oral metronidazole is the recommended first-line therapy. However, prolonged therapy or oral vancomycin might be necessary for patients who

experience relapse or recurrent episodes after primary treatment.

Candidemia also may occur in any of the settings in which bloodstream infections are observed after intestinal transplantation. Although the majority of episodes of candidemia take place in the first 3 to 6 months after transplantation, episodes may occur later. Intra-abdominal infection with *Candida* also is observed, typically as part of a polymicrobial infection related to technical problems occurring during the initial transplant surgery or subsequent laparotomies.

Invasive mycoses caused by fungal pathogens other than *Candida* seldom are observed. Rare infections with *Aspergillus*, *Alternaria*, and *Scedosporium* spp. have occurred in children undergoing intestinal transplantation at our institution. In general, these children have been receiving high levels of immune suppression, and the outcome of these infections has been poor. Guidelines for the diagnosis and management of fungal infection in children undergoing intestinal transplantation are provided in Table 83–4.

VIRAL INFECTIONS

Viral pathogens, especially herpesviruses, are a major source of morbidity and mortality after solid organ transplantation. Patterns of disease associated with individual viral pathogens generally are similar among all transplant recipients. However, frequency, mode of manifestation, and relative severity can differ according to the type of organ transplanted and the serologic status of the recipient.

CYTOMEGALOVIRUS

CMV continues to be the most common and one of the most important viral pathogens seen after organ transplantation in children. CMV infection can be asymptomatic or symptomatic and may be due to primary infection (from either the donor graft or blood products), reactivation of latent infection, or superinfection with a different CMV strain in a previously seropositive child. Before the use of prophylaxis, the incidence of symptomatic CMV infection was reported to be as high as 22 percent in adult[53] and 40 percent in pediatric[10] liver transplant recipients. Use of ganciclovir prophylaxis has resulted in a decreased rate and severity of CMV disease.[44] Intestinal transplantation was introduced after ganciclovir became available and, therefore, has been able to take advantage of using ganciclovir both as prophylaxis and as treatment. Nonetheless, CMV disease can be very severe after intestinal transplantation and has a high rate of recurrence.[12,71] Primary CMV infection, typically acquired from the donor organ (or passenger donor leukocytes that accompany the organ), is associated with the greatest degree of morbidity and mortality.[39,44] Accordingly, CMV-seronegative recipients of organs from CMV-seropositive donors are considered at high risk for the development of CMV disease. Reactivation of or superinfection with CMV tends to result in milder illness after liver transplantation but still can be severe after intestinal transplantation.[11,13] CMV disease appears to be more likely to develop in CMV-seropositive recipients of CMV-seropositive donor organs than in seropositive recipients of seronegative donor organs.[39] Patients treated with unusually high doses of immunosuppressants, especially antilymphocyte antibody preparations, experience an increased rate of CMV disease regardless of previous immunity.[10,70]

Symptomatic CMV disease typically occurs between 1 and 3 months after transplantation. An important note is that the use of prophylactic regimens may delay the onset of CMV disease. A characteristic constellation of fever (which may be high-grade, prolonged, and hectic) and hematologic abnormalities (including leukopenia, atypical lymphocytosis, and thrombocytopenia) frequently is seen. This "CMV syndrome" occurs in 25 to 50 percent of patients with symptomatic CMV infection. Invasive CMV disease is characterized by visceral organ involvement; common sites include the gastrointestinal tract, liver, and lungs. CMV hepatitis appears to be the most common site in liver transplant recipients, whereas CMV enteritis is a frequent finding in intestinal transplant recipients. CMV chorioretinitis is a rare development in organ transplant recipients.

The diagnosis of CMV disease may be confirmed by positive buffy coat culture, pp65 antigenemia assay,[117] or the presence of CMV DNA in the blood of a patient with a compatible clinical syndrome.[71] However, clinicians must be aware that the results of viral culture of urine and even bronchoalveolar lavage specimens are difficult to interpret in previously infected patients because CMV frequently is shed asymptomatically in these secretions. Similarly, the presence of pp65 antigen and CMV DNA in blood can be misleading because these assays often are positive in asymptomatic patients. The specificity of this approach may be improved by quantitative determination of pp65 antigen or CMV DNA. Because of the lack of specificity of these assays, histologic examination of involved organs to confirm the presence of CMV is critical when the diagnosis of invasive CMV is being entertained.

Antiviral agents with activity against CMV (e.g., ganciclovir, foscarnet, and cidofovir) have improved the survival of transplant recipients with CMV disease. Fatal, disseminated CMV disease occurred in 19 percent of infected children[10] and 5 percent of infected adults undergoing liver transplantation in the preganciclovir era.[105] For clinical CMV disease, ganciclovir therapy is given in conjunction with reduction of immunosuppression unless evidence of rejection is present. Clinical response usually occurs 5 to 7 days after initiation of therapy. Baseline immunosuppression levels typically are restored at the time of initial clinical response or upon recognition of rejection. Recent evidence supports serial monitoring of CMV load in peripheral blood as a guide to the duration of treatment of CMV disease.[26,104] The role of CMV hyperimmune globulin in combination with ganciclovir for the treatment of CMV disease is controversial, although some evidence of improved outcome has been reported in the treatment of CMV pneumonia in adult liver transplant recipients.[37] Finally, because of the relatively high rates of nephrotoxicity associated with their use, foscarnet and cidofovir should be restricted to patients with apparent or proven resistance to ganciclovir.

In approximately 25 percent of patients treated with ganciclovir for an initial episode of symptomatic CMV disease, one or more episodes of recurrent CMV disease will develop.[104,114] Recurrences are observed approximately 1 month after the initial infectious episode occurs and may be associated with invasive disease. More commonly, however, these recurrent episodes tend to be milder than is the initial episode. Factors associated with an increased risk for the development of recurrent CMV disease include being a CMV-seronegative recipient of a CMV-seropositive organ, having disseminated CMV disease, and having a history of multiple treatment courses for rejection.[104] In addition, one center has demonstrated a correlation between the height of the CMV viral load in peripheral blood leukocytes before treatment and also at the end of treatment and the likelihood of recurrent CMV disease developing.[104] These results are the basis for the recommendation to use the results of CMV viral load measurement to guide the duration of therapy for CMV disease.[26] A summary of our suggested approach to the diagnosis and management of CMV infection is provided in Table 83–5.

TABLE 83–5 Overview of Diagnosis and Management of Viral Infections after Liver and Intestinal Transplantation in Children

	Cytomegalovirus	Epstein-Barr Virus	Respiratory Syncytial Virus	Influenza	Parainfluenza	Adenovirus
Frequency*	Common	Common	Uncommon	Uncommon	Uncommon	Liver: uncommon Intestine: common
Diagnostic tests	Culture pp65 antigen Histology	EBV PCR Histology Serology	NP aspirate for antigen detection and culture	NP aspirate for antigen detection and culture	NP aspirate for culture	Viral culture Histology
Treatment						
Primary	Ganciclovir (5 mg/kg bid)	Decrease IS	Supportive care	Supportive care	Supportive care	Decrease IS
Secondary	Foscarnet† Cidofovir		Aerosolized ribavirin	Amantadine Rimantadine Zanamivir Oseltamivir		IV ribavirin
Adjunctive	Decrease IS CMV IVIG	Ganciclovir IVIG	RSV IVIG Decrease IS	Decrease IS	Decrease IS	IVIG
Duration of therapy	Site-dependent	Individualized	Individualized	Individualized	Individualized	Individualized
Follow-up	Monitor pp65 antigen or CMV PCR (treat until negative)	Monitor EBV PCR Repeat imaging studies if positive at outset	None	None	None	None

**Common, frequency greater than 5 percent; uncommon, frequency of 1 to 5 percent; rare, frequency of less than 1 percent.*

†Foscarnet is used for CMV infection when ganciclovir resistance is suspected or proven. Experience from patients infected with human immunodeficiency virus suggests that a synergistic benefit will be obtained from the combined use of both of these agents when ganciclovir resistance is present.

CMV, cytomegalovirus; EBV, Epstein-Barr virus; IS, immune suppression; IVIG, intravenous immunoglobulin; NP, nasopharyngeal; PCR, polymerase chain reaction; RSV, respiratory syncytial virus.

EPSTEIN-BARR VIRUS

EBV infection, including EBV-associated PTLD, is an important cause of morbidity and mortality after liver transplantation and intestinal transplantation,[60,81,113,119,128] particularly in children undergoing intestinal transplantation, who experience the highest rates of EBV-related disease among transplant recipients.[91]

Symptomatic EBV infection in general and PTLD in particular most commonly occur in transplant recipients experiencing primary EBV infection, especially those who receive organs from seropositive donors. Accordingly, children undergoing transplantation are disproportionately affected by EBV when compared with their adult counterparts.[51] In as many as 80 percent of children who are EBV-seronegative before undergoing liver transplantation, primary EBV infection will develop after this procedure.[108,111] Although primary infection occurs in the vast majority of seronegative patients, clinical disease develops in less than a third of these children.[108,111] In one study, PTLD developed in 4 percent of children undergoing solid organ transplantation and 10 percent of children with primary EBV infection between 1 month and 5 years after transplantation[60]; 75 percent of cases occurred during the first postoperative year in patients receiving cyclosporine-based immune suppression. The cumulative incidence can be as high as 12 to 20 percent by 7 to 12 years after liver transplantation.[78,87]

Pediatric recipients of intestinal transplants appear to behave differently from children undergoing other types of organ transplantation in that the rate of EBV disease, including PTLD, appears to be similar in both patients who are EBV-seronegative and those who are EBV-seropositive before undergoing intestinal transplantation. Rates of EBV disease and PTLD after intestinal transplantation as high as 30 to 40 percent were reported during the initial experiences with intestinal transplantation. More recently, these rates have declined to approximately 10 percent as a result of improved immune suppression regimens and EBV-monitoring protocols. However, these rates still remain higher than those seen in other organ recipients.

A wide spectrum of EBV disease is recognized and includes nonspecific viral illness, mononucleosis, and PTLD, including lymphoma. Histologic evaluation is important in differentiating among these categories; manifestations can evolve in individual patients, and asymptomatic seroconversion also occurs. Variation in severity and extent of disease is related to the degree of immunosuppression and adequacy of the host immune response. Although EBV disease and PTLD may affect many different clinical sites, the tendency of EBV disease is to involve the transplanted organ. Thus, EBV hepatitis and PTLD of the liver are observed more commonly in liver transplant recipients.[18] Similarly, the intestine is the most frequently observed site of involvement of EBV disease in intestinal transplant recipients. Of interest, involvement of sites beyond the gastrointestinal tract is an uncommon occurrence in intestinal transplant recipients.

The onset of viral syndrome, mononucleosis, and PTLD takes place primarily within the first year, whereas lymphoma tends to occur later. Immunosuppressive regimens based on the use of tacrolimus appear to have induced a shift in the timing of PTLD; only rare cases develop more than 18 months after transplantation.[16,128] This pattern of timing of EBV disease and PTLD appears to apply to all pediatric organ recipients, including children undergoing liver or intestinal transplantation. However, the impact of newer immunosuppressive agents and regimens on EBV disease remains to be determined.

The diagnosis of EBV-associated PTLD is made on the basis of clinical, laboratory, and histopathologic examination and should be suspected in patients with protracted fever, exudative tonsillitis, lymphadenopathy, organomegaly, leukopenia, or atypical lymphocytosis.[46,91] Gastrointestinal involvement should be suspected in patients with persistent fever and diarrhea. Accordingly, EBV must be considered in the differential diagnosis of rejection in intestinal transplant recipients. Serologic diagnosis often is confounded by the presence of passive antibody acquired at the time of transplantation or during subsequent transfusions. Detection of increased EBV viral load in peripheral blood by polymerase chain reaction (PCR) has gained wide acceptance as

a means of predicting the risk for or presence of EBV or PTLD.[48,69,91,97,100] Though extremely sensitive, these assays are limited by their lack of specificity; they often are elevated in asymptomatic patients.[3] Accordingly, every effort should be made to confirm the diagnosis of EBV or PTLD histologically. Occult sites of PTLD are assessed by performance of computed tomography of the chest and abdomen. Palpable nodes or lesions (or both) identified by radiographic surveillance should undergo biopsy. Endoscopic evaluation should be considered in patients with diarrheal illnesses and elevated viral loads. Histologic evaluation for typical features may be augmented through use of the Epstein-Barr–encoded RNA (EBER) probe.[95] Use of the EBER probe may be particularly helpful in differentiating between the presence of rejection and EBV infection in intestinal transplant recipients.

Management of patients with PTLD is controversial.[14,16,46,91] Reduction of immunosuppression is recommended widely. Antiviral agents typically are used,[54.55,98] although their role has not been studied formally. Reduction of immunosuppression, alone or in combination with antiviral agents, results in an approximate 67 percent cure rate of EBV disease and PTLD. The potential impact of monoclonal antibodies,[33] interferon,[103] and chemotherapy[35] awaits formal clinical trials. Resection of tumor also may be of value for patients with lymphoma. Recent experience has focused on several novel approaches to the management of EBV disease and PTLD. These newer strategies generally have been used for patients who fail to respond to reduction of immune suppression (with or without the use of antiviral agents). Rituximab, an anti-CD20 antibody, has been used increasingly for the treatment of EBV disease. Experience to date suggests that as many as two thirds of patients who fail initial withdrawal of immunosuppression will respond to a 4-week course of this agent.[47] However, relapse of EBV disease has been observed in 20 to 25 percent of treated patients 6 to 8 months after completion of therapy, at the time that rituximab no longer is present in the body. An alternative, chemotherapy-based approach for patients who fail to respond to initial reduction or withdrawal of immunosuppression also has been proposed.[52] This strategy, which uses modified doses of cyclophosphamide and prednisone, likewise has achieved success in approximately two thirds of treated patients. Unfortunately, as with rituximab, relapse of PTLD has been seen in 22 percent of treated patients, and outright treatment failures have occurred in patients with fulminant disease. Definitive studies comparing these two second-line therapies are needed to determine the best option for children who fail to respond to initial therapeutic modification of immunosuppression. A summary of our suggested approach and management of EBV/PTLD is provided in Table 83–5.

OTHER HERPESVIRUSES

Other herpesviruses also can be hazardous after transplantation. Herpes simplex can reactivate early after surgery or after augmentation of immunosuppression. Prophylaxis with acyclovir has been beneficial in these situations. A summary of the suggested approach to the diagnosis and management of infection with herpes simplex virus is provided in Table 83–5. Varicella in nonimmune transplant recipients can lead to disseminated fatal disease[82] and should be treated early and aggressively with intravenous acyclovir.

More recently, interest has focused on determining what role, if any, the recently recognized human herpesvirus-6 (HHV-6) and HHV-7 may play in causing disease in organ transplant recipients in general and abdominal transplant recipients in particular. Several groups of investigators have identified a potential interaction between the development of HHV-6 and HHV-7 and CMV infection in organ transplant recipients.[65,84] Reactivation of HHV-6 infection after liver transplantation has been associated with the development of an increased CMV viral load in peripheral blood, as well as a greater likelihood of symptomatic CMV disease developing.[65] In addition, some investigators have suggested that some or all of the symptoms typically associated with CMV syndrome (e.g., fever, leukopenia) in patients with proven CMV infection may be attributable in part to HHV-6 or HHV-7. Studies in children have suggested that infection with HHV-6 alone is a relatively common cause of unexplained fever in pediatric liver transplant recipients.[126,127] Interest also has begun to focus on what role, if any, HHV-8 may have in causing infection and disease in organ transplant recipients, particularly recipients from countries with moderate to high rates of HHV-8 prevalence, such as Africa, the Middle East, and the Carribean.[86] The full spectrum of disease caused by these newer viruses and their potential therapies remain to be determined.

ADENOVIRUS

Adenovirus has been reported to be the third most important virus affecting pediatric liver transplant recipients; it was found in 10 percent of our series of 484 children undergoing liver transplantation under cyclosporine-based immunosuppression.[85] Symptomatic disease (ranging from self-limited fever, gastroenteritis, or cystitis to devastating illness with necrotizing hepatitis or pneumonia) occurred in more than 60 percent of infected patients. Infection developed within the first 3 months after transplantation. The frequency of invasive adenovirus infection after pediatric liver transplantation appears to have decreased markedly with the use of tacrolimus-based immunosuppression.[50] In a more recent report, McLaughlin and colleagues[83] found that only 4.2 percent of pediatric liver recipients receiving tacrolimus-based therapy had adenovirus disease. This rate is in contrast to a significantly higher incidence of adenovirus infection of 20.8 percent in pediatric intestinal transplant recipients at the same institution. The increased prevalence of adenovirus in intestinal transplant recipients is illustrated further by the report of Pinchoff and colleagues,[92] who found adenovirus in all 14 of their pediatric intestinal transplant recipients. However, this exceptionally high rate might be attributable to the fact that viral cultures were performed as part of routine screening of graft biopsy specimens and not all of the patients were symptomatic. In both these studies, high volume of stool output, alone or in the presence of fever, was the most common symptom found in the patients.

Presumptively diagnosing infection caused by adenovirus in pediatric abdominal transplant recipients is very difficult inasmuch as fever, hepatitis, and pneumonia may be due to a variety of other pathogens and high volume of stool output after intestinal transplantation is nonspecific and can occur with rejection as well. The presence of high-grade fever and symptoms suggestive of adenovirus infection should prompt obtaining serial cultures for viruses (including adenovirus) or PCR investigation and evaluation of graft biopsy tissue. Unexplained elevations in hepatocellular enzymes suggestive of hepatitis should warrant consideration of liver biopsy. Similarly, an increase in stool output, with or without fever, should prompt endoscopic evaluation of the intestinal allograft. Histologic examination for the presence of adenoviral inclusions, as well as immunohistochemical staining of biopsy specimens from either site, should be undertaken to help confirm this diagnosis.

Unfortunately, no definitive treatment is available for adenoviral infection at this time. The most important component of therapy is supportive care along with a decrease in immunosuppression. The role of antiviral agents is unproven. A small number of case reports describe the use of ribavirin[8,68,76,83,88] and ganciclovir[125] in the treatment of single patients with adenoviral infection

after undergoing solid organ or bone marrow transplantation. In vitro evidence supports the theoretical role of ribavirin but not that of ganciclovir in the treatment of these infections. In addition to these published reports, an adult lung transplant recipient with disseminated adenovirus type 7 improved after treatment with cidofovir and pooled, high-titer immunoglobulin against RSV (Respigam) along with decreased immunosuppression.[43] Several other case reports have described the successful use of cidofovir for the treatment of adenoviral disease in a bone marrow transplant recipient, as well as a patient with acquired immunodeficiency syndrome.[56,96] A single case report also raised the possibility of a role for intravenous immunoglobulin (IVIG) as treatment of adenovirus infection.[27] Unfortunately, no conclusive evidence of the efficacy of these antiviral agents or IVIG can be drawn from these reports. A summary of a suggested approach to the diagnosis and management of adenovirus infection is provided in Table 83–5.

COMMON COMMUNITY-ACQUIRED VIRUSES

Although the course of illness has been poorly documented, most children who undergo liver and intestinal transplantation experience the usual respiratory viruses and gastrointestinal illness without significant problems. However, infection by influenza, parainfluenza, or RSV leads to more severe disease in young children, especially if the infection occurs soon after transplantation and during periods of maximal immunosuppression.[6,31,66,93] Likewise, transplant recipients may have prolonged viral shedding even after resolution of symptoms. A summary of suggested strategies for the diagnosis and management of these community-acquired viruses can be found in Table 83–5.

OTHER VIRUSES

Other viruses, including both donor-associated viral infections (e.g., hepatitis B and C) and community-acquired viral pathogens (e.g., enterovirus, rotavirus) are relatively uncommon causes of infection or disease after abdominal transplantation. Suggested approaches to the diagnosis and management of each of these viral pathogens are provided in Table 83–5.

OPPORTUNISTIC INFECTIONS

P. jiroveci is a well-documented cause of pneumonia in immunocompromised patients, including liver and intestinal transplant recipients. Prophylactic trimethoprim-sulfamethoxazole (TMP-SMX) is safe, inexpensive, and effective.[64] Use of this strategy has eliminated PCP in these patients at our center. Alternative prophylactic regimens for sulfa-allergic patients include aerosolized pentamidine (for patients >5 years of age)[74] or dapsone.[63]

Tuberculosis (TB) is a particular concern in immunosuppressed hosts, including recipients of liver and intestinal transplants. The incidence of TB after liver transplantation in Europe and the United States has been reported to range from 0.9 to 2.3 percent, with most cases reported in adults.[106,121] In contrast, TB may develop in as many as 15 percent of organ transplant recipients in areas of high-level endemicity.[106] However, development of TB after pediatric liver transplantation is an extremely uncommon event, with only 11 cases reported thus far.[79,106,121] To date, no cases have been reported in recipients of intestinal transplants. The development of TB in solid organ transplant recipients is associated with mortality rates ranging from 25 to 40 percent, with additional morbidity and mortality associated with the development of rejection in patients receiving antituberculous therapy.[106,121] The diagnosis of TB in transplant recipients is complicated by the fact that extrapulmonary disease occurs frequently and purified protein derivative (PPD) testing is likely to be unreliable after transplantation. Management of TB in liver transplant recipients is difficult because of both the side effects of antituberculous agents and their potential interactions with immunosuppressive agents.[106,121] Limited published experience in pediatric liver transplant recipients suggests that most infections caused by *Mycobacterium tuberculosis* in all probability are due to a primary infection, often associated with family contacts who have positive skin tests.[79,121] In contrast, experience with adult transplant recipients suggests that the development of TB is more likely to be due to reactivation of latent TB.[58,106,121] Despite the limited published information describing TB in these patients, transplant recipients known to have a positive PPD test or who come from an area endemic for TB appear to be at increased risk for symptomatic reactivation after transplantation.[58,121] Similar data are not available for recipients of intestinal transplants. Additional factors predisposing to the development of TB after transplantation include severe hepatic failure at the time of transplantation, aggressive anti-rejection therapy, and HIV infection.[58,115] Experience with adult renal transplant recipients suggests that even though the risk appears to be greatest in patients who received inadequate or no previous TB therapy,[75,78] it also can occur in patients who received appropriate anti-TB therapy in the pretransplant period.[75,78,94] Although TB has been encountered only rarely in pediatric liver transplant recipients[121] and not in intestinal recipients, screening for TB by history and PPD testing, along with review of a chest radiograph for lesions consistent with healed TB, is highly recommended. Patients with a positive TB history or a positive PPD test, or both, should receive isoniazid for 6 to 12 months after undergoing transplantation, although some experts recommend continuing isoniazid indefinitely while patients remain on immunosuppression. Attempts at establishing a more definitive diagnosis are indicated for patients from endemic areas with a negative PPD test but a suspicious chest radiograph. Careful evaluation for evidence of side effects, particularly hepatotoxicity, is recommended, and isoniazid is discontinued if unacceptable toxicity is identified.

Additional potential opportunistic infections include cryptococcosis, coccidioidomycosis, and histoplasmosis, although these pathogens have not been reported frequently in pediatric liver or intestinal transplant recipients. Previous infection with these pathogens is common in geographic areas where they are endemic. Because patients often travel to transplant centers distant from their homes, it is imperative that transplant physicians be aware of the local environmental risks for each patient. Experience with coccidioidomycosis in transplant recipients suggests that a minimum of 4 months of antifungal therapy, such as fluconazole, should be given to transplant recipients with this history.[53] Similarities between coccidioidomycosis and other fungal pathogens suggests that similar strategies may be necessary for patients with a positive history of previous fungal infection with pathogens known to recur after resolution of the primary infection.

MANAGEMENT

PRETRANSPLANT EVALUATION

Pretransplant evaluation is helpful in the management of infectious complications in liver and intestinal transplant recipients. A complete history and physical examination should be performed with particular attention given to previous infections, immunizations, and drug allergies. Attention should be paid to a history of infection with multiply antibiotic-resistant bacteria, which may provide guidance for any future empiric treatment that may be required after transplantation. An intermediate-strength tuberculin skin test should be performed on all patients.

We recommend serologic evaluation of all candidates for CMV; EBV; varicella; herpes simplex virus; hepatitis A, B, and C; and HIV. Serologic tests on the donor should include HIV, hepatitis B and C viruses, CMV, and EBV. Donors positive for HIV or hepatitis B virus should be excluded. The use of organs from donors positive for hepatitis C probably would be contraindicated except in the circumstance in which the recipient also is positive for hepatitis C. Knowledge of donor and recipient status for these viruses allows one to anticipate infection, identify patients who might benefit from prophylactic regimens, and narrow the differential diagnosis in a patient with fever.

PROPHYLACTIC REGIMENS

Prophylactic regimens vary among transplant centers. These strategies have been divided into perioperative and long-term prophylaxis and often evolve to reflect the infectious complications seen at individual institutions.

Perioperative prophylaxis is used to prevent intraoperative sepsis and wound infection. It is based on individual patient characteristics and the expected normal flora. We consider piperacillin-tazobactam an appropriate agent for perioperative prophylaxis. If sepsis is suspected in the donor, antibiotics are chosen to cover organisms identified from the donor, and treatment usually is extended to a therapeutic course of 10 to 14 days. In the absence of proven or suspected infection in the donor, perioperative prophylaxis generally is limited to the first 48 hours after transplantation.

Considerations regarding long-term prophylaxis against infections occurring beyond the perioperative period include the risk and severity of infection, as well as the toxicity, cost, and efficacy of a given prophylactic strategy. Nystatin is recommended for all pediatric transplant recipients in an effort to prevent oropharyngeal candidiasis. TMP-SMX is used to prevent PCP. Although some centers recommend using TMP-SMX for only the first 6 months after liver transplantation, anecdotal experience of PCP occurring long after patients receive their transplants and the relative safety of this agent have led us to recommend its use indefinitely after liver and intestinal transplantation in children.

The frequency and severity of CMV infections in transplant recipients prompt consideration of prophylactic strategies, and optimization of the target population and timing of interventions require further study.[7,26,32,90,101,123] Potential roles exist for intravenous and oral ganciclovir[34,42,72] and oral valganciclovir.[109,110] Currently, we recommend the use of intravenous ganciclovir alone (for varying durations) for liver and low-risk intestinal transplant recipients (recipient CMV-positive before transplantation or donor and recipient seronegative) and ganciclovir plus IVIG with a high titer of antibody against CMV for high-risk (donor CMV-positive/recipient CMV-negative) intestinal transplant recipients if it is available. At the present time, oral valganciclovir is an alternative to prophylaxis with intravenous ganciclovir for adolescents. Pharmacokinetic studies of dosing of oral valganciclovir suspension in children after transplantation are ongoing. On completion of these studies, the use of valganciclovir suspension probably will be of merit as an alternative to intravenous treatment in younger children.

Serial monitoring of the blood CMV viral load with either the pp65 antigen assay or quantitative CMV PCR as an indicator for the use of preemptive antiviral therapy has been proposed as an alternative to these chemoprophylactic and immunoprophylactic strategies.[45] In this approach, only patients demonstrating increased risk because of increased viral load are treated with intravenous or oral ganciclovir. Although this strategy has gained acceptance at some centers, experience in pediatric transplant recipients in general and abdominal transplant recipients in particular remains limited. The use of viral load monitoring after completion of chemoprophylaxis is gaining increasing acceptance.

The growing recognition of the importance of EBV infection in pediatric organ transplant recipients has led to an interest in the prevention of EBV infection and PTLD. Numerous strategies (e.g., immunoprophylaxis, monitoring, and preemptive therapy) are being explored,[47,80] the efficacy of these approaches has not been established. The use of viral load monitoring to inform preemptive reductions in immunosuppression appears to be the most promising of these strategies.[73,80] Because reduction of immune suppression is not always possible for intestinal transplant recipients with a rising EBV load, preemptive intravenous ganciclovir and IVIG have been used in addition to reduction of immune suppression (when possible) for this cohort of patients. Although prospective comparative data are not available, the use of this strategy appears to have resulted in a decreased risk for disease and better outcome than in historical controls who were not managed with this strategy.[41]

SUMMARY

Infections remain an important problem after liver and intestinal transplantation. Knowledge of the type, timing, and predisposing risk factors for these infectious complications allows for timely and appropriate diagnosis and management.

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CHAPTER 84

Opportunistic Infections in Kidney Transplantation

Gail J. Demmler-Harrison

Renal transplantation is the therapy of choice for end-stage renal disease (ESRD) in children and adolescents.[139] It is successful in 90 percent of recipients and allows most children the best opportunities for normal growth and development and an almost normal lifestyle.[74,142,221] Despite the overall success of renal transplantation, infection remains the major cause of morbidity, graft loss, and mortality in renal transplant recipients.[50,214] Newer and more effective immunosuppressive regimens have reduced the rates and hospitalizations for graft rejection after renal transplantation, but rates and hospitalizations for bacterial and viral infections seem to be unchanged or increasing.[53,143,185] Prednisone-free maintenance immunosuppressive combinations with tacrolimus, mycophenolate mofetil, and sirolimus help maintain graft function, but they also may increase the risk for development of post-transplantation lymphoproliferative disorders (PTLD) and invasive fungal diseases.[104]

These infections can be managed successfully if detected early and treated appropriately.[104] This chapter presents a general approach to renal transplant recipients from the perspective of the infectious disease specialist. Specific, detailed information regarding the diagnosis and management of each particular pathogen can be found in the respective chapters in the section of this textbook dedicated to infections with specific microorganisms.

PRE-TRANSPLANT EVALUATION

The role of the pediatric infectious disease specialist in the care of a renal transplant recipient ideally begins during the pre-transplantation period.[168,169] Before the patient undergoes transplantation, a thorough history and physical examination should be performed, with a focus on evaluation for evidence of an active infection that may require immediate therapy or, rarely, preclude transplantation (Table 84–1). The history should be comprehensive but focus on the details of any history of previous infections that may re-emerge during the post-transplant period, including urinary tract infections (UTIs); mucocutaneous diseases such as herpes simplex virus (HSV); systemic illnesses such as tuberculosis; chronic infections such as hepatitis B virus (HBV), hepatitis C virus (HCV), or human immunodeficiency virus (HIV); and diarrheal diseases. Infection with HIV previously was regarded as a reason to exclude a potential recipient from undergoing rental transplantation. More recent successes with highly active antiretroviral therapy have improved long-term survival in HIV-infected pediatric patients, however, and have led clinicians, research scientists, and family members themselves to investigate the role of renal transplantation as a treatment for ESRD.[60,167]

Renal transplant candidates undergo peritoneal dialysis or hemodialysis, so a history of previous dialysis catheter–associated infections should be documented, and any current infection should be treated and eliminated before transplantation is performed. Another focus of the history should include an exposure history for the patient's country of origin or foreign travel, especially to areas endemic for organisms such as *Strongyloides stercoralis*, *Coccidioides immitis*, *Blastomyces dermatitidis*, or *Histoplasma capsulatum* and for diseases such as malaria and tuberculosis.[87,185] Other important exposures include blood product transfusions; exposure to animals or plants; well water as a source of drinking water; and dietary habits, especially consumption of raw or undercooked eggs or meat or unpasteurized dairy products.[168,169]

TABLE 84–1 Guidelines for Pre-transplant Evaluation in Pediatric Kidney Transplant Candidates

History
Past infectious diseases
Routine childhood illnesses
Travel to, birth or residence in areas endemic for fungal or parasitic diseases
Tuberculosis exposure
Animal exposure
Diet preferences and water resources
Vaccinations
Reactions or allergies to antimicrobials
Current or past immunosuppression
Physical
Search for active or latent focus of infection
Nutritional status
Laboratory and Other Testing
PPD
Chest radiograph
Urinalysis and urine culture
Viral serology for HSV, CMV, EBV, VZV, human *Erythrovirus* (human parvovirus B19), HAV, HBV, HCV, HIV, BK virus, WNV, and others depending on the history
Baseline HSV, CMV and EBV DNA PCR or CMV antigenemia, parvovirus B19, HCV, BK virus if seropositive or post-transplant monitoring is anticipated
Fungal and parasitic testing if travel or exposure history positive; sputum or stool tests as indicated
Anticipatory Guidance
Update vaccines
Counsel regarding measures to reduce infection risk
Consider antimicrobial prophylaxis if at risk

CMV, cytomegalovirus; EBV, Epstein-Barr virus; HAV, hepatitis A virus; HBV, hepatitis B virus; HCV, hepatitis C virus; HIV, human immunodeficiency virus; HSV, herpes simplex virus; PCR, polymerase chain reaction; PPD, purified protein derivative; VZV, varicella-zoster virus; WNV, West Nile virus.

In children, a careful history of routine childhood illnesses, including varicella, measles, mumps, and rubella, should be documented.[107]

The immunization history of the patient should be documented carefully and, if needed or indicated, updated before the patient undergoes transplantation. Ideally, children with chronic renal disease should be fully immunized before progression to ESRD, for optimal immune response and protection during outbreaks of vaccine-preventable diseases.[41] Vaccinations against tetanus, pertussis, and diphtheria; polysaccharide vaccines, such as those against pneumococcus and *Haemophilus influenzae* type B; inactivated vaccines against polio and hepatitis A; and recombinant vaccines, such as those for hepatitis B, may be given or updated at any time before transplantation is performed.[28,208,220] Meningococcal vaccine is indicated routinely now for specific age groups and for special outbreak situations. The live varicella vaccine is recommended for renal transplant candidates who have not had varicella; it should be given at least 2 to 4 weeks before transplantation is performed.[238] Measles, mumps, rubella vaccine should be administered even earlier, preferably months before the patient undergoes transplantation. In addition, annual influenza vaccine and a tetanus booster every 10 years are recommended for patients who are renal transplant candidates or recipients.

If the child is unable to be fully immunized before undergoing transplantation, the routine immunization schedule for the inactivated vaccines may be reinstituted after immunosuppression is decreased, approximately 6 to 12 months after an uncomplicated transplant procedure.[28,208,220] Live virus vaccines should be avoided in the post-transplant period in most instances. Reports of safe and effective vaccination with varicella vaccine in selected post-transplant patients have been published, however.[26,34,71,161,162] Close contacts and family members of renal transplant candidates and recipients also should be fully immunized and should receive the annual influenza vaccine.[10] Other important points in the pre-transplant evaluation history include allergies or reactions to medications, especially antibiotics, and the use of immunosuppressive agents.

Pre-transplant laboratory and diagnostic imaging evaluations for most renal transplant candidates should include a tuberculin skin test, chest radiograph, urinalysis, and urine culture for bacteria. Baseline renal and liver function tests also should be performed.

Serologic screening of the transplant recipient's status regarding organisms that may reactivate in the recipient or infect the recipient via the donor organ should be performed; such screening should include tests for HSV; cytomegalovirus (CMV); varicella-zoster virus (VZV); Epstein-Barr virus (EBV); human *Erythrovirus* (human parvovirus B19); syphilis; toxoplasmosis; hepatitis A, B, and C viruses; and HIV. Transplant recipients who are seropositive for HSV or CMV usually receive antiviral prophylaxis during the peritransplant period, whereas recipients seropositive for EBV may undergo post-transplant monitoring. It may be helpful to save an aliquot of the recipient's (and donor's) serum in case unusual circumstances occur, such as infection with West Nile virus (WNV). In CMV-seropositive transplant candidates, baseline CMV antigenemia or quantitative DNA polymerase chain reaction (PCR) may be useful, and in EBV-seropositive candidates, detection of EBV DNA by quantitative PCR may be helpful to document pre-transplant viral load, especially if post-transplant virologic monitoring is to be performed. Other laboratory tests or imaging studies may be useful, depending on the patient's exposure history and physical examination.

The pre-transplant evaluation also is an opportunity for the infectious disease specialist to counsel the patient and family about measures that may reduce the transplant recipient's risk for development of infectious disease complications post-transplant. Patients who have an exposure, or even a suspected exposure, to varicella (chickenpox) or zoster (shingles) should contact their physicians immediately to see whether passive immunoprophylaxis with varicella-zoster immunoglobulin or post-exposure antiviral therapy with acyclovir or valacyclovir is indicated. Plans for foreign travel to remote areas also should be discussed with the physician. The transplant recipient should consume only thoroughly cooked meat and seafood and thoroughly washed fresh fruits and vegetables. In addition, drinking water should be pure. Transplant recipients should avoid, if possible, changing cat litter boxes, aquariums, and birdcages and should avoid close contact with people who have viral respiratory illnesses. Finally, medical attention should be sought if fever occurs, especially if it is significant or persistent.

POST-TRANSPLANT INFECTIOUS COMPLICATIONS

Infections occurring in the post-transplant period can be grouped into three main time frames: the first month post-transplant (early), 2 to 6 months post-transplant (middle), and 6 months post-transplant and onward (late).[187,204] Although almost any organism or pathogen can infect a transplant recipient at any time, these time periods provide the clinician with a guide to the organisms and disease processes most commonly encountered (Table 84–2).

INFECTIONS OCCURRING DURING THE EARLY POST-TRANSPLANT PERIOD

Infections occurring during the first month after renal transplantation has been performed usually are bacterial.[193] Common sites of early infections include the wound, urinary tract, lungs, and indwelling intravascular catheters.[174]

Wound Infections

As with any surgical procedure, wound infections may develop in a renal transplant recipient. They occur in approximately 2 percent of renal transplant recipients and range in severity from a superficial wound infection, easily treated with wound care and antimicrobial therapy, to deep perinephric abscesses that may be difficult to treat and result in transplant nephrectomy.[188,193] Wound infections are more likely to occur in patients with technical problems associated with the transplant surgery, including urinary leaks, vesicoureteral reflux, wound hematomas, or lymphoceles.[148,239] Malnutrition during the pre-transplant period may impair wound healing and predispose the patient to development of wound infection.

Open Penrose drains may increase the likelihood of introducing microorganisms into the wound, whereas closed suction drainage, such as with a Jackson-Pratt drain, may reduce this risk. In addition, prompt removal of all drains, usually within 5 days in most uncomplicated cases, and administration of prophylaxis with perioperative antibiotics may decrease the incidence of wound infections; such measures are performed routinely in most renal transplant centers. The regimen usually is aimed at uropathogens and staphylococci. One dose generally is given pre-transplant, and the regimen is continued for only 24 hours post-transplant.

The diagnosis of a wound infection should be suspected if erythema, warmth, or discharge is present at the wound site or if an unexplained fever develops. Fluid may drain from the wound persistently, or a fluid collection or abscess may be seen on imaging of the deeper operative sites. The patient will be receiving immunosuppressive agents, however, and the findings may be unusual, or the symptoms may be blunted. Any fluid or pus

TABLE 84–2 Timetable for the Occurrence of Common Infections and Usual Pathogens after Kidney Transplantation

Early Period
Wound infections
UTI
Bacteremia and sepsis syndrome
Pneumonia
HSV
HBV
Human *Erythrovirus* (human parvovirus B19)
WNV
Seasonal viruses
Drug reactions
Middle Period
Herpesviruses
CMV
EBV
VZV
HHV types 6, 7, and 8
Polyomaviruses
JC virus
BK virus
Papillomaviruses
Adenoviruses
Human *Erythrovirus* (human parvovirus B19)
Listeria monocytogenes
Mycobacterium tuberculosis
Atypical mycobacteria
Nocardia
Fungal diseases
Pneumocystis carinii
Parasitic diseases
Late Period
Community-acquired respiratory viruses and bacterial pathogens
UTI
Streptococcus pneumoniae
CMV
EBV
VZV
HBV
HCV
HIV/AIDS

AIDS, acquired immunodeficiency syndrome; CMV, cytomegalovirus; EBV, Epstein-Barr virus; HBV, hepatitis B virus; HCV, hepatitis C virus; HHV, human herpesvirus; HIV, human immunodeficiency virus; HSV, herpes simplex virus; UTI, urinary tract infection; VZV, varicella-zoster virus; WNV, West Nile virus.

obtained should be stained and cultured for bacterial, mycobacterial, and fungal organisms. Organisms most likely to be identified as causes of wound infection include staphylococci (*Staphylococcus aureus*, especially methicillin-resistant *S. aureus*, but also coagulase-negative staphylococci), streptococci, and gram-negative enteric organisms. Unusual, multidrug-resistant, or health care–associated bacterial pathogens and yeast, such as *Candida albicans*, also may cause wound infections post-transplant. In addition, case reports of wound and perinephric fluid collections infected with *Mycoplasma hominis* have been published.[206]

Appropriate antimicrobial therapy, initially broad-spectrum and then ultimately tailored to the isolated organism and its susceptibility pattern, should be administered. The duration of appropriate antimicrobial therapy usually is 10 to 14 days, or until the wound infection has resolved and the patient has been afebrile for 3 to 5 days. Deep abscesses or unusual organisms may require longer therapy.

Urinary Tract Infections

UTIs are common developments after renal transplantation and may affect 35 to 79 percent of renal transplant recipients.[116,239] Post-transplant UTIs are associated with acute and chronic graft dysfunction and may threaten renal graft survival.[2,11,30,48,106,151,213] They may occur during the early, middle, or late post-transplant period. A UTI that occurs during the early or early/middle post-transplant period often is a severe illness complicated by pyelonephritis, urosepsis, metastatic foci of infection, allograft dysfunction, rejection, and relapse.[152,189] UTIs occurring in the early post-transplant period are associated more often with graft loss than are UTIs that occur later.[48]

The risk for developing an invasive UTI after undergoing renal transplantation seems to be increased in patients with prolonged bladder catheterization (most catheters can be removed during the first few days post-transplant), dysfunctional bladder, ureterovesical disorders with reflux, malnutrition, underlying disorders, renal stones, obstructive uropathy, or a contaminated cadaveric kidney.[2,168] Surgical complications, such as hematoma, reflux, or obstruction at the urinary anastomosis, or inability of the bladder to empty completely are associated with UTIs. In addition, young infants, especially infants who have vesicoureteral reflux, seem to have a high incidence of complicated UTIs post-transplant.[152] Procedures that address the etiology and correct underlying physiologic and anatomic abnormalities of the bladder and ureters should be performed before transplantation to avoid or lessen development of complications associated with these problems.[2,11,30,213] Newer surgical techniques allow children with reconstructed bladders to undergo successful renal transplantation with minimal risk for post-transplant development of UTIs.[148,189]

The organisms most commonly isolated from patients with early-onset UTIs include not only the typical gram-negative enteric bacteria, but also enterococci, staphylococci, and *Pseudomonas aeruginosa*. Unusual organisms, such as *Streptococcus mitis*, *Serratia marcescens*, and *Corynebacterium urealyticum*, also can be found. Antimicrobial therapy should be tailored to the susceptibility pattern of the organism isolated from the urine. Because 30 percent of patients experiencing a UTI during the early post-transplant period may have recurrent UTIs, a prolonged 6-week course of antibiotics usually is recommended to reduce the risk of developing a relapsing kidney infection.[148,189] Antimicrobial prophylaxis may reduce the risk of developing a UTI. Trimethoprim-sulfamethoxazole administered for the first 4 months after renal transplantation is effective in preventing most UTIs and can provide cross-cover prophylaxis against *Pneumocystis carinii* pneumonia and other diseases.[69]

Pneumonia

Pneumonia can occur during the first month after renal transplantation and often is associated with prolonged endotracheal intubation. Gram-positive and gram-negative bacterial pathogens acquired from normal oropharyngeal flora and unusual or health care–associated multidrug-resistant organisms predominate during the early post-transplant period.[169] *Legionella pneumophila* and unusual *Legionella* spp., such as *Legionella micdadei*, *Legionella bozemanae*, and *Legionella dumoffii*, also have caused outbreaks of serious, life-threatening pneumonia in transplant recipients in some centers.[29,39,115,169] *P. carinii* can cause pneumonia in a transplant recipient during the early post-transplant period, but more often it is associated with disease after the first month post-transplant.[89] Rare or unusual pathogens, such as *Rhodococcus equi*, may cause pneumonia in these patients.[128]

Patients with pneumonia usually present with fever, chills, chest pain, malaise, change in tracheal secretions, cough, dyspnea, tachypnea, change in ventilatory status, rales or rhonchi on auscultation of the lungs, and pulmonary infiltrates on chest radiograph. Pneumonia may be complicated by pleural effusion, empyema, or pulmonary abscess, and death may occur if pneumonia is severe and not diagnosed and treated promptly.

Treatment with antibiotics effective against the bacterial pathogens isolated from culture of tracheal aspirates, bronchoalveolar lavage fluid, or lung tissue is appropriate.

Bacteremia, Fungemia, and Sepsis

Bacteremia, fungemia, and sepsis occurring during the early post-transplant period often are associated with indwelling catheters.[167] The urinary tract, surgical wound, or transplanted or native kidney also may be a source.[138] Usual bacterial organisms such as coagulase-negative staphylococci or *S. aureus*, unusual bacterial organisms, and yeasts such as *C. albicans* and other *Candida* spp. may be involved. *Listeria monocytogenes* also may cause primary bacteremia or sepsis at any time after a kidney transplantation has been performed, but the greatest risk occurs during the early period. Complications include meningitis, and 10 percent of these patients may die.[150,199] Renal transplant recipients also are at increased risk for development of bacteremia with *Salmonella* nontyphoidal species.[47,100,167]

Complications such as UTI, graft infection, peritonitis, abscesses, and meningitis can occur, and recurrences are common. Therapy for bacteremia, sepsis, or its complications is tailored to the susceptibility pattern of the organism isolated from the patient's blood. The duration of therapy for uncomplicated bacteremia usually is 10 to 14 days, but a longer course of treatment may be indicated if abscesses occur or an unusual organism is isolated. Removal of the indwelling catheter may be necessary to clear persistent bacteremia, and abscesses and other foci of infection should be drained.

Other Bacterial Diseases

Pediatric renal transplant recipients also seem to be at increased risk for development of antibiotic-associated colitis caused by *Clostridium difficile*.[232] Infection of a lymphocele with *Pasteurella multocida* has been reported, as has systemic infection with *Bartonella henselae*.[3,37,52,132,167] A high index of suspicion always must be maintained when evaluating a transplant recipient for infection because the immunosuppression required to maintain the transplanted kidney predisposes the recipient to development of infection with unusual organisms.

Viral Infections

HERPES SIMPLEX VIRUS

HSV is the most common virus encountered during the early post-transplant period, although antiviral prophylaxis significantly reduces the risk of this infection.[56,57] It occurs less frequently in pediatric (8%) than in adult renal transplant recipients (30%).[167,168] Most HSV infections encountered post-transplant are due to reactivation of the recipient's strain; however, primary or recurrent infection acquired from the renal allograft may occur, and primary infection from person-to-person transmission has been documented.[56,167,168,189]

Post-transplant HSV infection may be asymptomatic or associated with disease, most frequently oral ulcers in pediatric patients.[83] Genital and perianal ulcers may occur in adolescents and adults. Rarely, HSV may cause disseminated cutaneous lesions or zosteriform eruptions. HSV esophagitis may be manifested as dysphagia, refusal to eat, irritability, and substernal chest pain, and it may complicate oral HSV disease, especially if the oral mucosa has been traumatized by orogastric or nasogastric tubes. Acute, severe hepatitis with hepatic necrosis, often accompanied by hypotension and disseminated intravascular coagulation, also can occur. Tracheobronchitis and pneumonitis may occur as a result of HSV, primarily in patients with pneumonia caused by another pathogen and whose mucosa has been traumatized by endotracheal intubation. It is often severe and life-threatening, even with appropriate supportive care and antiviral therapy.[167] Encephalitis also has been reported in renal transplant recipients.[79]

The diagnosis is made by isolation of HSV in cell culture or detection of viral antigen by immunofluorescence or viral DNA by PCR in the end-organ involved. Detection or isolation of HSV in body secretions may represent asymptomatic shedding or disease and should be correlated clinically. Treatment with acyclovir is recommended for patients with disease, and HSV-seropositive transplant recipients should receive acyclovir or valacyclovir prophylaxis during the peritransplant period to prevent development of HSV infection. Most patients receiving ganciclovir, valganciclovir, foscarnet, or cidofovir for CMV prophylaxis also are protected against HSV.

OTHER VIRUSES

Seasonal viruses, especially winter respiratory viruses such as respiratory syncytial virus, influenza viruses, parainfluenza viruses, and adenoviruses; winter diarrhea viruses, such as rotavirus; and late summer/early fall viruses, such as the enteroviruses, can infect the transplant recipient during the early post-transplant period and cause disease. Such infections may be acquired from the family or the community, or they may occur from health care-associated exposures. The illness associated with these viruses typically is not as severe as the illness seen with the herpes family of viruses or adenoviruses.

Renal transplant recipients who are chronically infected with HBV or HCV may experience liver dysfunction during the early transplant period, but the late post-transplant period, beyond the first year, carries the greatest risk for progression of liver disease to cirrhosis. If a renal transplant recipient acquires HBV soon after undergoing transplantation, acute hepatitis can develop, often with death from liver failure.[46,167]

Non-infectious Causes of Fever

The most common non-infectious cause of fever in the first post-transplant month is allograft rejection.[188] Fever often is the first sign of rejection, especially in children, and rejection should be considered if an infectious source of the fever is not identified. Another common non-infectious cause of fever early after transplantation is antilymphocyte antibody therapy (OKT3). The first two or three doses of OKT3 produce a release of cytokines, which cause fever and chills. In most patients, these symptoms resolve after the third dose. Other non-infectious causes of fever during this period include drug reactions and pulmonary emboli.

INFECTIONS OCCURRING DURING THE MIDDLE POST-TRANSPLANT PERIOD

The cumulative effects of immunosuppression begin to be revealed during the period 2 to 6 months after transplantation. If a significant amount of antirejection therapy is required for multiple episodes of rejection, the effects may be more pronounced. Such immunosuppression allows classic opportunistic pathogens, such as CMV, *P. carinii*, *Toxoplasma gondii*, *L. monocytogenes*, *Aspergillus*, and *Nocardia*, to evade immune surveillance and cause disease.[89,167,231,233] Reactivation of organisms previously infecting the transplant recipient or the donor allograft, including *Mycobacterium tuberculosis*, HBV, HCV, HIV, *H. capsulatum*, and *C. immitis*, also may cause disease. In addition, an occult bacterial focus of infection that was not adequately identified and treated pre-transplant may become apparent at this time and cause significant disease.[188]

Herpesviruses

The herpes family of viruses (HSV types 1 and 2, CMV, EBV, VZV, and human herpesvirus [HHV] types 6, 7, and 8) share the biologic properties of latency, reactivation, cell association, and oncogenicity, which renders them the most important group of pathogens that affect renal transplant recipients.[167,194] HSV is more important during the early post-transplant period, whereas the other herpes family members are important causes of morbidity and mortality during the middle and late post-transplant periods.[90]

CYTOMEGALOVIRUS

CMV may cause primary infection in a CMV-seronegative transplant recipient through a renal allograft or blood product transfusion from a seropositive donor.[16,45,186] Person-to-person transmission within the family or close community also is possible. Recurrent CMV infection develops in seropositive transplant recipients if the recipient's CMV strain becomes reactivated. The renal allograft from a seropositive donor also can be a source of re-infection to the recipient and produce active CMV infection or disease.[36,84] CMV infection in renal transplant recipients may cause silent or asymptomatic infection; end-organ diseases such as hepatitis, esophagitis, colitis, encephalitis, vasculitis, and retinitis; and systematic disease with persistent fever and leukopenia. A case report also linked CMV infection with post-transplant occurrence of atypical hemolytic-uremic syndrome that resolved with plasma exchange and ganciclovir antiviral therapy.[160] It has been linked in some studies to allograft dysfunction and nephropathy.

CMV also causes depressed cell-mediated immunity and impaired alveolar macrophage function, rendering the host more vulnerable to other opportunistic infections, such as fungal disease and *P. carinii* pneumonia, and it serves as a cofactor for other viruses, such as EBV and HHV-6 and HHV-7.[111,163,175,216,217] In addition, CMV is associated with acute and chronic rejection and allograft nephropathy and decreased long-term patient survival.[1,58,121,167,186]

Disease caused by CMV can be documented by isolation of CMV from blood or tissue; detection of CMV antigen pp65 in circulating leukocytes; or detection of CMV DNA by PCR or similar assays in blood, bronchoalveolar fluid, or tissue.[21,22,171,176,186] Isolation of CMV in urine or saliva documents active infection but has little significance in predicting disease; serologic tests may document seroconversion in primary infections but generally should be reserved for pre-transplant screening only. Most transplant patients at risk for acquiring CMV disease (i.e., CMV-seropositive recipients [D^+/R^+] or CMV-seronegative recipients [D^+/R^-] who received a renal allograft from a seropositive donor) should be monitored by viral surveillance, usually by testing blood weekly for CMV antigen pp65 or for CMV DNA by PCR.[20,94]

Detection of significant levels of the virus by quantitative or semiquantitative assay, or an increase from baseline levels, usually predicts CMV disease.[217] Reduction of immunosuppression, along with preemptive antiviral therapy, generally is indicated and results in a decrease or resolution of CMV levels detected in blood.[7,22,40,43,77,81,129] Despite receiving adequate antiviral therapy, some patients have persistently positive CMV DNA by PCR, however, which should be interpreted within the clinical context.[21] In some patients who otherwise appear well, the DNA may be fragmented and nonreplicating, whereas in other patients with persistent symptoms, a strain of CMV resistant to one or more antiviral agents may be the cause.

The antiviral agents currently available for the treatment of CMV disease and preemptive therapy for positive CMV markers include ganciclovir, valganciclovir, foscarnet, and cidofovir.[230] For renal transplant patients with moderate to severe CMV-associated disease, 2 to 3 weeks of therapy with intravenous ganciclovir usually is adequate to treat disease; however, patients with CMV retinitis or repeat episodes of rejection may require maintenance therapy, usually with oral ganciclovir or valganciclovir.[101,134,153] Asymptomatic or mild CMV infections may respond to oral ganciclovir or valganciclovir administered for 1 to 3 months.[135,149] Preemptive therapy should be continued throughout the period of immunosuppression in patients who are severely immunosuppressed and at risk for development of CMV disease and recurrence (D^+/R^-).[81,94,135,149] Foscarnet and cidofovir have significant renal toxicity and should be used with extreme caution in renal transplant recipients. Prophylaxis for CMV disease is indicated in high-risk transplant recipients (D^+/R^-); options include intravenous CMV immune globulin, oral and intravenous acyclovir and ganciclovir, and oral valacyclovir and valganciclovir.[25,66,77,124,153,198,205,226]

EPSTEIN-BARR VIRUS

EBV infection in children who have received a renal transplant may be asymptomatic or may be associated with a variety of different syndromes, including a nonspecific viral syndrome, mononucleosis, smooth muscle tumors (leiomyoma), post-transplant PTLD, and lymphoma.[23,24,54,86,88,96] Infection and disease may occur after primary and recurrent EBV infection, but primary infection is more likely to occur and produce PTLD in younger children than in adults who have received solid organ transplants.[35,96] EBV disease may affect 10 percent of pediatric renal transplant recipients and may develop 1 month to 5 or more years post-transplant, with the risk accumulating every year of post-transplant survival.[50,200]

Knowledge about post-transplant EBV infection and disease is evolving; as graft survival has improved, with more intense immunosuppressive regimens that preserve graft function, and other opportunistic infections such as HSV, CMV, and *P. carinii* being successfully managed, EBV has emerged as a formidable obstacle to successful solid organ transplantation in children.[23,49,51,54,86,109,200] The estimated overall risk for development of serious life-threatening EBV-associated illness is at least 4 percent and increases to 10 percent in children who experience a primary infection with EBV after undergoing renal transplantation.[49,96] Other risk factors for PTLD in children who have received solid organ transplants include receipt of antilymphocyte therapy such as OKT3 for rejection; receipt of tacrolimus, sirolimus, or mycophenolate mofetil, rather than cyclosporine, for immunosuppression; and CMV or EBV donor/recipient (D^+/R^-) mismatch.[51,103,104]

Nonspecific viral syndromes and mononucleosis occur during the earlier post-transplant period, whereas lymphoma and leiomyoma are more likely to be a manifestation during the late post-transplant period.[25,54,70,86] Uncomplicated post-transplant mononucleosis is characterized by the self-limited illness of fever, pharyngitis, cervical adenopathy, and splenomegaly. The signs and symptoms of PTLD vary but often include persistent fever, weight loss, and generalized adenopathy.[96] The disease frequently is multisystemic and progressive, and involvement of the neck, chest, lungs, gastrointestinal tract, liver, spleen, eyes, and brain, as well as lesions, may be detected by computed tomography of the head, neck, chest, and abdomen.[4,5,168,195] The renal allograft also may be involved and show dysfunction. Lymphoma may be manifested as solid tumors in the renal allograft, lung, liver, spleen, brain, and soft tissues.[70,88] Smooth muscle tumors (leiomyoma) frequently are multicentric and multifocal; they often occur in lung, liver, and spleen but also may occur in unusual locations, including the brain and renal allograft.[23,86,95]

Laboratory diagnosis of post-transplant, EBV-associated disease is based on detection by PCR of EBV DNA in circulating lymphocytes.[5,43,62] Quantitative or semiquantitative PCR assays

may show increasing or persistently high copies of EBV DNA in the circulating lymphocytes of patients who are at risk for developing PTLD.[5,85,184] If end-organ disease is observed, the diagnosis can be confirmed histopathologically by detection by PCR of EBV DNA in tissue or by in situ hybridization with an EBV-encoded RNA probe.[4,217]

The diseases associated with EBV also may be classified in the laboratory as polyclonal or monoclonal. Polyclonal illnesses seem to be more benign than are monoclonal diseases, which often are associated with chromosomal abnormalities and malignant transformation.[168] The virus cannot be cultivated by routine means; when studied by special culture techniques, EBV has been found frequently in the oropharyngeal secretions of seropositive transplant recipients and is not predictive of EBV-associated disease.[168,184] Similarly, serologic approaches to establishing the diagnosis of EBV infection post-transplant also are nonspecific and difficult to interpret in most patients, unless the recipient clearly has seroconverted during the post-transplant period.

Aggressive or intense treatment of uncomplicated EBV-associated viral syndrome or mononucleosis usually is unnecessary because these illnesses seem to be self-limited in most patients. Immunosuppression may be reduced, and acyclovir may be administered; the patient should be monitored carefully. If symptoms in the patient or EBV DNA levels in the blood persist or increase, PTLD should be suspected, and the diagnosis should be confirmed.[211,218]

Treatment of established PTLD is challenging.[82] Mortality rates are high, and the best results seem to occur if the disease is diagnosed by quantitative PCR viral-load monitoring and serial imaging studies and treated aggressively.[217] Preemptive therapy instituted when viral surveillance monitoring detects an increase in EBV DNA in circulating lymphocytes before end-organ disease is evident also may be helpful in some patients.[43,134] Reduction in immunosuppression remains the most widely recommended strategy, but a variety of regimens have been studied as well.

Antiviral agents such as acyclovir and ganciclovir seem to reduce EBV replication early in the course of the disease process and may halt the progression of disease in some patients.[51,169,230] Antiviral agents are ineffective, however, against latent EBV or cells that have been transformed by the virus. Interferon-α, immunoglobulin, and anti–CD20 monoclonal antibody preparations such as rituximab have been used successfully in patients with established PTLD. Experimental protocols evaluating adoptive immunotherapy in transplant recipients are under way. In addition, some experts suggest that administration of intense anti-CMV therapy with ganciclovir and CMV hyperimmune globulin may improve chances of survival in certain patients with PTLD because CMV may serve as a cofactor in progression of disease caused by other members of the herpes family.[1,16,24,163,203,216] Treatment of patients with lymphoma or leiomyoma includes a reduction in immunosuppression, chemotherapy, radiation therapy, and surgical resection of tumors.

VARICELLA-ZOSTER VIRUS

VZV can cause primary (varicella, chickenpox) or reactivation (zoster, shingles) disease in a renal transplant recipient.[168,204] Infection with VZV can occur at any time but does so most often during the middle post-transplant period. Patients present with fever and painful or pruritic vesicular skin lesions.[153] Hepatitis, encephalitis, acute or chronic recurrent cerebral vasculitis and vasculopathy, and pneumonia also may occur, even in the absence of skin lesions, especially in an immunocompromised host.[97] Varicella occurs more frequently in children, and zoster occurs more frequently in adolescent and adult transplant recipients.[35]

In contrast to other herpes family viruses, VZV almost always is transmitted person-to-person or by aerosol in health care, family, and community settings; it rarely, if ever, has been linked to the transplanted allograft.[141,168] Before the advent of routine immunization and antiviral therapy, infection with VZV was a major cause of morbidity and mortality in children receiving solid organ transplants.[35,64,189] Untreated primary varicella may continue for several weeks and result in visceral dissemination, pneumonitis, hepatic necrosis, encephalitis, vasculitis with stroke, disseminated intravascular coagulation, hemorrhagic skin lesions, and death.[64,169,194] Zoster in solid organ transplant recipients may remain localized to a dermatome, but it often disseminates beyond the dermatomal distribution and produces widespread skin lesions and even visceral dissemination.

The diagnosis of VZV disease often is clinical, but it should be confirmed by isolation of VZV from fresh, vesicular skin lesions or detection of viral antigen by direct immunofluorescence assay on cells obtained by scraping the base of the skin lesion.[169] Tzanck smear may show multinucleated giant cells, suggesting a viral infection, but it cannot differentiate VZV from HSV. Other pathogens, especially HSV, may mimic VZV disease, so establishing an accurate viral diagnosis is important. VZV also may be detected by PCR-based DNA detection methods, which also may distinguish wild-type strains from OKA vaccine strain of VZV; VZV PCR tests may be performed by reference and research laboratories. Establishing a serologic diagnosis of active VZV infection is difficult. Routine serologic screening performed in the pre-transplant period identifies patients who are seronegative and at risk for developing primary infection with VZV.

Treatment of established VZV disease should be instituted as early as possible during the course of the illness because survival is improved if treatment begins before the fifth day of illness.[64] Acyclovir is recommended for most renal transplant recipients experiencing either primary infection with varicella or zoster with dissemination; it is administered intravenously in high doses (500 mg/m^2 per dose every 8 hours if renal function is normal or adjusted for renal function as needed) for 5 to 10 days, or until new lesions have ceased to occur for 24 to 48 hours, old lesions have crusted, the fever has resolved, and disease has abated.[26,161,168,230] Uncomplicated zoster or very mild primary varicella may be treated with oral acyclovir, famciclovir, or valacyclovir, provided that the patient is monitored carefully for clinical response. Ganciclovir, foscarnet, or cidofovir also provide cross-cover protection for most VZV strains, if these antiviral agents are being administered for difficult CMV infections.

Prevention of post-transplant VZV disease can be accomplished by several effective strategies, which should be discussed during the pre-transplant evaluation. Transplant recipients who were seronegative for VZV during the pre-transplant evaluation should receive varicella vaccine before undergoing transplantation, if possible.[71,112,238] Pre-transplant varicella vaccination is safe, beneficial, and cost-effective when administered to pediatric renal transplant candidates and in selected renal transplant recipients post-transplant.[26,34,71,72,161]

Seronegative, unimmunized transplant recipients who are exposed to varicella or zoster during the post-transplant period should receive passive immunoprophylaxis with varicella-zoster immunoglobulin. Preemptive therapy with oral acyclovir also is recommended by some experts in this situation because varicella-zoster immunoglobulin does not prevent, but only attenuates, the postexposure disease process. Finally, acyclovir, valacyclovir, ganciclovir, and valganciclovir administered prophylactically to transplant recipients who are seropositive for HSV or CMV also may provide protection against VZV disease.[124,168]

HUMAN HERPESVIRUSES 6, 7, AND 8

HHV-6, HHV-7, and HHV-8 also infect renal transplant recipients by primary infection and by reactivation.[32,33,37,61,92,187,188,203] Infection with these viruses becomes evident during the middle

post-transplant period, but their roles in specific disease processes are unclear.[169,197,212] HHV-6 and HHV-7 may act as cofactors in the progression of a disease, especially diseases caused by CMV and EBV.[61,111,153,163,216] HHV-8 is associated with Kaposi sarcoma post-transplant in severely immunosuppressed adult renal transplant recipients, but it has not been appreciated as a major opportunistic pathogen in pediatric renal transplant recipients to date.[32,35,63,168,181,193] HHV-8 may be transmitted through the renal allograft or by blood product transfusion, or it can become reactivated in the recipient post-transplant.[181]

Polyomaviruses and Papillomaviruses

POLYOMAVIRUSES

Human polyomaviruses (JC, BK, and SV40) frequently infect children and are present in urine and stool.[224,225] They can be detected in the urine of 33 to 58 percent of renal transplant recipients and detected serologically in 56 percent, and they are found histopathologically and by PCR in blood, body fluids, and tissue in 8 to 13 percent.* Knowledge about the roles that these viruses play in the outcome of renal transplant patients is evolving.

The JC and BK polyomaviruses seem to have a significant impact on adult and pediatric renal transplant recipients.[18,73,133,154-156,182,190] BK virus has been implicated in various syndromes, including ureteral stenosis, hemorrhagic or chronic cystitis, interstitial nephritis with graft failure, allograft nephropathy (BK-associated nephropathy [BKAN]), and rejection, in renal transplant recipients.[18,38,95,98,155,166,176,188,190] Disease associated with these viruses most often occurs during the middle and late post-transplant periods. BK virus may be asymptomatic or cause increasing serum creatinine levels, cystitis, tubular necrosis, graft dysfunction, or allograft rejection.[179] Risk factors for BKAN seem to include BK serostatus at the time of transplant, greater immunosuppression with use of mycophenolate mofetil, and concurrent CMV infection.[59,75,76,91,127]

BK virus often is detected in urine by the presence of "decoy cells," which are cells containing intranuclear viral inclusions, or more recently by quantitative PCR assays that detect and quantify BK viral DNA. BK viruria precedes BK viremia, which precedes BK nephropathy.[93] Patients with BK-associated nephropathy may have BK viral DNA detected in the urine, blood, or plasma, and renal biopsy specimens may show characteristic viral inclusions. Most recently, quantitative PCR viral surveillance in blood or plasma has been shown in renal transplant recipients to be useful in predicting the development of BK nephropathy and in guiding preemptive antiviral and immunosuppressive strategies.

Pediatric kidney transplant recipients who have increasing BK viremia levels should be managed preemptively with carefully monitored reduction of immunosuppression to prevent development of more serious complications of BKAN and graft rejection. This strategy usually results in reduction and then clearance of BK viremia and development of BK-specific cellular immunity coincident with BK virus clearance.[127] Treatment of established BKAN includes a reduction in immunosuppression. Cidofovir has activity against BK virus and has been used anecdotally to treat severe disease caused by BKAN.[93,230]

JC virus causes a rare syndrome called *progressive multifocal leukoencephalopathy*. It may be diagnosed by viral DNA detection using PCR-based assays available in reference and research laboratories or histopathologically by brain biopsy or autopsy examination. Successful treatment options for progressive multifocal leukoencephalopathy caused by JC virus are very limited, and most, if not all, patients die of progressive encephalopathy.

PAPILLOMAVIRUSES

Human papillomaviruses are common viruses that infect healthy children, adolescents, and adults. Adult renal transplant recipients are at increased risk for development of human papillomavirus–associated disease, such as cervical cancer and anogenital papillomas, whereas pediatric patients may develop numerous disfiguring warts post-transplant, especially if they require severe immunosuppression.[158,168,188] Malignant transformation of cutaneous warts caused by high-risk types has been documented.[188] Treatment options are limited but include physical removal of papillomas with laser and cryotherapy and careful reduction of immunosuppression. Information on the use of human papillomavirus vaccines in patients with ESRD or renal transplant recipients has not been published at this time.

Adenoviruses

Adenoviruses may infect pediatric renal transplant recipients at any time, but they are most likely to cause significant disease during the middle post-transplant period. They continue to emerge as a significant pathogen in pediatrics, including transplant recipients. They do not seem to play as important a role in renal transplant outcome, however, as do other viruses, such as the herpes family of viruses, and they currently are not as prominent in renal transplant recipients as they are in other transplant recipients, such as recipients of liver, lung, bone marrow, or stem cell transplants.[80,137,159,168,169] Nonetheless, infections with adenoviruses in pediatric renal transplant recipients, when they occur, can be serious and include hemorrhagic cystitis, diarrhea, allograft nephropathy, pneumonia, hepatitis, and disseminated disease with multisystemic involvement.[19,110,188,214,233,235]

The diagnosis is made by isolation of the virus in respiratory secretions, stool, urine, blood, or tissue. Rapid diagnosis may be accomplished within 30 to 60 minutes, using a rapid immunochromatographic assay that detects adenovirus antigens in eye secretions, respiratory samples, urine, and stool. Viral DNA also may be detected by PCR or quantitative PCR available in reference or research laboratories, and characteristic changes may be seen by histopathology in tissue. Adenovirus serotyping by neutralization assays and genotyping by PCR sequence-based assays are available in reference laboratories.[80] Treatment of serious adenoviral disease primarily is supportive. Immunosuppression also may be reduced, when possible. Antiviral agents, such as ribavirin, ganciclovir, and cidofovir, have activity against adenoviruses. Clinical reports of use of these agents in children with serious adenovirus disease post-transplant are published, but clinical trials documenting efficacy have not been performed.[31]

Human *Erythrovirus* (Human Parvovirus B19)

Human *Erythrovirus* (human parvovirus B19) has been reported to cause acute and chronic infection, manifesting as chronic anemia, red blood cell aplasia, and pancytopenia in renal transplant recipients.* It also has been implicated in collapsing glomerulopathy and thrombotic microangiopathy in renal transplant recipients.[228] Unusual manifestations, such as hepatic necrosis or central nervous system infection with vasculitis, also have been reported.[15,17,118,119] Transmission most likely is person-to-person; however, some reports indicate that the virus may be transmitted

*See references 6, 55, 59, 67, 68, 73, 75, 76, 91, 93, 95, 98, 127, 147, 154-156, 164, 166, 176, 177, 190, 219.

*See references 9, 14, 15, 17, 118, 126, 131, 144, 165, 209, 228, 234.

by blood product transfusion or in the renal allograft.[14,15,117] More recent reports suggest that the role that this virus plays in the outcome of renal transplant recipients may be more important than previously recognized, but systemic studies in pediatric patients have not been done.[14]

The diagnosis of acute infection with human parvovirus B19 is supported by parvovirus B19–specific IgG seroconversion; detection of parvovirus B19–specific IgM antibodies in serum; or detection of viral DNA in plasma, blood, body fluids, bone marrow, or tissue. The virus is not cultivatable in clinical virology laboratories that use routine cell cultures, but laboratories using special erythroid precursor cell lines may be able to cultivate parvovirus B19 for research purposes. No specific antiviral therapy is available, but anecdotal experience and case reports support a beneficial role for intravenous immunoglobulin and carefully monitored reduction in immunosuppression as management strategies to resolve parvovirus B19 viremia and associated end-organ disease.[149]

WEST NILE VIRUS

WNV is a single-stranded RNA flavivirus that has emerged as an important pathogen worldwide. It usually is transmitted by mosquitoes and causes a febrile illness, viral meningoencephalomyelitis, and acute flaccid paralysis in the normal host. Renal transplant recipients may become infected with WNV that is transmitted by the organ donor, by blood product transfusions, or naturally from mosquitoes.[44,102,112,180,227] Infection with WNV may manifest in the early post-transplant period if it is transmitted by the graft or blood products and, because of immunosuppression, produce severe febrile illnesses, seizures with status epilepticus, encephalitis, paralysis, and movement disorders. Death also may occur.[44] A high index of suspicion is required, and if WNV is suspected, serum and CSF should be sent for WNV-specific IgG and IgM antibody testing. The virus also may be isolated in special cell cultures and detected by reverse transcriptase PCR-based assays. Immunohistochemical staining may show WNV proteins in tissues from biopsy or autopsy examination. Outcome in renal transplant recipients with WNV disease improves with early establishment of the diagnosis and carefully managed reduction of immunosuppression.[180]

Mycoplasma

Mycoplasma spp. commonly infect school-aged children. Children and adolescents who undergo renal transplantation also may experience respiratory tract infections with *Mycoplasma pneumoniae* and *M. hominis*. The infection also may disseminate to extrapulmonary sites, causing hepatitis, septic arthritis, or perinephric fluid collections in pediatric renal transplant recipients.[108,136,206] *Mycoplasma* spp. may be detected using special biphasic culture media or PCR-based assays. Serologic diagnosis is difficult to accomplish, owing to cross-reactivity of antigens producing the possibility of false-positive reactions in some patients. Antimicrobial therapy with macrolide antibiotics, along with carefully managed reduction of immunosuppression, may be beneficial in selected patients.[108,136]

Bacterial and Mycobacterial Diseases

Bacteria that commonly produce disease in renal transplant recipients during the middle post-transplant period include *L. monocytogenes*, which often causes sepsis or meningitis.[168,199] Routine bacterial illnesses that were not identified and properly treated during the pre-transplant period also may emerge at this time and cause abscesses, sepsis syndrome, and death.

Mycobacterial disease caused by *M. tuberculosis* may occur at any time post-transplantation, but it occurs most frequently during the middle post-transplant period.[122,125,168,178] *M. tuberculosis* causes disease in approximately 1 percent of renal transplant recipients in the developed continents, such as North America and Europe, and in 15 percent of renal transplant recipients in developing countries with a high prevalence of tuberculosis, such as India or Pakistan.[105,168,178,185,191,192] Tuberculosis may develop in renal transplant recipients as a result of primary and reactivation infection in almost any site, and transmission from the renal allograft and reactivation in the native kidney have occurred.[114,145,146,185] *M. tuberculosis* may cause various diseases, including pulmonary infiltrates, cavitary lesions, adenopathy, cutaneous lesions, bone and joint disease, liver or spleen granulomata, and meningitis. Fever frequently occurs, and miliary or disseminated disease may occur, especially in young children.

Because the purified protein derivative (PPD) test is negative in most renal transplant recipients with active tuberculosis, the diagnosis is determined best by detection of acid-fast bacilli in smears from tissue or sputum and by isolation of *M. tuberculosis* in cultures of gastric aspirates, sputum, tracheal secretions, spinal fluid, or tissue.[125,168] The presence of granulomata in tissue also strongly suggests the diagnosis of tuberculosis.

Treatment of tuberculosis in a renal transplant recipient usually includes isoniazid, rifampin, and pyrazinamide for 1 year, although shorter courses may be acceptable in some patients.[122,168] Other antituberculous drugs may be added to or substituted for this standard regimen, depending on the disease process, the susceptibility pattern of the organism, and potential drug interactions. Because many antituberculosis drugs are excreted by the kidney, doses may need to be adjusted in a renal transplant recipient. Because rifampin may interact with cyclosporine, levels of cyclosporine should be monitored closely to avoid rejection of the allograft.[157] If a history of tuberculosis exposure is elicited during the pre-transplant evaluation, the transplant candidate should be evaluated for tuberculosis, including having a PPD and chest radiograph.[145,146,178] All close contacts also should be investigated for evidence of having tuberculosis. Treatment is recommended if disease is discovered, and prophylaxis with isoniazid is indicated for most patients who have a recently positive PPD on pre-transplant evaluation.[122,168,169,192,237] A transplant recipient who receives an allograft from a donor with a history of tuberculosis or a positive PPD also may be a candidate for prophylaxis with isoniazid post-transplant.[122,168,169]

Atypical mycobacteria are ubiquitous nontuberculous mycobacteria that can infect and produce disease in renal transplant recipients. They can cause disease during the middle post-transplant period and so are included here. Their effects usually do not become evident, however, until many years after the patient has undergone transplantation, during the late post-transplant period.[42,146,170] Atypical mycobacteria that have been documented to cause disease in solid organ transplant recipients include *Mycobacterium kansasii*, *Mycobacterium avium-intracellulare*, *Mycobacterium fortuitum*, *Mycobacterium xenopi*, *Mycobacterium haemophilum*, *Mycobacterium marinum*, *Mycobacterium chelonae*, *Mycobacterium abscessus*, *Mycobacterium gastri*, *Mycobacterium scrofulaceum*, and *Mycobacterium thermoresistibile*.[105,168,169]

A high index of suspicion is necessary to detect these elusive pathogens. They should be considered as the cause of disease in patients with persistent cutaneous ulcers, abscesses, adenopathy, pulmonary nodules, chronic wound infections, or bone and joint disease after negative routine bacterial cultures have been obtained, and the patient has failed to respond to standard antimicrobial therapy.[188] Disseminated, multisystem disease also can occur. Environmental sources of atypical mycobacteria include contaminated dialysis equipment, soil, and contaminated water in aquariums and pools. The diagnosis is established by isolation of nontuberculous mycobacteria in fluid or tissue. Granulomata are not observed consistently in tissue, and acid-fast stains may be negative.[188] Atypical nontuberculous mycobacteria are difficult

to treat, and treatment must be individualized to each patient. Strategies include reduction of immunosuppression and surgical débridement of localized disease. Antimicrobial therapy based on in vitro susceptibility testing of the isolate also should be administered, often for a prolonged period.

Nocardia

Nocardia asteroides is the most common *Nocardia* spp. causing illness in renal transplant recipients; however, other, more unusual *Nocardia* spp., including *Nocardia transvalensis*, *Nocardia brasiliensis*, *Nocardia nova*, *Nocardia otitidiscaviarum*, and *Nocardia farcinica*, also have been shown to cause disease in solid organ transplant recipients.[168,169,233] The most common manifestations of nocardial disease in transplant recipients are fever, cough, and pulmonary infiltrates.[233] Pleural effusion, pulmonary nodules, and cavitary lesions also may occur. Cutaneous infection, adenitis, arthritis, meningitis with brain abscesses, and infection of the renal allograft also may occur. *Nocardia* spp. can be seen on Gram and modified acid-fast stains of sputum, bronchoalveolar lavage fluid, abscess fluid, and tissue. They can be isolated on routine media but may take longer than conventional bacteria to grow. Prolonged treatment with sulfonamides, alone or in combination with trimethoprim, is recommended. Amikacin and other antimicrobials may be added in selected patients with severe disease, provided that renal function is monitored closely.[188] Trimethoprim-sulfamethoxazole prophylaxis for UTI and *P. carinii* also may be effective in preventing disease caused by *Nocardia*.

Fungal Diseases

Fungal infections occur infrequently in renal transplant recipients relative to other solid organ transplant recipients.[168,169,188] This lower rate of infection probably is related to technical procedures performed at the time of transplantation and the lower level of immunosuppression required to maintain most renal allografts.[188] When they do occur, however, fungal infections often are serious and life-threatening.[87,90,99] Fungal disease in a renal transplant recipient usually is manifested in one of two ways: (1) pulmonary or disseminated disease caused by one of the environmental mycoses, such as *H. capsulatum* or *C. immitis*, or (2) opportunistic infection with fungi that rarely cause disease in a normal host, such as *Candida* spp., *P. carinii*, *Aspergillus* spp., *Cryptococcus neoformans*, *Nocardia* spp., and others.[87,201]

Travel to endemic areas increases a transplant recipient's risk for development of histoplasmosis and coccidioidomycosis and may be identified during the pre-transplant evaluation. Factors that increase a transplant recipient's risk for acquisition of opportunistic fungal disease include underlying conditions such as diabetes mellitus, repeated episodes of rejection, long-term administration of steroids, prolonged use of antibiotics, and CMV infection. Similar to tuberculosis, invasive fungal disease can be a result of primary infection or reactivation infection with secondary dissemination.[188]

P. carinii, currently classified as a fungus, may cause pneumonia in 10 percent of solid organ transplant recipients, most often during the first 6 months post-transplant.[8,168,169] It seems to occur more commonly in children than in adults and usually causes fever, dyspnea, tachypnea, hypoxemia, and a nonproductive cough.[57,168] Interstitial pulmonary infiltrates are typical findings, but almost any radiographic picture can be observed, and pneumothorax is common in severe disease. The diagnosis can be suspected clinically but is documented best by organisms being shown in lung biopsy specimens; bronchoalveolar lavage may provide the diagnosis in some patients.

Treatment with high-dose oral or intravenous trimethoprim-sulfamethoxazole for 14 to 21 days is used most often. Intravenous pentamidine may be administered to selected patients, but renal function should be monitored carefully.[188] Corticosteroids may be helpful in treating severe disease if given early in the course. Low-dose oral trimethoprim-sulfamethoxazole provides effective prophylaxis against *P. carinii* pneumonia and UTIs and usually is administered to renal transplant recipients for at least 6 months post-transplant.[69] Transplant recipients experiencing repeated episodes of rejection, allograft dysfunction, or CMV disease may require a longer period of prophylaxis.[168,169] Aerosolized pentamidine is an alternative prophylaxis strategy for some patients.[188]

Candida spp., especially *C. albicans*, are the opportunistic fungi most frequently isolated in renal transplant recipients.[168,169] Other *Candida* spp. isolated include *Candida krusei*, *Candida glabrata*, and *Candida tropicalis*. Most fungal infections caused by *Candida* spp. occur during the first 2 months post-transplant, usually at the site of indwelling intravascular and urinary catheters.[188] Esophagitis, abscesses, and arthritis also can develop, and endocarditis with metastatic foci can occur if the fungemia persists. Other opportunistic fungi such as *Aspergillus* most often cause sinusitis and pulmonary disease, and, because of its angioinvasive nature, *Aspergillus* often disseminates and causes lesions in the liver, spleen, and brain.[168,169,201,202]

A thorough search for metastatic foci always should be undertaken when a primary focus of invasive fungal disease is documented. *C. neoformans* usually causes cutaneous lesions or abscesses and pneumonia with pleural effusion, but meningitis, arthritis, and pyelonephritis also can occur.[168,169,196] Exposure to soil or bird droppings provides an epidemiologic clue to the diagnosis. In addition, the Zygomycetes, including *Rhizopus*, *Mucor*, *Rhizomucor*, and *Absidia* spp., can cause disease in solid organ transplant recipients.[201,202] They most often cause cutaneous, rhinocerebral, or pulmonary disease or brain abscesses in renal transplant patients. Cutaneous and soft tissue infections, wound infections, and gastrointestinal disease with perforation also have been described. The Zygomycetes, similar to *Aspergillus*, are angioinvasive and disseminate via the bloodstream to cause disease, which often is severe. A variety of unusual fungi, such as *Paecilomyces*, *Fusarium*, and *Bipolaris*, have been shown to cause cutaneous infection, usually at the site of indwelling catheters, and *Hansenula anomala* reportedly has caused UTI in a renal transplant recipient.[78]

The endemic dimorphic fungi include *H. capsulatum*, *C. immitis*, *B. dermatitidis*, and *Paracoccidioides brasiliensis*. Infection with these organisms can occur anytime after the patient has undergone transplantation but usually occurs during the intermediate post-transplant period.[215] These organisms usually are noted in renal transplant recipients who reside in endemic areas. Histoplasmosis is endemic in the central part of the United States and many foreign countries, and nosocomial outbreaks have occurred during hospital construction projects. Fever, chills, and cough are the usual initial signs. Skin lesions, hepatosplenomegaly, and meningitis also can occur. Pancytopenia often is present as well, and the organism frequently is found in the bone marrow of patients with disseminated disease. Coccidioidomycosis is endemic in the southwestern portion of the United States and northern Mexico. It usually is manifested as fever, cough, and pulmonary infiltrates, but extrapulmonary dissemination frequently occurs. Blastomycosis is endemic in the southern United States, along the Mississippi and Ohio River valleys, and in the Great Lakes area. It is a rare development after renal transplantation, but most often causes lung and skin lesions. Paracoccidioidomycosis rarely has been reported in renal transplant recipients.[210]

The diagnosis of invasive fungal disease is established best by isolating the fungus from sputum or tracheal aspirate, bone marrow, tissue, or fluid. *C. neoformans* also may be isolated from urine. Fungal serology and tests for cryptococcal antigen may support the diagnosis.[210]

Fungal identification and susceptibility testing now can be performed in reference laboratories and should be used to guide therapy whenever possible. Amphotericin B usually is used to treat most invasive fungal disease in transplant recipients, and the deoxycholate, lipid complex, or liposomal forms can be used, provided that renal function is monitored closely.[123] Flucytosine may have additive or synergistic effects against many yeasts and fungi. The azole antifungal agents are not nephrotoxic and are used in renal transplant recipients whenever possible. Many *Candida* spp. are susceptible to fluconazole, although resistance is emerging in all *Candida* spp. and is present in a high percentage of *C. krusei* and *C. glabrata*. Itraconazole and voriconazole may be effective against other fungi, such as *Aspergillus.* Voriconazole has emerged as a treatment of choice for most *Aspergillus* infections. Itraconazole should be used with caution, however, because it has variable oral absorption and may interact with cyclosporine.[215] Caspofungin also has activity against most *Candida* spp. and a variety of opportunistic fungi, including *Aspergillus.* Currently, experience using the newest triazole, posaconazole, and the newest echinocandin, micafungin, in pediatric renal transplant recipients is limited.

Surgical resection or drainage of abscesses, avascular cavities, or effusions may be required to treat some forms of invasive fungal disease of the lungs, liver, or spleen. Carefully monitored reduction in immunosuppression also may help the host recover from invasive fungal disease.

Parasitic Infections

The parasites most commonly encountered in renal transplant recipients during the intermediate post-transplant period are *T. gondii*, *S. stercoralis*, and *Trypanosoma cruzi*.[65,168] These infections occur most often in adults. Children also may be infected with routine parasites such as *Enterobius vermicularis*, *Ascaris*, *Giardia*, and others pre-transplant. Other parasitic infections that may be found in renal transplant recipients include leishmaniasis, schistosomiasis, and malaria. Renal transplant recipients seem to be at much lower risk for acquisition of parasitic diseases than are other solid organ transplant recipients.

T. gondii usually occurs as a result of reactivation of latent disease in the donor allograft or recipient.[130] It is encountered most frequently in heart transplant recipients, but any solid organ recipient, including renal, may be affected. Clinical manifestations of infection with *T. gondii* include focal meningoencephalitis, brain abscesses, pneumonia, myocarditis, pericarditis, hepatitis, and retinochoroiditis.[140,207,215,222] The diagnosis may be made by demonstration of the organism in tissue by histopathology, detection of DNA by PCR in body fluids or tissue, or serologically by detection of high titers of IgG and specific IgM antibody to *T. gondii.* Treatment with pyrimethamine, sulfadiazine, and folinic acid usually is recommended; however, some patients also may respond to clindamycin. Routine prophylaxis with trimethoprim-sulfamethoxazole for UTI and *P. carinii* infection may help prevent disease with *T. gondii.*

S. stercoralis is an important pathogen in adult transplant recipients, but it is rarely, if ever, a significant problem in pediatric patients. Because *S. stercoralis* can be maintained in the human intestinal tract for decades, it can disseminate and cause serious disease in transplant recipients who were infected pre-transplant. A complete blood count for eosinophilia should be obtained, and stool and other specimens should be examined for rhabditiform larvae if the pre-transplant evaluation reveals travel to endemic areas. Thiabendazole should be given before transplantation is performed if *S. stercoralis* infection has been documented or is suspected. *T. cruzi*, the cause of Chagas disease, rarely has been transmitted by renal transplantation.[65,120]

INFECTIONS OCCURRING DURING THE LATE POST-TRANSPLANT PERIOD

Infections occurring 6 months or more after the patient has undergone renal transplantation usually are less severe than infections experienced in the earlier periods, especially if the level of immunosuppressive therapy is low and the allograft is functioning well.[187] Constant vigilance for health care–associated and community-acquired viral and bacterial diseases and unusual infections should continue, however.

Chronic rejection or repeated episodes of acute rejection complicated by allograft dysfunction predispose the patient to development of more serious opportunistic infections, similar to infections encountered during the first 6 months post-transplant. UTIs that develop during this period, in contrast to UTIs in the early post-transplant period, usually are benign and may be treated with conventional antimicrobial therapy in most instances, if they do not seem to threaten the function of the allograft or the patient's survival. Patients also may experience community-acquired infections with respiratory viruses, such as influenza virus and respiratory syncytial virus, and with bacteria, especially *Streptococcus pneumoniae* (pneumococcus) or *S. aureus*. These common community-acquired bacterial pathogens may be resistant to usual β-lactam antibiotics.[208,220] In addition, patients vaccinated with 7-valent or 23-valent pneumococcal vaccines still may acquire invasive pneumococcal disease with emerging, non-vaccine serotypes, such as 19A and 3A. These serotypes often are also multidrug-resistant. Opportunistic viral infections encountered during this period are zoster or, rarely, CMV retinitis.[149] The risk for developing PTLD also persists during this late post-transplant period.

Renal transplant recipients may be chronically infected with HBV or HCV pre-transplant. Increased risk of mortality from fulminant hepatitis with hepatic failure may occur during the early pre-transplant period, and chronic liver disease, cirrhosis, and liver failure may be seen 10 years or more after renal transplantation has been performed in hepatitis B surface antigen (HBsAg)–positive recipients.[236] All transplant recipients who are not immune to HBV should receive HBV vaccine before transplantation.[229] Most experts agree that renal allografts from HBsAg-positive donors should not be used, but the decision to perform renal transplantation in recipients who are HBsAg-positive is controversial and must be made on an individualized basis.

Renal transplant recipients who are infected with HCV pre-transplant usually do well during the early post-transplant period, but long-term survival is poorer than in recipients who are not infected with HCV. Not only can chronic liver disease develop, but also membranoproliferative glomerulonephritis has been reported in renal transplant recipients infected with HCV.[27] Despite these risks, however, most experts do not consider infection with HCV a contraindication for renal transplantation in patients with ESRD.[173] Hepatitis G virus also has been detected in the serum of renal transplant recipients, and at least one association with membranous glomerulonephritis has been reported.[12,13] The role of HGV in clinical disease in renal transplant recipients is not established, however.

HIV may be transmitted by kidney transplantation despite routine screening of donors of blood and organs.[117,183] A transplant recipient also may become infected with HIV after undergoing transplantation.[168] Shortly after a patient has undergone transplantation, HIV infection may cause fever and a mononucleosis-like syndrome. Complications of acquired immunodeficiency syndrome may develop during the late transplant period. HIV-infected patients with ESRD traditionally have been excluded from receiving renal transplantation. More recent advances in HIV therapy have caused some experts to reconsider this policy on an individual basis, however.[168]

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CHAPTER 85

Infections Related to Prosthetic or Artificial Devices

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The development of biomaterials used in the manufacturing of temporary or permanent implantable prosthetic devices has been one of the greatest advances in modern medicine.[64,85,86] These devices have become an integral and important part of the current practice of medicine and have improved considerably the lives and health of countless patients. In the United States, the number and types of permanent prosthetic devices implanted to replace diseased or damaged body parts has increased substantially during the last several decades. An estimated 3 million or more people in the United States currently have some type of long-term bio-

TABLE 85–1 Temporary or Permanent Implantable Devices Currently in Use

Temporary Devices
Intravascular catheters
Urinary catheters
Endotracheal and tracheostomy tubes
Permanent Devices
Central nervous system shunts
Deep-brain stimulators
Peritoneal dialysis catheters
Orthopedic prostheses (artificial hip, knee, and other joints; screws, pins, plates, and rods)
Intracardiac and intravascular prostheses (heart valves, vascular grafts)
Ventricular assist devices
Pacemakers and defibrillators
Ocular prostheses (artificial globes, intraocular lenses, ocular explants)
Implantable pump devices (baclofen, insulin)
Cochlear implant devices
Tissue expander devices

medical implant.[80,322,377] Table 85–1 is a listing of the implantable prosthetic devices in use today.

One of the major medical complications associated with the use of implantable prosthetic devices is infection, which may result in serious tissue destruction and dysfunction of the prosthetic device or in local and systemic consequences that may be life-threatening. In most cases, these infections are very difficult to cure with antimicrobial agents alone, and removal of the device usually is required for resolution of the infection.

INTERACTION OF THE HOST WITH A PROSTHETIC DEVICE

The prosthetic devices currently in use are composed of a variety of biomaterials, including cobalt-chromium-molybdenum alloy, titanium alloy, and complex polymers such as polytetrafluoroethylene, silicone, and polyethylene, which in general are chosen for their inert, nonreactive, and nontoxic qualities. The interplay of both implant and host factors determines the risk of acquiring and the severity of infection. The human body has numerous well-developed defense mechanisms to protect it against possible invasion by various microorganisms. Such mechanisms include cellular and humoral immune systems, anatomic barriers, and an elaborate network of cells that phagocytize and destroy invading organisms. The presence of a foreign body may compromise one or more of these defenses and elicit a complex acute or chronic inflammatory response (or both) from the host. Many of these devices breach cutaneous and mucosal barriers, thereby creating a direct route by which bacteria and fungi may invade. In addition, implanted devices may alter the local immunity of the host directly or indirectly.[18,85,322,377,389]

Shortly after being implanted, hydrophobic polymeric materials such as polyethylene, Dacron, polydimethylsiloxane, and polyether urethanes become coated with a layer of host proteins such as plasma and interstitial fluid proteins (fibronectin, albumin, laminin, collagen, immunoglobulin G, fibrinogen) that bind to and are absorbed readily into the surface of the implant.[21,291,377] This protein layer (especially fibrinogen) has a major influence on the body's response to and the biocompatibility of the implant. The presence of fibrinogen attracts a large number of phagocytic cells (neutrophils, monocytes/macrophages) to the implant; these cells interact with the implant surface and initiate an acute inflammatory response.[85,377] Some of these host proteins also serve as a receptor for various colonizing microorganisms. Collagen, laminin, and fibrinogen have been reported to play a role in adherence of bacteria,[393] whereas fibronectin has been found to be the major receptor for gram-positive cocci, especially *Staphylococcus aureus*.[314,382]

Chronic inflammatory responses, also known as *foreign body reactions*, are seen around many types of biomaterial implants and arise from interactions among the protein-coated surfaces of the implant and host tissues and adhering phagocytic cells such as macrophages and foreign body giant cells. These interactions may result in degradation and damage to the implant from the continuous generation of toxic catabolites and release of inflammatory mediators such as hydrolases, activated complement components, tumor necrosis factor (TNF), interleukins, prostaglandins, coagulation factors, and plasminogen activator by the phagocytic cells.[375,377,389]

INTERACTION OF MICROORGANISMS WITH A PROSTHETIC DEVICE

Once a prosthetic device is implanted, the surface of the device provides a potential area for adherence and multiplication of bacteria. Adherence is a complex process that involves electrostatic attachment of the bacteria to the surface of the implant, bacterial mechanisms that function specifically in attachment, and host-derived substances that coat the prosthetic device and serve as receptors for various bacteria. Bacteria arrive at the surface of the implant by many different routes: they may be inoculated at the time of implantation, the patient may have episodes of transient bacteremia, the device may be exposed by local trauma or infection, or the device may be implanted within an area in which the organism is part of the normal flora.[80,85]

Adherence of bacteria to the surface of an implant is influenced by numerous different factors, including the material used to make the device, the source of the device material (adherence is greater with synthetic material than with biomaterial), the surface of the device (irregular more than regular, textured more than smooth, hydrophobic more than hydrophilic), and the shape of the device.[80] Cell surface molecules or structures known as adhesins also play a role in adherence by attaching or binding an organism to specific receptors on implant surfaces; different bacteria use different adhesins to attach to and colonize medical implants. For example, *Staphylococcus epidermidis* uses proteinaceous autolysin and capsular polysaccharide intercellular adhesin for initial adherence to the implant surface and for adherence of bacteria to each other. These bacteria also produce a biofilm that increases cell-to-cell association and allows accumulation of bacteria.[330] *Streptococcus pyogenes* uses lipoteichoic acid as its adhesin, whereas *S. aureus* uses both lipoteichoic acid and host-tissue ligands (e.g., fibronectin, fibrinogen, collagen) for adherence. Binding of *S. aureus* to host-tissue ligands is mediated by genetically defined microbial surface proteins known as *microbial surface components recognizing adhesive matrix molecules* (MSCRAMM).[81] *Escherichia coli* and other bacteria use fimbriae as an adhesin to mediate binding to receptors on the surfaces of target cells.

Bacteria also can protect themselves from host defenses by synthesizing and excreting numerous complex polysaccharides, known as glycocalyces, that function either as part of the bacterial capsule or as the slime layer. This slime layer is known to play a major role in keeping an organism attached to an implant surface by coalescing with the polysaccharides of other bacteria and with host products to produce a thick, adherent, and somewhat impenetrable biofilm.[390] The biofilm functions by trapping nutrients and protecting the organism from phagocytosis, antimicrobial agents, and competing microflora. It also plays a role in inhibiting the response to chemotactic stimuli, increases both *N*-formyl-methionyl-leucyl-phenylalanine (FMLP)-induced

superoxide generation and release of specific granules, and impairs natural killer cell function while also altering the composition of T-lymphocyte cell subpopulations.[136,184,390] Therefore, the biofilm aids bacteria in evading host cellular and humoral defense mechanisms and thereby allows the organism to colonize and infect an implanted device effectively.

Substantial progress has been made in our understanding of the pathogenesis, prevention, and treatment of foreign body infections; this increased knowledge has resulted in a dramatic decrease in the morbidity associated with these infections, as well as subsequent improvement in the patient's quality of life. In the following sections we discuss specific device-related infections and the suggested treatment and management of these infections.

TISSUE EXPANDERS

The use of soft tissue expansion in reconstructive surgery was reported in the literature first in 1957, and since that time it has been used widely for the correction of multiple problems in plastic and reconstructive surgery in the adult and pediatric populations.[12,19] These expanders consist of an alloplastic prosthesis with a filling port that is implanted into a subcutaneous pocket. The expander is filled with saline through the filling port at various intervals to create adequate expansion of the skin. In children, tissue expansion is used most commonly to provide coverage for skin defects caused by burns, trauma, hemangiomas, and other congenital deformities.[237]

The most common complication of tissue expansion is infection of the subcutaneous expander pocket, usually with skin organisms introduced during insertion of the expander. Cellulitis of the overlying skin and hematogenous or lymphatic seeding of the expander pocket are seen as well but occur much less frequently.[12,123,171,235,237,272] In several pediatric case series, the infection rate ranged from 3.4 to 11 percent.[123,237] *S. aureus*, *S. epidermidis*, and group A streptococci are the microorganisms recovered most commonly from these infections.[123,171,236,237] Less commonly, nontypable *Haemophilus influenzae*,[237] *Pseudomonas aeruginosa*, *E. coli*, and *Actinomyces* spp.[252] have been isolated from infections of expander pockets.[236]

In cases of infection of subcutaneous pockets, treatment consists of removal of the tissue expander, débridement and drainage of the subcutaneous pocket, and administration of intravenous antibiotic therapy tailored to the organism isolated. For cellulitis of the overlying skin of an expander pocket, treatment with intravenous antibiotics but without removal of the tissue expander has been shown to be successful.[12]

The most common empiric antimicrobial therapeutic regimen consists of a first-generation cephalosporin or an extended-spectrum, penicillinase-resistant penicillin. For all of the infections of prosthetic devices discussed in the chapter, empiric antistaphylococcal coverage should include agents effective against community strains of methicillin-resistant *S. aureus* (MRSA) if warranted by their frequency in the area.

Therapy is tailored once the organism has been identified and its antibiotic susceptibility has been determined.

COCHLEAR IMPLANTS

During the last several decades, cochlear implantation has emerged as one of the best methods of providing auditory rehabilitation for the profoundly deaf (congenital or acquired). The goal of this surgery in young children is to provide hearing that is adequate to facilitate the development of receptive and expressive language. The surgical technique involves the creation of a C-shaped flap in the postauricular and parietal-occipital scalp skin areas, elevation of the flap, implantation of a multichannel prosthesis, and insertion of an electrode array into the cochlea through openings drilled into the temporal bone.[68,71,230]

The most common infectious complications associated with these implants are cellulitis of the overlying skin flap, meningitis, otitis media, and delayed cochlear implant infections leading to extrusion of the implant.* Rates of infection range from 0.3 to 0.5 percent for meningitis, 2 to 3 percent for cellulitis of the skin flap and delayed cochlear implant infections, to 36 percent for otitis media. The reported cases of meningitis have occurred either in association with leakage of cerebrospinal fluid (CSF) in persons with a malformed cochlea who undergo cochlear implantation or as a consequence of intracranial spread of a developing middle ear infection along the electrode pathway. In June 2002, the U.S. Food and Drug Administration received numerous reports of bacterial meningitis in children with cochlear implants who were younger than 6 years old when they received the implants. The most common causative organism identified was *Streptococcus pneumoniae*, followed by nontypable and type b *H. influenzae*. The incidence of pneumococcal meningitis in this group of patients was calculated to be 138.2 cases per 100,000 person-years—more than 30 times the incidence in the same-aged cohort in the general population. This increased incidence of meningitis was found to be associated strongly with the use of a cochlear implant with a positioner (a wedge-shaped insert that facilitates transmission of the electrical signal by pushing the electrode against the medial wall of the cochlea) in conjunction with the presence of radiographic evidence of a malformation of the inner ear and leakage of CSF. Cochlear implants with a positioner were voluntarily recalled in the United States in July 2002, although removal of existing implants containing a positioner was not recommended; use of appropriate vaccination against *S. pneumoniae* and type b *H. influenzae* was strongly recommended.[17,131,321] Children with cochlear implants may have a higher risk for the development of middle ear infections because of several factors, including the naturally high incidence of acute otitis media (AOM) in this population, the presence of a foreign body in the area of the infection, and the potential for spread of the infection into the cochlea along the electrode pathway. In a study of 50 children who received cochlear implants between 1991 and 1995, researchers found that children prone to the development of otitis media before undergoing implantation were at higher risk for developing postimplantation AOM but responded well to routine oral antimicrobial therapy. The overall prevalence and the severity of AOM were not found to be increased in children with cochlear implants.[227]

In cases of cellulitis of the skin flap, intravenous antimicrobial therapy commonly consists of a first-generation cephalosporin or an extended-spectrum, penicillinase-resistant penicillin to provide coverage for *S. aureus* and group A streptococci. Empiric antimicrobial therapy for meningitis usually consists of vancomycin and a third-generation cephalosporin that is tailored to the organism isolated. For delayed development of cochlear implant infections, therapy consists of removal of the implant and administration of intravenous antibiotics.

OCULAR PROSTHESES

This group of prosthetic devices includes artificial globes used primarily for cosmetic purposes, orbital implants, ocular explants, intraocular lenses (IOLs), and contact lenses.

ORBITAL IMPLANTS

Orbital implants are made of hydroxyapatite or porous polyethylene and frequently are used in orbital reconstruction after

*See references 32, 69-71, 82, 158, 159, 168, 175, 176, 227, 277, 289.

enucleation or evisceration surgery. Infection of these implants is a rare event, with only a handful of cases reported in the literature.* Patients most commonly complain of anophthalmic socket pain, discomfort, and irritation while wearing an artificial globe. Papillary conjunctivitis of the socket with exudate and sometimes dehiscence of the overlying conjunctiva also may be seen.[125,261] Infection may develop months to years after placement of the implant, and severity ranges from cellulitis to the development of an abscess around the implant itself.

Radiographic studies that can aid in the detection of these types of infection include technetium 99m–labeled leukocyte scintigraphy, which is most useful in detecting early low-grade graft infection,[186] and computed tomography (CT) and magnetic resonance imaging (MRI), which are useful later in the course of the infection to detect the presence of abscesses and structural tissue changes. Gram-positive cocci, primarily *S. aureus* and coagulase-negative staphylococci (CoNS), are the organisms associated most commonly with these infections; however, *H. influenzae*, *S. pneumoniae*, alpha-hemolytic streptococci, *Capnocytophaga*, *Pseudomonas*, and *Aspergillus fumigatus* have been cultured as well.[321,365,419,427] To cure the infection effectively, treatment involves both removal of the implant and institution of topical and parenteral antibiotic therapy directed against the organism isolated. Empiric therapy directed against gram-positive organisms may be started initially until the results of culture and sensitivity testing are available. The most common empiric regimens include a first-generation cephalosporin, a second-generation cephalosporin, or an extended-spectrum, penicillinase-resistant penicillin.

INTRAOCULAR LENSES

Insertion of polymethyl methacrylate (PMMA) IOLs at the time of removal of a cataract is the standard surgical therapy for this disorder. Even though the rate of postoperative infection of IOLs is low (ranging from 0.10% to 0.30%), the infection usually is serious and results in endophthalmitis and permanent loss of vision.[187,206] The predisposing factor for infection is bacterial adhesion to IOLs during their insertion. After adhesion is accomplished, the bacteria replicate, congregate, and form multiple layers of microcolonies that represent a biofilm in which the bacteria are embedded in a layer of slime. The major pathogens associated with this infection are *S. aureus* and *S. epidermidis*, which account for 90 percent of all isolates, although gram-negative bacilli, fungi, *Chlamydia trachomatis*, and rapidly growing mycobacteria are isolated on occasion.[6,93,206,239,348,428] IOL-associated endophthalmitis is a serious infection that is difficult to diagnose and treat.[412] In a few cases, use of topical and systemic antibiotics alone has been successful in eradicating the infection, but in most cases surgical débridement and systemic antibiotics are required for cure. Empiric therapy for these infections consists of an extended-spectrum, penicillinase-resistant penicillin, a second-generation cephalosporin, or, in some instances, clindamycin or vancomycin. In cases of fungal endophthalmitis, treatment with the later-generation azoles alone and in combination with an echinocandin has been shown to have clinical success[93]; in rapidly growing mycobacterial endophthalmitis combinations of topical and intravenous antibiotics to which the organism is susceptible have been used for treatment.[239]

CONTACT LENSES

Primarily three different types of contact lenses are available. Hard lenses are made of PMMA, a substance that is impermeable to water and gas. This type of lens is designed to be worn only during waking hours because it limits oxygen flow to the cornea to that present in tears. Gas-permeable hard lenses are composed of silicone, cellulose acetate butyrate, or PMMA-silicone copolymers; they allow gas but not fluid to pass through the lens. Hydrophilic or soft lenses are made of a cross-linked hydrogel polymer or copolymer and consist of between 38 and 85 percent water by weight; these lenses are permeable to both gas and water and allow the user to wear them continuously. However, for all these types of lenses, infection may result in damage to the corneal epithelium.

The two main infections that occur in association with contact lenses are conjunctivitis and keratitis.[55] The causative bacteria seen most commonly with these infections are *S. aureus*, streptococci, *Pseudomonas* spp. (found in improperly stored cleansing solutions), and fungi. Improper cleaning of soft or hydrophilic lenses may cause them to become a source of infection when bacteria penetrate the lens matrix. Treatment usually consists of removal of the lens and application of topical antibiotics. To prevent the development of infections associated with contact lenses, users should adhere strictly to the manufacturer's suggested guidelines for wearing and cleaning the lenses.

A rare and often devastating infection associated with the use of contact lenses is infection with the fresh-water protozoan *Acanthamoeba*.[67,157,261,367] This organism contaminates the lens when sterility of the cleansing solutions is not maintained; it usually is associated with users who prepare their own solutions. *Acanthamoeba* is very difficult to eradicate because it is not susceptible to standard antiparasitic agents, and it produces a chronic keratitis that can be complicated by corneal perforation and loss of the eye. Corneal transplantation may be necessary in many cases to restore vision.[157,367]

LEFT VENTRICULAR ASSIST DEVICES

The development plus use of mechanical circulatory assist devices has grown very rapidly in the last decade, especially with the shortage of available donor hearts. Such devices have improved considerably the hemodynamic status and quality of life of patients with heart failure who are awaiting cardiac transplantation. Figure 85–1 shows a left ventricular assist device (LVAD), a pneumatically driven pump located outside the heart that draws blood from an inflow cannula in the left ventricular apex and ejects the blood through an outlet into the ascending aorta. Within the pump are several sections of Dacron graft material and two trileaflet porcine valves in the inflow and outflow positions to ensure that blood flow is unidirectional. The pump is encased in titanium and implanted via an extended median sternotomy into the left rectus sheath. A percutaneous driveline connects to an exterior power pack for venting or for pneumatic actuation and exits the body, after passing through a subcutaneous tunnel, in the left lower quadrant. The interior surface of the pump is textured to prevent the formation of thrombi and encourage the deposition of a biologic pseudo-intimal lining. Once the device is implanted, in most cases it cannot be removed without performing concurrent cardiac transplantation.[154,193,243,245,288]

The approach taken by many centers for infection of an LVAD includes both preventive and interventional steps that are instituted before, during, and after implantation. Preventive strategies are focused on prevention of infections related primarily to the driveline and device pocket through the use of clean implantation techniques and limited traffic in the operating room in which the procedure is being performed. Antibiotic prophylaxis usually is given for 48 hours near the time of implantation of the LVAD and may include intravenous trimethoprim-sulfamethoxazole, rifampin, and fluconazole, along with the application of mupirocin ointment to the nares. Additional

*See references 3, 67, 126, 158, 165, 186, 187, 192, 206, 261, 426.

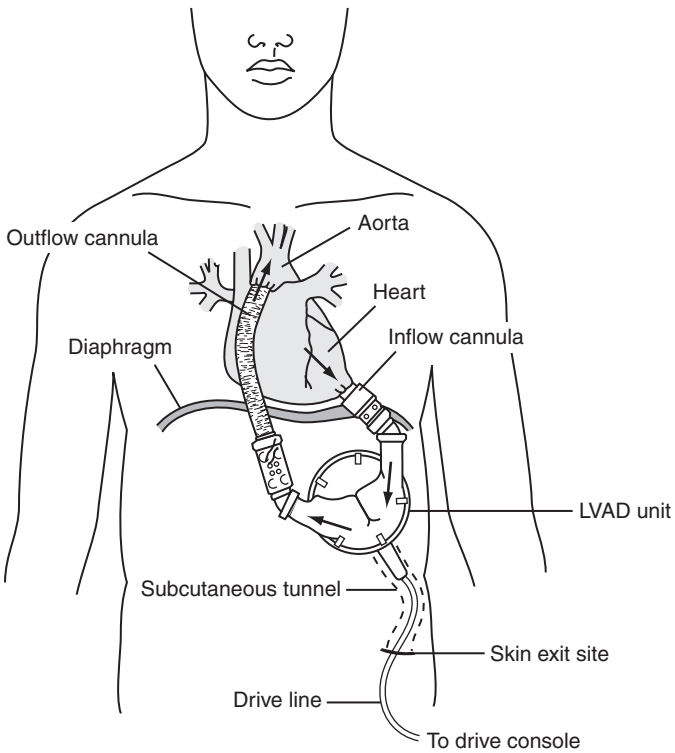

Figure 85–1 Left ventricular assist device (LVAD). *(Adapted from Fisher, S. A., Trenholme, G. M., Costanzo, M. R., and Piccione, W.: Infectious complications in left ventricular assist device recipients. Clin. Infect. Dis. 24:18-23, 1997.)*

preventive measures include soaking the surfaces of the LVAD in vancomycin and gentamicin for 30 minutes before placing it, irrigating the device pocket with povidone-iodine (Betadine), and meticulously placing the subcutaneous tunnel in the appropriate position. Postoperative care with sterile and semi-sterile dressing changes around the driveline is emphasized.[105,163,355]

Despite the preventive measures taken, infection, thromboembolism, and hemorrhage at the time of implantation remain the most common complications associated with the use of mechanical circulatory support devices. Of these complications, infection has the most significant impact on morbidity and mortality.[116] Predisposing factors for development of device-related infections in these patients include contact of blood with the prosthetic surfaces, device-related pockets and cavities, transcutaneous drivelines, and power cables; extent of surgery necessary for implantation; hemorrhage; reoperation; frequent occurrence of multiple organ dysfunction or failure; duration of mechanical circulatory support; poor health; and comorbid conditions. The incidence of infection in patients on LVAD support for longer than 60 days is reported to be two times higher than that in patients with an LVAD in place for less than 30 days; initial episodes of infection after 90 days are rare.[271,306,354] In addition, some evidence indicates that implantation of an LVAD itself may lead to defects in cellular immunity secondary to an aberrant state of T-cell activation that predisposes LVAD recipients to the development of candidal and other systemic infections.[11,128,174,306] The incidence of infection after implantation of an LVAD is reported to range between 13 and 80 percent, with most studies documenting an infection rate of 30 to 50 percent.* Most series report that the device pocket and drivelines are the device-related sites that account for most of the infections. Placement of the LVAD in the abdominal cavity instead of the preperitoneal area has been shown to decrease the chance for development of infection.[271]

Driveline infections are thought to result from a lack of integration of tissue around the driveline that allows the driveline to move and irritate surrounding tissues, which can result in an infection in the exit site and, in some cases, bacteremia. Such infections generally are defined by pain, erythema, warmth, drainage, or purulent discharge at the driveline exit site in the presence of a positive culture. Bacteremia developing after implantation of a pump and infections of the pump itself are additional reported complications.[14,244] In a study of 205 patients who underwent placement of LVADs at the Cleveland Clinic, positive blood cultures were noted in 52 percent, infections of the driveline site occurred in 22 percent, and infections of the pump pocket developed in 9.2 percent.[193] Sun and colleagues[373] reported their experience with 95 patients who underwent insertion of an LVAD. Twenty-six (27%) of these patients experienced device-related infections involving the driveline, device pocket, or blood-contacting surfaces; 15 of the 26 (57.7%) had infections of the driveline site. Persistent bacteremia and progression of infection of the device pocket and driveline site can lead to the serious infection of LVAD endocarditis, which mimics prosthetic valve endocarditis, is very difficult to treat, and is associated with a high mortality rate. LVAD endocarditis is defined as positive cultures of the LVAD surface in conjunction with clinical signs and symptoms of infection during LVAD support. Manifestations of this condition are varied and range from persistent fever and bacteremia or fungemia to cerebral thromboembolism, LVAD inlet obstruction with hemorrhage, or LVAD outflow graft rupture.[14] In a report by Weyland and associates[414] of 27 patients who underwent insertion of LVADs, infections of the driveline developed in 8 (30%) of the 27 recipients, and LVAD endocarditis, defined as positive cultures from the pump chamber, was found in 12 (44%) of the patients; 8 of the 12 (67%) died of endocarditis.

Despite a high potential for the development of serious morbidity after placement of an LVAD, studies have shown that the presence of an infection after insertion of an LVAD does not seem to have very much influence on eventual cardiac transplantation and post-transplant outcome. During a 5-year period, Argenziano and coworkers[14] compared the effect that infection after insertion of an LVAD had on mortality, the development of LVAD endocarditis, and eventual heart transplantation. The study found that infection subsequently developed in 29 of 60 (48%) patients who underwent insertion of an LVAD. The most frequent sites of infection were the blood (27%), LVAD driveline (13%), LVAD surface (13%), and central venous catheter (10%); these sites thus represented 63 percent of all infections.[11,14] The overall mortality rate, the rate of successful cardiac transplantation, and the rate of infection after transplantation were not influenced by the presence of infection during LVAD support.[14] In another study, Sinha and colleagues[355] reviewed their experience with 86 patients who received LVADs; device-related infections developed in 6 patients, 5 (83%) of whom had infections of the pocket. This study also found that that the presence of infection during LVAD support did not influence successful cardiac transplantation or patient survival after transplantation.

MICROBIOLOGY OF LEFT VENTRICULAR ASSIST DEVICE INFECTIONS

Numerous organisms have been isolated from LVAD infections. In most series, *S. aureus*, *S. epidermidis*, and *Enterococcus* spp. are the organisms most commonly isolated from these infections, usually within the first 4 weeks after implantation. Other organisms such as *Enterobacter*, *P. aeruginosa*, *Serratia marcescens*, other gram-negative organisms, and polymicrobial

*See references 14, 105, 114, 163, 244, 246, 298, 346, 373, 414.

organisms were seen more commonly later in the clinical course.[14,87,105,130,163,242,244,355,414] Fungal infections (e.g., *Candida albicans*) were reported as well and were responsible for only approximately 16 percent of the infections.[87,163,242] However, colonization with fungi occurs in 35 to 39 percent of patients with LVADs.[128] Investigators suggested that treatment with broad-spectrum antibiotics (common in these patients) rendered them more susceptible to the development of fungal infection. When a fungal LVAD infection is suspected, signs of thrush, esophagitis, retinal changes, peripheral embolization, and unexplained decrease in LVAD output should be investigated. The presence of yeast on Gram stain of the fluid surrounding the device and ultrasound showing increasing amounts of fluid around the device can be used to help in confirming the diagnosis.[128] Management of these infections, especially LVAD endocarditis, presents a major challenge, given that the device usually cannot be removed unless simultaneous heart transplantation is performed; moreover, if transplantation is accomplished, multiple issues regarding the immunosuppressed condition of the patient are raised. In most cases, management involves a prolonged course (usually 4 to 6 weeks) of aggressive antimicrobial or antifungal therapy (or both) appropriate for the organism or organisms isolated, with meticulous attention given to skin care around the driveline exit site; débridement, drainage, surgical revision, and irrigation with povidone-iodine of infected wound sites; or in certain instances, replacement of the LVAD unit or cardiac transplantation before completing antibiotic therapy if a donor heart becomes available and all blood cultures are negative.[105,163,244]

EXTRACORPOREAL MEMBRANE OXYGENATION CIRCUITS

Extracorporeal membrane oxygenation (ECMO) is the method used most commonly for treating severe cardiac and pulmonary failure in neonates and children. Almost 1 percent of all children treated by open heart surgery will undergo ECMO. In addition to these postcardiotomy patients, a growing number of children receive ECMO for acute decompensation of myocarditis or cardiomyopathy. ECMO also is used to support children after sudden cardiac arrest when conventional closed or open heart massage is unsuccessful. In children, perfusion through the neck venoarterial vessels is the preferred method, but the trans-sternal approach is used when direct decompression of the distended left ventricle is required. Although the overall survival rate of children managed by ECMO has improved in recent years, it still is only 50 to 70 percent,[23,408] in contrast to 88 percent in neonates treated for respiratory failure.[9,313] Multiple cardiac and noncardiac factors are associated with increased morbidity and mortality rates. Patients with a single ventricle or residual cardiac defect after surgery have a less favorable ECMO outcome.[34,79,202] Renal dysfunction, multiple organ system failure, initiation of ECMO in the operating room, blood product transfusion, mediastinal bleeding, ECMO circuit problems, and duration of ECMO for longer than 10 days also were predictors of increased risk of mortality.[202,260] The presence of infection (mostly nosocomial) while being supported by ECMO was associated with increased mortality rates.[207,260,315,431] Of interest, none of the patients who had a positive blood culture in the first 24 hours of ECMO survived.[262]

The same organisms that cause LVAD-associated infections also are associated with ECMO infections.[47,260] The distribution of these pathogens is similar to their distribution in other patients with infections treated in the intensive care unit, except for an increase in the incidence of *Enterobacter* and *Acinetobacter* infections.[47]

The signs and symptoms of infection often are subtle and nonspecific. Fever develops in only half of these patients. The value of tachycardia and elevated cardiac output also is limited because these patients frequently are tachycardic with increased cardiac output caused by a systemic inflammatory response (e.g., complement; cytokines such as TNF, interleukin-1 [IL-1], and IL-6), which is characteristic of cardiopulmonary bypass procedures.[257] For the same reason, leukocytosis (unless developing acutely) and an elevated erythrocyte sedimentation rate (ESR) should be interpreted with caution. Consequently, a high index of suspicion with random surveillance of blood and urine cultures and chest radiographs (to rule out pneumonia) is needed to identify these infections.

Most patients undergoing ECMO are treated with multiple broad-spectrum antibiotics. This practice probably is one of the reasons for the increased incidence of multidrug-resistant, gram-positive (e.g., *Enterococcus*) and gram-negative bacteria, as well as fungal (e.g., *Candida*), infections. In addition, many of these patients will be colonized with these same bacteria. Therefore, if a deep bronchial suction or urine culture (performed routinely) becomes positive but without supporting evidence of infection (e.g., increased white blood cell [WBC] count, fever), serious effort to distinguish between colonization and infection should be made before initiating an unnecessary change in antimicrobial therapy. If a decision to treat is made, appropriate coverage for both gram-positive and gram-negative organisms should be chosen. If blood, urine, and bronchial cultures remain negative and the patient's condition is not improving or the culture becomes positive for a fungus, antifungal therapy should be added.

PERMANENT CARDIAC PACEMAKER AND IMPLANTABLE CARDIOVERTER-DEFIBRILLATOR INFECTIONS

PERMANENT CARDIAC PACEMAKER INFECTIONS

Cardiac pacemakers were developed in the late 1950s, and since that time the field of cardiac pacing has made great strides with regard to the development and design of multiple types of pacemakers and the implantation of millions of devices into both adults and children worldwide. In the United States alone, the number of patients who have permanent pacemakers is estimated to be greater than 1 million.[95,403] The two types of permanent pacemaker generators in use include those with transvenous electrodes and those with epicardial electrodes. Both types are implanted in either the chest or the abdominal wall; pacemakers with epicardial electrodes are the ones used most commonly in the pediatric population.[403]

Infection is the most common medical complication of implantation of permanent pacemakers, with reported rates ranging from 0.13 percent to as high as 20 percent and a mortality rate of 27 to 66 percent.[36,49,62,95,113] An estimated 25 percent of cardiac pacemaker–associated infections develop within 1 to 2 months of placement of the device, but delays of up to 12 months may occur before diagnosis is established. However, since the 1980s, the incidence of infection associated with permanent pacemakers has decreased significantly because of improved surgical techniques and better design of the devices themselves; most series since that time report infection rates no higher than 5.7 percent.[13,36,95,156]

Studies have shown that infections of permanent cardiac pacemakers seem to occur more commonly in patients with both local and systemic underlying medical problems, especially diabetes mellitus or an underlying malignancy, or in those who are undergoing treatment with corticosteroids, anticoagulants, or other types of immunosuppressive therapy.[60,313] Other well-characterized independent risk factors include surgery related

to any part of the pacemaker (especially replacement of a battery or upgrade of the pacemaker),[60,149,150,156,210] the presence of a hematoma after implantation,[50,60,153,201] temporary transvenous pacing,[156,268] and operative time.[268] Multiple (two or more) pacemaker insertions and physician inexperience with insertion also represent significant risks for development of infection.[149]

Infections of permanent cardiac pacemakers are subdivided into different groups depending on the specific site of involvement and whether they are early infections (<1 to 2 months after implantation) or late infections (>2 months after implantation). The different groups include (1) local inflammation, infection, and the formation of an abscess in the generator pocket or subcutaneous portion of the lead, or in both sites; (2) secondary infection involving either the generator or the electrodes (including pacemaker endocarditis)[13,50,95,201]; (3) fever plus associated bacteremia, with or without concomitant infective endocarditis, in a patient without any apparent focus of infection[13,95]; and (4) mediastinitis, pericarditis, bronchopleural cutaneous fistulas, and mixed infections.[95]

The generator pocket and the subcutaneous portions of the leads (transvenous and epicardial) are the most common sites of infection. In a case series of permanent pacemaker–associated infections, Choo and associates[60] reported that abscesses in the generator pocket were present in 72 percent of the patients and were the only manifestation of infection in almost 40 percent. Infections of the pocket often are difficult to diagnose and may develop at any time after implantation; however, most tend to develop early and frequently are the result of contamination by skin flora at the time of the procedure.[258] Late infection usually occurs as a consequence of erosion of the device through the subcutaneous tissue and skin. The infection may remain localized to the pocket or may spread to the adjacent electrodes and lead to the development of bacteremia.[26,36] Infection of transvenous pacemaker leads may occur in as many as 17 percent of patients who receive permanent pacemakers. These infections often are not apparent initially but have been associated with significant morbidity and mortality. Bluhm[36] and others[140] reported a 25.3 percent mortality rate in 1734 patients with permanent pacemakers who had retained infected transvenous leads. Infection generally develops along the subcutaneous portion of the leads and, if unrecognized, may progress centrally and result in sustained bacteremia, endocarditis, or both.[36,140] Infections of the leads tend to occur later than infections of the pocket, with the median time of occurrence being 7 to 8 months after implantation of the pacemaker.[36,37] Endocarditis as a complication of unrecognized infected transvenous leads usually develops an average of 37 months after placement of the pacemaker.[263] Infections of permanent epicardial leads generally occur as a complication of infections of the generator pocket, skin erosion, or direct contamination at the time of placement. However, in contrast to infections of the transvenous leads, infections of the epicardial leads usually result in only local symptoms; rarely, they may lead to more severe disseminated disease such as pericarditis, mediastinitis, bronchopleural cutaneous fistulas, bacteremia, or sepsis.[26]

Clinical Findings

Diagnosing a pacemaker-associated infection can be difficult because of its nonspecific symptomatology, and months may elapse after the onset of symptoms before the diagnosis is established. Symptoms may be confined to a local area or may be more widespread with the development of bacteremia or other systemic effects. Fever (84-100%) and chills (75-84%) are considered the most common systemic symptoms and are indicators of local infection, especially if they occur after the second postoperative day in association with other signs; however, they may be the only clinical manifestation in more than a third of patients.[50,201,259,263,269,416] Early infections are more likely to be accompanied by both local and systemic clinical findings and are manifested more commonly as infections of the generator pocket, bacteremia, or septicemia[50,217,229,432]; late infections, on the other hand, typically cause vague symptoms that evolve over the course of time and usually are lead-related infections and endocarditis.[60,194,210,397]

Infections of the generator pocket typically cause local swelling (21%), erythema (34%), pain (32%), drainage (through an incompletely healed incision or fistulous tract) over or adjacent to the generator pocket (25%), and warmth (11.5%).[62] Sterile breakdown of the pacemaker pocket develops in an estimated 5 percent of patients with permanent pacemakers, and skin or soft tissue erosion over the electrodes occurs in an additional 2 to 4 percent. Because these complications are associated with a high risk for the subsequent development of infections, the presence of any erosion is considered a potential indicator of device-related infection, especially if the erosion occurs more than 24 months after placement of the pacemaker, at which time the infection rate may be as high as 80 percent.[33,138,143] Pacemaker-related endocarditis is a relatively rare complication that occurs in 0.05 to 0.5 percent of cases after implantation of a pacemaker. Such endocarditis is associated with a mortality rate as high as 34 percent in some series.[50,115,170,201] It tends to be a late complication; only 27 to 36 percent of patients are seen within 6 to 12 weeks after the last procedure at the pacemaker implant site, with symptoms developing in most patients a mean of 25 months after the last procedure at the implant site. The diagnosis of pacemaker-related endocarditis is difficult to establish, and usually a delay in making the diagnosis, with a mean interval of 5 to 8 months after the onset of symptoms, occurs.[50,201] Pulmonary symptoms are found in 20 to 45 percent of patients with pacemaker-related infections and may consist of bronchitis, lung abscess, pneumonia, or pulmonary embolism; these symptoms are seen more commonly in late-onset infection and in patients with intravenous lead–related infection.[50,201]

A variety of laboratory and imaging studies may be performed to aid in establishing the diagnosis of a pacemaker-related infection. An elevated ESR is found in 82 to 97 percent of patients, and peripheral leukocytosis is seen in 50 to 66 percent. The presence of a collection of fluid around the device as seen on ultrasound is suggestive of device-related infection. Gallium scanning may be performed in an attempt to determine the nature of the fluid (inflammatory versus infectious). Transthoracic echocardiography has emerged as a major tool in diagnosis; studies have shown that this test was able to demonstrate vegetations and other lesions on the electrodes, ventricular endocardium, and tricuspid valve in 90 to 96 percent of patients with pacemaker-related infections.[50,201,397]

Microbiology

Staphylococci (*S. aureus* and CoNS) are the most common causes of pacemaker-related infections, and they account for more than 85 percent of such infections; however, a wide variety of organisms have been isolated.[13,36,50,51,60,201,217,397] A higher proportion of *S. aureus* is isolated from early infections, whereas CoNS are isolated more commonly from late infections.[36,78,217] An increasing number of these organisms are methicillin-resistant. Other gram-positive organisms such as viridans streptococci, other streptococci, enterococci, *Listeria*, and *Corynebacteria* have been isolated as well, but each causes less than 1 percent of cases. Gram-negative organisms such as *E. coli*, *Proteus*, *Enterobacter cloacae*, *P. aeruginosa*, *Klebsiella*, and *S. marcescens* are isolated from 5 to 20 percent of device-related infections. In addition, *Candida*, *Aspergillus*, and other fungi have been isolated rarely; however, infections with these organisms generally are associated with poor clinical outcomes.[13,50,69,72,201,205,262,397,416,417]

Management and Treatment of Infection

Appropriate therapy for pacemaker-related infections is tailored to the specific clinical situation and depends on several different factors: (1) whether the infection is limited to the generator pocket or subcutaneous electrodes without bacteremia, (2) whether bacteremic pacemaker-related endocarditis is present with or without involvement of the subcutaneous electrodes, and (3) what organism is involved.

If the infection is limited to the generator pocket or subcutaneous electrodes, optimal treatment consists of both medical and surgical intervention in which all the parts of the infected device are removed. Studies have shown that failure to remove completely all portions of the infected device results not only in failure to cure the infection but also in higher mortality rates.[51,60,324,400] The patient is given parenteral antimicrobial therapy, and an exchange of the pacing system with total removal of the infected device and simultaneous insertion of a new pacemaker at a different site is performed surgically. The infected subcutaneous pocket is drained, débrided, and packed open, and local wound care is instituted. Initial antibiotic therapy should provide coverage for both staphylococci and gram-negative bacteria; therapy is tailored once the organism is identified and its antibiotic susceptibility is known. Typical regimens include vancomycin plus an aminoglycoside for a duration of 2 to 4 weeks, or oxacillin or nafcillin plus an aminoglycoside may be used. In certain instances, a new pacemaker cannot be placed at the time of removal of the infected device; in such situations, a period of temporary transvenous pacing is instituted until a new pacemaker can be implanted.[217]

The consensus approach to the management of pacemaker-related endocarditis and bacteremia involves removal of both the generator unit and the electrodes in conjunction with prolonged administration of parenteral antibiotic therapy designed to treat endocarditis (usual duration, 4-6 weeks). Mortality rates may be as high as 50 percent for patients in whom total removal of the pacemaker unit is not performed.[13,50,51,201] If the electrodes have been in place for longer than 18 months or large vegetations (>10 mm) are associated with them, extraction of the electrodes by traction may be difficult and risky to perform, so surgical extraction by cardiotomy may be necessary. In patients who require a pacemaker, temporary transvenous pacing may be implemented until a new permanent pacemaker can be placed. Antimicrobial therapy targeted at the causative organism should be administered for a minimum of 2 weeks, and sterility of blood cultures should be ensured before a new pacemaker generator and electrode are placed. Typical empiric therapy consists of vancomycin plus gentamicin and rifampin; therapy may be tailored once the organism has been identified and its antibiotic susceptibility is known.

INFECTION OF IMPLANTABLE CARDIOVERTER-DEFIBRILLATORS

The first implantable cardioverter-defibrillator (ICD) was placed in 1980, and during the last several decades it has become a successful therapeutic modality for the treatment of patients (both adults and children) with life-threatening ventricular arrhythmias. Older devices required surgical placement of a pulse generator, extrapericardial or epicardial defibrillation patches, and a transvenous rate-sensing electrode. The systems placed today use a rate-sensing lead that is implanted transvenously, a superior vena cava coil electrode, and additional subcutaneous or epicardial electrodes all connected to a pulse generator that is placed subcutaneously or submuscularly and usually is located in a pocket created in the abdominal region.[195,362,420]

Infection is the most serious complication of placement of an ICD.[50,211] Infection rates associated with placement of ICDs ranged from 1 to 7 percent.[13,363,383] However, infection rates have decreased substantially with the advent of transvenous non–thoracotomy-placed systems, with rates ranging from 0.8 to 1.5 percent.[127,286,357] Several risk factors that have been identified as increasing the likelihood for development of infection include steroid use, diabetes, malignancy, and renal failure.[35] Most ICD-related infections are clinically apparent within 3 to 6 months after placement, with infections of the generator pocket, subcutaneous patch wound site, or epicardial patches or bacteremia and endocarditis being the most common manifestations. An increased WBC count and ESR, anemia, and microscopic hematuria are the most common laboratory findings. Echocardiography may be helpful in half of the cases. Infection of the generator pocket or subcutaneous patch wound site usually causes local findings of pain, erythema, and collection and drainage of fluid from the site. Occasionally, these patients also may be bacteremic or hypotensive. In contrast, infection of the epicardial patches generally results in more systemic symptoms, bacteremia, or pericarditis.[286] The diagnosis of ICD-related infection typically is made on clinical grounds and confirmed by culture of the fluid or drainage around the device.

Microbiology

S. aureus and CoNS are the major pathogens seen with ICD-related infections, and they account for 60 to 80 percent of cases.[211,286,336,363] However, a broad spectrum of other gram-positive, gram-negative, and fungal organisms, including *P. aeruginosa*, corynebacteria, streptococci, *E. coli*, *Klebsiella*, *Bacteroides fragilis*, *Propionibacterium acnes*, atypical mycobacteria, and *Candida* spp., have been isolated from ICD-related infections. Polymicrobial infections may occur in the generator pocket.[211,336,357]

Management

To eliminate ICD-related infections effectively, optimal management involves a combination of medical and surgical interventions, especially if the patient is bacteremic or has systemic findings or if the causative organism is *S. aureus*. Several studies have suggested that conservative management (i.e., antibiotic therapy without removal of the hardware) may be sufficient. However, such an approach should be tried in cases in which only the generator pocket is infected (management may consist of the administration of parenteral antibiotics, wound care, and removal of only the generator portion of the device, with implantation of another generator at a different site) or in patients whose condition prevents removal of the entire device. In these situations, treatment of the ICD-related infection should include a prolonged course of parenteral antibiotics, followed by continuous suppressive oral antibiotic therapy.[286,386] The optimal management of ICD-related infections is parenteral antibiotics and removal of the entire ICD system.[13,62,211,336] The duration of antibiotic therapy varies from 2 to several weeks.

PROSTHETIC JOINT AND ORTHOPEDIC IMPLANT INFECTIONS

The implantation of prosthetic joints along with the use of other implantable orthopedic devices (e.g., pins, screws, plates, rods, external fixators, Ilizarov apparatus) has improved the quality of life greatly and restored function to patients suffering from debilitating bone and joint disease, tumor, or injury. Based on conservative estimates, millions of people worldwide have some form of prosthetic joint or other implantable orthopedic device. Of the possible complications associated with implantation, infection is the second most common cause of prosthetic

joint failure.[10] It occurs in 1 to 13 percent of cases[213] and results in postoperative prosthesis failure, chronic pain, immobility, and, in some cases, loss of the affected limb or, in the worst-case scenario, loss of life.[2,31,90,119,366] The health care cost in the United States for treating a single infection of a prosthetic joint is estimated to be more than $50,000, with an extrapolated expenditure of greater than $100 million per year nationwide.[28,152,344,366]

Prosthetic joints and implantable orthopedic devices may become infected by two major mechanisms: (1) the prosthetic device may be contaminated by microorganisms at the time of implantation, either as a result of airborne contamination in the operating room or by direct inoculation at the time of surgery, or (2) the prosthetic device may become infected as a result of hematogenous seeding from bacteremia or by direct contiguous spread from an infection adjacent to the prosthesis. Twenty to 40 percent of infections of prosthetic joints arise by hematogenous seeding, with the remainder occurring as a result of airborne or direct inoculation.[1,41,220,221,233] Infections may remain asymptomatic for years before symptoms become apparent, and usually a long delay occurs between onset of the infection and the appearance of symptoms and confirmation of the diagnosis. The overall rate of infection of prosthetic joints has been shown to be highest in the first 6 months postoperatively, with a steady decline occuring after this time. The incidence rate for infection of total-hip and total-knee arthroplasties during the first 2 years postoperatively is reported to be 5.9 infections per 1000 joint-years; in contrast, during postoperative years 2 to 10, it is 2.3 infections per 1000 joint-years.[366] The risk of acquiring infection and the incidence of infection depend on the anatomic location of the implanted orthopedic device or prosthetic joint, with the hips having the highest risk followed in descending order by the knees, elbows, shoulders, wrists, and ankles.[65,143,317,371] For implantable orthopedic devices, rates of infection range from 2 to 30 percent.[234,396,422]

RISK FACTORS

Numerous factors have been identified as increasing a patient's risk for developing infection of a prosthetic joint or orthopedic implant. These factors include rheumatoid arthritis, diabetes mellitus, obesity, poor nutritional status, the use of steroids, immunocompromised status, psoriasis, hemophilia, sickle-cell hemoglobinopathy, solid-organ transplant, dialysis-dependent renal failure, joint dislocation, and extremes of age.* In addition, previous surgery at the site of the prosthesis or implant also increases the risk of acquiring an infection. For example, several studies showed that the risk of deep infection developing in patients undergoing revision of a hip or knee arthroplasty was twofold to eightfold higher than that in patients with primary arthroplasty.[312,316,425] The relative risk for the development of prosthesis-related infections in patients with poor healing and wound complications increases from 13- to 20-fold after total-knee replacement and from 22- to 52-fold after total-hip replacement.[425]

The implanted metal prosthetic device and the PMMA cement that binds the prosthetic device to adjacent bone also predispose the joint space and bone to infectious processes, given that both are foreign bodies. In vitro studies have shown that the unpolymerized form of PMMA cement predisposes to infection by inhibiting phagocytic, lymphocytic, and complement function; the risk of acquiring infection seems to be enhanced further once the cement has polymerized in the body.[300,301] Cementless prostheses have been designed in an attempt to overcome the problem of infection associated with the PMMA cement. For certain orthopedic implants, the integrity of the skin is compromised chronically, thus providing ready access to organisms from the external environment.

*See references 31, 41, 109, 124, 139, 148, 182, 226, 292, 312, 370.

MICROBIOLOGY

More than 65 percent of infections associated with prosthetic joints and implanted orthopedic devices are caused by *S. aureus*, CoNS, beta-hemolytic streptococci, viridans streptococci, and enterococci. Antibiotic resistance in these organisms is increasing, and multiple different strains of staphylococci may be present in a single prosthetic joint infection.[173,180,234,337,396,421] Less commonly, aerobic gram-negative bacilli, including *E. coli*, *Proteus mirabilis*, *Klebsiella* spp., *Salmonella* spp., *S. marcescens*, other Enterobacteriaceae, and *P. aeruginosa*, may cause infection. In addition, 4 to 10 percent of infections are caused by anaerobic organisms such as peptostreptococci and *Bacteroides* spp.; polymicrobial infections occur in approximately 12 percent of cases.[173] Infections with fungi, particularly *Candida*, *Aspergillus*, and *Penicillium* spp., or with mycobacteria (i.e., *Mycobacterium tuberculosis*, *Mycobacterium avium* complex [MAC], and other rapidly growing mycobacteria) also have been described.[20,30,41,236,385] Rarely, a wide spectrum of other organisms, including *Corynebacterium*, *Propionibacterium*, *Bacillus* spp., and *Mycoplasma hominis*, have been reported to cause infection.[41] Zoonotic bacteria such as *Brucella* spp., *Yersinia* spp., and *Pasteurella* spp. rarely can cause infection and should be considered in the correct epidemiologic setting.[236]

Certain clinic situations may predispose a patient to particular organisms as the cause of infection. Pyogenic skin infections commonly result in staphylococcal and streptococcal infections of prosthetic joints, whereas infections of the teeth and gums are frequent causes of viridans streptococcal and anaerobic infections in prostheses. Genitourinary and gastrointestinal tract procedures or infections frequently are associated with enterococcal and gram-negative bacillary infections of prostheses.[41]

CLINICAL MANIFESTATIONS

The clinical findings and the severity of symptoms seen with infections of prosthetic joints are highly variable and determined primarily by three factors: (1) the route of infection—the hematogenous route versus direct inoculation; (2) the virulence of the infecting pathogen—*S. aureus* and, to a lesser extent, beta-hemolytic streptococci and gram-negative bacilli seem to be particularly virulent pathogens capable of producing a fulminant clinical picture, whereas infection with organisms such as CoNS is associated with a more chronic, indolent course; and (3) the nature of the tissue in which the microorganism proliferates—hematomas, seromas, ischemic wounds, and the tissues of diabetic patients and those receiving steroids all enhance the ability of the bacteria to proliferate and spread, thereby promoting the development of a more deep-seated fulminant infection.

The most common initial symptom is joint pain, which occurs in 95 percent of cases. Such pain can range from an acute fulminant illness with erythema, severe joint pain, swelling (38%), high fever (43%), and systemic symptoms to, more frequently, a chronic, slowly increasing pain in the joint that may be associated with the formation of a cutaneous draining sinus (32%) but no systemic symptoms.[173] The presence of constant joint pain is more indicative of infection than is the presence of pain occurring only with movement or weight bearing, which is indicative of mechanical loosening and inflammation.[139]

For implantable orthopedic devices, the most common initial symptom of infection is erythema, swelling, pain, or drainage from the area around and adjacent to the implant. Local symptoms also may be associated with fever, especially if the device is extensive or deep-seated.

DIAGNOSTIC STUDIES

Laboratory screening tests commonly used to diagnose infection of a prosthetic joint or implanted orthopedic device are the peripheral WBC count, C-reactive protein (CRP), and the ESR. Elevation of one or more of these factors may help support the diagnosis of an infection.[22,360]

The principal radiologic studies used for detection of an infected prosthetic joint include plain radiographs, arthrograms or sinograms, and radioisotope scans (indium and technetium diphosphate). Abnormalities that may be suggestive of infection and can be seen on plain radiographs include radiolucency at the bone-cement interface, motion and changes in position of the prosthetic components, evidence of osteomyelitis, cement fractures, and periosteal reaction. Intra-articular injection of dye (arthrogram or sinogram—in the presence of a sinus tract) may demonstrate abnormal communication between the joint space and the bone-cement interface. These radiographic abnormalities are present in approximately 50 percent of infected prostheses.[77,139,231] Nuclear scans with indium or technetium diphosphate may be used to detect periprosthetic inflammation. Both these scanning techniques are very sensitive but lack specificity. Technetium diphosphate (^{99m}Tc) shows increased uptake in areas of bone with an enhanced blood supply or increased metabolic activity. Increased uptake normally is seen around uninfected prostheses within the first 6 months after implantation; positive findings after this time are abnormal and reflect inflammation (which could be due to a variety of causes) but not specifically infection.[94,160,191,287] Indium 111–labeled leukocyte scanning has been shown to have a specificity of only 50 to 80 percent for knee and hip prosthetic infections.[278] In contrast, ^{99m}Tc-labled ciprofloxacin (which probably binds to live bacteria at the infection site) was shown to be 94 percent sensitive and 83 percent specific for the diagnosis of chronic infection.[359] Use of this technique allowed 11 of 12 infections of prosthetic joints to be identified, whereas ^{99m}Tc-WBC scintigraphy was positive in only seven patients.[359] CT and MRI are not used routinely in the evaluation of a patient with a suspected infection of a prosthetic implant because of the large amount of imaging artifact created by prosthetic devices.[40,256]

Aspiration of joint fluid for culture and culture of tissue obtained intraoperatively are the optimal ways of establishing a specific diagnosis of infection of a prosthetic joint. Although joint fluid findings indicative of an infectious process are a high leukocyte count (consisting mainly of polymorphonuclear leukocytes [PMNs]), a high protein content, and a low glucose concentration, these changes are not specific for bacterial infection and are present in only some patients. Nonetheless, infection should be suspected if there are five or more neutrophils per high-power field in a periprosthetic specimen.[360] The fluid obtained should be cultured for a variety of organisms; Gram stain is positive in a third of the cases, and a causative pathogen can be identified in only two thirds of patients.[297,361] Intraoperative cultures should include, if possible, any purulent discharge, devitalized bone, and tissue from the bone-cement interface. Vortexing or bath sonication (or both) of the explanted prosthesis may increase the yield.[294] Histopathologic examination of this tissue usually reveals an infiltration of PMNs consistent with an acute inflammatory reaction but not specific for infection.[141] More rapid, specific, and sensitive tests (e.g., in situ hybridization, immunofluorescence, polymerase chain reaction) are needed to differentiate between noninfectious inflammation and bacterial infection.

TREATMENT

Successful treatment of an infected prosthetic joint involves extensive surgical débridement of all devitalized bone and tissue, removal of the prosthesis and all associated cement, and prolonged parenteral antibiotic therapy.[22] For optimal results, the débridement should be performed within 1 to 2 weeks.[213] Microbiologic cure has been found to correlate well with the extent of débridement and the completeness of removal of all residual methylmethacrylate cement.[52] Historically, attempts at simple surgical débridement without removal of the prosthetic device in conjunction with parenteral antibiotic therapy have been successful in only 20 percent of cases, with relapse rates being as high as 88 percent by 2 to 4 years after therapy.[107,338,418] However, recent studies have shown that in patients with a short duration of prosthetic joint infection caused by penicillin-susceptible streptococci, débridement and antibiotic therapy alone appear to be sufficient, with a low risk of relapse.[251]

Two protocols have been used for the treatment of these infections. A two-stage surgical procedure with prolonged parenteral antibiotic therapy has been shown to be one of the most successful treatment regimens with the best functional results. The first stage involves complete removal of the prosthesis and cement followed by a 6-week regimen of antibiotic therapy empirically chosen to cover the most likely organism and then tailored once the organism has been identified and its antibiotic susceptibility known. The second stage involves reimplantation of a new prosthetic device at the end of the antibiotic course. Success rates with this procedure range from 53 to 100 percent.[104,119,124,222,270] An alternative method of therapy involves a one-stage exchange operation in which the infected prosthetic device and cement are removed, all devitalized tissue and bone are débrided, and a new prosthesis is reimplanted immediately, followed by 6 weeks of parenteral antibiotic therapy. Antibiotic-impregnated (either tobramycin or gentamicin) methylmethacrylate cement is used in these situations, and success rates range from 33 to 80 percent.[46,53,97,148,253,319,388] This procedure is appropriate only for patients with infections caused by less virulent microorganisms because of the high failure rates seen when a more virulent organism such as *S. aureus* or a gram-negative bacillus is the cause of the infection.[106]

The Ilizarov method allows simultaneous treatment of infection, bone and joint deformities, bone loss, and shortening of the limb. This device includes proximal and distal circular external rings with wires passing through the bone and soft tissue from one side of the limb to the other. The wires are placed in several planes and orientations to stabilize the bone. The rings are connected with threaded rods and nuts to allow for lengthening or shortening of the limb. Although this method is used more often for the treatment of bone nonunion or bone loss with or without infection,[265,290,296,350] David and colleagues[83] used this technique successfully to treat 12 patients who failed total-knee arthroplasty because of infection. Wound infection and chronic osteomyelitis caused by infection of the wire tract occur infrequently and should be treated by débridement of the infected soft tissue and curettage of the infected bone.

Currently, a two-stage prosthetic removal-reimplantation procedure coupled with the incorporation of antibiotic-impregnated cement during reinsertion of the implant, in combination with a 6-week antibiotic regimen, is the mainstay of therapy for infections of prosthetic joints.[120,270] Selection of antibiotics should take into account the recent increase in methicillin resistance among staphylococci for the following reasons: MRSA infections of the prosthetic joint result in a higher incidence of treatment failure than do infections with methicillin-susceptible *S. aureus*, and the former result in longer durations of hospitalization and a low survival rate free of treatment failure.[332] Thus, vancomycin (with and without rifampin) should be the initial drug of choice for gram-positive bacteria until the susceptibility of the infecting organism is known. Recent reports of treatment failures (even with vancomycin susceptibility within the treatment range [i.e., 2 to 4 μg/mL])[169,379] suggest that other

antibiotics (e.g., trimethoprim-sulfamethoxazole, linezolid) should be considered. Several reports have shown that linezolid is a reasonable alternative that results in control of the infection.[394]

For infected implanted orthopedic devices, the success of therapy is based on total removal of the device together with parenteral antibiotic therapy. In cases in which the foreign body cannot be removed, extended parenteral antibiotic therapy should be instituted and continued until the device can be removed. The duration of therapy varies with the severity of the infection and ranges from several weeks to several years.

CENTRAL NERVOUS SYSTEM SHUNTS

Infection is a major cause of morbidity in children who undergo CSF shunting procedures. Such procedures are performed to divert CSF in symptomatic hydrocephalic patients and are used commonly in patients with anatomic abnormalities (e.g., meningomyelocele and Chiari malformations), in premature newborns with intraventricular hemorrhage or intracranial infections (e.g., congenital cytomegalovirus, congenital toxoplasmosis, bacterial meningitis), and in patients with central nervous system (CNS) tumors or head trauma. Usually, the proximal end of the shunt is placed in the frontal or fourth ventricle and the distal end is inserted into the peritoneal cavity (i.e., ventriculoperitoneal [VP] shunt). Other compartments such as the right atrium (i.e., ventriculoatrial [VA] shunt), the pleural cavity, or the gallbladder can be used to place the distal end. CSF shunts are prone to complications, with a 10-year failure rate greater than 50 percent.[88,305] The most common complication is mechanical (such as obstruction or overdrainage from siphoning), followed by shunt-related infection. The incidence of shunt-related infection varies considerably, from 0.3 to 12 percent.[45,61,74,99,147,197,208,395] These infections increase morbidity rates and, in some cases, significantly affect patients' outcomes.[25] Recent understanding of some of the factors that contribute to the development of these infections has helped reduce their incidence.[61,102,198,409]

EPIDEMIOLOGY

Almost two thirds of shunt-related infections occur within 1 month after placement of the shunt, and 90 percent of infections are manifested within 6 months.[146,204,248] The incidence of shunt-related infections is significantly higher in infants in the first 6 months of life than in older children.[98,310,398] The infection rate is even higher in newborns with intraventricular hemorrhage who undergo shunting in the first week of life[7] and in premature infants.[208,249] Reasons for the increased incidence of shunt-related infection in very young patients are multifactorial. Several mechanisms that have been suggested include delayed wound healing, higher skin density of bacteria that are more resistant to antibiotics and more adherent to the shunt than in older children, longer duration of hospitalization, surgical technique, and increased exposure to antibiotics just before the shunt is placed.

No significant difference in infection rate is seen in patients with VP or VA shunts,[284,352] but a lower infection rate was noted in those with lumboperitoneal or cholecystic shunts. An increased risk of development of infection was reported for shunts placed immediately after removal of a previously infected shunt, probably because of incomplete eradication of bacteria.[99,199,346] Several other factors, including the underlying cause of the hydrocephalus,[98] the surgeon's experience,[66,196] previous shunt infection,[249] the duration of surgery, the number of people in the operating room, operative technique (e.g., prophylactic antibiotics, skin preparation, shaveless operation),[166] operative time, open surgery to insert the abdominal catheter versus direct puncture of the abdominal wall with a trocar, and postoperative CSF leakage,[208] have been reported to be associated with an increased incidence of shunt-related infection. Although a trend toward more shunt-related infections has been reported with these factors, it has not been demonstrated consistently. A few studies suggest that tapping the shunt may result in an infection, with an incidence ranging from negligible to greater than 30 percent in premature infants with shunts tapped multiple times.[45]

ETIOLOGY

Staphylococcal species are the most common cause of shunt-related infection, with CoNS (e.g., *S. epidermidis*, *Staphylococcus capitis*, *Staphylococcus hominis*, *Staphylococcus lugdunensis*)[96] being isolated in 25 to 70 percent of cases (Table 85–2).[96,204,264,352] *S. aureus*, the second most common gram-positive bacterium, is responsible for 10 to 40 percent of cases. Streptococci (e.g., viridans, group B or C, *S. pyogenes*, *S. pneumoniae*, enterococci) are identified less commonly (3% to 7%). Other gram-positive bacteria such as *Propionibacterium*[381] and *Corynebacterium* (diphtheroids)[15,137] are isolated as well. The seemingly increased incidence of shunt-related infections caused by these two groups of bacteria probably is the result of poor culture technique (e.g., failure to use anaerobic culture media, less than 5 to 7 days' incubation period) or misinterpretation of culture results (e.g., culture contamination) leading to under-reporting (or both).

Gram-negative bacteria (e.g., *E. coli*, *Klebsiella* spp., *Proteus* spp.) together are the cause of 5 to 25 percent of shunt-related infections.[364] *Pseudomonas* spp. and *Acinetobacter* spp. are reported as well, but less frequently. Shunt-related infections caused by gram-negative bacteria occur more commonly in patients with myelomeningocele and those in whom the distal part of the VP shunt is inserted into the peritoneal cavity via a percutaneous trocar (i.e., with an inadvertently perforated intestinal tract). Many other etiologic agents, including fungi (e.g., *Candida*,[76,122,133] *Histoplasma*,[342] *Cryptococcus*,[172] *Torulopsis*[405]), *Pasteurella multocida*,[214] *Neisseria* spp.,[167,372] *S. marcescens*,[44] nontuberculous myco-

TABLE 85–2 Pathogens Causing Cerebrospinal Fluid Shunt Infection

Pathogens	Incidence (%)
Gram-Positive Bacteria	
Staphylococcus, coagulase-negative (e.g., *Staphylococcus epidermidis*, *Staphylococcus capitis*, *Staphylococcus hominis*, *Staphylococcus warneri*, *Staphylococcus lugdunensis*, *Staphylococcus haemolyticus*)	25-70
Staphylococcus aureus	10-40
Streptococci (e.g., *Streptococcus pyogenes*, group B or C streptococci, Enterococcus, *Streptococcus pneumoniae*)	3-7
Propionibacterium species	Rare
Corynebacterium species	1-2
Gram-Negative Bacteria	
Escherichia coli	5-25
Klebsiella species	5-10
Proteus species	2-6
Pseudomonas species	2-4
Acinetobacter species	1-3
Other gram-negative bacteria (e.g., *Neisseria* species, *Haemophilus influenzae*, *Pasteurella*)	<1
Fungi	<1
Candida species	
Histoplasma	
Cryptococcus	
Torulopsis	

bacteria,[56] and others, have been reported less commonly as causing shunt-related infections. With the increase in the number of patients who are immunocompromised for various reasons (e.g., neutropenia, chronic intravenous catheters, prolonged administration of broad-spectrum antibiotics, hyperalimentation), the incidence of these rare infections will increase. Bacteria that traditionally cause meningitis, such as *H. influenzae*,[323,369] *S. pneumoniae*,[283] and *Neisseria meningitidis*,[215] were reported as causing shunt-related infections. Whether these cases were isolated shunt-related infections or extension of meningitis into the ventricular system (i.e., ventriculitis) is not clear. Therefore, if such bacteria are isolated from a suspected shunt and the patient has a communicating hydrocephalus, lumbar puncture should be performed to rule out meningitis.

PATHOGENESIS

Several observations suggest that most CNS shunt–related infections are caused by inoculation of the organism during surgery or contamination of the device by ward personnel during manipulation.[91,310,406] These observations include the facts that common skin flora (e.g., CoNS, *S. aureus*) are the pathogens most frequently encountered, most of the infections occur within the first few weeks after surgery, and irrigation of the system is a risk factor for the development of infection. Another common mechanism (occurring with gram-negative bacterial infections) is retrograde progression of bacteria from the gastrointestinal tract (i.e., bowel perforation)[164,328] or from the urinary tract (in the case of a ventriculo-ureteral shunt).[118] Other mechanisms by which shunts become infected include (1) hematogenous infection in which a distant site of infection produces bacteremia leading to a shunt infection (this type of infection occurs quite rarely and in most cases represents meningitis with secondary infection of the shunt, such as *S. pneumoniae* or *N. meningitidis* CNS infection in patients with a shunt) and (2) wound or skin infection (e.g., cellulitis, decubitus ulcer), with direct extension from the infection site to the shunt.

The predominant role of CoNS and *S. aureus* in CNS shunt–related infection is the result of their being the major constituents of normal cutaneous flora, especially in young children,[218,347] and their having the ability to adhere directly to the shunt (e.g., *S. epidermidis*) or to host proteins covering the shunt (e.g., *S. aureus*). In addition, CoNS (and some *S. aureus* strains) produce large amounts of extracellular slime (i.e., biofilm) that completely covers the organism.[42] More than 60 percent of staphylococci isolated from infected shunts produce biofilm.[84,101,142] The biofilm of *S. epidermidis* is a mixture of teichoic acid and protein.[170] Production of biofilm also was reported in corynebacterial infection, which may explain the increasing importance of this bacterium in CNS shunt–related infections.[24] The biofilm facilitates attachment of these organisms to the surface of the shunt and protects the bacteria from the host's immune defenses (i.e., reduces phagocytosis). Once the organisms are attached to the shunt material, they are extremely difficult to remove except by completely replacing the shunt. In addition, penetration of antibiotic into the biofilm is variable, and the biofilm antagonizes the antimicrobial activity of some antibiotics (e.g., vancomycin).[89,103] The importance of production of biofilm for the establishment of shunt-related infection caused by CoNS was shown by Younger and colleagues,[429] who found that 88 percent of the CoNS strains isolated from true shunt-related infections produced slime. Moreover, infections caused by nonadherent organisms were significantly more likely to be cured by antibiotics alone (without removal of the colonized shunt) than were infections caused by adherent organisms. Similarly, Diaz-Mitoma[84] and coworkers found that both obstruction of VP shunts and failure to cure the infection with antibiotics alone occurred more frequently when infectious episodes were caused by biofilm-producing CoNS. Therefore, complete removal of the shunt should be considered in patients with CoNS or *S. aureus* infection because biofilm-producing organisms may not be treated effectively when the shunt is in situ.

S. aureus infection is established primarily by the production of adhesin proteins. The most important proteins are fibronectin-binding (finb A and finb B) and fibrinogen-binding (Clf A and Clf B) proteins.[110] The ability to bind to fibronectin is very common in isolated strains of *S. aureus*, and its efficiency depends on the amount of finb A and finb B expressed on the cell surface of the individual isolate. The two fibrinogen-binding proteins attach to different parts of the host ligand, which suggests that they are acting synergistically or allow the bacteria to adhere to the ligand even during unfavorable conditions (e.g., antibodies against one of them).

The immature humoral immune system of young infants is not likely to explain the increased incidence of shunt-related infections in patients younger than 6 months old because these infants mount antistaphylococcal antibody responses that are comparable to those of older children.[310] Although levels of immunoglobulins and complement proteins are lower in this young group, levels of these proteins normally are very low in the CSF of older individuals (CSF levels of IgG and IgA are between 0.25% and 0.5% of those in serum). In addition, the types of bacteria causing CNS shunt–related infections are not associated commonly with humoral immunodeficiency states, thus suggesting that humoral protection is less important in CNS shunt–related infections. Little is known about the possible role of reduced tissue immunity in these infections.

The foreign body nature of the shunt apparatus plays an important role in the local host defense defect.[39] Electron-microscopic findings demonstrate irregularities in catheters that allow microorganisms to be buried in the catheter. In addition, the function of neutrophils is suboptimal because phagocytic and bactericidal activities are reduced as a result of the loss of lysosomal contents. Therefore, even when pathogens are phagocytized, they may not be killed and are protected from antibiotics that do not penetrate the cell membrane.

Other mechanisms that may contribute to shunt-related infections include (1) abnormal CSF flow (not being absorbed by the venous sinuses, thought to be important for prevention of infection in the CNS) and (2) interruption of the blood-brain barrier by the shunt catheter, with the creation of a direct tract between the subcutaneous tissues and the ventricles resulting in significant compromise in host defenses.[406]

CLINICAL MANIFESTATIONS

The initial signs and symptoms of most patients with shunt-related infections are nonspecific and include mild to moderate fever, malaise, irritability, nausea, vomiting, vague abdominal pain, and headache. With such nonspecific findings, the physician must be careful to differentiate between the possibility of a shunt-related infection and an intercurrent viral or bacterial infection of the upper respiratory, urinary, or gastrointestinal tract. Examination of the CSF (from a shunt tap) may be of help (see "Diagnosis"). Only a minority of patients have the classic signs and symptoms of CNS inflammation, such as a stiff neck, bulging fontanelle, change in mental status, cranial nerve palsy, or papilledema. In some patients the shunt tract may be infected, with evidence of cellulitis or dehiscence (or both) of the surgical wounds. Tenderness, edema, or erythema along the tract itself may be the only sign.

The type of shunt affects the nature of the infection. For example, VP shunt–related infections may cause symptoms and signs confined to the abdominal cavity, such as abdominal

pain, tenderness (with or without guarding), intestinal obstruction,[325] or spontaneous bacterial peritonitis.[121] Rarely, a distal shunt-related infection will be manifested as frank ascites as a result of CSF malabsorption.[209] A relatively common complication of VP shunts is an inflammatory peritoneal exudate that may lead to CSF loculation and the subsequent formation of a peritoneal pseudocyst.[8,326] These pseudocysts often are palpable and can be visualized by ultrasonography or CT. Bacteria are isolated in a third of cases, suggesting that infection may play a role in the pathogenesis of pseudocysts. In most cases, however, a high index of suspicion is required because the initial symptoms frequently are abdominal only, with no signs of shunt malfunction.

A unique complication of patients with VA shunts is the development of immune complex disease such as "shunt nephritis" (a form of acute glomerulonephritis), arthritis, or rash. In most of these cases, the infecting organism has been *S. epidermidis*, but other bacteria such as *Corynebacterium* can cause this complication.[38] In the case of shunt nephritis, the patient has fever, edema, malaise, hepatosplenomegaly, hypocomplementemia, anemia, azotemia, hematuria, and proteinuria.[78] Pathologic findings consist of mesangial hypercellularity and granular deposits of immunoglobulins and complement along the glomerular membrane. Rarely, arthritis may be the initial symptom because it may develop by the same immunologic mechanisms that cause nephritis.[216]

DIAGNOSIS

Although shunt-related infections are not common occurrences, the nonspecific signs and symptoms and the insidious onset in many cases render establishing a diagnosis very difficult. Therefore, any patient with a CNS shunt and fever without an obvious source should be suspected of having a shunt-related infection, especially if the symptoms continue for longer than a week. A higher index of suspicion for shunt-related infection is needed in young patients, in whom fever develops within 3 to 6 months after placement of the shunt. The only definitive diagnostic test is direct observation and culture of CSF. Tapping the shunt or sampling fluid in direct contact with the shunt should be performed if no signs or symptoms of increased intracranial pressure (ICP) are noted. CT of the head is recommended before the tap is done if such symptoms exist. The shunt tap should be performed with utmost attention given to sterile technique. The tap should be done by a neurosurgeon or physician who is familiar with the technique and the underlying hardware.

When percutaneous needle aspiration is performed, the area around the shunt reservoir should be scrubbed with antiseptic soap and the surrounding hair shaved (2 inches in each direction). The scalp area should be prepared by repeated application (at least three times) of povidone-iodine solution followed by alcohol. A 21- or 23-gauge butterfly needle is placed into the reservoir (or valve if no reservoir is present). Measurement of opening pressure can help in diagnosing a distal malfunction (i.e., increased pressure) or proximal shunt obstruction (i.e., less than expected pressure). CSF sample aliquots then should be allowed to drip into sterile vials. Gentle aspiration of CSF sometimes is performed if no fluid returns spontaneously. If only a few drops of CSF can be obtained, the more important tests, Gram stain and culture, should be performed first. Culturing the shunt wound, blood, or CSF obtained by lumbar puncture (which usually is not communicating with the ventricular fluid) often is unrevealing, misleading, or both. Although bacteremia frequently is present in patients with VA shunts and may help in diagnosing the etiologic agent, blood cultures generally are negative in patients with all other shunts (e.g., VP or pleural shunts). In addition, CoNS are the most common contaminants of blood cultures, and, therefore, interpreting a positive blood culture in these patients would be difficult.

CSF should be tested for glucose concentration, differential cell count, Gram stain, and culture. Protein concentration is requested often, but it is of very limited help in evaluating the presence or absence of an infection because high protein levels are found in many patients with shunt malfunction and no infection. In contrast, normal protein levels have been reported in many patients with shunt-related infections. A low glucose level suggests an infection, but one should confirm that the CSF sample was not diluted before the test was performed. Some physicians use saline to get a better flow of CSF (because of an occluded tube), which may affect the biochemical results. Of importance is to note that in many cases of shunt-related infection, the glucose level is within the normal range. Usually, pleocytosis with a predominance of PMNs is indicative of a shunt-related infection. Although in some cases the finding of a positive CSF culture is interpreted as a shunt-related infection despite a normal WBC count (<10 WBCs/mm^3), the absence of clinical symptoms (e.g., fever) in many of these patients suggests that the positive culture probably represents colonization or contamination. Other cells such as mononuclear cells[204,364] or eosinophils[247,384,399] may predominate during an infection. If eosinophilia is the predominant cellular response, an allergy to the shunt (e.g., silicone[183]) or the materials used for sterilization (e.g., ethylene oxide[307]) or intraventricular administration of antibiotics (e.g., gentamicin,[255] vancomycin[135]) should be considered.

Interpretation of the WBC count should be done cautiously if the red blood cell count is high because the increased number of WBCs can be the result of blood spilling into CSF without any infection or be part of the inflammatory response to the presence of blood (i.e., chemical ventriculitis). A positive Gram stain with an increased CSF WBC count or reduced glucose level (or both) is helpful in making the diagnosis of a shunt-related infection. A negative Gram stain does not exclude an infection, and one should wait for the results of culture. Ventricular fluid always should be cultured anaerobically as well as aerobically. Although most bacteria causing shunt-related infections grow within 48 to 78 hours, cultures should be held for 7 days (if still negative) for fastidious organisms such as *Propionibacterium*. The possibility of contamination or colonization of the shunt without infection should be considered when the culture is positive but other CSF parameters are normal. If such a scenario occurs and bacteria are growing only from one sample (e.g., a shunt tap) and not from follow-up cultures (i.e., from extraventricular drainage), a shorter course of therapy (see later) may be sufficient.

Blood cultures, a peripheral complete blood count (CBC), and ultrasound of the abdomen (for VP shunts) are of limited value. For example, although 90 percent of patients with VA shunt–related infection will have a positive blood culture, less than 10 percent of patients with other shunt-related infections will have a positive culture. In addition, in more than a third of patients with shunt-related infections, no elevation in the peripheral WBC count was found. Some investigators suggest that blood but not CSF CRP levels may be helpful in establishing the diagnosis of shunt-related infection when other concurrent infections (e.g., sinusitis, pneumonia) were excluded.[212,340] One suggestion is that if the CRP level is less than 7 mg/L, the shunt should not be removed because no infection is present. More data are needed to verify this observation. In patients with a VA shunt–related infection, measurement of serum anti-staphylococcal antibodies or the C3 and C4 components of the complement cascade may aid in establishing the diagnosis.[25,334] The triad of fever, abnormal CSF WBC count, and greater than 5 percent eosinophilia is highly predictive of a shunt-related infection.

TREATMENT

A variety of medical and surgical approaches to treatment of an infected shunt have been suggested.[415] Regimens include (1) the use of antibiotics alone (systemically, with or without intraventricular administration) without replacement of the shunt; (2) removal of the infected shunt followed by immediate insertion of a new shunt and the administration of systemic or intraventricular antibiotics, or both; (3) removal of the infected shunt and insertion of an extraventricular device (EVD) to monitor the patient's response to the accompanying antibiotic therapy, with a new shunt inserted only when the ventricular system is sterilized; (4) removal of the infected shunt followed by a stereotactic third ventriculostomy and administration of antibiotics; and (5) externalization of only the distal (e.g., peritoneal) catheter along with the administration of systemic or intraventricular antibiotics, or both.

The use of antibiotics alone without surgery was justified by the need to maintain CSF drainage and avoid costly operations and lengthy duration of hospital stay. The low success rate of this approach (33%) and the higher mortality rate associated with it suggest that it should not be used (Table 85–3). Of interest, the failure rate was much higher with infections caused by slime-producing organisms than with infections caused by non–slime-producing bacteria.[84] Only in shunted patients with purulent meningitis caused by *S. pneumoniae*, *N. meningitidis*, or *H. influenzae* did the administration of systemic antibiotics alone without removal of the shunt seem to be an acceptable option.[215,288,323,369]

Combining immediate replacement of the infected shunt with a new shunt and antibiotic therapy has a higher rate of success (70%, see Table 85–3) than does the use of antibiotics alone. Nonetheless, it is less effective than removal of the infected shunt accompanied by insertion of an EVD and administration of antibiotic therapy (88% success rate, see Table 85–3). A decision analysis of 17 studies reached the same conclusion and suggested that "this treatment option has the highest cure rate and the lowest failure and mortality rates."[339] In addition, lack of the ability to monitor when the ventricular fluid is sterilized results in a longer period of systemic antibiotic therapy (e.g., 4 to 8 weeks), which may lead to an increase in iatrogenic infections and cost. Some surgeons suggest removing the shunt and delaying replacement (i.e., a few days after removal to allow sterilization of the shunt's tract), but this approach is associated with increased morbidity.[108,407]

For infection that involves only the distal part of the shunt (e.g., pseudocyst, appendicitis,[295] erythema or swelling along the shunt tract, surgical wound infection), externalization of only the distal end of the shunt along with the administration of antibiotic therapy is recommended by some neurosurgeons. Potential advantages of this technique include (1) diversion of CSF from an infected area to avoid ascending infection, (2) maintenance of CSF flow to prevent increased ICP, (3) the ability to perform frequent CSF sampling, and (4) the capability of monitoring therapy. The disadvantage is that early infection or colonization of the proximal portion of the shunt may be obscured by the antibiotic treatment and become active after discontinuation of therapy and reinsertion of the distal part.

Internal shunting by a third ventriculostomy (with avoidance of a prosthetic device) was shown to be effective in managing patients with refractory shunt-related infections who have a noncommunicating hydrocephalus, patent subarachnoid space, and adequate CSF absorption.[108,185,264,275,352] Shunt independence for extended periods was documented in many patients without myelomeningocele. The success rate is lower in those with myelomeningocele or hemorrhage or after meningitis. Disadvantages of this technique include increased morbidity (e.g., hypothalamic injury, subarachnoid hemorrhage), technical difficulty in younger children, and, if the stereotactic technique is used, cost and availability of the necessary equipment.

The most effective treatment of shunt-related infections is to remove the entire infected shunt and insert an EVD to control ICP and monitor the infection (i.e., provide CSF access). After

TABLE 85–3 Shunt Infection Cure Rates in Relation to the Therapeutic Approach

Author	Antibiotics Alone*	Antibiotics and Immediate Replacement with a New Shunt	Antibiotics, Removal of the Shunt, and Insertion of an EVD
Schoenbaum[338a]	5/30[†]		25/26
Nelson[275]	10/13		46/46
Salmon[333]		5/10	
Sells[345]	1/8	1/6	9/9
James[179]	3/10		9/10
Venes[392]		6/9	3/3
James[178]	4/11	11/13	16/17
Wald[404]	15/20		
Mates[238]	7/8		
Shurtleff[351]	2/27	6/20	19/19
Morrice[266]	4/14	19/23	14/18
Nicholas[276]			
Frame[112]	8/11	21/27	
Forward[108]	8/15	2/2	13/13
Luthardt[228]	1/17		
O'Brien[281]	11/11	15/19	9/9
Walters[406]	13/92	11/21	44/71
Swayne[376]			19/20
Ronan[327]	3/4	4/7	21/22
Stamos[364]			23/23
Morissette[264]	3/6		3/3
Younger[429]	4/11	42/46	
Total (success rate)	**102/308 (33%)**	**143/203 (70%)**	**273/309 (88%)**

**With and without intraventricular antibiotics.*
[†]Number cured/number treated.
EVD, extraventricular device.

antibiotic therapy has been successful, a new shunt is placed. With this approach, treatment success is very high (see Table 85–3), with more rapid clearance of the infection and a shorter duration of therapy. The choice of intravenous antibiotic depends on local patterns of antimicrobial susceptibility and the ability of the antibiotic to penetrate the blood-brain barrier. With the increasing rate of methicillin-resistant staphylococci, vancomycin should be used as initial therapy while awaiting bacteriologic identification and the results of antibiotic sensitivity testing. Effort should be made to discontinue vancomycin as soon as the infecting bacteria are found to be sensitive to the semisynthetic penicillins so that the chance of the bacteria becoming resistant to vancomycin is reduced. In addition, even if in vitro data suggest that the bacteria are highly sensitive to the first-generation cephalosporins (e.g., cephalothin, cefazolin, cephapirin), they should not be used because they penetrate the blood-brain barrier poorly. Linezolid has been used successfully in a few cases of shunt infection.[54] This drug has a good CSF penetration, with concentrations above the minimal inhibitory concentration (MIC) of most gram-positive bacteria, including MRSA. It is also effective against bacteria living in biofilms. The limited data available suggest that linezolid should be considered cautiously when vancomycin fails or cannot be given.

To achieve more consistent and efficient eradication of bacteria, the CSF drug level should be at least 10 times higher than the MIC of the pathogen. Therefore, the dosage of antibiotic or antibiotics and the dosing interval should be maximized (i.e., "meningeal schedule"). If the selected antibiotic does not clear the infection within 2 to 3 days and no improvement occurs in CSF biochemical and WBC parameters (i.e., seemingly no control of the infection), measurement of the bactericidal titer of ventricular fluid should be considered. To determine bactericidal titer, 1 mL of CSF (if CSF production is >5 mL/hr) at the expected peak antibiotic level (i.e., 2 to 3 hours after the antibiotic is given parenterally) is diluted serially with culture media to produce dilutions of 1:2, 1:4, 1:8, and 1:16. To these dilutions, an equal amount of medium with 10^5 colonies per milliliter of the offending bacteria is added (the final CSF dilution is 1:4, 1:8, 1:16, and 1:32). After 24 hours of incubation, the tubes are observed for turbidity, which reflects the growth of bacteria. If no turbidity is seen in the 1:8 and 1:16 or higher dilution tubes, the CSF level of the antibiotic probably is sufficient, and continued growth of bacteria from CSF may be caused by colonization of the EVD or contamination. On the other hand, if turbidity is noted in the tube with less than a 1:8 dilution (i.e., a 1:4 dilution), the CSF level of antibiotic may not be sufficient to combat the infection, and the addition of another antibiotic or change to a different antibiotic is warranted.

In vitro synergy studies can help determine the best drug combination. Rifampin should be considered as one of the drugs in the combination for gram-positive bacteria for three reasons. First, most staphylococci are still sensitive to this antibiotic and their MIC usually is 10-fold lower (i.e., 0.05 μg/mL) than that of other anti-staphylococcal drugs (e.g., vancomycin, 0.5 μg/mL). Unfortunately, the use of rifampin alone may be followed rapidly by rifampin-resistant variants because they already are present in small numbers in any staphylococcal population. Second, rifampin penetrates CSF well and easily achieves a greater than 10-fold level over the MIC of most staphylococci. Third, rifampin has demonstrated good bactericidal activity even when staphylococci were embedded in slime. In contrast, staphylococcal slime inhibits the antimicrobial activity of vancomycin.[103]

Selection of the initial antibiotic before culture results are known can be based on the patient's clinical and CSF findings. An algorithm for initial antibiotic therapy before CSF culture results are available is presented in Figure 85–2. This algorithm should not be used for neonates or immunocompromised patients with suspected shunt-related infections because they often have less severe clinical symptoms and CSF response. The decision-making process starts with assessment of the patient's clinical condition. Usually, patients with infection in the CNS are only mildly symptomatic, whereas patients with *S. aureus* or gram-negative bacterial infection often are more seriously ill.

The next step is evaluation of the Gram stain. If the Gram stain is positive for gram-positive bacteria (e.g., *Staphylococcus*, *Streptococcus*), the drug of choice is vancomycin. If the Gram stain shows gram-negative bacteria, the drug of choice is cefotaxime or ceftriaxone. Treatment with an aminoglycoside is acceptable, but the outcome seems to be less favorable because of poorer penetration into CSF.[364] If the Gram stain is negative, other CSF parameters (e.g., WBC count and glucose) should be examined. If either is abnormal and the patient is not severely sick, vancomycin alone should be started because the chance of having a gram-negative infection is low.[364]

In contrast, if the patient is more seriously ill, coverage for gram-negative bacteria should be added (e.g., cefotaxime or ceftriaxone). In nontoxic patients with normal CSF parameters and no distal symptoms (e.g., peritonitis, wound or tract infection), antibiotic therapy can be withheld until the results of culture are known. On the other hand, if distal signs or symptoms exist, therapy is tailored according to the site. In patients with skin involvement, vancomycin is the drug of choice, whereas in febrile patients with abdominal symptoms, the combination of cefotaxime (or ceftriaxone) and clindamycin (to cover both gram-positive and anaerobic bacteria) is preferred.

Direct instillation of antibiotics into the ventricular system to increase their levels is recommended by some experts. Unfortunately, the suggested doses for intraventricular treatment have been determined empirically on only a small number of patients, and their pharmacokinetics and pharmacodynamics have not been studied well. This therapy is not without hazard, especially when the recommended doses often are much higher than those found to cause neurotoxicity.[200,356,411,413] Pleocytosis and eosinophilia also have occurred in patients receiving intraventricular vancomycin[224] or gentamicin.[255] In addition, preservative-containing preparations should be checked for appropriateness for intraventricular instillation. Limited pharmacokinetic data suggest that clearance of the instilled intraventricular antibiotic is sufficiently slow to allow once-daily administration. If possible, the EVD should be closed for 30 to 60 minutes after the drug is administered. If the EVD cannot be closed and the amount of CSF drainage exceeds 7 to 10 mL/hr, the frequency of intraventricular antibiotic instillation should be increased to twice daily.

Antibiotics commonly recommended for intravenous and intraventricular use according to the etiologic agent are shown in Table 85–4. A recent small study in adults showed that administration of 10 mg of vancomycin intraventricularly once daily was safe and achieved levels above 5 μg/mL (the recommended CSF trough level needed to achieve cure) for up to 21 hours.[303] Because of the potential toxicity of the empirically recommended intraventricular antibiotic doses and the unpredictability of CSF levels, irrigation of the ventricular system with a known concentration of an antibiotic solution is preferred when systemic antibiotics fail to eradicate the bacteria. To achieve irrigation, two EVDs must be inserted to produce a continuous flow of solution. The concentration of antibiotic in the solution should be equal to the highest safe plasma level when the drug is given intravenously. For example, for treating gram-negative bacteria sensitive to amikacin, amikacin at a dose of 30 to 40 mg/L of saline solution (producing a concentration of 30 to 40 μg/mL) is recommended. Gentamicin at a dose of 10 to 12 mg/L (using a special intrathecal preparation) is an acceptable alternative when the gentamicin MIC for the bacteria is less than 1 μg/mL. The antibiotic solution is administered through one EVD at a rate of 10 mL/hr, and the second EVD is left open to drain the fluid and to remove debris and pus from the ventricles.

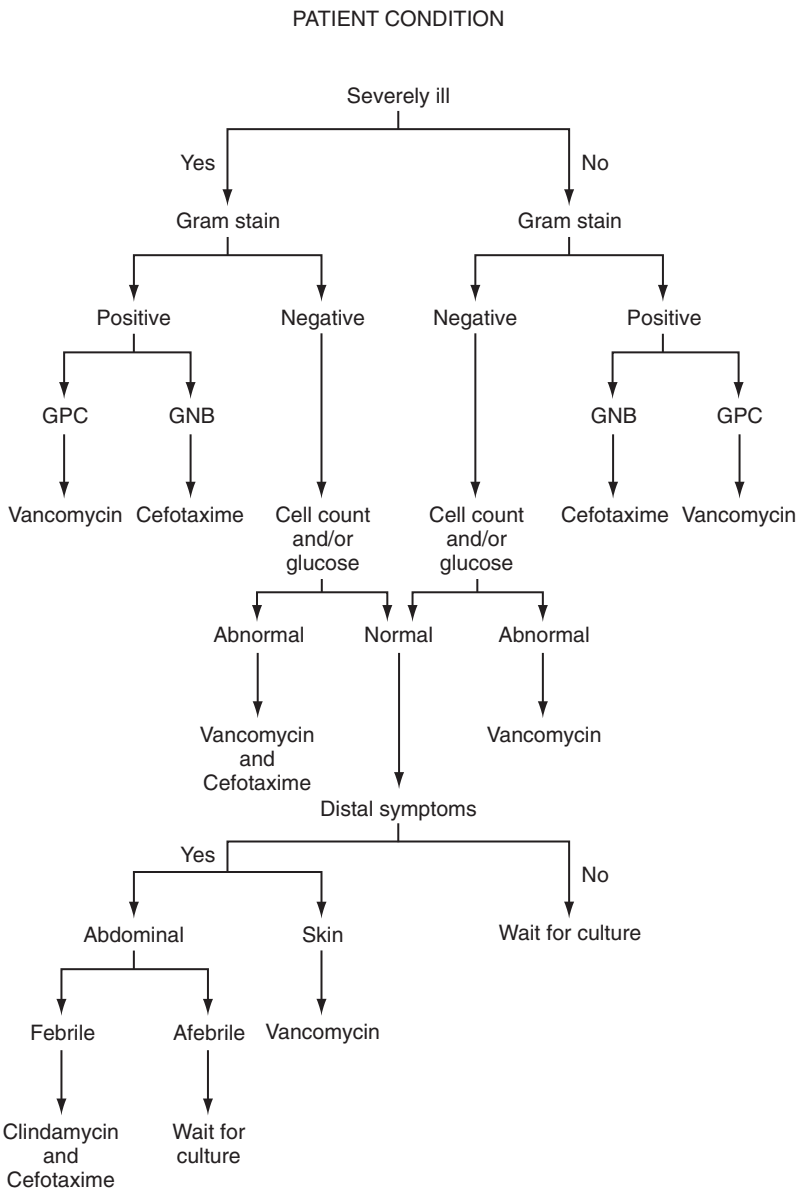

Figure 85–2 Algorithm for selection of antibiotic therapy before culture results are known. GNB, gram-negative bacillus; GPC, gram-positive coccus.

When antibiotic therapy is completed, abnormal CSF findings such as low glucose or mildly elevated protein or cell counts should not delay reshunting. The duration of treatment is empiric and depends on the etiologic agent, the CSF parameters at initial evaluation, and the time to sterilization. In our institution, we found the following schedule to be successful. If CSF parameters are *normal* but culture yields CoNS only from the operating room (i.e., the initial sample), therapy should be given for only 3 to 4 days. If subsequent cultures also are positive, therapy should continue until negative cultures have been obtained for 7 days. If CSF parameters are *abnormal* in the operating room and culture is positive *only* from that specimen, therapy should continue for 7 days. If subsequent samples show abnormal CSF findings and positive cultures, therapy should be extended until negative cultures have been obtained for 10 days.

A longer duration of antibiotic therapy is recommended with other bacteria (e.g., *S. aureus*, gram-negative bacteria). If culture is positive *only* on samples from the *operating room* and CSF findings are normal, treatment should be given until negative cultures have been obtained for 7 days. In all other situations, therapy should continue until negative cultures have been achieved for 10 days. Reshunting should take place immediately at the end of treatment. No benefit is found in observing the patient for a time without antibiotics for relapse of the infection.[409]

COMPLICATIONS

Shunt-related infections are associated with increased morbidity and mortality rates. Patients with shunt-related infection have an increase in shunt-related operations, which contributes to the increased morbidity and cost. In addition, these patients have been shown to have an increase in mortality rates in comparison to patients without shunt-related infection.[356] Even when the original infection is treated successfully, secondary infection (or contamination) of the EVD occurs in 5 to 10 percent of cases. To minimize the risk of development of a secondary infection, a sterile closed-drainage system should be maintained carefully. Only trained personnel should be allowed to drain CSF samples from the system, and injections into the system should be avoided. Continuous external drainage of CSF also causes loss of electrolytes and fluid. Therefore, routine assessment of serum

TABLE 85–4 Recommended Intravenous and Intraventricular Antibiotic Therapy for Shunt Infection according to the Etiologic Agent

Etiologic Agent	Antibiotic	Intravenous Dose (mg/kg/day)	Intraventricular Dose (mg/day, 1 Dose)
Bacteria			
Staphylococcus aureus or coagulase-negative staphylococci			
Methicillin-sensitive	Oxacillin*,†	200 (q8h)	NA
	or		
	Nafcillin*,†	200 (q8h)	50-75
Methicillin-resistant	Vancomycin†	60 (q8h)	5-10
	or		
	Linezolid	15 (q12)	
Streptococcal species	Penicillin*	400,000 U (q6h)	—
or	*or*		
Diphtheroids	Ampicillin*	400 (q6h)	10-25
Enterococcus faecalis	Ampicillin*	Doses as above	
	or		
	Penicillin*		NA
	plus		
	Aminoglycoside‡		
Anaerobic bacteria	Metronidazole	30 (q8h)	NA
Escherichia coli, *Klebsiella*, *Proteus*, *Enterobacter*	Cefotaxime	200-300 (q6h)	NA
	or		
	Ceftriaxone	100 (q12h)	NA
	or		
	Amikacin§	22.5 (q8h)	2-8
	or		
	Tobramycin§	7.5 (q8h)	1-4
	or		
	Gentamicin§	7.5 (q8h)	1-4
Pseudomonas species	Ceftazidime	200 (q8h)	NA
	or		
	Aminoglycoside		
	plus		
	Broad-spectrum penicillin		
Fungi			
Candida species	Amphotericin B¶	1 (q24h)	0.1-0.25
	AmBisome or Amphotec	5 (q24h)	

**In patients allergic to penicillin, use vancomycin.*
†If cerebrospinal fluid levels are not sufficient and the bacteria are sensitive to rifampin, add rifampin, 20 mg/kg/day divided every 12 hours.
‡See doses (for the specific aminoglycoside) for treatment of E. coli below.
§The addition of a broad-spectrum penicillin (e.g., piperacillin or ticarcillin, 300 to 400 mg/kg/day divided every 6 hours) may add to the bactericidal activity.
¶If ventricular fluid remains positive after 5 to 7 days of therapy with amphotericin, add flucytosine (150 mg/kg/day divided every 6 hours).
NA, not available.

electrolytes once or twice weekly is recommended, and the total daily amount of drained CSF should be replaced. Recurrent shunt-related infection after completion of treatment is common, with two thirds of such infections being caused by the same organism.[197] Unusual complications of ventricular shunt infections include brain abscess and subdural empyema.[100,402]

PROGNOSIS

Long-term morbidity occurring after a shunt-related infection includes seizures, psychomotor retardation, and cognitive deficiency. The intelligence quotient (IQ) scores of children with myelomeningocele who had a shunt-related infection were found to be significantly lower (mean IQ, 72) than the scores of children with shunts but no infection (mean IQ, 95).[250] A trend was observed in which younger children with shunt-related infections had a lower IQ than older children with shunt-related infections did, especially if the infection was caused by gram-negative bacteria. In addition, shunt-related infection adversely affected school performance.[398] With appropriate combined medical and surgical therapy, the mortality rate from shunt-related infection is low, but any episode of shunt-related infection appears to increase the probability of another episode developing.

PREVENTION

Preventive measures to reduce the incidence of shunt-related infection include attention to preoperative and intraoperative technique and the prophylactic use of antibiotics. Bactericidal shampoos (e.g., chlorhexidine) should be used to reduce the bacterial density of the scalp before surgery is performed. Shaving the scalp seems to be a risk factor, so shaveless surgery should be considered.[166] During the operation, only essential personnel should be present, and the skin should be cleaned with a fat solvent, followed by solutions that reduce the number of bacteria (elemental tincture of iodine, 20 ppm, is preferred over 10% povidone-iodine, which contains <1 ppm free iodine)[58] or their ability to adhere to the shunt (bacitracin A).[134] Careful attention should be paid to surgical technique, with contact between the shunt and the skin being avoided. A detailed preoperative protocol developed by Choux and associates[61] has reduced the infection rate from greater than 7 percent to less than 1 percent. This

protocol emphasizes a shorter operating time, less operating room staff and traffic, and fewer manipulations of the shunt. Even though several investigators were able to reduce the infection rate by using measures similar to those suggested by Choux and coworkers,[59,198] other studies have failed to show that all the factors emphasized by Choux and associates have a positive effect on the infection rate.[305,310] Although a few recent studies suggested that use of an antibiotic-impregnated shunt significantly reduces the incidence of shunt infections in comparison to the use of a standard shunt,[132,343] no significant reduction in the overall shunt-related infection rate was observed in other studies.[151,189] Large prospective, randomized studies are needed to evaluate the real contribution of an antibiotic-impregnated shunt to the reduction in infection rate.

Multiple studies have examined the effect of prophylactic antibiotics for the shunt insertion procedure on reducing the infection rate. Major variations in study design and the number of patients in each study preclude arriving at definitive conclusions. Previous meta-analyses of well-designed studies suggest that the use of prophylactic antibiotics is associated with a significant reduction in the incidence of infections *if* the baseline rate of shunt-related infections is greater than 5 percent.[145] If the infection rate is lower than 5 percent, prophylactic antibiotics are *not* recommended. A recent meta-analysis of 2134 patients (in 17 trials) found a statistically significant benefit for either systemic antibiotic prophylaxis (15 studies) or antibiotic-impregnated catheters (2 studies) in reducing the incidence of shunt-related infections.[318] The choice of antibiotics should be based on the local antibiotic sensitivity pattern of the pathogens commonly causing shunt-related infections in that area. Preferably, prophylaxis should be started 8 to 12 hours before surgery to allow higher levels of drug to accumulate in skin tissue than would do so if treatment were given just before the operation. The duration of prophylaxis should not exceed 24 to 36 hours. Further studies are needed to identify factors important in the development of shunt-related infections so that better techniques can be developed to further reduce or even eliminate this devastating complication of CNS shunting.

INTRACRANIAL PRESSURE MONITORS

Monitoring of ICP has become an important part of the evaluation, treatment, and management of children with a variety of intracranial pathologic processes,[225] including congenital anomalies (e.g., cranial or craniofacial dysostosis), metabolic diseases (e.g., Reye syndrome), trauma, intraventricular or subarachnoid hemorrhage, intracranial infections (e.g., encephalitis), and other ischemic or hypoxic insults. Several studies have demonstrated the therapeutic and prognostic benefit of monitoring ICP in children with high ICP[111,181,219,380]; nonetheless, this procedure is not without complications such as hematoma, bleeding, leakage of CSF, and infection. Infections for which monitoring of ICP is used include ventriculitis, meningitis, brain abscess, subdural empyema, skin infection, and cranial bone infections.[201,224,241,273,423]

Several methods are available for monitoring ICP. Intraventricular placement of a catheter with the distal end connected to a pressure transducer is the method used most frequently because of its accuracy and ease of calibration. This procedure also allows drainage of CSF for biochemical or dynamic testing. Disadvantages of ventriculostomy are penetration of the meninges and brain, technical difficulties if the ventricles are small, and greater risk for the development of infection. Other methods used to monitor ICP include a subdural bolt or catheter (only the meninges are penetrated, but the brain remains intact), an epidural transducer (the dura remains intact), and a continuous intraparenchymal monitor (i.e., a fiberoptic cable is inserted through the dura into the brain parenchyma, not intraventricularly). Although both the epidural transducer and the intraparenchymal monitor have fewer complications (e.g., infection) than the intraventricular devices do, they are prone to inaccuracy, which limits their use. Selection of the appropriate ICP-monitoring method depends on the patient's condition and the risk associated with the procedure.[232]

EPIDEMIOLOGY

Assessing the true infection rate of ICP monitoring devices is very difficult, mainly because the methods used to define infection (i.e., inclusion and exclusion criteria) and antibiotic use vary widely among reported studies. The incidence of infection ranged from less than 1 percent to 40 percent, with an average incidence of 8.8 percent.[63,73,223,233,346,368,401] A much higher rate of infection was reported in patients who had devices that penetrated the meninges[18,162,273] than in those who had parenchymal monitors.[311]

Multiple factors were suggested as increasing the risk for development of ICP device–related infection (Table 85–5). Patients with intracerebral hemorrhage usually do not have a higher risk for acquiring infection than do those without such hemorrhage. In contrast, if the bleeding is intraventricular, the incidence of infection increases dramatically.[241] The rate of infection also is higher in patients with open head trauma than in those with closed head trauma or intracranial malignancy.[168] Neurosurgical operations contributed significantly to the risk of acquiring infection,[241,374] and the same was noted if ICP was greater than 20 mm Hg.[241]

Interruption of the monitoring system's integrity (e.g., in-line stopcocks, number of times that the system was open, blockage of drainage, and irrigation) was identified by several investigators as increasing patients' risk of acquiring infection.[18,241,254,280] The effect of the duration of monitoring on the risk for infection is controversial, especially in patients with ventriculostomy catheters. Although several studies found a correlation between the length of time that the monitoring device was in place and the infection rate,[18,162,190,273] some investigators found no relationship with the duration of monitoring.[282,358,374,422] The different conclusions probably were the result of differences in the populations studied, the types of devices used to measure ICP, the definitions of infection, and analyses of the data. One critical review of the

TABLE 85–5 Risk Factors for Infection of Devices for Monitoring Intracranial Pressure

Factors Associated with Increased Risk for Infection
Intraventricular hemorrhage
ICP >20 mm Hg
Open head trauma
Neurosurgical procedure/operation
Perforation of the dura
Duration of catheterization
Problems with the system
Disconnections
Leaks
Irrigations
Blockage
Factors Not Associated with Increased Risk for Infection
Head trauma
Intracerebral hemorrhage
Intracranial malignancy
Underlying disease
Drainage of cerebrospinal fluid
Previous ICP monitoring device in the intensive care unit
ICP device dressing changes
ICP device component changes

literature found a correlation between the duration of ICP monitoring and the rate of infections.[223]

The need to replace the device at a certain time has been challenged. Analysis of the outcome in 584 patients (receiving 712 ventriculostomy catheters) who had 61 infections showed a steady increase in the daily incidence of infection, with a peak occurring at day 10. The average time until the onset of infection was 6.8 days.[162] In addition, replacing the ventriculostomy catheter before day 5 did not affect the daily rate of infection. The authors concluded that the lowest rate of infection occurred in the first 4 days of monitoring but that replacement of the catheter by day 5 did not reduce the infection rate, which continued to rise until day 10. The authors recommended that "ICP monitoring [devices] should be removed as quickly as possible," but if "prolonged monitoring is required, there appears to be no benefit from or need for catheter exchange."[162]

Several potential risk factors for ICP monitor–related infections that do not increase the infection rate are shown in Table 85–5.

ETIOLOGY

In general, the microorganisms that cause infections related to ICP monitoring devices are the same as those causing CNS shunt–related infections (see Table 85–2). The major difference is that gram-negative bacteria are isolated more often in ICP monitor-related infection than in CNS shunt–related infections. The more common bacteria include *Enterobacter* spp., *Klebsiella* spp., *S. marcescens*, and *Acinetobacter* spp. These bacteria are found in water and cause widespread colonization of hospitalized patients. Colonization of the respiratory tract is an especially common occurrence in patients in the intensive care unit and in those with serious underlying disease. Thus, these organisms are occurring increasingly frequently in ICP monitor–related infections. Few infections are caused by *Corynebacterium*, *Propionibacterium*, and fungi.[144]

CLINICAL MANIFESTATIONS

ICP monitor–related infections often occur in patients with an altered sensorium; therefore, the signs and symptoms of meningeal irritation usually are not present. The clinical diagnosis is complicated further by the fact that these patients frequently are critically ill and their signs and symptoms are caused by the underlying condition and are not a result of the ICP monitor–related infection. In addition, they may be receiving multiple antibiotics for other sources of infection (e.g., pneumonia, bacteremia, urinary tract infection), both nosocomial and nonnosocomial. Although fever is the most frequent indication of infection, the presence of infections or inflammatory processes at other body sites causes the predictive value of fever to be low. Therefore, providing close follow-up, having a high index of suspicion, and obtaining frequent cultures of CSF when available (e.g., ventriculostomy device) are recommended.

DIAGNOSIS

The predictive value of the peripheral WBC count and differential count is very low, and the only definitive diagnostic tests are the CSF WBC count and culture.[304] CSF biochemistry (i.e., protein and glucose) has a very low predictive value. Although a low glucose level may be of help in establishing the diagnosis, a normal level does not exclude the possibility of an infection. Calculation of the ratio of leukocytes to erythrocytes in CSF versus their ratio in peripheral blood was suggested as a potential diagnostic tool for early infection.[302] Most patients with ICP monitor–related infection will have an increased CSF WBC count with a predominance of PMNs. In some cases, with low growth of bacteria, no elevation in CSF WBC counts ($<10/mm^3$) was reported. Most of these cases were caused by CoNS. In such cases, if no other indicators of infection (e.g., clinical symptoms, low glucose) are present, the possibility that the positive cultures represent colonization or contamination should be considered before treatment is initiated.

TREATMENT AND PROPHYLAXIS

The same principles used for the management of CNS shunt–related infections can be applied to infections associated with ICP monitoring devices. The use of antibiotics alone without removal of the device may be adequate for the treatment of infections associated with devices placed in the subdural or epidural space, unless an abscess or empyema has formed. In the case of ventriculostomy, the infected device should be removed and appropriate antibiotic therapy instituted. Because gram-negative bacteria are almost as common as are gram-positive bacteria in causing ICP device-related infections, initial antibiotic therapy should include both vancomycin and cefotaxime or ceftazidime if the Gram stain is negative. When the etiologic agent is identified, the specific antibiotic or antibiotics can be chosen from those listed in Table 85–4. The duration of therapy depends on the etiologic agent and the CSF parameters. In patients with positive CSF culture but minimal CSF pleocytosis, if the CSF culture becomes negative immediately after removal of the catheter, therapy should be given for only 3 or 4 days because the cultures before removal of the catheter reflect colonization and not infection. If subsequent cultures are positive, therapy should continue for 10 days of negative cultures.

Removal of the ICP monitor (if longer than 5 days) and reinsertion at an alternate site was recommended as a prophylactic approach to reduce the infection rate. This recommendation is controversial, and several studies showed that routine changes of the ICP monitor did not reduce the risk for development of infection.[223,424]

Many physicians use prophylactic antibiotics for the duration of ICP monitoring in the hope of preventing infection. Unfortunately, the few studies that have evaluated the utility of antimicrobial agents in preventing such infections did not find any efficacy.[24,320] The optimal duration of antibiotic prophylaxis is controversial. Jacobs and Westerband[177] showed that patients who received prophylaxis had statistically significantly higher rates of septic morbidity and pneumonia. Yet a decreased rate of ICP monitor infection was reported in patients who received prophylaxis for the duration of the ICP monitoring.[309] Most studies found that the use of prophylaxis has no effect on infection rates. In addition, broad-spectrum antibiotic prophylaxis was associated with shifting to resistant gram-negative pathogens.[240]

INTRATHECAL PUMP INFUSION DEVICES

The intrathecal pump infusion system currently is used to infuse morphine to treat refractory pain,[285] to deliver baclofen (a γ-aminobutyric acid agonist), or to treat spasticity of spinal or cerebral origin.[4,16,297] In addition, patients with generalized dystonia also benefited from intrathecal baclofen.[5] More than 50,000 patients are being treated with implanted intrathecal pumps for pain or spasticity. Several pump systems are used. The simpler systems consist of an externalized catheter system similar to the Hickman catheter[89] or a subcutaneous reservoir system similar to an intravascular implanted port.[43] The more sophisticated systems include a programmable pump that usually is implanted in an

abdominal subcutaneous pocket and connected via a subcutaneous catheter to the subarachnoid space.[27,43] An optional sideport in some of these implanted pumps allows aspiration of CSF from the subarachnoid space.

Complications of the intrathecal pump system are relatively rare events[129,391] and include mechanical problems with the catheter, such as breaking, kinking, dislodging, or leaking.[378] Skin necrosis also has been reported.[117,378] Complications (especially wound complications such as CSF fistula, dehiscence, granuloma formation, and infection) occur more frequently in children than in adults, probably because of their decreased muscle and subcutaneous tissue.[391] The pump itself is very durable and only very rarely causes a mechanical problem. Overdose that may result in coma, respiratory depression, apnea, cardiac conduction abnormalities, hypotension or hypertension, and abnormalities of the pupils was reported as well.[299] Intrathecal baclofen can induce both recurrent and new-onset seizures.[16,203] In addition, acute withdrawal syndrome was reported when treatment was stopped.[353] Infectious complications included suppuration at the exit site,[48,92,161,308] along the subcutaneous catheter (tunnel infection),[27,48,92,161] or around the pump.[27,48,57] Furthermore, more severe infections such as epidural abscess and meningitis also were reported.[29,48,75,92,274,335,430]

EPIDEMIOLOGY

The infection rate of intrathecal pump infusion devices ranges from 0 to 27 percent (median, 4%). Of note, in general the infection rate was lower in studies that included a larger number of patients. A more accurate description of the incidence would be the infection rate per 1000 catheter-days, which ranged from 0 to 2.5. The infection rate was higher in patients with an externalized catheter (0.65 per 1000 catheter-days) than in those who had an implanted system (0.48 per 1000 catheter-days). Most of the infections occurred at the exit site, along the catheter, or in the subcutaneous pocket of the pump, but the infection in as many as 16 percent of cases involved the epidural space, the meninges, or both. The incidence of infections can be reduced by using the subfascial pump implementation technique and perioperative antibiotic prophylaxis.[267]

Infections occur more often within 2 to 4 weeks after insertion of the catheter, which suggests that the initial surgery to place the intrathecal pump system may be a risk factor. Byers and coworkers[48] found that the only risk factor associated with infection during this surgery was a prolonged duration of the procedure (i.e., >100 minutes). Multiple other factors, such as the patient's age, underlying diseases, immune deficiency, other concurrent infections (e.g., pneumonia), previous intrathecal catheter, number of pump refills, intraspinal anesthetics, surgeon's or anesthesiologist's experience with the procedure, and operative complications (e.g., operative loss of blood), were not associated significantly with an increased risk for the acquisition of infection.[48] Very thin or malnourished patients may be prone to wound dehiscence, which may increase their vulnerability to infection. In addition, patients with narrowed intervertebral spaces tended to have operations of longer duration, which puts them at risk for the development of infection.

ETIOLOGY

The most common bacteria causing intrathecal device–related infection are from the skin flora. CoNS and *S. aureus* lead the list, but *Streptococcus mitis*,[48,279] *Streptococcus* group G,[48] *Corynebacterium striatum*,[48] and *Enterococcus faecalis*[115] infections are observed as well. In addition, several studies have reported gram-negative bacteria as the cause of the pump-related infection. Such bacteria include *P. aeruginosa*,[48,115] *Pseudomonas paucimobilis*,[297] *E. coli*,[92] and *Klebsiella pneumoniae*.[297] One case each of *C. albicans*,[92] *Mycobacterium* spp.,[92] and *Acinetobacter baumanii*[387] also has been reported. A rare case of baclofen vial contamination with the fungus *Wangiella* that caused infection was reported as well.[249,297]

CLINICAL MANIFESTATIONS

Clinical findings depend on the site of the infection. Patients with more severe infections (i.e., epidural abscess, meningitis) may have symptoms of these infections (e.g., fever, irritability, headache, nerve root pain, meningeal signs). In some patients, pain experienced during the injection may be the clue, but in many patients, a high level of awareness for possible infection is important because they will have nonspecific signs and symptoms.

Exit-site and superficial catheter-related infections usually cause local inflammation (e.g., swelling, redness), tenderness, and drainage. The more serious deep-tract (tunnel) infections are more difficult to assess. Visible inflammation along the tract or a soft fluctuant fluid collection around it may be the only clue. Fever is not always present.

DIAGNOSIS

Performing a complete physical and neurologic examination may be helpful in some cases. A routine CBC is relatively unhelpful because leukocytosis is not always present. MRI may be useful for diagnosing an epidural abscess and evaluating its extent. The only definitive diagnostic tests are Gram stain and culture of exudate/drainage from the exit site (if available) or an aspirate of the fluid collection along the catheter tract if tunnel infection is suspected or CSF for suspected meningitis. Irrigation of the epidural space with 1 or 2 mL of saline after removal of the pump filter (which should be sent for culture) may help in diagnosing an epidural abscess or empyema.

TREATMENT AND PROPHYLAXIS

If infection has occurred only at the exit site and the superficial catheter tract, local drainage with aggressive cleansing (i.e., topical antibiotics and antiseptics) but without removal of the catheter was suggested.[92] Unfortunately, more severe infections developed (e.g., deep-tunnel and epidural abscess) in some of these patients. In addition, failure of "catheter-sparing" treatment also was reported.[161,308] Therefore, removal of the catheter should be considered even for mild infections if they do not respond promptly to therapy. In patients with pocket, epidural, and meningeal infections, the intrathecal pump system should be removed as soon as possible and systemic antibiotic therapy initiated (for selection of drugs and doses, see Table 85–4). With the increasing rate of multidrug-resistant staphylococci and the low CSF levels of vancomycin (only 20% to 30% of serum levels), linezolid should be considered as an alternative. Linezolid has very good CSF penetration (60-70% of the serum level) and was effective in treating severe CNS infections,[331] including intrathecal infusion pump infection.[188] Although a few studies have reported clinical improvement (and sometimes cure) of tunnel infection or meningitis with the administration of intravenous antibiotics and retention of the catheter, in many cases, the infection recurred when the treatment was stopped.[48,92] A few reports suggest successful treatment of severe pump infections (including meningitis) without removal of the pump system.[29,43,115,308,335,391,430] In some of these cases, the pathogen was considered to be of low virulence (e.g., CoNS) or colonization. In most of these cases, however, instillation of antibiotics into the

reservoir with cautious flushing of the catheter was combined with systemic antibiotics. In one protocol, "intrapump therapy is started by filling the pump to the total reservoir volume with a 5-mg vancomycin/185-µg baclofen/ml solution and programming the pump to deliver an initial volume of 1 mL per day and oral baclofen therapy is initiated to prevent withdrawal. Intravenous vancomycin therapy is used concomitantly and serum trough levels are drawn to optimize the dose and to avoid toxicity."[391] The main reason for the intrathecal administration of antibiotics is to increase the level of drug in CSF. Before antibiotics are added to the baclofen infusion, their compatibility with baclofen should be verified or tested (vancomycin is compatible and can be used safely according to Zed and associates[430]).

No guidelines exist on how to treat severe infections of intrathecal pump systems, but in most cases, removal of the pump system while systemic antibiotics are given and then reimplantation appear to be the treatment of choice. In rare cases in which removal of the pump will be detrimental to the patient, a trial of intrathecal administration of antibiotics may be justified, with close observation to ensure that the patient is not getting worse.

Although many physicians use perioperative (or longer) antibiotics for prophylaxis, their efficacy in preventing infection has not been proved. Therefore, prophylactic antibiotics should not be used or, if given, should not be administered for longer than 24 to 36 hours after the operation.

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CHAPTER 86

Infections Related to Craniofacial Surgical Procedures

Marc A. Mazade

Advances in plastic and reconstructive surgery have created the opportunity for surgeons to offer patients with complications of facial neoplasia, craniosynostosis, fibrous dysplasia, Crouzon syndrome, Apert syndrome, Treacher Collins syndrome, craniofacial clefts, and other anomalies relief from associated mechanical complications and restoration of a more normal cosmetic appearance. Operative procedures to address these problems involve cranial vault remodeling or reconstruction (or both) or advancement of the midface and maxillary block and, occasionally, correction of malocclusion by repositioning of the mandible. The overall incidence of infection associated with these procedures was 14.7 percent in one study,[10] but it varies greatly and may range from 3 to 45 percent, depending on the number of procedures attempted during a single anesthetic regimen, the duration of surgery, and the structures involved. A review published in 2005 demonstrated a rate of 3.2 infections per 100 craniofacial surgical procedures and found that surgical duration longer than 426 minutes, closure of skin under tension, use of bovine pericardium, and the presence of more than four surgeons in the operating suite were common risks for development of infection.[24] Many patients suffering infection were categorized as having complicated diagnoses.

PROCEDURES AND OSTEOTOMIES

Some familiarity with a few of the more common procedures in craniofacial surgery, beyond basic craniotomy and cranial vault remodeling, is necessary to understand the pathogenesis of associated infectious complications. Although every patient has a unique set of problems, several corrective procedures are used frequently and then are modified as specifically needed for each circumstance.

In young children with midfacial retrusion, frontofacial monoblock advancement may be performed.[17] This procedure involves detachment and advancement of the entire facial bony mask, excluding the mandible. Exposure for this procedure commonly requires a large frontal craniotomy, the bone from which may be shaped to complete the repair or used as needed to form a suitable foundation to which the advanced block can be secured. Retraction of the frontal lobes is necessary to provide access to the roof of the orbits, and much of the procedure is performed intracranially. The boundaries of the advancement block are formed by osteotomies performed horizontally along the lower portion of the frontal bone and posteriorly along the roof of the orbits, vertically along the lateral and medial inferior walls and horizontally along the inferior walls of the orbits, and vertically through the zygomatic arches, with subsequent dissection and pterygomaxillary disjunction. Finally, a frontoethmoidal osteotomy divides the posterior portion of the nasal septum to free the monoblock so that it can be advanced and secured in place, perhaps by wiring it anteriorly to a slightly fore-tilted strip of frontal bone and stabilizing it laterally with wired-in strips of calvarial bone grafts to bridge gaps in the zygomatic arches. Bone blocks in the pterygomaxillary area help to hold the maxillary portion forward. The temporary dead space created in the anterior cranial fossa after this procedure eventually is obliterated in a growing, developing child, although its persistence is problematic in some patients.[23] This intracranial procedure is associated with an inherent risk of developing meningitis, which is associated less frequently with the extracranial procedures described later, although meningitis still may occur.[21]

A subcranial Le Fort III osteotomy sometimes is used for the treatment of midface retrusion in children.[11,17] Exposure is provided through a coronal incision, but craniotomy is not necessary. Neither the orbital roof nor any portion of the frontal bone is advanced with this procedure. Osteotomies are required to free part of the medial, lateral, and inferior walls of the orbit and the nasal bridge, and the remainder of the block is freed by pterygopalatine disjunction and osteotomies of the zygomatic arch and posterior nasal septum. The entire inferior orbital and nasomaxillary unit is brought forward, and interposition grafts, hydroxyapatite, microplates, and wires secure the block in the advanced position. An intraoral stab incision may be required for pterygopalatine disjunction. As in the frontofacial advancement, split calvarial bone grafts bridge the space in the zygomatic arches.

If further advancement of the maxilla is desired, a subsequent Le Fort I osteotomy, which is an isolated maxillary advancement procedure that changes the position of the upper teeth, is performed.[11,14,17] This entirely intraoral procedure involves making various buccal sulcus and upper vestibular incisions. The palate and maxillary arch are freed for advancement by transverse osteotomies at the level of the nasal floor, through the nasal septum, and then posteriorly.

Another procedure that may be performed in some cases of Treacher Collins syndrome is the integrale (simultaneous midfacial and mandibular osteotomies).[17] In this complicated procedure requiring a tracheostomy, the midface osteotomies exclude the temporal, lateral walls of the orbits from the advanced block. In addition, C-shaped or inverted V-shaped osteotomies through the mandibular rami with placement of the interposition bone grafts and wiring provide advancement of the mandible. Split calvarial bone grafts subsequently reestablish the zygomatic arches. Incidentally, the bone grafts are harvested either by performing a true craniotomy or by removing the outer cortical bone table from the donor site.

More thorough descriptions of these and other operations, such as facial bipartition, are provided in the referenced texts,[11,17] to which the reader is referred for a better grasp of the inherent risks of bacterial contamination from intraoral, sinus, and skin sources that are specific to each craniofacial procedure. Nonetheless, suffice it that the infections encountered usually are caused by bacteria that are resident or pathogenic in the clean-contaminated sites through which incisions and osteotomies are performed and that each additional site that is surgically violated increases the risk of developing an infection.

Many reconstructive materials are used for various procedural applications. Some materials include bone autograft and solvent-treated allograft, titanium plates, and alloplasts such as polymethylmethacrylate (PMMA) and hydroxyapatite cements, some of which readily accept bone ingrowth. In an effort to limit postoperative infection, antibiotics such as tobramycin sometimes are mixed into hydroxyapatite cements, which are released into the surrounding field within the first 24 hours of placement.[15] Efficacy data regarding prevention of infection using this practice are not widely available.

EPIDEMIOLOGY

Infections that may be encountered include cellulitis and dehiscence of the wound, infection of subgaleal fluid, osteomyelitis, focal soft tissue abscesses, epidural abscesses,[23,24] and septicemia. In many instances, infection permeates the entire operative field and encompasses any number of these specific entities.[10] Donor bone graft sites may be involved in some instances. Bacteria may infect the wound or soft tissue area, may spread by contiguous extension through cortical bone during long periods of contact, or may extend through osteotomies that have disrupted the integrity of the periosteum and cortical bone barriers. Additionally, surgical alteration of the local blood supply to the cranial bones through disruption of medullary channels and removal of the adherent periosteum, as well as the presence of hardware required to secure the bony structures in their new locations, can render infection difficult to treat. Repeated or prolonged hospitalizations for previous surgical procedures, often for co-morbid conditions such as syndactyly, gastroesophageal reflux, or dysphagia, result in colonization of the skin and sinopulmonary tracts and subsequent postoperative infection with multidrug-resistant bacteria. Attempts to reduce the incidence of infection have made the use of parenteral prophylactic perioperative antibiotic regimens for 2 to 5 days commonplace.

The development of meningitis always is a concern when craniofacial procedures are performed. The presence of a cerebrospinal fluid leak is a predisposing factor, and meningitis may be manifested years after the procedure in the case of a leak.[21] However, the dura provides a significant barrier to infection when painstaking neurosurgical technique is applied during repair. In fact, no cases of meningitis were identified from a combined report of complications after 567 procedures spanning 6.5 years at Medical City Dallas Hospital and at the Division of Plastic Surgery at the University of Pennsylvania in Philadelphia, primarily for cranial vault remodeling.[9] Similarly, meningitis was not identified from an earlier report of 170 transcranial operations spanning 10 years at the South Australian Cranio-Facial Unit in which 53 accidental dural tears occurred and 32 planned dural openings were performed.[8] Nonetheless, meningitis has been documented to occur in association with a frontal abscess with osteomyelitis, subgaleal fluid infection, and contamination of cerebrospinal fluid drains.[10]

The Australian group just mentioned documented an average operative time of 10.5 hours for patients in whom a postoperative infection developed, 2.5 hours longer than operations on patients in whom an infection did not develop.[8] However, these observations were confounded by variables such as type of procedure and whether the procedure was a primary or subsequent operation. Longer and more complicated monoblock advancement procedures have been associated with infection rates as high as 45 percent,[10] whereas anterior cranial vault remodeling may be associated with infection rates as low as 2.5 percent.[9] In staged or subsequent procedures, the dura encountered during primary craniofacial procedures is manipulated more easily and has better vascularization than does the scarred dura encountered in more complicated and laborious secondary procedures, which are associated with higher rates of infection.[9] Additionally, craniofacial postoperative infections develop in infants far less frequently than in adults.[8,9] Whether this age difference is a reflection of the types of surgeries performed in infants or other microbial or host factors is not clear. Of the common procedures, infection remains a relatively rare complication of the less complicated Le Fort I maxillary osteotomy. In a large review of outcomes of 1000 such procedures, only 1.1 percent developed abscess or related infection.[12]

As experience in craniofacial surgery has grown, the reduction in the frequency of infection has been attributed to shortened operative time, attempts to avoid entrance into the contaminated sinus cavities (an easier task in young children, in whom the sinuses often are still poorly developed), and mucosal repair at the end of surgery.[22] The mean time to diagnosis of infection is approximately 10 days.[10]

MICROBIOLOGY

Organisms causing infection include flora of the skin such as *Candida albicans*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, beta-hemolytic streptococci, and *Propionibacterium acnes.* Resident flora of the oropharynx such as *Bacteroides* spp., *Corynebacterium* spp., various alpha-hemolytic streptococci, *Streptococcus pneumoniae*, *Morganella morganii*, *Eikenella corrodens*, *Haemophilus influenzae*, and *Haemophilus parainfluenzae* can be causative, as can nosocomial gram-negative bacilli such as *Pseudomonas aeruginosa*, *Escherichia coli*, *Klebsiella* spp., and *Acinetobacter calcoaceticus.*[6,9,24] Procedures that disrupt the oropharynx and sinus cavities are more apt to be complicated by polymicrobial infections. Procedures that do not violate these spaces are more inclined to be complicated solely by infection with skin flora. The frequency of infection with *Candida* spp. may rival the frequency of infection with more common bacteria.[9,23,24]

PREOPERATIVE PREPARATION, INTRAOPERATIVE IRRIGATION, AND PERIOPERATIVE ANTIBIOTIC THERAPY

Because wounds created during craniofacial surgery fall between the clean-contaminated category and the contaminated category,[6,7] giving attention to the treatment of dental caries, periodontal disease, and acute sinusitis is prudent before embarking on procedures involving intraoral incisions and trans-sinus osteotomies.[21] Protocols for the administration of perioperative antibiotics and for preoperative preparation of the oral cavity have been developed. Many of these protocols involve irrigation of the oral cavity with povidone-iodine (Betadine) solutions. Nonetheless, the sinus cavities do not lend themselves to preoperative antiseptic washing. Some surgeons make use of intraoperative antibiotic-containing irrigants to help reduce the incidence of infection. These irrigants may be flushed over the intact cranium after subperiosteal exposure and again before skin closure. Other surgeons prefer to use a solution of one part povidone-iodine to four parts saline for intraoperative irrigation.[21] No strong evidence supports any one approach over others for reducing infection.[10]

The choice and duration of perioperative antibiotics vary among surgical centers and their surgical teams. Antibiotic regimens may consist of the following: penicillin; ampicillin-sulbactam; a first-, second-, or third-generation cephalosporin; clindamycin; or any two of these drugs in combination.[6,7,9,10,12] Erythromycin is used in penicillin-allergic patients in some centers.[1]

Continuation of antibiotics may be necessary for 0 to 5 days postoperatively. The optimal duration of treatment is not clear. Higher infection rates occurred in some centers that tried restricting antibiotics to intraoperative use only.[1] Reports from one orthognathic surgical center showed a 10-fold reduction in infection rates with a 5-day antibiotic regimen in comparison with a single-day regimen.[2] Furthermore, isolation of a few infecting organisms that were susceptible in vitro to an antibiotic given 6 hours preoperatively until 48 hours postoperatively suggested that longer durations of treatment with postoperative antibiotics are needed for patients with more heavily contaminated wounds.[6] Some advocates of a shorter duration of treatment with perioperative antibiotics suggested that longer administration of peri-

operative antibiotics contributes to infection with gram-negative organisms or may result in infection with more resistant gram-negative bacilli such as *P. aeruginosa* or other pathogens such as *C. albicans*.[7-10]

Nonetheless, although the use of antibiotics indeed selects for these organisms, many single-organism infections occurring after initiation of antibiotics would have been polymicrobial were it not for intraoperative and immediate postoperative treatment. From an infectious disease perspective, continuation of antibiotics beyond the intraoperative period is similar to treatment of an open fracture and not very much like antibiotic prophylaxis in the traditional sense. Perhaps better stated is that the use of intraoperative antibiotics and immediate postoperative treatment may kill bacteria that have contaminated operative sites, including surgically fractured bone.

POSTOPERATIVE FEVER

Pyrexia is thought to be a normal physiologic response to craniofacial surgery. In a study of 136 transcranial surgical procedures performed for nonsyndromic craniosynostosis, postoperative temperatures of 38° C or higher were encountered in 76 percent of subjects, and hyperpyrexia exceeding 39° C was encountered in 11 percent. Temperature elevations usually are encountered in the first 48 hours but occasionally occur up to 5 days postoperatively.[18] Close clinical evaluation is warranted in all cases of pyrexia.

EVALUATION

Signs of postoperative craniofacial surgical infection include the following: fever (although not in all cases)[18]; the rapid development of unilateral local soft tissue warmth, tenderness, and erythema; and rapid recurrence of swelling.[5] Reasons for the delay in establishing the diagnosis and for providing definitive treatment of some infections are multifactorial. Postoperative swelling may persist for some time, often improving and worsening in dependent areas according to the patient's sleeping or resting position. Periorbital tissues are particularly prone to fluctuations in swelling. Violaceous discoloration and bruising of the overlying skin may complicate assessment further. In addition, the overall facial appearance of the child may be so altered by the surgical intervention that parents may not recognize subtle signs of infection immediately. In some instances, large distances may separate the child from the craniofacial surgery referral center after discharge from the hospital, and the surgeon must rely on verbal descriptions of the child's postoperative appearance from concerned caregivers. Therefore, patients who live far from the referral center should be educated that prompt local medical evaluation along with a phone call to the craniofacial surgeon at the referral center can assist in early diagnosis, determination of bacteriology by needle aspiration, and initiation of preliminary treatment during travel back to the referral center. Prescription of oral antibiotics in the hope of preserving the tenuous bone grafts without aspiration may render the interpretation of subsequent surgical culture results difficult.

Evaluation of extraocular movement may be limited by the age of the child, by postoperative swelling, and, frequently, by the limited capacity of the patient to comply interactively with the examination. Few operations, however, limit the ability to assess the presence of meningismus. Some patients may exhibit lassitude, lethargy, or increased irritability, subtle signs in children who have limited capacities to communicate.

The wound, often a coronal incision, should be examined closely. Purulent or seropurulent wound drainage or persistent serous wound drainage usually is indicative of underlying soft tissue and possibly bone infection, although infection may not be limited to the structures located directly beneath an area of wound dehiscence.[5] Drainage at one location along a wound may be the result of inflammatory fluids that originate elsewhere and are following a hydrodynamic gravity flow pattern or an established route to a low-pressure efflux portal at a nonhealing area of the wound, especially when the infection has become well established. Similarly, fluid collections in suborbital areas of the face such as the malar or submalar areas may suggest a local abscess when, in fact, the primary site of infection is cranial and the collection is only a dependent pool.

Laboratory abnormalities may be subtle. Leukocytosis may be present. The erythrocyte sedimentation rate has been shown to peak approximately 5 days after major orthopedic surgery and to decline slowly and irregularly for a period of 3 to 9 weeks, but it provides little diagnostic value initially.[3,13,19] C-reactive protein (CRP) levels peak within 2 to 3 days but usually normalize within 21 postoperative days.[3,13,16,19] Because an abrupt increase in CRP may be indicative of infection, some orthopedic centers monitor CRP levels to assist in early detection of infection after elective procedures such as total-joint replacement and spinal surgery.[13,19] Similarly, monitoring serial CRP levels is helpful when trying to discriminate between infection and shifting postoperative dependent swelling and in assessing the response to therapy in a craniofacial surgical patient.

Needle aspiration of underlying fluid collections is the best primary means of diagnosing infection, especially if significant residual postoperative swelling has rendered clinical assessment of a particular area difficult. In most instances, needle aspiration cultures are superior to surface cultures of wound drainage, which often yield coagulase-negative staphylococci of uncertain significance. However, cultures of wound drainage sometimes grow obvious pathogens and may be helpful.

Diagnostic imaging such as computed tomography with contrast or magnetic resonance imaging may identify occult fluid collections, but diagnosing bone infection, especially in the flat bones of the cranium, is difficult with these modalities. Not infrequently, focal enhancement of the meninges in regions where the cranium has been manipulated is noted on postoperative imaging. Corresponding postoperative cerebral gliosis also can be difficult to differentiate from cerebritis developing near an area of infection above the dura. Serial weekly or biweekly imaging can provide a means of performing ongoing evaluation of these abnormalities inasmuch as neurologic manifestations of cerebritis in the frontal lobes may not be elucidated easily. Surgical exploration of suspected areas of infection remains the most helpful source of diagnostic information regarding infection.

TREATMENT

Management of infections complicating craniofacial procedures is primarily surgical. Open débridement, inspection and scraping of contiguous bone, and copious pressurized saline irrigation are essential. The value of adding antibiotics to the irrigant is questionable. Some physicians argue that irrigation with povidone-iodine may devitalize tissues that participate in the healing process and should not be used, whereas other physicians avoid using povidone-iodine irrigation because of concern about systemic iodine absorption.[9] Removal of all hardware provides the best chance for eradicating the infection, but occasionally, as in the case of procedures that involve maxillary distraction devices, removing all the hardware initially is impractical. Furthermore, distraction pins may dislodge as the integrity of the bone is compromised by infection.[20]

Placement of several drains ensures an opportunity for drainage of what otherwise would remain sequestered focal soft tissue fluid collections. Continuous subgaleal flow-through irrigation

with saline at a rate of 15 to 30 mL/hr (with or without antibiotic additives) is an adjunctive measure instituted by some surgeons; other surgeons question the advisability of this approach.[9] Removal of devitalized bone and of any bone grafts in the infected surgical bed is an unpopular but necessary part of treatment. Failure to do so may result in multifocal osteomyelitis, possible meningitis or cerebritis, and persistence of chronic infection with potentially multidrug-resistant organisms.

Reluctance by the surgical team to excise infected bone aggressively and widely and to remove devitalized bone is understandable because the initial surgical procedure itself involves extensive planning, operative time, and anesthetic risk. In addition, interposition grafts may provide architectural platforms for the advanced structures, and successful treatment of these infections was reported in some surgical centers in which the initial bony débridement was limited to areas of visibly apparent osteitis.[9]

With regard to post-craniotomy wound infections, traditional dogma suggested operative débridement and complete removal of devitalized bone flaps followed by delayed cranioplasty.[4] However, some patient groups may fare better with attempted preservation of bone flap with minor surgical débridement and systemic antibiotic therapy. Within a small group of five patients who had undergone craniotomy without a history of previous craniotomy, radiation therapy, or skull-base surgery, operative débridement accompanied by culture and susceptibility data-driven systemic antibiotic therapy successfully treated infection with a mean of 35 ± 20 months of follow-up. Several other patients required second-look operations with more débridement, but they did not require removal of the bone flap and were cured. In the same study, the two other subjects had undergone more extensive craniofacial surgery and had recurrent infection requiring removal of the bone flap at 2 months after presentation in one patient and at 29 months in the other one, respectively; this finding bears out the concern that contamination of the bone flap can result in persistent, long-standing infection.[4]

As an advisor, the infectious disease consultant should point out evidence of persistent infection, suggest the possibility of deeper or more serious infection when concern arises, alert the surgical team when a point of failure of medical therapy has been reached, and emphasize that antimicrobial therapy is only an adjuvant to bony débridement and copious intraoperative irrigation, which may improve the outcome and limit the spread of infection. Recurrent wound dehiscence and continued wound drainage should prompt thorough exploration of the soft tissues and bone for evidence of osteomyelitis or retained hardware, including wires. Advanced infection in devitalized bone cannot be treated effectively in situ, and often entire bone plates must be removed, with cranioplasty performed months later.

For infections that complicate procedures not involving the oropharynx or sinus cavities, an antistaphylococcal antibiotic such as vancomycin, cefazolin, or nafcillin may be an appropriate empiric antibiotic choice pending the results of intraoperative drainage and débridement cultures. A third-generation cephalosporin with or without an aminoglycoside may be considered if the risk of developing an infection with a nosocomial pathogen is high because of prolonged hospitalization or persistent tracheal colonization with gram-negative bacilli such as *Pseudomonas*[8] or if infection of the central nervous system threatens. Infections complicating procedures involving the oropharynx or sinuses often are caused by organisms resistant to the perioperative prophylactic antibiotic chosen.[6] Thus, it may be reasonable to consider antibiotic therapy with clindamycin or metronidazole when a cephalosporin has been used or treatment with a second- or third-generation cephalosporin or ampicillin-sulbactam when clindamycin has been used prophylactically. The duration of antibiotic therapy must be individualized to each case. A period of 6 weeks of antibiotic therapy has been suggested for patients with suspected osteomyelitis.[21]

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CHAPTER 87

Infections in Burn Patients

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According to the American Burn Association, approximately 500,000 persons are treated for burns every year in the United States; of these, approximately 40,000 are hospitalized and approximately 4000 die from the burn injury.[4] Improvement in the rate of burn-associated mortality is a direct result of advancement in burn care, comprising developments in fluid resuscitation, wound care, early excision and grafting, nutritional support, infection control, and antimicrobial therapy. The mortality rates and lengths of stay of burned children have been reduced greatly in the past several decades. In the 1960s, the likelihood of survival was only 50 percent for pediatric patients with burns covering 35 to 44 percent of the total body surface area (TBSA), and few children with burns covering more than 45 percent of TBSA survived. The average length of stay was 103 days. In 2008, the LA_{50} (lethal burn size for 50% of patients) for children exceeded 95 percent of TBSA, and the average length of hospital stay for most serious burn injuries can be expected to be only 0.5 days per percent TBSA that is burned. Since 1998, the mortality rate at the Shriners Burns Hospital for Children (Galveston, Texas) has ranged from 1 to 3 percent.

BURN WOUND

1. **Burn wound depth:** Burn wounds are categorized by their depth (Fig. 87–1).[42]

a. *First-degree burns* consist of epidermal damage only. These wounds are painful and erythematous resulting from local vasodilation. They heal spontaneously, usually without forming scars, within 7 days.

b. *Second-degree burns* are injuries with partial thickness and are further categorized as *superficial* or *deep*. The epidermis and superficial portions of the dermis are injured in superficial second-degree burns. These wounds are painful and often result in formation of blisters. Healing occurs via epithelial migration from the wound edges, hair follicles, and sebaceous glands. Relatively little scarring occurs, and reepithelization occurs within 2 weeks. Deep second-degree burns are much more serious. The majority of the dermis is destroyed, leaving the bases of the epidermal appendages spared. The nerve endings also are destroyed, rendering the wound insensate. Blisters usually are not present owing to the thicker formation of eschar. These wounds are treated as full-thickness injuries. Reepithelialization is tenuous and slow. The protracted inflammatory phase often results in excessive deposition of collagen and extensive scarring.

c. *Third-degree burns* are full-thickness injuries to the skin. Healing occurs by contraction and reepithelialization from the edges of the wound. As with deep second-degree burns, these wounds are insensate and without blistering. Infants and young children have a much thinner dermal layer to their skin, resulting in increased propensity for deeper burn injury. Treatment for third-degree burns is with excision and skin grafting.

d. *Fourth-degree burns* extend into the deep tissue, which includes muscle, bone, and viscera. Treatment is débridement and possible amputation. Closure of these wounds may vary from primary closure post amputation to skin grafting and possibly flap reconstruction.

2. **Cytologic findings:** The effects of extreme heat on the skin lead to cellular and subcellular impairment. The determining factors of how severe a burn will be are the temperature, the length of exposure, and the actual burning agent. Moritz and Henrique[83] showed that the skin is able to withstand temperatures up to 40°C (104°F) for relatively long periods of time before an injury becomes apparent.[83] Increase in temperature leads to cell membrane dysfunction as ion channels are disrupted, resulting in sodium and water intake. As temperatures exceed 45°C, protein denaturation supersedes the cell's reparative capabilities and oxygen radicals are liberated. Plasma membrane necrosis has been observed in cells exposed to 45°C for 1 hour. Other cytologic findings in thermal injury include the redistribution of solid and fluid components of the cell nuclei. Imbibition of fluid results in nuclear swelling, rupture of membranes, and pyknosis. As denaturation proceeds, vital cellular metabolic processes are injured. If enzyme activity is decreased to less than 50 percent of its normal level, cell death occurs. In lesser degrees of enzyme impairment, cell recovery may be possible.

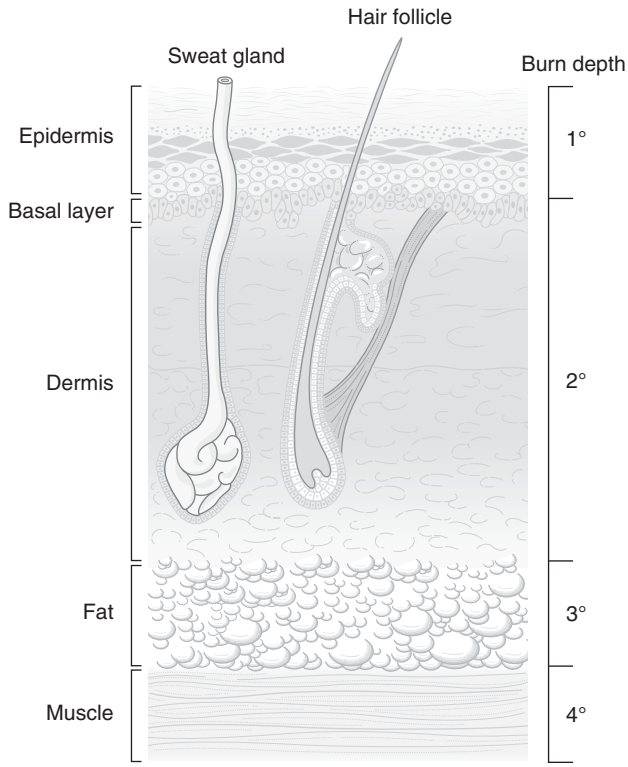

Figure 87–1 The burn wound depth. (See companion Expert Consult web site for color version.) *(With permission from Greenhalgh, D.: Wound healing.* In *Herndon, D. N. [ed.]: Total Burn Care. Philadelphia, WB Saunders, 2007, p. 579 [Fig. 46.1]).*

3. **Local tissue changes:** Local burn injury is classically described by Jackson[54] in three concentric zones. As temperature increases, protein denaturation results in coagulation. The protein architecture is destroyed and new aberrant macromolecules are formed. The central area of a burn wound is that which is in direct contact with the source of heat. Cell necrosis is com-

plete and is called the *zone of coagulation*. Cellular recovery is impossible, and the severity of injury decreases from the surface to the deeper levels. This zone is called the *burn eschar*. At the peripheral margins of the zone of coagulation, a less injured zone is present. The cells in this *zone of stasis* show direct injury from the heat, but the damage is not lethal. However, blood flow becomes progressively impaired to this area. Ischemia to the already compromised cells may lead to necrosis and conversion to dead eschar. Circulatory impairment occurs via adherence of neutrophils to the vessel wall, deposition of fibrin, formation of platelet microthrombus, vasoconstriction, and endothelial swelling. Heat-compromised erythrocytes lose their ability to deform, and their passage through microvessels is impeded. The circulatory embarrassment may be delayed for up to 24 hours, and the ischemia may progress for up to 48 hours after the burn has occurred. If stasis conditions are minimal, the injury may be halted and cell recovery may occur within 1 week. However, this tissue is fragile, and further insults such as infection, hypovolemia, pressure, and over-resuscitation can lead to further necrosis. Finally, the *zone of hyperemia* lies peripheral to the zone of stasis. This zone sustains minimal injury and often recovers within 7 to 10 days. Notable vasodilation is caused by potent vasoactive mediators secondary to the inflammatory response. Complete recovery is expected in this zone barring further trauma or infection.

4. **Burn inflammation:** Many of the above processes are either part of or result from the inflammatory process. Cellular infiltration, initiated by local inflammatory mediators such as prostanoids and leukotrienes, as well as proinflammatory cytokines from the burn wound, begins with the arrival of neutrophils at 4 to 5 days postburn, followed by macrophages.[106] The neutrophils further mediate damage by releasing oxygen free radicals.[36] Reestablishment of blood flow in the zone of stasis is yet another setting wherein oxygen free radicals are produced, leading to further injury. This phenomenon of ischemia-reperfusion injury occurs as oxygen is restored to the tissues.[59] Inflammation becomes prominent at 7 to 10 days. Consequently, blood flow is maximal at this stage, creating a troublesome and hazardous setting for surgical excision of the eschar. Along with local inflammatory responses, several systemic responses occur with burns of more than 15 percent of the TBSA.

5. **Inhalation injury:** Burn victims, especially those trapped in enclosed areas, injure the respiratory tract upon inhalation of toxic gases from surrounding burning materials. An actual thermal airway injury is quite rare. The upper airway is rather effective in cooling and warming inspired air. Also, air has a very low heat capacity. In order to have direct injury to the airway, the flames must come into direct contact with them. Injury to the oropharynx after inhalation of toxic gases resembles thermal injury elsewhere in the body.[1] Protein denaturation, release of inflammatory mediators, and increased cellular and microvascular permeability all occur, leading to airway edema and consequent obstruction of the airway.

The chemical injury from inhalation of toxic gases can damage the tracheobronchial tree. First, separation of ciliated epithelial cells from the basement membrane occurs.[11] Next, the circulation of blood to the lung, as well as to the bronchial tree, is increased due to vasodilation. Shortly thereafter, edema is evident. The inflammatory phase is followed by an exudative phase.[52] Furthermore, the protein component of this fluid is composed of lung lymph and induces bronchoconstriction. As postburn time increases, fibrin casts are formed from the exudates, resulting in obstruction of the airway. As the epithelium sloughs and formation of fibrin casts increases, susceptibility to infection also increases. Pneumonia leading to sepsis and death are well-known sequelae at this stage. Finally, formation of pseudomembranes proceeds and then squamous metaplasia follows.[92] Healing may take weeks to initiate, and permanent damage to the airway (e.g., stenosis and formation of tracheal granulomas) may occur.

INFLAMMATORY AND IMMUNE RESPONSES IN BURNS

Intact human skin is vital for preservation of the host's protection against infection. A combination of impaired local and systemic host defenses and loss of the skin barrier are major factors responsible for the increased susceptibility to infections in patients with burns. The major elements initially contributing to the inflammatory response that occurs after burns are incurred include the plasma proteins, mast cells, tissue macrophages, and systemically recruited neutrophils and monocytes.

Alterations in the host defenses include induction of local and systemic cytokine synthesis, decreased immunoglobulin levels, changes in the concentration and activity of both the classical and alternative complement pathways, reduced levels of circulating plasma fibronectin, depressed serum opsonic activity, and impairment of the macrophages, lymphocytes, neutrophils, and the reticuloendothelial system. However, many mechanisms of immune alterations remain unknown; for example, an association of the volume of blood transfusions with increased mortality rates and infectious episodes in patients with major burns was observed in two reports.[56,92] The immunologic status of the burned patient has a measurable impact on survival, death, and major morbidity.

1. The cytokine response: After burn injury occurs, numerous cytokines are induced rapidly. Many cytokines correlate with the severity of the burn injury and the prognosis. Recent studies at our center have shown a specific pattern of systemic cytokine responses in children with thermal injury. Compared with unburned healthy children, children with burns covering more than 40 percent of TBSA had significant increases in serum levels of 15 cytokines and immunoregulatory molecules during the first week after incurring the thermal injury: interleukin (IL)-1β, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12 p70, IL-13, IL-17, interferon gamma, monocyte chemoattractant protein-1 (MCP-1), macrophage inflammatory protein (MIP)-1β, and granulocyte colony-stimulating factor (G-CSF).[31] Granulocyte-macrophage colony-stimulating factor (GM-CSF) was significantly increased during the second week after burn occurred. Within 5 weeks, the serum concentrations of most cytokines decreased, approaching normal levels. In another study of children, serum IL-8, tumor necrosis factor (TNF), IL-6, IL-12p70, MCP-1, and GM-CSF were significantly increased in those with large burns.[56] In general, the notion was that the smaller the burn size, the lower the cytokine concentration.

In children with inhalation injury, the serum cytokine studies at enrollment showed significant reduction in level of IL-7 and elevation of IL-12p70, as compared with children without inhalation injury.[30] However, 5 to 7 days later, the levels were comparable. Host genetic factors also may affect the cytokine response in patients with burns. For example, single nucleotide polymorphisms for TNF-α (308G), Toll-like receptor 4 (+896G), IL-6 (174C), and CD14 (159C) were significantly associated with an increased risk for severe sepsis following burns.[7]

2. Neutrophils: Thermal injury induces neutropenia and myeloid maturation arrest despite elevated G-CSF levels.[108] The degree of neutropenia correlates with the reduction in bone marrow G-CSF receptor expression. The neutrophils of patients with burns also are functionally altered[84]: The expression of the Fc receptor is decreased, and intracellular killing capacity is depressed (this is a differential suppression, more for some organisms than others) and is accompanied by a brief increase in neutrophil respiratory burst response. Failure of initial alkalization

of the phagolysosome and alteration of subsequent kinetics of acidification also occur.[14] This depression of oxygen-independent bactericidal mechanism may impair the capacity of the neutrophil for intracellular killing after a thermal injury occurs. Expression of CD16 (Fc receptor [FcR], Fc immunoglobulin G [IgG] receptor) and CD11 (adhesion molecule) on neutrophils is impaired after a major injury occurs; this reduction appears to be related directly to the appearance of bacteremia or pneumonia.[5] These changes in expression of adhesion molecules, which is closely related to chemotaxis, may play a part in the failure of delivery of neutrophils in adequate numbers to the local site of a burn. Furthermore, a defect is present in actin polymerization in the neutrophils of patients with burns; as a basic mechanism of chemotaxis, it also may contribute to a failure of motility.

Generation of leukotrienes from the neutrophils of severely burned patients also is impaired and appears to be based on the availability, or lack of availability, of the metabolizable substrate-free arachidonic acid.[62] Because leukotriene B also is a potent neutrophil chemotactic agent, this impairment may further contribute to the failure of neutrophil function.

3. Complements: The fluid of the burn blister shows much lower opsonic activity for bacteria such as *Pseudomonas aeruginosa* than the patient's own serum.[90] A mild impairment of production of C3 and release by macrophages of burn patients in vitro also occurs. Systemically, both the classical and the alternative pathways are depleted, but the alternative pathway is more profoundly perturbed. After the development of bacteremia, additional complement activation and depletion occur.[115]

4. Macrophages: Suppression of the ability of the reticuloendothelial system to take up particulate material was among the original observations of burn immunology made in the 1960s. More recent reports, however, have described the demonstration of a differentially increased uptake of colloid in alveolar macrophages compared with other organs, perhaps indicating alveolar macrophage activation. Macrophages and monocytes appear to be activated in a fashion similar to that of lymphocytes after thermal injury occurs. Activation of macrophages, as measured by the serum neopterin level, is increased after thermal injury occurs.[6] This activation is confirmed by increased expression of the monocyte cell surface antigens C3b and iC3b.[82] At the same time, expression of human leukocyte antigen (HLA)-DR, HLA-DQ, and HLA-DP by monocytes is reduced, and these class II antigens are obligatory for many cell-mediated immunologic processes, thereby implying that a possible loss of function of monocytes occurs after thermal injury.[39] Production of C3 by macrophages is suppressed in patients with burns, but the synthetic ability for key cytokines such as IL-6 is increased.[126]

Peripheral blood monocytes are superstimulated to produce large amounts of IL-1, leading to exhaustion of the function on monocytes.[73] Reduced production of IL-1 by monocytes was found in patients with complicated organ injury, multiorgan failure, and systemic infection. Blood monocytes of patients with burns produce significantly more IL-10 at 7 to 10 days after burn injury, which correlates significantly with subsequent septic events.[61]

5. T lymphocytes and cell-mediated immunity: Early studies of T cells in patients with burns showed a variety of changes: impairment in mitogenic and antigenic responsiveness of lymphocytes, suppression of graft-versus-host reactivity related to the size of the burn, suppression of delayed cutaneous sensitivity tests, and diminution in both numbers of peripheral lymphocytes and concentration of thoracic duct lymphocytes. Whether the failure of T cell functions is due to an intracellular defect related to thermal injury, the result of "overuse," or indirectly the result of down-regulation by the cytokine cascade or other products of the inflammatory reaction remains controversial.

Analysis of peripheral blood T cells supports the theory that, rather than an absolute reduction in CD4 and an increase in CD8 cells, a redistribution of lymphocyte traffic may occur.[91] Suppression in the numbers of the total population of lymphocytes is the only consistent overall change. Further, not only does lymphocyte traffic between stores of central lymphocytes and the peripheral blood occur after a thermal injury, but the responsiveness of these populations of lymphocytes also vary according to the site; for example, splenic lymphocytes of experimentally burned animals remain most profoundly depressed in response to antigenic stimulation, compared with the peripheral blood and other organs.[25] In addition, in peripheral blood, the appearance of "activation" antigens on CD4 and CD8 cells (HLA-DR, IL-2 receptor [IL-2R], and transferrin receptor) is depressed significantly as early as 1 day after burn injury occurs.[75]

Addition of recombinant IL-2 does not appear to reverse the suppression of the appearance of surface markers such as IL-2R in burned patients, although it does not improve the response of natural killer (NK) cells to stimulation.[34] In experimental preparations, at least some of the observed T-cell suppression can be alleviated by early removal of the burn wound, thereby creating one further argument for promptly closing the burn wound.[45]

6. B lymphocytes and humoral immunity: The function of B cells after the occurrence of a thermal injury is less well documented than is that of macrophages or T cells. The expression of this major histocompatibility complex is impaired and, therefore, some diminution of B-cell function can be expected as a result of diminished recognition of antigenic presentation.[87] Under the influence of stress-induced corticosteroids, the number of circulating B cells is relatively increased compared with T cells in peripheral blood.[58] Spontaneous cytokine (IL-4 and IL-2)-induced expression of the activation antigen CD23 is reduced significantly during the second to fifth week after burn injury occurs.[103]

If the products of B-cell activation, namely the immunoglobulins, are measured in vivo, the results are somewhat difficult to interpret because of the increased catabolism of protein and the leakage through the burn wound. Briefly, marked diminution of serum IgG concentration, total and all subclasses, is present; these levels return to normal between 10 and 14 days after the burn injury has occurred. Extremely low levels of IgG on admission (300-400 mg/dL) are predictors of a poor prognosis. Levels of IgM and IgA appear to be relatively unaffected. Overall, the defective production of immunoglobulin after a thermal injury occurs appears to be a factor of macrophage/lymphocyte interaction rather than a failure of intrinsic activity by B cells.[29]

BURN WOUND MICROBIOLOGY

A working knowledge of the common flora of burn wounds is essential to appropriately tailor therapy. Pathogens peculiar to thermal injuries are basically no different from the normal flora of the environment. Table 87–1 shows the types of microorganisms found in various body tissues and secretions as either normal flora or as pathogens. However, the organisms that predominate as causative agents of infections of burn wounds in any burn-treatment facility change over the course of time. Gram-positive organisms prevail in the early postburn period and then are replaced by gram-negative bacteria and fungi.[27]

1. **Gram-positive bacteria:** The gram-positive predominance is consistent with the normal inhabitants of the skin prior to the thermal injury. *Staphylococcus* spp., *Micrococcus* spp., *Streptococcus* spp., *Pediococcus* spp., and *Enterococcus* spp. are gram-positive cocci commonly encountered in burn wounds. Erol and associates[27] recently demonstrated that coagulase-negative staphylococci and *Staphylococcus aureus* were the most prevalent isolates in admission cultures, followed by diphtheroids. These organisms can be life-threatening as invasive infections or simply be locally

TABLE 87–1 Tissue Association of Microorganisms Most Commonly Found in Burn Wound Infection*

Organism	Soft Tissue Skin	Upper Respiratory	Lower Respiratory	Endocardial	Gastrointestinal	Urogenital	Bone and Joint
Staphylococcus aureus	NF, P	P, NF	P	P	P	NF, P	P
Staphylococcus epidermidis	NF, P	NF, P	P	P	P	NF, P	P
Other *Staphylococci* spp.	NF	NF, P	P	P	NF, P	NF, P	P
Streptococcus pyogenes	P, NF	P, NF	P	P	P	P	P
Other *Streptococci* spp.	NF	NF	P	P	NF	NF, P	P
Enterococcus spp.	P	P	P	P	NF, P	NF, P	P
Escherichia coli	NF, P	NF, P	P	P	NF, P	NF, P	P
Klebsiella pneumoniae	NF, P	NF, P	P	P	NF, P	NF, P	P
Enterobacter cloacae	NF, P	NF, P	P	P	NF, P	NF, P	P
Enterobacter aerogenes	NF, P	NF, P	P	P	NF, P	NF, P	P
Proteus spp.	NF, P	P	P	P	NF, P	NF, P	P
Serratia marcescens	NF, P	(–)	P	P	P	P	P
Other enterics	NF, P	NF, P	P	P	NF, P	P	P
Pseudomonas aeruginosa	P	NF, P	P	P	NF, P	P	P
Acinetobacter spp.	NF	NF	P	(–)	NF	NF	P
Candida albicans	NF	NF	P	P	NF	P, NF	P

**NF, normal flora; P, pathogens; (–), normally not found, but these organisms should not be ignored when encountered.*
Modified from Heggers, J.: Microbiology for surgeons. In Kerstein, M. D. (ed.): Management of Surgical Infections. Mt Kisco, NY, Futura Publishing, 1980, pp. 27-55.

TABLE 87–2 Bacteria Isolated from 304 Acute Care Pediatric Burn Patients Admitted to Shriners Burns Hospitals for Children—Galveston, Texas (January 2006 to December 2006)

Gram-Positive Organisms	No. (%) of Isolates
Methicillin-resistant *Staphylococcus epidermidis*	196 (23.7)
Methicillin-resistant *Staphylococcus aureus*	152 (18.4)
Enterococcus faecium	119 (14.4)
Methicillin-sensitive *S. epidermidis*	84 (10.2)
Methicillin-sensitive *S. aureus*	82 (9.9)
Enterococcus faecalis	80 (9.7)
Other gram-positives*	113 (13.7)
Total no. of gram-positive isolates	826
Gram-Negative Organisms	
Carbohydrate Fermenters (Enterics)	
Escherichia coli	103 (17.4)
Enterobacter cloacae	67 (11.3)
Klebsiella pneumoniae	66 (11.1)
Other fermentors†	132 (22.3)
Noncarbohydrate Fermenters	
Pseudomonas aeruginosa	122 (20.6)
Acinetobacter spp.	48 (8.2)
Stenotrophomonas maltophia	16 (2.7)
Other nonfermentors‡	38 (6.4)
Total no. of gram-negative isolates	592
Total no. of bacterial isolates	1418
Gram-positive organisms (%)	(58.3)
Gram-negative organisms (%)	(41.7)

**Includes other Staphylococcus, Enterococcus spp.; Micrococcus, Pediococcocus and Streptococcus spp.*
†Includes other Enterobacter, Klebsiella, Serratia, Proteus, and Citrobacter spp.
‡Includes other Aeromonas and Achromobacter spp.

colonized. Table 87–2 shows the distribution of gram-positive bacteria at Shriners Burns Hospital for Children in Galveston, Texas, which receives pediatric burn patients from all over the world. For the year 2006, the gram-positive cocci accounted for 58.3 percent of bacterial isolates. Within this group, the staphylococci are more prevalent (62.2%) than are the enterococci, which account for 24.1 percent. Among staphylococci, a predominance of methicillin-resistant (42.1%) versus methicillin-sensitive (20.1%) isolates was noted. The rate of staphylococcal methicillin resistance has increased in this patient population since 2000. This increase is reflective of a worldwide emergence of such antimicrobial resistance in both hospitalized-acquired and community-acquired infections.

Because of such resistance patterns, Cook[21] stresses the importance of microbial surveillance and epidemiologic studies. This approach is thought to reduce the prevalence of methicillin-resistant *S. aureus* (MRSA), yet it may be inadequate for eradicating or preventing outbreaks. Minimizing transmission and infection is emphasized. However, Reardon and colleagues[95] suggest that this process is time-consuming and requires extensive resources for little gain. Colonization with methicillin-sensitive *S. aureus* and MRSA in 86 patients was studied and found to have no significant changes on length of stay, number of operations, or mortality between these two organisms. However, the presence of either type of *S. aureus* significantly increased the number of surgical procedures performed and the lengths of stay. Many burn units report frequent colonization of burn patients with toxic shock toxin (TSS-1)–producing strains of staphylococci; however, their presence does not correlate well with increased morbidity or mortality rates.[20]

In the past, group A β-hemolytic *Streptococcus* frequently was the cause of epidemics in burn units, but it seldom is encountered today because of the frequent emperic use of antibiotics for manipulations of burn wounds. Other β-hemolytic streptococci belonging to groups B, C, E, F, and G can be encountered as well.[71] Significant infection-control vigilance still is necessary, as occasional clusters of outbreaks of group A streptococcal infection continue to be reported.[43,98] Fortunately, group A *Streptococcus* remains uniformly sensitive to penicillins; hence, prophylaxis and treatment are easily accomplished.

Although enterococcal infections (*Enterococcus faecalis* and *Enterococcus faecium*) account for only 24.1 percent of burn-wound infections caused by gram-positive bacteria (see Table 87–2), a significant cause for concern is the emergence of vancomycin-resistant *Enterococcus* (VRE) in burn units.[67,69] Although additional morbidity associated with VRE itself is not clear, when it occurs as a polymicrobial bacteremia, a mortality rate as high as 20 percent has been noted.[69]

Other gram-positive bacilli include the aerobic *Corynbacterium* spp. and *Listeria* spp., as well as the spore-forming *Bacillus* spp. (aerobe) and *Clostridium* spp. (anaerobe). *Bacillus* and *Clostridium* spp. are associated with burn wounds that have come in contact with contaminated soil. In avascular muscle injuries (e.g., electrical injuries or crush injuries combined with burns), the risk for

developing tetanus *(Clostridium tetani)* is high[68] and has led to the practice of using tetanus immunoprophylaxis and booster vaccines.[105]

2. **Gram-negative bacteria:** The presence of the gram-negative bacteria in burn wounds is due in part to translocation of the bacteria from the gastrointestinal tract of the patients.[8] In a study of children with burns at our center, patients with large wounds (>50% TBSA) were found to have significantly higher colonization with their fecal gram-negative bacteria than were those with smaller wounds.[32] At our center, although the gram-negative bacteria account for only 41.7 percent of the total bacterial isolates of the wound, they represent a formidable adversary (see Table 87–2). The majority of this group are extended β-lactam–resistant (EBLR) bacteria. Although the carbohydrate fermenting enterics seem to account for 62.1 percent of the gram-negative isolates as a whole, *P. aeruginosa*, a nonfermentor, is the most common gram-negative pathogen in burned patients. Other important gram-negative bacteria include the Enterobacteriaceae such *as Escherichia coli*, *Enterobacter cloacae*, *Klebsiella pneumonia*, and *Serratia marcescens.* The Enterobacteriaceae also are encountered as a cause of nosocomial pneumonia in patients with inhalation injury who are on ventilators, and they are a cause of urinary tract infection in patients with indwelling urinary catheters. *Acinetobacter*, an increasingly more common cause of gram-negative infections, has been found more frequently in burned patients with glucose intolerance or preexisting diabetes mellitus.[33] *Acinetobacter* infections also are found more often in patients with more severe burns and comorbidities.[3]

Even without invasive infections, gram-negative bacteria have been implicated in systemic inflammatory diseases, including shock and disseminated intravascular coagulation, secondary to the circulation of bacterial endotoxin from the gut and the burn wound.[64,74] Often gut decontamination is instituted to reduce the incidence of endotoxin-mediated disease.

3. Fungi: Until the advent of topical antimicrobials and systemic antibiotics, fungal infections were not common developments in patients with burns. The burn wound is the site most commonly infected, although fungemia and dissemination to the respiratory tract in patients on ventilators and to the urinary tract in patients with indwelling catheters are encountered frequently. *Candida* spp. are the most common fungal colonizers of the wound (Table 87–3); however, less than 20 percent of patients develop widespread candidiasis. Overall, the rate of candidemia in the burn population is 3 to 5 percent, and burn-wound invasion has a comparable rate. A study of burned children at our center showed that those developing candidemia did so during the first week postburn and 7 days after excision of burn eschar.[24] One hypothesis is that massive burns with immunosuppression are further suppressed by repeated surgical intervention, anesthesia, and perioperative use of broad-spectrum antibiotics, further predisposing these patients to early development of *Candida* septicemia. With early recognition of invasion of burn wounds by routine biopsies, wound swabs, and early amphotericin therapy, the mortality rate has been reduced to less than 10 percent, as compared with 60 to 90 percent reported in earlier series.[104]

TABLE 87–3 Medically Important Fungi Isolated from 304 Acute Care Pediatric Burn Patients Admitted to Shriners Burns Hospitals for Children—Galveston, Texas (January 2006 to December 2006)

Organism	No. (%) of Isolates
Candida species	86 (83.5)
Fusarium species	6 (5.8)
Trichosporon beigelii	5 (4.9)
Rhizomucor species	2 (1.9)
Aspergillus species	2 (1.9)
Geotrichum species	1 (1.0)
Curvularia species	1 (1.0)
Total no. of fungal isolates	**103**

Unlike *Candida*, true fungal infections caused by *Aspergillus*, *Penicillium*, *Rhizopus*, *Mucor*, *Rhizomucor*, *Fusarium*, and *Curvularia* occur early in the hospital course, specifically in those exposed to the spores on the ground or in water at the time the injury occurred. Once colonized, broad nonbranching hyphae extend into subcutaneous tissue and stimulate an inflammatory response. Vascular invasion occurs frequently and often is accompanied by thrombosis and avascular necrosis, which is clinically observed as rapidly advancing dark discolorations of the wound margin. Systemic dissemination occurs with invasion of the vasculature.

4. Viruses: Linnemann and MacMillan[72] performed a retrospective survey of serum for viral antibodies in pediatric burn patients, 22 percent had fourfold increases in antibodies to cytomegalovirus (CMV), 8 percent had increases to herpes simplex virus (HSV) and to Epstein-Barr virus, and 5 percent had increases in antibodies to varicella-zoster virus (VZV). None of the patients had evidence of adenovirus or hepatitis B virus infection. On the basis of these observations, a prospective study of viral infections, using both serologic and viral culture techniques, was performed. This study showed that CMV infection developed in 33 percent of the children; herpes simplex infection in 25 percent, and adenovirus infection in 17 percent. CMV infections developed in all of the most severely burned children, and both primary and reactivation infections were observed. Most primary CMV infections that develop during treatment for burns are likely to occur from transfusion of blood products. CMV infection typically occurs approximately 1 month after the burn occurs and clinically presents as fever of unknown origin with lymphocytosis; however, it rarely alters the patient's clinical course.[23] Kealey and colleagues[60] have shown that 56 percent of burned patients who initially were seropositive for CMV had a fourfold or greater rise in CMV antibodies as evidence of CMV reactivation. These patients tended to be younger, to have a larger burn area, and to have a longer hospital stay. No patient who experienced CMV infection, whether primary or reactivated, had serious complications attributable to CMV. On the basis of these observations, despite the availability of anti-CMV agents such as ganciclovir and valganciclovir, treatment for CMV infection remains controversial.[96]

Since the screening of blood began, hepatitis C virus (HCV) has been an important risk. Coursaget and colleagues[22] screened 45 burn patients for anti-HCV antibodies, at the time of burn injury and more than 6 months postburn. HCV infection was detected in 18 percent of these patients, as a consequence of the numerous transfusions of blood or blood derivatives used during the postburn treatment. Five patients displayed evidence of anti-C100, anti-C33c, and anti-core antibodies together; two patients had only anti-C100 and anti-C33c antibodies, and the last one showed only anti-core antibodies. Chronic hepatitis was observed in 83 percent of HCV infections. Kinetics of appearance of anti-HCV antibodies varied among patients. Anti-core generally is the first to be detected at high levels; however, in at least one case, it was detected only 2.5 months after C100 and C33c antibodies were detected. The incidence of HCV using polymerase chain reaction (PCR) technique to detect the viral genome has not been evaluated in burn patients. Nonetheless, the current blood banking procedures have decreased the transmission of HCV by blood products.

Another transfusion-related agent is human immunodeficiency virus (HIV), which has become extremely rare as a result of the screening of donors that began in 1987 in the United States. However, significant risk existed prior to that period. A retrospective review of burned children at our center who had received blood/blood products between 1978 and 1985 identified

52 patients at risk for developing HIV infection.[100] More than 50 percent of the identified population had received three or more units of blood/blood products during their acute hospital stay. A total of 214 patients (36.8%) were tested for HIV seroconversions: five tested HIV-positive by enzyme-linked immunosorbent assay (ELISA) and four were confirmed by Western blot, yielding a 1.9 percent incidence. The four confirmed patients received two to nine total body blood volume turnovers during their postburn period in the hospital. HIV may affect the outcome of burn wound injury. A study of Malawian children showed that with burns affecting 11 to 30 percent of the body surface area, HIV-positive children had a mortality rate approximately twice that of the of HIV-negative children.[55]

HSV is of significant concern in burn units because it is a dermatopathologic virus. A review of the literature suggests that patients younger than 10 years of age were at greater risk of acquiring an HSV infection when the size of the burn wound was greater than 15 percent of TBSA.[46] However, the role of HSV in the healing of wounds is unclear. Bourdarias and colleagues[15] showed that in 11 patients with burns, local areas of active epidermal regeneration were affected most often. Acyclovir therapy was not used, and the duration of hospitalization was normal when compared with other children. Nonetheless, HSV in lungs may worsen morbidity. Byers and associates[17] showed that the relative risk for developing HSV infection was higher for cases with adult respiratory distress syndrome but not with pneumonia. Disseminated HSV infection also can be fatal.[17]

Another dermatopathologic virus is VZV. Mini-epidemics of VZV have occurred within pediatric burn units.[122] With the routine VZV vaccination of young children (1 to 2 years of age) in the United States, the outbreaks of varicella in burn units are now uncommon. The characteristic fluid-filled lesions appear in partial-thickness burns that are healed or healing, as well as in uninjured epithelium and mucous membranes. The vesicles are much more destructive in the injured than uninjured skin and may present as hemorrhagic, oozing pockmarks that are prone to development of secondary infection and subsequent scarring. Neovascularized skin grafts may be lost; therefore, further grafting procedures should be delayed until the lesions are quiescent.

The morbidity due to respiratory virus infections, particularly in those with inhalation injury, has not been studied well. More than 100 pediatric burn patients at our center were tested for respiratory syncytial virus (RSV) during the winter seasons of 1995 and 1996 (unpublished observations). Only six patients were found to be positive for RSV, with one death (1% mortality rate).

5. **Parasites:** Parasitic infestation also is seen especially in children from the developing world. Because many patients in our center originate from Mexico, where such infestation is endemic, parasitemia has been found to complicate burn injuries. Parasites that are asymptomatic in sites such as the intestinal tract and the respiratory tract can become symptomatic as a result of the stress of a burn injury. At the Shriners Burns Hospital for Children in Galveston, Texas, we have described three cases of ascaris pneumonitis that exacerbated the smoke-induced lung injury.[47] In 2006 the parasites isolated most frequently were *Giardia lamblia* and *Blastocystis homini*.

CLINICAL MANIFESTATIONS OF INFECTION

LOCAL SIGNS

An open burn wound is a favorable target for bacterial colonization. The progression from simple eschar colonization to the invasive process is favored by a series of factors related to the patient, such as extension and depth of the burn, age, presence of previous disease, and local conditions of the wound; to the microorganism such as density, motility, toxins and antimicrobial resistance; to iatrogenic causes such as prosthetic devices; and to the nosocomial spread of bacteria. It is essential to recognize the early signs of infection of a local burn wound by examining the wound at least once a day.

The local signs of burn wound infection include black or dark brown focal areas of discoloration, conversion of partial-thickness injury to full-thickness necrosis, hemorrhagic discoloration of subcutaneous tissue, enhanced sloughing of burned tissue or eschar, and purplish discoloration or edema of skin around the margins of the wound. Presence of *Pseudomonas* infection can lead to ecthyma gangrenosa and green pigmentation of subcutaneous fat. In fungal infection, centrifugal advance of subcutaneous edema with central ischemic necrosis and hemorrhagic saponification of subcutaneous fat in fungal infection can be seen. In viral infection, vesicular lesions in healing or healed partial-thickness burns and crusted serrated margins of partial-thickness burns may be observed.

SYSTEMIC SIGNS

Progression from local to systemic invasion can occur rapidly, which correlates with the size of the burn wound, the extent of environmental contamination, and the surgical procedures. Early recognition of systemic invasion is critical to avoid the high rates of mortality. Many of the signs of sepsis resemble complications of the burn itself; for example, fevers, tachycardia, shock, and elevated or depressed neutrophil count can occur in burned patients with or without infection. However, certain patterns of clinical signs and symptoms may help recognize the systemic bacterial invasion (Table 87–4).[35] A rise in levels of C-reactive protein serum has been found useful in predicting systemic infection, although increases in the first 2 days after the burn or the day after surgery may occur without infection.[86] When sepsis did occur, it always was preceded by increased levels of C-reactive protein approximately 2 days before the patient was deemed septic clinically. Elevated levels of certain cytokines may be useful markers, but they largely remain research tools.

TABLE 87–4 Signs and Symptoms of Progression from Local Invasion to Systemic Illness*

Gram-Negative Sepsis	Gram-Positive Sepsis
Burn wound biopsy $>10^5$ organisms/g tissue and/or histologic tissue invasion	Same
Rapid onset, well to ill in 8-12 hr	Gradual
Temp. 37-39°C, can be normal, followed by hypothermia (34°-35°C), plus decrease in WBC	Temp. >40°C
WBC may be elevated	WBC 20-50 $\times 10^3$, hematocrit decrease
Ileus	Same
Decreased blood pressure and urinary output	Same
Wounds develop focal gangrene, satellite lesions away from burn wound	Macerated wounds, Ropy and tenacious exudate
Mental obtundation	Anorexic and irrational

Five or more signs or symptoms are definitive diagnostic parameters.
**Modified from reference 35.*
WBC, white blood cell count.

INFECTION COMPLICATIONS

Other than the primary infection of the burned skin, several types of infectious complications in the burned patients have been recognized. Bacteremia is a frequent complication. Surgical burn wound manipulations are responsible for development of bacteremias in approximately 50 percent of cases, but routine instrumentation and intravascular catheter devices also can cause bacteremia.[13,44] The risk of developing bacteremia also correlates with the TBSA affected by burns; Sasaki and colleagues[101] showed that those patients with a positive blood culture had an average total body surface area injury of 47 percent, whereas those with a 26 percent injury had negative blood cultures. TSS caused by TSS toxin–producing strains of *S. aureus* has been identified in acutely burned children. Childs and colleagues[20] found that 13 percent of children developed a toxic shock–like illness; however, its effect on overall burn mortality was not clear.

Subacute bacterial endocarditis is a risk associated with persistent bacteremia caused by any cause, including repeated instrumentation, surgical intervention, and placement of central venous catheters.[12,18] *S. aureus* and gram-negative bacilli are the most frequent cause. In most cases, the antemortem diagnosis rarely is suspected in burned children.[2,12] In addition to causing local valvular damage, infected vegetations may dislodge septic emboli. Suppurative thrombophlebitis occurs at the site of the insertion of the catheter. It may occur in as many as 5 percent of patients with burns covering 20 percent of the TBSA.[109]

Suppurative chondritis occurs in patients with full-thickness burn of the ear. Because of the auricle's relatively low level of blood supply, chondritis frequently follows the progression of tissue ischemia, usually 3 to 5 weeks after burn injury occurs.[80] *P. aeruginosa* and *S. aureus* are the most common pathogens. However, with use of mafenide acetate as a topical agent, the incidence of suppurative chondritis has decreased significantly.

Suppurative sinusitis is seen in patients with long-term nasotracheal intubation. In one study, 8 percent of patients with burns who had nasotracheal intubation for more than 7 days developed sinusitis.[16] Pneumonia may occur with or without inhalation injury, although those with inhalation injury have a substantially higher risk.[107] Bronchopneumonia is the most common type of pulmonary infection, usually occurring in the second week of burn injury. Predisposing factors are size of the burn wound (hematogenous spread), aspiration, presence of tracheostomy or nasotracheal tube (nosocomial spread from burn wound), existence of inhalation injury, and disturbances of fluid and electrolyte balances. Currently, most infection-related deaths in burned patients are caused by pneumonia rather than wound infection.[107]

Urinary tract infection occurs in association with prolonged and often unnecessary catherization.[102] Osteomyelitis can occur when bones are exposed by the burn or by open fracture accompanying the burn; from extension of infection from a septic joint, introduction of organisms along traction pins, and internal fracture fixation devices; or by bacteremia. However, clinically significant osteomyelitis in burned patients is rare.[28] Septic arthritis occurs when a joint is exposed by a burn or by removal of burn eschar.[28] The joints most frequently exposed are the knee, the elbow, the proximal interphalangeal joints of the hand, and the metacarpophalangeal joints on the dorsal surfaces of the hand. The incidence of septic arthritis is obscured by its frequent association with signs and symptoms of severe burns that rarely are separable. Rarely in burns, a joint may become infected from adjacent metaphysical osteomyelitis. In children, most joints can be salvaged. Adult joints are less resilient.

Central nervous system infections in burned patients include meningitis, microabscesses, and septic infarcts. In one review, *Candida* spp., *S. aureus*, and *P. aeruginosa* caused almost 80 percent of infections, occurring most frequently in patients with extensive burns with wound infection or endocarditis.[123]

MICROBIOLOGIC INVESTIGATIONS

The three major approaches to determine burn wound infection are (1) quantitative burn wound cultures (BWCs), (2) histologic assessment of bacterial invasion, and (3) bronchioalveolar lavage (BAL).

1. **Quantitative BWC by biopsy:** Teplitz[112] demonstrated that quantitative bacterial counts of BWCs correlated with histologic specimens showing invasion or colonization. Burn wound infection (often referred to as "burn wound sepsis" in surgical literature) is suspected when proliferating microorganisms exceed 10^5/g tissue and when there is invasion of subjacent unburned tissue has occurred (Fig. 87–2). The presence of microorganisms within the necrotic eschar cannot be considered evidence of burn wound infection. Furthermore, although a bacterial count of 10^5/g tissue is likely to indicate bacterial invasion, this is not invariably true. Only the histologic sections can indicate the level of infection. Therefore, BWCs always should be accompanied by histologic sections from the same area.[93,94]

At the time that surgery is performed, the potentially infected tissues should be excised with a punch biopsy (Fig. 87–3) and divided into equal aliquots.[50] One aliquot should be placed into saline and delivered to the microbiology section for quantitative assessment. These biopsies are weighed aseptically, homogenized in a sterile tube in 3 mL of sterile saline. Known dilutions of the homogenate then are plated using precalibrated loops (10 μL) onto blood agar, colistin-neomycin agar, MacConkey agar, and Sabourands agar for identification in the initial dilutions of 0.1 mL with a 1-mL sterile pipette. After 24 hours, the number of colonies are counted and the quantitative wound culture (QWC) is calculated according to the following formula.

Colony forming unit (CFU) × g of tissue = *number of colonies* × *volume* × *dilution* weight of biopsy in grams

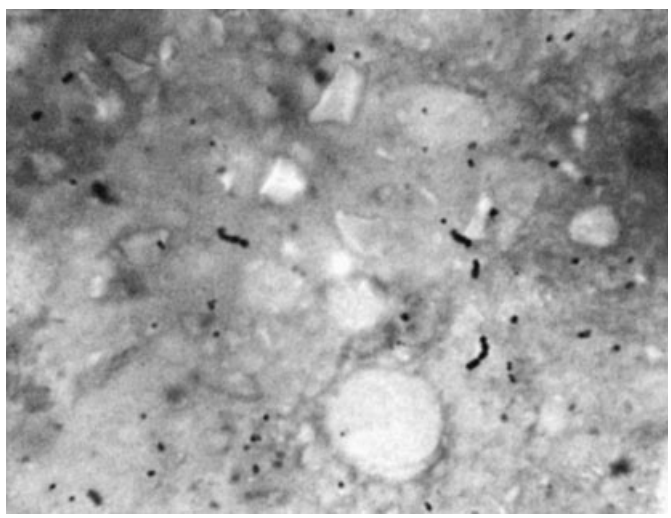

Figure 87–2 Photomicrograph of a homogenized biopsy stained with Gram stain. Gram-positive cocci are seen (cultures grew group A *Streptococcus* at $>10^5$ colony-forming units/gram tissue). *(With permission from Hunsicker, L., Heggers, J. P., Patel, J. A.: Infections in burn patients. In Patrick, C. C. [ed.]: Clinical Management of Infections in Immunocompromised Infants and Children. 2nd ed. Philadelphia, Lippincott Williams & Wilkins, 2001, p. 338 [Fig. 16.2]).*

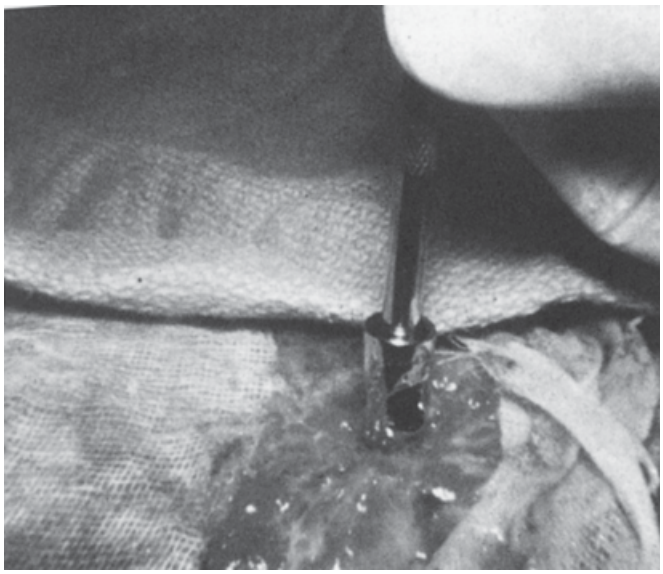

Figure 87–3 Photograph showing the use of a 6-mm punch for collecting a skin biopsy for tissue histology and quantitative culture. *(With permission from Hunsicker, L.; Heggers, J. P.; Patel, J. A.: Infections in burn patients.* In *Patrick, C. C.: Clinical Management of Infections in Immunocompromised Infants and Children. 2nd ed. Philadelphia, Lippincott Williams & Wilkins, 2001, p. 339 [Fig. 16.3]).*

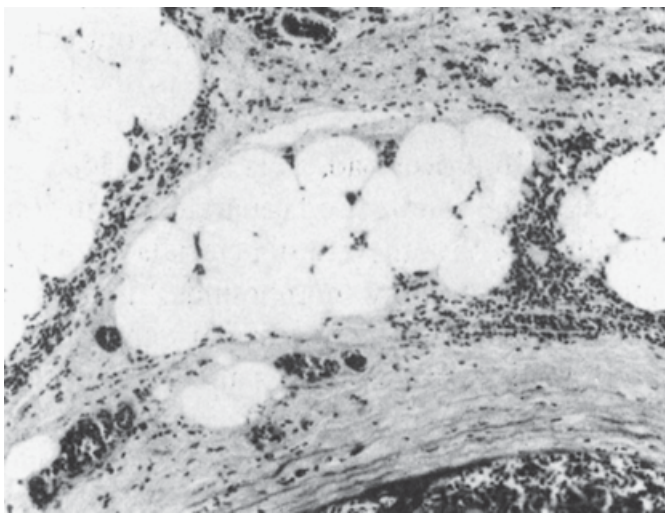

Figure 87–4 Photomicrograph of a cross-section of tissue stained with hematoxylin and eosin showing invasion of *Pseudomonas aeruginosa*. *(With permission from Hunsicker, L., Heggers, J. P., Patel, J. A.: Infections in burn patients.* In *Patrick, C. C. [ed.]: Clinical Management of Infections in Immunocompromised Infants and Children. 2nd ed., Lippincott Williams & Wilkins, 2001, p. 338 [Fig. 16.4]).*

Despite the dependence on quantitative tissue cultures, reliability of this procedure has been questioned because of the high degree of variability in quantitative counts. In a study published in 1981, Woolfrey and colleagues[124] found that when biopsy samples were divided and cultured separately, only 38 percent of paired quantitative results agreed within the same $\log_{10}$ unit, whereas 44 percent differed by +2 $\log_{10}$ units or more.

2. **Histologic procedures:** Standard histologic procedures, as well as cyrostat examination, are necessary.[94] The tissues should be examined for morphologic changes and for the presence of pathogens. Many bacterial and fungal pathogens can be identified by staining with Gram stain and Gomori–methamine silver stains (Fig. 87–4). More specialized stains may be necessary to identify other fastidious bacteria and fungi. Tzanck stain can be used to identify inclusion bodies suggestive of invasion of HSV or VZV. Viral-specific antibodies also can be used to detect these viruses by immunohistochemistry. The microbial status of the burn wound (i.e., colonization or invasion), as assessed by histologic examination of a biopsy specimen, can be graded on the basis of the density and depth of penetration of microorganisms (Table 87–5).[94]

TABLE 87–5 Staging of Microbial Status of the Burn Wound by Biopsy Histology*

Stage I: colonization
A. Superficial: microorganisms present only on burn wound surface
B. Penetrating: variable depth of microbial penetration of eschar
C. Proliferating: variable level of microbial proliferation at nonviable-viable tissue interphase (subeschar space)
Stage II: invasion
A. Microinvasion: microorganisms present in viable tissue immediately subjacent to subeschar space
B. Deep invasion: penetration of microorganisms to variable depth and expanse within viable subcutaneous tissue
C. Microvascular involvement: microorganisms within small blood vessels and lymphatics (thrombosis of vessels common)

**Modified from reference 94.*

3. **BAL:** BAL fluid needs to be collected by personnel experienced in the evaluation of patients with inhalation injury who are at risk for developing airway edema and obstruction. Ventilator-associated pneumonia can be diagnosed if 10^4 or more organisms/mL are cultured. In a study reported by Wahl and colleagues,[118] BAL eliminated the unnecessary antibiotic treatment of 21 percent of patients. In winter months, BAL fluids also can be processed for prevalent respiratory viruses such as RSV and influenza by rapid antigenic detection by immunoassays that are within the capability of most laboratories, or by culture that requires specialized virology laboratory.

PREVENTION AND TREATMENT OF INFECTION

Care of the wound is the mainstay of burn therapy. The goal is to minimize infection and facilitate healing of the burn wound. Wound care addresses both partial- and full-thickness burn injuries until all wounds are closed.

1. Wound dressing: The superficial epidermal layer provides a barrier to microorganisms while the deeper lipid epidermal layer provides protection against loss of water vapor. In full-thickness burns, the eschar may extend beyond the skin into the subcutaneous fat and muscle. Closure of the wound cannot occur until the eschar is removed. Bacterial proteases lead to eschar pseudo-separation and are slowed with antiseptic therapies. Then, the eschar is allowed to fragment and slough, with resultant decreased size. This process is called *wound contraction.* This can also be excised and skin grafted. Full-thickness skin grafts have the least contraction rates. Deep dermal wounds allowed to heal spontaneously lead to hypertrophic scarring. Compression garments are used for prevention and treatment.

When approaching burn wound care, a plan must first be decided. Outer burn dressings provide comfort, metabolic enhancement, and protection. First, superficial burns are exquisitely sensitive to air currents, as are deeper burns after some healing. Also, dressings provide splinting and drainage containment. Next, occlusive dressings help eliminate shivering, cold stress, and evaporative heat loss. In open, granulating wounds, loss of water vapor is maximal. Finally, the protective component

deals with the control of topical organisms. Supplies consist of large 9 × 9-inch burn gauze, Kerlex wraps, Ace wraps, and topical antimicrobials. Gentle cleansing of the wound and daily débridement also are important. Buttock burn wounds should be examined carefully and frequently for the presence of deep stool staining, an ominous predictor of burn wound sepsis and death.[97] Such wounds should be emergently excised.

2. Topical antimicrobials: Topical antimicrobial agents are used extensively in burned patients. Most patients with stage I and stage II A and B wounds (Table 87–5) can be treated with topical or subeschar antimicrobials alone.

Silver sulfadiazine (Silvadene, SSD, Thermazine, Flamazine, Burnazine): Silver sulfadiazine is a 1 percent water-soluble cream combining sulfadiazine with silver (Ag^+). The Ag^+ ion binds with the DNA of an organism and consequently releases the sulfonamide that interferes with the intermediary metabolic pathway of the microbe. It is most effective against *P. aeruginsosa*, the gram-negative enteric bacteria, and *C. albicans*. *S. aureus*, and some strains of *Klebsiella* spp. have been less effectively controlled.[35] Antimicrobial effectiveness has been observed to last for up to 24 hours. More frequent changes are required if a creamy exudate forms on the wound. The benefits of this topical agent are its ease of use and its ability to reduce pain. It has some tissue-penetrating ability, but it is limited to the epidermis.

Silver sulfadiazine can be used separately or in combination with other antibacterials or enzymatic escharotomy compounds. It can be combined with nystatin, which enhances the antifungal activity of this agent. By itself, silver sulfadiazine has been shown to retard wound healing; however, in conjunction with nystatin or *Aloe vera*, the wound-retardant effect is reversed.

Cerium nitrate–silver sulfadiazine (Flammacerium): The lanthanide salt cerium nitrate has been added to silver sulfadiazine since the 1970s and has been shown to have increased bacteriostastic effect in large burn wounds. The antimicrobial spectrum is similar to that of silver nitrate. Methemoglobinemia has been seen only rarely, and no associated electrolyte disturbances occur. Only minimal cerium absorption has been noted in patients with large burns treated for weeks. No complications were noted. Its use is somewhat limited because it is not commercially available in the United States. It is, however, available in several western European countries. Koller and Orsag[63] studied the effects of cerium sulfadiazine in 20 burn patients and found it to be safe and effective in the treatment of deep, extensive burn wounds. Caution is advised for use of sulfadiazine products as cases of *P. aeruginosa* resistance to sulfadiazine have been reported.[49]

Silver nitrate: Silver nitrate is available as a 0.5 percent solution that does not injure the regenerating epithelium in the wound and provides bacteriostatic effect against *S. aureus*, *E. coli*, and *P. aeruginosa*.[35] Silver nitrate is most effective when the wound is cleansed carefully of all emollients and other debris. Multilayered coarse mesh dressings should be placed over the wound and saturated with the silver nitrate solution. Like silver sulfadiazine, silver nitrate has limited penetration because the Ag^+ ion is bound rapidly to the body's natural anions such as Cl^-. Because it is hypotonic in nature, it can cause osmolar dilution, resulting in hyponatremia and hypochloremia. Serum electrolytes should be monitored very carefully.

Notable detriments to the use of silver nitrate include its high expense and light sensitivity. Furthermore, it also requires special handling because if it is allowed to dry or if it is covered with an impervious dressing, hyperpyrexia could occur. *Klebsiella*, *Providencia*, and other *Enterobacteriaceae* spp. are not as susceptible to it as are other bacteria. *E. cloacae* and other nitrate-positive bacteria can cause methemoglobinemia by converting nitrite to nitrate.

Mafenide acetate (Sulfamylon): Mafenide acetate is available both in a 10 percent water-soluble cream or a 5 percent solution and has more substantial bacteriologic data to support its efficacy than do any of the other topical antimicrobials.[66,78] It has been shown to be effective against a broad range of microorganisms, especially against all strains of *P. aeruginosa* and *Clostridium* spp.[101] After a wound has been cleansed of debris, mafenide acetate is applied to the wound like "butter." The treated burn surface is left exposed for maximal antimicrobial potency. The cream is applied a minimum of twice a day and can be reapplied as needed. The 5 percent solution is applied every 8 hours.

Additionally, mafenide acetate has the ability to permeate burn eschar and thereby reduce the risk of bacterial colonization of a deep burn wound, and it is especially effective after the dead tissue is removed from the granulating bed. Unfortunately, several detrimental aspects are associated with the use of mafenide acetate. Protracted use with the low environmental pH favors the growth of *C. albicans*. Mafenide acetate is converted to *p*-sulfamyl vanzoic acid by monamine oxidase, which is a carbonic anhydrase inhibitor; it subsequently causes metabolic acidosis in the patient. Another detrimental problem is that it is painful when applied to superficial, partial-thickness burns with intact free nerve endings. Also, its requirement to remain uncovered for antimicrobial activity may be considered a disadvantage when a dressing is required.

Membrane dressings: Acticoat and Aquacel are silver-coated dressings that have a broad antibacterial activity. Compared with silver nitrate or silver sulfadiazine use, Acticoat and Aquacel have been found easier to apply, were associated with less pain on removal, and have faster rates of reepithelialization.[19,114] Mepitel is another gridlike, silicone-coated, nylon dressing used in partial-thickness burn wounds. Gotschall and colleagues[40] found this product to decrease the pain experienced during dressing change, the time needed for the wound to heal, and the overall duration of the hospital stay as compared with silver sulfadiazine. This study did not find any difference in the incidence of infections between Mepitel and silver sulfadiazine treatments.

Topical antibiotics: Several topical antibiotics have been tried in the management of burn infections. These agents are highly discouraged in the setting of a burn unit because of rapid emergence of resistance:

a. Gentamicin sulfate: It is available as a 0.1 percent water-soluble cream and is chemically similar to the other aminoglycosides, such as kanamycin and neomycin. It has a broad spectrum of antimicrobial activity. It is used for its activity against *P. aeruginosa;* however, its topical use is highly limited because of the high level of gentamicin resistance in burn units.

b. Bacitracin/polymyxin: This antibiotic combination has little or no effect on localized infections of burn wounds.

c. Nitrofurantoin: It is used topically as adjunctive therapy in patients with second- and third-degree burns. With good eschar penetration, it can be used in the treatment of invasive burn wound infections with sensitive agents. The drug presents some advantages for the ambulatory patient. Tissue granulation begins sooner and crusts separate more rapidly.

d. Mupirocin (Bactroban): Studies at our center have shown mupirocin to be superior to silver sulfadiazine in treating methicillin-susceptible *S. aureus (*MSSA) or MRSA infection.[110] Although mupirocin is weaker against gram-negative bacteria, it is also comparable to silver sulfadiazine and mafenide acetate against *P. aeruginosa*, *E. coli*, and *K. pneumonia*.

Nystatin (Mycostatin, Nilstat): Studies at our center have noted that combining nystatin and silver sulfadiazine or nitrofurantoin results in effective prevention of local and systemic *Candida* infections, as well as burn wound sepsis.[48] However, in combination therapy of silver sulfadiazine and nystatin, mafenide acetate actually loses its antimicrobial activity. Therefore, use of nystatin with mafenide acetate is discouraged. Our investigators also have studied concentrated nystatin powder on the effect of angioinvasive fungi *(Fusarium*, *Aspergillus)* refractory to systemic amphotericin B and serial excisions, including amputations.[10] A

concentrated form of nystatin (6,000,000 units/g) was used as dry aerosol every 6 hours and wet to dry dressings laid overtop. Within 14 days, all four of the children studied recovered, with the eradication of their invasive fungal infections. Also, all areas previously autografted underneath the nystatin powder healed nicely.

Sodium Hypochlorite (0.025% Heggers Solution): Currently, the most effective topical antibacterial agent for cleansing a wound is sodium hypochlorite (NaOCl). It transcends the topical antimicrobial effects and tissue toxicity of such products as povidone-iodine, acetic acid, and hydrogen peroxide. The efficacy of NaOCl has been determined to be at a concentration of 0.025 percent, which is bactericidal, nontoxic to fibroblasts, and does not inhibit wound healing, provided buffers are used.[51] It is a broad-spectrum antiseptic and is bactericidal for *P. aeruginosa*, *S. aureus* (MRSA and MSSA), enterococci, and other gram-negative and gram-positive organisms.[101]

Povidone-iodine (Betadine): The active antimicrobial component in this compound is the iodine. It has a broad spectrum of antibacterial and antifungal activities. However, there are disadvantages that limit its use in a burn center. It is painful when applied and is inactivated by wound exudates. Renal dysfunction and acidosis have been noted in association with systemic absorption when applied to open wounds.[65]

Chlorhexidine: Chlorhexidine when combined with 0.5 percent silver nitrate has efficacy similar to that of silver sulfadiazine.[70] Some variants of this product have broader antimicrobial activities, but pain experienced by patients on application has limited its use. Unlike the sulfonamides, plasma-mediated resistance has not occurred.

Subeschar antibiotics: Moncrief[81] has described the technique of subeschar antibiotic infusion. It is used upon microbial invasion into unburned tissue, when topical therapies have not been effective, or in cases in which treatment has been delayed. The most common drugs used are tobramycin, gentamicin, and kanamycin. However, development of antimicrobial resistance is likely substantial, so subeschar infusion of antibiotics should be used infrequently. The antibiotic solution is administered via multiple needle infusions by subcutaneous lysis. The results of this technique are more rapid separation of eschar. The fluid infusion limit is 2000 mL (adult dose) and should be accounted for in patient's fluid requirements. The infusion fluid should consist of 0.25 to 0.45 N saline to avoid salt overload. Lactated Ringer solution should be avoided because some of the drugs are incompatible with calcium. Erythromycin has been studied but is too painful to use. Colistin, novobiocin, and cephaloridine have been used but found to be ineffective.

3. **Systemic anti-infective agents:** Drug pharmacokinetics are significantly altered in the burned patient and show significant inter- and intrapatient variation.[121] In 1976, altered aminoglycoside pharmacokinetics and the need for increased dosage in burned patients were reported, but despite this early study, a review of the currently available literature shows that for many drugs there is a paucity of information to support current dosage recommendations, specifically in children of various age groups. In addition, many reports are based on small numbers of patients, and even in larger studies, no standardization of the study population exists with regard to the important variables known to affect drug handling. For the subpopulation of burned patients who eliminate drugs extremely rapidly, a concern exists over the adequacy of antibiotic dosing. Researchers have suggested that antibiotic serum concentrations be measured for all drugs in every patient to ascertain whether a significant problem exists with dosing.

Whereas stage I and stage II A and B wounds (see Table 87–6) can be treated with topical or subeschar antimicrobials alone, stage II C wounds require the immediate institution of systemic anti-infective agents. Systemic agents also are indicated for the treatment of various systemic infectious complications as discussed earlier. Detailed information on selection of the appropriate systemic antimicrobials for specific bacterial, fungal, and viral pathogens is available in other chapters of this book. Nonetheless, the following general principles can help guide the use of antimicrobial therapy:

a. Each antimicrobial agent must be selected for its specificity for the microbe present.

b. Such decisions should be made on appropriately collected culture and susceptibility data.

c. Colonizing flora should be distinguished from those responsible for inflammation and invasion.

d. The time, dosage, route of administration, and duration of treatment should be in accordance with what is required to make the organism nonpathogenic.

e. The need for broad-spectrum antibiotics should be balanced with the risk of promoting fungal infections.

Treatment of multidrug-resistant, gram-negative bacteria: This issue deserves specific mention because it most severely affects the burn units. In many centers, pan-resistant *P. aeruginosa* strains and *Acinetobacter* spp. are identified frequently. In these cases, colistin and polymyxin B often remain the only susceptible antimicrobial agents. In a recent study, Goverman and colleagues[41] summarized their experience in 14 children treated with intravenous colistin: favorable response was obtained in 79 percent and the overall mortality rate was 14 percent. Both colistin and polymyxin B are associated with significant elevation in serum creatinine or renal failure in 15 to 25 percent of recipients. Neurotoxiciy in children is an infrequent occurrence. Intravenous and/or aerosol polymyxin B, doxycycline, and ampicillin/sulbactam (the active component is thought to be sulbactam) have been tried in multidrug-resistant *Acinetobacter* infections as well.[88] Other studies have demonstrated in vitro susceptibility of multidrug-resistant *Acinetobacter* to various synergistic combinations of antimicrobials, including carbapenems, colistin, rifampin, tigecycline, and ampicillin-sulbactam.[38,53,125] The clinical utility of these combinations against pan-resistant *Acinetobacter* remains to be determined.

4. Antibiotic prophylaxis: Prophylactic use of antibiotics remains a highly controversial topic in the literature. Penicillin prophylaxis is used commonly in many burn centers during outbreaks of group A streptococcal infections.[43,98] Prophylactic antibiotics also are used before surgical manipulation or instrumentation because the risk of developing bacteremia is as high as 50 percent. The choice of perioperative antibiotics is dependent on the knowledge of existing microorganisms, which are present not only on injured skin but also in the wound that is ready for surgery. Nonetheless, carefully controlled studies are necessary to identify the value of prophylactic antibiotics. In a placebo-controlled study of cefazolin use in burned children, researchers found that in children with less than 35 percent burn, cefazolin was not necessary, and in those with 35 percent burn or more, it was not effective.[99] In a retrospective review study, antibiotic prophylaxis was not associated with any reduction in the rate of wound infection; instead, the duration of hospitalization was found to be longer.[26]

5. Wound excision and grafting: Studies at our center have shown that early excision of wounds leads to significantly decreased rates of bacterial colonization and wound infection.[9] A meta-analysis of six trials found that early excision of burns is beneficial in reducing mortality (in patients without inhalational injury) and length of hospital stay. The only drawback is the greater volume of blood loss.[89]

6. Gut support and decontamination: The gut microflora have been implicated in the development of multiorgan failure when the gut barrier fails. Hence, one theory is that early enteral feeding of the gut, which promotes function of the gut barrier, is important for preventing multiorgan failure. A decontaminated gut with nonabsorbable, broad-spectrum antibiotics might dimin-

ish the impact of failure of the gastrointestinal barrier. Although the suggestion has been made that the rate of pneumonia may be decreased by such maneuvers, no apparent impact on mortality rates have been noted.[75] Overall, convincing human data on the beneficial effect of enteral feeding and decontamination of the gut on sepsis and multiorgan failure are lacking.

7. **Immunomodulators:** In view of the intricate relationship between altered immune defenses in burned patients and increased susceptibility to infections, various types of immunotherapies have been tried in burned patients. In an Italian study, treatment with intravenous pooled immunoglobulins (IVIG) was found to have a beneficial effect on septic phenomena and recovery.[79] However, in two U.S. studies, no change in rates of infection or mortality was shown. Overall, the beneficial role of IVIG as prophylaxis or treatment remains unproven.[85,119] In a German study, prophylaxis with intravenous *Pseudomonas* immunoglobulin did not appear to be beneficial to burned patients in general; however, it was shown to be effective in burned patients with inhalation injury.[111] In a South African study, prophylaxis with high-titer anti-lipopolysaccharide immunoglobulin G (IgG) reduced the incidence of burn wound infection but did not affect mortality.[57] Benefits of plasmapheresis or fresh frozen plasma infusion have not been proven.

INFECTION CONTROL

Hospital-acquired infections in burn patients are common occurrences. In burned children, wound infections, ventilator-related pulmonary infections, central-line bacteremias, and catheter-associated urinary tract infections are the most common nosocomial infections.[102,120] The rates of infections may be lower than those seen in adults, but urinary tract infections occur more frequently in children.[102] In a German study of children with burns, the overall infection rate was 59.7 nosocomial infections per 1000 inpatient days, and the device-associated infections per 1000 device days were 55.2 for pneumonia, 8.9 for primary bloodstream infections, and 41.7 for urinary tract infections.[37] The incident density of burn wound infections was 18.5 per 1000 inpatient days, which was associated with the percentage of TBSA of the burn wound. On the other hand, the rate of device-associated infections was not associated with the percentage of TBSA.

Surveillance of infection in a burned patient is performed to implement prompt treatment on the basis of surveillance cultures and antimicrobial sensitivities at the earliest sign of invasion. Such surveillance requires cultures of sputum, urine, and wounds about three times weekly; however, the need for such cultures and the frequency of monitoring remain controversial. Additional infection control measures involve surveillance of spread of pathogens among burned patients of a unit. In dealing with the burn wound, strict infectious disease precautions must be maintained to prevent contamination and worsening infection in these already immunocompromised patients.

In the past, poor handwashing and shared hydrotherapy tubs were the source of many infections, some of which were life-threatening. Strict enforcement of handwashing and use of gowns, gloves, and masks have led to decreased rates of patient contamination. Also, disposable hydrotherapy tub liners are used without the risk of patient-to-patient transmission of infections.[113] Besides this, isolation of burned patients in a single room compared with an open ward scenario has dramatically affected infection rates. McManus and colleagues[77] studied this measure in 2519 patients and found significantly lower incidence and mortality rates associated with gram-negative bacteremia in the isolated patients. Also, the open ward bacterial isolates showed significantly more antimicrobial resistance compared with those of the patients in isolation.

Additional infection control measures include the use of germicidal solutions to clean daily all of the hardware, such as intravenous poles and pumps, monitoring equipment, bedside tables, and beds in patients' rooms. Upon discharge of the patient, the whole room, including floors, walls, ceilings, and mattresses, needs to be cleaned with germicidal solutions. Air filters should be monitored repeatedly for fungal and bacterial growth.

Lai and colleagues[67] studied the effects of strict isolation of patients with VRE, the use of vancomycin, and the cost-to-benefit analysis of barrier precautions. Their findings showed that pharyngeal swabs were poor for surveillance but that rectal swabs were more useful. Also, vancomycin-use guidelines were adhered by 85 percent of the staff. The overall cost for these implementations, including the barrier supplies and cleaning protocols, was $11,000. Nonetheless, VRE was not eradicated. On the other hand, van Rijn and colleagues[116] have shown that isolation in a quarantine unit was highly effective in preventing outbreaks of multidrug-resistant bacteria.

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SECTION XV

Unclassified Infectious Diseases

CHAPTER 88

Kawasaki Disease

Stanford T. Shulman

Kawasaki disease is a multisystem acute febrile vasculitic syndrome of unknown (presumably infectious) origin that affects predominantly infants and young children. The diagnosis is based on characteristic clinical features (Table 88–1). Serious complications include coronary arteritis, coronary artery aneurysms and stenoses, coronary thrombosis leading to myocardial infarction, and, very rarely, rupture of a coronary aneurysm. Kawasaki disease has become the leading cause of acquired heart disease in children in most developed countries including the United States and Japan.[364,365] It has been reported in children of all racial groups and from all continents. Although the origin of Kawasaki disease remains unknown and a specific diagnostic test is lacking, establishing a timely diagnosis is very important because administration of intravenous immunoglobulin (IVIG) and aspirin before the 10th day of illness generally has a dramatic effect on the clinical manifestations and markedly reduces the likelihood of development of coronary abnormalities (see the later sections on treatment).[99,238,247,250-252,322,367]

Synonyms for Kawasaki disease include *Kawasaki syndrome* and *mucocutaneous lymph node syndrome* (MCLS, MLNS, or MCLNS). It also has been referred to as *lymphomucocutaneous syndrome* and similar terms. An earlier term used in autopsy reports was *infantile periarteritis nodosa* (IPAN), which is pathologically indistinguishable from fatal Kawasaki disease. The International Classification of Diseases (ICD-9) designates the condition as both Kawasaki disease and mucocutaneous lymph node syndrome (acute) (febrile) (infantile) under rubric 446.1. Before 1983, the National Library of Medicine listed Kawasaki disease publications under various subject headings, particularly "Lymphatic Diseases." Since 1984, publications have been listed under "Mucocutaneous Lymph Node Syndrome," a term very rarely used today.

HISTORY

The illness now bearing his name was first recognized as a clinical entity in 1961 by Dr. Tomisaku Kawasaki, who subsequently became Chairman of the Department of Pediatrics at Tokyo's Japan Red Cross Medical Center. Between 1961 and 1967, Kawasaki identified 50 infants and young children who manifested a distinctive constellation of signs that included prolonged high fever, unilateral cervical lymphadenopathy, bilateral conjunctival injection, a polymorphous erythematous rash, changes of the mucosa of the upper respiratory tract, and edema and erythema of the extremities, with subsequent desquamation of the finger and toes. Although the syndrome was impressive, its signs were nonspecific. Laboratory tests ruled out other disorders. A series of the first seven cases was presented by Kawasaki at the 61st Chiba General Meeting of the Japan Pediatric Society in 1962.[158] Convinced that he was observing a distinct clinical syndrome, Kawasaki published a report of his experience with 50 cases of "febrile oculo-oro-cutaneo-acrodesquamatous syndrome with or without acute nonsuppurative cervical lymphadenitis" in 1967.[159,160,162] Other Japanese physicians quickly recognized the syndrome after Kawasaki's report, although considerable discussion ensued about whether it was a distinct entity or an illness such as Stevens-Johnson syndrome.[161]

Cardiac involvement in this illness was suspected first in 1968, when Yamamoto and Kimura reported an infant with Kawasaki disease who had transient tachycardia with a gallop rhythm, cardiomegaly, and minor abnormalities on the electrocardiogram (ECG).[394] In 1970, Kawasaki succeeded in obtaining funding to establish the Research Committee of Mucocutaneous Lymph Node Syndrome, sponsored by the Japanese Ministry of Health and Welfare, which was organized with Dr. Fumio Kosaki as chair.[162] In the first national survey of this committee, four autopsied and six non-autopsied cases of children who had died of coronary artery complications after having apparent Kawasaki disease were identified.[175] These children were predominantly younger than 2 years of age and had died suddenly within 30 days of onset of disease, with evidence of coronary aneurysms and acute thrombosis. The first biennial national epidemiologic survey was conducted by this committee in 1970 under the leadership of Dr. I. Shigematsu and was published in 1972.[317] By this time, it had become well established that some patients who recovered apparently uneventfully from this acute illness were at risk for sudden cardiac death, with findings of acute myocardial infarction secondary to thrombosis within coronary arteries damaged by a severe vasculitic process.[175]

In 1971, physicians at the University of Hawaii who were unaware of the Japanese experience began to recognize patients with an unusual Reiter syndrome–like illness. When information about Kawasaki disease was published in the English-language literature,[162] the illness in Hawaii was recognized clearly as Kawasaki disease. Information exchanged between Japanese and U.S. investigators led to the 1974 publication of English-language articles by both groups[162,221,222] and triggered worldwide recognition of cases. In the early 1970s, death from myocardial infarction was reported to occur in approximately 2 percent of cases of Kawasaki disease; more recent data reflect much lower mortality rates of less than 0.1 percent.[120,241,400] With the availability of echocardiography in the late 1970s, researchers determined that 20 to 25 percent of patients will develop evidence of coronary artery abnormalities.[147]

In the decades before Kawasaki recognized the clinical features of the illness, many individual reports of fatal coronary arteritis in children (usually labeled IPAN) were published in the non-Japanese pediatric and pathology literature.[54,77,234,271,283,290] Clinical details of these cases generally are highly suggestive of Kawasaki disease, and the pathologic features of IPAN are indistinguishable from those of Kawasaki disease, as demonstrated

TABLE 88–1 Diagnostic Criteria for Kawasaki Disease

Fever for at least 5 days*
PLUS
Presence of four of the following features:
Bilateral conjunctival injection
Polymorphous exanthem
Changes in the lips and oral cavity (erythema, cracking of lips; oropharyngeal erythema; strawberry tongue)
Peripheral extremity changes (erythema and swelling of hands and feet; later periungual desquamation, Beau lines)
Cervical lymphadenopathy (≥1.5 cm in diameter)
Exclusion of other diseases with similar features

Note: The finding of fever plus three criteria in the presence of coronary abnormalities qualifies.

**In the presence of classic features, experienced clinicians may be able to establish the diagnosis before the fifth day of illness.*

conclusively by Landing and Larson in 1977.[184] Almost 100 years before Kawasaki's description was published, Samuel Gee of St. Bartholomew's Hospital in London in 1871 reported the case of a 7-year-old boy who at death ("following scarlatinal dropsy") had three coronary aneurysms, each filled with a fresh clot; histologic examination of that patient's cardiac tissue is compatible with inactive Kawasaki disease with extensive coronary myointimal proliferation and fibrosis.[103,327] Shibuya and colleagues identified cases of illnesses compatible with Kawasaki disease that occurred in Japan up to 2 decades before Kawasaki's description was published.[316] Likely, patients with Kawasaki disease in previous decades were misdiagnosed as having measles, scarlet fever, rubella, or other once common conditions, and reductions in the numbers of cases of those illnesses helped to facilitate recognition of Kawasaki disease.[161]

Kawasaki's clinical description of the syndrome has remained the foundation of diagnosis and the basis of the clinical and epidemiologic case definitions in use today (see Table 88–1). The American Heart Association Committee on Rheumatic Fever, Bacterial Endocarditis, and Kawasaki disease published guidelines for the management of patients with incomplete (or atypical) Kawasaki disease (see later).[252]

EPIDEMIOLOGY

SOURCES OF EPIDEMIOLOGIC DATA

As with most notifiable diseases, passive reporting of Kawasaki disease cases is incomplete. Passive surveillance data may help to monitor secular trends and to identify epidemics, but these data are of little value in estimating the incidence of disease. Outbreak investigations are more sensitive in determining local disease incidence, and they enable investigators to study potential risk factors.

In the absence of a confirmatory diagnostic test, the epidemiologic case definitions of Kawasaki disease are strict and exclude from surveillance data other exanthematous conditions that could dilute "true" cases and thus could obscure secular trends. However, the original epidemiologic case definitions were not intended for clinical application, a very important point since effective treatment became available. Thus, less strict application of clinical case criteria is appropriate for management of patients. Clinicians must be aware that children often present with clinical illnesses that do not completely fulfill the diagnostic criteria for Kawasaki disease but who are nonetheless at risk for having coronary artery sequelae and therefore warrant therapy. These patients generally are considered to have *incomplete* or *atypical* Kawasaki disease.[28,91,252,299] Incomplete presentations of Kawasaki disease are particularly common occurrences in young infants in whom clinical signs often are subtle or fleeting but who are at the highest risk for development of coronary artery abnormalities.[40,293] In the United States, the Centers for Disease Control and Prevention (CDC) case definition usually is used for epidemiologic purposes, and the American Heart Association published an algorithm to aid in the diagnosis of incomplete (atypical) Kawasaki disease.[252] The current Japanese diagnostic guidelines were revised for the fifth time and also reflect the importance of incomplete cases.[16]

INCIDENCE RATES

The incidence of Kawasaki disease varies throughout the world and reflects primarily the racial composition of various countries. Rates in Japan have climbed steadily, with an annual rate of 151 per 100,000 children younger than 5 years of age in 2002 (17th National Survey)[400] and as high as 175 per 100,000 children younger than 5 years.[34] In countries with predominantly white populations, the rate is approximately 15 per 100,000 children younger than 5 years of age.[128]

GENDER

In virtually all population-based studies in many countries, the ratio of male to female patients with Kawasaki disease approximates 1.5 : 1.[400,402] In addition, serious and fatal complications also are significantly more common findings among male patients with Kawasaki disease compared with female patients.[241,401] Examination of fatal Japanese cases indicated almost three times as many Kawasaki-related fatalities among male patients compared with female patients, with a higher ratio in infancy.[241] The basis for the preponderance of Kawasaki disease in male patients and for the even greater predominance of serious coronary artery disease in male patients with Kawasaki disease remains unclear. Of interest is that a male predominance is observed in many infectious diseases.

RACE OR ETHNIC BACKGROUND

The first cases of Kawasaki disease were recognized in Japanese children and in Hawaiian children of predominantly Japanese ethnicity, and subsequent data consistently supported higher rates in those of Asian background. Annual incidence rates in Japan have climbed steadily to more than 150 cases per 100,000 children younger than 5 years of age,[400] from 102.6 in 1995 and 108 in 1996, reaching 184.6 in 2005-2006,[243a] and are among the highest in the world, exceeded only by rates for Japanese-American children in Hawaii (≈197/100,000 <5 years).[129] In epidemic years in Japan, the annual age-specific incidence rates have reached or exceeded 200 per 100,000 children younger than 5 years of age.[396] Incidence rates in white children in many communities are much lower, most often approximating 10 to 15 per 100,000 children younger than 5 years.[60,128,329] Surveys in countries with almost exclusively white populations often yield rates of 5 to 10 cases per 100,000 children younger than 5 years of age.[29,32,45,121,276,333] In Washington state, ethnic group–specific incidence rates per 100,000 children younger than 5 years of age were estimated to be 33.3 for Asian Americans, 23.4 for blacks, and 12.7 for whites.[60] In Hawaii, with its complex racial-ethnic make-up, data showed the overall annual incidence to be 45 per 100,000 children younger than 5 years of age.[129] The yearly incidence for Japanese children in Hawaii approaches 200 cases per 100,000 children, and for whites it is 35 per 100,000, with intermediate rates for those of native Hawaiian and Chinese, Filipino, and other Asian ancestry.[129] In New Zealand, differences in incidence between white and Polynesian children were not

apparent,[104] but in Singapore, a higher rate of Kawasaki disease in Chinese children compared with Malay children was suggested.[253] Extrapolation of data from surveys of U.S. hospitals with large children's services led to estimates of approximately 2500 cases per year in the United States from 1984 to 1993,[364,365] with other estimates as high as 5000 cases annually.[34] A study of Kawasaki disease in the United States identified approximately 4200 hospitalizations in the year 2000, with highest rates among Asian and Pacific Islanders, lowest in whites, and intermediate in blacks and Hispanics.[128] Recent data from Japan indicated that a steady increase to more than 10,000 patients with Kawasaki disease diagnosed yearly has occurred,[243a] with occasional local clusters rather than the nationwide outbreaks as seen in 1979, 1982, and 1985 to 1986.[400]

The higher rates of Kawasaki disease in those of Japanese and some other Asian backgrounds suggest a genetic rather than an environmental basis, as supported by increased rates among third- and fourth-generation immigrants from Japan to Hawaii.[129] The increased incidence of Kawasaki disease among siblings and parents of patients (see later) also supports a genetic predisposition.[383,399] The genetic basis is complex in that no single human leukocyte antigen (HLA) is common to most patients with Kawasaki disease. Early reports that Kawasaki disease was associated with HLA-Bw 22 (or subtype Bw 54)[157,177,217] were not confirmed by subsequent studies in Japan, Hong Kong, or Boston, where HLA-Bw 51 and HLA-B 44 were found to be more common.[46,178] HLA-Bw 51 also was increased in a series of Israeli patients.[164] A small Maryland study suggested that the A2 B44 Cw5 haplotype was a risk factor for epidemic Kawasaki disease.[146] Studies of HLA major histocompatibility complex (MHC) class II genes have detected no clear association.[19,81] Immunoglobulin allotypic markers were studied as possible genetic markers for Kawasaki disease.[330] The kappa chain allotype Km1, which is very common in Asian populations, and the combination of Km1 with Gm heterozygosity were present in significantly greater proportions of white patients with Kawasaki disease than in the control white population. In addition, the haplotype G1m(a), G3m(t) was found significantly more often in Japanese and Japanese-American patients with Kawasaki compared with race-matched control populations. This study supports a complex genetic basis for susceptibility to Kawasaki disease.[330] Susceptibility has been linked to other allelic variations in small studies, but larger and more definitive studies are in progress.[35] Transmission disequilibrium studies involving children with Kawasaki disease and their parents may provide better insights into the genetic basis of susceptibility, with functional polymorphisms in the inositol triphosphate kinase-3 gene[268a] and in a network of gene single nucleotide polymorphisms.[32a]

AGE

Kawasaki disease occurs almost exclusively in children. In the United States and Japan, adult cases are quite rare, although some reports of adults diagnosed by accepted diagnostic criteria have been published.* Many of these adult patients have been infected with human immunodeficiency virus (HIV).[141] Many of the early reported adult cases, however, appear to have been associated with toxic shock syndrome or drug hypersensitivity reactions. Perhaps the best-documented adult case was that of a 31-year-old Japanese man (confirmed by Dr. Kawasaki) who later developed bilateral coronary artery aneurysms.[343] Because the signs and symptoms are nonspecific, adults suspected to have Kawasaki disease should be evaluated carefully for infectious, toxic, and other possible causes of illness.

The distribution of Kawasaki disease by age in childhood is characteristic. The disease occurs most frequently in young children: 50 percent are younger than 2 years of age, 80 percent are younger than 5 years of age, and cases seldom occur in those older than 12 years of age.[128,336,400] Infants in the first 3 to 6 months of life have a low incidence of Kawasaki disease, but the incidence rises rapidly from that point. In the United States, the peak age is approximately 15-18 months, whereas more recent data from Japan identified 26 percent as younger than 1 year old, 89 percent as younger than 5 years, and the peak age incidence as 9 to 11 months of age.[400] In Hawaii, 29 percent of cases are in children younger than 1 year old, and 85 percent of patients are younger than 5 years.[129] The lower peak age in Japan and perhaps in Hawaii may reflect better recognition of Kawasaki disease in infants, in whom Kawasaki disease may be more difficult to diagnose, or it may indicate a biologic difference. The age-incidence curve may be helpful in elucidating risk factors for developing Kawasaki disease. Such a pattern is compatible with highly transmissible infectious agents, particularly respiratory agents, and suggests possible transplacental immunity. The features of 28 patients with Kawasaki disease who were aged 8 years and older at the time of diagnosis at our Chicago institution were reported.[336] Delays in establishing the diagnosis and in providing treatment were common occurrences and were at least partially related to the prominence of arthritic and gastrointestinal symptoms in this population. We recently cared for a 19-year-old woman with classic Kawasaki disease.

Japanese mortality data suggest that fatality rates are approximately three times higher in children younger than 1 year old at the time of onset of disease, compared with older children, and that fatalities occur predominantly in the first several months after onset of Kawasaki disease.[399] The overall mortality rate in Japan has dropped from the initial report of approximately 2 percent to more recent Japanese estimates of approximately 0.08 percent.[399] Male patients account for a disproportionate number of deaths in infants and older children.[120,243,399] Long-term mortality rates in a large Japanese Kawasaki disease cohort study were increased over background rates, particularly during and shortly after the acute stage of illness.[241,243]

RECURRENT KAWASAKI DISEASE

A recurrence of Kawasaki disease is defined generally as a new episode of illness meeting clinical criteria for Kawasaki disease that begins at least 3 months after the initial episode and after inflammatory markers such as the erythrocyte sedimentation rate or serum C-reactive protein level have normalized. The frequency of recurrences after first cases of Kawasaki disease in Japan was estimated to be approximately 1.9 percent during a 3-year follow-up period, with approximately 0.07 percent of patients experiencing a third episode.[240] This corresponds to a rate of 5.21 per 1000 person-years for one or more recurrences.[240] With longer follow-up, recurrence rates in Japan may approach 3 percent.[401] Data from the United States suggest a recurrence rate of approximately 2 percent.[23] Recurrences occur most frequently within the first 2 years after the initial episode, especially in male patients and in children who have their initial episode before reaching their second birthday.[240] The frequency of recurrences in Chicago appears to be approximately 1 percent, although recurrences were documented in about 2 percent of Hawaiian cases from 1996 to 2000,[129] a finding possibly reflecting a racial difference. The true recurrence rate will be determined only when a specific diagnostic test becomes available for Kawasaki disease and thus minimizes recognition bias.

FAMILY CASES

Simultaneous or sequential cases of Kawasaki disease in siblings, twins, or other family contacts also have been reported,

*See references 12, 42, 44, 76, 109, 141, 191, 226, 278, 282, 313.

particularly during outbreaks in Japan.[86,132,215,402] Japanese epidemiologists have documented secondary sibling cases in approximately 1 percent of cases, a rate that is approximately 10 times greater than that in the general child population.[86] However, such figures are difficult to interpret because they may be influenced by recognition and reporting biases. Sibling cases are reported to occur more frequently in twins than in non-twins. Only three sibling pairs were recognized in approximately 1300 Chicago patients, and none were reported in 400 children in Los Angeles.[213] A report from two U.S. medical centers noted 18 families with multiple affected Kawasaki disease patients, including 9 families with 2 affected siblings and 9 with Kawasaki disease in 2 generations or in multiple affected members.[64]

EPIDEMICS AND OUTBREAKS

Japanese investigators noted large nationwide outbreaks of Kawasaki disease in 1979, 1982, and 1985 to 1986, with wavelike spread occurring from one prefecture (state) to the next, suggesting an infectious origin.[242,395,397] The 1982 Japanese epidemic started simultaneously in four areas and spread outward from each region, similar to the way that epidemic influenza spreads in Europe and America.[242,395] In the 1985 to 1986 Japanese epidemic, investigators identified epidemic "waves" that spread outward from an initial focus in the Tokyo metropolitan area and extended simultaneously northward and southward to involve most of the country within approximately 4 months.[242,395,397] A similar but less distinctive pattern of interprefectural progression in waves was noted in the 1982 Japanese epidemic. Within the northern Tohoku District, for example, Kawasaki disease spread from prefecture to prefecture over a period of approximately 7 months.[395] Korean epidemics were detected 7 and 15 months after the Japanese epidemics of 1979 and 1985 to 1986, respectively.[190,395] More localized outbreaks also have been observed. In the United States and elsewhere, community-wide outbreaks have been documented beginning in 1977.[24] Investigation of outbreaks provides opportunities to study potential risk and etiologic factors. Clustering of cases within families, schools, or neighborhoods is quite unusual, even during large-scale epidemics. Japanese investigators have associated epidemics with a significantly increased likelihood of second cases occurring in families, fatalities, and recurrent cases, but the implications of these findings are uncertain. It is striking that since 1986 no further nationwide Japanese outbreaks have been identified, a finding suggesting that the epidemiology of Kawasaki disease may have changed.[399,404]

GEOGRAPHY

Kawasaki disease has been diagnosed throughout the United States and Japan and in virtually all developed and many developing countries on all continents, including temperate and tropical zones.[32,45,78,210,279,305,307,374] No striking rural-urban differences have been noted. Elevation, longitude, and latitude have not been implicated. Travel histories of patients are unremarkable, although an anecdotal report of a 7-month-old infant in Australia documented Kawasaki disease onset 17 days after leaving Japan during that country's 1982 epidemic.[333]

SEASONALITY

In Japan, Kawasaki disease occurs year round but is most prevalent in the winter and spring, with peaks usually occurring in December or January, with a lower peak in June, and the lowest number of cases in October.[36,398,401] In the United States and other temperate areas, the number of cases peaks in the winter and early spring and is least in late summer; nonetheless, cases occur throughout the year.[25,128,213,284] Winter predominance has been observed in at least some Southern Hemisphere countries. That sporadic cases are recognized year round is somewhat different from the pattern usually observed with highly transmissible respiratory viral diseases, incidences of which peak sharply in the winter and spring (e.g., measles, rubella, influenza).

COMMUNICABILITY

Although little direct evidence exists that Kawasaki disease is transmissible from person to person, considerable circumstantial evidence supports an infectious origin. Secondary or co-primary cases in families occur but are not common. Several outbreak investigations found a higher rate of antecedent respiratory tract illness in patients with Kawasaki disease compared with matched controls.[24,375] This finding is of particular interest in view of the finding that immunoglobulin A (IgA) plasma cells infiltrate the proximal respiratory tract in fatal cases of Kawasaki disease, and it supports a respiratory mode of spread of an inciting agent (see later).[300] A history of previous exposure of children with Kawasaki disease to food or objects from Japan or elsewhere in Asia is a common factor, but the ubiquity of such exposures renders interpreting the data difficult. Japanese family data suggest that sibling cases cluster either on the same day as the index case or 7 days later.[86] Because these results are based on questionnaire data, however, the possibility of ascertainment bias is great; thus, determining the degree to which Japanese familial cases represent co-primary or secondary cases is difficult.

OTHER RISK FACTORS

In addition to demographic risk factors for Kawasaki disease, specific exposures that could be related to an etiologic agent have been linked to the disorder by some epidemiologic investigations. As noted earlier, a history of more frequent recent antecedent respiratory illnesses in cases compared with controls was documented in the early 1980s.[24] Because many viral agents are prevalent in the winter and spring, when Kawasaki disease is most prevalent, "background" isolation of various viruses from patients and from matched controls is expected.

Other past Kawasaki disease associations include exposure to recent carpet cleaning or shampooing, exposure to house dust mites, and residence near bodies of water. The association of Kawasaki disease with exposure to recently cleaned or shampooed carpets was observed in some reports,[27,79,110,131,264,275,284,286] but not in others.[59,209,230,284,291,292,366] In three carpet shampoo-associated clusters investigated by the CDC, the exposures clustered significantly in the 2- to 4-week interval before onset of disease, with few exposures in the 2-week interval immediately preceding the onset. The significance of a possible association with shampoo is unclear, because it is absent entirely in many well-studied outbreaks, and carpets are relatively uncommon in Japan.[59,230] Conceivably, rug shampooing could lead to aerosolization of a microbial or sensitizing agent present in the carpet, such as mites or microbial agents. A possible link between Kawasaki disease and house dust mites (chiefly *Dermatophagoides farinae* and *D. pteronyssinus*), initially proposed as an allergic hypothesis,[100] gained support when the presence of mites and mite antigens in carpets was realized. A Japanese group reported *Rickettsia*-like bodies in the digestive systems of mites in house dust of patients with Kawasaki disease.[115] Other investigators suggested that Kawasaki disease could result from an infectious agent in dust mites.[85] However, counts of dust mites were not significantly different in the houses of case and control patients,[110,135] and no

evidence of increased anti-mite IgE, IgG, or antibodies to *D. pteronyssinus* was found in patients with Kawasaki disease.[110,142]

In a small number of outbreaks, CDC investigators found that patients with Kawasaki disease lived closer to "bodies of water" compared with control patients.[286,287] However, other studies, including one in Washington State, did not find living in proximity to water to be a risk factor.[60]

ETIOLOGY

The origin of Kawasaki disease remains unknown. However, clinical and epidemiologic features strongly suggest that the disease has an infectious cause. A self-limited, generally nonrecurring illness manifested by fever, rash, enanthem, conjunctival injection, and cervical adenitis fits well with an infectious cause. The epidemiologic features noted earlier, including the age distribution (Fig. 88–1), the winterspring seasonality, the occurrence of community outbreaks with wavelike geographic spread, and apparent epidemic cycles, resemble those of a transmissible disease of childhood. The laboratory features, including leukocytosis with a "left shift," elevated acute-phase reactants, and pyuria, also suggest infection. Vasculitis with inflammatory cell infiltration (including IgA plasma cells)[300] may be infectious or may represent an immune reaction to infection. A very attractive hypothesis is that Kawasaki disease is caused by a ubiquitous infectious agent that produces clinically apparent disease only in selected, genetically predisposed individuals, particularly Asians. Its rarity in the first few months of life and in older children and adults suggests an agent to which virtually all adults are immune and from which very young infants are protected by passive maternal antibody. Consistent with this hypothesis is the paucity of evidence of person-to-person spread of Kawasaki disease, because most infections would be asymptomatic, and only a very few of those infected would develop clinical features of Kawasaki disease. This would be analogous to acute poliomyelitis, the relatively uncommon complication of poliovirus infection that occurs in only approximately 1 in 200 of those infected. However, efforts to identify an infectious agent of Kawasaki disease using conventional bacterial and viral culture and serologic methods, as well as inoculation of primates, mice, and guinea pigs, have failed to yield an infectious cause.[326]

Two major theories to explain the etiology of Kawasaki disease have gained prominence in the past decade: the specific respiratory pathogen theory[295] and the superantigen theory.[201] A very long list of microorganisms, including bacteria, leptospires, spirochetes, fungi, chlamydia, rickettsiae, and viruses, have been suggested at one time or another to cause Kawasaki disease, without confirmation.[303] Chlamydial studies eliminated this possibility.[116,314,337] The hypothesis that Kawasaki disease may be

Figure 88–1 Evaluation of suspected incomplete Kawasaki disease (KD). *1*, In the absence of a gold standard for diagnosis, this algorithm cannot be evidence based but rather represents the informed opinion of the expert committee. Consultation with an expert should be sought anytime assistance is needed. *2*, Infants up to 6 months old on day 7 or later of fever without any other explanation should undergo laboratory testing and, if evidence of systemic inflammation is found, an echocardiogram, even if the infants have no clinical criteria. *3*, Patient characteristics suggesting Kawasaki disease are listed in Table 88–1. Characteristics suggesting disease other than Kawasaki disease include exudative conjunctivitis, exudative pharyngitis, discrete intraoral lesions, bullous or vesicular rash, or generalized adenopathy. Consider alternative diagnoses (see Table 88–2). *4*, Supplemental laboratory criteria include albumin less than or equal to 3.0 g/dL, anemia for age, elevation of alanine aminotransferase, platelets after 7 day 450,000/mm^{3}or greater, white blood cell count 15,000/mm^3 or higher, and urine with 10 or more white blood cells/high-power field. *5*, The patient can be treated before an echocardiogram is performed. *6*, Echocardiogram is considered positive for purposes of this algorithm if any of three conditions are met: Z score of left anterior descending (LAD) or right coronary artery (RCA) 2.5 or greater, coronary arteries meet Japanese Ministry of Health criteria for aneurysms, or ≥3 other suggestive features exist, including perivascular brightness, lack of tapering, decreased left ventricular function, mitral regurgitation, pericardial effusion, or Z scores in LAD or RCA of 2 to 2.5. 7. If the echocardiogram is positive, treatment should be given to children within 10 days of fever onset and to those beyond day 10 with clinical and laboratory signs (C-reactive protein [CRP], erythrocyte sedimentation rate [ESR]) of ongoing inflammation. *8*, Typical peeling begins under nail bed of fingers and then toes. See the text for further details. f/u, follow-up. *(Newburger, J. W., Takahashi, M., Gerber, M. A., et al.: Diagnosis, treatment, and long-term management of Kawasaki disease. (American Heart Association Scientific Statement). Circulation* 110*:2747-2771, 2004.)*

caused by *Rickettsia*-like agents also seems very unlikely.[143] *Yersinia*, particularly *Yersinia pseudotuberculosis*, causes a systemic illness resembling Kawasaki disease in selected areas of Japan,[50,188,310] but convincing evidence of an etiologic link between *Yersinia* and Kawasaki disease is lacking, despite the use of newer molecular tools.[357]

That Kawasaki disease may be caused by a novel retrovirus was suggested when two groups reported reverse transcriptase activity in cultured peripheral blood mononuclear cells from patients with acute Kawasaki disease.[37,325] Other studies failed to confirm this finding.[223,268] Serologic studies for the retroviruses HIV-1, human T-cell lymphotropic virus types I and II, simian immunodeficiency virus, and feline T-cell lymphotrophic virus have been negative,[254,268,285,301] although features resembling Kawasaki disease have been described in adults with HIV infection.[141]

The hypothesis that Kawasaki disease may be the result of a bacterial superantigenic toxin was suggested because of possible selective expansion of $V\beta_2$ and $V\beta_8$ T-cell receptor families among circulating mononuclear cells.[1] Superantigens such as bacterial toxins bind to MHC class II molecules on monocytes and B cells and to the T-cell receptor, with resultant activation of large numbers of immunoreactive cells with release of inflammatory cytokines. Superantigens potentially cause expansion of autoreactive T cells.[123,176] Features of immune cell activation characterize the acute phase of Kawasaki disease. Toxic shock syndrome and Kawasaki disease share the features of fever, rash, conjunctival infection, and convalescent desquamation to some degree, but differences are present, such as the rarity of hypotension in Kawasaki disease and the absence of maculopapular or erythema multiforme–like rashes in toxic shock syndrome.[117,277] Although one group of investigators reported selective expansion of the $V\beta_2$ and $V\beta_8$ T-cell receptor families in acute Kawasaki disease,[1,2,56] this finding was not confirmed by others.[3,256,280,376] Although 11 of 16 studied patients with Kawasaki disease and only 1 of 15 controls were colonized with toxic shock syndrome toxin I (TSST-I)–producing staphylococci,[201] *Staphylococcus aureus* colonization rates, including by TSST-I–producing staphylococci, were not significantly different among patients with Kawasaki disease and controls in other studies.[202,211,370] That most studies failed to show *any* disturbance of Vβ T-cell receptor expression in Kawasaki disease substantially challenges the superantigen hypothesis.[61]

More recent work indicated that the immune response in Kawasaki disease is oligoclonal (antigen driven, i.e., a response to a conventional antigen) rather than polyclonal (as typically found in superantigen-driven responses), with both B-cell and T-cell oligoclonality demonstrated.[51,302] In a series of studies, Rowley and colleagues[298,300] demonstrated that IgA plasma cells infiltrate peribronchial, coronary arterial, and other tissues in patients who have died of Kawasaki disease in the acute stage and that the IgA plasma cells are oligoclonal,[302] findings strongly suggesting an immune response driven by a conventional agent. Using the techniques of modern molecular biology, Rowley and colleagues[295] synthesized oligoclonal antibodies from the IgA genes from a fatal acute-stage case of Kawasaki disease and identified an antigen in acute Kawasaki disease (but not control) bronchial epithelial cells and in a subset of macrophages in inflamed acute Kawasaki disease tissues, including coronary arteries. This antigen was localized to distinctive perinuclear, primarily apical, intracytoplasmic "spheroidal bodies" in acute Kawasaki disease ciliated bronchial epithelium. Light microscopic and transmission electron microscopic studies showed that the bodies are homogeneous electron-dense perinuclear inclusion bodies up to 1.4 μg in diameter that contain both protein and RNA and are consistent with aggregates of viral proteins and RNA, possibly related to the etiologic agent of Kawasaki disease.[296] The specific agent is still under very active investigation.

Reports of an association between a novel human coronavirus (dubbed HCoV-NH but later shown to be HCoV-NL63) appeared in 2005,[75] thus raising the possibility that this virus could be the agent observed by Rowley and colleagues noted earlier. However, several additional studies from four countries were unable to confirm any relationship between Kawasaki disease and this agent,[22,47,67,72,318] and Rowley's synthetic antibodies do not bind to HCoV-NL63.

Researchers have suggested that Kawasaki disease may be an immunologic response triggered by any of several different microbial agents. Consistent with this hypothesis is documented infection by different microorganisms in different individual cases, failure to detect a single microbiologic or environmental agent after 4 decades of study, and analogies to other multifactorial syndromes (e.g., aseptic meningitis). However, this hypothesis is difficult to reconcile with the distinctive clinical and laboratory pictures of Kawasaki disease, with its epidemiologic features such as epidemics and wavelike geographic spread, and with the studies of Rowley and colleagues.[295,296]

Efforts to associate Kawasaki disease with exposure to drugs[130] or to such environmental pollutants as toxins, pesticides, chemicals, and heavy metals have failed. However, clinical similarities between Kawasaki disease and acrodynia (mercury poisoning) are notable.[15,269]

Clearly, conventional culture and serologic methodologies have failed to yield the causative agent of Kawasaki disease. In addition, establishing the diagnosis can be difficult, particularly in young infants and in those with incomplete or atypical presentations.[28,40,299] A useful diagnostic test probably cannot be developed unless it is based on the etiologic agent, and more specific therapy and preventive efforts also await discovery of the cause of this disease. The hope is that the use of modern molecular biology techniques[295,296,298,302,324] will finally clarify the origin of Kawasaki disease, as well as provide insights into the mechanisms of disease pathogenesis.

PATHOLOGY AND PATHOGENESIS

RELATIONSHIP WITH INFANTILE PERIARTERITIS NODOSA

When the first Japanese survey in 1970 yielded reports of 10 deaths, the pathologic diagnosis was IPAN.[139,163,175] Japanese and Western investigators quickly recognized the pathologic similarities between fatal Kawasaki disease and IPAN, long known to have an unusual predilection for the coronary arteries.[4,20,21,359-362] In the most definitive study, Landing and Larson[184,189] showed clearly that IPAN and fatal Kawasaki disease were indistinguishable pathologically. Published case reports of autopsies of children with IPAN frequently contain clinical histories that include many or all features of acute Kawasaki disease and the development of severe vasculitis affecting primarily the coronary arteries.[290] The failure of some reported patients with IPAN to meet Kawasaki disease case criteria applied retrospectively is likely related to documentation bias and to the young age of many of the patients. Young infants with Kawasaki disease are less likely to have a classic presentation than are older children (see later). When pathologic and clinical criteria are combined, the two diseases appear indistinguishable. Adult-type PAN was described first in 1866 by Kussmaul and Maier,[183] and it differs from IPAN primarily by the presence of hypertension and involvement of small and medium muscular arteries, especially in the lung, kidney, and intestines.[88,184,361,362] An early recorded case of possible IPAN dates from 1899.[180] In 1959, Munro-Faure[234] very clearly delineated a syndrome of infantile necrotizing arteritis with coronary artery involvement, fever, rash, conjunctival and pharyngeal infection, and cervical adenitis, distinct from classic PAN. Roberts and Fetterman[290] and others expanded on these

observations to define a distinct clinical-pathologic syndrome of IPAN shortly before Kawasaki recognized the clinical syndrome.[159,160]

Coronary artery aneurysms were described as early as the early 19th century, with a male-to-female ratio of roughly 3:1, including a male preponderance in childhood cases. Childhood death from multiple coronary artery aneurysms was known to occur at least as early as 1871, as reported by Samuel Gee.[103] The cardiac specimen from this case, formalin fixed for more than 120 years in the pathology museum at St. Bartholomew's Hospital, London, recently was sectioned and examined histologically. We found that the coronary arteries showed the characteristic histologic findings of inactive Kawasaki disease and IPAN.[327] Whether these early cases with childhood coronary artery aneurysms truly represented early examples of Kawasaki disease is not certain; details of the clinical histories usually were scant.[103,283] What is clear, however, is that most of these cases greatly resemble (and very likely do, in fact, represent) Kawasaki disease.[54,77,234,283] From the features of many such early case reports, a reasonably accurate picture of Kawasaki disease as recognized today emerges. It is highly likely that in the decades before the introduction of the measles vaccine in the 1960s, cases of Kawasaki disease were misdiagnosed as measles, scarlet fever, drug hypersensitivity, or other common conditions.

PATHOLOGIC FEATURES OF KAWASAKI DISEASE

Cardiac death in Kawasaki disease generally occurs in the subacute or convalescent stages of illness.[241,243] Autopsy findings usually reveal evidence of active or inactive vasculitis, which is most severe in the medium-sized arteries, with a marked predilection for the coronary arteries.[8,9,87,184,353,361,362] Small arterioles, larger arteries, capillaries, and veins also are affected to a lesser extent.[9,88] In more than 80 percent of fatal cases in the acute stage, the immediate cause of death is acute thrombosis of inflamed coronary arteries, with resultant myocardial infarction. In a few early deaths, pancarditis with inflammation of the atrioventricular conduction system apparently caused a fatal arrhythmia or intractable congestive heart failure, whereas small numbers of other deaths may have been associated with acute coronary rupture, usually within a few weeks of the onset of illness. Deaths that occur months to years after the acute episode of Kawasaki disease often are secondary to coronary stenosis, with or without thrombosis with myocardial ischemia, or, very rarely, to rupture of a coronary aneurysm. In some patients with coronary artery abnormalities (usually patients with quite severe coronary disease), aneurysms of other major medium-sized arteries, including the brachial, renal, and iliac arteries, also may be present. Although phlebitis may be found, vascular inflammation more typically and more severely affects medium-sized musculoelastic arteries in their extraparenchymal portions. In the acute stage of Kawasaki disease, systemic inflammatory changes also are evident in many other organs, including myocardium, pericardium, cardiac valves, cerebrospinal fluid, lung, lymph nodes, pancreas, spleen, joints, and liver.[10,88]

In the early stages of the vasculitis of Kawasaki disease, edema of endothelial cells with nuclear degeneration and mild adventitial inflammation are seen.[8,87,359] Mononuclear cells predominate even in the acute stage of Kawasaki disease, and neutrophils also are prominent in the first 10 days after onset.[354] In more severely involved vessels such as the coronary arteries, panvasculitis is present, with inflammation of the endothelium, the media with edema and necrosis of smooth muscle cells, and the adventitia. Fragmentation and destruction of elastin and collagen fibers and the internal and external elastic laminae are seen in severely affected vessels.[8] These changes eventually obscure the various layers of the wall. Structural integrity may be lost, resulting in an aneurysm. In larger arteries with vasa vasorum, inflammation is seen in and around these vessels.[8]

Within 1 to 2 months after the onset of Kawasaki disease, inflammatory cells disappear, and fibrous connective tissue, collagen and elastic fibers begin to form within the vessel wall. The intima proliferates and thickens. In time, the vessel wall may become stenotic, and occlusion by stenosis or by superimposed thrombus can result. Calcification may occur, and intraluminal thrombus may become organized, remodeled, and recanalized.[8,245,309,349,350] Several groups investigated the mechanisms of vascular remodeling in Kawasaki disease. Suzuki and colleagues[351] showed by immunohistochemistry that active remodeling continues for at least several years after the onset of Kawasaki disease, with luxuriant intimal proliferation and neoangiogenesis. Extensive expression of vascular growth factors, including vascular endothelial growth factor (VEGF) within smooth muscle cells, was evident particularly in newly formed microvessels in the intima.[351] Gavin and colleagues[102] showed the prominence of matrix metalloproteinases in this process, particularly within the intima and media. Suzuki and associates[352] found evidence of inflammatory changes in the intact coronary artery of a child who died 13 months after having Kawasaki disease and who had no evidence of coronary artery involvement during the acute stage.

Fujiwara and associates[89,90] identified significant atrioventricular conduction system lesions in 5 of 10 autopsy specimens, with a strong correlation found between findings on the ECG, especially PQ prolongation, and acute inflammation of the atrioventricular conduction system. Severe acute changes were most pronounced at 21 to 31 days after onset. In a unique study of 201 right ventricular endomyocardial biopsies in uncomplicated acute Kawasaki disease, Yutani and associates[406,407] noted some degree of myocarditis and cellular infiltration, ranging from very mild (subclinical) to quite severe myocardial inflammation, in all 201 patients studied.

A histologic/immunochemical study examined the intercostal arteries to compare the nature and developmental processes of lesions with those of the coronary arteries.[214] Although similar lesions were seen, the lesions appeared somewhat later in the intercostal arteries.

The precise nature of the inflammatory infiltrate in the vessel wall or myocardium has been studied. Terai and colleagues[368] demonstrated helper (CD4) T cells and monocytes/macrophages in the arterial wall of one patient with fatal acute Kawasaki disease, as well as expression of class II MHC antigen (an activation marker) in coronary vascular endothelium, findings supporting the importance of immune responses in this form of vasculitis. Terai[371] also reported evidence of eosinophils in epicardial microvessel lesions in acute Kawasaki disease leading to death.

Rowley and colleagues[298] demonstrated the presence of many plasma cells of the IgA isotype in the vascular walls of patients who died in the acute stage. These investigators also showed that the IgA plasma cells are oligoclonal (i.e., they appear to be responding in an antigen-driven process)[302] and that they can be observed also in peribronchial, pancreatic, and renal tissues.[300] A predominance of CD8 cytotoxic/suppressor T cells over CD4 helper T cells in seven of eight acute-stage deaths also was observed in our laboratory by immunohistochemistry.[31] Takahashi[354] reported $CD68^+$ monocytes and macrophages to be most prominent in acute Kawasaki disease, but $CD3^+$ cells (T cells), $CD20^+$ cells (B cells), and neutrophils also were present in autopsy specimens of children who died in the first month of the illness.

Other less commonly reported pathologic findings include renal infarcts and glomerular histologic changes, possible evidence of immune complex deposition,[304] including mesangial deposition of IgM and C3, and multifocal periglomerular infiltration of lymphocytes and IgA plasma cells.[300] Changes have been

noted in other arteries, the thymus, and lymph nodes. Tanaka[359] described thymic atrophy and nondiagnostic lymph node changes at autopsy (although most patients likely had been treated with steroids). Naoe and colleagues[246] reported lymph node biopsies to show thrombotic arteriolitis, severe lymphadenitis with necrosis, and postcapillary venules with endothelial cell and reticular cell hyperplasia. Other reports described early lymph node biopsies showing multiple foci of necrosis and fibrin thrombi within the microvasculature, as well as T-zone hyperplasia, B-zone macrophage infiltration, and immunoblast proliferation.[108] Rowley and colleagues[300] identified IgA plasma cells not only in vascular and renal tissues but also in pancreatic and peribronchial locations. Miura and associates[228] found prominent macrophage infiltration of pancreatic acini and islets in 3 of 10 fatal cases.

CLINICAL MANIFESTATIONS

CLINICAL PHASES OF ILLNESS

The clinical course of Kawasaki disease can be divided into acute, subacute, and late or convalescent phases. The *acute* febrile phase begins with fever, rash, conjunctival injection, "strawberry" tongue, red swollen lips, edema and erythema of the hands and feet, lymphadenitis, and sometimes aseptic meningitis and mild hepatic dysfunction. Young children often are quite irritable. Evidence of myocarditis, rarely including congestive heart failure or arrhythmias, may develop during this time. Pericardial effusion, mitral regurgitation, or depressed myocardial function may be detected by echocardiogram. Without aspirin and IVIG treatment, the acute phase generally lasts for 8 to 30 days (mean, 11 days). After defervescence, the physical findings rapidly disappear, but during this *subacute* phase, the child may remain irritable and anorectic, with decreased activity. Some conjunctival injection may persist. Arthritis or arthralgia, mainly of larger joints, may develop in the subacute phase. Desquamation of fingers and toes, typically beginning in the periungual region, and thrombocytosis are very common manifestations during this period. The subacute phase persists until the child has returned to his or her normal state of health at approximately 3 to 6 weeks after the onset of fever, with normalization of inflammatory markers. The time of greatest risk of sudden death occurring from acute coronary artery thrombosis in patients with coronary lesions is during the subacute phase and the early convalescent phase. The *convalescent* phase begins when all clinical signs and symptoms have disappeared and inflammatory markers are normal, usually at about 6 to 8 weeks after onset.

When manifested completely, Kawasaki disease is a distinctive clinical entity with a fairly predictable course.[57,252] However, children who do not fulfill the criteria for diagnosis of Kawasaki disease, in fact, may have the illness and are at risk of developing complications, particularly coronary artery disease (see "Incomplete or Atypical Kawasaki Disease" later).[252,299] The principal clinical diagnostic criteria are presented in Table 88–1. Kawasaki disease should be considered in the differential diagnosis of infants and children with fever for at least 5 days associated with two or more of the five classic features: generalized polymorphous erythematous rash, conjunctival injection, characteristic changes of the lips and mouth, bilateral redness and swelling of the hands and feet, or unilateral nonfluctuant cervical lymph node enlargement greater than 1.5 cm.[252] All features are not necessarily present at the same time. A diagnosis of typical Kawasaki disease, according to accepted clinical criteria (see Table 88–1), is made in patients with fever and at least four of the five clinical criteria and with exclusion of other illnesses that mimic Kawasaki disease. Each of the five clinical features is present in 80 to 90 percent of patients with typical cases, except cervical lymphadenopathy, which is present in approximately 50 percent of patients. The most commonly encountered diseases to be excluded are as follows: (1) febrile exanthems, presumably viral, including measles; (2) acute streptococcal and staphylococcal infections; and (3) drug hypersensitivity reactions. Japanese diagnostic guidelines for Kawasaki disease are similar to the U.S. guidelines with rare exception; they consider fever and the other five major features to be six equal criteria and require at least five of those six criteria for diagnosis. Thus, a child could have no fever but all five other criteria and be considered a typical case by the Japanese criteria. The Japanese criteria also accept a course of fever lasting less than 5 days if it is shortened by early IVIG treatment.[16]

Certain scoring systems using clinical and laboratory features have been developed, primarily in Japan.[13,119,172,244] The goal of these systems generally has been to identify those patients with Kawasaki disease at highest risk for development of coronary abnormalities and who, therefore, would benefit most from receiving IVIG therapy, as well as those at low risk for coronary changes who could be spared IVIG treatment. This practice is decreasing in Japan because no such scoring system appears sufficiently sensitive and specific to enable selective therapy (i.e., to allow non-treatment of patients predicted to be at low risk for development of coronary abnormalities). Patients who are at greatest risk are as follows: those younger than 1 year old; male patients; those with prolonged or recurrent fever; and those with anemia, hypoalbuminemia, hyponatremia, and thrombocytopenia. Nonetheless, we recommend that all patients diagnosed with Kawasaki disease within the first 10 days after onset of disease and those diagnosed later who are still manifesting significant inflammation be treated with IVIG and aspirin.[252] Newer scoring systems have been developed to help predict those patients who are likely to be unresponsive to IVIG therapy, but confirmation is necessary.[172]

In Kawasaki disease, fever typically is high-spiking and remittent, with peak temperatures generally exceeding 39° C (102° F) and in many cases exceeding 40° C (104° F). Unless treated with aspirin or IVIG, fever persists for a mean of 11 days,[125] but it may continue for 3 to 4 weeks, rarely longer. In patients treated with 80 to 100 mg/kg/day of aspirin and a single 2 g/kg dose of IVIG, fever generally resolves within 1 to 2 days.[249]

Bilateral painless vascular infection of the bulbar conjunctivae, clearly more severe than infection of the palpebral conjunctivae and sparing the limbic region around the cornea, generally is seen in the first week of illness, usually beginning shortly after onset of fever. It generally is not associated with exudate, conjunctival edema, or corneal ulceration, thus distinguishing the eye findings of Kawasaki disease from purulent conjunctivitis and from Stevens-Johnson syndrome. Mild acute iridocyclitis or anterior uveitis, which may be noted by slit-lamp examination, resolves rapidly and only rarely is associated with photophobia or eye pain.[33,105,187,265,332] Less common ocular findings include superficial punctate keratitis, vitreous opacities, vitreous and chorioretinal inflammation, lateral rectus palsy, periorbital vasculitis, and papilledema and other optic disk changes.[11,80,137,391]

Changes of the mouth and lips consist of the following: (1) erythema, dryness, fissuring, peeling, cracking, and bleeding of the lips; (2) a strawberry tongue indistinguishable from that associated with streptococcal scarlet fever, with erythema and prominent papillae; and (3) diffuse erythema of the oropharyngeal mucosae. Oral ulcerations, pharyngeal exudates, and Koplik spots are rarely, if ever, found in Kawasaki disease and when present help to exclude the diagnosis.

Changes in the extremities are among the most distinctive features of Kawasaki disease. The hands and feet become indurated and swollen with stretched, shiny skin, sometimes with painful induration. The palms and soles become erythematous, often with an abrupt change to normal skin at the wrist and ankle. Infants and young children frequently refuse to hold objects or

to bear weight. In the subacute phase, a distinctive pattern of periungual desquamation of fingers and toes may occur from 2 weeks to 2 months after onset of Kawasaki disease in 50 to 70 percent of affected patients. Beau lines, which are transverse grooves across the nails, may appear at the nail base 1 to 2 months after a case of acute Kawasaki disease and may grow out over several months.

The erythematous rash associated with Kawasaki disease may take many forms. Most common is a nonspecific, diffuse, macular-papular, primarily truncal erythematous rash. Occasionally, diffuse scarlatiniform erythroderma, urticaria, or an erythema multiforme–like rash with target lesions develops. Vesicles and bullae are not seen, although very fine pustules occur rarely. Perineal erythema and then desquamation are quite common manifestations in diapered as well as toilet-trained children in the acute stage of illness. Rashes in Kawasaki disease tend to be most prominent on the trunk, with perineal accentuation, but they frequently also involve the face and extremities.

Cervical lymphadenopathy is the least common of the principal diagnostic criteria, but it sometimes is the dominant clinical feature along with fever.[335] It usually is unilateral and confined to the anterior cervical triangle. To fulfill diagnostic criteria, the enlarged node or mass of nodes exceeds 1.5 cm, is not fluctuant, usually is not associated with erythema of the overlying skin, and is not tender or only moderately tender. Lymphadenopathy generally is benign and transient. Clinicians should be aware that children with suspected acute cervical adenitis that is unresponsive to antibiotic therapy may have Kawasaki disease. Because other features of Kawasaki disease often are present but overlooked, Kawasaki disease should be considered in febrile children with suspected acute bacterial cervical adenitis that is unresponsive to antibiotics and without an alternate diagnosis.[335] The ultrasound appearance of lymph nodes in Kawasaki disease has been described as similar to that of acute Epstein-Barr virus (EBV) infection and distinct from bacterial adenitis.[363] The absence of changes on imaging (e.g., ultrasound or computed tomography [CT]) suggesting suppuration of such lesions should strengthen the suspicion of possible Kawasaki disease in this setting. Impressive cervical adenopathy in patients with Kawasaki disease generally resolves remarkably promptly after administration of appropriate therapy.

The associated features of Kawasaki disease reflect its multisystemic nature (Table 88–2). Sterile pyuria as a manifestation of urethritis, occasionally with meatitis, is found in approximately half of patients. Arthritis appearing during the first week of illness can be polyarticular or oligoarticular, including the small interphalangeal joints as well as large weight-bearing joints, with a prevalence of approximately 7.5 percent.[111] Arthrocentesis manifesting during this early phase yields purulent-appearing fluid, with a mean white blood cell count of 125,000 to 300,000/mm^3, normal glucose levels, and negative Gram stain and bacterial cultures. Arthritis developing after the 10th day of illness has a predilection for large weight-bearing joints, especially the knees and ankles, with a somewhat lower synovial fluid white blood cell count.[125] Gastrointestinal complaints occur in approximately one third of patients with acute cases; these complaints may be severe, leading to laparotomy, and they include nausea, abdominal pain, and some diarrhea. These findings may be related to hydropic gallbladder, pancreatitis, or appendicular vasculitis.[14,408] Obstructive jaundice and acute hydrops of the gallbladder are not uncommon findings, whereas mild to moderate elevations of serum transaminases occur in almost half of patients. Central nervous system involvement including aseptic meningitis occurs in almost half of patients.[62] Transient unilateral lower motor neuron facial nerve palsy occurs rarely,[281] as does sensorineural hearing loss.[345] Characteristic extreme irritability is very common, especially in young infants. Reactivation of inflammation at the site of a previous bacillus Calmette-Guérin (BCG) vaccination coincident with acute Kawasaki disease is a common finding in Japan, where BCG is used widely,[331] and we observed a child in whom a BCG site and purified protein derivative (PPD) test site reactivated with acute Kawasaki disease.[371a] Some patients experience a flare of atopic dermatitis or psoriasis during or after experiencing acute Kawasaki disease.[30,71] Various pulmonary manifestations of Kawasaki disease, including isolated pulmonary nodules,[84] pleural effusions, acute respiratory distress syndrome,[273] and pulmonary infiltrates,[384] all of which are interesting in view of the increased numbers of IgA plasma cells found in respiratory tract tissue, have been observed.[298,300]

TABLE 88–2 Associated Noncardiac Features of Kawasaki Disease

Musculoskeletal System
Arthritis or arthralgia
Central Nervous System
Aseptic meningitis
Facial nerve palsy
Marked irritability
Sensorineural hearing loss
Gastrointestinal System
Hydrops of gallbladder
Abdominal pain, diarrhea
Hepatic dysfunction, obstructive jaundice
Pancreatitis
Genitourinary System
Urethritis, meatitis
Respiratory System
Pulmonary infiltrates, nodules
Preceding respiratory illness
Other
Erythema and induration of bacille Calmette-Guérin vaccine site
Anterior uveitis (mild)
Peripheral gangrene (young infants)
Desquamating groin rash
Flare of atopic dermatitis or psoriasis

By far, the most important associated feature of Kawasaki disease is cardiac involvement. Cardiac manifestations can be prominent in acute Kawasaki disease, and they certainly are the major cause of long-term morbidity and mortality. In addition to the coronary artery abnormalities that develop in 20 to 25 percent of untreated children, pericardial effusion and myocarditis with congestive heart failure, tachycardia, gallop rhythm, nonspecific changes on the ECG, or arrhythmia may occur.[147,150,152,252] An imperfect correlation exists between clinically apparent cardiac involvement and echocardiographic evidence of coronary abnormalities, although in the pre-IVIG era, echocardiographic evidence of mitral regurgitation or pericardial effusion in the acute stage was shown to be predictive of subsequent coronary abnormalities.[106] Acute Kawasaki disease may involve pericardium, myocardium, endocardium, coronary arteries, and cardiac valves. Clinical and auscultatory features may include a hyperdynamic precordium, tachycardia out of proportion for the child's age and temperature, a gallop rhythm, and a flow murmur. Some infants may manifest very low cardiac output, and ECG changes (ST-segment and T-wave changes, prolonged PR interval, and arrhythmias) may be present.

INCOMPLETE OR ATYPICAL KAWASAKI DISEASE

A substantial subset of children presents with illnesses that do not completely fulfill diagnostic criteria for Kawasaki disease but that

include at least 5 days of fever and some features of the disease. Incomplete or atypical Kawasaki disease (incomplete is the preferred usage) is associated with a substantial risk for development of coronary artery aneurysms,[28,91,205,252,299] but it can be very difficult to diagnose. Incomplete Kawasaki disease occurs most frequently in young infants, who unfortunately are at greatest risk of developing coronary disease with Kawasaki disease,[40,293,299] and fatalities have occurred. The laboratory profile of incomplete cases is similar to that of classic cases, and laboratory results can increase or decrease the likelihood of the presence of Kawasaki disease in a particular patient. Echocardiographic findings in the acute stage, including perivascular brightness, coronary ectasia, decreased myocardial contractility, pericardial effusion, and mild mitral regurgitation, can support the diagnosis of Kawasaki disease. Individual manifestations of Kawasaki disease in young infants tend to be more subtle than those in older children and can be fleeting.[252,293] Kawasaki disease should be considered in the differential diagnosis of prolonged fever in infants because patients are described in whom such fevers are virtually the sole manifestation of Kawasaki disease.

A committee of the American Heart Association developed an algorithm to assist in the evaluation of patients with suspected incomplete Kawasaki disease (see Fig. 88–1), with emphasis placed on clinical assessment and measurement of acute-phase reactants (C-reactive protein [CRP], erythrocyte sedimentation rate [ESR]) in patients with 5 or more days of fever and two or three features of Kawasaki disease, or in infants 6 months old or younger with 7 days or more of fever without other explanation.[252] In addition to elevated ESR (≥40 mm/hour) and CRP (≥3.0 mg/dL), a set of six supplementary laboratory criteria that can be useful in this regard is as follows: albumin, 3.0 g/dL or less; anemia for age; increased alanine transaminase (ALT); platelets after day 7 of more than 450,000/mm^3; white blood cell counts of 15,000/mm^3 or more; and 10 or more white blood cells/high-power field in the urine.

Retrospective diagnosis of Kawasaki disease often is based on finding coronary abnormalities by echocardiogram, although the real goal is to identify patients with incomplete Kawasaki disease before detection of coronary changes. The existence of these patients again emphasizes the need to identify the etiologic agent of Kawasaki disease so that a diagnostic test can be developed. When possible, patients with illnesses suggesting incomplete Kawasaki disease should be referred to physicians with considerable experience in making the diagnosis.

LABORATORY FINDINGS

A specific diagnostic test for Kawasaki disease is not available and awaits discovery of the etiologic agent of the illness (Table 88–3). The laboratory features of Kawasaki disease, albeit quite nonspecific, are nonetheless characteristic of the illness. Leukocytosis, especially with neutrophilia, is typical in the acute stage, with a predominance of immature and mature granulocytes. White blood cell counts in excess of 30,000/mm^3 occur in approximately 5 percent of patients and in excess of 15,000/mm^3 in approximately 50 percent. Leukopenia is quite rare. Toxic granulations and Döhle bodies occasionally are seen on peripheral blood smear.[26] Anemia may develop, usually with normocytic red blood cell indices, particularly in patients with more prolonged duration of active inflammation. Severe hemolytic anemia requiring transfusions occurs but is unusual and usually can be related to IVIG therapy.[53,323] Curiously, Kawasaki's first patient in 1961 (and only rare subsequent patients) manifested Coombs-positive hemolytic anemia.[159,327]

TABLE 88–3 Laboratory Features of Kawasaki Disease

Leukocytosis with neutrophilia
Elevated erythrocyte sedimentation rate
Elevated C-reactive protein (and other acute-phase reactants)
Anemia
Thrombocytosis after week 1
Sterile pyuria
Hypoalbuminemia
Hyponatremia
Elevated serum transaminases, gamma-glutamyltransferas
Plasma lipid abnormalities
Cerebrospinal fluid pleocytosis
Synovial fluid pleocytosis

Elevation of acute-phase reactants such as ESR, CRP, and alpha$_1$-antitrypsin is nearly universal in Kawasaki disease, with the ESR usually returning to normal by 6 to 10 weeks after illness onset. CRP values rise and fall much more quickly than do ESR values. Additionally, IVIG therapy per se leads to elevation of the ESR (but not CRP) for several weeks, and, thus, it is of limited value in assessing the degree of inflammatory activity in IVIG-treated patients; CRP or other acute-phase reactants clearly are superior for this purpose.

A very characteristic feature of the later phases of illness is thrombocytosis, with platelet counts ranging from 500,000 to more than 1,000,000/mm^3. Thrombocytosis rarely is present in the first week of illness, usually appears in the second week, and peaks in the third week, with a gradual return to normal by 4 to 8 weeks after onset in uncomplicated cases. The mean peak platelet count is approximately 700,000/mm^3. In one study, infants younger than 1 year old with fever without a source who had platelet counts greater than 800,000/mm^3 were 17 times more likely to be diagnosed with Kawasaki disease ultimately than were infants with platelet counts lower than 800,000/mm^3.[255] No differences exist in chromium-65–labeled autologous platelet survival between cases and controls, and little correlation exists between thrombocytosis and increased platelet aggregation. The latter situation has been detected in patients with Kawasaki disease from a few days until a year after onset.[155,392] The rare patients with thrombocytopenia in the acute stage of Kawasaki disease, most often young patients, appear to be at increased risk for development of coronary artery disease and myocardial infarction.[257,259] The mechanism of thrombocytopenia appears to be consumptive coagulopathy.[259]

Plasma lipids are markedly perturbed in acute Kawasaki disease, with depression of plasma cholesterol, high-density lipoprotein (HDL) cholesterol, and apolipoprotein A-I (apo A-I).[43,248,308] Similar changes are observed in other conditions associated with an acute-phase response.[43] Marked appearance of serum amyloid A (SAA) protein in plasma, associated with HDL3-like lipoprotein particles, is seen acutely.[43] Cabana and colleagues [43] also showed that total cholesterol, HDL cholesterol, apoA-I, and triglyceride levels normalize over the course of several weeks, and that SAA disappears from plasma. The core composition of HDL normalizes more slowly than do plasma HDL cholesterol and apoA-I levels, a finding suggesting that Kawasaki disease has a profound effect on the lipoprotein profile acutely and a more subtle sustained effect on HDL composition.

Mild to moderate elevations in serum transaminase levels are present in as many as 40 percent of patients, and mild hyperbilirubinemia occurs in approximately 10 percent.[38] Plasma gamma-glutamyl transpeptidase levels are elevated in most patients.[372] Hypoalbuminemia and hyponatremia[386] are associated with more prolonged and more severe disease. Hypoalbuminemia appears to indicate more severe and more prolonged acute disease.[119] Urinalysis reveals intermittent mild to moderate sterile pyuria in approximately one third of patients, although suprapubic urine generally does not show pyuria, a finding suggesting urethritis.[63,221] In those children who undergo lumbar puncture,

evidence of aseptic meningitis, with a predominance of mononuclear cells, normal glucose, and normal to mildly elevated protein levels, is a common finding.[62]

Laboratory tests, even though nonspecific, can provide diagnostic support in patients with clinical features that are suggestive, but not diagnostic, of Kawasaki disease[252] and may aid in prediction of nonresponder patients.[172] A moderately to markedly elevated CRP (>3.0 mg/dL) or ESR (>40 mm/hour), almost universal in Kawasaki disease, is an uncommon finding in viral exanthems and hypersensitivity reactions. Platelet counts higher than 450,000 mm^3 usually are present in patients with Kawasaki disease after the seventh day of illness. In cases of incomplete Kawasaki disease associated with coronary abnormalities, thrombocytosis and elevated ESR are very common events in the acute stage. Clinical experience suggests that Kawasaki disease is very unlikely if platelet counts and a full panel of acute-phase inflammatory reactants (e.g., ESR, CRP) are essentially normal after the seventh day of illness.

IMMUNOLOGIC FINDINGS

Studies of children with acute Kawasaki disease reveal widespread immune perturbations[328] and have focused on immune activation, endothelial activation, and vascular infiltration with a variety of immune cells. Evidence of marked immune activation is present and is reflected by increased levels of a wide variety of pro-inflammatory cytokines, including tumor necrosis factor-α (TNF-α), interferon-γ, interleukin-1 (IL-1), IL-2, IL-4, IL-6, IL-8, IL-10, and soluble IL-2 receptor.* Increased chemokine and selectin activity also have been reported in acute Kawasaki disease.[358,389] The most intense immune activation and cytokine production occur during the acute and early subacute phases, the period of most intense vascular inflammation and of aneurysm formation. The immune perturbations subside as the illness resolves either spontaneously[197] or more promptly in response to anti-inflammatory therapy, and the benefit of therapy appears to result from immune modulating effects, although precise details are lacking.[194,321]

In acute Kawasaki disease, serum IgG levels are lower than normal for age,[251] whereas in the subacute stage, polyclonal elevation of all immunoglobulin classes generally is found.[204] Serum IgE and IgM levels particularly are frequently elevated.[179,181] The increase in serum immunoglobulins is associated with a very high proportion of circulating activated B cells,[168] which is reversed by aspirin/IVIG treatment.[196] Shingadia and colleagues[320] found decreased numbers of circulating B lymphocytes and plasma cells with surface or cytoplasmic IgA in the acute and subacute stages of Kawasaki disease, thus complementing the finding of IgA plasma cell infiltration in tissues.[298,300]

Circulating immune complexes can be detected in the subacute and convalescent (but not the acute) stages; these complexes appear to be unrelated to the development of coronary abnormalities by virtue of their time course of detection[124,204,206,212,266,270,306,387] and to be unaffected by IVGG infusions.[212] Some investigators have detected IgA in these immune complexes.[266] Most circulating IgG immune complexes in Kawasaki disease contain IgG1 and IgG3 antibodies,[206] with Fc portions that bind to monocytes and platelets. Immune complexes in Kawasaki disease may promote aggregation of platelets, with release of vasoactive factors.[206]

Several autoantibodies have been found in Kawasaki disease sera, but antinuclear antibody and rheumatoid factor are very uncommon findings. Antibodies to type III collagen have been detected,[171] but without clear relation to coronary complications. Some acute-phase sera contain IgM anti-myosin antibodies directed against epitopes that differ from those reactive with acute rheumatic fever sera, a finding suggesting a possible relationship with myocarditis in Kawasaki disease.[55] Both IgM and IgG anti-neutrophil cytoplasmic antibodies (ANCAs)[288,312,334] and anti-myeloperoxidase antibodies[288] have been reported in Kawasaki disease. Several reports have noted IgM or IgG anti-endothelial cell antibodies in Kawasaki disease, which may injure endothelial cells directly or participate in complement-mediated injury or in antibody-mediated cytotoxic reactions.[145,195,198-200] Among the endothelial cell antigens of interest are adhesion molecules such as intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), E-selectin, P-selectin, and endothelin, all of which may be up-regulated in Kawasaki disease.[35,93,166,232,261] The precise role of immunologic reactions to endothelial cells in the pathogenesis of Kawasaki disease is unclear; Miura and colleagues[229] found focal but not widespread expression of VCAM-1 or E-selectin on endothelial cells of acute Kawasaki disease coronary arteries, especially in areas of neovascularization. However, the temporal correlation of immune activation and endothelial cell activation with secretion of monocyte chemoattractant protein-1 (MCP-1)[35,200,368] and the development of coronary abnormalities and their suppression by IVIG are consistent with an immune stimulant that triggers an immune response leading to vascular damage in part through cytokine-induced exposure of endothelial epitopes, inflammatory cell infiltration, myointimal proliferation, matrix metalloproteinase release, and other mechanisms resulting in vascular wall damage.[102,315] A less than optimal animal model of Kawasaki disease induced by injected cell wall fragments of *Lactobacillus casei* has been reported.[193]

Investigation of the distribution of circulating T cells in acute and subacute Kawasaki disease has yielded conflicting results that range from no significant change in distribution of CD3, CD4, CD8, and CD19 cells at any stage of illness[51] to significant decreases in circulating CD8[203,369] or CD4[369] numbers acutely. An immunohistochemistry study of eight autopsied acute-stage Kawasaki hearts found activated T cells, a predominance of CD8 cells and macrophages, but no B cells,[31] whereas Terai and colleagues'[368] study of one autopsied child showed predominance of activated CD4 cells and macrophages as well as activated coronary endothelial cells. The presence of activated T cells in peripheral blood in acute Kawasaki disease is controversial.

As discussed earlier under etiology, expansion of Vβ2 and Vβ8 T-cell receptor families in acute Kawasaki disease has been reported,[1,2,56] but this finding has not been reproduced by other investigators.[3,211,224,256,280,370,376] Such expansion would suggest the possible role of a superantigen or could reflect clonal expansion of certain Vβ families in response to a conventional antigen. However, clonal expansion of CD8 T cells in acute Kawasaki disease supports the hypothesis that a conventional antigen rather than a superantigen is involved in the pathogenesis,[51,326] and compelling evidence of an oligoclonal IgA response in the vascular wall also strongly supports this hypothesis.[302]

Evidence for activation of neutrophils in Kawasaki disease is of interest because these cells, along with monocytes and macrophages, are postulated to mediate endothelial cell damage by production of oxygen radicals.[258,354,382] Kawasaki disease also is associated with widespread activation of monocytes and macrophages,[95-97] T lymphocytes,[98,311] and B lymphocytes.[168] Jejunal mucosal changes similar to delayed-type hypersensitivity in the epithelium and lamina propria, including decreases in $CD8^+$ cells and increases in $CD4^+$ and $HLA\text{-}DR^+$ cells, have been detected.[239] Delayed hypersensitivity skin test responsiveness to *Candida*, streptokinase/streptodornase, phytohemagglutinin, and PPD (tuberculin) is suppressed during the acute stage of Kawasaki disease but normalizes within 1 to 2 months.[403] Acute Kawasaki disease is associated with inflammatory reactivation of previous

*See references 17, 70, 94, 127, 165, 167, 185, 186, 197, 207, 208, 216, 218, 219, 301.

BCG vaccination sites, apparently reflecting both cellular and humoral responses.[405] We cared for a patient with acute Kawasaki disease and reactivation of a BCG site and the site of a recent tuberculin skin test.[371a] Plasma fibronectin levels are reported to be decreased early in illness but to rebound to high levels by the fourth week of illness.[319]

Circulating complement components C3 and C4 commonly are elevated, probably as acute reactants in the first several weeks of illness, and then normalize.[289] Nonetheless, complement appears to be activated through the classical pathway,[173] with high plasma C4D levels.[237]

Production of the chemoattractant leukotriene B_4 by polymorphonuclear cells is increased from 13 to 29 days after onset of Kawasaki disease,[114] and it may attract more inflammatory cells to the site. Leukotriene E_4 and prostaglandin E_2 also are elevated in Kawasaki disease,[92,192,220] as are increased platelet synthesis of thromboxane A_2 and plasma levels of thromboxane B_2.[126]

The strongest evidence of the importance of immunologic factors in the pathogenesis of vasculitis in Kawasaki disease is provided by the remarkably beneficial effect of IVIG on the acute febrile illness and the prevention of the development of coronary aneurysms.[238,247,251,262,321] It correlates with the effect of IVIG to reduce B-cell activation, more rapidly normalize T-cell activity, down-regulate cytokine secretion, and induce disappearance of endothelial cell activation antigens.[194,200] Potential mechanisms of the beneficial action of IVIG in Kawasaki disease include modulation of cytokine production, suppression of endothelial cell activation, inhibition of immunoglobulin production by a negative feedback mechanism, specific neutralization of an unknown etiologic agent or toxin, nonspecific blockade of a receptor for immune complexes, and augmentation of T-cell suppressor activity.[321]

TREATMENT IN THE ACUTE STAGE

INITIAL THERAPY

Patients diagnosed with Kawasaki disease should be admitted to hospital, undergo a baseline echocardiogram, and receive IVIG 2 g/kg over the course of 10 to 12 hours, with high-dose aspirin at 80 to 100 mg/kg/day in four divided doses (Table 88–4).[57,252] When administered by the 10th illness day, this regimen is highly effective in reducing the risk of development of coronary abnormalities.[249] Because few data exist regarding therapy of patients treated later than the 10th illness day, the goal is to treat by day 10 whenever possible. Patients who are diagnosed after the 10th illness day and who are still febrile may benefit from therapy, but the ability to prevent coronary changes is less certain. IVIG and aspirin have been shown to prevent the development of giant coronary aneurysms[52,297] and to have direct benefits on cardiac function.[249] The mechanism of action of IVIG in Kawasaki disease is unknown. The single high-dose schedule is superior to the earlier regimen of 400 mg/kg/day IVIG for 4 days with high-dose aspirin with respect to rapidity of defervescence and normalization of acute-phase reactants.[247,251,252,262] The large single-dose infusion generally is well tolerated, even in patients with decreased myocardial function.[249] Patients should remain hospitalized until they have been afebrile for at least 24 hours, to ensure that they are available for re-treatment if necessary.

TABLE 88–4 Treatment for Kawasaki Disease

Acute and Subacute Stages
IVIG, 2 g/kg infusion over 10-12 hr
PLUS
Aspirin, 80-100 mg/kg/day in four divided doses (until 14th illness day and patient afebrile at least 3-4 days), then 3-5 mg/kg once daily for 6-8 wk
IVIG may be repeated if fever persists or recurs together with at least one classic sign of Kawasaki disease (see text for other alternative "rescue therapies")
Convalescent Stage
No coronary abnormalities: no therapy
Transient coronary abnormalities: aspirin, 3-5 mg/kg once daily at least until resolution of abnormalities
Persistent small to medium coronary aneurysms: aspirin, 3-5 mg/kg once daily
Giant or multiple small coronary aneurysms: aspirin, 3-5 mg/kg once daily, with or without clopidogrel, with warfarin or low-molecular-weight heparin for most patients
Coronary obstruction: thrombolytic therapy, surgical or interventional procedures

IVIG, intravenous immunoglobulin.

Whether treatment with IVIG and aspirin earlier than the fifth illness day leads to poorer outcomes remains unclear. Tse and colleagues[378] compared 89 children treated on or before the fifth day of illness with controls treated on illness day 6 to 9. These investigators found significantly fewer patients with coronary ectasia at 1 year after Kawasaki disease and shorter total fever duration in the former group, without differences in frequency of recrudescence, need for additional therapy, need of steroids, length of hospitalization, or development of coronary aneurysms within 3 months. Zhang and associates[409] also concluded that patients treated before the sixth illness day had a significantly lower risk of developing cardiac sequelae compared with patients treated later than the sixth day. In contrast, Muta and colleagues[235] evaluated 4731 patients treated on days 1 to 4 and 4020 treated on days 5 to 9 and found that patients treated earlier were somewhat more likely to require additional therapy (odds ratio, 1.12, 1.10 to 1.16); no significant differences in cardiac sequelae were found between groups. Another study also found that treatment before illness day 5 was associated with increased need for re-treatment but no difference in coronary outcome.[82]

Single infusions of IVIG at doses less than 2 g/kg have not been demonstrated to be as effective as 2 g/kg.[18,74] A comprehensive meta-analysis of all Japanese and North American IVIG treatment trials showed that the coronary artery outcome is correlated directly with the *total* dose per kilogram of IVIG administered, with 2 g/kg superior to 1.6 g/kg, which is superior to 1.2 and 1.0 g/kg; the initial dose of aspirin used did not appear to influence coronary outcome.[367] Another study confirmed this finding.[69]

Patients diagnosed after the 10th illness day who are still febrile or who manifest other signs of active disease may benefit from IVIG and aspirin therapy because this treatment may result in prompt clinical improvement, with subsidence of fever and other signs of inflammation. No evidence supports that this approach results in lower rates of development of coronary abnormalities, however. Patients beyond the 10th to 12th illness day who have become afebrile and have resolved features of Kawasaki disease without therapy are unlikely to benefit from IVIG. Such children should be treated instead with aspirin, 3 to 5 mg/kg once daily, and should be evaluated carefully by serial echocardiograms. In patients who have already developed coronary aneurysms and whose acute manifestations of illness have resolved, no evidence suggests a beneficial effect of IVIG.

Various brands of IVIG differ in manufacturing processes and, therefore, in composition (e.g., proportion of IgG monomers, presence of proteins other than IgG). Although adverse reaction rates differ among products, clinical efficacy does not seem to differ.[294] An exception is a report that showed IVIG prepared with β-propiolactone, which can affect the biologic activity of the Fc portion of IgG, to be less effective in Kawasaki disease.[377] Measles and varicella immunizations (but not others)

should be deferred for 9 to 11 months after administration of high-dose IVIG because of impaired immune responses to these live virus vaccines.

The aspirin dosage for acute Kawasaki disease that is most thoroughly studied in the United States is 80 to 100 mg/kg/day in four doses. This therapy should be maintained until approximately the 14th day of illness (longer if necessary to ensure that the patient has been afebrile for ≥3 to 4 days) and then reduced to a daily dose of 3 to 5 mg/kg and continued until 2 to 3 months after onset of illness in those who have not developed coronary abnormalities.[252] Patients who develop coronary abnormalities should continue to take low-dose aspirin. High-dose aspirin is used for its anti-inflammatory activity, whereas the much lower doses inhibit platelet aggregation. Japanese clinicians generally use an intermediate anti-inflammatory dose of 30 to 50 mg/kg/day[182] because of perceived higher rates of untoward effects in Japan. Difficulty in achieving what are usually considered therapeutic anti-inflammatory serum salicylate levels during the acute phase of illness (20 to 25 mg/dL) may complicate aspirin treatment,[138] and selected refractory patients may require salicylate doses in excess of 100 mg/kg/day to achieve anti-inflammatory benefit. This situation probably reflects impaired absorption and bioavailability and enhanced salicylate clearance in acute Kawasaki disease.[174] An important note is that, with clinical improvement, patients with Kawasaki disease who are receiving very high doses can suddenly increase their salicylate absorption and may become salicylate toxic. Serum salicylate levels should be monitored if symptoms of vomiting, hyperpnea, tinnitus, lethargy, or striking liver function abnormalities develop in children receiving aspirin.

One study that randomized patients to receive salicylates at 80 to 100 mg/kg/day or 3 to 5 mg/kg/day for initial therapy (each regimen with 2 g/kg of IVIG) concluded that no difference in coronary outcome occurred, but a more prompt anti-inflammatory benefit was noted in the high-dose aspirin group.[225] In the absence of IVIG, aspirin therapy does not decrease the frequency of coronary abnormalities.[69] Reye syndrome has been reported rarely in children taking high-dose aspirin for Kawasaki disease, but whether low-dose aspirin poses this risk is unclear.[356]

The potential value of adding corticosteroid therapy to IVIG or aspirin for primary therapy was addressed in several trials.[250] For example, Jibiki and colleagues[140] compared 3 days of intravenous dexamethasone (0.3 mg/kg/day) plus heparin and IVIG, with subsequent low-dose aspirin, to IVIG plus higher-dose aspirin. These investigators found more prompt decreases in fever and inflammatory markers such as CRP in the dexamethasone-treated group, but no difference in rates of coronary abnormalities. Okada and associates[267] showed that the addition of intravenous prednisolone followed by a long taper of oral prednisolone to IVIG and aspirin was associated with shorter duration of fever and more prompt fall in CRP and circulating IL-2, IL-6, IL-8, and IL-10 levels. Inoue and colleagues[134] compared 88 patients treated with IVIG and aspirin with 90 patients who received in addition intravenous prednisolone (2 mg/kg/day in 3 divided doses) until afebrile and then orally until the CRP normalized, with a subsequent taper over 15 days, for a median of 23 days of steroids. The results indicated fewer coronary abnormalities, shorter durations of fever, more rapid decline in CRP level, and fewer initial treatment failures in the steroid recipients. Caution must be exercised in the interpretation of the findings of these Japanese trials because the IVIG regimens and aspirin doses differ from those used in the United States, and echocardiograms were not interpreted by investigators blinded to the treatment group.

The most definitive trial in this regard showed little if any direct benefit of the addition of a single dose of intravenous methylprednisolone (30 mg/kg) to the current IVIG and aspirin regimen, with the possible exception of patients who failed to respond to standard therapy and who required retreatment.[250] This observation will require additional study.

RESCUE THERAPY FOR TREATMENT FAILURES

Most patients with acute Kawasaki disease respond promptly to treatment with IVIG and aspirin, with defervescence and subsidence of inflammatory manifestations occurring within 48 hours.[57,247,322] A subgroup of approximately 5 to 15 percent, however, fails to show significant clinical response; these patients remain febrile 24 to 48 hours after receiving IVIG, or they manifest only transient improvement, with recurrent fever and clinical evidence of inflammatory signs. These patients need additional anti-inflammatory therapy, and specific guidelines or controlled treatment trials do not exist. Of course, when treating apparently treatment-refractory patients, it is also prudent to reconfirm the diagnosis.

In these patients, administration of a second dose of 2 g/kg of IVIG generally is effective in suppressing disease activity.[83,252,344] In a retrospective study of 179 patients with Kawasaki disease, 89 percent responded to the first dose of IVIG, and 67 percent of the nonresponders responded to a second IVIG dose; thus, only 3 to 4 percent of these patients failed to respond after a second dose of IVIG.[118] Carefully increasing the dose of aspirin to 120 mg/kg/day or higher, with monitoring of serum levels, may be helpful. Some physicians recommend a course (usually 3 days) of intravenous pulse methylprednisolone at 30 mg/kg/day instead of a second dose of IVIG.[390] We generally reserve pulse steroid therapy for the quite rare patient with highly refractory acute disease, who has failed to respond to at least two doses of IVIG, because early Japanese data suggested that steroids may predispose to development of coronary disease.[155] In addition, a more recent study noted coronary dilatation shortly after pulse steroids were administered in three of nine treatment-refractory patients.[122] No direct comparison between repeat IVIG dosing and corticosteroids has been performed. In a few particularly treatment-refractory patients, we employed a slow oral steroid taper over the course of several months once inflammatory activity appeared to have been controlled.

Infliximab (Remicade), a monoclonal anti–TNF-α antibody, has been reported in an open-label experience to be effective in most patients with acute Kawasaki disease who are refractory to standard therapy[39]; this agent is being studied in a randomized trial of patients with refractory Kawasaki disease. Even less published experience exists regarding other therapies for IVIG-refractory Kawasaki disease. Cyclophosphamide with or without methotrexate has been used in a very small number of patients.[385]

PREDICTION OF TREATMENT FAILURES

Several attempts have been made to develop scoring systems to predict, at the time of initial presentation, those patients who are at increased risk for failure to respond to standard therapy[73,172] or to develop coronary lesions after therapy.[231] In a retrospective analysis of 193 patients, Mori and colleagues[231] found that elevations in white blood cell counts, neutrophil counts, and CRP levels after IVIG treatment predicted increased risk for subsequent development of coronary lesions. Egami and colleagues[73] compared 279 patients who responded to initial standard treatment with 41 patients who were treatment resistant. These investigators developed a scoring system giving 1 point each for age less than 6 months, treatment before 4 days of illness, platelet count less than or equal to 300,000/mm^3, and CRP 8 mg/dL or higher, and 2 points for ALT 80 IU/L or higher; a score

of 3 points or higher identified the IVIG-resistant group with 78 percent sensitivity and 76 percent specificity.[73] A similar predictive scoring system for IVIG unresponsiveness was developed by Kobayashi and colleagues,[172] who gave two points each for day of illness at initial treatment 1 to 4 days, serum sodium less than 133 mmol/L, aspartate aminotransferase (AST) 100 IU/L or higher, and neutrophils 80 percent or greater, and two points each for CRP 10 mg/dL or higher, platelet count up to 300,000/mm^3, and age ≤12 months; this provided 86 percent sensitivity and 67 to 68 percent specificity for patients with 4 or more points.

SEQUELAE OF KAWASAKI DISEASE

The major (and virtually the only) sequelae of Kawasaki disease are cardiovascular, particularly involving the coronary arteries. Therefore, appropriate cardiac imaging is critical for the evaluation of patients suspected to have acute Kawasaki disease and for their subsequent follow-up. Echocardiography is considered the ideal imaging modality because it is noninvasive and has high sensitivity and specificity for detection of abnormalities of the proximal left main, left anterior descending, circumflex, and right coronary arteries.[252] This procedure should be performed under the supervision of an echocardiographer experienced with children, and internal arterial diameters should be measured and compared with normal standards for body surface area.[252] The American Heart Association classifies coronary aneurysms as small (<5 mm), medium (5 to 8 mm), or giant (>8 mm), and de Zorzi and colleagues[65] showed that adjusting coronary dimensions for body surface area may identify more accurately those patients with enlarged coronaries. Giant coronary aneurysms, defined as having 8 mm or larger internal diameter, are associated with worse prognosis, and these patients require particularly close follow-up.[52] The sensitivity and specificity of echocardiography to identify coronary thrombi and coronary stenosis are unclear, and visualization of coronary vessels is more difficult as body size increases. Therefore, angiography, magnetic resonance angiography (MRA), and ultrafast CT may be useful for selected patients with Kawasaki disease.[252]

MANAGEMENT BEYOND THE ACUTE STAGE

Patients with Kawasaki disease should be re-evaluated within 2 weeks after hospital discharge and again approximately 6 to 8 weeks after onset of illness because echocardiography at these time points is most likely to detect coronary artery aneurysms should they develop. If a baseline study and these two follow-up echocardiograms fail to detect evidence of coronary abnormality, performing further echocardiograms probably is unnecessary,[381] although a 6- to 12-month follow-up echocardiogram is performed at many centers.[58,252] Low-dose aspirin therapy (3 to 5 mg/kg/day) can be discontinued after the 6- to 8-week follow-up echocardiogram unless evidence of coronary abnormalities is present. To reduce the theoretical (and extremely low) risk of Reye syndrome in patients receiving low-dose aspirin, clopidogrel can be substituted for aspirin for a brief time in patients who develop varicella or influenza. Clopidogrel also can be used in the rare patient who is allergic to, or intolerant of, aspirin.

CARDIAC COMPLICATIONS

MYOCARDIAL INFARCTION

Myocardial infarction is the most common cause of death in Kawasaki disease (Table 88–5). In a cooperative Japanese study of 195 cases, the first myocardial infarction usually occurred in the first year after onset of disease and was fatal in 22 percent and asymptomatic in 37 percent of these patients. Major symptoms were shock, vomiting, and abdominal pain, with chest pain complaints only from children older than 4 years of age. Of those patients who survived a first infarct, 16 percent had a second myocardial infarct.[149,151] Patients with giant (>8 mm) coronary aneurysms are at greatest risk for having infarcts. Most fatal infarctions were the result of obstruction of the left main coronary artery or both the right coronary and left anterior descending coronary arteries; survivors were most likely to have isolated right coronary involvement. Approximately half the survivors of acute myocardial infarction had one or more complications, including ventricular dysfunction, mitral regurgitation, and arrhythmias. Parents of all children with Kawasaki disease with coronary abnormalities should be instructed to seek emergency medical care if chest pain, dyspnea, lethargy, or syncope develops. Prompt fibrinolytic therapy should be attempted at a tertiary care center if acute coronary thrombosis is diagnosed.[148] The degree of reversibility of coronary thrombosis in children with Kawasaki disease may be somewhat less than that in adults with atherosclerotic disease. Late cardiac sequelae of Kawasaki disease may not manifest until adulthood.[154]

TABLE 88–5 Cardiac Abnormalities in Kawasaki Disease

Acute Stage
Pericardial effusion
Decreased myocardial function
Mitral regurgitation
Brightness of coronary artery wall
Enlargement (ectasia) of coronary arteries
Arrhythmia (rare)
Subacute Stage
Coronary aneurysms, irregularity, ectasia
Mitral or aortic regurgitation, or both (rare)
Coronary aneurysm rupture (very rare)
Myocardial infarction (rare)
Convalescent Stage
Persistent coronary aneurysms
Regressed coronary aneurysms (residual fibrosis)
Coronary artery stenosis
Coronary aneurysm rupture (very rare)
Myocardial infarction (rare)

OTHER CARDIOVASCULAR COMPLICATIONS

Other cardiac complications include myocardial fibrosis, valvulitis, and coronary rupture. Some researchers have suggested that valvular disease occurs in as many as 1 percent of patients with Kawasaki disease; most of these cases result in mitral regurgitation.[5,170] Patients with well-documented aortic regurgitation also have been observed. At least one patient with Kawasaki disease developed severe aortic and mitral regurgitation that necessitated two valve replacements.[107] In an autopsy study, coronary rupture was a quite rare finding but was noted more commonly among older children who died of Kawasaki disease, whereas myocardial infarction was seen more commonly in younger fatal cases.[300]

Peripheral artery aneurysms develop in fewer than 1 percent of patients with Kawasaki disease, virtually always in those who have significant coronary abnormalities.[156,348] These abnormalities generally involve medium-sized muscular arteries, such as subclavian, brachial, axillary, iliac, or femoral arteries, and occasionally the hepatic or renal arteries or the abdominal aorta. In patients with Kawasaki disease who are undergoing their first coronary arteriography, abdominal aortography and subclavian arteriography are, therefore, recommended.[252]

PERIPHERAL GANGRENE

A rare but very serious complication in the acute febrile stage of Kawasaki disease is severe peripheral ischemia and dry gangrene of distal extremities.[373] Virtually all these patients have been young infants up to approximately 7 months of age with giant coronary aneurysms, and some have developed peripheral (especially axillary or iliac) arterial aneurysms as well. This complication is virtually unknown in Japan[161] and has been reported primarily in non-Asian children in North America.[68,373] Possible pathogenic mechanisms of peripheral gangrene include the following: severe arteritis of digital or other small peripheral arteries; arteriospasm of peripheral arteries, perhaps in association with severe vasculitis; thrombosis of inflamed or spastic arteries as a result of stasis and damaged endothelium; thrombosis of a more proximal aneurysm (especially axillary) with embolism distally; rarely, cardiogenic shock; and, most likely, a combination of these factors.[373,388] This process has led to amputations in a small number of infants. Therapy has been empiric, primarily because the precise mechanisms of disease are unclear, and has included aggressive use of anti-inflammatory agents, prostaglandin infusion, and anti-platelet, anti-coagulant, and vasodilation therapies.[68,373]

NONVASCULAR COMPLICATIONS

As many as 10 percent of patients with Kawasaki disease develop painful arthritis or arthralgia in the acute stage of disease, often in the ankles or elsewhere in the lower extremities, and they may require treatment with a nonsteroidal anti-inflammatory agent. Earlier-onset arthritis (≤10 days of illness) tends to be polyarticular, whereas later-onset arthritis involves primarily larger weight-bearing joints. We have used naproxen, usually 10 to 15 mg/kg/day divided into two or three doses, for several weeks with considerable success.

Abdominal pain and diarrhea in the early acute stage usually respond to intravenous hydration and supportive care. Acalculous distention (hydrops) of the gallbladder manifests clinically as right upper quadrant tenderness or a mass with or without obstructive jaundice and can be confirmed by ultrasonography.[338] Performing a cholecystectomy is not necessary. Hepatic involvement appears to be entirely self-limited and has not been associated with chronic liver disease.

Rare patients with Kawasaki disease develop hemophagocytic syndrome (HPS), also known as *macrophage activation syndrome* (MAS), as a complication. This syndrome manifests as persistent fever associated with cytopenias, hepatosplenomegaly, hepatic dysfunction, often hyperferritinemia, elevated serum lactate dehydrogenase, hypofibrinogenemia, and hypertriglyceridemia.[233,263,274] Therapy with high-dose prednisone or other immune modifiers are indicated for this rare but serious complication.

Rare events that occur in association with Kawasaki disease include sensorineural hearing loss,[345] transient unilateral facial nerve palsy,[101,281] and pneumonitis or pulmonary nodules.[84] We have cared for two older children who had sufficient abdominal findings to warrant performing appendectomies before establishing the diagnosis of Kawasaki disease. Consultation with a center that treats large numbers of patients with Kawasaki disease should be sought by the physician faced with rare or serious complications.

LONG-TERM FOLLOW-UP AND PROGNOSIS

Kawasaki disease normally is an acute and self-limited illness. However, cardiac abnormalities that develop when the disease is active may be progressive, and the prognosis clearly is related to the coronary artery status. Approximately 20 to 25 percent of patients not treated with IVIG develop coronary abnormalities that are detectable by two-dimensional echocardiography or angiography. The risk of development of coronary aneurysms is reduced to approximately 2 to 3 percent overall when IVIG is given in the first 10 days of illness.[247,251] However, the rates for coronary abnormalities for young infants are somewhat higher, even with timely IVIG therapy. Patients who develop moderate to severe coronary abnormalities are at risk of myocardial ischemia, myocardial infarction, and sudden death for at least 5 years after onset of illness.[151]

Children without apparent cardiac sequelae during the first month after onset of Kawasaki disease appear to return to their previous states of health, without cardiac signs or symptoms. However, some reports suggest the possibility of generalized endothelial dysfunction in patients with Kawasaki disease who never had coronary abnormalities. These dysfunctions include altered lipid metabolism,[43] increased brachial-radial artery mean pulse wave velocity,[49] lower myocardial flow reserve,[236] and abnormal endothelium-dependent brachial artery reactivity,[66,227,393] but the data conflict somewhat, and additional studies are needed.

Regression of small and medium aneurysms is a common occurrence. Overall, approximately half of all children with coronary aneurysms at 4 to 8 weeks after onset demonstrate regression by 1 to 2 years, with apparently normal vessels on angiography or echocardiography.[149] The likelihood that an aneurysm will regress is higher with smaller aneurysms, age younger than 1 year at the onset of Kawasaki disease, fusiform rather than saccular morphology, and involvement of a more distal coronary segment.[7,355] Regression of the internal diameter of the vessel to normal may occur by myointimal proliferation or by thrombus organization and recanalization.[87,309] If regression occurs, it generally does so within 2 years of the onset of disease.[152] Intravascular ultrasound studies show that thickened intima and media sometimes with calcifications are present in areas of regressed coronary aneurysms.[113,341] Regressed aneurysmal segments are histologically abnormal (intimal fibrosis) and have abnormal functional responses, with decreased vascular reactivity in response to exercise or pharmacologic agents such as isosorbide dinitrate or acetylcholine.[66,309,340] Only a small number of regressed aneurysms progress to stenosis.[153,340,347,350]

Functional abnormalities of coronary vessel endothelium relaxation have been reported years after the onset of Kawasaki disease[66,227] and warrant further investigation. Newer imaging methods have demonstrated vascular wall changes sometimes even in patients with no history of abnormalities in the acute phase. The meaning of these findings in patients thought to have escaped coronary abnormalities with acute Kawasaki disease and their long-term significance are uncertain.

Patients with giant coronary aneurysms are at the greatest risk for the development of significant stenosis with resultant myocardial ischemia.[133,156] The risk of significant stenosis, usually developing at the inlet or outlet of a moderate to large coronary aneurysm, shows a steady rise over 15 to 20 years of observation.[144,156,339,347] These markedly abnormal vessels are subject to calcification and thrombosis, which may lead to myocardial ischemia or infarction.

In 10- to 20-year follow-up studies of patients with Kawasaki disease, researchers found that the arteries most likely to develop stenosis are the right main and left anterior descending coronary arteries.[144,156,339,347,349] A limited number of studies of young adults with ischemic heart disease and history of diagnosed Kawasaki disease or a compatible clinical illness has been performed.[154] A survey of Japanese adult cardiologists identified 130 adult patients with coronary aneurysms detected by angiography to evaluate myocardial infarction or ischemia.[154] Twenty-one of these patients

(mean age, 34 years; range, 20 to 63 years) had a history compatible with Kawasaki disease in childhood. These patients had severe coronary disease with myocardial infarction, angina pectoris, mitral regurgitation, arrhythmias, congestive heart failure, and need for coronary bypass grafting. This study indicates that the coronary artery sequelae of Kawasaki disease likely are important causes of ischemic heart disease in young adults. Fatty deposits and advanced changes resembling atherosclerotic disease sometimes have been found at autopsy of children with a history of Kawasaki disease who died of other causes.[353] This finding raises the important but unanswered question of whether patients with Kawasaki disease are at increased risk for developing premature atherosclerosis or of having a more severe form of this disease.

LONG-TERM MANAGEMENT

The risk of coronary artery thrombosis or stenosis that may result in myocardial ischemia and infarction remains the most important long-term clinical problem in the subset of patients with Kawasaki disease who develop significant coronary abnormalities (see Table 88–5). Patients with medium (6 to 8 mm) and giant (>8 mm) aneurysms are at substantial risk for development of stenosis years after having the acute illness, compared with patients with small aneurysms or no aneurysmal changes,[7,144,149,152,156,348,380] and patients with giant aneurysms are at the highest risk for thrombosis.[252] Echocardiography and electrocardiography are not sufficiently sensitive to detect stenotic lesions. Various stress tests for detection of reversible myocardial ischemia, including nuclear perfusion scans with exercise, exercise echocardiography, and stress echocardiography with agents such as dobutamine, dipyridamole, or adenosine, have been used in children to detect stenosis.[252,260,272] Newer techniques, including stress magnetic resonance imaging (stress MRI), are being developed. Coronary arteriography remains the most definitive method to determine the degree of stenosis and the adequacy of collateral circulation. Intravascular ultrasound is an effective method to evaluate vascular wall morphology in selected patients during angiography.[342,346] All patients with evidence of myocardial ischemia or infarction should be studied by angiography to determine the need for intervention.[252] Most experts agree that patients with moderate to severe coronary artery aneurysms, a single large aneurysm, or multiple aneurysms should have their coronary anatomy defined by angiography at least once after the acute stage of illness (after the inflammatory process has subsided) to define fully the extent of involvement and to identify potential sites of thrombotic or stenotic complications.[252] Because aneurysms can occur in non-coronary arteries, especially the subclavian, brachial, axillary, iliac, and femoral arteries, and occasionally in the abdominal aorta and renal arteries,[348] in patients with coronary abnormalities, abdominal aortography and subclavian arteriography are recommended for patients with Kawasaki disease who are undergoing coronary angiography the first time.[252]

PATIENTS WITH NO EVIDENCE OF CORONARY ARTERY ABNORMALITIES AT ANY TIME (RISK LEVEL I)

Patients who have never manifested coronary artery abnormalities have no need for aspirin or other anti-platelet medication beyond 2 to 3 months after onset of illness or for restriction of physical activities in the convalescent stage. Only routine pediatric follow-up beyond 1 year, with routine cardiovascular risk assessment, is indicated.

PATIENTS WITH TRANSIENT CORONARY ECTASIA OR DILATATION (RISK LEVEL II)

Patients with transient coronary artery abnormalities that resolve by 6 to 8 weeks should be treated with aspirin, 3 to 5 mg/kg/day, until resolution of abnormalities. No restrictions are indicated after 6 to 8 weeks, angiography is not indicated, and risk assessment and counseling are recommended at 3- to 5-year intervals.

PATIENTS WITH ISOLATED (SOLITARY) SMALL TO MEDIUM (3- TO 6-MM) CORONARY ANEURYSM IN ONE OR MORE CORONARY ARTERIES (RISK LEVEL III)

Patients with solitary small to medium coronary artery aneurysms should be maintained on daily low-dose aspirin (3 to 5 mg/kg) at least until regression is documented with annual echocardiographic follow-up. For patients younger than 11 years old, no restriction on physical activity is indicated, but for those 11 years old and older, physical activity should be guided by a biennial stress test or myocardial perfusion test. Angiography should be performed if stenosis or ischemia is suggested.

PATIENTS WITH ONE OR MORE LARGE (>6-MM) OR GIANT (>8-MM) CORONARY ANEURYSM, OR MULTIPLE (SEGMENTED) SMALLER OR COMPLEX ANEURYSMS WITHOUT OBSTRUCTION (RISK LEVEL IV)

Long-term anti-platelet therapy with aspirin (3 to 5 mg/kg once daily) or clopidogrel (1 mg/kg/day up to adult dose of 75 mg) is indicated for these children and should be continued indefinitely. Anti-coagulant therapy with warfarin, with the international normalized ratio (INR) maintained at approximately 2.0 to 2.5, should be added for patients with giant aneurysms because these patients are at substantial risk for having coronary thrombosis. Daily subcutaneous low-molecular-weight heparin is an alternative to warfarin. All such patients should be under the care of a pediatric cardiologist with experience in managing patients with Kawasaki disease. Cardiac evaluation with echocardiogram and ECG should be performed every 6 months, with stress testing performed approximately annually. Angiography should be performed 6 to 12 months after the patient has recovered from the acute stage of disease to define the coronary anatomy, and it should be repeated whenever symptoms or stress tests suggest the presence of myocardial ischemia. Physical activity should be regulated on the basis of annual stress test results and level of anti-coagulation, and strenuous athletics should be discouraged.

PATIENTS WITH CORONARY ARTERY OBSTRUCTION (RISK LEVEL V)

Patients with obstructive lesions or signs of ischemia should be evaluated urgently for possible intervention. Balloon angioplasty, rotablator angioplasty, coronary artery bypass grafting, stent placement, and even cardiac transplantation all have been employed for patients with Kawasaki disease and particularly serious coronary artery disease.[6,48,112,136,379] Researchers have shown that arterial bypass grafts are superior to venous grafts in these patients.[169] Balloon angioplasty procedures have been associated with high rates of recurrent stenosis in patients with Kawasaki disease and coronary stenosis.[6]

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CHAPTER 89

Chronic Fatigue Syndrome

Leonard R. Krilov

"*All cases are unique and very similar to others.*" T.S. Eliot, *The Cocktail Party*, 1949.

Chronic fatigue syndrome (CFS) is an illness complex characterized by a prolonged (>6 months) period of constant or intermittent debilitating fatigue in association with multiple, often nonspecific, symptoms that may include new-onset headaches, decreased ability to concentrate, recurrent complaints of sore throat and tender cervical or axillary lymphadenitis, reports of low-grade fever, diffuse muscle or joint pain, postexertional increase in fatigue, and unrefreshing sleep. To date, no specific cause for this syndrome has been identified, and, despite similar arrays of signs and symptoms, patients with CFS likely represent a heterogeneous population. Still, evaluation of groups of patients with this symptom complex has provided information about pathophysiologic changes occurring in patients with CFS, and potentially beneficial, although not curative, approaches to therapy for affected individuals have been developed.

In an attempt to provide a degree of uniformity as a basis for research into the evaluation of such patients, the U.S. Centers for Disease Control and Prevention (CDC) developed a definition of CFS in 1988.[38] These criteria were revised in 1994, with physical signs removed from the definition because they appeared to be unreliably documented in studies, and the required number of symptoms for the diagnosis of CFS decreased from 8 of a list of 11 to 4 of 8 components (Table 89–1).[33] These changes, being less restrictive, may serve to increase the sensitivity of the diagnosis, but they also decrease the specificity. However, these criteria are purely speculative in the absence of any gold standard or definitive diagnostic test for CFS. These 1994 criteria also suggest subdivisions of patients with CFS for research purposes and provide guidelines for non-research clinicians as well. Other international groups have generated similar definitions of CFS for evaluating this condition.[12,54,80]

TABLE 89–1 Centers for Disease Control and Prevention 1994 Workshop Case Definition of Chronic Fatigue Syndrome

1. Fatigue (persistent or relapsing) that has new or definite onset, is of >6 months' duration, and leads to a substantial (>50%) reduction in level of activity
   PLUS
2. At least four of the following:
   Impaired memory or concentration
   Sore throat
   Tender lymph nodes: cervical or axillary
   Myalgias
   Arthralgias (multiple joints, without swelling or erythema)
   New onset (or in severity) of headaches
   Unrefreshing sleep (hypersomnia or insomnia)
   Postexertional fatigue lasting >24 hours
   AND
3. The absence of another diagnosis for the individual's signs and symptoms

Modified from Fukuda, K., Straus, S. E., Hickie, I., et al.: The chronic fatigue syndrome: A comprehensive approach to its definition and study. Ann. Intern. Med. 121:953-959, 1994.

The primary manifestations of CFS are severe fatigue of more than 6 months' duration that limits activity to less than 50 percent of premorbid function and the association of multiple other symptoms, as outlined in the case definitions of CFS.[33,38,54,80] These symptoms include new or more intense headaches, decreased ability to focus or concentrate, recurrent sore throats, a sensation of tender cervical or axillary lymph nodes, low-grade temperature elevations, myalgias and arthralgias, postexertional fatigue lasting longer than 24 hours, and sleep disturbances (hypersomnia or insomnia). The severity and persistence of these findings vary among individual patients with CSF. In addition, although not included in the case definitions, many patients report dizziness, especially with changes in position, feeling hot when others are cold or vice versa, chronic costochondritis, and a Raynaud-like phenomenon.

HISTORICAL OVERVIEW

In all likelihood, CFS is not a new illness. Numerous conditions with features comparable to those of CFS have appeared in the medical literature during the past several centuries.[91] Many of these descriptions attempted to associate an illness characterized by prolonged debilitating fatigue and numerous other symptoms with an infectious agent. They have included chronic brucellosis,[84] chronic enteroviral syndrome,[17,21,36] chronic candidiasis,[74] myalgic encephalomyelitis,[2] chronic mononucleosis (Epstein-Barr virus [EBV] infection),[3,40,96,97] human herpesvirus type 6 infection,[7,102] human herpesvirus type 7 infection,[26] chronic Lyme disease,[85] parvovirus B19 infection,[45] and a new retroviral infection.[23] Noninfectious conditions described with similarities to CFS include total allergy syndrome, hypoglycemia, neurasthenia, Iceland disease, Royal Free disease, and fibromyalgia rheumatica.[48] In addition to clinical similarity to CFS, all these conditions, at least at the time they were described, lacked a diagnostic test to confirm a definitive causative agent and, in their acute form, manifested with fatigue in association with multiple other complaints.

A review of the experience in the mid-1980s associated groups of patients with CFS-like illness with patients with chronic EBV infection.[3,40,96] These reports described elevated and aberrant

patterns of EBV antibody responses in individuals with prolonged fatigue and multiple symptoms consistent with CFS. Acute infectious mononucleosis also often manifests with fatigue, fever, malaise, sore throat, lymphadenitis, and multiple systemic complaints, although typically in a more pronounced manner. Subsequent studies, however, revealed that the elevation or pattern of EBV antibody responses in such patients were not consistently different from findings in others who resolved symptoms of acute EBV infection.[60] Additionally, shedding of EBV in secretions was not increased in these individuals, and no association was found between viral shedding and severity of symptoms.[98] Furthermore, no response to the antiviral drug acyclovir occurred in a group of such patients in a placebo-controlled, double-blind, crossover study, in terms of improvement of clinical symptoms.[93] After these observations were made, the name *chronic fatigue syndrome* was chosen to define this illness,[38] at least until a specific cause or marker for this illness is identified.

Cameron and colleagues recently reported on a cohort of patients with prolonged fatigue after having infectious mononucleosis. These investigators demonstrated no difference in EBV load in eight patients with 6 months or more of disabling symptoms after the diagnosis of infectious mononucleosis was established, and again they demonstrated a lack of correlation between persistent symptoms and viremia or altered host responses to EBV.[10]

TABLE 89–2 Symptoms Reported by 58 Children and Adolescents Evaluated for Chronic Fatigue Syndrome (1989 to 1994)

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Please refer to the printed publication.

From Krilov, L. R., Fisher, M., Friedman, S. B., et al.: Course and outcome of chronic fatigue in children and adolescents. Pediatrics 102:*360-366, 1998.*

EPIDEMIOLOGY

The definitions of CFS allow for attempts to characterize the prevalence and demographic features of this condition. Still, given the vagaries of the symptom complex, possible variations in application of the diagnosis by different health care providers, and the potential differences within groups of people to seek medical attention for this condition, data reported on these issues may not be a complete representation of the epidemiology of this condition. CFS has been reported in all age groups, including children as young as 5 years of age, and in all ethnic, racial, and socioeconomic groups, but most CFS cases have been reported to occur in middle- to upper-class white women with a median age of 35 to 40 years.[47] Based on a study of physician-diagnosed patients from four cities using the 1988 case definition of CFS,[1] the CDC estimates a minimum prevalence rate of CFS of 2 to 10 cases per 100,000, or higher, in adults aged 18 years and older in the United States.[8,57,71] A population-based descriptive epidemiologic study conducted by the CDC in Kansas estimated the prevalence of CFS to be 235 per 100,000 persons and a 1-year incidence to be 180 per 100,000 persons.[75] Studies from outside the United States have reported similar or higher prevalence rates of CFS in adults.[27,54]

Although the CDC definition does not include age criteria for the diagnosis of CFS, the prevalence of CFS in the pediatric age range has not been studied well. Some researchers have suggested that the diagnosis of CFS should not be used in children, to avoid potential delay in making an alternative medical or psychological diagnosis.[70] However, many referral centers have reported groups of pediatric patients, primarily adolescents, with features and a clinical course similar to those reported in adults.[5,13,28,50,58,83] Furthermore, in these studies, missed or alternative diagnoses were not found, despite years of follow-up. Rimes and colleagues reported prevalence rates of CFS in an adolescent population similar to those reported in adults.[76]

In these pediatric studies, a predominance of female patients with a median age of 14 years at the time of diagnosis was noted. Additionally, these patients were from predominately middle- to upper-class families, and, as discussed later, their signs and symptoms at presentation (Table 89–2) and their course of illness were similar to those reported in adults with CFS. CDC studies suggest that the prevalence of CFS in adolescents approaches that reported in adults, but cases in children younger than 12 years of age occur much less commonly.[33] One suggestion is that a shorter duration of symptoms (3 to 4 months versus 6 months) may be appropriate for establishing the diagnosis of CFS in pediatric patients.[33,41] Multiple family members with CFS may be seen as well, but to date, evidence that CFS is contagious does not exist.

ETIOLOGY AND PATHOGENESIS

INFECTION

As noted previously, an infectious origin of CFS has not been demonstrated to date, and a single microorganism as the cause of CFS is unlikely. However, an acute infection appears possibly to play a role in precipitating CFS because most (as many as two thirds) patients with CFS relate a sudden onset of their symptoms to an acute infection, most often infectious mononucleosis. Lyme disease[56,85] and an influenza-like illness[39] also have been reported in association with the onset of CFS.

IMMUNOLOGIC DYSFUNCTION

A role for immunologic dysfunction in CFS has been suggested, based on numerous studies demonstrating abnormalities in lymphocyte function or cytokine production.[9,46,53,61,92,95,99] However, these findings have not been reproducible in different groups of patients with CSF. Some evidence suggests that nonspecific elevation of antibody titers and an increase in allergic symptoms occur in patients with CSF.[94] However, these findings are mild, and similar immunologic changes may be seen in other conditions, including depression. Individuals with CFS may report an increased frequency and duration of infections compared with their pre-CFS state, but they tend to be mostly routine viral illnesses. Neither unusual or opportunistic infection nor increased risk for developing malignant disease has been observed in these individuals. Intravenous immunoglobulin infusions have not

demonstrated long-term clinical benefit when they are administered to patients with CFS and may cause significant adverse reactions.[55,69,90]

Some studies have focused on potential genetic markers of CFS using DNA microarray technology.[86] An analysis of cytokine gene polymorphisms in 80 Italian patients with a diagnosis of CFS assessed using the 1994 CDC definition reported significant difference in tumor necrosis factor and interferon-γ phenotypes compared with controls. The authors hypothesized a potential altered immunologic or inflammatory response based on these differences as potentially contributing to the pathogenesis of CFS.[11] An analysis of prolonged post-infectious fatigue in patients who had had mononucleosis identified certain genes involved in immune responses, and hormonal and neurologic pathways that were altered in a subset of patients with mononucleosis were associated with the development of prolonged fatigue.[10] The CDC has developed a molecular epidemiology program to standardize assays that can be applied to understanding the potential of alterations in gene expression in CFS (available at http://www.cdc.ncidod/diseases/cfs/publications/molecular_epi.htm; accessed 7/12/2007).

NEUROLOGIC FEATURES

Many patients with CFS report cognitive impairment manifest as a decreased ability to concentrate and focus, difficulty in processing information, and trouble with word recall. Complaints of headache (new onset or altered pattern) and other neurologic symptoms, such as paresthesias and dysequilibrium, also are reported commonly by patients with CFS.

Despite these complaints, physical examination does not reveal abnormal neurologic signs. Formal neuropyschometric testing also may not reveal objective abnormalities to the extent reported by the patient. Whether this discrepancy reflects a problem with testing methods or altered perception on the part of the individual is uncertain at this time.[7,66]

Magnetic resonance imaging (MRI) studies have been reported to show an increase in cerebral white matter abnormalities in patients with CFS.[7,65,78] Similarly, in many studies, single photon emission computed tomography (SPECT) scanning has demonstrated changes in perfusion in certain areas of the brain in patients with CFS.[19,30,79] In other studies, however, abnormalities specific to CFS have not been observed consistently, and methodologic concerns have been raised regarding the interpretation of abnormal findings cited previously.[18,37] An abnormality at the base of the fourth ventricle detected on focal computed tomography (CT) scan of the base of the brain and responding to neurosurgical intervention was reported in a group of patients with CFS,[35] but this defect is not seen on routine CT or magnetic resonance imaging (MRI) views, and this observation requires confirmation by other researchers before determining its significance for these patients. At present, imaging of the central nervous system is not indicated routinely in the evaluation of a patient with CFS.

ENDOCRINOLOGIC FACTORS

Subtle defects in the hypothalamic-pituitary-adrenal axis have been described in cohorts of patients with CFS. These abnormalities include decreased free urinary cortisol levels and exaggerated adrenal responsiveness to corticotropin infusion.[25] These changes are quantitatively minor, and mean values are still within normal for age and sex, thus rendering these values unreliable as a diagnostic test for CFS. Similar changes have been described in patients with fibromyalgia and post-traumatic stress disorder.

CARDIOVASCULAR FACTORS

In 1995, investigators at Johns Hopkins Hospital in Baltimore described a cohort of seven adolescents with chronic fatigue and autonomic dysfunction as assessed by tilt-table testing.[77] Furthermore, these patients reported improvement or resolution of symptoms, including fatigue, with treatment of their orthostatic intolerance. Further studies from this group demonstrated some component of orthostatic hypotension in 92 percent of patients with CFS.[6] Studies in groups of adults, in contrast, showed tilt-table test abnormalities in only 25 to 40 percent of patients.[31,82] Whether this finding reflects true age-related differences in this phenomenon in patients with CFS is uncertain, given potential methodologic differences in the performance of the tests.

The pattern of orthostatic intolerance among adolescents with CFS was characterized further by Stewart and colleagues and is consistent with the postural orthostatic tachycardia syndrome (POTS).[87-89] Symptoms of POTS may develop after an acute infectious illness or with severe deconditioning and include fatigue, light-headedness, impaired cognition, inappropriate sweating, headache, palpitations, nausea, vomiting, and tremulousness. These authors suggest that CFS may be an extreme expression of POTS in at least some cases.

The importance of these cardiovascular findings in CFS remains unsettled. Subjective components may contribute to defining a tilt-table test as abnormal because subjects are not blinded during these studies. Some healthy individuals also may experience hypotension during these tests. Further studies defining the effect of treatment of POTS on the course of CFS should help to answer this question. In this context, evaluation and therapy of orthostatic changes, especially if dizziness is a significant component of the patient's complaints, may be indicated.[109]

SLEEP PHYSIOLOGY

Sleep disturbances have been described in selected groups of patients with CFS, and some of these patients may be amenable to therapy.[52,73] However, no single pattern of sleep abnormality has been reported for these patients, and in our experience, the pattern of hypersomnia or insomnia tends to improve as the patient recovers.

PSYCHOLOGICAL COMPONENTS

Psychological factors have been considered in the origin and perpetuation of CFS. Adults with CFS have demonstrated a higher frequency of depression and other psychiatric disorders before the onset of their CFS compared with age-matched controls.[1,51] Certainly, many of the symptoms reported in CFS also are reported commonly in depression. They may include sleep disturbances, loss of energy, difficulty in concentrating, changes in appetite, and musculoskeletal complaints. Studies in children and adolescents with CFS also have shown significant psychological features, especially depression and somatization, compared with both healthy controls and those with other chronic illnesses (e.g., juvenile rheumatoid arthritis, cancer, cystic fibrosis).[13,14,41,67,104] Still, certain features of CFS argue against its being solely a variant of depression. Individuals with CFS do not have the mood-related symptoms reported in patients with clinical depression. These mood-related symptoms include negative affect, anhedonia, low self-esteem, and suicidal ideation. Furthermore, patients with CFS and their families have a firm belief that an infectious, immunologic, or other medical cause for their symptoms exists, and the patients desire a return to normal activities.

In considering the possible link between depression or psychological stress and CFS, several possible relationships may exist. Preexisting depression or stress may create a psychological vulnerability that allows for the development of CFS in combination with any of numerous other factors discussed previously. Studies have demonstrated a role for psychological factors predicting the response to mononucleosis and influenza.[39,43] Similar factors may be involved in the development of CFS. Alternatively, depression may be a physiologic consequence of the central nervous system changes that occur in those who develop CFS, just as decreased concentration and memory occur as part of the syndrome.[41,104] Furthermore, reactive depression may occur in these patients in response to the inability to participate in their usual activities and to absence from school and separation from friends. Finally, CFS may be, at least in some cases, a manifestation of separation anxiety or school phobia in which secondary gain, as a conversion reaction, is playing a major role. Certainly, at least a subset of adolescents and children with CFS in our experience does not appear eager to return to school or activities, and secondary gain may play a role in the perpetuation of their illness.[48]

In summary, each of these potential links between depression or stress and CFS likely plays a role, with the relative contribution of each feature varying for different people. This suggestion is consistent with the overall hypothesis that many different factors appear to contribute to the development and perpetuation of CFS. The relative contribution of these different features may vary from individual to individual and even for the same patient over the course of the illness. The assessment and management of the patient with CFS should attempt to consider these issues and their relative importance for that individual.

DIAGNOSIS, DIFFERENTIAL DIAGNOSIS, AND EVALUATION

The diagnosis of CFS is one of exclusion and requires a comprehensive history. In the pediatric population, this information is obtained best from the patient and parents. Additionally, we request that families bring prior medical records, test results, and pertinent school records, to facilitate a complete evaluation. This process may be lengthy because the details are complicated, long standing, and often a source of debate between the patient and parents. The history should focus on the onset of illness, the duration and severity of symptoms, prior evaluations (often multiple) as well as medical history before the illness, family history, academic performance, and social history. The physical examination of the individual with suspected CFS almost always is essentially normal despite the multitude of symptoms. Findings of mild pharyngeal erythema and cervical adenopathy commonly are reported. However, finding fever, weight loss, significant adenopathy, or organomegaly should alert the clinician to the possibility of an alternative diagnosis.

DeMeirleir and colleagues reported increased detection of a 37-kd, 2-5A-synthetase binding protein in peripheral blood mononuclear cells from patients with CFS compared with healthy controls.[24] These binding proteins are related to the ribonuclease L antiviral pathway of these cells. This finding requires further confirmation before it can be considered a potential diagnostic test for CFS. At present, no specific tests for diagnosing CFS exist, and laboratory testing is aimed primarily at eliminating other possible diagnoses. Most patients have undergone multiple laboratory tests before a diagnosis of CFS has been considered, but these tests may be repeated or completed as part of the initial CFS evaluation. Additionally, interpreting previously performed tests may be part of the initial patient assessment. Screening studies may include complete blood count with differential and platelets, erythrocyte sedimentation rate, hepatic and renal function studies, urinalysis, and thyroid function tests. Additional tests that may be indicated based on history and physical examination may include toxicology screening, human immunodeficiency virus serology, antinuclear antibody, rheumatoid factor, tuberculin skin test, and cortisol level. Serologic evaluations for EBV, Lyme disease, and group A streptococcal infection may be requested based on history and physical infection. In most instances, these tests are not indicated; however, they often are obtained before CFS is suspected, and they need to be appropriately reviewed or repeated as part of the initial evaluation for CFS. Screening radiographic studies may include a chest radiograph or imaging of the paranasal sinuses based on the patient's symptoms.

Elimination of every possible disorder that may cause a patient to experience prolonged fatigue is impossible, but when guided by history, physical examination, and laboratory screening tests as outlined previously, the clinician can make a reliable diagnosis of CFS. Follow-up for periods of 4 to 13 years for pediatric patients diagnosed with CFS have not identified cases of missed or alternative diagnoses for their complaints.[4,50] In general, the longer the duration and the greater the number of symptoms, the less the need for extensive laboratory evaluations to suggest the diagnosis of CFS. Conversely, alternative diagnoses should be considered if a single symptom dominates the clinical presentation or if physical examination or laboratory tests reveal significant abnormalities.

Psychosocial assessment is indicated for all children and adolescents presenting for CFS evaluation. The extent of such evaluation may be limited to assessment by primary care personnel or may include collaboration with a social worker, psychologist, or psychiatrist, based on the individual's needs and the comfort level of the examiner. Cardiopulmonary and neurologic evaluations may be used in some cases, both to consider possible alternative diagnoses and to assist in assessing factors that may be contributing to the symptoms of CFS.

MANAGEMENT

"One of the essential qualities of the clinician is interest in humanity, for the secret of care of the patient is in caring for the patient."

Dr. Francis Peabody, 1926 (wall plaque, lobby of the Massachusetts General Hospital, Boston)

Management of patients with CFS is aimed at providing a combination of supportive treatment and emotional support.[32] Such an approach is outlined in Table 89–3.

This process can be initiated during the initial evaluation, with a discussion of the diagnostic criteria for CFS and a review of previously obtained laboratory tests. The relationship between physical and psychological symptoms can be explained, and the patient and family can be reassured that symptoms are real, even if the symptoms have psychiatric components. Additionally, the patient and family can be advised that most children and adolescents with CFS do well over time[4,28,49,50] and have a better long-term outlook than that described for adults with CFS.[42,108] Furthermore, emphasizing the frequent ups and downs in symptoms that generally characterize the course of CFS is important in assisting the patient and family to develop coping skills. This assistance may include guidance on how to modify lifestyle most appropriately and how to set realistic schedules and goals. Studies from the United Kingdom suggest that formal cognitive-behavioral therapy[22,72,81,107] or graded exercise programs, or both, may be beneficial for patients with CFS.[15,34,105]

Many other therapies have been advocated by different groups for patients with CFS. These treatments may include supplements, such as essential fatty acids,[103] magnesium,[20] liver extract

TABLE 89–3 Approach to Management of Chronic Fatigue Syndrome in Children and Adolescents

Evaluation and explanation of the diagnosis, including overview of multifactorial components
Reassurance that symptoms are real
Anticipatory guidance regarding secondary problems, up-and-down course of syndrome, secondary gain
Coping skills: lifestyle modification, decreased stress, and realistic expectations and schedule
Cognitive behavioral approaches: gradual increases in activity, an exercise program, attention to sleep patterns, attention to nutrition
Psychological support for individual and family
Educational issues: return to classes, home tutors, neuropsychiatric testing as indicated
Relationship issues: friends and family
Follow-up plan: monitoring of physical symptoms and psychological issues, ongoing guidance and reassurance (follow-up visits every 4 to 6 weeks)
Minimize: shopping for a doctor; unnecessary testing; family strain; unconventional, unproven, or experimental therapies

Modified from Krilov, L. R., and Fisher, M.: Chronic fatigue syndrome in children and adolescents. Contemp. Pediatr. 19*:61-68, 2002.*

injections,[44] and vitamin and nutritional supplements,[59] as well as pharmacologic treatments, such as steroids,[16,62,68] reduced nicotinamide adenine dinucleotide,[100] antidepressants,[64,101] and growth hormone.[63] Homeopathic therapies, osteopathy, and massage therapy[29] also have been reported to be beneficial in the management of CFS. Most of these approaches have not been studied adequately to allow definitive comment on their potential benefit for a given patient. Still, the clinician should be aware of them, to help guide patients who are likely to hear about them from other patients or outside sources, including the Internet.[35,106,107]

PROGNOSIS AND FUTURE DIRECTIONS

CFS likely affects heterogeneous groups of patients, and a single cause or definitive treatment modality most likely will not be uncovered. Nonetheless, studying groups of such patients yields helpful information on the pathophysiology of this condition, and useful steps to address and alleviate symptoms for patients have been reported. In recognition of the significance of this entity, the CDC launched an awareness campaign, including the development of an Internet site for health care professionals and the public (http://www.cdc.gov.cfs; accessed 7/12/2007). In addition, long-term follow-up data demonstrating improvement over the course of time, especially in children and adolescents, without emergence of significant other conditions, are encouraging for patients, families, and clinicians caring for such individuals.

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SECTION XVI

BACTERIAL INFECTIONS

CHAPTER 90

NOMENCLATURE FOR AEROBIC AND ANAEROBIC BACTERIA

David A. Bruckner

Table 90–1 represents an update of the current nomenclature, taxonomy, and classification of various microbial agents. Taxonomic methods have evolved from the use of biochemical testing to molecular characterization using 16S or 23S rRNA. Molecular methods have helped to define groupings of organisms and have led to considerable changes in bacterial nomenclature. The classification process is not complete until molecular and phenotypic descriptions of the studied taxa are provided. The primary purpose of nomenclature is to permit us to know as exactly as possible what another clinician, microbiologist, epidemiologist, or investigator is referring to when describing an organism responsible for infecting individuals or for causing an outbreak. The *International Code of Nomenclature of Bacteria*[20] includes rules on how to name bacteria and use the name. The most comprehensive taxonomic information available for bacteriologic classification can be found in *Bergey's Manual of Determinative Bacteriology*, ninth edition,[9] and in *Bergey's Manual of Systematic Bacteriology*,[7,8] volumes 1 through 4. Leading journals that contain up-to-date information on nomenclature and new species include *Anaerobe*, *International Journal of Systematic and Evolutionary Microbiology*, *Annales de Microbiologie* (Institut Pasteur), *Current Microbiology*, *Journal of Clinical Microbiology*, and *Systematic and Applied Microbiology*. An overview of validly published names can be obtained at http://www.bacterio.cict.fr or http://www.dsmz.de/microorganisms/bacterial_nomenclature.php.

Taxonomic ranks for naming bacterial organisms include kingdom, division, class, order, family, genus, species, and subspecies. All these ranks have official standing in nomenclature. Ranks below subspecies have no official standing but are used to indicate groups of strains or isolates that can be distinguished by some special characteristics (Table 90–2). Each taxonomic name should be represented by a nomenclature type. The species is represented by a type strain that is deposited in a recognized culture collection.

Nomenclature priorities for bacteriologic names date back to May, 1753. Because of difficulties in searching literature and limited available information on described species, approved lists of bacterial names were published in the *International Journal of Systematic Bacteriology* in 1980. Names not included on those lists have lost all standing in nomenclature status.

Historically, bacterial classification has been based on phenotypic characteristics. Multivariate analysis has played a large role in classification since the 1950s. This analysis used biochemical, cultural, and morphologic characteristics and susceptibilities to antibiotics and inorganic compounds to define the degrees of similarities among organisms. More recently, molecular techniques (e.g., DNA hybridization, rRNA-DNA hybridization, gene sequence analysis) have played a major role in determining phylogenetic relationships.

The DNA molecular weight for most bacteria is 1×10^9 to 8×10^9 daltons, enough to specify 1500 to 6000 genes. Using nucleic acid analysis, researchers have developed numerous parameters to determine taxonomic relationships. These parameters include genome size, mole percent guanine plus cytosine content, DNA relatedness under optimal and supraoptimal conditions for DNA reassociation, and rRNA oligonucleotide sequences. By correlating phenotypic results with DNA homology and rRNA sequence analysis, researchers have been able to select phenotypic tests that can be used more accurately to identify organisms belonging to specific groups.

The bacterial classification shown in Table 90–1 is based on the organism's morphologic and stain characteristics. Organisms included are those that often are associated with pathologic processes or that are medically significant. The current names are those either officially recognized or proposed for recognition and currently used in the literature.[1-21] The type species for *Salmonella* is *Salmonella enterica* rather than *Salmonella choleraesuis*, although in the United States, Public Health uses the genus *Salmonella* and the serovar for reporting purposes.[4]

Text continued on p. 1196

TABLE 90–1 Current Bacterial Nomenclature, Taxonomy, and Classification

I. Aerobic Gram-Positive Cocci

Characteristics: aerotolerant anaerobes that can occur singly or in pairs, tetrads, chains, or clusters; they can be catalase-positive or catalase-negative. Organisms positive for coagulase or clumping factor include *Staphylococcus aureus*, *Staphylococcus intermedius*, *Staphylococcus lugdunensis*, and *Staphylococcus schleiferi* subspecies *coagulans*.

Current Name	Synonym
Catalase-Positive Organisms	
Alloiococcus otitidis	*Alloiococcus otitis*
Kocuria kristinae	*Micrococcus kristinae*
Kocuria rosea	*Micrococcus roseus*
Kocuria varians	*Micrococcus varians*
Kytococcus schroeteri	
Kytococcus sedentarius	*Micrococcus sedentarius*
Micrococcus luteus	
Micrococcus lylae	
Nesterenkonia halobia	*Micrococcus halobius*
Staphylococcus arlettae	
Staphylococcus aureus subspecies *anaerobius*	
Staphylococcus aureus subspecies *aureus*	
Staphylococcus auricularis	
Staphylococcus capitis subspecies *capitis*	
Staphylococcus capitis subspecies *ureolyticus*	
Staphylococcus caprae	
Staphylococcus carnosus subspecies *carnosus*	
Staphylococcus carnosus subspecies *utilis*	
Staphylococcus casei	*Staphylococcus succinus* subspecies *casei*
Staphylococcus chromogenes	*Staphylococcus hyicus* subspecies *chromogenes*
Staphylococcus cohnii subspecies *cohnii*	
Staphylococcus cohnii subspecies *urealyticus*	
Staphylococcus condimenti	
Staphylococcus delphini	
Staphylococcus epidermidis	*Staphylococcus albus*
Staphylococcus equorum	
Staphylococcus felis	
Staphylococcus fleurettii	
Staphylococcus gallinarum	
Staphylococcus haemolyticus	
Staphylococcus hominis subspecies *hominis*	
Staphylococcus hominis subspecies *novobiosepticus*	
Staphylococcus hyicus	
Staphylococcus intermedius	
Staphylococcus kloosii	
Staphylococcus lentus	*Staphylococcus sciuri* subspecies *lentus*
Staphylococcus lugdunensis	
Staphylococcus lutrae	
Staphylococcus muscae	
Staphylococcus nepalensis	
Staphylococcus pasteuri	
Staphylococcus piscifermentans	
Staphylococcus pseudintermedius	
Staphylococcus saccharolyticus	*Peptococcus saccharolyticus*
Staphylococcus saprophyticus subspecies *saprophyticus*	*Micrococcus* subgroup 3
Staphylococcus schleiferi subspecies *coagulans*	
Staphylococcus schleiferi subspecies *schleiferi*	
Staphylococcus sciuri subspecies *rodentium*	
Staphylococcus sciuri subspecies *sciuri*	
Staphylococcus simulans	
Staphylococcus succinus subspecies *succinus*	
Staphylococcus vitulinus	
Staphylococcus warneri	
Staphylococcus xylosus	
Catalase-Negative Organisms	
Abiotrophia defectiva	*Streptococcus defectivus* Nutritionally variant streptococci
Aerococcus christensenii	
Aerococcus sanguicola	*Aerococcus sanguinicola*
Aerococcus urinae	
Aerococcus viridans	
Dolosicoccus paucivorans	
Dolosigranulum pigrum	
Enterococcus avium	*Streptococcus avium* Group D *Enterococcus*
Enterococcus caccae	
Enterococcus casseliflavus	*Enterococcus flavescens* *Streptococcus casseliflavus*
Enterococcus cecorum	*Streptococcus cecorum*
Enterococcus dispar	
Enterococcus durans	*Streptococcus durans* Group D *Enterococcus*
Enterococcus faecalis	*Streptococcus faecalis* Group D *Enterococcus*
Enterococcus faecium	*Streptococcus faecium* Group D *Enterococcus*
Enterococcus gallinarum	*Streptococcus gallinarum*
Enterococcus gilvus	
Enterococcus hirae	
Enterococcus italicus	*Enterococcus saccharominimus*
Enterococcus malodoratus	
Enterococcus mundtii	
Enterococcus pallens	
Enterococcus pseudoavium	
Enterococcus raffinosus	
Facklamia hominis	
Facklamia ignava	
Facklamia languida	
Facklamia sourekii	
Gemella bergeriae	
Gemella haemolysans	*Neisseria haemolysans*
Gemella morbillorum	*Streptococcus morbillorum* *Peptostreptococcus morbillorum*
Gemella sanguinis	
Globicatella sanguinis	Salt-tolerant viridans streptococci
Granulicatella adiacens	*Abiotrophia adiacens* *Streptococcus adiacens* Nutritionally variant streptococci
Granulicatella elegans	*Abiotrophia elegans* Nutritionally variant streptococci
Granulicatella para-adiacens	
Helcococcus kunzii	
Helcococcus pyogenica	
Helcococcus sueciensis	
Ignavigranum ruoffiae	
Lactococcus garviae	*Streptococcus garvieae* Lancefield group N
Lactococcus lactis	
Leuconostoc citreum	
Leuconostoc cremoris	
Leuconostoc dextranicum	
Leuconostoc lactis	
Leuconostoc mesenteroides	
Leuconostoc pseudomesenteroides	
Oenococcus oeni	*Leuconostoc oenos*
Pediococcus acidilactici	
Pediococcus damnosus	
Pediococcus dextrinicus	
Pediococcus equinus	*Streptococcus equinus*
Pediococcus parvulus	
Pediococcus pentosaceus	
Streptococcus acidominimus	

TABLE 90–1 Current Bacterial Nomenclature, Taxonomy, and Classification—cont'd

Current Name	Synonym
***Streptococcus bovis* group**	Group D streptococci
Streptococcus alactolyticus	
Streptococcus bovis	
Streptococcus equines	
Streptococcus gallolyticus subspecies *gallolyticus*	
Streptococcus gallolyticus subspecies *macedonicus*	
Streptococcus gallolyticus subspecies *pasteurianus*	
Streptococcus infantarius	
***Streptococcus milleri* group**	Viridans streptococci
Streptococcus anginosus	
Streptococcus constellatus	
Streptococcus intermedius	
***Streptococcus mitis* group**	Viridans streptococci
Streptococcus australis	
Streptococcus infantis	
Streptococcus mitis	*Streptococcus mitior* *Streptococcus sanguis* II
Streptococcus oralis	
Streptococcus peroris	
Streptococcus pneumoniae	*Diplococcus pneumoniae*
Streptococcus pseudopneumoniae	
***Streptococcus mutans* group**	Viridans streptococci
Streptococcus cricetus	
Streptococcus mutans	
Streptococcus ratti	
Streptococcus sobrinus	
***Streptococcus pyogenes* group**	
Streptococcus agalactiae	Group B streptococci
Streptococcus canis	
Streptococcus dysgalactiae subspecies *equisimilis*	Group C streptococci *Streptococcus equi* *Streptococcus equi* subspecies *zooepidermidis* *Streptococcus equisimilis* Group G streptococci
Streptococcus iniae	*Streptococcus shiloi*
Streptococcus porcinus	
Streptococcus pyogenes	Group A streptococci
***Streptococcus salivarius* group**	Viridans streptococci
Streptococcus salivarius	
Streptococcus thermophilus	
Streptococcus vestibularis	
***Streptococcus sanguinis* group**	Viridans streptococci
Streptococcus cristatus	*Streptococcus crista*
Streptococcus gordonii	
Streptococcus massiliensis	
Streptococcus parasanguis	
Streptococcus sanguis	
Streptococcus suis	
Tetragenococcus halophilus	*Enterococcus solitarius*
Vagococcus fluvialis	
Weissella paramesenteroides	*Leuconostoc paramesenteroides*

II. Anaerobic Gram-Positive Cocci
Characteristics: occur singly or in pairs, chains, or clumps

Anaerococcus octavius	*Peptostreptococcus octavius* *Peptostreptococcus prevotii* *Peptostreptococcus tetradius*
Anaerococcus hydrogenalis	*Peptostreptococcus hydrogenalis* *Peptostreptococcus lactolyticus*
Gemella morbillorum	*Streptococcus morbillorum*
Micromonas micros	*Peptostreptococcus micros*
Peptococcus niger	*Micrococcus niger*
Peptoniphilus asaccharolyticus	*Peptostreptococcus asaccharolyticus*
Peptoniphilus harei	*Peptostreptococcus harei*
Peptoniphilus ivorii	*Peptostreptococcus ivorii*
Peptoniphilus lacrimalis	*Peptostreptococcus lacrimalis*
Peptostreptococcus anaerobius	
Peptostreptococcus indolicus	*Peptococcus indolicus*
Peptostreptococcus magnus	*Peptococcus magnus* *Peptococcus variabilis*
Peptostreptococcus massiliae	
Peptostreptococcus stomatis	
Peptostreptococcus trisimilis	
Ruminococcus hansenii	*Streptococcus hansenii*
Ruminococcus productus	*Peptostreptococcus productus*

III. Aerobic Gram-Negative Cocci
Characteristics: occur singly or in pairs or clumps; catalase and oxidase positive

Lautropia mirabilis	*Sarcina mirabilis*
Neisseria canis	
Neisseria bacilliformis	
Neisseria cinerea	*Micrococcus cinereus* *Neisseria pharyngis*
Neisseria elongata subspecies *elongata*	*Neisseria elongata*
Neisseria elongata subspecies *glycolytica*	*Neisseria elongata*
Neisseria elongata subspecies *nitroreducens*	*Neisseria elongata* CDC group M-6
Neisseria flavescens	
Neisseria gonorrhoeae	
Neisseria kochii	
Neisseria lactamica	*Neisseria lactamicus*
Neisseria meningitidis	
Neisseria mucosa	
Neisseria parelongata	
Neisseria polysaccharea	
Neisseria sicca	
Neisseria subflava biovar *flava*	*Neisseria subflava*
Neisseria subflava biovar *perflava*	*Neisseria subflava*
Neisseria subflava biovar *subflava*	*Neisseria subflava*
Neisseria weaveri	*Moraxella* sp. M-5 CDC group M-5

IV. Anaerobic Gram-Negative Cocci
Characteristics: occur in pairs or clumps

Acidaminococcus fermentans	
Anaeroglobus geminatus	
Megasphaera elsdenii	*Peptostreptococcus elsdenii*
Megasphaera micronuciformis	
Subdoligranulum variabile	
Veillonella atypica	
Veillonella dispar	
Veillonella montpellierensis	
Veillonella parvula	

V. Aerobic Gram-Positive Bacilli
Characteristics: rodlike; catalase-negative or -positive; some are acid-fast stain-positive, and some have branching. Only *Bacillus*, *Brevibacillus*, and *Brevibacterium* spp. produce spores.

Actinomadura latina	
Actinomadura madurae	
Actinomadura pelletieri	
Amycolata autotrophica	
Amycolatopsis orientalis	*Nocardia orientalis* *Streptomyces orientalis*
Arcanobacterium bernardiae	*Actinomyces bernardiae* CDC coryneform group 2
Arcanobacterium haemolyticum	*Corynebacterium haemolyticum*
Arcanobacterium pyogenes	*Actinomyces pyogenes* *Corynebacterium pyogenes*
Arthrobacter albus	

TABLE 90–1 Current Bacterial Nomenclature, Taxonomy, and Classification—cont'd

Current Name	Synonym	Current Name	Synonym
Arthrobacter creatinolyticus		*Corynebacterium minutissimum*	
Arthrobacter cumminsii		*Corynebacterium mucifaciens*	
Arthrobacter luteolus		*Corynebacterium nigricans*	CDC fermentive coryneform group 4
Arthrobacter nicotianae			
Arthrobacter oxydans		*Corynebacterium pilosum*	
Arthrobacter scleromae		*Corynebacterium propinquum*	CDC coryneform group ANF-3
Arthrobacter woluwensis			
Aureobacterium spp.	*Corynebacterium aquaticum*	*Corynebacterium pseudodiphtheriticum*	*Corynebacterium hofmanii*
		Corynebacterium pseudotuberculosis	
Aureobacterium resistens		*Corynebacterium resistens*	
Bacillus anthracis		*Corynebacterium riegelii*	
Bacillus cereus		*Corynebacterium sanguinis*	
Bacillus circulans		*Corynebacterium simulans*	
Bacillus coagulans		*Corynebacterium singulare*	
Bacillus licheniformis		*Corynebacterium striatum*	
Bacillus massiliensis		*Corynebacterium sundsvallense*	*Corynebacterium thomssenii*
Bacillus megaterium			
Bacillus mycoides			*Rothia dentocarrosa*
Bacillus pumilus		*Corynebacterium tuscaniae*	
Bacillus sphaericus		*Corynebacterium ulcerans*	
Bacillus subtilis		*Corynebacterium urealyticum*	*Corynebacterium* group D2
Bacillus thuringiensis			
Brevibacillus agri	*Bacillus agri*		CDC coryneform group D2
Brevibacillus brevis	*Bacillus brevis*		
Brevibacillus laterosporus	*Bacillus laterosporus*	*Corynebacterium xerosis*	
	Brevibacterium casei	*Curtobacterium* spp.	
	CDC coryneform groups B-1 and B-3	*Dermabacter hominis*	CDC fermentative coryneform groups 3 and 5
Brevibacterium epidermidis			
Brevibacterium mcbrellneri		*Dermatophilus congolensis*	*Actinomyces congolensis*
Brevibacterium otitidis		*Erysipelothrix rhusiopathiae*	*Erysipelothrix insidiosa*
Brevibacterium sanguinis		*Exiguobacterium acetylicum*	*Brevibacterium acetylicum*
Callatomonas turbata	*Oerskovia turbata*	*Exiguobacterium aurantiacum*	*Brevibacterium aurantiacum*
Cellulomonas hominis	CDC coryneform group A-3	*Gardnerella vaginalis*	*Haemophilus vaginalis*
Cellulosimicrobium cellulans	*Cellumonas cellulans*		*Corynebacterium vaginalis*
	Oerskovia xanthineolytica	*Gordonia aichiensis*	*Rhodococcus aichiensis*
	CDC coryneform groups A-1 and A-2	*Gordonia bronchialis*	*Tsukamura aichiessii*
			Rhodococcus bronchialis
Corynebacterium accolens		*Gordonia polyisoprenivorans*	
Corynebacterium afermentans subspecies *afermentans*	CDC coryneform group ANF-1	*Gordonia rubropertincta*	*Rhodococcus rubiopertincta*
		Gordonia sputi	*Rhodococcus sputi*
Corynebacterium afermentans subspecies *lipophilum*	CDC coryneform group ANF-1		*Rhodococcus chubuensis*
		Gordonia terrae	*Rhodococcus terrae*
Corynebacterium amycolatum	*Corynebacterium xerosis*	*Kurthia* spp.	
	Corynebacterium minutissimum	*Listeria bulgaria*	
		Listeria grayi subspecies *grayi*	*Listeria grayi*
	Corynebacterium striatum	*Listeria ivanovii* subspecies *ivanovii*	*Listeria monocytogenes* serovar 5
	CDC coryneform groups F-2 and I-2	*Listeria ivanovii* subspecies *londoniensis*	
Corynebacterium aquaticum		*Listeria monocytogenes*	
Corynebacterium argentoratense		*Microbacterium* spp.	CDC coryneform groups A-4 and A-5
Corynebacterium aurimucosum	CDC fermentive coryneform group 4	*Microbacterium arborescens*	CDC coryneform group A-4
Corynebacterium auris	CDC coryneform group ANF-1	*Microbacterium imperiale*	CDC coryneform group A-4
Corynebacterium confusum			
Corynebacterium coyleae		*Microbacterium lacticum*	
Corynebacterium diphtheriae		*Microbacterium oxydans*	
Corynebacterium durum		*Microbacterium phyllosphaerae*	
Corynebacterium falsenii		*Mycobacterium abscessus*	*Mycobacterium chelonae* subspecies *abscessus*
Corynebacterium freneyi			
Corynebacterium glucuronolyticum		*Mycobacterium africanum* subtype I	
Corynebacterium imitans		*Mycobacterium africanum* subtype II	
Corynebacterium jeikeium	*Corynebacterium* group JK	*Mycobacterium alvei*	
		Mycobacterium arupense	
	CDC coryneform group JK	*Mycobacterium asiaticum*	
		Mycobacterium aurum	
Corynebacterium kroppenstedtii		*Mycobacterium avium* subspecies *avium*	
Corynebacterium lipophiloflavum		*Mycobacterium avium* subspecies *paratuberculosis*	
Corynebacterium macginleyi	CDC coryneform group G-1	*Mycobacterium barassie*	
Corynebacterium matruchotii	*Bacterionema matruchotii*	*Mycobacterium bohemicum*	

TABLE 90–1 Current Bacterial Nomenclature, Taxonomy, and Classification—cont'd

Current Name	Synonym
Mycobacterium bovis	
Mycobacterium branderi	
Mycobacterium brumae	
Mycobacterium celatum	
Mycobacterium chelonae	*Mycobacterium chelonae* subspecies *chelonae*
	Mycobacterium chelonei
Mycobacterium chubuense	
Mycobacterium colombiense	
Mycobacterium conceptionense	
Mycobacterium confluentis	
Mycobacterium conspicuum	
Mycobacterium cookii	
Mycobacterium flavescens	
Mycobacterium fortuitum	*Mycobacterium fortuitum* subspecies *fortuitum*
	Mycobacterium fortuitum (third complex) sorbitol-positive biovariant
	Mycobacterium fortuitum (third complex) sorbitol-negative biovariant
Mycobacterium gadium	
Mycobacterium gastri	
Mycobacterium genavense	
Mycobacterium goodii	
Mycobacterium gordonae	*Mycobacterium aquae*
Mycobacterium haemophilum	
Mycobacterium hassiacum	
Mycobacterium heckeshornense	
Mycobacterium heidelbergense	
Mycobacterium immunogenum	
Mycobacterium intracellulare	
Mycobacterium interjectum	
Mycobacterium jacuzzii	
Mycobacterium kansasii	
Mycobacterium kubicae	
Mycobacterium lacticola	
Mycobacterium lentiflavum	
Mycobacterium leprae	
Mycobacterium mageritense	
Mycobacterium maichiense	
Mycobacterium malmoense	
Mycobacterium marinum	*Mycobacterium balnei*
Mycobacterium massiliense	
Mycobacterium microgenicum	
Mycobacterium microti	
Mycobacterium monacense	
Mycobacterium mucogenicum	*Mycobacterium chelonae*–like organism (MCLO)
Mycobacterium moriokaense	
Mycobacterium neoaurum	
Mycobacterium nonchromogenicum	
Mycobacterium novocastrense	
Mycobacterium parascrofulaceum	
Mycobacterium peregrinum	*Mycobacterium fortuitum* biovar *peregrinum*
Mycobacterium phlei	
Mycobacterium ratisbonense	
Mycobacterium scrofulaceum	
Mycobacterium senegalense	*Mycobacterium peregrinum* type II
Mycobacterium sherrisii	
Mycobacterium shimoidei	
Mycobacterium simiae	*Mycobacterium habana*
Mycobacterium smegmatis	
Mycobacterium szulgai	
Mycobacterium terrae	
Mycobacterium thermoresistibile	
Mycobacterium triplex	
Mycobacterium triviale	
Mycobacterium tuberculosis	
Mycobacterium ulcerans	*Mycobacterium buruli*
Mycobacterium vaccae	
Mycobacterium wolinskyi	
Mycobacterium xenopi	
Nocardia abscessus	
Nocardia aoensis	
Nocardia africana	
Nocardia anaemiae	
Nocardia araoensis	
Nocardia arthritidis	
Nocardia asiatica	
Nocardia asteroides type IV	
Nocardia asteroides type VI	
Nocardia beijingensis	
Nocardia brasiliensis	
Nocardia brevicatena	*Mycropolyspora brevicatena*
Nocardia camea	
Nocardia carnea	
Nocardia corynebacteroides	
Nocardia cyriacigeorgica	*Nocardia cyriacigeorgici*
Nocardia exalbida	
Nocardia farcinica	
Nocardia higoensis	
Nocardia ignorata	
Nocardia inohanensis	
Nocardia kruczakiae	
Nocardia mexicana	
Nocardia niigatensis	
Nocardia nova	
Nocardia otitidiscaviarum	*Nocardia caviae*
Nocardia paucivorans	
Nocardia pneumoniae	
Nocardia pseudobrasiliensis	
Nocardia puris	
Nocardia sienata	*Nocardia senatus*
Nocardia testaceus	*Nocardia testaceus*
Nocardia thailandica	
Nocardia transvalensis	
Nocardia vermiculata	
Nocardia veterana	
Nocardia vinacea	
Nocardia yamanashiensis	
Nocardiopsis dassonvillei	*Actionmadura dassonvillei* *Nocardia dassonvillei*
Nocardiopsis synnemataformans	*Oerskovia turbata* CDC coryneform groups A-3 and A-4
Paenibacillus alvei	*Bacillus alvei*
Paenibacillus amylolyticus	
Paenibacillus macerans	*Bacillus mascerans*
Paenibacillus massiliensis	
Paenibacillus polymyxa	*Bacillus polymyxa*
Paenibacillus sanguinis	
Paenibacillus stellifer	
Parastreptomyces abscessus	
Rhodococcus equi	*Corynebacterium equi*
Rothia dentocariosa	*Nocardia dentocariosus*
Rothia mucilaginosa	*Stomatococcus mucilaginosus*
Streptomyces anulatus	*Streptomyces griseus*
Streptomyces lydicus	
Streptomyces paraguayensis	
Streptomyces sampsonii	
Streptomyces somaliensis	
Tropheryma whipplei	*Tropheryma whippelii*
Tsukamurella inchonensis	
Tsukamurella paurometabola	*Gordona aurantiaca*
Tsukamurella pulmonis	
Tsukamurella tyrosinosolvens	
Turicella otitidis	*Rhodococcus aurantiacus*
Williamsia deligens	

TABLE 90–1 Current Bacterial Nomenclature, Taxonomy, and Classification—cont'd

Current Name	Synonym
VI. Anaerobic Gram-Positive Bacilli (Non–Spore-Forming) **Characteristics: may be long-branching bacilli or pleomorphic coccobacilli**	
Actinobaculum massiliae	
Actinobaculum schaalii	
Actinobaculum timonae	
Actinobaculum suis	*Actinomyces suis*
Actinobaculum urinale	
Actinomyces cardiffensis	
Actinomyces europaeus	
Actinomyces funkei	
Actinomyces georgiae	*Actinomyces* DO8
Actinomyces gerencseriae	*Actinomyces israelii* serotype II
Actinomyces graevenitzii	
Actinomyces hongkongensis	
Actinomyces israelii	
Actinomyces meyeri	
Actinomyces nasicola	
Actinomyces naeslundii	
Actinomyces neuii subspecies *anitratus*	
Actinomyces neuii subspecies *neuii*	CDC coryneform group 1
Actinomyces odontolyticus	
Actinomyces oricola	
Actinomyces radicidentis	
Actinomyces radingae	CDC coryneform group E; APL1
Actinomyces turicensis	CDC coryneform group E; APL10
Actinomyces viscosus	
Anaerofustis haemolyticum	
Anaerofustis stercoribominis	
Atopobium fossor	*Eubacterium fossor*
Atopobium minutum	*Lactobacillus minutus*
Atopobium parvulum	*Streptococcus parvulus* *Peptostreptococcus parvulus*
Atopobium rimae	*Lactobacillus rimae*
Atopobium vaginae	
Bifidobacterium adolescentis	
Bifidobacterium angulatum	
Bifidobacterium bifidum	
Bifidobacterium breve	
Bifidobacterium catenulatum	
Bifidobacterium dentium	*Bifidobacterium appendicitis*
Bifidobacterium eriksonii	
Bifidobacterium longum subspecies *infantis*	*Bifidobacterium infantis*
Bifidobacterium longum subspecies *longum*	*Bifidobacterium longum*
Bifidobacterium pseudocatenulatum	
Collinsella aerofaciens	*Eubacterium aerofaciens*
Cryptobacterium curtum	
Dorea formicigenerans	*Eubacterium formicigenerans*
Dorea longicatena	
Eggerthella hongkongensis	
Eggerthella lenta	*Eubacterium lentum*
Eggerthella sinensis	
Eubacterium brachy	
Eubacterium combesii	
Eubacterium contortum	
Eubacterium infirmum	
Eubacterium limosum	
Eubacterium minutum	*Eubacterium tardum*
Eubacterium moniliforme	
Eubacterium nitritogenes	
Eubacterium nodatum	
Eubacterium saburreum	
Eubacterium saphenum	
Eubacterium sulci	*Fusobacterium sulci*
Eubacterium tenue	
Eubacterium timidum	
Eubacterium yurii subspecies *margaretiae*	
Eubacterium yurii subspecies *schtitka*	
Eubacterium yurii subspecies *yurii*	
Holdemania filiformis	*Eubacterium* S14
Lactobacillus acidophilus	
Lactobacillus brevis	
Lactobacillus casei	
Lactobacillus catenaforme	
Lactobacillus colehominis	
Lactobacillus crispatus	
Lactobacillus fermentum	
Lactobacillus gasseri	
Lactobacillus iners	
Lactobacillus jensenii	
Lactobacillus leichmannii	
Lactobacillus oris	
Lactobacillus paracasei subspecies *paracasei*	
Lactobacillus paraplantarum	
Lactobacillus plantarum	
Lactobacillus rhamnosus	*Lactobacillus* GG
Lactobacillus salivarius	
Lactobacillus vaginalis	
Mobiluncus curtisii subspecies *curtisii*	
Mobiluncus curtisii subspecies *holmesii*	
Mobiluncus mulieris	*Falcivibrio grandis*
Olsenella profuse	
Olsenella uli	*Lactobacillus uli*
Parascardovia denticolens	*Bifidobacterium denticolens*
Propionibacterium acnes	
Propionibacterium avidum	
Propionibacterium granulosum	
Propionibacterium Iymphophilum	
Propionibacterium propionicus	*Propionibacterium propionicum* *Arachnia propionica* *Actinomyces propionicus*
Pseudoramibacter alactolyticus	*Eubacterium alactolyticum*
Scardovia inopinata	*Bifidobacterium inopinatum* *Bifidobacterium dentium*
Slackia exigua	*Eubacterium exigaum*
Slackia heliotrinireducens	*Peptostreptococcus heliotrinreducens*
Solobacterium moorei	
Varibaculum cambriensis	
VII. Anaerobic Gram-Positive Bacilli (Spore-Forming) **Characteristics: broad, short bacilli with blunt ends. Most organisms readily produce spores, except *Clostridium perfringens*.**	
Clostridium absonum	
Clostridium aldenense	
Clostridium amygdalinum	
Clostridium argentinense	*Clostridium botulinum* group G *Clostridium subterminale* *Clostridium hastiforme*
Clostridium baratii	*Clostridium barati* *Clostridium paraperfringens* *Clostridium perenne*
Clostridium bartlettii	
Clostridium bifermentans	
Clostridium beijerinckii	
Clostridium bolteae	
Clostridium botulinum	*Clostridium putrificum*
Clostridium butyricum	*Clostridium pseudotetanicum*
Clostridium cadaveris	
Clostridium carnis	
Clostridium celatum	
Clostridium celerecrescens	
Clostridium citroniae	
Clostridium clostridioforme	*Clostridium clostridiiforme*

TABLE 90–1 Current Bacterial Nomenclature, Taxonomy, and Classification—cont'd

Current Name	Synonym
Clostridium coccoides	
Clostridium cochlearium	*Clostridium lentoputrescens*
Clostridium cocleatum	
Clostridium difficile	*Clostridium difficilis*
Clostridium fallax	*Clostridium pseudofallax*
Clostridium ghonii	*Clostridium ghoni*
Clostridium glycolicum	
Clostridium haemolyticum	*Clostridium novyi* type
Clostridium hastiforme	
Clostridium hathewayi	
Clostridium hiranonis	
Clostridium histolyticum	
Clostridium indolis	
Clostridium innocuum	
Clostridium irregulare	*Clostridium irregularis*
Clostridium leptum	
Clostridium limosum	CDC group P-1
Clostridium malenominatum	
Clostridium neonatale	
Clostridium novyi	
Clostridium oroticum	*Zymobacterium oroticum*
Clostridium paraputrificum	
Clostridium perfringens	*Clostridium welchii* *Welchia perfringens*
Clostridium piliforme	*Bacillus piliformis*
Clostridium putrefaciens	
Clostridium ramosum	*Eubacterium filamentosum* *Ramibacterium ramosum* *Actinomyces ramosus* *Eubacterium ramosum*
Clostridium scindens	
Clostridium septicum	
Clostridium sordellii	
Clostridium sphenoides	
Clostridium sporogenes	Nontoxigenic *Clostridium botulinum*
Clostridium subterminale	
Clostridium symbiosum	*Fusobacterium symbiosum* *Fusobacterium biacutus* *Bacteroides symbiosus*
Clostridium tertium	
Clostridium tetani	
Filifactor alocis	*Fusobacterium alocis*
Filifactor villosus	*Clostridium villosum*

VIII. Aerobic Gram-Negative Bacilli: Enterobacteriaceae

Characteristics: ferment sugars; are oxidase-negative; most reduce nitrate to nitrite. Diagnostic laboratories may report *Salmonella* serovars by name (e.g., *Salmonella typhi* or *Salmonella* serovar *typhi*).

Current Name	Synonym
Budvicia aquatica	
Buttiauxella noackiae	CDC enteric group 59
Cedecea davisae	CDC enteric group 15
Cedecea lapagei	
Cedecea neteri	*Cedecea* subspecies 4
Cedecea subspecies 3	
Cedecea subspecies 5	
Citrobacter amalonaticus	*Levinea amalonatica*
Citrobacter braakii	*Citrobacter freundii*
Citrobacter farmeri	*Citrobacter amalonaticus* biogroup 1
Citrobacter freundii	*Colobactrum freundii*
Citrobacter gillenii	*Citrobacter* genomospecies 10 *Citrobacter freundii*
Citrobacter koseri	*Citrobacter diversus* *Levinea malonatica*
Citrobacter murliniae	*Citrobacter* genomospecies 11 *Citrobacter freundii*
Citrobacter rodentium	*Citrobacter* genomospecies 9 *Citrobacter freundii*
Citrobacter sedlakii	*Citrobacter* genomospecies 8 *Citrobacter freundii*
Citrobacter werkmanii	*Citrobacter* genomospecies 7 *Citrobacter freundii*
Citrobacter youngae	*Citrobacter* genomospecies 5 *Citrobacter freundii*
Edwardsiella hoshinae	
Edwardsiella tarda	
Enterobacter aerogenes	*Aerobacter aerogenes*
Enterobacter agglomerans group	
Enterobacter amnigenus	
Enterobacter asburiae	CDC enteric group 17
Enterobacter cancerogenus	*Enterobacter taylorae* *Erwinia cancerogena* CDC enteric group 19
Enterobacter cowanii	
Enterobacter cloacae	
Enterobacter gergoviae	
Enterobacter hormaechei subspecies *hormaechei*	CDC enteric group 75
Enterobacter hormaechei subspecies *oharae*	
Enterobacter hormaechei subspecies *steigerwaltii*	
Enterobacter intermedius	*Enterobacter intermedium*
Enterobacter kobei	
Enterobacter sakazakii	
Erwinia persicinus	
Escherichia blattae	
Escherichia coli	
Escherichia fergusonii	CDC enteric group 10
Escherichia hermannii	CDC enteric group 11
Escherichia vulneris	CDC enteric group 1
Ewingella americana	CDC enteric group 40
Hafnia alvei	*Enterobacter hafniae*
Klebsiella granulomatis	*Calymmatobacterium granulomatis*
Klebsiella ornithinolytica	*Klebsiella oxytoca* ornithine positive
Klebsiella oxytoca	
Klebsiella planticola	*Klebsiella travisanii*
Klebsiella pneumoniae subspecies *ozaenae*	*Klebsiella ozaenae*
Klebsiella pneumoniae subspecies *pneumoniae*	*Klebsiella pneumoniae*
Klebsiella pneumoniae subspecies *rhinoscleromatis*	
Klebsiella terrigena	
Kluyvera ascorbata	CDC enteric group 8
Kluyvera cryocrescens	
Kluyvera georgiana	CDC enteric group 36/37 *Kluyvera* spp. group 3
Leclercia adecarboxylata	*Escherichia adecarboxylata* CDC enteric group 41
Leminorella grimontii	CDC enteric group 57
Leminorella richardii	
Moellerella wisconsensis	CDC enteric group 46
Morganella morganii subspecies *morganii*	*Proteus morganii*
Morganella morganii subspecies *sibonii*	*Proteus morganii*
Pantoea agglomerans	*Enterobacter agglomerans*
Pantoea ananatis	
Pantoea dispersa	
Photorhabdus luminescens	*Xenorhabdus luminescens*
Pragia fontium	
Proteus hauseri	*Proteus vulgaris* biogroup 3
Proteus mirabilis	
Proteus penneri	*Proteus vulgaris* biogroup 1
Proteus vulgaris	*Proteus vulgaris* biogroup 2
Providencia alcalifaciens	*Proteus inconstans*
Providencia heimbachae	

TABLE 90–1 Current Bacterial Nomenclature, Taxonomy, and Classification—cont'd

Current Name	Synonym
Providencia rettgeri	*Proteus rettgeri*
Providencia rustigianii	*Providencia alcalifaciens* biogroup 3
Providencia stuartii	*Proteus inconstans*
Rahnella aquatilis	
Salmonella bongori	*Salmonella* subgroup 5
Salmonella enterica	
Salmonella enterica subspecies *arizonae*	*Salmonella choleraesuis* subspecies *arizonae*
	Salmonella subgroup 3a
Salmonella enterica subspecies *diarizonae*	*Salmonella choleraesuis* subspecies *diarizonae*
	Salmonella subgroup 3b
Salmonella enterica subspecies *enterica*	*Salmonella choleraesuis* subspecies *choleraesuis*
	Salmonella subgroup 1
Salmonella enterica subspecies *houtenae*	*Salmonella choleraesuis* subspecies *houtenae*
	Salmonella subgroup 4
Salmonella enterica subspecies *indica*	*Salmonella choleraesuis* subspecies *indica*
	Salmonella subgroup 6
Salmonella enterica subspecies *salamae*	*Salmonella choleraesuis* subspecies *salamae*
	Salmonella subgroup 2
Serratia ficaria	
Serratia fonticola	
Serratia grimesii	*Serratia liquefaciens*
Serratia liquefaciens	*Enterobacter liquefaciens*
Serratia marcescens	
Serratia odorifera	
Serratia plymuthica	
Serratia proteamaculans subspecies *proteamaculans*	*Serratia liquefaciens*
Serratia proteamaculans subspecies *quinovora*	*Serratia liquefaciens*
Serratia rubidaea	
Shigella boydii	*Shigella* biogroup C
Shigella dysenteriae	*Shigella* biogroup A
Shigella flexneri	*Shigella* biogroup B
Shigella sonnei	*Shigella* biogroup D
Tatumella ptyseos	CDC group EF-9
Trabulsiella guamensis	CDC enteric group 90
Yersinia aldovae	
Yersinia bercovieri	*Yersinia enterocolitica* biogroup 3b
Yersinia enterocolitica	*Pasteurella enterocolitica*
Yersinia frederiksenii	
Yersinia intermedia	
Yersinia kristensenii	
Yersinia mollaretii	*Yersinia enterocolitica* biogroup 3a
Yersinia pestis	*Pasteurella pestis*
Yersinia pseudotuberculosis	*Pasteurella pseudotuberculosis*
Yersinia rohdei	
Yokenella regensburgei	*Koserella trabulsii* CDC enteric group 45

IX. Aerobic Gram-Negative Bacilli: Nonenterobacteriaceae; Fermentative
Characteristics: ferment sugars; are oxidase-positive

Aeromonas allosaccharophila	
Aeromonas bestiarum	
Aeromonas caviae	
Aeromonas enteropelogenes	
Aeromonas hydrophila	*Pseudomonas hydrophila*
Aeromonas jandaei	
Aeromonas media	
Aeromonas popoffii	
Aeromonas salmonicida	
Aeromonas schubertii	
Aeromonas trota	
Aeromonas veronii biotype *sobria*	
Aeromonas veronii biotype *veronii*	
Chromobacterium violaceum	*Bacillus violaceus*
Pasteurella aerogenes	
Pasteurella bettyae	CDC group HB-5
Pasteurella canis	*Pasteurella multocida* biotype 6
Pasteurella dagmatis	*Pasteurella* new sp. 1 *Pasteurella* "gas"
Pasteurella gallinarum	
Pasteurella haemolytica	
Pasteurella multocida subspecies *gallicida*	*Pasteurella septica*
Pasteurella multocida subspecies *multocida*	
Pasteurella multocida subspecies *septica*	
Pasteurella pneumotropica	
Pasteurella stomatis	
Pasteurella-like	CDC group EF-4
Photobacterium damsela	
Plesiomonas shigelloides	*Aeromonas shigelloides*
Vibrio alginolyticus	*Vibrio parahaemolyticus* biotype 2
Vibrio carchariae	
Vibrio cholerae	*Vibrio comma*
Vibrio cincinnatiensis	
Vibrio damsela	CDC group EF-5
Vibrio fluvialis	CDC group EF-6
Vibrio furnissii	*Vibrio fluvialis* biogroup 2
Vibrio hollisae	CDC group EF-13 CDC enteric group 42
Vibrio metschnikovii	CDC enteric group 16 *Vibrio cholerae* biovar *proteus*
Vibrio mimicus	*Vibrio cholerae* sucrose negative
Vibrio parahaemolyticus	
Vibrio vulnificus	CDC group EF-3 *Beneckea vulnifica*

X. Aerobic Gram-Negative Bacilli: Nonenterobacteriaceae; Nonfermentative
Characteristics: may or may not oxidize sugars; are catalase positive; are oxidase variable

Achromobacter piechaudii	*Alcaligenes piechaudii*
Achromobacter xylosoxidans subspecies *denitrificans*	*Alcaligenes denitrificans* *Alcaligenes xylosoxidans* subspecies *denitrificans* CDC group Vc
Achromobacter xylosoxidans subspecies *xylosoxidans*	*Alcaligenes xylosoxidans* *Alcaligenes xylosoxidans* subspecies *xylosoxidans* *Alcaligenes denitrificans* subspecies *xylosoxidans* *Achromobacter xylosoxidans* CDC groups IIIa and IIIb
Acidovorax delafieldii	*Pseudomonas delafieldii*
Acidovorax facilis	*Pseudomonas facilis*
Acidovorax temperans	*Pseudomonas temperans*
Acinetobacter baumannii	*Acinetobacter anitratus*
Acinetobacter baylyi	
Acinetobacter bouvetii	
Acinetobacter calcoaceticus	*Acinetobacter anitratus* *Acinetobacter calcoaceticus* subspecies *calcoaceticus*
Acinetobacter gerneri	
Acinetobacter grimontii	
Acinetobacter haemolyticus	*Acinetobacter anitratus*
Acinetobacter johnsonii	
Acinetobacter junii	*Acinetobacter anitratus*
Acinetobacter lwoffii	*Acinetobacter calcoaceticus* subspecies *lwoffi*

TABLE 90–1 Current Bacterial Nomenclature, Taxonomy, and Classification—cont'd

Current Name	Synonym
Acinetobacter parvus	
Acinetobacter radioresistens	
Acinetobacter schindleri	
Acinetobacter tandoii	
Acinetobacter tjernbergiae	
Acinetobacter towneri	
Acinetobacter ursingii	
Agrobacterium radiobacter	*Agrobacterium tumefaciens*
	CDC group Vd-3
Alcaligenes faecalis subspecies *faecalis*	*Alcaligenes odorans*
	Pseudomonas odorans
	CDC group VI
Asaia bogorensis	
Azospirillum brasilense	*Roseomonas fauriae*
	CDC "pink coccoid" group
Balneatrix alpica	
Bergeyella zoohelcum	*Weeksella zoohelcum*
	CDC group IIj
Brevundimonas diminuta	*Pseudomonas diminuta*
	CDC group Ia
Brevundimonas vesicularis	*Pseudomonas vesicularis*
	Corynebacterium vesiculare
Burkholderia ambifaria	
Burkholderia anthina	
Burkholderia cenocepacia	
Burkholderia cepacia	*Pseudomonas cepacia*
	Pseudomonas multivorans
	Pseudomonas kingae
	CDC group EO-1
Burkholderia dolosa	
Burkholderia gladioli	*Pseudomonas gladioli*
	Pseudomonas marginata
Burkholderia mallei	*Pseudomonas mallei*
	Actinobacillus mallei
Burkholderia multivorans	*Burkholderia cepacia* genomovar II
Burkholderia oklahomensis	
Burkholderia pseudomallei	*Pseudomonas pseudomallei*
Burkholderia pyrrocinia	
Burkholderia stabilis	*Burkholderia cepacia* genomovar IV
Burkholderia thailandensis	
Burkholderia vietnamiensis	*Burkholderia cepacia* genomovar V
Chryseobacterium gleum	*Flavobacterium gleum*
	CDC group IIb
Chryseobacterium indologenes	*Flavobacterium indologenes*
	CDC group IIb
Chryseobacterium massiliae	
Chryseobacterium timonae	
Comamonas terrigena	CDC group EF-19
Comamonas testosteroni	*Pseudomonas testosteroni*
Delftia acidovorans	*Comamonas acidovorans*
	Pseudomonas acidovorans
Elizabethkingia meningoseptica	*Chryseobacterium meningosepticum*
	Flavobacterium meningosepticum
	CDC group IIa
Elizabethkingia miricola	*Chryseobacterium miricola*
Empedobacter brevis	*Flavobacterium breve*
Flavobacterium group IIe	CDC group IIe
Flavobacterium group IIh	CDC group IIh
Flavobacterium group IIi	CDC group IIi
Granulibacter bethesdensis	
Inquilinus limosus	
Laribacter hongkongensis	
Massilia timonae	
Methylobacterium spp.	
Methylobacterium aminovarans	
Methylobacterium extorquens	
Methylobacterium fujisawaense	
Methylobacterium mesophilicum	
Methylobacterium organophilum	
Methylobacterium podarium	
Methylobacterium radiotolerans	
Methylobacterium rhodesianum	
Methylobacterium rhodinum	
Methylobacterium zatmanii	
Moraxella atlantae	CDC group M-3
Moraxella canis	
Moraxella catarrhalis	*Branhamella catarrhalis*
	Neisseria catarrhalis
Moraxella lacunata	*Moraxella liquefaciens*
Moraxella lincolnii	
Moraxella nonliquefaciens	
Moraxella osloensis	
Myroides odoratimimus	
Myroides odoratum	*Chryseobacterium odoratum*
	Flavobacterium odoratum
	CDC group M-4f
Ochrobactrum anthropi	*Achromobacter* spp. biotypes 1 and 2
	CDC groups Vd-1, Vd-2
Ochrobactrum intermedium	
Oligella ureolytica	CDC group IVe
Oligella urethralis	*Moraxella urethralis*
	CDC group M-4
Pandoraea apista	
Paracoccus yeei	*Paracoccus yeeii*
	CDC group EO-2
Photorhabdus asymbiotica	*Xenorhabdus luminescens*
Pseudomonas aeruginosa	*Pseudomonas pyocyanea*
	Bacterium aeruginosum
Pseudomonas alcaligenes	
Pseudomonas chlororaphis	*Pseudomonas aureofaciens*
Pseudomonas fluorescens	
Pseudomonas luteola	*Chryseomonas luteola*
	CDC group Ve-1
Pseudomonas mendocina	CDC group Vb-2
Pseudomonas oryzihabitans	*Flavimonas oryzihabitans*
	CDC group Ve-2
Pseudomonas otitidis	
Pseudomonas pertucinogena	*Bordetella pertussis* rough phase IV
Pseudomonas pseudoalcaligenes	*Pseudomonas alcaligenes* biotype B
Pseudomonas putida	
Pseudomonas stutzeri	CDC group Vb-1
Pseudomonas stutzeri–like	CDC group Vb-3
Psychrobacter immobilis	*Micrococcus cryophilus*
Psychrobacter phenylpyruvicus	*Moraxella phenylpyruvicus*
	CDC group M-2
Ralstonia spp.	CDC group IVc-2
Ralstonia pickettii	*Burkholderia pickettii*
	Pseudomonas pickettii
	CDC groups Va-1, Va-2, Va-3
	Pseudomonas thomasii
Roseomonas cervicalis	CDC "pink coccoid" group
Roseomonas genomospecies 4	CDC "pink coccoid" group
Roseomonas genomospecies 5	CDC "pink coccoid" group
Roseomonas genomospecies 6	CDC "pink coccoid" group
Roseomonas gilardii subspecies *gilardii*	
Roseomonas gilardii subspecies *rosea*	
Roseomonas mucosa	
Shewanella algae	
Shewanella putrefaciens	*Alteromonas putrefaciens*
	Pseudomonas putrefaciens
	CDC groups Ib-1, Ib-2

TABLE 90–1 Current Bacterial Nomenclature, Taxonomy, and Classification—cont'd

Current Name	Synonym
Sphingobacterium mizutae	*Flavobacterium mizutaii*
Sphingobacterium multivorum	*Flavobacterium multivorum*
	CDC group IIk-2
Sphingobacterium spiritivorum	*Flavobacterium spiritivorum*
	Sphingobacterium versatilis
	CDC group IIk-3
Sphingobacterium thalpophilum	*Flavobacterium thalpophilum*
Sphingobacterium yabuuchiae	
Sphingomonas parapaucimobilis	
Sphingomonas paucimobilis	*Pseudomonas paucimobilis*
	CDC group IIk-1
Sphingomonas sanguis	
Sphingomonas yanoikuyae	
Stenotrophomonas africana	
Stenotrophomonas maltophilia	*Xanthomonas maltophilia*
	Pseudomonas maltophilia
Wautersiella falsenii	
Weeksella virosa	*Flavobacterium genitale*
	CDC group IIf

XI. Anaerobic Gram-Negative Bacilli

Characteristics: may appear as rods with rounded ends, curved rods, coccobacilli, or slender, spindle-shaped rods with tapered ends. *Dialister* and *Johnsonella* belong to the *Clostridium* subphylum.

Alistipes finegoldii	
Alistipes onderdonkii	
Alistipes shahii	
Anaerobiospirillum succiniciproducens	
Anaerobiospirillum thomasii	
Anaerorhabdus furcosus	*Bacteroides furcosus*
Anaerostipes caccae	
Atopobium parvulus	
Bacteroides caccae	
Bacteroides capillosus	
Bacteroides coagulans	
Bacteroides dorei	
Bacteroides eggerthii	
Bacteroides finegoldii	
Bacteroides forsythus	
Bacteroides fragilis	
Bacteroides fragilis group	True *Bacteroides*
Bacteroides intestinalis	*Bacteroides putredinis*
	Alistipes putredinis
	Bacteroides furcosus
Bacteroides massiliae	
Bacteroides nordii	
Bacteroides ovatus	
Bacteroides putredinis	
Bacteroides pyogenes	*Bacteroides tectus*
Bacteroides salyersae	
Bacteroides splanchnicus	
Bacteroides stercoris	*Bacteroides fragilis* subspecies a
Bacteroides tectus	*Bacteroides tectum*
Bacteroides thetaiotaomicron	
Bacteroides uniformis	*Bacteroides corrodens*
Bacteroides ureolyticus	
Bacteroides vulgatus	
Bilophila wadsworthia	
Butyrivibrio fibrisolvens	
Catonella morbi	
Centipeda periodontii	
Cetobacterium somerae	
Desulfobulbus spp.	
Desulfomonas pigra	
Desulfovibrio fairfieldensis	
Desulfovibrio desulfuricans	
Desulfovibrio vulgaris	
Dialister invisus	
Dialister pneumosintes	*Bacteroides pneumosintes*
Dichelobacter nodosus	*Bacteroides nodosus*
Fusobacterium gonidiaformans	
Fusobacterium mortiferum	
Fusobacterium naviforme	
Fusobacterium necrogenes	
Fusobacterium necrophorum subspecies *funduliforme*	
Fusobacterium necrophorum subspecies *necrophorum*	
Fusobacterium nucleatum subspecies *fusiforme*	
Fusobacterium nucleatum subspecies *nucleatum*	
Fusobacterium nucleatum subspecies *polymorphum*	
Fusobacterium nucleatum subspecies *vincentii*	
Fusobacterium periodonticum	
Fusobacterium russii	
Fusobacterium sulci	
Fusobacterium ulcerans	
Fusobacterium varium	*Fusobacterium pseudonecrophorum*
Johnsonella ignava	
Leptotrichia amnionii	
Leptotrichia buccalis	
Leptotrichia goodfellowii	
Leptotrichia hofstadii	
Leptotrichia shahii	
Leptotrichia trevisanii	
Leptotrichia wadei	
Mitsuokella multacida	*Mitsuokella multiacida*
	Bacteroides multiacidus
Olsenella profuse	
Olsenella uli	
Parabacteroides distasonis	*Bacteroides distasonis*
Parabacteroides goldsteinii	*Bacteroides goldsteinii*
Parabacteroides merdae	*Bacteroides merdae*
	Bacteroides fragilis T4-1
Porphyromonas asaccharolytica	*Bacteroides asaccharolyticus*
	Bacteroides melaninogenicus subspecies *asaccharolyticus*
Porphyromonas cangingivalis	
Porphyromonas canoris	
Porphyromonas cansulci	
Porphyromonas catoniae	*Oribaculum catoniae*
Porphyromonas circumdentaria	
Porphyromonas crevioricanis	
Porphyromonas endodontalis	*Bacteroides endodontalis*
Porphyromonas gingivalis	*Bacteroides gingivalis*
Porphyromonas gingivicanis	
Porphyromonas ulae	
Porphyromonas levii	*Bacteroides levii*
	Bacteroides melaninogenicus subspecies *levii*
Porphyromonas macacae	*Bacteroides macacae*
	Porphyromonas salivosa
Porphyromonas salivosa	
Porphyromonas somerae	
Prevotella bergensis	
Prevotella bivia	*Bacteroides bivius*
Prevotella buccae	*Bacteroides buccae*
	Bacteroides ruminicola subspecies *brevis*
	Bacteroides capillus
	Bacteroides pentosaceus
Prevotella buccalis	*Bacteroides buccalis*
Prevotella corporis	*Bacteroides corporis*

TABLE 90–1 Current Bacterial Nomenclature, Taxonomy, and Classification—cont'd

Current Name	Synonym
Prevotella dentalis	*Mitsuokella dentalis*
	Hallella seregens
Prevotella denticola	*Bacteroides denticola*
Prevotella disiens	*Bacteroides disiens*
Prevotella enoeca	
Prevotella heparinolytica	*Bacteroides heparinolyticus*
Prevotella intermedia	*Bacteroides intermedius*
	Bacteroides melaninogenicus subspecies *intermedius*
Prevotella loescheii	*Bacteroides loescheii*
Prevotella massiliensis	
Prevotella melaninogenica	*Bacteroides melaninogenicus*
	Bacteroides melaninogenicus subspecies *melaninogenicus*
Prevotella nigrescens	*Prevotella intermedia*
Prevotella oralis	*Bacteroides oralis*
Prevotella oris	*Bacteroides oris*
	Bacteroides ruminicola subspecies *brevis*
Prevotella oulorum	*Bacteroides oulorum*
	Prevotella oulora
Prevotella pallens	
Prevotella salivae	
Prevotella shahii	
Prevotella tannerae	
Prevotella veroralis	*Bacteroides veroralis*
Prevotella zoogleoformans	*Bacteroides zoogleoformans*
Selenomonas artemidis	
Selenomonas dianae	
Selenomonas flueggei	
Selenomonas infelix	
Selenomonas noxia	
Selenomonas sputigena	
Sneathia sanguinegens	*Leptotrichia sanguinegens*
Sutterella wadsworthensis	
Synergistes cluster I	
Synergistes cluster II	
Tannerella forsythus	*Bacteroides forsythus*
	Tannerella forsythensis
Tissierella praeacuta	*Bacteroides praeacutus*

XII. Aerobic Gram-Negative Fastidious Coccobacilli

Characteristics: small, curved or straight gram-negative bacilli or coccobacilli. They may require carbon dioxide and enriched media or special conditions for adequate growth.

Actinobacillus arthritidis	*Actinobacillus lignieresii*	
Actinobacillus equuii		
Actinobacillus hominis		
Actinobacillus ureae	*Pasteurella ureae*	
Afipia broomeae		
Afipia clevelandensis		
Afipia felis		
Aggregatibacter actinomycetemcomitans	*Actinobacillus actinomycetemcomitans*	CDC groups HB-3, HB-4
Aggregatibacter aphrophilus	*Haemophilus aphrophilus*	CDC group HB-2
Aggregatibacter paraphrophilus	*Haemophilus paraphrophilus*	
Aggregatibacter segnis	*Haemophilus segnis*	
Arcobacter butzleri	*Campylobacter butzleri*	
Arcobacter cryaerophilus	*Campylobacter cryaerophila*	
Bartonella alsatica		
Bartonella bacilliformis		
Bartonella clarridgieae		
Bartonella elizabethae	*Rochalimaea elizabethae*	
Bartonella grahamii		
Bartonella henselae	*Rochalimaea henselae*	
Bartonella koehleras		
Bartonella quintana	*Rochalimaea quintana*	
Bartonella vinsonii subspecies *arupensis*	*Rochalimaea vinsonii*	
Bartonella vinsonii subspecies *berkhoffii*		
Bartonella washoensis		
Bordetella bronchiseptica	CDC group IVa	
Bordetella hinzii		
Bordetella holmesii	CDC group NO-2	
Bordetella parapertussis		
Bordetella pertussis		
Bordetella trematum		
Brucella abortus		
Brucella canis		
Brucella melitensis		
Brucella suis		
Campylobacter coli		
Campylobacter concisus	CDC group EF-22	
Campylobacter curvus	*Wolinella curva*	
Campylobacter fetus subspecies *fetus*	*Vibrio fetus*	
Campylobacter fetus subspecies *venerealis*		
Campylobacter gracilis	*Bacteroides gracilis*	
Campylobacter hominis		
Campylobacter hyointestinalis subspecies *hyointestinalis*		
Campylobacter jejuni subspecies *doylei*		
Campylobacter jejuni subspecies *jejuni*		
Campylobacter lari	*Campylobacter laridis*	
Campylobacter mucosalis		
Campylobacter rectus	*Wolinella recta*	
Campylobacter showae		
Campylobacter sputorum subspecies *paraureolyticus*		
Campylobacter sputorum subspecies *sputorum*		
Campylobacter upsaliensis		
Campylobacter ureolyticus	*Bacteroides ureolyticus*	
Capnocytophaga canimorsus	CDC group DF-2	
Capnocytophaga cynodegmi	CDC group DF-2	
Capnocytophaga gingivalis	CDC group DF-1	
Capnocytophaga granulosa		
Capnocytophaga haemolytica		
Capnocytophaga ochracea	CDC group DF-1	
Capnocytophaga sputigena	CDC group DF-1	
Cardiobacterium hominis	CDC group IId	
Cardiobacterium valvarum		
Chlamydophila avium		
Chlamydophila pneumoniae	*Chlamydia pneumoniae*	TWAR
Chlamydophila psittaci	*Chlamydia psittaci*	
Chlamydophila trachomatis	*Chlamydia trachomatis*	
Chromobacterium violaceum		
Coxiella burnetii		
Dysgonomonas capnocytophagoides	CDC group DF-3	
Dysgonomonas gadei		
Ehrlichia spp.	Human granulocytic ehrlichiosis	
Ehrlichia canis		
Ehrlichia chaffeensis	Human monocytic ehrlichiosis	
Ehrlichia ewingii		
Ehrlichia sennetsu		
Eikenella corrodens	CDC group HB-1	
Francisella philomiragia	*Yersinia philomiragia*	
Francisella tularensis biovar *mediaasiatica*		

TABLE 90–1 Current Bacterial Nomenclature, Taxonomy, and Classification—cont'd

Current Name	Synonym
Francisella tularensis biovar *novicida*	*Francisella novicida* *Pasteurella tularensis* *Bacterium tularense*
Francisella tularensis biovar *palearctica*	*Francisella tularensis* type B *Bacterium tularense*
Francisella tularensis biovar *tularensis*	*Francisella tularensis* type A *Pasteurella tularensis* *Bacterium tularense*
Haemophilus ducreyi	
Haemophilus haemolyticus	
Haemophilus influenzae	*Haemophilus aegyptius*
Haemophilus parahaemolyticus	
Haemophilus parainfluenzae	
Helicobacter bilis	
Helicobacter cinaedi	*Campylobacter cinaedi*
Helicobacter bizzozeronii	
Helicobacter canadensis	
Helicobacter canis	
Helicobacter fennelliae	*Campylobacter fennelliae*
Helicobacter heilmannii	*Gastrospirillum hominis*
Helicobacter pullorum	
Helicobacter pylori	*Campylobacter pylori*
Helicobacter rappini	*Flexispira rappini*
Helicobacter westmeadii	
Helicobacter winghamensis	
Kingella denitrificans	CDC group TM-1
Kingella kingae	*Moraxella kingae* *Moraxella kingii*
Kingella oralis	
Kingella potus	
Legionella anisa	
Legionella birminghamensis	
Legionella bozemanii	*Fluoribacter bozemanae*
Legionella cincinnatiensis	
Legionella dumoffii	*Fluoribacter dumoffii*
Legionella feeleii	
Legionella gormanii	
Legionella hackeliae	
Legionella israelensis	
Legionella jordanis	
Legionella lansingensis	
Legionella longbeachae	
Legionella maceachernii	
Legionella micdadei	*Tatlockia micdadei*
Legionella oakridgensis	
Legionella pneumophila	
Legionella sainthelensi	
Legionella tucsonensis	
Legionella wadsworthii	
Orientia tsutsugamushi	*Rickettsia tsutsugamushi*
Rickettsia aeschlimannii	
Rickettsia africae	
Rickettsia akari	
Rickettsia amblyommii	
Rickettsia australis	
Rickettsia canadensis	
Rickettsia conorii subspecies *caspia*	
Rickettsia conorii subspecies *conorii*	
Rickettsia conorii subspecies *indica*	
Rickettsia conorii subspecies *israelensis*	
Rickettsia felis	
Rickettsia helvetica subspecies *heilongjiangensis*	
Rickettsia helvetica subspecies *mongolotimonae*	
Rickettsia helvetica subspecies *sibirica*	
Rickettsia honei	
Rickettsia japonica	
Rickettsia marmionii	
Rickettsia massiliae	
Rickettsia parkeri	
Rickettsia prowazekii	
Rickettsia rickettsii	
Rickettsia sibirica	
Rickettsia slovaca	
Rickettsia texiana	
Rickettsia typhi	
Streptobacillus moniliformis	*Haverhillia multiformis*
Suttonella indologenes	*Kingella indologenes*
XIII. Mycoplasma (Pleuropneumonia-like Organisms [PPLO]) **Characteristics: small, highly pleomorphic organisms that are difficult to observe with routine stains; require complex medium for growth**	
Acholeplasma laidlawii	
Mycoplasma amphoriforme	
Mycoplasma buccale	
Mycoplasma faucium	
Mycoplasma fermentans	*Mycoplasma incognitus*
Mycoplasma gallisepticum	
Mycoplasma genitalium	
Mycoplasma hominis	
Mycoplasma lipophilum	
Mycoplasma orale	
Mycoplasma penetrans	
Mycoplasma pirum	
Mycoplasma pneumoniae	
Mycoplasma primatum	
Mycoplasma salivarium	
Mycoplasma spermatophilum	
Mycoplasma synoviae	
Ureaplasma parvum	
Ureaplasma urealyticum	T-mycoplasma
XIV. Treponemataceae (Spiral Organisms) **Characteristics: filamentous, spiral organisms that may or may not stain with usual laboratory stains; require complex media or animal host for growth**	
Borrelia afzelii	
Borrelia andersonii	
Borrelia anserina	
Borrelia bissettii	
Borrelia burgdorferi	
Borrelia caucasica	
Borrelia crocidurae	
Borrelia duttoni	
Borrelia garinii	
Borrelia hermsii	
Borrelia hispanica	
Borrelia japonica	
Borrelia latyschewii	
Borrelia lonestari	
Borrelia lusitaniae	
Borrelia mazzottii	
Borrelia miyamti	
Borrelia parkeri	
Borrelia persica	
Borrelia recurrentis	
Borrelia spielmanii	*Borrelia spielmani*
Borrelia tanukii	
Borrelia turdae	
Borrelia turicatae	

TABLE 90–1 Current Bacterial Nomenclature, Taxonomy, and Classification—cont'd

Current Name	Synonym
Borrelia valaisiana	
Borrelia venezuelensis	
Brachyspira aalborgi	
Brachyspira intermedia	*Serpulina intermedia*
Brachyspira murdochii	*Serpulina murdochii*
Brachyspira pilosicoli	*Serpulina pilosicoli*
Leptospira alexandria	
Leptospira borgpetersenii	
Leptospira broomii	
Leptospira fainei	
Leptospira inadai	
Leptospira interrogans	
Leptospira interrogans serogroup *australis*	
Leptospira interrogans serogroup *autumnalis*	
Leptospira interrogans serogroup *ballum*	
Leptospira interrogans serogroup *bataviae*	
Leptospira interrogans serogroup *bulgarica*	
Leptospira interrogans serogroup *canicola*	
Leptospira interrogans serogroup *copenhageni*	
Leptospira interrogans serogroup *grippotyphosa*	
Leptospira interrogans serogroup *hardjo*	
Leptospira interrogans serogroup *icterohaemorrhagiae*	
Leptospira interrogans serogroup *pomona*	
Leptospira interrogans serogroup *pyrogenes*	
Leptospira kirschneri	
Leptospira noguchii	
Leptospira santarosai	
Leptospira weilii	
Spirillum minus	*Spirillum minor*
Treponema amylovorum	
Treponema carateum	
Treponema denticola	
Treponema maltophilum	
Treponema medium	
Treponema minutum	
Treponema pallidum subspecies *endemicum*	*Treponema pallidum*
Treponema pallidum subspecies *pallidum*	*Treponema pallidum*
Treponema pallidum subspecies *pertenue*	*Treponema pertenue*
Treponema parvum	
Treponema pectinovorum	
Treponema phagedenis	
Treponema putidum	
Treponema refringens	
Treponema skoliodontum	
Treponema socranskii	
Treponema vincentii	

CDC, Centers for Disease Control and Prevention.

TABLE 90–2 Bacterial Ranks Below Subspecies

Preferred Name	Synonym	When Applied
Biovar features	Biotype	Special biochemical or physiologic features
Serovar	Serotype	Distinct antigenic features
Pathovar	Pathotype	Host-specific pathogenic features
Phagovar	Phagotype	Lysis by distinct bacteriophages
Morphovar	Morphotype	Special morphologic features

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SUBSECTION 1

Gram-Positive Cocci

CHAPTER 91

STAPHYLOCOCCUS AUREUS INFECTIONS (COAGULASE-POSITIVE STAPHYLOCOCCI)

Sheldon L. Kaplan ❂ Kristina G. Hulten ❂ Edward O. Mason

Staphylococcus aureus is a gram-positive coccus that occurs in pairs, chains, and grapelike clusters (Fig. 91–1). *S. aureus* is ubiquitous in nature and can be pathogenic for humans and animals. Staphylococci are nonmotile, aerobic, or facultative anaerobic and are readily cultivated on routine laboratory media.

These organisms are part of the normal human flora. *S. aureus* is responsible for an impressive variety of diseases ranging from minor skin and soft tissue infections to major life-threatening and fatal infections such as bacteremia, endocarditis, pericarditis, pneumonia, empyema, osteomyelitis, myositis, and septic arthritis.

On blood agar, *S. aureus* forms round, convex, shiny opaque colonies 1 to 4 mm in diameter, often with a zone of clear beta-hemolysis (Fig. 91–2) surrounding the colony. Production of pigment is variable, with strains exhibiting a yellow or golden pigment on primary isolation; yellow pigment actually is an *S. aureus* virulence factor.[132] *S. aureus* secretes free coagulase, the basis for the most definitive and most accepted method for identifying pathogenic staphylococci associated with human and animal infection. Free coagulase reacts with coagulase activator in plasma and converts fibrinogen to fibrin, with the formation of a fibrin clot. Coagulase also can be evaluated by testing for bound coagulase or clumping factor in a rapid slide test (Fig. 91–3). Clumping factor bound to the organism acts directly on fibrinogen and converts it to fibrin; it is detected by visible clumping or agglutination when a suspension is incubated with plasma. The bound coagulase test is used frequently in clinical laboratories and is faster than the tube coagulase test. However, the slide test for clumping factor may be falsely negative in 10 to 15 percent of cases. Thus, a negative slide coagulase test with an isolate that is highly suggestive of *S. aureus* should be confirmed with a tube coagulase test for free coagulase.[120,174] A limited number of biochemical reactions can differentiate *S. aureus* from other staphylococci (Table 91–1).

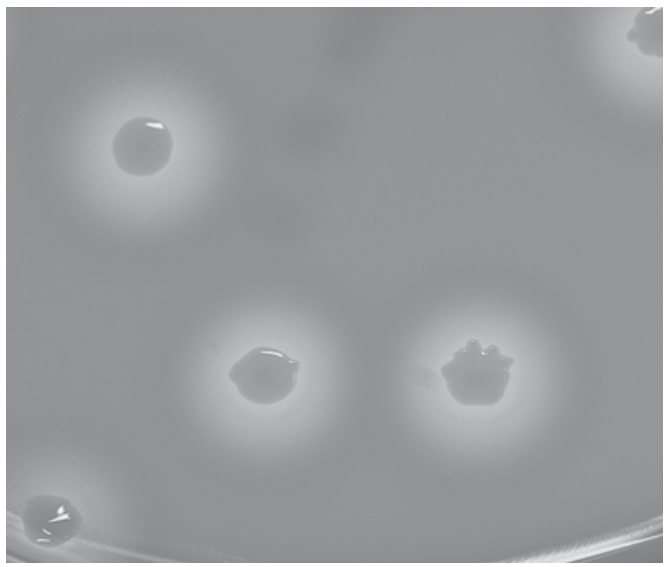

Figure 91–2 Typical hemolysis produced by *Staphylococcus aureus* on sheep blood agar. (See companion Expert Consult web site for color version.)

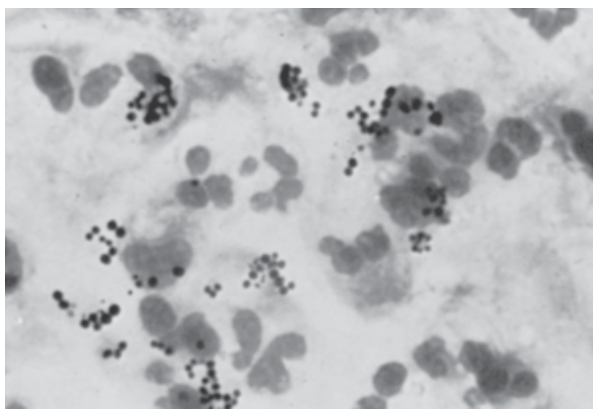

Figure 91–1 Staphylococci in pus. The organisms tend to form clusters, are round, and stain purple with Gram stain (positive), similar to bunches of grapes.

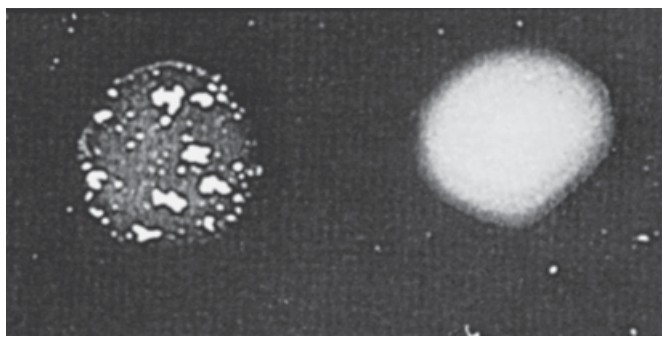

Figure 91–3 Bound (slide) coagulase test. A suspension of organisms is mixed with plasma. Immediate clumping (reaction on the *left*) indicates both the presence of bound coagulase and the fact that the organism is coagulase-positive.

STRUCTURE

CAPSULE

Capsule production often is considered a virulence factor and frequently is used by bacteria to hinder phagocytosis by the host.[159] Karakawa and colleagues described a scheme of eight *S. aureus* capsular types in 1982.[110] Subsequently, Sompolinsky and coworkers described three more serotypes, bringing the total number to 11.[199] Most *S. aureus* organisms are encapsulated to a varying degree (Fig. 91–4). Two serotypes (1 and 2) produce mucoid colonies on agar media but are rare among clinical isolates.[199] Serotypes 5 and 8 account for approximately 75 percent of strains recovered from infections in humans. In practice, strains that do not belong to serotypes 1, 2, 5, or 8 are referred to as *nontypeable*.[93] The role of capsule polysaccharide (CP) in virulence comes primarily from studies of CP5 and CP8 and is not completely clear. These studies show that inhibition of phagocytosis by capsule can result in persistence on surfaces but modulates adherence to endothelial surfaces.[159] A protein conjugate vaccine composed of CP5 and CP8 conjugated to nontoxic recombinant *Pseudomonas aeruginosa* exotoxin A (StaphVAX) initially appeared promising in prevention of *S. aureus* bacteremia in patients undergoing hemodialysis.[192] However, in a larger study, this vaccine was not found to be efficacious.[190]

PROTEIN A

Staphylococcal protein A, a major component of the cell wall of coagulase-positive staphylococci, has been found to bind to the Fc portion of immunoglobulin G (IgG). This binding is nonimmune. It differs from specific antigen-antibody reactions in that the reaction is nonspecific, although both precipitation and agglutination are observed. Protein A binds the IgG of many mammalian species. This property has rendered protein A a major reagent in many immune assays. Protein A also has been found to bind to platelets through the platelet gC1qr, a 33-kd cell protein[158] as well as to von Willebrand factor,[87] both of which may be important in the pathogenesis of endovascular infections caused by *S. aureus*. Protein A plays a central role in the pathogenesis of staphylococcal pneumonia in that TNFR1, a receptor for tumor necrosis factor-α (TNF-α), also is a receptor for protein A, and thus protein A is a principal staphylococcal proinflammatory factor in the lung.[74]

TABLE 91–1 Identification of Staphylococci

Characteristics	*S. aureus*	*S. epidermidis*	*S. saprophyticus*
Coagulase	+	–	–
Acid aerobically from			
Sucrose	+	+	+
Trehalose	+	–	+
Mannitol	+	–	+
Phosphatase	+	+	–
Novobiocin	Sensitive	Sensitive	Resistant

EXTRACELLULAR PRODUCTS

S. aureus elaborates a wide variety of extracellular toxins, many of which have potent biologic effects in the isolated state on intact animals, tissues, cells, and membranes. These toxins generally are thought to be responsible for the virulence of *S. aureus*, and the role of several of these products in staphylococcal infections has been proved definitively in humans. The important staphylococcal extracellular products include the following: alpha-, beta-, and delta-hemolysins; coagulases; leukocidin; hyaluronidase; staphylokinase; bacteriocins; the epidermolytic toxins; toxic shock syndrome toxin type I (TSST-I); and the enterotoxins.

Almost half of the known toxins and virulence genes in *S. aureus* are found in pathogenicity islands. These genetic clusters, commonly 15 to 20 kb in size, also contain genetic elements such as integrases and transposases. A high genetic instability is associated with many of these islands, and mosaic structures are common findings. Seven pathogenicity islands (νSa) have been associated with *S. aureus*.[68] Table 91–2 illustrates some of the features described for these islands, although allelic variations in gene content occur. All sequenced strains of *S. aureus* contain variations of the νSaα, νSaβ, and νSaγ.[47,68,94] In addition to the pathogenicity islands, virulence genes are also located on prophages (e.g., *lukS-PV* and *lukF-PV* on SLT).

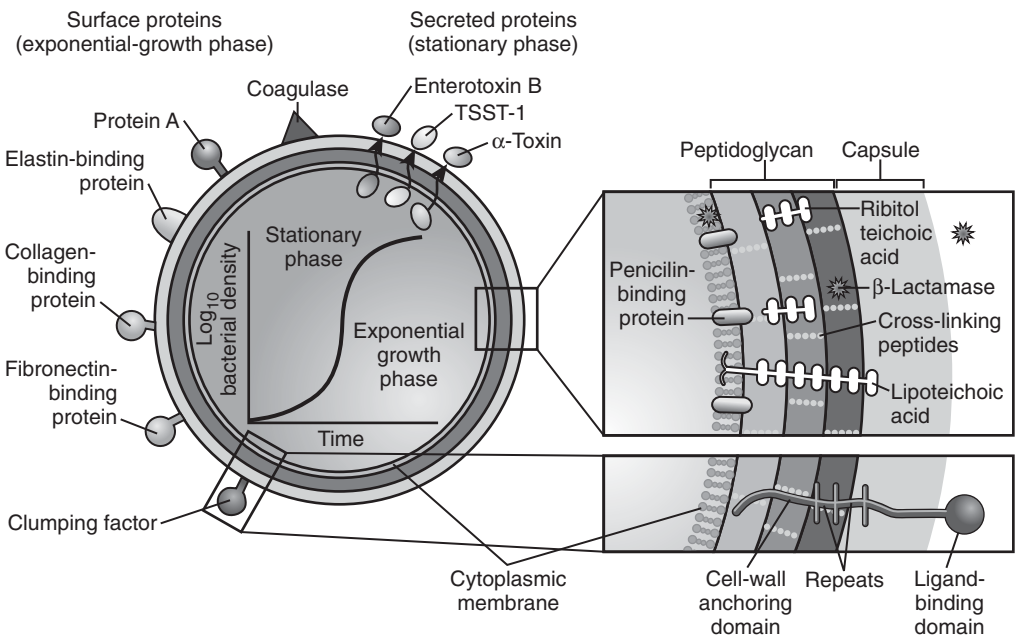

Figure 91–4 Structure of *Staphylococcus aureus.* TSST-1, toxic shock syndrome toxin type I. *(From Lowy, F. D.: Medical progress: Staphylococcus aureus infections. N. Engl. J. Med. 339:520-532, 1998.)*

TABLE 91–2 Pathogenicity Islands of *Staphylococcus aureus**

Island	Alleles	Genes	Reference
νSa1	SaPI1, SaPI3	*seb*, *tst*, *sek*, *seq*, *ear*	131
νSa2	SaPIbov	*tst*, *sec*	60
νSa3	SaPI3	Type I: *fhu*D; type II: *sel*2, *sec*4, *ear*	232
νSa4	SaPI2	Type I: *sel*, *sec*3, *tst*; Type II: 4 unknown ORFs	130
νSaα		Type I: *set* 6-15, *lpl* 1-9; Type II: *set* 16-26, *lpl* 10-14 Type III: *set* 1-5, *lpl* 2,7,8,11,13	68, 235
νSaβ		Type I: *spl* A-F, *lukDE*, *ear*, epidermin gene cluster; Type II: *spl* A-F, *lukDE*, *ear*, *seg*, *sen*, *sei*, *sem*, *seo*, epidermin gene cluster	68
νSaγ		*set* 1,3,4, *eta*, phenol-soluble modulin,	68

**Enterotoxins: seb, sec, seg, sei, sel, sem, sen, seo; exotoxins: set 1-26, toxic shock syndrome toxin I (tst), β-lactamase (ear), tandem lipoprotein (lpl), ferric hydroxamate uptake (fbuD), leukocidin (lukDE), serine protease (IA-F), exfoliative toxin A.*
ORF, open reading frame.

HEMOLYSINS

Alpha-hemolysin is produced by most *S. aureus* isolates and is a classic pore-forming bacterial toxin. The purified toxin has impressive hemolytic, dermonecrotic, and lethal properties. Advances in protein purification have allowed pure toxin to be produced; this development, in turn, has led to a better understanding of its properties.

The protein interacts with and damages a variety of cell membranes, releases hemoglobin from erythrocytes of various mammalian and avian species, and is cytotoxic to numerous cell lines in tissue culture. It lyses rabbit and human platelets and disrupts lysosomes. It causes contraction in skeletal and vascular smooth muscle, an action that perhaps explains its property of causing localized dermal necrosis. In a rat model, alpha-toxin damages the air-blood barrier in vivo during *S. aureus* pneumonia.[143] Alpha-toxin forms pores in the membranes of endothelial cells; this process leads to vasoconstriction and increases vascular permeability, as well as having effects that cause apoptosis of numerous different cells.[49]

Injection of alpha-toxin is lethal to mammals and reptiles; death occurs within 2 to 5 minutes. Human death has been attributed to preformed alpha-toxin on at least one occasion. Anti–alpha-toxin can be demonstrated in physiologically normal persons; however, the level of anti–alpha-hemolysin is high in approximately 70 percent of patients with staphylococcal osteomyelitis.[208] In humans and experimental animals, the presence of anti–alpha-hemolysin neither modifies nor prevents staphylococcal infection.

The other hemolytic toxins, beta- and delta-hemolysin, also possess hemolytic and cytotoxic activities. In addition, beta-toxin produces lethal and dermonecrotic effects. Eighty to 100 percent of adults possess antibody to beta-hemolysin.

Despite evidence that these toxins are produced during infection, their roles in production of the typical staphylococcal tissue lesion remain unclear.[21,49] Although these toxins may be important in the establishment of infection by interacting with each other and with other biologically active staphylococcal products, this theory has not been proved; infection is not prevented by antitoxin antibody, and virulent staphylococci that lack one or more of these toxins are encountered.[197]

A recent in vitro study using proteomics (two-dimensional gel electrophoresis coupled with automated direct infusion-tandem mass spectrometry [ADI-MS/MS] analysis) showed that alpha-toxin was present in culture supernatants seven- to ninefold greater in the USA300 compared with the USA400 strain when grown at mid-exponential and stationary phases of growth.[31] Further studies of the way in which exotoxins influence strain-induced disease and host response are warranted.

LEUKOCIDIN

Although certain hemolysins are toxic to various leukocytes, the Panton-Valentine leukocidin (PVL) is the only known extracellular toxin that attacks the leukocyte exclusively.[166] It consists of two protein components, LukS-PV and LukF-PV, encoded by the genes *lukS-PV* and *lukF-PV* carried on a bacteriophage, that synergistically cause pores in polymorphonuclear leukocytes and macrophages.[26] Leukocidin injected into rabbits causes a striking fall in levels of circulating and bone marrow leukocytes, followed by marked granulocytosis; these changes occur without death of the rabbits. Leukocidin interacts with the membrane phospholipid and causes depolarization, increased permeability, and cell death. Local secretion of leukocidin may appear to confer an advantage to the staphylococcus by killing leukocytes and thereby preventing phagocytosis and intracellular killing. Levels of anti-leukocidin rise rapidly in the course of infection,[71,99] and some evidence indicates that infants and mothers with high levels of anti-leukocidin antibody are less likely to contract staphylococcal disease in high-risk epidemiologic situations.[17]

In early studies, only a few *S. aureus* isolates recovered from humans were *pvl*-positive.[171] Interest in PVL greatly increased following reports that *S. aureus* isolates carrying *pvl* genes were associated with severe furunculosis[41] and, particularly, necrotizing pneumonia in children and adolescents that frequently was fatal.[70,128] Furthermore, the independent emergence worldwide of community-acquired methicillin-resistant *S. aureus* (CA-MRSA) clones that generally carry the *pvl* genes led to further studies of the role of PVL in the pathogenesis of *S. aureus* infections.[215] The USA300 CA-MRSA clone especially has been linked to *pvl*.[150,151] In children with invasive *S. aureus* osteomyelitis, the presence of *pvl* genes was associated with greater local and systemic inflammation, longer duration of fever, greater frequency of positive blood cultures, and more frequent complications such as the development of chronic osteomyelitis or venous thrombosis.[24,140] Pulmonary manifestations also occurred more frequently among children with invasive *S. aureus* infections caused by *pvl*-positive isolates.[75] In adults with staphylococcal pneumonia, *pvl*-positive isolates were associated with increased rates of mortality.[134] Thus, the presence of *pvl* genes may be a marker for isolates capable of causing more severe disease.[56]

The contribution of PVL to the severity of *S. aureus* infections has been examined in mice using isogenic *pvl*-positive or *pvl*-negative isolates. One study found no difference in lethality for sepsis or abscess volume in a skin abscess model,[219] as well as no difference in the destruction of human neutrophils by the isogenic strains. The investigators concluded that PVL was not a major virulence determinant of CA-MRSA. The other study found that *pvl*-positive isolates caused much greater lung inflammation and necrosis in a mouse acute pneumonia model.[118] PVL was detected in the infected lung tissues. These investigators also discovered that staphylococcal protein A is more highly expressed in *pvl*-positive strains and postulated that PVL coupled with increased staphylococcal protein A could result in greater tissue inflammation than that found with *pvl*-negative strains. The discrepancy in these studies may be explained by the use of different *S. aureus* isolates as well as different models of infection.

Although the exact role of PVL in *S. aureus* infections has not been fully established, treatment measures directed against PVL have been proposed and include the preferred use of

protein-inhibiting antibiotics rather than or in addition to bactericidal antibiotics to decrease production of PVL,[51,202] as well as adjunctive administration of intravenous immunoglobulin preparations containing antibody to PVL.[67]

ENZYMES

Staphylococci elaborate a variety of enzymes, including hyaluronidase, nuclease, proteases, lipase, catalase, lysozyme, and lactate dehydrogenase, that may play a role in the spread of infection in local tissues or the establishment of a nidus of infection. Other biologically active extracellular products, as yet unidentified, undoubtedly are produced by the staphylococcus. The potency and number of these weapons in the armamentarium of the coagulase-positive staphylococcus, in contrast to the small number of extracellular products associated with the much less virulent coagulase-negative staphylococcus, suggest that the synergistic action of these toxins and enzymes may explain the superior ability of coagulase-positive staphylococci to establish infection and cause tissue necrosis. Several extracellular toxins have been proved to have specific roles in staphylococcal infection or disease: the epidermolytic toxins, TSST-I, and the enterotoxins.

EPIDERMOLYTIC TOXINS

Two biochemically and immunologically distinct exotoxins, epidermolytic toxins A and B (epidermolysins, exfoliatins), can separate adjacent cell layers within the epidermis and can cause the various skin manifestations of the staphylococcal scalded skin syndrome (SSSS). The toxin acts extracellularly; it does not induce cell death directly or lysis of the cell membrane or elicit an inflammatory response. This toxin does not damage any organ or cell, except those of the upper epidermis.[53,127,147] These low-molecular-weight (≈24,000 d) protein exotoxins now are known to hydrolyze desmoglein-1, an important structural skin protein.[9] Exfoliatin toxin D has been described but has not been associated with bullous impetigo or SSSS.[231]

TOXIC SHOCK SYNDROME TOXIN TYPE I

TSST-I was discovered independently in 1981 by two research groups. It was documented to be an excellent marker for staphylococci associated with vaginal toxic shock syndrome (TSS), in which it correctly identified more than 90 percent of strains in blinded testing. In experimental animal models, purified TSST-I can induce the major physiologic changes of TSS: fever, mucous membrane suffusion, renal impairment, hepatic damage, hypocalcemia, lymphocytopenia, and hypotension. TSST-I can be detected in blood, pus, urine, and tissues during human and experimental model TSS. USA300, the major CA-MRSA clone in the United States, does not carry the gene encoding TSST-1.[151] TSS is discussed in Chapter 71.

ENTEROTOXINS

Multiple different staphylococcal exotoxins (enterotoxins A to Q) have been identified, and most of them cause emesis in primates.[49] The toxins are heat-stable and resist boiling. Therefore, once sufficient toxin has formed within food, even heating or boiling will not inactivate the toxin. Foods involved in outbreaks of food poisoning frequently have been inoculated by a lesion on the hand of a food handler in situations in which the food is then held at temperatures that allow bacterial growth (between 25° C and 60° C) to occur for some time before the food is served. Foods implicated in particular are ham, salads with starch and mayonnaise, salami, poultry, cream sauces, pastries, and dairy products. The mode of action of enterotoxins is not understood completely, but vomiting appears to be induced through receptors in the abdominal viscera that lead to an emetic response.[29] Intravenous injection of purified enterotoxins in laboratory animals causes hypotension, cardiovascular collapse, and death. The significance of these properties of enterotoxins in naturally occurring human infections is unknown, although they may be linked to clinical syndromes, including TSS. Enterotoxin A often is elaborated with TSST-I in menstrual-associated TSS, but it also is elaborated without TSST-I in patients with septicemia in whom TSS does not develop. Staphylococcal enterotoxin B, most often isolated in phage group V organisms, is associated with local infections and pneumonia.[139] USA300 strains typically do not contain the genes encoding most enterotoxins, but they frequently have the genes for enterotoxins Q and K.[46] In contrast, USA400 isolates may carry many of the enterotoxin genes, including enterotoxin H, which is unique among the other *S. aureus* genomes.[14,156]

STAPHYLOCOCCUS AUREUS MSCRAMMs

Bacterial adherence to host tissues is mediated by numerous bacterial surface components or adhesins that recognize and bind to host extracellular matrix and cell surface molecules.[98] The subfamily of adhesins that bind to the extracellular matrix is known as Microbial Surface Components Recognizing Adhesive Matrix Molecules (MSCRAMMs). For *S. aureus*, important MSCRAMMs are protein A, fibronectin-binding proteins (A and B), clumping factors A and B (fibrinogen-binding proteins), and collagen-binding protein (cna).[63] Many other less well characterized adhesins also have been identified from the *S. aureus* genome.[181] The MSCRAMMs are linked to bacterial wall peptidoglycan following sortase cleavage of the LPXTG (LeuProX-ThrGly) motif that is a conserved C-terminal cell wall sorting signal among the adhesins.[36] Fibronectin-binding proteins play a role in *S. aureus* invasion into endothelial cells.[24,98] Collagen adhesin protein may contribute to the pathogenesis of *S. aureus* musculoskeletal infections,[52,164] although both animal studies and clinical studies are conflicting in this area.[100,184] USA300 CA-MRSA isolates do not carry the gene for cna, yet osteomyelitis is the most common CA-MRSA invasive infection in children.[140]

Clumping factor A has been the target for protective immunity in the prevention of *S. aureus* infections, and this approach has been successful in experimental models.[217] The safety and pharmacokinetics of an intravenous immunoglobulin preparation enriched for antibody to clumping factor A were established in low-birth-weight infants; however, a large randomized trial did not document efficacy in prevention of staphylococcal infections in this population.[23] A monoclonal antibody (tefibazumab [Aurexis]) directed against clumping factor A has undergone preliminary evaluation as adjunctive therapy of *S. aureus* bacteremia in adults.[224]

REGULATION

Bacterial pathogenesis is reliant on cell population, density-dependent gene expression. This quorum sensing acts to adjust the production of accessory genes (e.g., virulence genes), depending on the needs of the cell. Studies have shown that in low-density populations, when the bacterial cells are multiplying, accessory genes are either turned off or are expressed at a low level to the advantage of other genes, such as genes involved in cell wall formation. In high-density bacterial populations, expression of the accessory genes is increased. The accessory gene

regulator, agr, is genus specific, and the effector molecule is a regulatory RNA molecule, named RNAIII. Many staphylococcal toxins and virulence factors are under the regulation of agr.[1,13,28] The staphylococcal accessory regulator, sar, is another global regulator in *S. aureus*. The effector peptide, SarA, is a DNA-binding protein. In animal models of infection, both RNAIII and Sar A have been shown to affect virulence. In addition to agr and sar, several other factors have been described that affect accessory gene regulation.[22] Thus, the complex network of regulatory operons that act on each other or directly on specific genes is not completely understood.

STAPHYLOCOCCAL L-FORMS AND SMALL-COLONY VARIANTS

L-forms are staphylococcal variants with impaired or absent cell walls. L-forms can be induced in vitro by growing staphylococci in hypertonic media, in the presence of the muralytic enzyme lysostaphin, or with the addition of antibiotics that inhibit the formation of cell walls, such as the penicillin-methicillin group, cycloserine, and vancomycin. These L-forms, or protoplasts, require hypertonic environments for survival, do not accept Gram stain, and are resistant to cell wall–inhibiting antibiotics. Under favorable conditions, L-forms revert to complete bacteria with cell walls.

Clinical interest in L-forms has arisen because of concern that antibiotics may induce these forms in vivo. L-forms then could persist in the host in a latent or less virulent phase and revert to a fully virulent state at some time when antibiotics no longer are present. Identification of L-forms in specimens from human infection or in infected animals treated with antibiotics has been reported exceedingly rarely, despite considerable effort.[220] The presence of latent L-forms remains an attractive hypothesis to explain the well-known tendency of staphylococcal infections to persist and recur. Despite extensive laboratory and clinical investigation, no evidence has demonstrated that L-forms are important causes of bacterial persistence in clinical infections.

Small-colony variants of *S. aureus* are isolates with atypical colony morphology, decreased hemolysis and pigmentation, decreased growth rate, and unusual biochemical characteristics that result in difficulty isolating and identifying these organisms.[216] Small-colony variants also have been implicated in persistent and recurrent *S. aureus* infections including osteomyelitis,[172] brain abscesses, and pulmonary infections in patients with cystic fibrosis.[104,188] Small-colony variants can persist intracellularly and often are more resistant to antibiotics than is the parent strain, and antibiotics that penetrate cells (e.g., rifampin) may be advantageous in their treatment.[173]

TYPING METHODS

Staphylococcal strains were characterized historically by bacteriophage typing (World Health Organization method).[18] In the 1990s, pulsed-field gel electrophoresis (PFGE) became the gold standard for strain typing used by both reference and research laboratories.[210] The method has proven to be reliable and reproducible, and the use of digital imaging and band analysis has enhanced the quality of inter-gel comparisons. The Centers for Disease Control and Prevention (CDC) used the PFGE method to define and interrelate staphylococcal clones circulating in the United States and designated these 11 clones USA100 to USA1100 (available through the Network on Antimicrobial Resistance of *Staphylococcus aureus* [NARSA] at http://www.narsa.net).[142]

Multilocus sequence typing (MLST) is a nucleotide sequence-based method, adapted from multilocus enzyme electrophoresis (MLEE). In MLST typing, the alleles at each locus of seven housekeeping genes (*arc*C, *aro*E, *glp*F, *gmk*, *pta*, *tpi*, and *yqi*L) are determined through polymerase chain reaction (PCR) amplification, nucleotide sequencing, and submission of sequence data through computer Web site to the MLST database (http://www.mlst.net).[54] In this database, strains can be compared with strains submitted by other investigators, and within-laboratory comparisons with control strains thus have become obsolete.

spa typing was developed based on the polymorphic X or the short sequence repeat (SSR) region of the protein A gene.[194] *spa* typing has some advantages over PFGE in terms of speed, interpretation, and exportability; the main limitation is the single locus-based design.[84] Less commonly used methods include repetitive element PCR (rep-PCR), restriction fragment length polymorphism (RFLP), and toxin typing.

STAPHYLOCOCCUS AUREUS CLONES IN THE HOSPITAL AND IN THE COMMUNITY

Of the methods mentioned earlier, MLST has been especially useful in dividing *S. aureus* strains into lineages (or clonal clusters). Frequently, these clones are described in the literature by their sequence type followed by the staphylococcal cassette chromosome *mec* (SCC*mec*) type (e.g., ST8-IV). Most human *S. aureus* strains belong to 1 of 10 independent lineages. Five major clonal clusters (CCs) that have been described to contain a majority of the human MRSA strains are CC5, CC8, CC22, CC30, and CC45. Of these, MRSA clones within the CC5 and CC8 clusters have been recognized as the main causes of hospital outbreaks worldwide.[38] Examples of widespread hospital MRSA clones are EMRSA15-ST22, EMRSA16 (ST36-II), the Archaic (ST250-I or 247-I), the Pediatric (ST5-IV), and the Brazilian (ST239-III) clones.[73]

Although a few hospital MRSA clones have circulated around the world, genetically distinct community clones have been reported from different geographic areas. Commonly, these strains contain the smaller SCC*mec* elements, type IV or V.[179] Why these different clones emerged in parallel at this point in time is unknown.

In the United States, the two most commonly described community MRSA clones are USA300-ST8 and USA400-ST1. USA400, originally described as MRSA strain MW2 and responsible for four pediatric deaths in Minnesota and North Dakota, has been observed more commonly in the Midwestern States.[10] USA300 is the strain most frequently reported causing community-acquired disease in both pediatric and adult settings in the United States. Like many other epidemic strains, it has been associated with a spectrum of disease presentations ranging from simple skin and soft tissue infections[105,108] to severe sepsis syndrome,[76] sometimes with a fatal outcome. USA300 is a highly virulent strain that has been distributed across the continent and has become the most prevalent cause of community-acquired infections in less than a decade.[151] It also has become the most common source of hospital-acquired *S. aureus* infection in many hospitals, where it has acquired one or more antibiotic resistance markers.[45,77,189] The special features and adaptability of this strain are discussed further in the genome section of this chapter.

MLST typing has enabled the ancestry of many of these clones to be traced phylogenetically. For example, phage type 80/81 (ST30-MSSA) was epidemic in the 1960s and is the likely ancestor to the widely circulated Southwest Pacific clone, ST30-MRSA-IV, which has been successful both as a colonizer and as a cause of diseases ranging from skin and soft tissue infections to invasive manifestations.[180] Similarly, the USA300 (ST8-MRSA-IV) clone is postulated to have descended from an archaic ST8-MSSA.[55]

S. aureus isolates are susceptible to lysis when they are exposed to bacteriophages. The particular pattern of bacteriophage lysis

can be used to identify strains of staphylococci, but bacteriophage typing has been used primarily as an epidemiologic tool for identifying related strains in epidemics. An international system for identifying strains by bacteriophage typing has been established, but it is not widely used currently. Staphylococci of phage group 80/81 were widespread during the pandemic of staphylococcal disease in the late 1950s. At present, many different phage types are involved in staphylococcal disease.[180]

GENOMES

To date, nine genomes of *S. aureus* have been sequenced (Table 91–3). MW2,[14] FPR3757,[47] and TCH1516[90a] are the three CA-MRSA isolates with sequenced genomes, designated PFGE USA400 and USA300, respectively. *S. aureus* COL[68] is an early hospital-associated MRSA strain, and N315 and Mu50 are hospital-associated MRSA strains from Japan.[117] The Mu50 strain also has intermediate resistance to the glycopeptides(GISA/VISA). Strains 476[94] and NCTC8325[69] are methicillin-susceptible strains from the community and hospital environment, and EMRSA-16 is an MRSA strain that is a major hospital pathogen in Europe.[101] Genome sizes range from 2.8 to 2.9 Mb, and the structures of the chromosomes are well conserved. The core genome (the part of the genome that is the same in all strains) represents 75 percent of the genome.[129] Only approximately 40 to 50 percent of the predicted proteins have a known function.

EPIDEMIOLOGY

S. aureus isolates are responsible for both sporadic infections and epidemics of varying extent ranging from the intrafamily outbreaks of commonly encountered staphylococcal disease to large and often prolonged hospital-associated outbreaks, such as those emanating from a newborn nursery or a surgical service. Epidemic spread of staphylococci of phage type 80/81 was so widespread in hospitals worldwide in the period encompassing the mid-1950s through the early 1960s that it constituted a pandemic. Researchers have suggested that a few "epidemiologically virulent" strains, such as 80/81, are particularly capable of spreading widely and causing disease. Because we are largely ignorant of the factors responsible for pandemic spread, we cannot prevent or predict recurrence. Robinson and colleagues concluded that descendants of 80/81 have acquired methicillin resistance to emerge as a CA-MRSA clone, closely related to the southwest Pacific ST30 clone.[180]

Staphylococci may be transmitted by multiple routes, including contact with infected persons, contact with asymptomatic carriers, airborne spread, and contact with contaminated objects. Of these mechanisms, contact with a person who has a staphylococcal lesion appears to be particularly important in the spread of staphylococci. Persons with open draining lesions disseminate organisms into their environment and to others by direct contact. In a hospital, staphylococci may be spread from an infected patient to another on the hands of caretakers, such as physicians or nurses. Hospital personnel with mild or inapparent lesions, such as styes, furuncles, or paronychia, may themselves spread organisms. In family and small community outbreaks, multiple secondary cases frequently can be traced to an individual with a draining lesion. Secondary cases tend to appear for months after the initiating case within these small epidemiologic units.[200]

Staphylococci also may be spread by asymptomatic carriers who have staphylococci in one or more body sites, including the nose, skin, hair, nails, axillae, and perineum. At any one time, as many as 30 percent of individuals are colonized in their anterior nose with *S. aureus*.[114] The rate of nasal carriage of MRSA appears to have increased in the past decade. Nasal carriage of MRSA was 0.8 percent in 2001[157] and 9 percent in 2004[40] for children seen in an emergency center in Nashville, Tennessee. For children admitted to the hospital in Corpus Christi, Texas, in 2005, the rate of MRSA colonization was 22 percent.[6] Why nasal colonization of children by CA-MRSA has increased during the past decade is unclear but likely is related to unique properties of the major CA-MRSA clone, USA300.[47] Detailed studies have been performed to delineate factors regulating the carrier state and its establishment and perpetuation, as well as factors responsible for dispersion of organisms from carriers. Asymptomatic carriers may be a source of disease for themselves and for others. For example, a wound infection appears to be more likely to develop in a hospitalized patient who becomes a carrier than in a noncarrier.[225] Much attention has been devoted to detecting and attempting to treat nasal carriers of staphylococci; however, studies of nursery outbreaks have indicated that nasal carriage is not as important as is hand transmission in the dispersal of staphylococci.[226] Transmission by hand contact can be minimized by effective handwashing.[72] The problem of hair carriage of staphylococci in operating room personnel has been minimized by using improved head coverings.

Staphylococci are widespread in the environment and can be cultured from clothing, carpets, toiletries (e.g., hairbrushes, razors), and virtually all environmental surfaces. Airborne dissemination of organisms is possible, particularly in operating rooms with poor ventilation and heavy traffic; improved methods of ventilating operating rooms may have reduced the rates of sepsis or colonization. Environmental staphylococci may serve as an important reservoir, but direct human-to-human transfer probably is a much more important means of transmission in epidemic situations than is airborne spread or contact with contaminated objects. This means of transmission is particularly true for spread of CA-MRSA among athletes.[112] Various methods that

TABLE 91–3 Complete Genomes of *Staphylococcus aureus*

Strain	Class	Meth	Van	ST	PFGE	SCC*mec*	PVL	*spa*	agr
COL	HA	R	S	250		I	Pos	YHGRMBQBLO	1
252/MRSA16	HA	R	S	36	200	II	Neg	WCKAKAOMQQQ	3
476	CA	S	S	30		N/A	Pos	UKJFKBPE	3
Mu50	HA	R	I	5	100	II	Pos	MDMGMK	3
MW2	CA	R	S	1	400	IV	Pos	UJJJJFE	3
N315	HA	R	S	5		II	Pos	MDMGMK	3
NCTC8325	HA	S	S	8		n/a	Pos	YHGGFMBQBLO	1
FPR3757	CA	R	S	8	300	IV	Pos	YHGFMBQBLO	1
TCH1516	CA	R	S	8	300	IV	Pos	YHGFMBQBLO	1

agr, accessory gene regulator; CA, community-acquired; HA, hospital acquired; I (under vancomycin), intermediated; meth, methicillin; N/A, not applicable; neg, negative; pos, positive; PVL, Panton Valentine leukocidin; PFGE, pulsed field gel electrophoresis USA pattern; R, resistant; S, susceptible; SCCmec, staphylococcal cassette chromosome; spa, spa type; ST, ST-sequence type; van, vancomycin.

are used to identify epidemiologic markers in nosocomial outbreaks of staphylococcal infection include molecular typing techniques, plasmid DNA analysis, restriction endonuclease fingerprinting of chromosome DNA, and rapid-field inversion gel electrophoresis. Molecular methods of typing offer adjunctive information useful in characterizing strains involved in outbreaks.[48,54]

The neonatal nursery has been an area of particular concern in the transmission of staphylococci. Until the 1980s, *S. aureus* was the major concern. During the pandemic of strain 80/81 disease, outbreaks of serious neonatal disease were commonplace, high colonization rates were found in infants on discharge from the nursery, and the subsequent incidence of disease in some outbreaks was as high as 50 to 70 percent. Skin disease and infant and maternal mastitis usually appear within 1 to 4 weeks after discharge. Staphylococcal pneumonia, far more common during that period than at present, may not be seen for months after delivery, even though the infecting strain was acquired in a hospital. During the experience with strain 80/81 disease, a particularly high staphylococcal attack rate was seen in the families of colonized infants. The same epidemiology has been noted for the USA300 CA-MRSA clone, which has been associated with staphylococcal infections in otherwise healthy newborns up through 30 days of life.[61] The mother in approximately 20 percent of cases had a presumed or concomitant *S. aureus* infection around the time of her infant's infection in one study.[61]

Various control measures were used successfully to terminate individual outbreaks during the 1980s. Measures to protect the infant from colonization and subsequent development of disease by establishing a barrier at the site of initial colonization included hexachlorophene bathing, application of antibiotic ointment to the umbilicus and circumcision site, and application of an antiseptic dye to the umbilicus. Another approach, deliberate colonization of the umbilicus with an interfering "avirulent" strain, was successful in controlling several epidemics but itself caused skin disease and, in one case, fatal septicemia.[193] Hexachlorophene bathing became a widespread practice during the period in which nosocomial staphylococcal infections were declining.[72,169] After approximately a decade of using hexachlorophene, researchers recognized that cutaneous absorption of hexachlorophene could result in potentially toxic hexachlorophene blood levels in infants, particularly premature ones, subjected to repeated daily baths over time. Brain stem abnormalities associated with the use of hexachlorophene were demonstrated in premature infants. For these reasons, controls were placed on the sale of hexachlorophene, and its routine use was discouraged in 1973. In one neonatal nursery outbreak of MRSA, the use of 0.3 percent triclosan (Bacti-Stat) was associated with cessation of an outbreak.[233]

Coincident with the widespread discontinuation of hexachlorophene bathing, but not necessarily causally related to it, was the reported increased incidence of nursery outbreaks of *S. aureus* disease. Many of these outbreaks were caused by epidermolytic toxin-producing strains of staphylococci belonging to phage group II. In these outbreaks, limited areas of bullous impetigo developed in most children within 1 to 4 weeks after discharge, and a few children showed the more dramatic manifestations of generalized exfoliative disease. Contemporary epidemics appear to have a much lower incidence of septicemia, pneumonia, and osteomyelitis and less potential for spread to other family members.

Patterns of crowding of infants and understaffing contribute to outbreaks. Active clinical and bacteriologic surveillance and cohorting of infants may be effective in reducing the number of outbreaks.[16,83]

The frequency of bacteremia related to intravenous catheters and prosthetic devices has increased considerably.[137,138] Methicillin-resistant staphylococci have become more prevalent in the community and are responsible for widespread nosocomial outbreaks of disease in hospitals.[25,29,39,43,57,113,186,204,223]

HOST DEFENSES

S. aureus is ubiquitous in the environment, and approximately 30 percent of the population can be classified as carriers. A major defense against staphylococcal infection is intact skin. Minor wounds frequently become colonized and infected and serve as portals to deeper, more significant staphylococcal infection. In some cases, the integumentary infection may be of major importance; in others, minor skin punctures may serve to introduce infection to distant internal sites. Burns, varicella virus and cutaneous herpesvirus infections, insect bites, minor lacerations or abrasions, primary skin diseases (e.g., atopic eczema), epidermolysis bullosa, and surgical wounds are important portals of entry for staphylococci. A deficiency in peptides (defensins and cathelicidins) important for innate immunity of skin against *S. aureus* has been shown in biopsied skin of patients with atopic dermatitis and likely contributes to the increased susceptibility of the skin to *S. aureus* infections in these patients.[115,163] In hospitalized patients, intravenous needles and catheters may be sources of staphylococcal infection.[137,138]

Foreign bodies reduce local resistance to staphylococcal engraftment and are important in the pathogenesis and perpetuation of infection. Noteworthy foreign bodies are cerebrospinal fluid shunts, prosthetic cardiac valves, nonabsorbable sutures, vascular prostheses (including arteriovenous shunts for hemodialysis), and orthopedic prostheses, nails, and wires. In neonates, the umbilicus and circumcision sites, which may be colonized within the first few hours of life and from which both local and distant infections may be established, are important portals of entry.

Viral respiratory diseases such as measles and influenza[82,170] predispose the individual to development of pulmonary infection, again predominantly by damaging the integrity of the barrier at the portal of entry. Disruption of the respiratory epithelium and impairment of ciliary motion and other local defenses may allow secondary staphylococcal invasion to occur.

Once the integumentary barrier has been breached, the polymorphonuclear leukocyte appears to be the most important line of defense. Successful phagocytosis involves chemotaxis, opsonization, and intracellular killing. The incidence of difficulty with staphylococcal infection is highest in patients with defects in this area of host defense. Granulocytopenia of any origin predisposes one to the development of infection, particularly with the host's endogenous bacteria, including staphylococci.[15]

Patients with disorders of neutrophil chemotaxis are particularly subject to the development of recurrent and severe staphylococcal infections. Patients with lazy leukocyte syndrome have recurrent respiratory infections, gingivitis, and stomatitis. Their leukocytes exhibit normal phagocytosis and intracellular killing but are deficient in both motility toward a chemotactic stimulant and random motility. Leukocyte adhesion deficiency is a rare autosomal recessive disorder of leukocytes in which patients suffer recurrent skin infections. Patients with Chédiak-Higashi syndrome have recurrent infection in association with neutrophils that are morphologically abnormal, with giant lysosomes present in decreased number.[230] Hyper-IgE syndrome or Job syndrome is associated with severe, recurrent staphylococcal infections of the subcutaneous and deep skin tissues. Even for patients without defects in neutrophil chemotaxis, approximately 60 percent of *S. aureus* isolates secrete the chemotaxis inhibitory protein of staphylococci (CHIPS), and almost all have the extracellular adherence proteins that interfere with neutrophil chemotaxis and extravasation.[62]

Once the staphylococcus and the leukocyte are close to one another, opsonization of the bacterium must proceed for phagocytosis to occur. Two systems for opsonization of staphylococci have been described. Serum from normal adults has good opsonic activity when unheated but generally is inactive after heating at 56° C for 1 hour. The heat lability of normal opsonin and the observation of normal or only slightly decreased opsonic activity in unheated sera from patients with agammaglobulinemia[227] indicate that the major opsonin is complement. A few sera, generally from patients convalescing from serious staphylococcal disease, contain heat-stable opsonins, presumably antibody directed at the staphylococcal cell wall components.[121] Therefore, either complement or antibody can provide opsonins for staphylococci; specific antibody is helpful but not required. The clinical correlate to these observations may be found in patients with defective defenses; patients with agammaglobulinemia and defective antibody generally have more severe problems with organisms other than staphylococci, whereas patients with deficiencies in complement components have been reported to have repeated and severe staphylococcal infections.[3,7,8,64,149,167]

Once the bacterium has been ingested, intracellular killing proceeds normally. Coagulase-positive staphylococci can survive within polymorphonuclear leukocytes for a considerably longer period than can coagulase-negative organisms.[148] Prolonged intracellular survival may be an important virulence factor separating coagulase-negative from coagulase-positive strains and may provide a mechanism whereby surviving organisms may be carried to distant body sites to establish metastatic foci of infection. Some *S. aureus* exotoxins outlined earlier, such as PVL, contribute to the ability of *S. aureus* to evade human neutrophils. In addition, many *S. aureus* genes are regulated upward or downward after being exposed to human neutrophils, and differences exist in gene expression among various strains in response to human innate host response that may account for differences in the ability of the isolate to cause infection.[218]

Patients with chronic granulomatous disease of childhood have an inborn error in intraleukocytic killing of catalase-positive bacteria and fungi. These patients have an early onset of recurrent purulent infections of the skin, subcutaneous tissue, lungs, and reticuloendothelial organs, particularly the liver, that often are caused by *S. aureus*.[176] The response of these patients to therapy is slow and poor, a finding further demonstrating the absolute necessity of an intact polymorphonuclear bactericidal system in host defense against staphylococci. In contrast to the primacy of polymorphonuclear defense against staphylococci, evidence for an important role of specific humoral and cellular immunity in host defense against staphylococci is either lacking or contradictory.

Specific antibody is not required for opsonization of unencapsulated strains of staphylococci. The opsonic activity of serum from patients recovering from staphylococcal endocarditis or other serious disease is greater than that in physiologically normal persons.[121,228] Despite the presence of humoral antibodies to cell wall teichoic acids and to various toxins and enzymes, which are found regularly in those convalescing from serious staphylococcal infection, the patient remains susceptible to recurrence of infection with the same strain of staphylococci.

Staphylococcal infections generally occur and progress in the presence of some degree of humoral immunity. Specific antibody to one or more staphylococcal components or products does not protect against infection. Clinical evidence from patients with deficient cell-mediated immunity (combined immunodeficiency, thymic aplasia, Nezelof syndrome) suggests that staphylococcal infections are not among the most important pathogens in these patients.

Present evidence, therefore, suggests that intact local skin and mucous membrane barriers are the most important defenses against the establishment of staphylococcal infection. Once infection is established, an intact polymorphonuclear response is essential for containment of the infection and clearance of the organisms.

PATHOGENESIS

Staphylococci cause disease by two mechanisms: direct invasion of tissues with liberation of toxins, which may have effects at sites distant from the focus of infection, and colonization. The hallmark of a staphylococcal lesion is the abscess. Local tissue destruction at the site of inoculation is followed rapidly by hyperemia and a vigorous inflammatory response marked by the accumulation of large numbers of polymorphonuclear leukocytes. Tissue necrosis in the center of the lesion occurs next. At the site of intensive hyperemia surrounding the lesion, a fibrin wall is formed. Liquefaction necrosis occurs centrally; the mature lesion consists of a fibrin wall surrounded by inflamed tissues enclosing a central core of pus consisting of organisms and leukocytes. Live bacteria may persist within these lesions for a considerable period of time. As pus accumulates, it may drain toward the skin surface or into adjacent tissues, where it forms sinus tracts and secondary abscesses. In the presence of an intact host inflammatory response, this type of reaction may be seen in diverse areas, including the skin and subcutaneous tissues, lymph nodes, joints, renal tissues, liver, parotid glands, muscles, lungs, and long bones.

In addition to local extension, *S. aureus* may disseminate hematogenously from this focus of infection, even from abscesses that are trivial in size. Hematogenous dissemination may result in infection of bones, joints, and heart valves. Given the ubiquitous nature of staphylococci, skin and wounds, which are the usual ports of entry, appear to be remarkably resistant to infection. This natural resistance is affected dramatically by the presence of foreign bodies within the wound, such as sutures and bits of soil or gravel. Natural resistance also is affected by the tissue compromise that develops after ecchymosis or hemorrhage occurs and by vascular insufficiency. Poor personal hygiene also predisposes to development of staphylococcal skin infection. Moist, macerated skin is invaded more easily and thus contributes to the increased frequency of staphylococcal skin infection in intertriginous areas and in tropical climates.

Toxigenic staphylococcal disease includes SSSS, TSS, and staphylococcal food poisoning. The major manifestations of these diseases are caused by the effects of specific toxins. Clinical manifestations of infections caused by *S. aureus* in children are discussed in several chapters, and the reader is referred to the specific infection of interest.

DIAGNOSIS

The diagnosis of significant staphylococcal infections should be pursued with vigor. Collections of pus, whether superficial or deep, should be aspirated or drained surgically for diagnostic and therapeutic purposes. Gram stain and culture should be performed. An aggressive approach to establishing the diagnosis of osteomyelitis by bone aspiration and bone biopsy provides an etiologic security that is helpful during the prolonged treatment phase that necessarily follows. When infection is associated with a foreign body, such as an intravenous catheter or suture, removal and culture of the foreign body help in determining the cause, and removal almost always is required for successful resolution of infection.

At least two blood cultures should be obtained before starting therapy for all serious infections. One need not wait for fever spikes or delay therapy to obtain specimens. Blood cultures frequently are negative in serious staphylococcal infection, a finding that demonstrates the need for performing other cultures. Blood cultures are positive in most cases of staphylococcal endocarditis,

approximately half the cases of osteomyelitis and septic arthritis, and fewer than half the cases of pneumonia and deep-tissue abscesses. Measurement of nonspecific indicators of inflammation (e.g., erythrocyte sedimentation rate and C-reactive protein), although of limited value in establishing the diagnosis, can be helpful in monitoring the clinical course of infection and response to intervention. Accurate, sensitive serologic methods for determining a diagnosis of serious staphylococcal infection are not available.

Enterotoxins can be identified by a variety of methods. Immunoassay is used routinely but may lack the sensitivity required to detect levels seen in staphylococcal food poisoning. DNA oligonucleotide probes are highly sensitive, but their clinical utility is limited by the identification of nonexpressed genes.[160] Numerous molecular tests are available to identify MRSA rapidly once it is isolated in the microbiology laboratory and to detect MRSA colonization in patients at the time of hospital admission.[214]

TREATMENT

Successful treatment of staphylococcal infection depends on adequate drainage of collections of pus and the rational use of antibiotic therapy. (Strategies for clinical management of MRSA in the community are outlined in a document developed by the CDC and available at http://www.cdc.gov/ncidod/dhqp/pdf/ar/CAMRSA_ExpMtgStrategies.pdf.) Staphylococcal infections have a particular tendency to persist and recur; for these reasons, prolonged courses of antibiotic therapy usually is required for all but minor infections. Surgical drainage is extremely important and, in some patients with minor superficial abscesses, may be all that is required. In one study, an abscess less than 5 cm in diameter was associated with successful outcome of incision and drainage, regardless of whether the antibiotic administered was active against the isolated pathogen, which was CA-MRSA.[124] For most infections, a course of antibiotic therapy administered after surgical drainage better ensures that the infection has been contained. In an adult study, antibiotics active against CA-MRSA were associated with significantly greater cures than achieved with agents not active against CA-MRSA in patients who had undergone incision and drainage of skin and soft tissue infections.[183] Failure to provide surgical drainage is an important reason for persistence or recurrence of organisms. Antibiotics cannot be expected to penetrate the avascular center of abscess cavities. When abscess cavities are undrained or when antibiotic therapy is discontinued before an area is sterilized, live bacteria may persist and disseminate to cause later recurrence at that site or at distant sites.

For moderate to severe staphylococcal infection, the patient should be hospitalized initially for intravenous therapy. This strategy ensures peak antibiotic levels, which may allow greater penetration into relatively avascular areas. Intramuscular injections rarely are indicated in children. Once the patient is afebrile, has negative blood culture, and shows definite evidence of clinical improvement, therapy can be completed with home administration of antibiotics or with oral agents, as deemed appropriate by the treating physicians.

ANTIBIOTICS FOR *STAPHYLOCOCCUS AUREUS* INFECTIONS

Since the mid-1960s, most *S. aureus* isolates from most sections of North America and Europe have been penicillinase producers and, therefore, are penicillin-resistant. When *S. aureus* isolates are likely to be the cause of infection, the most appropriate agents to administer for empiric treatment are based on the relative frequency of CA-MRSA isolates in the particular community. In areas where CA-MRSA isolates are not a concern, treatment with a penicillinase-resistant penicillin or cephalosporin should be initiated before isolating the bacteria and performing susceptibility testing (Tables 91–4 and 91–5). Methicillin-resistant organisms

TABLE 91–4 Therapy for Staphylococcal Infections in Infants and Children (Excluding Neonates)

	Oral (Mild-Moderate Infection)		Parenteral (Moderate-Severe Infection)	
Agent	**Children <40 kg**	**Children >40 kg and Adults**	**Children <40 kg**	**Children >40 kg and Adults**
Penicillins				
Oxacillin			150-200 mg/kg/24 hr in 4-6 doses q4-6h IV	4-8 g/24 hr in 4-6 doses q4-6h IV
Nafcillin			150-200 mg/kg/24 hr in 4-6 doses q4-6h IV	4-8 g/24 hr in 4-6 doses q4-6h IV
Cloxacillin	50-100 mg/kg/24 hr in 4 doses	2-4 g/24 hr in 4 doses		
Dicloxacillin	25-75 mg/kg/24 hr in 4 doses	1-2 g/24 hr in 4 doses		
Cephalosporins				
Cefazolin (Ancef, Kefzol)			75-100 mg/kg/24 hr in 3 doses q8h	4-6 g/24 hr in 3 doses
Cephalexin (Keflex)	25-100 mg/kg/24 hr in 4 doses	1-4 g/24 hr in 4 doses		
Cefadroxil (Duricef, Ultracef)	30 mg/kg/24 hr in 2 doses	1-2 g/24 hr in 2 doses		
Other Agents				
Erythromycin	35-50 mg/kg/24 hr in 4 doses	1-2 g/24 hr in 4 doses		
Clindamycin	20-40 mg/kg/24 hr in 3 doses	600-1200 mg/24 hr in 3-4 doses	30-40 mg/kg/24 hr q6-8h IV	600-3000 mg/24 hr q6-8h IV
Vancomycin			40-60 mg/kg/24 hr q6h drip over 1 hr or by continuous drip IV	1-2 g/24 hr q6h over 1 hr or by drip continuous IV
Linezolid (Zyvox)	10 mg/kg/dose q8h*	10 mg/kg/dose q12h†	10 mg/kg/dose q8h*	10 mg/kg/dose q12h†
Doxycycline	2-4 mg/kg/24 hr 1or 2 doses for children >7 yr of age	100-200 mg/24 hr		
Trimethoprim-sulfamethoxazole	8-12 mg/kg/24 hr of TMP in 2 doses	160 mg of TMP q12h		

**The dose for children younger than 12 years.*
†*The dose for children ≥12 years of age, not to exceed 600 mg/dose.*
IV, intravenously; TMP, trimethoprim.

TABLE 91–5 Anti-staphylococcal Therapy in Neonates with Moderate to Severe Infection

	Premature Infants (<2000 g)		Term Infants	
Agent	**<1 wk**	**1-4 wk**	**<1 wk**	**1-4 wk**
Oxacillin or nafcillin IV	25-50 mg/kg/dose q12h	25-50 mg/kg/dose q8h	25-50 mg/kg/dose q12h	100-200 mg/kg/24 hr nafcillin IV in 4 doses
Linezolid IV	10 mg/kg q 8-12h*	10 mg/kg/dose q8h	10 mg/kg/dose q8h	10 mg/kg/dose q8h
Vancomycin IV	15 mg/kg/dose q12-24h†	15 mg/kg/dose q8-24h†	30 mg/kg/24 hr in 2 doses q12h†	45 mg/kg/24 hr in 3 doses q8h†

**For infants <34 weeks' gestation and <1 week of age, administer every 12 hours.*
†Administer over a 1-hour period. Monitor serum concentrations during therapy.
IV, intravenously.

cannot be treated adequately with any β-lactam antibiotic, including cephalosporins. In some locations, methicillin resistance is widespread in community isolates; thus, alternative agents (discussed later) are indicated for empiric treatment. When a penicillin-resistant organism that is susceptible to oxacillin is isolated, the semisynthetic penicillinase-resistant penicillins are the drugs of choice. Oxacillin and nafcillin are available for parenteral use. Dicloxacillin and cephalexin are the preferred oral agents.

If clinical response appears to be slow, nothing is to be gained by switching to another antibiotic within this category. Instead, microbiologic data are reviewed, and the dose of antibiotic is rechecked to be certain it is correct. The patient is reassessed to be certain that all sites requiring drainage have been addressed, and compliance with therapy is addressed, especially if the antibiotics have been administered orally.

ALTERNATIVE DRUGS

The cephalosporin antibiotics are active against penicillinase-producing staphylococci and cause less irritation of veins with intravenous infusion than do penicillinase-resistant penicillins. A potential disadvantage lies in their broader spectrum of activity, which may promote superinfection with cephalosporin-resistant, gram-negative organisms in a debilitated patient with serious staphylococcal disease. Cephalosporins have been advocated widely for use in patients allergic to penicillin, but because of considerable cross-reactivity, they should be used extremely cautiously, if at all, in patients with a clear history of serious penicillin allergy or anaphylaxis. Among this group of antibiotics, cefazolin is the agent of choice for parenteral use.[168] It is well tolerated and can be administered every 8 hours intravenously. Serum concentrations of cefazolin are higher, and effective tissue levels appear to be easier to attain. Cefazolin also is associated with less bone marrow suppression than is nafcillin or oxacillin. The efficacy of the second- and third-generation cephalosporins against *S. aureus* is reduced. Therefore, these drugs, especially cefotaxime, ceftriaxone, and cefuroxime, should be given in addition to specific anti-staphylococcal agents if *S. aureus* is suspected strongly. Clindamycin and trimethoprim-sulfamethoxazole are alternative agents for patients who are seriously allergic to or intolerant of β-lactam antibiotics.[12,58,109]

The β-lactam resistance of MRSA is caused by the production of a novel penicillin-binding protein (PBP) designated PBP-2′ or 2a, which, unlike the intrinsic set of PBPs (PBP-1 to PBP-4) of *S. aureus*, has remarkably reduced binding affinities to β-lactam antibiotics. Despite the presence of otherwise inhibitory concentrations of β-lactam antibiotics, MRSA can continue to synthesize cell walls solely through the uninhibited activity of PBP-2′ or 2a.[111,141] PBP2′ or PBP2a is encoded by the *mecA* gene, which is carried by a unique mobile genetic element integrated into the *S. aureus* chromosome designated SCC*mec*.[92]

Some cases of MRSA actually may be caused by infection with *S. aureus* that lacks the *mecA* gene responsible for true methicillin resistance. The mechanism of resistance in these cases may be hyperproduction of β-lactamase.[102] Frequently in these cases, methicillin resistance is borderline, with minimal inhibitory concentrations (MICs) of 8 μg/mL or less. Hence, high doses of β-lactam antibiotics may be inhibitory. The clinical significance of these isolates is unknown.

STAPHYLOCOCCAL CASSETTE CHROMOSOME *mec*

SCC*mec* is a region spanning between 20 and 70 kilobases approximately 30 kb downstream from the chromosomal origin of replication. SCC*mec* contains the *mecA* gene encoding a PBP (PBP2a or PBP2′) with poor affinity to β-lactam antibiotics, thus allowing essential functions of cell wall formation to proceed in the presence of β-lactam antibiotics. The *mecA* gene is regulated by mecR1-mecI, in which activated mecR1 (signal transducer) inactivates mecI (repressor) through site-specific proteolytic cleavage, thereby initiating production of PBP2a or PBP2′. In vitro studies have suggested that the β-lactamase regulators blaR1-blaI may also be effective regulators of *mecA*.[146]

The SCC*mec* classification systems are based on several genetic elements including the class of *mec* and the *ccr* genes, which are recombination genes involved in the mobility of the genetic cassette. Other elements that may be present are IS431, IS1272, Tn554, pUB110, and pT181. SCC*mec* types II and III also contain other antibiotic resistance genes such as tet (tetracycline resistance). All strains contain a J(unk)-region of variable size and composition. Several schemes for SCC*mec* typing have been published.[116,161,162] Historically, nosocomial MRSA isolates contained the SCC*mec* types I to III, and when community isolates were first described, three new types (IV, V, and VI) were introduced. The smaller size (<30 kb) of SCC*mec* types IV to VI is thought to explain an increased mobility between strains. Because the community strains have been brought into the health care setting, SCC*mec* typing cannot be used reliably to predict whether an infection is related to health care or to the community.[77,88,189]

MRSA was described first within 1 year of the introduction of penicillinase-resistant penicillins.[97] Initial reports of infection appeared in England in the early 1960s and subsequently were followed by reports from other European countries.[119,203] In the United States, only sporadic cases were observed initially,[113,119] and not until 1968 was the first nosocomial outbreak described.[19] Since then, the prevalence of MRSA in the hospital setting has increased steadily. In a 1989 survey of U.S. hospitals, 97 percent reported the presence of MRSA.[25] The SCOPE (Surveillance and Control of Pathogens of Epidemiologic Importance) prospective surveillance project conducted between 1995 and 2001 identified more than 3400 episodes of nosocomial bacteremias in children. *S. aureus* accounted for 9 percent of isolates.[229] The proportion of *S. aureus* isolates that were MRSA increased from 10 percent in 1995 to 29 percent in 2001. Risk factors associated with development of infection or colonization with MRSA

include recent or prolonged hospitalization, exposure to antibiotics, and stay in an intensive care unit.[133] Nosocomially acquired MRSA appears to be fully virulent, with in vitro characteristics similar to those of methicillin-susceptible staphylococci. It has equivalent virulence in studies of experimental infection in mice, and clinical studies confirm comparable mortality rates.[90,213] These strains characteristically are multiresistant and usually show little or no susceptibility to cephalosporins, aminoglycosides, erythromycin, clindamycin, and tetracyclines.[4,222] Strains that appear to be susceptible to cephalosporins by standard disk sensitivity tests are proved resistant in quantitative dilution tests. The clinical efficacy of cephalosporins against methicillin-resistant strains has been poor.[222]

Epidemic outbreaks of nosocomially acquired MRSA have been reported. In these outbreaks, nasopharyngeal colonization with MRSA often occurs before infection develops. A high rate of nasal and hand carriage has been observed in health care workers associated with units that have MRSA outbreaks.[153] The usual approach to outbreaks has been to emphasize handwashing between examining and caring for patients. Strict isolation in a private room generally is advocated.[154] Single-room isolation usually is impractical in neonatal and pediatric intensive care units, where isolation facilities are in short supply and a single MRSA-colonized patient may occupy a room for months. Strict adherence to universal precautions (body substance isolation) with all moist body fluids and strict handwashing between seeing patients appear to be rational alternatives to "strict isolation."[135] Intranasal application of mupirocin ointment has been found to be capable of eliminating nasal and hand carriage of both colonized patients and hospital staff.[178] Interventions to control outbreaks of MRSA in intensive care or other units are multifaceted, and the reader is referred to major guidelines for recommendations.[154,196]

Occasional cases of MRSA infection apparently acquired in the community were observed sporadically in the 1970s. However, these infections were from patients who were chronically ill, and many patients had a history of nursing home residence, recent admission to acute or chronic health care facilities, previous receipt of antibiotics, or intravenous drug abuse.[125,145,185,206] Hence, in these cases, infections usually were traceable to the hospital setting. By contrast, since the 1980s, cases of true CA-MRSA infection in patients without identified risk factors have appeared in the literature. The first reports in children arose from small MRSA outbreak investigations.[85] Subsequently, Rathore and Kline described three patients with deep-seated infections caused by MRSA acquired in the community.[177] In the 1990s, reports of CA-MRSA in patients without known risk factors continued to appear sporadically in the literature. However, most of the infections described occurred in adults.[79,122,152] Four pediatric deaths in Minnesota and North Dakota in the period 1997 to 1999 demonstrated the potential for severe disease resulting from CA-MRSA infections.[10] The landmark study describing the changing epidemiology of CA-MRSA in children was published in 1998.[89] Herold and colleagues performed a retrospective review of medical records and compared the frequency of *S. aureus* isolation in hospitalized children during two time periods: between 1988 and 1990 and between 1993 and 1995. The prevalence of CA-MRSA in children without identified risk factors was 25.9 times higher in 1993 to 1995 than in 1988 to 1990.[89] After the publication of this article, several reports of CA-MRSA in children without risk factors from different parts of the United States and many regions of the world appeared.[30,37,103,108,175,187,234] This explosive increase in reporting that occurred in just a few years suggests that clones of CA-MRSA have unique properties that allow rapid spread once these pathogens are introduced into the community.[106]

All reports of the clinical characteristics of CA-MRSA infection in children without risk factors document a predominance of superficial infections, including subcutaneous abscesses and cellulitis.[30,89,108,175] However, many children with abscesses require admission to the hospital for incision and drainage with the patient under anesthesia.[108] Invasive disease with CA-MRSA, including severe, life-threatening infection, has been increasing and accounted for about 5 percent of cases in children seeking care in an emergency center of a large children's hospital in Houston.[5,10,75,76,108]

Unlike hospital-acquired MRSA, CA-MRSA isolates usually are susceptible to most non–β-lactam antibiotics, including clindamycin, gentamicin, trimethoprim-sulfamethoxazole, and doxycycline or minocycline, in addition to vancomycin, daptomycin, and linezolid.[105] Erythromycin susceptibility is somewhat more variable; the proportion of susceptible isolates varied from 29 to 80 percent in various studies.[80,89,95,155] Macrolide (erythromycin, azithromycin, clarithromycin)-resistant strains remain susceptible to clindamycin if the macrolide resistance is caused by an efflux pump (MEF). However, resistance to clindamycin, lincosamide, and streptogramin B can be constitutive (macrolide-lincosamide-streptogramin B] MLS_B]) or inducible ($iMLS_B$). Both mechanisms can be detected with molecular methods and also reliably in the routine laboratory by the "D test" (Fig. 91–5).[59] In vitro as well as some in vivo evidence indicates that, in the presence of erythromycin resistance, *S. aureus* could become resistant to clindamycin during therapy with this antibiotic.[50,144,165,221] This phenomenon, $iMLS_B$ resistance, which consists of modification of the target rRNA of *S. aureus*, is mediated by the presence of the resistance-conferring *erm* (erythromycin resistance methylase) gene, which encodes a 23S rRNA methylase.[96,123] Although 14-membered ring macrolides such as erythromycin are the most potent inducers, lincosamides such as clindamycin also can act as weaker inducers.[123] This ability may have clinical relevance in the setting of infections in which the bacteria are not eliminated quickly and may be exposed to subinhibitory concentrations of clindamycin for any amount of time. Examples include therapy for undrained deep-seated abscesses or treatment of osteoarticular infections. In patients infected with a strain having the $iMLS_B$ resistance genotype and expressing

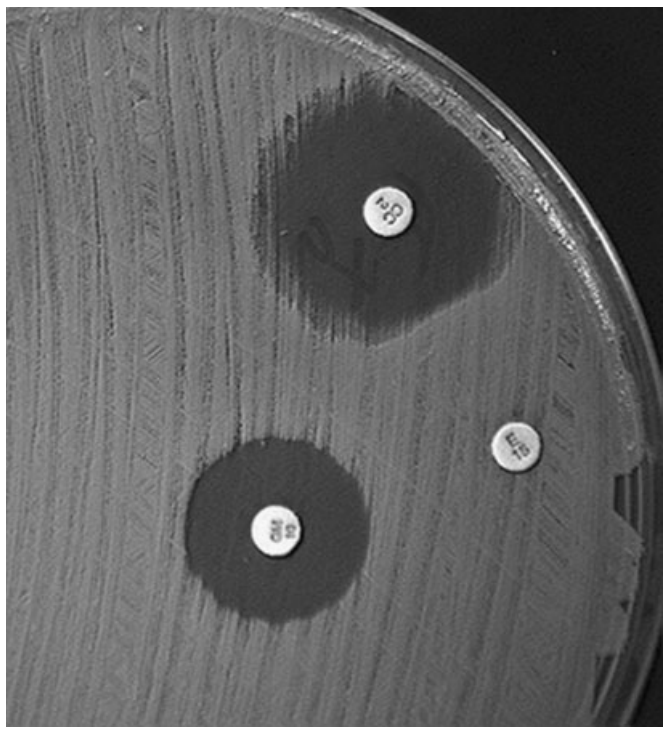

Figure 91–5 D test.

the erythromycin-resistant, clindamycin-susceptible phenotype, cases of clindamycin therapeutic failure have been reported.[66,126,195] Therefore, for MRSA isolates that are erythromycin-resistant and clindamycin-susceptible, detection of the presence of inducible MLS_B resistance should be performed by the disk approximation method, in which clindamycin- and erythromycin-impregnated disks are set 15 to 20 mm apart over Mueller-Hinton agar containing a standard inoculum of bacteria. After a 24-hour incubation period, if the zone of inhibition around the clindamycin disk is flattened or blunted (D shaped, as in Fig. 91–5) on the side facing the erythromycin disk, the isolate is classified as having an $iMLS_B$ resistance phenotype.[123]

Vancomycin, alone or together with an aminoglycoside or rifampin, is the drug of choice for serious MRSA infections. Linezolid, the first of a new family of antimicrobial agents known as oxazolidinones, has demonstrated significant activity against MRSA, vancomycin-resistant enterococci, and penicillin-resistant *S. pneumoniae*. It is available in both oral and intravenous formulations. Quinupristin-dalfopristin is an intravenous streptogramin antibiotic combination that has been found to be effective in the treatment of MRSA and vancomycin-resistant *Enterococcus faecium* infections in patients intolerant of therapy or in whom previous therapy failed. The frequent development of phlebitis with parenteral administration through a peripheral vein mandates the use of central venous access whenever possible. Both linezolid and quinupristin-dalfopristin are expensive, so their use should be restricted to situations for which other alternatives are not feasible. Daptomycin is another newer agent with the most rapidly bactericidal activity in vitro against MRSA.[27]

For severe infections caused by MRSA, the antibiotic of choice is vancomycin. In areas where CA-MRSA has been isolated from children without identified risk factors, severe, life-threatening infections suspected to be caused by *S. aureus* should be treated empirically with both nafcillin and vancomycin because nafcillin is a more active antibiotic than is vancomycin for the treatment of methicillin-susceptible isolates.[78] The addition of gentamicin should be considered for synergistic purposes. Antibiotic therapy can be adjusted subsequently after antibiotic susceptibility testing results are available. Clindamycin and linezolid are options for therapy depending on the susceptibility of the isolate, the desired route of administration, and the absence of endovascular involvement. Daptomycin is approved for serious *S. aureus* infections including bacteremia in adults, but pharmacokinetics and safety studies in children are lacking.[11,65] Tigecycline is another newer agent most useful for polymicrobial infections including MRSA in adults, but it has not been studied in children.[201] Nafcillin, oxacillin, and cefazolin are the recommended agents for treatment of severe MSSA infections.

For mild to moderate skin and soft tissue infections caused by CA-MRSA, empirical treatment may include other antibiotics such as clindamycin or trimethoprim-sulfamethoxazole. Doxycycline or minocycline are options for children older than 8 years of age.[182] If more than 90 percent of community *S. aureus* isolates are susceptible to clindamycin, this agent can be used for empiric treatment of moderately invasive infections, such as in children with osteomyelitis or pneumonia and empyema who do not require intensive care. This approach is not recommended when the clindamycin rate exceeds 10 percent of the CA-MRSA and CA-MSSA isolates combined.[44] Vancomycin or linezolid would be considerations in such circumstances.[107] Once an organism is isolated, therapy is modified based on susceptibility patterns. Nafcillin, oxacillin, and cefazolin are the preferred parenteral agents, and dicloxacillin and cephalexin are the preferred oral agents for moderate MSSA infections.

The origin of CA-MRSA is not known. The absence of health care exposure in patients harboring the bacterium, the unique antibiotic susceptibility characteristics of these isolates, and distinctive pulsed-field electrophoresis patterns that are different from the patterns of hospital-acquired MRSA isolates for a given institution suggest that the origin may be the community and that these isolates were not merely transferred from the hospital setting.[2,81] The unique combination of the gene complex and the much smaller SCC*mec* size than identified in the SCC*mec* of hospital-acquired MRSA also suggest a different origin.[136] Whether the origin is the hospital or the community, the changing epidemiology of MRSA is remarkably similar to the emergence of penicillin-resistant *S. aureus* that occurred in the 1940s and 1950s.[34] Finally, the increasing rate of isolation of MRSA will mandate the use of alternative antibiotics, which, in turn, may promote the development of additional resistance to other antibiotic classes, the most concerning of which is resistance to glycopeptide antibiotics such as vancomycin. Although vancomycin is the antibiotic of choice for severe MRSA infection, its use must remain monitored and controlled because clinical isolates with decreased susceptibility to this antibiotic, vancomycin- or glycopeptide-intermediate *S. aureus* (VISA or GISA), have been reported.[198]

In June of 2002, the first documented infection by vancomycin-resistant *S. aureus* (VRSA) in the United States was reported.[32] The isolate, obtained from a catheter exit site of a 40-year-old diabetic patient undergoing chronic dialysis, had high MICs for vancomycin (1024 μg/mL) and oxacillin (>16 μg/mL) but was susceptible to chloramphenicol, linezolid, minocycline, quinupristin-dalfopristin, and trimethoprim-sulfamethoxazole.[32,35] Since then, six additional isolates have been reported from Michigan, Pennsylvania, and New York.[33,209,212] All the VRSA strains are resistant to the glycopeptides by virtue of the *van*A gene found in the enterococci. VISA strains (MIC of 4 or 8 μg/mL) do not harbor the *van*A gene and are intermediately resistant to vancomycin because of a thickened cell wall containing vancomycin-binding dipeptides.[42,86,91] Vancomycin (and other glycopeptides) intermediate resistance detection in the laboratory is not detected by disk diffusion techniques and may not be consistently detected by automated systems.[211] In response to Clinical and Laboratory Standards Institute guidelines, microbiology laboratories have lowered the breakpoints for vancomycin susceptibility to 2 μg/mL or less, intermediate to 2 to 8 μg/mL, and resistant to 16 μg/mL or more. In actuality, very few *S. aureus* have MICs greater than 4 μg/mL.[209]

PREVENTION

Staphylococcal infections are so common that virtually everyone has had at least some minor encounters. Skin infections occur more commonly in tropical climates or during warm, humid weather in temperate areas and are likely to arise in moist areas of the body such as the axillae and skin creases. High standards of personal hygiene, careful cleaning, and adequate protection of abrasions and minor lacerations reduce the likelihood of skin infection developing.[207] Early attention given to minor infections in these small wounds with careful cleaning and antibiotic ointment may help to prevent more serious or invasive infections. We have been impressed repeatedly with the minor nature of the cutaneous source of infection in serious staphylococcal osteomyelitis, pneumonia, and endocarditis.

Person-to-person spread from an overt lesion is a major route for dissemination of infection within families, in hospitals, and in schools. A person with an infected, purulent wound should receive prompt treatment and be excused from school and from such occupations as hospital worker or food handler while the infection is open or draining. At home, special precautions should be taken in care and dressing of the wound. Disposable gauze pads should be used to wash and dry it, and towels and washcloths should not be shared with other members of the family. For children with recurrent infections or for families with multiple

family members with infections, we have found that having the patients take a bath twice a week in water to which bleach (1 teaspoon/gallon of bath water) has been added decreases the frequency of recurrences.

To date, no vaccine has been successfully developed for the prevention of *S. aureus* infections.[191] As noted earlier, a vaccine targeting types 5 and 8 polysaccharide capsule initially appeared promising but was not efficacious in a larger study.[192] One trial using immune globulin enriched for antibody to clumping factor A failed to prevent staphylococcal infections in low-birth-weight infants,[23] and a monoclonal antibody directed against clumping factor A was investigated preliminarily in adults with *S. aureus* bacteremia.[224] A polyclonal IgG preparation containing high levels of antibody to *S. aureus* capsular polysaccharides 5 and 8 has been developed by collecting plasma from healthy donors immunized with StaphVAX.[20] The pharmacokinetics and safety of this product in very low-birth-weight infants were established in a phase II trial, but further development of this antibody preparation is questionable. The safety and pharmacokinetics of human chimeric monoclonal antibody directed against the lipoteichoic acid, an integral component of the cell wall of gram-positive organisms, including *S. aureus*, were evaluated in a phase II study in very low-birth-weight infants.[23] Whether this antibody (pagibaximab) will be efficacious in preventing invasive *S. aureus* infections in these infants is to be determined.

Perhaps taking advantage of the *S. aureus* genome with a reverse vaccinology approach will lead to a safe and efficacious vaccine.[205] Until such time, however, simple measures such as washing hands frequently, giving attention to skin breaks, and avoiding contact of obviously infected skin lesions are the most effective measures to prevent *S. aureus* infections.

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CHAPTER 92

Coagulase-Negative Staphylococcal Infections

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Coagulase-negative staphylococci are among the most common causative agents of nosocomial infections and are among the bacteria most frequently isolated in the microbiology laboratory. Their normal habitat includes skin and mucous membranes of humans and animals. This ecologic niche hampers studies on this group of bacteria because they also are frequent contaminants in the clinical microbiology laboratory. Coagulase-negative staphylococci infections occur mainly in immunocompromised patients, particularly those with indwelling medical devices.[160,178,203,249] New epidemiologic and molecular techniques can be useful to identify characteristics of strains in nosocomial outbreaks. Understanding the pathogenesis of infections caused by these organisms has advanced, and several possible virulence factors have been identified. The clinical spectrum of the disease is characterized by a more indolent presentation when compared with other gram-positive cocci. Removal of catheters or prosthetic devices may be necessary for patients with persistent infections. Furthermore, effective therapy can be difficult to achieve because of the high proportion of isolates resistant to antibiotics. This chapter reviews the microbiology, epidemiology, pathogenesis, clinical manifestations and diagnoses, and treatment of infections caused by these ubiquitous organisms.

HISTORICAL BACKGROUND

Staphylococcus albus was the descriptive term used to define all coagulase-negative staphylococci before 1960 because so few techniques for species definition existed. Rosenbach in 1884 used the term *S. albus* for coagulase-negative staphylococci to denote the white color imparted by the colony on an agar plate, in contrast to the pathogenic *Staphylococcus aureus*, which had a yellow colony color.

In the 1960s, coagulase-negative staphylococci clearly were identified in association with infections in certain patient populations. Coagulase-negative staphylococci were implicated as the etiologic agent of infections in patients with atrioventricular shunts and peritoneal catheters and in neonatal sepsis.[30,32,137,217]

During the 1960s, *Staphylococcus saprophyticus* was recognized as a singular species and found to be a pathogen in urinary tract infections. Additionally, *Staphylococcus epidermidis* supplanted *S. albus* as a generic term for all coagulase-negative staphylococci other than *S. saprophyticus*. The association of disease with coagulase-negative staphylococci highlighted the need for further specification. Work in the 1970s focused on coagulase-negative staphylococci biotyping (differentiation based on biochemical reactions), and this work continues. Currently, more than 32 species of coagulase-negative staphylococci are recognized. Now that individual species can be identified, *S. epidermidis* is used as a specific species term.

MICROBIOLOGY

Staphylococci are nonmotile, non–spore-forming, gram-positive bacteria. The genus *Staphylococcus* is related most closely to the

newly described genus *Macrococcus*, and it has a relatively close relationship with the genera *Bacillus*, *Salinicoccus*, *Gamella*, *Listeria*, *Planococcus*, and *Brochothrix*.[129] Members of the genus *Staphylococcus* have a low DNA G + C content (30 to 39 mol %), whereas members of the genus *Micrococcus* have a G + C content within the range of 66 to 75 mol percent.[127] Staphylococci can be divided by the ability to produce or not produce coagulase, an extracellular enzyme that promotes the congealing of rabbit plasma. The thermonuclease reaction is particularly useful for rapidly differentiating *S. aureus* (positive) from other staphylococcal species (negative) and is more accurate than are tests based on coagulase production.[101] *S. aureus* also can be differentiated from most coagulase-negative staphylococci by the fermentation of mannitol. Currently, 32 species of staphylococci are recognized, 29 of which are coagulase-negative staphylococci, and are identified by the following criteria: (1) colony morphology, (2) oxygen requirements, (3) novobiocin resistance, (4) aerobic acid production from carbohydrates, and (5) selected liability to enzymatic activities.[127,129] Susceptibility to novobiocin is a convenient assay to differentiate *S. saprophyticus* from most coagulase-negative staphylococci from human specimens, including *S. epidermidis*. *S. saprophyticus*, the uncommon pathogen *Staphylococcus cohnii*, and the rare pathogen *Staphylococcus xylosus* are novobiocin resistant.[186] Commercial kits used to identify species of coagulase-negative staphylococci have various degrees of confidence: an identification is made 60 to 95 percent of the time, depending on the species. Most systems are developed to identify especially *S. epidermidis* and *S. saprophyticus* because these are the species clearly associated with clinical diseases.[189] Additionally, DNA-pairing studies have identified intraspecies differences with strains considered to be *S. epidermidis* by biotyping.[265]

Coagulase-negative staphylococci are prototypic gram-positive bacteria. The outermost structure is a cell wall composed primarily of peptidoglycan with teichoic acid molecules and an assortment of interspersed proteins. The teichoic acid has a glycerol backbone, compared with the ribitol of *S. aureus*.[167] Approximately 20 to 30 proteins are located within the cell wall; 15 to 20 of these are surface exposed and thus are able to interact with the host.[180]

S. epidermidis and other coagulase-negative staphylococci produce a capsule that appears to be a virulence factor in animal models.[106,168,261] However, its presence has been demonstrated in only 9 percent of fresh clinical isolates.[105] A glycocalyx or slime-layer substance, produced by most strains of *S. epidermidis*, is considered a virulence factor that inhibits phagocytosis.[39,41,52,53]

TABLE 92–1 Staphylococci That Are Part of the Normal Flora, Including Common Sites of Habitation and Pathogenic Potential

Species	Common Anatomic Site of Habitation	Pathogenic Potential
S. aureus	Nares	Common
S. epidermidis	Nares; axillae; skin of head, arms, and legs	Common
S. saprophyticus	Occasionally skin	Common
S. haemolyticus	Skin of head, arms, and legs	Uncommon
S. hominis	Axillae; skin of head, arms, and legs	Uncommon
S. lugdunensis	Widely distributed on body	Uncommon
S. simulans	Occasionally skin	Uncommon
S. cohnii	Occasionally skin	Uncommon
S. warneri	Occasionally skin	Uncommon
S. saccharolyticus	Rarely skin	Uncommon
S. caprae	Occasionally skin	Rare
S. capitis	Skin of head, face, ears, and arms	Rare
S. auricularis	Ears	Rare
S. schleiferi	? Skin	Rare
S. xylosus	Occasionally skin	Rare

Modified from Pfaller, M. A., and Herwaldt, L. A.: Laboratory, clinical, and epidemiological aspects of coagulase-negative staphylococci. Clin. Microbiol. Rev. 1:281-299, 1988.

TABLE 92–2 Current Methods of Epidemiologic Analysis of Coagulase-Negative Staphylococci

Conventional	Molecular
Biotyping[94,175]	Multilocus enzyme electrophoresis[166,239,249,267]
Colony morphology[127]	Plasmid analysis[8,127,172,173,213,214,236,253]
Antibiograms[91,92,127,136,149,155]	Chromosomal analysis[20,75,107]
Serology[1,182,263]	Polymerase chain reaction amplification[127]
Polypeptide analysis[31,33,44,57,239]	
Slime-layer production[40,47]	
Phage typing[39,50,175,176,230,234]	
Pyrolysis mass spectrometry[68]	

EPIDEMIOLOGY

Epidemiologic studies involving strain delineation of coagulase-negative staphylococci have proved difficult to perform because of the organism's commensal nature on the human body. *S. epidermidis* is the prominent species, accounting for 60 to 90 percent of all staphylococci recovered from humans. The ecologic niches of coagulase-negative staphylococci have allowed a classification, as shown in Table 92–1.

Coagulase-negative staphylococci, except for *S. saprophyticus*, cause primarily nosocomial infections. Antibiotic resistance of coagulase-negative staphylococci is a common occurrence because of selective antibiotic use in the hospital setting. In 1997, the National Nosocomial Infections Surveillance Report noted an 87 percent increase in the rate of oxacillin resistance among coagulase-negative staphylococci isolates from patients in intensive care units when compared with the same period 5 years earlier.[36] Coagulase-negative staphylococci gain access to the bloodstream primarily by breakdown of skin or mucocutaneous barriers, by following a prosthetic catheter tract, or through the hub of a central venous catheter. Two studies addressed the acquisition of *S. epidermidis* as a colonizing organism in low-birth-weight neonates.[46,90] Both reports described rapid colonization, in which 75 percent of neonates were colonized by 2 weeks of age, but differed in their findings of increased production of slime-layer or antibiotic-resistant organisms with increased colonization time.[46,90] Although most infants acquire coagulase-negative staphylococci from environmental sources, including hospital personnel, a few are colonized by vertical transmission.[89,181]

Epidemiologic techniques are of paramount importance in coagulase-negative staphylococci infections to identify a strain causing either a common-source outbreak or repeated infections within an individual.[166,189,249] Techniques currently available are divided into conventional or molecular and are listed in Table 92–2. Conventional methods are fraught with poor standardization, sensitivity, and specificity. However, a combination of these techniques has been used for strain delineation. Genotyping by pulse-field gel electrophoresis (PFGE) has been shown to be highly discriminatory when investigating patterns of transmission and sources of outbreaks.[26,134,156,238,249] Random amplification of polymorphic DNA (RAPD) by polymerase chain reaction (PCR) also has been successful in identifying clonal outbreaks and transmissions. Although it is less discriminatory than is PFGE, RAPD

Figure 92–1 Scanning electron microscopy of slime-producing coagulase-negative staphylococci. *(From Peters, G., Locci, R., and Pulverer, G.: Adherence and growth of coagulase-negative staphylococci on surfaces of intravenous catheters. J. Infect. Dis.* 146*:479-482, 1982.)*

is less costly and time-consuming.[23,195] Multilocus sequence typing (MLST) can be used to delineate clonal relationships between strains and has been used to identify predominant clones from a group of clinical isolates.[144,266]

Antimicrobial susceptibility testing is the method most readily available for typing using a phenotypic characteristic. Methicillin resistance is mediated by the *mecA* gene, which is carried on a chromosomal element called *staphylococcus chromosomal cassette mec* (SCC*mec*).[266] This mobile element is thought to be capable of horizontal transfer between species and to confer methicillin resistance in community-acquired *S. aureus*. Typing of SCC*mec* can be achieved by PCR.

PATHOGENESIS

Several risk factors for development of coagulase-negative staphylococci infection are caused by changes in host defense. Initial risk factors include a breakdown of the mucocutaneous barrier, immunosuppression,[64,119] and prior antibiotic therapy.[120,218] In addition, the presence of a prosthetic device, such as an indwelling central venous catheter, cerebrospinal fluid (CSF) shunt, or peritoneal dialysis catheter, increases the susceptibility to infection.[12,19,59,138] Opsonophagocytosis is the most important immune defense against coagulase-negative staphylococci.[64,120,227] An increased rate of infection has been noted in patients with a dysfunctional opsonophagocytosis system, including neonates[64,227] and patients receiving continuous ambulatory peritoneal dialysis.[77,120]

Biolfilm formation (Fig. 92–1 and Table 92–3) is considered to be a major virulence factor for *S. epidermidis* and other coagulase-negative staphylococci, especially in nosocomial and prosthetic device infections.[5,56,144,241,247] It consists of multiple layers of cells embedded in an amorphous extracellular material often referred to as *slime*. It provides a mechanism for staphylococci to evade antibiotics and host defense.[34,60,79,111,131,220,221,248,252]

Biofilm formation is initiated by attachment of the organism to a surface such as polystyrene.[144,145] This adhesion is mediated by cell wall teichoic acids[102] and proteins, including autolysins AltE (surface-associated autolysin) and Aae,[98,102,204,240] that interact and bind with extracellular matrix proteins such as fibronectin, fibrinogen, and vitronectin. Nonspecific electrostatic and hydrophobic interaction also promote attachment.[95,100,243] Initial

TABLE 92–3 Biofilm Formation

Mechanisms
Initial Attachment
Physicochemical forces (charge, van der Waals, hydrophobic interactions)
Staphylococcal surface proteins (SSP-1 and SSP-2)
Surface-associated autolysin (AtlE)
Capsular polysaccharide/adhesin (PS/A)
Host factor–binding proteins: fibrinogen-binding protein (Fbe); AtlE*
Adhesin/autolysin (Aas)†
Accumulation Process
Polysaccharide intercellular adhesin (PIA)
Capsular polysaccharide/adhesin (PS/A)
Accumulation-associated protein (AAP)

**AtlE exhibits vitronectin-binding activity.*
†Aas binds fibronectin and hemaglutinates sheep erythrocytes.
Data from Heilman, C., and Peters, G.: Biology and pathogenesis of Staphylococcus epidermidis. In Fischetti, V. A., Novick, R. P., Ferretti, J. J., et al. (eds.): Gram-Positive Pathogens. Washington, D.C., ASM Press, 2000, pp. 442-449.

attachment is followed by accumulation of biofilm, which is primarily mediated by polysaccharide intercellular adhesin (PIA).[97,205] PIA also acts as a hemaglutinin[144,202] and protects the bacteria from phagocytosis and killing by human polymorphonuclear leucocytes.[222,252] The *ica*ADBC operon, which is found in both *S. epidermidis* and *S. aureus*, encodes enzymes necessary for PIA production.[97]

In numerous epidemiologic studies, the presence of the *ica* operon has been associated more frequently with *S. epidermidis* strains isolated from hospitalized patients with prosthetic device infections than with community strains isolated from healthy individuals. Eighty-one to 89 percent of isolates from hospitalized patients have been reported to carry the *ica* operon. In contrast, only 6 to 38 percent of community-acquired isolates were found to carry the *ica* operon.[67,130,244] The utility of *ica* operon detection in differentiating between invasive strains and commensal strains of *S. epidermidis* obtained from patients in neonatal intensive care units, bone marrow transplant units, and oncology units has been investigated. Although both groups of strains demonstrated high carriage rates of the *ica* operon, no significant difference was found.[27,51,183,198] However, higher proportions of invasive strains demonstrated biofilm production in quantitative assays compared with commensal strains.[27,198] Most of the commensal strains were biofilm-negative despite possessing the *ica* operon, a finding suggesting that regulation of gene expression plays a large role in virulence.

Numerous extracellular enzymes are produced by coagulase-negative staphylococci; an extracellular metalloprotease with elastase activity; a cysteine protease that degrades human secretory immunoglobulin A, immunoglobulin M, serum albumin, fibrinogen, and fibronectin; and an extracellular serine protease involved in epidermin processing.[96,251,254] Two lipases have been postulated to facilitate skin colonization.[96,251] Unlike *S. aureus*, which is capable of producing various cytolytic and superantigenic toxins, *S. epidermidis* produces very few toxins.[251] *Delta* toxin, an enteropathogenic toxin, encoded by the *hld* component of the regulatory system agr, has been linked to necrotizing enterocolitis in infants.[215] *S. epidermidis* also produces antibacterial peptides called *lantibiotics* that may play a role in bacterial interference on skin and mucous membranes and in the creation of an ecologic niche for *S. epidermidis*.[132]

S. saprophyticus can cause urinary tract infections in the absence of indwelling catheters.[132] Several virulence factors have been described to explain its pathogenic potential to adhere and persistently grow in the urinary tract. A surface-exposed protein with hemagglutinin and adhesive properties[74] and a surface fibrillar

protein,[73] designated *ssp*, are associated with attachment to urinary tract epithelium. Urease production also has been associated with invasiveness of this organism by causing damage to bladder tissues.[71,72] The whole genome of *S. saprophyticus* has been sequenced and compared with *S. aureus* and *S. epidermidis*.[132] Analysis revealed a single, unique cell wall anchored protein with properties of hemagglutination and adherence to human bladder cells. A high population of ionic transport systems capable of providing osmotolerence in the highly variant ionic environment of urine also was demonstrated.

CLINICAL MANIFESTATIONS

Coagulase-negative staphylococci have been implicated in a variety of clinical infections in immunocompetent and immunocompromised patients (Table 92–4).[126,178,206] A basic difficulty in interpreting clinical studies of coagulase-negative staphylococci exists because of the different criteria used to define a clinically significant culture.[54] In neonates, immunocompromised patients, and patients with prosthetic implants, repeated isolation of the same phenotypic strain of coagulase-negative staphylococci from blood cultures facilitates interpretation. For catheter-related bacteremia, quantitative cultures from the catheter exhibit a five- to tenfold increase in the number of colony-forming units as compared with cultures from a peripheral vessel. The Committee on Infectious Diseases of the American Academy of Pediatrics suggested the following considerations to distinguish pathogenic from contaminant coagulase-negative staphylococci: (1) two or more positive blood cultures from different sites, (2) a positive culture from blood and another usually sterile site with identical or nearly identical antimicrobial susceptibility patterns, (3) growth in continuously monitored blood culture system within 15 hours of incubation, (4) clinical findings of infection in the patient, (5) an intravascular catheter that has been in place for 3 days or more, and (6) similar or identical genotypes among all isolates.[4]

Clinical presentation markedly differs from that of S. *aureus* infection. In general, infections caused by coagulase-negative staphylococci are more indolent and can manifest with a subacute or even chronic course.

TABLE 92–4 Clinically Important Coagulase-Negative Staphylococcal Infections

Bacteremia
Neonates
Patients with leukemia and lymphoma
Bone marrow transplant recipients
Infections in Patients with Indwelling Medical Devices
Central venous catheters
Cerebrospinal fluid shunts
Peritoneal dialysis catheter
Prosthetic values
Other Infections
Prosthetic joints
Vascular grafts and prostheses
Hemodialysis shunt
Pacemaker
Scalp electrode
Native Valve Endocarditis
Urinary Tract Infections
Miscellaneous Infections
Endophthalmitis after ocular surgery
Postoperative wound infections
Osteomyelitis (sternal wound or hematogenous)
Toxic shock syndrome

Modified from Patrick, C. C.: Coagulase-negative staphylococci: Pathogens with increasing clinical significance. J. Pediatr. 116:497-507, 1990.

BACTEREMIA

Coagulase-negative staphylococci, particularly *S. epidermidis*, have become the major nosocomial pathogens in most studies.[54,197] The analysis of 6290 nosocomial infections, including 110,709 patients from 61 pediatric intensive care units in the United States during 1992 to 1997, showed that bloodstream infections were the most common types of nosocomial infection; coagulase-negative staphylococcus was the causative agent in 38 percent (717 of 1887) of the cases.[197] In a large surveillance study between 1995 and 2001 among 49 hospitals in the United States, coagulase-negative staphylococci were the most common cause of nosocomial bacteremias in pediatric patients and accounted for 43 percent of the isolates.[258] These infections occur primarily with the use of indwelling vascular catheters. In febrile, immunocompromised pediatric patients with cancer, coagulase-negative staphylococci can account for 35 percent of all positive blood culture isolates.[2,11,133]

NEONATAL BACTEREMIAS

Coagulase-negative staphylococci are the single most frequent cause of late-onset septicemia among premature infants, especially in low-birth-weight infants.[16,65,80,87,88,165,179] Table 92–5 lists the salient features from six large studies.[65,88,114,115,158,179] These infections are found predominantly in premature infants with a gestational age of less than 35 weeks who have an immature immune system, particularly in quantitative and qualitative neutrophil function.[35,227,257] The presence of indwelling peripheral or umbilical catheters has been implicated in approximately half of neonatal coagulase-negative staphylococci bacteremias.[16,88,164,175]

Signs and symptoms of coagulase-negative staphylococci bacteremia *usually* are subtle and most commonly include bradycardia, apnea, and temperature instability (see Table 92-5). Skin abscesses were noted in more than 40 percent of neonates with coagulase-negative staphylococci bacteremia in one study.[179] Frequency of fulminant late-onset sepsis caused by coagulase-negative staphylococcosis bacteremia is very low (1%).[118]

Laboratory studies usually are not helpful in identifying patients with coagulase-negative staphylococci bacteremia.[161] Leukocytosis is an inconsistent finding. Reports have conflicted about production of slime, a possible virulence factor, as a marker for infection.[88,179] One study successfully correlated quantitative blood cultures with specific clinical information to distinguish a true pathogen.[233] Peripheral blood cultures yielding more than 50 colony-forming units per milliliter occurred exclusively in infants with proven septicemia. In contrast, low colony counts were observed in both septicemia and culture contamination. Those patients with low colony counts and septicemia were more likely to have a central venous line or an abnormal hematologic value (leukocytosis, decreased platelets, or increased immature to total neutrophil ratio).[233]

Persistence of coagulase-negative staphylococci bacteremia (mean duration, 12 to 13 days), despite administration of adequate antibiotic therapy, was described in neonates.[122,179] This persistence was observed in patients with and without central venous catheters and in patients with normal findings on cardiac echocardiography. Neonates with persistent bacteremia had a significantly higher incidence of severe thrombocytopenia requiring platelet transfusions than did those with nonpersistent bacteremia.[122]

Rarely, coagulase-negative staphylococci meningitis has been reported in low-birth-weight neonates with normal CSF

TABLE 92–5 Salient Aspects of Six Studies of Coagulase-Negative Staphylococci Bacteremia in Neonates

Characteristics	Munson et al., 1982[158] (*n* = 27)	Fleer et al., 1983[65] (*n* = 30)	Hall et al., 1987[88] (*n* = 29)	Patrick et al., 1989[179] (*n* = 32)	Kacica et al., 1994[114] (*n* = 47)	Kallman et al., 1997[115] (*n* = 27)
Patient						
Mean birth weight (g)	1130	1564	1607	1172	1259	NR
Gestational age	28.9	32.1	31	28.6	29	<30
Central lines (%)	85	NR	34	19	NR	NR
TPN (%)	78	77 (20/26)	NR	91	81	NR
Clinical						
Apnea or bradycardia (%)	>50	100	62	78	NR	NR
Temperature instability (%)	<50	70	7	22	NR	NR
Tachycardia (%)	>50	100	NR	6	NR	NR
Mortality (%)	0	0 (0/13)	0	0	NR	7.4
Laboratory						
Slime production, patient isolates (%)	NR	NR	79	54 (6/13)	65	NR
Antibiotic susceptibility	100% S to cephalothin	100% S to cephalothin	83% S to methicillin; 100% S to vancomycin	100% S to vancomycin	48% S to oxacillin; 100% S to vancomycin	86% S to methicillin*; 55% S to methicillin†

**Period: 1981-1986.*
†Period: 1987-1994.
NR, not reported; S, susceptible; TPN, total parenteral nutrition.

TABLE 92–6 Clinical Characteristics of Coagulase-Negative Staphylococcal Bacteremia in Patients with Childhood Cancer*

Characteristics	Friedman et al., 1984[69] (*n* = 150)	Langley and Gold, 1988[133] (*n* = 100)	Aledo et al., 1998[2] (*n* = 140)	Auletta et al., 1999[11] (*n* = 102)
Coagulase-negative staphylococci among total bacteremias (%)	12.7	35	31.4	35
Patients with central venous catheters (%)	32	53	95	95
Mortality (%)	10.5	38	<5	19
Patient isolates susceptible to				
Methicillin (%)	17	38	NR	NR
Vancomycin (%)	100	100	NR	NR

**Percentages are based on the number of episodes of coagulase-negative staphylococcal sepsis.*
NR, not reported.

profiles.[85] Thus, CSF examination should be considered in neonates with coagulase-negative staphylococci bacteremia. Abnormal CSF findings or a positive CSF culture may influence the duration of therapy. Coagulase-negative staphylococci also have been implicated as etiologic agents of necrotizing enterocolitis,[84,215] although one report showed no association.[201]

LEUKEMIA AND LYMPHOMA

Immunocompromised patients with leukemia, lymphoma, or both are at risk for the development of coagulase-negative staphylococci bacteremia (Table 92–6).[2,11,69,133,190,191,219] The most common portals of entry are the gastrointestinal tract, where chemotherapy causes defects, and the skin in association with catheter devices. In several reports, coagulase-negative staphylococci were the isolates most commonly causing bacteremia in pediatric patients with cancer and accounted for 35 percent of all initial isolates.[2,11,133] One third of these patients did not have central venous catheters at the time of their bacteremia.[69]

RAPD analysis and PFGE of DNA macrorestriction fragments of blood culture coagulase-negative staphylococci isolates obtained from 40 neutropenic hemato-oncologic patients related to 61 episodes of Hickman catheter–related infections allowed the characterization of clonal coagulase-negative staphylococci types that successfully and persistently colonized patients; this finding confirmed that the skin flora was the likely source of catheter-related infections (CRIs) in 75 percent of the patients.[166]

Morbidity from coagulase-negative staphylococci bacteremia is appreciable because of the organism's acquisition of multiple antibiotic resistance, which requires the use of vancomycin or combination antibiotic therapy, with potentially toxic effects.[69,119,133,142,143,192,212] Mortality rates have varied from 0 to 11 percent.[69,133]

HEMATOPOIETIC STEM CELL TRANSPLANTATION

Coagulase-negative staphylococci have become the primary pathogen causing bacteremia in hematopoietic stem cell transplant recipients.[31,154] These episodes occur most often during periods of agranulocytosis before marrow engraftment,[31] and they can be fatal.[18] Most of these episodes are a result of the universal use of central venous catheters and the use of broad-spectrum antibiotics. Bacteremia that occurs during the neutropenic phase after hematopoietic stem cell transplantation appears to be associated with early death from invasive fungal infection.[232]

INDWELLING MEDICAL DEVICES

Central Venous Catheters

Infections associated with central venous catheters are caused primarily by coagulase-negative staphylococci, which also can cause infections in peripheral catheters composed of steel or polyethylene.[242] Central venous catheters often are used in children for long-term hyperalimentation or administration of medication.[104] Two types of devices are in common use: (1) Broviac and Hickman catheters, which have an external port, and (2) totally implanted vascular access devices.[29,99,200] Broviac and Hickman catheters have an exit site where the catheter enters the skin, whereas the totally implanted vascular devices have a subcutaneous tract; all three types have a Dacron cuff that promotes fibrosis, thereby limiting the trafficking of potential pathogens, and an insert site into the major vessel. Thus, infection can occur at the exit site, along the catheter tunnel, or at the catheter vessel insertion site. Totally implanted catheters (e.g., port-A-Cath) have the lowest rates of bacteremia (0 to 0.04 per 100 catheter days), followed by long-term Hickman or Broviac catheters (0.14 per 100 catheter days).[101] Infection at the vessel insertion site can lead to bacteremia or septic thrombophlebitis, with further complications caused by metastatic spread.[193]

Infectious complications involving central venous catheters have a variable reported frequency of 2.7 to 47 percent.[19,76,93,141,157,229,250] Coagulase-negative staphylococci are the pathogen in approximately half these cases. This variability depends on the definition of a CRI, the type of catheter used, and the presence of hyperalimentation fluid and lipid in the infusion.[146,221,223] The use of a guide wire for catheter placement or of a multilumen catheter leads to higher infection rates.[225]

S. epidermidis is the species associated most often with central venous CRIs; it is identified in approximately 70 percent of coagulase-negative staphylococci central venous CRIs.[19,93] This prevalence is not unexpected because the organism is the predominant species colonizing the skin.[128] Other implicated coagulase-negative staphylococci species include *Staphylococcus haemolyticus*, *Staphylococcus warneri*, and *Staphylococcus hominis*.[93]

Differentiating between a true infection caused by *S. epidermidis* and a contaminated specimen is difficult. Establishing the diagnosis depends on the patient's clinical status and the isolation of identical isolate strains in repeated cultures. Proper therapy, possibly including removal of a catheter, requires accurate diagnosis.[194] Additionally, distinguishing central venous catheter–related bacteremia from bacteremia not associated with a central venous catheter is important. The use of quantitative blood cultures using the DuPont isolator system has shown that catheter-related bacteremias have a 5- to 10-fold difference in bacterial concentration in blood cultures drawn through the catheter compared with peripheral cultures.[66,196] Maki and associates[147] determined that catheter-related bacteremia can be confirmed after removal of the catheter by rolling the distal 5 to 7 cm of the catheter on a culture plate and finding more than 15 colony-forming units.

Therapy for coagulase-negative staphylococci infections involving central venous catheters should include removal of the catheter if the catheter no longer is necessary. Exit-site infections usually can be managed without removing the catheter.[194] Approximately one third of tunnel tract infections can be managed without removing the catheter, but the remaining patients have continued bacteremia or relapse of their bacteremia that requires removal of their catheters. Catheters should be removed immediately if the patient's clinical status deteriorates. Duration of therapy depends on whether the catheter has been removed and on the patient's underlying immune status.

Central Nervous System Shunts

Central nervous system (CNS) shunts divert or shunt CSF to relieve hydrocephalus.[121] Other prosthetic devices within the CNS have been used to monitor ventricular pressure or to administer chemotherapy.[53] Initial shunts diverted CSF into the right atrium; this technique is used infrequently because of its high complication rate.[169] Ventriculoperitoneal shunts that divert CSF into the peritoneal cavity have been used since the 1960s because of their lower rate of mechanical complications.[121,159,169] However, the incidences of infection involving both the ventriculoperitoneal and the ventriculoatrial shunts are comparable.[121,169,224] Infection rates are higher in neonates. Coagulase-negative staphylococci account for 60 to 75 percent of all bacterial causes of shunt infections.[121,162,224]

The pathogenesis of CSF shunt infections occurs primarily at the insertion site by contamination of the catheter from the patient's skin flora[224] or during subsequent revisions, with a short-term infection rate of 13 percent and a 10-year infection rate of 27 percent.[199] Seventy percent of ventriculoperitoneal shunt infections occur within 2 months of placement of the shunt.[162] The pathogenesis of this infection appears to be similar to that of CRI, described in the pathogenesis section.

The diagnosis of CNS shunt infections caused by coagulase-negative staphylococci may be difficult to make, owing to the differentiation between a true infection and a contaminating organism. The Gram stain of ventricular fluid often is negative, but culture is sensitive. Subtle changes are noted in CSF and ventricular fluid cell counts or cytochemical findings.[262] Patients with ventriculoatrial shunts often have signs and symptoms compatible with septicemia and the additional complications of glomerulonephritis secondary to immune complexes. In patients with ventriculoperitoneal shunts, because of the distal placement of the catheter into the peritoneum, an intra-abdominal cyst may develop at the distal end of the catheter. Dysfunctions of the shunt secondary to development of infections often lead to signs and symptoms consistent with increased intracranial pressure. Standard therapy for ventriculoperitoneal shunt infections has been removal of the shunt system and administration of systemic antibiotics.[224,262,264]

Peritoneal Dialysis Catheters

Infection remains the most common complication of peritoneal dialysis, and *S. epidermidis* is the bacterial pathogen most commonly isolated,[83,187] representing as many as 50 percent of infecting organisms.[187] The pathogenesis of infections involving the peritoneal dialysis catheter is similar to that of CRIs, with infections involving the exit site, infections along the subcutaneous catheter tunnel, and peritonitis.[187] The prevalence of peritonitis in patients undergoing continuous ambulatory peritoneal dialysis is approximately 60 percent. Signs of peritonitis include fever, abdominal pain, and cloudy peritoneal dialysis; fever is variable. Removal of the catheter usually is not necessary for successful therapy but may be required in refractory cases or when the catheter malfunctions. Antibiotics are administered in the dialysis fluid, and systemic antibiotics also can be used.[62]

Prosthetic Devices

Coagulase-negative staphylococci are the second most common cause of prosthetic valve endocarditis; these pathogens account for 17 percent of contemporary cases.[255] The in-hospital mortality rate from prosthetic valve endocarditis is reported to be 26 percent. Commonly, coagulase-negative staphylococcus prosthetic valve endocarditis occurs within 60 days of implanting the device and is defined as early prosthetic valve endocarditis. It can

occur in the first year after surgery, probably caused by inoculation of the organism at the time of surgery, as noticed by the multiresistant phenotype analysis from the isolates in these patients.[26] The condition finally leads to an abscess of the mechanical valve ring. No sign or symptom is consistently diagnostic of prosthetic valve endocarditis, but fever is the most common finding. Classic endocarditis findings such as peripheral emboli and multiple positive blood cultures may be lacking in coagulase-negative staphylococci endocarditis. Anemia is the abnormality identified most commonly by laboratory tests. Obtaining blood cultures is imperative in diagnosing prosthetic valve endocarditis. Therapy should include institution of vancomycin and rifampin or an aminoglycoside, with surgical intervention as indicated.[13]

Other Indwelling Medical Devices

Coagulase-negative staphylococci are involved in an expanded spectrum of infections involving indwelling medical devices.[52,81] They include infections of prosthetic joints,[63,116] vascular grafts,[14,55] hemodialysis shunts,[174,216,217] and pacemaker pockets.[38,108,148,185] Additionally, osteomyelitis secondary to *S. epidermidis* has been reported to occur after hemodialysis and in neonates after the use of a monitoring scalp electrode.[170]

NATIVE-VALVE ENDOCARDITIS

Native-valve endocarditis, along with *S. saprophyticus* urinary tract infection, is the only non-nosocomial infection caused by coagulase-negative staphylococci.[110] Approximately 7 percent of cases of native-valve endocarditis are caused by coagulase-negative staphylococcus, and *S. epidermidis* is the most frequently isolated species.[42] These infections usually are subacute and arise from transient bacteremia. Pathogenesis is thought to involve seeding of a previously damaged valve or endocarditis that previously had not been identified.[6,17]

Staphylococcus lugdunensis can cause acute, severe, and destructive endocarditis similar to *S. aureus* endocarditis,[246] with most favorable outcomes occurring in patients who undergo valve replacement.[48] Most patients with *S. lugdunensis* endocarditis have been older than 50 years of age. Native aortic or mitral valves are involved most frequently.[245] The perineum appears to have a high colonization rate of *S. lugdunensis.*[112]

SURGICAL SITE INFECTIONS

Coagulase-negative staphylococci are the second most common cause of postoperative surgical site infections, according to National Nosocomial Infections Surveillance Report survey data.[37] Most infections probably are caused by the patient's own endogenous skin flora, but outbreaks originating from operating room personnel have been reported.[26] Outbreaks of *S. epidermidis* surgical site infections, such as mediastinitis and endocarditis, among patients who have undergone cardiac valve replacements also have been reported.[25,195]

URINARY TRACT INFECTIONS

S. saprophyticus is the most common coagulase-negative staphylococcus that causes infections in both the upper and the lower urinary tracts.[135] These infections occur predominantly in young, healthy, sexually active women,[113,135,150] during late summer and fall, with a pattern similar to that of sexually transmitted diseases.[71] Recent sexual intercourse, outdoor swimming, and occupational meat processing have been identified as risk factors.[71] *S. epidermidis* and other coagulase-negative staphylococci rarely cause urinary tract infections, but they have been noted to produce disease in older adults with urinary tract complications.[86,139,163] McDonald and Lohr[152] and Hall and Snitzer[87] described healthy children with pyelonephritis caused by *S. epidermidis*.

MISCELLANEOUS

Endophthalmitis secondary to *S. epidermidis* has been reported in patients undergoing ocular surgery or trauma.[15,28,188] Postoperative mediastinitis that develops after median sternotomy for open heart surgery can be caused by *S. epidermidis*.[24,58,82] Primary osteomyelitis secondary to coagulase-negative staphylococci is a rare occurrence in healthy children.[171] *Staphylococcus caprae*[226] and *S. lugdunensis* [211] have been linked to bone and joint infections. Coagulase-negative staphylococci have been implicated as a possible cause of toxic shock syndrome.[45]

TREATMENT

The treatment of coagulase-negative staphylococcal infections[2] depends on the patient's immunologic status, the presence of an indwelling medical device, and the results of antimicrobial susceptibility testing. Infections are more difficult to treat when they are associated with a thrombosed vessel or an intra-atrial thrombus. When an intravascular catheter becomes infected, the presence of vegetation or a thrombus in the heart or great vessels always should be considered.

Many coagulase-negative staphylococci, particularly *S. epidermidis* and *S. haemolyticus*, are resistant to many antimicrobiotics.[7,9,39,49,61,78,218,231,256] Methicillin resistance, especially, is prevalent in nosocomial infections and occurs in most cases.[208,228] Methicillin-resistant coagulase-negative staphylococcus has a high degree of resistance to other antibiotics, such as clindamycin, erythromycin, and gentamicin.[7]

The mechanism of methicillin resistance in coagulase-negative staphylococci is similar to that of *S. aureus* because of the production of an additional non-native penicillin-binding protein (PBP), PBP2a, encoded by the *mecA* gene.[96,103] PBP2a does not allow the correct binding of β-lactams to the bacterial cell wall. Phenotypic methicillin resistance can be difficult to detect because of the heterogeneous expression of the *mecA* gene by many strains of staphylococci.[103] *S. aureus* breakpoints have been seen to fail to detect many coagulase-negative staphylococci that contained the *mecA* gene involved in staphylococcal resistance. As a consequence, the Clinical and Laboratory Standards Institute in 2007 recommended specific breakpoints for coagulase-negative staphylococci (Table 92–7).[43] In some species other than *S. epidermidis*, the differentiation between *mecA*-negative and *mecA*-positive strains may not be possible by susceptibility methods,[238] and isolates may falsely be designated as oxacillin resistant. Detecting the *mec*A gene is an accurate method to predict resistance to oxacillin.[103] Testing of coagulase-negative staphylococci using oxacillin screen plate no longer is recommended because of its failure to detect many resistant strains.[238]

Coagulase-negative staphylococci (*S. haemolyticus* and *S. epidermidis*) were the first organisms in which acquired glycopeptide resistance was recognized in 1986.[218] In addition, *S. epidermidis*, *S. haemolyticus*, and *S. hominis* are more likely to be multiresistant to antimicrobial agents than are other coagulase-negative staphylococcal species. A prolonged course of vancomycin is a well-recognized risk factor for the emergence of strains with decreased susceptibility to glycopeptides.[21,260] Although they are uncommon

TABLE 92–7 Breakpoints for Antibiotic Susceptibility for Coagulase-Negative Staphylococci

MICs	Oxacillin-Susceptible	Oxacillin-Resistant	
CoNS	≤0.25 μg/mL	≥0.5 μg/mL	
S. aureus	≤2 μg/mL	≥4 μg/mL	
Zone Size	**Oxacillin-Susceptible**	**Oxacillin-Intermediate**	**Oxacillin-Resistant**
CoNS	≥18 mm	No intermediate zone	≤17 mm
S. aureus	≥13 mm	11-12 mm	≤10 mm

CoNS, coagulase-negative staphylococci; MICs, minimal inhibitory concentrations. Data from Clinical and Laboratory Standards Institute: Performance testing for antimicrobial susceptibility testing, 17th informational supplement M100-S17, 2007.

occurrences, infections caused by these strains have been described and may become more common. To prevent or delay development of resistance, the Centers for Disease Control and Prevention published recommendations for the prudent use of vancomycin.[36] Decreased susceptibility occurs more frequently to teicoplanin than to vancomycin. The minimal inhibitory concentrations of teicoplanin usually fall over a wide range, whereas vancomycin minimal inhibitory concentrations tend to remain more stable over a narrower range within the limits of susceptibility.[21] The peptidoglycan of these strains is highly cross-linked, containing additional serine in place of glycine, an alteration that may interfere with glycopeptide binding.[96]

The mechanism for vancomycin-intermediate resistance of *S. aureus* and coagulase-negative staphylococci is unknown. Vancomycin-intermediate *S. aureus* and vancomycin-intermediate, coagulase-negative staphylococcal strains have thickened cell walls, as shown by electron microscopy. Vancomycin resistance may be related to an increased ability of these organisms to bind vancomycin at sites other than those to which they normally bind.[96]

For the few isolates that are established to be penicillin susceptible and β-lactamase negative, penicillin is a suitable drug. For organisms that are resistant to penicillin but susceptible to semisynthetic penicillins, nafcillin and oxacillin are the most active antibiotics.[189,209] A degree of cross-resistance exists between the semisynthetic penicillin-resistant penicillins and cephalosporins.[208] Thus, routine susceptibility testing can indicate that a strain is susceptible to a cephalosporin when, in fact, it is resistant.[83,136] Methicillin resistance means resistance to all β-lactam antibiotics.[109]

Vancomycin is the drug of choice for methicillin-resistant organisms and is recommended for treating severe infections.[3,124] Coagulase-negative staphylococci can exhibit heteroresistance, defined as a culture population comprising two subpopulations, one susceptible to methicillin and the other resistant.[208,260] Enhanced detection of this admixture of organisms can be obtained by change in the culture conditions.[153] If these strains are incubated overnight on plates of increasing concentrations of methicillin or vancomycin, a small fraction of the total population will be able to grow at much higher antibiotic concentrations. If these highly resistant subclones are recultured, they may maintain this high level of resistance, a finding suggesting a mutant strain. However, in some cases, the highly resistant subclone will revert to methicillin or vancomycin susceptibility when it is replated, even though it was grown from a single colony. No satisfactory model can explain the mechanism governing heterogeneous resistance. Strains of coagulase-negative staphylococci and *S. aureus* with heterogeneous resistance are stable in the absence of antibiotic selective pressure. These strains may constitute 10 to 15 percent of any coagulase-negative staphylococci or *S. aureus* collection of isolates. In addition, to avoid overlooking these organisms, the clinical microbiology laboratory should attempt to optimize expression of resistance by culturing on salt-containing media at 30° C to 35° C.[207] The clinical significance of this heteroresistance in coagulase-negative staphylococci is unknown, but a study of *S. aureus* showed it did not affect outcome.[125]

The quinolones and teicoplanin have shown activity against coagulase-negative staphylococci and could be useful in therapy of highly selected multiresistant organisms.[140,221] Teicoplanin is significantly less active than is vancomycin against coagulase-negative staphylococci, in particular *S. haemolyticus*, and, to a lesser extent, *S. epidermidis*. Resistance to these agents also has been observed.[9,218,231,256]

Gentamicin and rifampin are active against coagulase-negative staphylococci, but rapid emergence of resistance has limited their use as single drugs.[39,151,209] These two antibiotics have been shown to be synergistic with vancomycin against methicillin-resistant, coagulase-negative staphylococci.[142] Additionally, rifampin has been used in neonates with persistent bacteremia to eradicate the organisms.[235] The association of vancomycin and rifampin is the mainstay of therapy for deep-seated, foreign body infections.

Excellent in vitro activity of quinupristin-dalfopristin, a streptogramin combination,[10] and linezolid, an oxazolidinone compound that inhibits bacterial growth through inhibition of protein synthesis,[206] has been shown against staphylococcal species tested, regardless of the resistance pattern, particularly resistance to methicillin. Linezolid is an option for vancomycin-intermediate susceptible isolates or for completing therapy with oral administration.[117] Daptomycin is a lipopeptide antibiotic with excellent in vitro activity against coagulase-negative staphylococci.[210]

PREVENTION

Preventing the development of coagulase-negative staphylococci infection is difficult because of these organisms' ubiquitous nature as predominant skin commensals. A 3-year analysis by molecular typing of coagulase-negative staphylococci nosocomial infections in a neonatal intensive care unit showed that many of the infections were caused by clonal dissemination and, thus, potentially were preventable by handwashing, thus reducing the transmission rate from staff to patient or from patient to patient.[249] Additionally, proper surgical techniques help to minimize these infections associated with installation of indwelling medical devices.

The effort toward developing catheters that are both inert and resistant to bacterial colonization adherence has increased; however, these attempts have been only marginally successful.[123,177,184] Catheters with antibiotics or disinfectants impregnated in their surfaces appear promising.[237] Prophylactic antibiotics are administered routinely for surgical procedures involving placement of a prosthetic device or material. However, the most appropriate agent to prevent infections caused by coagulase-negative staphylococci varies among institutions.[3] Vancomycin often is recommended for CSF shunt or prosthetic valve placement. Polyclonal antibody (intravenous gammaglobulin) and polyclonal antibody enriched for antibody to clumping factor A have not been found to be efficacious for preventing coagulase-negative staphylococcal infections in neonates.[22]

In reference to infection control policies, standard precautions should be used for methicillin-resistant, coagulase-negative staphylococci. For vancomycin-intermediate, coagulase-negative staphylococci, contact transmission precautions for multidrug-resistant organisms should be used.[4]

CONCLUSION

Coagulase-negative staphylococci, particularly *S. epidermidis*, are a major source of nosocomial infection in a variety of clinical situations. Most infections occur in patients who are immunosuppressed or have an indwelling medical device. Epidemiologic studies are difficult to perform because of the organisms' commensal existence, but newer molecular tools will help investigators to gain insight with regard to the epidemiology of these infections. The pathogenesis of coagulase-negative staphylococci infection is being defined in the context of a CRI. Therapy can be complex because of the usual presence of an indwelling medical device and the multiple antimicrobial resistance of the organisms.

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CHAPTER 93

Group A, Group C, and Group G Beta-Hemolytic Streptococcal Infections

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GROUP A STREPTOCOCCAL INFECTIONS

Group A beta-hemolytic streptococci (*Streptococcus pyogenes*) are common pathogenic bacteria isolated from children. They are associated with a wide variety of infections and disease states (Fig. 93–1). Although uniformly sensitive to penicillin and still exquisitely sensitive to many other antibiotics, group A streptococcal infections present formidable clinical and public health problems for pediatricians and primary care physicians. Although most group A streptococcal infections are of short duration and relatively benign, they may be fulminant and life-threatening. The importance of group A streptococcal infections was reinforced in

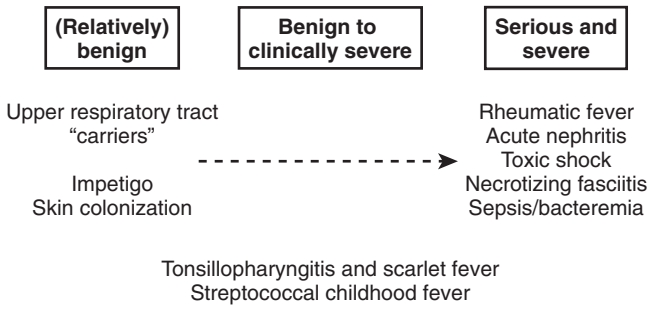

Figure 93–1 Spectrum of group A streptococcal infections.

the late 1990s by a resurgence of acute rheumatic fever in the United States[114] and the appearance of a group A streptococcal toxic shock syndrome (TSS) with very high morbidity and mortality rates.[153,154] Additionally, this bacterium is different from other pyogenic bacteria because of the potential for development of delayed, nonsuppurative sequelae (e.g., acute glomerulonephritis, acute rheumatic fever, reactive arthritis) to follow uncomplicated infections.

ORGANISM

S. pyogenes (group A streptococcus) is a gram-positive coccus, forming either short or long chains. Group A streptococci produce clear (beta) hemolysis on blood agar, a bacteriologic feature important in their recognition and in their differentiation from nonhemolytic (gamma) streptococci and from viridans (alpha) streptococci, which cause partial or green hemolysis on sheep blood agar. Although hemolysis is produced on culture plates containing blood from a variety of mammalian species, sheep or horse blood gives the clearest differentiation. Some strains of group A streptococci hemolyze red blood cells slowly or result in almost greenish hemolysis on the surface of blood agar plates incubated aerobically. These strains can be recognized by their ability to produce clear hemolysis under anaerobic conditions, which is achieved readily by routinely making a short cut or stab into the blood agar at the time of inoculation.[85] Incubation with carbon dioxide or in a candle jar also can be helpful in enhancing beta-hemolysis. Rare strains of group A streptococci are not hemolytic.[77,84]

Approximately 124 different types of group A streptococci have been recognized either on the basis of a series of serologically distinct surface proteins, the M proteins, or by sequencing of the *emm* gene, which encodes for M protein.[56,57,85,108] The M serotypes of streptococci associated with impetigo and pyoderma often are different from the serotypes associated with clinical pharyngitis, although a few M types have the capacity to produce both kinds of infection.[6] The M protein renders the group A streptococcus resistant to phagocytosis and is a major virulence factor for these organisms. Additional evidence indicates that more M-types exist that have not been identified yet; new *emm* sequences are continuing to be described (see http://www.cdc.gov/ncidod/biotech/strep/strepindex.htm).

The group A streptococcal cell is a complex structure. In rapidly dividing strains (e.g., young cultures, epidemic strains), the cell is covered with a hyaluronic acid capsule, which gives the colonies a mucoid or water drop appearance. Protruding from the cell surface and into the hyaluronic capsular layer are microscopic hairlike fimbriae, which are responsible for adherence of group A streptococci to epithelial cells. A basic chemical component of these fimbriae is lipoteichoic acid.[12] The M protein also is associated with these fimbriae.[60] Other surface proteins of interest are the T and R proteins, the serum opacity factor (SOF) proteins, and proteins that bind nonspecifically to the Fc fragment of gamma-globulins. The function and the exact location of these other proteins on the surface of the organism have not been identified precisely. Strains of a particular M type generally are associated with a particular T agglutination pattern.[82] In strains producing serum opacity, the serologically specific SOR protein usually correlates closely with the M type of the strain. At present, more than 50 opacity factor–positive types of group A streptococci have been recognized; others undoubtedly have not been identified yet. All of these characteristics are useful in epidemiologic studies of streptococcal infections, either in an individual patient or in a community.

In addition to these surface proteins, the carbohydrate moiety responsible for group specificity (e.g., group A carbohydrate) is found in the cell wall in a position sufficiently superficial to permit reaction with antibody specifically directed toward it. The group A carbohydrate is a polymer of rhamnose units with side chains of *N*-acetylglucosamine, which is responsible for its group (e.g., A) specificity.[118] The structure providing rigidity for the cell wall is another large polymer, a peptidoglycan, consisting of glycan strands cross-linked by peptide bridges. Its role in the pathogenesis of infection remains incompletely defined.

Within the cell wall of the group A streptococcus lies the cell membrane, composed mainly of lipoprotein or lipid-protein complexes. This membrane is the outer surface of the osmotically fragile protoplasts or L forms of streptococci. These wall-less forms of group A streptococci are resistant to penicillin.[62]

Intracellular constituents of the group A streptococci include, in addition to DNA and RNA, many enzymes and hemolysins.[26] Plasmids have been identified that control resistance to certain antibiotics (e.g., erythromycin).[113] Bacteriophages play an important role in the genetics of group A streptococci, including the transfer of the determinants of antibiotic resistance and the control of pyrogenic exotoxin production.[169,174]

Group A streptococci produce and release into the surrounding medium numerous biologically active extracellular products. Some of these are toxic for human and other mammalian (eukaryotic) cells. Streptolysin O (the oxygen-labile hemolysin) and streptolysin S (the oxygen-stable hemolysin) injure cell membranes, not only lysing red blood cells, but also damaging other eukaryotic cells (including myocardial cells) and membranous subcellular organelles.[15] Streptolysin O is antigenic; streptolysin S is not. The latter hemolysin is bound loosely to the streptococcal cell and is released in a complexed, stable form with a variety of carrier molecules. The pyrogenic exotoxins resemble endotoxin in exhibiting a primary or intrinsic toxicity and a secondary toxicity resulting from the acquisition of host hypersensitivity.[103] The outbreak of streptococcal TSS, which became pronounced in the late 1990s, was reported to be associated with the reappearance of strains making pyrogenic exotoxin A, but many unanswered questions remain about the precise pathogenesis.[153] Group A streptococci also produce bacteriocins,[155] low-molecular-weight proteins that can kill a variety of other gram-positive bacterial species and may play a role in promoting infection or even persistence of colonization.

Many of the other extracellular products of group A streptococci are specific enzymes that do not seem to be directly toxic for mammalian or bacterial cells, but digest or initiate the breakdown of important biologic substrates. Included are the deoxyribonucleases (nucleases A, B, C, and D), the streptokinases (which activate the fibrinolytic or plasmin-plasminogen system), a hyaluronidase, an amylase, a proteinase, an esterase, a nicotinamide adenine dinucleotidase (NADase), and C5a peptidase. Several of these enzymes are antigenic (e.g., DNAse B, streptokinase, hyaluronidase, NADase, C5a peptidase). Among group A streptococcal surface virulence factors, C5A peptidase plays a major role by inactivating the complement chemotaxin C5A, reducing the early

phagocytic response in allowing group A streptococci to become locally established. This extracellular product has been shown to be antigenic in animals and in humans.[34]

TRANSMISSION

Humans are the only known reservoir for group A streptococci. The mechanism of spread of streptococci from one person to another and from one body site to another varies according to the clinical manifestations of the infection. Epidemiologic studies of patients with streptococcal sore throat indicate that airborne routes of spread (by small droplet nuclei, dust) and environmental contamination (e.g., contaminated clothing or bedding) play little, if any, role in transmission of this kind of group A streptococcal infection.[132] Close personal contact is required for transmission of streptococcal pharyngitis to occur, apparently by direct projection of large droplets or by physical transfer of respiratory secretions containing the infectious bacteria. Spread within homes, school rooms, or crowded facilities such as military barracks is a common occurrence.[49] Residential nursing facilities also have proven to be highly susceptible to spread among staff members and patients.

Contaminated food or milk also may result in group A streptococcal infection of the throat, producing a common-source outbreak.[74] Salads containing hard-boiled eggs (e.g., egg salad) seem to be a special problem. Anal carriers have been identified as the source of contagion in several hospital outbreaks of streptococcal wound infections. Some studies have suggested that rectal or anal carriers may be more common than suspected.[104]

The period of greatest contagiousness of streptococcal pharyngitis and scarlet fever is during the acute stage of the illness. Most antibiotic therapies (especially penicillin) rapidly suppress the growth of group A streptococci and, if continued, most often eradicate them from the upper respiratory tract. A patient can be considered much less contagious after 24 hours of antimicrobial therapy. Most physicians concur that children can return to school by that time, especially if they are afebrile, with reduced risk of spread of the organism to close contacts.[150]

Although humans with active but subclinical infection also may contribute to the spread of group A streptococci, the role of throat "carriers" in the spread of this organism apparently is less important. Most secondary spread occurs during the first 2 weeks after acquisition occurs.[163] Rarely do streptococcal upper respiratory tract carriers spread the organism.[87] In contrast to carriage in the throat, which may persist for weeks or months, the prolonged presence of group A streptococci in the anterior nares is unusual.

In contrast to the upper respiratory tract, where group A streptococcus can establish infection readily on an intact epithelial surface, the production of streptococcal impetigo or pyoderma seems to be facilitated by prior disruption of the skin by trauma, insects, or some preexisting skin disorder. Group A streptococci may be found on the normal skin for several days to 2 weeks before infection develops,[59] requiring some other means of access. It does not seem likely that the source of infection for streptococcal skin infections is the upper respiratory tract. Group A streptococci causing impetigo may be found in the nose or throat, but they usually do not reach this site until several weeks after cutaneous infection has been established. One possible source is a skin lesion in another child, with spread occurring by direct contact. Some data suggest that spread may occur even by small flies that feed on such lesions.[11] The exact role of environmental contamination in the spread of streptococcal impetigo and pyoderma, and in secondary infection of wounds, burns, and eczema and other dermatoses, is unknown. The mechanism of transmission of erysipelas also is poorly understood, but may involve spread via the respiratory tract.

Measures to prevent spread of group A streptococcal infections vary in their effectiveness. Spread of throat or skin infection within a family often occurs before the index case is identified and isolated or treated. In epidemic situations, especially situations involving cases of rheumatic fever or acute nephritis, a culture survey with treatment of all individuals with positive cultures (mass prophylaxis) may be indicated. Reduction of crowding, especially in sleeping quarters, seems to be an effective long-term method of minimizing the incidence of transmission of streptococcal sore throat among some groups.

In families in which persistence or recurrence of streptococcal infection is a problem, simultaneous throat culture and culture of skin lesions of all members and treatment of all who have positive results have been successful in eradicating the organism. Some investigators have advocated a role for family pets (dogs) in transmission of streptococcal infections. Available data do not support such transmission as a common occurrence, however.[17,39,172] Control of environmental contamination would be expected to have little or no influence on the spread of group A streptococcal respiratory infections, although it possibly has an effect in controlling skin or wound infections. It also is suggested that family toothbrushes may have a role in the intrafamilial transmission of group A streptococci. More recent guidelines have suggested, however, that obtaining cultures of all family members in instances in which invasive group A streptococcal infections occur is not always necessary. Because of reported instances in which secondary cases have occurred, this guideline remains controversial.[132,136]

EPIDEMIOLOGY

Group A streptococci have a narrow range of hosts. They are one of the pathogenic bacteria identified almost exclusively in humans and only extremely rarely are found in other species.[108] In considering the epidemiology of group A streptococcal infections, one must recognize that significant differences exist between throat and skin infections.[133,164]

Streptococcal impetigo occurs with greatest frequency in preschool-aged children, whereas streptococcal pharyngitis predominantly is a disease of school-aged children. Outbreaks of streptococcal respiratory tract infections also have been observed in daycare centers.[149] On average, streptococcal respiratory infections occur at the rate of one every 3 to 5 years during childhood. One comprehensive long-term study suggested that the average child has three documented group A streptococcal infections (range of one to eight per child) before reaching age 13.[49] Among preschool and early school–aged children of certain populations, streptococcal impetigo is a recurrent problem.

The seasonal occurrence and geographic distribution are different for throat and skin infections. Tonsillitis and pharyngitis caused by streptococci are common occurrences in temperate and cold climates; streptococcal pyoderma and impetigo seem to occur with greater frequency in hot or tropical climates.[28] Streptococcal sore throat occurs more frequently in late autumn, winter, and early spring months. In tropical climates, pharyngeal colonization seems to occur more frequently during the rainy season. Streptococcal impetigo usually is a disease of the summer months in temperate climates, but it may occur with equal frequency year-round in tropical countries. In some tropical climates, groups C and G beta-hemolytic streptococci are isolated more frequently from the upper respiratory tract than is group A.[69] This finding has led some researchers to speculate about a possible etiologic role for groups C and G streptococci in the pathogenesis of rheumatic fever.[69]

PATHOGENESIS

No complete explanation is available for the predilection of certain body sites for infection by group A streptococci, or for the ability of strains of certain M types to produce pharyngitis or tonsillitis and of others to produce impetigo or pyoderma. In the establishment of throat infection, a primary requisite is a method of attachment to the epithelial cells of the pharynx. Group A streptococci attach by means of their fimbriae. To initiate an infection, group A streptococci also must compete with the resident pharyngeal flora, notably the alpha-hemolytic or viridans streptococci, which may interfere with the colonization of group A streptococci in the throat,[40] perhaps as a result of the production of a bacteriocin-like substance.[45] The relative importance of bacterial interference in preventing colonization of the human upper respiratory tract with group A streptococci is unclear, however. The influence of producers of a bacteriocin-like substance may be minimal in certain situations.[76]

In the production of impetigo, group A streptococci also must vie with other local bacterial flora. Removal of the normal flora increases the time of survival of group A streptococci applied to the skin.[3] Skin lipids, some of which are lethal for group A streptococci in vitro, also may provide a natural barrier against the establishment of streptococcal infection.

Invasion of the tissues by group A streptococci may be facilitated by a combination of bacteriologic properties. Damage to leukocytes and to fixed tissue cells may result from any of the several toxins produced, and the spread of infection may be aided by specific enzymes that attack hyaluronic acid and fibrin. M protein is antiphagocytic and contains a moiety that is cytotoxic in the presence of non–type-specific antibody.[14] The protective role of mucosal immunity remains incompletely defined. The hyaluronic acid capsule of group A streptococcus may serve as a camouflage because it resembles mammalian hyaluronic acid. In addition, several streptococcal substances (streptococcal pyrogenic exotoxins and peptidoglycan) have been shown to have endotoxin-like properties. A role for pyrogenic exotoxins has been postulated for streptococcal TSS and for necrotizing fasciitis.[152,153]

The factors responsible for the early host defense against group A streptococci (before the development of antibody) are incompletely understood. Type-specific antibody against M protein, which greatly promotes phagocytosis, usually is not detectable until 6 to 8 weeks after the infection has occurred[47]; its primary role may not be in the limitation or termination of active infection, but rather in the prevention of re-infection by the same serologic type. Reports indicate that type-specific immunity may be to specific strains within a given serotype, rather than to all strains within a given serotype.[46,161] The significance of this potentially important observation in the epidemiology of streptococcal infections and in the pathogenesis of nonsuppurative sequelae has not been defined fully. Surface phagocytosis, first by monocytes and later by polymorphonuclear leukocytes, may be the primary mechanism of defense in the early stages of infection.[141] In streptococcal skin infections, an increase in the leukotactic activity of polymorphonuclear leukocytes has been reported.[65]

Approximately 30 minutes after ingestion by a polymorphonuclear leukocyte has occurred, the streptococcus may be killed. Occasionally, the reverse occurs because of a phenomenon known as *leukotoxicity*, which apparently is related to the production of streptolysin S.[124] Degradation of the streptococcus within phagocytes or in tissues is a much slower process, suggesting that the human host may be unable to break down the streptococcal cell wall in an efficient manner.[65] This feature seems to be in contrast to the reported engulfment of group A streptococci by respiratory epithelial cells; in the latter instance, evidence suggests intracellular survival of the streptococci occurs, perhaps accounting for the persistence of these organisms in individuals thought to be carriers.[109]

Spread of streptococci to the regional lymph nodes can occur, especially in infections of the pharynx and tonsils.[45] Bacteremia occurs in the absence of underlying systemic disease, such as leukemia or other malignancies,[52,68] but it is an uncommon occurrence in older children and adults. The reason for the apparent increase in prevalence of severe systemic group A streptococcal infections beginning in the 1980s and 1990s and continuing into the 21st century remains incompletely explained. Although certain M types seem to be isolated more frequently from such severe infections (e.g., M-1, M-3), these strains apparently are no different from similar M types causing uncomplicated infections. Many M/*emm* types have been recovered from patients with severe systemic infections.

The rash and other toxic manifestations of scarlet fever have been attributed to the development of hypersensitivity to the pyrogenic toxins.[119] Toxic manifestations that have been noted in group A streptococcal TSS also may result from a direct influence of the pyrogenic exotoxins on lymphokines, such as tumor necrosis factor.[152,153] Hypersensitivity to other streptococcal products also may contribute to the manifestations of streptococcal disease.

Theories abound about the pathogenetic mechanism leading to the development of the nonsuppurative complications of streptococcal infections, acute rheumatic fever, and acute glomerulonephritis. Most of these hypotheses invoke immunologic processes in one way or another.[75,119,159] A major impediment toward clarifying the pathogenetic mechanism responsible for the development of nonsuppurative sequelae has been the lack of an appropriate animal model for study.

CLINICAL MANIFESTATIONS

Streptococcal pharyngitis or tonsillitis usually is a short-lived clinical illness with a brief incubation period (12 hours to 4 days). It varies greatly in its severity, from a subclinical or almost subclinical form, occurring in 30 to 50 percent of infections, to a very toxic form with high fever, nausea, and vomiting. Extreme toxicity may occur more frequently in epidemic situations, especially food-borne outbreaks, suggesting the importance of the rapid passage of the infecting organism from person to person in determining the severity of infection. The onset is acute and may be marked by fever, sore throat, headache, or abdominal pain (more common in children). The tonsils and pharynx may appear inflamed or infected but may look pale in the presence of marked edema. Exudate is a common manifestation (50%-90%). It usually appears by the second day and typically is discrete and whitish yellow and may become confluent by the following day. Swollen, tender anterior cervical lymph nodes (adenitis) also can be observed in 30 to 60 percent of patients.

The clinical manifestations usually subside spontaneously in 3 to 5 days, unless suppurative complications (otitis media, sinusitis, peritonsillar abscess) develop. Patients who develop nonsuppurative sequelae have a latent period of 1 to 3 weeks during which they seem completely well. After streptococcal infection of the upper respiratory tract develops, the average latent period for acute glomerulonephritis is 10 days; for acute rheumatic fever, the average latent period is 18 days.[164]

An infantile form of streptococcal infection, referred to as *streptococcal childhood fever*, may take a more prolonged course, with chronic low-grade fever, generalized lymphadenopathy, and a persistent serous nasal discharge; little or no evidence of localized inflammation is present in the pharyngeal area. The term *streptococcosis*, which sometimes has been used to refer specifically to this infantile form, should be employed more correctly to indicate the broad spectrum of clinical pictures that change with age in a manner analogous to tuberculosis.[131]

Scarlet fever is a rare disease in infants. This observation may be because of the possibility of placental transfer of maternal antibody to the pyrogenic toxins. A more complete explanation may relate to a necessity for hypersensitization to these exotoxins to develop before this manifestation of streptococcal disease can be expressed.[99] In the mid-20th century, the severe toxic form of scarlet fever was a rare occurrence in most industrialized countries; milder forms of the illness were prevalent. In the late 1980s, numerous reports of an illness characterized by scarlet fever–like rash but with severe systemic manifestations, including fasciitis, myositis, adult respiratory distress syndrome, and very high mortality rate (up to 30%), became more prevalent in the United States.[22,91]

The characteristic rash is red and finely punctate, appearing initially on the trunk and spreading peripherally within several hours to several days to cover almost the entire body in full-blown cases. A typical feature of the rash is that it fades on pressure and almost always leads to desquamation. Linear red lines may develop in the skin folds of the joints (Pastia lines) or in other areas of the extremities. The strawberry tongue of scarlet fever has a swollen, red, and mottled appearance and eventually peels. A scarlatiniform rash also may occur with streptococcal impetigo and streptococcal wound infections. An enanthema of stippled, bright red or hemorrhagic spots may appear on the soft palate or the anterior pillars of the tonsillar fossae. Exudate and tender cervical nodes may be present as in streptococcal pharyngitis without a rash, but the pharyngeal signs sometimes are minimal.

Streptococcal impetigo may develop a few days to several weeks after deposition of the infecting strain on the normal skin; the average latent period is 10 days.[59] In contrast to pharyngitis, this form of group A streptococcal infection frequently is painless, and the patient usually is afebrile. The initial lesion is a superficial vesicle with little surrounding erythema. This lesion rapidly develops into a pustule and then into a thick, honey-colored crust; this stage may last for a few days to several weeks. Secondary infection with staphylococci is a common development in the pustular and crust stages.[44,48] Removal of the crusts by trauma or as part of local therapy reveals a moist or purulent undersurface in the earlier stages. The infection does not involve the dermis. On healing, depigmentation may be seen, but permanent scarring rarely occurs. The lesions develop most frequently on the lower extremities but may occur on other exposed portions of the body, such as the upper extremities and the face.

Acute poststreptococcal glomerulonephritis may develop after impetigo or other forms of cutaneous streptococcal infection produced by a nephritogenic strain have occurred; rheumatic fever has not been proven to be associated with group A streptococcal skin infections.[166] This concept has been challenged by investigators working in populations in which group A streptococcal upper respiratory tract infections are rare compared with streptococcal pyoderma; these populations have very high incidence rates for acute rheumatic fever.[28] At present, however, a role for group A streptococcal skin infection in the pathogenesis of rheumatic fever remains unproven. The latent period for acute nephritis is longer after a skin infection (average, 3 weeks) than after a throat infection (average, 10 days).[92] The serologic types associated with nephritis after skin infection usually differ from the types causing nephritis after throat infection.[164] M-12 has been the classic nephritogenic serotype associated with pharyngitis, whereas serotypes such as M-49, M-55, and M-57 have been associated more frequently with nephritis occurring after skin infection.[164]

Impetigo and more nondescript forms of streptococcal pyoderma may be superimposed on scabies, eczema, other dermatoses, burns, and wounds, which afford a means of access through the cutaneous barrier. Ecthyma is a more deep-seated and chronic form of streptococcal pyoderma found predominantly in tropical climates.[1]

Erysipelas is an unusual type of streptococcal infection involving the skin and sometimes the adjacent mucous membranes. It is an elevated erythematous lesion, sometimes exhibiting blebs filled with yellowish fluid, which may crust over after rupture. The lesion is characterized by a well-demarcated advancing border, more reddened and edematous than is the central area, which may fade and become more normal in appearance as the lesion progresses. Erysipelas most often involves the face (especially in children), the extremities, or the body. The lesion may surround a surgical or traumatic wound, an area of dermatosis, or the umbilical stump in a newborn infant. Erysipelas tends not to spread from one body region to another. In erysipelas, the onset is acute and often is accompanied by the manifestations of systemic toxicity characteristic of other febrile forms of streptococcal infection. The lesion may last for a few days to several weeks. Relapses are common occurrences, with recurrences frequently at the same body site.

In addition to the infections described earlier, group A streptococci may produce a variety of other clinical pictures. Other infections associated with upper respiratory tract infections by these organisms include otitis media, retropharyngeal abscess, sinusitis, mastoiditis, pneumonia, and empyema. Beta-hemolytic streptococci are recoverable from approximately 50 percent of patients with peritonsillar abscess and may act in concert with anaerobic bacteria in the production of this clinical picture.[61] Acute puerperal sepsis, now a rare development, classically has been associated with group A streptococci. Outbreaks of omphalitis, bacteremia, and meningitis in nurseries continue to be reported occasionally.[156] Fatal gangrene,[66] disseminated intravascular coagulopathy,[80] and purpura fulminans[38] may be associated with infection by group A streptococci. These bacteria also are a common cause of perianal cellulitis and vaginitis in children.[4,104] Subpectoral abscesses and empyemas may develop as complications of streptococcal infections of the thumb and index finger as a result of the lymphatic drainage of that part of the hand.[4] Septic complications of varicella, including varicella gangrenosa,[25,148] osteomyelitis (especially in infants),[70] hand-foot syndrome,[71] blistering distal dactylitis,[72] necrotizing fasciitis, and TSS, are associated with beta-hemolytic streptococci. Some investigators have suggested that streptococcal infections may be responsible for episodes of acute guttate psoriasis.[8]

STREPTOCOCCAL UPPER RESPIRATORY TRACT CARRIER STATE

A puzzling aspect of the relationship of group A streptococci and the human host is the streptococcal "carrier" state. Not only does it represent a diagnostic and a therapeutic enigma for clinicians and public health authorities, but also the theoretical implications relating to pathogenesis of nonsuppurative sequelae are intriguing.[87] Data in the literature suggest that group A upper respiratory tract carriers are less dangerous to others because carriers only rarely spread the organism to close contacts. In addition, the risk of developing nonsuppurative sequelae, such as rheumatic fever, seems to be significantly reduced in carriers.[88] The epidemiologic and immunologic reasons for establishment and continuation of the carrier state are not understood.

Much of this confusion has resulted from the definition of the carrier state. In contrast to true infection, in which the patient has the presence of an organism and evidence of a host immune response, group A streptococcal upper respiratory tract carriers may harbor the organism in the upper respiratory tract for prolonged periods without evidence of an immunologic response, as measured by an increase in antibody to streptococcal antigens.[87]

The explanation for this prolonged persistence of group A streptococci in the upper respiratory tract is unknown. Whether

it is attributable to bacterial or host factors is unexplained. Hypotheses have been proposed to explain persistence of the organism in the upper respiratory tract. Bacterial data suggest that internalization of the group A streptococci into epithelial cells may explain their ability to continue to survive. Researchers have seen in vitro that stationary phase organisms are internalized easily by epithelial cells, and others have associated internalization with the presence of fibronectin-binding proteins in specific strains of group A streptococci.[109,122] Because of the inability of penicillin to penetrate the epithelial cell and to eradicate the organism from the carrier, this is an attractive explanation for clinical findings.[94]

For clinicians, the carrier has proven to be particularly problematic.[82,168] The ability to identify prospectively carriers and to separate them from individuals with bona fide upper respiratory tract infections, and the reported difficulty of eradicating the organism from the upper respiratory tract of carriers remains a perplexing and frustrating problem, especially considering that 5 to 20 percent of children may carry group A streptococci in their upper respiratory tract during the late autumn, winter, and early spring.

TABLE 93–1 Upper Limits of Normal for Anti-streptolysin O and Anti-deoxyribonuclease B for Children 2 to 12 Years Old

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Please refer to the printed publication.

From Kaplan, E. L., Rothermel, C. D., and Johnson, D. R.: Antistreptolysin O and anti-deoxyribonuclease B titers: Normal values for children ages 2-12 in the United States. Pediatrics 101:*86-88, 1998.*

IMMUNOLOGIC RESPONSE

The numerous somatic constituents and extracellular products of group A streptococci, most of which are antigenic, accounts for the complex nature of the host immune response after group A streptococcal infection develops. Humoral and cellular immune responses have been studied, the former more thoroughly than the latter.[167]

Skin and in vitro tests suggest that most adults are hypersensitive to a variety of streptococcal antigenic preparations, whereas infants more often are nonreactive. Lymphocyte transformation responses to most streptococcal substances probably are specific in nature, resulting from prior sensitization. Some studies indicate, however, that a nonspecific (mitogenic) response may occur with certain extracellular and cellular fractions. Inhibition of migration of leukocytes has been shown with fractions of streptococcal culture supernates and with cell membrane and cell wall fractions.[135,144] Some evidence from research in humans suggests that the cellular immune response to a streptococcal extracellular antigen is controlled genetically.[67,144]

In humans, humoral immune responses have been shown to numerous somatic components of the group A streptococcal cell. Of particular interest are antibodies to the group A carbohydrate that serologically are cross-reactive with the glycoprotein of human and bovine heart valves and antibodies to protein components of the group A cell wall or cell membrane that have been reported to be cross-reactive with the sarcolemma of heart muscle.[9]

Antibody to the M protein (type-specific antibody) is of special importance because it is the basis of immunity and protection against re-infection with the same serologic type.[13,107,163] Type-specific antibody may be transferred across the placenta from mother to fetus.[176] The development of type-specific antibody can be inhibited partially by prompt administration of penicillin for the streptococcal infection.[42]

Humoral antibodies to specific streptococcal extracellular products can be shown readily by neutralization assays.[21] These assays have been especially useful as a more precise method of defining streptococcal infection in clinical and epidemiologic studies and of documenting the occurrence of a preceding streptococcal infection in patients with a suspected nonsuppurative complication.

The anti-streptolysin O assay is the streptococcal antibody test used most frequently.[145] Because streptolysin O also is produced by group C and G streptococci, the test is not specific for group A infection. The antistreptolysin O response can be feeble in patients with streptococcal impetigo or pyoderma[93]; its usefulness for this latter condition is limited. In contrast, the anti-deoxyribonuclease B (anti-DNAse B) and the anti-hyaluronidase responses are reliable after skin and throat infections have occurred.[93]

Another antibody test, the Streptozyme agglutination test, is based on antibody agglutination of erythrocytes coated with a mixture of streptococcal extracellular antigens. It has the theoretical appeal of simplicity, speed, and reaction with numerous streptococcal antigens.[78] Peak titers for an immune response as measured by the Streptozyme test have been shown within the first 7 to 10 days after onset of infection,[125] whereas neutralizing antibody titers to streptolysin O (3-6 weeks) or anti-DNAse B (6-8 weeks) do not peak until later. Because of documented problems of standardization of this reagent (variable results may be obtained with different lots) and because of problems with group specificity, this test should be interpreted with caution.[97,167] Some studies have indicated problems in interpretation of this test, and the World Health Organization has recommended that it not be used.[5]

Antibody titers reported by clinical immunology laboratories may vary. Upper limits of normal are higher for children than for adults, and these values, even for the same age group, are higher in some populations than in others. Interpretation of antibody titers for clinical purposes must take these factors into consideration.[145] Often, values given by laboratories for upper limits of normal are determined using adult sera; these values often are much too low to be used in a pediatric population. Table 93–1 gives results of normal levels of antibody titers in children 2 to 12 years old.[99]

DIAGNOSIS AND DIFFERENTIAL DIAGNOSIS

In patients presenting with acute pharyngitis or tonsillitis, the clinician must rely on a combination of the clinical appearance, identification of the organism, and epidemiologic findings to confirm the probability of group A streptococcal infection. The clinical difficulty is compounded because most of the clinical manifestations of streptococcal pharyngitis can be associated with a variety of other etiologic agents.[19] Group A streptococci often are found in the throats of normal children and in children whose clinical findings are due to one of these other agents (see also Chapter 10).[165] Exudative sore throat may be caused by many

viruses, *Corynebacterium diphtheriae*, gonococci, and groups C and G in addition to group A streptococci.[19] The clinical syndrome associated with *Arcanobacterium haemolyticus* can be quite similar and clinically confusing.

Viral pharyngitis may closely mimic streptococcal pharyngitis, which can be ruled out only by the absence of a positive culture for group A streptococci. In children, the white blood cell count may be elevated in viral infections, but a low count renders it unlikely that the infection is streptococcal. The C-reactive protein test is only marginally useful in the acute phase of the illness.[101]

Because most streptococcal infections are short-term illnesses and antibody responses can be slow in appearing, streptococcal antibody titers are useful only retrospectively in diagnosing acute group A streptococcal infection. Little or no reason exists to use streptococcal antibody titers in the management of acute pharyngitis. In addition to their primary role in supporting the diagnosis of nonsuppurative complications (acute nephritis and acute rheumatic fever), however, occasionally they may be useful clinically in diagnosing infections in which obtaining cultures from the primary site is difficult (e.g., streptococcal pneumonia or osteomyelitis) or the infections have been treated or partially treated with antibiotics. The presence of an elevated streptococcal antibody titer does not, by itself, confirm a diagnosis of rheumatic fever, however. A more reliable approach is to use an increase in titer to confirm group A streptococcal infection.

Numerous clinical schemes have been proposed for differentiating streptococcal from nonstreptococcal pharyngitis, but none of them is entirely satisfactory in making this distinction or in differentiating streptococcal carriers with an intervening nonstreptococcal pharyngitis from individuals with active streptococcal disease.[86,165] Clinical manifestations that are most suggestive of a group A streptococcal cause include the scarlatiniform rash (which, however, occasionally is associated with staphylococcal, rather than streptococcal, infection), excoriated nares (especially in infants), tender (not merely enlarged) anterior cervical lymph nodes, and a history of close contact with a well-documented case of group A streptococcal infection. The presence of cough, hoarseness, or conjunctivitis renders the diagnosis of streptococcal pharyngitis unlikely. Exudative pharyngitis in infants usually is nonstreptococcal in etiology.[2] In addition, numerous clinical scoring systems have been found to be helpful to the clinician in some circumstances, but even they are not entirely reliable.[168]

Cultures may yield invalid results unless they are obtained and processed carefully. For cultures of the throat, the affected areas (tonsils and posterior pharynx) should be rubbed firmly with the rayon or cotton culture swab. Impetiginous lesions should be cleansed with alcohol, and the vesicle should be punctured or the crust lifted by a sterile needle so that purulent material or the moist base can be touched by the swab.[85] Group A streptococci sometimes can be recovered even from dry crusted lesions if the swab is moistened with culture broth before touching the exposed base of the lesion. Because streptococcal impetigo lesions commonly contain secondarily invading staphylococci, which may overgrow and obscure the colonies of streptococci, cultures should be examined carefully with a hand lens; alternatively, gentian violet or other inhibitors of normal flora may be incorporated on the blood agar plates as an inhibitor of staphylococci.

Presumptive differentiation of group A from other hemolytic streptococci can be achieved by the sensitivity of group A streptococci (but relatively few of other hemolytic streptococci) to bacitracin, but only when tested by a disk designed specifically for this purpose.[85] Definite identification of group A streptococci can be accomplished by several serologic or immunologic techniques, including (1) extraction of the organism by boiling in hydrochloric acid or by several other extraction methods with examination of the resulting extract in a precipitin test, (2) fluorescent antibody test on isolated colonies or broth cultures, and (3) agglutination of the organisms by group-specific antisera bound to protein A–containing staphylococci.

Numerous rapid techniques for direct identification of group A streptococci from the upper respiratory tract are available commercially. Direct and rapid identification of group A streptococcal antigens from throat swabs (e.g., latex agglutination, enzyme-linked immunosorbent assay) has become quite popular. The specificity of these tests usually is very good, but published reports indicate that the sensitivity varies widely.[90,146] Guidelines promote a back-up throat culture if a rapid antigen detection test is negative.[20] These techniques employ extraction of the group-specific carbohydrate from the cell wall of the organism. Available data suggest that the specificity for these tests generally is greater than 90 percent, but the sensitivity ranges from less than 60 percent to greater than 90 percent.[90]

The advantages of these tests include the ability to identify group A streptococci rapidly and to treat the patient at the time the patient is in the physician's office or emergency department. This advantage has appeal, especially in view of the data suggesting that in children, the more quickly the patient is treated, the more rapid is the clinical response.[134] Studies also have shown that rapid antigen detection tests can be useful in the detection of group A streptococci in streptococcal pyoderma-like lesions.[95]

Data suggest that the sensitivity of rapid antigen detection tests is improved, leading to the suggestion that a positive rapid antigen detection test is sufficient proof of group A infection, but that a negative rapid antigen detection test should be confirmed with a conventional throat culture on sheep blood agar. The throat culture remains a "gold standard" for identifying group A streptococci in the upper respiratory tract. Although they rarely are encountered, false-positive rapid antigen detection tests also have been documented, owing to a cross-reaction with antigens present in some *Streptococcus milleri*.[88] Just as with any other laboratory test, a "learning curve effect" exists with streptococcal rapid antigen detection tests.[98]

On clinical findings alone, it generally is safer to make a diagnosis of streptococcal impetigo than it is to make a diagnosis of streptococcal pharyngitis. Impetigo in which staphylococci are the primary invader generally is bullous rather than vesicular in type, and on rupture of the vesicle a crust appears that is paper-thin and white, rather than thick and honey-colored. Some confusion may result from reports of cultures performed on patients with primary streptococcal impetigo. Staphylococci, often present as secondary invaders, may overgrow the streptococci, which consequently may be missed, unless the colonies are well isolated and the bacteriologist has an unusually sharp eye, or unless a culture medium inhibitory for staphylococci is used.

The vesicles of varicella infection may resemble those of streptococcal impetigo superficially, but they are less transient, are surrounded by a red areola, are more centripetal in distribution (tending to involve the trunk and the proximal portions of the extremities), frequently itch, occur in crops, and often are accompanied by constitutional symptoms. The crusts are not as thick as are those of streptococcal impetigo. The lesions of chickenpox can be infected with streptococci secondarily, and varicella has been identified as an important risk factor in the development of severe and invasive group A streptococcal infections.

PROGNOSIS

Patients with streptococcal sore throat or streptococcal impetigo recover spontaneously. A very few may develop suppurative complications, and an occasional patient may have a nonsuppurative sequela, particularly in industrialized countries. There is more

chance of developing complications in socially and economically disadvantaged populations.

In the general population, the risk of developing rheumatic fever after an untreated bona fide group A streptococcal infection of the upper respiratory tract has been shown to be approximately 3 percent under epidemic conditions but is apparently considerably less (about 0.3%) in endemic situations,[147] owing partly to differences in definition of infection.[100] Patients who have had one attack of rheumatic fever are at high risk of a rheumatic fever recurrence when re-infected with group A streptococci. There seems to be no risk of developing rheumatic fever after having a streptococcal infection only of the skin, but this concept has been challenged if not yet substantiated.[28]

The risk of acute glomerulonephritis depends on whether the infection is caused by a nephritogenic strain. With a nephritogenic strain, the attack rate is about 10 to 15 percent, and acute glomerulonephritis can occur after either throat or skin infection.[164]

In contrast to the usual short, often benign course of throat and superficial skin infections, streptococcal cellulitis spreads rapidly locally and to the regional lymph nodes and bloodstream. In immunosuppressed patients and patients with streptococcal infection superimposed on leukemia, lymphoma, or other malignancies, bacteremia may develop, and patients may have serious life-threatening problems. Patients with puerperal sepsis, neonatal infection, streptococcal toxic shock–like syndrome, or gangrene owing to group A streptococci also have a high mortality rate despite timely administration of and high doses of penicillin therapy.[153]

The prognosis for complete recovery in patients with group A streptococcal TSS and in patients with necrotizing fasciitis varies. Morbidity and mortality rates have been reported to be greater in adults, especially the elderly, than in pediatric patients. Several series reported a mortality rate of at least 30 percent in patients with TSS.[154] The mortality rate with necrotizing fasciitis can be even greater.[152,153]

TREATMENT

Although group A streptococci generally are susceptible to many antibiotics,[37,53,117] penicillin remains the drug of choice for treatment except in patients allergic to it.[20] No clinical isolates of group A streptococci have been identified yet that are resistant to penicillin.[112] Although tolerance to penicillin has been described in group A streptococci, its clinical significance has not been determined.[102]

Eradicating group A streptococci from the upper respiratory tract (especially from carriers) with penicillin or other antibiotics sometimes is difficult.[89,95] This observation has not been explained adequately, but possible explanations include the presence of β-lactamase–producing organisms in the upper respiratory tract, the presence of group A streptococci tolerant to penicillin, and the production by certain of the normal upper respiratory tract flora of inhibitory substances that reduce persistence of the organism. In addition, some evidence suggests that persistence frequently is associated with the upper respiratory tract carrier state.[105] The presence of intracellular group A streptococci also has been noted in this regard.

Erythromycin remains the drug of choice in patients allergic to penicillin. Resistance to erythromycin is a rare occurrence in many countries (<5%). More recent data from Europe, where macrolides are widely used, show resistance rates in some countries of greater than 30 percent. In some countries, noticeable increases in resistance to macrolide by group A streptococci has been associated with increased use of macrolides. Although the prevalence of macrolide-resistant group A streptococci generally has been low in North America and of little clinical significance,[73,173] isolated examples have shown the ability of the organism to become resistant to macrolides.[115] The incidence was high in Japan in the 1960s,[50] and this observation also has been noted in Finland.[143] The use of broad-spectrum antibiotics for group A streptococcal infections has no advantage and potential disadvantages.[6-20] Group A streptococci most frequently are resistant to tetracyclines and the sulfonamides. β-lactamase–resistant antibiotics have been advocated in some studies[23]; a role for production of β-lactamase by normal flora in penicillin treatment failures has not been clarified yet.

Some reports have pointed out that cephalosporins may be more efficacious than is penicillin, especially in carriers. This form of oral therapy has been recommended by some clinicians.[30,31]

In the treatment of bona fide streptococcal sore throat, the group A streptococcus must be eradicated to prevent the development of acute rheumatic fever.[32] Many guidelines recommend administration of 10 days of oral penicillin V, or erythromycin for a penicillin-allergic individual, as being optimal for eradication.[20] Administration of a single intramuscular injection of benzathine penicillin G (1,200,000 U in adults and in children weighing >60 lb; 600,000 U in children weighing <60 lb) is one method of accomplishing this objective. If a combination of benzathine penicillin and procaine penicillin is used, the total dosage should be based on the amount of benzathine penicillin used.

If oral medication (penicillin or erythromycin) is used, the parent must be impressed with the importance of continuing the medication for a full 10 days. Oral penicillin V (250 mg two to three times a day) has been the treatment of choice for children. Many pediatricians prefer amoxicillin because of its taste. For adolescents, 250 mg given either three or four times a day or 500 mg twice a day has been suggested.[20] More recent studies have reported that amoxicillin given once daily (750 to 1000 mg) is as effective as is amoxicillin given twice daily (two doses of 375 mg or 500 mg) in eradicating group A streptococci in children.

In patients with a suspected allergy to penicillin, erythromycin (in adults, 250 mg four times a day; in children, 40 mg/kg/day in four doses, not to exceed the adult dose) has been used.[43] Two other macrolide/azalide preparations, azithromycin and clarithromycin, have been used as substitutes for erythromycin. The exact dose of erythromycin varies with the preparation used (e.g., stearate, estolate). Other antibiotics that have been used successfully in the therapy of group A beta-hemolytic streptococcal pharyngitis or tonsillitis include clindamycin, amoxicillin, a mixture of amoxicillin with clavulanic acid, and other cephalosporins. For patients who have *not* had an immediate type reaction to penicillin, many clinicians feel safe using a first-generation cephalosporin.

The problem of patient adherence to a 10-day course of oral antibiotics is well recognized. Because of this problem, several antibiotics have been approved for short-course therapy (<10 days) for treatment of group A streptococcal pharyngitis. These antibiotics include some cephalosporins and one of the newer macrolides/azalides. Some studies show equivalence of short-course therapy to 10 full days of conventional penicillin V oral therapy. Because of conflicting data, some guidelines still promote caution in using short-course therapy for the treatment of group A streptococcal upper respiratory tract infection.[20]

In patients with strong clinical or epidemiologic evidence of streptococcal infection, the physician may decide to begin therapy before the result of the throat culture is available. If oral antibiotic therapy is used for initiating treatment, the therapy may be continued or discontinued, depending on the culture report. Alternatively, an intramuscular injection of benzathine penicillin G may be administered at that time if the laboratory culture report is positive for group A streptococci. For patients who may not return for a culture report, or who may be difficult to contact, making an immediate clinical judgment whether to prescribe penicillin therapy may be important. In these situations, the use

of the rapid direct techniques for detection of group A streptococci is advantageous. Because such patients also may be less reliable with respect to completing a course of oral therapy, the use of intramuscular benzathine penicillin G may be preferable. Even if the decision to treat or not to treat must be made on clinical grounds, throat cultures can be useful in indicating to the physician the current prevalence and clinical features of streptococcal and nonstreptococcal respiratory illnesses. Intramuscular benzathine penicillin G also is advantageous in epidemic situations.

At one time, obtaining repeat throat cultures after completion of oral therapy was thought to be appropriate to ensure that the group A streptococci had been eradicated. Studies have shown that many penicillin treatment failures seem to occur in carriers of group A streptococci in the upper respiratory tract.[87] In areas where rheumatic fever has not reappeared, re-culturing asymptomatic individuals routinely after they have received antibiotic therapy usually is unnecessary unless unusual epidemiologic circumstances (a rheumatic individual in the household or epidemic streptococcal disease in the community) exist. Other regimens that have been used for treating patients with persistently positive cultures include clindamycin, a mixture of amoxicillin and clavulanic acid, and rifampin along with either an injection of benzathine penicillin G or 10 days of oral penicillin.[20]

For patients who show a repeated clinical pattern of treatment failure, determining the serologic group and type of the strains recovered may be helpful to ascertain whether the isolates are the same or are different M protein types. Such testing is not done routinely in hospital laboratories, however, and in those circumstances contact with a state health department or streptococcal research laboratory would be required. It is important in "problem" families, in which intrafamilial spread is problematic, to culture the throats of all members of the family simultaneously and to treat all family members with positive results. The main problem of the persisting carrier is that this carrier status may complicate the interpretation of throat cultures obtained at the time of future nonstreptococcal respiratory tract infections.

Some researchers have suggested that the rapid treatment of group A streptococcal upper respiratory tract infection tends to promote recurrent streptococcal infections in the future because of the suppression of the type-specific antibody response. Some studies have shown no difference, however, in the frequency of recurrences of streptococcal infections whether therapy is started on diagnosis or delayed 48 hours.[63]

In contrast to group A streptococcal pharyngitis or tonsillitis, no authoritative guidelines have been developed for the treatment of streptococcal impetigo.[170] The effectiveness of hygienic measures and local skin care (removal of crusts and use of antibacterial soaps) probably depends on the thoroughness and perseverance with which they are carried out. These measures and the use of local antimicrobial ointments can be sufficient for the management of patients with only a few lesions. Systemic antibiotics have been associated with rapid clearing of the lesions, however. Oral or parenteral penicillin or oral erythromycin (in the amounts prescribed for the treatment of streptococcal pharyngitis) should be administered to patients with more severe or persistent infections. In the absence of microbiologic data, many clinicians use antibiotics that are effective against streptococci and staphylococci. First-generation cephalosporins and semisynthetic penicillins also are effective. Antibiotic therapy probably does help prevent the spread of streptococcal impetigo in family members. In an era when methicillin-resistant *Staphylococcus aureus* are common findings, special care must be exercised when selecting antibiotic therapy.

Whether penicillin or other antibiotic treatment reduces the risk of development of acute nephritis is unclear.[171] One study suggests that penicillin therapy may reduce the risk of this complication occurring in patients with streptococcal sore throat caused by a nephritogenic strain.[151] No definitive proof that penicillin treatment reduces the frequency of acute nephritis after treatment of skin infections exists, however. Clinical experience indicates that patients with cutaneous infections caused by a nephritogenic strain of group A streptococci may develop this complication despite receiving adequate penicillin therapy.[92]

Otitis media or cervical adenitis caused by group A streptococci usually responds to regimens prescribed for treatment of streptococcal sore throat. Patients with peritonsillar abscess require surgical drainage in addition to vigorous parenteral antibiotic therapy. Patients with more serious infections (e.g., mastoiditis, pneumonia, empyema) also should be given intensive systemic therapy. Patients with meningitis, arthritis, or osteomyelitis require high-dose intravenous penicillin administered for a long time (see Chapters 37 and 61). Patients with streptococcal TSS and patients with necrotizing fasciitis may be treated with parenteral penicillin, but more recent evidence suggests that clindamycin combined with penicillin has advantages.[152] In patients allergic to penicillin, clindamycin is an excellent alternative. Because of cross-reactions with penicillin, the cephalosporins always should be used cautiously in penicillin-allergic patients, especially in patients who have had immediate-type reactions.

PREVENTION

Antimicrobial agents have been helpful in controlling group A streptococcal infections and their sequelae, but they do not provide an encompassing solution for this group of diseases, either in industrialized countries or in the developing world—hence the "resurgence" of rheumatic fever and the appearance of streptococcal TSS in the United States during the 1980s and 1990s. Penicillin's greatest impact has been on the prevention of recurrences of rheumatic fever (see Chapter 35).[18] Prevention of first attacks of rheumatic fever is a problem of greater dimensions because it involves detection, diagnosis, and appropriate treatment in the general population. One cost-effective program can be developed from well-conceived secondary prevention programs in defined rheumatic patients and perhaps from primary prevention programs in school-aged children, especially programs in socially and economically disadvantaged populations.[127]

The prevention of spread by isolation, limiting the density of the population, and antibiotic treatment of known cases is discussed in the section on transmission (see earlier). Mass penicillin prophylaxis has been used in epidemics with a well-defined streptococcal etiology,[24] but in actual practice, the epidemic often is subsiding by the time a large-scale prophylactic effort can be mounted. Intramuscular benzathine penicillin G is very effective for this purpose.[24] The dose is the same as recommended for treating streptococcal pharyngitis. In populations in which streptococcal infections occur at epidemic or near-epidemic levels over a long period of time (e.g., certain military populations), it may be necessary to repeat the injections of benzathine penicillin at monthly intervals and to administer them to all new arrivals.

Although guidelines for management of contacts of patients with severe streptococcal infections are available, and routine surveillance cultures among family members and close contacts are not recommended universally,[132] this approach remains controversial because of documented secondary cases in families.[136] In the family studies carried out several decades ago, researchers found that approximately 25 percent of family contacts harbored the organisms in families where an index case was identified.[49] Because of documented instances in which secondary cases have occurred, some clinicians choose to culture close contacts and to use long-acting benzathine penicillin, long-acting benzathine penicillin plus rifampin, or clindamycin to treat individuals who are positive for group A streptococci. The effectiveness of such

regimens has not been studied; contacts need to be monitored closely even after being treated.

The question of the possible advantages of tonsillectomy for the prevention of streptococcal infections and their sequelae also has not been settled by well-controlled studies. Available information indicates that tonsillectomy may reduce the frequency of clinically apparent streptococcal infections,[128] perhaps rendering it less likely that these patients receive appropriate treatment.[53] One study indicates, however, that recurrences are more frequent in individuals who have had rheumatic fever and who have large tonsils.[58]

The inability of antibiotics to influence significantly the epidemiology of group A streptococcal infections and their sequelae consistently and favorably is reflected by the concentration of sequelae occurring more recently in middle-class populations with ready access to medical care.[160] Control measures would be much more effective if a group A streptococcal vaccine were available. Several different approaches to streptococcal vaccines that are being investigated include a multivalent, type-specific vaccine based on M-protein type, a vaccine based on conserved regions of the M-protein that are present in all M-protein types, and a third group of candidate vaccines that are based on immunity to extracellular or somatic antigens of the organism not associated with M protein. Early studies in animals have shown immunogenicity in several of these candidate vaccines, and some vaccines based on type-specific immunity have undergone early clinical trials in humans. Although one cannot make a prediction with certainty, the availability of a cost-effective group A streptococcal vaccine to the general public still does not seem imminent. Large-scale clinical trials in different geographic areas and among different populations are required ultimately to determine safety and efficacy.

GROUP C AND GROUP G STREPTOCOCCAL INFECTIONS

ORGANISMS

The characterization of beta-hemolytic streptococci on the basis of group-specific carbohydrate antigens is complicated by the presence of similar antigens among streptococci that, on the basis of biochemical and genetic testing, have been shown to be different species. Organisms that possess either the group C or group G Lancefield antigens can be divided into groups based on colony size. The strains that produce small or minute colonies (<0.5 mm in diameter) have been placed in the *Streptococcus anginosus* group (classified as *Streptococcus milleri* by British taxonomists). Although these small-colony-forming strains of group C and group G streptococci may produce human infections, with abscesses being the most common form, they also are found as commensals and have considerably less pathogenic potential than do the large-colony-forming group C and group G streptococci. Despite the existence of these groupable, beta-hemolytic strains, members of the *S. anginosus* species group are considered to be viridans group streptococci, most of which display alpha-hemolytic or nonhemolytic reactions.

In contrast, the strains that produce large-sized colonies (≥5 mm in diameter) have been referred to as true or large-colony-forming group C or group G streptococci, depending on the nature of their carbohydrate antigen. The heterogeneity of large-colony streptococci with the Lancefield group C or group G polysaccharides has produced considerable confusion, however. The taxonomic classification of these streptococci still is unsettled, and more changes are likely to occur before a universally accepted scheme is established; however, at least three species have been found in human infections: *Streptococcus dysgalactiae* subsp. *equisimilis*, *Streptococcus equi* subsp. *zooepidemicus*, and *Streptococcus canis*.[54,137,140]

Most group C streptococci are beta-hemolytic on blood agar plates, but all types of hemolysis have been observed. Group C streptococci are aerobic, facultatively anaerobic, coprophilic, and catalase-negative organisms. Rhamnose-*N*-acetylgalactosamine is the group C antigenic determinant in the cell wall. Traditionally, four species possessing this determinant have been differentiated on the basis of their ability to ferment various carbohydrates: *S. equisimilis*, *S. zooepidemicus*, *S. equi*, and *S. dysgalactiae*. More recent genetic studies have led, however, to the reclassification of group C streptococci into two species—*S. dysgalactiae* and *S. equi*—each with two subspecies. Although most group C streptococci are resistant to bacitracin, at least a third (in one study, 62%) of group C streptococci are bacitracin-sensitive.[7]

S. dysgalactiae subsp. *equisimilis* is the species of group C streptococci and group G streptococci that most often colonizes and causes infections in humans.[54] The actual frequency is unknown, however, because clinical laboratories do not perform phenotypic tests on group C or group G streptococcal isolates other than the hemolytic reaction and the Lancefield group determinations. *S. dysgalactiae* subsp. *equisimilis* has been isolated from the nose, throat, and genital tract of asymptomatic children and adults and from the umbilicus of asymptomatic newborns. This species produces streptokinase and streptolysin O, and infection may elicit an antibody response to these extracellular antigens similar to that seen with a group A streptococcal infection.

S. dysgalactiae subsp. *equisimilis* are pyogenic streptococci that are similar to *S. pyogenes* with respect to some virulence traits. They express homologues of the M virulence protein of *S. pyogenes* that are antiphagocytic (encoded by the *emm* gene), and some strains contain superantigen genes first characterized in *S. pyogenes*.[111,129] The *emm* genes of *S. dysgalactiae* subsp. *equisimilis* isolates with either the group C or the group G Lancefield antigen can be used for sequence-based typing. Pulsed-field gel electrophoresis also can be used to identify specific stains. This organism can cause infections in a variety of domestic animals (e.g., horses, cattle, pigs, chickens). *S. dysgalactiae* subsp. *dysgalactiae* has not been found to cause human infections but does cause a serious mastitis in cows and a suppurative polyarthritis in lambs.

S. equi subsp. *zooepidemicus* possess the group C Lancefield antigen and can cause significant, often epidemic, infections in domestic animals (e.g., horses, cattle, pigs, sheep), but it is an uncommon pathogen in humans. Most human infections have been associated with consumption of homemade cheese or unpasteurized cow's milk. *S. equi* subsp. *equi* possess the group C Lancefield antigen and have not been found to cause human infections but do cause a serious and highly contagious respiratory disease in horses, known as *strangles*.

Most group G streptococci are beta-hemolytic on blood agar plates. L-rhamnose is the group G antigenic determinant in the cell wall. Most of the group G streptococci isolated from human infections are thought to be *S. dysgalactiae* subsp. *equisimilis*. *S. canis* with the group G Lancefield antigen also cause infections in humans.

Several schemes for typing group G streptococci have been described based on biochemical properties or bacteriocin typing. Although no association between particular types and infections in humans has been identified, these schemes have been useful in distinguishing human from animal strains and in epidemiologic investigations. T-typing and M-typing schemes similar to those used for group A streptococci also have been devised for group G streptococci. Newer methods, such as *emm*-typing, pulsed-field gel electrophoresis, and multilocus enzyme electrophoresis,

also are being used to type specific strains of group G streptococci.[16,129] Although most group G streptococci are resistant to bacitracin, various reports have determined that 8 to 67 percent of group G streptococci are bacitracin-sensitive.[140]

In addition to M protein, human isolates of group G streptococci share other virulence factors with group A streptococci, such as a streptokinase, a hyaluronidase, and a C5a peptidase. Group G streptococci also produce a streptolysin that is similar antigenically to the streptolysin O produced by group A streptococci. Patients with group G streptococcal infections may show a significant increase in levels of antistreptolysin O titers.

EPIDEMIOLOGY

Group C streptococci are an uncommon cause of human infections but more commonly are pathogenic in animals. Humans infected with this organism often have had some animal contact. Group C and group G streptococci often can be part of the normal human flora of the nasopharynx, skin, or genital tract. Group C streptococci also can be cultured from the umbilicus of asymptomatic newborns and from routine puerperal vaginal cultures. Group G streptococci also can be cultured from the gastrointestinal tract. The low virulence of group C and group G streptococci is indicated by the fact that most humans infected with either of these organisms have some underlying medical disorder (e.g., diabetes mellitus, malignancy, alcohol abuse, immunosuppression).[29,106]

CLINICAL MANIFESTATIONS

The clinical features of group C and group G streptococcal pharyngitis are similar to those of group A streptococcal pharyngitis and include fever, mild to moderate sore throat, pharyngeal exudate, and cervical adenitis.[110] In temperate climates, 1 to 18 percent of asymptomatic individuals have been reported to harbor group C streptococci in the upper respiratory tract. The proportion of carriers among individuals living in the tropics is even greater.[69,126] Such carrier rates render establishing the etiologic role of group C streptococci in acute pharyngitis difficult. Several earlier studies compared the isolation rates of group C streptococci from patients with acute pharyngitis with rates in asymptomatic controls; the results were contradictory.[7,35] Several more recent investigations have established a strong epidemiologic association between group C streptococci and endemic acute pharyngitis. In two investigations, group C streptococci were isolated significantly more often from college students with acute pharyngitis than from asymptomatic controls.[157,158] In the other investigation, group C streptococci were isolated significantly more often from adults who came to an emergency department with acute pharyngitis (6%) than from asymptomatic controls (1.4%).[121]

In addition to endemic pharyngitis, group C streptococci can cause epidemic food-borne pharyngitis after contaminated products, such as unpasteurized cow's milk, have been ingested. Epidemics have been reported from Great Britain, Romania, the United States, and Israel.[7,35] In one report of a milk-borne epidemic of pharyngitis caused by *S. equi* subsp. *zooepidemicus*, approximately one third of the patients developed signs of acute glomerulonephritis.[51] This outbreak was related to the consumption of unpasteurized milk from cattle with mastitis. No cases of acute rheumatic fever occurred. More recently, a large outbreak of acute glomerulonephritis in Brazil was attributed to the consumption of unpasteurized cheese containing *S. equi* subsp. *zooepidemicus*.[10] Family outbreaks of group C streptococcal pharyngitis and an outbreak in a residential school for boys also have been described.

Group C streptococci also have been reported as the cause of numerous other uncommon infections, including skin and soft tissue infections, septic arthritis, osteomyelitis, pneumonitis, infective endocarditis, bacteremia and septicemia, meningitis, epiglottitis, pericarditis, urinary tract infections, and sinusitis. These organisms also have been associated with epidemic and nonepidemic cases of puerperal sepsis and endometritis.[81] Several reports have suggested that an association exists between group C streptococci and reactive arthritis and a toxic shock–like syndrome.[54,79]

Some studies have reported that 1 to 23 percent of asymptomatic individuals may carry group G streptococci in their upper respiratory tract. As with group C streptococci, the carrier rates of group G streptococci seem to be even higher in the tropics.[69,126] Such carrier rates also render establishing the etiologic role of group G streptococci in acute pharyngitis difficult. Several studies have been performed comparing the isolation rates of group G streptococci from patients with acute pharyngitis with rates from asymptomatic controls. The results of these studies showed little difference in the isolation rates, suggesting that group G streptococci may not play an important role in endemic acute pharyngitis. Few of these studies were adequately controlled, prospective investigations, however, and in many of them, the incidence of group G streptococci in the symptomatic group was so low as to preclude any possibility of showing a statistically significant difference.

Support for an etiologic role of group G streptococci in acute pharyngitis comes primarily from anecdotes, small case clusters, and a few large outbreaks, most of which were food-borne. To date, several food-borne outbreaks of group G streptococcal pharyngitis, all of which occurred in semiclosed populations, including one outbreak at a college cafeteria, have been reported.[116] In the first reported respiratory outbreak of group G streptococcal pharyngitis in the United States, McCue[120] described 68 cases of acute pharyngitis on a college campus seen during a 9-day period in 1981. The possibility of food-borne spread could not be eliminated completely but seemed unlikely, and airborne droplet transmission seemed to be the most likely mechanism of spread.

Despite the evidence supporting the etiologic role of group G streptococci in epidemic pharyngitis, the role of group G streptococci in acute, endemic pharyngitis remains unclear. Previous outbreaks of group G streptococcal pharyngitis had been reported in college-age or older patients, and all had occurred in semiclosed communities. A community-wide, respiratory outbreak of group G streptococcal pharyngitis in a pediatric population was described, however.[64] During a 6-month period, group G streptococci were isolated from 56 of 222 (25%) consecutive children with acute pharyngitis seen at a private pediatric office. Results of DNA fingerprinting of the group G streptococcal isolates suggested that 75 percent of them were the same strain. The patients with group G streptococcal pharyngitis were comparable to patients with group A streptococcal pharyngitis with respect to clinical findings, antistreptolysin O titer response, and clinical response to antibiotic therapy. Patients with group G streptococci were significantly older, however. The findings suggested that antibiotic therapy may have an impact on the clinical course of group G streptococcal pharyngitis. These findings lend support to the belief that group G streptococci may be a more important cause of acute, endemic pharyngitis than was recognized previously.

The actual role of group C and group G streptococci in acute pharyngitis may be underestimated for several reasons. Anaerobic incubation increases the yield of these organisms, but most clinicians do not use anaerobic incubation for throat cultures routinely.[142] Because clinicians generally disregard beta-hemolytic streptococci that are bacitracin-resistant (and most strains of

group C and group G streptococci are bacitracin-resistant), many group C and group G streptococci would be missed.

Acute rheumatic fever has not been described as a complication of either group C or group G streptococcal pharyngitis, and although there have been reports attempting to link acute glomerulonephritis with group G streptococcal pharyngitis, the evidence is anecdotal, and a causal relationship has not been established.[130,137] Although acute glomerulonephritis has been reported as a complication of group C streptococcal pharyngitis, it is extremely unusual.[10,51,123] The primary reason to identify either group C or group G streptococci as the etiologic agent of acute pharyngitis is to initiate antibiotic therapy that may reduce the clinical impact of the illness. No convincing evidence as yet has emerged from controlled studies of a clinical response to antibiotic therapy in patients with acute pharyngitis and either group C or group G streptococci isolated from the upper respiratory tract.

Group G streptococci also have been reported to be an uncommon cause of puerperal sepsis, and they occasionally may cause a neonatal infection that is clinically very similar to early-onset group B streptococcal infections. Other infections occasionally caused by group G streptococci include bacteremia, endocarditis, septic arthritis, osteomyelitis, pneumonia, erysipelas and other skin and soft tissue infections, and meningitis.[55,81] Group G streptococci also have been associated with a toxic shock–like syndrome.[54,162]

TREATMENT

Penicillin is the antibiotic of choice for treating infections caused by either group C or group G streptococci.[139] Some strains of group C and group G streptococci have been shown to be tolerant to penicillin in laboratory studies, but the clinical significance of this finding is unknown.[123,138] Synergism in producing in vitro killing of group C and group G streptococci has been shown with gentamicin and various β-lactam antibiotics, but no controlled trials have been performed to establish the clinical significance of this finding. Group C and group G streptococci also are susceptible to most β-lactam antibiotics and to macrolides, vancomycin, clindamycin, and chloramphenicol. Pharyngitis usually is treated in a manner similar to that for group A streptococcal upper respiratory infections. More severe infection requires parenteral therapy.

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CHAPTER 94

Group B Streptococcal Infections

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HISTORY

The organism we know as group B *Streptococcus*, or *Streptococcus agalactiae*, was isolated first by Nocard in 1887[233] and for decades was recognized as a cause of bovine mastitis[220] but not human infection. Serologic techniques for differentiating beta-hemolytic streptococci were developed by Lancefield,[173] who also described isolation of group B streptococci from parturient women in 1935.[174] In that same year, Congdon[82] included one fatal puerperal case of group B streptococcal sepsis and pneumonia in a report of streptococcal infections associated with childbirth. The significance of this organism as a human pathogen was reported first by Fry[115] in 1938, who described three cases of fatal puerperal sepsis. Group B streptococcal infections continued to be reported sporadically until the 1960s, when maternal and neonatal infections increasingly were ascribed to this pathogen.[68,101,147] In the 1970s, group B *Streptococcus* emerged as the predominant organism causing bacteremia and meningitis in neonates.[12,26,113,150,241] The incidence of neonatal infection remained stable, with reported attack rates ranging from 0.2 to 5.4 per 1000 live births, until the late 1990s, when maternal intrapartum chemoprophylaxis gained wide acceptance and incidence rates fell.[33,75,280] Invasive infection also occurs beyond the neonatal period in pregnant women, nonpregnant adults with underlying medical conditions, and elderly persons.[95,103,235,311]

MICROBIOLOGY

ISOLATION AND IDENTIFICATION

Group B streptococci are facultative gram-positive diplococci that grow on a variety of bacteriologic media. Colonies are 3 to 4 mm in diameter, grayish white, flat, and somewhat mucoid. Colonies are surrounded by a narrow zone of beta-hemolysis that for some strains is detectable only when the colony is lifted from the agar. Nonhemolytic strains account for 1 to 2 percent of isolates and may cause human disease.[12,269] Definitive identification of group B streptococci relies on detection of the group B-specific antigen, a carbohydrate cell wall antigen common to all strains. The standard method, as described by Lancefield,[173] requires acid treatment of the bacteria to solubilize the carbohydrate group B antigen, followed by capillary precipitation with hyperimmune rabbit serum. Several newer methods using hyperimmune antisera have been developed, but latex agglutination is used widely because of the commercial availability of test kits, the ease of performing the assay, and the specificity of the results when organisms in pure culture are tested.[292] Other laboratory methods for presumptive identification include testing for resistance to bacitracin or trimethoprim-sulfamethoxazole, hydrolysis of sodium hippurate broth, failure to hydrolyze bile esculin, production of orange pigment when cultured under certain conditions, and CAMP (Christie, Atkinson, Munch, Peterson) testing. CAMP is an acronym of the names of the authors who first described the production of CAMP factor by group B streptococci in the presence of the beta-toxin of *Staphylococcus aureus* that results in synergistic hemolysis on sheep blood agar.[81]

SEROLOGIC CLASSIFICATION AND ANTIGENIC STRUCTURE

Group B streptococci possess two carbohydrate cell wall antigens, the group B–specific antigen and type-specific capsular polysaccharide. Group B streptococci are classified into serotypes based on type-specific capsular polysaccharides. Nine such polysaccharides are characterized: Ia, Ib, and II to VIII. The

type-specific polysaccharides of group B streptococci are repeating units of five to seven monosaccharides (glucose, galactose, glucosamine, and *N*-acetylneuraminic acid). All the characterized polysaccharides include an *N*-acetylneuraminic acid (sialic acid) residue, which is important in the pathogenesis of type III human infection and perhaps other types.[31,287,325] Despite structural relatedness, antibody directed against the capsular polysaccharide of one type does not provide cross-protection against other capsular polysaccharide types.[175,317,328] A few strains isolated from patients with systemic infection contain type-specific capsular polysaccharide genes by genotypic methods but produce very low levels of capsule or have modified capsular structures that do not react with hyperimmune sera to the characterized capsular polysaccharides.[260] These strains are called *nontypeable*.

Further differentiation of type Ia strains was based on the presence or absence of a protein antigen known as C, which led to the nomenclature of Ia and Ia/c serotypes. The C protein also is present in many other serotypes except type III strains and consists of two components, alpha (trypsin resistant), found in 70 percent of non–type III capsular polysaccharide isolates, and beta (trypsin sensitive), found in approximately 20 percent of isolates.[109,157,202]

Pili, an essential virulence factor in many gram-negative pathogens such as adhesins, was discovered in group B streptococci, and its role in pathogenesis is being studied.[179] Analyses of the group B *Streptococcus* genome sequenced in 2002 will provide further insights into its antigenic structure, genes contributing to its virulence, and targets for treatment.[123,305]

EXTRACELLULAR PRODUCTS

Several bacterial products are elaborated by group B streptococci. Type-specific capsular polysaccharide is released from cells, and the amount elaborated has been correlated with virulence.[169,338] These soluble polysaccharides inhibit opsonophagocytic killing in vitro, thereby providing a mechanism for the documented increase in virulence.[181] Most strains possess C5a-ase, an enzyme of the serine esterase class that inactivates complement component C5a.[144] Because C5a is a potent chemoattractant for neutrophils, this enzyme helps the bacteria to evade the host immune system by hindering the accumulation of neutrophils at the site of infection. The beta-hemolysin elaborated by group B streptococci was characterized in 1980, but only more recently has its role in virulence been explored through the creation of nonhemolytic and hyperhemolytic mutants.[205,322] Expression of beta-hemolysin correlated with tissue damage in an in vivo arthritis model,[256] activation of neutrophil signaling pathways in brain endothelium leading to development of meningitis,[92] and increased virulence in pulmonary infection in rats and rabbits.[140,232] Other bacterial products of group B streptococci, including CAMP factor,[163] lipoteichoic acid,[228,229] pigment,[303] hippuricase, neuraminidase, hyaluronidase,[111,213] and nucleases,[110] have been described, but the contributions of these substances to pathogenesis are not clear.

ANTIMICROBIAL SUSCEPTIBILITY

To date, human isolates of group B streptococci have remained uniformly susceptible to penicillin G. However, approximately 10-fold greater concentrations are required for inhibition and killing of group B streptococci than for group A streptococci. Group B streptococci also are susceptible to other β-lactam agents, cephalosporins, vancomycin, and carbapenems. The prevalence of resistance to the macrolides (erythromycin, clindamycin, clarithromycin) is increasing; from 1970 to 1990, it was reported in 3.4 to 7.4 percent of isolates, but more recent studies reported resistance to erythromycin in 17 to 29 percent of isolates and resistance to clindamycin in 7 to 26 percent.[55,86,107,186] Resistance is related to the presence of *ermTR*, *ermB*, or *mefA* genes.[86] Macrolide resistance is highest in type V strains.[186] Ninety-five percent of strains are resistant to tetracycline, and resistance to bacitracin, nalidixic acid, trimethoprim-sulfamethoxazole, and metronidazole is uniform.[249] Low-level gentamicin resistance is typical, but when gentamicin is combined with either penicillin G or ampicillin, synergistic killing of group B streptococci occurs in vitro and in vivo.[275,276,300]

As many as 5 to 17 percent of group B streptococcal isolates have been reported to be tolerant to penicillin.[48,167] Expression of tolerance requires laboratory conditions that promote a greater than 16-fold discrepancy between minimal inhibitory concentrations and minimal bactericidal concentrations. Tolerant strains are characterized in vitro by delayed penicillin killing, similar rates of killing by penicillin whether growth is exponential or stationary, an additive rather than a synergistic response to the combination of penicillin and gentamicin, and deficient autolysis. The clinical significance, if any, of these laboratory-induced properties remains unknown.[22,166,290]

EPIDEMIOLOGY

MATERNAL COLONIZATION

Asymptomatic colonization occurs at vaginal, rectal, urethral, and pharyngeal sites in one third of healthy young women.[52,217] Much effort has been exerted trying to define groups of women at enhanced risk for colonization with group B streptococci. Factors found to be associated with vaginal acquisition include African-American ethnicity, multiple sex partners, frequent sexual intercourse, male-to-female oral sex, tampon use, and infrequent hand washing.[52,203,217] Women younger than 20 years of age have higher prevalence of colonization.[14,29] Several studies have found significantly higher colonization rates among African Americans,[71,143,217] whereas others found higher rates in Hispanics.[263] Asians have the lowest colonization rates.[71,217]

Maternal vaginal or rectal colonization rates during pregnancy vary from 18 to 35 percent in reported studies.[19,23,59,88,93,212,249,263] These rates relate to the body sites sampled, microbiologic techniques employed, and the period of gestation in which the cultures are performed. Specimens from the distal portion of the vagina yield group B streptococci more frequently than do specimens from the proximal vagina or cervix.[197] Concomitant sampling of the rectal site results in a 10 percent increase in detection over culturing the vaginal site alone.[19,88] Several investigators have suggested the gastrointestinal tract as the principal reservoir for this organism, and, not uncommonly, the rectal site is the only one that yields group B streptococci.[19,88,148,249] The urinary tract is an important site of infection (asymptomatic bacteriuria) because it is a surrogate for a high or "heavy" ($>10^5$ colony-forming units per milliliter) genital inoculum and a marker of increased risk for development of early-onset sepsis in neonates.[225,334] Bacteriuria mandates therapy during pregnancy.[278]

The manner in which swab specimens from the vagina and rectum are processed also is important in accurately assessing colonization.[74] Anogenital culture swabs collected by patients themselves are as accurate as those performed by physicians.[307] Swabs can be placed in transport media at environmental temperatures for as long as 96 hours.[278] Specimens then should be placed in a selective antibiotic-containing broth medium, rather than on solid media, because solid media fail to detect as many as 50 percent of group B streptococcal carriers.[27,30] The addition of antibiotics to the broth limits the growth of competing flora, especially gram-negative enteric organisms, and enhances detection.[23,27,30,209] Todd-Hewitt broth with gentamicin and nalidixic

acid (selective broth medium)[27] or with colistin (Lim broth)[185] and nalidixic acid is recommended for detection of group B streptococcal colonization.[278] After overnight incubation, the broth is subcultured onto a 5 percent sheep blood agar plate and is processed conventionally.

Ninety-two percent of culture-positive women are identified if lower vaginal and rectal cultures are obtained at a single visit with correct laboratory detection methods.[94] The proximity to delivery affects the accuracy of predicting colonization at delivery. Cultures obtained at 35 and 37 weeks' gestation predict colonization at delivery with 87 percent sensitivity and 96 percent specificity.[59,248,337]

INFANT COLONIZATION

Colonization and infection in the neonate are associated significantly with the presence of group B streptococci in the maternal genital tract at delivery. Vertical transmission from colonized mothers to their infants occurs in 41 to 72 percent of cases (mean, ~50%).[7,15,23,57,93,143,212,340] Only 1 to 12 percent (mean, ~5%) of colonized infants are born to noncolonized mothers.[15,23,93,212,340] Acquisition is presumed to occur either by the ascending route through ruptured membranes or from contact with the organism in the genital tract during parturition. Heavy maternal inocula in the genital tract (>10^5 colony-forming units per milliliter) greatly increases the rates of vertical transmission to neonates and rates of heavy infant colonization.[9,15,59,148,159] Other factors associated with increased colonization rates in infants include prolonged rupture of membranes (>12 to 18 hours)[15,143] and vaginal delivery.[143] Maternal intrapartum antibiotic therapy substantially diminishes the vertical transmission of group B streptococci.[7,57,60,143,341] The body sites in neonates most likely to yield group B *Streptococcus* are the rectum and throat, findings reflecting replication of organisms at the respiratory or gastrointestinal tract sites after the ingestion of infected amniotic fluid or genital secretions.[143]

Horizontal transmission from nosocomial and community sources has been described.[1,93,118,234] Infant-to-infant or colonized staff member–to-infant spread may occur through hands of hospital personnel, but epidemics are unusual.[93,234] The rate of community acquisition, presumably by an oral-fecal route, appears to be low.[118,285] Breast milk also has been proposed as a mode of transmission for late-onset infection; most reported cases involved a mother with postpartum mastitis.[50,171,265]

INCIDENCE OF DISEASE

Before the widespread use of maternal intrapartum chemoprophylaxis to prevent early-onset disease, reported attack rates for group B streptococcal disease in infants ranged from 1.8 to 4.0 per 1000 live births.[235,323,343] Early-onset disease (onset within the first week of life) accounted for approximately 80 percent of cases.[280] Late-onset disease (onset between 7 days and 3 months of life) rates ranged from 0.3 to 1.8 per 1000 live births.[89,241] With the 2002 guidelines for universal screening of pregnant women at 35 to 37 weeks' gestation and administering of intrapartum chemoprophylaxis to carriers, the incidence of early-onset disease decreased to 0.3 cases per 1000 live births in 2004, a finding representing a decline of 81 percent from 1990.[75,280] However, the incidence of late-onset disease has remained unchanged.[75] Approximately 30 percent of cases of early-onset disease and up to 55 percent of those of late-onset and late, late-onset disease now occur in preterm infants.[188,280,283] Late, late-onset group B streptococcal disease occurs in infants older than 3 months of age and accounts for 7 to 13 percent of childhood group B streptococcal infections.[151,280,336] Affected infants typically were born before 34 weeks' gestation or have an underlying immunodeficiency or concomitant infection with human immunodeficiency virus (HIV).[87,151,336] Case-fatality rates for all infants with group B streptococcal disease have dropped from 46 percent in the 1970s to 6.5 percent in 2004.[75,156]

Group B streptococcal disease also is a common finding in pregnant women, with clinical manifestations that include urinary tract infection (usually asymptomatic bacteriuria but also cystitis or pyelonephritis), intra-amniotic infection, postpartum endometritis (often with bacteremia), and puerperal sepsis.[240,283,342] Occasionally, meningitis, septic thrombophlebitis, and other serious complications occur.[342] Attack rates of 2 per 1000 deliveries have been reported, and 28 percent of maternal cases are associated with pregnancy loss or birth of an infant with early-onset group B streptococcal disease.[240,342] Nonpregnant women and men account for 68 percent of invasive group B streptococcal disease in adults.[103] These patients typically are more than 65 years old or have underlying medical conditions, including diabetes mellitus, malignant disease (especially breast cancer), HIV infection, liver disease, stroke and other neurologic disorders, decubitus ulcers, and neurogenic bladder.[103,154,235,311]

RISK FACTORS FOR INFANT DISEASE

Vertical transmission is a prerequisite for the development of invasive, early-onset infection.[23,211,241] Evidence suggests that approximately 50 percent of late-onset infections also occur after vertical transmission.[89] The degree of colonization or inoculum also increases the likelihood of infant disease; heavily colonized mothers are more likely to have infants with invasive infection, and heavily colonized infants are more likely to have either early- or late-onset disease.[59,89,159,184] Other maternal factors associated with the development of early-onset disease include delivery before 37 weeks' gestation, premature rupture of membranes (rupture of membranes before onset of labor) or rupture of membranes more than 18 hours before delivery at any gestation, and intrapartum fever.[12,58,59,89,113,241] Women colonized with group B *Streptococcus* also have a higher incidence of premature rupture of membranes and preterm labor, a finding suggesting a causal relationship between group B streptococci and events leading to preterm birth.[6,210,214,262] Other maternal factors associated with an increased attack rate of early-onset infection are African-American ethnicity, age younger than 20 years, history of previous fetal loss, history of urinary tract infection with group B streptococci, and primiparity.[282,283] Late-onset disease also is associated with young maternal age and African-American ethnicity.[283] Siblings of infected infants who are the product of a multiple pregnancy (e.g., twins, triplets) have enhanced risk for development of early- and late-onset group B streptococcal disease.[98,242]

CAPSULAR POLYSACCHARIDE TYPES CAUSING DISEASE

Reports from the 1970s indicated that the group B streptococcal capsular polysaccharide types colonizing pregnant women and neonates were divided fairly evenly among types I, II, and III.[24,327,329] The current distribution of capsule polysaccharide types in invasive disease from multiple North American studies [84,134,187,324,342] is shown in Figure 94–1. Serotypes Ia and III are the most common cause of early-onset infection, whereas serotype III is the most common cause of late-onset infection. In Europe, type III predominates in both early- and late-onset disease.[112,308,324] Serotype III is implicated in 80 to 93 percent of meningitis cases, either as a focus of early-onset disease or as a manifestation of late-onset infection (80 to 93%).[24,26,329] Capsular polysaccharide type V emerged in the 1990s.[53,71,143,266] Type V causes both early- and late-onset infant disease and is the

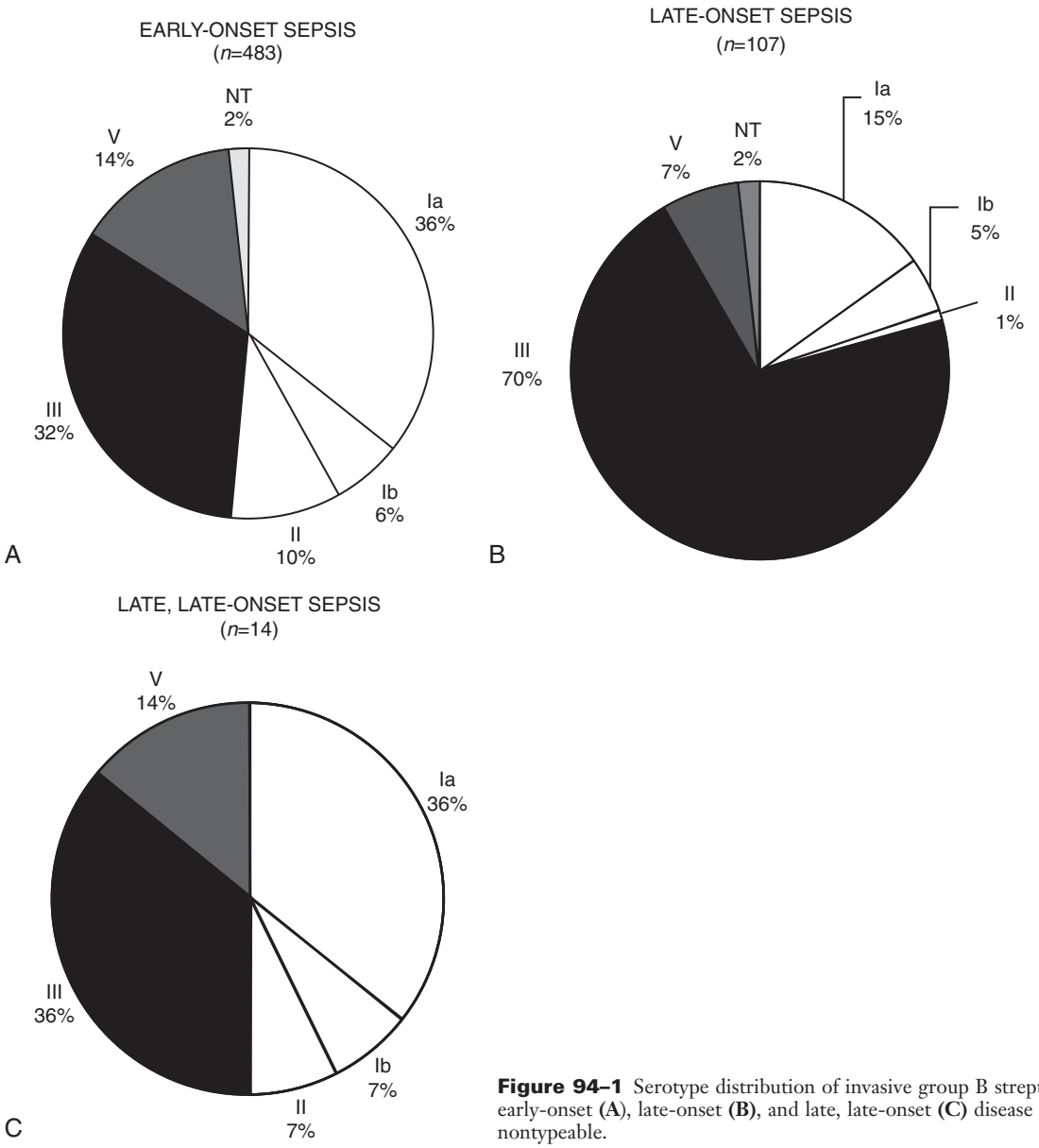

Figure 94–1 Serotype distribution of invasive group B streptococcal strains causing early-onset **(A)**, late-onset **(B)**, and late, late-onset **(C)** disease in infants.[53,84,134,187,342] NT, nontypeable.

type most commonly isolated from adults with invasive disease.[53,266] In Japan, types VI and VIII are the most common isolates from pregnant women.[172] The least common serotypes causing human disease are types Ib/c, IV, VI, VII, and VIII.

PATHOGENESIS

For pediatricians, group B streptococcal disease predominantly afflicts neonates, a predilection that results from a unique combination of maternal, bacterial, and host factors. Figure 94–2 shows a simplified flow chart of group B streptococcal transmission. First, an organism carried by an asymptomatically colonized mother is transmitted vertically to her neonate. Evidence suggests that this transmission may occur in utero shortly before birth or during parturition. This continuum in time of acquisition is reflected in the time of onset of symptoms; infected neonates often are ill at or within 12 hours of birth (≤90% of cases), whereas others may not have evidence of disease for a few days.[26,283] The organism successfully colonizes approximately 50 percent of all infants born to group B streptococcal carriers, yet disease develops in only 1 to 2 percent of these infants.[12,281,325] A breach in the delicate interplay of maternal, infant, and bacterial factors allows the organism to invade. Once invasion occurs, a combination of host defenses and therapeutic interventions may halt the progression of disease, or the infant's defenses may fail and the disease may progress and result in tissue damage or death.

MATERNAL FACTORS

The bacterial inoculum in the maternal genital tract determines the likelihood that the organism will be transmitted vertically. Infants born to heavily colonized women are more likely to be colonized themselves and are more likely to have early-onset disease.[9,15,59,89,148,159] Infants delivered before 37 weeks of gestation have an increased risk for development of early-onset disease. Heavy maternal colonization may induce preterm delivery,[211,262] and disease is more likely to develop in the infant in this setting

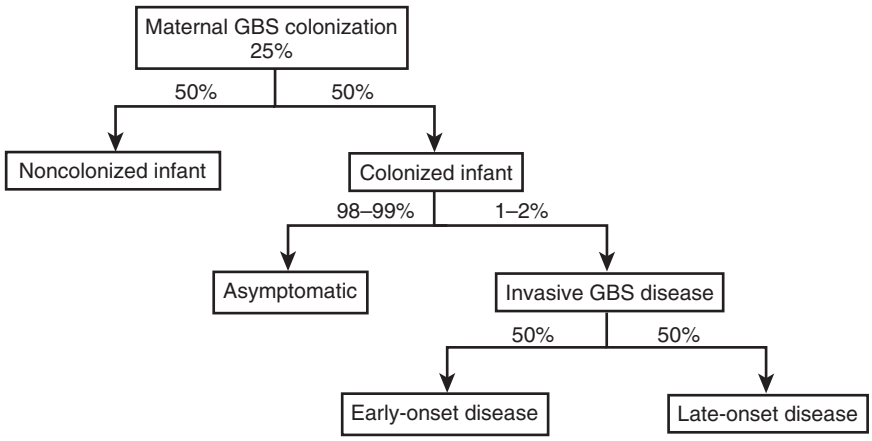

Figure 94–2 Flow chart of vertical transmission and disease manifestation of group B streptococcal disease in the era of intrapartum chemoprophylaxis.

of high bacterial inoculum and immunologic immaturity. Invasive disease also has been reported in term neonates delivered by elective cesarean section with intact membranes, but this is a rare event.[12,101,240]

In addition to bacterial inoculum, another critical maternal factor is the concentration of serum antibody to the serotype-specific polysaccharide capsule of the colonizing strain of group B *Streptococcus* at delivery. Antibody to the type-specific capsular polysaccharide is protective against the homologous serotype in the mouse model of lethal infection; antibody to the group B polysaccharide is not.[176] Baker and associates[28,32] determined that invasive disease with type III group B streptococci occurred primarily in infants born to women with low serum concentrations of antibodies directed against the type III capsular polysaccharide. Several other investigators also reported a correlation between low concentrations of antibody to types Ia, Ib, II, and III capsular polysaccharide in maternal delivery sera and the occurrence of early- and late-onset group B streptococcal infant disease.[125,127] A correlation between antibody concentration and maternal age may explain the epidemiologic association of young maternal age and increased likelihood of neonatal disease.[13] Animal models have demonstrated that antibody directed against the alpha or beta determinants of C protein is protective,[176,218] but definitive human studies are lacking. Invasive disease, but not colonization, elicits immunoglobulin M (IgM) and IgG antibodies to beta C protein in humans.[236] Antibody to other protein determinants in some serotypes has been postulated to be partially protective. Titers of antibody to R protein were found to be higher in maternal serum from mothers colonized with R protein–bearing strains whose infants were healthy than from mothers whose infants were ill; such antibodies were protective in a mouse model against type II, but not type III strains.[189,190] Presence of maternal antibody to Rib also was associated with protection in neonates against Rib-expressing group B streptococcal strains.[178]

BACTERIAL FACTORS

To colonize the genital tract or cause disease effectively, the organisms must be able to adhere to host tissue. Group B streptococci adhere well to many types of epithelium, including vaginal epithelium and chorioamnionic membranes.[116,196,293,302] Type III strains adhere more avidly to vaginal cells than do other serotypes in vitro.[56] Furthermore, invasive strains adhere better to epithelial tissue than colonizing strains do.[138] This capacity to adhere to and possibly invade chorioamnionic cells may explain the association of group B streptococcal colonization with preterm labor and premature rupture of membranes.[145] If a breach occurs in the membranes, the bacteria can multiply in amniotic fluid.[139] After transmission and colonization occur, the capacity of the bacteria to adhere to neonatal epithelial cells may allow them to invade and disseminate. The lung, after aspiration of infected maternal fluids, is a frequent initial site of infection in a newborn with early-onset disease. Group B streptococci can adhere to and invade respiratory epithelial cell lines.[272] In addition, they can invade endothelial cells, which may be a mechanism for some of the pathologic features of disseminated disease.[121]

Several different factors may confer adherence properties of group B streptococci including bacterial cell wall component lipoteichoic acid[229] or surface proteins.[67,302] One study showed that translocation across epithelial barriers was mediated by alpha C protein binding to host cell surface glycosaminoglycan.[42] Several proteins with potential roles in adherence and invasion were identified by detecting up-regulation during cell invasion using proteomics and include an undefined surface antigen, penicillin-binding protein 2b, glyceraldehydes-3-phosphate dehydrogenase, and an iron-binding protein.[158] Mouse antisera to these five proteins inhibited binding of group B streptococci to cervical epithelial cells. Translocation also may occur by a paracellular route.[296] Adherence to and invasion of the microvascular endothelial cells of the blood-brain barrier has been associated with proper anchoring of lipoteichoic acid,[91] beta-hemolysin/cytolysin,[92] fibrinogen binding protein FbsA,[304] and the newly discovered pilus proteins.[199]

A well-defined virulence structure of group B streptococci is the capsular polysaccharide. Mouse virulent strains are able to synthesize greater amounts of surface-bound type-specific polysaccharide than avirulent strains.[169,338] An unencapsulated mutant, created by inserting a transposon into the gene regulating capsule expression, has significantly less virulence in neonatal rats than does the parent type III strain.[273] As with other encapsulated organisms, the capsule is thought to confer virulence primarily by interfering with opsonophagocytosis.[181] In vitro, the capsule of type III group B streptococci has been shown to prevent the deposition of C3,[207] and the presence of the terminal sialic acid residue on the repeating unit of the polysaccharide is crucial to this interference.[99] Removal of sialic acid residues from the polysaccharide leads to diminished virulence, and a desialylated mutant loses virulence when compared with the parent strain.[326] Furthermore, the presence of this sialic acid moiety prevents activation of the alternative complement pathway, the predominant pathway used by the human host when minimal type-specific antibody is present.[99] In addition, when bacteria grow in the presence of human serum, the quantity of sialic acid increases, thereby potentiating its contribution to virulence.[244] Opsonization may be affected by cell surface components other than capsular sialic acid. C protein also lends relative resistance to

opsonization to strains bearing the antigen.[157,244] Beta-hemolysin/cytolysin contributes to cytolysis and apoptosis of phagocyte while protecting group B streptococci by the linked carotenoid pigment against oxidative damage.[194]

Sialic acid residues on the capsule may interfere with another component of the immune system. In a serum-free system, the desialylated mutant of type III group B streptococci elicited much larger quantities of leukotriene B_4 from macrophages than did the parent strain.[270] Leukotriene B_4 is a potent neutrophil chemoattractant, so this effect may result in diminished influx of effector cells. Similarly, C5a-ase may disable C5a, another host product capable of eliciting neutrophil influx. By enhancing phagocytosis and killing of group B streptococci, C5a also has direct stimulatory effects on neutrophils.[301] Bacterial elaboration of C5a-ase may affect both the accumulation of neutrophils and the efficiency of neutrophil function.

Once invasive infection is established, ongoing replication and digestion of bacteria can instigate host inflammatory responses that may be deleterious. Neonates recovering from group B streptococcal disease have circulating immune complexes for a prolonged period of time, and immune complexes can contribute to end-organ damage.[312] Additionally, immune complexes containing group B streptococcal components elicit inflammatory mediators such as leukotriene B_4 and interleukin-6 (IL-6). The cytokine response to gram-positive pathogens is not delineated as clearly as is that to gram-negative bacteria, partly because of the greater heterogeneity in structure of the latter.[54] Both group B and type III capsular polysaccharides induce the release of IL-6 from monocytes[296]; group B antigen also causes the release of tumor necrosis factor-α (TNF-α).[314] As with other gram-positive pathogens, the cell walls of group B streptococci contain peptidoglycan and lipoteichoic acid. Peptidoglycans from other gram-positive organisms elicit a variety of proinflammatory cytokines such as TNF-α, IL-1, IL-6, and granulocyte colony-stimulating factor.[90,142,264] Lipoteichoic acid also induces the release of IL-1β, IL-6, and TNF-α.[49,164] Group B streptococcal cell wall components likewise exert similar effects. Elaboration of these cytokines has been implicated in the clinical and hemodynamic effects of sepsis.[54] Specifically, blockade of IL-1 activity by administration of an IL-1 receptor antagonist in a piglet model of group B streptococcal sepsis ameliorated systemic hypotension and prolonged survival.[315]

INFANT HOST FACTORS

Neonates have several domains of immune dysfunction that affect their ability to mount a sufficient defense against group B streptococci. Neutrophils are the primary effector cell in host defense against extracellular bacterial pathogens, and neutrophils from neonates have many functional abnormalities.[182] The generation of chemotactic activity in neonatal serum in response to group B streptococci is diminished, as is the release of chemotactic factors by neonatal monocytes.[10,11,271] Thus, the diminished ability of neutrophils to migrate is amplified by a diminished level of chemotactic stimulation. Phagocytosis and bacterial killing also are impaired when opsonic activity is poor, as is the usual pattern in neonates with sepsis.[182] The neutrophil storage pools of neonates rapidly become depleted during invasive infection, thereby leading to profound neutropenia,[80] an ominous prognostic indicator.[245] These defects in neonatal neutrophils are even more pronounced in infants born prematurely.[182]

Cells of the monocyte/macrophage lineage also may play a role in host defense against group B streptococci, especially in the lungs, where the alveolar macrophage is the first effector cell to encounter pathogens. Defects in the functions of these cells have been described in neonates. Cord blood monocytes have impaired phagocytosis and killing of group B streptococci.[206] In animal models, oxidative metabolism, bacterial uptake, and migration to the site of infection by neonatal macrophages are diminished in response to group B streptococci.[208,284,286]

Humoral immunity is compromised in neonates. Both the alternative and classic complement pathways are affected, and preterm neonates are impaired more severely than are term neonates.[85,97] Both pathways are important in the opsonization of group B streptococci.[33] The concentration of type-specific antibody passively acquired from the mother is an important protective factor. Because passive transfer of IgG increases dramatically during the final 8 weeks of pregnancy, premature neonates are again at a disadvantage because they may not receive sufficient amounts of antibodies if they are born before protective levels are transferred.[79]

The pattern of production of cytokines by neonatal immune cells frequently is altered when compared with that of adults. For example, in response to group B streptococci, the level of TNF-α and IL-6[313] released by neonatal monocytes is increased relative to adults,[314,330] and IL-6[313] but levels of IL-8 and leukotriene B_4 are decreased.[271,277] IL-12 and interferon-γ are associated with improved outcome in an animal model of group B streptococcal infection, but lower quantities of these mediators are released by neonatal mononuclear cells.[162,201] Cytokine networks are important both in the manifestations of sepsis and in the stimulation of an appropriate immune response, so alterations in expression of cytokines may affect the neonate's response to this pathogen both clinically and immunologically.

CLINICAL MANIFESTATIONS

EARLY-ONSET DISEASE

Group B *Streptococcus* remains the leading cause of neonatal sepsis, and it accounts for 40 to 50 percent of early-onset sepsis cases.[40,51,152] The bimodal distribution of group B streptococcal infections corresponding to age of neonates and young infants was described first in 1973 and now is divided into two types, termed *early-* and *late-onset disease*.[26,113] The syndromes of early- and late-onset disease differ in epidemiologic characteristics, pathogenesis, clinical findings, and prognosis. Their major clinical features are detailed in Table 94–1. Early-onset disease is defined as onset of infection in the first 6 days of life, but most neonates (61-95%) become ill within the first 24 hours of life (median, 1 hour).[58,64,283] Premature infants often are ill at birth or within 6 hours of birth and account for 17 to 32 percent of neonates with early-onset group B streptococcal disease.[20,75,257,280,283] In very-low-birth-weight infants, group B *Streptococcus* has dropped to the second most common cause of early-onset sepsis, after *Escherichia coli*, since the implementation of intrapartum antibiotic chemoprophylaxis.[298]

Early-onset disease often occurs in the setting of maternal complications, including labor before 37 weeks' gestation, prolonged rupture of membranes for more than 12 hours, intrapartum fever greater than 100.4° F, chorioamnionitis, and early postpartum febrile morbidity.[25,45,58,59,89,323] In one study, infants born to a mother with one or more of these risk factors were 12.5 times more likely to develop early-onset group B streptococcal disease compared with those born to a mother with no risk factors.[58] Term infants can develop group B streptococcal disease without definable maternal risk factors except vaginal/rectal colonization.[33] In another study, 76 percent of mothers of infants with early-onset disease had deliveries complicated by at least one of the foregoing identifiable risk factor or was known to be a group B *Streptococcus* carrier.[257]

The three most frequent clinical manifestations are bacteremia without a focus, pneumonia, and meningitis.[12,323,336] The

TABLE 94–1 Features of Group B Streptococcal Disease in Infants

Feature (References)	Early-Onset Disease	Late-Onset Disease	Late, Late-Onset Disease
Age at onset (58, 64, 283)	≤6 days; mean, 8 hr; median, 1 hr	7-89 days; mean, 36 days; median, 27 days	≥90 days
Infants affected (75, 132, 283, 323)	Premature neonates, births after maternal obstetric complications	Term infants predominate	Premature neonates <32 wk gestation, immunodeficiency
Clinical findings (33)	Acute respiratory distress, apnea, and hypotension common	Fever, irritability, nonspecific signs, occasionally fulminant	Fever, irritability, nonspecific signs
Manifestations (12, 21, 96, 113, 323, 336)	Bacteremia (40-55%), pneumonia (30-45%), meningitis (6-15%)	Bacteremia without a focus (55%), meningitis (35%), osteoarthritis (~5%), cellulitis/ adenitis (~2%)	Bacteremia without a focus, focal infections as in late-onset disease
Case-fatality rate (75, 280, 283, 323, 336)	5-15%	2-6%	<5%

presentation for each may range from shock and respiratory failure at delivery to a healthy infant who has infection detected during evaluation because of maternal risk factors known to increase the risk for development of invasive infection.[257,323] Respiratory distress, including apnea, grunting respirations, tachypnea, and cyanosis, is the most common initial manifestation in all forms of early-onset group B streptococcal disease.[20,26,113,316,336] Other signs include lethargy, poor feeding, abdominal distention, pallor, jaundice, tachycardia, and hypotension. Fever usually is present in term neonates, but preterm infants often are normothermic or hypothermic.[323] Administration of intrapartum antibiotic prophylaxis to the mother does not alter the findings in infants, in whom early-onset disease develops despite administration of prophylaxis; most still become clinically ill in the first 24 hours of life.[40,64]

Bacteremia without a focus is present in 27 to 87 percent of neonates with early-onset disease.[283,323,336] Signs of septicemia, such as respiratory distress and poor perfusion, often are present, especially in neonates born at less than 37 weeks' gestation.[323] Bacteremia in healthy-appearing term infants also may develop, and in one series it occurred in 22 percent of cases.[150,323,336] These infants probably remained healthy because of early evaluation and institution of empiric antimicrobial therapy.[323] More often, bacteremic infants are mildly ill and have fever and nonspecific signs that should prompt bacteriologic evaluation. Bacteremia also frequently accompanies pneumonia and meningitis.

Radiographic findings of pulmonary involvement include infiltrates suggestive of congenital pneumonia, small pleural effusions, a pattern similar to that of hyaline membrane disease or respiratory distress syndrome, and increased vascular markings as seen in transient tachypnea of the newborn; radiographs also may appear normal despite abnormal pulmonary signs.[183,192,316] In preterm neonates, the findings frequently are identical to those of respiratory distress syndrome, and at autopsy, hyaline membranes containing bacteria and minimal inflammatory infiltrates have been described.[2,316]

Meningitis is documented in 6 to 15 percent of neonates with early-onset disease (Fig. 94–3).[283,323,332] As a rule, no signs specifically indicate its presence; this situation underscores the need to evaluate all neonates with presumed early-onset disease for the possibility of meningeal involvement.[323,332] Early in the course, the cerebrospinal fluid (CSF) white blood cell count may be normal despite isolation of group B streptococci.[14] Again, respiratory distress is the most frequent clinical finding.[26,323] Seizures rarely are the initial feature but do occur in as many as 50 percent of affected neonates early in the course of therapy; often these seizures are focal and subtle.[26] Postmortem evaluation of infants who die of early-onset group B streptococcal meningitis reveals hemorrhage, prominent basilar involvement, and abundant bacteria with relatively sparse inflammation.[113]

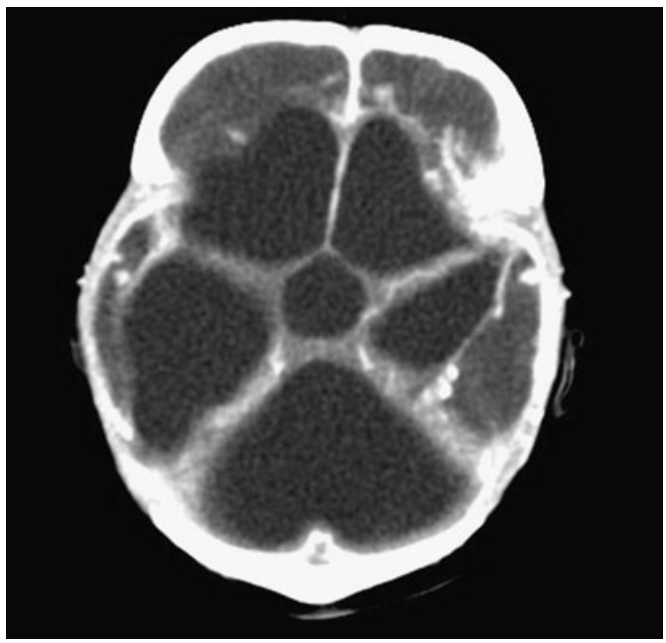

Figure 94–3 Head computed tomography scan at 6 weeks of age from a term infant with early-onset group B streptococcal (GBS) meningitis shows significant loss of normal brain matter.

LATE-ONSET DISEASE

Late-onset disease is defined as infection in infants 7 to 90 days of age, and it primarily affects term infants with an unremarkable maternal history and early neonatal course.[26,89] However, more recent case series suggested that late-onset infection develops in a larger proportion of infants born quite prematurely (<34 weeks' gestation).[188] Bacteremia without a focus and meningitis are the two most common manifestations of late-onset disease.[113,117,336] Osteoarticular infections and cellulitis are additional clinical foci.[21,96,336] Whereas early-onset disease most frequently occurs acutely with apnea and hypotension, late-onset disease often is manifested by fever, irritability, and other nonspecific signs.[33] More fulminant, rapidly progressive cases do occur, however.[26]

Meningitis is a frequent complication, occurring in 35 to 40 percent of late-onset disease cases.[26,323,336] Late-onset meningitis most typically manifests with fever and lethargy, although respiratory distress, coma, and shock also may occur.[26] Classic signs of meningitis, such as a bulging fontanelle and nuchal rigidity, occur more commonly in neonates with late-onset than early-onset meningitis. Subdural effusions develop in as many as 20 percent of cases, but subdural empyema is a rare occurrence.[12,26,242] Infants who die of late-onset meningitis have purulent leptomeningitis at postmortem examination.[113]

Sequelae occur in 20 to 30 percent of survivors of early- and late-onset meningitis. These sequelae include mental retardation, spastic quadriplegia, cortical blindness, deafness, uncontrolled seizures, hydrocephalus, and speech and language delay.[100,318] Signs at admission that are correlated with a high likelihood of a poor outcome include hypotension, coma or semicoma, status epilepticus, neutropenia, CSF protein levels greater than 300 mg/dL, and high concentrations of bacterial capsular polysaccharide antigen in CSF.[39,100,245]

LATE, LATE-ONSET DISEASE

Contemporary studies show increasing incidence of group B streptococcal infection in infants older than 3 months of age, defined as late, late-onset disease.[151,280,336] These infections tend to occur in infants who were born extremely prematurely and whose corrected age is less than 3 months.[151,280,336] Late, late-onset disease also occurs in children co-infected with HIV and those with immunodeficiencies,[87,151,336] a finding supporting the recommendation that immunodeficiency be considered in any child infected beyond the usual period of risk.[87] The clinical manifestations in these older infants are similar to those in patients with typical late-onset infection; bacteremia without a focus and meningitis are the most common clinical features.[87,151] Endocarditis and central venous catheter infection also have been noted.[151]

SEPTIC ARTHRITIS AND OSTEOMYELITIS

Osteoarthritis occurs in approximately 5 percent of infants with late-onset infection.[336] Among neonates and young infants with osteomyelitis, group B streptococci have been identified as the causal agent in 7 to 38 percent of cases.[96,333] The osteoarticular disease usually is more indolent than is the osteomyelitis caused by other etiologic agents in young infants. Diagnosis usually is established after a mean of 9 days of findings at a mean age of 31 days.[33,96] Decreased motion of the involved extremity and pain with manipulation are common signs. Warmth and redness are uncommon findings but have been described.[96] Systemic signs and symptoms, including fever, are unusual manifestations.[96,214]

Group B *Streptococcus* has a predilection for the proximal humerus.[96] The femur is the second most commonly affected bone. Involvement of more than one bone is an unusual finding.[96] However, involvement of the adjacent joint is described.[214] Blood cultures infrequently are positive, unlike neonatal osteomyelitis caused by other etiologic agents. A lytic lesion often is seen on radiographs at first evaluation (Fig. 94–4). This observation suggests that lytic lesions may be a late phenomenon that develops after seeding of the metaphysis during an episode of asymptomatic, early-onset bacteremia several weeks before presentation.[33]

The mean age at diagnosis in infants who have septic arthritis without osteomyelitis is 20 days. The clinical picture typically is acute, with a mean of 2 days of abnormal findings before diagnosis is established. The lower extremities are involved most often, with the hip joint predominating. Infants typically present with local joint signs in the absence of systemic symptoms.[215]

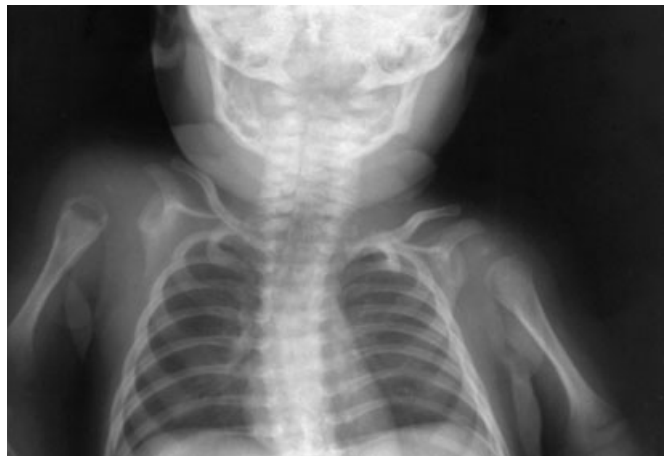

Figure 94–4 Radiograph of the right arm of a 26-day-old infant with a 2-day history of diminished movement of that extremity. A well-defined, lytic lesion is present in the proximal end of the humerus. Necrotic material from this area was débrided surgically and grew group B streptococci on culture. *(Courtesy of Morven S. Edwards, M.D.)*

CELLULITIS/ADENITIS

In one case series, 2 percent of late-onset group B streptococcal infections were associated with cellulitis/adenitis syndrome.[336] Diagnosis is made at a mean age of 5 weeks, with a preponderance of cases in male patients.[21] Typical presentation includes fever, irritability, poor feeding, and swelling of the affected soft tissue area. Enlarged adjacent lymph nodes become palpable within a few days. The most frequent sites of cellulitis are the face and neck,[21,136,243] with enlarged lymph nodes in the submandibular area. Ipsilateral otitis media was noted in four of five infants with facial or submandibular cellulitis in one case series.[139] Less commonly affected areas reported include the genital or inguinal region (Fig. 94–5), the hand, and the prepatellar bursa.[21,33,61] Aspiration of the affected area of cellulitis often yields group B streptococci, and concomitant bacteremia almost always is present.

OTHER MANIFESTATIONS

Nearly every organ has been reported to be a site of group B streptococcal infection in young infants. Table 94–2 lists these reported unusual manifestations of early- and late-onset disease.

RECURRENT INFECTIONS

Second (and sometimes third) episodes of group B streptococcal infection occur in approximately 1 to 2 percent of cases after early- and late-onset infection.[128] In a case series from Houston, the mean age at recurrence was 44 days, and the mean duration between the first course of therapy and recurrence was 19 days.[128] Molecular epidemiologic techniques have indicated that most, but not all, of these recurrent infections are caused by the strain implicated in the first episode; this finding suggests that persistent mucosal colonization after treatment of the first episode is followed by invasion of the bloodstream.[227] Contaminated breast

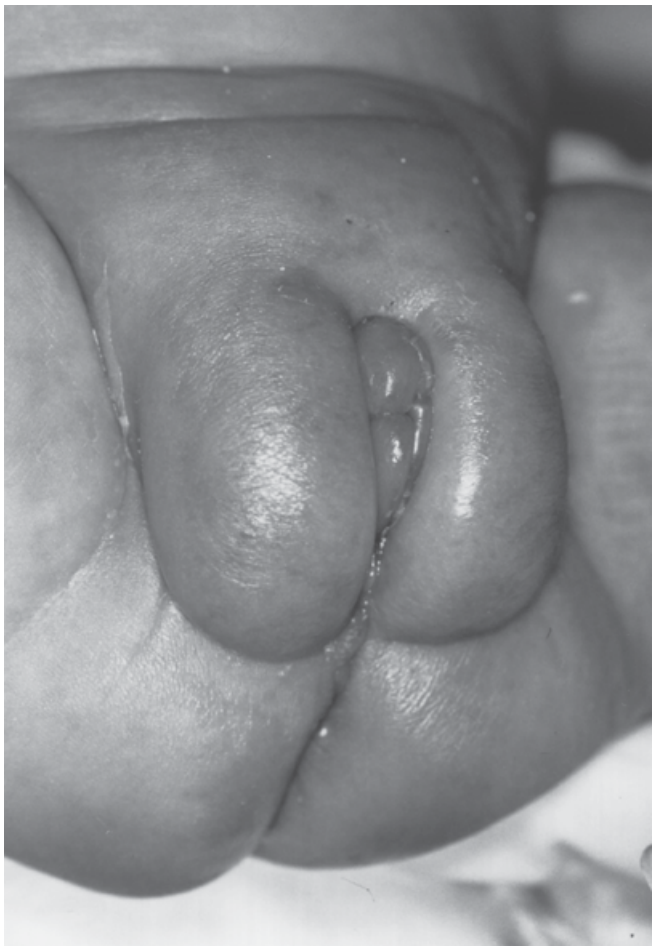

Figure 94–5 This 3-month-old female infant who was born after 28 weeks' gestation had fever, lethargy, swelling of the external genitalia, and erythema that extended to the lower part of the abdomen and the thigh. Blood culture grew type Ib/c group B streptococci. *(Courtesy of Morven S. Edwards, M.D.)*

TABLE 94–2 Unusual Clinical Manifestations of Group B Streptococcal Disease in Infants and Children

Site and Manifestation	References
Brain	
Abscess	290
Cerebritis	168
Chronic meningitis	295
Diabetes insipidus	198
Eosinophilic meningitis	221
Subdural empyema	106
Ventriculitis	222
Eye	
Conjunctivitis	16, 113, 254
Endophthalmitis	129, 180
Ear and Sinus	
Ethmoiditis	150
Otitis media/mastoiditis	21, 274, 288
Cardiovascular/Hematologic	
Asymptomatic bacteremia	117, 150, 261
Endocarditis	8, 43, 149, 234, 321
Myocarditis	44
Mycotic aneurysm	3
Pericarditis	133
Respiratory Tract	
Epiglottitis	339
Supraglottitis	191
Tracheitis	240
Pleural empyema	150, 294
Skin and Soft Tissue	
Breast abscess	230, 265
Bursitis	63
Cellulitis/adenitis	21, 136, 150, 177, 243, 331
Dactylitis	114
Fasciitis	124, 259
Impetigo neonatorum	46, 170, 193
Purpura fulminans	153, 195
Omphalitis	47, 155, 336
Rhabdomyolysis	310
Scalp abscess	104, 132
Abdomen	
Adrenal abscess	18, 72, 319
Delayed-onset diaphragmatic hernia	17, 41, 251, 299
Gallbladder distention	246
Peritonitis	70, 76
Urinary Tract	
Renal abscess	319, 335
Urinary tract infection	297, 336

Adapted from Baker, C. J., Nizet V, and Edwards, M. S.: Group B streptococcal infections. In *Remington, J. S., and Klein, J. O. (eds.): Infectious Diseases of the Fetus and Newborn Infant. 6th ed. Philadelphia, W. B. Saunders, 2005, pp. 403-464.*

milk also has been implicated as a source of recurrent infection.[171] No specific risk factors are evident in these infants, but most of them are born prematurely.[128]

DIAGNOSIS AND DIFFERENTIAL DIAGNOSIS

LABORATORY STUDIES

The diagnosis of group B streptococcal infection is confirmed by isolation of this pathogen from a normally sterile body site including blood, CSF, pleural fluid, bone aspirate, joint fluid, or soft tissue. Cultures of the skin, umbilicus, or mucous membranes do not have clinical significance. Tracheal aspirate cultures that grow group B streptococci indicate neonatal colonization but do not prove pulmonary invasion (pneumonia). Rather, isolation of the organism from blood (or in rare circumstances, lung tissue or the pleural space) is required.

Meningitis in early-onset disease is clinically indistinguishable from bacteremia without a focus, and 10 to 38 percent of neonates with meningitis have negative blood culture results,[332] so lumbar puncture is necessary to determine the presence or absence of meningeal involvement. Lumbar punctures also are indicated in patients with a focal site of group B streptococcal infection resulting from its propensity for involving the CNS. For example, group B streptococci grew from CSF cultures in 24 percent of infants with cellulitis/adenitis who underwent lumbar puncture.[5] One study found that if selected criteria were used instead of routine lumbar punctures, the diagnosis of bacterial meningitis would have been missed or delayed in 17 percent of infants.[332]

Several methods for detecting group B polysaccharide antigen in body fluid specimens have been developed. These techniques include countercurrent immunoelectrophoresis, latex particle agglutination, and enzyme immunoassay.[39,131,226,247,267]

The advantages of these methods are their simplicity, rapidity, and ability to detect antigen, even after cultures are rendered sterile by antimicrobial therapy. The disadvantage is the frequency of false-positive results, especially when they are used to "screen" asymptomatic infants for sepsis.[247] Studies also have shown false-negative results from specimens from infants with subsequent death from group B streptococcal disease.[231]

CSF has had detectable group B antigen in 72 to 89 percent of neonates with meningitis; serum is much less likely to be positive. The only specimens recommended for antigen testing are blood and CSF; urine specimens should not be tested because they are unreliable. Antigen testing should not be used as a substitute for bacterial culture. Their proper use should be limited to the setting of symptomatic infants in whom rapid diagnosis may alter therapy or allow proper tailoring of antibiotic choice, for example, a sick neonate with CSF pleocytosis and previous antibiotic administration. These tests are never useful in infants who appear healthy.

A limited study of real-time polymerase chain reaction (PCR) to detect DNA in blood of eight neonates with culture-proven group B streptococcal sepsis showed positive corresponding results.[160] However, larger, prospective studies are required to evaluate sensitivity and specificity.

The white blood cell count of a neonate with proven group B streptococcal sepsis may reflect leukopenia, neutropenia, or leukocytosis.[204,245,316] Manroe and associates[204] found the ratio of absolute immature neutrophils to absolute total neutrophils (I:T index) to be the most reliable index for distinguishing respiratory distress caused by group B streptococcal infection from that with a noninfectious origin. Most infected neonates had an elevation greater than 0.20 (91% versus 4% of uninfected infants). Other markers of inflammation such as C-reactive protein may also rise with acute infection and decrease in response to therapy.[250,255]

DIFFERENTIAL DIAGNOSIS

The signs and symptoms of early-onset group B streptococcal disease are clinically indistinguishable from neonatal sepsis caused by other bacterial pathogens such as *E. coli* and *Listeria monocytogenes*. The timing may be somewhat different, with group B streptococcal disease appearing earlier and leading to death earlier in fatal cases.[267] The prominence of respiratory signs in early-onset disease has led to confusion with noninfectious causes of respiratory distress such as transient tachypnea of the newborn, persistent fetal circulation, and respiratory distress syndrome.[2,20,245,316] Clinical features that suggest group B streptococcal infection include a history of prolonged rupture of membranes, apnea, shock in the first 24 hours of life, a 1-minute Apgar score of 5 or less, and rapid progression of pulmonary disease.[2,20,316]

The differential diagnosis of late-onset disease depends on the focus of infection. Meningitis in infants of this age also is caused by *Streptococcus pneumoniae*, *Neisseria meningitidis*, *Listeria monocytogenes*, *Haemophilus influenzae* (type b and nontypeable), and viruses. If the results of Gram stain analysis of CSF are inconclusive, other potential pathogens should be considered when therapy is selected. The findings of osteomyelitis may be subtle; refusal to move the arm may be ascribed to neuromuscular disease or Erb palsy.[96] Careful physical examination usually reveals tenderness over the involved area, and radiographs generally show a lytic defect in the metaphyses.[96] If the organism is isolated from a bone aspirate, the diagnosis is definitive. Finally, as seen in Table 94-2, many unusual manifestations of group B streptococcal infection have been described, so this organism should be included in the differential diagnosis of any focal infection occurring in the age group at risk.

TREATMENT

EMPIRIC TREATMENT

The antimicrobial regimens recommended for the treatment of group B streptococcal infection in infants are summarized in Table 94–3. Penicillin G remains the drug of choice because susceptibility is uniform. In the usual circumstance, however, antimicrobial therapy for group B streptococcal infection is started before culture results are known. Initial empiric therapy for early-onset disease would include ampicillin and gentamicin for the treatment of neonatal pathogens in addition to group B streptococci. Irrespective of gestational age, neonates with suspected meningitis and those whose clinical condition will not permit lumbar puncture should receive high doses of ampicillin (300 mg/kg/day) plus gentamicin.[12] This combination is more effective than is either ampicillin or penicillin G alone in killing most group B streptococcal strains in vitro[275] and in vivo.[300] For suspected late-onset disease, the usual initial therapy includes intravenous ampicillin in combination with cefotaxime or ceftriaxone.[252] If an infant is receiving empiric vancomycin therapy and group B streptococcal meningitis has not been excluded, penicillin G or ampicillin should be added to the regimen because vancomycin is inhibitory in vitro rather than bactericidal, and CSF concentrations may not exceed the minimal inhibitory concentration if a high inoculum of group B streptococci is present.[33]

TABLE 94–3 Treatment of Group B Streptococcal Infections in Infants

Focus of Infection	Antibiotic	Daily Dose	Duration
Suspected meningitis (initial empiric therapy)	Ampicillin *plus* gentamicin	300-400 mg/kg 7.5 mg/kg	Until cerebrospinal fluid is sterile
Suspected sepsis* (initial empiric therapy)	Ampicillin *plus* gentamicin	150-200 mg/kg	Until bloodstream is sterile
Meningitis	Penicillin G	450,000-500,000 units/kg	14 days minimum†
Bacteremia	Penicillin G	200,000 units/kg	10 days
Arthritis	Penicillin G	200,000-300,000 units/kg	2-3 wk
Osteomyelitis	Penicillin G	200,000-300,000 units/kg	3-4 wk
Endocarditis	Penicillin G	400,000 units/kg	4 wk‡

**Assumes that lumbar puncture has been performed and the cerebrospinal fluid has no abnormalities.*
†Should be extended to 21 days or longer if ventriculitis, cerebritis, subdural empyema, or other suppurative complications occur.
‡In combination with low-dose gentamicin for the first 14 days.

SPECIFIC TREATMENT

Once group B streptococci have been identified in cultures of blood, CSF, or other normally sterile body sites and blood and CSF cultures are shown to be sterile, penicillin G alone should be used to complete therapy. Recommendations concerning the optimal dose and duration have varied, but they should be dictated by the focus and severity of the infection (see Table 94–3). Several issues should be considered when selecting the appropriate dose: (1) the usual minimal bactericidal concentration of penicillin for group B streptococci ranges from 0.04 to 0.8 μg/mL,[165] (2) only 10 percent of penicillin serum levels reach the CSF, (3) the inoculum of group B streptococci in the CSF of infants with meningitis may reach 10^7 to 10^8 colony-forming units per milliliter,[105] and (4) high doses of penicillin G and ampicillin are safe in neonates.[33] To ensure rapid bacterial killing, especially in infants with meningitis, relatively high doses of penicillin are recommended for both early- and late-onset infections.

The infant with meningitis should undergo a second lumbar puncture 24 to 48 hours into therapy to document CSF sterility.[33] Infants with continued positive CSF cultures may have very high inoculum, ventriculitis with obstruction, severe infection with cerebritis and vasculitis, subdural empyema, or septic thrombophlebitis, or they may be receiving an insufficient dose of antibiotics; in this circumstance, appropriate studies should be initiated to determine which of these conditions is present. When CSF sterility and penicillin G susceptibility are verified, penicillin G alone is given for a minimum of 14 days.[252] Longer treatment is indicated if the course is severe, the infant has ventriculitis, or CSF sterilization is delayed. Another lumbar puncture to evaluate CSF cell count and protein should be considered at the anticipated completion of therapy. Findings of polymorphonuclear cells greater than 30 percent or protein higher than 200 mg/dL may warrant further diagnostic evaluation and longer duration of therapy.[33] In addition, a contrast-enhanced computed tomography scan of the head should be performed near completion of therapy in complicated cases, including infants who have prolonged fever (>5 days), cerebritis, abscess, subdural empyema, or venous thrombosis. Such complications often correlate with neurologic abnormalities that lead to a poor prognosis for complete central nervous system recovery.[100,318]

Infants with bacteremia without a focus should receive intravenous therapy for a total of 10 days.[252] A shorter duration has not been documented to be efficacious, and relapses, although rare, have been reported in these circumstances.[65,320] Patients with septic arthritis, osteomyelitis, or endocarditis should be treated for the durations summarized in Table 94–3. Oral therapy has no place in the management of infants with group B streptococcal disease.[33,252] Alternative agents such as the cephalosporins and vancomycin are active against group B streptococci in vitro.[107,186] The efficacy of these agents is unknown, however, and these drugs are not recommended in most circumstances.

SUPPORTIVE TREATMENT

The importance of prompt, vigorous, and careful supportive therapy in the successful treatment of infant group B streptococcal infections cannot be overemphasized. Neonates with early-onset disease accompanied by pneumonia should be suspected of having early respiratory failure, and ventilatory support should be initiated before the onset of apnea, septic shock, or frank respiratory failure occurs. Persistent metabolic acidosis and delayed capillary refill should prompt treatment for shock. All patients with signs of impending respiratory or circulatory failure or meningitis should be treated in an intensive care unit. When present, hypoxemia, severe anemia, and acidosis should be corrected, and seizures should be controlled with anticonvulsants. In addition, fluid and electrolyte status should be monitored meticulously. Surfactant should be used as per nursery protocol; improved gas exchange is seen in infected premature neonates who receive surfactant, although the response is slower than in patients with respiratory distress syndrome.[141] Finally, if an infant has persistent pulmonary hypertension or if conventional ventilatory therapy has failed, extracorporeal membrane oxygenation may be considered, if available.

ADJUNCTIVE TREATMENT

Adjunctive treatment of life-threatening group B streptococcal disease is aimed at correcting poor host defenses and is being investigated. Such treatment includes intravenous human immunoglobulin, monoclonal antibodies to group B streptococcal polysaccharide antigen, leukocyte transfusion, and growth factors such as granulocyte colony-stimulating factor and granulocyte-monocyte colony-stimulating factor for neutropenia.[69,78,122] The efficacy of these agents has not been established, and, although they may be used occasionally, they should be considered experimental.

RECURRENT INFECTIONS

In the few infants who experience a recurrence, suppurative foci, HIV infection, or humoral immune deficiency should be excluded or treated. Immunoglobulin levels should be determined to exclude humoral immune deficiency. Although it may be too early to document humoral immune deficiency unequivocally, total IgG usually is significantly lower than would be expected for age in weeks.[128] Tube dilution susceptibility of isolates from the first and recurrent episodes should be determined to ensure in vitro susceptibility to penicillin. If the reason for the recurrence remains unknown, persistent mucous membrane colonization with group B streptococci probably is the source.[227] β-Lactam antibiotics, even when administered by the parenteral route, do not eradicate group B streptococcal colonization reliably.[239] Some studies have shown the benefit of rifampin (20 mg/kg/day) to eradicate mucosal group B streptococcal colonization when given orally during the last 4 days of parenteral therapy.[128,219] However, a more recent study showed failure of rifampin to eradicate group B streptococcal colonization in infants.[108]

PROGNOSIS

The outcome of group B streptococcal disease is related closely to the severity and site of infection at initial evaluation. Improved outcomes after early-onset infection have resulted from greater awareness among pediatricians and improved obstetric management. Improvements in obstetrics include the use of intrapartum antibiotic prophylaxis in women at risk for delivering an infant with early-onset group B streptococcal infection. However, the mortality rate remains substantial at 2 to 8 percent, especially in neonates born at less than 37 weeks' gestation, in whom the mortality rate often exceeds 20 percent.[75,281]

Little information is available on the long-term prognosis of survivors of group B streptococcal sepsis without meningitis. In infants with septic shock, the development of periventricular leukomalacia has been reported and associated with neurodevelopmental sequelae.[102] However, the frequency of this association is not known.

For infants with early- or late-onset meningitis, 20 to 30 percent will have permanent neurologic sequelae, and 20 percent of these impaired survivors have global mental retardation, cortical blindness, hearing loss, spasticity, or paresis.[100,135,318] Whether

these rates reported over 20 years ago can be applied to infants currently being treated is unknown. Improvements in both specific and supportive therapy may have diminished the frequency of lasting impairments.

To date, the prognosis for infants with osteoarticular or soft tissue infections with group B streptococci has been excellent.[21,96] However, omission of early surgical intervention for infections that involve either the hip or shoulder joints may result in epiphyseal injury.

PREVENTION

The continuing magnitude and severity of group B streptococcal disease and its attendant mortality and morbidity have led to investigations aimed at its prevention. Several general approaches have been proposed: maternal chemoprophylaxis to decrease transmission, infant chemoprophylaxis to decrease colonization, and immunoprophylaxis to increase protection against disease.

CHEMOPROPHYLAXIS

Maternal Chemoprophylaxis

Epidemiologic studies during the 1980s showed that women with group B streptococcal colonization during pregnancy had greater than 25 times likelihood of delivering infants with early-onset disease compared with women with negative prenatal cultures.[278] The idea to give antibiotic therapy to the mother to prevent vertical transmission to her fetus was suggested first by Franciosi and colleagues.[113] The first strategy to be evaluated was antenatal treatment of group B streptococcal maternal carriers with oral or intramuscular penicillin. This method temporarily suppresses colonization density but does not eradicate it or interrupt vertical transmission of group B streptococci to neonates.[119,130,258]

The next strategy, maternal intrapartum chemoprophylaxis, has been demonstrated to be efficacious in the prevention of vertical transmission of group B streptococci from colonized mothers to their neonates, the development of early-onset disease, and maternal febrile morbidity. Infants are less frequently colonized with group B *Streptococcus* if they are born to mothers treated with ampicillin during labor than to mothers not given antibiotics.[59,60,341] Several controlled trials involving thousands of deliveries indicated that intrapartum penicillin G or ampicillin given intravenously to group B streptococcal carriers during labor prevents early-onset disease in neonates.[57,211,309] Early-onset infection occurred in 0 to 1.1 percent of infants whose mothers received intrapartum ampicillin compared with 5 to 9 percent of infants of mothers in the groups not receiving treatment. A large trial involving more than 50,000 live births in Australia showed similar results.[120]

In 1996, guidelines supported by the Centers for Disease Control and Prevention (CDC), the American College of Obstetricians and Gynecologists, and the American Academy of Pediatrics recommended a risk-based approach or culture-based approach.[278] The risk-based approach was based on the presence of one of more of the following: premature labor at less than 37 weeks' gestation, prolonged rupture of membranes (>18 hours), group B streptococcal bacteriuria, intrapartum fever (≥100.4° F [38° C]), or delivery of a previous infant with group B streptococcal infection. The culture-based approach required intrapartum chemoprophylaxis for all women with positive vaginal and rectal cultures routinely obtained at 35 to 37 weeks' gestation regardless of risk factors. Use of these methods to define women who were candidates for intrapartum chemoprophylaxis led to a 70 percent decline in the incidence of early-onset group B streptococcal disease.[75,280]

In 2002, a population-based study compared the two approaches and found the culture-based approach to be 50 percent more effective than the risk-based approach in preventing early-onset group B streptococcal disease.[279] Sixty-two percent of infants with early-onset disease were born to mothers who did not have any clinical risk factors. Therefore, revised guidelines published by the CDC in 2002 recommended universal vaginal and rectal screening cultures of all pregnant women between 35 and 37 weeks of gestation, with intrapartum chemoprophylaxis for all women identified as group B streptococcal carriers.[278] The risk-based approach was to be reserved for any women in labor whose colonization status is unknown. Colonization during a previous pregnancy is not an indication for intrapartum chemoprophylaxis. Women with group B *Streptococcus* isolated from urine in any concentration during pregnancy should receive intrapartum chemoprophylaxis, because these women usually are heavily colonized. Women who previously gave birth to an infant with invasive group B streptococcal disease always should receive chemoprophylaxis. An algorithm of these current guidelines is shown in Figure 94–6.

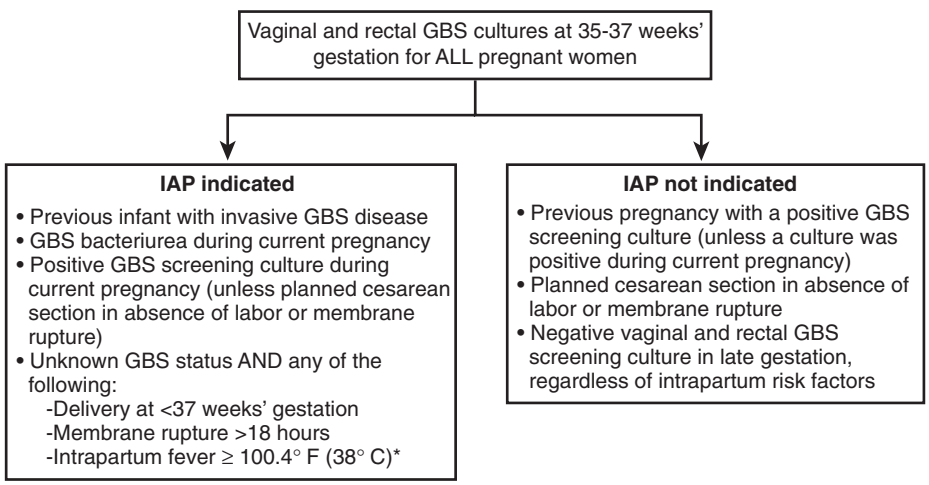

Figure 94–6 Recommendations for intrapartum antimicrobial prophylaxis (IAP) to prevent early-onset group B streptococcal (GBS) disease using a universal culture screening strategy. *(Adapted from Centers for Disease Control and Prevention: Prevention of perinatal group B streptococcal disease: Revised guidelines from CDC. M. M. W. R. Morb. Mortal. Wkly. Rep.* 51:*1-22, 2002.)*

Optimal culture techniques are discussed earlier in the chapter. Many rapid tests are being studied because they offer the advantage of detecting colonization status in women who present for preterm labor or who have not had prenatal care. These tests include latex agglutination, optical immunoassay, DNA hybridization, enzyme immunoassay, Islam starch medium tests, and PCR.[146] With the exception of PCR, all tests showed poor sensitivity or specificity compared with the standard culture method and are not sufficiently accurate to aid in decision making concerning intrapartum antibiotic prophylaxis. None of these tests can replace routine culture at this time. A prospective multicenter study evaluated a real-time PCR assay that gave results in 40 minutes.[83] Compared with intrapartum culture, the PCR assay had a sensitivity of 94 percent and a specificity of 96 percent.

Maternal chemoprophylaxis begins at hospital admission for delivery or at rupture of membranes and consists of intravenous penicillin G (initial dose, 5 million units; subsequent doses, 2.5 million units every 4 hours recommended for intrapartum chemoprophylaxis).[278] Although penicillin is preferred because of its narrow spectrum of activity, ampicillin (initial dose, 2 g; subsequent doses, 1 g every 4 hours) can be given as an alternative. For women who are allergic to penicillin, intrapartum chemoprophylaxis must take into account increasing resistance rates to clindamycin and erythromycin.[55,86] Women at low risk of developing anaphylaxis should be given cefazolin (initial dose, 2 g; subsequent doses, 1 g every 8 hours). Women at high risk of developing anaphylaxis should have susceptibility testing performed on their isolates obtained during screening at 35 to 37 weeks of gestation. If the isolate is susceptible, clindamycin (900 mg every 8 hours) or erythromycin (500 mg every 6 hours) can be used. If susceptibility testing is not available, the results are not known, or the isolate is resistant to clindamycin and erythromycin, vancomycin (1 g every 12 hours) can be given as the alternative. The efficacy of the latter two regimens is not known.

Since the widespread adoption of chemoprophylaxis, the incidence of early-onset disease has plummeted.[75,280] Racial disparities in early-onset disease have declined significantly.[73,280] Opportunities to prevent early-onset disease still are missed, however, because of laboratory practices,[74] hospital or procedural errors in communication of the screening results, or failure to administer intrapartum chemoprophylaxis to a mother known to be colonized.[257] The incidence of late-onset disease remains unaffected by intrapartum chemoprophylaxis.[75]

Management of an infant born to a mother given intrapartum penicillin G prophylaxis depends on the clinical findings at birth, the gestational age, and the timing of doses administered to the mother (Fig. 94–7). Amniotic fluid levels of penicillin that will kill group B streptococci are not achieved until at least 3 hours after administration of the first dose.[62] If the infant appears healthy, has a gestational age of 35 weeks or more, and has a mother given at least one dose of intrapartum chemoprophylaxis at least 4 hours before delivery, neither diagnostic evaluation nor empiric antimicrobial therapy will be required. However, to ensure their ongoing stability, such infants should be observed in the hospital for at least 48 hours. Keeping the infant hospitalized through day of life 2 has been challenging because the associated expense affects the cost-to-benefit ratio of intrapartum prophylaxis.[223] However, an analysis of all live births in the state of Florida for the years 1992 through 1994 demonstrated a 115 percent increase in the rate of readmission for group B streptococcal infection in infants discharged on day of life 1; this finding supports the recommendation to observe infants for 48 hours.[126]

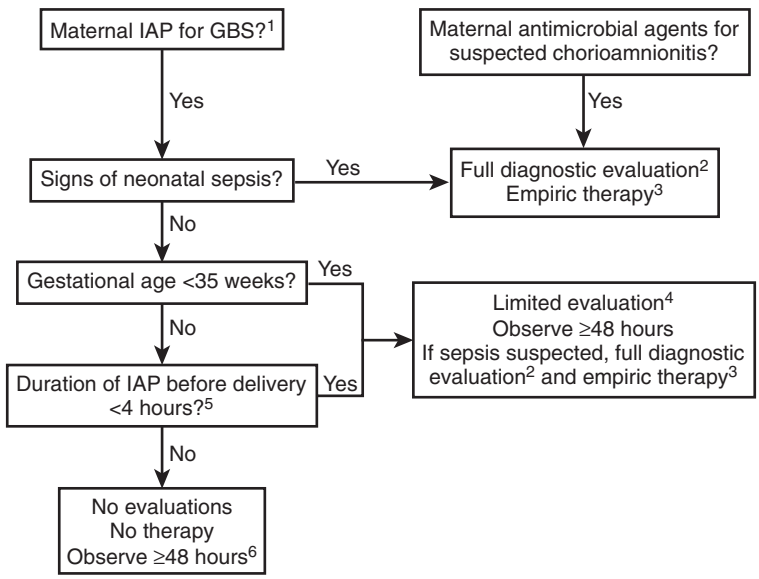

Figure 94–7 Empiric management of a newborn exposed to intrapartum antimicrobial prophylaxis (IAP) for group B streptococcal (GBS) infection. *1*, When no maternal intrapartum prophylaxis for GBS was administered despite the presence of an indication, data are insufficient on which to recommend a single management strategy. *2*, Includes complete blood cell count (CBC) and differential, blood culture, and chest radiograph if respiratory abnormalities are present. When signs of sepsis are present, a lumbar puncture, if feasible, should be performed. *3*, Duration of therapy varied depending on results of blood culture, cerebrospinal fluid findings, if obtained, and clinical course of the infant. If laboratory results and clinical course do not indicate bacterial infection, duration may be as short as 48 hours. *4*, CBC and differential and blood culture. *5*, Applies only to penicillin, ampicillin, or cefazolin and assumes recommended dosing regimens. *6*, A healthy-appearing infant who was at least 38 weeks' gestation at delivery and whose mother received at least 4 hours of IAP before delivery may be discharged home after 24 hours if other discharge criteria have been met and a person able to comply fully with instructions for home observation will be present. If any one of these conditions is not met, the infant should be observed in the hospital for at least 48 hours and until criteria for discharge are achieved. *(Adapted from Centers for Disease Control and Prevention: Prevention of perinatal group B streptococcal disease: Revised guidelines from CDC. M. M. W. R. Morb. Mortal. Wkly. Rep.* 51:*1-22, 2002.)*

Neonates with signs of sepsis are evaluated and treated empirically for sepsis. Healthy-appearing neonates who either are born at less than 35 weeks' gestation or are born to women given no intrapartum chemoprophylaxis or a single dose of intrapartum chemoprophylaxis less than 4 hours before delivery should undergo limited laboratory evaluation (complete blood count and blood culture) and observation in the hospital for 48 hours without therapy. However, if the subsequent clinical course or the laboratory results suggest infection, full diagnostic evaluation and therapy is initiated.

Concern that widespread use of intrapartum ampicillin use will result in a greater incidence of ampicillin-resistant neonatal infection has been supported by some case series.[161,216] Development of penicillin- or ampicillin-resistant group B streptococci has not been seen.[77] In most studies, incidence rates of *E. coli* and other gram-negative pathogens have remained stable.[4,161,216,268] The rate of ampicillin-resistant *E. coli* infections is increasing in preterm infants. However, rates of ampicillin resistance in term neonates before and after widespread intrapartum chemoprophylaxis are the same.

Infant Chemoprophylaxis

The two controlled trials evaluating neonatal prophylaxis reached contradictory conclusions.[256,289] In the first trial, more than 16,000 newborns received either intramuscular penicillin G or topical tetracycline (prophylaxis for gonococcal ophthalmia neonatorum) within an hour of birth during alternating weeks.[289] In penicillin-treated newborns, the incidence of proven early-onset group B streptococcal disease was less common, but death rates were similar, and an increase in the number of penicillin-resistant infections in the penicillin group was noted. In the second controlled study, blood cultures were obtained before initiation of penicillin therapy, and rates of group B streptococcal bacteremia were similar in the treatment and control groups.[256] In this study of nearly 1200 neonates weighing 2000 g or less at birth, intramuscular penicillin (or no treatment) was given in the first hour of life and was continued for 3 days. Rates of group B streptococcal bacteremia were similar in the treatment and control groups. Penicillin prophylaxis probably was ineffective because almost 90 percent of neonates with early-onset group B streptococcal disease were bacteremic immediately after birth. The results of the second study are supported by numerous observations, thus indicating that early-onset disease often begins in utero.[60,241,253,281,282] Infant chemoprophylaxis currently is not recommended.[278]

IMMUNOPROPHYLAXIS

Although efforts to implement intrapartum chemoprophylaxis further are ongoing, the most promising and potentially lasting method for prevention of both early- and late-onset infant infections is immunoprophylaxis. This approach remains investigational, and several reviews have summarized the rationale.[25,137,237] It is based on the observation that immunity to group B streptococci correlates with antibody directed against the type-specific capsular polysaccharides of these organisms. These IgG class antibodies and complement promote opsonization, phagocytosis, and bacterial killing of group B streptococci and protect animals against lethal challenge.[238,325] Thus, provision of protective levels of type-specific immunity to the infant could be achieved through active immunization of the mother. Baker and associates[36] immunized women at a mean gestation of 31 weeks with purified type III polysaccharide vaccine. Although the immune response was not optimal (63%), placental transport of maternal antibodies was 90 percent in neonates born to women who did respond to vaccination, and most of these infants had protective levels of antibodies in their sera at 3 months of age. A more recent study immunizing women with a type III polysaccharide-tetanus toxoid conjugate reported a 95 percent immune response, with persistence of opsonophagocytic antibodies at 2 months of age in 100 percent of infants born to maternal responders.[38] Other studies immunizing nonpregnant adults with purified capsular polysaccharide-tetanus toxoid conjugate vaccines showed the safety and immunogenicity of these vaccines.[34,35,37]

Availability of the complete genome sequences for group B *Streptococcus* has led to the discovery of many potential surface proteins and secreted proteins that can be expressed in recombinant form and used for production of antibodies.[200,306] A set of proteins shared by the disease-causing serotypes theoretically can be used in combination to create a vaccine capable of providing protection against multiple serotypes. Group B streptococcal pili with at least one of the pilus islands present in all sequenced group B streptococcal genomes may be included in a future vaccine.[66]

Because most pregnant women (estimated at 85-90%) have nonprotective levels of these antibodies in their sera at delivery, active immunization of childbearing-age women before or during pregnancy would optimize strategy to induce high maternal antibody levels. Human studies have demonstrated efficient antibody transfer to the neonate.[36,38] Transfer of high levels of maternal antibody would provide protection of the neonates from early- and late-onset group B streptococcal disease. In addition, immunization also would offer protection for the mother against preterm deliveries and fetal loss caused by group B streptococcal infection. Finally, vaccination of elderly or nonpregnant adults with defined medical conditions could reduce the disease burden significantly. Because immunoprophylaxis should be the most cost-effective and beneficial prevention strategy for group B streptococcal disease,[224,291] it should be promoted by physicians, public health officials, parents, pharmaceutical manufacturers, and legislators.

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CHAPTER 95

Enterococcal and Viridans Streptococcal Infections

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ENTEROCOCCAL INFECTIONS

The enterococci are gram-positive ovoid bacteria that are related closely to the streptococci but now are known to be phylogenetically distinct and make up the genus *Enterococcus*. These organisms are found in the normal bowel flora of humans and many animals and commonly are isolated from environmental sources. Enterococci generally are considered to be of low virulence but have been known to cause human infection for more than 100 years (reviewed by Murray[164]). These ubiquitous bacteria have become recognized increasingly as important causes of both nosocomial and community-acquired infection in adults[62,152,153,164,166,168,180,185] and children,* yet the role of *Enterococcus* spp. as a pathogen (or co-pathogen) in certain clinical settings, particularly intra-abdominal and pelvic infections, often remains uncertain.

Enterococci are intrinsically resistant to many antimicrobial agents (including the cephalosporins, oxacillin, clindamycin, and the aminoglycosides). Since the emergence of high-level aminoglycoside resistance in *Enterococcus* spp. more than 35 years ago,[157] increasing percentages of these organisms have acquired clinically significant resistance to β-lactam antibiotics and to vancomycin and other glycopeptides, as well as to aminoglycosides. Vancomycin-resistant enterococcal (VRE) infections, especially those caused by vancomycin-resistant *Enterococcus faecium* (VREF), are of particular concern because VREF isolates frequently are resistant to other bactericidal antimicrobial agents.[166,168] The dramatic increase in nosocomial VRE infections[62,166,180] has resulted in failure of antimicrobial therapy[125] and has inspired comparisons with the pre-antibiotic period[5] and even speculation about a "post-antimicrobial era."[44] Fortunately, new classes of antimicrobials active against most VRE isolates have become available recently, but pediatric experience with these agents is limited, and enterococci resistant to these agents already have been detected (see later). Concern about the continued nosocomial spread of VRE isolates and the documented transfer of vancomycin resistance determinants to *Staphylococcus aureus*[27,28,242,252] has led to the development of stringent hospital infection control guidelines designed to interrupt the spread of vancomycin-resistant organisms.[193] Although the impact of VRE infections in children has been less dramatic, increasing numbers of pediatric centers are reporting infections caused by these organisms.*

*See references 18, 19, 20, 30, 61, 117, 132, 159, 180, 194, 199, 202, 215, 216, 220, 221.

*See references 18, 30-38, 58, 59, 78, 91, 132, 159, 193, 202.

MICROBIOLOGY

The genus *Enterococcus* consists of gram-positive cocci that are catalase-negative and occur singly, in pairs, and in short chains. Morphologically, enterococci are indistinguishable from streptococci and traditionally were classified as members of the genus *Streptococcus*. Sherman's early classification scheme[222] divided the streptococci into four groups: pyogenic, viridans, lactic, and enterococcal. By the Lancefield criteria, enterococci were classified as group D streptococci, along with the "nonenterococcal" *Streptococcus bovis* group. However, recent genetic evidence has indicated that the enterococci are sufficiently different from the streptococci to merit establishment of a separate genus.[68]

Figure 95–1 is a scheme for the differentiation of enterococci from other gram-positive cocci. Catalase-negative gram-positive cocci that have been isolated from human sources include the streptococci, the enterococci, *Lactococcus* spp., *Leuconostoc* spp., *Pediococcus* spp., and *Gemella* spp. Most enterococci produce no (gamma) or partial (alpha) hemolysis on blood agar; differentiation between enterococci and certain alpha-hemolytic or nonhemolytic streptococci and other nonstreptococcal gram-positive cocci may require a series of biochemical tests.[67,68] Clinical laboratories may identify an organism presumptively on a primary isolation plate as an enterococcus based on colony morphology, Gram stain, and the pyrrolidonyl arylamidase (PYR) test. Most enterococci produce PYR, as do *Streptococcus pyogenes* and nutritionally variant streptococci (*Abiotrophia*), but not other streptococci. The PYR test is particularly useful for differentiating enterococci from group D streptococci and *Leuconostoc* spp. (see Fig. 95–1). *S. pyogenes* and *Abiotrophia* spp. are distinguished easily from enterococci by colony morphology, hemolysis, and special growth requirements.

Enterococci are able to hydrolyze esculin in the presence of 40 percent bile salts; of the true streptococci, only group D streptococci (*S. bovis* group) and approximately 5 to 10 percent of viridans streptococci share this characteristic.[68] Enterococci are facultatively anaerobic and grow under harsh conditions that inhibit the growth of streptococci; growth in 6.5 percent sodium chloride at 45° C is a useful confirmatory test. Enterococci produce leucine aminopeptidase, as do streptococci, lactococci, pediococci, and some *Gemella* strains. The presence of group D streptococcal antigen is of limited value because the *S. bovis* group, most pediococci, and half of the clinical *Leuconostoc* spp. isolates share this antigen.[68]

Occasional clinical isolates of *Leuconostoc* spp., *Pediococcus* spp., and *Lactococcus* spp. may be difficult to distinguish from the enterococci. Some strains of *Leuconostoc* spp. and *Pediococcus* spp. may grow in 6.5 percent NaCl at 45° C, but they are PYR-negative. Lactococci are PYR-positive, and some isolates will grow in 6.5 percent NaCl; however, most lactococci fail to grow (or grow very slowly) at 45° C. Consequently, definitive confirmation of an organism as an enterococcus may require complete identification to the species level. Molecular techniques allow rapid, reliable identification and speciation of enterococci,[50,57,60] but currently they are available only in the research setting.

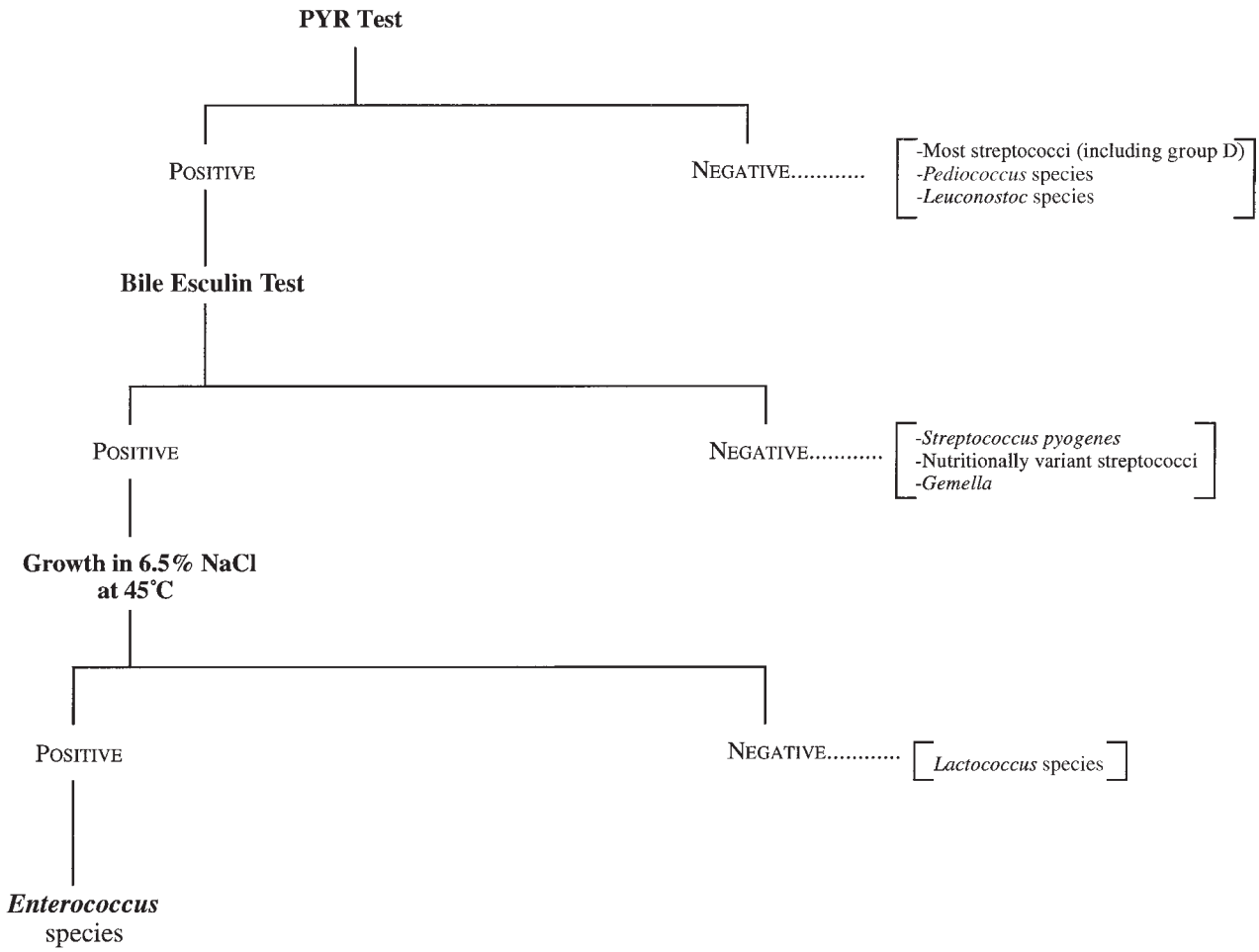

Figure 95–1 Differentiation of enterococci from other catalase-negative, gram-positive cocci. PYR, pyrrolidonyl arylamidase.

TABLE 95–1 *Enterococcus* Species

Typical Enterococci (PYR$^+$)
E. faecalis
E. faecium
E. avium
E. casseliflavus
E. durans
E. raffinosus
E. gallinarum
E. malodoratus
E. hirae
E. mundtii
E. pseudoavium
E. dispar
E. flavescens
E. sulfureus
Atypical Enterococci (PYR$^-$)
E. cecorum
E. columbae
E. saccharolyticus

PYR, pyrrolidonyl arylamidase.

The genus *Enterococcus* now includes at least 14 "typical" species and 3 additional "atypical" species (the latter are PYR-negative and grow very slowly in the presence of 6.5% NaCl) (Table 95–1). However, most human clinical isolates are either *Enterococcus faecalis* (50-90%) or *E. faecium* (5-37%), although clusters of human infection caused by *Enterococcus raffinosus*,[33] *Enterococcus casseliflavus*,[172] *Enterococcus avium*,[184] and *Enterococcus durans*[216] and occasional human infections attributable to *Enterococcus gallinarum*, *Enterococcus mundtii*, and *Enterococcus flavescens* have been reported.[66] Even though *E. faecalis* and *E. faecium* continue to account for most clinical isolates, the percentage of *E. faecium* isolates has been increasing, and relatively more "other" (non-*faecalis*, non-*faecium*) enterococci are being identified by clinical laboratories.[110,112,138]

Speciation of enterococci has been useful primarily for epidemiologic purposes, but distinction between the more antibiotic-susceptible *E. faecalis* and the more antibiotic-resistant (see later) *E. faecium* may be helpful in selecting optimal therapy for endocarditis and other serious enterococcal infections. A panel of biologic tests can differentiate these two common enterococcal species readily.[68,118,164] Most *E. faecalis* isolates (unlike those of *E. faecium*) grow in the presence of 0.04 percent tellurite, reduce tetrazolium to formazan, and produce acid from sorbitol and glycerol.

A variety of molecular techniques, including a modification of pulsed-field gel electrophoresis known as the CHEF (contour-clamped homogeneous electric field electrophoresis) technique[39,40,56,160,255] and polymerase chain reaction (PCR), are available to assist in the identification of enterococci and determination of the relatedness of enterococcal isolates.[60] These molecular techniques have been particularly valuable in investigations of possible nosocomial transmission of multiresistant enterococci.[39,40,57,60,68,160,182,205,255]

EPIDEMIOLOGY

Enterococci are normal flora of the gastrointestinal tract of most humans and have been found in as many as 97 percent of fecal samples from adults in Europe, Asia, and North America (reviewed by Murray[164]). Approximately half of newborn infants have become colonized with enterococci by the time they are 1 week of age.[175] Enterococci are isolated less commonly (<20% of specimens) from other sites, including the vagina, oral cavity, and skin. These organisms also are common inhabitants of the bowel flora of many animals and frequently are present in soil, water, and food. Enterococci are hardy organisms and may persist for long periods on environmental surfaces, thereby contributing to potential nosocomial spread of these bacteria.

Human infection caused by enterococci was reported before the beginning of the 20th century, but the initial reports included patients with infections that seldom are associated with enterococci today: enteritis, meningitis, and appendicitis (reviewed by Murray[164]). The ubiquitous presence of enterococci in fecal samples led to the mistaken impression that these organisms cause enteritis and food poisoning. Enterococci are isolated commonly as part of mixed flora in intra-abdominal and pelvic infections, but the contribution of these organisms to the pathogenesis of such infections remains uncertain.[81,173] However, enterococci were identified as pathogens causing urinary tract infection and endocarditis as early as 1906,[4] with later confirmation by many studies. Subsequently, enterococci have been documented to cause invasive infection in neonates,[56,77,121] patients with malignancies,[85,86,159,221] recipients of bone marrow and solid organ transplants,[9,116,119,183,210] burn victims,[127] patients with indwelling catheters,[9,51,153,164,185,199] and other immunosuppressed or debilitated patients.[30,153,164,185,199]

In general, enterococcal infections occur less frequently in children (outside the neonatal period) than in adults, and enterococci are less common causes of pediatric (versus adult) urinary tract infection[259] and endocarditis.[231] Much of the published experience with enterococcal infection in children focuses on the neonatal period,[47,56,140,194] and some evidence suggests that late-onset (but not early-onset) neonatal enterococcal infection may be increasing in frequency.[39,56] Relatively few series of pediatric enterococcal infection have been published.[14,17,22,39,51,85,202]

Enterococci recently have emerged as important nosocomial agents, and they rank as either the second or third most common hospital-acquired pathogens in this country.[62,103,152,180,200,211,261,262] Evidence of nosocomial spread of enterococci is relatively recent. Initially, all enterococci isolated from hospitalized patients were thought to have originated from the patient's endogenous bowel flora. However, the emergence of multiantibiotic-resistant enterococci prompted the performance of careful epidemiologic studies that documented the nosocomial spread of these organisms.* Many groups have reported that VRE and other enterococcal isolates may persist for long periods of time in the environment and may be spread either by direct patient-to-patient contact via the hands of colonized health care personnel[194] or from contaminated beds or other hospital material or contaminated patient equipment, including thermometers.[18,117,132,153]

The growing problem of nosocomial infection by VRE organisms is multifactorial; the diversity of isolates found in many hospitals indicates that resistant organisms may be introduced via multiple sources.[39,61,78,161,167,199,221] Consequently, outbreaks of VRE infection in institutions may be caused by single or multiple clones, although the first recognized outbreak of VRE infection or colonization in a hospital usually is associated with a single clone.[30,78]

In many reports, distinguishing risk factors for VRE colonization from those for invasive disease caused by these organisms has not been possible, and certain factors probably increase the risk for both colonization and development of infection caused by VRE organisms. Major risk factors for VRE colonization or the development of infection, or for both, appear to be the severity of the underlying illness or immunosuppression; the proximity to patients colonized with VRE organisms (e.g., admission to an intensive care unit [ICU] or a transplant unit); receipt of a bone marrow or solid organ (especially liver) transplant; increasing length of hospital stay; recent cardiothoracic or abdominal

*See references 10, 11, 40, 87, 93, 194, 199, 200, 202, 229, 251, 262.

surgery; the presence of indwelling central venous or urinary catheters; and previous treatment with vancomycin, broad-spectrum antibiotics (particularly those lacking appreciable anti-enterococcal activity, such as the third-generation cephalosporins), or agents with broad anti-anaerobic activity.* Patients infected with human immunodeficiency virus (HIV) also appear to be at higher risk for the development of VRE bacteremia.[9] Factors contributing to nosocomial spread of these organisms probably differ from those responsible for the gradual overall increase in resistant strains.[199]

Since the first report of a vancomycin-resistant isolate in 1988,[128] infection and colonization with VRE bacteria in the United States have been observed primarily in the ICUs of large teaching hospitals.[166,168,180] For example, data reported to the National Nosocomial Infection Surveillance (NNIS) system of the Centers for Disease Control and Prevention (CDC) from January 1989 through March 1993 revealed no vancomycin resistance in hospitals with fewer than 200 beds, a resistance rate of 1.8 percent in hospitals with 200 to 500 beds, and a rate of 3.6 percent in hospitals with more than 500 beds.[180] A 1995 report from the multicenter *Enterococcus* Study Group analyzed 1936 isolates collected from 97 laboratories in 47 states during the last quarter of 1992 and generally confirmed the findings of the NNIS survey.[110] However, VRE infections are not limited to ICUs. A 1999 report from the Surveillance and Control of Pathogens of Epidemiologic Importance (SCOPE) group's ongoing surveillance of nosocomial bloodstream infections in 49 U.S. hospitals revealed no overall differences in rates of vancomycin resistance in enterococci isolated from patients located in critical care versus hospital ward settings.[62] Fortunately, VRE infections have occurred much less frequently in children than in adults, and overall rates of vancomycin resistance in enterococci isolated from children's hospitals remain very low. However, increasing numbers of pediatric centers have reported VRE infections.*

In the United States, VRE isolates generally have not been detected in environmental sources or in patients who have not had exposure to hospitals,[166] with rare exceptions.[30] However, the ecology of VRE organisms in Europe differs: they have been detected in the feces of nonhospitalized patients and healthy volunteers in several European studies, with rates of VRE colonization as high as 28 percent in adults living in some parts of Belgium (reviewed by Murray[166,168]). The more widespread occurrence of VRE strains in Europe probably was related to the use of oral glycopeptides such as avoparcin in animal feed and the oral administration of bacterial preparations (possibly contaminated with resistant enterococci) to humans and animals for therapeutic purposes.[166-168]

Initial reports of VRE organisms in the United States were clustered in the northeast region, particularly New York, Maryland, and Pennsylvania,[180] and geographic differences in rates of VRE colonization and infection persist. However, subsequent reports indicate that VRE strains are found in most parts of the United States[109,138] and that rates of vancomycin resistance among enterococci isolated from patients in this country generally are higher than those found in isolates from Canada, western Europe, or Latin America.[108,111,138] The reasons for these geographic differences remain uncertain, but the high rates of resistance in the United States may be related to the marked increase in the use of vancomycin in this country during the past 3 decades.[120]

PATHOGENESIS AND VIRULENCE

Enterococci are organisms of low virulence, and their ubiquitous presence in the human gastrointestinal tract probably has contributed to both the spurious association of these organisms with some illnesses (e.g., enteritis) and the failure to recognize other situations in which enterococci are true pathogens (e.g., immunosuppressed and debilitated patients, neonates). When compared with organisms such as *S. pyogenes* and *S. aureus*, enterococci are much less virulent in animal models of infection,[104,152] but they are capable of causing disease at a higher inoculum.

Enterococci rarely cause primary cellulitis or abscesses, although these organisms are isolated frequently as components of polymicrobial wound infections and intra-abdominal and pelvic infections. The contribution of enterococci to the pathogenesis of these polymicrobial infections remains uncertain.[81,173] In animal models, synergy between enterococci and a variety of other organisms (particularly anaerobes) can be demonstrated, but enterococci injected alone have little propensity to cause either peritonitis or subcutaneous infection.[104,105,164,181] Many clinical trials have concluded that provision of anti-enterococcal therapy generally is not necessary to effect a cure of human intra-abdominal and pelvic infections, even though *Enterococcus* frequently (14-33% of cases) is isolated from primary peritoneal cultures.[81]

Enterococci are an important cause of urinary tract infections in adults (particularly in elderly men, patients with structural abnormalities of the urinary tract, and those with indwelling urinary catheters), but they are associated less frequently with urinary tract infections in children.[7,259] Enterococci also are important but less frequent (than in adults) causes of bacterial endocarditis in children.[151,231]

Adherence of bacteria to tissue is a necessary first step in the pathogenesis of both urinary tract infection and endocarditis, and evidence suggests that pathogenic enterococci produce factors that mediate adherence to urinary epithelial cells and endocardial tissue (reviewed by Jett and colleagues[104] and Johnson[105]). Enterococcal adhesins include an enterococcal surface protein known as aggregation substance[36,123] and surface carbohydrates. Lipoteichoic acid, an important adhesin for *S. pyogenes*, does not appear to mediate adherence of enterococci,[228] but it may trigger the host inflammatory response to these organisms.[238] Recent studies have implicated an enterococcal surface protein, Esp, in biofilm formation by *E. faecalis*.[24,240] In addition, many virulent *E. faecalis* isolates express a cytolysin in the presence of target cells.[43,48]

Nosocomial enterococcal bacteremia in adults frequently is associated with urinary tract and wound infections, but catheter-related bacteremia is of increasing importance. Enterococcal bacteremia without a source occurs relatively more commonly in children,[17,199] although Bonadio[14] reported that many children with enterococcal bacteremia had an identifiable focus of infection. Enterococcal bacteremia occurs more frequently in patients with severe underlying disease and may be life-threatening. However, the precise contribution of enterococcal bacteremia to morbidity and mortality in severely ill patients remains controversial. Adult ICU patients with enterococcal bacteremia have a very high mortality rate,[83] and some early studies suggested that isolation of *Enterococcus* from blood merely serves to identify a very high-risk group of patients.[164] In several studies, clearance of VRE organisms from blood was not associated with a reduced mortality rate in this high-risk population. In other studies, spontaneous resolution of VRE bacteremia has been reported, thus suggesting that transient VRE bacteremia or pseudobacteremia (or both) may occur.[246] However, a series of recent studies has clearly associated vancomycin resistance with an increased risk for mortality in certain patients and has demonstrated that effective anti-enterococcal therapy reduces the mortality rate in patients with VRE bacteremia.[53,54,61,97,168,185] Taken together, these findings indicate that at least a subset of high-risk patients with VRE bacteremia are at risk for the development of significant morbidity and mortality directly related to the infection.

*See references 14, 17, 47, 56, 77, 121, 140, 147, 194, 226.

CLINICAL MANIFESTATIONS

Enterococcal infections generally occur less frequently in children than in adults, but *Enterococcus* is a relatively frequent cause of neonatal infections.[56] The types of infections caused by enterococci in children are similar to those in adults,[14,17,51,199] although enterococcal bacteremia may be associated with fewer sequelae in children.[51] In children, as well as in adults, enterococci have become increasingly important nosocomial pathogens.[17,39,147,152,180,200,262] They are important causes of endocarditis, urinary tract infection, and bacteremia (particularly catheter-related bacteremia) in pediatric patients, and these organisms commonly are isolated as components of polymicrobial wound, intra-abdominal, and pelvic infections in children.[14,17,153,164] Meningitis[234] and septic arthritis[192] are rare manifestations of enterococcal infection. Respiratory infections caused by enterococci are extremely uncommon occurrences, although many neonates with enterococcal infection have pulmonary symptoms.[55]

URINARY TRACT INFECTION

Urinary tract infections are the most common enterococcal infections in adults,[153] and enterococci are important urinary pathogens in children.[52] Most enterococcal urinary tract infections occur in elderly men after they have undergone urinary catheterization, instrumentation, or both.[70,162] Enterococci are infrequent (<5% of isolates) causes of cystitis and pyelonephritis in otherwise healthy children, infants, and neonates[7,259] and are infrequent causes of urinary tract infection in young women.[164] However, some centers are reporting an increasing incidence of both community-acquired and nosocomial urinary tract infection caused by enterococci.[70]

Most enterococcal urinary tract infections in children[7,52,134] and adults[70,152] are nosocomial. Risk factors for the development of urinary tract infection by *Enterococcus* include indwelling urinary catheters, instrumentation of the urinary tract, structural abnormalities of the urinary tract, and previous broad-spectrum antimicrobial therapy.[162,164] The increasing problem of nosocomial enterococcal urinary tract infection[70] is compounded by the growing problem of multiantibiotic-resistant enterococci.[180]

The genitourinary tract reportedly is the most common entry site leading to enterococcal bacteremia in adults,[83,124] but it is implicated much less frequently in the cause of enterococcal bacteremia in children.[14,17,51] Christie and colleagues reported that urosepsis was the cause of 12 percent of episodes of nosocomial enterococcal bacteremia in one children's hospital,[39] but Das and Gray[51] failed to detect any cases of urosepsis in 75 consecutive cases of enterococcal bacteremia in another pediatric hospital during a 3-year period. Other complications of enterococcal urinary tract infection in adults include prostatitis and perinephric abscess.[153]

ENDOCARDITIS

Enterococcal endocarditis was reported first in 1906,[4] and these organisms are important causes of native- and prosthetic-valve endocarditis in children and adults.[2,150,197] Either normal or previously damaged valves may be involved. Enterococci cause approximately 5 to 20 percent of cases of native-valve endocarditis in adults (excluding cases in intravenous drug users),[150] approximately 6 to 7 percent of prosthetic-valve endocarditis,[150,243] and approximately 5 to 10 percent of endocarditis in intravenous drug users.[164] Enterococcal endocarditis in intravenous drug users generally involves the aortic or mitral valves, unlike staphylococcal endocarditis in this setting, which usually involves the tricuspid valve.[164] As with other enterococcal infections, *E. faecalis* causes most cases of endocarditis.

Enterococcal endocarditis primarily is a disease of older men, and the genitourinary tract is the most commonly identified source of the initial bacteremia. Enterococci are relatively less frequent causes of endocarditis in children—less than 5 percent of cases in most series.[106,107,230,231,239,263] Typically, enterococcal endocarditis occurs after a subacute course that is clinically indistinguishable from that caused by streptococci.[150] Although the enterococcus is a common neonatal pathogen,[56] reports of neonatal enterococcal endocarditis are extremely rare.[239] The prognosis of enterococcal prosthetic-valve endocarditis is somewhat better than that of native-valve endocarditis caused by these organisms.[2,197] Aortic-valve involvement is associated with increased rates of morbidity and mortality.[150] Endocarditis rarely complicates nosocomial enterococcal bacteremia.[142,164,185] Consequently, VRE-associated endocarditis is a rare development, although a number of cases have been reported recently.[233]

BACTEREMIA

Enterococcal bacteremia often represents a conundrum. Bacteremia in severely ill hospitalized patients, particularly that caused by VRE organisms, is associated with considerable morbidity and mortality. However, only a portion of that morbidity and mortality can be attributed directly to enterococcal bacteremia *per se*. Nosocomial enterococcal bacteremia frequently occurs as a component of polymicrobial bacteremia, with 21 to 45 percent of bloodstream isolates of *Enterococcus* being accompanied by one or more other pathogens.[51,142,185,224] As many as half of cases of catheter-related enterococcal bacteremia are polymicrobial.[147,185] Many,[90,142] but not all,[96,147] series of patients with enterococcal bacteremia have reported increased mortality rates in patients with polymicrobial bacteremia (including *Enterococcus*) versus isolated enterococcal bacteremia. Mortality in adults with nosocomial enterococcal bacteremia has ranged from 23 to 46 percent,[142,143,185] but patients at risk for the acquisition of nosocomial enterococcal bacteremia are severely ill and have a poor prognosis independent of the bacteremic event. However, hospital-acquired enterococcal infections can be life-threatening,[61,164,185,189] and specific therapy does appear to reduce the overall mortality rate.[61,97,185] Furthermore, a recent meta-analysis concluded that vancomycin resistance is independently correlated with increased mortality in patients with enterococcal bloodstream infections.[53,54] Nonetheless, many episodes of enterococcal bacteremia in high-risk patients apparently resolve in the absence of specific therapy.[97,246] The mortality rate associated with enterococcal bacteremia in children has been lower than that reported in adults in most studies but has ranged from 7.5 percent[51] to 12 percent[17] to 20 percent[14] to 26 percent,[39] depending on the population studied.

In adults, many cases of enterococcal bacteremia are associated with a primary focus, most commonly a urinary tract infection.[55,85] In contrast, few children with enterococcal bacteremia have urosepsis, and most episodes of enterococcal bacteremia in children have not been associated with any identifiable focus.[17,199] However, Christie and coworkers identified a primary focus in 21 of 57 children (37%) with nosocomial enterococcal bacteremia, including 7 patients with urosepsis and 6 with peritonitis.[39] Many children with enterococcal bacteremia do have underlying disease involving the gastrointestinal or respiratory tract.[14,17] Central venous catheter–related enterococcal bacteremia is a growing problem in both children[14,17,39,51,56,185] and adults.[82,185] In earlier series, infections of vascular catheters were reported to account for only 2 to 14 percent of cases of enterococcal bacteremia (reviewed by Graninger and Ragette[83]), but more recent

reports have implicated intravascular devices in as many as 22 to 28 percent of these episodes in adults and children.[51,185]

Most episodes of enterococcal bacteremia do not lead to endocarditis,[142,164,185] and endocarditis is particularly uncommon in the setting of nosocomial enterococcal bacteremia. Maki and Agger[142] identified only one case of endocarditis in 118 episodes of hospital-acquired enterococcal bacteremia, whereas endocarditis was diagnosed in 12 of 35 patients with community-acquired enterococcal bacteremia.

INTRA-ABDOMINAL INFECTIONS

Most of the published information about the role of *Enterococcus* in intra-abdominal and pelvic infections comes from series of adult patients. Enterococci commonly are isolated as components of polymicrobial infections involving the abdomen or pelvis,[81] and animal models suggest that these organisms can play a synergistic role in the pathogenesis of such infections.[104] However, evidence that the addition of specific anti-enterococcal therapy improves the outcome of human intra-abdominal and pelvic infections, even when enterococci are isolated from peritoneal cultures, is not compelling.[81]

Children with enterococcal bacteremia frequently have underlying conditions related to the gastrointestinal tract.[14,17,39] Bonadio reported five cases of enterococcal bacteremia in previously healthy infants with gastroenteritis, six cases associated with bowel obstruction, and one case associated with acute appendicitis without perforation.[14] Boulanger and coworkers[17] identified underlying conditions affecting the gastrointestinal system in 8 of 32 pediatric patients, but they were unable to specifically implicate any of these conditions as the source of the bacteremia. Das and Gray detected underlying chronic gastrointestinal pathology (short-gut syndrome, congenital anomalies of the gastrointestinal tract, ulcerative colitis, chronic liver disease) in fully a third (25 of 75) of pediatric patients with enterococcal bacteremia.[51]

MENINGITIS

Enterococci are rare causes of bacterial meningitis in adults and children. Stevenson and colleagues[234] found only four cases of enterococcal meningitis among 493 episodes (0.8%) of bacterial meningitis in adults, and they identified an additional 90 cases in a literature search of the interval from 1966 to 1992. These authors reviewed 16 published cases of enterococcal meningitis in children: 11 of these 16 pediatric cases were complications of central nervous system (CNS) trauma or surgery, but 4 children (3 of the 4 were neonates) had primary meningitis.[234] Enterococci are uncommon but well-recognized causes of infection involving cerebrospinal fluid (CSF) shunts and related devices.[122,170,214] Meningitis rarely complicates nosocomial bacteremia in adults,[142,185] but it has been reported more frequently in children (particularly neonates) with bacteremia. For example, meningitis developed in 7 percent (4 of 57) of episodes of nosocomial enterococcal bacteremia in children in Cincinnati[39] and in 15 percent (4 of 26) of premature neonates with late-onset enterococcal sepsis in Houston.[56]

Stevenson and associates found that most adults with enterococcal meningitis were immunocompromised (most were receiving steroids) or had a history of CNS trauma or surgery (or both).[234] Enterococcal meningitis has been reported in an adult patient with HIV infection who had completed a course of steroids for presumed *Pneumocystis* pneumonia.[187] Although many children with enterococcal meningitis do have a history of CNS trauma or surgery or are premature infants, most do not have other identifiable predisposing conditions or a history of immunosuppressive therapy.

As with the majority of enterococcal infections, most isolates from CSF are *E. faecalis*, but meningitis and ventriculoperitoneal shunt infections caused by *E. faecium* also have been reported in children.[170,171]

NEONATAL INFECTIONS

Enterococci are important neonatal pathogens,* and the published experience with neonatal enterococcal infections constitutes much of the pediatric experience with these organisms. Although several large series of neonatal sepsis included few cases of enterococcal infection (reviewed by Klein and Marcy[121]), many centers have reported that *Enterococcus* is a relatively frequent cause of neonatal bacteremia. Siegel and McCracken[226] found that enterococci were second only to group B streptococci as causes of neonatal sepsis at Parkland Hospital in Dallas from 1974 to 1977, with an incidence rate of approximately 1.0 case per 1000 live births. Gladstone and colleagues[77] reported that *Enterococcus* caused 18 of 270 (6.7%) episodes of neonatal sepsis at Yale–New Haven Hospital during the period 1979 to 1988, which ranked fourth in incidence behind group B streptococci (64 cases, 23.7%), *Escherichia coli* (46 cases, 17.0%), and coagulase-negative staphylococci (36 cases, 13.3%). During the 1990s, reports from Houston,[57] Cincinnati,[39] and New York City[147] documented sharp increases in the rate of late-onset neonatal infection with enterococci in both hospitalized high-risk premature neonates and infants[57,147] and otherwise healthy term newborns with "community-acquired" infection.[57]

Enterococci cause both early-onset (<7 days of age) and late-onset (>7 days of age) neonatal sepsis. Early-onset disease is indistinguishable from that caused by other neonatal pathogens, but it tends to be less severe.[56] Rates of early-onset disease have remained relatively stable; however, several centers are reporting increasing rates of late-onset infection. Dobson and Baker identified 56 neonates with enterococcal sepsis during a 10-year period at Jefferson Davis Hospital in Houston; 18 of 56 (32%) had early-onset sepsis, 26 of 56 (46%) had late-onset sepsis, and 12 of 56 (21%) had sepsis associated with necrotizing enterocolitis (2 early onset, 10 late onset).[56] In this study 25 of the 26 (96%) infants with late-onset enterococcal sepsis were premature infants. Christie and colleagues[39] identified 83 cases of enterococcal bacteremia between 1986 and 1992 at Children's Hospital of Cincinnati; 58 of the 83 episodes occurred in neonates. Most cases (57 of 83, 68.7%) were nosocomial, but many (26 of 83, 31.3%) were community acquired. Young infants (<3 months of age) accounted for almost all (24 of 26, 92.3%) of the community-acquired episodes and for many (34 of 57, 59.6%) of the nosocomial infections. Bonadio[14] and Boulanger and colleagues[17] also have reported community-acquired enterococcal bacteremia in young infants.

McNeeley and associates[147] identified 138 episodes of enterococcal bacteremia in a New York City neonatal ICU during a 20-year period and reviewed 100 of the episodes in detail. These authors noted a sharp increase in the rate of enterococcal bacteremia during the second decade (1984 to 1994) of this study and found that most cases occurred in older infants; during this decade, the mean age at onset was 44.7 days (versus 16.1 days during the first decade of the study), and 65 percent (51 of 78) of the episodes occurred after the infants reached 30 days of age. Most of the infections occurred in neonates with indwelling central venous catheters (77%), and more than half of the patients had evidence of gastrointestinal disease (necrotizing enterocolitis in 33% and abdominal distention in an additional 21%). The

*See references 5, 29, 30, 32, 47, 61, 69, 78, 89, 91, 117, 129, 139, 153, 161, 164, 166, 168, 174, 196, 199, 202.

overall mortality rate in this study was 28 percent, although many of the deaths were not attributed to the enterococcal infection. Most (64%) of the episodes of enterococcal bacteremia in this series were polymicrobial.[147]

Nosocomial outbreaks of enterococcal infection, including VRE infection, have been reported in several neonatal units in the United States.[39,47,140,194] Indwelling central venous catheters, necrotizing enterocolitis, and intra-abdominal surgery are important predisposing factors for the development of nosocomial enterococcal bacteremia in neonates, whereas the genitourinary tract is implicated much less frequently as a source.[39,147]

Enterococcal bacteremia in neonates and young infants has been associated with diarrhea[14,56] and with respiratory disease,[14,56] although a causative role for *Enterococcus* in the pathogenesis of gastroenteritis or pneumonia remains undefined. Enterococci rarely cause urinary tract infections in neonates,[14,39,56,147] but nosocomial and community-acquired cases have been reported. Enterococci have been noted to cause a variety of other neonatal infections, including focal skin and soft tissue infections such as scalp abscess,[56] brain abscess,[201] omphalitis,[56] and conjunctivitis.[250]

Most enterococcal infections in neonates are caused by *E. faecalis*, but outbreaks of infection with *E. faecium* have been reported.[47] In the series from Cincinnati reported by Christie and associates and consisting largely of neonates, 82 percent of enterococcal isolates were *E. faecalis* and 14 percent were *E. faecium*.[39] McNeeley and colleagues reported the isolation of *E. faecalis* from 94 of 100 patients and *E. faecium* from 15 of 100 patients in their series (both organisms were isolated from 9 of the patients).[147] Six of the *E. faecium* isolates were resistant to vancomycin, and all six patients with VRE bacteremia died, although only one death appeared to be related directly to the VRE infection.

SEPTIC ARTHRITIS

Enterococci rarely have been reported to cause septic arthritis, but they can infect native or prosthetic joints. Raymond and colleagues[192] reported a case of enterococcal septic arthritis involving a prosthetic hip and reviewed an additional 18 cases from the literature. Eleven of these 19 episodes involved prosthetic joints (2 hips, 9 knees), and 8 involved native joints; only 1 of the 8 individuals with native-joint arthritis had an underlying abnormality of the joint. In 7 of the 19 episodes, enterococcus was isolated from synovial fluid along with a second organism (3 with coagulase-negative staphylococci, 1 with group B streptococci, 1 with *Pseudomonas*, 1 with *Streptococcus*, and 1 with *Kingella kingae*). Only one pediatric case was identified: a 21-month-old girl with septic arthritis of the wrist whose joint aspirate grew *Enterococcus* spp. and *K. kingae*.[225]

DIAGNOSIS

Enterococcal infections usually are diagnosed by isolation of *Enterococcus* from a culture of blood or another normally sterile site. As discussed earlier, enterococci are ubiquitous inhabitants of the human gastrointestinal tract, and isolation of these organisms from stool or surface cultures is not evidence of invasive infection. Although enterococci are uncommon blood culture contaminants, transient bacteremia or pseudobacteremia (or both) may be caused by these organisms.[97,246]

More problematic is the interpretation of a positive culture for enterococcus as a component of polymicrobial infections, particularly intra-abdominal and pelvic infections. In this setting, the role of the enterococcus in pathogenesis is uncertain, and therapeutic regimens that do not include anti-enterococcal agents usually suffice to effect a clinical cure.[81] However, rare cases of breakthrough enterococcal bacteremia have been reported with these regimens, and one study found a decreased rate of abdominal surgical wound infections when anti-enterococcal coverage was provided in a prophylactic regimen.[253]

ANTIMICROBIAL SUSCEPTIBILITY AND RESISTANCE

The increasing importance of *Enterococcus* as a human pathogen, especially in the nosocomial setting,[62,112,147,152,164,180,200,261,262] is of particular concern because of the concomitant development of antimicrobial resistance by these organisms. Furthermore, antibiotic resistance probably has played a critical role in allowing the organism to persist and eventually cause disease in high-risk patients despite its relatively low virulence.

Some enterococci have acquired high-level resistance to all three classes of antimicrobial agents that have been used to treat life-threatening enterococcal infections—β-lactams, aminoglycosides, and glycopeptides. This acquired resistance has occurred in the context of intrinsic (usually lower-level) resistance of enterococci to many antibiotics, and physicians have been confronted with the possibility that invasive disease caused by these organisms might not respond to any available antibiotics. Some enterococci also have acquired resistance to recently developed agents (e.g., quinupristin-dalfopristin, linezolid, daptomycin) that are active against most antibiotic-resistant gram-positive cocci (see later).

INTRINSIC RESISTANCE

β-Lactam Antibiotics

Relative resistance to β-lactam antibiotics is an intrinsic characteristic of enterococci that occurs even in human populations without previous exposure to antibiotics[154]; such resistance is due to the lower affinity of enterococcal (versus streptococcal) penicillin-binding proteins, especially PBP-5.[153,256] In general, the minimal inhibitory concentration (MIC) of penicillin for most *E. faecalis* isolates (2 to 8 μg/mL) is at least 10 to 100 times higher than that of most streptococci, and *E. faecium* is even more resistant (MIC of 8 to 32 μg/mL or higher).[164] Ampicillin is the most active of the β-lactam antibiotics against enterococci, with average MIC values approximately twofold lower than those for penicillin.[153,164] Nafcillin generally is less active than is penicillin, and methicillin is much less active (MIC > 50 μg/mL for *E. faecalis*), as are carbenicillin and ticarcillin. Importantly, enterococci exposed to β-lactam antibiotics rapidly become tolerant of the killing effects of these agents.[153,164] Along with intrinsic resistance to these agents, tolerance limits the utility of β-lactam monotherapy for the treatment of endocarditis or other severe enterococcal infections.

Imipenem has some activity against *E. faecalis* but is much less active against *E. faecium*.[153,164] None of the cephalosporins currently available has clinically useful activity against the enterococci, and frequent use of broad-spectrum cephalosporins and imipenem has been identified as a risk factor for the acquisition of nosocomial enterococcal infection.

Aminoglycosides

Enterococci are intrinsically resistant to all aminoglycosides because of diminished uptake of these drugs. For most *E. faecalis* isolates, the MIC for gentamicin or tobramycin ranges from 8 to 64 μg/mL, and that for streptomycin ranges from 12 to 250 μg/mL.[149,186,244] Moellering and colleagues first demonstrated that the addition of a cell wall–active antibiotic results in dramatically

increased aminoglycoside uptake by enterococci[156,266] and that combinations of β-lactam and aminoglycoside antibiotics can lead to synergistic killing of these organisms. All *E. faecium* strains exhibit higher MICs (than *E. faecalis* does) to certain aminoglycosides, including tobramycin, netilmicin, kanamycin, and sisomicin, and these aminoglycosides do not exhibit synergy with β-lactam antibiotics against *E. faecium*.[34,164]

Other Antibiotics

Under carefully standardized laboratory conditions, enterococci are inhibited by the combination of trimethoprim and sulfamethoxazole (TMP-SMX). However, *Enterococcus* isolates should be considered resistant to TMP-SMX because these organisms are capable of using exogenous folinic acid to evade the antimicrobial action of TMP-SMX.[265] TMP-SMX fails to eradicate enterococci in animal models of infection,[31,88] and breakthrough enterococcal bacteremia has occurred in patients being treated with TMP-SMX for enterococcal urinary tract infection.[80] Enterococci also are intrinsically resistant to clindamycin, a drug with excellent activity against many other gram-positive cocci. Most enterococci have a clindamycin MIC of 12.5 to 100 μg/mL.[164] As with low-level β-lactam resistance, clindamycin resistance is found in enterococcal isolates from human populations with no previous antibiotic exposure.[154]

ACQUIRED RESISTANCE

Enterococci have acquired resistance to antibiotics by the acquisition of both narrow– and broad–host range plasmids and via the exchange of conjugative transposons (reviewed by Murray[164]). Resistance mediated by broad–host range plasmids is of particular concern because glycopeptide resistance encoded by broad–host range plasmids has been transferred to staphylococci in vitro[176] and in vivo.[27,28,242,252]

High-Level Resistance to Aminoglycosides

Intrinsic resistance of enterococci to aminoglycosides is caused by poor drug uptake by these organisms and can be overcome effectively both in vitro and in vivo by the addition of cell wall–active antibiotics. In contrast, high-level resistance to aminoglycosides is mediated either by the acquisition of plasmids encoding aminoglycoside-modifying enzymes (affecting all aminoglycosides via several different enzymes) or by ribosomal mutations (streptomycin only). High-level aminoglycoside resistance is of great clinical importance because it eliminates synergism between the affected aminoglycoside or aminoglycosides and β-lactam or glycopeptide antibiotics.[34,164,186] All *E. faecium* strains produce a chromosomally encoded aminoglycoside acetyltransferase that eliminates synergistic killing between cell wall–active antibiotics and certain aminoglycosides (including tobramycin, kanamycin, netilmicin, and sisomicin),[34] but it does not result in high-level resistance to these compounds. Consequently, these particular aminoglycosides should not be used to treat infections caused by *E. faecium*.

Enterococci with high-level resistance (MIC usually ≥2000 μg/mL) to streptomycin, kanamycin, and several other aminoglycosides (excluding gentamicin) were identified more than 35 years ago[157] and were widely prevalent in the United States by the mid-1970s.[23] High-level resistance to streptomycin occurs via two mechanisms, ribosomal mutation or enzymatic modification by 6′-adenylyltransferase. Initial reports of high-level resistance to kanamycin were associated with the production of 3′-phosphotransferase.[34,164]

Horodniceanu and colleagues first reported high-level resistance to gentamicin in *E. faecalis* in 1979,[99] and this resistance was shown later to be mediated by a fusion enzyme containing both 6′-acetyltransferase and 2″-phosphotransferase activity. Expression of this fusion enzyme conferred resistance to all clinically useful aminoglycosides except streptomycin.[129,130] Enterococci expressing this fusion protein usually have gentamicin MICs that are 2000 μg/mL or higher. Thus, enterococci expressing this fusion enzyme and the 6′-adenylyltransferase mediating streptomycin resistance (or chromosomally mediated streptomycin resistance) are highly resistant to all available aminoglycosides and generally fail to be killed synergistically by any combination of β-lactam antibiotics and aminoglycosides. Strains of *E. faecalis* resistant to both streptomycin and gentamicin (and thus to all aminoglycosides) were detected first in Houston, Bangkok, and Santiago in 1983.[149,164] Subsequently, strains of *E. faecalis*, *E. faecium*, and other enterococci resistant to gentamicin, streptomycin, or both (or all) aminoglycosides have become increasingly prevalent[82,108,110,111,138,186] (Table 95–2). Although high-level resistance to both streptomycin and gentamicin was described first in *E. faecalis*, it now is at least as common in *E. faecium*.[110]

Enterococci resistant to all aminoglycosides have been isolated at an increasing rate from clinical specimens. Jones and colleagues and the *Enterococcus* Study Group[109] found that fully 20 percent of 1936 enterococcal isolates from late 1992 (from 97 participating laboratories in 47 states) exhibited high-level resistance to both gentamicin and streptomycin. Low and associates and the SENTRY Antimicrobial Resistance Surveillance Program[138] reported similar rates of high-level resistance to both gentamicin and streptomycin from 1997 to 1999 (see Table 95–2). Almost a third (32.8%) of enterococci collected by the SENTRY group from 1997 to 2005 were highly resistant to gentamicin.[111] Many medical centers in the United States now report that most enterococcal isolates exhibit high-level resistance to all aminoglycosides.[168]

Several new aminoglycoside-resistant genes were identified in enterococci during the late 1990s (reviewed by Chow[34]). Some enterococci produce three or more enzymes. Strains expressing some of the recently described resistant genes may fail to exhibit synergy between gentamicin and β-lactams despite MICs below those usually associated with high-level resistance. For example, the aph (2″)-Ic gene[37] found in clinical isolates of both *E. faecalis* and *E. faecium* results in gentamicin MICs of approximately 256 to 384 μg/mL, lower than the standard screening cutoff for high-level resistance to gentamicin (500 μg/mL). Nonetheless, these organisms are resistant to ampicillin-gentamicin synergism and would not be detected by standard screening methods.

High-Level Resistance to β-Lactams and Production of β-Lactamase

The mechanisms of resistance to penicillin, ampicillin, and other β-lactam antibiotics differ among enterococcal species. The high-level β-lactam resistance (ampicillin MIC ≥ 16 μg/mL) of *E. faecium*[19,87,146] and some other non-*faecalis* enterococcal strains[19] has increased considerably during the past decade and is mediated by additional alterations in PBPs, particularly PBP-5.[71,87,198] Thus, high-level resistance to ampicillin and other β-lactams in *E. faecium* (and other non-*faecalis* strains) represents an exaggerated form of intrinsic β-lactam resistance. *E. faecalis* isolates also are intrinsically resistant to β-lactams (though less so than *E. faecium*), but little change has occurred in the level of this resistance in recent years. However, some strains of *E. faecalis*[110,165,169] and *E. faecium*[46] have acquired clinically significant resistance to ampicillin and penicillin via the plasmid-mediated, constitutive production of a β-lactamase enzyme identical to that of *S. aureus*.[267] β-Lactamase–producing strains of *E. faecalis* and *E. faecium* will not be detected by routine susceptibility testing because of a pronounced inoculum effect. Therefore, enzymatic methods

TABLE 95–2 Antimicrobial Resistance Patterns of Enterococci (United States)

Agents	Gordon et al.[82] (July 1988–April 1989)			Jones et al.[110] (Enterococcus Study Group) (October 1992–December 1992)		
	E. faecalis (*N* = 632) (%)	*E. faecium* (*N* = 58) (%)	*Total* (*N* = 705) (%)	*E. faecalis* (*N* = 1428) (%)	*E. faecium* (*N* = 306) (%)	*Total* (*N* = 1936) (%)
Ampicillin resistance (MIC ≥ 16 μg/mL)	0*	41†	4	0.6-0.7‡	58.7-59.3	12
Streptomycin high-level resistance (MIC > 2000 μg/mL)	14	33	16	31.5	55.7	36
Gentamicin high-level resistance (MIC > 500 μg/mL)	11	2	10	26.0	30.8	27
Vancomycin resistance (MIC > 4 μg/mL)	0.3	0	0.3	2.0	21.9	5.6

**However, 11 of 632 (1.7%) E. faecalis isolates were β-lactamase producers.*
†No β-lactamase–producing strains were identified.
‡Only two β-lactamase–producing isolates were identified.

such as the nitrocefin test must be used to screen for β-lactamase–producing strains.[118,165] Fortunately, such strains remain very uncommon.[153]

Glycopeptide Resistance

Enterococci resistant to vancomycin were identified first in 1988[128,247] and rapidly have become a major nosocomial problem. Data collected by the NNIS of the CDC revealed a dramatic increase in the rate of vancomycin resistance in nosocomial isolates of *Enterococcus* during the interval 1989 to 1993.[180] The NNIS survey documented a 26-fold increase in vancomycin resistance among all nosocomial isolates (from 0.3% of enterococcal isolates in 1989 to 7.9% in 1993) and a 34-fold increase in vancomycin resistance among isolates obtained from adult patients in ICUs (from 0.4% of isolates in 1989 to 13.6% in 1993). Rates of vancomycin resistance in enterococci continued to increase during the next decade (see Table 95–2). The NNIS reported that fully 25 percent of enterococci associated with nosocomial infections in adult patients in ICUs in the United States were resistant to vancomycin in 1999 and 2000; by 2003 to 2004, approximately 30 percent of the enterococcal isolates from these patients were vancomycin-resistant (*http://www.cdc.gov/ncidod/dhqp/pdf/ar/ICU_RESTrend1995-2004.pdf*). Isolates of *E. faecium* are particularly likely to be vancomycin-resistant. Jones and colleagues in the SENTRY Antimicrobial Resistance Surveillance Program reported that 69.4 percent of 1512 U.S. bloodstream isolates of *E. faecium* collected between 1997 and 2005 were resistant to vancomycin.[111] Similarly, Wisplinghoff and colleagues in the SCOPE surveillance study found that 60 percent of U.S. bloodstream isolates of *E. faecium* collected between 1995 and 2002 were resistant to vancomycin, whereas only 3 percent of *E. faecalis* bloodstream isolates were vancomycin-resistant.[261]

Enterococci were the second most common organisms associated with pediatric bloodstream infections in both the 1992-1997 NNIS database[200] and in the pediatric component of the 1995-2001 SCOPE survey.[261] However, VRE organisms were detected much less frequently in these pediatric patients; only 11 percent of *E. faecium* and 1 percent of *E. faecalis* bloodstream isolates (of 357 total enterococcal isolates) obtained from pediatric patients in the SCOPE study were vancomycin-resistant.[262] This finding is important because vancomycin-resistant isolates, particularly those of *E. faecium*, also may exhibit high-level resistance to both β-lactam antibiotics and aminoglycosides, thus rendering treatment of these infections extremely challenging.*

Vancomycin resistance among enterococci is phenotypically and genotypically heterogeneous[6,78,167,168] and may or may not be associated with resistance to other glycopeptides, including teicoplanin. Five major phenotypes of vancomycin resistance (VanA, VanB, VanC, VanD, and VanE) have been characterized. The VanA and VanB phenotypes are most common, and both are transferable. The VanA phenotype is characterized by high-level resistance to vancomycin and teicoplanin, whereas strains with the VanB phenotype exhibit variable levels of resistance to vancomycin but not teicoplanin.[6,78,167,168] The VanC phenotype is limited to *E. gallinarum* and *E. casseliflavus* and is associated with constitutive, low-level, chromosomally mediated (nontransferable) resistance to vancomycin but not teicoplanin and is not transferable.[78,167,168,199] VanD and VanE phenotypes occur uncommonly and are not transferable.

High-level resistance to both vancomycin and teicoplanin (the VanA phenotype) is found primarily in strains of *E. faecium*, whereas most vancomycin-resistant strains of *E. faecalis* express the VanB phenotype and remain susceptible to teicoplanin. Jones and associates and the *Enterococcus* Study Group found that 10 of 11 (91%) vancomycin-resistant strains of *E. faecalis* remained susceptible to teicoplanin (VanB phenotype) whereas 49 of 62 (79%) vancomycin-resistant strains of *E. faecium* were resistant to teicoplanin (VanA phenotype).[110]

The biochemical mechanisms responsible for the major vancomycin-resistant phenotypes are the subject of intense study (reviewed by Murray[168] and Gold[78]). Both VanA and VanB phenotypes are mediated by homologous enzymes that catalyze the formation of an altered, vancomycin-resistant depsipeptide that is incorporated into cell wall peptidoglycan.[6,168,199] The *vanA* and *vanB* gene clusters share functional similarities but are regulated quite differently.[78,168] VanA resistance is transferable by either transposition (e.g., via the transposon Tn1546) or conjugative plasmids. VanB resistance often is encoded by chromosomal DNA but may be transferred by at least two different transposons (Tn1547 and Tn5382).[78,168] Vancomycin induces the production of enzymes encoded by both the *vanA* and *vanB* gene clusters, whereas teicoplanin induces VanA but not VanB enzymes.[78]

The peculiar phenomenon of infection caused by vancomycin-dependent enterococci has been described. Fraimow and colleagues[72] reported a urinary tract infection attributable to a strain of *E. faecalis* that would grow only in the presence of vancomycin, and Green and coworkers[89] reported breakthrough bacteremia with a vancomycin-dependent strain of *E. faecium* occurring during therapy for bacteremia. These organisms are progeny of VanA or VanB enterococci that undergo mutations preventing them from growing in the absence of glycopeptides.

Other Antibiotics

Recently licensed antimicrobials active against most antibiotic-resistant, gram-positive cocci (including VRE organisms) include

*See references 18, 74, 91, 113, 132, 159-161, 196, 199, 202, 220, 221.

Edmond et al.[62] (April 1995–April 1998) (Bloodstream Isolates)			Low et al.[138] (SENTRY Antimicrobial Surveillance Program) (1997-1999)	Jones, et al.[111] (SENTRY Antimicrobial Resistance Surveillance (1997-2005)
E. faecalis (*N* = 598) (%)	*E. faecium* (*N* = 303) (%)	*Total* (*N* = 1354) (%)	*Total enterococcal Isolates* (*N* = 2303) (%)	*E. faecium* (*N* = 1512) (%)
2.7	81.1	NA	24	89
NA	NA	NA	40	NA
NA	NA	NA	31	32.8
3.2	50.5	17.7	17	69.4

quinupristin-dalfopristin, linezolid, and daptomycin. Enterococcal isolates resistant to each of these new agents already have been identified, although overall resistance rates remain very low in the United States.

Streptogramins are protein synthesis inhibitor antibiotics and are natural combinations of two chemically unrelated molecules (referred to as streptogramin A and B, respectively).[137] Quinupristin-dalfopristin is a streptogramin agent that is active against *E. faecium* (including most strains of VREF) but lacks activity against *E. faecalis* at clinically achievable concentrations.[45] An early study found that more than 95 percent of 875 initial patient isolates of VREF were susceptible to quinupristin-dalfopristin,[65] but both emergence of resistance to this agent[155,207] and superinfection with *E. faecalis* and other organisms[35,260] have been reported during therapy with this drug. Multiple molecular mechanisms, including the expression of modifying enzymes (acetyltransferases), active transport via an efflux pump, and activation of the target site, account for resistance to quinupristin, dalfopristin, or both.[96] In the United States, overall rates of enterococcal resistance to quinupristin-dalfopristin remain low, in the range of 1 to 2 percent in most areas.[96] However, resistance to quinupristin-dalfopristin was observed in 10 percent of *E. faecium* isolates obtained from European patients in one recent survey.[55]

The oxazolidinones are a novel class of synthetic protein synthesis inhibitors that act to inhibit the formation of ribosomal initiation complexes in bacteria.[41] Linezolid, the first oxazolidinone antimicrobial approved for human use, is active against both *E. faecalis* and *E. faecium*, including VRE isolates. Initial studies demonstrated virtually uniform activity of this agent against clinical isolates of enterococci,[16,64,179] with 100 percent of 180 strains (representing multiple resistance profiles) inhibited by linezolid concentrations of 1 to 4 μg/mL in one study.[64] Furthermore, initial in vitro studies suggested that the development of resistance would occur very rarely.[41,112,268] However, resistance to linezolid has been reported in several patients receiving prolonged courses of therapy with this drug[79,144,151,217] and in some patients who have not previously received the agent,[15] and it has been associated with clinical failures.[79] Resistance to linezolid is conferred by single nucleotide changes in varying numbers of copies of the bacterial genes encoding 23S ribosomal RNA.[151] Resistance increases in strains containing multiple copies of these mutations.[144,151] Although some centers have reported increasing rates of linezolid resistance in VRE isolates[151] and nosocomial spread of linezolid-resistant VRE strains,[95] overall rates of linezolid resistance in enterococci remain low. For example, only 0.9 percent of 1512 U.S. bloodstream isolates of *E. faecium* collected from 1997 to 2005 by the SENTRY group were resistant to linezolid.[111]

Daptomycin is a novel cyclic lipopeptide antimicrobial that exhibits rapid, concentration-dependent bactericidal activity against gram-positive pathogens, including methicillin-resistant *S. aureus* (MRSA) and VRE strains.[26,232] Daptomycin has a novel method of action in which binding to bacterial membranes and triggering of rapid depolarization of membrane potential lead to inhibition of protein and nucleic acid synthesis.[1,74] Daptomycin was found to have potent activity against a panel of VRE isolates from the United States and Europe—all 75 VRE isolates tested (55 *E. faecium*, 20 *E. faecalis*) were susceptible to this agent.[206] Daptomycin also was reported to be active against a collection of VRE strains resistant to linezolid or quinupristin-dalfopristin.[3] However, daptomycin resistance has developed during therapy with this agent,[135,163,204] although resistance rates remain very low. The mechanism or mechanisms responsible for daptomycin resistance are not known.

TESTING FOR ANTIMICROBIAL RESISTANCE IN ENTEROCOCCI

All enterococci isolated from cultures of blood, CSF, or other normally sterile sites (with the possible exception of urine) should be tested for resistance to β-lactam antibiotics (ampicillin or penicillin, or both, including a test for β-lactamase production), vancomycin, and high levels of aminoglycosides (streptomycin and gentamicin)[68,193,199] by using the methodology and interpretative guidelines published by the Clinical and Laboratory Standards Institute (CLSI).[42] For multiantibiotic-resistant isolates, testing for susceptibility to alternative agents, including linezolid and daptomycin, should be performed.

Testing of enterococci for ampicillin (or penicillin) resistance must involve determination of the MIC of these agents and a test for β-lactamase production.[42] Jones and colleagues and the *Enterococcus* Study Group compared three techniques that are used commonly to determine the ampicillin MIC of enterococcal isolates—disk diffusion, broth microdilution, and E-test strips—and found excellent agreement among the three methods.[109] Enterococcal isolates with an ampicillin or penicillin MIC of 16 μg/mL or greater are considered resistant to these agents.[42] β-Lactamase-producing enterococci cannot be detected by these methods but are identified routinely by performance of the chromogenic nitrocefin assay.[237]

High-level resistance to gentamicin and streptomycin usually may be detected by either agar dilution (high-level resistance to gentamicin, MIC > 500 μg/mL; high-level resistance to streptomycin, MIC > 2000 μg/mL) or broth microdilution (high-level resistance to gentamicin, MIC > 500 μg/mL; high-level resistance to streptomycin, MIC > 1000 μg/mL).[42,235,237] These isolates also may be identified by disk diffusion with the use of high-content aminoglycoside disks (120-μg gentamicin disk, 300-μg streptomycin disks, ≥10-mm zone = susceptible)[237] or high-range E-test strips.[109,209] However, the recent identification

of novel genes encoding gentamicin resistance in both *E. faecalis* and *E. faecium* has raised concern about continued use of the 500-μg/mL cutoff for detection of high-level resistance to gentamicin (reviewed by Chow[34]). These enterococcal isolates are not killed synergistically by combinations of ampicillin/penicillin and gentamicin, even though gentamicin MICs are 256 to 384 μg/mL. If these isolates become more widely prevalent, modification of the standard screening procedures for high-level aminoglycoside resistance may be required.

Commercially available automated antimicrobial susceptibility testing methods may fail to detect vancomycin resistance in enterococci, particularly the VanB phenotype of moderate resistance,[241] although newer versions of these systems appear to be much more reliable.[76,248] Routine disk diffusion testing is more dependable but requires an extended incubation time and the use of transmitted light for examination of zone size to be highly accurate.[236,241] Agar dilution screening using brain-heart infusion agar supplemented with 6 μg/mL vancomycin reliably identifies VRE strains,[42,237,254] as does the standard broth microdilution method.[110,241] E-test glycopeptide strips are a useful alternative to the more cumbersome broth dilution methodology.

THERAPY FOR ENTEROCOCCAL INFECTIONS

Before the emergence of VRE infection as a major nosocomial problem in high-risk patients, the optimal management of serious enterococcal infections (especially endocarditis) was well established. Successful treatment of enterococcal endocarditis required the administration of combination therapy consisting of a cell wall–active agent (usually ampicillin) and an aminoglycoside (gentamicin or streptomycin). Combination therapy also was recommended for other potentially life-threatening enterococcal infections (e.g., sepsis, meningitis) based on the extensive experience with endocarditis. Other enterococcal infections, including urinary tract infections, responded well to monotherapy with a variety of antimicrobials.

In contrast, the optimal therapy for many infections caused by VRE strains remains uncertain. Older agents such as chloramphenicol are no longer used.[177,188,195] Several new agents (quinupristin-dalfopristin, linezolid, daptomycin) have been used for the treatment of VRE infections in adults. However, only one of these antibiotics (linezolid) has been approved for use in children, and enterococci resistant to these new agents already have been detected.[96,150,163] In addition, the prognosis for patients with VRE infections (including bacteremia) often is related more closely to the underlying disease than to the infection, thus complicating the management of these high-risk patients.

TREATMENT OF INFECTIONS CAUSED BY ANTIBIOTIC-SUSCEPTIBLE ENTEROCOCCI

Any discussion of the treatment of serious enterococcal infection must begin with a review of the large experience in the treatment of enterococcal endocarditis. The difficulty of treating enterococcal endocarditis has been apparent since early reports showed that penicillin alone failed to cure as many as two thirds of patients with enterococcal endocarditis but was highly effective in the treatment of streptococcal endocarditis (reviewed by Murray[164]). The early failures of penicillin therapy stimulated studies of the in vitro and in vivo effects of β-lactam antibiotics on enterococci; these studies led to the discovery that enterococci were "tolerant" of the killing effects of cell wall–active agents and provided evidence that bactericidal therapy was required to cure bacterial endocarditis reliably.

For more than half a century, standard therapy for enterococcal endocarditis has included an aminoglycoside plus a cell wall–active agent. Hunter[101] first reported clinical evidence of synergism between penicillin and streptomycin in the treatment of enterococcal endocarditis in 1947, and this synergism subsequently was confirmed for combinations of penicillin or ampicillin and streptomycin or gentamicin both in vitro and in vivo (reviewed by Murray[164]). Moellering and Weinberg[156] first demonstrated that synergy between β-lactams and aminoglycosides was a consequence of increased aminoglycoside uptake by enterococci exposed to cell wall–active agents. Glycopeptides and aminoglycosides also exhibit in vitro and in vivo synergy against "susceptible" (not expressing high-level resistance) strains of enterococci.[98,151]

The preferred therapy for endocarditis caused by "susceptible" strains of enterococci in both adults[13,151,153,197,257] and children[49,231] consists of combination therapy with parenteral ampicillin (or penicillin G) plus parenteral gentamicin (or streptomycin) for a minimal duration of 4 to 6 weeks. Patients with severe penicillin allergy should be treated with vancomycin plus gentamicin or streptomycin. Selected adult patients with a short duration of symptoms and an uncomplicated course may be treated with 4-week regimens[257,258]; most other patients, including those with mitral-valve involvement, a longer duration of symptoms (especially those with symptoms for >3 months), or prosthetic-valve endocarditis, probably should receive 6-week courses of therapy.[151,257] Interestingly, several reports indicate that the prognosis of enterococcal prosthetic-valve endocarditis is better than that of native-valve disease, perhaps because of the generally shorter duration of symptoms before the diagnosis is made.[151,197] Many patients with prosthetic-valve endocarditis caused by enterococci can be cured without surgery. Rice and colleagues[197] reported a 69 percent cure rate with medical therapy in patients with enterococcal endocarditis involving prosthetic valves.

The general consensus, based on experience with enterococcal endocarditis, is that other life-threatening enterococcal infections, including meningitis and septicemia, should be treated with bactericidal regimens.[152,164,234] The duration of therapy for uncommon enterococcal infections such as meningitis must be individualized, although 2- to 3-week courses of antibiotics have been reported to cure enterococcal meningitis.[234] The optimal treatment of enterococcal bacteremia, particularly that occurring in the nosocomial setting, remains controversial.

The prognosis for these patients varies widely and often is related more closely to the underlying disease than to the enterococcal infection. In adults, single-drug regimens generally are successful in the treatment of enterococcal bacteremia, thus indicating that bactericidal therapy often is not required in all cases.[152,164]

Considerable clinical experience supports the routine use of single-drug therapy for uncomplicated enterococcal urinary tract infection and for soft tissue infection caused by enterococci. Urinary tract infections by susceptible strains of enterococci generally respond promptly to ampicillin, penicillin, nitrofurantoin, or vancomycin.[152,162,164]

TREATMENT OF INFECTIONS CAUSED BY ANTIBIOTIC-RESISTANT ENTEROCOCCI (INCLUDING VANCOMYCIN-RESISTANT ENTEROCOCCI)

Unfortunately, the emergence of enterococci with clinically significant resistance to aminoglycosides, β-lactams, and glycopeptides has complicated the management of endocarditis and other serious infections caused by these organisms to a considerable extent. This discussion focuses on the management of endocarditis caused by drug-resistant enterococci because the need to provide bactericidal therapy has been well established in this setting. The general principles probably apply to the management of other life-threatening enterococcal infections (see earlier).

Endocarditis caused by enterococci with high-level resistance to either streptomycin or gentamicin may be treated by substitut-

ing the other aminoglycoside in a combination regimen, but isolation of strains with high-level resistance to both aminoglycosides means that no available aminoglycoside will provide synergistic killing in concert with cell wall–active agents.[151,164] No reliably bactericidal regimen is available for the treatment of endocarditis caused by these strains, even if the isolates remain susceptible to other classes of antimicrobials. Based on animal studies, some experts recommend prolonged treatment (8 to 12 weeks or more) with high-dose intravenous ampicillin given by continuous infusion in this situation (if the isolate is susceptible).[63,153] Surgical excision of infected valves may be required in such patients.[197]

Endocarditis and other serious infections caused by enterococci resistant to β-lactam antibiotics also are increasing in frequency.[87] Endocarditis caused by enterococci (usually *E. faecium*) highly resistant to β-lactams may be treated with vancomycin plus an aminoglycoside (if the strain is susceptible to vancomycin). Endocarditis caused by β-lactamase–producing strains of *E. faecalis* (or rarely, *E. faecium*) would be expected to respond to ampicillin-sulbactam because this combination is highly effective in animal models.[63] Higher-dose or continuous-infusion ampicillin may be effective in the treatment of endocarditis caused by some strains of enterococci that are "resistant" to ampicillin by current guidelines (MIC ≥ 16 μg/mL) because sustained plasma concentrations in excess of 100 μg/mL may be achieved with these regimens.[168] However, even high-dose ampicillin therapy probably will fail to cure endocarditis caused by enterococci with ampicillin MICs greater than 64 μg/mL.[21,78]

Enterococci resistant to vancomycin may remain susceptible to teicoplanin (VanB phenotype). Teicoplanin with or without the addition of an aminoglycoside has been used successfully in the treatment of serious enterococcal infections caused by susceptible isolates,[213] including some cases of endocarditis[190,213] and meningitis.[136] However, teicoplanin is not available in the United States, treatment of endocarditis with this agent has been associated with both treatment failure and relapse,[190,213] and high-level teicoplanin resistance may develop even without exposure to the drug.[92] Endocarditis caused by VanB strains of enterococci should be treated with high-dose ampicillin plus gentamicin or streptomycin if resistance to these agents is not present.

Similarly, endocarditis caused by enterococci highly resistant to both vancomycin and teicoplanin (VanA phenotype) but susceptible to β-lactams should be treated with ampicillin plus an aminoglycoside (if high-level resistance to aminoglycosides is not present). Unfortunately, the VanA phenotype is associated primarily with strains of *E. faecium*, which increasingly are resistant to β-lactams.[71,87,109] Endocarditis caused by enterococci highly resistant to both glycopeptides and β-lactams is especially difficult to treat. Combinations of ampicillin and vancomycin are not bactericidal against these isolates but may[223] or may not[29] provide additive or synergistic inhibition in vitro. If high-level resistance to aminoglycosides is not present, triple-combination therapy with a β-lactam, a glycopeptide, and an aminoglycoside may achieve bactericidal activity; such combinations are reported to be highly effective in animal models of endocarditis caused by ampicillin- and vancomycin-resistant *E. faecium*.[25] For endocarditis caused by enterococci exhibiting high-level resistance to both gentamicin and streptomycin along with resistance to β-lactams and glycopeptides, no proven effective therapies are available.

Newer Options for Treatment of Vancomycin-Resistant Enterococcal Infections

Three new antimicrobials with activity against most VRE strains recently have been licensed: quinupristin-dalfopristin, linezolid, and daptomycin. Of these drugs, only linezolid has been approved for use in children.

The streptogramin antibiotic quinupristin-dalfopristin[137] was approved by the Food and Drug Administration (FDA) in September 1999 for the treatment of adults with infections caused by VREF. This agent is not approved for the treatment of *E. faecalis* infections, nor for pediatric use. Although time-kill curves indicate that quinupristin-dalfopristin is not reliably bactericidal against strains of *E. faecium*,[45] it may provide bactericidal activity against some multiresistant clinical isolates.[141,170,207] Quinupristin-dalfopristin has been reported to effect clinical cures of several serious infections caused by multiresistant *E. faecium*, including ventriculoperitoneal shunt infections in two patients,[170,245] an infected aortic graft in one patient,[210] and VREF peritonitis in a series of adults.[141] Several case series suggest that treatment with quinupristin-dalfopristin reduces the mortality rate associated with VREF bacteremia in adults[131,155,260] and children.[84,133,249] Clinical response to quinupristin-dalfopristin has been reported in approximately 74 to 80 percent of patients with VREF bacteremia.[84,155,249,260] The lack of a consistent bactericidal effect may limit the utility of this agent in the treatment of endocarditis and other life-threatening infections. Therapy with quinupristin-dalfopristin for 10 weeks was reported to cure one patient with VREF endocarditis, although bacteremia persisted until the eighth week of therapy.[75] A second patient with VREF endocarditis failed treatment with 2 weeks of quinupristin-dalfopristin monotherapy but was cured by treatment with a combination of quinupristin-dalfopristin, doxycycline, and rifampin.[145] Other patients with VREF endocarditis have failed to respond to quinupristin-dalfopristin.[8,155]

Quinupristin-dalfopristin therapy generally has been tolerated fairly well in both adults and children, although a high rate of venous phlebitis in recipients has led to a recommendation that the agent be administered through a central venous catheter. Myalgias and arthralgias are other commonly reported side effects of this agent. The potential utility of quinupristin-dalfopristin for the treatment of pediatric patients is limited by its venous toxicity, its lack of a consistent bactericidal effect against VREF, and its lack of clinically useful activity against *E. faecalis*.[45]

Linezolid, the first oxazolidinone antibiotic, was approved by the FDA in April 2000 for the treatment of selected infections caused by antibiotic-resistant, gram-positive bacteria in adults, including those caused by VRE strains (*E. faecium* or *E. faecalis*). In December 2002, the FDA approved this agent for the treatment of pediatric patients with pneumonia, skin and skin structure infections, and VREF infections. Linezolid is available in both parenteral and oral formulations (with comparable bioavailability). Linezolid is active against many antibiotic-resistant, gram-positive pathogens, including MRSA, penicillin-resistant *S. pneumoniae*, and vancomycin-resistant isolates of both *E. faecium* and *E. faecalis*.[16,41,64,179] This agent is bacteriostatic against most susceptible organisms, including the enterococci, but it does exhibit bactericidal activity against some strains of pneumococci. Initial studies demonstrated virtually uniform activity of linezolid against clinical isolates of enterococci.[16,64,179]

Linezolid has been reported to effect both bacteriologic and clinical cure in patients with life-threatening VRE infections, including endocarditis,[8,12,32,233] bacteremia,[32,148,178] and meningitis,[219,264] although clinical experience with this agent in the treatment of endocarditis or meningitis remains limited. Several patients, including those with endocarditis[8] and persistent VREF bacteremia, who responded to linezolid therapy previously had failed therapy with quinupristin-dalfopristin.[148] Linezolid therapy has been reported to cure several patients with VRE endocarditis,[8,12,32,233] including 10 of 22 patients in one study[12] and all of 3 patients who received at least 6 weeks of the drug in another report.[233] These results are encouraging, but enthusiasm for the use of linezolid to treat serious VRE infection must be tempered by its generally bacteriostatic activity and the potential for increasing resistance to this agent. Pediatric experience with line-

zolid is limited, but the drug was demonstrated to be well tolerated and as effective as vancomycin in the treatment of children with serious gram-positive infections in a large multicenter trial.[114]

Linezolid generally has been tolerated well in both adults and children,[41,73,208] with fewer side effects occurring than with quinupristin-dalfopristin. Adverse effects most frequently reported in patients receiving linezolid have included nausea, diarrhea, and headache in adults and diarrhea and vomiting in children.[73] Reversible myelosuppression has been reported in adults.[73] Longer courses of linezolid therapy have been associated with more serious adverse effects, including optic neuropathy that usually is reversible and peripheral neuropathy that may be irreversible.[102,203]

Daptomycin is a novel cyclic lipopeptide antimicrobial that exhibits rapid, concentration-dependent bactericidal activity against enterococci, including VRE strains.[26,232] It was approved by the FDA in September 2003 for the treatment of adult patients with complicated skin and soft tissue infections caused by gram-positive bacteria, including staphylococci, streptococci, and vancomycin-susceptible strains of *E. faecalis*. Daptomycin is available in an intravenous formulation. It is not approved for use in children, and pediatric experience with the drug is very limited. Daptomycin should not be used for the treatment of pneumonia because it is inactivated by pulmonary surfactant.[227]

The published experience with daptomycin therapy in patients with VRE bloodstream infections is limited. Half (11 of 22) of the patients with VRE bacteremia or endocarditis treated with daptomycin summarized in three recent reports were cured.[126,189,218] Additional studies are under way, and pediatric trials are needed.

Numerous other promising new antibiotics with in vitro activity against VRE isolates recently have been licensed for use in adults (e.g., the glycylcycline antibiotic tigecycline) or are being evaluated in clinical trials. Agents in clinical trials include the lipoglycopeptides oritavancin and telavancin, as well as new members of the oxazolidinone and streptogramin classes of antibiotics.

PREVENTION OF ENTEROCOCCAL INFECTIONS

The explosive increase in nosocomial infections caused by vancomycin-resistant and multiantibiotic-resistant enterococci[61,180] has rendered prevention of enterococcal infections, particularly those caused by VRE strains, a public health priority in the United States.[30,78,168,193,220] In 1995, the Hospital Infection Control Practices Advisory Committee (HICPAC) recommended a series of overlapping strategies designed to prevent the development of serious enterococcal infections.[193] These strategies are aimed at simultaneous interruption of the considerable increase in vancomycin resistance in enterococci and prevention of nosocomial infection with these organisms. Implementation of such strategies has been reported to reduce or eliminate the transmission of VRE organisms in health care facilities.[182] However, overall rates of nosocomial infection by VRE continued to increase in the United States from 1995 to 2004 (*http://www.cdc.gov/ncidod/dhqp/pdf/ar/ICU_RESTrend1995-2004.pdf*), perhaps because the HICPAC guidelines have been followed inconsistently (reviewed by Cetinkaya and colleagues[30]).

REVERSING THE TREND TOWARD VANCOMYCIN AND MULTIPLE-ANTIBIOTIC RESISTANCE IN ENTEROCOCCI

The use of vancomycin[193,212,220] and treatment with broad-spectrum antibiotics, including the third-generation cephalosporins and carbapenems,[61,86,161,166,173,196] are risk factors for colonization and the development of infection with VRE strains. Consequently, the HICPAC and the CDC have recommended that all hospitals, even those at which VRE organisms have not been isolated, should (1) develop a comprehensive antimicrobial utilization plan, (2) oversee surgical prophylaxis, and (3) develop institution-specific guidelines for the proper use of vancomycin.[193]

Efforts to eliminate unnecessary use of vancomycin are of critical importance[193,220] but may be inadequate unless the use of other classes of antimicrobials is reduced as well (see later). In 1995, the HICPAC provided recommendations regarding the acceptable and appropriate use of vancomycin (Table 95–3), as well as recommendations regarding situations in which vancomycin use should be discouraged[193] (Table 95–4). These recom-

TABLE 95–3 Situations in Which Vancomycin Use Is Appropriate or Acceptable

Treatment
Treatment of serious infections caused by β-lactam–resistant, gram-positive microorganisms
Treatment of gram-positive infections in patients with serious allergies to β-lactams
Treatment of antibiotic-associated colitis only when it fails to respond to metronidazole or is severe and potentially life-threatening
Prophylaxis
Endocarditis prophylaxis after certain procedures in high-risk patients according to the American Heart Association guidelines
Prophylaxis for major surgical procedures involving implantation of prosthetic materials or devices (single-dose prophylaxis usually is adequate)

Modified from Recommendations for preventing the spread of vancomycin resistance: Recommendations of the Hospital Infection Control Practices Advisory Committee (HICPAC). M. M. W. R. Recomm. Rep. 44*(RR-12):1-13, 1995.*

TABLE 95–4 Situations in Which Vancomycin Use Should Be Discouraged

Treatment
Empiric therapy for febrile neutropenic patients (unless there is presumptive evidence of an infection caused by gram-positive organisms, such as a Hickman catheter exit-site infection, and the local prevalence of methicillin-resistant *Staphylococcus aureus* strains is substantial)
Treatment of an isolated, single blood culture positive for coagulase-negative *Staphylococcus*
Treatment (chosen for dosing convenience) of infections caused by β-lactam–susceptible, gram-positive microorganisms in patients with renal failure
Continued empiric use of presumed infection in patients whose cultures are negative for β-lactam–resistant gram-positive microorganisms
Primary treatment of antibiotic-associated diarrhea
Eradication of methicillin-resistant *S. aureus* colonization
Prophylaxis
Routine surgical prophylaxis except in patients with life-threatening allergy to β-lactams
Systemic or local prophylaxis for infection of indwelling intravascular catheters
Routine prophylaxis for very-low-birth-weight infants
Routine prophylaxis for dialysis patients
Use of vancomycin solution for topical application or irrigation
Selective decontamination of the gastrointestinal tract

Modified from Recommendations for preventing the spread of vancomycin resistance: Recommendations of the Hospital Infection Control Practices Advisory Committee (HICPAC). M. M. W. R. Recomm. Rep. 44*(RR-12):1-13, 1995.*

mendations remain useful, but the recent emergence of community-acquired MRSA infections[94,100,115] has resulted in increased empiric and definitive use of vancomycin for a HICPAC-approved indication: "treatment of serious infections caused by β-lactam–resistant, gram-positive microorganisms."[193]

In addition, efforts should be undertaken to reduce the unnecessary use of broad-spectrum antibiotics and certain agents with potent anti-anaerobic activity, particularly in settings with high rates of nosocomial infection (such as ICUs). These efforts are necessary because many studies indicate that nonglycopeptide antibiotics also exert selective pressure for VRE strains and that limitation of the use of vancomycin alone has only a modest effect on reducing VRE colonization and infection (reviewed by Rice[196]). Exposure to agents with broad-spectrum activity (but lacking anti-enterococcal activity), such as the extended-spectrum cephalosporins, appears to predispose to colonization with VRE organisms,[59,196] whereas exposure to antimicrobials with potent anti-anaerobic activity (even if they are also active against enterococci) may promote high-density, prolonged VRE colonization (by eliminating gut anaerobes that may interfere with colonization by VRE strains).[58,191,196]

PREVENTING AND CONTROLLING THE SPREAD OF NOSOCOMIAL INFECTION BY VANCOMYCIN-RESISTANT ENTEROCOCCI

The increasing prevalence of *Enterococcus* in nosocomial infections is related to the intrinsic and acquired antimicrobial resistance of these organisms, but the factors leading to increased antibiotic resistance in enterococci are not identical to those that predispose to enterococcal colonization or infection, or both. Enterococcal infections are concentrated increasingly in debilitated and immunocompromised patients, including those with malignancies, recipients of bone marrow and solid organ transplants, burn victims, premature neonates, and critically ill patients with indwelling intravascular catheters. Consequently, special attention should be paid to potential outbreaks of VRE infection in hospital wards caring for these high-risk patients.[30]

Efforts to prevent the spread of VRE colonization or infection (or both) probably will be more successful if the VRE isolates are confined to a few patients in a single area of the hospital. Widespread colonization with VRE strains may precede identification of infections by these organisms. Therefore, all hospitals should implement active VRE surveillance and formulate a multidisciplinary plan to prevent nosocomial spread of VRE strains if such organisms are identified. In hospitals that have not isolated VRE strains, periodic antimicrobial susceptibility testing should be performed on enterococcal isolates from all sources, particularly from high-risk patient populations such as those in intensive care or transplant units. If VRE organisms are identified, a comprehensive plan to prevent nosocomial spread of these bacteria should be instituted immediately. The HICPAC guidelines summarized the essential elements of such a plan.[193] Hospital infection control staff and clinical staff must be notified promptly when VRE strains are isolated from a clinical sample, and isolation precautions should be implemented immediately to prevent patient-to-patient transmission of VRE organisms. These precautions include gown and glove isolation, vigorous handwashing, dedicated use of noncritical patient items such as thermometers and stethoscopes, and prompt surveillance of any possibly exposed patients for VRE colonization. Additional measures may be necessary in hospitals with endemic or continued VRE transmission despite implementation of the aforementioned measures.[193] Ostrowsky and colleagues[182] reported that an active infection control program that included surveillance cultures and prompt isolation of infected patients successfully reduced the spread of VRE strains in health care facilities throughout the Sioux land region of Iowa, Nebraska, and South Dakota.

Once VRE organisms become endemic in a hospital unit, achieving complete eradication is very difficult. The duration of VRE colonization in individual patients may be weeks or months,[30,159] although spontaneous resolution of colonization occurs frequently.[30,158] Attempts to eradicate colonization in individual patients generally have been unsuccessful.[30,158] Consequently, the measures recommended by HICPAC and others are directed at preventing the initial establishment of VRE strains in hospitals. Implementation of these policies will require the involvement of hospital pharmacy and therapeutics committees, quality assurance programs, and medical staff. Ongoing monitoring of the efficacy of these programs will be required.

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VIRIDANS STREPTOCOCCAL INFECTIONS

A group of *Streptococcus* spp. known as viridans, alpha-hemolytic, or oral streptococci are ubiquitously present on the oral mucosa of virtually all humans. These organisms are important pathogens in children and adults alike and cause infections ranging from caries and bacterial endocarditis in immunocompetent hosts to fatal sepsis in neutropenic persons.

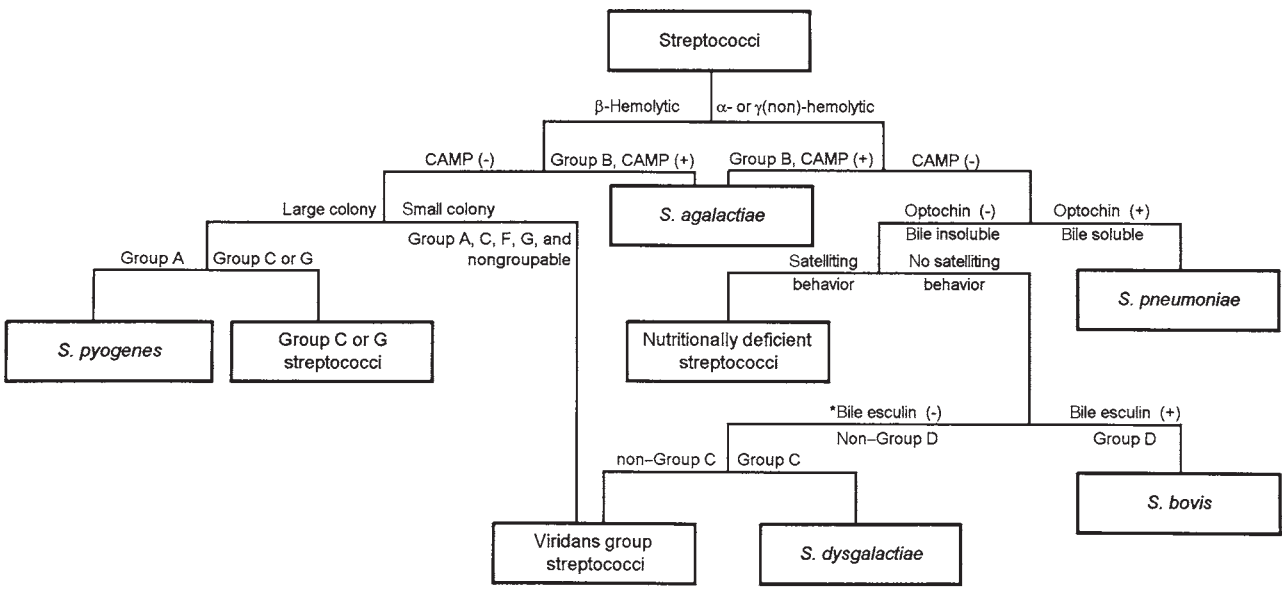
Each of the terms applied to this group is wanting. Not all members of the *alpha-hemolytic* streptococci are alpha-hemolytic, some being gamma-hemolytic (nonhemolytic) or even beta-hemolytic. *Streptococcus pneumoniae*, which is alpha-hemolytic, is considered to be a separate group. The term *viridans streptococci* likewise is inadequate because it is derived from the Latin *viridis*, or green, and refers to the sheen caused by partial hemolysis around alpha-hemolytic colonies on sheep blood agar. The term *oral streptococci* circumvents the dilemma of outliers in the hemolytic classification schema, but it also is confusing because viridans streptococci are found in sites other than the oral cavity and nonviridans streptococcal species frequently are present in the oral cavity. In accordance with the American Society for Microbiology's most recent efforts in this field,[219] the term *viridans streptococci* will be used here in referring to this diverse group of bacteria in recognition that the member organisms typically, but not invariably are alpha-hemolytic. Also of emphasis is that viridans does not refer to a species of streptococci but rather to a group of species, erroneous references to "*Streptococcus viridans*" notwithstanding.[238]

Streptococci have been reclassified on the basis of molecular and genetic studies[55,219]; this reclassification adds to the clinical confusion, at least temporarily. For example, under the new classification system, certain small-colony, beta-hemolytic streptococci, including some that are in Lancefield group A, now are considered to be viridans streptococci. The hope is that classification based on molecular relatedness eventually will lead to a clearer understanding of the infectious diseases associated with these organisms. One should recognize, however, that the current knowledge of viridans streptococcal infections is based predominantly on study of the alpha-hemolytic members of the viridans group.

MICROBIOLOGY

Streptococci are gram-positive, catalase-negative bacteria that are spherical or ovoid and less than 2 μm in diameter. They are facultatively anaerobic and nonmotile and do not produce spores or gas. Some strains require an atmosphere enriched with carbon dioxide (5%). The enterococci (distinguished by their ability to grow in 6.5% sodium chloride) and lactococci (formerly Lancefield group N streptococci) once were considered to be streptococci but now are classified as separate genera.

Figure 95–2 is a schema for classifying the clinically important streptococcal species. Hemolysis of blood agar remains a key tool



*Occasional viridans streptococcal strains are positive or weakly positive.

Figure 95–2 Schema for the identification of clinically important streptococcal species. Viridans group species are, in general, those remaining after identification of other streptococcal species. CAMP, Christie, Atkins, and Munch-Peterson test.

for classifying streptococci. Strains that are beta-hemolytic are characterized further according to colony size and Lancefield group (a serologic classification system based on cell wall carbohydrate). Group B *Streptococcus agalactiae* strains typically can be identified by beta-hemolysis and a positive result of the CAMP (Christie, Atkins, and Munch-Peterson) test[219]; however, some strains of *S. agalactiae* that are alpha- or gamma-hemolytic also are recognized by a positive CAMP test result.

Large-colony, beta-hemolytic, group A streptococci make up the species *Streptococcus pyogenes*. Other large-colony, beta-hemolytic streptococci occasionally are pathogenic, and most of them are group C or G streptococci. Small-colony, beta-hemolytic streptococci, including groups A, C, G, and F and nongroupable strains, partially constitute the *Streptococcus milleri* group of organisms within the viridans streptococci group. Among the alpha- or gamma-hemolytic streptococci, *S. agalactiae* (group B streptococci) and *S. pneumoniae* generally are identified by positive results on the CAMP test and optochin test, respectively. Bile solubility confirms an optochin-susceptible isolate as *S. pneumoniae*.

Nutritionally deficient streptococci are recognized by a requirement for the presence of a second bacterial species (*Staphylococcus aureus* typically is used in testing) to maintain growth on agar. These streptococci generally will grow in blood culture media in the absence of the reduced form of nicotinamide adenine dinucleotide (NADH) produced by a second bacterial species and may demonstrate some growth on agar in the absence of other bacteria. Nutritionally deficient streptococci once were classified as viridans streptococci, but more recent studies have classified these organisms in a new genus: *Abiotrophia adjacens* and *Abiotrophia defectiva*.[132] Excluding *Enterococcus* spp., streptococci that are alpha- or gamma-hemolytic, possess Lancefield group D antigen, and are bile esculin–positive may be identified tentatively as *Streptococcus bovis*. Though once included among viridans streptococci, this species now is classified separately from the viridans group.

Strains of one species of group C streptococci, *Streptococcus dysgalactiae*, are alpha- or gamma-hemolytic. These strains also must be distinguished from the viridans group of streptococci. A former streptococcal species included in the viridans group, *Streptococcus morbillorum*, was reclassified as *Gemella morbillorum*.[136] The remaining streptococcal organisms tentatively may be identified as viridans group streptococci. Thus, in practice, viridans streptococci continue to be characterized by the absence of features that distinguish the other major streptococcal pathogens. No characteristics can be used to confirm the identity of viridans streptococci definitively in the standard microbiology laboratory.

Classification of species within the viridans streptococci group also has been challenging. Over the years, several schemata that have been developed include those of Carlsson,[41] Coleman and Williams,[51] Facklam,[85] Ruoff and Kunz,[220,221] and Coykendall.[55] All these classification schemata lack reliable markers for member species and therefore result in inconsistent classification of clinical isolates. Consequently, efforts to characterize the clinical features of infections according to individual species of viridans streptococci have had marginal results. Molecular and polymerase chain reaction (PCR)-based taxonomies have been used to classify viridans streptococci,[4,55,183,218,290] but these techniques generally are not available in the clinical laboratory. Given the current flux in the taxonomy of these organisms, a simplified, practical approach to classifying viridans streptococci as outlined in Table 95–5 has been advocated.[219] The biochemical tests available in many clinical microbiology laboratories can be used to assign clinical isolates to one of five groups that encompass the 14 clinically important viridans streptococcal species listed in Table 95–5. Rapid molecular and PCR-based tests are available for tentative identification of individual viridans streptococcal species[89,98,189,221]; however, at this time, identification of precise species has little clinical significance except as an epidemiologic tool.

EPIDEMIOLOGY

Viridans streptococci are the predominant microorganisms in the oral flora of humans. These organisms also are found commonly in other areas of the upper respiratory tract, throughout the gastrointestinal tract, and in the female genital tract. Occasionally, viridans streptococci are found as members of the skin flora.

Viridans streptococci begin colonizing neonates shortly after birth. By 1 month of age, virtually all infants are colonized with at least one species of viridans streptococci.[186] The mix of colonizing viridans streptococcal species varies with ontogeny. For example, *Streptococcus mutans*, a species that plays an important role in the development of caries, rarely is found in predentate children but commonly is present after the eruption of teeth.[42,73,256] The ecology of other viridans streptococcal species also appears to be affected by the eruption of teeth.[43,73,239,256] In addition to these temporal factors in the colonization of infants and children, viridans streptococci have species-specific predilections for

TABLE 95–5 Simplified Classification Schema for Viridans Streptococci

Group	Species	Hemolysis	Sorbitol	Arginine	Voges-Proskauer	Mannitol	Esculin
S. milleri	*S. anginosus*	α, β, γ	–	+	+	±	±
	S. constellatus					-	
	S. intermedius					±	
S. mutans	*S. mutans*	β, γ, α	+	–	+	+	+
	S. sobrinus						
	S. rattus						
	S. cricetus						
S. salivarius	*S. salivarius*	γ, α	–	–	+	-	+
	S. vestibularis						
S. sanguis	*S. sanguis*	a	-	+	-	-	+
	S. gordonii						+
	S. parasanguis						±
	S. crista						-
S. mitis	*S. mitis**	a	-	-	-	-	-

**Previously included S. mitis, S. sanguis II, and S. oralis.*

Data from Coykendall, A. L.: Classification and identification of the viridans streptococci. Clin. Microbiol. Rev. 2:315-328, 1989; and Ruoff, K. L.: Streptococcus. In *Murray, P. R., Baron, E. J., Pfaller, M. A., et al. (eds.): Manual of Clinical Microbiology. 6th ed. Washington, D.C., American Society for Microbiology, 1995, pp. 299-307.*

certain anatomic areas of the oral cavity and pharynx. For example, *Streptococcus sanguis* is the predominant isolate of the buccal mucosa but rarely is found on the dorsum of the tongue, where *Streptococcus mitis* is the predominant species.[93] Diet also may affect the viridans streptococcal ecology. For example, consumption of sugar-containing beverages and the low pH that can result from carbohydrate intake favor colonization with caries-producing *S. mutans*.[106,254]

Little is known about the transmission of viridans streptococci, but studies of the transmission of *S. mutans* within families indicate that intrafamily transmission commonly occurs.[3,110,222] Toothbrushes may be an important vehicle of transmission of viridans streptococci in children.[153] The hands of hospital personnel, especially those with skin disorders such as eczema, also may be a vehicle of transmission of viridans streptococci.[48] In addition, dental records have been suggested as a vehicle in the transmission of viridans streptococci.[56]

Viridans streptococci have an important role in the ecology of the oral flora in that they protect against potentially more invasive pathogens by resistance to colonization with these organisms.[17,97] This mechanism has been demonstrated by a double-blind study in which patients who had received antibiotic therapy for group A streptococcal pharyngitis were treated with either a preparation that contained four species of viridans streptococci or a placebo. In the treated group, none of 17 patients experienced recurrence of group A streptococcal pharyngitis. In contrast, pharyngitis recurred in 7 (37%) of 19 patients in the placebo group.[211] A second double-blind, placebo-controlled multicenter trial by the same group confirmed the finding of the first study.[212] Viridans streptococci also may have a role in resistance to colonization by pathogens such as methicillin-resistant *S. aureus* (MRSA) in the oral cavity of infants[269]; nontypeable *Haemophilus influenzae*, *S. pneumoniae*, and *Moraxella catarrhalis* at the eustachian tube orifice[255]; and *Neisseria gonorrhoeae* in the female genital tract.[162] In addition to competition for mucosal adherence sites, viridans streptococci produce hydrogen peroxide and bacteriocins that are bactericidal to certain competing bacteria and may contribute to resistance to colonization.[57,59,270,275] Conversion of hydrogen peroxide to ozone in the oral cavity may play a role in the resistance to colonization by other bacterial species and *Candida*.[268]

PATHOGENESIS

Viridans streptococci are organisms of low virulence that usually cause nonpyogenic infection when they do cause infection. They are involved most often in localized infections of the sinuses and oral cavity, including the teeth, in which the tissues are invaded directly by colonizing organisms. Caries is a disease of teeth that develops over a period of years, often in association with *S. mutans* infection. Viridans streptococci also have a role in a variety of gingival diseases, possibly including gingival hyperplasia accompanying the chronic administration of phenytoin. In an experimental animal model, phenytoin-induced gingival hyperplasia was enhanced in rats infected with *Streptococcus sobrinus* when compared with uninfected control animals.[175] Occasionally, viridans streptococci, usually organisms of the *S. milleri* group, cause pyogenic infections, including abscesses in the brain, lung, and abdomen. An intriguing, but preliminary study suggested that viridans streptococcal–related dental disease is associated with the subsequent development of coronary artery heart disease.[146] Viridans streptococci are among the microorganisms isolated from atherosclerotic plaque, although the role of these microbes in the pathogenesis of atherosclerosis has not been determined.[47] Typically, viridans streptococci cause life-threatening infection only in settings in which the oral mucosa is disrupted and the host's mechanisms of clearance are compromised, such as in patients with neutropenia or cardiac-valve disease.

The preponderance of viridans streptococci in the oral flora rather than its virulence accounts for the domination of viridans streptococci in infections originating from the mouth. In a study of 36 children who underwent extraction of normal or abscessed teeth, 11 (30%) had postextraction positive blood cultures.[245] In all 11, viridans streptococci were isolated exclusively. Bacteremia occurred more commonly after removal of diseased teeth (37%), but it also occurred after removal of normal teeth (23%).

A separate study of 58 children who underwent dental extraction included 26 who received penicillin, amoxicillin, or erythromycin prophylaxis because of a risk of endocarditis developing.[54] Bacteremia was detected in only 9 (35%) of the 26 children who received prophylaxis and in 20 (63%) of 32 children who did not ($p < 0.05$). In this study, viridans streptococci accounted for 37 percent of the blood isolates, strict anaerobes accounted for 26.5 percent, and a variety of organisms accounted for the remainder. The number of colony-forming units per milliliter of blood ranged from 2 to 12. Even within a single colony-forming unit, more than one bacterial species sometimes was found after subculture.

Although these two studies were dissimilar in the diversity of organisms isolated, both indicated that viridans streptococci are the organisms most likely to invade the blood after trauma to the oral cavity. Also reflecting the predominance of these organisms in oral and salivary flora, bacteremia with viridans streptococci occurs commonly during orthodontic banding[84] and dilation of esophageal strictures.[296]

The ability of viridans streptococci to bind to oral mucosa, tooth surfaces, and dental plaque via interaction of specific microbial and host "receptors" accounts for the preferential colonization of the oral cavity by these organisms.[273] This interaction also helps explain localization of the various viridans streptococcal species to distinct anatomic sites, as well as the involvement of ontologic factors in colonization. The precise nature of bacterial adherence to the oral mucosa is not defined but appears to involve streptococcal lipoteichoic acid.[118] Through release of modulin protein I/II, viridans streptococci induce the expression of adhesion molecules such as E-selectin and intercellular (ICAM-1) and vascular cell (VCAM-1) adhesion molecules on endothelial cells, promote transendothelial migration of neutrophils in vitro, and induce release of cytokines in epithelial (interleukin-6 [IL-6]) and endothelial cells (IL-6 and IL-8).[277,278] The host's immunologic status also may be an important determinant of adherence and subsequent invasion. For example, immunocompetent persons produce secretory IgA to *S. mutans*,[11] and such antibody is capable of preventing caries.[169] Viridans streptococci within the biofilm present in dental plaque are at least 500 times more resistant to antibiotic treatment than predicted on the basis of their susceptibility in culture medium; therefore, this finding suggests that antibiotic treatment cannot eradicate viridans streptococci from dental plaque, even temporarily.[140]

At the University of Texas M. D. Anderson Cancer Center in Houston, the incidence of viridans streptococcal bacteremia increased extraordinarily between 1972 and 1989, from 1 to 47 cases per 10,000 admissions.[75] Analysis of risk factors associated with viridans streptococcal bacteremia indicated that prophylactic administration of trimethoprim-sulfamethoxazole (TMP-SMX) or a fluoroquinolone, profound neutropenia, and the administration of antacids or histamine type 2 receptor antagonists each significantly predisposed patients to the development of bacteremia.[75] Presumably, administration of the implicated antibiotics and antacids favored the overgrowth of viridans streptococci and their proliferation throughout the gastrointestinal tract, which in turn would favor the development of viridans streptococcal bacteremia in an immunocompromised host, especially if the mucosal barrier was disrupted by cytotoxic

chemotherapy and the host was neutropenic. Of note, administration of cytarabine (cytosine arabinoside or ara-C) was not associated with viridans streptococcal sepsis in this study despite being identified as a major risk factor in many other studies.[28,29,39,67,123,157,167,205]

The ability to adhere to damaged cardiac valves and vegetations is a principal factor in the predominance of viridans streptococcal endocarditis. Strains of viridans streptococci carried by healthy children adhered less well to buccal and endocardial cells than did disease-producing strains isolated from children with endocarditis.[229,230] Lipoteichoic acid is thought to help mediate adhesion of viridans streptococci to endocardium, and penicillin prophylaxis may be effective in part because of its reduction of lipoteichoic acid on the bacterial cell surface.[148]

The ability of antibiotics to alter the surface properties of bacteria, even when the bacteria are resistant to the bactericidal action of the drug, may be an important determinant in the effectiveness of prophylactic antibiotics.[148,236] In the host, fibronectin is an important determinant of binding of viridans streptococci to damaged endothelium. Viridans streptococci do not bind to soluble fibronectin, but a reactive domain becomes available for binding when the fibronectin molecule is immobilized, as is the case in endocarditis.[147] Mutant viridans streptococci that cannot bind to fibronectin were significantly less virulent in an animal model of endocarditis.[147]

The ability of viridans streptococci to induce platelet aggregation also may be involved in the pathogenesis of endocarditis.[90,154] In viridans streptococci–challenged, anticoagulated rabbits, only microscopic vegetations developed despite fulminant sepsis. In contrast, viridans streptococci–challenged, non-anticoagulated animals tended to have large vegetations and a subacute course.[121] These findings are concordant with those of a separate study in which viridans streptococci–challenged thrombocytopenic rabbits had a greater density of bacteria within vegetations than did nonthrombocytopenic control animals; these results suggest that platelets limit progression of the disease.[250] Surprisingly, neutropenia appears to have little effect on susceptibility to endocarditis in animal models but affects containment of the infection.[165,166]

In neutropenic patients with cancer and in patients who have received bone marrow transplants, viridans streptococci cause septic shock and adult respiratory distress syndrome (ARDS). Viridans streptococci also induce nephritis.[5,259] In these circumstances, little is known about the mechanisms involved. Viridans streptococci produce no endotoxin, and specific exotoxins have not been identified. Nonetheless, products of these organisms can activate complement[173] and induce the production of tumor necrosis factor-α (TNF-α) and TNF-β; IL-1, IL-2, IL-6, and IL-8; interferon-γ; and nitric oxide.[18,80,112,161,182,240,242,253] Strains isolated from patients with sepsis were found to be more active in inducing TNF-β and IL-8 production than were colonizing strains isolated from healthy subjects.[243] In a study of two neutropenic patients with fatal viridans streptococcal sepsis, high blood levels of IL-6 were detected, especially late in the course, whereas IL-1 and TNF-α blood levels were not remarkable.[78] Viridans streptococcal lipoteichoic acid induces cytokine and nitric oxide production in vitro,[80,173] but the clinical significance of these observations is not known. In contrast to group A streptococci isolated from patients with septic shock, viridans streptococci isolated from the blood of pediatric cancer patients with septic shock do not express superantigens in vitro.[180] Thus, intravenous immunoglobulin, which has been suggested as an adjuvant therapy for group A streptococcal shock, may not be effective in patients with viridans streptococcal sepsis.

CLINICAL MANIFESTATIONS

The viridans streptococcal species are a diverse group of bacteria, and consequently, a variety of clinical manifestations are associated with the infections that they cause. Typically, the viridans streptococci cause nonpyogenic infections such as bacteremia and endocarditis, whereas the *S. milleri* group, also referred to as the *Streptococcus intermedius* group, tends to cause invasive pyogenic infections, including bone infections, brain abscesses, appendicitis, and pulmonary and abdominal abscesses.[176]

SEPSIS IN IMMUNOCOMPROMISED HOSTS

For obscure reasons, during the past 2 or 3 decades the relative incidence of gram-positive bacterial infections, especially viridans streptococcal infection, has increased in immunocompromised hosts. In 1978, viridans streptococci first were perceived as an important cause of sepsis in neutropenic patients with cancer when 29 episodes in adults and children at the National Cancer Institute[192] and 6 episodes in children at the M. D. Anderson Cancer Center[117] were reported. Before these reports, the significance of blood isolates of viridans streptococci in this setting generally was not appreciated. The fact that no deaths occurred in the original National Cancer Institute series or in a subsequent series from that center[216] suggested that viridans streptococci produce a benign bacteremia similar to that seen with coagulase-negative staphylococci. In contrast, three of the six children at M. D. Anderson died. Several other centers in Europe and North America have reported fulminant, sometimes fatal viridans streptococcal sepsis in patients with cancer and in those who have received transplants.* Overall, the death rate associated with viridans streptococcal sepsis has ranged from 0 to 50 percent, with most centers reporting mortality rates of approximately 10 percent. In some centers, viridans streptococci are the most common cause of fatal sepsis.[21,28,105] The incidence of viridans streptococcal sepsis appears to be higher in children than in adults.[160,248]

The oral cavity is the most common portal of entry in immunocompromised hosts. Catheter-related viridans streptococcal bacteremia is an unusual occurrence. However, because administration of antacid is a risk factor,[75] the lower gastrointestinal tract probably is a portal of entry in some patients. Several factors predispose patients to the development of viridans streptococcal sepsis. Profound neutropenia clearly is a predisposing factor,[28,75] although viridans streptococcal bacteremia occasionally develops in patients with cancer and absolute neutrophil counts greater than 1000 cells/mm^3. Mucositis, especially oral mucositis, is a definitive risk factor.[28,29,75,105,157,167] Cytarabine appears to be a risk factor even beyond its predisposition to produce clinically evident mucositis,[28,29,39,67,79,123,157,167,205] although at least one study has not found this association.[99] The use of prophylactic TMP-SMX or quinolones also is an important risk factor.[28,29,49,75,105,188] The observation that the administration of acyclovir may decrease the incidence of viridans streptococcal bacteremia in transplant patients suggests that herpes simplex virus infection may be a risk factor as well.[206] Allogeneic bone marrow transplantation likewise has been associated with an increased risk for the development of viridans streptococcal sepsis, with a greater than fourfold increase in the mortality rate.[157] An association between the development of viridans streptococcal sepsis and menstruation has been noted.[75]

The hallmark clinical feature of viridans streptococcal sepsis in an immunocompromised host is fever that typically is high, occurs in the presence of neutropenia and mucositis, and frequently lasts for several days after viable organisms are cleared from the blood. Most patients recover uneventfully. However, fulminant septic shock may occur. Shock may appear early,

*See references 10, 13, 21, 28, 29, 39, 50, 52, 75, 105, 107, 114, 141, 157, 160, 188, 203, 205, 235, 241, 248, 281.

although it often is delayed for 2 or 3 days after the onset of sepsis, and it occurs despite prompt sterilization of blood by effective antibiotics.[248] ARDS frequently occurs in severe cases, usually 2 or 3 days after the initial bacteremia.[10,75,120,257] Focal complications, including pneumonia and meningitis, are uncommon occurrences. Rash and palmar desquamation may be present but are not common manifestations.[75] For unexplained reasons, the incidence of aspergillosis is increased after the development of viridans streptococcal sepsis in children with cancer.[181]

Several studies have implicated *S. mitis* as a more pathogenic species of viridans streptococci with a predilection to cause shock and ARDS in patients with cancer,[10,29,50,71,111,163,248,257] but other studies have not found a clear relationship between clinical features and species.[75] *S. mitis* isolated from cancer patients with sepsis also has been found to be more resistant to antibiotics than other species of viridans streptococci isolated from the same patient population are.[111]

NEONATAL SEPSIS, MENINGITIS, AND OTHER INFECTIONS

Viridans streptococci are normal inhabitants of the female genital tract and are a common cause of chorioamnionitis and subsequent abortion,[9] as well as a frequent cause of neonatal sepsis and meningitis.[1,25,34,94,145,174,284] Viridans streptococci ordinarily do not colonize newborns' skin and should not be dismissed as contaminants when isolated from normally sterile sites.[1] In some newborn centers, the incidence of viridans streptococcal bacteremia and meningitis has approached or exceeded that attributable to group B streptococci, although viridans streptococcal infections tend to be less severe.[34,174] *Streptococcus oralis* (*S. mitis*) caused more than half of the cases in one study.[284] The portal of entry of the organism generally is unknown in this setting, but a fetal scalp electrode was implicated in one case.[95] Unusual manifestations in newborns include pharyngitis and epiglottitis,[31] endocarditis,[164] and conjunctivitis.[138]

ENDOCARDITIS

Viridans streptococci are a common cause of endocarditis at all ages because of the organism's ability to adhere to diseased endocardium and its frequent implication in bacteremia during dental procedures and routine mouth care. A recent study at a teaching hospital in Finland found that starting in 1995, *S. aureus* became more prevalent than viridans streptococci as a cause of endocarditis[113]; however, a study from Minnesota found no significant change in the etiology of endocarditis from 1970 to 2000, with viridans streptococci being the most common cause throughout.[263] Viridans streptococci tend to cause subacute endocarditis; blood cultures may be positive only intermittently. *S. sanguis* and *S. mitis* are the species identified most commonly.[68,201,207,251,286] Complications of viridans streptococcal endocarditis include septic pulmonary emboli, congestive heart failure, pericarditis, myocardial abscess, meningitis, osteomyelitis, and glomerulonephritis.[139,201] The shock and ARDS that develop in immunocompromised patients do not occur in immunocompetent patients with endocarditis.

PNEUMONIA

Pulmonary infiltrates frequently complicate viridans streptococcal sepsis in neutropenic hosts. In most cases, these infiltrates represent ARDS, not primary pneumonia. However, several cases of primary viridans streptococcal pneumonia that developed in previously healthy persons have been reported.[96,104,155,177,198,226] In some cases the diagnosis was supported by multiple isolates of viridans streptococci obtained from blood in the absence of endocarditis. The incidence of viridans streptococcal pneumonia may be underestimated significantly because tracheal isolates of viridans streptococci usually are discounted as contaminants and tracheal isolates probably do represent contamination of the specimen with oral flora in many cases.[213] Given the increasing incidence of viridans streptococcal infection and the escalating prevalence of antibiotic resistance among these organisms, clinicians should consider viridans streptococci as a potential cause of pneumonia when they are isolated in the absence of other pathogens.

OSTEOMYELITIS AND SEPTIC ARTHRITIS

Viridans streptococci are unusual causes of osteomyelitis and septic arthritis. Extension of an oral infection into the mandible or maxilla is the most common circumstance in which viridans streptococci cause osteomyelitis,[185] but vertebral osteomyelitis caused by these organisms has been described on several occasions.[2,38,62,217,271,282] Infection of the long bones[204] and septic arthritis[14] occur infrequently.

CARIES

Although caries was recognized as an infectious disease in 1890 by workers trained in Robert Koch's laboratory, its infectious nature still is not accepted universally.[8,12,72] Evidence that *S. mutans* is the major cause of caries in children and adults alike is substantial,[8,12,32,233,258,267,274] but other viridans streptococci and *Actinomyces* spp. also have cariogenic potential.[233,274] *S. mutans*, which colonizes the oral cavity only after the eruption of teeth, has a predilection for the dental surfaces, metabolizes sucrose, and produces a strong acid that weakens the mineral matrix of teeth and allows the organisms to penetrate the structure of the teeth.[44,233] Fluoridation of the water supply has been credited with strengthening tooth enamel, thereby fostering resistance to the harsh acids produced by *S. mutans*.[233] Fluoride has potent antibacterial action against *S. mutans*, particularly at low pH.[44,276] Thus, fluoridation of water supplies may represent, albeit in a unique form, the most widespread and successful use of antibacterial prophylaxis. Topical treatments also provide protection against the cariogenic actions of *S. mutans* and in some circumstances depend on the antibacterial action of fluoride.[116,262,272,294] Short courses of oral antibiotics can reduce colonization with *S. mutans* substantially and may be an important adjunct to the treatment of caries.[247] Specially designed culture systems are available to detect the presence and measure the concentration of *S. mutans* in plaque and thus permit the effects of therapy to be monitored.[32,283] Because the development of cavities typically requires a few years of infection, opportunity to interrupt the pathogenesis of this disease is ample.[8]

ABSCESSES AND OTHER INFECTIONS

The suppurative infections produced by viridans streptococci typically are caused by the *S. milleri* group: *Streptococcus anginosus*, *Streptococcus constellatus*, and *S. intermedius*. Like *S. aureus*, members of this group resist killing by polymorphonuclear leukocytes and stimulate less chemotaxis than other viridans streptococci do.[280] Because *S. milleri* bacteria inhabit the upper respiratory tract and the gastrointestinal tract and are relatively invasive, these organisms cause sinusitis, otitis media, meningitis, and abdominal abscesses and often are present in brain abscesses.[24,53,77,170,172,176,224] *S. milleri* group organisms may infect the brain via a hematogenous route that originates in the oral

cavity or intestinal tract or by direct invasion through the upper respiratory tract. Sepsis in non-neutropenic patients occurs uncommonly, usually in association with an intra-abdominal source.[151,224]

Among other unusual infections caused by viridans streptococci was a large outbreak of pharyngitis caused by *S. mitis* accompanied by a toxic shock–like syndrome in half of the cases.[150] Meningitis resulting from both hematogenous and direct spread has been observed in healthy adults.[149] Iatrogenic meningitis caused by contamination with oral viridans streptococci may complicate lumbar puncture or other central nervous system procedures.[82,228,291] Lung abscesses caused by viridans streptococci may result from the aspiration of saliva.[128,198] Empyema and mediastinitis are other reported thoracic infections associated with viridans streptococci.[19,128] Myositis complicated by rhabdomyolysis in children with leukemia has been reported.[225] Viridans streptococci also are found occasionally in liver abscesses,[16,91,100] peritonitis,[23] and appendicitis.[193]

DIAGNOSIS

Infections caused by viridans streptococci cannot be distinguished clinically from infections caused by other gram-positive and gram-negative bacteria. Collection of adequate culture specimens is essential for establishing the diagnosis. Viridans streptococcal infections typically are diagnosed by culture of blood or other normally sterile tissue. The organisms may be present in low concentration. A volume of 30 mL of blood has been suggested as the optimal volume for culture in an adult-sized patient.[232] The addition of agents that neutralize the antibacterial effects of fresh blood, such as sodium polyanethole sulfonate, significantly improves the yield of blood cultures.[231] Chemotherapeutic agents may interfere with the detection of viridans streptococci in blood.[187]

Viridans streptococci normally are not part of the skin flora and ordinarily should not be considered contaminants. Viridans streptococci have been reported to cross-react with *S. pneumoniae* Omniserum, and this cross-reaction has the potential to lead to false-positive results of the Omniserum test.[120]

ANTIBIOTIC SUSCEPTIBILITY

In the past, viridans streptococci have been considered penicillin-sensitive. Today, penicillin-resistant and penicillin-tolerant viridans streptococci are found worldwide as causes of sepsis, endocarditis, meningitis, and other infections, including conjunctivitis of the newborn.* Penicillin resistance occurs commonly, particularly in patients receiving long-term penicillin therapy,[7,26,184,246,249] although even short courses of antibiotics may predispose patients to colonization with resistant viridans streptococci.[83,101,171] Infection with penicillin-resistant viridans streptococci is associated with a higher mortality rate than infection with penicillin-susceptible strains is.[64,244] Antibiotic resistance may develop in all species of viridans streptococci, although *S. mutans* rarely exhibits resistance.[127]

Resistance to a variety of antibiotic classes has developed during the past 2 decades,[196] although resistance still is an uncommon finding in patients who live in the community and have not been treated recently with antibiotics.[292] Resistance to cephalosporins is widespread, with the general pattern of susceptibility being the following: cefotaxime and ceftriaxone usually are more active than cefepime and cefuroxime, both of which are more active than ceftazidime and cephalexin.[65,156,190,287] Previous cephalosporin therapy is a risk factor for cephalosporin resistance, an observation with particular relevance for patients with cancer.[36,133] Resistance to fluoroquinolones, especially ciprofloxacin and ofloxacin, is a common finding.[46,60,63,134,227,290] Levofloxacin has better activity against viridans streptococci than the older fluoroquinolones do, and the newer agents garenoxacin, gatifloxacin, and moxifloxacin have enhanced activity against most gram-positive pathogens, including viridans streptococci.[208-210] However, resistance develops rapidly when patients receive quinolones prophylactically—levofloxacin, gatifloxacin, and moxifloxacin included.[197,260] At the Mayo Clinic in Rochester, Minnesota, viridans streptococcal sepsis developed in 6 (16%) of 37 transplant patients receiving levofloxacin prophylaxis (3 experienced shock); blood isolates were resistant to levofloxacin, as well as the newer quinolones gatifloxacin and moxifloxacin.[202] Resistance was associated with mutations in the quinolone resistance-determining region of GyrA or ParC. Resistance of viridans streptococci to aminoglycosides,[131] tetracycline,[60,65,290] TMP-SMX,[60,63,65,290] clindamycin,[63,266,290] erythromycin,[33,60,63,65,290] other macrolide antibiotics,[6] and vancomycin[237] also has been reported.

Resistance of viridans streptococci to penicillin appears to involve chromosomally mediated alterations in the organisms' PBPs.[200,295] Initially, researchers suspected that genes conferring penicillin resistance might have been acquired from *S. pneumoniae*.[70] Subsequent studies, however, indicated that penicillin resistance may have evolved first in *S. mitis* and that *S. pneumoniae* acquired genes mediating penicillin resistance from this and other closely related viridans streptococcal species.[45,69,195] Quinolone resistance determinants also are transmitted efficiently between viridans streptococci and *S. pneumoniae*,[126] and the *mef*(E) gene that confers resistance to macrolides is transmitted readily from viridans streptococci to *S. pyogenes*.[129]

The clinical impact of the development of penicillin resistance among viridans streptococci has been far-reaching. The emergence of penicillin-resistant viridans streptococci incidentally may be encouraging the development of vancomycin-resistant enterococci because of the increased use of vancomycin to prevent and treat viridans streptococcal infection in patients with cancer.

Overall, vancomycin is the antibiotic with the most reliable activity against viridans streptococci. Teicoplanin also has been used successfully to treat patients with endocarditis caused by viridans streptococci.[288] Daptomycin, dalbavancin, quinupristin-dalfopristin, and linezolid are active in vitro against most clinical isolates of viridans streptococci.[137,223,285]

TREATMENT

Empiric antibiotic therapy for viridans streptococci should be based on the local pattern of antibiotic susceptibility among recent clinical isolates. Antimicrobial susceptibility testing of viridans streptococcal isolates is necessary. Limited data indicate that antibiotic susceptibility testing performed with the E-test correlates well with agar dilution susceptibility testing.[214] Although vancomycin-resistant clinical isolates currently are very rare findings, inclusion of vancomycin in susceptibility testing is advisable. For infections other than endocarditis and meningitis, single-antibiotic therapy usually is preferred. An exception is infection in neutropenic patients with cancer because restricting antibiotic therapy to drugs active against gram-positive bacteria exclusively may predispose these patients to the development of gram-negative bacterial infections.[191] Some investigators have advocated reserving vancomycin for neutropenic patients with shock or ARDS,[265] whereas others have advocated inclusion of vancomycin in the initial empiric therapy for patients with fever and neutropenia.[76,235] Viridans streptococcal sepsis may occur in neutropenic

*See references 30, 35, 74, 87, 103, 119, 138, 144, 194, 200.

patients despite ongoing antibiotic therapy with β-lactam agents to which the infecting organisms are susceptible in vitro[235]; the fact that this phenomenon has not been observed with vancomycin therapy supports the use of this antibiotic for the treatment of viridans streptococcal sepsis in neutropenic patients.

Combination therapy often is advocated for the treatment of viridans streptococcal endocarditis[27,92,159,279] and may be considered for the treatment of meningitis, especially when the infecting organism is tolerant of penicillin.[81] Combinations of either penicillins and aminoglycosides or vancomycin and aminoglycosides are used most commonly. Penicillin and vancomycin are thought to increase uptake of aminoglycosides, thereby leading to synergistic bactericidal activity.[293] However, a recent meta-analysis of five clinical trials failed to identify any overall improvement in the outcome of patients who had endocarditis and were treated with β-lactam and aminoglycoside combination therapy versus β-lactam monotherapy.[86] Vancomycin monotherapy has been used to treat patients with penicillin-resistant viridans streptococcal endocarditis successfully,[122] and endocarditis also has been treated successfully with linezolid[179] or ceftriaxone[234] monotherapy.

Frequent dosing of antibiotics generally has been recommended; however, viridans streptococci exposed to penicillin or cephalosporin plus aminoglycoside combinations appear to be susceptible to a post-antibiotic effect.[40,135,142] Consequently, longer dosing intervals may be satisfactory, but data are insufficient currently to support a recommendation. Viridans streptococcal endocarditis usually is treated for 4 to 6 weeks; the duration of therapy for infections at other sites has not been studied but generally can be guided by site-specific practice and individual clinical response.

In the treatment of endocarditis, penetration of antibiotics into the fibrin vegetation may be impeded markedly. Viridans streptococci produce an exopolysaccharide composed predominantly of dextran, which may limit penetration. Experimental studies indicate that the degree of exopolysaccharide production by viridans streptococcal strains affects the success rate of antimicrobial therapy.[199] In accord with this observation, administration of dextranase to animals with experimental viridans streptococcal endocarditis enhances antibiotic efficacy.[168] In the future, such adjuvant therapies designed to reduce the size or density of valvular vegetations may offer promise for patients not helped by conventional antibiotic therapy for endocarditis.

Another setting in which adjuvant therapy may be considered is viridans streptococcal sepsis in neutropenic patients who have received cytarabine chemotherapy. One uncontrolled trial suggested that the early addition of high doses of corticosteroids to the antimicrobial therapeutic regimen may reduce the incidence of associated ARDS and death.[66] However, data are insufficient to recommend this approach routinely.

PREVENTION

Attempts to prevent viridans streptococcal infection have focused on three distinct settings: prevention of caries, prevention of endocarditis, and prevention of sepsis in neutropenic patients with cancer. Efforts have been successful in the former two settings. However, the emergence of penicillin-resistant viridans streptococci and concern about the possible emergence of vancomycin resistance highlight the need for new approaches to prevent infection with these ubiquitous organisms. In developing such methods, investigators should not forget that the resistance to colonization provided by viridans streptococci can protect the host from more virulent pathogens (see "Epidemiology").

The incidence of caries in the United States has been reduced sharply by fluoridation of water supplies, inclusion of fluoride in toothpaste, and modification of diet (e.g., use of sugar substitutes). Fluoride acts as an antibacterial agent that also strengthens resistance of the teeth to invasion by bacteria. The use of dental varnishes, gels, and rinses that contain fluoride or other antibacterial agents such as chlorhexidine or vancomycin may be beneficial in selected cases.[88,130,158,178]

The American Heart Association has led a successful effort to prevent the development of endocarditis by systemic antibiotic prophylaxis of patients with known endocardial defects who are undergoing dental procedures.[58] These efforts are aimed especially at preventing viridans streptococcal endocarditis, and penicillin is the antibiotic used most commonly. The mechanism or mechanisms by which antibiotic prophylaxis prevents endocarditis are not understood completely. In animals, endocarditis can be prevented by the administration of bacteriostatic antibiotics and by maintenance of serum levels of bactericidal antibiotics that are well below the minimal inhibitory concentration for the colonizing viridans streptococci.[102] Vancomycin has been observed to prevent the development of vancomycin-tolerant *S. sanguis* endocarditis in experimentally challenged animals without reducing the incidence or level of bacteremia; thus, antibiotics may prevent endocarditis by reducing bacterial adherence to endocardium.[20] This hypothesis is supported by a study in which bacteremia, in some cases with antibiotic-resistant organisms, developed in 21 percent of children receiving antibiotic prophylaxis, but endocarditis rarely occurred.[115] However, studies in animals have indicated that the probability of preventing endocarditis is correlated with the antibiotic susceptibility of the challenging streptococcal strains.[115]

Prophylaxis should be targeted carefully[264] and administered immediately before dental procedures are initiated. Increased numbers of antibiotic-resistant viridans streptococci can be detected within 6 hours of administration of antibiotic treatment, and they persist for 9 days or longer.[143] Experimental studies of the prevention of endocarditis by the administration of antibiotics *after* challenge with bacterial inocula have yielded inconsistent results,[22,125,152] and the clinical utility of this approach is not known. Topical treatment with vancomycin or chlorhexidine has been advocated as an adjuvant to prevent the development of endocarditis, but the efficacy of this approach has not been proved.[252,289]

In recent years, viridans streptococcal infections have become a major problem in neutropenic patients with cancer and in recipients of bone marrow transplants. Because penicillin-resistant viridans streptococci are widespread, some cancer centers include vancomycin in the initial empiric antibiotic regimen for neutropenic patients with unexplained fever.[235] In addition, despite concern about the possibility of inducing vancomycin-resistant bacterial strains, physicians managing bone marrow transplant units are administering intravenous vancomycin prophylactically to high-risk patients in an effort to prevent the development of viridans streptococcal sepsis; the results of an observational cohort study support this practice.[124]

Two noncontrolled trials of oral vancomycin paste[15] or vancomycin mouthwash[37] in children who were receiving cytotoxic chemotherapy suggested efficacy in the prevention of viridans streptococcal infection. However, increased colonization and infection with vancomycin-resistant enterococci are a predictable consequence of increased vancomycin use, which has prompted the Centers for Disease Control and Prevention (CDC) to recommend that empiric vancomycin therapy be avoided when feasible and has led investigators to explore alternatives to the empiric or prophylactic use of vancomycin.

In a comparative trial, penicillin prophylaxis was superior to TMP-SMX prophylaxis in preventing viridans streptococcal infections in patients with cancer despite extensive colonization with penicillin-resistant streptococci.[109] In other studies, oral administration of penicillin or roxithromycin, a macrolide antibiotic, also appeared to reduce the incidence of viridans strepto-

coccal infection in patients with cancer in comparison to historical controls.[61,215,244] In contrast, in a study of prophylactic administration of ampicillin to patients receiving autologous bone marrow transplants, no reduction in the incidence of viridans streptococcal sepsis occurred, whereas the incidence of penicillin resistance increased.[26] Increased penicillin resistance associated with penicillin prophylaxis has been noted in other prophylactic trials as well.[244] The CDC does not recommend the routine use of penicillin prophylaxis in patients receiving bone marrow transplants.[108]

Levofloxacin prophylaxis currently is used commonly to prevent bacterial infections in adults with cancer, but viridans streptococci rapidly develop quinolone resistance, a point of concern.[261] Innovative prophylactic methods and carefully designed clinical trials are needed to identify effective prophylactic measures, especially for patient cohorts at high risk such as children receiving chemotherapy for acute myeloid leukemia.

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CHAPTER 96

Pneumococcal Infections

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The pneumococcus (*Streptococcus pneumoniae*) continues to be a leading cause of morbidity and mortality in persons of all ages. Most children experience some form of pneumococcal infection (e.g., otitis media or pneumonia), and sepsis or meningitis develops in some cases. Despite more than a century of research, many of the aspects of pneumococcal disease remain obscure. The continued frequency and severity of pneumococcal disease, coupled with the knowledge that antimicrobial therapy invariably does not prevent illness or death, and the high and still increasing prevalence of strains of pneumococci resistant to antimicrobial agents serve to underscore the need for better understanding of pneumococcal infections. Currently, attention is concentrated on efforts to prevent these infections by the development and use of appropriate vaccines.

HISTORY

Pasteur and Sternberg, working independently in 1880 and 1881, discovered the pneumococcus. Pasteur called the organism *microbe septicémique de la salive*, and Sternberg called it *Micrococcus pasteri*. Each researcher recovered pneumococci from rabbits injected with human saliva. Friedlander demonstrated pneumococci in tissue from humans with pneumonia in 1882 and, in the following year, found them in most cases of acute pneumonia. Friedlander described both the characteristic capsule and colonial morphologic features of pneumococci and, in 1884, recovered pneumococci from the blood of patients with pneumonia for the first time. During the next few years, pneumococci were found in virtually all types of infection, including meningitis and otitis media. By 1890, researchers had established the pneumococcus as the most common cause of acute pneumonia, and, hence, the term *pneumococcus* emerged. In addition, the pneumococcus became recognized as a principal cause of meningitis and other serious infections.

During the next decade, researchers immunized animals with cell-free filtrates of pneumococci, demonstrated that serum from immune animals could protect against experimental pneumococcal infection, deduced the role of immunity in promoting phagocytosis, and noted agglutination of pneumococci by serum from immune animals. In 1897, Pane treated humans suffering from pneumonia with serum from such animals. By 1900, researchers had laid the foundation for immunotherapy for pneumococcal pneumonia, the only effective treatment until the advent of chemotherapy.

During the next few years, investigators noted that agglutination of pneumococci appeared to depend on the strain isolated. In 1910, Neufeld and Haendel classified pneumococci into several discrete serotypes on the basis of the appearance of capsular swelling (the quellung reaction). Only strains exposed to homologous serum showed capsular swelling. Their work made possible all subsequent epidemiologic investigations of pneumococcal infection, immunotherapy with type-specific serum, and the development of vaccines.

After these discoveries were made, researchers concentrated on several aspects of pneumococcal disease, including identification of additional serotypes and their roles in disease, production and clinical use of antisera, and development of pneumococcal vaccines.

In 1926, the pneumococcus was called *Diplococcus pneumoniae* because it usually appears in pairs. In 1974, it was renamed *Streptococcus pneumoniae* because it forms long chains when grown in liquid medium. The original classification of pneumococci was limited to types I, II, III, and IV (others). Currently, 90 serotypes have been identified, and certain serotypes have proved to be more virulent than others, with virulence depending, to some extent, on the species of animal infected.

The use of antisera for the treatment of pneumococcal pneumonia proved strikingly effective when type-specific sera were administered. As early as 1913, Cole and associates showed that treatment with antisera lowered fatality rates from 25 to 30 percent to 10.5 percent. In addition to allergic reactions, difficulties associated with this treatment included the necessity of identifying the causative serotype, the need for the earliest possible administration of antisera, and the availability of antisera to only types I, II, and III. White compared the efficacy of early antisera therapy and found that 403 of 1614 (25.0%) who did not receive any therapy died, 32 of 377 (8.5%) who received therapy within 3 days of onset died, and 24 of 127 (18.9%) who received therapy 4 or more days after onset died. Unfortunately, therapy with antisera had no beneficial effect on other pneumococcal infections such as meningitis and endocarditis. Despite these

drawbacks, the use of antisera soon became widespread. The advent of chemotherapy—first sulfa compounds, then penicillin—was followed by a precipitous decline in the use of antisera. Antimicrobial agents killed or inhibited pneumococci, regardless of serotype, and cured patients with previously incurable localized infections.[778]

Coincident with research resulting in the general use of antisera came research into the efficacy of pneumococcal vaccines. Proof of efficacy lagged, and indisputable evidence of protection induced by vaccination was not available until 1945. The ability of pneumococci to cause epidemic pneumococcal pneumonia in young men crowded into army camps or gold mines allowed large-scale trials to be performed. Highlights of the development of effective vaccines include the trial of Wright and associates[789] in South Africa beginning in 1911. Using a vaccine made with whole, killed pneumococci, this trial produced inconclusive results. Many trials followed, with some showing trends toward protection. In 1923, Heidelberger and Avery[317] published their classic article in which they stated that protective antibodies were reactive with surface capsular polysaccharides. In 1930, Francis and Tillett[238] showed capsular polysaccharides to be immunogenic for humans. Ekwurzel and colleagues[203] used a vaccine containing such polysaccharides during 1933 to 1937 and showed it to be effective. Smillie and associates used a preparation of serotype 1 polysaccharide to abort a hospital epidemic of pneumonia at State Hospital in Worcester, Massachusetts.[694]

Although many of these studies suggested that specific pneumococcal polysaccharide antigens could confer protection against severe pneumococcal infection, not until 1945, in a trial performed on U.S. Army and Air Force recruits, were they finally proved by MacLeod and associates[485] to do so. This trial showed vaccination to be strikingly effective in preventing pneumococcal pneumonia caused by serotypes contained in the vaccine but not in preventing disease caused by other serotypes, thus showing serotype-specific protection.

Regrettably, interest in vaccination waned rapidly with the general availability of penicillin, and manufacturers voluntarily withdrew their vaccines from the market. This unfortunate attitude persisted for the next 2 decades until the inability of chemotherapy to prevent many deaths from pneumococcal disease was recognized.[42] The rapid development and spread of antibiotic resistance among many clinically important strains further emphasized that prevention could be more effective than treatment for pneumococcal disease. Fortunately, a few farsighted individuals continued to maintain surveillance of the serotypes causing human disease, and their work allowed the reintroduction of pneumococcal vaccines. The current status of vaccines is discussed later in this chapter.

Interested readers should consult both White's *The Biology of Pneumococcus* and Heffron's *Pneumonia, with Special Reference to Pneumococcus Lobar Pneumonia*, as well as a comprehensive review by Watson and colleagues, for a complete account of the long and fascinating history of this organism.[316,774,778]

THE ORGANISM, HOST DEFENSE MECHANISMS, AND PATHOGENESIS

STRUCTURE OF THE PNEUMOCOCCUS

Pneumococcal cells are surrounded by a trilamellar, lipopolysaccharide, cytoplasmic membrane that has two electron-dense bands, each 25 to 30 Å wide. A cell wall surrounding the plasma membrane has two bands—an inner 30- to 40-Å-wide band and an outer 60- to 80-Å-wide band. Numerous bridges connect the cell wall and the plasma membrane. The polysaccharide capsule covers the cell wall in encapsulated strains and is seen as a wider, less structured band.[735] A schematic representation of the major structural components and selected cell wall components is shown in Figure 96–1.

Cell Wall Structure

The predominant structural components of the pneumococcal cell wall are peptidoglycan, teichoic acid (TA), lipoteichoic acid (LTA), and several choline-bound proteins. Choline is a lipid that is an essential growth factor for *S. pneumoniae*.

PEPTIDOGLYCAN. Peptidoglycan, which accounts for approximately half of the cell wall mass, is a cell wall polymer

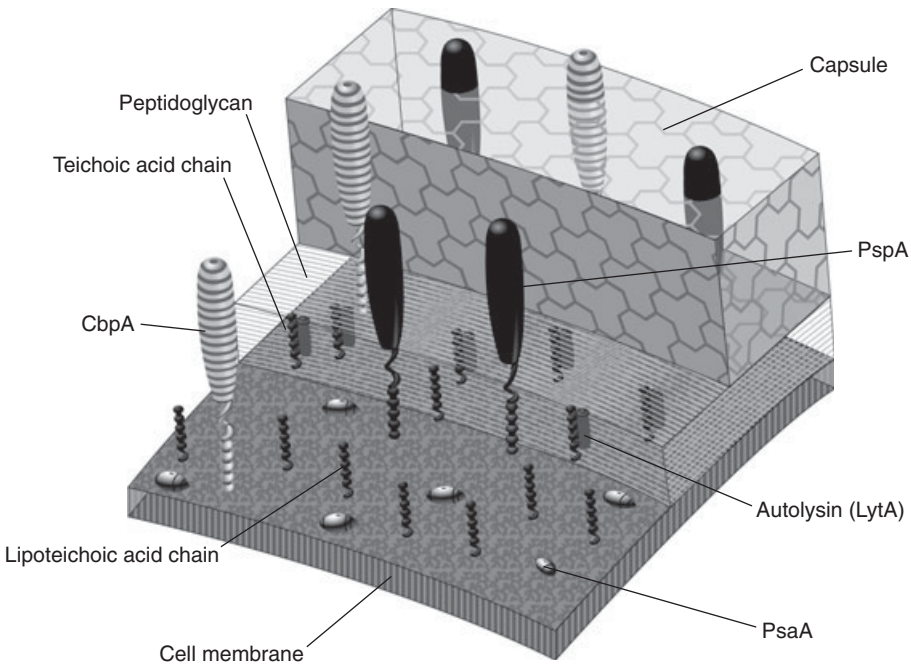


Figure 96–1 Schematic three-dimensional representation of the major structural components of the cell membrane, cell wall, and capsule of *Streptococcus pneumoniae*. The locations of selected major virulence factors of the organism are shown as well. Chains of lipoteichoic acid are attached to cell membrane glycolipid, whereas surface proteins, such as PspA and CbpA, in turn are attached to the lipoteichoic acid chains via phosphocholine links. Chains of teichoic acid are attached to the peptidoglycan layer via phosphodiester bonds. Autolysin (LytA) is attached to teichoic acid chains via phosphocholine links. PsaA is found on the outer surface of the cell membrane. *(Adapted from references 229, 349, 740. Copyright Michael R. Jacobs, used with permission.)*

linked by stem peptides to form a complex, three-dimensional structure.[670] Stem peptides are formed when transpeptidases (also known as *penicillin-binding proteins* [PBPs]) link pentapeptide chains into linear stem peptides in penicillin-susceptible strains.[670] However, in non–penicillin-susceptible strains, branched and other variant stem peptides are produced.

LIPOTEICHOIC ACID. LTA also is known as *pneumococcal Forssman* (*F*) *antigen*. The LTA of pneumococci possesses identical repeat and chain structures linked to a cell membrane glycolipid that anchors LTA to the cell.[229,230] Phosphocholine is attached to saccharide residues. PspA and other proteins (see later) also are attached to choline residues on LTA.

TEICHOIC ACID. TA, also known as *pneumococcal C polysaccharide*, has a chain structure similar to that of LTA, except that the saccharide differs. TA chains are attached to the cell wall peptidoglycan. Some phosphocholine residues of both LTA and TA are expressed on the cell wall surface, where they are thought to serve three functions: (1) activation of the pneumococcal autolysin (LytA) enzyme, which is responsible for the autolysis of pneumococci; (2) binding of the choline-binding domain of LytA to choline on TA, which may regulate the activity of LytA; and (3) a function associated with transformability (choline-deficient cells lack transformability).[683]

SURFACE PROTEINS. Pneumococci have several surface proteins, with the four most important being *pneumococcal surface protein A* (PspA), *pneumococcal surface adhesin A* (PsaA), *choline-binding protein A* (CbpA), and *hyaluronate lyase* (Hyl).

PspA is a cell wall protein with a molecular size of 67 to 99 kd that is bound to TA and LTA by phosphocholine links.[373] This protein extends through the cell wall and capsule to the surface of the organism.[794] PspA exists in various antigenic forms, and epitopes within one PspA molecule can recombine into different types.[97] However, PspA variants usually are sufficiently cross-reactive that immunization with one PspA serotype elicits immunity to other PspA serotypes.

PsaA is a 37-kd surface protein thought to be anchored to the cell membrane and associated with magnesium and zinc transport.[187] It appears to be a lipoprotein and is common to virtually all *S. pneumoniae* isolates.[606] Considerable variation in the amino acid sequences of PsaA from different strains has been detected.[570] The relationship of this protein to other cell wall components has not been determined.[721]

CbpA is a protein similar to PspA and has a mass of 75 kd. It is an adhesin involved in the adherence of pneumococci to cytokine-activated human cells.[632] Several other choline-binding proteins also have been identified.

Hyl is a hyaluronidase that results in breakdown of the hyaluronan and chondroitin sulfate present in the extracellular matrix of human tissues.[373] It is bound to peptidoglycan in the cell wall.

Capsule

The capsule of pneumococci consists of polysaccharides that vary in the make-up of monosaccharides, the sequence of monosaccharides in polysaccharides, the linkage of monosaccharides to each other, and the presence of nonsaccharide components.[732] Currently, 90 serotypes consisting of 25 individual serotypes and 65 serotypes grouped into 21 serogroups are known (see the section "Microbiology" later) (Table 96–1). Each serotype has a specific capsular structure, and serotypes within a serogroup often have the same oligosaccharide sequences linked differently. The capsular structure of many serotypes has been determined.[250] Several epidemic clones have been shown to have different serotypes, and extensive genetic changes involving the replacement of entire cassettes of genes related to capsule production are required for capsular switching to take place.[604]

Genetics and the Pneumococcal Genome

The pneumococcal genome has been mapped recently, and 90 to 95 percent of its DNA sequences are known. The genome has been estimated to be 2.0 to 2.1 megabases (Mb) in size, approximately half that of *Escherichia coli*.[51,192] The locations of more than 100 genes, including 20 tRNA synthetase and 20 ribosomal protein genes, have been mapped. Genes involved in cell wall synthesis also have been identified.

S. pneumoniae is a naturally transformable bacterium, which means that it is able to take up single-stranded DNA from its environment and incorporate this exogenous DNA into its genome. This process is known as *transformational recombination*.[521,703] Recombination is a powerful means of genome evolution and provides a great degree of genome flexibility to this organism. Transformation occurs only at high cell densities (10^5 to 10^8 colony-forming units per milliliter), and a peptide pheromone quorum-sensing signal called *activator* or *competence factor* is required.[602] This factor also is termed *competence-stimulating peptide*.

The genes associated with capsule synthesis have been characterized for several serotypes as well. The complete nucleotide sequence of 24 of these genes of several *S. pneumoniae* serotypes has been determined,[35,249] as has the genetic basis for the structural diversity of capsule polysaccharides within *S. pneumoniae* serogroups.[517,518] The abundance of transposable elements at the gene locus favors genetic variability of the capsule.[249]

VIRULENCE FACTORS

Animal models of pneumococcal infection have provided considerable insight into the pathogenesis of disease and the association of virulence factors with disease. However, the pneumococcus is primarily a human pathogen, and the host defenses of animal models can vary significantly from those in humans. For example, pneumococci adhere to human but not to rabbit polymeric immunoglobulin receptor (pIgR).[802] Additionally, virulence in mice varies considerably with the strain of pneumococcus: pneumococci belonging to serogroups 6, 14, 19, and 23 rarely are virulent in mice, whereas serotypes 1, 2, and 3 usually are virulent.[60,95,434] Virulence also may vary according to the mouse strain.[1,45,729] Some serotypes can be virulent to one species of animal but not to others. An example is serotype 19F, which rarely is virulent in mice[45] but is highly virulent in guinea pigs.[40] In addition, penicillin resistance appears to be linked to decreased virulence by virtue of the fact that isogenic mutants of a virulent, penicillin-susceptible strain were reduced significantly when transformed into a penicillin-resistant strain with an abnormal *pbp2x* gene.[614] Therefore, many animal models of pneumococcal virulence may not be representative of virulence in humans or representative of all pneumococcal serotypes or antimicrobial-resistant strains.

Although the polysaccharide capsule has been recognized as the major determinant of virulence, relatively little is known about the molecular basis of the pathogenesis of pneumococcal disease. A library of 1786 pneumococcal mutants created by insertion-duplication mutagenesis was analyzed for their ability to survive and replicate in murine models of pneumonia and bacteremia.[440] One hundred eighty-six mutant strains exhibited attenuated virulence; 56 of these strains were genetically characterized, and genomic DNA inserts were sequenced and subjected to database searches. Most of the insertions were in probable operons, but no pathogenicity islands were found. Forty-two novel virulence loci were identified. Five strains showed

TABLE 96–1 Capsular Serotypes of *Streptococcus pneumoniae*

Serogroup	Danish Serotype	U.S. Serotype
	1	1
	2	2
	3	3
	4	4
	5	5
Group 6	6A	6
	6B	26
Group 7	7A	7
	7B	48
	7C	50
	7F	51
	8	8
Group 9	9A	33
	9L	49
	9N	9
	9V	68
Group 10	10A	34
	10B	NA
	10C	NA
	10F	10
Group 11	11A	43
	11B	76
	11C	53
	11D	NA
	11F	11
Group 12	12A	83
	12B	NA
	12F	12
	13	13
	14	14
Group 15	15A	30
	15B	54
	15C	77
	15F	15
Group 16	16A	NA
	16F	16
Group 17	17A	78
	17F	17
Group 18	18A	44
	18B	55
	18C	56
	18F	18
Group 19	19A	57
	19B	58
	19C	59
	19F	19
	20	20
	21	21
Group 22	22A	63
	22F	22
Group 23	23A	46
	23B	64
	23F	23
Group 24	24A	65
	24B	60
	24F	24
Group 25	25A	NA
	25F	25
	27	27
Group 28	28A	79
	28F	28
	29	29
	31	31
Group 32	32A	67
	32F	32
Group 33	33A	40
	33B	42
	33C	39
	33D	NA
	33F	70
	34	41
Group 35	35A	47, 62
	35B	66
	35C	61
	35F	35
	36	36
	37	37
	38	71
	39	69
	40	45
Group 41	41A	74
	41F	38
	42	80
	43	75
	44	81
	45	72
	46	73
Group 47	47A	84
	47F	52
	48	82

mutations in genes involved in gene regulation, cation transport, or stress tolerance; the virulence of these strains was shown to be highly attenuated in a murine respiratory tract infection model. Additional experiments also suggest that induction of competence for genetic transformation has a role in virulence.[440] This approach has revealed several previously unrecognized genes required for virulence.

A similar genomic approach was used to look for genes coding for surface-localized proteins that could be targets for protective humoral immunity. By exploiting the whole genome sequence of *S. pneumoniae*, researchers found 130 open-reading frames encoding proteins with secretion motifs or similarity to predicted virulence factors.[787] Mice were immunized with 108 of these proteins, and 6 conferred protection against disseminated pneumococcal infection. Each of the six protective antigens showed broad strain distribution and immunogenicity in human infections. Some of these proteins have been identified as LytB, LytC, and a cell wall–anchored serine protease. Another genomic-based study used a genomic expression library of *S. pneumoniae* screened with convalescent-phase serum for immunoreactive proteins.[805] Six known and 17 unknown pneumococcal proteins were detected. Five of the known proteins, including PspA and SpsA (CbpA), were surface-located virulence factors, and 8 of the unknown proteins were putative membrane proteins. Use of these genomic approaches for the identification of novel microbial targets to elicit a protective immune response has been validated, and these new antigens may play roles in the development of improved vaccines against *S. pneumoniae*.

CAPSULE. The capsule is the major determinant of virulence in pneumococci. It prevents phagocytosis by polymorphonuclear leukocytes (PMNs) and macrophages, thereby allowing unrestricted extracellular multiplication of the organism. Because the pneumococcus has 90 antigenically distinct serotypes, production

of anticapsular antibody in response to one serotype provides protection against only that serotype or serogroup, whereas nonencapsulated strains are considerably less virulent.[527] The importance of the capsule as a virulence factor is emphasized by the fact that protection from pneumococcal infection can be achieved by capsular-specific antibodies. Despite the large number of additional virulence factors (see the following paragraphs), the capsule remains the single most important determinant of virulence in avoiding host defenses after the epithelial barriers have been breached. Other virulence factors are important in breaching host defenses such as epithelial barriers.

NEURAMINIDASES. Neuraminidases are enzymes that cleave terminal sialic acid residues from glycolipids, glycoproteins, and oligosaccharides on eukaryotic cell surfaces; such cleavage may unmask cell surface receptors for pneumococcal adhesins.[570] The neuraminidase NanA has been implicated in the ability of *S. pneumoniae* to colonize and persist in the nasopharynx and middle ear.[736] A second neuraminidase, NanB, has much weaker activity than NanA does but exhibits optimal activity at pH 5, whereas NanA is most active at pH 7.[570]

PNEUMOLYSIN. Pneumolysin is a 53-kd cytoplasmic protein produced by all pneumococci. It is essential for the initial binding to membrane cholesterol and the interaction leading to subsequent membrane damage.[46] Functions of pneumolysin include the following:

1. Pore formation in host epithelial cell membranes. Pneumolysin binds to cholesterol in host epithelial cell membranes, where oligomers of pneumolysin molecules assemble to form 35- to 45-nm pores in the cell membrane, which results in lysis of the targeted cell. Pneumolysin is, therefore, cytotoxic to epithelial cells, and it also slows ciliary beating of bronchial epithelial cells and disrupts the tight junctions between epithelial cells. In addition, pneumolysin disrupts alveolar epithelial cells and the alveolar-capillary boundary, thereby facilitating entry of pneumococci into the bloodstream and through the blood-brain barrier.[269,806]
2. Effects on phagocytic and immune cell function. Pneumolysin attracts neutrophils in the early phases of disease and lymphocytes at a later stage.
3. Direct activation of the complement system. Expression of pneumolysin by pneumococci reduces serum complement levels and serum opsonic activity.[11]
4. Promotion of nitric oxide (NO) production by macrophages. NO is produced by an inducible NO synthase (iNOS) during inflammation as an essential element of antimicrobial defense, but it also can contribute to host-induced tissue damage.[93]

SURFACE-LOCATED CHOLINE-BINDING PROTEINS. The virulence of members of the choline-binding protein (Cbp) family and two recently described cell wall hydrolases, LytB and LytC, has been characterized.[281] Cbp-, LytB-, and LytC-deficient mutants show significantly reduced colonization of the nasopharynx. The following proteins of the Cbp family and their virulence mechanisms have been described:

1. *PspA* is a serologically variable protein that has undergone extensive recombination.[332] It is thought to exert its virulence function in systemic infection by interfering with deposition of the complement component C3b onto pneumococci or by blocking recruitment of the alternative pathway, thereby reducing the effectiveness of complement receptor–mediated pathways of clearance.[739] PspA recently has been shown to bind to lactoferrin, an iron-sequestering glycoprotein found in mucosal secretions, when the level of free extracellular iron is not sufficient for the growth of pneumococci. This binding is thought to overcome the iron limitation at mucosal surfaces and might represent a potential virulence mechanism for colonization of mucosal surfaces.[303]
2. CbpA, or *SpsA*, is a surface protein adhesin that acts as a bridging element between pneumococci and host-cell glycoconjugates on cytokine-activated host cells.[304] This process is thought to be associated with change from nasopharyngeal colonization to invasion of epithelial and endothelial cells.[632] One mechanism by which this process occurs, wherein CbpA binds to the pIgR of human epithelial cells, has been described.[800] The pneumococcus co-opts the transcytosis machinery and gains entry into and across airway epithelial cells. This process is a novel example of a pathogen co-opting the transcytosis machinery to promote translocation across a mucosal barrier.[384]
3. *PsaA* is a surface protein that also is associated with virulence via adhesion to epithelial cells.[605]
4. *Pneumococcal histidine-containing protein A* (*PhpA*) is a 20-kd protein with putative proteolytic activity against the human complement component C3. PhpA is a potential candidate for use as a vaccine against systemic pneumococcal disease and otitis media.[802]
5. The *pneumococcal histidine triad* (*Pht*) proteins PhtA, PhtB, PhtD, and PhtE constitute a novel family of homologous surface proteins associated with virulence; they are also potential vaccine candidates.[7] Although antibodies targeting PhtA, PhtB, or PhtD are protective, the function of these proteins remains unknown. The number of histidine and tyrosine residues in these proteins suggests that they may be involved in metal or nucleoside binding.

PHASE VARIATION. Phase variation in the colonial opacity of *S. pneumoniae* has been implicated as a factor in bacterial adherence, colonization, and invasion.[776] On clear media, colonies can appear as opaque or translucent when viewed under magnification with oblique, transmitted light. All strains of *S. pneumoniae* are thought to be capable of phase variation. Opaque colonies are less likely to autolyze, contain less TA but more PspA in their cell walls, and colonize the nasopharynx poorly in animal models but are more virulent when inoculated into sterile sites. Conversely, translucent colonies are more likely to autolyze, contain more TA but less PspA in their cell walls, and colonize the nasopharynx well but are less virulent when inoculated into sterile sites. Translucent colonies become umbilicated as a result of autolysis, whereas opaque colonies remain domeshaped.

PHOSPHORYLCHOLINE ESTERASE. This enzyme has activity that removes phosphorylcholine residues from cell wall TA and LTA.[759] Inactivation of the gene encoding for the enzyme in pneumococcal strains caused a change in colony morphology from translucent (colonizing) to opaque (virulent) and a striking increase in virulence in the intraperitoneal mouse model. Phosphorylcholine esterase therefore appears to be a regulatory element involved in the interaction of *S. pneumoniae* with its host.[759]

PNEUMOCOCCAL AUTOLYSIN. Pneumococcal autolysin (LytA) is a 36-kd cell wall protein attached to choline residues on TA and LTA. It is associated with unlinking of cell wall glycan from stem peptides during cell remodeling and division. Autolysin initially was considered to be a significant virulence factor, but more recent work has shown that it plays only a minor role and that immunization with autolysin does not provide protection.[570]

CELL WALL STEM PEPTIDES. The peptidoglycan of gram-positive bacteria triggers the release of cytokine from peripheral

blood mononuclear cells.[489] However, 100 to 1000 times more gram-positive peptidoglycan than gram-negative lipopolysaccharide endotoxin is required to release the same amount of cytokine. Simple stem peptides were 10-fold less active than was undigested peptidoglycan in stimulating tumor necrosis factor (TNF). In contrast, complex branched peptides such as tripeptides were at least 100-fold more potent than was the native material. These complex branched peptides represented 2 percent or less of the total material, but their activity in stimulating TNF was almost equal to that of endotoxin.

IRON TRANSPORT. The availability of iron is a major requirement for the growth and survival of many organisms, including *S. pneumoniae.* Two *S. pneumoniae* genetic loci, *pit1* and *pit2*, which encode homologues of ABC iron transporters, are required for uptake of iron by this organism.[105] Virulence in mouse models of pulmonary and systemic infection is attenuated moderately with a *pit2*-disrupted strain and attenuated strongly with a *pit1/pit2*-disrupted strain.

IgA PROTEASE. IgA proteases belong to a family of proteins used by a diverse group of bacteria, including *S. pneumoniae*, for colonization and invasion. IgA1 protease allows bacteria to cleave human IgA1 in the hinge region. The exact role of these enzymes in bacterial pathogenesis is not understood completely, but they are important in bacterial colonization of mucosal membranes in the presence of secretory IgA antibodies by causing local IgA deficiency.[401] The IgA protease genes of *S. pneumoniae* and *Streptococcus mitis* show extensive polymorphism, which results in enzymes with considerable antigenic diversity.[591]

PHOSPHOGLUCOMUTASE. Phosphoglucomutase is an enzyme that is necessary in one of the early steps in capsular polysaccharide synthesis, and *S. pneumoniae* mutants lacking this enzyme do not produce a capsule and are avirulent in immunocompetent but not in immunosuppressed mice.[307] Other metabolic pathways also are thought to be affected by this enzyme.

FREE OXYGEN RADICALS. Release of free oxygen radicals has been implicated in the pathogenesis of otitis media by *S. pneumoniae*, and antibiotic killing of bacteria leads to the release of further free oxygen radicals, which results in tissue damage despite the administration of appropriate antibiotic therapy.[719] Reactive oxygen intermediates also mediate brain injury in bacterial meningitis.[38,477]

NADH OXIDASE. Reduced nicotinamide adenine dinucleotide (NADH) oxidase has been shown to be a virulence factor necessary for *S. pneumoniae* infection. The basis of NADH oxidase as a virulence factor is the conversion of O_2 to H_2O. If O_2 is not reduced fully, it can form superoxide anion (O_2^-) and hydrogen peroxide (H_2O_2), both of which can be toxic to cells.[796]

PYRUVATE OXIDASE. Pyruvate oxidase decarboxylates pyruvate to acetyl phosphate plus H_2O_2 and CO_2 and appears to be associated with regulation of the multiple adhesive properties of pneumococci.[702] A pneumococcal mutant lacking the gene encoding pyruvate kinase showed a greater than 70 percent loss of the ability to attach to all cell types.

PLASMINOGEN BINDING AND PENETRATION OF THE BASEMENT MEMBRANE. Binding of plasminogen plus penetration of the basement membrane is thought to be an essential step in the pathogenesis of bacterial meningitis.[199] Most strains adhere to reconstituted basement membrane, as well as to its purified laminin and collagen IV components, and to bound plasminogen. Penetration of the basement membrane was achieved within 3 to 4 hours in the presence of plasminogen, whereas without plasminogen, no penetration occurred.

HYALURONIDASE. Virtually all pneumococcal strains produce the enzyme hyaluronidase, a 107-kd protein. Models used to simulate human meningitis generally involve the direct intracerebral route of infection. However, intranasal inoculation would provide a more realistic model, and it recently was achieved by intranasal administration of *S. pneumoniae* with hyaluronidase. This model induced meningitis in 50 percent of inoculated mice, whereas meningitis did not develop in any of the mice inoculated without hyaluronidase. Hyaluronidase was found to facilitate pneumococcal invasion of the bloodstream after colonization of the upper respiratory tract. This murine model mimics important features of human disease, which allows the model to be used to study issues related to the pathophysiology and treatment of pneumococcal meningitis.[804]

PEPTIDOGLYCAN *N*-ACETYLGLUCOSAMINE DEACETYLASE A. The glucosamine and muramic acid residues of the pneumococcal cell wall traditionally are regarded as being *N*-acetylated. However, more than 80 percent of the glucosamine and 10 percent of the muramic acid residues have been shown to be deacetylated, thereby explaining the resistance of peptidoglycan to the hydrolytic action of lysozyme, a muramidase that cleaves the glycan backbone.[758] A gene that encodes for peptidoglycan *N*-acetylglucosamine deacetylase A has been identified. This gene may, therefore, contribute to pneumococcal virulence by providing protection against host lysozyme, which is known to accumulate in high concentration at sites of infection.

PHAGES. Whereas transformation is recognized as occurring in pneumococci, another mechanism of DNA transfer in pneumococci is transduction of DNA carried by bacteriophages (lysogeny). A high proportion (76% of 791 isolates) of clinical isolates of pneumococci were found to carry multiple copies of LytA, thus indicating the widespread occurrence of lysogeny in pneumococci.[603] The LytA hybridization pattern of a strain has been found to be stable during extensive serial culturing; it is specific for the clonal type of the strain and can be used as a molecular epidemiologic marker.[671] In addition, phage DNA integrated into the pneumococcal genome acts as an integrase to facilitate the introduction of foreign genes into the pneumococcal chromosome.[270]

TOLERANCE. The ability of *S. pneumoniae* to escape lysis and killing by vancomycin and penicillin, a property termed *tolerance*, has been described recently.[324] Among 116 clinical isolates of pneumococci, 3 percent and 8 percent were tolerant to vancomycin and penicillin, respectively. Tolerance may contribute to treatment failure, particularly in meningitis, in which bactericidal activity is critical for eradication. A vancomycin- and cephalosporin-tolerant strain of *S. pneumoniae*, the Tupelo strain, has been isolated from the cerebrospinal fluid (CSF) of a patient in whom recrudescence of meningitis developed despite treatment with vancomycin and a third-generation cephalosporin.[503] The defect leading to tolerance in this strain involves the control pathway for triggering of autolysis.

HOST DEFENSE MECHANISMS

Although anticapsular antibody is the most prominent protective mechanism against pneumococcal infection, many host responses to infection occur and many other factors are associated with protection against disease.[527] Pneumococcal infection and disease have been modeled in several animal species. Most are models of sepsis arising from intravenous or intraperitoneal inoculation of

bacteria, and only a few were designed to study disease arising from intranasal infection. Chinchillas provide the only animal model of middle ear pneumococcal infection in which the disease can be produced by very small inocula injected into the middle ear or intranasally. This model, developed at the University of Minnesota in 1975, has been used to study pneumococcal pathogenesis at a mucosal site, the immunogenicity and efficacy of pneumococcal capsular polysaccharide vaccine antigens, and the kinetics and efficacy of antimicrobial drugs.[265]

ANTICAPSULAR SERUM IgG ANTIBODY. IgG to the capsular polysaccharide of *S. pneumoniae* is thought to provide the greatest degree of protection against systemic pneumococcal disease, as well as limited protection against colonization. The reference method for measurement of antibody is the opsonophagocytosis assay, which involves serial dilutions of serum, viable pneumococci, complement, and viable PMNs incubated together for 1 hour.[626] An infant mouse assay system for assessment of protective concentrations of human serum pneumococcal anticapsular antibodies correlated well with opsonophagocytic titer but not with naturally occurring IgG antibody concentrations or IgG produced in response to nonconjugated polysaccharide vaccines, as determined by enzyme-linked immunosorbent assay (ELISA).[379,532] However, the ELISA method of serotype-specific antibody assay with absorption of cross-reacting antibody to cell wall polysaccharides does correlate well with protection after vaccination with conjugated vaccine, and it is the method used most commonly to predict serotype-specific immunity.[27,749,750] The development of a phagocytosis assay based on flow cytometry has not overcome the limitations of the ELISA method and is inferior to the opsonophagocytosis method.[371] Investigation of polymorphisms in the variable region of IgG that affect protective function has indicated that the capsular polysaccharide antibody repertoire in adults is derived from memory B-cell populations that have switched class and undergone extensive hypermutation.[483] Functionally disparate anticapsular polysaccharide antibodies can arise within individuals both by activation of independent clones and by intraclonal somatic mutation, which illustrates the complexity of assaying and interpreting serum capsular polysaccharide antibody levels.

ANTICAPSULAR IgA ANTIBODY. The role of IgA in the control of invasive mucosal pathogens such as *S. pneumoniae* is understood poorly. Human pneumococcal capsular polysaccharide–specific IgA initiates dose-dependent killing of *S. pneumoniae* in the presence of complement and phagocytes. The majority of specific IgA in serum is of the polymeric form, and the efficiency of killing initiated by this polymeric form exceeds that of monomeric IgA–initiated killing. In the absence of complement, specific IgA induces minimal bacterial adherence, uptake, and killing. Killing of *S. pneumoniae* by resting phagocytes with immune IgA requires complement, predominantly via the C2-independent alternative pathway, which in turn requires factor B but not calcium. Pneumococcal capsule–specific IgA may have distinct roles in effecting the clearance of pneumococci in the presence or absence of inflammation, and the polymeric form may control pneumococcal infections locally and after the pathogen's entry into the bloodstream by several mechanisms.[367]

PHAGOCYTOSIS AND LEUKOCYTE IgG RECEPTORS. IgG-mediated phagocytosis by PMNs is the main defense against *S. pneumoniae*. Two leukocyte IgG receptors, FcγRIIa and FcγRIIIb, are expressed constitutively on PMNs. Blocking experiments have shown that FcγRIIa is crucial for opsonophagocytosis of serum-opsonized *S. pneumoniae*. In adults, serum-induced phagocytic activity depends mainly on antipneumococcal IgG2 antibodies.[370,623] However, in infants and young children, the main response to pneumococcal conjugate vaccines occurs in the IgG1 subclass.[26,205,755] Investigators have suggested that IgG1 subclass antibodies are at least as highly functional as is IgG2.[205] Recruitment and function of neutrophils also are important host defenses. In a pneumococcal infection model in immunocompetent and immunodeficient mice intranasally infected with *S. pneumoniae* type 2, immunocompetent BALB/c mice were resistant and immunodeficient CBA/Ca mice were susceptible to infection. BALB/c mice recruited significantly more neutrophils in the lungs, and inflammatory lesions were visible much earlier than in CBA/Ca mice.[271]

ANTIBODIES TO SURFACE PROTEINS AND PNEUMOLYSIN. PspA, PsaA, and pneumolysin are common to virtually all pneumococcal isolates. The development of antibodies to PspA, PsaA, and pneumolysin as a result of pneumococcal infection and carriage in young children was determined by measurement of serum antibodies to these proteins by ELISA in children at ages 6, 12, 18, and 24 months and in their mothers. All age groups were shown to produce antibodies to the three proteins, which increased with age and were associated strongly with pneumococcal exposure as a result of carriage or acute otitis media (AOM).[605] IgA to PspA, PsaA, and pneumolysin has been detected by ELISA in the saliva of children aged 6 to 24 months.[685] This finding was associated with pneumococcal carriage and otitis media.

Serum antipneumolysin IgG at the time of hospital admission has been found to be higher in patients with nonbacteremic pneumococcal pneumonia than in those with bacteremic pneumococcal pneumonia or uninfected control subjects.[531] Serum antipneumolysin IgG levels also rose significantly during convalescence in patients with bacteremic pneumonia, and the levels attained were equal to those observed in nonbacteremic patients. Children aged 6 to 24 months were shown to produce antibodies to pneumolysin, and antibody concentrations increased with age and were associated strongly with pneumococcal exposure, whether by carriage or infection such as AOM.[605] Infants also have been shown to mount a specific antibody response to pneumolysin during AOM.[606]

DEFENSE MECHANISMS OF THE SPLEEN. The spleen is the principal organ that clears pneumococci from the bloodstream.[527] Opsonized particles are removed from the circulation by the liver, but with decreasing opsonization, the spleen increasingly assumes the role of clearance. The slow passage of blood through the spleen and the prolonged contact time with reticuloendothelial cells in the cords of Billroth and the splenic sinuses allow time for the removal of nonopsonized particles. Overwhelming pneumococcal infection occurs in children and adults whose spleens have been removed or do not function normally. Pneumococcal disease progresses so rapidly in such individuals that pneumonia is not detectable clinically or by chest radiographs, although it is seen at autopsy. The increase in the incidence of pneumococcal bacteremia and meningitis in children with sickle-cell disease is due largely to splenic dysfunction.

VITAMIN A. The association of nasopharyngeal colonization with *S. pneumoniae* and vitamin A supplementation in infants in an area with endemic vitamin A deficiency in southern India showed that neonatal vitamin A supplementation delayed the age at which colonization occurs; therefore, it may play a role in lowering morbidity rates associated with pneumococcal disease.[139]

C-REACTIVE PROTEIN. C-reactive protein (CRP) is a normal constituent of human serum that is synthesized by hepatocytes and induced by proinflammatory cytokines. The function of this acute-phase reactant includes activation of complement and enhancement of opsonophagocytosis. CRP binds to phos-

phorylcholine, a constituent of eukaryotic membranes that also is found on the cell surface of the major bacterial pathogens of the human respiratory tract, including *S. pneumoniae* and *Haemophilus influenzae*. CRP is present in inflamed (0.17 to 42 mg/mL) and uninflamed (<0.05 to 0.88 mg/mL) secretions from the human respiratory tract in sufficient quantities to have an antimicrobial effect. In addition, the CRP gene was expressed in human respiratory epithelial cell cultures. The complement-dependent bactericidal activity of normal nasal airway surface fluid and sputum was abolished when the secretions were pretreated to remove CRP. Human respiratory epithelial cells are capable of expressing CRP, and this protein may contribute to bacterial clearance in the human respiratory tract.[282]

PLATELET-ACTIVATING FACTOR RECEPTORS OF AIRWAY EPITHELIAL CELLS. Adherence of pneumococci to cultured human tracheal epithelial cells increased after exposure to acid and decreased after exposure to a specific inhibitor of the receptor for platelet-activating factor.[348] Exposure to acid thus may stimulate the adherence of *S. pneumoniae* to airway epithelial cells via increases in platelet-activating factor receptors. The clinical significance of these findings is not clear.[348]

CYTOKINES. Polymorphonuclear granulocytes, which provide a major defense against *S. pneumoniae* infection, are attracted to and activated by various cytokines, including interleukin-1β (IL-1β), IL-6, TNF-α, IL-8, IL-10, IL-12, interferon-γ, and granulocyte-macrophage colony-stimulating factor.[36] The inflammatory response in bacterial meningitis also is mediated by TNF-α and IL-1, which are produced in the subarachnoid space by cells such as leukocytes, astrocytes, and microglia.[559] Inoculation of pneumococcal cell wall components directly into the CSF of rabbits also results in the induction of an inflammatory response with pleocytosis and increased levels of CSF TNF-α and IL-1.[283] Both TNF-α and IL-1α have been shown to increase mucosal adhesion of pneumococci to tracheal epithelium in a chinchilla trachea whole-organ perfusion model.[737]

Respiratory Viral Infections. The role of respiratory viral infection in predisposing the host to secondary bacterial infection, including pneumonia, empyema, and lung abscess, is well recognized.[462] The lungs of immunocompetent mice infected with influenza A virus on day 1 and *S. pneumoniae* on day 8 demonstrate greater *S. pneumoniae* colony counts, more extensive neutrophil infiltration, and higher lung levels of IL-1β and TNF-α after exposure to *S. pneumoniae* than do the lungs of control mice not pre-infected with influenza virus.[462]

L-ASCORBIC ACID (VITAMIN C). Degradation of the connective tissue component hyaluronic acid by the hyaluronate lyase produced by *S. pneumoniae* is inhibited competitively by L-ascorbic acid (vitamin C).[465] One L-ascorbic acid molecule was found to bind to the active site of the enzyme. The high concentration of L-ascorbic acid in human tissues probably provides a low level of natural resistance to pneumococcal invasion by this mechanism.

LEUKOTRIENES. Leukotrienes are produced by macrophages and are considered important for antibacterial defense in the lung. Leukotrienes comprise a group of highly potent lipid mediators synthesized by the enzyme 5-lipoxygenase. Multidrug-resistance protein 1 (mrp1) is a transmembrane protein responsible for the cellular extrusion of leukotrienes from macrophages. In a mouse pneumonia model, mrp1-deficient mice display diminished growth of pneumococci in the lungs and low mortality by a mechanism that involves increased release of leukotriene B_4.[661] Pneumococci also induce the production of leukotrienes in the middle ear, which has been related to up-regulation of two genes that govern the lipoxygenase pathway.[469]

HUMAN ALVEOLAR MACROPHAGE BINDING AND PHAGOLYSOSOMES. Human alveolar macrophages are the major resident phagocytic cells of the lung. After contact with macrophages, bacteria enter phagosomes, which gradually acquire the characteristics of terminal phagolysosomes, with incorporation of lysosome-associated membrane protein. Opsonization with serum containing immunoglobulin resulted in significantly greater binding of pneumococci to macrophages than did opsonization with immunoglobulin-depleted serum.[279] Binding, intracellular localization, and killing of pneumococci by macrophages all are increased significantly by opsonization with serum containing immunoglobulin, complement, or both.

INTRACELLULAR KILLING. Once pneumococci undergo phagocytosis by "professional" phagocytes (leukocytes and macrophages), they are killed.[527] However, researchers have shown that pneumococci can enter and survive inside A549 cells, a human lung alveolar carcinoma (type II pneumocyte) cell line.[720] Not all clinical *S. pneumoniae* isolates were capable of penetrating these cells, and the presence of a polysaccharide capsule also reduced their capacity to penetrate A549 cells significantly. The intracellular activity of various antibiotics against pneumococci in A549 cells showed that in the presence of antibiotics for 18 hours, more than 98 percent of the A549 cells were viable and less than 3 percent of the pneumococci that initially were phagocytosed could be detected intracellularly after exposure to peak serum concentrations of penicillin G, azithromycin, moxifloxacin, trovafloxacin, rifampin, and telithromycin.[494] In the absence of antibiotics, pneumococci were phagocytosed efficiently but then paradoxically went on to kill all the A549 cells within 18 hours. The clinical significance of these findings is unknown.

COMPLEMENT. The second component of complement (C2) is an important factor associated with host defense against encapsulated organisms. Homozygous deficiency of C2 is the deficiency of complement most commonly inherited. Although C2 deficiency can be asymptomatic, patients usually have either autoimmune disease or recurrent pyogenic infection caused by encapsulated bacteria such as *S. pneumoniae*, *H. influenzae* type b (Hib), and *Neisseria meningitidis*. An association between C2 deficiency and IgG subclass deficiency also has been described previously.[37] *S. pneumoniae* challenge of mice deficient in the third component of complement (C3) results in a 2000-fold increase in organism load in the bloodstream in comparison to controls.[130] Binding of pneumococcal CbpA to epithelially produced C3 results in adhesion of pneumococci to type II pulmonary epithelial cells. CbpA-deficient pneumococcal mutants and lysates, therefore, fail to bind C3 and demonstrate a moderate decrease in adhesion to type II pulmonary epithelial cells, thus confirming the interaction of CbpA and C3 in adhesion.[696]

POLYMERIC IMMUNOGLOBULIN RECEPTOR. pIgR plays a crucial role in mucosal immunity against microbial infection by transporting polymeric immunoglobulins such as IgA across the mucosal epithelium. Polymeric IgA consists of two IgA molecules joined by a small polypeptide J chain. The J chain shows high affinity for the glycoprotein pIgRs of epithelial cells, which are responsible for externalization of polymeric IgA across cell membranes.[376] However, pIgR also can act as a "Trojan horse" and participate in the pathogenesis of invasive pneumococcal disease as pIgR binds to a major pneumococcal adhesin, CbpA.[800] Expression of pIgR in human nasopharyngeal cells greatly enhances pneumococcal adherence and invasion; this effect is abolished either by insertional knockout of CbpA in pneumococci or by antibodies against either pIgR or CbpA.

BASIC FIBROBLAST GROWTH FACTOR. Basic fibroblast growth factor is a neurotrophic factor in the central nervous system that is expressed at high levels in response to seizure or stroke. It also occurs in pneumococcal meningitis, as shown in experimental bacterial meningitis in mice and in children with bacterial meningitis.[341] Patients with meningitis in whom major sequelae or death occurred had much higher levels of CSF basic fibroblast growth factor than did those who survived. In patients with bacterial meningitis who survived, basic fibroblast growth factor decreased significantly in CSF after 24 to 50 hours of administration of antibiotic therapy. However, its biologic role in the pathophysiology of bacterial meningitis is not known.

GRANULOCYTE COLONY-STIMULATING FACTOR. In a limited study of 22 non-neutropenic adult patients with pneumococcal meningitis, granulocyte colony-stimulating factor (G-CSF), in addition to cefotaxime and dexamethasone, was administered subcutaneously for 6 days. All patients survived, and in only one patient did a complication develop (bilateral hearing deficit). Improvement of inflammation indices in CSF was rapid.[182] However, controlled clinical trials are needed. In a rabbit meningitis model, G-CSF increased the percentage of granulocytes in blood but not in CSF and increased CSF TNF-α and IL-1β concentrations.[658] However, G-CSF did not reduce the density of apoptotic neurons in the dentate gyrus of the hippocampus. A second study in a rabbit meningitis model used longer pretreatment with G-CSF and showed more positive results.[558] G-CSF pretreatment attenuated meningeal inflammation and enhanced systemic killing of bacteria. Pretreatment with recombinant human G-CSF in a murine model of pneumococcal pneumonia resulted in improved survival with low, but not high, bacterial inocula. Therefore, the benefits of using G-CSF are limited inasmuch as pneumococci already have recruited large numbers of neutrophils in the lungs by this time.[176]

INTRACELLULAR SIGNALING PATHWAYS. Pneumococcal cell walls activate multiple intracellular signaling pathways in microglial brain cells, with induction of an outwardly rectifying K^+ channel; suppression of the constitutively expressed inwardly rectifying K^+ current; and release of TNF-α, IL-6, IL-12, and other inflammatory mediators.[596] The presence of serum strongly facilitated these effects. The mechanisms involved in microglial activation by pneumococcal cell walls were different from those activated by gram-negative lipopolysaccharide.

LACTOFERRIN. Human lactoferrin is an iron-binding glycoprotein that is particularly prominent in exocrine secretions and leukocytes and also is found in serum, especially during inflammation. It is able to sequester iron from microbes and has immunomodulatory functions, including inhibition of both activation of complement and production of cytokines. Binding of human lactoferrin to the surface of *S. pneumoniae* depends entirely on PspA.[298] Prevention of the binding of lactoferrin to pneumococcal PspA could be an important host defense mechanism.

MANNOSE-BINDING LECTIN. Mannose-binding lectin (MBL) is a key mediator of innate host immunity that activates the complement pathway and directly opsonizes some infectious pathogens. Mutations in three codons in the MBL gene have been identified, and individuals homozygous for a mutant genotype have very little or no serum MBL. In a study conducted in the United Kingdom of 229 patients in whom *S. pneumoniae* was isolated from sterile sites, 28 (12%) were homozygous for MBL codon variants versus only 18 of 353 (5%) controls (odds ratio, 2.59; 95% confidence interval, 1.39 to 4.83).[638]

PATHOGENESIS OF DISEASE

To cause disease, pneumococci, like other extracellular bacterial pathogens, must adhere to mammalian cells, replicate in situ, be carried to and replicate in parts of the body that normally are free of them, escape phagocytosis, and damage tissue by causing inflammation or producing substances that directly damage cells and, in some cases, invade the bloodstream.[527] As discussed earlier, the vast array of virulence mechanisms available to pneumococci are countered by numerous host defense mechanisms, although some host responses facilitate infection.

Even though colonization with a pneumococcal strain can progress to disease, it usually does not occur, and the development of anticapsular type-specific antibodies occurs within 30 days,[287,288,529,530] at least in older children and adults. If organisms find their way into the eustachian tubes, sinuses, or bronchi, clearance mechanisms, chiefly ciliary action, lead to their rapid removal. After the development of humoral immunity, colonization with a strain may persist for 1 to 12 months, during which time disease may occur in contiguous sites, but the host is protected from invasive disease by circulating type-specific anticapsular IgG. Loss of colonization with a strain is followed, after a variable colonization-free interval, by colonization with a different serotype. Because 90 antigenically distinct serotypes exist, this cycle of colonization accompanied by the development of humoral immunity occurs many times.

Progression of colonization to disease usually requires the combination of two events: first, acquisition of a serotype to which the host is not immune and, second, a concurrent respiratory viral infection, chronic damage to respiratory epithelium (e.g., smoking or occupational exposure), allergy, or other conditions that result in the development of disease rather than just colonization.[527] Many of these concurrent conditions initiate cytokine activation of the respiratory epithelium, which facilitates increased adhesion of pneumococci to respiratory epithelial cells, as well as invasion of these cells.[462] Mechanisms by which cytokine activation results in these effects include expression of platelet-activating factor and pIgR as discussed earlier. This combination of factors leads to a higher density of colonizing organisms and enables pneumococci to cause infection, including pneumonia, acute exacerbations of chronic bronchitis, sinusitis, otitis media, and mastoiditis, in contiguous respiratory tract sites. Adherence of *S. pneumoniae* to host cells involves an array of surface adhesin molecules such as CbpA, PspA, PspC, Hyl, Ply, PsaA, and both neuraminidases. As discussed earlier, these proteins are involved in interactions with the host complement system (PspA), degradation of hyaluronan of the extracellular matrix (Hyl), lysis of cholesterol-containing membranes (pneumolysin), and binding of metals (divalent cations) such as Mn^{2+} or Zn^{2+} (PsaA), followed by their transport inside the cytoplasm of pneumococci.

Additionally, transepithelial and transendothelial transport of organisms into the bloodstream results in bacteremia, and subsequent transport across other epithelial cells leads to infection of noncontiguous sites such as the leptomeninges, peritoneum, and joint spaces. Such infection occurs in nonimmune hosts by virtue of the fact that pneumococci are able to escape ingestion and killing by host phagocytic cells in the absence of type-specific antibody because the capsule is the major determinant of virulence. *S. pneumoniae* produces few toxins and largely causes disease by its capacity to replicate in host tissues and generate an intense inflammatory response. Cell wall TA and peptidoglycan stimulate the production of cytokines (IL-1, IL-6, IL-8, and TNF) and activate complement by the alternative pathway. The polysaccharide capsule also activates the alternative complement pathway in vitro. Such activation is associated with the release of C5a, a potent attractant for PMNs. The classic complement pathway also is activated by antibody to cell wall polysaccharides

in the absence of anticapsular antibody, and an intense inflammatory response fueled by vigorous activation of both the alternative and classical complement pathways accompanies pneumococcal infection of an immunologically naive host.[527] The disease process is largely a result of this inflammation, and its severity is in direct proportion to its intensity. Pneumolysin also is associated with the severity of disease, and injection of pneumolysin into rat lung causes all the histologic findings of pneumonia, whereas immunization of mice with pneumolysin before infection or challenge with pneumolysin knockout pneumococci is associated with a significant reduction in virulence.[49,221] Numerous other factors contribute to the ability of strains to cause disease and to the severity of disease, as discussed under virulence mechanisms.

A 2006 study evaluated 189 isolates from blood or CSF, 3200 isolates from middle ear fluid, and 348 isolates from the conjunctiva of children aged younger than 36 months with pneumococcal infection. A positive association with invasive pneumococcal disease was demonstrated for serotypes 1, 5, and 12F; with AOM for serotypes 1, 3, 5, 12F, 19A, and 19F; and with acute conjunctivitis for serotype 3 and nontypeable *S. pneumoniae*.[678]

Although pneumococci most commonly cause bacteremia, otitis media, pneumonia, and meningitis, they can produce disease in virtually any organ. Before the advent of immunotherapy and chemotherapy, such "unusual" infections were relatively common. Today, they are less so.

Survival of a patient with a pneumococcal disease depends on numerous variables, including the site of infection, the underlying disease, and the patient's age. Before the advent of chemotherapy, pneumococcal meningitis was universally fatal, whereas pneumococcal pneumonia killed approximately 25 percent of patients. Austrian and Gold[42] in 1964 dramatically illustrated the role of age and underlying disease when their survey of mortality associated with bacteremic pneumococcal pneumonia destroyed the complacency produced by the use of antimicrobial agents. The aged and infirm were likely to die despite receiving immediate and appropriate therapy with penicillin. Today, normal children rarely die of pneumococcal disease; thus, the prognosis must be related to the likelihood of permanent sequelae occurring. Pneumococci do not cause necrosis in pulmonary tissue, and survivors rapidly regain normal pulmonary function.[372] Many children recovering from pneumococcal meningitis are found to have neurologic sequelae. Some investigators suggest that the first attack of pneumococcal otitis media in some way predisposes the individual to subsequent attacks of otitis media.[338]

Before the availability of antimicrobial agents, recovery of a patient with pneumococcal pneumonia depended on the development of type-specific antibody. Although serum and white blood cells (WBCs) from nonimmune children kill pneumococci, probably by activation of the alternative complement pathway, they do so slowly. The importance of this pathway is illustrated best by the inability of children with sickle-cell disease to handle pneumococcal infection.[186,378,572] These children and others with asplenia may die rapidly despite the administration of prompt, vigorous therapy.[382,406,666]

MICROBIOLOGY

S. pneumoniae is a gram-positive coccus that replicates in pairs and chains in liquid medium. The shape of the individual organism is a lanceolate coccus, usually in pairs with the long axis forming a straight line. Elongated or pointed forms are common findings.

Pneumococci are cultured readily on blood and chocolate agar media, as well as in suitable liquid culture media for isolation from blood. Isolates are facultative anaerobes, and most strains require atmospheric enrichment with 5 to 10 percent CO_2 for primary isolation[41]; occasional strains are strict anaerobes. Strains can be adapted for growth without CO_2 supplementation by repeated subculture. Detection of nasopharyngeal carriage of pneumococci is a problem because of the presence of other flora; the use of antimicrobial-containing media, such as blood agar supplemented with gentamicin (5 μg/mL), has led to improvements in isolation of pneumococci, particularly resistant strains.[574,599] However, the sensitivity of current in vitro methods is poor in comparison to the sensitivity of mouse inoculation, although not all serotypes are virulent in mice.[330,423]

IDENTIFICATION OF PNEUMOCOCCI

Pneumococci usually are identified readily by standard features such as colonial morphology, alpha-hemolysis, negative catalase reaction, optochin susceptibility, bile solubility, and specific reactions with antisera to capsular polysaccharides.[353] *S. pneumoniae* produces an autolytic intracellular enzyme, LytA, that causes the organism to autolyze rapidly when grown on artificial media. Bile salts accelerate this natural autolytic process by combining with the pneumococcal cell and activating its autolysin. Strains with atypical features, such as rounded rather than flat or concentrically ringed colonies, optochin resistance, or lack of capsules, do occur and can result in misidentification of such strains as viridans streptococci. Atypical strains are more likely to be encountered from normal flora sites and with penicillin-resistant strains.

Strains with optochin zones greater than 14 mm can be identified presumptively as pneumococci, strains with 7- to 14-mm zones require confirmation by bile solubility, and strains with no zone usually are not *S. pneumoniae*. However, incubation in carbon dioxide has been recognized for a long time as decreasing the size of the zone around optochin disks, and incubation in room air generally results in an increase in zone size if the strain is a pneumococcus or a decrease if the strain is a member of the viridans group of streptococci.[600] Optochin-resistant variants of pneumococci can occur and usually are seen as a subpopulation within the zone of inhibition of an optochin disk, and optochin-resistant mutants can be selected by passage of strains in the presence of optochin.[525] Strains with equivocal optochin zones or atypical colonial morphology can be tested for bile solubility, either directly by placing a drop of bile salt solution (10% sodium deoxycholate) onto colonies and observing for lysis of the colonies or by suspension of organisms in a bile salt solution with a bile salt–free control. Care must be taken in the tube bile solubility test to avoid obtaining false-positive results, which can be caused by the organism suspension being too light or by organisms being suspended in broth rather than saline.[353]

Identification of the capsular polysaccharide serotype or serogroup also is useful in characterizing strains and confirming the identity of problem strains. Such identification is performed by the capsular swelling technique, in which equal volumes of an organism suspension, 0.3 percent methylene blue dye solution, and antiserum are mixed on a glass slide, covered with a coverslip, and read at 1000× magnification by phase-contrast microscopy. Alternatively, organism suspensions can be dried on slides and antiserum and methylene blue dye solution mixed on a coverslip, which then is placed on the slide. The polysaccharide capsule of the pneumococcal organism binds with type-specific antiserum, and organisms can be seen to clump or agglutinate; the resulting change in the refractive index of antibody-coated capsule causes the capsule to appear swollen. Currently, antisera to each serotype or serogroup are available commercially, and factoring antisera to subtype serogroups are available from Statens Serum Institut, Copenhagen, Denmark. The numbering system for the 90 pneumococcal serotypes is shown in Table 96–1.

Currently, 90 serotypes have been identified and are divided into 25 individual serotypes and 21 serogroups in the Danish

classification, which now is used universally.[323] Most serotypes have one antigenic determinant, whereas serogroups have one or more antigenic determinants common to the group and one or more determinants unique to each serotype. Serotypes within a serogroup usually are identified by the serogroup number followed by a letter indicating the serotype to which a strain belongs in the Danish system, or they are identified by a unique number in the U.S. system. Except for serogroups 6 and 9, the letters in the Danish system are F for the first subtype, followed by A, B, and so on. Each serogroup contains 2 to 5 related types, and the 21 serogroups include 65 individual subtypes. Serotype numbers 26 and 30 are not in use. Omniserum containing antibodies to all 90 serotypes is available and can be used to confirm the identity of isolates as pneumococci. Because many serotypes or serogroups are included in this reagent, reactions may not always be optimal and usually are stronger in pool or monovalent reagents. Nine antiserum pools classified from A to I, each containing four to seven serotypes or serogroups, also are available and can be used to identify strains in a group of serotypes before individual serotype or serogroup reagents are tested. Other methods of capsular typing, such as latex agglutination, coagglutination, and capillary precipitation, can be used, but these methods are not available commercially.

The ability of a polymerase chain reaction (PCR) method to identify the capsular serotype of pneumococci has been developed on the basis of polymorphisms in two genes common to the different capsule loci.[441] In a limited study, the correct serotype or serogroup was identified in 92 of 93 strains, but this method did not differentiate serotype 6A from 6B strains. PCR holds promise as a noncultural method for determining serotype.

DETECTION OF CLONALITY

In addition to phenotypic features, such as serotype and antimicrobial resistance markers, the various DNA fingerprint methods for epidemiologic typing of *S. pneumoniae* that have been applied include ribotyping, BOX fingerprinting with the BOX repetitive sequence of *S. pneumoniae* used as a DNA probe, PCR fingerprinting with a primer homologous to the enterobacterial repetitive intergenic consensus sequence, pulsed-field gel electrophoresis of large DNA fragments digested by restriction enzymes, and restriction fragment end labeling to detect restriction fragment length polymorphisms of small DNA fragments.[325,504] The discriminatory power of the individual techniques differed significantly. BOX fingerprinting, pulsed-field gel electrophoresis, and restriction fragment end labeling provided the highest degree of discriminatory power. Ribotyping, BOX fingerprinting, and restriction fragment end labeling were very suitable techniques for computerized data analysis. Pulsed-field gel electrophoresis of large DNA fragments digested by restriction enzymes such as Sma1 is the method used most frequently. Descriptions and nomenclature for the 16 major pneumococcal clones that have contributed to the increase in antimicrobial resistance worldwide were published recently.[504]

DIAGNOSIS OF PNEUMOCOCCAL DISEASE

Definitive diagnosis of pneumococcal infection is based on recovery of pneumococci from the site of infection or documentation of pneumococcal bacteremia, whereas presumptive diagnosis is based on detection of pneumococcal cellular components, such as capsular polysaccharide, and on species-specific DNA and RNA sequences from the site of infection or from remote sites such as urine.[353] Definitive diagnosis is confounded in many instances by the need for invasive procedures to obtain specimens (e.g., from the middle ear space) and by nasopharyngeal carriage of pneumococci when sputum is cultured. Pneumococci almost invariably are isolated from CSF in pneumococcal meningitis, even in patients receiving oral antibiotics.[635] However, establishing the diagnosis of pneumococcal pneumonia is more challenging because sputum rarely is available from children and direct lung puncture is performed very infrequently. Detection of pneumococcal bacteremia to confirm the diagnosis of pneumococcal pneumonia or other localized infection is valuable, but it does not occur frequently and the actual prevalence of bacteremic pneumococcal pneumonia in children is not known,[728] although a recent study suggests that the prevalence is approximately 17 percent (11/64).[509] Therefore, pediatricians must use other methods to diagnose pneumococcal pneumonia. Signs and symptoms significantly associated with bacteremic pneumococcal pneumonia in children include high temperature (>38.9° C [>102° F]), leukocytosis (>15,000/mm^3), and lobar or segmental consolidation.[728] However, the frequency with which these findings are associated with nonbacteremic pneumococcal pneumonia is not known.

With the widespread deployment of pneumococcal vaccine, occult bacteremia caused by *S. pneumoniae* occurs less commonly. The likelihood that a positive blood culture will be a false-positive result is increased if the WBC count is less than 15,000/mm^3, the time for the blood culture to become positive exceeds 24 hours, or the Gram stain result is suggestive of a contaminant.[655] Overall, the frequency with which positive blood cultures are obtained from children with occult bacteremia has declined in comparison to the pre-pneumococcal vaccine era and has declined precipitously since the era before the deployment of vaccine for Hib.

Direct examination of Gram-stained smears of clinically appropriate material remains the fastest diagnostic method and can be augmented if necessary by direct demonstration of capsular swelling of organisms in the presence of anticapsular antisera. The availability of antigen-detection systems that can be used in urine, serum, CSF, and other specimens, such as capsular antigen detection by counterimmunoelectrophoresis and latex agglutination with polyvalent pneumococcal reagent, generally has not improved patient management because of the low sensitivity and specificity of these methods and the fact that they usually are positive only when a Gram stain also is positive. The concentration of pneumococcal capsular antigen in saliva was evaluated by latex agglutination in a study consisting of children with community-acquired pneumonia and healthy controls. None of the children with pneumonia in this study had a positive blood culture, and pneumococcal capsular antigen was detected in the saliva of 27 percent of children with pneumonia versus 17 percent of controls. More cases (20%) than controls (2%) had a pneumococcal capsular antigen titer of 10 or greater ($p < 0.01$). Quantitative measurement of pneumococcal capsular antigen in saliva may be valuable in helping make an etiologic diagnosis in children with pneumonia, but its sensitivity is poor and it is confounded by false-positive results caused by pneumococcal carriage.[233]

A rapid (15-minute) immunochromatographic membrane test to detect pneumococcal polysaccharide capsular antigen in urine samples (Binax NOW) has been developed and was evaluated in the diagnosis of bacteremic and nonbacteremic pneumococcal pneumonia. Urine samples were studied in 51 patients with bacteremic and nonbacteremic pneumonia caused by *S. pneumoniae*; the pneumonia was diagnosed by blood culture, and pneumococcal polysaccharide capsular antigen was detected by counterimmunoelectrophoresis in urine samples. Pneumococcal antigen was detected in urine by the immunochromatographic membrane test in 41 of 51 patients with pneumococcal pneumonia (80.4%), including 23 of 28 bacteremic cases (82.1%) and 18 of 23 nonbacteremic cases (78.3%). Antigen also was detected in 7 of 16 patients with a diagnosis of presumptive pneumococcal pneumonia (43.7%) and in 1 of the 16 patients with pneumonia but in

whom no pathogen was identified. The specificity of the immunochromatographic membrane test was 97.2 percent, but its sensitivity was only approximately 80 percent, thus limiting its value.[191] Furthermore, the usefulness of this test is questionable because the antigen was detected in the urine in 30 of 138 (22%) healthy children with nasopharyngeal carriage of *S. pneumoniae* versus only 3 of 71 (4%) noncarriers ($p < 0.001$).[302] Thus, the test was shown to be often positive in healthy pneumococcal carriers.

One recent study found that immunochromatographic testing for *S. pneumoniae* in CSF was both 100 percent sensitive and specific. The simplicity of the test and the longevity of the CSF antigen even after treatment suggest potential utility of this method in identifying *S. pneumoniae* meningitis in resource-poor countries with widespread prehospital antimicrobial use.[646] Another study showed immunochromatographic testing to be more sensitive than culture in detecting *S. pneumoniae* as the causative agent in thoracic empyema.[587]

Newer molecular-based methods for establishing the diagnosis of pneumococcal disease include the use of DNA probes to detect pneumolysin, autolysin, and PsaA protein, but considerable practical problems must be overcome before these methods will be applicable clinically.[173] Examples include a commercial method involving real-time PCR for simultaneous detection of *N. meningitidis*, *H. influenzae*, and *S. pneumoniae* in patients suspected of having meningitis and septicemia. This method is based on detection of the pneumolysin gene for *S. pneumoniae* and uses a single-tube, 5′-nuclease multiplex PCR assay on samples of CSF, plasma, serum, and whole blood. Amplified products are monitored with sequence-specific, fluorescent dye–labeled probes. The sensitivity of using clinical samples (CSF, serum, plasma, and whole blood) from culture-confirmed cases of *S. pneumoniae* infection was 91.8 percent. The multiplex assay also was used to test a large number of culture-negative samples, which resulted in the detection of numerous cases of meningococcal, *H. influenzae*, and pneumococcal disease that had not been detected by culture.[141] However, whether these results are true positives or false positives is not known, and in another study, although the sensitivity of PCR amplification of the pneumolysin gene in the serum and CSF of infants and children with culture-proven pneumococcal bacteremia and meningitis was 100 percent, the specificity was poor, with 17 percent of healthy controls having positive results.[173] The prevalence of false-positive reactions was highest (33%) in 2-year-old children, the age group with the highest rate of nasopharyngeal carriage of pneumococci. Therefore, although PCR of serum and CSF is a sensitive test for the detection of *S. pneumoniae* in these sites, its high rate of positivity in healthy controls as a result of nasopharyngeal carriage limits its utility in detecting systemic pneumococcal infection.[173]

Another rapid PCR method involving the use of a set of primers that amplify 273 base pairs of the autolysin gene has been developed to identify *S. pneumoniae*. In addition, three sets of primers were designed to amplify a 240–base pair fragment of the PBP-2B gene (*pbp2b*) of penicillin-susceptible *S. pneumoniae* and two common *pbp2b* mutations present in penicillin-resistant *S. pneumoniae* in order to simultaneously identify the penicillin susceptibility of strains. The autolysin gene was identified in all 1062 clinical isolates of *S. pneumoniae* evaluated. In addition, 98.9 percent of 621 penicillin-susceptible isolates were shown to have DNA fragments amplified by the penicillin-susceptible primers, whereas 72.1 percent of 441 penicillin-resistant isolates were detected by the penicillin-resistant *S. pneumoniae* primers.[741] Although further refinement of this method is required, this study has shown that it is possible to identify pneumococci and differentiate penicillin-susceptible from penicillin-resistant *S. pneumoniae* by applying PCR and a combination of primers to detect the susceptible *pbp2b* gene, resistant *pbp2b* gene mutations, and the autolysin gene. In another study, a 208–base pair region of the pneumolysin gene was amplified by PCR in blood specimens from hospitalized children with pneumonia. Whole blood, buffy coat, or plasma samples from 67 children (44%) tested positive by PCR. The sensitivity was 100 percent in 11 culture-confirmed children, and the specificity was 95 percent in control subjects. Age, previous administration of oral antibiotic therapy, and pneumococcal nasopharyngeal colonization did not influence the PCR results, which were more specific than were serologic and urinary antigen testing.[509]

A gene probe for the gene encoding the PsaA protein also has been developed on the basis of PCR assay. PsaA was confirmed to be present in representative strains of all 90 serotypes of *S. pneumoniae*. The specificity of the assay was verified by the lack of signal from analysis of heterologous bacterial species ($n = 30$) and genera ($n = 14$), including viridans group streptococci. The potential of the assay for clinical application was shown by its ability to detect pneumococci in culture-positive nasopharyngeal specimens.[520]

SUSCEPTIBILITY TESTING

Susceptibility testing of pneumococci has been well standardized, and testing can be performed by determination of the minimal inhibitory concentration (MIC) and, for selected agents, by disk diffusion.[356] MICs can be performed by macrodilution or microdilution in cation-supplemented Mueller-Hinton broth enhanced with 5 percent whole defibrinated sheep or horse blood or 5 percent lysed and centrifuged horse blood.[537] If sulfonamides are tested, only the latter supplement should be used to avoid the presence of sulfonamide antagonists. MICs also can be determined by dilution in Mueller-Hinton agar supplemented as just described; agar dilution generally is regarded as the reference method for pneumococci and often is used for developmental work.[353]

Many systems based on frozen or dried microdilution trays are available commercially and used extensively for surveillance testing.[354] As with any system, commercial microdilution panels should be validated and used with appropriate quality controls.

A new method for determination of MICs, the E-test (A. B. Biodisk, Solna, Sweden), is much simpler to use than are the other methods for MIC determination. The E-test consists of a calibrated antibiotic-impregnated plastic strip that is applied to the surface of an inoculated agar plate. An antibiotic gradient is produced that results in an elliptical zone of inhibition after incubation. The MIC is read at the point where the ellipse of inhibition meets the strip. Evaluation of the E-test has shown that this method generally is reliable, although problems are encountered with some agents because acidification of the medium occurs during incubation of the plates in CO_2, which is required to ensure the growth of clinical isolates. Agents particularly affected are macrolides and some quinolones.

Disk diffusion also has been standardized well for testing pneumococci against selected agents. Distinction between susceptible and resistant strains is accomplished readily by using the current National Committee for Clinical Laboratory Standards (NCCLS) method involving macrolides, tetracycline, chloramphenicol, trimethoprim-sulfamethoxazole, and clindamycin.[353] For testing penicillin and other β-lactams, disk diffusion is used best as a screening method with 1-μg oxacillin disks that have a susceptible cutoff zone of 20 mm or larger. Strains with zones of 20 mm or larger are fully susceptible to penicillin and other β-lactams. However, strains with zones that are less than 20 mm need to have MICs of penicillin and other appropriate β-lactams determined.[537] Penicillin-susceptible strains with MICs of 0.06 μg/mL usually screen out with resistant strains.

Although interpretative categories for clarifying the significance of MIC values are available, many limitations to the

currently available NCCLS pneumococcal breakpoints exist, particularly for agents that can be administered in multiple-dosing regimens and by multiple routes of administration and that are used for infections in different body sites. Breakpoints for parenteral β-lactam agents generally are based on the use of agents in meningitis, whereas those of oral β-lactam agents are based on nonmeningeal infections such as otitis media.[537] Nonmeningeal breakpoints for some parenteral β-lactam agents were introduced in 2002, which to some extent will avoid the use of non–β-lactam agents for serious nonmeningeal pneumococcal infections. These breakpoint changes classify strains previously interpreted as intermediate in sensitivity to penicillin G, cefotaxime, and ceftriaxone as susceptible if the agents are administered parenterally to treat pneumonia and other nonmeningeal infections. Pharmacokinetic and pharmacodynamic parameters recently have been shown to correlate with clinical outcome and offer a more rational approach to predicting antimicrobial efficacy and determining clinically relevant susceptibility breakpoints.[145,150,361]

ANTIBIOTIC RESISTANCE

Mechanisms of Antibiotic Resistance

RESISTANCE TO β-LACTAM DRUGS. Widespread resistance to β-lactam and other drug classes has evolved in the most common pathogens, including *S. pneumoniae*. Although pneumococci are naturally transformable organisms, β-lactamase production never has been described in this organism. Instead, a much more complex resistance mechanism has evolved in *S. pneumoniae* that is mediated by sophisticated restructuring of the targets of the β-lactams, the PBPs, and by other newly described mechanisms.[300] The PBP targets in penicillin-resistant strains of *S. pneumoniae* are modified, low-binding affinity versions of the native PBPs. PBP targets may be modified by mutation or by transformation and homologous recombination with DNA from the PBP genes of viridans streptococci. The level of resistance is determined by how many and to what extent targets are modified.[120] Restructuring of PBPs is mediated by stepwise alterations in PBPs. The high-molecular-weight PBPs—types 1A, 2X, and 2B—that usually are detected in *S. pneumoniae* are involved in transpeptidase activity and play an important role in resistance.[301] Alterations in PBP-2B are associated with low-level resistance to penicillin, and alterations in PBP-2X mediate low-level resistance to cephalosporins. The additional alterations in PBP-1A raise penicillin MICs to 1 μg/mL or greater and cefotaxime MICs to 0.5 μg/mL or greater. Genomic comparison between *S. pneumoniae* and commensal *S. mitis* and *Streptococcus oralis* strains has documented the mosaic nature of PBPs among these species, with pneumococci acquiring their altered PBP genes from *S. mitis* and *S. oralis*.[299] Many other mosaic gene clusters not associated with penicillin resistance also have been found.[255] The capacity to produce branched cell wall stem peptides encoded by altered *murM* and *murN* genes, as well as altered PBPs, is required for expression of penicillin resistance in *S. pneumoniae*.[224] The *fibA* and *fibB* genes, which are homologous to the *Staphylococcus aureus femA/B* genes required for expression of methicillin resistance in this organism, encode proteins involved in the formation of interpeptide bridges and also are required for expression of PBP-mediated penicillin resistance.[775] Other mechanisms of β-lactam resistance have been described in laboratory mutants and in a clone of Hungarian pneumococcal strains with notably high levels of β-lactam resistance (penicillin MIC, 16 μg/mL; cefotaxime MIC, 4 μg/mL).[300,695]

RESISTANCE TO NON–β-LACTAM DRUGS. The molecular and genetic mechanisms of resistance to macrolides, chloramphenicol, tetracycline, fluoroquinolones, and trimethoprim-sulfamethoxazole in *S. pneumoniae* also have been determined. Resistance genes for several agents are carried on a transposon, Tn1545.[144] It confers resistance to three antimicrobial classes—kanamycin (*aphA-3*), macrolide-lincosamide-streptogramin B–type antibiotics (*ermB*), and tetracycline (*tetM*). This transposon has been conjugated and transposed to the chromosome of *Enterococcus faecalis*, oral streptococci, and *Listeria monocytogenes*. The properties of this transposon account for the sudden emergence, rapid dissemination, and stabilization of resistance to multiple antibiotics in *S. pneumoniae* in the absence of plasmids.

Resistance mechanisms include the production of chloramphenicol acetyltransferase, an enzyme capable of catalyzing the conversion of chloramphenicol to nonfunctional derivatives. Chloramphenicol acetyltransferase is encoded by a chloramphenicol acetyltransferase (*cat*) gene identical to the *cat* gene from the *S. aureus* plasmid pC194. Tetracycline resistance occurs through ribosomal protection encoded by the genes *tetM* and *tetO*. The tetM and tetO proteins are thought to cause tetracycline to be released from the ribosome. Resistance to fluoroquinolones primarily involves mutations in the DNA gyrase gene *gyrA* and in the topoisomerase IV genes *parC* and *parE*, as well as an efflux mechanism that affects some fluoroquinolones. Resistance to trimethoprim is mediated through a single amino acid substitution in the chromosomal dihydrofolate reductase gene of *S. pneumoniae*, which is thought to disrupt the bond with trimethoprim without affecting the action of dihydrofolate reductase. Sulfonamide resistance appears to result from repetitions of one or two amino acids in the chromosomal dihydropteroate synthase.[782]

Two major mechanisms have been described for resistance to erythromycin. Co-resistance to macrolides, clindamycin, and streptogramin B–type antibiotics is a result of modification of the ribosome through methylation of an adenine residue in domain V of the 23S rRNA. Methylation is encoded by a methylase gene, *ermB* (previously called *ermAM*). Resistance to 14- and 15-membered macrolides (erythromycin, azithromycin, and clarithromycin) but not to 16-membered macrolides (roxithromycin, josamycin, and spiramycin), ketolides, or clindamycin is a result of efflux of the antibiotic from the cell; such resistance is encoded by the gene *mefE* in *S. pneumoniae* and appears to be emerging rapidly as the predominant mechanism of resistance to erythromycin in many countries.[255] Other macrolide resistance mechanisms that have been described recently include mutations in position 2059 of the 23S rRNA and in genes encoding ribosomal protein L4.[717]

VANCOMYCIN TOLERANCE. Although vancomycin resistance has not been described in pneumococci, antibiotic tolerance, or the ability of bacteria to survive but not grow in the presence of antibiotics, has been described. It has been shown to be caused by loss of function of the VncS histidine kinase of a two-component gene expression sensor-regulator system in *S. pneumoniae* that produces tolerance to vancomycin and other classes of antibiotics.[543]

Evolution of Antibiotic Resistance among Pneumococci

Pneumococci initially were susceptible to many antimicrobial agents, but they became resistant with varying degrees of rapidity to many of these agents. The earliest example was the development of resistance to optochin (ethylhydrocupreine) when this agent was used experimentally in mice in the early part of the 20th century. With the introduction of sulfonamides in 1939, pneumococci similarly exhibited an ability to acquire resistance in experimental infections in mice, as well as in a human case of meningitis.[418] Sulfonamide resistance was identified sporadically

thereafter, and a trimethoprim-sulfamethoxazole–resistant strain was recognized first in 1972. Trimethoprim-sulfamethoxazole resistance subsequently has become widespread in virtually all serotypes throughout the world, including developing countries, and resistance to this agent is greater than that to any other antimicrobial class worldwide.[328] Tetracycline resistance emerged in the 1960s and chloramphenicol resistance in 1970. However, little attention was paid to the development of resistance in this species until 1977, when isolates resistant to several antimicrobial classes, including penicillins, chloramphenicol, tetracyclines, macrolides, clindamycin, and trimethoprim-sulfamethoxazole, were detected in South Africa.[29,360] Subsequently, multiresistant clones of pneumococci have spread throughout many regions of the world. Noteworthy is that multiresistant clones are confined mostly to serotype 14 and serogroups 6, 9, 19, and 23.[179] Whereas resistance to penicillins occurs in a stepwise fashion and can be overcome by using β-lactams with appropriate pharmacokinetics, resistance to other drug classes usually is absolute, and distinct populations of strains are found to be susceptible and resistant to agents such as macrolides, clindamycin, tetracyclines, trimethoprim-sulfamethoxazole, and chloramphenicol. Unlike enterococci, resistance to vancomycin has not developed in pneumococci yet, although vancomycin-tolerant strains have been detected.[52,324]

Cross-resistance among *S. pneumoniae* to macrolides and other classes of antibiotics usually increases with increasing MICs to penicillin[357] (Fig. 96–2). Whereas only 6 percent of penicillin-susceptible pneumococci are resistant to macrolides and 14 percent to trimethoprim-sulfamethoxazole, approximately half of the penicillin-intermediate isolates were resistant to these agents. In the case of penicillin-resistant strains, three quarters were resistant to macrolides, 90 percent to trimethoprim-sulfamethoxazole, and 28 percent to clindamycin. However, this pattern is not the case in all countries, and at least one multiresistant clone resistant to chloramphenicol, tetracycline, erythromycin, clindamycin, and trimethoprim-sulfamethoxazole has remained susceptible to penicillin.[184,716]

Strains of *S. pneumoniae* were exquisitely susceptible to penicillin (MICs of 0.01 to 0.03 μg/mL) when this agent initially was used clinically in the 1940s and 1950s, and this MIC range is referred to as the baseline activity of penicillin against *S. pneumoniae*.[353] Evolution of resistance to this class of agents was noted first when a few strains of *S. pneumoniae* were isolated in the 1960s in Australia and New Guinea. These strains had decreased susceptibility to penicillin, with MICs of 0.1 to 0.25 μg/mL, approximately 10-fold higher than the MICs of baseline strains. Strains with penicillin MICs of 2 to 4 μg/mL, approximately 100-fold higher than, the baseline strains, were isolated in South Africa in 1977, and, subsequently, strains with even higher MICs (16 μg/mL, approximately 1000-fold higher than baseline strains) were described in Hungary.[695] Pneumococci conventionally are classified as penicillin-susceptible if the MICs are 0.06 μg/mL or less, intermediate if the MICs are 0.12 to 1.0 μg/mL, and resistant if the MICs are 2.0 μg/mL or greater. This classification is useful mainly in characterizing strains as fully susceptible to β-lactams if susceptible or as having decreased susceptibility if intermediate or resistant. Strains with such decreased susceptibility are better referred to as β-lactam drug-challenged because the mechanism of resistance can be overcome if the pharmacokinetics of the β-lactam drug used in serum or at the site of infection exceeds the MIC for 40 to 50 percent of the dosing interval.[146] Similar variations in MIC ranges are seen with all β-lactams, although MIC ranges for many β-lactams are much higher than that for penicillin itself. Agents such as ampicillin, amoxicillin, cefotaxime, and ceftriaxone have MIC ranges similar to that of penicillin, whereas agents such as cefazolin, cefaclor, cefprozil, ceftazidime, and cefixime have much higher MIC ranges. For example, the baseline activity of cefaclor against *S. pneumoniae* is 0.5 to 1 μg/mL, which is a concentration approximately 20- to 30-fold higher than that required for penicillin to inhibit the most susceptible strains. Changes in susceptibility that occur over the course of time were illustrated in a study of recent versus archived otitis media strains. In this study, the MIC_{90} for cefaclor against archived isolates was 1 μg/mL, whereas the MIC_{90} against recent isolates was greater than 64 μg/mL.[355] A few agents, such as imipenem and meropenem, have slightly lower MIC ranges than those of penicillin. Currently, 50 to 60 percent of pneumococci in the United States are penicillin-susceptible, 15 to 20 percent are penicillin-intermediate, and 20 to 30 percent are penicillin-resistant. The proportions of strains in each group vary considerably throughout the world.

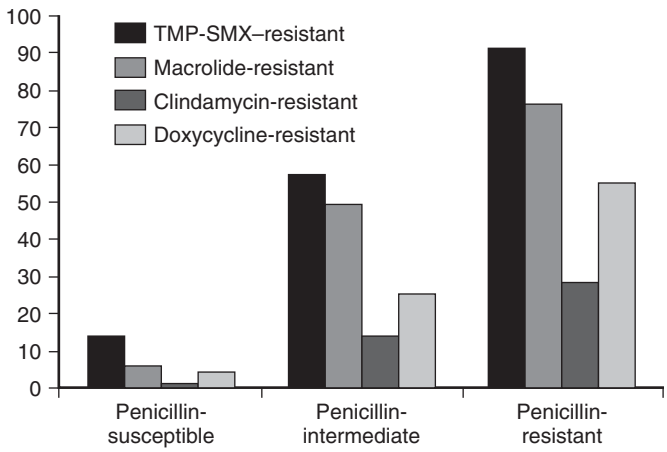


Figure 96–2 Pneumococci often are resistant to several drug classes, and cross-resistance to macrolides and other classes of antibiotics increases as minimal inhibitory concentrations of penicillin increase. TMP-SMX, trimethoprim-sulfamethoxazole.[357,687]

Resistance to macrolides in strains of *S. pneumoniae* was noted first in 1964 and was detected sporadically in the United States until it became widespread in the latter half of the 1990s.[328,344,418] The baseline activity of macrolides (0.03 μg/mL) and MIC distributions (1000-fold concentration range, 0.03 to >32 μg/mL) against *S. pneumoniae* are somewhat similar to those of penicillin. The MIC distribution of macrolides is trimodal, with strains being exquisitely susceptible (erythromycin MIC, ≤0.03 μg/mL) or highly resistant (erythromycin MIC, ≥32 μg/mL) or demonstrating intermediate resistance (MICs of 1 to 16 μg/mL).[216] These distributions closely correlate with macrolide ribosomal methylase and efflux resistance mechanisms. The prevalence of macrolide resistance and reports of clinical failure resulting from strains with efflux and ribosomal methylase resistance mechanisms continue to increase.* Some authors, however, have argued that isolates with efflux-mediated resistance could be susceptible to the high intracellular concentrations that these agents achieve in phagocytic cells and in epithelial lining fluid of the alveoli.[18,398] However, no clinical or animal data support these arguments for extracellular pathogens such as *S. pneumoniae*, whereas considerable clinical and animal data support the use of current breakpoints.[50,145,146,512] The rising incidence of macrolide-resistant pneumococci was directly proportional to the increasing use of macrolides in various communities and age groups.[251,344,586] In a recent report, 18 to 23 percent of pneumococci from the United States were macrolide-resistant[328,733] as compared with 10 percent from Canada, 11 percent from Latin America, 20 percent from Europe, and 39 percent from the Asia-Pacific region.[328]

Strains with multiple antibiotic resistance have greater selective advantages than do strains resistant to just one antibiotic because the opportunity for positive selection is increased as the

*See references 80, 232, 351, 399, 479, 480, 516, 586, 651, 773.

number of drug classes to which isolates are resistant increases.[419] Exposure to different classes of antibiotics allows more opportunity for selective advantage to a multiple antibiotic–resistant organism than to a monoresistant strain, which must wait to encounter the one antibiotic to which it is resistant and is likely to be killed by agents of other antibiotic classes. Thus, the increasing prevalence of antibiotic-resistant pneumococci is associated with the increasing prevalence of multidrug-resistant strains.[779] Therefore, one is not surprised that the use of one class of antibiotics (mainly macrolides and trimethoprim-sulfamethoxazole) can be associated with an increase in resistance to other classes of antibiotics (mainly β-lactam drugs).[5,30,169,277] Many authorities now think that antibiotic agents such as the newer macrolides (e.g., clarithromycin and azithromycin) and trimethoprim-sulfamethoxazole are stronger promoters of antibiotic resistance among *S. pneumoniae* strains than are the β-lactam drugs.[30,169,251,277,586] Researchers also have suggested that among the β-lactam drugs, cephalosporins are stronger promoters of resistance in *S. pneumoniae* than are the aminopenicillins.[251,650]

Although many strains are resistant to tetracyclines and macrolides, they are susceptible to the new tetracycline derivatives, the glycylcyclines, as well as to streptogramins, ketolides, glycopeptides, and rifampin. Many strains are resistant to trimethoprim-sulfamethoxazole worldwide, with more than 40 percent being resistant in the United States. Fluoroquinolones with antipneumococcal activity (e.g., gatifloxacin, levofloxacin, moxifloxacin) are active against most strains of *S. pneumoniae*; however, in several countries where fluoroquinolones have been prescribed widely, clinically relevant levels of resistance have been described.[122,328,613] No doubt, antibiotic resistance will continue to evolve and challenge us.

EPIDEMIOLOGY

Pneumococcal infection remains a serious problem at the beginning of the 21st century in both the developed and developing worlds. It still is a leading cause of death worldwide and a very important cause of morbidity in all countries.

In the United States, since the introduction of Hib vaccination, *S. pneumoniae* has become the leading cause of bacteremia and bacterial meningitis and has remained a major cause of otitis media. This organism causes more deaths than does any other vaccine-preventable organism.[252] The burden of pneumococcal disease in the United States has been estimated to be 125,000 to 500,000 cases of pneumonia, 50,000 cases of bacteremia, 3000 cases of meningitis, and 7 million cases of otitis media, with 40,000 deaths occurring annually.[56,315,594] In the developing world, pneumococcal infections are among the leading causes of death in children younger than 2 years old, with the estimated 1.2 million deaths per year accounting for 9 percent of all deaths.[409]

The only reservoir of *S. pneumoniae* is the human nasopharynx. From there, the organism can (1) enter the bloodstream and cause invasive infections such as sepsis and meningitis and infections in remote foci such as joints, bones, and soft tissues; (2) spread to adjacent mucosal tissue and cause mucosal infections such as otitis media, sinusitis, and pneumonia; and (3) be transmitted by direct contact and through aerosols to other individuals.[154] Acquisition and nasopharyngeal carriage of *S. pneumoniae* are associated with the occurrence of pneumococcal AOM,[210,442,478,799] bacteremia,[286,448,473,497] and pneumonia.[330]

The most common diseases caused by *S. pneumoniae* are related to the upper respiratory tract (mainly otitis media, conjunctivitis, and sinusitis). The least common are invasive infections such as bacteremia and meningitis, whereas pneumonia is of intermediate frequency. Figure 96–3 shows the difference in the order of magnitude of these diseases. The yearly incidence of invasive infections is reported to be less than 10 to greater than 1000 per 100,000 children younger than 5 years old in various populations. The incidence of pneumonia usually is reported as a few dozen to a few thousand per 100,000 children younger than 5 years of age. Pneumococci are responsible for 25 to 50 percent of cases of otitis media in children, which translates to more than 10,000 per 100,000 children younger than 5 years old. Thus, pneumococcal otitis media is roughly up to 1000-fold more common than is invasive pneumococcal infection, and pneumococcal pneumonia is 10- to 100-fold more common than is invasive infection.

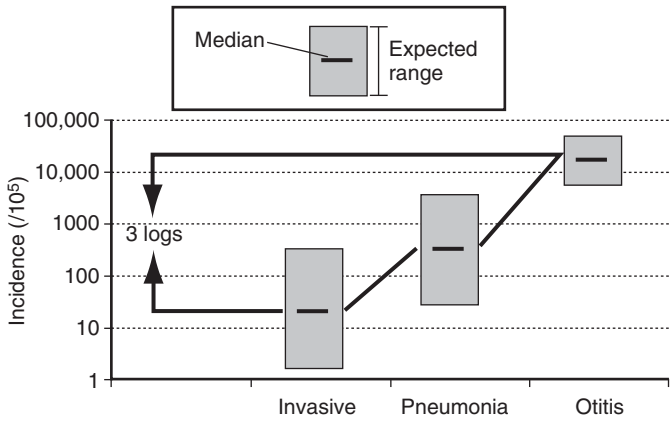


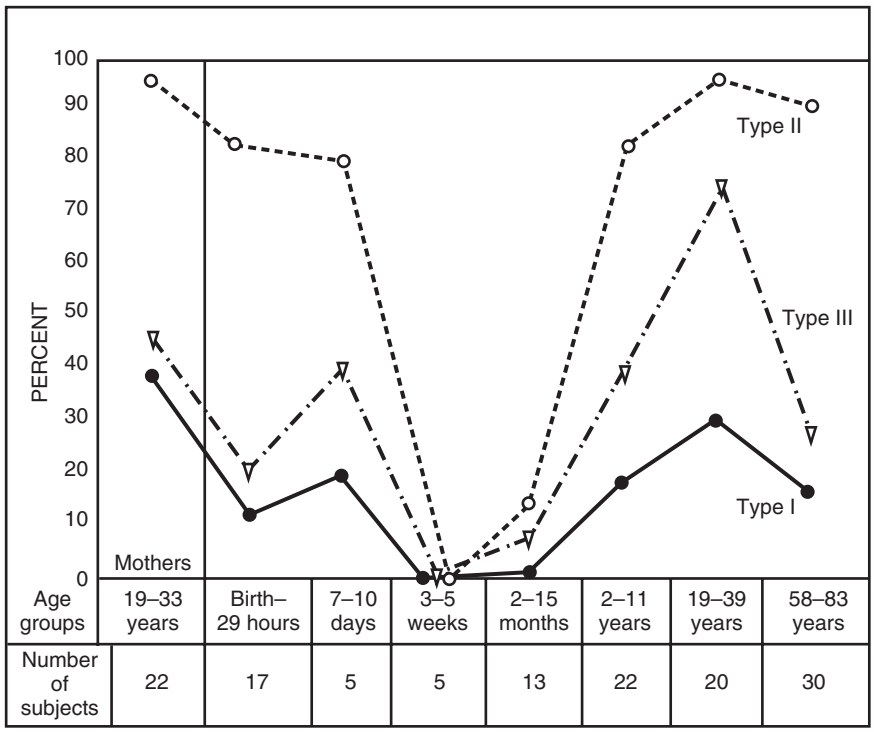
Figure 96–3 Relative incidence of pneumococcal invasive infections, pneumonia, and otitis media in children. The figure is based on estimates from global data that have been published or presented at meetings. The *horizontal line* in each box presents the estimated average value and the upper and lower limits of the presumed range.

RISK FACTORS FOR PNEUMOCOCCAL INFECTION

In general, decreased host defenses, especially in humoral immunity, or increased exposure to the organism can be considered the main risk factors for acquiring pneumococcal infection. Risk factors can be divided into various categories, although such division is arbitrary because numerous predisposing conditions can be present.

YOUNG AGE. Young age is perhaps the most important risk factor for acquiring pneumococcal infection because natural immunity is highly age-dependent. One of the best demonstrations of the relationship between age and immunity to pneumococcal infection was published in 1932 by Sutliff and Finland (Fig. 96–4). Immunity in the first few months of life, derived from maternal antibody passively transferred to the fetus, protects against invasive infection. These antibodies disappear rapidly, and, within a few months, the incidence of invasive infection increases. The incidence then starts to decline sharply after the child is 18 months of age and able to mount an immune response to most pneumococci, coupled with cumulative exposure to the various pneumococcal strains.

ABSENCE OR MALFUNCTION OF THE SPLEEN, INCLUDING HEMOGLOBINOPATHIES. The spleen is the principal organ that clears pneumococci from the bloodstream.[710,769] Patients in whom the spleen has been removed or does not function normally are at risk for the development of overwhelming pneumococcal infection. Children with invasive *S. pneumoniae* from eight children's hospitals in the United States were studied during the period 1993 to 1999. Of 2581 cases, 1 percent occurred either in children with congenital asplenia or in children who had undergone surgical splenectomy.[662] The mortality rate was high (6/22 [27%]), especially in those with meningitis.



Age groups	19–33 years	Birth–29 hours	7–10 days	3–5 weeks	2–15 months	2–11 years	19–39 years	58–83 years
Number of subjects	22	17	5	5	13	22	20	30

Figure 96–4 Percentage of subjects in different age groups whose blood is pneumococcidal.

Splenic malfunction is considered to be the most important reason for the increased incidence and severity of pneumococcal bacteremia in sickle-cell disease and other sickle hemoglobinopathies (e.g., hemoglobin SC disease or S-β-thalassemia).[569,571,662,797] Although the incidence of pneumococcal infection in children with hemoglobin SC disease is lower than in persons with sickle-cell disease, it is higher than in healthy children.[439,738] The incidence of overall bacterial sepsis in other hemoglobinopathies (e.g., S-β-thalassemia) is estimated to be intermediate between that for hemoglobin SC and hemoglobin SS disease.[560] The high risk for the development of pneumococcal infection in persons with sickle-cell disease is thought to be due to the combination of low levels of circulating antibodies, splenic dysfunction, and complement deficiency, which results in decreased clearance of encapsulated bacteria from the bloodstream.[571,784] Although the use of prophylactic penicillin has reduced the risk for acquiring pneumococcal disease, children younger than 5 years old with sickle-cell disease still have increased rates of invasive disease (range, 1230 to 1500/100,000 population).[226,569,788,797]

DEFECTIVE ANTIBODY FORMATION. As reviewed in the "Pathogenesis" and "Prevention" sections of this chapter, defective antibody production against pneumococcal polysaccharides is the rule for most serotypes in individuals younger than 18 months old and may be seen at even older ages for some serotypes. However, at all ages, conditions associated with reduced antibody formation constitute a high risk for acquiring pneumococcal infection in comparison to peers. Such conditions are congenital agammaglobulinemia, acquired common variable hypogammaglobulinemia,[147] selective IgG subclass deficiency,[746] and secondary defective antibody production in diseases such as malignancies and human immunodeficiency virus (HIV) infection. HIV infection predisposes individuals to the acquisition of secondary bacterial infection by several mechanisms, but defective antibody production is the most important factor in pneumococcal infection. The ability to produce antipneumococcal capsular antibody is inversely proportional to the peripheral blood $CD4^+$ lymphocyte count, especially if it falls below $500/mm^3$.[368,625] *S. pneumoniae* is the most common cause of invasive bacterial infection in HIV-infected children; it accounts for 35 to 50 percent of such episodes, with the relative risk of acquiring disease being 3- to 22-fold higher than that in children without HIV infection.[22,64,214,239,487,515] In one study, the incidence of invasive pneumococcal disease was 6.1 cases per 100 patient-years in HIV-infected children through the age of 7 years.[495]

DEFECTS IN COMPLEMENT. Congenital or acquired deficiencies in C1, C2, and C4 may be associated with increased susceptibility to pneumococcal infection, although cases documenting these associations are rare.[222]

NEUTROPENIA OR NEUTROPHIL DYSFUNCTION. The primary neutropenias, such as cyclic neutropenia, as well as secondary ones, such as drug-induced neutropenia and aplastic anemia, are associated with an increased incidence of severe pneumococcal infection. In some neutrophil dysfunction states, such as seen in alcoholism, liver cirrhosis, glucocorticoid treatment, and renal insufficiency, an increased incidence of pneumococcal infection is found. However, the incidence is not increased in other granulocyte dysfunction syndromes such as leukocyte adhesion deficiency syndromes[19] and chronic granulomatous disease.[248] In chronic granulomatous disease, although intracellular bacterial killing by polymorphonuclear WBCs is defective, the absence of catalase renders the pneumococcus susceptible to interaction between its endogenous H_2O_2 and myeloperoxidase and the halides present in PMNs.

DIABETES MELLITUS. Diabetes is associated with a high incidence of pneumococcal infection in adults.[113,213,601,772] However, because diabetes can predispose to pneumococcal infection by several mechanisms, some of which are found only in adults, whether diabetes mellitus in children also is a risk factor for the development of pneumococcal infection is not clear.

CONDITIONS ASSOCIATED WITH DECREASED PULMONARY CLEARANCE. Inflammatory conditions such as

asthma, chronic bronchitis, and chronic obstructive lung disease predispose to the development of bacterial infections of the lung, including pneumococcal pneumonia. Both active smoking and passive smoking are associated with chronic lung damage and inflammation and have been shown to be important risk factors for development of serious pneumococcal infection.[545] Respiratory viral infections also contribute to decreased pulmonary clearance. However, as described in the "Pathogenesis" and "Prevention" sections of this chapter, viral infections contribute to pneumococcal infection by several additional mechanisms.

CROWDING. Crowding contributes to many factors that increase the risk for acquiring pneumococcal infection, including viral infections, poor hygiene, and increased person-to-person transmission of *S. pneumoniae*. Attendance at daycare centers was the most important risk factor for acquiring invasive pneumococcal infection in children and infants in several studies.[68,124,261,463,608,718] In a recent population-based, case-control study, adults aged 18 to 64 years who lived in households that included children attending daycare were at greater risk of acquiring invasive pneumococcal infection.[545] In developed countries, attendance at daycare centers is also the most important risk factor for acquiring AOM, including pneumococcal otitis.[535,637,744]

NON–BREAST-FEEDING. Breast-feeding may be protective against pneumococcal infection. Human milk has been shown to block the attachment of pneumococci to pharyngeal cells, whereas bovine milk demonstrated only a weak effect.[305] This antibacterial effect could be mediated through several components of the immune system, including secretory IgA, lactoferrin, and lysozyme. Some authors have suggested that breast-feeding protects against otitis media,[24,104,197,744] but others have failed to show this effect.[58] Studies in the United States demonstrated a protective effect of breast-feeding against invasive pneumococcal infection in children in the general population[463] and in Alaskan natives.[261] In Finland, in contrast, no such protective effect was seen.[718]

GENETIC VARIATION IN MANNOSE-BINDING LECTIN. Approximately 5 percent of the population in Europe and North America and an even larger population in many developing countries are homozygous for MBL codon variants. These subjects have a greater than 2.5-fold increased risk for the acquisition of pneumococcal infection than do those who do not have this variant or are only heterozygous for this variant.[638]

MALE GENDER. Males are affected more often than are females in most studies on pneumococcal disease, including studies on pneumococcal bacteremia, pneumonia, meningitis, and otitis media.* The reason for the male preponderance is not understood.

SEASONALITY. The occurrence of pneumococcal infection is related to season. Clustering of invasive infections, pneumonia, and otitis media occurs from September to October through April to May in the Northern Hemisphere (with the opposite picture in the Southern Hemisphere), with peaks usually occurring from December through February.† Variation in the seasonality of carriage of pneumococci also occurs and is lowest in the summer months.[290,476] This seasonality probably is related, at least in part, to two factors: (1) it parallels the seasonal variations in viral respiratory infections, which play an important role in predisposing to pneumococcal carriage and infection, and (2) the peak pneumococcal infection season coincides with attendance at school and daycare centers, which often excludes the summer months. However, many issues regarding seasonality remain unclear, as exemplified by data from an ongoing surveillance of invasive infections in southern Israel, where two different populations live side by side and have two distinct seasonal patterns (Fig. 96–5). In this example, the Jewish population in southern Israel, with a lifestyle equivalent to that of a middle/lower social class Western population, has a seasonality of invasive pneumococcal infection similar to that of developed populations, as represented in Figure 96–5 by children from Houston, Texas.[413] In contrast, the Bedouin population in southern Israel, a population in transition from semi-nomadism to a Western lifestyle and who live in crowded and poor hygienic conditions with a high birth rate and a disease pattern similar to that in the developing world,[464] does not demonstrate a clear pattern of seasonality. The relative abundance of cases in the spring and summer is speculated to be related to the peak of diarrheal illness in this population.[464] Further understanding of seasonality patterns may contribute to prevention of pneumococcal disease in the future.

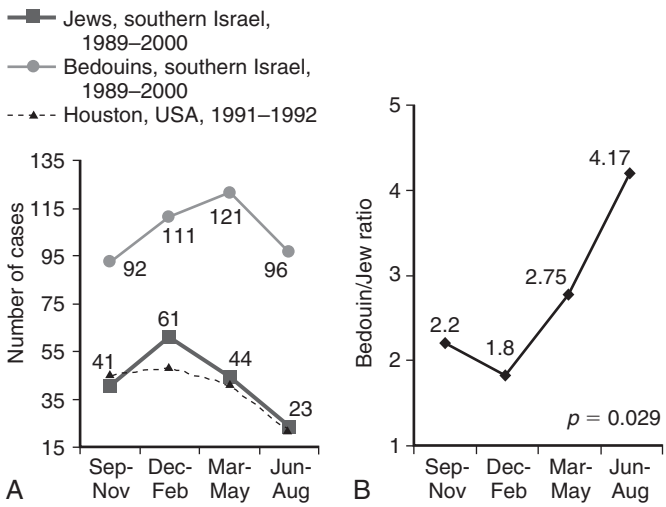


Figure 96–5 Seasonality of invasive pneumococcal infection in children from two populations in southern Israel during the years 1989 to 2000 and in Houston during the years 1991 to 1992.[405] **A,** Average number of cases for each season. **B,** Ratio of the average number of cases per season among Bedouin versus Jewish children in southern Israel.

NASOPHARYNGEAL CARRIAGE

Because acquisition and carriage of *S. pneumoniae* are associated with infection, that the higher incidence of pneumococcal infection in children than adults is associated with a higher incidence of nasopharyngeal carriage is not surprising. In various parts of the world, the carriage rate is approximately 5 to 10 percent in adults but can reach levels greater than 90 percent under various circumstances in infants and young children. Virtually all individuals carry pneumococci belonging to several serotypes during their lifetime. In a study conducted in the United States,[321] the prevalence of nasopharyngeal carriage in preschool children was 38 to 60 percent versus 29 to 35 percent and 9 to 25 percent in elementary school and junior high-school students, respectively. The prevalence in adults with no children at home was 6 percent. In studies in closed populations such as kibbutzim in Israel[87] or a poor and crowded black community in southern Israel,[160] the same differences between children and adults were noted.

Contact with young carriers increases the carriage rates in older children and adults. In the United States, although adults

*See references 92, 103, 112, 153, 207, 240, 350, 360, 701, 726.
†See references 98, 153, 207, 243, 285, 286, 288, 387, 405, 411, 522, 706, 754.

with no children had a carriage rate of 6 percent, the carriage rate increased to 29 percent when children were present at home. Similarly, primary school–aged children who have siblings younger than 2 years old carried *S. pneumoniae* more often than did those without young siblings.[481] In a study in Costa Rica, mother-child paired cultures showed an increased prevalence of carriage: from 6 percent at 1 month of age to 39 percent at 12 months of age. At the same time, carriage in mothers increased from 0 to 9.8 percent, thus confirming the influence of infants on adult carriage.[757] In Finland, the increased prevalence of carriage in mothers, fathers, and siblings when the index child grew older resulted in increased rates of pneumococci in the family.[459] In a Swedish study, the observed average duration of carriage was longer in children who had family carriers of the same serotype and, therefore, was suggestive of rapid recirculation in the family.[202]

Acquisition of *S. pneumoniae* may occur during the very first days of life. In infants aged 2 months, the prevalence of carriage ranges from less than 15 percent in some developed countries[23,82,171,286,459,476] to greater than 60 percent in developing countries.[138,241,284,498,514] Colonization peaks toward the second to third year of life.[171,290,320] The relationship of age to carriage is not understood, but it depends, at least in part, on the development of specific anticapsular antibodies.[288,289] In toddlers vaccinated with a pneumococcal conjugate vaccine, nasopharyngeal acquisition of new *S. pneumoniae* serotypes was related inversely to serum levels of specific antipolysaccharide IgG antibodies.[158] In another study, the presence of both circulating IgG and secretory IgA antibodies to the surface pneumococcal protein PsaA was associated with a lower prevalence of nasopharyngeal pneumococcal carriage.[397,605,685] A 2004 study showed that mucosal anticapsular IgA developed in response to colonization in preschool-aged children, regardless of vaccination status.[83,800] This phenomenon has been hypothesized to contribute to the falling carriage rates observed with increasing age.[801] The relative role of innate immunity with increasing age is not clear.

Crowding is an important factor that facilitates the spread of *S. pneumoniae*. Therefore, one is not surprised that in the developed world, the nasopharyngeal carriage rate and spread are highest in infants and toddlers attending daycare centers, with levels exceeding 90 percent in some studies,* followed by those living with one or more sibling at home.[23,171,481,595,753] In addition, the viral infections that are very prevalent in infants and toddlers attending daycare centers enhance colonization of the nasopharynx with *S. pneumoniae*.[290,476,763] The combination of young age, poor hygienic behavior, and increased incidence of respiratory viral infections renders daycare centers the ideal site for promotion and spread of *S. pneumoniae*. In addition, because of widespread antibiotic use, carriage of antibiotic-resistant *S. pneumoniae* is highest in daycare center attendees.† Daycare centers, thus, are a nucleus of high carriage of *S. pneumoniae*, particularly antibiotic-resistant strains. This nucleus is then responsible for the dissemination of such resistant organisms to other children, mainly to young siblings of daycare center attendees.[156,171]

The duration of carriage depends on age and serotype and may be related to additional factors such as antibiotic treatment, the immune status of the child, and other unknown factors. Carriage lasts longer in infants and young toddlers than in older children and adults.[159,202,288] In a study in Sweden,[202] infants were colonized for an average of 30 days, and after 3 months, 17 percent were carrying the same organism. In a study in the United States in adults, individual serotypes usually persisted for 2 to 4 weeks. The study showed that the first pneumococcal

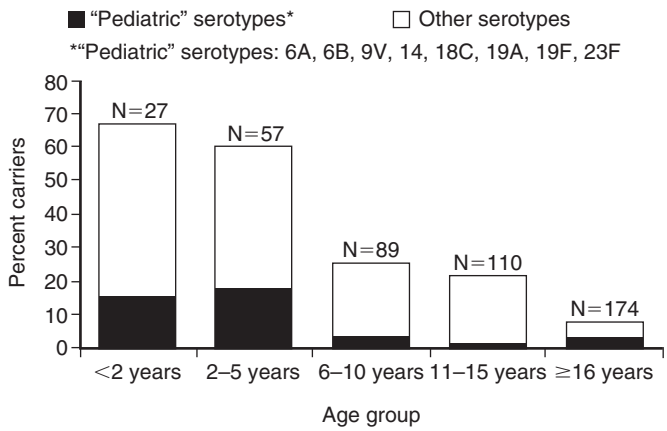


Figure 96–6 Nasopharyngeal carriage of *Streptococcus pneumoniae* in a closed black community living in crowded conditions in southern Israel. *(Adapted from Dagan, R., Gradstein, S., Belmaker, I., et al.: An outbreak of Streptococcus pneumoniae type 1 in a closed community in southern Israel. Clin. Infect. Dis.* 30*:319-321, 2000.)*

serotype that colonizes infants (typically before they reach 6 months of age) can be detected for as long as 12 months (mean, 4 months).[288]

The relationship between the ability of a specific serotype to colonize the nasopharynx of a child and its ability to cause respiratory or invasive infection is not clear. Some serotypes, such as 6A, 6B, 9V, 14, 18C, 19A, 19F, and 23F, are among the most frequent colonizers in infants and young children in most parts of the world and thus often are considered "pediatric" serotypes.[90,160] Some of these "pediatric" serotypes, such as serotypes 6B and 23F, also are less immunogenic than are others, especially in children younger than 2 years old, but some can show a reduced response even late in childhood. These serotypes are acquired frequently by infants and young children and often are carried for a prolonged period. After the child reaches the age of 2 years, carriage of these "pediatric" serotypes declines rapidly. Although pneumococcal carriage decreases overall with age, the proportion of the "nonpediatric" serotypes increases with age. This phenomenon is demonstrated in Figure 96–6.

In contrast to the "pediatric" serotypes, serotypes such as 1, 5, 7F, and 12 are carried infrequently and are eliminated from the nasopharynx rapidly. However, these serotypes are able to cause disease and even epidemics.[160,331,526,529,694] The different distribution of serotypes among colonized children, children with invasive infections, and adults with pneumonia is exemplified in Figure 96–7.

Although most colonization occurs without the development of disease, some prospective, longitudinal studies have suggested that most systemic infections develop soon after colonization by a new pneumococcal serotype.[210,286,288] Carriers are protected from invasive disease by the development of circulating antibodies. However, such antibodies do not always protect against invasion of contiguous sites, and other studies have shown that a substantial number of pneumococcal otitis media cases occur at any time during nasopharyngeal colonization by a specific serotype.[43,714]

The epidemiology of nasopharyngeal carriage of antibiotic-resistant pneumococci is important. The most significant promoter of carriage of antibiotic-resistant *S. pneumoniae* is antibiotic use.* Prolonged carriage of azithromycin-resistant *S. pneumoniae* was observed after a single dose of azithromycin was administered to Australian aboriginals for the treatment of trachoma.[443]

*See references 23, 84, 135, 159, 171, 274, 541, 595, 648, 791.

†See references 73, 159, 171, 185, 274, 400, 611, 648, 649, 715, 748, 791.

*See references 30, 33, 133, 159, 171, 195, 256, 419, 422, 595, 799.

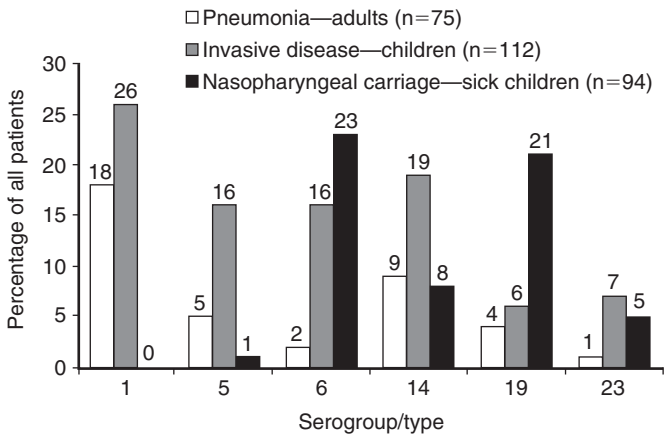


Figure 96–7 Distribution of selected serogroups/serotypes in *Streptococcus pneumoniae* isolates from adults with pneumonia, children with invasive infection, and nasopharyngeal specimens from sick children in Kenya. *(Adapted from Scott, J. A. G., Hall, A. J., Hannington, A., et al.: Serotype distribution and prevalence of resistance to benzylpenicillin in three representative populations of Streptococcus pneumoniae isolates from the coast of Kenya. Clin. Infect. Dis. 27:1442-1450, 1998.)*

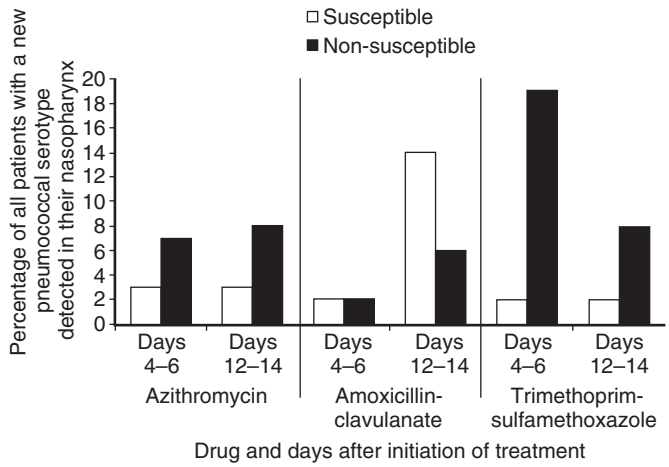


Figure 96–8 Nasopharyngeal acquisition of new pneumococcal serotypes during antibiotic treatment of acute otitis media based on susceptibility or resistance to the antimicrobial that was administered. Material for culture was obtained 3 to 5 days and 11 to 13 days after initiation of treatment. The regimens were azithromycin, 10 mg/kg as a single dose on day 1, followed by 5 mg/kg once daily for an additional 4 days; amoxicillin-clavulanate, 45/6.6 mg/kg/day in two divided doses for 10 days; and trimethoprim-sulfamethoxazole, 8/40 mg/kg/day in two divided doses for 10 days.[169]

Prophylaxis with amoxicillin increased the carriage of penicillin-resistant *S. pneumoniae*.[101] An association between trimethoprim-sulfamethoxazole prophylaxis and nasopharyngeal colonization with both trimethoprim-sulfamethoxazole– and penicillin-resistant *S. pneumoniae* has been demonstrated.[5,30,169]

A series of prospective studies revealed some of the early processes that occur in the nasopharynx during and in the immediate post-treatment period in cases of AOM caused by antibiotic-resistant *S. pneumoniae*.[133,137,148,167,169] These events can be summarized as follows: (1) most drugs studied had a substantial effect on the nasopharyngeal flora and eradicated or reduced the carriage of pneumococci that were susceptible to the drugs; (2) little, if any effect was seen when the organism had reduced susceptibility to the drugs administered; (3) some drugs, such as azithromycin and trimethoprim-sulfamethoxazole, appeared to promote colonization with resistant *S. pneumoniae*; and (4) β-lactam drugs with higher activity against *S. pneumoniae* in general and against non-penicillin-susceptible *S. pneumoniae* in particular, such as amoxicillin-clavulanate and cefuroxime axetil, decreased colonization better than did drugs with poor antipneumococcal activity, such as cefaclor, cefpodoxime, and cefixime.

Even more intriguing than the differential effect of various drugs on carriage of *S. pneumoniae* is the ability of these agents to alter nasopharyngeal colonization. Such alteration occurs by selection of pneumococcal strains that were masked by other organisms or selection of strains that were acquired after initiation of treatment, as was exemplified when children suffering from trachoma received one dose of azithromycin and were monitored for nasopharyngeal carriage of *S. pneumoniae*.[443] Before treatment, 68 percent were colonized with *S. pneumoniae*, but only 1 percent had azithromycin-resistant *S. pneumoniae*. Within 2 to 3 weeks after they received treatment, colonization decreased to 29 percent, but 16 percent were colonized with azithromycin-resistant *S. pneumoniae*. Two months later, 78 percent were colonized with *S. pneumoniae* and 27 percent with azithromycin-resistant *S. pneumoniae*. The prevalence of pneumococcal colonization and azithromycin susceptibility returned to pre-exposure values only after 6 months. In another study, cultures performed shortly after initiation of treatment and immediately after cessation of treatment showed that new serotypes, either susceptible or resistant to the treatment drug, appeared in 21, 24, and 31 percent of those treated with azithromycin, amoxicillin-clavulanate, and trimethoprim-sulfamethoxazole, respectively[169] (Fig. 96–8). The proportion of new strains of pneumococci resistant to these drugs was 15, 8, and 27 percent, respectively.

A study looking at more than 31,000 *S. pneumoniae* isolates between 2000 and 2003 found that 29.4, 22.5, and 0.9 percent were resistant to erythromycin, penicillin, and levofloxacin, respectively. Thirty-one percent of the isolates exhibited multidrug resistance. Among macrolide-resistant isolates, *mefA* was the most prevalent resistant gene identified. Ninety percent of isolates that contained both the *mefA* and the *ermB* resistance genes were resistant to penicillin, tetracycline, or trimethoprim-sulfamethoxazole. However, 98.6 percent of those isolates were susceptible to levofloxacin.[374]

Prolonged and low-dose antibiotic regimens are suggested to be important contributors to promotion of carriage and spread of antibiotic-resistant *S. pneumoniae*.[136,294,329,482,659]

One of the serious problems with pneumococcal strains clearly is the emergence of resistance to more than one antibiotic class.[355] This phenomenon explains why the use of one antibiotic may result in carriage of *S. pneumoniae* resistant not only to the antibiotic to which the host was exposed but also to other, unrelated classes of antibiotics. In 1993, approximately a fifth of the pneumococcal strains in Iceland were not penicillin-susceptible and 80 percent of them were multidrug-resistant. When the risk for carriage of penicillin-resistant *S. pneumoniae* was investigated, a clear association was found not only with use of β-lactam drugs but also with the use of trimethoprim-sulfamethoxazole and erythromycin.[30] Other authors likewise have shown an association between the use of trimethoprim-sulfamethoxazole and carriage of penicillin-resistant *S. pneumoniae*.[82,507]

When a group of children who did not carry trimethoprim-sulfamethoxazole–resistant *S. pneumoniae* were studied longitudinally during therapy for AOM,[169] carriage of trimethoprim-sulfamethoxazole–resistant *S. pneumoniae* was found in 23 percent of patients by day 6 and in 33 percent by day 40. Additionally, non–penicillin-susceptible *S. pneumoniae* was carried at these times by 26 and 43 percent of children, respectively. This remarkable promotion of colonization with penicillin-resistant strains by trimethoprim-sulfamethoxazole

treatment occurred because many strains were resistant to both penicillin and trimethoprim-sulfamethoxazole.

Mass chemoprophylaxis and therapeutic campaigns now are being conducted in different regions against a variety of diseases, which raises concern about the widespread development of resistance. Examples are mass azithromycin treatment campaigns to eradicate trachoma, mass sulfadoxine-pyrimethamine (Fansidar) treatment (shown to be associated with an increased rate of resistance to trimethoprim-sulfamethoxazole in pneumococci),[218] increasing use of trimethoprim-sulfamethoxazole for prophylaxis of patients infected with HIV, and mass treatment with fluoroquinolones and tetracyclines after exposure to *Bacillus anthracis*.

The dramatic change in nasopharyngeal flora after initiation of antibiotic therapy has two important consequences. First, the phenomenon described earlier predisposes patients to new acquisition of infection with more resistant organisms.[157] Antibiotic treatment not only can increase nasopharyngeal carriage of antibiotic-resistant *S. pneumoniae* but also can induce superinfection of the middle ear with a resistant strain within a few days.[167] Second, the increased prevalence of antibiotic-resistant *S. pneumoniae* in the nasopharynx may increase transmission in crowded conditions such as extended families and daycare centers.[171,262,274,320,595,627,791] A recent study found that reducing the number of antibiotic prescriptions written in a French community resulted in an 18 percent decrease in colonization ($p < 0.05$). Physicians participating in the study were instructed not to write prescriptions for presumed viral respiratory tract infections even with purulent rhinitis. In addition, the physicians were asked to document actual streptococcal pharyngitis with an antigen test.[295] Therefore, the widespread use of antibiotics is likely to be responsible for the increase in antibiotic-resistant *S. pneumoniae*, especially in crowded populations, thus creating a vicious cycle that is difficult, if not impossible, to overcome (Fig. 96–9). The presence of this vicious cycle poses a real challenge to society.

Since the use of pneumococcal vaccine has become widespread in the United States, the serotypes found most commonly in nasopharyngeal colonization have been replaced by nonvaccine serotypes. A study evaluating nasopharyngeal colonization in 16 Massachusetts communities during 2001 to 2004 showed a decrease in serotypes found in the PCV-7 vaccine from 36 percent to 14 percent along with an increase in non–PCV-7 serotypes from 34 percent to 55 percent.[342] Another study showed that 25 percent of children colonized with pneumococci carried antibiotic-resistant, nonvaccine serotypes, including serotypes 19A and 35B, rarely detected before introduction of the pneumococcal conjugate vaccine. Seven percent of the 19A isolates tested in the study were resistant to all oral agents tested (penicillin, amoxicillin, cefuroxime, ceftriaxone, azithromycin, and trimethoprim/sulfamethoxazole), and 12 percent of the 35B isolates were not susceptible to penicillin or cefuroxime.[359]

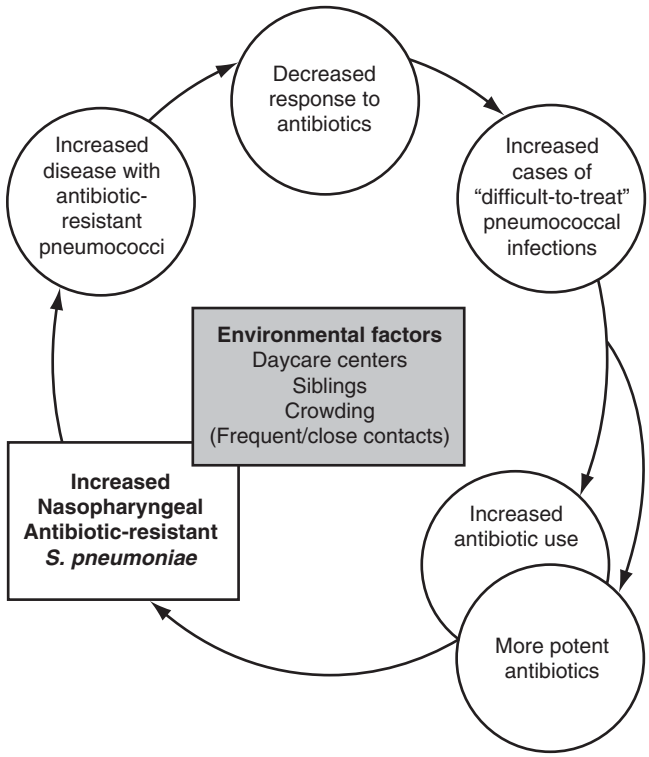


Figure 96–9 Chain of events that create a vicious cycle in which antibiotic treatment increases the carriage of antibiotic-resistant pneumococci, which cause more disease with resistant organisms. This situation results in reduced response to antibiotic treatment and an increase in the number of "difficult-to-treat" cases. As a result, the use of antibiotics is increased, especially more potent ones, thus again promoting a further increase in nasopharyngeal carriage of antibiotic-resistant pneumococci.

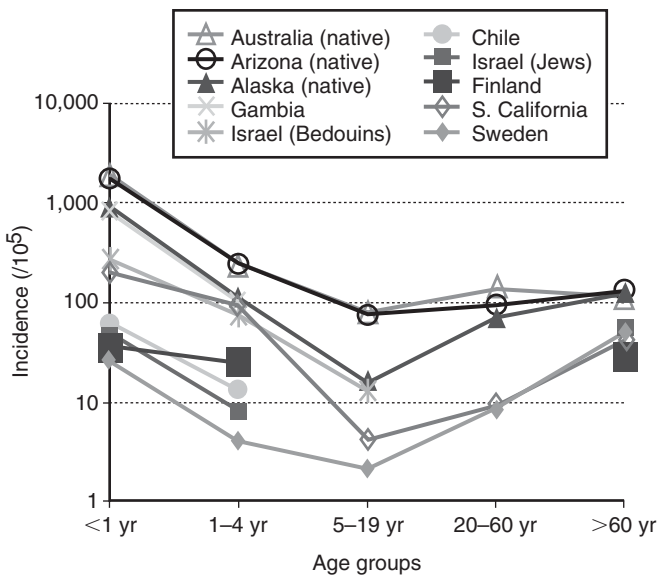


Figure 96–10 Age-specific incidence of invasive pneumococcal disease in various populations. Data were obtained from various presentations and publications.

INVASIVE PNEUMOCOCCAL DISEASE

Although invasive pneumococcal infections occur far less commonly in early childhood than does pneumonia or otitis media, they are an important cause of morbidity and mortality during childhood worldwide. Pneumococci are more common than are Hib and *N. meningitidis* as causes of bacteremia in most countries and rank second after Hib or third after *N. meningitidis* in causing bacterial meningitis. Pneumococci are estimated to be responsible for 25 to 50 percent of cases of bacterial meningitis in children in the United States,[660] Europe,[140] and Africa.[575]

The incidence and severity of invasive pneumococcal disease vary in different populations. Figure 96–10 exemplifies the differences in incidence in various populations by age group. Several important points can be drawn from this figure: (1) an age-dependent pattern exists that is similar in all populations: the highest rate is in infants, the rate decreases rapidly toward the age of 5 years, and then it increases again toward the age of 60 years, with a second peak occurring in persons older than 65; (2)

a marked difference exists among various populations in the incidence of invasive infection, and this difference can be up to 100-fold; and (3) in general, the incidence in the more industrialized countries is lower than that in less industrialized countries or in less privileged populations, but the U.S. figures seem to be higher than those of other developed populations.

Because invasive pneumococcal infections are detected by blood culture in most cases, variations in rates of performing blood cultures in young children could be responsible, at least in part, for differences in the reported incidence of invasive pneumococcal infection.[217,312] In contrast to Europe, where most pediatric blood isolates were obtained from hospitalized children, many blood cultures are performed on outpatients in the United States.[312] This difference arises because U.S. practice guidelines recommend blood cultures for children aged 3 to 36 months with high fever and WBC counts of 15,000/mm^3 or greater.[53] Indeed, several European investigators have noted that the recent rises in reported invasive pneumococcal disease rates might be related to an increasing likelihood of taking blood for culture.[48,387,539,697] Preliminary evidence from Chile also suggests that a considerable proportion of relatively mild invasive infections routinely go unrecognized.[438] This hypothesis can be supported by the fact that the incidence of meningitis is similar in the United States and Europe.[312]

Thus, the incidence of invasive infection in children younger than 5 to 6 years in western Europe (e.g., Finland, the United Kingdom, Germany, Switzerland, Denmark, Spain) was lower than 25 cases per 100,000 population per year in various studies; in Chile, Australia, and New Zealand, it was 25 to 60 per 100,000; and during the same period in the United States, the range was approximately 65 to 75 cases per 100,000.[312] In contrast, the incidence of pneumococcal meningitis in these countries was similar: 3.6 per 100,000 in the United States versus a mean of 4.6 per 100,000 in Europe (range, 2.1 to 7.0).[312]

Differences in the incidence of invasive pneumococcal infection among populations are not due solely to different blood culture practices because true significant differences can be found within the same country. This difference can be exemplified by comparing the incidence of invasive pneumococcal disease in different ethnic groups in the United States, where a higher incidence of invasive pneumococcal disease occurs in African Americans, Alaskan Natives, and specific American Indian populations than in whites (Table 96–2). The incidence of pneumococcal bacteremia and meningitis in Alaskan Native children younger than 5 years ranges from 598 cases per 100,000 population in those 6 to 11 months of age to 56 cases per 100,000 population in those 36 to 47 months of age, which is approximately four times the incidence in similarly aged non–Alaskan Native/non–American Indian children.[115] The highest incidence for any ethnic group in the United States is found in Navajo and Apache populations living on reservations in the southwestern United States. The incidence in children aged 1 to 2 years in these populations is 557 to 2396 per 100,000.[142,549] Among children younger than 5 years, the incidence of invasive pneumococcal disease in African American children in the United States is two to three times higher than that in white children of the same age.[61,94,309,622] In a case-control study of risk factors for the development of invasive disease in young children, the association of race with disease risk was not statistically significant in an analysis that controlled for socioeconomic status.[463] However, other studies have reported persistence of increased risk even when controlling for income.[94,123,223,309,322] The reasons for the increased incidence in some populations in comparison to others in the United States are unclear.

In other geographic regions, differences among populations also can be observed, again being higher in populations that live in underprivileged conditions, which raises the question of the importance of genetic versus environmental risk factors. In southern Israel, the incidence of pneumococcal invasive infection during the first year of life in Bedouin infants (a population with a lifestyle, birth rate, and general disease incidence similar to that of the developing world) was 270 cases per 100,000 versus 53 per 100,000 in Jewish infants (a population with standards of living comparable to the middle/low social class in the developed world) ($p < 0.001$).[240] This difference also was found in children aged 1 to 4 years: 75 and 21 cases per 100,000, respectively ($p < 0.001$). In New Zealand, when Maoris, Pacific Islanders, and others were compared, incidence rates of invasive pneumococcal disease in the first year of life per 100,000 population were 153, 276, and 52, respectively. The respective rates in children younger than 5 years were 67, 117, and 36 per 100,000, respectively.[760]

Studies on the incidence of invasive pneumococcal infection in developing regions are scanty. In one study in The Gambia, the annual incidence in children younger than 1 and 5 years was 554 and 240 per 100,000, respectively.[554] Another study in The Gambia documented the incidence of invasive pneumococcal infection to be 224, 139, and 82 cases per 100,000 children during the first, second, and third years of life, respectively.[747] *S. pneumoniae* plays a more important role in the acquisition of meningitis in developing countries than in developed countries. Reports from several African countries show that *S. pneumoniae* accounts for 20 to 50 percent of all cases.[575] Data from many other developing populations, especially from countries in Southeast Asia, are lacking but are needed because data from Africa cannot be extrapolated to other developing parts of the world.

TABLE 96–2 Incidence (Cases/100,000 Population) of Invasive Pneumococcal Disease in Selected U.S. Pediatric Populations—Selected Years

Age Group	United States, All Races, 1998[593]	United States, Whites, 1998[593]	United States, African Americans, 1998[593]	Alaska, Natives, 1986-1990[178]	Alaska, Natives, 1986-1997[115]	Navajo American Indians, 1989-1996[549]	Apache American Indians, 1983-1990[142]
0-5 mo	73.4	60.9	163.5	624*	276.8	629*	1820*
6-11 mo	227.8	178.2	542.2	↓	597.7	↓	↓
12-23 mo	184.2	137.2	440.7		453.0	557	
24-35 mo	64.7	54.6	116.4	98*	125.2	73*	227*
36-47 mo	26.7	23.9	46.1	↓	56.2	↓	↓
48-59 mo	14.3	9.1	20.6		73.2		
5-9 yr	5.7	4.8	9.3	23	—	—	54
10-19 yr	2.9	2.5	4.8	5	—	—	35
All ages	23.2	19.7	49.7	—	—	63	207

**Average of all age groups indicated by arrows.*

The fatality rate of pneumococcal meningitis is higher than that of meningitis caused by other organisms.[575] Mortality rates for invasive pneumococcal infection in developed populations vary between less than 2 percent and 6.6 percent.[153,180,293,511,622,760] In contrast, in the developing world, mortality rates for invasive pneumococcal infection, including meningitis, vary from 19 percent in South Africa[343] to as high as 67 percent in Mali and Niger.[573,575]

Most invasive pneumococcal diseases in children are caused by a limited number of pneumococcal serotypes. However, despite the many studies reporting on serotypes or serogroups published or presented thus far, the data are not yet complete. Data on serotypes are important, especially with regard to the question of coverage of the various conjugate vaccines, as discussed in the section on prevention in this chapter. The extensive literature was summarized by Hausdorff and colleagues[310,311] in two review articles. The results of their exhaustive review, despite showing many gaps in our knowledge, did lead to certain conclusions (Fig. 96–11): (1) in children younger than 5 years, the great majority of invasive infections are covered by the 11 serotypes that will be included in future conjugate vaccines (or the cross-reacting serotypes in the same serogroup [serogroups 1, 3, 4, 5, 6, 7, 9, 14, 18, 19, 23]); (2) in some regions, 4 serogroups not present in the 7-valent vaccine (serogroups 1, 3, 5, and 7) are of great importance; (3) in older children and adults (mainly those older than 18 years), the 7-valent vaccine often covers only a minority of cases, whereas the 11-valent vaccine covers a greater percentage; and (4) the 23-valent nonconjugate vaccine provides considerably wider coverage than the conjugate vaccines do, although it may not be immunogenic in infants, young children, or immunocompromised hosts.

A recent study evaluating the invasiveness of different *S. pneumoniae* serotypes found that serotypes 4, 14, 7F, 9V, and 18C were associated with rates of greater than 20 invasive pneumococcal disease cases per 100,000 acquisitions, whereas serotypes 23F, 6A, 19F, 16F, 6B, and 15B/C were associated with fewer than 10 invasive pneumococcal disease cases per 100,000 acquisitions. The study also found an inverse relationship between the duration of carriage and the likelihood of causing invasive disease ($p < 0.0001$).[690]

Since the PCV-7 vaccine was introduced, the rate of invasive pneumococcal disease in young infants has decreased significantly.[588] In a study involving eight U.S. children's hospitals, the incidence of invasive pneumococcal disease has decreased more than 75 percent in children younger than 24 months. Replacement of vaccine serotypes by nonvaccine serotypes was noted, particularly serogroups 15 and 33.[278,393] Furthermore, penicillin resistance has increased among nonvaccine serogroups.[393] A study in northern California clinics showed an 84 percent reduction in *S. pneumoniae* bacteremia and a 67 percent reduction in overall bacteremia since routine vaccination was implemented. In this same study, a third of the pathogens identified in positive blood cultures were *E. coli*, a third were nonvaccine serotype *S. pneumoniae*, and the remaining third were mostly *S. aureus*, *Salmonella*, *N. meningitidis*, or *Streptococcus pyogenes*.[326] Despite the use of pneumococcal vaccine, children younger than 1 year and children with comorbid illnesses such as malignancy, HIV infection, immune deficiency, or nephrotic syndrome continue to remain at risk for acquiring invasive pneumococcal disease.[340]

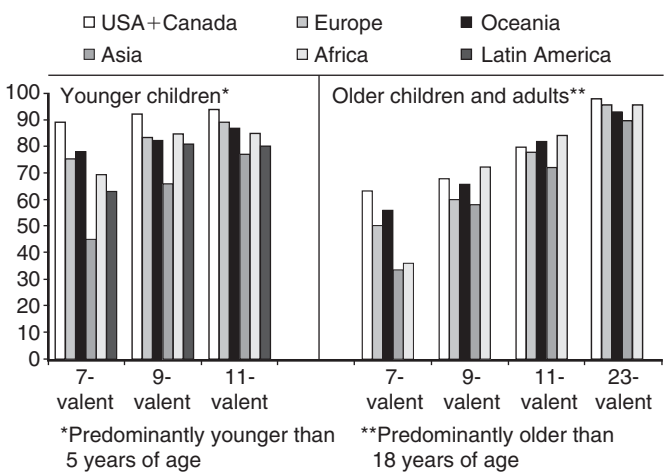


Figure 96–11 Coverage of 7-valent, 9-valent, and 11-valent conjugate pneumococcal vaccines and 23-valent nonconjugated pneumococcal polysaccharide vaccine in younger children (predominantly 5 years of age) and older children and adults (predominantly >18 years of age) in various regions of the world.[310,311]

Serotype 19A is, at present, the most important cause of invasive pneumococcal disease by replacement serotypes since the widespread use of pneumococcal vaccine, and it is becoming increasingly drug-resistant. Between July 1999 and June 2004, the overall rate of invasive pneumococcal disease decreased from 23.3 to 13.1 cases/100,000 population ($p < 0.00001$); during this same period, the rate of invasive pneumococcal disease caused by serotype 19A in children younger than 5 years old increased from 2.6 to 6.5 cases/100,000 population.[562]

In the first 3 years after routine vaccination with heptavalent pneumococcal conjugate vaccine was introduced, the incidence of overall invasive pneumococcal disease decreased 67 percent in Alaska Native children younger than 2 years old. However, between 2001 to 2003 and 2004 to 2006, an 82 percent increase occurred in the rate of invasive disease in Alaska Native children younger than 2 years old. Since 2004, the invasive pneumococcal disease rate caused by nonvaccine serotypes has increased 140 percent in comparison to the prevaccine period.[686]

Many reasons, such as differences in living conditions and socioeconomic status and genetic differences among populations, can be cited for the diversity of serotype/serogroup distribution in populations. However, Hausdorff and associates[312] suggest that the differences in serotype/serogroup distribution among various geographic regions may be related to differences in the testing and reporting practices of various countries. Some serotypes/serogroups may be associated with more severe disease than others are. In regions where blood cultures are performed for mildly ill children, such as the United States, the predominance of serogroups such as 6, 9, 14, 18, 19, and 23 can be accentuated, whereas serotypes such as 1, 3, 5, and 7F, which are found in more severe disease, appear to be less important. The importance of having local data on serotype coverage for evaluating disease burden that can be prevented by vaccination always should be considered.

PNEUMONIA

In adults, *S. pneumoniae* is by far the predominant cause of community-acquired bacterial pneumonia. However, this cause is more difficult to assess in children because obtaining bacteriologic specimens such as sputum from infants and young children is difficult. Blood cultures usually are positive in less than 5 percent of the children with any pneumonia and, thus, cannot provide an accurate representation of the pathogens in childhood pneumonia. Nasopharyngeal cultures, as well as antigen detection and PCR, also are difficult to correlate with bacterial pneumonia (see the section on diagnosis). Studies using lung aspirates are helpful but scant,[761] and most of the recent studies were performed in one developing country (The Gambia). However, data derived from these studies show an important role of *S. pneumoniae* as a causative agent of pneumonia in children.[8,234-236,673]

In a recent study conducted in Finland, transthoracic needle aspiration disclosed an etiology in 18 of 26 patients from whom a representative sample specimen was obtained.[762] Of these patients, 10 of 26 (38%) had lung aspirate cultures positive for *S. pneumoniae*, and 1 additional child had a positive blood culture for *S. pneumoniae*. An additional six patients (23%) had a positive PCR test. Thus, in total, 17 of 26 (65%) had at least one test that was positive for *S. pneumoniae*.

Studies with the newly developed pneumococcal conjugate vaccines in children suggest that the role of *S. pneumoniae* in lower respiratory infections in general and pneumonia in particular may be more important than previously demonstrated. In a study in California,[71] administration of a 7-valent conjugate pneumococcal vaccine reduced the number of cases of clinical pneumonia in children younger than 3.5 years by 6.0 percent ($p = 0.13$) (intention-to-treat analysis). A reduction in the incidence of pneumonia of 8.9 percent ($p = 0.03$) was found in patients who had clinical features of pneumonia and had a chest radiograph performed, regardless of the findings. The incidence of pneumonia was reduced by 22.7 percent in patients with a positive finding on the chest radiograph (defined as parenchymal infiltrates, consolidation or effusion [or both], but not perihilar infiltrates only). In South Africa, administration of three doses of 9-valent conjugate pneumococcal vaccine reduced the incidence of radiologically proven pneumonia by 22.1 percent ($p = 0.049$) in children younger than 2 years old (Klugman, K., and Madhi, S.: Data presented at the Third International Symposium on Pneumococci and Pneumococcal Diseases, May 2002, Alaska). This ability of a pneumococcal vaccine to reduce the incidence of clinical and radiologically documented pneumonia suggests that (1) *S. pneumoniae* is the causative agent in most cases of pneumonia, with consolidation being seen in infants and toddlers in the population studied, and (2) *S. pneumoniae* is a causative agent in many cases usually considered to be viral. Supportive evidence for the latter suggestion can be derived from a study conducted in Israeli toddlers attending daycare centers.[174] In these toddlers, a 9-valent pneumococcal vaccine reduced the incidence of lower respiratory disease, including bronchiolitis, cough, and pneumonia, by 16 percent and reduced antibiotic treatment days for these entities by 47 percent. More studies are being undertaken to investigate the potential of pneumococcal conjugate vaccines to reduce the incidence of pneumonia and other lower respiratory tract infections in developing populations (Native Americans and those in The Gambia and the Philippines).

Although pneumonia usually is not a fatal disease in developed countries, it is an important cause of morbidity and hospitalization. Pneumococcal infections are thought to be rare causes of serious lower respiratory tract disease requiring hospitalization in children younger than 6 months.[181] However, *S. pneumoniae* is an important cause of hospitalization for community-acquired pneumonia in older children.[131,189,258,429,542,640]

In developing countries, acute respiratory infections in general and pneumonia in particular are the leading causes of morbidity and mortality.[505] The mortality rate from respiratory infections in developing countries is estimated to be 10- to 30-fold higher than that in developed countries.[6,461] In addition, of the 15 million children younger than 5 years old who die annually, an estimated 4 million die of pneumonia.[296,461] When obtained, rates of positive sputum culture for *S. pneumoniae* in developing countries can be as high as 88 percent in children.[63] Using a variety of methods, including antigen detection, cultures from blood, sputum, and pleural and lung aspirates, and antipneumococcal antibody testing, a series of investigations in The Gambia associated *S. pneumoniae* with severe acute lower respiratory tract infection in 20 percent of hospitalized infants and in 61 percent of children aged 1 to 9 years.[235,236] In another study in The Gambia[234] conducted on ambulatory children younger than 5 years old with lower respiratory tract infections, *S. pneumoniae* was associated with 8.6 percent of all cases of clinical acute lower respiratory tract infection, 12.3 percent of episodes of radiologically proven acute lower respiratory tract infection, and 28.0 percent of episodes of lobar pneumonia. Despite the limitations of these diagnostic tests, these results demonstrate the importance of *S. pneumoniae* in severe respiratory infection.

No accurate information exists about the pneumococcal serotypes involved in pneumonia in infants and young children. The only accurate data are for bacteremic pneumococcal pneumonia. In these cases, the serotype distribution is not different from that for other invasive infections. However, the bacteremic cases represent only a minority of pneumococcal pneumonia cases, and one cannot extrapolate these data to nonbacteremic cases. The finding of an impressive reduction in pneumonia after the use of a conjugate pneumococcal vaccine, as reviewed earlier, suggests that most pneumonia cases in the population studied are pneumococcal and caused by serotypes included in the vaccine.

OTITIS MEDIA

Otitis media is the diagnosis that accounts for most office visits in pediatric clinics in the United States. It results in more than 15 million visits per year.[425,706] In the United States, 10 percent of children have one or more episodes of otitis media by the time that they are 3 months of age, approximately 60 percent by 1 year of age, and more than 80 percent by 3 years of age.[412,727] More recent statistics show even higher figures: a study involving 2253 infants in the Pittsburgh area showed that 48 percent had one or more episodes of otitis media between 2 and 6 months of age, 79 percent between 2 and 12 months of age, and 91 percent by 24 months of age.[566] Finnish studies in the 1980s showed an incidence of 0.47 to 1.05 episodes per year in infants aged 0 to 12 months.[394,598,688] Otitis media often is not perceived as a severe problem in developing countries, but community studies have shown perforation of the tympanic membrane in 0.4 to 6.1 percent and mastoiditis in 0.19 to 0.74 percent of all children.[62] Although serious complications rarely occur, the economic cost of otitis media is estimated at more than $3.5 billion each year in the United States.[254,706] During 1996, approximately 500,000 tympanostomy tubes were placed in children's ears in the United States.[536] AOM also is the leading reason for prescribing antibiotics to children.[593]

In a prospective study conducted in southern Israel that examined the burden of AOM on patients and their families,[291] the average number of days of severe crying, temperature higher than 38° C, loss of appetite, and insomnia was 2.9, 7.8, 7.0, and 6.6, respectively. An average of 18.6 days was required for a family to return to normal activity. The number of visits, use of emergency rooms, and care by otolaryngologists averaged 2.6, 0.3, and 0.35 per episode, respectively. The average antibiotic and over-the-counter drug treatment days per episode were 9.1 and 6.6, respectively. Parents lost an average of 1.6 working days, and children lost an average of 3.5 daycare days per episode. The parents thought that during a 1-month follow-up period, an average of 18.6 days were nonroutine days versus only 3.4 such days in controls.

The role of *S. pneumoniae* in otitis media has been studied extensively. During the last decades, numerous studies with various designs have been performed in many geographic regions. *S. pneumoniae* was the major bacterial cause of otitis media and accounted for 25 to 60 percent of cases.* However, in some recent studies, *H. influenzae* was found more commonly than was *S. pneumoniae* in AOM.[161,257,402] *H. influenzae* is a rare finding in

*See references 72, 77, 162, 183, 195, 206, 264, 358, 377, 402, 410, 484, 528, 629.

first episodes of AOM, but it becomes increasingly common from the third episode onward, whereas *S. pneumoniae* is a common finding in all episodes.[402]

Although the cost of each AOM episode may be relatively low when compared with other infections caused by *S. pneumoniae*, the highest overall cost in all pneumococcal infections is due to otitis media because of the large number of episodes.[466] The estimated cost for meningitis was 11,081 U.S. dollars per case in 1997, for bacteremia it was $2313, and for pneumonia with consolidation it was $1464. In contrast, for simple otitis media, the cost was $294 per episode, and for complex otitis media it was $1339 per episode. The cost of tympanostomy tube placement was $2390 per case. The estimated annual cost in the United States was $3 to $5 billion for simple pneumococcal otitis media and approximately $1.3 billion for complex pneumococcal otitis media. In contrast, the annual cost of pneumococcal meningitis was approximately $9 million, that of pneumococcal bacteremia was $35 million, and that of pneumococcal pneumonia was $113 million.

Antibiotic resistance in *S. pneumoniae* strains causing otitis media is rising sharply, and in some countries, resistance to at least one antibiotic class (in most cases penicillin and other β-lactams) is now the rule, not the exception. Figure 96–12 exemplifies the rapidity of the increase in antibiotic resistance among *S. pneumoniae* isolates from the middle ear fluid of children with AOM in France and Israel in the last decade. A well-established fact is that higher antibiotic resistance rates are found in pneumococci from patients with nonresponsive AOM and recurrent AOM, in whom recent antibiotic treatment is common, as well as in daycare center attendees.[55,73,135,157,164,458,583,799] In children younger than 18 months old, the prevalence of antibiotic-resistant *S. pneumoniae* in AOM is higher than that in older age groups.[55,73,159,358]

Studies of pneumococcal serotypes causing AOM in the last 60 years have demonstrated relatively similar patterns, with the most common serotypes being 6A, 6B, 9V, 14, 19A, 19F, and 23F.[43,73,114,157,225,257,556,577,680] (Fig. 96–13). As mentioned in the section on bacteriology, these serotypes also are the most frequent antibiotic-resistant serotypes. Furthermore, the great majority of highly penicillin-resistant and multiply-resistant *S. pneumoniae* belong to five serotypes (6B, 9V, 14, 19F, 23F) and, to a lesser extent, to related serotypes (6A and 19A).[157,380] This finding is somewhat reassuring with regard to vaccination because the serotypes most frequently associated with otitis media are included in the PCV-7 vaccine. The serotypes included in the PCV-7 vaccine are 4, 6B, 9V, 14, 18C, 19F, and 23F. A recent study found that 60 to 70 percent of all *S. pneumoniae* isolates that caused AOM in children in the 6- to 59-month age range were represented in the PCV-7 vaccine.[313] Most of the data with regard to AOM are obtained from studies involving middle ear puncture (tympanocentesis or myringotomy) or spontaneously draining ears; therefore, the spectrum of cases studied probably could be skewed toward the most severe cases or treatment failures.

Immunization with conjugate pneumococcal vaccine is associated with replacement of vaccine serotypes by other serotypes. Widespread immunization with conjugate vaccine may increase the importance of some nonvaccine serotypes while decreasing that of the serotypes included in the vaccine. A study in children showed a shift in pneumococcal colonization toward nonvaccine serotypes (11, 15 and 23B)[86] and an increase in *S. aureus*–related AOM after immunization.[85]

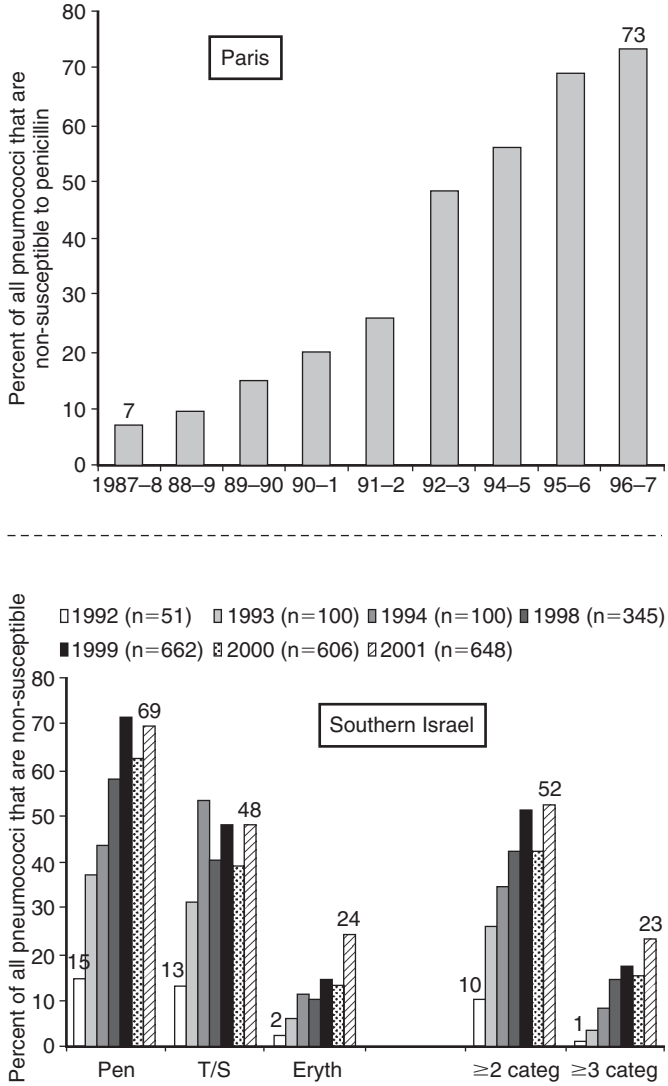


Figure 96–12 Rate of increase in non–antibiotic-susceptible pneumococci isolated from middle ear fluid during acute otitis media episodes in France and Israel during a 10-year period. **Top,** Non–penicillin-susceptible *Streptococcus pneumoniae* in Paris from 1987 through 1997. A 10-fold increase in resistance to penicillin occurred during this period.[259] **Bottom,** Non–penicillin-susceptible *S. pneumoniae* isolated from middle ear fluid during acute otitis media episodes in children in southern Israel from 1992 through 2001.[168] Categ, antibiotic drug category; Eryth, erythromycin; Pen, penicillin; T/S, trimethoprim-sulfamethoxazole. (Data from 1999 to 2001 are unpublished.)

CLINICAL SYNDROMES

S. pneumoniae can spread from the nasopharynx, which is its natural niche, to adjacent mucosal surfaces and cause mucosal infections such as AOM, sinusitis, and pneumonia, or it can invade the bloodstream. Infection may spread from the bloodstream to other sites and cause sepsis or meningitis or result in focal infection in heart valves, bones, joints, and soft tissues and within the peritoneal cavity. In rare cases, such as after penetrating trauma or fracture of the base of the skull, pneumococci can invade the brain directly from the upper respiratory tract. Organisms also can spread through the fallopian tubes to the peritoneal cavity after colonizing the perineum.

The clinical signs and symptoms of patients with pneumococcal infection are diverse. In general, invasive diseases such as bacteremia and meningitis are manifested as fever with temperatures that can exceed 40° C.[47] A peripheral blood leukocyte count of greater than 15,000/mm^3 can be found in most patients with bacteremia, and in one study, the average WBC count was 20,000/mm^3, with a range of 16,100 to 24,600/mm^3.[47] In patients

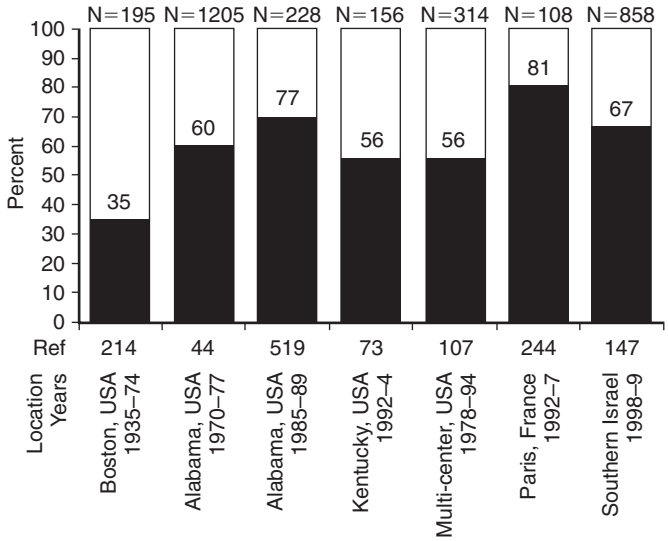


Figure 96–13 Relative proportion of serotypes 6A, 6B, 9V, 14, 19A, 19F, and 23F as a group in relation to all other serotypes causing acute otitis media.

with pneumococcal pneumonia, cough and tachypnea are the most common manifestations besides high fever.[722] Pneumococcal pneumonia and other focal infections such as cellulitis, peritonitis, and bone and joint infections often cause similar high temperatures and elevated WBC counts. Other infections such as AOM and sinusitis frequently have minimal systemic manifestations.

BACTEREMIA. The most common clinical manifestation of invasive pneumococcal disease in children younger than 3 years old is *occult bacteremia*, defined as a positive blood culture without a known focus of infection. *S. pneumoniae* caused an estimated 90 percent of all cases of occult bacteremia after the introduction of Hib conjugated vaccine.[446] Severe complications such as meningitis or sepsis are relatively rare occurrences after occult bacteremia—approximately 6 percent of all cases.[10] The risk for the development of occult bacteremia is related to age, with approximately 75 percent of all patients being between 3 and 24 months of age.[362] Signs and symptoms vary among patients with occult bacteremia. In most patients with pneumococcal bacteremia, fever appears within 24 hours after the diagnosis has been made. In one study, initial diagnoses at the time of occult bacteremia were AOM in 43 percent of patients, fever without a source in 30 percent, and viral infection in 22 percent.[47] Forty-one (8.7%) patients had complications: persistent bacteremia developed in 4 percent, meningitis in 1.7 percent, pneumonia in 1.7 percent, and cellulitis in 2.5 percent.

Febrile patients with focal infections such as otitis media or upper respiratory tract infection have a lower incidence of bacteremia than do febrile patients without an obvious source of infection (3.3% vs. 9.9%, respectively).[501] The height of the fever is associated with a greater risk for the development of bacteremia, with temperatures above 40° C being associated with a 25 percent risk.[57] A higher peripheral WBC count (>15,000/mm^3) also is associated with the presence of occult bacteremia.[365,437] These findings can help in categorizing high- and low-risk patients for the presence of occult bacteremia.

Occult bacteremia resolves spontaneously without complication in most children. However, complications such as meningitis, pneumonia, osteomyelitis, arthritis, cellulitis, and fulminant sepsis develop in approximately 10 percent of patients.[446] The case-fatality rate usually is low and generally less than 1 percent in children without underlying immunologic problems.[392,622]

Persistent bacteremia and new focal infections were associated with higher temperature (mean of 38.8° C vs. 37.7° C), greater elevation in WBC count (18,900/mm^3 vs. 14,900/mm^3), age younger than 20 months, and no antibiotic treatment being prescribed during the initial visit.[47]

MENINGITIS. *S. pneumoniae* is either the most frequent or the second most frequent (after *N. meningitidis*) cause of bacterial meningitis in countries in which routine vaccination against Hib has been introduced. *S. pneumoniae* most commonly invades the meninges via the bloodstream.[198] However, in rare cases it can invade the meninges directly, especially after penetrating trauma or fracture of the base of the skull.[660] Patients with underling conditions such as CSF leak, HIV infection, sickle-cell anemia, or asplenia are predisposed to the development of pneumococcal meningitis.[31]

The initial signs and symptoms are variable, and a rapid onset (<24 hours of illness) occurs in less than 20 percent of children with pneumococcal meningitis.[89] Patients commonly have the typical signs of meningitis, including fever, nuchal rigidity, irritability or lethargy, and poor feeding. The anterior fontanelle often is bulging. The duration of meningeal signs before admission averages 28 hours but can be as short as 4 hours and as long as 52 hours. Seizures develop in approximately a quarter of patients. Decreased consciousness and septic shock occur in 11 and 16 percent, respectively.[31]

In one study, the mean total CSF WBC count was 1136/mm^3, whereas it was 4612/mm^3 with Hib disease and in *N. meningitis* disease it reached 5476/mm^3.[657] In another study, significantly higher median blood leukocyte counts, lower median CSF protein concentrations, and higher CSF glucose concentrations were found in children with meningitis caused by penicillin-resistant *S. pneumoniae* isolates than in patients with penicillin-susceptible *S. pneumoniae*.[227] However, in a different study, patients with penicillin-susceptible *S. pneumoniae* did not differ from those with non–penicillin-susceptible *S. pneumoniae* with regard to blood and CSF leukocyte counts and CSF glucose or protein concentrations.[110]

Children with CSF pleocytosis are admitted routinely to the hospital and treated with antibiotics, although few have bacterial meningitis. Patients are at very low risk for acquiring bacterial meningitis in the post–pneumococcal vaccine era if they lack all of the following criteria: (1) positive CSF Gram stain, (2) CSF absolute neutrophil count of at least 1000 cells/μL, (3) CSF protein of at least 80 mg/dL, (4) peripheral absolute neutrophil count of at least 10,000 cells/μL, and (5) a history of seizure before or at the time of evaluation.[540]

The course of pneumococcal meningitis can be associated with prolonged fever that can last for more than 10 days or with secondary fever higher than 38° C that occurs at least 1 day after the primary fever has resolved.[89] This course can be attributed to complications of the primary infection, such as subdural effusion or empyema, but these events are now relatively rare. More commonly, secondary fever is related to intercurrent conditions such as urinary tract infection, otitis media, phlebitis, pneumonia, drug fever, or a nosocomial viral infection.[177] In patients treated with corticosteroids in the first 2 to 4 days, a second spike of fever can be seen after discontinuation of the steroids.[582,597] The outcome of patients with prolonged or secondary fever is similar to that of other patients with pneumococcal meningitis.[208]

The outcome and long-term complications of pneumococcal meningitis include severe hearing loss (in as many as 46% of patients), seizures, learning or mental difficulties, or paralysis (in as many as 22%).[31] Patients with *S. pneumoniae* meningitis have a higher mortality rate and are at greater risk for the development of neurologic sequelae than are patients with *N. meningitidis* or Hib infection.[389,538] The mortality rate varies from 6.3 to 20 percent.[198,392,660] A peripheral WBC count of less than 5000/mm^3

at initial evaluation was recognized as a poor prognostic factor with a high mortality rate.[428] A trend toward a higher mortality rate also was noted if the CSF WBC count was less than 1000/mm^3.[428] A similarly poor outcome can be predicted in patients with pneumococcal meningitis when low CSF glucose concentrations (<1.11 mmol/L)[237] or high CSF protein concentrations (>250 mg/dL) are present.[428]

A recent study evaluating outcomes of pneumococcal meningitis in pediatric patients in the intensive care unit over a 12-year period revealed a hospital mortality rate of 49 percent (24 of 49 patients) and neurologic deficits in 47 percent of survivors.[770]

Recent studies have shown that patients who have severe to profound hearing loss with cochlear implants are more than 30 times more likely than children in the general U.S. population to acquire pneumococcal meningitis. Children with implants with positioners were at a higher risk than were those with other implant models. The higher risk of bacterial meningitis developing continued for 24 months after implantation.[66,610]

Delayed sterilization of CSF cultures after antimicrobial treatment also has been associated with a poorer outcome.[444]

PNEUMONIA. Pneumococcal pneumonia in its classic form is manifested as an acute illness with rigors, fever with temperatures often exceeding 40° C, general malaise with a productive cough, dyspnea, and chest pain. However, the signs and symptoms may be subtle in young infants. Fever and cough occur in 90 and 70 percent of all patients, respectively. Lethargy, emesis, rhinorrhea, abdominal pain, and chest pain occur in 10 to 50 percent of patients.[722] Pneumococcal pneumonia can have an atypical and incomplete course, without respiratory symptoms, and in some cases even an absence of fever or the presence of extrathoracic manifestations. Severe abdominal pain with or without vomiting may be the only initial symptom, especially in left lower lobe pneumonia. Irritation of the meninges in cases of upper lobe pneumonia may elicit meningeal signs without meningitis.

When serologically diagnosed pneumococcal pneumonia was compared with pneumonia caused by respiratory syncytial virus (RSV), the serologically diagnosed pneumococcal pneumonia overlapped with RSV pneumonia. However, pneumococcal pneumonia was associated significantly less often with tachypnea (17% vs. 45%, respectively) and a high WBC count (mean of 20,800/mm^3 vs. 12,000/mm^3, respectively), as well as a higher CRP level (mean of 137 vs. 28 mg/L, respectively). Alveolar infiltrates were found in 76 versus 15 percent of chest radiographs, respectively.[381] Children with bacteremic pneumococcal pneumonia appeared ill more often than did those with serologically proven pneumococcal pneumonia without bacteremia (79% vs. 50%, respectively), and they had typical pneumococcal pneumonia with high fever, leukocytosis, and lobal infiltrates on chest radiographs more often (70% vs. 34%, respectively).

Pleural effusion can be found in as many as 40 percent of patients with pneumococcal pneumonia, but only approximately 10 percent have a significant amount of effusion and only approximately 2 percent exhibit empyema.[467] These patients can have a pleural friction rub, abdominal pain, chest pain, and dullness on percussion. In patients with pleural effusion, fever persists longer than in patients with pneumonia but without pleural effusion despite adequate treatment.[722]

In one study conducted in Salt Lake City during the years 1993 to 1999, 153 of 540 (28%) children hospitalized with community-acquired pneumonia had empyema.[117] Pathogenic bacteremia, 26 (41%) cases of which were caused by *S. pneumoniae*, was detected in 64 (42%) of the patients. In nine additional patients, gram-positive cocci were revealed by Gram stain but did not grow in culture. *S. pneumoniae* was the most common pathogen identified in patients with empyema. When compared with patients who had pneumonia only, patients with empyema were more likely to be aged 3 years or older, to have had fever for 7 days or longer, to have varicella, and to have received antibiotics and ibuprofen before admission to the hospital.

Another study in Utah after widespread use of the PCV-7 vaccine found that pneumococcal parapneumonic empyema was more common. Serotype 1 was the most common cause, but serotypes 3 and 19A also were prevalent.[116]

CRP, the erythrocyte sedimentation rate, and the peripheral blood absolute neutrophil count are significantly higher in pneumococcal pneumonia than in viral pneumonia.[430] Blood culture is positive in no more than 10 percent of patients with pneumococcal pneumonia.[773] Children with bacteremic pneumococcal pneumonia usually have a high temperature and leukocytosis on admission, with peripheral WBC counts exceeding 15,000/mm^3, similar to those without bacteremia.[734] Body temperature, CRP, the erythromycin sedimentation rate, and leukocyte counts are of limited value in differentiating pneumococcal from nonpneumococcal pneumonia in individual patients.

No strict radiologic definition of pneumococcal pneumonia in children is accepted broadly. The classic chest radiographic finding in pneumococcal pneumonia is thought to be an alveolar infiltrate, usually confined to one lobe or a part of it.[557] Such infiltrates are thought to be present in approximately 85 percent of all cases. In studies performing lung aspiration in cases of radiologically proven lobar infiltrates, the proportion of patients in whom *S. pneumoniae* was detected was high.[212,761,768] In most instances in which patients with lobar consolidation were studied, the predominant bacterial pathogen was *S. pneumoniae*.[8] In addition, a 63 percent reduction in incidence of pneumonia with alveolar infiltrates was demonstrated in a study using this definition when children were vaccinated with a 7-valent pneumococcal conjugated vaccine versus placebo,[71] thus suggesting a major role of *S. pneumoniae* when alveolar infiltrates are present.

A recent study found that the incidence of human metapneumovirus (hMPV) lower respiratory tract infection was reduced by 45 percent ($p < 0.002$) and the incidence of clinical pneumonia was reduced by 55 percent ($p < 0.003$) in non–HIV-infected children who had received three doses of conjugate pneumococcal vaccine. In fully vaccinated HIV-infected children, the incidence of hMPV-associated lower respiratory tract infection was reduced by 53 percent and that of clinical pneumonia by 65 percent.[486]

In the pre-antibiotic era, the natural course of pneumococcal pneumonia consisted of 7 to 9 days of a stationary phase of symptoms followed by worsening of the systemic signs ("pre-crisis") and subsequently, in those who recovered, defervescence, sweating, polyuria, and a brief febrile attack ("post-crisis").[39,557] Today, long-term complications are rare developments, and the mortality rate is extremely low—less than 1 percent even in cases of bacteremic pneumonia.[392]

OTITIS MEDIA. *S. pneumoniae* causes 25 to 60 percent of cases of AOM.* The bacteriologic outcome of *S. pneumoniae* AOM is less favorable than is the outcome of AOM caused by other organisms when untreated or treated inappropriately.[414,528,555] A recent study found that as many as 79 percent of patients with pneumococcal AOM developed the disease in association with newly acquired carriage of pneumococcus.[713] Also recognized is that commonly used oral antibiotics are not as effective as they were in the past because of the increased antibiotic resistance among *S. pneumoniae* isolates.[149,150,152]

Some studies have suggested that pneumococcal AOM is more severe than that caused by other pathogens. In addition, a suggestion was made in 1970 that AOM caused by *S. pneumoniae*

*See references 72, 77, 162, 195, 206, 264, 358, 377, 402, 410, 484, 528, 629.

was accompanied more frequently by pain and fever than was AOM caused by other pathogens.[337] Moreover, on the first day of diagnosis, WBC counts in the middle ear fluid of patients with *S. pneumoniae* AOM were significantly higher than those in the case of AOM caused by other bacterial pathogens.[100] *S. pneumoniae* AOM was associated with significantly higher fever and more redness of the tympanic membrane than was AOM caused by *H. influenzae* or *Moraxella catarrhalis*.[624] The presence of *S. pneumoniae* in the nasopharynx during a case of otitis media was associated with a higher tympanic membrane severity score and higher rates of persistent symptoms on day 5, persistence of tympanic membrane abnormalities on day 28, and recurrence before day 28.[211,306] Spontaneous remission of the infection occurs less commonly in patients with pneumococcal AOM than in those with other pathogens.[335]

MASTOIDITIS. In the pre-antibiotic era, acute mastoiditis was the most common complication of otitis media and was observed in as many as 20 percent of cases without appropriate treatment. The infectious process may lead to reabsorption of the bony septa of mastoid air cells and subsequently to the formation of empyema and anatomic loss of the air-cell system.[231] *S. pneumoniae* is the most common cause of acute mastoiditis in children and accounts for 25 to 46 percent of all culture-proven cases.[272,333] A recent international study suggested that in countries in which otitis media cases are seldom treated with antibiotics, the rate of mastoiditis is greater than in those in which antibiotic treatment of otitis is the rule.[752]

SINUSITIS. The true incidence of pneumococcal sinusitis in children is unknown. It is estimated to be present in 10 percent of all upper respiratory tract infections, but the diagnosis usually is subjective.[765] *S. pneumoniae* is the pathogen most commonly isolated from patients with sinusitis, and it causes 35 to 42 percent of all bacterial cases.[767] The disease can occur at any age, and clinical signs and symptoms depend on the maturation of the different sinuses affected. The ethmoid and maxillary sinuses are the sites most commonly involved in children younger than 5 years old. Sinusitis is manifested initially as a viral infection, followed by a nasal discharge that frequently is purulent and accompanied by cough. The symptoms are aggravated at night because of postnasal drip from the sinus to the pharynx and larynx. Malodorous breath can be noted, as can vomiting.[347,385] In cases of sinusitis, isolation of *S. pneumoniae* from the nasal discharge strongly suggests its involvement at the site of infection.

CONJUNCTIVITIS. Bacterial conjunctivitis often is purulent and tends to be bilateral more frequently than is the case with viral conjunctivitis.[764] *S. pneumoniae* causes 12 to 32 percent of all cases of bacterial conjunctivitis.[75,268,777]

The syndrome of "conjunctivitis-otitis" was described first in 1982.[81] *H. influenzae* is the most common pathogen and can be isolated in as many as 73 percent of cases from the conjunctiva and the middle ear concomitantly. This rate is higher than the rate when conjunctivitis is present without AOM.[81]

In one study in the United States, the rate of non–penicillin-susceptible *S. pneumoniae* in isolates taken from children with bacterial conjunctivitis was 28 percent, with most isolates being fully resistant.[75]

A recent study in Minnesota evaluated 735 children with conjunctivitis in two cities. Forty-nine percent of positive cultures were *S. pneumoniae*. All isolates were nontypeable. Pulsed-field gel electrophoresis identified three clonal groups, with 84 percent of the isolates belonging to one clonal group. Multilocus sequence typing revealed that isolates had the same multilocus sequence type as that of isolates from a 2002 pneumococcal disease outbreak at a New England college. It has been suggested that certain pneumococcal strains have a predilection for causing conjunctivitis.[108] Another study suggested that the absence of a capsule might provide *S. pneumoniae* with a selective virulence advantage in conjunctivitis.[590]

BONE AND JOINT INFECTIONS. Septic arthritis caused by *S. pneumoniae* is a relatively rare condition that accounts for 2.2 to 9.7 percent of all cases.[297,641] Its main initial clinical features are single or multiple swollen, warm, and painful joints. The joints most commonly involved are the knee and the ankle.[349] In one study, the mean duration of symptoms before hospitalization was 2 days, and patients were hospitalized for an average of 11 days. The mean peripheral WBC count was 20,600/mm^3, the erythrocyte sedimentation rate usually was greater than 90 mm/hr, and the CRP concentration generally was higher than 100 mg/L. *S. pneumoniae* can be isolated from synovial fluid or blood in most patients.[641] The outcome is favorable in the majority of cases unless underlying diseases or sepsis occurs concomitantly.[349]

SOFT TISSUE INFECTIONS. *S. pneumoniae* is an uncommonly recognized etiologic agent of soft tissue infections, but it can cause serious infections of soft tissues, especially in patients with connective tissue diseases such as systemic lupus erythematosus. Other risk factors are HIV infection and corticosteroid treatment. The organism can be isolated from the infected tissue, as well as from blood.[188] *S. pneumoniae* can cause facial cellulitis as well. Periorbital infection can spread and cause infection of the orbit (orbital cellulitis or abscess) manifested as proptosis and ophthalmoplegia, but these sequelae are rare occurrences and usually a complication of purulent sinusitis, or they occur after bacteremia in infants and young children without another apparent source.[674] Most patients are younger than 3 years of age and previously were healthy. Fever and peripheral WBC counts higher than 15,000/mm^3 can be observed in most patients, and blood cultures frequently are positive. Overall, response to therapy generally is good, and patients usually recover.[273]

PERITONITIS. The term *primary peritonitis* is associated with organisms that spread from the blood or via the lymphatic system to the peritoneal cavity and cause infection. Primary peritonitis is a rare condition that accounts for only 17 percent of all cases of peritonitis, and *S. pneumoniae* is the major pathogen in primary peritonitis and is responsible for 38 percent of all cases.[280] Primary pneumococcal peritonitis should be considered in the differential diagnosis of children with an acute abdominal syndrome.[242] This condition can be associated with underlying medical conditions such as nephrotic syndrome, immunocompromised status, or sickle-cell disease.[280] Colonization of the perineum and subsequent spread via the fallopian tube to the peritoneal cavity have been suggested to be important sources in young girls.[689] Indeed, 87 percent of all patients with pneumococcal primary peritonitis are females,[669] in contrast to other invasive infections, for which a male preponderance is observed.[3,15,92,103,112,207,240,350,701,726] Most of the patients are between 4 and 10 years of age.[669] Symptoms include diffuse abdominal pain, fever, vomiting, and diarrhea. Abdominal tenderness can be maximal in the right lower quadrant, which can lead to confusing it with acute appendicitis. In some cases, *S. pneumoniae* can be isolated as the only cause of peritonitis secondary to appendicitis.[319] Cultures should include blood, peritoneal fluid, and a vaginal swab. Some patients need laparotomy or laparoscopy to exclude other pathologies such as appendicitis or a tubo-ovarian abscess.[689] Morbidity and mortality rates can be high when the period without treatment exceeds 1 week, but in most cases the outcome is favorable when treated adequately.[669]

ENDOCARDITIS. Infection of native valves can lead to distal embolization and heart failure as a result of valve destruction. Cases of *S. pneumoniae* endocarditis, including those caused by

non–penicillin-susceptible strains, were reported with involvement of the aortic and mitral valves.[683] The triad of pneumococcal meningitis, pneumonia, and endocarditis was described first by Osler, but this triad also is known as *Austrian syndrome*. Today, this entity is an uncommon occurrence because the incidence of *S. pneumoniae* endocarditis has decreased significantly from 10 to 15 percent of all endocarditis cases in the pre-antibiotic era to less than 3 percent today.[352,522,724]

Only 32 cases of pediatric pneumococcal endocarditis have been reported in the English medical literature since 1900. A fourth of them have been reported since 1990. Clinical features differed from adult cases, with mitral-valve involvement being more frequent and Osler's triad rarely present in children. A history of congenital heart disease was the only identifiable risk factor. Medical therapy alone resulted in a high mortality rate that was improved in a group of patients receiving combined medical and surgical interventions.[127]

PERICARDITIS. In the pre-antibiotic era, *S. pneumoniae* was the pathogen most commonly isolated from purulent pericarditis in children. These infections were common complications of pneumococcal pneumonia. After the introduction of antibiotics, pneumococcal cases decreased from 51 to 9 percent of all pericarditis cases and occurred mainly in adults.[275,643] Currently, infections of the pericardium are rare findings in children and often are related to underlying medical conditions such as immunodeficiency.[578]

INFECTION IN IMMUNOCOMPROMISED HOSTS. Patients with functional asplenia, including those with hemoglobinopathies, are at high risk for the acquisition of invasive pneumococcal infection. In a study of 19 asplenic patients with invasive pneumococcal infection, the clinical findings included fever (86%), shock (27%), petechiae or purpura (27%), disseminated vascular coagulopathy (18%), and respiratory distress (18%). Clinical illness included bacteremia alone (52%), meningitis (26%), bacteremia with otitis/sinusitis (13%), and bacteremia with pneumonia (9%). The mortality rate was 32 percent. No association was noted between antimicrobial resistance and mortality.[662]

HIV-infected children with pneumonia usually have the typical symptoms of fever, shortness of breath, and productive cough associated with pleuritic pain. The chest radiograph shows unilobar or multilobar infiltrates, and peripheral WBC counts often are elevated. A comparison between HIV-infected and non–HIV-infected children with community-acquired pneumonia showed no significant differences between groups with regard to the duration of hospitalization and mortality rates.[488] In contrast, when only patients from the aforementioned groups infected with isolates belonging to the "pediatric serotype group" (see the sections on epidemiology and prevention) were compared, marked differences, such as a higher rate of pneumonia with or without concurrent meningitis in the HIV-infected group and a higher rate of septic shock without focus in the non-HIV-infected group, were observed.[488] Recurrence of pneumococcal infection within 6 months from the initial episode is common in patients infected with HIV, particularly children.[260] Most HIV-infected patients recover without significant sequelae, and the clinical course of their systemic infection does not appear to be markedly different from that in healthy children.[369]

Patients with immunodeficiency disorders consisting of antibody and complement deficiencies are at high risk for acquisition of invasive and recurrent pneumococcal infection.[369] Children with nephrotic syndrome have a particularly increased risk for the development of pneumococcal peritonitis, mainly during relapse of their renal disease.[220] Other underlying diseases such as hematologic malignancies, diabetes mellitus, and cirrhosis, as well as conditions such as alcoholism, are associated with an increased risk for the development of pneumococcal bacteremia and other invasive diseases, mainly in adults.[369]

Children with sickle-cell disease are prone to the development of overwhelming *S. pneumoniae* bacteremia and sepsis; in children younger than 3 years of age, the incidence of bacteremia is 6.1 events per 100 patient-years, and the case-fatality rate is 24 percent for *S. pneumoniae* sepsis.[798]

Transplant recipients have been recognized for some time as being at high risk for the development of invasive pneumococcal disease. Of 42 children who had undergone bone marrow or solid organ transplantation and became infected with *S. pneumoniae*, 8 (19%) had two or more pneumococcal infections.[663] Solid organ recipients were more likely to have recurrent invasive disease than were recipients of bone marrow transplants. Death occurred in 2 of 42 recipients (5%). Cardiac transplant patients have an incidence of 39 cases of invasive pneumococcal infection per 1000 patients per year,[709] the highest risk of which occurs in African American heart transplant recipients who undergo transplantation because of idiopathic dilated cardiomyopathy.

TREATMENT

Penicillin G is the parenteral drug of choice for disease caused by penicillin-susceptible *S. pneumoniae* strains (defined as those with a penicillin MIC < 0.1 μg/mL). The usual doses of penicillin G result in serum concentrations that exceed this level in blood and most other body fluids for an adequate period. Penicillin G is the most common parenteral drug used for the treatment of pneumococcal infection, with doses ranging from 50,000 U/kg/day for minor infections to 300,000 U/kg/day for meningitis. For penicillin-susceptible strains, other parenteral β-lactams such as ampicillin, cefuroxime, cefotaxime, and ceftriaxone provide no advantage over penicillin G, even for serious infections. Macrolides and cephalosporins are an alternative treatment for penicillin-allergic patients if regional data do not show macrolide and cephalosporin resistance. Other agents such as clindamycin, tetracycline, and trimethoprim-sulfamethoxazole are active against *S. pneumoniae*, but resistance to these agents is increasing rapidly in most parts of the world.

In many regions, treatment of non–penicillin-susceptible *S. pneumoniae* has become a challenge, and reports of treatment failure have increased, especially in patients with invasive multidrug-resistant *S. pneumoniae* infection.[691] Rates of antibacterial resistance are highest in infants and decrease with increasing patient age.[106] Because isolates of *S. pneumoniae* that are resistant to penicillin frequently are resistant to other drug classes such as cephalosporins (mainly oral), macrolides, and trimethoprim-sulfamethoxazole,[779] the challenge of treatment is becoming complex.

The critical time for treatment of severe pneumococcal infection is during the first few hours after initial evaluation of the patient, before culture results are available and, thus, before knowledge of the presence or susceptibility of the pathogen is obtained. Therefore, the choice of antibiotics must rely on considerations based on the most recent epidemiologic data and the clinical status of the child, as well as the site of infection. Furthermore, because treatment is empiric, the choice of antibiotics must take into account other potential pathogens. Knowledge of the patient's antimicrobial use in the 3 months before infection developed is crucial for determining the appropriate therapy for a patient who has a possible pneumococcal infection.[751]

The proportion of *S. pneumoniae* isolates from U.S. pediatric patients covered by the PCV-7 vaccine decreased substantially in the 4 years after the vaccine was introduced. Resistance to commonly used antibiotics, including β-lactams and macrolides, as well as multidrug-resistant strains, also increased significantly among respiratory tract isolates of nonvaccine serotypes.[215]

Because treatment of pneumococcal infection is not necessarily similar in each of the clinical entities, specific details are provided for each clinical entity separately.

BACTEREMIA. Results of studies are conflicting with regard to the role of antibiotic treatment of occult pneumococcal bacteremia. In one study, the bacteremia resolved without any need for parenteral antibiotic treatment in 95.7 percent of patients.[12] No significant difference was found between amoxicillin and placebo recipients regarding complications such as meningitis in bacteremic patients, but faster reduction of fever and improvement in clinical appearance were observed in the group treated with amoxicillin.[366] A meta-analysis showed that rates of serious bacterial infection and meningitis did not differ between children treated with oral antibiotics and those treated with parenteral antibiotics.[634] In contrast, in another meta-analysis conducted by the same authors, treated patients had fewer serious bacterial infections than did untreated patients (3.3% versus 9.7%), and meningitis developed in only 0.8 percent of the treated group versus 2.7 percent of the untreated group.[636] In one study, the rate of persistent pneumococcal bacteremia was significantly higher in patients receiving no therapy for pneumococcal bacteremia than in those receiving either oral or parenteral treatment. A higher prevalence of persistent bacteremia in the orally treated group than in the parenterally treated group also was noted.[47] Thus, in patients suspected of having occult bacteremia, treatment with a β-lactam drug can be initiated, especially if the patient looks toxic or has a high fever. However, if the patient has been immunized with both Hib and pneumococcal conjugate vaccines, does not look toxic, and has no predisposing risk factors such as immunodeficiency, the risk of significant bacteremia resulting in complications is extremely low, and in this case, withholding treatment is reasonable.[792]

For proven *S. pneumoniae* bacteremia in a previously healthy child who is not critically ill, treatment, including oral amoxicillin, amoxicillin-clavulanate, second-generation oral cephalosporins, or parenteral third-generation cephalosporins such as ceftriaxone, can be initiated at the usually recommended dosages.[91] In most cases of penicillin-susceptible and penicillin-intermediate *S. pneumoniae* (penicillin MICs ≤1.0 μg/mL), most parenteral and some oral β-lactam antibiotic agents achieve serum concentrations that exceed the MIC of the organism for an adequate period.[421] The exceptions are drugs such as loracarbef, cefaclor, and cefixime, which have little activity against non–penicillin-susceptible *S. pneumoniae*.

In studies looking at the outcome of children with bacteremia caused by non–β-lactam–susceptible *S. pneumoniae* (not susceptible to penicillin, cefuroxime, or ceftriaxone), no difference in outcome was observed when compared with those with susceptible organisms.[126,392,684] However, in critically ill infants and children with invasive *S. pneumoniae* infection, initial antimicrobial therapy should include a third-generation cephalosporin (cefotaxime or ceftriaxone) alone or with vancomycin. Vancomycin should be discontinued as soon as antimicrobial susceptibility test results demonstrate effective alternative agents.[14,731] For children with severe hypersensitivity to β-lactam antibiotics (i.e., penicillins and cephalosporins), initial management of a potential pneumococcal infection could include clindamycin or vancomycin, in addition to antimicrobial drugs for other potential pathogens, as indicated.

MENINGITIS. Penicillin G, 250,000 to 400,000 U/kg/day, is an excellent treatment regimen for meningitis caused by penicillin-susceptible pneumococci, and ceftriaxone, 100 mg/kg/day, or cefotaxime, 300 mg/kg/day, is an excellent choice for meningitis caused by *S. pneumoniae* susceptible to these drugs.[14] However, treatment failures have been reported in penicillin- and ceftriaxone-nonsusceptible pneumococcal meningitis in patients, as well as in animal models.[245,246] Therefore, because of the increased prevalence of penicillin-, cefotaxime-, and ceftriaxone-resistant *S. pneumoniae*, combination therapy consisting of vancomycin (60 mg/kg/day) and cefotaxime (300 mg/kg/day) or ceftriaxone (100 mg/kg/day) should be administered initially to all children 1 month of age or older with definite or probable bacterial meningitis. One recent study showed that delaying administration of the first dose of vancomycin until 2 or more hours after the first dose of parenteral cephalosporin has been administered was associated with a decrease the incidence of hearing loss related to the use of vancomycin.[111] This finding requires confirmation. However, vancomycin does not need to be used if compelling evidence indicates that the cause is an organism other than *S. pneumoniae* (e.g., gram-negative diplococci on a CSF smear or during an outbreak of meningococcal disease).[14] Vancomycin should not be given alone because bactericidal concentrations in CSF are difficult to sustain, clinical experience to support its use as monotherapy is minimal, and clinical failure and inadequate CSF drug concentrations have been reported.[9,756] Therapy should be modified after the organism has been isolated and based on the results of susceptibility testing. If the organism is susceptible to the β-lactam agent being used, vancomycin use should be discontinued. Antibiotic treatment in proven pneumococcal meningitis should not be shorter in duration than 10 days, and in complicated cases with a suspected focus, it may need to be provided for an even longer period.

When appropriately treated, the clinical manifestations and outcomes of meningitis caused by non–antibiotic-susceptible pneumococci are not significantly different from those caused by antibiotic-susceptible pneumococci.[31,110,227,723]

The combination of ceftriaxone and rifampin in an animal meningitis model had a bactericidal effect in meningitis caused by ceftriaxone-resistant *S. pneumoniae*.[567] The addition of rifampin to vancomycin after 24 to 48 hours of therapy should be considered in the following cases if the isolate is susceptible to rifampin: (1) when the patient's clinical condition has worsened despite therapy with vancomycin and cefotaxime or ceftriaxone, (2) when the subsequent Gram-stained smear or culture of CSF indicates failure to eradicate or substantially reduce the number of organisms, or (3) when the organism has an unusually high cefotaxime or ceftriaxone MIC (>4 μg/mL).[14] Although rifampin resistance is a rare event, rifampin should not be given as monotherapy because resistance may develop during therapy.[731]

Other β-lactam antimicrobial agents that can be used for the treatment of pneumococcal meningitis include meropenem (120 mg/kg/day) and cefepime (150 mg/kg/day).[390,644,645]

In patients with severe hypersensitivity to β-lactam antibiotics (i.e., penicillins and cephalosporins), the combination of vancomycin and rifampin should be considered.[246]

Chloramphenicol (75 to 100 mg/kg/day) is an acceptable alternative to β-lactam antibiotics in patients hypersensitive to these drugs. However, for unknown reasons, failure occurred in a patient with non–penicillin-susceptible pneumococcal meningitis treated with chloramphenicol.[244] Thus, treatment with chloramphenicol should be reserved for patients with β-lactam hypersensitivity who have pneumococcal meningitis caused by penicillin-susceptible strains.

Much debate has ensued on the role of dexamethasone as adjunctive therapy for pneumococcal meningitis. On the one hand, it might exert a positive effect in decreasing inflammatory reactions, but on the other hand, the same decrease in inflammation may reduce penetration of antibiotic agents into CSF. A meta-analysis of three randomized, double-blind, placebo-controlled studies combined with one retrospective study demonstrated a potential benefit of reducing hearing loss and neurologic sequelae after having pneumococcal meningitis.[656] Some studies had suggested a beneficial effect on pneumococcal meningitis when dexamethasone was given for 2 days with or

before administration of parenteral antibiotics.[506] Thus, for infants and children aged 6 weeks and older, adjunctive therapy with dexamethasone should be considered after weighing the potential benefits and potential risks. When given in the recommended dosages to children with meningitis treated with dexamethasone, CSF concentrations of vancomycin, ceftriaxone, cefotaxime, and rifampin usually are adequate to treat meningitis caused by most strains of *S. pneumoniae*, but in some parts of the world, the CSF concentration of the drugs might be inadequate because of the high MIC values of ceftriaxone and cefotaxime. Dexamethasone may lead to decreased fever and a misleading impression of clinical improvement, even though CSF sterilization may not have been achieved. In some cases, after discontinuation of the steroids, secondary fever may develop, which also can be misleading and give the impression of a nonresponsive case and lead to use of additional lumbar punctures or other procedures.[208]

Repeat lumbar puncture should be considered after 24 to 48 hours of therapy if the following apply: (1) the organism is not susceptible to penicillin, (2) the results of cefotaxime and ceftriaxone susceptibility testing are not yet available, (3) the patient's condition has not improved or has worsened, or (4) the child has received dexamethasone, which might interfere with the ability to interpret a clinical response, such as the resolution of fever.[14,208]

PNEUMONIA. *S. pneumoniae* accounts for most cases of bacterial pneumonia in children. Thus, empiric treatment of pneumonia should cover this pathogen despite the fact that an organism-specific diagnosis seldom is established in children with pneumonia. Oral treatment with a β-lactam antibiotic such as amoxicillin, cefuroxime axetil, or amoxicillin-clavulanate is an appropriate option for the first-line treatment of ambulatory community-acquired pneumonia in children younger than 5 years old in most countries.[315]

In most cases of pneumonia caused by non–penicillin-susceptible *S. pneumoniae*, the outcome is favorable if the penicillin MIC is between 0.1 and 2.0 μg/mL and treatment consists of standard doses of parenteral penicillin G (100,000 to 300,000 U/mg/day), oral amoxicillin or amoxicillin-clavulanate (40 to 50 mg/kg/day), or cefuroxime (30 to 50 mg/kg/day).[315,722] No difference in outcome was found between patients with community-acquired pneumococcal pneumonia treated with oral amoxicillin-clavulanate and those treated with parenteral ceftriaxone.[633]

For immunocompetent hospitalized patients who are not critically ill on admission, parenteral treatment with β-lactam antibiotics such as cefuroxime, cefotaxime, or ampicillin (with or without β-lactamase inhibitors) is an appropriate option because both *S. pneumoniae* and most other potential organisms are covered.[315]

In adult and pediatric patients with pneumococcal pneumonia treated with standard regimens of parenteral penicillin G or a cephalosporin, the outcome was similar when patients infected with penicillin- or cephalosporin-resistant organisms were compared with those infected with susceptible organisms.[91,119,209,219,391,519,563,771,790]

In one retrospective cohort study, adult patients with bacteremic pneumonia caused by isolates that were not penicillin-susceptible had a significantly greater risk of dying in the hospital and of having more suppurative complications than did patients with susceptible isolates.[508] However, the mortality rate was not significantly different after adjustment for baseline differences in severity of illness. A population-based active surveillance study of pneumococcal disease in the United States found that 12 percent of 5837 cases were fatal.[219] In this study, a higher mortality rate was noted after the fourth hospital day in patients with invasive pneumococcal pneumonia caused by isolates with penicillin MICs of 4 μg/mL or greater. However, potential limitations of this study included absence of data on the severity of illness at initial evaluation, as well as lack of information on the antibiotics used.

Treatment failure and breakthrough meningitis were reported in a patient who was infected with highly resistant *S. pneumoniae* (penicillin MIC of 2 μg/mL, cefuroxime MIC of 2 μg/mL, and cefotaxime MIC > 8 μg/mL) and treated with cefotaxime and cefuroxime.[109] Because of such cases, some authorities recommend that in critically ill patients with highly resistant *S. pneumoniae*, vancomycin (or in older patients, fluoroquinolones active against *S. pneumoniae*) be added initially to third-generation cephalosporins.[315]

In a series of 32 children with pleural empyema caused by *S. pneumoniae*, those with non–penicillin-susceptible strains were significantly younger and more frequently had been treated previously with antibiotics than were children with penicillin-susceptible strains.[561] However, no significant differences were found between the two groups in the duration of fever and tachypnea, need for surgical treatment, presence of bacteremia, mean duration of therapy, or length of hospital stay.[561] In cases of pleural fluid or empyema in patients infected with highly resistant organisms, vancomycin or rifampin may be added if no clinical response is achieved within 48 to 72 hours.[91]

For β-lactam–allergic patients, macrolides are favored.[390] However, in some areas, macrolide resistance occurs commonly and may lead to treatment failure.[232,344,399,479,480,516,711,773] Breakthrough pneumococcal bacteremia has been reported during treatment with clarithromycin or azithromycin for community-acquired pneumonia.[399]

Drugs that are not appropriate for treating critically ill patients with pneumococcal pneumonia are the first-generation cephalosporins, ceftazidime, and ticarcillin because most penicillin-resistant *S. pneumoniae* also are resistant to these drugs.[190,565] Trimethoprim-sulfamethoxazole is not recommended because of the high prevalence of resistance to this drug.[315]

The duration of treatment is related to the clinical findings, clinical response to treatment, and underlying diseases of the patient and generally is 7 to 14 days. For hospitalized patients, 5 to 7 days of parenteral treatment followed by 7 days of oral therapy is recommended,[91,364] but many clinicians use a shorter course of 7 to 10 days total.

OTITIS MEDIA. Antibiotics are the standard of care for the treatment of AOM in the United States and in many other countries. Although antibiotic therapy is required in only 20 to 30 percent of all cases of AOM, most patients are treated with antibiotics because these cases cannot be identified quickly and easily.[415] As with other infections, the main goal of antibiotic therapy is to eradicate the causative pathogens from middle ear fluid.[166,386,453,631]

S. pneumoniae is responsible for 30 to 50 percent of all cases of acute bacterial otitis media, and it is the least likely pathogen to resolve without the administration of appropriate antibiotic treatment.[624] After 2 to 7 days, if not treated, approximately 50 percent of all *H. influenzae* organisms are eradicated spontaneously, but less than 20 percent of pneumococci are eradicated spontaneously in AOM.[334] Therefore, *S. pneumoniae* generally is considered the most important organism against which antibiotic treatment should be directed in AOM.

Studies in which tympanocentesis is performed before treatment and a second tympanocentesis procedure during treatment to document bacteriologic efficacy ("double-tympanocentesis" bacteriologic outcome studies) have demonstrated that pharmacodynamic models can predict bacteriologic outcome.[146,163] Studies have shown that the increasing resistance of *S. pneumoniae* to various drugs has increased the complexity of antibiotic treatment of otitis media. In addition to having activity against antibiotic-resistant pneumococci, a drug appropriate for empiric treatment of AOM should be active against the two other main

pathogens of otitis media, namely, *H. influenzae* and *M. catarrhalis*. Table 96–3 summarizes the activity of drugs used commonly against otitis media pathogens, with the pharmacodynamic properties (the relationship between the drug concentration in plasma and the concentration at the site of infection) and the MIC of the drug to the infecting organism taken into account. The in vitro activity of oral agents has not always been predictive of their in vivo activity because of incorrect or unavailable interpretative breakpoints, but this deficiency has been rectified for *S. pneumoniae* in the United States since 2000.

Most of the drugs used for the treatment of AOM belong to the β-lactam and macrolide classes; these drugs act against pathogens by a "time-dependent killing" mechanism,[79,146] which means that an effective dosing regimen for AOM would require that the unbound plasma concentration exceed the MICs of the drug against the causative pathogens for at least 40 to 50 percent of the dosing interval. Although the drug exerts its effect in the middle ear cavity, penetration of the drug into this space is driven by the unbound plasma concentration, which is in equilibrium with the extracellular fluid compartment of tissues and sites such as the middle ear space.

For penicillin-susceptible *S. pneumoniae*, most β-lactam drugs (with the exception of penicillin V and the first-generation oral cephalosporins) reach that goal and are appropriate drugs for otitis media caused by penicillin-susceptible *S. pneumoniae*.

In the case of non–penicillin-susceptible *S. pneumoniae*, on the other hand, the picture is different. The most active oral β-lactam drugs against penicillin-intermediate isolates are amoxicillin and amoxicillin-clavulanate (clavulanate has no effect on pneumococci, but it is used often to empirically add coverage against β-lactamase–producing organisms). However, most of the oral cephalosporins, with the exception of cefuroxime axetil, cefpodoxime proxetil, and cefprozil, cannot reach the goal of unbound plasma concentrations exceeding the MIC_{90} values for most penicillin-intermediate organisms for 40 to 50 percent of the dosing interval and thus are not appropriate for the treatment of AOM in areas where penicillin-intermediate strains occur commonly.[146,195] For fully penicillin-resistant *S. pneumoniae* strains, of the β-lactam drugs commonly used against AOM, only high-dose amoxicillin (80 to 100 mg/kg/day), amoxicillin-clavulanate (80 to 100 mg/kg/day of the amoxicillin component), and intramuscular ceftriaxone (50 mg/kg/day) reach plasma or middle ear fluid concentrations that are above the MIC values for an appropriate duration. A recent study found that 90 mg/kg of amoxicillin (given as amoxicillin-clavulanate) significantly decreased nasopharyngeal colonization when compared with 45 mg/kg

TABLE 96–3 In Vivo Activity of Antibiotic Drugs in Common Use for the Treatment of AOM That Are Effective against the Major AOM Pathogens*

	Antimicrobial Activity					
	Streptococcus pneumoniae†			*Haemophilus influenzae*		*Moraxella catarrhalis*
Drug	***Penicillin-Susceptible***	***Penicillin-Intermediate***	***Penicillin-Resistant***	***β-Lactamase–Negative***	***β-Lactamase–Positive***	
Amoxicillin, 40-50 mg/kg/day	+++	+++	+	++	–	–
Amoxicillin, 80-90 mg/kg/day	+++	+++	+++	+++	–	–
Amoxicillin-clavulanate, 45/6.4 mg/kg/day	+++	++	+	++	++	+++
Amoxicillin-clavulanate, 90/6.4 mg/kg/day	+++	+++	++	+++	+++	+++
Cefaclor	+++	–	–	+	+	++
Cefdinir	+++	+	–	++	+++	+++
Cefixime	++	–	–	+++	+++	+++
Cefpodoxime proxetil	+++	++	+	+++	+++	+++
Cefprozil	+++	++	+	–	–	++
Ceftriaxone (50 mg/kg/day)—1 day	+++	++	+	+++	+++	+++
Ceftriaxone (50 mg/kg/day)—3 days	+++	+++	+++	+++	+++	+++
Cefuroxime axetil	+++	++	+	++	++	+++
Clindamycin	+++	++‡	++‡	–	–	–
Erythromycin-azithromycin-clarithromycin	+++	++‡	–‡	+/–	+/–	++
TMP-SMX	++	–‡	–‡	++	++	++

	S. pneumoniae	
	Macrolide-Susceptible	**Macrolide-Resistant**
Clindamycin	+++	+
Erythromycin-azithromycin-clarithromycin	+++	–
	TMP-SMX–Susceptible	**TMP-SMX–Resistant**
TMP-SMX	+++	–

**In vitro activity, pharmacodynamic properties, and, if available, the results of bacteriologic outcome studies are taken into account.*
†Penicillin-susceptible (MICs ≤ 0.06 μg/mL), penicillin-intermediate (MICs of 0.1 to 1 μg/mL), penicillin-resistant (MICs ≥ 2 μg/mL).
‡When Streptococcus pneumoniae is not penicillin-susceptible, the prevalence of macrolide, clindamycin, and TMP-SMX resistance among the strains is higher than in penicillin-susceptible ones. This rate can be very high in penicillin-resistant S. pneumoniae. (See "Bacteriology.")
+++, Appropriate; ++, may not be appropriate for some strains; +, frequent bacteriologic failures are likely; –, usually associated with bacteriologic failure.
AOM, acute otitis media; MICs, minimal inhibitory concentrations; TMP-SMX, trimethoprim-sulfamethoxazole.

($p = 0.0261$).[102] To eradicate *S. pneumoniae* with penicillin MICs of 2.0 μg/mL or higher, an amoxicillin or amoxicillin-clavulanate dose of 80 to 100 mg/kg/day (divided into two or three doses) is necessary.[161,453,667]

Among the parenteral cephalosporins, ceftriaxone and cefotaxime have the lowest MICs, but high-level resistance of *S. pneumoniae* to these agents has been reported.[700] Intramuscular ceftriaxone (50 mg/kg/day) given once daily for 1 to 3 days is effective for pneumococcal otitis caused by penicillin-susceptible strains. For otitis caused by non–penicillin-susceptible *S. pneumoniae*, 3 days of treatment may be required.[456,457]

Resistance to macrolides is significant and affects bacteriologic and clinical outcomes in AOM.[28,52,165] All macrolides are efficacious against otitis caused by macrolide-susceptible *S. pneumoniae*. Efficacy was shown for erythromycin,[336] clarithromycin,[334] and azithromycin.[165] However, when the organism is resistant to macrolides, these drugs are not effective because the MIC is too high to be exceeded by drug concentrations at the site of infection, which has been demonstrated best for azithromycin[162,165] but can be generalized to other macrolides. In one child, pneumococcal bacteremia and meningitis were reported to have developed during azithromycin therapy for otitis media.[351]

Clindamycin is active against most non–penicillin-susceptible *S. pneumoniae* strains and may be used to treat pneumococcal AOM that does not respond to β-lactam antibiotics. However, prospective, controlled studies on the bacteriologic and clinical efficacy of clindamycin in the treatment of AOM have not been performed. Furthermore, as described in the section on microbiology, an increasing proportion of *S. pneumoniae* isolates resistant to macrolides also are resistant to clindamycin.

Trimethoprim-sulfamethoxazole no longer is an appropriate choice for empiric treatment of AOM because of the high prevalence of resistance to this drug among *S. pneumoniae* strains in many regions. In the case of AOM caused by *S. pneumoniae* resistant to trimethoprim-sulfamethoxazole, the effect of this drug is no better than that of placebo.[452]

Treatment of nonresponsive AOM (cases refractory to one or more courses of antibiotics) was studied in double-tympanocentesis studies, with bacteriologic outcome being the major endpoint.[415] Cefaclor, cefixime, loracarbef, ceftibuten, azithromycin, and trimethoprim-sulfamethoxazole clearly have been shown to be ineffective against nonsusceptible pneumococci and no longer can be recommended for the empiric treatment of nonresponsive AOM.[195] Second-line treatment preferably should be based on the results of middle ear fluid cultures and pathogen susceptibility tests.

For patients with clinically defined treatment failure after receiving 3 to 5 days of initial therapy, suitable second-line agents active against non–penicillin-susceptible pneumococci, as well as β-lactamase–producing *H. influenzae* and *M. catarrhalis*, should be administered. Three antibiotic drugs fulfill these criteria: amoxicillin-clavulanate, intramuscular ceftriaxone, and, to a lesser extent, cefuroxime axetil.[72,195,502] In these cases, amoxicillin-clavulanate should be given at 80 to 100 mg/kg/day of the amoxicillin component. Intramuscular ceftriaxone given for 3 days was found to be superior to a 1-day regimen in cases caused by penicillin-resistant *S. pneumoniae*.[457] A recent study found that gatifloxacin, 10 mg/kg once daily, was as effective and well tolerated in children with AOM treatment failure or recurrent otitis media.[675] Use of gatifloxacin was not associated with the development of arthropathy, and, in fact, an observational study of more than 6000 children treated with fluoroquinolones found the incidence of joint disorders comparable to that after treatment with erythromycin.[793]

Erythromycin-sulfisoxazole, clarithromycin, and azithromycin may be used as alternatives in penicillin-allergic patients.[454] One recent study found that azithromycin was as effective as was high-dose amoxicillin for the treatment of children with AOM and that rates of adverse events were lower and compliance improved with the simplified single dosing regimen.[32] However, macrolide resistance among middle ear *S. pneumoniae* isolates has been increasing steadily.[496] Therefore, in such cases, tympanocentesis is recommended either before initiation of treatment or after 48 to 72 hours of treatment if no clinical response is observed.

The recommended duration of treatment usually is 10 days for most antibiotic drug regimens in children younger than 2 years.[416] Recent studies suggest that a shorter treatment course (5 to 7 days) may be adequate, at least for a subgroup of children older than 2 years of age with uncomplicated otitis.[435,580,584] However, data evaluating the bacteriologic outcome and clinical efficacy of shortened antibiotic treatment of AOM are not complete in children younger than 2 years old, children with severe or complicated AOM, and those with a history of recurrent or chronic otitis media, although such studies are being undertaken. Clinical studies suggest that 5 to 7 days of treatment may not be adequate for these groups of patients.[134,136,196,585] Children attending daycare centers are at particular risk of having a poor response and recurrence if treated for 5 rather than 10 days.[135] Therefore, until the results of more studies are available, shortened antibiotic treatment in AOM cannot be recommended for children younger than 2 years old, children with severe or complicated AOM, children with chronic or recurrent AOM, and children attending daycare centers.[136,151]

Prophylactic use of amoxicillin or trimethoprim-sulfamethoxazole at half the therapeutic dose may reduce the number of new episodes significantly in patients with recurrent AOM.* However, this approach may promote carriage of drug-resistant *S. pneumoniae* and lead to the development of new infections with resistant organisms.[164] Thus, antibiotic prophylaxis is not recommended except in children with frequently recurrent AOM episodes, defined as three or more documented episodes in 6 months or four or more in 12 months.[455]

Although most non–penicillin-susceptible *S. pneumoniae* are included in PCV-7, AOM caused by these strains will not disappear completely. Therefore, clinicians must continue to consider non–penicillin-susceptible pneumococci when prescribing antibiotics for AOM.[308]

SINUSITIS. Although studies on the bacteriology of acute sinusitis are sparse, they suggest that the pathogens causing sinusitis are similar to those causing AOM. Acute bacterial sinusitis usually is a complication of viral rhinosinusitis and occurs in 0.5 to 2 percent of cases. In a study comparing antimicrobial therapy with placebo for the treatment of children with a clinical and radiographic diagnosis of acute bacterial sinusitis, children receiving antimicrobial therapy recovered more quickly and more often than did those receiving placebo.[766] Thus, antimicrobial agents that are effective for the treatment of AOM also are likely to be effective and are recommended for acute sinusitis.[14,550,687,765]

In the selection of appropriate therapy for bacterial sinusitis, physicians should consider the following parameters: (1) the severity of disease: mild (healthy patients with 10 days of persistent anterior and posterior rhinorrhea and fatigue), moderate (patients with 10 days of nasal congestion in whom a low-grade fever developed during the past 3 days, as well as increasing unilateral tenderness over the frontal or maxillary sinuses that becomes aggravated while the patient bends forward), or severe (life-threatening infection); (2) antibiotic use during the 4 to 6 weeks before the onset of infection, which is an important risk factor for the selection of resistant organisms; (3) age younger than 5 years; (4) attendance at a daycare center; and (5) underlying condition predisposing to invasive infection, such as immunocompromised status.

*See references 65, 318, 413, 493, 499, 579, 592, 618, 630, 783.

Amoxicillin is recommended for the initial treatment of children who have uncomplicated acute bacterial sinusitis that is of mild severity, do not attend daycare centers, and have not been treated recently with an antimicrobial.[132] If the patient is allergic to amoxicillin, cefdinir, cefuroxime axetil, or cefpodoxime can be used (if the allergic reaction was not a type I hypersensitivity reaction). In children with serious allergic reactions, macrolides or clindamycin can be used in an effort to select an antimicrobial of an entirely different class.[132] In patients with disease moderate in severity or those attending daycare, therapy should be initiated with high-dose amoxicillin-clavulanate. Parenteral antibiotic treatment should be considered in patients with severe disease. For patients with mild or moderate disease who are not improving after receiving 72 hours of treatment, therapy should be changed to amoxicillin-clavulanate unless this agent was used initially, in which case treatment options are very limited.[132,363] In addition, re-evaluation of deteriorating or nonresponsive patients may include computed tomography, fiberoptic endoscopy, or sinus aspiration with culture.[687]

Acute sinusitis should be treated for 7 days after improvement is noted and for at least 10 to 14 days.[550] However, the duration of treatment has not been studied adequately and, therefore, is arbitrary.

CONJUNCTIVITIS. When compared with placebo, topical therapy with polymyxin-bacitracin ointment has been shown to reduce the duration of symptoms by half and to achieve a 2.5-fold increase in the rate of bacteriologic eradication after 8 to 10 days of therapy (31% vs. 79%, respectively).[267] Polymyxin B–trimethoprim, applied four times daily for 1 week, also can be an effective broad-spectrum therapy for nonsevere conjunctivitis, although the coverage against gram-positive organisms provided by trimethoprim is less than optimal for some species. Aminoglycoside drops (0.3% gentamicin or 0.3% tobramycin) are inherently less active against gram-positive organisms and have a low therapeutic-to-toxic ratio as topical agents. Neomycin–polymyxin B–gramicidin drops or neomycin–polymyxin B–bacitracin ointment provides broad-spectrum coverage, but neomycin poses a 10 percent risk for hypersensitivity reactions such as contact dermatitis.[764]

Most organisms causing conjunctivitis are susceptible to chloramphenicol.[75,621] However, significant public and professional concern regarding the use of chloramphenicol eyedrops has been raised because of the associated risk of development of bone marrow aplasia.[99,125]

Increased resistance to polymyxin B–sulfamethoxazole for the treatment of conjunctivitis caused by *S. pneumoniae* in children was reported recently. Topical tetracycline and fluoroquinolones are active against penicillin-resistant *S. pneumoniae* and are considered suitable for the treatment of patients with conjunctivitis that is not responsive to the other treatment.[75]

BONE AND JOINT INFECTIONS. In a review of 13 cases of septic arthritis or osteomyelitis caused by penicillin-susceptible *S. pneumoniae*,[4] the drug used most commonly was penicillin, followed by ampicillin. Nafcillin and cefotaxime also were used to treat these patients. In addition, three patients with non–penicillin-susceptible *S. pneumoniae* were treated with combinations of vancomycin and cefotaxime or ceftriaxone for septic arthritis and osteomyelitis.

Rifampin and clindamycin also were used to treat a patient with septic arthritis of the left hip and osteomyelitis of the proximal end of the femur caused by highly resistant *S. pneumoniae* (penicillin and cefotaxime MICs of 8 μg/mL); this patient also experienced prolonged fever (21 days). The patient had been treated initially with cefotaxime and nafcillin, as well as with drainage and irrigation of the infected site.[4]

The duration of treatment with parenteral antibiotics in patients with *S. pneumoniae* bone infection varied from 14 to 196 days and, in those with joint infection, from 12 to 67 days.[4,54] Parenteral treatment may be followed by oral antibiotic therapy once the patient has improved considerably.

ENDOCARDITIS. *S. pneumoniae* endocarditis is a rare event that accounts for less than 3 percent of all cases of endocarditis.[352,524,724] Cases caused by penicillin-resistant *S. pneumoniae* have been reported exclusively in adults.[683] Treatment is based on a combination of antibiotics and surgery because *S. pneumoniae* endocarditis is associated with rapid destruction of heart valves. Parenteral second- or third-generation cephalosporins are recommended as initial treatment, and the addition of vancomycin is advised in patients with penicillin- or cephalosporin-resistant organisms.[420] Newer fluoroquinolones may serve as an alternative treatment in older patients.[683]

TREATMENT OF PNEUMOCOCCAL INFECTION IN IMMUNOCOMPROMISED HOSTS. Children with underlying conditions such as HIV infection, nephrotic syndrome, sickle-cell disease, other congenital hemoglobinopathies, or congenital immunoglobulin deficiencies, as well as children receiving immunosuppressive drugs and those with congenital or acquired asplenia, are at increased risk for acquiring *S. pneumoniae* infection in general and infection with drug-resistant organisms in particular.[346,488,664,731] The results of two studies[665,740] suggested that in HIV-infected adults, the outcome is worse in patients with nonsusceptible *S. pneumoniae* than in those with susceptible *S. pneumoniae*. However, both studies had limitations that preclude arriving at firm conclusions. In regions where penicillin-resistant *S. pneumoniae* occurs commonly, consideration should be given to initiating therapy with vancomycin and cefotaxime or ceftriaxone in critically ill patients until susceptibility results are available; subsequent therapy should be based on these results and the patient's clinical course.

For infants with sickle-cell anemia, oral penicillin prophylaxis against invasive *S. pneumoniae* disease should be initiated as soon as the diagnosis is established, preferably by the time that the infant is 2 months of age. Although the efficacy of antimicrobial prophylaxis has been proved only in patients with sickle-cell anemia, other asplenic children at particularly high risk, such as those with malignant neoplasms or thalassemia, also should receive daily chemoprophylaxis. In general, antimicrobial prophylaxis (in addition to immunization) should be considered strongly for all asplenic children younger than 5 years old and for at least 1 year after they undergo splenectomy.[13]

The age at which chemoprophylaxis is discontinued often is empiric. Based on a multicenter study, prophylactic penicillin can be discontinued in children with sickle-cell anemia who are receiving regular medical attention and have not had a severe *S. pneumoniae* infection or surgical splenectomy when they reach approximately 5 years of age.[13] The appropriate duration of prophylaxis for children with asplenia caused by other conditions is unknown. Some experts continue administering prophylaxis throughout childhood and into adulthood for high-risk patients with asplenia.[13]

For antimicrobial prophylaxis, oral penicillin V (125 mg twice a day for children younger than 5 years old and 250 mg twice a day for children 5 years and older) usually is recommended. Some experts recommend amoxicillin (20 mg/kg/day). Breakthrough infections caused by drug-resistant *S. pneumoniae* may occur. Therefore, parents should be aware that all febrile illnesses are potentially serious in immunocompromised children and that immediate medical attention should be sought because the initial signs and symptoms of fulminant bacteremia can be subtle.[13]

In addition to receiving chemoprophylaxis, children aged 2 years and older who are at increased risk of acquiring invasive *S. pneumoniae* infection should be immunized with a pneumococcal conjugate vaccine followed by a 23-valent nonconjugate polysaccharide vaccine 2 months or longer after the last dose of the conjugate vaccine is taken.[593] Indications for immunization are the following: (1) sickle-cell disease; (2) functional or anatomic asplenia; (3) nephrotic syndrome or chronic renal failure; (4) conditions associated with immunosuppression, such as organ or bone marrow transplantation, drug therapy, or cytoreduction therapy (including long-term systemic corticosteroid therapy); (5) HIV infection; and (6) CSF leaks.[14] For details on immunization, see the section on prevention.

MISCELLANEOUS INFECTIONS. Other infections, such as primary peritonitis and orbital cellulitis, should be treated with antibiotic agents that are recommended for bacteremia. Parenteral penicillin, ampicillin and cefotaxime, or ceftriaxone is suitable treatment in most cases, and the addition of vancomycin for drug-resistant *S. pneumoniae* is recommended. Treatment should be adjusted according to susceptibility results.[731]

PREVENTION

Prevention of pneumococcal infection in all ages is, without doubt, a more effective approach to reducing the burden of pneumococcal disease than any successful treatment modality is. In general, prevention can be divided into nonimmunologic and immunologic strategies (immunoprophylaxis).

NONIMMUNOLOGIC STRATEGIES

This category includes interventions that aim at (1) reducing risk factors predisposing to the development of pneumococcal infection, (2) providing chemical substances (e.g., antibiotics) to abort or prevent pneumococcal colonization or disease, and (3) modifying anatomic abnormalities that predispose to the development of pneumococcal infection.[417]

A factor that is important not only for pneumococcal infection but also for many other serious bacterial infections is the need to improve general health and nutrition worldwide, particularly in developing countries. Many risk factors for the acquisition of pneumococcal infection that can be alleviated include poor living conditions, overcrowding, poor hygiene, malnutrition, and a high prevalence of viral infections, particularly respiratory viruses, measles, and HIV. In the developed world, carriage of pneumococci, especially antibiotic-resistant strains that result in sporadic infections as well as outbreaks, is related to daycare attendance and is proportional to the number of children per group.[124,135,168,171,463,608,718] Therefore, reducing the number of children per group in daycare centers and developing alternative forms of childcare may reduce the rate of pneumococcal morbidity.

The importance of passive smoking (namely, being in close contact with smokers in the same household) has been highlighted as a risk factor for the development of pneumococcal disease.[545] Thus, effort to prevent smoking in the home may have an important role in the prevention of pneumococcal infection. Breast-feeding may protect against some infections related to *S. pneumoniae*, such as otitis media.[23,104,197,628,744] Therefore, breast-feeding should be encouraged, especially in families in which otitis media occurs commonly, although the precise role of prolonged breast-feeding in protecting against pneumococcal infection has not been established.

Antimicrobial chemoprophylaxis is used predominantly for two indications: prevention of recurrent AOM and prevention of pneumococcal sepsis in children with anatomic or functional asplenia. Data now exist to support the common practice of prescribing regular doses of penicillin for children with asplenia or sickle-cell disease.[253] Chemoprophylaxis is used commonly for the prevention of middle ear infections. Controlled clinical trials have compared antimicrobial chemoprophylaxis with placebo, surgery, or historical controls.[76] Various antibiotics were tested (either as ongoing daily prophylaxis or as intermittent treatment during viral infections). Most of the studies, but not all, showed a benefit of chemoprophylaxis over controls, especially for amoxicillin, ampicillin, sulfonamides, and trimethoprim-sulfamethoxazole.* However, those studies were performed before the era of increased antibiotic resistance in *S. pneumoniae*. The dosing schedule recommended for prophylaxis usually is half the daily therapeutic dose given once a day, but the optimal dosing regimen has not been defined.

The potential benefits of otitis media chemoprophylaxis have been weighed against the ability of chemoprophylaxis to alter the nasopharyngeal flora, foster colonization with resistant organisms, and thereby compromise the long-term efficacy of the prophylactic drug and contribute to the propagation of resistant organisms throughout the community. As a result, the practice of otitis media chemoprophylaxis has been reduced greatly. In one study,[101] prophylaxis with amoxicillin induced a dramatic increase in the carriage of penicillin-resistant *S. pneumoniae*, as well as increased carriage of other resistant pathogens. The fear of increasing resistance led the Committee on Infectious Diseases of the American Academy of Pediatrics to issue a warning against the widespread use of otitis media prophylaxis and to state in its recent report that "Antimicrobial prophylaxis should be reserved for control of recurrent acute otitis media, defined by 3 or more distinct and well-documented episodes during a period of 6 months or 4 or more episodes during a period of 12 months."[15]

Surgical otitis media prophylaxis by myringotomy and insertion of tympanostomy tubes or adenoidectomy (with or without tonsillectomy) has been recommended for otitis-prone children.[411] Some authorities now prefer this modality to the chemoprophylactic approach because it is not associated with altered flora. However, surgical risks also need to be considered.

A novel approach to prevention of pneumococcal disease is the potential use of oligosaccharides to prevent attachment of organisms, including *S. pneumoniae*, to the respiratory mucosa. Because colonization of the respiratory mucosa can result in local and systemic disease, as well as spread to other individuals, prevention of colonization is a reasonable approach to prevent disease. Human milk oligosaccharides can prevent attachment of *S. pneumoniae* and other organisms to the mucosa of the respiratory tract[19,803] and have been shown to interfere with the establishment and progression of experimental pneumococcal pneumonia in infant rats.[345] Natural oligosaccharides act as decoys in mucosa (also in saliva, tears, urine, sweat, and breast milk) and bind the carbohydrate-binding proteins of the microbial pathogens and thereby prevent mucosal attachment.[417] Despite this promise, a large-scale study examining the efficacy of such an oligosaccharide (3′-sialyllacto-*N*-neoteratose) given intranasally as prophylaxis for AOM and nasopharyngeal carriage of bacteria failed to show any beneficial effect on either outcome.[745]

Other experiments conducted in Finland showed that xylitol (a five-carbon sugar alcohol used extensively as a sweetener in toothpaste, chewing gum, and various foods) inhibited the growth of streptococci, including *S. pneumoniae*, in vitro[426,427] and prevented the development of otitis media in daycare center attendees when provided as chewing gum, lozenges, or syrup.[742,743]

*See references 65, 318, 413, 493, 499, 579, 592, 618, 630, 783.

Whether this approach eventually will develop into a practical strategy to prevent respiratory infections caused by *S. pneumoniae* and other organisms is not clear. Obviously, the concept of reducing nasopharyngeal colonization and thereby the number of episodes of bacterial respiratory infection through competitive inhibition remains "cutting-edge" medicine. Additional new agents, altered doses, or the existing agents or different methods of administration still should be studied.[576]

IMMUNOPROPHYLAXIS

As discussed in the section on pathogenesis in this chapter, *S. pneumoniae* infections typically are opportunistic infections complicating viral respiratory infection. Recent advances in the development of vaccines against respiratory viruses, especially influenza virus, RSV, and parainfluenza virus, could, therefore, reduce the incidence and severity of pneumococcal infection by reducing these preceding viral infections. Some of these vaccines are expected to be licensed and widely used in the next decade. Inactivated influenza vaccines are licensed already and used in children. The expected addition of live attenuated influenza vaccines may herald a new era in the prevention of influenza disease in children.

The reader is referred to the sections on the structure of the pneumococcus, virulence factors, and host defense mechanisms in this chapter for an understanding of the basis for the immune-based strategy of prevention of disease.

Unconjugated Capsular Polysaccharide Vaccines

Efforts to prevent pneumococcal infection by providing specific immunity started more than 100 years ago, as described in the section on history. That both passive immunization (administration of specific antibodies) and active immunization can protect against many pneumococcal infections is well established.

Passively administered serotype-specific antibodies can protect animals and humans against diseases caused by pneumococci. Administration of antisera was associated with improved clinical outcome, including reduced mortality rates.[118] Human immunoglobulins can prevent experimental pneumococcal bacteremia in mice[128,639] and otitis media in chinchillas.[679] The finding of low cord blood IgG antibodies, mainly of the IgG1 subset, is predictive of early-onset AOM in infancy.[59,474,647] In several clinical studies, bacterial polysaccharide immune globulin (BPIG) obtained by immunizing healthy adults with a 14-valent pneumococcal vaccine (in addition to group C meningococcal and Hib polysaccharide vaccines) decreased the prevalence of AOM[654,680] and invasive infection[682] in children. High-dose BPIG administered to infant rats resulted in high serum concentrations of serotype-specific IgG against serotype 3 (geometric mean concentrations of 8.2 and 1.4 μg/mL on days 1 and 7, respectively) and, consequently, protection against nasopharyngeal carriage of this serotype.[492]

Immunization with pneumococcal polysaccharide antigens, mainly in adults, has been studied for a long time. First, hexavalent polysaccharide vaccines were introduced in the late 1940s, followed by 14-valent vaccines in the late 1970s and 23-valent vaccines in the early 1980s. The last, produced by several manufacturers, contains 25 μg of purified, nonconjugated polysaccharide antigens per dose for serotypes 1, 2, 3, 4, 5, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19A, 19F, 20, 22F, 23F, and 33F. These 23 serotypes account for approximately 90 percent of the serotypes responsible for invasive pneumococcal infection in all age groups in both developed and developing countries.[17,310,311,619,698] The 23-valent polysaccharide vaccines are tolerated well by healthy children for primary[447,668] or repeated immunization.[88] However, the presence of preexisting antibodies was associated with an increased incidence of adverse events at the site of infection in adults but less pronounced effects in children.[78,432,552]

Generally speaking, these bacterial polysaccharide-based vaccines are poorly immunogenic in infants and toddlers for important disease-causing serotypes, which is to be expected because bacterial capsular polysaccharides induce antibody production primarily by T-cell–independent mechanisms that still are not fully developed in this age group.[615] Polysaccharide-specific IgG concentrations are relatively high in very young infants because they are acquired transplacentally and consist mainly of IgG1. Nasopharyngeal colonization with various serotypes of *S. pneumoniae* results in the natural production of serotype-specific antibodies.[107,314,730,786] The immune response to pneumococcal polysaccharides is serotype-dependent, and some serotypes commonly associated with disease are especially poor immunogens until the child reaches the age of approximately 5 years.[194] Serotypes 6A, 6B, 12, 19A, 19F, and 23F are examples of this phenomenon.[170,194,431,447,460,599,699,730,786] As for other species, the IgG subclasses that are produced after exposure to polysaccharide antigens are mainly IgG2 and IgG4.[468] A second dose of polysaccharide vaccine does not provide a booster effect and even may result in a reduced immune response when compared with the response after the first dose, which may suggest an antigenic tolerance effect.[78,754]

Children with certain underlying conditions that predispose to the development of pneumococcal infection respond more poorly to pneumococcal polysaccharide vaccines than do otherwise normal children. Published studies of children with recurrent respiratory tract infections,[204,327,652,653] HIV infection,[34,263,581] sickle-cell disease,[67,754] splenectomy,[3,612] malignancies,[609] and chronic renal disease[247,630,642,703] all have demonstrated this phenomenon.

In addition to a relatively poor systemic immune response, the polysaccharide vaccines have been shown to induce only minimal mucosal immune responses. Serotype-specific antibodies to pneumococcal serotypes 6A, 14, 18C, and 23F were measured in the sera and middle ear effusions of 14 children who had received a 14-valent pneumococcal capsular polysaccharide vaccine and in controls.[431] Serotype-specific antibody concentrations in middle ear effusions correlated with serum concentrations and generally were higher in pneumococcal vaccine recipients than in control vaccine recipients. IgM class antibodies frequently were seen only in the serum samples, thus suggesting that antibodies diffuse into the middle ear space rather than being synthesized in situ in response to the vaccine. This finding refutes previous theories that local production of antibodies occurs in the middle ear after vaccination.[433,692,693]

Limited data exist regarding the efficacy of polysaccharide pneumococcal vaccines in preventing disease in infants and children. A large study conducted in Papua New Guinea on more than 7000 children showed a reduction in mortality rates from acute lower respiratory tract disease.[449,616,617] In this study the effect was dramatic: a 59 percent reduction in mortality rates in all children vaccinated (5 months to 5 years of age) and a 50 percent reduction in children vaccinated before they reached 2 years of age. However, the vaccine did not protect against nonfatal disease. In the United States, researchers have suggested that the polysaccharide vaccines would be 62 percent effective in preventing invasive pneumococcal disease caused by vaccine serotypes in children aged 2 to 5 years.[226] The effectiveness of polysaccharide pneumococcal vaccines in preventing otitis media is not clear. Several studies have shown some reduction in the incidence of otitis media in vaccinated children,[339,490] but other studies have failed to show this effect.[193,394,725] No effect of the use of polysaccharide pneumococcal vaccine on pneumococcal nasopharyngeal carriage could be demonstrated.[160,170]

The aforementioned data on T-cell–independent polysaccharide pneumococcal vaccines clearly show that the benefit in children was at best marginal.

Conjugated Capsular Polysaccharide Vaccines

In contrast to the T-cell–independent nature of the immune response that occurs after the administration of bacterial polysaccharides, when these polysaccharides are conjugated to protein, the antibody response changes to a T-cell–dependent one.[44,200,396,620,681,705] These conjugate vaccines induce helper T cells to stimulate polysaccharide-specific B cells that not only produce antibodies but also mature into memory cells (Fig. 96–14). Polysaccharide–protein conjugate products characteristically are immunogenic in infancy and result in the production of high concentrations of antibodies. These antibodies have improved functional capacity (determined by avidity and opsonization assays), are long lasting, and induce a brisk and rapid elevation in highly functional antigen-specific antibodies with re-exposure (booster effect).[205]

The development of pneumococcal conjugate vaccines initially used the technology developed for Hib conjugate vaccines. Increasing experience and challenges have led to modifications of that technology, the development of new technologies, and the addition of new protein carriers. Studies were initiated first on monovalent pneumococcal conjugate vaccines. Currently, this technology has brought about vaccines with 7 to 13 different conjugated pneumococcal polysaccharide antigens. Table 96–4 shows the pneumococcal conjugate vaccines that are licensed in at least one country, are being tested in phase III efficacy studies, or are in advanced phase II (safety and immunology) stages. The rationale for choosing among the 7-, 9-, 11-, or 13-valent conjugate vaccines is presented in the section on epidemiology in this chapter.

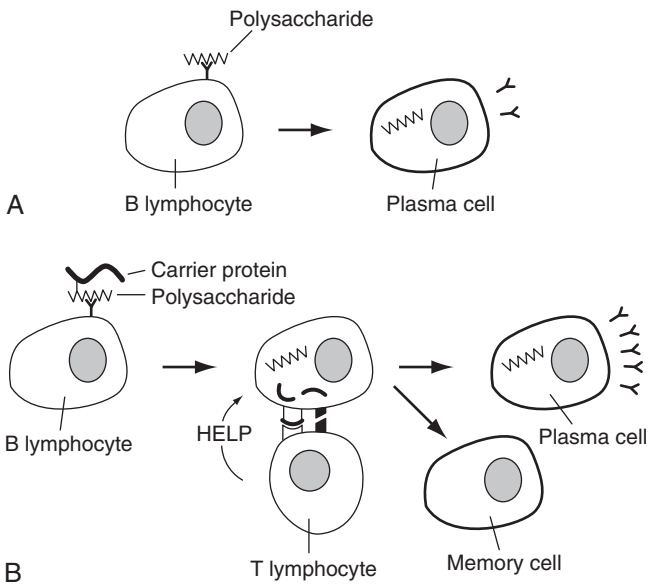


Figure 96–14 T-cell–independent **(A)** and T-cell-dependent **(B)** antibody responses to polysaccharide or polysaccharide–protein conjugate antigens. *(From Eskola, J., and Anttila, M.: Pneumococcal conjugate vaccines. Pediatr. Infect. Dis. J.* 18*:543-551, 1999.)*

The main target populations for the development of conjugate pneumococcal vaccines were infants and young children. Conjugate pneumococcal vaccines have been studied in these target populations since the early 1990s. Pneumococcal conjugate vaccines have been found to be safe and well-tolerated. The reactions reported usually were local ones, such as pain, redness, and swelling at the injection site. The incidence of fever and irritability was somewhat higher than reported in studies with the Hib conjugate vaccines or hepatitis B vaccines, perhaps because these vaccines represent 7 to 11 separate monovalent vaccines administered together as opposed to the monovalent Hib conjugate vaccine or hepatitis B vaccine. The 7-valent pneumococcal vaccine conjugated to CRM_{197} protein (PnCRM7) was found to be safe even when administered to low-birth-weight and preterm infants.[677]

The conjugate vaccines tested were able to elicit a T-cell–dependent immune response in normal infants and toddlers, namely, priming with immunologic memory and maturation of the functional antibody response, as measured by the predominance of IgG1 subclass antibodies, opsonophagocytic activity assays, and antibody avidity assays.[205,217] The immunogenicity of one vaccine (PnCRM7) also was studied in various high-risk groups. It was immunogenic in children with sickle-cell disease,[544,552,754] HIV infection,[407,408,419,534] solid organ transplants,[471] or allogeneic bone marrow transplants,[513] as well as in Alaska Natives and American Indians.[510] A 9-valent CRM (PnCRM9) pneumococcal vaccine also was immunogenic in children with sickle-cell disease.[276]

Determining the efficacy of pneumococcal vaccines is complex. Pneumococci cause a range of clinical diseases, and, therefore, several end-points of efficacy trials should be considered. The end-points thought to be of most importance in evaluating pneumococcal vaccines have been (1) prevention of invasive pneumococcal disease as defined by isolation of pneumococci from a normally sterile site (e.g., bacteremia or septicemia, meningitis, osteomyelitis/septic arthritis, and soft tissue infections); (2) prevention of mucosal infections such as otitis media, sinusitis, and nonbacteremic pneumonia; and (3) reduction of nasopharyngeal colonization, which in turn results in a reduction in spread of the organisms (the biologic basis for indirect effects, also known as *herd immunity*).

TABLE 96–4 Pneumococcal Conjugate Vaccines That Are Licensed in at Least One Country, Are Being Tested in Phase III Trials, or Are in Advanced Stages of Phase II Development

Vaccine	Valency	Pneumococcal Polysaccharides	Carrier Protein	Manufacturer
PnOMPC7	7-valent	4, 6B, 9V, 14, 18C, 19F, 23F	Meningococcal outer-membrane protein complex	Merck Research Laboratories
PnCRM7	7-valent	4, 6B, 9V, 14, 18C, 19F, 23F	CRM_{197} protein	Wyeth Pharmaceuticals
PnCRM9	9-valent	1, 4, 5, 6B, 9V, 14, 18C, 19F, 23F	CRM_{197} protein	Wyeth Pharmaceuticals
PncT/D	11-valent	1, 3, 4, 5, 6B, 7F, 9V, 14, 18C, 19F, 23F	A mixture of tetanus and diphtheria toxoids	Aventis Pasteur
Pn-PD	11-valent	1, 3, 4, 5, 6B, 7F, 9V, 14, 18C, 19F, 23F	*Haemophilus influenzae*—protein D	GlaxoSmithKline
PnCRM13	13-valent	1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F	CRM_{197} protein	Wyeth Pharmaceuticals

By mid-2001, the 7-valent pneumococcal CRM vaccine had been licensed in the United States and in more than 30 other countries. The vaccine's trade name is Prevnar in the United States and Prevenar in other countries. In the United States, the Committee on Infectious Diseases of the American Academy of Pediatrics has made the following recommendations with regard to pneumococcal conjugate vaccines. PCV-7 is recommended for routine administration as a four-dose series for infants at ages 2, 4, 6, and 12 to 15 months; catch-up immunization is recommended for all children up to 23 months of age. The PCV-7 vaccine may given along with other age-appropriate childhood immunizations in a separate syringe and at a separate injection site. Infants of very low birth weight may be immunized when they reach a chronologic age of 6 to 8 weeks, regardless of their calculated gestational age. The PCV-7 vaccine also is recommended for all children younger than 60 months who are at high risk of acquiring invasive pneumococcal infection (patients with sickle-cell disease, asplenia, HIV/acquired immunodeficiency syndrome, diabetes, cancer, liver disorders, lung diseases, and cardiac diseases). For some high-risk children, supplemental protection should be given by administration of the PPV23 vaccine. The only contraindication to receiving the vaccine is a serious allergic reaction to a previous dose of this vaccine or one of its components. Children with minor illnesses such as colds may be vaccinated.[16]

In the United States, the practice of giving three primary doses and a booster dose is known as a "3 + 1 schedule." Some countries routinely immunize with fewer doses. Some countries give two primary doses and a booster, a "2 + 1 schedule," whereas others give three primary doses without a booster, a "3 + 0 schedule." Some serotypes, namely serotypes 6B and 23F,[395] produce less antibody when a two–primary dose series is used as opposed to a three–primary dose series.[475] One study found that three infant doses with a booster were more protective against vaccine-type disease than were two doses alone ($p = 0.0323$).[781] The booster dose may play a role in reducing nasopharyngeal carriage; therefore, absence of a booster dose may decrease indirect protection.[475]

Widespread use of the vaccine in the United States began in the first half of the year 2000. Such widespread use has provided additional data on the safety of PnCRM7. Common side effects of the vaccine include redness, tenderness, or swelling at the site of injection, fever, fussiness, drowsiness, rashes, and urticaria.[785] Furthermore, the incidence of invasive pneumococcal disease has decreased sharply in infants and toddlers and even in their household contacts.[70,676,780]

Three efficacy studies with the end-point of a reduction in the number of invasive infections were completed by mid-2002, two with PnCRM7 and one with the PnCRM9 vaccine. The first study was a prospective double-blind study of 37,868 healthy infants in northern California to whom either the PnCRM7 vaccine or a control vaccine was administered at 2, 4, 6, and 12 to 15 months of age.[69] The vaccine was 97.4 percent efficacious (95% confidence interval, 82.7-99.9%) against invasive disease caused by the serotype included in the vaccine in fully vaccinated infants and 93.9 percent efficacious (95% confidence interval, 79.6-98.5%) in partially vaccinated infants. No evidence of an increase in invasive disease caused by serotypes that were not included in the vaccine was detected, and thus the overall effect was a reduction in total invasive pneumococcal disease by 89.1 percent (95% confidence interval, 73.7-95.8%). This trial was the pivotal efficacy one that led to U.S. licensure of the vaccine by the Food and Drug Administration in February 2000 and subsequent licensure in many other countries. Additional analysis[677] has shown that the vaccine was as effective in the subset of low-birth-weight and premature infants as it was in the full study cohort.

A second large-scale efficacy study with the PnCRM7 vaccine was conducted among American Indian (Navajo and White Mountain Apache) children in the United States. This population has rates of invasive pneumococcal infection that are approximately five times those of the general U.S. population.[142,549] In a double-blind, community-randomized study, infants and young children aged 2 months to 2 years received the PnCRM7 vaccine or a control vaccine.[551] A total of 8292 infants from 43 communities were enrolled; of these, 8091 lived in 38 communities that were randomized to the pneumococcal or the control vaccine. During the study period, two cases of invasive pneumococcal infection caused by the serotypes included in the vaccine occurred in the vaccine group versus eight in the control group. After controlling for community randomization, the primary efficacy of the vaccine was 76.8 percent (95% confidence interval, 9.4-95.1%), and the intent-to-treat efficacy was 86.4 percent (95% confidence interval, 11-96.1%). These results are not statistically different from those of the trial in northern California.

A third efficacy study was conducted with the PnCRM9 vaccine among black infants in South Africa. In a double-blind, randomized, placebo-controlled study, PnCRM9 was administered to children at the ages of 6, 10, and 14 weeks according to the World Health Organization's Expanded Programme on Immunization. The infants were monitored until they reached 2 years of age, and the study was completed by the end of 2001. The intent-to-treat analysis showed a reduction of 82.5 percent (95% confidence interval, 39.0-96.7%) in the number of invasive infections caused by the serotypes included in the vaccine for children not infected with HIV and a reduction of 65.4 percent (95% confidence interval, 23.8-85.7%) in children infected with HIV.[424]

Otitis media is caused by various organisms in addition to *S. pneumoniae* (mainly *H. influenzae* [usually untypeable], *M. catarrhalis*, and, less frequently, *S. pyogenes* and enteric bacteria). Therefore, a positive evaluation of conjugate pneumococcal vaccines must demonstrate not only a reduction in the incidence of disease caused by the pneumococcal serotypes included in the vaccine but also a parallel increase in episodes caused by other pathogens, especially the pneumococci of serotypes not present in the vaccine. The latter were shown to be isolated with increased frequency from the nasopharynx of children vaccinated with conjugate pneumococcal vaccine (the so-called replacement phenomenon), as will be discussed further in the section.

Pneumococci are found in 25 to 50 percent of cases of AOM occurring in children.[77,378,484] The serotypes causing otitis media are not always identical to those causing invasive infection, but the most common serotypes found in otitis media worldwide are included in the 7-, 9-, or 11-valent conjugate vaccines. They include serotypes 3, 6B, 9V, 14, 19F, and 23F.[74,114,157,394] Serotypes 6A and 19A also are important causes of otitis media.[157,206] These two serotypes are not included in the vaccine, but cross-protection of serotype 6A by antibodies to serotype 6B has been demonstrated.[2,25,159,206,266,548,568] The pneumococcal serotypes included in the various vaccine formulations would account for 50 to 85 percent of all serotypes causing otitis media. Assuming 100 percent efficacy of pneumococcal conjugate vaccines against the serotypes included in the vaccines and no increase in otitis media caused by other pneumococcal serotypes or other pathogens, conjugate pneumococcal vaccines could reduce the incidence of all causes of otitis media by at most 10 to 25 percent. The results of analysis of four efficacy/effectiveness studies confirmed these expectations.

The northern California study, conducted primarily to determine efficacy of the PnCRM7 conjugate vaccine against invasive pneumococcal infection,[69] also looked at the reduction in the number of clinic visits for otitis media in 18,927 pneumococcal vaccine recipients and 18,941 control meningococcal vaccine

recipients at the ages of 2, 4, 6, and 12 to 15 months. During the 30-month follow-up period, a total of 73,041 visits related to otitis media and 52,789 distinct episodes of otitis media had occurred in the study population. Of those, 5451 subjects had frequent episodes of otitis media (three episodes in 6 months or four episodes in 1 year). The pneumococcal vaccine reduced the number of clinically diagnosed otitis media episodes by 7 percent (95% confidence interval, 4.1-7.9%). The effectiveness of the PnCRM7 vaccine against frequent otitis media was 9.3 percent (95% confidence interval, 3.0-15.1%) when three episodes in 6 months or four in 12 months were counted, and it was 22.8 percent (95% confidence interval, 6.7-36.2%) when a frequency of five episodes in 6 months or six in 12 months was considered. Children who received the pneumococcal conjugate vaccine were 20.1 percent (95% confidence interval, 1.5-35.2%) less likely to require placement of a pressure-equalizing tube than controls were. This study was not designed to evaluate the effect of conjugate pneumococcal vaccine on otitis media as a primary outcome, and, therefore, no attempts were made to standardize or validate the clinical diagnoses of otitis media. Hence, the study was able to assess only the effect of PnCRM7 vaccine on otitis media as it is diagnosed and managed in the routine clinical setting. As a result, the authors were not able to examine the efficacy of the vaccine against otitis media caused by pneumococcal serotypes included in the vaccine, except for cultures performed on fluid obtained from spontaneously draining ears. In 23 children, culture of fluid from spontaneously draining ears was positive for pneumococci of the vaccine serotypes, with a 66 percent calculated efficacy against disease caused by serotypes included in the vaccine. However, its efficacy against clinically diagnosed otitis media indicated that the vaccine reduced morbidity attributed to otitis media and medical services used for the management of clinical otitis.

A second study, conducted in Finland, looked at the efficacy of PnCRM7 vaccine against AOM in general and more specifically against otitis media caused by serotypes in the conjugate pneumococcal vaccine under study.[206] A total of 1662 infants were enrolled and randomized in a double-blind manner to receive either the PnCRM7 vaccine or a hepatitis B vaccine at the ages of 2, 4, 6, and 12 months. The clinical diagnosis of AOM was based on predefined criteria, and bacteriologic diagnosis was based on culture of middle ear fluid obtained by myringotomy. The children were monitored through 24 months of age. A total of 2596 episodes of AOM occurred in children who had received three doses of vaccine by the time that they reached 6 months of age. The efficacy of the vaccine in reducing cases caused by the serotypes included in the vaccine was 57 percent (95% confidence interval, 46-67%). The PnCRM7 vaccine also provided cross-protection against serotypes 6A and 19A. The efficacy of the vaccine in reducing all culture-confirmed pneumococcal infections (including serotypes not included in the vaccine) was 34 percent (95% confidence interval, 24% to 45%). However, the vaccine was not effective against nonvaccine pneumococcal serotypes that are not cross-reactive with the ones included in the vaccine. The vaccine also was ineffective against otitis media caused by *H. influenzae* and *M. catarrhalis*, and a trend toward an increase in the incidence of otitis media among vaccinated children (replacement disease phenomenon) was noted. Thus, the overall high protection provided against the serotypes included in the vaccine was offset partially by an increase in the number of episodes of otitis media caused by other serotypes of pneumococci and other pathogens. The overall reduction in the number of episodes of AOM of any cause in the pneumococcal vaccine group was 6 percent (95% confidence interval, –4-16%). This difference did not reach statistical significance, but it was within the expected range when all theoretical considerations listed earlier were taken into account, and it also was strikingly similar to the 7 percent reduction in the number of cases of otitis media observed in the California study.[69] In addition, similar to the California study, by the time that they reached the age of 4 to 5 years, the vaccinated subjects had undergone tympanostomy tube placement at a rate that was reduced by 39 percent (95% confidence interval, 4% to 61%) in comparison to controls.[564]

A third study was conducted by the same Finnish group in parallel with the study just described, but this time with a different 7-valent vaccine (serotypes 4, 6B, 9V, 14, 18C, 19F, and 23F conjugated to the meningococcal outer-membrane protein complex [OMPC]—PnOMPC7).[403,404] In this randomized, double-blind, controlled trial, 1666 children were randomized to receive either PnOMPC7 or hepatitis B vaccine at 2, 4, and 6 months of age. At 12 months of age, approximately 22 percent of the PnOMPC7 conjugate vaccine recipients received a nonconjugate 23-valent polysaccharide vaccine as a booster, and the others received a fourth dose of PnOMPC7. Follow-up continued through 24 months of age. The methodology was similar to that described earlier for the PnCRM7 conjugate vaccine, including cultures for the diagnosis of otitis media episodes. A total of 2709 otitis media episodes occurred in evaluable children who had completed their three-dose schedule, 360 of which were caused by pneumococci of the serotypes included in the vaccine. The efficacy of the vaccine in reducing the number of cases of otitis media caused by the serotypes included in the vaccine was 56 percent (95% confidence interval, 44-66%). In reducing the number of all pneumococcal otitis media episodes, the efficacy was 25 percent (95% confidence interval, 11-37%). However, no overall reduction in the number of episodes of otitis media was seen because of an increase in the number of episodes caused by nonvaccine serotype pneumococci and other nonpneumococcal organisms (replacement disease).

The results of the two studies in Finland and the study in northern California are strikingly consistent. They all have shown that (1) the efficacy of the two conjugate vaccines tested thus far in the prevention of otitis media caused by pneumococcal serotypes included in the vaccine was greater than 50 percent and that efficacy on the order of magnitude observed for invasive infections (i.e., 90%) cannot be achieved against otitis media, (2) replacement otitis media with pneumococci not included in the vaccine and other organisms such as *H. influenzae* and *M. catarrhalis* occurs and reduces the overall efficacy of the pneumococcal conjugate vaccines against otitis media, and (3) better protection is provided against episodes of more severe otitis media and recurrent otitis media than against simple otitis media. The more severe and recurrent otitis media episodes often are associated with pneumococcal serotypes 6B, 9V, 14, 19F, and 23F, which tend to persist both in the nasopharynx and in the middle ear. These serotypes also tend to be more resistant to antibiotics.[157,380]

A fourth study was conducted in toddlers attending daycare centers in southern Israel. In this double-blind study, the efficacy of a 9-valent CRM conjugate pneumococcal vaccine (PnCRM9) in reducing nasopharyngeal carriage of *S. pneumoniae* and respiratory infections was compared with that of a control vaccine. This study showed, in conjunction with an extensive reduction in carriage of the serotypes included in the vaccine, especially serotypes 6B, 9V, 14, 19F, and 23F,[155,159,174] a 17 percent (95% confidence interval, –0.2-33%) reduction in the number of cases of otitis media and a 20 percent (95% confidence interval, 14-36%) reduction in antibiotic use for otitis media.[174] These findings support the notion that conjugate pneumococcal vaccines can reduce the morbidity of otitis media, not only by reducing the number of episodes or reducing the severity of otitis media in general but also by selectively reducing otitis media in high-risk groups such as attendees at daycare centers.

TABLE 96–5 Studies* on the Effect of Conjugate Pneumococcal Vaccine on Carriage of *Streptococcus pneumoniae* and Antibiotic-Resistant *S. pneumoniae*

Author	Conjugate Vaccine (Valence)	Site	Age (mo) at Vaccination	Reduction in Serotypes Included in the Vaccine	Reduction in Resistant Pneumococci	Increase in Nonvaccine Serotypes
Dagan[170]	PnOMPC7	Israel	12-18	Yes	Yes	No
Dagan[172]	PnT, PnD (4-valent)	Israel	2, 4, 6	Yes	Yes	+/–
Obaro[546]	PnCRM5	The Gambia	2, 3, 4	Yes	ND	Yes
Kristinsson[436]	PnT, PnD (8-valent)	Iceland	3, 4, 6	Yes	ND	Yes
Mbelle[500]	PnCRM9	South Africa	1.5, 2.5, 3.5	Yes	Yes	Yes
Edwards[201]	PnCRM9	USA	2, 4, 6, 12	Yes	ND	Yes
Dagan[175]	PnT/D (11-valent)	Israel	2, 4, 6, 12	Yes	Yes	No
Dagan[155,159]	PnCRM9	Israel	12-35	Yes	Yes	Yes
O'Brien[548]	PnCRM7	USA (Native American)	2, 4, 6, 12-15	Yes	ND	Yes
Kilpi[404]	PnCRM7	Finland	2, 4, 6, 12	Yes	ND	ND

PnOMPC, 7-valent pneumococcal vaccine conjugated to the outer-membrane complex of Neisseria meningitidis B; PnT, pneumococcal vaccine conjugated to tetanus toxoid; PnD, pneumococcal vaccine conjugated to diphtheria toxoid; PnT/D, pneumococcal vaccine conjugated to a mixture of tetanus and diphtheria toxoid; PnCRM, pneumococcal vaccine conjugated to CRM_{197} protein (PnCRM5, 5-valent; PnCRM7, 7-valent; PnCRM9, 9-valent).

Since the use of PCV-7 has become common practice, several studies have shown that the PCV-7 vaccine is not extremely efficacious in decreasing the total number of children who have AOM because of an increase in cases caused by nonvaccine serotypes or other organisms.[707,708] The vaccine should be promoted because of its primary indication of preventing invasive pneumococcal disease and not decreasing the incidence of AOM.[375] However, some papers have reported a decline in the number of visits to primary care physicians for AOM[292] by up to 19 percent.[475] Another study found that the incidence of tympanostomy tube placement decreased by 24 percent after introduction of the vaccine.[228]

Protection against pneumococcal pneumonia by the conjugate pneumococcal vaccines was shown in the PnCRM7 vaccine efficacy trial conducted in northern California.[69,71] However, pneumococcal pneumonia cases are associated only infrequently with bacteremia, and the PnCRM7 vaccine should provide protection against these invasive episodes to the same degree as they do against all other invasive episodes. Because most cases of pneumonia are nonbacteremic, the great majority remain without a clear bacteriologic diagnosis. In these cases, the role of *S. pneumoniae* can be demonstrated indirectly if the use of a vaccine significantly reduces the occurrence of pneumonia cases. Thus, the conjugate pneumococcal vaccines can be used as surrogates to estimate more accurately the proportion of pneumonia cases attributable to vaccine serotype pneumococci.

In the northern California study conducted on 37,868 infants given the PnCRM7 vaccine, all clinically diagnosed episodes of pneumonia identified through hospital, outpatient, and emergency records were collected.[71] In total, 3711 clinical episodes of pneumonia that occurred before the children reached the age of 3.5 years were identified; the efficacy of the PnCRM7 vaccine in reducing the incidence of disease (intent-to-treat analysis) was 6.0 percent (95% confidence interval, 1.5-11.0%). Of the 3711 clinical episodes, a chest radiograph was obtained in 2249 episodes, and among these children, vaccine efficacy was 8.9 percent (95% confidence interval, 0.9-16.3%). Of the 2249 children in whom a chest radiograph was obtained, 737 had a positive chest radiograph (defined as parenchymal infiltrates, consolidation or effusion [or both], but not perihilar infiltrates alone). In these 737 patients, efficacy was 22.7 percent (95% confidence interval, 8.7-34.5%). These findings have some limitations because they were not derived from a study in which the primary objective was to evaluate protection against pneumonia. However, the findings suggest two important points: (1) many cases of clinically and radiologically proven pneumonia in children are caused by *S. pneumoniae* by virtue of the fact that vaccination was associated with a marked reduction in not only the "classic" lobar pneumonia usually associated with *S. pneumoniae* but also clinical pneumonia, with negative or minimal findings on the chest radiograph, and (2) the 22.7 percent efficacy observed for pneumonia with radiologically documented findings suggests that at least in the developed world where Hib vaccines are used widely, *S. pneumoniae* causes a high proportion of pneumonia with parenchymal infiltrates.

A recent study from South Africa showed that administration of three doses of PnCRM9 at the ages of 6, 10, and 14 weeks reduced the incidence of radiologically proven pneumonia by 22.1 percent (95% confidence interval, 0.1-39.5%) in children who were not infected with HIV.[424]

Additional supportive evidence that *S. pneumoniae* may play a more important role in respiratory infections than usually attributed to this pathogen can be derived from a study in southern Israel involving toddlers aged 12 to 35 months who attended daycare centers.[174] In this study, 263 children were randomized to receive either PnCRM9 vaccine or a control vaccine (meningococcus CRM conjugate) in a double-blind fashion. The children were monitored for 5556 child-months. A total of 906 episodes of non–otitis media upper respiratory tract infection were reported, for an efficacy estimate of 15 percent (95% confidence interval, 4-24%); 596 episodes of lower respiratory tract problems occurred, including bronchiolitis, cough, and pneumonia, and the efficacy of pneumococcal vaccine was 16 percent (95% confidence interval, 2-28%). For these two clinical illness categories, children received a total of 3678 days of antibiotics. A reduction of 10 percent in the number of days that antibiotics were given for upper respiratory tract infections was achieved, as was a reduction of 47 percent for lower respiratory tract problems in the pneumococcal vaccine group ($p < 0.001$ versus control children). The incidence of bronchiolitis and pneumonia, often regarded as viral in this age group, was decreased significantly by the administration of this conjugate pneumococcal vaccine, thus suggesting that *S. pneumoniae* plays a role as a pathogen or co-pathogen for these entities.

As stated earlier, control of nasopharyngeal carriage of pneumococci is the key to managing pneumococcal disease and person-to-person spread of *S. pneumoniae*. The nonconjugate pneumococcal vaccines do not have a significant effect on carriage of *S. pneumoniae* in children and adults.[170,485] In contrast, the conjugate vaccines do have a significant effect on carriage.* Table 96–5 shows the studies conducted and published thus far to

*See references 155, 159, 170, 172, 175, 201, 436, 500, 546, 548.

document the effect of pneumococcal vaccines on carriage of *S. pneumoniae*. Despite variations in the nature of the conjugate vaccines, populations, and the ages at which the vaccines were administered, a significant reduction in carriage of the serotypes included in the vaccine clearly was observed in all studies. However, in most of the studies, a "replacement" phenomenon occurred: an increase in the carriage of *S. pneumoniae* serotypes not included in the vaccine was observed in conjunction with a decrease in the carriage of serotypes included in the vaccine. Although this replacement phenomenon is remarkable, its clinical significance is not clear. In theory, such a phenomenon could be simply an artifact of "unmasking," in which nonvaccine serotypes are detected more readily in vaccinees than in controls because the vaccine serotypes are not present. However, the use of mathematic modeling and testing in controlled vaccine studies strongly suggests that a true "replacement phenomenon" does exist in which non–vaccine-type pneumococci truly are replacing vaccine-type pneumococci.[472] This suggestion is supported by the observation of an increase in the incidence of otitis media caused by organisms not included in the vaccine after vaccination with PnCRM7 and PnOMPC7 in Finland.[206,403]

The reduction in nasopharyngeal carriage of vaccine serotypes of pneumococci is important because it certainly will reduce the spread of these serotypes. Two studies in different settings clearly have shown the existence of this phenomenon. In one double-blind comparative study conducted in southern Israel, toddlers attending daycare centers were vaccinated with a PnCRM9 vaccine or a control vaccine.[156] In this study, attendees at daycare centers and their younger siblings who stayed home were monitored after the daycare attendees were vaccinated. A marked reduction in the incidence of vaccine-type pneumococcal carriage was seen in the young siblings of those who were vaccinated with the PnCRM9 vaccine when compared with siblings of the controls.[156]

In a second study, Navajo and White Mountain Apache children younger than 2 years old were randomized according to their community of residence to receive either PnCRM7 or meningococcus C conjugate vaccine. Nasopharyngeal swabs were cultured for *S. pneumoniae* from 598 nonimmunized infants residing in both vaccinated (by PnCRM7 vaccine) and control communities.[547] A 24 percent reduction in carriage of vaccine-associated serotypes was noted in vaccinated infants residing in the PnCRM7 communities versus those living in control vaccine communities. The reduction was found both in infants who lived with a PnCRM7-vaccinated sibling and in those who did not have direct contact with a PnCRM7-vaccinated child. This study demonstrated the indirect protective effect of pneumococcal vaccination on those in the household.

Because most antibiotic-resistant *S. pneumoniae* strains belong to only a few serotypes that are included in the conjugate pneumococcal vaccines or are related to these serotypes (see the section on epidemiology and microbiology), vaccines are expected to reduce disease, carriage, and spread of antibiotic-resistant *S. pneumoniae* and may have an impact on the use of antibiotics. Indeed, in all vaccine studies that investigated the effect of conjugate vaccines on nasopharyngeal carriage of antibiotic-resistant *S. pneumoniae*, a reduction in carriage of such strains was observed[155,170,172,175,500] (Table 96–5). Furthermore, the use of conjugate vaccines reduced the use of antibiotics in two prospective double-blind studies. In a study conducted in northern California, administration of the PnCRM7 vaccine reduced the use of antibiotics by 5.3 percent in patients given vaccines versus controls; the reduction was 5.0 percent for drugs generally used as first-line agents (such as amoxicillin and ampicillin) and 11.2 percent for those often used as second-line agents (cephalosporins, amoxicillin-clavulanate, and azithromycin) (Black, S., Shinefeld, H.: Presented at the 19th Annual Meeting of the European Society for Pediatric Infectious Diseases, April 26 to 28, 2001, Istanbul, Turkey). In another study performed in toddlers attending daycare centers in southern Israel, the PnCRM9 conjugate vaccine reduced the use of antibiotics in vaccine recipients versus controls by 20 percent,[174] in parallel with the reduction in carriage of antibiotic-resistant *S. pneumoniae* and respiratory diseases. The reduction in both these factors may contribute to reducing antibiotic resistance in the community. However, the overall effect remains to be determined.

Although invasive infections are the most dramatic part of the pneumococcal disease spectrum, respiratory infections such as otitis media occur far more frequently.[466] In a study investigating current estimates of pneumococcal disease burden, clinical outcome, and vaccine efficacy, researchers estimated that for each annual U.S. birth cohort, routine use of a 7-valent vaccine could prevent 12,000 cases of pneumococcal bacteremia and meningitis, 53,000 cases of pneumococcal pneumonia, and more than 1 million clinical cases of otitis media per year.[466]

Pneumonia has a greater impact globally than do otitis media or even invasive infections because of the high mortality rate in children in the developing world.[63,296,461] However, it is more difficult to study because it does not occur as commonly as does otitis media, and bacteriologic documentation rarely is available for this entity in children.

Thus, the protection conferred by the conjugate pneumococcal vaccines with regard to reduction in the number of episodes of otitis media, pneumonia, and other mucosal infections, if proven by additional studies, could have an even greater impact on the burden of disease than on prevention of invasive infections.

Potential Future Vaccines and Future Strategies

Studies have been conducted to determine whether immunization of pregnant women with pneumococcal conjugate or nonconjugate vaccines can protect their offspring from acquiring pneumococcal diseases during their first few months of life, before they achieve immunity through active vaccination. This suggestion is based on studies that have shown that naturally acquired IgG antibodies are transferred transplacentally to the fetus readily,[21,129,143] and clinical studies of maternal immunization with pneumococcal polysaccharide vaccines have resulted in similar observations.[450,553,672] In experimental studies, a correlation has been demonstrated between higher concentrations of vaccine-specific antibodies at birth and greater response to specific serotypes after subsequent active immunization in the offspring of vaccine recipients.[445,470] In one study, administration of a conjugate pneumococcal vaccine to pregnant women[523] resulted not only in efficient transplacental passage of vaccine-induced pneumococcal antibodies (mainly of the IgG1 subtype) but also in prevention of pneumococcal carriage in the offspring of vaccinated mothers. A recent Cochrane review found no evidence that pneumococcal vaccination administered during pregnancy reduces the risk for neonatal infection. Although the data did suggest an effect in reducing pneumococcal colonization in infants by the time they reached 16 months of age, no evidence was found of this effect in infants at 2 months of age or by 7 months of age.[121] A recent study in New Guinea found that breast milk IgA was 1.1 to 1.8 times higher in women immunized with pneumococcal polysaccharide vaccine than in unimmunized women for 6 months post partum.[451] Thus, maternal immunization during pregnancy may prove to be a successful strategy to protect against pneumococcal infection in early infancy, but additional serotypes need to be included in these vaccines.

Adding more polysaccharide capsular antigens to the conjugate pneumococcal vaccines to increase serotype coverage is being investigated. In addition, much effort is being invested in combining the present conjugate pneumococcal vaccine with other childhood vaccines to be given in the same syringe in

order to reduce the number of injections needed to immunize infants.

Despite the great potential benefit of the pneumococcal conjugate vaccines, they have two important limitations: (1) inclusion of a limited number of serotypes, which results not only in noncoverage of some other important serotypes causing disease but also in potential replacement disease by serotypes not related to the vaccine, and (2) their high price as a result of complex production and quality control processes.

Many of the proteins mentioned in the section on pathogenesis appear to be suitable antigens for candidate vaccines, and such vaccines that include proteins immunogenic in infants are being developed. The main drawback of protein vaccines is the antigenic variability of many pneumococcal proteins. However, some of the most important proteins considered essential for bacterial virulence have epitopes that are common to many pneumococcal strains. Among these proteins are pneumolysin, PspA, and PsaA.[96] Plasma concentrations of antibodies to these proteins increase with age and are associated strongly with pneumococcal exposure, whether by carriage or infection such as AOM.[605] One suggestion is that antibodies to PsaA may prevent pneumococcal otitis media,[607] but such prevention has not been confirmed. Preliminary studies show that PspA and PsaA given as single antigens or as a mixture of antigens are immunogenic and protective in mice and safe and immunogenic in humans.[533] Further studies in humans were expected to begin in 2002. Other candidate protein vaccines are being investigated in animals, but as of mid-2002, they had not yet been administered to humans. An additional novel approach to providing immunization against *S. pneumoniae* infection is to immunize subjects with killed whole-cell *S. pneumoniae* intranasally with an adjuvant. This approach was studied in animal models and showed promising results.[491]

During the next decade, other pneumococcal vaccine prototypes probably will be discovered and developed. Whether they will equal or surpass the beneficial effects of the pneumococcal conjugate vaccines remains to be seen.

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CHAPTER 97

Miscellaneous Gram-Positive Cocci

Randall G. Fisher

This chapter discusses relatively uncommon gram-positive cocci that are of importance because of their unusual antimicrobial sensitivities and because of their increasing recognition as pathogens in hospitalized patients. The reader is referred to a review of these organisms for more details.[16]

LEUCONOSTOC SPECIES

BACTERIOLOGY

Leuconostoc spp. are facultatively anaerobic, gram-positive cocci that usually appear in pairs or chains. They are catalase-negative,

Vogues-Proskauer–positive, and leucine aminopeptidase–positive. In addition, colonies often are alpha-hemolytic on blood agar and also may react with group D streptococcal antiserum. These properties are shared by viridans streptococci, with which *Leuconostoc* spp. often are confused.[18] They also may resemble enterococci, except that they are pyrrolidone carboxylyl peptidase–negative. Differences include the production of gas from glucose and high-level vancomycin resistance.

EPIDEMIOLOGY

Leuconostoc spp. are found commonly on plants, especially sugar cane and leafy vegetables. They also are present in dairy products and wine.[44] They are used in the food industry as starter cultures in food production.[30,55] Studies using culture methods have suggested that, although they occasionally are recovered from vaginal swabs in healthy individuals[48] and from mucosal surfaces in some hospitalized individuals,[26] *Leuconostoc* spp. are not part of the normal human flora.[3] However, a study of the acquisition of colonizing bacteria in the newborn gut involving phylogenetic analysis of 16S recombinant DNA sequences showed that *Leuconostoc citreum* generally was acquired by newborns on their first day of life. Case reports of pediatric infection began to appear in the 1980s.[12,27,29,41,60]

PATHOPHYSIOLOGY

Leuconostocs rarely are pathogenic. Underlying disease states or compromised immune system, gastrointestinal tract disease (especially short-gut syndrome), previous or current antibiotic therapy, venous or gastrointestinal tract access devices, recent invasive procedures, and infancy are thought to be risk factors.[14,15,26,44] Frequently, leuconostocs are isolated as part of a polymicrobial infection after a patient has been treated with vancomycin.[16] Of the first 21 cases reported in the English literature, 12 were in children, 10 of which occurred in patients younger than 1 year old. Documented portals of entry include central lines,[3,27] peritoneal dialysis and urinary catheters,[6] and gastrostomy tubes.[29] Contaminated enteral formula also has been implicated.[7,29,39] Occasional sporadic cases without a known risk factor have been described,[12,26] which underscores the pathogenic potential of *Leuconostoc* infection.

CLINICAL MANIFESTATIONS

Bacteremia, heralded by fever and usually leukocytosis in patients with the risk factors outlined earlier, is by far the most common clinical manifestation of *Leuconostoc* infection. Gastrointestinal disturbances, especially diarrhea, are common manifestations.[3] Infants are prone to emesis. *Leuconostoc* bacteremia, in association with cough and chest x-ray findings consistent with pneumonia, was reported in a child with acquired immunodeficiency syndrome (AIDS).[44] *L. citreum* has been isolated from the lung tissue of a 33-year-old patient with AIDS who also had *Pneumocystis carinii* pneumonia.[21] Small necrotic cavities scattered through both lungs were associated with gram-positive cocci in tissue. Patients with dental infections,[60] peritonitis,[22] osteomyelitis,[37] and meningitis[12,19] also have been described. Meningitis has been reported in an otherwise healthy 16-year-old patient and in a neonate with fatal infection despite high cerebrospinal fluid (CSF) bactericidal antibiotic titers.[12,19] A nosocomial cluster of five cases of urinary tract infection caused by *Leuconostoc pseudomesenteroides* (strictly a species of a new genus, *Weissella*)[11] has been reported,[6] as has a cluster of three cases of *Leuconostoc* septicemia in critically ill postsurgical patients.[51]

DIAGNOSIS

Cultures usually are positive within 24 to 48 hours. Leuconostocs are somewhat fastidious. Identification of vancomycin-resistant *Streptococcus* should raise suspicion of *Leuconostoc* infection and prompt additional biochemical studies,[44] including evaluation for the production of gas in MRS broth, failure to hydrolyze arginine, and delayed esculin hydrolysis.

TREATMENT

Treatment with relatively high doses of penicillin or ampicillin frequently is successful in eradicating infection. When possible, access devices should be removed. Leuconostocs are most sensitive to the primitive β-lactam antibiotics, especially penicillin and ampicillin, although penicillin tolerance is a common finding.[29,57] They also frequently are sensitive to erythromycin, cephalothin, and the aminoglycosides. *Leuconostoc* spp. are variably resistant to clindamycin and trimethoprim-sulfamethoxazole. Resistance increases with later generations of cephalosporins.[26,57] *Leuconostoc* spp. are intrinsically vancomycin-resistant because their pentapeptide cell wall precursors end in D-Ala-lactate rather than the usual D-Ala-alanine.[25] Vancomycin cannot bind the lactate. This mode of resistance is the same as that possessed by vancomycin-resistant enterococci. However, whereas resistance in enterococci is plasmid derived and transferable, in leuconostocs it is chromosomally mediated, constitutional, and not transferable.

PEDIOCOCCUS SPECIES

BACTERIOLOGY

Pediococci also are intrinsically vancomycin-resistant, facultatively anaerobic gram-positive cocci. They appear most characteristically in tetrads on Gram stain, although they may appear in pairs or clusters.[23] The genus name *Pediococcus* is derived from the Greek word *pedium*, which means "plane." The name, therefore, suggests that they are a genus of cocci that grow in a single plane. It is a misnomer, however, because pediococci are the only lactic acid bacteria that divide in two planes.[20] They are catalase- and oxidase-negative. They do not reduce nitrates, and no gas is produced in MRS broth.[53] They are pyrrolidone carboxylyl peptidase–negative.[16] Most isolates react with Lancefield group D streptococcal antibodies.[53] They are leucine aminopeptidase–positive, which distinguishes them from *Leuconostoc* spp.[17] They produce white, opaque, nonhemolytic colonies on sheep's blood agar. A new broth medium, a combination of 90 percent Iso-Sensitest broth and 10 percent deMan-Rogosa-Sharpe broth and called LAB susceptibility test medium, has been found to provide optimal support of growth and to yield the most accurate minimal inhibitory concentration values.[33]

Pediococci produce powerful bacteriocins, which are substances that kill other bacteria. The bacteriocins of pediococci are active against other gram-positive organisms in particular[10] but also are active against *Clostridium botulinum* spores,[40] some gram-negative organisms,[54] and *Listeria monocytogenes*. Reports have suggested that some species of *Pediococcus* have antifungal activity[35]; however, this activity in other studies has been found to be present only at low pH and has been attributed to acetic acid in the medium.[5]

EPIDEMIOLOGY

Like leuconostocs and other lactic acid bacteria, pediococci are found on plants, in dairy products, and in alcohol-containing

beverages, where certain species are associated with spoilage.[58] They also are used in the formation of silage[53] and as starter cultures for some meat products. They are not thought to be part of the normal flora. They have been isolated from saliva and stool on rare occasion.[49] Though formerly thought to be nonpathogenic, they now are considered rare opportunistic pathogens with minimal virulence.

PATHOPHYSIOLOGY

Pediococci rarely are pathogenic. Many cases of blood isolates are found in patients without symptoms of infection or in polymicrobial cultures in which the significance of the isolate could not be assessed adequately.[36] One patient with a clinical picture of septic shock in whom the only organism recovered was *Pediococcus pentosaceus* has been reported.[13] Risk factors for bacteremia, with or without symptoms, appear to be the extremes of age, recent abdominal surgery or tube feeding, broad-spectrum antimicrobial therapy, and the presence of severe underlying disease states.[36] However, because the overall number of reported cases is small, the relative risks of these factors are uncertain.

CLINICAL MANIFESTATIONS AND DIAGNOSIS

Most patients either are asymptomatic or have fever as the only symptom. Six of the first 12 reported cases of *Pediococcus* bacteremia had concomitant pneumonia. Fifty-six percent of adult patients either were receiving tube feeding or had undergone abdominal surgery within 30 days of isolation of the organism.[36] The three reported pediatric cases occurred in infants, and all had underlying gastrointestinal tract anomalies. A 16-day-old infant with congenital jejunoileal atresia had undergone surgical repair just 8 days before being evaluated.[36] Her acute illness was characterized by emesis and a 200-g weight loss. She was afebrile when initially examined. The second patient was a 64-day-old infant with gastroschisis who had undergone two abdominal surgical procedures.[1] His fever reached 101.5° F, and his peripheral white blood cell count was 38,000 with a significant left shift. The third patient was a 3-month-old girl who had undergone surgical repair of gastroschisis on the first day of life.[2] Lethargy, direct hyperbilirubinemia, and a CSF pleocytosis were noted. Blood cultures grew *Pediococcus* spp., and the spinal fluid (obtained after initiation of antibiotics) was sterile. She responded to a 21-day course of ampicillin and gentamicin.

Pediococci are isolated frequently in localized infections, especially from abdominal sites, but they are virtually always part of a polymicrobial process. The relative importance of pediococci in these sites is difficult to assess. A case of *Pediococcus* bacteremic pneumonia was reported in a previously healthy pregnant woman.[50] Among 31 cases of *Pediococcus* infection submitted to the Centers for Disease Control and Prevention laboratory, 17 were from blood culture isolates (including 2 reported cases of endocarditis), and 4 strains were associated with urinary tract infections. Other sources included catheter tips, wounds, peritoneal fluid, CSF, lung, and bone.[16]

Diagnosis of infection with pediococci is established by identifying catalase-negative, vancomycin-resistant, gram-positive cocci in the characteristic tetrads. Many pediococci are misidentified initially as *Streptococcus equinus*, *Streptococcus constellatus*, or group D *Streptococcus*, not *Enterococcus*. Reported cases of pediococcal infection may, therefore, represent only a fraction of the total number of infections.

TREATMENT

Pediococci generally are susceptible to penicillin, ampicillin, imipenem, clindamycin, and first- and second-generation cephalosporins. Although both imipenem and penicillin are highly active against pediococci, they do not appear to be bactericidal. Pediococci are moderately resistant to the quinolones, tetracycline,[58] and trimethoprim-sulfamethoxazole.[47] Pediococci, like leuconostocs, are intrinsically resistant to vancomycin. Resistance is not plasmid-mediated, nor can it be transferred to other bacteria.[58] Sensitivity to ticarcillin and cefotaxime, when measured by agar dilution, is poor despite large zones of inhibition on disk susceptibility testing.[58] Occasionally, inducible resistance to erythromycin is found, although most isolates remain sensitive. Aminoglycoside sensitivity is variable.

AEROCOCCUS SPECIES

BACTERIOLOGY

The genus *Aerococcus* contains at least five species, but only two, *viridans* and *urinae*, have known clinical significance. In earlier papers, *A. urinae* was referred to as *Aerococcus*-like organism, or ALO.[9] Aerococci are catalase-negative, nonmotile, gram-positive cocci that appear preferentially in tetrads but sometimes in pairs or clusters. These relatively slow-growing organisms produce small, well-delineated, translucent, alpha-hemolytic colonies on blood agar.[8] They also are weakly bile esculin–positive and pyrrolidone carboxylyl peptidase–negative. They ferment mannose and mannitol.[45] Like enterococci, most aerococci will grow in 6.5 percent salt.[34]

EPIDEMIOLOGY

Aerococci are distributed throughout the world and are contaminants of air and dust.[28] They also have been found on meat, on raw vegetables, and in small numbers on human skin.[4] In hospitals, aerococci have been cultured from all areas, including operating suites and delivery rooms.[31] They also are found in salt water, where they cause a fatal disease in lobsters.[42]

Disease in humans is an uncommon event, and the organism usually is recovered from the bloodstream of patients with infective endocarditis. Most patients are elderly, but infection of infants and neonates also has been reported.[38,42] A rapidly fatal bacteremia has been described in patients with profound neutropenia.[32]

PATHOPHYSIOLOGY

In most circumstances, aerococci are saprophytic. The exact conditions that favor the development of infection have not been elucidated clearly. Some cases of *A. urinae* infection have occurred after genitourinary tract surgery. One case of septic arthritis developed after an elective abortion.[59] Immunocompromised patients are at higher risk, but infection in otherwise well persons has been described.[45] One report of meningitis in newborns found adherence of aerococci to inflammatory cells and suggested a role of as-yet-undefined adhesion factors.[38] Work in laboratory animals also has shown that a protease isolated from *A. viridans* cleaves the hemagglutinin of influenza virus and potentiates both viral replication and disease in mice.[52]

CLINICAL MANIFESTATIONS

Most cases of *A. viridans* bacteremia have been found in association with signs and symptoms of subacute infective endocarditis, although septic emboli and cardiac failure have not been described. In a recent series of four cases of *A. viridans* infective endocarditis, two of the patients required surgical intervention.[46] *A. urinae* causes urinary tract infection with dysuria and frequency, usually in the absence of fever.[8] *Aerococcus* infection in childhood is an uncommon occurrence. One case of bacteremia in a 1-month-old infant has been reported.[42] The patient had a 2-day history of loose stools, irritability, and drowsiness and on physical examination was noted to have mottled skin, circumoral cyanosis, and agitation. CSF and urine were normal, but blood cultures exhibited pure growth of *A. viridans*. No predisposing factors were identified.

Nathavitharana and associates[38] reported three cases of meningitis caused by *A. viridans*: a 7-month-old girl with jerking movements of the extremities, irritability, and a bulging fontanelle; a 5-month-old girl with fever, decreased appetite, and generalized convulsions; and a 24-month-old girl whose illness was manifested as fever, vomiting, and the neurologic signs of truncal ataxia, flaccidity, and hypoactive deep-tendon reflexes. All three patients had elevated CSF white blood cell counts with 55 to 93 percent segmented forms. Remarkably, all these patients had a history of prolonged illness (1 week to 2 months) before being evaluated, in contrast to patients with other causes of bacterial meningitis. No risk factors for infection were identified in any of these patients, who were thought to have normal immune function.

Swanson and colleagues[56] reported a case of penicillin-resistant *A. viridans* bacteremia in an 11-month-old girl receiving prophylactic penicillin for sickle-cell disease. The patient responded clinically to a 10-day course of therapy with cephalosporins.

Bone and joint infections and wound or other localized infections are exceedingly rare occurrences and not distinguishable from similar syndromes caused by more common organisms.

DIAGNOSIS

Careful observation of both appearance on Gram stain and growth in culture is key to establishing the diagnosis of aerococcal infection. On Gram stain, aerococci resemble staphylococci. On blood agar, they resemble viridans group streptococci. One series revealed that 1 percent of 719 cultures called streptococci actually were aerococci[43]; another study reclassified 3 percent of 168 cultures.[4] In one study of 24 alpha-hemolytic, nonenterococcal bacterial isolates from urine, standard identification methods were compared with 16S rRNA sequencing and special biochemical profiling. Thirteen of the isolates were aerococci; all were identified correctly by sequencing, but typical procedures failed to differentiate them from nonaerococcal isolates. Ciprofloxacin and trimethoprim susceptibility and the ability to grow at 45° C improved the discrimination of standard testing.[24] In their propensity toward tetrad formation, they mimic pediococci. However, all aerococci are vancomycin-sensitive. In their bile esculin hydrolysis and growth in 6.5 percent salt, they resemble enterococci; unlike enterococci, however, aerococci are pyrrolidone carboxylyl peptidase–negative and do not form chains.

TREATMENT

Aerococci generally are sensitive to penicillin, ampicillin, the cephalosporins, chloramphenicol, and the macrolides. They usually are intermediately susceptible or resistant to sulfonamides and aminoglycosides.[4,28] One report suggests that *A. viridans* and *A. urinae* have distinct antibiograms[34]; that is, *A. urinae* is more sensitive to penicillin and resistant to sulfonamides, whereas *A. viridans* is more resistant to penicillin and sensitive to the sulfonamides.[4] Individual case reports do not confirm these in vitro observations, and no large clinical trials of antimicrobial susceptibility have been conducted.

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SUBSECTION 2

Gram-Negative Cocci

CHAPTER 98

MORAXELLA CATARRHALIS

Barbara W. Stechenberg

Once thought to be an unimportant commensal organism in the human respiratory tract, *Moraxella catarrhalis* now is recognized as an important pathogen in respiratory tract diseases, particularly otitis, sinusitis, and lower tract disease. The initial paper by Ghon and Pfeiffer[25] published in 1902 described gram-negative cocci in sputum referred to as *Micrococcus catarrhalis*. Since then, the organism has undergone several changes in nomenclature, first to *Neisseria catarrhalis* because of its resemblance to other *Neisseria* organisms. In 1970, in recognition of the contributions of Sarah Branham, the name was changed to *Branhamella catarrhalis*.[11] More recently, that this organism should be a member of the genus *Moraxella* has become clear; thus, the name was changed to *M. catarrhalis*. With the transition in nomenclature, the last 2 decades have seen a parallel rebirth of this organism as a mucosal pathogen.

MICROBIOLOGY

M. catarrhalis is a gram-negative diplococcus that is morphologically indistinguishable from *Neisseria*. It has a tendency to resist decolorizing.[1] The organism is kidney-shaped, with the flat sides abutting each other. The size varies, but it may be larger than meningococcus or gonococcus. However, the resemblance on sputum Gram stain or conjunctival smear to gonococcus can be striking and clinically confusing.

The organism grows well on blood or chocolate agar and forms small, opaque, grayish colonies that are circular and nonhemolytic. They have poor adhesion to the agar surface, on which they act like hockey pucks when pushed over the surface of the agar plate.[17] The use of selective media such as modified Thayer-Martin or TV broth (Mueller-Hinton broth supplemented with trimethoprim and vancomycin) increases the likelihood of recovering *M. catarrhalis* from the complex flora of the mucosal surfaces.

Isolates of *M. catarrhalis* cannot use maltose, glucose, lactose, or sucrose as carbohydrate sources. *M. catarrhalis* is oxidase-positive and produces deoxyribonuclease. Valuable differentiating tests are hydrolysis of DNA and tributyrin. Several rapid tests such as butyrate hydrolysis, Tween 80 hydrolysis, and selective DNase agar have been described.[50,57] Fatty-acid analysis also has been used to identify atypical strains.

The surface of *M. catarrhalis* is composed of outer-membrane proteins, lipo-oligosaccharide, and pili. The organism does not appear to have a capsule, and the role of the pili is not defined well. Initial attempts to isolate outer-membrane proteins by detergent fractionation of cell envelopes were unsuccessful. By using a technique involving the collection of outer-membrane vesicles, which are released into the culture media, and then examination by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE), Bartos and Murphy[6] identified eight proteins designated outer-membrane proteins A through H. The outer-membrane protein patterns of strains from diverse geographic and clinical sources were strikingly homogeneous.

Preliminary studies of the lipo-oligosaccharide of *M. catarrhalis* show that it is more antigenically conserved than are those of other gram-negative bacteria, thus rendering it a reasonable candidate for vaccine studies.[39,53] A recent study suggests that the lipo-oligosaccharide is not an adhesin, but its surface charge plays a critical role in the initial stage of attachment to human epithelial cells.[2]

Restriction endonuclease analysis has been used as an epidemiologic tool to distinguish strains of *M. catarrhalis*. Patterson and associates[42] used the technique to evaluate a nosocomial outbreak and to demonstrate the lack of association of other strains. Dickinson and coworkers[16] used a similar technique to study isolates from children with otitis media.

PATHOGENESIS

The ability of *M. catarrhalis* to produce disease, particularly in sequestered areas such as the ear and lung, indicates that this organism possesses virulence mechanisms that allow it not only to grow in these anatomic sites but also to produce pathologic effects. The presence of endotoxin in the *M. catarrhalis* outer membrane undoubtedly is important for its pathogenic potential, especially in situations in which inflammation plays a major role. Very little is known about the mechanisms involved in colonization and disease production. However, a subset of strains of *M. catarrhalis* associated with selected virulence traits suggests that some strains are more virulent than are others.[9,56]

A body of literature concerning the immune response to *M. catarrhalis* and the antigenic composition of this organism is accumulating rapidly.[12,13,34] Goldblatt and associates[26] demonstrated that children younger than 4 years old possess IgG1 and IgG2 that recognize an 82-kd outer-membrane protein exclusively but that older children mount an IgG3 response to a broad range of outer-membrane proteins. Faden and colleagues[23] developed a technique to measure opsonic antibody with the use of outer-membrane, antigen-coated beads. Convalescent sera opsonized homologous, antigen-coated sera significantly more often than acute sera did.

Unhanand and coworkers[52] used a murine model to study pulmonary clearance of *M. catarrhalis* from infected lungs. They investigated 10 strains and found marked variability in clearance rates and recruitment of phagocytic cells. With this model, Maciver and associates[36] actively immunized animals with the outer-membrane vesicles of *M. catarrhalis* and passively immunized animals with rabbit antiserum raised against these vesicles. Both experiments resulted in enhanced pulmonary clearance of both homologous and heterologous strains. The same model has been used to evaluate the role of a large, antigenically conserved protein of *M. catarrhalis* in pulmonary clearance, as well as the role of outer-membrane protein B in defense mechanisms of the lung.[30] The contribution of these proteins, as well as lipo-oligosaccharide, in host defense continues to unfold.

EPIDEMIOLOGY

M. catarrhalis is a normal inhabitant of the upper respiratory tract and is recovered exclusively from humans. The rate of colonization is highest in the early years and then declines steadily to less than 5 percent in adult life.[54] Prevalence studies report that as many as 50 percent of children are colonized with *M. catarrhalis*.[32,37] Faden and associates[22] monitored a large cohort of children from birth to 2 years of age. Sixty-six percent became colonized by the time that they reached 1 year of age, and 77.5 percent did so by 2 years of age. The frequency of nasopharyngeal colonization was higher during visits for otitis media than during well-child visits. Otitis-prone children were colonized by 6 months of age. Restriction endonuclease analysis showed marked heterogeneity, with children acquiring and eliminating many different strains over time.[22,42]

Numerous studies have documented the increased prevalence of *M. catarrhalis* as a pathogen in otitis media.[32,55] Shurin and colleagues[48] noted that isolation of *M. catarrhalis* from middle ear exudates increased from 6.4 percent between 1979 and 1980 to 26.5 percent between 1980 and 1982. A similar increase in *M. catarrhalis* as a pathogen in sinusitis has been reported.[58] In the pneumococcal conjugate vaccine (PCV7) era, a significantly higher proportion of PCV7-immunized children exhibit nasopharyngeal colonization with *M. catarrhalis*.[44]

Once colonization of the oropharynx occurs, colonization of the tracheobronchial tree may follow, but usually only if other risk factors are operative. In adults, such risk factors include previous cardiorespiratory disease, smoking, use of corticosteroids, use of immunosuppressive agents, malignancy, and intercurrent viral illnesses.[28,57] In children, many cases of pneumonia are associated with a preceding viral illness, prematurity, underlying lung disease, IgG deficiency, or other risk factors.

Nosocomial transmission has been well documented by DNA restriction endonuclease analysis.[14,42] In pediatric intensive care units, endotracheal intubation and frequent suctioning have been identified as risk factors for the development of pneumonia and bacterial tracheitis.[14,21]

Other interesting associations need to be investigated further. Seddon and associates[47] noted that colonization with *M. catarrhalis* occurred more commonly in asthmatic than in normal children. Whether the organism might be a trigger or pathogen in these children remains to be seen. Gottfarb and Brauner[27] found

a high carriage rate of *M. catarrhalis* in children with severe persistent cough.

Infection with *M. catarrhalis* exhibits seasonality in both adults and children.[28,48,55] Infection is much more likely to occur in the winter and spring months.

CLINICAL MANIFESTATIONS AND DIAGNOSIS

Otitis media caused by *M. catarrhalis* is indistinguishable clinically from otitis caused by other pathogens such as *Streptococcus pneumoniae* and *Haemophilus influenzae*. *M. catarrhalis* may be isolated as a single agent or in combination with other organisms. In one study, children with *M. catarrhalis* were less likely to have a serum C-reactive protein level greater than 1.0 mg/dL than were children with either of the other two major pathogens.[55]

Because tympanocentesis usually is not performed in routine cases, the exact cause of an episode of acute otitis media generally is not known. One study found that *Moraxella* was an unusual organism as a single agent in children in whom acute otitis media had been treated recently.[29] Nonetheless, in a study of persistent otitis, Pichichero and Pichichero[43] demonstrated *Moraxella* in 7 percent of specimens. The spontaneous resolution rate of *M. catarrhalis* may be high,[4] and this fact should be considered when therapy is planned.

The clinical manifestations of acute sinusitis caused by *M. catarrhalis* are similar to those of other organisms; therefore, selection of antibiotics should be made with consideration of this organism as a potential pathogen.

Lower respiratory tract disease caused by *M. catarrhalis* may have a broad clinical spectrum; however, because sputum samples often are not available in children, many cases documented in the literature have been severe. Berg and Bartley[7] described five premature infants, all younger than 6 months old, in whom precipitous clinical deterioration developed after 2 to 4 days of a prodrome consisting of cough, tachypnea, and intercostal retractions. They all required assisted ventilation, which is common in young infants with severe pneumonia.[7,14,20] Some cases of *M. catarrhalis* pneumonia have been associated with bacteremia.

Underlying conditions such as leukemia, acquired immunodeficiency syndrome, and trauma have been reported to predispose a patient to the development of infection with *M. catarrhalis*.[37,59] An association with immunoglobulin deficiency has been demonstrated in some patients with disease caused by this organism.[12] In adults, *M. catarrhalis* pneumonia occurs more commonly in patients with human immunodeficiency virus infection, malignancy, or chronic lung problems.[26,57] The role of this organism in less severe manifestations probably is limited.[33]

Bacterial tracheitis caused by *M. catarrhalis* has been reported in both immunocompromised hosts and those with normal immune systems.[21]

M. catarrhalis also has been responsible for a wide variety of other infections. Urethritis caused by this organism can be mistaken for infection with *Neisseria gonorrhoeae*.[49] Several cases of conjunctivitis have been reported; when present in the newborn period, *M. catarrhalis* can mimic the ophthalmia neonatorum of *N. gonorrhoeae*.[51] The relationship of neonatal infections to maternal vaginal carriage of *Moraxella* has not been established.[41]

More severe infections with *M. catarrhalis* include meningitis and bacteremia, so this organism should be considered in appropriate circumstances, especially in immunocompromised children.

Children with *M. catarrhalis* bacteremia may have many different manifestations. Some patients have petechial or purpuric rashes, thus rendering the clinical picture indistinguishable from that of meningococcemia. Others have been neutropenic and have required mechanical ventilation.[8] Baron and Shapiro[5] reported two cases of unsuspected bacteremia in children with nonspecific symptoms. Their manifestations were similar to those of children with occult pneumococcal bacteremia. An immunocompetent child with *M. catarrhalis* bacteremia and preseptal cellulitis has been reported.[46]

Meningitis can occur from hematogenous spread of *M. catarrhalis* from the nasopharynx or as a consequence of ventriculoperitoneal shunt placement or surgery. Rarely, suppurative arthritis has been seen in children and adults.[31,38] Endocarditis is a rare occurrence, as is peritonitis.

TREATMENT

In the past, *M. catarrhalis* was susceptible to all β-lactam antibiotics. In the late 1970s, however, β-lactamase–producing strains of *M. catarrhalis* were isolated in Europe. In the United States, a parallel increase in the frequency of isolation of β-lactamase–producing *M. catarrhalis* has been noted, so it is not unusual for a laboratory to report that more than 90 percent of strains are β-lactamase producers.

The β-lactamases BRO-1, BRO-2, and BRO-3 are of chromosomal origin. Laboratory detection of β-lactamase activity depends on the assay used; the chromogenic cephalosporin nitrocefin usually is recommended.[19] Polymerase chain reaction methods can be used. BRO-1 is the most common β-lactamase in North American strains.[15] The β-lactamase inhibitors clavulanic acid, sulbactam, and tazobactam are active against the enzymes produced by *Moraxella*. Most β-lactamase–positive isolates will respond to achievable concentrations of β-lactam/β-lactamase inhibitor antimicrobial combinations, cephalosporins, and cephamycins.[18,24] Because of the high prevalence of β-lactamase–producing strains, many laboratories choose not to do any such testing.

Among the oral cephalosporins active against β-lactamase–positive strains of *Moraxella*, the minimal inhibitory concentration increases twofold to fourfold in the presence of the β-lactamase BRO-1 but not the β-lactamase BRO-2; cefixime is more active than are the older cephalosporins.[40] Resistance to tetracycline (by the nontransferable tetB determinant) and erythromycin has been reported, but such resistance is a rare finding.[10,45]

In vitro, *M. catarrhalis* usually is susceptible to ampicillin-sulbactam, amoxicillin-clavulanate, erythromycin, azithromycin, clarithromycin, trimethoprim-sulfamethoxazole, tetracyclines, chloramphenicol, fluoroquinolones (e.g., ciprofloxacin), aminoglycosides, and second- or third-generation cephalosporins such as cefuroxime, cefprozil, cefpodoxime, cefixime, cefdinir, and loracarbef.[35] Levofloxacin also exhibits good activity against *M. catarrhalis*.[3] The *Moraxella* organism is resistant to clindamycin, vancomycin, and oxacillin.

PREVENTION

Prevention of nosocomial *M. catarrhalis* infection is dependent on sound practices of infection control, especially with regard to pulmonary toilet. Prevention of other infections with this organism has not been attempted, except for the use of prophylactic antibiotics for recurrent otitis media.

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CHAPTER 99

Meningococcal Infections

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Epidemic meningococcal meningitis was described first by Gaspard Vieusseux in Geneva in the spring of 1805. In a monograph titled "The Disease Which Raged During the Spring of 1805," he wrote:

> It commences suddenly with prostration of strength, often extreme: the face is distorted, the pulse feeble. There appears a violent pain in the head, especially over the forehead; then there comes pain of the heart or vomiting of greenish material, stiffness of the spine, and in infants, convulsions. In cases which were fatal, loss of consciousness occurred. The course of the disease is very rapid, termination by death or by cure. In most of the patients who died in 24 hours or a little after, the body is covered with purple spots at the moment of death or very little time afterward.[200]

Today, *Neisseria meningitidis* continues to be a cause of endemic and epidemic disease. In the United States, approximately 1400 to 2800 cases occur each year, and 95 to 97 percent of these cases are sporadic.[10,170] In 2005 and 2006, 1904 and 1742 U.S. cases were reported to the Centers for Disease Control and Prevention (CDC), respectively.[35] However, epidemics in Brazil in the early 1970s and in Finland in 1975 serve as a reminder of the potential virulence of this organism. In São Paulo, Brazil, invasive meningococcal disease developed in almost 1 in every 300 inhabitants during the epidemic in a 1-year period.[49]

The reader is referred to several excellent reviews of meningococcal disease that have been published recently.[72,111,171]

MICROBIOLOGY

The *Neisseria* genus was named for Dr. Albert Neisser, who described the organism of gonorrhea in 1879. The genus *Neisseria* contains two species important in human disease, *Neisseria gonorrhoeae* and *N. meningitidis*. In addition, several species are part of the normal flora of humans and animals. *Neisseria sicca*, *Neisseria lactamica*, *Neisseria subflava*, *Neisseria flavescens*, *Neisseria mucosa*, *Neisseria cinera*, *Neisseria polysacchreae*, and *Neisseria elongate* are human commensals; they only occasionally cause human disease.[121]

N. meningitidis is a gram-negative coccus, usually less than 1 μm in diameter. It classically occurs in pairs, with adjacent sides flattened, similar to kidney beans. Occasionally, the organism divides into two planes at right angles to one another, which results in the formation of tetrads. The organisms are nonmotile and aerobic (but facultatively anaerobic), produce catalase and oxidase, and may be encapsulated. *N. meningitidis* oxidizes glucose and maltose to acid. This feature distinguishes it from *N. gonorrhoeae*, which oxidizes only glucose. However, certain rare strains of *N. meningitidis* have been reported that fail to produce acid from either glucose or maltose. Fresh isolates have complex nutritional requirements and grow best on chocolate or blood agar. Incubation in a humidified 10 percent carbon dioxide environment is not essential but enhances growth. Morphologically, the colonies are bluish gray in appearance and will produce beta-hemolysis after 48 to 72 hours of incubation on 5 percent horse blood agar.[24]

N. meningitidis can be transformed by DNA originating in other species of *Neisseria*. Thus, when a *Neisseria* spp. cohabiting the nasopharynx with *N. meningitidis* dies and releases its DNA, the co-resident meningococcus can take up the DNA and incorporate it into its genome. Although this sequence of events occurs very rarely, if the new DNA confers a selective advantage to the recipient meningococcus, the trait encoded by the incoming DNA may be retained in future progeny. This is the mechanism by which sulfonamide resistance and penicillin G resistance have arisen in *N. meningitidis*.[121] Many commensal *Neisseria*, which also inhabit the nasopharynx, are relatively resistant to penicillin G. DNA fragments from these commensal strains that encode penicillin resistance can be detected in penicillin-resistant *N. meningitidis*.[173] Transformation of new DNA into *N. meningitidis* also can introduce genes that modify the structure of the bacterium. Serotyping of groups A, B, and C meningococci has provided valuable macro-epidemiologic information.[71] Clearly, as a population develops immunity to one capsular polysaccharide, the organism is capable of acquiring DNA encoding for an alternative capsule. Switching from serogroup B to serogroup C has been observed in a population[188] and a contact of a case.[201] There also appears to be horizontal gene transfer from commensal *Neisseria* to *N. meningitidis* of genes whose products aid the bacterium in surviving in the host or are involved in housekeeping functions.[119] This phenomenon probably is responsible for the occasional report of commensal *Neisseria* causing meningitis.[6] In one case, the strain was a virulent meningococcus that acquired a gene for the synthesis of polysaccharide from sucrose; this additional property led it to be identified as *N. subflava* rather than *N. meningitidis*.

In addition to having the transformation route for varying the antigenic phenotype, meningococci also may exhibit phase variation, which allows variance of the number of surface antigens. Investigators have estimated that at least 65 genes in *N. meningitidis*, including *por*A, are subject to phase variation.[64,194] Capsule synthesis may be switched on and off by this mechanism.

Like other gram-negative bacteria, the outer and inner cell membranes of *Neisseria* are phospholipid bilayers. These membranes sandwich a layer of peptidoglycan. Lipo-oligosaccharide (LOS), which is similar to the lipopolysaccharide seen in gram-negative enteric bacteria, is associated with the outer leaflet of the outer cell membrane. Outer-membrane proteins, which function as porins, are an integral part of the outer membrane. Some of these outer-membrane proteins are opacity proteins and facilitate adherence and invasion.[138] Outside the outer membrane, meningococci possess a polysaccharide capsule that protects the organism from phagocytosis.

Meningococci also have pili, which seem to be important in some phases of adherence to host cells, colonization, and invasion. The genes that code for pili also can be turned on and off, so at times the organism may not express pili. This feature may help the meningococci detach and allow the organism to be transmitted to another site or another host.[87] Antigenic variation of pili by a cassette mechanism allows the bacterium to escape the host's immune system.[82] In addition, both outer-membrane proteins and lipo-oligopolysaccharide display antigenic differences by phase variation.

For epidemiologic purposes, meningococci are divided into serogroups, serotypes, and subtypes. Differences in capsular polysaccharides, outer-membrane proteins, and LOS constitute the basis for these classifications. The capsular polysaccharides are antigenic and the basis for serogroup designation. Thirteen

serogroups currently are recognized: A, B, C, D, H, I, K, L, X, Y, Z, W-135, and 29E.[209] Serogroups A, B, C, Y, and W-135 are the most common causes of invasive disease worldwide.

Serotyping of organisms is based on antigenic differences in outer-membrane proteins called *porins*. Porins are protein channels in the outer membrane that permit nutrients and certain antibiotics to diffuse into the periplasmic space. A single locus encodes porin B, but two mutually exclusive alleles have antigenic differences. All meningococci have either antigenic class 2 or class 3 porin B. These differences permit serotyping. Differentiation of subtypes is based on antigenic differences in porin A. This highly variable antigen is called a class 1 outer-membrane protein. Multiple serotypes and subtypes have been identified. Antigenic specificity is achieved by the generation of monoclonal antibodies to the major protein (primarily class 2 or 3), LOS, and class 1 protein. The designation B:2a:P1.1:L3,7 indicates that the serogroup B strain is an "a" subtype of class 2 proteins, is a "one" of class 1 protein, and possesses two LOS antigens: 3 and 7.[77]

Type 2 protein antigen is isolated from more than 50 percent of cases in the United States and Canada and is an antigenic determinant in group B and C strains. These protein antigens not only are important as epidemiologic markers but also can induce protective bactericidal antibodies.[70]

EPIDEMIOLOGY

The incidence of meningococcal disease varies significantly by geographic location. Endemic disease, which occurs in developed countries such as the United States and Europe, ranges in incidence from 0.35 to 3.0 cases per 100,000 population per year.[30-32,170] The U.S. Active Bacterial Core Surveillance (ABC) system tracks invasive disease caused by encapsulated organisms in selected counties of 10 states geographically dispersed throughout the country. This reporting system covers a U.S. population of 39 million people. In 2005, the overall incidence of invasive meningococcal disease for the United States was estimated to be 0.35 per 100,000 population. Fifty-six percent of cases reported through the ABC system were meningitis with a fatality rate of 1.1 percent, and 31 percent of cases were bacteremia with a fatality rate of 15.1 percent. The rate of invasive disease was the same for African Americans and whites.[32]

In developing countries, the incidence is approximately 10 to 20 times higher than that in the United States (10-25 per 100,000 inhabitants per year).[80] The highest rate of meningococcal disease is found in a multicountry belt across sub-Saharan Africa, which has been termed the *meningitis belt*. Serogroup A meningococcal disease is prevalent there and occurs in epidemics during the dry season.[128] During epidemics, the incidence of meningococcal disease is as high as 1000 per 100,000 inhabitants (1%). Spread of clonal strains of group A meningococcus may be a factor in Africa's unusually high rate of epidemic disease. Clonal strains can migrate transcontinentally and cause epidemics, such as the clonal group A meningococcus designated electrophoretic type III-1 (ET III-1), which caused epidemics in China, Nepal, Saudi Arabia, Chad, and Kenya in the 1980s.[133,152]

International outbreaks of *N. meningitidis* infection occurred in 1987 and 2000 and were associated with Muslims making the annual pilgrimage to Mecca. The disease spread to persons of all nationalities represented, some of whom returned home before becoming ill.[133,156] In some cases, close contacts of travelers were infected. Several cases in U.S. citizens were attributed to the outbreaks. The 1987 outbreak was linked to serogroup A, whereas the 2000 outbreak was caused by serogroup W-135.[28,131,156] Saudi Arabia now requires meningococcal vaccine for travelers entering the country to participate in the annual pilgrimage to Mecca.[107]

Serogroups A, B, C, and Y account for more than 90 percent of meningococcal disease worldwide. Currently in the United States, serogroups B, C, and Y each accounts for approximately a third of the cases.[21,31] Serogroup A, which causes periodic epidemics in developing countries, is responsible for only occasional cases of meningococcal disease in the United States. Serogroup B usually causes sporadic disease but occasionally is associated with outbreaks.[29] Serogroup C, though also a cause of sporadic disease, has been associated with numerous outbreaks in the United States, Canada, and Europe.[96] In addition, school-related outbreaks of serogroup C meningococcal disease have been reported.[21,134,214] The incidence of serogroup C and serogroup Y disease has increased in the United States during the last few years as a result of antigenic shifts involving outer-membrane protein genes.[86] Disease caused by serogroup W-135 occurs infrequently and, like serogroup Y, has been associated with meningococcal pneumonia.[171,203] Recently, serogroup X has been identified as a predominant serogroup causing epidemic meningitis in Niger, with a case-fatality rate of 12.2 percent.[14]

In 2006, Brooks and colleagues reported on an analysis of 69 outbreaks of meningococcal disease that occurred in the United States from 1992 to 2002. Outbreak-associated cases represented less than 2 percent of the total cases of invasive disease. Additionally, outbreak-related cases were associated with a higher case-fatality rate than sporadic cases were (21% versus 11%; odds ratio, 3.3; 95% confidence interval, 2.0 to 5.5).[21]

Meningococcal disease in the United States peaks during the months November through March.[168,171] The highest attack rate is seen in February, and the lowest attack rate occurs in September. In Africa, epidemic disease occurs during the dry season and decreases once the rainy season starts.[152] Males and females are affected equally.

The risk of acquiring meningococcal disease is related inversely to age, with the highest rates of disease occurring in children younger than 1 year old.[31] As many as 49 percent of cases occur in children younger than 2 years old.[95] However, in epidemics, an age shift occurs, with older children, adolescents, and young adults more often being affected.[29] A progressive increase in the development of protective antibodies against meningococci occurs between the ages of 2 and 12 years. Neonates usually are protected by passive IgG transfer in utero if the mother has antimeningococcal antibody. In general, the development of bactericidal antibodies to meningococci increases in children at a rate of approximately 5 percent per year.[77]

The precise epidemiology of meningococcal disease was defined elegantly in a series of observations in New Jersey military recruits. An inverse correlation was noted between the age-related incidence of meningococcal disease in the United States and the prevalence of serum bactericidal activity against three pathogenic strains of *N. meningitidis*.[77,78] The investigators prospectively studied all incoming recruits to Fort Dix and demonstrated that those who lacked bactericidal activity to case strains had a 38 percent attack rate if they were exposed and acquired a pathogenic meningococcus strain.[77] Usually, nasopharyngeal carriage was an immunizing process. Carriage induced the formation of protective antibodies against homologous strains but also produced cross-reacting antibodies to heterologous strains of pathogenic meningococci. Antibody production typically was seen within 2 weeks of the acquisition of carriage.[77,78]

In addition to nasopharyngeal carriage, other processes may contribute to meningococcal antibody formation. Certain strains of cross-reacting bacteria, such as *Escherichia coli* and *Bacillus*, produce capsular polysaccharides that are immunologically identical to the capsules of meningococci serogroups A, B, and C. They represent a potential immunizing source against invasive meningococcal disease in the general population.[77,102,198]

Nasopharyngeal carriage of *N. meningitidis* is lowest in infants and children and highest in adolescents and young adults. One study determined that 2.4 percent of asymptomatic infants and children had a positive nasopharyngeal culture for *N. meningitidis*

during a nonepidemic period.[123] A study of 1500 Norwegians found the carriage rate to be 32.7 percent in persons 20 to 24 years of age and 10 percent in individuals older than 25.[27] Other investigators have shown that approximately 20 percent of children harbor meningococcal species in their nasopharynx, but most of these isolates are atypical, nontypeable strains that ferment lactose in addition to glucose and maltose.[77,78]

Nasopharyngeal meningococcal carriage in household contacts of persons with documented meningococcal disease is approximately 10 percent.[123,144,164] Several studies have shown that the carriage rate increases if infants or children are in the home.[102,144] In one outbreak of serogroup C meningococcal disease, carriage rates were 37.8 percent in households with a case in an infant, 17.5 percent in households with a case in a child, and 6.9 percent in households with disease in an adult.[102]

Nasopharyngeal carriage is a common finding in those who have had household contact with an index case, and colonization can persist for weeks to months. Without chemoprophylaxis, as many as 35 percent of contacts become colonized by the eighth week. The median duration of carriage is 9 months.[123] However, in nearly 40 percent of individuals, it may exceed 16 months.[79] An association between smoking and an increased rate of meningococcal carriage has been demonstrated.[181,185] In addition, passive smoke has been implicated in increasing the risk for acquiring meningococcal disease in children younger than 5 years old by 7.5 times that of the general population.

The factors responsible for converting nasopharyngeal colonization to invasive disease have not been established firmly. Many people in whom invasive disease subsequently develops are colonized shortly before the illness begins. Probably, these individuals lack bactericidal antibody, and, for some reason, protective antibody does not develop after acquiring the pathogenic strain. Other risk factors associated with invasive disease include young age, crowding (new military recruits, college freshmen in dormitories),[23,127,159] lower socioeconomic class,[170] concurrent upper respiratory infections,[132,213] intimate kissing with multiple partners,[192] specific immune deficiencies (properdin or terminal complement),[65] functional or anatomic asplenia,[69] and active or passive smoking.[67,181]

Speculation regarding the role of other upper respiratory pathogens in meningococcal disease prompted several observations. The peak incidence of meningococcal infection has been noted to mirror the peaks of such agents as influenza and *Mycoplasma*. Several studies have shown an association between colonization or infection with respiratory viruses or *Mycoplasma* and an increased risk for development of meningococcal disease.[26,92,132,176] Simultaneous outbreaks of meningococcal and influenza or echovirus infections have been documented.[117,213] In addition, one study suggested that the incidence of meningococcal disease and the resultant morbidity and mortality increased in the 5 weeks after individuals had influenza-like syndromes.[132] Researchers have postulated that viral pathogens may affect the immune response temporarily and facilitate meningococcal disease. Also possible is that disruption of the normal respiratory epithelium occurs with viral infection and thereby increases the likelihood that colonizing meningococci might become invasive.[132] Preceding viral respiratory disease is not a prerequisite for the establishment of meningococcal carriage or disease; however, its occurrence may increase the risk for invasive meningococcal disease.

A variety of host genetic factors also may influence susceptibility to invasive meningococcal disease. Individuals with late complement component deficiencies (C5, C6, C7, C8, or C9) and properdin pathway deficiencies are at increased risk for acquiring meningococcal disease.[65] This defect usually is inherited, and other members of the family may have a history of meningococcal disease or repeated meningococcal infections. Acquired complement deficiencies associated with diseases such as systemic lupus erythematosus, nephrotic syndrome, and chronic liver disease also are associated with an increased risk of acquiring meningococcal disease.[113] A Russian study found the incidence of complement deficiency in first episodes of meningococcal disease to be approximately 1 percent.[154] In Italy, the prevalence of complement deficiency in patients with meningococcal meningitis was 17 percent.[45] The decision of whom to screen for complement deficiency should be based partly on the prevalence of meningococcal disease in a given country. In countries where the incidence of meningococcal disease is high, complement deficiency is less likely to be found. In countries such as the United States, where the incidence is relatively low, complement screening may be justified.

The chance of finding a complement or alternative pathway (properdin) deficiency substantially increases in patients with recurrent meningococcal disease or with uncommon serogroups. The prevalence of complement or properdin deficiency in a patient infected with an unusual serogroup was found to range from 31 to 50 percent.[66,141] Therefore, screening all individuals with unusual serogroups is reasonable. CH_{50} testing generally is available and will screen for the combined activity of C1 to C9. If complement deficiency is found, the individual should receive the meningococcal vaccine. In addition, family members should be screened for complement deficiency, and all family members should be educated about the disease and immunized with quadrivalent polysaccharide or protein conjugate meningococcal vaccine. Most experts concur that the conjugate vaccine is preferred in the age groups for which the vaccine is licensed (2 to 55 years).

Some investigators have shown that although persons with complement deficiency are more at risk for acquiring meningococcal disease, the disease that they get often is mild. Meningococcal infection activates the complement system, and the LOS present in the bacterial outer membrane probably is responsible for the activation. In fatal cases, intense activation has continued until the time of death.[20] The case-fatality rate in complement-deficient individuals is approximately 3 percent.[172] Complement-deficient individuals are thought to be unable to maintain the high-level activation of the complement pathway and, therefore, have less severe disease.

Properdin deficiency, which impairs activation of the alternative complement pathway, is X-linked and has been associated with fulminant meningococcal disease.[51,66] The case-fatality rate in one kindred was 75 percent for persons with meningococcal disease.[12] Screening of individuals with exceptionally fulminant disease or those who have a family history of meningococcal infection should be considered. AP_{50} will screen for properdin deficiency in the alternative complement pathway. If a deficiency is documented, those individuals and their family members should receive the quadrivalent meningococcal vaccine to prevent disease caused by serogroups of meningococcus in the vaccine (conjugate vaccine is preferred in the age groups for which the vaccine is licensed, 2 to 55 years).

Mannose-binding lectin (MBL) is a pattern recognition receptor and a component of the innate immune system. Patients with meningococcal disease during childhood have a significantly increased incidence of structural mutations in the genes coding for MBL; such mutations lead to deficient MBL function when compared with healthy age-matched controls.[62] Similarly, mutations in genes coding for toll-like receptor 4 also may increase susceptibility to meningococcal disease. Human toll-like receptor 4 mutations are associated with susceptibility to invasive meningococcal disease in infancy.[61]

PATHOLOGY AND PATHOGENESIS

The mechanisms by which meningococci invade humans are understood only partially. We do know that encapsulated, type-

able meningococci are virulent, whereas nonencapsulated strains are relatively nonpathogenic. Even among encapsulated strains, differences in virulence between case and carrier strains of *N. meningitidis* have been demonstrated in an animal model.[91] The presence of a polysaccharide capsule enhances invasiveness by resisting opsonization and subsequent phagocytosis.

The human nasopharynx is the only natural reservoir of *N. meningitidis*.[183] Once pathogenic meningococci have colonized the respiratory tract of susceptible persons, either they become invasive or antibody to the organism develops and confers immunity. Meningococci adhere to nonciliated, columnar epithelial cells in the nasopharynx via pili. Binding induces endocytosis of the organism into the epithelial cell, and the bacteria may penetrate the epithelial barrier via phagocytotic vacuoles.[182] If antibody is insufficient and invasion occurs, the individual may become bacteremic. Occasionally, unsuspected meningococcal bacteremia has been detected on blood culture and spontaneously cleared on a follow-up culture without antibiotics.[186] However, in most cases, such clearance does not occur, and the individual becomes progressively ill. Bacteria in blood may seed the meninges and cause meningitis.

Meningococci are gram-negative organisms, and LOS is a major component of the bacterial cell membrane. Meningococci release blebs from their surfaces that contain outer membrane laden with LOS. LOS is a potent endotoxin, and concentrations of LOS correlate with the severity of disease.[18-20] LOS induces the release of a host of inflammatory and anti-inflammatory mediators whose concentrations also are correlated with the severity of disease: tumor necrosis factor-α (TNF-α), interferon-γ, interleukin-1 (IL-1), IL-6, IL-8, IL-10, and IL-1 receptor antagonist.[75,114,195,196,205] TNF-α down-regulates thrombomodulin expression on endothelial cells, thereby leading to decreased activity of proteins S and C.[39,139] Like TNF-α, many of the mediators directly or indirectly contribute to the formation of a procoagulant state, which results in the formation of microthrombi characteristically found in the skin, digits, extremities, and organs.

Cytokines play an important role in development of the shock frequently seen in meningococcemia. Release of cytokines causes activation of neutrophils and up-regulation of adhesion molecules that may promote endothelial damage and capillary leakage.[59] Vasodilation also may occur secondary to increased production of nitric oxide by endothelial cells.[130] Subsequent compensatory vasoconstriction of splanchnic, skin, and renal vessels may not be sufficient to maintain adequate blood pressure. Endotoxin-related or cytokine-mediated cardiac dysfunction also may contribute to the development of heart failure and hypotension.[146] Profound capillary leakage in the pulmonary bed may lead to the development of acute respiratory distress syndrome.

The disseminated intravascular coagulation (DIC) commonly seen in meningococcemia also is thought to be a consequence of activation of the coagulation system by endotoxin.[167] Experimental work in animals has suggested a synergistic effect of meningococcal endotoxin and materials egested from leukocytes containing meningococci in the initiation of DIC.[53]

The pathologic purpuric lesions seen in fulminant meningococcal disease are similar to those that the generalized Shwartzman reaction induces in rabbits by endotoxin. Pathologically, these lesions show evidence of microthrombi in small dermal vessels, endothelial damage, and hemorrhage. Inflammatory vasculitis is mediated by up-regulation of endothelial adhesion molecules, activation of neutrophils, and effects of cytokines and bacterial endotoxin.[4,44,90] Circulating concentrations of endotoxin in patients with meningococcemia may be 50 to 100 times that of other gram-negative infections.[155] In addition, meningococcal endotoxin is more potent than is endotoxin from enteric bacilli.[46]

A pathologic study of 200 fatal meningococcal infections[85] illustrates the ability of the meningococcus to affect virtually any organ, either directly or indirectly. Approximately 40 percent of the patients had both meningococcemia and meningitis. Except for adults with meningitis, the average survival for all patients was 72 hours or less. The major organ systems involved at autopsy in these 200 cases were the heart, central nervous system (CNS), skin, mucous and serous membranes, and adrenals. Myocarditis developed in 78 percent of cases. Cutaneous hemorrhage occurred in 69 percent of the fatal infections and ranged from isolated petechiae to diffuse purpura. Acute meningitis was noted at autopsy in 68 percent, with brain abscesses found in two patients. In almost half the patients with acute meningococcemia, *N. meningitidis* was isolated from otherwise normal cerebrospinal fluid (CSF). Adrenal hemorrhage and necrosis were found in 48 percent of autopsy cases. Diffuse adrenal hemorrhage occurred in approximately 50 percent of adult cases and in more than 80 percent of pediatric cases. Focal areas of inflammation and petechial hemorrhage were seen in many other tissues, including the synovium, skeletal muscle, and the tracheobronchial tree. An association was noted between the pathologic findings and the infecting serogroup of *N. meningitidis*. Serogroup A infections most frequently were associated with encephalitis; serogroup B and C infections were associated with necrotizing myocarditis.

CLINICAL MANIFESTATIONS AND DIFFERENTIAL DIAGNOSIS

The spectrum of disease caused by *N. meningitidis* ranges from asymptomatic transient bacteremia, which clears spontaneously, to fulminant sepsis resulting in death only a few hours after the first symptoms occur.[186] In 2006, Kaplan and colleagues (U.S. Multicenter Meningococcal Surveillance Study Group) reviewed 159 episodes of invasive meningococcal disease seen at 10 children's hospitals in the United States between January 2001 and March 2005.[101] Meningitis was the most common manifestation of disease, seen in 70 percent of cases. Twenty-seven percent of children had bacteremia alone, and 4.4 percent had fulminant disease. Fifty-five percent of the children had petechiae, and 38 percent had purpura. Hypotension was present in 10 percent and thrombocytopenia in 6 percent on admission. The overall mortality rate was 8 percent, and it was significantly higher in children who were 11 years of age or older than in children younger than 11 years ($p < 0.001$). Hearing loss occurred in 12.5 percent of children with meningitis. Skin necrosis occurred in 9.4 percent of children, and 2.4 percent required skin grafts. One percent of children underwent amputations; one child lost all four limbs. Only one penicillin-intermediate isolate was identified in this study, and no penicillin-resistant isolates were found.

MENINGOCOCCEMIA/MENINGITIS

Serious or invasive disease usually is manifested in one of two ways: meningococcemia or meningitis (either with or without meningococcemia). It is important to consider other causes in the differential diagnosis of meningococcemia (Table 99–1).

The signs and symptoms of meningococcemia are variable. Early in the course, evidence of an upper respiratory infection, including coryza, pharyngitis, tonsillitis, and laryngitis, may be present. Patients generally are febrile, with complaints of headache, lethargy, and vomiting. Severe myalgia with muscle tenderness and joint pain also may be the initial complaint.[93] The typical patient with meningococcemia has a short history of upper respiratory symptoms, fever, and a hemorrhagic rash. Signs of severe circulatory collapse often develop. It is not unusual for purpura and shock to develop within hours of the onset of symptoms.

The skin manifestations of meningococcemia range from diffuse mottling to extensive purpuric lesions (Figs. 99–1 and

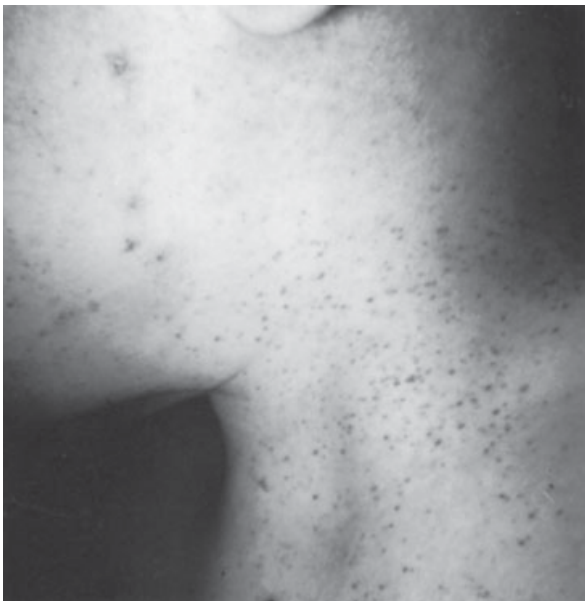

Figure 99–1 Petechial lesions are seen on the face and neck of a young child with meningococcemia.

TABLE 99–1 Differential Diagnosis of Meningococcemia

INFECTIOUS
Rocky Mountain spotted fever
Ehrlichia
Streptococcal pneumoniae sepsis
Haemophilus influenzae type b sepsis
Group A *Streptococcus* sepsis
Other bacterial sepsis with disseminated intravascular coagulation (i.e., enteric gram-negatives)
Subacute bacterial endocarditis
Gonococcemia
Rat-bite fever
Typhus
Secondary syphilis
NONINFECTIOUS
Henoch-Schönlein purpura
Idiopathic thrombocytopenic purpura
Collagen vascular disease
Neoplastic processes

99–2). Unfortunately, some variation in the type of rash is seen. Petechiae or purpura is present in 50 to 60 percent of patients.[103,212] Maculopapular rash alone is reported in 10 to 13 percent of patients.[124,212] Twenty to 30 percent of children may have no rash at initial evaluation.[212] This variance renders differentiating meningococcemia from a viral exanthem particularly difficult. A pink macular rash resembling early varicella is another variant sometimes seen in children with meningococcemia; these lesions often are tender. They may occur in crops, as do petechiae, and are seen most frequently on the trunk and extremities.[55,89]

The finding of petechiae or purpura in a febrile child should increase the index of suspicion for meningococcemia or other serious disease (e.g., infectious, neoplastic, immunologic). Acral distribution of a rash, in particular, is worrisome for meningococcemia or another infectious vasculitic process. In two studies involving more than 200 patients with fever and petechiae (not purpura), 8 percent to 20.2 percent had invasive bacterial disease.[199,212] Seven to 10 percent of the total group had infections

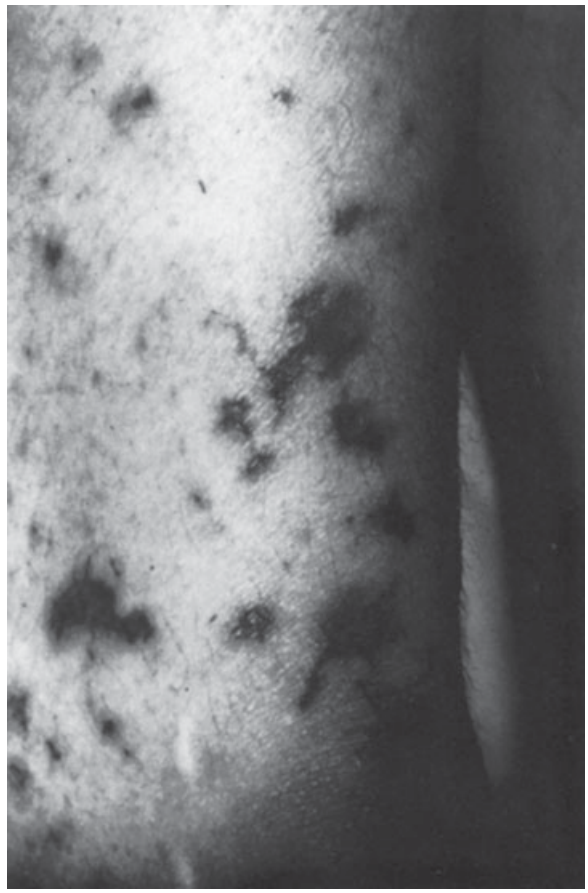

Figure 99–2 Extensive purpuric lesions occurring in a child with overwhelming meningococcemia and disseminated intravascular coagulation.

caused by *N. meningitidis*. Therefore, obtaining blood cultures for most febrile patients with petechiae seems reasonable. Lumbar puncture should be performed if clinically indicated or if the blood culture ultimately is positive. Antibiotic therapy in these patients should include coverage for meningococcus.

Purpura is noted in 16 to 24 percent of patients.[157,168,212] When the purpura is extensive and accompanied by shock, it is referred to as *purpura fulminans*. Acquired deficiencies of protein S and protein C have been described in some patients with purpura fulminans.[157,158] One study showed a mortality rate of 50 percent in patients in whom purpura developed. Progressive purpura was accompanied by declining protein C levels, and the protein C level was related inversely to the clinical severity of the disease.[157]

Patients with meningitis often are febrile, with headache, vomiting, irritability, stiff neck, and sometimes seizures. They may have a history of lethargy, or they may be obtunded at initial evaluation. In neonates, physical findings of meningismus, such as the Kernig and Brudzinski signs, often are absent. The anterior fontanelle, if open, may be full and tense.

The most common neurologic complications of meningitis are hydrocephalus, cranial nerve palsy (especially hearing loss), subdural effusion or empyema, cerebral edema, cortical vein thrombosis, and cerebral infarction. Neurologic sequelae occur much more commonly in patients with meningitis, but complications such as cerebral infarction also can be seen in children with meningococcemia and shock.[145]

Cerebral edema and cranial nerve palsies may be seen initially or develop shortly thereafter. Sixth nerve or third nerve palsies

are suggestive of increased intracranial pressure (ICP) and impending herniation, respectively. The development of either of these signs is indicative of an urgent intracranial process. Hearing loss occurs in 5 to 10 percent of patients with meningitis. Auditory testing should be performed after recovery on all patients with meningitis.

Subdural effusion or empyema should be considered in patients with fever persisting after 8 days of therapy, vomiting, or the development of signs of increased ICP after the initial few days of treatment. Drainage is recommended only if the effusion is infected (empyema) or is large enough to produce either focal neurologic signs or increased ICP.[145]

Vascular thrombosis, cerebral infarction, or both can be caused by arterial or venous thrombosis. Venous thrombosis is more common and generally is not seen before the second week. Hemorrhagic infarction of the brain then may occur. Cerebral infarction also may be seen in patients without arterial or venous thrombosis who present in shock with prolonged hypotension and cerebral ischemia. Cerebral infarction in these individuals is an early event.

Hydrocephalus occurs most frequently in young children and those with a delayed diagnosis or severe disease. It tends to occur 3 to 4 weeks after the onset of illness. Inflammation, with collagen deposition and proliferation of fibroblasts in the meninges, produces an obstruction to the flow of CSF. A progressive increase in head circumference should alert the clinician to the possibility of hydrocephalus.[145] Imaging (computed tomography, magnetic resonance imaging) studies are diagnostic.

Neonatal meningococcemia or meningitis (or the presence of both) is an uncommon finding but has been reported. In one report, a 2-week-old infant died after having a brief febrile illness with both CSF and blood cultures positive for meningococcus. Maternal endocervical colonization was documented. In addition, the mother's pharyngeal culture grew *N. meningitidis*.[99] If endocervical colonization by *N. meningitidis* is detected prenatally, treatment with antibiotics probably should be initiated in an attempt to eradicate colonization before delivery, or intrapartum antibiotics should be given. Ceftriaxone would seem to be a reasonable choice. Rifampin and ciprofloxacin are not advocated for use during pregnancy.

CHRONIC MENINGOCOCCEMIA

Chronic meningococcemia, first described in 1902, is defined as meningococcal septicemia without meningeal symptoms in which fever has persisted for at least a week before any antibiotic therapy is initiated.[57,143] Benoit[8] reviewed 148 cases of chronic meningococcemia in the United States in 1963; patients ranged in age from 3 months to 62 years. The major symptoms included fever and chills (present in 100% of patients), rash (93.2%), arthralgias (70.3%), and headache (61.5%). The patients generally did not appear toxic and were in good health before becoming infected. The mean duration of illness before diagnosis was 6 to 8 weeks (range, 1 to 40 weeks). Symptoms tended to be intermittent; the rash often appeared in association with fever and then disappeared over the next several days. Bacteremia also was intermittent in some patients. In the Benoit study, five blood cultures were obtained for the average patient before meningococci were isolated. However, after a blood culture yielded the organism, most subsequent cultures were positive. In children, some investigators report that the organism frequently is isolated in the first blood culture.[115] The arthralgias also tended to be intermittent in nature.

In Benoit's series,[8] localizing complications developed in almost 40 percent of the patients with chronic meningococcemia. The meninges were the most common site of localization, and meningitis developed in 15.5 percent of these patients. Other localized infections included carditis, nephritis, epididymitis, conjunctivitis, iritis, and retinitis. In only one instance was an organism recovered from the joint. The average duration of meningococcemia in patients with complications was 10.2 weeks, as opposed to 4 to 8 weeks in patients without any localization.

The diagnosis is established by identifying the organism in blood cultures. Antibiotic therapy results in prompt defervescence and dramatic recovery.

The pathophysiologic mechanisms permitting chronic meningococcemia remain unclear. No evidence exists that the organisms are less virulent than other meningococci are; thus, a defect in host immunity has been suggested. A hypersensitivity basis for this disease has been postulated, and researchers have theorized that the skin changes and arthritis may be secondary to antigen-antibody complexes.[143] In contrast to acute meningococcemia, bacteria almost never are found by biopsy or culture of skin lesions. The histology of the skin lesion is distinct from that seen in skin lesions of patients with acute meningococcemia.[8,85,143]

MENINGOCOCCAL PNEUMONIA

Meningococcal pneumonia occurs in conjunction with meningococcemia or meningitis in 8 to 15 percent of cases.[106] However, the meningococcus also can play a role as a primary respiratory pathogen.

Primary meningococcal pneumonia, once considered a rare disease, now is recognized as the most common form of meningococcal disease in certain military recruit populations and has been reported to be responsible for 4.5 percent of all cases of bacterial pneumonia in a general hospital population.[106,118]

Patients with preceding viral pneumonias are at risk for the development of meningococcal pneumonia; more than 100 cases of meningococcal pneumonia occurred during the influenza pandemic of 1918 and 1919. In addition to disease among military recruits, nosocomial acquisition of meningococcal pneumonia in hospitalized patients has been reported.[38]

The diagnosis of meningococcal pneumonia is difficult to establish because isolation of the organism from sputum does not distinguish a meningococcal carrier from an individual with meningococcal pneumonia. In addition, routine sputum cultures do not include media selective for the meningococcus. Blood cultures are positive in only 15 percent.[106]

Koppes and associates[106] reported on 68 cases of meningococcal pneumonia; diagnostic criteria included a compatible clinical syndrome and isolation of *N. meningitidis* from pleural fluid or blood. All 68 meningococcal pneumonia cases were group Y; during the same period, 10 cases of meningococcemia and 6 cases of meningitis caused by group Y occurred. The high ratio of pneumonia to meningitis in this study suggested that group Y organisms may be more likely than are other serogroups to cause pneumonia, and this finding has been substantiated by other investigators.[160,170] Pneumonia caused by group B or C meningococci has been reported, usually in association with meningococcemia or meningitis.[210]

Primary meningococcal pneumonia usually is associated with a gradual onset of symptoms and a history of an antecedent upper respiratory infection. Rales and fever are found in most patients, and 80 percent have pharyngitis. The radiograph often shows involvement of the lower lobes with patchy alveolar infiltrates. More than one lobe is involved in 40 percent of these patients. Twenty-five percent of them have pleural effusions. Petechiae, purpura, or shock was not present in any of the patients with pneumonia reported by Koppes and colleagues.[106]

The pathogenesis of this disease is thought to be pulmonary infection via inhalation of droplets. The epidemiologic importance of meningococcal pneumonia was emphasized in a report

from the CDC that discussed nosocomial transmission of group Y *N. meningitidis* in oncology patients.[38] The index case had meningococcal pneumonia. Meningococcal bacteremia developed in one other patient in an adjacent room; in three additional patients, group Y *N. meningitidis* was isolated from nasopharyngeal cultures. Airborne dissemination seemed to be the mode of transmission. Respiratory isolation is indicated for a patient with suspected meningococcal pneumonia.

Penicillin therapy for penicillin-sensitive meningococcal pneumonia results in a prompt clinical response. Ninety-three percent of the patients reported by Koppes and colleagues[106] were afebrile after 3 days of therapy. Third-generation cephalosporins, such as ceftriaxone and cefotaxime, may be the current drugs of choice pending definitive identification.

OTHER MENINGOCOCCAL SYNDROMES

Conjunctivitis

Primary meningococcal conjunctivitis is indistinguishable clinically from acute bacterial conjunctivitis caused by other organisms. Usually, it is manifested in children as an acute onset of unilateral purulent conjunctivitis.[7] It has been reported in individuals aged 2 days to adulthood.[8] Gram stain of the purulent material typically shows gram-negative diplococci that sometimes may be confused with gonococcal conjunctivitis. Barquet and colleagues[7] showed that 44 percent of the isolates were serogroup B meningococci.

The complications of primary meningococcal conjunctivitis reported by Barquet and associates[7] included sepsis or meningitis in approximately 18 percent. The symptoms of systemic meningococcal disease occurred 3 to 96 hours after onset of the conjunctivitis (mean, 41 hours). In patients treated only with topical therapy when initially evaluated for conjunctivitis, systemic disease was 19 times more likely to develop than in patients treated with systemic therapy ($p = 0.001$). Ocular complications occurred in 15.5 percent of patients and included corneal ulcers (10.7%), keratitis, hemorrhage, and iritis.

Pharyngitis

The diagnosis of meningococcal pharyngitis is difficult to establish because, like group A beta-hemolytic streptococci, isolation of meningococcus from the pharynx does not establish that this organism is the etiologic agent. In fact, most individuals who harbor meningococci in their nasopharynx are asymptomatic carriers. However, Banks[5] noted overt nasopharyngitis in a third of patients with meningococcal sepsis or meningitis. Pizzi,[153] in describing a severe epidemic of meningococcal meningitis, noted that individuals with sore throats often had a pure culture of meningococci. Olcen and associates[144] performed cultures on family members of 21 consecutive patients with meningococcal disease and found that 61 percent of family members with a sore throat or other upper respiratory symptoms were meningococcal carriers, as opposed to 14 percent of asymptomatic family members.

On occasion, individuals in the community with symptomatic pharyngitis have cultures performed and the laboratory reports the growth of *N. meningitidis*. If the patient is febrile but otherwise appears well, we recommend obtaining blood for culture and administering intramuscular or intravenous ceftriaxone. If the blood culture is negative, the patient should be treated with an antibiotic that will eradicate the meningococcus (see "Chemoprophylaxis"). If the patient is afebrile and asymptomatic at the time that the culture results are obtained, we recommend eradication of the organism with one of the antibiotics suggested for chemoprophylaxis.

Arthritis

Meningococcal arthritis occurs primarily in adults. The overall incidence, as a complication of bacteremia, is approximately 2 to 14 percent.[81,212] There are two forms of meningococcal arthritis. The first is seen within the first few days of treatment and is characterized by severe arthralgias and few objective signs of joint inflammation. The pathogenesis of this arthritis has been suggested to be an inflammatory response to viable organisms that have seeded the synovium during the initial bacteremia. The second, more common form appears to be an immune complex phenomenon. It usually is noted 3 to 7 days after recognition of meningococcemia, often at a time when the patient appears to be improving from the meningitis or sepsis. The knee, wrist, elbow, and ankle joints are involved most commonly.[81]

In both forms, the arthritis usually is monarticular or oligoarticular with an effusion, minimal pain, erythema, and limitation of motion. Organisms seldom are cultured from the effusion; culture of joint fluid yields meningococci in less than 10 percent of cases. The exception is a child with suppurative arthritis on initial evaluation. In one study, 8 percent of patients with meningococcal infection had arthritis; 75 percent were culture-positive when culture was performed before receiving antibiotics.[210,212]

Synovial fluid leukocyte counts vary widely, but counts greater than 100,000/mm^3 have been reported.[151] The appearance of arthritis often is accompanied by a rise in temperature; in 7 of 47 patients with arthritis, a characteristic skin lesion appeared at the same time.[82] These lesions began as skin hyperpigmentation but progressed to vesiculation and ulceration; biopsy showed vasculitis. Additional evidence of concurrent vasculitis is suggested by reports of episcleritis and mild proteinuria developing simultaneously with arthritis.[82]

On histologic examination, the synovium is infiltrated by mononuclear cells that contain IgM, C3, and meningococcal antigen,[82] which strongly suggests an immune complex–mediated disease. No specific therapy is indicated, and the arthritis resolves spontaneously. Controversy exists regarding the role of intermittent closed drainage of the joint space.[13] Permanent joint deformity is an uncommon feature of this disease that occurs in approximately 10 percent of cases.[175] Edwards and Baker[58] reported allergic complications of meningococcal disease in 10 percent of the 86 children monitored prospectively. More than 83 percent were serogroup B, although late-onset arthritis and vasculitis also have been reported with serogroup A and C disease.

Permanent joint damage is an unusual event that occurs in approximately 1.5 percent of patients with arthritis. Potential sequelae include ankylosis, decreased range of motion, and bone necrosis.[113]

Pericarditis and Myocarditis

Pericarditis occurs in 3 to 5 percent of cases as a complication of meningococcal disease, although one series reported a 19 percent incidence in 32 patients with meningococcal meningitis.[56,135] It generally occurs in patients with meningococcemia but has been reported as an isolated event without septicemia or meningitis.[88]

Pericarditis is presumed to be a late complication of meningococcal disease because clinical symptoms such as fever, dyspnea, or substernal chest pain (or even cardiac tamponade) usually do not appear until the fourth to seventh day of illness. However, several investigators have noted early evidence of pericarditis based on the electrocardiographic or roentgenographic data of patients examined at the time of hospital admission.

Because most symptomatic pericardial effusions develop late in the course of the illness, are serous in nature, and are sterile, the pathophysiologic mechanism is presumed to be an immune

complex reaction. Uncontrolled studies report the successful use of steroids for the treatment of this complication.[101] However, one report documented pericarditis and the development of tamponade in a patient receiving steroids.[147]

The clinical course of meningococcal pericarditis usually is benign, but pericardial compression requiring pericardiocentesis can occur.[147] Early relapses also have been reported, but they were self-limited. Cases of constrictive pericarditis requiring pericardectomy have been reported.[147,177,204]

Myocarditis was noted at autopsy in 78 percent of patients with fatal meningococcal disease.[85] Myocarditis was seen most often in adults but was more severe when it occurred in children. Rosenblatt and colleagues[168] noted myocarditis at autopsy in 10 of 12 children with fatal meningococcal infection. Pathologic examination revealed collections of inflammatory cells in the myocardial interstitium and focal extravasation of erythrocytes with acute vasculitis. Abscesses and endocarditis were not seen. Inflammation occasionally may involve the atrioventricular node and has been reported as a cause of sudden death in a patient recovering from meningococcal meningitis.[166]

Miscellaneous Meningococcal Infections

Several unusual syndromes, including primary meningococcal pericarditis,[199] mesenteric adenitis,[109] peritonitis,[109] and genitourinary infections, have been reported.[63]

LABORATORY FINDINGS AND DIAGNOSIS

Wong and colleagues[212] reviewed 100 cases of meningococcal infection in children seen at their institution between 1985 and 1988. Leukopenia (white blood cell count <5000/mm^3) was present in 21 percent, and thrombocytopenia was noted in 14 percent. Fifty-five percent of patients had meningitis. Eleven percent of those with culture-positive meningitis had no CSF abnormalities detected on chemistry panels or examination.

Hyponatremia is seen in some patients with meningitis. Inappropriate secretion of antidiuretic hormone is the mechanism. In one study of 43 children with meningococcal meningitis, the syndrome of inappropriate secretion of antidiuretic hormone developed in 7 percent.[60]

Other laboratory abnormalities seen in patients with sepsis or shock include abnormal coagulation panels (DIC), acidosis, and abnormal liver function studies.

The gold standard for diagnosis is based on recovering the organism from blood, CSF, or petechiae. Blood culture alone is positive approximately 50 percent of the time in patients who have not received antibiotics.[212] Rapid diagnosis often can be made by Gram stain of CSF in patients with meningitis. Characteristic gram-negative diplococci are seen. Caution should be exercised in relying solely on the Gram stain to determine initial antibiotics. Broad-spectrum antibiotic therapy should be initiated pending identification of the organism by culture. Overdecolorized gram-positive cocci of *Streptococcus pneumoniae*, on occasion, have been confused with meningococci on Gram stain.

In patients who have skin lesions, a rapid presumptive diagnosis of meningococcemia frequently can be made by needle aspiration and Gram stain of a skin lesion. Needle aspiration yields gram-negative diplococci on the Gram-stained specimen in approximately 50 percent of patients with acute meningococcal infections.[197] Culture of the aspirate increases the yield further. Correlation of the Gram stain with clinical findings is important because disseminated gonococcal infection also may be accompanied by skin lesions that yield gram-negative diplococci on Gram stain.

Counterimmunoelectrophoresis and latex agglutination have been used to detect circulating antigen in the serum, CSF, and urine of patients with meningococcal disease.[207] Latex agglutinin tests using serum and urine are not recommended.[2] Cross-reactions with certain *E. coli* K1 (cause of neonatal meningitis) or *Bacillus* strains may occur. Latex agglutinin tests using CSF may be helpful in patients pretreated with antibiotics and with a compatible clinical syndrome.[2] These tests are of particular benefit in cases of partially treated meningococcal meningitis, in which culture and Gram stain may be negative.

Polymerase chain reaction (PCR), one of the newer tests for detection of *N. meningitidis*, may be very helpful in establishing the diagnosis in patients with partially treated meningococcemia or meningitis. Once antibiotics have been given, the chance that a blood culture will be positive decreases to less than 5 percent.[25] One study showed the sensitivity and specificity of PCR for *N. meningitidis* to be 91 percent with CSF specimens. Treatment with antibiotics before the test did not decrease the test's sensitivity or specificity.[140] Use of this test in confirming meningococcal infections in patients pretreated with antibiotics may be valuable in subsequent patient management, follow-up, and prompt institution of chemoprophylaxis in contacts. One report from the United Kingdom describes the use of a multiplex PCR assay for the simultaneous detection of *N. meningitidis*, *Haemophilus influenzae*, and *S. pneumoniae* from clinical samples of CSF and whole blood. Corless and colleagues[41] found that sensitivity for detection of the three organisms ranged from 88.4 percent to 91.8 percent with 100 percent specificity. Commercially available assays such as this would greatly facilitate establishing the etiologic diagnosis in children with sepsis or meningitis. Currently, meningococcal PCR is in use in the United Kingdom, but it is available in the United States only as a research test.

MORTALITY AND PROGNOSIS

The overall mortality associated with invasive meningococcal disease in the United States is 7 to 19 percent.[101,142,169,184] Numerous different scoring systems have been devised in an attempt to predict prognosis in patients with meningococcal disease. These scoring systems were reviewed in an article by Kirsch and colleagues.[105]

The Glasgow Meningococcal Septicaemia Prognostic Score (GMSPS) is a validated scoring system developed to assess clinically patients and facilitate admission of the most severely ill children to intensive care units.[178,189] This scoring system, which evaluates seven key items (hypotension, difference in skin core temperature, coma, acute deterioration, absence of meningismus, progressive purpura, and base deficit), has been used by many investigators to define criteria for entry into clinical trials.

In a review of 100 cases of meningococcal disease at the Los Angeles Children's Hospital, five features were identified that correlated with poor prognosis: shock or seizures on initial evaluation, hypothermia, total white blood cell count less than 5000/mm^3, platelet count less than 100,000/mm^3, and the development of purpura fulminans. The overall mortality in this series was 10 percent.[212]

Most prognostic scoring systems and clinical reviews agree that purpura fulminans and shock uniformly are poor prognostic signs. Individuals with only meningitis have a case-fatality rate lower than that of those with bacteremia or an isolate from another source (2% vs. 12%).[169] Presumably, this is a function of virulence of the organism, ability of the immune system to contain the infection, or both. Case-fatality rates also differ by serogroup and are higher in W-135 disease (21%) than in serogroups C (14%), Y (9%), or B (6%).[169]

Evidence suggests that a genetic component of host cytokine production may be associated with the severity of disease. Westendorp and coworkers[206] reported that families with low TNF production or high IL-10 production are at increased risk for

having fatal outcomes of meningococcal disease. Recently, investigators have been studying variants of proinflammatory host genes (TNF-α, IL-1) to see whether polymorphisms in these genes might be linked to the severity of disease. Read and associates[163] found that homozygosis of certain alleles at the IL-1 locus increased the risk of death occurring in individuals with meningococcal disease and suggested that the IL-1 genotype may be associated with fatal outcomes.

TREATMENT

Therapy for meningococcal disease has evolved during the last century. In the early 1900s, treatment consisted of the administration of intravenous and intrathecal horse serum and CSF drainage. The mortality rate associated with this therapy was 26 percent.[89] The use of sulfonamides lowered the death rate to 5 or 10 percent.[83] With the emergence of sulfa-resistant strains, penicillin was added to the regimen and still may be used for susceptible meningococcal infections. In the late 1980s, reports began to appear of meningococci relatively resistant to penicillin. The mechanism of resistance is reduced binding affinity of penicillin-binding proteins. Relatively resistant strains have been reported in the United States, Canada, Europe, South Africa, Romania, and Croatia.[16,97,148,187,213] Isolates that are absolutely resistant to penicillin (minimal inhibitory concentration >1.0 μg/mL) have been documented from Spain and the United Kingdom.[148,187] Strains with resistance to fluoroquinolones also have been reported on rare occasion.[42,122] Non–ceftriaxone-susceptible strains have been reported from India.[122] Routine susceptibility testing of meningococcal strains is not currently recommended.

Genotypic and phenotypic studies of penicillin-resistant isolates from Spain revealed that the strains were genetically diverse and did not arise from a single clone.[215] In addition, Mendelman and colleagues[126] demonstrated that these strains have a penicillin-binding protein (PBP-3) with less penicillin-binding capacity than seen in sensitive strains. These strains appear to have arisen by the acquisition of segments of genes (by transformation) from the naturally resistant *Neisseria* commensals *N. flavescens* and *N. lactamica*.[180] The gene encoding PBP-2, *penA* in commensal *Neisseria*, encodes a protein that has less avidity for penicillin G. Transformation of *N. meningitidis* with *penA* from these strains leads to a slight decrease in penicillin G susceptibility; repeated transformation will yield a penicillin-resistant strain.

In 1988, penicillin-resistant meningococci also were reported from South Africa and the United Kingdom; these strains had acquired a gonococcal plasmid encoding for the production of β-lactamase.[187]

Prompt institution of antibiotic therapy for suspected meningococcal infection may be lifesaving. Children with fever and purpura should be considered to have meningococcemia until proved otherwise while recognizing that other pathogens may cause fever and purpura. A purpura fulminans–like manifestation also has been reported rarely to be caused by *Staphylococcus aureus*,[108] group A *Streptococcus*,[54] group B *Streptococcus*, and overt sepsis, with other organisms causing DIC. Antibiotics should be administered as soon as possible. A systematic review of the effectiveness of antibiotics given before admission concluded that "the data are consistent with benefit when a substantial proportion of cases are treated."[84] If possible, blood should be drawn for culture before antibiotics are administered, but collection of specimens should not delay such administration. A lumbar puncture can be performed in stable patients, but the procedure also should not delay the administration of antibiotics. CSF obtained after antibiotic therapy has been administered may be sterile, but pleocytosis will be apparent. In patients who are unstable or have significant coagulopathy, lumbar puncture should be deferred. Shortly after receiving bactericidal antibiotics, some patients exhibit marked clinical deterioration, including hypotension and sometimes death. Rapid liberation of endotoxin (and resultant stimulation of cytokine release) from lysing organisms may be the cause of this phenomenon.[125]

The clinical features of *N. meningitidis* meningitis may be similar to those of meningitis caused by *S. pneumoniae* or *H. influenzae*. Empiric antibiotic therapy should, therefore, take into consideration the most likely pathogens. In children older than 1 month with meningitis, vancomycin plus cefotaxime (or vancomycin plus ceftriaxone) is an appropriate regimen until a definitive diagnosis has been established. Similarly, empiric therapy for children younger than 1 month includes ampicillin and cefotaxime, with consideration given to the addition of vancomycin.[171]

For penicillin-susceptible meningococcemia or meningitis, intravenous penicillin G, 250,000 to 300,000 U/kg/day (maximum, 12 million U/day) given in divided doses every 4 to 6 hours for 7 days, is effective.[2] Third-generation cephalosporins, ceftriaxone (100 mg/kg/day intravenously in two divided doses) or cefotaxime (200 mg/kg/day intravenously in three or four divided doses), also are effective. Cefotaxime or ceftriaxone is recommended for travelers from Spain, Italy, and parts of Africa because of reports of penicillin resistance in these areas.[2]

In confirmed cases of meningococcal disease, comparisons of penicillin G with ceftriaxone have shown ceftriaxone to be as efficacious. Necrotic skin lesions were seen more commonly in the penicillin group, but otherwise, complication and mortality rates were equivalent.[193] Meningococcal disease has been treated successfully with ceftriaxone intravenously in both once-a-day (80 to 100 mg/kg/day) and twice-a-day (100 mg/kg/day in two divided doses) dosing regimens.[193] Chloramphenicol is an alternative for penicillin-allergic patients. Currently, routine susceptibility testing of meningococcal isolates is not recommended. However, in selected cases in which the patient is not responding as expected, susceptibility testing may be warranted. With all cases of meningococcal disease, eradicating colonization of the index case is important (see "Chemoprophylaxis").

Administration of steroids to patients with septic shock and meningococcemia is controversial, but several studies have reported adrenal insufficiency (or partial adrenal insufficiency) in 10.3 to 16.9 percent of severely ill children with meningococcemia.[15,48,50,165] In those patients with Waterhouse-Friderichsen syndrome, treatment with steroids is indicated. However, treatment of meningococcal meningitis with steroids has been debated in the literature for years. Steroid proponents point to *H. influenzae* type b meningitis and *S. pneumoniae* studies, in which treatment with steroids decreases hearing loss and may reduce neurologic sequelae. They postulate that steroids, through anti-inflammatory effects, may decrease polymorphonuclear neutrophils, macrophages, and cytokines in the CNS and thus decrease CNS immune-mediated damage and hearing loss. Steroid opponents say that no conclusive studies show steroids to be of benefit in meningococcal meningitis and that risks include gastrointestinal ulceration, decreased penetration of antibiotics into the CNS (because of decreased meningeal inflammation), and steroid psychosis. If steroids are used, it seems prudent to administer them early (preferably close to the time that the first dose of antibiotics is administered). However, antibiotics should never be withheld while waiting for steroids to be given.

EXPERIMENTAL/ADJUNCTIVE THERAPIES

Many adjunctive and experimental therapies have been tried or are being evaluated presently. Anti-endotoxin therapies and infusion of protein C concentrate are the most recent additions (Table 99–2). Two anti-endotoxin therapies have been evaluated

TABLE 99–2 Recent Studies of Alternative Therapies for Severe Meningococcemia or Sepsis Syndrome

Year	Study	Authors	Randomized/Placebo Controlled	Eligible Patients	Patient Number (*N*)	Results
1997	Activated protein C	Smith, White, Vaughan, et al.[179]	No/No	3 months to 27 years old, severe meningococcemia with septic shock and purpura fulminans	12	0 deaths, 2 patients with amputations. Favorable results in comparison to the historical mortality rate of 50% in patients with shock, purpura fulminans, and Glasgow meningococcal septicemic prognostic score indicating an expected mortality of >80%
1999	HA-1A	Derkx, Wittes, and McCloskey[52]	Yes/Yes	3 months to 18 years old, petechiae or purpura, hypotension, toxicity, or end-organ dysfunction	269	No statistically significant reduction in 28-day mortality demonstrated. Mortality rate with HA-1A, 18%; with placebo, 28% ($p = 0.11$)
2000*	rBPI	Levin, Quint, Goldstein, et al.[116]	Yes/Yes	2 weeks to 18 years, petechiae or purpura, and severe disease	393	No statistically significant reduction in mortality (7.4% with rBPI, 9.9% with placebo). The treatment group had fewer amputations and a trend toward improved outcomes
2001†	Activated protein C	Bernard, Vincent, Laterre, et al.[9]	Yes/Yes	Severe sepsis (any organism, known or suspected) with organ dysfunction	1690	Significant reduction in mortality (30.8% with placebo versus 24.7% in the treatment group) and increased risk of bleeding in the treatment group
2005‡	Activated protein C	Nadel, Goldstein, Williams, et al.[137]	Yes/Yes	Pediatric patients with severe sepsis	477	Study stopped after review by Data Safety and Monitoring Committee. No difference in mortality or time to resolution of complete organ failure

**Fifty-seven patients died before receiving drug or placebo (after randomization) and were not included in this analysis.*
†This study enrolled adults with severe clinical sepsis (meningococcemia not a criterion for entrance).
‡This study enrolled pediatric patients with severe clinical sepsis of all types.
rBPI, recombinant bactericidal/permeability increasing protein.

in clinical trials: HA-1A and recombinant bactericidal/permeability increasing protein (rBPI). HA-1A, a human monoclonal antibody to endotoxin, was evaluated in a randomized, double-blind, placebo-controlled trial in 269 children with severe meningococcemia. Although the 28-day mortality rate in the treatment group was 18 percent as compared with 28 percent in the placebo group, this difference was not statistically significant ($p = 0.11$).[52] BPI is a naturally occurring protein in neutrophil azurophilic granules. BPI binds to and neutralizes the effects of endotoxin. Administration of rBPI was studied in 393 children with presumed meningococcal disease. There was no difference in mortality rates (7.4% with rBPI versus 9.9% with placebo). A trend in reduction of amputations in the treatment group that approached statistical significance ($p = 0.067$) was noted.[116]

Infusions of recombinant protein C concentrate have been studied in adults in a large randomized, placebo-controlled trial of 1690 patients with severe sepsis. A reduction in the mortality rate was demonstrated, but the treatment group had an increased risk of bleeding.[9] Smith and colleagues[179] compared 12 children with severe meningococcemia and purpura fulminans treated with protein C concentrate and historical controls. No deaths occurred in the treated patients versus a 50 percent rate in historical controls. A large study published in 2007 compared activated protein C with placebo in children with presumed meningococcal infection. The study was terminated by the Data and Safety Monitoring Board because of a low likelihood of improved outcome in the treatment group.

Additional supportive measures such as prophylactic low-dose heparin are used by some clinicians.[208] Many investigators have studied the effect of heparin on survival and DIC in patients with meningococcemia, but no consistent beneficial effect on these parameters has been noted. Serious side effects directly attributed to heparin therapy have been reported rarely.[73] Difficulty adjusting the dose in small infants may lead to heparin intoxication. A retrospective chart review of 24 patients with purpura fulminans showed less necrosis of the digits and extremities in patients treated with heparin, but the results were not statistically significant.[110]

Many other experimental therapies have been attempted in patients with fulminant meningococcemia. Anecdotal reports or small case series describe the use of tissue plasminogen activator,[1,136] antithrombin III infusion,[37] topical nitroglycerin,[94,129] plasmapheresis,[36] and extracorporeal membrane oxygenation.[112] Continuous caudal block has been used to restore lower extremity perfusion.[190] Evidence is insufficient to state that any of these therapies has a significant impact on outcomes in meningococcal disease. Larger, multicenter studies are needed to evaluate efficacy.

CHEMOPROPHYLAXIS

The ability of *N. meningitidis* to spread from person to person and cause epidemic disease has been recognized since the 1800s. The secondary attack rate in households with an index case is approximately 1000 times the attack rate in the general population.[3,181] Household crowding and young age are factors that increase the secondary attack rate. The mode of transmission is direct contact with respiratory droplets or secretions. For these reasons, chemoprophylaxis is recommended for household contacts of an index case and for young daycare center contacts. Persons who have had significant contact with the oral secretions of an index case also should be considered for prophylaxis. Several epidemiologic studies suggest that casual acquaintances (such as school-aged classmates) are not at increased risk, although a number of authors have reported secondary cases in this population.[98,100] Prophylaxis is indicated for health care workers who have had intimate exposure to nasopharyngeal secretions (e.g., mouth-to-mouth resuscitation).[74] The period of communicability of the index patient is not well established. Most public health authorities recommend that persons in contact with the patient up to 7 days before the onset of illness be considered for prophylaxis. Prophylaxis should be instituted as soon as possible after identification of the index case. It is not necessary to wait for laboratory confirmation of a case if the clinical picture is most consistent with meningococcal infection. Nasopharyngeal cultures are not recommended because many nonpathogenic *Neisseria* spp. may colonize the nasopharynx.

The index patient should receive chemoprophylaxis before being discharged unless the patient has been treated with ceftriaxone (or cefotaxime). Bilukha and Rosenstein[9] reported that 4 of 14 (29%) patients with meningococcal infection treated with intravenous penicillin had positive cultures of the respiratory tract 1 week after completion of therapy.

Secondary cases originally were defined as cases occurring more than 24 but fewer than 31 days after onset in the index case. According to this definition, approximately 50 percent of secondary cases will develop in the first 7 days after occurrence in the index case. Prophylaxis must be initiated as soon as possible based on clinical findings in the index case (e.g., fulminant meningococcemia) or on laboratory culture data if the infection is manifested as bacterial meningitis, septic arthritis, or other disease.

The original drug used for chemoprophylaxis was sulfadiazine. However, a large percentage of strains now are resistant to sulfa. Table 99–3 lists four antibiotics that are highly effective in eradicating meningococcus from the nasopharynx. Rifampin generally is the drug of choice for chemoprophylaxis of children. It usually is tolerated well but has a number of side effects, including orange urine and sweat, orange staining of contact lenses, and stimulation of liver microsomal enzymes leading to a reduction in levels of other concurrent medications (e.g., oral contraceptives, anticoagulants, digoxin, phenytoin). The development of resistant strains has been reported rarely.[40] Ciprofloxacin, ceftriaxone, and rifampin have been evaluated in a randomized, comparative study.[43] All three drugs were highly effective in eradicating carriage. Ceftriaxone probably is the drug of choice for a pregnant contact and also may be indicated for children because a single intramuscular injection may result in greater compliance than do four doses of rifampin. Ciprofloxacin is contraindicated in individuals younger than 18 years old because of evidence of cartilage damage in juvenile beagles. Three cases of fluoroquinolone-resistant *N. meningitidis* disease were recently reported in North Dakota and Minnesota.[35a] Azithromycin was studied in a randomized, controlled trial that compared 500 mg of azithromycin given once orally with rifampin, 600 mg given twice daily orally for 2 days. Carriage was eradicated in 56 of 60 (93%) colonized adults treated with azithromycin versus 95 percent of the 59 colonized adults treated with rifampin.[76]

TABLE 99–3 Chemoprophylaxis Regimens for Eradication of *Neisseria meningitidis*

Drug	Age	Dose/Duration
Rifampin*	Children <1 month	5 mg/kg per dose PO bid for 2 days (4 doses)
	Children ≥1 month	10 mg/kg per dose PO bid for 2 days (4 doses)
	Adults	600 mg PO bid for 2 days (4 doses)
Ceftriaxone	Children <15 years	125 mg IM, single dose
	Children >15 years	250 mg IM, single dose
	Adults	250 mg IM, single dose
Ciprofloxacin†	Children	Not recommended
	Adults	500 mg PO, single dose
Azithromycin‡	Adults	500 mg PO, single dose (only studied in adults)

**Rifampin is not recommended for pregnant women (teratogenicity in laboratory animals). The reliability of oral contraceptives may be affected by rifampin therapy; therefore, alternative contraceptive measures should be used during and for the month after rifampin administration.*

†Ciprofloxacin is not recommended for children, pregnant women, or lactating women. Three cases of fluoroquinolone-resistant N. meningitidis disease were reported in North Dakota and Minnesota. The CDC recommends the use of an alternative agent for chemoprophylaxis in affected counties.[35a]

‡Azithromycin is not currently listed in the Centers for Disease Control and Prevention's recommendations for prophylaxis.

As mentioned in the section on meningococcal vaccines, secondary disease also can be prevented by immunization of contacts. We recommend both immunization and chemoprophylaxis whenever possible (realizing that no protection against group B meningococci currently is provided by immunization).

The single most important element of chemoprophylaxis is education of contacts regarding the need for immediate medical attention if signs or symptoms of a febrile illness develop. No prophylactic strategy is 100 percent effective, and ill contacts should be evaluated medically with maintenance of a high suspicion for meningococcal disease.

MENINGOCOCCAL VACCINES

Primary prevention of meningococcal disease is essential for several reasons. The manifestation may be fulminant, with no opportunity for antibiotics to influence the course of the disease. Antibiotic-resistant strains now are recognized, and chemoprophylaxis of contacts is a cumbersome and often ineffective public health measure. Mass immunization offers the opportunity to prevent both endemic and epidemic disease worldwide.

Two vaccines, meningococcal polysaccharide vaccine and meningococcal conjugate vaccine, currently are licensed for prevention of meningococcal disease in the United States. Both vaccines are quadrivalent and contain antigens from serogroups A, C, Y, and W-135.

MENINGOCOCCAL POLYSACCHARIDE VACCINE

Meningococcal polysaccharide vaccine (MPSV4, Menomune) is a capsular polysaccharide vaccine licensed for use in individuals older than 2 years who are at increased risk for acquiring meningococcal disease. This vaccine was licensed in 1981 and has been the only meningococcal vaccine widely available in the United States until the licensing of meningococcal conjugate vaccine. MPSV4 has several limitations. First, children younger than 2 years old do not develop high levels of antibody to most pure polysaccharide antigens, so MPSV4 vaccination in young

children is not reliably protective. Second, like other polysaccharide antigen–based vaccines, MPSV4 is unable to stimulate T-cell–dependent immunity to produce memory cells. Third, repeated boosting with MPSV4 has resulted in blunting of the antibody response, termed *hyporesponsiveness*.[17,120] Finally, meningococcal polysaccharide vaccines do not substantially decrease nasopharyngeal carriage. For these reasons, although MSPV4 remains licensed and available, most experts agree that meningococcal conjugate vaccines have significant advantages in indicated groups. MSPV4 is given subcutaneously as a single dose.

MENINGOCOCCAL CONJUGATE VACCINES

Meningococcal conjugate vaccines have been developed via technology similar to that used for the highly effective pneumococcal conjugate and *H. influenza*e type b conjugate vaccines. This technology couples polysaccharide antigens to a protein carrier. The United Kingdom has developed a monovalent serogroup C conjugate vaccine, and the U.S. vaccine is quadrivalent.

MENINGOCOCCAL CONJUGATE VACCINE IN THE UNITED KINGDOM. Meningococcal C conjugate vaccination in the United Kingdom has demonstrated efficacy,[161,191] ability to produce herd immunity (67% disease reduction in unvaccinated 1- to 17-year-olds),[162] and a 66 percent reduction in adolescent nasopharyngeal carriage. These studies and the dramatic reduction of disease in the United Kingdom after rollout of a comprehensive meningococcal vaccine program in children bolstered hopes that the MCV4 vaccine would have similar results.

MENINGOCOCCAL CONJUGATE VACCINE IN THE UNITED STATES. A meningococcal conjugate vaccine (MCV4, Menactra) was licensed by the U.S. Food and Drug Administration in 2005 for use in persons aged 11 to 55 years. This vaccine is a conjugate vaccine that couples polysaccharide capsular antigens (serogroups A, C, Y, W-135) to a diphtheria toxoid protein carrier. Protein conjugate vaccines stimulate both B- and T-cell–dependent immunity. The T-cell response leads to a good amnestic response.

The safety and immunogenicity of MCV4 were evaluated in adolescents and adults by several studies. In the United States, adolescent and adult immunogenicity studies comparing MCV4 and MPSV4 demonstrated no difference in the number of subjects (80%) achieving a fourfold rise in serum bactericidal antibody for all four serogroups.[104] Efficacy was inferred by demonstration of the development of serum bactericidal antibodies to each of the A, C, Y, and W-135 antigens at a level that was not inferior to the response generated by the polysaccharide vaccine (MPSV4).

The safety of MCV4 was evaluated before licensure in six clinical studies and included more than 7500 MCV4 vaccine participants and more than 3000 MPSV4 recipients. In general, the overall safety profile is similar to that of MPSV4. Local reactions occurred more commonly in the MCV4 group in both adolescents and adults; most reactions were graded as mild. The frequency of local reactions after MCV4 was similar to that reported after vaccination with tetanus and diphtheria toxoids (Td).[10,68] Some systemic symptoms (fever, headache, arthralgia, fatigue) were slightly more common in the MCV4 group than in the MPSV4 group, and most were mild in severity.

Close monitoring for potential adverse events has suggested a possible association between recent meningococcal conjugate vaccine receipt and Guillain-Barré syndrome (GBS). As of March 2007, 17 cases of GBS with onset occurring 2 to 33 days after administration of the vaccine have been reported through the Vaccine Adverse Event Reporting System since licensure of MCV4 in June 2005.[34] Researchers calculated the rate of GBS after MCV4 administration to be 0.20 per 100,000 person-months as compared with a background incidence rate of 0.11 per 100,000 person-months.[47] This study suggests that there *may* be a slightly higher risk of GBS developing in MCV4 recipients than in the general population. Because GBS is such a rare event, this association is difficult to study. The Advisory Committee for Immunization Practices (ACIP) and the American Academy of Pediatrics continue to recommend MCV4 because if the risk does exist, it is small. The observation that GBS has been reported rarely after meningococcal conjugate vaccine has been added to the Vaccine Information Statement for this vaccine.

Concomitant administration of Td and MCV4 produced similar frequencies of adverse events as did sequential administration (in which Td and MCV4 were separated by 28 days). Concomitant administration of Td and MCV4 did not result in decreased antibody titers of diphtheria, tetanus, or the meningococcal antibody responses. However, bactericidal antibody titers for serogroups C, Y, and W-135 were higher when MCV4 and Td were administered concomitantly versus sequentially (MCV4 given 28 days after Td). The clinical significance of this finding is unknown.[174] Diphtheria antibody titers were observed to be higher with concomitant administration of Td and MCV4 than with sequential administration. The clinical significance of this finding also is unknown.[174]

Meningococcal conjugate vaccine is administered intramuscularly (as opposed to MPSV4, which is administered subcutaneously). Since MCV4 has been licensed, there have been numerous reports of misadministration of it. The CDC studied 100 such cases and found that misadministration of MCV4 by the subcutaneous route still resulted in a protective immune response. Although this route of administration is not recommended for MCV4, persons who received the vaccine by this route do not need to be revaccinated.[33]

The duration of protection afforded by MCV4 is unknown. However, a study in adolescents compared persistence of serum bactericidal antibody for each of the four vaccine serogroups 3 years after vaccination with MPSV4 versus MCV4. MCV4 was associated with greater persistence of antibody titers to serogroups C and W-135 at 3 years than MSP4 was.[202]

Meningococcal conjugate vaccine studies are currently being conducted and analyzed in younger children. A U.S. study of the immunogenicity and safety of MCV4 in 2- to 10-year-old children showed higher seroconversion and antibody titers against all four vaccine serogroups in MCV4 recipients than in MPSV4 recipients ($p < 0.001$).[149] A follow-up study of these children at 23 to 36 months after receipt of MCV4 showed persistence of antibody activity, and a booster response was demonstrated by exposure to meningococcal polysaccharide antigens (small amount of MPSV4).[150]

RECOMMENDATIONS FOR USE OF MENINGOCOCCAL VACCINES

Table 99–4 summarizes recommendations of the ACIP for the use of vaccines to prevent meningococcal disease. Meningococcal vaccine is recommended for all U.S. children (who have not previously received it) at the 11- to 12-year-old pre-adolescent visit, with catch-up immunization of all older teens who have not received vaccine yet. The ACIP recommends that MCV4 be used as the preferred vaccine, but MPSV4 is an alternative.[10]

Entering college students who will be living in dormitories are at increased risk for acquiring meningococcal disease and should receive a dose of MCV4 if they have not been immunized previously (MCV4 preferred, MPSV4 is an alternative).[22] Other persons at increased risk for acquiring meningococcal disease and recommendations for vaccination are listed in Table 99–4.

TABLE 99–4 Current Recommendations for Meningococcal Vaccines in the U.S.

Age Group (Yr)	Recommendations for Healthy Persons	Recommendations for "At Risk Persons"*
0 to <2	No meningococcal vaccines are licensed in the U.S. for this age group. Phase III clinical trials of conjugate vaccines in progress.	No meningococcal vaccines are licensed in the U.S. for this age group.
2-10	Not routinely recommended	MCV4 intramuscularly† (preferred product) *or* MPSV4 subcutaneously†
11-18	Routine administration at 11-12 years recommended (catch up vaccination for older teens that have not previously been vaccinated): MCV4 intramuscularly (preferred product) or MPSV4 subcutaneously	MCV4 intramuscularly† (preferred product) *or* MPSV4 subcutaneously†
19-55	Not routinely recommended	MCV4 intramuscularly† (preferred product) *or* MPSV4 subcutaneously†

MCV4, meningococcal conjugate vaccine; MPSV4, meningococcal polysaccharide vaccine.

**"At risk persons" include: individuals with functional or anatomic asplenia, complement or properidin deficiency, travelers to areas where meningococcal disease is epidemic or hyperendemic, military recruits, college freshmen living in dormitories, persons infected with HIV, and military recruits.*

†*MCV4 is licensed for a single dose. Second doses of MCV4 are not currently recommended (studies pending). Persons who previously received MPSV4 and who continue to be "at risk"* for meningococcal disease should receive a single dose of MCV4 three years after prior MPSV4 administration.*

Persons 2 to 55 years of age who are at continued increased risk for acquiring meningococcal disease and previously have received MPSV4 should be vaccinated with a single dose of conjugate vaccine 3 years after receiving the last MPSV4 dose. Studies are currently under way regarding the issue of reimmunization with a second dose of conjugate vaccine in persons who remain at increased risk of acquiring meningococcal disease. Development of an immunogenic serogroup B meningococcal vaccine has been problematic because the polysaccharide capsule of group B meningococcus is not immunogenic in animals or humans. In 1983, Finnish investigators reported that horse antiserum to meningococcus group B reacted with glycoproteins isolated from human and rat brain.[11] Therefore, good immunogenic responses may not be achievable with group B polysaccharide antigen vaccines because the body may see these antigens as "self" antigens (similar to neural tissue). However, a serogroup B outer-membrane vesicle vaccine has been studied recently in 16- to 24-month-old children in New Zealand, and 75 percent demonstrated seroconversion after receiving three doses.[211]

Deeper understanding of the pathophysiologic mechanism of meningococcal disease and the bacterial structures critical for antigenicity and immunogenicity in humans is needed. A safe and effective vaccine that could induce protection against all encapsulated meningococci in all age groups is the ultimate goal.

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CHAPTER 100

Gonococcal Infections

Charles R. Woods, Jr.

Gonorrhea, the foremost manifestation of human disease caused by *Neisseria gonorrhoeae* (the gonococcus), is one of the oldest known human diseases. Hippocrates called the disease "strangury" in the fifth and fourth centuries BCE. Galen coined the name *gonorrhea*, meaning "flow of semen," in the second century CE. The association of the disease with sexual activity was recognized, and sexual abstinence plus washing of the eyes of newborns was prescribed by Greco-Roman physicians for treatment of the disease. Gonorrhea also has been known as *clap* since the late 1300s. The origin of this term is unclear but may have derived from the Les Clapier district of Paris, where prostitutes were housed during the Middle Ages.[186,229]

After syphilis appeared in Europe in the late 15th century, the two diseases were thought to represent different manifestations of the same infection, and they often coexisted then as today. The description by Neisser of *N. gonorrhoeae* in stained smears of urethral and other exudates in 1879 and culture of the organism by Leistikow and Löffler in 1882 provided the foundation for modern understanding of the clinical spectrum of gonococcal diseases. The advent of safe and effective antimicrobial agents, first sulfonamides in 1936 and then penicillin in 1943, was the next major advance in combating gonorrhea.[186,229] Further understanding of the clinical aspects of the disease was facilitated by development of the Thayer-Martin medium in 1964.[322] Modern knowledge of the molecular pathogenesis of *N. gonorrhoeae* infection began in 1963 with the observation by Kellogg and colleagues that gonococcal strains with differences in colony morphology also varied in virulence.[188]

The potential reproductive sequelae of gonococcal infections (e.g., infertility), the ever-growing resistance to antimicrobial agents, and disproportionate case burdens in public clinics continue to cast *N. gonorrhoeae* infection as a major international public health problem.[57,213,276] The purpose of many current investigations remains the development of an effective vaccine.[81,308]

In infants, the gonococcus causes primarily ophthalmia neonatorum. Wound infections, including scalp abscess (often associated with fetal monitoring), funisitis, and vaginitis may occur. Infection can disseminate from any colonized or infected skin or mucous membrane site and may result in sepsis, meningitis, septic arthritis, or endocarditis. In prepubertal children, gonococcal infection usually involves the genital tract and almost always is transmitted sexually. Vaginitis is the most common manifestation. Extension to the upper genital tract in girls does occur but very seldom. Urethritis can develop in boys but rarely does so. Anorectal and tonsillopharyngeal infections also can occur in prepubertal children.

In adolescents, as in adults, the most common clinical manifestations are urethritis, endocervicitis, and salpingitis in females and urethritis in males. Epididymitis, bartholinitis, pelvic inflammatory disease (PID), and perihepatitis can occur as a result of extension from primary genital infections. Rectal and pharyngeal infections and disseminated disease, which may include reactive or septic arthritis, also are seen. Additional information on gonococcal infections is provided in Chapter 48.

EPIDEMIOLOGY OF GONOCOCCAL INFECTION

Gonococcal infections remain second only to *Chlamydia* infections in incidence among reportable diseases for 15- to 24-year-olds in the United States.[60,68] Gonococcal infections are relatively rare occurrences in Canada and much of western Europe but remain common in developing countries.[164] Rates of gonococcal infection in the United States and western and central Europe declined steadily in the 1980s and 1990s. Rates of gonorrhea increased in eastern Europe in the early 1990s.[326] These rates have declined but remain above those in other European regions.[342] Regions with higher rates of adult disease have higher numbers of pediatric cases,[109,131] a finding that reflects the adult origin of virtually all pediatric cases.

An estimated 1 million new cases of gonococcal infection occur annually in the United States, only approximately a third of which are reported. The 339,593 reported cases in the United States in 2005 (115.6 cases per 100,000 population) represented a slight increase in the national rate for the first time since 1999 (Fig. 100–1). This rate remains well above the Healthy People 2010 goal of 19 per 100,000. At least half the cases are thought to go undiagnosed or unreported. After World War II, reported cases of gonorrhea in the United States declined from 204 per 100,000 in 1950 to 129 per 100,000 in 1958. Rates rose in the 1960s and early 1970s, in association with changing sexual mores, before peaking at 472 per 100,000 in 1975. Gonococcal infections rarely are fatal (approximately four deaths per year in the United States), with death being caused by gonococcal sepsis.[58,60,65,213,276]

Adolescent females 15 to 19 years of age have the highest reported incidence of infection in the United States (625 cases per 100,000 in 2005). Among those aged 25 years and younger (including children), rates of gonorrhea are higher for females than for males. Rates for males are higher in those aged 25 years and older. Rates in African American adolescents of both genders greatly exceed those of white adolescents, and this trend continues into adulthood. The disparity between African Americans and non-Hispanic whites narrowed slightly from 26-fold in 2001 to 18-fold in 2005.[60,65]

Low socioeconomic status, early onset of sexual activity, unmarried marital status, and past gonococcal infections are risk factors for acquiring gonococcal infection, as are prostitution and illicit drug use.[16,39] Limited access to quality health care also may contribute to an increased prevalence of disease in impoverished populations.[65] The prevalence of gonococcal infection in adolescents attending private clinics probably is lower than that in those seeking care in publicly funded settings.[22] For women, the use of

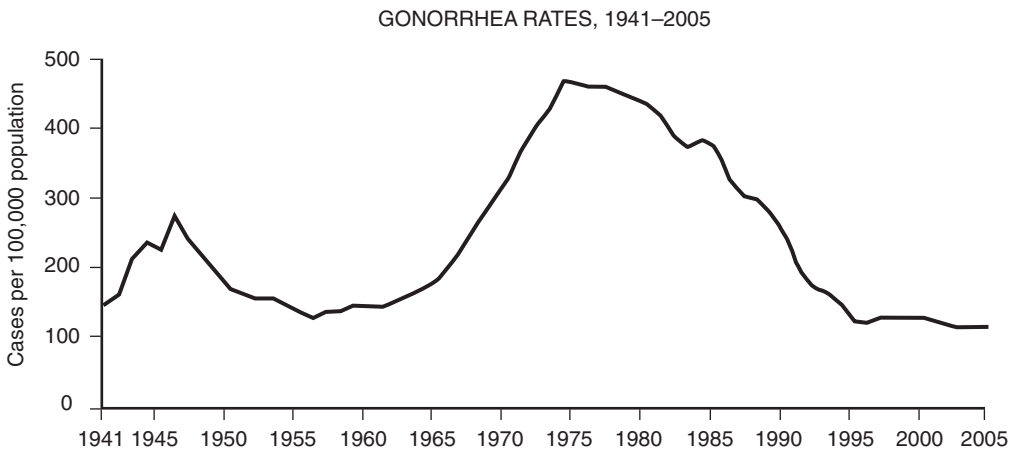

Figure 100–1 Rates of gonorrhea in the United States, 1941 to 2005. After a 74 percent decline from 1975 through 1997, overall gonorrhea rates have plateaued in recent years. Gonorrhea is substantially underdiagnosed and underreported. About twice as many new infections are estimated to occur each year as reported. *(From Centers for Disease Control and Prevention: Trends in reportable sexually transmitted diseases in the United States, 2005. December 2006. Available at* http://www.cdc.gov/std/stats/05pdf/trends-2005.pdf.*)*

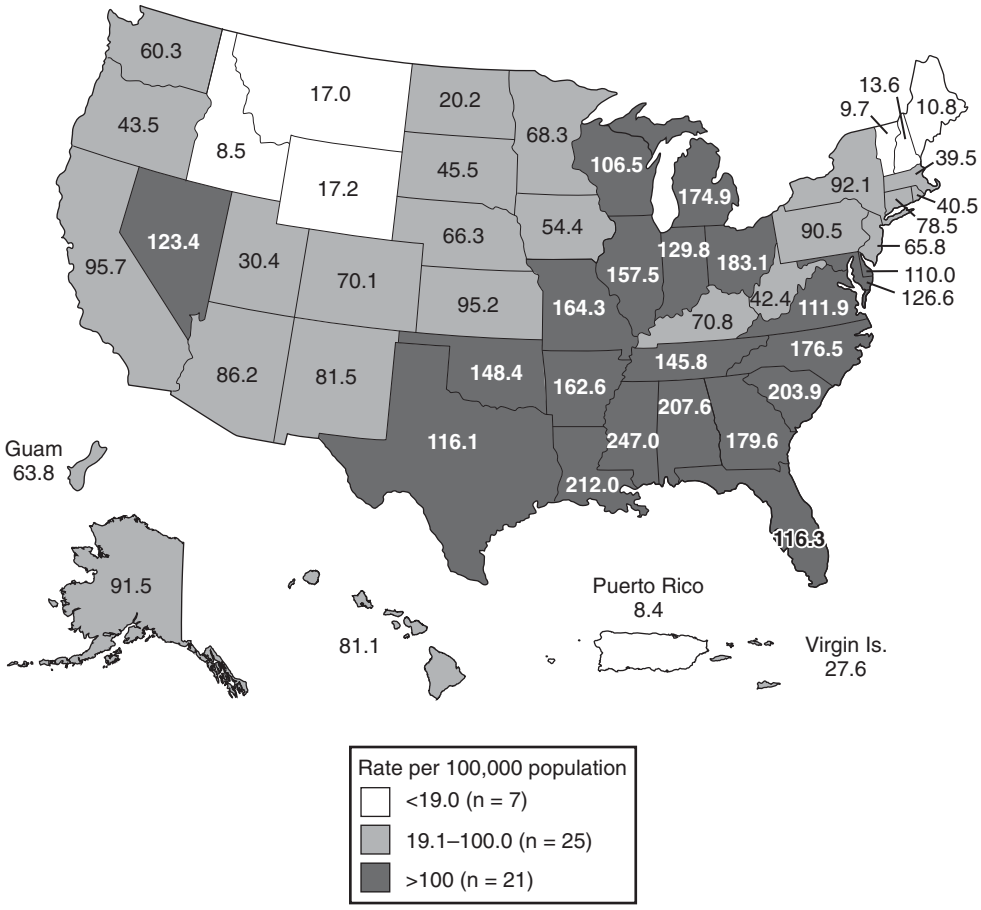

Figure 100–2 Rates of gonorrhea by state in the United States and outlying areas, 2005. *(From Centers for Disease Control and Prevention: Sexually Transmitted Disease Surveillance 2005 Supplement, Gonococcal Isolate Surveillance Project (GISP) Annual Report 2005. Atlanta, U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, January 2007.)*

hormonal contraceptives may increase the risk of acquiring infection, whereas the use of spermicides or diaphragms appears to be at least partially protective.[210,211]

Rates of gonococcal infection among men who have sex with men (MSM) are lower than those in past years but remain higher than those among males who are exclusively heterosexual.[196] Among human immunodeficiency virus (HIV)-infected persons included in the Adult/Adolescent Spectrum of HIV Disease Project in the United States from 1991 to 1998, 9.5 cases occurred per 1000 person-years. A trend toward increasing rates of gonorrhea was noted during that period among MSM, whereas a decline occurred in those with heterosexual contact as their HIV exposure risk.[112] In a prospective study from 2001 to 2003, the prevalence of pharyngeal gonorrhea among HIV-negative MSM was 5.5 percent; 92 percent of cases were asymptomatic.[227]

The southeastern United States continues to have rates of gonorrhea higher than those of other regions of the country, but rates per 100,000 population in the Southeast fell from 175 to 140 between 2001 and 2005 (Fig. 100–2). Rates in the western United States increased during this period from 72 to 82 per 100,000.[65] This increase occurred in all age, gender, and racial/ethnic groups, regardless of sexual orientation, and was explained only in part by increased testing and the use of more sensitive tests.[66]

Rates of gonorrhea are based primarily on detection of symptomatic infection. Asymptomatic chronic infection in adults is well documented and may account for 5 percent of cases.[164] Such persons can transmit the infection. Some untreated cases will resolve over the course of time. Data also indicate that asymptomatic infection occurs in children, including prepubertal children.[3,123,158,171]

Humans are the only reservoir of *N. gonorrhoeae*. Exudate and secretions from infected mucosal surfaces allow transmission to occur during the intimate contact of sexual acts, parturition, and, rarely, household exposure. The incubation period usually is 2 to 7 days. No evidence of airborne transmission of gonococci exists; contact with viable microbes is required for transmission. Gonococci survive on surfaces outside the human body for only short periods of time (probably minutes). Although organisms can be cultured from environmental sources (e.g., toilet seats) for up to 3 hours after artificial inoculation in large numbers, viable gonococci have not been recovered from random samplings in public restrooms.[133]

Gonococcal conjunctivitis can be acquired nonvenereally.[203] Vulvovaginitis potentially could be acquired when a child sleeps in the same bed with an infected family member,[133] and sharing of bath towels and other similar objects has been suspected as the cause of epidemics in prepubescent girls living together.[308] However, nonsexual transmission rarely occurs and never should be presumed without extensive investigation of the social setting of the infected child.[239] Although persistence of apparently perinatally acquired gonococcal infection for up to 1 year of age has been reported and fomite or maternal nonsexual transmission to young infants is plausible,[34] these possibilities should not be generalized beyond infancy.

The risk of a male acquiring urethral infection after a single episode of vaginal intercourse with an infected female is estimated to be 20 percent. With four exposures, the risk increases to 60 to 80 percent. The prevalence of infection in females named as sexual contacts of males with gonococcal urethritis has been reported to be 50 to 90 percent.[162,167]

The annual direct medical cost of treating sexually transmitted diseases (STDs), including HIV, in the United States alone has been estimated at $11 to $17 billion in 2003 U.S. dollars. Almost half of these costs accrue to persons aged 15 to 24 years. The reduction in gonorrhea and syphilis rates in the United States between 1990 and 2003 has been estimated as being equivalent to $5 billion during this period.[78]

MICROBIOLOGY

Neisseria spp. are aerobic, gram-negative, nonmotile, non–spore-forming cocci that occur in pairs (diplococci), with adjacent sides flattened. They have the typical gram-negative microbial outer membrane overlying a thin peptidoglycan layer and cytoplasmic membrane. The species lacks a true polysaccharide capsule but produces a surface polyphosphate that provides a hydrophilic, negatively charged surface.[246] In Gram stains of clinical specimens, the microbes frequently are observed within phagocytes.[303]

Gonococci are able to use glucose, lactate, and pyruvate as carbon sources but cannot use other carbohydrates, which is the basis for the carbohydrate utilization tests for speciation of *Neisseria* (Table 100–1). Catalase and cytochrome oxidase are produced by the gonococcus, as in most *Neisseria* spp., but gonococci do not produce appreciable amounts of superoxide dismutase. Growth is optimal in a 5 percent CO_2 atmosphere at 35° C to 37° C and a pH of 6.5 to 7.5. Gonococci do not survive below pH 6.0, grow poorly below 30° C, and do not survive above 40° C.

Neisseria spp. require enriched media, including free iron, to support their growth. Multiple colony types are evident when a single isolate is grown on clear agar. Small convex glistening colonies are piliated, whereas larger, flatter colonies are nonpiliated. In vitro passage usually results in loss of pili. Under low-power microscopy, some colonies appear opaque or granular and others are transparent. The former represent colonies in which most cells express one or more opacity-associated proteins (Opa), whereas in the latter colonies, most cells do not express these proteins.[37,42,188,310,318,319,331]

CULTURE FROM CLINICAL SPECIMENS

The organisms usually are cultured on chocolate blood agar in an atmosphere enriched by carbon dioxide. If the clinical specimen has been obtained from a highly contaminated site (e.g., rectum, cervix), a selective medium containing nystatin, vancomycin, trimethoprim, and colistin (e.g., modified Thayer-Martin medium) to suppress contaminating flora allows the growth of most *Neisseria* spp. A few strains of gonococci can be inhibited by vancomycin (see later).[37,225] Chocolate agar without antimicrobial agents is preferred if the culture is taken from a usually sterile area such as blood, cerebrospinal fluid (CSF), synovial fluid, or a skin lesion. Gonococcal colonies usually are evident on agar plates within 24 to 48 hours after inoculation. Frequent propagation is necessary to maintain isolates because viability is lost rapidly after 48 hours of growth.

Because gonococci cannot tolerate drying, clinical specimens must be plated as soon as possible onto appropriate media. Transport bottles that contain medium and a CO_2-enriched atmosphere should be used if definitive processing of the specimen must be delayed. Transport bottles should be maintained upright to preserve the CO_2 atmosphere.

TABLE 100–1 Biochemical Characteristics Differentiating Species of the Genus *Neisseria*

	N. gonorrhoeae	*N. meningitidis*	*N. sicca*	*N. subflava*	*N. flavescens*	*N. mucosa*	*N. lactamica*
Acid from							
Glucose	+	+	+	+	−	+	+
Maltose	−	+	+	+	−	+	−
Sucrose	−	−	+	±	−	+	−
Lactose	−	−	−	−	−	−	+
Polysaccharide produced from 5% sucrose	0	0	+	±	+	+	0
Reduction of							
Nitrate	−	−	−	−	−	+	−
Nitrite	−	±	+	+	+	+	+
Pigment	−	−	±	+	+	+	−
Extra CO_2 for growth	+	+	−	−	−	−	−

In addition to sugar fermentation patterns (see Table 100–1) and oxidase positivity, other biochemical reactions also can be used to differentiate between *Neisseria* spp. or confirm that an isolate is *N. gonorrhoeae*.[71] Enzyme substrate tests that identify the presence or absence of 1-hydroxyprolylaminopeptidase, gamma-glutamyl aminotransferase, and beta-galactosidase are available as an adjunctive means of confirmation.[97] Gonococci possess only the former. These reactions alone will not distinguish *N. gonorrhoeae* from several commensal *Neisseria* spp. Antigen-detection–based tests, including fluorescent antibody staining, also have been used to confirm the identity of an isolate as *N. gonorrhoeae*.[189] Tests based on detection of gonococcal nucleic acid sequences, which are being used increasingly for diagnostic purposes, also can be used to confirm the identify of gonococcal isolates from cultures (see "Diagnostic Testing" and "Medicolegal Issues" later).

GENETIC CHARACTERISTICS

Gonococci have a circular chromosome consisting of 2219 kilobases (kb), which is about half the size of the *Escherichia coli* genome.[107] The gonococcal genome has approximately 2250 predicted open-reading frames (ORFs).[35] The entire *N. gonorrhoeae* strain FA1090 has been sequenced. A macrorestriction map with the positions of many genetic markers has been available since 1991.[199,257] More than 1300 ORF sequences have been validated.[35]

Piliated gonococcal cells (the natural in vivo state) are competent for genetic transformation by exogenous DNA at all stages of growth.[25] Only homologous DNA is taken into the cell.[114] Gonococci are highly autolytic and release DNA in a biologically active form. Thus, different strains are able to exchange genetic material readily. Such exchange can lead to further genetic and phenotypic diversity, which helps maintain the species in its human hosts and facilitates transfer of chromosomal antibiotic resistance genes.[291]

A 36-kb conjugal plasmid is present in many gonococci. It efficiently mobilizes its own transfer and other non–self-mobilizable plasmids (e.g., the 4.5- and 7.5-kb penicillinase plasmids), but not chromosomal genes.[24,253,279] Extrachromosomal, non-plasmid DNA circles recently have been identified in wild-type gonococcal isolates. They may play a role in gene recombination, amplification of chromosomal genes, and transformation.[18]

Gonococci possess multiple restriction endonucleases and their corresponding DNA methylases.[317] No bacteriophages for *N. gonorrhoeae* are known, and no drug resistance transposon systems have been identified.[308] The species is relatively nonmutagenic and lacks photoreactivation and error-prone repair systems.[48]

STRAIN TYPING

The ability to differentiate one strain from another allows researchers to investigate the epidemiology of transmission of gonorrhea and assessment of virulence factors. Numerous methods have been applied to gonococci.

Auxotyping classifies strains according to their ability to grow or not grow in the absence of 11 specific compounds, including amino acids (e.g., arginine, proline), purines, pyrimidines (e.g., hypoxanthine, uracil), and other nutrients (e.g., thiamine). Approximately 20 auxotype phenotypes are recognized. Genetic studies have shown that multiple mutations in the same biochemical pathway can lead to the same phenotype, such that a single auxotype can represent many genotypes.[298] Auxotypes are stable in vitro, however, and organisms cultured from sexual partners are of a similar auxotype.[51,55] The auxotype of greatest epidemiologic importance is designated arginine-, hypoxanthine-, and uracil-negative (AHU^-). Such strains are unable to grow in the absence of these compounds. AHU^- strains typically are more resistant to killing by normal human serum, are more likely to cause asymptomatic infections in males, and are found more frequently in patients with disseminated infection.[117] AHU^- strains often are susceptible to vancomycin.[225]

Numerous serotyping schemes have been attempted,[72] but the best and most widely available serologic technique is based on antigenic heterogeneity of the porin protein (formerly called protein I) contained in the outer membrane of the gonococcus.[10,179,192,288,289] Two immunochemically distinct serogroups, PorA and PorB, exist and can be subdivided into serovars by using sets of monoclonal antibodies. Serovars remain stable in vitro and are designated IA-x or IB-x, where *x* is the numeral of the serovar. At least 26 IA and 31 IB serotypes have been identified.[62] Serotyping can be combined with auxotyping to further discriminate among gonococcal strains.[166,263] Limitations of auxotype-serovar classification include restricted supplies of reagents and batch-to-batch variation in monoclonal antibodies. In addition, common serovars can have such significant genetic diversity that this typing method may not provide sufficient discrimination for some epidemiologic evaluations.

Antimicrobial susceptibility patterns (antibiograms) have been used in the past as an adjunctive tool, but the usefulness of this method for long-term epidemiologic studies has been compromised by the ability of gonococci to transfer genetic elements for antibiotic resistance between strains.[308]

Genotyping methods have been applied to *N. gonorrhoeae* in the past 2 decades. Simple restriction endonuclease methods with rare cutting enzymes[122,262] have been supplanted by polymerase chain reaction (PCR)-based methods and pulsed-field gel electrophoresis (PFGE). Arbitrarily primed PCR (AP-PCR) has been used, but the reproducibility of this method can be problematic.[47,208] PFGE and Opa typing, which involves PCR primers that are able to generate DNA bands from each of the 11 *opa* genes, followed by restriction enzyme digestion, each appear to be able to produce higher discrimination among gonococcal isolates than does serotyping combined with AP-PCR.[208]

Sequencing of the *por* gene also appears to be highly discriminatory among strains,[87] and PCR amplification of the *por* gene with restriction enzyme digestion provides discrimination similar to that of combined auxotyping-serotyping.[111] Repetitive-element sequence-based PCR (rep-PCR), which uses longer primers than AP-PCR does and is a more reproducible method, correlates well with PFGE for analysis of gonococcal strains.[263] Rep-PCR is a rapid method that can be performed directly on colonies without having to purify microbial DNA, which is required with most other genotyping methods.[341]

PATHOGENESIS

A continuously expanding body of literature is devoted to the pathogenesis of *N. gonorrhoeae*. Gonococci are able to survive in the urethra in the face of hydrodynamic forces that tend to wash other microbes away and persist there despite close proximity and even attachment to the hoards of neutrophils that respond to their presence. These observations suggest that they have an ability to adhere to mucosal epithelia and evade the host's acute innate immune response. Individuals also can have repeated infections by the same gonococcal strain, thus suggesting that the organism can thwart established local immune responses, probably through frequent antigenic variation. The tissue damage that occurs in the fallopian tubes during salpingitis implies that one or more directly toxic moieties or factors can trigger a deleterious host response.[124,308] Much progress has been made in understanding the mechanisms that account for these properties.

Clinical studies have focused largely on the adaptation of organisms to specific anatomic areas (e.g., rectum, blood, endocervix) in older adolescents and adults. This information probably holds true for most infections in infants and children. Recent insight into the molecular pathogenesis of gonococcal infection has been derived from in vitro molecular studies involving cell lines and fallopian tube organ cultures and experimental infections in human males.[81,215,216,272] Currently, no adequate animal models of gonococcal infection exist.

After adhering to host epithelial cells, some gonococcal microbes are able to invade, replicate intracellularly inside phagosomes/vacuoles, and exit the basal (but not lateral) surface of the cell via exocytosis[216,221,237] (Fig. 100–3). Adjacent nonepithelial cells are sloughed, probably because of the toxicity of lipo-oligosaccharide (LOS) and peptidoglycans.[142] Mucosal cell damage and submucosal invasion are followed by an influx of neutrophils along with the formation of submucosal microabscesses and exudation of purulent material into the lumen of the infected organ.[339]

The vast majority of invading gonococci are ingested and killed by neutrophils. Gonococci are susceptible to the oxidative burst products of phagocytes, as well as to nonoxidative products such as cathepsin G.[52,296] A few gonococci are able to escape the innate and early adaptive immune responses such that infection and contagion can persist for weeks and sometimes months in the absence of treatment.

Adherence of gonococci to mucosal epithelial cells causes activation of nuclear factor κB and activator protein 1, which in turn leads to up-regulation and release of multiple cytokines and chemokines, including macrophage colony-stimulating factor, tumor necrosis factor-α (TNF-α), tumor growth factor-β, monocyte chemoattractant protein 1, interleukin-1β (IL-1β), IL-6, and IL-8.[238,266]

The initial steps in pathogenesis may result from the synergistic effects of pili, Opa proteins, and porin proteins.[19,144] Close interactions of *N. gonorrhoeae* with mucosal cells, erythrocytes, spermatozoa, and polymorphonuclear cells are well described. Species specificity is demonstrated by greater adherence of gonococci to human cells than to nonhuman cells. The best-characterized microbial factors are listed in Table 100–2 and discussed in the following sections.

TABLE 100–2 Pregnancy Complications and Outcomes of Mothers Who Were Infected with *Neisseria gonorrhoeae* at Delivery

Pregnancy Complications and Outcomes	Charles et al.[74] (*N* = 14)*	Sarrel and Pruett[290] (*N* = 37)	Israel et al.[175] (*N* = 39)	Amstey and Steadman[7] (*N* = 222)*	Edwards et al.[116] (*N* = 19)*	Handsfield and Holmes[154] (*N* = 12)*	Totals†
Normal or term infant	—	13 (35%)	30 (77%)	142 (64%)	7 (37%)	—	192/317 (61%)
Aborted	—	13 (35%)	1 (2%)	24 (11%)	—	—	38/298 (13%)
Perinatal death	—	3 (8%)	1 (2%)	15 (7%)	2 (11%)	—	21/317 (7%)
Premature	—	6 (16%)	5 (13%)	49 (22%)	8 (42%)	8 (67%)	76/329 (23%)
Perinatal distress	—	—	2 (5%)	—	2 (11%)	—	4/58 (7%)
Premature rupture of membranes	6 (43%)	8 (22%)	—	52 (23%)	12 (63%)	9 (75%)	87/304 (29%)

**Data were provided in which the outcomes of pregnancies of mothers not infected with N. gonorrhoeae were shown to be significantly more favorable.*
†Percentages in all columns may sum to greater than 100 because pregnancies could have more than one of the listed outcomes.

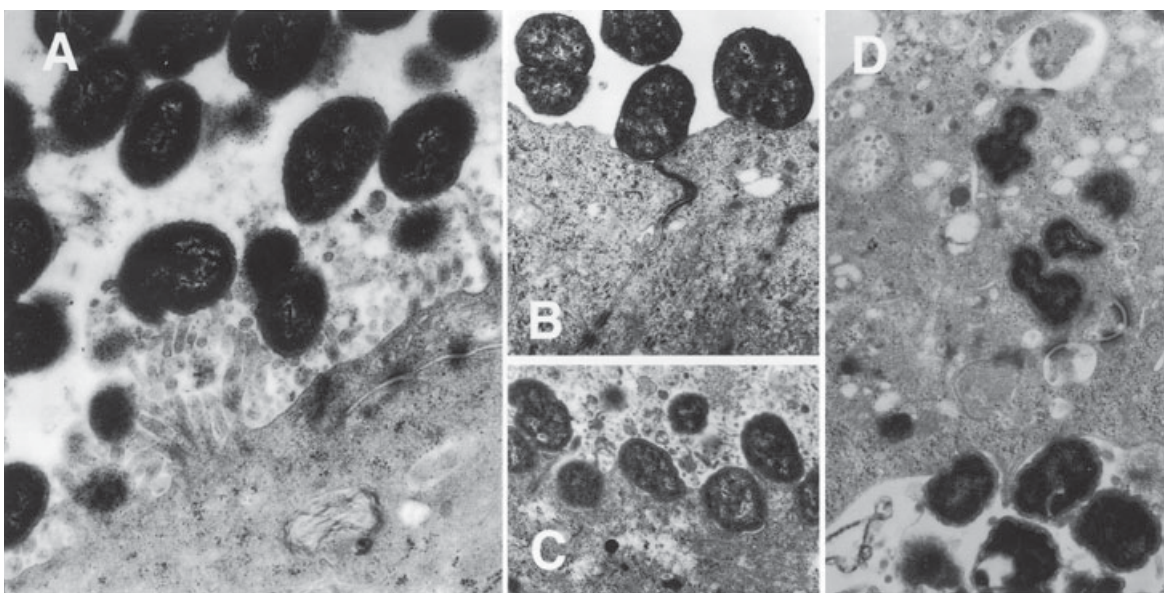

Figure 100–3 Electron micrographs of *Neisseria gonorrhoeae* (strain MS11) interactions with polarized T84 human epithelial cell monolayers. At early stages of infection, the microbes adhere to the apical plasma membrane as microcolonies. Adherent bacteria are surrounded by a matrix of microvilli (**A**). Bacteria subsequently disperse from the microcolony and adhere as a monolayer in which bacterial and host-cell membranes are tightly apposed (**B** and **C**). The region of contact between the bacteria and the host-cell membrane enlarges (**C**), with subsequent internalization of the microbes (**D**). Bacteria then traverse the host cell and exit via the basolateral membrane (**D**). *(Photographs by Magdalene So, from the* Annual Review of Cell and Developmental Biology, *Volume 16. © 2000 by Annual Reviews.* www.AnnualReviews.org.*)*

VIRULENCE FACTORS

Characteristics of *N. gonorrhoeae* strains that appear to have a role in virulence include (1) pili, (2) opacity proteins, (3) porin protein, (4) the ability to survive in low iron environments, (5) IgA protease, (6) LOS, (7) cell wall peptidoglycan, and (8) reduction modifiable protein (Rmp). Many other gonococcal gene products and molecular systems have been identified, and a more detailed and complex understanding of virulence at the molecular level probably is forthcoming.

THE PRESENCE OF PILI. Gonococci are piliated predominantly in vivo and express type IV pili. Pili cover essentially the entire surface of the cell and are arranged in individual fibrils or fibrillar aggregates. Pili consist primarily of polymers of an 18-kd subunit now denoted as PilE. A single pilus is approximately 6 nm in diameter and up to several microns in length.[221] Each may contain thousands of PilE subunits.[44] The PilC protein is present in lower numbers in the pilus but appears to be the key pilus adhesin. PilC interacts with CD46 molecules, which are present on most human cells and serve as receptors for C3b, C4b, and measles and other viruses. PilC-CD46 attachment appears to be a key step in the initiation of microbial adherence to host cells.[45,185,190,283,285]

Binding of pili to a host-cell membrane induces at least two responses in the host cell: release of Ca^{2+} from intracellular stores[184] and cytoskeletal rearrangements with the formation of cortical plaques that represent an accumulation of actin, ezrin, and other phosphotyrosine-containing proteins.[219,220] The latter results in elongation of the microvilli that embrace the microbe. Formation of plaque appears to be induced by retraction of the attached pilus, and retraction is dependent on another pilus protein designated PilT.[222]

Gonococcal pili undergo both phase variation (switching from piliated to nonpiliated states) and high-frequency antigenic variation.[44] These variations occur during natural infection and in vitro passage.[293] Pili have a common N-terminal domain, semivariable domains in the midportion, and two hypervariable regions in the carboxyl-terminal of the pilin protein subunit.[309] Relatively invariant regions occur between the variable domains. The pilin protein consists of 159 to 160 amino acids. A disulfide bridge is formed by cysteine residues at amino acids 121 and 151.

The genetic mechanism of pilus antigenic variation has been studied extensively. The chromosome contains either one or two complete pilin genes. The expressed gene contains an intact promoter region, a ribosome binding site, and a seven–amino acid signal sequence in addition to the pilin sequence. Scattered around the chromosome are six to eight loci that contain varying portions of the pilin sequence without the promoter region or the 5′ end of the structural gene. Some loci may contain many incomplete pilin sequences arranged head to tail that differ slightly from one another. Movement of these sequences into the active expression site through nonreciprocal combination events results in antigenic variation of the pilus protein. If recombination leads to a faulty pilin subunit product that cannot be processed into a mature pilus, the progeny of that organism become nonpiliated (phase variation). Subsequent recombination events can allow reversion back to the piliated phase.[150,223,224,243,292,309,320]

In addition to promoting adhesion to epithelial cells, pili also provide a twitching motility, are involved in DNA transformation, and may play a role in resistance to ingestion by phagocytic cells.[221] The presence of pili, perhaps mediated through their promotion of adhesion, affects the activation of $CD4^+$ T-lymphocytes and the production of IL-10. This property may help gonococci diminish the T-cell response to their presence.[259]

OPACITY-ASSOCIATED PROTEINS. Opa proteins also influence adherence of the gonococcus to host cells. The isolation of particular Opa protein phenotypes from different anatomic sites or at different times during the menstrual cycle has suggested that these proteins contribute to the ability of the organism to succeed in a given niche. Opa proteins (formerly designated *protein II*) are a set of as many as 12 related proteins that are variably present in gonococcal outer membranes. Opa proteins are 24 to 28 kd in size and confer increased opacity to colonies of organisms by promoting adherence of the organisms to one another. A given strain has the capacity to make at least 10 different Opa proteins but appears to express no more than 5 at the same time. Some cells express no Opa proteins. Like pili, Opa proteins undergo both antigenic and phase variation. Both types of variations can occur within a single colony, so sector variations in opacity can be seen.[27,308,318,319]

Each *opa* gene is present as a complete gene with its own promoter and is transcribed at all times. Phase variation in expression occurs at the translational level. Each gene has a varying number of pentameric CTCTT repeat units adjacent to the ATG start codon. When the number of repeats is divisible by three, the transcribed mRNA is translationally in frame and the Opa protein is expressed. The number of pentameric repeats is subject to high-frequency variation. Antigenic variation results from recombinations between the two hypervariable regions in each of the *opa* genes.[86,313-315]

Two classes of host-cell receptors for Opa proteins have been identified. Heparan sulfate–proteoglycan (HSPG) receptors are present on epithelial cells and interact with one particular Opa protein variant (Opa_{50}). HSPG binding can stimulate the lipid hydrolysis enzymes phosphatidylcholine-specific phospholipase C and acid sphingomyelinase and thereby lead to clathrin-independent endocytosis. HSPG-Opa binding also can lead to interactions with serum factors such as vitronectin and fibronectin, which then mediate endocytosis via host-cell integrin receptors. The CD66 family is present on epithelial cells and neutrophils, recognizes many different Opa proteins, and mediates nonopsonic phagocytosis, a process distinct from antibody- and complement-mediated phagocytosis.[102,103,140,197,221,328]

PORIN PROTEIN. Formerly designated protein I, porin protein is the most common gonococcal outer-membrane protein. Porin is 34 to 36 kd, is exposed on the surface, exists as a trimer in the membrane, and is physically proximate to LOS and Rmp.[28,159] The trimer forms an anion-specific pore in the bacterial membrane that allows small water-soluble molecules to enter the cell.[343] The *por* locus has two alleles that encode chemically and immunologically distinct classes of porin protein—PorA and PorB—that are similar to porin proteins in other gram-negative bacteria. A given strain expresses only PorA or PorB, and many antigenic variants of both exist (thereby forming the basis for serotyping).[50,192]

The porin trimer appears to translocate into the host-cell membrane and is able to disrupt neutrophil degranulation, the oxidative burst, and phagosome maturation,[151,209,230] in addition to induction of apoptosis in epithelial cells and neutrophils in vitro.[234] Exposure to gonococcal porin protein induces the typical structural and biochemical changes seen in apoptotic cells.[233] Porin translocation permits rapid influx of Ca^{2+} into the host cell from the external environment.[234] Porin proteins also may play a role in endocytosis mediated by the binding of HSPG and Opa protein[19] and in down-regulation of complement by binding with C4b-binding protein.[265]

THE ABILITY TO USE IRON. Iron is an essential nutrient for *N. gonorrhoeae*, and in host tissues, iron is sequestered in hemin compounds as ferritin or is bound to lactoferrin or trans-

ferrin. The species does not produce any siderophores but relies on an iron-repressible system that scavenges iron from transferrin, lactoferrin, and hemoglobin. This system is composed of numerous proteins, some of which serve as receptors for the aforementioned iron-bearing ligands.[26,75,92,333] The ability to use iron from transferrin is a general property of pathogenic *Neisseria* spp. Transferrin- and lactoferrin-binding proteins are required for gonococci to cause experimental urethritis.[91]

IgA PROTEASE. All gonococci (and meningococci, but not nonpathogenic *Neisseria*) make a protease that cleaves both serum and secretory IgA1 (but not IgA2) at the hinge region with the release of Fab and Fc fragments. This protease may help the organism evade host IgA at the mucosal surface, especially early in secondary infections, when preexisting antibodies may be present. However, mutant strains without the IgA protease have limited ability to grow inside epithelial cells. The protease appears to cleave a host-cell intracellular protein (LAMP1) involved in phagosome compartmentalization.[205] IgA1 protease is not required for gonococci to cause experimental urethritis,[178] thus suggesting that its intraphagosome function may be more important in pathogenesis.

LIPO-OLIGOSACCHARIDE. Gonococci express LOS complexes of 3 to 7 kd on their cell surface. LOS consists of a lipid A moiety and a core polysaccharide composed of ketodeoxyoctanoic acid, heptose, glucose, galactose, glucosamine, galactosamine, or any combination of these constituents.[146] The lack of a long polymeric sugar attached to the core distinguishes LOS from the lipopolysaccharides of other gram-negative bacteria. The core sugar antigens of LOS are subject to intrastrain and interstrain variation, and a single strain may express as many as six variants of LOS.[105,145] Numerous genes are involved in LOS synthesis; these genes undergo high-frequency phase variation similar to *opa* genes.[139] LOS terminal sugars mimic the structure of certain human glycosphingolipids.[146]

Gonococci with predominately short LOS molecules appear to be more sensitive to killing by human serum but also more able to invade eukaryotic cells. Strains with longer LOS are more serum-resistant but noninvasive.[329] Longer LOS moieties are sialylated readily with host neuraminic acid by a bacterial sialyltransferase that appears to shield both LOS and porin molecules from antibody binding, thus providing protection from complement-mediated killing in serum.[33,334]

LOS from serum-sensitive strains is able to activate the classical complement pathway and may do so in the absence of antibody.[295] LOS induces the production of cytokines (TNF-α, IL-1β, IL-6, IL-8) by urethral epithelial cells[157] and can mediate host defensin-enhanced adherence to epithelial cells.[138]

CELL WALL PEPTIDOGLYCAN. Gonococci shed membrane fragments with peptidoglycan into their environment during exponential growth. Peptidoglycan monomers have numerous biologic properties, including activation of complement and modulation of mononuclear cell proliferation. These fragments also damage fallopian tube mucosa in organ culture, thus suggesting a pathogenic role for the compounds in invasive disease.[142,216]

REDUCTION MODIFIABLE PROTEIN. Rmp (formerly designated protein III) is an antigenically conserved, 30- to 31-kd protein that is present in all pathogenic *Neisseria*. Rmp is located proximate to LOS and porin in the outer membrane. Antibodies that bind to Rmp epitopes block the bactericidal effect of the complement-fixing IgM antibodies that recognize LOS. Women with preexisting anti-Rmp antibodies appear to be more susceptible to the development of infection than do those without such antibodies.[274,275,308]

RIBOSOMAL PROTEIN L12. Ribosomal protein L12 mimics the structure of human chorionic gonadotropin, the natural ligand for lutropin receptors. It is able to bind to lutropin receptors in the upper female genital tract, which may facilitate ascending infection in females.[115,312]

CHARACTERISTICS OF STRAINS CAUSING DISSEMINATED DISEASE

The increased virulence of some strains is suggested by observations such as a micro-epidemic of gonorrhea involving one asymptomatic infected male and eight female contacts.[154] Seven of the women were infected symptomatically, and four experienced disseminated infection. Clinical data repeatedly have shown that most infected women have asymptomatic infection and dissemination occurs relatively rarely.

Strains of *N. gonorrhoeae* obtained from adult patients with disseminated disease usually are less susceptible to the bactericidal activity of sera[38,83,272,273,297] (see discussion of LOS earlier). These strains have an atypical growth pattern on agar[226] that reflects the absence of Opa protein expression, which suggests that the genetic variation capacity described earlier allows adaptation to different niches and probably helps organisms elude the host response.[23] Unlike most gonococcal strains, many invasive strains (designated AHU^-) require arginine, uracil, and hypoxanthine for growth.[191] A high degree of sensitivity to penicillin also has been characteristic of most strains from patients with disseminated disease.[338] *Many also are susceptible to vancomycin*, which may prevent them from being detected in selective media but not in the usual media used for blood culture.

HOST RESPONSE

The adult response to local and systemic infection with *N. gonorrhoeae* has been investigated extensively, but no such studies have been performed on infected children. Gonococcal infections usually are characterized by intense inflammation that involves neutrophilic and mononuclear granulocytes. The vast majority of gonococcal cells ingested by neutrophils are killed.[52] Gonococci are susceptible to the oxidative products produced by neutrophils but also can be killed efficiently by nonoxidative products such as cathepsin G.[296]

An intact complement system is essential for successful eradication of the organism. Persons with inherited or acquired complement deficiencies may be predisposed to contracting disseminated gonococcal infection, as is the case with meningococcal infection. Approximately 13 percent of patients with disseminated disease have complement deficiencies.[119,246]

Adherence of gonococci in vitro induces the activation of nuclear factor κB and activator protein 1, which leads to up-regulation of mRNA for and release of numerous cytokines and chemokines: macrophage colony-stimulating factor, TNF-α, tumor growth factor-β, monocyte chemoattractant protein 1, IL-1β, IL-6, and IL-8.[238] Intra-urethral challenge leads to increased levels of IL-8, IL-6, and TNF-α in urine before the onset of symptoms and in plasma at the onset of symptoms.[266]

Multiple episodes of gonorrhea may occur in a single individual in a short period of time and even may be caused by the same strain. The antigenic diversity and ease of altering the antigenicity of both pili and opacity proteins undoubtedly contribute to the insufficiency of the host response in preventing reinfection.

Circulating humoral antibody to the infecting strain is measured by an assay of bactericidal antibody and is present in most persons who have prolonged mucosal colonization with *N. gonor-*

rhoeae.[172,187] In addition, women in whom PID develops usually produce bactericidal antibody during the infection. IgA- and IgG-blocking antibodies against some gonococcal antigens appear to block killing of the bacteria mediated by otherwise bactericidal IgG and IgM antibodies (see Rmp information earlier).[272] Serum antibody responses are greater in patients with invasive disease (e.g., bacteremia, salpingitis).

Secretory IgA antibody is present in the urethral exudate of men with gonorrhea and in the genital secretions of women with gonorrhea.[247] The development of local IgA antibody occurs more rapidly and is more transient than is development of the serum bactericidal response. Microbial IgA1 protease is not required for infection but may play a role in reinfection.

A cellular immune response to gonococcal antigens in patients with uncomplicated gonorrhea has been demonstrated in vitro.[200] The significance of this response in controlling or preventing infection is unknown.

PERINATAL GONOCOCCAL INFECTIONS

Since the 1970s, health clinics have encouraged the routine screening of sexually active women for gonorrhea. Such screening is especially important during pregnancy, and rates of gonorrhea in pregnant women range from exceedingly low to approximately 10 percent. Adolescent girls have a higher prevalence of gonorrhea than do older women of child-bearing age, and this prevalence probably translates into higher rates of gonorrhea in pregnant adolescents. Rates of gonococcal infection in neonates reflect the frequency of infection in pregnant women. Recognition of gonorrhea early in pregnancy identifies a population at risk that should be monitored sequentially for reinfection throughout pregnancy.[180]

The spectrum of infection with *N. gonorrhoeae* appears to be similar in pregnant and nonpregnant women. Most women are asymptomatic. Pharyngeal infection seems to occur more commonly during pregnancy, perhaps reflecting altered sexual practices. One study reported that 39 percent of patients with *N. gonorrhoeae* at any site had concurrent involvement of the pharynx and that 30 percent had pharyngeal infection as the sole manifestation.[90] In adolescent girls, pregnancy and menstruation are associated with disseminated disease.[161]

Gonorrheal infection puts both the mother and infant at risk for the development of other forms of gonococcal disease. Pregnant women have an increased risk for the development of gonococcal septic arthritis, and most such cases occur during the third trimester or in the immediate postpartum period.[40,161] Although the incidence of gonococcal arthritis is low, many cases have occurred in pregnant adolescents.

Gonococcal PID and acute salpingitis also may complicate the pregnancy of an infected woman.[132] These complications usually occur during the first trimester and have been associated with a high rate of fetal loss. An increased incidence of postpartum fever in women with untreated gonorrhea likewise has been noted. A new mother with complications of peripartum gonorrhea may have difficulty providing care for her child.[175]

Maternal gonococcal infection has been associated with abnormalities in labor and delivery that may affect the infant adversely. Prolonged rupture of membranes, premature delivery, chorioamnionitis, funisitis, and a clinical diagnosis of sepsis occur frequently in infants with *N. gonorrhoeae* detected in the gastric aspirate during delivery.[7,116,153,280]

Hazards to the fetus posed by maternal gonorrhea include septic abortion, perinatal death, prematurity, perinatal distress, and premature rupture of membranes. Table 100–2 tabulates the proportions in six studies of infants born to infected mothers who experienced these problems. A controlled study in an area with a high prevalence of gonorrhea found that maternal infection with *N. gonorrhoeae* was associated significantly with preterm birth and that 14 percent of preterm births in this population were attributable to gonococcal infection.[118]

GONOCOCCAL OPHTHALMIA NEONATORUM

Epidemiology

Researchers have recognized for centuries that ophthalmia neonatorum occurs in infants born to women with a vaginal discharge. Recommendations for flushing the eyes of a newborn commonly were given before the 20th century.[218] In the late 1800s, Neisser helped establish the relationship between the gonococcus and neonatal ophthalmia.

By 1881, Dr. Carl Sigmund Franz Credé had recognized that asymptomatic disease in the mother was a potential source of infection.[96] Gonorrhea was highly prevalent in Europe, and Credé described an increased incidence in patients from lower socioeconomic backgrounds. Mechanical cleansing of the birth canal failed to protect the infant from gonococcal ophthalmia neonatorum (GON), which was occurring in approximately 10 percent of newborns in major cities. The infection was the cause of a large proportion of admissions to schools for the blind.[17] Credé described the technique of instillation of 2 percent silver nitrate ($AgNO_3$) into the infant's conjunctival sac, which remains one method recommended for preventive procedures today.

By 1930, most states in the United States required that all newborns receive $AgNO_3$ prophylaxis (i.e., the Credé procedure). At present, prophylaxis of some form is required by most states. GON decreased as a cause of admission to schools for the blind in the United States, from an average of 24 percent from 1906 to 1911 to 0.5 percent from 1951 to 1955.

The worldwide decline in adult gonorrhea in the 1950s probably contributed to the decreased recognition of disease in newborns during that period. The incidence of GON rose in the 1960s and 1970s along with the increased incidence of gonorrhea in the general population and was very high in some developing nations.[197] In Los Angeles, California, the rate rose from 9 per 100,000 live births in 1957 to 1958 to 56 per 100,000 live births in 1962 to 1963. In a New York hospital between 1970 and 1973, a rate of 145 per 100,000 births was reported, and in a hospital in North Carolina during 1969 and 1970, a rate of 265 per 100,000 live births was proved by culture.[307] In the late 1970s and 1980s, GON again subsided in developed countries, but it has remained a major problem in underdeveloped nations.

Factors associated with higher rates of GON include lower socioeconomic class of the mother. An increased incidence of GON in infants of unwed mothers and mothers who have not had prenatal care also may exist.[304] Previous treatment of gonorrhea during pregnancy likewise is associated with an increased incidence of GON.[180]

The incidence of prematurity in infants with GON often is reported to be higher. Whether it is due to an adverse effect of gonorrhea on the pregnancy or because GON may be recognized more readily in premature infants has not been established. Premature rupture of membranes appears to increase the risk of GON occurring.

Prevention

Prophylaxis for GON has been accomplished mainly through the local instillation of either 1 percent $AgNO_3$ or one of several antimicrobial agents. In the United States, prophylaxis for GON is recommended for all infants immediately after birth and is required by law in most states. Prophylaxis regimens using a 1 percent solution of $AgNO_3$, 1 percent tetracycline ointment, or 0.5 percent erythromycin ophthalmic ointment have been con-

sidered equally effective for GON. Each is available in single-dose units, which are preferred over multidose tubes. One unit should be instilled into the eyes of every neonate as soon as possible after delivery. Instillation may be delayed for as long as 1 hour to facilitate parent-infant bonding. Povidone-iodine 2.5 percent solution also may be effective, but this agent is not recommended for use in the United States at this time.[5] Bacitracin is not effective for prevention of GON.[141]

The efficacy of tetracycline and erythromycin in the prevention of tetracycline-resistant *N. gonorrhoeae* ophthalmia is unknown. Both probably are effective because of the high concentration of drug in these preparations. Studies of these agents for prevention of *Chlamydia trachomatis* ophthalmia have been conflicting. Povidone-iodine may be more effective in preventing *C. trachomatis* ophthalmia than is topical $AgNO_3$ or erythromycin is. None of these agents prevents chlamydial colonization of the nasopharynx. Some Western countries with low rates of gonococcal and chlamydial infection do not recommend routine ophthalmic prophylaxis.[5,149]

CURRENT PROPHYLAXIS RECOMMENDATIONS IN THE UNITED STATES. In the United States, the 2006 version of the Sexually Transmitted Diseases Treatment Guidelines of the Centers for Disease Control and Prevention (CDC) no longer includes the use of silver nitrate. This change reflects the lack of availability of this agent in the United States at that time and not concerns about safety or effectiveness. The recommended agents are erythromycin 0.5 percent ophthalmic ointment or tetracycline 1 percent ophthalmic ointment, either one administered as a single application to each eye as soon as possible after delivery. None of the topical agents prevents mother-to-infant transmission of *C. trachomatis*.[64]

SILVER NITRATE AND GONOCOCCAL OPHTHALMIA NEONATORUM. Numerous studies have compared the efficacy of $AgNO_3$ and no prophylaxis or prophylaxis with another agent in the prevention of GON, and these studies have been reviewed elsewhere.[76,248,281] Until the late 1900s, most studies of the efficacy of $AgNO_3$ were not randomized and did not document rates of perinatal gonococcal exposure in the infants studied. Such studies involved large numbers of infants and consistently have shown lower rates of GON in treated groups than in untreated controls. Observations of a decreased frequency of blindness from GON in the population during the last half century, even during periods of increased rates of gonorrhea in women of child-bearing age, further support the benefits of $AgNO_3$ prophylaxis.

In a randomized trial of Kenyan infants born to mothers with gonorrhea reported in 1988, rates of GON were 42 percent in the control group, 7 percent in the group treated with 1 percent $AgNO_3$ (83% reduction), and 3 percent in the group treated with 1 percent tetracycline (93% reduction). The two treatment arms were not statistically different.[199] In a randomized trial in the United States involving infants born to women without gonococcal infection, infants treated with 1 percent $AgNO_3$ had a 39 percent lower rate (statistically significant) of conjunctivitis of any type in the first 2 months of life than did infants receiving no prophylaxis. Infants treated with 0.5 percent erythromycin ointment in this trial had a 31 percent reduction in comparison to control infants, but this result was not statistically significant.[20]

The major advantages of the use of $AgNO_3$ for prophylaxis are the lack of allergic potential, the absence of development of bacterial resistance to the compound, and very low cost. Two outbreaks of erythromycin-resistant staphylococcal conjunctivitis in newborn nurseries have been associated with the use of erythromycin eye ointment as ocular prophylaxis, but the outbreaks remitted when $AgNO_3$ was substituted. Disadvantages include the occurrence of conjunctival irritation along with the development of exudate in many babies and lack of effectiveness if infection already is established before drops are instilled into the eye. A solution of $AgNO_3$ that becomes concentrated may cause ophthalmic injury, but this problem largely has been alleviated by dispensing doses in ampules, which prevents evaporation.

Failure of $AgNO_3$ prophylaxis (as well as other agents for prevention of GON) does occur. Improper administration of $AgNO_3$ such that the solution does not reach the conjunctival sac, irrigation performed too quickly after instillation, and inadvertent omission of prophylaxis can lead to apparent failure. When gonococcal infection of the eye has begun before birth, $AgNO_3$ is not expected to prevent further progression. The increased risk of GON associated with premature rupture of membranes is due to exposure to and establishment of infection before actual delivery. In some cases of premature rupture of membranes, clinically apparent GON can be present at the time of birth.

Despite these problems, the use of 1 percent $AgNO_3$ remains a widely accepted, carefully evaluated, and safe form of GON prophylaxis. The occasional failure of $AgNO_3$ prophylaxis emphasizes that it is preferable to prevent GON through identification and treatment of pregnant women.

Clinical Features

The newborn eye is subject to colonization with numerous bacterial organisms that cause infections that usually are clinically mild, nonprogressive, and characterized by conjunctival discharge. The bacteria most commonly associated with conjunctival discharge are *Haemophilus influenzae*, *Streptococcus pneumoniae*, *Staphylococcus aureus*, *Neisseria cinerea*, *Klebsiella pneumoniae*, enterococci, and *C. trachomatis*.[110,195] Viral causes include herpes simplex virus and adenoviruses. In contrast, infection of the eye of a newborn with *N. gonorrhoeae* results in disease of a severity that, despite frequently being mild, can be rapidly destructive and lead to scarring and blindness. The chemical conjunctivitis that can result from $AgNO_3$ prophylaxis typically starts within 6 to 24 hours after administration and disappears within 24 to 48 hours.

Colonization during delivery is followed by an incubation period of 2 to 5 and usually less than 3 days, but cases occasionally may be recognized for 2 to 3 weeks after delivery.[130] A discharge typically develops that initially is watery but usually becomes thick and mucopurulent within a short time and may contain blood (Fig. 100–4). The disease generally is bilateral. Early findings include prominent edema of the conjunctivae and lids, followed by edema and later ulceration of the cornea or spread to wider areas in severe cases. Rapid arrest of the disease is essential because the degree of corneal involvement determines whether vision will be preserved. Some cases are self-limited and have benign outcomes, and occasional cases of asymptomatic GON have been discovered during routine screening.[261] Perforation of the globe and panophthalmitis can result from extensive local disease. The conjunctivae may serve as a portal of entry for gonococcal septicemia, arthritis, or other manifestations of invasive disease, but such events seldom occur.

A presumptive diagnosis may be made by demonstrating typical gram-negative diplococci by Gram stain of conjunctival exudate. Because other organisms also may cause exudative infection of the conjunctivae, laboratory confirmation of the diagnosis of GON depends on culture results of *N. gonorrhoeae*.

Invasive disease rarely occurs in association with GON. However, in the United States, the recommendation is that neonates with GON or any other manifestation of gonococcal infection have cultures performed on blood and CSF (as well as any local sites of infection) and be hospitalized pending culture results.[5]

GONOCOCCAL SCALP ABSCESS AND OTHER LOCAL INFECTIONS

Gonococcal infections of scalp wounds occur especially after fetal monitoring electrodes have been used on infants born to infected mothers.[99] The lesions may produce extensive local inflammatory disease and necrosis and can be a focus for disseminated infection. A scalp wound in a neonate should be cultured for gonococci, as well as other likely pathogens, which include *Staphylococcus* spp., group B streptococci, *H. influenzae*, and gram-negative enteric flora. Herpes simplex also may be present in areas of injury to the scalp of newborns. Overall, approximately 1 in 200 births that are monitored with fetal scalp electrodes has been complicated by infection at the monitoring site.[260]

Neonatal gonococcal vulvovaginitis, proctitis, rhinitis, funisitis, and urethritis have been described but are infrequent findings.[149] Gonococcal colonization of the oropharynx, gastric fluid, or both occurs relatively frequently in perinatally exposed infants, however. Pharyngeal colonization is present in as many as 35 percent of neonates who have GON.[127] A summary of selected studies on the incidence of neonatal gonococcal colonization and disease in exposed infants is presented in Table 100–3.

Infants with localized gonococcal infection at any site should have cultures obtained of blood and CSF, as well as the local site, and should be hospitalized pending the availability of culture results (see "Treatment" later).

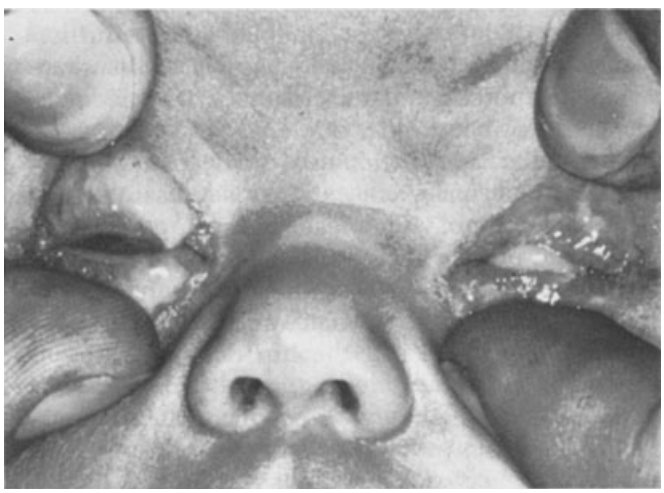

Figure 100–4 Gonococcal ophthalmia neonatorum. The usual clinical finding is bilateral conjunctivitis that becomes progressively purulent if untreated. *(From Gutman, L. T.: Gonococcal infections. Semin. Pediatr. Infect. Dis.* 16*:4, 2005.)*

SYSTEMIC DISEASE IN THE NEONATE

Disseminated disease occurs in 1 percent or less of infants who are perinatally exposed to gonococcal infection[13] (see Table 100–3). Septic arthritis is the most common form of disseminated gonococcal infection in neonates. Gonococcal arthritis of the newborn was described extensively between 1900 and 1930 by Cooperman[88,89] and Wehrbein,[332] and recent case reports suggest that the disease remains similar. Clinical findings usually become evident when the infant is 1 to 4 weeks old. Although a few infants with gonococcal arthritis will have evidence of GON or other sites of mucosal or skin infection at the time of onset of arthritis, most do not.

The signs and symptoms of gonococcal septic arthritis are similar to those of joint infection caused by other microbes in the neonatal period, including a predominance of polyarticular involvement. Infection most frequently involves the ankles, knees, wrists, and hands.[88] The hip may be infected with minimal signs other than pseudoparesis. Involvement of a joint may include suppurative arthritis, inflammatory disease of the periarticular structures, and tenosynovitis. Leukocytosis usually occurs, and most infants have a positive culture and compatible Gram stain results from the synovial fluid of the involved joint.[135]

In a hospital-associated outbreak of gonococcal polyarthritis that occurred in 1927, involvement of the joint was noted in 53 of 67 infected infants (79%). Other studies of outbreaks have indicated that perhaps 15 percent of children with gonorrhea acquire gonococcal arthritis. These rates probably are considerably higher than the estimated 1 to 3 percent incidence in adults and suggest an increased risk of dissemination in infants and children. For example, case reports have described gonococcal arthritis in both mother and newborn infant.[143] Prompt drainage of septic hips, along with initiation of antimicrobial therapy, is necessary because of the risk of development of aseptic necrosis of the femoral head. Long-term dysfunction from gonococcal infection of other joints seldom occurs.[89,149,163,193]

Although gonococcal sepsis can develop in neonates with or without associated septic arthritis, the bacteremic phase of spread to the joints from the initial sites of infection generally is clinically silent. Premature infants seem to be more at risk for the development of sepsis with bacteremia than term infants are. Meningitis has been documented but appears to be an exceedingly rare manifestation of neonatal gonococcal infection.[32,149,153] In the newborn period, the gastric aspirate may be cultured to determine colonization from a maternal source.

GONOCOCCAL DISEASE BEYOND THE NEONATAL PERIOD

LOWER GENITAL TRACT INFECTION IN PREPUBERTAL GIRLS

Gonococcal vaginitis or vulvovaginitis is the most common form of gonorrhea in prepubertal girls beyond the neonatal period. In

TABLE 100–3 Incidence of Neonatal Gonococcal Disease in Exposed Infants

Site of Neonatal Infection	Rate of Positive Cultures (%)	Population	Reference
Conjunctiva	0-10	Exposed infants who underwent $AgNO_3$ ocular prophylaxis	Edwards et al.,[116] Allen and Barrere,[4] Armstrong et al.,[11] Laga et al.[197]
	2-48	Exposed infants who had no ocular prophylaxis	Rothenberg,[281] Fransen et al.,[127] Laga et al.[197]
Orogastric fluid	26-40	Infants of infected mothers	Handsfield et al.,[153] Edwards et al.[116]
Oropharynx	35	Infants with gonococcal ophthalmia	Laga et al.[197]
Disseminated disease as a proportion of all neonatal gonorrhea	0-1 (rare)	Reported series of neonatal gonococcal disease	Folland et al.,[125] Tomeh and Wilfert,[323] Wald et al.,[330] Edwards et al.,[116] Fransen et al.[127]

contrast to that of postpubertal females, the anestrogenic vaginal mucosa of prepubertal girls creates an alkaline environment that is colonized and infected more readily with *N. gonorrhoeae*. Infection of the endocervix, urethra, paraurethral and Bartholin glands, and upper genital tract occurs only rarely.

Gonococcal vaginitis in prepubertal girls almost always is symptomatic, with vulvar erythema and a profuse vaginal discharge. The girl may complain of dysuria, urinary frequency, vulvar discomfort, or pain while walking.[235,297,300] Asymptomatic cases may occur but are uncommon.

Symptoms and signs should resolve promptly within a few days after treatment is initiated, but acute manifestations may persist for several weeks if the child is not treated. The natural course of disease is for the inflammation to subside and the discharge to become scant and seropurulent. Infection may resolve spontaneously but occasionally may persist until the girl reaches puberty.

Prepubertal vulvovaginitis can be caused by numerous irritative and infectious agents, including pinworms, foreign bodies, group A streptococci, *Neisseria meningitidis*, *Neisseria sicca*, and *Moraxella catarrhalis* (see also Chapter 48). Vulvovaginitis can mimic urinary tract infection, and pyuria can be seen on urinalysis with gonococcal and other causes of vulvovaginitis.[128,207]

Although ascending infection seldom occurs, it may result in salpingitis or peritonitis. One study found that 10 percent of girls with gonorrhea had signs compatible with peritonitis, including fever, diffuse abdominal pain, leukocytosis, and decreased bowel sounds.[46] Salpingitis and periappendicitis may cause findings similar to those of appendicitis. Therefore, perineal examination for vaginal irritation or discharge should be done before performing abdominal surgery in young girls.[12]

In children with gonorrheal infection of the genitourinary tract, concomitant anorectal and tonsillopharyngeal colonization is a common findng[240] because sexual abuse usually is the means of infection.

LOWER GENITAL TRACT INFECTION IN POSTMENARCHEAL FEMALES

Gonococcal infections in adolescent girls are similar to those in adults. The endocervix is the primary site of urogenital infection, although the external genitalia, urethra, vulvar mucosa, and vestibular glands also may be infected. Symptoms of acute infection usually begin 3 to 5 days after exposure with the development of a profuse, purulent vaginal discharge. The vulvar tissues are inflamed, with resultant pruritus and a burning sensation. Urethritis often is present initially and can lead to dysuria with urinary frequency and urgency. Urethral discharge can be seen but is much less prominent than in males. A purulent discharge from the vestibular and paraurethral glands also may be noted.

The endocervical mucosa is edematous, inflamed, and often friable. A profuse yellow-green discharge is present and detaches easily from the surface. The zone of endocervical ectopy that normally is present in as many as 50 percent of adolescents may appear bright red, does not bleed easily when touched with swabs, and should not be mistaken for cervicitis. Ectopy that appears swollen and friable suggests cervicitis. Nabothian inclusion cysts (transparent and grayish) also are normal findings.

Infection of the Bartholin (major vestibular) gland or paraurethral (Skene) gland ducts may develop during acute gonorrhea. Bartholin gland abscesses appear as large, circumscribed painful swellings of the dorsal aspect of the labium minus (see Fig. 48–12). They occur in 5 percent of females with endocervical gonorrhea.[269] Paraurethral duct abscesses (see Fig. 48–11) appear as small painful swellings in the urethrovaginal septum and may cause dysuria. Rupture into the urethra can create a urethral diverticulum. Bartholin gland or paraurethral duct abscesses should be incised and drained, but small asymptomatic nodules do not require drainage.

If the gonococcal infection goes untreated, the acute symptoms generally subside in 8 to 10 weeks. Persisting acute symptoms are more likely to represent reinfection than chronic infection. Chronic urethritis, thickening of the vestibular glands and paraurethral ducts, and chronic cervicitis can occur. A secondary nonspecific vaginitis can develop as a result of irritation of the mucosa from persisting profuse endocervical discharge.

Mucopurulent cervicitis can be caused by *N. gonorrhoeae*, *C. trachomatis*, *Gardnerella vaginalis*, *Mycoplasma hominis*, *Ureaplasma urealyticum*, and group B streptococci. The overlap in clinical manifestations of infections caused by these microbes and the frequency of infection by two or more simultaneously preclude establishing the diagnosis by clinical findings alone.

Adolescents may be unaware of their infection, especially if they have a preexisting profuse discharge as a result of nonspecific vaginitis or trichomoniasis. Early recognition and treatment of lower genital tract gonococcal infection may prevent extension to and complications of upper genital tract infection. Therefore, sexually active adolescent females should be examined for gonococcal infection as part of their routine health care.[158,286]

A review of reported complications in 1232 cases of gonococcal vaginitis in the pre-antibiotic era revealed that 35 percent had urethritis, 19 percent had proctitis, and 6 percent had peritonitis.[21]

Urinalysis may provide clues to gonococcal infection in some cases. In a cross-sectional study of 296 sexually active females 14 to 22 years of age, gonorrhea or trichomoniasis was present in 65 percent who had sterile pyuria.[168]

UPPER GENITAL TRACT INFECTION IN POSTMENARCHEAL FEMALES

PID will develop in 10 to 17 percent of females with endocervical gonococcal infection. Conditions encompassed by the term PID include endometritis, parametritis, salpingitis, oophoritis, tuboovarian abscess, and pelvic peritonitis. PID can be caused by a variety of bacteria. Anaerobes, including *Bacteroides*, *Peptococcus*, and *Peptostreptococcus* spp., are the organisms usually recovered. *N. gonorrhoeae* is present in 25 to 50 percent of cases, similar to the proportion of cases from which *C. trachomatis* is recovered. Coliform organisms, *G. vaginalis*, *M. hominis*, *U. urealyticum*, and various streptococcal species also may be involved. PID develops when these organisms are able to ascend into the uterus, fallopian tubes, and beyond from the lower genital tract. Multiple species may be involved in a single episode. Gonococcal PID often occurs during or just after menses.[130,161] Identification of the specific microbial cause or causes of PID is complicated by the difficulty of obtaining fallopian tube specimens before initiating therapy.

Common findings in PID include an acute onset of lower abdominal pain with tenderness, fever, tenderness on lateral motion of the cervix, adnexal tenderness that generally is bilateral, and adnexal fullness. Vaginal discharge, urinary symptoms, and irregular vaginal bleeding also may be present. In moderate to severe cases, abdominal pain frequently is bilateral, exacerbated with movement, and continuous. Nausea, vomiting, marked abdominal tenderness, an abdomen that appears tense, and fever exceeding 39° C may be present. Such patients often appear to be ill and may have tachycardia consistent with the height of fever.[294] Leukocytosis and elevated erythrocyte sedimentation rates are common findings. Alternatively, PID may be clinically silent or cause only mild pain without discernible tenderness, vaginal discharge, or leukocytosis. Such mild cases can go unrecognized by patients and physicians.

Establishing the diagnosis of PID may be difficult, and the differential diagnosis includes numerous other lower abdominal conditions such as appendicitis, ectopic pregnancy, cholecystitis, mesenteric adenitis, pyelonephritis, and septic abortion. Sonography and pregnancy testing may be helpful in diagnostic decision making. No single symptom, sign, or laboratory finding is sensitive and specific for the diagnosis of PID. Combinations of findings can improve the sensitivity but do so at the expense of reduced specificity, and vice versa.

Initiation of empiric therapy for PID should be considered if the minimal criteria of lower abdominal tenderness, adnexal tenderness, and cervical motion tenderness all are present and no other cause for these findings is readily apparent. More elaborate evaluation may be needed in some cases because incorrect diagnosis and management might lead to unnecessary morbidity. Additional criteria that support the diagnosis of PID include an oral temperature higher than 38.3° C (101° F), an abnormal cervical or vaginal discharge, elevated erythrocyte sedimentation rate, increased C-reactive protein level, and laboratory documentation of cervical infection with *N. gonorrhoeae* or *C. trachomatis*. Some cases may require pursuit of definitive criteria for diagnosing PID, which include histopathologic evidence of endometritis on endometrial biopsy, transvaginal or abdominal ultrasonography or other imaging studies that show thickened fluid-filled fallopian tubes with or without free pelvic fluid or a tubo-ovarian abscess, and laparoscopic abnormalities consistent with PID.[59,176]

The outcome for fertility probably is improved with prompt and vigorous therapy. In the past, physicians have recommended that all adolescents suspected of having PID be hospitalized for therapy. More recently, inpatient observation and treatment have been recommended when a surgical emergency (e.g., ectopic pregnancy or appendicitis) cannot be excluded; when compliance with or tolerance of an outpatient treatment regimen and follow-up within 72 hours cannot be ensured; when the illness appears to be severe (e.g., pelvic or tubo-ovarian abscess, overt peritonitis); or when the patient is immunocompromised, is pregnant, or has failed to respond to outpatient therapy.[5] All courses of therapy should include treatment that is appropriate for *C. trachomatis* and other causes of PID, in addition to *N. gonorrhoeae*.

Complications of PID include tubo-ovarian abscess, perihepatitis (Fitz-Hugh-Curtis syndrome), future ectopic pregnancy, and infertility. Perihepatitis is characterized by right upper quadrant abdominal pain that may radiate to the shoulder. Nausea, fever, and other symptoms and signs of PID may be present as well. Leukocytosis occurs commonly, and liver enzymes are elevated in some cases. The differential diagnosis of perihepatitis includes pleuritis, cholelithiasis, subphrenic abscess, and perforated ulcers.[206]

An estimated 15 percent of women may become sterile after a single episode of PID and 50 percent after three infections. Because of scarring and fibrosis in the fallopian tube, patency is compromised and fertility is jeopardized.[53,120,121] PID in adolescents is particularly likely to result in infertility and ectopic pregnancy, and PID is the single most common cause of infertility in young women.[43,231] Between 1970 and 1980, the rate of ectopic pregnancies per 1000 live births increased from 4.8 to 14.5, and between 1975 and 1981, the rate of hospitalization of females 15 to 19 years of age for salpingitis was 4 per 100,000.[330] These increases correlated with rising rates of gonococcal infection in the affected populations. Data from several studies of gonococcal disease in adolescents are presented in Table 100–4.

Risk factors for the development of PID include young age at the time of acquisition of gonococcal disease or other STDs, a history of previous PID, multiple sexual partners, and the use of an intrauterine device (IUD) for contraception. The immature cervix may be at particular risk for progression to upper tract disease.[302] Disseminated gonococcal infection may accompany asymptomatic infection of an IUD,[84] and the rate of acute PID in those who use IUDs may be increased.

GENITAL TRACT INFECTION IN MALES

Gonococcal urethritis in prepubertal boys is a less frequent event than is vaginitis in girls because of gender differences in rates of sexual abuse. In children with gonorrheal infection of the genitourinary tract, concomitant anorectal and tonsillopharyngeal colonization is a frequent finding.[240]

Urethritis is the primary manifestation of gonococcal infection in males of all ages beyond the neonatal period. Even in young boys, the disease usually is symptomatic and resembles gonococcal urethritis in men.[95,132] Dysuria, purulent discharge, or both develop 2 to 7 days after exposure. Patients generally are afebrile. Purulence typically is greater than with nongonococcal urethritis, but symptoms often are mild enough that patients (or their parents) may delay seeking medical care for weeks. Associated inguinal adenopathy occurs rarely.

At least 5 percent of cases of gonococcal urethritis in males are asymptomatic, and asymptomatic infection can occur at all ages. Asymptomatic pyuria is a manifestation with which the pediatrician should be familiar.[100] It may be the only finding in some cases and should raise suspicion of gonococcal or chlamydial infection in boys who may have been sexually abused or in sexually active adolescent males.

Males with asymptomatic infection are a major reservoir for transmission to their sexual partners.[100,125] Untreated male urethral infection may persist for as long as 6 months.

Complications of gonococcal infection in males now occur much less frequently than in the pre-antibiotic era. Epididymitis is the most common complication. Unilateral swelling, pain, and erythema in the posterior aspect of the scrotum are the usual features. Fever may occur. If untreated, the infection may progress to involve the ipsilateral testis. Hydrocele may result from secretion of fluid into the potential space of the tunica vaginalis. Epididymitis can lead to testicular infarction, abscess, infertility, prostatitis, paraurethral abscesses, and penile lymphangitis, but these local complications are rare findings. Perihepatitis in males has been described but occurs extremely infrequently.[194]

TABLE 100–4 Prevalence of Gonorrhea and Other Sexually Transmitted Diseases in Clinics for Adolescents

Reference	Location	Total Clinic Population Studied	Gonorrhea (%)	*Chlamydia* Infection (%)	Other
Shafer et al., 1984[295]	California	366	15	4	*Trichomonas* infection
Golden et al., 1984[136]	New York	186	10	10	Syphilis, *Trichomonas* infection
Demetriou et al., 1984[106]	Oklahoma	839	14	—	
Mulcahy and Lacey, 1987[232]	Leeds	210	14	16	*Trichomonas* infection
Jamison et al., 1995[177]	Colorado	632	7	—	Human papillomavirus infection

DISSEMINATED DISEASE

Dissemination requires invasion of the bloodstream from local infection of the mucous membranes of the genital tract, rectum, pharynx, or conjunctiva. Bacteremia then can lead to infection of other sites. The risk of dissemination in children after mucosal infection appears to be higher than the 1 to 3 percent rate in adults. Dissemination in adults seems to be more common with asymptomatic infections, many of which are caused by AHU$^-$ gonococcal strains (see "Pathogenesis" earlier).[189] Deficits in the complement system have been associated with disseminated disease in adults, but the frequency of such deficiencies in children is unknown.

As in neonates, gonococcal arthritis is the most common form of disseminated disease in older children, adolescents, and adults. The ankles, knees, wrists, and hands are involved most frequently. The clinical findings are not distinct from those of other microbial causes of septic arthritis. Polyarticular involvement occurs less frequently than in neonates.[93] Gram stain and synovial fluid cultures often are negative. Therefore, empiric coverage for *N. gonorrhoeae* must be considered when septic arthritis develops in a sexually active adolescent or a child who may have been sexually abused.

Typically, the patient has a single most severely affected joint, and associated myositis and tenosynovitis may be prominent findings. Gonococcal arthritis in older children and adolescents resembles that in adults and may be accompanied by cutaneous lesions (Fig. 100–5).[4] Osteomyelitis rarely occurs but has been reported in all age groups, usually in association with septic arthritis.[9]

Treatment of gonococcal arthritis depends on prompt recognition of the disease. Cultures of all mucous membranes (nasopharyngeal, rectal, vaginal or endocervical, conjunctival), blood culture, and aspiration of the involved joint should be performed. The local signs of gonococcal arthritis may not respond to antibiotic therapy for several days. Serial needle aspiration rather than open drainage usually is sufficient for relief of pain and recovery without sequelae (the hip may be an exception).

Gonococcemia is clinically silent generally but can cause a syndrome of migratory polyarthralgia, fever, and rash that precedes the onset of arthritis by several days to a week. The symptomatic course normally is only mild to moderate in severity. Blood cultures often are negative by the time that care is sought. Gonococcemia-related symptoms may resolve after several days, even without treatment. Rare cases can resemble meningococcemia with purpura and fulminant sepsis with disseminated intravascular coagulopathy. These cases sometimes can be fatal.[253]

The skin lesions associated with gonococcemia usually are pustules on an erythematous base (Fig. 100–5), but petechiae, papules, and hemorrhagic bullae can develop. The development of skin lesions in conjunction with septic arthritis also has been called the arthritis-dermatitis syndrome. The lesions usually arise on the extremities and are fewer than 20 in number. Low-grade fever is most common, but high fever with shaking chills may occur. Tenosynovitis is present in a fourth of these patients. Leukocytosis, pyuria, and elevated liver enzyme test results may be seen. The resultant septic joints generally become clinically apparent during the second week after the onset of disseminated infection.

ANORECTAL GONORRHEA

Gonococcal anorectal infection (proctitis) frequently is asymptomatic but can be associated with pruritus, tenesmus, purulent discharge, or rectal bleeding. Rectal infection can occur as a result of rectal intercourse or inoculation from vaginal secretions. Approximately 40 percent of females with genital gonococcal infection have positive anorectal cultures. Rectal infection is an unusual finding in males who have not engaged in rectal intercourse.[311]

PHARYNGEAL GONORRHEA

Pharyngeal gonococcal infection in all age groups beyond the neonatal period is acquired by orogenital contact. Pharyngeal gonorrhea may be asymptomatic, without evidence of inflammation, or may cause an exudative tonsillopharyngitis that can mimic group A streptococcal or viral infection. Cervical adenopathy also may be present in some cases. In sexually abused children, the pharynx may be the only site of infection. It may be the sole culture-positive site in some cases of disseminated gonococcal infection, and pharyngeal infection possibly is a factor predis-

Figure 100–5 Skin lesion typical of the arthritis-dermatitis syndrome of disseminated gonococcal infection: necrotic pustule with an erythematous halo. *(Courtesy of Daniel P. Krowchuk, M.D.)*

posing to dissemination. Pharyngeal infection usually resolves spontaneously within 10 to 12 weeks but should be treated when recognized.

Throat cultures for *N. gonorrhoeae* should be considered in sexually active adolescents with pharyngitis, asymptomatic orogenital contacts of infected persons, patients with disseminated disease in whom other sites of initial infection are not readily apparent, and children who have been sexually abused.

Current treatment regimens for genital gonococcal infection are effective in eradicating gonococcal infection from the pharynx. Routine throat cultures for screening all sexually active adolescents are not cost-effective, but throat culture may be considered for those who give a history of frequently engaging in orogenital sexual activity.*

CONJUNCTIVITIS BEYOND INFANCY

Gonococcal ophthalmia occasionally is seen in children and adults. Direct inoculation of the eye can occur as a result of transmission of fomites from infected persons. Clinical findings typically include a profuse purulent discharge, chemosis, eyelid edema, keratitis, and fever. The initial ocular discharge may be watery before turning purulent. The acute phase may mimic orbital cellulitis. Untreated infection, as with neonates, can lead to corneal opacification, ulceration, and rupture of the globe with resultant visual loss. Some cases may be minimally symptomatic with a minimal inflammatory response and spontaneous resolution.[203,207,261]

OTHER FORMS OF GONOCOCCAL DISEASE

Gonococcal meningitis is a rare condition that may occur with or without associated signs of gonococcemia or septic arthritis. Pyomyositis of the biceps and soft tissue abscesses remote from the genital area have been reported. A gonococcal abscess arising in an area of blunt trauma to a hand has been described in an adolescent with associated endocervical infection.[137] Ventriculoperitoneal shunt–associated infection, endocarditis, and myocarditis caused by *N. gonorrhoeae* have been reported in adults and can be expected to occur occasionally in children and adolescents.[161] These manifestations of gonococcal infection seldom are seen in children.

DIAGNOSTIC TESTING

Isolation of *N. gonorrhoeae* in culture remains the standard for diagnosing gonococcal infection, but non-culture, DNA-based tests have become widely used in recent years. Uses of these tests are listed in Table 100–5. Only culture should be used for rectal or pharyngeal specimens. Serologic tests based on complement fixation, latex agglutination, enzyme-linked immunosorbent assays, and other techniques have been developed, but the sensitivity of these methods is only about 70 percent, which limits their use primarily to studies of immune response and pathogenesis.[160,164,287]

A diagnosis of *N. gonorrhoeae* infection at any site should prompt evaluation for the presence of other common STDs, if not already done[286] (see also Chapter 48).

CULTURE

In adults, culture of clinical specimens is 80 to 95 percent sensitive when promptly inoculated and incubated. Cultures of adult male urethral specimens, blood, and other normally sterile body sites tend to have sensitivities in the higher range.[164] Because false-positive cultures (with appropriate laboratory confirmation; see later) are not thought to occur, specificity and positive predictive values are 100 percent. Culture in adolescents and children probably has sensitivity similar to that of adults. Vaginal specimens are adequate for diagnosis in prepubertal girls, for whom obtaining endocervical specimens is unnecessary.

The use of selective media such as modified Thayer-Martin (which contains nystatin, vancomycin, and trimethoprim, and colistin) is required for culture of endocervical, rectal, and pharyngeal specimens to suppress contaminating flora. Selective or nonselective media (chocolate agar) can be used with equal sensitivity for male urethral cultures. Plating of specimens on both types of media may improve the sensitivity, but the incremental yield is small and probably not cost-effective for routine practice.[30,98,270]

Gonococcal colonies become evident on agar plates within 24 to 48 hours after inoculation. Isolates are considered presumptively positive if Gram stain shows gram-negative diplococci and colonies are oxidase-positive. Further testing that demonstrates the gonococcal phenotypic pattern of acid production from selected carbohydrates or a positive result by a nucleic acid method (or both; see later) is required for confirmation as *N. gonorrhoeae* (Table 100–5).[63]

Gonococci do not tolerate drying, so clinical specimens must be plated onto appropriate media as soon as possible. Transport bottles containing medium and a CO_2-enriched atmosphere should be used if definitive specimen processing will be delayed. Transport bottles should be maintained upright during inoculation to preserve the CO_2 atmosphere.

In sexually active females with endocervical gonococcal infection, the urethra, Bartholin gland ducts, and Skene gland ducts usually are infected also. Cultures of these sites may improve the overall yield/sensitivity, in part by avoiding the sampling errors that can occur with any single culture. However, the incremental yield is small enough to render obtaining such cultures unnecessary in routine clinical settings. Culture of the rectum and pharynx in females can be considered optional except when evaluating for sexual abuse.[30,182]

In sexually active males, sites to be cultured depend on the sexual practices and the anatomic sites exposed. Among MSM, rectal infection occurs almost as frequently as urethritis, and pharyngeal infection is not an uncommon finding.[36,164,217,227]

GRAM-STAINED SMEARS

In symptomatic males, a Gram stain of a urethral specimen that demonstrates polymorphonuclear leukocytes with intracellular gram-negative diplococci can be considered diagnostic of infection with *N. gonorrhoeae*. Gram stain in this population has greater than 99 percent specificity and greater than 95 percent sensitivity.[64] Negative Gram stain results are not sufficient to exclude the diagnosis of gonorrhea. Although nonpathogenic *Neisseria* spp. and *N. meningitidis* are morphologically indistinguishable from gonococci, the former rarely are cell-associated.

Gram stains of endocervical, pharyngeal, and rectal specimens are not recommended because of lower sensitivity in detection of infection at these sites. The specificity of a negative Gram stain appears to be at least 95 percent for specimens from the endocervix and rectum.[164,282]

Gram stains of eye exudates, joint fluid, and pustular fluid should be performed because the presence of gram-negative diplococci may help guide initial therapeutic interventions pending culture results.

*See references 1, 41, 73, 94, 104, 147, 165, 240, 250, 301, 335, 339.

TABLE 100–5 Laboratory Testing of Specimens for the Presence of *Neisseria gonorrhoeae*

Clinical Indication for Testing	Testing Method and Specimen Type
Suspected female genitourinary tract infection	1. Culture performed on an endocervical swab specimen (adolescents or adults) or vaginal swab specimen from prepubescent females* *If transport and storage conditions are not conducive to maintaining the viability of* N. gonorrhoeae, *a nucleic acid amplification test (NAAT) or nucleic acid hybridization test (NAHT) can be performed on an endocervical swab specimen*† (NAAT or NAHT generally is not acceptable for prepubescent children undergoing evaluation for sexual abuse) 2. NAAT performed on urine (generally not acceptable for prepubescent children undergoing evaluation for sexual abuse)
Suspected male urethral infection	1. Culture performed on an intraurethral swab specimen if collecting such a specimen is acceptable and transport and storage conditions are suitable for culture†,‡ 2. Gram stain of urethral discharge (positive if intracellular gram-negative diplococci are seen) 3. NAAT or NAHT performed on an intraurethral swab specimen if collecting such a specimen is acceptable 4. NAAT performed on urine
Suspected male or female rectal or pharyngeal infection	1. Culture performed on rectal or pharyngeal swab specimens* 2. No other tests recommended
Suspected gonococcal ophthalmia (neonatal or childhood) or disseminated infection	1. Culture performed on conjunctival exudate specimens, blood, synovial fluid, cerebrospinal fluid, or pustular skin lesions with the use of nonselective media (e.g. chocolate agar) 2. Gram stain on exudates or fluid samples (synovial, cerebrospinal fluid) can be useful as an initial point-of-care test that can help guide early treatment considerations pending culture results. Should be accompanied by culture
Scenario for Confirmatory Testing	**Recommendations and Comments**
Presumptively positive culture (identification in the laboratory of typical gram-negative, oxidase-positive diplococci consistent with *N. gonorrhoeae*)	1. Preferred methods for confirmation of *N. gonorrhoeae* are (a) acid production from carbohydrates§ or (b) positive NAHT results (use of molecular method to confirm culture results) 2. Requiring both methods to be positive ensures high specificity 3. If an isolate cannot be conclusively identified as *N. gonorrhoeae* at a local laboratory, it should be sent to a reference laboratory for confirmation (especially in cases of alleged sexual abuse, sexual assault, or rape)
Positive non-culture test (e.g., NAAT, NAHT)	1. Culture with confirmation as above is the preferred additional test after a positive non-culture test if specimen transport and storage conditions are suitable. Confirmatory testing is not routinely required unless medicolegal issues exist or there is need for antibiotic susceptibility testing 2. Competitive probe format after a positive NAHT (probe) test, but this approach theoretically is less likely to detect a false-positive result 3. A different NAAT as an additional test after NAAT or NAHT has received limited evaluation; certain NAATs sometimes cross-react with nongonococcal *Neisseria* species 4. Antibody tests that detect gonococcal antigens are not recommended for detection of *N. gonorrhoeae*

**A selective medium (e.g., modified Thayer-Martin) should be used for culture of specimens from these sites.*
†*Transport cultures with an enriched CO_2 atmosphere can be used in some situations.*
‡*Selective or nonselective media may be used.*
§*N. gonorrhoeae produces acid reactions with glucose but not maltose, lactose, sucrose, or fructose. The species also does not reduce nitrates or hydrolyze tributyrin.*
Compiled from Centers for Disease Control and Prevention: Screening tests to detect Chlamydia trachomatis and Neisseria gonorrhoeae infections—2002. M. M. W. R. Recomm. Rep. 51(RR-15):1-38, 2002.

NON-CULTURE DIAGNOSTICS

Non-culture diagnostic tests have become widely used in the United States for establishing a rapid diagnosis of gonococcal (and chlamydial) infection. Many offer the advantage of using urine samples rather than the more invasive swabs and also allow *C. trachomatis* to be evaluated with the same specimen. Numerous gonococcal antigen-detection tests have been developed, but they have been supplanted largely by nucleic acid detection methods.

Nonamplified DNA-DNA hybridization probe tests have been the non-culture tests used most commonly in the United States in recent years. These tests are based on a single-stranded DNA probe complementary to gonococcal rRNA. Sensitivities are about 95 percent (less than culture), with a specificity of 99 percent.[152,164,204,252] Nucleic acid amplification tests (NAATs) use standard PCR-based assay and actually are more sensitive than is culture (Table 100–5). False-positive reactions can occur as a result of the carry-over contamination that occurs during processing or because of occasional cross-reactions with commensal

Neisseria spp. that may be present in some clinical specimens. Specificities are generally greater than 98 percent. False-negative results can occur as a result of target sequence differences (or absence) in some gonococcal clones. NAATs can be used to evaluate first-void urine specimens, which permits screening for gonococcal infection in males and females when genital examination is impractical.[79,256,306,325,327,336]

NAATs and DNA probe tests can detect the presence of nonviable gonococcal microbes, thereby possibly reducing need for the stringent transport conditions often required for culture. Currently available NAATs are multiplex tests that also can detect *C. trachomatis*. NAATs generally are approved by the U. S. Food and Drug Administration for use with endocervical, vaginal, and urethral swab specimens and with urine specimens. They are not approved for rectal or pharyngeal specimens—culture remains the best testing method for detecting gonococcal infection at these sites.[64] Some NAATs can be used for conjunctival specimens.[5]

NAATs and DNA probe tests are adequate for rapid screening in clinical settings, and their results are adequate for making treatment decisions and public health case identifications. Culture remains the definitive test for medicolegal purposes (see the next section). In addition, because of ever-increasing resistance to the available antimicrobial agents, monitoring of gonococcal susceptibility by culture is necessary at the local level, at the very least in patients who do not respond to apparently adequate therapy.[63,336]

MEDICOLEGAL ISSUES RELATED TO DIAGNOSTIC TESTS

N. meningitidis and other members of the Neisseriaceae family are morphologically and often biochemically similar to *N. gonorrhoeae* and may be isolated from sites such as the vagina, blood, and nasopharynx.[108,113,337] Accurate identification of *Neisseria* organisms from any pediatric specimen is essential because misidentification of nongonococcal species as *N. gonorrhoeae* may lead to very serious social consequences for children and their families by precipitating concern regarding sexual abuse. Nonculture methods consistently identify more clinical specimens as positive than standard cultures do.[327] Determining whether such results indicate greater sensitivity, increased false positivity, or a combination of both has been difficult.

Because of this potential for obtaining false-positive results with non-culture NAAT or DNA probe tests, culture remains the medicolegal standard for children who require evaluation for suspected sexual victimization, including prepubertal girls with obvious vaginal discharge. Sequential testing using non-culture tests followed by culture if the former is positive has been proposed as a more sensitive approach than is culture alone.[251] Further study of this approach is required before it can be recommended for adoption.

The CDC also has defined three levels of diagnosis based on clinical and laboratory findings. They are more stringent than are the case definitions used for public health surveillance. A *suggestive diagnosis* is defined by presence of mucopurulent endocervical or urethral exudate and sexual exposure to a person with gonococcal infection. A *presumptive diagnosis* requires two of three criteria: (1) typical gram-negative *intracellular* diplococci on a Gram stain of urethral exudate from males or endocervical secretions; (2) growth of apparent *N. gonorrhoeae* from such specimens on culture medium, defined as typical colonial morphology, positive oxidase reaction, and typical gram-negative morphology; and (3) detection of *N. gonorrhoeae* by a non-culture laboratory test. A *definitive diagnosis* requires (1) isolation of *N. gonorrhoeae* from clinical specimens by culture, as in the second criterion for a presumptive diagnosis, and (2) confirmation of identity by biochemical, enzymatic, serologic, or nucleic acid testing (Table 100–5).[71]

PROPER COLLECTION OF CLINICAL SPECIMENS

Urethral exudate from males may be obtained by passing small swabs or bacteriologic loops 2 to 4 cm into the urethra.[155] Endocervical specimens from postpubertal females are obtained by speculum examination with swabs inserted 1 to 2 cm into the external os after the cervix has been cleansed of external exudate and vaginal secretions.[278] The swab should be rotated one full revolution and withdrawn. Self-obtained vaginal swabs also provide adequate specimens for non-culture methods.[305] This technique has been studied in adolescents. Vaginal specimens collected with tampons also can be used with non-culture methods.[321]

In prepubertal girls, cultures can be obtained from the vaginal introitus by gently swabbing the hymenal opening. Deeper insertion is not necessary. Swabs of exudates emanating from the urethral meatus or vaginal orifice also are sufficient for culture.

In persons with symptomatic anorectal infection, rectal specimens should be obtained by anoscopic means, which increases the sensitivity. In asymptomatic persons, rectal specimens can be procured by blindly inserting a swab 2 to 3 cm into the anal canal and applying lateral pressure to avoid entering any fecal mass. Swabs that are grossly contaminated with fecal matter should be discarded.[101,340]

When urine specimens can be used for non-culture methods, the first 15 to 30 mL of voided urine should be collected.[212,236] Collection of urine should be delayed at least 1 hour after the most recent void to maximize sensitivity. The posterior pharynx, tonsillar areas, and faucial pillars should be swabbed to obtain adequate specimens for the diagnosis of pharyngeal infection.[164]

ANTIMICROBIAL RESISTANCE AMONG GONOCOCCI

Gonococcal strains have been able to acquire resistance to antibiotics since the first agents were introduced. Sulfanilamide became available in 1936 and represented a major improvement over previous therapies that included local genital irrigation with solutions of silver nitrate or potassium permanganate.[255] Widespread resistance to sulfanilamide occurred by 1944. As successive agents have become available, multidrug-resistant strains are being seen with increasing frequency in many parts of the world. Ongoing analysis of gonococcal isolates for antibiotic susceptibility to the available antimicrobial agents remains essential for maintaining effective empiric therapeutic regimens.

In the 1940s, virtually all gonococcal isolates were highly susceptible to penicillin. Despite a gradual increase in the mean minimal inhibitory concentration (MIC) to penicillin from the mid-1950s through the mid-1970s, almost all strains had penicillin MICs of less than 0.5 μg/mL. This low-level resistance is mediated by alterations at a genetic locus called *penA* that result in modification of its product, penicillin-binding protein 2 (PBP-2). Two other loci designated *mtr*, which encodes an efflux pump that reduces concentrations of multiple antibiotics, and *penB*, which is an allele of *por*, the gene of the porin protein, also mediate low-level resistance to penicillins. *penB* encodes a porin with a mutation that decreases permeability to hydrophilic antibiotics.[134]

In 1976, strains of *N. gonorrhoeae* were discovered that had acquired plasmid-conferring resistance to penicillin through the production of penicillinase (a TEM-1 β-lactamase). These strains were found in many parts of the Far East and London, and in the former they constituted approximately 30 percent of the isolates in some cities. The strains caused the expected spectrum of clinical disease, and treatment with penicillin was not effective. Penicillinase-producing gonococci contain one of two closely related 5.3- or 7.2-kb plasmids (Pc^r) that carry a Tn2 transposon

system. This plasmid appears to have been acquired from *Haemophilus ducreyi*.[8,49,77,258] In 1983, an outbreak of chromosomally mediated, penicillin-resistant (MICs of 2 to 4 μg/mL), non–penicillinase-producing gonococci was reported from North Carolina.[124] Such strains have been seen subsequently in other areas of the country.

Resistance to tetracycline antibiotics emerged in the 1980s and subsequently increased. Three chromosomal loci designated *mtr*, *penB*, and *tet* mediate low-level resistance. High-level resistance to tetracycline is conferred by *tetM*, which resides on a 38-kb plasmid (Tc^r) that is a derivative of the 36-kb conjugal plasmid. *tetM* produces a cytoplasmic protein that protects ribosomes from tetracycline. Tc^r gonococci can transfer this plasmid, as well as Pc^r, efficiently to other gonococcal strains.[228,308]

Spectinomycin resistance was described first in 1987 in U.S. military personnel in Korea. This agent had been introduced as the drug of choice there in 1981 because of high rates of penicillin resistance. Treatment failures actually began to occur in 1983. Resistance is chromosomally mediated and results in alteration of the ribosomal target site of the drug.[31,49] The widespread use of spectinomycin was associated with a decline in the rate of penicillin resistance.[31] Many gonococcal strains in some parts of the world also are resistant to streptomycin.[244]

Ciprofloxacin and other fluoroquinolone antibiotics became widely available for the treatment of gonococcal infection in the 1980s. Low-level resistance to fluoroquinolones is associated with mutations in the DNA gyrase gene *gyrA*, and high-level resistance has been linked to mutations in the topoisomerase gene *parC*.[174] Resistance to these agents has been noted since the early 1990s in areas of Southeast Asia.[126] In Hawaii in 1999, 9.5 percent of isolates were resistant to ciprofloxacin, but resistance elsewhere in the United States remained low at 0.2 percent.[61,62]

By the year 2000, fluoroquinolones no longer were recommended for initial treatment of gonococcal infection in Asia, the Pacific Islands, or Hawaii. In 2002 this recommendation was extended to the state of California and in 2004 to MSM throughout the United States. During the first 6 months of 2006, 13.3 percent of 3005 isolates collected through the CDC's Gonococcal Isolate Surveillance Project (GISP) were resistant to ciprofloxacin (and thus all other quinolone antibiotics). Among gonococcal isolates from MSM and heterosexual males from U.S. states other than California and Hawaii, ciprofloxacin-resistant rates were 30.7 and 5.1 percent, respectively. Therefore, as of April 2007, the CDC no longer recommends the use of fluoroquinolones for the treatment of gonococcal infections in the United States.[67]

In 1986, the CDC initiated the GISP to monitor the antimicrobial sensitivities of *N. gonorrhoeae* in STD clinics in 21 cities in the United States.[56] This system has been expanded to 27 sites.[62,68] In 1989, 13 percent of the *N. gonorrhoeae* isolates evaluated were resistant to penicillin, tetracycline, or both.[57] In 1999, such strains accounted for 28 percent of all isolates.[62] Rates of resistance to these two agents have fallen slightly in the United States since that time, with 9 percent being resistant to penicillin and 17 percent resistant to tetracycline in 2005.[68]

Strains resistant to azithromycin and erythromycin have been identified in the United States.[61] The distribution of azithromycin MICs has shifted upward in the last decade, although the proportion of strains that probably were resistant in 2005 was less than 3 percent.[68] Rifampin resistance is also high in some areas of the world.[244]

TREATMENT

Because of ever-changing gonococcal susceptibility patterns and variations in susceptibility in different international regions, practitioners must remain alert for modifications of treatment guidelines for their respective geographic locations. Indeed, based on the current patterns of antimicrobial resistance in prevalent *N. gonorrhoeae* strains in the United States, extended-spectrum, third-generation cephalosporins are the only remaining antibiotics routinely recommended as initial therapy in children and adults as of April 2007.

The recommendations for treatment of childhood gonorrhea in the United States discussed in this section and listed in Tables 100–6 and 100–7 are based on 2006 guidelines from the CDC[64,67,69] and the 2006 Report of the Committee on Infectious Diseases of the American Academy of Pediatrics.[5] The CDC guidelines are based on the stringent clinical efficacy criterion of an expected

TABLE 100–6 Treatment of Gonococcal Infections in the Neonatal Period

Disease Category	Treatment Regimen	Comments
Infants born to mothers with gonococcal infection	Ceftriaxone, 25 to 50 mg/kg IV or IM, not to exceed 125 mg (single dose) *or* Cefotaxime, 100 mg/kg IV or IM (single dose)	Ceftriaxone should be given cautiously to hyperbilirubinemic infants, especially premature ones
Gonococcal ophthalmia neonatorum (GON) or other focal sites of infection (i.e., rectum, pharynx, vagina, urethra)	Ceftriaxone, 25 to 50 mg/kg IV or IM, not to exceed 125 mg (single dose) *or* Cefotaxime, 100 mg/kg IV or IM (single dose)	Infants with GON should receive eye irrigation with saline solution immediately upon recognition and at frequent intervals subsequently until the discharge is eliminated Topical agents alone are inadequate and unnecessary when recommended systemic antibiotics are given Some experts prefer to continue parenteral therapy with one of these agents until blood (with or without cerebrospinal fluid) cultures have been negative for 48 to 72 hours
Disseminated gonococcal infection (septic arthritis, sepsis, meningitis) or scalp abscess	Ceftriaxone, 25 to 50 mg/kg IV or IM once daily for 7 days (10-14 days for meningitis) *or* Cefotaxime, dosed according to neonatal algorithms, IV or IM for 7 days (10-14 days for meningitis)	Cefotaxime is preferred for infants with hyperbilirubinemia who require more than a single dose of therapy If meningitis is present, higher doses in the recommended ranges may be needed

Data largely compiled from Centers for Disease Control and Prevention: Sexually transmitted diseases treatment guidelines 2006. M. M. W. R. Recomm. Rep. 55(RR-11):1-94, 2006; and American Academy of Pediatrics: Gonococcal infections. In Pickering, L. K. (ed.): 2006 Red Book: Report of the Committee on Infectious Diseases. 27th ed. Elk Grove Village, IL, American Academy of Pediatrics, 2006, pp. 301-309.

TABLE 100–7 Treatment of Gonococcal Infections in Children beyond the Neonatal Period*,†

	Prepubertal Children Who Weigh <100 lb (45 kg)	Patients ≥8 Years Old and Who Weigh >100 lb (45 kg)
Uncomplicated Gonococcal Infections‡		
Vulvovaginitis Endocervicitis Urethritis Proctitis Pharyngitis	Ceftriaxone, 125 mg IM in a single dose *Alternative regimen* Spectinomycin,§ 40 mg/kg (maximum, 2 g) IM in a single dose (Cefixime has been used by some experts because it can be administered orally. There are no published data regarding safety or efficacy for this purpose)	Ceftriaxone, 125 mg IM in a single dose¶ *or* Cefixime,¶ 400 mg orally in a single dose *Alternative regimens* Spectinomycin,§ 2 g IM in a single dose *or* Single-dose cephalosporin regimens (see Note)
Conjunctivitis**	Ceftriaxone, 50 mg/kg (maximum, 1 g) IM in a single dose	Ceftriaxone, 1 g IM in a single dose
Disseminated Gonococcal Infection††,‡‡		
Arthritis, sepsis Arthritis-dermatitis syndrome	Ceftriaxone, 50 mg/kg/day (maximum, 1 g/day) IV or IM once a day for 7 days§§	Ceftriaxone, 1 g IV or IM every 24 hours for 24-48 hours after clinical improvement, followed by cefixime, 400 mg twice daily orally, or cefpodoxime, 400 mg twice daily orally to complete at least 7 days of total antimicrobial therapy§§ *Alternative regimens* Cefotaxime, 1 g IV every 8 hours, or ceftizoxime, 1 g IV every 8 hours, with switch to oral therapy and duration as for ceftriaxone§§ *or* Spectinomycin, 2 g IM every 12 hours *or* Fluoroquinolones if antimicrobial susceptibility can be documented by culture¶¶
Meningitis Endocarditis	Ceftriaxone, 50 mg/kg/day (maximum, 2 g/day) IV or IM given every 12 hr; for meningitis, the duration is 10-14 days; for endocarditis, the duration is at least 28 days	Ceftriaxone, 1-2 g IV every 12 hr; for meningitis, the duration is 10-14 days; for endocarditis, the duration is at least 28 days
Epididymitis		Ceftriaxone, 250 mg IM in a single dose
Treatment of potential concurrent *C. trachomatis* infection that has not been ruled out†	Azithromycin, 20 mg/kg (maximum, 1 g) in a single dose *or* Erythromycin base or ethylsuccinate, 50 mg/kg/day (maximum, 2 g/day) in 4 divided doses for 14 days	Doxycycline,¶¶ 100 mg orally twice a day for 7 days *or* Azithromycin, 1 g orally in a single dose

Note: Other single-dose cephalosporin therapies that are considered alternative treatment regimens for uncomplicated urogenital and anorectal gonococcal infections include ceftizoxime, 500 mg IM, or cefoxitin, 2 g IM, administered with probenecid, 1 g orally, or cefotaxime, 500 mg IM. Some evidence indicates that cefpodoxime, 400 mg, and cefuroxime axetil, 1 g, might be adequate oral alternatives. Ceftriaxone and cefixime are preferred.

**See text for discussion of treatment options for patients with allergies to the recommended regimens.*

†In addition to the recommended treatment of gonococcal infection, therapy for Chlamydia trachomatis is recommended because of the common occurrence of co-infections with these microbes.

‡Hospitalization should be considered for children (1) who are unlikely to receive the prescribed treatment because of personal or parent/guardian failure to adhere to the regimen or (2) whose infection has not responded to outpatient therapy.

§Spectinomycin currently is not available in the United States. This agent is not recommended for the treatment of pharyngeal infections. If spectinomycin must be used for pharyngeal infection, follow-up cultures should be performed.

¶Alternative regimens for uncomplicated infections include spectinomycin (2 g IM in a single dose), ceftizoxime, cefotaxime, cefotetan, and cefoxitin. Spectinomycin is not recommended for pharyngitis.

¶Experience in adults suggests that cefixime can be considered for use in children with uncomplicated gonococcal infections, but few data are available to confirm its effectiveness for gonococcal infections in children.

***Eyes should be lavaged with saline initially and at regular intervals until secretions no longer continue to accumulate.*

††Hospitalization is required. For older children and adolescents, parenteral therapy can be discontinued 24 to 48 hours after improvement occurs and the 7-day course completed with an appropriate oral antimicrobial agent. Cefotaxime also can be used for disseminated infections in children.

‡‡Persons with disseminated gonococcal infections also should receive one of the age-appropriate regimens for treatment of possible C. trachomatis co-infection listed in this table as part of treatment for persons with uncomplicated gonococcal infections.

§§Some experts advise a 10- to 14-day course of therapy for gonococcal sepsis or septic arthritis.

¶¶Fluoroquinolones are no longer recommended in the United States because of an increasing prevalence of antibiotic-resistant strains. Ciprofloxacin, ofloxacin, and levofloxacin are acceptable for use in adults and adolescents when gonococcal isolates have documented susceptibility. These agents generally are not used in children but may be considered depending on clinical circumstances and susceptibility of the infecting strain. Quinolones are contraindicated for women who are pregnant or nursing.

¶¶Doxycycline is not recommended for routine use in children younger than 8 years.

Data from Centers for Disease Control and Prevention: Sexually transmitted diseases treatment guidelines 2006. M. M. W. R. Recomm. Rep. 55(RR-11):1-94, 2006; Centers for Disease Control and Prevention: Updated recommended treatment regimens for gonococcal infections and associated conditions—United States, April 2007. Available at http://www.cdc.gov/std/treatment/2006/updated-regimens.htm *(accessed May 2007); and American Academy of Pediatrics: Gonococcal Infections.* In *Pickering, L. K. (ed.): 2006 Red Book: Report of the Committee on Infectious Diseases. 27th ed. Elk Grove Village, IL, American Academy of Pediatrics, 2006, pp. 301-309.*

95 percent efficacy with a lower bound of the 95 percent confidence interval of 95 percent or greater.[241] Children who are postpubertal or who weigh more than 45.4 kg (100 lb) may be treated with dosage regimens as defined for adults (including fluoroquinolones for infecting strains known to be susceptible to these agents).[64] The recommended regimens generally have not been studied in populations of prepubertal children with either uncomplicated or complicated gonococcal infection, but these regimens are likely to be highly effective in most cases, as they are for older adolescents and adults.

As of April 2007, recommendations for treating uncomplicated urogenital and anorectal gonorrhea in adolescents and adults in the United States are limited to a single 125-mg intramuscular dose of ceftriaxone or a single 400-mg oral dose of cefixime. Up-to-date information on treatment regimens for gonorrhea and other STDs can be obtained from the CDC website *http://www.cdc.gov/std/treatment*.[67,69]

In adults with an uncomplicated gonococcal infection at any site, 99 percent of cases are cured by ceftriaxone. As a general rule, children treated with ceftriaxone do not require follow-up cultures, but if other treatment regimens are used, follow-up cultures may be indicated. Cefixime has an antimicrobial spectrum similar to that of ceftriaxone. Experience in adults, including pregnant women,[267] suggests that oral cefixime may be considered for the treatment of uncomplicated gonococcal infections in children, provided that follow-up is ensured. The 400-mg oral dose in adults does not provide as high or as sustained a bactericidal concentration as that provided by a 125-mg parenteral dose of ceftriaxone, but clinical trials in adults with uncomplicated gonococcal infection have shown cure rates of 97 percent with cefixime.

Other parenteral cephalosporins that are safe and highly effective against uncomplicated gonococcal infection in adults—and probably in children as well—include cefotaxime, ceftizoxime, cefotetan, and cefoxitin. Some evidence indicates that cefpodoxime and cefuroxime axetil might be adequate oral alternatives in adults,[69] but no data have been reported regarding their use for gonococcal infections in children.

The fluoroquinolone antibiotics ciprofloxacin, ofloxacin, and levofloxacin have been used widely for treating gonococcal infection in adults. Cure rates for uncomplicated gonococcal infection with both agents exceed 98 percent in adults with susceptible isolates. These agents no longer are recommended for routine treatment of gonococcal infections in the United States and many other parts of the world because of substantial resistance rates.[67,69] They remain an option for adults and potentially for children in selected circumstances when the infecting gonococcal strain is known to be susceptible to them.

Resistance to spectinomycin remains rare in the United States, and this compound still is an effective alternative in most cases when other agents cannot be used.[5] However, spectinomycin remains unavailable in the United States at this time. Azithromycin may be useful in circumstances in which patients are unable to take standard regimens because of allergies, pregnancy, or concerns of age-related toxicity (e.g., dental staining with tetracyclines) or when no other alternatives are available.

PRESUMPTIVE TREATMENT OF CONCURRENT *CHLAMYDIA TRACHOMATIS* INFECTION

Persons with gonococcal infection, including children, are at high risk for acquiring concurrent chlamydial infection. Co-infections frequently occur and were present in 50 of 5877 students attending an urban U.S. high school in 1998 to 1999 who participated in a school-based screening program.[245] Among adolescents in 14 U.S. juvenile detention centers from 1997 through 2002, more than 50 percent of the teens with gonorrhea were co-infected with *C. trachomatis*.[183] In adolescents, treatment of gonococcal cervicitis with drug regimens that are effective against gonococci but not *Chlamydia* has been associated with a high incidence of residual salpingitis in females and urethritis in males because of the ongoing presence of *C. trachomatis*.[254,268,271,294]

Therefore, treatment recommendations for gonococcal infections beyond the neonatal period include agents active against both organisms (Table 100–7). Neonates with gonococcal infections also should be evaluated for *C. trachomatis* co-infection. Penicillin, amoxicillin, ceftriaxone, or spectinomycin alone will fail to eradicate *C. trachomatis*. Trimethoprim-sulfamethoxazole, tetracycline, doxycycline, azithromycin, and erythromycin are effective in vitro and in many clinical forms of chlamydial disease.

TREATMENT OF INFANTS BORN TO MOTHERS WITH GONOCOCCAL INFECTION

Infants born to mothers with untreated gonorrhea are at high risk of acquiring infection (e.g., ophthalmia, disseminated gonococcal infection); consequently, even without overt signs of infection, such infants should be treated with a single injection of ceftriaxone (25 to 50 mg/kg intravenously or intramuscularly, not to exceed 125 mg). Ceftriaxone should be given cautiously to hyperbilirubinemic infants, especially premature ones (see Table 100–6). A single dose of cefotaxime (100 mg/kg intravenously or intramuscularly) is an acceptable alternative. Topical prophylaxis for neonatal ophthalmia is not adequate therapy for documented infections of the eye or other sites.

TREATMENT OF NEONATES WITH GONOCOCCAL INFECTION

Neonates with clinical evidence suggestive of gonococcal infection at any site (including the eye) should be evaluated for disseminated disease. The evaluation should include a thorough physical examination, especially of the joints. Exudate from the eyes or other sites of apparent local infection should be sent for Gram stain and culture on appropriate media. Blood and CSF generally should be cultured, and the infant should be admitted to the hospital for parenteral antibiotics and any needed supportive care. Most but not all experts recommend that CSF studies be performed even in afebrile, otherwise well-appearing infants whose only clinical manifestation is apparent GON.

The presence of typical gram-negative diplococci in Gram-stained specimens is sufficient justification to begin treatment of GON or gonococcal infection in other sites. The absence of gram-negative diplococci in Gram-stained specimens is not sufficient to abrogate presumptive treatment of GON in a neonate with conjunctival exudate.

Tests for concomitant *C. trachomatis* infection, HIV infection, and congenital syphilis should be performed in infants with gonococcal infection at any site. The mother and her partners should be evaluated for gonococcal infection (and other STDs) and treated according to the recommendations for gonococcal infection in adolescents and adults (see the next section).

Infants with GON can be treated with a single dose of ceftriaxone (25 to 50 mg/kg intravenously or intramuscularly, not to exceed 125 mg) (Table 100–6). This regimen also may be used for infants with other sites of nondisseminated gonococcal infection, including the rectum, pharynx, vagina, and urethra. A single dose of cefotaxime (100 mg/kg given intravenously or intramuscularly) is an alternative treatment of GON. Some experts prefer to continue parenteral therapy with one of these agents until blood cultures (with or without CSF cultures) have been negative for 48 to 72 hours. Infants with GON also should receive eye

irrigation with saline solution immediately on recognition and at frequent intervals until the discharge is eliminated. Topical antimicrobial agents alone are inadequate and unnecessary when the recommended systemic antibiotics are given.[5,128,198]

Simultaneous infection with *C. trachomatis* should be considered a potential explanation for neonates who do not respond satisfactorily to the recommended treatment.

DISSEMINATED GONOCOCCAL INFECTION OR SCALP ABSCESS. The recommended therapy for gonococcal arthritis, scalp abscess, and sepsis is ceftriaxone (25 to 50 mg/kg intravenously or intramuscularly given once a day) for 7 days or cefotaxime for 7 days (50 mg/kg/day given intravenously or intramuscularly in two divided doses will be adequate for many infants; dose and interval adjustments may be needed depending on gestational and chronologic age and other clinical considerations). Cefotaxime is preferred for infants with hyperbilirubinemia. For gonococcal meningitis, treatment should be continued for 10 to 14 days, with consideration of the use of higher daily doses of these agents (see Table 100–6).

TREATMENT OF GONOCOCCAL INFECTIONS BEYOND THE NEONATAL PERIOD

Treatment recommendations based on weight for uncomplicated and complicated gonococcal infection are outlined in Table 100–7. Treatment recommendations for adolescents are the same as those for adults. For treatment recommendations for PID, see Tables 100–7 and 100–8. All treatment regimens include agents active against *C. trachomatis* in addition to *N. gonorrhoeae*. Treatment issues in a number of circumstances are discussed in more detail in the following text.

UNCOMPLICATED PHARYNGEAL GONORRHEA. Gonococcal infection of the pharynx is more difficult to eradicate than is infection of urogenital and anorectal sites. Chlamydial infection of the pharynx is an unusual occurrence, but genital co-infection may be present. A single dose of ceftriaxone (125 mg, intramuscularly) is the preferred treatment of uncomplicated pharyngeal gonococcal infection. A single dose of an oral fluoroquinolone may be used if the isolate is known to be susceptible to these agents. Spectinomycin is unreliable against *N. gonorrhoeae* in the pharynx; it eradicates the organism in only approximately 50 percent of cases. If spectinomycin is required because of allergies or contraindications to the other recommended treatments, pharyngeal culture should be performed 3 to 5 days after completion of therapy to verify eradication of the infection.

SEVERE ALLERGIES TO CEPHALOSPORINS. Selection of alternative antibiotic therapies for persons with a history of a reaction to a cephalosporin must be guided by the severity of the reaction and the availability of suitable alternative regimens.[63] Azithromycin is probably the best available choice at this time for persons with documented severe allergies to penicillins or cephalosporins.[67,69] If circumstances permit delay in treatment until antibiotic susceptibility test results can be obtained, other agents such as fluoroquinolones or doxycycline may be used if the isolate is shown to be susceptible.

Azithromycin, 2 g orally, is effective against uncomplicated gonococcal infection in adolescents and adults, but it is expensive and frequently associated with gastrointestinal problems. Because of these issues and concern about rapid emergence of resistance, it is not recommended for routine use.[64] A single dose of 20 mg/kg (maximum, 1 g) is recommended for the treatment of chlamydial infection in children, but no dose recommendations for azithromycin are available for the treatment of gonococcal infection in children.[5]

Patients should be observed for at least 30 minutes after the ingestion of a dose of 2 g of azithromycin to monitor tolerance of the medication.[70] An oral dose of 1 g of azithromycin cures approximately 93 percent of uncomplicated gonococcal infections in adults. Though better tolerated, this dose is not sufficiently effective for routine use.

Spectinomycin (40 mg/kg given intramuscularly, with a maximal dose of 2 g) remains effective but no longer is available in the United States. Updated information regarding its potential availability can be obtained from the CDC website *http://www.cdc.gov/std/gonorrhea/org*.

INFECTION WITH HUMAN IMMUNODEFICIENCY VIRUS. Children and adolescents infected with HIV who acquire a gonococcal infection should receive the same treatment as persons without HIV infection.

CONCURRENT SYPHILIS INFECTION. A single dose of ceftriaxone is not adequate for treating syphilis. Longer courses of therapy are required (see Chapter 156). *Treponema pallidum* is not susceptible to fluoroquinolones or spectinomycin.

PREGNANCY. Pregnant women should not be treated with quinolone or tetracycline agents. Those unable to take one of the recommended cephalosporin regimens can be treated with spectinomycin. Azithromycin may be considered if the recommended cephalosporins or spectinomycin cannot be used.

FOLLOW-UP

The recommended regimens have such high cure rates that routine repeat testing for cure no longer is recommended.[59,64,80] Obtaining follow-up cultures may be prudent if alternative thera-

TABLE 100–8 Sexually Transmitted Diseases in Prepubertal Children Evaluated for Suspected Sexual Abuse

Reference	No. of Children Evaluated	Number (%) Who Had a Diagnosis of				
		Gonorrhea	Chlamydiosis	Syphilis	Trichomoniasis	Condylomata Acuminata
Wald et al., 1980[330]	189	28 (14.8%)	ND	ND	ND	ND
Rimsza and Niggemann, 1982[277]	285	21 (7.4%)	ND	0	ND	ND
White et al., 1983[335]	409	46 (10%)	ND	6 (5.5%)	4 (18%)	3 (5.6%)
Ingram et al., 1984[172]	50	10 (20%)	3 (6%)	ND	2 (4%)	ND
DeJong, 1986[104]	532	25 (4.7%)	ND	1 (0.2%)	ND	3 (0.6%)
Ingram et al., 1992[171]	1469	41 (2.8%)	17 (1.2%)	1 (0.1%)	3 (2%)	28 (2%)
Siegal et al., 1995[300]	855*	12 (1.4%)	11 (1.3%)	0	4 (0.5%)	ND
Muram et al., 1996[235]	865	12 (1.4%)	ND	ND	ND	ND

**These children also were evaluated for HIV infection, and all were found to be uninfected.*
ND, no data.

pies are used or adherence is uncertain. Children who receive treatment regimens other than ceftriaxone or who remain in at-risk environments for reinfection should have follow-up evaluations.[5]

Treatment failures can occur, and cultures should be repeated for antibiotic susceptibility testing when symptoms persist after treatment. This precaution may lead to recognition of an antibiotic-resistant strain that could have public health implications.

Apparent treatment failure usually is the result of reinfection. Reinfection may be associated with a higher risk for PID and other complications of gonococcal infection. Therefore, retesting in 3 months for reinfection (not test of cure) is advisable for sexually active adolescents and adults, regardless of whether patients think that their sex partners have been treated.[64]

GONOCOCCAL INFECTION AND SEXUAL ABUSE OF CHILDREN

When gonococcal infection or any other STD is identified in a prepubertal child or in adolescents who are not sexually active within their peer groups, sexual abuse must be considered to have occurred unless proved otherwise.[5] Nonsexual transmission never should be assumed without extensive investigation of the social setting of an infected child.[5,239] Isolation of *N. gonorrhoeae* in cultures from children should be reported to local public health authorities and also to child protective services to ensure that the child is not exposed to further abuse. Sexual abuse has been reported in approximately 10 percent of all self-reporting populations of girls during childhood in numerous studies, and rates in boys are around 3 percent.[14,284] Approximately 1 percent of children appear to experience serious forms of sexual abuse yearly.[6]

STDs occur in 3 to 20 percent of sexually abused children.[104,171,172,235,277,300,330,335] Gonococcal infections have been found in 5 to 7 percent of sexually abused children.[104] The absence of STDs does not rule out sexual abuse when other findings suggest that it has occurred. Table 100–8 summarizes the STD diagnoses from eight studies involving children who had been evaluated for suspected sexual abuse.

The prevalence and clinical manifestations of gonococcal infection in sexually abused children are determined by the prevalence of infection in the adults who constitute the population with whom the child resides, the age of the child, and the type and frequency of sexual contact.[251] Gonorrhea was the STD recognized most frequently in abused children when gonococcal infection was highly prevalent in the general population. As a result of improved control of gonorrhea in the 1980s, fewer pediatric cases have been recognized in the United States in recent years. Nonetheless, girls who are exposed to an infected male appear to have a high rate of acquired disease. In an outbreak in an orphanage, 53 of 95 abused girls were found to have contracted infection.[2]

Among prepubertal girls who have a vaginal discharge after being sexually abused, 9 to 11 percent have been found to have gonococcal infection. No cases of genital tract gonorrhea without discharge have been reported in prepubertal girls.[171,297,300] The oropharynx also is a common site of gonococcal infection associated with sexual abuse of children and may be the only site of infection in children forced to perform orogenital sexual acts with an infected abuser. Pharyngeal infection may be asymptomatic. Although few children who suffer sexual abuse subsequently contract an STD, the diagnosis of an STD is a very important indication that the child probably has been abused, and an STD may be the sole finding on physical examination.[169]

Children with suspected gonococcal infection (or suspected to have suffered sexual abuse) should undergo a thorough physical examination by someone familiar with the medicolegal and clinical aspects of sexually abused children. Genital, rectal, and pharyngeal cultures should be obtained. Vaginal cultures are satisfactory in prepubertal girls. Endocervical cultures should be obtained only after puberty. Blood culture should be performed if disseminated disease is a consideration. Other sites (conjunctivae, joint fluid) should be cultured if clinically indicated. Cultures should be handled in a manner that will ensure legal acceptance if needed (see later).

Non-culture methods may be used in conjunction with—but not in lieu of—routine cultures to increase sensitivity in detecting gonococcal infection. These test results, when rapidly available, may permit more rapid initiation of therapy than possible with standard culture results.[251]

Selective criteria based on the observations that most sexually abused children do not have gonococcal infection or other STDs and that the vast majority of prepubertal girls with gonococcal infection will have clinical signs of vulvovaginitis have been developed to determine which children undergoing evaluation for sexual abuse should be tested for the presence of gonococcal infection and other STDs.[170,235,297,299,300] These criteria are (1) the known presence of any STD in the child, a sibling, another household member, a close associate of the child, or the apparent perpetrator or (2) a history or physical findings suggesting that oral, genital, or rectal contact has occurred.[170]

It is highly unlikely that a sexually abused child with gonococcal or chlamydial infection will be missed through the use of these criteria. Meanwhile, the number of potentially sexually abused children who must undergo culture is greatly decreased.[170,173] One caveat is that rectal and pharyngeal gonococcal infections in girls and boys and genital infections in boys often are asymptomatic, so these selective criteria may apply only to the issue of vaginal cultures in girls.[57]

All children with gonococcal infections (including neonates) should be evaluated for other STDs, including chlamydial disease, syphilis, hepatitis B, and HIV infection.[5,148,286]

The following are general issues for evaluation and management of children who may have been sexually abused:

1. Many institutions have a clinical service or clinician with training and skill in interviewing and examining children suspected of having experienced sexual abuse. Direct involvement by or consultation with persons with such expertise should be sought at the time of initial evaluation.[249]

2. If the child is symptomatic, all available and appropriate cultures and examinations should be completed before the child receives therapy. Because asymptomatic gonococcal infections occur commonly, strong consideration should be given to obtaining cultures from the rectum and oropharynx, even in the absence of symptoms, especially for children and adolescents who meet the selective criteria described earlier.[29,123,158]

3. A chain-of-custody policy that ensures proper handling, labeling, and delivery of diagnostic samples to the appropriate laboratory is essential for test results to be used in any court procedures when issues of the child's safety are being considered.

4. If sexual abuse is confirmed or suspected by the social history, physical findings, or laboratory results, the child should not be allowed to leave the evaluation site until the child's safety has been ensured. This determination usually is made by the local department of social services and may require involvement of law enforcement agencies. Temporary hospitalization of the child may be needed to permit sufficient time for any additional investigations to be performed. Reporting of suspected and confirmed cases to the social service of the county in which the child resides is required by child abuse reporting laws.

5. Most children who have been victims of sexual abuse will not have specific physical findings. Oral-genital contact is a common form of abuse, as are fondling and external genital contact. These forms of abuse may occur without physical signs of injury.

6. If the child is identified with an STD, other members of the family may be infected as well. Other female children are especially likely to be infected and should be examined.[123,131]

7. Internal pelvic examination of prepubertal children rarely is necessary unless there has been major trauma or there are concerns regarding foreign bodies. Internal examination of prepubertal girls should be performed by an experienced examiner with appropriate sedation or anesthesia.

8. Children often have compelling reasons to deny abuse and frequently do not disclose sexual abuse, even to skillful forensic interviewers.[202] Denial, therefore, is not compelling disproof of sexual abuse.

9. Sexual abuse of children can be a repeated event if the perpetrator has ongoing access to the child. If the child remains unprotected and the perpetrator has an STD, the child may be subject to recurrent episodes of that STD.[201]

10. Counseling with providers skilled in helping children who have been sexually abused should be part of long-term follow-up care. The likelihood of the child having serious emotional harm is greater when abuse has been more intrusive or violent, occurred repetitively over longer periods, or was committed by someone in a close relationship with the child.[85] Parents of victims also may need treatment and support.

11. Behavioral changes such as developmentally unusual sexual behavior or acting out can be a manifestation of child sexual abuse.[129] New onset of symptoms such as abdominal pain (which may lead to medical attention when it becomes persistent), enuresis, encopresis, sleep disturbances, and phobias can be manifestations of child sexual abuse. These issues also can arise from physical or emotional abuse or from non–abuse-related social stressors and do not indicate sexual abuse per se.[85]

PREVENTION AND CONTROL OF GONOCOCCAL INFECTIONS

All gonococcal infections must be reported to public health officials. Effort should be made to evaluate, counsel, and treat all sexual partners who were exposed to the index case within 60 days before the onset of symptoms or diagnosis in the index case. If a patient's last sexual intercourse occurred more than 60 days before the onset of symptoms, the most recent sexual partner of the patient should be evaluated and treated. Patients should be instructed to avoid engaging in sexual activity until therapy is completed and any symptoms have resolved.[69] Cases in prepubertal children must be investigated to identify the source of infection (and potential perpetrator of abuse).[5]

Condoms provide a high degree of protection against the acquisition and transmission of genital gonococcal infection.[181,324] Other barrier contraceptive measures and topical spermicidal and bactericidal agents likewise can reduce the likelihood of acquiring gonococcal (and chlamydial) infection.[15,54,214,316] Postexposure prophylactic antibiotics also reduce the risk of infection developing but are unlikely to be cost-effective.[156]

When patients, including infants with ophthalmia neonatorum,[5] with gonococcal infection are hospitalized, standard precautions are recommended.

PREVENTION OF NEONATAL INFECTION

Pregnant women should have an endocervical culture for *N. gonorrhoeae* as part of their initial prenatal care visit. A second culture late in the third trimester should be performed for those at high risk of exposure during pregnancy. Pregnant adolescents are at higher risk for contracting gonococcal infection than older women are. Treatment options are listed in Table 100–8. Fluoroquinolone and tetracycline antibiotics should not be used during pregnancy because of potential fetal toxicity.[5]

Infants born to mothers known to have active gonococcal infection are at risk of acquiring local and disseminated infection from colonization during birth. Such infants should be treated as listed in Table 100–7. Standard preventive measures for GON were described earlier.

VACCINE DEVELOPMENT

Research into the development of vaccines for gonococcal infection is ongoing. Although natural infection induces antibody responses directed primarily against pili, Opa, porin protein, and LOS, two factors render efforts to use these gonococcal components for vaccines a difficult challenge: (1) the rapid antigenic variation in gonococcal surface proteins and (2) the reality that natural genital infection does not induce a sufficient immune response to prevent later reinfection by the same strain. The development of a gonococcal vaccine shares the difficulties of similar efforts that are under way to produce nonpolysaccharide vaccines effective against serogroup B strains of *N. meningitidis*.[82,242]

Molecular analysis of gonococcal transferring binding proteins (Tbps) may have potential as vaccine antigens because of their exposure on the microbial surface. These proteins are expressed in all strains and have highly conserved gene sequences. Natural infection, however, generates little systemic or local antibody response to Tbps.[264] An oligosaccharide epitope (2C7 OS) of gonococcal LOS that is expressed on most strains and is widely conserved has been identified. A peptide that mimics the structure of 2C7 OS was able to induce antibodies in mice that possess dose-responsive complement-dependent bactericidal activity against $2C7^+$ strains.[242] This approach, as well as the potential use of combinations of recombinant antigens from multiple porin or Opa proteins, may have promise for future gonococcal vaccine development.

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SUBSECTION 3

Gram-Positive Bacilli

CHAPTER 101

DIPHTHERIA

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Diphtheria is an acute infectious disease caused by *Corynebacterium diphtheriae* or, less commonly, *Corynebacterium ulcerans*. *Corynebacterium pseudotuberculosis*, which primarily causes infections in sheep and goats, is not discussed here because it only rarely causes a diphtheria-like disease in humans. Infection by toxigenic strains of *C. diphtheriae* causes disease that is mediated by the production of an extracellular protein. Nontoxigenic strains also can cause disease, but it is usually less severe.

Before the discovery of antitoxin at the turn of the 20th century, the "strangling angel of children," as diphtheria once was called, was a significant cause of mortality in children and adults.[104] Apparent reference to diphtheria can be traced to the 5th century B.C. in the works of Hippocrates, *Epidemics III*, case 7.[74] In 1821, it was recognized as a specific entity by Brettoneau, who suggested that the disease was caused by a germ and that it could be transmitted from person to person. Brettoneau coined the origin of the modern term *diphtheria* from the Greek root *diphthera*, which means "skin" or "hide."[46] In 1883, the causative agent was identified by Klebs in stained smears from diphtheritic membranes; in 1884, Löffler grew the organism on artificial media and showed that in guinea pigs it caused a fatal infection closely resembling human disease.

The toxin was purified in 1889 by Roux and Yersin, who found that toxin alone could cause the disease. Shortly thereafter, Behring and Kitasato discovered antitoxins when they immunized animals with toxins rather than bacteria. The use of antitoxin to treat children with diphtheria at the turn of the 20th century resulted in one of the largest decreases in mortality rates by a therapeutic intervention. In Germany alone, an estimated 45,000 lives were saved each year.[57]

ETIOLOGY

Corynebacteria (Klebs-Löffler bacilli) are irregularly staining, gram-positive, nonmotile, nonsporulating, pleomorphic bacilli.[49] The club-shaped appearance of the bacillus is not a true morphologic feature, but results from attempting to grow the bacillus on media that are nutritionally inadequate (Löffler media). The organism can be recovered most readily on media containing selective inhibitors that retard the growth of other microorganisms; a sheep blood agar–based medium containing fosfomycin (for selectivity) and Tindale medium (tellurite medium with cystine) are ideal.[39,40] *C. diphtheriae* and *C. ulcerans* grow on

supplement-free blood agar, chocolate agar, and other standard media.[39]

Colonies of *C. diphtheriae* (with the exception of the lipophilic, gray *intermedius*) and *C. ulcerans* appear grayish white on Löffler medium. On tellurite medium, three diphtheria colony types can be distinguished: *mitis*, *gravis*, and *intermedius*. *Mitis* colonies are smooth, black, and convex; they do not ferment starch or glycogen and are hemolytic. *Gravis* colonies are gray, radially striate, and semirough; they ferment starch and glycogen and usually are not hemolytic. *Intermedius* colonies are small and smooth and have a black center; they do not ferment starch or glycogen and are not hemolytic. *C. ulcerans* colonies resemble *gravis* on Tindale medium but differ in that they are hemolytic. Similar to *gravis*, they ferment starch and glycogen. All diphtheria biotypes and *C. ulcerans* are characterized by cystinase activity and absence of pyrazinamidase activity. *C. ulcerans* may be distinguished from *C. diphtheriae* by its urease activity and ability to liquefy gelatin. Biotype *belfanti*, which does not occur in a toxigenic form, may be distinguished from the three potentially toxigenic diphtheria biotypes by its inability to reduce nitrate on Tindale medium and from *C. ulcerans* by its lack of production of urease.[39] Ribotyping and pulsed-field gel electrophoresis, both of which involve restriction digestion of genomic bacterial DNA followed by gel electrophoresis and Southern blotting, permit more specific typing within each diphtheria biotype and aid in the epidemiologic study of outbreaks.[36,91]

C. diphtheriae biotypes *intermedius*, *gravis*, and *mitis* and *C. ulcerans* all have been observed in a toxigenic form. *Intermedius* was the biotype isolated most commonly in the United States between 1971 and 1981. Of the strains isolated, *intermedius* was found to be toxigenic more often than was either *mitis* or *gravis*.[31] In the United Kingdom between 1993 and 1998, and similarly in other parts of Europe, the biovar *gravis* has represented most nontoxigenic isolates, followed by *mitis* and *belfanti*.[40] Of the four nontoxigenic isolates obtained during a surveillance study of a U.S. Northern Plains Native American Community in 1996, two were of biotype *mitis*, and two were *gravis*.[54] According to a United Kingdom diphtheria reference laboratory, between 1993 and 1998, the toxigenic isolates originating from Asia, Africa, and the Middle East were reported to be of the biotype *mitis* or *gravis*, with the exception of one *intermedius* isolate.[40] Golaz and associates[54] reported similar findings when they surveyed the different biotypes in South Dakota. A significant overall increase in the proportion of nontoxigenic isolates has been observed in Europe and Australia in recent years.[52] The reason for this increase is unclear, but one hypothesis is that increased immunity to toxigenic strains secondary to immunization has altered this epidemiology.

The complete genome sequence of *C. diphtheriae* biotype *gravis* has been elucidated.[28] The genome is approximately 2.48 Mb with a G+C content of 53 percent and has approximately 2320 predicted coding sequences. Metabolic analysis of the genome has revealed that *C. diphtheriae* has a complete set of enzymes for glycolysis, gluconeogenesis, pentose-phosphate pathways, anaerobic and aerobic respiration, amino acid biosynthesis, and purine nucleotide biosynthetic pathway. Most of the enzymes for the tricarboxylic acid cycle are present with the exception of an enzyme succinyl–coenzyme A (CoA) synthetase that catalyzes the conversion of succinate to succinyl-CoA. An alternative enzyme present in *C. diphtheriae* may fulfill this conversion, however. The pyrimidine pathway in *C. diphtheriae* lacks an enzyme that interferes with the production of cytidine; the pathway to the biosynthesis of thymidine is complete. The genome has revealed 13 regions that are unique to *C. diphtheriae* and may serve as pathogenicity islands.[28]

No functional or significant differences have been detected in the exotoxins elaborated by the three strains of *C. diphtheriae* or by *C. ulcerans*. Only strains that are lysogenic for beta-prophage or a closely related phage carrying the gene for toxin production produce diphtheria toxin. One or more *tox* gene sequences may exist in the bacterial genome, and the most highly toxigenic strains contain three or more copies.[77] Phage multiplication is not a prerequisite for the production of toxin. The capacity to synthesize toxin depends on genetic and nutritional factors. Toxin-producing cells apparently are cells in which spontaneous induction of the prophage to the phage occurs.[48] The most important factor controlling the yield of toxin is the concentration of inorganic iron in the culture medium.[34] Growth of *C. diphtheriae* in iron-deficient media prolongs the duration of induction lysis and is associated with a high yield of toxin. High concentrations of iron inhibit the production of toxin. Production of toxin also can be increased by the use of ultraviolet radiation. Conversion to a toxigenic strain occurs in nature, as has been shown by restriction enzyme studies of carriers of toxigenic and nontoxigenic strains in Manchester, England.

The ability of a strain of *C. diphtheriae* to elaborate diphtheria toxin can be shown by using several methods. In vivo studies involving necrosis of tissue in guinea pigs has been replaced by the widely used Elek[42] or modified Elek test.[45] Enzyme immunoassay[44] and polymerase chain reaction (PCR)[90] have been used to detect toxigenicity.

Diphtheria toxin is lethal to humans in an amount of approximately 130 μg/kg body weight. Cytoplasmic internalization of one molecule of toxin has been shown to cause cell death.[11] Toxigenic and nontoxigenic strains of *C. diphtheriae* can cause disease, but only strains that produce toxin cause disease with symptoms of myocarditis and neuritis.

EPIDEMIOLOGY

Asymptomatic human carriers serve as the reservoir for *C. diphtheriae*. Infection by *C. diphtheriae* is acquired by contact with either a carrier or an individual with active disease. The bacteria may be transmitted via droplets during coughing, sneezing, or talking. Rarely, transmission of *C. diphtheriae* occurs from skin lesions or fomites. Some reports suggest that skin carriers of *C. diphtheriae* are more infectious than are either nose or throat carriers and that skin carriers may serve as potential reservoirs for the initiation of epidemic spread.[12,63] In areas in which skin infections are endemic, levels of natural immunization may be high.[21] This phenomenon is illustrated particularly well in a survey of tetanus and diphtheria immunity in a rural Kenyan community, where age was not found to be predictive of immunity and no correlation was found between levels of antibody for tetanus and diphtheria.[72]

Person-to-person transmission of *C. ulcerans* is not known to occur, although *C. ulcerans* was isolated from the siblings of two patients reported in the United Kingdom between 1995 and 1997.[18] Cases of respiratory diphtheria caused by *C. diphtheriae* and by *C. ulcerans* have been documented in association with contaminated unpasteurized milk taken from cows with infected teats.[19,55,101] *C. diphtheriae* has been isolated from horses, dogs, and other domestic animals. *C. ulcerans* has been reported to infect ground squirrels in the United States, but transmission to humans has not been reported.[62,89]

According to World Health Organization reports, diphtheria is distributed worldwide and remains endemic in many developing areas of the world, including Asia, Africa, South America, and the Mediterranean regions.[20] Worldwide, diphtheria epidemics have occurred in a cyclical manner since the 16th century. The most recent epidemic began in 1990 in the Russian Federation, Ukraine, and the other newly independent states of the former Soviet Union.[23,103]

In the United States, the incidence and mortality rates from diphtheria in the 1920s were 140 to 150 cases per 100,000, with

13,000 to 15,000 deaths each year. The number of cases gradually declined to 15 per 100,000 population in 1945 with the extensive use of diphtheria toxoid vaccine. From 1970 through 1979, the average number of cases of diphtheria reported annually in the United States was 196.[8] From 1980 to 2004, only 57 cases of diphtheria were reported in the United States. Approximately 75 percent of the cases were in patients older than 15 years who were unimmunized or inadequately immunized.[8] From 1980 to 1995, only four fatal cases involving diphtheria and unimmunized children were reported.[14,83,92] In 1996, *C. diphtheriae* was isolated from a Native American population in South Dakota.[54] Only five cases of diphtheria have been reported since 2000. Maintenance of immunity in adults requires a booster vaccination every 10 years. The Centers for Disease Control and Prevention estimated in the mid-1990s that less than 50 percent of adults in the United States had received their 10-year boosters and that 40 to 50 percent of adults were susceptible to diphtheria.[81,92] In addition, the toxoid vaccine does not provide any protection against nontoxigenic strains.

The incidence peaks during the cooler autumn, winter, and spring months. Several epidemics, primarily in the southern United States, have occurred in late summer and fall and corresponded to a high prevalence of *C. diphtheriae* skin infections. Between the years 1971 and 1981, the incidence of diphtheria was highest in the western United States. A 100-fold greater incidence of diphtheria occurs in Native Americans than in the general population.[31] This difference may be attributable to socioeconomic factors more than to race.

Evidence that diphtheria is diagnosed more frequently in chronic alcoholics and the indigent than in the general population is significant. In a 1993-1994 outbreak in St. Petersburg, Russia, 69 percent of a total of 42 deaths occurred in individuals classified as chronic alcoholics.[94] Between 1972 and 1982, three outbreaks occurred in the indigent alcoholic population living in Seattle's Skid Road.[61] Cutaneous infections accounted for 86 percent of the 1100 total cases. The first outbreak was caused by a single toxigenic *intermedius* biotype clone, whereas the other two involved nontoxigenic *mitis* and *gravis* strains. The incidence was highest in winter and spring.

Native Americans also represent a disproportionate number of diphtheria cases, the reasons for which are unclear, but apparently infection is endemic in some closely knit communities. During 1974 and 1975, 27 percent of Native Americans in the Skid Road population were affected, compared with 5 percent of the white population. In 1996, a surveillance study was conducted in a Northern Plains American Indian community after *C. diphtheriae* was isolated from the skin of a resident of the community. The woman was a chronic alcoholic admitted to the hospital for detoxification and treatment of severe necrotizing leg ulcers, from which a toxigenic *mitis* biotype was isolated. In the following 4 months, 11 positive cultures were obtained from the community: 6 pharyngeal isolates from patients with pharyngitis, 1 ear isolate from a patient with suppurative otitis media, and 4 positive throat swabs from asymptomatic household contacts of the index cases. Of the 11 total isolates, 6 were biotype *mitis* and 5 were *gravis*; 9 isolates were toxigenic or weakly so. The two nontoxigenic isolates were biotype *gravis*. Ribotyping indicated that the isolates were related closely to each other genetically and to strains obtained from past cases from the same area; they were different from organisms obtained from other parts of the United States and from the former Soviet Union, where an ongoing epidemic was occurring at the time.[54,102]

A similar study was conducted in a Koorie (Aborigine) community in Victoria, Australia, in 1994, after three cases of nontoxigenic *C. diphtheriae* endocarditis were diagnosed in the community. After screening 359 asymptomatic (with the exception of 4 people who had chronic skin ulcers swabbed) contacts of the index cases, 12 produced positive cultures for nontoxigenic *C. diphtheriae*. Five of them were of the same biovar *gravis* clone as the three index cases.[64]

A major epidemic began in 1990 in the new independent states of the former Soviet Union and spread throughout the area. Between 1990 and 1997, approximately 150,000 cases of diphtheria were reported, with approximately 4000 fatalities.[60,91] The epidemic was attributed to decreasing immunization rates and immunity in adults and children and to movement of large numbers of people during the collapse of the former Soviet Union.[23,103] Apparently multiple foci of infection existed across the continent. Most of the epidemic isolates from Ukraine and Russia were biotype *gravis*, but further molecular characterization of the *tox* genes from these areas revealed distinct epidemic strains in each location.[65] Superimposed over the steady increase in the number of cases from 1990 through 1994, a seasonal variation typical of the Northern Hemisphere also was observed, with a significant peak in the number of diagnoses occurring in October and November and a trough from April to July.[60]

A mass immunization program was initiated in Russia in 1993, with a resultant 10 percent decrease in the number of new cases reported between 1994 and 1995 (versus twofold to threefold increases in the number of cases each year for the preceding 3 years).[52] The World Health Organization also held training workshops and assembled laboratory kits to assist in establishing the proper diagnosis of diphtheria. As cases also began to appear in Europe with increasing frequency during this period, the European Working Group on Diphtheria (ELWGD) and reference laboratories were assembled to assist in an effort to increase routine screening for diphtheria. The ELWGD since has expanded and currently includes 20 participating countries, including representatives in Western and Eastern Europe, the United States, Australia, and Southeast Asia.[41]

PATHOGENESIS AND PATHOLOGY

Diphtheria is initiated by entry of *C. diphtheriae* into the nose or mouth, where the bacilli remain localized on the mucosal surfaces of the upper respiratory tract. Occasionally, the ocular or genital mucous membranes serve as the site of localization. The bacilli are unable to invade intact skin but may infect preexisting skin lesions. After a 2- to 4-day period of incubation, lysogenized strains may elaborate toxin.

Diphtheria toxin is secreted as a single polypeptide of 535 amino acids with a molecular weight of 58,342 D.[65] The toxin is composed of two subunits: a large B subunit that is involved in receptor binding and an A subunit that is the enzymatically active portion of the toxin. The toxin initially is absorbed onto the target cell membrane by binding a receptor on the cell surface and then undergoes receptor-mediated endocytosis. When it is in the endolysosomal complex, it undergoes a conformational change with subsequent release of the A subunit into the cytoplasm. The A subunit transfers an adenosine diphosphate (ADP)–ribosyl group from nicotinamide adenine dinucleotide (NAD) to elongation factor 2. This ADP-ribosylation inactivates elongation factor 2 and inhibits protein synthesis in the cell. Cholera and pertussis toxins also mediate target protein ribosylation by this mechanism. In addition to the inhibition of cellular protein synthesis, an independent mechanism of cytolysis has been described. In the presence of calcium and magnesium, diphtheria toxin has a nuclease-like activity that causes DNA fragmentation that results in cytolysis.[29,30,65,73]

Marked toxin-mediated tissue necrosis occurs in the vicinity of *C. diphtheriae* colonization and induces a robust local inflammatory response. The inflammatory response coupled with the necrotic tissue produces a patchy exudate that initially can be removed. As the infection progresses, the increased production of toxin causes a centrifugal widening of the area of infection,

and eventually a fibrinous exudate develops. A tough adherent membrane results from coagulation of the exudate. The color of the pseudomembrane initially is white but over the course of time becomes dirty gray. Late in the course of the infection, green or black spots appear on the membrane, representing areas of necrosis. Histologic analysis of the pseudomembrane differs, based on the site of formation and maturation of the membrane. Analysis of the pharyngeal pseudomembrane shows fibrin; inflammatory cells, primarily composed of neutrophils, red blood cells, and colonies of organisms; and superficial epithelial cells.

With severe infections, significant vascular congestion, interstitial edema, fibrin exudate, and intense neutrophilic infiltration develop.[59] Profuse bleeding can occur when the membrane is torn off. The edematous tissue and the diphtheritic membrane may encroach on the airway. The membrane sloughs spontaneously during the recovery period, although sloughing can occur during the acute phase of the illness, leading to aspiration. Occasionally, secondary bacterial infection (classically caused by *Streptococcus pyogenes*) develops. Respiratory embarrassment or suffocation may occur, with involvement of the larynx or tracheobronchial tree. Bronchopneumonia may develop if the exudate enters the small airways and alveoli. Infection of these sites is an uncommon occurrence, however. Infections of the esophagus and stomach, with pseudomembranous lesions indistinguishable from lesions found in the respiratory tract, have been reported.[67]

Toxin produced at the site of infection is distributed throughout the body via the bloodstream and the lymphatics. This distribution occurs most readily when the pharynx and tonsils are covered by a diphtheritic membrane. Any organ or tissue can be damaged as a result of diphtheria toxin, but lesions of the heart, nervous system, and kidneys are particularly prominent. Clinical manifestations appear after a variable latent period of 10 to 14 days for myocarditis and 3 to 7 weeks for manifestations in the nervous system, such as peripheral neuritis. In a study of 102 patients who died of diphtheria caused by *C. diphtheriae*, the hearts appeared dilated, flabby, and pale, with a characteristic "streaky" appearance in the myocardium. The most prominent pathologic findings are necrosis and hyaline degeneration of the myocardium. The myocardium also appears edematous and is infiltrated with mononuclear cells with eosinophilic cytoplasm. In a significant proportion of cases, fatty accumulation in muscle fibers and the conducting system may be observed.[86] Burch and associates[22] showed mitochondrial damage with depletion of glycogen and accumulation of lipid droplets in the damaged myofibrils. Toxin may be observed within the myocardial cells with fluorescent antibody staining.[59] If the patient survives, muscle regeneration and interstitial fibrosis can be seen.

Peripheral neuropathy occurs secondary to *C. diphtheriae* infections. Histologic studies have shown that affected nerves have significant degeneration of myelin sheaths and axons. A toxic neuritis with fatty degeneration of paranodal myelin can be noted early in the disease course; segmental demyelination occurs later.[9] Axonal damage is secondary to the application of external pressure from the swollen Schwann cell cytoplasm and myelin.[76]

C. diphtheriae infections also can lead to necrosis and hyaline degeneration of the liver, which can lead to hypoglycemia. Adrenal hemorrhage and acute tubular necrosis of the kidney also have been known to occur secondary to *C. diphtheriae* infections.[59]

CLINICAL MANIFESTATIONS

The signs and symptoms of diphtheria depend on the site of infection, the immunization status of the host, and whether toxin has been distributed to the systemic circulation. The incubation period is 1 to 6 days (range, 1 to 10 days). Diphtheria can be classified clinically on the basis of the anatomic location of the initial infection and the diphtheritic membrane (nasal, pharyngeal/tonsillar, laryngeal or laryngotracheal, skin, and others) involved. More than one anatomic site may be involved simultaneously.

Nasal diphtheria initially resembles a common cold and is characterized by mild rhinorrhea and a paucity of systemic symptoms. Gradually, the nasal discharge becomes serosanguineous and then mucopurulent. A foul odor may be noticed, and careful inspection reveals a white membrane on the nasal septum. In severe cases, the infection may excoriate the nares and upper lip. Nasal diphtheria is a mild form of the disease because absorption of toxin usually is slow from this site. Frequently, delays in establishing an accurate diagnosis of nasal diphtheria occur because of the lack of systemic symptoms. The nasal form of the disease occurs most often in infants.

Pharyngeal and tonsillar diphtheria begin insidiously with anorexia, malaise, low-grade fever, and pharyngitis. Within 1 or 2 days, a membrane appears. The extent of membrane formation correlates with the immune status of the host; in some partially immune individuals, a membrane may not develop. The white or gray adherent membrane may cover the tonsils and pharyngeal walls and extend on to the uvula and soft palate or down on to the larynx and trachea. Attempts to remove the membrane are followed by bleeding. Cervical lymphadenitis varies. In some cases, it is associated with edema of the soft tissues of the neck and may be so severe that it gives the appearance of a "bull neck." In a 1970 epidemic, "erasure" edema of the neck was noted in patients with pharyngeal diphtheria.[80] Patients with erasure edema did not have a classic bull neck appearance, but the edema was characterized by obliteration of the sternocleidomastoid muscle border, the mandible, and the median border of the clavicle. The edema was brawny, pitting, warm to the touch, and tender to palpation. Erasure edema was noted in 29 percent of immunized patients and 30 percent of nonimmunized or inadequately immunized patients. It occurred most commonly in children older than 6 years and generally was associated with infection by the *gravis* or *intermedius* strain of *C. diphtheriae*.

The course of pharyngeal diphtheria depends on the degree of elaboration of the toxin and the extent of the membrane. In severe cases, respiratory and circulatory collapse may occur. The pulse rate is increased disproportionately to body temperature, which generally remains normal or slightly elevated. The palate may be paralyzed. This paralysis may be unilateral or bilateral and associated with difficulty swallowing and nasal regurgitation of swallowed fluids.[37] Stupor, coma, and death may occur within 7 to 10 days. In less severe cases, recovery may be slow and may be complicated by the development of myocarditis or neuritis. In mild cases, the membrane sloughs off in 7 to 10 days, and recovery is uneventful.

Laryngeal diphtheria generally reflects a downward extension of the membrane from the pharynx. Rarely, laryngeal diphtheria is primary and does not reflect an extension of disease from the pharynx. In these cases, toxicity and signs of toxemia generally are less prominent. Two cases of isolated diphtheritic tracheitis have been reported in the literature.[13,99] The clinical findings of laryngeal diphtheria are indistinguishable from other types of infectious croup. Noisy breathing, progressive stridor, hoarseness, and a dry barking cough may be noted. Suprasternal, subcostal, and supraclavicular retractions reflect severe laryngeal obstruction, which may be fatal unless alleviated. Occasionally, in a mild case, an acute and fatal obstruction may occur because of a partially detached piece of membrane that occludes the airway. In severe cases of laryngeal diphtheria, the membrane may extend downward and invade the entire tracheobronchial tree.

Cutaneous disease, in contrast to pharyngeal disease, is more common in warmer climates and often is caused by nontoxigenic

strains. In some countries with tropical and subtropical climates, such as Uganda, Tanzania, Sri Lanka, and Samoa, *C. diphtheriae* has been isolated from 60 percent of skin lesions in children.[63] Cutaneous diphtheria is more contagious than is respiratory diphtheria. Cutaneous diphtheria may be an important source of person-to-person transmission of diphtheritic organisms and outbreaks in indigenous populations in which overcrowding and poor hygiene are important risk factors.[12,21,63] The skin lesions begin as vesicles or pustules that progress to typical ulcers with sharply defined borders, membranous bases, and surrounding erythema and edema. They may be covered with a dark pseudomembrane. The lesions occur most commonly on the legs, feet, and hands. For the first 1 to 2 weeks, the lesions are painful. Spontaneous healing generally takes 6 to 12 weeks, but lesions have been reported to persist for 1 year.[63]

Conjunctival, aural, and vulvovaginal diphtheria also may occur. Conjunctival lesions usually are limited to the palpebral conjunctiva, which appears red, edematous, and membranous. Rarely, conjunctival lesions have been associated with corneal erosion.[96] Diphtheria infections of the ear are characterized by the development of otitis externa with a persistent purulent and frequently foul-smelling discharge.

Clinical presentations other than typical diphtheria have been associated with isolation of the organism from patients with meningitis, endocarditis, osteomyelitis, and hepatitis. In most cases, these infections have occurred in patients with underlying problems, such as structural or valvular heart disease or intravenous drug use, or in individuals from poor socioeconomic backgrounds.[35,101]

Several cases of septic arthritis caused by nontoxigenic *C. diphtheriae* have been described.[1,58,101] Afghani and Stutman[1] reported the case of a 27-month-old child who had septic arthritis of the hip and skin lesions on the lower extremities. In this case, the skin lesions were presumed to be the portal of entry for nontoxigenic *C. diphtheriae*. Although the child had received four doses of diphtheria and tetanus toxoids and pertussis vaccines, immunization with toxoid does not provide protection against nontoxigenic strains of *C. diphtheriae*. In this case, the organism was sensitive to penicillin, cefuroxime, cephalothin, and clindamycin but was resistant to oxacillin, an antistaphylococcal antibiotic often used for the treatment of septic arthritis when the causative organism cannot be identified. A similar case was described in an immunocompetent, fully vaccinated 2-year-old child who had skin lesions from which *C. diphtheriae* was isolated. The skin lesions also were assumed to be the portal of entry for the organism because pan-sensitive *C. diphtheriae* were isolated from the skin and the articular aspirate.[58]

Within a 12-month period, in New South Wales, Australia, four cases of septic arthritis complicating endocarditis caused by the nontoxigenic *gravis* variety of *C. diphtheriae* were reported. In addition, the same strain caused three cases of endocarditis without the development of septic arthritis. Demographic distribution of these seven cases included a 12-year-old boy who died, five patients who were in their 20s, and a patient who was 49 years old. Three of the patients had underlying cardiac abnormalities, and one had a history of intravenous drug use.[101] This same clone of nontoxigenic *C. diphtheriae* was isolated from three patients in Koorie, an aborigine community in Victoria, Australia, who had with endocarditis and five asymptomatic contacts.[64] Two of the three patients with endocarditis were members of the same family, and one of them had a history of alcohol abuse. The third patient had a septic sternoclavicular joint, in addition to endocarditis, with isolation of the same organism. Nontoxigenic *C. diphtheriae* sepsis can lead to splenic and hepatic abscesses, as reported in a patient with chronic lymphocytic leukemia in British Columbia, Canada.[66]

Complications secondary to elaborated diphtheria toxin may affect any system, but myocarditis and involvement of the nervous system are most characteristic. Myocarditis may occur after mild and severe cases of diphtheria. Generally, it develops in patients in whom administration of antitoxin is delayed. Myocarditis most commonly appears in the second week of the disease, but it can appear as early as the first or as late as the sixth week of illness. Tachycardia, a muffled S_1, murmurs, and arrhythmias such as atrioventricular dissociation indicate myocardial involvement. Echocardiography may show left ventricular dysfunction.[3,56,75] Although some cases may result in cardiac failure, most myocardial complications are temporary.

Neurologic complications appear after a variable latent period. Approximately 75 percent of all patients with severe diphtheria develop neuropathies. The incidence of neurologic sequelae has been shown to correlate with the severity of respiratory symptoms; 20 percent of all patients with respiratory problems develop polyneuritis. Neurologic complications from diphtheria infections predominantly are bilateral, are motor rather than sensory, and usually resolve completely. Paralysis of the soft palate is the most common occurrence and generally appears in the third week. It is manifested by a nasal quality in the voice, nasal regurgitation, and difficulty swallowing. Ocular paralysis usually occurs around the fifth week of illness and is characterized by blurring of vision and difficulty with accommodation. Internal strabismus also may be noted. Paralysis of the diaphragm, peripheral neuropathy involving the limbs, and loss of deep tendon reflexes likewise are reported as complications of diphtheria. When they occur, along with an elevated cerebrospinal fluid protein, the syndrome is clinically indistinguishable from Guillain-Barré syndrome.

Rarely, 2 or 3 weeks after the onset of illness, involvement of the vasomotor centers results in hypotension and cardiac failure. Gastritis, hepatitis, nephritis, and hemolytic-uremic syndrome also have been reported as complications of diphtheria.[98]

Information on the effects, if any, of diphtheria on the fetus during pregnancy was unavailable until more recently. El Seed and associates[43] reported a case of pharyngeal diphtheria in a pregnant woman that occurred during the first trimester of pregnancy. Apart from vaginal bleeding, no complications of pregnancy were noted. Severe diphtheritic toxemia in the mother was characterized by quadriparesis, from which she fully recovered. A physically normal female infant was delivered at term. In this single case, severe diphtheritic toxemia during pregnancy was not associated with any teratogenic effect in the fetus and did not impair intrauterine fetal growth.

DIAGNOSIS

The diagnosis of diphtheria should be based on clinical findings because any delay in initiating therapy poses a serious risk to the patient. Isolation of the organism is used to confirm the clinical diagnosis. Material obtained from beneath the membrane, where organisms are concentrated most highly, or a portion of the membrane itself should be obtained for culture.[5,33]

C. diphtheriae is relatively resistant to drying. The use of a non-nutritive, moisture-reducing transport medium helps prevent the overgrowth of other microorganisms. The laboratory should be notified about the possibility of diphtheria so that appropriate culture media are inoculated. A Löffler slant, a tellurite plate, and a blood agar plate should be inoculated. Tellurite-containing media inhibit the growth of normal oral flora, allowing *C. diphtheriae* to grow into characteristic black colonies. Other corynebacteria, staphylococci, and yeast also can reduce tellurite and grow into black colonies.[14,33] Although examination of direct smears of colonies or diphtheritic lesions remains an important supplement to clinical examination, it often is inaccurate. Screening the colonies from the tellurite plate for catalase, urea, nitrate, pyrazinamidase, and cystinase is important. Most biotypes of

C. diphtheriae are catalase-positive, urease-negative, nitrate-positive (except biotype *belfanti*), pyrazinamidase-negative, and cystinase-positive.[39]

All diphtheria bacilli that are recovered should be tested for toxigenicity. In 1949, the Elek immunoprecipitation assay replaced in vivo testing for toxigenicity using guinea pigs or rabbits.[42] The Elek test is based on gel diffusion and immunoprecipitation of toxin, from organisms inoculated onto agar adjacent to an antitoxin-containing well. A strain that is positive for toxin is indicated by the formation of a precipitin band between the toxin and antitoxin.[40,42] The Elek test would take 48 hours to yield results on the toxigenic nature of a *C. diphtheriae* strain. A modified Elek test that consists of placement of an antitoxin-impregnated disk onto an agar plate surrounded by inoculates of clinical specimen and positive control has been described. Compared with the conventional Elek test, the modified test has the advantage of using "spot" inoculations of numerous colonies directly from the primary plate. In addition, the modified Elek test has fewer false-positive and false-negative results and yields results more rapidly (16 to 24 hours).[39]

A rapid enzyme immunoassay is another test available for the detection of diphtheria toxin. This method uses equine polyclonal antibody to capture the diphtheria toxin and an alkaline phosphatase-labeled monoclonal antibody to detect fragment A of the toxin. It is a rapid test that takes 3 hours and has a limit of detection of 100 pg/mL.[44]

Rapid testing for diphtheria toxin by PCR specific for the "A" or "B" portion of the toxin gene, *tox*, is sensitive and has produced positive results in specimens stored for 12 months before performance of the assay.[7,69,70,87,88,90] The absence of the *tox* gene by PCR excludes the diagnosis of diphtheria. PCR may give false-positive results, however, because it does not differentiate between partial or nonfunctional *tox* genes and functional *tox* gene products. An increasing number of cases of nontoxigenic diphtheria that are positive for *tox* gene by PCR have been reported from Ukraine and Russia.[53,78]

The immune status of patients can be determined by toxin neutralization in Vero cells.[85] This method is used frequently, although it is difficult to standardize and relies heavily on individual interpretation of results. Enzyme-linked immunosorbent assay is a more rapid and quite sensitive method, but it detects some nonspecific antibodies; when antitoxin levels are in the low range, the assay may generate falsely elevated results.[82] Finally, a delayed fluorescence immune assay method was developed by Aggerbeck and colleagues[2] in 1996 and has been reported to have good sensitivity, specificity, and reproducibility.[17] Levels of diphtheria antitoxin of 0.01 IU/mL or greater generally are accepted as protective. A skin-testing method, the Schick test, also has been used to assess immunity.

The Schick test was used previously to determine the immune status of the patient. It is not helpful in establishing an early diagnosis because it cannot be read for several days, and currently it is not widely used. In the Schick test, a measured amount of purified diphtheria toxin (0.1 mL) is injected subcutaneously. A hypersensitivity reaction indicates an inadequate presence of antitoxin. A toxoid control also is injected, in the opposite arm, to help distinguish between a reaction to toxin and a reaction to other antigens in the toxin preparation.

Other laboratory studies are of little diagnostic value. The white blood cell count may be normal or elevated. Rarely, anemia develops as a result of rapid hemolysis of red blood cells. Examination of cerebrospinal fluid may reveal a minimal elevation of protein and, rarely, a mild pleocytosis in patients with diphtheritic neuritis. Hypoglycemia, glucosuria, or both may occur and reflect hepatic toxicity. An elevation in blood urea nitrogen may develop in patients with acute tubular necrosis. An electrocardiogram should be obtained and may reveal ST-segment and T-wave changes or arrhythmias indicative of myocarditis.

DIFFERENTIAL DIAGNOSIS

Mild forms of nasal diphtheria in a partially immunized host may resemble the common cold. When the nasal discharge is more serosanguineous or purulent, nasal diphtheria must be distinguished from a foreign body in the nose, sinusitis, adenoiditis, or the snuffles of congenital syphilis. Careful examination of the nose with a nasal speculum, sinus radiographs, and appropriate serologic tests for syphilis are helpful in excluding these disorders.

Tonsillar or pharyngeal diphtheria must be differentiated from streptococcal pharyngitis. Generally, streptococcal pharyngitis is associated with more severe pain on swallowing, higher temperature, and a nonadherent membrane limited to the tonsils. In some patients, pharyngeal diphtheria and streptococcal pharyngitis coexist.

Tonsillar and pharyngeal diphtheria also must be differentiated from infectious mononucleosis (lymphadenopathy and splenomegaly are common findings, atypical lymphocytes are generally present, and heterophile antibody may be present), nonbacterial membranous tonsillitis (white blood cell count generally is low, throat cultures reveal normal flora, and the course is unaffected by antibiotics), primary herpetic tonsillitis (presence of gingivitis, stomatitis, and discrete lesions of the tongue and palate may be helpful), Vincent angina (may be indistinguishable), and thrush (constitutional symptoms are absent, and lesions are present on the buccal mucosa and tongue). Tonsillar and pharyngeal diphtheria also must be differentiated from blood dyscrasias such as agranulocytosis and leukemia (complete blood count and bone marrow study are helpful); post-tonsillectomy faucial membranes (membranes are stationary and do not spread); and oropharyngeal involvement by toxoplasmosis, *Arcanobacterium*, cytomegalovirus, tularemia, and salmonellosis (associated signs and symptoms and appropriate cultures and serologic tests may be diagnostic).[50]

Laryngeal diphtheria must be differentiated from spasmodic or nonspasmodic croup; acute epiglottitis; laryngotracheobronchitis; aspirated foreign bodies; peripharyngeal and retropharyngeal abscesses; and laryngeal papillomas, hemangiomas, or lymphangiomas. A careful history, followed by careful visualization in the hospital under controlled conditions, aids in making a correct diagnosis.

PREVENTION

Diphtheria is prevented on a community-wide basis most effectively by active immunization. Diphtheria toxoid is available in combination with tetanus toxoid as pediatric DT or adult Td and in combination with acellular pertussis as DTaP and Tdap. Combination regimens with DTaP and inactivated poliovirus and hepatitis B (Pediatrix) and inactivated poliovirus and *H. influenzae* type B (Pentacel) are also available. Pediatric formulations of diphtheria toxoid vaccines contain three to four times more diphtheria toxoid but the same tetanus toxoid compared with the adult formulations. Children younger than 7 years should be given the pediatric formulations of vaccine, whereas children older than 7 years should receive the adult Td. Two forms of Tdap are available: Boostrix, which is approved for children 10 to 18 years old, and Adacel, which is approved for individuals 11 to 64 years old.[24-27,38]

Primary immunization is carried out conveniently and effectively by giving diphtheria and tetanus toxoids and pertussis vaccine, DTaP, at 2, 4, and 6 months of age, with booster doses given at 15 to 18 months and again when the child is 4 to 6 years of age. Booster doses with adult-type diphtheria and tetanus toxoids adsorbed (Td) should be given at 10-year intervals to all immunized individuals. Current recommendations are to give

Tdap as the first booster dose in patients who are 11 to 12 years old. Td and Tdap contain 2 to 2.5 Lf (flocculation units) diphtheria toxoid per dose compared with 7 to 25 Lf in the pediatric diphtheria, tetanus toxoid, and pertussis vaccine preparations (DTaP, DT). Primary immunization of children older than 7 years may be performed with Td. Two doses are given intramuscularly at least 4 weeks apart, with a booster dose provided 1 year later. Children and adults who are severely immunocompromised or undergoing long-term hemodialysis should use the standard immunization schedule, although response may be suboptimal.[47,51,71]

Most local and systemic reactions to the diphtheria and tetanus toxoids and whole cell pertussis vaccine (DTP), including fever, were related to the pertussis component.[10,32] Administration of tetanus and diphtheria toxoids is not followed by the high incidence of reactions associated with the use of pediatric DTP vaccines. At least one study showed that 7.5 Lf toxoid can be given safely to adults without a higher risk of reactions occurring.[15] Primary immunization against diphtheria for infants with progressive neurologic disorders and completion of the primary immunization series in patients who had experienced an untoward reaction to an earlier DTP vaccine injection may be performed with diphtheria and tetanus toxoids rather than diphtheria and tetanus toxoids and pertussis vaccine.[4]

A report of 97 preterm infants who received diphtheria and tetanus toxoids and acellular pertussis vaccine (DTaP) (94 of these infants also received *Haemophilus influenzae* type b vaccine) showed that most infants tolerated the vaccination without side effects, although a subgroup of very-low-birth-weight infants (mean 873 g) had either recurrence or an increase in the number of apneic and bradycardic episodes in the 48 hours after receiving vaccination. The apneic and bradycardic episodes were present before immunization in every case.[97]

Booster doses of tetanus and diphtheria toxoids should be given at 10-year intervals to all immunized individuals. As mentioned earlier, levels of diphtheria antitoxin of 0.01 IU/mL or greater generally are accepted as protective.

Diphtheria immunization is not always followed by complete protection.[79] Immunization is directed against the phage-mediated toxin, not against infection. Fully immunized individuals may be carriers or may have disease caused by nontoxigenic strains. An investigation conducted during an epidemic in Texas showed no statistical difference in the risk of diphtheria infection developing in individuals with full, lapsed, inadequate, or no previous diphtheria immunization; however, a 30-fold increased risk of development of symptomatic diphtheria in individuals with no immunization and an 11.5-fold increase in individuals with inadequate immunization was noted.[84]

The most important health problem in the United States today is inadequate immunization of the population. Immunization rates in adults are poorer than those in infants and children because of failure to maintain adequate immunity through appropriate booster immunization. A 70 to 80 percent immunization level is thought to be required to prevent epidemic spread.[31]

Prevention of diphtheria also depends on management of the contacts of known cases of diphtheria and carriers of the organism and on isolation of patients to minimize the spread of disease. Individuals at risk of contracting the disease from the index case include those who have had close respiratory or physical contact or prolonged close proximity with the infected individual, including members of the index case's household.[18] Specifically, family members who share body towels and cups or eating utensils, share a bed or a bedroom with more than two people, or take a bath less than once a week have a significantly greater risk of contracting the disease from the infected patient. A history of eczema in the contact also has been associated with a significantly increased risk of contracting diphtheria from the index case.[93] The patient is infectious until diphtheria bacilli no longer can be cultured from the site of infection. Two or three consecutive negative cultures at least 24 hours apart are required, and antibiotic therapy must be complete for 24 hours before the patient is released from isolation. If obtaining cultures is impossible, isolation may be ended after the completion of 14 days of appropriate antibiotic treatment.[6]

Cultures should be taken from the nose and throat of all close contacts, who should be kept under surveillance for 7 days (surveillance as an outpatient is acceptable).[5] Regardless of their immunization status, contacts should be treated with a single intramuscular dose of benzathine penicillin G (600,000 U for individuals weighing <30 kg and 1.2 million U for individuals weighing >30 kg) or a 7-day course of erythromycin, 40 to 50 mg/kg/day (maximum 2 g/day) divided into four doses.[5] The immune status of each contact should be determined; individuals for whom immune status is inadequate, including individuals who have had the primary series but more than 5 years has elapsed since they received their last booster dose, should receive an injection of diphtheria toxoid. In addition, patients with diphtheria should be immunized during convalescence because infection may not confer immunity.[6]

Asymptomatic carriers who previously were not immunized against diphtheria should have cultures taken, receive diphtheria toxoid and penicillin or erythromycin (as described earlier), and be seen daily by a physician. Asymptomatic contacts who are found to carry a toxigenic strain should be subjected to the same isolation and treatment measures as the index case.[6] If daily surveillance is impossible, benzathine penicillin is preferred over erythromycin for treatment because failure to adhere to an oral drug regimen is a concern. If a contact is experiencing symptoms when seen, treatment of diphtheria is indicated. It is important to initiate prophylactic therapy in contacts who have not been immunized, before the results of culture are received. Management of carriers is described in the next section. Contacts whose occupations involve close contact with unimmunized children or food handling (especially milk) should refrain from working until cultures are confirmed to be negative.[6]

TREATMENT

Treatment of diphtheria is predicated on neutralization of free toxin and eradication of *C. diphtheriae* or *C. ulcerans* by the use of antibiotics. Disease caused by *C. ulcerans* should be treated in the same manner as is disease caused by *C. diphtheriae*.[3,95] The decision to administer equine antitoxin should be based on the site and size of the membrane, the degree of toxicity, and the duration of illness.[16]

Antitoxin can neutralize circulating toxin or toxin that is absorbed onto cells, but is ineffective when cells have been penetrated. Early treatment is essential to limit tissue damage. An adequate dose of antitoxin must be administered intravenously as early as possible to neutralize all free toxins. A single dose is used to avoid the risk of developing sensitization from repeated doses of horse serum. Tests for sensitivity to horse serum must be performed before antitoxin is administered. For this purpose, 0.02 mL of a 1:1000 dilution of antitoxin in saline can be given intracutaneously. Positive (histamine) and negative (isotonic saline) controls should be applied similarly. A positive reaction consists of a wheal at least 3 mm larger than the negative control, with surrounding erythema at the site of injection, developing within 15 to 20 minutes, and necessitates desensitization. The histamine control must be positive if test results are to be considered valid. Alternatively, the test may be done with a drop of serum diluted 1:100 and applied to the site of a superficial scratch, prick, or puncture on the volar aspect of the forearm.

Controls should be applied and results interpreted as described for intracutaneous administration.[5]

If a patient has been shown to be sensitive to horse serum, the serum should be provided in a slowly increasing dosage given at 15-minute intervals. Several regimens have been used. One recommended regimen is as follows:[5]

- 0.1 mL of a 1:1000 dilution intravenously
- 0.3 mL of a 1:1000 dilution intravenously
- 0.6 mL of a 1:1000 dilution intravenously
- 0.1 mL of a 1:100 dilution intravenously
- 0.3 mL of a 1:100 dilution intravenously
- 0.6 mL of a 1:100 dilution intravenously
- 0.1 mL of a 1:10 dilution intravenously
- 0.3 mL of a 1:10 dilution intravenously
- 0.6 mL of a 1:10 dilution intravenously
- 0.1 mL undiluted intravenously
- 0.3 mL undiluted intravenously
- 0.6 mL undiluted intravenously
- 1 mL undiluted intravenously

The intravenous route for desensitization is considered safest because it offers good control, but protocols involving the intradermal, subcutaneous, and intramuscular routes also have been used frequently.[5]

If no reaction has occurred, the remaining material is given by slow intravenous infusion. Intravenous administration results in more rapid excretion of antitoxin into saliva, rendering it atoxic and preventing further absorption of toxin in the oropharynx, but it does not result in more rapid systemic elimination of antitoxin than intramuscular administration.[100] Reactions should be treated with aqueous epinephrine (1:1000) provided intravenously.

The antitoxin dosage is empiric. Pharyngeal or laryngeal disease of 48 hours' duration or less should be treated with 20,000 to 40,000 U, nasopharyngeal disease with 40,000 to 60,000 U, and severe pharyngeal or laryngeal diphtheria with 80,000 to 120,000 U of antitoxin. The last dose also should be given to patients with mixed clinical symptoms and to patients with brawny edema or disease of longer than 48 hours' duration. The value of antitoxin in the treatment of cutaneous disease is debated, but some experts recommend 20,000 to 40,000 U because toxic effects have been reported.[5]

Although antibiotics are not a substitute for treatment with antitoxin, they should be given when diphtheria is suspected clinically. Penicillin and erythromycin are still effective against most strains of *C. diphtheriae*. Penicillin and erythromycin also are effective in eradicating group A hemolytic streptococci, which may complicate 30 percent of cases of diphtheria. Treatment consists of a 14-day course of penicillin or erythromycin. Penicillin may be given as aqueous penicillin G, 100,000 to 150,000 U/kg/day in four divided doses intravenously, or as procaine penicillin, 25,000 to 50,000 U/kg/day (maximum of 1.2 million U) in two divided doses intramuscularly. Patients who are sensitive to penicillin should be given erythromycin in a daily dosage of 40 to 50 mg/kg (maximum of 2 g/day) in four divided doses for 14 days. When the patient is able to tolerate oral medications, erythromycin or penicillin V may be used in place of the intravenous antibiotics.[5]

Follow-up cultures should be obtained at least 2 weeks after antibiotic therapy is complete; if they are positive, erythromycin should be given for an additional 10 days.[5] Some resistance to erythromycin has been observed, but it is uncommon, and its epidemiologic significance is unknown.[5,6] Penicillin is recommended as first-line treatment in Vietnam, based on sensitivity data.[68] Amoxicillin, rifampin, and clindamycin provided in appropriate dosages also may be effective. Lincomycin and tetracycline have proved to be less effective, and cephalexin, oxacillin, and colistin have been shown to be ineffective against *C. diphtheriae*. The end-point of therapy is two to three consecutive negative cultures at least 24 hours apart. In addition to receiving antibiotic therapy, patients with diphtheria should be immunized during convalescence because infection may not confer immunity.[5]

The carrier state has been treated effectively with a single intramuscular dose of benzathine penicillin G (600,000 U for children weighing <30 kg or 1.2 million U for individuals weighing ≥30 kg) or oral erythromycin (40 to 50 mg/kg/day for children and 1 g/day for adults) for 7 to 10 days.[5] Carriers should have repeat pharyngeal cultures performed a minimum of 2 weeks after antibiotic therapy is complete; if the repeat cultures are positive, carriers should receive an additional course of antibiotics.

SUPPORTIVE TREATMENT

Bed rest is extremely important and should be required for 2 to 3 weeks. Serial electrocardiograms should be obtained two or three times each week for 4 to 6 weeks to detect myocarditis as early as possible. Absolute bed rest must be enforced if myocarditis is detected because sudden death has been precipitated by excessive activity. A patient with myocarditis may receive digitalization if congestive heart failure develops. Digitalization for arrhythmias caused by diphtheria may be contraindicated, however. In severe disease, prednisone, 1 to 1.5 mg/kg/day for 2 weeks, has been shown to lessen the incidence of myocarditis.

Hydration should be maintained, and a high-calorie liquid or soft diet should be provided. Secretions should be suctioned as needed to prevent aspiration. Palatal and pharyngeal paralysis increases the risk of aspiration occurring, so gavage via a nasogastric tube is indicated in these patients.

The quality of the voice and the gag reflex should be checked regularly for assessment of progression of the disease. Laryngeal diphtheria may require relief of obstruction with a tracheostomy. This procedure should be performed before the patient has become exhausted.

Adequate immunity does not develop in at least half of patients who recover from diphtheria, and they remain subject to reinfection. Immunization is indicated after the patient recovers.

PROGNOSIS

Many factors affect the prognosis in cases of diphtheria, the most important being the immunization status of the host. Morbidity and mortality rates are increased significantly in patients who are unimmunized or inadequately immunized. The rapidity with which medical care is sought and the diagnosis of diphtheria is suggested has a great impact on outcome. If specific treatment is provided on the first day of disease, the mortality rate may be reduced to less than 1 percent; delay in providing treatment until day 4 may be associated with a 20-fold increase in the mortality rate.

The virulence of the infecting organism and the location of infection are important prognostic factors. Infection with a nontoxigenic *C. diphtheriae* strain may cause disease but does not lead to myocarditis, neuritis, and other toxin-related phenomena. Toxigenic disease may vary from mild to severe. In cases of mild diphtheria, membrane sloughing and full recovery generally occur within 7 days. Disease caused by toxigenic *gravis* strains tends to be more severe and carries a poorer prognosis. Although diphtheria may affect the skin, nasopharynx, and other mucous membranes, involvement of the larynx heralds a more complicated course. Laryngeal diphtheria increases the risk of development of airway obstruction and promotes systemic absorption of

the toxin. These patients require close monitoring of respiratory function and for involvement of other organ systems. Laryngeal diphtheria is more likely to be fatal in infants.

Few laboratory parameters indicate the severity of diphtheria. The development of amegakaryocytic thrombocytopenia and leukocytosis with counts of greater than 25,000 cells/mm^3 has been associated with a poor outcome.

The prognosis in a patient with diphtheria remains guarded until recovery is complete. At any time during the course of the illness, complications such as laryngeal obstruction, shock, and ventricular fibrillation may occur suddenly and unexpectedly. In patients with myocardial involvement, permanent damage to the heart, specifically fibrosis, may occur and lead to later complications. In addition, potentially severe neurologic manifestations, such as phrenic nerve paralysis, may appear late in the course of the disease.

Persistence of *C. diphtheriae* may be noted in the nasopharynx of 5 to 10 percent of convalescing patients. Recovery from diphtheria is followed by immunity that is demonstrable for at least 1 year after illness in 50 percent of patients. Second attacks are rare; nonetheless, immunization should be performed after the patient recovers.

Before the use of antitoxin and the availability of antibiotics, the mortality rate from diphtheria was 30 to 50 percent. Death was most common in children younger than 4 years old and was the result of suffocation. At present, the worldwide mortality rate is 5 to 10 percent, with no clear association with age.

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CHAPTER 102

Anthrax

Morven S. Edwards

Anthrax is a toxigenic disease of herbivores for which humans are an incidental host. The term *anthrax*, derived from the Greek *anthrakos*, which means "coal," refers to the black eschar characteristic of cutaneous anthrax. The three clinical manifestations of anthrax are cutaneous, which accounts for 95 percent of infections in the United States; inhalational; and gastrointestinal. Each manifestation can occur in children,[22,30,42,48] and all can be complicated by meningitis.

HISTORICAL ASPECTS

Anthrax has been recognized since antiquity. The earliest reference to a disease thought to be anthrax is a description in *Exodus* of the plague that caused the death of all of the Egyptians' cattle.[7] Hippocrates described carbuncles that are thought to represent the cutaneous form of anthrax. An account of anthrax in animals is detailed in the third of the Roman poet Virgil's four *Georgics*.[39] In the early 17th century, an anthrax pandemic referred to as the "Black Bane" caused 60,000 human deaths in Europe.[7] By the 18th century, several excellent descriptions of the clinical disease in humans had been published.

Bacillus anthracis has a unique niche in the history of infectious diseases. The organism, seen microscopically by Delafond in 1838, was isolated and cultivated in 1877 by Koch, who showed its proliferation in vivo, establishing a model for the causation of infectious disease. In 1881, both Pasteur and Greenfield[45] showed that a live-attenuated anthrax bacillus vaccine protected against subsequent challenge in animals, demonstrating the efficacy of immunization for the prevention of an infectious disease. In the late 1800s, anthrax was proved to be the cause of woolsorter's disease, or inhalation anthrax, contracted by factory workers handling anthrax-contaminated animal hides.

During World War I, *B. anthracis* was manufactured as an agent for biologic warfare. Because anthrax is transmissible by the respiratory route, inhalational anthrax usually is fatal, and because *B. anthracis* spores are stable in the environment, anthrax continues to be a focus of biologic warfare research programs.[12] The accidental release of anthrax spores from a military research facility in Sverdlovsk in the former Soviet Union in 1979 resulted in at least 68 deaths from inhalational anthrax.[32] One estimate is that the aerosolized release of 100 kg of anthrax spores upwind of Washington, D.C., would result in 130,000 to 3 million deaths.[23]

The first confirmed outbreak associated with intentional anthrax release in the United States occurred in October and November 2001.[24] Anthrax spores were disseminated by mail, resulting in 5 deaths from inhalation and 22 total cases of cutaneous and inhalational anthrax. Envelopes containing anthrax spores were sent through the U.S. Postal Service to offices of newspaper and broadcast media and U.S. senators. The spores were "weaponized," or finely milled, and treated with chemicals to prevent clumping so that they dispersed when the envelopes were opened and leaked from sealed envelopes as they passed through mail sorting machines. A single anthrax strain was implicated. This outbreak has had a substantial impact on the health care system and has revealed the potential impact of a bioterrorism event on society.[5]

BACTERIOLOGY

B. anthracis is an aerobic, nonmotile, spore-forming rod in the family Bacillaceae. Optimal growth occurs at 36° C in nonselective media. Colonies are gray-white, rough, and flat and may have comma-shaped projections caused by the outgrowth of chains of bacilli from the edges of the colony, giving it a "Medusa head" appearance. Individual colonies are 4 to 5 mm in diameter with a ground-glass appearance and exhibit tenacity or a "beaten egg white" appearance when lifted with an inoculating loop.[16] A capsule, which is associated with virulence, can be delineated by electron microscopy. Encapsulation occurs with growth on enriched media. Gram stain of clinical material reveals large, square-ended, gram-positive rods singly or in short chains without a visible capsule. The equatorial or paracentral spores are invisible in smears fixed promptly after collection. After 24 to 48 hours of aerobic incubation, strands of rods are arranged in "boxcar" or "bamboo" fashion, and sporulation occurs. Small numbers of bacteria are pathogenic for mice, guinea pigs, and rabbits, and death usually occurs 2 to 5 days after inoculation. Currently, no method exists for serologically classifying strains of *B. anthracis*.

EPIDEMIOLOGY AND TRANSMISSION

Domestic herbivores—cattle, sheep, horses, goats, and swine—are the most important agricultural sources of anthrax, but all domestic and many wild animals can serve as hosts. Animals are infected by ingestion of spores from infected pastures. Spores germinate in vivo, and death, associated with massive septicemia, usually occurs in 1 or 2 days. Anthrax is endemic in areas where an animal-soil-animal cycle is established because the spores can survive indefinitely in a dry environment. Uncultivated soil with a pH greater than 6.0 and ambient temperature greater than 15.5° C provides an environment favorable for persistence of spores.[47]

An estimated 20,000 to 100,000 human cases of anthrax occur yearly worldwide.[40] Regions of high endemicity include South and Central America, southern Europe, eastern Europe, Asia, Africa, the Caribbean, and the Middle East. The number of human cases probably correlates with the enzootic status of the disease in livestock in these countries. Familial clustering can occur in association with exposure to diseased animals.[1,35] Children who live in endemic areas often contract cutaneous anthrax from direct animal contact.[34] More than half of 448 patients from the Gambia with cutaneous anthrax were younger than 15 years of age, and 11 percent were younger than 2 years.[20] In the United States, epizootics of anthrax occur in the lower Mississippi valley and in parts of California, Texas, Missouri, Nebraska, and South Dakota[49]; sporadic cases have been reported from almost every state. Cases of illness were identified in four states and in Washington, D.C., during the 2001 bioterrorism-related outbreak.

The incidence of human anthrax in the United States has decreased markedly since the early 1900s, when more than 100 cases were reported annually. Cutaneous infections are reported sporadically.[9] Cases often are associated with exposure to contaminated animal products in commercial preparations, but exposure to indigenous animal anthrax does occur in the United

States.[43] Two members of a farmer's family ate hamburgers made from an anthrax-infected steer in Minnesota in 2000. The family members received antibiotic prophylaxis and anthrax vaccine and remained well.[9]

Direct contact with contaminated animal meat or carcasses or with contaminated animal products, such as hides, hair, wool, bone meal, and animal feed, are sources of transmission of anthrax spores to humans. Children of industrial workers have acquired infection, presumably from contact with their parents' contaminated clothing.[7] Processed products, such as shaving brushes and saddle blankets, have been implicated as sources of infection. Cutaneous anthrax is transmitted by deposition of spores or bacilli into abrasions or cuts in the skin. Blood-sucking insects, including mosquitoes and stable flies, can be vectors.[31,46,47]

The specific mode of acquisition may be difficult to determine in children; in one child with cutaneous anthrax, the only potential exposure was proximity to a bone meal factory that he walked past on his way to school.[27] Tying the umbilicus with a dirty thread was the presumed portal of entry for a neonate with *B. anthracis* sepsis.[34] Rubbing with contaminated fingers or deposition by an insect vector can lead to cutaneous involvement of the eyelids.[51] Discharge from cutaneous lesions theoretically is infectious, but person-to-person transmission has not been confirmed. Skin-to-skin contact through his mother's exposure at her workplace was proposed as the route of transmission to an infant in the bioterrorism-related outbreak in the United States in 2001.[19] Maternal anthrax has been associated with preterm delivery.[25]

Inhalational anthrax results from inhalation of spores. The estimated infectious dose in humans is 8000 to 50,000 spores. Cases of inhalational anthrax in the United States have occurred in a weaver whose imported yarn was contaminated and in a drum maker whose imported hand-dried animal hide was contaminated with *B. anthracis*.[8,13] Eleven adults acquired inhalational anthrax during the 2001 bioterrorism outbreak of anthrax in the United States. Gastrointestinal anthrax is caused by ingestion of contaminated meat. A newborn was reported to have contracted anthrax meningitis from his mother, who was septicemic at the time of delivery.[20]

PATHOGENESIS AND PATHOLOGY

Anthrax toxicity results from the activity of three polypeptides—protective antigen, edema factor, and lethal factor—that combine to form two toxins, lethal toxin and edema toxin. Protective antigen is integral to the action of both toxins. Toxin entry into cells is initiated when protective antigen binds to membrane receptors of susceptible cells. Two related proteins, tumor endothelial marker 8 and capillary morphogenesis protein 2, function as receptors. Cleaved protective antigen oligomerizes, and a heptamer of protective antigen provides binding sites for edema factor or lethal factor. The complex is endocytosed as edema toxin or lethal toxin.

Edema factor is a calmodulin-dependent adenylate cyclase that increases intracellular cyclic adenosine monophosphate levels and is responsible for the massive edema that occurs in cutaneous anthrax.[28] Lethal factor is a zinc metalloproteinase that cleaves mitogen-activated protein kinase kinases so that they are unable to activate their downstream substrates, the mitogen-activated kinases.[18] Lethal factor stimulates production of tumor necrosis factor-α and interleukin-1β. The capsule of *B. anthracis* also contributes to virulence by inhibiting phagocytosis, and the toxins inhibit neutrophil priming by lipopolysaccharide, modulating the inflammatory response.[24,50]

Cutaneous infection is initiated when anthrax spores are ingested at the site of entry by macrophages, and germination occurs in skin tissues. Production of toxin results in local edema. Regional lymphangiitis and lymphadenopathy can occur.[15] Interstitial edema, lymphatic dilation, and thrombosis and necrosis of blood vessels are characteristic microscopic features of cutaneous anthrax lesions. Erythrocytes extravasate freely into interstitial fluid. Few neutrophils or other inflammatory cells are present unless a lesion is infected secondarily. Hemorrhagic lymphadenitis involving regional lymph nodes occurs in all forms of anthrax.

In inhalation anthrax, spores entering the alveoli are phagocytosed by alveolar macrophages and dendritic cells and carried to hilar lymph nodes, where they germinate. Primary focal hemorrhagic necrotizing pneumonia can occur at the pulmonary portal of entry.[2] Massive hemorrhagic mediastinal lymphadenitis can block lymphatic drainage routes and can be causally related to the pulmonary edema and respiratory distress observed clinically.[48]

Gastrointestinal anthrax results from ingestion of spores or vegetative bacilli in contaminated meat. The primary sites of infection are the epithelium of the stomach or bowel wall.[6] Edema and small necrotic ulcers of the mucosa of the gastrointestinal tract, especially the ileum or cecum, are characteristic findings. Dissemination to the central nervous system can occur by hematogenous or lymphatic routes from any site of primary involvement. Hemorrhage involving the meninges and intense arteritis are uniform findings in patients who die of anthrax meningitis.

CLINICAL MANIFESTATIONS

CUTANEOUS ANTHRAX

The lesions of cutaneous anthrax occur mainly on exposed areas of the body. In one report, the distribution in young children was 52 percent of lesions on the head and neck, 28 percent on the trunk, and 20 percent on the extremities, whereas in older children, the distribution was 70 percent on the head and neck, 16 percent on the trunk, and 14 percent on the extremities.[22] Spores are introduced through abraded or injured skin. Children can have one or several lesions, and they are associated with regional adenitis.[29] After an incubation period of 2 to 5 days, a small, nontender, but frequently pruritic, papule develops at the site of inoculation. The lesion progresses to a serous or serosanguineous vesicle with surrounding nonpitting edema within 36 hours. Satellite vesicles, sometimes referred to as a "pearly wreath,"[51] may be seen occasionally.

The lesion undergoes central necrosis, with a black eschar left behind (Fig. 102–1).[38] The term *malignant edema* is used to describe severe lesions, particularly lesions involving the head and neck, which can be associated with systemic toxicity and occlusion of the airway. Small children can appear acutely ill with a temperature of 39° C to 40° C and leukocytosis with counts of 20,000 to 30,000 cells/mm^3.[19] Approximately 5 percent of patients are bacteremic. With appropriate therapy, the edema usually resolves within 2 to 3 days, but the central lesion continues its evolution unaffected. The eschar usually is 1 to 3 cm in diameter, with sharply defined margins seen 1 week to 10 days after onset. Separation of the eschar may take several weeks, and healing occurs with variable central scarring.[27]

INHALATIONAL ANTHRAX

Inhalational anthrax is a biphasic illness. Symptoms in the initial stage—malaise, low-grade fever, myalgia, and nonproductive cough—are nonspecific and resemble the symptoms of a respiratory viral illness or bronchitis. Adults who have inhalational anthrax are more likely to present with tachycardia, high hematocrit, low albumin, and sodium levels, and are less likely to present with myalgias, headache, and nasal symptoms than are

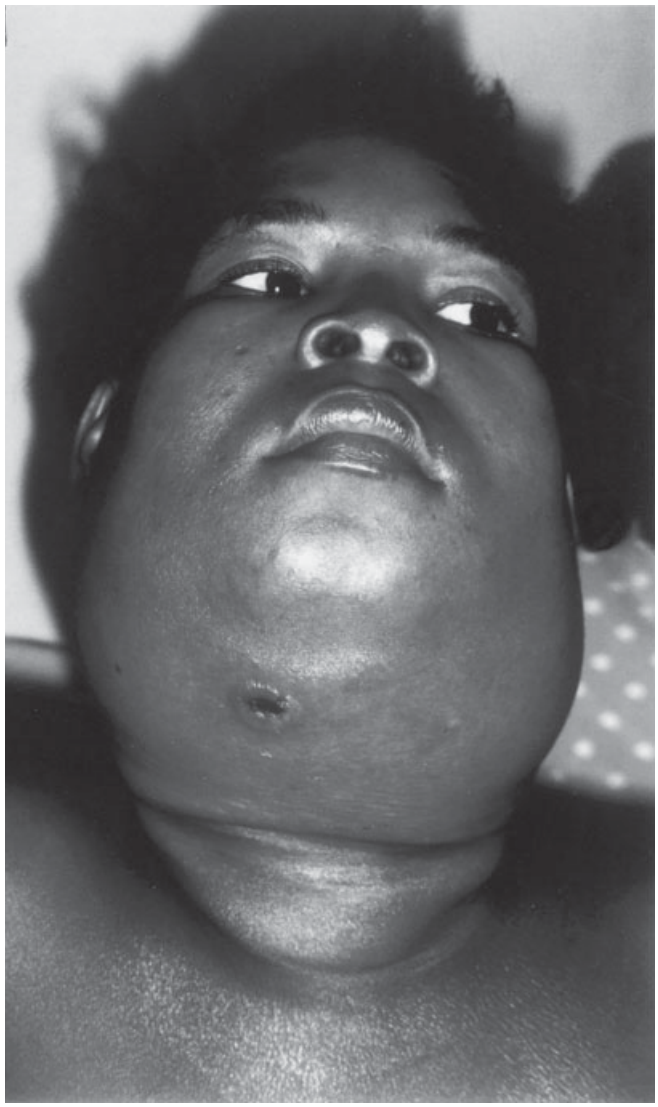

Figure 102–1 Cutaneous anthrax with associated massive submental edema. The lesion is located at the site of a small, initially trivial laceration that served as the portal of entry for the anthrax endospores. The patient received ampicillin and recovered completely. *(Courtesy of M. Thomas Casey, M.D.)*

adults who have influenza-like illnesses.[26] After several days of illness, dyspnea and stridor initiate onset of the second stage, which usually terminates fatally within 24 hours. Chest radiographs show a widened mediastinum with smooth borders and evidence of hemorrhagic mediastinitis and pleural effusions.[48] The pleural effusions often are large and usually contain bloody fluid. Pulmonary infiltrates usually are the result of superimposed bacterial infection. In the more recent cases from the United States, computed tomography scans of the chest showed the characteristic findings of hemorrhagic mediastinal and hilar lymph nodes and mediastinal edema and pleural effusions.[5]

GASTROINTESTINAL ANTHRAX

Gastrointestinal anthrax can manifest with oropharyngeal or intestinal manifestations. Each has an incubation period of 2 to 5 days after the ingestion of contaminated meat. The oropharyngeal form results in an oral or esophageal ulcer.[6,24,37] Presenting features include fever, severe sore throat, neck swelling caused by edema and enlargement of the cervical lymph nodes, dysphagia, and respiratory difficulty. Lesions on the tonsils or posterior oropharynx progress over 1 to 2 weeks from an area of edema to a pseudomembrane-covered ulcer. The oropharyngeal form of anthrax, although uncommon, has a more favorable prognosis than does intestinal anthrax.

Initial features of intestinal anthrax are fever, nausea, anorexia, vomiting, and diffuse abdominal pain that progresses rapidly to severe abdominal pain with rebound tenderness. Vomiting of blood-tinged or coffee ground-like material and melena are common symptoms and are secondary to ulceration of the intestinal mucosa.[6,24,33] The pain decreases, and massive ascites develops 24 to 48 hours after the onset of symptoms. Abdominal radiographs at this time show edematous loops of bowel and decreased air. If the abdomen is explored, findings include enlarged, erythematous mesenteric lymph nodes and straw-colored to purulent ascitic fluid in which organisms are readily visible.[3] Death usually occurs in association with significant blood loss, fluid and electrolyte imbalances, and subsequent shock. If the patient survives the acute illness, the edema and melena subside in 10 to 14 days.

MENINGITIS

Most reports of anthrax meningitis are associated with cutaneous disease, likely due to the higher frequency of cutaneous disease. Anthrax can disseminate to the meninges from any site of primary involvement. In the 2001 bioterrorism event, one adult with inhalation anthrax presented with meningitis, and several others had features suggestive of central nervous system involvement.[36] In a report of 70 patients with anthrax meningitis who ranged in age from newborn to 71 years, no primary focus could be found in 12 percent of patients.[21] Young and middle-aged men are affected most often as a consequence of occupational exposure.

Anthrax meningitis is characterized by a sudden onset and fulminant course. Initial symptoms include intense headache, nausea and vomiting, myalgia, chills, dizziness, and, occasionally, a petechial rash. Meningismus is usually, but not invariably, present because of the acuity of the course. Progressive neurologic deterioration with delirium, convulsions, and coma can occur in hours or over the course of 2 to 4 days. Overall survival has been only approximately 5 percent, but survival without apparent neurologic sequelae has been reported in at least three children, two of whom had cutaneous lesions at the time of diagnosis.[36,41,42,44]

Examination of cerebrospinal fluid reveals (1) gross or microscopic hemorrhage, (2) leukocytosis consisting predominantly of polymorphonuclear leukocytes, (3) elevated protein, and (4) depressed glucose levels. Gram-positive rods can be seen easily on smears of cerebrospinal fluid. Peripheral leukocytosis is a common finding, and the white blood cell count may be 60,000 to 80,000 cells/mm^3. Blood cultures yield the organism in 70 percent, and cerebrospinal fluid cultures yield the organism in virtually 100 percent of patients.[20] Neuroimaging findings are notable for multiple hemorrhages in the ventricles, subarachnoid space, and deep gray matter.[36]

DIAGNOSIS AND DIFFERENTIAL DIAGNOSIS

B. anthracis can be visualized by direct smear and cultured from vesicular fluid or exudate from cutaneous lesions and from pleural fluid, blood, and cerebrospinal fluid in systemic infections. The commercially available QuickELISA Anthrax-PA Kit (Immunetics Inc., Boston, MA) can be used as a screening test.[4] Definitive diagnosis can be obtained through the Laboratory Response

Network for Bioterrorism in each state. Time-resolved fluorescent assay, real-time polymerase chain reaction, serologic tests to detect antibodies against protective antigen, and tissue immunochemistry also are available through state health laboratories. The enzyme-linked immunosorbent assay used to detect IgG to *B. anthracis* protective antigen is highly sensitive, has good specificity, and yields a positive result 10 days after the onset of symptoms.[5]

The lesion of cutaneous anthrax must be differentiated from ecthyma gangrenosum and from ulcerative skin lesions with regional lymphadenopathy, including rat-bite fever, ulceroglandular tularemia, plague, glanders, scrub typhus, rickettsialpox, cowpox, and orf. Staphylococcal lymphangitis can be distinguished from anthrax by the discharge of purulent material and by the inflammatory response observed microscopically. Noninfectious causes of eschars include arachnoid bites and vasculitides.[24] The first stage of inhalational anthrax and the clinical features of the intestinal form are nonspecific, so a history of exposure is crucial for establishing the diagnosis. The mediastinal widening that occurs early in the course of inhalational anthrax can cause confusion with that seen in acute bacterial mediastinitis and in fibrous mediastinitis caused by *Histoplasma capsulatum*.[15] Gastrointestinal anthrax must be differentiated from other causes of acute abdominal illness and, if bleeding is present, from duodenal ulcer, typhoid, and intestinal tularemia. Anthrax meningitis must be differentiated from subarachnoid hemorrhage.[20]

TREATMENT

Most, but not all, strains of *B. anthracis* are susceptible to penicillin and tetracycline, but naturally resistant strains occur, and penicillin resistance can be induced. Resistance occurs sufficiently uncommonly in naturally occurring cutaneous disease that penicillin or tetracycline for 7 to 10 days is adequate treatment. Treatment for bioterrorism-associated cutaneous anthrax should be initiated with ciprofloxacin, 10 to 15 mg/kg every 12 hours (not to exceed 1 g/day) orally until susceptibility data are available. Doxycycline is an alternative antimicrobial for initial therapy. The dosage is 100 mg orally every 12 hours (children >8 years old) or 5 mg/kg/day in divided doses given every 12 hours (children ≤8 years old). Treatment should continue for 60 days in the context of bioterrorism-associated cutaneous anthrax.[4]

The initial regimen for treatment of inhalational anthrax, gastrointestinal anthrax, anthrax meningitis, or cutaneous anthrax if (1) systemic signs are present, (2) lesions are located on the head and neck, and (3) extensive edema is present should be ciprofloxacin intravenously (400 mg every 8-12 hours) or doxycycline intravenously (200 mg every 8-12 hours).[4,10,11] One or two additional antimicrobials should be administered intravenously with either ciprofloxacin or doxycycline as initial therapy until susceptibility testing is available. Other agents with in vitro activity include levofloxacin, gatifloxacin, penicillin, ampicillin, clindamycin, vancomycin, rifampin, imipenem, meropenem, clarithromycin, and chloramphenicol.[17] If the organism is determined to be susceptible to penicillin, penicillin may be administered at a dosage of 300,000 to 400,000 U/kg/day intravenously or 50,000 U/kg/day orally. Therapy should be continued for at least 60 days.

Supportive therapy includes attention to details of fluid and electrolyte balances, endotracheal intubation if indicated to maintain a patent airway, and local care for cutaneous lesions. Systemic steroids may reduce the severity of infections in patients with massive edema or meningitis.[42] A specific anthrax antitoxin is not available.

PROGNOSIS

Before penicillin was introduced, cutaneous anthrax was fatal in approximately 20 percent of patients. With effective treatment, the mortality rate is less than 1 percent. Cutaneous anthrax of the eyelid may be complicated by ectropion of the upper lid and corneal scarring with blindness.[51] Immunity probably is lifelong in most patients. Although second attacks of cutaneous anthrax have been recorded, they have not been confirmed serologically and usually are mild.[14,22] Fatality rates are high for systemic anthrax and range from 50 to 100 percent for gastrointestinal anthrax to virtually 100 percent for inhalation anthrax, but children who have survived these infections have no apparent sequelae.

PREVENTION

The only human anthrax vaccine licensed in the United States is BioThrax (BioPort Corporation, Lansing, MI), formerly known as *anthrax vaccine adsorbed*, which is prepared from a cell-free filtrate of *B. anthracis* that contains no dead or live bacteria.[12] It is an aluminum hydroxide–precipitated preparation of protective antigen from an attenuated, nonencapsulated anthrax strain. Routine vaccination is indicated for individuals engaged in work involving the production of quantities or concentrations of *B. anthracis* in culture and in activities involving a high potential for aerosol production. The primary immunization series consists of three subcutaneous injections at 0, 2, and 4 weeks and three booster vaccinations at 6, 12, and 18 months. To maintain immunity, an annual booster dose is recommended.[12]

The limited data available suggest that the best means of preventing inhalational anthrax after exposure to spores is administration of ciprofloxacin (10 to 15 mg/kg orally every 12 hours to a maximum of 500 mg orally every 12 hours) or doxycycline (100 mg orally every 12 hours for children >8 years old or 5 mg/kg/day in divided doses given every 12 hours for children ≤8 years old) in conjunction with a three-dose regimen of vaccine (given at 0, 2, and 4 weeks after exposure).[4] In the event of a biologic anthrax attack, children exposed to spores should receive ciprofloxacin as postexposure prophylaxis for 60 days. If penicillin sensitivity is established, prophylactic therapy can be changed to amoxicillin, 80 mg/kg divided into three doses taken every 8 hours (maximum dose 500 mg orally every 8 hours). Although no data in children are available, the vaccine probably would be safe and effective.[23]

Worldwide, anthrax is controlled through livestock immunization programs. Procedures to prevent the spread of anthrax in animals include disposal of contaminated carcasses by burning and annual vaccination of livestock in known enzootic areas. All suspected or proven cases of anthrax should be reported to public health officials. Hospitalized patients should be kept under isolation until the lesions are bacteriologically sterile. Contaminated dressings and clothing must be burned or sterilized, and the patient's room must be disinfected to destroy spores.

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CHAPTER 103

BACILLUS CEREUS

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Recognition of *Bacillus cereus* pathogenicity was delayed until clarification of the taxonomy of the genus *Bacillus* in the early 1950s. Multiple early reports of food poisoning and other infections including gastroenteritis, bacteremia-septicemia, cellulitis, ear and eye infections, endocarditis, and urinary tract infections that were attributed to *Bacillus subtilis* or to other *Bacillus* spp. probably were caused by *B. cereus.*[91] *B. cereus* can give rise to two distinct forms of food-borne diseases related to different toxins, the emetic and the diarrheal syndromes, and occasionally to localized and systemic disease.[36,53]

The diarrheal syndrome was recognized first by Hauge[43] in 1955 after four clinically similar outbreaks in Norway occurred. This common form of disease was related to a great variety of foods, such as meat and vegetable soup, poultry, pudding, sauce, pasta, cake, and milk. In 1974, Mortimer and McCann[69] described a vomiting syndrome associated with the consumption of fried rice in Chinese restaurants. Despite widespread recognition in Europe, *B. cereus* outbreaks have been reported infrequently in the United States. The first documented outbreak in the United States occurred in 1970.[66]

BACTERIOLOGY

Members of the genus *Bacillus* are aerobic or facultative anaerobic, gram-positive or gram-variable, spore-forming rods. They are distributed widely in the environment because of the high resistance of their endospores to extreme conditions, including heat, cold, desiccation, salinity, and radiation.[28,79] Based on the high variability in guanine and cytosine content (32 to 69%), the debate ensues regarding the classification of *Bacillus* spp.[96]

B. cereus belongs to the group of gram-positive rods that produce central or terminal ellipsoid or cylindric spores that do not distend the sporangia.[96] Studies of DNA-DNA hybridization and 16S and 23S rRNA sequencing and enzyme electrophoretic patterns have shown a close relationship among *B. cereus*, *Bacillus anthracis*, *Bacillus mycoides*, and *Bacillus thuringiensis*; these organisms are so closely related that they all may be considered variants of *B. cereus*. Differentiating, particularly between *B. cereus* and the insect pathogen *B. thuringiensis*, sometimes is difficult in the diagnostic laboratory. Polymerase chain reaction technology has been used for identifying species.[16]

B. cereus is a flagellated, motile, gram-positive rod, typically 1 to 1.2 µm in diameter by 3 to 5 µm in length. The organism sporulates freely on many media under well-aerated conditions, but vegetative cells also can grow anaerobically. It is able to metabolize glucose, fructose, and sucrose but not pentose and other sugar alcohols. It produces acid from glucose but not from arabinose, xylose, or mannitol. Starch hydrolysis and catalase production are similar to those of the other members of the genus. The presence of lipid globules or protoplasts is a characteristic that it shares with *Bacillus megaterium*.[54] Colonies on blood agar are large, flat, granular, and slightly green-tinged. *B. cereus* is differentiated from *B. anthracis* by motility, hemolysis, lack of lysis by gamma-phage, penicillin resistance, and absence of a capsule in *B. cereus*.[28] Identifying morphologic differentiation with nonmotile *B. cereus* strains and *B. anthracis* strains that are occasionally weakly hemolytic may be difficult.

Growth and multiplication of vegetative cells occur in a temperature range of 10° C to 50° C, with an optimum of 28° C to 35° C. Variations in toxin levels found in certain foods also can be related to pH levels, sugar content, the presence of other lactic acid bacteria, and aeration.[85,86] Some strains responsible for milk spoilage can grow at temperatures of 5° C,[54,79] but few strains are able to produce toxin at temperatures less than 7° C.[79] Ribosomal DNA characterization and genotyping based on the major cold shock protein homologue cspA have defined a novel species, *Bacillus weichenstephanensis*, which can grow at 4° C to 7° C, but not at 43° C.[56] These strains form a new fifth subgroup of *B. cereus* organisms described as psychrotolerant or psychrotropic, meaning they are able to grow at temperatures of 7° C and less.[55,75,87] Spore formation allows some *B. cereus* strains to survive pasteurization and heating.[55] More recently, a crystalline cell surface protein layer or "S-layer" has been described as a determinant of cell hydrophobicity. S-layer–producing cells are hydrophilic and able to bind to human matrix proteins, and presence of the S-layer in some *B. cereus* strains is associated with resistance to phagocytosis and increased radiation resistance against gamma irradiation.[53]

Serologic differentiation of *Bacillus* spp. is hampered by cross-reactive antigens and autoagglutination of spores caused by hydrophobic surface properties. Serologic typing based on the flagellar (H) antigen can be used during an outbreak to distinguish among strains and to determine the similarity of isolates obtained from humans with the strains isolated from suspect foods.[28] The serotype scheme is based on 42 H antisera raised against prototype strains. A common flagellar antigenic epitope has been suggested. Detection of the flagellar antigen by enzyme-linked immunosorbent assay is perhaps more sensitive than is detection by the agglutination method.[70] In addition to serology and biotyping (based on biochemical typing), plasmid analysis and phage typing have proved useful epidemiologically.[2,54,83,100] Techniques such as pyrolysis mass spectrometry and gas-liquid chromatography of whole-cell fatty acids are showing promise.[28]

Diagnosis of the rare extraintestinal infection is made by isolation from normally sterile sites (blood or tissue) after overnight incubation on nutrient or blood agar; clinical specimens from normally nonsterile sites (feces, vomitus) and food or environmental samples require selective techniques. Polymyxin B is used as a selective agent, and the lecithinase reaction of the organism on egg yolk and its inability to catabolize mannitol permit presumptive identification to be made with a variety of media: mannitol–egg yolk–polymyxin B, Kim and Goepfert medium, and polymyxin B–pyruvate–egg yolk–mannitol with bromothymol blue or bromocresol purple.[28]

EPIDEMIOLOGY

B. cereus in the spore and vegetative form is a ubiquitous organism found in soil, water, vegetation, and food products, especially cereals, dairy products, dried foods, spices, meat products, and vegetables.[54] The emetic syndrome typically is associated with cooked rice, usually fried, from Chinese restaurants.[52,69] Saving portions of boiled rice at room temperature overnight until required for frying previously was a common practice. Refrigerating boiled rice makes the grains stick together, which is less convenient for frying. The spores of *B. cereus* survive cooking and are capable of germination and outgrowth.[37,39] The optimal temperature for growth in boiled rice is 30° C to 37° C, although growth does occur during storage at 15° C to 43° C.[37] Most samples of uncooked rice contain multiple serotypes of *B. cereus*, and little difference occurs in the growth rate of the various serotypes in boiled rice at 22° C, but spores of serotype 1 strains are more resistant to heating at 95° C, which probably is the reason that this serotype usually is implicated in outbreaks.[72]

Starchy dried foods other than rice, such as cereals, frequently are contaminated.[15] Tortillas can be contaminated with the water used in preparation and from the hands of producers.[19] Specific foods incriminated in outbreaks in the United States include Chinese food (accounting for 50% of reported outbreaks between 1973 and 1987), followed by Mexican food, beef, fruits, and vegetables. Other foods implicated, particularly in the enterotoxin syndrome, include beef stew, turkey loaf, barbecued pork, macaroni and cheese, potatoes, spaghetti and other pastas, dairy and dried milk products, and seafoods.[27,35,46,50,53,59] In a study of 96 milk and milk product samples collected from retail shops in Nairobi, Kenya, 57 percent of samples were contaminated with *B. cereus* and 81 percent of the bacterial isolates (38 of 47) produced non-hemolytic enterotoxins, including 6 of 43 pasteurized milk products (43%).[71] Among the factors thought to contribute to the outbreaks, the most frequent were improper storage or holding temperature (94%), contaminated equipment (53%), inadequate cooking (32%), and poor personal hygiene (24%).[7]

Outbreaks of *B. cereus* have been recognized widely in Europe but rarely in the United States. In the Netherlands, *B. cereus* reportedly was the cause of 22.4 percent of food-borne disease outbreaks of known bacterial cause. Similarly, in Finland, it accounted for 11.9 percent of outbreaks. In most places, *B. cereus* is incriminated in 0.9 to 7 percent of outbreaks of food-related disease and 0.7 to 3 percent of cases.[54] In the United States between 1998 and 2002, *B. cereus* was identified as the cause of 37 of 6647 (0.6%) foodborne outbreaks reported to the Centers for Disease Control and Prevention, with a total of 571 cases and no deaths recorded.[62] The location of the outbreak is known for 24 of these outbreaks, and most of these (15 of 24 [62.5%]) occurred in commercial food outlets, such as restaurants, delicatessens, or workplace cafeterias. In outbreaks for which the impli-

cated source was known, cereal products were the most common vehicle.[62] The susceptibility of children to this pathogen is evident from the report of an outbreak involving two daycare centers[52] and other reports that included neonates, children, and adolescents.[31,45,49,73,78,92,104] Only a small fraction of outbreaks are reported to the Centers for Disease Control and Prevention; small outbreaks of mild, brief illness are less likely to be reported. It is thought that 10^5 to 10^8 organisms per gram need to be ingested to cause the emetic syndrome and 10^5 to 10^7 cells or spores (total dose) ingested to cause the enterotoxin syndrome.[40]

For infections not related to food, groups at risk include neonates,[31,73] immunocompromised hosts,[4,20,30,39,45,49,83,89] intravenous drug abusers,[23,89] and patients with intravascular devices or artificial prostheses.[4,6,30,31,83,84] Studies in three different populations in South Africa and London, including school-aged children, found the organism in 18 to 43 percent of fecal samples.[95] The organism can be part of the normal intestinal flora. *B. cereus* does not persist in the intestine after ingestion.[34]

PATHOGENESIS

B. cereus produces an enormous range of extracellular metabolites, including peptides with antibiotic properties (biocerin, cerein, thiocillins), β-lactamases, hydrolases, nuclease, urease, and proteases.[91] Two different groups of toxins, known as diarrheal enterotoxins and emetic toxin, are responsible for the clinical syndromes of food poisoning.[64,97] Some strains may be able to produce both toxins based on the evidence that culture filtrates derived from strains isolated from emetic poisoning occasionally are able to produce a positive rabbit ileal loop assay.[79]

Biologic activities of diarrheal enterotoxins can be shown in multiple different assay systems. Three enterotoxins are produced. Purification plus isolation of multiple toxic fractions with some, but not all, of these activities has caused confusion.[28,90] Evaluation of the toxic activity of whole-cell suspension, cell-free culture filtrates, and the purified enterotoxin complex classically has included the rabbit ileal loop fluid accumulation assay, vascular permeability in rabbit skin, dermonecrosis and intestinonecrosis, mouse lethality, cytotoxicity, and hemolysis.[12,18,58,88,93,98] Hemolytic *B. cereus* enterotoxin has been characterized as a complex called *hemolysin BL* or *HBL*, composed of a binding component B (35 kd) and two lytic components, L1 and L2 (36 kd and 45 kd)[8]; all three are needed for fluid accumulation in the rabbit ileal loop.[10] Another *B. cereus* enterotoxin complex that is non-hemolytic and is composed of three distinct proteins has been described.[61] Several other single proteins with enterotoxic diarrhea–producing activity also have been described.[53]

The emetic toxin cereulide is a heat-stable (126° C for 90 minutes), ring-shaped peptide with a molecular weight of 1.2 kd[65]; it is stable between pH 2 and 11 and is protease-resistant. In contrast to the diarrheal toxins, it is preformed in foods, so the presence of living organisms at the time of ingestion is not necessary to cause symptoms.[36,91] Rice culture filtrates derived from emetic syndrome–associated strains cause cytoplasmic vacuolation and swollen mitochondria in HEp-2 cells, characteristics suggestive of uncoupling of oxidative phosphorylation.[76] Emetic activity occurs through the serotonin 5-HT_3 receptor and stimulation of the vagus afferent nerve.[1]

Among the multiple other substances with relevant activity, two groups are associated with local infection.[93,94] Cereolysin, or hemolysin I, is a thiol-activated cytolysin. Phospholipase C–like or lecithinase-like substances, including a sphingomyelinase and two hydrolases with preference for phosphatidylcholine and phosphatidylinositol, also exist. These enzymes induce the release of lysosomal enzymes from neutrophils that probably are involved in tissue damage, especially in wound and ocular infections.[28,106]

CLINICAL MANIFESTATIONS

FOOD POISONING

Diarrheal Syndrome

The enterotoxins preformed in food or produced in vivo in the intestine after the ingestion of bacilli cause profuse, watery, nonbloody diarrhea accompanied by abdominal pain and cramps, nausea, and, occasionally, vomiting or low-grade fever.[3,27,35,50,54,60,82] The typical incubation period is 8 to 16 hours, and the clinical characteristics resemble the food poisoning of *Clostridium perfringens*. The interval between ingestion and the onset of symptoms reflects the time required for production of toxin in the gut. The symptoms resolve within approximately 12 to 24 hours but occasionally can last 2 days to 2 weeks.[35] The diarrhea (3 to 10 bowel movements per day) rarely leads to dehydration in healthy individuals. In the elderly, bloody stools can occur.[35] The most prevalent flagellar H serotypes are 1, 2, 6, 8, 10, 12, and 19. Serotyping is available at research laboratories.[15,27,59,95]

Emetic Syndrome

The usual illness is characterized by a rapid onset (within 1 to 5 hours after the ingestion of contaminated food) of nausea, vomiting, and malaise, occasionally followed by diarrhea hours later.[69] Infrequently, the diarrhea is reported to last several days.[101] The short incubation reflects the ingestion of preformed emetic toxin.

EXTRAINTESTINAL INFECTIONS

Eye Infection

Keratitis, conjunctivitis, endophthalmitis, and panophthalmitis can be produced by *B. cereus*. Endophthalmitis that develops after the occurrence of penetrating wounds is caused by this organism in 27 to 46 percent of cases.[25,99] A history of soil contamination or the presence of a metal foreign body should raise clinical suspicion. Less frequently, corneal ulcers and surgical procedures are predisposing factors.[44,99] Exogenous endophthalmitis usually progresses rapidly, with deterioration of vision occurring in less than 48 hours. Severe pain is accompanied by chemosis, periorbital swelling, proptosis, and pus in the anterior chamber. The classic lesion is a corneal ring abscess, similar to that produced by *Pseudomonas* and *Proteus* spp. Endogenous cases may have subretinal exudation, retinal hemorrhage, and perivasculitis. Associated systemic symptoms are not unusual manifestations.[23] The outcome is poor, with almost half of patients left with visual acuity no better than simple light perception.[99] Many patients require enucleation. Endogenous ophthalmitis is associated with the use of illicit intravenous drugs or transfusion of contaminated blood products and subsequent bacteremic seeding of one eye.[23,83,89,99]

The pathogenesis of *B. cereus* on ocular tissue has been linked to the lecithinase activity of phospholipase C. A toxin fraction, hemolysin BL, which is formed by three separate components, produces similar destruction of retinal tissue in vitro and in animal experiments.[9] Its relationship with the diarrheal enterotoxin is unclear.[12]

Wound and Soft Tissue Infections

Wound infections of variable severity related to trauma, burns, or postsurgical complications occasionally are reported.[92-94,104] Because *Bacillus* is a common environmental contaminant, proof of the relevance of a *B. cereus* isolate is clearer if the organism is obtained from deep tissue in heavy pure growth. Severe infec-

tions in individuals involved in motor vehicle accidents can be complicated by necrotizing fasciitis and require extensive débridement.[104] Superficial, benignly infected wounds are common findings in the tropics.[29] Immunosuppressed patients may contract a severe gas gangrene–like infection with myonecrosis similar to disease caused by *Clostridium* spp.,[65] requiring amputation,[42] or a less severe primary cutaneous infection manifested by vesicles, pustules, or cellulitis.[45]

Skeletal Infections

Cases of chronic osteomyelitis occur rarely and result from accidental or surgical trauma. Radical débridement and antibiotic treatment are required.[83] Because *B. cereus* can be found as a copathogen with other more frequent pathogenic bacteria, resolution of symptoms is delayed until eradication of the organism.[78] Acute osteomyelitis may occur in drug abusers.[83,89]

Bacteremia and Septicemia

Bacteremia occurs with indwelling catheters and other foreign bodies, contaminated intravenous drugs (e.g., heroin), and blood products, particularly platelets.[17,30,107] Immunosuppression and impaired neutrophil killing, such as occur in individuals with neutropenia secondary to malignancy or chemotherapy and in neonates with immaturity of the immune system, are major contributors to morbidity in systemic *B. cereus* infection.[4,18,47,73,74] Disseminated intravascular coagulation, multiorgan failure, and a fulminant course may occur in neonates and compromised hosts. Intestinal perforation with abdominal infection in neonates also has been described.[38] Outbreaks of sepsis in newborn nurseries have been linked to contaminated ventilation balloon devices.[102] Endocarditis is an infrequent complication of bacteremia that usually occurs in intravenous drug abusers or individuals with a long-term intravascular device.[83,89] Vegetations can form over mechanical prosthetic valves or pacemaker wires, requiring their replacement.[83,84] Morbidity and mortality rates are high in patients with valvular heart disease. Fatal serosanguineous pericarditis in a patient undergoing hemodialysis has been reported.[32]

Pneumonia

Primary pulmonary disease rarely has been recorded. The manifestation can be subacute and consist of cough, fever, dyspnea, chest pain, and hemoptysis, with progression to necrotizing pneumonia, cavitation, and empyema. Pleural fluid may appear serosanguineous or sanguinopurulent.[13,47] Underlying predisposing conditions include leukemia, alcohol abuse, chronic hepatitis, and steroid use.[13,30,47,83] Multiorgan disease in premature neonates with necrotizing pneumonia usually is fatal.[51] Outbreaks of *B. cereus* pneumonia in neonates have been linked to contaminated ventilators.[41] Blood culture is positive in 75 percent of cases, followed by pleural fluid and sputum culture in 40 to 60 percent.[13] The pleural space may become contaminated with *B. cereus* by mishandling of the thoracic drainage system in patients with other causes of pleuritis.[48]

Infection of the Central Nervous System

Intracranial shunts and penetrating surgical or traumatic cranial wounds expose the central nervous system to environmental *B. cereus*.[31,81] Spinal anesthesia[30] was associated with *Bacillus* infection in the past. Contamination in the operating room through the linen is a potential source.[5,6] Premature neonates are susceptible to meningeal seeding by dissemination of intravascular infection related to catheters.[73] As in patients with other serious infections, patients who are immunosuppressed are at higher risk.[47] The cerebrospinal fluid in *B. cereus* meningitis is purulent, with white blood cell counts of more than 1000/mm^3, a predominance of polymorphonuclear leukocytes, and moderate increments in protein content. Gram stain is positive in 70 percent of cases. Multiple brain abscesses may result from hematogenous spread in patients with leukemia.[47,49] Sequelae include hydrocephalus and brain damage. Often, the infection is fatal unless caused by a contaminated spinal anesthetic, in which case the course is usually more benign.

Liver Failure

Fulminant hepatitis and rhabdomyolysis have been associated with the emetic toxin. The toxin inhibits hepatic mitochondrial fatty-acid oxidation. Liver changes include fatty infiltrates and midzonal necrosis.[63]

Pseudoinfections

Pseudoepidemics with *B. cereus* are a challenge for the clinical microbiologist.[67] Because these spore formers are so hardy, they are common laboratory contaminants. *Bacillus* spp. isolated from clinical specimens usually should be considered a contaminant, although their pathogenic potential is well known.[24,30,47,74,83,89,93,94] Pseudoinfections have been associated with contaminated blood culture media, syringes, blood culture analyzers, and fiberoptic bronchoscopes.[24] Colonization of umbilical cord stumps and eye surfaces by contaminated diapers may cause pseudo-outbreaks in nurseries.[105]

COMPLICATIONS

Death rarely ever results from the food-poisoning syndromes. Rapidly spreading wound infections may require amputation of extremities,[39] and ocular infections can lead to loss of vision with or without enucleation.[44,99,106] The risk of death in patients with septicemia, endocarditis, and meningitis is related to the underlying condition and severity of the disease.[4,45,68,73,84]

DIAGNOSIS

The laboratory finding of *B. cereus* in a foodstuff without quantitative cultures and without epidemiologic data is insufficient to establish its role in an outbreak.[101] In practice, appropriate specimens often either are unavailable or are submitted long after the incident, rendering their microbiologic significance questionable. During analysis of foods not involved in food-borne illness, the bacteria may be found in counts of 10^1 to 10^6 organisms per gram.[14,15,19,37,72,85] Diagnosis of the diarrheal form of *B. cereus* food poisoning is supported by the isolation of 10^5 or more organisms per gram from epidemiologically incriminated food.[68] The levels of *B. cereus* found in the implicated foods usually are in the range of 5×10^5 to 9.5×10^8 colony-forming units per gram. With the exception of milk, the products rarely appear to be spoiled, despite the bacteria's high density. Isolation of more than 10^5 organisms per gram in feces during an acute attack provides supportive evidence for the presumed diagnosis and confirms the association if the same serotype is isolated from incriminated food.

The organisms that produce emetic toxin are present in concentrations of 1×10^3 to 5×10^{10} colony-forming units per gram. Reheating may decrease or eliminate the organisms and leave the toxin intact, but render isolation of the organism difficult to accomplish.[28,101]

Commercial immunoassay kits (reversed passive latex agglutination test, enzyme-linked immunosorbent assay, and microslide

TABLE 103–1 Management and Complications Associated with *Bacillus cereus* Infections

Condition	Management	Complications
Food poisoning	Supportive measures; hydration	Usually none; liver failure rarely reported
Ocular	Systemic, topical, intravitreal antibiotics—vancomycin or clindamycin ± aminoglycosides; surgical—early vitrectomy	Severe decreased visual acuity, blindness, enucleation
Septicemia	Removal of IV device, foreign body; IV antibiotics (according to sensitivity)—vancomycin	Localization of infection—endophthalmitis, endocarditis, meningitis; death
Pneumonia/pleuritis	IV antibiotics; drainage of pleural space; resection of necrotic tissue	Death
Meningitis	IV antibiotics; removal of infected intracranial shunts or cerebrospinal fluid reservoirs	Hydrocephalus, brain damage, death
Wound/subcutaneous infection	IV antibiotics; surgical débridement	Amputation, death

IV, intravenous.

immunodiffusion assay) are available for the detection of *B. cereus* diarrheal toxin. The kits may detect a variety of proteins, however.[11,26] Experimental techniques based on the detection of genes for phospholipase C and sphingomyelinase by polymerase chain reaction have been developed for the identification of *B. cereus* in food products.[65]

The relevance of clinical isolates in extraintestinal syndromes can be assessed by the degree of growth (heavy versus scanty), the number of occasions that growth was obtained, the source of the material cultured, and predisposing or underlying conditions.[94] For suspected endophthalmitis, aqueous and vitreous samples should be obtained if possible before the initiation of antibiotics.[25]

TREATMENT

During a mild, self-limited attack of food poisoning, patients require only supportive therapy. Usually, oral fluid and electrolyte replacement is adequate (Table 103–1).[35]

No antibiotic therapy is required except in cases of nongastrointestinal infection. The production of three different β-lactamases by most of the organisms renders penicillin derivatives, including third-generation cephalosporins, ineffective against *B. cereus*. Most strains are susceptible to chloramphenicol, vancomycin, clindamycin, aminoglycosides, erythromycin, tetracycline, imipenem, and ciprofloxacin.[4,21,93,94,103] Definitive antibiotic therapy should be based on the antibiogram susceptibility, but initial empiric therapy with clindamycin or vancomycin, with or without an aminoglycoside, is appropriate, pending susceptibility data.[4,13,24,49,78,104] Ciprofloxacin has been reported to be useful in the treatment of recurrent pneumonia and bacteremia[33] and may have advantages in the penetration of respiratory and eye secretions,[57] but other options for children younger than 18 years are preferred.

Immediate empiric coverage for *B. cereus* is indicated for endophthalmitis in groups at risk. Selection of the antibiotic and the appropriate means of administration are controversial.[25] Clindamycin and an aminoglycoside[106] or vancomycin[99] are the best options. Together with antibiotics administered parenterally, topical and periocular antibiotics are considered adjunctive therapy. In cases of penetrating trauma, early vitrectomy and intravitreal antibiotics (vancomycin in combination with amikacin) should be considered.[25,99]

Surgical débridement of necrotic tissue, drainage of closed-space infections, and prompt removal of foreign bodies and indwelling catheters are important aspects of successful therapy.[13,22,78] Some bacteremic patients may be cured by removal of the intravenous catheter only.[83]

PREVENTION AND CONTROL

Low-level contamination in food products is difficult to avoid, but proper food handling should diminish the proliferation of bacilli.[79] Practical precautions for handling cereals and rice include not preparing large quantities at a single time and maintaining the food at a hot temperature (>63° C) or cooling it quickly. The food must not be stored under warm conditions, especially in the range of 15° C to 50° C.[15,37]

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CHAPTER 104

ARCANOBACTERIUM HAEMOLYTICUM

Natascha Ching

Arcanobacterium haemolyticum is a pleomorphic gram-positive coryneform rod that causes pharyngitis and exanthem in children and young adults.[18,37,41,46]

HISTORY

This diphtheroid was noted first by MacLean and colleagues[41] in association with exudative pharyngitis in American servicemen in the South Pacific during World War II. The organism originally was named *Corynebacterium haemolyticum* but was reclassified in 1986 as *A. haemolyticum* on the basis of phenetic, peptidoglycan, fatty acid, menaquinone, and DNA data.[16-18] The association between infection with *A. haemolyticum* and pharyngitis was observed repeatedly over the years, but a cause-and-effect relationship between the organism and illness has been established only more recently.[10,40,46]

ORGANISM

MICROBIOLOGY

A. haemolyticum is a gram-positive to gram-variable pleomorphic rod.[18,20,25,64,67] The laboratory characteristics are presented in Table 104–1. *A. haemolyticum* grows best at 37° C on a blood-enriched or serum-enriched medium with the addition of 5 percent carbon dioxide. Alternatively, it grows well anaerobically. On rabbit or human blood agar, colonies are pinpoint (0.5 mm) at 24 hours; they increase to 1 to 1.5 mm after 48 hours. At this time, a unique black opaque dot is noted in the center of the colony, and this dot remains on the agar when the colony is scraped away.

TABLE 104–1 Identification Characteristics of *Arcanobacterium haemolyticum*

Test or Characteristic	Finding
Catalase	Negative
Beta-hemolysis	Positive (narrow zone of slight hemolysis after 48 hr on sheep blood)
Nitrate reduction	Negative
Pigment production	White or gray
Urease	Negative
Gelatin hydrolysis	Negative
Motility	Negative
Esculin hydrolysis	Negative
Carbohydrate use	
Glucose	Positive
Maltose	Positive
Sucrose	Positive (requires rabbit serum for growth in peptone water)
Mannitol	Negative
Xylose	Negative

Data from Collins, M. D., and Cummins, C. S.: Genus Corynebacterium. In *Sneath, P. H. A., Main, N. S., Sharpe, M. E., et al. (eds.): Bergey's Manual of Systemic Bacteriology. Vol. 2. Baltimore, Williams & Wilkins, 1986, pp. 266-276.*

At 24 hours, a 1-mm zone of hemolysis develops around colonies grown on human or rabbit blood agar. The hemolytic zone increases to 3 to 5 mm by 48 hours. Growth and red blood cell hemolysis are minimal on horse and sheep blood agar. Because throat cultures usually are performed on sheep blood agar plates, the hemolytic activity of *A. haemolyticum* may be missed.

Colonies of *A. haemolyticum* can be of either the smooth or the rough biotypes on horse blood agar.[12] These biotypes also

differ in their hemolysis and biochemical properties. Smooth colonies predominate in wound infections and frequently use sucrose and trehalose, are beta-hemolytic, and lack β-glucuronidase. Rough colonies are found almost exclusively in the respiratory tract and do not use sucrose and trehalose, are non-hemolytic, and are β-glucuronidase-positive.

A. haemolyticum resembles *Actinomyces pyogenes* (formerly *Corynebacterium pyogenes*), a common cause of bovine mastitis and a rare cause of skin ulcers in children.[33] These organisms can be differentiated by several means. *A. pyogenes* is able to hydrolyze gelatin and ferment xylose. In addition, *A. haemolyticum* has a positive reverse CAMP (Christie, Atkins, and Munch-Petersen) test in that it inhibits the beta-hemolysis of *Staphylococcus aureus*, whereas *A. pyogenes* shows slight enhancement of beta-hemolysis.[24] *A. haemolyticum*'s poor growth on tellurite medium and lack of catalase help distinguish it from *Corynebacterium diphtheriae*, which also causes pharyngitis.[29,35,37]

TOXIN PRODUCTION

A. haemolyticum liberates three toxins: phospholipase D (PLD), a hemolysin, and neuraminidase.[18] PLD is a dermonecrotic toxin that causes local hemorrhagic necrosis after intradermal inoculation in rabbits and guinea pigs; injection of PLD also is lethal in rabbits.[54] The PLD gene of *A. haemolyticum* has a high degree of homology to that of *Corynebacterium pseudotuberculosis*.[19,43] PLD has been shown to be involved in the virulence of *C. pseudotuberculosis*; PLD mutants are less pathogenic in experimental infections in goats.[42] The PLD of *C. pseudotuberculosis* is similar biochemically and shares some biologic activity with the PLD that is found in brown recluse spider venom and that plays a role in the venom's toxicity.[6,37] *A. haemolyticum* carries a gene similar to the gene encoding the erythrogenic toxin of *Streptococcus pyogenes*.[18]

ANTIMICROBIAL SUSCEPTIBILITY

Almost all *A. haemolyticum* strains are highly susceptible to erythromycin (minimal inhibitory concentration <0.06 μg/mL). Erythromycin is not bactericidal, however.[9,41,64] Carlson and colleagues[11] reported an *A. haemolyticum* isolate from a diabetic foot ulcer that was exceptionally resistant to macrolides, clindamycin, tetracycline, and ofloxacin. Waagner[64] noted that of 100 pharyngeal isolates, all were inhibited by concentrations of 0.25 μg/mL or less of penicillin G and by 1 μg/mL of penicillin V. Tolerance to penicillin has been observed, however.[41,51] In one study, the minimal bactericidal concentration-to-minimal inhibitory concentration ratio varied from 1:1 to 1:8.[41] In addition to being sensitive to penicillin and erythromycin, *A. haemolyticum* is sensitive to other β-lactams, clindamycin, chloramphenicol, azithromycin, vancomycin, ciprofloxacin, tetracyclines, and rifampin; most strains are resistant to sulfonamides and trimethoprim-sulfamethoxazole.[9,11,64]

Carlson[8] compared E Test (AB Biodisk, Sweden) and agar dilution methods for susceptibility testing of 12 antimicrobial agents in 70 *A. haemolyticum* isolates. E test and agar dilution method results for benzylpenicillin, clindamycin, erythromycin, imipenem, levofloxacin, rifampin, and vancomycin were in 100 percent agreement. Tetracycline, ciprofloxacin, and ofloxacin were in 97 to 99 percent agreement, but a lower percentage of agreement was found for cefotaxime and cefuroxime, 93 percent and 84 percent, respectively. Because there are no universal standards for the organism with regard to susceptible, intermediate, or resistant categories, results should be interpreted with caution for clinical care. Almuzara and colleagues[1] also evaluated the susceptibility of 19 strains of *A. haemolyticum* according to National Committee for Clinical Laboratory Standards interpretative standards for aerobic organisms. Most isolates were susceptible to penicillin, cephalosporins, clindamycin, ciprofloxacin, vancomycin, and macrolides (erythromycin and azithromycin). Only 68 percent of isolates were found to be sensitive to tetracycline.

Although the Clinical and Laboratory Standards Institute has no standardized susceptibility testing method for *Arcanobacterium* spp., it has been suggested that the broth microdilution method used for testing *Corynebacterium* spp.[15] is appropriate for *Arcanobacterium* spp. (Janet Hindler, MCLS, UCLA Medical Center, Clinical Microbiology Laboratory, Los Angeles, CA, and Guido Funke, MD, Gartner & Colleagues Laboratories, Ravensburg, Germany, personal communication). This method involves testing in cation-adjusted Mueller-Hinton broth supplemented with lysed horse blood (2.5 to 5% v/v). Some investigators have used agar dilution or E test satisfactorily.[8]

A vancomycin-resistant *A. haemolyticum* isolated from stool during an outbreak of vancomycin-resistant enterococci has been described. It contained the *vanA* gene primarily found in *Enterococcus faecium*.[47] Two other case reports have described organisms resistant to ciprofloxacin[63] and tetracycline.[36]

EPIDEMIOLOGY

Although similar organisms are common causes of infection in animals, humans seem to be the primary host of *A. haemolyticum*.[56,64] The organism is isolated primarily from throat specimens in patients with pharyngitis,[41,46,64] although it also may be a commensal of human skin.[40] It likewise may be a commensal in the throat, but it often is overlooked because laboratories frequently do not differentiate diphtheroids, which are considered "normal flora." In addition, because *A. haemolyticum* often is found in polymicrobial respiratory infections with classic respiratory pathogens, including *S. pyogenes*, it sometimes is missed when these more classic pathogens are identified.[37]

Although no definitive data are available, person-to-person spread is assumed to be from the throat discharge of an infected individual to the throat of a susceptible host. Transmission could occur directly or indirectly by fomites. Secondary cases in families indicate that spread is from person to person rather than from an environmental source.[23,46]

In an 8-year study, the organism was found to be isolated from throat specimens in each year, with isolation rates varying from 0.2 to 0.7 percent.[46] The peak age for contracting illness caused by *A. haemolyticum* is during the second decade of life; in contrast, the peak age for developing pharyngitis caused by *S. pyogenes* is during the first decade of life.[23,41,46] In two studies, illnesses occurred more commonly in young women than in young men.[23,46] In a review of *A. haemolyticum* systemic and deep-seated infections, a preponderance of boys over girls was found among adolescents with no risk factors for development of invasive disease.[60] No seasonal prevalence has been reported.

Screening throat cultures for pharyngotonsillitis in northern Israel were performed in 518 patients aged 1 to 90 years old.[14] Only one culture was positive (0.2%) for *A. haemolyticum* compared with 26 percent recovery of group A streptococcus. The one case was in a 9-year-old patient, however, and only 58.7 percent of patients were aged 1 to 18 years old, the more common age of isolation for this organism.

PATHOGENESIS AND PATHOLOGY

Few data are available regarding pathogenesis and pathology. The dermonecrotic toxin probably plays a role in pharyngitis. Skin biopsy specimens were taken from the exanthem in two

TABLE 104–2 Signs and Symptoms in Children, Adolescents, and Young Adults with *Arcanobacterium haemolyticum* Infection

	Frequency (%)
Symptoms	
Sore throat	100
Rash	40-70
Pruritus	50
Fever	40-75
Hoarseness	60
Cough (nonproductive)	40-60
Vomiting	30
Signs	
Pharyngitis or tonsillitis	100
Exudative	50-70
Palatal petechiae	30
Glossitis	25
Cervical lymphadenitis	40-75
Rash	40-70
Scarlatiniform	50
Urticarial	5
Maculopapular	25

Data from references 23, 32, 41, 46, and 64.

patients, and both showed only a mild lymphohistiocytic perivascular infiltrate.[46] Cultures of both samples were negative, and no IgG, IgA, or IgM deposition was noted. These findings suggest that the rash may be toxin-mediated, similar to the rash in group A streptococcal infections.

A. haemolyticum has been found to persist intracellularly,[52] which might account for failure of penicillin treatment in some cases. In one study, 5 of 42 patients were found to have apparent dual infections with Epstein-Barr virus (EBV) and *A. haemolyticum*.[41] The authors of this study suggested that immunosuppression by the virus contributes to a more marked effect of the bacterial infection in the throat. With regard to the immune response to *A. haemolyticum*, paired acute and convalescent sera showed the development of a humoral response to four distinct cell wall–associated proteins on Western blot analysis in seven of eight patients with culture-confirmed infection.[49]

CLINICAL MANIFESTATIONS

PHARYNGITIS

Pharyngitis* is the most common finding in *A. haemolyticum* infection. Signs and symptoms associated with pharyngitis are listed in Table 104–2. The illness is indistinguishable from that caused by group A streptococci; frequently, it resembles EBV infectious mononucleosis. Several patients with typical infectious mononucleosis had laboratory evidence of infection with EBV and *A. haemolyticum*.[27,28,41] Peritonsillar abscess caused by *A. haemolyticum* has been reported on several occasions.[4,35,38,45] The most common exanthem is scarlatiniform, the onset of which occurs 1 to 4 days after the beginning of pharyngeal symptoms. The rash is most prominent on the extensor surfaces of the arms and legs (see Fig. 64–19). Circumoral pallor, which is seen with group A streptococcal scarlet fever, does not seem to occur with *A. haemolyticum* infection. The rash may progress to involve the chest and back; it usually spares the palms, soles, and face, and it rarely involves the abdomen and buttocks.

The rash frequently is pruritic and may be urticarial. Erythema multiforme has been described.[3] Gaston and Zurowski[26] reported a 20-year-old man who had, in addition to pharyngitis, a rash involving mainly his hands and feet. His feet were swollen, and on the soles were erythematous macules, petechiae, and vesicles. His palms were tender, and 2- to 4-mm erythematous macular lesions that contained small central vesicles were present. Mehta[44] reported a 19-year-old woman who presented with pharyngitis and a pruritic rash on her arms and legs 4 days before evaluation. Physical examination revealed an erythematous, urticarial rash with large and small annular rings over the whole body with confluence on the upper thighs and smaller lesions in groups on distal dorsal parts of the hands and feet.

Carlson and colleagues[13] also reported a 19-year-old man who presented with a 2-day history of a pruritic exanthem on the upper back and chest, which spread centrifugally. The patient had pharyngitis that worsened to exudative tonsillitis, and a throat culture was positive for *A. haemolyticum*. He also had swollen fingers and worsening erythematous maculopapular exanthem over his trunk and proximal extremities. Pastia lines were observed on the third day. Two weeks after initiation of antimicrobial treatment and discharge, a mild late desquamation was noted on his palms and around his fingernails. The duration of the exanthem has not been described adequately in the literature. In one study, exanthem was noted to persist for longer than 2 days in 69 percent of patients.[46]

Occasionally, *A. haemolyticum* infection has been manifested as a grayish white pharyngeal pseudomembrane that has been confused with diphtheria.[3,29,31,35] *C. diphtheriae* and *A. haemolyticum* are diphtheroids that cannot be distinguished on Gram stain but can be differentiated by their biochemical properties.

SKIN INFECTIONS

In the initial report of infections with *A. haemolyticum* in 1946, MacLean and associates[41] noted pharyngitis in U.S. servicemen and skin infections in the native populations of the South Pacific Islands. Cutaneous infections have been observed mainly in tropical countries.[64] The most common manifestations are ulcerative lesions that resemble ecthyma. Cellulitis, wound infections, and paronychia all have been noted.[7,22,23,34,45,47,64,68] In wound infections, mixed infections with *A. haemolyticum* and other organisms are common.

Tan and colleagues[62] reported five cases of *A. haemolyticum* bacteremia associated with soft tissue infections in adults. These patients, two elderly bedridden patients with decubitus ulcers and three diabetic patients with foot ulcers with extensive involvement that required surgical débridement, had a history of soft tissue infections. Four patients also had *A. haemolyticum* isolated from the suspected soft tissue focus of infection. A 3-year retrospective review of clinical samples over the same period as the five reported cases revealed 25 isolates of *A. haemolyticum*. Isolates from wound infections or cellulitis or both were found in 96 percent of cases; 20 percent of those patients also had bacteremia. Cultures from nonsterile sites often were polymicrobial, but the authors reported that *A. haemolyticum* usually was the predominant isolate.

OTHER MANIFESTATIONS

Isolated instances of septicemia, brain abscess, meningitis, meningoencephalitis, orbital cellulitis, endocarditis, osteomyelitis, deep soft tissue infections, pleural empyema, cavitary pneumonia, pyothorax, Lemierre syndrome, and sinusitis have been attributed to *A. haemolyticum* infection.* Most of these serious infections

*See references 3, 10, 23, 26, 28, 29, 32, 40, 41, 46, 48, 50, 57-59, 64.

*See references 2, 5, 21, 24, 27, 28, 30, 36, 39, 53, 60, 62, 64-66, 69, 70.

occurred in adults and frequently were associated with underlying conditions, such as diabetes, a malignancy, or intravenous drug use. In a 1998 review of systemic and deep-seated infections caused by *A. haemolyticum*, Skov and associates[60] identified two groups of patients. The first group comprised middle-aged to elderly adults who were immunocompromised or had other known risk factors for development of serious infectious disease. The second group consisted of preteens to young adults with no known risk factors except for one individual receiving steroid treatment for EBV infection.

A pyothorax with multiloculated hydropneumothorax was reported in a 19-year-old man in India with no history of pharyngitis.[53] Pure growth of *A. haemolyticum* was obtained from the thoracentesis. Treatment consisted of a therapeutic thoracentesis and continued chest tube drainage for 1 month. Ceftriaxone and metronidazole were given for 1 month, and the patient had clinical and radiologic resolution.

A brain abscess was documented in an 18-year-old man in Venezuela with a history of multiple periodontal problems, including periodontitis, dental caries, and multiple teeth extractions.[63] The patient presented with headache, vomiting, and neurologic deficits in the left extremities. Computed tomography of the chest revealed a left-sided hypodense frontoparietal lesion with cystic, contrast ring enhancement; edema; and midline mass effect. When his neurologic status worsened, a craniotomy was performed, and aspiration of purulent material from the encapsulated mass revealed positive cultures with pure *A. haemolyticum* growth. Therapy consisted of ceftriaxone and metronidazole for 1 week, and he was switched after susceptibility testing to penicillin for 3 weeks. Limjoco-Antonio and colleagues[36] reported a 9-year-old girl who presented with a swollen right eye after incuring a blunt trauma. She had ethmoid and maxillary sinusitis and orbital cellulitis that required surgical drainage, débridement of a subperiosteal abscess, and a second procedure with external ethmoidectomy and endoscopic sinus surgery. Cultures from the ethmoid sinus grew *A. haemolyticum.* She was treated with ceftriaxone and clindamycin.

Younus and colleagues[70] reported Lemierre syndrome with co-infection of *A. haemolyticum* and *Fusobacterium necrophorum* bacteremia. A 21-year-old man presented with fever, sore throat, tenderness along the border of the left sternocleidomastoid muscle, crackles, a blanching maculopapular rash, sepsis, and worsening pulmonary symptoms. The initial blood culture had *F. necrophorum*, and repeat blood cultures from day of admission had *A. haemolyticum* before initiation of antimicrobial therapy. Computed tomography of the chest revealed multiple nodular densities in the lungs with evidence of central necrosis in one area. Therapy consisted of anticoagulation with heparin and initial antimicrobial therapy of gatifloxacin and metronidazole, which was switched to vancomycin and piperacillin-tazobactam for 2 weeks followed by an oral course of amoxicillin-clavulanic acid for 3 months; no surgical intervention was needed.

DIFFERENTIAL DIAGNOSIS

Pharyngitis caused by *A. haemolyticum* must be differentiated from all other causes of pharyngitis (see Chapter 10). Of particular importance is distinguishing *A. haemolyticum* pharyngitis from *S. pyogenes* pharyngitis. Such differentiation can be achieved with certainty only by specific culture. In *A. haemolyticum* infection, the rapid group A streptococcal antigen tests would be negative, as would the usual group A streptococcal culture. These negative tests in specimens from adolescents should suggest strongly the possibility of *A. haemolyticum* pharyngitis.

When exanthem occurs, the confusion with illness caused by *S. pyogenes* is more pronounced. In many cases, the rash in patients infected with *A. haemolyticum* is scarlatiniform. The lack of typical circumoral pallor and a tendency for more discrete lesions in *A. haemolyticum* infection occasionally may help in establishing the clinical diagnosis. Other common causes of pharyngitis and exanthem in adolescents and young adults are *Mycoplasma pneumoniae* and EBV infections. As noted by Mackenzie and colleagues,[40] *A. haemolyticum* and EBV co-infections are common findings. Concurrent *M. pneumoniae* pneumonia and *A. haemolyticum* empyema and bacteremia have been reported in a previously healthy 20-year-old man.[61] Cutaneous infections, including subacute ulcerations, wound infections, cellulitis, and paronychia caused by *A. haemolyticum*, must be differentiated from cutaneous infections caused by other organisms, such as staphylococci and streptococci.

SPECIFIC DIAGNOSIS

A specific diagnosis is made by culturing *A. haemolyticum* from the pharynx, a skin lesion, or a sterile body site in invasive infections. Culturing is done best with rabbit or human blood agar and the addition of 5 percent carbon dioxide.[18,20,64,67] Horse or sheep blood agar, which generally is used for culture of *S. pyogenes*, is not satisfactory for the growth and identification of *A. haemolyticum*. Using biochemical identification systems such as the API (RAPID) Coryne strip (API bioMérieux, La-Balme-les-Grottes, France) or the Biolog system (Biolog, Hazelwood, CA) can help differentiate *A. haemolyticum* from other coryneform bacteria. The Biolog system can make the identification in 4 hours.[25]

TREATMENT

A. haemolyticum is highly sensitive to numerous antibiotics.[9,12,41,50,64] Although no specific treatment studies have been done, the experience in several large studies suggests that penicillin and erythromycin are effective.[9,32,41,46] Clinical failure with penicillin has been noted.[3,52,64] Erythromycin has been suggested for first-line therapy for certain indications, such as *A. haemolyticum* tonsillitis.[11] Carlson and coworkers[11] suggest using either a broad-spectrum β-lactam antibiotic or clindamycin or macrolides for serious systemic *A. haemolyticum* infection, although these authors acknowledge that macrolides do not provide anaerobic coverage. An alternative approach would be to use high-dose penicillin plus an aminoglycoside.[60,64]

PROGNOSIS

The prognosis in cases of *A. haemolyticum* pharyngitis is good, even in untreated patients. Invasive disease can be fatal, however, and peritonsillar abscess requires prompt surgical intervention and appropriate antimicrobial therapy.

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CHAPTER 105

ERYSIPELOTHRIX RHUSIOPATHIAE

Randall G. Fisher

Erysipelothrix rhusiopathiae (insidiosa) was identified definitively first by Rosenbach[29] in 1884 as a cause of the cutaneous disease erysipeloid. Although most commonly associated with localized skin infection in humans, this organism has been associated with sepsis,[13,27,37] chronic skin eruption,[9,16] and endocarditis.[3,13,21,22,28,37]

BACTERIOLOGY

E. rhusiopathiae is a slender, pleomorphic, gram-positive, unencapsulated rod that produces 0.1-mm bluish colonies on blood agar. On Gram staining, they may appear singly, in short chains, or, rarely, in long, branching filaments. Although they are gram-positive, they decolorize readily, sometimes producing a spotted appearance. Some strains produce alpha-hemolysis in 48 to 72 hours. Gelatin inoculated by stab inconsistently forms a "test-tube brush" or "pipe-cleaner pattern" appearance diagnostic for this organism.[13] *Erysipelothrix* is differentiated from morphologically similar *Listeria monocytogenes* and diphtheroids by the absence of motility and catalase production, and the presence of hydrogen sulfide production in triple-sugar iron medium.[13,37]

EPIDEMIOLOGY

First isolated from mice in 1880 by Koch, *Erysipelothrix* is a common commensal of wild and domestic mammals, birds, and fish.[13,37] The *Erysipelothrix* organism may lead a saphrophytic existence in soil. First identified by Loeffler in 1882 as the causative agent of swine erysipelas, it remains an important epidemic cause of disease in these animals, with losses greater than $25 million annually to this industry.[13] Sheep, rabbits, cattle, turkeys, and rats are subject to infection with this organism. *Erysipelothrix* has been recovered from wild moose and domestic emus.[5,20] *E. rhusiopathiae* survives salting and smoking procedures. Pieces of meat may contain the organism for 170 days after pickling, but exposure to moist heat for 15 minutes at 55° C[35] kills most strains.[13] Fish handlers, meat processors, poultry workers, veterinarians, abattoir workers, and food handlers are at risk for exposure to *Erysipelothrix*.[25] Isolates of the same serotype may show genetic diversity, so serotyping may not be completely reliable as an epidemiologic tool for tracking outbreaks.[6]

PATHOPHYSIOLOGY

Human infection is largely accidental and results from contamination of skin abrasions during handling of infected material. Males are infected more commonly than are females, perhaps because of an increased risk of exposure. The presence of an antiphagocytic capsule may be a virulence factor for *E. rhusiopathiae*.[32] In vitro study shows that encapsulated strains are poorly phagocytized by macrophages, unless immune serum is provided; ingested bacteria are able to replicate within the macrophage. The ability to survive and replicate within macrophages probably is due to failure of the encapsulated strains to induce the oxidative burst.[31] Disease usually is self-limited, most often involving the hands. Biopsy of skin lesions shows a marked inflammatory response. Difficulty of establishing bacteriologic confirmation has been attributed to the organism's location in the deep part of the pars reticularis of the corium.[7]

CLINICAL MANIFESTATIONS

Human disease typically manifests as a mild, localized cutaneous eruption; a more severe, generalized cutaneous form; or a septicemia often associated with endocarditis. Localized cutaneous infection, the erysipeloid of Rosenbach,[29] is the most common manifestation of *Erysipelothrix* disease.[25] After a 1- to 4-day incubation period, an acute localized lesion appears at the site of an abrasion contaminated with *E. rhusiopathiae*–colonized material. Slowly progressive, purplish red, painful induration is typical. Absence of suppuration and involution without desquamation help to distinguish this lesion from streptococcal or staphylococcal infection. Occasionally, the skin may show sharply circumscribed bluish red lesions, which are similar to the cutaneous manifestations in swine.[13,37] Fever and other constitutional symptoms are uncommon manifestations, occurring in less than 10 percent of cases, unless bacteremia supervenes.[9,25] Untreated infection usually is self-limited, with an average duration of 3 weeks.

Lymphangitis and adenitis occur in 10 percent of cases; in 20 percent of cases, progression of disease extends from lesions on the hand to the wrist and forearm.[17] In one case, a patient with type 2 diabetes was discovered at the time of surgery to have necrotizing fasciitis; *E. rhusiopathiae* was the predominant but not only organism isolated from surgical specimens.[33] A 7-week-old infant with localized *E. rhusiopathiae* infection of the knee without a known source of exposure has been reported,[19] and a 6-year-old girl with *Erysipelothrix* pyopneumothorax has been described.[26] Septic arthritis has been described in a healthy 18-year-old man in whom disease developed after he underwent arthroscopic knee surgery,[2] in association with infective endocarditis,[30] and in a patient with systemic lupus erythematosus and chronic monarthritis without associated systemic symptoms.[36]

Cutaneous eruptions rarely may occur in areas distant from the site of inoculation,[18] appearing as violaceous lesions with advancing pink borders. Bullous vesiculation has been described.[9] In 1921, Prausnitz[27] reported the first case of apparent septicemia in childhood, isolating the organism from the blood of a 10-year-old boy.

E. rhusiopathiae rarely is associated with the bite of a domestic dog or cat.[1] In one prospective study of infected domesticated animal–bite wounds, two patients who had cat-bite wounds infected with *E. rhusiopathiae* were identified.[34] Cultures were carefully processed and were performed in reference laboratories.

An uncommon but important complication of *Erysipelothrix* infection is endocarditis. Presumed or proven endocarditis accounts for 90 percent of serious *E. rhusiopathiae* infection.[12] Patients with congenital heart disease or heart valve damage secondary to acute rheumatic fever are at the greatest risk for development of endocarditis. However, previously normal heart valves can be infected.[13,21] Valvular and myocardial abscesses have

been described.[24] In contrast to diphtheroid endocarditis, *E. rhusiopathiae* endocarditis usually does not involve prosthetic valves, and in contrast to *Bacillus* spp. endocarditis, it is not associated with intravenous drug abuse.[12] In a review of 1989 cases of endocarditis from 13 series,[3] *Erysipelothrix* was documented in two patients. *Erysipelothrix* endocarditis commonly involves the aortic or mitral valves or both. Overall mortality in reported cases is 38 percent, which is considerably higher than that associated with other pathogens of endocarditis.[11] Many patients with endocarditis are treated empirically with vancomycin, a drug to which all *Erysipelothrix* isolates are constitutively resistant.[15] No history or physical evidence of cutaneous lesions is found in 50 percent of cases of endocarditis, and history of exposure to contaminated material often is lacking. Although immunocompromised individuals[22] may be at increased risk, serious infection also occurs in otherwise normal hosts,[4,13,21] particularly in association with occupational exposure.

DIAGNOSIS

For localized disease, diagnosis depends largely on clinical appearance of the lesion in association with an appropriate history of exposure. Attempts to culture the organism from material collected by swab or aspirate of a local lesion almost always are unsuccessful, presumably because of the bacteria's location deep within the skin.[7,37] Biopsy specimens of affected skin cultured in broth generally yield the offending bacteria. Amplification and detection of *Erysipelothrix* DNA by polymerase chain reaction show promise in animal models of infection.[20] *Erysipelothrix* is isolated commonly from the blood of patients with septicemia or endocarditis and can be found in affected heart valves at autopsy or at the time of valve replacement.[9] A high index of suspicion is important for establishing the diagnosis of endocarditis. The organism has been misidentified as a viridans group streptococcus because of its pleomorphic coccoid appearance, alpha-hemolysis, and catalase-negative character. Abbreviated identification schema that do not include testing for hydrogen sulfide production sometimes lead to misidentification as *Lactobacillus* spp. or enterococci.[8]

TREATMENT

E. rhusiopathiae is exquisitely sensitive to penicillin. Most isolates also are sensitive to ceftriaxone.[10] Clindamycin or ciprofloxacin may be used for patients who are allergic to penicillin.[10] Localized disease usually can be treated with oral medication, but high parenteral doses occasionally are necessary, particularly for disseminated disease.[13] Treatment of endocarditis is similar to treatment of endocarditis caused by viridans streptococci. At least 12 million U of penicillin administered for 4 weeks has been curative in adult patients, but many cases have been treated for 6 weeks or longer. Concomitant administration of an aminoglycoside has been used in some cases. *Erysipelothrix* is resistant to vancomycin.[15] Prompt microbiologic differentiation of *E. rhusiopathiae* from other gram-positive organisms is important in guiding antimicrobial choice because vancomycin often is employed in empiric therapy for endocarditis. Hyperimmune serum, which at one time was advocated for therapy, is of little value. Risk of acquiring disease is minimized by protecting individuals exposed to potentially contaminated materials.

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CHAPTER 106

LISTERIOSIS

Robert Bortolussi ✪ Timothy Mailman

Listeria monocytogenes was described first by Murray and associates[105] in 1926 while they were investigating an epidemic of perinatal infection in laboratory rabbits. Human disease caused by *Listeria* was first published 3 years later, in 1929.[111] Neonatal infection was not described until 1936.[22] Since then, the organism has been isolated with increasing frequency from elderly individuals, pregnant women, immunocompromised individuals, and neonates. A large body of information has accumulated, and several comprehensive reviews have been published.[16,45,118,128] More recently, food-borne outbreaks of *Listeria* causing gastroenteritis have refocused attention on this organism.[6,112,124]

ORGANISM

Listeria is a regular, short, facultatively anaerobic, non–spore-forming, gram-positive rod that is motile and forms bluish gray colonies on nutrient agar. The genus is thought to be named in honor of Lister, the father of antiseptic technique. Of the six *Listeria* spp. (*L. monocytogenes*, *Listeria innocua*, *Listeria grayi*, *Listeria welshimeri*, *Listeria seeligeri*, and *Listeria ivanovii*), only *L. monocytogenes* and *L. ivanovii* have been reported to infect humans.[59,85] Various features of *L. monocytogenes* have been used to separate it from nonpathogenic *Listeria* spp. and other gram-positive rods.[130] The following four characteristics are used commonly for differentiation: (1) *Listeria* exhibits a characteristic tumbling motility at 25° C (77° F), with reduced motility at 37° C (98.6° F); (2) it grows with a narrow zone of beta-hemolysis (nonhemolytic strains exist but rarely appear in clinical material) and exhibits a rectangular area of increased hemolysis when streaked on blood agar in proximity to *Staphylococcus aureus* (Christie, Atkins, Munch-Peterson [CAMP] test); (3) it is catalase-positive; and (4) it ferments α-methyl-D-mannoside and L-rhamnose but not D-mylose.

Listeria organisms tolerate low temperatures, high salt concentrations, and high pH, which allows replication to occur in soil, water, sewage, manure, animal feed, and, more importantly, refrigerated foods. Cold enrichment procedures have been used to improve the isolation rate from clinical material, but generally they are not recommended.[12,45,128]

After the early work of Paterson,[113] Seeliger and Finger[131] performed an extensive serologic characterization of *L. monocytogenes*. At least 17 serotypes have been identified on the basis of somatic and flagellar antigens, but three (1/2a, 1/2b, 4b) account for most clinical isolates and are the serotypes most commonly found in food.[97,114]

TRANSMISSION

The factors involved in transmission are not well understood. Although contamination of food and human exposure to *Listeria* are common occurrences,[51] most infections are sporadic and have no epidemiologic explanation.[21,104,146] In investigations of outbreaks, a source that is linked to food often is discovered. An outbreak described by Schlech and colleagues[125] involving the consumption of *Listeria*-contaminated cabbage was the first investigation to prove that food-borne transmission can occur. Since then, numerous outbreaks have been linked to food, particularly contaminated dairy products.[45,66,86,124,145,146] Pate, pork, ready-cooked chicken, and hot dogs also have been implicated.[6,66,69,98,124,129] The incubation period for food-borne listeriosis is approximately 3 weeks. Molecular techniques involving polymerase chain reaction and multilocus enzyme electrophoresis are being used to track epidemic and sporadic strains found in food.[4,66,92,107,114,120]

EPIDEMIOLOGY

L. monocytogenes colonizes humans and a variety of mammals worldwide.[130] In the developed world, approximately 1 to 5 percent of the population carry the organism in their feces.[51,77] Nonetheless, listeriosis remains an uncommon infection. In the United States, estimates of its incidence range from 0.4 to 0.7 case per 100,000 population.[138] Listeriosis reported in the United States has a nonuniform age distribution, with most cases occurring in newborns and elderly individuals (Fig. 106–1). Although cases can occur in otherwise healthy subjects,[128,148] immunocompromised individuals with acquired immunodeficiency syndrome (AIDS), individuals with cancer (related to chemotherapy, particularly fludarabine), or organ transplant recipients are at significantly increased risk.[30,104,110,135] Other host factors associated with listeriosis include diabetes mellitus, renal failure requiring dialysis, hemochromatosis, and cirrhosis.

Perinatal attack rates during outbreaks are extremely high, reaching 1 to 2 percent of all deliveries.[125] Vertical transmission from mother to infant through the placenta is the mode of transmission for early-onset newborn infection; the epidemiologic factors contributing to late-onset infection are unknown. Nosocomial spread rarely has been reported in nursery outbreaks.[40,50,81,98,127]

PATHOGENESIS AND PATHOLOGY

The gross and microscopic findings in listeriosis are well documented. Although multiple granulomata are characteristic of disseminated disease, the basic pathologic change of suppurative inflammation is nonspecific.[75] Examination of the placenta of infants with early-onset infection reveals macroabscesses, funisitis, and villitis in most cases.[140]

L. monocytogenes is a facultative intracellular pathogen that has been used extensively to study cell-mediated immunity and the biochemical and pharmacologic abnormalities associated with

Figure 106–1 Human listeriosis cases by age group for the United States, 1970-1971 and 1980-1981. *(From Albritton, W. L., Cochi, S. L., and Feeley, J. C.: Overview of neonatal listeriosis. Clin. Invest. Med. 7:311-314, 1984.)*

infection.[7,13,64,78,93,96,137,151] Factors involved in mucosal colonization are crucial for invasion of the host to occur.[112] The cycle begins with adhesion to a eukaryotic cell and subsequent internalization. This process is mediated partly by an *L. monocytogenes* surface protein known as internalin[8,54,82] and its mammalian cell receptor, E-cadherin.[82] The internalin–E-cadherin interaction is necessary for the organism to cross the intestinal epithelium barrier. The intestinal mucosa is a depot for *Listeria*-specific effector $CD8^+$ T cells, which accumulate during and after infection.[63] Spread of the organism to the liver and bloodstream is the usual mode of infection. Resident hepatocytes, enterocytes, and fibroblasts are invaded by the organism. Intracellular bacteria are shielded from macrophage and polymorphonuclear phagocytic cells, permitting intracellular proliferation to occur.[27,43,56,70,79,117] Numerous virulence genes and their roles in the pathogenesis (internalization, vacuole escape, intracellular proliferation, and intercellular spread) are known to exist.[57,115] When inside the host cell, *Listeria* uses a hemolysin to escape into the cytoplasm.[116] The hemolysin secreted by *L. monocytogenes*, termed *listerolysin O*, has been purified, and its gene, *hly*, has been sequenced.[24,115] It is a pore-forming protein (antigenically similar to streptolysin O produced by group A streptococci) that causes lysis of host-cell vacuoles with the subsequent release of *Listeria* into the host cytoplasm. Monoclonal antibody to listerolysin O provides resistance to *Listeria* infection in a murine model of infection.[17,32,33] When in the cell, *Listeria* uses its ActA protein to promote actin polymerization for intracellular movement. The intracellular cycle is complete after escape from the host cell.[71,146]

The mechanism for passage through the placenta has provided new insights into pathogenic mechanisms in utero. In a transgenic mouse model that expresses human E-cadherin, the internalin–E-cadherin interaction was found to mediate crossing of the placental barrier by *L. monocytogenes*. The crucial role of internalin for placental passage was not present in a guinea pig model of perinatal infection model.[8] ActA-mediated, cell-to-cell spreading also plays a role in the vertical transmission of *Listeria* to the fetus in the murine model.[83]

For many years, immunity to *L. monocytogenes* and other facultative intracellular bacteria has been attributed almost exclusively to a repertoire of T cells and cytokines.[93,99] We now realize that phagocytic cells also play a role in resistance to *Listeria* infection.[31,88,152] Peak immunity to *Listeria* in adults is expressed after 5 to 6 days of infection, coinciding with the maximal T-cell responses.[15,76,99,33] Cytokines such as granulocyte colony-stimulating factor, tumor necrosis factor-α, and interleukin-6 (IL-6), IL-10, IL-12, IL-15, and IL-18 are induced and mediate clearance of *Listeria*.*

In newborn animals, susceptibility to *L. monocytogenes* is associated with delayed production of cytokines and activation of T cells and natural killer cells.[14,36,90] In particular, monocyte,[152] T-cell,[91] and natural killer cell activity[36,91] is immature in animal models. Delayed cytokine and cell activation processes seem to operate in humans.[65,152] Synthesis of tumor necrosis factor-α, interferon-γ, interferon-α, and IL-2, all of which modulate the immune response and macrophage activation, is deficient in newborns.[132,152] Pretreatment of newborn animals with interferon or inducers of interferon seems to enhance immune responsiveness and protects against *L. monocytogenes* infection.[16,20,24]

In older children and young adults, *Listeria* infection is rare; however, underlying diseases or medications that interfere with cell-mediated immunity may increase susceptibility.[30,133] Cyclosporine, which blocks production of cytokine, is associated with increased susceptibility.[62]

CLINICAL MANIFESTATIONS

Although a variety of clinical manifestations are described in *L. monocytogenes* infections, most pediatric infections occur in the first months of life. The initial clinical features are similar to those of the more prevalent group B streptococcal infection.[9] The serotype distribution of *L. monocytogenes* seems to depend on the age at onset. Serotypes 1a and 1b occur more commonly in the early-onset form of disease (<7 days of age), and serotype 4b occurs in the late-onset form (>7 days of age).[3]

The early-onset form of neonatal listeriosis usually is diagnosed within the first 24 hours of life, and affected infants have respiratory distress or pneumonia, septicemia, and, occasionally, meningitis (Table 106–1).[2,3,39,86,139,148] Mothers of these infants frequently have an influenza-like illness with fever, malaise, headache, gastrointestinal symptoms, or pharyngitis in the few days

*See references 28, 29, 73, 80, 87, 108, 109, 144, 149, 153.

TABLE 106–1 Features of Early-Onset and Late-Onset Neonatal Listeriosis*

	Early Onset[2,3,39,86,140]	Late Onset[3,41,74,128,148]
Age at onset (median in days)	<0.1 (<0.1-1.3)	28 (7-140)
Birth weight (median in g)	2250 (1800-2540)	3100 (3000-3150)
Percentage of isolates from		
Cerebrospinal fluid	5 (2-7)	63 (30-87)
Blood and cerebrospinal fluid	10 (6-26)	25 (13-40)
Blood alone	74 (45-88)	12 (0-30)
Other only†	5 (0-24)	0
Newborn and maternal	72 (54-93)	4 (0-8)
Percent mortality	38 (22-63)	3 (0-10)
Percentage with obstetric complications	63 (38-87)	3 (0-7)

**This table shows the median values and ranges of results from various publications.*
†Sources other than blood and cerebrospinal fluid included cutaneous, gastric, throat, urine, and rectal sources.

preceding delivery.[60,86] During labor, maternal fever and green-stained or brown-stained amniotic fluid may be seen.[2,84] More severely affected infants are infected in utero, born prematurely, and often critically ill at birth. Widespread microabscesses and macroabscesses may occur and are demonstrable externally as discrete roseolar or pustular lesions on the skin and pharynx. The rash has been termed *granulomatosis infantisepticum* (Fig. 106–2). Depression at birth, respiratory distress, apnea, lethargy, and fever are common manifestations; diarrhea, conjunctivitis, and myocarditis also have been described.[84] The respiratory symptoms may mimic symptoms of respiratory distress syndrome. Patchy bronchopneumonic infiltrates, probably caused by aspiration of infected amniotic fluid, may be seen on chest radiographs. In addition, intrauterine infection may result in spontaneous abortion or stillbirth.[60] Colonized asymptomatic adults and neonates have been reported, however.[126]

The late-onset form of neonatal listeriosis occurs less commonly than does the early-onset form and usually affects term infants, who appear healthy until the onset of meningitis or, less commonly, septicemia and colitis 1 to 8 weeks after birth.[3,41,60,74,81,127] Clinical manifestations of late-onset meningitis may be subtle and include fever, irritability, lethargy, and poor feeding.[74,127] Cerebrospinal fluid findings vary. Although pleocytosis usually is significant, not all infections have a polymorphonuclear cell predominance. The maternal history in these cases usually is negative. An outbreak of late-onset neonatal listeriosis associated with mineral oil, reported by Schuchat and associates,[127] provides insight into the pathophysiology of this disease. During the outbreak, infants were bathed at birth with mineral oil. A strain of *L. monocytogenes* identical to the one causing the outbreak was isolated from a mineral oil container in the delivery room. Despite intensive investigation, no other source of infection was identified. The incubation period before the development of symptoms was 5 days, and the median age at first positive culture was 7 days. The symptoms and signs in infants were those of the late-onset form of *Listeria* infection, including fever and meningitis, and, in 7 of 10 infants, a positive cerebrospinal fluid culture.

Immunocompromised patients can have a variety of clinical findings, most commonly meningitis or septicemia. Although patients receiving immunosuppressive therapy have a high incidence of infection, the clinical features are similar to those of non-immunosuppressed patients with *Listeria* infection.[133] Rhombencephalitis, brain abscess, arthritis, osteomyelitis, endocarditis, endophthalmitis, liver abscess, and peritonitis also have been reported in adult patients.* Rarely, invasive listeriosis has been seen in children who are otherwise well.[94,97,128,148] *Listeria* also is recognized as a cause of febrile gastroenteritis.

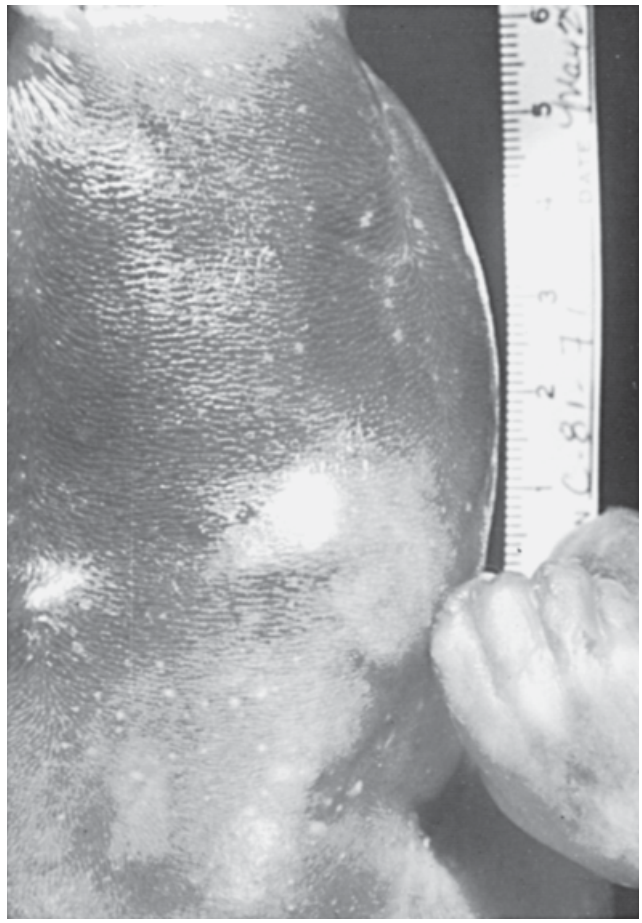

Figure 106–2 Typical pustular rash on the abdomen of a stillborn infant with listeriosis. Note the small pale granuloma measuring 1 to 3 mm and the dark erythema surrounding these lesions.

DIAGNOSIS

Appropriate specimens for staining and culture vary with the clinical syndrome, but investigation of the usual sources, such as blood, cerebrospinal fluid, amniotic fluid, and genital tract secretions, is most productive. Because prompt recognition is essential, examination of Gram-stained material (ear, meconium, and placenta) from newborns is recommended in suspected, early-onset sepsis.[83] Pathologic specimens (e.g., biopsy material, placental or fetal tissue) may reveal the characteristic Gram-stain morphology and pathologic features, such as microabscesses and granulomata. Selective culture media, such as PALCAM (polymyxin B–acriflavine–lithium chloride–ceftazidime–esculin–mannitol agar) or modified Oxford agar, may be helpful for isolating from contaminated material, such as stool or vaginal secretions.[95,114] Laboratories undertaking primary isolation must be aware of the similarities between *L. monocytogenes* and frequently discarded "commensals."[97]

After 48 hours of incubation at 37° C on 5 percent sheep blood agar, colonies are gray with a narrow zone of beta-hemolysis. The appearance on Gram stain of short rods has been confused with lancet-shaped pneumococci. Traditionally,

*See references 1, 5, 19, 23, 35, 44, 52, 89, 94, 103, 106, 135.

identification is based on morphology; tumbling motility; beta-hemolysis; positive catalase, esculin, and CAMP tests; and carbohydrate use pattern. Commercial kits are available for the rapid identification of *Listeria* from culture. These kits include DNA probes, latex agglutination, and enzyme immunoassay methods.

Despite extensive attempts to develop serologic techniques for the diagnosis of listeriosis, none has proved satisfactory, and few centers attempt serodiagnosis.[10,61] At present, a confirmed diagnosis requires isolation of the organism.

Histologic diagnosis from pathologic material should be attempted if the organism is not cultured. Specific fluorescent antibody staining,[25] nucleic acid hybridization,[136] and polymerase chain reaction[4,67] generally are not available in the routine diagnostic laboratory, but they offer the potential for development in the future, especially in patients pretreated with antibiotics and for rapid food surveillance.

TREATMENT

Prompt administration of antibiotic therapy is needed to prevent death or severe sequelae caused by *Listeria* infection. Antibiotic resistance in *L. monocytogenes* is low.[53,150] The bactericidal activity of the antibiotics commonly used is influenced in vitro by such factors as size of inoculum, type of medium, and definition of end-points.[37,89] Similar to a few other bacteria, *L. monocytogenes* shows tolerance to some antibiotics in vitro; the minimal bactericidal concentration is more than fourfold the minimal inhibitory concentration.[37,102] The antibiotics commonly recommended for treatment—ampicillin, penicillin, erythromycin, and tetracycline—are bacteriostatic only at concentrations usually achieved in blood.[37,46,47,142,147] Relapse of *L. monocytogenes* infection after apparent therapeutic success has been documented in immunocompromised patients, suggesting that bactericidal regimens may be needed in such patients.[42,122]

Other antibiotics have been considered for possible therapy. Some studies have shown trimethoprim-sulfamethoxazole and rifampin to be effective in eradicating *Listeria* in vivo.[42,55,58,100,123] These drugs seem to be bactericidal and have been used successfully in a few cases of human listeriosis.[51,135] Rifampin and trimethoprim-sulfamethoxazole offer a theoretical advantage over other drugs because of their better intracellular penetration.[42] Many newer antibiotics, such as the quinolones, macrolides, and imipenem, have only moderate activity in vitro against *Listeria*. Most quinolone and macrolide antibiotics are bacteriostatic only, and in vivo studies with these agents are not promising.[101,121] In addition, *L. monocytogenes* is resistant to cephalosporin antibiotics.[37] Vancomycin has been used in a few penicillin-allergic and sulfa-allergic patients with some success, but experience with this drug is limited.[119]

Combinations of antibiotics for therapy and in vitro testing also have shown variable results. Ampicillin plus gentamicin has a synergistic effect on most *Listeria* strains.[34,100,141] Partial synergy seems to occur with combinations of ampicillin or vancomycin and rifampin, but combinations of penicillin G and rifampin have shown activity ranging from synergy to antagonism.[100] In an in vivo model of *L. monocytogenes* encephalitis, the combinations ampicillin/gentamicin and co-trimoxazole/rifampin were highly active against intracerebral bacteria.[11] Antagonism also seems to occur between some antibiotic combinations (erythromycin and penicillins, erythromycin and aminoglycosides, penicillin and chloramphenicol, and penicillin and tetracycline).[37,46,100] On the basis of results of in vitro susceptibility testing and in vivo models, ampicillin plus gentamicin has proved to be the most reliable synergistic combination and remains the recommended initial therapy for patients suspected to have listeriosis. Trimethoprim-sulfamethoxazole may be considered for use in non-perinatal listeriosis, particularly in the presence of penicillin allergy, but it cannot be recommended for use in perinatal infections because of the concern of bilirubin toxicity developing with sulfonamides.[42] The duration of therapy depends on the clinical syndrome, the presence of underlying disease, and the response to treatment. In newborns, 2 weeks of antibiotic therapy usually seems to be adequate. Liposomal encapsulation also results in marked enhancement of the therapeutic activity of ampicillin.[8]

PROGNOSIS

Precise morbidity and mortality data for *L. monocytogenes* infection are unavailable. Maternal listeriosis may result in abortion or stillbirth. Fetal mortality rates probably are high with gestational listeriosis, although the relative risk of intrauterine death occurring is unknown. Convincing evidence that *L. monocytogenes* is associated with repeated abortions is lacking.[49]

Among reported cases of early-onset sepsis, the more recent mortality rate in North America is approximately 40 percent (see Table 106–1).[2,39] Most survivors seem to be normal.[38,39,139] Sequelae are related to the associated complications of prematurity, pneumonia, and sepsis; hydrocephalus and cerebral palsy also have been reported.[38,72] Early treatment of maternal disease would seem to affect fetal and neonatal outcome favorably.[39,72]

Late-onset *Listeria* meningitis has a mortality rate of less than 10 percent. The outcome after *Listeria* meningitis may be more favorable than the outcome associated with other types of bacterial meningitis.[74,148] Major sequelae are hydrocephalus and mental retardation. Beyond the newborn period, the outcome of listeriosis depends on the nature of any underlying disease and the availability of intensive medical care.

PREVENTION

The sporadic nature of the disease in North America emphasized the need for collaborative investigation and reporting of infections by physicians and veterinarians to public health authorities.[12,45] In the early 1990s, epidemiologists in the United States and Canada endorsed making listeriosis a notifiable disease.[114,138] Since then, the important role of food in the transmission of sporadic cases has been linked firmly.[128] Aggressive investigation of cases and close inspection and testing of food and food-handling facilities in the United States have been under way since the early 1990s.[92,138] By tracking strains of *L. monocytogenes* from patients' refrigerators to retail sources, specific foods have been identified. Strict adherence to regulations for pasteurization of raw milk is important to inactivate the organism and prevent listeriosis.[143] Contamination of food can occur during the preparation and processing of pasteurized milk products and ready-to-eat meat or poultry products. Recommendations for individuals at high risk, such as pregnant women and immunocompromised patients, include avoiding soft cheeses and delicatessen meats and avoiding reheating leftover foods or ready-to-eat foods (e.g., hot dogs).[114,138] Preventive strategies involving inspection of food and facilities and dissemination of recommendations and educational materials seem to have reduced the incidence of listeriosis in the developed nations.[4,48,138]

During an outbreak of *Listeria*, prompt investigation and treatment of pregnant women with a febrile "influenza-like" illness have been advocated.[12,72] The attack rate for late-onset disease in colonized infants is unknown, and no data show that treatment of colonized infants can either eradicate asymptomatic carriage or prevent infection. Careful attention given to the handling of infected infants in a neonatal unit in an attempt to prevent transmission is of the utmost importance for preventing nosocomial infection.[40,127]

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CHAPTER 107

TUBERCULOSIS

Jeffrey R. Starke

Tuberculosis still ranks as one of the three most important infectious diseases in the world in terms of morbidity and mortality. Recognizable in skeletons from the Stone Age and in mummified corpses from the Egyptian Old Kingdom, tuberculosis became more widespread in western Europe after the plague years of the Middle Ages and the epidemic during the era of urbanization and industrialization in the 18th and 19th centuries.[153] At that time, scrofula affected more than half the young inhabitants of workhouses and orphanages.

As similar social trends developed outside Europe, tuberculosis followed. In the eastern cities of the United States (Boston, New York, and Philadelphia), the mortality rate from tuberculosis was about 400 per 100,000 population. With improving socioeconomic conditions, the mortality rate fell to 200 per 100,000 around 1900 and to 26 per 100,000 by 1950. Stress in all its forms—famine, war, rationing, long working hours, child labor, population displacement, crowded living and working conditions—favors the spread of tuberculosis in human beings, whereas years of peace and plenty favor its rapid decline.[152,463] The decrease in the Western world in the incidence of tuberculosis was accentuated by the discovery, development, and widespread use of antituberculosis drugs beginning in the late 1940s.

Another important factor leading to the decline of tuberculosis in Western countries was the recognition in the 1920s of the importance of bovine tuberculosis and its successful eradication as a public health problem in the United States by gradual slaughter of infected cattle and almost universal pasteurization of milk.

Tuberculosis was recognized as a clinical entity in the early 19th century by Schönlein, who first used the term *tuberculosis* in 1830, and by Laennec in Paris, among others. Credit for extensively detailed descriptions of the primary focus goes to Anton Ghon (1866-1936), professor of pathology in Prague. In 1882, Koch identified *Mycobacterium tuberculosis*. The special diagnostic tools essential to understanding the disease in children were provided by Escherich, who in 1898 set up the first diagnostic radiography for children; by von Pirquet, Mantoux, Mandel, and Moro, who developed tuberculin testing between 1907 and 1910; and by Meunier and DeLille, who in 1898 taught the usefulness of gastric lavage in children. Revealing, long-term studies on the natural history of tuberculosis in children and on chemotherapy and prevention came principally from Scandinavia (Wallgren, Ustvedt, Holm, Hyge) and the United States (Brailey, Hardy, Lincoln, Hsu, Ferrebee).

TERMINOLOGY: EXPOSURE, INFECTION, DISEASE[12]

The pathophysiologic process of tuberculosis is complicated, and the delay between acquisition of infection and manifestation of disease renders certain pathophysiologic events less distinct. This chapter considers three major stages of tuberculosis: exposure, infection, and disease.[561]

Exposure means that the child has had significant contact with an adult or adolescent with infectious pulmonary tuberculosis. The contact investigation—examination of individuals close to a person suspected of having tuberculosis by performing a tuberculin skin test, chest radiograph, and physical examination—is the most important activity in a community to prevent cases of

tuberculosis in children.[10,50,234] The most frequent setting for exposure of a child is the household, but it can occur in a school, daycare center, or other closed setting.[136,227] In this stage, the tuberculin skin test result is negative, the chest radiograph is normal, and the child lacks signs or symptoms of disease. Some exposed children may have inhaled droplet nuclei infected with *M. tuberculosis* and have early infection, but the clinician cannot know it because delayed hypersensitivity to tuberculin—a positive skin test response—takes up to 3 months to develop. Children younger than 5 years old who are in the exposure stage should be treated to prevent the rapid development of disseminated or meningeal tuberculosis, which can occur before the skin test becomes reactive.[160,338,416,444,576]

Infection occurs when the individual inhales droplet nuclei containing *M. tuberculosis*, which becomes established intracellularly within the lung and associated lymphoid tissue. The hallmark of latent tuberculosis infection is a reactive tuberculin skin test. In this stage, the child has no signs or symptoms and the chest radiograph is either normal or reveals only granuloma or calcifications in the lung parenchyma or regional lymph nodes or in both tissues. In developed countries, virtually all children with tuberculosis infection should receive treatment, usually with isoniazid, to prevent the development of disease in the near or distant future.

Disease occurs when signs or symptoms or radiographic manifestations caused by *M. tuberculosis* become apparent. The word *tuberculosis* refers to disease. Not all infected individuals have the same risk of contracting disease. An immunocompetent adult with untreated tuberculosis infection has approximately a 5 to 10 percent lifetime risk for development of disease; half the risk exists in the first 2 to 3 years after infection occurs. Adults with tuberculosis infection who then become infected with human immunodeficiency virus (HIV) have a 5 to 10 percent annual risk for development of tuberculosis disease.[508] Historical studies have shown that disease, often serious, life-threatening forms, will develop within 1 to 2 years in as many as 40 percent of immunocompetent infants with untreated tuberculosis infection.

EPIDEMIOLOGY

INCIDENCE AND PREVALENCE

Between 20 and 45 percent of the world's population (approximately 2 billion people) are infected with *M. tuberculosis*, and more than 90 percent of new cases occur in the developing world, where resources are very limited. According to the World Health Organization (WHO), in 1997, only 32 percent of the world's population lived in areas where effective tuberculosis control programs were fully operational.[412] The WHO estimates that 8 million new cases and 2 million deaths from tuberculosis occur annually worldwide.[464,644] Approximately 11 percent (884,000) of new cases and about 5 percent of deaths are predicted to occur in children younger than 15 years.[295,405] Approximately 75 percent of childhood cases occur in the 22 highest burden countries. The inability to control tuberculosis despite the availability of effective, relatively inexpensive therapy is one of the greatest medical failures of our time.[210,560]

Between 1953, when reporting began, and 1985, the number of annually reported tuberculosis cases in the United States fell by 74 percent from 84,304 to 22,201.[92,93] In 1985, the overall case rate was approximately 10 per 100,000 population. At that time, the incidence curve flattened out, and for the first time since 1953, case numbers and rates increased to a peak of 26,673 cases in 1992. Between 1985 and 1992 in the United States, the total tuberculosis case numbers rose 20 percent.[92] During that same time, the number of pediatric tuberculosis cases rose 40 percent[264,612] (Fig. 107–1). Most experts cite four probable causes

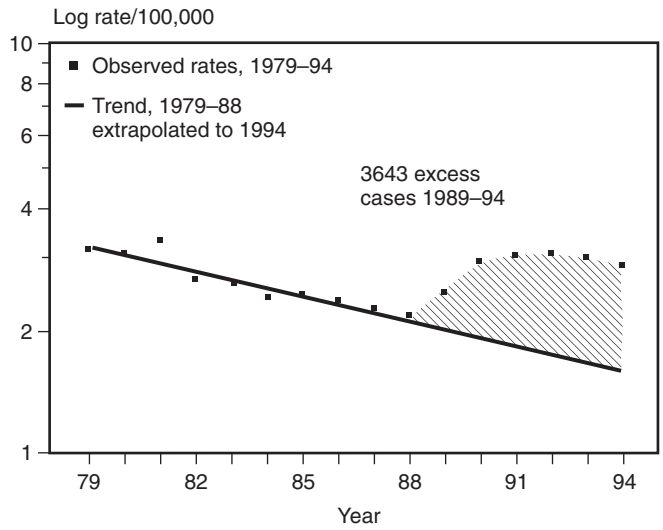

Figure 107–1 Observed and expected tuberculosis cases in children younger than 15 years old in the United States, 1979 to 1994.

for the increases: (1) the co-epidemic of HIV infection; (2) the increasing rates of tuberculosis in foreign-born individuals in the United States[71]; (3) the increased transmission among adults in congregate settings, including jails and prisons, nursing homes, homeless shelters, HIV treatment facilities, hospitals, and, rarely, schools;[35,38,52,376,442] and (4) a decline in the public health infrastructure in many areas of the country.[66,112,465,562] After several years of intense and expensive effort were expended, the number of tuberculosis cases in the United States declined again. In 2006, a total of 13,779 tuberculosis cases (4.6 cases per 100,000 population) were reported in the United States, a 21 percent decrease from 1999 and 48 percent decrease from 1992.[92] Rates have decreased consistently in the United States in all groups except immigrants from high-risk countries. However, the annual cost of tuberculosis in the United States still exceeds $1 billion.[65] An estimated 4 to 6 percent of the U.S. population, or approximately 11 million people, are infected with *M. tuberculosis*. This group represents a large reservoir from which cases of tuberculosis disease will emerge in the future if these individuals are not treated.[6] The resurgence of tuberculosis in the United States between 1985 and 1992 was associated with the emergence of multidrug-resistant tuberculosis (MDR-TB), strains that are resistant to at least isoniazid and rifampin and may be resistant to other antituberculosis medications as well.[654] MDR-TB is difficult and expensive to treat, and the mortality rates may be as high as 50 percent in complicated cases.[193,253] Since 1993, the incidence of resistance to isoniazid has remained stable, and the incidence of MDR-TB has decreased. In 2006, 8 percent of isolates were resistant to at least isoniazid, and 1 percent were resistant to at least isoniazid and rifampin (MDR-TB) in the United States.[92]

MDR-TB has become an important problem in many areas of the world.[159,654] Global surveys conducted between 1996 and 1998 in 58 geographic areas showed a median prevalence of MDR-TB of 1 percent, but the rates in some regions were alarming: 14 percent in Estonia, 10 percent in the Henan Province in China, and 9 percent in Latvia and parts of Russia.[171,654] There has been an emergence recently of extremely drug-resistant tuberculosis, isolates resistant to isoniazid, rifampin, fluoroquinolones, and at least one injectable drug.[514] In adults, drug resistance in *M. tuberculosis* often is secondary, the resistance emerging during therapy because treatment is inadequate or interrupted.[180] In children, drug resistance usually is primary in that the child is infected with a strain that already has become resistant.[413,494,496,591]

Rates of drug resistance in children tend to mirror those in adults in the same population.[567-569] Rates of drug resistance may be higher in developing countries because of difficulty in completing therapy, inadequate supply of medications, and use of over-the-counter cough medications that often contain isoniazid or rifampin.

Since treatment became available in the late 1940s, tuberculosis has been concentrated in certain high-risk groups in the United States[94] (Table 107–1). Tuberculosis occurs most commonly in areas with ethnically diverse populations, including large urban areas, coastal states, and states bordering Mexico[85,95] (Fig. 107–2). Tuberculosis disproportionately affects ethnic and minority populations in the United States. In 2006, the Centers for Disease Control and Prevention (CDC) reported the highest overall number of cases in Latinos, 4066 cases, but the case rate was highest among Asians, with 25.6 cases per 100,000 population.[92,414]

During the 1990s, immigration from a high-prevalence country was the single largest risk factor for tuberculosis, with rates among foreign-born persons being four to six times higher than those for U.S.-born persons.[108] In 2006, the CDC reported that 50 percent of all cases occurred in foreign-born individuals.[92] Tuberculosis is endemic in most developing countries, and between 30 and 50 percent of recent immigrants to the United States have latent tuberculosis infection on entry into the country.[108,588] During the immigration process, adults are screened for tuberculosis disease with a chest radiograph, but skin testing to detect tuberculosis infection is not required. Children younger than 15 years receive neither a chest radiograph nor a tuberculin skin test.[506] Among children in the United States, being born in a country with a high rate of tuberculosis is the most important risk factor for having tuberculosis infection. Foreign-born adopted children also are at risk for having tuberculosis infection and disease.[179,309,482] People who enter the United States illegally may be unable or afraid to seek medical treatment, even when they are ill. Studies have shown that in most immigrants in whom tuberculosis develops, it does so within 5 years of immigration, thus indicating that many cases could be prevented if appropriate screening and treatment programs were conducted.

Other important risk factors for tuberculosis in adults include lower socioeconomic status, migrant work,[87] HIV infection, drug use, homelessness, travel to high-prevalence countries, history of incarceration, and occupations with exposure to high-risk populations (see Table 107–1). Children from high-risk population groups or children who have contact with adults in these groups may be at increased risk for development of tuberculosis infection.[359] Age has an important influence on tuberculosis case rates.[404] During 2006, 6 percent of cases in the United States occurred in children younger than 15 years, 11 percent in persons 15 to 24 years old, 34 percent in persons 25 to 44 years old, 29 percent in persons 45 to 64 years old, and 19 percent in persons older than 64 years.[92] The highest case rates among children are in those younger than 5 years. Children aged 5 to 14 years, the so-called favored age, have a consistently lower case rate than that of any other segment of the population.[353,404] In early childhood, the incidence is not significantly different in girls and boys, although adolescent girls generally experience higher rates of disease than adolescent boys do.[59]

The age at which tuberculosis disease initially develops currently varies among ethnic groups in the United States.[92] White persons develop tuberculosis more commonly as older adults, whereas African Americans and Hispanics have a higher incidence of disease as young adults or children (Fig. 107–3).[577] Many of the risk factors for development of tuberculosis, such as HIV

TABLE 107–1 High-Risk Groups for Tuberculosis Infection and Disease

Groups at High Risk of Exposure or Infection
Close contacts of person with tuberculosis
Foreign-born persons from high-risk countries (Asia, Africa, Latin America, Russia, eastern Europe)
Residents and employees of high-risk congregate settings (correctional institutions, nursing homes, homeless shelters, hospitals serving high-risk populations, drug treatment centers)
Medically underserved, low-income populations
High-risk racial or ethnic minority populations
Injection drug users
Children exposed to adults in high-risk categories
Groups at Higher Risk for Disease Once Infected
Immunosuppressed patients, including HIV infected
Recent tuberculosis infection (within past 2 years)
Persons with certain medical conditions (diabetes mellitus, silicosis, cancer, end-stage renal disease, gastrectomy, body weight ≤90% of ideal)
Injection drug users
History of inadequately treated tuberculosis
Children ≤4 years, especially infants

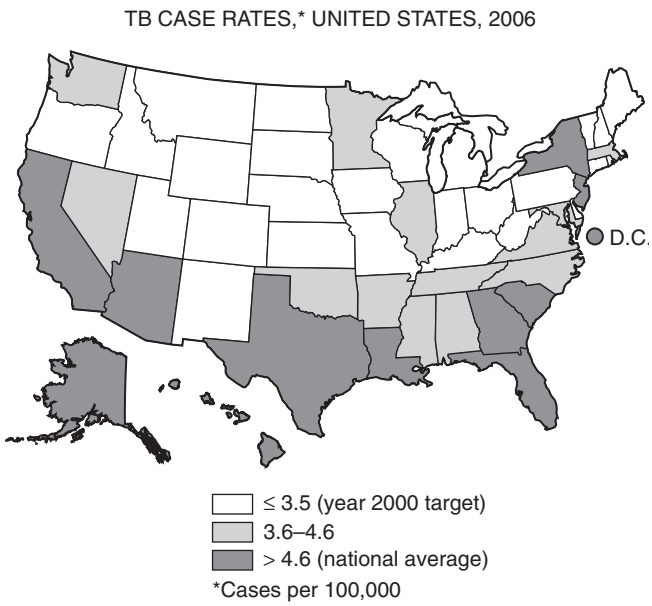

Figure 107–2 Tuberculosis case rates (cases per 100,000) by state, 2006. *(From Centers for Disease Control and Prevention: Reported Tuberculosis in the United States, 2006. Atlanta, GA, U.S. Department of Health and Human Services, CDC, October 2007.)*

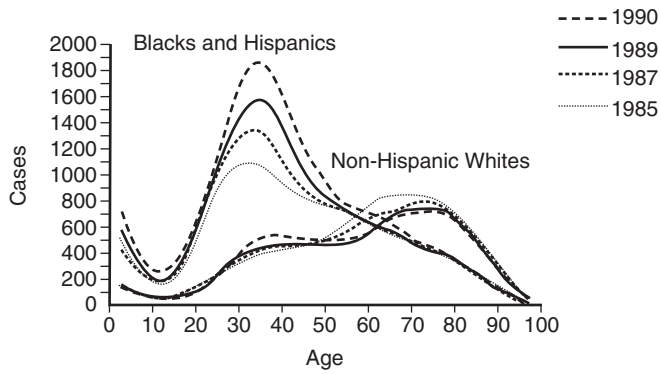

Figure 107–3 Tuberculosis cases in Hispanics and blacks versus non-Hispanic whites in the United States, 1985 to 1990.

infection, drug use, history of incarceration, and recent immigration, occur more commonly in young adults in their 20s, 30s, and 40s, a time when contact with children is more likely. Other factors, including recent immigration, socioeconomic status, high-risk behavior, and possibly genetic susceptibility, may influence the peak age distribution of tuberculosis among different groups.[349] Inherited susceptibility to tuberculosis may contribute to differences among various ethnic groups.[349] Laboratory animals and humans have been shown to differ in genetic susceptibility to tuberculosis.[343,511] In the United States, highly urbanized immigrants, such as Jews from European ghettos, fared much better than did their rural Irish and African counterparts, presumably because generations of exposure to tuberculosis in previous European epidemics had selected in favor of more resistant individuals.[454] As the disease spread to other continents, previously unexposed populations may have been more susceptible to tuberculosis and experienced higher rates of disease.

In some U.S. locales that have had recent increases in tuberculosis, the demographic groups with the greatest tuberculosis morbidity also have large numbers of HIV-infected persons.[104,226,401] The HIV epidemic has had a profound effect on the epidemiology of tuberculosis in children by two mechanisms[490]: (1) most important, in the United States, HIV-infected adults with tuberculosis may transmit *M. tuberculosis* to children in their environment, and tuberculosis disease will develop in some of them; and (2) important in many developing countries, children with HIV infection are at increased risk of progressing from asymptomatic tuberculosis infection to disease.[244,342] Several studies have demonstrated increased rates of childhood tuberculosis associated with increased rates of disease among HIV-infected adults in the community.[209,270] Tuberculosis probably is underdiagnosed in HIV-infected children, especially in the developing world, because of the similarity of its clinical manifestations with other opportunistic pulmonary diseases and the difficulty of confirming the diagnosis with the skin test or culture. All children with suspected tuberculosis disease should have HIV serotesting because the two infections are linked epidemiologically and many experts prolong treatment in HIV-infected children with tuberculosis.[204,640]

The site of manifestation of tuberculosis disease differs between adults and children. Although pulmonary disease is most common for all ages, extrapulmonary tuberculosis occurs more often in children.[404] Because HIV co-infection increases the risk for development of extrapulmonary disease, rates have increased in adults during the past 15 years. In general, 25 percent of pediatric cases are extrapulmonary, with 75 percent being pulmonary. In 1985, before widespread HIV infection occurred, 85 percent of adults had pulmonary disease and 15 percent had extrapulmonary disease. With the spread of HIV infection, these numbers have shifted, and in 2006, the CDC reported that 79 percent of all new cases, including those in adults and children, were pulmonary and 21 percent were extrapulmonary.[92] Comparison of the site of extrapulmonary disease in children and adults in 1985, before the acquired immunodeficiency syndrome (AIDS) epidemic, shows some important differences. First, approximately 70 percent of extrapulmonary tuberculosis in children involved the lymph nodes, as opposed to 25 percent in adults (Fig. 107–4). Second, tuberculous meningitis accounted for 13 percent of extrapulmonary disease in children versus 4 percent in adults. Genitourinary involvement occurred in 16 percent of adults, but it was a rare finding in children. Although the proportion of extrapulmonary cases in adults has increased as a result of the HIV-AIDS epidemic, extrapulmonary disease still occurs more commonly in children.

The global epidemiology of childhood tuberculosis is not well described.[405] In developing countries where the burden of disease is greatest, the only available diagnostic test often is an acid-fast smear of sputum, the result of which rarely is positive from

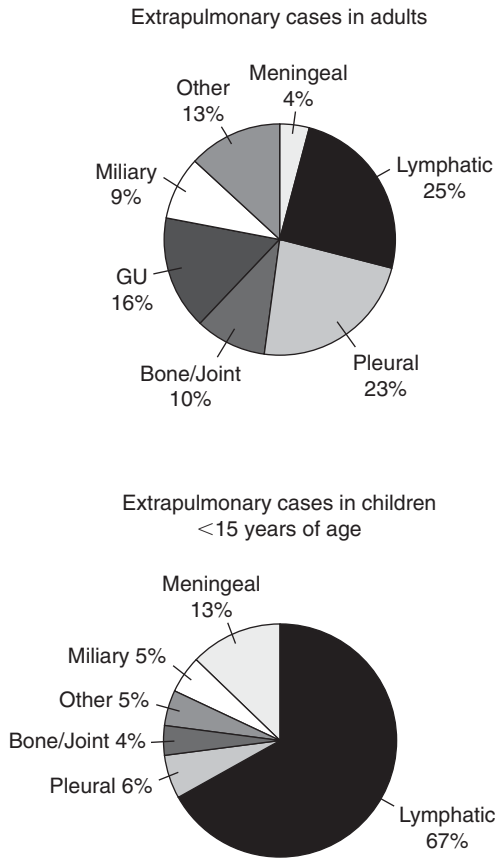

Figure 107–4 Extrapulmonary disease by site in adults and children in the United States. *(From the Centers for Disease Control and Prevention.)*

infants and children with pulmonary tuberculosis. In many developing countries, more than 50 percent of the population is younger than 18 years, so the true burden of childhood tuberculosis has been underestimated.

TRANSMISSION

Transmission of tuberculosis is from one human to another, usually through infected droplets of mucus that become airborne when an individual coughs, sneezes, or laughs.[103] The droplets dry and become droplet nuclei, which may remain suspended in air for hours. Only particles less than 10 μm in diameter are small enough to reach the alveoli.[476] Transmission sometimes occurs by direct contact with infected discharges (sputum, saliva, urine, or drainage from an open sinus or abscess); it occasionally occurs by means of heavily contaminated fomites, such as shoes, gastric lavage tubes, bronchoscopes, or syringes prepared by someone with positive sputum.[223] Rare cases of tuberculosis transmitted by a lung or kidney transplant have been reported.[381,400,473] Dogs may be a source of infection for children because dogs are susceptible to the human type of tubercle bacillus.[470]

The collective experience of many clinicians is that children usually are infected by an adult or adolescent in the immediate household, most often a parent, grandparent, older sibling, boarder, or household employee.[37] Casual extrafamilial contact is the source of infection much less often, but physicians, babysitters, schoolteachers, music teachers, school bus drivers, parishioners, nurses, gardeners, and candy store keepers have been implicated in individual cases and in hundreds of mini-epidemics.[23,57,155,320,326,635] Attention has been drawn to the preva-

lence of active tuberculosis among residents of nursing homes for the elderly. Children visiting their grandparents have contracted tuberculosis in this setting. Within the household of an infectious adult, infants and toddlers most often become infected. Adults with pulmonary disease who are receiving regular, appropriate chemotherapy probably rarely infect children; much more dangerous are those with chronic tuberculous disease that is unrecognized, inadequately treated, or in relapse because of the development of resistance.

Wallgren[631] was the first to point out that children with tuberculosis rarely if ever infect other children. Many children with the disease have tuberculin-negative siblings and parents. Children with tuberculosis often have been cared for by their families or in hospitals and institutions without infecting their contacts.[399] When transmission of *M. tuberculosis* has been documented in children's hospitals, it almost invariably has come from an adult with undiagnosed pulmonary tuberculosis.[24,187,277,278,391,636] Adults accompanying a child with suspected tuberculosis disease should be screened as soon as possible for pulmonary tuberculosis.[61,82,107,279,280,559] In tuberculous children, tubercle bacilli in endobronchial secretions are relatively sparse, and cough is not characteristic of endothoracic tuberculosis or miliary disease. When young children cough, they lack the tussive force of adults. Specimens collected by bronchoalveolar lavage or early-morning gastric aspiration from children with clinically suspected tuberculosis seldom if ever have acid-fast bacilli seen on smears. Only approximately 40 percent eventually will grow *M. tuberculosis* by culture.[546] Young infants with congenital tuberculosis or advanced, postnatally acquired pulmonary disease are more likely to have smear- or culture-positive specimens (80%) than older children are.[493,613] Therefore, specimens and secretions from infants, children with cavitary lesions, and intubated patients should be handled as potentially infectious.[82,366] Most preadolescent children with tuberculosis, however, are not contagious and do not require isolation. Tuberculosis in adolescents may be more typical of adult-type reactivation disease, including the presence of cavitary lesions with smear- and culture-positive sputum. Children or adolescents who have symptomatic pulmonary tuberculosis with features of adult-type tuberculosis should be treated as potentially contagious until mycobacterial smears and cultures are negative.[74,120,175]

Children, nonetheless, play an extremely important role in the transmission of tuberculosis, not so much because they are likely to contaminate their immediate environment but rather because they may harbor a partially healed infection that lies dormant, only to be reactivated as infectious pulmonary tuberculosis many years later under the social, emotional, and physiologic stresses arising during adolescence, pregnancy, or old age. Thus, children infected with *M. tuberculosis* constitute a long-lasting reservoir of tuberculosis in the population.

The risk of infection developing in child contacts of adults receiving antituberculosis chemotherapy often is a matter of practical concern. Several studies reveal that most contacts are infected by the index case before the diagnosis is made and treatment is initiated. Although it is not possible to carry out a definitive clinical study, evidence indicates that patients receiving effective chemotherapy rarely transmit *M. tuberculosis*. Nonetheless, it seems prudent to avoid exposure of children to adults with positive sputum smears or positive cultures and to assume that adults positive by smear or culture remain infectious for at least several weeks after the start of therapy.

MYCOBACTERIOLOGY

The genus *Mycobacterium*, closely related by its cell wall antigens to the genera *Corynebacterium* and *Nocardia*, presently is classified in the order Actinomycetales and the family Mycobacteriaceae. Mycobacteria are nonmotile, non-spore-forming, pleomorphic, weakly gram-positive rods measuring 1 to 5 μm long, typically slender and slightly "bent." Some appear beaded, and some are clumped. In general, species pathogenic for humans are more acid fast, have more exacting nutritional requirements, grow more slowly, form less pigment, and are more sensitive to chemotherapeutic agents than are the saprophytic species.

The cell wall constituents of mycobacteria determine their most striking biologic properties. The cell walls contain 20 to 60 percent lipids by dry weight, largely bound to proteins and carbohydrates. These organisms are more resistant than are most others to light, alkali, acid, and the bactericidal action of antibodies. Their growth is slow, with a generation time of 14 to 24 hours, perhaps because of the slow metabolic exchange through the waxy "capsule." Their hydrophobic properties render them difficult to study.

Acid-fastness, that is, the capacity to form stable mycolate complexes with certain aryl methane dyes (specifically, carbolfuchsin, crystal violet, auramine, and rhodamine, which then are not removed readily even by rinsing with 95 percent ethanol plus hydrochloric acid), is the hallmark of mycobacteria. The cells appear red when stained with fuchsin (as with the Ziehl-Neelsen or Kinyoun stains), appear purple with crystal violet, or exhibit yellow-green fluorescence under ultraviolet light (when stained with auramine and rhodamine, as in Truant stain). Truant stain, in experienced hands, is considered the best stain for specimens expected to contain small numbers of organisms.

Identification of mycobacteria depends on their staining properties and on their biochemical and metabolic characteristics. Mycobacteria are obligate aerobes. On the whole, their growth requirements are simple. *M. tuberculosis* can grow in "classic" media, whose essential ingredients are egg yolk and glycerin (Löwenstein-Jensen, Petragnani, Dorset); often, a dye such as malachite green to inhibit contaminants; and, sometimes, potatoes, charcoal, and so on, which probably neutralize growth inhibitors. They also can grow in simple synthetic media, frequently with an admixture of asparagine, glutamate, or amino acid mixtures (Middlebrook 7H9, Tween-albumin). Once grown, they can be replated on media also containing antituberculosis drugs to determine drug susceptibility patterns. Isolation on solid media often takes 3 to 6 weeks, followed by another 2 to 4 weeks for drug susceptibility results. Improvements in laboratory methods have permitted more rapid culture, identification, and drug susceptibility testing of mycobacteria, such as by an automatic radiometric method known as the BACTEC (Becton-Dickinson, Towson, MD) method, in which a decontaminated, concentrated specimen is inoculated into a bottle of medium containing carbon 14–labeled palmitic acid as the substrate.[524] As mycobacteria metabolize the carbon 14–labeled palmitic acid, carbon dioxide 14 accumulates in the head space of the bottle, where radioactivity can be measured. Unfortunately, cross-contamination of bottles has been reported and has resulted in false-positive culture results.[154] The addition of appropriate dilutions of antituberculosis drugs permits an evaluation of drug susceptibility to be made. The time for identification and drug susceptibility testing can be reduced to 1 to 3 weeks, depending on the size of the inoculum.

Bacteriophage typing to determine the relatedness of isolates has advanced slowly. Progress is being made, however, in standardization of techniques, and phage typing already is of use in strain identification for epidemiologic purposes. However, a newer technique, restriction fragment length polymorphism analysis of mycobacterial DNA, has become a powerful tool for determining strain relatedness in both outbreaks and routine epidemiology of tuberculosis in a community.[8,529]

RESISTANCE AND IMMUNITY

Natural resistance to tuberculosis infection varies greatly among animal species; humans, guinea pigs, and rabbits are highly susceptible. However, Lurie[343] experimentally bred resistant rabbits and showed that although the virulent tubercle bacilli disseminated just as well in the resistant rabbits, multiplication within tissues was inhibited. Thus, the differences between resistant and susceptible rabbits appeared to lie in the ability of the resistant rabbits to produce an effective immune response, and this ability seemed to be controlled genetically.

Additional evidence that genetic factors influence susceptibility comes from data involving twins. Kallmann and Reisner[273] noted that when one homozygous twin suffered from tuberculosis, the other twin had a higher chance of being affected than was the case with heterozygous twins. Gender affects resistance, and females appear to be especially susceptible during adolescence. The negative nitrogen and calcium balance that can arise during adolescence may account in part for susceptibility at this age.[268]

Likewise, young age appears to predispose one to tuberculosis.[404,492,613] However, one cannot be sure that the apparent susceptibility is not due to a larger dose of bacteria because of more intimate contact between very young children and their infectors. Although diabetes mellitus affects the resistance of adults, whether it affects that of children is not clear. Many viral infections depress tuberculin reactivity, but only measles and perhaps influenza have been incriminated in lowering resistance to tuberculosis.[39,64,215,564,657] Unfortunately, natural resistance is ill-defined and poorly understood.

Cell-mediated immunity is regarded as most important in host defense against *M. tuberculosis*.[126,323,418] The T cell–mediated immune response involves a variety of cell subsets that are involved in numerous functions, including protection, delayed hypersensitivity, cytolysis, and establishment of memory immunity.[428] The functions also involve an array of cytokines, several of which direct cells of the monocyte-macrophage axis to contain and destroy the invading bacilli.[14,582] The exact role of individual cytokines is not yet clear, but an emerging concept is that much of the clinical response to the presence of *M. tuberculosis* is determined by the balance of the cellular-cytokine response, which to some degree is under genetic influence.[428] In general, T-helper 1 (T_H1) responses are more beneficial to the host than are T_H2 responses, but unfortunately, infants and young children have increased propensity to develop T_H2 responses to mycobacterial immunogens.[323] More details about the immune response to *M. tuberculosis* are emerging as new immunotherapies are developed. During the past decade, several monoclonal antibodies directed against tumor necrosis factor–α have been used to manage inflammatory bowel disease and rheumatologic disorders. Unfortunately, use of these agents, particularly infliximab, has been associated with a high incidence of serious tuberculosis in patients who had existing tuberculosis infection or who acquire it during treatment.[60,275,458] All immunocompromised patients, including those who will undergo any form of immunotherapy, should be evaluated carefully for tuberculosis infection or disease.

PATHOGENESIS

PORTAL OF ENTRY

The tubercle bacillus usually is inhaled. The observations of Riley[476] suggest that a single tubercle bacillus can initiate infection. Ghon, Kuedlich, and their associates (Table 107–2) reported that the primary focus found in 2114 autopsies on children was the lung in 95.93 percent of cases. Especially significant is that their study was done at a time when bovine tuberculosis, which might have produced many primary gastrointestinal foci, was much more common than it is today. Ingestion probably accounts for a small percentage of primary pulmonary foci and for some gastrointestinal foci, particularly in infants who have consumed milk containing bovine tubercle bacilli. Contamination of a superficial skin or mucous membrane lesion, such as an abrasion of the sole of the foot or the elbow, insect bite, ritual circumcision, or infection of the vulva, may lead to infection. Infection by inoculation with a sputum-contaminated syringe has been reported in more recent years.[223] True congenital infection, although rare, may be a result of either lymphohematogenous spread in the mother during pregnancy or smoldering endometritis.[615]

TABLE 107–2 Portal of Entry of Tubercle Bacilli

Respiratory (%)		Nonrespiratory (%)	
Lung	95.93*	Bowel	1.14
Tonsils	0.09	Skin	0.14
Nose	0.09	Eye	0.05
Middle ear	0.09	Parotid	0.05
Total†	**96.20**	**Total**	**1.38**

**Of 2114 autopsies on children.*
†Undetermined, 2.4%.
Data from Ghon, A., and Kuedlich, H.: Die Eintrittspforten der Infektion. In *Engel, S., and Pirquet, C. (eds.): Handbuch der Kindertuberkulose. Stuttgart, Germany, Georg Thieme Verlag, 1930.*

INCUBATION PERIOD

The incubation period from the time that the tubercle bacillus enters the body until cutaneous sensitivity develops has been found to be 3 weeks to 3 months.[355] With both bacille Calmette-Guérin (BCG) and experimental infections, the incubation period is shorter when the inoculum is large, and clinical experience suggests that the same is true in humans. Debré, for example, noted long ago that tuberculosis acquired by an infant from its mother was likely to be much more severe than was an infection acquired from a visitor to the home. Animal experiments support this concept. The end of the incubation period coincides with the onset of tuberculin hypersensitivity and may be accompanied by a period lasting 1 to 3 weeks that Wallgren called fever of onset or fever of invasion. At this time, the tissue reaction intensifies throughout the primary complex and may permit the complex to be visible on x-ray films.

THE "TIMETABLE" OF TUBERCULOSIS

Wallgren's tremendous experience with tuberculous children in institutions permitted him to recognize and to describe the usual early course and timing of the initial infection and each of its best-known complications.[634] His timetable concept is an extremely useful one for clinicians because it permits a realistic prognosis, an understanding of what complications to look for and when, and a more productive approach to finding the infectious source case (Fig. 107–5).[355]

Symptomatic, massive lymphohematogenous spread (i.e., miliary or acute meningeal tuberculosis) is seen in only 0.5 to 3 percent of infected children. When it does occur, the usual onset is 2 to 6 months after initial infection. Endobronchial tuberculosis, possibly with segmental pulmonary lesions, develops slightly later on average. The metastatic lesions of bones and joints, which can be expected in 5 percent of untreated infected children, usually do not appear until approximately 1 year after infection

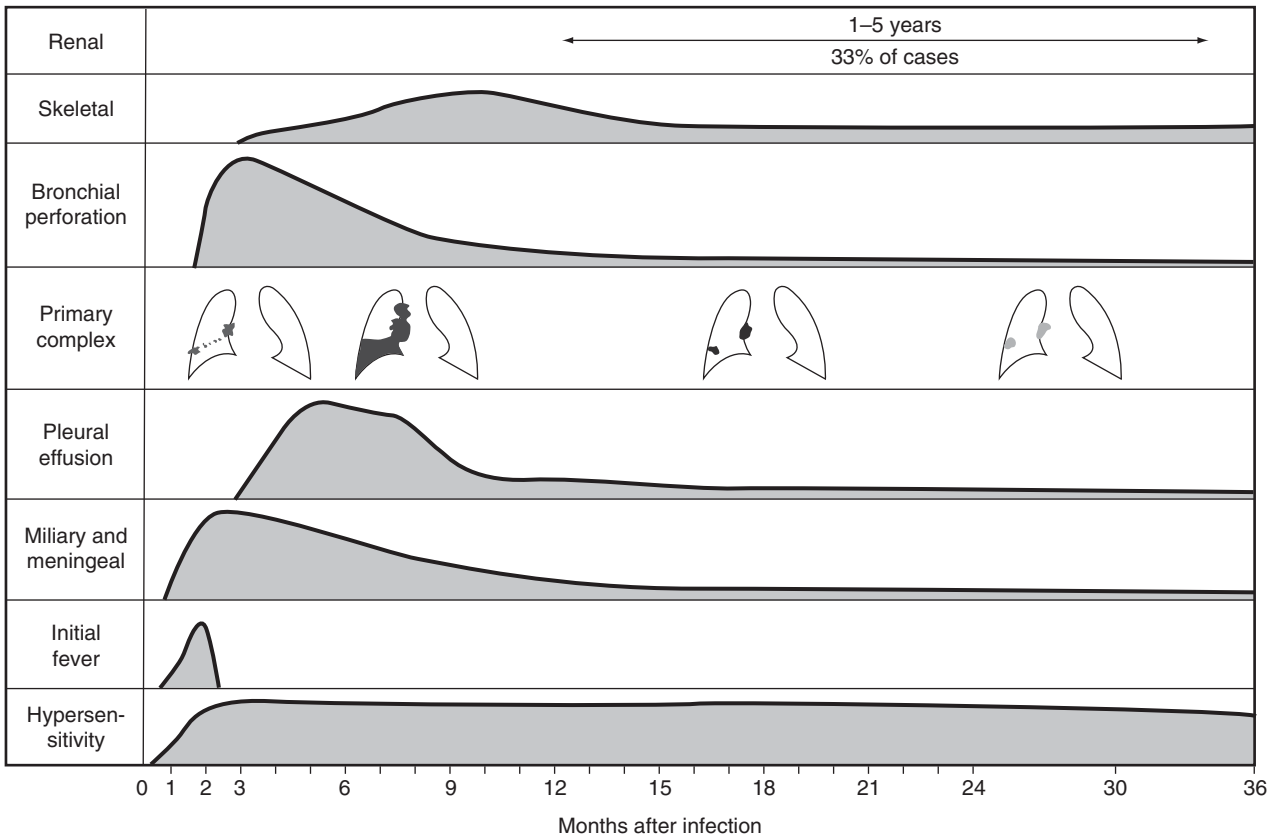

Figure 107–5 The timetable of tuberculosis.

TABLE 107–3 Median Age of Children* with Tuberculosis by Predominant Site of Involvement, United States, 1988

Site	No. of Cases (%)	Median Age (yr)
Pulmonary	1213 (77.5)	6
Lymphatic	209 (13.3)	5
Pleural	49 (3.1)	16
Meningeal	29 (1.9)	2
Bone or joint	19 (1.2)	8
Other	15 (1.0)	12
Miliary	14 (0.9)	1
Genitourinary	13 (0.8)	16
Peritoneal	4 (0.3)	13
Not stated	1 (0.1)	—
Total	1566 (100.0)	6

**Younger than 20 years.*

occurs, at the earliest. Renal lesions come later still, 5 to 25 years after initial infection. The relationship between the anatomic site of tuberculosis and the median age at onset in children is shown in Table 107–3. The interval between the acquisition of initial infection and the appearance of chronic pulmonary tuberculosis is extremely variable but can be months to decades, depending mainly on the age of the child at the time of infection. The interval is likely to be short in adolescents but much longer in infants.

In summary, the first 5 years after initial acquisition of tuberculosis infection in childhood, especially the first year, is when complications usually occur. Later in life, during times of stress, a previously silent or arrested lesion may reactivate and become dangerous to the patient as well as highly infectious to others.

CLINICAL FORMS OF TUBERCULOSIS IN CHILDREN

ENDOTHORACIC

Asymptomatic Tuberculosis Infection

Asymptomatic (or latent) infection can be defined as infection associated with tuberculin hypersensitivity and a positive tuberculin test result but with no striking clinical or radiographic manifestations. Computed tomography may reveal enlarged lymph nodes in the chest, even though the plain radiograph is normal.[19,132] On occasion, low-grade fever is found at the onset, usually by chance. If the child has been in recent contact with a person who has contagious tuberculosis and the tuberculin test result is positive, disease should be ruled out immediately with a chest radiograph and a thorough physical examination. Asymptomatic tuberculosis infection occurs more frequently in children of elementary school age than in adolescents or infants. Some 40 to 50 percent of infections in infants younger than 1 year and 80 to 95 percent in older children can be expected to cause no specific recognizable symptom or radiographic findings.[441] Gastric washings in these patients, even when performed with great care, yield a very low percentage of positive results.

A clinician making a diagnosis of tuberculosis infection in a child must assume, however, that the patient might be in the earliest stage of infection and at risk for the development of symptomatic disease in the near future.[355] A careful history and investigation of contacts should be undertaken immediately for determination, if possible, of the date of exposure. Chemotherapy must be started, and the patient closely monitored not only to detect any toxic effect of chemotherapy and to monitor adher-

ence with treatment but also to be sure that disease does not develop.

The Endothoracic Primary Complex and Its Complications

The primary complex, described by Ghon,[188] includes three elements: the primary focus, lymphangitis, and regional lymphadenitis. This complex holds true for every primary infection, regardless of the portal of entry. Ghon noted that at least 70 percent of primary pulmonary foci are subpleural. Thus, pleurisy is almost a regular feature of the primary complex.

Evolution of the primary pulmonary focus begins with an acute inflammatory reaction around tubercle bacilli inhaled into an alveolus, with the localized alveolar consolidation varying from the size of a pea to the size of a walnut. Macrophages appear within hours in the inflammatory exudate and change into clusters of epithelioid cells to form tubercles. In turn, these tubercles may resolve and disappear, or central caseation consisting of incomplete cell autolysis may develop. The caseous lesion contains large numbers of multiplying tubercle bacilli that spread rapidly from the primary focus through the regional lymphatic vessels to the regional lymph nodes, with areas of inflammation being set up along the way that later may caseate and calcify.[452]

The primary pulmonary focus has been studied carefully by numerous investigators (Table 107–4). Plotting their locations on a normal chest radiograph creates a pattern resembling the scatter of birdshot on a paper target. Thus, all parts of the lung apparently are at equal risk of being seeded. The thought that the primary focus has a predilection for the lower fields of the lung probably arises from the fact that the lung is pyramid shaped, with more basilar than apical lung tissue.

Many investigators concur that 70 to 85 percent of primary infections are initiated by one focus.[188,585] In a study of 170 cases, Ghon[188] found two foci in 15 percent, three in 7 percent, four in 3 percent, and five in 2 percent. Multiple lung foci can result, although rarely, from the ingestion and inhalation of tubercle bacilli. This pattern of disease was shown clearly in the results of pathologic studies of the 71 infants who died in the Lübeck disaster of the 1930s, an incident in which 251 newborn infants mistakenly had been given live tubercle bacilli by mouth instead of BCG vaccine. Fifteen of the infants were found to have primary lung lesions at autopsy, and all 251 had primary intestinal lesions.

Although the lymphadenitis cannot be detected clinically and rarely is apparent even on radiographs, the hallmark of initial tuberculosis infection is the relatively large size and importance of the adenitis as opposed to the relatively insignificant size of the initial focus in the lung, skin, or elsewhere. The development of tuberculin hypersensitivity within 3 to 12 weeks after initial acquisition of infection enhances the cellular reaction throughout the primary complex, but with a particularly prominent effect on the primary focus and the regional lymph nodes. At this time, the infection may spread along nearby lymphatic chains to involve more distant nodes. The lymphatic drainage of the lungs is outlined in Table 107–5; it occurs predominantly from left to right. One is not surprised, therefore, that the nodes in the right upper paratracheal area appear to be the ones most often affected. Many primary lesions are subpleural, and the lymphatic drainage of the apical pleura is to the cervical nodes. Moreover, the paratracheal chains have communications with both the deep cervical nodes and the abdominal nodes, as shown in Figure 107–6. Among 54 patients with the primary lesion in the right upper lobe, Blacklock found that 14 had involvement of the deep cervical nodes on the same side and three had involvement of the abdominal nodes.

Bronchial obstruction as a result of enlargement of the peribronchial lymph nodes was reported by Ghon[188] and others. Not until the 1920s did Wallgren[632] and others elucidate the role of these large nodes in producing the radiographic shadows variously called epituberculosis, collapse-consolidation, and segmental lesions that so often are seen in cases of childhood tuberculosis.[329,354,632] At the end of the incubation period, as tuberculin sensitivity develops, the hilar lymph nodes enlarge greatly, and in many cases, caseous foci appear within them. Acid-fast studies of smears and sections have confirmed that this caseum has few tubercle bacilli. As the nodes enlarge, they fre-

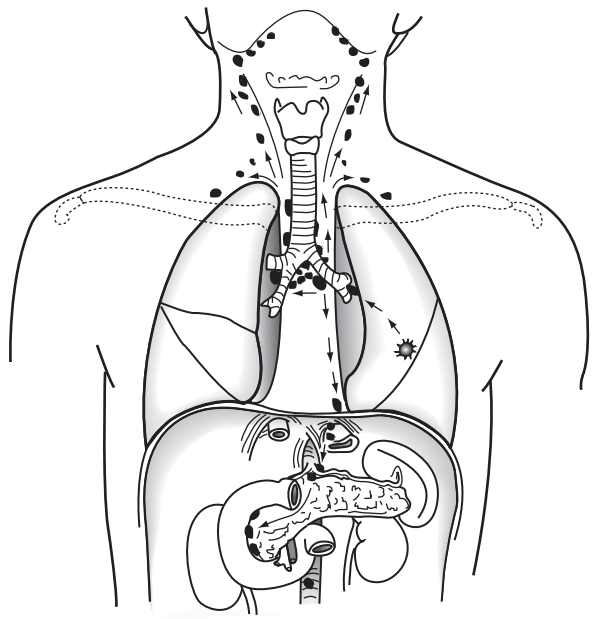

Figure 107–6 Schematic composite drawing illustrating wide lymphogenic spread of the tuberculosis infection from a primary pulmonary focus in the base of the left upper lobe. The infection extended cephalad to the submandibular nodes in the head and caudad as far as the pancreatic nodes in the abdomen. *(From Caffey, J.: Pediatric X-Ray Diagnosis. 7th ed. Chicago, Year Book, 1978.)*

TABLE 107–4 Location of the Primary Pulmonary Focus

Location	No. of Patients	%
Right upper lobe	138	27
Right middle lobe	40	7
Right lower lobe	107	20
Left upper lobe	122	24
Left lower lobe	104	20
Total	511	98

Data from Ghon, A., and Kuedlich, H.: Die Eintrittspforten der Infektion. In *Engel, S., and Pirquet, C. (eds.): Handbuch der Kindertuberkulose. Stuttgart, Germany, Georg Thieme Verlag, 1930.*

TABLE 107–5 Lymphatic Drainage of the Lung

Right upper lobe	→	Right paratracheal chain
Right middle lobe	→	Right and left paratracheal nodes
Right lower lobe	→	Subcarinal nodes
Left upper lobe	→	Left paratracheal nodes
Left lower lobe	→	Left paratracheal nodes
Lingula	→	Subcarinal nodes
Subcarinal nodes	→	Right paratracheal nodes

Based on data of Rouviere, quoted by Courtice, F. C., and Simmonds, W. J.: Physiological significance of lymph drainage of the serous cavities and lungs. Physiol. Rev. 34:419-442, 1954.

quently impinge on the neighboring regional bronchus and compress it and cause diffuse inflammation of its wall, even to the point of obstructing the lumen.[329,505,553] Daly and colleagues,[123] in their study of endobronchial tuberculosis in children at Bellevue, found this mechanism to be the cause of obstruction in half their patients. Other mechanisms of obstruction include damage to the bronchial cartilage leading to gradual (or, rarely, abrupt) perforation of the bronchus and the formation of plugs of semiliquid toothpaste-like caseum that partially or completely occludes the bronchus. In some cases, endobronchial granulomatous tissue forms around the stoma of the fistula and obstructs the lumen.

Three immediate results of bronchial obstruction are possible.

The first is sudden death by asphyxia, fortunately an extremely rare event.[311]

The second is obstructive hyperaeration (also called *obstructive emphysema*) of a lobar segment, a lobe, or even an entire lung. This unusual result usually affects children younger than 2 years old, and it may be accompanied by wheezing. Physical examination generally is of little help; radiographs, best taken on expiration, show hyperaeration, which usually is not accompanied by mediastinal displacement, probably because of fixation by the tuberculous mediastinal nodes (Fig. 107–7). Aspiration of a foreign body always must be considered in the differential diagnosis. The obstruction ultimately resolves by itself; however, corticosteroids may be added to the chemotherapeutic regimen to hasten recovery.[406,407,602] Surgical removal of the obstructing nodes has been successful but rarely is performed.[129]

The third possible result of bronchial obstruction is the appearance of a segmental lesion, fan-shaped on a radiograph, representing mainly atelectasis and almost always involving the very segment occupied by the primary pulmonary focus[307,354,393] (Fig. 107–8). Actually, the radiographic opacity results from a combination of several elements: the primary pulmonary focus,

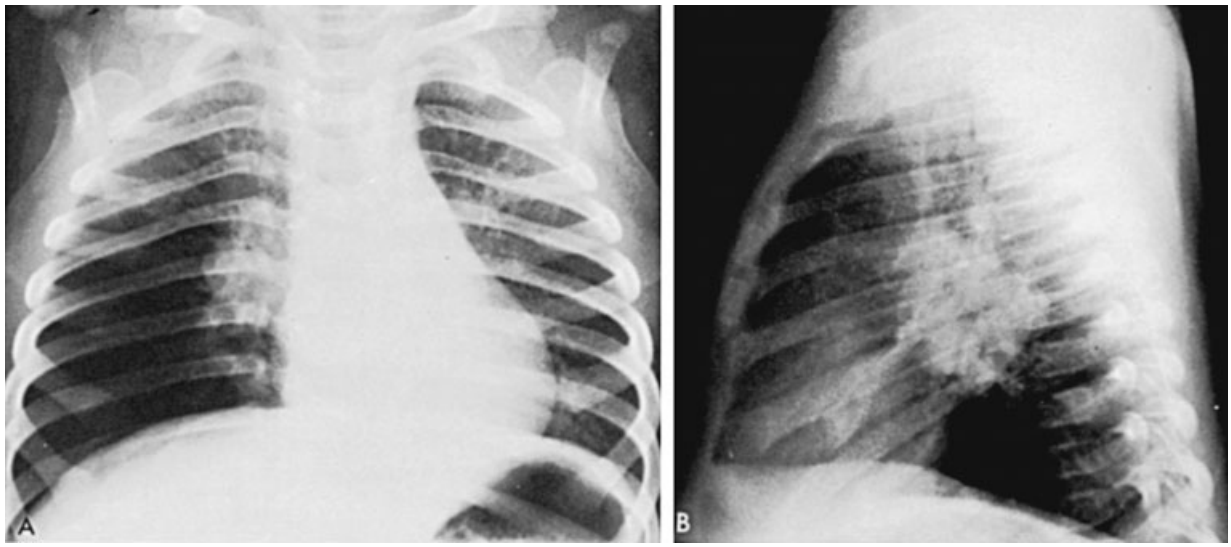

Figure 107–7 X-ray film of an 8-month-old girl with obstructive hyperaeration of the right lower lobe as a result of tuberculosis. **A,** Note the hyperlucent right lower lobe and shift of the heart and mediastinum away from the ball-valve obstruction to the left. **B,** Note the large hilar lymph nodes, which are compressing the right lower lobe bronchi. Tuberculosis should be considered in patients with hyperaeration of unknown etiology.

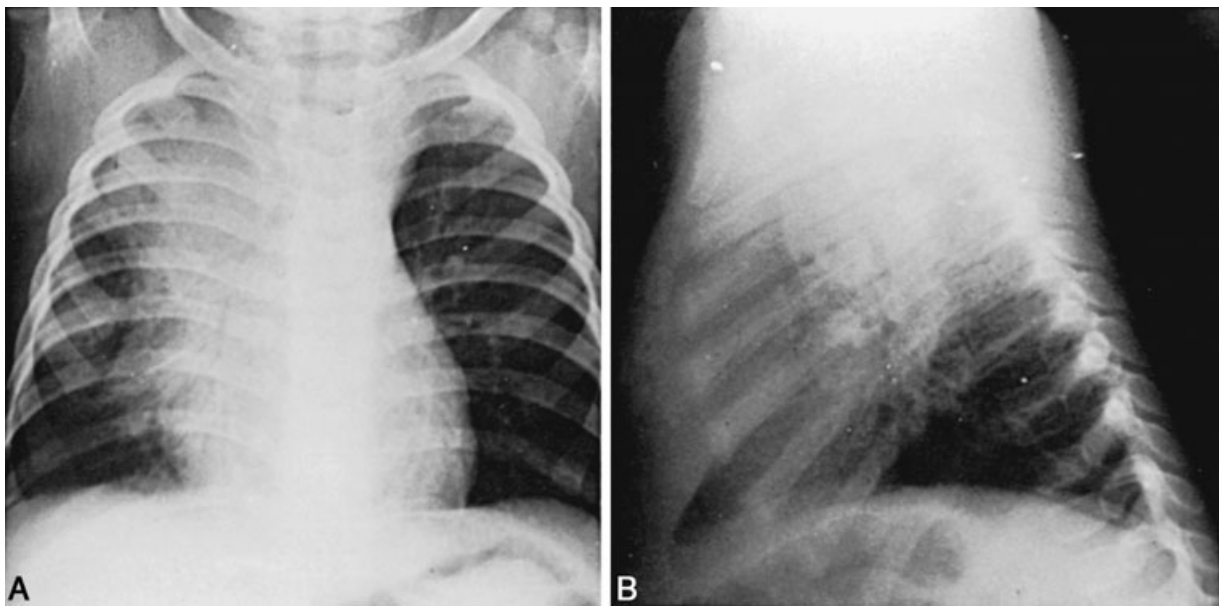

Figure 107–8 **A** and **B,** Radiographs of an 8-month-old boy with primary tuberculosis infection. The posteroanterior film shows collapse-consolidation of the right upper lobe, hilar and paratracheal adenopathy, and pleural reaction. Note the narrowed right bronchi.

the caseous material from an eroded bronchus, the inflammatory response elicited by the caseum, and atelectasis. In some instances, acute secondary infection plays a role. Children with secondary bacterial pneumonia often initially have high fever, cough, and rales; the signs and symptoms respond to conventional antibiotics, but the chest radiographic findings usually do not clear because of the underlying tuberculosis. The relative roles of these various elements often cannot be assessed; sometimes atelectasis is conspicuous, and, at other times, consolidation is the salient pathologic process, with the volume of the segment undiminished or even increased (hence the descriptive term *collapse-consolidation lesion*, preferred by some clinicians to the less accurate term *atelectasis*).

The percentage of infected children in whom segmental lesions develop has been estimated by several investigators. All agree that the younger the child, the more frequently collapse-consolidation lesions occur (Table 107–6). The segmental lesion is likely to form during the first 3 to 6 months after acquisition of infection (50 of 65 in Payne's series[441]). Multiple segmental lesions can occur simultaneously (18 of 160 children in Payne's experience). When multiple lesions develop, the segmental lesions usually form in one lung, but occasionally, the lobes or segments of both lungs are affected. Sometimes, segmental lesions and obstructive hyperaeration occur simultaneously. The physical signs and symptoms of segmental lesions—cough, rales, localized wheezing, egophony—are surprisingly meager but are seen more frequently in infants because of the smaller size of their airways.

Although segmental lesions and hyperaeration are the most common findings produced by enlarging thoracic lymph nodes, others occur. Enlarged peritracheal nodes may cause stridor and respiratory distress.[358] Subcarinal nodes may impinge on the esophagus and cause difficulty swallowing, followed occasionally by the formation of an esophageal diverticulum, or the nodes may rupture directly into the esophagus and produce a bronchoesophageal fistula. Enlarged lymph nodes may compress the subclavian vein and produce edema of the hand and arm, or they may erode major blood vessels, including the aorta. They also may rupture into the mediastinum and point in the left or more often the right supraclavicular fossa. Compression of the left recurrent laryngeal nerve has been reported. Compression of the left phrenic nerve leads to paralysis of the left leaf of the diaphragm in an estimated 0.1 to 0.3 percent of tuberculous children. Rupture into the pericardial sac is described later.

The late results of bronchial obstruction include the following possibilities: complete re-expansion of the lung and resolution of the radiographic findings; disappearance of the segmental lesion, with residual calcification of the primary focus or the regional lymph nodes; or scarring and progressive contraction of the lobe or segment, usually associated with bronchiectasis.[329,355] Permanent anatomic sequelae result from segmental lesions in approximately 60 percent of all cases, even though the abnormality usually is not apparent on plain radiographs. Cylindrical (rarely saccular) bronchiectasis, sometimes stenoses, and elongation or shortening can be demonstrated on bronchography. Fortunately, most of these abnormalities are asymptomatic in the upper lobes. However, secondary infection may occur in the middle and lower lobes and cause the middle lobe syndrome.[63] On occasion, the chronic vascularity that accompanies bronchiectasis leads to poor oxygen saturation during exercise and to restricted body growth. In addition, bronchogenic carcinoma may arise years later in the old scarred lesions remaining in the bronchus.

Calcification of the primary complex, when it appears, always results from caseation. In calcified caseum, as in bone, the predominant calcium salt is tribasic calcium phosphate. Calcification of caseous lesions occurs much more readily in children than in adults, probably because children's calcium and phosphorus plasma levels are higher. In the infants who were victims of the Lübeck disaster, autopsy showed calcification to be present as early as 58 days after the onset of massive infection. Payne,[441] in a study of calcification in 299 children from Newcastle-on-Tyne, reported calcification visible on the chest radiographs of one child within 6 months of infection, 38 within 12 months, 104 within 18 months, 165 within 24 months, 252 within 3 years, and 299 within 4 years.

Calcium usually is deposited as fine particles and creates a stippled effect (Fig. 107–9), but it may be deposited in large, even enormous masses. Calcification may persist without much change, or it may start resorbing within 5 years and eventually disappear completely. It occasionally progresses to ossification with the formation of true bone and functional bone marrow. Calcification, if it is visible at all on radiographs, most often involves the regional lymph nodes.[58] Sometimes, however, the primary pulmonary focus or the entire primary complex, including the lymphangitis, calcifies (Fig. 107–10).

Calcification took place in 75 to 80 percent of the 525 children with pulmonary tuberculosis monitored by Payne.[441] Currently, extensive calcification occurs uncommonly in the Western world, probably because tuberculous lesions treated early with isoniazid rarely caseate, and caseation is a prerequisite for calcification.

TABLE 107–6 Percentage of Tuberculin Converters and Age at Which Segmental Lesions Develop

Rights were not granted to include this table in electronic media. Please refer to the printed publication.

After Payne, M., quoted by Miller, F. J. W., Seale, R. M. E., and Taylor, M. D.: Tuberculosis in Children. Boston, Little, Brown, 1963.

Pleural Effusion

Pleural effusion can be localized or generalized, unilateral or bilateral.[327] Localized pleural effusion so frequently accompanies the primary pulmonary focus that it is practically a component of the primary complex. All tuberculous serous effusions probably originate in the discharge of bacilli into the cavity from an adjacent lesion—in the case of the pleura, from a subpleural pulmonary focus or from subpleural caseous lymph nodes. The breakthrough may be small and the pleuritis localized and asymptomatic, or it may occur in the form of a generalized effusion, usually 3 to 6 months after infection occurs (Fig. 107–11). In 75 percent of Wallgren's[632] cases, later calcification of the pulmonary focus or regional lymph nodes proved the effusion to be on the same side as the original primary focus. Seemingly without logical explanation are the following clinical observations: tuberculous pleural effusion is a rare occurrence in children younger than 2 years and an uncommon one in children younger than 5 years (perhaps because sensitivity to tuberculin is lower in the very young), occurs more frequently in boys than in girls, almost never is associated with a segmental lesion, and rarely is associated with miliary tuberculosis.

The onset of pleurisy usually is abrupt and resembles bacterial pneumonia, with fever, chest pain, shortness of breath, and, on physical examination, dullness to percussion and diminished breath sounds. Fever may be high and, in untreated cases, last for several weeks. On occasion, differentiation of an effusion from an extensive pneumonic lesion is difficult; lateral decubitus radio-

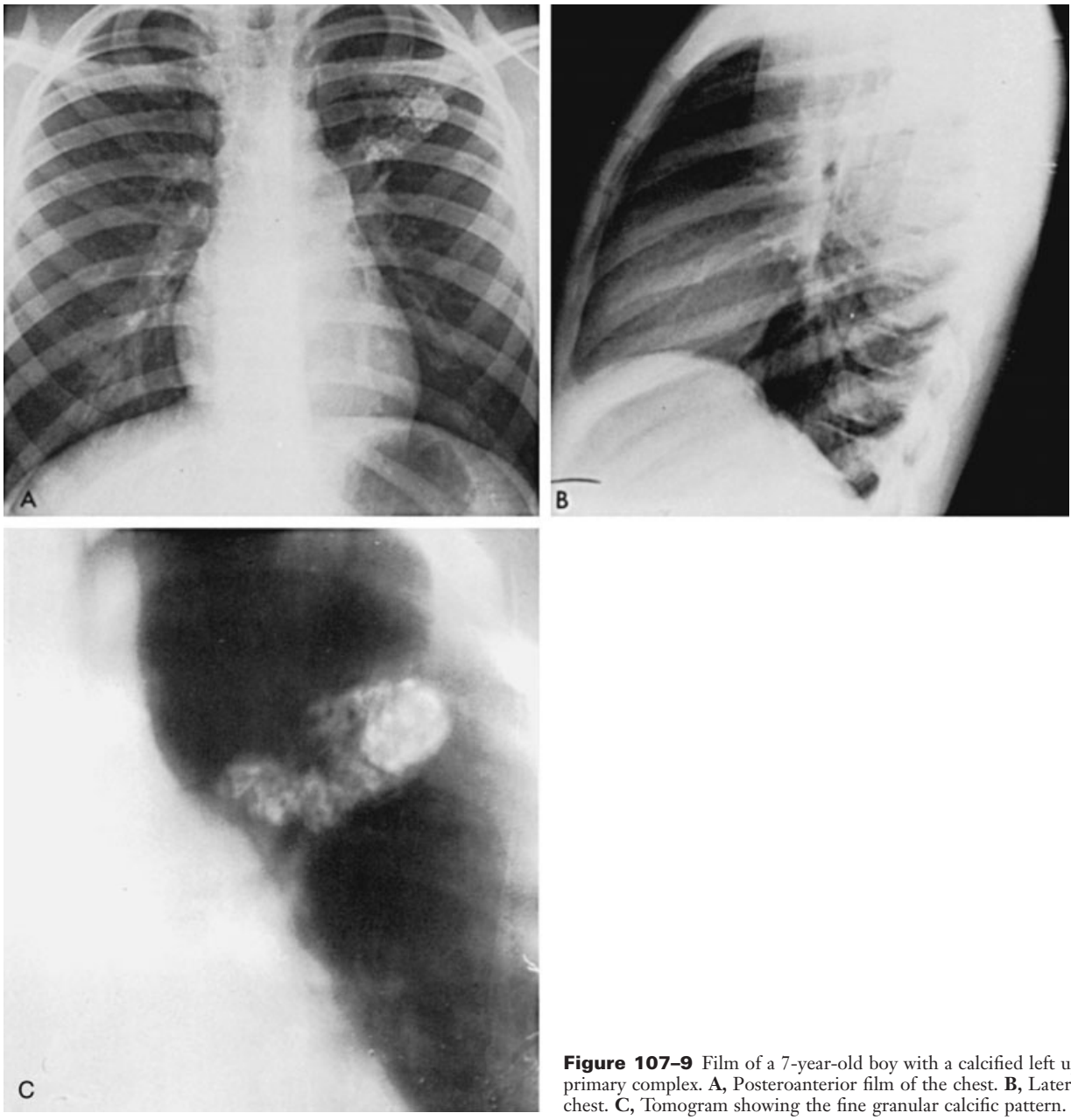

Figure 107–9 Film of a 7-year-old boy with a calcified left upper lobe, primary complex. **A,** Posteroanterior film of the chest. **B,** Lateral film of the chest. **C,** Tomogram showing the fine granular calcific pattern.

graphic views are helpful in confirming the presence of pleural fluid.

Thoracentesis is, of course, the essential diagnostic procedure. The puncture should be made in the area shown on the radiograph to have the greatest accumulation of fluid. No more than 30 mL of pleural fluid should be withdrawn; otherwise, the protein loss from this usually protein-rich fluid may be considerable. The fluid generally is greenish yellow, occasionally tinged with blood, with a specific gravity of 1.012 to 1.022, a high protein content, and often a low glucose level (<30 mg/dL), and it has several hundred white cells per cubic millimeter with a predominance of neutrophils or lymphocytes, depending on the age of the effusion. The cells in tuberculous pleural effusion are predominantly T lymphocytes, which are present in a higher proportion than in blood.[167,520] Tubercle bacilli generally are present in such small numbers that results from direct smears and cultures are likely to be disappointing; smears almost always are negative, and pleural fluid cultures are positive in less than 30 percent of cases. Pleural biopsy is a useful diagnostic procedure because both the finding of typical tubercles on histologic study and culture of the tissue are much more likely to establish the diagnosis than is culture of pleural fluid.[322]

The prognosis for children with tuberculous effusion always has been relatively good compared with that for other overt forms of tuberculosis, even in the days before chemotherapy.[331] Permanent impairment of pulmonary function is a surprisingly uncommon event after pleural effusion.[178] The development of scoliosis is a remote possibility and should be guarded against while the patient recovers.

Progressive Pulmonary Tuberculosis

In this serious complication of the primary complex, the primary pulmonary focus, instead of resolving or calcifying, enlarges steadily and develops a large caseous center. This center then

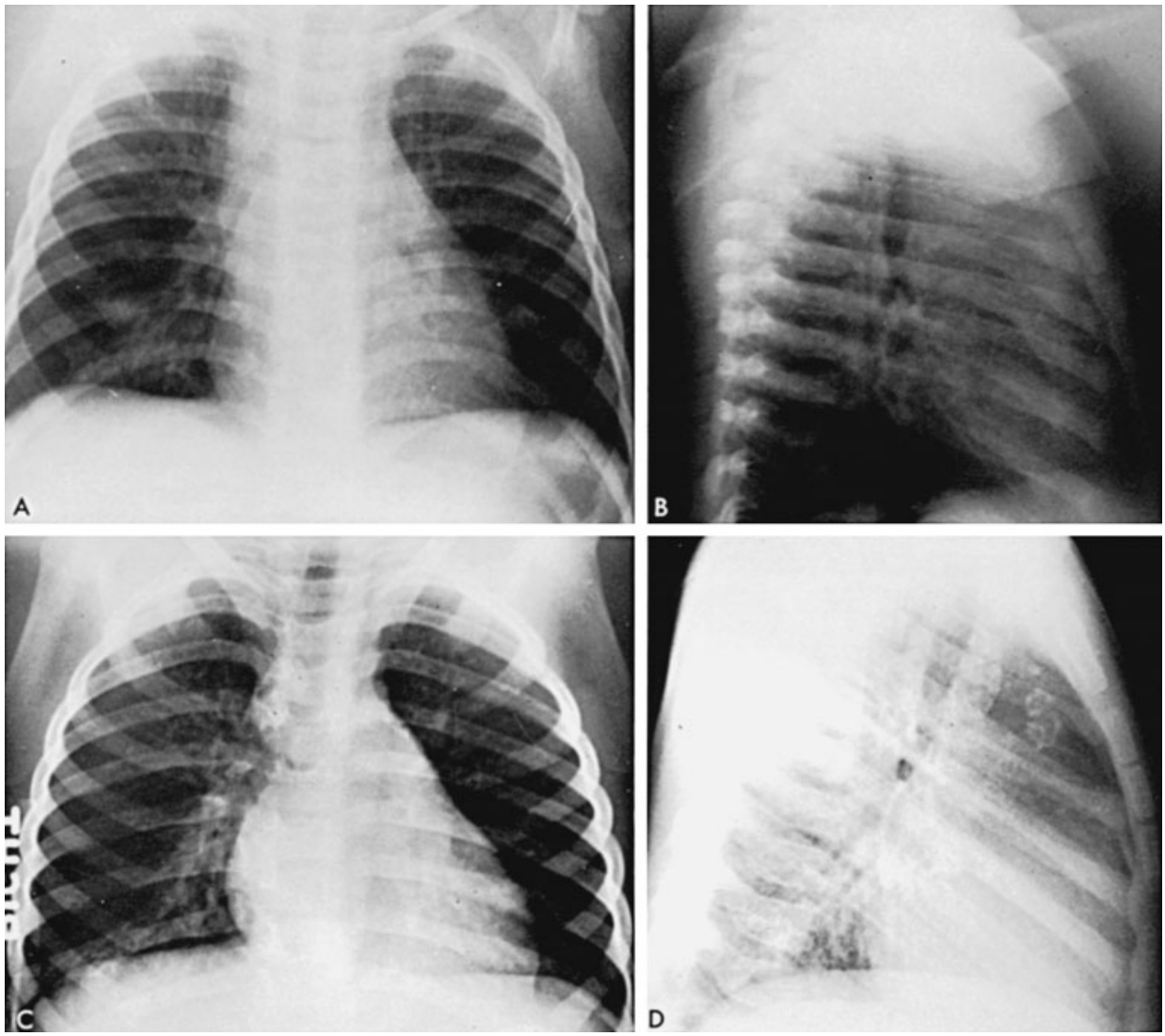

Figure 107–10 **A** and **B,** Radiographs of a 7-month-old girl with mild fullness of the right upper mediastinum and a hazy infiltrate in the right lower lobe. The result of a skin test for tuberculosis was positive. She was treated for tuberculosis and clinically improved. **C,** The same patient 3.5 years later has a calcified primary complex in the right upper and lower lobes over the diaphragm. **D,** The anterior location of the upper lobe complex is seen clearly on the lateral chest film.

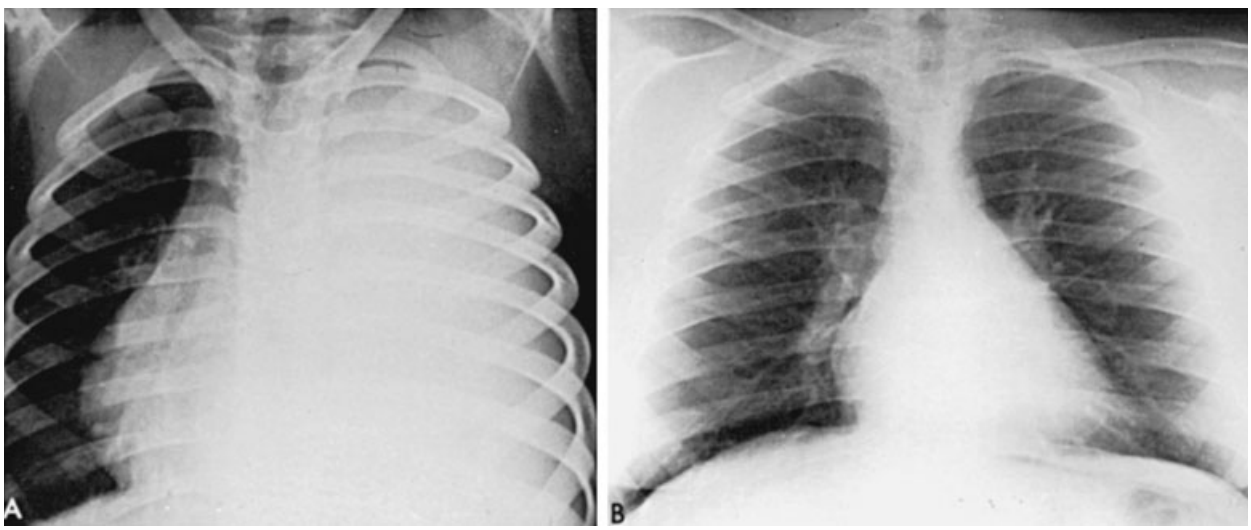

Figure 107–11 Radiographs of a 5-year-old boy with massive left pleural effusion caused by tuberculosis. **A,** Posteroanterior film of the chest. **B,** The same patient 6 years later with a normal chest film and no physical complaint.

liquefies and empties into an adjacent bronchus to create a "primary cavity"[590,619] (Fig. 107–12); the liquefaction is associated with particularly large numbers of tubercle bacilli. The tubercle bacilli further disseminate to other parts of the lobe and to the entire lung, where other foci of infection form. On rare occasion, an enlarging primary focus ruptures into the pleural cavity and creates a pneumothorax, bronchopleural fistula, or caseous pyopneumothorax or into the pericardial sac or the mediastinum.

Whereas clinical symptoms often are minimal when the primary focus is uncomplicated, a progressive lesion often is accompanied by more severe fever, cough, malaise, and weight loss as well as classic signs of cavitation, such as egophony.[202,493] Before chemotherapy was available, the inability to contain the primary focus was associated with a grave outlook; 25 to 65 percent of patients thus affected died. Now, with appropriate treatment, the prognosis is good.

Distinguishing between progressive pulmonary tuberculosis and a simple tuberculous focus with a superimposed acute bacterial pneumonia caused by *Staphylococcus*, *Klebsiella*, or anaerobes may be difficult. Antimicrobial agents effective against these pathogens may be indicated, in addition to appropriate antituberculosis drugs. Sometimes, especially during convalescence from pulmonary parenchymal lesions, bullous lesions appear and persist for several months. They seem to be associated, in children as in adults, either with "tears" in damaged alveolar walls or with the emptying of caseum out of cavities.[567]

Chronic Pulmonary Tuberculosis

Chronic pulmonary tuberculosis, sometimes referred to as adult or reactivation tuberculosis, is the type of disease seen in pulmonary tissue sensitized and immunized by an earlier tuberculosis infection. For many years, ongoing debate centered around whether the lesions of chronic pulmonary tuberculosis, more localized than those of the initial tuberculous lesion and less likely to spread to lymph nodes and the bloodstream, were due to "endogenous reinfection." Evidence that has accumulated during subsequent decades indicates that endogenous reinfection is the

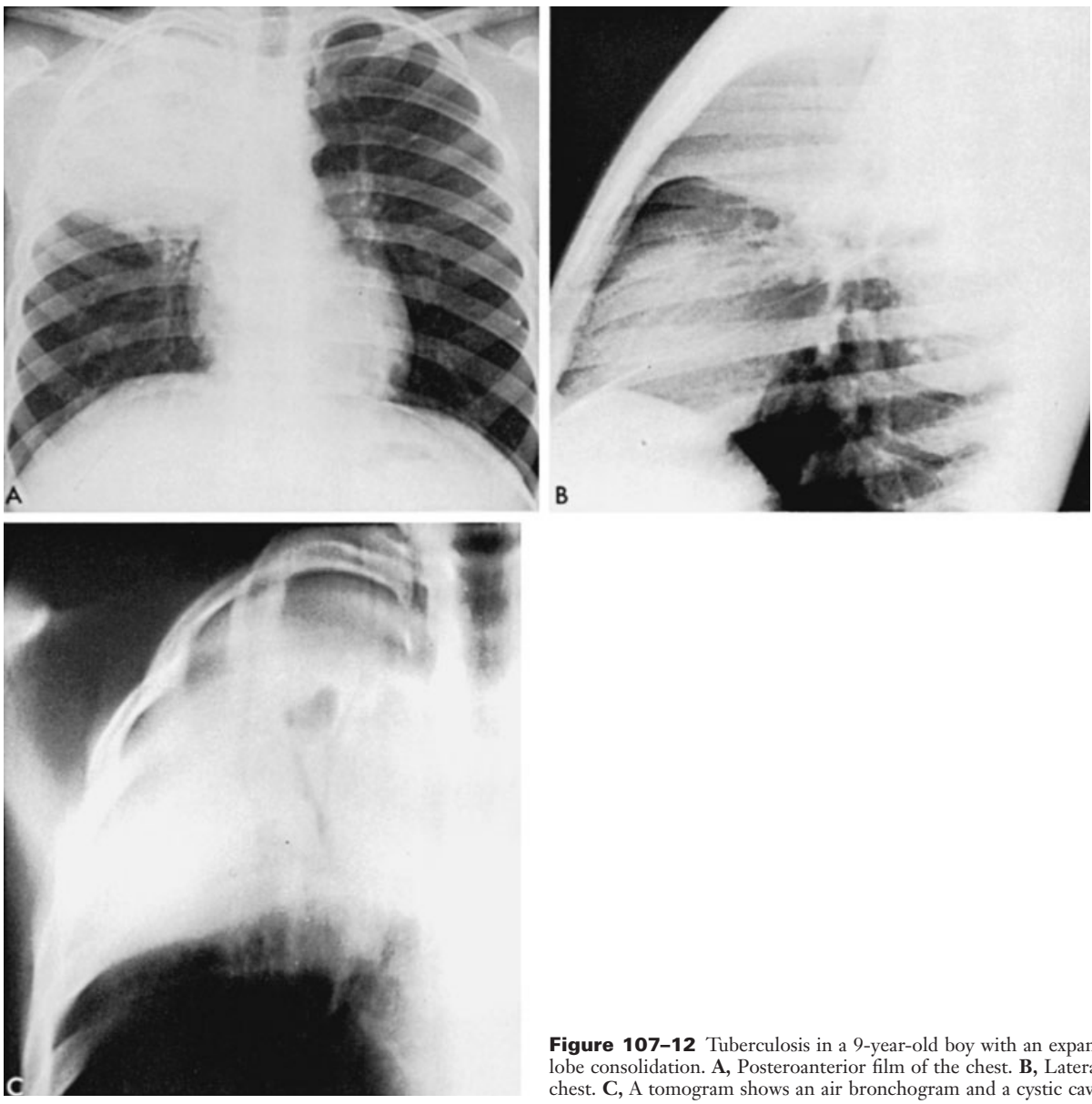

Figure 107–12 Tuberculosis in a 9-year-old boy with an expanding right upper lobe consolidation. **A,** Posteroanterior film of the chest. **B,** Lateral film of the chest. **C,** A tomogram shows an air bronchogram and a cystic cavitary lesion.

usual event.[565] However, reinfection with a different strain of *M. tuberculosis* has been documented and may be more common in areas where tuberculosis is prevalent.[530]

Careful long-term studies of children have revealed a continuum of involvement in many cases: first the primary focus, followed within a few years in some patients by infraclavicular small round foci in the lung apices that often were calcified (Assmann foci, Simon foci) and thought to result from hematogenous spread at the time of initial infection. Later, these foci disappear spontaneously or remain visible as tiny calcifications or as larger "round foci," which may, if untreated, progress to the typical lesions of chronic pulmonary tuberculosis.

Even before antituberculosis drugs were discovered, chronic pulmonary tuberculosis was a rare occurrence in children (6-7% in the series of Lincoln and colleagues[328] of closely monitored patients at Bellevue Hospital). It appears more frequently in children in the lower socioeconomic strata of society and more frequently in girls than in boys. Chronic pulmonary tuberculosis rarely develops in children who survive with a healed, untreated tuberculosis infection acquired before they have reached 2 years of age, but it is much more frequent in children who acquire their initial infection after reaching 7 years of age and particularly if they become infected close to the onset of puberty. In that case, the "adult" type of lesion often develops in the very lobe where the primary lesion occurred. In this situation, progressive pulmonary tuberculosis cannot be differentiated from chronic pulmonary tuberculosis. Now, with effective treatment, such differentiation is unimportant.[211]

Cough, fever of unknown origin, chest pain, hemoptysis, and supraclavicular adenitis are the most common clinical manifestations. Essential diagnostic procedures are the tuberculin skin test and appropriate chest radiographs, often including such special procedures as tomograms and lordotic views. An intense search for tubercle bacilli must be made in sputum, gastric washings, and, if necessary, secretions obtained by bronchoscopy.

Myocardial and Pericardial Tuberculosis

Tubercles often are found in the heart in miliary tuberculosis. Although it is exceedingly rare, myocardial caseation has been described, usually secondary to direct spread from mediastinal glands and accompanied by paroxysmal tachycardia or arrhythmias.[633]

Tuberculous pericarditis, although more common than symptomatic tuberculous myocarditis, occurred in only 0.4 percent of 2500 children monitored by Lincoln and Sewell[331] at Bellevue and in 4 percent of 200 children in Boyd's series.[56] It occurs more commonly in males than in females. In most cases, tuberculous pericarditis probably arises by direct invasion or by lymphatic drainage from caseous lymph nodes in the subcarinal area or from nodes close to the ductus arteriosus, with resulting exudation of hemorrhagic fluid and the development of granulation tissue on both the parietal and visceral surfaces of the pericardium.[477] Pericardial fluid may be serofibrinous or hemorrhagic; tubercle bacilli rarely are found on smears.[242] Sometimes, extensive fibrosis leads to obliteration of the pericardial sac, with the development, usually years later, of constrictive pericarditis.

The initial symptoms generally are nonspecific: low-grade fever, poor appetite, failure to gain weight, and, rarely, chest pain. On examination, a pericardial friction rub may be heard, or if a large effusion already is present, distant heart sounds, tachycardia, and a narrow pulse pressure may suggest the diagnosis. The diagnosis then is confirmed by radiography, echocardiography, electrocardiography, tuberculin skin test, and aspiration of fluid for culture. Before the advent of chemotherapy, approximately half the patients succumbed. Now, with appropriate drugs and possibly corticosteroids to diminish the size of the effusion[578] and also occasional partial pericardiectomy, the outlook is excellent.

Lymphohematogenous Spread

Tubercle bacilli from the lymphadenitis of the primary complex are disseminated during the incubation period in all cases of tuberculosis infection. The results of liver biopsy of young asymptomatic tuberculin converters (indicating recent infection) show that the liver always is involved.[106] Tubercle bacilli can reach deep, distant organs through the bloodstream or lymphatic channels. Autopsies on individuals who have died soon after development of initial infection show that bacilli often are deposited in the liver, spleen, skin, and apical pulmonary tissue.

The clinical picture produced by lymphohematogenous spread probably is determined by host susceptibility at the time of spread and by the quantity of tubercle bacilli released.[516,621] Three clinical forms can be recognized:

1. The lymphohematogenous spread may be occult, in which case it usually remains so, or it may be occult initially with metastatic, extrapulmonary lesions appearing months or years later (e.g., renal tuberculosis).[470]
2. So-called protracted hematogenous tuberculosis, rarely seen today, is characterized by high, spiking fever, marked leukocytosis, hepatomegaly and splenomegaly, and general glandular enlargement, sometimes with repeated evidence of metastatic seeding in the choroid, kidneys, and skin. Calcifications may appear subsequently, often in large numbers, in the pulmonary apices (Simon foci) and in the spleen, thus attesting to the earlier dissemination of tubercle bacilli through blood. The tuberculin skin test result usually is strongly positive. Bone marrow biopsy may confirm the clinical impression, but treatment often must be started on a presumptive basis. Although this type of tuberculosis in past years often ended tragically in tuberculous meningitis, today it is completely treatable if it is diagnosed in time.
3. The third form of lymphohematogenous spread, analogous to sepsis with pyogenic bacteria, is miliary tuberculosis.[244,516] It usually arises from discharge of a caseous focus, often a lymph node, into a blood vessel such as a pulmonary vein; it may be self-propagating, with repeated discharge arising at various sites. Most common during the first 2 to 6 months after infection in infancy, it can arise even in adults who have apparently well-healed, calcified lesions.[290,503]

Miliary disease provides a striking illustration of the difference in susceptibility of tissue to tubercle bacilli; tubercles tend to be larger and more numerous in the lung, spleen, liver, and bone marrow than in the heart, pancreas, and brain. The number of fixed intravascular phagocytes, as well as the relative tortuosity of the smaller blood vessels themselves, must play an important role in determining tissue susceptibility. In acute caseating miliary tuberculosis, the lesions are likely to be numerous and sometimes almost coalescent.

The clinical picture of miliary tuberculosis varies greatly, probably depending on the number of bacilli in the bloodstream. Sometimes, the patient is afebrile and appears to be well, and the condition is diagnosed by chance during contact investigation of another individual with infectious tuberculosis. The onset can be insidious, often occurring after the patient has had another precipitating infection. In rare cases, the onset is abrupt. Drowsiness, loss of weight and appetite, persistent fever, weakness, rapid breathing with a rustling sound on auscultation of the lungs, occasionally cyanosis, and almost always a palpable spleen are the clinical manifestations that lead the clinician to obtain a chest radiograph.

Usually within no more than 3 weeks after the onset of symptoms, tubercles, sometimes tiny and at times large, can be seen evenly distributed throughout both lung fields[427]; in the early stages, they often are detected best on a lateral view of the retrocardiac space (Fig. 107–13). The incidence of choroidal tuber-

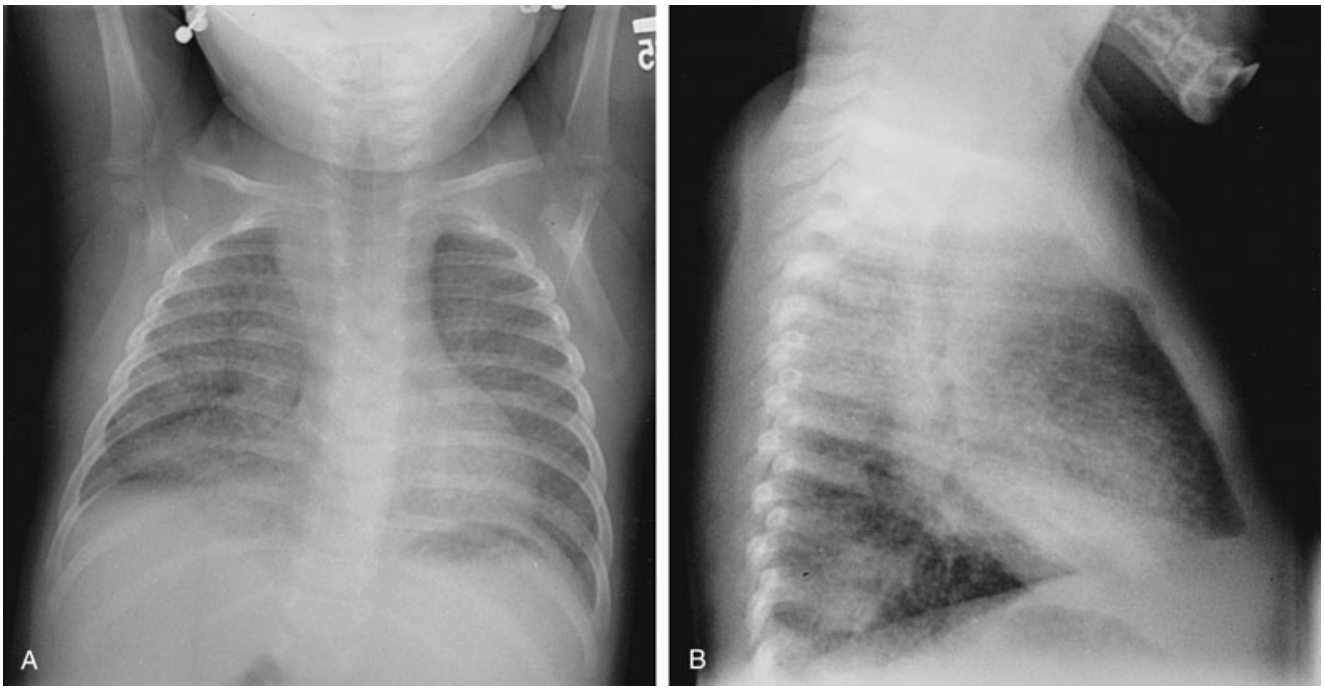

Figure 107–13 Miliary tuberculosis in an infant. The numerous tubercles can be seen on both the posteroanterior (**A**) and lateral (**B**) views.

cles varies greatly; it has been reported variously as 13 and 87 percent. Recurrent pneumothorax, subcutaneous emphysema, pneumomediastinum, and pleural effusion are less serious but well-recognized complications of miliary tuberculosis. Second attacks have been reported.[647] Cutaneous lesions, including painful nodules, papulonecrotic tuberculids, and purpuric lesions, may appear in crops.[286]

The diagnosis usually is established by means of the clinical picture and a chest radiograph. Sometimes it is confirmed by a liver or skin biopsy; by culture of *M. tuberculosis* from the gastric aspirate, urine, or bone marrow[106,214]; or by fiberoptic bronchoscopy and transbronchial biopsy.[67] Treatment usually is successful.

EXTRATHORACIC SPREAD

Central Nervous System Tuberculosis[558]

Tubercle bacilli are distributed by the bloodstream into all parts of the central nervous system (CNS) during lymphohematogenous spread.[330] Surprisingly, they do not multiply as well in nervous tissue as in other areas such as the lung. Thus, CNS tuberculosis, although an early manifestation of infection, usually does not appear simultaneously with miliary spread, but days later. The tubercle bacilli can affect the CNS in various ways[611,628] and produce tuberculous meningitis,[248,581] serous meningitis,[325,611] tuberculoma,[603,611] or tuberculous brain abscess, or it can affect mainly the spinal cord and cause spinal tuberculous leptomeningitis.[420]

Tuberculous meningitis arises from caseous foci, often very small ones, situated in the brain or meninges.[471] These lesions arise from hematogenous spread early in the infection.[143,260] In time, the caseous foci discharge tubercle bacilli directly into the subarachnoid space. The thick, gelatinous exudate lies in the meshes of the pia-arachnoid in the brain, where it infiltrates the walls of meningeal arteries and veins and produces inflammation, caseation, and obstruction; the exudate can extend along small vessels into the cortex, where it causes occlusion and produces infarcts. This same exudate interferes with normal flow of cerebrospinal fluid in and out of the ventricular system and with absorption of the fluid by the pacchionian bodies. The predilection of the exudate for the base of the brain accounts for the frequent involvement of the third, sixth, and seventh nerves and the optic chiasm. The combination of vascular lesions producing infarcts,[321] interference with flow of cerebrospinal fluid resulting in hydrocephalus, and direct cranial nerve involvement, especially of the eye, causes the devastating damage that all too often results from tuberculous meningitis.[20]

Tuberculous meningitis has been estimated to develop in one of every 300 untreated infections.[258] Virtually never seen in infants younger than 4 months, it occurs most frequently in children younger than 6 years, usually appears within 2 to 6 months after initial infection, and accompanies miliary tuberculosis in approximately 50 percent of cases. Tuberculosis should be suspected as the cause of meningitis that is accompanied by cranial nerve involvement, hydrocephalus, or evidence of inflammation at the base of the brain.[616]

The onset of tuberculous meningitis usually is gradual and occurs during a period of approximately 3 weeks; in some cases, it seems to be precipitated by a viral infection, a fall, or a blow on the head. On occasion, the onset is abrupt and marked by a convulsion. A convenient approach is for the clinician to divide the course into three stages. The first stage is characterized by personality change, irritability, anorexia, listlessness, and some fever. After 1 or 2 weeks, the disease passes into the second stage, when signs of increased intracranial pressure and cerebral damage appear: drowsiness, stiff neck, cranial nerve palsies, inequality of the pupils, vomiting, tache cérébrale, absence of the abdominal reflexes, and convulsions that may be tonic or clonic, focal or generalized. The third stage is characterized by coma, irregular pulse and respirations, and rising fever. Papilledema occasionally is noted.

Aids in establishing the diagnosis are a history of contact with an adult who has tuberculosis (however, the family history of tuberculosis often is "negative" because the incubation period of

meningitis is short and the contagious adult has not been discovered yet)[139]; a tuberculin skin test (positive result in only 50% of cases); positive tuberculin skin test results in siblings; a chest radiograph, which often reveals pulmonary disease; and changes in and the characteristic findings from spinal fluid. However, ventricular cerebrospinal fluid may be relatively normal because it is obtained proximal to the site of inflammation. The lumbar spinal fluid usually is clear and under substantially increased pressure. It will contain 50 to 500 white blood cells/mm^3, with polymorphonuclear leukocytes predominant early and lymphocytes predominant later. The spinal fluid glucose level may be at the lower limits of normal if the patient is examined early in the second stage, and it falls by 5 mg or so each day; by the third stage, it usually is very low. The protein content may be normal at the time of the first spinal tap, but it rises steadily to very high concentrations, at which time a pellicle will develop on the fluid on standing. Tubercle bacilli may be found in the pellicle but are scarce at best. Only approximately 50 percent of cases of tuberculous meningitis in children can be confirmed by culture of spinal fluid. Gastric washings should be cultured, not only to confirm the diagnosis in retrospect but also to permit drug susceptibility testing of the organisms.[651]

Computed tomography or magnetic resonance imaging[17,20,203,423] is recommended for the evaluation of all patients with tuberculous meningitis. Both permit recognition and follow-up of tuberculomas,[610,646] infarction or vasculitis, and hydrocephalus that might require shunting.[20,119,265,572] Involvement of the basal ganglia often is a diagnostic clue for the presence of tuberculosis.[17] Profound abnormalities in the electrolyte pattern of intracellular and extracellular fluid and spinal fluid have been reported in patients with tuberculous meningitis. These abnormalities consist mainly of low sodium and chloride levels, with low or normal plasma potassium levels, accompanied by high sodium levels in red cells and skeletal muscle.[212] High levels of antidiuretic hormone (syndrome of "inappropriate" antidiuretic hormone secretion) may cause hypotonic expansion of the extracellular fluid. Because intense vomiting and dehydration usually accompany tuberculous meningitis, the electrolyte disturbances usually include severe hypochloremia. Salt wasting in the urine occurs in rare cases. Determination of the spinal fluid chloride concentration is useless because it merely reflects the plasma level.

A good prognosis depends on immediate initiation of treatment without awaiting epidemiologic and bacteriologic confirmation of the diagnosis.[243,484] Before chemotherapy was used, every case of true tuberculous meningitis was fatal within 3 to 4 weeks, whereas today, appropriate treatment during the first stage allows survival in nearly every case, although the patient's intelligence may never return to its previous level. When it is initiated during the second stage, treatment results in survival in 75 percent or more of patients. If patients are in coma when treatment is begun, they rarely recover unscathed. Very young age and the occurrence of convulsions generally are poor prognostic factors.[484,501] Hydrocephalus, usually of the communicating type, occurs frequently (38% in one series) and is largely responsible for poor outcomes. Relieving the hydrocephalus surgically appears to improve the sensorium, vision, and neurologic deficits. Studies support the early use of ventriculoperitoneal shunting in patients severely ill at the time of admission.[308,435] Infarcts caused by vasculitis also can leave catastrophic residua. The presence of drug-resistant tuberculous meningitis greatly worsens the prognosis,[431] as does co-infection with HIV.[618] The role of corticosteroids in the treatment of intracranial tuberculosis is discussed later in this chapter.

The long-term sequelae of tuberculous meningitis are numerous and include blindness, deafness, intracranial calcification, diabetes insipidus, obesity, paraplegia, and mental retardation.[501]

Serous tuberculous meningitis is an uncommon occurrence (13% of 500 cases in the experience of Udani and associates[611]). Apparently, it develops when a tuberculous focus close to the subarachnoid space causes a lymphocytic reaction in the subarachnoid space without the actual presence of tubercle bacilli.[655] In the days before the advent of chemotherapy, "serous" meningitis was differentiated readily from "true" tuberculous meningitis because the serous type was the only nonfatal form of tuberculous meningitis. Today, such differentiation no longer is possible because treatment is begun immediately, so true tuberculous meningitis does not develop.

Isolated tuberculoma is manifested clinically as a brain tumor.[26,128,140] As many as 30 percent of brain tumors are tuberculomas, depending on the incidence of tuberculosis in the particular population under study (in India, for instance). Tuberculomas occur most often in children younger than 10 years old and often are located at the base of the brain around the cerebellum.[630] In contrast, tuberculomas in adults more often are supratentorial. Headache, convulsions, fever, and other symptoms and signs usually associated with brain abscess or tumor also characterize tuberculoma. Only careful evaluation, including inquiry about exposure to tuberculosis, a tuberculin skin test, chest radiography, and computed tomography, will permit these cases to be recognized in time to begin appropriate chemotherapy before neurosurgical intervention is needed. The widespread use of neuroimaging has revealed that small, multiple tuberculomas are a frequent feature of tuberculous meningitis in infants and young children.[17,20] A phenomenon recognized fairly recently is a symptomatic intracranial tuberculoma appearing and enlarging during treatment of meningeal, miliary, and even pulmonary tuberculosis.[4,319,462,518,595] This phenomenon appears to be mediated immunologically; it usually responds to corticosteroids and does not necessitate a change in antituberculosis chemotherapy. In addition, some small children with severe pulmonary or disseminated tuberculosis have one or several tuberculomas but normal findings on spinal fluid evaluation.[139,261] Computed tomography or magnetic resonance imaging of the head should be performed whenever neurologic signs or symptoms accompany tuberculosis.

Tuberculous brain abscess is a rarely reported form of CNS tuberculosis that tends to occur at an older age than tuberculoma does.[303,641] On pathologic examination, the lesion lacks the giant-cell and granulomatous reaction associated with tuberculoma. Focal neurologic signs occur commonly. Computed tomography and magnetic resonance imaging, if used routinely in cases of tuberculous meningitis, may permit this type of abscess, which requires surgical intervention as well as chemotherapy, to be recognized more frequently. Intramedullary tuberculoma of the spinal cord is exceedingly rare.[266,345] It can be manifested as recurrent abdominal pain.[45,420]

Spinal tuberculous leptomeningitis occurs more often in older children and adults than in infants[340]; in the series of 500 cases studied by Udani and coworkers,[611] only 2 percent fell into this last age group. Protein levels in the spinal fluid are elevated substantially, and sometimes partial or total block may be noted on myelography. Exudate may completely surround the spinal cord.

Cutaneous Tuberculosis

The manifestations of cutaneous tuberculosis can be classified in children according to a modification of the earlier classifications designed for adults[301]: (1) lesions produced by inoculation from an exogenous source[448,517] in a previously uninfected child, in a previously infected child, or after inoculation with BCG vaccine; (2) lesions resulting from hematogenous dissemination[286]; (3) lesions arising from an endogenous source; and

(4) erythema nodosum. A newer classification has been proposed and perhaps is more useful for adults than for children.[44]

Skin lesions associated with the primary complex may be caused by direct inoculation of tubercle bacilli into a traumatized area, such as a lesion on the sole of a child's bare foot, a mosquito bite on the face, an abraded elbow, or the foreskin at the time of ritual circumcision. The initial skin focus usually is a small, painless nodule, sometimes with tiny satellite lesions that soon turn into indolent ulcers without surrounding inflammation. The most striking feature is regional lymphadenitis, which often is what convinces the patient to see a physician. Fever and systemic reactions generally are minimal; low-grade pyogenic infection and cat-scratch fever (*Bartonella* infection) always must be considered in the differential diagnosis. A strongly positive tuberculin skin test result usually is obtained. Needle aspiration and culture may differentiate disease caused by *M. tuberculosis* from that caused by environmental mycobacteria.

Scrofuloderma indicates tuberculosis of the skin overlying a caseous lymph node (most often in the cervical area) that has ruptured to the outside and left either a shallow ulcer or a deep sinus, sometimes surrounded by a cluster of nodules. In the past, scrofuloderma was a frequent manifestation of tuberculosis in children and usually left extensive scars. Today, it is a rare occurrence because the diagnosis generally is made before the node ruptures. Administration of chemotherapy often forestalls the need for surgical excision.

Manifestations that result from hematogenous dissemination are papulonecrotic tuberculids and tuberculosis verrucosa cutis. Papulonecrotic tuberculids[527] are miliary tubercles in the skin that usually appear as tiny papules with "apple-jelly" centers, most often on the trunk, thighs, and face. They frequently are similar to papular urticaria or early varicella lesions, or they may be confused with the skin lesions of Letterer-Siwe disease. Skin biopsy provides a reliable diagnosis. Lupus vulgaris is a rare form of chronic, indolent tuberculosis, usually on the face, that often seems to evolve from tuberculids, very rarely at the site of BCG inoculation.

Tuberculosis verrucosa cutis is a condition characterized by large (several centimeters in diameter) papulonecrotic tuberculids. The lesions usually appear on the arms, legs, or buttocks, suggesting that trauma may play some part in their causation. Fungal infection is the main consideration in the differential diagnosis.

Erythema nodosum formerly was a common manifestation of hypersensitivity to tuberculin. It occurs mostly in young teenage girls. Usually beginning with fever and systemic toxicity manifesting soon after initial infection develops, erythema nodosum is characterized by large, deep, painful, indurated nodules on the shins and sometimes on the thighs, elbows, and forearms. The nodules gradually change from light pink to a bruise-like color. Erythema nodosum is not specific for tuberculosis but also occurs with streptococcal and meningococcal infections, histoplasmosis, coccidioidomycosis, sarcoidosis, drug sensitivity reactions, and perhaps cat-scratch disease. Tuberculin hypersensitivity is pronounced in children with tuberculosis underlying erythema nodosum, and tuberculin skin testing should be performed with caution. These patients may or may not have associated tuberculous lesions, but some clinicians hold that these patients have a greater chance of suffering complications. Skin biopsy reveals only nonspecific changes regardless of cause and, therefore, is useless in establishing the diagnosis. Both fever and nodules clear within 2 or 3 weeks.

Skeletal Tuberculosis

Bone and joint tuberculosis can be expected in 1 to 5 percent of children whose initial infection with tubercle bacilli is untreated. Usually, the tubercle bacilli are disseminated to skeletal structures during lymphohematogenous spread of the initial infection.[31] The disease becomes symptomatic during the first 1 to 3 years after infection occurs. Each of the most frequently involved bones and joints has a characteristic "incubation period" (e.g., 1 month for dactylitis but about 30 months for tuberculosis of the hip). In very young children, blood flow through growing bone is intense; consequently, they suffer from skeletal tuberculosis more often than older children do. The lesion usually starts as an area of endarteritis in the metaphysis of the long bone, where the blood supply is particularly abundant; lesions can be single or multiple.[460,515] Bone infection can be initiated by two other mechanisms, particularly in the vertebrae: (1) direct extension through the lymphatics from a caseous paravertebral lymph node[68] and (2) direct local hematogenous or lymphatic extension from a neighboring bone. As bone is destroyed progressively by pressure necrosis and formation of a cold abscess, a nearby joint may become involved.[461,614,629]

The bones most often affected are the vertebrae.[426] In one study of 1074 patients with the disease, 440 had involvement of the vertebrae; 89, the knee; 81, the hip; and 51, the elbow.[608] The upper extremities and non–weight-bearing bones, such as the skull, clavicle, and mandible, rarely are involved.[213,216] Often a history of trauma is present and may play some part in activation of an underlying lesion or may serve simply to draw attention to the process.

Tuberculous spondylitis, or tuberculosis of the vertebrae, frequently affects the thoracic vertebrae, particularly the 12th one.[225] In one series of 64 cases of spinal tuberculosis in children, the lesions were in the thoracic area in 24 children, in the lumbar area in 19, and in both areas in 13. Cervical involvement is a rare finding.[238] Usually, two vertebrae are involved, but sometimes three, four, or even as many as 11 (usually contiguous, but at times with "skips") are affected. The body of the vertebra is affected far more often than the spinous processes or the arch.

The progression of tuberculous spondylitis, as seen on radiographs, is from slight narrowing of the disk space with only minimal disk involvement and slight collapse and "wedging" of the vertebral body; to marked narrowing of the disk space with collapse, wedging of the bodies, and resulting angulation of the spine (gibbus); to extensive destruction of the bodies with severe kyphosis (Pott disease).

Paravertebral abscess (Pott abscess), retropharyngeal abscess, psoas abscess, and neurologic lesions are serious complications to be expected in 10 to 30 percent of cases of spondylitis. Neurologic complications most often arise from cervical and lumbar vertebral lesions and consist of various degrees of neuroplegia, paraplegia, or even quadriplegia. The complications are caused by inflammation of the spinal cord secondary to a neighboring cold abscess, by caseum or granuloma in the extradural space, or by spinal vessel thrombosis.

The signs and symptoms of spondylitis include "night cries" and restless sleep, a low-grade daily fever, and a peculiar position (such as torticollis with cervical lesions) or gait. Findings on physical examination may include marked "guarding" because of dorsal muscle spasm, pain when the back is "pounded," a deformity (such as gibbus), or reflex changes (including clonus). On occasion, the presence of referred chest pain leads to discovery of a paravertebral abscess on the chest radiograph. With chemotherapy and, when necessary, surgery, the outlook for both eventual clinical healing and neural recovery is in the range of 80 to 90 percent, although patients may not gain their full expected height.

Tuberculosis of the knee can be divided into several clinical types[224]: effusion into the joint without bone erosion and with little restriction of motion; thickening and fibrosis of the synovial membrane without bone erosion but often with considerable restriction of motion; synovial disease and a bone focus but an intact joint space; and synovial disease with diminished range of

motion.[629] Some degree of pain, stiffness, and limping, usually intermittent in milder cases, first calls attention to the problem.

Tuberculosis of the hip, in some series of patients more common than tuberculosis of the knee, should be considered in the differential diagnosis when a child refuses to walk or a limp develops. Today, when the disease still is limited at the time of discovery to the acetabulum or to the head of the femur and no intra-articular disease is present, good mobility of the joint usually can be preserved.

Tuberculous dactylitis (spina ventosa)[208] is the most common form of skeletal tuberculosis in infants. Endarteritis is followed by swelling (often painless), and cystic bone lesions are seen on radiographs. A cold abscess may form and drain spontaneously. The prognosis for recovery without deformity is surprisingly good and was so even before chemotherapy was available.

Tuberculous arthritis is a rare finding in children. Usually monarticular, it involves primarily the weight-bearing joints and only exceptionally the joints of the upper extremities. Bacterial cultures and histologic examination of the synovium establish the diagnosis.[629] Poncet disease is a rare form of rheumatic joint disease associated with tuberculosis; it is an exceedingly rare finding in children.[122] The diagnosis of skeletal tuberculosis should be considered immediately in any child who is known to have been infected with tubercle bacilli and in whom a bone or joint lesion develops and in any child with a persistent, not otherwise explained bone or joint lesion.[625] The differential diagnosis must include low-grade infections caused by *Staphylococcus*, *Haemophilus influenzae*, *Salmonella*, and *Brucella*; fungal infections; rheumatoid arthritis; malignant disease of bone; eosinophilic granuloma, particularly with skull or pelvic lesions; and osteochondrosis resulting from aseptic necrosis of bone, particularly Legg-Calvé-Perthes disease and tuberculosis of the hip.

Children with bone and joint tuberculosis usually react strongly to tuberculin. Although the number of tubercle bacilli in an active bone lesion is much lower than in a lung lesion, the organisms almost invariably can be recovered on culture, and a great effort to do so should be made by means of aspiration or open biopsy.[201]

Treatment of skeletal tuberculosis includes both chemotherapeutic and orthopedic interventions, the chemotherapeutic interventions by far the more important.[457] Orthopedic procedures can be used for several purposes: establishment of the diagnosis, evacuation of caseum and necrotic bone, immobilization of a joint, and reconstruction or strengthening of damaged bone. Since the advent of chemotherapy, any indicated surgical procedure can be performed without fear of subsequent development of sinus formation. However, the trend has been toward reliance on drug therapy with increasingly conservative surgical management. Controlled trials of treatment undertaken in different parts of the world (Southeast Asia) by the Medical Research Council Working Party on Tuberculosis of the Spine and others have demonstrated the remarkable effectiveness of ambulatory treatment with different regimens of chemotherapy in children and adults,[250,371-373] including short-course chemotherapy.[206]

Tuberculosis of the Superficial Lymph Nodes (Scrofula)

Striking enlargement of the superficial regional lymph nodes is an integral component of the primary tuberculous complex. The tonsillar and submandibular nodes are involved most often and probably represent extension from the paratracheal lymph nodes (and not from a primary lesion in the tonsil, as once was thought); occasionally, these nodes are involved when the primary lesion occurs in the mucous membranes of the mouth, a rare event. Enlarged supraclavicular nodes may accompany a primary pulmonary lesion in the upper lung fields. Enlarged axillary and epitrochlear nodes can result from a primary skin lesion of the elbow or hand, often a small, insignificant-looking area that has, however, been present for some time. Preauricular adenitis suggests a focus on the scalp or forehead or in the lacrimal sac, sometimes attributed to an insect bite. With inguinal adenitis, careful examination of the sole of the foot may reveal a small ulcer, often at the base of the toes.

When the superficial lymph nodes are involved early in the course of infection—the normal event—the enlargement usually is painless, and the node or nodes are rubbery and discrete.[360] Low-grade fever generally is present, sometimes unnoticed by the parents. On occasion, acute respiratory infections seem to precipitate or to aggravate superficial tuberculous lymphadenitis, and the patient has high fever, some local pain, and perilymphadenitis. Rarely, the patient is seen first with a fluctuant mass, and the overlying skin is shiny and erythematous.[124,550]

The diagnosis usually is not obvious, so it is wise in all cases of superficial cervical and supraclavicular lymphadenitis to perform a throat culture, radiography of the chest, and a Mantoux tuberculin test. If the node is fluctuant, it should be aspirated and cultured for mycobacteria as well as for pyogenic bacteria.[312] In preauricular, axillary, and inguinal adenitis, a possible primary skin lesion should be sought diligently. If a lesion is found, a biopsy should be performed, in addition to a tuberculin skin test, chest radiography, and a careful history.

Currently, in addition to pyogenic infection, cat-scratch disease, tularemia, malignant tumors, sarcoid, and infection caused by mycobacteria other than *M. tuberculosis* also must be considered as a possible cause.[30,306] If the adenitis is caused by infection with *M. tuberculosis*, the induration from a Mantoux tuberculin skin test usually is greater than 15 mm, whereas mycobacteria other than *M. tuberculosis* produce a less intense reaction. The key to distinguishing tuberculosis from infection by another mycobacterium generally is epidemiologic—has the child been exposed to tuberculosis?

Because infection with pyogenic bacteria often enhances mycobacterial adenitis, frequently a wise approach is to institute therapy with a conventional antibacterial agent while awaiting the results of the skin test, chest radiography, and cultures. If the adenitis persists and evidence of mycobacterial infection is elicited, antituberculosis drug therapy should be initiated and surgical excision seriously considered.[306,504] Surgical excision currently is the treatment of choice for adenitis caused by mycobacteria other than *M. tuberculosis*. In the case of lymphadenitis caused by *M. tuberculosis*, the response to antituberculosis drugs is likely to be good. On the other hand, the adenitis is more likely to extend down into the mediastinum and to be difficult to remove. Under these circumstances, surgical excision probably is unwise, whereas a few weeks of corticosteroid and antituberculosis therapy may be effective.[263,306]

Superficial tuberculous lymphadenitis sometimes occurs early in the course of lymphohematogenous spread, in which case it often is manifested as "general glandular enlargement" accompanied by swinging fever, malaise, and weight loss. The tuberculin test result almost always is positive in immunocompetent patients, and the tuberculin test should be included in the investigation of every patient with general glandular enlargement.

Finally, tuberculous adenitis, either localized or generalized, can occur in adolescents or young adults who were infected months or even years earlier, whose infection has been quiescent, and in whom the appearance of lymphadenitis heralds reactivation of the tuberculosis infection.[305]

Ocular Tuberculosis

Ocular tuberculosis is an uncommon finding in children.[136] When it does occur, the conjunctiva and the cornea are the areas most often involved.

The conjunctiva can serve as the initial portal of entry for tubercle bacilli, especially after trauma. Unilateral lacrimation and reddening may lead to the discovery of yellowish gray nodules, usually on the palpebral conjunctiva. Preauricular adenitis appears early; the submandibular and cervical nodes also may enlarge. A tuberculin skin test and biopsy with culture can be performed to confirm the diagnosis.

Phlyctenular conjunctivitis probably is one of the hypersensitivity phenomena of childhood tuberculosis. Tubercle bacilli have, on rare occasion, been isolated from the small, grayish, jelly-like nodules usually clustered on the limbus and surrounded by dilated conjunctival vessels. Pain and photophobia are intense, and the lesions may recur in crops for weeks and affect one or both eyes. In the differential diagnosis, foreign body, herpes simplex virus conjunctivitis, vernal conjunctivitis, and trachoma should be considered. Tuberculin sensitivity is likely to be pronounced, and 1 tuberculin unit of purified protein derivative (PPD) should be used in the initial tuberculin skin test. Fortunately, hydrocortisone drops are effective in controlling both a strong local reaction to the diagnostic tuberculin test and the discomfort from the underlying disease. The prognosis for complete recovery is excellent, provided the phlyctenules do not ulcerate and leave corneal scars. Systemic chemotherapy should be started immediately after the diagnostic procedures are completed.

Although tuberculosis of the ciliary body and iris has been reported rarely in children, tubercles of the choroid often have been found in patients with miliary tuberculosis (up to 70% of patients in some series) and occasionally in children with a seemingly uncomplicated infection.[394,424] Frequently multiple, the tubercles heal slowly with deposition of retinal pigment; residual scarring apparently can be prevented with steroid therapy. Tuberculous uveitis and tuberculosis manifested as an orbital mass are rare clinical entities.[519]

Tuberculosis of the Middle Ear

Tuberculosis of the middle ear is a relatively rare manifestation of the disease.[168,341,344,398,488] It occurs as a primary focus in the area of the eustachian tube (because of reflux up the tube) in neonates who have aspirated infected amniotic fluid or in older infants who have ingested tuberculous material. It can occur as a metastatic lesion in older children who have a primary focus elsewhere. If it is a primary focus, regional lymphadenitis involves the preauricular lymph node or the anterior cervical chain, and facial paralysis occurs frequently. The primary focus always is unilateral. Otorrhea is a common occurrence and painless but may become foul smelling because of bacterial contamination with enteric organisms. Older patients may complain of tinnitus and "funny noises." The eardrum often is damaged extensively. A large central perforation or several perforations are characteristic findings. A tuberculin skin test, biopsy, and careful cultures for tubercle bacilli are essential. Once greatly feared for the almost inevitable loss of hearing and the frequent occurrence of tuberculous meningitis, tuberculous otitis media now heals well with appropriate chemotherapy.[398]

Gastrointestinal and Abdominal Tuberculosis

Tuberculosis of the mouth occurred more commonly in the days of bovine tuberculosis than it does today; at that time, scrofula often represented a primary complex in the mouth or tonsils, with associated submandibular or cervical lymphadenitis.[380] A primary focus in the mouth generally consists of a painless ulcer or mass of granulation tissue around a tooth socket or in the gingivolabial sulcus, with enlarged submental or submandibular nodes. Primary tuberculosis of the tonsils begins as a painless swelling of one tonsil, sometimes with an ulcer or yellowish node and, of course, enlargement of the regional lymph nodes. If tubercles found on histologic examination of a tonsil removed at tonsillectomy are unaccompanied by lymphadenitis, the lesion is considered a metastatic rather than a primary lesion. Tuberculosis of the esophagus occurs rarely if ever in children; sometimes, dysphagia is produced by a mass of mediastinal nodes, which may rupture into the lumen and later heal, possibly leaving an esophageal diverticulum.

Abdominal tuberculosis may occur after the ingestion of tubercle bacilli or as a part of generalized lymphohematogenous spread, but tuberculous enteritis always has been uncommon.[18,46,480,586,589] When tubercle bacilli penetrate the gut wall, they usually do so through the Peyer patches or the appendix, where they give rise to local ulcers followed by mesenteric lymphadenitis and sometimes peritonitis.[16] On occasion, especially in older children, tuberculous enteritis accompanies extensive pulmonary cavitation. Symptoms and signs include vague abdominal pain, intussusception, blood in the stool, and sinus formation after a seemingly routine appendectomy.

The spleen is seeded during the initial lymphohematogenous spread. Only rarely are the tubercles numerous and large enough to undergo caseation and to calcify.[296] The reticuloendothelial system of the liver is involved also. Symptoms rarely are manifested except in miliary tuberculosis, in which the liver may be enlarged markedly, or in congenital tuberculosis, in which both the liver and spleen usually are enlarged.[347]

Mesenteric lymphadenitis can arise as part of an intra-abdominal primary complex or by extension from tuberculous thoracic or pelvic lymph nodes; often asymptomatic, it may be discovered later when calcified. It can cause ascites and dilatation of the superficial abdominal veins, but the symptom most frequently attributed to mesenteric lymphadenitis is colicky abdominal pain after exercise, probably because adhesions are stretched.

Tuberculous peritonitis can be a result of direct extension from a primary intestinal focus, adjacent mesenteric lymph nodes, or tuberculous salpingitis.[100,137,186] It may be "plastic" or accompanied by a serous effusion. On palpation, a mass of lymph nodes often can be felt, and the abdomen may have a characteristic "doughy" feeling. Paracentesis should be performed with care because the intestine may be immobilized by adhesions. Even with a large effusion, absorption usually occurs within a month. Malignant disease must, of course, be considered strongly in the differential diagnosis, and it may be necessary to obtain a biopsy specimen even if the tuberculin skin test result is positive. Laparoscopy frequently is useful, as is fine-needle aspiration[335] and ultrasonography.[276] The ascitic fluid–blood glucose ratio of the aspirated peritoneal fluid can be helpful in differentiating tuberculous peritonitis from ascites of other causes.[645]

Renal Tuberculosis

Renal tuberculosis is a late and uncommon complication of pulmonary disease; it rarely occurs less than 4 or 5 years after primary infection and is therefore likely to be diagnosed during adolescence.[162,531] However, tubercle bacilli can be recovered from the urine in many cases of miliary tuberculosis and some cases of pulmonary tuberculosis in young children. Hematogenous dissemination can give rise to tubercles in the glomeruli, with resultant caseating and sloughing but tiny lesions, which then discharge tubercle bacilli into the tubules. An encapsulated caseous mass occasionally develops in the zone between the renal pyramid and cortex; it may calcify in situ or discharge into the pelvis of the kidney and form a cavity analogous to a pulmonary cavity. Infection can be unilateral or bilateral and can spread downward to involve the bladder. Frequently, dysuria, hematuria, and "sterile" pyuria are the initial findings in the urine; they may occur grossly but often not until late in the course of a disease that causes strikingly few symptoms. Appropriate examination and culture of

single early-morning urine specimens rarely fail to reveal tubercle bacilli. Intensive chemotherapy renders surgical intervention a rare necessity.[531] Urine from patients with renal tuberculosis is highly infectious, and such children should be isolated until their urine is sterile.

Dialysis- and Renal Transplant–Associated Tuberculosis

Infections are a common cause of morbidity and mortality in patients with end-stage renal disease. Depressed cellular immunity, as manifested by cutaneous anergy, delayed homograft rejection, and depressed lymphocyte count, has been demonstrated in uremic patients, whereas cell-mediated immunity appears to recover in stable patients treated by long-term hemodialysis. Thus, it is not surprising that tuberculosis occurs more frequently in patients maintained on dialysis than in the general population and that when it does become active, it does so before or early in the course of dialysis. Extrapulmonary involvement (mediastinal, meningeal, pleural, osseous, and renal) and miliary tuberculosis appear relatively frequently in this group. In these patients, fever of unknown origin should lead to suspicion of active tuberculosis.[15] Tuberculosis also can originate in the transplanted organ.[400,624]

Genital Tuberculosis

Tuberculosis of the genital tract is an uncommon finding in both sexes before puberty.[531] It usually arises as a metastatic lesion during lymphohematogenous spread and occasionally by direct extension from an adjacent lesion of bone, gut, or the urinary tract. Genital tuberculosis is a particular hazard for adolescent girls with tuberculosis infection. Frequently, other forms of tuberculosis associated with initial infection, such as a pleural effusion, also are present. The fallopian tubes are involved in 90 to 100 percent of cases, the endometrium in 50 percent, the ovaries in 20 to 30 percent, and the cervix in 2 to 4 percent.[498] With tubal involvement, peritoneal tuberculosis occurs frequently.

Lower abdominal pain and amenorrhea usually prompt the patient to seek medical care. A lower abdominal mass, free peritoneal fluid, and constitutional symptoms may or may not be present. Chemotherapy is effective, but infertility remains a potential sequela of the disease.

Tuberculosis of the external genitalia has been seen as a manifestation of child abuse.

Genital tuberculosis can occur in males as primary tuberculosis of the penis after ritual circumcision; many such instances were reported in the past.[228] Massive inguinal lymphadenopathy in a circumcised infant should arouse suspicion of a possible tuberculous etiology. Epididymitis or epididymo-orchitis can occur in early childhood.[196] These disorders are characterized by nodular, painless swelling of the scrotum and a dragging pain in the groin and have a gradual onset rather than the acute onset that results after trauma, torsion of the testis, mumps orchitis, or epididymitis associated with bacterial infection.

Inoculation Tuberculosis

More than 200 cases of syringe-transmitted tuberculosis have been described in the world literature.[223] It has become more common since the widespread use of injectable penicillin and routine childhood immunization and usually has been a result of contamination of the syringe or the solution by an individual with infectious sputum. If the recipient has not been infected previously, the lesion in the muscle or subcutaneous tissue will be the primary focus, and the regional lymph nodes will enlarge, caseate, and, under favorable circumstances, calcify. However, at least 10 infants thus infected have died as a result of generalized tuberculosis, and in others, bone tuberculosis has developed. If the recipient already is tuberculin positive, a tuberculous abscess will form without regional lymphadenitis. In this case, an injection abscess must be differentiated from a deep tuberculous abscess arising in the stage of hematogenous dissemination.

Perinatal Tuberculosis (Congenital and Postnatal)

Transmission of *M. tuberculosis* from mother to infant through the placenta or amniotic fluid has been reported in approximately 300 patients.[73] Infection of the placenta has been demonstrated, and tubercle bacilli have been grown from the tissues of stillborn infants and from living infants within a few days of birth. Perinatal tuberculosis can be acquired by the infant through one of several routes:

1. By transplacental spread through the umbilical vein from a mother with primary hematogenous tuberculosis occurring during pregnancy (i.e., true congenital tuberculosis). The liver is enlarged, enlarged lymph nodes may be present at the porta hepatis, and evidence of widespread miliary disease may be seen in the infant (i.e., the infant may have a primary liver complex or primary lung complexes).
2. By in utero aspiration of amniotic fluid infected from endometritis in the mother or from the placenta. This route of infection also constitutes true congenital tuberculosis. In both these situations, clinical onset of signs and symptoms is likely to be rapid (by 2 to 3 weeks of age) and to include failure to thrive, fever, respiratory distress, and hepatosplenomegaly.
3. By ingestion of infected amniotic fluid or secretions during delivery. This mechanism would seem to be less well documented than the first two routes, but it certainly is a possibility.
4. By inhalation of tubercle bacilli at or soon after birth from the mother, other relatives, or attendants with infectious pulmonary tuberculosis. This route is the most common mode of transmission to newborns.
5. By ingestion of infected breast milk or cow's milk.

One cannot always be sure of the type of infection in a particular neonate; only clear-cut evidence of a primary complex in the liver establishes a definite diagnosis of congenital tuberculosis. However, the presence of early forms of tuberculosis (such as pleural, miliary, or meningeal) in the mother during pregnancy or the puerperium also is strong evidence of true congenital tuberculosis in the infant.

Routes 4 and 5 are really "postnatally" acquired tuberculosis and need, for epidemiologic purposes, to be distinguished from routes 1, 2, and 3. Neonates with these types of tuberculosis usually lack striking clinical features until they are 1 or 2 months of age.

The diagnosis of perinatal tuberculosis is likely to be difficult to establish and often delayed.[318] Disease in the mother often is overlooked; the mother may have pleural effusion, fever of unknown origin, cough, endometritis, and other symptoms without the tuberculous etiology being recognized. The early symptoms and signs in the neonate likewise are overlooked frequently and may be similar to those caused by other congenital infections. Once the diagnosis is suspected, treatment should be started immediately, and diagnostic procedures should be carried out rapidly and aggressively.

The clinical manifestations vary, probably depending on the size of the infecting dose of bacilli and the site and size of the caseous lesions.[368,409] In many of the reported cases, tuberculosis disease was discovered only at autopsy.[241] Symptoms usually appeared during the second week of life and included loss of appetite and failure to gain weight, fever, nasal or ear discharge, cough, bronchopneumonia, jaundice, hepatomegaly, and spleno-

megaly occurring later.[73] Wasting has been noted frequently and, in one case, clearly was shown to be caused by hypoadrenocorticism.[392]

Because the result of the tuberculin skin test very rarely is positive in infants, demonstration of tubercle bacilli in gastric washings, middle ear fluid, lymph node biopsy specimens, skin biopsy specimens, bone marrow aspirate, endotracheal aspirate, or lung biopsy specimens is essential.[205] Examination of the placenta for organisms or characteristic histopathologic changes can be extremely helpful and should be done when tuberculosis is diagnosed in the mother around the time of delivery. Successful treatment of congenital tuberculosis has been reported by several investigators, although clinical response may be very slow, and extensive calcification of the lungs, liver, spleen, and muscles may result.[281,284,409,538,552]

Tuberculosis in Adolescents

Tuberculosis in adolescents has become relatively more important as the incidence of infection in childhood has lessened.[134,357,408,536] Logically, it should be considered in two ways: first, tuberculosis acquired as an initial infection during the adolescent years and, second, tuberculosis infection acquired in early life and reactivated during adolescence.[353,411] In actual practice, separating the two often is difficult or impossible, and most clinical reports and studies do not.

Tuberculosis in adolescents may occur exactly as it does in young children, or a classic primary complex may progress rapidly to chronic pulmonary tuberculosis while the hilar lymph node involvement characteristic of childhood tuberculosis remains present. The Medical Research Council in 1963 published a report of 504 cases of tuberculosis in adolescents, including 316 cases of pulmonary tuberculosis, 44 cases of pleural effusion, 44 with hilar lymph node enlargement, 6 cases of miliary and 5 of meningeal tuberculosis, 13 with bone and joint disease, and 8 with genitourinary tuberculosis. In a series of cases of primary tuberculosis in adults, Stead[565] included 11 adolescents, 5 with simple primary tuberculosis, 5 with pleurisy and effusion, and 1 with progressive pulmonary tuberculosis.

From the extensive clinical experience of Lincoln and Sewell[331] and others, several observations emerge. Tuberculosis infection in early infancy rarely leads to pulmonary tuberculosis in adolescence, perhaps because it has several years in which to heal, whereas tuberculosis infection acquired after 7 years of age and, in particular, acquired after 10 years of age is likely to progress. When *M. tuberculosis* is acquired during adolescence, chronic pulmonary tuberculosis may develop within 1 to 3 years. Moreover, the risk of acquiring pulmonary tuberculosis is two to six times greater for adolescent girls than for adolescent boys. In both sexes, the adolescent growth spurt is the time of greatest risk. The work of Johnston[268] suggests that the depressant effect of puberty on calcium and nitrogen retention, at least in girls, may be correlated with the failure of tuberculosis infection to heal.

Tuberculosis and Pregnancy

In the era before chemotherapy, whether pregnancy and tuberculosis affected each other adversely was an ongoing controversy. Since the advent of chemotherapy, however, the prognosis has improved greatly.[177] The main problems now are serious unrecognized tuberculosis in a pregnant woman, sometimes with a fatal outcome, and serious unrecognized disease in her infant. Another problem is whether pregnancy influences the risk of progression of tuberculosis infection to disease; the data are conflicting in this regard. Tuberculin skin testing probably is valid during pregnancy; chest radiographs (with shielding) should be obtained for all tuberculin-positive pregnant patients. Therapy, when indicated, can safely include isoniazid (which crosses the placenta but apparently without ill effects), rifampin, and ethambutol. The safety of pyrazinamide in pregnancy has not been established, but a growing number of experts recommend it because anecdotal data have not shown it to be harmful to the mother or fetus. Streptomycin, because of fetal ototoxicity, is not recommended.[544] However, one study of seven pregnant women with multidrug-resistant tuberculosis showed no adverse effects on their newborns when mothers were treated with second-line drugs, including aminoglycosides.[150,521] Should a mother receiving antituberculosis therapy breast-feed?[545] It is probably safe for her to do so because the drugs, although found in milk, are present only in small amounts.

Tuberculosis and Human Immunodeficiency Virus Infection

In adults infected with both HIV and *M. tuberculosis*, the rate of progression from asymptomatic infection to disease is increased greatly.[491] The clinical manifestations of tuberculosis in HIV-infected adults are typical when the $CD4^+$ cell count is higher than 500/mm^3. As the $CD4^+$ cell count falls, manifestations become "atypical." Extrapulmonary foci occur in as many as 60 percent of profoundly immunocompromised patients.[27] Pulmonary cavities are rare findings; lower lobe infiltrates or nodules often accompanied by thoracic adenopathy are common occurrences, especially if the patient's tuberculosis infection is recent. Of course, many patients have a nonreactive tuberculin skin test. Sputum is less likely to be produced or to contain visible acid-fast organisms on stained smears; more invasive procedures, such as bronchoscopy, often are required to isolate *M. tuberculosis* and to rule out other causes of opportunistic lung disease.[361] Malabsorption of antituberculosis drugs in HIV-infected patients can lead to prolonged symptoms and disease.[446]

When tuberculosis develops in HIV-infected children, the clinical features tend to be fairly typical of childhood tuberculosis in immunocompetent children, although the disease often progresses more rapidly and the clinical manifestations are more severe.[53,97,105,164,289,395,434,598] Many children have sizeable pulmonary radiographic findings with minimal respiratory signs or symptoms. An increased tendency for extrapulmonary disease may be noted. Recurrent disease occurs more frequently in HIV-infected children.[497] Establishing the diagnosis can be very difficult; a diligent search for an infectious adult in the child's environment may yield the best clues to the correct diagnosis.[293] The prognosis generally is good if tuberculosis disease is not far advanced when the patient is diagnosed and appropriate antituberculosis drugs are available.[218]

The introduction of highly active antiretroviral therapy (HAART) into regions where tuberculosis is prevalent has had a profound effect on the epidemiology and clinical expression of both diseases. During the initial months of HAART, immune reconstitution can be complicated by adverse clinical phenomena in which either previously subclinical infections are "unmasked" or preexisting, partly treated opportunistic infections clinically deteriorate. These phenomena are referred to as the immune reconstitution inflammatory syndrome (IRIS).[555] Sometimes it is difficult to tell if a deterioration in the clinical course of a patient with an opportunistic infection who is started on HAART is due to IRIS, poor compliance with treatment for the opportunistic infection, or drug resistance.[314-316] Factors to be considered that suggest IRIS are temporal association (within 3 months of starting HAART), unusual clinical manifestations, unexpected clinical course, exclusion of alternative explanations, evidence of preceding immune restoration (rise in CD4 lymphocyte count), and fall in HIV viral load. In adults, the risk of developing IRIS in patients with tuberculosis who are started on HAART is greatest if they have extrapulmonary tuberculosis, high initial HIV viral loads

and low CD4 lymphocyte counts, and rapid response to HAART.[54] Published reports of IRIS in children have been rare,[274,403,456,649] although anecdotal evidence from pediatric acquired immune deficiency clinics in Africa suggest it is becoming common as HAART is introduced to more children with HIV infection. IRIS in children has been associated with nontuberculous mycobacteria[456] and previous BCG vaccination.[299,455,523] The finding that IRIS occurs in children with tuberculosis and HIV infection who receive HAART is not surprising. For decades, so-called paradoxical reactions have been described in HIV-uninfected children with tuberculosis after antituberculosis therapy is started. The most common manifestations are fever, enlarging lymph nodes in the thorax or neck, and appearance or enlargement of tuberculomas in the brain, with or without accompanying meningitis.

The best management of IRIS is unknown. As with paradoxical reactions that cause tissue damage or obstruction, corticosteroids may be beneficial to "quiet" the local immune response, although no randomized clinical trials have been reported. The high frequency of IRIS associated with tuberculosis has led some experts to recommend delay of HAART until appropriate (for drug susceptibility pattern) antituberculosis chemotherapy has been given for at least 2 months.[324] No consensus on this approach has been reached, however, and data from children may indicate that they are more likely to die of complications of HIV than of tuberculosis when they are dually infected.[218]

DIAGNOSIS

HOW CHILDREN WITH TUBERCULOSIS ARE DISCOVERED

In the developing world, the only way that children with tuberculosis disease are discovered is passively, when they have a profound illness that is consistent with one manifestation of tuberculosis. Having an ill adult contact is an obvious clue to establishment of the correct diagnosis. The only available laboratory test usually is an acid-fast smear of sputum, which the child rarely produces. In many regions, chest radiography is not available. To aid in establishing the diagnosis, a variety of scoring systems based on available tests, clinical signs and symptoms, and known exposures have been devised.[379,638] However, no single clinical scoring system has been validated, and the sensitivity and specificity of these systems can be very low and lead to both overdiagnosis and underdiagnosis of tuberculosis.[288,379]

In industrial countries, children with tuberculosis usually are discovered in one of two ways.[148,291,492] Obviously, one way is to consider tuberculosis as the cause of a symptomatic illness.[485] Discovering an adult contact with infectious tuberculosis is an invaluable aid to establishing the diagnosis; the "yield" from a contact investigation usually is higher than that from cultures from the child.[56] Culture from the infectious adult case may yield the only drug susceptibility results for the child because cultures from children with tuberculosis frequently are negative. The second way is to discover a child with pulmonary tuberculosis during the contact investigation of an adult with tuberculosis.[362,388,547,576,579] Typically, the affected child has few or no symptoms, but investigation reveals a positive tuberculin skin test result and an abnormal chest radiograph. In some areas of the United States, as many as 50 percent of children with pulmonary tuberculosis are discovered in this manner, before significant symptoms have begun. The recent use of molecular epidemiology techniques has shown, however, occasional discordance between the isolate from the child and that from the presumed source, meaning that the real source of transmission to the child is undiscovered.[642,648] It is less common to find tuberculosis disease in a U.S.-born child as the result of a community-or school-based tuberculin skin testing program, although foreign-born children with tuberculosis may be found in this manner.[149,444]

TUBERCULIN SENSITIVITY AND THE SKIN TEST

Sensitization to tuberculin is induced by infection with living tubercle bacilli. Specific tuberculin sensitivity to either *M. tuberculosis* or other mycobacteria can be transferred in humans by injection of lymphocytes from sensitized donors and also by injection of certain purified mycobacterial protein antigens.

The time of appearance of sensitivity in animals after infection has occurred with tubercle bacilli depends on the number of tubercle bacilli in the infective dose and on the virulence (i.e., the rate of multiplication of the organisms), and such is also likely to be the case in humans.[28] For all practical purposes in humans, tuberculin reactivity seems to appear in 3 to 6 weeks, rarely a few days earlier, and occasionally as long as 3 months after initial infection.

The tuberculin sensitivity reaction has been studied best in the skin, although it can be elicited in any tissue of a sensitive subject (conjunctiva, lung, meninges, kidney, and so on) after tuberculoprotein has been injected. At first, an inflammatory reaction appears at the site of injection, with predominance of segmented neutrophils, followed by immigration of macrophages and T lymphocytes, until the entire area of induration consists of mononuclear cells.

The size of the induration depends on the amount of tuberculoprotein injected and the availability of sensitized T lymphocytes in sufficient number. Multiple tuberculin skin tests given simultaneously result in smaller individual reactions, probably because of a finite number of sensitized cells in the body. Corticosteroids,[55,346,499] adrenocorticotropic hormone, nitrogen mustard, irradiation, and viral infections such as measles, influenza, and mumps diminish tuberculin reactivity, perhaps simply by inducing lymphopenia.[561] The size of the induration seems to depend on at least two additional factors: the local behavior of the skin (the disappearance time for wheals of normal saline solution, the so-called Aldrich-McClure test, is accelerated during fever, pregnancy, cachexia, and extreme malnutrition) and the number of actively multiplying tubercle bacilli in the body, which can be demonstrated in animals and probably in humans as well. That still other factors must be involved is clear because 10 to 20 percent of immunocompetent patients with proven tuberculosis are tuberculin-negative during initial disease, with tuberculin sensitivity often regained during treatment.[450] Decreased T-lymphocyte blastogenesis has been demonstrated in some cases, and inhibition by B lymphocytes may play a role.

Temporary desensitization to tuberculin occurs most strikingly during measles and has been studied carefully. Full reactivity diminished during the incubation period and returned within approximately 1 month after appearance of the exanthem.[215] Influenza and administration of influenza and measles vaccines tend to depress sensitivity but rarely suppress it entirely.[64,657] Whether other viral diseases and vaccines regularly are less active in this regard and what the important factors may be are not known.[39] If a temporary desensitizing effect occurs in bacterial infections such as scarlet fever, it probably depends on factors such as hyperthermia and dehydration.

Sensitization to tuberculin as a result of infection with *M. tuberculosis* tends to persist undiminished for life.[161,207,235] The likelihood of disappearance of tuberculin sensitivity seems to be greater when the lesion is negligible. That low degrees of sensitivity to *M. tuberculosis* are induced by mycobacteria other than *M. tuberculosis* also is clear. Previous receipt of BCG vaccine can cause increased reactivity to tuberculin, but the association is weaker than many clinicians suspect.[9,78,339,396,510] Less than 50 percent of infants given BCG vaccination have a reactive tuberculin skin test at 9 to 12 months of age, and the great majority will have a nonreactive skin test by the time they are 5 years of age. Older children and adults who receive BCG vaccination have a reactive skin test and keep it longer, but by 10 to 15 years after

vaccination, most individuals have lost tuberculin skin test reactivity.[114,378] Repeated administration of BCG vaccine can maintain tuberculin reactivity.[249] Repeated tuberculin skin tests in a person sensitized previously by BCG vaccination, infection with *M. tuberculosis*, or probably infection by an environmental mycobacterium may increase the reaction to subsequent tuberculin skin tests (called the booster phenomenon).[41,182,309,599]

The antigens currently used in tuberculin testing are crude extracts consisting of a mixture of many antigens, some species-specific, some shared among many species. Standardized, isolated, purified mycobacterial antigens are needed in clinical practice. The major antigen used in tuberculin testing is PPD, obtained from filtrates of heat-killed tubercle bacilli. Batch 49608, prepared by Dr. Florence Seibert in 1939, was designated by the WHO as the international standard tuberculin, the only one to be called PPD-S. All other PPD preparations are referred to simply as PPD or as PPD-T. However, each batch now must be stabilized with Tween 80 at 5 ppm to minimize adherence to glass and plastic and must be identified by manufacturer and lot number.

A tuberculin unit is the activity contained in a specified weight of PPD-S. The standard test dose, 5 tuberculin units, refers to the equivalence in biologic activity, determined in the guinea pig, of a commercial PPD preparation with that contained in 5 tuberculin units of PPD-S. Products labeled 1 tuberculin unit and 250 tuberculin units are calculated dilutions based on 5 tuberculin units. However, the potency of PPD doses varies from batch to batch.

The Mantoux test is the reference test. A graduated syringe and a 26- or 27-gauge needle are used to inject 0.1 mL of PPD into the most superficial layer of the epidermis of the forearm, which raises an immediate wheal (Fig. 107–14). Under optimal circumstances, the needle should not be withdrawn for a few seconds to minimize leakage. The reference test uses a dose of 5 tuberculin units. One tuberculin unit very rarely should be used, for example, if extreme hypersensitivity is suspected, as when a tuberculous eye lesion or erythema nodosum is present. The reading should be made at 48 to 72 hours, with the forearm slightly flexed. Any induration (not erythema) should be measured, preferably with calipers, and the diameter at right angles to the axis of administration recorded in millimeters. Use of the words "negative" and "positive" should be avoided because interpretation can change as more epidemiologic information becomes known. The previous widespread use of multiple-puncture tests led to the practice of allowing parents to interpret the results and to report them to the clinician. This practice assumes parental knowledge of and adherence to a broad range of motivational behavior and skills. No study has demonstrated that parents can read positive skin test results accurately, but that they may not correctly interpret or report positive results has been well documented.[101,102,199,233] Two studies have demonstrated that pediatric care providers tend to under-read Mantoux tuberculin skin tests.[77,285]

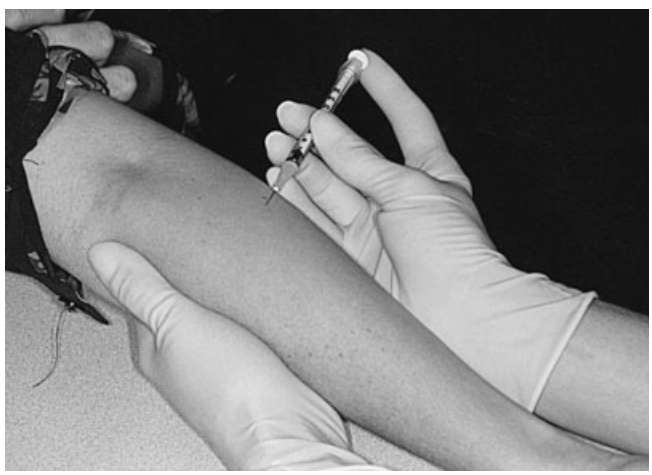

Figure 107–14 A useful technique for performing a Mantoux tuberculin skin test on a child. The needle is applied perpendicular to the long axis of the arm to attain better control.

Several studies have shown great variability in readings of Mantoux tests.[33] Such variability can be minimized only by ongoing training of both testers and readers and by concentrating the responsibility of testing and reading to a small number of trained individuals.

The importance of the tuberculin test cannot be overemphasized as the main criterion for diagnosis of tuberculosis infection in an individual child. Only rarely is the tuberculin test response negative in an infected child, usually as a result of anergy from overwhelming infection, from viral infection, from HIV infection, from the use of immunosuppressive drugs, or because of factors not yet understood.[571] Anergy in tuberculosis can be selective for tuberculin; results of "control" skin tests may be positive but the Mantoux test result negative in a child with tuberculosis disease.[563]

The Mantoux test has a sensitivity and specificity of only approximately 95 percent.[239] When a test with these characteristics is applied to a population with a 90 percent prevalence of tuberculosis infection, the positive predictive value of the skin test is 99 percent, an excellent result. However, if the same test is applied to a population with only a 1 percent prevalence of infection, the positive predictive value drops to 15 percent; 85 percent of the "positive" results are false-positives created by biologic variability, nonspecific reactions, and infection with environmental mycobacteria. These false-positive results lead to unnecessary treatment, cost, and anxiety for the patient, family, and clinician. In short, the low sensitivity and specificity of the tuberculin skin test render the test undesirable for use in persons from low-prevalence groups. The trend in the United States is to reduce or to eliminate routine testing of low-risk children but to target children with specific risk factors for one-time or periodic tuberculosis skin testing.[10,90,389]

Several recently published studies have shown that a questionnaire can be used in the United States to identify children with significant risk factors for tuberculosis infection, who then can receive a tuberculin skin test.[183,337,430,483] Factors that consistently correlate with having a positive tuberculin skin test result include contact with a case of tuberculosis, other family members with a positive tuberculin skin test result, and foreign birth in or extensive travel to a high-prevalence country.[502]

The CDC and the American Academy of Pediatrics (AAP) have recommended varying the size of induration considered positive in various groups, according to their risk factors (Table 107–7), in an attempt to minimize the incidence of false-negative results in children most likely to have rapid progression of asymptomatic tuberculosis infection to disease and the false-positive results in persons with no known risk factors for tuberculosis. In general in the United States, previous receipt of BCG vaccine should not influence interpretation of the initial tuberculin skin test of a child.[267]

INTERFERON RELEASE ASSAYS

Advances in molecular biology and genomics have led to use of alternatives to the tuberculin skin test.[432,472,554] Two new in vitro formats have been developed that measure interferon-γ release by sensitized lymphocytes after stimulation by *M. tuberculosis*

TABLE 107–7 Amount of Induration in Reaction to a Mantoux Tuberculin Skin Test Considered Positive (Indicating Probable Infection with *Mycobacterium tuberculosis*)

Reaction Size	Risk Factors
≥5 mm	Contact with infectious cases
	Abnormal chest radiograph
	HIV infection or other immunocompromise
≥10 mm	Birth or previous residence in a high-prevalence country
	Residence in long-term care or corrections facility
	Certain medical risk factors: diabetes mellitus, silicosis, renal disease
	Occupation in health care field, exposure to patients with tuberculosis
	Member of a local high-risk group
	Close contact with a high-risk adult (except health care workers)
	Age <4 years
≥15 mm	No risk factors

antigens. Both formats use two proteins—early secreted antigen target 6 and culture filtrate protein—that are found on *M. tuberculosis* and only a few fairly rare species of nontuberculous mycobacteria but not on the bacilli BCG or the *Mycobacterium avium* complex. The first test licensed in the United States was QuantiFERON-TB (Cellestis, Carnegie, Australia). The test measures whole-blood interferon production.

The second format licensed in the United States is the enzyme-linked immunospot (ELISPOT, T-SPOT.TB; Oxford Immunotec, Oxford, UK). This technique measures the number of mononuclear cells that produce interferon.

Both formats of interferon release assays have been studied primarily in adults.[432,472] In many clinical situations, these tests have a higher specificity than that of the tuberculin skin test, better correlation with surrogate measures of recent exposure to *M. tuberculosis* in low-incidence settings, and less cross-reactivity than does the tuberculin skin test because of previous BCG vaccination. Like the tuberculin skin test, interferon release assays cannot differentiate between tuberculosis infection and disease. Two clear advantages of the interferon release assays are the need for only one patient encounter (versus two with the tuberculin skin test) and the lack of possible boosting of the result, as the patient is not exposed to any biologic material.

Studies of interferon release assays in children are fewer for both ELISPOT[221,222,548] and QuantiFERON-TB.[22,117,173,606] Studies of child household contacts of adults with pulmonary tuberculosis have shown that children who previously received a BCG vaccination were more likely to have a positive tuberculin skin test result but a negative ELISPOT result.[173] A study from the Gambia showed an 83 percent agreement between tuberculin skin test and ELISPOT results in child household contacts, with a nonsignificant trend toward the ELISPOT being less sensitive than the tuberculin skin test.[25] In this study, previous BCG vaccination did not significantly affect the results of either test.

Although interferon release assays are becoming widely used[433] and are recommended by the CDC,[83] studies in adults have questioned their sensitivity even in patients with tuberculosis disease. At present, although the interferon release assays show great promise for improving the diagnosis of tuberculosis infection, too little is known about their characteristics in young children and immunocompromised hosts for their routine use to be recommended in these groups.

DIAGNOSTIC MYCOBACTERIOLOGY IN CHILDREN[487]

The demonstration of acid-fast bacilli in stained smears of sputum is presumptive evidence of pulmonary tuberculosis in most patients. However, in children, tubercle bacilli usually are relatively few in number. Gastric washings, which often are used in lieu of sputum, can be contaminated with acid-fast organisms from the mouth. However, fluorescence microscopy of gastric washings has been found to be useful, particularly in a setting where malnutrition and tuberculin-negative tuberculosis are rampant.[313] Tubercle bacilli in cerebrospinal fluid, pleural fluid, lymph node aspirate, and urine are sparse; thus, only rarely are direct-stained smears for tubercle bacilli of any use in pediatric practice.[573] Cultures for tubercle bacilli are of great importance, not only to confirm the diagnosis but increasingly to permit testing for drug susceptibility. If culture and drug susceptibility data are available from the associated adult case and the child has classic features of tuberculosis (positive skin test result, consistent abnormal chest radiograph), obtaining cultures from the child adds little to management.

Painstaking collection of specimens is essential for diagnosis of children because fewer organisms usually are present than in adults.[522] Gastric lavage should be performed in the very early morning, when the patient has had nothing to eat or drink for 8 hours and before the patient has a chance to wake up and start swallowing saliva, which could dilute the bronchial secretions that were brought up during the night and made their way into the stomach. The stomach contents should be aspirated first. Then, no more than 50 to 75 mL of sterile distilled water (not saline) should be injected through the stomach tube and the aspirate added to the first collection. The gastric acidity (poorly tolerated by tubercle bacilli) should be neutralized immediately. Concentration and culture should be performed as soon as possible after collection. However, even with optimal, in-hospital collection of three early-morning gastric aspirate samples, *M. tuberculosis* can be isolated from only 30 to 40 percent of children and 70 percent of infants with pulmonary tuberculosis.[69,563,613]

Bronchial secretions (sputum) produced by stimulation of a cough with an aerosol solution can be obtained commonly from older children.[76] The aerosol is heated in a nebulizer at 46° C to 52° C (114.8° F to 125.6° F) and administered to the patient for 15 to 30 minutes. This method gives good results and may be superior to gastric lavage both in yield of positive cultures and in acceptance by the patient.[76,517] Recent improvements in technique have led to improved induction of sputum in even young children, with culture yields as high as from inpatient gastric aspirates.[252,650] Nasopharyngeal aspiration also has been used.[429] The bronchial aspirate obtained at bronchoscopy often is thick, and the laboratory will process it with a mucolytic agent such as *N*-acetyl-L-cysteine. In most studies, the yield of *M. tuberculosis* from bronchoscopy specimens has been lower than from properly obtained gastric aspirates.[1,96]

Cerebrospinal fluid, pleural fluid, and synovial fluid (as much fluid as possible should be collected) usually are centrifuged, and the sediment is used for stained smear and culture. An overnight urine specimen should be obtained in the early morning and taken immediately to the laboratory for processing because the organisms tolerate the low pH of urine poorly. Lymph node aspirates and bits of biopsy tissue can be inoculated directly into a fluid medium such as Middlebrook 7H9.

Staining and examination of smears as well as inoculation of special media, incubation in a carbon dioxide environment, strain differentiation based on many cultural characteristics, and drug susceptibility testing require equipment, skills, and experience beyond those available in the usual clinic or hospital laboratory.[217] Thus, most laboratories depend on regional or reference laboratories for procedures beyond their scope.

Despite an enormous amount of research and thousands of publications on the subject, the only definite way to diagnose active tuberculosis is to demonstrate tubercle bacilli in tissues or secretions. No single species–specific antigen of *M. tuberculosis* ever has been identified. The search for quick, simple, inexpensive, specific, sensitive immunologic and chemical detection techniques is ongoing.

NUCLEIC ACID AMPLIFICATION

The main form of nucleic acid amplification studied in children with tuberculosis is polymerase chain reaction (PCR), which uses specific DNA sequences as markers for microorganisms.[163,352,390] Various PCR techniques, most using the mycobacterial insertion element IS6110 as the DNA marker for *M. tuberculosis* complex organisms,[11,81] have a sensitivity and specificity of more than 90 percent in comparison to sputum culture for detecting pulmonary tuberculosis in adults. However, test performance varies even among reference laboratories.[417,501] The test is relatively expensive, calls for fairly sophisticated equipment, and requires scrupulous technique to avoid cross-contamination of specimens. In the United States, it is approved for use only on acid-fast stain–positive specimens.

Evaluation of PCR in childhood tuberculosis has been limited. Compared with a clinical diagnosis of pulmonary tuberculosis in children, the sensitivity of PCR has varied from 25 to 83 percent, and specificity has varied from 80 to 100 percent.[133,390,440,451,533] PCR of gastric aspirates may be positive in a recently infected child even when the chest radiograph is normal, thus demonstrating the occasional arbitrariness of the distinction between tuberculosis infection and disease in children. PCR may have a useful but limited role in evaluating children for tuberculosis. A negative PCR analysis never eliminates tuberculosis as a diagnostic possibility, and a positive result does not confirm it. The major use of PCR will be in evaluating children with significant pulmonary disease when the diagnosis is not established readily by clinical or epidemiologic grounds. PCR may be particularly helpful in evaluating immunocompromised children with pulmonary disease, especially those with HIV infection, although published reports of its performance in such children are lacking. PCR also may aid in confirming the diagnosis of extrapulmonary tuberculosis, but only a few reports have been published.[194,351,383]

SEROLOGY AND ANTIGEN DETECTION

Despite hundreds of studies published during the past century, serology has found little place in the routine diagnosis of tuberculosis in adults or children.[125] Some studies have used enzyme-linked immunosorbent assay to detect antibodies to whole bacterial cells or to various purified or complex antigens of *M. tuberculosis* in children.[125,271,272] In general, both the sensitivity and specificity of the various tests have been unacceptably low.[42,245] Tests using the mycobacterial antigen A60 have shown both good and poor results in children.[131] No available serodiagnostic test for tuberculosis is adequate under various clinical conditions to be useful for children.

Detection of mycobacterial antigen has been evaluated in clinical samples from adults but rarely from children.[481] Most of these techniques require technically advanced equipment (such as high-pressure liquid chromatography apparatus) and expertise that are not available where tuberculosis in children is common.

TREATMENT

MANAGEMENT OF TUBERCULOUS CHILDREN

Treatment of cavitary tuberculosis in adults is one of the most scientifically accurate areas in all of medicine and one of the finest examples of international professional cooperation in all of history. Because tubercle bacilli in adult patients with tuberculosis can be seen and cultured, their numbers quantified, and the size of the cavities that they produce measured and because so many cases of tuberculosis exist in the world, researchers have been able to ask precise questions about treatment and to design prospective cooperative studies that yield accurate answers about the effect of individual drugs, multiple drug regimens, drug dosage, duration of chemotherapy, rest, and surgical procedures on the course of the disease. Although chemotherapy, without doubt, has been extremely effective in treating childhood tuberculosis, recommendations for treatment historically have been based to a great extent on analogy with adults, on "custom," and on "experience" because in children the tubercle bacilli are fewer in number and not readily accessible and the lesions are not as easy to evaluate as are cavities. However, during the past 3 decades, a large number of treatment trials for children have been reported, which has led to dramatic changes in the therapeutic approach to childhood tuberculosis.

As recently as the early 1980s, the recommended treatment duration for children with tuberculosis disease was 12 to 18 months. Although these regimens are effective when they are used properly, actual failure rates are high because of poor adherence during the long period of treatment. Newer regimens often are called short-course chemotherapy because treatment durations as short as 6 months are routinely successful. However, the key to the new approach is not the short duration but the intensive initial therapy with three or more antituberculosis drugs.

Antituberculosis Drugs (Tables 107–8 and 107–9)

Isoniazid (INH) is the mainstay of therapy for tuberculosis in children. Inexpensive, highly effective in preventing the multiplication of tubercle bacilli, of low molecular weight and therefore readily diffusible to all tissues in the body,[141] and relatively nontoxic to children, INH is one of the most nearly perfect drugs in the pediatrician's armamentarium. It can be administered orally, intramuscularly, or intravenously. When INH is taken orally, high plasma, sputum, and spinal fluid levels are reached within a few hours and persist for at least 6 to 8 hours.[310,425] Because of the slow multiplication of *M. tuberculosis*, the total daily dose can be given at one time. The usual level necessary to inhibit multiplication of tubercle bacilli is 0.02 to 0.05 μg/mL.

Human variation in the acetylation rate of INH to an inactive compound is known to be determined genetically.[365] Rapid acetylation occurs more frequently in black people and Asians than in whites. Although a simple method, specifically the use of a urine sample, now is available for classifying patients as slow or rapid inactivators of INH, the normal way of coping with the problem in children has been to give a sufficiently large dose of INH to ensure an adequate level even in rapid inactivators.[364,623]

The principal toxic effects of INH are peripheral neuritis and hepatitis. Peripheral neuritis resulting from competitive inhibition of pyridoxine utilization is a rare event in North American children because both milk and meat are the main dietary sources of pyridoxine.[48] In some well-nourished children, serum pyridoxine concentrations are depressed by INH, but clinical signs are not apparent.[445] In the case of most children, therefore, the use of supplementary pyridoxine is not necessary. However, in teenagers whose diets may be inadequate, in children from ethnic groups with low milk and meat intake, and in breast-fed babies,

TABLE 107–8 Commonly Used Drugs for the Treatment of Tuberculosis in Children

Drug	Dosage Forms	Daily Dose (mg/kg/day)	Twice-Weekly Dose (mg/kg/dose)	Maximal Daily Dose
Ethambutol	Tablets 100 mg 400 mg	20-25	50	2.5 g
Isoniazid*†	Scored tablets 100 mg 300 mg Syrup‡ 10 mg/mL	10-15†	20-30	Daily: 300 mg Twice weekly: 900 mg
Pyrazinamide	Scored tablets 500 mg	20-40	50	2 g
Rifampin*	Capsules 150 mg 300 mg Syrup Formulated in syrup from capsules	10-20	10-20	Daily: 600 mg Twice weekly: 900 mg
Streptomycin (IM administration)	Vials 1 g 4 g	20-40	20-40	

**Rifamate is a capsule containing 150 mg of isoniazid and 300 mg of rifampin. Two capsules provide the usual adult (>50 kg body weight) daily doses of each drug.*
†When isoniazid is used in combination with rifampin, the incidence of hepatotoxicity increases if the isoniazid dose exceeds 10 mg/kg/day.
‡Most experts advise against the use of isoniazid syrup because of instability and a high rate of gastrointestinal adverse reaction (diarrhea, cramps).

TABLE 107–9 Drugs for Treatment of Drug-Resistant Tuberculosis in Children

Drugs	Dosage Forms	Daily Dosage (mg/kg/day)	Maximum Daily Dose
Capreomycin	Vials: 1 g	15-30 (IM)	1 g
Ciprofloxacin	Tablets 250 mg 500 mg 750 mg	Adults: 500-1500 mg in 2 divided doses	1.5 g
Clofazimine	Capsules 50 mg 100 mg	50-100 mg/day	200 mg
Cycloserine	Capsules 250 mg	10-20	1 g
Ethionamide	Tablets 250 mg	15-20 given in 2 or 3 divided doses	1 g
Kanamycin	Vials 75 mg/2 mL 500 mg/2 mL 1 g/3 mL	15-30 (IM)	1 g
Levofloxacin	Tablets 250 mg 500 mg 750 mg	Adults: 500-750 mg total/day	750 mg
p-Aminosalicylic acid	Packets: 4 g	200-300 given in 2 to 4 divided doses	12 g

pyridoxine supplementation (25 to 50 mg/day) is important. Peripheral neuritis, when it does occur, usually is manifested by "pins and needles" sensations in the hands and feet.

The risk for development of hepatotoxicity from INH, rare in children, increases in frequency with age.[34,80,298,333,402,422,459,551,566] Its cause is unclear.[300] Rapid acetylators are no more susceptible than are slow acetylators.[165,364] Simultaneous use of alcohol, phenytoin, piperazine, and especially rifampin (RIF) seems to increase the likelihood of hepatotoxicity.[80,304] Monitoring of aspartate aminotransferase and alanine aminotransferase activity sometimes reveals transient increases during treatment with INH, but the levels usually return spontaneously to normal without interruption of treatment. Liver enzyme abnormalities in adolescents receiving INH are common occurrences and usually disappear spontaneously, but severe hepatitis can occur.[184,334,436,539,617]

The possible occurrence of hepatitis raises the question of routine monitoring of liver enzyme levels once a month in all children receiving INH. The advantage of doing so has to be weighed not only against the expense but particularly against the difficulty of ensuring regular monthly visits if the patient and parents know that every clinic visit entails a venipuncture. Most experts prefer to substitute routine questions about appetite and well-being, determination of weight, and a check of the appearance of the sclera and the size of the liver.[70,444] Patients should be counseled to stop taking INH and to contact the clinician immediately if significant nausea, vomiting, abdominal pain, or jaundice occurs during the use of INH.

Allergic manifestations of INH hypersensitivity are extraordinarily rare. Convulsions have been reported after doses of 100 mg/kg or more, as in suicide attempts.[369,415,437,513]

The usual dosage in children is 10 to 20 mg/kg/day, to a maximum of 300 mg/day. INH is available in tablets of 100 and 300 mg. The original liquid preparation of INH in syrup was abandoned when investigators found that the drug was unstable in sucrose. A syrup of INH in sorbitol (10 mg/mL) is now on the market and appears to be satisfactory; however, it is unstable at 37° C (98.6° F) and should be kept cool. Significant gastrointestinal intolerance (nausea, diarrhea) develops in many children while they are taking the INH suspension. If tablets are used, they are crushed easily in a dessert spoon, to which then is added in the same spoon a vehicle such as applesauce, mashed banana, thawed undil frozen orange juice, or another palatable medium. The crushed tablets must never be added to the nursing bottle or offered in milk or water because they will be ingested only partially. If INH is given concurrently with RIF, the dose should not exceed 10 mg/kg/day.[422] If the intramuscular form is used, for example, in a child with meningitis who is vomiting, the daily dose is the same as the daily oral dose but usually is divided and given every 8 to 12 hours. INH can interact with several other drugs, particularly theophylline, and the dosage of each may need to be modified in a patient taking several drugs.[25] INH also can increase serum phenytoin levels by blocking its metabolism in the liver, thereby leading to toxicity.[382]

RIF is a semisynthetic drug derived from *Streptomyces mediterranei*. Active against a wide variety of both intracellular and extracellular organisms, it is more effective against mycobacteria than is any other drug except INH. Most clinical isolates are susceptible to 5 μg/mL or less. The drug is absorbed readily from the gastrointestinal tract in the fasting state; peak serum levels of 6 to 10 μg/mL are achieved within 2 hours, and the drug is distributed widely in body fluids and tissues, including spinal fluid. Excretion is mainly through the biliary tract; however, effective levels are achieved in the kidneys and urine. In many patients receiving RIF treatment, tears, saliva, urine, and stool turn orange as the result of a harmless metabolite, but patients always must be warned in advance. Drawbacks include the relatively high cost of treatment; the rare occurrence of explosive hypersensitivity reactions with hemolytic anemia, which, however, usually accompany intermittent (separated by weeks or months) rather than daily RIF therapy[190]; the occasional occurrence of leukopenia or thrombocytopenia while the patient is taking daily RIF[200]; the fact that RIF can render birth control pills inactive when both are used (an alternative method of birth control must be used); and finally—most serious of all for children—a "therapeutic orphan" clause in the United States for children younger than 5 years, which also means that no formulation is commercially available for young children. However, RIF easily can be made into a suspension for use in children.

RIF should be used alone only in treating tuberculosis infection with an INH-resistant organism. If one uses INH, 20 mg/kg, and RIF, 15 to 20 mg/kg, the incidence of hepatotoxicity is appreciable. Therefore, when the two are used together, one would be wise to approximate INH, 10 mg/kg, and RIF, 15 to 20 mg/kg. Rifamate is a capsule containing both INH (150 mg) and RIF (300 mg). Two capsules supply the usual adult (more than 50 kg) daily dose of each drug. Rifamate may be appropriate for older children and adolescents.[3] Rifater contains INH, RIF, and pyrazinamide (PZA) together in one pill in varying concentrations. Rifapentine is a new rifamycin with a very long half-life. Pharmacokinetic studies have been performed in adolescents, but no data on its effectiveness in adolescents or children have been published.[363]

PZA contributes to the killing of *M. tuberculosis*, particularly at a low pH, such as within macrophages.[486] The exact mechanism of action of PZA is a subject of controversy. PZA has no effect on extracellular tubercle bacilli in vitro but clearly contributes to the killing of intracellular bacilli. Primary resistance is very rare, except that *Mycobacterium bovis* is resistant. The drug diffuses readily into all areas, including spinal fluid.[166] The usual adult daily dose is 30 to 40 mg/kg. The adult dose is tolerated well by children, results in high cerebrospinal fluid concentrations, and clearly is effective in therapy trials for tuberculosis in children.[144,478,489,556] However, serum concentrations are lower in children than in adults taking a weight equivalent dose.[197] PZA appears to exert its maximal effect during the first 2 months of therapy. Hepatotoxicity can occur at high doses but rarely does at the usual dose. PZA routinely causes an increase in serum uric acid concentration by inhibiting its excretion through the kidneys. Toxic reactions in adults include flushing, cutaneous hypersensitivity, arthralgia, and overt gout; however, the considerable experience with this drug in children in Latin American countries, Hong Kong, and the United States has revealed few problems. It plays a major role in intensive, short-course treatment regimens.[191,556]

Ethambutol (EMB) has been used for many years as a companion drug for INH in adults. The usual oral dose is 20 mg/kg/day.[142] At this dose, the drug primarily is bacteriostatic, its major role being to prevent the emergence of resistance to other drugs. However, at doses of 25 mg/kg/day or 50 mg/kg given twice a week, EMB has some bactericidal action.[118,185] Unfortunately, at these higher doses, optic neuritis or red-green color blindness has occurred in some adults. Regular visual field and color chart testing should detect these reversible effects early. The incidence of ophthalmologic toxicity in children is extremely low, if it occurs at all.[142] It is used frequently and safely in children with life-threatening forms of tuberculosis or who are at risk for drug-resistant tuberculosis.

Ethionamide is an effective and well-tolerated drug in children at a dose of 15 to 20 mg/kg/day divided into two or three doses given after meals. Children rarely complain about its sulfurous taste, which is repulsive to adults. Related to INH, it likewise diffuses readily into spinal fluid.[145,146,240] Ethionamide is used in cases of drug-resistant tuberculosis and tuberculous meningitis. Unfortunately, no convenient pediatric dosage form is available.

Streptomycin (STM) is used in conjunction with INH and RIF in life-threatening forms of tuberculosis. It is bactericidal and tolerated well in children in the usual dose of 20 to 40 mg/kg/day intramuscularly up to 1 g. Usually, STM can be discontinued within 1 to 3 months if clinical improvement is definite, whereas the other two or three drugs are continued by mouth.

p-Aminosalicylic acid, either the sodium or the potassium salt, formerly was part of the standard treatment of tuberculosis. However, it is a purely bacteriostatic drug that has been superseded by more powerful drugs (RIF, PZA). It is used only for the treatment of drug-resistant tuberculosis.

Other antituberculosis drugs that may be needed for patients whose mycobacteria are resistant to INH or RIF are the aminoglycosides kanamycin, amikacin, and capreomycin, each of which has a spectrum of activity that differs from that of STM with respect to individual mycobacterial strains. Cycloserine and viomycin are other drugs sometimes used in patients with multidrug resistance. Clofazimine[259] and rifabutin (related to RIF) are newer drugs that have antimycobacterial activity but have been used mainly in children who have AIDS and are suffering from *M. avium-intracellulare* infection. Clofazimine also is used for infection with *Mycobacterium leprae*.

Several of the fluoroquinolones, especially levofloxacin and ciprofloxacin, have significant antituberculosis activity,[287,387] but they cannot be used routinely in children because of the possible destruction of growing cartilage seen in animal models. However, the dire consequences of drug-resistant tuberculosis lead many experts to use them successfully and with little apparent toxicity in children with MDR-TB disease.[246]

TABLE 107–10 In Vivo Location of *Mycobacterium tuberculosis*: A Model

	Population Size	Metabolism and Replication	pH	Most Effective Drugs
Cavity	10^7-10^9	Active and rapid	Neutral or alkaline	INH, RIF, STM
Closed caseous lesions	10^5-10^7	Slow and intermittent	Neutral	RIF, INH
Within macrophages	10^4-10^6	Very slow	Acid	PZA, RIF, INH

INH, isoniazid; PZA, pyrazinamide; RIF, rifampin; STM, streptomycin.

Microbiologic Basis for Treatment[384,537]

Laboratory observations of *M. tuberculosis* and the results of clinical therapy trials have led to a hypothesis concerning the actions of various drugs and drug combinations.[157,200,384,385] The tubercle bacillus can be killed only during replication, which occurs in organisms that are active metabolically. In one model, bacilli in a host exist in different populations (Table 107–10). They are active metabolically and replicate freely where oxygen tension is high and the pH is neutral or alkaline. Environmental conditions for growth are best within cavities, and such conditions can lead to a large bacterial population. Adults with reactivation-type pulmonary tuberculosis usually have all three populations of tubercle bacilli. Children with pulmonary tuberculosis and patients of all ages with only extrapulmonary tuberculosis are infected with a much smaller number of tubercle bacilli because the cavitary population is not present.

Naturally occurring drug-resistant mutant organisms occur within large populations of tubercle bacilli even before chemotherapy is started. All known genetic loci for drug resistance in *M. tuberculosis* are located on the chromosome; no plasmid-mediated resistance is known. The rate of resistance within populations of organisms is related to the rate of mutations at these loci.[375,584,587,592,652,653] Although a large population of bacilli as a whole may be considered drug susceptible, a subpopulation of drug-resistant organisms occurs at a fairly predictable rate. The mean frequency of these drug-resistant mutants is about 10^{-6} but varies among drugs: STM, 10^{-5}; INH, 10^{-6}; and RIF, 10^{-7}.[124] A cavity containing 10^9 tubercle bacilli has thousands of single drug–resistant mutant organisms, whereas a closed caseous lesion contains few if any resistant mutants.

The two microbiologic properties of population size and drug resistance mutation explain why single antituberculosis drugs cannot cure cavitary tuberculosis.[370] In the mid-1940s, STM alone was given to adults with cavitary pulmonary tuberculosis.[338] Within 3 months, 80 percent of patients had significant numbers of STM-resistant organisms. This phenomenon has been observed for every antituberculosis drug developed subsequently. However, the natural occurrence of resistance to one drug is independent of resistance to any other drug because the resistance loci are not linked. The chance of having even one organism "naturally" resistant to two drugs is on the order of 10^{-11} to 10^{-13}. Populations of this size in patients are extremely rare, and mutants naturally resistant to two drugs are nonexistent.

The population size of tubercle bacilli within a patient determines the appropriate therapy. For patients with large bacterial populations (adults with cavities or extensive infiltrates), many single drug–resistant mutants are present, and at least two antituberculosis drugs must be used.[127] Conversely, for patients with tuberculosis infection but no disease, the bacterial population is small (about 10^3 to 10^4 organisms), drug-resistant mutants are rare findings, and a single drug can be used. Children with pulmonary tuberculosis and patients of all ages with extrapulmonary tuberculosis have medium-sized populations in which drug-resistant mutants may or may not be present. In general, these patients should be treated with at least two drugs.

Some antituberculosis drugs, such as INH, RIF, and STM, are bactericidal against *M. tuberculosis*. Other drugs, including ethionamide, *p*-aminosalicylic acid, and low-dose EMB, are bacteriostatic. The earliest treatment regimens for tuberculosis combined the killing action of a bactericidal drug with a bacteriostatic drug that would suppress replication of drug-resistant mutant organisms. A small number of organisms survived despite administration of chemotherapy, and 18 to 24 months of treatment was necessary to permit host defenses to eliminate persisting organisms. Despite the prolonged treatment period, relapse rates were 5 to 15 percent, mostly a result of poor adherence to treatment.

The availability of RIF and the rediscovery of PZA in the early 1970s effected radical change in antituberculosis chemotherapy. These two drugs have the most potent sterilizing action, the ability to kill tubercle bacilli within lesions as quickly as possible. The addition of RIF to INH for the treatment of pulmonary tuberculosis leads to cure rates approaching 100 percent with only 9 months of treatment. The further addition of PZA shortens the necessary treatment duration to only 6 months.

TREATMENT OF THE STAGES OF TUBERCULOSIS

EXPOSURE

Children exposed to potentially infectious adults with pulmonary tuberculosis should begin treatment, usually with INH only, if the child is younger than 5 years or has other risk factors for the rapid development of tuberculosis disease, such as immunocompromise of some kind.[576,643] Failure to do so may result in the development of severe tuberculosis disease even before the tuberculin skin test becomes reactive; the "incubation period" of disease may be shorter than that for the skin test. The child is treated for a minimum of 3 months after contact with the infectious case is broken (by physical separation or by effective treatment of the case). After 3 months, the tuberculin skin test is repeated. If the second test result is positive, infection is documented and INH should be continued for a total duration of 9 months; if the second skin test result is negative, treatment can be discontinued. If the exposure was to a person with an INH-resistant but RIF-susceptible isolate, RIF is the recommended treatment.

Two special circumstances of exposure deserve attention. A difficult situation arises when exposed children are anergic because of HIV infection. These children are particularly vulnerable to rapid progression of tuberculosis, and it will not be possible to determine whether infection has occurred. In general, these children should be treated as though they have tuberculosis infection.

The second situation is potential exposure of a newborn to a mother (or other adult) with a positive tuberculin skin test result or, rarely, a nursery worker with contagious tuberculosis.[324,570] Management is based on further evaluation of the mother:

1. *The mother has a normal chest radiograph:* no separation of the infant and mother is required. Although the mother should receive treatment of tuberculosis infection and other household members should be evaluated for tuberculosis infection or disease, the infant needs no further work-up or treatment unless a case of disease is found.

2. *The mother has an abnormal chest radiograph:* the mother and child should be separated until the mother has been evaluated thoroughly. If the radiograph, history, physical examination, and analysis of sputum reveal no evidence of pulmonary tuberculosis in the mother, a reasonable assumption is that the infant is at low risk of acquiring infection. The radiographic abnormality is due to another cause or a quiescent focus of previous tuberculosis infection. However, if the mother remains untreated, contagious tuberculosis may develop later, and the infant will be exposed. Both the mother and infant should receive appropriate follow-up care, but the infant does not need treatment. If the radiograph and clinical history are suggestive of pulmonary tuberculosis, the child and mother should remain separated until both have begun appropriate chemotherapy. The infant should be evaluated for congenital tuberculosis. The placenta should be examined. If the mother has no risk factors for drug-resistant tuberculosis, the infant should receive INH and close follow-up care. The infant should have a tuberculin skin test at 3 or 4 months after the mother is judged to be contagious no longer; evaluation of the infant at this time follows the guidelines for other exposure of children. If no infection is documented at this time, repeating the tuberculin skin test in 6 to 12 months would be prudent. If the mother has tuberculosis caused by a multidrug-resistant isolate of *M. tuberculosis* or she has poor adherence to therapy, the child should remain separated from her until she is no longer contagious or the infant can be given BCG vaccine and be kept separated until the vaccine "takes" (marked by a reactive tuberculin skin test).

INFECTION

The recommendation for treatment of asymptomatic tuberculin-positive individuals is based on data from several well-controlled studies; it applies particularly to children and adolescents who are at high risk for the development of overt disease but at very low risk for development of the main toxic manifestation of INH therapy, which is hepatitis.[147,176,410,422,541] The large, carefully controlled U.S. Public Health Study of 1955, followed by others both in this country and abroad, demonstrated the favorable effect of 12 months of INH on the incidence of complications as a result of both lymphohematogenous and pulmonary spread.[237] The younger the tuberculin reactor, the greater the benefit.[116]

The American Thoracic Society and CDC,[13] recommend that INH treatment of tuberculosis infection be given to the following groups:

1. Household members and other close associates of potentially infectious tuberculosis cases. All contacts of any age with a Mantoux tuberculin skin test reading of 5 mm or greater and without a documented history of reaction in the past should be considered recently infected and receive therapy.

2. Newly infected people, regardless of age, who have had a tuberculin skin test conversion within the past 2 years.

3. People with HIV infection or at risk for development of HIV infection who have a reaction of 5 mm or greater to a Mantoux test.

4. People of any age with tuberculosis in the past who received inadequate treatment.

5. People of any age with a significant tuberculin reaction and an abnormal but stable chest radiograph.

6. People with significant tuberculin reactions who have special clinical situations, including silicosis, diabetes mellitus, prolonged corticosteroid therapy, immunosuppressive therapy, hematologic malignant disease, and end-stage renal disease.

7. All children and adolescents with a "positive" tuberculin skin test reaction or interferon release assay result.

The question arises regarding how long the protective effect can be expected to last. Comstock and associates,[113] in their final report on INH prophylaxis in Alaska, demonstrated the protective effect of 1 year of chemoprophylaxis to be 19 years at least. Hsu[236] described 2494 patients monitored for up to 30 years and showed that adequate drug prophylaxis prevented reactivation of tuberculosis infection during adolescence and into young adulthood. It seems reasonable to hope that the decreased risk of active tuberculosis after INH prophylaxis may in fact be lifelong in individuals infected with INH-susceptible tubercle bacilli. Failure of INH after exposure to INH-resistant *M. tuberculosis* has been documented. No controlled study of an alternative regimen has been reported. RIF alone is recommended and widely used, although failures have been reported.[336]

The dosage of INH to be used has had little study. Most investigators have used a regimen based on 4 to 8 mg/kg of body weight per day, usually taken all at once, for a period of 6 to 12 months. A dose of 5 mg/kg/day was found satisfactory in one study.[115] Most clinicians prescribe a dose of 10 to 15 mg/kg/day to a total of 300 mg/day for treatment of infection to be sure of achieving therapeutic levels even in patients who inactivate the drug rapidly by acetylation.

The duration of INH treatment initially was set arbitrarily at 12 months.[251] A large trial comparing regimens of daily INH taken for 12, 24, and 52 weeks with placebo for their ability to prevent tuberculosis disease was conducted in eastern Europe with adults who had old fibrotic lesions caused by tuberculosis. Therapy for 1 year was most effective, especially if the patients were adherent. However, therapy for 24 weeks afforded a fairly high level of protection. A subsequent analysis concluded that the 24-week duration of preventive therapy was more cost-effective for adults than was the 52-week duration.[540] Subsequently, many health departments have accepted 6 months of INH preventive therapy as their standard regimen for adults. However, the cost-effectiveness analysis does not apply to children. A recent review of all published studies concluded that the effectiveness of INH therapy increased up to 9 months' duration, but no additional benefit was achieved with a longer duration.[13] A duration of 9 months is recommended for children and adults by the AAP and CDC.[13] INH is taken daily under self-supervision or can be taken twice weekly under directly observed therapy. When the child is infected with an INH-resistant but RIF-susceptible strain of *M. tuberculosis*, RIF should be substituted for INH and given for 6 months' duration. If the infecting strain is resistant to both INH and RIF, usually two other drugs are used; an expert in tuberculosis should be consulted in this situation.

A shorter duration of effective treatment for tuberculosis infection is highly desirable to improve compliance and effectiveness.[359] In England, a 3- to 4-month course of INH plus RIF is used frequently. There have been published case-control series, and a recent meta-analysis of available data suggests that especially when compliance is considered, a 3- to 4-month course of INH plus RIF may be as effective as is a 9-month course of INH.[169] One small, randomized study also yielded favorable results with this regimen.[549] However, this regimen is not yet recommended by the CDC, AAP, or WHO.

DISEASE IN ADULTS

A shorter duration of antituberculosis chemotherapy is desirable for several reasons: it may be significantly less expensive than

traditional therapy; the patient is exposed to potentially toxic drugs for shorter periods of time; more time and resources can be allotted to ensure adherence with treatment; and if a patient absconds from treatment, a greater likelihood will exist that bacteriologic cure already has been achieved as a result of the early and rapid sterilizing activity of the newer regimens.

A 9-month regimen of INH and RIF cures more than 98 percent of cases of drug-susceptible pulmonary tuberculosis in adults.[528] Both drugs are given daily for the first 2 weeks to 2 months and then can be given daily or twice weekly under directly observed therapy for the remaining 7 to 8 months with equivalent results and rates of adverse reactions. When it is given twice weekly, the RIF dose is the same as the daily dose, but the INH dose is increased to 900 mg in adults. Twice-weekly administration is supported by pharmacologic and animal model data determining the area-under-the-curve characteristics for these antituberculosis drugs. Unfortunately, durations of therapy with only INH and RIF for less than 9 months are unacceptable because failure and relapse rates exceed 10 percent.

When three or more antituberculosis drugs are used initially, treatment durations of 6 months are routinely successful.[109] Regimens using INH, RIF, PZA, and STM during the initial phase (2 months), followed by INH and RIF in the continuation phase (4 months), routinely yield cure rates greater than 98 percent and relapse rates below 4 percent.[21,525] If PZA is excluded from the initial phase, the rate of bacteriologic failure rises to 7 to 10 percent.[229] However, exclusion of STM does not affect cure or relapse rates appreciably.[229,542] Use of PZA beyond the first 2 months of therapy does not add any benefit.[230] Regimens of 4 months' total duration have unacceptably high relapse rates of 10 percent or greater.[262] On the basis of all reported studies, the American Thoracic Society and CDC currently recommend for the treatment of pulmonary tuberculosis in adults a 6-month regimen using INH, RIF, PZA, and EMB for 2 months, followed by 4 months of daily or twice-weekly doses of INH and RIF.[51,88]

CHEMOTHERAPY FOR CHILDREN

Clinical trials of antituberculosis drugs in children are difficult to perform, mostly because of the difficulty in obtaining positive cultures at diagnosis or relapse and the need for long-term follow-up.[556] Historically, recommendations for treating children with tuberculosis have been extrapolated from clinical trials of adults with pulmonary tuberculosis.[535] However, during the past 2 decades, a large number of clinical trials involving only children have been reported. In 1983, Abernathy and colleagues[2] reported successful treatment of 50 children with tuberculosis in Arkansas using INH and RIF daily for 1 month, then twice weekly for 8 months. The success rate virtually was 100 percent. Most pulmonary infiltrates cleared by the end of therapy, but hilar adenopathy usually was still present radiographically and then gradually cleared during a period of 2 to 3 years. Patients with only hilar adenopathy can be treated successfully with INH and RIF for 6 months.[256,467]

Several major studies of 6-month therapy in children with at least three drugs in the initial phase have been reported.[7,47,247,302,583,596,605] The regimen used most commonly was 6 months of INH and RIF supplemented during the first 2 months with PZA. The overall success rate has been greater than 98 percent and the incidence of clinically significant adverse reactions less than 2 percent. Regimens not using STM were as successful as those that included it. Use of twice-weekly medications (under directly observed therapy) during the continuation phase was as effective and safe as was daily administration. Three studies used twice-weekly therapy throughout the treatment regimen with excellent success.[302,596,622] The 6-month, three-drug regimen is successful, tolerated well, and less expensive.[574,607] It also effects a cure faster, so the likelihood of successful treatment is greater if the child becomes nonadherent later in therapy.

EXTRAPULMONARY TUBERCULOSIS

Controlled treatment trials for various forms of extrapulmonary tuberculosis are rare. In most reports, extrapulmonary cases have been combined with pulmonary cases and often are not analyzed separately. Several of the 6-month, three-drug trials in children included extrapulmonary cases.[47,302] Most non–life-threatening forms of extrapulmonary tuberculosis respond well to a 9-month course of INH and RIF[156,158] or to a 6-month regimen including INH, RIF, and PZA.[254] One exception may be bone and joint tuberculosis, which may have a high failure rate when 6-month chemotherapy is used, especially if surgical intervention has not occurred.[156] Tuberculous meningitis usually is not included in trials of extrapulmonary tuberculosis therapy because of its serious nature and low incidence. Treatment with INH and RIF for 12 months generally is effective.[626] In the 1950s, Lorber[340] treated children with tuberculous meningitis for only 6 months with good results. A more recent study from Thailand showed that a 6-month regimen that included PZA for serious tuberculous meningitis led to fewer deaths and better outcomes than did longer regimens that did not contain PZA.[257] Most children are treated initially with four drugs (INH, RIF, PZA, and ethionamide or STM). Treatment with PZA and the fourth drug is stopped after 2 months, and INH and RIF are continued for a total of 6 to 9 months.[146]

DRUG-RESISTANT TUBERCULOSIS IN CHILDREN

The incidence of drug-resistant tuberculosis is increasing in the United States and the world because of poor adherence by the patient, the availability of some antituberculosis drugs in noncontrolled over-the-counter formulations, and poor management by physicians.[49,181,348,419] In the United States, approximately 10 percent of *M. tuberculosis* isolates are resistant to at least one drug.[86,92] Initial drug resistance rates of up to 80 percent have been noted in adults with pulmonary tuberculosis in some countries,[350] and rates of 20 to 30 percent are common findings. Resistance is most common to STM and INH and still is relatively rare to RIF.[130,231,386,656] Certain epidemiologic factors—disease in an Asian or Hispanic immigrant to the United States, homelessness in some communities, and history of previous antituberculosis therapy—correlate with drug resistance in adult patients.[5,29,439] Patterns of drug resistance in children tend to mirror those found in adult patients in the population.[84,475,495,512,543,567,569] Outbreaks of drug-resistant tuberculosis in children occurring at schools have been reported.[84,474] Individual cases also have been recognized. The key to determining drug resistance in childhood tuberculosis usually comes from the drug susceptibility results of the infectious adult contact case's isolate.

Therapy for drug-resistant tuberculosis is successful only when at least two bactericidal drugs are given to which the infecting strain of *M. tuberculosis* is susceptible.[72,193,438,494,593] If only one effective drug is given, secondary resistance will develop. When INH resistance is considered a possibility on the basis of epidemiologic risk factors or the identification of an INH-resistant source case isolate, an additional drug—usually EMB or STM—should be given initially to the child until the exact susceptibility pattern is determined and a more specific regimen can be designed.[584] Exact treatment regimens must be tailored to the specific pattern of drug resistance. The duration of therapy

usually is extended to at least 9 to 12 months if either INH or RIF can be used and to at least 18 to 24 months if resistance to both drugs is present.[253] Children tend to tolerate second-line antituberculosis drugs well, and community-based treatment usually is successful.[151,397] On occasion, surgical resection of a diseased lung or lobe is required.[255,453] An expert in tuberculosis always should be involved in the management of children with drug-resistant tuberculosis infection or disease.

ADHERENCE AND DIRECTLY OBSERVED THERAPY

Nonadherence with drug treatment by patients is a major problem in control of tuberculosis because of the long-term nature of its treatment.[377,580,620,627] As treatment regimens become shorter in duration, adherence assumes an even greater importance.[43] Suspected cases of tuberculosis must be reported to the local health department so that it can perform the necessary contact investigations and assist both patients and health care providers in overcoming barriers to adherence. To comply, the patient and family must know what is expected of them through verbal and written instructions in the patient's first language. An assessment of potential nonadherence should be made at the beginning of therapy.[32] Missed appointments should be brought quickly to the attention of the responsible public health officials, who may be able to use incentives or enablers, behavior modification, or, rarely, confinement to ensure adherence. The success of twice-weekly therapy, especially after a period of daily administration of medications, allows directly observed therapy to be given by a health care professional in cases of proven or suspected nonadherence.[99,254] Most experts hold that twice-weekly medication should be administered only under the direct observation of a health care worker.[297,637] Direct observation means that a health care worker or other nonrelated third party (e.g., teacher, school nurse, social worker) is physically present while the patient ingests the medication. As many as 50 percent of patients taking long-term antituberculosis medications will have significant nonadherence, and its occurrence is not predictable by the physician. In most communities in the United States and in an increasing number of other nations, directly observed therapy is the standard of care for all patients with tuberculosis disease.[90,170] However, when directly observed therapy cannot be used, structured behavior interactions can increase compliance with treatment.[79,597]

SUMMARY OF TREATMENT RECOMMENDATIONS

1. A regimen of INH and RIF for 6 months, supplemented with PZA during the first 2 months, is standard therapy for children with drug-susceptible intrathoracic tuberculosis in the United States and Canada.

2. An alternative regimen is INH and RIF for 9 months. The disadvantages of this regimen include a longer duration, the potential for increased drug resistance during therapy, and less effectiveness if the patient absconds from treatment. This regimen should be used only if PZA cannot be tolerated.

3. After an initial 2 weeks to 2 months of daily drug administration, drugs can be given twice weekly under directly observed therapy with excellent effectiveness.[449] With patients for whom social or other restraints prevent reliable daily self-administration even during the initial phase of therapy, drugs can be given two or three times per week from the beginning under directly observed therapy.

4. In most cases, extrapulmonary tuberculosis can be treated with the same regimens as used for pulmonary tuberculosis, although data for tuberculous meningitis and bone or joint disease are relatively lacking.

5. In cases of possible initial INH resistance, EMB or STM should be added to the initial phase of all regimens until drug susceptibilities are known.[87]

6. Optimal therapy for tuberculosis in children with HIV infection has not been established.[575] Most HIV-infected adults with tuberculosis respond well to antituberculosis drugs but may require longer durations of treatment.[269] Immunosuppressed children with tuberculosis, including those with HIV infection, should be treated with at least three drugs initially, and treatment should be continued for a minimum of 9 months. HIV testing is recommended for all infants and children with tuberculosis disease.

7. Tuberculosis disease occurring during pregnancy should be treated with a 9-month regimen of INH and RIF supplemented during the initial phase with EMB (STM should not be used). The use of PZA in pregnant patients is controversial although probably safe.

CORTICOSTEROIDS

Corticosteroids have a place in the treatment of patients with tuberculosis. They should never be used except under cover of effective antituberculosis drugs. Corticosteroids would be expected to be beneficial in situations in which the host inflammatory reaction is contributing to tissue damage or is impairing function.

Corticosteroids often are a useful addition to antituberculosis drugs if suppression of inflammatory reaction is desired, such as in the following situations[534]:

1. In patients with tuberculous meningitis in whom increased intracranial pressure is present.[600] The major actions are to reduce vasculitis, inflammation, and, ultimately, intracranial pressure. Not only is reduction of pressure desirable, but lowering of the pressure also probably favors the circulation of chemotherapeutic drugs through the brain and meninges.[174,189] One study demonstrated lower rates of mortality and long-term neurologic sequelae in patients with tuberculous meningitis treated with corticosteroids than in control patients not treated with steroids.[189]

2. In patients with acute pericardial effusion in whom tamponade is occurring. Relief of symptoms takes place within hours.[477,578]

3. In patients with pleural effusion, a shift of the mediastinum, and acute respiratory embarrassment.[317,537] The long-term course probably is the same with or without steroids, but symptomatic improvement usually is dramatic.

4. In patients with miliary tuberculosis if the inflammatory reaction is so severe that it produces alveolocapillary block with cyanosis.

5. In patients with enlarged mediastinal lymph nodes that are causing respiratory difficulty or a severe collapse-consolidation lesion, particularly in the middle or lower lobes, when bronchiectasis is likely to be a troublesome sequela.[407,408] Under either of these circumstances, a course of corticosteroids is warranted, with the realization that it will be more successful in a younger infection because inflammation characterizes the early stages of tuberculosis. If caseation already is advanced, steroids will be of little benefit.

The dosage of corticosteroids should be in the anti-inflammatory range, that is, prednisone, 1 to 2 mg/kg/day for 4 to 6 weeks with gradual withdrawal. Some experts prefer dexamethasone, but no comparative trials have been published.

ACTIVITY

Activity need not be restricted in children with tuberculosis, except when a particular complication is inevitable (shortness of breath in pleural effusion, immobilization for a vertebral lesion). During the early months of treatment, patients probably should avoid engaging in competitive sports, excessive study, fatigue, and sunburn.

ISOLATION

Isolation should be maintained for children with cavitary lesions, productive cough with acid-fast, stain–positive sputum, draining sinuses, or renal tuberculosis until their secretions are negative on smear and preferably on culture. Young children are virtually noninfectious because they rarely cough and because their bronchial secretions contain few bacilli compared with those of adults with tuberculosis. Guidelines issued by the CDC state that most children with typical tuberculosis do not require isolation in the hospital.[82,399] Children with possible pulmonary tuberculosis should be treated as potentially infectious if they have a cavity or extensive upper lobe infiltrate, if they have a productive cough (especially if the sputum is acid-fast smear positive), or during high-risk procedures such as bronchoscopy.

FOLLOW-UP

Follow-up of children treated with antituberculosis drugs has become somewhat more streamlined in recent years. While receiving chemotherapy, the patient should be seen monthly, both to encourage regular intake of the prescribed drugs and to check, by a few simple questions (concerning appetite, well-being) and a few observations (weight gain; appearance of the skin and sclerae; palpation of the liver, spleen, and lymph nodes), that the disease is not spreading and that toxic effects of the drugs are not appearing.[639] Repeated chest radiographs should be obtained 1 to 2 months after the onset of chemotherapy to ascertain the maximal extent of disease before chemotherapy takes effect; thereafter, radiographs rarely are necessary.[292] Chemotherapy has been so successful that follow-up beyond its termination is not necessary, except for children with serious disease, such as tuberculous meningitis, or those with extensive residual chest radiographic findings at the end of chemotherapy.

CASE REPORTING

Every case of definite or suspected tuberculosis, by law,[89] must be reported immediately by telephone to the health authority[192] to ensure prompt contact investigation[234] and free antituberculosis drugs, which are available for diagnosed cases and for intimate contacts in almost every state of the United States and in many countries.

PREVENTION

Prevention of tuberculosis can be subdivided logically to consider the following circumstances:

1. Protection against exposure to the disease.
2. Use of antituberculosis drugs in tuberculin-negative individuals at high risk for development of infection.
3. Immunization of tuberculin-negative individuals.

Protection against exposure to disease is the ideal form of prevention.[356] It presupposes thorough pre-employment and ongoing case-finding programs among all who come in contact with children, including daycare center and school personnel, Sunday school personnel, music and art teachers, hospital nurses, babysitters, household servants, food handlers, beauticians, and barbers. Numerous epidemics, mini-epidemics, and mass exposures in newborn nurseries have been traced to such infected individuals.

IMMUNIZATION

Immunization against tuberculosis theoretically would be a tremendous boon to humanity, but in practice, it has been fraught with very great difficulty. Various strains of mycobacteria and diverse nonliving immunogenic fractions have been studied. The impossibility of standardizing vaccines in the early days, the lack of any clinically useful test reflecting the immune status of the individual, and the relatively slow course of the disease have handicapped epidemiologic studies considerably. Furthermore, the very lack of adequate scientific data has intensified national and individual emotional responses to the point that rational approaches to data gathering and interpretation are often impossible. Although the use of tuberculosis vaccines for control of tuberculosis has waned in recent years in industrialized countries because of the falling incidence of the disease, new interest in them has cropped up because of their beneficial effect in certain types of malignant disease. New insights into their mode of action may prove fruitful in the long run in understanding immunity to tuberculosis, as well as to neoplasia, and might lead to a greatly improved and clinically useful vaccine.

BCG was developed at the Institut Pasteur in Paris by Calmette and Guérin, who, starting in 1908, made 231 passages of a strain of *M. bovis* on a beef bile medium, thereby producing marked attenuation. Injected into laboratory animals, this strain was shown to increase resistance to challenge with virulent *M. tuberculosis*. In 1921, it first was administered orally to newborn infants and since then has been given to more than 4 billion people.

BCG vaccine attempts to replace the potentially dangerous primary infection with *M. tuberculosis* with an innocuous primary infection with the bacillus of Calmette and Guérin, thus activating host cell-mediated immunity with minimal chance of causing progressive disease so that an infection with *M. tuberculosis* will be of the "reinfection" type.[332]

Variations in strains and lack of standardization are basic problems in evaluating the results of immunization.[198,594] BCG was maintained for many years by serial passage at the Institut Pasteur and distributed to hundreds of laboratories all over the world. Not until 1966 did the WHO Expert Committee on Biological Standardization adopt formal requirements for the maintenance of frozen "seed lots" to minimize the inevitable mutations that have produced BCG vaccines with widely varying characteristics. Routine quality control measures carried out by the production laboratory include an identity test, a test for contamination, a safety test in guinea pigs, an estimate of the total bacillary mass, viability, and a test of heat stability. Periodic assessment of the allergenic capacity in humans is part of quality control testing. The WHO, through its International Reference Preparation for BCG vaccine and through quality control testing on request carried out in its several cooperating laboratories, has helped decrease the gross variations in BCG vaccine found until recent years.

Vaccination techniques and dosages are variable. Intradermal injection is the most precise technique. Multiple-puncture techniques are popular because they are easy, but reported results consistently are inferior to those obtained with intradermal injection. Oral vaccination, the original method of administration,

largely has been abandoned because of poor results. The actual dose of BCG at present usually is approximately 10^6 culturable particles. Because in animals large doses produce better resistance to challenge than small ones do, the largest convenient dose is used. However, in neonates, who have a higher incidence of untoward reactions, the customary approach is to halve the dose generally used in older infants and children to prevent local complications.

The usual local reaction to intradermal BCG vaccine is the development of a papule at the site of vaccination, and this papule reaches its maximal diameter (10 to 20 mm) in the sixth week. A small crust that may form on the papule detaches at about this time, with only a small ulcer remaining that may discharge a surprising amount of pus. Most ulcers are healed by the 10th week. A small scar is visible in almost all BCG-vaccinated individuals. Enlargement of the regional lymph nodes occurs regularly and is painless, sometimes ending in calcification. Formation of an abscess with breakdown is a rare event, but it occurs more often in infants.

Untoward reactions to BCG rarely have been a problem.[609] Fatalities caused by progressive disease have been reported in no more than 60 vaccine recipients (of an estimated 3+ billion), usually (but not always) children with well-documented immunodeficiency.[75,135,172,195,443,447] No return of the attenuated strain to virulence has ever been noted. In countries where BCG is used routinely for immunization of neonates, osteomyelitis has been diagnosed in some 5 per 100,000 neonates. It usually becomes manifested when the child is between 5 and 33 months of age, when a tender swelling is noted near a joint; bone destruction is well localized and responds to conservative treatment. On the whole, BCG is one of the safest vaccines in use.

In many countries where BCG is given routinely, the incidence of HIV infection in adults and children is high.[62,479] Reports of local and systemic complications from BCG vaccine in HIV-infected people are increasing, but the true magnitude of the interaction is not known yet.[40,219,220,232,421,466] However, in more recent studies, the rate of adverse BCG reactions in HIV-infected children appears to be higher than thought previously.[219,220] In most cases, BCG complications occur shortly after vaccination, but in one man with AIDS, adenitis as a result of BCG occurred 30 years after inoculation.[469] Routine treatment of patients with previous BCG vaccination who subsequently become immunocompromised is not recommended, but the clinician should be aware of previous BCG vaccination if signs or symptoms of mycobacterial infection occur.

The effectiveness of revaccination has never been evaluated scientifically and is not recommended.

The efficacy of BCG vaccines in humans has been evaluated in several large, well-controlled studies (Table 107–11). Three of these trials showed excellent protection, two showed mediocre protection, and two showed little or no effect of BCG. Another study, not tabulated because its numbers are relatively small, is the "experiment of nature" reported by Hyge[246a] in 1957. Hyge[246a] observed an epidemic of tuberculosis in a school for girls, where 105 girls initially were tuberculin negative, 130 were tuberculin positive, and 133 were BCG immunized. In this group, the total incidence of tuberculosis was 23 times as high in the tuberculin-negative as in the BCG-immunized girls. Explanations for these differences in outcomes among trials must be sought in the quality and characteristics of the BCG vaccine used in the particular trial, the possible immunizing effect (in Georgia and Alabama) of infections with other mycobacteria,[532] the possibly greater effectiveness of BCG vaccine in areas of high tuberculosis prevalence, and methodologic variations among the trials.

The most recent large study of BCG effectiveness is the Chingleput study, started in 1968 in Chingleput District near Madras, South India, an area where sensitization with environmental mycobacteria is prevalent. People of all ages were vaccinated with one of two BCG vaccines or a placebo; only the incidence of adult-type pulmonary tuberculosis in the three groups was compared (i.e., not the forms usually found in children). During the ensuing years, no difference in incidence was noted among the three groups.[20a] This disturbing result has been the subject of several WHO investigations because BCG is one of the vaccines recommended for all children in the Expanded Program of Immunization sponsored by the WHO itself.[468] Another study of neonatal vaccination with BCG in England reported very favorable results with BCG.[121]

A group at the Harvard School of Public Health reviewed all published studies of BCG efficacy in a meta-analysis.[111] Most published trials were not analyzed because of serious flaws in their experimental design or reporting. Among all trials and case-control studies included, the average protection against tuberculosis disease by various BCG preparations was 50 percent. The protective levels were higher for disease in children, particularly for meningitis and tuberculosis-associated death.[110,604] However, ascertainment bias and lack of standardized case definitions render the results of these analyses very difficult to interpret. The BCG vaccines prevent many cases of tuberculosis in children, but the effect is quite variable. That BCG vaccines are not an instrument of tuberculosis control also has become apparent because they do not prevent infection with *M. tuberculosis*, their protective effect is short-lived, and vaccination of infants does little to prevent future cases of contagious tuberculosis among adults in a community.

The role of BCG vaccine in the United States today is very limited. The Advisory Committee on Immunization Practices of the U.S. Public Health Service and the Advisory Council for the Elimination of Tuberculosis recommend BCG only for tuberculin-negative infants and children in the United States who (1) are at high risk for having intimate and prolonged exposure to persistently untreated or ineffectively treated adults with infectious pulmonary tuberculosis, cannot be removed from the source of infection, and cannot be prescribed long-term preventive

TABLE 107–11 Summary of Seven Large Controlled Trials of Bacillus Calmette-Guérin (BCG) Immunization Against Tuberculosis

Trial	Investigators	Intake Period	Vaccine Laboratory	Duration of Observation (yr)	% Protection from BCG
North America: Native Americans	Stein and Aronson, 1953	1935-1938	Phipps	9-11	80
Chicago: infants	Rosenthal et al., 1961	1937-1948	Tice	12-23	75
Britain: schoolchildren	Medical Research Council, 1971	1950-1952	Copenhagen	15	78
South India: rural population	Frimodt-Moller et al., 1964	1950-1955	Madras	2.5-7	60/31*
Puerto Rico: children	Palmer et al., 1958	1949-1951	New York State	5.5-7.5	31
Georgia, Alabama: population	Comstock and Palmer, 1966	1950	Tice	14	14
Georgia: schoolchildren	Comstock and Webster, 1969	1947	Tice	20	0

**The initial estimate of efficacy was 60 percent. Subsequently, when follow-up was extended to 9 to 14 years, the efficacy figure declined to 31 percent.*
Modified from Sutherland, quoted by Eickhoff, T. C.: The current status of BCG immunization against tuberculosis. Annu. Rev. Med. 28:411-423, 1977.

therapy or (2) continuously are exposed to people with tuberculosis resistant to INH and RIF.[91] A few experts, however, are more inclined toward the use of BCG in neonates who are at any risk of exposure to tuberculosis whatsoever.[282,283,526,601]

Contraindications to the use of BCG for prevention of tuberculosis include congenital immunodeficiency, known HIV infection (in the United States; the WHO recommends giving BCG to asymptomatic HIV-infected infants who reside in areas with high tuberculosis rates), leukemia, lymphoma, and generalized malignant disease as well as treatment with corticosteroids, alkylating agents, antimetabolites, and radiation.

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CHAPTER 108

Other Mycobacteria

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The definition of mycobacteria other than tubercle bacilli is quite confusing. Runyon,[135] in his address to the International Conference on Atypical Mycobacteria, probably defined them best: "Tubercle bacilli include *Mycobacterium tuberculosis*, *Mycobacterium bovis*, and *Mycobacterium africanum*. Together with *Mycobacterium microti* (not pathogenic for humans), these organisms constitute the tubercle bacillus complex." Any mycobacterium not listed in this group is considered to be in the "other" grouping. Mycobacteria other than those causing tuberculosis and leprosy were not recognized as causes of disease in humans until the 1950s.[165] The incidence of disease caused by these organisms remained stable until the acquired immunodeficiency syndrome (AIDS) epidemic began in the 1980s. The most common forms of the disease are chronic pulmonary disease resembling tuberculosis (occurring mainly in adults), cervical adenopathy in children, skin and soft tissue infection, and disseminated disease in immunocompromised individuals.[120] In the mid-1980s, the incidence of infections with atypical mycobacteria increased markedly, probably because of the increased number of immunocompromised patients (e.g., because of AIDS and organ transplantation) and the significant improvement in microbiologic methods for cultivating these organisms.[186,191]

EPIDEMIOLOGY

The atypical mycobacteria are ubiquitous in nature. They are found in soil, animals, milk,[42] and food. Of importance in some hospital-acquired infections or infections in immunocompromised hosts is the presence of the organisms in common tap water.[16,59,109,157] Exposure to environments (especially soil) colonized by these organisms seems to be important for acquisition of disease in children. Organisms commonly found in soil include *Mycobacterium scrofulaceum*, *Mycobacterium flavescens*, *Mycobacterium avium–intracellulare* (MAI), *Mycobacterium gastri*, *Mycobacterium terrae*, *Mycobacterium fortuitum*, and *Mycobacterium chelonae*. Water is an important source for all of the previously named organisms and for *Mycobacterium kansasii*, *Mycobacterium marinum*, *Mycobacterium gordonae*, and *Mycobacterium xenopi*.

In contrast, humans are the only known reservoirs for *M. tuberculosis*. In a survey from the Centers for Disease Control and Prevention (CDC), 35 percent of mycobacteria isolated in laboratories were nontuberculous.[53] MAI accounted for 66 percent of the nontuberculous isolates, followed by *M. fortuitum* (19%), *M. kansasii* (9%), and *M. scrofulaceum* (6%). These data were collected in 1980 before the present AIDS epidemic and before the rapid increase in the number and types of immunocompromised patients; therefore, current rates probably are much higher.

Geography also apparently has some bearing on the prevalence of these infections. The southeastern part of the United States has much higher rates in children than does the Northeast or the Northwest. *M. avium* complex (MAC) was seen most commonly along the coastal borders of the United States and in the states bordering Canada. The highest rates were seen in Hawaii (10.9 cases per 100,000 population), Connecticut (8.9 cases per 100,000 population), and Florida (8.4 cases per 100,000 population). High rates also were seen in Kansas and the desert

Southwest, however, showing the widespread nature of this organism in causing disease. In contrast, *M. kansasii* was seen most commonly in the Midwest of the United States and almost never was seen in the Southeast.[53] States with rates of *M. kansasii* greater than 0.75 case per 100,000 included Missouri, Illinois, Kentucky, Indiana, Kansas, Nebraska, Louisiana, Texas, Arizona, and Florida. North Carolina, a state with one of the highest rates of MAC (>4.8 cases per 100,000 population), had very few cases of *M. kansasii* (0 to 0.25 case per 100,000 population). *M. marinum* frequently was isolated from coastal areas, whereas *M. xenopi* was scattered across the United States, with 50 percent of cases being found in just three states (Connecticut, Wisconsin, and California).

For all the nontuberculous species, males and rural residents had a much higher incidence of infection.[141] Extrapolation of these rates to determine disease patterns is not without problems, however. The number of mycobacterial isolates could be skewed easily by the presence of multiple isolates from a single patient, or it may just represent colonization. Nonetheless, these rates can help predict which species of nontuberculous mycobacteria are most likely to be encountered in a particular geographic region while the physician awaits final identification and sensitivity testing on an isolated nontuberculous organism.

The site of isolation from the human source can be helpful in determining the type of mycobacteria that may be involved in the disease process. *M. avium* and *Mycobacterium intracellulare* are responsible for lymphadenitis, particularly in children. These two organisms also are responsible for pulmonary disease and disseminated disease to bones and occasionally to the meninges. *M. kansasii* is associated most commonly with infections of pulmonary origin and disseminated lesions in adults; rarely, it is associated with adenitis and skin granuloma in children. *M. scrofulaceum* also causes lymphadenitis in children, and *M. marinum* is responsible for skin granuloma and ulcers after exposure to certain salt-water beaches, swimming pools, and tropical fish tanks. The rapid growers (*M. fortuitum* and *M. chelonae*) rarely are responsible for pulmonary and disseminated disease in adults and children.

A familial immune defect predisposing to disseminated atypical mycobacterial infection in childhood has been reported.[96] The six children studied had disseminated infection with atypical mycobacteria and no obvious evidence of immunodeficiency. Clinical and immunologic features seem to indicate that these children acquire infections similar to Lsh/Ity/Bcg-susceptible mice. Ongoing studies to determine the defect may provide insight into the mechanisms by which certain children are susceptible to mycobacterial infections, whereas others exposed to the same environmental factors do not develop disseminated disease.

MICROBIOLOGY

Runyon and Timpe[133,134,165] in their monumental work on atypical mycobacteria suggested a useful classification system based on three characteristics of the organisms: production of pigment, rate of growth, and colonial characteristics. The four groups are as follows: group I—photochromogens, which produce bright yellow to red pigment in the presence of light; group II—scotochromogens, which produce yellow to orange pigment in the dark; group III—nonphotochromogens, which are nonpigment producers; and group IV—rapid growers, which generally grow in less than 1 week. Kubica[89] published an updated version of Runyon's classification in 1978, and it was useful for many years. Subsequently, a more "simplified" classification based on the growth rates of the organisms alone was proposed.[186]

The atypical mycobacteria still are differentiated by most clinical laboratories on the basis of various morphologic, physiologic, and biochemical characteristics (Tables 108–1 and 108–2). The difficulty with identifying many of these organisms is their slow growth rates with standard techniques. Beginning sensitivity testing as soon as an organism is cultivated and thought to be the responsible pathogen is useful because several months may be required for proper identification and susceptibility testing. Serologic tests can be helpful in identifying some of the

TABLE 108–1 Characteristics of Slow-Growing Pathogenic Mycobacteria

Organism	Growth Present (° C)			Growth Rate (Days)	Niacin	Nitrate Reduction	Pigment Dark	Pigment Light	Growth in 5% NaCl
	25	*37*	*45*						
Mycobacterium tuberculosis	–	+	–	12-28	+	+	–	–	–
Mycobacterium bovis	–	+	–	21-40	–	–	–	–	–
Photochromogens, Runyon group I									
Mycobacterium kansasii	+	+	–	10-21	–	+	–	+	–
Mycobacterium marinum	+	±	–	7-14	–	–	–	+	–
Scotochromogens, Runyon group II									
Mycobacterium scrofulaceum	+	+	–	10-28	–	–	+	+	–
Mycobacterium szulgai	+	+	–	12-28	–	+	+	+	–
Mycobacterium gordonae	+	+	–	10-28	–	–	+	+	–
Nonchromogens, Runyon group III									
Mycobacterium avium	+	+	+	10-21	–	–	–	–	–
Mycobacterium intracellulare	+	+	–	10-21	–	–	–	–	–
Mycobacterium ulcerans	–	–	–	28-60	–	–	–	–	?
Mycobacterium xenopi	–	+	–	14-28	–	–	–	–	–

TABLE 108–2 Characteristics of Rapid Mycobacterial Growers

Runyon Group IV	Optimal Temperature	Growth Rate	Niacin	Nitrate Reduction	Growth in 5% NaCl
Mycobacterium fortuitum	37° C	3-7 days	–	+	+
Mycobacterium abscessus	37° C	3-7 days	–	–	+
Mycobacterium chelonae	37° C	3-7 days	–	–	–

organisms.[35,90,133,168,176,186,187,191] In clinical medicine, they rarely are valuable, however, for establishing a diagnosis in an individual patient. A study using humoral immunoglobulins against mycobacterial antigens failed to diagnose tuberculosis or nontuberculous adenitis in children.[169]

Blood cultures for mycobacteria are best performed with the Isolator lysis-centrifugation system (Wampole Laboratories, Cranbury, NJ) or the radiometric BACTEC 13A blood culture bottle (Becton-Dickinson Diagnostic Instrument Systems, Cockeysville, MD).[1,81,82] Blood collected in ethylenediaminetetraacetic acid or coagulated blood is unacceptable. Body fluids such as cerebrospinal fluid, pleural fluid, and peritoneal fluid can be inoculated directly into BACTEC or Septi-Chek (Becton-Dickinson) broth, particularly if only small volumes are available. Gene probes became commercially available in the late 1980s. They involve DNA probes complementary to species-specific sequences of rRNA for the identification of *M. tuberculosis*, MAC, *Mycobacterium gordonae*, and *M. kansasii* (AccuProbe; Gen-Probe, San Diego, CA).[55]

Polymerase chain reaction (PCR) assay has been evaluated for the routine detection of *M. tuberculosis* in the clinical laboratory and compared with fluorochrome smear and culture. Two large studies compared PCR with the standard technique and found that the sensitivity of PCR assay was 84 percent in both studies.[28,50] In another study, a multiplex PCR assay for immediate identification of various *Mycobacterium* spp. was shown to be 97.9 percent sensitive and 96.9 percent specific.[87] The PCR testing offered by commercial laboratories at present is not approved by the U.S. Food and Drug Administration for in vitro diagnosis. The sensitivity and specificity of this test vary widely among laboratories.[118]

MANIFESTATIONS OF NONTUBERCULOUS MYCOBACTERIAL INFECTION IN CHILDREN

LYMPHADENITIS

Lymphadenitis is the most common manifestation of atypical mycobacterial infection in children. It also can occur rarely in adults.[37,94,101] All nodes in the cervical chain can be affected, but the nodes of the submandibular region seem to be the ones most commonly involved.[39] The parotid gland also can be affected.[32] The differential diagnosis frequently centers on deciding whether a malignant process versus a nonmalignant process is present. Nonmalignant processes to consider include mononucleosis, bacterial adenitis, cat-scratch disease, toxoplasmosis, and *M. tuberculosis* infection.

Most mycobacterial cases of lymphadenitis are caused by the nontuberculous organisms. The clinician also must consider the possibility of *M. tuberculosis*, however.[30] No clinical features help the clinician discern between nontuberculous mycobacterial infection and tuberculosis. The use of histopathology has been postulated to help differentiate between atypical and tuberculous infections.[124] In this retrospective study, the findings of ill-defined (nonpalisading) granulomas, irregular or serpiginous granulomas, a predominantly nonspecific granulomatous response, predominantly sarcoid-like granulomas, or lack of significant caseation were seen more commonly with nontuberculous lymphadenitis. Additionally, nontuberculous infections had neutrophils predominantly in the center of the necrosis, whereas in tuberculous infections, neutrophils were scattered throughout the specimen. A prospective study investigating these findings has not been published to date, and other authors have not noted similar patterns.[11]

In a Canadian study, the rate of atypical mycobacteria as a cause of lymphadenitis was 1.21 cases per 100,000 children, whereas the rate for tuberculosis was 0.3 case per 100,000 children.[147] Investigators from San Diego reported a marked increase in the number of infections in children.[125] Other authors around the world have reported a markedly increased incidence of infection with these organisms in immunocompetent children.[52] In one study, the incidence increased from one case between 1987 and 1990 to 85 cases between 1991 and 1993.[93] In a large study compiled by Lincoln and Gilbert[100] involving 243 children, more than 50 percent were younger than 3 years of age, and 80 percent were younger than 5 years. In contrast, one study showed that the mean age had increased to 5.2 years.[183]

Most patients have no systemic symptoms and normal chest radiographs; other laboratory studies generally are not helpful. The mean duration of swelling is approximately 6 weeks. Historically, the cervical nodes are affected most commonly in children, although a more recent study from Greece reported submandibular prominence.[107]

Infections are caused mainly by MAI and *M. scrofulaceum*. A large prospective study spanning 32 years from 1958 to 1990 showed that MAC has become the predominant etiologic agent and has surpassed *M. scrofulaceum* from earlier in the study.[189] A series of 190 patients from India showed, however, that 60 percent of the nontuberculous adenitis cases were caused by *M. scrofulaceum* followed by 40 percent by *M. avium* intracellulare.[77] Case reports involving *M. fortuitum* also have been published.[131] Lymphadenitis caused by *Mycobacterium haemophilum* is being diagnosed in immunocompetent children in increasing numbers.[7,138]

Samra and colleagues[138] showed that the BACTEC radiometric system or MB Redox broth (Heipha Diagnostika Biotest, Heidelberg, Germany) is superior to Löwenstein-Jensen (Heipha Diagnostika Biotest) media for isolation of *M. haemophilum*. This organism is thought to have been underdiagnosed previously because of the widespread use of Löwenstein-Jensen media. *Mycobacterium malmoense* also has been described as an etiologic agent of cervical lymphadenitis in children.[167] Haas and associates[62] have described *Mycobacterium heidelbergense*, a new agent of mycobacterial lymphadenitis in children.

The usefulness of mycobacterial antigens in skin testing for the diagnosis of atypical mycobacterial infection versus tuberculous lymphadenitis is controversial. The use of purified protein derivative type B (PPD-B) (*M. intracellulare*), PPD-Y (*M. kansasii*), PPD-G (*M. scrofulaceum*), and PPD-T (*M. tuberculosis*) was compared to discern whether children with active lymphadenitis caused by the atypical mycobacteria could be distinguished from children with tuberculosis.[74] Children with confirmed nontuberculous adenitis were six times more likely to have greater than 10-mm induration to PPD-B than were children with negative culture or biopsy results. In all groups except those with confirmed *M. tuberculosis*, responses to PPD-T were significantly smaller than responses to the nontuberculous mycobacterial antigens. The results of the study seem to indicate that the use of nontuberculous mycobacterial antigens could be helpful in diagnosing mycobacterial cervical adenopathy. The specificity for nontuberculous organisms is unknown, however.

Needle aspiration of an affected node can be a valuable diagnostic tool. Cultures for bacterial and mycobacterial etiologies should be performed, and recovery rates in children with cervical lymphadenitis range from 60 to 88 percent.[9,14,80,193] The aspirate should be inoculated onto aerobic and anaerobic media and Sabouraud agar and mycobacterial media (Löwenstein-Jensen slants or Middlebrook media). Use of the BACTEC system can be very helpful; mycobacteria can be isolated 12 to 17 days after inoculation.[152]

More recently, preoperative diagnosis of *M. avium* lymphadenitis was accomplished with the use of PCR of gastric aspirates in two children.[61] Further studies are needed to conclude whether PCR could be a noninvasive method of diagnosing this infection.

The best treatment of lymphadenitis caused by atypical mycobacteria is complete excision of the involved lymph node.[108,172] Incision and drainage without excision result in a high rate of secondary drainage, and subsequent excision of the remaining tissue is required for cure.[4,137,144] Small uncontrolled trials have attempted to use antimicrobials alone for lymphadenitis caused by nontuberculous mycobacteria.[102] Preliminary results have been successful, but use of toxic agents for prolonged periods was required. Controlled trials have not been published.

ACQUIRED IMMUNODEFICIENCY SYNDROME AND ATYPICAL MYCOBACTERIA

Surveys have noted an increasing rate of nontuberculous infection in children with AIDS.[73] Approximately 6 percent of adults and 4 percent of children with AIDS reported to the CDC had disseminated MAC infection as AIDS-defining disease.[20] Autopsy studies show that MAC infection is present in 20 to 50 percent of adult human immunodeficiency virus (HIV)–infected patients.[129,182,185] In a retrospective study from 1990 to 1996 in New York City, 26 percent of children had evidence of MAC infection at the time of their deaths.[78] MAC infection was thought to be the cause of death in 13 percent of the 54 children with HIV infection, and the mean age at death was 7.8 years. In two autopsy studies of opportunistic infection in HIV-infected children in Latin America and Argentina, only 2.7 percent and 3.4 percent had evidence of disseminated disease.[41,127] In these studies, 62 percent (Argentina) and 72 percent (Latin America) of the children studied were younger than 1 year of age. These data indicate that MAI infection is seen less commonly in younger children with HIV.

In the CDC study, MAC was the nontuberculous mycobacterium most commonly isolated. *M. kansasii* and *M. scrofulaceum* were found in one case each. In adult studies, the incidence of infection with *M. kansasii* and *M. scrofulaceum* also is quite low.[38,72] More than 70 percent of children with MAC and AIDS had evidence of disseminated disease. Almost all had $CD4^+$ counts less than 100 cells/mm^3. Clinical findings included failure to maintain growth curves, anorexia, fever, abdominal pain, and anemia. The median age at diagnosis was 46 months, with a median of 9 months elapsing between the onset of symptoms and positive cultures. When nontuberculous mycobacterial infection was diagnosed in these patients, they survived less than 10 months. Blood cultures have 90 to 95 percent sensitivity in detecting disseminated MAC infection in adult patients with AIDS.[65]

MAI has been shown to infect the esophagus, stomach, and intestine of pediatric patients with AIDS.[79] Patients with extensive MAI infection of the small and large intestines have severe, persistent diarrhea.

Mycobacterium genavense has been shown to cause infection in children with HIV infection.[117] The children were febrile and had abdominal cramps and diarrhea. $CD4^+$ lymphocyte counts were less than 400/mm^3. The organism was found in numerous stool samples and lymph node specimens. Multiple-drug regimens that include amikacin, ethambutol, rifampin, and clarithromycin may be useful in treating this infection.

For treatment of MAC infection in HIV-infected children, combination therapy is recommended.[6] Clarithromycin (15 mg/kg/day divided into two oral doses, maximum of 500 mg) and ethambutol (15 to 20 mg/kg/day in a single dose, maximum of 1600 mg) should be included. Additionally, the use of rifabutin (5 to 10 mg/kg/day once daily, maximum of 300 mg), ciprofloxacin (20 to 30 mg/kg/day intravenously or orally once daily, maximum of 1.5 g), or azithromycin (10 mg/kg once daily) can be considered. Because of their liquid form, clarithromycin and azithromycin have become available therapy for many infections in pediatric patients, including otitis media, pharyngitis, and skin infections.[85] The use of granulocyte colony-stimulating factor as an adjunct to antimicrobial therapy for disseminated MAC infection has been reported,[121] but additional data are needed to determine the usefulness of this agent.

For secondary prevention of recurrent disease, lifelong prophylaxis with clarithromycin is recommended (15 mg/kg/day in two divided doses, maximum of 500 mg), in combination with at least one of the following: ethambutol (15 to 20 mg/kg/day once daily), rifabutin (5 mg/kg/day once daily, maximum of 300 mg), or ciprofloxacin (20 to 30 mg/kg/day in two divided doses, maximum of 1.5 g).

The use of rifabutin or azithromycin (5 mg/kg/day once daily, maximum of 250 mg, or 20 mg/kg once weekly) for prophylaxis has been recommended based on adult studies in an attempt to delay and prevent MAC bacteremia in adults with $CD4^+$ cell counts less than 100 cells/mm^3. The U.S. Public Health Service and the Infectious Disease Society of America have published guidelines for the prevention of opportunistic infections, including disseminated MAC.[170] These guidelines incorporate age-specific $CD4^+$ counts at which prophylaxis should be used: children 6 years or older, less than 50 cells/μL; children 2 to 6 years old, less than 75 cells/μL; children 1 to 2 years old, less than 500 cells/μL; and children younger than 12 months, less than 750 cells/μL. Azithromycin (20 mg/kg once weekly) seems to be effective. A phase I/II study of prophylactic rifabutin for prevention of disseminated MAC infection in children showed a side effect of bilateral, stellate, corneal deposits without associated uveitis in 6 of 25 children.[149]

The emergence of resistance is a concern with the MAC prophylactic regimens in patients with AIDS. In some trials, 9 percent of adults receiving azithromycin prophylaxis and 5 percent of adults receiving clarithromycin prophylaxis had breakthrough MAC bacteremia. Of these cases, 11 percent and 58 percent of the azithromycin and clarithromycin, respectively, breakthrough isolates were macrolide-resistant.[5,64]

PULMONARY INFECTIONS

Reviews from the late 1970s showed that pulmonary disease caused by atypical mycobacteria usually was caused by *M. kansasii* and MAC.[26,54,132] MAC infection most commonly occurred during the sixth decade, whereas *M. kansasii* infection occurred in individuals a decade younger. Men were affected most commonly, in ratios as high as 4:1. Chronic obstructive pulmonary disease as noted by radiographic findings was found in 50 to 60 percent of patients with atypical mycobacterial pulmonary infections. Bullous lung disease was seen in 24 to 39 percent. The lobar distribution and severity of disease were similar for atypical mycobacterial agents and *M. tuberculosis*, with one exception: *M. kansasii* was much more prone to produce unilateral disease, which occurred nearly 60 percent of the time compared with 35 percent of the time in MAC or *M. tuberculosis* infection. Typically, disease begins in the posterior portions of the upper lobes. Progression to cavitary disease occurred in 87 percent of patients with *M. tuberculosis* and MAC infections and in 96 percent of patients with *M. kansasii* infection. Rare case reports of mediastinal mass lesions in children caused by nontuberculous mycobacteria have been published.[48]

Hilar and mediastinal adenopathy was an uncommon finding, particularly with MAC (4%) and *M. kansasii* (0.5%). Pleural effusions were rare, occurring in 6 percent of patients with MAC and 4 percent with *M. kansasii*. Treatment at that time generally

involved three to four courses of drug therapy, including regimens of isonicotinic acid hydrazide, *p*-aminosalicylic acid, streptomycin, and rifampin. The American Thoracic Society recommends a four-drug regimen for MAC pulmonary infection in adults: isoniazid (300 mg), rifampin (600 mg), ethambutol (25 mg/kg for 2 months, then 15 mg/kg), and streptomycin (0.5 to 1 g five times each week for 8 to 12 weeks, then 0.5 to 1 g two or three times each week for 3 months, as tolerated).[177] Drugs are administered for 18 to 24 months, with a minimum of 12 months of culture negativity required while receiving therapy. A previous clinical investigation revealed success rates of 25 to 80 percent, and the best response rate was noted with a three-drug regimen of ethambutol, ethionamide, and cycloserine.[44] Resectional surgery seemed to have poor outcomes. For *M. kansasii* pulmonary infection, the American Thoracic Society recommendations include isoniazid (300 mg/day), ethambutol (15 mg/kg/day), and rifampin (600 mg/day) for 18 months in adults.

Patients with cystic fibrosis have been noted to have an increased incidence of infection with nontuberculous mycobacteria. Prevalence rates of 2 to 20 percent have been reported.[3,67,68,84] A study from France involving children aged 1 to 18 years old indicated that mycobacterial species were isolated from 6.6 percent of routine sputum samples, and that 1.9 percent had documented mycobacterial lung infection.[47] Mycobacterial infections are seen more commonly in older patients with cystic fibrosis. Frequent intravenous antibiotic use is a possible risk factor for colonization with nontuberculous mycobacteria.[166] Organisms most commonly found are MAC, *M. kansasii*, *M. fortuitum*, and *M. chelonae*. *Mycobacterium abscessus* has emerged as a clinically persistent pathogen in patients with cystic fibrosis, especially patients on steroid therapy for allergic bronchopulmonary aspergillosis.[46,116] Bacterial contamination, particularly with *Pseudomonas aeruginosa*, of the acid-fast bacilli cultures from patients with cystic fibrosis has been a major problem that has rendered isolation of mycobacteria more difficult.[150] Another confounding problem is the difficulty in differentiating infection from colonization in these patients.[84]

The American Thoracic Society recommends radiographic changes, isolation of multiple colonies of the same species, and the absence of other potential pathogens as criteria for the diagnosis of pathogenic infection with nontuberculous mycobacteria.[2] Discernment is even more difficult in the setting of the chronic lung disease seen in patients with cystic fibrosis. Kilby and associates[84] suggest that repeated isolation of nontuberculous mycobacteria associated with pulmonary cavities or infiltrates that do not improve with aggressive standard antibacterial treatment could indicate active mycobacterial disease in patients with cystic fibrosis.

M. xenopi has been described in adults as a cause of infection of the pulmonary tract.[148] The mean age at infection was 62 years, and it occurred in a mainly Canadian population. Eighty-six percent of the patients had underlying pulmonary pathology, including chronic obstructive pulmonary disease, previous pulmonary tuberculosis, carcinoma of the lung, sarcoidosis, and cystic fibrosis. Additionally, a case was reported of a 7-year-old boy with leukemia in whom pneumonia with *M. xenopi* developed and was treated successfully with 2 years of therapy that included ethambutol and clarithromycin.[95]

Mycobacterium simiae has been isolated in adults with underlying pulmonary abnormalities.[10,88] *M. simiae* is the most drug-resistant of all the nontuberculous mycobacteria. Some isolates are resistant even to all drugs tested.

Mycobacterium szulgai has been described in several case series as a cause of lung disease.[106,180] Disease occurred in elderly white men and resembled chronic tuberculosis. Therapeutic regimens effective against *M. avium* seemed to provide good clinical outcomes.

SKIN INFECTIONS

Mycobacterium marinum

M. marinum is photochromogenic and was identified as a pathogen in fish in 1926 by Aronson.[100] The skin lesions usually result from light trauma (abrasions) in swimming pools or other bodies of water when the surfaces of the pool are colonized by *M. marinum*.[34,113] Fish tanks also have been implicated and generally involve the upper limb or a finger.[8,162] Most cases occur in children aged 10 to 16 years old. The most common sites are the elbows, knees, and ankles. Cooler superficial portions of the body are affected most frequently. Other exposed body areas can be involved, depending on what part of the body has made contact with the surface containing the mycobacterium (e.g., the nose in divers).[114] Regional spread of lesions has been reported.[154]

The incubation period from exposure to formation of a small indurated area that ulcerates generally is 3 weeks. The lesion then crusts and forms a granuloma with a small crater. The lesions usually are painless and resolve in several months; occasionally, they can last longer. In contrast to other mycobacterial diseases, regional nodes are not involved.

Infection with this organism usually is benign. A main consequence is, however, that patients with *M. marinum* infection frequently have conversion of their PPD-T test to positive.[100] The natural reservoir for *M. marinum*, which requires a cool incubator (32° C), is in fish and other cold-blooded animals. Generally, only a few organisms may be isolated in some granulomas.

Treatment of *M. marinum* has been successful with rifampin and ethambutol,[171,190] and one report showed good results with rifampin alone.[40] An accompanying editorial cautioned against the use of rifampin alone, however, and recommended using rifampin with ethambutol.[17] The duration of therapy, according to the literature, varies from several weeks to 18 months. Generally, response to therapy is rapid, and treatment should be continued for 4 to 6 weeks after clinical resolution.[40]

Mycobacterium ulcerans

In 1948, MacCallum[105] reported the first cases of disease caused by *Mycobacterium ulcerans*. Most cases since then have occurred in remote, tropical, or subtropical areas of the world, including parts of Africa and Australia.[104,130] In a 1-year period, 23 cases of Buruli ulcer caused by *M. ulcerans* occurred in Lambarene (Gabon).[19] Cases also have been reported in Mexico.[100] The natural reservoir for *M. ulcerans* is unknown, although one report suggests the spines of a tall prickly grass known as *Echinocloa pyrimidalis*.[155] The lesions caused by *M. ulcerans* occur mainly on the cooler superficial portions of the body. Patients harboring this organism are in otherwise good health without underlying immunodeficiency.[27] Scraping of the skin by thorns or pieces of wood has been implicated as the route of inoculation in many of the cases. The organism is very fastidious, with growth seen only between 30° C and 35° C.

The incubation period for this painless infection also is approximately 3 weeks. Regional lymphadenitis rarely occurs with this organism. The infection has three distinct stages, and knowledge of them can be helpful in making the diagnosis and providing treatment. The disease begins as a hard, mobile nodule. It frequently is associated with pruritus, and in Zaire is termed *mputa matadi* (the itching stone). This stage is known as the preulcerative stage.

In some patients, the infection resolves on its own, but in others, it progresses to the ulcerative stage. In contrast to *M. marinum*, in which the lesions are short-lived and do not progress past the ulcerative stage, the lesions of *M. ulcerans* usually last 6 to 9 months and frequently progress and lead to deformities of

limbs that may require amputation.[100] The organisms can be isolated in large numbers from the periphery of ulcers adjacent to normal tissue, which again contrasts with infection by *M. marinum*, in which very few organisms are found in the lesions.

The infection generally involves subcutaneous adipose tissue and leads to areas of fat necrosis, which then proceeds to overlying necrosis of the adjacent skin. The lesions can become enormous, sometimes involving a complete limb. Although numerous organisms are seen in the progressing edge of the infection, little evidence of a cellular immune response is apparent. Additionally, anergy to skin reagents prepared from *M. ulcerans* (burulin) frequently is noted at this stage.[156] Finally, the next stage, called the *reactive phase*, is reached. Cellular infiltrates with granuloma formation occur in the lesion. The number of organisms in the lesion decreases dramatically, and a positive skin test reaction develops to the burulin. Infection with *M. ulcerans* also can cause conversion of a patient's PPD response to positive; however, conversion is seen in only approximately 50 percent of cases.[27] Finally, healing may occur, but with fibrosis left in its wake.

Treatment of *M. ulcerans* infection is anecdotal at best. Treatment choices are based on the stage of infection. Lesions in the pre-ulcerative stage are best treated with excision and primary closure.[57] Successful therapy in the anergic progressive stage is considered the most difficult and involves appropriate antimycobacterial therapy for the infection. Success has been reported with isonicotinic acid hydrazide and streptomycin or diaminodiphenylsulfone and oxytetracycline combinations[27] and sulfamethoxazole, rifampin, minocycline,[153] and clofazimine.[104] In the final stage, healing should be promoted with as little deformity or loss of function as possible, the use of skin grafting and splinting, and excision of fibrous tissue.[57,130] Disseminated infection in an immunocompetent child along with the development of multifocal osteomyelitis has been described.[69] (See also Chapter 109.)

Other Mycobacteria in Skin Disease

M. haemophilum has produced painful subcutaneous nodules in immunocompromised patients, particularly patients with renal transplants.[36,188] In addition, *M. haemophilum* has produced disseminated disease, including bacteremia, osteomyelitis, and pulmonary disease, in immunocompromised patients.[160] *M. chelonae* has been found to be a cause of disseminated cutaneous infection.[175] Steroid use is the predisposing factor for infection with this organism. *M. fortuitum* has been implicated in cutaneous lesions in a child involved in a motor scooter accident, with the subsequent development of lesions at the site of knee lacerations; regional adenopathy of the inguinal nodes also developed.[146] *M. fortuitum-chelonae* complex has been responsible for superficial skin abscesses in children.[13] *M. fortuitum* in adult studies also has been implicated in severe infections in immunocompromised hosts; these infections usually are rapidly disseminating, with high mortality.[188] *M. avium* has been isolated from an eyelid abscess with drainage.[143] Treatment of cutaneous disease caused by these organisms is difficult at best. Four- or five-drug therapy has been tried, but with poor results.

ORGANISMS SEEN IN CHILDREN

Specific organisms and treatment guidelines are discussed in the following sections. Table 108–3 provides a quick guide to some of the more commonly used antimycobacterial agents.

MYCOBACTERIUM AVIUM–INTRACELLULARE COMPLEX

MAC consists of *M. avium* and *M. intracellulare*. These organisms are slow-growing, obligate aerobes that require 2 to 6 weeks for colony formation on solid media. Colonies usually are smooth but may be rough and can be transparent or opaque. These organisms grow on routine bacterial media, but growth is achieved best on selective mycobacterial media, such as Löwenstein-Jensen medium or Middlebrook 7K10 and 7K11 agar. Nucleic acid hybridization probes using target sequences of ribosomal RNA are available commercially for rapid identification of clinical isolates.[99,115] MAC infection is diagnosed most commonly by culture of blood or bone marrow.

TABLE 108–3 Antimycobacterial Agents*

Drug	Dosage	Form
Amikacin (A)	15-20 mg/kg/day divided q8h	IV or IM
Azithromycin (Z)	500 mg bid (adults/adolescents); 10-12 mg/kg/day (children)	PO
Cefoxitin (X)	80-160 mg/kg/day divided q4-6h	IV or IM
Ciprofloxacin (C)	20-30 mg/kg/day divided q12h (adults only in U.S.)	PO or IV
Clarithromycin (CL)	15-30 mg/kg/day divided q12h	PO
Clofazimine (CLO)	1-2 mg/kg/day	PO
Doxycycline (D)	2-4 mg/kg/day divided q12h (>8 yr old)	PO, IV
Ethambutol (ETB)	15-25 mg/kg/day	PO
Ethionamide (ETH)	10-20 mg/kg/day divided q12h	PO
Isoniazid (I)	10-14 mg/kg/day	PO
Pyrazinamide (PZA)	15-30 mg/kg/day	PO
Rifabutin (RIB)	5-10 mg/kg/day; maximum of 300 mg/day (adults)*	PO
Rifampin (RIF)	10-20 mg/kg/day divided q12-24h	PO or IV
Streptomycin (S)	20-30 mg/kg/day	IM

Infections
Disseminated MAC: HIV infected: CL (or Z) + ETB (± RIB)
Disseminated MAC: HIV-negative, immunocompromised: RIF + ETB + INH + S or A
Mycobacterium abscessus: CL alone or A alone
Mycobacterium chelonae: A ± CLO or CL alone
Mycobacterium fortuitum: A + X + probenicid or A + C + sulfonamide
Mycobacterium kansasii: RIF + ETB + I
Mycobacterium marinum: ETB + RIF, or D or TMP-SMX

**Not approved for use in children.*
HIV, human immunodeficiency virus; MAC, M. avium complex; TMP-SMX, trimethoprim-sulfamethoxazole.
Data based on references 60, 73, 97, 98, 136, 171, 177, 179, 188, and 190.

In a study of 56 isolates from pediatric patients involving sequence analysis of the ribosomal internal transcribed spacer, Hazra and colleagues[66] showed that the closely related Mav-B and Mav-A sequevars caused most disease. Patients from geographically diverse areas of the United States (Boston, Miami, and Los Angeles) had isolates with closely related patterns. The finding of related strains causing disease in epidemiologically unrelated patients is most consistent with two hypotheses: a similar subset of *M. avium* strains is more virulent and more likely to cause disease in humans, and pathogenic strains are more prevalent in the environment.

M. avium was recognized in 1890 as the causative agent of disease in chickens.[188] *M. intracellulare* was designated in 1967 and at the time was difficult to distinguish routinely from *M. avium*-thus the name *M. avium-intracellulare*. Today, with the use of DNA probes, most seroagglutination types have been discerned between the two groups. *M. avium* is the most common nontuberculous mycobacterium causing disease in humans, but isolates from environmental sources are more likely to be *M. intracellulare*. Both organisms can be found in birds, soil, dust, and fresh or salt water. Infections caused by MAC strains isolated from adult patients with AIDS could be identified as either serotype 4 or 8, in contrast to patients without AIDS, in whom no predominant serotype has been identified.[83,192] Nearly all isolates of MAC from patients with AIDS have been identified as *M. avium*; in patients without AIDS, the rate of *M. avium* is approximately 55 percent, and the rate of *M. intracellulare* to 32 to 40 percent.[60,192]

Lung disease has been the major manifestation of MAC infection in nonimmunocompromised adults. Most investigators thought that MAC infection occurred mainly in patients with deficient immunity or underlying lung disease. Later reports seemed to indicate, however, that normal adult hosts are at risk for development of infection with MAC, and that rates are increasing.[76,126] Case reports involving children are lacking in detail because they usually appear within discussions of adult patients.[128]

Pediatric case reports of disseminated disease caused by MAI/MAC have appeared in the literature. Children have had ulcerative lesions of the colon[33]; mesenteric disease with abscess formation[143]; hematogenous spread to the liver, spleen, kidneys, and adrenal cortex; lesions of the epididymis[151]; bone lesions[174]; and skin lesions. Disseminated osteomyelitis rarely is caused by nontuberculous mycobacteria, but if it occurs, *M. intracellulare* most commonly is isolated.[25,86] Septic arthritis also has been reported in association with osteomyelitis.[51] Immunocompromised patients with disseminated MAI infection historically require multiple-drug therapy, including a combination of isoniazid, ethambutol, clofazimine, and rifabutin.[97] Some reports indicate, however, that disseminated disease in HIV-infected patients may respond to only two agents, as mentioned earlier in the AIDS section. The addition of other agents may be necessary because of the high incidence of resistant organisms. A preliminary report showed that interferon-γ may be effective when combined with conventional therapy in some patients who are refractory to standard chemotherapy alone.[71]

Bacterial peritonitis is a common occurrence in patients regularly undergoing ambulatory peritoneal dialysis for chronic renal failure. Reports of nontuberculous mycobacteria causing peritonitis have been noted.[63,122,184] In cases involving nontuberculous mycobacteria with foreign bodies, such as Tenckhoff catheters, the development of infected sinus tracts is frequent. Additionally, antituberculous drug regimens in these cases generally are unsuccessful. Although the mycobacteria were sensitive to the agents used, the patients continued to have sinus tract drainage without improvement, even after removal of the foreign body.

MYCOBACTERIUM SCROFULACEUM

M. scrofulaceum has many characteristics similar to *M. avium* and *M. intracellulare* and can be found in soil, water, and dairy products. This organism is associated most commonly with lymphadenitis in children 1 to 5 years old and rarely causes other manifestations in humans. Skin and bone lesions have been reported in two children chronically infected with *M. scrofulaceum* for 10 years.[43,181] Few data are available on chemotherapeutic agents for treatment of infection with this organism. Based on susceptibility patterns, three or more drugs may be necessary for treatment of serious disease. Lymphadenitis can be cured with complete excision of the lymph node.

MYCOBACTERIUM KANSASII

Of the photochromogens, *M. kansasii* is the one most commonly isolated in humans. In contrast to MAI and *M. scrofulaceum*, *M. kansasii* rarely is isolated in soil, but has been cultured from water and milk.[22,23] Chronic pulmonary infection is the most common manifestation of this disease and is seen mainly in adults, particularly adults with AIDS. A survey from Israel of 56 adults with *M. kansasii* pulmonary infection showed that 64 percent occurred in men, 59 percent had underlying lung disease, and none were HIV-infected.[145] Pulmonary disease occurs infrequently in children. Some children have a course similar to that of adults, with underlying pulmonary disease caused by previous tuberculosis or chronic pulmonary disease.[12,112] In contrast, other children have acute symptoms of classic bacterial pneumonia with an abrupt onset of fever and sputum production and lung consolidation on physical examination and radiographs.[12,21] Pleural effusions also can occur.

In contrast to some other nontuberculous organisms, *M. kansasii* is sensitive to most of the antituberculous drugs, particularly rifampin. Most authorities recommend the use of three drugs, including rifampin, isoniazid, and ethambutol. The treatment course usually requires a minimum of 12 months, with therapy for 24 months needed in some patients. A study of 18 patients showed that thrice-weekly clarithromycin, ethambutol, and rifampin was effective, with a mean follow-up of 46 months.[58] Patients with AIDS and *M. kansasii* infection have responded to this three-drug regimen, but the total duration of therapy is unknown at this time. In children with AIDS, the diagnosis of *M. kansasii* infection is rare, and the clinical response to therapy has been poor.[73] Cases have been reported in other immunocompromised children, including a 7-month-old boy from Texas with disseminated *M. kansasii* infection and numerous organisms found in his spleen at autopsy.[111] *M. kansasii* also has been reported to cause meningitis; the patients died despite the use of antimycobacterial therapy.[75,140]

MYCOBACTERIUM MALMOENSE

Buchholz and coworkers[18] reviewed infections with *M. malmoense* in the United States from 1993 to 1995. Only one of 73 patients was younger than 10 years. This patient had cervical lymphadenitis and was cured with surgical excision alone. This organism, which frequently is overlooked on standard Löwenstein-Jensen egg medium, grows at 25° C to 37° C. The organism is slow growing, and it may require 8 to 12 weeks for colonies to become visible on solid media. The BACTEC system was shown to be superior in isolating *M. malmoense* in one study of children with lymphadenitis.[70] Bone marrow involvement also was described in a patient with chronic granulocytic leukemia.[45] The aforementioned study by Buchholz and colleagues[18] showed that prolonged combination therapy with isoniazid, rifampin,

ethambutol, and pyrazinamide after surgical excision was effective in some cases.

MYCOBACTERIUM CHELONAE AND *MYCOBACTERIUM FORTUITUM*

M. chelonae is the most important rapidly growing pathogenic mycobacterium, but its taxonomy is quite confusing.[56] Since Grange[56] made a presentation in 1981, the organism's taxonomy has continued to be in flux. The *M. chelonae* group consists of *M. chelonae* (formerly *M. chelonae* subsp. *chelonae*), *M. abscessus* (formerly *M. chelonae* subsp. *abscessus*), and a third biovariant known as *M. chelonae*–like organisms.[92] The *M. fortuitum* group consists of *M. fortuitum*, *Mycobacterium peregrinum*, and a third unnamed biovariant.[92] *M. fortuitum* is associated closely with *M. chelonae*. The two groups can be differentiated on the biochemical basis of nitrate reduction and iron uptake. Wallace and associates[175] have provided the largest series of patients with skin, soft tissue, and bone involvement with *M. chelonae*. Steroid use seemed to be the factor associated most commonly with the development of disease.

M. fortuitum and *M. chelonae* have been implicated in sternal wound infections and endocarditis and have occurred in outbreak-type settings.[91,136,163] Patients responded to surgical débridement and amikacin with cefoxitin. A case series from Hong Kong reported successful treatment of *M. fortuitum* sternotomy infections with the use of single daily dose ofloxacin as monotherapy in three patients.[194] Adult renal transplant patients also have been described with skin and subcutaneous tissue involvement caused by *M. chelonae*.[31] *M. chelonae*, likewise, has been described as an etiologic agent for otitis media, probably from contamination of ear, nose, and throat instruments with colonized water sources.[103]

M. abscessus, as stated earlier, is related closely to *M. chelonae* and should be designated as a separate species.[92] Manifestations of infection with this organism usually are related to pulmonary, cutaneous, or disseminated infections.[178] Clarithromycin may be effective for *M. chelonae* infection.[179] Maxson and associates[110] reported a case of osteomyelitis caused by *M. abscessus* that was controlled with long-term clarithromycin monotherapy.

Mycobacterium smegmatis, which resembles *M. fortuitum* except for the absence of a positive 3-day arylsulfatase test result, is a rapid grower that is responsible for skin and soft tissue infections.[176] A case of disseminated infection has been reported in a child with inherited interferon-γ receptor deficiency.[123]

Mycobacterium septicum is a newly described, rapidly growing species associated with catheter-related bacteremia.[142] It resembles *M. fortuitum* and *Mycobacterium senegalense*.

OTHER SITES OF INFECTION

Carpal tunnel syndrome in adults has been reported as being caused by *M. szulgai*, an uncommon scotochromogenic mycobacterium.[159] Effective treatment included débridement, ethambutol, and rifampin. Other infections in humans include choroiditis,[29] panniculitis,[139] genitourinary tract infection,[15,164] and synovitis.[161] Ear infections with nontuberculous mycobacteria also have been reported, as has mastoiditis.[49,119,158] More recently, infection of Broviac catheters in pediatric patients with leukemia and hemodialysis catheters has been described.[24,95] In all cases, removal of the catheter was required for resolution of the infection.

With the continued proliferation of immunocompromised patients because of AIDS and new treatment modalities that induce an immunocompromised state (e.g., organ transplantation, new immunosuppressive drugs), the atypical mycobacteria probably will continue to remain important pathogens. With newer isolation techniques and new technology such as DNA probes, the ability to diagnose these infections and understanding of the pathogenesis of the infections that these organisms produce should improve, as should the ability to treat these infections.

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CHAPTER 109

Leprosy and Buruli Ulcer: The Major Cutaneous Mycobacterioses

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Mycobacterial infections in humans date to at least the 10th millennium BCE. Bartels[15] in 1907 detected convincing evidence of tuberculosis in a Neolithic skeleton found near Heidelberg, Germany, and Ruffer[263] in 1910 noted Pott disease in an Egyptian mummy from approximately 1000 BCE. The origins of mycobacterial infections in humans are unknown, but most authorities speculate that domestication of animals in the Neolithic era promoted the transmission of mutants of *Mycobacterium tuberculosis* from livestock to humans. The origins of leprosy and Buruli ulcer and their respective etiologic agents are understood less well but may involve interplay among environmental mycobacteria, animals, and humans.[273] After tuberculosis, leprosy and Buruli ulcer are the second and third most common mycobacterial infections in humans. Leprosy and Buruli ulcer are the two most common cutaneous mycobacterioses.

Tuberculosis and other cutaneous mycobacterial infections are discussed in Chapters 107 and 108. This chapter is devoted to the two cutaneous mycobacterioses that are of greatest medical importance: leprosy and Buruli ulcer.

LEPROSY

Leprosy is a chronic infectious disease that is caused by *Mycobacterium leprae* and that principally affects the cooler parts of the body, especially the skin, upper respiratory tract, testes, eyes, and superficial segments of peripheral nerves.[27] Although leprosy has affected nearly every part of the world at some time, its geographic origin is only now being unraveled.[210]

The World Health Organization (WHO) reported that in 1999, approximately 800,000 patients were being treated for active leprosy and that also in 1999, 738,000 new patients were reported.[349] By 2005, the numbers had decreased to 220,000 and 296,000.[352] Many authorities consider that the total global prevalence of patients with active leprosy is much higher (1.5 to 2 million), however, and that new case rates are not rapidly declining.[11] Several more million patients experience serious sequelae.[168]

In the Middle Ages, leprosy was common in Europe and may have been transported to the Western Hemisphere by Portuguese and Spanish explorations beginning in the 15th century and later by slaves from Africa. At least two foci in the United States were established in the 19th century by specific immigrations: Asians brought leprosy to the Hawaiian Islands and started an epidemic in the highly susceptible Hawaiians[213] and Scandinavians introduced leprosy into the northern Midwest region of the United States.[178]

Patients with leprosy frequently experience severe stigmas, and, in Western cultures, this is attributable at least partially to a misunderstanding of what is called leprosy in the Old Testament.[28] Other cultures not influenced by Judaic laws and traditions have similar or more severe attitudes toward patients with leprosy. The Chinese literature indicates that in the 8th century BCE, patients with symptoms now recognized as symptoms of leprosy were stigmatized.[288] Because of enduring irrational attitudes based on the premise that relevant Old Testament references are to the same single disease now called leprosy, a brief explanation of "Old Testament leprosy" follows.

The Hebrew word *tsara'ath* was rendered *lepra* when the Old Testament was translated into Greek in the 3rd and 2nd centuries BCE. In preparing the Latin Vulgate version in AD 405, Jerome used the word *lepra* directly from the Greek. In the first English translation from the Vulgate in 1384, Wycliffe translated *lepra* as *leprosy*, perhaps because leprosy, then common in Europe and Great Britain, seemed to portray an image of an unholy and loathsome human condition. In the original text, *tsara'ath* was not a specific disease but probably a group of diseases, the identities of which are obscure, and the word more generally referred to ceremonial uncleanness. Old Testament *tsara'ath*, as described, for example, in Leviticus 13 and 14, had none of the distinctive clinical features of leprosy.

No rationale exists for attitudes toward leprosy that are based on Old Testament *tsara'ath*. Continuing efforts must be made to minimize the stigma peculiarly associated with leprosy. To help achieve this goal, the Fifth International Leprosy Congress in 1948 adopted a resolution to abandon the word *leper* for leprosy patient.[166] Some physicians prefer Hansen disease as a synonym for leprosy. Because of the stigma of leprosy, physicians must consider carefully the social implications of a diagnosis of leprosy, especially in children.

ORGANISM

M. leprae is a species in the order Actinomycetales and the family Mycobacteriaceae. This bacillus was seen first by Hansen in 1873 in Bergen, Norway, in lepromas from Norwegian patients, and this organism was the first reported bacterium causing chronic disease in humans.

M. leprae is an acid-fast bacillus (AFB) 0.3 to 0.5 μm wide by 4 to 7 μm long. The acid-fastness of *M. leprae* is weaker than that of other mycobacteria, but as in other mycobacteria, the acid-fastness is related to mycolic acid in the cell wall.[14] Viable, undamaged *M. leprae* organisms stain solidly, but degenerating bacilli first stain irregularly, then become granular, and eventually lose acid-fastness completely. The persistence of bacillary carcasses can be verified by silver staining techniques.[323] Staining quality provides a rapid method for determining the effectiveness of therapy. In vitro cultivation of *M. leprae* frequently is claimed, but all claims have been refuted or are as yet unsubstantiated.[152,256] Because *M. leprae* still cannot be cultivated, identification depends on criteria other than those used routinely for cultivable mycobacteria. Current criteria for *M. leprae* are the following: (1) It does not grow on routine laboratory media, (2) it infects the footpads of mice in a characteristic manner,[167,280] (3) acid-fastness is extractable with pyridine,[47] (4) the organism invades nerves of the host, (5) suspensions of dead bacilli produce a characteristic pattern of reactions when injected into the skin of patients (lepromin reaction) with the various clinical forms of leprosy, (6) it produces the species-specific antigen phenolic glycolipid-1 (PGL-1),[94] and (7) it exhibits species-specific DNA sequences.[344]

Electron micrographs of *M. leprae* reveal a cell wall 15 to 20 nm thick around a cytoplasmic membrane that gives rise to mesosomes extending into the cytoplasm. *M. leprae* divides by transverse fission. Its cell walls contain arabinogalactan, mycolates, peptidoglycan, and protein.[185]

The genome of *M. leprae* is small (3,268,203 base pairs) compared with that of *M. tuberculosis* (approximately 4.4 million base pairs).[41] Gene deletion and decay have markedly limited the metabolic activities of *M. leprae* and may contribute significantly to failure to cultivate the organism and to its long generation time in the mouse footpad (14 days).[25,76] In suitable hosts, the generation time of *M. leprae* has been speculated to be considerably shorter than 14 days.[117] Very young infants may have highly bacilliferous leprosy.[30,103] Localization of infections to the cooler parts of the body,[23] selective growth in the footpads of immunologically intact mice and in the ears of hamsters, and the high susceptibility of the armadillo (central body temperature of 32° C to 35° C [89.6° F to 95° F]) to disseminated infections all suggest that the optimal temperature for growth of *M. leprae* is less than 37° C (98.6° F).[189]

TRANSMISSION

The modes of transmission of *M. leprae* in nature have not been fully established. The frequency in children of a single early lesion in skin that usually is covered by clothing argues against the development of such lesions at the site of contact with *M. leprae*.[16] For many years, skin-to-skin contact between the patient and healthy subjects was considered the most important means of transmission, and this concept cannot be abandoned readily.[165] Intact skin of heavily infected patients discharges a few *M. leprae*, but ulcers in the skin may be a source of numerous bacilli. Skin-to-skin contact and fomites containing *M. leprae* could be sources of infection, but this mode has been minimized in recent years in favor of the nasorespiratory route.

That the nasal mucosa of lepromatous patients harbors massive numbers of *M. leprae* has been known since Hansen's original discovery, and studies suggest that the respiratory passages could be an important source of infecting bacilli.[66] *M. leprae* may bind to nasal mucosal cells by first binding fibronectin and attaching to fibronectin receptors on mucosal cells.[35] *M. leprae* organisms ejected in nose blowing remain viable under ambient conditions

for 1 week,[58] and disseminated leprosy develops in immunosuppressed mice after the inhalation of aerosol that contains *M. leprae*.[250] Breast tissue and milk from lepromatous patients contain *M. leprae*, and infants may acquire infection from this source.[230]

Placental transmission of leprosy has been a subject of conjecture for some time, but evidence is growing for a significant influence of leprosy on fetal development and for intrauterine infection of the fetus. In a study of 116 pregnant leprosy patients in Ethiopia, the placentas were small, birth weights were low, and growth rates of the infants were retarded.[72] Mean birth weights of infants of lepromatous and healthy control mothers were 2558 and 3280 g. Estrogen excretion levels at 32 to 40 weeks' gestation are reduced in patients with leprosy, which suggests fetoplacental dysfunction.[74] Immunoglobulin A (IgA) and IgM antibodies for *M. leprae* are present in the cord blood of 30 to 50 percent of infants delivered by mothers with lepromatous leprosy.[186] Evidence is strong for synthesis of fetal antibodies to *M. leprae* or antigens thereof. Occasionally, *M. leprae* has been shown in placentas and cord blood.[127,313] *M. leprae*–specific IgA and IgM levels increased in infants of lepromatous mothers during the 3- to 24-month period after birth,[187] and two such infants had clinical leprosy at 9 and 17 months of age.[73]

Leprosy in young infants may be a common occurrence in areas of high endemicity.[103] In a report combining cases on file in the Leprosy Registry at the Armed Forces Institute of Pathology, cases cited in the literature, and personal observations by experienced leprologists, a total of at least 49 patients with leprosy younger than 1 year of age were identified.[30] In only half of these infants did the mother have leprosy or a history of leprosy. The youngest infant was 2.5 months old at the time the diagnosis was established. The fact that many of the mothers never had clinical leprosy suggests that they had an evanescent *M. leprae* bacteremia during gestation. A substantial bacteremia is a common finding in multibacillary disease[70,159] and is detectable in 15 percent of paucibacillary patients.[153]

The discoveries of a naturally acquired leprosy-like disease in recently captured wild armadillos in Louisiana,[331,333,334] chimpanzees,[69,107] a mangabey monkey from West Africa,[200] and a cynomolgus macaque from the Philippines provide reason to consider that leprosy is a zoonosis.[191,314,332] Reports of naturally acquired leprosy in armadillos range from 3 to 53 percent in the southern region of the United States.[137,305-307] In all these species, the histopathologic features resemble those in leprosy in humans, and the bacilli that cause the infection cannot be distinguished from *M. leprae*.[22,192,199] Leprosy has been transmitted successfully from the mangabey monkey to other mangabey, rhesus, and African green monkeys.[139,346]

Some authorities suggest that insects may ingest *M. leprae* during a blood meal from lepromatous patients and harbor viable bacteria. The natural transmission of leprosy by insects remains unproved, however, and generally is disregarded.

EPIDEMIOLOGY

The highest prevalence rates of leprosy are found in tropical Africa, South America, and Southeast Asia. Approximately 73 percent of all patients live in Southeast Asia (65% in India), 12 percent live in Africa, and 8 percent live in the Americas.[27] Based on limited whole-population surveys in endemic areas, the total number of active patients may exceed the number reported by WHO by a significant margin. The stigma of the disease and inefficiency in health care delivery systems contribute to this disparity in statistics.[164] In 1995, approximately 6000 patients with a history of leprosy resided in the United States,[129] with 101 new patients (L. Pfeifer, National Hansen's Disease Programs, Baton Rouge, LA, personal communication) reported in 1998, down from an annual high in recent times of 361 in 1985.

Most patients in the United States are immigrants, but a few indigenous patients regularly come from Hawaii, Louisiana, Texas, and other southeastern states.[160] No instances of secondary transmission from imported cases within the United States have been reported; immigrants with leprosy present no known public health risk to the population of the United States. The same situation probably is true for other nonendemic countries that receive many immigrants from endemic areas.

Hansen's discovery of the leprosy bacillus developed from his conviction that leprosy was a specific contagious disease, based on clinical and anatomic findings and, more importantly, on epidemiologic observations. In 1871 and 1872, he studied 69 families in western Norway in which several members had leprosy. The prevailing concept of that era was that leprosy was hereditary, but from data gathered on these families, Hansen showed that patients always had contact with another leprosy patient. Members of the same families with no such contacts were free of leprosy.[114] Hansen reasoned, after his pioneering observation of the leprosy bacillus in 1873, that the spread of leprosy depended on dissemination of this etiologic agent in a susceptible population.

The leprosy epidemic in Nauru in the central Pacific area shows how rapidly leprosy can spread in a leprosy-naive population.[108] Leprosy was introduced into this small island in 1912, and by 1924, one third of the 2500 inhabitants had leprosy.

The prevailing concept has been that an individual becomes infected only after experiencing repeated exposure. This concept now is doubted, and a single exposure may be sufficient in optimal conditions. One report describes leprosy transmission occurring after a single exposure from a patient to a surgeon who practiced in a leprosy non-endemic area.[2] In any patient-contact situation, the number of viable *M. leprae* being shed by the patient and the degree of susceptibility of the contact both may vary. Long periods of association may be necessary before optimal conditions for infection exist.

Lymphocyte transformation studies show that occupational contacts of leprosy patients in Ethiopia have the highest rate of sensitization (58%) to *M. leprae*, followed closely by household contacts (47%). Noncontacts living in endemic areas have a lower rate of sensitization, but approximately 29 percent of the population still is sensitized.[104]

Geographic, ethnic, and socioeconomic factors may contribute to the spread of leprosy by affecting the number of untreated or ineffectively treated bacillary-positive patients and the opportunities for exposure. The percentage of patients who harbor large numbers of bacilli—generally, patients with lepromatous leprosy—is related to ethnic background. In some Asian populations, 50 percent or more of patients with leprosy have lepromatous leprosy; in Africans, this figure is 5 to 10 percent. Socioeconomic factors are difficult to assess, and their relationship to the prevalence or clinical severity of leprosy is unknown. Nutritional status may or may not be important. The Nauru leprosy epidemic, indolent from 1912 through 1920, became rampant after a devastating epidemic of influenza (30% mortality) left a debilitated population with marked dietary deficiencies. During the next 4 years, the incidence of leprosy increased from 4 to 346 patients, but the role played by malnutrition is obscure.[108] Ryrie[264] in Malaya noted that during the Japanese occupation, the severity of leprosy worsened, which he attributed to a combination of malnutrition and psychic trauma. Skinsnes and Higa[289] drew similar conclusions from a study of mortality in leprosaria in China during World War II. Nonetheless, convincing evidence that the prevalence of leprosy is unusually high in chronically malnourished populations is lacking.

Improvements in housing and other living conditions may play a role in the declining prevalence of leprosy. No other factor satisfactorily explains the virtual disappearance of leprosy from northern Europe after the Middle Ages and from Scandinavia in

the 20th century, long before any effective chemotherapy was available. If the disease is predominantly airborne, the construction of dwellings that provide less confined sleeping quarters in this era could have contributed in a major way to the disappearance of leprosy in northern Europe and Scandinavia. Consistent with this concept is the inadequate housing that prevails in all geographic areas in which leprosy is common today.

The presumed increased susceptibility of children is difficult to establish and may depend more on exposure to contagious patients and genetic predisposition than on other factors. The proportion of children among all detected patients is 20 to 30 percent.[95,222] Of the 615 known patients who were diagnosed in Louisiana between 1855 and 1970, 5 percent had disease onset at 0 to 9 years of age, and 19 percent were in the 10- to 19-year-old age group.[84] Lara,[162] in a study of 2000 children who lived in a leprosarium in the Philippines in an era when effective chemotherapy was unavailable, noted that leprosy developed in 470 (23%). Of these 470 patients, 254 were monitored closely, and in approximately 75 percent, the lesions healed spontaneously. Active, persistent disease developed in approximately 6 percent of the children who were heavily exposed to leprosy. In most populations studied, only 5 to 10 percent of individuals are susceptible to leprosy.

In adults, leprosy occurs more commonly in men than in women (2:1 to 3:1). In children, the sex ratio is approximately 1:1.

Genetic factors likely influence the susceptibility of some individuals to leprosy and the form of disease that develops.[27,85,88,89,282] Earlier studies found that if one twin has leprosy, the chance of leprosy developing in a monozygotic twin is 60 to 85 percent versus a 15 to 25 percent risk for dizygotic twins.[115] More recently, genome screening in Indian patients found an association with leprosy susceptibility on chromosome 10p13, near the gene for mannose receptor C, a phagocytic receptor on macrophages, and on chromosome 6, within the major histocompatibility complex (MHC).[284] Within the MHC, associations exist between leprosy and MHC class II genes in Indian patients, and with the tumor necrosis factor (TNF) gene in Brazilian patients.[279] Polymorphisms in the "promoter regions" of the interleukin-10 (IL-10) and TNF genes are associated with leprosy,[268] and the TNF promoter especially for the development of lepromatous or multibacillary leprosy.[262]

Certain human leukocyte antigens (HLA-DR) seem to be associated with specific forms of leprosy. HLA-DR2 and HLA-DR3 alleles are associated with tuberculoid disease, and HLA-DQ1 is associated with lepromatous disease.[50] In Suriname, HLA-DR3 is found frequently in mixed populations with tuberculoid leprosy and rarely in lepromatous patients; however, in Indians with tuberculoid leprosy, HLA-DR2 predominates.[317] HLA-DR antigens may influence the presentation of antigens of *M. leprae* to T cells and may affect the immune response to leprosy.[225]

Growing genetic evidence supports inter-population heterogeneity in leprosy susceptibility. On chromosome 6, within the leprosy susceptibility locus region q25-q26,[202] further investigation revealed a significant association between leprosy and 17 markers near the Parkinson disease gene *PARK2* and the co-regulated gene *PACRG* in Vietnamese and Brazilian families.[201] Having two of the alleles was associated with increased susceptibility to leprosy. In Malawi, a large case-control candidate gene study of leprosy susceptibility found that homozygotes for a silent T-to-C change in codon 352 of the vitamin D receptor gene were at higher risk, whereas homozygotes for the McCoy b blood group defining variant K1590E of the complement receptor 1 gene seemed to be protective.[88]

Toll-like receptors, molecules present on the surface of innate or native immune cells, mediate cytokine production on encountering a foreign invader, including mycobacteria, and ultimately may influence the pattern of specific immunity, which is especially relevant in leprosy.[180,258] Studies in Korea found that lepromatous leprosy occurred more commonly in patients with a mutation in the Toll-like receptor-2, associated with altered production of IL-10 and IL-12, underscoring the role early cytokine responses against *M. leprae* may play.[142-144] In African patients, polymorphisms in the *NRAMP1* gene, a gene that also is associated with cellular immunity to *M. leprae*, are associated with lepromatous leprosy,[184] as shown in the Mitsuda reaction.[7,245]

In Texas and Louisiana, the ratios of autochthonous to imported leprosy patients are the highest in the continental United States. Indigenous leprosy is highly prevalent in armadillos in only those two states,[23,290,332,334] and contact with such wild infected armadillos probably transmits leprosy to humans.[160,170,341] No cases of transmission of leprosy to humans from naturally infected mangabey monkeys or chimpanzees have been reported, but this potential exists.[107,193]

PATHOGENESIS AND PATHOLOGY

M. leprae causes disease by its ability to survive and multiply in macrophages (Fig. 109–1).[273] If macrophages of the host digest the bacilli early, disease is undetectable, or the patient has only minimal lesions. If the macrophages are totally incapable of destroying the organisms, a widely disseminated lepromatous leprosy follows. Survival of *M. leprae* in macrophages depends on the immune response of the patient; knowledge of immunity to *M. leprae* is necessary for understanding the mechanism of pathologic changes in leprosy.

IMMUNITY

The immunopathogenesis of leprosy can be understood by examining the spectral or polar nature of the condition, whereby polar tuberculoid disease is characterized by one or several well-demarcated lesions, borderline disease manifests with a modest number of medium-sized lesions, and lepromatous disease manifests with widespread poorly demarcated lesions (Table 109–1). Each type is associated with a different immunologic profile, especially within the lesions.

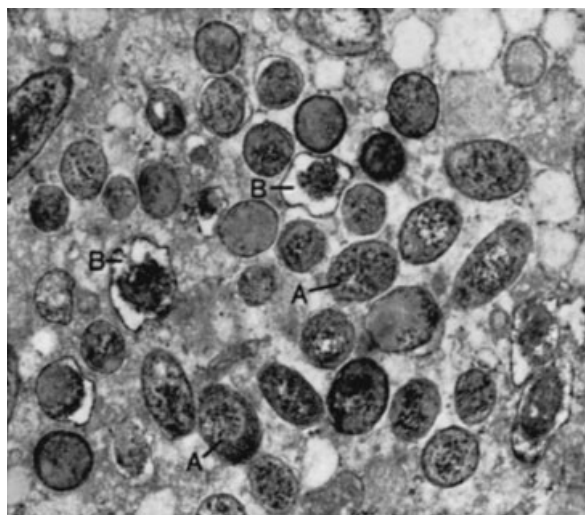


Figure 109–1 Electron micrograph of a portion of a globus of *M. leprae* within a histiocyte in a leproma in the skin. Cross sections of well-preserved (A) and degenerated (B) bacilli are presented (×45,000). *(Courtesy of Dr. S. C. Chang.)*

TABLE 109–1 Criteria for Classification of Leprosy

Group	Clinical Features	Histologic Features	Lepromin Reaction (Mitsuda)	Bacillary Density
Tuberculoid (TT)	A single or few anesthetic macules or plaques Borders well-defined Peripheral nerve involvement common	Epithelioid-lymphocyte granulomas, with or without giant cells, in skin and nerves No subepidermal clear zone Bacilli in nerves, but rare	Strongly positive	Rare
Borderline-tuberculoid (BT)	Lesions similar to those of TT, but more numerous Borders of lesions less distinct Satellite lesions sometimes present around larger lesions Peripheral nerve involvement common	Granulomas similar to those in TT Nerves are infiltrated Bacilli frequently found in nerves	Positive	Scanty
Borderline (BB)	More lesions than in BT Borders more vague Satellite lesions often seen Peripheral nerve involvement common	Epithelioid cells and histiocytic infiltrations focalized by lymphocytes Nerves show increased cellularity Bacilli readily found in nerves	Negative or weakly positive	Moderate
Borderline-lepromatous (BL)	Lesions are numerous and similar to those of BB Some nerve damage	Histiocytic infiltrations show a tendency to evolve toward epithelioid cells and foamy cells Lymphocytes present Nerves have less cellular infiltration Bacilli plentiful in nerves	Negative	Heavy
Lepromatous (LL)	Multiple, nonanesthetic, macular or papular, symmetrically distributed lesions No neural lesions until late Late complications of madarosis, leonine facies, testicular damage	Foamy histiocytes containing large numbers of bacilli Few or no lymphocytes Subepidermal clear zone Numerous bacilli in nerves and perineurium without significant intraneural cellular infiltration	Negative	Very heavy
Indeterminate (I)	Vaguely defined hypopigmented or erythematous macule	Often indistinguishable from "mild nonspecific dermatitis" Lymphocytes and histiocytes around skin appendages and nerves	Weakly positive or negative	Negative or scanty

The ability of an individual to resist *M. leprae* is assessed readily by the induration provoked by an intradermal injection of a suspension of killed *M. leprae* prepared from lepromatous tissue. Lepromatous nodules of patients were the traditional source, but now infected tissue from armadillos commonly is used.[194] The reagent is known as "lepromin," and the response is known as the "lepromin reaction." This reaction, first studied by Hayashi and later evaluated by Mitsuda,[204] has two components: an early response at 48 hours (Fernandez reaction) and a late response at 3 to 4 weeks (Mitsuda reaction). The Mitsuda reaction is the most consistent and is used by clinicians as an aid in classifying the clinical forms of leprosy.

Mitsuda reactions are strongly positive (>5 mm in diameter) in tuberculoid patients, weak or negative (0 to 2 mm) in lepromatous patients, and intermediate (3 to 5 mm) in borderline patients. The reactions are a direct measure of delayed hypersensitivity or cell-mediated immunity (CMI) to *M. leprae* antigens; lepromatous patients are anergic to *M. leprae*. The lepromin reaction has no value in establishing the diagnosis because a high percentage of any population is Mitsuda-positive. Even in children without leprosy, the Mitsuda reaction is positive in 20 percent of children younger than 5 years old and in two thirds of children aged 7 to 9 years old.[112] Modifications of the lepromin reaction with the use of concentrated lepromin show that macrophages at the test site in lepromatous patients cannot clear *M. leprae* from the skin, whereas in tuberculoid patients, the bacilli are destroyed efficiently.[46]

Nonspecific factors participate in host defense against *M. leprae*. Complement promotes phagocytosis of leprosy bacilli.[271] After phagocytosis occurs, phagolysosomal fusion and intracellular killing ensue, perhaps by oxygen-independent mechanisms.

Although the precise mechanisms of specific immunity remain elusive, abundant experimental evidence indicates that in a lepromatous patient, CMI to *M. leprae* is markedly suppressed. Delayed hypersensitivity skin test reactions to many antigens often are depressed, but they are depressed most consistently and most severely to *M. leprae*.[32] The degree of suppression is gradually less pronounced in clinical forms of disease that are progressively nearer tuberculoid leprosy.[330]

A continuous decrease occurs in the sensitivity of peripheral blood lymphocytes to *M. leprae* that proceeds from tuberculoid to lepromatous patients.[217] Many investigators consider that the defect in CMI to *M. leprae* is in T-lymphocyte function or in the

interaction of T lymphocytes with macrophages. Total numbers of circulating T lymphocytes are decreased in lepromatous patients,[75] but no consistent alteration occurs in the percent distribution of circulating T-cell subsets, particularly in the helper-suppressor (T_H1-T_H2) cell ratio.[246]

Within tuberculoid and lepromatous lesions, immunophenotypic and functional profiles of the infiltrating T cells differ markedly and are important factors in understanding the immunopathogenesis of leprosy.[208,209,244] In tuberculoid lesions, the CD4-to-CD8 T cell ratio is about 2:1, and the lesions are characterized by a T_H1 or T_H1-like profile, containing abundant mRNA transcripts for numerous proinflammatory cytokines that confer strong CMI, including IL-2, interferon-γ (IFN-γ), and IL-12.[356] Notably, $CD4^+$ T cells in tuberculoid lesions produce IFN-γ.[266] In lepromatous lesions, the CD4-to-CD8 ratio is approximately 1:2, and the lesions are characterized by a T_H2 or T_H2-like profile, containing mRNA transcripts for anti-inflammatory cytokines that include IL-4 and IL-10, factors associated with weak CMI, but strong humoral responses.[356] The $CD8^+$ T cells in lepromatous disease produce IL-4.[266] In contrast to Buruli ulcer (see later section on Buruli ulcer), induction of apoptosis in the lesions does not seem to play a major role in pathogenesis.[326] This immunologic dichotomy between tuberculoid and lepromatous lesions is consistent with the paradigm that robust CMI limits disease progression, and that humoral immunity has little or no effect.

The factors that determine whether a host generates a T_H1 or T_H2 response after having an infection with *M. leprae* are not completely understood. In addition to genetic predisposition (see section on epidemiology), an important immunologic factor may relate to host innate responses, when *M. leprae* is first encountered. Toll-like receptors on innate immune cells may recognize mycobacterial lipoproteins, generating cytokines that mediate specific or adoptive responses toward a T_H1 or T_H2 direction.[26,205]

In lepromatous lesions, T_H1 and T_H2 cells are admixed among the macrophages. Suppressor activity is generated by lepromin in vitro in peripheral leukocytes from lepromatous patients but not in cells from tuberculoid patients.[183] This suppressor activity also may be induced by the unique phenolic glycolipid (PGL-1) of *M. leprae*,[123,182] but it is unrelated to the type of leprosy.[241] PGL-1 abounds in the tissues of lepromatous patients. Secondary immunosuppression in advanced lepromatous leprosy may result from blockade of thymus-dependent areas of lymph nodes by *M. leprae*–laden macrophages.[309] Specific suppressor T-cell activity in immunosuppression in leprosy remains controversial.[147]

Macrophages of lepromatous patients are thought to have the capacity to kill, digest, and clear *M. leprae* if they are activated.[122] Patients with lepromatous leprosy fail to produce IL-2, but IL-2 restores the proliferation of lymphocytes in response to specific antigens.[116] Defective IFN-γ is produced by lymphocytes from lepromatous patients on stimulation by *M. leprae* antigens.[221] IL-2-bearing lymphocytes are reduced markedly in lepromatous infiltrations in tissues.[207] Suppressor T cells may influence IL-2 production in situ and reduce the proliferation of specifically sensitized T cells to release IFN-γ, with the result that macrophages are not activated. The injection of IFN-γ or recombinant IL-2 into the skin of lepromatous patients causes a local influx of $CD4^+$ T cells along with the formation of epithelioid and giant cells and a reduction in bacillary load.[146,320] IL-2 and IFN-γ, when available in quantity, may prove to be important immunotherapeutic agents for lepromatous leprosy. Therapy with IL-2 or IFN-γ induces the secretion of TNF-α; however, the activity of this toxic molecule may be inhibited by thalidomide or pentoxifylline.[145,302] Whether the increasing use of TNF-α antagonists such as infliximab, generally used for autoimmune disorders such as rheumatoid arthritis, would be associated with the development of leprosy from latent infections and perhaps be useful in the management of leprosy reactions remains unclear.[80,274]

Immunoglobulin production (IgG, IgA, and IgM) usually is elevated only slightly in tuberculoid patients but is elevated markedly in lepromatous patients, consistent with the predominant T_H1 versus T_H2 responses.[33] The total number of B lymphocytes is increased in the blood of lepromatous patients, as is the circulating antibody to mycobacterial antigens.[92,219]

The role of immunologic processes in damage to nerves in leprosy is poorly understood. Some observations suggest that antineural antibodies in the sera of many patients, especially patients with lepromatous disease, are related to such damage.[228] TNF-α is associated with macrophage infiltration of peripheral nerves in reversal reactions.[155] Infected Schwann cells present antigens to T cells, rendering them targets for immune attack.[273]

HISTOPATHOLOGY

Biopsy specimens from well-defined lesions of leprosy should be taken from the active border and fixed in buffered 10 percent formalin or other suitable fixative. The Fite-Faraco staining method is used because the Ziehl-Neelsen stain does not show *M. leprae* optimally in tissue sections. A histopathologic diagnosis of leprosy must not be made unless the evidence is convincing. The pathologist must avoid making ambiguous evaluations, such as "consistent with leprosy." DNA probes specific for *M. leprae* are available and are useful in identifying leprosy bacilli in tissue or nasal secretions.[66,344] Specimens for DNA evaluation or polymerase chain reaction (PCR) amplification, techniques that have been steadily improved over the past decade, should be preserved in 70 percent ethyl alcohol.[37,86,109]

Indeterminate Leprosy

In indeterminate leprosy (Fig. 109–2), the immune potential of the patient is not portrayed clearly in the cellular reaction. Only a mild chronic inflammation is found, with small infiltrations of lymphocytes or histiocytes along neurovascular channels and sometimes around appendages (Fig. 109–3). If leprosy is suspected, all nerves in the dermis and subcutaneous tissue in numerous sections of the biopsy specimen must be searched for AFB, even if no inflammatory changes within the nerves are present. Sometimes AFB appear only in the arrector pili muscles or subepidermal zone.[252] A histopathologic diagnosis of leprosy cannot be made unreservedly in indeterminate leprosy without showing AFB. Molecular biologic studies are rarely helpful in diagnosing indeterminate leprosy.

Tuberculoid Leprosy

Patients with tuberculoid (TT) leprosy (Fig. 109–4) have a high level of CMI to *M. leprae*, which is reflected in the cellular reaction. Granulomata composed of epithelioid cells, Langhans giant cells, and lymphocytes are present in the dermis or subcutaneous tissue (Fig. 109–5A). Frequently, upper dermal granulomata invade the lower layers of the epidermis (see Fig. 109–5A). Damage to nerves is a distinctive feature—in old advanced lesions, all cutaneous nerves may be damaged beyond recognition (see Fig. 109–5B). Occasionally, S-100 immunostaining helps reveal a neural pattern of Schwann cells if a question exists of whether or not nerves are damaged. Schwann cells are increased in number in early lesions, and the nerves are invaded by mononuclear cells. Bacilli rarely are found, and often many sections must be searched to locate a single bacillus. The bacilli usually are within remnants of dermal nerves but sometimes are located just beneath the epidermis.

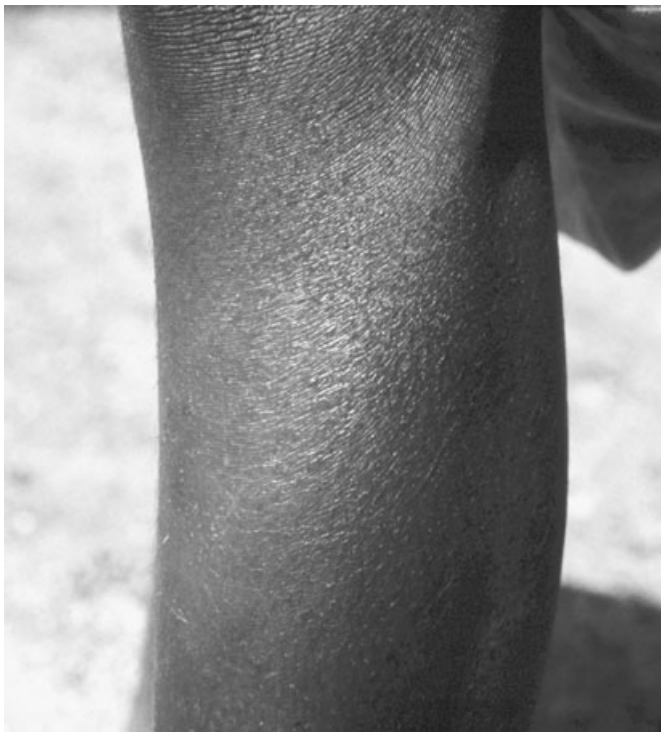


Figure 109–2 Hypopigmented macule of indeterminate leprosy on the anterior surface of the leg of an Indian girl. (See companion Expert Consult web site for color version.)

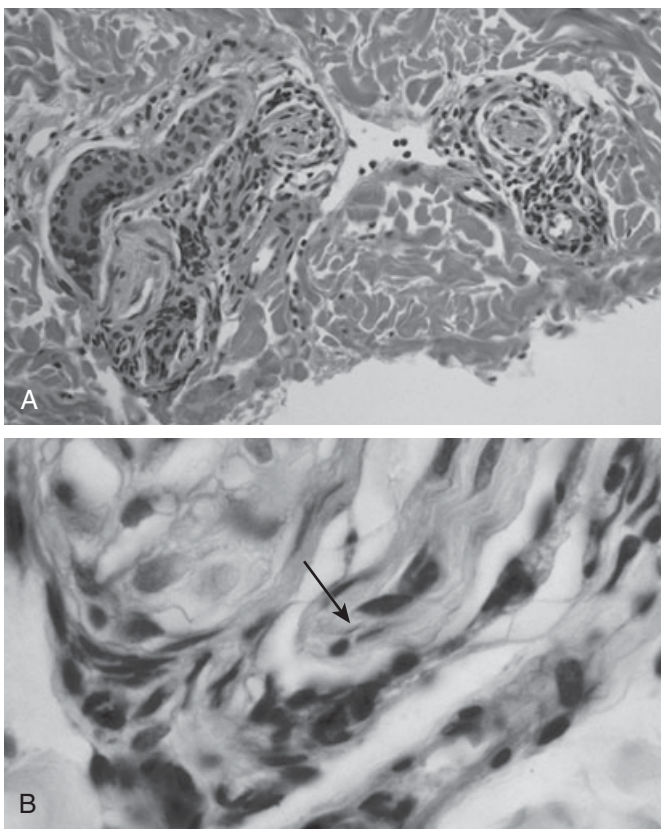


Figure 109–3 **A,** Indeterminate leprosy showing only mild infiltrations along neurovascular channels in the deep dermis (hematoxylin and eosin stain). **B,** Only a single acid-fast bacillus was found in one deep dermal nerve *(arrow)* after a search of multiple sections (Fite-Faraco stain). (See companion Expert Consult web site for color version.)

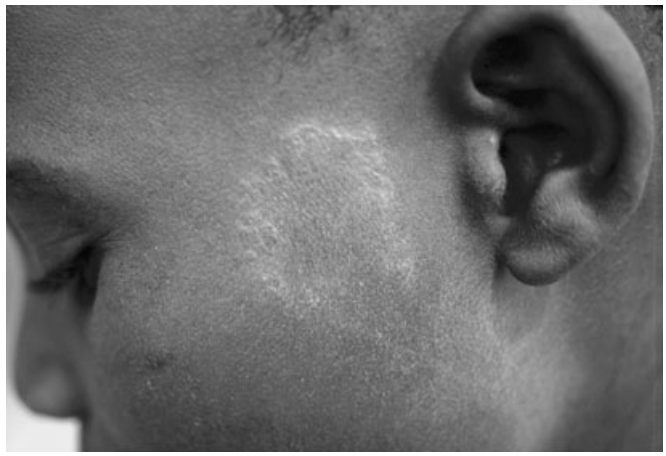


Figure 109–4 Tuberculoid leprosy in a 12-year-old Congolese boy. This was the only lesion, and it has a well-defined papulated border with central healing. (See companion Expert Consult web site for color version.)

When major nerve trunks are involved, they contain typical tuberculoid infiltrates that eventually may replace the entire nerve. Occasionally, caseous "abscesses" are present in large nerves, but they are rare in children.

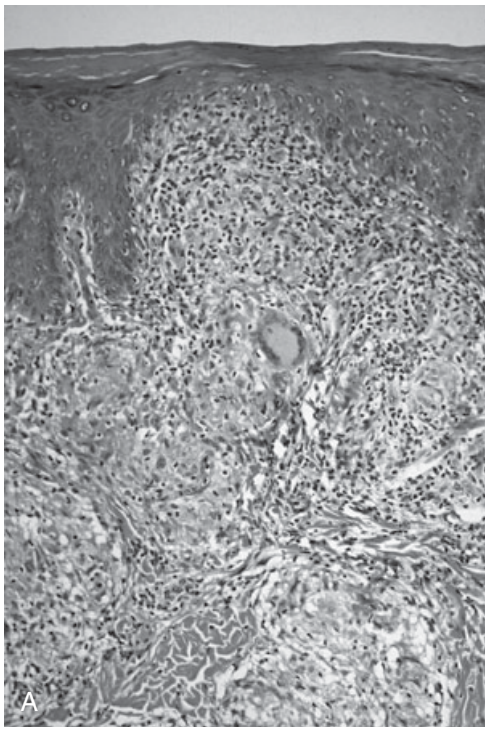
Borderline Leprosy

Borderline leprosy represents a broad spectrum of clinical (Fig. 109–6) and histopathologic variations (see Table 109–1). In the borderline-tuberculoid (BT) variety, strong CMI still is evident by the large numbers of epithelioid cells and lymphocytes. The nerves usually are damaged less and are identifiable more readily than in TT leprosy. *M. leprae* often remain rare but are seen more readily than in TT in nerves (Fig. 109–7) or in the subdermal zone. In the central portion of the borderline area of leprosy (BB), the epithelioid cells are not surrounded by large numbers of lymphocytes, and Langhans giant cells are uncommon. The subepidermal area is free of infiltrating cells, and nerves are not damaged severely, but the perineurium often is thickened by epithelioid cells. Some histopathologists do not recognize BB leprosy as an entity because there is usually a suggestion that the lesion is either on the BT or the borderline-lepromatous (BL) side of the spectrum of the disease.[136] BL lesions reveal a low level of CMI. The granulomata are composed mostly of macrophages, but contain many irregularly distributed lymphocytes. A few nests of epithelioid cells may be present. The perineurium of the nerves is infiltrated with cellular exudates. Nerves are easily identified and contain many bacilli.

Lepromatous Leprosy

Pre-lepromatous lesions show only a mild proliferation of macrophages around vessels, nerves, and appendages. AFB are few and often difficult to show.

Anergy to *M. leprae* becomes apparent early in the lepromatous lesion (Fig. 109–8), with the bacilli-laden macrophage (Virchow cell or lepra cell) being the predominant inflammatory cell. In early lesions, they tend to accumulate around vessels, nerves, and appendages, but they eventually may replace the entire dermis (Fig. 109–9A). The infected macrophages are supported by a delicate stroma and supplied by a rich network of capillaries. As the macrophages age, they become vacuolated (foamy), largely from their lipid content. In developing lesions, the intracellular bacilli are arranged in small bundles (see Fig.



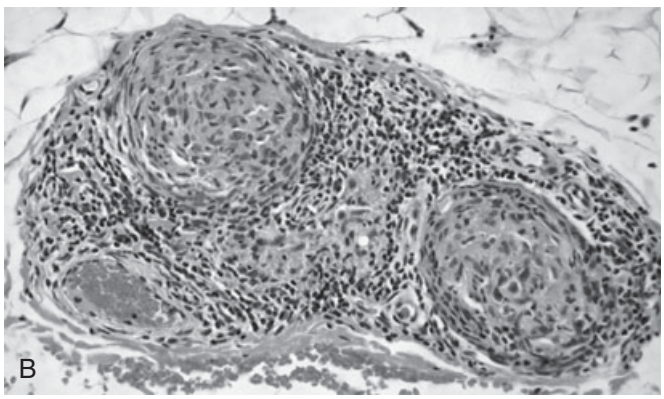


Figure 109–5 **A**, Tuberculoid leprosy with a dense granulomatous infiltration that invades the epidermis. Granulomata contain epithelioid cells, Langhans giant cells, and lymphocytes (hematoxylin and eosin stain). **B,** High magnification of a nerve bundle in the subcutaneous tissue of section in **A**. Granulomata have nearly completely destroyed the nerves. Remnants of nerves contained rare acid-fast bacilli (hematoxylin and eosin stain). (See companion Expert Consult web site for color version.)

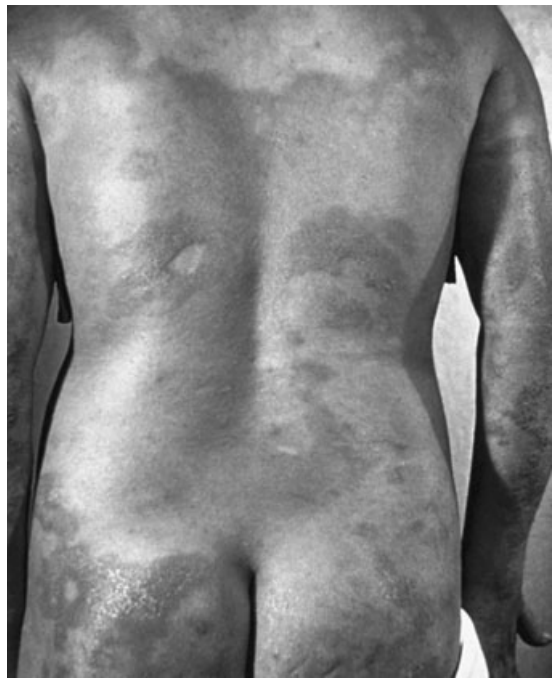


Figure 109–6 Borderline lepromatous leprosy in a Filipino. There are many plaques, some with well-defined borders and others vaguely defined. The erythematous plaques indicate that the patient is undergoing an upgrading reversal reaction (type 1). (See companion Expert Consult web site for color version.)

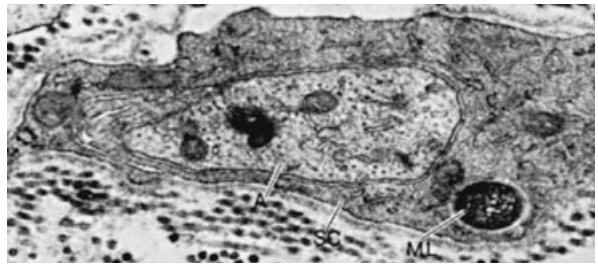


Figure 109–7 Electron micrograph of a portion of a damaged dermal nerve in borderline leprosy. A nonmyelinated axon (A) is surrounded by a Schwann cell (SC) that contains a single *M. leprae* bacillus (ML) (×60,000). *(Courtesy of Dr. S. C. Chang.)*

109–9B); in advanced lesions, dense masses of bacilli termed *globi* may replace nearly the entire cytoplasm of the macrophage. Infiltrating cells do not invade the epidermis but leave a narrow subepidermal clear zone. Many bacilli are found in the dermal nerves and frequently in endothelial cells, walls of blood vessels, arrector pili muscles, and epithelial cells of hair follicles.[215] Plasma cells vary in number and probably reflect B-lymphocyte hyperactivity.[92] Few lymphocytes are in a lepromatous lesion. Large nerve trunks may show typical lepromatous infiltrations.

Occasionally, in patients with lepromatous (LL) leprosy, elevated firm nodules form in the skin, especially in relapsing disease. Because of the characteristic histologic pattern of these lesions, in which the histiocytes resemble fibrocytes, this form is called *histoid leprosy*.[324] LL leprosy is disseminated widely, and lepromatous infiltrations frequently are found in the upper respiratory tract as far down as the larynx and in the eyes and testes. In adults, the testes sometimes are densely infiltrated, with subsequent development of sterility and gynecomastia, but these complications are rare findings in children. Lymph nodes often are infiltrated by bacilli-laden macrophages, especially in the medulla and paracortical areas.

CLINICAL MANIFESTATIONS

The incubation period varies (usually 2 to 5 years), and no prodromal manifestations are well established. Some experienced clinicians working in areas of high prevalence recognize early signs of nerve involvement (localized paresthesia, itching, or numbness) before any visible lesions develop.

After the incubation period, various lesions appear. The nature of the lesions depends on the immune response of the patient to *M. leprae*. So far, no strain variations of the bacillus except in drug sensitivity have been detected.[342,343] Most clinicians

Figure 109–8 Advanced lepromatous leprosy in an adolescent Filipino. Skin of most of the face, especially the alae nasi, is diffusely thickened. Eyebrows are thinned. (See companion Expert Consult web site for color version.)

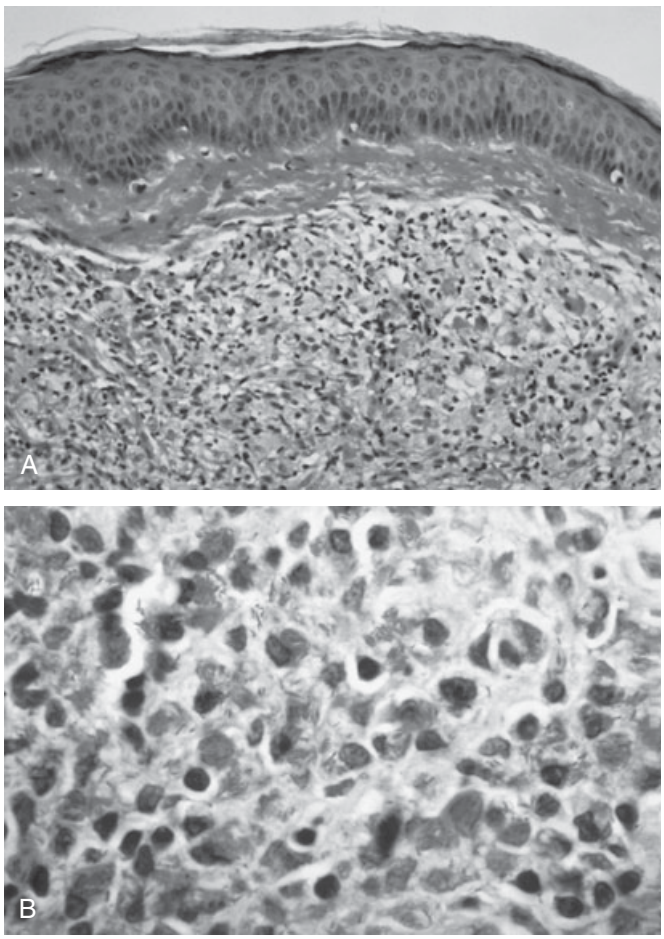


Figure 109–9 **A,** Advanced lepromatous leprosy showing replacement of dermis by foamy histiocytes and very few lymphocytes. Note thin subepidermal clear zone (hematoxylin and eosin stain). **B,** Higher magnification of same biopsy specimen showing clumps of *M. leprae* (globi) in histiocytes in a leproma in the skin (Fite-Faraco stain). (See companion Expert Consult web site for color version.)

today follow the classification scheme outlined by Ridley and Jopling (see Table 109–1).[253] Classification is important because it aids in establishing the prognosis and treatment program for the patient.

Virtually all patients with leprosy have peripheral neuropathy if cutaneous sensory changes are included, and approximately 25 percent have significant deformity. In experimental studies, the pathogenesis of peripheral neuritis in leprosy involves bacillation of the endothelial cells of epineural and perineural blood vessels and lymphatics.[272,273,275] Surface proteins of *M. leprae* may bind the bacillus to Schwann cells via lamimin.[243,283] Detailed discussions of peripheral neuropathy in leprosy can be found elsewhere.[265]

Ocular complications in leprosy are well known.[51,56,57,120] All patients with leprosy should be evaluated by an ophthalmologist at diagnosis and periodically thereafter, especially during any reactional episodes.

INDETERMINATE LEPROSY

An indeterminate lesion is the first manifestation of leprosy in most patients and may heal spontaneously, remain unchanged for months or years, or gradually progress toward TT or LL disease. Patients with indeterminate leprosy have a single or a few macules in the skin (see Fig. 109–2). The macule is defined poorly and is mildly hypopigmented in deeper pigmented skin and slightly erythematous in lighter skin. Skin texture, sensation, and sweating within early macules are normal or only slightly altered. Peripheral nerves are normal, and skin smears from lesions rarely contain bacilli. The definitive diagnosis can be made only by finding AFB in histopathologic sections.

TUBERCULOID LEPROSY

Patients with TT leprosy have a single or several asymmetrically distributed hypopigmented skin lesions (see Fig. 109–4). Tuberculoid lesions arise de novo or evolve from indeterminate macules. The lesion may be macular or infiltrated, but the borders always are sharply demarcated from the surrounding normal skin and frequently are finely papulated. Lesions range in size from less than 1 cm to large enough to cover entire regions such as the thigh or buttock. Many TT lesions heal spontaneously. In large, active lesions, the centers often are healed and repigmented, although somewhat atrophic.

In TT lesions, sensory loss with impaired sweating and eventual loss of hair occurs. On the face, because of its rich innervation, the detection of hypoesthesia in early lesions requires discriminating tests. Conversely, clinicians may mistakenly diagnose leprosy in areas of the body that normally are hypoesthetic (e.g., over the elbows or knees).

Involvement of peripheral nerves commonly occurs in TT leprosy (Fig. 109–10), and cutaneous nerves often can be palpated adjacent to or within lesions. The regional nerve trunks most commonly enlarged are the ulnar from the olecranon groove to midarm, lateral popliteal just distal to the head of the fibula, and posterior tibial in the medial aspect of the ankle. Enlarged or tender nerves anywhere should alert the clinician to the possibility of leprosy. Any readily palpable cutaneous nerve probably is enlarged, but evaluating the size of nerve trunks requires experience because of the wide range in normal size.

BORDERLINE LEPROSY

Borderline leprosy, sometimes called *dimorphous* or *intermediate leprosy*, has features of the LL and the TT forms and

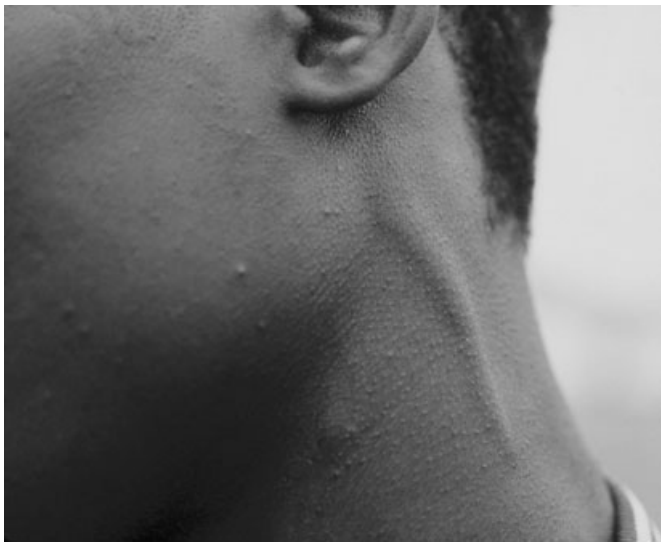


Figure 109–10 Enlargement of the great auricular nerve in an adolescent Congolese boy. A large macule of tuberculoid leprosy over the angle of the mandible is now nearly inactive and barely visible. (See companion Expert Consult web site for color version.)

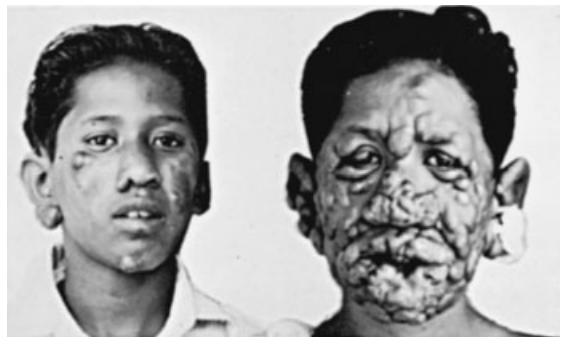


Figure 109–11 Progression of lepromatous leprosy in a Hawaiian boy. The photograph on the *left* was taken in 1931, when the patient was 13 years old, and the photograph on the *right* was taken 2 years later. No effective chemotherapy was available in that era.

represents a continuous spectrum of disease ranging from near-tuberculoid to near-lepromatous. It is an unstable form of leprosy and may evolve gradually toward TT leprosy by undergoing reversal reactions or be downgraded toward LL leprosy. Table 109–1 describes the three major subgroups of borderline leprosy: borderline-tuberculoid (BT), borderline (BB), and borderline-lepromatous (BL).

In BT leprosy, the number of lesions usually is greater than in TT leprosy, and the borders of each lesion, macule, or plaque are defined less sharply than in TT leprosy. Small satellite lesions may develop around larger macules or plaques. BL leprosy often manifests with widespread nodular infiltrations or plaques of varying size (see Fig. 109–6).

Damage to nerves and the resulting deformity develop early and often are widespread. Pain in nerves or neurotropic changes (e.g., sensory changes that lead to damaged hands or feet or a muscular weakness such as footdrop) frequently bring the patient to the physician. Severe damage to nerves occurs infrequently in young children, but can be disastrous. Prevention of this complication is an important goal of leprosy detection programs and of treatment of every patient with leprosy.

LEPROMATOUS LEPROSY

In LL leprosy, the bacilli multiply freely, and the disease disseminates widely, often before striking cutaneous manifestations develop, in contrast to the strict localization of lesions in TT leprosy. LL leprosy may evolve from indeterminate or BB leprosy or may be the first recognizable form. In its earliest form, LL leprosy manifests as "juvenile leprosy," a clinical entity delineated from observations of large numbers of children in homes for children of patients with leprosy in India.[214] This form, also called *pre-lepromatous leprosy*, is difficult to detect and frequently goes unrecognized until a more advanced stage develops. Skin texture may be altered slightly, but the vague macules with indistinct borders are detected only under appropriate lighting, preferably daylight. No changes in sensation or sweating occur in the macules, and frequently AFB are not detectable in smears from skin. Histopathologic sections may reveal a few bacilli to confirm the diagnosis; however, if leprosy is suspected, the patient should be monitored until an explanation for the mild skin changes is found. If leprosy is present and not detected and treated, advanced forms of LL leprosy develop in many of these patients (Fig. 109–11).

The hypopigmented or slightly erythematous macules of early LL leprosy, similar to those of juvenile leprosy, are missed easily because they are vague and have slight, if any, sensory changes. These macules usually are small but gradually may coalesce and cover large areas of skin, even nearly the entire body. Clinical diagnosis often is missed, and over the course of a few years, advanced LL leprosy develops. If skin smears or biopsy specimens are taken in the macular stage, diagnosis is virtually ensured. If the disease is not diagnosed and treated in the macular stage, infiltration of the skin increases gradually, and nodules may develop. The skin is infiltrated most heavily in the cooler portions of the body, notably the ears (pinnae) and face. By this time, nerves usually are enlarged, with early signs of sensory loss in the hands and feet. Eyebrows are thinned and eventually lost, beginning at the lateral edges. These advanced changes of LL leprosy are not common findings in young children but are well known.

Patients of Latin American ancestry, especially patients from Mexico and Costa Rica, may contract the highly anergic diffuse form of LL leprosy called *Lucio leprosy*. The disease may be so diffuse that it is not recognized until sensory changes appear in the hands and feet, and the eyebrows and other body hair begin to disappear. In advanced forms of Lucio leprosy, a marked obstructive vasculitis in the skin is present, with the production of dermal infarcts and irregular ulcers (Lucio phenomenon).[163,247] Lucio leprosy has been reported in children 7 years old.[270]

NEURITIC LEPROSY

Rarely, leprosy involves one or more major nerve trunks unaccompanied by cutaneous lesions. These patients have anesthesia, paresis, or wasting of muscles in the affected area. Nerve trunks frequently are painful, enlarged, and tender. Leprosy must be suspected in patients with any peripheral neuritis that has these features. Chronic neuritis with pain and enlargement and tenderness of peripheral nerves often persists for years after the patient has completed chemotherapy for leprosy.[119] Large leprotic nerve abscesses are rare occurrences but are exquisitely painful and may require surgical intervention for drainage.[278] Most clinicians prefer to treat such lesions with corticosteroids.

REACTIONS

The course of leprosy, treated or nontreated, often is interrupted by acute episodes known as reactions, which fall into two general categories: reversal reactions (or type 1) and erythema nodosum leprosum (ENL) (or type 2).

REVERSAL REACTIONS

Reversal reactions complicate borderline leprosy and represent delayed hypersensitivity reactions, with an upgrading of CMI toward TT leprosy. Lesions become erythematous and edematous, and neuritis is a common manifestation (Fig. 109–12). Patients who are lepromin-positive and have IgM antibodies to PGL-1 are most at risk for having reversal reactions.[254] Proliferation of sensitized T lymphocytes initiates reversal reactions, releasing lymphokines that amplify the inflammatory response, calling in and activating macrophages.[259] Immunohistopathologic evidence has shown that effective chemotherapy for paucibacillary and multibacillary patients may activate CMI and provoke clinical or subclinical reversal reactions. Expression of HLA-DR is increased, which may enhance production of IFN-γ by lymphocytes in granulomata.[53] Histopathologically, edema is accompanied by an increase in the number of lymphocytes, often with epithelioid cells and giant cells. In severe reactions, necrosis may occur, almost always in nerves. Increased levels of TNF-α may partially explain this necrosis.[20] Cyclooxygenase-2 levels are elevated in nerves and blood vessels during reversal reactions, a finding that explains why nonsteroidal anti-inflammatory drugs, including selective cyclooxygenase-2 inhibitors, often improve the condition.[231]

Patients experiencing such reactions must be observed closely so that sensory loss and deformities are minimized. By repeated reversal reactions, borderline leprosy, even cases close to LL disease, may be upgraded gradually to TT leprosy, often with disastrous peripheral neuropathy.

Differentiating reversal reactions from relapsing lesions frequently is difficult and requires careful correlation of clinical and histopathologic findings. This correlation is becoming increasingly important in endemic areas, where shorter term chemotherapeutic regimens of fixed duration are used.[90,188] The following criteria for differentiating relapses and reversal reactions are suggested: A relapse involves an increased number of lesions, positive skin smears for AFB (for patients with BB and BL leprosy), tissue reaction inconsistent with a reversal reaction, and a favorable response to chemotherapy; a reversal reaction involves an exacerbation of existing lesions, skin smears negative for AFB, tissue reaction consistent with a reversal reaction, and a rapid response to anti-inflammatory drugs.

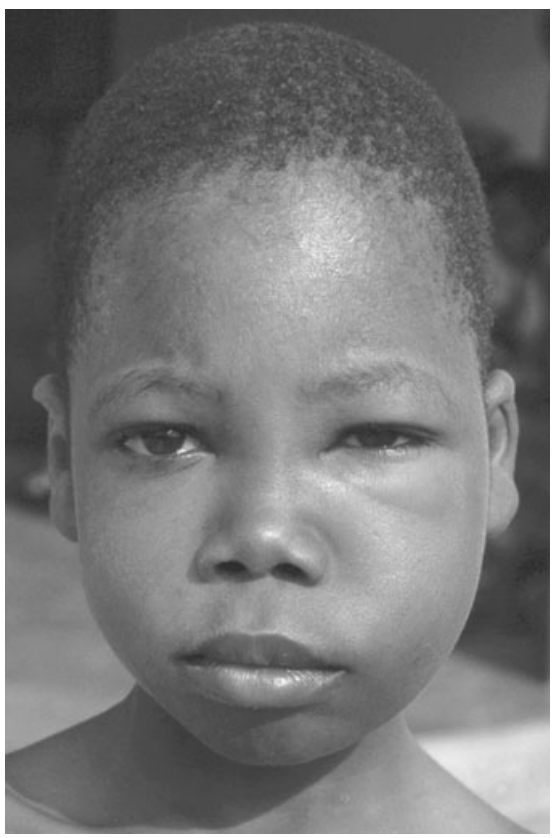


Figure 109–12 Reversal reaction (type 1) in an 8-year-old Congolese boy with borderline-tuberculoid leprosy. The left side of his face is swollen and displays mild palsy from facial nerve damage. The patient responded rapidly to oral steroid therapy. (See companion Expert Consult web site for color version.)

ERYTHEMA NODOSUM LEPROSUM

Formerly, ENL developed in approximately 50 percent of LL patients after they had undergone a few months of chemotherapy; however, with the addition of clofazimine to the standard therapeutic regimen, frequency of ENL is much reduced.[82] Tender erythematous subcutaneous nodules develop rapidly (Fig. 109–13); the nodules often are accompanied by fever and occasionally by synovitis and iridocyclitis.[234] ENL resembles the Arthus reaction and is thought to result from immune complex formation.[96] Immune complexes may form within lesions by the local release of antigens of *M. leprae* and could modulate the development of T-cell populations in situ. Numbers of helper T lymphocytes are increased in lesions of ENL.[206] Serum TNF-α is elevated in ENL.[20,269]

In the nodule, neutrophils and sometimes an intense vasculitis are present. Ulceration of the skin frequently accompanies severe ENL. Glomerulonephritis sometimes complicates ENL; secondary amyloidosis is a late sequela of repeated reactions and may be a consequence of the neutrophilic leukocytosis.[177] Although neutrophilic infiltration is considered by many physicians to be the hallmark of the tissue reaction, tissue from a rare patient with clinically typical ENL may not show neutrophils. In such patients,

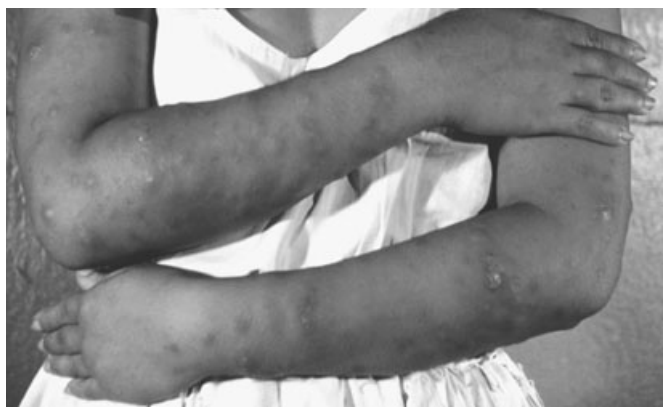


Figure 109–13 Erythema nodosum leprosum in a Filipino adolescent girl with lepromatous leprosy. (See companion Expert Consult web site for color version.)

demonstration of serum amyloid A and C-reactive protein may aid in establishing the diagnosis of ENL.[124] Occasional patients may have mixed reversal (upgrading) reactions and ENL. These patients usually have leprosy in the BL area of the spectrum.

DIAGNOSIS AND DIFFERENTIAL DIAGNOSIS

The cardinal signs of leprosy are hypoesthetic lesions of the skin, enlarged peripheral nerve or nerves, and AFB in skin smears. In the absence of another clear explanation, any one of these signs strongly suggests leprosy.

An experienced observer can establish a clinical diagnosis in most patients, except those with early leprosy, with a high degree of accuracy. Because leprosy is considered one of the "great imitators,"[329] however, histopathologic evaluation is strongly recommended to supplement and confirm the clinical diagnosis.

Patients with leprosy may be found in almost any geographic area, an awareness of which would minimize missed and delayed diagnoses, especially in areas of low prevalence. In the United States, the usual delay in establishing the diagnosis after the patient's first visit to a physician for symptoms related to leprosy is approximately 1.5 to 2 years. This delay often significantly worsens the prognosis.

History is important. Contact with patients with leprosy or residence in an endemic area raises suspicion of leprosy in a patient with a chronic lesion of the skin. Sensory loss or unexplained damage to hands or feet suggests damage to nerve trunks. Sometimes, a footdrop or clawhand brings the patient to a physician. Occasionally, patients with LL leprosy consult an otolaryngologist first because of a chronic stuffy nose.

Clinicians must evaluate sensory changes in a lesion by using the precautions already mentioned. The modalities usually tested are light touch with the use of a few fibers of cotton or calibrated nylon threads and heat-cold discrimination with the use of warm and cold water in test tubes. Much patience and repeated testing often are necessary in evaluating young children. Spontaneous sweating can be observed directly, or induced sweating can be evaluated. Hair may be completely preserved in early lesions but lost in advanced lesions.

The main nerve trunks must be palpated for tenderness and enlargement. Skin in the area of discrete lesions also must be palpated gently to detect enlargement of cutaneous nerves. In the world population, leprosy is the most common cause of peripheral neuropathy and must be considered in any patient with peripheral neuropathy.[265]

Obtaining and examining smears for AFB is an important diagnostic procedure and should be controlled carefully by experienced laboratories. Briefly, smears are made from the edge of discrete macules or plaques, nodules, ear lobes, and nasal mucosa. Skin smears are made by squeezing and holding a fold of skin between the thumb and forefinger to avoid getting blood in the smear and by making a short, shallow slit in the skin with a razor blade or scalpel. The instrument is turned at a right angle to the slit, and the edges of the incision are scraped. The cells and fluid thus obtained are spread on a slide, heat-fixed, and stained by the Ziehl-Neelsen method.[319] Evaluation of smears should not be done by researchers unfamiliar with their interpretation. An occasional AFB may be a harmless contaminant in the staining reagents.

The lepromin reaction is useless for the diagnosis of leprosy. Currently available skin tests with soluble *M. leprae* antigens are unreliable.[113] Enzyme-linked immunosorbent assays and gelatin particle agglutination tests for antibodies to the PGL-1 of *M. leprae* are available.[38,106,128] Although specificity for *M. leprae* is high, these tests detect antibodies to PGL-1 in only approximately 50 percent of paucibacillary patients. Other serologic tests for antibodies to *M. leprae*–specific epitopes on protein moieties of the bacillus are being evaluated.[229] PGL-1 antigen is detectable in the serum and urine of most multibacillary patients.[223,357]

Reliance on DNA probes and PCR technology may prove useful in establishing the diagnosis of leprosy in tissue sections, skin smears, and nasal smears.[66,301,342,344] Because these methods can detect a single leprosy bacillus, interpreting results, particularly in highly endemic areas, is difficult. Careful clinicopathologic correlation is essential when basing a diagnosis on DNA findings.[315]

The differential diagnosis of leprosy in children is an extensive subject and can be discussed only briefly here. Superficial mycoses and postinflammatory changes commonly are confused with early leprosy. Changes in pigmentation may be caused by scars, birthmarks, and actinic dermatitis. In areas where dermal filariasis is endemic, vague macules in a black-skinned patient may appear identical to the early macules of leprosy.[29,179,190] Among the many infiltrated lesions of the skin that can resemble leprosy are leishmaniasis, lymphoma, granuloma annulare, granuloma multiforme (Mkar disease), lupus erythematosus, psoriasis, pityriasis rosea, sarcoidosis, and neurofibromatosis. Peripheral neuropathies that may simulate leprosy are those seen in Morvan syndrome, syringomyelia, lead intoxication, diabetes mellitus, primary amyloidosis of nerves, and familial hypertrophic neuropathy. Cardiolipin antibody assays of sera from patients with advanced LL leprosy frequently give false-positive reactions for syphilis.

PROGNOSIS

Without chemotherapy, the prognosis in all patients except those with limited and self-healing disease potentially is poor. Patients with borderline or advanced TT leprosy frequently become mutilated because of damage to nerves. Borderline patients can downgrade toward LL leprosy. In patients with LL leprosy, the disease is progressive and can cause death from laryngeal obstruction. Secondary amyloidosis is a frequent late sequela. Blindness may result from lagophthalmic keratitis or repeated episodes of iridocyclitis. General debility and deformity eventually prevent gainful employment in many patients.

With adequate specific chemotherapy and control of reactions, the prognosis is good in nearly all patients. If therapy is started early, the prognosis usually is excellent, and deformity and mutilation are prevented. Even after receiving successful chemotherapy, however, some patients continue to experience significant neuritis and loss of peripheral nerve function. Sometimes this "silent neuropathy" goes unnoticed by the patient and the physician.[59,338] Appropriate early attention to anesthetic hands and feet and restoration of function by reconstructive surgery can prevent most mutilation.

The lepromin test is a valuable prognostic tool because it measures the CMI potential of the host to acquisition of infection by *M. leprae*.[135] Patients with early macular lesions who are lepromin-negative have a poorer prognosis than patients who are lepromin-positive if treatment cannot be administered. Histopathologic evaluation made before instituting chemotherapy is important for determining the prognosis and, if available, should not be neglected.

TREATMENT

When a diagnosis of leprosy is established, chemotherapy must be initiated, and appropriate measures must be instituted for preventing or correcting deformity in patients with neurotropic changes.[40,91,161] The three chemotherapeutic agents most commonly used are dapsone, clofazimine, and rifampin.[212] Although WHO recommends only clinical findings for assessment of the therapeutic response in field programs, clinicians may wish to use

additional evaluations. The effectiveness of chemotherapy is assessed readily in patients with LL or near-LL leprosy by the staining quality of *M. leprae* in skin smears. The response to chemotherapy in TT patients and most borderline patients is determined by the clinical response and by histopathologic evaluation. Viability of *M. leprae* in tissues is assessed in unusual cases in the mouse footpad.

Combined chemotherapy is being accepted increasingly for all forms of active leprosy. Although experimental data are limited,[281] the growing body of clinical data indicates that multidrug therapy has replaced monotherapy.[67,164] Monotherapy with any chemotherapeutic agent no longer is advised. The regimens recommended currently for multidrug therapy are given later, after a brief discussion of the most used individual drugs.

DAPSONE

In 1941, Faget and associates[81] at Carville, Louisiana, introduced the sulfones as the first chemotherapeutic agents regularly effective for leprosy and revolutionized the care of leprosy patients. The sulfone in common use is diaminodiphenylsulfone, or dapsone, available in the United States as Avlosulfon and Dapsone. Dapsone is an antimetabolite for *M. leprae* and is a bacteriostatic agent. The drug usually is given orally at 6 to 10 mg/kg body weight per week, divided into equal daily doses. The effect of dapsone on the bacilli is slow; 3 to 6 months of treatment is necessary to render bacilli from patients with LL leprosy noninfectious for the mouse footpad. Sulfone-resistant strains of *M. leprae* are being detected with increasing frequency,[337,338] and monotherapy with dapsone is not recommended. Dapsone usually is well tolerated but may provoke one or more of the following side reactions: dermatitis, anemia, hepatitis, or psychosis.[5]

CLOFAZIMINE

Clofazimine (Lamprene) is a bacteriostatic riminophenazine dye that has anti–*M. leprae* and anti-inflammatory activities, which render it a useful drug for the treatment of patients who are prone to having ENL reactions.[82] The mechanism of bacteriostasis may involve enhancement of oxygen-dependent killing of *M. leprae* and binding to bacterial DNA. The adult dose of clofazimine ranges from 50 to 300 mg daily, and some authorities recommend 1 mg/kg/day for children. The lower dosages are used for maintenance therapy when a good clinical response has been achieved, and the higher dosages may be needed to control ENL. The major side reactions to clofazimine are hyperpigmentation of skin and enteritis. Enteritis is experienced only at the higher dosages generally used for ENL. The hyperpigmentation subsides with the clinical improvement in leprosy, and hyperpigmentation and enteritis resolve after drug withdrawal. Although two instances of clofazimine-resistant *M. leprae* infection have been reported, the validity of these observations is uncertain.[148,336] For some time, clofazimine has been considered safe in pregnancy, but one report described three neonatal deaths in 15 observed pregnancies.[83] Whether the drug was associated with the deaths was not established. Monotherapy with clofazimine is not recommended.

RIFAMPIN

Rifampin is an antibiotic that inhibits bacterial DNA-dependent RNA polymerase and is rapidly bactericidal for *M. leprae*. A single large dose can render a highly positive LL patient noninfectious within 1 week. Dosages of 15 to 20 mg/kg/day have been given to children (maximal dose 600 mg/day). Optimal doses for children with leprosy have not been reported. Adult doses range from 300 to 600 mg daily.[347] Many side reactions to rifampin have been described, and the relevant literature must be consulted.[32] Rifampin-resistant leprosy has been reported,[130,345] so monotherapy with rifampin is not recommended.

MULTIDRUG THERAPY

Because of the existence of drug-resistant *M. leprae*, combined drug regimens are mandatory for the treatment of all forms of leprosy.[77,281,337,338] The first large-scale multidrug therapy for leprosy was initiated in Malta in 1972. Rifampin, dapsone, prothionamide, and isoniazid were used. Evaluation of the patients approximately 20 years later revealed a single relapse; however, presumed "persisting" *M. leprae* organisms were detected.[93,141]

Combined drug therapy minimizes development of drug-resistant strains of *M. leprae* and may eliminate some "persisting" organisms. Persisting *M. leprae* are viable bacilli that can be isolated in small numbers from patients who are clinically responding well to therapy. These persisting *M. leprae* bacilli are sensitive to the drug in question when tested in the mouse footpad and may account for relapses when treatment is discontinued. Persisting *M. leprae* organisms have been detected after 5 years of therapy with rifampin, 6 years of clofazimine therapy, and 22 years of dapsone therapy.

In 1982, a WHO study group recommended the multidrug therapy regimens that are described hereafter.[348] Patients were divided into paucibacillary and multibacillary groups. Paucibacillary patients (usually indeterminate, TT, and BT) were defined originally as patients with negative skin smears at all sites or patients who have fewer than four lesions and no clinical peripheral neuritis. Subsequently, for field programs, paucibacillary patients are classified only as patients who have fewer than four lesions, without reference to skin smear evaluation. All other patients are multibacillary.

The multidrug therapy regimens were designed primarily for field programs, and they use pulsed supervised monthly rather than daily rifampin.[18] Multidrug therapy is well tolerated, and compliance in large-scale control programs has been satisfactory. The efficacy of multidrug therapy has been promising.[36,338] In two surveys involving approximately 112,000 multibacillary patients monitored for 9 years after receiving therapy, the cumulative risk of relapse was 0.77 percent. Anecdotal descriptions of certain groups of highly bacilliferous patients with relapse rates of 20 percent and recurrences developing 5 years or more after therapy have been reported.[132] These and other results suggest that therapeutic regimens for multibacillary patients should be given for longer than 2 years.

In most reports, relapse rates in paucibacillary patients exceed those in multibacillary patients. In our experience in evaluating histopathologic specimens, many patients classified clinically as paucibacillary were multibacillary. The potential for relapse after receiving multidrug therapy regimens must await long-term, large-scale follow-up results.[36,131] Peripheral neuropathy sometimes persists after completion of these therapeutic regimens.[4,119]

The trend since 2001 has been to reduce the duration of treatment and change the therapeutic regimens—even to the extreme of a single-dose regimen composed of rifampin, ofloxacin, and minocycline for single-lesion therapy. Many of these innovations are interwoven into WHO Elimination of Leprosy Program and have provoked critical concern by some authorities.[87,168,218,291]

Regimen for Paucibacillary Patients (Indeterminate, Tuberculoid, and Borderline-Tuberculoid)

In adults, rifampin, 600 mg once a month, plus dapsone, 100 mg daily, is given for 6 months, and treatment then is stopped. After

the conclusion of multidrug therapy, the patient should be seen every 3 to 6 months. All apparent relapses require histopathologic examination for establishing whether the lesions represent relapses or reversal reactions. Relapsing patients must be treated again. The aforementioned multidrug therapy is not used alone in patients with concurrent tuberculosis.

Regimen for Multibacillary Patients (Lepromatous, Borderline-Lepromatous, and Borderline)

In adults, rifampin, 600 mg monthly; dapsone, 100 mg daily; and clofazimine, 50 mg daily, are given. If patient compliance is questionable, the rifampin and 300 mg of clofazimine should be given monthly under supervision, in addition to the 50 mg daily. These drugs must be given for 2 years. For patients in whom the hyperpigmentation caused by clofazimine is unacceptable, daily doses of 250 to 375 mg of prothionamide or ethionamide may be substituted for clofazimine. Another alternative therapy suggested by WHO for patients unwilling to accept the hyperpigmentation of clofazimine or who are noncompliant with other regimens consists of rifampin, 600 mg; ofloxacin, 400 mg; and minocycline, 100 mg, coined "ROM," all of which are administered on a single day, once monthly, for at least 2 years.[321]

Dosages of Multidrug Therapy for Children

Pediatric dosages of multidrug therapy are given in Table 109–2.[140]

Recommendations for the United States

The National Hansen's Disease Programs, Baton Rouge, Louisiana, recommends the following variations of WHO's recommended multidrug therapy regimens for adult patients in the United States:

- Paucibacillary disease: dapsone, 100 mg daily, plus rifampin, 600 mg daily, for 1 year.
- Multibacillary disease: dapsone, 100 mg daily, plus rifampin, 600 mg daily, and clofazimine, 50 mg daily, for 2 years. Minocycline, 100 mg daily, is substituted for clofazimine in patients who refuse to take clofazimine.
- Because the National Hansen's Disease Programs may change their recommendation for patient management, we advise clinicians in the United States to consult it before treating any patient with leprosy (telephone, 1-800-642-2477 or 1-225-756-3709, or e-mail, Mtemplet@hrsa.gov; it also has a website available at www.bphc.hrsa.gov/nhdp/).

OTHER DRUGS UNDER INVESTIGATION

Other potential antileprosy drugs that are undergoing advanced clinical evaluation and may gain general use include fluoroquinolones (pefloxacin and ofloxacin), the macrolide clarithromycin, and the tetracycline minocycline.[97,99,110,134]

TABLE 109–2 Dosage of Multidrug Therapy for Children with Leprosy

Rights were not granted to include this table in electronic media. Please refer to the printed publication.

From Jopling, W. H.: Handbook of Leprosy. London, William Heinemann, 1984.

TREATMENT OF REACTIONS

Patients experiencing a reaction should be observed daily in the early stages and hospitalized if the symptoms are severe. Formerly, specific therapy was stopped or the dosage reduced during reactions, but these measures no longer are recommended.[348] Damage to eyes and neurotropic changes may ensue rapidly without immediate attention. Nerve tenderness and function must be assessed frequently during reactions. Acute inflammation of isolated lesions without damage to nerves is likely to be of little consequence except for cosmetic considerations, but the patient should be monitored closely.

Reversal (Type 1) Reaction

Patients with painful, tender nerves must receive immediate care, usually in the hospital. Analgesics are given, and the affected area is rested. Large daily doses of corticosteroids are started and tapered to a minimal effective dose until the reaction subsides. Conversion to alternate-day steroid regimens may be attempted when long-term treatment is necessary. Some clinicians use clofazimine for chronic reversal reactions, but it is not recommended for the initial treatment of reactions with acute neuritis. For reactions, clofazimine probably is consistently efficacious only for ENL.[126]

Erythema Nodosum Leprosum (Type 2) Reaction

Mild ENL is treated with analgesics; more severe ENL is treated with thalidomide or corticosteroids.[322] Pediatric doses of thalidomide in ENL have not been established, but the initial adult dose is 100 mg four times daily followed by a minimal effective dose, usually 100 mg daily. The teratogenic action of thalidomide demands that appropriate measures be taken in the treatment of fertile women. For the rare patient who does not respond to thalidomide or in fertile women, corticosteroids or clofazimine is used. Corticosteroids, if used, are administered in the usual dosage schedules, beginning with large doses and tapering to a minimal effective level. Some clinicians use an alternate-day regimen when long-term steroid therapy is necessary, minimizing the well-known side effects. A few studies suggest that pentoxifylline or pentoxifylline plus clofazimine is effective for ENL.[220,302,340]

Clofazimine is effective in most patients with ENL and does not have the disadvantages of thalidomide or corticosteroids. The anti-inflammatory action of clofazimine is not manifested until after 4 to 6 weeks of continuous use. The dosage must be adjusted to the minimal effective level.

Iridocyclitis requires emergency measures. Local corticosteroids must be added to systemic anti-inflammatory regimens. Ophthalmologic consultation should be obtained.

PREVENTION

Precise recommendations for the prevention of leprosy in individuals have not been formulated. Control programs today are based on the general principles that (1) the number of contagious patients is reduced by chemotherapy and (2) the surveillance of contacts detects early leprosy. To accomplish these goals, appropriate education of the public and medical personnel and population surveys in areas of higher prevalence must be implemented. In endemic areas, improved housing probably is a highly important preventive measure by reducing close contact of patients with healthy individuals.

The most important obstacles to improving control of leprosy include persistence of *M. leprae* in treated patients, the cost and toxicity of antileprotic medications, the long-term nature of

therapeutic regimens, patient compliance, and the social stigma of leprosy.[249] For the total eradication of leprosy, zoonotic sources of *M. leprae* must be taken into account.[193]

CHEMOPROPHYLAXIS

Chemoprophylaxis with dapsone for close contacts has limited usefulness, but it is not recommended for large populations.[59] This recommendation is based on the probability that long-term use would be irregular, and dapsone-resistant *M. leprae* may develop.[347] Rifampin prophylaxis may have some value, at least in the short-term,[10] but the risk of increasing the rates of drug-resistant tuberculosis, or even leprosy, must be considered.

VACCINATION, IMMUNOPROPHYLAXIS, AND IMMUNOTHERAPY

WHO initiated an Immunology of Leprosy Program (IMMLEP) in 1974 with two primary goals: (1) development of a vaccine against leprosy and (2) development of reagents for detecting subclinical leprosy. Achievement of both goals could diminish profoundly the incidence of leprosy. *M. leprae*, or specific antigens thereof, for the IMMLEP studies were obtained from experimentally infected armadillos. Vaccines composed of heat-killed whole *M. leprae* alone or in combination with live bacille Calmette-Guérin (BCG) have been found to be safe and to induce delayed-type hypersensitivity to *M. leprae* in a high percentage of lepromin-negative individuals. Several other vaccines based on cultivable mycobacteria (*Mycobacterium vaccae*, *Mycobacterium* "w," and the Indian Cancer Research Centre bacillus) induce similar responses.[19,149,293]

Field trials of these vaccines for the immunoprophylaxis of leprosy have been conducted; however, because of the chronicity and low prevalence of the disease, meaningful evaluation of their efficacy would require extended follow-up observations.[21] Because infection-induced immunity is not observed regularly in leprosy, a reasonable doubt exists that vaccines containing only *M. leprae* would be protective. Combined vaccines of killed *M. leprae* and live BCG have been studied. Such vaccines convert lepromin-negative contacts of patients with leprosy to positive reactors and upgrade LL patients toward the tuberculoid region of the disease spectrum.[45,195] Vaccines based on cell wall fractions of *M. leprae* are being studied.[98] WHO does not recommend BCG vaccination for the prevention of leprosy.[17] This decision was based on the highly variable results of extensive studies in Burma, Papua New Guinea, and Uganda.[348] Another trial in India involving 270,000 individuals confirmed that during a 12.5-year follow-up, BCG vaccination was shown to be only approximately 25 percent effective against leprosy.[304]

Initial evaluations of a large-scale immunoprophylaxis trial of heat-killed *M. leprae* plus BCG vaccine in humans in Venezuela showed no better protection than did BCG alone 5 years after vaccination.[48] A randomized trial of a single BCG vaccination, repeated BCG, or BCG plus killed *M. leprae* involving 121,020 individuals in Malawi gave the following results over a 5- to 9-year follow-up: A single BCG vaccination afforded 50 percent protection against leprosy, a second BCG vaccination added appreciably to this protection, but the addition of killed *M. leprae* to BCG did not enhance protection against leprosy.[150]

LEPROSY AND ACQUIRED IMMUNODEFICIENCY SYNDROME

Because *M. tuberculosis*, *Mycobacterium avium–intracellulare*, and *Mycobacterium kansasii* are frequent opportunistic pathogens in patients with acquired immunodeficiency syndrome (AIDS), observations of AIDS in patients with leprosy are of interest.[312] In one study in Zambia, antibodies to human immunodeficiency virus (HIV) were found in 33 percent of new leprosy patients versus 7 percent of controls.[181] Perhaps the positivity of some of these patients with leprosy can be explained by cross-reactivity between antibodies to HIV-1 and the lipoarabinomannan of *M. leprae*.[151] In other populations in Africa, HIV infection constituted an overall risk factor of 2.2 for leprosy, with 4 to 23 percent of cases of multibacillary leprosy attributable to HIV co-infection.[24]

Other prospective studies show that HIV infection may not be a risk factor for acquisition of leprosy in some populations.[154,276] Only a few detailed clinicopathologic reports on individuals co-infected with *M. leprae* and HIV exist.[133,158,211] In these patients, no consistent deleterious effect of HIV infection on the clinical or pathologic findings of leprosy occurred. These observations are supported by a study of the parameters of CMI in co-infected patients in Brazil.[267] In these patients with borderline leprosy, the quality of the granulomata in the infiltrations of leprosy were not altered significantly despite low $CD4^+$ T-cell counts. The incidence of reversal reactions (type 1) and neuritis is increased in multibacillary patients co-infected with HIV.[34] Perhaps, as previously and perceptively suggested,[169] observations in patients co-infected with HIV and *M. leprae* will lead to some revisions of the immunopathogenesis of leprosy.

BURULI ULCER

Mycobacterium ulcerans causes indolent, necrotizing cutaneous ulcers that are known classically as *Buruli ulcers*.[171,287,316] Other names for these lesions include Bairnsdale or Searles' ulcer in Australia and Kumusi ulcer in Papua New Guinea. Buruli ulcer seems most appropriate, however, because Clancey and colleagues[39] were the first to name the disease, after the site of the first large epidemic, which was located in Buruli County, Uganda.[68] Cook[49] in Uganda described lesions that fit Buruli ulcer in 1897. Today, researchers recognize that many infections with *M. ulcerans* are not manifested as ulcers, and *M. ulcerans* infection is a technically more appropriate name for the disease. A few infections have been acquired by North American or European travelers to endemic countries,[44,78,277] and patients with Buruli ulcers frequently come to European medical centers for treatment.[12,242]

WHO has recognized Buruli ulcer as a re-emerging infectious disease in western Africa with an important public health impact. WHO Global Buruli Ulcer Initiative has held annual meetings to evaluate all aspects of the disease since 1998, and in 2004, the World Health Assembly adopted a resolution for all member states to intensify research to develop tools for diagnosing, treating, and preventing Buruli ulcer.[351]

ORGANISM

MacCallum and associates[172] in 1948 in Australia were the first to isolate the etiologic agent in culture. *M. ulcerans* is strongly acid-fast, with an optimal growth temperature of 30° C to 32° C on routine mycobacteriologic media such as Löwenstein-Jensen medium. *M. ulcerans* is a slow-growing organism, and several months may be required to achieve isolation in primary culture. Microaerophilic conditions promote the growth of *M. ulcerans*, and the organism is strikingly sensitive to temperatures of 37° C or greater.[196,227] This temperature sensitivity often reduces rates of cultivation of the etiologic agent in laboratories remote from endemic areas.

M. ulcerans is the only mycobacterial pathogen of humans known to elaborate a necrotizing and immunosuppressive toxin.[55,233,248] This toxin is a polyketide and has been named *mycolactone*.[100,101] The genomic sequences of the plasmid-encoded synthases that produce mycolactone have been described more recently.[299] Animal studies and immunohistologic evaluations of human lesions indicate that at least one mechanism by which mycolactone mediates tissue destruction is by triggering massive levels of apoptosis.[102,328]

Although mycolactone is viewed as the dominant virulence factor of *M. ulcerans*, other factors may participate (e.g., phospholipases).[105] The phenolic mycosides of *M. ulcerans* and *Mycobacterium marinum* are identical, and sequences for the 16S rRNA gene differ by only one base pair.[54,255]

Specific insertion sequences for *M. ulcerans* have been characterized and are available for identification of the organism by PCR.[1,285,297] Variations in the 3′ end of the 16S rRNA gene sequence are related to geographic origin and are used to divide the organism into African, American, Asian, and Australian strains.[240]

TRANSMISSION AND EPIDEMIOLOGY

Endemic foci of *M. ulcerans* infections usually appear in rural settings near permanent wetlands in warm countries, especially in terrain subject to seasonal flooding. Reports of patients with Buruli ulcers have come from at least 27 countries, principally in the tropics.[335] A few patients live in nontropical climates, such as China,[79] Japan,[308] and southern Australia.[318] Today, the largest numbers of reported patients live in West Africa (Benin, Burkina Faso, Côte d'Ivoire, Ghana, Guinea, Liberia, and Nigeria).[238,316,335] In these countries, the disease is re-emerging rapidly, with an estimated total annual incidence exceeding 7000 patients. Other countries in which the disease is well known to be endemic include Malaysia, Indonesia, Papua New Guinea, Peru, Suriname, French Guiana, Cameroon, Equatorial Guinea, Gabon, Angola, Democratic Republic of Congo, and Uganda.[60,121,188,232]

Observers attribute this re-emergence to environmental factors such as deforestation, artificial topographic alterations (dams and irrigation systems), increasing populations engaged in basic manual agriculture in wetlands, and possibly climactic changes.[198] In North America, two cases of Buruli ulcer were reported in 2005 in central Mexico, the nearest location to the United States to date.[42]

Individuals of all ages are affected, but the highest frequencies are in children 15 years of age or younger—usually approximately 75 percent of all cases.[176] The sexes are affected equally, and a racial predilection is unknown.[61] Anecdotal observations of children in families of multiple parentage have suggested a possible genetic predisposition, now supported by molecular studies.[295] Seasonal variations in incidence occur in some foci.[118,251] Approximately 80 percent of the lesions are on the limbs, with the highest frequencies involving the lower extremities.

Although Buruli ulcer long has been associated with riverine environments, repeated long-term studies (1960 through 2005) on a large variety of flora, fauna, water, and mud samples from many endemic foci failed to reveal *M. ulcerans* in nature by culture.[13,71,235] After the development of molecular biologic techniques for the identification of *M. ulcerans*, the organism was detected in the environment in Australia by PCR.[260,261,296] With the increased understanding of this fastidious organism now available, researchers expected in 2001 that successful culture of *M. ulcerans* from nature would soon be achieved.[238] In 2006, two strains of *M. ulcerans* cultured from nature were reported.[287]

Although the ultimate source of *M. ulcerans* remains obscure, the organism has been discovered in aquatic insects, such as water bugs, firefly larvae, and beetles, obtained from stagnant water in endemic areas of West Africa.[175,238,239] In some cases, the organisms were found only in the salivary glands of aquatic insects, supporting the notion that insect bites may play a role in transmitting *M. ulcerans*.[173,174]

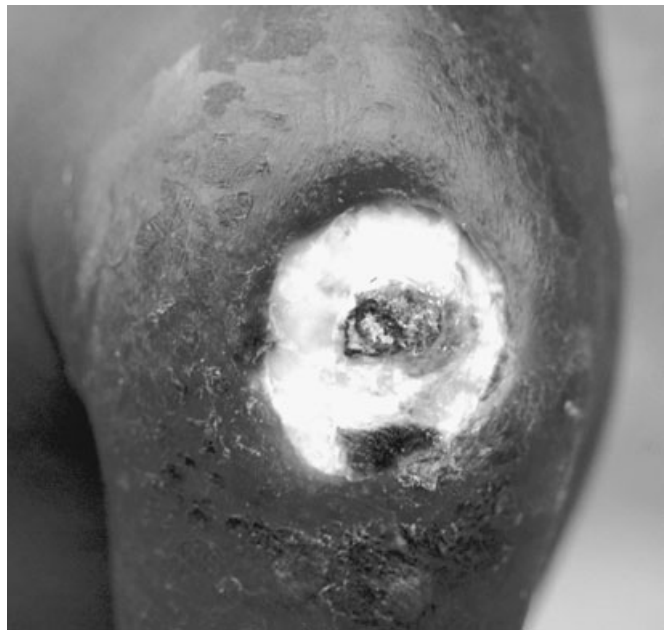


Figure 109–14 A major Buruli ulcer in the deltoid area of a 12-year-old Angolan boy. This pristine ulcer developed 3 months after a documented hypodermic anticholera vaccine injection at this site. Typical Buruli ulcer features are represented—undermining of borders, necrotic base, and induration of the adjacent skin. (See companion Expert Consult web site for color version.)

Some authorities speculate that *M. ulcerans* is a saprophytic or commensal organism that thrives in the mud, flora, or fauna of the cool microaerophilic environment of the bottom of stagnant water, well protected from the lethal ultraviolet radiation that prevails in the tropics. Koalas and possums in Australia acquire infections in the wild that are typical of Buruli ulcer.[203,238]

Buruli ulcer rarely, if ever, is contagious. The distribution of patients, even in highly endemic foci, is random, suggesting that each patient is exposed to environmental sources, such as swamps where villagers work their gardens and obtain water for domestic use and especially where children play.

The mode or modes of transmission to humans have not yet been delineated completely; however, the most plausible route is by trauma at sites of skin recently contaminated by *M. ulcerans*.[71,197] Many patients give a history of specific antecedent penetrating trauma at the site of their initial lesion, which may include wounds from a gunshot or land mine, thrown stones, hypodermic injection, and even a human bite (Fig. 109–14).[64] The organism may be spread by aerosol from the surface of ponds or be carried by fomites or insects to skin surfaces. Insects may introduce *M. ulcerans* into the skin, but this means of transmission has not been proved. Although proposed by some authorities, transmission by nasorespiratory passage with a subsequent bacteremia seems to be unlikely.

PATHOGENESIS AND PATHOLOGY

The pathologic features of early *M. ulcerans* infection are determined primarily by two properties of the etiologic agent: optimal growth at 30° C to 33° C and elaboration of the toxin mycolactone. The temperature requirement tends to favor the development of lesions in the skin, and the toxin destroys tissues and

suppresses immune responses. The spectrum of clinical and histopathologic forms of infection suggests that some patients have innate resistance, or resistance develops soon after infection is acquired, whereas others acquire resistance later or, occasionally, never.

A skin test with burulin, a purified sonicate of *M. ulcerans*, reveals that most patients do not have delayed-type hypersensitivity to *M. ulcerans* early in the course of the infection but mount a cellular response as healing begins.[292] Very few studies have investigated the immunology of Buruli ulcer. *M. ulcerans* profoundly suppresses B and T lymphocytes in vitro.[233] Mycolactone, a toxin of *M. ulcerans*, is thought to prevent sensitization of T lymphocytes to mycobacterial antigens and to inhibit production of TNF-γ by monocytes and IL-2 by T lymphocytes.[3,52,100,101,226] In a study of tissue sections of Buruli ulcer lesions, IL-10 was increased in ulcerative lesions without granulomata, whereas IFN-γ was abundant when granulomata were present.[156] These activities partly explain the immunologic unresponsiveness and reduced inflammatory reaction at the site of the lesion.

Based on the current understanding of the natural history of *M. ulcerans* infection, pathogenesis may proceed as follows: After inoculation of the etiologic agent deep into the skin or subcutaneous tissue, a latent phase occurs during which the mycobacterium proliferates slightly, probably initially intracellularly,[224] and begins to elaborate small amounts of toxin that causes necrosis, especially of fatty tissue. This necrosis provides a microaerophilic environment and perhaps nutrients favorable for the accelerated growth of *M. ulcerans* and elaboration of increased amounts of toxin. During this necrotic phase, no significant cellular response occurs.[43,44,68]

In some patients, at this stage, a subcutaneous nodule begins to develop, with clusters of *M. ulcerans* in the center surrounded by a zone of necrosis. In highly resistant patients, this lesion may self-heal, perhaps without ulceration, or form a small, sharply delineated ulcer. In others, the skin is undermined by the necrosis and eventually breaks down into larger ulcers with widely undermined skin. In the least resistant individuals, a nodule never develops, and the necrosis spreads rapidly and widely to cover large body surface areas, but ulceration, if it occurs at all, is a late event.

Eventually, the necrotic stage ceases in most patients, either because the toxin is neutralized or because production is interrupted. At this time, a granulomatous stage begins to develop, followed by healing and scarring. Some of the variability in clinical presentation and progression of the disease may be related to heterogeneity in the *M. ulcerans* genome and in the plasmid that encodes the production of mycolactone.[1,216,298,300]

Microscopically, the active ulcer shows extensive coagulation necrosis of the subcutaneous tissue down to and often including the fascia (Fig. 109–15).[111] The deep layers of necrosis reveal masses of AFB, often with mineralization and extensive vasculitis. Marked edema is present, and fat cells enlarge and die, with only their cellular ghost outlines remaining (Fig. 109–16). The dermis and surrounding tissue seldom contain AFB. Lesions sometimes provoke a reactive (contiguous) osteitis that leads to necrosis of cortical bone and osteomyelitis. Metastatic lesions may develop in the skin and bone from bacteremia and produce skin lesions distant from the original lesion and frequently focal or multifocal osteomyelitis. Regional lymph nodes may show massive necrosis and contain large numbers of AFB, especially in the capsule. Visceral organs are not known to be involved, but no necropsies of patients who died of disseminated disease have been reported.

An understanding of the pathogenesis and developing improved therapies for Buruli ulcer has been slowed by the lack of an experimental animal model that replicates the spectrum of features found in human disease. Laboratory rats and mice develop skin lesions after intradermal inoculation with *M. ulcerans*, but they lack the extensive ulceration found in human disease. Multimammate rats (*Mastomys natalensis*) rapidly develop systemic infections and die.[286] In the mouse footpad, *M. ulcerans* multiply, but necrosis without ulceration destroys the limb and causes death.[248] Guinea pigs develop inflammatory lesions at the inoculation sites that may resolve without formation of ulcers.[157,248] The nine-banded armadillo is susceptible to *M. ulcerans* and develops cutaneous lesions after experimental intradermal infection, approximating those of the human disease but is phylogenetically distant.[327] The cynomolgus monkey is moderately susceptible to experimental *M. ulcerans* infection and develops some of the clinical and histologic features of Buruli ulcer.[325]

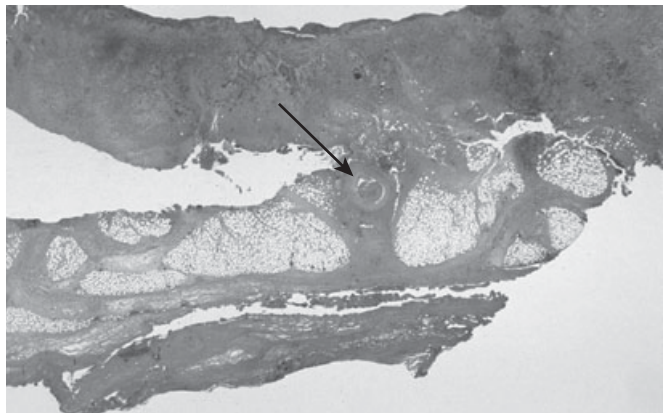


Figure 109–15 Histopathologic section of the undermined edge of a major Buruli ulcer. Note the coagulation necrosis of the entire panniculus and fascia, and vasculitis with thrombosis of a blood vessel *(arrow)* (hematoxylin and eosin stain). (See companion Expert Consult web site for color version.)

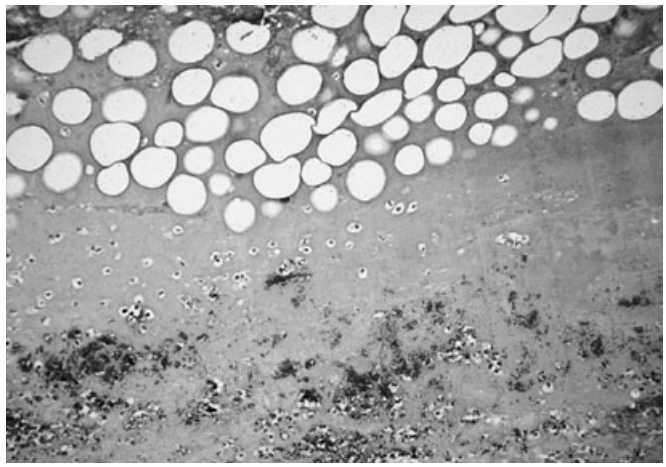


Figure 109–16 High magnification of the necrotic panniculus of a Buruli ulcer showing the dead fat cell "ghosts," coagulation necrosis, and masses of acid-fast bacilli (Ziehl-Neelsen stain). (See companion Expert Consult web site for color version.)

CLINICAL MANIFESTATIONS

INCUBATION AND FORMS OF LESIONS

In one study of specific trauma related to lesions, the incubation period ranged from 2 weeks to 3 years, with a mean of 3 months.[197]

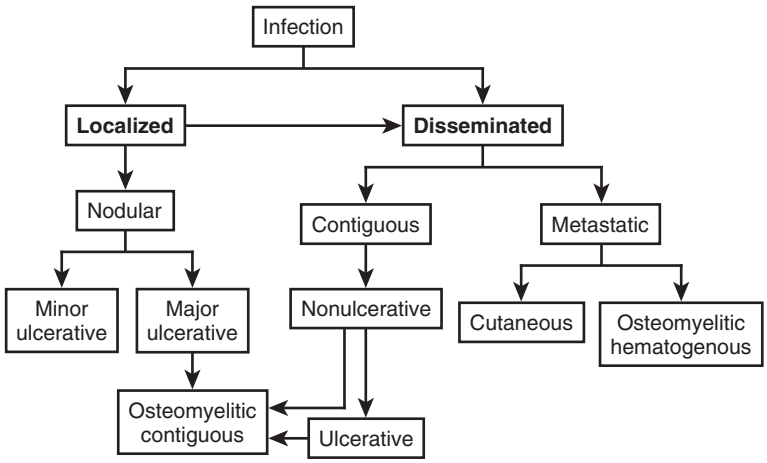


Figure 109–17 Proposed classification of clinical forms of *M. ulcerans* infections, indicating possible progression of lesions after the initial cutaneous infection. Without treatment, lesions may self-heal at any stage, or advance according to the schema shown at left.

Figure 109–18 Edematous form of *M. ulcerans* infection in a 6-year-old Ghanaian girl after surgery to remove all necrotic tissue. There was never any ulceration. Note ablation of breast tissue. (See companion Expert Consult web site for color version.)

The following are the various forms of lesions of *M. ulcerans* infection according to WHO designations.[353,354] Figure 109–17 presents a proposed classification of the clinical forms of *M. ulcerans* disease and their possible inter-relationships.

Papule

The papular stage has been described only in patients from Australia. These papules are painless and elevated and measure up to 1 cm in diameter.

Nodule

Nodules are primarily subcutaneous and firm, measure approximately 2 cm in diameter, and are painless, although they are often pruritic. The overlying skin may be discolored. This stage is the initial one in most Africans, and in the Kikongo language, the disease is called *mputa matadi* because the nodule is a "rock-hard lesion."

Plaque

Plaques are firm, elevated, painless, well-defined lesions more than 2 cm in largest dimension. Their borders are irregular. This stage often is the one in which physicians initially see the patient, but the lesion may or may not arise from a nodule. The skin over the lesion is reddened or discolored. These lesions may ulcerate in the late stages.

Edematous Form

Most edematous lesions do not begin in the nodular stage but spread directly from the initial nidus of infection. Spread often is rapid and wide and covers entire limbs or major portions of the trunk (Fig. 109–18). This type of lesion is characterized by diffuse nonpitting swelling and vague margins. Lesions are firm and frequently painful, with changes in color and scaling on the skin surface.

Ulcerative Forms

Classically, when fully developed, pristine ulcers have undermined edges surrounded by a zone of induration and often desquamation of the epidermis (see Fig. 109–14). In the base of the ulcerated area, a whitish necrotic slough and sometimes eschar develop. An oily exudate frequently oozes from the dependent area. Old ulcers tend to begin healing in the uppermost part while activity continues in the dependent portion. Collections of fluid in this area probably continue to support the growth of *M. ulcerans*, which sustains progression of the lesion.

The ulcerative forms are divided into major and minor ulcers. Both forms tend to self-heal. A major ulcer is large and chronic. Minor ulcers are small (1 to 2 cm in diameter), are sharply delineated, and heal early. Both ulcerative forms begin as subcutaneous nodules.

BONE INVOLVEMENT

Contiguous Osteomyelitis

Reactive osteitis occasionally develops beneath destroyed overlying skin and soft tissue. Bone becomes devitalized and necrotic, with the development of sequestra (Fig. 109–19).

Metastatic Osteomyelitis

M. ulcerans–specific osteomyelitis develops in approximately 10 percent of all patients, underscoring that Buruli ulcer should always be considered a potentially systemic disease.[242] Most likely, metastatic osteomyelitis is a result of lymphohematogenous spread of *M. ulcerans* from a distant, earlier cutaneous lesion (Fig. 109–20). The overlying skin ordinarily is intact, but swelling and inflammation occur over the site of bone involvement. If the

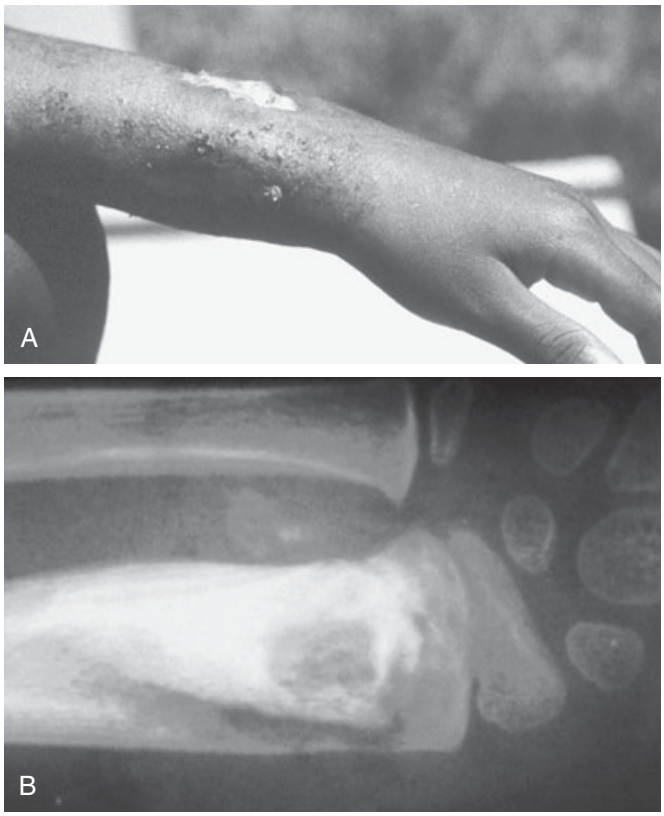


Figure 109–19 **A,** *M. ulcerans* infection in a young Congolese boy. Note swelling and ulcer on dorsum of forearm. (See companion Expert Consult web site for color version.) **B,** Radiograph of forearm shows reactive osteitis, necrosis of cortex of distal end of the radius, and early sequestration with osteomyelitis.

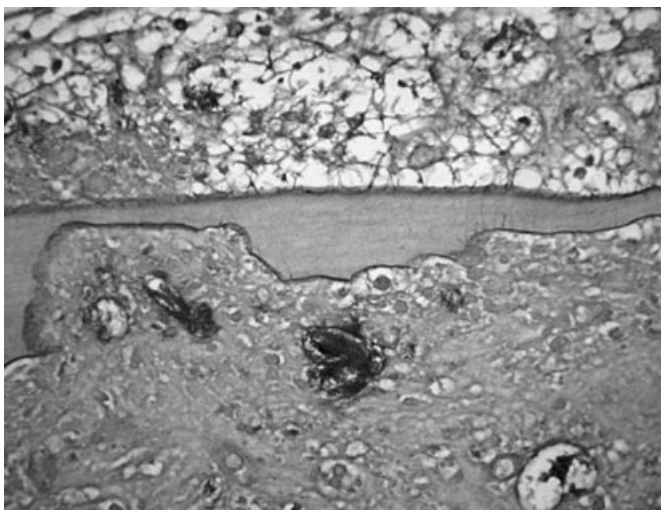


Figure 109–20 Ziehl-Neelsen–stained section shows metastatic osteomyelitis of the tibia in a 6-year-old boy in Benin. This lesion developed after *M. ulcerans* infection of the contralateral leg. Note necrosis of marrow, erosion of a trabecula, and many clusters of acid-fast bacilli (Ziehl-Neelsen stain). (See companion Expert Consult web site for color version.)

Figure 109–21 This 13-year-old girl in Benin initially had a cutaneous lesion of Buruli ulcer. Subsequently, metastatic spread to bones of the lower extremities occurred, resulting in bilateral high-thigh amputations. Note also the spread to bones of both wrists and left hand. (See companion Expert Consult web site for color version.)

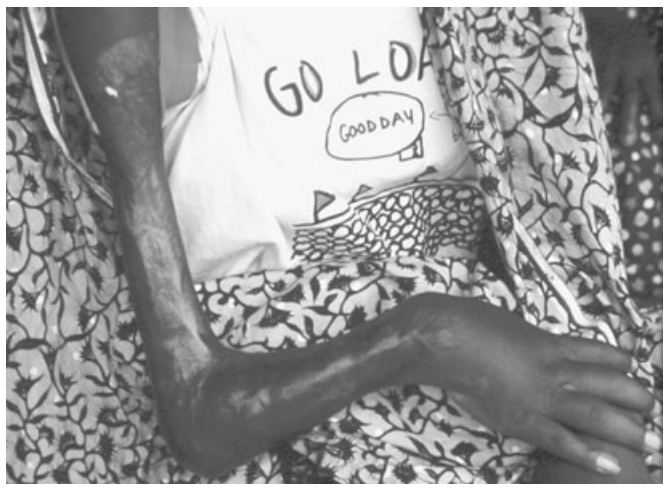


Figure 109–22 Ghanaian adolescent girl with healed *M. ulcerans* infection that extended from the wrist to the shoulder. The scarring resulted in contraction deformities of the wrist and elbow and disuse atrophy of the entire extremity. Appropriate physiotherapy would have minimized these sequelae. (See companion Expert Consult web site for color version.)

condition is suspected, and where available, bone scans may be helpful in diagnosis.[242] If the condition goes untreated, a draining fistula usually develops. Osteomyelitis often requires an amputation (Fig. 109–21).

Complications

Infection may traverse the deep fascia and damage tendons, nerves, joints, genitalia, and periorbital tissues, requiring subsequent enucleation of the eye. Healing leads to fibrosis and scarring and can severely limit movement, with attendant lifestyle alterations.[294] The scar may form keloids and often causes major contraction deformities, especially in lesions that cross joints (Fig. 109–22). Squamous cell carcinoma may develop in healed lesions, especially nonpigmented lesions. Skin grafting and physiotherapy prevent most of these complications. Most disease-related deaths result from septicemia, gas gangrene, or tetanus.

DIAGNOSIS AND DIFFERENTIAL DIAGNOSIS

To an experienced observer, an accurate clinical diagnosis often can be made with ease.[353,355] In the ulcerated forms, a Ziehl-Neelsen stain of exudate from the undermined edge obtained with a cotton swab reveals clusters of extracellular AFB. The same material, obtained by swab after decontamination, may be used for culture on Löwenstein-Jensen or other suitable mycobacterial media. The incubation temperature must be 30° C to 32° C. If culture cannot be performed locally, transport media may be inoculated with material from the cotton swab and maintained at 4° C while in transport to a specialized laboratory. The transport medium is composed of Middlebrook 7H9 broth supplemented with polymyxin B, amphotericin B, nalidixic acid, trimethoprim, azlocillin, and 0.5 percent agar. Molecular biologic analysis techniques consisting of PCR for the identification of *M. ulcerans* are available and increasing in popularity and convenience.[1,285,296] Tissue for histopathologic analysis should be obtained from the edge of the ulcer and must include all levels, including the fascia. Fixation in 4 percent buffered formalin is adequate.

Following are some differential diagnostic possibilities:

1. Papules—insect bites, verruca vulgaris, pityriasis, granuloma annulare
2. Nodules—lipoma, sebaceous cyst, onchocerciasis, furuncle
3. Plaques—leprosy, mycosis, necrobiosis, psoriasis
4. Edema—bacterial cellulitis, actinomycosis, elephantiasis, pyomyositis
5. Ulcers—tropical phagedenic ulcer, noma, stasis ulcer, leishmaniasis

PROGNOSIS

Without treatment, Buruli ulcer often leads to deforming depressed scars, contraction deformities, or amputation. The stigma of the deformities and the socioeconomic burden of the disease often are marked.[9] With early appropriate treatment, including excision and grafting, the prognosis usually is excellent. Metastatic lesions and local recurrences occur frequently enough, however, to warrant vigilant follow-up.[6]

TREATMENT

Treatment options for Buruli ulcer include antibiotics and surgical intervention. The choice is usually based on the morphology and extent of the lesions and the availability of antibiotics and surgical facilities. Current WHO guidelines are available.[350,354]

Briefly, antibiotic treatment as recommended by WHO, although still empiric, currently includes at least 8-week courses of daily oral rifampin (10 mg/kg) and streptomycin (15 mg/kg) given as an intramuscular injection. Rifampin is supplied in tablet and syrup forms, the latter being helpful for small children. Streptomycin and, to a lesser extent, rifampin, are associated with rare but important side effects, some of which require stopping treatment. For streptomycin, treatment should be stopped if hearing impairment or vertigo with nystagmus develops. For rifampin, treatment should be stopped if hepatitis, jaundice, or acute renal failure occurs, side effects that generally are associated with intermittent therapy of more than 10 mg/kg. Streptomycin also is contraindicated during pregnancy. In our opinion, antibiotic therapy for advanced lesions remains a subject for inquiry, and the above-described regimens do not constitute established recommendations at this time.

If surgical intervention is chosen, to minimize the danger of development of *M. ulcerans* bacteremia, many surgeons prescribe clarithromycin and rifampin 1 to 2 weeks before performing surgery and have the patients continue antibiotic therapy for several weeks after surgery. Although reasonable, this antibiotic complement to surgery is not of proven efficacy. Papules and pre-ulcerative nodules seldom are diagnosed, even in endemic areas; however, wide excision and primary closure usually are curative.[311]

Plaques and edematous forms are excised widely down to the fascia, or through the fascia if it is necrotic. Muscle usually is not damaged, but if so, the excision is extended into muscle. The lateral extent of excision often is difficult to determine. By careful palpation, the physician can establish an approximate limit of the disease. Exploratory incisions and blunt dissection may help determine the limit of induration and necrosis. Use of real-time PCR for determining the extent of disease is being evaluated.[257]

Very small ulcers can be excised and closed primarily, as for nodules. Large ulcers are excised widely. The required extent of surgery may be determined by exploratory lateral excision and blunt dissection. Split-skin autografting of surgical defects usually is performed after a bed of granulation tissue has formed. Postoperative care, including physiotherapy, should be designed to prevent contractures.

Bone lesions should be referred to specialists. Heat therapy without surgical excision has been successful for appropriate lesions, but must be applied assiduously with all necessary controls.[196] Recurrences after surgical treatment are frequent, but rates are not well established.[62]

PREVENTION

In an endemic tropical rural setting where children usually are scantily attired, prevention of contamination of the skin from environmental sources is virtually impossible. Wearing long trousers seems to prevent development of infection.[176] Protected water supplies in villages would reduce exposure; however, such protective measures usually are futile in rural areas of developing countries. Other risk factors have been analyzed extensively.[63]

Vaccination with BCG has a moderate protective effect against *M. ulcerans* infections for 6 to 12 months.[310] Studies are projected to determine whether repeated BCG vaccination may render the population more immune to *M. ulcerans* infection,[236,237] as was found for leprosy.[150] Other vaccines based on virulence factors of *M. ulcerans* (e.g., the toxin) also are being studied.[125]

HUMAN IMMUNODEFICIENCY VIRUS AND BURULI ULCER

Because Buruli ulcer primarily is a disease of children in rural areas, very few patients have been reported with Buruli ulcer and HIV infection.[8] Relevant reports available indicate, however, that HIV infection may render Buruli ulcer disease highly aggressive; rapidly spreading *M. ulcerans* osteomyelitis has developed in several patients with AIDS.[138,303]

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CHAPTER 110

NOCARDIA

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Nocardia spp. are obligate aerobic bacilli that exist throughout the world as soil and dust saprotrophs. These organisms are non–spore-forming, thin, branching, gram-positive, partially acid-fast, filamentous bacteria. Humans become infected with *Nocardia* by two primary routes: inhalation of contaminated airborne dust particles or traumatic implantation of the bacterium into subcutaneous tissue. Pulmonary disease is the form of nocardiosis recognized most commonly in the United States.[2] The pulmonary event may be subclinical or transient, or it may provoke an acute or chronic process mimicking staphylococcal or fungal pneumonia, tuberculosis, or carcinoma. Hematogenous dissemination may occur, especially in immunocompromised hosts. The central nervous system (CNS) is the most common site of dissemination, with involvement manifested most often as a brain abscess. Cutaneous nocardiosis may be acute, subacute, or chronic. This form of disease is seen predominantly in immunocompetent hosts, with *Nocardia brasiliensis* being the agent identified most frequently.

In 1888, Nocard, a veterinarian, noted an aerobic actinomycete in bovine farcy, a chronic wasting disease in cattle characterized by pulmonary abscesses and draining cutaneous sinus tracts. Eppinger first described human disease in 1890. The earliest pediatric case was documented in 1895 in a boy with pulmonary and subcutaneous infection.[5]

ORGANISM

Nocardia spp. are included among the aerobic actinomycetes. They are gram-positive bacteria that are filamentous and branched and grow more slowly than other aerobic and facultatively anaerobic bacteria. They commonly produce a fungus-like mycelium that fragments or breaks up into rod-shaped and short coccoid forms. *Nocardia* spp. grow well aerobically on a variety of simple media (e.g., blood agar, brain-heart infusion agar); adding carbon dioxide (10%) promotes more rapid growth. The organisms are inhibited by antibiotics and antifungal agents, so media containing such agents do not support the growth of *Nocardia*. Because of their growth on commonly used fungal media (e.g., Sabouraud dextrose agar) and on some mycobacterial media (e.g.,

Löwenstein-Jensen medium), many *Nocardia* samples may be misdirected to the mycology or mycobacteriology section of clinical laboratories for identification.

In the past, microscopic and colonial morphology, biochemical tests, chemotaxonomic methods, or antimicrobial susceptibility patterns were used to speciate *Nocardia*. 16S rRNA gene sequencing and polymerase chain reaction (PCR) restriction fragment length polymorphism (RFLP) analysis are the current methods most frequently used for speciation; use of these molecular techniques has resulted in changes in the nomenclature of *Nocardia* spp.[10,44]

More than 30 species of *Nocardia* of human clinical significance have been identified.[10] The term *Nocardia asteroides* complex formerly was used to account for the heterogeneity in antimicrobial resistance patterns seen among *N. asteroides.* Detailed molecular analyses and susceptibility testing have established clearly that *N. asteroides* complex represented many different *Nocardia* spp.[10] The most medically important species of *Nocardia* include *N. asteroides*, *Nocardia abscessus*, *Nocardia brevicatena/paucivorans* complex, *Nocardia farcinica*, *Nocardia nova* complex, *Nocardia brasiliensis*, *Nocardia otitidiscaviarum* complex, *Nocardia pseudobrasiliensis*, and *Nocardia transvalensis* complex.

Nocardia spp. grow best at temperatures of 25° C to 45° C; growth at higher temperatures may be used for differentiation. In pure culture, small, chalky white, heaped, wrinkled, or verrucous colonies appear in 3 to 5 days. Mature colonies usually are light orange and have a velvety appearance caused by the production of rudimentary aerial mycelia. They have the odor of a musty basement or freshly turned soil. Detection of colonies from clinical specimens, such as respiratory secretions, may require 2 to 4 weeks. In mixed culture, rapidly growing bacteria may obscure small *Nocardia* colonies. Use of modified Thayer-Martin medium may enhance recovery.[39]

Gram stain of a portion of a colony shows delicate, branching filaments no more than 1 μm in diameter. The delicate filaments may fragment and produce bacillary or coccoid forms. Many *Nocardia* spp. are partially acid-fast (i.e., compared with *Mycobacterium* spp., they retain fuchsin less tenaciously). A modified Ziehl-Neelsen or Kinyoun stain that decolorizes with 1 percent sulfuric acid instead of the more active acid alcohol is best for showing acid-fast *Nocardia* in clinical specimens. Acid-fastness is characteristic of *Nocardia* in tissue or primary colonial isolates but is lost quickly on subculture; not all pathogenic strains of *Nocardia* are acid-fast.[6] *N. asteroides* often survives the *N*-acetylcysteine digestion procedure (without sodium hydroxide) that is performed on sputum or bronchial washings; however, some positive sputum specimens may be rendered falsely negative.[38] Cultures of sputum and bronchial washings for isolation of *Nocardia* should be performed before and after the digestion procedure.

EPIDEMIOLOGY, TRANSMISSION, AND PATHOGENESIS

Nocardia spp. occasionally can be skin contaminants or upper respiratory tract saprotrophs.[21,44,53] Bronchial obstruction or decreased bronchociliary clearance predisposes to colonization.[44] The presence of signs and symptoms that correlate with nocardiosis can help ascertain the clinical significance of a positive *Nocardia* culture.

Most infections occur in the lungs, presumably via inhalation, with dissemination to the CNS occurring in one third of affected patients. Although nocardiosis occurs in immunocompetent individuals, 70 percent of patients are immunosuppressed by medications or underlying disease.[23] The typical patient has compromised cellular immunity (secondary to steroids, organ transplantation, cytotoxic chemotherapy, chronic granulomatous disease, chronic alcoholism, diabetes mellitus, or human immunodeficiency virus [HIV] infection). Nocardiosis also is seen more commonly in patients with chronic pulmonary disease or a history of surgery or trauma.[35] Person-to-person communicability has not been a problem. The incidence of nocardiosis in the United States has been estimated to be approximately 500 to 1000 new cases per year.[3] This number most likely is an underrepresentation, however, given the marked increase in the number of immunocompromised individuals. In addition, nocardial infections can be difficult to recognize, resulting in underestimation of the true incidence of nocardiosis. Presumed nosocomial nocardiosis has been reported.

Cutaneous nocardiosis usually is an infection of immunocompetent hosts. *N. brasiliensis* is isolated from approximately 80 percent of cases of primary cutaneous or subcutaneous nocardiosis.[46] Traumatic implantation of aerobic actinomycetes into deep subcutaneous tissue may result in an indolent condition termed *actinomycotic mycetoma* (to distinguish it from the eumycotic mycetomas caused by true fungi). Mycetoma is a chronic, progressive infection that can extend to underlying bone. Actinomycotic mycetomas usually involve the lower extremities or hands and typically are caused by *Actinomadura* spp., non–acid-fast aerobic actinomycetes, or *Streptomyces* spp. Actinomycotic mycetomas caused by *Nocardia* frequently involve the chest and back. Mycetomas, the cause of which may be *N. brasiliensis*, have been described in Mexican and South American field workers. This *Nocardia* spp. also has been documented as an opportunistic pathogen prevalent in Florida, with a predilection for diabetics.[49]

In addition to the classic mycetoma, traumatic introduction of *Nocardia* spp. from soil may result in wound infections that occur after a more acute or subacute course. Over a 5-year period, 31 cases of localized cutaneous nocardiosis with *N. brasiliensis* were identified in immunocompetent children in South Texas.[19] The organism can be introduced into the skin by tick[31] and other insect bites[19,41] or by a cat scratch,[19,45] and result in cellulitis, pustules, or pyoderma; these conditions occasionally disseminate in immunocompromised individuals.[26,27] Post-traumatic endophthalmitis[20] and post-sternotomy mediastinitis[54,61] have been described.

Nocardiosis previously was considered a rare disease in humans; however, it is being recognized more frequently.[37] It has been diagnosed in individuals ranging from 4 weeks to 82 years of age, and, except in cases of cutaneous nocardiosis, almost all patients have one or more severe underlying diseases (e.g., lupus erythematosus, asthma, glomerulonephritis, ulcerative colitis, bronchiectasis, tuberculosis, rheumatoid arthritis, sarcoidosis). Patients with lymphoreticular neoplasms and transplant recipients seem to be particularly at risk. In addition, the risk of acquiring infection is increased in patients with chronic pulmonary disease and in any patient who is receiving long-term corticosteroid treatment. Infliximab treatment has been associated with the development of nocardial infection.[60] Severe infection with this catalase-positive organism may develop in children with chronic granulomatous disease.[24] Although *Nocardia* is not a surveillance organism for acquired immunodeficiency syndrome (AIDS), patients with AIDS may contract nocardiosis.[25] In a review of all cases ($n = 25$) of nocardiosis at an urban adult teaching hospital from 1999 to 2004, 76 percent of patients with nocardiosis also were infected with HIV; all had $CD4^+$ counts less than 100 cells/mm^3.[12]

In a retrospective review of cases of pediatric nocardiosis admitted to Arkansas Children's Hospital, 5 children with nocardiosis were identified from more than 100,000 admissions in a 10-year period. Four of the five patients had received a transplant within the previous year. Three of these patients had pulmonary disease caused by *N. asteroides*, and one transplant recipient had skin involvement with *N. brasiliensis* at a central venous line site.

One immunocompetent 5-year-old boy contracted CNS infection caused by *N. asteroides* 2 months after incurring a penetrating brain injury.[22]

N. farcinica is a medically important pathogen.[48,59] In a series of 200 cases of *Nocardia* infection reported by Wallace and associates,[59] the isolates designated *N. farcinica* were from patients with severe illness, 56 percent of whom had disseminated infections. *N. farcinica* isolates were characterized by increased resistance to antimicrobials, specifically third-generation cephalosporins. Evidence from mouse models also indicates that *N. farcinica* seems to be more virulent than are other *Nocardia* spp.[16] Because the various species of *Nocardia* seem to have different pathogenicities, determination of the infecting species of *Nocardia* is important.

The immune response to *Nocardia* is multifaceted.[2] Neutrophils are mobilized to the site of infection and are the predominant cell type found in lesions. Neutrophils only inhibit the organisms, however, and limit the spread of infection until an adequate cell-mediated immune response develops, or until effective antimicrobial agents are administered. Immune T cells are vital in clearing *Nocardia* from the lung and preventing dissemination; many predisposing conditions for nocardiosis involve inadequate cell-mediated immunity. Activated macrophages induce cytotoxic T cells effective against *N. asteroides*. *Nocardia* may survive inside neutrophils and macrophages by inhibiting phagosome-lysosome fusion and by the production of catalase and superoxide dismutase, which inactivate the myeloperoxidase system. *Nocardia* organisms exhibit a differential ability to evade phagosome-lysosome fusion that depends on their state of growth, possibly related to specific cell wall mycolic acids detected only in log-phase cells.[4] Differential cell wall characteristics also may influence the ability of *Nocardia* organisms to exhibit specific organ tropism (e.g., the brain).[7] Antibody may play a role in host defense through enhancement of macrophage activities. Although antecedent conditions of nocardiosis frequently involve dysfunctional cellular immunity, other preconditions include neutrophil and immunoglobulin disorders.

PATHOLOGY

The lesions of nocardiosis are suppurative, whether in the lung or in subcutaneous tissue, and they involve primarily proliferation of neutrophils rather than formation of granulomas. Pulmonary nocardiosis in immunocompetent patients often resembles pulmonary actinomycosis in that it results in a chronic localized pneumonia that often abuts the pleura.[23] Indolent progressive fibrosis resembling fibronodular tuberculosis may occur. Nocardiosis is more aggressive in immunocompromised patients and is manifested as multifocal necrotizing pneumonia with confluent abscess formation. *Nocardia* spp. tend to invade the pleura and chest wall, with tissue planes disregarded in the process. Little evidence of encapsulation is characteristic of all organs invaded and probably accounts for the ready dissemination of organisms from the initial pulmonary focus.

N. asteroides organisms appear as delicate, beaded, branching filaments in tissue stained with Gram stain or modified acid-fast stain (Fig. 110–1). *Nocardia* spp. are invisible in hematoxylin and eosin preparations or in sections stained with periodic acid–Schiff for fungi. Methenamine silver preparations sometimes detect tissue organisms; overstaining with silver enhances visualization.

CLINICAL MANIFESTATIONS AND DIAGNOSIS

The most common manifestation of nocardiosis (>40% of all cases) in the United States is pulmonary disease in a patient with underlying immunosuppression.[14,42,44] Infection may remain localized to the lung or may disseminate hematogenously to the CNS and skin and, more rarely, to almost any organ in the body. In high-risk patients, the diagnosis should be suspected when CNS manifestations, particularly signs of a brain tumor, abscess, or soft tissue swelling, develop in conjunction with a current or recent subacute or chronic pulmonary infection. Although sulfonamides are the most effective drugs for treatment of nocardiosis, invasive infections have been described in immunocompromised individuals receiving oral trimethoprim-sulfamethoxazole (TMP-SMX) as prophylaxis for *Pneumocystis* pneumonia[58] and in patients with chronic granulomatous disease who are receiving TMP-SMX prophylaxis.[50]

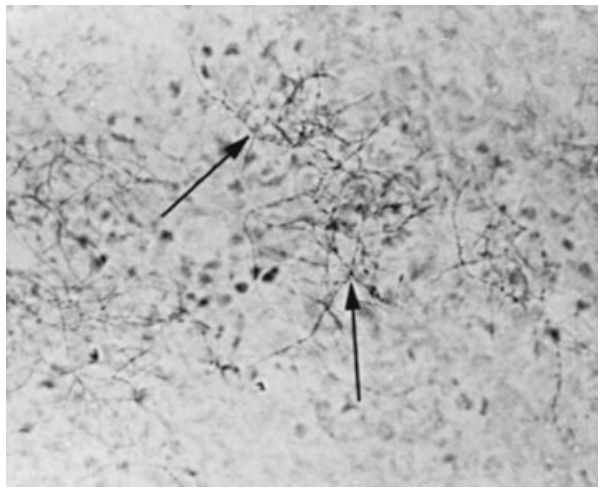

Figure 110–1 Appearance of *Nocardia asteroides* and *Nocardia brasiliensis (arrows)* in a properly decolorized acid-fast smear. Organisms appear as fragmented bacilli with stain concentrated in a beaded fashion along portions of the filaments (×160).

Clinical manifestations of pulmonary nocardiosis are not specific and include anorexia, weight loss, productive cough, pleural pain, dyspnea, and, occasionally, hemoptysis.[14] Untreated pulmonary nocardiosis usually runs a chronic course, similar to tuberculosis, but it also may clear spontaneously and obscure the source of subsequent metastatic infection. The diverse clinical and radiographic manifestations, including acute bronchopneumonia, lobar pneumonia or necrotizing pneumonia with single or multiple abscesses, and pleural empyema, may mimic more common pulmonary infections, such as mycobacterial, staphylococcal, and fungal pneumonia. Normal hosts or patients with only slightly impaired host defenses may have only mild respiratory tract symptoms of several months' duration.[17]

The CNS is the most common secondary site of infection, and such infection occurs in one third of patients.[2] Most experts recommend performing routine cranial computed tomography for patients with pulmonary nocardiosis even when they are asymptomatic because of the frequency of involvement of the CNS. Brain abscesses are the most common manifestation; meningitis is reported less frequently.[9]

Other reported clinical manifestations include tracheitis, peritonitis, iliopsoas abscess, hematogenous endophthalmitis, endocarditis, mediastinitis, septic arthritis, and osteomyelitis.[14] Traumatic inoculation may result in localized disease manifested as cellulitis, subcutaneous abscess, or a lymphocutaneous syndrome in which one or more cutaneous nodules are associated with regional adenopathy or suppurative lymphadenitis.[30] *Nocardia* spp. may cause cervicofacial disease and cervical adenitis in children.[29]

Bacteremic nocardiosis is reported rarely and usually is associated with endovascular foreign bodies in patients receiving long-

term steroid therapy.[28] In a report of six cases of central venous catheter–related *Nocardia* bacteremia, four cases were in children 18 years or younger.[18] All six patients received antibiotic therapy; the catheter was retained in three of the six. Relapse occurred in one of the three patients in whom the catheter was not removed.[18]

The diagnosis of nocardiosis is established in one third of cases by sputum analysis and culture. Humoral methods used to diagnose nocardiosis generally lack specificity because of the high degree of serologic cross-reactivity that occurs among *Nocardia* spp. and between *Mycobacterium* and *Streptomyces* spp.[8] The probable decreased sensitivity for detection of an antibody response in immunocompromised individuals also likely is a limitation of serologic testing. It is important to notify the laboratory when nocardial infection is suspected so that steps can be taken to optimize its recognition and recovery. Although *Nocardia* colonies usually are evident by 3 to 5 days after culture, prolonged incubation may be necessary.

Although *Nocardia* spp. sometimes can be respiratory saprotrophs, withholding therapy from immunocompromised patients when cultures repeatedly are positive is difficult. Bronchoalveolar lavage or lung biopsy may be required to establish the diagnosis. Although these procedures are invasive, given the overlap in symptoms with other infectious and neoplastic processes and potential for rapid progression of disease, bronchoalveolar lavage or lung biopsy should be considered early in immunodeficient patients to establish a definitive diagnosis. The demonstration of tissue invasion confirms active infection. Tentative species identification of most *Nocardia* can be made by biochemical methods, by chemotaxonomic methods, or via antimicrobial susceptibility patterns. Only *N. brasiliensis*, *N. farcinica*, and *N. pseudobrasiliensis* can be identified reliably by these methods, however.[10] Molecular confirmation (e.g., DNA probes, PCR and PCR-RFLP molecular analyses, DNA sequencing, pyrosequencing, ribotyping) of species identification is the method of choice. Molecular testing by *hsp65* PCR and 16S restriction enzyme analysis identifies greater than 90 percent of currently recognized clinical species.[10]

TREATMENT AND PROGNOSIS

Sulfonamides have been the best-studied drugs for the treatment of nocardiosis.[47,50,57] In the United States, TMP-SMX, the only intravenous sulfonamide formulation available, has been the agent of choice for treatment of nocardiosis. TMP-SMX has been used successfully at doses of 15 mg/kg/day of TMP and 75 mg/kg/day of SMX, either parenterally or orally.[50,55,57] No prospective randomized trials have established the optimal antimicrobial therapy for nocardiosis, however. Recommended antimicrobial therapy regimens are based on efficacy in animal models of nocardiosis and in vitro susceptibility and synergy testing.[10] TMP-SMX as a single agent usually is sufficient for patients with cutaneous nocardiosis.

Many infectious disease specialists administer empiric combination drug therapy, consisting of TMP-SMX, amikacin, and a third-generation cephalosporin, or a carbapenem, for patients with severe disease, CNS nocardiosis, or disseminated disease, and for immunosuppressed patients because of documented treatment failure and mortality associated with sulfonamide monotherapy in those settings.[33,37,51] Currently, no *Nocardia* spp. are resistant to amikacin and a β-lactam in vitro; the use of both (plus TMP-SMX) ensures that all clinical isolates would be susceptible to at least one other drug.[10] Antimicrobial therapy can be narrowed to two agents when results of in vitro susceptibility testing become available. Because antimicrobial resistance patterns vary by species, clinical isolates identified initially as *Nocardia* should be speciated further using molecular techniques.

The 2003 Clinical and Laboratory Standards Institute document on susceptibility testing of mycobacteria and other aerobic actinomycetes recommends that all clinically significant isolates of *Nocardia* spp. be tested for susceptibility to multiple antimicrobial agents.[40] No published prospective clinical trials have correlated in vitro susceptibilities with clinical outcome for nocardiosis. Susceptibility testing is the best available guide for selecting appropriate combination therapy and alternative therapies if sulfonamides fail or cannot be given because of patient intolerance or allergy.

Clinical experience with minocycline, the tetracycline with best in vitro activity against *Nocardia*, has been encouraging. Amoxicillin-clavulanic acid has been effective in treating infections with *N. brasiliensis*, commonly a β-lactamase producer.[56] A case of acquired resistance to β-lactamase inhibitor antibiotics has been reported, however.[52] Other species with susceptibility to amoxicillin-clavulanic acid include *N. abscessus* and *N. farcinica*, whereas isolates of the *N. nova* complex are ampicillin susceptible/intermediate but resistant to amoxicillin-clavulanic acid.[10] Susceptibility of some *Nocardia* spp. to fluoroquinolones has been reported. In an in vitro study, the newer fluoroquinolones, gatifloxacin and moxifloxacin, generally were more active than was ciprofloxacin against isolates of *Nocardia*, especially against isolates of *N. farcinica* and *N. brasiliensis*.[10] A patient with disseminated *N. farcinica* nocardiosis initially treated with aspiration, TMP/SMX, and imipenem had recurrence of brain lesions after a change to monotherapy with moxifloxacin despite in vitro susceptibility to moxifloxacin.[15]

The oxazolidinone linezolid is the first antimicrobial shown to be active in vitro against all clinically important *Nocardia* spp.[11] Linezolid, which is available for oral administration, was used successfully to treat six patients with nocardiosis.[36] Linezolid-associated hematologic toxicity was seen in three of the six patients.[36] Myelosuppression may occur more commonly in individuals receiving linezolid for longer than 2 weeks. The use of linezolid for nocardial infection is limited by the prolonged treatment required for nocardiosis.

Clinical improvement typically is seen within 7 to 10 days after initiation of appropriate therapy.[35] Patients with unchanged or worsening symptoms after receiving 2 weeks of therapy should be reassessed thoroughly. Intravenous antibiotic therapy may be changed to an oral regimen after at least 3 to 6 weeks if clinical improvement is noted. The variable and chronic course of nocardiosis precludes determining precise therapeutic end-points. Metastatic lesions can appear during or after an otherwise effective course of sulfonamide therapy with maintenance of the recommended 100- to 150-μg/mL level in serum or plasma. Because the tendency for relapse or the late appearance of metastatic disease is a concern, therapy often is continued for many months. Patients with mycetomas may require 6 to 12 months of therapy; localized cutaneous nocardiosis usually is treated for 3 to 6 months. Therapy for 6 to 12 months is suggested for patients with serious infection. Therapy for 12 months is indicated for patients with CNS nocardiosis and for immunocompromised patients.[32] Patients with CNS disease should be monitored with serial neuroimaging studies. Patients with AIDS probably should be treated with suppressive therapy indefinitely.

Surgical drainage of abscesses is important because metastatic abscesses can appear in the face of adequate therapy until surgical drainage is achieved.[21] Select brain abscesses may respond to antimicrobial treatment without surgery.[34]

Despite specific therapy, the overall mortality rate is 25 to 40 percent.[43,44] Most reported cases involving dissemination to the CNS have been fatal.[43] Disseminated nocardiosis has a poor prognosis, with a mortality rate of greater than 85 percent in immunocompromised hosts.[3] Factors associated with increased mortality rates in one reported patient series were treatment with corticosteroids or antineoplastic agents, underlying Cushing

disease, disseminated disease involving two or more noncontiguous organs or the CNS, and the presence of symptoms for less than 3 weeks before initial evaluation.

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CHAPTER 111

Miscellaneous Gram-Positive Bacilli

Randall G. Fisher

The gram-positive rods encompass a vast number of species. They are widespread in the environment and are part of the normal flora of animals and humans. This chapter focuses on gram-positive bacilli seldom encountered as pathogens in healthy individuals. Many of these organisms show increasing prominence as causes of disease in immunocompromised patients.[12] Bacteria from the following genera are discussed: *Corynebacterium* (other than *Corynebacterium diphtheriae*) and *Rhodococcus*.

CORYNEBACTERIUM

The genus *Corynebacterium* comprises a wide number of organisms possessing little pathogenic potential, with some notable exceptions. The most infamous member of this genus, *C. diphtheriae* (see Chapter 101), is a cause of a potentially lethal pharyngeal infection with systemic manifestations. *Corynebacterium jeikeium* (formerly *Corynebacterium* group JK) can be a major nosocomial agent of bacteremia and endocarditis; other species commonly are associated with infections in animals and rarely cause invasive infection in humans.[70,98] For an exhaustive consideration of coryneform bacteria, the reader is referred to the review by Funke and colleagues.[41]

BACTERIOLOGY

Corynebacterium spp. derive their name from their club-like shape. Because of their resemblance to *C. diphtheriae*, they are included in the heterogeneous group of diphtheroids. Snapping division produces the angular and palisade arrangement of cells responsible for their characteristic gram-positive, "Chinese letters" microscopic appearance.[21] Organisms are facultatively anaerobic or aerobic and do not produce spores. They are nonmotile and catalase-positive and contain mycolic acid in their cell walls. Clinically relevant species include *C. diphtheriae*, *C. jeikeium*, *C. pseudotuberculosis*, *C. xerosis*, *Corynebacterium amycolatum*, *Corynebacterium pseudodiphtheriticum*, *Corynebacterium minutissimum*, *Corynebacterium striatum*, *Corynebacterium ulcerans*, and *Corynebacterium urealyticum*. Species can be differentiated according to biochemical tests and fermentation of sugars.[21,41,95]

EPIDEMIOLOGY

Corynebacterium spp. can be part of the normal skin and upper respiratory tract flora and commonly colonize hospitalized patients. Nosocomial acquisition of *C. jeikeium* has been characterized most completely. Thirty-five percent of hospitalized patients may be colonized with *C. jeikeium*, and the organism may be isolated at the time of admission.[42] The skin, groin, and rectum are common sites of recovery. Wounds and suppurative sites quickly become colonized.[42] Longer duration of hospitalization, receipt of broad-spectrum antimicrobial agents, and impaired skin integrity are risk factors for invasive infection. Hospital personnel caring for oncology patients have higher rates of hand colonization with pathogenic *Corynebacterium* spp. than do nurses caring for dermatology patients.[55] Transmission from patient to patient in the hospital environment can occur, and selective antibiotic pressure has been shown to augment colonization with *Corynebacterium* spp.[68,78,128] Outbreaks of bacteremic infection have been reported in hematology wards,[92,110] and DNA restriction fragment analysis and hybridization techniques have been used to document spread.[68,89]

PATHOPHYSIOLOGY

C. jeikeium and *C. urealyticum* are lipophilic, which may account for their ability to proliferate on skin that has a higher lipid content; organisms have been isolated from sebum-filled eccrine gland biopsy specimens in an adolescent with malignancy.[59] Breach of the integrity of the skin is an important risk factor for acquisition of local infection and bacteremia with *Corynebacterium*. Plastic intravascular catheters increase the risk of infection.[93] Certain isolates of *C. pseudotuberculosis* and *C. ulcerans* can express diphtheria-like toxins,[126] which may confer virulence.

Some *Corynebacterium* spp., notably *C. jeikeium* and *C. urealyticum*, commonly show resistance to penicillins, cephalosporins, aminoglycosides, erythromycin, and tetracycline.[30,42,70,78,84,95,108] Strains with a significant DNA relationship to the *C. jeikeium*–type strain show penicillin resistance.[94] Resistant organisms have been noted to have significantly thickened cell walls compared with susceptible strains, but the functional importance of this feature is unknown.[14]

CLINICAL MANIFESTATIONS

Systemic infections with *Corynebacterium* spp. generally are not clinically distinguishable from serious infections caused by other pathogens. Immunocompromised patients are at the highest risk of developing disease caused by *Corynebacterium*,[51,97] but infections also have been described in neonates and immunocompromised older children.[13,30,62] Risk factors include male gender, neutropenia, broad-spectrum antibiotic exposure, and prolonged hospital stay.[92,110] Catalase production may be responsible for the rare infection with non-JK *Corynebacterium* spp. in children with chronic granulomatous disease.[62] *Corynebacterium* spp. are responsible for approximately 9 percent of early-onset and 4 percent of late-onset prosthetic valve endocarditis.[125]

C. jeikeium is a pathogen of particular concern. In 1976, this agent first was described as a cause of serious infection in four patients, including an 11-year-old boy with a ventriculoatrial shunt.[51] The other three patients had hematologic malignancies and were neutropenic at the time of infection. Immunocompromised patients, particularly patients with leukemia, are vulnerable to bacteremia. These high-risk subjects may show a local inflammatory lesion at the site of infection or a disseminated, hemorrhagic, or necrotic papular exanthem. Subcutaneous nodules also may be seen. *C. jeikeium* has been recovered from disseminated lesions in a 14-year-old boy with leukemia.[59] A literature review of 83 neutropenic patients (nearly all adults) with *C. jeikeium* sepsis found the following risk factors for development of infection: presence of a central venous catheter, being male, profound and prolonged neutropenia, and exposure to multiple antibiotics. Skin lesions were reported in 48 percent and pulmonary

infiltrates in 36 percent of patients. The overall case-fatality rate was 34 percent, but it was reduced to 5 percent in patients with recovery of neutropenia.[119]

Infections with *C. jeikeium* also have been reported after trauma, ventriculoperitoneal shunting procedures,[2,64] orthopedic procedures,[20] bone marrow aspiration,[30] and central venous catheter placement.[30] *C. jeikeium* now is recognized to be one of the most common causes of prosthetic valve endocarditis in adults.[84] Cutaneous findings of bacteremia so commonly observed in patients with cancer generally are absent in patients with endocarditis.[26] In a series of 38 patients with *C. jeikeium* endocarditis, 74 percent of patients had prosthetic valves. The mortality rate was 33 percent and was not altered by valve replacement surgery.[82] Exit-site infections in patients on continuous ambulatory peritoneal dialysis also may be caused by *Corynebacterium* spp.; in the largest series published to date, *C. striatum* caused almost twice as many of these infections as did *C. jeikeium*. Most infections are manageable without removal of the dialysis catheter.[102]

Although primarily a pathogen in farm animals, *C. pseudotuberculosis* can cause localized suppurative granulomatous lymphadenitis in humans[70]; almost all cases are associated with animal contact, particularly sheep and goats.[43] Presentation and histology may mimic the much more common cat-scratch disease, and Gram stain and culture are required to differentiate the two entities.[70] Most cases have been reported from Australia.[88]

C. xerosis has been reported as a rare cause of endocarditis, arthritis, and ventriculoperitoneal shunt infection.[7,15] Most individuals with endocarditis have a prosthetic heart valve.[34,72] *C. xerosis* also can be a cause of eye infections in patients with superficial corneal foreign bodies; in one study of 101 patients, *C. xerosis* caused 3 of 15 culture-positive cases.[73] It also is an extremely rare cause of post-LASIK (laser-assisted in-situ keratomileusis) infectious keratitis.[29] Using chemical testing and molecular genetic investigation, Funke and colleagues[40] showed that many isolates tentatively identified as *C. xerosis* are actually *C. amycolatum*. *C. amycolatum* is much more likely to be multidrug-resistant than *C. xerosis*.[41] A case of fatal sepsis in a premature newborn caused by *C. amycolatum* has been reported.[13]

C. pseudodiphtheriticum, a commensal of the oropharynx, can cause pneumonia, bronchitis, or tracheitis.[1,22,25] Lung disease usually occurs in the context of underlying cardiopulmonary pathology or immunocompromise.[49] Onset typically is acute, but fever may be noticeably absent.[74] At least 18 cases of *C. pseudodiphtheriticum* endocarditis, including three infections in children with congenital heart disease, have been reported.[83] Infection of allograft material is a common finding; native heart valves may be involved but usually in the context of preexisting lesions or intravenous drug abuse. Three cases of exudative pharyngitis with a pseudomembrane mimicking diphtheria, including one in a 4-year-old girl from Arkansas, have been reported to be caused by *C. pseudodiphtheriticum*.[58] In one case, a surface swab from a draining wound after arthroscopy was reported to harbor "normal skin flora," but joint fluid grew the same organism, and it was finally identified as *C. pseudodiphtheriticum*. Treatment resulted in resolution of symptoms of septic arthritis.[63] Patients who are immunocompromised and have corneal epithelial defects can get severe eye infections with this organism.[69]

C. minutissimum traditionally has been regarded as the cause of the mild cutaneous disease erythrasma, which is characterized by scaly, pruritic red-brown patches, usually occurring in the axilla or groin. Although *C. minutissimum* may play a role, erythrasma probably is a polymicrobial process.[41] *C. minutissimum* is recognized as a rare nosocomial infectious complication of malignancy and dialysis and has been implicated in a case of pyelonephritis in an 8-month-old infant with posterior urethral valves.[24] An adult who developed native-valve endocarditis caused by *C. minutissimum* has been reported.[6]

C. striatum has been recovered from purulent sputum of hospitalized patients and from infected central venous catheter sites.[103] It also is a cause of exit-site infections in patients on long-term ambulatory peritoneal dialysis.[102] It has been reported as a cause of fatal pulmonary infection and endocarditis.[68,77,98] *C. striatum* was responsible for a nosocomial outbreak among 14 patients over a 12-month period in a surgical intensive care unit. Endotracheal intubation for longer than 24 hours was the only risk factor found to predispose to acquisition of infection.[17] *C. striatum* also has been reported as a cause of meningitis and ventriculoperitoneal shunt infections.[56,123]

C. ulcerans derives its name from its association with ulcerative pharyngitis. Although it is more commonly a pathogen of nonhuman primates,[87] infection can occur in humans after contact with an animal or consumption of contaminated raw milk.[11,27] Toxigenic strains of *C. ulcerans* can produce a syndrome indistinguishable from that caused by toxigenic *C. diphtheriae*.[4,5] Skin lesions that exactly mimic cutaneous diphtheria, caused by diphtheria toxin–secreting strains of *C. ulcerans*, also have been reported.[121]

C. urealyticum (formerly *Corynebacterium* group D2) is a cause of alkaline-encrusted cystitis and pyelitis, primarily in the elderly.[36,108] A case series of four children with encrusted cystitis and pyelitis has been published. The mean age was 9 years (range, 4 to 13 years). Treatment was with prolonged antibiotics and endoscopic debulking. Cure was effected in three of the four patients; the other patient was a renal transplant recipient who lost the graft.[80] Routine urine culture may miss the organism; it may grow with prolonged incubation of sheep's blood agar.[105] It is associated less commonly with infection at other sites and with bacteremia.[107] An 8-year-old boy with chemotherapy-induced neutropenia and a necrotic soft tissue infection of the scrotum caused by *C. urealyticum* has been reported.[99]

DIAGNOSIS

Diagnosis of *Corynebacterium* infection is based on isolation of the organism from clinical material. This organism commonly is accompanied by other pathogens. Similar to *Mycobacterium* and *Nocardia* spp., *Corynebacterium* organisms have mycolic acids in their cell wall. The chains are shorter, however, and the organisms are not acid-fast. *Corynebacterium* spp. may be difficult to distinguish from some *Rhodococcus* spp.[21] Colonies of *C. jeikeium* may show a metallic sheen when grown on agar.[51] Growth of *C. jeikeium* is enhanced by the addition of lipids to the medium. Most *Corynebacterium* spp. can be differentiated quickly from each other by sugar fermentation, hydrolysis of urea, and reduction of nitrate.[116] Selective media containing kanamycin or trimethoprim-sulfamethoxazole have been useful in the recovery of multidrug-resistant strains of *Corynebacterium*.[50] Erythrasma usually is diagnosed by the typical coral red fluorescence under Wood lamp examination.

TREATMENT

Empiric therapy for infection must account for the frequency of infection with *C. jeikeium*, which often are multiresistant to antibiotics but are susceptible to vancomycin.[41] In some series of immunocompromised patients, this organism is the most common *Corynebacterium* spp. encountered.[109,124] Vancomycin is recommended for empiric therapy of suspected *Corynebacterium* infection until susceptibilities are known. Treatment can be changed to a penicillin or cephalosporin, if appropriate. In vitro, *C. jeikeium* is sensitive to linezolid,[45] and most strains also are sensitive to quinupristin-dalfopristin[100] and daptomycin.[44] Linezolid is bacteriostatic against all species of *Corynebacterium*, and in vitro its activity is slower against *C. jeikeium* than it is against

C. striatum and *C. amycolatum*.[45] Clinical experience using these newer antimicrobial agents is lacking.

Two-drug therapy generally is recommended for treatment of *Corynebacterium* endocarditis; for gentamicin-susceptible strains, penicillin-gentamicin combinations have been shown to be synergistic, regardless of whether the strains are susceptible to penicillin.[84] Rarely, resistance to vancomycin is encountered. A woman with prosthetic valve endocarditis caused by a vancomycin-resistant *Corynebacterium* spp. was treated successfully with imipenem and ciprofloxacin.[10] Removal of infectious sources, such as central nervous system shunts and central venous catheters, may be required for cure. Scrupulous attention given to skin hygiene may reduce colonization of hospital personnel and the incidence of patient-to-patient transmission of pathogenic strains.[32,92] Management of toxigenic *C. ulcerans* infection is identical to that for infection caused by toxigenic *C. diphtheriae*, including the use of antitoxin.[5] Erythrasma usually responds to treatment with a macrolide.

RHODOCOCCUS

The genus *Rhodococcus* contains at least 15 species, of which *Rhodococcus equi* is the most clinically relevant to humans. Infections with other species are rare. Suppurative keratitis caused by *Rhodococcus ruber* after penetrating trauma has been described.[65] This organism derives its name from its role as a cause of pyogranulomatous pneumonia in young horses to occur.[90] It has assumed a prominent role as a cause of human pulmonary disease in immunocompromised patients, particularly in patients with acquired immunodeficiency syndrome (AIDS).[35,52,103] For a comprehensive discussion of this organism, the reader is referred to the review by Cornish and Washington.[23]

BACTERIOLOGY

R. equi is a catalase-positive, urease-positive, oxidase-negative, gram-positive rod. The organism assumes a more coccoid morphology in solid media and a more bacillary form in liquid media. Its cell wall contains mycolic acid, rendering it acid-fast when grown on Lowenstein-Jensen media and stained with Kinyoun stain.[46]

EPIDEMIOLOGY

R. equi is a soil organism, and its growth is enriched by the manure of herbivores. Despite the common occurrence of this pathogen as a cause of veterinary infections, exposure to animals apparently is not necessary for human infection to occur[90]; most reported human patients have not had farm or animal exposure.[52] Hospital outbreaks of infection associated with patient-to-patient transmission have not been reported. In a retrospective analysis of 24 cases of *R. equi* infection, however, six patients had shared a hospital room with a patient with *R. equi* pneumonia, raising the possibility of nosocomial transmission.[8] Most patients diagnosed with *Rhodococcus* infection are immunocompromised; AIDS is the most common underlying diagnosis, but infection also has been reported in transplant patients[9] and rarely in patients thought to be immunocompetent.[118]

PATHOPHYSIOLOGY

The prominence of pulmonary infection suggests that the respiratory tract is a common portal of entry. After gaining access to the lower respiratory tract, organisms are taken up by alveolar macrophages; Mac-1 macrophage receptors and complement are required for binding.[53] The appearance of pyogranulomatous lesions is consistent with the role of *R. equi* as an intracellular parasite containing mycolic acid, a possible virulence factor in the cell wall.[47,91] Surface 15- and 17-kd antigens expressed by an 85-kb plasmid seem to confer virulence in mice and foals,[111] and virulent strains seem to have an increased capacity for intracellular survival in macrophages.[54]

Most *R. equi* isolates from patients with AIDS express either the 15- to 17-kd antigens or a 20-kd antigen that seems to confer intermediate virulence. Most isolates from patients without AIDS express none of these antigens, however.[112] Other factors may play a role in promoting *Rhodococcus* disease in humans.[113] Death of parasitized macrophages may release enzymes, which contribute further to tissue damage. $CD4^+$ lymphocytes are essential for pulmonary clearance of *R. equi* in a mouse model,[61] which may help to explain the high risk of infection associated with cellular immunodeficiency.

CLINICAL MANIFESTATIONS

Infection typically manifests as a subacute pneumonia developing over several weeks. Symptoms such as cough and fever are common, but progression of disease may be silent. Although most infections currently occur in patients with AIDS, malignancy and transplantation also pose risks. Pulmonary infection in children with leukemia has been described.[3,79] Infection may be accompanied by other pathogens, particularly in patients with AIDS.

Pulmonary infection often is pleura-based and associated with cavitation.[52] Empyema may occur as a complication. Computed tomography scan most frequently reveals pneumonia with cavitation, but other patterns, including "ground-glass" opacities, peribronchial nodules, and centrilobular nodules, may be seen.[75] Lung tissue showing malakoplakia, an unusual-appearing granulomatous inflammation with aggregates of histiocytes that contain concentrically layered basophilic inclusions, should raise suspicion of the presence of *R. equi* infection.[19,48,101]

Extrapulmonary disease is seen at diagnosis in 7 percent of patients with pneumonia.[120] Manifestations of infection include otitis/mastoiditis,[3,57,71] abscesses,[37] osteomyelitis,[16,38,86] meningitis,[28,104] pericarditis,[67] lymphadenitis,[67] and endophthalmitis.[33] The organism has been grown from a biopsy of a granulomatous skin lesion in an immunocompetent 7-year-old girl.[76] *R. equi* also has been reported as a cause of peritonitis in patients receiving long-term peritoneal dialysis.[18,114]

DIAGNOSIS

Diagnosis relies on isolation of *Rhodococcus* from clinical material. Although sputum specimens may be positive, bronchoalveolar lavage or lung biopsy may be required. Blood cultures may be positive in half or more of patients with AIDS and with focal pneumonic disease.[117] The physician should be alert to the possible coexistence of *R. equi* with other pathogens. In the laboratory, confusing this organism with *Corynebacterium*, acid-fast organisms, and other gram-positive coccobacilli has been shown to delay establishment of the diagnosis.[31] Positive findings on Gram stain and Kinyoun stain should be interpreted in the context of clinical information.[103] Organisms appear salmon-pink when grown on blood agar and orange on Lowenstein-Jensen medium.[12] Differentiating from acid-fast bacteria on smear sometimes can be difficult. Combined use of a siderophore detection medium, ethylene glycol degradation, and β-galactosidase activity may help differentiate *Rhodococcus* from *Nocardia* and

rapid-growing mycobacteria.[39] DNA restriction fragment analysis and ribotyping show promise in aiding the identification and tracking of *Rhodococcus* spp.[66] A *Rhodococcus equi*-specific polymerase chain reaction has been used to confirm infection in some cases in which identification was difficult.[127]

TREATMENT

Clinical isolates commonly are resistant to penicillins and cephalosporins. Even if susceptible in vitro, β-lactam antibiotics should be avoided because of rapid development of resistance.[60] Erythromycin, clindamycin, rifampin, aminoglycosides, vancomycin, fluoroquinolones, and imipenem are active against *R. equi*.[85] Moxifloxacin is more active in vitro than is either ciprofloxacin or levofloxacin.[96] Synergy has been shown with various combinations of these agents. Including rifampin or erythromycin in a two-drug combination has been recommended because of penetrance of macrophages.[12,106] Combinations of antibiotics that included vancomycin were found to be most effective in clearing infection in a mouse model.[85] Cure rates in adults with lung infection are approximately 60 percent when antibiotic therapy alone is employed but may reach 75 percent when combined with surgical resection of infected pulmonary tissue.[52] Surgery has not been shown to increase survival rates, however.[8]

Pediatric patients generally have fared better than adults, but most reported cases in children have been in non-AIDS patients.[12] Relapse is a common occurrence, but the optimal duration of therapy to prevent relapse is unknown. For patients with AIDS, some authors recommend a minimum of 2 months of therapy followed by long-term suppressive therapy.[60] Relapse has been reported to occur at extrapulmonary sites in 13 percent of immunocom-promised patients,[120] often without reappearance of pulmonary disease. Treatment of *R. equi* peritonitis in patients receiving peritoneal dialysis has been reported to be successful with intraperitoneal imipenem or vancomycin for 14 days.[18,115] Removal of the peritoneal dialysis catheter may be required for cure.

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SUBSECTION 4

Enterobacteria

CHAPTER 112

CITROBACTER

Randall G. Fisher

Citrobacter, a genus of enteric gram-negative rods closely related to *Salmonella*, has been associated increasingly with human disease. Although *Citrobacter* strains usually are not considered normal inhabitants of the intestinal tract of humans and animals,[44,76] a study using 16S rDNA polymerase chain reaction showed that normal newborns are colonized with these organisms by day 6 of life.[52] They have been associated with urinary tract infections,[6,19,34,40,43,44] osteomyelitis,[43] diarrhea,[15,25,79] and invasive disease in the immunocompromised host.[32,34,43,44] As detailed in a review by Doran,[13] *Citrobacter* commonly is associated with sepsis, meningitis, and brain abscess in neonates.

BACTERIOLOGY

In 1931, Werkman and Gillen[76] proposed the generic term *Citrobacter* for citrate-positive coliaerogenes intermediates isolated from stool. This genus now includes 11 species, of which the most commonly identified are *Citrobacter freundii*, *Citrobacter koseri* (formerly *Citrobacter diversus*), and *Citrobacter farmeri* (formerly *Citrobacter amalonaticus*).[7] *Citrobacter* are straight, facultatively anaerobic bacilli possessing peritrichous flagellae that confer motility. In addition to using citrate, these organisms hydrolyze urea and ferment glucose, with production of gas.[65] They grow on ordinary media as gray, opaque, round colonies that produce a strong, fetid odor. In contrast to *Salmonella*, *Citrobacter* grows in the presence of potassium cyanide. Indole-negative strains that produce hydrogen sulfide are classified as *C. freundii*. Indole-positive, hydrogen sulfide–negative strains are differentiated by their ability to ferment malonate; *C. koseri* ferments malonate, whereas *C. farmeri* does not.[2] Antigenic schemata have been developed to classify the O somatic antigens of *Citrobacter*[20,24,79]; these antigens show cross-reactivity with O antigens of other Enterobacteriaceae.

EPIDEMIOLOGY

Meningitis caused by *Citrobacter* was reported first in 1960, with two cases of *C. freundii*.[29] In the decade from 1970 to 1979, 69 cases of *Citrobacter* meningitis were reported,[21] and 4 percent of neonatal meningitis cases reported in the First Neonatal Meningitis Cooperative Study Group were caused by *Citrobacter*.[48] *C. koseri* is the species usually isolated with meningitis; central nervous system (CNS) infection caused by *C. freundii* occurs less commonly.[13,21] Most of the cases in the United States are reported from southern states; biotype d or serotypes O2 and O1[21] are the most common isolates of *C. koseri* encountered.

Most cases of neonatal meningitis caused by this organism have been sporadic. The source of sporadic cases usually is unknown, but nine cases clearly have been documented to be vertically transmitted from mother to infant.[13] In addition, several well-documented nosocomial outbreaks of infection have been reported.[16,19,20,42,53,60,79] The source usually is the gastrointestinal tract or hands of nursery staff. One cluster was associated with contaminated formula.[71] When *Citrobacter* is introduced into the neonatal nursery, colonization may exceed 79 percent.[20] Parry and associates[53] described a nursery outbreak in which 11 of 128 infants were colonized with *C. koseri* over an observation period of 3 months; two of the colonized infants developed meningitis. Additional colonization of neonates seemed to be eliminated by removal of a nurse with persistent hand carriage of the organism.

In another outbreak,[79] introduction of *C. koseri* into the nursery was linked to an infant admitted with meningitis. Thirty-one percent of infants in the nursery subsequent to the index case were found to be colonized with *C. koseri* of the same serotype and biotype. A second infant from this study developed meningitis with the organism during the observation period. Umbilical colonization of infants in this cluster was more common than was rectal colonization, but rectal colonization was more persistent, lasting 4 months. Two nurses were found to have hand colonization with the organism, and the reintroduction of these bacteria into the nursery was linked to a pregnant nurse who had perineal cultures at delivery yielding the epidemic strain. She was implicated in the colonization of her own infant and three other neonates. Corresponding culture data from a reference hospital revealed an overall neonatal colonization rate with *Citrobacter* of 1 to 10 percent over a 5-year period, with no invasive disease.

Although *Citrobacter* has been isolated increasingly from debilitated adult patients,[77] particularly as a urinary tract,[34,43,44] soft tissue,[22,43,44] and bone[44] pathogen, *Citrobacter* infection in children is unusual after the first 2 months of life. One case of meningitis was reported in a teenager who lacked apparent risk factors or immunodeficiency.[59] In older children, *Citrobacter* spp. are more likely to cause opportunistic infection in an immunocompromised host or, occasionally, urinary tract infection. One study showed *Citrobacter* spp. to be responsible for 37 (1.4%) urinary tract infections in children over a 3-year period.[17] One quarter of infections were nosocomially acquired, and one third occurred in children with urinary tract abnormalities.

PATHOPHYSIOLOGY

Citrobacter infrequently colonizes the intestinal tract and perineum of humans.[34] Vertical transmission of strains shown to be identical by DNA typing shows that the newborn can acquire colonization at the time of passage through the birth canal of a colonized mother.[30,51] Onset of disease beyond the first week of life is related commonly to colonization of the infant in the nursery. As with other types of gram-negative neonatal meningitis, CNS infection results from bacteremia in a colonized infant, leading to seeding of the meninges.

The basis for the particular invasiveness of *Citrobacter* in the neonate and its propensity to cause multiple brain abscesses are largely unexplained. *Citrobacter* spp. possess the ability to invade, transcytose, and multiply within human brain microvascular endothelial cells in vitro.[3] Additionally, in the neonatal rat model, after being taken up into macrophages, *C. koseri* is able to survive phagolysosomal fusion and replicate therein.[72] Many strains of *C. koseri* seem to be able to produce brain pathology in the mouse, but the degree of damage seems to be related to virulence of the strain and the age of the mouse.[39,66] Differences in strains, related to the presence of an outer-membrane protein with a molecular weight of 32,000, have been associated with differences in brain histopathology in one infant rat model of *C. koseri* meningitis.[38] Strains isolated from cerebrospinal fluid (CSF) of infants with meningitis more commonly possess this outer-membrane protein than do strains isolated from other body sites.[39]

In an immunocompromised patient, broad use of antimicrobial agents may produce selective pressure, leading to increased colonization with *Citrobacter*. Increased bacterial density combined with a blunted immune response may result in invasive disease. Some strains of *C. freundii* have been shown to produce Shiga toxins (verotoxins) nearly identical to those produced by enterohemorrhagic strains of *Escherichia coli*.[63] At least one outbreak of gastroenteritis and the hemolytic-uremic syndrome caused by Shiga-toxin–producing *C. freundii* has been reported.[73]

CLINICAL MANIFESTATIONS

Citrobacter, similar to other neonatal pathogens, can cause early-onset and late-onset infection. In a review of 74 cases of neonatal meningitis caused by these bacteria,[21] the mean age of onset reported for early disease was 7 days; 85 percent of patients were included in this group. Fifteen percent of cases occurred after 3 weeks of age. Twenty-three (31%) of 74 patients were younger than 36 weeks' gestational age at birth, suggesting that preterm infants are at increased risk for acquisition of *Citrobacter* infection. Prematurity is even more common (71%) in cases that are proven to be vertically acquired.[13]

Clinical signs and symptoms are typical of neonatal sepsis. Fever, lethargy, poor feeding, vomiting, irritability, bulging fontanelle, seizures, and jaundice are common presenting features. Umbilical infection and surgical manipulation of colonized umbilical stumps occasionally have preceded development of bacteremia and meningitis.[53] The white blood cell count may show leukocytosis or leukopenia. CSF fluid findings are consistent with most types of neonatal bacterial meningitis and usually show polymorphonuclear cell elevation, elevated protein, and depressed glucose; gram-negative rods may be seen on smear. Although growing the organism in culture normally is not difficult, in one reported case standard cultures were negative, but the organism was recovered by direct inoculation of CSF into a BacTec blood culture bottle.[11] Of *Citrobacter* meningitis cases in which the results of blood cultures are reported, 80 percent document concurrent bacteremia.[13]

Citrobacter is a particularly devastating cause of neonatal meningitis. The most common *Citrobacter* spp. causing neonatal meningitis is *C. koseri*, which accounts for more than 80 percent of the cases.[13] CNS infection with this organism produces multiple brain abscesses with unusually high frequency.[21,27,35,39,41] In the extensive reviews by Graham and Band[21] and by Doran,[13] three quarters of *Citrobacter* meningitis cases resulted in intracerebral abscesses. By comparison, the incidence of abscess formation in non-*Citrobacter* gram-negative meningitis is reported to be as low as 10 percent.[21] The case-fatality rate for *Citrobacter* meningitis is approximately 30 percent, and at least three quarters of surviving infants have neurologic sequelae, such as mental retardation, hemiparesis, seizures, and developmental delay.[13]

The presence of brain abscess seems to contribute significantly to morbidity and mortality.[12,20,21] For unknown reasons, neonates with vertically acquired *Citrobacter* meningitis seem to be less likely to develop intracerebral abscesses.[13] At least three cases in which diffuse pneumocephalus developed in association with *Citrobacter* meningitis have been reported; in one, gas was noted to accumulate within the brain and in the anterior chamber of the eye, a condition known as *pneumatosis oculi*.[1,58] Rarely, *Citrobacter* infection in the neonatal period may lead to focal infection not involving the CNS. A case of septic arthritis and osteomyelitis of the shoulder in a 3-week-old infant has been reported.[31]

In adults, *Citrobacter* is isolated most commonly from the urinary tract.[34,43,44] In earlier studies, 5 to 12 percent of bacterial isolates from urinary tract infections in adult patients were *Citrobacter*.[15,78] More recently, in a health maintenance organization, *Citrobacter* spp. accounted for only 0.8 percent of 4342 isolates from women with acute uncomplicated cystitis.[26] *Citrobacter* spp. are a similarly uncommon cause of urinary tract infection in children.[17] Sputum is the second most common clinical specimen to yield *Citrobacter* in adults[34]; lung abscess,[18] pneumonia,[43,44] bronchitis,[32] and septic arthritis[43] have been reported. *Citrobacter* is an occasional cause of bacteremia in hospitalized patients, accounting for approximately 0.5 percent of blood culture isolates.[56,64] In one series, all 45 patients had at least one underlying disease, with malignancies (particularly intra-abdominal tumors) and hepatobiliary stones being the most frequent coexisting conditions.[64] Polymicrobial bacteremia occurred in one third of patients. The case-fatality rate was 18 percent.

Gastrointestinal disease occasionally has been attributed to *Citrobacter*, but frequent isolation of this agent from normal stools often renders this diagnosis equivocal. This genus was implicated first in an outbreak of mild gastroenteritis by Barnes and Cherry[5] in 1946, and an outbreak of watery diarrhea in a Virginia infant care unit included two infants in whom isolates of enterotoxin-liberating *Citrobacter* were obtained from the stool.[25] Some studies have found higher incidences of *Citrobacter* isolation from the stool of patients with enterocolitis syndrome than from stools of control patients.[79] Shiga-toxin (verotoxin)–producing *C. freundii* isolated from organically grown parsley was associated with an outbreak of diarrhea and hemolytic-uremic syndrome in a daycare setting.[73] *C. freundii* has been found as a cause of appendicitis in a healthy adult,[43] peritonitis in adults with liver disease or pancreatitis,[43] neutropenic colitis following chemotherapy for breast cancer,[10] and meningitis in adults as a complication of neurosurgery.[70] A case of Meleney gangrene occurring after cesarean section delivery has been reported.[69] A patient with diabetes developed necrotizing fasciitis caused by *C. freundii* associated with injury from a fish fin.[9] Bone and soft tissue infections occur[8,67]; 3 percent of *Citrobacter* pathogens were isolated from joint or bone in one adult series.[43]

DIAGNOSIS

Biochemical characteristics of *C. koseri* include lack of hydrogen sulfide production on triple-sugar iron agar, negative Voges-Proskauer reaction, use of citrate, motility, production of indole, decarboxylation of ornithine but not lysine, and production of acid from adonitol.[2] Identification of this organism as a pathogen in a nursery setting should heighten suspicion of its possible role in subsequent neonatal infections. *C. freundii* is indole-negative and hydrogen sulfide–positive, which differentiates it from *C. koseri*. *C. farmeri* differs from *C. koseri* because of the former's inability to ferment malonate. *C. freundii* and *C. farmeri* account for a significant portion of disease caused by *Citrobacter* in immu-

nocompromised individuals and should be suspected particularly in this group of patients.[32]

Infants with invasive *Citrobacter* disease present in a similar fashion to infants with sepsis and meningitis of other causes. Such infants should undergo a thorough evaluation, including blood and urine culture and CSF studies. Brain imaging studies should be done when the diagnosis is established. Computed tomography is the most common test used, although ultrasound often is more feasible for an unstable neonate and may be nearly as sensitive in detecting abscesses.[41,49,80] Serial imaging studies should be done because abscesses may develop during the first few weeks of illness. In cases in which the CSF cultures are negative because of prior antimicrobial therapy, surgical aspiration of abscesses sometimes enables identification of the organism.

TREATMENT

Most *C. koseri* organisms are resistant to ampicillin (97% in one series)[43] and sensitive to aminoglycosides and third-generation cephalosporins.[13] A 4-year experience with neonatal septicemia caused by *C. koseri* has been described, however, in which all of 13 isolates were resistant to gentamicin, but susceptible to third-generation cephalosporins.[16] The resistance patterns of *C. freundii* were reported in a national surveillance study of nosocomial bloodstream infections.[56] Of the 23 *C. freundii* isolates tested, resistance to piperacillin, piperacillin/tazobactam, ceftriaxone, and ceftazidime was a common (39-48%) finding. Isolates generally were susceptible to the aminoglycosides and ciprofloxacin (91-96%), and all *C. freundii* tested were susceptible to cefepime and imipenem.

Some *Citrobacter* isolates contain chromosomally mediated group I β-lactamases. These bacteria possess a gene that, when triggered by exposure to cephalosporins or by spontaneous mutation, produces a cephalosporinase capable of inactivating cephalosporins.[33] Clinically, it manifests as treatment failure and emergence of drug resistance to various cephalosporins despite initial susceptibility.[45] In one study, the presence of group I β-lactamases was much more common with *C. freundii* (9 of 22 isolates) than with *C. koseri* (0 of 7 isolates).[33] Resistance was associated with previous receipt of an extended-spectrum, β-lactam antibiotic. Reliable estimates of the percentage of *Citrobacter* strains that contain the chromosomal resistance gene are difficult to obtain because most studies lump *Citrobacter*, *Enterobacter*, and *Serratia* isolates together. In one Korean study, of 152 *Enterobacter/Citrobacter/Serratia* isolates, 45 (30%) were derepressed AmpC mutants.[52] In an in vitro study, AmpC production was inducible in eight of nine clinical isolates of *C. freundii* and in one of three isolates of *C. koseri*. AmpC synthesis was stably derepressed in one of the nine *C. freundii* isolates.[14] Cefepime seems to be less likely to induce production of these β-lactamases and more resistant to hydrolysis by them,[62] although a highly cefepime-resistant strain has been described.[4] Ninety-nine percent of 3030 ceftazidime-resistant Enterobacteriaceae in a U.S. study retained susceptibility to imipenem, and 96.7 percent of ceftazidime-resistant *Citrobacter* isolates were susceptible to cefepime.[57] Many different types of β-lactamases, including a novel TEM-type (TEM-134),[54] the class A β-lactamase CKO,[55] and a VIM-1 metallo-β-lactamase, have been described in various isolates of *Citrobacter*.[75]

Treatment of *Citrobacter* meningitis often requires a multidisciplinary effort involving the neurosurgeon and the pediatrician. Although cerebral abscesses usually are aspirated or drained surgically, some patients are treated with antibiotics alone, and neither approach is clearly shown to be superior. When abscesses are inaccessible or small and not progressive, conservative management may be considered.[13] Ventriculostomy and craniectomy with open drainage of abscesses have been required in some children to effect bacteriologic cure, and placement of a shunt for hydrocephalus often is required.

Generally, antibiotic therapy for gram-negative neonatal meningitis has proved disappointing (see Chapter 78).[46,47] No evidence supports one combination of antibiotics over another in the treatment of *Citrobacter* meningitis. Usually a third-generation or fourth-generation cephalosporin or a carbapenem (usually meropenem) is used in combination with an aminoglycoside initially.[13] Chloramphenicol,[12,20,28] imipenem/cilastatin,[15,28] and trimethoprim-sulfamethoxazole[23] also have been used successfully.

Poor meningeal penetration of aminoglycosides in addition to the presence of intracranial abscesses renders antibiotic therapy for *Citrobacter* meningitis especially difficult. The ability of this organism to persist in the brain is shown by its recovery 4 years after neonatal infection.[15] Cranial computed tomography usually is used for evaluation of complications such as hydrocephalus and multicystic encephalomalacia (Fig. 112–1). Administration of antibiotics intrathecally or directly into abscess cavities has been tried but has not been shown convincingly to be beneficial.[23,24,27,37,42,50,61,68,74] In a randomized controlled trial, intraventricular administration of gentamicin in the treatment of neonates

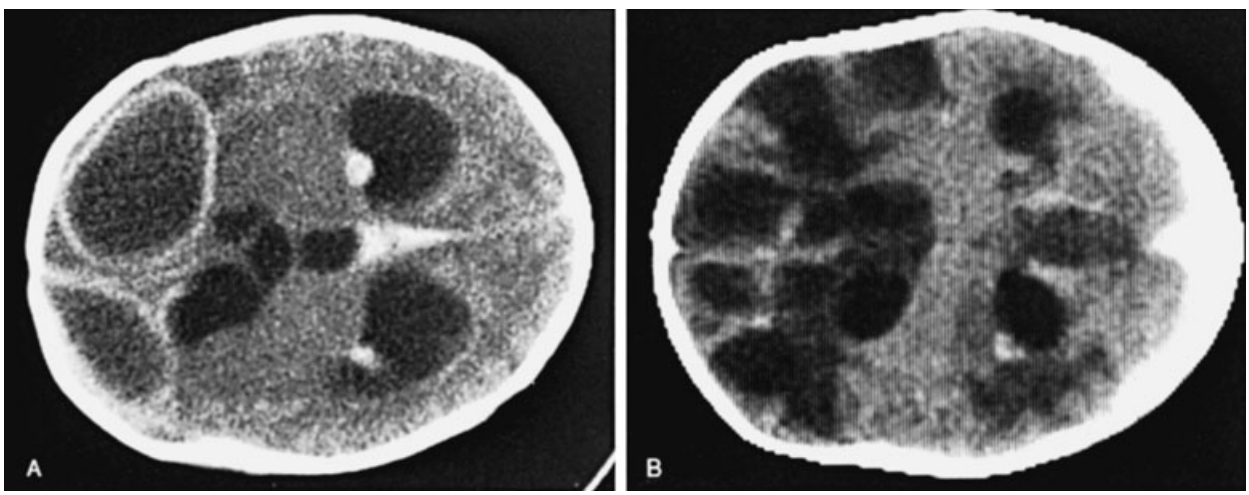

Figure 112–1 **A** and **B,** Computed tomography scans showing progressive abscess formation and encephalomalacia in an infant at 3 weeks of age **(A)** and 6 weeks of age **(B)**, despite bacteriologic "cure" of *Citrobacter* meningitis.

with gram-negative meningitis was associated with a poorer outcome.[48]

Neonates with gram-negative meningitis should undergo repeat lumbar puncture approximately 72 hours after beginning therapy to document sterilization of CSF. Duration of therapy with intravenous antibiotics generally is a minimum of 21 days for gram-negative neonatal meningitis. For cases complicated by intracranial abscesses, prolonged therapy (usually 4 to 6 weeks after sterilization of CSF) is indicated.[13,56]

Scrupulous attention given to preventive infection control practices has been recommended to stem nursery outbreaks.[20,53,79] These prophylactic measures include care of skin and the umbilical cord, elimination of crowding with isolation of infected infants and carriers, and good handwashing practices. Exclusion of colonized personnel and temporary closing of the nursery have been followed by a reduction in neonatal *C. koseri* colonization. Although cohorting of colonized infants is a reasonable practice, multiple sources of introduction of *Citrobacter* may limit the efficacy of this approach in some outbreaks.[19]

Treatment of *Citrobacter* infection beyond the neonatal period requires choice of an appropriate antibiotic, with drainage of abscesses and appropriate débridement of wounds. Therapy should be guided by antimicrobial susceptibility testing. Outcome depends largely on the preceding debility of the host and location of the infection. Significant mortality is associated with immunocompromised patients with septicemia or pulmonary disease.[32,34,43,44,64]

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CHAPTER 113

ENTEROBACTER

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Enterobacter is a genus of Enterobacteriaceae that is an increasingly frequent cause of nosocomial pediatric infection. *Enterobacter* can cause infection of postsurgical wounds; meningitis; and infection of the gastrointestinal, urinary, and respiratory tracts. Development of resistance to antibiotics commonly used for treatment of infection is an increasingly common challenge. For a more detailed overview of *Enterobacter* spp., the reader is referred to the review by Sanders and Sanders.[132]

BACTERIOLOGY

Enterobacter spp. are named for their enteric recovery as gram-negative bacteria.[128] They commonly are found in soil, water, and sewage. They also are causes of botanical disease. These organisms are facultatively anaerobic and motile by peritrichous flagella, with the exception of *Enterobacter asburiae*. Most species yield positive results on malonate, citrate, sucrose fermentation, and Voges-Proskauer tests. *Enterobacter sakazakii* is distinguishable from the other species by its yellow-pigmented colonies. Taxonomic studies have led to several classifications of species contained in this genus. With the reclassification of *Enterobacter agglomerans* as *Pantoea agglomerans* (see Chapter 124), *Enterobacter cloacae*, *Enterobacter aerogenes*, and *E. sakazakii* are the most common species recovered from clinical material.[5,17,29,41,50,69,156,158]

Additional species in the *Enterobacter* genus rarely recovered from human infections include *Enterobacter amnigenus*, *E. asburiae*, *Enterobacter gergoviae*, *Enterobacter cancerogenus* (formerly *Enterobacter taylorae*), *Enterobacter kobei* (formerly NIH group 21), *E. cloacae* subsp. *dissolvens* (formerly *Enterobacter dissolvens*), *Enterobacter nimipressuralis*, and *Enterobacter hormaechei*.[1,53,99,100,109,128] NIH group 42 strains now have been proposed to become *Enterobacter cowanii*.[67] *Enterobacter intermedius* now has been reclassified

to *Kluyvera intermedia* (formerly *Kluyvera cochleae*).[116] *Enterobacter ludwigii* is another new species isolated from clinical specimens that is closely related to *E. cloacae* complex.[65]

EPIDEMIOLOGY

Enterobacter is encountered most commonly as a hospital-acquired pathogen in patients with chronic illness.[5,17,29,41,69,156,158,165] A surveillance study at 50 American medical centers showed *Enterobacter* as the eighth most common cause of nosocomial bloodstream infections, accounting for 230 (5%) of 4725 isolates.[121] Of these, 71 percent were *E. cloacae*, 23 percent were *E. aerogenes*, and 6 percent were other species of *Enterobacter*. *Enterobacter* infections are particularly common in intensive care units (ICUs).[102] A report from the Centers for Disease Control and Prevention describing the epidemiology of health care–associated infections in 61 pediatric ICUs in the United States revealed that *Enterobacter* spp. were isolated with increasing frequency from patients with pneumonia and were the most common gram-negative isolates from bloodstream infections.[69] The frequency of *Enterobacter* spp. increased from 7 to 12 percent of reported pathogens over a 6-year period. *Enterobacter* spp. also were found to be second only to *Pseudomonas aeruginosa* in prevalence in health care–associated sinusitis.[129]

In another Centers for Disease Control and Prevention survey focusing on data from the National Nosocomial Infections Surveillance system, *Enterobacter* was one of the five pathogens most commonly encountered in ICUs, and accounted for 8.6 percent of reported infections.[69] During a 10-year period in six Brazilian neonatal ICUs, gram-negative pathogens accounted for more than half of all bloodstream infections, with *Enterobacter* spp. representing 2 percent.[32] In one pediatric hospital, *Enterobacter* spp. (primarily *E. cloacae*) were the most common cause of enteric bacteremia, and accounted for 14 percent of all bacteremic episodes occurring during a 3-year period.[5] Multiple outbreaks caused by *Enterobacter* have been described in neonatal ICUs.* *E. cloacae* has been found in some neonatal ICUs as the predominant pathogen, representing almost 40 percent of the bloodstream isolates.[93] In some series, one third of *Enterobacter* bactere-mias are polymicrobial, which represents a greater prevalence than that of bacteremia caused by other gram-negative organisms.[20,50]

The most frequently cited risk factor for *Enterobacter* infection is the recent receipt of antibiotics, particularly third-generation cephalosporins.[3-5,16,23,50,70,154] Other risk factors include prolonged hospital stay, especially in an ICU, presence of serious underlying illness (e.g., burns, malignancy, and diabetes), prematurity in a neonate, immunosuppression, and the presence of a foreign device.[34,132,151]

Vertical spread of *Enterobacter* from mother to infant may occur at the time of birth.[48] Environmental sources implicated in outbreaks of infection have included intravenous fluids,[6,25,94,96,142,152,155,157] chronic hand dermatitis of a care provider,[11] contaminated infant formula,[7,14,30,33,108] blood gas machines,[2,82] contaminated transesophageal echocardiography probes,[72] cardioplegia ice,[22] therapeutic bed mattresses and mattress covers,[147] and rectal thermometers.[146]

Ribotyping, pulsed-field electrophoresis, and restriction-fragment polymorphism analysis of DNA from clinical isolates have been useful in discriminating possible sources of contamination and patient-to-patient transfer of individual strains.[15,22,29,30,33,57,60,84,132] Plasmid profiles often are of little value because many strains of *Enterobacter* possess few, if any, plasmids.[132] Integrons, discrete mobile units of DNA that can confer antibiotic resistance, have been reported in *Enterobacter* spp. and are more common findings in infections acquired in a health care setting.[34]

Most cases are not outbreak-associated, and endogenous origin of infection is a more common means of acquisition than is the patient's environment.[121] This factor explains why recent antimicrobial use by the patient is such a strong predictor of infection.

PATHOPHYSIOLOGY

Newborns often are colonized by *Enterobacter* spp. in the gastrointestinal tract soon after birth,[13,113] and acquisition of hospital strains in immunocompromised newborns is common.[13,44,49,149] *Enterobacter* may contaminate the compromised respiratory tract. The oropharynx commonly is colonized by *Enterobacter* by the time the infant is 1 month of age; colonization rates generally are lower in breast-fed infants.[10] Newborns with gastrointestinal abnormalities requiring prolonged parenteral nutrition have higher rates of colonization with *Enterobacter* and a higher incidence of sepsis.[122] Other risk factors for increased *Enterobacter* colonization in neonates include prematurity and prior antibiotic use.[117] Enteric organisms are recovered less frequently from the oropharynx of healthy older children and adults, but increased colonization, including increased risk for third-generation cephalosporin–resistant strains, especially with prior cephalosporin treatment of at least 3 days, is noted during illness (see also Chapter 115).[160]

The ability of *Enterobacter* spp. to develop inducible resistance to penicillins and cephalosporins increases their pathogenic potential. All *Enterobacter* spp. possess an inducible chromosomally encoded class C (Bush group I) β-lactamase (*ampC*), which usually is produced in small amounts.[132] An inducible plasmid-encoded *ampC* (gene $bla_{\text{ACT-1}}$) also has been described and can be present in *Enterobacter* spp. and other Enterobacteriaceae.[130] Both enzymes have a high affinity for third-generation cephalosporins but a low maximum hydrolysis rate.[99] Consequently, β-lactamase mediates resistance to these antibiotics only when it is produced in large quantities or large numbers of plasmids are present.[88,127]

Resistance emerges after a mutation occurs in the *ampD* gene, which normally prevents high-level expression of β-lactamase.[68,71] Such mutants are considered "stably derepressed" and may be induced by exposure to β-lactam antibiotics, especially ampicillin and cephalosporins. Mutations in the *ampR* gene produce similar effects.[81] The addition of a β-lactamase inhibitor (e.g., clavulanic acid) does not increase the activity of β-lactam antibiotics against *Enterobacter* spp; rather, it may decrease the activity against the organism by inducing the class C β-lactamase, as can the presence of *ampR* mutants.[71,81,150]

Emergence of resistance can occur during therapy for an isolate that initially is shown to be susceptible.[62,132] Resistance can be detected 24 hours after initiation of therapy or may be delayed 2 to 3 weeks.[28] In addition, more reports of resistant strains of *Enterobacter* that produce plasmid-mediated, extended-spectrum β-lactamases (ESBL) have been published.[66,95,123] Various ESBL genes, including genes from the TEM, SHV, and CTX-M families, have been found.[26,114,138,171] This variation can complicate choosing antimicrobial agents because discerning the source of the β-lactam resistance in the clinical microbiology laboratory often is difficult without performing specific testing, such as the double disk diffusion method. A survey of 11 clinical laboratories in Germany to determine ESBL prevalence found that 40 percent of *E. cloacae* complex organisms were resistant to extended-spectrum cephalosporins, and only 6 percent carried ESBL

*See references 8, 46, 61, 82, 91, 120, 133, 147, 148, 161.

genes.[66] This finding is in contrast to other reports with prevalences of 36 percent in some Asia-Pacific regions.[12]

In one study, gram-negative fecal aerobic flora was eradicated completely after 24 hours of ceftriaxone therapy, only to be replaced within 10 days (mean 6.7 days) of therapy by *Pseudomonas aeruginosa*, *Enterobacter*, and *Citrobacter* resistant to all β-lactam antibiotics.[58] Although uncommon, the combination of reduced outer-membrane permeability and high-level β-lactamase production renders some clinical isolates of *Enterobacter* resistant to carbapenems (imipenem, meropenem, and ertapenem) and fourth-generation cephalosporins (cefepime).[18,37,85,87,99,119,139,144]

Other than the presence of endotoxin, factors responsible for the virulence of *Enterobacter* spp. are not well defined.[76,112] In vitro, it is possible to transfer and express an *Escherichia coli* enterotoxigenic plasmid in *Enterobacter*, implying the potential for transfer of virulence factors to *Enterobacter* spp. from other species.[170] Shiga-toxin–producing *E. cloacae* has been isolated from the stool of a 5-month-old girl with hemolytic-uremic syndrome.[115] *E. coli* OR:H9, which also produced Shiga toxin, also was isolated from the child's stool, rendering the cause of the child's symptoms unclear. Similar to other organisms associated with central venous catheter infections, *Enterobacter* spp. may adhere irreversibly to catheter material, promoting colonization and infection.[77,118] Certain ribotypes have been encountered more commonly as community or bloodstream isolates, indicating the presence of as yet undefined factors affecting virulence.[159] The propensity of *E. sakazakii* to produce neonatal meningitis complicated by abscesses and cerebral infarction is unexplained.[14,30,112,164] Proposed mechanisms have included brain capillary and intestinal endothelial cell invasion, persistence in human macrophages, and biofilm formation.[77,107,141]

CLINICAL MANIFESTATIONS

Infection caused by *Enterobacter* commonly is indistinguishable from illness caused by other enteric pathogens. Sources of infection include central venous catheters and the urinary and biliary tracts.[5,9,50,63,69,90] *Enterobacter* commonly colonizes the respiratory tract secretions of intubated patients, and it is implicated increasingly as a cause of nosocomial pneumonia.[69,129] It also is one of the most common causes of pneumonia after lung transplantation[92] and can be found as part of the oral flora in patients with strokes.[56] Neonatal infection warrants special mention because of the prominence of *E. sakazakii* as a cause of devastating meningitis.

Similar to *Citrobacter diversus* (see Chapter 112), *E. sakazakii* causes neonatal meningitis complicated by cerebral abscesses or infarctions.[14,30,39,74,164,166] Poor feeding, irritability, jaundice, a full anterior fontanelle, and fever or hypothermia are presenting features that are shared with other gram-negative causes of bacterial meningitis. The mortality rate has been reported to range from 33 to 80 percent, and almost all survivors experience severe neurologic complications, including quadriplegia, developmental impedance, and impaired sight and hearing.[39,164] This severe morbidity is consistent with the development of multiple cystic lesions of the brain in 50 percent of surviving neonates. Serial computed tomography scans commonly reveal evolution of lesions most consistent with initial cerebral infarction, rather than primary abscess formation.[51,83,164] Subsequent cystic lesions may be purulent abscesses, from which the organism can be cultured; alternatively, they sometimes represent sterile fluid collections.[24] Meningitis tended to develop in infants of greater gestational age and birth weight than in infants with only bacteremia, but they tended to be younger in chronologic age.[21]

Enterobacter also may be associated with necrotizing enterocolitis in the newborn, which became more apparent during the outbreaks of *E. sakazakii* associated with contaminated powdered infant formula.[83,145] *Enterobacter* spp. were the third most common isolate recovered from peritoneal fluid in a series of patients with necrotizing enterocolitis.[83,105]

In older immunocompromised children, bacteremia complicated by sepsis is a significant risk. Central venous catheterization and gastrointestinal tract pathology seem to pose greater risks for development of bacteremia than infection of the urinary tract.[5,29,50,90] Bacteremia is accompanied by shock in almost one third of patients, but disseminated intravascular coagulation occurs in less than 5 percent.[16,20,50] Seeding of metastatic foci is uncommon. Overall case-fatality rates with bacteremia vary but usually are approximately 30 percent.[132] Factors associated with a poor prognosis include age younger than 18 months, inadequacy of antimicrobial chemotherapy, septic shock, type of underlying disease, presence of pulmonary infection, thrombocytopenia, unknown primary site of infection, and requirement for intensive care.[16,20,36,50,73] Absence of fever during the course of infection may be a particularly ominous sign; four of five afebrile subjects died in one series.[16] One study found that the use of an aminoglycoside as part of the empiric antibiotic management was a factor contributing to survival of *Enterobacter* or *Citrobacter* bacteremia.[36]

Some *Enterobacter* spp. recovered from children with diarrhea have been reported to produce enterotoxin,[27] although a causative role in enteritis has not been established. Other presentations of *Enterobacter* infection include endophthalmitis,[78,101,103] endometritis,[54] wound infections,[50] diskitis,[124,136] endocarditis,[143] and osteomyelitis.[31,40,75,137] *Enterobacter* also has caused syndromes classically associated with other agents, such as gas gangrene,[45] childhood purpura fulminans,[59] ecthyma gangrenosum,[125] and necrotizing fasciitis.[80]

DIAGNOSIS

Diagnosis of infection caused by *Enterobacter* relies primarily on isolation of the organism in culture from clinical material. Motility, production of ornithine decarboxylase, and the absence of deoxyribonuclease help to distinguish the genus from *Klebsiella* and *Serratia*. The presence of yellow pigmented colonies can help identify *E. sakazakii*. Patterns of sugar fermentation and production of decarboxylase also distinguish the species. The ability to detect pathogens directly in tissue using biotinylated probes offers promise for rapidly establishing the diagnosis but remains primarily in the clinical research arena.[97] Other molecular techniques becoming available for rapid identification of bacteria include polymerase chain reaction (PCR)–single strand conformation polymorphism analysis using 16S ribosomal DNA. This technique allows the potential identification of pathogens to be made from a wide range of clinical specimens with low numbers of bacteria and shows promise for clinical applications.[162,168] In light of the increase of *E. sakazakii* invasive disease after ingestion of contaminated powdered infant formula, several techniques have been introduced for the rapid identification of this pathogen clinically and in foodstuffs. The use of a specific PCR amplification of the outer membrane protein A gene (*ompA*) is being investigated,[104] as is reverse-transcriptase PCR[89] and the use of selective media.[38] Ribotyping is a highly discriminatory and reproducible method for the typing of *E. cloacae*, the most common cause of infection.[52] Other useful molecular techniques include restriction endonuclease analysis of chromosomal DNA, pulsed-field gel electrophoresis, random amplification of polymorphic DNA, and amplification of short interspersed repetitive sequences.[132] Most often, molecular typing methods are used in concert with biotyping, serotyping, or bacteriocin typing.

TREATMENT

Treatment of *Enterobacter* infection is made problematic by inducible resistance to cephalosporins and intrinsic resistance to aminopenicillins, cefazolin, and cefoxitin.[19] The increasing recognition that *Enterobacter* spp. can carry ESBL plasmids in addition to expressing chromosomal *ampC* β-lactamases can be problematic using automated ESBL detection systems. The use of the double-disk synergy method for ESBL detection can indicate the presence of plasmid-mediated resistance but is challenging because high production of *ampC* can mask the inhibition of ESBL by clavulanic acid.[163]

Antibiotic resistance may be present at the time of initial isolation or may develop during therapy.[29,62] This problem is compounded by the common observation of resistance to extended-spectrum penicillins and, to a lesser extent, aminoglycosides. The risks for development of resistance to cephalosporins, piperacillin, and aminoglycosides have been reported to be higher at a tertiary care center than at a primary care hospital.[43] Previous administration of third-generation cephalosporins increases the risk of having multiresistant *Enterobacter* isolates in an initial positive blood culture.[5,28,106] Hospitalized newborns quickly may acquire multiresistant strains, even though they themselves have not been treated with cephalosporins.[13] Isolation of multiresistant *Enterobacter* spp. in blood culture is associated with a higher case-fatality rate compared with mortality after isolation of a more sensitive *Enterobacter*.[28,134] Although less common, resistance to imipenem has been reported.[42,85,140] Cefepime, a fourth-generation cephalosporin, generally maintains activity against *Enterobacter* spp. that possess the class C β-lactamase,[131] although minimum inhibitory concentrations may be higher.[99]

Emergence of cefepime resistance during therapy has been described in a liver transplant recipient with a hepatic abscess caused by *E. aerogenes*.[87] The accompanying editorial warns about the risk of failure using cefepime in patients who have high-density infections (e.g., poorly drained liver abscess) caused by ceftazidime-resistant strains of *Enterobacter*.[99] The presence of an SHV-type ESBL has been associated with cefepime resistance, highlighting the need to identify those strains.[138]

In a nationwide survey of nosocomial bloodstream infections, rates of *Enterobacter* resistance to third-generation cephalosporins (ceftazidime, ceftriaxone) and broad-spectrum semisynthetic penicillins (piperacillin) with or without a β-lactamase inhibitor (tazobactam) was high, ranging from 35 to 50 percent.[121] Cefepime and imipenem inhibited 97 to 100 percent of isolates. Susceptibility to aminoglycosides and fluoroquinolones ranged from 92 to 98 percent, and 85 to 96 percent of isolates were susceptible to trimethoprim-sulfamethoxazole. Fluoroquinolone resistance is now being reported and likely is plasmid-mediated.[111,169] Because resistance patterns may vary based on geographic location, local susceptibility data should be used to guide initial therapy.

Some investigators recommend initial combination therapy that includes an aminoglycoside plus either cefepime or a carbapenem (imipenem or meropenem), until results of susceptibility testing are available. Third-generation cephalosporins should be used with caution, even when initial susceptibility results seem favorable. When strains are resistant to gentamicin and tobramycin, amikacin may be a suitable alternative. Good responses to therapy and return of gentamicin susceptibility of hospital *Enterobacter* strains have occurred after routine substitution of amikacin for gentamicin.[126,135] Trimethoprim-sulfamethoxazole alone (or combined with an aminoglycoside) and quinolones seem to be good alternatives for the treatment of *Enterobacter* infections, including meningitis.[5,35,55,100,166] Fluoroquinolones may be another option, depending on local resistance rates. They have been reported to be effective in bone and joint infections, in combination with cefepime.[86] As newer antimicrobial agents become available, more testing will be required to determine their effectiveness. Tigecycline may have promise, but further studies are needed to determine its usefulness in clinical treatment.[153,167]

Enterobacter meningitis creates special concerns. As is common with other forms of neonatal enteric meningitis, even susceptible organisms often persist in cerebrospinal fluid for 5 days or longer.[74] Monotherapy with a cell wall–active agent seems to be less effective than combination therapy with an aminoglycoside. Trimethoprim-sulfamethoxazole also has been used successfully.[47,166] Intrathecal administration of antibiotics does not seem to be beneficial in infants,[98] but it has been used with some success in adults.[110] The physician should anticipate the potential development of cerebral abscesses, infarctions, and cysts in newborns with *E. sakazakii* infection. Serial computed tomography scans should be considered, and a neurosurgeon should be sought for drainage of abscesses and management of fluid accumulation.

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CHAPTER 114

Diarrhea-Causing and Dysentery-Causing *Escherichia coli*

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Escherichia coli has long been recognized as the most common facultative anaerobe in the human gastrointestinal (GI) tract. Virtually all *Homo sapiens* (and most other mammals) harbor this bacterium. Yet within the *E. coli* biomass lurk highly evolved pathogenic subtypes that have adapted themselves to a new niche—human pathogenicity. These pathotypes may prefer the GI tract, the genitourinary tract, or disseminated sites (including the meninges)—the last almost exclusively in neonates. This chapter focuses on *E. coli* associated with enteric infections.

The first implication of *E. coli* with diarrhea occurred more than 75 years ago, when Adam[3] postulated the existence of a group of "dyspepsia" *E. coli* responsible for neonatal and infantile diarrhea. In the 1940s, Bray reported that many, but not all, *E. coli* from severe infant diarrhea cases in summer agglutinated with antiserum prepared from a diarrheal isolate, whereas *E. coli* organisms from other sources did not.[7] The isolate used by Bray to develop the antiserum was later serotyped by the Kauffman scheme as O111:B4, one of the now classic serogroups of enteropathogenic *E. coli* (EPEC) (Table 114–1). Although this initial description was made possible by serologic fingerprinting, the advent of molecular biology rapidly produced a plethora of reports describing the several discrete *E. coli* pathotypes.

CAUSATIVE ORGANISMS

Six distinct diarrheogenic pathotypes are recognized currently on the basis of clinical, biochemical, and molecular/genetic criteria (Table 114–2), as follows:

1. Enterotoxigenic *E. coli* (ETEC), a major cause of traveler's diarrhea and infant diarrhea in developing countries, elaborates the heat-stable (ST) enterotoxin or the heat-labile (LT) enterotoxin, or both, and causes infection of the small intestine.

TABLE 114–1 Serotypes Characteristic of the Diarrheogenic Pathotypes of *Escherichia coli*

Enteropathogenic	Enterotoxigenic	Enteroinvasive	Enterohemorrhagic	Enteroaggregative
O44:H34	O6:H -, 12, 16, 40	O28:H -	O26:H11	Nontypeable
O55:H6, 7, 32	O8:H -, 42	O29:H -	O77:H18	Rough
O86:H2, 34	O25:H -, 42	O32:H -	O103:H2	O3:H2
O111:H2, 7, 12	O27:H7	O42:H -	O104:H21	O6:H1
O114:H2	O29:H21	O89:H -	O111:H8	O11:H16
O119:H6	O63:H -, 12	O112:H -	O113:H21	O15:H21
O124:H?	O78:H11, 12	O121:H -	O128:H2	O44:H18
O125:H21	O117:H4	O124:H -	O145:H -	O92:H23
O127:H4, 6, 21	O125:H30	O136:H -	O157:H -, 7	O111:H21
O128:H2, H21	O126:H10, 27	O144:H -	O178:H19	O126:H2, 27, H -
O142:H6, 34	O127:H2	O152:H -		
O158:H23	O128:H8, 35			
	O143:H -			
	O146:H39			
	O148:H28			
	O153:H45			
	O159:H4, 21			

TABLE 114–2 Relationship of *Escherichia coli* Virulence Genes to Clinical Patterns of Diarrhea

	Virulence Genes†									
Pathogen Group*	*EAF*	*A/E*	*LA*	*AA*	*LT/ST*	*CFA*	*Invasion Factors*	*Stx*	*ShET-2*	Clinical Disease
ETEC	–	–	–	–	+	+	–	–	–	Watery
EPEC	+	+	+	–	–	–	–	–	–	Watery
EHEC	–	+	–	–	–	–	–	+	–	Bloody (hemorrhagic colitis)
EIEC	–	–	–	–	–	–	+	–	+	Bloody (dysentery)
EAEC	–	–	–	+	+‡	–	–	–	–	Watery (persistent)

**ETEC, enterotoxigenic E. coli; EPEC, enteropathogenic E. coli; EHEC, enterohemorrhagic E. coli; EIEC, enteroinvasive E. coli; EAEC, enteroaggregative E. coli.*
†EAF, EPEC adherence factor; A/E, attaching and effacing changes; LA, localized adherence pattern; AA, autoaggregative attaching pattern; LT/ST, heat-labile and heat-stable toxins; CFA, colonization factor antigens; invasion factors, chromosomal and plasmid factors mediating cell invasion; Stx, Shiga family cytotoxins; ShET-2, plasmid-encoded Shigella enterotoxin-2, highly homologous to the plasmid-encoded EIEC toxin.
‡Enteroaggregative stable toxin (EAST) is a member of the ST family.

2. EPEC, the original pathotype defined by Bray,[7] causes infant diarrhea in less-developed countries. The bacteria induce a characteristic attaching and effacing lesion in the small intestine. Classic EPEC requires the chromosomally encoded locus of enterocyte effacement (LEE) and a high-molecular-weight virulence plasmid termed EPEC adherence factor (EAF) plasmid.

3. Enterohemorrhagic *E. coli* (EHEC) causes outbreaks of hemorrhagic colitis and hemolytic-uremic syndrome (HUS) in temperate climates. The essential virulence factor is the Shiga toxin (Stx), which circulates through the bloodstream and acts by inhibition of protein synthesis in the target cell.

4. Enteroinvasive *E. coli* (EIEC) is an unusual cause of diarrhea and dysentery. It is similar to *Shigella* epidemiologically and pathogenetically and shares similar virulence genes.

5. Enteroaggregative *E. coli* (EAEC) causes diarrhea in individuals of all ages in industrialized countries and in less-developed areas and in travelers. The organism adheres to the intestinal mucosa and secretes several enterotoxins and cytotoxins.[95]

6. Diffusely adherent *E. coli* (DAEC) is the sixth category. The epidemiologic scenario for this organism has not been elucidated.

TRANSMISSION AND EPIDEMIOLOGY

Diarrheogenic *E. coli* strains are worldwide in distribution. The route of infection is fecal-oral, predominantly via contaminated food and water, although person-to-person transmission may occur with EHEC and possibly EPEC in infants.[111] As discussed subsequently, environmental contamination by EHEC is an emerging problem in industrialized countries, whereas ETEC continues to be ubiquitous worldwide. Table 114–3 lists the age-specific predilections of the various *E. coli* pathogens.

TABLE 114–3 Age-Related Patterns of *Escherichia coli* Diarrhea

Pathogen Classification	Age at Highest Risk	Characteristics of Diarrhea		
		Bloody	*Watery*	*Inflammatory*
EAEC	<6 mo	–	+++	–
EPEC	<1 yr	–	+++	–
ETEC	<1 yr	–	+++	–
EIEC	>2 yr	++	+	+++
EHEC	2-10 yr	+++	+	–

EAEC, enteroaggregative E. coli; EHEC, enterohemorrhagic E. coli; EIEC, enteroinvasive E. coli; EPEC, enteropathogenic E. coli; ETEC, enterotoxigenic E. coli.

ENTEROTOXIGENIC *ESCHERICHIA COLI*

The infectious dose for ETEC is approximately 10^8 bacteria.[47] The incubation period is 14 to 50 hours.[107] Transmission typically occurs via contamination of food and water,[145] and person-to-person transmission is thought to be uncommon. In developing

countries, contamination of food and water sources occurs commonly.[132] ETEC has been detected in the United States in numerous food samples, including cheese, hamburger, sausage, and seafood.[103,145] A domestic U.S. outbreak of diarrhea at Crater Lake National Park in Oregon was traced to ETEC organisms in the water supply.[143] Because large numbers of organisms are required for experimental infection, transmission probably would require multiplication of the inoculum within the vehicle, which probably contributes to the well-documented propensity of ETEC infection to occur in warm months.[132]

ETEC is a major cause of diarrhea in infants and children in the developing world.[64,132] The incidence typically increases during the first 6 months of life and decreases after 12 months. ETEC has been estimated to cause approximately 20 percent of diarrheal episodes in the developing world,[132] although this percentage varies substantially by location. A study in rural Egypt[134] found that ETEC accounted for 66 percent of all first episodes of diarrhea, with an incidence of 1.7 episodes per child-year during the first 6 months of life and 2.3 during the second 6 months. In Tehran,[148] researchers found that ETEC could be isolated from 15.5 percent of patients younger than 5 years old with diarrhea. The prevalence was 18 percent in Bangladesh[131] and 33 percent in Mexico.[36] A study in Hanoi[117] found, however, that only 2.2 percent of diarrheal episodes could be attributed to ETEC, and the frequency in Mongolia[150] was similarly low.

ETEC disease is primarily a childhood problem, with the incidence decreasing after the child reaches 5 years of age.[131,132] After infection with a particular ETEC strain, the host is resistant to disease but still may excrete the organism asymptomatically, a characteristic that contributes to the environmental burden.[111,162] Adults still may be susceptible to ETEC diarrhea.[64,132] In a study of elderly individuals in Dhaka, Bangladesh,[54] researchers found that 13 percent of adults older than 60 years with diarrhea were noted to have ETEC. Although the incidence of ETEC diarrhea decreases after age 5 years, the incidence seems to increase again in patients older than 15 years, and 25 percent of ETEC infections are seen in adults.[131,132]

ETEC is the most common cause of traveler's diarrhea, typically accounting for approximately 30 to 40 percent of cases.[2,84,152] Illness is acquired by ingestion of contaminated food or water. The diarrhea is acute and watery and typically resolves spontaneously without severe limitation of activities.[2] ETEC also has been implicated as the cause of diarrhea among cruise ship travelers, introduced when the ship docks in a foreign port.[40]

ETEC organisms are extremely diverse, and children in endemic areas experience multiple infections. The basis of immunity, which increases with repeated exposure, is unknown.[162] Strains producing ST cause more severe disease than strains producing only LT, and the former is not immunogenic. Colonization of the small intestinal mucosa is mediated by surface fimbriae called *colonization factor antigens* (CFAs), which are immunogenic and which may elicit some element of protective immunity.[135] Despite the existence of more than 20 CFAs, a few are overrepresented among clinical isolates; in a study from Bangladesh, 7 CFAs constituted greater than 75 percent of the CFAs isolated.[131] In an analysis of multiple studies, Wolf[180] suggested that most ETEC express CFA I, II, or IV. In some epidemiologic studies, most strains do not express known CFAs, however.[156]

ETEC is predominantly an infection of warm weather months in endemic areas, coincident with conditions promoting bacterial replication in the environment. Some sites report that a second peak occurs after the rainy season.[131,132]

ENTEROPATHOGENIC *ESCHERICHIA COLI*

Neter and associates[116] first coined the term EPEC to denote a group of *E. coli* serotypes associated with dramatic and highly lethal nursery outbreaks of diarrhea in the United States and the United Kingdom (see Table 114–1). We now recognize that these serotype antigens serve as surrogate markers for a package of chromosomal and plasmid-borne virulence genes, which together orchestrate enteric pathogenesis. The epidemiology of EPEC has changed since its original description was provided. Gone are the lethal outbreaks formerly reported in industrialized countries, to be replaced by sporadic endemic disease in resource-poor countries. The infection retains its dramatic predilection for infants and children younger than 24 months, is rarely seen in older children and adults, and is not a cause of traveler's diarrhea.[35,36,65,170] The basis of this age-dependent incidence is unknown.

Globally, EPEC is among the most common causes of infant diarrhea.[35,36,65,140] EPEC has a propensity to cause persistent infection and wasting, suggesting that its burden may comprise more than simple dehydration. Breast-feeding offers some protection against EPEC because of the presence of immune factors and oligosaccharides that inhibit adherence of EPEC to epithelial cells.[38] In industrialized countries such as the United States, EPEC generally is no longer considered to be a cause of endemic diarrhea, although EPEC may be present in infants immigrating from or visiting resource-poor settings. Rare indigenous cases may occur.[67] In an outpatient setting in the United States, 2 of 147 cases of non-dysenteric acute diarrhea were caused by EPEC.[27] In 166 Swiss children admitted to hospitals with diarrhea, 13 (7.8%) had EPEC isolated from stool cultures.[53]

As noted previously, EPEC strains of the classic serotypes (also known as typical EPEC) require the LEE chromosomal locus and the EAF plasmid to cause diarrhea. Controversy exists over whether or not so-called atypical EPEC (which are LEE-positive and EAF-negative) are a cause of diarrhea.[109] The description of an outbreak of diarrhea in Japan caused by an atypical EPEC strain suggests that at least some of these strains are pathogenic.[182]

ENTEROHEMORRHAGIC *ESCHERICHIA COLI*

Since its discovery in 1977, EHEC has emerged as an important and increasingly common human pathogen. The organisms may be excreted asymptomatically by cattle herds, and large outbreaks of human infection have been associated with the ingestion of undercooked hamburger and other foods contaminated indirectly by cattle manure. Careful prospective epidemiologic surveillance in Minnesota has revealed an increase in the incidence of EHEC from 0.5 to 2 per 100,000 children younger than 18 years old during the period 1979 to 1988,[101] similar to the incidence of EHEC determined in the state of Washington in the late 1980s: 2.1 per 100,000. In a multicenter study of mild acute diarrhea in outpatients in the United States, 5 (3.4%) of 147 cases were caused by EHEC,[27] a frequency similar to that in Swiss children hospitalized for community-acquired diarrhea.[53] Very young children are not commonly infected; rather, infection occurs most commonly and severely in children 2 to 10 years old or in the elderly.

Ground beef is identified as the most common source of EHEC infection. Several outbreaks have been associated with fast-food consumption.[133] In meat-processing plants where bulk ground beef is prepared for the fast-food industry, meat from one contaminated carcass can contaminate and distribute the organisms to a huge number of beef patties. Several other food sources of transmission that have been documented include other beef; produce, including spinach and lettuce; milk; water; and processed foods, including unpasteurized apple cider, mayonnaise, and dry fermented sausage. Person-to-person, cattle-to-person, and waterborne outbreaks have been described. Person-to-person spread as sequelae of point-source epidemics is common.[155] Many

unusual modes of transmission linked with cattle have occurred and include poorly washed apples dropped in cow pastures and used to make cider, surface water supplies in proximity to areas where cattle graze, spread of aerosolized particles at agricultural fairs, and transmission by contact at petting zoos. Contaminated swimming holes have been implicated in several outbreaks.

A multistate outbreak of O157:H7 EHEC infections associated with the consumption of fresh bagged spinach occurred in the United States in 2006, affecting 26 states and resulting in 205 confirmed illnesses, 103 hospitalizations, 31 cases of HUS, and three deaths.[11,28] This outbreak exemplifies the widespread effect of a foodborne outbreak caused by the mass production and distribution of produce.

Strong epidemiologic evidence associates EHEC with HUS. In one series of patients from Canada, 60 percent of patients with HUS had either neutralizable free Shiga toxin (Stx) or Stx-producing *E. coli* in their stool, and 75 percent had serum antibody against Stx.[124] A review of O157:H7 outbreaks (defined as two or more cases) reported to the Centers for Disease Control and Prevention in the United States from 1982 to 2002 found 350 outbreaks representing 8598 cases, 1493 (17%) hospitalizations, 354 (4%) cases of HUS, and 40 (0.5%) deaths.[133] During a large outbreak of *E. coli* O157:H7 infection associated with fast-food restaurant hamburgers on the West Coast of the United States in 1993, of the 732 affected individuals identified, 195 were admitted to the hospital, HUS developed in 55 (7.5%), and 4 died.[67] Investigation of this outbreak showed that small numbers of bacteria (in the range of a few hundred) constituted an infectious dose. Among 93 cases of O157:H7 infection reported in Washington State in 1987, HUS or thrombotic thrombocytopenic purpura (TTP) developed in 11 (12%), for an approximate incidence of 0.23 per 100,000.[122]

The highest incidence of HUS occurs in children younger than 5 years, in whom the age-specific incidence ranged from 2.6 to 5.8 per 100,000 in Minnesota,[101] Washington,[122] and Oregon.[142] In the 2000 FoodNet Annual Report, *E. coli* O157:H7 accounted for 2 cases of HUS per 100,000, and more than 40 percent of cases with confirmed infection were hospitalized. The incidence of HUS in Argentina, 21.7 per 100,000, is four to eight times higher and is the highest reported incidence of HUS in the world.[99]

In the United Kingdom and the United States, HUS and TTP are associated predominantly with Stx2-producing *E. coli* O157:H7[101,123]; however, this finding may represent more facile detection of this serotype. In addition, the incidence of non-O157:H7 infections in the United States is increasing (serotypes associated with EHEC infection are presented in Table 114–1).[25,85,179] In Argentina, non-O157:H7, Stx2-producing strains are isolated most commonly.[99] Similarly, in Australia, other serotypes, especially O111, have been associated with major outbreaks and a significant number of HUS cases.[9] Many of these serotypes have been implicated in sporadic and outbreak disease in the United States[24] (e.g., an outbreak caused by O104:H21 acquired from contaminated milk[10]).

Non-O157:H7 EHEC infections have increased in prevalence in the United States and are now known to account for 20 to 50 percent of all EHEC infections.[85] Although some non-O157 strains are not pathogenic, others have been shown to cause disease as severe as that caused by O157:H7 strains, suggesting that a clinical suspicion for EHEC should be maintained even when testing for O157:H7 is negative.[85] Non-O157, Stx-producing *E. coli* infection was made a reportable disease in the United States in 2000.[25]

HUS occurs seasonally in the United States and Canada (most common during the summer months), although no specific seasonal risk factor or factors for infection with Stx-producing *E. coli* are known. Other epidemiologic data suggest that poor children are at lower risk for the development of HUS, perhaps because they already have immunity to EHEC or Stx toxins through early contact with the organisms in their environment.[32]

ENTEROINVASIVE *ESCHERICHIA COLI*

EIEC is closely related to *Shigella* microbiologically and pathogenetically. The pathotype typically is found in areas with a high burden of *Shigella*. The infectious dose may be higher than that of *Shigella*, and person-to-person transmission seldom is reported. Food-borne outbreaks occur sporadically.[66,100] The first description of EIEC as a pathotype occurred in 1971, when bloody diarrhea was traced to the consumption of French Camembert cheese contaminated with *E. coli* O124.[100] Small outbreaks or sporadic cases involving a limited number of serotypes have been reported from numerous countries, including the United States, France, Japan, and Brazil.[100,120,160,171,174] Prospective studies in Thailand using DNA probes for EIEC pathogenicity genes indicated that 6 percent of cases of dysentery may be caused by EIEC strains.[48] The epidemiology of the cases is complicated by the fact that in contrast to the initial descriptions of EIEC-associated disease, most EIEC infections probably are neither dysenteric nor characterized by bloody diarrhea but instead manifest as watery diarrhea with low-grade fever, similar to that caused by viral agents and ETEC.

Infection with 10^8 bacteria has been shown to be necessary for experimental dysentery in human volunteers,[47] a dose substantially higher than that for *Shigella* spp. ($\leq 10^4$ organisms). In a food-borne outbreak caused by a nontypeable EIEC, secondary person-to-person transmission was not reported.[160]

ENTEROAGGREGATIVE *ESCHERICHIA COLI*

EAEC was implicated first as a cause of diarrheal disease in studies of children in Chile, Mexico, and India.[18,19,37,112] The pathogenicity of at least some EAEC strains has been established by several observations. First, volunteer studies by Nataro and associates[108] revealed that some EAEC could elicit diarrhea in healthy subjects, whereas other strains may not be pathogenic. Second, EAEC strains have been implicated in outbreaks of diarrhea, the largest of which involved nearly 2700 schoolchildren in Japan.[72,82,183] Lastly, but most emphatically, a meta-analysis performed by Huang and colleagues[79] of more than 20 years of studies revealed convincingly that EAEC is a cause of diarrhea in infants; adults, especially those with human immunodeficiency virus and acquired immunodeficiency syndrome (AIDS) in developing countries; children in industrialized countries; and adult travelers to less-developed areas. In addition, a study from the United States by Nataro and colleagues[113] suggested that EAEC was the most frequent bacterial cause of diarrhea among all ages in Baltimore, Maryland, and New Haven, Connecticut.

Nonetheless, a persisting controversy surrounding EAEC epidemiology and pathogenesis is the definition of the pathotype. The meta-analysis described earlier implicated EAEC, as defined by the characteristic stacked-brick adherence pattern to epithelial cells in culture.[79] A study of infant diarrhea in Cincinnati suggested, however, that only strains hybridizing with an EAEC gene probe (see later) were truly associated with clinical diarrhea.[33] A full understanding of what constitutes a truly pathogenic EAEC strain is unavailable.

CLINICAL MANIFESTATIONS

Diarrhea-causing *E. coli* may be responsible for a variety of clinical syndromes because virtually all known mechanisms of diarrhea, including secretory toxins, cytotoxic toxins, invasion, and

pathogenic adherence, are manifested by the various *E. coli* pathotypes. The clinical manifestations are largely a function of the complement of virulence genes (see Table 114–2), and identification of the essential pathogenicity genes provides the definitive diagnostic maneuver. As with other pathogens, invasive *E. coli* strains (i.e., EIEC) produce inflammatory diarrhea with fever, abdominal pain, nausea, vomiting, and leukocytes and blood in the stool. Noninvasive, cytotoxin-producing EHEC strains cause a frankly bloody diarrhea associated with leukocytosis but without fecal leukocytes or fever. Producers of LT or ST enterotoxins (i.e., ETEC) elicit brisk, watery diarrhea with the potential for significant dehydration.

ENTEROTOXIGENIC *ESCHERICHIA COLI*

ETEC causes watery, nonmucoid, nonbloody diarrhea in infants, older children, and adults, and it is a common cause of traveler's diarrhea.[132] The onset of diarrhea is abrupt, with an incubation period of 14 to 50 hours.[107] The frequency of stools varies from a few to more than 10 per day, and a striking absence of leukocytes is noted when the diarrheal stool is examined by light microscopy. Young affected children may vomit and commonly are febrile (38° C to 40° C), although adults typically are afebrile. ETEC disease cannot be distinguished clinically from most other causes of acute nonspecific watery diarrhea. The illness usually is self-limited to 3 to 5 days but occasionally lasts more than 1 week. Severely dehydrating illness that resembles clinical cholera can occur but is uncommon.

Variability in the clinical picture may be due to age differences and preexisting immunity, but it also may be attributed to differences in the infecting inoculum. DuPont and colleagues[47] produced mild diarrhea (three watery stools per day for 2 to 3 days) when 10^8 bacteria were fed to adult volunteers, whereas 10^{10} organisms caused more pronounced diarrhea (more than five stools per day for 4 to 5 days), with mucus but no blood observed in some subjects. Many subjects also had abdominal cramping, but no tenesmus was present, and all remained afebrile. This clinical picture is typical of traveler's diarrhea in adults.[104] ETEC diarrhea can result in severe dehydration necessitating aggressive fluid therapy.

ENTEROPATHOGENIC *ESCHERICHIA COLI*

EPEC diarrhea typically is copious and watery, without blood or fecal leukocytes.[97] Patients may have low-grade fever. In a Swiss study conducted with modern diagnostic methods, the mean age of 13 hospitalized children with EPEC diarrhea was 1.4 years (range 0.5-3 years).[53] Fever was low-grade, and vomiting occurred in 69 percent. Volume depletion was considered moderate in five and severe in two children. Although nursery outbreaks no longer occur commonly, severe illness still can develop in infants infected with EPEC,[83] with high mortality rates ranging from 25 to 70 percent.[43] The cause of death of patients in nursery outbreaks is not well documented. Investigations using an experimental EPEC infection model in adult volunteers (who are not naturally susceptible) revealed that watery diarrhea occurred 3 to 16 hours after inoculation and generally lasted less than 2 days. Diarrhea occasionally was copious and in some cases was associated with abdominal cramps, nausea, vomiting, malaise, and fever.[45] This clinical picture is consistent with disease caused by outbreaks of EPEC in adults and traveler's diarrhea caused by EPEC.

EPEC also has been implicated in chronic diarrhea in the United States and elsewhere, with serious nutritional consequences that may require total parenteral nutrition and hospital stays of 120 days.[144] In prospective studies of EPEC diarrhea in young infants in Brazil[65] and Ethiopia,[169] fever, vomiting, and dehydration all were observed commonly. The clinical significance of chronic diarrhea may be increased by the underlying malnutrition of infants in resource-poor countries. In many settings, especially where effective oral rehydration programs are in place, chronic diarrhea and associated malnutrition are now more important causes of diarrheal deaths than are acute diarrhea and dehydration.[22]

In the course of the classic, severe nursery outbreaks caused by typical EPEC strains, the first signs and symptoms were mild and nonspecific, but they commonly were followed by vomiting and diarrhea of increasing severity. Stools contained neither mucus nor blood, and the volume fluctuated over a period of weeks. Plain abdominal radiographs revealed only nonspecific dilation of small bowel loops, although severe ileus not attributable to hypokalemia developed in many patients. Infected infants lost as much as 15 percent of body weight, leading to profound electrolyte disturbances and severe dehydration along with central nervous system manifestations, such as irritability, hypertonicity, convulsions, and coma. Circulatory collapse occurred despite adequate replacement of fluids and achievement of electrolyte balance. In an epidemic in Virginia, Belnap and O'Donnell[16] described fatal infections caused by *E. coli* O111:B4 occurring after 3 weeks of illness and associated with renal failure, coma, and signs of disseminated intravascular coagulation, but blood cultures typically remained negative. The fatality rate was age-dependent; although the overall mortality was 16 percent, the rate in neonates was 40 percent.

ENTEROHEMORRHAGIC *ESCHERICHIA COLI*

In 1971, a distinctive clinical diarrheal syndrome was recognized that was characterized by afebrile bloody diarrhea in addition to cramping and colonic inflammatory changes, usually right-sided.[138] In 1983, this illness was associated with an otherwise rare *E. coli* serotype, O157:H7, and a characteristic syndrome, hemorrhagic colitis, was defined.[139] O157:H7 subsequently was recognized as the most virulent prototype of a Stx-producing pathotype designated EHEC. EHEC now has been shown to cause a wide spectrum of diseases that may be confined to the GI tract or, in a sizable proportion (typically 5-10%), can become systemic.[15,165] After an incubation period of 3 to 4 days (range 1 to 8 days), the local GI illness usually begins with nonbloody diarrhea that can progress to bloody diarrhea after 1 or 2 days. Hemorrhagic colitis occurs in 50 to 90 percent of O157:H7 infections.[122,165]

Further progression may occur over the course of the next day or two and result in the passage of frank blood, the pathognomonic clinical feature of hemorrhagic colitis. Associated manifestations include vomiting in approximately 50 percent of patients and abdominal pain. Fever may occur but typically is low-grade. This clinical picture may be confused with other conditions, such as appendicitis, intussusception, inflammatory bowel disease, ischemic colitis, and diverticulitis. The difficulty in establishing the diagnosis can lead to inappropriate drug therapy or even surgery.[68,80]

A serious complication of hemorrhagic colitis is the development of HUS or TTP.[68] HUS is characterized by the triad of acute renal failure, thrombocytopenia, and hemolytic anemia and develops in approximately 10 to 20 percent of infected children younger than 10 years.[111] HUS is the most common cause of acquired renal failure in the United States.[172,173] The acute mortality rate is approximately 5 percent, and renal failure is expected to develop eventually in many more patients during the next several decades. After renal failure, cerebrovascular accident and colonic perforation are the most common serious sequelae. TTP and HUS have overlapping clinical features, but TTP

generally occurs in older adults and is associated with prominent neurologic findings, including behavioral changes, altered consciousness or coma, and seizures, and fever.[77]

A prospective study in Canada identified *E. coli* O157:H7 in 15 percent of 125 patients with grossly bloody diarrhea over a 6-month period.[124] The age range was 15 months to 73 years; however, almost half of the patients were younger than 10 years old. The illness was similar to that described earlier, with a mean duration of 7.8 days, but it was significantly longer in children (9.1 ± 2 days) than in adults (6.6 ± 1.1 days). Sigmoidoscopy findings were abnormal in seven of eight adults examined, with hyperemic mucosa in six and superficial ulcerations in one. Biopsy samples showed mild mucosal inflammation in four of five patients. In another study, the findings on colonoscopy in 10 patients with hemorrhagic colitis caused by *E. coli* O157:H7 included severe inflammation (predominantly right-sided), marked edema, easy hemorrhage, and the frequent appearance of longitudinal ulcer-like lesions.[158]

Because local and systemic manifestations in EHEC infection are caused, at least partially, by Stx, the presence of these same toxins in the intestinal lumen in patients with non-O157:H7 infection puts them at risk for development of hemorrhagic colitis and HUS. No comparative data are available to assess the relative risk in O157:H7 and non-O157:H7 EHEC infection. Non-O157:H7 EHEC may cause similar clinical manifestations, with less aggressive transmission potential.

ENTEROINVASIVE *ESCHERICHIA COLI*

Naturally acquired EIEC infection causes a mild to moderately severe dysentery syndrome consisting of fever, malaise, diarrhea, tenesmus, and abdominal cramping.[47,174] Watery diarrhea usually occurs at the onset of the illness and progresses to mucoid diarrhea with streaks of blood or microscopic hematochezia but rarely to the classic small-volume, grossly bloody, dysenteric stool seen with *Shigella* infection. Of 204 cases reported in one study, grossly bloody stool occurred in only 4.[65] In two of these patients, sigmoidoscopy revealed superficial ulcerations in one and hyperemia alone in the second patient. As in *Shigella* infection, the stool reveals abundant polymorphonuclear cells, compatible with the inflammatory, invasive nature of the organism. Vomiting and dehydration can occur; the latter generally is mild in nature. Fever of 38° C to 39.5° C is a typical symptom that occurs early in association with malaise, myalgia, and headache and lasts for 2 to 3 days. In most instances, the diarrhea ceases in 1 week or less, but in some patients, it may continue for 2 weeks or more.

In adult volunteers with experimentally induced EIEC disease, febrile illness developed approximately 11 hours (range 8 to 24 hours) after the inoculum was ingested.[47] Chills, myalgia, headache, and profuse diarrhea or abdominal cramps and tenesmus rapidly followed. In 2 of 13 subjects, this picture was associated with systemic toxicity and transient hypotension consistent with bacteremia, even though blood cultures were negative for all patients. Clinical dysentery with bloody stools occurred in several subjects, and reddened, friable mucosa with multiple bleeding points was seen by sigmoidoscopy. Clinical illness was controlled quickly with parenteral ampicillin therapy.

ENTEROAGGREGATIVE *ESCHERICHIA COLI*

Descriptions of EAEC clinical features are derived from outbreak investigations, volunteer studies, and studies of traveler's diarrhea.[79] Most EAEC infections are accompanied by watery diarrhea, which may progress to persistent diarrhea that lasts 14 days or longer.[19,112] Bloody diarrhea is observed in a subset of patients.[37] An EAEC strain was implicated in a school lunch outbreak in Tajimi City, Japan, in 1993.[82] Of 6636 children who ate the school lunch at 16 schools, 2697 (rate of attack 40.6%) reported GI symptoms. Major symptoms were abdominal pain (73.6%), nausea (51.1%), and diarrhea (39.9%). The incubation period for the onset of GI illness was 40 to 50 hours on average. Duration of symptoms was not reported. A nursery outbreak in Serbia involved 19 normal infants. All experienced watery diarrhea, which persisted beyond 2 weeks in three patients. A report of 17 pediatric patients with illness caused by EAEC of serotype O126:H27 in Israel suggested that patients typically manifested diarrhea (in all), vomiting (in 8), and fever (in 12, ≤40° C).[157] In this report, one 6-week-old infant developed diarrhea persisting for 40 days. Studies in travelers suggest that EAEC disease may be mildly inflammatory, although fever and fecal leukocytes are present in a few patients.[79]

PATHOGENESIS

ENTEROTOXIGENIC *ESCHERICHIA COLI*

ETEC was identified first as a porcine pathogen,[71,159] which elicited fluid secretion when injected into ligated ileal loops.[71] Two secretogenic activities were characterized: one could be inactivated by heating, and the other could not; the activities could be found both in the same strain or independently. These activities now have been assigned to two well-characterized enterotoxins, LT and ST. LT is genetically, structurally, and mechanistically similar to cholera toxin produced by *Vibrio cholerae* O1 strains.[63,111] ST is actually two distinct toxins: STa is associated with human disease, whereas STb causes disease only in animals.[90,130]

The essential pathogenic strategy for ETEC involves colonization of the proximal small intestine, followed by release of one or both of the enterotoxins. Colonization of the intestinal epithelium (Fig. 114–1) is mediated by proteinaceous, hair-like bacterial surface appendages called *fimbriae* (or *pili*), of which greater than 25 antigenic types are known to exist.[180] The adherence factors are termed *colonization factor antigens* (CFA) or *coli surface antigens* (Fig. 114–2).[60]

Human STa is a small, non-immunogenic molecule that is translated as a 72-amino acid polypeptide.[161] The first 19 amino acids serve as a signal sequence for secretion into the periplasmic space; a further cleavage event occurs after secretion, which results in the final 18- to 19-amino acid active peptide.[111,136] The sequence of the mature ST has six cysteine residues that form stabilizing disulfide bonds, contributing to the high stability of the toxin to heat and proteases.[184] The ST receptor on the apical surface of the intestinal epithelial cells is guanylate cyclase C. This receptor is a large transmembrane protein with an extracellular ligand-binding domain and intracellular catalytic domain; binding to guanylate cyclase C results in its activation, which is responsible for an increase in cellular cyclic guanosine monophosphate (cGMP).[153] cGMP is an intracellular signaling molecule that induces activation of apical chloride channels and inhibition of absorptive mechanisms.[57,69,105] ST exploits an endogenous signaling pathway, the natural agonist of which is the peptide guanylin, a natural controller of electrolyte homeostasis.[39]

The LT family of toxins includes LT-I, which has significant homology in structure and function with cholera toxin, and LT-II, which does not seem to cause human or animal disease.[111] LT-I shares 80 percent identity with cholera toxin and similarly has an A_1-B_5 stoichiometry.[62,111,163] The 28-kd A subunit is the catalytically active moiety, whereas the 11-kd B subunit pentamer mediates cell binding and entry. LT interacts mainly with G_{M1} gangliosides on the surface of the epithelial cells (although additional potential receptors have been identified).[111,168] The A

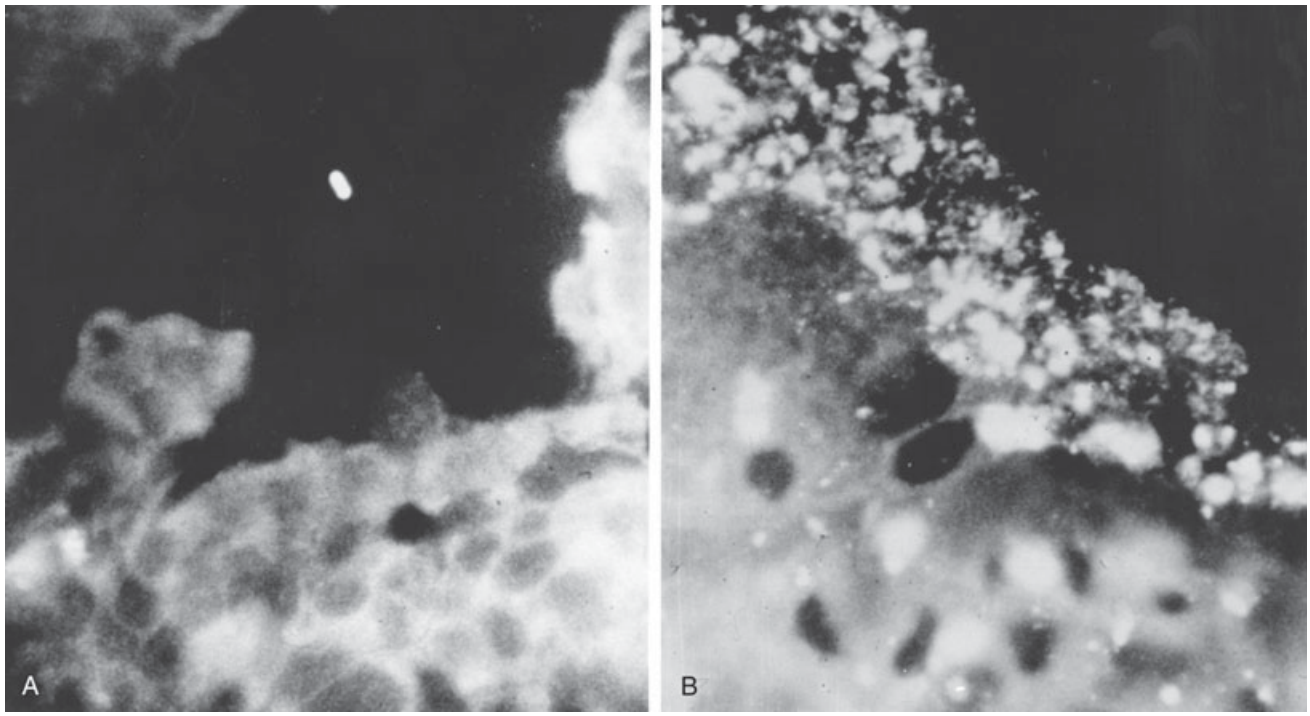

Figure 114–1 **A,** Laboratory-passaged avirulent *Escherichia coli* H10407 in infant rabbit intestine. No adherent, colonizing bacteria are seen. **B,** Fresh, virulent H10407 organisms at the same time interval in infant rabbit intestine are closely adherent to the brush border (indirect immunofluorescent stained section, ×1000). *(Courtesy of Drs. Dolores and Doyle Evans, Department of Microbiology, Baylor College of Medicine.)*

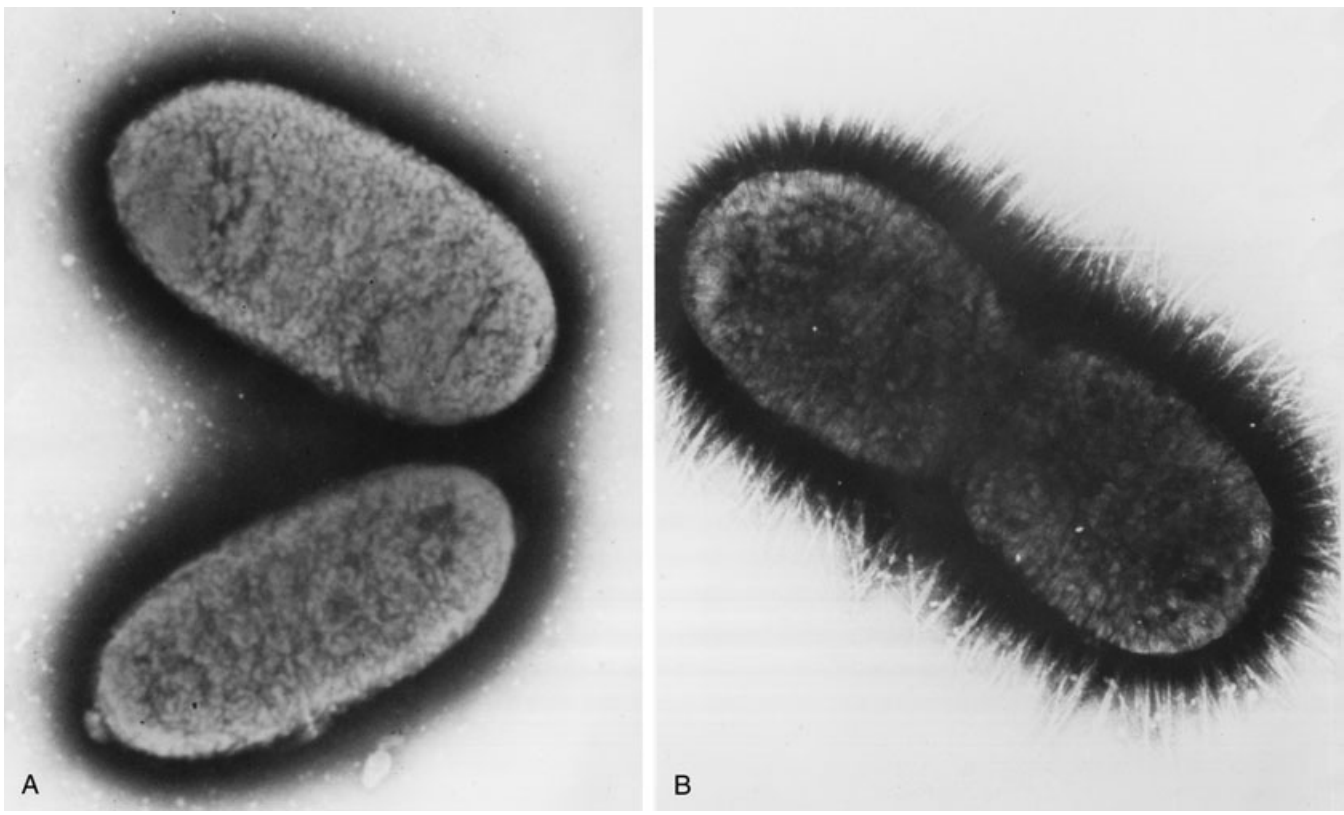

Figure 114–2 **A,** Electron micrograph of negatively stained cells of avirulent plasmid-cured *Escherichia coli* H10407. Note the bald appearance of the outer surface of the organism. **B,** Similar view of virulent, fresh *E. coli* H10407. Note the hairy surface of the organism as a result of CFA/I (×20,000). *(Courtesy of Drs. Dolores and Doyle Evans, Department of Microbiology, Baylor College of Medicine.)*

subunit is composed of two domains, termed A1 and A2, which are bound via a disulfide bond. A1 has an adenosine diphosphate–ribosyl transferase, which targets the cellular guanosine triphosphate binding protein G_s, a regulator of cellular cyclic adenosine monophosphate (cAMP) levels. Ribosylation of G_s leads to constitutive activation of adenylate cyclase, which greatly increases cAMP levels. Increased cAMP leads to increased phosphorylation of chloride channels, especially CFTR, by protein kinase A.[90,154]

ENTEROPATHOGENIC *ESCHERICHIA COLI*

EPEC pathogenesis is complex and only partly understood. In contrast to ETEC, it possesses no potent secretogenic toxin but rather induces changes to the enterocyte signal transduction environment, which transforms the enterocyte from an absorptive cell to a secretory cell. EPEC pathogenesis has been described as a multistep process. The first step comprises attachment to the small bowel mucosa, an event mediated by the EAF plasmid-encoded, bundle-forming pilus (BFP). BFP is a complex surface fimbrial structure that may undergo contraction, not only tethering the bacteria to the cell but also pulling itself close to the cell's apical membrane.[92] Approach of the bacteria is followed by induction of a characteristic lesion, called *attaching and effacing*. Under electron microscopy, the normal microvillus architecture is dissolved, and the bacterium adheres tightly (<20 nm distance) to the now undulating membrane. The bacteria may be observed sitting atop a cup or pedestal of protruding cell membrane (Fig. 114–3). This deceivingly simple phenotype is mediated and accompanied by a dizzying array of invisible changes to the cell's internal environment.[43]

Cellular changes are induced largely by a platoon of protein toxins that the bacterium injects directly into the cytoplasm of the target host cell. This injection process is mediated by a complex bacterial organelle termed an *injectisome*, also known as a *type III secretion system*. The first toxin injected is called the *translocated intimin receptor* (Tir). Tir inserts itself into the apical plasma membrane, where it serves as the receptor for the bacterial outer membrane adhesion protein called *intimin*. As its name suggests, intimin mediates very close attachment of the bacterium to the cell and contributes to the resulting cytoskeletal damage.

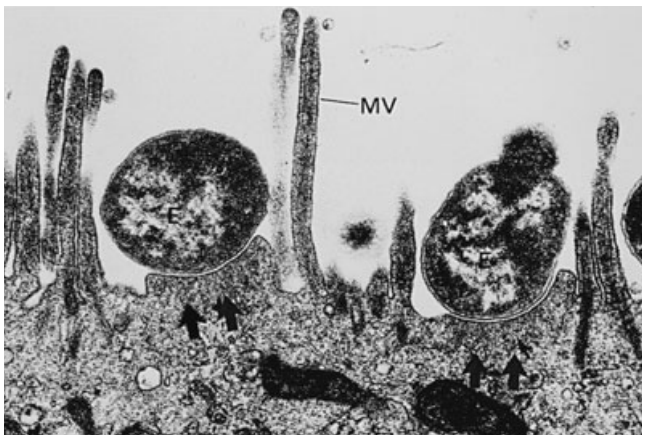

Figure 114–3 High-power electron micrograph of intestinal epithelium infected by typical enteropathogenic *Escherichia coli* strains *(E)*, with occasional intact microvilli *(MV)* and pedestal formation indicated by *double arrows*. This figure shows not only the structural alterations and loss of the brush border, but also the close apposition of the organisms to the epithelial cells. *(Courtesy of Ralph A. Gianella, Department of Medicine, University of Cincinnati School of Medicine.)*

The net effect of Tir, intimin, and other injected toxins is dissolution of the microfilament and microtubule networks, phosphorylation of membrane ion transporters, and disruption of tight junctions at the cell's periphery. Diarrhea apparently is caused by a combination of ion transport and paracellular leakage. Release of proinflammatory cytokines may complete the pathogenic cascade. The relative contributions of each of these events to EPEC disease are unknown.

EPEC adhere to HEp-2 epithelial cells in a characteristic localized pattern, which has been suggested as a diagnostic assay (Fig. 114–4).[43] Also, EPEC causes actin polymerization of target cells at sites of attachment, a virulence property readily detected in tissue culture (e.g., HEp-2 cells) by a fluorescent actin-staining test (Fig. 114–5).[91] As discussed subsequently, these phenotypic assays, although useful for pathogenesis studies, have largely been replaced by molecular diagnostic tests.

ENTEROHEMORRHAGIC *ESCHERICHIA COLI*

The cardinal pathogenic feature of the EHEC pathotype is the production of Stx, leading to the alternate name of Shiga-toxin–producing *E. coli*, accompanied by a cohort of accessory virulence factors. As with EPEC, these factors are encoded by the bacterial chromosome and a high-molecular-weight virulence plasmid. Stx is encoded on a lysogenic phage, which apparently is capable of infecting some nonpathogenic *E. coli* strains. The full package of virulence genes has been well characterized in serotype O157:H7; some evidence suggests that non-O157 pathogens share some, but not all, of the virulence traits, accounting for their reduced virulence and frequency. Stx toxins were discovered by Konowalchuk and associates,[93] who reported finding Vero cell toxic activity in culture filtrates of certain strains of *E. coli* isolated from patients with diarrhea (Stx occasionally has been referred to by the archaic synonym *Vero toxin*).

Stx toxins constitute a family of protein toxins that share identical enzymatic action and target cell binding specificity. Stx are subdivided into two families, Stx1 and Stx2, each consisting of the major Stx type and variants (i.e., Stx1c, Stx1d, Stx2c, Stx2c2, $Stx2d_{EH250}$, $Stx2d_{activatable}$, Stx2e, and Stx2f).[20] Stx2 and Stx2c have been associated with severe disease, including hemorrhagic colitis and HUS.[59,123] $Stx2d_{activatable}$ can be activated by mouse and human intestinal mucus and is associated with a severe clinical phenotype.[20] The greater pathogenicity of Stx2 may be due to greater accessibility of the active site on the Stx2 structure.[58] The *stx1* gene differs from the *Shigella stx* gene by three nucleotide changes, which result in a single conservative amino acid substitution in the A subunit: threonine to serine.[77]

Stx toxins have the same structure and mechanism of action. They have an A_1-B_5 stoichiometry, comprising a single enzymatically active A subunit and a pentamer of B subunits responsible for toxin binding. The B subunits bind to globotrioacyl ceramide (Gb_3) and related glycolipids on host cells, including epithelial enterocytes, vascular endothelial cells, smooth muscle cells, renal endothelial cells, and erythrocytes.[46,94] The catalytic A subunit cleaves the *N*-glycosidic bond in a specific adenosine of the 28S rRNA in the 60S ribosomal subunit.[49] This single cleavage event results in irreversible cessation of protein synthesis and ultimately leads to cell death via multiple signal transduction pathways.[34] Intestinal villus cells are susceptible to the action of Stx, which includes reduced absorption of sodium. Data suggest that Stx induces local inflammatory cytokine production, with potential effects on epithelial cell and mucosal integrity. This host response may induce a vicious cycle because both hydrogen peroxide and neutrophils augment production of Stx.[178]

Epidemiologic evidence strongly links Stx-producing strains to hemorrhagic colitis and to the associated systemic complica-

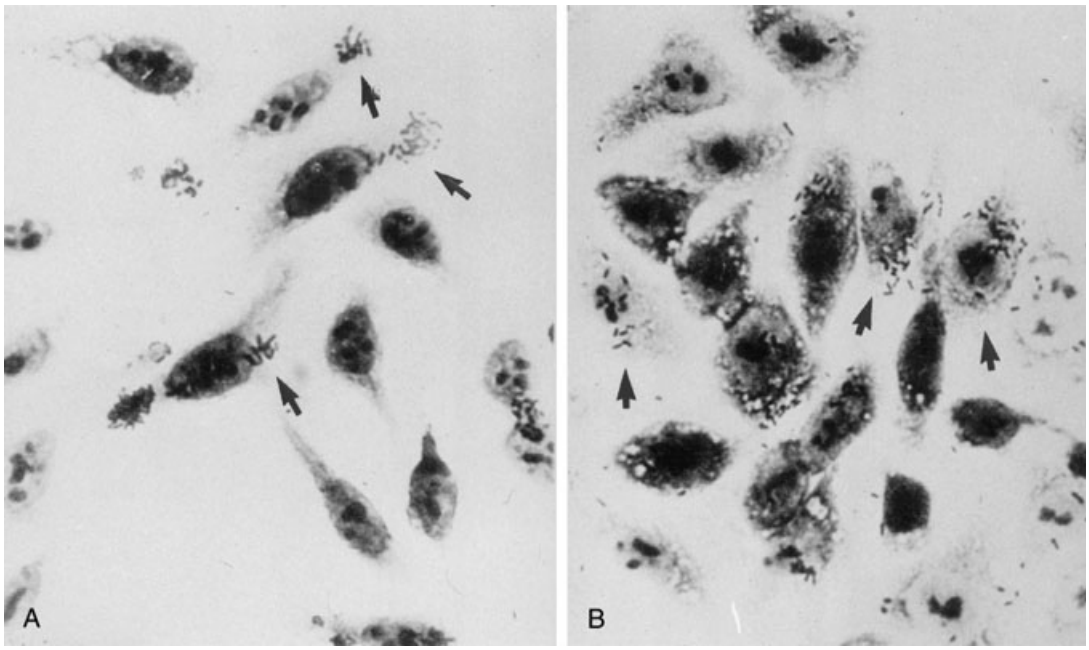

Figure 114–4 **A** and **B,** Patterns of adherence of *Escherichia coli* to HEp-2 cells in tissue culture. Typical enteropathogenic *E. coli* associates with HEp-2 cells in the pattern shown in **A,** termed localized adherence (LA). *Arrows* point to cells where growing microcolonies of bacteria are attached to focal areas of the cell membrane. Other *E. coli* organisms isolated from patients with diarrhea adhere over the entire HEp-2 cell membrane, as shown in the cells identified with *arrows* in **B**. This pattern of interaction is termed diffuse adherence (DA). Although the LA phenotype has been associated definitively with the capacity to induce diarrhea in experimental models, no convincing evidence of virulence in DA strains has been obtained to date.

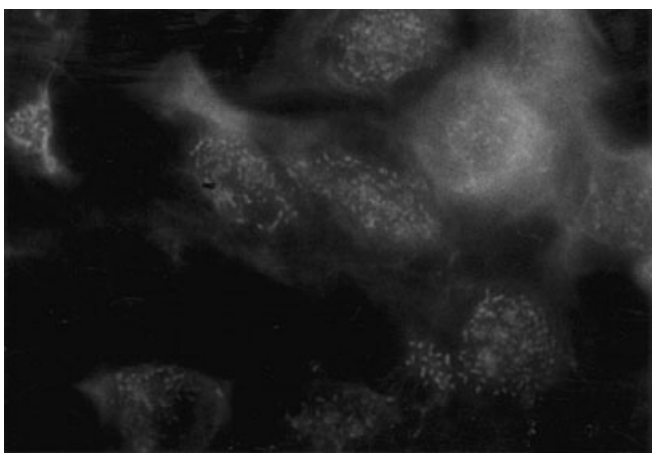

Figure 114–5 Some *Escherichia coli* strains have the capacity to attach to the eukaryotic cell membrane and induce polymerization of actin beneath the membrane. This property may be shown by the fluorescent actin staining test, in which polymerized F-actin is detected by a fluorescein-tagged mushroom toxin specific for this form of actin. In this figure, the bacteria are attached to HEp-2 cells, and the induced actin polymerization revealed by the fluorescent reagent highlights the organisms, which are seen as bright rods on the infected cells.

tions of HUS and TTP.[67,87,139] The mechanisms underlying HUS and TTP are uncertain but seem to be due to the effects of toxin on vascular endothelial cells, possibly in concert with lipopolysaccharide and a variety of cytokines, which initiate events resulting in endothelial cell injury and platelet thrombi and, subsequently, the characteristic thrombotic microangiopathy.[32,88,119] Other possible initiation factors include abnormal levels of von Willebrand factor, but whether this abnormality is a cause or a consequence of disease is unknown.[106] Stx1 has been shown to decrease production of prostacyclins; however, the role of this event in the pathogenesis of HUS is uncertain.[88]

Similar to EPEC, EHEC express the intimin outer membrane protein and a type III secretion apparatus.[111] EHEC are capable of inducing complex cytoskeletal alterations similar to those caused by EPEC infection, although certain subtle differences are observed. EHEC infects the human colon, whereas EPEC is an infection of the small bowel. The genes encoding intimin, Tir, the type III secretion system, and secreted proteins reside on a 35-kb pathogenicity island called the LEE, which may be more crucial for EPEC than for EHEC for virulence.[46] A 60-Md plasmid, pO157, which contains genes encoding an enterohemolysin, is commonly found in O157:H7 strains, but its role in production of disease is unclear.[111] Subtilase cytotoxin (SubAB), a novel cytotoxin with an A_1-B_5 stoichiometry, was described more recently and associated with HUS in diverse EHEC strains in Australia and the United States.[90,127] SubAB is heat-stable, more toxic to vero cells than Stx, and lethal when injected in mice.[90,127] Cytotoxicity is the result of direct cleavage of an endoplasmic reticulum chaperone protein, BiP.[126]

ENTEROINVASIVE *ESCHERICHIA COLI*

In 1967, Trabulsi and coworkers[171] in Brazil and Sakazaki and associates[147] in Japan described the isolation of certain *E. coli* serotypes from patients with a disease resembling bacillary dysentery, but with cultures negative for *Shigella*. These isolates possessed a critical virulence hallmark associated with *Shigella*—the ability to invade intestinal and other epithelial cells (Fig. 114–6).[119] They have been called EIEC, and a limited number of serotypes, distinct from the EPEC O groups, but often cross-reactive with *Shigella* O antigens, have been found to possess this property (see Table 114–1). The genetic and molecular basis of

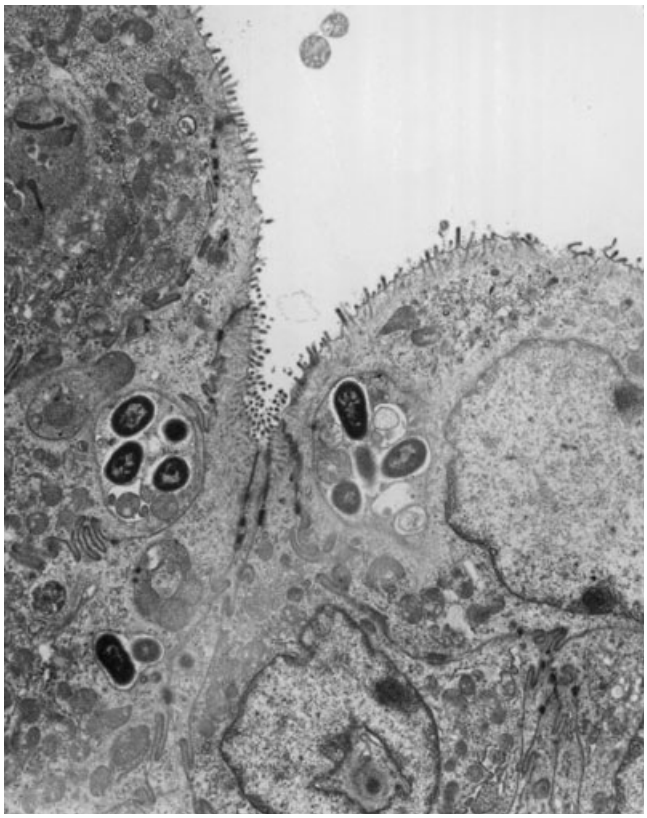

Figure 114–6 Electron micrograph of enteroinvasive *Escherichia coli* infection of intestinal mucosa. The intracellular location of the bacteria clearly is seen within membrane-bound vesicles in two adjacent infected cells. The pathogenesis of enteroinvasive *E. coli* infection involves the invasion of intestinal cells and local cell-to-cell spread, as shown here, by a mechanism identical to that of *Shigella* spp. *(Courtesy of Saul Tzipori, D.V.M., Department of Comparative Medicine, Tufts University School of Veterinary Medicine.)*

invasion by *Shigella* was well defined in the 1990s, and the same plasmid and chromosomal genes encoding invasion properties and mechanisms seem to be present in EIEC as well.[78,98,125,149]

The invasive process for EIEC is thought to be the same as that for *Shigella* spp. It has been well characterized and involves four main steps: (1) initial entry into cells, (2) intracellular multiplication, (3) intracellular and intercellular spread, and (4) host-cell killing. The process is complex and involves multiple genes on the invasion plasmid and the chromosome.[1] EIEC produces toxins reported to be structurally distinct from the Stx of *Shigella dysenteriae* type 1 and the Stx1 and Stx2 of EHEC.[55] Nonetheless, studies suggest that EIEC toxins possess many properties in common with the Stx family of toxins, including the ability to cause fluid secretion in animal models.[55] EIEC may contain a plasmid-borne gene that encodes a 63-kd protein called *ShET2* (Shigella enterotoxin 2); a mutation in this gene substantially decreases the enterotoxic activity of the parent strain.[114]

ENTEROAGGREGATIVE *ESCHERICHIA COLI*

The pathogenesis of EAEC includes adherence to the intestinal epithelium as a thick biofilm, accompanied by mucus hypersecretion, intestinal damage, and induction of an inflammatory response. Most of these phenotypes seem to be mediated by a set of bacterial cytotoxins and enterotoxins.[73]

Adherence and colonization of the intestinal epithelium are mediated by pili called *aggregative adherence fimbriae*, of which several allelic variants exist. EAEC may colonize the large and small intestines.[76] All known aggregative adherence fimbriae variants are encoded on high-molecular-weight virulence plasmids collectively termed *pAA*. Also encoded on pAA is a transcriptional activator of the fimbrial genes, called *AggR*. AggR is emerging as a global regulator of virulence functions in EAEC; also under AggR control is a gene encoding a surface protein nicknamed *dispersin*. In the absence of dispersin, the aggregative adherence fimbriae adhesins collapse onto the surface of the bacterium, rendering it unable to colonize the intestine (J. Nataro, unpublished). Several cryptic genes, including a large chromosomal locus, also are under AggR control.

Damage to the intestinal mucosa has been linked to the presence of a secreted enterotoxin called *Pet* (plasmid-encoded toxin).[52] Pet is a protease that is internalized by target epithelial cells, where it cleaves cytoskeletal proteins including spectrin. The net effect of these cleavage events is induction of fluid, secretion of electrolytes, and, ultimately, exfoliation of cells from the mucosal surface.[75]

EAEC strains also harbor a homologue of the ETEC heat-stable toxin, enteroaggregative ST,[151] and a chromosomally encoded toxin called *Shigella* enterotoxin 1.[56] Inflammation by EAEC has been linked to the expression of the bacterial flagellum, although studies in polarized epithelial monolayers suggest that additional factors under control of AggR also are required.[73]

DIAGNOSIS AND DIFFERENTIAL DIAGNOSIS

Diarrheogenic *E. coli* can be identified best by detecting their defining virulence genes. Although many molecular tests are described for this purpose, few laboratories in the United States use them. For most pathotypes, consultation with an expert, such as the senior author or the *E. coli* reference laboratories at the U.S. Centers for Disease Control and Prevention, is recommended. Detection of *E. coli* generally is indicated for severe and persistent diarrhea lacking any other diagnosis (particularly in returning travelers), or in the setting of diarrheal outbreaks of unknown cause. With the exception of EHEC, most infections caused by diarrheogenic pathotypes are self-limiting.

Because of the potential of EHEC to cause serious sequelae, establishing a specific diagnosis is of clinical and epidemiologic importance. The earlier in the course of illness that a stool specimen is obtained, the more likely that *E. coli* O157:H7 will be recovered[166]; recovery more than 7 days after onset of diarrhea is unusual. The laboratory can use sorbitol-MacConkey (SMAC) agar to screen colonies for lack of sorbitol fermentation or substrates such as 4-methylumbelliferyl-β-D-glucuronide (MUG) for the production of β-glucuronidase; *E. coli* O157:H7 is almost uniquely sorbitol-negative and glucuronidase-negative. These techniques have limited sensitivity, with the sensitivity of SMAC agar reported as 50 to 60 percent.[89] Some sorbitol-fermenting O157:H7 isolates and many other sorbitol-fermenting, non-O157 EHEC are capable of causing severe illness and HUS that are missed by sorbitol-MacConkey agar.

Detecting *Shiga* toxin in stool specimens, either directly or after overnight broth enrichment, currently is the most sensitive nonmolecular method for identifying EHEC.[89] These tests are more sensitive than are culture techniques and are not dependent on serotype. Commercial enzyme immunoassays for Stx have been approved by the U.S. Food and Drug Administration for either confirmation of isolates as toxin producers or rapid direct diagnosis based on the detection of Stx1 or Stx2 in stool.[17,110] Despite the detection of *Shiga* toxin by these rapid assays, isolation of the organism, usually performed at reference laboratories, remains crucial for serotyping and other epidemiologic purposes.

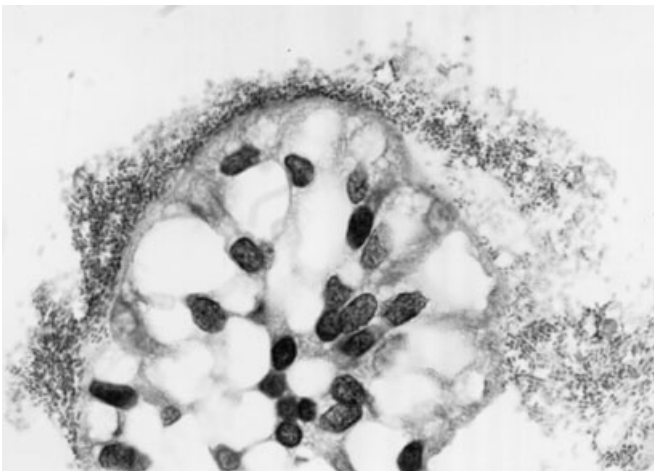

Figure 114–7 Enteroaggregative *Escherichia coli* (EAEC) infection of the gnotobiotic pig intestine. This photomicrograph shows the characteristic "stacked-brick" appearance of the aggregative organisms over the surface of the intestinal epithelium in vivo in the same manner described for EAEC adherence to HEp-2 cells in tissue culture. *(Courtesy of Saul Tzipori, D.V.M., Department of Comparative Medicine, Tufts University School of Veterinary Medicine.)*

Serotype-specific commercial antigen immunoassays also are available.[110]

Diagnosis of ETEC would require identifying the LT or ST genes in an isolate or, after isolation and growth in vitro, detecting their products by enzyme-linked immunosorbent assay. Polymerase chain reaction techniques are available that allow LT or ST genes to be detected directly from stool without the need for performing culture.[26] A retrospective diagnosis can be made by detecting an increase in antitoxin antibody, especially for LT. Enzyme-linked immunosorbent assay for CFA antigens of ETEC also could be useful.

EPEC can be detected reliably by DNA probes for the 60-Md EAF adherence plasmid or by adherence to cells in culture. The ability of *E. coli* to adhere to HEp-2 cells in culture in distinct morphologies led to the description of EPEC, EAEC, and DAEC as different pathotypes (see Fig. 114–3). After a 3-hour incubation of bacteria with cells, EPEC adheres to the cell in tight microcolonies, called the *localized adherence* pattern. Although it remains sensitive and specific for EPEC, this method has been replaced by molecular detection methods to detect the LEE island (via the intimin-encoding gene *eae*) and the EAF plasmid (often via detection of the BFP-encoding genes).[44]

EAEC can be detected by the presence of the aggregative adherence pattern in the Hep-2 adherence assay (Fig. 114–7). DNA probes and polymerase chain reaction are available to detect the AggR regulon, and these tests are likely to provide more specific detection of EAEC pathogens.[33]

EIEC is detected best using molecular techniques that are specific for detection of pathogenesis-related genes. Several multiplex polymerase chain reaction assays that allow the user to screen for multiple *E. coli* pathotypes simultaneously have been described.[13,128] They can be introduced readily into any molecular biology laboratory and produce excellent results.

PROGNOSIS

EPEC, EAEC, and ETEC do not cause systemic infections or complications except complications resulting from dehydration or the consequences of nutritional depletion. The prognosis is related directly to the availability and adequacy of fluid therapy. When this need is dealt with correctly in an otherwise healthy and well-nourished patient, the principal complication is the rare instance of monosaccharide intolerance. In infants or young children in developing countries with protein-energy malnutrition, however, chronic diarrhea and progressive worsening of nutritional status frequently are observed, sometimes resulting in mortality. When food is withheld from either well-nourished or poorly nourished children, hypoglycemia may occur and produce seizures, coma, or death. Rarely, loss of water in excess of salt causes hypertonic dehydration, with serum sodium concentrations greater than 160 mEq/L, a situation that may cause seizures, coma, and death.

As noted earlier, EAEC has been associated with persistent diarrhea lasting longer than 14 days in young infants and children. Persistent diarrhea leads to nutritional deterioration, may be difficult to control, and may culminate in death from sepsis or other infections. Inflammatory diarrhea caused by EIEC also results in nutritional deterioration, with significant protein losses occurring via the gut.

EHEC often is followed by HUS, neurologic and intestinal complications, and, especially in adults, TTP. Ten percent of patients may die early in the course of the systemic phase of either HUS or TTP. Although the renal failure of HUS generally is reversible with good management of fluid and electrolytes and the use of dialysis as needed, permanent damage to the kidneys is, contrary to earlier more optimistic assessments, likely to occur in 25 percent or more of patients over the course of 1 or 2 decades, and many of them require permanent dialysis or transplantation in the future.[61] The prognosis in TTP is related directly to initiation of plasmapheresis, which has been documented to reduce mortality rates significantly.[177] No such benefit has been noted in HUS.[177]

TREATMENT

In all age groups, the principal treatment of the intestinal manifestations of *E. coli* enteric infection is replacement of fluids and electrolytes; with maintenance of fluid balance, the disease is self-limited to 1 week or less in most patients and lasts no more than 2 weeks in nearly all patients. The earlier that fluid replacement therapy is begun, the better the prognosis, particularly when one considers that clinical signs of dehydration do not develop until a 5 percent loss of body weight occurs, and that sustained loss of more than 10 percent of body weight is incompatible with survival.

When shock is present (usually with altered consciousness and an absent or thready pulse) or oral rehydration is unsuccessful because of persistent vomiting, patients must be rehydrated by intravenous infusion of an isotonic electrolyte solution, such as lactated Ringer solution or normal saline. Three 20 mL/kg fluid boluses can be given back-to-back, with close monitoring of the patient's clinical status. When dehydration is less severe, initial replacement of losses usually can be accomplished by oral rehydration. Patients not in shock, who may have normal findings on physical examination or may manifest poor skin turgor, tachycardia, postural hypotension, and oliguria (along with irritability and a sunken fontanelle in the very young), should receive 50 to 120 mL/kg of fluid, depending on the severity of dehydration, as rapidly as they can be encouraged to drink, over 4 hours. Pulse, blood pressure, urine volume, skin turgor, general appearance, and thirst are monitored as indicators of response. Adults may need 1 L/hr to establish rehydration; adults as well as children may tire of drinking and fail to keep up with requirements.

Several prerequisites must be ensured for oral therapy: (1) the patient must not be in shock; (2) the patient must be fully conscious; (3) the patient must be able to drink (vomiting, par-

ticularly common in children, is not an absolute contraindication because frequent small oral feedings usually are largely retained; when the metabolic abnormality begins to reverse toward normal, vomiting ceases); (4) bowel sounds must be present; and (5) renal function must be normal. Current recommendations in the United States and Europe suggest the use of hypotonic fluid containing 30 to 60 mEq of sodium per liter, although specific attention given to sodium and potassium deficits may be required. In the developing world, where cholera is a common occurrence, the World Health Organization recommends that a solution containing 90 mEq of sodium be used for all cases of diarrhea because of the large sodium losses that occur in cholera, and the desire to avoid the need to choose among formulations because of difficulty in making an etiologic diagnosis. Rice-based solutions for oral rehydration currently are favored in many diarrheal centers because of their reduced osmotic load.[5]

A prospective cohort study comparing *E. coli* O157:H7 HUS patients who developed oligoanuric renal failure requiring dialysis with patients with non-oligoanuric renal failure found that the patients who required dialysis had received less intravascular volume expansion and sodium than had the patients who did not require dialysis, suggesting that intravascular volume expansion with isotonic fluid was protective for oligoanuric renal failure when given early in the course of O157 infection.[4] The authors advocate hospitalizing all children with suspected EHEC infection for volume expansion with isotonic fluids and close monitoring.

Although rehydration therapy is the cornerstone of management, antibiotic therapy should be considered in some cases. In most *E. coli*–associated diarrheal illnesses, the disease is mild and of short duration, and no specific antimicrobial therapy is required. Studies to address this issue have found antibiotics to be beneficial in some circumstances. In traveler's diarrhea secondary to ETEC, antibiotic therapy can shorten the duration of illness and decrease its severity.[8,51] Prophylactic antibiotic therapy probably carries more risk than benefit, however, and generally is not recommended. When antibiotics are used, trimethoprim-sulfamethoxazole (TMP-SMX) therapy presently is recommended for children with traveler's diarrhea caused by susceptible isolates; fluoroquinolones are the drugs of choice for adults when chemoprophylaxis is indicated.[137] A more recently approved antibiotic for adults, rifaximin, is not absorbed from the GI tract and shows promise as an effective treatment of ETEC.[50] The data in children are very limited, however, and use of rifaximin is not yet recommended in this population.

Children in developing countries with ETEC diarrhea also may benefit from receiving antibiotic therapy.[118] Resistance of ETEC to TMP-SMX was noted in U.S. troops in Saudi Arabia, where 44 percent of isolates were resistant.[121] EIEC infection theoretically would benefit even more from antibiotic therapy, given its pathogenic similarity to shigellosis and the known benefits of the early use of antibiotics. No controlled studies have validated this benefit for EIEC, however. In experimentally induced disease, DuPont and colleagues[47] reported that parenteral ampicillin produced a bacteriologic cure and rapid clinical response with defervescence and improvement of diarrhea in adults. TMP-SMX and ampicillin are the current drugs of choice, unless resistance is a problem.

Immunocompromised hosts, especially children with AIDS, may require prolonged antibiotic therapy for protracted or recrudescent diarrhea, even when it is caused by bacteria that normally produce only self-limited disease. Malnourished children and children with other serious underlying illness also fit this category. In these patients, systemic invasion may develop along with associated complications, including shock and renal failure. In addition, prolonged carrier states are common findings, with frequent relapses requiring long-term antibiotic therapy for suppression of relapse.

Epidemic EPEC infection, especially in newborns, seems to be affected favorably by antibiotic therapy.[115] The potential for this pathogen to cause prolonged disease and a history of high rates of mortality in neonates suggest the need for antibiotic trials in this age group. Based on limited data, either TMP-SMX or oral nonabsorbable antibiotics, such as gentamicin or colistin, usually are recommended, although antibiotic resistance is common and may alter the choice of antibiotics based on local susceptibility patterns.[14,176]

Antibiotic treatment of EHEC remains controversial but is not recommended. Antibiotics in clinical use do not improve the course or prevent sequelae, and they may exacerbate the illness by promoting release of Stx.[6,21,30] In vitro data suggest that subinhibitory concentrations of TMP-SMX and other antibiotics may increase expression of Stx,[86] perhaps related to enhanced release of toxin from damaged organisms.[14] The most rigorous prospective study to address this question found that the cohort who received antibiotics had a higher incidence of HUS (of 9 patients who received antibiotics, 5 developed HUS) than the cohort who did not (of 62 patients who did not receive antibiotics, 5 developed HUS; $p < 0.001$).[181] Other studies have found no association.[122]

Notably, no studies have used a randomized placebo-controlled design, so the association may reflect selection bias because both the use of antibiotics and systemic complications are associated with more severe disease. A meta-analysis[146] did not show a higher risk of HUS associated with antibiotics; however, this meta-analysis has been criticized because it included a study in which all of the patients received antibiotics[81] and because comparisons were made among different antibiotics, which may not induce similar sequelae.

Antimotility agents such as loperamide generally are not needed and should be used cautiously if prescribed, with great attention paid to dosage, especially in very young patients.[167] Dysentery is a contraindication to the use of antimotility agents, which may be a risk factor for the development of ileus and abdominal distention.[3] The prolonged use of antimotility agents is reported to be associated with more serious systemic complications of EHEC infection and is not recommended.[30,31]

In most instances of *E. coli* diarrhea, the clinician is left to make therapeutic decisions without knowing the etiologic agent responsible. For reasons already outlined, routine diagnostic microbiology laboratories cannot distinguish pathogenic from nonpathogenic *E. coli*, with the exception of classic EPEC serotypes and O157:H7 EHEC. Clinical decisions regarding the administration of antibiotic therapy are made on purely clinical grounds with criteria such as the history, duration, and severity of the illness; the age and immunologic competence of the patient; and the nature of the diarrheal stool (e.g., watery, inflammatory, bloody, or dysenteric). Empiric antimicrobial therapy is more justifiable for immunocompromised hosts, for patients with prolonged or severe illness or a history of relevant risk factors (e.g., specific food ingestion, travel, exposure to known contacts), and for cases of inflammatory or dysenteric illness.

A novel treatment approach was reported by DiCesare and associates,[42] who evaluated SP-303, a plant-derived product with antisecretory properties, in a randomized placebo-controlled study of acute diarrhea in 184 U.S. travelers to Mexico and Jamaica. SP-303 shortened the duration of traveler's diarrhea by 21 percent; ETEC was the etiologic agent of traveler's diarrhea in 19 percent of the subjects. Human breast milk contains oligosaccharides that can partially bind to ETEC and presumably block attachment to intestinal epithelial cells.[102] Bovine hyperimmune milk from cows immunized against ETEC and EPEC is not effective treatment or prophylaxis for ETEC or EPEC diarrhea.[29,164]

Research on alternative treatments of EHEC and prevention of HUS is ongoing. A synthetic verotoxin receptor, SYNSORB

Pk, was not shown to prevent death, dialysis, or serious extrarenal events in a trial of 145 children with EHEC-induced HUS.[175] Although antibodies against *E. coli* O157 intimin were protective for colonization and disease in a pig model,[41] plasmapheresis and intravenous immunoglobulin have not been shown to ameliorate HUS in humans.[141] Investigation of monoclonal antibodies directed against Stx are under way.[175] The complete genome sequence of *E. coli* O157:H7 is now known and may lead to greater understanding of the pathogenesis of hemorrhagic colitis and HUS and perhaps innovative approaches to treatment and prevention.[74]

PREVENTION

Epidemic nursery outbreaks of diarrhea in newborns can be controlled by the application of antiquated but still valid principles of preventive medicine. Prompt diagnosis and treatment and scrupulous attention given to details of handwashing and environmental sanitation to eliminate person-to-person transmission are still effective, whereas prophylactic antimicrobials have no role. Outbreaks in neonatal nurseries can be contained by epidemiologic control measures such as cohorting; by screening staff for carriage; and, if necessary, by closing the unit until it is decontaminated. In contrast, preventing sporadic cases of *E. coli* diarrhea is difficult. In communities with obvious deficits in water supply and feces disposal, correction of these problems would lead to a diminished incidence of diarrheal diseases in general.

For traveler's diarrhea, several studies indicate that prophylactic antibiotics can protect adult travelers, at least for a limited time.[70] The risk of selection of resistant organisms and drug side effects limits the use of antibiotic prophylaxis to short-term travelers with business or diplomatic missions that would be hindered significantly by an episode of diarrhea. Fluoroquinolones, tetracyclines, azithromycin, and rifaximin have been evaluated for prophylaxis and presumptive treatment, and all are efficacious. The use of tetracyclines is not recommended for children younger than 8 years, and toxicity concerns for fluoroquinolones are still present.[6] Some evidence has been presented for the efficacy of bismuth compounds, such as bismuth subsalicylate, for the prevention of ETEC diarrhea in adults. The concern for bismuth toxicity with prolonged use in young children would render it a problematic solution for pediatric *E. coli* diarrhea and its prevention.

ETEC vaccine development has been propelled by the observation that ETEC infection confers some protection from reinfection with the same strain,[162] also evident in the decreasing rates of infection in children older than 5 years.[132] The challenge is that greater than 25 CFAs have been identified, and strain serotypes vary tremendously across the world.[64] As noted previously, however, it also is known that certain serotypes and CFAs are most common (CFA I, II, and IV) and tend to co-segregate.[60,64,180] This factor would limit the antigenic variability needed in an effective vaccine. Most isolates of ETEC expressing only LT do not express fimbria colonization factors and would be unaffected by a CFA-based vaccine. Although no vaccine currently is licensed and available, several approaches have been attempted. Among the most successful to date is a vaccine composed of recombinant cholera toxin B (as a surrogate for the LTB toxin subunit) with four strains of formalin-killed ETEC expressing the most prevalent CFAs in developing countries. This vaccine was found to prevent 80 percent of ST ETEC diarrhea in travelers to developing countries.[132] It is available commercially in Europe and may provide limited, short-term protection in travelers.[96] In a study of Egyptian children, however, it was not efficacious in preventing disease.[23,132]

Other avenues of vaccine development that have been pursued include subunit vaccines. ST does not create a significant immune response; however, LT is a known adjuvant similar to cholera toxin and has been conjugated to ST.[23,64] Concerns for human use of this vaccine come, however, from the homology of ST to guanylin, which is produced endogenously.[23,39] Live, attenuated *Shigella* vaccine vectors also are being studied in an attempt to elicit a mucosal immune response. Researchers are studying new methods of delivering antigens to the mucosa, including encapsulation in microspheres and delivery in recombinant foods. A transcutaneous vaccine, composed of fimbrial antigens mixed with LT, has been shown to elicit serum antibodies in adult volunteers.[64] Work continues in this field in an attempt to decrease the incidence and severity of morbidity from dysentery in the developing world and from traveler's diarrhea.

Vaccine development for other diarrheogenic *E. coli* strains lags behind that for ETEC. Because EHEC-induced HUS is a rare disease, widespread human vaccination may not be cost-effective. Vaccine development for cattle, the major reservoir, is ongoing. A vaccine composed of type III secreted proteins resulted in decreased shedding of *E. coli* O157:H7 compared with controls, although prevalence of shedding increased over the study period. Repeat vaccination of cattle may serve as a mode to decrease human exposure to EHEC.[129]

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CHAPTER 115

KLEBSIELLA

Randall G. Fisher

Klebsiella is a genus of Enterobacteriaceae, a frequent cause of nosocomial pediatric infection. Classically described by Friedländer[38] as a cause of pneumonia, *Klebsiella* can cause infections of the urinary tract, lung, and central venous catheters in high-risk newborns and immunocompromised older children.[18]

BACTERIOLOGY

Klebsiella organisms were named for Edwin Klebs, the noted German bacteriologist.[86] Distinguishing features of *Klebsiella* spp. include the absence of motility and the presence of a polysaccharide capsule that gives rise to large mucoid colonies on solid media. The organisms are oxidase-negative and citrate-positive; they ferment inositol and hydrolyze urea but do not produce ornithine decarboxylase or hydrogen sulfide. Acetoin and 2,3-butanediol predominate over acidic end-products during sugar fermentation (positive result on the Voges-Proskauer test). Four species of *Klebsiella* commonly are agreed on by microbiologists: *Klebsiella pneumoniae* (the most common human pathogen), *Klebsiella oxytoca* (a less common human pathogen), *Klebsiella terrigena*, and *Klebsiella planticola*. Previously, *K. planticola* was recovered almost exclusively from soil and aquatic environments; reports now suggest that this organism may be a relatively common neonatal pathogen in some parts of the world.[92,113] *K. planticola* may express virulence factors similar to those of *K. pneumoniae*.[93] Organisms are defined serologically by their capsular polysaccharide (K antigens) and lipopolysaccharide (O antigens). Significant cross-reactivity exists between the capsule of some pneumococci (e.g., 19F) and *Klebsiella*.[68] The reader is referred to a review by Podschun and Ullman[95] for a detailed description of *Klebsiella* spp.

EPIDEMIOLOGY

Friedländer[38] proposed that *K. pneumoniae* was the most common cause of community-acquired pneumonia, an observation that was refuted by Fraenkel's[36] observations on pneumococcal pneumonia. *K. pneumoniae* accounts for less than 10 percent of hospitalized cases of pneumonia in adults.[20] *Klebsiella* spp. now are in greatest evidence as opportunistic nosocomial pathogens of the urinary tract, respiratory tract, biliary tract, and bloodstream. In one survey of the Centers for Disease Control and Prevention, the infection rate of nosocomial *K. pneumoniae* was 16.7 infections per 10,000 patients discharged.[54] Hand-carriage generally is regarded as the common mode of transmission.[42] Environmental sources of *Klebsiella* spp. include contaminated blood-pressure monitoring equipment,[98] ventilator traps,[42] dialysate,[64] ultrasound coupling gel,[40] dextrose solution,[66] and hand disinfectant.[101] The emergence of plasmid-mediated, β-lactamase resistance can be responsible for the rapid spread of resistant organisms to susceptible patients in intensive care units (ICUs).[10,12] Outbreaks may be complex; patient-to-patient transmission of epidemic strains containing different plasmids may be interspersed with sporadic, nonepidemic *Klebsiella* infections.[12] *Klebsiella* spp. are second only to *Escherichia coli* as causes of sepsis,[41] with the highest rates of infection being reported from larger hospitals affiliated with medical schools.

Klebsiella spp. commonly are highlighted as pathogens of debilitated adults and alcoholics,[60] but by 1985, nearly 50 percent of reported outbreaks of *Klebsiella* were in neonatal ICUs.[54] Outbreaks in newborns continue to occur frequently worldwide.[2,7,24,30,49,90,100] Most outbreaks in newborns have been associated with *K. pneumoniae* infection, but scattered outbreaks of *K. oxytoca* infection in nurseries also have been reported.[6,112] A high percentage of infants in ICUs may become colonized with hospital strains of *Klebsiella*.[46] In one longitudinal study in which weekly rectal swabs were cultured, 80 (22%) of 368 neonates in an ICU harbored extended-spectrum β-lactamase (ESBL)–producing *Klebsiella* spp.[15] Infecting organisms have been isolated from care providers and from mothers of colonized infants.[24] One report described an outbreak among newborns associated with infestation of a neonatal unit by cockroaches colonized with infecting *Klebsiella* strains.[25] *Klebsiella* may spread from newborn units to adult units; interhospital and international spread of resistant strains has been described.[23,30,106]

Ribotyping, pulsed field gel electrophoresis, and DNA amplification techniques have proven valuable in characterizing *Klebsiella* strains associated with outbreaks.[70,108] Different ribotypes that share plasmids conferring antibiotic resistance can be responsible for pediatric infections in a particular institution.[12] Strains expressing ESBL may become endemic and may present a complex and diverse pattern of production of enzymes with resistance to β-lactamase inhibitors.[31,35] Although broad-spectrum resistance to β-lactams and carbapenems[72] has been described, some longitudinal studies have shown that the frequency of ESBLs in *K. pneumoniae* isolates is decreasing.[103]

PATHOPHYSIOLOGY

Pneumonias caused by *Klebsiella* most commonly arise from colonization of the upper respiratory tract, followed by aspiration of organisms to the lower respiratory tract. Some degree of gram-negative oropharyngeal colonization is a normal finding in newborns. The oropharynx of nearly one third of healthy newborns is colonized by gram-negative rods, including *Klebsiella*, by the time infants reach 1 month of age; colonization rates generally are lower in breast-fed infants.[9] Antibiotic pressure in high-risk newborns and older children has been observed to promote overgrowth of *Klebsiella*.[11,105] Enteric organisms are recovered less frequently from the oropharynx of healthy older children and adults; oral colonization with gram-negative rods is increased during illness,[57] after postoperative viral infections,[56,97] and in debilitated adults.[73] Increased adherence of gram-negative rods to oropharyngeal cells contributes to increased colonization.[56] Elastase made by polymorphonuclear cells contributes to such colonization by reducing the fibronectin coating of sugar receptors.[27] The capsule plays an initial role in interactions of epithelial cells but is not required for an adhesin interaction with the cell surface.[34] Adherence properties may be affected by plasmid content[29] and may be transferred between *E. coli* and *K. pneumoniae*.[53]

In animal models of sepsis, capsular polysaccharide (K antigens) is a virulence factor; monoclonal antibodies to the K antigens reduce the severity of illness in mice.[67] In a mouse model of urinary tract infection, the K antigens seemed to be more important in infection than was the lipopolysaccharide (O antigens), and clinical strains deficient in lipopolysaccharide retained virulence by resistance of capsule to complement.[3,19] In one series of adult patients, capsular type K2 frequently was associated with

asymptomatic bacteriuria and cystitis, but not pyelonephritis; the presence of type 1 fimbriae bore a closer relationship to upper urinary tract infection.[94] In a retrospective study, patients bacteremic with hyperviscous strains were more likely to develop invasive localized disease, such as liver abscess, meningitis, pleural empyema, or endophthalmitis.[69] Neutrophils play an important role in clearance of *Klebsiella*, and phagocytosis is augmented by leukotrienes.[74]

CLINICAL MANIFESTATIONS

Klebsiella infection shows little clinical distinction from diseases produced by other enteric pathogens. The organism generally is less common than is group B streptococcus or *E. coli* as a cause of "early-onset" or "late-onset" infection in newborns.[43,77] Investigators from Spain,[49] however, reported one 7-year interval in which *K. pneumoniae* was the most common cause of bacteremia in newborns. Risk factors for neonatal *Klebsiella* infection include prematurity, presence of indwelling catheters, previous antibiotic treatment, and parenteral nutrition.[100] Infection in newborns is characterized by typical features of pneumonia, sepsis, and meningitis.[77] In one unusual case, *K. pneumoniae* bacteremia manifested on day 4 of life as a morbilliform maculopapular exanthem, more severe on the face. The infant was afebrile, but the mother had had fever in the peripartum period, and the amniotic fluid had been meconium stained.[21]

Although hepatic abscesses have been associated principally with adults with poorly controlled diabetes mellitus, one case report described a 32-week-gestation neonate with *K. pneumoniae* sepsis who developed a liver abscess large enough to be noted on physical examination as an abdominal "lump."[110] In adults, hepatic abscess occasionally is complicated by endophthalmitis or central nervous system infection, with devastating outcomes. A study from Taiwan found that infection with genotype K1 was the only significant risk factor for these complications.[33] This series also revealed that 8 (42%) of the 19 complicated K1 strain infections occurred in patients without identifiable underlying medical disease.[33] Other investigators have emphasized that serotype K2 also can be virulent in patients with liver abscess.[39] Careful study of the pathophysiology and epidemiology of complicated *K. pneumoniae* liver abscesses is ongoing, and the picture is likely to continue to be clarified.

Klebsiella spp. have been isolated commonly from blood and peritoneal fluid in outbreaks of necrotizing enterocolitis.[44,80] Less common manifestations in infants include toxic epidermal necrolysis,[47,89] conjunctivitis,[65] parotitis,[22] retropharyngeal abscess,[26] subdural hematoma,[84] psoas abscess,[4] and renal abscess.[111]

Klebsiella is an unusual cause of infection in an otherwise healthy older child. The classic Friedländer pneumonia that occurs in debilitated adults[38] is rare in children. The identification of pulmonary infection should suggest the possibility of underlying immunodeficiency or significant malnutrition, if not suspected previously.[52,58] If pneumonia caused by *Klebsiella* does occur, progression to lung abscesses should be anticipated.

Lung abscesses may develop within days to weeks after *Klebsiella* infection. Formation of abscesses occurs more frequently during *Klebsiella* pulmonary infection than during any other community-acquired infection.[20] A rare but devastating outcome is massive pulmonary gangrene, the rapid total destruction of part of the lung presumed to be due to vascular compromise. This complication is heralded by radiographs that show small cavities that later coalesce into a large cavity with an intracavitary mass of necrotic lung.[82,85] Some researchers have speculated that *Klebsiella* lung infection is accompanied by coincident anaerobic infection that contributes to or is primarily responsible for the pathology.[20]

Catheterization of the urinary tract can be associated with urinary tract *Klebsiella* infection, but bacteremia is an uncommon complication in an immunocompetent child.[28,71] Approximately 10 percent of nosocomial urinary tract infections observed in infants after surgery are caused by *Klebsiella*.[28] Focal renal infection progressing to renal abscess has been described.[63] *K. pneumoniae* bacteremia has been associated with lesions of the gastrointestinal tract, presence of an indwelling central venous catheter, and neutropenia. Patients with short-bowel syndrome seem to be at greater risk than are patients with inflammatory bowel disease or malignancy for development of catheter-associated *Klebsiella* or *Enterobacter* bacteremia. *K. pneumoniae* was a constituent of polymicrobial bacteremia in 15 such patients (26%).[14] Mortality rates have ranged from 5 to 20 percent, with higher death rates occurring in children infected with an aminoglycoside-resistant strain.[13,54] Pneumonia, shock, and disseminated intravascular coagulation are poor prognostic factors in children with underlying malignancy. Rare clinical presentations include multifocal osteomyelitis[62] and endophthalmitis.[75] Pediatric recipients of solid organ transplants may have high rates of acquisition of drug-resistant *Klebsiella*.[99] *Klebsiella* spp. have been described as a frequent pathogen in children and adults with sickle cell disease in West Africa.[1]

Of six patients with clinical and colonoscopic findings suggestive of antibiotic-associated hemorrhagic colitis (pseudomembranous enterocolitis) but negative evaluation for *Clostridium difficile*, five had stool cultures positive for *K. oxytoca*.[51] All five patients had been receiving penicillin therapy. *K. oxytoca* was cultured from only 1.6 percent of 385 healthy controls. Additionally, the disease could be reproduced in a rat model when rats were inoculated with *K. oxytoca* and administered amoxicillin-clavulanate, but disease was not seen if rats were not given the injection and the antibiotic treatment.[51]

DIAGNOSIS

Klebsiella spp. characteristically grow as large mucoid colonies on MacConkey agar. Citrate-containing media can be used to facilitate isolation of *Klebsiella* strains because these organisms can use citrate as a sole carbon source.[86] Serotyping with specific antisera usually is determined by countercurrent immunoelectrophoresis or a Quellung test.[8] In situ hybridization techniques have been used to identify *Klebsiella* in phagocytes from blood specimens,[76] and restriction-enzyme analysis and ribotyping of clinical isolates have been used to characterize nosocomial spread of antibiotic-resistant strains.[12,45] Conventional, commonly used microbiologic methods may misidentify some *Klebsiella* spp., particularly *K. planticola* and *K. terrigena*.[81] Rarely, blood cultures have required longer than 72 hours of incubation for radiometric detection of *Klebsiella*.[78]

TREATMENT

Empiric antimicrobial therapy should be guided by an understanding of antimicrobial susceptibilities of *Klebsiella* in the hospital. Therapy with a cephalosporin plus an aminoglycoside (rather than a cephalosporin alone) has been associated with a more favorable outcome in patients with cancer who are infected with susceptible strains.[13] Antimicrobial therapy of *Klebsiella* spp. is made problematic, however, by the resistance to penicillins and cephalosporins conferred by ESBL.[10,12,16,48] *Klebsiella* with ESBL are widely distributed, and regional differences in susceptibility occur.[59] Outer membrane protein changes and porin deficiencies of some strains can augment resistance to third-generation cephalosporins.[5,102] Some investigators have reported significant correlation between production of ESBL and resistance to

ciprofloxacin.[88,107] Plasmid-mediated resistance to aminoglycosides also is common.[6,24,41,83] Endocarditis isolates that developed resistance to piperacillin-tazobactam and to ciprofloxacin during therapy have been reported.[114]

Effective treatment of multidrug-resistant isolates sometimes requires creativity or use of antibiotics no longer commonly employed. In one case report, a 15-year-old girl developed bacteremia with a meropenem-intermediate strain; bacteremia persisted despite thrice-daily administration of meropenem combined with gentamicin, to which the isolate was sensitive. Clearance was achieved eventually with meropenem administered as a continuous infusion.[32] In another case, bacteremia and peritonitis developed in a patient on continuous ambulatory peritoneal dialysis; infection persisted despite removal of the catheter and treatment with meropenem and amikacin (to which the isolate was intermediately resistant). Cure required a switch to intravenous polymyxin B.[87]

Antibiotic pressure is important in increasing the risk for development of resistant isolates.[96] In some nursery outbreaks, switching from gentamicin to amikacin has been associated with return of the susceptibility of *Klebsiella* isolates to gentamicin.[6,50] Imipenem or the combination of piperacillin and tazobactam may show good antimicrobial activity against multiply-resistant organisms.[55,91,96] The combination of β-lactamase and porin deficiency has been associated with resistance to imipenem,[17] and hyperproduction of some β-lactamases can limit the effectiveness of β-lactam–β-lactamase inhibitor combinations.[37] Experience with use of ciprofloxacin in young children is limited because of observations of irreversible cartilage injury in laboratory animals after administration of this quinolone. Successful treatment of a multidrug-resistant *K. pneumoniae* has been reported in a preterm infant, however, without observable short-term adverse effects.[61] Strict adherence to infection control policies that promote restricted antibiotic use, cohorting, and handwashing may help to prevent the spread of resistant *Klebsiella* strains.[6,24,46,79,104]

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CHAPTER 116

MORGANELLA MORGANII

Randall G. Fisher

Similar to *Proteus* and *Providencia* spp., *Morganella morganii* has emerged as an important nosocomial pathogen, most often associated with urinary tract or wound infections. Although most descriptions of infection are in adults, infections in children do occur.

BACTERIOLOGY

M. morganii (formerly *Proteus morganii*) is a motile gram-negative bacillus commonly found in the feces of humans, other mammals, and reptiles.[27] The organism was elevated to genus rank because of genetic differences from *Proteus*, with which it is otherwise biologically similar.[7] Most strains do not ferment lactose. *M. morganii*, *Proteus* spp., and *Providencia* spp. are distinguished from other Enterobacteriaceae by their ability to deaminate phenylalanine and lysine. Similar to *Proteus* and *Providencia* spp., *M. morganii* produces urease, but this enzyme is unrelated genetically and serologically to urease produced by the other two genera.[19,27] In contrast to *Proteus* spp., neither *Providencia* spp. nor *M. morganii* shows swarming activity on 1.5 percent agar[27]; *M. morganii* is ornithine-positive, whereas *Providencia* spp. are ornithine-negative. *M. morganii* organisms do not hydrolyze gelatin and do not produce hydrogen sulfide. For a detailed discussion of taxonomy and characterization of *M. morganii*, the reader is referred to the review by O'Hara and colleagues.[26]

EPIDEMIOLOGY

Similar to *Proteus* and *Providencia* spp., *M. morganii* organisms commonly are found in soil, sewage, and manure. Similar to *Proteus mirabilis*, *M. morganii* commonly invades the instrument-fitted urinary tract and surgical wounds; adults in inpatient surgical units have the greatest risk for colonization and infection.[1] Institutionalized elderly patients also are infected frequently with these organisms. Urinary tract colonization with *M. morganii* accompanying groin skin colonization in elderly individuals may account for the greater frequency of urinary tract infections in this population.[15] *M. morganii* accounted for nearly 10 percent of 145 consecutive complicated, multidrug-resistant urinary tract infections.[11] *Escherichia coli* and *P. mirabilis* account for most urinary tract infections in children, but *M. morganii* has been implicated in some cases of cystitis and pyelonephritis. In contrast to well-described nursery epidemics of *P. mirabilis* infection,[3,9] no *M. morganii* neonatal outbreaks have been described, and infections of the central nervous system are rare in newborns.[13,37] Ribotyping is a sensitive method for molecular characterization of isolates that may aid in analyzing outbreaks.[29]

PATHOPHYSIOLOGY

Factors that predispose the urinary tract to invasion by *P. mirabilis* and *Providencia* spp. also may favor *M. morganii* colonization and infection. These organisms all split urea, forming ammonium hydroxide and increasing local pH, which results in toxicity to renal cells and potentiation of urolithiasis.[6,25] The ability of *P. mirabilis* to regenerate more rapidly in urine with faster generation of alkaline pH compared with *M. morganii* may provide a selective advantage for the former organism in establishing itself as a urinary tract pathogen.[34]

CLINICAL MANIFESTATIONS

Urinary tract infection with *M. morganii* often is associated with an elevated urinary pH. Urolithiasis can occur, although perhaps less frequently than during *P. mirabilis* infection.[4] *M. morganii* has been recovered from less than 10 percent of adult bacteremic episodes, but mortality rates have exceeded 20 percent.[1] Approximately three fourths of all cases of adult *M. morganii* bacteremia

are hospital-associated.[22] In a series of 132 patients with bacteremia from tribe Proteeae, preexisting biliary or hepatic disease (especially the presence of biliary drainage catheters) was associated with *Morganella* bacteremia.[22] Other reported complications identified in immunocompromised or instrument-fitted patients include meningitis,[17,23] arthritis,[21,33] empyema,[18] peritonitis,[18] and skin infection.[2] Many cases of postoperative *M. morganii* endophthalmitis and panophthalmitis have been reported, as have rare cases of endogenous endophthalmitis, all in adults.[10,36,38,39] *M. morganii* has been recovered alone or in combination with other organisms from surgical wounds[11] and soft tissue abscesses in children.[8] Perinatal bacterial sepsis and brain abscess have been described.[30-32,37] In three more recently reported cases of early-onset neonatal sepsis, premature rupture of membranes and maternal receipt of antepartum amoxicillin were documented.[5,14]

DIAGNOSIS

M. morganii produces a reddish brown pigment when cultured on nutrient media supplemented with 5 percent tryptophan.[27] Production of urease and deamination of tryptophan help to distinguish this bacterium from other organisms. In contrast to the closely related *Proteus* and *Providencia* spp., *M. morganii* generally ferments only glucose and mannose and does not produce a red color on lysine iron agar.[27] *M. morganii* may be missed or mistaken for other organisms in the common circumstance of polymicrobial infection in a catheterized patient. In one series, *M. morganii* was among the most common bacteriuric species in patients on long-term catheterization but commonly was missed by reference laboratories.[12] The clinical laboratory should be directed to look for this organism, particularly in circumstances of nosocomial urinary tract infection and sepsis.

TREATMENT

Effective treatment of local infections or septicemia relies on appropriate choice of an antibiotic, often including an aminoglycoside, combined with surgical débridement and drainage of abscesses, as necessary. Antimicrobial susceptibility can vary widely among *M. morganii* and the related *Proteus* and *Providencia* strains, emphasizing the importance of species identification and susceptibility testing.[28] Complex combinations of aminoglycoside resistance that differ by hospital and geographic region can occur.[24] Most isolates are intrinsically resistant to ampicillin and first-generation and second-generation cephalosporins. Some carry a chromosomal broad-spectrum AmpC β-lactamase that can be stably derepressed on treatment with third-generation cephalosporins, similar to *Enterobacter* spp. Most urinary tract infections respond to third-generation cephalosporins. Failure to clear bacteriuria should alert the physician to the possibility of urolithiasis or structural abnormality[4]; stone removal or surgical correction of anatomic defects often is required for cure. The approach to the treatment of sepsis or serious infection of non–urinary tract sites should be considered carefully in light of local susceptibility patterns, keeping in mind that isolates with AmpC β-lactamases may seem to be sensitive to cephalosporins on initial testing, only to develop resistance during therapy. Treatment failure, with prolonged culture-positive ventriculitis, has been described in an infant with *M. morganii* meningitis after initial therapy that included cefotaxime led to stable derepression of an AmpC β-lactamase, showing the potentially devastating clinical relevance of this phenomenon.[35] Aztreonam has shown effectiveness in therapy for 98 percent of multidrug-resistant strains.[11]

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CHAPTER 117

PROTEUS

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Proteus spp. are pathogens that are associated increasingly with pediatric illness. Neonatal meningitis and pediatric urinary tract infections are the most common childhood conditions in which these organisms are isolated, but infections of other organ systems have been described.

BACTERIOLOGY

Proteus spp. are motile, gram-negative bacilli that do not ferment lactose and are distinguished from other Enterobacteriaceae by their ability to deaminate phenylalanine and lysine. Rapid and abundant production of urease further differentiates *Proteus* spp. from *Providencia* spp.[33] *Proteus vulgaris* and *Proteus mirabilis* tend to form a thin, spreading growth (swarm) on the surface of moist agar media, often overgrowing other bacterial isolates. They also produce hydrogen sulfide and liquefy gelatin. *P. mirabilis* is distinguished from other *Proteus* spp. (e.g., *P. vulgaris*) by its inability to produce indole from tryptophan. Disparate DNA content and anomalous biochemical and serologic reactions have caused *Proteus morganii* to be renamed *Morganella morganii* (see Chapter 116),[33] and both terms for this organism appear in the clinical literature. For detailed discussion of taxonomy and characterization of *Proteus* spp., the reader is referred to the review by O'Hara and colleagues.[44]

EPIDEMIOLOGY

Proteus spp. commonly are found in soil, sewage, and manure. Although they are normal inhabitants of the colon and perineum, their numbers can be increased in individuals receiving antibiotic therapy.[30]

First reported by Buisine and Henninot in 1949,[12] neonatal meningitis caused by *P. mirabilis* accounts for approximately 4 percent of all neonatal meningitis cases.[41] Outbreaks in nurseries have been attributed to contaminated equipment and human carriers. Vertical transmission from mother to infant has been confirmed by DNA fingerprinting and ribotyping methods.[9] In Becker's series[6] of *P. mirabilis* neonatal meningitis, all affected infants came from the same nursery, and all were exposed to mist from an apparatus that yielded *P. mirabilis.* The importance of hand carriage was well documented by Burke and associates[13]; newborn umbilical colonization and invasive disease were linked to a single nurse from whom *P. mirabilis* was cultured from her hands, rectum, and vagina. Ribotyping is a sensitive method for molecular characterization of isolates and may aid in analyzing outbreaks.[48]

Proteus infection after the first few months of life most commonly involves the urinary tract. Although *Escherichia coli* accounts for most urinary tract infections in children, *Proteus* spp. commonly are implicated in reported series of cystitis and pyelonephritis[8,22,31,65]; in a large pediatric series, they were the third most common cause of urinary tract infection, after *E. coli* and *Klebsiella pneumoniae*.[10] *P. mirabilis* is the most common species of *Proteus* isolated. *P. mirabilis* has been cultured more frequently from the urethra of uncircumcised than of circumcised male infants and has replaced *E. coli* as the most prevalent pathogen in one consecutive series of male patients presenting with initial urinary tract infection.[31,66]

Urinary tract infection with *Proteus* spp. is one of the most common presenting signs of urolithiasis in children,[18] and *P. mirabilis* supplants *E. coli* as the major urinary tract pathogen in children prone to renal stone formation.[8] Diagnosis of greater than 50 percent of pediatric urolithiasis cases is based on preceding urinary tract infection, and *Proteus* is responsible for 65 percent of these infections.[8] Isolation of this organism as a pathogen on urine culture should alert the physician to the possible presence of a urinary tract stone.

PATHOPHYSIOLOGY

Most cases of central nervous system infection caused by *Proteus* spp. occur in neonates and are thought to arise by bacteremic spread of the organism to the brain or meninges. Contiguous spread to the brain from localized infections is reported occasionally.[40] As is the case with *Citrobacter* meningitis, a propensity for formation of abscesses of the central nervous system remains unexplained. Rabbit models of *P. mirabilis* meningitis have shown that in vivo concentrations of gentamicin necessary to produce bacterial killing are 10 to 30 times higher than the concentrations predicted from in vitro susceptibility testing.[63] Reduced aminoglycoside effect may be secondary to depressed cerebrospinal fluid pH associated with *P. mirabilis* infection.[63] Whatever the mechanism, lack of effective antimicrobial activity in the ventricles accounts partly for the persistence of organisms at these sites.

Crystallization of urinary catheters and stents leads to obstruction of outflow of urine. Urease produced by *Proteus* infection

causes calcium phosphate and magnesium phosphate to precipitate and accumulate in the catheter lumen.[62] This process is less likely to occur when the urine is dilute and acidic.[62] Mannose-resistant/*Proteus*-like fimbriae (often shortened to MR/P fimbriae) are important in the formation of biofilms on catheter material.[52] Methods of preventing this crystallization, including the use of low-energy acoustic waves generated by electrically activated piezo elements, which interferes with docking and attachment, are being studied.[26] More conventional approaches include coating the catheters with agarose[61] and impregnating catheters with chlorhexidine and triclosan.[21]

Numerous factors may predispose the urinary tract to invasion by *Proteus* spp. *Proteus* spp. split urea, forming ammonium hydroxide and increasing local pH, which results in toxicity to renal cells and potentiation of urolithiasis.[11,43] The ability of *P. mirabilis* to regenerate more rapidly in urine with faster generation of alkaline pH when compared with *M. morganii* (*P. morganii*) may provide a selective advantage for the former organism in establishing itself as a urinary tract pathogen.[55] Biochemically complex struvite ($MgNH_4PO_4$) stones provide a refuge for *Proteus* organisms and form a barrier to effective antimicrobial therapy.[8] Formation of struvite stones is a major cause of urinary bacterial persistence in women without azotemia,[60] and a similar case probably can be made for pediatric patients with urolithiasis. *P. mirabilis* ureases showed lower affinities for substrate, but hydrolyzed urea 6 to 25 times faster than enzymes from other species, which may explain the frequent association of this species with formation of stones.[27]

Organisms have been shown to be taken up by human renal epithelium, by an actin-independent mechanism.[14] Pili may enhance the virulence of *Proteus* in pyelonephritis by increasing adherence of organisms to the renal pelvis.[57] Flagella have been implicated in the spread of this organism in the urinary tract[36]; the ability to invade host uroepithelial cells is coupled closely with the ability of *P. mirabilis* to differentiate into hyperflagellated, filamentous swarm cells capable of rapid spread on the surface of moist agar media.[3] The role of fimbriae as a factor predisposing to ascending infection is less clear.[5,39] Swarming behavior might inherently assist ascending colonization of the urinary tract, as shown in a mouse model of infection.[4] A putative gene regulator for swarming behavior (RsbA) may act to identify environmental conditions that favor swarming.[7] The reader is referred to the review by Rozalski and colleagues[53] for a more in-depth review of *Proteus* virulence factors.

A growing body of literature suggests that adult rheumatoid arthritis may be triggered by infection of the urinary tract with *Proteus* spp. In one study, increased levels of total and class-specific IgG antibodies directed at three *Proteus* peptides were found in patients with rheumatoid arthritis, but not in controls.[51] Patients with active disease had measurable IgM, and a positive correlation was found between antibody indices and inflammatory markers.[51] These same investigators proposed measuring antibodies to *Proteus* hemolysin and urease as diagnostic tests for rheumatoid arthritis.[50] This association is still speculative and is being investigated; at this time, no suggestion has been made that juvenile rheumatoid arthritis is related in any way to *Proteus* infection.

CLINICAL MANIFESTATIONS

P. mirabilis can produce a broad spectrum of symptoms associated with neonatal infection. Most patients present with typical symptoms of early-onset neonatal sepsis, including nonspecific lethargy, fever, and poor feeding; manifestations of sepsis may include septic arthritis and osteomyelitis. A few patients present after the first week of life. Meningitis may occur with either early-onset or late-onset disease. Brain abscesses associated with subtle clinical abnormalities rarely develop for weeks to months before presentation.[13] *Proteus* brain abscesses are associated with a high degree of mortality, frequent complications, and increased risks of neurologic deficits in survivors.[28,35,58] Hydrocephalus is a particularly frequent complication and should be anticipated. Destruction of the brain may progress to porencephaly or compartmentalization of ventricles and often requires surgical intervention.[28] Computed tomography is useful, especially in diagnosing and following progression of cerebral complications.[58]

Urinary tract infection with these bacteria involves predominantly younger patients and often is associated with an elevated urinary pH; clinical findings and urine abnormalities often are less striking than in patients with *E. coli* urinary tract infections.[31] Thirty percent of patients may show recurrent infection during the 12 months after receiving initial treatment.[31] Indwelling urinary catheters increase the risk of *Proteus* colonization and infection. Long-term indwelling urinary catheters or stents may become blocked by encrustations of aggregated struvite crystals; prolonged colonization with urease-producing *P. mirabilis* is associated with this complication.[34] *P. mirabilis* is the most common pathogen associated with xanthogranulomatous pyelonephritis,[2] which can mimic Wilms tumor and usually is not seen in childhood except in malnourished patients.

Proteus spp. often are implicated as agents of septicemia in adult patients and account for approximately 8 percent of gram-negative bacteremias in this group.[19,32] In 60 percent of *Proteus* bacteremic episodes in adults, the urinary tract has been determined to be the source[19]; no anatomic source is identified in 20 percent of cases of *Proteus* bacteremia. *P. mirabilis* and *P. vulgaris* are the species responsible for most cases of *Proteus* bacteremia. The overall incidence of gram-negative enteric bacteremia in pediatric patients is lower than that of adults; 5 percent of such cases are caused by *Proteus* spp.[19] As in adults, the genitourinary tract is the source identified most frequently.[19] Mortality rates average less than 40 percent and strongly depend on the severity of underlying disease in the host.[19,32]

Osteomyelitis,[29,47,63] pneumonia,[19,32,49] mastoiditis,[40] and wound infections[32] also occur. Rarely, native-valve endocarditis has been caused by *P. mirabilis*.[15] Severe multifocal osteomyelitis requiring bilateral above-knee amputation for cure has been reported in a patient with human immunodeficiency virus infection despite a reasonably good $CD4^+$ cell count.[46] *Proteus* spp. have been isolated commonly from chronic suppurative otitis media on several different continents.[23,64] Reports that *Proteus* spp. are major pathogens of otogenic brain abscess in pediatric and adult patients support this association.[24,50] Pediatric osteomyelitis secondary to contiguous infection of traumatized soft tissue often is polymicrobial, and *Proteus* spp. have been implicated as co-pathogens in at least 10 percent of such cases.[47] Sickle cell anemia is a risk factor.[1,29]

DIAGNOSIS

Proteus is suspected readily because of its ability to swarm on the surface of moist agar. A selective medium developed for the isolation of protei relies on the ability of all members to produce a dark brown pigment in medium containing DL-tryptophan.[25] Production of urease, lack of indole production from tryptophan, and a positive result with ornithine decarboxylase testing distinguish *P. mirabilis* from *Providencia* spp. and other *Proteus* spp.[33]

TREATMENT

Treatment of meningitis caused by *Proteus* spp. should conform to standard regimens recommended for gram-negative meningi-

tis. *P. mirabilis* usually is sensitive to ampicillin, however, and this drug alone or combined with an aminoglycoside often is suitable therapy when the identity and susceptibilities of the infecting organism are known.[54] A third-generation cephalosporin often is an alternative, but resistant extended-spectrum β-lactamase–producing strains and β-lactamase inhibitor–resistant strains have been described in pediatric patients.[17,36] Consecutive lumbar punctures should be performed for *Proteus* meningitis until cerebrospinal fluid cultures are sterile. A minimum of 2 weeks of antibiotic therapy is recommended after bacteriologic cure has been achieved. Ventricular aspiration or drainage of abscesses may be required to direct therapy, based on persistence of organisms at these sites. Open drainage of abscesses often is necessary, but resolution of abscess formation with the use of antibiotic therapy alone has been reported.[59] Intraventricular antibiotics are not of proven benefit in terms of mortality or morbidity but have been used to clear ventricular colonization. A 15-year-old boy with *Proteus* mastoiditis and meningitis has been treated successfully with intravenous trimethoprim-sulfamethoxazole.[40]

Effective treatment of local infections or septicemia relies on appropriate choice of antibiotics, often including an aminoglycoside, combined with surgical débridement and drainage of abscesses as necessary. Many experts recommended "double coverage"—a cell wall–active agent plus an aminoglycoside—especially when the infection is severe or is caused by an indole-positive strain. In a large, three-continent longitudinal survey, approximately 5 percent of *P. mirabilis* strains exhibited phenotypic extended-spectrum, β-lactamase resistance patterns.[20] In France, 59 percent of 1008 urinary *P. mirabilis* isolates were resistant to amoxicillin, 48 percent were resistant to piperacillin, 34 percent were resistant to amoxicillin-clavulanate, and 2.8 percent were resistant to piperacillin-tazobactam.[37] One third of isolates were resistant to trimethoprim-sulfamethoxazole.[37] Complex combinations of aminoglycoside resistance that differ by hospital and geographic region can occur.[42] In one study, amikacin retained better activity than did other aminoglycosides.[37] Most *P. mirabilis* urinary tract infections respond to ampicillin, but some organisms have been shown to acquire a plasmid-mediated β-lactamase.[38] Failure to clear bacteria should alert the physician to the possibility of urolithiasis or structural abnormality; removal of stones or surgical correction of anatomic defects often is required for cure.

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CHAPTER 118

PROVIDENCIA

Randall G. Fisher

The genus *Providencia* comprises pathogens most commonly associated with urinary tract infection. *Providencia* spp. are encountered most often as pathogens in hospitals or long-term care facilities and can be responsible for outbreaks of multidrug-resistant infection.[34]

BACTERIOLOGY

Providencia spp. (named after the city of Providence, RI) are motile gram-negative bacilli that do not ferment lactose and are distinguished from other Enterobacteriaceae by their ability to deaminate phenylalanine and lysine.[8,27] The genus distinguishes "urease-negative" organisms, *Providencia rettgeri*, *Providencia stuartii*, *Providencia alcalifaciens*, *Providencia rustigianii*, and *Providencia heimbachae*, from the otherwise biochemically similar "urease-positive" *Proteus* spp.[15,27] Urease is produced by most strains of *P. rettgeri* and by 15 percent or less of *P. stuartii* strains.[27] *Providencia* spp. also differ from other Proteeae in their ability to produce acid from inositol. Strains are differentiated further by reactivity with straight-chain hydroxy alcohols.[27] For a detailed discussion of taxonomy and characterization of *Providencia* spp., the reader is referred to the review by O'Hara and colleagues.[24]

EPIDEMIOLOGY

Providencia organisms are recovered uncommonly from stool in healthy humans, but they frequently colonize indwelling or condom urinary catheters, particularly in patients receiving antibiotic therapy.[5,10,16,34,35] In a Japanese study of patients with traveler's diarrhea, *Providencia* spp. were recovered, using a new selective medium, from 23 of 130 specimens tested.[36] *Providencia* spp. have been recognized as pathogens for more than 50 years[7]; *P. rettgeri* and *P. stuartii* are the most common species implicated in urinary tract infection.[10,12,13,21,35] Multiple biotypes of *P. stuartii* have been identified in hospital outbreaks, indicating the probability of multiple sources of colonization.[2] Ribotyping is a sensitive method for molecular characterization of isolates that may aid in analysis of outbreaks.[29]

PATHOPHYSIOLOGY

P. stuartii does not seem to have greater access to the urinary tract compared with other bacteria; in patients with long-term catheters, the incidence of bacteriuria caused by this organism is equivalent to that caused by other uropathogens.[34] Rather, *P. stuartii* manifests an extraordinary ability to persist within the

catheterized urinary tract.[35] Bacteriuria may take weeks to months to clear. A mannose-resistant, *Klebsiella*-like hemagglutinin may play an important role in the persistence and adherence of *P. stuartii* to urinary tract catheters.[23]

Despite the similarities between *Proteus mirabilis* (the major pathogen responsible for urolithiasis in children)[4] and urease-producing *Providencia* spp., the latter organisms rarely are associated with formation of stones. *P. stuartii* occasionally produces urease with a higher affinity for substrate, but *P. mirabilis* ureases hydrolyze urea 6 to 25 times faster.[14] Restriction-enzyme analysis of genes coding for the respective enzymes shows significant divergence.[14] These differences may explain the more frequent association of *P. mirabilis* with formation of stones.

Some strains of *P. alcalifaciens* have been isolated more commonly in children with diarrhea, and enteropathogenicity has been shown in Hep 2 cells and a rabbit model.[2,3] Ex vivo *P. alcalifaciens* strains show the ability to translocate and to resist complement-mediated lysis.[33] In children with diarrhea, *P. alcalifaciens* often is associated with other enteric pathogens, so its role in pathogenesis remains unclear.[1]

CLINICAL MANIFESTATIONS

Although *Escherichia coli* and *Proteus* spp. account for most urinary tract infections in children, *Providencia* spp. have been reported as a cause of infection in children with spinal injury and long-term urinary tract catheterization.[17,21] Most infections have been described, however, in elderly patients or adults with spinal injury who require long-term urinary tract catheterization.[34] Clinical findings are typical of those associated with urinary tract infection. Bacteremia is uncommon but devastating.[18] In a study of 132 cases of bacteremia caused by members of the tribe Proteeae, only 8 were caused by *Providencia* spp., but four of those patients died of the infection.[18] A single case of endocarditis caused by *P. stuartii* has been reported.[19]

Eye infections caused by *P. rettgeri*, including conjunctivitis, dacryocystitis, keratitis, and endophthalmitis, have been reported. These infections generally occurred in patients with compromise in ocular surface or in immunity.[25]

Providencia spp. have been implicated in the so-called purple urine bag syndrome, in which an enzyme from the organism causes 3-indoxyl sulfate to be formed, which discolors the urine blue or blue-violet. This syndrome usually occurs in patients with indwelling cystotomy or nephrostomy tubes.[20,32]

DIAGNOSIS

Infection with *Providencia* spp. should be suspected when indole-positive, urease-negative, gram-negative rods, which oxidatively deaminate tryptophan, are isolated in culture. Because patients with long-term catheterization may be colonized with multiple organisms, *Providencia* spp. frequently are overlooked or misidentified.[7] Clinical laboratories should be encouraged to identify all bacterial colonies in patients with long-term catheterization in whom infection is suspected. Identification is particularly important because of the marked differences in susceptibility of uropathogens.[28]

TREATMENT

Empiric therapy should be guided by antimicrobial susceptibility testing of the patient's isolate and knowledge of susceptibilities of previously identified *Providencia* within the care facility. Removal of urinary tract catheters speeds eradication of these pathogens. Strains of *P. stuartii* and *P. rettgeri* commonly are resistant to many antibiotics. In a longitudinal Italian study of 223 *P. stuartii* isolates, 116 (52%) were extended-spectrum β-lactamase positive. The rate of extended-spectrum β-lactamase–positive isolates increased from 31 percent in 1999 to 62 percent in 2002.[31] Since the 1970s, multidrug resistance has emerged[26]; many strains are resistant to sulfonamides, trimethoprim, nitrofurantoin, nalidixic acid, penicillins, cephalosporins, and aminoglycosides; some singular strains are resistant to most antibiotics in common use.[28] Imipenem-resistant strains have now been described.[30] Much of the observed resistance seems to be plasmid-based[11]; quinolones and aztreonam have shown some promise in the treatment of such cases.[6,9] Complex combinations of aminoglycoside resistance that differ by hospital and geographic region can occur.[22] Organisms that are resistant to gentamicin and tobramycin may remain susceptible to amikacin.

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CHAPTER 119

SHIGELLA

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HISTORICAL BACKGROUND

The term *dysentery* classically has been used to describe the frequent, painful passage of stools containing blood and mucus. The syndrome has been recognized since the time of Hippocrates. The differentiation of dysentery into bacillary and amebic forms followed the recognition by Shiga in 1898 that one form of dysentery was associated with a bacterium in the stools of affected individuals; their sera also were found to agglutinate the bacillus. In recognition of his achievement, the genus was eventually named after Shiga.

The most important subsequent advance has been the recognition of the molecular basis of *Shigella* virulence. In the 1960s, researchers showed that shigellae invade the corneal epithelium of guinea pigs and cause keratoconjunctivitis (Sereny test). Subsequently, Formal and colleagues[70] showed that *Shigella flexneri* invades the intestinal epithelium. Since 1980, Sansonetti and other investigators[201,203,204] have identified multiple plasmid and chromosomal virulence genes. *Shigella*-like enteroinvasive *Escherichia coli* have the same or closely related genes and produce a similar clinical syndrome.

ORGANISM

Shigellae are small, nonencapsulated gram-negative rods that are members of the family Enterobacteriaceae. Technically, they are *E. coli*, but for reasons of tradition and clinical usefulness, the designation as a separate genus has been preserved. Shigellae do not ferment lactose or do so slowly and are nonmotile (lack the H [flagellar] antigen). They lack urease and do not produce hydrogen sulfide on triple sugar iron media or gas during metabolism of carbohydrate.[62] The somatic antigen (or O antigen) side chains that determine serotype and serogroup are attached as multiple repeating units to the lipid A core and core oligosaccharides shared with other members of Enterobacteriaceae. Envelope or K antigens that are heat-labile also have been described, although their clinical relevance is uncertain.

SEROGROUP CLASSIFICATION

Four serogroups, or species, of *Shigella* are defined on the basis of serologic similarities and biochemical reactions. Group A (*Shigella dysenteriae*), mannitol nonfermenters, includes 13 serotypes having O antigens that do not cross-react immunologically. Two additional provisional serovars, types 14 and 15, also are pathogenic.[9] Serogroup D (*Shigella sonnei*), ornithine decarboxylase–positive, slow lactose fermenters, share the same lipopolysaccharide. *Shigella* strains that ferment mannitol (in contrast to *S. dysenteriae*) but do not decarboxylate ornithine or ferment lactose (in contrast to *S. sonnei*) are classified as serogroups B and C. Of these, the strains that express lipopolysaccharides that are related to each other immunologically are group B (*Shigella flexneri*), whereas the strains whose O antigens are unrelated to each other or to other shigellae are group C (*Shigella boydii*). Multiple serotypes of *S. flexneri* (15 serotypes and subtypes) and of *S. boydii* (18 serotypes) exist.

EPIDEMIOLOGY

An estimated 164.7 million cases of shigellosis occur annually, of which 99 percent occur in developing countries, with 1.1 million deaths, 61 percent of which involve children younger than 5 years old.[116] Generally, *Shigella* spp. are associated with 5 to 10 percent of all diarrhea cases and 30 percent of dysentery cases.[162] Although epidemic *Shigella* dysentery is the most dramatic manifestation of *Shigella* infections in developing countries, most infections are caused by endemic shigellosis. Endemic shigellosis is responsible for approximately 10 percent of all diarrheal episodes among children younger than 5 years of age in developing countries.[63] Despite the fact that severe dehydration usually is not seen in shigellosis, 75 percent of diarrheal deaths may be due to these infections.[162] According to surveillance reports of the Centers for Disease Control and Prevention, 10,000 to 15,000 cases of shigellosis have been documented each year during the last 30 years in the United States, for annual incidence rates of 5 to 10 per 100,000.[45]

Because shigellae are spread through a fecal-oral route, they are especially prevalent where hygiene is poor. The organisms can be cultured from around toilets in homes where shigellae have caused disease. Toilet paper does not prevent contamination of fingers. Shigellae are transmitted easily from person to person because the inoculum size required to cause disease is only 10 organisms in the case of *S. dysenteriae* serotype 1[60] and a few hundred organisms in the cases of *S. sonnei* and *S. flexneri*.[59] Handwashing and wearing gloves are mandatory procedures for individuals caring for patients with bacillary dysentery. Patients who lack the acid barrier provided by a normally functioning stomach because of prior gastrectomy or use of antacid are at increased risk of acquiring infection.

Shigellae survive for 30 days in foods such as milk, whole eggs, oysters, shrimp, and flour.[147] Epidemics usually are associated with exposure to contaminated water or food, although as might be predicted from the inoculum size, outbreaks related to swimming also occur.[137] Houseflies can be colonized with shigellae in their guts without illness and pass the organisms in their feces. Feces adherent to their legs can lead to contamination of food. Flies have been implicated in epidemics of shigellosis, particularly where the fly population is large. *Shigella* infection shows seasonal variation. In North America, few cases occur in the winter, whereas in tropical regions, the peak is during the rainy season. Shigellae are worldwide and thrive where susceptible individuals are grouped together (including institutions for retarded or mentally ill individuals, prisoner-of-war camps, Indian reservations, the military, daycare centers, and the developing world).[110,178,212] Spread within families is a common occurrence.

The species of *Shigella* causing most infections vary according to regions. In developed countries, *S. sonnei* is the most common species, followed by *S. flexneri* (approximately 70% *S. sonnei* and 25% *S. flexneri*); however, in the developing world, this pattern is reversed, with *S. flexneri* being more common than *S. sonnei*. *S. dysenteriae* serotype 1 occurs primarily in Africa, India, and Bangladesh. *S. boydii* is found primarily on the Indian subcontinent.[162]

Humans and other primates can be infected with shigellae, and an age-related risk of acquiring symptomatic shigellosis exists. In contrast to *Salmonella* spp., which cause disease most frequently in the first few months of life, shigellae infrequently cause illness in the first 6 months of life. The peak incidence occurs in children between 1 and 4 years of age, with fewer cases occurring in children aged 5 to 9 years old. Adults are at lower risk.

PATHOGENESIS

INVASIVENESS AND TOXIN PRODUCTION

The ability to invade mammalian cells is the most important virulence trait of *Shigella* spp.[74,122,129,159,169] Uptake by M cells overlying Peyer patches with ingestion by macrophages under the M cells induces production of cytokines and recruitment of polymorphonuclear leukocytes. Apoptosis is induced in macrophages after ingestion of shigellae; these events are accompanied by release of interleukin-1 (IL-1), IL-1β, and IL-18, which triggers other inflammatory events.[203,227,229] Polymorphonuclear leukocytes enter the gut lumen by moving between epithelial cells. The gaps between epithelial cells may be the portal of entry of bacteria into the epithelium. After penetration of intestinal epithelial cells, shigellae are located in vacuoles derived from the cytoplasmic membrane of the mucosal cells. The bacteria lyse these vacuoles, move intracellularly, multiply, kill the epithelial cells, and infect adjacent cells. Cell death is followed by formation of ulcerations and microabscesses in the colon. In contrast to *Salmonella* infection, *Shigella* infection rarely spreads beyond the lamina propria, so bacteremia and metastatic infections are uncommon.

The genetic basis of virulence has been studied extensively.[170,174] Invasiveness is the result primarily of genes on a large 120- to 140-MDa (200- to 220-kb) virulence plasmid.[81,82,88,200-202,204] These genes encode a molecular apparatus called a type III secretion system that is capable of injecting bacterial proteins through bacterial and host membranes into host cells (translocation) or the extracellular milieu (secretion) to influence host biochemistry and cell physiology directly.[174] The invasive plasmid antigen (ipa) region includes genes for four polypeptides needed for invasion: *ipaA*, *ipaB*, *ipaC*, and *ipaD*. The proteins produced by these loci are recognized by the humoral immune system. The product of the *ipaB* locus is essential for induction of apoptosis in macrophages.[228] The mxi-spa region of the virulence plasmid is necessary for orientation of the ipa-encoded proteins into the outer membrane of the bacteria. The *virG* gene encodes a protein that causes intracellular and intercellular spread of shigellae after invasion of epithelial cells. The *virF* gene regulates a locus (*virB*) that is responsible for positive regulation of the ipa genes.[192]

Chromosomal loci also regulate virulence.[173] Chromosomal loci regulate expression of the virulence plasmid. The keratoconjunctivitis provocation (*kcpA*) gene is a positive regulator of the *virG* virulence plasmid gene that determines ability to spread within and between cells. The chromosomal *virR* gene is a temperature regulator that represses expression of ipa genes at 30° C, but not at 37° C.

Lipopolysaccharides (LPSs) play a role in resistance to nonspecific host defense mechanisms that are encountered during invasion of tissue. The *Shigella* LPS induces the trafficking of Toll-like receptor 4, the dominant mediator of the innate immune response.[42] Smooth colonies express the complete complex of LPS O side chains required for full virulence—ability to invade epithelial cells, to multiply within them, and to resist phagocytosis.[77,171] Rough colonial variants that lack complete LPS do not penetrate epithelial cells efficiently and are avirulent.

Some strains of *Shigella* spp. produce toxins that injure mammalian cells. A chromosomal locus in *S. dysenteriae* serotype 1 encodes a protein synthesis–inhibiting exotoxin (Shiga toxin) that is a major virulence factor in this serotype (and in the enterohemorrhagic *E. coli* serotypes that have the same or closely related genes).[25,207] This toxin is composed of a single copy of an A subunit (32,000 daltons) that is linked to five copies of B subunits (7790 daltons).[167] The B subunits bind to a glycolipid cell receptor, globotriaosylceramide, followed by internalization. The A subunit cleaves an adenine residue from the eukaryotic 28S ribosomal subunit. The resulting block in elongation factor 1–dependent binding of aminoacyl tRNA to the ribosome causes cell death through inhibition of protein synthesis.[167] Shiga toxin previously was considered to be a neurotoxin because its administration to mice or rabbits caused paralysis and death[37,44,92]; it now is thought to target primarily the vascular endothelium. It causes fluid accumulation in rabbit ileal loops[64,111] that probably is related to reduced fluid uptake by damaged villus cells.

The severity of *S. dysenteriae* serotype 1 infection relative to other *Shigella* serotypes is thought to be caused by production of Shiga toxin. Enterohemorrhagic *E. coli* serotypes, such as *E. coli* O157:H7, produce an identical (Shiga toxin 1) or similar (Shiga toxin 2) toxin and, similar to *S. dysenteriae* serotype 1, cause bloody diarrhea and hemolytic-uremic syndrome. Enteroinvasive *E. coli* serotypes do not produce these toxins.[50]

Shigella spp. also produce two enterotoxins, ShET-1 and ShET-2.[218] All *S. flexneri* 2a strains produce ShET-1, whereas only 3.3 percent of other *Shigella* serotypes and no enteroinvasive *E. coli* serotypes possess the gene for this toxin.[163] ShET-2 is produced by all *Shigella* spp.[218] Genes of an additional toxin, designated *Shigella* enterotoxin, or Sen, have been found in 75

percent of enteroinvasive *E. coli* serotypes and 83 percent of *Shigella* strains.[156] These enterotoxins may contribute to the high-volume, watery diarrhea often seen in the initial stages of the disease.[19] Vaccine studies using a mutant with deletions of these toxin genes have shown that one or both enterotoxins play a role in human disease.[117]

IMMUNE RESPONSE

Serum IgG, IgM, and IgA responses occur to the LPS and invasion plasmid antigens of shigellae.[166] Secretory IgA specific to both of these sets of antigens occurs in human milk and feces.[51] Protection is thought to be serotype-specific. The level of IgG antibodies to LPS present in an individual before infection occurs determines whether symptomatic shigellosis develops.[55] No clear proof currently exists about the role of antibodies to the invasion plasmid virulence proteins or to the toxins. Cell-mediated immunity against shigellae also may play a role in resolution of infection. Production of αβ T cells and natural killer cells mediates interferon-γ production that may be essential to defense from *Shigella*.[124,222] Antibody-dependent cellular cytotoxicity against shigellae has been shown.

During infection, shigellae use effectors secreted via the type III secretion system to direct various cellular signaling pathways and to modify the innate immune activation of the host. More recent evidence indicates that a large part of the mucosal inflammation is initiated by intracellular sensing of bacterial peptidoglycan by cytosolic leucin-rich receptors of the NOD family in epithelial cells.[170,175] This causes activation of the nuclear factor κB and other terminal kinase pathways, with IL-8 appearing as a major chemokine mediating the inflammatory burst that is dominated by massive infiltration of the mucosa by polymorphonuclear leukocytes.[175] The levels of cytokines tumor necrosis factor-α, IL-1β, IL-1RA, IL-6, IL-8, and granulocyte-macrophage colony-stimulating factor in stool correlate with the severity of shigellosis. In contrast to other cytokines, interferon-γ and its receptor[186] are depressed early and increase during recovery. Fecal concentrations of tumor necrosis factor-α, IL-1β, IL-1RA, IL-6, IL-8, and granulocyte-macrophage colony-stimulating factor are significantly higher in patients with *S. dysenteriae* serotype 1 than in patients with *S. flexneri* infection.[189] Elevated tumor necrosis factor-α and IL-6 levels in stool and serum have been associated with complications during infection with *S. dysenteriae* serotype 1.[85] Infiltration of polymorphonuclear leukocytes and lymphocytes into the intestine is controlled by these cytokines. Epithelial cell production of IL-8[199] and a low IL-1RA-to-IL-1 ratio[11] are responsible for the severe inflammation. T lymphocytes with suppressor-cytotoxic and helper-inducer phenotypes are recruited into the epithelium and lamina propria during infection with *Shigella*, related partly to the induction of HLA-DR expression in the rectal mucosa.[187] Peripheral blood lymphocytes of infected individuals respond to *Shigella* antigens in vitro with production of interferon-γ and IL-10.[198]

Shigella spp. elicit an inflammatory response during multiplication within macrophages and epithelial cells. *Shigella* multiplication in macrophage cytoplasm induces cell death, which is achieved by two distinctive pathways: activation of caspase-1 by IpaB secreted by the type III secretion system, and translocation of lipid A from the cytosol, which occurs independent of caspase-1 and Toll-like receptor 4 activities.[170]

PATHOLOGY

The main morphologic changes of shigellosis (superficial ulcerations, focal hemorrhages, mucosal edema, erythema, and friability) occur in the colon where the organisms invade.[97,208] The rectosigmoid and distal segments of the colon typically are involved more severely than is the proximal colon. The epithelial cell damage may cause development of a pseudomembrane composed of thick, fibropurulent exudate tightly adherent to the necrotic ulcerated colonic mucosa. Pseudopolyposis also has been reported.[39] On microscopic examination, damage to epithelial cells, ulcerations, goblet cell depletion, and intense polymorphonuclear and mononuclear infiltration with crypt abscesses are seen. Rectal mucosa shows increased numbers of $CD8^+$ and $\gamma\delta^+$ T cells.[95] Vessels in the lamina propria are congested or thrombosed. Perforation of the colon usually does not occur as part of the colitis.[23] Evidence of inflammation and proinflammatory cytokines persists for at least 1 month after clinical resolution.[185] Histologic changes generally are more severe and persistent[96] with *S. dysenteriae* than with *S. flexneri*.[8]

CLINICAL MANIFESTATIONS

The incubation period ranges from 12 hours to a few days (if a low number of organisms is ingested). Onset of high fever, toxicity, and crampy abdominal pain is sudden. During the first 48 hours, high-volume watery diarrhea may occur (small bowel phase of disease); subsequently, low-volume, bloody, and mucous diarrhea develops in association with urgency and tenesmus (large bowel or "dysenteric" disease). In some cases, the watery diarrhea persists for several days, without subsequent development of dysentery. Other children present with bloody or mucous diarrhea.

Physical examination shows fever, signs of toxicity, tenderness over the lower abdominal quadrants, and hyperactive bowel sounds. Signs of dehydration may be present. Rectal examination reveals severe tenderness. Rectal prolapse may be present, particularly when diarrhea is associated with malnutrition.

The course without therapy typically lasts 7 to 10 days. Protein-losing enteropathy occurs during shigellosis; this enteropathy is severe with *S. dysenteriae* serotype 1.[32] In addition, anorexia related to fever and abdominal pain contributes further to malnutrition, particularly in *S. dysenteriae* type 1 infection.[184] These facts may explain partly the hypoproteinemia and adverse effect on growth of severe shigellosis. Malnutrition is associated with a more severe course of shigellosis. Fever may not develop, even when severe dysentery is present. Chronic infection may last for months, despite administration of appropriate antibiotic therapy. It may cause further deterioration of the nutritional status, which together with other complications (e.g., ileus, bacteremia, and pneumonia) is associated with overall increased mortality rates from shigellosis. Surgical complications of shigellosis are rare but are associated with significant morbidity and mortality because of delay in establishing the diagnosis. In a series of 57 children with surgical complications of shigellosis,[148] intestinal obstruction (53%), appendicitis (28%), and colonic perforation (17%) were the most common (Table 119–1).

Although the organism usually is excreted for only a few days or weeks (range 1 to 30 days), carriage for many months sometimes occurs.[52,130] The illness usually is acute; a chronic carrier state can occur in malnourished individuals. Asymptomatic infection of toddlers living in an endemic area occurs commonly. Most *Shigella* infections in Mexican children who were cultured each week from birth were not associated with symptoms.[80]

EXTRAINTESTINAL MANIFESTATIONS AND COMPLICATIONS

Multiple extraintestinal complications have been described with *Shigella* infection (see Table 119–1). Seizures have been reported in 10 to 45 percent of hospitalized children with culture-proven shigellosis.[17,21,24,57,68] In outpatient settings, the frequency of seizures is very low. Children who develop neurologic complaints

TABLE 119–1 Complications of Shigellosis

Abdominal
Persistent diarrhea
Post-dysenteric irritable bowel syndrome
Ileus, toxic megacolon, intestinal perforation
Protein-losing enteropathy, malnutrition
Surgical complications: intestinal perforation and obstruction, appendicitis, intra-abdominal abscesses

Neurologic
Seizures
Headache, lethargy, disorientation, hallucinations
Coma
Severe toxin encephalopathy or ekiri syndrome

Bacteremia
In malnourished children, young infants, and AIDS patients

Hemolytic-uremic syndrome
Only with *Shigella dysenteriae* serotype 1

Urogenital
Vulvovaginitis, urinary tract infections

Other
Conjunctivitis, keratitis, corneal ulcers
Reactive arthritis
Reiter syndrome
Hepatitis
Myocarditis

may have lethargy, severe headache, disorientation, hallucinations, or self-limited convulsions lasting less than 15 minutes.[15,18,21,152] Seizures are most likely to occur in very young patients, patients with a high peak body temperature, and patients with a family history of convulsive disorders.[17,118] Seizures can be focal, although usually they are generalized.

When symptoms related to the nervous system occur, they are likely to appear early in the illness, even preceding development of diarrhea. Death rarely has been described[90]; most children recover completely with no residual neurologic deficits.[18] In contrast, in Bangladesh, where seizures in shigellosis typically are associated with factors known to alter consciousness (e.g., hypoglycemia, hyponatremia, fever), mortality rates are high.[114] The pathogenesis of neurologic signs and symptoms during episodes of shigellosis is unclear. Hypoglycemia and electrolyte abnormalities are found in a few patients.[18] Direct invasion of the central nervous system during *Shigella* bacteremia is very rare.[223] Simple febrile seizures might explain convulsions in a few children with dysentery, but some children who have seizures during episodes of shigellosis do not experience seizures during other febrile infections and are outside the age range usually associated with febrile seizures.[18] Shiga toxin formerly was thought to cause the neurologic symptoms because it was considered to be a neurotoxin; however, data now clearly show that Shiga toxin is not responsible.[16]

Severe toxic encephalopathy has been described. This syndrome (ekiri), as originally described in Japan, was characterized by dysentery with hyperpyrexia, convulsions, sensory disturbances, and rapid progression to death.[193] The children died with cerebral edema early in the course of disease (6 to 48 hours after onset). Mild hyponatremia has been a common finding.[76] Children with ekiri did not have sepsis, disseminated intravascular coagulation, hemolytic-uremic syndrome, or severe dehydration. This toxic encephalopathy is rare. Whether this syndrome is part of a continuum of central nervous system dysfunctions with seizures and other encephalopathic symptoms or has a completely different pathogenesis is unclear.

Hemolytic-uremic syndrome (microangiopathic hemolytic anemia, thrombocytopenia, and acute renal failure) or isolated hemolysis has been reported, mainly after infections with *S. dysenteriae* serotype 1 and rarely after infection with *S. flexneri*.[182] Vascular endothelial cell damage by Shiga toxin is considered to be the initial event, although endotoxin absorbed from the gut also may play a role.[115]

Ileus that progresses to toxic megacolon with distended loops and eventual intestinal perforation occurs.[22] It is seen mainly with *S. dysenteriae* serotype 1 infection.

Septicemia in shigellosis is a rare occurrence except in malnourished children, young infants, and children with *S. dysenteriae* serotype 1 infections.[84,143,205] The mortality rate is at least twice as high in dysentery-associated sepsis as in shigellosis uncomplicated by bacteremia. The serogroup-related mortality risk in shigellemia has been reported to be 85 percent for infection with *S. dysenteriae*, 43 percent for infection with *S. flexneri*, and 25 percent for infection with *S. sonnei*; bacteremia rarely is reported with *S. boydii* infection.[141] In Bangladesh, *Shigella* bacteremia was found in 4 percent of patients.[213] When bacteremia occurs with dysentery, it is as likely to be caused by other enteric bacteria as by the *Shigella* itself. The occurrence of *Klebsiella*, *E. coli*, and other enteric pathogens in blood cultures of children with shigellosis presumably reflects loss of the barrier function during severe colitis.[158] *Shigella* bacteremia may be complicated by disseminated intravascular coagulation and multiorgan failure. Bronchopneumonia may develop in septicemic children,[4] but its pathogenesis is unclear. Children who die with shigellosis often have pneumonia at autopsy.[39]

Other extraintestinal infections rarely are caused by *Shigella* spp. Vaginitis with a bloody discharge (sometimes lasting for months in the absence of specific therapy) may occur, usually without concurrent or recent diarrhea.[56,154] *Shigella* cystitis, not always associated with diarrhea, has been described, usually in girls.[24] Conjunctivitis, keratitis, corneal ulcers, and iritis are other uncommon manifestations of shigellosis that usually are assumed to occur after autoinoculation.[24,216] Reactive arthritis or Reiter syndrome (arthritis, urethritis, conjunctivitis) may develop after episodes of shigellosis,[41] especially in adults who are HLA-B27-positive; reactive arthritis is uncommon in children. Hepatitis with mildly abnormal liver function test results has been described.[211] Myocarditis manifested clinically by hypotension despite fluid replacement, arrhythmia, heart block, or low voltage on electrocardiography and pathologically by interstitial lymphocytic infiltrates and focal necrosis has been described.[191] Post-dysenteric irritable bowel syndrome occurs in some patients after recovery.[221]

SHIGELLOSIS IN THE NEONATAL PERIOD

Bacillary dysentery is rare in newborns.[25,61,66,83,123] More than half of the reported neonatal cases occurred during the infants' first 3 days of life, consistent with fecal-oral transmission during delivery,[78,160] usually from a symptomatic mother. Although a neonate with shigellosis usually has only low-grade fever with diarrhea of variable severity,[5,120,150] septicemia[127,181] and chronic diarrhea are more common than in older children. Intestinal perforation has been reported in neonatal shigellosis.[210] Diarrhea more often is nonbloody in infants; fever also occurs less commonly than in older children.[94] Data on age-related mortality caused by shigellosis in developing countries suggest that the mortality rate of the neonate is more than twice that of older children.

SHIGELLOSIS IN ACQUIRED IMMUNODEFICIENCY SYNDROME

In patients with acquired immunodeficiency syndrome (AIDS), shigellosis not only is more common and more severe, but also is associated more often with bacteremia.[26,93] In contrast to the

usual short, self-limited course of shigellosis, *Shigella* infection in patients with AIDS may be chronic and relapsing, despite appropriate antibiotic treatment.[121,206]

LABORATORY FINDINGS

The fecal leukocyte examination is helpful in evaluating a patient with fever and watery diarrhea. Direct microscopic examination of fecal mucus stained with methylene blue shows many polymorphonuclear leukocytes in patients with colitis, including most patients with shigellosis.[87] The blood leukocyte count in patients with shigellosis often is normal, although leukopenia or leukocytosis may occur.[13] The differential of the blood leukocyte count typically shows an increased percentage of band forms. Approximately one third of children with shigellosis have more bands than segmented neutrophils in their peripheral blood smear. A leukemoid reaction, with a peripheral leukocyte count greater than 50,000/mm^3, has been reported, mainly with infections caused by *S. dysenteriae* serotype 1.[40] A leukemoid reaction has been reported in 10 percent of patients infected with the Shiga bacillus. When examination of cerebrospinal fluid is performed in children with neurologic symptoms, normal results usually are obtained, although some patients have a mild lymphocytic pleocytosis. Likewise, when electroencephalography is performed, the results usually are normal.[18]

DIAGNOSIS

Bacillary dysentery usually is suspected in children who present with bloody diarrhea, high fever, and generalized toxicity. Approximately half of children do not develop bloody diarrhea during the course of their disease. This fact is especially relevant in developed countries, where most infections are caused by *S. sonnei*. The presence of only watery diarrhea does not exclude the possibility of shigellosis in an ill patient with high fever.

ISOLATION TECHNIQUES

Proof of the diagnosis of suspected bacillary dysentery often is problematic. Definite diagnosis of *Shigella* infection depends on isolation of the organism from stool specimens or rectal swabs. The bacteria may not survive in fecal specimens during transit, however, and special selective media are necessary for isolation. Recovery of shigellae is easier early in the course of the disease than later because the number of viable organisms in stools decreases significantly during late stages of the disease. Even in adult volunteer studies, when appropriate stool cultures were obtained daily, cultures failed to isolate shigellae in approximately 20 percent of volunteers who had ingested the organism and developed diarrhea.

Several measures increase the likelihood of isolating *Shigella*. Specimens should be processed without delay. If a specimen cannot be processed immediately, a transport medium, such as buffered glycerol saline, should be used. More than one stool culture or rectal swab should be obtained and inoculated promptly onto at least two different culture media. Specimens should be plated lightly onto MacConkey, xylose-lysine-deoxycholate, or eosin–methylene blue agar, whereas a heavier plating is necessary for the more inhibitory *Shigella-Salmonella* medium.[151,215] After overnight incubation at 37° C, lactose-negative colonies are transferred to triple sugar iron and lysine iron agar slants and incubated again overnight. Slants showing characteristic reactions of alkaline red slants, acid butt, and production of gas are tested biochemically for presumptive identification and then serologically for definitive identification.

OTHER DIAGNOSTIC METHODS

Because presumptive identification of *Shigella* takes at least 48 hours and definite identification takes approximately 72 hours, attempts to develop rapid diagnostic methods are being made, especially because institution of early treatment is important in shigellosis. Identification of shigellae by specific DNA probes with use of either restriction fragments or synthetic oligonucleotides[161] based on detecting virulence genes located on the 120- to 140-MDa plasmid has been described. Because the invasion plasmids of shigellae, similar to other plasmids, may be lost spontaneously, a DNA probe derived from *S. flexneri ipaH* gene, a multicopy element that is found on the chromosome and on the invasion plasmid of shigellae, has been developed and found to be more sensitive than are probes used previously.[219] Use of polymerase chain reaction (PCR) in vitro amplification of nucleic acids, to detect shigellae directly from stool specimens, has been reported.[71] PCR can detect as few as 10 colony-forming units of *S. flexneri* in stool specimens, whereas the sensitivities of DNA probe hybridization (with no amplification) and standard biochemical methods are 10^3 and 10^6 colony-forming units. PCR for *ipaH* genes seems to be more sensitive than culture; 28 percent of patients with dysentery with a positive PCR assay result are culture negative.[73,99] Another new method for the identification of *Shigella* is microarray analysis using the *gyrB* genes.[106] This novel approach also renders it possible to identify simultaneously other enteric pathogens, such as *Salmonella* spp. and diarrheogenic *E. coli*.[106]

Serologic studies are not helpful in establishing the diagnosis of shigellosis in individual patients; humoral antibodies develop after clinical recovery. Serologic studies may be helpful, however, in epidemiologic studies to define spread of the disease in a population.[24]

Sigmoidoscopy and barium enema study are unnecessary, unless they are indicated to rule out other conditions. When these procedures are performed in patients with a possibility of having shigellosis, caution is necessary because of the diffuse acute colitis.

DIFFERENTIAL DIAGNOSIS

Colitis of any etiology manifesting with acute-onset bloody diarrhea with fever and abdominal cramps can mimic shigellosis. Etiologic agents to be considered include *Campylobacter* spp., *Salmonella* spp., *Clostridium difficile*, *Yersinia enterocolitica*, *Shigella* spp., *Vibrio parahaemolyticus*, enteroinvasive *E. coli*, enterohemorrhagic *E. coli* (e.g., serotype O157:H7), *Balantidium coli*, and *Entamoeba histolytica*. The initial presentation of inflammatory bowel disease can mimic shigellosis.

An etiologic diagnosis of acute colitis syndrome on the basis of clinical presentation is difficult to make, although some data suggest a specific causative agent. In developed countries, *Campylobacter* is the most common cause of acute infectious colitis. Shigellosis should be suspected when evidence exists of person-to-person spread, and when convulsions or other neurologic symptoms develop. In the first few months of life, *Salmonella* is the most common infectious cause of bloody diarrhea, and *Shigella* is very rare.

A history of previous antibiotic treatment suggests diarrhea related to *C. difficile*, and previous consumption of seafood suggests *V. parahaemolyticus*. *Yersinia* infections are found mainly in the cooler regions of Europe and North America; the disease may mimic acute appendicitis because of the right lower quadrant pain associated with mesenteric lymphadenitis.

Enterohemorrhagic *E. coli* infection often causes bloody diarrhea with little or no fever, in contrast to shigellosis, in which high fever is typical. Negative stool cultures for the mentioned

bacterial pathogens may suggest infection by enteroinvasive *E. coli*. Amebiasis causes a colitis similar to the colitis caused by *Shigella*,[209] although it is of slower onset, with a lower degree of fever; the findings on fecal leukocyte examination are negative. The involvement of the colon with amebiasis is less diffuse than in shigellosis; areas of normal mucosa are found between ulcerations.

A prolonged course with negative cultures should raise concern about the possible presence of either ulcerative colitis or Crohn disease. When watery diarrhea is present, the list of possible etiologic agents is even longer, although many agents that cause watery diarrhea are associated with little or no fever and are not confused with shigellosis. The diagnosis usually cannot be made by clinical presentation alone and depends on culture results or other specific laboratory assays.

TREATMENT

FLUID ADMINISTRATION

Dehydration is less a problem with shigellosis than with rotavirus or toxigenic *E. coli* infection. Some children with shigellosis, particularly young infants, have dehydration during the course of the disease. The high-volume watery diarrhea seen early in the course of the disease may cause excess losses of fluids and electrolytes; likewise, in patients with severe colitis, systemic toxicity and vomiting may cause anorexia that interferes with fluid intake.

Assessment of the hydration status of the patient on admission is mandatory, with early institution of appropriate fluid and electrolyte therapy needed. The World Health Organization's oral rehydration therapy with glucose-electrolyte solutions usually is effective.[155] This solution contains 90 mEq/L of sodium, 20 mEq/L of potassium, 80 mEq/L of chloride, 30 mEq/L of bicarbonate or citrate, and 20 g/L of glucose. Oral rehydration therapy should be given with additional water containing no electrolytes to prevent hypernatremia; this therapy is particularly important in children too young to express their need for additional free water. In infants, 2 parts of oral rehydration solution should be followed by 1 part of water without electrolytes. Oral rehydration with commercially available solutions (e.g., Pedialyte) also is acceptable. Administration of intravenous fluid therapy is necessary in children who are comatose, have an ileus, or are in shock. Early (12 to 24 hours after oral fluids are begun) reinstitution of breast milk or other food is mandatory.

ANTIBIOTIC THERAPY

Children who have dysentery always should be treated empirically with antimicrobials. Antibiotic therapy for milder illness is controversial. World Health Organization guidelines suggest that presumptive shigellosis should be treated with antibiotics, the choice being decided by the antimicrobial susceptibility pattern of locally circulating *Shigella* strains (WHO/CDR/95.3). If after 2 days of therapy, the patient's condition improves, a full course of 5 days should be given. If the patient does not improve, the antibiotic should be changed. If with the second antibiotic, the patient does not show signs of improvement, the diagnosis must be reviewed, and stool microscopy, culture, and susceptibility testing should be performed.[162]

Appropriate antimicrobial therapy of shigellosis shortens the duration of fever and diarrhea, and it apparently also reduces the risk of developing complications.[12] Shedding of the organisms in stools stops within 1 to 2 days, so that intrafamilial spread may be decreased. Although use of antibiotics may favor emergence of resistant organisms, most authorities recommend that antibiotics be started when shigellosis first is suspected clinically, before culture confirmation of the infection is obtained. Therapy should be stopped or changed on the basis of culture results (e.g., another pathogen or a resistant *Shigella* strain is isolated) and clinical response.

The choice of antimicrobial agent is complicated by the increasing frequency of plasmid-mediated antibiotic resistance.[63,67,197] Organisms resistant to ampicillin, trimethoprim-sulfamethoxazole, tetracycline, and chloramphenicol have been reported from the Middle East,[1,6,10,19,64,86,105,224] Africa,[1,35,43,108,142,157] South America,[132,156,164,180] Europe,[25,46,101,107,119,139,220] Eurasia,[23,172,225] Asia,[2,7,34,112,131,133,135,138] and the South Pacific. Currently, ampicillin and trimethoprim-sulfamethoxazole are inappropriate for empiric therapy; they should be used only if the organism has been shown to be susceptible.[12]

Multiresistant strains also are found commonly in some parts of the United States. Among 344 *Shigella* isolates, from 1999 to 2001, 95 percent were resistant to one or more antimicrobial agents, and 70 percent were resistant to two or more agents. Ampicillin resistance occurred in 80 percent of isolates, and trimethoprim-sulfamethoxazole resistance occurred in approximately 50 percent of isolates. None of the isolates was resistant to ceftriaxone or gentamicin, and only one isolate (0.3%) was resistant to ciprofloxacin. Susceptibility testing against azithromycin was not performed.[176]

Multiresistant *Shigella* spp. are particularly likely to emerge in individuals who are exposed to multiple antibiotics[214] (e.g., patients with AIDS) and individuals who recently have traveled to areas with known resistance. Likewise, children in daycare centers are at risk of acquiring resistant organisms because the frequent use of antibiotics for otitis media may favor selection and emergence of resistant enteric organisms, and because crowding and poor hygiene facilitate transmission.[36]

Generally, shigellae are susceptible in vitro to azithromycin, ceftriaxone, cefotaxime, cefixime, nalidixic acid, and quinolones in the United States and elsewhere. In vitro susceptibility does not always predict clinical efficacy or superiority of one drug over another, however. The first-generation and second-generation cephalosporins are active in vitro but have been ineffective in clinical trials. Clinical studies have shown some drugs to be clearly superior to others when susceptibility data have suggested that either might work well.

Prevalent serotypes and resistance patterns vary from year to year in a given locale. Typically, rates of resistance are related to the severity of disease caused by a given serotype. The more likely an organism is to cause severe disease, the more likely that resistant strains will emerge. *S. dysenteriae* serotype 1 is more likely to be multiply resistant than *S. flexneri*; *S. flexneri* is more likely to be resistant than *S. sonnei*. In Africa[35,108] and Asia,[28,30,49,138,153] Shiga bacilli resistant to nalidixic acid and ciprofloxacin have been reported. Nalidixic acid–resistant *S. flexneri* also have been recognized occasionally.[48] Local resistance patterns, history of travel to an area of frequent resistance,[214] and severity of illness should determine treatment.

Given the frequent occurrence of resistant organisms, optimal empiric therapy in children with dysentery should be azithromycin, a third-generation cephalosporin, nalidixic acid, or ciprofloxacin (Table 119–2).[12,27,100,126,162,176] Although less well studied, ampicillin-sulbactam and pivmecillinam also have been shown to be effective in children. In children, oral cefixime seems to be superior to ampicillin-sulbactam.[89] Adults do not respond well to usual doses of cefixime.[196] Ampicillin, tetracycline, and chloramphenicol are used infrequently now for shigellosis.

Use of quinolones has been controversial in children. An oral fluoroquinolone (ciprofloxacin, norfloxacin) seems to be optimal for adults.[14,33,89,125,140,179,195] Limited data suggest that not all of these agents are equally effective, even when in vitro data suggest

TABLE 119–2 Antibiotic Treatment of Shigellosis in Children

Antibiotic	Dosage*	Comments
Ampicillin	100 mg/kg/day divided q6h PO, IV, or IM (maximum 4 g/day)	Currently of limited value because of the high frequency of resistance; it can be used when the organism is known to be susceptible
Trimethoprim-sulfamethoxazole	10 mg/kg/day of trimethoprim divided q12h PO (maximum 320/1600 mg/day)	Currently of limited value because of the high frequency of resistance; it can be used when the organism is known to be susceptible
Azithromycin	12 mg/kg/day once daily PO on day 1, followed by 6 mg/kg/day once daily on days 2-5 (maximum 500 mg/day)	Although limited published data in children, its use is recommended by many experts
Nalidixic acid	55 mg/kg/day divided q6h PO (maximum 2 g/day)	Not available in the United States; contraindicated in infants <3 mo old
Ciprofloxacin	15-30 mg/kg/day divided q12h PO (maximum 1.5 g/day)	Alternative when other options unavailable
Cefixime	8 mg/kg/day divided q12-24h PO (maximum 400 mg/day)	
Ceftriaxone	50 mg/kg/day IM or IV q24h (maximum 2 g/day)	Current drug of choice for empiric therapy of severe dysentery in children

**Treatment should be given for 5 days.*

susceptibility. Norfloxacin is clinically superior to nalidixic acid in children and adults with shigellosis.[33,190] Nalidixic acid therapy is effective treatment,[194] although plasmid-mediated resistance to nalidixic acid of *S. dysenteriae* serotype 1 strains in Bangladesh[28,30,153] has emerged and spread rapidly. Various other quinolones have been effective in adult patients with shigellosis, but they are not approved for use in children younger than 17 years old because of potential damage to the cartilage of epiphyseal plates.[190] A considerable body of evidence from the long-term use of these drugs in children with cystic fibrosis has been accumulated, however, as has evidence from short-term use to treat typhoid fever and dysentery in children; no evidence of bone or joint toxicity or growth impairment has been found. Nalidixic acid does not cause arthropathy or limit growth when it is used for a short time.[165]

As resistance continues to increase, situations are likely to occur in which a quinolone is the only option for treatment of a child with severe shigellosis. Data for children suggest that norfloxacin at a dose of 10 to 15 mg/kg/day or ciprofloxacin at a dose of 10 mg/kg every 12 hours (maximum 500 mg/dose) for 5 days is effective therapy.[79,125,136,195] In adults, short-course therapy (1 or 2 days) with ciprofloxacin has been effective for treatment of infections caused by *Shigella* spp. other than *S. dysenteriae*, for which a 10-dose, 5-day regimen is superior.[31] Pediatric data suggest that even for *S. dysenteriae* serotype 1, a short course of ciprofloxacin (15 mg/kg/dose every 12 hours for 3 days) is as effective as a 5-day course.[226]

Because the patterns of antibiotic resistance of shigellae change, susceptibility testing should be performed on all clinical isolates, and the treatment should be changed accordingly. The recommended duration of antibiotic therapy for shigellosis usually is 5 days. Studies in adults and children have shown, however, that short-course treatment is nearly as effective as are multiple doses in terms of symptomatic improvement,[75,140] although eradication of the organism from stools is less likely to occur with a single-dose regimen.[102] Because a major goal of antibiotic therapy is to reduce person-to-person transmission, multiple doses are preferred.

After initiation of therapy, a resistant organism can be suspected in the event of persistence of fever, grossly bloody stools, or unchanged frequency of stools by day 3 of therapy.[98] Persistent presence of numerous fecal leukocytes (>50 per high-power field) and erythrocytes (>5 per high-power field) at day 5 also suggests resistance. These findings are important because morbidity and mortality rates are higher when the organism is not susceptible to the initial drug of choice.[105] Protein-losing enteropathy is a more likely occurrence with resistant *Shigella* spp. if an inadequate agent has been used.[32]

ADJUNCTIVE THERAPY

As with other forms of infectious colitis, antimotility agents should be avoided. Antimotility drugs, such as diphenoxylate (Lomotil), prolong the duration of fever, diarrhea, and excretion of the organism.[58] It has been speculated that intestinal motility and the constant fluid flow are important host defense factors for clearing of the organism and recovery from the infection.

A high-protein diet during convalescence may be important, particularly in settings in which malnutrition, growth retardation, and hypoproteinemia is a major complication of shigellosis.[103,104,145] Vitamin A (200,000 IU) has been found to speed resolution of illness in a population in which vitamin A deficiency is a common finding.[91] Zinc supplementation in a population that is commonly deficient in this mineral improves development of shigella-specific antibody responses.[183,188]

PROGNOSIS

Most patients recover eventually with or without specific antimicrobial therapy, although illness may be prolonged and severe if it is not treated.[38] The mortality rate in developed countries is less than 1 percent, and life-threatening complications are rare events. With appropriate antibiotic therapy, defervescence usually occurs within 24 hours, and the diarrhea decreases dramatically in 2 or 3 days. If left untreated, the disease usually lasts 1 week or more. In developing countries, childhood shigellosis is associated with significant morbidity and mortality (10 to 30%),[29] particularly if it is caused by *S. dysenteriae* serotype 1. Children with malnutrition are particularly likely to have a complicated course.[47] Shigellosis in malnourished children often causes a vicious cycle of further impaired nutrition, and repeated infections may be associated with impaired growth. Young infants and children whose course is complicated by bacteremia also are at increased risk of dying.[168]

PREVENTION

In developed countries where person-to-person transmission of shigellae is the major mode of infection, personal hygiene mea-

sures are most important.[113] Special attention to hygiene should be given in daycare centers, which sometimes play a central role in community-wide outbreaks of shigellosis.[149] The close contact among children too young to control their excretions renders this setting ideal for fecal-oral spread of the organism. Children attending daycare centers frequently transmit infection to their families. Washing hands after defecating and before eating or preparing meals is important and helpful in preventing spread.[113] Daycare personnel who prepare food should avoid performing diaper-changing duties. Sick children should be excluded from the daycare center or cohorted, and mothers should be educated regarding the possibility of being infected by their children and the use of the necessary precautions. Proper cooking of potentially infected food, appropriate refrigeration, and exclusion of individuals with diarrhea from handling food are important precautions. Education of staff members in proper hygiene is essential to infection control.[177]

Patients with diarrhea in institutional and hospital settings should be isolated for prevention of outbreaks. Aggressive investigation and early initiation of appropriate antibiotic therapy in cases of bacillary dysentery are important measures in reducing excretion of virulent shigellae and stopping spread of the disease. Use of antibiotics for prophylaxis is not recommended.

In developing countries, a safe water supply and appropriate sanitation systems are important measures for reducing the risk of shigellosis. Chlorination of drinking water is important. Water stored in vessels that permit hand dipping has been defined as a risk factor.[217] Food prepared by street vendors also has been recognized as a risk factor. Prolonged breast-feeding is the best practical strategy for prevention of shigellosis (and most other enteric infections) in infants in most of the developing world.[3,53,144] Educational efforts to promote breast-feeding in these areas are key to children's survival. Human milk contains specific secretory IgA antibodies against *Shigella* LPS and virulence plasmid-coded antigens.[51] Lactoferrin and other nonspecific (non-antibody) factors in human milk, the effect of human milk on the type of intestinal flora, and the supply of an uncontaminated food source all may contribute to the protective effect of breast-feeding against diarrheal disease.

Epidemiologic data suggest that prior infection with shigellae confers resistance to subsequent illness caused by organisms of the same serotype. Serotype-specific (LPS-based) vaccines have been produced.[69,70] Although early studies showed that immunization by the parenteral route with killed vaccines was ineffective, interest in this approach continues. Polysaccharide conjugate vaccines[20,54] are now in phase 3 clinical trials.[128] Several oral, live organism–based *Shigella* vaccines have been studied. Avirulent mutants of *S. flexneri* that lack the ability to invade the intestinal mucosa are safe and effective in monkeys. Multiple doses of large numbers of organisms were required to protect humans, however. Attenuated vaccines prepared from streptomycin-dependent mutant strains were effective, but unstable.[146] Genetically attenuated *S. flexneri* strains conferred protection but caused diarrhea when fed to some volunteers. Prototype-attenuated *S. flexneri* strains CVD 1208[117] and WRSS[109] have provided encouraging results in early clinical trials. Two nonliving vaccine approaches include the oral administration of proteosomes[72] or of inactivated *Shigella* bacteria. No effective, licensed vaccine against shigellosis is available.[128,134]

Whatever prototype *Shigella* vaccines prove to be well tolerated, immunogenic, and protective, the final formulation will have to confer protection against multiple epidemiologically important serotypes, including *S. dysenteriae* 1 (the cause of epidemic Shiga), all *S. flexneri* serotypes and subtypes (the most important agents of endemic disease in developing countries), and *S. sonnei* (responsible for 5 to 15% of shigellosis in developing countries, an important cause of traveler's shigellosis, and of shigellosis in diarrhea in daycare centers in industrialized countries).[128]

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CHAPTER 120

SERRATIA

Randall G. Fisher

Similar to other members of Enterobacteriaceae, the genus *Serratia* contains species increasingly associated with opportunistic infection in the compromised host. One of the oldest bacterial organisms to be named,[105] S*erratia marcescens* is the chief species associated with disease in humans and has been associated with infection of the urinary tract, the respiratory tract, local wounds, and central venous catheters. Illness may be complicated by bacteremia and meningitis. Treatment of infection may be made exceptionally difficult because these organisms frequently are resistant to penicillins, cephalosporins, and aminoglycosides.

BACTERIOLOGY

S. marcescens can produce a red pigment resembling blood on contaminated foodstuffs. In the 6th century, the "miraculous" appearance of blood on food provoked superstition and scientific investigation. Troops were goaded into battle, and religious beliefs gained support because of the fortuitous growth of the saprophyte in bread.[105] In vitro, the red pigment produced by these strains is toxic to cancer cells but not to nonmalignant cell lines.[31]

In 1819, *S. marcescens* was named by Bizio, who correctly interpreted the discoloration of cornmeal to be due to a living organism.[69] The genus name honors the Italian physicist Serrafino Serrati, who Bizio thought had been slighted in favor of Robert Fulton as inventor of the steamboat; the species name *marcescens* was drawn from the Latin word meaning "to decay."

We now recognize the genus *Serratia* as straight, motile, catalase-positive, gram-negative rods. On solid agar, colonies are opaque; iridescent; and white, pink, or red. Organisms are Voges-Proskauer test-positive.[48] The genus may be distinguished from other enterobacteria (genera) by its use of caprylate or L-fucose as a sole carbon source, and its hydrolysis of gelatin.[48,100] Clinically relevant strains include *S. marcescens*, *Serratia liquefaciens*, *Serratia odorifera*, *Serratia ficara*, and *Serratia plymuthica*.[18,37] For a detailed review of the properties of *S. marcescens*, the reader is referred to the review by Hejazi and Falkiner.[49]

EPIDEMIOLOGY

S. marcescens was thought to be nonpathogenic in earlier times and was used as a biologic marker of transmission in 1906. In that year, N. H. Gordon, commissioned to study the atmospheric hygiene of the British House of Commons, gargled a liquid culture of *S. marcescens* and then quoted Shakespeare to an audience of agar plates in the otherwise empty House.[43,105] The organism subsequently was recovered from the plates, documenting the possibility of aerosol transmission of bacteria (Gordon reported no ill effects). The importance of *S. marcescens* as a biologic marker for hand-to-hand bacterial transmission, ascension of bacteria in the urinary tract in catheterized patients, and bacteremia after dental extraction is reviewed in detail by Yu.[105] Most remarkably, in investigations in 1950 and 1952 to judge the threat of biologic warfare to the United States, the Navy released *S. marcescens* into the Pacific, where it became aerosolized and drifted 80 m inland.[6] Although an epidemic of *S. marcescens* infection in a San Francisco hospital coincided with this event, subsequent serotype and biotype analyses cast doubt on any relationship to the Navy experiments.[36] Rather, the early San Francisco hospital experience heralded the increased frequency of nosocomial infections that would be observed in subsequent years.[105]

In some studies, *S. marcescens* is the organism most frequently isolated from contact lens solutions and contact lens cases.[82,107] Ex vivo mouse cornea studies show that the organism adheres to injured but not to intact corneas.[82] Commercial multipurpose disinfectant solutions all are active against the type strain but vary in their activity against actual clinical isolates. Polyquaternium-1–based solutions fared the worst in their activity against circulating strains.[52]

Sporadic nosocomial outbreaks of infection were reported first in the 1950s and 1960s.[66,83,103] Early outbreaks in a pediatric ward and neonatal nursery were attributed to contaminated intravenous solution and caps of bottles containing saline used to moisten umbilical cords.[66] As reviewed by Yu,[105] environmental sources before 1979 included disinfectants, water from ultrasonic nebulizers, respirators, arterial pressure monitors, and fiberoptic bronchoscopes. Environmental sources since have included suction traps,[74] intra-aortic pressure transducers,[11,101] contaminated handwashing brushes,[5] illicit intravenous drug paraphernalia,[27] contaminated urologic instruments,[34] colonized disinfectants or soaps,[35,64,67] contaminated infant parenteral nutrition fluid,[38] contaminated whole blood or blood products,[42,84] and inadequately sterilized breast milk pumps.[45]

Hand-to-hand transmission seems to be the primary mechanism of nosocomial spread. In one dramatic outbreak, spread of an organism with the same serotype, phage type, and antimicrobial sensitivity pattern was documented among four geographically separated teaching hospitals in the same region[89]; spread likely was due to passive carriage of *S. marcescens* on the hands of rotating personnel. By 1979, it was apparent that nosocomial

increase in *S. marcescens* infection was becoming a worldwide concern.[18]

Outbreaks in neonatal units and pediatric wards have been widespread, persistent, and associated with high morbidity and mortality rates.[5,15,74,97] At the peak of one epidemic of invasive *S. marcescens* disease in a neonatal nursery, more than 90 percent of infants were colonized with the epidemic strain.[33] Increased rates of colonization have been associated with nearly 10-fold increases in rates of *S. marcescens* bacteremia and meningitis.[108] In a case-control study, neonates with *S. marcescens* bloodstream infection were at least three times more likely to have associated meningitis than were neonates whose blood cultures grew *Escherichia coli*.[13] Outbreaks of multidrug-resistant strains have been especially troublesome in surgical subspecialty wards, and an outbreak has been reported in a bone marrow transplant unit.[19,57]

Biotyping may be successful in characterizing isolates, which can be traced in the hospital environment.[47,96] Ribotyping or identification of a unique biochemical characteristic has proved useful for showing cross-contamination across hematology, gastroenterology, and neonatology units in a pediatric hospital.[12,39] Use of typing methods may be particularly important because drug-resistant and drug-susceptible isolates of *Serratia* organisms may co-circulate.[25] Banding differences from pulse field gel electrophoresis of DNA digests are restricted in number from outbreak strains; increases in banding pattern may reflect genetic drift over time.[8] Such DNA techniques have aided decisions regarding cohorting and closure of neonatal units and have been used to show cross-contamination of wards.[51,72] DNA amplification techniques offer promise for characterizing isolates in future outbreaks and defining antimicrobial susceptibility.[50,64,92]

PATHOPHYSIOLOGY

Pathologic findings of sepsis are similar to the findings of other gram-negative enteric bacilli. Postmortem examination of the lungs of patients with radiologic findings of *S. marcescens* pneumonia reveals a focal necrotizing pneumonia in most cases and hemorrhagic manifestations in some.[10]

Several properties may enhance virulence of *Serratia* organisms in human infection. The 56-kd protease of *S. marcescens* seems to possess properties of a virulence factor. It enhances vascular permeability through activation of the Hageman factor–kallikrein–kinin pathway in vivo.[65] The protease also has the capacity to degrade host proteins important in humoral immune response, such as immunoglobulins and fibronectin,[73] and inactivates the chemotactic effect of C5a.[78] *Serratia* hemolysin indirectly may increase vascular permeability, local edema, and granulocyte accumulation. Clinical strains seem to possess increased adherence properties compared with environmental isolates.[9] Compared with some enteric organisms, *Serratia* organisms adhere better to epithelial cells of the bladder, which may facilitate development of urinary tract infection.[29]

Cell-mediated immunity and humoral immunity may be important in protection from *Serratia* infection and illness. In a murine model of immunization against *S. marcescens*, only the transfer of antiserum and spleen cells from vaccinated mice increased bacterial clearance from the liver and survival after infection developed.[61]

Resistance of *S. marcescens* to aminoglycosides generally is plasmid-mediated. Resistance to aminoglycosides may be conferred by one of several genes producing acetylating, phosphorylating, or adenylating enzymes.[1,2,19,53,70] Risk of acquiring infection with aminoglycoside resistance increases with exposure to these agents.[44,106] In some patients, repeated hospitalizations have shown greater importance, however, than aminoglycoside use as a risk factor for developing infection with resistant strains.[7] High levels of resistance to penicillins and cephalosporins are mediated by one or more plasmids. Cephalosporin resistance also may be derived chromosomally (see also Chapter 113).[25,40,68,81] Chromosomally mediated β-lactam resistance may be inducible in the presence of high levels of penicillin or cephalosporins, particularly when plasmid-derived β-lactamase is blocked by clavulanic acid.[20]

A group of researchers found and cloned a multidrug efflux pump of the major facilitator superfamily from *S. marcescens*.[94] Transposable plasmid elements may seem partly responsible for the rapid spread of multidrug resistance.[85,86,91] In one survey, 2.6 percent of *S. marcescens* isolates were deemed "multidrug resistant," defined as resistant to three or more classes of antimicrobials.[32] Plasmids conferring multidrug resistance are transferable from *S. marcescens* to *Klebsiella* spp. and may be responsible for sequential nosocomial outbreaks of different genera sharing common drug resistance patterns.[99]

CLINICAL MANIFESTATIONS

First described in a patient with bronchiectasis as a cause of "blood-tinged" sputum colored by the organism,[104] *S. marcescens* commonly is associated with urinary tract, respiratory tract, central venous catheter, and bacteremic infections.[2] Other species, including *S. liquefaciens*, *S. ficara*, *S. odorifera*, and *S. plymuthica*, are less common causes of disease.[13,23,30,37,93]

Chromogenic *S. marcescens* was responsible for the historically interesting and reportedly benign "red diaper syndrome," which persisted for 7 months in the infant of a genetics professor.[102] In at least one series, the organism has been identified as one of the top five causes of neonatal sepsis[46] and now is recognized as a major pathogen of compromised newborns. Disease in newborn intensive care units is associated commonly with high rates of underlying respiratory illness.[75] Other preexisting risk factors include necrotizing enterocolitis, surgical procedures, intravenous catheters, prolonged intubation, and cardiac disease.[13] Clinical illness shares features in common with other neonatal enteric pathogens; apnea, hypotension, and respiratory distress are seen frequently. Pneumonia with empyema has been reported.[56] Meningitis occurs as a complication in 24 percent of cases of neonatal bacteremia,[13] and antibiotic resistance may emerge during therapy for bacteremia or localized infection.[21] Significant brain injury caused by ventriculitis, brain abscesses, or porencephalic cysts is observed in most infants with meningitis.[21,62]

In older children and adults, *Serratia* spp. are isolated most frequently from the urinary tract.[2,89] Instrumentation, catheterization, and clustering of susceptibles are important risk factors.[7,22,53,87,90] By the 1970s, increased frequencies of serious infection, such as endocarditis, were noted in intravenous drug abusers.[27] In some series of respiratory and urinary tract infections, *S. marcescens* was observed to be associated more commonly with the complication of bacteremia than was any other enteric pathogen.[55,60] As is true of other causes of gram-negative sepsis, *Serratia* sepsis characterized by shock, pneumonia, or hemorrhage confers a substantially poorer prognosis.[16,88] The risk of these complications in cancer patients was observed by Saito and associates[88] to be lower, however, than their previous experience with other pathogens, such as *E. coli* and *Pseudomonas aeruginosa.* When predictive factors of mortality were sought in 385 subjects with nosocomial bacteremia, *S. marcescens* was not an independent predictor of death.[71] Other infections caused by *Serratia* include soft tissue infections, abscesses, endophthalmitis (including a case occurring after septicemia in an infant),[3,4] osteomyelitis

and arthritis,[98] spinal epidural abscess, and peritonitis in dialysis patients.[26]

DIAGNOSIS

The diagnosis of *Serratia* infection relies primarily on isolation of organisms from clinical material. Nonspecific laboratory tests occasionally may be misleading. *Serratia* meningitis in a neonate may be accompanied by a normal cerebrospinal fluid white blood cell count or only a modest cerebrospinal fluid pleocytosis.[21] Although *S. marcescens* is famous historically because of its chromogenic potential, most strains are nonpigmented. Hydrolysis of gelatin distinguishes *S. marcescens* from *Klebsiella* and *Enterobacter* in the clinical microbiology laboratory. The presence of ornithine decarboxylase and fermentation of sorbitol but not arabinose helps to differentiate further *S. marcescens* from other *Serratia* spp.[48] Biotyping,[47] DNA and RNA detection techniques,[64] and antimicrobial susceptibilities[96] can be used to characterize strains.

TREATMENT

Empiric decisions about antibiotic treatment of *Serratia* infection should rely on knowledge of hospital flora. Therapy should be tailored when susceptibilities are known. In newborns, meningitis and its complications should be suspected, and interventions should be guided by imaging of the central nervous system. Imaging techniques may be useful in guiding needle aspiration of abscesses.[77] Recommended antibiotic therapy for neonatal meningitis is a cephalosporin and an aminoglycoside for susceptible strains. Mortality rates remain high (>45%) even with appropriate antibiotic management.[21]

In older children and adults, reported response rates for bacteremic infection have been 75 percent for patients who received appropriate antibiotics, 22 percent for patients who received inappropriate antibiotics, and 29 percent for patients who received no antibiotics.[88] Patients who continue to have positive blood culture results while receiving appropriate antibiotic therapy have a poor prognosis. Inclusion of a penicillin or cephalosporin for susceptible strains should be considered. In the review by Saito and associates[88] of 118 patients with *Serratia* bacteremia, patients who received only an aminoglycoside had the poorest response rate among those who received appropriate therapy; patients who received a cephalosporin, alone or in combination, fared better. *Serratia* organisms generally have proven susceptible to third-generation cephalosporins in more recent surveys of hospitals from North America.[54] The physician needs to be wary, however, of potential resistance to cephalosporins and penicillins owing to the production of extended-spectrum β-lactamases. An extended-spectrum, metallo-β-lactamase–mediating resistance to imipenem has been described,[80] but it is uncommon. Physicians should consider strongly empiric use of a carbapenem in combination with an aminoglycoside in critically ill patients.

Amikacin historically has been effective in the treatment of gentamicin-resistant strains.[28,63] In 1985, amikacin was recommended as a first-line antibiotic for treatment of pediatric nosocomial infection when *Serratia* spp. or other enterics with resistance potential were suspected.[95] Since the 1980s, however, outbreaks of *S. marcescens* infections caused by amikacin-resistant strains have been reported.[79] Quinolones have been used successfully for treatment of organisms resistant to other agents,[41] but resistance to these drugs also has been identified.[59]

Cohorting and attempts to remove environmental sources of infection have been successful in ending epidemics but typically require several months.[24] Rarely, neonatal intensive care units have been closed to admissions to halt epidemics.[17,74]

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CHAPTER 121

SALMONELLA

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MICROBIOLOGY

The classification of *Salmonella* is confusing because multiple nomenclature systems are used (Table 121–1). In this chapter, we use the current designation of the Centers for Disease Control and Prevention, rather than either the complete name or the traditional clinical shorthand that referred to each of the 2463 serovars of *Salmonella* as though they were separate species. In hospital laboratories, *S.* ser. *Choleraesuis* and *S.* ser. *Typhi* are distinguished biochemically from other *Salmonella* spp. Serogroup, based on O (somatic) antigen, also usually is determined on initial isolation, and organisms that are not *S.* ser. *Typhi* or *S.* ser. *Choleraesuis* are reported as *Salmonella* serogroup A, B, C1, D1, and so on. Common *Salmonella* spp. and their serogroups are shown in Table 121–2.

TABLE 121–1 Examples of Current *Salmonella* Nomenclature

CDC Designation	Complete Name	Previous Designation
S. ser. Typhi	*S. enterica** subsp. *enterica* ser. Typhi	*S. typhi*
S. ser. Enteritidis	*S. enterica* subsp. *enterica* ser. Enteritidis	*S. enteritidis*
S. IIIa 18:z_4,z_{23}: -	*S. enterica* subsp. *arizonae* ser. 18:z_4,z_{23}: -	*Arizona hinshawii* ser. 7a, 7b:1,2,5: -
S. ser. Marina	*S. enterica* subsp. *houtenae* ser. Marina	*S. marina*

**S. choleraesuis and S. enteritidis also are designations commonly used for the species. CDC, Centers for Disease Control and Prevention.*

Salmonella serotype is defined by the serogroup antigens, the flagellar (H) antigens, and the virulence (Vi) antigen. H antigens can be either phase 1 (nonspecific) or phase 2 (specific). The Vi antigen, a heat-labile polysaccharide found on *S.* ser. *Typhi*, *S.* ser. *Dublin*, and *S.* ser. *Paratyphi C*, may block agglutina-

TABLE 121–2 *Salmonella* Species Included in Major Serogroups

Serogroup*	Representative Serotypes
A	*S.* ser. *Paratyphi A*
B	*S.* ser. *Paratyphi B*
	S. ser. *Saint-Paul*
	S. ser. *Agona*
	S. ser. *Derby*
	S. ser. *Typhimurium*
	S. ser. *Heidelberg*
C1	*S.* ser. *Paratyphi C*
	S. ser. *Choleraesuis*
	S. ser. *Montevideo*
	S. ser. *Infantis*
C2	*S.* ser. *Newport*
C3	*S.* ser. *Santiago*
D1	*S.* ser. *Typhi*
	S. ser. *Enteritidis*
	S. ser. *Dublin*
D2	*S.* ser. *Strasbourg*
E1	*S.* ser. *Anatum*
E2	*S.* ser. *Newington*
E3	*S.* ser. *Illinois*

**Human infections with organisms in serogroups E4, F, G1, G2, H, and I and the O antigens not given serogroup designation (O17 through O67) are uncommon.*

tion caused by antibodies to O antigen. Serotyping generally is done in public health department laboratories. Although serotyping is an important epidemiologic tool for defining outbreaks, it is most useful when an unusual type is associated with disease. When a common serotype is associated with an outbreak (e.g., *S.* ser. *Typhimurium*), biochemical phenotype, antibiogram, plasmid characterization,[199] bacteriophage typing,[26,87] outer-membrane protein analysis, pulsed field gel electrophoresis, and randomly amplified polymorphic DNA may help determine whether a single-strain, common-source outbreak is in progress.

Six subgroups of *Salmonella* have been proposed on the basis of DNA relatedness. Most serotypes, including almost all of the serotypes important in human and animal disease, belong to subgroup I. The genus *Arizona* now is classified as a *Salmonella*.

Salmonellae are motile (owing to peritrichous flagella), nonencapsulated, gram-negative bacilli of the Enterobacteriaceae family. Most ferment glucose, maltose, and mannitol but do not use lactose or sucrose. All pathogenic *Salmonella* spp. other than *S.* ser. *Typhi* produce gas. *Salmonella* spp. are facultative anaerobes. Blood agar or chocolate agar supports their growth when they are present as the sole organisms in blood, cerebrospinal fluid, or joint fluid. For specimens containing mixed flora (e.g., stool), selective media such as Salmonella-Shigella (SS) agar or bismuth sulfate agar must be used.

EPIDEMIOLOGY

NONTYPHOIDAL *SALMONELLA*

Public Health Issues

In most of the world, the prevalence of *Salmonella* varies according to the water supply, waste disposal, food preparation practices, and climate. The incidence of nontyphoidal salmonellosis in the United States has been increasing steadily, however, despite implementation of good public health measures. During the last 40 years, a greater than sixfold increase in reported nontyphoidal *Salmonella* infection in the United States has occurred, with an estimate of 168,000 physician office visits, 15,000 hospitalizations, and 400 deaths annually during 1996 to 1999.[252] This increase reflects industrial-scale food production and distribution,[38] misuse of antimicrobial agents (in humans and animals) that alter the gastrointestinal flora and increase host susceptibility to *Salmonella*, and probably an increasing number of immunocompromised individuals in the population. More recently, an important decrease has occurred in the incidence of *S.* ser *Enteritidis* infections in the United States, as a result of targeted interventions, including on-farm control measures, refrigeration, and education,[153] highlighting the importance of these efforts to reduce the rate of *Salmonella* infection further.

Significance of Animal Reservoirs

In contrast to *Shigella* spp., which infect only primates, nontyphoidal *Salmonella* spp. infect a variety of animals (including poultry, livestock, and pet reptiles and rodents).[232] Animals and animal products (including meat and dairy products), water, and infected humans can be the source of infection. *Salmonella* is the number one cause of foodborne illness in the United States, and the second most common cause of death from foodborne pathogens.[146] Spread of resistant organisms from food animals to humans has been shown.[166] *Salmonella* spp. have been isolated from 50 percent of poultry,[28] 16 percent of pork, 5 percent of beef, and 40 percent of frozen egg products in retail stores. Undercooked eggs (e.g., in Caesar salad, egg-dipped bread, homemade eggnog) may be contaminated by organisms on the shell surface or transovarially directly through the egg yolk. Grade A shell eggs have been implicated in more than 40 percent of more recent outbreaks.[146] Even in the absence of recognized outbreaks, eggs probably are important vehicles of infection; foods containing eggs that have been undercooked are more likely to have been consumed during the 3 days before illness in sporadic cases than in control cases.[112]

The risk of outbreaks occurring was shown when milk contaminated with *S.* ser. *Typhimurium* was distributed in Chicago, Illinois. Reports estimated that more than 150,000 people became ill, with more than 16,000 culture-confirmed cases, 2777 individuals hospitalized, and 14 fatalities.[21] Ice cream, cream cakes, and mayonnaise commonly have been incriminated as the source of infections. Fruits and vegetables rarely are vehicles.[36]

Some serotypes are associated with particular reservoirs. *S.* ser. *Dublin* is associated with dairy cattle and frequently is found in individuals who drink raw milk.[237] *S.* ser. *Choleraesuis* is associated with pigs. *S.* ser. *Typhimurium* is associated with contact with pet rodents.[232] Infection with *S.* ser. *Marina* is associated with contact with pet iguanas. *Salmonella* group F, *S.* ser. *Typhimurium*, *S.* ser. *Muenchen*, and *S.* ser. *Java* infections have been traced to pet turtles. Reptiles, including rattlesnakes, are important *S.* IIIa $18{:}z_4{,}z_{23}{:}$ (*Arizona hinshawii*) reservoirs.

Humans as a Reservoir

After infection occurs, nontyphoidal *Salmonella* spp. are excreted in feces for a median of 5 weeks. Children younger than 5 years old may excrete the organisms for 20 weeks after having an illness, but older children and adults usually excrete *Salmonella* for less than 8 weeks. *S.* ser. *Typhi* may be excreted chronically, particularly in the presence of gallbladder disease. Food handlers who are excreting *Salmonella* spp. represent an important risk group.

Bacterial Characteristics Favoring Survival

Salmonella spp. are hardy. They survive refrigeration and sometimes heating; they may remain viable at ambient or reduced temperatures for weeks. When contaminated foods are cooked for less than 12 minutes at temperatures less than 150° F (<65.5° C), salmonellae may remain viable. *Salmonella* spp. are killed by heating to 130° F (54.4° C) for 1 hour or 140° F (60° C) for 15 minutes. Salmonellae survive for hours on the hands of slaughterhouse workers.[183] The organisms have been found to survive in flour for nearly 1 year. *S.* ser. *Tennessee* has been reported to remain viable for 2 to 8 days on glass, stainless steel, enameled surfaces, rubber mattress, linen, and a rubber tabletop.[257] Nosocomial infections have been related to contaminated medical equipment (e.g., endoscopes) and diagnostic or pharmacologic preparations, particularly those of animal origin (e.g., pituitary extracts, bile salts, pancreatic extracts, pepsin, vitamins).

Relationship of Age to Risk of Disease

The highest incidence rates occur in children younger than 5 years of age, especially infants younger than 1 year, and in individuals older than 70 years. Nursery outbreaks often can be traced to an infected mother,[1,2,16,137] with subsequent spread occurring through health care personnel.[217,258] The mother of the index case can be symptomatic[82,155,207] or asymptomatic,[258] recovering from recent infection,[2,173,221] or a chronic carrier.[214] Low-birth-weight infants seem to be at higher risk than are full-term infants for acquiring *Salmonella* infection.[19,217,258] The source of infection occasionally is contaminated food, but more often it is fomites (delivery room resuscitators,[208] rectal thermometers,[123,158] suction devices,[133] water baths for heating formula,[199] soap dis-

pensers,[165] scales,[6,20,258] tables,[258] air-conditioning filters,[258] and plumbing[162]). Outbreaks in nurseries often are extraordinarily difficult to stop. They have been reported to last months[158,177,258] to years.[82,162,234] Contamination sometimes can become so widespread that other areas of the hospital also experience cases.[156,219] These outbreaks occur far more commonly with *Salmonella* than with other bacterial enteropathogens. Such outbreaks sometimes are caused by multiresistant *Salmonella*.[137]

Seasonality

Salmonella infection occurs in warm months.

Inoculum Size Required to Cause Disease

The estimated inoculum size required to cause symptomatic disease in healthy adult volunteers is 10^5 to 10^{10} organisms,[25] but the number of organisms required to cause symptoms in infants and children probably is much lower. In contrast, large inocula are not required for *Shigella* infection, which occurs in adult volunteers exposed to only 10 organisms. In some outbreaks, very small inocula of *Salmonella* seem to have caused disease. Large inocula (e.g., 10^9 organisms) may cause severe symptoms, even in healthy children.[238] The incubation period usually is 6 to 72 hours, but it depends on inoculum size, bacterial virulence, and host immunocompetence. Communicability parallels the duration of fecal excretion. The probability of salmonellosis occuring is increased when a member of the household is infected. Infants especially may be susceptible to acquiring *Salmonella* infection directly or indirectly from ill family members. Infants also may acquire infection via exposure to raw meat while riding in a grocery cart or while having contact with infected pets.[127] In a retrospective review of 187 infants younger than 1 year old with *Salmonella* gastroenteritis, 39 percent had at least one family contact with diarrhea, and 71 percent of the contacts had stool cultures positive for *Salmonella*.[259] *Salmonella* spp. rarely have been isolated during studies of gastroenteritis in daycare centers, suggesting that larger inocula are required to cause illness in toddlers and older children.[44,142,186]

Antibiotic Selection Pressure

Since the mid-1960s, *Salmonella* spp. have become increasingly resistant to ampicillin, chloramphenicol, and trimethoprim-sulfamethoxazole (TMP-SMX). Multiresistant strains have included *S.* ser. *Typhimurium*, which is the most common serotype in Europe and the United States, and *S.* ser. *Heidelberg*, *S.* ser. *Agona*, *S.* ser. *Muenchen*, *S.* ser. *Enteritidis*, and *S.* ser. *Hadar*. Antibiotic resistance usually is transferable between organisms through plasmids that carry genes encoding resistance factors. More recent studies have revealed that some serotype-specific virulence plasmids form hybrid plasmids through recombination with resistance plasmids or acquire gene cassettes consisting of multiple resistance genes. Such evolutionary events provide a virulent strain with the advantage of survival in an unfavorable drug environment.[231] Patients who are infected with antibiotic-resistant strains are more likely to be hospitalized, to be very young, to be black, and to have been exposed recently to antibiotic agents.[138] Previous use of antimicrobial agents for treatment of other illnesses is a significant risk factor for acquiring multiresistant *Salmonella* infection.[148,200]

Perhaps the most important factor is the overuse and misuse of antibiotics in animals raised for food.[49,117,118,141,229] Subtherapeutic concentrations of antibiotics used to enhance growth and to prevent infection promote intestinal colonization by antibiotic-resistant bacteria, including *Salmonella*; these organisms may be found in feces and may contaminate meat at the time of slaughter. Plasmid analysis and antibiotic susceptibility patterns have linked *Salmonella* outbreaks to specific farms and slaughterhouses.[117,118,166]

SALMONELLA SER. *TYPHI*

The Centers for Disease Control and Prevention estimates that 21 million typhoid cases occur annually in the world, with an annual incidence varying from 100 to 1000 cases per 100,000 population.[55] The global mortality estimates from typhoid also have been revised downward from 600,000 to 200,000, on the basis of regional extrapolations.[55] *S.* ser. *Typhi* is the most common *Salmonella* isolate in many developing countries. Although the overall ratio of disease caused by *S.* ser. *Typhi* to disease caused by *S.* ser. *Paratyphi* is about 10:1, the proportion of *S. paratyphi* infections is increasing in some parts of the world.[23] The human immunodeficiency virus (HIV) and acquired immunodeficiency syndrome (AIDS) epidemic in Africa has been associated with a concomitant increase in community-acquired bacteremia caused by nontyphoidal *Salmonella*.[22,102] In the United States, approximately 1700 total cases were reported (1 case per 100,000) in 1955. In 1988, approximately 400 total cases were reported (0.018 case per 100,000). Approximately 28 percent of infections occurred in individuals 19 years old or younger. Frequently, the highest incidence is stated as occurring in individuals 5 to 12 years old. More recent population-based studies from South Asia and India suggest, however, that the incidence is highest in children younger than 5 years, with higher rates of complications and hospitalization.[23,224]

In the United States, individuals traveling to developing countries are a high-risk group; 62 to 81 percent of infections are related to foreign travel, especially to Mexico, India, the Philippines, Pakistan, El Salvador, and Haiti. Of these areas, the Indian subcontinent has the highest incidence of typhoid among travelers.[5,37,163,212]

Reservoir

Humans are the reservoir for *S.* ser. *Typhi*; infection implies direct or indirect contact with an infected person. Animal products transmit *S.* ser. *Typhi* if they are contaminated by infected humans during processing. The most common mode of transmission is food or water contaminated by human feces. Water-borne typhoid fever epidemics are especially important. Congenital transmission can occur from a bacteremic mother to her fetus transplacentally or at the time of delivery.

Relevance of Inoculum Size to Disease

As with nontyphoidal *Salmonella*, more than 10^5 organisms are required to cause clinical illness in adults.[120]

Antibiotic Resistance

The worldwide frequency of antibiotic-resistant *S.* ser. *Typhi* has been increasing since the 1960s[226] but remains much lower than that of nontyphoidal *Salmonella*. Extensive protracted outbreaks have been reported throughout Asia, the Middle East, and Central and South America. These outbreaks may have been related to widespread availability and inappropriate use of antimicrobial agents (especially chloramphenicol) as over-the-counter drugs in these areas. After sporadic outbreaks of chloramphenicol-resistant typhoid between 1970 and 1985, many strains of *S.* ser. *Typhi* developed plasmid-mediated, multidrug resistance to the three primary antimicrobials used (ampicillin, chloramphenicol, and co-trimoxazole).[205] This resistance was countered by the advent of oral quinolones, but chromosomally acquired quinolone resistance in *S.* ser. *Typhi* and *S.* ser. *Paratyphi* has been

described more recently in various parts of Asia, possibly related to the widespread and indiscriminate use of quinolones.[197,223]

PATHOPHYSIOLOGY

Host susceptibility is understood most easily in terms of specific events in pathogenesis. Tables 121–3 and 121–4 show the relevance of specific host and bacterial virulence factors in salmonellosis. The outcome of ingestion of *Salmonella* depends on the bacteria and the host.

TABLE 121–3 Susceptibility to *Salmonella* Species Infection

Patient Group at Risk	Mechanism
Newborn	Achlorhydria, rapid gastric emptying Poorly developed cell-mediated immunity Complement deficiency Immunoglobulin deficiency in premature infants
Sickle-cell anemia	Reticuloendothelial system overload owing to hemolysis Functional asplenia Tissue infarcts Defective opsonization
Neutropenia (congenital or acquired)	Polymorphonuclear neutrophils needed for killing
Chronic granulomatous disease	Defective killing by polymorphonuclear neutrophils
AIDS	Low CD4 Effects of malnutrition on cell-mediated immunity Survival of organisms in macrophages (owing to *Salmonella* genes PhoP/PhoQ, spvA-D, R)
Organ transplantation, immunosuppression	Defective cell-mediated immunity
Gastrectomy	Loss of stomach acid barrier
Malaria	Reticuloendothelial overload during hemolysis Abnormal complement levels Abnormal macrophage function
Bartonellosis	Reticuloendothelial overload during hemolysis
Schistosomiasis	*Salmonella* sequestered in schistosomes protected from host defenses and antibiotics

TABLE 121–4 Putative Pathophysiologic Basis of Selected Clinical Features of Salmonellosis

Disease Manifestation	Mechanisms and Bacterial Genes
Bloody diarrhea	*sip* A-D mediated invasion and interleukin-8 mediated inflammation
Watery diarrhea	*stn* enterotoxin (cholera-like toxin) *SopB*-mediated intestinal inflammation and fluid secretion Serotypes that induce transepithelial polymorphonuclear leukocyte migration (e.g., *S.* ser. *Typhimurium*) are more likely to cause diarrhea than are serotypes that do not (e.g., *S.* ser. Typhi)
Bacteremia	*viaB* (Vi synthesis) capsular antigen interferes with C3 binding (*S.* ser. *Typhi*, *S.* ser. *Dublin*, *S.* ser. *Paratyphi C*) *rck* resistance to serum complement (virulence plasmid encoded) *rfb* encodes lipopolysaccharide synthesis; lipopolysaccharide contributes to persistence of bacteremia
Relapses, prolonged fever, failure of certain antibiotics	Survival in macrophages (*sseABC*, *spiC*, *mgtCB*, cytotoxin and virulence plasmid genes *spvRABCD*)

Various *Salmonella* strains can (1) adhere to, invade, and multiply in intestinal epithelium; (2) produce cholera toxin–like enterotoxin that increases cyclic adenosine monophosphate levels within intestinal crypt cells, causing a net efflux of electrolytes and water into the intestinal lumen; (3) be taken up by M cells overlying Peyer patches of the distal ileum and proximal colon; (4) survive in macrophages of Peyer patches, mesenteric lymph nodes, and the extraintestinal reticuloendothelial system; and (5) survive in the bloodstream.[80,81,90] Specific genes encode virulence factors necessary for each step in these processes.

Pathologic findings include hypertrophy and hyperplasia of the intestinal and mesenteric lymphoid tissues, liver, and spleen in *S.* ser. *Typhi* infection. In contrast, *S.* ser. *Typhimurium* and other nontyphoidal serotypes cause diffuse colitis, mucosal edema, and crypt abscesses as the major pathologic abnormalities.[28,58] Some virulence genes are shared by all *Salmonella* strains, whereas others are serotype-specific. Differences in invasiveness of various serotypes exist. *S.* ser. *Typhi*, *S.* ser. *Choleraesuis*, *S.* ser. *Heidelberg*,[122,160] and *S.* ser. *Dublin*[237] are more likely to enter the blood and to seed distant sites. Virulence plasmids have been identified in *S.* ser. *Typhi*, *S.* ser. *Typhimurium*, and *S.* ser. *Dublin*.[17] The presence of virulence plasmids seems to be more common in blood isolates of *S.* ser. *Typhimurium* than in fecal isolates (76% versus 42%).[79]

Nursery outbreaks of *Salmonella* have shown dramatically the variability in severity of illness related to strain or serotype. In nursery outbreaks of *S.* ser. *Oranienburg*[234] and *S.* ser. *Newport*,[137] grossly bloody stools were found in 76 to 90 percent of infected infants, with 10 to 11 percent febrile and only 9 to 11 percent asymptomatic. Watery, green, nonbloody diarrhea has been common with *S.* ser. *Typhimurium*,[2] *S.* ser. *Virchow*,[204] and *S.* ser. *Nienstedten*.[219] A high frequency of asymptomatic infections has been seen during nursery outbreaks with *S.* ser. *Heidelberg* (38% asymptomatic),[19] *S.* ser. *Virchow* (42% asymptomatic),[204] and *S.* ser. *Tennessee* (100% asymptomatic).[258]

Which genes are required for disease in humans versus animals remains unclear. *S.* ser. *Typhimurium* has genes that allow it to cause a nondiarrheal typhoidal illness in mice; in humans, it typically causes symptoms related primarily to intestinal involvement. An estimated more than 200 genes determine *S.* ser. *Typhimurium* virulence in mice. The clinical variability in host range and disease manifestations is due to the fact that *Salmonella* strains vary in their possession and expression of virulence genes.[154]

Of critical importance for in vivo virulence are the *Salmonella* pathogenicity islands (SPI), in particular SPI-1 and SPI-2. Both SPIs encode a molecular apparatus called *type III secretion system*, capable of injecting bacterial proteins through bacterial and host membranes into host cells (translocation) or the extracellular milieu (secretion) to influence host biochemistry and cell physiology directly.[47]

Genes relevant to the intestinal phase of illness are encoded primarily in SPI-1. The *invA-H* chromosomal genes are necessary for adherence to and invasion of intestinal mucosal cells[80]; most of the genes described so far seem to be involved in secretion or transport of virulence proteins.[104,248] Genes related to the *Shigella* invasion plasmid antigens (*ipaA-D*) have been described in *Salmonella* spp.[129,130]; these *Salmonella* genes (*sipA-D*) encode the proteins that interact with host cells to cause bacterial uptake and intracellular movement. Although initially characterized as an invasiveness island, SPI-1 has additional functions related to the activation of innate immune pathway, including (1) induction of polymorphonuclear recruitment across intestinal epithelium by the SPI-1 secreted effect SipA, (2) activation of nuclear factor-κB signaling by the concerted activity of SPI-1 translocated effec-

tors, and (3) activation of caspase-1–mediated interleukin-1β (IL-1β)/IL-18 activation and proinflammatory cell death by the SPI-1 translocator effector SipB.[47]

The role of host cells in the invasion process is complex. After *S.* ser. *Typhimurium* comes in contact with epithelial cells, activation of epidermal growth factor receptor occurs, which activates a kinase that turns on phospholipase A_2 so that arachidonic acid is generated. Arachidonic acid is converted to leukotriene D_4, which opens calcium channels and causes membrane ruffling, cytoskeletal changes, and uptake of bacteria.[80] Nonphagocytic cells, including epithelial cells, are adapted poorly for killing of internalized bacteria. Not only do *Salmonella* spp. survive in vacuoles within epithelial cells, but they also can replicate actively.[81] *S.* ser. *Typhi* survives better in human than in mouse macrophages, whereas *S.* ser. *Typhimurium* survives better in mouse macrophages.[31,218] *Salmonella* is capable of infecting a wide variety of cells, including dendritic cells, macrophages, hepatocytes, neutrophils, colonocytes, and other epithelial cells. In vitro, within minutes of contact with cells, *Salmonella* organisms are internalized and take up residence in a unique membrane-bound compartment distinct from a phagosome or lysosome, the *Salmonella*-containing vacuole.[101]

SPI-2 is essential for intracellular parasitism and systemic virulence in murine typhoid and for evasion of phagocyte oxidase machinery of the host.[250] SPI-2 has additional roles in inflammatory disease, such as induction of cyclooxygenase and modulation of host cytokine expression and signaling.[244,245] SPI-2 is crucial for early and complete induction of enterocolitis and systemic disease.[47]

Several pathogen-associated molecular patterns of pathophysiologic importance are presented by *Salmonella* during infection, principally lipopolysaccharides and flagellin. The activation of toll-like receptor 4 in response to *Salmonella* lipopolysaccharides is essential for inducing host responses.[249] *Salmonella* flagellin is a potent inducer of host inflammation in polarized epithelial monolayers when delivered to the basolateral surface of the epithelium. When delivered there, *Salmonella* flagellin induces IL-8 secretion by stimulating basolateral toll-like receptor 5.[266] Flagellin stimulation of innate immune responses is crucial for intestinal inflammation but not for murine typhoid.[47]

The development of diarrhea depends on host and pathogen factors. An influx of polymorphonuclear leukocytes into the mucosa must occur for diarrhea to develop.[256] Neutropenic animals fail to develop fluid secretion when they are infected with *Salmonella*[255]; infiltration of leukocytes is thought to trigger production of prostaglandin because fluid secretion can be blocked by indomethacin.[91] A cholera toxin–like enterotoxin is made by approximately two thirds of *Salmonella* strains, including *S.* ser. *Typhimurium* and *S.* ser. *Typhi*.[125]

For most nontyphoidal *Salmonella* strains, infection does not extend beyond the lamina propria and the local lymphatics. In contrast, *S.* ser. *Typhi*, *S.* ser. *Dublin*, and *S.* ser. *Choleraesuis* rapidly invade the bloodstream with little intestinal involvement. Some virulence genes confer a survival advantage to the organisms if they get into the extraintestinal milieu. Vi capsular antigen present in *S.* ser. *Typhi*, *S.* ser. *Dublin*, and *S.* ser. *Paratyphi C* interferes with C3 binding. Mutations in lipopolysaccharide genes decrease invasiveness of *S.* ser. *Typhi* and *S.* ser. *Choleraesuis*, but not of *S.* ser. *Typhimurium*.[81,168] *S.* ser. *Dublin*, *S.* ser. *Typhimurium*, and *S.* ser. *Enteritidis* have virulence genes that confer resistance to complement by preventing the formation and insertion of the C5b-9 membrane attack complex. Patients with sickle-cell anemia have complement defects and defects in opsonization of *S.* ser. *Typhimurium*.[110] Newborns also have complement deficiencies that may explain the high frequency of *Salmonella* infection in newborns and the susceptibility of newborns to bacteremic complications seldom seen in normal hosts.

Multiple host defense strategies have evolved to deal with these virulence factors; host susceptibility often can be related directly to defects in these defense mechanisms. The host tries to kill ingested organisms in the stomach, to inhibit their growth in the gut, to limit their spread beyond the intestine, and to clear them by immune mechanisms.

At a pH of 2.0, most *Salmonella* spp. are killed rapidly.[89] When gastric pH is increased by oral administration of antacid, susceptibility increases.[88,120,200] A *Salmonella* inoculum ingested in water passes through the stomach more rapidly than when the same inoculum is ingested in food. Rapid transit through the small bowel decreases the contact time of organisms with the mucosa. Patients with decreased intestinal motility caused by medication or anatomic factors have increased severity and complications and may have a prolonged carrier state. Prior antimicrobial exposure increases the risk of incurring infection with antimicrobial-susceptible and antimicrobial-resistant strains of *Salmonella*.[182] The normal flora may compete for substrates, lower the local pH by production of short-chain fatty acids, and produce antibacterial substances such as colicins. Some patients with gastroenteritis have progression or exacerbation of symptoms when antibiotics are given.[203]

Salmonella organisms are able to survive in macrophages but not in polymorphonuclear leukocytes. Patients with neutropenia (e.g., congenital, related to chemotherapy) or neutrophil dysfunction (e.g., chronic granulomatous disease) are at high risk for development of disseminated infection. Patients who have been bacteremic with a nontyphoidal *Salmonella* strain are at increased risk for having a relapse if leukopenia is present.[85]

Cell-mediated immunity generally is thought to be more important than is humoral immunity in clearance of *Salmonella* organisms. T-cell activation of macrophages is necessary to kill intracellular *Salmonella* organisms.[150] Oral immunization with an attenuated typhoid vaccine primes lymphocytes to produce cytokines typical of a T_H1 response (high interferon-γ/low IL-4) to the flagellar antigen.[236] Healthy individuals vaccinated with either oral or parenteral typhoid vaccines develop antibody-dependent cellular cytotoxicity mediated by IgA, IgG, or both.[56] Studies of serum and secretory antibodies to O and H antigens have not shown protection, however; relapses of typhoid fever have occurred despite high antibody titers. Immunity may be short-lived. In a study of 14 individuals (17 to 28 years old) with acute typhoid fever, cell-mediated immunity persisted for 16 weeks; intestinal secretory IgA persisted for 48 weeks; and IgG, IgM, and anti-O and anti-H agglutinins persisted for 2 years, 16 weeks, 16 weeks, and 36 weeks, respectively.[216]

Cytokines play a crucial role in initiating and regulating the innate and adaptive immune response against *Salmonella*. These bacteria can trigger synthesis of cytokines and chemokines in epithelial cells, macrophages, and dendritic cells. The consequences of cytokine activation vary. Although interferon-γ, IL-12, tumor necrosis factor-α, IL-18, transforming growth factor-β, and CCL2 have protective functions during *Salmonella* infection, IL-4 and IL-10 interfere with host defenses.[70]

Impaired cell-mediated immunity probably explains the high frequency of bacteremia with nontyphoidal *Salmonella* strains in children with HIV infection[209] and with malnutrition.[225] Defective cell-mediated immunity can be congenital or acquired (tumors,[107] collagen vascular disease, organ transplantation,[72] chemotherapy, glucocorticosteroids).[195] Inherited deficiency in the IL-12/IL-23/interferon-γ pathway results in susceptibility to recurrent *Salmonella* and *Mycobacterium* infections.[64] Defects have been described in which patients have mutations in IL-12, IL-12 receptor, IL-23, IL-23 receptor, interferon-γ receptor, and STAT (signal transducer and activator of transcription). Complete and partial deficiency syndromes have been described.[10,11,128]

An increased risk of acquiring disease exists in settings in which reticuloendothelial function or cell-mediated immunity is impaired[171,228] or immature.[172] Hemolytic anemias are thought to cause reticuloendothelial overload. Children with sickle-cell anemia are at risk for developing bacteremia and osteomyelitis.[152,233,262,265] Children with sickle-cell and S-Thal also sometimes develop osteomyelitis.[48] Malaria predisposes to salmonellosis by multiple mechanisms.[147] Schistosomiasis predisposes to development of *Salmonella* infections and prolonged bacteremia[201]; reticuloendothelial cell killing of *Salmonella* is impaired, and *Salmonella* colonizes the schistosomes. Pili on *Salmonella* adhere to the surface of *Schistosoma mansoni* and *Schistosoma haematobium*.[144] In Gabonese children with bacteremic nontyphoidal *Salmonella* infection, rectal biopsy specimens showed the eggs of *Schistosoma intercalatum* in 90 percent of cases.[86]

Humoral immunity is less important. Preterm neonates who are infected with *S.* ser. *Typhimurium* may have a lower risk of developing complications (e.g., intestinal perforation, meningitis, endophthalmitis, sepsis, pyelitis), however, if they are given intravenous immunoglobulin plus cefoperazone than if given cefoperazone alone (16% versus 82%); the mortality rate also is decreased (12% versus 41%).[98]

CLINICAL MANIFESTATIONS

Salmonella spp. may cause acute or chronic asymptomatic infection. Symptomatic infections include acute gastroenteritis, bacteremia with or without local suppuration, and enteric fever.

ACUTE ASYMPTOMATIC INFECTION

Asymptomatic infections usually are identified by stool cultures obtained during prospective research studies or epidemiologic investigations. A study of Mexican infants showed that 74 percent of nontyphoidal *Salmonella* infections were asymptomatic.[54]

ACUTE GASTROENTERITIS

The most common clinical illness caused by *Salmonella* infection is gastroenteritis. Nausea, vomiting, and crampy abdominal pain begin 6 to 72 hours (median 24 hours) after ingestion of contaminated food or water. The abdominal pain may be severe enough to suggest appendicitis. Diarrhea usually is moderate in volume and, depending on the serotype, may contain blood. Headaches, malaise, myalgias, and fevers are common. These symptoms usually resolve in approximately 1 week without antibiotic therapy; symptoms may persist in very young patients and patients with underlying diseases. In neonates, loose, green, mucous stools or, less often, bloody diarrhea is seen; fever is common with *Salmonella* gastroenteritis during the first months of life.[122] Reactive arthritis develops in some adults after having otherwise uncomplicated *Salmonella* gastroenteritis; this complication rarely occurs in children.

BACTEREMIA WITH OR WITHOUT METASTATIC FOCAL INFECTION

Some *Salmonella* serotypes (e.g., *S.* ser. *Typhi*; *S.* ser. *Choleraesuis*; *S.* ser. *Paratyphi* A, B, and C; *S.* ser. *Heidelberg*; *S.* ser. *Typhimurium*; *S.* ser. *Enteritidis*; *S.* ser. *Saint-Paul*; *S.* ser. *Newport*; *S.* ser. *Panama*; *S.* ser. *Dublin*) have a propensity to invade the bloodstream; others (e.g., *S.* ser. *Tennessee*, *S.* ser. *Weltevreden*[261]) rarely seem to cause bacteremia. Fever, chills, diaphoresis, myalgias, anorexia, and weight loss may last for days or weeks. Stool cultures may be negative. A child sometimes can have afebrile diarrhea and yet be bacteremic for several days.[131] The true frequency of bacteremia is uncertain. Depending on the patient's age, geographic location, and nature of the study (prospective versus retrospective), 2 to 45 percent of infections are bacteremic.[57,122,160,172,225,241,261,263] Bacteremia probably occurs more commonly in newborns (in some studies 30 to 50%) than in older children,[122] although not all studies have reached this conclusion.[160] The true risk of bacteremia developing in the first year of life is likely to be 2 to 6 percent.[57,241]

Hemolytic anemia, especially sickle-cell anemia, is associated with a high risk of development of *Salmonella* bacteremia. Persistent or recurrent bacteremia occurs in patients with AIDS, schistosomiasis, and intravascular focal infection. Immunocompromised adults who become bacteremic with *Salmonella* are more likely to do so without having had a preceding gastroenteritis and to have a high mortality rate.[109] Children more typically are relatively immunocompetent; most often, they develop bacteremia associated with diarrhea and have a much better prognosis.[139,160] In a large study in African children, nontyphoidal *Salmonella* spp. were the second most common cause of community-acquired bacteremia in all children and in HIV-positive patients.[22] Even children with neoplastic disease may have a benign course when they are bacteremic with *Salmonella* spp.,[175] although the risk for development of focal infections during bacteremia is higher (36%) in children with underlying conditions than in previously healthy children (2.5%).[264]

Focal suppurative infections may occur almost anywhere; the most common sites are bones (particularly in sickle-cell anemia)[26,233,262] and the central nervous system.[29,66,134,145,202,264] Meningitis has a high morbidity, with acute hydrocephalus, seizures, ventriculitis, abscesses, subdural empyema, and cerebral infarction. Long-term neurologic sequelae include mental retardation, hemiparesis, chronic hydrocephalus, epilepsy, visual impairment, and athetosis.[48] Neurologic sequelae are particularly common in patients who have prolonged fever (>10 days) while receiving antibiotic therapy.[121] Mortality rates from meningitis have been 40 to 60 percent in the past, even with appropriate treatment; more recent data suggest that the mortality rate is now much lower. Relapses even after prolonged therapy occur commonly (reflecting the intracellular localization of *Salmonella* and the difficulty of achieving adequate intracellular levels of antibiotics). Of nontyphoidal *Salmonella* meningitis, 50 to 75 percent occurs in the first 4 months of life.[48]

The serotypes causing meningitis, including *S.* ser. *Typhimurium*, *S.* ser. *Heidelberg*, *S.* ser. *Enteritidis*, *S.* ser. *Saint-Paul*, *S.* ser. *Havana*, *S.* ser. *Oranienburg*, *S.* ser. *Newport*, and *S.* ser. *Panama*,[48,259] are serotypes commonly associated with bacteremia. In infants, complications include pneumonia,[19] osteomyelitis,[62,136,262] septic arthritis,[19,217] pericarditis,[108,159] pyelitis,[235] peritonitis,[2] otitis media,[2] mastitis,[169] cholecystitis,[106] endophthalmitis,[53] cutaneous abscesses,[192] and infected cephalohematoma.[62] In adults and occasionally in older children, femoral and distal aorta (mycotic aneurysms),[48] heart valves,[48] scrotum,[246] testicles,[48] prostate,[215] ovaries,[48] and fallopian tubes[215] also may be infected.

Hemolytic-uremic syndrome associated with *S.* ser. *Typhimurium*[67,157] and *S.* ser. *Typhi*[18] has been reported. Because the cytotoxins produced by various *Salmonella* strains are distinct immunologically from Shiga toxin produced by *Shigella dysenteriae* 1 and the Shiga toxins produced by enterohemorrhagic *Escherichia coli*,[12] the association of hemolytic-uremic syndrome with salmonellosis may represent undiagnosed dual infection with toxin-producing organisms.

ENTERIC FEVER

Enteric fever usually is caused by *S.* ser. *Typhi* and, less often, other invasive *Salmonella*, including *S.* ser. *Paratyphi* and *S.* ser.

Choleraesuis. In contrast to sepsis caused by other gram-negative bacilli, the onset of symptoms in enteric fever is insidious.[120] After an incubation period of 10 to 14 days (range 6 to 21 days), which generally is related to the inoculum size, fever, malaise, anorexia, and abdominal pain develop over 2 to 3 days. The incubation period tends to be shorter with paratyphoid fever. The temperature rises in small increments, usually reaching 40° C to 40.5° C by the end of the first week of illness. The temperature does not return to normal but rather rises to higher peaks each afternoon, with higher nadirs occurring each subsequent morning during the first week. Eventually, the fever is unremitting; spikes in temperature occur without return to normal.

Constipation occurs in approximately 50 percent of cases, whereas diarrhea occurs in approximately 30 percent of patients. When diarrhea develops, it usually does so after the patient has been febrile for several days. It is small in volume, resembles pea soup, and contains erythrocytes but usually is not grossly bloody. Fecal leukocytes are present in nearly all patients with diarrhea. Diarrhea occurs more commonly with paratyphoid than with typhoid fever.[242] Vomiting is mild and not sustained.

A dull, continuous frontal headache begins during the first 2 days of fever; headache is present in approximately 75 percent of patients. In adults, confusion or delirium occurs more commonly than does a normal mental status. Children commonly complain of headache; they often are drowsy, irritable, or delirious.[61] Mild arthralgia involving multiple joints and vague, poorly localized back pain occur in nearly 60 percent of patients.

Physical examination during the first week may show a relative bradycardia for the degree of fever. The patient has a dull, expressionless, toxic facies; coated tongue; a musty, "damp hay–like" odor; and a tender, doughy abdomen with slight guarding. Occasionally, a child may have a cough; it tends to be minimal and unimpressive. The skin is dry with little sweating. Meningismus may occur early in the illness.

During the second week, rose spots may appear on the abdomen or chest, and less often on the back, upper arms, and thighs. The spots typically begin between days 7 and 10 as crops of 10 to 15 lesions, each measuring 2 to 4 mm. More lesions may occur in paratyphoid. They are blanching, erythematous, slightly raised lesions that last approximately 3 days. Rose spots occur in a few patients and are difficult to recognize in dark-skinned individuals. New crops of rose spots may continue for 1 to 2 weeks.

The spleen becomes palpable, soft, and tender by early in the second week of illness. Respiratory symptoms may progress, and epistaxis occasionally may occur. If left untreated, enteric fever has a prolonged course, with continuous high temperature of 39.5° C to 40.5° C for up to 4 weeks, followed by a gradual return to normal, beginning during the third or fourth week. A rapid decrease in temperature late in illness suggests intestinal hemorrhage or perforation[24]; such a decline in temperature typically is followed by an increase a few hours later as peritonitis develops. Intestinal hemorrhage and intestinal perforation[24,33,92] may occur in the second to fourth week in 3 percent of patients with typhoid fever.[33] Late in the course of untreated typhoid, the mental status changes to a "coma vigil," in which the patient lies with open eyes, mutters, and is oblivious to the surroundings.

Complications occur in 10 to 15 percent of patients; most of these develop during the second or third week of illness. Many complications, of which gastrointestinal bleeding, intestinal perforation, and typhoid encephalopathy are the most important, have been described (Table 121–5).[140,178,180,194,260] Gastrointestinal bleeding is the most common, occurring in 10 percent of patients.[180] It results from erosion of a necrotic Peyer patch through the wall of an enteric vessel. Suppurative lymphadenitis,[126] tonsillitis,[126,215] infected prosthetic heart valves,[10] and pancreatitis[211] rarely occur. Patients who have thalassemia or glucose-6-phosphate dehydrogenase deficiency may have hemolysis during typhoid fever.[242]

TABLE 121–5 Complications of Typhoid Fever

Abdominal
Gastrointestinal hemorrhage
Intestinal perforation
Hepatitis
Cholecystitis
Pancreatitis
Neuropsychiatric
Encephalopathy
Delirium
Psychosis
Meningitis
Impairment of coordination
Cardiovascular
Asymptomatic electrocardiographic changes
Myocarditis
Infected prosthetic valve
Shock
Respiratory
Bronchitis
Pneumonia
Hematologic
Anemia
Disseminated intravascular coagulation
Other
Focal abscess
Suppurative lymphadenitis
Pharyngitis
Tonsillitis
Osteomyelitis
Arthritis
Parotiditis
Orchitis
Pyelonephritis
Miscarriage
Relapse
Chronic carriage

Modified from Parry, C. M., Hien, T. T., Dougan, G., et al.: Typhoid fever. N. Engl. J. Med. 347:*1770-1782, 2002.*

The relapse rate is 5 to 20 percent, even when appropriate therapy has been given. Relapses typically occur 2 to 3 weeks after the resolution of fever and are milder than the initial illness. The *Salmonella* isolate from a patient in relapse usually has the same antibiotic susceptibility pattern as the isolate obtained from the patient during the original episode. Re-infection also may occur and can be distinguished from relapse by molecular typing.[253]

In some geographic areas, such as Indonesia, where an exceptionally virulent *S.* ser. *Typhi* is endemic, toxemia, delirium, obtundation, coma, and shock sometimes occur.[120,140] Some serotypes in Indonesia (e.g., H1-j) seem to be less virulent than others are, suggesting that properties of the flagellar antigen may be important to virulence.[105]

Typhoid fever varies in its clinical course. Variations on the classic theme include a completely afebrile course occurring in debilitated patients, a high spiking fever from the first day (particularly in children), a focal presentation (e.g., pneumonia, nephritis), and a severe course during relapses. Infants are said to be at higher risk for development of massive hepatomegaly, thrombocytopenia, and other complications.[193] The mortality rate is high in the neonatal period.[196] Children younger than 5 years old may have a nonspecific illness that is not recognized clinically as typhoid[224]; infants and toddlers often have a febrile illness misinterpreted as a "viral syndrome." In children younger

than 2 years old, the fever may last for only 1 to 5 days, despite the presence of *S.* ser. *Typhi* or *S.* ser. *Paratyphi* in the blood; low-grade fever (temperature of 38.3° C to 38.8° C) and cough may be the only findings in such children.[77] The case-fatality rates are highest among children younger than 1 year and among elderly patients. The most important contributor to a poor outcome is probably a delay in instituting effective antibiotic treatment.[180]

Typhoid and nontyphoidal *Salmonella* infections during pregnancy increase the risk of spontaneous abortion.[165,230] Spontaneous abortion or premature labor usually can be prevented by early treatment.[222] Transmission of *S.* ser. *Typhi* rarely occurs in utero.[40] Typically, premature delivery occurs during the second to fourth week of untreated maternal typhoid fever.[103] In the pre-antibiotic era, 40 percent of women with typhoid delivered prematurely; the remainder carried to term, although only 17 percent of infants survived.[60] If infection occurs late in gestation and is treated appropriately, the infant may survive.

ASYMPTOMATIC CHRONIC CARRIER STATE

Chronic carriers excrete *Salmonella* organisms in stools for longer than 1 year after having gastroenteritis-enterocolitis or enteric fever. Approximately 1 to 4 percent of patients who recover from enteric fever caused by *S.* ser. *Typhi* chronically excrete the organism[164]; less than 1 percent of patients with nontyphoidal *Salmonella* excrete for a prolonged period.[30,39] Twenty-five percent of prolonged carriers have no history of typhoid.[180] Nontyphoidal infection is associated with excretion for a mean of 5 weeks, although children younger than 5 years old,[30] female patients, elderly patients, and patients with biliary tract disease are more likely to become carriers. The biliary tract is infected in almost all chronic carriers of *S.* ser. *Typhi*. As many as 10^6 organisms per 1 g of feces may be excreted.[164]

The significance of chronic excretion is that such patients serve as a source of infection to their contacts. Chronic carriers represent an epidemiologically important reservoir of *S.* ser. *Typhi*; they often are the source of outbreaks of typhoid fever. In the United States, although typhoid fever generally is imported, 30 percent of infections result from exposure to previously diagnosed or newly diagnosed chronic carriers.[212]

Patients who have a history of *S. haematobium* or tuberculous infections of the urinary tract may develop chronic urinary carriage after a bout of typhoid fever.[73,201] Other predisposing conditions include hydronephrosis, strictures, and kidney stones.

DIAGNOSIS

The symptoms in *Salmonella* gastroenteritis overlap sufficiently with symptoms seen in other diarrheal illnesses that laboratory studies generally are required to prove the diagnosis. On the rare occasions when proctoscopy has been done, typical findings have included mucosal edema, hyperemia, friability, and hemorrhages.[58] The fecal leukocyte examination is positive for polymorphonuclear leukocytes in 36 to 82 percent of nontyphoidal cases,[111,187] but this finding is nonspecific. Stool culture is preferable to swab culture, particularly for evaluation of long-term carriers. Overnight enrichment in selenite broth increases the yield from stool cultures. The optimal agar for isolation of the organism (SS agar, Hektoen enteric agar, MacConkey agar, xylose-lysine-deoxycholate agar, xylose-lysine-Tergitol 4 agar, brilliant green agar, or modified semisolid Rappaport Vassiliadis medium) is debated. Modified semisolid Rappaport Vassiliadis medium has a high yield but cannot be used for isolating *S.* ser. *Typhi* or nonmotile strains and has a lower specificity than SS agar.[210]

Salmonella organisms usually can be isolated readily from blood by use of conventional media if the patient is bacteremic. In patients with extraintestinal focal nontyphoidal infection, specimens from the affected areas may have positive Gram stains and grow the organism.

Enteric fever should be suspected on the basis of the setting and clinical course. Laboratory abnormalities are common but nonspecific findings. A normocytic, normochromic anemia and leukopenia or neutropenia, perhaps caused by hemophagocytosis in the bone marrow, often are present.[151] Clotting abnormalities consistent with disseminated intravascular coagulation (e.g., thrombocytopenia, hypofibrinogenemia) may occur[32] but usually are transient and not associated with clinically significant bleeding. In enteric fever, electrolyte values usually are normal, but increases in alkaline phosphatase, serum lactate dehydrogenase, serum aspartate aminotransferase, and serum cholesterol occur frequently. A transient proteinuria sometimes occurs during the first week of enteric fever.

Cultures from multiple sites should be submitted for suspected enteric fever; culture of bone marrow has the highest yield,[114,116,247] particularly if the patient has had antibiotic pretreatment. During the first week of typhoid fever, approximately 90 percent of patients have positive blood and bone marrow cultures, but negative stool and urine cultures. During subsequent weeks, the yield of blood and bone marrow cultures decreases as the yield of stool and urine cultures increases. Culture of duodenal fluid obtained by string capsules can be as sensitive as culture of bone marrow aspirates.[15,116,247] The overall frequencies of positive cultures during the course of typhoid are found in blood (40 to 54%), urine (7 to 10%), stool (approximately 35%), bone marrow (80 to 90%), rose spots (approximately 65%), and duodenal string test culture (58 to 85%).[93,116]

The Widal test measures antibodies against the O and H antigens of *S.* ser. *Typhi*. Although many patients with enteric fever may have a fourfold increase in the titer of paired sera during the second week of illness, false-negative and false-positive test results occur. Patients with acute or chronic liver disease and patients infected with other gram-negative enteric bacilli may develop cross-reacting antibodies. Recipients of the typhoid vaccine show positive Widal test results, which can be misleading. These titers may be more useful in children with typhoid who are living in a nonendemic area, such as the United States. Although patients who have a negative titer early in infection tend to maintain a negative titer, most develop titers of 1 : 80 or more.[50] Interpretation of Widal test results is aided by information about seropositivity in the population to which the patient belongs.[45,243] Some centers have found Widal's test helpful when it is used with locally determined cutoff points.[46,181]

Various diagnostic kits, including serologic tests such as passive hemagglutination, passive bacterial agglutination, latex particle agglutination slide tests, counterimmunoelectrophoresis, radioimmunoassay, and enzyme-linked immunosorbent assay with use of monoclonal antibodies, have been developed.[124] These commercial assays (Typhidot, Typhidot-M, Tubex) have not proved to be sufficiently robust in large-scale evaluations in community settings.[23] Molecular techniques used primarily in epidemiologic studies include DNA hybridization studies, phage typing, chromosome analysis, and plasmid analysis.

DIFFERENTIAL DIAGNOSIS

Salmonella gastroenteritis cannot be distinguished clinically from other infectious causes of acute diarrhea reliably, although history and epidemiology sometimes may suggest an etiologic agent. Bloody diarrhea with mucus can be caused by *Salmonella*, *Shigella*, enteroinvasive *E. coli*, enterohemorrhagic *E. coli*, *Campylobacter* spp., *Yersinia enterocolitica*, *Clostridium difficile*, *Trichuris trichiura*,

and *Entamoeba histolytica*. Watery diarrhea may be caused by rotavirus or other viral enteropathogens or enterotoxin-producing bacterial pathogens. When abdominal pain and tenderness are severe, the differential diagnosis includes appendicitis, perforated viscus, and mesenteric adenitis.

Enteric fever can mimic other infections of the reticuloendothelial system, including Epstein-Barr virus infection, disseminated histoplasmosis, tuberculosis, ehrlichiosis, brucellosis, leptospirosis, tularemia, plague, malaria, systemic *Bartonella henselae* infection, and typhus. Noninfectious illnesses with prolonged fever that sometimes can be confused with typhoid include juvenile rheumatoid arthritis and other collagen vascular diseases, Kawasaki syndrome, and lymphomas. An early diagnosis often is difficult to establish because the findings are nonspecific. Findings that are particularly helpful in discriminating typhoid fever from other prolonged febrile illnesses include severe cough and chest pain (more typical of lobar pneumonia), diarrhea with grossly obvious blood (more typical of dysentery), acute onset of chills (more typical of malaria), and marked lower abdominal pain early in the febrile illness (more typical of bacillary dysentery, *Y. enterocolitica* infection, and salpingitis). For patients in countries where typhoid is not endemic, a history of travel is crucial.

TREATMENT

For children with salmonellosis for whom antibiotic treatment is appropriate, the interpretation of antimicrobial susceptibility studies is important. Drugs such as aminoglycosides, polymyxins, tetracyclines, and first-generation and second-generation cephalosporins (e.g., cephalothin, cefazolin, cefuroxime, cefamandole) have a poor clinical track record, despite apparent in vitro susceptibility. Table 121–6 shows the drugs that typically are useful in treatment of children with *Salmonella* infections. The emergence of *S.* ser. *Typhimurium* DT104 in the United States has led to a dramatic increase in multidrug-resistant (ampicillin, chloramphenicol, streptomycin, sulfonamides, tetracycline) organisms.[97] Most nontyphoidal *Salmonella* strains in the United States still are sensitive to ampicillin, chloramphenicol, amoxicillin–clavulanic acid, and TMP-SMX; ceftriaxone resistance, although described, is rare.[113]

GASTROENTERITIS

As with all forms of gastroenteritis, fluid and electrolyte replacement and maintenance are the first order of business. For most patients, oral rehydration is all that is necessary to treat *Salmonella* gastroenteritis. Generally, *Salmonella* gastroenteritis should not be treated with antibiotics because these agents do not shorten the course of illness. Multiple agents,[11] including ampicillin, amoxicillin,[132,170,184] neomycin,[13,184] chloramphenicol,[149] TMP-SMX,[132,213] azithromycin,[43] cefixime,[43] ceftriaxone,[42] and ciprofloxacin, have been shown to be ineffective.[213] Antibiotics prolong excretion of *Salmonella*.[11,13,63,132,170,184] *Salmonella* serotypes typically have been grouped together for these treatment studies, however, as though they were all the same organism. Given the variability in expression of virulence genes, whether treatment may be useful for some serotypes that possess particular virulence traits remains an open question.

TABLE 121–6 Antibiotics Commonly Used in the Treatment of *Salmonella* Infections

Drug	Dose
Ampicillin	200 mg/kg/day in 4 doses PO, IM, or IV
Trimethoprim-sulfamethoxazole (TMP-SMX)	10 mg/kg/day TMP, 50 mg/kg/day SMX in 2 doses PO or IV
Cefotaxime	100-200 mg/kg/day in 3 doses IM or IV
Ceftriaxone	50-100 mg/kg/day in 1 or 2 doses IM or IV
Cefixime	10-20 mg/kg/day in 1 or 2 doses PO
Chloramphenicol	50-75 mg/kg/day in 4 doses PO
Azithromycin	8-12 mg/kg/day in 1 dose PO
Ciprofloxacin	10-20 mg/kg/day in 2 doses PO or IV

Exceptions to the generalization that *Salmonella* gastroenteritis should not be treated include children at high risk for developing complications, including children with underlying diseases or receiving therapies that impair host defenses. Examples of children who probably ought to be given antibiotics are infants 3 months old or younger; children with AIDS or malignant diseases; and children with hemolytic anemias, particularly sickle-cell anemia. Treatment of these patients is debatable; the data from neonates suggest that antibiotics make little difference in the course.[2,71,132,198,234] Because the risk of development of bacteremia is high, however, antibiotics likely will continue to be used in such settings. Because bacteremia occurs in a small fraction of infections, determining whether treatment of gastroenteritis prevents bacteremia is impossible without conducting a massive study.

When treatment is given, probably 5 days or fewer of antibiotics is indicated, barring complications. Although antibiotic resistance is an increasingly important problem, patients who require antibiotic therapy for *Salmonella* gastroenteritis not thought to be life-threatening usually should be given ampicillin or amoxicillin, pending susceptibility testing.

EXTRAINTESTINAL INFECTIONS

Any child who appears to be sufficiently toxic that bacteremia is suspected also should be started on antibiotic treatment until blood cultures exclude the diagnosis. For children with bacteremic nontyphoidal *Salmonella* and focal extraintestinal complications, a third-generation cephalosporin (e.g., ceftriaxone, cefotaxime) or chloramphenicol is an appropriate choice. If the patient seems to have a life-threatening infection, ampicillin should be used only if evidence exists that the pathogen is not ampicillin resistant. Children at high risk of having recurrence of bacteremia (children with congenital or acquired immunodeficiencies, such as AIDS) may require a third-generation cephalosporin or a fluoroquinolone to achieve cure; frequent recurrences of life-threatening infection sometimes necessitate use of lifelong maintenance therapy.[51,119]

Meningitis should be treated with a third-generation cephalosporin because these agents have good penetration into CSF; ampicillin and chloramphenicol use has been associated with higher relapse rates and lower cure rates than are third-generation cephalosporins.[134] Meningitis must be treated for at least 4 weeks; approximately three fourths of patients who have relapses have been treated for 3 weeks or less.[48] A bactericidal agent, such as ampicillin or a third-generation cephalosporin, is preferred for treatment of endovascular infections (e.g., endocarditis, mycotic aneurysm).

For extraintestinal infections, the duration of antibiotic treatment usually is 10 to 14 days in children with bacteremia, 4 to 6 weeks in children with acute osteomyelitis, and 4 weeks in children with meningitis. Collections of pus should be drained. Schistosomiasis, when present, must be treated to achieve resolution of the coincident *Salmonella* infection.

TYPHOID FEVER

The response to treatment with antibiotics is slow. Fever may persist for many days, even after bacteremia has resolved. The

emergence and spread of multidrug-resistant *S.* ser. *Typhi* (MDRST) since 1989 has caused a shift in empiric therapy from chloramphenicol, TMP-SMX, or ampicillin to a fluoroquinolone in adults and a third-generation cephalosporin, such as ceftriaxone, azithromycin, or a fluoroquinolone, in children (Table 121–7). MDRST is particularly common in the Indian subcontinent, Southeast Asia, and Africa; strains resistant to ciprofloxacin are being recognized increasingly.[205]

In the United States, MDRST is less problematic than it is elsewhere; most strains are sensitive to ampicillin and chloramphenicol.[212] More recent data suggest that *S.* ser. *Typhi* is still sensitive consistently to ceftriaxone and ciprofloxacin, although nalidixic acid–resistant isolates are becoming more common in individuals who have traveled to the Indian subcontinent.[5] Such strains respond poorly to fluoroquinolones and may require several courses of treatment.[254] When the patient has a history of recent travel to an area with MDRST or of contact with an individual returning from such an area, the choice of empiric treatment should take this information into account. Third-generation cephalosporins are effective against *S.* ser. *Typhi* strains resistant to ampicillin, chloramphenicol, and TMP-SMX,[74,124,134,161,167,179,227,240] and are appropriate for children with suspected or proven MDRST. Some studies suggest that cefoperazone may have advantages in typhoid fever over chloramphenicol treatment (more rapid sterilization and defervescence),[179] perhaps related to its biliary excretion.[59] Data suggest that TMP-SMX may not be as effective as is ampicillin or chloramphenicol in typhoid fever.[94] Aztreonam also is less effective than is chloramphenicol for strains susceptible to both agents.[99]

Although 2 weeks of antibiotic treatment usually is given, data suggest that shorter courses with some drugs may be adequate. A short course of ceftriaxone (once daily for 3 to 5 days) is as effective and safe as is a 2- to 3-week course of chloramphenicol in adults and, on the basis of relatively small numbers, probably in children as well.[3,167] A 5-day course of ceftriaxone (50 to 70 mg/kg/day as a single dose) was associated with a significantly more rapid defervescence (average 3.9 days until afebrile) than was oral cefixime (7.5 mg/kg/dose twice daily for 14 days) or intramuscular aztreonam (50 to 70 mg/kg/dose every 8 hours for 7 days); relapse rates were similar (approximately 5%).[96]

TABLE 121–7 Recommended Antibiotic Treatment of Typhoid Fever

Susceptibility	Antibiotic	Daily Dose (mg/kg)	Days
Full susceptible	Chloramphenicol, *or*	50-75	14-21
	Amoxicillin, *or*	75-100	14
	Trimethoprim-sulfamethoxazole, *or*	8/40	14
	Third-generation cephalosporin (e.g., cefixime), *or*	15-20	7-14
	Fluoroquinolone (e.g., ofloxacin or ciprofloxacin)	15	5-7
Multidrug-resistant	Azithromycin	8-10	7
	Third-generation cephalosporin (e.g., cefixime), *or*	15-20	7-14
	Fluoroquinolone (e.g., ofloxacin or ciprofloxacin)	15	5-7
Quinolone-resistant	Azithromycin, *or*	8-10	7
	Third-generation cephalosporin (e.g., cefixime), *or*	20	7-14
	Parenteral third-generation cephalosporin (e.g., ceftriaxone), *or*	75	10-14
	Fluoroquinolone (e.g., ofloxacin or ciprofloxacin)	20	10-14

Strong evidence supports fluoroquinolones as the most effective drugs for the treatment of typhoid fever. These drugs have proved safe in all age groups, are rapidly effective, and are associated with lower rates of stool carriage than traditional first-line drugs. There are three main issues regarding the use of fluoroquinolones: (1) the potential for toxic effects in children, (2) the cost, and (3) the potential emergence of resistance.[180] In preclinical testing, the fluoroquinolones damaged the articular cartilage of young beagles. A considerable body of evidence exists from the long-term use of these drugs in children with cystic fibrosis and from short-term use to treat typhoid fever and dysentery in children. No bone or joint toxicity or growth impairment has been found. The production of generic fluoroquinolones in many countries has reduced the price considerably. The emergence of quinolone resistance in areas where the drugs are inexpensive and readily available is likely to be the greatest limitation on their use.[180]

Concerns about toxicity of fluoroquinolones in children have limited their use to situations in which infection is caused by an organism proved to be resistant to all of the usual antibiotics but sensitive to a fluoroquinolone. If a fluoroquinolone is used, ciprofloxacin or ofloxacin is superior to norfloxacin because it has inadequate oral bioavailability. Ciprofloxacin (500 mg twice daily for 10 days in adults) causes defervescence in an average of 4.2 days with infrequent relapses, even with MDRST.[7] Children with MDRST who have been treated with ciprofloxacin (10 mg/kg/day) became afebrile in 3.3 days, and 94 percent achieved clinical cure, with no relapses or carriers detected on follow-up.[68] Ofloxacin (20 mg/kg twice daily for 10 days) is associated with more rapid defervescence than TMP-SMX.[220] Ofloxacin is associated with more rapid defervescence and better cure rates than cefixime.[35] Three days of ofloxacin (15 mg/kg/day in two doses for 3 days) causes defervescence more quickly than chloramphenicol (50 mg/kg/day in four doses for 14 days).[185] Very short courses of ofloxacin (2 or 3 days) may be followed by relapse,[251] however, especially if the organism is resistant to nalidixic acid.[174]

Other agents have been described that occasionally may be useful. Furazolidone (7.5 mg/kg/day) is nearly as effective as is chloramphenicol in strains susceptible to both drugs (86% versus 90% cure).[69] Despite low serum levels, azithromycin seems to be equivalent to chloramphenicol or ciprofloxacin; the high intracellular concentration in macrophages (>100 times serum levels) of azithromycin presumably accounts for its efficacy.[34,95] When MDRST is resistant to nalidixic acid, azithromycin is more effective than is ofloxacin.[41]

A 5-day course of oral azithromycin (20 mg/kg/day, maximum 1000 mg/day) is as effective as intravenous ceftriaxone (75 mg/kg/day, maximum 2.5 g/day for 5 days).[84] Various nonantimicrobial measures should be considered as part of the management of *S.* ser. *Typhi* infections. Dexamethasone, although potentially increasing the relapse rate,[52] is indicated for patients with severe typhoid fever presenting with delirium, stupor, shock, or coma; the dose is 3 mg/kg initially and then eight doses of 1 mg/kg every 6 hours for 48 hours. This therapy reduces mortality rates from 35 to 55 percent to 10 percent.[115,191] Parenteral fluoroquinolones are probably the antibiotic of choice for severe infections, but no randomized trials of such treatment have been performed.[68] Antipyretics were thought at one time to be dangerous in typhoid fever[65]; whether this concern is correct is doubtful on the basis of more recent experience.[176]

Intestinal hemorrhage or perforation during enteric fever generally is considered to be an indication for surgical intervention.[24,33,92,143] Resection of 10 cm of intestine proximal and distal to the perforation seems to improve outcome compared with other surgical approaches.[14] Antibiotic coverage should be broad-

ened to include anaerobes and gram-negative enterics when perforation occurs.[24]

CHRONIC CARRIERS

Generally, patients who are not food handlers probably should not be cultured or given special treatment after having a bout of gastroenteritis caused by a nontyphoidal *Salmonella* strain. Carriers of *S.* ser. *Typhi* should be decolonized to decrease the risk to close contacts. Carriers who have a normal gallbladder can be treated with high-dose intravenous ampicillin, oral ampicillin, or amoxicillin combined with probenecid for 6 weeks or, when a multiresistant organism is present, with a fluoroquinolone, such as norfloxacin[100] or ciprofloxacin.[78] Chronic carriers who cannot be decolonized are treated with cholecystectomy if cholelithiasis or cholecystitis is present; such patients should receive ampicillin intravenously for 7 to 10 days before and 30 days after cholecystectomy.

PREVENTION

PUBLIC HEALTH MEASURES

Recognition of an increased frequency of human infections with an unusual serotype should be followed by an epidemiologic investigation aimed at detecting the source and vehicle. Intervention to stop such outbreaks then can be attempted. Judicious use of antibiotics in dairy and livestock animals,[117] careful food processing and storage, and proper preparation of foods are helpful in decreasing transmission of infection. Appropriate sewage disposal, assurance of a safe water supply, prevention of sale of pet turtles, inspection of cosmetics for contamination, and adequate cleaning of medical equipment are important public health strategies. Families with small children should be informed of the risks associated with pet reptiles and encouraged to avoid such unnecessary risks.

PERSONAL HYGIENIC MEASURES

Person-to-person spread can be decreased by handwashing after defecating or changing diapers, frequent handwashing during preparation of foods that might be contaminated (e.g., meat), and excluding of infected individuals from food-handling tasks.

INFECTION CONTROL

Hospitalized children with *Salmonella* gastroenteritis should be isolated (enteric precautions) until stool cultures are negative. Children with extraintestinal infections should be isolated until stool studies exclude intestinal infection or colonization.

NURSERY OUTBREAKS

Neonatal *Salmonella* infection outbreaks should be investigated to determine the source. Cultures of fomites sometimes reveal a removable focus. Neonates and staff members caring for them should be cohorted during outbreaks, with use of enteric precautions in dealing with infants who are excreting the organism. Surveillance cultures should be done on feces of not only sick infants but also well infants to cohort more appropriately. With current early postpartum discharge policies, reporting *Salmonella* infections in infants is important in detecting outbreaks. Isolation and cohorting can be effective in controlling such outbreaks.[198]

BREAST-FEEDING

In the developing world, breast-feeding is key in prevention because human milk contains secretory IgA and other factors that protect infants from *Salmonella* spp.[27,76,83,188,189] In a case-control study conducted in the United States, breast-feeding was found also to have a strong protective effect against sporadic *Salmonella* infections.[206] Pediatric health care providers and community education programs should encourage mothers to breast-feed.

VACCINATION

Several vaccines have been developed for typhoid fever. Vaccination of children is indicated when the risk for development of typhoid fever is high (e.g., living with a chronic carrier or in an endemic area) but probably is underused.[239] Two vaccines are available: (1) an oral live attenuated Ty21a vaccine (Vivotif Berna; Swiss Serum and Vaccine Institute) and (2) a parenteral purified Vi capsular polysaccharide vaccine (Typhim Vi; Aventis Pasteur).[135,190]

The Ty21a vaccine has been evaluated in liquid and capsule forms. Ty21a oral vaccine is well tolerated; abdominal pain, nausea, vomiting, and rashes occur rarely. The form licensed in the United States is an enteric-coated capsule preparation meant to be given in four separate doses on alternate days taken 1 hour before meals. It is not recommended for children younger than 6 years of age. The four doses should be completed 1 week before potential exposure. Revaccination with the entire four-dose series is recommended every 5 years in high-risk settings. Because the Ty21a oral vaccine is a live-attenuated *Salmonella*, it should not be used in immunocompromised hosts or in individuals taking antibiotics at the time of receiving vaccination.[37] The antimalarial mefloquine inhibits growth of the attenuated organism, and vaccination should be delayed for 24 hours after its use. Malaria prophylaxis with atovaquone and proguanil does not interfere with immune response to Ty21a.[75]

Several large field trials suggest that the Vi capsular vaccine as a single 25-μg dose (0.5 mL) has an efficacy of 55 percent and 75 percent in adults and children older than 5 years.[4,190] Although fever, malaise, local pain, and tenderness occur with this vaccine, it has two major advantages over the Ty21a oral vaccines: It does not require refrigeration, and only a single dose is required for protection. It may be used in children 2 years of age. Vaccination must occur at least 2 weeks before exposure.

PROGNOSIS

Salmonella gastroenteritis usually is a self-limited disease in the normal host, although chronic diarrhea sometimes develops after an acute episode. Extraintestinal focal infections with nontyphoidal *Salmonella* strains are difficult to cure, particularly if they involve the meninges or occur in compromised hosts. *Salmonella* spp. meningitis may relapse if the course of treatment is too short. Likewise, bacteremia and focal infection recur after treatment in severely compromised hosts, particularly patients with AIDS. Relapse after typhoid fever has long been recognized as a risk.

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CHAPTER 122

PLAGUE *(YERSINIA PESTIS)*

Gary D. Overturf

HISTORY

Yersinia pestis has been responsible for the most devastating epidemics in human history. Possibly the first mention of plague dates back to approximately 1320 B.C.E., in I Samuel (Old Testament), Chapters 5 and 6. The next recorded outbreak may have been in 542 during Justinian's reign, when an estimated 100 million people died.[48] In 1346, plague appeared during the siege of the city of Kaffa in the Crimea, thereafter spreading throughout most of Europe, where it became known as the *black death*. One third of the population of Europe died in its aftermath. Between the 14th and 20th centuries, plague remained endemic in most of Europe and Russia, with resultant frequent outbreaks.[43]

In 1894, Yersin and Kitasato, working independently, first described the plague bacillus,[47] and the role played by rats and fleas in the spread of the disease became known. In 1900, plague was introduced into San Francisco by rats aboard ships docking there. The disease spread to ground squirrels and then to other wild animals of the American Southwest.[46] In 1943, effective antibiotics against *Y. pestis* became available. In the United States today, plague is rare, but it continues to be endemic in many parts of the world, leading to more recent outbreaks in Madagascar, Peru, and India. Although antibiotics have been effective in the treatment and prophylaxis of plague, plasmid-borne antibiotic resistance is being noted increasingly.[19] The potential of aerosolized *Y. pestis* as a biologic weapon is being scrutinized, leading to renewed interest in the understanding and study of this organism.[29]

BACTERIOLOGY

Y. pestis belongs in the family Enterobacteriaceae and is one of 10 *Yersinia* spp. It is a small, pleomorphic, nonmotile, gram-negative bacillus. With Wayson, Giemsa, and Gram stains, the bacillus takes on bipolar or safety-pin morphologic features.[17] *Y. pestis* grows at temperatures ranging from 0° C to 40° C (32° F to 104° F); the optimal temperature is 28° C (82.4° F). On first isolation at 35° C (95° F) on 5 percent blood agar, the colonies are pinpoint in size, growing to 1 to 2 mm after 2 days. They are nonhemolytic on 5 percent sheep blood agar.

Depending on the clinical nature of the disease, blood cultures, sputum samples, or aspirates of enlarged nodes should be examined for typical bacilli. The isolated bacilli can be identified by the following criteria. *Y. pestis*, the sole *Yersinia* spp. that is nonmotile at 37° C and 22° C (98.6° F and 71.6° F), also is the sole *Yersinia* spp. that is urease-negative, but it may be positive in freshly isolated strains. The organism is positive for esculin, β-galactosidase, catalase, and methyl red. Oxidase, indole, and Voges-Proskauer reactions are negative. It ferments glucose, maltose, salicin, xylose, arabinose, dextrin, trehalose, and mannitol. It does not produce acid from lactose, sucrose, rhamnose, melibiose, adonitol, cellobiose, sorbose, or dulcitol. It does not use citrate, and it does not grow in potassium cyanide. *Y. pestis* is negative for lysine, ornithine decarboxylase, and arginine dihydrolase.[44,53]

Positive cultures show pinpoint colonies within 24 to 48 hours after inoculation. Many laboratories use fully automated or semiautomated identification systems (e.g., Vitek) that may not detect *Y. pestis*, often confusing *Y. pestis* and *Yersinia pseudotuberculosis* or *Yersinia enterocolitica* with plague bacillus. Nonautomated laboratories may require 6 days to identify the organism. Because of lack of standarized susceptibility testing procedures, suspected isolates must be referred to public health or other reference laboratories to comply with the select agent requirements of the Title II Enhanced Controls for Dangerous Biological Agents and Toxins Act.[29]

Several chromosomal-mediated virulence factors are responsible for the virulence of *Y. pestis*, including (1) an antiphagocytic capsular material known as fraction 1, (2) the endogenous purine synthesis that allows the organism to grow within macrophages, and (3) the ability to absorb iron from the medium. Several plasmids have been implicated in the development of other virulence factors. A plasmid of 9-kb pairs contains the determinant of secretory protein that kills other bacterial strains. A plasmid of 72-kb pairs, which all pathogenic *Y. pestis* strains contain, confers the requirement for environmental calcium to be present for the organism to grow at 37° C (98.6° F). When grown under this condition, *Y. pestis* produces V and W antigens that are necessary for virulence.[21] An exotoxin and an endotoxin have been found to contribute to the lethal effects of plague.[53]

TRANSMISSION

HOST

More than 200 mammalian species have been reported to be naturally infected with *Y. pestis*. Epidemics of plague usually are transmitted by the fleas of infected domestic rats, however. This form of spread is more likely to occur in urban, rat-infested, and crowded dwellings and may result in epidemics. In the United States, plague is transmitted sporadically to humans after contact with an enzootic sylvatic focus.[31] Infected wild rodents perpetuate the plague bacillus in a given ecosystem by virtue of their ability

to withstand an inoculum of *Y. pestis* many times greater than that necessary to cause disease in humans or domestic animals. After inoculation, wild rodents may become bacteremic and infect fleas that feed on them; these fleas transmit the plague bacillus to another rodent. Hibernating animals are especially resistant to clinical infection. Animals inoculated before going into hibernation may survive through the winter and not die until after they come out of their burrows, reintroducing the bacillus in the new season.[26] Carnivores are relatively resistant to infection but contribute to the spread of the organism by transporting infected fleas from one area to another.[31]

The role of domestic animals in bridging the gap between sylvatic plague and human infection has been studied extensively.[41,47,51] Cats and dogs are susceptible to natural and experimental plague. Epizootics in cats have been observed in conjunction with plague epidemics in humans.[47] Experimentally infected cats develop severe systemic illness, with bacteremia and abscess formation at the site of the inoculum. Between 1977 and 1998, 23 cases of feline-associated human plague infection were reported.[22] Five of these cases were fatal; two of the patients presented with primary pneumonic plague, and one presented with septicemic plague. Many of these cases were misdiagnosed at presentation, leading to delays in treatment and, in some cases, fatalities. This diagnosis should be considered especially in the western states of Arizona, California, Colorado, and New Mexico. No seasonal variation in the occurrence of cat-associated illness was noted.[22]

Ten other cases reported in the literature of feline transmission of plague to humans involved four veterinarians.[18] Dogs also are susceptible, but the disease is milder.[51] Swine are resistant to plague, the only evidence of subclinical infection being the presence of antibodies to the fraction 1 antigen of *Y. pestis*. Domestic animals, by virtue of their intimate contact with wildlife and humans, may be responsible for some cases of human plague. This danger is accentuated by a dearth of symptoms in some animals.[41]

VECTOR

Plague is transmitted to humans by the bite of an infected flea, the skinning and evisceration of infected animals,[32] or the inhalation of infected droplets from a case of pneumonic plague.[35] Infrequent portals of entry include the conjunctiva[41] and the pharynx.[40,49]

The efficiency of the flea as a vector for human disease depends on the likelihood that the infected flea will feed on a person and that the flea will regurgitate the bacillus into the victim's bloodstream in the process of feeding.[31] Flea species vary in both of these attributes. Wild rodent fleas are reluctant to feed on humans and do it only under duress (e.g., when the natural host dies). Fleas of domestic animals are more likely to bite humans. The Oriental rat flea, *Xenopsylla cheopis*, is the most efficient transmitter of plague because of its willingness to bite people and its propensity for regurgitating large numbers of bacilli in the process.[31] When the Oriental rat flea ingests infected blood, the actions of a coagulase produced by *Y. pestis* and a trypsin-like enzyme present in the flea's stomach result in the formation of an infected clot that blocks the flea's proventriculus. In obstructing the flea's intestinal tract, the clot allows further replication of bacteria. When the flea tries to feed again, it regurgitates large numbers of plague bacilli. The formation and dissolution of the fibrin clot are temperature-dependent. At temperatures greater than 27° C (>80.6° F), a fibrinolytic enzyme is activated that dissolves the clot and allows the flea to dispose of the bacillus. One postulation is that this temperature-dependent phenomenon is responsible for the observed cyclic nature of urban plague epidemics, which tend to subside with the advent of hot weather.[7]

In contrast, fleas in which the intestinal tracts do not become blocked contribute to the endemicity of plague by harboring the organism and transmitting it in sublethal doses.[9] Sylvatic plague depends on the rodent flea as the vector. This flea, although not as efficient as the rat flea in transmitting the bacillus, may itself become a reservoir of *Y. pestis* by surviving for 12 to 15 months after the original host dies. In the new season, it reintroduces the plague bacillus into the new rodent population.[47] The observation that *Y. pestis* can survive in the soil during interepizootics suggests another possible mechanism of transmission of plague.[25]

EPIDEMIOLOGY

More recent studies have suggested that plague is an ancient disease of mammals; the plague bacterium emerged 1500 to 20,000 years ago as a clone that evolved from *Yersinia pseudotuberculosis* that first caused outbreaks in Africa.[1] The plague bacillus exists in enzootic cycles involving wild animals or domestic rats. In urban plague, the course of events usually is initiated by the introduction of the plague bacillus from an enzootic focus into a susceptible rat population. With humans and rats living in proximity, an epizootic in rats may be followed by an epidemic in humans.[47] The epidemic may subside with the advent of hot, humid weather[7] or the obliteration of the rat population.[47] Such epidemics rarely occur today but have been described in the former South Vietnam[4,33] and more recently in India.[13] Between 1985 and 1999, 23 countries reported a total of 29,020 cases of plague to the World Health Organization, with an average mortality rate of 11 percent; major epidemics and outbreaks occurred in Tanzania (1991), Zaire (1992, 1993), Peru (1993, 1994), India (1994), and Madagascar (1995), with fatality rates ranging from 4.6 to 22.3 percent.[55]

In the United States today, humans become infected most frequently by direct contact with a sylvatic reservoir of infection. Sporadic human cases usually result from working or hunting in a plague-infested area[31] and increasingly from living near foci of infection as suburban spread encroaches on the natural habitats of rodents.[14] In recent years, domestic animals, especially cats, have been responsible for a significant proportion of human cases. During 1990 to 2005, 107 cases of plague were reported in the United States, a median of 7 cases a year. By September 2006, 13 plague cases, with 2 deaths (case-fatality 15%), had been reported from four states (New Mexico, Colorado, California, and Texas). Such resurgences often occur after ecologic changes resulting in population increases in ground rodents such as prairie dogs, ground squirrels, mice, and rats in the American Southwest.[15]

Sylvatic plague epizootics occur in the summer. Most cases of rural plague occur between April and September. The rare occurrence of human plague in the winter usually is associated with hunting and direct exposure to infected tissues.[12,32]

The continental United States has a large enzootic focus that includes 130 counties in 15 western states. Surveillance for plague in rodents during the 1990s has identified infected animals farther east than ever before reported. The plague bacillus now has been isolated in wild rodents in eastern Montana, western Nebraska, western North Dakota, and eastern Texas.[14] Between 1925 and 1965, the number of reported cases in the United States averaged between two and three per year.[8,10] During the 1970s, 105 cases were reported.[32] The number of cases reported in 1980 through 1982 showed a similar increasing trend.[10] Between 1970 and 1979, 53 percent of cases were in females, in contrast to the period 1926 to 1969, when only 27 percent were in females. Approximately 60 percent of cases occur in individuals younger than 20 years.[32] Of 10 confirmed cases reported in the United States in 1993, however, the age distribution was 22 to 96 years. Five of the patients were older than 65 years.[14] Native Americans

living on reservations in Arizona, New Mexico, and Utah are at increased risk. In the period 1970 to 1979, 35 percent of cases in these three states occurred in Native Americans.[10,32] Many of the patients were infected within 1 mile of their residence and almost all within their state of residence.[27] Seven of the 10 patients described in 1993 were exposed in their home sites, and one, a veterinarian, was exposed at work.[14]

Occasionally, plague has been acquired by a traveler in an endemic area who then traveled during the incubation period to a plague-free region of the country. This set of circumstances shows why all physicians need to be aware of the presenting symptoms and signs of plague and to obtain an accurate travel history.[34]

PATHOGENESIS AND PATHOLOGY

The portal of entry of the plague bacillus determines, to some extent, the form of disease. The most common portal of entry is the skin when it is bitten by an infected flea. Broken skin may provide access for direct inoculation while infected animals are being handled. After overcoming the skin barrier, the organisms move through the lymphatics to the regional lymph nodes, where they elicit an inflammatory response. The infection may be localized at this site, with subsequent formation of antibody and recovery. This clinical form is known as *pestis minor*. The bacillus commonly is disseminated through the bloodstream. Distant organ involvement may include the liver, spleen, kidneys, lungs, and meninges. Disseminated intravascular coagulation is common in fatal cases. Coagulation defects, including thrombocytopenia and elevated fibrin split products[4] and fibrin deposits in the glomeruli,[21,47] may be present. Bacteremia is not synonymous with severe disease and occurs commonly in mild cases.[47]

The major determinant of severity seems to be the presence of high levels of endotoxin. The toxin of *Y. pestis* has the biologic properties of typical endotoxin. When injected into experimental animals, it can cause the clinical symptoms and signs and pathologic changes characteristic of endotoxic shock and death. The quantity of endotoxin necessary to kill is estimated to be comparable to that present in a lethal dose of live bacteria.[2,54] The murine toxin of *Y. pestis* has a direct inhibitory effect in vitro on the respiration of heart mitochondria of rats and mice, whereas it has little or no effect on the mitochondria of rabbits, chimpanzees, dogs, and monkeys. The differing sensitivities in vitro correlate with the susceptibilities in vivo of these species to *Y. pestis* infection.[50]

Achieving high levels of toxin depends on the ability of the bacillus to replicate in the infected host. Resistance to phagocytosis had been assumed to be related to virulence. More recent experimental evidence has shown that virulent *Y. pestis* organisms are phagocytosed, but, in contrast to avirulent ones, are not killed. They continue to replicate freely in macrophages, allowing the accumulation of endotoxin.[30,50]

When the lung is the portal of entry, the disease usually is more fulminant. After being inhaled, bacilli replicate freely in the alveolar spaces. Severe pneumonia, endotoxemia, and septicemia ensue and, if untreated, cause death. In fatal cases, the thoracic lymph nodes show infarction, necrosis, and liquefaction, with pus formation. Edema and inflammation of the surrounding tissue are common.[47] The mucosa of trachea and bronchi is covered by bloody, frothy exudate. Submucosal hemorrhages and areas of necrosis may surround the trachea. The pleural surfaces contain hemorrhagic lesions and fibrinous adhesions. The lung parenchyma may be consolidated or show signs of acute edema.[47] The predominant histologic feature is an alveolar exudate consisting of histiocytes and polymorphonuclear leukocytes.[21]

Other organs also are involved. The kidneys may appear grossly hemorrhagic and contain areas of necrosis. Microscopic examination reveals leukocytic infiltrates of congested veins and capillaries. Glomeruli with fibrin thrombi frequently are found in patients with disseminated intravascular coagulation.[21] Biopsy of purpuric skin lesions reveals subepithelial hemorrhages and fibrin deposit in the capillaries. These changes are indistinguishable from the changes seen in a generalized Shwartzman reaction.[4]

CLINICAL MANIFESTATIONS

The incubation period of *Y. pestis* generally is 3 to 4 days, but ranges from a few hours to 10 days. The onset of illness usually is abrupt, beginning with fever, malaise, weakness, and headache.[47,48] Fever is high, frequently accompanied by shaking chills.[41] The appearance of a visible and palpable bubo may be preceded by pain and tenderness at that site.[48]

On physical examination, the patient is "toxic," apprehensive, and tachycardic. The inoculation site in the skin may not be evident, or it may be marked by a carbuncle. In bubonic plague, typical large, fixed, edematous, and exquisitely tender nodes are present at one anatomic site.[33] In decreasing order of frequency, the areas of nodal involvement are the groin (including femoral and inguinal nodes), axilla, and neck.[47] Any lymph node may suppurate, sometimes presenting an atypical picture (e.g., if intra-abdominal nodes are involved, an acute abdominal emergency may be suspected).[48] Septicemia as an initial presentation of *Y. pestis* infection is common.[27] Twenty-five percent of the patients presented without adenopathy in the 71 confirmed cases of plague in New Mexico from 1980 to 1984. All patients with septicemic presentation had fever and chills, and most had tachycardia, tachypnea, and relative hypotension. Seventy-two percent had gastrointestinal symptoms. Plague pneumonia was twice as likely to occur among septicemic as among patients with bubonic plague. Septicemic patients were significantly older and more likely to die than were patients with a bubonic presentation. Although septicemic plague occurred more often in older patients, patients younger than 30 years with septicemic presentation were more likely to die.[27]

As a result of its nonspecific presentation, septicemic plague is difficult to diagnose early. Of 27 patients with plague admitted to Indian Medical Center in Gallup, New Mexico, between 1965 and 1989, 5 presented with a nonspecific febrile syndrome with upper respiratory symptoms. They were prescribed penicillin. Three of the five patients died. Another five patients presented with a nonspecific febrile syndrome associated with chills, myalgias, and anorexia. These patients were not treated initially with antibiotics, and three of the five died.[18] The index of suspicion must be high because early diagnosis is imperative for avoiding a high risk of mortality. Individuals presenting with what seems to be community-acquired, gram-negative sepsis and who reside in or have a history of recent travel to endemic areas of plague must be evaluated for and treated with antibiotics effective against *Y. pestis*.[39]

Gastrointestinal symptoms occur in patients with plague, especially patients with septicemic plague.[26] Between 1980 and 1984, more than half of the 71 patients with plague in New Mexico presented with gastrointestinal symptoms that sometimes preceded the appearance of the buboes in the bubonic cases. Common symptoms are abdominal pain, nausea, vomiting, and diarrhea. These symptoms are thought to be a general response of the body to gram-negative septicemia. Occasionally, hepatosplenomegaly and mesenteric or retroperitoneal lymphadenopathy have masqueraded as an acute abdomen.[28,34]

Neurologic manifestations caused by the effects of toxin on the brain are common. A patient with plague may have insomnia, delirium, stupor, weakness, staggering gait, vertigo, disorders of speech, and loss of memory.[38] *Y. pestis* meningitis is a rare occur-

rence, but it does occur. Children younger than 15 years old seem to be more susceptible, and septicemic patients are four times more likely to develop meningitis than are patients with bubonic plague. It often manifests while the patient is well into a course of antibiotic therapy for bubonic or septicemic plague.[3] When intravascular coagulation supervenes, renal involvement may be manifested by acute cortical or tubular necrosis. Hepatic involvement may be evidenced by mildly elevated liver enzymes.[47] Hantavirus pulmonary syndrome may mimic septicemic or pneumonic plague; they share similar geographic distribution, and patients for whom this diagnosis is contemplated should be treated with antibiotics to cover the possibility of plague.[45]

Primary pneumonic plague has identical constitutional symptoms but follows a fulminant course with a more pronounced pulmonary component. Within 20 to 24 hours after the onset of the illness, tachypnea, dyspnea, and cough productive of bloody mucopurulent sputum supervene. If early and effective treatment is not instituted, the patient usually dies.[47]

DIFFERENTIAL DIAGNOSIS

Because of the rare incidence of plague today, the diagnosis often is delayed or missed. Bubonic plague may be confused with other diseases affecting the skin and lymph nodes. The diagnosis of staphylococcal or streptococcal adenitis can be established easily by culture. Lymphogranuloma venereum is more indolent, has milder systemic symptoms, and is associated with anogenital ulcer. Syphilitic adenitis usually is nontender. With cat-scratch disease and *Pasteurella multocida* infections, the constitutional symptoms are few, and the patient typically has a history of animal exposure. Tularemia has a more gradual onset.[47] In their later stages, the ulcerated skin lesions of plague may resemble anthrax.[33]

DIAGNOSIS

The most important factor in promptly establishing the diagnosis of plague is having a high index of suspicion. Suspicion should trigger immediate notification to the local or state health department. The state reference laboratory can arrange for rapid diagnostic tests.

Bacterial staining of lymph node material by Giemsa, Wayson, or Wright stain often shows the typical bipolar plague organisms. In the septicemic form of the disease, similar bacterial staining of venous blood frequently permits visualization of the plague bacillus.[36] Fluorescent antibody staining of direct smears and tissues may provide a rapid, presumptive diagnosis of plague.[29] More recent rapid tests, such as enzyme-linked immunosorbent assay for F1 antigen and polymerase chain reaction, have shown promise in the laboratory or in outbreaks, but they are not widely available,[12] (*pla* and *caf1* genes).

TREATMENT

Therapeutic decisions cannot await culture results. All patients suspected to have plague should receive prompt antimicrobial therapy after appropriate blood and tissue have been obtained for cultures, fluorescent antibody staining, and serologic testing.

The sulfonamides and streptomycin proved effective when they were introduced first in the 1940s. Resistant strains to one or the other of these antibiotics soon appeared.[6,49] Despite the paucity of published trials in humans on antibiotic effectiveness, other than streptomycin and tetracycline, the Working Group on Civilian Biodefense Consensus Statement[29] recommends gentamicin as an effective alternative to streptomycin for patients needing parenteral antibiotics. Gentamicin has been used frequently in recent years and has shown comparable outcomes to streptomycin in one case series.[3] In vitro and in vivo studies in mice corroborate its effectiveness against *Y. pestis* infections.[53]

For acutely ill patients thought to have plague infection, streptomycin and gentamicin are the drugs of choice. If available, streptomycin is given intramuscularly, 20 to 30 mg/kg/day in two divided doses.[38] Gentamicin is administered intravenously or intramuscularly, 7.5 mg/kg/day to children and 3 to 5 mg/kg/day to adults in three divided doses. Antibiotic susceptibility testing should be done because *Y. pestis* plasmid-mediated, multiple-antibiotic resistance has been described.[23]

When plague meningitis develops, chloramphenicol, 50 to 100 mg/kg/day (after administration of an initial dose of 25 mg/kg) intravenously in four divided doses, is the treatment of choice. Duration of therapy is determined by the length and severity of the illness. Treatment is continued for at least 7 days in patients with uncomplicated disease.[13]

Patients older than 8 years who do not require hospitalization receive tetracycline at a dose of 25 to 50 mg/kg/day every 4 to 6 hours up to a total daily dose of 1 g in children and 2 g in adults. When outpatient treatment is given, the patient should be observed closely for the first 3 days to ensure resolution of the disease.[13] Sulfonamides may be used for prophylaxis in pediatric patients as an alternative to the tetracycline class of antibiotics.[29] Doxycycline at a dose of 4 mg/kg/day (up to a maximum of 200 mg) divided into two doses may be substituted for tetracycline).

PROGNOSIS

In outbreaks of untreated plague, the mortality rate has ranged from 40 to 70 percent. Pneumonic plague almost invariably is fatal without treatment. With prompt specific antimicrobial therapy, the overall mortality rate for plague has decreased to 5 percent.[35] Complications during convalescence include polyarthritis, small lung abscesses, delayed suppuration of buboes,[33] and meningitis. *Staphylococcus aureus* and *Pseudomonas* spp. may superinfect involved lymph nodes.[48] Immunity usually ensues after clinical or asymptomatic infection occurs, but natural re-infection rarely has been observed.[48]

PREVENTION AND CONTROL

Institution of hygienic measures and eradication of rats from areas of human habitations have all but eliminated epidemics of urban plague. When epizootics occur in wild rodents, control measures must be directed against rodents and fleas. Vector control can be achieved by the use of insecticides in fields and housing areas. In plague-endemic areas, the public must be instructed to avoid burrows, not to handle sick or dead rodents, to deflea household pets, and to eliminate trash near living areas.[10] The immune status of domestic animals can be used as a surveillance tool to ascertain the presence of *Y. pestis* in the community. Dogs, cats, and swine develop antibodies to the fraction 1 antigen of *Y. pestis*.[41,51]

Patients with plague should be isolated with respiratory precautions until they are bacteriologically sterile. Contacts of patients with pneumonic plague should receive chemoprophylaxis with tetracycline at 25 to 50 mg/kg/day up to 2 g in adults and up to 1 g in children 8 years and older. Younger children receive trimethoprim-sulfamethoxazole at 40 mg/kg/day (sulfamethoxazole) in two equal doses orally.[29] The 6-day quarantine period for international travel for contacts of patients with plague does not guarantee the clearance of the bacillus from asymptomatic pharyngeal carriers.[6] Public and professional education in endemic zones is paramount for ensuring prompt reporting of human and animal cases.

Plague vaccines had been used since the late 19th century for individuals at high risk for occupational exposure. They are no longer being manufactured in the United States. Research in this area is continuing.[29]

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CHAPTER 123

OTHER *YERSINIA* SPECIES

Charles R. Woods, Jr.

Yersinia spp. are gram-negative, coccobacillary organisms that are primarily zoonotic. The genus is a member of the family Enterobacteriaceae and consists of 11 species, 3 of which clearly are human pathogens:[69] *Yersinia pestis*, *Yersinia pseudotuberculosis* (both formerly included in the genus *Pasteurella*), and *Yersinia enterocolitica*. *Y. pestis*, the causative agent of plague, is found in rodents and insect vectors and is discussed in Chapter 122.

Y. enterocolitica and *Y. pseudotuberculosis* are responsible for a variety of syndromes, some of which originally were called *pseudotuberculosis*. Infections caused by these enteropathogenic *Yer-*

sinia spp. now are collectively called *yersiniosis*, which is the focus of this chapter. Molecular phylogenetic analysis suggests that *Y. pestis* evolved from *Y. pseudotuberculosis* 1500 to 20,000 years ago.[4] The genomes of representative strains of the three pathogenic *Yersinia* spp. have been sequenced.[194]

During the past 3 decades, *Y. enterocolitica* has been recognized as an important human pathogen worldwide and is a common cause of gastroenteritis in some pediatric populations of the industrialized world.[1,47,56,118] *Y. enterocolitica* also has drawn attention because of its immunologic or postinfectious manifestations, which include reactive arthritis and erythema nodosum.[47] *Y. pseudotuberculosis*, although widespread in nature, is a much less common cause of human disease.[69] In Japan, it has been associated with clinical illness that sometimes has resembled Kawasaki disease.

HISTORICAL ASPECTS

In 1883, Malassez and Vignal[130] described a bacterium that produced a disease they named *pseudotuberculosis*. When injected into guinea pigs, the organism produced tuberculosis-like lesions. It grew at 4° C and multiplied better at 22° C than at 37° C. This observation was confirmed in 1910 by Albrecht,[11] who labeled the disease *enteritis follicularis suppurativa*. The first case of mesenteric adenitis, the most common syndrome produced by *Y. pseudotuberculosis*, was reported in 1913 by Saisawa.[173] A second instance of disease that indicated its capacity to produce death (bacteremia and multiple hepatic abscesses) was recorded in 1949 by Hassig and colleagues.[79] Masshoff[132] was the first to recover the organism from a culture of mesenteric lymph nodes of a patient with the clinical picture of acute appendicitis. Masshoff and Dolle[133] subsequently described the histologic picture produced by *Y. pseudotuberculosis*. In 1954, Knapp and Masshoff[106] first reported the clinical features of infection produced by this organism. Only 14 cases of this infection had been described up to that time.[55]

The existence of a species of *Yersinia* other than that causing pseudotuberculosis was suggested in 1933 by Gilbert,[62] who reported an unusual infection in animals. Schleifstein and Coleman[174,175] examined numerous organisms that were isolated between 1933 and 1957 from stool cultures of human cases of diarrhea from which *Salmonella* and *Shigella* organisms could not be recovered and that resembled the infections in animals reported by Gilbert. These investigators identified an organism that had not been described previously and named it *Bacterium enterocoliticum*. In 1964, it was named *Y. enterocolitica* by Frederiksen.[59]

The genus is named for A. J. Yersin, the French bacteriologist who first isolated the plague bacillus.[186] During the past 40 years, an extensive literature detailing the microbiology, pathology, epidemiology, molecular pathogenesis, and clinical features of disease caused by *Y. pseudotuberculosis* and *Y. enterocolitica* has accumulated.*

MICROBIOLOGY

Yersinia organisms are large (0.5 to 1 μm × 1 to 2 μm or larger), gram-negative, and ovoid or rod-shaped. *Y. enterocolitica* and *Y. pseudotuberculosis* are motile at 22° C to 25° C but not at 37° C. Similar to other members of Enterobacteriaceae, *Yersinia* organisms are facultative anaerobes and grow well on ordinary media. On Gram staining, *Y. pseudotuberculosis* appears as a large coccobacillus. Staining with methylene blue and carbol fuchsin discloses a bipolar (safety pin) morphology of most, but not all, strains. *Y. enterocolitica* is smaller and shows little, if any, bipolarity.[183,186]

Yersinia organisms may be confused with coliforms, such as *Escherichia coli*, *Morganella*, *Proteus*, *Shigella*, *Salmonella*, and *Providencia*, or with *Y. pestis*, *Brucella*, and *Achromobacter*, unless careful biochemical and physiologic studies are conducted. *Yersinia* organisms reduce nitrates and are oxidase-negative, catalase-positive, urease-positive, and citrate-negative. They ferment glucose, maltose, mannitol, glycerol, xylose, and fructose, producing acid but no gas with each sugar. *Yersinia* organisms usually do not ferment lactose but produce α-galactosidase. They do not ferment dulcitol, inositol, raffinose, or rhamnose. On lysine-iron agar slants, *Yersinia* organisms produce an alkaline slant with an acid butt. They do not produce hydrogen sulfide. The Voges-Proskauer reaction is negative at 37° C but may be positive at 25° C for some strains of *Y. enterocolitica*. All strains of *Y. pseudotuberculosis* and most strains of *Y. enterocolitica* isolated in Europe are indole-negative. Most strains of *Y. enterocolitica* found in the United States have been indole-positive.[50,66,88,146,151,183,186]

Although these two species of *Yersinia* share many properties, they are distinguishable on the basis of several biochemical activities, antigenic structure, and sensitivity to various *Yersinia* phages.[171] *Y. enterocolitica* produces an acid slant and acid butt on triple sugar iron agar caused by fermentation of sucrose, whereas *Y. pseudotuberculosis* produces an alkaline slant and an acid butt. *Y. enterocolitica* elaborates ornithine decarboxylase and ferments sucrose and amygdalin. *Y. pseudotuberculosis* does none of these, but it ferments adonitol, which *Y. enterocolitica* does not.[88,186]

Commercially available tests used to identify Enterobacteriaceae in clinical laboratories may not contain the biochemical reactions needed to identify specific *Yersinia* spp. Traditional macroscale biochemical testing may be required to distinguish *Y. enterocolitica* and *Y. pseudotuberculosis* from nonpathogenic *Yersinia* spp.[73,122] On solid culture media, colonies of the pathogenic species typically are small, smooth, opaque colonies. The colonies of nonpathogenic species are larger and more translucent.[100]

TYPING OF *YERSINIA* STRAINS

Biotyping and serotyping have been the predominant methods used to characterize strains of *Y. enterocolitica*. At least 54 to 60 serotypes of *Y. enterocolitica* exist on the basis of variability of somatic O antigens, but only 11 typically are associated with human disease.[21,23,56,69,197] Six biotypes of *Y. enterocolitica*, designated 1A, 1B, and 2 to 5, have been defined on the basis of differences in the following: lipase activity; salicin acid production; esculin hydrolysis; indole production; ornithine decarboxylase; Voges-Proskauer test; pyrazinamide activity; nitrate reduction; and fermentations of xylose, trehalose, sorbose, and inositol.[23,33,196]

Only a few serotype : biotype combinations are regarded as human pathogens: O:8, O:4, O:13a,13b, O:18, O:20, and O:21 (biotype 1B); O:9 and O:5,27 (biotype 2); O:1,2,3 and O:5,27 (biotype 3); O:3 (biotype 4); and O:2,3 (biotype 5).[202] These pathogenic strains, which carry *Yersinia* virulence plasmids (pYV), generally have negative test results for pyrazinamidase activity, esculin hydrolysis, and salicin fermentation. Nonpathogenic strains, generally of biotype 1A, have positive test results for each of them.[41,54,98] Data suggest that some biotype 1A strains that lack pYV and other *Yersinia* virulence factors also may cause gastroenteritis.[68,179]

Strains of *Y. enterocolitica* can be typed genetically by repetitive element-based (inter-repeat) polymerase chain reaction (PCR), arbitrarily primed PCR,[148] pulsed-field gel electrophoresis,[141] and

*See references 14, 21, 23, 24, 45, 47, 53, 55, 81, 82, 87, 92, 104-107, 118, 125, 137, 169, 183, 185, 186, 189, 200, 212.

ribotyping.[123] These methods allow distinction among strains within biotypes and serotypes and may be useful for outbreak and other epidemiologic investigations.

At least 11 antigenic groups of *Y. pseudotuberculosis* exist on the basis of variation of somatic O antigens. They have been labeled 1a, 1b, 2a, 2b, 2c, 3, 4a, 4b, 5a, 5b, and 6.[84,91,129] Type 2 is related antigenically to *Salmonella* group B, and type 4 is related to *Salmonella* groups D and H.[66] *Y. pseudotuberculosis* strains also can be typed by arbitrarily primed PCR.[129]

EPIDEMIOLOGY

Although most of the early reports of yersiniosis caused by *Y. pseudotuberculosis* and *Y. enterocolitica* came from northern Europe, these microbes have been identified with increasing frequency in all parts of the world,[13] with the possible exception of South America. During 1996 to 1998, the rate of foodborne disease caused by yersiniosis microbes in five areas of the United States was 1 per 100,000 population.[35]

YERSINIA ENTEROCOLITICA

Y. enterocolitica is distributed worldwide but is isolated most frequently in cooler climates.[138] Whether such geographic differences reflect differences in reservoirs or culinary practices that may enhance the risk of acquisition of this organism, or rather represent differences in surveillance for the disease and use of more sensitive culturing techniques in these areas, is unclear.[47] Increased frequency of infections during fall and winter has been reported from Europe[192] and the United States,[1] but no seasonality is evident among outbreaks of disease where more than three cases of *Y. enterocolitica* disease have been identified.[47]

Geographic differences in serotype distribution and frequency also exist. Sporadic infections caused by serotypes O:3 and O:9 are common in Europe,[9,85] but outbreaks rarely have occurred.[47] In North America, multiple serotypes have been responsible for sporadic disease,[18,22,33,65,177,181] but more recently serotype O:3 has become predominant.[33,51,118] Five outbreaks in the United States have been caused by serotype O:8, and two outbreaks in Canada have been caused by serotypes O:5 and O:5,27.[47] Disease caused by serotype O:8 has been reported in Europe.[85]

The true incidence and prevalence of *Y. enterocolitica* infection are unknown.[47] The reported proportional frequency of isolation of *Y. enterocolitica* from stool cultures from patients with diarrhea has ranged from 0 to 3.2 percent in series from Europe, the United States, and New Zealand (Table 123–1).[49,56,85,118,131,136,177] Symptomatic infection occurs more commonly in children. Most series show a slight male predominance of approximately 1.3 : 1.[51,56,131,196] Surveillance at five FoodNet sites in the United States during 1996 to 1999 found that African-American infants had far higher rates than white infants, with a peak occurrence from November to February (Fig. 123–1).[162]

Animals and water sources are the primary environmental reservoirs for *Y. enterocolitica*, but the biotypes and serotypes of the strains found in them usually differ from those causing human disease.[33,47,177] Blood transfusions also may be a source of *Y. enterocolitica* infection.[34]

Animal Reservoirs

Y. enterocolitica strains have been isolated from a wide variety of mammals (dogs, pigs, sheep, rabbits, guinea pigs, cows, horses, chinchillas, monkeys), frogs, fish, flies, fleas, snails, crabs, and oysters. Birds do not seem to be a major reservoir for *Y. enterocolitica*, although avian isolates have been reported.[47,88,125]

Pigs seem to be an important reservoir for the human pathogenic serotypes O:3 and O:9 in Europe and Japan and serotype O:3 in North America and South Africa.[47,118,158] The biochemical and phage typing profiles of isolates from pigs are similar to those of strains commonly responsible for human infections.[200] *Y. enterocolitica* has been isolated from the tongue, tonsils, and cecal contents of swine and from pork, ham, and butcher shop cutting boards.[47,66] Pig farmers in Finland were 3 times and 2.4 times as likely to have seropositivity to serotypes O:3 and O:9 than were berry farmers.[176]

Wild rodents captured in areas of Japan where human infections caused by *Y. enterocolitica* serotype O:8 had occurred were shown to harbor isolates of the same serotype.[43] Two distinct serotype O:8 strains, defined by restriction enzyme analysis of the virulence plasmids, were isolated from humans and rodents. This finding suggests that rodents are a potential source of sporadic human infection in Japan.

Apparent transmission to humans from dogs and cats also has been reported. A fecal-oral or oral-oral route has been postulated but not confirmed. Little evidence supports airborne or insect vector–borne transmission.[47]

Foods and Water

In countries with high numbers of cases of yersiniosis, ingestion of raw pork has been common. Infection with serotypes O:3 and O:9 was highly associated with ingestion of raw pork during the 2 weeks preceding the illness in a case-control study.[192] In Belgium, laboratory surveillance that began in 1967 showed yearly increases in cases through 1986. After a media campaign was launched to dissuade people from eating raw or undercooked

TABLE 123–1 Percentage of Stool Cultures Yielding *Yersinia enterocolitica*

Country	Years	Population	Total Cultures	Percentage *Y. enterocolitica*
Canada[131]	1977-1978	Symptomatic children	6364	2.8
The Netherlands[85]	1982-1984	Enteritis patients <40 years old	827	2.9
Italy[136]	1981-1985	Children with diarrhea	2500	1.4*
New Zealand[56]	1988-1993	Patients with gastroenteritis	231,128	0.6
U.S. (Detroit, MI)[49]	May-November 1977	Children with diarrhea	1262	0
U.S. (New York State)[177]	1976-1980	Survey of cultures from a state laboratory, 6 hospitals, and several daycare centers	2487	0.9†
U.S.‡ (7 cities)[118]	November 1989-January 1990	All stool cultures submitted to 7 hospitals	4841	0.8
U.S. (St. Louis, MO)[103]	1998-2001	Children presenting to an emergency department with diarrhea	1626	0.1

**Yearly percentages during the 5 years ranged from 0 to 4.4 percent.*
†This increased to 4 percent of 3035 isolates when cultures from an outbreak and other screenings were included.
‡Includes Detroit, MI.

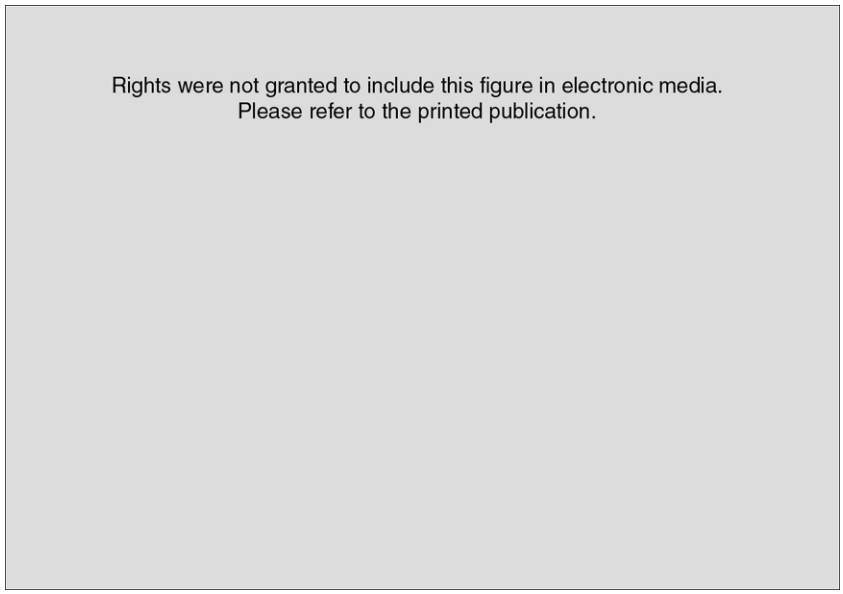

Figure 123–1 Adjusted incidence of *Yersinia enterocolitica* (YE) infection, by race and age. Average incidences are given over the 4-year surveillance period (1996 to 1999) by age group and adjusted race/ethnicity. *(From Ray, S. M., Ahuja, S. D., Blake, P. A., et al.: Population-based surveillance for* Yersinia enterocolitica *infections in FoodNet sites, 1996-1999: Higher risk for disease in infants and minority populations. Clin. Infect. Dis.* 38:*S181-S189, 2004.)*

pork or pork products and to educate consumers regarding good hygiene practices during food preparation, the number of isolations of *Y. enterocolitica* decreased from a high of 1469 in 1986 to 707 in 1996.[202] Changes in techniques for slaughtering also may have contributed to this decline. Preparation of chitterlings in the household was a risk factor for *Y. enterocolitica* infection among children in Michigan[1] and Illinois.[36]

Ingestion of water contaminated with serotype O:8 has led to sporadic cases and outbreaks.[47] Bean sprouts that had been immersed in contaminated water were the source of an outbreak in Pennsylvania in 1982. Ingestion of tofu (bean curd) packed in untreated spring water that subsequently was found to be contaminated with *Y. enterocolitica* caused 44 cases of symptomatic infection in Washington state in 1981 and 1982.[191] Serotypes commonly found in water samples rarely are isolated from humans with symptomatic disease, however.[33]

Contaminated milk has been implicated as the source of several large outbreaks of *Y. enterocolitica* infection.[19,47,197] Whipped cream and ice cream may harbor the organism. Contamination of milk products after pasteurization has been documented. *Y. enterocolitica* has been found in raw milk samples from cows and goats. Samples of beef, lamb, poultry, oysters, and a variety of vegetables also have been found to be contaminated with *Y. enterocolitica*.[30,47,66]

Serotypes O:3, O:4,33, O:5,27, O:7,8, O:8, O:10, O:13, and O:16 cause most human disease in North America but rarely are isolated in surveillance of water or food samples. Serotype O:8 strains have been cultured from cattle, milk, and water samples[146]; serotype O:4,33 strains have been isolated from pigs and cattle; and serotype O:4,32 strains have been found in cheese, ham, sausage, raw beef, and one pancake specimen.[33,177,197]

Incubation, Carriage, and Transmission in Humans

The incubation period of *Y. enterocolitica* enterocolitis ranges from 1 to 14 days, with a median of approximately 4 days.[33,47,118] The minimal infective dose of *Y. enterocolitica* is unknown. Ingestion of 3.5×10^9 organisms by a volunteer resulted in diarrhea in less than 1 day, but such large inocula are unlikely to be encountered clinically. The duration of excretion of the organism after development of infection in children ranges from 14 to 97 days (mean 42 days).[131] The impact, if any, of antibiotic treatment on the duration of carriage is unknown.

Transmission to household members occurs uncommonly, even among young children, who are at higher risk for development of symptomatic disease.[19,131,191] Six percent of household contacts developed disease in one outbreak,[191] but several large outbreaks with no secondary household cases have been reported.[47]

Yersinia enterocolitica and Blood Transfusion–Related Sepsis

Sporadic cases of *Y. enterocolitica* sepsis related to contamination of transfusions of red blood cells have been recognized since 1987 and have occurred in the United States, Europe, and Australia.[34] *Y. enterocolitica* is the most common cause of transfusion-related sepsis.[97] Among 20 cases from the United States, chills occurred in 16, fever occurred in 14, hypotension occurred in 13, and disseminated intravascular coagulation occurred in 7.[42] Death attributable to *Y. enterocolitica* infection occurred in 12, half of which occurred within 25 hours of receipt of the contaminated transfusion. Among the 20 donors, 13 had had gastrointestinal symptoms within the month before receiving a blood donation, and 16 had titers equal to or greater than 1:128 (considered positive).

In many cases of transfusion-related sepsis, the contaminated red blood cell units had been stored for 25 days or more.[80] After experimental inoculation of small numbers of *Y. enterocolitica* into packed cells kept at 4° C, the organisms continue to replicate, reaching concentrations of 100 colony-forming units (CFU)/mL in 7 days and 10^6 CFU/mL in 21 days. High levels of endotoxin can result from such replication and have been documented in samples from red blood cell units that led to transfusion-related sepsis.[42]

Human Outbreaks

Outbreaks of *Y. enterocolitica* disease have involved communities, families (with interfamily spread), hospitals, and schools. The sources of the organism have been various foods and animals, especially dogs. A review of these outbreaks suggests that infection may occur more commonly than has been recognized. Yersiniosis also resembles disease caused by *Salmonella* and *Shigella* organisms in many respects, including the environmental sources

of the organisms, the clinical syndromes, and the occurrence of asymptomatic infection.

OUTBREAKS IN SCHOOLS AND COMMUNITIES

A community outbreak caused by a serotype O:8 strain occurred in New York state in 1976.[19] At least 222 children and employees had a yersiniosis-like illness during a 10-week period. Illness was associated with drinking chocolate milk that was contaminated after pasteurization during hand mixing with chocolate syrup. Transmission of infection from ill children to household contacts was not observed. School-related outbreaks also have occurred in Japan.[15,215]

In the summer of 1982, an estimated several thousand individuals in several southern states who consumed milk from a single dairy developed yersiniosis. A total of 172 culture-positive infections were confirmed, many as a result of hospitalization for illness. Seventeen patients underwent appendectomy; 24 others had extraintestinal spread of infection. The strain involved was designated serotype O:13a,13b.[197] An outbreak associated with consumption of pasteurized milk in Vermont and New Hampshire in 1995 probably resulted from contamination after pasteurization with a serotype O:8 strain, possibly from rinsing bottles with untreated well water.[5]

During November to December 2002, nine infants in the Chicago area developed gastroenteritis caused by *Y. enterocolitica*. Eight were African-American and were exposed to caretakers who had prepared chitterlings for holiday meals. All recovered, although six were hospitalized for a median of 5 days.[36]

FAMILY EPIDEMICS

In North Carolina, 21 individuals from four families were involved in an outbreak of yersiniosis that had an unusually high attack rate.[70] Of the 21 affected individuals, 18 were children aged 3 to 13 years; 16 of the 21 had diarrhea and fever, and 5 were asymptomatic. *Y. enterocolitica* was recovered from the spleen of the youngest child at autopsy. The diagnosis was established serologically in the others. A dog that had given birth to puppies that had died of a diarrheal illness a week before the families became ill seemed to be the source of the infection.

NOSOCOMIAL OUTBREAKS

A young child who was hospitalized in Finland with acute gastroenteritis was the source case of infection in a housekeeping worker and four nurses who cared for her.[195] The infecting strain was serotype O:9. Another hospital outbreak involved nine patients in Canada.[161] It was caused by a serotype O:5 strain. Person-to-person contact was considered the likely mode of transmission.

Prevention of Disease

During outbreaks, efforts should be made to identify environmental sources and vehicles of transmission.[47] A single environmental source can harbor multiple serotypes of *Y. enterocolitica*, such that resulting outbreaks may be polyclonal in nature.[191] Enteric precautions should be used for hospitalized patients with diarrhea caused by *Y. enterocolitica* (as with other causes of gastroenteritis).[47] At the population level, decreased consumption of raw or undercooked pork products potentially can reduce the incidence of infection.[192,202]

YERSINIA PSEUDOTUBERCULOSIS

Y. pseudotuberculosis may infect individuals of all ages, but at least 75 percent of patients with clinically apparent disease are children younger than 15 years old.[91,171] Infection in young infants has been reported.[91,208] Of 130 cases diagnosed in Great Britain from 1959 to 1970, boys were involved three times more frequently than girls.[125] Infections occur more commonly during the cold months of the year.[91,171] The seasonal winter peak of human infection produced by *Y. pseudotuberculosis* is similar to that seen in wild and domesticated animals.[104,105,171]

The attack rates for children living in rural and urban areas seem to be the same. *Y. pseudotuberculosis* occasionally has been recovered from healthy individuals. Exposure to the organism seems to be uncommon; antibody to *Y. pseudotuberculosis* was detected in only 1 of 2000 sera from individuals with no history of yersiniosis.[59,171]

Y. pseudotuberculosis is distributed worldwide in a large variety of animals and birds, but infection seldom occurs.[63] Guinea pigs, rodents, and rabbits are infected most often[125] and may experience a plaguelike illness.[63,88] Lesions in guinea pigs may be confused easily with lesions caused by *Y. pestis*. Rats and other rodents also may have plaguelike disease caused by *Y. pseudotuberculosis*. The microbe can be cultured from tongue, tonsils, intestines, and large organs of infected animals.[145]

Infection has been reported in various domestic animals (cattle, sheep, goats, cats, dogs, hamsters), commercially raised fur bearers (chinchillas, mink, coypu), and other wild or captive animals (rabbits, raccoons, foxes, deer, beavers, monkeys, puma, kangaroos). *Y. pseudotuberculosis* has been found in more than 50 species of birds,[75,99,125] and epizootics have occurred among turkeys, ducks, pigeons, and doves and in aviaries of canaries and finches. Strains obtained from animals and birds in the United States are predominantly of serotypes 1a, 1b, and 3.[125,171]

The incubation period for human disease ranges from 41 hours to 20 days.[91] In a food-associated outbreak in Finland, the median incubation period was 8 days (Fig. 123–2).[94] The organism can survive in fresh tap water for 46 days at room temperature and for 8 months at 4° C. It can survive at 4° C in meat for 145 days and in milk and bread for 2 to 3 weeks.[125]

A family outbreak of mesenteric adenitis caused by *Y. pseudotuberculosis* that involved four siblings aged 7 to 14 years has been reported.[160] A pet dog was shown to have increasing antibody titers at the time the children were ill.

Periodic outbreaks have been reported in Japan, northern Europe, and areas of the former Soviet Union. Sandwiches prepared by a single bakery were the primary risk factor for 67 cases that occurred in a 3-week period in Japan.[91] Drinking unchlorinated well water or mountain stream water has been the source of other outbreaks. Children were much more likely to have clinical disease than adults. More recent outbreaks in Finland have been associated with contaminated lettuce and grated carrots.[94]

PATHOLOGY

The diseases produced by *Y. enterocolitica* and *Y. pseudotuberculosis* are similar and share the histopathologic theme of involvement of the lymphoid tissues of the intestinal mucosa and mesentery.

YERSINIA ENTEROCOLITICA

Y. enterocolitica infection predominantly affects the gastrointestinal tract. The most severe clinical symptoms correlate with an acute terminal ileitis. The mucosal surface of the ileum and other involved sites may be inflamed diffusely. Ulcerations may occur throughout the gastrointestinal tract and may be small and superficial or extend to the muscularis propria. Mucosal and submucosal hyperplasia of Peyer patches occurs with scattered microabscess formation.

Figure 123–2 Distribution of incubation periods for *Yersinia pseudotuberculosis* infection to onset of gastrointestinal (GI) symptoms and erythema nodosum after point source exposure to contaminated carrots. Of 72 case patients with a known date of onset, 51 had GI symptoms and 38 had erythema nodosum only; 28 had GI symptoms and erythema nodosum and appear twice in the graph. *(From Jalava, K., Hakkinen, M., Valkonen, M., et al.: An outbreak of gastrointestinal illness and erythema nodosum from grated carrots contaminated with Yersinia pseudotuberculosis. J. Infect. Dis.* 194*:1209-1216, 2006.)*

Ulcers occur primarily over the sites of lymphoid tissue within the mucosa, which accounts for their more longitudinal appearance in the small intestine and an oval or punctate appearance in the stomach and colon. Ulcerations are characterized by necrosis of the epithelial layer. In the colon, the necrosis also may extend through the superficial third of the crypts. Large colonic ulcerations covered by pseudomembranes or mucoid debris are seen occasionally. Ulcerations may progress to perforation, with subsequent development of peritonitis or gastrointestinal hemorrhage in severe cases.[27,64,200,201]

The inflammatory response in the mucosa consists mainly of neutrophils and mononuclear cells. Lymphocytes and plasma cells also may be seen. Giant cells are not seen, although a granulomatous appearance can be imparted by the presence of plump epithelioid histiocytes. Numerous colonies of gram-negative bacteria often can be seen beneath the mucosal ulcerations and within the microabscesses that occur in the lymphoid tissues.[64,200,201]

The appendix usually appears normal on gross inspection, but small focal ulcerations frequently are present.[7] Large areas of necrosis are found occasionally, and acute, suppurative appendicitis has been reported.[27,96,131] Periappendicular inflammation may result from a true appendicitis or an adjacent terminal ileitis.[200]

Mesenteric adenitis, the hallmark of infection caused by *Y. pseudotuberculosis*, also is a common feature of enterocolitis caused by *Y. enterocolitica*. The lymph nodes usually show numerous large pyroninophilic cells and mitotic figures in the cortical area and marginal sinuses. Small collections of leukocytes in the germinal centers are seen in some cases and suggest formation of microabscesses. In severe cases, extensive areas of necrosis circumscribed by a neutrophilic infiltrate may be seen. The sinusoids can become filled with neutrophils and mononuclear cells. The germinal centers often appear reactive.

The histopathologic appearance of the mesenteric adenitis of infection caused by *Y. enterocolitica* can resemble the adenitides caused by cat-scratch disease (*Bartonella henselae*), toxoplasmosis, infectious mononucleosis, and *Y. pseudotuberculosis*.[7,200,211] The necrotizing epithelioid granulomata that may be present in mesenteric adenitis caused by *Y. pseudotuberculosis* have not been described in infection caused by *Y. enterocolitica*. A more recent study of six cases suggests that biotype 1B strains may be more likely to cause suppurative inflammation, whereas biotype 2 through 5 strains may be more likely to cause granulomatous infection.[115]

YERSINIA PSEUDOTUBERCULOSIS

Numerous reports* collectively have described the pathology of infection caused by *Y. pseudotuberculosis*. Grossly enlarged, soft, and inflamed mesenteric lymph nodes are the predominant finding on laparotomy. They frequently are located at the ileocecal angle. Punctate hemorrhages and small, yellow microabscesses may be present on the surfaces of the nodes at the height of infection. The appendix usually appears normal, but the terminal ileum and cecum occasionally appear inflamed. A necrotic purulent mass sometimes is seen in the mesentery.

Histopathologic findings in the mesenteric lymph nodes include enlarged follicles and small abscesses; hyperplasia of reticulum cells; necrosis of the nodes with infiltrates of neutrophilic leukocytes and plasma cells; and, in some instances, punctate hemorrhages. Scattered clusters of neutrophils may be seen within the sinusoids and germinal centers without formation of abscess or necrosis. Atypical mononuclear cells, some of which may be mitotic, may be present in the sinusoids. When necrosis is absent, large numbers of eosinophilic leukocytes occasionally are seen surrounding reticulogranulocytic infiltrates in the nodes. Giant cells can occur and may cause the histologic picture to be confused with that of tuberculosis.

The mesenteric adenitis produced by *Y. pseudotuberculosis* seems to progress through histopathologic stages akin to those of other pyogenic infections that can lead to formation of abscesses.[50,71] Reticulogranulocytic infiltration is followed by formation of an abscess, with subsequent organization and clearing of the abscess. The inflammatory process of *Y. pseudotuberculosis* seems to remain confined to the lymph nodes without rupture through the nodal capsule.

Focal mucosal ulcerations may be seen in the ileum and are more likely to be found at the site of Peyer patches. Aggregates of neutrophilic leukocytes similar to those in mesenteric nodes may be seen in germinal centers within mucosal lymphoid tissue. Fibrinoid material may be a prominent feature at these sites. Small areas of necrosis surrounded by reticulum cells and leukocytes also may be present in the submucosal follicles.[57,105,107,207] Ulcerated lymphoid follicles in the intestinal wall are connected to the regional lymph nodes by a lymphangitis. This anatomic situation is analogous to the primary complex of tuberculosis.

Despite a clinical picture of acute appendicitis as the presenting feature of infection by *Y. pseudotuberculosis*, the appendix typically is grossly and microscopically normal. Inflammatory changes, when present, usually are in the form of a periappendicitis. Phlegmonous appendicitis can result from infection caused by *Y. pseudotuberculosis*, but is rare.[50,84]

PATHOGENESIS

The pathogenesis of infection by the two enteropathogenic *Yersinia* spp. has been studied extensively. After ingestion and successful transit to the small intestine have occurred, these *Yersinia* spp. are able to penetrate into the lamina propria primarily by passing through the cytoplasm of M cells that reside on the mucosal surface of Peyer patches. The bacteria are internalized in membrane-bound vacuoles in which they survive, but do not replicate. After reaching the lamina propria, the microbes multiply as extracellular microcolonies in lymph follicles and Peyer patches, where they may reach densities of 10^9 CFU/g of tissue.[52]

Neutrophils and macrophages infiltrate these sites in response to the infection, ultimately creating microabscesses, but *Y. enterocolitica* is able to resist phagocytosis and intracellular killing by neutrophils and macrophages. *Yersinia* spp. also inhibit production of tumor necrosis factor-α and interferon-γ and induce apoptosis in host macrophages. The microbes disseminate to the mesenteric lymph nodes, apparently through lymphatic vessels. Infection usually is contained at this point, although abscesses often develop within the nodes. Systemic spread occurs occasionally, more commonly with infection by serotype O:8 strains.

Control of infection caused by *Y. enterocolitica* is the result of T-cell responses that lead to restriction of bacterial growth in infected organs through stimulation of production of *Yersinia*-specific antibody by B cells and cytokine-mediated activation of macrophages. These responses ultimately overcome the antiphagocyte strategies of *Yersinia*. Production of tumor necrosis factor-α and interferon-γ seems to be an essential part of the host response to *Yersinia* infections. *Yersinia* microbes generally do not survive within granulocytes after they have been successfully phagocytosed.[43,45,46,149]

Only a few of the many serotypes of *Yersinia* spp. are capable of infecting humans. Numerous virulence factors have been identified among these "pathogenic" strains (Table 123–2). Most of them are encoded on the *Yersinia* virulence plasmid pYV. At least eight chromosomal loci that encode novel *Yersinia* virulence factors have been found by tagged mutagenesis studies.[67] Much of this knowledge has been gained through mouse and rabbit models of human infection and observations of interactions of *Yersinia* organisms with in vitro cell culture models.[26,43,83,127,156]

The distinctions in pathogenesis between the two enteropathogenic *Yersinia* spp. and *Y. pestis* seem to be caused partly by the presence in *Y. pestis* of (1) two additional plasmids that encode a plasmin activator and a mouse exotoxin and (2) a hemin storage locus on its chromosome. These additional factors may enable *Y. pestis* to survive in and transmit between fleas and rodents. Such transmission does not occur with the enteropathogenic *Yersinia* spp.[167]

BACTERIAL DETERMINANTS OF MUCOSAL INVASION

Two outer-membrane proteins encoded by chromosomal genes permit entry into various mammalian cell types in vitro and are likely to be responsible for the ability of *Yersinia* to invade into and through the intestinal mucosal epithelium. They have been named *invasin* and the *attachment invasin locus protein* (Ail). All isolates of *Y. enterocolitica* that are virulent in humans contain DNA sequences that encode for invasin and Ail. Nonpathogenic strains do not contain the genetic code for Ail but most contain invasin genes that cannot be expressed because of chromosomal rearrangements. These proteins apparently are unique to the genus *Yersinia*.[92,135,153]

Invasin attaches to receptors in the β_1 integrin family and induces an actin-mediated endocytosis of the microbe. It binds to β_1-chain integrin receptors with approximately 100-fold higher affinity than do natural ligands, such as fibronectin. Such high-affinity binding triggers endocytosis-like internalization that involves clathrin.[46] Invasin is expressed maximally at ambient temperatures when the organism is in stationary phase. *Y. enterocolitica* microbes living in the environment probably exist in a stationary phase–like state and may be primed maximally for invasion of the host after ingestion. Invasin expression also can remain elevated at 37° C when the pH is 5.5, and such conditions are encountered during passage through the intestinal tract. Ail is expressed maximally at 37° C, functions as an adhesin and an invasion factor, and plays a role in the resistance of *Y. enterocolitica* to the bactericidal activity of human serum.[92,93,153]

High levels of β_1-chain integrin receptors are found on the luminal surface of M cells, which are antigen-sampling epithelial cells found in highest numbers overlying Peyer patches in the intestinal tract.[76] Flagellum-dependent motility seems

*See references 50, 55, 57, 87, 105, 126, 132, 133, 137, 160, 207.

TABLE 123–2 Known and Putative *Yersinia* Virulence Factors*

Stage of Pathogenesis	Determinant	Genomic Origin†	Function	Conditions of Expression
Gastric transit	Urease	Chromosome	Aids survival of gastric acidity	
Mucosal invasion	Invasin (inv)	Chromosome	Attachment, invasion via β_1 integrins; broad host/cell range	28° C
	Attachment invasion locus (AiL)	Chromosome	Attachment, lesser invasion factor; more host-specific than invasin; serum resistance	37° C
	Yersinia adhesin A (YadA)	Plasmid	Attachment, invasion; reduces opsonization by C3b by binding complement factor H; ? role in resistance to antibacterial polypeptides (e.g., bactericidal permeability-increasing protein)	37° C and *any* calcium concentration
Disruption of phagocyte function	Effector proteins (act on host cells)	Plasmid	Tyrosine phosphorylase that prevents phagocytosis by macrophages by preventing assembly of focal adhesion structures of phagocytes	37° C and low calcium concentration
	YopH[d]		Phagocytosis resistance via induction of host cell actin filament rearrangements	
	YopE		Cysteine protease that inhibits mitogen-activated protein kinase and nuclear factor κB signaling via interruption of post-translational covalent additions of ubiquitin-like molecules to these enzymes. This prevents production of cytokines (tumor necrosis factor-α), inhibits the host immune response, and triggers apoptosis of macrophages. Inhibits kinase phosphorylation via an acetyltransferase function	
	YopJ/P		Serine kinase, causes rounding up of cells, specific target unknown	
	YpkA/YopO		Disruption of actin filaments	
	YopT		Role unclear. Inhibits thrombin-induced platelet activation in vitro but is delivered intracellularly during infection. Leads to depletion of natural killer cells and alters expression of interleukin-15 and interleukin-15 receptors	
	YopM			
	Host cell membrane attachment, pore insertion proteins	Plasmid	Pore formation in host cell membrane to allow translocation of effector proteins into the host cell. ? Suppression of interleukin-8 secretion by epithelial cells	37° C and low calcium concentration
	YopB and YopD		Controls translocation via modulating the size of the putative YopB, YopD pore in the host cell membrane	
	YopQ (YopK)		Involved in control of translocation, perhaps stabilizing contact between the bacterium and host cell membranes	
	YopN		Assists extrusion of Yops B and D to the host cell membrane. Homologue of the *Y. pestis* V antigen. Antibodies against V antigen seem to be protective at the serotype level	
	LcrV[e]			
	Yop secretion apparatus (Ysc) (28 proteins in total)	Plasmid	Form a complex that spans the inner bacterial membrane (? gated pore/channel)	37° C and low calcium concentration
	YscD, YscR, YscU, and LcrD/YscV		Forms a pore in the outer bacterial membrane that may connect with the inner membrane pore	
	YscC secretin		ATPase that energizes Yop transfer across the membranes	
	YscN		Negative *yop* gene regulators that, when secreted, decrease in concentration in the bacterium, allowing *yop* expression	
	LcrQ (in *Y. pseudotuberculosis*), YscM1 and YscM2 (in *Y. enterocolitica*)			
	Cytosolic chaperones (Syc) (6 proteins in total)	Plasmid	Secretion/translocation pilots or antidegradation roles for effector proteins	37° C and low calcium concentration
Colonization of Peyer patches and lymph nodes	*Yersinia* phospholipase A	Plasmid	? Serum resistance, ? inhibition of phagocytosis	37° C and low calcium concentration
Iron metabolism	Yersiniabactin	Chromosome (high pathogenicity island)	Siderophore	37° C, iron starvation

TABLE 123–2 Known and Putative Yersinia Virulence Factors*—cont'd

Stage of Pathogenesis	Determinant	Genomic Origin†	Function	Conditions of Expression
Diarrhea	*Yersinia* heat-stable enterotoxin (Yst)	Chromosome	Fluid secretion in intestine. Precise role in pathogenesis of disease in humans is unclear, but found in clinical isolates from children with diarrhea	28° C (in vitro)
? Systemic invasion	O antigen	Chromosome	Component of lipopolysaccharide. ? Complement/serum resistance	

**The pathogenesis/virulence roles of several gene products encoded on the Yersinia virulence plasmid pVY have yet to be determined, and additional functions may be identified for other gene products. Additional chromosomally encoded virulence factors are suspected, with investigations ongoing.*
†Plasmid refers to the Yersinia virulence plasmid pYV.
Lcr, low calcium response; Yop, Yersinia outer membrane protein (although not all such designated proteins reside in the outer membrane).
Data from references 23, 44, 45, 67, 83, 102, 111, 119, 140, 149, 167, 179, 180, 214.

to be required for invasin-mediated invasion of cells by *Y. enterocolitica*.[213]

YERSINIA ADHESIN A

Yersinia adhesin A (YadA) is a multifunctional virulence factor of *Y. enterocolitica*. YadA is approximately 50 kd and probably forms tetrameric fibrillae on the microbial surface. Its gene resides on the pYV plasmid and is transcribed at 37° C independently of the calcium concentration. YadA binds to extracellular matrix proteins, such as collagen, and mediates adhesion to cells. It may play a role in translocation of Yop effector proteins into eukaryotic phagocytic cells. YadA also inhibits the terminal complement attack complex, reduces opsonization by C3b through binding of complement factor H, and contributes to the ability of *Y. enterocolitica* to resist killing by antimicrobial polypeptides of human granulocytes. YadA knockout mutants are far less virulent in mice than is the parent wild-type strain.[45,82,205]

VIRULENCE PLASMID AND TYPE III SECRETION SYSTEM

Pathogenic strains of *Y. enterocolitica* harbor a plasmid that consists of approximately 70 kb (denoted pYV) and encodes the low calcium response system of *Yersinia* spp., which involves a complex response to environmental conditions of 37° C and calcium concentrations of less than 2.5 mM, both of which describe the intracellular compartment of mammalian cells.[45,180] Plasmid-encoded factors are required for survival and extracellular multiplication after reaching Peyer patches. Plasmid-cured derivatives are ingested rapidly and killed by neutrophils in Peyer patches, whereas wild-type strains are able to proliferate and spread through the lamina propria to adjacent villi. Plasmid-encoded factors are not required for *Y. enterocolitica* to penetrate the intestinal mucosa.[82]

In DNA cross-hybridization studies, the pYVs of *Y. enterocolitica* serogroups O:9, O:3, and O:5 show 90 percent nucleotide identity with one another, 75 percent identity with the pYV of serogroup O:8, and 55 percent identity with the pYV of *Y. pestis* and *Y. pseudotuberculosis*. Despite the nucleotide divergence among the pYVs of the three *Yersinia* spp., the overall structures and most of the genes are highly similar among them. The *Yersinia* low calcium response plasmids are nonconjugative.[45,180]

The *Y. enterocolitica* pYV contains approximately 70 genes, of which approximately 53 have protein products with known or putative functions (many of these are listed among the virulence factors in Table 123–2). pYV encodes *Yersinia* adhesin A and a system of six effector proteins that are delivered into host cells (primarily phagocytic granulocytes) through a complex mechanism of regulatory proteins, secretion chaperones, and proteins that act as secretion channels or pores in the bacterial and host cell membranes. This system, called a type III secretion system, denotes secretion of the bacterial proteins only in response to contact with a mammalian cell. The effector proteins and several others involving pore formation are designated Yops (*Yersinia* outer-membrane proteins), although we now know that most of them do not reside in the outer membrane. Similar type III secretion systems are found in *Salmonella* and *Shigella* spp.[45,180]

The secretion system is composed partially of syringe-like organelles—called *Ysc injectisomes*—that develop when *Yersinia* organisms are incubated at mammalian body temperatures. These injectisomes are protein pumps that span the peptidoglycan layer and the two bacterial cell membranes, topped by a needle-like structure protruding outside the bacterium. Twenty-seven proteins are involved in the structure; 10 of them are located internally and appear to be similar to the basal body of a flagellum. The structure also includes an ATPase that functions as a proton pump. A homomultimeric ring-shaped structure extends through the outer membrane, with a central pore diameter of about 50 Å.[31]

As the injectisomes assemble, stocks of intracellular Yops are synthesized. Active transcription of Yop genes is limited to bacteria in close contact with eukaryotic cells. The secretion channel of the injectisome remains closed, with negative feedback mechanisms to prevent overproduction of Yops. On close contact with a phagocyte, the injectisome channels open, and secretion of effector Yops into the target cell begins.[31]

After secretion into a phagocytic cell occurs, Yop H, Yop E, Yop T, and YopO/YpkA disrupt its cytoskeletal dynamics, blocking phagocytosis (see Table 123–2). Other Yops inhibit production of tumor necrosis factor-α, interleukin-8, and other inflammatory chemokines and induce apoptosis of macrophages.[31]

SUMMARY OF *YERSINIA* PATHOGENESIS

Pathogenic strains of *Y. enterocolitica* require calcium concentrations equivalent to those of serum and extracellular fluids in humans for growth at body temperature. Plasmid proteins are synthesized maximally, however, at 37° C under conditions of low calcium concentrations, such as those found intracellularly. These regulatory effects of environmental calcium concentrations permit free growth of *Yersinia* organisms during extracellular life and production of factors that inhibit phagocytosis by macrophages and neutrophils when *Yersinia* organisms pass through the intracellular compartment during mucosal invasion or come into contact with these granulocytes.[43,45,82]

A model of how pathogenic *Yersinia* organisms living in cold (≤28° C) environmental reservoirs are able to establish infection in the mammalian host can be described at the molecular level (see Table 123–2). *Yersinia* microbes living in the environment

express invasin, which renders them ready to attach and invade the intestinal mucosal M cells after ingestion. Expression of invasin continues at body temperature during gastric passage to the intestines. Survival of gastric acidity is assisted by a urease-producing system.

During intracellular passage through M cells in the small intestine, pYV genes are likely to be activated (conditions of low calcium, 37° C). YadA can be produced at body temperature and may assist in mucosal invasion. Transcytosis from the intestinal lumen to the lamina propria seems to occur by a clathrin-mediated process that probably is a normal function of the host cell, after internalization is triggered. Ail also is synthesized after ingestion occurs and probably promotes attachment to migrating cells in the lamina propria that may facilitate extracellular spread of the microbes to regional lymph nodes and perhaps the liver and spleen.

To survive this journey, *Yersinia* organisms must evade phagocytosis by macrophages and neutrophils. YadA can inhibit complement opsonization, reducing the likelihood that phagocytosis will occur. When contact between the microbe and a host granulocyte occurs, the previously activated type III secretion system (described earlier) encoded by the pYV plasmid comes into play. A contiguous pore is inserted into the granulocyte cell membrane (involving Yops B, D, Q, and N and LcrV). Effector proteins (Yops E, H, J/P, O/A, M, and T) then are translocated into the granulocyte cytoplasm, disrupting its abilities to ingest the bacterium and produce cytokines and triggering apoptotic cell death (see Table 123–2). Although this system of virulence factors is robust enough to facilitate short-lived infection that often causes mild to moderate clinical symptoms, the microbes that do not succumb to the innate immune response ultimately are contained and then eliminated by the adaptive host response.

IRON METABOLISM AND VIRULENCE

Iron is an essential growth factor for most bacteria, many of which release siderophores (high-affinity chelators) that bind ferric iron and then are taken up again through receptors by the microbe. *Y. enterocolitica* serotype O:8 and other biotype 1B strains synthesize a chromosomally encoded siderophore, designated *yersiniabactin*, which sits on the outer membrane of the bacterium. The presence of this siderophore decreases the concentration of environmental iron required for optimal growth and probably accounts for the increased virulence observed for serotype O:8 strains.[47,82] The gene encoding yersiniabactin and the genes required for its biosynthesis, transport, and regulation compose the core of what is termed a *high-pathogenicity island* because of the high lethality for mice that its presence confers.[159]

Serotypes O:8, O:4, O:13, O:18, O:20, and O:21 (all biotype 1B and historically considered "American" strains) are highly lethal to mice after intraperitoneal injection and are able to evoke a keratoconjunctivitis after inoculation into the conjunctival sac of guinea pigs (the positive Sereny test result). Oral infection in mice predominantly produces the features of mesenteric adenitis and systemic infection, rather than simple gastroenteritis, which is characteristic of human infection by American strains. The "European" serotypes, O:3, O:9, and O:5,27, yield a negative Sereny test result and cause mild diarrhea but not death in mice. The European serotypes of *Y. enterocolitica* do not produce yersiniabactin, but are able to use siderophores synthesized by other organisms.[43,82,135]

Deferoxamine, a *Streptomyces*-derived siderophore used clinically to treat iron overload states, can be used by *Y. enterocolitica* strains as a source of iron. The increased availability of ferric iron that exists in iron overload states, such as hemochromatosis and diseases such as thalassemia, that require frequent red blood cell transfusions also facilitates survival and growth of *Y. enterocolitica*. Iron overloading and deferoxamine are independent risk factors for development of systemic disease after intestinal infection with *Y. enterocolitica*.[47]

ENTEROTOXIN PRODUCTION

All enteropathogenic strains of *Y. enterocolitica* produce a heat-stable enterotoxin that closely resembles the heat-stable toxin of *E. coli*. Both enterotoxins induce increases in levels of cyclic guanosine monophosphate levels in intestinal epithelial cells. The *Y. enterocolitica* enterotoxin is not plasmid-encoded, and its presence does not correlate with the expression of other virulence phenotypes.[63] Because the enterotoxin is not produced in vitro at temperatures exceeding 30° C, production in the gastrointestinal tract and a causative role in diarrhea were thought to be unlikely.[47] Observations in the young rabbit oral infection model have shown, however, that enterotoxin-negative mutants did not induce diarrhea, and that the wild-type strain did. Clinical biotype 1A isolates from children with diarrhea in India produce *Y. enterocolitica* stable toxin-b. These findings suggest that *Yersinia* enterotoxins may play a role in causing the diarrhea frequently associated with *Y. enterocolitica* infection in children.[82,152,179]

GASTRIC ACIDITY AS A PROTECTIVE HOST FACTOR

Although *Y. enterocolitica* is able to grow under conditions at pH 5.0 to 9.0, optimal growth occurs at pH 7.0 to 8.0. Gastric acidity may play a protective role against some *Yersinia* inocula, although pathogenic *Yersinia* organisms produce a urease enzyme that facilitates survival. Therapeutic agents or clinical conditions that result in reduced gastric acidity may predispose patients to development of infection. *Y. enterocolitica* bacteremia has been reported after gastrectomy.[47,111]

CLINICAL MANIFESTATIONS

Clinical disease caused by *Y. enterocolitica* occurs far more frequently than that caused by *Y. pseudotuberculosis*.[56,120,121] Historically, diarrheal illness has been considered the hallmark of *Y. enterocolitica* and the pseudoappendicular syndrome of mesenteric adenitis indicative of *Y. pseudotuberculosis*. Each species can cause enterocolitis and mesenteric adenitis, however. Various other clinical infections and postinfection syndromes also are caused by these microbes. In a series of patients presenting with acute abdominal pain suggestive of appendicitis, the incidence of serologic evidence of *Yersinia* infection has ranged from 7 to 31 percent.[16]

YERSINIA ENTEROCOLITICA

The clinical features of the disease caused by *Y. enterocolitica*, primarily an acute enteritis, have been described by many investigators.* The clinical manifestations depend partly on the age and physiologic condition of the host.[47,131,160] Enterocolitis is the most common presentation and occurs most often in young children. The pseudoappendicular syndrome, which results primarily from mesenteric adenitis and mimics acute appendicitis, occurs more commonly in older children and young adults.[19,47,86,96,147,166]

*See references 17, 38, 47, 51, 74, 110, 117, 118, 127, 128, 131, 161, 166, 196, 212.

TABLE 123–3 Clinical Features of *Yersinia enterocolitica* Infection in Children

	Sweden,[17] 1967-1973	Finland,[128] 1974-1978	Canada,[51] 1972	Canada,[131] 1977-1978	U.S.,[118] 1989-1990	U.S.,[142] 1988-1991	Combined Totals
No.	31	35	40	57	37*	48	248
Age ≤5 yr	28	26	19	NS†	≥28	NS	≥101/142 (≥71%)
Fever (>38° C)	15	6	36	39	35	44	175/248 (71%)
Diarrhea	31	26	32	56	37	45	227/248 (92%)
Grossly bloody diarrhea	2	NS	7	NS	14	22	45/156 (29%)
Abdominal pain	4	6	20	31/48	NS	NS	61/154 (40%)
Vomiting	NS	12	12	22	18	23	87/217 (40%)
Rash‡	NS	2	2	NS	NS	NS	4/75 (5%)
Appendectomy	0	0	4	1	0	0	5/200 (2%)
Serotype O:3	31	NS	34	57	34	NS	156/165 (94%)

**All were black children; 7 different cities, 3-month period.*
†More than half of these children were <2 years old.
‡Maculopapular rash or urticaria.
NS, not specified.

Asymptomatic infection can occur, but the relative frequency compared with symptomatic disease is unknown. The predominant clinical features of *Y. enterocolitica* infection in children are summarized in Table 123–3.

Enterocolitis

Y. enterocolitica enterocolitis is characterized by diarrhea and abdominal pain. The diarrhea usually persists for 7 to 14 days.[118,131,200] A range of 1 to 46 days of diarrhea has been reported.[109] Ten percent of cases may persist for 30 days or more,[131] and chronic diarrhea persisting for several months has been described.[51]

During the first week of symptoms, patients commonly have 3 to 10 stools per day, with a gradual decrease in frequency thereafter. Stools typically are greenish, exhibit variable consistency (usually watery or mucoid), and are not remarkably malodorous. Gross blood is noted in approximately 25 to 50 percent of patients. Vomiting occurs in 40 percent of cases. Nausea is a common symptom. The abdominal pain can be colicky, diffuse, or localized to the right lower quadrant or epigastrium. Fever occurs commonly and usually is low-grade, but may exceed 40° C; it usually resolves within 1 week.[1,128]

Most cases are self-limited, but some children require hospitalization. Among 60 children hospitalized in Michigan between 1990 and 1997, the mean number of hospital days required was 4 (range 1 to 17 days).[1]

Fecal leukocytes are present commonly but not universally. The peripheral white blood cell count may range from 5600/mm^3 to more than 30,000/mm^3. Most counts are greater than 15,000/mm^3. Band forms often exceed 15 percent of the total. Infants frequently exhibit an immature-to-total neutrophil ratio greater than 0.5.[1,118,128] An absolute monocytosis may be seen in two thirds of patients.[113] Culture-negative cerebrospinal fluid pleocytosis can occur.[1]

Radiologic examination by upper gastrointestinal barium studies in 24 adult patients with severe diarrhea caused by *Y. enterocolitica* showed abnormalities of the terminal ileum in 21 cases.[201] Diffuse thickening of the mucosal folds was seen in 16 cases, and nodular filling defects were seen in 11. The radiographic appearance suggested the presence of one or more ulcerations of the terminal ileum in 11 patients. Dilation of the terminal ileum was noted in 12 patients, and extrinsic compression, presumably from enlarged lymph nodes, was present in 4. In some instances, the findings suggested the terminal ileitis of Crohn disease. Follow-up studies performed 2 months after acute illness showed decreased but persistent thickening of mucosal folds in eight patients. Barium enema studies were done in 15 patients and showed no striking abnormalities other than mucosal ulcerations, which were seen best on air-contrast studies.[200]

Figure 123–3 Mesenteric lymphadenopathy (*solid arrow*) and thickened bowel wall (*open arrow*) seen on computed tomography scan of a toddler with a 1-week history of severe abdominal pain, fever, vomiting, and nonbloody diarrhea. Stool culture on cefsulodin-irgasan-novobiocin agar yielded growth of *Yersinia enterocolitica*, serotype O:8. *(From Tuohy, A. M., O'Gorman, M., Byington, C., et al.: Yersinia enterocolitis mimicking Crohn's disease in a toddler. Pediatrics 104[issue 3]: e36, 1999.)*

Mesenteric lymphadenopathy can be seen on computed tomography scans (Fig. 123–3).[199] Among 13 adults who had colonoscopy or sigmoidoscopy for severe diarrhea caused by *Y. enterocolitica*, abnormalities were seen in 8; the mucosa appeared diffusely swollen, erythematous, and friable in 6 individuals, and 2 had only small, 1- to 2-mm aphthoid ulcerations. Serial procedures showed macroscopic and microscopic healing of ulcers within 4 to 5 weeks.[201] Aphthoid ulcerations may be seen throughout the colon (Fig. 123–4).[199]

Pseudoappendicitis-Mesenteric Adenitis

The syndrome of pseudoappendicitis, characterized in most cases by a normal appendix and an intense suppurative mesenteric adenitis, has attracted considerable attention since it was reported first in 1953.[132,133] The first recognized cases were caused by *Y.*

Figure 123–4 Multiple mucosal aphthoid ulcerations, 2 to 3 mm in size, as seen during colonoscopy in the child described in Figure 123–3. *(From Tuohy, A. M., O'Gorman, M., Byington, C., et al.:* Yersinia *enterocolitis mimicking Crohn's disease in a toddler. Pediatrics* 104*[issue 3]: e36, 1999.)*

pseudotuberculosis, but most cases reported in recent years have been caused by *Y. enterocolitica*.[28,95,117]

Fever, abdominal pain, right lower quadrant tenderness, and leukocytosis are the primary features of *Y. enterocolitica*–induced pseudoappendicular syndrome.[47,96] Some patients also have features of enterocolitis (nausea, vomiting, and diarrhea). The clinical presentation often is highly suggestive of acute appendicitis, such that laparotomy is required. Among a series of 581 patients in Scandinavia who underwent laparotomy for suspected appendicitis, 3.8 percent of cultures of stool or operative specimens yielded *Y. enterocolitica*.[147] Another 284 patients with similar symptoms were observed, and 5.6 percent of stool cultures from these cases yielded *Y. enterocolitica*. In a similar Scandinavian series of 205 patients who underwent appendectomy, 22 subsequently were diagnosed by serology as having *Y. enterocolitica* infection.[96] The findings on laparotomy usually are mesenteric lymphadenitis, terminal ileitis, and a normal or slightly inflamed appendix.[19,47,96,147]

A study of 40 cases of granulomatous appendicitis found evidence suggesting infection caused by *Y. enterocolitica* in 4 specimens, *Y. pseudotuberculosis* in 4 specimens, and both in 2 specimens by PCR analysis.[116] Two patients in the series subsequently were diagnosed as having Crohn disease, but causation cannot be inferred.

Asymptomatic Infection

In an unknown number of cases, infection by *Y. enterocolitica* is entirely asymptomatic. In a study of the distribution of antibodies to *Y. enterocolitica* in sera collected for various purposes in Ontario, Canada, from 4209 individuals who had no evidence of infection by this organism, specific antibody was present in 199 (serotype O:3 in 158 and serotype O:9 in 41).[196]

Other Presentations of Acute Infection

Bacteremia can occur[32,39,47,139] and may result in spread of infection to virtually any body site. Such events occur uncommonly and are seen more often in adults than in children. In children, bacteremia occurs more commonly in infants than in older children.[1] The risk for development of bacteremia during gastroenteritis in infants younger than 3 months may be 30 percent.[142] Bacteremias may be transient and asymptomatic or lead to septic shock and death. Septic cases tend to occur among patients with underlying illnesses and are associated with mortality rates of 34 to 50 percent.[47]

Y. enterocolitica may cause focal infections in many extraintestinal sites, even in the absence of detectable bacteremia. Pharyngitis has been reported and occurs primarily in adults. Cervical adenopathy may be associated with *Y. enterocolitica* pharyngitis, and gastrointestinal symptoms may be absent.[47] One adult with pharyngitis died of associated septic shock.[168] Conjunctivitis and panophthalmitis caused by *Y. enterocolitica* have been described. Parinaud oculoglandular syndrome,[40] inguinal adenopathy,[208] and suppurative lymphadenitis[198] have been reported.

Cellulitis, soft tissue abscesses, and wound infections also have been reported. Cellulitis may have associated vesiculobullous lesions. An erysipelas-like rash, maculopapular rash, and urticaria also have been described in association with infection caused by *Y. enterocolitica*.[17,28,47,72,177,191,209]

Pancreatitis, cholecystitis, diverticulitis, and intestinal perforation have been described.[120,157] Peritonitis also can occur but is extremely rare,[163] especially considering the frequency of mesenteric adenitis. Pneumatosis intestinalis has been reported in an infant.[1] Pneumonia, pleural empyema, lung abscess, hepatic and splenic abscesses, urinary tract infection, and renal abscess also have been reported.[47] Glomerulonephritis that usually is transient has been reported.[48,60] Cases of meningitis, osteomyelitis, septic arthritis, pyomyositis (including psoas muscle abscess), endocarditis, mycotic aneurysm, and intravenous catheter–related infection caused by *Y. enterocolitica* have been described.[12,29,47,83,184,186] Thrombocytopenia[65] and hemolytic anemia[108] have occurred in association with infection caused by *Y. enterocolitica*.

Underlying Conditions That Predispose to Bacteremia

Y. enterocolitica bacteremias that occur in patients beyond early infancy most often occur in patients with chronic illnesses or iron overload states. Thalassemias are such conditions that occur most commonly in children.[38,58,101] Among 144 Italian children with thalassemia who were receiving deferoxamine therapy and frequent blood transfusions, 14 developed infection caused by *Y. enterocolitica* during a 12-month period.[38] Septicemia occurred in 5 of the 14 and was preceded by enterocolitis or mesenteric adenitis in each case. All 14 recovered after receiving 2 weeks of therapy with intravenous trimethoprim-sulfamethoxazole. A similar proportion of children with thalassemias observed in two centers in Canada between 1979 and 1994 also developed invasive disease caused by *Y. enterocolitica*.[6] Bacteremia can be associated temporally with blood transfusions in such patients, suggesting that transfusions may be the source of infection or predispose to development of infection in some cases.[120]

Hemochromatosis, cirrhosis, and other liver diseases may facilitate development of *Y. enterocolitica* bacteremia, also on the basis of excess availability of serum iron. Deferoxamine therapy itself is a risk factor for development of sepsis caused by *Y. enterocolitica* because of the ability of the microbe to extract iron from this compound. Immunosuppressive therapies, diabetes mellitus, and malnutrition also may predispose to development of *Y. enterocolitica* bacteremia.[47]

Postinfectious Syndromes

A reactive arthritis may occur 1 to 14 (usually 4 to 10) days after the cessation of acute illness.* Most such events occur in adults,

*See references 7, 8, 10, 13, 83, 86, 166, 193, 201, 210.

with a slight female predominance, but 8 of 74 cases in one series from Sweden were in children aged 11 to 20 years old (5 boys, 3 girls).[210] In a series from the Netherlands, 10 percent of children with yersiniosis, most of whom were aged 7 years old or older, developed arthritis.[86] The knees, ankles, and wrists are affected most commonly, and in approximately 50 percent of cases, only one or two joints are involved. Hands, fingers, toes, shoulders, hips, and elbows also may be involved. Pain usually is severe, and the arthritis is additive and usually not migratory. The inflammatory process is self-limited and may persist for 2 months or longer in two thirds of cases, with one third persisting for 4 months or longer.

The erythrocyte sedimentation rate exceeds 60 mm/hr in approximately 50 percent of cases. Joint effusions usually are inflammatory, but cell counts and differentials vary and occasionally mimic septic arthritis. Immune complexes have been found in joint fluid. Nonsteroidal anti-inflammatory drugs and corticosteroids, intra-articular and systemically administered, have been used for symptomatic relief for this process.[165]

Erythema nodosum also occurs as a postinfectious manifestation, more often in adults than in children. It can occur alone or in association with arthritis.[86] Tendinitis, myositis, myocarditis, urethritis, uveitis, and conjunctivitis also can occur in association with arthritis. Many but not all patients who develop these postinfectious reactions are HLA-B27 positive.[1] Some patients manifest full Reiter syndrome.[86,109,170,182] Acute glomerulonephritis has been linked to infection with serotype O:3 strains in one series of adults.[60]

Yersinia antigens, but not intact bacteria, have been found in synovial tissue obtained several weeks to months after onset of reactive arthritis. *Y. enterocolitica* is able to survive within in vitro cultures of human synovial cells for 6 weeks, with resultant deposition of residual antigen aggregates within the cells.[90] Synovial fluid–derived T cells from patients with *Y. enterocolitica*–induced reactive arthritis have been shown to respond to several *Y. enterocolitica* antigens: heat shock protein 60, urease beta subunit, ribosomal L2 protein, and a region of the plasmid-encoded tyrosine phosphatase YopH that is highly homologous to the catalytic domain of eukaryotic protein tyrosine phosphatases.[114,134,155]

Epitopes on *Yersinia*-produced proteins likely trigger cross-reactive immunologic recognition of host proteins that leads to chronic inflammation in susceptible individuals.[95] High levels of antibodies that react with 60 kd recombinant *Yersinia* heat shock protein have been found in HLA-B27–positive individuals with acute anterior uveitis or pars planitis.[31]

Antibodies against *Y. enterocolitica* have been detected in patients with disorders of the thyroid, including Graves disease, thyroid adenoma, and Hashimoto thyroiditis.[178] A Danish twin study found no increased risk of thyroid antibodies after *Y. enterocolitica* infection, however.[77] These observations may reflect autoantibodies that cross-react with *Yersinia* epitopes, rather than a causal link between yersiniosis and thyroid disease.[47,164]

Brachial plexus neuropathy and transverse myelitis, each occurring after the resolution of gastrointestinal symptoms caused by *Y. enterocolitica*, have been described in one patient.[187] Various chronic ailments have been described among a group of 160 Scandinavian patients observed for 4 to 14 years after having acute yersiniosis.[172] Ailments included persistent joint complaints, ankylosing spondylitis (in HLA-B27–positive patients), iridocyclitis, chronic hepatitis, chronic abdominal pain, rheumatoid arthritis, chronic nephritis, thyroid disease, and neurologic ailments. Observed deaths among these patients exceeded the expected number. These findings require confirmation before causal links can be considered.

YERSINIA PSEUDOTUBERCULOSIS

The pseudoappendicular syndrome that results from mesenteric adenitis is the primary disease produced by *Y. pseudotuberculosis*.* The chief complaint is abdominal pain, either diffuse or localized to the right lower quadrant. Fever (38° C to 40° C) almost always is present. Tenderness over the McBurney point usually is present. All of these symptoms are highly suggestive of acute appendicitis. Diarrhea may occur but often is absent. Mild leukocytosis occurs, but white blood cell counts usually are less than 20,000/mm^3. The clinical course almost always is benign, with recovery usually beginning approximately the fifth day of illness. On laparotomy, the appendix is normal in most cases but occasionally appears inflamed or suppurative. The mesenteric lymph nodes are enlarged and may appear necrotic.

Efforts have been made to distinguish the pseudoappendicular syndrome caused by *Y. pseudotuberculosis* from that caused by *Y. enterocolitica*.[24,66] *Y. pseudotuberculosis* adenitis is less likely to have associated enterocolitis and may have a shorter febrile course, but no clear distinction can be made on clinical grounds alone.

A fulminant typhoidal or septicemic form of infection caused by *Y. pseudotuberculosis* can occur but seems to affect primarily older adults with debilitating conditions, such as diabetes or liver disease. This syndrome often is fatal.[105,171] Isolated cervical adenitis, liver abscess, and terminal ileitis have been described.[79,207] Subacute and recurrent disease can occur.[126]

Erythema nodosum[171] and nonsuppurative arthritis[37] also have been reported in association with infection caused by *Y. pseudotuberculosis*. In an outbreak related to eating contaminated grated carrots in Finland in 2003, erythema nodosum was seen in numerous patients with gastrointestinal illness.[94] Median time from exposure to development of gastrointestinal symptoms was 8 days. Median time from exposure to development of erythema nodosum was 19 days (see Fig. 123–2). One patient developed reactive arthritis in a similar time frame.

Yersinia pseudotuberculosis and Kawasaki Disease–like Illness

In Korea and Japan, *Y. pseudotuberculosis* strains have been responsible for a clinical syndrome that can mimic Kawasaki disease.[37] This manifestation of disease occurs primarily in outbreaks and has been described as scarlet fever–like.[150] A transient (2 to 3 days) erythematous maculopapular rash, strawberry tongue, conjunctivitis, and desquamation can occur, generally in association with gastrointestinal symptoms and fever. Erythema nodosum, lymphadenopathy, uveitis, and coronary aneurysms have occurred in some of these cases, as has acute interstitial nephritis, which can lead to transient renal failure.[3,112,150] Among a series of 33 patients (median age, 5 years) with such presentations, 20 had elevated antibody titers against *Y. pseudotuberculosis*–derived mitogen, which can function as a superantigen.[3] As in Kawasaki disease, Vβ3 T lymphocytes were increased in many of these patients compared with healthy control subjects.

DIFFERENTIAL DIAGNOSIS

The differential diagnosis of *Yersinia* enterocolitis includes viral and other bacterial causes of acute gastroenteritis. When the symptoms of mesenteric adenitis are predominant and severe, appendicitis and other causes of an acute abdomen must be considered. The acute terminal ileitis caused by *Yersinia* infections

*See references 20, 50, 55, 57, 66, 84, 89, 104, 105, 126, 150, 169, 171, 203, 207.

also can be similar to the gastrointestinal manifestations of Crohn disease, ulcerative colitis, cat-scratch disease, anisakiasis, amebiasis, actinomycosis, typhoid fever, and lymphoma.[64,86,150,200,207] Cases of infection caused by *Y. enterocolitica* with concomitant recovery of *Salmonella*, *Campylobacter*, and rotavirus antigen in stool specimens have been observed.[1]

DIAGNOSIS

The most effective approach to the diagnosis of yersiniosis is isolation of the organism from the stool of patients with enteritis caused by *Y. enterocolitica* or from the infected mesenteric lymph nodes of patients infected by *Y. pseudotuberculosis*. *Y. enterocolitica* occasionally can be recovered from involved mesenteric lymph nodes or the distal ileum.[47] Cultures of feces from individuals with acute suppurative mesenteric adenitis usually fail to grow either organism.

Isolating *Yersinia* organisms from extraintestinal specimens such as lymph nodes and blood is not difficult because they grow on ordinary media (e.g., blood agar) and on several selective and differential media employed for enteric bacteria. Isolating from fecal specimens is more difficult, however, because *Yersinia* multiply more slowly than other enteric bacteria at 37° C and have no characteristic colony morphology. Selective media have been developed, but many clinical laboratories culture stool specimens for *Yersinia* spp. only on request because of the costs of these media and the relatively low frequency of occurrences of these pathogens in the community.

Yersinia organisms grow well on MacConkey agar but are much smaller than are other enteric bacteria after standard incubation at 37° C. Cefsulodin-irgasan-novobiocin agar plates have been designed specifically for the isolation of *Yersinia* spp. from stool specimens. After being incubated for 48 hours, *Yersinia* colonies appear dark pink with translucent borders and occasionally are surrounded by a zone of precipitated bile. Cefsulodin-irgasan-novobiocin agar inhibits the growth of most other bacteria except for *Citrobacter* spp. (positive citrate reactions of which allow their distinction). If a dedicated medium for *Yersinia* isolation is not used, MacConkey agar can be examined after 24 hours at 35° C to 37° C for small colorless colonies that become much larger after an additional 24 hours of incubation at room temperature. Most *Y. enterocolitica* strains are lactose-negative.[69] *Yersinia* spp. can be differentiated readily from *Salmonella* spp. because the latter are motile at 37° C, urease-negative, citrate-positive, and lysine-positive. Most *Salmonella* strains also produce gas during fermentation and produce hydrogen sulfide. *Shigella* are urease-negative and lack motility at room temperature.[186]

Yersinia spp. grow faster at 37° C than at room temperature. Growth occurs readily at 22° C to 28° C, however, and these lower temperatures are recommended for primary isolation.[69] Because of the ability of *Yersinia* spp. to grow at even colder temperatures, specimens can be inoculated into phosphate-buffered saline, refrigerated at 4° C to 6° C, and subcultured periodically (up to 4 weeks) if the routine plates that were inoculated with the specimen remain negative. Such "cold enrichment" greatly enhances the isolation rate of *Yersinia* spp. and may be the most reliable method for isolating these organisms from fecal specimens. Many of the *Yersinia* isolates recovered by cold enrichment, however, represent either *Y. enterocolitica* serotypes, which usually are not associated with human disease, or other *Yersinia* spp., which have roles that remain unclear.

Pathologic or virulent *Yersinia* strains can be distinguished in most instances from nonpathogenic strains by three biochemical tests that are associated with absence of the virulence plasmid. The virulent strains lack pyrazinamidase activity, do not ferment salicin, and do not hydrolyze esculin. On Congo red–magnesium oxalate agar during incubation at 36° C, fresh pathologic isolates (but not those that have been subcultured serially) grow as small red colonies, showing the virulence plasmid-determined properties of Congo red dye uptake and calcium-dependent growth.[54]

SEROLOGY

Serology can be performed with microtiter techniques and is most reliable for serotypes O:3 and O:9 of *Y. enterocolitica*. Antibody to the infecting serotype usually is absent at the onset of disease. Peak titers usually are reached 3 to 4 weeks after onset of clinical illness and decrease during the next 3 to 5 months. Low postconvalescent titers may persist for months. Microhemagglutination, complement fixation, and enzyme immunoassays are available in a few commercial laboratories.[25,69,200]

Agglutinin titers of 1:128 or higher for *Y. enterocolitica* in previously normal healthy individuals suggest infection. Titers of 1:200 or greater were present within 3 weeks of onset of illness in 62 of 65 Canadian children who had infection with serotype O:3. Fourfold increases in titer rarely were seen. Negative or minimal titers (≤1:32) do not rule out yersiniosis in infants or immunosuppressed patients. Serologic responses occur more commonly and are of higher titer among patients with extraintestinal systemic infection. Prozone reactions may occur at dilutions of 1:32 or lower. Marked cross-agglutination occurs between *Y. enterocolitica* serotype O:9 and *Brucella abortus*, *Morganella morganii*, and *Salmonella*.[25,131] Antibodies to *Y. pseudotuberculosis* often are detectable at the onset of clinical signs of infection and may be highest during the acute phase of illness.[84]

MOLECULAR TECHNIQUES

PCR assays have been developed for *Y. enterocolitica* and *Y. pseudotuberculosis* with use of primers that allow assessment of 16S rRNA sequences and of specific plasmid-encoded and chromosome-encoded genes.[37,116,143,144,204,206] PCR techniques have been used to identify *Yersinia* organisms in blood, tissue, stool, water, and food samples.[78,115]

TREATMENT

Most patients with yersiniosis do not require treatment because the disease usually is self-limited. Seriously ill patients generally have responded to treatment with chloramphenicol, gentamicin, or tetracyclines, but clinical success has not been uniform. Of these agents, tetracyclines have been the traditional agent of choice.[66,200] *Y. enterocolitica* isolates resistant to the tetracyclines have been reported in recent years, however. Two percent of a sample of *Y. enterocolitica* isolates from Canada in 1992 were resistant to tetracycline,[154] and 10 percent of a sample of *Y. enterocolitica* isolates from the Netherlands from 1982 to 1991 were resistant to doxycycline.[188]

More than 99 percent of 1060 isolates of *Y. enterocolitica* collected in Canada in the years 1972 to 1976, 1980, 1985, and 1990 were susceptible in vitro to piperacillin, cefotaxime, aztreonam, gentamicin, tobramycin, amikacin, trimethoprim-sulfamethoxazole, chloramphenicol, and ciprofloxacin. No evidence of decreasing susceptibility to any of these agents was found across the periods that were sampled. These results were mirrored by a study of 335 isolates obtained in the Netherlands from 1982 to 1991. All of these isolates were susceptible to ceftazidime, cefepime, imipenem, trimethoprim-sulfamethoxazole, ciprofloxacin, and ofloxacin. Aminoglycosides were effective against more than 99 percent, chloramphenicol was effective against 94 percent,

and cefuroxime was effective against 90 percent. Seven multidrug-resistant isolates were present among the isolates from Canada, but none was found among the isolates from the Netherlands.[154,188]

Most *Y. enterocolitica* isolates, regardless of serotype, are resistant to ampicillin, ticarcillin, and first-generation cephalosporins. Most of them also are resistant to amoxicillin–clavulanic acid. Azithromycin was active in vitro against 50 percent of the 335 isolates in the Netherlands, but almost all isolates were resistant to erythromycin and clarithromycin.[154,188]

The decreasing effectiveness of tetracyclines in vitro raises the question of whether these agents should be the first choice for treatment of infection caused by *Y. enterocolitica*. A retrospective review of 43 cases (with patient ages ranging from 3 to 89 years) treated for *Y. enterocolitica* septicemia in France between 1985 and 1991 showed that third-generation cephalosporins were effective in 85 percent of cases in which they were used, although aminoglycosides or fluoroquinolones usually were administered concurrently.[61] Fluoroquinolones alone or in combination with other agents cured all 15 patients. Seven children with bacteremia and diarrhea in Michigan responded well to cefotaxime.[1]

In a double-blind, placebo-controlled trial of trimethoprim-sulfamethoxazole for treatment of children with gastroenteritis caused by *Y. enterocolitica*, the clinical course of illness was not shortened.[151] The children had been ill for a mean of 12 days before treatment was begun, however.

Systemic infections, extraintestinal focal infections, and enterocolitis in compromised hosts should be treated with antibiotics.[47] The in vitro susceptibilities and limited clinical data suggest that children with such infections should be treated with a third-generation cephalosporin, an aminoglycoside, or both. Trimethoprim-sulfamethoxazole also may be used.[38,86] Fluoroquinolones also probably would be effective. Cefotaxime and ceftriaxone were effective in treating bacteremia in a series of 12 children.[2]

Even fewer clinical and in vitro susceptibility data are available for *Y. pseudotuberculosis*. These infections probably can be managed identically to *Y. enterocolitica* infections. All isolates of *Y. enterocolitica* and *Y. pseudotuberculosis* should be examined for susceptibility to a variety of antibacterial drugs.

OTHER *YERSINIA* SPECIES

Eight other "non-*pestis*" *Yersinia* spp. (*Yersinia frederiksenii*, *Yersinia intermedia*, *Yersinia kristensenii*, *Yersinia aldovae*, *Yersinia bercovieri*, *Yersinia mollaretii*, *Yersinia rohdei*, and *Yersinia ruckeri*) occasionally have been isolated from clinical specimens, but their roles as human pathogens remain unclear.[69,177,189] Each except *Y. aldovae* has been isolated from humans worldwide, including individuals with gastrointestinal disorders.[189] These microbes also can be found in fresh-water sources, sewage, dogs, pigs, cattle, wild mammals, birds, reptiles, fish, and some foods, especially milk and meat products.

These organisms are similar biochemically to one another and have been termed atypical *Y. enterocolitica*.[47] None contains the *Yersinia* virulence plasmid pYV.[135] Some contain other large plasmids distinct from pYV, carriage of which may be associated with the ability to cause diarrhea in humans.[152,189] A heat-stable enterotoxin has been found in isolates of *Y. bercovieri*.[190] These *Yersinia* spp. can grow at 4° C and on cefsulodin-irgasan-novobiocin agar and can multiply in refrigerated foods.

These species occasionally may cause diarrhea and other gastrointestinal symptoms, especially in immunocompromised hosts or individuals with gastric acid suppression.[124] Use of cold enrichment techniques may enhance recovery of these organisms. Their isolation from clinical specimens neither should be disregarded nor should be deemed causative of clinical disease without careful epidemiologic considerations.[69] *Y. bercovieri*, *Y. mollaretii*, and *Y. rohdei* can be misidentified as *Y. enterocolitica* by commercial identification systems used in many clinical laboratories. Differences in colony morphology, pyrazinamide reactions (negative for *Y. enterocolitica*), and other biochemical tests can be used to distinguish them from *Y. enterocolitica*.[73,100]

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CHAPTER 124

Miscellaneous Enterobacteria

Randall G. Fisher

This chapter focuses on three less commonly isolated organisms of the family Enterobacteriaceae: *Edwardsiella tarda*, *Hafnia alvei*, and *Pantoea agglomerans*. Each of these organisms, although uncommon, can cause significant disease in certain clinical circumstances.[51]

EDWARDSIELLA TARDA

BACTERIOLOGY

E. tarda is a non–lactose-fermenting, gram-negative bacillus that is indole-positive and produces hydrogen sulfide. It ferments only glucose and maltose. The species name *tarda* reflects its biochemical inactivity. It usually is lysine-positive and ornithine decarboxylase–positive.[21] The organism resembles *Salmonella* biochemically and clinically.[33] *Salmonella* usually ferments mannitol, sorbitol, and rhamnose, however. The innate resistance of *Edwardsiella* to colistin also distinguishes it from *Salmonella*.[54] *E. tarda* grows well on usual differential media in the laboratory and produces smooth, glistening, semitranslucent colonies.

EPIDEMIOLOGY

E. tarda is an organism associated with fresh-water and marine life and has been isolated from turtles, fish, pelicans, alligators, seals, and toads.[33] It also is found in snakes and lizards. Case reports of human disease have implicated ornamental fish,[76] pet turtles,[55] snakes,[64] and catfish,[6,13,29] and injuries in fresh and brackish water.[70]

Patients with chronic liver disease, chronic ethanol abuse, steroid therapy, and hemoglobinopathy particularly are prone to developing infection with *E. tarda*.[33] Any condition associated with iron overload also is a potential risk factor. Serious infection with *E. tarda* in otherwise well individuals also can occur.[70]

Infection with *E. tarda* is global but occurs more commonly in tropical and subtropical climates, particularly Southeast Asia, Africa, and Latin America.[34] The elderly and the very young seem to be at increased risk for developing severe illness.[9] Asymptomatic carrier states have been documented well,[60] but no true epidemic has been reported, and person-to-person transmission has not been established directly.[19]

PATHOPHYSIOLOGY

Invasiveness of the organism in HeLa and HEp-2 cells,[61] siderophore production, the elaboration of a cell-associated hemolysin,[30] and resistance to complement-mediated lysis may contribute to virulence, although no clear associations have been made.[37] Hemolytic activity requires the presence of two genes, *ethA* and *ethB*. The *ethA* gene codes for the hemolysin, whereas the *ethB* gene codes for an activation/secretion protein, which is necessary for activation of the hemolysin. Transcription of the *ethB* gene is regulated by iron, which may explain the association between iron overload states and severe *E. tarda* infection.[30] Hemolysin activity seems to be necessary for cell entry and cytotoxicity.[73]

CLINICAL MANIFESTATIONS

Infections with *E. tarda* can be divided broadly into two types: intestinal and extraintestinal. Approximately 80 percent of infections are intestinal.[70] Gastrointestinal infection usually causes self-limited enteritis, with intermittent watery diarrhea and low-grade fever.[39] Nausea and vomiting usually are not seen. Occasionally, enterocolitis or a dysentery-like illness is noted.[47] Because most laboratories do not culture stool specimens specifically for *Edwardsiella*, most cases are unrecognized.

Wound infection is the most common extraintestinal infection. Most of the wounds are caused by fish fins or snakes; wounds sustained in automobile accidents also have been implicated.[33] Cellulitis or abscesses may be produced. Coinfection with other organisms, particularly *Aeromonas hydrophila*, is common.[29,70] One case of necrotizing fasciitis and myonecrosis in an immunocompetent man has been reported. Cultures obtained at surgical débridement yielded a pure growth of *E. tarda*.[70]

Septicemia with *E. tarda* is a rare but serious infection that carries a mortality rate of approximately 45 percent. Most patients with septicemia have underlying conditions, such as liver disease, iron overload, and immunosuppression. Septicemia occasionally follows a mild diarrheal illness.[13] Infants without other risk factors have been reported.[78] Septicemia manifests with high fever, shock, and, often, disseminated intravascular coagulation. Meningitis also has been reported.[59,68] Death sometimes occurs despite administration of appropriate antimicrobial therapy.

Other syndromes associated with *E. tarda* infection include an enteric fever–like illness,[13] multiple or solitary liver abscesses,[45,86] osteomyelitis,[64] septic arthritis,[6] cellulitis,[24] myonecrosis,[70] necrotizing fasciitis,[50] endocarditis,[56] uterine pyomyoma,[84] tubo-ovarian abscess,[62] and peritonitis.[13] A case of puerperal intrauterine infection in a woman without obvious risk factors has been reported; her infant was not affected.[52] Patients with sickle-cell disorders may be predisposed to bony infection with *E. tarda*, as they are with *Salmonella*.[68,83] Reported cases all are in patients with sickle-cell hemoglobinopathy, rather than homozygous sickle-cell disease.

DIAGNOSIS AND TREATMENT

Diagnosis rests on identification of *E. tarda* in culture. The major pitfall is mistaking it for *Salmonella*.

E. tarda is sensitive in vitro to most antibiotics used routinely in the treatment of gram-negative infections, including β-lactams, cephalosporins, aminoglycosides, and fluoroquinolones.[12] It also is sensitive to chloramphenicol, trimethoprim-sulfamethoxazole, and tetracycline.[72] Resistance to polymyxin B, colistin, and, occasionally, penicillin has been shown.[12,13] The organism almost universally elaborates a β-lactamase, but no resistance to β-lactams other than penicillin[12] and oxacillin[72] has been reported.

Gastrointestinal disease usually does not require treatment. Severe disease, such as septicemia or meningitis, probably should be treated with the combination of a β-lactam and an aminoglycoside,[33] even though synergy has not been shown.

HAFNIA ALVEI

BACTERIOLOGY

H. alvei is a facultatively anaerobic, gram-negative bacillus, formerly referred to as *Enterobacter hafnia*.[69] This indole-negative, catalase-positive, and oxidase-negative organism is positive for lysine and ornithine decarboxylases. It is motile at lower temperatures, but may be immotile at temperatures of 35° C and greater. *H. alvei* ferments mannitol, maltose, and sucrose. It grows well on blood or MacConkey agar as a nonlactose fermenter, producing gray-white, slightly elevated, glistening colonies.[20]

EPIDEMIOLOGY

H. alvei has been found in soil, dairy products, sewage, and the feces of humans and animals. Some question exists as to whether it is part of the endogenous microflora of the gut[65] or whether an asymptomatic "carrier" state exists. One study from Japan cultured *H. alvei* from 13 percent of healthy subjects[49]; other epidemiologic surveys have shown the incidence to be less than 2 percent.[66] Long regarded as a nonpathogen, *H. alvei* now has been associated clearly with enteritis and rarely has been isolated in pure culture from other sites, including blood, cerebrospinal fluid, peritoneal fluid, and urine. Infection seems to be opportunistic.

PATHOPHYSIOLOGY

The pathophysiology of *H. alvei* has been investigated most thoroughly with regard to its production of gastrointestinal symptoms,[2] although whether *H. alvei* is actually a diarrheagenic pathogen remains controversial. Albert and associates[3] showed that although *H. alvei* elaborated neither enterotoxins nor a Shiga-like toxin and was not invasive in HeLa cell assays or by Sereny test, it did produce diarrhea in experimental animals whether given parenterally or by mouth. Sections of intestines from infected animals showed lesions indistinguishable from those caused by enteropathogenic *Escherichia coli*. "Diarrheagenic strains" of *H. alvei* also were thought to differ from other strains in that they possessed the *eaeA* gene.[32] Janda and colleagues[36] first presented data suggesting that diarrheagenic *H. alvei* isolates were actually either unusual biotypes of *E. coli* or a new species in the genus *Escherichia*. Subsequently, the same group showed that organisms originally called *H. alvei* reported to produce attaching-effacing lesions and to possess the *eaeA* gene are actually a novel species, *Escherichia albertii*.[31] Information about the pathophysiology of extraintestinal infection is sparse.[35]

CLINICAL MANIFESTATIONS

Cases of diarrhea caused by *H. alvei* (possibly *E. albertii*) have been reported primarily in children.[2,3] Most patients with gastroenteritis secondary to *H. alvei/E. albertii* report 6 to 12 episodes of watery diarrhea per day, low-grade or no fever, and nausea with or without vomiting. Mucus sometimes is found in stools, but blood is not.[65,82] In most patients, symptoms last for a few days, but in some patients, symptoms persist for more than a week.[65] One patient with a reactive arthritis from *H. alvei/E. albertii* enteritis has been reported.[57] A unique case has been reported of an 11-year-old girl who had a 3-day history of red lesions on the forehead, neck, and trunk associated with abdominal pain, emesis, and decreased urinary output, with one bloody diarrheal stool the day before admission, who then developed hemolytic-uremic syndrome. Stool cultures repeatedly were negative for *E. coli* O157:H7, but positive for a toxigenic strain of *H. alvei* from two consecutive cultures.[15]

Traditionally, extraintestinal *H. alvei* infections were thought to occur mostly in hospitalized patients and to be hospital-acquired. In one large series, the organism was isolated from 80 samples collected from 61 patients; 57 (93%) patients had underlying illnesses, most commonly malignancies.[28] Nearly half of isolates were from the respiratory tract. Other sites included blood, skin, wounds, urine, and intra-abdominal abscesses. In 60 (75%) samples, other organisms were cultured concomitantly. In a more recent, larger study from Canada, two thirds of 138 isolates were of community onset, 112 (81%) were from the urinary tract, and 94 (68%) were from monomicrobial infections.[40] Older age and female gender were risk factors for development of infection.

One 20-day-old premature infant with necrotizing enterocolitis grew *H. alvei* from blood and stool.[25] Yeager and associates[85] reported four cases of pneumatosis intestinalis in patients after they had undergone bone marrow transplantation, and one of the four grew *H. alvei* in pure culture from blood. An 8-year-old boy with acquired immunodeficiency syndrome (AIDS) developed recurrent episodes of *H. alvei* bacteremia.[14] Both episodes were associated with fever and diarrhea; the second episode also was associated with pneumonia and pleural effusion, the cause of which is unclear. A well-documented case of pneumonia in a 54-year-old woman with AIDS has been reported in which the pleural fluid grew a pure culture of *H. alvei*.[22]

Wound abscess caused by *H. alvei* has been reported.[1] A 2-year-old liver transplant recipient developed hepatic abscess and bacteremia with *H. alvei* and *Enterococcus faecalis*.[7] One case of meningitis in a 1-year-old infant without known predisposing risk factors has been reported.[53] A case of a woman with rheumatoid arthritis who contracted endophthalmitis with *H. alvei* in mixed culture has been described; the woman used snake powder as a food seasoning.[11] Two case reports are described in the older literature of persistent bacteremia with this organism, alone and in mixed cultures. One of these patients was a previously well 13-year-old girl.[27]

DIAGNOSIS AND TREATMENT

Diagnosis is established by isolation of the organism from stools or from normally sterile body fluids. Gunthard and Pennekamp[28] reported the susceptibility results of 80 *H. alvei* isolates recovered from 61 patients over a 2.5-year period. All isolates tested were susceptible to ciprofloxacin. Isolates also were generally susceptible to tobramycin (99%), imipenem (99%), piperacillin (92%), trimethoprim-sulfamethoxazole (90%), ceftriaxone (88%), and ceftazidime (88%). Nearly all isolates were resistant to ampicillin and to amoxicillin-clavulanate.

H. alvei is constitutively cephalothin-resistant.[82] One report describes an inducible β-lactamase that rendered the isolate ceftazidime-resistant.[74] In one study, the antimicrobial susceptibility of *H. alvei* was compared with that of *E. albertii*, the organism with which *H. alvei* is confused most frequently. *E. albertii* isolates were naturally susceptible to all β-lactams except for penicillin and oxacillin; they also were susceptible to azithromycin. In contrast, *H. alvei* strains were naturally resistant to tetracycline, amoxicillin, amoxicillin-clavulanate, ampicillin-sulbactam, azithromycin, and the narrow-spectrum cephalosporins.[72]

Treatment probably is unnecessary for most cases of gastroenteritis. Treatment for invasive infection should be based on susceptibility testing. Empiric therapy with a third-generation cephalosporin and an aminoglycoside is reasonable pending susceptibility results.

PANTOEA AGGLOMERANS

First identified as a plant pathogen and named after Erwin Smith in 1917, the genus *Erwinia* has an extended domain as an infectious microbe, including production of disease in humans.[10,63,71] A member of this group of organisms, *Pantoea agglomerans* (*Enterobacter agglomerans*, *Erwinia herbicola*) has been established as a cause of conjunctivitis,[10] central nervous system infections,[10,69] urinary tract infections,[62,68] pneumonia,[4] and nosocomial infections secondary to contaminated intravenous fluids.[24,38]

BACTERIOLOGY

Species definition within *Erwinia* has been controversial and confusing. It has been suggested to rename the anaerogenic clinically relevant *Erwinia* of the *herbicola-lathryi* group *E. agglomerans* or *P. agglomerans*.[21,33,71] In clinical microbiology, both of the latter terms tend to be used interchangeably. This species consists of facultatively anaerobic, fermentative, hydrogen sulfide–negative, gram-negative rods; they do not possess oxidase, phenylalanine deaminase, proteinase, or arginine dehydrolase.[21] None decarboxylates ornithine.[33] They are motile, have peritrichous flagella, and produce a yellow pigment. Strains grow well at 37° C (98.6° F) on standard agar. When colonies are viewed microscopically after growth for 18 to 20 hours, characteristic biconvex spindle-shaped bodies and bacterial aggregates often can be seen.[10,69] Almost all strains isolated from clinical specimens and grown on agar slants show characteristic elongated, spheroidal aggregates,[69] called *symplasmata* by Cruickshank,[16] who first described them.

EPIDEMIOLOGY

Microorganisms of the genus *Erwinia* long have been recognized as phytopathogens producing dry necrosis, wilts, and soft rots in plants, and more recently they have been associated with disease in trout and leafhoppers.[63,71] *P. agglomerans* (*E. herbicola*) first was isolated in humans from stool specimens of patients with typhoid fever in the 1920s and given the name *Bacterium typhi flavum* because of the organism's alleged capacity to be transformed into *Salmonella typhi* in subculture. Identification of saprophytic human strains followed, and the biochemical and cultural identity of *B. typhi flavum* with the *E. herbicola-lathryi* group finally was established.[21]

The first reports of *P. agglomerans* as a human pathogen appeared in the 1960s. Subsequently, a nationwide outbreak of infection caused by this organism was traced to contaminated liners from caps of parenteral solution bottles.[43,44] The importance of this organism as a nosocomial cause of bacteremia is emphasized by the 17 percent mortality rate in patients receiving an infusion with the contaminated intravenous fluid.[43] A trend was noted for an increased mortality rate in infected individuals younger than 20 years old. In neonatal intensive care units, the situation seems to be even more dire; in one study, the mortality rate for infants with blood cultures positive for *P. agglomerans* was 88 percent (seven of eight).[77] Lipid-based medications support rapid bacterial growth at room temperature and, in the absence of strict aseptic handling have been implicated in more recent nosocomial *P. agglomerans* bloodstream infections.[8] *Pantoea* outbreaks also have been traced to contaminated blood products; prolonged storage of packed red blood cells at 4° C provides conditions that allow these organisms to grow and subsequently produce high concentrations of endotoxin.[5] Cotton used to filter heroin has been implicated as a source of infection ("cotton fever") in intravenous drug users.[23]

Outbreaks in pediatric hospitals caused by contaminated intravenous solutions also have been described.[48] At least one retrospective review of *Erwinia* organisms isolated from clinical specimens suggested a predisposition to infection in the pediatric age group[10]; however, other studies have noted no preference based on season, sex, age, or residence in hospitals.[71] A large pseudo-outbreak traced to contamination of cotton pledgets also has been reported.[38]

PATHOPHYSIOLOGY

The true incidence of clinical infection caused by *P. agglomerans* is difficult to ascertain because of the common association of this microbe with other organisms when obtained from clinical specimens. Nonetheless, the accumulation of case reports in which this organism is isolated in pure culture from infected material leaves little doubt that *P. agglomerans* can be a human pathogen, apart from its role as a nosocomial contaminant. This organism has little inherent invasiveness. Evidence for animal pathogenicity of *Erwinia* strains isolated from plants and humans is limited; 10^{13} washed organisms injected intraperitoneally into mice or guinea pigs do not cause symptoms, whereas inoculation of 10^{27} organisms leads to death within 36 hours.[16]

Most strains seem to act as saprophytes in humans,[79] but the organism has been isolated from purulent wounds of the extremities acquired through lacerations or thorn pricks, which suggests agricultural injury as a possible mode of infection.[79] Thorn injuries also have led to septic arthritis and osteomyelitis.[17] A delay of 4 to 6 weeks often occurs between the injury and the bone or joint infection. Most serious infections have occurred in individuals with a breach of host defenses (e.g., immunocompromised individuals or patients who received contaminated intravenous fluid).

CLINICAL MANIFESTATIONS

P. agglomerans bacteremia often is associated with fever, shaking chills, and systemic toxicity characteristic of gram-negative sepsis. These symptoms frequently have been misinterpreted, however, in hospitalized patients who unknowingly were administered contaminated intravenous fluids.[44] Premature neonates with sepsis owing to *P. agglomerans* tend to have a rapidly progressive sepsis with prominent pulmonary involvement, including hemorrhage and acute respiratory distress syndrome. In the largest pediatric series reported to date, 21 of 53 (about 40%) *P. agglomerans* isolates from normally sterile sites were grown from central venous lines; of these, two thirds were polymicrobial.[17]

Eye and skin infections caused by *P. agglomerans* are particularly prominent. Bottone and Schneierson[10] included six cases of conjunctivitis, five of which occurred in infants, from whom this organism was isolated. Only two of the isolates were in pure culture, however, and a description of the clinical course of these children was not included in the report. *Erwinia* endophthalmitis has been associated with foreign body penetration of the eye in a 14-year-old boy.[58]

Skin infection in association with a casted fracture has been described in an elderly patient,[79] and wound infections from which this organism was isolated have been described subsequently, most often in association with agricultural injury.[79] Four isolates were obtained in mixed cultures from skin lesions of children younger than 5 years old, but their possible role in those infections was not confirmed.[10] In a recent series, 14 cases of abscesses required drainage; all 14 were polymicrobial.[17] *P. agglomerans* was the only organism isolated from six consecutive blood

cultures in a 9-year-old boy with osteomyelitis.[46] A 13-year-old boy developed septic arthritis caused by *P. agglomerans* 1 month after sustaining a plant thorn injury to his knee.[18] A case of *P. agglomerans* spondylodiskitis has been reported.[63] In many cases of *P. agglomerans* osteomyelitis or septic arthritis, the patient is afebrile and blood cultures are negative. In addition, measures of inflammation and joint fluid findings may be largely unremarkable.[17] A high index of suspicion of *P. agglomerans* must be maintained when bone or joint infection follows plant thorn injury.

Primary lung disease caused by these bacteria is a rare occurrence; it has been reported in an adult with chronic bronchitis.[4] This organism is a very rare cause of meningitis. A contaminated incubator has been implicated in two cases of neonatal central nervous system infection,[75] and a cisternal tap revealed the presence of *P. agglomerans* in an unrelated newborn case whose clinical course was not described.[10] A 57-year-old man with tetralogy of Fallot and cyanosis had a brain abscess in which *P. agglomerans* organisms grew in pure culture.[80] Presenting manifestations included headaches, seizures, and left-sided weakness, which occurred over a 2-week period before admission. The patient recovered after undergoing drainage of the abscess and gentamicin therapy. One case of peritonitis in a patient on continuous ambulatory peritoneal dialysis has been reported; it was thought to be secondary to a rose thorn injury.[41]

DIAGNOSIS

Difficulty in identifying this organism is common; in March 1972, as part of a quality control program, *P. agglomerans* was sent as an unknown organism to 250 U.S. hospitals and was identified incorrectly 45 percent of the time.[44] Even when fully identified in isolates from human sources, *P. agglomerans* often is considered a contaminant or saprophyte. The organisms have been identified mistakenly as *Citrobacter*, *Escherichia coli*, *Flavobacterium*, and *Klebsiella*. In addition to routine microbiologic studies, identification of yellow-pigmented colonies and observation of the characteristic spindle-shaped bodies and symplasmata can aid in this differentiation. Symplasmata (elongated, spheroid aggregates) are seen best in the condensation water of slant cultures.[79] Spindle-shaped bodies termed *Wetzsteinformen* by German authors are observed best on standard agar with a low-power microscope lens.[71]

TREATMENT

Most localized infections respond to treatment that includes an aminoglycoside. The presence of persistent localized infection with this organism should prompt a search for an organic foreign body, given the organism's tendency to live as a saprophyte or as a pathogen in vegetable material. In addition to appropriate antimicrobial therapy, treatment of bacteremia should include removal of any potentially contaminated intravenous access. The physician should be alert to the possibility of a common source of infection in nosocomial outbreaks.[44] In view of the rarity of bacteremia caused by *P. agglomerans*, single sporadic cases should be investigated, and clusters of two or more cases should lead to immediate inquiry into possible sources of contamination. Formal surveillance programs have been the key to early recognition and abortion of epidemics.[44]

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CHAPTER 125

AEROMONAS

Ralph D. Feigin ✪ Roger K. Nicome

Aeromonas spp. were recognized for their role in disease first by Sanarelli in 1891. His findings associated aeromonads with bacteremic "red leg" disease in frogs.[131] Studies by Hill and associates in 1954 further linked the first human disease, acute fulminating metastatic myositis, with *Aeromonas* infection.[23] Generally, *Aeromonas* spp. have been considered opportunistic pathogens for humans; however, these organisms have been identified with increasing frequency as primary pathogens in normal individuals and in compromised hosts.

Aeromonas bacteria are ubiquitous. They are found as normal flora in nonfecal sewage and are isolated from tap water, canals, streams, and rivers. They also have been isolated from clinical and food samples. Aeromonads are pathogens for cold-blooded animals (fish, amphibians, and reptiles).

EPIDEMIOLOGY

Ewing and associates[46] and other investigators have isolated *Aeromonas* from tap water and from the water or sediments of rivers, especially during periods when the water temperature was relatively warm.[75,97,139] Hazen and colleagues[61] recovered *Aeromonas hydrophila* from 135 of 147 natural water sources in 30 states of the United States and reported that its density was higher in flowing (lotic) than in calm (lentic) water systems and lower in fresh-water than in saline systems; however, *Aeromonas* could not be recovered from waters in which the saline content approached that of sea water or from extremely polluted waters. Leclerc and Buttiaux[83] found *Aeromonas* in 30 percent of more than 9000 samples of drinking water in France. *Aeromonas* also have been recovered from hospital water supplies.[97,113,114] More recently, Pin and colleagues[116] isolated these organisms from clinical and food samples, including poultry, shellfish, pork, and beef, where they thrived even at low temperatures.

Aeromonas organisms survive readily on surfaces such as bench tops and moistened paper towels. Slotnick[144] recovered *Aeromonas* organisms from a moistened paper towel that had been allowed to dry 24 hours after the application of the organisms. When the towel was placed in a humidified closed environment, the period was extended to 2 weeks.

A. hydrophila may be found in the mouths of fish, alligators, turtles, tadpoles, and frogs.[65] It also has been found in the feces of guinea pigs and laboratory mice.[40] Ticks are another source of *Aeromonas*. Unusual sources of *Aeromonas* infection include contamination of home or hospital hemodialysis equipment,[71,121] tsunami-associated wound contamination,[64] and contamination of blood or blood products.[118,122] San Joaquin and colleagues[133] noted that *Aeromonas* organisms also have been found in ornamental aquaria belonging to patients with *Aeromonas*-associated gastroenteritis; however, they found that isolates from the aquaria differed in susceptibility testing from the gastrointestinal isolates, so the aquaria probably were not the sources of infection.

Aeromonas also have been isolated from a large proportion of ready-to-eat salads.[93] Of *Aeromonas* salad isolates, 35 percent were *A. hydrophila* or *Aeromonas sobria*. All isolates tested in this study had at least one marker of enteropathogenicity, including hemolysin and cytotoxin production. Nonetheless, few if any, cases of diarrhea have been associated with ingestion of these salads in immunocompetent hosts.

In 1988, California made infection by *Aeromonas* a reportable condition, permitting the first population-based study of the epidemiology of infection caused by this organism. The overall incidence rate for *Aeromonas* isolation was 10.6 cases per 1 million population. The gastrointestinal tract was the most common site of infection (81% of cases), followed by wounds (9%). Five (2%) of 219 patients with *Aeromonas* infection died; all had serious underlying medical conditions.[78] *A. hydrophila* has been placed on the U.S. Environmental Protection Agency Contaminant Candidate List of emerging pathogens in drinking water because it has the potential to grow in water distribution systems and to be resistant to chlorination.[14]

ETIOLOGIC AGENT

Aeromonas organisms are motile, asporogenic, gram-negative rods that contain a single polar flagellum. The organisms are oxidase-positive and catalase-positive and produce acid or gas during carbohydrate fermentation. They can grow at temperatures between 0° C and 41° C. Growth may occur within a pH range of 5.5 to 9. Lipase, gelatinase, DNase, and other exoenzymes are formed by these organisms.[45,138,156]

Aeromonads grow well on blood agar; most strains produce a large zone of beta-hemolysis on this medium, although nonhemolytic strains exist. *Aeromonas* colonies on blood agar have a ground-glass appearance and a fruity odor.[158] Aeromonads also grow on MacConkey, eosin-methylene blue, *Salmonella-Shigella*, and triple sugar iron media.

The sensitivity and specificity of various media for detection of *Aeromonas* spp. in fecal specimens have been evaluated.[103,126,155] Isolation is achieved readily on any of the following: prilxylose-ampicillin agar, xylose-sodium, deoxycholate citrate agar, alkaline peptonic water, inositol–brilliant green bile salts agar, trypticase soy broth with ampicillin, and dextrin-fuchsin-sulfite agar.[155] The colonies appear almost colorless on these media, with the exception of growth on dextrin-fuchsin-sulfite agar, on which they appear dark red.

Mishva and associates[99] evaluated five selective agars. Of these, sheep blood agar with 30 mg of ampicillin/L (ASBA 30) permitted the greatest number of *Aeromonas* colonies to grow, while also inhibiting the competing fecal flora. They recommend that ASBA 30 be used with DNase–toluidine blue agar (DNTA) to detect the ampicillin-susceptible strains and the nonhemolytic strains. The combination of ASBA 30-DNTA allowed 98 percent of all isolates to be detected.

Strains of motile *Aeromonas* isolates can be identified to species level by use of the following tests: production of nitrate reductase; fermentation of D-glucose and trehalose; failure to use mucate; and the inability to produce acid from D-arabitol, dulcitol, erythritol, and xylose.[1] Detection of *Aeromonas* organisms by use of strain-specific fluorescent antibody has been described.[155] The technique is not satisfactory; only a small percentage of isolates react with prepared antisera, which suggests the presence of numerous serogroups. The use of a monoclonal antibody against *A. hydrophila* has been shown to overcome this problem, however.[38] This monoclonal antibody could prove beneficial in the future as a screening tool. Additionally, the study of selected housekeeping genes has been helpful in establishing phylogenetic relationships among members of the aeromonads.[147]

Aeromonas previously was considered a member of the Vibrionaceae family, which included the genera of *Vibrio* and *Plesiomonas*.[119] Molecular genetic techniques have identified that these three genera are not related evolutionarily, however, and aeromonads represent a family of their own.[34] *Aeromonas* have long been viewed as belonging to one of two major groups on the basis of their ability to grow at different temperatures. Mesophiles were strains that grew at 35° C to 37° C, and psychrophilic strains were more prevalent at lower temperatures (22° C to 28° C). These two groups could be distinguished not only by optimal growth conditions but also by elaboration of pigmentation on tyrosine agar and production of indole. The mesophilic strains are motile, and the psychrophilic strains are nonmotile.

Fourteen *Aeromonas* spp. have been recognized, and this number is expected to increase as new strains are isolated and identified.[1] Although the number of strains of psychrophilic *Aeromonas* spp. has remained stable, the number of various strains of mesophilic species isolated has increased. Several classification schemes within the genus are in use. The strains are classified based on biochemical assays or the use of genotyping techniques such as DNA-DNA hybridization and restriction fragment length polymorphism of the 16S rRNA, or both. Genetic techniques have been used to differentiate the genus into genomospecies or hybridization groups (HG), allowing more precise identification.[15]

Abbott and associates[1] classified *Aeromonas* based on the results of the Møeller decarboxylase and dihydrolase reactions. They also were able to place the strains they studied into complexes based on certain biochemical reactions. The *Aeromonas* strains that produced elastase, pectinase, and stapholysin were grouped into the *A. hydrophila* complex (*A. hydrophila*, *Aeromonas bestiarum*, *Aeromonas salmonicida*). Similarly, the *Aeromonas carviae* complex includes *A. caviae*, *Aeromonas media*, and *Aeromonas eucrenophil*, and the *Aeromonas sobria* complex comprises *Aeromonas veronii* HG8, *Aeromonas jandaei*, *Aeromonas schubertii*, and *Aeromonas trota*.

In addition, other species, including *Aeromonas media* (similar to *A. salmonicida*),[5] *A. veronii*,[63] *A. schubertii*,[22,62] *A. jandaei*,[20] *A. trota*,[21] *Aeromonas allosaccharophila*,[91] *Aeromonas encheleia*,[44] *A. bestiarum*,[6] *Aeromonas popoffii*,[68] and *A. eucrenophila*,[138] have been described. A new species, *Aeromonas culicicola*, was isolated more recently.[115] Other isolates include *Aeromonas enteropelogenes* and *Aeromonas ichthiosmia*, which seem to be similar to previously identified species.[33] Of the species isolated currently, *A. hydrophila*, *A. caviae*, and *A. veronii* have been associated most often with human infections.[69]

Aeromonas is confused most frequently with Enterobacteriaceae. The oxidase test always should be performed to aid in differentiation; *Aeromonas* organisms generally are oxidase-positive, whereas Enterobacteriaceae organisms are oxidase-negative. McGrath and associates[95] described oxidase-variable strains of *A. hydrophila*. These strains are oxidase-positive when grown on nonselective media but oxidase-negative when grown on differential or gram-negative media. Organic acid end-products of lactose fermentation can inhibit the oxidase reaction. Paik[110] also has shown that a platinum wire loop should be used for oxidase testing; use of an iron-containing loop can cause false-positive oxidase results.

Aeromonas is susceptible to ceftriaxone (8 μg/mL), chloramphenicol (2 to 4 μg/mL), gentamicin (0.5 to 2 μg/mL), streptomycin (16 μg/mL), and trimethoprim-sulfamethoxazole (<0.5/9.5 μg/mL).[46,47,108,109,123,155] Reinhardt and George[124] determined that the most active on a weight basis were ciprofloxacin, enoxacin, and norfloxacin; nalidixic acid and trimethoprim-sulfamethoxazole also possessed good activity. Neither sulfamethoxazole nor trimethoprim alone was active. In addition, they did not note appreciable differences in susceptibility among species or in susceptibility between fecal versus nonenteric isolates. In contrast, Motyl and colleagues[102] noted higher levels of resistance to various antibiotics among *A. hydrophila* strains compared with *A. sobria* or *A. caviae*, as judged by minimal inhibitory concentration. Susceptibility to cephalothin may serve as a useful criterion in the identification of *A. sobria*.[102,133]

Aeromonas organisms consistently are resistant to penicillin, ampicillin, carbenicillin, cephalothin, erythromycin, clindamycin, and vancomycin. Sawai and associates[137] showed that resistance to β-lactam antibiotics is caused by the production of a species-specific, chromosome-mediated β-lactamase. It is not R plasmid–mediated. In 1980, McNicol and coworkers[96] reported the recovery of *Aeromonas* isolates from the Chesapeake Bay that were resistant to tetracycline and polymyxin B. They also noted that 57 percent of isolates from Bangladesh had a multiple streptomycin-chloramphenicol-tetracycline resistance phenotype that correlated with the presence of a larger plasmid.

PATHOGENESIS

In addition to its pili, *A. hydrophila* produces numerous virulence factors, including extracellular toxins and enzymes that are involved in the pathogenesis of *A. hydrophila* infection. Some of the toxins elaborated by *A. hydrophila* include hemolysins, aerolysin, and enterotoxins.

Alpha-hemolysin is released from cells during a stationary growth phase. Alpha-hemolysin has a molecular weight of 50,000[87,88] to 65,000[88] and is stable at room temperature between pH 3.5 and 9.5. It is heat-labile. Alpha-hemolysin is cytotoxic to HeLa cells and human embryonic lung fibroblasts.[154] When injected into rabbit skin, it causes dermonecrosis. Intraperitoneal injection of alpha-hemolysin is lethal for rabbits and mice.

Beta-hemolysin has a molecular weight of 49,000 to 53,000 and is released near the end of the logarithmic phase of growth of the *Aeromonas* organism. It is heat-labile and resists destruction by trypsin and pronase.[88] It causes dermonecrosis of rabbit skin and is lethal for rabbits, rats, and mice. It is cytotoxic to HeLa cells and to human diploid lung fibroblasts.[152] It also has been shown to induce impairment in the intestinal epithelial barrier and to stimulate active chloride secretion in human colon cell monolayers, contributing to enteritis.[43]

Alpha-hemolysin and beta-hemolysin may be significant virulence factors. Most clinical isolates are beta-hemolytic, and hemolysis is a common feature of infection caused by *Aeromonas* spp. Alpha-hemolysin and beta-hemolysin in high concentrations produce hemorrhagic enteritis in a rabbit ileal loop; however, neither alpha-hemolysin nor beta-hemolysin is clearly established as a virulence factor in diarrheal disease.[89] Antibodies to either hemolysin neutralize both toxins.

Aerolysin is a pore-forming toxin that shares some homology with beta-hemolysin.[43] After inserting into its target cells, it disrupts ion gradients and membrane potentials, leading to cell lysis.[82] Caco-2 cells derived from human intestine have been shown to be very sensitive to the effects of aerolysin.[2]

In 1975, Sanyal and associates[135] showed that the enterotoxins from *A. hydrophila* were enterotoxigenic. Subsequently, enterotoxigenic *A. hydrophila* has been isolated from humans,[19,55-58,75,157] water sources, fish, and pigs.[16,108] Sha and colleagues[140] have characterized three enterotoxins from *A. hydrophila*. The cytotoxic enterotoxin has hemolytic, cytotoxic, and enterotoxic activities and has a molecular weight of 52,000. It is a pore-forming toxin that has been shown to cause fluid secretion and tissue damage in mouse ileal loops.[82] The other two are cytotonic enterotoxins. One has a molecular weight of 44,000 and is heat-labile. The second has a predicted molecular weight of 71,000 and is heat-stable. These cytotonic enterotoxins cause elevation of cyclic adenosine monophosphate and prostaglandin levels in Chinese hamster ovary cells and fluid secretion in rabbit ileal loops.[140] All three enterotoxins have been implicated in *A. hydrophila*–induced gastroenteritis. *Aeromonas* spp. also produce proteinase A and B, endopeptidase, staphylolytic enzyme, fibrinolysin, and leukocidin.[87] The precise relationship of these toxins and enzymes to the pathogenesis of human infection is unclear.

Strains of *Aeromonas* produce proteases, DNase, lecithinase, and elastase.[3] These extracellular enzymes may have pathologic significance. *Aeromonas* strains that possess hemolytic and cytotoxic capabilities and that are characterized as HG1/BD-2 type strains have been related strongly to patients with diarrheal disease.[81]

Antihemolysin and agglutinating and precipitating antibodies to *A. hydrophila* have been detected in patients with systemic *Aeromonas* infections but not in patients with superficial infections. Increases in the antihemolysin titer to 1:1280 and in the agglutinin titer to 1:640 have been noted.[24]

Burke and colleagues[18] found that many strains associated with diarrhea were able to hemagglutinate cells from human, horse, rat, and guinea pig. Of these strains, 68 percent displayed fucose-resistant hemagglutination. The investigators suggested that these properties may contribute to virulence.

Ketover and associates[76] showed that normal serum promotes phagocytosis and intracellular killing of *Aeromonas* organisms by normal white blood cells. In contrast, sera from two patients with fatal *Aeromonas* infections failed to do so. One patient had an

increase in the serum opsonic antibody titer from less than 1:5 in the acute stage of disease to 1:5120 in the convalescent stage. These studies suggest that a specific opsonizing antibody that is present in normal serum and normal bactericidal activity of neutrophils are required to prevent invasive *A. hydrophila* infections.

The flexible type IV pili of *Aeromonas* are the predominant pili expressed on fecal isolates of diarrhea-associated species of *Aeromonas*. They represent a family of type IV pili that has been designated Bfp (bundle-forming pili). Kirov and associates[79] have presented compelling evidence to support the concept that Bfp are important intestinal colonization factors. More recently, this group also showed that *Aeromonas* flagella are adhesins for human intestinal cells, playing a role in its enteropathogenicity.

Serogroup analysis may be helpful in establishing which *Aeromonas* isolates can cause human disease. Serogroups O:11, O:34, and O:16 predominate as causes of clinical infection.[70]

Clear correlation between known virulence properties and enteropathogenicity in humans is lacking. This lack of a clear correlation has been attributed to the heterogeneity of *Aeromonas* spp., the great variety of *Aeromonas* virulence factors, and the loose association between virulence factors and the phenotype and genotype.[69] Consequently, many researchers have proposed a multifactorial pathogenesis with the involvement of numerous cellular and extracellular virulence factors.[29]

CLINICAL MANIFESTATIONS

Aeromonas spp. have been implicated as a cause of septicemia, gastroenteritis, peritonitis, skin and wound infections, osteomyelitis, septic arthritis, ocular infections, myositis, urinary tract infections, pneumonia, meningitis, and hemolytic-uremic syndrome in children. Most of these infections have been noted in normal and compromised hosts.[13,48,111,112]

Sepsis caused by *Aeromonas* spp. has been reported in children.* Because *Aeromonas* infection is not a reportable disease, the total number of affected children is unknown. Although septicemia caused by *Aeromonas* spp. has occurred in normal children, most children affected have had a disorder known to impair the normal host response to infection or a disorder in which the intact skin as a barrier to infection has been destroyed. *Aeromonas* infection has been noted in children with leukemia (particularly those with neutropenia), aplastic anemia, cirrhosis, hemoglobinopathies, malnutrition, burns, and renal failure.

The clinical manifestations of septicemia are similar to the manifestations noted in other gram-negative enteric bloodstream infections with high fever and shock. During the course of septicemia in some of these patients, ecthyma gangrenosum also has been noted. The reported case-fatality rate of 50 percent despite administration of antibiotic therapy presumably is related to the severity of the underlying disorders and not to an unusual virulence of the organism. The propensity for infection in the compromised host suggests that *Aeromonas* organisms are of low virulence for the human host. Bacteremia with *A. sobria* and *A. punctata* also has been described.[71,122,143]

Meningitis caused by *Aeromonas* spp. also has been reported in children.[49,50,159] In one reported review of 21 years' experience with gram-negative bacillary meningitis, *Aeromonas* spp. accounted for 2 percent of the cases.[154] The course was fulminant, and the patients died despite having received antibiotic therapy. All of these children could be considered immunocompromised as a result of sickle-cell anemia (a 23-month-old child) or age (neonates).

Gastrointestinal infections caused by *Aeromonas* spp. have been reported with greater frequency in pediatric patients. *A. hydrophila*, *A. sobria*, *A. caviae*, and *A. punctata* have been recovered from stool specimens of patients with gastroenteritis.[19,28,51,71,101] In 1961, Martinez-Silva and colleagues[92] described an epidemic of enteritis in a newborn nursery affecting nine infants (eight as newborns and one at 7 days old). *Aeromonas* spp. were found in the stools of six of these infants. One newborn, the only patient with a pure growth of *Aeromonas* in a stool specimen, died. In 1964, Rosner[129] reported severe gastroenteritis in a child with growth of *A. hydrophila* from four stool cultures. In 1991, one study reported 224 cases of *Aeromonas* gastroenteritis in Iowa from January to June.[120] Taneja and associates[150] described an outbreak of diarrhea caused by *Aeromonas* spp. on a hematology-oncology ward in a pediatric hospital during March-April 2001 where 6 of 18 children were infected. *Aeromonas* spp. were found to be the sole enteropathogen in five of the six children. Aeromonads have been detected worldwide as the sole pathogen causing diarrhea in 2 to 20 percent of affected children and in only 0 to 2 percent of children without diarrhea.[29,39,56,151]

In an attempt to assess the role of *Aeromonas* spp. in diarrheal disease, numerous investigators have evaluated the fecal carriage rate of the organism.[60] Freij[50] described a study performed by other investigators who recovered *Aeromonas* from 0 of 300 adults and from 31 of 4426 (0.7%) children younger than 2 years. Pitarangsi and coworkers[117] reported carrier rates of 16 to 27 percent in various districts in Thailand and noted the same frequency of *A. hydrophila* in stools of Thais with and without diarrhea. In contrast, *A. hydrophila* was recovered from the stools of American Peace Corps volunteers more frequently when they had diarrhea than when their stool frequency was normal. Only three *Aeromonas* isolates were reported from 1685 rectal swab cultures obtained from 1217 children hospitalized for gastroenteritis in Manitoba, Canada. Bhat and colleagues[10] recovered *Aeromonas* organisms from 7 of 133 patients with acute diarrhea in a valley in India and found *Aeromonas* and *Plesiomonas shigelloides* in the well water commonly used by these individuals. Soltan Dallal and Moezardalan[148] isolated *Aeromonas* spp. from 14 of 310 Iranian children in a case-control study.

Gracey and associates[57,58] described a prospective study in Australia of 1156 children with diarrhea and an equal number of age-matched and sex-matched control subjects. Enterotoxigenic *Aeromonas* organisms were isolated from 10.2 percent of children with diarrhea compared with 0.6 percent of healthy children. *Aeromonas* was the only potential pathogen recovered in 6.5 percent of children with diarrhea. Cases of *Aeromonas* infection peaked during the summer months. The mean duration of diarrhea was 15.3 days, and 33 percent of the children required hospitalization. These investigators described three clinical syndromes of *Aeromonas* gastroenteritis: (1) watery diarrhea, vomiting, and low-grade fever in 41 percent; (2) diarrhea with blood and mucus in 22 percent; and (3) prolonged diarrhea of more than 2 weeks' duration in 37 percent.

Many investigators[4,27,104,106,132] have attempted to describe gastrointestinal infections caused by *Aeromonas* spp. Gluskin and associates[53] performed a 15-year study of the rate of *Aeromonas* spp. in gastroenteritis in hospitalized children. A total of 146 strains of *Aeromonas* spp. were isolated from 32,810 fecal specimens from 13,820 hospitalized patients. These isolates constituted 4 percent of all pathogenic bacterial strains cultured. Most of the cases of diarrhea (94%) occurred in children younger than 3 years old. The peak incidence occurred in infants aged 2 to 6 months. Bloody diarrhea occurred in 7 percent of children. Several investigators[4,132] have detected a larger number of cases of *Aeromonas*-associated diarrhea in the summer months than in other months, whereas Challapali and colleagues[27] found no seasonal patterns of *Aeromonas* isolation. The greatest number of cases occurred in children younger than 12 months to 3 years of age.

Symptoms included diarrhea, bloody stools, vomiting, abdominal cramps, mild dehydration, and fever. *Aeromonas*-associated

*See references 17, 32, 36, 59, 67, 76, 105, 111, 128, 136, 158-160.

diarrhea resembled other types of bacterial diarrhea, except that fecal leukocytes were absent in children with *Aeromonas*-associated diarrhea; in contrast, fecal leukocytes were found in 60 percent of children with other types of bacterial enteritis.[27] A cholera-like illness caused by enterotoxigenic *A. sobria* has been described.[28] Gastroenteritis caused by *A. sobria* and *A. hydrophila* tended to be acute, whereas diarrhea associated with *A. caviae* frequently was chronic, lasting 4 to 6 weeks in untreated patients.

San Joaquin and Pickett[132] described three patients with *A. caviae* diarrhea who originally presented with failure to thrive presumed to be secondary to formula intolerance. Diarrhea in these patients improved after administration of trimethoprim-sulfamethoxazole. Moyer[104] noted that although *A. caviae* is considered nonpathogenic, five pediatric patients with otitis media who were treated with penicillin or ampicillin subsequently developed diarrhea in which *A. caviae* was the only potential enteric pathogen. The prior therapy with antibiotics to which *Aeromonas* spp. are known to be resistant probably contributed to colonization of the gastrointestinal tract with *A. caviae* and subsequent development of diarrhea. These four patients also were treated successfully with trimethoprim-sulfamethoxazole.

Outbreaks of diarrhea associated with *Aeromonas* in daycare centers have been described.[39] *A. caviae*, *A. hydrophila*, and *A. sobria* were the strains recovered most commonly.

Complications of *Aeromonas* intestinal infection included gram-negative bacteremia, intussusception, internal hernia strangulation, hemolytic-uremic syndrome,[13,125] and failure to thrive. Peritonitis has been reported in a 5-year-old patient with a ruptured appendix.[67] No additional clinical information about this patient was provided.

A. hydrophila has been recovered from the skin or from wound infections of children.* Most of these patients have been normal hosts; three had leukemia. In 40 percent of these cases, *Aeromonas* organisms were not recovered from the lesion in pure culture. The lower extremity was involved in 75 percent of these cases. Exposure to water was noted in 40 percent of these cases; alligator bites, snakebites, stepping on glass, and burns were other presumed predisposing factors. Clinical manifestations included cellulitis, hemorrhagic blebs, and purulent diarrhea with fever and leukocytosis. Secondary bacteremia and osteomyelitis have been reported.[12]

We (R.D.F.) have recovered *Aeromonas* organisms from skin lesions that have been the result of tick bites. In each of these cases, a circumscribed area of purple discoloration has surrounded the bite, and nonpurulent drainage from the center of the lesion yielded the organism. These patients sought medical attention because the local lesion had persisted or had increased in size over the course of 1 to 2 weeks. *A. schubertii* also has been isolated from traumatic wound infections.[22]

Ecthyma gangrenosum caused by *A. hydrophila* has been described in several children with leukemia.[76,105,141] We (R.D.F.) have seen ecthyma gangrenosum in several children with *Aeromonas* septicemia who have had malignant or hepatobiliary disease. Clark and Chenoweth[31] isolated *A. hydrophila* from an 18-month-old boy and a 2-year-old boy, both of whom had liver transplants for biliary atresia and subsequently developed cholangitis. In both instances, these were polymicrobial infections.

Lopez and associates[90] described an 8-year-old child with acute myelogenous leukemia with bacteremia and osteomyelitis; *Aeromonas* organisms grew from a bone aspirate of this patient. Blatz[12] reported finding osteomyelitis and *Aeromonas* bacteremia in a previously healthy 16-year-old patient; a bone aspirate was not attempted. Septic arthritis caused by *A. hydrophila* also has been described in a child with leukemia; the organism was recovered from the second metacarpophalangeal joint at autopsy.[36] Elwitigala and colleagues[42] also recovered *A. hydrophila* in a previously healthy 13-year-old girl who developed severe septic arthritis of the knee after injury sustained in a private fresh-water lake. This infection frequently is rapidly progressive.

Aeromonas has been recovered from the conjunctiva of a previously healthy 7-year-old boy whose eye had been penetrated by a safety pin[145] and from the anterior chamber of an 8-year-old boy who developed endophthalmitis after receiving a corneal laceration from a fishhook.[32] *A. sobria* endophthalmitis has been described in a 14-year-old patient after a penetrating eye injury occurred in which a cormorant pecked the patient's eye.[84]

Although numerous cases of *Aeromonas* urinary tract infections have been reported in adults, only a few cases have been reported in children. McCracken and Barkley[94] reported the recovery of *A. hydrophila* in pure culture from the urine of a 5-month-old boy with diarrhea. Bartolomé and colleagues[9] described a case of urinary tract infection associated with diarrhea in a newborn boy with bilateral ureterohydronephrosis and bladder involvement from posterior urethral valves. Ojeda-Vargas and associates[107] reported the case of an 18-year-old girl with severe pyelonephritis requiring admission to the intensive care unit. *A. hydrophila* and *Pseudomonas* were isolated from her urine cultures.

Myositis caused by *Aeromonas* also has been described in children. A 9-year-old girl and a 16-year-old boy required amputation of their legs as a result of *Aeromonas* myositis.[37,146] Necrosis of muscle was noted in both cases, and gas was seen on the radiographs before amputation in the 9-year-old girl. Fatal myofascial necrosis also has been reported in a patient with a history of aplastic anemia.[54] *A. hydrophila* also has been isolated from abscesses caused by snakebites.[72]

Pneumonia caused by *A. hydrophila* is a rare event in children. Kao and associates[74] reported that there have been a total of seven case reports in the literature so far, and four patients died of fulminant disease. Of the seven patients, four had accompanying medical conditions, including acute leukemia (two patients), cavernous hemangioma with thrombocytopenia (one patient), and nephrotic syndrome (one patient). One patient was the victim of a near-drowning, and two patients were previously healthy.[74,123,142] One of the previously healthy patients was described by Kao and associates[74] in their case report and literature review. The patient was a 5-year-old girl who presented with fulminant sepsis and pneumonia and quickly died. *A. hydrophila* was recovered from her blood, endotracheal aspirate, and postmortem pleural fluid. Neither a bone marrow aspirate nor an immunologic work-up was performed, so whether she had an underlying malignancy or immunodeficiency that might have contributed to her rapid demise is unknown. *A. hydrophila* has been isolated from lung abscess at autopsy of a 16-year-old girl with *A. hydrophila* septicemia and leukemia.[36]

DIAGNOSIS AND DIFFERENTIAL DIAGNOSIS

Aeromonas should be considered a possible cause of infection in children with any of the disorders previously noted. It always should be included as a possible cause of gastroenteritis, bacteremia, and skin infection in a compromised host. Generally, *Aeromonas* organisms are recognized only when they grow from body fluids or tissues that normally are sterile. The best methods for isolation of *Aeromonas* organisms have been described (see the section on etiologic agent).

A. hydrophila in food can be detected by an enzyme-linked immunosorbent assay and polymerase chain reaction.[11,98,153] These organisms also have been identified in environmental

*See references 9, 12, 37, 48, 67, 76, 94, 105, 112, 128, 141, 149, 155.

samples by use of 16S rDNA–targeted oligonucleotide primers.[41] Many sophisticated techniques, including rRNA sequencing, DNA-DNA reassociation techniques, and polymerase chain reaction, have been used to continue to identify and differentiate *Aeromonas* spp.[15,35,69,77]

TREATMENT AND PROGNOSIS

Aeromonas infection occurs infrequently; controlled studies to permit recommendation of one antibiotic over all others are unavailable. Penicillin-hydrolyzing β-lactamases have been detected in most strains of *Aeromonas*, rendering those strains resistant to ampicillin.[100] Piperacillin shows variable activity against *Aeromonas* spp., whereas ticarcillin-clavulanate generally is active. In vitro, *Aeromonas* organisms generally are susceptible to chloramphenicol, aminoglycosides, trimethoprim-sulfamethoxazole, aztreonam, quinolones, and the third-generation cephalosporins.[73,80,130]

In our (R.D.F.) experience, chloramphenicol or third-generation cephalosporins have proved efficacious. A drug to which the organism is sensitive should be provided (usually intravenously). The duration of administration depends on the site of infection and the clinical response to therapy. The occurrence of *Aeromonas* infections predominantly in compromised hosts accounts for the high case-fatality rate despite administration of an antibiotic agent to which the organism is susceptible.

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CHAPTER 126

PASTEURELLA MULTOCIDA

Barbara W. Stechenberg

In 1878, Kitt first isolated a bacterium of the *Pasteurella* group from wild hogs during an epidemic; 2 years later, Pasteur described the organism that causes fowl cholera. Since that time, the same organism has been implicated in rabbit septicemia, swine plague, hemorrhagic septicemia, and wildseuche (a fatal disease in deer). Hueppe applied the term *hemorrhagic septicemia* to this group of infectious diseases in lower animals because of the characteristic hemorrhagic areas scattered throughout most of the viscera. Original names for the causative organisms included *Pasteurella aviseptica*, *Pasteurella boviseptica*, *Pasteurella suiseptica*,

and *Pasteurella lepiseptica*, but these organisms now are classified under the name *Pasteurella multocida*, a small, nonmotile, gram-negative rod.[38]

Although *P. multocida* primarily is a pathogen in animals, recognition of its potential for infection in humans has been increasing. Brugnatelli reported the first bacteriologically proven case in a human in 1913. Schipper[39] did an extensive review of the literature from 1930 through 1947 and reported only 40 cases of infection with *P. multocida*. Since then, an increasing number of human infections with *P. multocida* have been reported.

ORGANISM

P. multocida generally appears as a short, ovoid, gram-negative rod; however, it also may appear as coccobacilli with convex sides and rounded ends. The length ranges from 0.3 to 1.25 μm, and the diameter ranges from 0.15 to 0.25 μm. *P. multocida* may appear singly or in pairs, chains, or clusters. Healthy organisms stain easily with aniline dyes and are gram-negative. They may show bipolar staining, especially when smears are made directly from animal tissue or fluids. They become increasingly pleomorphic on subculture and may resemble enterics in broth. They do not grow on eosin–methylene blue, MacConkey, deoxycholate, or any other bile-containing agar. They are facultatively anaerobic and nonmotile. They do not require X or V factor for growth, an important differential point in distinguishing them from *Haemophilus influenzae*.

Colonies are nonhemolytic and translucent, usually 1 to 2 mm in diameter, and generally low, convex, and butyrous. Occasionally, they may be larger and mucoid. In a study of 30 strains isolated from humans, Heddleston and Wessman[17] reported that 9 of the cultures produced watery mucoid colonies, 4 produced iridescent colonies, 10 produced blue colonies, and 3 produced a mixture of iridescent and blue colonies. Colonies on blood agar are smaller, opaque, and grayish white. In broth cultures, turbidity, often with a flocculent sediment, is present.

Cultures tend to autoagglutinate in saline and have a peculiar odor described as musty, similar to semen or burning hair. They are catalase-positive, usually oxidase-positive, and indole-positive. They usually ferment galactose, glucose, fructose, mannitol, mannose, and sucrose without production of gas. Some variability occurs in the fermentation of other sugars. They reduce nitrates but have negative urease, methyl red, and Voges-Proskauer reactions. Using fermentation reactions, Oberhofer[31] developed a biotyping system in which correlation of biotype, 61 percent A and B, was found with cat-bite isolates but not with dog-bite isolates.

The pathogenicity of the organism varies; most mucoid and smooth colony-forming strains produce a capsule and usually are highly pathogenic for mice and rabbits. The capsule is antiphagocytic, resisting intracellular killing by neutrophils.

Nielsen and Rosdahl[29] have developed a bacteriophage typing system for typing toxigenic and nontoxigenic strains. DNA hybridization studies have led to a reclassification of the genus *Pasteurella*. *P. multocida* now includes three subspecies—*P. multocida* subsp. *multocida*, *P. multocida* subsp. *septica*, and *P. multocida* subsp. *gallicida*. Of 159 strains recovered from 46 infected humans, 95 were identified as *P. multocida* subsp. *multocida*, and 21 were identified as *P. multocida* subsp. *septica*; the remainder were divided among multiple other species.[16] The use of serology in conjunction with DNA fingerprinting can classify isolates for epidemiologic studies.[51]

Immunity to *P. multocida* can be shown in many animals, and vaccines have been developed with known efficacy, especially in birds and cattle. The precise mechanisms involved in this immunity and, more important, in natural immunity are being elucidated. Antibodies to somatic and capsular antigenic determinants develop within 2 weeks of development of clinical infection, with the capsular antibodies being longer lasting.[5] The precise role of these antibodies in human host defenses is unclear. Woolcock and Collins[52] developed models in pathogen-free mice that may help uncover these mechanisms. The efficacy of heat-killed vaccine has been shown to be considerable when multiple doses are used. With the use of an aerogenic mouse model for the stimulation of respiratory spread, however, the protection is reduced. Local instillation of the vaccine can be used with this model to develop local antibodies, but preliminary results show protection only with modest challenge doses.[3] A footpad inoculation model may help elucidate the mechanisms in local human infections.

TRANSMISSION

The organism is found in the oral flora of many different animals; 67 percent of cats may harbor this organism in their mouths or throats. Smith[40] found that *P. multocida* could be recovered from the tonsils of 54 percent of dogs and from the nose of 10 percent of dogs. Schipper[39] grew *P. multocida* from 14 percent of wild rats trapped in the Baltimore area. Hansmann and Tully[15] showed that *P. multocida* may remain as a commensal for prolonged periods in the mouth of a cat. They described a patient who had been bitten on two occasions 3 years apart by the same healthy pet cat; each time, an abscess caused by *P. multocida* developed. *P. multocida* also has been isolated from lion, tiger, panther, buffalo, mink, and opossum.[4,20]

Although verifying the mode of transmission has been easiest when an infection has been related specifically to a pet or farm animal, many cases of infection caused by *P. multocida* have been documented despite a negative history of such exposure. Respiratory infection with this organism has been described in veterinarians, farmers, milkmen, and individuals employed where animal tissues are processed. Meningitis caused by *P. multocida* has been described in a patient after undergoing brain surgery in which rabbit muscle was used for hemostasis.

The possibility of a reservoir of infection in humans with resultant interhuman transmission rarely has been considered. In a study of veterinary students, Smith[40] described 2 of 71 with positive throat isolations. The organisms were present in one student for a few days and in the other for the full 4 months of the study; both patients were asymptomatic. Several other cases of isolation of the organism from the human respiratory tract, many without associated symptoms or known animal contact, have been made.

In addition, interhuman spread by nasopharyngeal excretions, feces, and urine also may occur because the organism has been recovered from these sites. The female genital tract is another potential source, especially for cases of septicemia and meningitis that occur during pregnancy and in the newborn period.

Investigators of an outbreak in a chronic disease hospital showed that *P. multocida* may be viable on a hand towel for 24 hours. Although the source in this outbreak was not established, this finding may have implications for spread in other situations, particularly for pet owners.[23]

EPIDEMIOLOGY

P. multocida has been isolated from humans in all areas of North America and Europe, with some reports coming from other areas. In the United States, it is not a reportable organism, so incidence

and prevalence data are unavailable. Lee and Buhr[26] have cited a seasonal variation in the number of reported cases related to dog bites, with the highest incidence being in the fall and winter months, possibly related to increased nasal carriage in dogs during that period. Other investigators have found no seasonal differences.[9,21]

No difference in attack rate has been found between the sexes. The attack rate is higher in individuals of both sexes in very young (0-4 years old) and in older (>55 years old) individuals.

PATHOGENESIS AND PATHOLOGY

In animals that are stressed, a benignly parasitic strain may invade the mucous membrane on which it is carried. With highly virulent strains, a picture of hemorrhagic septicemia may develop. It may be characterized by high fever, cardiac weakness, toxemia, and early death. Organisms can be cultured from the blood; autopsy findings may be minimal or include petechial hemorrhages on mucosal and serosal surfaces and in various organs. Less acute forms, such as a pneumonia with serofibrinous exudate in the interlobular septa of the lungs, a hemorrhagic gastroenteritis, and subacute and chronic infections such as otitis in the rabbit, may occur. The mechanisms by which these bacteria invade the mucosa and cause systemic disease continue to unfold. Key virulence factors are the capsule and lipopolysaccharide, with others still being identified.[16]

In humans, three major types of infections occur.[50] In the first and most common type, local infection occurs after a cat bite or scratch, a dog bite, or, rarely, the bite of another animal. Approximately 15 to 20 percent of dog-bite wounds and more than 50 percent of cat-bite wounds become infected. Many cases have been associated with non-bite exposure, such as licking.[11] These cases usually are characterized by a rapidly progressive, acute cellulitis with lymphangitis, local lymphadenitis, or both. Cat-bite wounds may progress to osteomyelitis of the underlying bone. This development is not because of any known predilection of *P. multocida* for the bone but because the sharp fangs of a cat deposit the organism on or under the periosteum.

The second type includes cases of chronic pulmonary infection in which the organism may be the only isolate or one of several organisms. Cases of bronchiectasis and empyema have been reported, usually in patients with underlying pulmonary disease. In a series of 28 cases of bronchiectasis in which the organism was recovered, it usually appeared as a secondary invader. It seems to have low pathogenicity in the respiratory tract until some other infection or physiologic disturbance decreases the natural resistance of the host, which enables active infection, most commonly liver disease such as cirrhosis, to occur.

Pasteurella infection also may be septicemic or occur with meningitis. The pathology and pathogenesis are similar to those of other organisms.

CLINICAL MANIFESTATIONS

In cases of local infection from a scratch or bite, the usual clinical pattern shows swelling, erythema, and tenderness within a few hours of the bite; most symptoms occur within the first 24 hours. A gray serous or sanguinopurulent discharge from the puncture sites may be present. Signs of systemic toxic effects, such as chills and fever, may or may not be present; regional lymphadenopathy often is evident. Less commonly, the infection may be lower grade and smoldering.[35] As noted, osteomyelitis and tenosynovitis most often occur after cat bites because of the sharpness of cats' teeth.

Lee and Buhr[26] found *P. multocida* to be the most common infecting organism in a report of 69 dog bites that had been cultured; 20 of the bites became grossly infected, and *P. multocida* was isolated from 10. Of 30 wounds that were sutured, 14 (47%) were infected with *P. multocida*. Other unusual localized infections include chronic skin ulcers, secondary infection of a gouty joint, and infection of a compound fracture site and an amputation site.[46] Preexisting joint disease seems to be a risk.

Clinical manifestations of patients having respiratory complaints are not unusual. Most of the isolates have been associated with chronic bronchitis, bronchiectasis, chronic sinusitis or otitis media, and pneumonia. Several cases of massive pulmonary abscesses, pleural effusion, and empyema also have been reported. Larsen and Holden[25] described a 14-year-old girl with chronic otitis media for 2 years who developed a *P. multocida* cerebellar abscess. A case of epiglottitis caused by this organism has been reported in an adult.[24] A child developed Ludwig angina after having a non-bite exposure.

In a report of 136 cases of *P. multocida* infection that were not related to animal bites, the most common site of infection after the respiratory tract was the abdomen; the organism was recovered from 10 patients with appendicitis. Eight isolates were from the female reproductive tract, four from the urine, and one from a chronic sacral abscess.[21] Whether these cases are secondary to ingestion of the organism or to hematogenous spread has not been determined. Raffi and colleagues[34] described three children with appendiceal peritonitis associated with *P. multocida*. The organism also has been seen in cases of peritoneal dialysis-associated peritonitis secondary to puncture of dialysis tubing.[27,28]

The disseminated infections are the other major clinical group of *Pasteurella* infections. Isolated bacteremia may be present[33]; however, most of these cases have been meningitis, many of which were mistaken for *Haemophilus influenzae* or *Neisseria meningitidis* infection because of the morphologic similarities among these organisms. In a review of the subject in 1967, Controni and Jones[7] noted 14 confirmed cases of *Pasteurella* meningitis; 11 occurred in adults, and 3 occurred in children. Eight of the 14 patients had a history of accidental or surgical trauma. The mortality rate was 50 percent, but only five patients were treated with antibiotics. Evaluation of the cerebrospinal fluid showed white blood cell counts ranging from 580/mm^3 to 5200/mm^3, all with a predominance of polymorphonuclear leukocytes. More recently, Green and coworkers[14] reviewed 29 cases of *P. multocida* meningitis in adults.

The first reported newborn with *Pasteurella* meningitis died at 88 hours of age.[1] The mother had a fever in the postpartum period, but her pretreatment cultures were lost, so verification of the source was impossible.[7] Since then, a case of *Pasteurella* chorioamnionitis associated with premature delivery and neonatal sepsis and death within 1.5 hours of delivery has been reported.[42] Gingival cultures of a pet cat that had scratched a mother numerous times during pregnancy also yielded *P. multocida*. Subsequently, several young infants with septicemia and meningitis caused by this organism survived without apparent sequelae after treatment with penicillin or ampicillin and gentamicin.[2,10,36,45] Pizey[32] reported another case of neonatal infection in a 3-week-old infant with septic arthritis. *P. multocida* infection may take a rapidly fatal course even in an older infant.[39] Clapp and associates[6] described two infants whose disease was associated with nontraumatic facial licking by pets, an avoidable exposure. In another report, a case of in utero infection at 12 weeks' gestation was described.[48] A newborn with meningitis and cervical spine osteomyelitis with full recovery has been reported.[18]

DIAGNOSIS AND TREATMENT

Although *P. multocida* is one of the more likely pathogens to cause infection of cat or dog bites, its clinical manifestations are not unusual. Diagnosis of *P. multocida* infection can be made definitively only by culture. It may resemble several other organisms morphologically, but identification should not be difficult to make. The fact that it does not require X and V factors for growth should distinguish it from *H. influenzae*. Its production of indole should differentiate it from the *Neisseria* group, and its inability to grow on MacConkey agar or a bile salt medium should distinguish it from *Acinetobacter* spp. and the enteric organisms.

The drug of choice for *P. multocida* infection is penicillin, to which the organism is exquisitely sensitive. This feature may be used as a rapid means of distinguishing it from *H. influenzae* or the enterics. Rare strains producing β-lactamase and resistance to penicillins have been recovered.[37] The organism usually is sensitive to a wide variety of other antibiotics, including ampicillin, other broad-spectrum penicillins (e.g., ticarcillin, piperacillin, mezlocillin), amoxicillin–clavulanic acid,[12] tetracyclines, parenteral cephalosporins (particularly second-generation and third-generation),[30] cefuroxime, cefpodoxime, and chloramphenicol. Semisynthetic penicillins (e.g., nafcillin, dicloxacillin), erythromycin, some orally administered cephalosporins (cephalexin, cefaclor), clindamycin, and aminoglycosides have relatively low activity against *P. multocida*.[8,13,41] Azithromycin seems to have acceptable activity,[6] as does ciprofloxacin.[22] Trimethoprim-sulfamethoxazole may be an alternative, particularly for patients unable to take a β-lactam antibiotic.[38] Surgical drainage or débridement also may be necessary. The duration of treatment depends on the primary disease process. Seven to 10 days generally is adequate for local infections.

PROGNOSIS AND PREVENTION

Proper cleansing and débridement of wounds caused by animal bites or scratches are important in prevention of *P. multocida* infection. Lee and Buhr[26] found that suturing wounds caused by dog bite was associated with a higher incidence of infection. Whether a wound is sutured often depends on the site and the potential cosmetic result. The use of prophylactic antibiotics to prevent infection after animal bites is controversial. Some experts recommend their use if a delay occurs in seeking medical assistance and for cat bites or for patients with immunocompromising conditions.

Limiting contact with wild and domestic animals that may harbor the organism probably is the only definitive way to prevent infection. Teaching proper handling of pets and keeping pets from licking infants and young children, particularly on the face, may help. No vaccine for human use is available.

Prognosis depends on the particular site of infection. With appropriate treatment, resolution usually occurs, but the healing process may be very slow, particularly in local infections with extension to the bone or tendons.[9,47]

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CHAPTER 127

Cholera

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Cholera is a toxin-mediated diarrheal illness caused by the gram-negative organism *Vibrio cholerae*. Cholera is invariably a watery diarrhea syndrome that can range from mild and self-limiting to the severe life-threatening manifestation known as *cholera gravis*. Severe cholera can cause rapid intravascular volume depletion, shock, and death. The remarkable efficacy of rehydration therapy in preventing cholera deaths is one of the great triumphs of 20th century medicine.

Cholera may have existed since before the time of Hippocrates, and the infection continues to cause significant morbidity and mortality in areas where sanitation is poor. The infection is particularly prevalent in the Indian subcontinent and in parts of Africa. In 2005, a 30 percent increase in the global incidence of cholera was noted, mostly resulting from outbreaks in West Africa that accounted for 58 percent of all confirmed cases. The case-fatality rate decreased from 2.3 percent in 2004 to 1.7 percent in 2005.[6] This improvement likely is attributable to improved diagnostic tools and treatment, including availability of oral rehydration therapy and access to intravenous fluids for severe cases. On the forefront of prevention are the oral cholera vaccines, which have emerged as useful public health tools to combat the disease, especially in highly vulnerable transient populations.

HISTORY

Cholera has long been indigenous to the Ganges river valley in northern India. Although it exhibits a seasonal regularity, the annual epidemics are inconsistent, at times ravaging the population and at other times tolerable. In the absence of a known vertebrate reservoir or carrier state, the cause of this erratic pattern has remained mysterious until more recently.

With increased European incursion into the Indian subcontinent, the cyclical nature of cholera epidemics took on global proportions, occurring in a series of discrete pandemics through the 19th and 20th centuries. These disastrous events are largely responsible for the evolution of public health as an effective discipline. The first recorded cholera pandemic began in 1817, extending from India to southern Russia. During the second cholera pandemic, beginning in 1826, many jurisdictions were induced to establish local sanitation and health boards, eventually evolving into the first organized public health apparatus. During the London cholera epidemic of 1854, Dr. John Snow first showed the association of the disease with contaminated drinking water derived from the city's infamous Broad Street pump; this episode is widely considered the first scientific public health investigation. In 1866, an epidemic in New York City led to the creation of the first Board of Health in the United States, and cholera became the first reportable disease.

During the fifth pandemic in 1884, the causative bacterium, *V. cholerae*, finally was identified. The seventh pandemic began in Indonesia in the 1960s and marked the introduction of biotype *El Tor* strains. The seventh pandemic was the most widespread on record, involving Asia, Africa, southern Europe, and the Americas.[54] A new cholera serogroup O139 Bengal emerged in India and Bangladesh in the 1990s; it represents the first non-O1 serogroup isolated in association with an epidemic.[54]

MICROBIOLOGY

V. cholerae is a curved gram-negative rod that is classified by biochemical tests and subdivided into serogroups based on the somatic O antigen. It belongs to the family Vibrionaceae, which differs from the related family Enterobacteriaceae in being oxidase-positive and motile with a single flagellum. Vibrios are fermentative and oxidative in metabolism. They have few nutritional requirements and can grow in glucose as the sole source of carbon and energy. Most vibrios are marine organisms, and most grow best in 2 to 3 percent sodium chloride under aerobic conditions. Although neither the typical comma shape of *V. cholerae* nor its motility can be observed on Gram stain, both are apparent in wet mount even on fresh fecal samples, a characteristic that can be useful for rapidly establishing the diagnosis. A presumptive diagnosis of cholera can be made immediately by

adding anti-*Vibrio* antiserum, which results in cessation of motility of only the homologous organism.

Numerous *Vibrio* spp. exist, most of which are nonpathogenic in humans. Within the species *V. cholerae* are more than 200 serogroups according to the antigenic characteristics of the lipopolysaccharide O antigen.[11] To date, only *V. cholerae* organisms carrying the somatic O antigens O1 and O139 are associated with epidemic disease. Some non-O1 *V. cholerae* serogroups are associated with sporadic diarrheal illness, which can be severe and sometimes invasive and inflammatory.

The O1 serogroup is divided further into two biotypes, Classical and El Tor, based on biochemical properties and phage susceptibilities. The Classical biotype traditionally is associated with a more severe disease, but the El Tor biotype may be better adapted to persistence in the environment and cause more persistent epidemics. The expression of genes conferring human virulence seems to be higher in the Classical biotype, whereas in the El Tor biotype, enhanced expression of genes for biofilm formation, chemotaxis, and transport of amino acids, peptides, and iron is higher.[11] Classical and El Tor biotypes are divided into three serotypes (Ogawa, Inaba, and Hikojima) based on their dominant heat-stable lipopolysaccharide somatic antigens. Media used to isolate cholera organisms in stool include thiosulfate citrate bile salts sucrose (TCBS) agar and tellurite taurocholate gelatin agar. The appearance of yellow ("popcorn") colonies on TCBS agar is considered to be highly suggestive of *V. cholerae*. The bacterium is confirmed further by its ability to grow in the presence (or absence) of salt and by serology.

PATHOGENESIS

The pathogenic paradigm of *V. cholerae* comprises colonization of the small bowel mucosa followed by release of one or more enterotoxins, the most potent of which is the oligomeric cholera toxin (CT) itself. The bacteria first must pass through the acidic gastric environment to reach the upper small intestine, and compromised gastric acidity is a classic risk factor for infection.[34] *V. cholerae* attaches to the intestinal mucosa by virtue of proteinaceous hairlike fimbriae, the most important of which apparently is the toxin-coregulated pilus.[54] Colonization is facilitated by chemotaxis and motility of the organism, accelerating its approach to the intestinal mucosa.

The infectious dose varies with gastric pH because the organism does not typically tolerate acidic conditions. When water is the source, 10^3 to 10^6 bacteria are needed to cause disease. Only 10^2 to 10^4 bacteria are required when ingested with food.[16,17]

V. cholerae organisms shed in the stool of an infected individual apparently are more virulent than are strains acquired from an environmental source, and serial passage through multiple patients may augment virulence further, partly accounting for the violent epidemics occurring throughout history.[42] On intestinal passage, genes required for acquiring nutrients and motility are up-regulated, whereas genes required for chemotaxis are down-regulated. This increased virulence is confirmed in an animal model in which *V. cholerae* shed by mice have a 10-fold lower infectious dose than organisms grown in vitro.[42] Reduced chemotaxis prevents dispersion in environmental waters and increases the clumping of the bacteria[14]; the resulting increased concentration of organisms also could contribute to the vigor of cholera outbreaks.

The principal secretogenic factor, the oligomeric protein toxin CT, is composed of five identical binding (B) subunits and one active (A) subunit. Similar in structure and function to the heat-labile toxin produced by enterotoxigenic *Escherichia coli*,[46] CT binds to the GM_1 ganglioside receptor on the surface of intestinal epithelial cells. The A subunit is transported into the cell, where it activates adenylate cyclase. As a result, cyclic adenosine monophosphate is up-regulated, causing an increase in chloride secretion from the intestinal crypt cells and a decrease in sodium chloride absorption by the villous cells. In addition to this mechanism, cholera toxin may have effects on prostaglandins and the enteric nervous system that may contribute to the voluminous diarrhea characteristic of the disease.[34] The loss of electrolytes is followed by water along the osmotic gradient into the intestinal lumen. The dramatic loss of fluid and electrolytes rapidly leads to decreased intravascular volume and the complications of hypovolemic shock and metabolic acidosis. Although CT is responsible for much of the pathogenicity associated with the organism, strains mutated in CT still elicit mild diarrhea.[34]

The genetics of *V. cholerae* provide insight into the organism's pathogenicity and spread. The genome comprises two circular chromosomes, the larger of which contains genes for growth and pathogenicity, whereas the smaller one contains elements of metabolism and regulation. The genes required for virulence can propagate laterally and among different strains. Required for pathogenicity are the CT genetic element and the vibrio pathogenicity island, which encodes the toxin-coregulated pilus. The CT-encoding genetic element can exist as a plasmid or as a prophage integrated into the chromosome. Transfer of genetic material can lead to the emergence of virulence in a previously avirulent organism, or it can lead to the emergence of a new epidemic strain. The O139 Bengal strain derived DNA from a more pathogenic O1 strain, resulting in the emergence of a new pathogen.[54]

EPIDEMIOLOGY

V. cholerae O1 is distinguished by its ability to cause explosive outbreaks and pandemics. Now truly pandemic, *V. cholerae* O1 occurs (although rarely) in the U.S. Gulf Coast region, where it is associated with consumption of shellfish. Cases were described in the wake of the severe hurricanes occurring in 2005.[5]

V. cholerae is isolated commonly from surface water of moderate salinity, where the bacterium associates itself with zooplankton and phytoplankton. Pathogenic *V. cholerae* exist in thick layers (biofilms) of partially dormant cells on plankton, which may be the infectious form first encountered by humans at the start of an epidemic. When human cases begin to occur in a community, direct contamination of food and water may occur, circumventing the need for the aquatic reservoir.[21] Although *V. cholerae* have been isolated from animals, their role in transmission is probably minimal.[56] Person-to-person transmission also seems to be uncommon.[47]

During acute cholera disease, infected individuals excrete 10^7 to 10^8 organisms per 1 g of stool.[38] If the infected individual survives the episode, shedding of organisms may continue for 1 to 2 weeks after recovery in the absence of antibiotic treatment, and in some cases may continue for even longer. Transient asymptomatic carriers often are found in the households of individuals affected with acute illness.[34] Although environmental reservoirs seem to be important in the initiation of epidemics, highly virulent strains seem to be more readily isolated from areas contaminated by human feces.[34] Clinical studies suggest that increased pathogenicity of organisms occurs after passage through the human intestine.[14]

A great deal of attention has been devoted to understanding the epidemiology of cholera during interepidemic periods, and findings suggest a complex interaction between humans and their environment. Seasonal patterns have long been recognized for cholera outbreaks, with a predilection for warmer months. *V. cholerae* can remain dormant in aquatic environments in a nonculturable but viable state.[32,61] When aquatic conditions are

conducive to growth, *V. cholerae* proliferates.[19] Environmental conditions play an important role in the seasonal variations. In a 33-year observational study in Bangladesh, outbreaks were predicted by an increase in temperature and concentrations of cyanobacteria in local waters.[30] More recent climate changes in Bangladesh (El Nino/Southern Oscillation) are associated with 70 percent of the variance in cholera incidence seen during specific time intervals of heightened activity of climate change.[53]

More recent studies suggest that an increase in ocean water temperatures as a result of global warming has had an effect on cholera epidemics.[19,27,51] The ecology of vibrio-infecting phages also may affect the abundance of *V. cholerae* in the environment.[22]

Cholera classically is described as a water-borne illness, but it also can be transmitted via contaminated food. The incubation period depends on the inoculum, with lower inocula corresponding to a longer incubation period and decreased stool volumes.[34] Incubation generally is 1 to 3 days, with a range of several hours to 5 days. Symptoms usually last 2 to 3 days, and patients treated with antibiotics may be infectious for several days. Patients who are untreated remain infectious for 1 to 2 weeks. Although water-borne transmission is typical in developing countries of Asia and Africa, *V. cholerae* that is found in marine environments can be transmitted via undercooked shellfish.[20] Person-to-person transmission is rare, owing to the high infectious dose.

Factors that predispose the human host to cholera include age, immunity, blood type, reduced gastric acid production, and other factors that have not been determined. Age groups commonly affected by cholera typically include young children because susceptibility depends on the level of preexisting immunity. In endemic areas such as Bangladesh, cases of cholera are concentrated in the 2- to 9-year-old age category, followed by women of childbearing age.[34] The increased susceptibility of children is supported further by a more recent prospective study in Kolkata, India, which found that the burden of cholera was greatest in children younger than 2 years old and that cholera cases were more likely to have a household member with diarrhea.[58] Individuals with blood type O also are at increased risk of experiencing severe cholera for reasons not completely understood.[23,57,59] Host factors in addition to reduced gastric acidity also are thought to play a role because volunteers who received the same inoculum had large variations in the amount of stool they produced.[34]

Because of the potential for epidemic disease, cholera outbreaks should be reported to local and national health authorities. In 2004, five laboratory-confirmed cases of cholera were reported to the Centers for Disease Control and Prevention. Four were acquired outside the United States, and one occurred in Hawaii, thought to have been acquired through imported seafood.[33]

CLINICAL MANIFESTATIONS

Most individuals infected with *V. cholerae* have only mild symptoms, indistinguishable from symptoms caused by other enteric infections. Individuals who develop severe disease urgently need fluid resuscitation and replacement of ongoing losses. In areas where cholera is indigenous, the populations have developed a (generally healthy) respect for the potential life-threatening nature of diarrheal illness and seek early medical treatment. Of all causes of infectious diarrhea, stool volumes passed and the rapidity by which dehydration develops are greatest with cholera.[55] In severe disease, stool volume can exceed 250 mL/kg body weight in a 24-hour period.[44,52]

The most distinctive clinical feature of cholera is the painless passage of voluminous, watery stools. Termed "rice water stools" because of the watery, colorless diarrhea dotted with mucus, cholera stools may have a fishy smell or may be nearly odorless. Other common symptoms include abdominal cramping and vomiting. Abdominal cramping is probably due to increased abdominal secretions and resulting small bowel distention. Vomiting commonly occurs a few hours after the onset of diarrhea, but it also occurs later in the course of the illness.[17,28,48]

Complications of cholera relate mostly to the massive loss of intravascular volume and electrolytes. If cholera is not treated promptly, diarrhea and vomiting eventually lead to dehydration, which is most often isotonic. Patients with severe cholera may experience vascular collapse, shock, and death rates of 50 percent,[54] starting within hours after the onset of diarrhea. Related to the massive loss of intravascular volume and hypoperfusion, bicarbonate losses in stool, and hyperphosphatemia, acidosis also may develop.[64,66] Compensatory tachypnea may be observed in patients with acidosis. In addition to bicarbonate losses in stool, potassium also is lost and may not be reflected in serum potassium levels; acidosis induces the shift of intracellular potassium into the intravascular space in exchange for hydrogen ions, resulting in decreased total body potassium and potentially normal serum potassium values. As the acidosis is corrected, serum hypokalemia may result. In cases complicated by severe malnutrition, in which body potassium stores already are depleted, hypokalemia may be quite severe and manifest as paralytic ileus. This hypokalemia rarely is associated with severe cardiac arrhythmias, but it may manifest as changes on electrocardiogram.

After dehydration, hypoglycemia is the second most common cause of death in pediatric patients with cholera.[10] At the International Center for Diarrheal Disease Research, Bangladesh, the mortality rate in pediatric patients with hypoglycemia caused by cholera was reported to be 15 percent compared with 1 percent mortality in patients with normoglycemia.[10] Although the precise mechanism of hypoglycemia is unknown, children who are malnourished and acutely ill have lower glycogen stores and impaired gluconeogenesis. Special attention should be given to monitor patients with concomitant malnutrition and cholera for hypoglycemia.

Severe cholera is diagnosed in individuals who present with clinical signs and symptoms of severe dehydration (loss of ≥15% of total body water, or loss of approximately 10% of body weight). Common signs of severe volume depletion include a combination of decreased skin turgor, depressed anterior fontanelle in infants, lack of tears and sunken eyes, dry mouth, anuria, tachycardia, decreased peripheral perfusion, and hypotension (Fig. 127–1). In

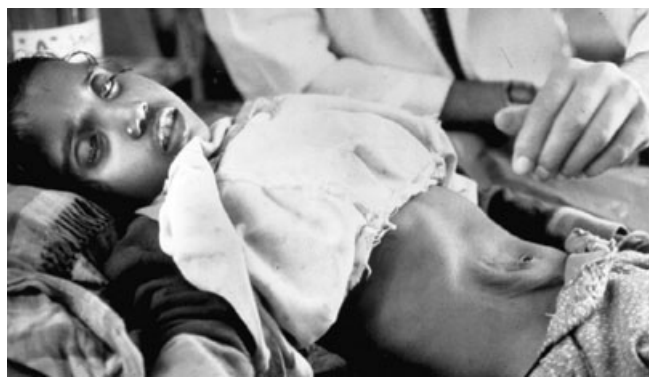

Figure 127–1 An adolescent girl with severe dehydration from cholera. Characteristic features include obtundation, sunken eyes, and "tenting" of the abdominal skin and subcutaneous tissues after firmly pinching the abdomen.

TABLE 127–1 Electrolyte Concentration in Cholera Stool and in Fluids Used for Rehydration and Replacement of Stool Losses

	Electrolyte Concentration (mmol/L)				
	Na^+	Cl^-	K^+	HCO_3^-	Osmolality
Cholera Stool					
Adults	130	100	20	44	300*
Infants and children	100	90	33	30	300*
Hydration Solutions					
WHO oral rehydration	90	80	20	30†	220‡
Intravenous					
Dhaka	133	98	13	48	273
Lactated Ringer	130	109	4	28§	251
5:4:1	129	97	11	44	281

**Osmolality includes unmeasured osmotically active molecules (primarily organic acids) in addition to electrolytes.*
†As citrate.
‡From electrolytes only; also contains 111 mmol/L of glucose.
§As lactate.
WHO, World Health Organization.

some cases, mental status changes may be a sign of volume depletion, but more common are somnolence, restlessness, and lethargy. Table 127–1 summarizes the clinical findings associated with various degrees of clinical dehydration.

LABORATORY FINDINGS AND DIAGNOSIS

Laboratory abnormalities in patients with cholera relate to the massive loss of volume and electrolytes. The hypovolemic state can lead to hemoconcentration, causing increased hematocrit, serum specific gravity, serum protein, serum creatinine, and blood urea nitrogen.[64,66] Because of the loss of sodium that accompanies water loss, serum sodium is either slightly low[52] or normal.[34] As noted earlier, although potassium loss occurs in the stool, serum potassium may be normal, whereas total body potassium may be decreased. Acidosis caused by loss of bicarbonate in stool and lactic acidosis may result in a serum bicarbonate concentration of less than 15 mmol/L.[64,66] Serum creatinine and blood urea nitrogen also are elevated as a result of a decreased glomerular filtration rate. The common abnormalities of sodium, potassium, bicarbonate, and glucose mandate early determination of serum glucose and electrolytes as a guide to therapy.

Although presumptive diagnosis is made on the basis of typical oxidase-positive colonies from stool samples that agglutinate with O1 or O139 antiserum, conventional culture methods remain the gold standard. The characteristically motile organisms can be visualized directly using darkfield microscopy,[9] but few laboratories except those in cholera-endemic regions are experienced with this technique. If cholera is suspected, special culture media, such as TCBS agar, should be used to culture the organism.

In epidemic settings or in areas of intense transmission, a rapid vibrio dipstick test may be used. This is a one-step immunochromatographic test developed by the Institut Pasteur for rapid detection of cholera O1 and O139 from stool samples. The method has been tested in Madagascar, Bangladesh, and Mozambique, where the specificity ranged from 84 to 100 percent and sensitivity ranged from 94 to 100 percent.[49,65] It is especially advantageous because it requires minimal technical skill and can be read in 10 minutes.[49] This test also has been shown to be useful in detecting cholera from rectal swabs.[12,65] Confirmation of *V. cholerae* in stool also can be done through polymerase chain reaction amplification and use of oligoprobes.[31,43,63]

TREATMENT

The cornerstone of cholera management is fluid resuscitation. When fluid resuscitation is initiated, previous losses must be estimated and replaced, and ongoing losses must be quantified and replaced. Replacement fluids in children with cholera should be similar to fluids that are lost, such as Ringer lactate solution (Table 127–1).[4,30] The ideal solution contains amounts of bicarbonate and potassium sufficient to replace what is lost in stool.[8]

At the International Centre for Diarrhoeal Disease Research, Bangladesh, where thousands of cholera patients are treated annually, patients of all ages present with severe dehydration of rapid onset. Many present in hypovolemic shock. The extent of dehydration is determined as shown in Table 127–1. One or two intravenous catheters are inserted, and isotonic resuscitation solution is administered rapidly. The composition of the preferred solution at the Center (Dhaka solution) is listed in Table 127–1. The solution contains Na^+, Cl^-, K^+, and HCO_3^-, but no glucose. The complete estimated deficit is administered over 4 hours, whereupon ongoing losses and maintenance fluids are provided. Serum glucose and electrolytes are determined rapidly, with administration of glucose as needed based on these results.

Because severely dehydrated patients characteristically are anuric, concern may exist over early administration of potassium. The rapid onset of dehydration in cholera patients reduces this risk, however, and acute tubular necrosis rarely is seen.[52,66] A greater concern may be the rapid shift of potassium from the circulation on correction of acidosis. Patients treated in this manner respond dramatically. After the 4-hour resuscitation phase, most patients can begin to accept oral rehydration solution (ORS). Close attention to fluids and electrolytes is required for the duration of the diarrhea.

Although oral and intravenous rehydration methods are acceptable in theory for the management of cholera, the severity of losses renders intravenous rehydration the mainstay for treating cases of severe cholera. Not only do intravenous fluids allow predictable and rapid expansion of intravascular fluid volume to occur, they also ensure timely replacement of ongoing losses that may be difficult because of excessive purging.[50] Mild cases can be managed with ORS (see subsequently).

Because of the massive volumes of fluid that are lost during cholera gravis, the volume deficit frequently is underestimated, and rehydration therapy often is inadequate. Dehydration is not clinically apparent until a child has lost at least 5 percent of his or her body weight and can be diagnosed from the parent's history.

The use of a "cholera cot" is extremely helpful in quantifying ongoing fluid losses and for patient comfort (Fig. 127–2). It is constructed of a simple cot with a hole in the center through which a plastic sheet drains via a funnel into a graduated bucket below. This allows for accurate quantification of ongoing losses and permits the patient to remain horizontal, an important advantage if the patient is initially hypotensive. Attention given to tachycardia and signs of hypoperfusion in prostrate patients is essential.

The World Health Organization–approved oral rehydration salts (see Table 127–1) are recommended for patients with no detectable dehydration or mild dehydration, provided that the patient can drink sufficient quantities to replace the deficit and keep pace with ongoing losses. ORS also can be used for patients with severe dehydration to replace ongoing losses after initial intravenous replacement therapy. For cholera, ORS that is made with rice rather than with glucose, as carbohydrate seems to be more effective because it reduces the purging rate.[45,67] Food should be offered to patients as soon as they are able to eat and should not be restricted.

Effective use of ORS requires extra effort in treating pediatric patients with cholera. Parents need to be instructed to encourage

and supervise ORS intake by the child, including taking small amounts frequently. Parents also need to be reassured that oral rehydration is still effective despite occasional vomiting.

A short course of antibiotics should be given to children with clinically significant cholera. This approach has been shown to shorten the duration of the illness and to decrease the amount of diarrhea.[25,54] Because of high resistance rates in some areas, the organism should be isolated and tested for susceptibility using standard methods for gram-negative enteric bacteria. Patients not treated with antibiotics may continue to shed organisms for 1 to 2 weeks after symptoms have abated.[34]

Treatment options for cholera in pediatric patients are derived from adult studies (Table 127–2). The treatment of choice for *V. cholerae* O1 and O139 is tetracycline for 3 days or a single dose of doxycycline for children older than 8 years.[3] This therapy generally is not recommended for children younger than 8 years old because of the risk of dental staining. This risk is cumulative, however, and the life-threatening nature of cholera may justify this risk. Trimethoprim-sulfamethoxazole is an alternative treatment for children younger than 8 years of age, although resistance may be present among O139 strains.[1] If tetracycline-resistant strains are encountered, other options include ampicillin, trimethoprim-sulfamethoxazole, erythromycin, and single-dose ciprofloxacin.[13,24,35,36] Single-dose azithromycin proved to be as effective as is a 3-day course of erythromycin in children 1 to 15 years old in Bangladesh.[37] Although transmission rates in endemic areas may decrease with prophylactic treatment of household members, this approach may promote resistance.[41] In the United States, where secondary transmission rates are low, prophylactic treatment of contacts is not recommended.

Antibiotic resistance to cholera seems to fluctuate with local usage patterns, showing alternating resistance and sensitivity. Conjugative plasmid–mediated, multiply-resistant *V. cholerae* O1 has been reported from several cholera endemic countries.[54] Appropriate antibiotics, usually prescribed before obtaining sensitivity testing results, should be administered according to local resistance patterns, and should be monitored for increased or decreased sensitivity patterns. *V. cholerae* O139 strains first appeared carrying a novel conjugative, self-transmissible, chromosomally integrating "SXT" element that conferred resistance to sulfamethoxazole, trimethoprim, chloramphenicol, and streptomycin. Multiple mechanisms of drug resistance are reported in *V. cholerae* and include proton-motive, force-dependent efflux and modifying enzymes.[7,26,62]

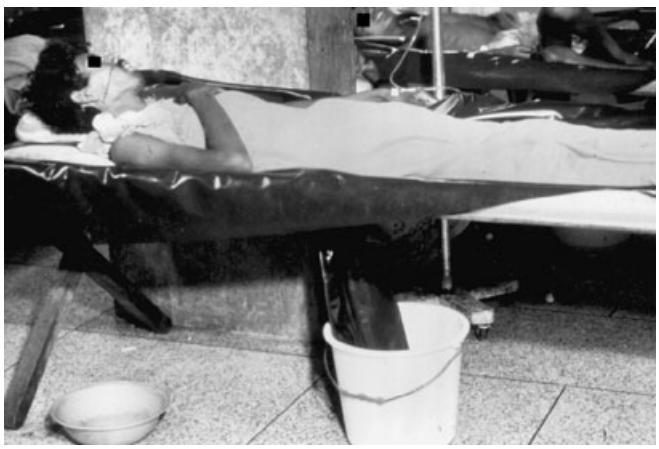

Figure 127–2 A cholera cot: a simple folding cot covered with plastic that has a hole and bucket for collecting the stool output, as used for the care of cholera patients at the International Centre for Diarrhoeal Disease Research, Bangladesh. The bucket is calibrated, and the volume of stool (and replacement fluids required) can be calculated easily. The plastic sheet is cleaned daily and between patients.

PREVENTION

Because contaminated food and water are the main routes of transmission of pathogenic *V. cholerae*, strategies to improve access to safe water sources and a clean food supply are the best methods of prevention. Proper sanitation methods also play a key role. During outbreaks, measures to increase awareness of cholera transmission and means to decrease spread should be communicated to the public; these measures include proper handwashing (especially after defecating), proper cooking of food, and identifying ill individuals so that they can be treated.

Vaccination is another method of preventing cholera. Parenteral and oral vaccines have been developed. The whole-cell, killed parenteral vaccines stimulate short-term immunity to cholera, but they are not effective in young children. Because of limited efficacy and frequent local adverse reactions, this vaccine no longer is available.[29]

Two oral cholera vaccines, one composed of killed cholera bacteria and the other composed of a living attenuated organism, currently are licensed for use in children at least 2 years old.[29]

TABLE 127–2 Antimicrobial Therapy of Cholera*

Agent	Single Dose	Multiple Dose
Erythromycin	Not evaluated	40 mg/kg/day erythromycin base divided into 3 doses for 3 days; maximal dose 1 g/day
Azithromycin	20 mg/kg	
Furazolidone	7 mg/kg; maximal dose 300 mg†	5 mg/kg/day divided into 4 doses for 3 days; maximal dose 400 mg/day
Ciprofloxacin‡	30 mg/kg; maximal dose 1 g	30 mg/kg/day divided into 2 doses for 3 days; maximal dose 1 g/day
Trimethoprim-sulfamethoxazole	Not evaluated	8 mg trimethoprim/40 mg sulfamethoxazole/kg/day divided into 2 doses for 3 days; maximal doses 320 mg trimethoprim and 1.6 g sulfamethoxazole daily
Ampicillin	Not evaluated	50 mg/kg/day divided into 4 doses for 3 days; maximal dose 2 g/day

**Antimicrobial therapy is an adjunct to fluid therapy for cholera and is not an essential component. It reduces diarrhea volume and duration, however, by approximately 50 percent. The choice of antimicrobial agent is determined by the susceptibility pattern of local strains of Vibrio cholerae O1 or O139. Resistance to all agents except fluoroquinolones, such as ciprofloxacin, has been reported and is common in some areas.*

†Single-dose therapy with these drugs has not been evaluated systematically in children, and recommendations are extrapolated from experience in adults.

‡The fluoroquinolones, such as ciprofloxacin, are not approved for use in children <18 years old in the United States because when given in high doses to juvenile animals, they cause arthropathy. Clinical experience indicates that this risk is very small in children when used for short courses of therapy.

The killed cholera vaccine (rBS-WC; marketed by SBL Vaccines [Sdna, Sweden] under the trade name Dukoral) consists of 1 mg of recombinant cholera toxin B subunit and approximately 1×10^{11} inactivated whole cells of the Classical and El Tor biotypes of *V. cholerae* O1, serotypes Inaba and Ogawa. The vaccine is approved for children older than 24 months of age and generally is well tolerated. The rBS-WC vaccine is supplied as 3-mL single-dose vials, each provided with a sachet of sodium bicarbonate buffer. The buffer solution is prepared by dissolving the sachet in 150 mL of water immediately before consumption. In one study, each dose of vaccine was mixed with 40 mL, 75 mL, or 150 mL of buffer solution for individuals 2 to 4 years, 5 to 11 years, or older than 11 years.[40] The vaccine must be refrigerated during shipping. Two doses administered at least 15 days apart are recommended. In a cohort of children and adults in Beira, Mozambique, studied during a cholera outbreak, receipt of one or more doses of rBS-WC vaccine was associated with 78 percent protection (95% confidence interval 39 to 92%; $P = 0.004$) against culture-confirmed cholera. Most vaccinees had received two doses. All of the cases were caused by infection with *V. cholerae* O1 El Tor Ogawa, and all occurred within 6 months of immunization. The vaccine was equally effective in children younger than 5 years old and older individuals. Protection induced by the killed vaccine persists for at least 3 years in adults, but it declines significantly after 6 months in children 2 to 5 years old.[18] The rBS-WC vaccine also may provide herd immunity in endemic settings.[2]

The live-attenuated cholera vaccine, CVD 103HgR, provided 100 percent protection against the homologous strain in challenge models for at least 6 months.[60] This vaccine also was proven to be effective in an outbreak of naturally occurring cholera in Micronesia[15]; a single dose of CVD 103HgR provided 79 percent protection (95% confidence interval 72.9 to 84.6%) against clinically defined cases of cholera during the 3-month post-vaccination period.

Both vaccines are available for travelers to developing countries with active transmission of cholera. The use of cholera vaccine in addition to initiation of proper sanitation methods should be considered in outbreaks in refugee camps, in relief and disaster workers, and in individuals who are traveling to endemic areas where access to medical care is limited.

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CHAPTER 128

VIBRIO PARAHAEMOLYTICUS

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Vibrio parahaemolyticus is recognized worldwide as a cause of foodborne disease associated with consumption of seafood, in particular crustaceans and mollusks, and occasionally as a cause of wound infections and sepsis in immunocompromised hosts. *V. parahaemolyticus* was isolated first in Japan during a foodborne outbreak in 1951. Almost 10 years after it was first described, the organism was classified correctly in the genus *Vibrio*,[8] and its distribution in coastal waters and estuaries of temperate climates around the world was recognized. The regional differences in the incidence of this disease are related to patterns of consumption of marine products and practices of food preparation. In Japan and Taiwan, where fish and shellfish are a major source of dietary protein and frequently consumed raw, *V. parahaemolyticus* accounts for 10 to 40 percent of foodborne outbreaks.[57,73] In the United States, the first confirmed foodborne outbreak occurred in Maryland in 1971, secondary to consumption of contaminated crabmeat.[17] Since then, sporadic outbreaks have been reported,[5,6,17,18,55] showing the potential risk for development of infectious and toxic syndromes from marine products in the United States.[24]

BACTERIOLOGY

V. parahaemolyticus is a gram-negative, non–spore-forming, curved rod with rounded ends that possess a polar flagellum.[45] On surfaces or in viscous environments, *V. parahaemolyticus* can differentiate into swarmer cells equipped with additional shorter lateral peritrichous flagella.[44] *V. parahaemolyticus* is a facultative anaerobe with respiratory and fermentative metabolisms. Similar to most vibrios, it is an oxidase-positive nonfermenter of lactose.[7] It is arginine dihydrolase–negative and ornithine decarboxylase–positive. The requirement of sodium and enhancement of growth in a specific range of concentration differentiate it from *Vibrio cholerae* and *Vibrio mimicus*. The optimal sodium concentration range is 2 to 4 percent; a concentration greater than 8 percent inhibits its growth.[72]

V. parahaemolyticus produces round, blue-green colonies on the widely used *Vibrio*-selective thiosulfate citrate bile salt sucrose (TCBS) agar. This medium inhibits most fecal flora by the presence of bile salts and a highly alkaline pH. Direct plating on TCBS agar may be used for feces and other clinical specimens,

but food samples that are not heavily contaminated require enrichment either in alkaline peptone water supplemented with 3 percent sodium chloride or in a strongly selective medium, such as glucose salt teepol broth.[72] The optimal pH for growth is in the neutral range, but *V. parahaemolyticus* can survive in alkaline media. Optimal growth temperatures are between 35° C and 39° C.[76] Growth is remarkable in conventional media and foodstuff, with generation times of only 8 minutes[36]; a significant number of organisms can be found after a short period of inappropriate storage. Growth is inhibited at temperatures of 44° C or greater. Degree of inactivation is greater with increased temperatures; a million-fold decline occurs in viable bacteria in shrimp homogenate kept at 100° C for only 1 minute.[70] In laboratory conditions with low temperature and nutrient supply, *V. parahaemolyticus* has been shown to enter a viable but nonculturable state, during which the organism no longer can be cultured on routine growth media, but its viability can be confirmed using direct microscopic assays.[25,74]

The genome of *V. parahaemolyticus* consists of two circular chromosomes. Genes for a type III secretion system (TTSS) were identified in a pathogenicity island on chromosome 2.[42] TTSS, which is considered a central virulence factor in salmonellae and shigellae, possesses needle-like structures, which facilitates direct injections of bacterial proteins into target host cell, and is related strongly to the pathogenicity of inflammatory diarrhea seen with *V. parahaemolyticus* infections. This finding suggests that the presence of TTSS in *V. parahaemolyticus* differentiates its pathogenicity from that of *V. cholerae*, which does not carry the genes.[42]

EPIDEMIOLOGY

In the United States, *V. parahaemolyticus* is found along the East, West, and Gulf coasts.[20,36] Water, sediments, suspended particulates, plankton, fish, and shellfish have been shown to harbor the organism.[4,47] The organisms are present in highest numbers in water at temperatures between 17° C and 35° C and containing 0.5 to 2.5 percent salinity.[34] Marked seasonal and geographic variations occur, with maximal mean concentrations occurring during late summer and spring along the Gulf Coast.[20] The seasonal distribution correlates with outbreaks, predominantly between June and October.[3]

Finfish and all types of shellfish products, including oysters, clams, crabs, and shrimp, may be involved in the transmission of the infection (Table 128–1).[34] The risk of acquiring infection is higher with molluscan shellfish because of their ability to concentrate contaminants and bacteria by filter feeding.[4,25,76] In oysters, the density of *V. parahaemolyticus* may be 100 times greater than in the surrounding water.[20] The levels of contamination of seafood generally are low in freshly collected oysters, with the highest mean density of only 160 bacteria/g found in the United States.[20] In Japan, higher counts (up to 10^3 to 10^4/g) have been reported in market shellfish.[13] The minimum infective dose is thought to be in the range of 10^5 to 10^7 organisms, based on volunteer feeding studies.[23] More recent outbreaks of illness caused by oysters that met bacteriologic safety standards have raised questions about current recommendations, however.[18]

During the period 1973 to 1987, only 23 of 1869 foodborne disease outbreaks with known bacterial etiology reported to the Centers for Disease Control and Prevention were caused by *V. parahaemolyticus*.[5] In 18 of these outbreaks, a shellfish was recognized as the food vehicle. No fatal cases were reported. Contributing factors included inadequate cooking (92%), improper holding temperature (75%), and food acquired from an unsafe source (75%).[5,6] Most of the affected individuals were adults, and no patterns of unusual susceptibility by age group or gender were noted. No evidence of secondary spread was found among family members. Between 1988 and 1997, 345 sporadic cases were reported to the Centers for Disease Control and Prevention. Most infections were related to eating raw oysters. Most of these infections (59%) were gastroenteritis.[19]

Until more recently, *V. parahaemolyticus* infections were casually associated with multiple serotypes and occurred as sporadic cases or localized outbreaks.[49] In 1996, a sudden increase in incidence was seen in India, and 50 to 80 percent of the cases were attributed to a single serotype, O3:K6.[54] Shortly thereafter, O3:K6 strains were isolated from clinical samples from other countries in Asia. In 1998, two outbreaks of gastroenteritis in the

TABLE 128–1 Selected Outbreaks of *Vibrio parahaemolyticus* Infection

Setting	Persons Ill/Exposed	Symptoms	Incubation	Resolution	Medical Attention/ Hospitalization	Vehicle	Risk Factor
Picnic[14]	351/631 (56%)	D (95%), C (82%), N (68%), V (61%), HA (41%), F (27%)	16 hr (4-42 hr)	4 days (<1-10 days)	60%/2%	Steamed crab	Cross-contamination, unrefrigerated food
Chronic hospital[14]	24/100 (24%)	D (100%), C (89%), N (72%), V (44%), HA (56%), F (33%)	18 hr (4-60 hr)	3 days (<1-7 days)	No data available	Crab salad	No data available
Shrimp boil[49]	~600/1200 (50%)	D, C, V, HA, F	23 hr (5-92 hr)	(few hr-1 wk)	1%/0%	Boiled shrimp	Storage temperature
International flight[43]	12/134 (9%)	D, C, V	(8-20 hr)	No data available	40%/25%	Cooked crab	Cross-contamination
Parish dinner[11]	~1000/1700 (59%)	D (95%), C (92%), N (72%), HA (47%), F (47%), V (12%)	16 hr (3-76 hr)	4.6 days (<1-8 days)	26%/7.4%	Shrimp	Cross-contamination, storage temperature
Pacific Northwest[47]	209 ill	D (99%), C (88%), N (52%), V (39%), F (33%)	15 hr (4-96 hr)	3 days mean	2 hospitalized, 1 death	Raw oysters	Uncooked oysters

C, cramps; D, diarrhea; F, fever; HA, headache; N, nausea; V, vomiting.

United States were associated with O3:K6.[19,56] By 2005, this pandemic clone was found in patients from Europe, South America, and Africa,[2,28,43] and other clones that genetically diverged from O3:K6, such as O4:K68 and O1:KUT, were isolated from clinical cases.[16]

PATHOGENESIS

The ability of certain strains of *V. parahaemolyticus* to produce beta-hemolysis on Wagatsuma agar, known as the Kanagawa phenomenon (KP), was first epidemiologically associated with human pathogenicity.[46] KP is observed in 88 to 96 percent of strains from clinical specimens and in 1 to 2 percent of strains from environmental sources.[23,36,68] Thermostable direct hemolysin (TDH) produces KP.[67] TDH has a molecular weight of 42,000, consisting of two subunits of 21,000,[47] and, in contrast to other hemolysins produced by *V. parahaemolyticus*, is not inactivated by heating at 100° C for 10 minutes. It is a pore-forming toxin that causes hemolysis by binding to and disrupting erythrocyte membranes.[30] TDH also has been shown to have enterotoxic activities, such as causing accumulation of fluid in rabbit ileal loop assays, invading intestinal mucosa,[15,71] and increasing intracellular calcium concentrations that trigger secretion of chloride by intestinal cells.[61,65,66] TDH is cytotoxic, lethal to small experimental animals, and cardiotoxic.[78]

Certain KP-negative isolates from outbreaks have been shown to produce TDH-related hemolysin (TRH).[32] Although TRH is heat-labile, it shares similarities with TDH, such as hemolysis, production of fluid accumulation in the rabbit ileal loop model, and induction of chloride secretion in human colonic epithelial cells.[31,65] Likewise, some variants closely related to the original TDH by molecular analysis (Vp TDH/I and Vp TDH/II) have been isolated from KP-negative strains.[48]

When the *tdh* and *trh* genes had been cloned and sequenced, researchers realized that certain strains possess the genetic material but are phenotypically incomplete. Strains with a typical hemolysin-positive phenotype carried two chromosome gene copies, whereas *tdh* gene–positive strains with weakly positive or negative hemolysin phenotype possessed only a single chromosome gene copy.[52] Less than 50 percent of *trh* gene–positive strains produce TRH when examined by enzyme-linked immunosorbent assay.[63] Low-level expression of the *tdh* genes may be the reason for the KP-negative phenotype.[53] The expression of the *tdh* genes is controlled by the Vp-toxRS operon. The basal production of mRNA and the degree of transcriptional activation seem to be related to differences in the nucleotide sequences and strength of the promoter region.[53]

Some studies suggest that virulence mechanisms in addition to TDH and TRH exist and that the mere presence of the *tdh* or *trh* genes does not reliably correlate with virulence of a given strain.[71,76] *V. parahaemolyticus* requires intestinal colonization factors to cause disease. A variety of pili and other potential colonization factors are present, but the evidence is too poor to implicate any of the candidate adhesins in virulence.[13] Adherence of *V. parahaemolyticus* to intestinal epithelial cells of rabbits[14] and human small intestine[27,75] seems to require binding to complex carbohydrates or hemagglutinins of the host. Also, *V. parahaemolyticus* appears to disrupt epithelial barrier function, regardless of TDH and TRH production.[41]

The occurrence of cases with grossly bloody stools[33,55] is indirect evidence of either invasiveness or production of cytotoxin. Invasion of Caco-2 cells in culture has been shown in approximately 20 percent of strains.[1] KP-positive and KP-negative strains from clinical specimens can invade and colonize the mucosal cells of the rabbit ileum, producing acute inflammation, degeneration, and erosion of the villi.[7] Vibrios can be cultured from tissue specimens of spleen, liver, and heart in experimental animals, indicating spread through the lymphatic or circulatory systems.[10]

CLINICAL MANIFESTATIONS

The spectrum of intestinal disease varies from a mild gastroenteritis to a dysenteric syndrome. The incubation period typically is 15 to 24 hours (range 4 to 96 hours)[3,64]; the variability presumably is related to the number of organisms ingested. Resolution is expected to occur in approximately 3 days,[47] but it varies from several hours to more than 10 days.[3,11] Fatigue may persist for a few days. Diarrhea (96%) and abdominal pain (95%) are the most frequent and earliest symptoms, accompanied by nausea, vomiting, and headache in 40 to 70 percent of cases. Chills and moderate fever are less frequent (20%) manifestations.[3,17] Diarrhea is watery and explosive,[12] with patients having up to 15 stools during the first day. Shock caused by loss of fluid is an exceptional event.[20] Mucus is observed frequently, but grossly bloody stool is seen less commonly.[8,33,40] Small superficial ulcerations on sigmoidoscopy may be present.[8] No difference in symptoms exists in cases associated with KP-negative strains.[30]

Extraintestinal infections occur. During the Vibrio Surveillance Program that started in 1989 in four Gulf Coast states, 18 to 34 percent of *V. parahaemolyticus* isolates were found to come from wound infections, and 3 to 5 percent were found to be associated with septicemia.[18,40] Wound infection occurs after contamination of skin lacerations with seawater or after direct trauma with pieces of shellfish, fishhooks, or utensils contaminated with seawater.[9] Superficial infection can extend to deeper soft tissue and may require radical surgical débridement. Septicemia is a concern in immunocompromised patients, particularly patients with leukemia[22] and patients with liver disease.[21,29] Bacteremia may develop after either wound infections[9,22] or ingestion of seafood.[29,60] Skin bullae,[29] intravascular hemolysis,[22] and disseminated intravascular coagulation[29] may complicate wound and bacteremic infections.

COMPLICATIONS

An acute diarrhea episode requires medical attention in 25 to 50 percent of cases during an outbreak, but less frequently requires hospitalization.[11,17,40,64] Children younger than 5 years old are more likely to require hospitalization compared with individuals older than 5 years.[69] Usually, no long-term sequelae occur. Severe dehydration, shock,[33,58] and death (0.04% of cases in Japan) can occur.[7,72] Primary septicemia,[38,40,60] septicemia secondary to wound infection,[9,22,38] and septicemia secondary to gastroenteritis[29,38] have higher mortality rates, especially in immunosuppressed patients or in patients with liver diseases.[9]

DIAGNOSIS

Epidemiologic data are the basis for the presumptive diagnosis. In a patient with compatible symptoms, a history of recent consumption of seafood should suggest this diagnosis. Leukocytosis and fecal leukocytes may be found.[8,33] A positive stool culture on selective media, such as TCBS agar, can confirm the clinical impression. Routine use of TCBS agar is not cost-effective, even in coastal areas, unless an appropriate clinical setting is available.[9,40,45] The use of a transport medium, such as Cary-Blair, is necessary if a delay in processing the sample is expected. Isolation of more than 10^5 *V. parahaemolyticus* from epidemiologically implicated food supports the diagnosis and identifies the vehicle.[45] A correlation between the serotype of the food and isolates from patients is not always present because multiple strains can con-

taminate a single food.[3] Adding an enrichment broth to the processing increases the yield of isolation.

For testing blood specimens, use of routine culture media followed by selective media is appropriate.[29,60] Immunoassays (enzyme-linked immunosorbent assay, immunoprecipitation in agar medium, reversed passive latex agglutination) are available in research laboratories and commercially in Japan (KAP-RPLA, Denka Seiken, Tokyo; BT test, Nissui Pharmaceutical, Tokyo) to detect the TDH. Strains producing TRH may be detected by cross-reactions.[77] Serologic methods (slide agglutination) can detect H antigens in lateral flagella.[62] Gene probe hybridization[51] and polymerase chain reaction with a sequence of a highly conserved DNA fragment[39] or targeting the *tdh* gene can be used to detect low numbers of the organism in environmental and clinical specimens. Histologic findings from duodenal and rectal biopsy samples from patients infected with *V. parahaemolyticus* were consistent with acute inflammatory response, showing epithelial degradation, polymorphonuclear neutrophil infiltration of villi and crypt cells, and hemorrhage.[59]

TREATMENT

Only supportive therapy and careful control of the fluid and electrolyte balances are required for the management of gastroenteritis. Oral rehydration usually is appropriate, although intravenous fluids may be required if massive losses of fluid occur.[8,33,58] Antibiotic therapy is unnecessary for this short-lived disease. In the unusual protracted episode, tetracycline or a fluoroquinolone may be beneficial for adults and older children.[7,8,36]

For wound infections and septicemia, antibiotics always are indicated. *V. parahaemolyticus* usually is susceptible to tetracycline. In addition, only a few strains are resistant to chloramphenicol, trimethoprim-sulfamethoxazole, third-generation cephalosporins, aztreonam, imipenem, fluoroquinolones, and aminoglycosides.[9,22,26,29] Penicillins are ineffective because of the presence of β-lactamases in 50 percent of isolates.[26,37] The older cephalosporins also have poor activity.[37]

PREVENTION AND CONTROL

Although ensuring the lack of contamination of seafood is impossible, avoiding food-handling errors should diminish the risk of infection. A recommendation for an acceptable upper limit of 100 colony-forming units per gram of *V. parahaemolyticus* in raw shrimp has been made by the International Commission on Microbiological Specification for Foods.[34] Thorough cooking eliminates the organism.[70] Heating seafood to 60° C for 15 minutes[23] or boiling for 7 minutes[11] seems to be adequate to reduce the risk of infection. When undercooked or raw seafood is consumed, adequate prior refrigeration to preclude multiplication of organisms is important. During the preparation of seafood, special attention should be paid to possible cross-contamination. Use of the same utensils, board surfaces, or containers for fresh and recently cooked seafood should be avoided.[58,70] Raw seafood consumption should be discouraged, particularly for individuals at high risk for development of septicemia.[21,38,40]

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CHAPTER 129

VIBRIO VULNIFICUS

Randall G. Fisher

BACTERIOLOGY

Vibrio vulnificus is a small, curvilinear, gram-negative rod of the family Vibrionaceae.[6] This facultative anaerobe is oxidase-positive and lysine-positive similar to other species of the genus *Vibrio*. Its major difference is that it ferments lactose, a feature that accounts for its original name, Lac + *Vibrio*. *V. vulnificus* is arginine-negative and ornithine-variable. This halophilic (salt-loving) organism grows in concentrations of sodium chloride of from 1 to 8 percent and seems to grow best at approximately 3 percent.[35]

EPIDEMIOLOGY

V. vulnificus, similar to *Vibrio parahaemolyticus*, is a marine organism that is a common inhabitant of off-shore waters, especially estuarial waters.[50] It has been isolated from sediment, plankton,

water, finfish, crabs, and oysters. Peak recovery occurs in the summer and early fall,[17] possibly because at higher water temperatures *V. vulnificus* is released and rises to surface waters. There it attaches to plankton and shore fish and then is taken up and concentrated by filter-feeding mollusks and crustaceans.

V. vulnificus has been isolated from wild and commercial oysters throughout the world. It especially is prevalent in warm coastal waters. Reports about the survival of the organism in oysters stored at low temperatures are conflicting. Nilsson and associates[48] revealed, however, that even if *V. vulnificus* is rendered nonculturable by storage at low temperatures, it can be "resuscitated" by allowing the oysters to warm up to room temperature; this cycle could be carried out twice without any reduction in bacterial count. Because of the ubiquity of the organism in the marine environment and its ability to thrive under even the most careful conditions of sanitation, storage, and transport,[16,33] ensuring that commercial shellfish do not contain viable *V. vulnificus* is very difficult.

So-called heat shock shucking, during which the internal meat temperature exceeds 50° C for 1 to 4 minutes, reduces the *V. vulnificus* and total bacterial levels in oysters 10-fold to 10,000-fold compared with conventional processing.[22] A separate study confirms that low-temperature pasteurization, which brings oyster temperatures to 50° C for 10 to 15 minutes, reduces colony counts from greater than 100,000/g of oyster meat to undetectable levels.[2] Three-hour ice immersion is ineffective at reducing *V. vulnificus* and actually increases coliform counts in oysters.[53] High-pressure processing is a promising new approach to eliminating *V. vulnificus* from oysters; in preliminary studies, *V. vulnificus* could be reduced to undetectable levels by processing at 586 MPa with a 7-minute "come up" time.[36]

Disease in humans is initiated by contact with the organism. Contact occurs either through marine contamination of a wound or in the gastrointestinal tract after ingestion of the organism in raw shellfish, most commonly oysters.[6]

PATHOPHYSIOLOGY

V. vulnificus causes three distinct diseases in humans: wound infection and gastrointestinal infection, which may be self-limited or progress to septicemia. Local infection occurs after a wound is exposed to contaminated sea water. Wound infections with *V. vulnificus* are marked by rapid spread, the formation of bullae, and necrosis of involved tissues.[6] Marked edema and vascular thromboses occur in experimental and natural infection. *V. vulnificus* elaborates a cytolysin,[20,21,35] a collagenase,[56] and a protease,[46] which enhance its rapid spread in tissues. The protease activates the plasma kallikrein-kinin system to produce bradykinin[45]; histamine also is released locally. These factors account for the intense inflammatory reaction seen in these lesions. One group of researchers constructed an isolate that lacked a flagellum and found that its ability to adhere to cells was decreased, as was its lethality in mice.[39] Hemorrhagic complications may be due to the presence of a metalloprotease that interferes with blood homeostasis through prothrombin activation and fibrinolysis.[11]

In otherwise healthy individuals, a mild, self-limited gastrointestinal illness similar to that caused by *V. parahaemolyticus* has been described.[34] The most serious infection produced by *V. vulnificus* is primary septicemia, which occurs most commonly in patients with liver disease, after they have ingested the organism in raw shellfish.[6,51,52]

For patients with hepatic disease, the risk of acquiring septicemia is 40 to 80 times greater, and the case-fatality rate is 2.5 times higher than for otherwise healthy individuals.[24] *V. vulnificus*, similar to many other gram-negative organisms, requires iron for growth and grows better in an excess of iron.[64] It is able to extract iron from hemoglobin, even if it is complexed completely to haptoglobin.[64,67] Liver damage, excess iron, and deferoxamine therapy all have been shown to decrease the median lethal dose (LD_{50}) of *V. vulnificus* in experimental animals. Deferoxamine alone decreased the LD_{50} by four orders of magnitude.[9] Iron overload almost certainly underlies *V. vulnificus* septicemia in patients who require repeated transfusions and deferoxamine therapy for anemia.[29,66] Some evidence indicates that alcoholism, even in the absence of demonstrable liver disease, is a risk factor for developing sepsis.[51] Fatal septicemia also has been reported in patients with other chronic diseases, such as diabetes mellitus and lymphomas.[52]

Virulence factors of the organism also have been described.[65] Virulent strains are resistant to the bactericidal activity of human serum,[37] probably because of the presence of a polysaccharide capsule.[1] Poor uptake of virulent strains into phagocytes[59] and opaqueness of the colony on agar have been correlated with the presence of the capsule.[55,65] No difference has been shown in the lipopolysaccharides of virulent versus avirulent strains.[5] The production of recalcitrant shock in patients with septicemia is secondary to toxins, loss of vascular tone, capillary leak, and possibly negative inotropy, as in other forms of gram-negative sepsis.

CLINICAL MANIFESTATIONS

Patients commonly present with wound infection or primary septicemia. Wound infection occurs after injury in sea water or after the contamination of a recently acquired wound with sea water. Often, the wound is caused by the shell of a crustacean or mollusk. Many patients with wound infections work in shellfish-related industries. Apparently, wound infection with *V. vulnificus* can develop from superficial wounds acquired within 24 hours of "uneventful" fish handling; the physiologic characteristics of human sweat may be conducive to the survival of the organism on the skin, allowing for later inoculation.[10] Cellulitis may develop and spread rapidly. Overlying skin often is covered with tense bullae. At débridement, the extent of necrosis may exceed presurgical expectations.[63] Primary wound infection may progress to systemic infection; for this reason, wound infection with *V. vulnificus* carries a mortality rate of 7 to 24 percent.[6,34]

Patients with primary septicemia generally give a history of recent (6 to 72 hours) consumption of raw seafood. Illness is marked by the rapid onset of fever, hypotension, and septic shock. Prodromal symptoms, such as malaise, chills, and fever, are common manifestations. Vomiting and diarrhea are seen in approximately 20 percent of patients.[7] Shock progresses quickly and is difficult to reverse. Secondary skin lesions develop in approximately half of patients[6] and may be bullous, petechial, or maculopapular. *V. vulnificus* frequently is isolated from cultures of secondary lesions, providing evidence of septicemic spread to those sites.[6] The mortality rate from primary septicemia has been reported to be 46 to 79 percent.[6,51]

In addition to causing the two above-mentioned syndromes, *V. vulnificus* has been reported as a cause of corneal ulcer,[60] myositis,[31] adult epiglottitis,[42] osteomyelitis,[61] endocarditis,[60] peritonitis,[26] tubo-ovarian abscess,[43] and meningitis.[29,51] In one case, a patient presented with endogenous endophthalmitis without systemic symptoms that developed after ingestion of raw seafood.[28] A case of fatal septicemia in which the presenting symptom was a compartment syndrome of the forearm has been described.[27]

Ninety percent of reported patients are 40 years old or older.[6,51] Childhood cases of septicemia have been associated with thalassemia major,[28] for which frequent transfusions and deferoxamine therapy are required. Wound infections in previously healthy children and adolescents have been reported. *V. vulnificus* also has been isolated from a premature infant's stool sample obtained on the infant's first day of life; the infant's mother worked as an oyster shucker.[4]

DIAGNOSIS

The diagnosis of *V. vulnificus* infection is made by isolating the organism from blood or tissue culture. It also may be recovered from stool specimens.[4,52] Agars designed specifically to aid in the growth and identification of *V. vulnificus* have been developed; cellobiose-colistin agar outperforms thiosulfate citrate bile salts sucrose agar (TCBS) for recovery of *V. vulnificus* from environmental samples.[25] From a practical standpoint, however, most hospital microbiology laboratories do not stock cellobiose-colistin agar. Of the commercially available media, *V. vulnificus* tends to grow best in thiosulfate citrate bile salts sucrose agar (TCBS),[6] but it also may be recovered from ordinary blood agar plates[3,54] or MacConkey plates.[6]

A very sensitive nested polymerase chain reaction technique that is capable of detecting 1 pg of bacterial DNA and one colony-forming unit of *V. vulnificus* has been described; it was positive in 94 percent of clinical samples that grew the bacteria in culture and was positive in 42 percent of culture-negative samples from patients with suspected *V. vulnificus* infection.[40] An enzyme immunoassay also has been developed.[59] Both of these methods are experimental and not yet available for routine clinical use.

The diagnosis of *V. vulnificus* wound infection or septicemia can be suspected on clinical grounds and appropriate therapy initiated while awaiting culture results. A history of ingestion of raw shellfish or contamination of a wound with either sea water or brackish inland waters[57] should be sought. *V. vulnificus* infection should be given high consideration in patients with hemosiderosis, anemia with transfusion therapy, liver disease, or other chronic diseases.

TREATMENT

For severe wound infections, performing surgical therapy as rapidly as possible is paramount. For primary septicemia, support of the patient's airway, along with aggressive pressor support and other adjunctive therapies for severe septic shock, is of primary concern. Secondarily, appropriate antibiotic treatment for *V. vulnificus* should be started as early as possible.

In vitro, the organism is susceptible to many antibiotics, including ampicillin, third-generation cephalosporins, tetracycline, chloramphenicol, and gentamicin.[6] Bowdre and associates[8] reported that, in mice, the minimum inhibitory concentrations obtained in the laboratory did not seem to correspond with response to therapy. In particular, the organism seemed to be exquisitely sensitive to cefotaxime in vitro (minimum inhibitory concentration 0.06 μg/mL); however, 9 of 10 mice treated with cefotaxime died of overwhelming infection. Anecdotal evidence indicates that this puzzling phenomenon may occur in humans as well; four of five patients reported by Chuang and associates[14] died, despite receiving therapy with third-generation cephalosporins at appropriate dosages. Similarly, although the organism is almost universally sensitive to gentamicin in the laboratory, case reports show a lack of response to this antibiotic. Of all the antibiotics to which the organism was sensitive in vitro, only tetracycline led to survival of mice in Bowdre's study; all 12 of the mice treated with tetracycline survived the infection.[8]

Case reports also show favorable outcomes with tetracycline or doxycycline[14] and chloramphenicol[14] and ciprofloxacin.[41] More recently, the combination of minocycline and cefotaxime has been shown to be synergistic in vitro, with the combination reducing the growth of *V. vulnificus* by six orders of magnitude compared with either drug alone.[13] Enhanced survival of mice infected with *V. vulnificus* and treated with minocycline plus cefotaxime provides an in vivo correlate of the in vitro results.[15] No data are available comparing the activity of minocycline versus that of tetracycline; nonetheless, the aforementioned evidence is sufficiently strong to suggest that either tetracycline or minocycline together with cefotaxime should be the treatment of choice for known or suspected *V. vulnificus* infection. Fluoroquinolones also may have a role in treatment. In vitro synergy studies show that the combination of ciprofloxacin and cefotaxime is synergistic for *V. vulnificus*,[32] and in a murine model, the newer fluoroquinolones (e.g., moxifloxacin) worked as well as combination therapy at preventing lethality in mice.[12] As yet, no human clinical data are available regarding the efficacy of third-generation fluoroquinolones.

The addition of modified Dakin solution (0.025% sodium hypochlorite) may have some utility in the treatment of skin and wound infections. An in vitro study of eight different topical antibiotic preparations showed that *V. vulnificus* was most sensitive to modified Dakin solution.[44] Although no controlled trial has been performed, a series of 10 patients with culture-proven *V. vulnificus* wound infections treated with doxycycline and topical modified Dakin solution, none of whom experienced progression of infection or required surgical débridement, has been reported.[62]

PREVENTION

Patients with severe anemia, liver disease, hemosiderosis, or other debilitating chronic diseases and patients on deferoxamine therapy should be advised against eating raw seafood of any kind. Patients with open wounds probably should avoid contact with sea water or brackish inland waters.

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CHAPTER 130

Miscellaneous Non-Enterobacteriaceae Fermentative Bacilli

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This chapter discusses fermentative bacilli that are not of the family Enterobacteriaceae. Specifically, it examines *Chromobacterium violaceum*, *Plesiomonas shigelloides*, and *Pasteurella* organisms other than *Pasteurella multocida*.

CHROMOBACTERIUM VIOLACEUM

C. violaceum is a facultatively anaerobic, gram-negative rod that is a saprophyte of soil and water, especially in tropical and sub-

tropical climates. It causes occasional illness in animals and, rarely, in humans. Infection with *C. violaceum*, when it does occur, is a serious disease with a high mortality rate.

BACTERIOLOGY

C. violaceum is a long, motile, gram-negative bacillus that appears singly or in pairs on Gram stain. It has a polar flagellum and one to four subpolar or lateral flagella, which antigenically are distinct from the polar flagellum.[73] Most isolates produce an insoluble pigment, violacein. This pigment is intense and makes colonies appear dark purple to black, especially on blood agar. Violacein induces apoptotic death in certain cell types in vitro, including leukemia and lymphoma cells.[57] The therapeutic potential of violacein and structural analogues is being explored. Violacein also has antibacterial, anti-trypanosomic, and weak antiviral effects.[7] *C. violaceum* grows readily on standard agar (any medium that contains tryptophan supports growth). The colonies are low convex, violet, smooth, and not gelatinous. Colonies produce hydrogen cyanide, so a faint almond odor may be present.[73]

C. violaceum is catalase-positive and oxidase-positive, although the latter may be difficult to interpret because of the production of pigment. Growing the organism anaerobically inhibits pigment formation.[45] Pigment also may be lost on subculture[65] or as effective treatment is initiated. *C. violaceum* has a fermentative, not oxidative, attack on carbohydrates. *C. violaceum* produces antimicrobial agents that have been shown to have activity against bacteria and trypanosomes.

EPIDEMIOLOGY

C. violaceum commonly is found in soil and water of areas with tropical or subtropical weather patterns. It also has been recovered from soil as far north as New Jersey.[17] Ten of the first 12 cases reported in the United States occurred in Florida; the other two were in Louisiana. Subsequently, one case from New Jersey[61] and one from Ohio[75] have been reported, along with more from southeastern states. All but one case occurred during summer.[65] Patients tend to be young, with a median age of 14 years.[65]

PATHOPHYSIOLOGY

Although *C. violaceum* is a common inhabitant of soil and water, human infection is rare. Disorders of neutrophil function are important risk factors. A disproportionately high number of *C. violaceum* infections have occurred in patients with chronic granulomatous disease.[52] *C. violaceum* infection in a child with another disorder of neutrophil function, polymorphonuclear leukocyte glucose-6-phosphate dehydrogenase deficiency, has been reported.[53] Organisms may show variable virulence, and differences in endotoxin activity, resistance to phagocytosis, and production of catalase and hydrogen peroxide have been observed between clinical and soil isolates.[58] An elastase activity, expressed through the entire life cycle and produced by a zinc metalloproteinase, may account for the propensity of *C. violaceum* to cause abscesses.[87] Because *C. violaceum* is a free-living organism that is exposed to diverse environmental conditions, it exploits a wide range of energy resources and is able to thrive in aerobic and anaerobic conditions.[16] It is equipped to be tolerant of harsh conditions, including acid and ultraviolet stress, temperature stress, heavy metal exposure, and antibiotic exposure.[37] The complete sequencing of its genome has allowed researchers to identify putative virulence genes that are likely involved in host cell adhesion, cell invasion, and cytolysis.[11]

The organism usually gains entrance to the body through cuts or abrasions that come in contact with contaminated soil or water. After entrance, a localized infection usually develops at or around the site of entry, which is commonly followed by dissemination of infection via the bloodstream to distant sites. Rapid débridement and appropriate antibiotic therapy may arrest the infection at the wound stage.[48] Two cases of systemic infection occurred in near-drowning victims. One case report describes fulminant infection and death in an immunocompetent host from *C. violaceum* sepsis that began as conjunctivitis after a fall that splattered mud into the patient's eye.[22] Numerous microabscesses are found in multiple organs, especially liver, lung, and kidneys. Spread to bone, joints, and the central nervous system also has been described.

CLINICAL MANIFESTATIONS

The pattern of illness in all reported patients is similar, with a contaminated inoculation site, localized disease, regional lymphadenopathy, and hematogenous spread to visceral organs. Progression of symptoms tends to be rapid after a variable incubation period.

Most patients have cutaneous lesions,[52] which are described as being nodular or pustular and sometimes with surrounding cellulitis.[80] They may progress to suppuration and drainage, or ulceration. Occasionally, classic ecthyma gangrenosum lesions have been described.[12] Regional lymphadenopathy is common; some of the nodes suppurate and require surgical drainage or removal.

Severe disease is heralded by high fever (39° C to 41° C), confusion or lethargy, abdominal pain, headaches, nausea and vomiting, and sometimes myalgias. Patients with systemic illness appear toxic. Hepatosplenomegaly develops frequently, and jaundice may be present. Progression from high fever and moderate toxicity to septic shock with disseminated intravascular coagulation and multisystem organ failure is precipitous. After the liver, the lung is the most common site of dissemination of infection, and evidence of pneumonia often is found. Adult respiratory distress syndrome is rare.[49] Brain and liver abscesses have been noted.[59] The overall mortality rate is 65 percent.[52] Rarely, there may be recurrence, which can prove fatal.[45,65] A review of all 25 pediatric cases reported between 1971 and 2005 revealed, counterintuitively, that the case-fatality rate was 12 of 16 (75%) in patients without chronic granulomatous disease and 0 of 9 in patients with a known diagnosis of chronic granulomatous disease.[71] Disseminated infection, bacteremia, and initiation of ineffective antibiotic therapy were associated with mortality.[71]

DIAGNOSIS

Diagnosis is made by recovery of the organism from blood, lymph nodes, skin lesions, or abscesses. Gram-negative bacilli sometimes can be seen in smears of material from skin lesions. Laboratory values reveal either very low or very high white blood cell counts with marked left shifts. Mild to moderate anemia is common. There may be elevated liver enzymes; evidence of early renal failure sometimes is present. The organism grows readily and is easy to identify if it produces the characteristic violet pigment. Nonpigmented forms exist in soil and have similar virulence in mice[72] but are recovered only rarely from clinical specimens.[75] Other cases may have been missed, however, because the nonpigmented forms often are misidentified as *Aeromonas hydrophila* or as pseudomonads.[72] Reports that *C. violaceum* infection causes a melioidosis-like illness were probably due to misidentification of *Burkholderia pseudomallei* by the API 20NE system.[38]

A clinical history of contamination of a wound with water or soil, especially in the southeastern United States or in Southeast Asia, with subsequent local infection, lymph node suppuration, and lack of response to conventional antibiotic therapy, should arouse suspicion of *C. violaceum* infection. The clinician particularly should be aware of the possibility of *C. violaceum* infection in patients with chronic granulomatous disease.

TREATMENT

C. violaceum is sensitive in vitro to chloramphenicol, gentamicin, fluorinated quinolones, tetracyclines, imipenem, trimethoprim-sulfamethoxazole, and semisynthetic penicillins. *C. violaceum* often is resistant to cephalosporins, penicillin, ampicillin, and the antistaphylococcal penicillins. All isolates are resistant to vancomycin and rifampin.[4] Some strains have been shown to elaborate a β-lactamase, which is chromosomal and inducible in vitro.[21] At least one case report gives in vivo evidence of inducible resistance to ceftazidime.[80] Laboratory evidence of susceptibility to erythromycin cannot be relied on. In vitro, the fluorinated quinolones have the highest activity.[4] Clinical experience with the fluoroquinolones in *C. violaceum* infection is scarce; an impressive case of a 4-month-old infant with severe disseminated *C. violaceum* infection who survived after therapy with trimethoprim-sulfamethoxazole and ciprofloxacin has been reported.[59] The review of 25 cases mentioned earlier found that all survivors had been given one or a combination of the following agents: ciprofloxacin, trimethoprim-sulfamethoxazole, chloramphenicol, or imipenem.[71]

Because infection with *C. violaceum* is rare and often rapidly fatal, optimal antimicrobial therapy is unknown. Duration of therapy also is unknown, but because of late recurrences, some authors recommend 3 to 4 weeks of intravenous therapy followed by 1 month or more of oral trimethoprim-sulfamethoxazole.[65]

PLESIOMONAS SHIGELLOIDES

Plesiomonas shigelloides is the only species for the genus *Plesiomonas*. These organisms are facultatively anaerobic, motile, gram-negative rods that are common inhabitants of surface water and fish. They have been implicated in gastrointestinal infections and, rarely, have been recovered from extraintestinal sites.

BACTERIOLOGY

These facultatively anaerobic, gram-negative rods are members of the family Vibrionaceae, although some authorities suggest they are related more closely to Enterobacteriaceae.[51] They are motile by means of a polar flagellum. They are lysine-positive, ornithine-positive, and arginine decarboxylase–positive. They can be distinguished from Enterobacteriaceae by oxidase positivity. They also are catalase-positive and indole-positive. They grow well on MacConkey agar but not on thiosulfate citrate bile salts sucrose. Growth may be enhanced by the use of selective media, such as trypticase soy broth with ampicillin[66] and inositol brilliant green–bile salts agar. Growth is maximal at 40° C to 44° C and completely inhibited at 8° C. Usually within 24 hours, 1- to 1.5-mm grayish, shiny, opaque colonies with a slightly raised center and a smooth surface are visible. A few isolates of *P. shigelloides* share a common O antigen with *Shigella sonnei*.

EPIDEMIOLOGY

The organism is a ubiquitous fresh-water inhabitant at temperatures greater than 8° C. It sometimes also is found in estuarial waters in temperate or tropical climates and can exist in sea water during the summer. It has been cultured from finfish, shellfish, pigs, birds, and dogs.[84] Although infection with *P. shigelloides* has been associated with ingestion of raw or improperly cooked fish (especially oysters), it is unknown what role, if any, other animals play in the ecology of the organism. Asymptomatic carriage of *P. shigelloides* is rare in developed countries,[68] but may be 15 percent in some parts of China.[84]

PATHOPHYSIOLOGY

Despite the ubiquity of the organism in nature, human infection is uncommon and has been recognized only more recently. Most often, infection is associated with gastroenteritis. Evidence for the role of *P. shigelloides* in the production of gastrointestinal symptoms is that it has been isolated much more frequently from patients with diarrhea than from healthy controls[35]; there have been some outbreaks, especially in Japan; it often is the only organism detected in the stools of patients with gastroenteritis[56]; and patients who have *P. shigelloides* growing from a stool culture recover more quickly with than without antibiotic therapy.[42] Acquisition of disease has been linked specifically to consumption of raw seafood or untreated water and to foreign travel, especially to Mexico.[33,55]

The mechanism by which the organism produces disease has been elusive. It is not enteroinvasive by laboratory tests, most investigators fail to find either a Shiga toxin or an enterotoxin,[1,33] no animal model of gastrointestinal disease has been found,[10] patients in the recovery phase do not show serologic evidence of infection, and inoculation of volunteers fails to produce illness.[33] In a suckling gnotobiotic piglet model in which the animals became septic, histology of the gastrointestinal tract showed neither destruction of cells nor invasion of tissues.[33] Neonatal BALB/c mice became chronically infected with *P. shigelloides*, however, and histopathologic findings included some cases of necrosis of the mucosal surface of the ileum and colon.[83]

Some potential virulence factors (i.e., a cholera-like toxin, a weak cytolysin, serum resistance, and a large [>150-kd] plasmid) have been described, but their exact roles in pathogenesis are uncertain; attempts to correlate these features with virulence have been fruitless.[1] Investigators have characterized a cytotoxin derived from *P. shigelloides* using strains from patients with a diarrheal illness. The cytotoxin is a complex of three lipopolysaccharide-binding proteins and lipopolysaccharide in a 6 : 5 ratio.[63] Cytotoxic activity was inhibited 80 percent in vitro by proteinase K or when incubated with anti-cholera toxin antibody. This cytotoxin produced a positive reaction in the suckling mouse assay, whereas the purified lipopolysaccharide exhibited almost no cytotoxicity.[63]

Transmission electron microscopy has provided investigators with the first definitive proof that *P. shigelloides* can adhere to and invade eukaryotic intestinal cells. Organisms attached to microvilli and plasma membranes of the cells; they also were seen within vacuoles in the cell cytoplasm, suggesting a phagocytotic entry mechanism.[78] In one in vitro system using Caco-2 cells, adherence occurred within 10 minutes, and internalization occurred within 60 minutes. Cytotoxicity was due to the induction of apoptosis.[81] In vitro, cell-free and cell-associated hemolysis is demonstrable,[29] and low levels of elastase, proteinase, histidine decarboxylase, and moderate levels of triacylglycerol lipase activity have been found.[15] Culture filtrates are capable of inducing vacuolation of a variety of mammalian cells in vitro.[20]

P. shigelloides rarely, but more clearly, is a pathogen in extraintestinal sites. Osteomyelitis, endophthalmitis, cholecystitis, pseudoappendicitis, spontaneous peritonitis,[3] meningitis,[77] and septicemia have been reported sporadically.[10] Most patients with septicemia have been immunocompromised hosts, but the organism has been isolated from blood cultures in otherwise healthy individuals.[39,64] The mode of infection in extraintestinal sites is unclear, but most cases are thought to arise from the gastrointestinal tract. The case of a newborn with *P. shigelloides* meningitis, septicemia, and endophthalmitis who was born to a mother who reported severe diarrhea after eating raw oysters 2 weeks before delivery raises the possibility of transplacental transmission.[54]

CLINICAL MANIFESTATIONS

Patients with *P. shigelloides* gastroenteritis complain of diarrhea, crampy abdominal pain, nausea and vomiting, headache, and fever. Symptoms usually begin 24 hours to 4 days after contact with the organism.[35] Diarrhea tends to be secretory, although some patients have symptoms more consistent with colitis.[42] Passage of blood, mucus, or both in the stools is a common manifestation, as is the presence of white blood cells by Wright stain.[35] Patients with *P. shigelloides* gastroenteritis tend to have disease that is more acute, associated with more severe abdominal pain, and of longer duration than patients with diseases caused by other enteropathogens.[42]

In one case-control study, 76 percent of patients were sick for more than 2 weeks, and 32 percent were sick for more than a month.[42] In contrast, a large Japanese study of returning travelers suggested that for most patients, the symptoms abated within approximately 3 days.[70] In a series of 38 pediatric cases in Bangladesh, 84 percent had secretory diarrhea and 71 percent had associated emesis. Only three patients (8%) had fever, and only five (13%) had diarrhea for 14 days or longer.[47] In a separate report, one child developed migratory polyarthritis during an otherwise typical case of culture-proven gastrointestinal infection. All symptoms and signs of arthritis disappeared when the gastrointestinal infection was treated with antibiotics.[30]

Localized extraintestinal infections have been reported but are rare. Two cases of severe polymicrobial endophthalmitis resulting from fishhook trauma progressed to enucleation.[13] Two cases of peritonitis associated with continuous ambulatory peritoneal dialysis were reported from Hong Kong. Both patients recovered with 10 days of receiving intraperitoneal cefazolin and tobramycin.[85] In one odd case, a woman presented with pyosalpinx from *P. shigelloides*, thought to have been acquired by swimming in contaminated water.[69]

Septicemia, meningitis, or both usually occur in immunocompromised hosts. Severe, rapidly progressive sepsis complicated by disseminated intravascular coagulation, adult respiratory distress syndrome, renal insufficiency, hepatic dysfunction, adrenal hemorrhage, and splenic abscess has occurred in patients with hemoglobinopathies and absence of splenic function, including thalassemia intermedia and sickle-cell disease.[6,82] Unusual sites of infection sometimes are noted in immunocompromised individuals, such as epididymo-orchitis in a patient with human immunodeficiency virus infection.[86] Newborns constitute most of the reported cases of *P. shigelloides* meningitis, in whom the mortality rate is 80 percent.[1] Septicemia also has a high mortality rate in adults, although otherwise well patients may recover with appropriate antimicrobial therapy.

DIAGNOSIS

A clinical history of foreign travel or of ingestion of raw seafood or untreated water should raise suspicion of possible *P. shigelloides* infection, especially when the clinical illness matches the description just mentioned. Oxidase tests should be done on any predominant or solitary organisms to distinguish them from Enterobacteriaceae.[35] The organisms can be shown not to be aeromonads or pseudomonads by production of ornithine decarboxylase and fermentation of inositol. Selective medium can be used if the index of suspicion is high.

TREATMENT

Most strains of *P. shigelloides* produce a β-lactamase,[67] which seems to be specific for the penicillins. In one study, all isolates were resistant to ampicillin, ticarcillin, carbenicillin, and piperacillin.[26] *P. shigelloides* is universally susceptible to trimethoprim-sulfamethoxazole, the fluoroquinolones, most cephalosporins, carbapenems,[76] tetracycline,[76] and chloramphenicol. It is variably susceptible to the aminoglycosides and mostly resistant to erythromycin.

P. shigelloides gastroenteritis resolves without therapy, but the illness may be prolonged. Treatment seems to shorten the course.[42] Extraintestinal infections carry a poor prognosis and should be treated aggressively. For meningitis, the cephalosporins have good cerebrospinal fluid penetration and are effective therapy against most isolates.

OTHER *PASTEURELLA* ORGANISMS

The genus *Pasteurella* consists of a group of pleomorphic, gram-negative coccobacilli that are part of the normal flora of many animals. These organisms are frequent animal pathogens. *P. multocida* is a common human pathogen; it is discussed elsewhere (see Chapter 126). The other species of the genus *Pasteurella* are rare but occasionally serious causes of infection in humans.

BACTERIOLOGY

These organisms, similar to *P. multocida*, grow readily on most common laboratory media, including blood agar. Most of the species do not grow on MacConkey agar. They are non–spore-forming, nonmotile, aerobic, and facultatively anaerobic. These glucose-fermenting organisms all are oxidase-positive. Most are nitrate-positive and catalase-positive, and all except *Avibacterium gallinarum* (formerly *Pasteurella gallinarum*, see later) produce indole. They are small, coccoid or rod-shaped bacilli that may show prominent bipolar staining on Gram stain. Colonies are small, translucent, and gray. They may be smooth or rough. A browning discoloration may develop around them. Colonies are nonhemolytic. They have a distinctive musty or "mushroom" odor.[14]

The taxonomy of these organisms is confusing and continues to be revised. *Pasteurella ureae* and *Pasteurella pneumotropica* have been moved into the genus *Actinobacillus*. *Pasteurella haemolytica* has been reclassified into a new genus, *Mannheimia*.[8] *Pasteurella dagmatis* is the name now given to what formerly was called *P.* new species, or *P.* gas.[60] *Pasteurella gallinarum*, *Pasteurella avium*, and *Pasteurella volantium* have 96.8 percent sequence similarity and are phenotypically separate from other *Pasteurella* spp.; this feature has led to a proposal that they be moved into a new genus, *Avibacterium*.[9] Most experts agree that the genus *Pasteurella sensu stricto* should include *P. multocida*, *Pasteurella canis*, *Pasteurella stomatis*, *P. dagmatis*, and *Pasteurella* spp. B.[43] Clinically recovered species other than *P. multocida* include *Actinobacillus ureae*, *Mannheimia haemolytica*, *Actinobacillus pneumotropica*, *P. dagmatis*, *P. canis*, *Pasteurella aerogenes*, *Pasteurella bettyae*, *P. "SP"* group,

and *P. stomatis*. *Pasteurella caballi* has caused wound infections after horse bites.[18] *A. gallinarum* is an extremely rare pathogen in humans.

PATHOPHYSIOLOGY

Infection with *Pasteurella* spp. has been divided clinically into three types: infection (1) from animal bites, (2) from animal contact,[25] and (3) without known animal contact.[16] Infections from animal bites include cellulitis, abscesses, tenosynovitis, and bone and joint infection, but infection can become generalized, especially in patients who are immunocompromised. Infections caused by animal contact can be similar to infections described earlier and are caused by animals licking broken skin or wounds. Sometimes pulmonary infections occur, possibly related to aerosolization of organisms. In some cases, these organisms have been reported as respiratory flora in patients with pets, but at present no proof exists that such colonization is an antecedent of infection.[23] Cases without known animal contact history constitute 3 to 30 percent[36,40] of all cases.

Infection usually occurs when the organisms are inoculated into deeper tissues either on animal teeth that break the skin or in animal saliva that comes in contact with nonintact skin. Bite wound infections often are polymicrobial. Infection in cases without known animal contact is harder to explain but definitely happens.[44] Most cases of serious infection occur in patients with underlying diseases, such as diabetes mellitus, chronic alcoholism, and other types of liver disease.[62] Central nervous system infection with these organisms has occurred after head trauma or neurosurgery in 10 of 11 reported cases.[44] One intrauterine death of the fetus of a 20-year-old woman who worked at a pig farm has been attributed to *P. aerogenes*.[79] Case reports of unusual infections with rare species of *Pasteurella*, such as fatal sepsis and meningitis in a 4-day-old infant caused by *A. (Pasteurella) gallinarum*,[2] should be considered doubtful unless molecular identification methods have been used.[32]

CLINICAL MANIFESTATIONS

Pasteurella infections produce pain, swelling, pus, and sometimes abscess formation at the site of inoculation, beginning within 24 to 36 hours. Clinically, these infections are indistinguishable from wound infections with *Staphylococcus aureus* or other gram-positive organisms. Gram stain may show the characteristic pleomorphic bacilli with bipolar staining. Growth on standard agar is rapid.

Patients with peritonitis,[62] meningitis,[44] osteomyelitis,[26] or infectious endocarditis[3] have symptoms typical of these diagnoses. Risk factors, such as household pet exposure, animal contact, animal bites, and comorbid conditions, should heighten suspicion of possible *Pasteurella* infection.

DIAGNOSIS

Establishing the diagnosis of *Pasteurella* infection can be difficult, not because the organism is fastidious or slow-growing, but because it often is misidentified. Other organisms of the same family (i.e., *Actinobacillus* spp. and *Haemophilus* spp.) have similar biochemical profiles and can be misidentified by commonly used systems, such as API. Lester and associates[50] reported that of 30 species firmly identified as *Pasteurella* by biochemical means, only three were identified correctly by the API 20E system. *Haemophilus aphrophilus* and *Actinobacillus actinomycetemcomitans* are sometimes misidentified as *P. gallinarum* by commercial systems.[24] Additionally, Hamilton-Miller[31] reported that four strains of *Haemophilus influenzae* and three strains of *Haemophilus parainfluenzae* were identified falsely as *Pasteurella* spp. by API, and suggested that if the clinical history renders *Pasteurella* infection unlikely, tests for X and V factor requirements should be performed (see Chapter 145). Clinical case reports corroborate these laboratory observations.[19,62,74] Notifying the bacteriology laboratory of suspicion of *Pasteurella* infection is helpful.

TREATMENT

Penicillin has been considered the drug of choice for *Pasteurella* infection in the past and, despite some reports of penicillin resistance, still is effective against most strains. *Pasteurella* spp. also are susceptible to ampicillin, β-lactamase inhibitor combination drugs, tetracycline, and chloramphenicol. Truncated but fully functional tetracycline resistance genes have been identified in isolates from animal sources.[46] The aminoglycosides, erythromycin, clindamycin, cefadroxil, and cefaclor are not recommended. Dicloxacillin and cephalexin have poor activity against *Pasteurella* spp. and should not be used as monotherapy for animal bite wounds.[27] In one study of bite wound infections, ertapenem was found to be active against all *Pasteurella* spp.[28] Of fluoroquinolones tested in vitro against 75 clinical isolates of *Pasteurella* spp., levofloxacin was the most active.[34]

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CHAPTER 131

ACINETOBACTER

Armando G. Correa

First recognized as a human pathogen in 1908,[66] the ubiquitous organism *Acinetobacter* has emerged as a rather common cause of nosocomial infections in immunocompromised hosts.[6] Some of the confusion regarding this organism may be attributed to the many changes in nomenclature that the members of this genus have undergone over the years. Names used in the past to identify this genus include *Herellea*, *Bacterium*, *Mima*, *Achromobacter*, *Alcaligenes*, *Neisseria*, *Micrococcus*, *Diplococcus*, *Moraxella*, and *Cytophaga*. Treatment of infection caused by *Acinetobacter* is complicated by its widespread multidrug resistance and the difficulty encountered in eradicating the organism.

THE ORGANISM

The genus *Acinetobacter* belongs to the family Neisseriaceae, which also includes the *Neisseria*, *Moraxella*, and *Kingella* genera. *Acinetobacter* is a gram-negative bacterium that typically appears as a rod 0.9 to 1.6 μm in diameter and 1.5 to 2.5 μm in length, but it may become spherical in the stationary phase of growth. It frequently occurs in pairs or short chains. Many strains are encapsulated. The organism has a strictly aerobic respiratory metabolism and does not grow under anaerobic conditions. It does not form spores or exhibit swimming mobility. *Acinetobacter* grows well in all common complex media between 20° C and 30° C, with optimal growth occurring between 33° C and 35° C, and it has no growth factor requirements.

Convex, grayish-white colonies 1 to 2.5 mm in diameter are typical findings. The colonies may appear mucoid if the strain is encapsulated. *Acinetobacter* is catalase-positive and may be differentiated readily from other closely related genera by virtue of its negative reaction to oxidase.

Formerly, the genus *Acinetobacter* contained the single species *Acinetobacter calcoaceticus* subdivided into two subspecies or biovars: *anitratus* and *lwoffii*.[31] However, in 1986, the taxonomy of the genus *Acinetobacter* was changed extensively on the basis of DNA hybridization studies,[10] and at least 32 genomic species have been proposed, of which 17 have been assigned species names.[17] Ten of these nomenspecies have been isolated from human specimens: *Acinetobacter baumannii*, *A. calcoaceticus*, *Acinetobacter haemolyticus*, *Acinetobacter johnsonii*, *Acinetobacter junii*, *Acinetobacter lwoffii*, *Acinetobacter radioresistens*, *Acinetobacter parvus*, *Acinetobacter schindleri*, and *Acinetobacter ursingi*.[17] Several unnamed genospecies also have been isolated from human clinical samples and include genomic species 3, 13TU, 10, and 11.[17] These species may be difficult to differentiate in the clinical laboratory on the basis of their growth characteristics and biochemical activity. Because of their clinical relevance and antimicrobial resistance, four of these species, *A. baumannii*, *A. calcoaceticus*, and genomic species 3 and 13TU, are grouped under the term *A. baumannii-calcoaceticus* complex. Under the new classification, most *A. baumannii* strains represent organisms that were classified formerly as biovar *anitratus*, whereas *A. junii* and *A. lwoffii* previously were listed under the biovar *lwoffii*.

EPIDEMIOLOGY

Acinetobacter strains are distributed widely in nature and can be found in soil, fresh water, and sewage.[8,28] *Acinetobacter* also can be isolated from many animals, fresh meats, poultry, contaminated milk, and frozen foods.[8,28] The organism can be part of the bacterial flora of the skin in healthy individuals,[8,12] and the skin frequently becomes a reservoir for *Acinetobacter* in hospitalized patients and the health care staff.[6,8] It occasionally forms part of the normal flora of the oral cavity and the upper respiratory, genital, and lower gastrointestinal tracts.[8,12] Colonization by *Acinetobacter* is particularly common in patients who have undergone a tracheostomy.[52] The organism frequently can be found in the hospital environment, particularly in moist areas, such as in humidifiers, water sinks, and ventilators.[8] Nosocomial outbreaks have been linked to colonized medical equipment such as ventilator tubing and other respiratory equipment,[1,26] intravenous catheters,[8] gloves,[46] and mattresses.[59]

The frequency of occurrence of nosocomial infections by *Acinetobacter* is not easy to assess because the pathogenic role of this organism often has been underestimated. However, a national surveillance study conducted from 1974 to 1977 identified *Acinetobacter* as a pathogen in 0.76 percent of nosocomial infections.[49] The estimated rate of nosocomial infections caused by this organism was 3.11 per 10,000 patients discharged, and approximately 15 percent of 1372 reported episodes occurred in the pediatric age group.[49] By 1978, this rate increased by 14 percent and accounted for 1 percent of the bacterial isolates associated with nosocomial infections.[12] An unusual seasonal pattern was observed, with most infections occurring in late

summer.[12,49] The cause of this increase is unknown. Pneumonia, tracheobronchitis, and infections of the urinary tract and surgical wounds were the entities observed most frequently.[49] In a national prospective survey of U.S. hospitals from 1995 through 2002, *Acinetobacter* spp. accounted for 1.3 percent of all nosocomial bloodstream infections and was associated with a crude mortality rate of 34 percent.[70] Data from SENTRY, an international surveillance program that monitors the frequency of occurrence and antimicrobial susceptibility of bacterial pathogens, revealed a significantly greater frequency of *Acinetobacter* infections in Latin America than in all other regions.[22] A higher prevalence rate of *Acinetobacter* infections was noted in patients ranging in age from birth to 10 years.[22] In the pediatric age group, neonates appear to be particularly susceptible to nosocomial infection with this organism (Table 131–1).[42]

More recently, *Acinetobacter* emerged as a particularly important pathogen in unusual situations such as earthquakes and war zones.[30] This prevalence was illustrated by reports of outbreaks of multidrug-resistant *Acinetobacter* infections associated with the U.S.-Iraq conflict.[56]

PATHOGENESIS

Limited information is available regarding the pathogenesis of *Acinetobacter* infections. The lipopolysaccharide of *Acinetobacter*, a normal constituent of the outer membrane of gram-negative bacteria, is capable of eliciting multiple pathogenic host responses. Strains producing exopolysaccharide have been shown to be more pathogenic than are nonproducers.[30] Nonspecific adherence factors, such as fimbriae, also have been described in *Acinetobacter*.[30] The role of quorum sensing as a regulatory mechanism for autoinduction of multiple virulence factors in *Acinetobacter* is currently under investigation.[30]

In animal models, *Acinetobacter* can enhance the virulence of other bacteria in mixed infections, perhaps by slime-induced inhibition of neutrophils.[44] Researchers have speculated that the ability of this organism to grow in an acidic pH at lower temperatures may enhance its ability to invade devitalized tissue.[2] The organism also may survive in a dry environment for as long as a week.[14]

CLINICAL MANIFESTATIONS

Acinetobacter can cause suppurative infection of virtually any organ, and the clinical manifestations typically are similar to those seen with other bacterial infections because no unique features are suggestive of *Acinetobacter* infection. The clinical manifestations also may depend on the underlying immune status of compromised hosts. Infections caused by *Acinetobacter* are rare occurrences in normal children.[18]

INTRACRANIAL INFECTION

Most cases of *Acinetobacter* meningitis are the result of a penetrating injury or occur after a neurosurgical procedure, although sporadic cases of meningitis have been reported in the absence of these factors. A cluster of eight children in whom *Acinetobacter* meningitis developed after the administration of intrathecal methotrexate was reported.[32] All patients had fever, headache, nausea, and vomiting, and lumbar puncture revealed cerebrospinal fluid (CSF) pleocytosis.

The earlier literature contains several reports of *Acinetobacter* meningitis developing in apparently normal children.[15,16,29,63,67] CSF pleocytosis with a predominance of segmented forms occurred commonly.[16] Because as many as 30 percent of these patients had a petechial rash and gram-negative diplococci on CSF smears, the diagnosis of meningococcal meningitis was made erroneously in most of these cases, thereby leading to a delay in the institution of appropriate therapy and possibly contributing to a mortality rate as high as 27 percent.[16]

Siegman-Igra and associates,[60] in a review of 25 cases of *Acinetobacter* meningitis secondary to invasive procedures that included some children, found that fever, leukocytosis, and neck stiffness, along with other clinical signs of central nervous system (CNS) infection, were common features. The CSF in these patients showed pleocytosis with a predominance of polymorphonuclear leukocytes, elevated protein concentration, and a low glucose level. Most of the infections were associated with indwelling ventriculostomy tubes or a fistula into the CSF space. Filka and colleagues[20] reported 10 cases of *Acinetobacter* meningitis that occurred over the course of a 7-year period in children who had undergone ventriculoperitoneal shunt insertion.

Researchers have suggested that an inherited or acquired complement deficiency may be associated with meningitis caused by *Acinetobacter*[19] because it is seen with *Neisseria meningitidis* and other related species. Treatment of CNS infections caused by *Acinetobacter* requires a minimum of 3 weeks of parenteral antibiotics.

BACTEREMIA

Acinetobacter bacteremia may occur as an isolated event or may be secondary to a primary infected site, such as the respiratory or urinary tract or a wound. Primary bacteremia appears to occur

TABLE 131–1 Illustrative Nosocomial Clusters of *Acinetobacter* Infection in Pediatric Patients

Country	Year	Type of Unit	Infected Children	Colonized Children	Presentation	Mortality (%)	Suspected Source
United Kingdom[42]	1981	NICU	4	0	Meningitis	0	None identified
United Kingdom[61]	1983	NICU	9	1	Pulmonary infection	22	Ambu bag
Japan[64]	1983-1986	NICU	19	52	Sepsis	11	Multiple sources
India[32]	1988	Oncology	8	N/A	Meningitis	38	Intrathecal needle
Germany[55]	1988	NICU	3	41	Sepsis	100	Humidifier
Israel[48]	1988-1990	NICU	9	N/A	Sepsis	44	None identified
United Kingdom[43]	1989	NICU	7	N/A	Sepsis	0	Intravenous fluids
Bahamas[40]	1996	NICU	8	1	Sepsis	37	Air conditioner
South Africa[47]	1997	NICU	9	N/A	Sepsis	22	Suction catheters
India[41]	1995	NICU	79	N/A	Sepsis	14	None identified
India[13]	1986-1990	NICU	26	N/A	Sepsis	42	None identified

N/A, data not available; NICU, neonatal intensive care unit.

more commonly in immunocompromised neonates, and clinical manifestations can vary from an absence of clinical signs of infection to fulminant septic shock and disseminated intravascular coagulation.[33,55] Thrombocytopenia has been reported to be a prominent feature in these neonates.[43,48] Pneumonia has been seen more commonly in early-onset sepsis.[41] Predisposing factors include low birth weight,[53,55] previous antibiotic therapy,[48,53,55] and the presence of indwelling catheters.[40,53]

Acinetobacter bacteremia in children with malignant diseases also has been noted to occur rarely. Fuchs and colleagues[21] reported 29 episodes of sepsis caused by this organism over the course of a 12-year period in an oncology center. All these children were febrile and appeared ill at the time of diagnosis, and a high association of *Acinetobacter* sepsis with the presence of intravascular catheters was noted. Surprisingly, no connection was found with the level of neutropenia.[21]

RESPIRATORY TRACT

Because *Acinetobacter* may be a transient colonizer of the pharynx in 7 percent of healthy children[5] and adults[23] and this rate is increased in hospitalized patients, the relative importance of *Acinetobacter* in comparison with other potential pathogens isolated from sputum is difficult to ascertain. The tracheobronchitis and pneumonia attributed to *Acinetobacter* are mostly nosocomial infections associated with the presence of an endotracheal tube or tracheostomy.[23] Pneumonias frequently are multilobar and occasionally may lead to cavitary destruction or pleural empyema.[23]

Community-acquired pneumonia caused by *A. baumannii* has been reported to occur in adults in the Northern Territory of Australia and other tropical regions.[4] This entity generally is seen in patients with diminished host defenses caused by alcoholism, cigarette smoking, or underlying pulmonary disease and is characterized by the rapid onset of fever, dyspnea, pleuritic chest pain, and purulent sputum. The mortality rate has been as high as 53 to 64 percent.[4]

MISCELLANEOUS

Urinary tract infections occur almost exclusively in patients with indwelling bladder catheters, usually are limited to the bladder, and generally are mild.[23] Burns, as well as traumatic and surgical wounds, frequently become colonized by *Acinetobacter* as a result of the ability of this organism to thrive on compromised tissue and foreign material.[23] Bacteremia may occur as a consequence of this colonization, which is often polymicrobial. *Acinetobacter* is a prominent cause of peritonitis in children undergoing peritoneal dialysis when gram-negative organisms are involved.[68]

Other rare infections caused by *Acinetobacter* that have been reported include suppurative otitis media,[50,63] cellulitis (frequently in association with trauma, a foreign body, or an animal bite),[23,50] synergistic necrotizing fasciitis,[3] native-valve and prosthetic-value endocarditis,[25] septic arthritis,[50] osteomyelitis,[63] and liver abscesses.[23] Ocular infections also have been documented.[38] A case of osteomyelitis occurring after a hamster bite in a child has been described.[39]

DIAGNOSIS

The diagnosis of *Acinetobacter* infection is made by culture of appropriate body fluids or tissue specimens. No serologic or antigen-detection tests are available. A selective medium containing MacConkey agar with cephaloridine has been used to culture skin specimens during investigation of outbreaks[61] because of its ability to inhibit most of the skin flora but not *Acinetobacter*.

Biotyping, phage typing, electrophoretic analysis of isoenzyme and cell wall proteins, plasmid analysis, polymerase chain reaction–based DNA fingerprinting, and restriction endonuclease digestion of DNA have been used for investigation of nosocomial outbreaks.[7] Antibiogram typing no longer is considered an effective method in the investigation of *Acinetobacter* epidemics because the susceptibility pattern may change rapidly within the same outbreak.[7,11]

TREATMENT

As with many other opportunistic gram-negative organisms, treatment of infections caused by *Acinetobacter* spp., particularly those of the *A. baumannii-calcoaceticus* complex,[57,65] have become more complicated by the rapid increase in resistance to the antibiotics used commonly in hospitals. Selection of an antibiotic regimen should be based on in vitro susceptibility testing and ideally should include both a β-lactam and an aminoglycoside, which may have synergistic activity[23] and prevent the emergence of resistance.[6]

Species of the *A. baumannii-calcoaceticus* complex have shown decreased susceptibility to ampicillin, broad-spectrum penicillins, cephalosporins, aminoglycosides, and ciprofloxacin.[22,57] Resistance to extended-spectrum cephalosporins may be the result of the presence of cephalosporinases (particularly the chromosomally encoded cephalosporinase AmpC),[9] other broad-spectrum β-lactamases, or changes in the outer-membrane porins and penicillin-binding proteins.[9,45] Resistance to aminoglycosides is mediated by aminoglycoside-modifying enzymes.[65] The carbapenems imipenem and meropenem appear to be the most active agents against *A. baumannii*,[60,65] but reports have found more than 10 percent of such strains to be resistant to these antibiotics in some areas,[22,37] and increasing numbers of nosocomial outbreaks of carbapenem-resistant *Acinetobacter* infections have been reported.[24,64,72] The incidence of carbapenem-resistant *Acinetobacter* strains has been particularly high in Latin America (11.4% of all isolates versus 4.8% in the United States).[22] Resistance to the carbapenems has been associated with the presence of serine and metallo-β-lactamases (carbapenemases). Of particular concern are the numerous carbapenem-hydrolyzing OXA enzymes that have emerged in *A. baumannii*.[9] Caution should be exercised when using imipenem at high dosage in children for the treatment of meningitis (i.e., 100 mg/kg/day) because of an unusually high rate of seizures.[71] Thus, meropenem is recommended for this indication. Ertapenem, a newer carbapenem antibiotic, has poor in vitro activity against *Acinetobacter*[35] and should not be used for the treatment of these infections. Combinations of a β-lactam antibiotic with a β-lactamase inhibitor, such as ampicillin-sulbactam, piperacillin-tazobactam, or ticarcillin-clavulanate, have been used for infections caused by carbapenem-resistant strains.[64,72]

The fluoroquinolones, in particular ciprofloxacin, have been used successfully to treat infections caused by multidrug-resistant *Acinetobacter* in children[41,47] and adults. Although ciprofloxacin has been approved for the treatment of complicated urinary tract infections in children, the other systemic fluoroquinolones are not approved for use in children younger than 16 years of age because of the theoretic concern for damage to growth cartilage. Of the quinolones currently available, gatifloxacin exhibits the best in vitro activity against *A. baumannii*, followed by levofloxacin and trovafloxacin.[27] Although 76 to 86 percent of sporadic isolates of *A. baumannii* are susceptible to the fluoroquinolones, only 32 to 55 percent of outbreak-related strains remain susceptible to this antibiotic class.[27] Topoisomerase mutations in both *gyrA* and *parC* have been identified in quinolone-resistant *A. baumannii*.[9] In some cases, the polymyxin drugs polymyxin B and colistin are the only therapeutic options for the treatment of multidrug-resistant *Acinetobacter*

infection.[24,34] Efflux pumps capable of conferring resistance to multiple antibiotic classes have been identified in *Acinetobacter* spp.[9,51]

Tigecycline is the first member of the glycylcycline antimicrobial class to gain approval in the United States. Because this agent demonstrates in vitro activity against multidrug-resistant strains of *A. baumannii*, its potential use as an alternative therapy for severe infections caused by this organism is being investigated.[51] Data regarding the use of this agent in pediatric patients are lacking.

Imipenem, meropenem, amikacin, ciprofloxacin, ceftazidime, and ceftriaxone have exhibited good in vitro activity against isolates identified as species other than the *A. baumannii-calcoaceticus* complex.[57] In addition to antimicrobial therapy, prompt drainage of focal suppurative sites and removal of infected indwelling catheters are essential. Intraventricular administration of amikacin has been used in the treatment of CNS infections caused by this organism.[69]

PROGNOSIS

Because *Acinetobacter* strains often are resistant to the antibiotics used commonly, prompt recognition of the specific cause and institution of effective antibiotic therapy are critical to achieving a successful outcome. The reported mortality rate in a series of pediatric patients ranged from 0 percent to more than 50 percent (see Table 131–1), and the outcome appeared to correlate more closely with the underlying condition than with other factors such as polymicrobial bacteremia.[62] In a series of 58 infections caused by this organism that occurred over the course of a 2-year period from 1973 through 1974 at the Massachusetts General Hospital in Boston, the mortality rate was 23 percent.[23] A 1999 prospective study in the Slovak Republic revealed that the case-fatality rate for 157 episodes of *A. baumannii* bacteremia was significantly higher in adults than in children (34% vs. 12%).[33]

The nosocomial acquisition of multiresistant *A. baumannii-calcoaceticus* complex has been associated with high mortality rates and prolonged hospitalization in adult patients in intensive care units,[36,54] in contrast to the more benign clinical outcome usually seen with other species of *Acinetobacter.*[58]

PREVENTION

Nosocomial acquisition of *Acinetobacter* by high-risk compromised hosts can be prevented by placing emphasis on the control measures routinely used for endemic infections, such as careful handwashing by personnel, limitation of the frequency and duration of use of devices, proper isolation of colonized and infected patients, application of strict techniques for invasive procedures, and restricted use of antibiotics.[14,36,60]

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CHAPTER 132

ACHROMOBACTER (ALCALIGENES)

Randall G. Fisher

Organisms of the genus *Achromobacter* are gram-negative bacilli that live in aqueous environments. Originally considered commensals, they increasingly are recognized as important, although rare, hospital pathogens. *Achromobacter* can be especially problematic in immunocompromised patients and in neonates, in whom infection can be life-threatening. These organisms have been isolated from such diverse clinical specimens as sputum, urine, feces, blood, cerebrospinal fluid, cornea, and peritoneal and pleural fluids.

BACTERIOLOGY

Achromobacter spp. are gram-negative, motile, indole-negative, obligate aerobes that are oxidase- and catalase-positive. They are considered to be nonfermenters because of their extremely limited action on carbohydrates. Most ferment xylose, and some ferment glucose. All reduce nitrate to nitrite. They are urease-, lysine-, and ornithine-negative. They grow well on both blood and MacConkey agar and produce colonies that are smooth and glistening and have a distinct edge. They alkalinize organic salts and amides, a property that led to the name *Alcaligenes*, which means *alkali producing*. *Alcaligenes faecalis* has a distinct, sweet odor that has been described as resembling that of green apples.[27]

Bacteriologically, these bacilli may be confused with other nonfermenting gram-negative organisms, especially *Pseudomonas* spp. Morphologically, however, they can be distinguished easily from pseudomonads by the presence of peritrichous flagella: *Pseudomonas* spp. have polar flagella.[23]

The taxonomy of these organisms is confusing and undergoes frequent changes. They were classified as *Achromobacter* spp., then reclassified as *Alcaligenes*; they have been reassigned the name *Achromobacter*. The genera *Achromobacter* and *Alcaligenes* are closely related and comprise many species, but clinically important ones are as follows: (1) *Achromobacter xylosoxidans*, which has two subspecies: *xylosoxidans* and *dentrificans*, the former of which is the most common cause of clinically recognizable infection; (2) *Alcaligenes faecalis*, which is a less common pathogen but has a distinct antimicrobial susceptibility pattern[5]; (3) and *Achromobacter piechaudii*, which has been isolated from clinical specimens[35] but is of doubtful significance. In the discussion that follows, the abbreviation *A. xylosoxidans* refers to *Achromobacter xylosoxidans* subspecies *xylosoxidans*.

EPIDEMIOLOGY

Like *Pseudomonas* spp., *Achromobacter* are water organisms and prefer aqueous environments and moist soil. They do not survive long on porous surfaces or fomites or if they become desiccated.[39] They also may be part of the normal flora of the ear and of the gastrointestinal and respiratory tracts of some people. These organisms establish a niche within the hospital environment and have been recovered from ventilators, humidifiers, "sterile" saline, intravenous fluids, and irrigation and dialysis solutions. *Achromobacter* spp. also have been recovered from infant formula,[16] children's soap bubbles,[32] well water,[45] and swimming pools.[25] Organisms also survive many disinfectants and have been cultured from chlorhexidine,[42] 1 percent eosin,[6] and alcohol- or quaternary amine–containing compounds.[39,42] Shigeta and associates[42] reported an outbreak of *A. xylosoxidans* ventriculitis secondary to contaminated chlorhexidine used on a surgical ward. Foley and colleagues[19] reported an outbreak accompanied by deaths in a neonatal intensive care unit secondary to contamination of saline used as an eyewash. Boukadida and coworkers[6] reported a neonatal death caused by meningitis contracted by dissemination after treatment of a diaper rash with 1 percent eosin. An outbreak of 37 cases (with 2 fatalities) that was caused by bacterial contamination of deionized water in a hemodialysis system was described by Reverdy and associates[36]. Surgical wound infection also has occurred wherein infection was suspected to be secondary to contaminated irrigation fluids used in surgery.[50] One outbreak of four cases in a hemodialysis unit was linked to an atomizer of 2.5 percent chlorhexidine used to disinfect the skin.[47] Three different kinds of pseudo-outbreaks have been described: in one, seven patients in a pediatric hospital were infected with *A. xylosoxidans*, but restriction fragment-length polymorphism analysis proved they all were genetically unrelated[4]; in another, *A. xylosoxidans* was isolated from three different clinical specimens but was later proven to be a contaminant of the saline used in their processing[22]; in a third, blood cultures were positive when drawn on the night shift because the nurses were cleaning the top of the blood culture bottles with a contaminated disinfectant.[44]

Studies have documented that *A. xylosoxidans* colonization of patients with cystic fibrosis is on the rise. In one large study, *A. xylosoxidans* was isolated from the sputum culture of 52 of 595 patients (8.7%).[7] In other studies, isolates of *A. xylosoxidans* at a single cystic fibrosis center were not genetically related, a finding implying no common source of infection and little or no patient-to-patient spread within the center.[14,49] However, colonization of multiple patients with identical strains also is well documented.[37] Environmental cultures at one cystic fibrosis center were positive for *A. xylosoxidans* only 0.8 percent of the time, whereas 22.8 percent of such cultures were positive for *Pseudomonas aeruginosa*.[18]

PATHOPHYSIOLOGY

Achromobacter spp. are weakly virulent bacteria. Medical care commonly provides the conduit through which organisms are introduced into their host, by way of indwelling catheters, endotracheal tubes, and so forth. The bacteria may take advantage of a weakened immune system and disseminate, causing sepsis, meningitis, and death. Preterm or small-for-gestational-age term infants are at particular risk for acquiring such severe *Achromobacter* infections.[19] Although most neonatal infections are considered to be nosocomial, vertical transmission from mother to baby may occur.[23] An increased incidence of infection has been reported for patients with neoplasms[28] and those receiving long-term steroid therapy.[26] Sporadic cases of *Achromobacter* infection in patients with idiopathic immunoglobulin M (IgM) deficiency,[15] Waldenström macroglobulinemia,[46] and systemic lupus erythematosus have been reported.[38] My colleagues and I have seen one boy with hyper-IgM syndrome in whom 14 separate episodes of *Achromobacter* bacteremia occurred; an exhaustive environmental search for a source was fruitless. The source was eventually proven to be deep infection of a cervical lymph node, and the episodes ceased when the node was removed.[51] *Achromobacter* infections occur in patients with acquired immunodeficiency syndrome (AIDS),[8,21] but whether this syndrome is an independent risk factor for infection is unclear.

In one study, patients with cystic fibrosis and colonized with *A. xylosoxidans* tended to be older (mean age, 20 years) and at baseline had worse lung function than did noncolonized controls; however, over the course of the study, the rate of decline of lung function did not differ between cases and controls.[10] In a second similar case-control study, lung function decline and growth parameters generally were not affected by colonization; however, a subset of patients had rapidly increasing antibody titers against *A. xylosoxidans*, and this group did experience more accelerated decline.[37]

In unusual circumstances, patients with neither overt underlying disease nor obvious immune deficiency will develop infection with *Achromobacter* spp. Most of these cases involve penetrating trauma. One case of corneal infection complicating epidemic keratoconjunctivitis in a normal host has been described.[33]

CLINICAL MANIFESTATIONS

Signs of sepsis or meningitis caused by *A. xylosoxidans* in the newborn are difficult to differentiate from other causes of bacterial sepsis. However, some babies may develop a distinctive rash in association with this infection, in which 1- to 2-cm, sharply demarcated red patches appear, especially in the head and neck region. This rash was noted in 29 of 33 newborns with *A. xylosoxidans* infection reported by Doxiadis and associates in 1960[12] and was seen again in a case reported in 1993.[6] *A. xylosoxidans* sepsis/meningitis tends to manifest later in life than do infections with the usual vertically acquired pathogens and may have a more insidious onset.[30] In some cases, cerebrospinal fluid profiles may resemble those usually associated with viral meningitis, with white blood cell counts in the hundreds and with monocytic predominance.[40] Neonatal *Achromobacter* sepsis or meningitis has an extremely poor prognosis; one series noted a mortality rate that approached 75 percent, and 36 percent of survivors had severe neurologic deficits.[19] The incidence of intracranial hemorrhage also was high.

Three large series of bloodstream infections with *A. xylosoxidans* all revealed a similar story: most cases are nosocomial, most patients have an underlying malignant disease, many are neutropenic or receiving high-dose steroids, and many patients have indwelling vascular catheters.[2,20,41] Polymicrobial bacteremia was not rare in these series. Mortality rates ranged from 15 percent[20]

to 48 percent.[41] Risk factors for mortality were age greater than 65 years at diagnosis, neutropenia, nosocomial acquisition, and polymicrobial bacteremia. An earlier review of reported cases revealed that the case-fatality rate was lowest in patients with catheter-associated bacteremia and highest (65%) in those with pneumonia, meningitis, or endocarditis.[13]

One child developed osteomyelitis caused by *A. xylosoxidans* after stepping on a nail through old sneakers (a clinical situation classically associated with *Pseudomonas* infection)[24,25]; another developed *Achromobacter* infection as a consequence of a gunshot wound.[9] In the setting of a patient with an artificial heart valve, *A. xylosoxidans* endocarditis has been described.[34] In another case, endocarditis was associated with an abandoned pacemaker lead.[1] A case series of liver abscesses included three patients from whom *A. xylosoxidans* was isolated, all of whom shared a clinical pattern: history of cholecystectomy, "coral-like" multilobulated appearance on computed tomographic scanning, and epithelioid granulomas at the periphery of the abscesses.[3] *A. xylosoxidans* infection in older patients usually is not suspected on clinical grounds but rather in the context of a common-source outbreak or because of microbiologic clues. *A. faecalis* infection is less common and usually is part of a polymicrobial process.

DIAGNOSIS AND TREATMENT

Generally, the diagnosis of *Achromobacter* infections rests on recovery of the organism from clinical samples, although newer methods sometimes have been employed. In one case of culture-negative endophthalmitis, fluid obtained from the anterior chamber was subjected to polymerase chain reaction using a 16S rDNA primer set, finding a 214 base pair sequence from *A. xylosoxidans*.[48] These organisms often are mistaken for pseudomonads, and the clinician should suspect *A. xylosoxidans* when the laboratory reports an organism as a *Pseudomonas* spp. that is resistant to all aminoglycosides.[39] Key differentiation features include the antibiogram and the morphology of the organism, with its distinctive peritrichous flagella.

Achromobacter spp. typically are resistant to a large number of antibiotics, including ampicillin, aztreonam, aminoglycosides, first- and second-generation cephalosporins, tetracyclines, and rifampin. They variably are resistant to chloramphenicol, fluoroquinolones, macrolides, ureidopenicillins, and β-lactamase combination drugs.[5] *Achromobacter* spp. have been shown to produce β-lactamases, some of which are chromosomal, constitutive, and inducible[11] and some of which are on plasmids.[29] Some isolates overproduce β-lactamase,[11] which stoichiometrically can render β-lactamase inhibitors useless. In addition, their porins are small, thus rendering antibiotic entry difficult. Although there is no antibiotic to which all isolates have been shown to be sensitive,[34] most are sensitive in vitro to trimethoprim-sulfamethoxazole, imipenem, ceftazidime, and cefoperazone. Imipenem-resistant strains also have been discovered. These strains produced VIM-2, OXA-30, and a chromosomal AmpC β-lactamase.[43] Two case reports describe treatment failures of ceftazidime[31] and piperacillin[11] in clinical isolates that were sensitive at the time of isolation but developed resistance during the course of therapy.

Because resistance patterns vary from isolate to isolate, the combination of a third-generation cephalosporin, piperacillin, or imipenem with trimethoprim-sulfamethoxazole is reasonable empiric therapy for suspected *Achromobacter* infection, pending susceptibility results. In general, in vitro susceptibilities seem to correlate well with in vivo results,[28] but the risk of inducible resistance to β-lactam antibiotics should be acknowledged. One report described synergy in microbial killing with an aminoglycoside, even though the isolate was resistant to the same aminoglycoside when it was tested alone.[8] This phenomenon was confirmed by two-disk Kirby-Bauer approximation methods using 11 clinical blood culture isolates; all were resistant to gentamicin, but 10 of 11 were inhibited synergistically when gentamicin was added to ticarcillin/clavulanate, and 9 of 11 displayed synergy when gentamicin was added to piperacillin or ceftazidime.[13]

Removal of infected catheters may speed recovery, although some patients have been treated successfully through indwelling lines.[8] Because of a high recurrence rate, experts in the care of patients with renal failure treated with continuous ambulatory peritoneal dialysis recommend removal of peritoneal catheters in patients who develop peritonitis with *A. xylosoxidans*.[17]

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CHAPTER 133

EIKENELLA CORRODENS

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Eikenella corrodens is a facultatively anaerobic, fastidious gram-negative rod that is part of the normal flora of the mouth and the gastrointestinal and genitourinary tracts. Long regarded as a commensal, its pathogenicity no longer is in doubt. It frequently is a pathogen of periodontitis in both adults and children and is a common isolate from wounds that have been contaminated by oral secretions. It also has been recovered from pleuropulmonary infections, central nervous system infections, orbital cellulitis, peritonsillar abscesses, abdominal infections, osteomyelitis, and bloodstream infections, including endocarditis.

BACTERIOLOGY

In 1948, Hendriksen[17] described the organism and called it the *corroding bacillus* because it pitted the agar. It was characterized more fully in 1958 by Eiken,[10] who named it *Bacteroides corrodens*. In 1972, Jackson and Goodman[19] separated two species of corroding bacteria; the strict anaerobe kept the name *B. corrodens* (now called *Bacteroides ureolyticus*), and the facultative anaerobe was classified as *Eikenella*. It is a small, straight, nonmotile gram-negative rod that occasionally is coccobacillary. It is oxidase-positive and catalase-negative. Most strains are lysine- and ornithine decarboxylase-positive. The organism is nonfermentative, reduces nitrate to nitrite, and is urease- and indole-negative. *E. corrodens* cell surface components vary from isolate to isolate; these differences probably relate to virulence.[6]

E. corrodens will grow either aerobically or anaerobically, but its growth is not rapid. Growth can be enhanced by 3 to 10 percent carbon dioxide. It grows on blood or chocolate agar but poorly or not at all on MacConkey agar. Selective medium, which contains clindamycin, may increase the yield. Colonies are small and grayish. They look slightly yellow when they are old. Although *E. corrodens* is nonhemolytic, a faint green appearance may be seen on blood agar. Approximately 50 percent will produce the characteristic pitting. They elaborate an odor that resembles that of bleach or hypochlorite.[18]

E. corrodens is a member of the so-called HACEK family of organisms, which have the following in common: (1) slow growth, (2) a requirement for carbon dioxide, and (3) a predilection for infecting heart valves. The other members of the family are *Haemophilus aphrophilus*, *Actinobacillus actinomycetemcomitans*, *Cardiobacterium hominis*, and *Kingella kingae*.

EPIDEMIOLOGY

Infection with *E. corrodens* occurs when mucosal or skin barriers are disrupted and the organism gains access to deeper tissues. Puncture of the skin with forks[32] or toothpicks[38] may result in

deep-seated infections. One case of vertebral osteomyelitis that occurred after a woman accidentally inoculated the organism into the paravertebral space by penetration of a fish bone through the posterior pharynx has been reported.[28] In a similar case, a fish bone stuck in the throat for 2 months eventually led to a spinal epidural abscess.[21] Infection commonly occurs after clenched-fist injury as a result of fistfighting.[13] Hand infections in children are more likely to be secondary to digital biting or sucking.[15] Intravenous drug abusers are at risk for injection site and soft tissue abscesses,[14] bacteremia, and endocarditis.[9,31] Elderly persons and patients with advanced carcinomas are the other high-risk groups. However, children are at particularly high risk for serious *E. corrodens* infections.[37] Reports of thyroid abscesses[7,46] and purulent thyroiditis[36] all have been in children. In one review,[23] more than 20 percent of *E. corrodens* pleuropulmonary infections occurred in children younger than 14 years of age, and more than 50 percent of abdominal infections were reported in patients younger than 25 years of age.[8] *E. corrodens* orbital cellulitis,[16] empyema,[41] peritonsillar abscess,[25] paronychia,[1] and osteomyelitis[34,40] have been observed in children. A review of 54 cases of *E. corrodens* infection in children and adolescents revealed that 41 percent of pediatric infections occurred in the head and neck. The most common single site was the thyroid gland.[32]

PATHOPHYSIOLOGY

E. corrodens infections often are polymicrobial[44] and may include other anaerobes or gram-negative rods. However, *E. corrodens* is accompanied most frequently by recovery of alpha-hemolytic streptococci. In most reports, the streptococci were not speciated further, but Jacobs and associates[20] made a case for the *Streptococcus anginosus* group because of similarities between the two organisms (i.e., both are found in the mouth and gastrointestinal tract, both produce local suppurative infection, and both thrive in carbon dioxide-rich, oxygen-poor environments). Brooks and colleagues[4] also reported synergy of the two organisms in a rabbit model of skin infection. In vitro studies of *E. corrodens* co-cultivated with members of the *S. anginosus* group showed that a significant degree of co-aggregation occurs. Additionally, exponential growth of *Streptococcus constellatus* and *Streptococcus intermedius* occurs 6 hours into incubation when these species are grown in the presence of *E. corrodens*; in its absence, exponential growth does not occur until 25 hours after inoculation.[48]

The possible role of *E. corrodens* in periodontitis has not been delineated precisely. However, soluble products of *E. corrodens* induce gene expression and protein production of vascular endothelial growth factor and cause phosphorylation of mitogen-activated protein kinase in vitro, a process that leads to increased production of interleukin-8 and adhesion molecules.[49] This cascade of inflammatory responses could promote chronic periodontitis.

E. corrodens has a propensity toward formation of an abscess in any location, whether alone or in concert with other organisms. Such formation is a hallmark of central nervous system infection.[3] Of intra-abdominal infections reported by Danziger and associates,[8] 15 of 19 patients had abscesses. In two cases of orbital cellulitis reported by Hemady and coworkers,[16] both patients had subperiosteal abscesses. Deep or superficial skin abscesses reported in drug addicts[14] or in clenched-fist injury from fistfighting[13] often recur, even after presumed adequate drainage.[35]

CLINICAL MANIFESTATIONS

Infections with *E. corrodens* are indolent. The time from inoculation to onset of symptoms generally is 1 week or longer.[4] Many cases show initial improvement with treatment but relapse days later, even with appropriate therapy.[15,25,33,37] The head and neck are the most common sites of infection at all ages.[32]

Infection of periodontal sites may be associated with rapid progression and bone resorption thought to be secondary to surface-associated materials of *E. corrodens* and other organisms of periodontitis.[29] Craniofacial and neck infections tend to have prolonged morbidity; many require repeated drainage procedures and long courses of antimicrobial agents.[37] Central nervous system infections often are preceded by sinus infections but also have been seen in children with congenital heart disease.[2,45]

Pleuropulmonary infections are marked by fever, cough, and chest pain. Necrotizing pneumonia with multiple abscesses sometimes is seen. Effusions or empyema are noted in 30 percent, and cavitation is seen in 8 percent. Children with a predisposition toward aspiration may be at higher risk.[23] In one case, a bedridden 16-year-old girl developed a bronchopleural-cutaneous fistula complicating necrotizing pneumonia with empyema.[47]

Endocarditis is associated with large, friable vegetations and frequent emboli and often requires valve replacement.[11] Intravenous drug use has been implicated in approximately half of reported cases.

Abdominal *E. corrodens* infections are seen most commonly as complications of ruptured appendicitis but also have been associated with abdominal trauma and surgery. The clinical course is protracted.[8]

Chorioamnionitis leading to premature delivery has been documented infrequently. In one case, the infection precipitated the birth of twins at 23 weeks' gestation and led to the demise of one twin.[22,24,43]

Soft tissue infections tend to be severe. Many require wide débridement and skin grafting. Infection of underlying joints, tendons, or bones is not infrequent and can be necrotizing and even lead to amputation.[35]

DIAGNOSIS

Definitive diagnosis rests on recovery of *E. corrodens* in culture, which can be a difficult task because of the organism's slow growth. *Eikenella* tends to be overgrown by hardier species when it is part of a polymicrobial process and may be missed, especially if the isolate does not pit the agar. All the HACEK organisms can pit agar,[5] although not with the regularity of *E. corrodens*.

Many bacteriology laboratories have difficulty identifying and separating catalase-negative, oxidase-positive, gram-negative rods. Not surprisingly, one report noted that of 100 isolates of *E. corrodens* identified by the National Collection of Type Cultures, only 21 were sent in as probable *E. corrodens*.[5] Organisms that *E. corrodens* may be mistaken for include the other HACEK organisms, *H. paraphrophilus*, *Moraxella atlantae*, and *Actinobacillus ureae*.

TREATMENT

E. corrodens has a very unusual antimicrobial susceptibility pattern, in that although most isolates are sensitive to penicillin and ampicillin, they are resistant to semisynthetic penicillins, such as methicillin and nafcillin.[42] Additionally, these organisms uniformly are resistant to clindamycin and metronidazole,[18] drugs commonly used to treat anaerobic infections. They also variably are resistant to aminoglycosides.

Most isolates are sensitive to piperacillin, second- and third-generation cephalosporins, and tetracycline. In one report, all of 31 *Eikenella* isolates derived from normal oral flora were sensitive to azithromycin in vitro.[30] Although penicillin often is cited as the drug of choice, some strains produce β-lactamases. One

report associated the β-lactamase with a transposon[26] and another with a plasmid[39]; one report found a chromosomal enzyme that was not inducible.[27] In addition, there are reports of intermediate resistance to penicillin, even in isolates that do not produce a β-lactamase.[12]

Incision and drainage of abscesses and débridement of necrotic tissue are essential to recovery from these infections. Therapy should be prolonged after patients apparently have recovered because early cessation of antibiotic therapy tends to be associated with relapse. If patients continue to have fever or other signs of infection days after appropriate therapy has been started, reimaging of the infected area may be prudent to detect early reaccumulation of purulence.

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CHAPTER 134

Elizabethkingia and *Chryseobacterium* Species

Randall G. Fisher

Members of the genus *Chryseobacterium* (formerly *Flavobacterium*) seldom are associated with human infection. Most disease occurs after exposure to a contaminated environmental source. In 1944, Shulmann and Johnson[50] reported a case of meningitis caused by a previously unidentified, gram-negative bacillus isolated from a 9-day-old premature infant. The term *Flavobacterium meningosepticum* was proposed for this organism by King[30] in 1959, based on her studies of bacterial isolates associated primarily with neonatal meningitis and septicemia. Although neonatal meningitis is the most common manifestation of human disease caused by this genus,[15,55] sepsis,[14,23,42,52] endocarditis, pneumonia,[44] septic arthritis with penetrating trauma or prosthesis,[22,31] and skin infection[19] (including one case of necrotizing fasciitis)[32] occur in individuals beyond the newborn period.[3]

BACTERIOLOGY

The taxonomy of these organisms is confusing because of frequent changes. All but one of the clinically relevant species of the original genus *Flavobacterium* were reclassified to the new genus *Chryseobacterium* in 1994.[59] Phylogenetic analysis based on 16S rRNA sequencing demonstrated that the organisms formerly classified as *Chryseobacterium meningosepticum* and *Chryseobacterium miricola* represent a separate lineage. Researchers proposed that these two organisms be moved to a new genus, named *Elizabethkingia* after the microbiologist who first described them.[29] The former *Flavobacterium odoratum*, rarely responsible for human disease, has been divided into two species (*odoratus* and *odoratimimus*) reclassified as members of the genus *Myroides*.[58] *Chryseobacterium indologenes* is the species isolated most frequently from human specimens, but it usually is not associated with significant disease. Therefore, this chapter focuses mainly on *Elizabethkingia meningosepticum*, which can cause severe infections, especially in newborns. These organisms are long, thin, catalase-positive, gram-negative rods with slightly swollen ends; they are nonmotile, oxidase-positive, weakly fermentative, and proteolytic and grow on solid agar as 1- to 2-mm, convex, glistening colonies of buttery consistency.[30] Yellow pigmentation is seen occasionally. Colonies do not demonstrate hemolysis on blood agar but may produce a lavender-green color in the surrounding media as a result of extensive proteolytic enzyme activity. *Elizabethkingia* is unable to grow on Salmonella-Shigella agar or Simmons citrate and lacks motility. These characteristics distinguish *Elizabethkingia* from *Pseudomonas*, with which it often is confused.[15] Similarly, utilization of glucose in an open tube of oxidation-fermentation media distinguishes *Elizabethkingia* from *Achromobacter faecalis*.[15] The clinically relevant species *E. meningosepticum* (also referred to interchangeably in the current literature by its old name *Chryseobacterium meningosepticum* and in the older literature as *Flavobacterium meningosepticum*) and *Flavobacterium IIB* (renamed *Flavobacterium balustinum*) are differentiated by the former's consistent liquefaction of gelatin and early utilization of mannitol and maltose and the latter's lack of these abilities.[30] *Myroides odoratus* and *Myroides odoratimimus* (*F. odoratum*), which have been identified most commonly as saprophytes in skin wounds,[26] characteristically are nonsaccharolytic and produce a fruity odor when they are grown on standard media.

EPIDEMIOLOGY

Elizabethkingia spp. and *Chryseobacteria* are distributed widely as saprophytes in fresh and salt water, as well as in the soil. *E. meningosepticum* has been identified as a pathogen in birds.[57] In hospitals, these organisms have been found to be ubiquitous colonizers of the patient's environment and have been isolated from flower vases,[54] ice machines,[52] vials of intravenous drugs,[42] and nebulizers.[16] In addition, tap water,[16] eyewashes,[45] tube feedings,[16] sink traps,[8] and hand cultures of hospital personnel[16] have yielded this organism. In some instances, these reservoirs have been implicated in nosocomial outbreaks of patient colonization and invasive disease.

Neonatal infection caused by *E. meningosepticum* has been reported frequently in the literature, often in association with nursery epidemics.[2,7,47] As with other neonatal pathogens, infants who are premature and small for gestational age seem to be at particular risk of infection. More than 50 percent of infected infants weigh less than 2500 g. Almost all cases occur within 3 weeks of birth, and more than 50 percent of these infants manifest illness before they are 7 days old.[15]

Nosocomial epidemics have occurred sporadically since the nursery outbreak reported by Brody and colleagues in 1958.[2,5,7,8] Cabrera and Davis[8] reported such an outbreak in detail in 1960. During a 3-month period, the bacteria were isolated from a total of 44 infants, of whom 14 had overt infection. Most colonized infants had organisms isolated from the nasopharynx. The only reservoir of infectious bacteria discovered was a faulty sink trap, beneath which cleaning materials for the nursery were stored. Repair of the defective trap and thorough cleansing and repainting of the nursery coincided with termination of the epidemic. More recently, clusters of neonatal and adult systemic infections caused by atypical strains of *E. meningosepticum* were reported from Taiwan. These strains initially were identified erroneously as *Aeromonas salmonicida*.[12]

Nursery outbreaks of *E. meningosepticum* have been traced to saline used to flush infants' eyes after administration of silver nitrate,[45] and organisms have been recovered from washbasins sinks, disinfectants, and suction devices in other epidemics.[8] Colonization of patients in a surgical intensive care unit was associated with tap water, sinks, ice machines, and washbasins yielding the bacteria.[16] Ribotyping and random amplified polymorphic DNA fingerprinting (RAPD) offer promise for more precise characterization of epidemics.[12,13]

PATHOPHYSIOLOGY

Elizabethkingia spp. and *Chryseobacterium* spp. generally are of low virulence. Rabbits administered 1-mL intravenous infections of 24-hour-old broth cultures demonstrated no mortality or morbidity; death rates were less than 30 percent in mice inoculated intracerebrally with "barely turbid" preparations.[30]

Most cases of invasive human disease are thought to be caused by environmental contamination with high numbers of *E. meningosepticum*, with spread to the compromised newborn or debilitated older patient. Some neonatal infections may be caused by colonization of the infant during passage through the birth canal of a colonized mother.[13] Intrapartum infection is supported by

occurrence of symptoms as early as 10 hours after birth.[15] However, only 0.3 percent of genital swabs submitted from patients with suspected venereal disease yielded the organism.[41] Continuing reports of *E. meningosepticum* as a cause of neonatal infection in developing countries have been speculated to be related to use of contaminated groundwater for bathing of newborn infants and feminine genital hygiene.[15] The propensity for this organism to produce meningitis in the newborn is not understood, but infection may occur in association with heavy nasopharyngeal colonization, leading to subsequent bacteremia and seeding of the meninges.

Cases in older children have been related to insulin-dependent diabetes mellitus,[9] thalassemia major with splenectomy,[43] and oncologic disease with indwelling catheters.[40] Peritonitis in patients undergoing continuous ambulatory peritoneal dialysis, including a case series of 30 patients, also has been reported.[44]

In older individuals, *E. meningosepticum* and chryseobacteria primarily play the role of opportunists.[38,91] Heavy nosocomial colonization combined with a blunted immune response probably accounts for the immunocompromised patient's poor capacity to handle this otherwise noninvasive bacterium.

CLINICAL MANIFESTATIONS

Neonatal sepsis and meningitis caused by *E. meningosepticum* share signs and symptoms in common with other forms of neonatal bacterial infection. However, the development of meningitis may be insidious, and several days of illness often pass before its presentation[15,47]; this factor is consistent with the low virulence of *E. meningosepticum* in comparison with other agents of neonatal sepsis. Prognosis is extremely poor, and mortality rates may exceed 60 percent.[33] Fifty percent of survivors develop significant neurologic complications, often in association with hydrocephalus.[34]

These organisms are uncommon pathogens in adults, and childhood disease occurring beyond the newborn period is extremely rare. Among the 24 initial isolates of *E. meningosepticum* identified by King,[30] organisms were identified in a throat culture from an adult patient and in cerebrospinal fluid (CSF) from an 8-month-old infant. Bacteria formerly classified as *Flavobacterium IIB* (now known as *F. balustinum*) were isolated from the blood and CSF of several adult patients without clinical information.[27] Since their initial identification in 1959, these agents have been implicated as causes of meningitis,[38,47] postoperative bacteremia,[3,41] bacterial endocarditis,[61] pneumonia,[53,54] catheter-associated infection, septic arthritis, and skin infection.[19] *E. meningosepticum* is the clinically relevant species most commonly isolated, but *C. balustinum*, *M. odoratus* and *M. odoratimimus* (*F. odoratum*), and other *Chryseobacterium* spp. have been implicated in human disease. *Elizabethkingia* and *Chryseobacterium* spp. accounted for 0.25 percent of infections in a large consecutive series of patients with human immunodeficiency virus infection. Risk factors included low CD4 counts and leukopenia.[37]

E. meningosepticum meningitis beyond the neonatal period typically occurs in immunocompromised patients. Adults with preexisting leukemia,[47] glomerulonephritis,[38] and squamous cell carcinoma[23] have been described as having meningitis caused by this organism. In a 56-year-old woman, meningitis with *E. meningosepticum* developed after she underwent transsphenoidal hypophysectomy[10]; in an 8-month-old male child with preceding severe neurologic damage, meningitis developed with bacteria designated by the Centers for Disease Control and Prevention as *Flavobacterium*-like organisms (IIE). In a 6-week-old infant, *E. meningosepticum* bacteremia and meningitis developed in association with a strangulated hernia.[15]

Elizabethkingia and *Chryseobacterium* spp. were isolated frequently from tracheal aspirates of patients in intensive care during a 70-month observation period; yet during that time, none of more than 2000 critically ill patients developed pneumonia attributable to these microbes.[16] However, true respiratory tract infection was identified in an intubated pediatric patient and in adults receiving aerosolized medications.[6,54]

Sporadic cases of bacteremia have been reported in adult patients.[23,35] Infection in immunocompromised patients can occur as a complication of relatively benign invasive procedures or as a localized infection.[35,51] Endocarditis was documented in intravenous drug abusers and in patients undergoing dialysis.[18,61] Postoperative bacteremia in eight adult patients was linked to contaminated intravenous medications infused during anesthesia.[42] Contaminated arterial catheters were implicated in an epidemic of *Chryseobacterium* bacteremia.[52] Four patients, including a 7-year-old boy, became bacteremic in an outbreak associated with contamination at the time of intracardiac surgery.[3] This organism also has been associated with bacteremia in pediatric burn patients.[49]

Chryseobacterium spp. also have been isolated from infected skin lesions manifesting as papules, sheet-like lesions, plaques, and deep panniculitis.[19] One fatal case of necrotizing fasciitis in a patient with diabetes mellitus and chronic heart failure was reported.[32] Infection may have been related to wound contamination during repair of an orthopedic injury. Chryseobacteria have been isolated from amputation stumps, but the bacteria may play a largely saprophytic role at these sites.[26]

DIAGNOSIS

Rapid identification of infection with these organisms is urgent, not only to ensure providing proper therapy for the patient, but also to hasten initiating appropriate infection control measures to forestall epidemic outbreaks. Identification of *E. meningosepticum* is hindered by characteristically long periods required for oxidation of carbohydrates and weak or delayed indole production. Cultures may be misidentified as species of *Achromobacter* or *Pseudomonas*.[15] Clinical isolation of an unidentified gram-negative rod that is catalase- and oxidase-positive and that shows multiple antibiotic resistance should raise suspicion of *E. meningosepticum* infection. Cultures should be kept for several days for observation for typical carbohydrate reactions, which confirm the diagnosis.[15,46] Pulse-field gel electrophoresis of DNA was used successfully to identify recurrence of intravenous catheter-associated infection in a 6-year-old boy with non-Hodgkin lymphoma.[4,48]

TREATMENT

Unfortunately, treatment of *E. meningosepticum* meningitis represents an especially difficult challenge for the physician. The organism is paradoxically likely to be sensitive to drugs commonly employed against gram-positive organisms (rifampin, vancomycin, clindamycin, trimethoprim-sulfamethoxazole) and resistant to those used to treat gram-negative infections (aminoglycosides, cephalosporins, tetracyclines, carbapenems). Most *Elizabethkingia* and *Chryseobacterium* spp. produce broad-spectrum metallo–β-lactamases. In *E. meningosepticum*, the enzyme is highly efficient at hydrolyzing carbapenems. Inactivation of cephalosporins displays a remarkable variability that is based on the affinity of the enzyme for the different compounds.[60] Delay in establishing a specific identification of the organism occurs commonly, so the physician knows only that the organism is gram-negative, often resulting in prolonged periods of suboptimal therapy because recommended empiric antimicrobial treatment of gram-negative neonatal meningitis usually consists of a third-generation cephalosporin or ampicillin and an aminoglyco-

side, drugs to which *E. meningosepticum* almost uniformly is resistant. Moreover, antimicrobial susceptibilities determined by disk diffusion must be interpreted with caution. Aber and associates[1] found clinical isolates in which specific strains were sensitive to gentamicin and rifampin by disk diffusion but were resistant by agar gel dilution susceptibility testing. Therefore, more direct methods of measuring the minimal inhibitory concentration than disk diffusion sensitivity should be used to determine the optimal microbial agents for therapy.

Probably as a consequence of difficulties encountered in providing rapid and effective antibacterial therapy, these organisms frequently persist for prolonged periods in CSF. Average persistence of *E. meningosepticum* in CSF is 19 days,[15] as compared with the 3.9 days described by McCracken[39] for most cases of gram-negative neonatal bacterial meningitis.

Studies of in vitro susceptibility data forced a reappraisal of the idea that vancomycin is a good first-line choice for the treatment of chryseobacterial infection. In one report, a literature review showed that only 65 percent of historic isolates were susceptible to vancomycin[4]; in another, a thorough search for a susceptible organism among 58 clinical isolates met with utter failure.[20] Ninety-seven percent of *E. meningosepticum* isolates were susceptible to rifampin. Clinically, drugs that have been used alone or in combination with some success have included erythromycin, vancomycin, trimethoprim-sulfamethoxazole, fluoroquinolones (especially sparfloxacin and levofloxacin), and rifampin.[56] Some of these agents have the potential disadvantage of poor penetration of CSF. Combined use of many of these drugs renders interpreting therapeutic response difficult. The 3 survivors among the 12 patients reported by George and associates[21] all received vancomycin intravenously, intrathecally, or both, as a part of their regimen. Hawley and Gump[24] reported a case of *E. meningosepticum* meningitis in a neonate who responded to systemic vancomycin after unsuccessful treatment with multiple antibiotics, including erythromycin.

Intraventricular erythromycin[17,47] or rifampin[11,33,34,47] has been used in conjunction with systemic administration of these drugs. In particular, Lee and associates[34] reported no deaths in seven infants with *E. meningosepticum* meningitis who were treated with intraventricular rifampin through an Ommaya reservoir at a dose of 2 to 5 mg every 24 hours combined with 40 mg/kg/day administered intravenously. Intraventricular administration continued until the CSF was sterile. However, colonization of the Ommaya reservoir was a common finding, and formation of a porencephalic cyst occurred in one patient. Chandrika and Adler[11] reported sterilization of ventricles in one afflicted neonate within 48 hours after institution of therapy with intraventricular and intravenous rifampin. Erythromycin has been used intraventricularly with limited success at 5 to 10 mg/day.[17,47] Rios and Associates[47] reported the successful addition of intraventricular rifampin to a failing regimen of intravenous and intraventricular erythromycin. Development of resistance during therapy has been demonstrated with erythromycin and rifampin[17,47]; persistence of organisms in the CSF despite administration of presumably adequate therapy should alert the physician to test for this possibility. Addition of trimethoprim-sulfamethoxazole may be of benefit with such an occurrence; this agent effected a bacteriologic cure in eight of nine infants with meningitis.[36] This agent usually is not recommended in the neonatal period because of possible displacement of bilirubin from albumin-binding sites. Bacterial eradication was achieved in 48 hours in two patients with meningitis who were treated with clindamycin, rifampin, and cefotaxime systemically and rifampin intraventricularly.[7]

As with other types of gram-negative meningitis, antimicrobial therapy should be continued for at least 2 weeks after sterilization of ventricular fluid has been achieved. Complications of hydrocephalus and the potential use of intraventricular therapy render the neurosurgeon an essential part of the management team. Historically, mortality has been in excess of 70 percent, no doubt in part because of delays in identifying the organism and the limited antibiotic spectrum available for effective therapy. More recent series of patients, although small, suggest some improvement in this statistic, but the rate of morbidity of hydrocephalus and neurologic deficits remains high.

Recovery has been the rule in immunocompetent older individuals infected with contaminated materials, often despite treatment with antibiotics to which the organism was insensitive, a finding highlighting the very low virulence of these organisms in immunocompetent adults.[38] However, significant mortality and morbidity often occur in immunocompromised individuals with bacteremia or meningitis. Use of chloramphenicol, vancomycin, levofloxacin, erythromycin, or rifampin has achieved some success in these patients, but the choice of antibiotic must be based on a detailed examination of the organism's susceptibility.[25,49]

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CHAPTER 135

PSEUDOMONAS AND RELATED GENERA

Michael T. Brady

The original classification of the genus *Pseudomonas* described a wide variety of aerobic gram-negative rods divided into five rRNA homology groups (homology groups I to V). The genus since has undergone extensive revision, with four of the five homology groups being reclassified into separate genera (Table 135–1).[83] This reclassification was accomplished by using DNA-rRNA hybridization, DNA-DNA hybridization, and 16S rRNA sequencing techniques.

Pseudomonas and related genera are aerobic, motile, non–spore-forming, nonfermentative, gram-negative bacilli that live in soil, in water, and on plants and animals. Most organisms of these genera are ubiquitous and rarely pathogenic in humans. Although the pseudomonads may produce disease in any individual, they usually are opportunists that more commonly cause disease in patients with burns, cystic fibrosis, malignant diseases, and immunodeficiency conditions; in recipients of immunosuppressive therapy; or in malnourished persons. The most important of the opportunistic pseudomonads is *Pseudomonas aeruginosa*. However, numerous other pseudomonads cause specific clinical syndromes in children.

TABLE 135–1 Recent Toxignomic Changes and Reclassification of *Pseudomonas* Homology Groups I to V That Cause Disease in Humans

Homology Group	Previous Designation	Current Designation
I		*Pseudomonas aeruginosa*
		Pseudomonas fluorescens
		Pseudomonas putida
		Pseudomonas stutzeri
		Pseudomonas mendocina
		Pseudomonas pseudoalcaligenes
		Pseudomonas alcaligenes
		Pseudomonas (CDC group 1)
	Chryseomonas luteola	*Pseudomonas luteola*
	Flavimonas oryzihabitans	*Pseudomonas oryzihabitans*
II	*Pseudomonas cepacia*	*Burkholderia cepacia*
	Pseudomonas gladioli	*Burkholderia gladioli*
	Pseudomonas mallei	*Burkholderia mallei*
	Pseudomonas pickettii	*Ralstonia pickettii*
	Pseudomonas pseudomallei	*Burkholderia pseudomallei*
III	*Pseudomonas acidovorans*	*Delftia acidovorans*
	Pseudomonas testosteroni	*Comamonas testosteroni*
	Pseudomonas delafieldii	*Acidovorax delafieldii*
	Hydrogenomonas facilis	*Acidovorax facilis*
IV	*Pseudomonas diminuta*	*Brevundimonas diminuta*
	Pseudomonas vesicularis	*Brevundimonas vesicularis*
V	*Xanthomonas maltophilia*	*Stenotrophomonas maltophilia*
		Stenotrophomonas africana

CDC, Centers for Disease Control and Prevention.
Data from Gilligan, P. H., and Whitier, S.: Burkholderia, Stenotrophomonas, Ralstonia, Brevundimonas, Comamonas, and *Acidovorax. In Murray, P. R., Baron, J., Pfaller, M. A., et al. (eds.): Manual of Clinical Microbiology. 7th ed. Washington, D.C., ASM Press, 1999, pp. 526-538.*

ETIOLOGY

Pseudomonas spp. usually are gram-negative obligate aerobes (oxygen as the terminal electron acceptor), but they can grow anaerobically in the presence of nitrates or arginine (terminal electron acceptors). They lack the phosphoenolpyruvate-hexose-phosphotransferase system and catabolize carbohydrates by the Entner-Doudoroff pathway. Because pseudomonads can use a wide variety of carbon sources (simple and complex carbohydrates, alcohols, and amino acids), they can survive and multiply in almost any moist environment containing minimal amounts of organic compounds.

P. aeruginosa is the most clinically important species of the genus *Pseudomonas*. It is an oxidase-positive, gram-negative rod varying in size from 0.5 to 0.8 μm by 1.5 to 3.0 μm. Most strains are motile by one or more polar, monotrichous flagella and display fine projections (pili or fimbriae). *P. aeruginosa* grows readily on standard laboratory media. It grows optimally at 37° C but not at 4° C. Clinical *Pseudomonas* isolates are oxidase-positive (except for *Pseudomonas luteola* and *Pseudomonas oryzihabitans*) and catalase-positive. On MacConkey agar, *Pseudomonas* spp. are identified as nonlactase fermenters. They do not ferment carbohydrates but do oxidize monosaccharides such as glucose and xylose, but not maltose. Strains from clinical specimens may produce beta-hemolysis on blood agar. More than 90 percent of *P. aeruginosa* organisms produce a bluish-green phenazine pigment (pyocyanin-blue pus), as well as fluorescein, a yellow-green fluorescent pigment. Occasional *Pseudomonas* strains also produce dark red (pyorubin) or black (pyomelanin) pigment. These pigments diffuse into and color the medium surrounding the colonies. Strains of *P. aeruginosa* can be differentiated from one another for epidemiologic purposes by serologic typing, phage typing, ribotyping, and pyocin (bacteriocin) typing.

EPIDEMIOLOGY

P. aeruginosa is a ubiquitous environmental organism found in soil, in water, and on vegetation, including the surface of many raw fruits and vegetables. It was recognized first as a pathogen when Gessard recovered it from green pus in 1882. Its minimal nutritional requirements and ability to grow in a wide variety of physical environments enhance the organism's ability to survive in numerous ecologic niches. *P. aeruginosa* is found infrequently as normal microflora of healthy humans. The gastrointestinal (GI) tract is the most frequent site of human colonization. As many as 5 to 30 percent of normal persons have *P. aeruginosa* in their GI tract, although it rarely is the predominant organism. The large intestine is the most frequent site of transient colonization after ingestion. Other moist body sites that may become colonized include the throat, nasal mucosa, axillae, perineum, and the respiratory tract of patients who have been hospitalized for extended periods, have foreign bodies in place (endotracheal tubes or tracheostomies), have poor mucociliary clearance, and have received broad-spectrum antibiotics or chemotherapy. In general, *P. aeruginosa* is an opportunistic and not a frank pathogen. Despite the ability to colonize skin and mucosal surfaces, it rarely results in persistent colonization, it does not produce specific toxic factors that damage host tissues, and it is not capable of invading normal skin or mucosa. *P. aeruginosa* proves most effective as a pathogen in situations in which the host's immune defenses are diminished or lacking (e.g., poor mucociliary clearance in children with cystic fibrosis, neutropenia in children receiving chemotherapy for cancer, damaged skin barriers in burned patients).

P. aeruginosa frequently enters the hospital environment on the clothes, skin, respiratory tract, or shoes of patients or hospital personnel; colonization of any moist environment ensues. Thus, these organisms may be found growing in distilled water, hospital kitchens and laundries, mops, shower heads, whirlpools, antiseptic solutions, eyedrops, irrigation fluid, dialysis fluid, and equipment used for dialysis and respiratory care or inhalation therapy. Transmission of *P. aeruginosa* from patient to patient or from hospital personnel to patient often is assumed but rarely is documented.[54,242] In hospitalized patients, the likelihood of *Pseudomonas* colonization increases with the duration of hospitalization.

Sources of *Pseudomonas* outside the hospital that may result in colonization with subsequent infection include swimming pools, water slides, hot tubs, contact lens solutions, cosmetics, illicit injectable drugs, and the inner soles of sneakers. *P. aeruginosa* commonly is found on the surface of many types of raw fruits and vegetables. Consumption of these foods by profoundly immunosuppressed children can result in GI colonization and potentially can lead to invasive disease.

The environmental distribution of most of the other *Pseudomonas* spp. and related genera is similar to that of *P. aeruginosa*. *Pseudomonas pseudoalcaligenes* has one of the more unusual habitats. This organism has been identified in metalworking fluid (a mixture of water and petroleum products) at concentrations as high as 10^8 organisms per milliliter or higher. Metalworkers are exposed to aerosols containing high concentrations (10^5 organisms per milliliter) of *P. pseudoalcaligenes*.[138] Despite the development of high antibody levels to *P. pseudoalcaligenes*, these metalworkers with long-term exposure do not have clinical evidence of any acute or chronic respiratory or systemic disease.

Burkholderia, formerly *Pseudomonas cepacia*, is a plant pathogen that is ubiquitous in nature and can be found in water, soil, and decaying organic matter.[124] *Burkholderia cepacia* is an obligate aerobic, gram-negative rod that is intrinsically resistant to a wide range of antimicrobial agents, including aminoglycosides and carboxypenicillins (e.g., ticarcillin, azlocillin, and piperacillin). Historically, it was regarded as an organism with low pathogenicity. However, *B. cepacia* has been identified increasingly as a cause

of sporadic nosocomial outbreaks of infection in medical intensive care units. Outbreaks are far less common than with *P. aeruginosa*. When they occur, they have been traced to contaminated automated peritoneal dialysis machines, blood gas analyzers, povidone-iodine, and chlorhexidine.[20,21,88,215] *B. cepacia* can survive for extended periods in moist environments and even in the presence of disinfectants, including povidone-iodine.[6] Colonization of the respiratory tract, sometimes associated with endobronchial infection in patients with cystic fibrosis, is becoming a more common occurrence and is associated with increased morbidity and mortality. This organism also has become more important as a cause of infection and complications in patients with chronic granulomatous disease[219] and sickle-cell hemoglobinopathies.[22]

Stenotrophomonas maltophilia (formerly *Xanthomonas maltophilia*) is an aerobic gram-negative rod that grows readily on most bacteriologic media. *S. maltophilia* is being isolated with increasing frequency in hospitalized patients. Colonization of nonsterile sites such as the respiratory tract and wounds in the absence of clinical disease occurs commonly in hospitalized patients receiving long-term or broad-spectrum antibiotics. However, clinical illnesses such as pneumonia, urinary tract infections, endocarditis, bacteremia, meningitis, and peritonitis have been reported.[16,61,73,149,231] Isolation of *S. maltophilia* from the respiratory tract of patients with cystic fibrosis is increasing; in some centers, it is the second most frequent gram-negative bacterium isolated from sputum.[13,117]

Burkholderia pseudomallei is most prevalent in the tropical and subtropical areas of Southeast Asia and northern Australia. *B. pseudomallei* has been recovered frequently from rice paddy surface water in rice-growing areas of northern Thailand.[47,216]

PATHOGENESIS

The pathogenesis of *Pseudomonas* infections is multifactorial.[17,133] Although *P. aeruginosa* is not uncommonly a human saprophyte, it usually causes disease as an opportunistic pathogen. *P. aeruginosa* is a significant cause of infection in compromised hosts. Its ability to adapt to a variety of environments, its minimal nutritional requirements, and its propensity for the development of antibiotic resistance allow *P. aeruginosa* to survive in compromised patients. The requirement of oxygen for growth may account for the lack of invasiveness of these organisms after they have colonized and even infected the skin. *P. aeruginosa* possesses a variety of virulence factors, including an endotoxin, an enterotoxin, numerous extracellular enzymes, and cell-bound organelles such as flagella and pili. *P. aeruginosa* endotoxin is not as potent as are the endotoxins produced by other gram-negative organisms (2 to 3 mg needed to kill a 20-g mouse). However, *P. aeruginosa* endotoxin does protect the organism from the effects of complement and triggers cytokine pathways that lead to the sepsis syndrome and shock. This endotoxin may produce a diarrheal syndrome. A *Pseudomonas* enterotoxin also has been described, but its role in causing diarrhea in humans remains unclear.

The extracellular enzymes of *P. aeruginosa* include lecithinase, collagenase, lipase, elastase (LasA and LasB), caseinase, gelatinase, fibrinolysin, hemolysin, alkaline protease, phenazines, phospholipase C, exoenzyme S, exoenzyme U, and exotoxin A. The proteolytic enzymes may be responsible for localized necrosis of the skin or lung and for corneal ulceration. Exotoxin A is an adenosine-5′-diphosphate ribosyltransferase enzyme that inhibits the synthesis of eukaryotic cell protein (same mechanism as diphtheria toxin). Specific exotoxin A–deficient mutants of *P. aeruginosa* have reduced virulence in producing infection of the cornea or lung in mice and rats.[161,245] Exotoxin A also diminishes the activity of host phagocytes. Passive or active immunization against exotoxin A significantly protects against experimental infection with exotoxin-producing strains of *P. aeruginosa*. Phospholipase C degrades phospholipids, which are plentiful in eukaryotic but not prokaryotic cell membranes. The hemolysis produced by *P. aeruginosa* may be caused by heat-labile phospholipase C and by a heat-stable moiety. Exoenzyme S is another virulence factor.

In patients with burn injuries, *P. aeruginosa* growing in the burned area produces exoenzyme S; *P. aeruginosa* on intact skin does not.[158] In burned patients in whom *Pseudomonas* sepsis develops, exoenzyme S can be found in the blood before the bacteria can be detected. *P. aeruginosa* strains that lack exoenzyme S production are less able to cause invasive disease.[198]

The various proteases also can degrade numerous plasma proteins such as complement and coagulation factors.[246] Solubilization and destruction of lecithin (surfactant) may play a role in the atelectasis seen in pulmonary infections caused by *P. aeruginosa.* A leukocidin also has been described that may in part be capsular material. The purified slime from *Pseudomonas* is nontoxic. Pigments produced by *P. aeruginosa* also are nontoxic.

Surface structures such as pili and fimbriae are involved in attachment of *P. aeruginosa* to the epithelial cells of mucosal surfaces. *P. aeruginosa* that lack flagella are less capable of dissemination from wounds and of causing bacteremia and pneumonia in animal models.[8,55,65] *P. aeruginosa* preferentially binds to normal respiratory mucin, in contrast to some members of the family Enterobacteriaceae.[234] Fibronectin may protect epithelial cells from bacterial attachment, but the ability to do so is reduced in patients with cystic fibrosis (high levels of protease in respiratory secretions) and after cellular injury (trauma after intubation and viral infection of the lower respiratory tract, especially influenza virus). The glycocalyx (extracellular slime layer) is important in allowing *P. aeruginosa* organisms to adhere to each other and form microcolonies, which impair phagocytosis and antibody and antibiotic activity.

The pathogenicity of *P. aeruginosa* also depends on its ability to resist phagocytosis. Fick and Reynolds[69] noted that in patients with cystic fibrosis, the opsonic function of immunoglobulin G (IgG) was reduced as a result of a molecular change in the Fc portion of the IgG molecule. This deficit was magnified in the lungs of patients with cystic fibrosis infected with *P. aeruginosa* because bacterial proteases can fragment IgG and further impair its opsonic activity, which already may be marginal. The persistent presence of *P. aeruginosa* in the lungs of patients with cystic fibrosis also may be related to the presence of one or more factors in their sputum that interfere with the bactericidal activity of fresh normal human serum against *P. aeruginosa*. These blocking factors have been shown to be IgG antibody that blocks the normal bactericidal IgM activity of human sera.[170,203]

Concentrations of IgG subclass immunoglobulins have been studied in patients with cystic fibrosis and compared with values obtained in age-matched healthy children and adults. Pressler and associates[176] noted that in 52 percent of patients with cystic fibrosis, at least one of the four IgG subclasses had an elevated serum concentration in comparison to controls. A significant correlation of elevated serum concentrations of IgG2 (and, to a lesser extent, IgG3) with decreased forced expiratory volume at 1 second was noted. Moss[147] found that patients with cystic fibrosis who were infected with *P. aeruginosa* had markedly elevated serum concentrations of IgG antibodies to the opsonic immunodeterminant, type-specific lipopolysaccharide. This elevation was distributed among all four IgG subclasses, with a significant shift toward IgG3. Sera from patients with cystic fibrosis who were colonized with *P. aeruginosa* had diminished opsonic capacity, but complement-dependent human neutrophil phagocytosis was not impaired. Serum concentrations of IgG4 but not IgG1, IgG2, or IgG3 were correlated inversely with opsonic capacity. On the basis of these data, Moss[147] suggested that high levels of IgG4

antibody to opsonic immunodeterminants may inhibit normal pulmonary clearance of *P. aeruginosa* by pulmonary macrophages in vivo.

P. aeruginosa produces two elastolytic enzymes: LasA and LasB. These enzymes are virulence factors produced during infection. They probably cause direct damage to lung tissue (elastin accounts for 30% of the protein of lung tissue) and interfere with immune clearance of *P. aeruginosa* from the lungs. Berger and associates[18] noted that elastase treatment of isolated polymorphonuclear leukocytes severely impaired the ability of the cells to kill opsonized *P. aeruginosa*. These investigators demonstrated proteolytic degradation of C3b receptors and suggested that it may contribute to the inability of patients with cystic fibrosis to eradicate *P. aeruginosa* from their lungs. Because several cell types, including macrophages, monocytes, B lymphocytes, and some T lymphocytes, all carry the same C3b receptor, the proteolytic activity may cleave this molecule from all these cells, thereby decreasing the phagocytic activity of monocytes and macrophages in these patients. Berger and colleagues[18] demonstrated that optimal interaction between the complement-derived opsonic ligands C3b and iC3b and their respective receptors does not occur in the milieu of the lungs of patients with cystic fibrosis who are infected with *Pseudomonas*. These workers suggested that both *Pseudomonas* and host proteases may contribute to the initiation of a cycle of events in which neutrophils entering the infected lung actually impair phagocytosis rather than eradicate the source of these infections. Breakdown of elastin in the walls of blood vessels possibly may be responsible for the intrapulmonary hemorrhage noted in individuals with cystic fibrosis.

The mucoid strains of *P. aeruginosa* isolated from the respiratory secretions of patients with cystic fibrosis produce large quantities of alginate (composed of acetylated D-mannuronic acid and L-glucuronic acid).[39,220] This polysaccharide polymer not only gives *P. aeruginosa* a mucoid appearance on agar but also has antiphagocytic activity. Alginate also can elicit a significant inflammatory immune response in the lungs of patients with cystic fibrosis that may contribute to the lung damage present after chronic *P. aeruginosa* lung infection.[140] Because of its viscous nature, alginate contributes to the thick bronchial secretions in the lungs of children with cystic fibrosis; these secretions obstruct small airways and impair mucociliary clearance and movement of phagocytic cells. Production of alginate by *P. aeruginosa* is regulated and inducible. Mucoid *P. aeruginosa* loses the mucoid trait when it is serially cultured on laboratory media. Nonmucoid isolates convert to the mucoid (alginate-producing) phenotype when they are inoculated into the lung in a rat model.[198]

The role of lipopolysaccharide in the virulence of *P. aeruginosa* also has been studied. The virulence of several strains of *P. aeruginosa* in burned mice was found to be related directly to lipopolysaccharide integrity.[44] Deficiency of the O side chain of lipopolysaccharide reduced virulence markedly.

S. maltophilia and *B. cepacia* are opportunistic organisms with virulence factors that include intrinsic resistance to many antimicrobials effective in treating infection with *Pseudomonas* spp., an ability to adhere to plastic materials,[111] and elaboration of exoenzymes such as elastase and gelatinase.[152] *B. cepacia* also resists nonoxidative neutrophil killing.[219] For both *S. maltophilia* and *B. cepacia*, the following factors increase the risk of colonization occurring with these organisms, as well as progression from colonization to infection: (1) prolonged hospitalization, especially in intensive care settings[138]; (2) administration of broad-spectrum antibiotics[221]; (3) malignant disease, particularly if associated with immunosuppressive therapy and neutropenia[112,221]; and (4) breaks in mucocutaneous defense barriers, chiefly by instrumentation or the use of invasive devices.[112]

CLINICAL MANIFESTATIONS

P. aeruginosa can produce disease in healthy, immunocompetent children.[64] Generally, when disease occurs in previously healthy children, the organism has been introduced into a minor wound contaminated with water, soil, or vegetative material. This contamination is followed by the development of localized cellulitis that typically progresses to an abscess that exudes green or blue pus. The skin lesions (whether caused by direct inoculation or secondary to septicemia) begin as pink macules that progress to small cutaneous hemorrhagic nodules and eventually to areas of necrosis with eschar formation surrounded by an intense red areola (ecthyma gangrenosum; Fig. 135–1). In addition to skin infections, previously healthy children may experience a number of localized and systemic infections, including septicemia, endocarditis, corneal infection, otitis externa, dacryocystitis, mastitis, mastoiditis, meningitis, pneumonia, diarrhea, necrotizing fasciitis, peritonitis, and urinary tract infection. *Pseudomonas* osteochondritis/osteomyelitis may develop after puncture wounds, particularly of the foot.[72] Systemic *P. aeruginosa* infections in healthy children can be life-threatening, with mortality rates reported as high as 55 percent.[232]

Outbreaks of dermatitis (folliculitis), plantar nodules (*Pseudomonas* hot-foot syndrome), otitis externa, mastitis, and urinary tract infections caused by *P. aeruginosa* have been reported in normal, healthy children after the use of community swimming pools, water slides, recreational whirlpools, or family-owned hot tubs.[63,64,71,85,197,214,236] Pruritic or painful skin lesions (5 to 30 mm) develop several hours to 5 days or longer (mean, 48 hours) after contact with these water sources. Skin lesions may be erythematous, macular, or pustular. In some cases, very tender nodules have been observed.[71] Illness may vary from a few scattered lesions in some patients to extensive truncal involvement in others. The rash is most severe in areas occluded by snug-fitting bathing suits. In some children, malaise, fever, otitis externa, vomiting, sore throat, conjunctivitis, rhinitis, pyuria, abdominal cramps, and swollen breasts may be associated with the dermal lesions.

Multiple serotypes of *P. aeruginosa* have been associated with these outbreaks. The use of whirlpool baths usually involves soaking in water for variable periods. Superhydration of skin and exposure to *P. aeruginosa* result in primary cutaneous infection.[92] Whirlpool water is heated to temperatures higher than 37.8° C (100° F) and frequently is not filtered, thereby allowing for the persistence of desquamated skin. Both these factors are conducive to growth of *P. aeruginosa*.

Otitis externa caused by *P. aeruginosa* has been reported in healthy competitive swimmers who swim repetitively in a pool contaminated with *P. aeruginosa*.[182] The organism also has been associated with a more malignant form of otitis externa manifested by high fever, necrosis of portions of the external ear, facial nerve paralysis, mastoiditis, and osteomyelitis of the temporal bone and basilar skull.[95,154] Rarely, *P. aeruginosa* meningitis results from progression of this infection.[173] Malignant otitis externa usually is associated with predisposing factors such as malnutrition, leukopenia (a disorder of leukocyte function), malignant disease, or diabetes mellitus. Successful management of this condition requires aggressive surgical débridement in addition to appropriate systemic antibiotic therapy.

P. aeruginosa is a common agent of chronic suppurative otitis media (with or without cholesteatoma) and acute and chronic mastoiditis.[33,109,159] Chronic suppurative otitis media is a complication of inadequately treated acute otitis media and is manifested by a perforated tympanic membrane with persistent otorrhea. Chronic suppurative otitis media also occurs in children with surgically induced perforations of the tympanic membrane by

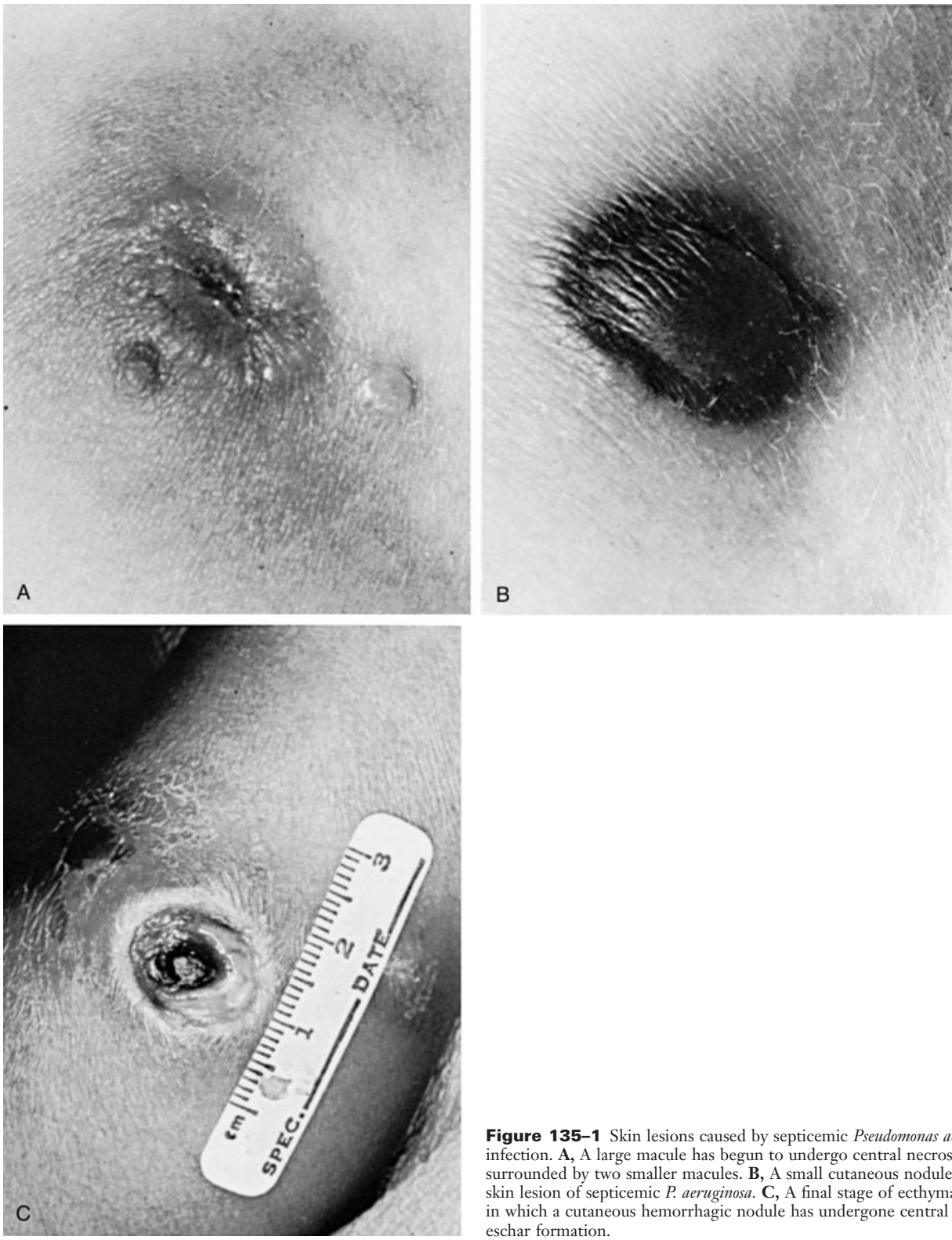

Figure 135–1 Skin lesions caused by septicemic *Pseudomonas aeruginosa* infection. **A,** A large macule has begun to undergo central necrosis and is surrounded by two smaller macules. **B,** A small cutaneous nodule representing the skin lesion of septicemic *P. aeruginosa*. **C,** A final stage of ecthyma gangrenosum in which a cutaneous hemorrhagic nodule has undergone central necrosis and eschar formation.

tympanostomy tubes and incompletely or inadequately treated otitis media. The etiologic microbial agents of acute otitis media in children with tympanostomy tubes differ from those in children with intact tympanic membranes. *P. aeruginosa* has been recovered from 12 percent of children with acute otitis media and tympanostomy tubes.[190] When tympanostomy tubes become colonized with *P. aeruginosa*, eradication of the infection may be complicated by the production of a biofilm.[26] The presence of a biofilm reduces the effectiveness of conventional systemic and topical antimicrobial therapy. Outpatient therapy with oral anti-

biotics frequently is unsuccessful because of the lack of oral antimicrobial agents with antipseudomonal activity. Topical ciprofloxacin with or without dexamethasone has been shown to be effective and superior to oral amoxicillin/clavulanic acid in the treatment of acute otitis media in children with otorrhea through tympanostomy tubes.[53] Intravenous antibiotics targeting the bacterial agents isolated from middle ear aspirates may be needed to cure chronic suppurative otitis media. This therapy may preclude the necessity of performing tympanomastoid surgery, which becomes essential in patients with extensive granulation tissue and osteitis in the mastoid.

P. aeruginosa ear infections were reported after commercial ear piercing.[107] In these patients, upper ear cartilage was involved more commonly than was lobe piercing. Re-use of a "single-use" disinfectant bottle contaminated at a sink harboring *P. aeruginosa* was implicated.[107]

P. aeruginosa infection of the eye usually occurs after trauma or surgery, after deposition of a large inoculum topically, or by hematogenous spread. Using contaminated contact lens solution, using tap water during contact lens care, and endotracheal suctioning without covering the eyes of sedated or comatose patients have been implicated.[91,94] Infection of the cornea can result in ulceration, which may progress to more invasive disease, including endophthalmitis. Loss of vision may result, even if appropriate antimicrobial therapy is administered promptly.

P. aeruginosa may produce serious infections during the neonatal period. Septicemia may be noted in the earliest hours of life and is associated with high morbidity and mortality rates. In utero acquisition of the organism resulting in intra-amniotic infection, neonatal sepsis, and death has been described.[160,194,252] The clinical course is similar to that of any other form of gram-negative septicemia, with hypotension, respiratory distress, and skin lesions the predominant manifestations. Mortality rates may be as high as 80 percent.[102] Late-onset neonatal *P. aeruginosa* infection usually occurs as a nosocomial infection (bacteremia, urinary tract infection, and pneumonia) associated with a foreign body (e.g., indwelling urinary or vascular catheter or endotracheal tube) in hospitalized infants. An outbreak in a neonatal intensive care unit of *P. aeruginosa* pneumonia and bloodstream infection was determined to be associated with intermittent otitis externa in a health care worker.[253] Maternal colonization after the use of a hot tub for relaxation during labor was responsible for *P. aeruginosa* meningitis and bacteremia in an 11-day-old infant.[235]

Increasing use of implantable devices has created new opportunities for health care–associated *Pseudomonas* infections. Infections of intrathecal baclofen pumps used to manage spasticity has been associated with wound infections and meningitis caused by *P. aeruginosa*.[248] Delayed infections in cochlear implant recipients with *P. aeruginosa* have been reported.[79] In these cases of device-associated *Pseudomonas* infection, removal of the device is important for successful management of the infection.

Other pseudomonads (except *B. pseudomallei*) rarely cause disease in healthy persons. Reports in normal, healthy children, particularly when these children had been hospitalized in an intensive care unit,[24,204] include the following: pneumonia, keratitis,[129] and abscesses caused by *B. cepacia*; otitis media caused by *Shewanella putrefaciens*; abscesses caused by *Pseudomonas fluorescens*; otitis media, pneumonia, and osteomyelitis[34,192] caused by *Pseudomonas stutzeri*; post-traumatic leg ulcers[169] and brain abscess[227] caused by *Sphingomonas paucimobilis*; and cellulitis, pneumonia, septicemia, endocarditis, peritonitis, and meningitis caused by *S. maltophilia*. *S. maltophilia* septicemia and endocarditis have been associated with intravenous abuse of illicit drugs.[256] Peritonitis and septicemia caused by *B. cepacia*, *S. putrefaciens*, and *S. paucimobilis* have been associated with contamination of equipment used for peritoneal dialysis.[11,20,46,84]

BURNS AND WOUND INFECTION

The surface of wounds or burns frequently is populated by pseudomonads and other gram-negative organisms.[64] Colonization does not imply infection necessarily, but it is a prerequisite to development of invasive disease. Septicemia with *P. aeruginosa* is a major problem in burned patients; the mortality attributed to *Pseudomonas*-associated burn wound sepsis approaches 78 percent.[141] Systemic involvement may be related to the multiplication of organisms in devitalized surface areas, followed by invasion, or it can be associated with the prolonged intravenous or urinary catheterization required for the care of these persons. Antibiotics may diminish the susceptible microbiologic flora but permit more resistant selected strains of *P. aeruginosa* to flourish. In addition, the hydrotherapy that commonly is provided to burned patients promotes colonization of the burned area, as well as other sites.[228]

In burn patients, abnormalities in neutrophil function that precede the onset of septicemia have been described.[3] Killing of *Pseudomonas* by neutrophils is impaired. Burn injury also is associated with abnormal responses to antigens, delayed rejection of homografts, abnormal vascular responses, impaired delayed hypersensitivity responses, diminished uptake of particles by the reticuloendothelial system, and altered antimicrobial pharmacokinetics. Contamination of wounds with high concentrations of bacteria ($>10^5$ colony-forming units [CFUs] per gram of tissue) impedes contraction and healing of the wound.[167] In addition, *P. aeruginosa* produces numerous substances that can further inhibit the natural healing process of burns and wounds. Secretion of exogenous plasminogen activators and proteases breaks down proteins such as fibrin and halts the contraction process.[172] *P. aeruginosa* exotoxin A, a protein synthesis inhibitor, is also a potent cause of retardation of wound healing.[87]

BONE AND JOINT INFECTIONS

P. aeruginosa is the most common cause of osteomyelitis after puncture wounds of the foot and is responsible for more than 90 percent of cases.[27,62,144] The calcaneus or metatarsal bones commonly are affected.[43] Symptoms may be present for 2 to 40 days (mean, 9 days) before diagnosis is established and hospitalization is needed.[43] Pain and swelling are the most common symptoms; fever and wound drainage rarely are noted. Leukocytosis (white blood cell count >10,000/mm^3) and an elevated erythrocyte sedimentation rate are present in most patients. Radiographs of the affected foot usually show evidence of osteomyelitis at some time during the period of evaluation and treatment. Bone scan results almost universally are abnormal and frequently yield evidence of osteomyelitis before positive findings on radiographs. The inner pad of sneakers has been implicated as a possible source of *P. aeruginosa* in these patients.[72] However, *P. aeruginosa* osteomyelitis of the foot bones has developed when the puncture occurred through other types of footwear or while the child was barefoot.

Other *P. aeruginosa* infections of bones and joints are uncommon findings in children. When they do occur, they are the result of hematogenous spread of *P. aeruginosa* in patients who are intravenous drug abusers or who have urinary tract or pelvic infections. Although any bone or joint may be affected as a result of *P. aeruginosa* bacteremia, *P. aeruginosa* has a unique predilection for the vertebrae, sternoarticular joints, pelvis, and symphysis pubis. The clinical course of *P. aeruginosa* osteomyelitis or septic arthritis is more indolent than that occurring after infection with *Staphylococcus aureus*. Contiguous spread can occur after penetrating trauma, surgery, or overlying soft tissue infections, especially decubitus ulcers.

CYSTIC FIBROSIS

Cystic fibrosis is one of the most common lethal inherited diseases of children.[64] It is a generalized disorder of salt and water transport that affects the exocrine glands and is caused by mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene.[118,191] The course and prognosis are determined largely by chronic infections of the airways with opportunistic bacteria. Death usually results from chronic obstructive pulmonary disease. *P. aeruginosa* can be recovered from cultures in most children with cystic fibrosis. Colonization with *P. aeruginosa* occurs more frequently in children with cystic fibrosis who require pancreatic enzyme supplementation. Recovery of *P. aeruginosa* from the sputum of a child with cystic fibrosis does not imply necessarily infection and the destructive pneumonitis related to this organism. Mucociliary clearance is impeded in patients with cystic fibrosis; as a consequence, they fail to cleanse the bronchopulmonary epithelium of inhaled particles, including bacteria.[118,146] Adherence of *P. aeruginosa* to the airways of children with cystic fibrosis may be enhanced by acidic environment that results from the hyperacidification of the trans-Golgi network caused by the dysfunctional CFTR in cystic fibrosis lung epithelial cells.[175] Colonization of the sputum of patients with cystic fibrosis may reflect the use of mist tents, inhalation therapy, exposure to colonized individuals in health care facilities, cystic fibrosis camps, or at home, and continuous use of broad-spectrum antibiotic therapy.[254] Colonization of the respiratory tract of patients with cystic fibrosis by mucoid strains of *P. aeruginosa* can be correlated with the patient's age, clinical score, extent of pulmonary function abnormalities, severity of changes on chest radiographs, and serum immunoglobulin levels.[68,70] Once *P. aeruginosa* is in the respiratory tract, it rarely is eradicated by antibiotic therapy.

Some observations, however, suggest that the relationship between *Pseudomonas* and patients with cystic fibrosis is more specific. Patients with cystic fibrosis almost always harbor an unusual mucoid *P. aeruginosa* phenotype that produces an excessive amount of capsular slime.[184] The tracheobronchial tree of 70 to 80 percent of these patients is colonized chronically, and the organism infrequently is eradicated either spontaneously or by antibiotic therapy.[80,210] The peculiar lung environment of a patient with cystic fibrosis is thought to trigger a switch to a cluster of genes that code for abundant production of the mucoid polysaccharide (alginate), thereby giving rise to the mucoid phenotype.[39,220] In contrast, mucoid strains of *P. aeruginosa* are recovered from only 0.5 to 1.7 percent of patients without cystic fibrosis. Chronic infection of the lungs in patients with cystic fibrosis causes additional genetic adaptations by *P. aeruginosa*. Chronic, persistent infection results in the loss of genes responsible for virulence factors required for initiation of acute infections.[213] The loss of these virulence factors is presumed to be beneficial for immune evasion. The immune system can recognize these virulence factors, thus leading to effective immune response. Loss of the virulence factors inhibits the immune response from eradicating *P. aeruginosa* from the airways.

Persistence of *P. aeruginosa* within the respiratory tract is aided by growth of the organism in microcolonies embedded in a biofilm of alginate.[93,168] This biofilm allows nutrients to pass while protecting the organism from host defense mechanisms, antibodies, and probably antibiotics.[118] In addition, investigators have noted that rabbit alveolar macrophages fail to phagocytize and kill the organism in the presence of serum from patients with cystic fibrosis. This phenomenon suggests that these patients have a specific, local defect in pulmonary resistance to *P. aeruginosa*.

A clustering of *P. aeruginosa* serotypes also occurs in isolates obtained from patients with cystic fibrosis. Homma type 8 strains may be recovered from 50 to 93 percent of patients with cystic fibrosis.

Bacterial infection in patients with cystic fibrosis is limited almost entirely to the respiratory tract, and pulmonary exacerbations with endobronchial disease are common findings. Infection of the pulmonary parenchyma rarely occurs. Rather, the epithelium of the airways and the submucosa are edematous and contain infiltrates of chronic inflammatory cells. Documentation of a pulmonary exacerbation in cystic fibrosis relies heavily on clinical impression (e.g., increase in the frequency of productive cough, increase in volume or a change in characteristics of the sputum, increase in the respiratory rate or dyspnea, and decrease in appetite, activity, or exercise tolerance). Fever and leukocytosis are present in a minority of patients and are associated with poorer pulmonary function test results and a worse prognostic score.[212] Concentrations of *P. aeruginosa*, DNA (derived from polymorphonuclear leukocytes and, to a lesser extent, from respiratory epithelial cells), and total protein in sputum are increased during pulmonary exacerbations and decrease significantly after administration of antimicrobial therapy.[212] Pulmonary infection in patients with cystic fibrosis generally is chronic. Bronchitis, bronchiolitis, and bronchiectasis can occur. Eventually, local necrotizing pneumonitis may be noted, in contrast to the overwhelming generalized necrotizing pneumonitis seen in immunosuppressed patients. Septicemia is a rare occurrence. However, bacteremia may develop in patients with indwelling venous catheters.

B. cepacia complex has emerged as an increasingly frequent agent of asymptomatic colonization, pneumonia, and septicemia in patients with cystic fibrosis. At least 10 distinct *Burkholderia* spp. are in the *B. cepacia* complex.[131] *Burkholderia cenocepacia* (genomovar III) is the most prevalent species, responsible for nearly half of all clinical isolates in the United States. This species is responsible for most of the cases of the "cepacia syndrome."[130] *Burkholderia multivorans* (genomovar II) accounts for approximately one third of clinical isolates in the United States and generally is thought to be less virulent than is *B. cenocepacia*.[105] A less common species, *Burkholderia dolosa* (genomovar IV), is associated with a rapid deterioration in lung function and decreased rates of survival.[105] Rates of colonization have been as high as 40 percent in some centers.[196] The increased frequency of colonization of the respiratory tract with *B. cepacia* in patients with cystic fibrosis has been associated with increased morbidity and mortality rates in some cystic fibrosis centers since the 1980s. The risk of colonization with *B. cepacia* complex increases with the severity of underlying disease and increasing age.[225] The source or mode of transmission of the organism has not been defined adequately.[131,226] However, more recent information supports person-to-person transmission.[103] Nosocomial transmission (patient-to-patient and contaminated inhalation therapy equipment) within cystic fibrosis centers and social contact, particularly at summer camps, appear to be important in the acquisition of new infection with *B. cepacia*. Once colonization with *B. cepacia* has been identified in patients with cystic fibrosis, three distinct clinical patterns have been noted: (1) chronic asymptomatic carriage (usually in association with *P. aeruginosa*); (2) progressive deterioration over the course of many months, with recurrent acute pulmonary exacerbations accompanied by fever, progressive weight loss, leukocytosis, and an elevated erythrocyte sedimentation rate; and (3) rapid, usually fatal, deterioration in pulmonary function associated with necrotizing pneumonia and, at times, bacteremia (cepacia syndrome).[98,130] The last complication can occur even in patients who were affected only mildly before acquiring *B. cepacia* infection.

B. cepacia has numerous important virulence factors, some of which may play a specific role in the clinical syndromes seen in patients with cystic fibrosis. *P. aeruginosa* enhances subsequent adhesion to epithelial surfaces by *B. cepacia*,[195] a property that may suggest a synergistic relationship between the two bacterial

pathogens, particularly in patients with cystic fibrosis.[218] Additionally, *B. cepacia* strains from patients with the most severe progression of cystic fibrosis bind most avidly to mucin.[196]

S. maltophilia was reported first in respiratory cultures of patients with cystic fibrosis in 1975.[76] Since that time, the prevalence of isolation of *S. maltophilia* from patients with cystic fibrosis has been increasing; rates in the United States range from 1.8 to 8.7 percent,[51,148] and many European centers report higher rates. One center in Spain reported a prevalence rate of 30 percent.[12] The prognostic significance of *S. maltophilia* colonization in patients with cystic fibrosis is not certain. In general, new acquisition of *S. maltophilia* does not seem to be associated with an adverse clinical outcome.[51] However, prolonged colonization with *S. maltophilia*, particularly with high bacterial counts in excess of 10^5 to 10^6 CFUs/mL of sputum, may be associated with progressive deterioration in pulmonary function.[12,106]

MALIGNANCY

Children with leukemia, particularly those receiving immunosuppressive therapy and those who are neutropenic, are extremely susceptible to septicemia caused by *P. aeruginosa* and other pseudomonads.[64] Most pseudomonads, including *Pseudomonas putida*, have been reported as causes of septicemia in children with malignant disease.[4] Generally, infection results from invasion of the bloodstream by a colonizing *Pseudomonas* organism (e.g., from the GI tract). Anorexia, malaise, nausea, vomiting, diarrhea, and fever may be noted. Generalized vasculitis develops, and hemorrhagic necrotic lesions can be found in all organs, including the skin, where the lesions appear as purple nodules or ecchymotic areas that become gangrenous.[181] Other invasive *P. aeruginosa* infections noted in children receiving immunosuppressive chemotherapy include hemorrhagic gangrenous perirectal cellulitis or abscess, typhlitis, pre-septal cellulitis, and necrotizing fasciitis.[134,142,233]

Children undergoing treatment of malignant diseases are particularly vulnerable to bacterial infection. Chemotherapy and radiation therapy can disrupt mucocutaneous barriers and result in moderate to severe immunosuppression. Fergie and associates[66] described 98 children and adolescents with cancer in whom *P. aeruginosa* bacteremia developed; the rate of bacteremia was highest in patients with leukemia. Most cases occurred when patients had absolute neutrophil counts of less than 100/mm^3. Mortality associated with *P. aeruginosa* bacteremia was higher in patients with solid tumors, an absolute neutrophil count of less than 100/mm^3, perineal skin lesions, and bacteremia during remission or induction therapy rather than during a relapse.

The single most important factor that predisposes children with cancer to development of infection is granulocytopenia. The bactericidal capacity of children with leukemia and other neoplasms also may be impaired. Heat-stable opsonins specific for *P. aeruginosa* similarly may fall precipitously in children with acute leukemia who are receiving intensive combination chemotherapy. Fatal infections with *P. aeruginosa* may be related in part to a deficiency of this specific opsonin.

IMMUNOSUPPRESSION

Immunosuppressive agents may be used in the management of malignant diseases, transplantation, or collagen vascular disease. The location of the infectious process and the type of causative organisms depend somewhat on the underlying disease. Infection by *P. aeruginosa*, particularly pneumonia and septicemia, occurs more commonly in children receiving immunosuppressive therapy than in the normal, healthy population.

OTHER CONDITIONS THAT PREDISPOSE TO *PSEUDOMONAS* INFECTION

P. aeruginosa is a major cause of hospital-acquired infections in children. It is the leading cause of nosocomial respiratory tract infection in children undergoing mechanical ventilation or receiving inhalation therapy. Asymptomatic colonization of the upper and lower airways occurs commonly and should be distinguished from respiratory tract disease, tracheitis, and pneumonia. A predominance of gram-negative bacilli with an abundance of polymorphonuclear leukocytes on Gram stain of lower respiratory tract secretions, in conjunction with a positive culture for *P. aeruginosa*, strongly supports the role of *P. aeruginosa* as the causative agent for the lower respiratory tract infection. Absence of *P. aeruginosa* from lower respiratory tract secretions markedly reduces the likelihood that *P. aeruginosa* is in the lower respiratory tract.

P. aeruginosa septicemia occurs with increased frequency in children with indwelling vascular or urinary catheters.[223] In addition, septicemia may occur in children with congenital or acquired neutropenia or in persons with a functional deficit in polymorphonuclear leukocyte function. Urinary tract infections also have been associated with cystoscopic examination. *Pseudomonas* is a common cause of abscesses and meningitis in children with dermoid sinus tracts or dermoids extending down to or communicating with the meninges or neural tissue and in children with meningomyeloceles. *P. aeruginosa* may produce acute or subacute endocarditis in children with congenital cardiac lesions before or after they undergo cardiac surgery and in adolescents who inject illicit drugs intravenously. *P. aeruginosa* supraglottitis was reported in a 6-month-old child with severe combined immunodeficiency syndrome.[122]

Severe *P. aeruginosa* infections have been reported in children infected with human immunodeficiency virus (HIV), primarily after severe immunodeficiency has developed.[74,189] Risks for acquiring *P. aeruginosa* infection in HIV-infected individuals include hospital exposure, declining CD4$^+$ cell count, and the use of dapsone or trimethoprim-sulfamethoxazole; azithromycin use was reported to be protective.[217] Bacteremia may occur with or without the presence of an indwelling vascular catheter. Fever, hypotension, skin lesions (papules or ecthyma gangrenosum), and pneumonia are common manifestations.[74] Mortality rates can be high, particularly when empiric antimicrobial therapy is inadequate for the treatment of severe, invasive *Pseudomonas* infection.

P. aeruginosa causes endocarditis on both native and prosthetic heart valves. Fortunately, it is an uncommon occurrence in children because its usual setting is in intravenous drug users. Obviously, *P. aeruginosa* needs to be considered in adolescents, particularly those with a history of intravenous drug use or at risk for acquiring HIV infection, and in infants, children, and adolescents with prosthetic valves or other intracardiac synthetic material. Tricuspid valve involvement occurs most commonly, but involvement of multiple valves is possible with *P. aeruginosa*; the manifestations are typical of subacute endocarditis. When the left side of the heart is involved (aortic or mitral valves), the patient has acute and more fulminant disease. Fever and heart murmur are almost universal findings.

DISEASE CAUSED BY OTHER PSEUDOMONADS

In addition to causing respiratory tract infection in patients with cystic fibrosis, *B. cepacia* can result in other infections, primarily in hospitalized or immunocompromised patients. Nosocomial pneumonia in ventilated patients, bacteremia, wound infections, urinary tract infections, meningitis, endocarditis, and skin lesions

may be caused by *B. cepacia*. Fortunately, all these infections are uncommon occurrences in children.

S. maltophilia has been reported with an ever-expanding spectrum of clinical manifestations, most notably bacteremia, endocarditis, lower respiratory tract infection, urinary tract infection, wound infection, meningitis, conjunctivitis, keratitis, dacryocystitis, otitis media, and bone and joint infections. Lower respiratory tract colonization, at times associated with symptomatic disease, is the most common manifestation in children. In addition to its occurrence in children with cystic fibrosis, *S. maltophilia* may be isolated from the respiratory tract of hospitalized children, especially those with endotracheal tubes or tracheostomies. Although colonization usually is asymptomatic, occasionally, lower respiratory tract infection with *S. maltophilia* can result in respiratory deterioration, pneumonitis, and an increased risk of mortality.[119,145]

Bacteremia with *S. maltophilia* usually results from the presence of an intravascular device or an infection of the respiratory, GI, or urinary tracts. Pseudobacteremia with *S. maltophilia* arising from contamination of blood cultures that were performed with blood used to fill nonsterile tubes designed for coagulation studies has been reported.[99] Other infections with *S. maltophilia* in children occur primarily after trauma or instrumentation.

Melioidosis is a rare disease of Southeast Asia and northern Australia that increased in frequency in the United States when Americans returned from Vietnam, Honduras,[37] or El Salvador,[36] and, rarely, after immigration by Southeast Asians.[47,163] The causative agent is *Burkholderia pseudomallei*, an environmental saprophyte of soil and water in the tropics, particularly in rice paddies. *B. pseudomallei* is a small, motile, pleomorphic gram-negative rod without a capsule that exhibits bipolar staining. It is an obligate aerobe that grows best at pH 7 and 37.0° C (98.6° F). Infection occurs after contact of abrasions or wounds with contaminated soil or water, inhalation of contaminated dust, or ingestion of contaminated water. Patients with poorly controlled diabetes, renal disease, or immunocompromised conditions caused by collagen vascular disease, hematologic malignant diseases, or immunosuppressive therapy appear to have an increased risk for development of disease after infection with *B. pseudomallei*.

Transmission most commonly occurs following percutaneous inoculation. Inhalation and ingestion are less common modes of transmission. Hematogenous spread occurs following local infection. Transmission from animals to humans has not been reported. The rat flea and the *Aedes aegypti* mosquito have been reported to infect animals with *B. pseudomallei*, but this route of infection has not been documented in human cases.[164] Human-to-human transmission has occurred during sexual or prolonged close contact.[121] Two cases of mother-to-child transmission of *B. pseudomallei* occurred through breast milk.[179] One mother had obvious mastitis.[179] An outbreak of melioidosis in Australia was traced to a potable water source from a water treatment plant that had irregularities in purification.[96]

Most infections with *B. pseudomallei* remain subclinical. Melioidosis can have a broad spectrum of clinical signs, and symptoms may be latent for months or years before the disease becomes clinically apparent. The initial clinical finding may be a single primary skin lesion (vesicle, pustule, bulla, urticaria) in a patient with no underlying disease. Septicemia occasionally occurs, and multiple abscesses may be noted in every organ of the body. The mortality rate associated with fulminant sepsis approaches 90 percent.[120] Meningitis, encephalitis, arthritis, nodular pulmonary densities, abscesses of liver,[7] lung, spleen, kidney, and bones, and endophthalmitis have been observed in both normal and compromised hosts after or concomitant with an episode of septicemia. The acute septicemic illness is indistinguishable from other types of septicemia caused by gram-negative organisms.

B. pseudomallei can cause myocarditis, pericarditis, endocarditis, intestinal abscess, cholecystitis, acute gastroenteritis, urinary tract infection, septic arthritis, paraspinal abscess, osteomyelitis, hilar lymphadenopathy, and cervical lymphadenopathy. Parotitis was documented in 38 percent of 126 children with melioidosis in Thailand.[48] None of the 126 children with melioidosis had any apparent predisposition to infection.

Subacute melioidosis generally is characterized by an illness lasting weeks to months. Pulmonary infection in this form of disease is a common occurrence and may mimic tuberculosis. The disease can vary from mild bronchitis to severe, fulminant pneumonitis.[120] Consolidation and cavitation occur frequently.

Neonatal melioidosis has been reported in Thailand.[135] Infants with neonatal septicemia, meningitis, or both, caused by *B. pseudomallei*, have been described. The mode of transmission of this organism to these newborn infants is not always clear. A clear case of mother-to-child transmission of *B. pseudomallei* was reported.[1] The mother had acute melioidosis treated with ofloxacin at 32 weeks' gestation. Consolidation of the right lung of the infant occurred, and an abscess eventually developed. Postpartum cultures of the mother's cervix yielded *B. pseudomallei*.

Chronic melioidosis occurs more frequently in whites than in Asians.[139] Chronic melioidosis may involve every organ in the body, including the brain.[120] Melioidosis may become dormant, with exacerbations occurring years after primary infection when host defenses are impaired as a result of steroid use, burns, diabetes mellitus, or other processes. The longest latent period (24 years) was reported by Kingston.[113]

Melioidosis should be considered in any person who has been to Southeast Asia or northern Australia at any time and has fever of unknown origin, overwhelming sepsis, single or multiple abscesses, or any tuberculosis-like illness. The diagnosis is established by culture of blood, skin lesions, or purulent material from an abscess cavity or from other sites of infection.[125] The organism grows in media commonly used for isolation of gram-negative bacteria. On solid media, the colonies develop slowly (over a week) and have a characteristic daisy-head appearance. Alpha-hemolysis is noted on sheep blood agar. A selective medium (Ashdown medium) can increase the recovery rate of *B. pseudomallei* from clinical specimens containing mixed bacterial flora, such as throat, rectal, and sputum specimens.[9] *B. pseudomallei* produces a dry, wrinkled, and violet-purple colony with a pungent, earthy odor on Ashdown medium.[81]

Serologic tests are more useful in establishing the diagnosis of melioidosis in latent or asymptomatic forms of this disease.[2,136,156] Hemagglutination (HA), indirect HA, complement-fixation (CF) tests, and an enzyme-linked immunosorbent assay (ELISA) are available. Diagnostic titers are 1:40 or greater for the HA test and 1:10 or greater for the CF test. Because the sensitivity of these serologic tests varies, both should be performed. HA antibodies generally are present within 7 to 14 days after onset of the illness; the CF test yields positive results in 4 to 6 weeks. Maximal titers for both tests are reached in 4 to 6 months. Both HA and CF antibodies persist for 9 months to 2 years after the onset of disease. In the United States, the indirect HA test is used by the Centers for Disease Control and Prevention for the diagnosis of melioidosis. An ELISA that detects specific IgG and IgM antibody to *B. pseudomallei* was developed.[10] This assay proved more suitable than was an IgG indirect fluorescent antibody test in screening for melioidosis and also was more sensitive than was the indirect HA test for melioidosis. Gold blot detection of IgM- and IgG-specific antibodies has been developed[122] and allows serodiagnosis of melioidosis to be made more rapidly. *B. pseudomallei* can be detected in serum by an ELISA method.[244]

DIAGNOSIS AND DIFFERENTIAL DIAGNOSIS

The diagnosis of *Pseudomonas* infection depends on recovery of the organism from blood, cerebrospinal fluid (CSF), and urine

obtained in a manner that avoids contamination by cutaneous flora (suprapubic aspiration or urethral catheterization is usually required for young children) and from joint fluid, peritoneal dialysis fluid, or purulent material obtained by aspiration of subcutaneous abscesses or areas of cellulitis. A diagnosis of *Pseudomonas* pneumonia can be made by needle aspiration of the lung and, less convincingly, by recovery of the organism from sputum obtained by postural drainage of a child with cystic fibrosis. Recovery of the organism from the surface of the skin or the throat, a tracheal aspirate, or bronchial secretions may reflect colonization and is not necessarily diagnostic of infection. The validity of a positive culture is enhanced when it is associated with a typical clinical syndrome (e.g., *P. aeruginosa* recovered from a skin lesion typical of whirlpool folliculitis). Isolation of *P. aeruginosa* from the respiratory tract, particularly when the specimen is obtained from an endotracheal tube in an intubated patient, is not an unusual occurrence. Differentiating colonization from infection is clinically important. Gram stain of respiratory secretions obtained by endotracheal suction typically reveals abundant gram-negative rods and polymorphonuclear leukocytes in the setting of a true lower respiratory tract infection caused by *P. aeruginosa* (tracheitis or pneumonitis). An absence of gram-negative rods or the presence of squamous epithelial cells, rather than polymorphonuclear leukocytes, indicates that either the patient does not have a lower respiratory tract infection or, if an infection is present, the etiologic agent is not likely to be *P. aeruginosa*.

Isolation of *Pseudomonas* spp. other than *P. aeruginosa* from clinical specimens occurs far less frequently. Recovery of these *Pseudomonas* spp. from sites that normally are sterile, such as blood or blood product containers, always should be considered clinically significant unless proved otherwise.

Pseudomonas and *Burkholderia* spp. are nutritionally versatile and grow well on most standard laboratory media (such as 5% sheep blood or chocolate agar). They grow optimally at 37° C and also at 42° C, but not at 4° C. All members of both genera grow in broth blood culture systems.[81] Isolation of *Pseudomonas* and *Burkholderia* from specimens with mixed bacterial flora is enhanced by using selective media such as MacConkey agar. Cetrimide, acetamide, nitrofurantoin, and 9-chloro-9[4-(diethylamino)phenyl]-9,10-dihydro-10-phenylacridine hydrochloride (C390) can be used for the isolation of *P. aeruginosa* from clinical as well as environmental specimens. Two media, PC (for *P. cepacia*)[81] and OFPBL (for oxidative-fermentative base-polymyxin B-bacitracin-lactose)[239] agar, inhibit *P. aeruginosa* and are useful for recovery of *B. cepacia* from the sputum of patients with cystic fibrosis.

P. aeruginosa usually is recognized easily on laboratory media by its characteristic colony morphology, diffusible pigment (when present), and odor (resembling sweet grapes or corn tacos). Colonies generally are spreading (sometimes overrunning other organisms in mixed infections) and flat. They usually have a metallic sheen. However, patients with cystic fibrosis typically have *P. aeruginosa* isolates with mucoid colony formation. *P. aeruginosa* can be identified reasonably by the presence of the following: (1) positive oxidase test, (2) triple sugar iron agar reaction of alkaline over no charge, (3) growth at 42° C, and (4) production of a bright blue to blue-green (and, to a lesser extent, red or brown) diffusible pigment on non–dye-containing agar such as Mueller-Hinton. Many laboratories rely on commercial systems for identifying *P. aeruginosa*. For pigmented *P. aeruginosa*, these systems are accurate 70 to 100 percent of the time (average, >90%).[77,186,188] The accuracy with nonpigmented *P. aeruginosa* is significantly less.[115]

The bluish, nodular skin lesions and the ulcers with ecchymotic and gangrenous centers and bright areolae (ecthyma gangrenosum) have been considered to be virtually pathognomonic of *P. aeruginosa* infection. Rarely, skin lesions that are clinically indistinguishable from those caused by *P. aeruginosa* develop after septicemia secondary to *Aeromonas hydrophila*.[208] Cutaneous or disseminated infections with *Aspergillus* and *Fusarium* in immunocompromised patients also can cause the necrotic skin lesions of ecthyma gangrenosum.

Immunoglobulin antibodies to *P. aeruginosa* surface antigens in serum have been detected reliably by ELISA.[28] Detection of specific IgG and IgA antibodies is not clinically useful for establishing the diagnosis of acute *P. aeruginosa* infection. However, antibody titer increases were associated with active disease caused by *P. aeruginosa* in patients with cystic fibrosis. Antibody titers returned to baseline when *Pseudomonas* infection was controlled by effective antimicrobial therapy. Thus, this assay appears to help in differentiating between early infection and colonization. Antibodies to *P. aeruginosa* also may be detected by immunoblotting (Western blotting).[209] These methods may be sensitive and useful for determining the onset of *P. aeruginosa* infection in patients with cystic fibrosis.

TREATMENT

Systemic infections with *Pseudomonas* should be treated promptly with antibiotics to which the organism is susceptible in vitro.[23,185] Community-acquired *P. aeruginosa* infections typically are sensitive to antipseudomonal penicillins, aminoglycosides, ciprofloxacin, ceftazidime, meropenem, and imipenem. Susceptibility is less predictable for aztreonam, a monobactam. Nosocomially acquired *P. aeruginosa* is more likely to be antibiotic-resistant than are community-acquired strains. Response to treatment may be impaired, and prolonged treatment may be required when systemic infection occurs in an immunocompromised host. Unfortunately, *P. aeruginosa* has been developing resistance to many of the classes of drugs that previously were used to treat this organism. Multidrug-resistant and panresistant *P. aeruginosa* are occurring with increasing frequency, especially in critical care settings. *Multidrug resistance* may be defined when *P. aeruginosa* has diminished susceptibility to more than one of the following five drug classes: aminoglycosides, antipseudomonal cephalosporins, antipseudomonal carbapenems, antipseudomonal β-lactam–β-lactamase inhibitor combinations, and antipseudomonal fluoroquinolones.[165] *Panresistance* may be defined when *P. aeruginosa* has diminished susceptibility to antipseudomonal cephalosporins, antipseudomonal carbapenems, antipseudomonal fluoroquinolones, and antipseudomonal β-lactam–β-lactamase inhibitor combinations.[165] If *P. aeruginosa* organisms retain susceptibility to aminoglycosides while being resistant to the other drug classes, they still should be considered panresistant because aminoglycoside monotherapy in serious infections, especially in critically ill or immunocompromised patients, has a high failure rate.

Prior use of a specific antibiotic may predict the development of resistance to that antibiotic.[60,165] However, use of fluoroquinolones and, to a lesser extent, antipseudomonal β-lactam antibiotics predisposes to multidrug-resistant *P. aeruginosa*.[50,165] Combination therapy has been suggested as a means of reducing the development of resistance while the patient is receiving treatment. Some studies supported the benefit of combination therapy to reduce the development of resistance.[56,237] However, other studies found that antipseudomonal β-lactam antibiotics alone are as effective as is an antipseudomonal β-lactam antibiotic-aminoglycoside combination for preventing the emergence of antibiotic resistance.[166]

Pseudomonas spp. not only exhibit intrinsic resistance to numerous antibiotics but also have the ability to acquire genes encoding resistance determinants. Mechanisms of resistance include production of β-lactamases [AmpC cephalosporinases, PSE β-lactamases, OXA β-lactamases, TEM β-lactamases, SHV-type β-lactamases, PER-1 β-lactamases, and metallo-carbapene-

mases] and aminoglycoside modifying enzymes [AAC (6′)-I and APH (3′)-II], mutations in topoisomerases II and IV, up-regulation of efflux pumps, and diminished expression of outer-membrane proteins (porin).[165]

Table 135–2 provides dosages of some of the more commonly prescribed antipseudomonal antibiotics.[151] These dosages are only guidelines because the doses of some of these antibiotics may vary with different clinical situations and patient populations. Once-daily administration of aminoglycoside is being evaluated as a way to decrease nephrotoxicity and improve clinical efficacy. Aminoglycoside doses must be decreased, preferably by increasing the dosing interval, in patients with diminished creatinine clearance (e.g., renal impairment, neonates). Significantly higher doses (e.g., 7 to 12 mg/kg/day for gentamicin or tobramycin) may be required for patients with increased total plasma clearance, such as those with cystic fibrosis and burns. Therefore, aminoglycoside therapy must be individualized and doses guided by pharmacokinetic information. Extended-infusion (4-hour infusion every 8 hours) of β-lactam antibiotics (piperacillin-tazobactam) has been considered to enhance the duration of drug concentration that remains in excess of the minimal inhibitory concentration (MIC) of *P. aeruginosa*.[134]

Invasive infections, including septicemia caused by proven or suspected *P. aeruginosa* infection, should be treated with a β-lactam antibiotic (antipseudomonal penicillin, third- or fourth-generation cephalosporin, or carbapenem) combined with an aminoglycoside (gentamicin, tobramycin, netilmicin, or amikacin). The combination of an aminoglycoside and a β-lactam antibiotic may be synergistic against the organism. Rifampin (synergistic in vitro with anti-*Pseudomonas* penicillins and aminoglycosides) may be added to the combination therapy if the clinical response is not adequate. Monotherapy with the cell wall–active β-lactam antibiotics, as well as the fluoroquinolones, frequently leads to the development of antibiotic resistance during therapy as a result of a mutation.[15,205]

P. aeruginosa may be responsible for lower respiratory tract infections in neonates, infants, and children. Involvement of the airways in patients with cystic fibrosis and nosocomial tracheitis or pneumonitis in intubated patients are the most common manifestations. In patients with cystic fibrosis and in critically ill intubated patients, previous use of antimicrobial therapy results in a high frequency of antibiotic-resistant *P. aeruginosa.* Combination therapy with a β-lactam antibiotic and an aminoglycoside is synergistic in vitro and has superior clinical efficacy when compared with monotherapy, particularly with aminoglycosides alone.[89] Quinolones do not appear to be synergistic with either β-lactam antibiotics or aminoglycosides. However, in postpubertal children, quinolones can be used in combination therapy when antibiotic resistance reduces the potential benefit of either the β-lactam antibiotic or the aminoglycoside.

For empiric treatment of *P. aeruginosa* lower respiratory tract disease before antibiotic susceptibility test results are available, the choice of which β-lactam antibiotic and which aminoglycoside will be used should be based on the patient's previous antibiotic experience and knowledge of the usual pattern of antibiotic susceptibility and resistance in the patient's clinical environment. Once susceptibility testing has been completed, at least two effective antibiotics should be included in the patient's regimen. The dose and pharmacotherapeutics of the chosen antibiotics should be optimized. If the patient fails to respond to therapy or if clinical deterioration is noted, acquisition of antibiotic resistance should be anticipated. If antibiotic failure is likely, more than one new antibiotic should be substituted. Selection of the new antibiotic should be guided by previous susceptibility testing and the probable changes that resulted in antibiotic resistance.

The route of administration also may be important with aminoglycoside antibiotics. Penetration of aminoglycosides from blood into respiratory secretions is poor.[19] Topical application of aminoglycosides by aerosolization, particularly through an endotracheal tube, provides much higher concentrations in respiratory

TABLE 135–2 Dosages of Commonly Prescribed Antipseudomonal Antibiotics

Generic Name	Dosage (mg/kg/day)	Route	Interval	Pediatric Precaution
Antipseudomonal Penicillins				
Carbenicillin indanyl sodium	30-50	PO	q6h	SNE
Ticarcillin disodium	200-300	IV	q4-6h	
Ticarcillin/clavulanate	200-300 (ticarcillin)	IV	q4-6h	
Piperacillin	200-300	IV	q4-6h	PDNE
Piperacillin/tazobactam	240 (piperacillin)	IV	q4-6h	PDNE
Cephalosporins				
Ceftazidime	200-300	IV, IM	q6-8h	
Cefepime	100-150	IV, IM	q8-12h	
Aminoglycosides				
Gentamicin sulfate	3-7.5	IV, IM	q8h or q24h	
Tobramycin sulfate	3-7.5	IV, IM	q8h or q24h	
Netilmicin sulfate	3-7.5	IV, IM	q8h or q24h	
Amikacin sulfate	15-22.5	IV, IM	q8h or q24h	
Quinolones				
Ciprofloxacin	20-30	PO, IV	q12h	>18 yr*
Monobactams				
Aztreonam	90-120	IV, IM	q6-8h	SNE
Carbapenems				
Imipenem/cilastatin sodium	60-100	IV, IM	q6h	SNE
Meropenem	60 (meningitis: 120)	IV	q8h	

**Safety in children younger than 18 years is not completely established, and use is recommended for specific infections (complicated urinary tract infections, pyelonephritis, and postexposure treatment of inhalation anthrax) and in circumstances in which the benefit outweighs the risk.*

IM, intramuscular; IV, intravenous; PDNE, pediatric dose not yet established; PO, oral; SNE, safety in children not yet established.

Data from Bradley J. S., and Nelson J. D.: Nelson's Pocket Book of Pediatric Antimicrobial Therapy. 16th ed. Buenos Aires, Alliance for World Wide Editing, 2006-2007, pp. 94-112.

secretions. This approach results in faster bacteriologic eradication, but clinical efficacy varied in different studies.[30] Administration of aerosolized aminoglycosides to patients with cystic fibrosis resulted in improvement in pulmonary function tests, decreased concentrations of *P. aeruginosa* in sputum, and no significant apparent toxicity. This aerosol treatment did not increase the isolation of *B. cepacia*, *S. maltophilia*, or *Alcaligenes xylosoxidans*; however, isolation of the fungi *Candida albicans* and *Aspergillus* did increase.[31]

Aztreonam, a monobactam antibiotic, has excellent antipseudomonal activity but is not approved for use in pediatric patients. Fluoroquinolones are approved for use in patients younger than 18 years of age to treat complicated urinary tract infections and pyelonephritis and for post-exposure treatment for inhalation anthrax. Polymyxin B and colistin (polymyxin E), used previously, have been superseded largely by less toxic agents, but they may be useful in selected patients who are infected with strains resistant to the other agents. However, all *Burkholderia* spp. are resistant to polymyxin B.

Ciprofloxacin and the other quinolones have been evaluated for the treatment of acute and chronic *P. aeruginosa* infections in teenagers and adults with cystic fibrosis.[100,193,222,224] These antibiotics, which may be given orally or intravenously, proved to be effective, as judged by clinical scores and results of pulmonary function tests. In the United States, quinolone (ciprofloxacin, ofloxacin, norfloxacin, enoxacin, levofloxacin, lomefloxacin, sparfloxacin, and trovafloxacin) use is limited to specific infections until after puberty because these antibiotics may bind cartilage and arrest growth. The information available from clinical trials in Europe suggests that ciprofloxacin and the other quinolones may not be as harmful in children as they are in other juvenile animal species.[41,200,201] However, pefloxacin, a fluoroquinolone that had been used extensively in France, does cause arthropathy in children and adults. In addition, two other quinolones, alatrofloxacin and trovafloxacin, cause acute liver failure, and some cases resulted in patients' deaths. Ciprofloxacin may be considered in selected children when the risks associated with the use of this antibiotic are outweighed by the potential benefits associated with its clinical efficacy (e.g., multiresistant strains of *Pseudomonas*, substitution of an oral quinolone to avoid long-term intravenous therapy requiring an indwelling catheter). If ciprofloxacin is used in a patient younger than 18 years of age for infections other than those for which it is approved, the balance of the risks and benefits of administering ciprofloxacin and alternatives should be explained to the patient and parents or guardians. Ciprofloxacin may be administered in a dosage of 15 mg/kg every 12 hours orally or 10 to 15 mg/kg twice a day intravenously. The oral dosage should not exceed 1000 mg/day in patients who weigh less than 40 kg or 1500 mg/day in patients who weigh more than 40 kg.

Determining the optimal antibiotic therapy for patients with cystic fibrosis may be very problematic. Respiratory cultures may yield *P. aeruginosa* with many different colony morphotypes. These different morphotypes may have significantly different antibiograms. The accuracy of susceptibility testing is improved when different morphotypes are tested individually. However, it is labor-intensive and expensive.

After years of antibiotic exposure, patients with cystic fibrosis commonly are infected with *P. aeruginosa* that is resistant in vitro to all available antimicrobial agents. For these patients, aerosolized tobramycin may be used to yield tobramycin concentrations in the range of 100 to 200 μg/mL of respiratory secretion. Some of these panresistant *P. aeruginosa* strains may be susceptible in vivo when concentrations of tobramycin reach these high levels in respiratory secretions. Susceptibility testing of these panresistant organisms should be performed by either determining MICs or performing an E-test to ascertain whether the organism is susceptible at these higher levels, 100 to 200 μg/mL.[82] In addition to management of resistant *P. aeruginosa*, aerosolized tobramycin given over a prolonged period (>1 year) may have the potential to eradicate *P. aeruginosa* temporarily from patients with cystic fibrosis who have newly acquired this organism.[180] Confirmation of the efficacy of prolonged antibiotic aerosol therapy may prove valuable because data indicate a poor outcome in children with cystic fibrosis who acquire *P. aeruginosa* by the age of 7 years.[157]

P. aeruginosa endocarditis requires aggressive medical and surgical therapy. Despite combination therapy with maximal β-lactam and aminoglycoside antibiotics, valve replacement (native and prosthetic) frequently is required for cure. When gentamicin or tobramycin is administered in three divided doses per day, peak concentrations should be maintained at 12 to 15 μg/mL. Quinolones should be reserved for patients intolerant of aminoglycosides or whose bacteria are resistant to aminoglycosides or for long-term suppression of *P. aeruginosa* prosthetic valve endocarditis.

Although fortunately uncommon, *P. aeruginosa* infection of the eye can be serious and sight-threatening. *P. aeruginosa* corneal ulcerations or keratitis may be seen in contact lens wearers or in intubated, sedated patients in intensive care units. Topical therapy with ticarcillin, piperacillin, tobramycin, gentamicin, amikacin, ciprofloxacin, or ofloxacin may be used and is effective. Clinical efficacy is improved by frequently clearing inflammatory debris and applying topical antibiotics. Initially, antibiotic solutions should be administered every 15 to 30 minutes. The frequency can be decreased gradually to four to six times a day when clinical improvement is apparent.

P. aeruginosa endophthalmitis frequently occurs after invasive eye surgery or penetrating injuries. Systemic, topical, and intraocular (anterior chamber and vitreous cavity) routes all are required. Even with aggressive medical intervention, return of retinal function seldom is achieved. The prognosis is worse when initiation of therapy is delayed. Ceftazidime, imipenem, and ciprofloxacin have greater intraocular penetration than do the aminoglycosides.[251]

P. aeruginosa meningitis or brain abscess should be treated with ceftazidime (200 mg/kg/day in four divided doses every 6 hours), and an aminoglycoside should be given intravenously. The initial empiric choice of the aminoglycoside should be guided by local susceptibility patterns. Concomitant intraventricular or intrathecal treatment with gentamicin may be required if initial intravenous therapy fails to sterilize the CSF. Gentamicin can be placed into the ventricular or lumbar CSF in a total dose of 1 to 5 mg once each day (the dose is independent of body weight). Meropenem has good penetration into CSF and can be used when the *P. aeruginosa* is ceftazidime-resistant. Meropenem is the preferred carbapenem because the high doses of imipenem required to treat central nervous system (CNS) infections may be associated with CNS toxicity. Fluoroquinolones such as parenteral ciprofloxacin or pefloxacin and aztreonam are possible alternatives in the treatment of *P. aeruginosa* CNS infections if more conventional therapy has failed. However, experience with these agents for CNS infections is limited.[112,127,158,207]

Skin abscesses or abscesses in other locations caused by *P. aeruginosa* should be incised and drained.[181] Failure to do so may result in a poor response despite prolonged systemic antibiotic treatment. Osteomyelitis of foot bones requires surgical débridement in every case. A course of 10 to 14 days of appropriate antibiotics appears to be adequate if surgery has removed the infected tissue effectively.[98] The adequacy of surgical débridement and clinical improvement can be monitored by serial sedimentation rates.[43] *P. aeruginosa* infection of foreign bodies (vascular, peritoneal, and CNS catheters) may require removal of the foreign material to cure the infection, particularly if a tunnel or exit-site infection exists.

Pseudomonas folliculitis and plantar nodules generally are self-limited and do not usually require specific antimicrobial therapy.

More severe cases can be treated by the topical application of any of the following: 2.5 percent acetic acid compresses (vinegar is 5% acetic acid), gentamicin ointment, topical 0.1 percent polymyxin B, or silver sulfadiazine (Silvadene).

The β-lactam antibiotics (antipseudomonal penicillins, cephalosporins, carbapenems), monobactams, and ciprofloxacin are rapidly bactericidal to *P. aeruginosa*.[75] The combination of an aminoglycoside with any of these antibiotics is unlikely to have a significant effect on the initial clinical response rate. However, a reduction in the emergence of drug-resistant *P. aeruginosa* clones is the major potential benefit of combination therapy that includes an aminoglycoside. Combination therapy with two β-lactam antibiotics is inappropriate for serious *Pseudomonas* infections because the induction of β-lactamase may result in resistance to both antibiotics.

Antibiotic resistance is an important factor in patients with serious *Pseudomonas* infections that do not respond to antibiotic therapy. As the quintessential opportunist, *Pseudomonas* has acquired various means to resist the activity of antibiotics. *P. aeruginosa* produces numerous different β-lactamase enzymes. Plasmid-mediated β-lactamases are responsible for resistance to antipseudomonal penicillins but not to the cephalosporins or carbapenems.[42] The most clinically relevant β-lactamases produced by *P. aeruginosa* are encoded primarily chromosomally rather than located on plasmids. Cephalosporinases, classified as class I β-lactamases,[187] produced by *P. aeruginosa* increase on exposure to any of the β-lactam antibiotics (de-repression of the β-lactamase gene).[199] However, the propensity to induce β-lactamase varies among the β-lactam antibiotics.[45] Imipenem and cefoxitin are strong inducers of β-lactamase.[57] All antipseudomonal penicillins, cephalosporins, and aztreonam are susceptible to the class I β-lactamase produced by *P. aeruginosa*.[155]

Clavulanate and tazobactam are β-lactamase inhibitors effective against plasmid-encoded class III and class V β-lactamases and the chromosomally encoded class II β-lactamases found in *P. aeruginosa* and some other gram-negative bacteria. However, these β-lactamase inhibitors not only are ineffective against the common class I β-lactamase of *Pseudomonas* but also are potent inducers of the β-lactamase gene. Use of these β-lactamase inhibitors will not enhance the activity of ticarcillin (Timentin) or piperacillin (Zosyn) against *Pseudomonas* and may actually increase the likelihood of emergence of resistant strains.

Pseudomonas strains with chromosomally encoded class I β-lactamase may not produce detectable β-lactamase until they are exposed to β-lactam antibiotics. These β-lactamase–encoded strains may appear sensitive to β-lactam antibiotics on in vitro sensitivity testing before antibiotic use. However, administration of β-lactam antibiotics to patients colonized or infected with these strains results in the induction of β-lactamase production and emergence of resistance. For this reason, repeat in vitro sensitivity testing of clinical isolates of *Pseudomonas* a few days after the administration of β-lactam antibiotics may reveal reduced sensitivity to β-lactams.

Whereas imipenem and meropenem resist the β-lactamases commonly produced by *Pseudomonas*, resistance to carbapenems can result through the loss of an outer-membrane porin that allows carbapenems to enter *Pseudomonas*.[32] The permeability of other β-lactam antibiotics also may be reduced when this outer-membrane porin is lost. Although chemically distinct from imipenem or meropenem, fluoroquinolones such as ciprofloxacin may induce decreased permeability to both antibiotics.[178] Plasmid-mediated metallo-β-lactamases that confer resistance to imipenem have been described.[143,206,238] Fortunately, these metallo-β-lactamases are identified only rarely in *P. aeruginosa*. Continued use of carbapenems may pressure an increase in these β-lactamases in the future. *S. maltophilia* is resistant innately to imipenem.

Tobramycin is the most active aminoglycoside against *P. aeruginosa*, whereas amikacin induces the lowest frequency of resistant strains. Resistance to aminoglycosides usually results from enzyme-mediated antibiotic modification.[52] The various aminoglycoside-modifying enzymes have different substrate affinities. Therefore, resistance to one aminoglycoside through aminoglycoside-modifying enzymes does not predict resistance to others necessarily. Resistance is less common to amikacin than to other aminoglycosides.[78,250] Aminoglycoside-modifying enzymes usually are coded by plasmid-mediated genes, but they occasionally can be coded by genes on the bacterial chromosome.[52] Plasmid-encoded resistance supports rapid transference among strains within an institution. *P. aeruginosa* also can become resistant to aminoglycosides by decreasing the intracellular uptake of aminoglycosides or by modification of intracellular ribosomal attachment.[49,57] These mechanisms of resistance generally cause cross-resistance for all aminoglycosides.

Ciprofloxacin, a bacterial DNA gyrase inhibitor, is the most effective of the quinolones used against *P. aeruginosa*. Alteration of the binding site of DNA gyrase and decreased penetration of ciprofloxacin through the *Pseudomonas* cell membrane can result in resistant strains.[57,127]

Multidrug resistance is not an unusual occurrence in *P. aeruginosa*, and it may arise after treatment with a single antibiotic. The induction and production of β-lactamase may act synergistically with diminished outer-membrane permeability.[251] In addition, facilitation of energy-dependent efflux of antibiotics by *P. aeruginosa* can result in simultaneous resistance to quinolones, β-lactams, tetracycline, and chloramphenicol.[174,251]

In addition to antibiotic resistance, other clinical factors can adversely affect aminoglycoside activity against *Pseudomonas*. The acidic environment in tissue infected with *P. aeruginosa* can inactivate aminoglycosides.[25] Aminoglycosides may fail to reach therapeutic tissue levels because of poor penetration into bronchial secretions and lung tissue.[19] For patients with tracheitis (e.g., intubated patients) or endobronchial disease (e.g., cystic fibrosis), aminoglycosides and, less frequently, colistin have been administered by aerosol.[202] This route of delivery allows for greater availability of the antibiotic at the site of the infection, with enhanced safety because of negligible absorption into the systemic circulation. Doses of gentamicin and tobramycin of 2.5 to 8 mg/kg can be given safely by aerosol three times a day, with a maximum of 300 mg/dose. Resistance may emerge after prolonged courses.

Antibiotic therapy for *B. cepacia* infection is very challenging and should be guided by results of in vitro susceptibility testing. Unfortunately, *B. cepacia* frequently is resistant to many commonly used antipseudomonal antibiotic agents, particularly the aminoglycosides. Antibiotics that may have activity against *B. cepacia* include ceftazidime, cefoperazone, ureidopenicillins, quinolones, trimethoprim-sulfamethoxazole, and chloramphenicol. Susceptibility to carbapenems and minocycline varies; meropenem has greater in vitro activity against *B. cepacia* than does imipenem.[58] Typically, *B. cepacia* isolates from patients with cystic fibrosis are more antibiotic-resistant than are isolates from other patient populations. Combination therapy with two or three antibiotics may be required to achieve a clinical response. Combinations of β-lactam agents with aminoglycosides may provide synergy clinically, even when the *B. cepacia* strain isolated is aminoglycoside-resistant. *B. cepacia* may be sensitive to minocycline.[119] Minocycline may be considered to have an adjunctive role in the management of *B. cepacia* infection in patients with cystic fibrosis. However, resistance to minocycline commonly develops with prolonged therapy (3 to 13 months).[119]

S. maltophilia also exhibits significant antibiotic resistance to the common antipseudomonal agents. Trimethoprim-sulfamethoxazole, chloramphenicol, ceftazidime, cefoperazone, ticarcillin plus clavulanic acid, and ciprofloxacin may be active

against this organism whether these drugs are used alone or in combinations. *S. maltophilia* is resistant to antipseudomonal penicillins, imipenem, and aminoglycosides. A combination of trimethoprim-sulfamethoxazole and ticarcillin-clavulanate has been recommended as the most appropriate initial therapy for serious infections that are suspected or known to be caused by *S. maltophilia*.[230] For patients with catheter-related infections, removal of the catheter offers the greatest opportunity for cure.[61]

The most active antibiotics against *B. pseudomallei* are imipenem, piperacillin-tazobactam, piperacillin, ceftazidime, ticarcillin-clavulanate, ampicillin-sulbactam, tetracycline, and chloramphenicol.[216] Piperacillin, ceftazidime, and imipenem are not bactericidal in vitro.[216] Ciprofloxacin seems to be of limited value because of a high rate of resistance.

Chronic melioidosis can be treated with chloramphenicol over a period of many months or with tetracycline. Trimethoprim-sulfamethoxazole was recommended previously, but most strains currently are resistant.

For acute systemic melioidosis, ceftazidime (120 mg/kg/day) or chloramphenicol (50 to 75 mg/kg/day) plus an aminoglycoside (amikacin, 15 to 20 mg/kg/day) and sulfisoxazole (120 to 150 mg/kg/day) should be administered for a period of 4 weeks. When third-generation cephalosporins have been used, cefoperazone and ceftazidime have shown greater activity against *B. pseudomallei* than have other third-generation cephalosporin agents. Ceftazidime was compared with chloramphenicol, doxycycline, and trimethoprim-sulfamethoxazole for the treatment of severe melioidosis.[241] Ceftazidime, in a dosage of 120 mg/kg/day intravenously in three divided doses every 8 hours, was associated with a 50 percent lower overall mortality rate when compared with other forms of therapy. These results suggest that ceftazidime combined with an aminoglycoside and sulfisoxazole now should be considered the treatment of choice for severe melioidosis.

Soft tissue infections should be treated for 4 to 6 months with tetracycline (in children older than 8 years) provided in a dosage of 50 mg/kg/day in four divided doses. In younger children, trimethoprim-sulfamethoxazole (8 mg/kg/day of trimethoprim and 40 mg/kg/day of sulfamethoxazole) in two divided doses may be used. Most penicillins are ineffective.[59,216] The duration of therapy must be guided by clinical and laboratory findings; therapy for 4 weeks to many months may be required in patients with osteomyelitis. Relapses are common occurrences and should be treated as one would treat the first episode.[38]

PREVENTION

Prevention of infection with pseudomonads depends, in part, on a continuous surveillance program of the hospital environment that is designed to identify and subsequently eradicate sources of pseudomonads as quickly as possible. Transmission of health care–associated *P. aeruginosa* frequently can be traced back to colonization of the water distribution system of the health care institution. Biofilm colonization of faucets can be particularly problematic. However, eradication of *P. aeruginosa* from the water distribution system has not been successful, even after using high water temperatures, copper and silver ionization, or microfilters at faucets or after removing colonized faucets. Because *P. aeruginosa* cannot be eradicated from the environment, other infection control measures, especially good hand hygiene and the use of sterile water to wash patients and patient supplies, should be emphasized. Pseudomonads can grow to a concentration of 10^6 organisms per milliliter in distilled water that appears to be perfectly clear. Growth of pseudomonads in distilled water, disinfectants, and medications is the factor most commonly incriminated in single-source outbreaks of *Pseudomonas* infection in hospitals. Prevention of the follicular dermatitis caused by *P. aeruginosa* contamination of whirlpools or hot tubs should be possible by maintaining the pool water at a pH of 7.2 to 7.8 and free allowable chlorine concentrations at 0.4 to 1.5 ppm.[35]

Outbreaks of *Pseudomonas* infection in newborn nurseries have been reported.[24] Generally, infection has been transmitted by the hands of personnel from washbasin surfaces and by suction catheter rinse solution to the newborn infants. Strict attention given to handwashing, particularly with a liquid iodophor handwashing agent before and between contact with newborn infants, may prevent or interdict epidemic disease. Growth of *Pseudomonas* on suction catheters can be prevented by rinsing the catheter in an acetic acid solution.

Daily replacement of all apparatus used for intravenous administration greatly reduces the hazard of extrinsic contamination by *Pseudomonas* and other gram-negative organisms. When intravenous administration is indicated, a small metal needle is preferable to a plastic catheter because these needles have been associated with a lower rate of septicemia and phlebitis. Meticulous care is required in the preparation of solutions for total parenteral alimentation and in the insertion and care of catheters.

The risk of developing *Pseudomonas* infection in a burned patient also can be minimized by careful protective isolation and by the topical application of silver nitrate (0.5%) solution or 10 percent mafenide acetate cream. Débridement for removal of devitalized tissue also is imperative.

Pseudomonas infection of dermal abnormalities that communicate with the cerebrospinal axis can be prevented by careful evaluation and early surgical repair. Providing antibiotic prophylaxis of *Pseudomonas* urinary tract infection is difficult without a suitable oral antipseudomonal antibiotic for children. Identification and surgical correction of obstructive lesions of the urinary tract minimize or prevent the development of *Pseudomonas* infection of the urinary tract.

Cohorting plus isolation of patients with cystic fibrosis who are colonized with multiresistant strains of *P. aeruginosa* or *B. cepacia* has been suggested as a means of reducing nosocomial transmission of these organisms.[67] However, the proper manner of handling patients with these organisms has not been established. Any attempt to reduce transmission should be based on measures with proven efficacy and must also consider the potential consequences of strict isolation or segregation on this population, which spends so much time at health care facilities.

In patients with cystic fibrosis and in certain immunocompromised patients, high rates of *P. aeruginosa* colonization and infection and ever-increasing antibiotic resistance render active or passive immunization (or both) against *P. aeruginosa* desirable. Since the 1980s, an understanding of the human immune response to *P. aeruginosa* and the immune responses that may provide protection against infection or disease has increased considerably. Naturally occurring immunity generally is ineffective. Certain naturally generated antibodies may even be detrimental.[240] These antibodies may form antigen-antibody complexes that increase pulmonary inflammation and direct lung damage. Even if neutralizing antibodies could be administered passively or developed after vaccine administration, the large quantities of mucoid exopolysaccharide produced by *P. aeruginosa* could possibly mask many of the antigens targeted for antibody neutralization or opsonization.

Despite the aforementioned difficulties, numerous investigations of candidates for *P. aeruginosa* vaccines are ongoing. Purified bacterial proteins, including flagellar antigen, outer membrane proteins, lipopolysaccharide-O, several inactivated bacterial toxins, high-molecular-weight polysaccharide antigen and glycoconjugate, and killed whole-cell vaccine preparations, have been tested. Many of the candidate vaccines have been shown to be safe, immunogenic, and capable of generating protective immunity in various animal systems.[177] To date, evidence of protective

efficacy in humans has not been established definitively for any of these vaccines. Some studies have demonstrated the efficacy of active immunization of burned patients with specific strains of *Pseudomonas* or the administration of hyperimmune globulin in the prevention of *Pseudomonas* septicemia.[3,103] *P. aeruginosa* vaccine also has been suggested as a possible method for preventing or delaying this disease in patients with acute leukemia or cystic fibrosis.[124,171]

The route of vaccine delivery may be important in creating the optimal immune response. Data from experimental animals suggest that vaccine antigens administered parenterally stimulate IgG and IgM antibodies; antigens administered orally induce IgA and IgG at mucosal surfaces. For patients whose disease occurs after mucosal colonization (e.g., cystic fibrosis), oral administration of a *P. aeruginosa* vaccine could be the preferred route of administration to develop the optimal immune response at the site of *P. aeruginosa* infection.

Purified bacterial proteins and lipopolysaccharide from *B. cepacia* are in the initial vaccine research and development stages. Whether these *B. cepacia* vaccine candidates will enter preclinical or phase I clinical trials is yet to be determined.

PROGNOSIS

The prognosis depends largely on the nature of the underlying disease process. Septicemia is the leading cause of death in children with leukemia; *Pseudomonas* is responsible for half of these deaths. Four variables independently influence the outcome of *Pseudomonas* septicemia: (1) the development of septic shock, (2) inappropriate antibiotic therapy, (3) granulocyte counts less than 500/mm^3, and (4) the development of septic metastases.[17] Most deaths in children with cystic fibrosis are caused by pulmonary insufficiency. *Pseudomonas* can be recovered from the lungs of almost every one of these patients, and, in many, it has been responsible for their deaths.

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CHAPTER 136

Stenotrophomonas (Xanthomonas) maltophilia

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Stenotrophomonas maltophilia is a gram-negative bacillus that previously belonged to the *Pseudomonas* and subsequently the *Xanthomonas* genus. In 1993, the new genus *Stenotrophomonas* was proposed, resulting in the most recent reclassification of this bacterium.[75] *S. maltophilia* is an opportunistic pathogen that has emerged as a significant cause of nosocomially acquired infection. It rarely is a cause of infection in healthy, immunocompetent children.

BACTERIOLOGY

S. maltophilia is an aerobic, nonfermentative, gram-negative bacillus that is oxidase-negative and lysine decarboxylase-positive. In addition, key features that allow identification of *S. maltophilia* include oxidation of glucose and maltose, as well as a positive DNase reaction.[40] Optimal growth occurs at 35° C; on sheep blood agar, colonies appear rough and lavender green and have a distinct ammonia-like odor. Morphologically, the organism is a straight bacillus, 0.7 to 1.8 μm long, that is motile by means of multiple polar flagella. It grows well on standard culture media, including blood agar and chocolate agar, as well as in broth blood culture systems, within the standard 5-day incubation period. Because of the hardy nature of this organism, standard collection, transport, and storage procedures are sufficient. The use of selective media such as MacConkey agar allows for *S. maltophilia* to be isolated from polymicrobial specimens, although for highly contaminated samples such as feces, the addition of antibiotics such as imipenem, vancomycin, and amphotericin B to the media should be considered.[54]

EPIDEMIOLOGY

S. maltophilia is a free-living, ubiquitous organism, the natural habitats for which include water, soil, and plants. The ability to survive in an aqueous milieu has allowed *S. maltophilia* to occupy a niche in the hospital environment. It has been cultured from dialysis fluids,[9] ventilators and other respiratory equipment,[24,55,81] and preoperative surgical brushes.[74] Several reports have linked nosocomial outbreaks of *S. maltophilia* to contamination of hospital water sources such as faucet aerators,[96] taps, and sinks,[55] as well as disinfectant solutions.[99] Intestinal colonization with *S. maltophilia* has been described in hospitalized oncology patients with diarrhea and could represent a potential source of nosocomial infection.[4] Other reports have described cases of nosocomial cross-infection occurring in neonatal and pediatric intensive care units.[36,44,59] In addition, cases of "pseudoinfection" caused by contamination of blood collection tubes have been described.[84]

Infection with *S. maltophilia* generally is hospital acquired.[37,83] Predisposing factors associated with colonization and infection by *S. maltophilia* include the presence of a severe, debilitating underlying illness, particularly malignancy; immune suppression (including human immunodeficiency virus infection); exposure to

broad-spectrum antibiotics (particularly to carbapenems, quinolones, or broad-spectrum cephalosporins); prolonged exposure to antibiotics; the presence of a central venous catheter; neutropenia; severe mucositis; prolonged hospital stay; stay in an intensive care unit; tracheostomy; mechanical ventilation; chronic obstructive pulmonary disease; or a combination of these factors.[5,12,13,21,30,65,72,73,89,94] Among children, predisposing factors are similar to those found in adults.[83] *S. maltophilia* is being isolated from clinical samples with increasing frequency.[5,29,56,64,68,83] This increase probably is related to advances in medical care that allow for a rise in the survival rate of severely ill patients, who are at highest risk for acquiring infection with this organism and who require more frequent use of invasive medical equipment, including indwelling lines, as well as broad-spectrum antimicrobials to which the organism is not susceptible.

PATHOPHYSIOLOGY

Like many pseudomonads, *S. maltophilia* is an organism with low virulence and limited invasiveness. An intact host immune system is an important deterrent to acquisition of a severe and even life-threatening infection; septicemia and death occur in patients with underlying debilitating illnesses. *S. maltophilia* elaborates a wide range of extracellular enzymes, including DNase, RNase, fibrinolysin, lipase, hyaluronidase, protease, and elastase, which may play a role in the pathogenesis of disease processes associated with *S. maltophilia*.[23] The pathogenesis of *S. maltophilia* infection also may be related to the development of cytotoxic activity.[33] In addition, *S. maltophilia* has been found to adhere to plastic materials, including intravenous catheters, and to produce biofilm, properties that may account for the relatively high incidence of catheter-related bloodstream infections caused by this organism.[20,23,31] *S. maltophilia* is inherently resistant to several classes of antibiotics. Colonization with *S. maltophilia*, especially in the respiratory tract, is not an uncommon finding. In debilitated patients, exposure to broad-spectrum antimicrobials, many of which are ineffective against this bacterium, may allow overgrowth of colonizing organisms that subsequently gain access to sterile body sites and cause infection. Portals of infection include indwelling devices such as central venous catheters, peritoneal dialysis or urinary tract catheters, the respiratory tract, and the gastrointestinal tract.

CLINICAL MANIFESTATIONS

S. maltophilia once was regarded as a microorganism with very limited pathogenicity that was unlikely to cause infection except in the most debilitated patients.[37] However, not only the incidence but also the severity of infection and the spectrum of clinical manifestations caused by this bacterium have increased. The most common site of isolation of *S. maltophilia* is the respiratory tract, particularly in hospitalized, mechanically ventilated patients. Attributing a causative role to *S. maltophilia* occasionally is difficult when the organism is isolated only from respiratory secretions, particularly as part of a mixed culture. Most critically ill, mechanically ventilated patients have abnormal chest radiographs, and differentiating infectious from noninfectious infiltrates may be very difficult. Nonetheless, *S. maltophilia* has unequivocally been associated with pneumonia, which may be severe and which may occur in patients who are not receiving mechanical ventilation.[1,34,39] Occasionally, the pathogen may be associated with massive, fatal pulmonary hemorrhage in adult patients with malignant disease,[28] and it is a cause of ventilator-associated pneumonia.[62] In adults with pneumonia, the development of secondary bacteremia signals a grave prognosis.[29]

S. maltophilia bloodstream infections usually are catheter-associated.[10,55,57,58,69,93] In a series of 32 episodes of bacteremia in children, all were related to the presence of a central venous catheter, although in 10 episodes, the primary source was not the catheter.[83] In a series of 217 episodes of *S. maltophilia* bloodstream infections occurring in patients with cancer who had central venous catheters, secondary bacteremia generally occurred in patients who were neutropenic, had concurrent pneumonia, or were critically ill, and it was associated with a worse outcome compared with patients who had central venous catheter–related bacteremia.[10] Frequently, *S. maltophilia* is isolated as part of a polymicrobial bloodstream infection, particularly when the central venous catheter is the origin of the infection. In children, the severity of illness appears to be similar regardless of whether *S. maltophilia* is isolated in a monomicrobial or mixed blood culture.[83] Malignant disease is the most common underlying illness in patients with bacteremia, and many patients have concomitant neutropenia. Bloodstream infections caused by *S. maltophilia* can be severe. In a pediatric series, 31 percent of the patients initially were seen in septic shock, and the attributable mortality rate was 6.3 percent.[83] In another pediatric series of 32 episodes of bacteremia occurring in 31 children, the crude mortality rate was 40.6 percent.[100] However, the death rate associated with *S. maltophilia* bloodstream infection is, in general, higher in adults.[55,56,66,69]

S. maltophilia endocarditis is a rare occurrence. It has been associated with intravenous drug abuse and frequently occurs after replacement of a prosthetic valve.[6,46,70,101] Surgical therapy often is required, although cure with medical treatment alone has been reported. No cases of endocarditis caused by this organism in children have been reported.

Urinary tract infections caused by *S. maltophilia* almost invariably occur in patients with structural abnormalities of the urinary tract, indwelling urinary catheters, or underlying illnesses. Infection may be severe and associated with sepsis and septic shock.[91]

Skin and soft tissue infections caused by *S. maltophilia* have occurred in patients who have had work-related injuries and wounds contaminated with soil and plant material, such as occur in injuries associated with use of lawn mowers.[18,27,41] In the hospital setting, *S. maltophilia* frequently is cultured from wounds and surgical sites, although determining the clinical significance of this organism in children, particularly when it is isolated in mixed culture, often is difficult.[83] In adult patients with cancer, tender, erythematous nodular skin lesions have been described in association with *S. maltophilia* bacteremia and probably represent metastatic infectious foci.[92] In 2006, the first reported cases of metastatic soft tissue involvement, including nodular skin lesions and pyomyositis, in pediatric patients with bacteremia were described.[87,100] All these patients had severe underlying diseases, particularly hematologic malignancies, associated with neutropenia.

S. maltophilia has been implicated as a cause of conjunctivitis and keratitis in the setting of ocular surface compromise resulting from trauma, the use of soft contact lenses, or previous infection with human herpes simplex virus.[79] Endophthalmitis occurring after ophthalmologic surgical interventions also has been described.[15,48,51]

Meningitis caused by *S. maltophilia* is an extremely rare occurrence and usually is nosocomial and associated with neurosurgical procedures and infection of intraventricular devices.[78] However, spontaneous meningitis in four infants was reported.[61,71,82]

S. maltophilia is the fourth most common organism isolated from the bronchial secretions of patients with cystic fibrosis, after *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Haemophilus influenzae*.[88] The incidence and prevalence of *S. maltophilia* isolated from the respiratory secretions of patients with cystic fibrosis are increasing,[53,86] although they are not uniform across all

cystic fibrosis centers.[21] *S. maltophilia* is recovered from the sputum of an estimated 10 percent of patients with cystic fibrosis in the Untied States and from as many as 25 percent of such patients in Europe.[95] The origin of the bacterium is uncertain; evidence suggests that it may be acquired both in the hospital and in the community.[21] Colonization may be transient or persistent, with chronic colonization occurring more frequently in older patients.[88] Case-control studies identified greater exposure to antipseudomonal antibiotics, oral ciprofloxacin, inhaled aminoglycosides, and oral corticosteroids, as well as more hospitalization days, and isolation of *Aspergillus fumigatus* from the sputum as risk factors for colonization with *S. maltophilia*.[26,63,86] Unlike with *Burkholderia cepacia* infection, no evidence of patient-to-patient transmission of *S. maltophilia* has been found, nor does the organism appear to be associated with rapid deterioration of pulmonary function in patients with cystic fibrosis.[25,42] Cases of *S. maltophilia* bacteremia have not been reported in patients with cystic fibrosis, with the exception of one patient who died of sepsis caused by this bacterium on the first day after undergoing lung transplantation.[52] The clinical significance of isolation of *S. maltophilia* from the sputum of patients with cystic fibrosis and its role in the deterioration in lung function is uncertain. Its presence may represent more a marker of severe, advanced disease than causally related respiratory deterioration.[22,43]

Other infections caused by *S. maltophilia* include peritoneal catheter exit-site infections and peritonitis in patients undergoing peritoneal dialysis,[19,85] as well as cholangitis,[76] osteochondritis,[7] mastoiditis,[47] bursitis,[77] sinusitis,[45] liver abscess,[80] osteomyelitis,[58] and necrotizing ulcerative gingivitis.[67]

DIAGNOSIS

The diagnosis of infection is established by isolating the organism from normally sterile sites in the presence of a compatible clinical picture. However, growth of *S. maltophilia* from normally sterile samples actually may represent an episode of pseudoinfection caused by the ability of this organism to contaminate and survive in hospital equipment such as blood collection tubes and antiseptic solutions. Because *S. maltophilia* is a common colonizer of hospitalized patients, isolation from nonsterile sites such as sputum or wounds is more difficult to interpret, particularly when the organism is isolated in mixed culture. Because nosocomial outbreaks have been reported, isolation of *S. maltophilia* from other patients in a hospital setting should alert the physician to the possibility of *Stenotrophomonas* infection. Infection control offices should be notified after the organism has been isolated.

Occasionally, *B. cepacia* is misidentified as *S. maltophilia*, especially in patients with cystic fibrosis.[11] *S. maltophilia* is oxidase-negative and DNase-positive. These tests should be repeated when identification of the organism is in doubt. Because of the serious clinical implications of misidentification in this group of patients, molecular analysis of the isolates should be considered if results remain uncertain.[98]

TREATMENT

Providing antibiotic therapy for *S. maltophilia* is difficult for several reasons. This organism displays multiple resistance mechanisms that confer broad antibiotic resistance, including resistance to β-lactam antibiotic agents. Mechanisms of resistance include the production of two chromosomally encoded, inducible β-lactamases designated L1 and L2. The former is a β-lactamase inhibitor–resistant metalloenzyme that hydrolyzes a broad range of β-lactam antibiotics, including carbapenems such as imipenem and meropenem. L2 is a cephalosporinase and, unlike L1, is susceptible to β-lactamase inhibitors.[23] Other mechanisms of resistance include reduced antibiotic uptake, the main mechanism providing aminoglycoside resistance, and an antibiotic efflux system that confers multidrug resistance.[3,103] In addition, in vitro antibiotic susceptibility testing is plagued by numerous methodologic problems. Several factors, including the time of incubation and the composition of the medium, affect the interpretation of test results. Furthermore, poor reproducibility among different testing methods has been described.[14] The Clinical and Laboratory Standards Institute (CLSI, formerly the National Committee for Clinical Laboratory Standards) has established standards for antibiotic susceptibility testing, including testing by both disk diffusion and broth or agar dilution (minimum inhibitory concentration [MIC] evaluation).[17] For the disk diffusion method, the CLSI recommends testing only trimethoprim-sulfamethoxazole, levofloxacin, and minocycline. In the case of broth or agar dilution, the CLSI recommends testing trimethoprim-sulfamethoxazole (susceptible: 2/38 μg/mL; resistant: 4/76 μg/mL), ceftazidime (susceptible: 8 μg/mL; intermediate: 16 μg/mL; resistant: 32 μg/mL), chloramphenicol (susceptible: 8 μg/mL; intermediate: 16 μg/mL; resistant: 32 μg/mL), levofloxacin (susceptible: 2 μg/mL; intermediate: 4 μg/mL; resistant: 8 μg/mL), minocycline (susceptible: 4 μg/mL; intermediate: 8 μg/mL; resistant: 16 μg/mL), and ticarcillin-clavulanate (susceptible: 16/2 μg/mL; intermediate: 32/2-64/2 μg/mL; resistant: 128/2 μg/mL). However, no controlled clinical studies have determined the most effective antibiotic regimen or duration of treatment.

Antibiotic susceptibility studies and clinical observation suggest that the most active antibiotic against *S. maltophilia* is trimethoprim-sulfamethoxazole, and most authorities agree that it is the drug of choice. It is bacteriostatic, however, a property that may have clinical implications in immunosuppressed patients. In addition, resistant strains are being identified with increasing frequency.[2,66,90] Other antibiotics that have shown good in vitro activity include ticarcillin-clavulanate, doxycycline, minocycline, and tigecycline, although clinical experience with the latter three drugs is limited.[23,49] The activity of early fluoroquinolones such as ciprofloxacin and ofloxacin varies widely, and emergence of resistance during treatment has been reported.[16] Newer quinolones, including levofloxacin and gatifloxacin, appear to have better activity in vitro against *S. maltophilia*,[32,50,60,97] but their activity decreases against multiresistant strains, and the development of resistance during the course of treatment has been described.[8,58] In addition, geographic variations in antibiotic resistance rates have been reported.[35]

Bloodstream infections can be severe, particularly in immunosuppressed patients. Combination antibiotic therapy with trimethoprim-sulfamethoxazole and ticarcillin-clavulanate for bloodstream infections by isolates that are susceptible has been advocated by some authorities and is supported by in vitro pharmacodynamic model data.[69,90,102] Other combinations have shown varying efficacy and correlation between in vitro and in vivo findings, which have not always been consistent.[23] In cases of catheter-related bloodstream infection, removal of the catheter has been associated with improved outcome, irrespective of the appropriateness of antibiotic therapy.[29,83] Nonetheless, successful treatment without removal of the catheter,[69] as well as an association of inappropriate antibiotic therapy and death,[66] has been described. In cases of serious infection such as bacteremia or severe pneumonia, combination antibiotic therapy should be considered when in vitro and in vivo evidence suggest that rapid emergence of resistance may occur during treatment.[38]

Appropriate management of patients from whom *S. maltophilia* is isolated from nonsterile sites, particularly in mixed culture, is not always clear because some isolates may represent colonization or contamination of the sample. In a pediatric study describing nonrespiratory infections in children, 6 of 16 patients with *S. maltophilia* that was cultured from sites other than the

bloodstream were treated with antibiotics not active in vitro, and 5 of these children were "cured."[83] Nonetheless, in adults, *S. maltophilia* has been associated unquestionably with severe infection at sites other than the bloodstream, and death caused by *S. maltophilia* infection has been associated with inappropriate initial antibiotic therapy.[72]

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SUBSECTION 5

Gram-Negative Coccobacilli

CHAPTER 137

ACTINOBACILLUS ACTINOMYCETEMCOMITANS

Suzanne Whitworth ❂ Richard F. Jacobs

Actinobacillus actinomycetemcomitans is a fastidious, non–spore-forming, nonmotile, facultatively anaerobic gram-negative rod that frequently complicates actinomycosis caused by *Actinomyces israelii*. In addition to being associated with actinomycosis, it has been implicated as a pathogen in periodontal disease and is part of the oral flora. Infection by this bacterium often is not resolved by the normal host, possibly because of inefficient phagocytosis and a weak oxidative burst response of neutrophils.[7] In addition, the bacterium has several virulence factors, including induction of apoptosis and tissue destruction.[3] This organism is characterized by slow growth in culture and a requirement for incubation in an atmosphere enhanced with carbon dioxide.[4] Other bacterial species concomitantly isolated in human actinomycosis are *Eikenella corrodens*, *Fusobacterium*, *Bacteroides*, *Capnocytophaga*, *Staphylococcus*, *Streptococcus*, and members of Enterobacteriaceae.

*A. actinomycetemcomitan*s is a pathogen in at least 30 percent of actinomycotic infections[4] (see Chapter 161). Failure to recognize this organism and to treat it adequately has resulted in clinical relapse and deterioration in patients infected with actinomycosis.[5,13] Severe forms of periodontitis, particularly localized aggressive periodontitis, also are associated with this pathogen, and studies have shown that this organism is related strongly to children in the 10- to 19-year age group.[9] This type of periodontitis is characterized by rapid loss of attachment and bone around the permanent incisors and permanent molars. Treatment consists of local débridement and antibiotic therapy, usually metronidazole alone or in combination with amoxicillin. *A. actinomycetemcomitan*s also is an important pathogen in Papillon-Lefèvre syndrome, an autosomal recessive disorder characterized by prepubertal periodontitis and palmar-plantar hyperkeratosis.[8] Additionally, it is one of the HACEK (HACEK also includes *Haemophilus aphrophilus*, *Cardiobacterium hominis*, *E. corrodens*, and *Kingella kingae*) organisms that have a propensity for infecting heart valves. The endocarditis caused by this organism usually is insidious, with fever occurring in fewer than 50 percent of cases.[4] This organism also has been reported to cause pericarditis, meningitis, brain abscess, parotitis, synovitis, osteomyelitis, urinary tract infection, pneumonia, and empyema.[4] Cases of endophthalmitis[1,11] and cavernous sinus infection also have been reported.[12]

A. actinomycetemcomitans can be cultured on blood and chocolate agar, but it grows poorly on MacConkey agar. Cultures require incubation in an enhanced carbon dioxide atmosphere. Growth of the organism in a blood culture may take as long as 9 days in patients with endocarditis, and thus cultures should be held longer. On Gram stain, the organism appears coccoid to coccobacillary. Molecular techniques based on non-amplification nucleic acid probes or on polymerase chain reaction can provide rapid and accurate identification of *A. actinomycetemcomitans*.[10] Because of the frequency of co-infection with this organism in cases of actinomycosis, attempts always should be made to isolate this organism in these patients.

A. actinomycetemcomitans is susceptible to the newer cephalosporins, rifampin, trimethoprim-sulfamethoxazole, aminoglycosides, quinolones, tetracycline, azithromycin, and chloramphenicol. It is susceptible also to penicillin and ampicillin in vitro, but test results do not necessarily correlate with clinical outcome. Vancomycin, erythromycin, and clindamycin have very little activity against this organism. Treatment of aggressive periodontal disease consists of local débridement and antibiotic therapy with metronidazole and amoxicillin, which appear to be effective at suppressing *A. actinomycetemcomitans* to less than the level of detection.[2] Endocarditis caused by this organism has been treated successfully with a combination of ampicillin and gentamicin.[6] Cefotaxime or ceftriaxone is also acceptable.

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CHAPTER 138

Bartonellosis

Barbara W. Stechenberg

Bartonellosis is a term that has been used to describe a geographically distinct disease caused by *Bartonella bacilliformis*. Recent advances in molecular biology have led to the reclassification of several bacterial pathogens, and the family Bartonellaceae, genus *Bartonella*, has expanded from a single species, *B. bacilliformis*, to include 16 validated species, 8 of which have been known to be pathogenic in humans. Of the members of the genus *Rochalimaea* that have been reclassified as *Bartonella* spp., *Bartonella henselae* is the cause of cat-scratch disease (see Chapter 151). Manifestations of *Bartonella* infection other than classic bartonellosis seldom occur in children and are discussed briefly.

BARTONELLOSIS (CARRIÓN DISEASE)

Bartonellosis is a disease unusual in its manifestations and rich in its history. The organism, *B. bacilliformis*, causes two illnesses that are both clinically and temporally distinctive. Besides producing subclinical asymptomatic infection, this organism can cause Oroya fever, a disease characterized by severe febrile hemolytic anemia, or verruga peruana, an eruption of hemangioma-like lesions. The eponym *Carrión disease* is used to designate the two forms collectively. The disease is restricted in its distribution to an area in South America that includes parts of Peru, Ecuador, and Colombia.

The origin of this disease in the history of the region probably considerably precedes the first written documentation of the disorder. The first written account of bartonellosis is attributed to Gago de Vadillo, who published a treatise on the subject in 1630, a century after the arrival of the first Spaniards. In 1764, Cosme Bueno first described the vector of this disease and of cutaneous leishmaniasis as the uta or sandfly.[13] The era of the mid-1800s was a period of increasing wealth in Peru because of a new industry, the mining of guano, or bird manure. With this increase came the building of a railroad from Callao to Oroya and nearly 10,000 workers from Chile, Bolivia, and China, none of whom had previous contact with or immunity to Carrión disease.[17] An epidemic that took the lives of hundreds of workers ensued. A cavalry unit of black soldiers sent to round up deserters quickly fell ill with the disease. A physician caring for them was so impressed with the rapidity and the profound anemia of the disease that he said, "It turned the blacks to whites," a remark henceforth frequently associated with the disease.[30]

In 1885, a Peruvian medical student (Carrión) was collecting data on the geographic distribution and clinical features of verruga peruana. Because of concern about the difficulty in diagnosing the pre-eruption period of verruga, he inoculated himself with material taken from a patient with verruga. He experienced his first symptoms 21 days after inoculation and then went on to exhibit the classic signs and symptoms of Oroya fever. Carrión realized the significance of his experiment 3 days before he died; he had proved the unitary origin of the two illnesses. In 1905, Alberto Barton, a Peruvian physician, described the etiologic agent *(B. bacilliformis)*, but several years passed before this organism was accepted as the cause of Oroya fever and was named in his honor.

THE ORGANISM

B. bacilliformis is small, 0.2 to 1.0 μm wide by 0.3 to 2.0 μm long. It stains easily with Giemsa (purple) and is a gram-negative and motile organism, with a brush of 10 or more unipolar flagella. In tissues, the organism stains black with silver-impregnated stains, such as Warthin-Starry stain. On electron microscopy, the contrast between *B. bacilliformis* and other members of the genus *Bartonella* is striking. Cultured *B. bacilliformis* organisms show the retracted cytoplasm and cell walls typical of bacteria.[22] They are rod shaped in young culture and become mostly coccoid in older culture. These organisms are obligate aerobes that grow best at 28° C in semisolid nutrient agar with 10 percent rabbit serum and 0.5 percent rabbit hemoglobin. Growth is subsurface, usually occurring in 7 to 10 days. The organism is pathogenic only for human beings and other primates, and only one antigenic type exists. Analysis of 16S rRNA sequences shows that *B. bacilliformis* is in the alpha$_2$ subgroup of proteobacteria and that its closest relatives are *Bartonella quintana* and *Brucella abortus*.[5]

EPIDEMIOLOGY

The distribution of the disease historically has been restricted to the mountain valleys of the Andes Mountains in Peru, Ecuador, and Colombia. Within these regions, the disease usually is seen only between the altitudes of 500 and 3200 m above sea level and primarily in valleys that are at right angles to the prevailing wind. This interesting geographic distribution reflects the habits of *Lutzomyia verrucarum*, the sandfly vector, which is seen only at these altitudes. One usually acquires the disease at twilight or soon thereafter because of the feeding habits of the insects. Within the region, the disease is endemic, with sporadic epidemic outbreaks that continue to occur.[12] Recent outbreaks have occurred in nonendemic populations in the surrounding area.[16] Several isolated reports of anemia with *Bartonella*-like organisms have been reported: three cases from Thailand in 1966 and one case from the Sudan in 1969.

PATHOPHYSIOLOGY

After inoculation by the sandfly, *Bartonella* organisms enter the endothelial cells of the blood vessels, where they proliferate during the incubation period. Microscopically, masses of organisms may be noted within the cytoplasm of the cells lining the blood vessels and lymph channels, and their numbers cause them to bulge into the lumen of the vessel. The organisms may be found within reticuloendothelial cells, particularly in the lymph nodes, but also in the liver, spleen, bone marrow, kidneys, adrenals, pancreas, and, more rarely, the skin, heart, and lungs.[11]

The organisms then re-enter the bloodstream and parasitize erythrocytes (red blood cells [RBCs]). Binding of *B. bacilliformis* to RBCs causes indentations and deformation of the membrane; membrane fusion is induced, and the organisms then enter the intracellular vacuoles, where they replicate.[4] The resulting anemia is caused primarily by destruction of these parasitized cells. Because as many as 90 percent of cells may be infected, profound, rapid anemia is a common symptom; the life span of infected RBCs is markedly shortened, particularly in the first few days. All parasitized cells are not destroyed, and no hemolysins or agglutinins have been recovered.[27] *B. bacilliformis* can be demonstrated easily with Giemsa stain. In earlier studies, considerable controversy ensued regarding whether the parasites were within or on the surface of RBCs; Cuadra and Takano[9] showed that they are located predominantly within the cells. In the recovery phase

of the anemia, the rod-shaped organisms change to a more coccoid form and rapidly disappear from the blood.

A patient who survives the acute phase of Oroya fever may or may not experience cutaneous manifestations of the disease, which appear as nodular, hemangiomatous lesions ranging in size from a few millimeters to several centimeters. Light microscopy reveals angioblastic and histiocytic hyperplasia of the dermis. Numerous newly formed small vessels with endothelial cell proliferation are found. Mast cells, lymphocytes, and macrophages are present.[1] Electron microscopy demonstrates that the bacterial organisms are located in the verruga, extracellularly in the fine fibrous interstitium. Two types of histiocytic cells are found in the verruga: a more numerous one, which is clear and has many lysosomes, ribosomes, mitochondria, and cytoplasm, and a darker one, with numerous lamellar membranous structures in the cytoplasm.[26] Studies have substantiated the presence of activity in *B. bacilliformis* that stimulates endothelial cells in vitro and is angiogenic in vivo. This finding may explain the similar pathogenesis of verruca and bacillary angiomatosis produced by other *Bartonella* spp.[1]

CLINICAL MANIFESTATIONS

The incubation period varies from 2 to 14 weeks, with a mean of 3 weeks. The difficulty in determining the duration of the incubation period results from the variable symptoms of the disease. Some patients are totally asymptomatic, and disease is detected only by blood culture or on serologic survey.[16] In a population-based cohort study, 0.5 percent of participants had asymptomatic bacteremia.[7] Other patients are not anemic, but symptoms such as headache, malaise, and occasional fever develop, and *B. bacilliformis* is recovered from blood cultures. Still others have severe anemia (Oroya fever). Patients with hemolytic anemia are febrile, and organisms may parasitize the RBCs. The anemia develops rapidly. Patients are deeply apathetic and have a peculiar discoloration of their skin and sclerae secondary to the combination of slight icterus and severe anemia.[28] Tachycardia and soft hemic murmurs are noted; occasionally, peripheral vascular collapse occurs. Headache, vertigo, restlessness, tinnitus, and occasionally angina pectoris may be present. Clouding of the sensorium and delirium are rather common findings; these effects usually are mild but may progress to overt psychosis. The temperature usually fluctuates between 37.5° C and 38.5° C (99.5° F and 101.3° F); higher elevations in temperature may be caused by intercurrent infection. Physical examination discloses generalized lymphadenopathy and nonpainful hepatomegaly.[18]

The anemia is macrocytic and usually hypochromic, with anisocytosis and poikilocytosis. The RBC count may drop to as low as 500,000/mm^3 in the first 2 to 4 weeks of illness. Reticulocytes may increase to 50 percent. The pathognomonic sign of the disease is the presence of *B. bacilliformis* within Giemsa-stained RBCs as red-violet rods. The leukocyte count may be normal, low, or elevated.

The "critical stage" of the anemia is the period of transition when the organism suddenly disappears from the RBCs.[28] During this time, *Bartonella* organisms change from the rod shape to more coccoid forms, the number of parasitized RBCs decreases, and the degree of anemia decreases; accordingly, the RBC count increases, and less hyperbilirubinemia is present. Clinically, fever decreases, and the patient stabilizes.[28] In some cases, the illness may become more severe, a finding that suggests the development of intercurrent infection (usually with *Salmonella*). Although this complication may occur at any time, it does so most commonly during the transitional period and may be noted in as many as 40 percent of patients.[8]

In the pre-eruptive stage, patients may complain of pain in their joints, bones, and muscles, as well as cramps and paresthesias. Inflammatory reactions such as phlebitis, parotitis, pleuritis, erythema nodosum, and encephalitis may occur. The anemia and lymphadenopathy of the invasive stage disappear.

The appearance of red cutaneous nodules, or verruga, is pathognomonic of the disease in the eruptive stage. Usually, these lesions are present in the skin, but they may be found in mesenchymatous tissue. They vary greatly in number and size, from small nodules to disfiguring zonular (hemangioma-like) lesions. They rarely cause symptoms; however, larger lesions may require surgical excision. This stage may last from several months to a year and may be the sole manifestation of the disease, particularly in school-aged children in endemic areas.

DIAGNOSIS

The diagnosis is based on clinical manifestations, in conjunction with a Giemsa-stained blood smear showing typical organisms, or on blood cultures. In the pre-anemic stage or in patients without the typical anemia who reside in an endemic area, the diagnosis can be based on blood culture alone. The presence of typical verruga in patients from an endemic area is pathognomonic of the disease. Immunoglobulin M antibody may be present in both stages of the disease, as well as in some healthy persons.[12,14] Persons with typical Oroya fever who are treated with antibiotics may not have an antibody response.[12,14] More recently, an indirect fluorescent antibody assay has shown promise for evaluating patients in both the acute and convalescent phases of the disease.[6] The differential diagnosis in the initial phase includes typhoid fever, malaria, tuberculosis, leptospirosis, brucellosis, and meningitis, as well as hematologic malignant disease and aplastic or hemolytic anemia. The eruptive phase may resemble hemangiomata, bacillary angiomatosis, Kaposi sarcoma, and other nodular diseases.

TREATMENT

B. bacilliformis is sensitive to many antibiotics, including penicillin, tetracycline, streptomycin, and chloramphenicol. With treatment, the fever usually abates by 24 hours; the rod-shaped organisms change to more coccoid forms and soon disappear from the blood.

The choice of antibiotic may be guided by considerations other than simple eradication of *B. bacilliformis*, including the risk of developing intercurrent infection. Chloramphenicol is considered the drug of choice because it also is useful in the treatment of salmonellosis.[33] Occasional patients need the addition of another antibiotic, usually a β-lactam.[29] Blood transfusions may be helpful during the period of severe anemia, especially if blood is obtained from patients who have recently recovered from the disease.[27]

Treatment of verruga peruana usually is not necessary unless particularly large zonular lesions interfere with function; in these persons, surgery may be necessary. Treatment is considered when patients have more than 10 cutaneous lesions, if the lesions are particularly erythematous or violaceous, or if the onset of lesions was less than 1 month before presentation.[18] Oral rifampin, ciprofloxacin (not approved for children), or tetracycline may be used to aid in healing of the cutaneous lesions.[17,29]

PROGNOSIS

The mortality rate in untreated bartonellosis was estimated in the past at approximately 40 percent. However, the more recent fatality rate has been approximately 9 percent in patients admitted to the hospital.[17] Intercurrent *Salmonella* infection increases

the mortality rate. In one population-based study, the fatality rate was 0.7 percent.[14,16] With the use of chloramphenicol, the prognosis is much improved. Permanent immunity develops in most patients.

PREVENTION

Dichlorodiphenyltrichloroethane (DDT) has been effective in controlling the disease by eliminating the vector *Lutzomyia verrucarum*. Persons can protect themselves by leaving endemic areas at night and using insect repellents. No vaccine of demonstrable efficacy has been developed.

TRENCH FEVER (*BARTONELLA QUINTANA*)

Trench fever was described first in Russia and was recognized as causing a severe epidemic during World War I, with more than a million troops infected.[3] The human body louse, *Pediculus humanus* variety *corporis*, is the vector, and humans are the only known reservoir.

The incubation period is extremely variable (from 4 to 35 days, with an average of 22 days). Symptoms also are highly variable. Four major fever patterns have been described: (1) a single febrile episode; (2) a single febrile period lasting 4 to 5 days; (3) three to eight recurrent febrile episodes, each lasting 4 to 5 days (sometimes for a year or more); and (4) persistent fever lasting 2 to 6 weeks.[10,31] Associated signs and symptoms may include conjunctival injection, retro-orbital pain, myalgias, arthralgias, headache, bone pain (especially in the shins), and splenomegaly. Chronic bacteremia following clinical improvement is common.[10]

In a non-epidemic situation, establishing the diagnosis of trench fever is impossible because the manifestations are not distinctive. The relapsing form can mimic malaria or *Borrelia recurrentis* relapsing fever. A history of body louse infestation or association with an epidemic should heighten suspicion. *B. quintana* can be cultured from blood by using a modification that includes culturing on epithelial cells. Serologic testing is available; however, cross-reactions with *B. henselae* occur. No controlled trials of treatment have been performed, but dramatic defervescence has been noted with the use of tetracycline and chloramphenicol.[15,31]

BACILLARY ANGIOMATOSIS AND BACILLARY PELIOSIS HEPATIS

Both *B. henselae* and *B. quintana* can cause disease in immunocompromised persons, primarily adults with acquired immunodeficiency syndrome (AIDS) or cancer or recipients of organ transplants. Lesions of bacillary angiomatosis are the most easily recognized form of *Bartonella* infection in immunocompromised patients. They are seen predominantly in patients with AIDS and with very low CD4 counts.[13,15] The vasoproliferative lesions may be cutaneous or subcutaneous and pathologically resemble the verruga of *B. bacilliformis*. Most characteristically, these lesions are red with a collarette of scale, but the clinical findings can be diverse. The differential diagnosis includes Kaposi sarcoma, pyogenic granuloma, and verruga peruana, and deep soft tissue masses may develop. Trauma may result in ulceration or bleeding. Osseous lesions occur in the long bones and can be very painful. A 12-year-old child with lymphocytic leukemia and bacillary angiomatosis has been reported.[19]

Bacillary peliosis hepatis was described first in 1990.[21] It is seen primarily in patients infected with human immunodeficiency virus (HIV) who have fever and abdominal pain. Vascular proliferative lesions develop, primarily in the liver and spleen. The differential diagnosis includes hepatic Kaposi sarcoma, lymphoma, extrapulmonary pneumocystosis, and infection with *Mycobacterium avium-intracellulare*. Both bacillary angiomatosis and bacillary peliosis hepatis have been treated successfully with antimicrobial therapy, including erythromycin, newer macrolides such as azithromycin or clarithromycin, or, as an alternative, doxycycline.[15,21,31]

ENDOCARDITIS

B. henselae, *B. quintana*, and *Bartonella elizabethae* all have been reported to cause bacteremia or endocarditis. The symptoms and signs are similar to those of other causes of endocarditis, although prolonged fever, night sweats, and profound weight loss may occur.[32] Many of the immunocompetent patients reported have been homeless. Cases also have been described in immunocompromised persons, particularly HIV-infected patients. *Bartonella* organisms may be a significant cause of culture-negative endocarditis.[2,23,24,32] Two children have been reported with central nervous system infection associated with *B. quintana*.[20]

Special culturing techniques, including the use of Isolator tubes, and prolonged incubation, as well as serology, histopathology of valvular tissue, and polymerase chain reaction, may be helpful.[34] Initial treatment of culture-negative endocarditis with ceftriaxone and gentamicin is effective for *Bartonella* organisms. A retrospective analysis of 101 patients with endocarditis[25] showed a benefit with the use of an aminoglycoside as part of a treatment regimen for a minimum of 14 days. If *Bartonella* is proven, doxycycline with gentamicin are recommended.

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CHAPTER 139

Brucellosis

Edward J. Young

Brucellosis is a disease of animals (zoonosis) that is transmittable to humans.[76] Humans are accidental hosts, and human-to-human transmission rarely occurs. Brucellosis is distributed worldwide, and an estimated 500,000 human cases occur annually.[78] Programs to eradicate the disease in animals have reduced the incidence of human infection in many countries; however, brucellosis remains enzootic in large parts of the world.[117] Although brucellosis was once considered a rare occurrence in children, it now is recognized that persons of all ages are susceptible, especially in areas where *Brucella melitensis* is endemic.[38]

HISTORY

Brucellosis was one of many indistinguishable fevers until 1859, when J. A. Marston, a Royal Army Medical Corps physician, provided the first accurate description of the disease among troops stationed on Malta during the Crimean War.[62] During the 19th century, brucellosis was known by various names, including Malta fever, Mediterranean fever, and undulant fever.[72] Although the disease caused considerable morbidity and mortality in British military personnel stationed throughout the Mediterranean, the cause was not immediately apparent. In 1886, David Bruce,[16] another Royal Army Medical Corps surgeon, isolated a microorganism from spleen tissue of victims of Malta fever. Termed *Micrococcus* (later renamed *Brucella*) *melitensis*, the organism could be found in the blood, urine, and feces of patients with Malta fever. Between 1904 and 1907, Bruce headed the Mediterranean Fever Commission, which studied aspects of the disease in Malta. Themistocles Zammit, a Maltese physician working with the Commission, first identified native goats as the reservoir of brucellosis and their unpasteurized milk as the vehicle of transmission to humans.[108] When fresh goat's milk was replaced with tinned condensed milk in the military mess, the incidence of brucellosis among military personnel declined precipitously.[97] In 1897, Almroth Wright applied the newly devised agglutination assay to the serologic diagnosis of Malta fever.[112]

Unlike *B. melitensis* that was initially isolated from human tissue, other *Brucella* spp. were recognized for the disease they caused in animals. In 1897, Bernhard Bang,[13] a Danish veterinarian, isolated the "abortion bacillus" (later, *Brucella abortus*) from placental tissue of cattle suffering contagious abortions. *Brucella suis* was isolated around 1914 by F. M. Hayes and J. Traum[45] at the Bureau of Animal Industry. The bacteriologist Alice Evans[36] finally recognized the relatedness of these disparate bacteria in 1918. After Evans' work was confirmed, K. F. Meyer and E. B. Shaw proposed the name *Brucella* for the genus to honor Bruce.[43]

Additional *Brucella* spp. were isolated from sheep (*Brucella ovis*) and from desert wood rats (*Brucella neotomae*), but to date they have not been shown to cause illness in humans. *Brucella canis* was isolated from kennel-bred dogs in 1968 by Carmichael and Brunner,[19] although it appears to be a rare cause of human brucellosis.[102,109] Since the late 1990s, at least two previously unrecognized species of *Brucella* have been isolated from marine mammals.[15,47] Tentatively named *Brucella cetaceae* (from cetaceans) and *Brucella pinnepediae* (from pinnepeds),[23] their role as human pathogens remains to be clarified.[63,91]

ETIOLOGY

The brucellae are small, fastidious, nonmotile, non–spore forming, gram-negative coccobacilli that lack native plasmids. The outer cell membrane resembles other gram-negative bacilli with a dominant lipopolysaccharide (LPS) component. Their metabolism is oxidative, and all strains are aerobic. Some strains require carbon dioxide for growth, especially for primary isolation. Brucellae are always catalase-positive, but oxidase activity varies among biovars. Most strains reduce nitrate to nitrite, but some do not. Production of hydrogen sulfide also varies, as does urease activity.[28] Various media, including chocolate, trypticase soy, and serum dextrose agars, support the growth of brucellae. Growth in vitro characteristically is slow, and when brucellosis is suspected, cultures should not be discarded before 28 days.

Brucellae belong to the alpha-2 subdivision of the Proteobacteria phylogenetically related to plant pathogens and symbionts, such as *Agrobacterium* and *Rhizobium*, and to intracellular animal parasites, such as *Bartonella* and *Rickettsia*.[22] On the basis of DNA-DNA hybridization and genome sequencing, the genus *Brucella* comprises a single species.[31,44,79] Despite genetic homology greater than 90 percent, the *Brucella* nomen species show a remarkable preference for certain natural hosts. Consequently, the traditional nomen species classification is retained for epidemiologic purposes (Table 139-1).

TABLE 139–1 Nomen-Species Classification of *Brucella* Species

Species	Biovars	Natural Hosts	Pathogenicity for Humans
B. abortus	1-6, 9	Cattle, other Bovidae	Yes
B. melitensis	1-3	Goats, sheep	Yes
B. suis	1-3	Swine	Yes
	4	Reindeer, caribou	Yes
	5	Rodents	Yes
B. canis	—	Dogs, other canines	Yes
B. neotomae	—	Desert wood rats	No
B. ovis	—	Sheep	No
*B. maris**			
*B. pinnipediae**		Pinnipeds	?
*B. cetaceae**		Cetaceans	?

**Tentative designations.*
?Still unresolved.

EPIDEMIOLOGY

Historically in the United States, brucellosis was linked to the livestock industry, and *B. abortus* was the predominant species causing human illness. Farmers, ranchers, meat inspectors, and veterinarians were at highest risk of acquiring infection, usually through direct contact with infected animals. With the virtual elimination of bovine brucellosis by way of test-and-slaughter practices and immunization of susceptible cattle, the epidemiology of brucellosis changed, especially in states that border Mexico. *Brucella melitensis* replaced *B. abortus* as the major cause of human disease, and foodborne transmission replaced direct contact with animals as the primary mode of infection.[20,105] On the Mexican border, human brucellosis occurs at a rate eight times higher than the national average in the United States,[33] and unpasteurized goat's milk cheese is a common vehicle of transmission.[104,121] Human infection caused by *B. suis* traditionally has been an abattoir-associated infection,[18] but in recent years, the hunting of feral swine has become a risk when hunters are contaminated with the blood of infected animals.[99] Personnel working in clinical or research laboratories also are at risk, and brucellosis continues to be the most commonly reported laboratory-associated bacterial infection.[85,113]

Brucellosis once was thought to be an uncommon or mild disease in children, but susceptibility now is recognized in persons of all ages.[90] Conditions for the transmission of brucellosis from animals to humans vary among countries and cultures. Where farm animals traditionally are raised in the home, contact with children occurs frequently. Moreover, foodborne infection is not limited to any age or sex and can occur without direct contact with animals. Childhood brucellosis occurs more commonly in locations where *B. melitensis* is enzootic.[38,60]

The clinical manifestations of brucellosis in children do not differ from those in adults,[101] although unfamiliarity with the disease can delay diagnosis.[3,21] Human-to-human transmission of brucellosis is a rare occurrence, but evidence of venereal transfer has been documented.[88,98] Rare cases associated with bone marrow transplantation have been reported.[35] Few cases of brucellosis have been reported in patients infected with the human immunodeficiency virus.[68]

Spontaneous abortion is a common manifestation of brucellosis in animals in which brucellae localize within the reproductive organs of both sexes. Brucellosis in pregnant women can lead to abortion if the infection is unrecognized and untreated.[75] In a retrospective study from Saudi Arabia, intrauterine death and spontaneous abortion occurred in 46 percent of 92 women with brucellosis.[52] This rate was significantly higher than the rate of spontaneous abortion in women without brucellosis. When the infection is diagnosed early, pregnant women can be treated successfully without compromising their ability to carry the pregnancy to term or to conceive again. On rare occasions, transplacental transmission can lead to neonatal brucellosis.[69]

PATHOGENESIS

The incubation period for brucellosis varies; however, symptoms generally appear within 2 to 3 weeks after acquisition of infection. Disease caused by *B. melitensis* and *B. suis* tends to be more severe than is illness caused by *B. abortus* or *B. canis*.[105] Morbidity can be considerable, but death occurs in only approximately 1 percent of cases, usually from complications such as neurobrucellosis or endocarditis.[117] Although no racial or genetic differences in susceptibility are known in humans, innate immunity has been demonstrated in various animals.[103] Normal human serum has limited bactericidal activity against brucellae, but complement opsonizes the organism for phagocytosis by neutrophils.[122] Brucellae are facultative intracellular pathogens that are able to survive and multiply within phagocytic cells of the host. The mechanisms by which brucellae evade killing by neutrophils is not completely understood; however, virulent strains contain a potent superoxide dismutase enzyme and nucleotides (adenine and guanosine monophosphate) that inhibit phagolysosome fusion, degranulation, and activation of the myeloperoxidase-halide system.[29] Brucellae that survive neutrophils killing enter the lymphatics, where they are ingested by monocytes and fixed macrophages of the reticuloendothelial system (RES). Survival within mononuclear phagocytes appears to depend on specific proteins, including stress proteins that block tumor necrosis factor-α (TNF-α), increase cyclic adenosine monophosphate, and prolong the life of the host cell by inhibiting apoptosis.[49,56] Intracellular killing of brucellae occurs when macrophages become "activated" by specifically committed T lymphocytes. This T-helper (T_H1) response involves cytokines, including interleukin-2, interferon-?, and TNF.[114]

CLINICAL MANIFESTATIONS

The spectrum of human brucellosis ranges from subclinical (diagnosed by serology) to chronic (characterized by recurrent symptoms over many years).[120] The disease is characterized by a plethora of nonspecific somatic complaints, such as fatigue, anorexia, nausea, weight loss, sweats, and depression. In contrast, there is a paucity of physical findings, notably fever, and occasionally hepatosplenomegaly. The infection can involve any organ or organ system of the body. Sometimes, symptoms related to a single organ predominate, in which case the disease is termed *localized*.[26] Not unexpectedly, localization often involves organs rich in elements of the RES.

Osteoarticular complications are the most frequent localized manifestations of brucellosis, and in children monoarticular arthritis involving the hips, knees, and sacroiliac predominates.[12,41,59,71] Spondylitis, osteomyelitis, and inflammatory arthropathy also have been described, but they occur less often in children than in adults.[53,93] Brucella is a rare cause of prosthetic joint infection.[110]

Neurobrucellosis comprises a variety of nervous system complications including meningitis/encephalitis, myelitis, radiculitis, peripheral and cranial neuropathies, and demyelinating syndromes.[64] Although most patients complain of headache and weakness, direct invasion of the central nervous system occurs in less than 5 percent of cases.[14,70] Analysis of cerebrospinal fluid (CSF) in cases of *Brucella* meningitis reveals lymphocytic pleocytosis, elevated protein, and normal or low glucose. Organisms are rarely seen on Gram stain or culture from CSF; however, anti-

bodies to *Brucella* are present, and their finding in CSF is diagnostic.[7]

Gastrointestinal complaints frequently reported include anorexia, nausea, vomiting, abdominal discomfort, and weight loss.[67,90] Rare cases of ileitis,[81] colitis,[48,100] and peritonitis[2] have been reported.

The liver is the largest organ of the RES, and it probably always is involved in brucellosis, even when transaminase levels are normal or only slightly elevated.[25] Rarely, acute hepatitis occurs with hepatic enzyme levels resembling viral hepatitis.[57] The liver histology in patients infected with *B. abortus* is characterized by epithelioid granulomas indistinguishable from sarcoidosis.[96] In contrast, infection with *B. melitensis* can result in a spectrum of lesions ranging from diffuse hepatitis to granulomas.[115,118] Infection with *B. suis* (and *B. melitensis*) also can result in suppurative abscess that may become chronic.[11,27,107]

Genitourinary tract involvement usually manifests as orchitis or epididymo-orchitis and can be mistaken for testicular cancer.[74] Rare cases of interstitial nephritis, membranous nephropathy, and glomerulonephritis also have been reported.[106]

Respiratory tract localization of brucellosis is reported in approximately 7 percent of cases.[77] Lung lesions attributed to brucellosis include hilar adenopathy, lobar pneumonia, lung nodules, pleural effusion, and thoracic empyema.[58]

Cardiovascular lesions of brucellosis include endocarditis, myocarditis, pericarditis, and aneurysms of the aorta and cerebral blood vessels.[84] Both native valve endocarditis and prosthetic valve endocarditis have been described, and the aortic valve is involved most often. Delays in establishing a diagnosis can result in life-threatening complications such as valve rupture, myocardial abscess, and sinus of Valsalva fistula. Treatment of *Brucella* endocarditis generally requires the combination of antibiotics in addition to valve surgery.[46,51]

Ocular lesions, most notably uveitis, have been described in patients with brucellosis. Other eye lesions that have been reported include endophthalmitis, optic neuritis, episcleritis, nummular keratitis, and chorioretinitis. The pathogenesis of some of the lesions is a matter of conjecture because brucellae rarely are isolated from the eye.[5,34,50]

Cutaneous lesions attributed to brucellosis include contact dermatitis, rashes, abscess, and vasculitis.[66] On occasion, brucellae have been cultured from subcutaneous papules.[60]

Hematologic abnormalities such as anemia, leukopenia, and thrombocytopenia are common occurrences in the course of brucellosis.[30] These changes generally are mild and resolve promptly with antimicrobial therapy. On occasion, thrombocytopenia can be severe, resulting in hemorrhage into the skin and from mucosal sites.[123] The origin of this complication likely is multifactorial, including hypersplenism, disseminated intravascular coagulation, bone marrow suppression and hemophagocytosis, and immune mechanisms.

DIAGNOSIS

The symptoms of brucellosis are nonspecific. Therefore, the importance of obtaining a detailed history including occupation, avocations, travel, animal exposure, and food habits cannot be overemphasized. Routine laboratory tests generally are not helpful; however, characteristic hematologic findings (normal or low white blood cell count) can suggest the possibility of brucellosis.[4]

A definitive diagnosis of brucellosis is made by isolating a *Brucella* sp. from blood, bone marrow, or other tissue. The rate of positive blood cultures varies from 15 to 70 percent depending on the methods used and the period of in vitro incubation.[42] Bone marrow culture was reported to be more sensitive than blood culture before the introduction of newer isolation techniques.[32,65] Nucleic acid amplification tests using a variety of gene sequences are being applied with some success to the rapid diagnosis of brucellosis.[73,83,111]

In the absence of bacteriologic confirmation, a presumptive diagnosis can be made by measuring the titer of specific antibodies in serum.[116] Human brucellosis is characterized by an initial production of immunoglobulin M (IgM) antibodies followed by a switch to IgG synthesis within the second week of infection. Treatment results in a gradual decline in both antibody isotypes; however, the persistence of IgG antibodies is associated with relapse or chronic infection.[80] Consequently, the pattern of immunoglobulin isotypes is important in differentiating active from treated disease[39,116] This distinction is made by performing the serum agglutination test with and without 2-mercaptoethanol or dithiothreitol, agents used to destroy the agglutinability of IgM while preserving agglutination by IgG.[17] Alternatively, a more direct way to measure IgM and IgG antibodies is the indirect enzyme-linked immunosorbent assay (ELISA) employing anti-IgM and anti-IgG conjugates.[61]

Most serologic tests for brucellosis employ smooth LPS (S-LPS), the immunodominant cell wall antigen capable of detecting antibodies to all smooth species (*B. abortus*, *B. melitensis*, *B. suis*). Because *B. canis* is naturally rough and lacks S-LPS, antibodies against this organism are detected with antigen prepared from rough species (*B. canis* or *B. ovis*).[82] Various cell wall and cytoplasmic proteins have been studied as potential serodiagnostic antigens.[40] However, none has proved superior to LPS-based assays. More recently, a latex agglutination assay using S-LPS has been shown to be simple to run and rapid and to have good sensitivity and specificity.[1]

TREATMENT

Antimicrobial therapy lessens morbidity, shortens the course of illness, and reduces the incidence of complications of brucellosis.[119] Numerous drugs are active against *Brucella* spp., but the results of in vitro sensitivity tests do not always correlate with clinical effectiveness. Because the rate of relapse with single-drug therapy is high, successful treatment of brucellosis requires combination therapy for prolonged periods of time.[93,94] The tetracyclines remain the most effective antibiotics against brucellae with minimal inhibitory concentrations (MICs) lower than 1 μg/mL. Aminoglycosides (streptomycin and gentamicin) have been shown to enhance the killing of brucellae in vitro by numerous antibiotics.[89] Traditionally, the combination of tetracycline HCl administered for 6 weeks and streptomycin given for 2 to 3 weeks provided the highest cure rates in patients with brucellosis.[10] Currently, the preferred regimen is doxycycline (for adults 200 mg/day orally for 45 days) in combination with gentamicin (5 mg/kg/day as a single daily dose intramuscularly for the first 7 days).[86,92]

Rifampin also shows good in vitro activity against *Brucella* spp., and in 1986 the World Health Organization recommended the combination of doxycycline (200 mg/day orally for 45 days) and rifampin (600 to 900 mg/day orally for 45 days) as the treatment of choice. Although this regimen has been used successfully, subsequent studies have shown the superiority of the combination of doxycycline and an aminoglycoside.[95]

Children who are older than 8 years can be treated as adults. Because tetracycline is contraindicated for children younger than 8 years old and for pregnant women, alternative treatments have been sought. A regimen of trimethoprim-sulfamethoxazole (TMP-SMZ; co-trimoxazole) in a fixed combination (80 mg TMP, 400 mg SMZ twice daily for 45 days) in addition to gentamicin (5 mg/kg/day as a single daily dose for 7 days) has yielded satisfactory results.[90] Alternatively, the combination of TMP-SMZ and rifampin (15 mg/kg once daily) administered for either 6 or 8 weeks gave comparable results.[87] Similarly,

pregnant women can be treated successfully with TMP-SMZ in combination with rifampin without causing adverse drug effects in the newborn.[52]

The quinolones are active against *Brucella* spp. in vitro, but the MIC values vary for each compound and to some extent for *Brucella* isolates.[37] In clinical practice, however, results have been disappointing.[54,119] Consequently, quinolones are not recommended alone for treating brucellosis, and their role in combination therapy awaits well-designed clinical trials.

The optimal treatment for complications of brucellosis such as meningitis and endocarditis has not been defined with certainty. Doxycycline crosses the blood-brain barrier more effectively than does generic tetracycline, and it has been used in combination with other drugs (e.g., rifampin, TMP-SMZ) for neurobrucellosis.[64,70] Third-generation cephalosporins achieve high concentrations in CSF, but the sensitivity of brucellae varies, and in general, β-lactam drugs are not effective when they are used alone.[55] On occasion, patients with *Brucella* endocarditis have been treated successfully with antimicrobial therapy alone; however, most patients have required valve replacement surgery.[6]

RELAPSE AND CHRONIC BRUCELLOSIS

Most patients with brucellosis recover completely within a few weeks to months after receiving adequate therapy. Despite receiving appropriate treatment, some patients suffer a relapse characterized by recurrence of symptoms and re-isolation of brucellae from their blood.[9] Obviously, relapse occurs more frequently if the full course of treatment is not completed, and taking oral antibiotics for 6 weeks can tax a patient's compliance. With few exceptions, relapse is *not* caused by antibiotic-resistant strains of *Brucella*.[8]

Even with appropriate treatment, some patients experience delayed recovery and continue to have nonspecific complaints, notably fatigue. Such patients have no objective evidence of infection, such as fever, and their antibody titers decline as expected. Whether this condition represents a variant of the chronic fatigue syndrome is not clear, but additional antibiotic therapy does not improve their recovery.[24] Rarely, chronic brucellosis results from a persisting focus of infection such as osteomyelitis or a deep tissue abscess. Such patients have fever or other objective signs of infection, and levels of IgG antibodies in the serum remain elevated. Scanning techniques (e.g., technetium 99m bone scan, gallium 67 scan, computed tomography, or magnetic resonance imaging) can be useful in localizing an occult focus of infection.

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CHAPTER 140

Pertussis and Other *Bordetella* Infections

James D. Cherry ✪ Ulrich Heininger

Pertussis (whooping cough) is an acute infectious illness of the respiratory tract caused by *Bordetella pertussis* and, less frequently, by *Bordetella parapertussis*.[49,55,68,174,257] The illness occurs worldwide and affects all age groups, but it is recognized primarily in children; it is most serious in young, unprotected infants.[85,168,257]

HISTORY

Unlike other severe epidemic infectious diseases of children (e.g., smallpox, poliomyelitis, and measles), pertussis lacks an ancient history.[184] The first observation of pertussis occurred in France in 1414, and the first epidemic was noted in Paris in 1578.[68,77,221] This epidemic and the clinical characteristics of the cases were described in 1640 by Guillaume de Baillou.

Pertussis was noted as *the kink* (a Scottish term synonymous with *fit* or *paroxysm*) and *kindhoest* (a Teutonic word meaning *child's cough*) in the Middle Ages.[58,184] In 1669, Sydenham named the illness *pertussis* (meaning *violent cough*).[221] Isolation of *B. pertussis*, the main causative agent of pertussis, was reported by Bordet and Gengou in 1906.[30,31]

Vaccines consisting of killed whole *B. pertussis* organisms were developed shortly after the bacterium was isolated, and the first results of protection were reported by Madsen in 1925.[246] The mouse protection test, developed and reported by Kendrick and collaborators[209] in 1947, allowed vaccine production to be standardized. Comprehensive studies conducted by the British Medical Research Council[264] in the 1940s and 1950s demonstrated a correlation between the potency of pertussis vaccines as determined by the mouse protection test and their clinical efficacy in children. As a consequence, immunization against pertussis, most commonly in combination with diphtheria and tetanus toxoids (DTP), became part of routine vaccination programs in many countries throughout the world.

Concern about a relationship between pertussis vaccination and temporally associated serious adverse events (e.g., sudden infant death syndrome and a variety of neurologic illnesses) led to a sharp decline in vaccination rates in Japan and several European countries during the 1970s.[49,55,68,213] This concern, along with well-documented high rates of unpleasant local and systemic reactions, led to the development of new acellular vaccines. These vaccines cause reactions less frequently and have been used in Japan since 1981 and in many developed countries since the mid 1990s.[162,213,391,408]

B. bronchiseptica, which causes cough illnesses in a number of animals as well as humans, was first isolated some time around 1910 by Ferry[115], McGowan, and perhaps others who were studying dogs with distemper.[257,260] *B. parapertussis* was isolated first from children with pertussis in the 1930s, and *Bordetella holmesii* was noted in nasopharyngeal specimens from patients with pertussis-like illnesses in Massachusetts during the period from 1995 through 1998.[32,104,105,258,427]

MICROBIOLOGY

The genus *Bordetella* contains nine species: *B. pertussis*, *B. parapertussis*$_{hu}$ (adapted to humans), *B. parapertussis*$_{ov}$ (ovine-adapted *B. parapertussis*), *B. bronchiseptica*, *Bordetella avium*, *Bordetella hinzii*, *B. holmesii*, *Bordetella trematum*, and *Bordetella petrii*.[81,174,210,257] *B. pertussis* infects exclusively humans. *B. parapertussis* also is a human pathogen, but it has been recovered from sheep ("ovine") as well.[322] Both *B. pertussis* and *B. parapertussis* are respiratory pathogens. *B. bronchiseptica* primarily is an animal pathogen that causes atrophic rhinitis and pneumonia in pigs, kennel cough in dogs, pneumonia in cats, and respiratory illnesses in other animals.[81,132,377] This organism also is the occasional cause of respiratory illness in humans.[72,89,143,299,344,361,377,378,423] *B. avium* is an important cause of respiratory illness in turkeys and other birds.[211]

Three additional species of *Bordetella* have been recognized to infect humans: *B. holmesii* and *B. hinzii* have been isolated from blood cultures, primarily in patients with underlying chronic illness.[80,234,380,414] *B. holmesii* also has been isolated from the human respiratory tract, and *B. trematum* has been found in wounds and ear infections.[258,395,427] *B. petrii* has been isolated from the environment and is capable of anaerobic growth.[403]

The genus *Bordetella* consists of gram-negative, pleomorphic, aerobic bacilli that are grouped together on the basis of genotypic characteristics, and species are differentiated by phenotypic characteristics. Selected differential characteristics of the four *Bordetella* spp. that cause respiratory illnesses in humans are presented in Table 140–1. All species have relatively simple requirements, but *B. pertussis* is quite fastidious and is inhibited by constituents in common laboratory media such as fatty acids, metal ions, sulfides, and peroxides.[239,394] For laboratory growth, *B. pertussis* requires the addition of "protective substances" such as charcoal, blood, or starch, whereas the other species are less fastidious and may grow in blood or MacConkey agars.

TABLE 140–1 Selected Differential Characteristics of *Bordetella* Species That Cause Respiratory Illnesses in Humans

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From Loeffelholz, M. J.: Bordetella. In Murray, P. R., Baron, E. J., Jorgensen, J. H. (eds.): Manual of Clinical Microbiology. 8th ed. Washington, D.C., American Society for Microbiology, 2003, pp.780-788.

The genomes of representative strains of *B. pertussis*, *B. parapertussis*, and *B. bronchiseptica* have been sequenced.[312,324] The genome sizes are as follows: *B. pertussis*, 4,086,186 bp; *B. parapertussis*, 4,773,551 bp; and *B. bronchiseptica*, 5,338,400 bp. *B. pertussis* has 3816 predicted genes, whereas *B. parapertussis* and *B. bronchiseptica* have 4404 and 5007 predicted genes, respectively. Pseudogenes occur most commonly in *B. pertussis* (358) and *B. parapertussis* (220) and are uncommon in *B. bronchiseptica* (18). *B. pertussis* has three insertion sequence elements (IS): IS 481 (238 copies), IS 1002 (6 copies), and IS 1663 (17 copies). *B. parapertussis* has IS 1001 (22 copies) and IS 1002 (90 copies). The sequenced RB50 *B. bronchiseptica* strain contained no IS elements, but studies of other *B. bronchiseptica* strains found strains with IS 481, IS 1001, and IS 1663.[95,122,328]

ETIOLOGY OF PERTUSSIS (WHOOPING COUGH)

B. pertussis and *B. parapertussis* are the etiologic agents of pertussis, but approximately 95 percent of illnesses are caused by *B. pertussis*.[133,174,325] In rare instances, *B. bronchiseptica*, which normally is enzootic in pigs, dogs, cats, rodents, and other animals, has been isolated from humans with pertussis-like cough illnesses.[72,89,143,299,344,361,377,378,423] From 1995 to 1998, *B. holmesii* was isolated from the nasopharynx of 33 individuals in Massachusetts suspected of having pertussis, most of them adolescents and young adults.[258] In contrast to all other *Bordetella* spp., this organism is susceptible to cephalexin, an antibiotic that frequently is added to *Bordetella* culture media.[302] This addition may explain why the organism has not been noted in patients with pertussis in other laboratory studies.

Adenoviruses have been isolated from children with pertussis, and some researchers have suggested that several adenoviral types on occasion may cause a pertussis-like illness.[14,76,78,295] However, the data of Nelson and associates[295] and Baraff and coworkers,[14] as well as our own observations, lead us to suggest that mixed infections are occurring and that the classic symptoms are caused by *B. pertussis* and not infection with an adenovirus. At variance with our view are data presented by Wirsing von König and associates.[421] These investigators noted pertussis-like illnesses caused by viral or *Mycoplasma pneumoniae* infections in 83 patients in whom pertussis laboratory studies were performed and were negative. These authors serologically identified 33 adenoviral illnesses, 18 illnesses caused by parainfluenza viruses, 15 illnesses caused by *M. pneumoniae*, and 14 caused by respiratory syncytial virus (RSV). In young infants, co-infection with an adenovirus and perhaps RSV may lead to more severe disease.[20,227] Infection with human bocavirus also may cause an illness with a paroxysmal cough.[9] Of 54 children with laboratory confirmed bocavirus infections, 85 percent had cough, and 19 percent had paroxysmal coughing episodes.

Physicians often suggest that *Chlamydia trachomatis* can cause a pertussis-like illness. However, in our opinion, the repetitive cough of *C. trachomatis* is distinctly different from the paroxysmal cough of *B. pertussis* infection, and thus illnesses caused by the two agents usually should not be confused clinically. Infections with *Chlamydia pneumoniae* and *M. pneumoniae* also cause long-lasting illness with cough.[87,149,421] Although infection with these agents in older children and adults can be confused with *B. pertussis* infection, true paroxysms typical of pertussis rarely occur.

ANTIGENIC AND BIOLOGICALLY ACTIVE COMPONENTS OF *BORDETELLA PERTUSSIS*

B. pertussis contains a variety of components that are antigenic or biologically active (Table 140–2).* With the exception of tracheal cytotoxin (TCT), all known virulence factors produced by *B. pertussis* are regulated by the single genetic locus *bvgAS*.[81] Under certain conditions, such as an environmental temperature of 37° C, *bvgAS* is active, toxins and adhesins are produced, and the organism is virulent in a mouse model (*bvg*$^+$ phase). In the *bvg*$^-$ phase, a different set of genes (*vrg*, *vir* repressed genes) are expressed, and *B. pertussis* is avirulent in mice in this phase.[411] The switch from *bvg*$^+$ to *bvg*$^-$ is a phenomenon common to all *Bordetella* spp. and is associated with a change in phenotype. An intermediate phase with reduced virulence and expression of specific proteins also has been characterized. Although still speculative, the intermediate phase may have some function in transmission of the organism.[81,367]

Fimbriae

Fimbriae (FIM) are protein projections on the surface of *B. pertussis*.[257,279,281,325,334,335] They are highly immunogenic, and antibody to them, as well as to other antigens, causes agglutination of the organism. Two fimbrial antigens (FIM 2 and 3) are the main agglutinogens; endotoxin and pertactin (PRN) also are agglutinogens.[52,334]

In the past, typing of *B. pertussis* strains was based on the agglutination patterns noted with specific antisera.[57,106,325,334] Six specific agglutinogens were recognized, and typing was based on the presence or absence of agglutination by each specific antiserum. More recently, researchers recognized that two of the agglutinogens (agglutinogens 2 and 3) are fimbrial in location (FIM 2 and 3) and that agglutinogens 4, 5, and 6 are minor antigens.[325,334] All *B. pertussis* strains contain agglutinogen 1. The nature of this agglutinogen is not known.[334] It could be lipopolysaccharide (LPS) or PRN, but because the original serotyping scheme was based on heat-labile antigens, it probably was not LPS.[33,229,382]

FIM function as adhesins, but some studies suggest that in infection they are not the primary adhesins but serve to sustain the attachment established by other attachment factors.[68,335,387,412] An in vitro study by Rodriguez and associates[336] suggested that the effect of FIM on attachment was the result of bacterial agglutination. In the mouse model system, immunization with purified FIM resulted in protection against infection when challenged with *B. pertussis*.[206] Data from two trials in which serologic correlates of immunity were studied indicated that antibody to FIM was important in protection.[69,372]

*See references 7, 52, 68, 81, 82, 130, 157, 162, 175, 179-181, 210, 238, 243, 248, 255, 257, 274, 281, 296, 345, 355, 387, 390, 405, 412, 432.

TABLE 140–2 Biologically Active and Antigenic Components of *Bordetella pertussis*

Component	Characteristic
Adhesions	
Fimbriae (FIM)	Two serologic types (types 2 and 3); antibody to specific types causes agglutination of the organism; organisms may contain fimbriae 2, fimbriae 3, fimbriae 2 and 3, or neither fimbriae 2 nor fimbriae 3
Filamentous hemagglutinin (FHA)	220-kd surface-associated and secreted protein; highly immunogenic
Pertactin (PRN)	A 69-kd outer-membrane protein that is the most important adhesin; antibody to pertactin causes agglutination of the organism
Vag8	95-kd outer-membrane protein
BrkA	73-kd surface-associated N-terminal passenger domain with 30-kd outer-membrane C-terminal protein; confers serum resistance and protection against antimicrobial peptides in *B. pertussis*
SphB1	Subtilisin-like Ser protease/lipoprotein required for FHA maturation
Tracheal colonization factor (TcfA)	60-kd secreted protein
Toxins	
Pertussis toxin (PT; also called lymphocytosis-promoting factor)	A classic bacterial toxin with an enzymatically active A subunit and a B oligomer-binding protein; effects in an animal model system include histamine sensitization, promotion of lymphocytosis, stimulation of insulin secretion, and adjuvant and mitogenic activity; it is an envelope protein that is also an important adhesin; it adversely affects host immune cell function
Adenylate cyclase toxin (ACT)	Calmodulin-activated RTX family toxin with dual adenylate cyclase/hemolysin activity; acts as an antiphagocytic factor during infection
Dermonecrotic toxin (DNT)	160-kd heat-labile secreted toxin; induces necrosis in vitro
Tracheal cytotoxin	Disaccharide-tetrapeptide monomeric byproduct of peptidoglycan synthesis; causes mitochondrial bloating, disruption of tight junctions, damage to cilia, and interleukin-1α and nitric oxide production
Lipopolysaccharide (LPS) (endotoxin)	An envelope toxin with activities similar to endotoxins of other gram-negative bacteria; a significant cause of reactions to whole-cell pertussis vaccines; antibody to LPS causes agglutination of the organism

Filamentous Hemagglutinin

Filamentous hemagglutinin (FHA) is a component of the cell wall of all *Bordetella* spp.[25,68,81,214,248,257,387] It is highly immunogenic and is the dominant attachment factor for *Bordetella* in animal model systems.[257,345,354] However, because FHA is released in large amounts from the cell surface, its role as an adhesin must be questioned. Adhesins typically remain associated with the bacterial surface to promote maximum attachment. FHA is a component of most acellular component (DTaP) vaccines.[102] However, the importance of antibody to FHA and protection from disease is not clear. Some data indicate that a vaccine containing both pertussis toxin (PT) and FHA had an efficacy greater than that of a vaccine containing only toxoided PT.[370] However, in two studies in which serologic correlates of immunity were evaluated, researchers found that FHA made no contribution to protection.[69,372] Finally, one whole-cell component DTP vaccine in which vaccinees did not mount an antibody response to FHA was, nonetheless, highly efficacious.[166,362]

Pertactin

PRN is a 69-kd outer-membrane protein that is, in our opinion, the most important adhesin of *B. pertussis*.[25,225,302,303] Antibody to PRN has a strong protective effect in aerosol-challenge studies in mice.[48,355] In the two vaccine efficacy trials in which serologic correlates of immunity were evaluated, researchers found that antibody to PRN was most important in protection.[69,372] In addition, the vaccine efficacy trials conducted in the 1990s, in which mild disease and typical disease were evaluated, revealed that DTaP vaccines that contained PRN in addition to PT and FHA clearly were significantly more effective.[56,65,145,257] Another study revealed that anti-PRN antibodies were required for efficient phagocytosis of *B. pertussis* by host immune cells.[176]

Other Autotransporters

In addition to PRN are several other surface-associated proteins (Vag8, BrkA, SphB1, and TcfA) that are considered to belong to the autotransformer family.[257] All likely contribute to attachment to host cells, but their potential adhesive functions have not been investigated directly.

Pertussis Toxin

PT is an adenosine diphosphate–ribosylating toxin synthesized and secreted exclusively by *B. pertussis*. It is an A-B toxin with an enzymatically active A subunit (S_1) and a B oligomer ($S_{2\text{-}5}$) binding portion.* PT inactivates G proteins, a process resulting in disruption of signaling pathways and leading to histamine sensitization, enhancement of insulin secretion in response to regulatory signals, and both suppressive and stimulatory immunologic effects in animal model systems.[257] The various effects of PT in animal model systems and in humans were reviewed in depth previously by one of us (J. D. C.).[68,257] In contrast to animal studies, histamine sensitization does not appear to happen in children, but PT does cause an increase in plasma insulin levels.[68,257] PT also is responsible for leukocytosis with lymphocytosis in *B. pertussis* infections. PT is a strong adjuvant in several immunologic systems in animals, but in DTP-vaccinated persons (DTP vaccines contain small amounts of active toxin), only the enhancement of serum antibody responses to antigens of other vaccines has been demonstrated. In mouse and rat models, PT inhibits chemotaxis and migration of neutrophils, monocytes/macrophages, and lymphocytes to infection sites, and PT also functions as an adhesin in the adherence of *B. pertussis* to human macrophages and ciliated respiratory epithelial cells.

In 1979, researchers suggested that pertussis was a single-toxin disease, a suggestion that led to the idea that pertussis could be prevented by a PT vaccine in a manner similar to the success achieved with diphtheria toxoid in diphtheria.[52,319,320] Although convincing arguments to the contrary have been presented, this idea persists.[332] The most compelling evidence that pertussis from *B. pertussis* infection is not a PT disease is as follows: identical illness results from *B. parapertussis* infection, and this organism does not express PT.[52,174]

PT does contribute to morbidity in *B. pertussis* infections, as indicated by the severity of illness, which tends to be greater than

*See references 7, 8, 68, 81, 125, 196, 225, 257, 319, 320, 389.

that caused by *B. parapertussis* infection. In particular, the frequent finding of extreme leukocytosis caused by PT in neonates and young infants with fatal pertussis is noteworthy.

Adenylate Cyclase Toxin

Adenylate cyclase toxin (ACT) is an extracytoplasmic enzyme that impairs host immune cell function and may contribute to local tissue damage in the respiratory tract.[68,81,181,212,257,413] ACT enters phagocytic cells (particularly polymorphonuclear neutrophils) and, once inside, is activated by calmodulin and catalyzes the production of supraphysiologic amounts of cyclic adenosine monophosphate from adenosine triphosphate, which intoxicates these cells.[257]

Dermonecrotic Toxin

Dermonecrotic toxin (DNT) is a heat-labile toxin described by Bordet and Gengou[31] in 1909. This cytoplasmic protein causes skin necrosis in laboratory animals,[293] and it may contribute to local tissue damage in the respiratory tract.

Tracheal Cytotoxin

TCT is a disaccharide-tetrapeptide monomer of peptidoglycan.[81,130] It causes local damage to respiratory epithelium and may affect host neutrophil function adversely.[86,130] The cytopathology caused by TCT probably is the result of increases in nitric oxide.[81]

Lipopolysaccharide (Endotoxin)

The LPS of *B. pertussis* is similar to the endotoxins of other gram-negative bacteria.[46,68,81] Its function in disease is unknown, but it may act as an adhesin.[89] LPS is a major cause of reactions to whole-cell pertussis vaccines.[13] LPS is a significant agglutinogen. Antibody to LPS reduces colonization of *B. pertussis* in the lungs and trachea of mice after aerosol challenge.[289]

EPIDEMIOLOGY

Today, considerable misinformation is circulating with regard to the epidemiology of pertussis. The main reason for misinformation is the failure to recognize the significant differences in the dynamics of reported pertussis compared with the dynamics of *B. pertussis* infections.[60,61] The two different epidemiologies are the (1) epidemiology of reported clinical pertussis and (2) the epidemiology of *B. pertussis* infection.

OBSERVED (REPORTED PERTUSSIS)

Pertussis is one of the most highly communicable diseases; when it has been introduced into a susceptible population, attack rates of 100 percent in susceptible individuals have been recorded.[220] Infants and young children have the highest risk of acquiring the disease.

Incidence

The incidence of reported pertussis and its mortality are affected markedly by the use of pertussis vaccine. In the prevaccine era in the United States, the average attack rate of reported pertussis was 157 per 100,000 population, versus 230 per 100,000 population in England and Wales.[49] Previous studies, however, suggested that reported cases represent only between 15 and 25 percent of cases that actually occur.[159,203,205,368]

With the introduction and widespread use of pertussis vaccines, the attack rate in the United State fell approximately 150-fold from 1943 to 1976. For the 7-year period from 1976 to 1982, the attack rate in the United States remained between 0.5 and 1.0 per 100,000 population (Fig. 140–1). From 1982 to 2005, the attack rate curve shifted modestly upward and reached a rate of 8.9 per 100,000 in 2004.[61] Possible reasons for the resurgence of reported pertussis that have been suggested are (1) increased vaccine failures resulting from genetic changes in *B. pertussis*, (2) increased vaccine failures related to vaccines of lessened potency, (3) greater awareness of pertussis, and (4) the availability of better laboratory tests.[60,61,65,74,138,145] Of these possibilities, it is our opinion that greater awareness is the most important. Also of probable importance is that, in general, DTaP vaccines are less efficacious than are DTP vaccines, which, in particular, may be a contributing factor relating to the increase in reported pertussis in older children and adolescents.

Pertussis epidemics in the prevaccine era occurred at 2- to 5-year intervals (average, 3.2 years), and these cycles have continued in the vaccine era. As noted by Fine and Clarkson,[116,118] this continuation of the same cycles today as occurred in the prevaccine era indicates that, although immunization has controlled disease, it has not reduced transmission of the organism in the population.[49,54,57]

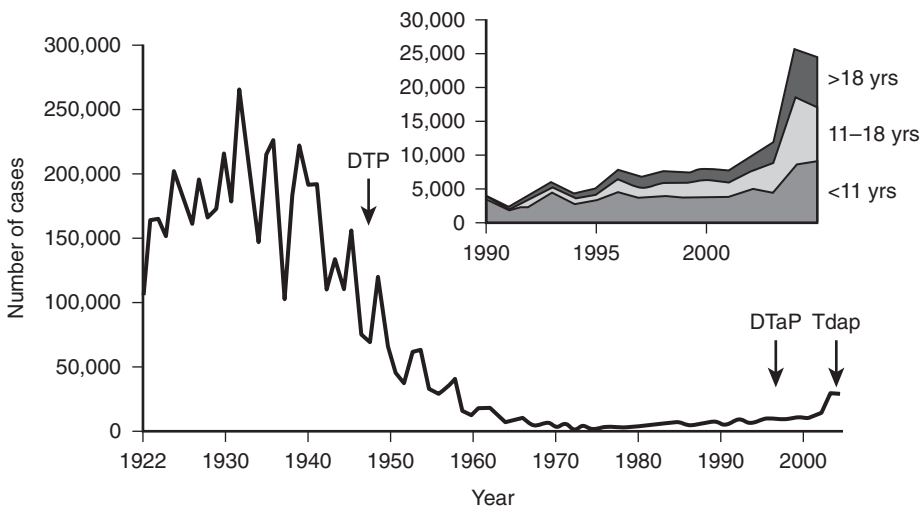

Figure 140–1 Number of reported cases of pertussis by year, United States, 1922 to 2005. DTaP, diphtheria-tetanus-acellular pertussis; Tdap, tetanus-diphtheria-acellular pertussis component vaccine. *(Data from Centers for Disease Control and Prevention, Atlanta.)*

In the prevaccine era, the following percentages of cases by age were noted in Massachusetts: younger than 1 year, 7.5 percent; 1 to 4 years, 41.1 percent; 5 to 9 years, 46.0 percent; 10 to 14 years, 4.1 percent; and 15 years and older, 0.9 percent.[30] Associated with the marked reduction in reported cases of pertussis in the United States resulting from widespread pediatric immunization, a major shift occurred in the percentages by age category.[30] During the period from 1978 to 1981, the age distributions were as follows: younger than 1 year, 53.5 percent; 1 to 4 years, 26.5 percent; 5 to 9 years, 8.2 percent; 10 to 14 years, 5.4 percent; and 15 years or older, 6.5 percent. In contrast, U.S. data for 2005 revealed the following: younger than 1 year, 16 percent; 1 to 4 years, 9 percent; 5 to 9 years, 10 percent; 10 to 19 years, 31 percent; and 20 years or older, 28 percent (unknown age, 6%). Today, pertussis in adolescents and adults is an important source of *B. pertussis* infection in unimmunized or partially immunized children.* Disease in adolescents and adults usually is not recognized as pertussis, even though the cough frequently is paroxysmal and the illness persists for weeks.[57,251,323,352]

In a study in university students, members of our group found that 26 percent of students with a cough illness of 6 days' duration or longer had *Bordetella* infections, none of which had been diagnosed correctly clinically.[278] The findings in this study led to the suggestion that *B. pertussis* infections are endemic in adults and are responsible for cyclic outbreaks in susceptible children. More recent studies in the United States, Germany, and elsewhere support this hypothesis.†

Morbidity and Mortality

During the first 30 years of the 20th century, pertussis was an important cause of death in the United States.[221] The number of deaths from pertussis in the United States between 1926 and 1930 was 36,013,[133] and most of these deaths occurred in children younger than 1 year of age. The pertussis death rate curve in the United States declined throughout the 20th century. In infants, the mortality rate decreased approximately fivefold from 1900 to 1944. During the next 35 years, it declined more than 85-fold.[286,287] Today, most of the deaths caused by pertussis occur in unimmunized infants younger than 6 months of age.[144,257] During 1990 to 1996, 57 deaths attributed to pertussis were reported in the United States.[144] Of these 57 patients, 49 were younger than 6 months old. Young maternal age and preterm delivery were risk factors for fatal disease. Currently in the United States, approximately 10 to 20 deaths are reported each year.[39,42,144,402,425]

Of importance is that deaths caused by pertussis frequently are misdiagnosed as deaths from other respiratory infectious illnesses.[49,171,300] For example, in England and Wales during the epidemic from 1977 to 1979 and at the beginning of the epidemic in 1982, 32 deaths were reported to be caused by pertussis.[49] However, when the excess deaths from other respiratory infectious illnesses were examined, approximately 362 additional deaths appeared to be caused by pertussis.

The morbidity caused by pertussis in recent years can be gleaned from numerous reports. The numbers of pertussis-related hospitalizations, complications, and deaths in the United States during 1990 to 1996 were reviewed in 1999 by Güris and colleagues.[144] Of the 31,837 cases analyzed, 31.7 percent of these patients were hospitalized, 9.5 percent had pneumonia, 1.4 percent had seizures, 0.2 percent had encephalopathy, and 0.2 percent died. The most severe morbidity occurred in infants younger than 6 months of age. Of this group, 72.2 percent were hospitalized, 17.3 percent had pneumonia, 2.1 percent had seizures, 0.5 percent had encephalopathy, and 0.5 percent died. During the period from 2000 to 2004, 12,174 cases in infants were reported.[44] Of this group, 62.8 percent were hospitalized, 55.8 percent had apnea, 12.7 percent had pneumonia, 1.5 percent had seizures, and 0.8 percent died.

In adolescents with reported pertussis, 0 to 2 percent are hospitalized, and 2 percent have pneumonia.[45] In a review of data from seven studies of reported adult pertussis, the following findings were noted: hospitalization, 3 to 12 percent; seizures, 0 to 0.6 percent; rib fracture, 0 to 4 percent; and pneumonia, 0.6 to 8 percent.[44]

A pertussis surveillance system was introduced in conjunction with a large pertussis vaccine efficacy trial in several regions of Germany in 1990. The initial results of this ongoing surveillance study were published in 1993.[164] Of 601 culture-proven cases, 12.3 percent occurred in infants, and 86.2 percent occurred in children younger than 6 years of age. Serious complications were reported in 22 of 275 patients with follow-up. These complications included pneumonia in 5.5 percent, apnea in 2.2 percent, and cardiorespiratory failure in 0.4 percent.

In a follow-up report of the same study, 1 of 185 culture-confirmed cases in infants was fatal.[168] In another German study from 1993 to 1996, Herzig and associates[178] noted 116 hospitalized pertussis patients with the following complications: pneumonia in 81 percent, apnea requiring assisted ventilation in 15 percent, seizures in 14 percent, encephalopathy in 5 percent, and death in 2 percent. In Canada from 1991 to 1997, Halperin and colleagues[156] analyzed the complications of pertussis in 1082 hospitalized children younger than 2 years of age and noted that 9.4 percent had pneumonia, 3 percent had atelectasis, 2.3 percent had seizures, 0.59 percent had encephalopathy, 0.8 percent had inguinal or umbilical hernias, 1.3 percent had more than a 5 percent weight loss, and 0.9 percent died.

Season, Geography, and Sex

Pertussis occurs throughout the world.[68] Historically, epidemic pertussis had no seasonal pattern.[49] However, in the present vaccine era in North America, pertussis usually occurs in the summer and fall.[110,134,144,257,294] In the past, the incidence of pertussis was greater in female than in male patients.[49,133,409] Between 1980 and 1996, this female preponderance was observed again, but only in older age groups.[110,144] In 2004, 25,827 cases were reported in the United States, and of these patients, 11,199 (45%) were male and 13,879 (55%) were female (sex not identified in 749 cases).[41] In the large study in Germany, 1263 (50.7%) of the 2493 subjects were female.[168]

Transmission

Transmission is thought to occur by droplets from a coughing patient that reach the upper respiratory tract of a susceptible person. Indirect spread also possibly occurs. A symptomatic patient could contaminate the environment with respiratory secretions. The hands of the new host-to-be make contact with the secretions and then may inoculate the respiratory tract.[68] Attack rates in susceptible household contacts range from 70 to 100 percent.[49,68] Antibody studies indicate that asymptomatic infections also occur in contacts.[91,240,241] These asymptomatic infections are likely to be short-lived and probably are not important with regard to contagion. Transmissibility is greatest early in the illness, that is, during the catarrhal and early paroxysmal phases.

BORDETELLA PERTUSSIS INFECTION

The cyclic pattern of reported pertussis is similar today to what it was in the pre-vaccine era.[257] This pattern is different from that

*See references 27, 44, 57, 60, 61, 66, 219, 236, 237, 285, 294, 392.
†See references 26, 57, 60, 61, 91, 94, 280, 352, 420, 426.

seen with other diseases that have been brought under control by universal immunization.[57,60,61,278] An example is measles. As this disease was brought under control, the interepidemic cycle lengthened because circulation of the measles virus in the population had been reduced. In contrast, with pertussis, immunization controlled disease but did not decrease the circulation of *B. pertussis*.[118,119] In the 1970s, Nelson[294] noted that the source of *B. pertussis* infection in hospitalized infants most often was an adult. This observation led many investigators to suspect that *B. pertussis* infections were endemic in adolescents and adults.[57,278] Sporadic infection in the adolescent and adult reservoir is the major source of *B. pertussis* infections in nonimmune children. The cyclic pattern occurs because it takes a few years for a significant number of susceptible members of the population to develop so that an outbreak will result from the sustained transmission.

Our present understanding of the importance of adolescent and adult *B. pertussis* infections has been the result of the ability to diagnose infection serologically by measuring immunoglobulin G (IgG) and IgA antibodies to PT by enzyme-linked immunosorbent assay (ELISA).[60,61,257,278] During the last 20 years, numerous studies in adolescent and adult populations have contributed to our understanding of *B. pertussis* epidemiology.

Studies of prolonged cough illnesses in adolescents and adults suggest that between 13 and 20 percent of the illnesses are caused by *B. pertussis* infection.* Many studies have looked at overall infection rates by determining significant IgG or IgA titer rises to PT in persons who have two or more serum samples collected over extended periods.[60,61,83,94,182,257,407] These studies suggest that between 1 and 6.7 percent of adolescents and adults have a *B. pertussis* infection each year. In another large study in the Netherlands, which used a combination of serologic surveys, an incidence of infection of 6.6 percent per year for persons between the ages of 3 and 79 years was estimated.[92] The incidence in children 3 to 4 years of age was 3.3 percent. It then increased gradually to a peak of 10.8 percent in those 20 to 24 years of age. Other age-specific incidences were 6.5 percent in persons 25 to 55 years old and 4.0 percent in those older than 55 years of age.

The specific rates of cough illnesses caused by *B. pertussis* were determined in two prospective specific population-based studies.[373,406] In the first study, the rate was 500 per 100,000 (0.5%), and in the second, it was 370 per 100,000 (0.37%). In addition, the retrospective serologic analysis of a respiratory disease study in adults older than 65 years suggested a rate as high as 1.5 percent.[182] This high value assumes that all respiratory illnesses with cough during the 4-month periods of seroconversion were the result of *B. pertussis* infection.

PATHOLOGY

Data on pathology of *B. pertussis* infections have been determined mainly by postmortem study in fatal cases.[49,68,73,221,235,249,311,358] However, information relating to uncomplicated pertussis can be gleaned from experiments using rhesus and ringtail monkeys.[221,347] In these studies, pure cultures of *B. pertussis* have been obtained from between the cilia of the smaller bronchi and bronchioli. Endobronchitis and peribronchitis are noted, and the bronchi contain leukocytes, mucus, and debris.

In a recent study of autopsy material from is infants (≤4 months old), the consistent histopathologic features were necrotizing bronchiolitis and pneumonia, intra-alveolar hemorrhage and fibrinous edema, and abundant intra-alveolar macrophages.[311] Angiolymphatic aggregates of mixed leukocytes in the intralobular septa and pleurae were seen in 86 percent of the specimens. Intact *Bordetella* were noted in cilia of the trachea, bronchi, and bronchioles and within airways and alveoli. They also were noted intracellularly in alveolar macrophages and respiratory epithelium. Six of the respiratory specimens had evidence of co-infections with one or more other agents (cytomegalovirus, 1 child; RSV, 2 children; *Streptococcus pneumoniae*, 2 children; *Streptococcus pyogenes*, 2 patients; *Moraxella catarrhalis*, 1 child; and viridans streptococci, 1 child).

Pathologic changes in the brain and liver also have been described. Microscopic or gross cerebral hemorrhage may be noted, and cortical atrophy has been observed. These changes most likely are the result of anoxic brain damage. In some studies of pertussis encephalopathy, findings suggested meningoencephalitis, with perivascular cuffs of lymphocytes within cerebral gray matter and pleocytosis.[422] However, the studies in which inflammation was demonstrated were performed before modern virologic techniques became available. Probably, the neurologic findings in these instances were caused by interactions with neurotropic viruses or other infectious agents and were not the result of *B. pertussis* infection.[68] Fatty infiltration of the liver has been noted in patients with pertussis encephalopathy.

PATHOGENESIS AND IMMUNITY

After the patient is exposed to *B. pertussis*, the pathogenesis of infection depends on four important steps: attachment, evasion of host defenses, local damage, and systemic disease.* The biologically active antigenic components of *B. pertussis* listed in Table 140–2 have various roles in pathogenesis.

Infection is initiated in the respiratory tract by the attachment of *B. pertussis* organisms to the cilia of ciliated epithelial cells.[412] Adhesins (FHA, PT, FIM, LPS, TcfA, Vag8, BrkA, and PRN) facilitate this attachment.[81,210,291,325,387,412] Because of the redundancy of protein adhesins, determining the importance of the individual proteins and ascertaining a primary adhesin or adhesins have been difficult.[257] In animal model systems, FHA is an important adhesin; however, data from two vaccine efficacy trials in which serologic correlates of immunity were studied suggested that FHA may not be necessary if other adhesins are present.[69,372] Data from one of these efficacy trials noted that a DTP vaccine that contained a minimal amount of FHA and generated a minimal anti-FHA response had greater efficacy than did a DTaP vaccine that contained a large amount of FHA and generated a vigorous antibody response to FHA.[166,362] However, in a study in which a PT toxoid vaccine was compared with a two-component PT/FHA vaccine, researchers found that the two-component vaccine was more efficacious.[1,369-371] This finding suggests that in the absence of antibody to other adhesins, antibody to FHA contributes to protection.

Of the other attachment proteins, data relating to human infection are available for PRN and FIM only. In the two trials that looked at serologic correlates of immunity, researchers found in both that antibody to PRN was most important and that antibody to FIM was also important.[66,372] Studies suggest that FHA and PRN may act synergistically.[25,214,225] Because the FIM of some gram-negative bacteria are important for attachment, researchers have assumed that *B. pertussis* FIM are important in attachment. However, in one tissue culture study, FIM did not mediate the attachment of organisms to cells.[390] In a more recent study, investigators found that FIM played a role in attachment in persistent infection.[281] Furthermore, antibodies against FIM were shown to provide protection against colonization with *B. pertussis* in the murine respiratory tract.[417]

*See references 26, 60, 61, 127, 202, 257, 278, 297, 338, 352, 373, 401, 420, 426.

*See references 68, 81, 130, 179, 257, 289, 291, 293, 325, 412.

Both ACT and PT adversely affect immune cell function and, therefore, allow infection, once initiated, to continue.[68,181,291] PT prevents migration of lymphocytes and macrophages to areas of infection and adversely affects phagocytosis and intracellular killing. ACT enters phagocytic cells and catalyzes excessive production of cyclic adenosine monophosphate, which intoxicates neutrophils and results in a decrease in phagocytosis.

TCT, DNT, and ACT all have been implicated as contributors to local tissue damage in the respiratory tract.[68,130,293] Of these toxins, TCT is likely to be the most important.[130] In hamster tracheal organ cultures, TCT selectively destroys ciliated cells in a manner similar to that seen in *B. pertussis* infection, and the pathologic process is similar to that noted in human pertussis autopsy studies. As noted in Table 140–2, several other *Bordetella* virulence factors have been described and have been characterized to some extent in mouse models.[81,114,120,210,257,428] Determination of their precise roles, however, requires further investigation.

Pertussis is a unique illness in that it has only one manifestation of systemic disease in uncomplicated infection: leukocytosis with lymphocytosis caused by PT.[68,174,194] T and B lymphocytes increase to a similar extent in the circulation.[24] In contrast to the situation in *B. pertussis* infection, lymphocytosis is not a characteristic of *B. parapertussis* infection because this organism does not liberate PT. The most important systemic complication of pertussis is encephalopathy, the mechanism for which is not known. The most likely explanation is anoxia associated with coughing paroxysms.

Some investigators have suggested that pertussis is a toxin-mediated disease caused by PT.[319,320] Although some researchers continue to entertain this idea,[332] little evidence supports it. Undoubtedly, PT is a fascinating protein with multiple activities in experimental animals, such as histamine sensitization, promotion of lymphocytosis, effects on glucose metabolism, and induction of adjuvant and mitogenic activity.[68,125,257,291] However, in infections in humans, the main effects that appear to be caused by PT are lymphocytosis and mild, compensated hyperinsulinemia. PT has been suggested to be the cause of the prolonged cough in pertussis. However, because persistent cough is a major manifestation of *B. bronchiseptica* infection in dogs and of *B. parapertussis* infection in children, and because neither organism liberates PT, this hypothesis should be refuted.[174,257,302]

Cell-mediated immune function is altered by *B. pertussis* infection. In some studies, cell-mediated immunity was depressed, whereas in others it was augmented.[307]

Various antibodies develop after exposure of the human host to infection with *B. pertussis*. The development of agglutinins, hemagglutination-inhibiting antibodies, and bactericidal antibodies has been described.[49] ELISA techniques have demonstrated class-specific antibodies (IgA, IgE, IgG, and IgM) to many of the specific proteins of *B. pertussis*.[161,250,308] These antibodies develop after infection and, with the exception of IgA antibodies, also after immunization. Neutralizing antibody to PT likewise develops after both infection and immunization.[152,308] Specific IgA antibodies to PT and FHA also can be demonstrated in nasopharyngeal secretions and saliva.[136,429]

At present, both *B. pertussis* infection and immunization with whole-cell or acellular pertussis vaccines clearly elicit protection of varying degree and duration against pertussis. The prevailing opinion throughout the last century was that immunity acquired from having *B. pertussis* infection is lifelong, whereas vaccine-induced immunity is relatively short-lived. Although the latter clearly is true,[118] studies performed by members of our research group suggest that the former opinion regarding infection-induced immunity is wrong.[55,58,67,257,352,399] Proceeding from the knowledge that IgA antibodies to pertussis antigens (PT, FHA, and PRN) result from infection and not from primary vaccination, our group studied the prevalence of these antibodies in the sera of young German and American men of similar ages.[67] In Germany, routine childhood immunization was not carried out during the 1970s and 1980s, and pertussis was epidemic. To our surprise, the rate and mean values of IgA antibodies in the two populations were similar, thus suggesting that adult infection rates were similar. In another study in Germany, we found that *Bordetella* infections were common occurrences in adults, often in persons with a known history of childhood pertussis.[352]

The nature of immunity in pertussis is not known. The consensus has been that serum antibodies greater than some unknown concentration to one or more of the pertussis antigens are responsible for protection.[91] Antibodies to PT, FHA, and PRN have been shown to be protective in animal model systems.[25,82,225,345,355] However, no serologic correlates of immunity had been established until recently, although several large vaccine trials have been performed since the late 1980s.[1,138,145,231,357,362,385] In a nested household contact study, our group was able to evaluate the roles of IgG antibodies to PT, FHA, PRN, and FIM 2 by determining pre-exposure imputed values in children at the time of household exposure to *B. pertussis* infection by using both classification tree and logistic regression methods.[69] The imputed geometric mean antibody values to PT, PRN, and FIM 2 were higher in non-cases than in cases. In the classification tree analysis, however, only antibodies against PRN contributed significantly to protection. Specifically, subjects with an imputed PRN value of less than 7 EU/mL had a 67 percent likelihood of infection. Logistic regression analysis also found that PRN values of 8 EU/mL or more were associated with prevention of illness after household exposure. In accordance with our findings, data from a Swedish study indicate that antibody values of 5 EU/mL or more to PRN and FIM 2/3 correlated with protection.[372]

In addition to humoral responses to several *B. pertussis* antigens, evidence exists that cell-mediated immune responses to PT, FHA, and PRN also occur.[10,247,275-277,340,341,384,415,431] Studies in a murine respiratory infection model suggest that cellular immunity plays an important role in bacterial clearance and augments the effects of antibody by predominantly T-helper 1 (T_H1) cell stimulation.[275,276] Studies in humans demonstrate a cellular immune response shortly after natural infection with *B. pertussis*, with PT, FHA, and PRN preferentially inducing the synthesis of T_H1 cells.[415] Immunization with a whole-cell pertussis vaccine results in a T_H1 response, whereas the response to acellular vaccines is more heterogeneous and involves both T_H1 and T_H2 cells.[315] Persistent memory T and B cells and anamnestic antibody responses are important in long-term immunity.[247]

Immunity developed after having *B. pertussis* infection or receiving vaccination with a whole-cell pertussis vaccine does not protect against illness caused by *B. parapertussis*, and, similarly, infection with *B. parapertussis* does not induce protection against disease caused by *B. pertussis*.[222,381] However, in our vaccine efficacy trial in Germany, the results showed some evidence that the acellular pertussis multicomponent vaccine, which contained a large amount of FHA, offered some protection against *B. parapertussis* infections, whereas the whole-cell vaccine, which contained minimal amounts of FHA, did not.[172] Although *B. pertussis* infection is a localized infection involving ciliated cells of the respiratory track, bacteremia has been noted in immunocompromised adults.[40,201,386]

CLINICAL MANIFESTATIONS

The *clinical* manifestations of *B. pertussis* infection have considerable variation that depends on age, previous immunization or infection, the presence of passively acquired antibody, and perhaps other factors such as the degree of exposure, host genetic and acquired factors, and the genotype of the organism. The

incubation period for pertussis generally varies between 6 and 20 days; most cases have an onset 7 to 10 days after exposure. However, after household exposure, 22 percent of secondary cases were noted to have an onset more than 4 weeks after the onset of illness in the primary case.[166]

CLASSIC ILLNESS

Classic illness occurs as a primary infection in unimmunized children between the ages of 1 and 10 years.[58,68,133,307] The illness usually lasts 6 to 12 weeks and has three stages: catarrhal, paroxysmal, and convalescent. The initial illness is characterized by rhinorrhea, lacrimation, and mild cough suggesting a common cold. Body temperature usually is normal. The severity of the cough gradually increases over a period of 1 to 2 weeks, but pertussis usually is not suspected until the cough becomes paroxysmal.

After the catarrhal period, the coughs increase in severity and number. Repetitive series of 5 to 10 or more forceful coughs during a single expiration can be noted. These paroxysms are followed by a sudden massive inspiratory effort, and a characteristic whoop may occur as air is forcefully inhaled through a narrowed glottis. Cyanosis, bulging eyes, protrusion of the tongue, salivation, lacrimation, and distention of neck veins occur during paroxysms. Several paroxysmal coughing episodes with their associated massive inspiratory effort may occur sequentially until the child succeeds in dislodging the obstructing mucus. The production of purulent sputum does not occur. Post-tussive vomiting is a common occurrence. Paroxysms may strike several times per hour, and they occur during both day and night.

The paroxysmal episodes are exhausting, and patients frequently appear dazed and apathetic. Weight loss may occur as a result of vomiting and also because eating and drinking may be resisted, given that they trigger attacks. Attacks also may be triggered by yawning, sneezing, or physical exertion. Between attacks, the patient may appear normal and usually is in no distress.

Common and important complications of classic pertussis include pneumonia, otitis media, seizures, and encephalopathy. Pneumonia may be caused by *B. pertussis* or secondary bacterial invaders. Atelectasis may develop as a result of the mucus plugs. The forcefulness of the paroxysms can cause rupture of the alveoli and can produce interstitial or subcutaneous emphysema.

Otitis media is a common occurrence and frequently is caused by *S. pneumoniae*. Pertussis also has been associated with activation of latent tuberculosis. Convulsions and coma may be observed. These findings may be a reflection of cerebral hypoxia related to asphyxia. Rarely, subarachnoid and intraventricular hemorrhage may occur. Tetanic seizures may be associated with the severe alkalosis that results from the loss of gastric contents caused by persistent vomiting.

Other complications that have been noted include ulcer of the frenulum of the tongue, epistaxis, melena, subconjunctival hemorrhage, subdural hematoma, spinal epidural hematoma, rupture of the diaphragm, umbilical hernia, inguinal hernia, rectal prolapse, dehydration, meningoencephalitis, the syndrome of inappropriate antidiuretic hormone secretion, apnea, rib fracture, and nutritional disturbances.[37,58,198,256,323,359,410]

The convalescent stage, which usually lasts 1 to 2 weeks, is characterized by a decreasing frequency and severity of coughing episodes, whooping, and vomiting. All patients with classic pertussis caused by primary infection have leukocytosis secondary to lymphocytosis. Fever and pharyngitis are not usual manifestations in pertussis, and, therefore, a search for a secondary cause should be undertaken when these findings occur. Except for the observation of typical paroxysms, physical examination in pertussis usually is unrewarding. Diffuse rhonchi may be noted on auscultation.

Infection with *B. parapertussis* causes an illness that is similar to that caused by *B. pertussis*, but generally it is less severe and of shortened duration.[174]

MILD ILLNESS AND ASYMPTOMATIC INFECTION

Mild, nonclassic illness is common in *B. pertussis* infection.[91,164,168,348] It occurs in previously vaccinated children and also as a primary infection in nonvaccinated children. In a study in which physicians sent nasopharyngeal specimens from children with cough illness, regardless of whether the illness was typical of pertussis, to our laboratory,[164] 247 culture-positive cases were noted. Of these patients, 47 percent had a total duration of cough illness of 28 days or less. In 26 percent, the duration of cough was less than 3 weeks. Most of these cases occurred in unvaccinated children. In a 6-year, similar study involving 2592 culture-positive, previously unvaccinated children, 38 percent had a duration of cough illness of 28 days or less, and in 17 percent, the duration was 21 days or less.[168] In a study in which both culture and polymerase chain reaction (PCR) were used for establishing the diagnosis of *B. pertussis* infection, many mild cases were found to be PCR-positive and culture-negative.[348] Of these cases, only 68 percent had a cough illness lasting 4 weeks or longer, and only 57 percent and 32 percent had paroxysmal cough and whoop, respectively.

In studies of household contacts, asymptomatic infections in family members are common occurrences.[91,240,241] Deen and associates[91] found that 52 (46%) of 114 household contacts who remained well had laboratory evidence of *B. pertussis* infection. In another study, 21 of 399 healthy infants who were controls had PCR-positive nasopharyngeal samples.[167]

INFANTS

Pertussis in infancy is a unique experience.[257] Its spectrum of clinical manifestations varies by age, immunization, and the presence or absence of transplacentally acquired antibody.* Most deaths resulting from *B. pertussis* infection occur in neonates and early infancy, and morbidity is most severe in infants.† From 1997 through 2000, 8276 cases of pertussis were reported in infants in the United States.[39] Of this group, 59 percent were hospitalized, 11 percent had pneumonia, 1 percent had seizures, 0.2 percent had encephalopathy, and 0.7 percent died. Eighty-seven percent of these infant cases occurred in children younger than 6 months of age.

The source of infection in infants usually is a family member.[16,27,85,91,107,217] In a study of 616 infant cases, the source was identified in 43 percent.[27] A family member was the source 75 percent of the time, and the mother was the most common source (32%). Of the source persons, 56 percent were adults, and 20 percent were 10 to 19 years of age.

B. pertussis infection in neonates is particularly severe, with a death rate of between 1 and 3 percent.[20,73,144,171,173,189,233,261] A common initial finding is apnea, and typical coughing is not observed. Seizures in association with apnea caused by hypoxia occur frequently. Severe pulmonary hypertension is a relatively common problem in pertussis in the first 4 months of life.[88,97,135,142,148,311,317,360,416] The severity of disease and the risk of death correlate directly with the white blood cell count and, in particular, the number of lymphocytes.[20,73,88,142,171,261,269,311,317] White blood cell counts in the range of 30,000 to more than 100,000 cells/mL are common findings. Co-infection with

*See references 20, 49, 68, 73, 107, 110, 171, 217, 261, 294, 300.
†See references 16, 20, 73, 84, 85, 88, 129, 135, 142, 144, 147, 148, 167, 171, 173, 189, 227, 257, 259, 261, 269, 294, 300, 311, 317, 321, 337, 358, 360, 379, 402, 416.

respiratory viruses (RSV, adenovirus, influenza viruses) and respiratory bacterial pathogens (*S. pneumoniae*, *Haemophilus influenzae*) are relatively frequent.[20,85,227,311,358]

Whoop is a rare manifestation of illness in infants, and other respiratory manifestations frequently are confused with those caused by respiratory viruses.[49] *B. pertussis* infection has been noted in association with sudden infant death syndrome (SIDS), but whether a cause-and-effect relationship exists is not clear.[167,173,233,300] Nichol and Gardner,[300] in a study in England, found that many deaths attributed to SIDS were, in fact, related to *B. pertussis* infection. Using PCR, we found *B. pertussis* DNA in nasopharyngeal specimens from 9 (18%) of 51 infants who had sudden, unexpected deaths.[173] In a subsequent study, we collected specimens for PCR from 254 infants who experienced sudden, unexplained deaths and from 441 healthy matched controls.[167] The rate of PCR-positive results in the sudden death cases was 5.1 percent; it was 5.3 percent in the controls. In a careful follow-up histopathologic study with unique immunohistochemical staining of specimens from a subset of these fatal cases, we could find no evidence of specific *B. pertussis* pulmonary infection or pathologic features.[310]

ADULTS

Increased awareness of adult pertussis has occurred since the 1970s.* Unrecognized pertussis cases in adults often are the source from which infants and children become infected.[20,27,57,73,171,261,294,352] All adults previously have been exposed to *B. pertussis* antigens by immunization, infection, or both,[67,91,94,257] and this exposure tends to modify their illness. In a U.S. study, the following cough characteristics were noted in 31 university students with laboratory evidence of *Bordetella* infection: the median duration was 21 days before initial evaluation, 94 percent had one or more coughing episodes per hour, and 90 percent of the coughs had a staccato or paroxysmal quality.[278] Despite these findings, pertussis was not suspected in any of the students, and clinical diagnoses by the primary care providers included upper respiratory tract infection (39%), bronchitis (48%), and other diagnoses (16%). Although specific records were not available, most of these students probably were vaccinated as children and almost certainly had previous, unrecognized infections.[67,91]

In contrast to these findings in the United States, in a study in Germany, adults were found to be more likely to have typical pertussis, even though the epidemiologic data suggested that all had a previous infection, and 26 percent of 64 patients with laboratory evidence of infection recalled having had pertussis during childhood.[67,352] Rates of clinical manifestations in the 64 laboratory-confirmed cases were as follows: paroxysms, 70 percent; whoop, 38 percent; post-tussive phlegm, 66 percent; and post-tussive vomiting, 17 percent. The clinical diagnosis in 39 percent was definite or probable pertussis; only 14 percent were thought not to have pertussis. Of note is that the clinical diagnosis was not made by primary care physicians but by a small team of specially trained central investigators with high awareness for pertussis.

In a German household contact study, similar findings were noted: 80 percent of 79 adults with laboratory-confirmed *B. pertussis* infection coughed for 3 or more weeks, and 63 percent had spasmodic cough for 3 or more weeks.[323] In addition, in 53 percent of patients, coughing was followed by choking or vomiting. However, only 8 percent of the adults in this study had whoops. Complications that were observed included pneumonia, rib fracture, inguinal hernia, and severe weight loss. Unique sweating episodes are reported in approximately 5 percent of adults, and fainting can occur in association with coughing.[93,323]

*See references 26, 27, 41, 42, 44, 45, 57, 60, 61, 91-94, 110, 127, 144, 182, 200, 202, 219, 257, 278, 280, 294, 297, 323, 333, 338, 352, 373, 383, 407, 420, 426.

DIAGNOSIS

DIFFERENTIAL DIAGNOSIS

In typical pertussis, the clinical diagnosis should be apparent based on the paroxysmal cough with post-tussive vomiting and whooping and lack of significant fever. However, the cause of the illness can be *B. pertussis* or *B. parapertussis*. A history of contact with a known case (laboratory confirmed) will help to establish the diagnosis in a patient with mild or atypical illness. The presence of leukocytosis with lymphocytosis in a child with a cough illness or the presence of apnea in an infant is a strong indication that the illness is caused by *B. pertussis* and not *B. parapertussis*. In a matched-control comparison, none of 11 children with culture-proven *B. parapertussis* infection versus 7 of 22 (32%) with *B. pertussis* infection had lymphocytosis of 10,000 or more cells/mL.[174] Lymphocytosis of 10,000 or more cells/mL is observed in few other diseases.

Many other infectious agents cause illnesses with prolonged and repetitive cough that can be confused with *B. pertussis* or *B. parapertussis* infections.[257] In particular, *M. pneumoniae*, *C. pneumoniae*, adenoviruses, bocavirus, and other respiratory viruses all can cause prolonged cough illnesses.* In addition, coughing episodes may be seen in children with asthma, bronchiolitis, bacterial pneumonia, cystic fibrosis, *Coccidioides immitis* infection, and other fungal pulmonary infections and tuberculosis. Another problem is the cough associated with sinusitis, which can be confused with *B. pertussis* infection. The cough associated with gastroesophageal reflux also can be confused with pertussis, as can cough associated with an airway foreign body.

SPECIFIC DIAGNOSIS

A laboratory diagnosis of pertussis caused by either *B. pertussis* or *B. parapertussis* can be made by culturing the organisms on appropriate media, by identifying their presence by direct fluorescent antibody testing (DFA) or PCR, and by demonstrating the presence of specific antibodies. *Bordetella* spp. can be recovered from nasopharyngeal specimens, with the highest rate of isolation occurring within the first 3 weeks of cough.[164,374] Specimens for culture can be collected by swabbing the nasopharynx, by nasopharyngeal washing, or by nasopharyngeal aspiration.[68,150] In general, nasopharyngeal aspiration gives the highest yield of positive cultures.

B. pertussis and *B. parapertussis* are recovered most easily by direct plating of the specimen from the patient onto selective media.[68,70,186,239] Specific swabs (calcium alginate or Dacron) and media (Regan-Lowe or Bordet-Gengou agar and modified Stainer-Scholte broth) are required, and laboratory personnel should be experienced in isolating the organisms. If cultures cannot be inoculated directly, the use of Regan-Lowe transport medium is recommended. In classic disease, the culture or a DFA study will be positive in approximately 80 percent of cases if the specimen is obtained within 2 weeks of the onset of cough and antibiotics have not been administered previously.[165,308] DFA has been used for the diagnosis of pertussis caused by *B. pertussis* or *B. parapertussis* during the last 40 years.[257,290,363] It is rapid and inexpensive, but it lacks sensitivity and specificity; it does not employ amplification for sensitivity, and it lacks specificity because

*See references 9, 14, 49, 68, 76, 78, 87, 149, 295, 421.

of cross-reactions with other organisms of the nasopharyngeal flora.[109]

Since the late 1980s, numerous PCR assays with primers derived from four different chromosomal regions have been developed for the diagnosis of *B. pertussis* and *B. parapertussis* infections, and they have been evaluated in multiple studies by comparison with culture and clinically typical pertussis.*

PCR has the advantage of having much higher sensitivity than that of conventional culture. In a prospective study in which swabs for PCR and culture were obtained simultaneously from 555 subjects with cough illnesses, the use of PCR increased the identification of *B. pertussis* infection almost fourfold, from 28 to 111.[348] Only a few studies have been reported in which the sensitivity and the specificity of PCR for the diagnosis of *B. pertussis* infection were determined by comparison with serologically identified cases.[169,230,232,329,397] In a study that our group performed, we compared PCR results with serologic diagnosis and found that PCR had a sensitivity of 61 percent and a specificity of 88 percent.[118] Similar findings have been noted in other studies.[230,232,330,397] At present, the most commonly used primers for the diagnosis of pertussis include IS 481 and IS 1001.[257] IS 481 occurs in the genomes of *B. pertussis* and *B. holmesii* but not in *B. parapertussis*. IS 1001 occurs in the genomes of *B. parapertussis* and *B. holmesii* but not in *B. pertussis*. The genome of *B. bronchiseptica* generally contains neither IS 481 nor IS 1001. However, in two recent studies, *B. bronchiseptica* strains were found to contain IS 1001 in their genomes.[122,328] False-positive results are a potential problem with the use of PCR for establishing the diagnosis of pertussis and other respiratory illnesses.[3,262,290] False-positive results can occur if specimens are opened in the pertussis laboratory before transport to the PCR laboratory.[257] False-positive results also can result from contamination of the air in a room in which the previous patient had pertussis. Moreover, as noted earlier, *B. bronchiseptica* can be mistaken for *B. parapertussis* when the IS 1001 primer is used.[122,328] Therefore, rigorous internal and external laboratory controls are necessary.

Obtaining a routine laboratory diagnosis of *B. pertussis* infection in adults or in other atypical cases is hampered by the problem that medical care usually is not sought until the third or fourth week of the illness, and antibiotics frequently have been administered before the possibility of pertussis was considered.[278,352] During the last 2 decades, the most significant advance in the diagnosis of pertussis has been the development of ELISA.[257]

Natural infection with *B. pertussis* is followed by a rise in serum concentrations of IgA, IgG, and IgM antibodies to specific antigens of the organism, as well as to preparations of the whole organism.[79,123,154,250,268,278,308,362,400] In contrast to natural infection, primary immunization of children induces mainly IgM and IgG antibodies.

Serologic testing for *B. pertussis* infection in the clinical setting is neither standardized nor widely available.[68,308] In the research setting, the use of ELISA has contributed significantly to establishing the diagnosis of *B. pertussis* infection in many patients with negative cultures.[94,151,240,241,278,280,352,426] Most useful has been the determination of IgG and IgA antibodies to PT and FHA. The most reliable proof of acute infection is the demonstration of a significant increase in antibody values between acute-phase and convalescent-phase serum specimens. Frequently, because collection of acute-phase specimens is delayed and, therefore, the acute-phase values already are elevated, significant increases between first and second serum specimens cannot be demonstrated. However, a diagnosis frequently can be established on the basis of a high value or values on a single serum specimen.[91,278,352,419,426] Because *B. parapertussis* infection induces cross-reacting antibodies to *B. pertussis* FHA, the use of this antigen alone cannot differentiate *B. pertussis* from *B. parapertussis* infection.[137,362]

Measurement of agglutinating antibodies also is useful for establishing the diagnosis of *B. pertussis* infection, and, because the test is simple, inexpensive, and accurate, it can be used in the clinical setting.[91,170,278,308,352] Unfortunately, its sensitivity is low.

Today in clinical practice, the laboratory diagnosis of pertussis should be approached as follows: In all cases in which the cough illness is of less than 2 weeks' duration in adolescents and adults or 3 weeks' duration in children, a nasopharyngeal specimen should be obtained for culture or PCR. In adults who have had cough for greater than 2 weeks' duration, single serum ELISA is the preferred method. This method also can be used in children if they have not been immunized within 2 years. At present, many commercial laboratories offer single serum diagnostic tests for *B. pertussis*, and almost all the offered tests lack specificity. Any test that employs the whole organism in the test is fraught with false-positive results. Tests that report specific IgM antibodies also are unreliable. The greatest sensitivity and specificity for the serologic diagnosis of *B. pertussis* infection are achieved by ELISA or an ELISA-like test with the measurement of IgG and IgA antibodies to PT. Single high values of IgG or IgA antibodies to PT are indicative of infection.[257] In our opinion, a sensitive and specific test is available from Focus Diagnostics, Cypress, California.[326]

Not all infected persons develop antibody responses to PT. In children, approximately 25 percent lack an adequate response, as do approximately 10 percent of adolescents and adults.[63,362]

An ELISA also has been developed for the detection of IgA antibody to *B. pertussis* in nasopharyngeal secretions and as an indicator of recent infection.[131,136,429] *B. pertussis* IgA appears in nasopharyngeal secretions during the second or third week of illness and persists for at least 3 months.[131] However, the appearance of secretory IgA may be delayed in children younger than 1 year of age.[292] This antibody is not induced by primary parenteral *B. pertussis* vaccination. Detection with the use of ELISA of *B. pertussis* IgA in secretions may be a diagnostic aid in culture-negative patients whose symptoms have persisted for longer than 3 weeks. To our knowledge, this test is not clinically available.

TREATMENT

Several antibiotics have in vitro efficacy against *B. pertussis*.[14,17-19,23,43,187,190-192] The first choice for treatment since the 1970s has been oral erythromycin; this ameliorates the symptoms if it is given early during the course of the illness and eliminates the organism from the nasopharynx within a few days, thereby shortening the period of contagiousness.[23] The dose for children is 40 to 50 mg/kg/day given every 6 hours for 14 days; the dose for adults is 2 g/day given every 6 hours for 14 days. A 7-day course of erythromycin estolate was shown in a large study in Canada to be as efficacious as 14 days of treatment.[153] The newer macrolides, azithromycin (10 mg/kg on day 1 and 5 mg/kg on days 2 to 5 as a single dose for 5 days for children and 500 mg on day 1 and 250 mg on days 2 to 5 for adults) or clarithromycin (15 to 20 mg/kg/day in two divided doses for 7 days for children and 1 g/day in two doses for 7 days for adults), also can be expected to be effective.[6,43] Although rare, the use of erythromycin in young infants is associated with hypertrophic pyloric stenosis, so parents need to be educated about the symptoms of this potential risk.[38,185,284] Because of this risk, the Centers for Disease Control and Prevention (CDC) recommends treating neonates with azithromycin rather than erythromycin.[43] Trimethoprim-sulfamethoxazole can be used as an alternative agent in those who

*See references 98, 108, 111, 128, 141, 160, 169, 193, 215, 228, 230, 232, 253, 262, 304, 329, 330, 348, 349, 396-398, 430.

cannot tolerate erythromycin.[192] The first erythromycin-resistant strain of *B. pertussis* was isolated from a 2-month-old male infant in Yuma County, Arizona, in June 1994.[227] The isolate was highly resistant, with a minimal inhibitory concentration (MIC) greater than 64 μg/mL (the usual MIC of erythromycin is 0.02 to 0.1 μg/mL). More recently, two more resistant *B. pertussis* strains were recovered from cases in California and Utah.[216,224] At present, no evidence of a pattern of emerging macrolide resistance has been seen, but because PCR rather than culture is becoming the diagnostic method of choice in many laboratories, the chance of missing resistant strains is a possible problem.

Patients infected with *B. parapertussis* and *B. holmesii* also can be treated with macrolides, but *B. bronchiseptica* usually is resistant to erythromycin so that alternative therapy is necessary.[257] *B. bronchiseptica* strains usually are sensitive to aminoglycosides, extended-spectrum, third-generation penicillins, tetracyclines, quinolones, and trimethoprim-sulfamethoxazole.

Historically, no infectious disease has a greater list of remedies lauded as beneficial but without objective evidence of effectiveness.[62] Supportive care includes avoidance of factors that provoke attacks of coughing and maintenance of hydration and nutrition. In the hospital, gentle suction to remove secretions and well-humidified oxygen may be required, particularly in infants with pneumonia and significant respiratory distress. In severe infections in neonates and young infants, assisted ventilation may be necessary.

However, infants who develop pulmonary hypertension with respiratory and cardiovascular failure respond poorly to aggressive therapy (pulmonary artery vasodilators and extracorporeal membrane oxygenation [ECMO]) and have high mortality rates. Because data suggest that the pulmonary hypertension results from the extreme leukocytosis with lymphocytosis, which is always present in fatal cases, our opinion is that leukocyte-reducing measures such as exchange transfusion should be implemented.[96,142,317,337]

The use of corticosteroids has received attention in the treatment of pertussis. Cortisone treatment in the murine model of pertussis increased the mortality rate.[195] In contrast, Zoumboulakis and associates[433] suggested that a 7-day course of steroids and erythromycin reduced the number of coughing paroxysms and episodes of vomiting significantly, in addition to shortening the duration of symptoms. Unfortunately, this study was not controlled rigorously.

The use of salbutamol has also been suggested as having some value, but definitive studies are needed to confirm the efficacy of this mode of treatment.[34] Pillay and Swingler[318] reviewed the symptomatic treatment of pertussis and found no statistically significant benefit for the use of diphenhydramine, dexamethasone, or salbutamol.

PROGNOSIS

The prognosis in pertussis is related to the patient's age. In older children and adults, the prognosis is good, but infants have a significant risk of death and development of encephalopathy.* In addition, long-term follow-up suggests that apnea or seizures at the time of disease may be associated with subsequent intellectual impairment.[376] The present availability of pediatric intensive care units and assisted ventilation has reduced the rate of mortality in infants who receive medical care.[171] Unfortunately, many deaths occur outside the hospital. No evidence has shown that pertussis impairs ventilatory function later in life.[204]

*See references 16, 20, 73, 84, 85, 110, 129, 142, 144, 189, 259, 261, 269, 321, 337, 358, 379, 402.

PREVENTION

VACCINE EFFICACY

Whole-Cell Vaccines

The first pertussis vaccines were developed in the 1920s, and effective vaccines have enjoyed worldwide use since the 1940s.[49,63,68,257] After World War II, extensive vaccine trials were organized by the British Medical Research Council.[263-265] Based on these studies, as well as smaller studies in England, the United States, and other countries, DTP vaccine use became routine in many countries.[119]

The pertussis attack rate was relatively constant in the United States in the pre-vaccine era between 1922 and 1942 (see Fig. 140–1). From 1943 to 1976, a 150-fold reduction in the attack rate was noted in association with widespread childhood pertussis immunization.

The existence of a relationship between vaccine use and disease control also was supported by data from England and Wales. The pertussis attack rate declined between 1958 and 1973 and increased dramatically between 1977 and 1983 after a marked decrease occurred in the number of vaccinations administered, beginning in 1974.[49] The attack rate decreased with the widespread use of vaccine and increased when vaccine use decreased. Moreover, the attack rate after the decrease in vaccine use was increased most markedly in the newly susceptible cohort of children younger than 4 years of age. Until recently, English children received their pertussis immunization only in the first year of life, and protection is not long-lasting.

In the 1980s, numerous household contact studies were performed.[91,309,313-315] In a study from 1982 to 1983 involving 440 household contacts aged 6 months to 9 years, the secondary attack rate in unvaccinated contacts was compared with the rate in children who had received three or more DTP doses. Vaccine efficacy was found to be 91.4 percent. A similar study conducted during the period 1979 to 1981 revealed an efficacy of 82.4 percent.

In another study, Onorato and associates[309] noted that the calculated efficacy varied markedly according to the clinical case definition. Efficacy against any cough illness was 63 percent, whereas it was 83 percent if a cough duration of 21 or more days was required.

In the 1990s, the most definite studies of DTP vaccine efficacy were carried out in four countries.[138,145,166,231,351,357,362] In these trials, the efficacy of the candidate DTaP vaccines was compared with the efficacy of DTP vaccines; the controls were subjects who received DT. With the exception of one lot of one DTP vaccine (Connaught, USA), which had poor immunogenicity, the DTP vaccines were more efficacious than were the DTaP vaccines. The poor efficacy of the Connaught DTP vaccine probably was the result of an unusual low potency lot of this vaccine rather than a generic problem with this vaccine.[257] In a case-control study in the United States, the efficacy of the Connaught vaccine was similar to that of the Wyeth-Lederle DTP vaccine, which, in the controlled trial in Germany, had a high level of efficacy.[28] Finally, in a large comparative trial, the DTP vaccine from England (Evans vaccine) had efficacy greater than those of the three DTaP vaccines with which it was compared.[146,306]

Acellular Vaccines

Research in the 1970s showed that three *B. pertussis* antigens (PT, FHA, LPS) were liberated into the medium during culture and that these antigens could be concentrated and separated by density gradient centrifugation.[68,179] This finding allowed for the development and production of vaccines by six manufacturers in Japan.[64,213,301,346] All six vaccines had minimal or no endotoxin but

different amounts of PT and FHA. In addition, some of the vaccines were found to contain FIM 2 and PRN.

Despite limited proof of efficacy, the six vaccines were put into routine use in Japan in 1981, and they have controlled, to some degree, epidemic pertussis during the ensuing decades. However, because adequate data were not available on any single vaccine or on vaccine use in young infants, many extensive trials were performed subsequently in Europe, Africa, and Japan.[1,288]

After extensive analysis of the data was conducted on the original efficacy trial in Sweden in the mid-1980s, calculated efficacy was found to vary significantly, depending on the clinical case definition and the laboratory methods.[1,29,151,369-371] Therefore, researchers decided that a universal primary case definition should be developed for use in all subsequent efficacy trials so that different vaccines in different trials could be compared.

A World Health Organization (WHO) committee met in Geneva in January 1991, and a primary case definition was developed.[424] This definition and minor variations of it were used in the efficacy trials in the 1990s. The WHO case definition is as follows: (1) an illness with 21 days or more of spasmodic cough and either culture-confirmed infection with *B. pertussis* or serologic evidence of infection with *B. pertussis* as indicated by a significant rise in IgA or IgG antibody by ELISA against PT or FHA in paired sera or (2) contact with a case of culture-confirmed pertussis in the household with onset within 28 days before or after the onset of cough in the study vaccinee. Not all members of the WHO committee, including one of us (J. D. C.), agreed with this primary case definition because its use results in the elimination of many laboratory-confirmed cases from efficacy calculations.[56,164,168,369-371] With this definition, vaccines that lessen the severity of disease but are poor at preventing mild disease will be overrated.

In 1994 and 1995, seven efficacy trials with candidate DTaP vaccines in four countries were completed,[138,145,231,351,357,362,385] and an additional trial in Sweden was completed in 1997.[306] As noted in Table 140–3, the nine vaccines are different in the number of antigens that they contain, as well as in the concentrations of the specific antigens. In all efficacy studies, confounding factors may affect the results. In general, double-blind studies with placebo and whole-cell vaccine controls are ideal. However, placebo control was not ethical in countries in which DTP vaccine was recommended. Therefore, studies in Germany and Senegal used various methods to obtain efficacy data in spite of the lack of a blinded diphtheria-tetanus (DT) toxoid group. Observer bias can affect the results of all studies, including those with double-blind control. For example, a less efficacious vaccine that prevents typical disease but not mild disease can be determined to be more efficacious than it actually is if the study observers believe they "know pertussis" and dismiss atypical cases as being other respiratory illnesses and do not obtain cultures or conduct prospective follow-up.[71]

In general, household contact studies, unless they are nested analyses in prospective cohort studies, also are subject to observer bias, and case-control studies result in significantly inflated efficacy percentages.[56,65,71,117,119,288] In cohort studies, observer bias by parents can be reduced by frequent prospective telephone contact with study families. Finally, serologic diagnosis, as well as diagnosis by culture, increases the identification of mild cases, which are more likely to occur in vaccinees than in control subjects.

A summary of the efficacy data for 10 acellular pertussis vaccines evaluated in the 8 trials performed in the 1990s and the earlier 1980s Swedish trial is presented in Table 140–4. The data

TABLE 140–3 Pertussis Antigens in Nine Diphtheria-Tetanus-Acellular Pertussis Vaccines Evaluated in Eight Efficacy Trials (1990 to 1997)

Vaccine*	Pertussis Toxin (μg/Dose)	Filamentous Hemagglutinin (μg/Dose)	Pertactin (μg/Dose)	Fimbriae (μg/Dose)
Certiva†	40			
Tripedia†	23.4	23.4		
Triavax	25	25		
SKB-2‡	25	25		
Acelluvax	5	2.5	2.5	
INFANRIX†	25	25	8	
Acel-Immune†	3.5	35	2	0.8§
Daptacel†	10	5	3	5‖
CCL DTaP5¶	20	20	3	5‖

**Product name.*
†Licensed in the United States.
‡No product name.
§Fimbriae 2.
‖Fimbriae 2 and 3.
¶No product name. However, is available in Canada with IPV as Quadracel and in Canada and the United States with IPV and Hib as Pentacel.

TABLE 140–4 Vaccine Efficacy Data for 10 Acellular Pertussis Vaccines Evaluated in Eight Trials Carried Out in the 1990s and the Earlier 1980s Swedish Trials

Location/References	Design	Vaccine	Schedule	Efficacy	
				Typical Pertussis (%)	Mild and Typical Pertussis (%)
Sweden, Stockholm[1,29,369]	Double-blind prospective cohort	JNIH-6	2 doses (2-3 mo apart starting at 5-11 mo of age)	84*	42
		JNIH-7		90	–7
Sweden, Göteborg†[385]	Double-blind prospective cohort	Certiva	3 doses (3, 5, 12 mo)	71	54
Sweden, Stockholm[145]	Double-blind prospective cohort	SKB-2	3 doses (2, 4, 6 mo)	59	42
		Daptacel		85	78
Italy, Rome[138]	Double-blind prospective cohort	Acelluvax	3 doses (2, 4, 6 mo)	84	71
		INFANRIX		84	71
Germany, Erlangen[362]	Prospective cohort	Acel-Immune	4 doses (3, 4½, 6, 15-18 mo)	83	72
Germany, Mainz[351]	Household contact	INFANRIX	3 doses (3, 4, 5 mo)	89	81
Germany, Munich‡[231]	Case control	Tripedia	4 doses (2, 4, 6, 15-25 mo)	80, 93	—
Senegal§[357]	Household contact	Triavax	3 doses	31, 74	—

**Efficacy against typical pertussis based on positive culture without serologic analysis.*
†Significant observer bias occurred in this trial.[56]
‡Laboratory diagnosis based on culture only; 80 percent efficacy was against cough illness of 21 or more days, and 93 percent efficacy was against the World Health Organization (WHO) case definition.
§Thirty-one percent efficacy based on 21 days or more of cough illness; 74 percent efficacy was against the WHO case definition.

in this table indicate that three- and four-component vaccines (vaccines containing PRN and FIM as well as PT and FHA) have greater efficacy against *B. pertussis* illness (mild and typical) than do the PT or PT/FHA vaccines. The apparent high efficacy of the two-component vaccine (Tripedia) in the Munich study[231] can be explained by the type of study (case-control study), significant observer bias, and the lack of serologic diagnosis. However, in the post-licensure effectiveness case-control study done in the United States from 1998 to 2001, investigators found that the two-component vaccine (Tripedia) was slightly more effective than the four-component vaccine (Acel-Immune).[28] This finding suggests that a vaccine without PRN was more effective than was a vaccine that contained PRN. However, it is our understanding that this "two-component" vaccine also contained some PRN and perhaps some fimbrial protein as well. The final study, done in Stockholm, was a comparative study without a DT control group.[305] In this trial, in which vaccines were administered to children at 3, 5, and 12 months of age, the efficacy of CCL DTaP5 (five-component) was significantly better than that of Acelluvax (three-component vaccine) and was not significantly different from that of the comparative DTP vaccine (Evans vaccine). Acelluvax had greater efficacy than did the two-component vaccine SKB-2.

Follow-up studies of the various efficacy trials have been done in Sweden, Italy, and Germany, and all suggest sustained protection.[146,242,305,342,343] Of particular importance in this regard is the most recent report by Gustafsson and associates,[146] in which the large cohort vaccinated at 3, 5, and 12 months in the 1993 to 1996 trial were followed from October 1997 to September 2004. Overall, good protection lasted for approximately 5 years. The tabular data in this report suggest that Acelluvax (three-component vaccine) had efficacy similar to that of CCL DTaP5 (five-component vaccine). However, when all the data from the original trial and the present follow up data are combined, the overall attack rate in those children who had received the five-component vaccine was lower (47.5/100,000) than that in children who had received the three-component vaccine (59.7/100,000). This difference was not significantly different, however. Most notable is that the Evans DTP vaccine had greater sustained efficacy than did either Acelluvax or CCL DTaP5. In summary, it appears that DTaP vaccines that contain PRN have relatively good efficacy for approximately 5 years, but that (with the exception of one lot of one DTP vaccine), in general, DTP vaccines are more efficacious.[138,145,146,231,306,342,351,357,362]

In recent years, two acellular pertussis component, diphtheria, and tetanus toxoid vaccines (Tdap vaccines) have been developed for use in adolescents and adults.[44,45,59,112,113,163] These two vaccines are Adacel and BOOSTRIX, and their components are presented in Table 140–5. Both these vaccines elicit vigorous antibody responses to the antigens that they contain after a single dose. These responses are significantly greater than those observed in infants at 7 months of age after they have received three doses of the DTaP vaccines (Daptacel and INFANRIX) of the two manufacturers.

TABLE 140–5 Antigenic Composition of Adacel and BOOSTRIX per 0.5 mL

Antigen	Adacel	BOOSTRIX
Diphtheria toxoid	2 Lf	2.5 Lf
Tetanus toxoid	5 Lf	5 Lf
Pertussis toxin toxoid (PT)	2.5 µg	8 µg
Filamentous hemagglutinin (FHA)	5 µg	8 µg
Pertactin (PRN)	3 µg	2.5 µg
Fimbriae 2/3 (FIM 2, 3)	5 µg	—

Lf, flocculation units.

In a double-blind efficacy trial in adolescents and adults, an acellular pertussis vaccine (without diphtheria and tetanus toxoids) with the same concentrations of PT, FHA, and PRN as BOOSTRIX had an efficacy of 92 percent.[406] No similar efficacy trial was done with Adacel or its acellular pertussis components. However, extensive epidemiologic data from Canada, where Adacel has been used since 2003, show a marked reduction of pertussis in adolescents.[207]

ADVERSE EVENTS

Whole-Cell Vaccines

Local reactions and relatively mild systemic complaints are frequent occurrences after pertussis immunization. Less commonly, severe neurologic illness and death have been noted in temporal association with DTP immunization.

The largest study in the United States designed to assess the risk of relatively common and uncommon reactions to pertussis vaccine was performed by Baraff and associates.[11-13,75] This study was conducted between January 1978 and December 1979. Reactions in children who received either DTP or DT immunization were compared. A total of 15,752 DTP immunizations and 784 DT immunizations were given to children aged birth to 6 years of age. These children were evaluated for reactions that occurred within 48 hours of vaccine administration. All common local and systemic reactions occurred more frequently in the DTP recipients than in the DT group. Differences between the common reactions in the two groups were all highly significant ($p < .005$).

Redness at the injection site occurred in 37.4 percent of DTP recipients and in 7.6 percent of DT vaccinees. Fever (≥38° C [100.4° F]) was noted in 46.5 percent of DTP recipients. A temperature of 39° C (102.2° F) or higher occurred in 6.1 percent of DTP recipients, but in only 0.7 percent of DT recipients. Drowsiness, fretfulness, vomiting, anorexia, and persistent crying were other reactions recorded in 3.1 percent (persistent crying) to 53.4 percent (fretfulness) of DTP recipients versus 0.7 percent (persistent crying) to 22.6 percent (fretfulness) of DT vaccinees. In general, rates of local reactions, but not those of systemic reactions (except fever), increase from dose to dose in an immunization series.

In addition to these reactions, 0.1 percent of DTP recipients in this study were reported by the parents to have a high-pitched, unusual cry; 0.06 percent had convulsions, and 0.06 percent had hypotonic-hyporesponsive episodes (shock, collapse). No children in the control group (DT recipients) had similar reactions; however, the control group was of modest size (784 DT recipients), so statistical significance could not be assigned to any of these relatively uncommon events.

Because convulsions in young children are the result of many different etiologic factors, the cause-and-effect relationship with pertussis vaccine is less clear. However, inasmuch as fever develops in almost half of all DTP vaccinees and febrile convulsions are not uncommon events, a reasonable assumption is that many convulsions that occur in temporal association with DTP vaccination are induced by the immunization. Three studies have noted a significant association between pertussis immunization and febrile convulsions.[15,356,404] Approximately 1 per 1000 vaccinees older than 6 months of age will have a first febrile seizure after receiving pertussis immunization. The concomitant use of acetaminophen (15 mg/kg per dose at the time of immunization and every 4 hours for 24 hours) and DTP vaccine has been suggested as a means of reducing the incidence of febrile convulsions in vaccinees.[226]

Neurologic disease and death occurring in temporal association with pertussis immunization have been of major concern

throughout the vaccine era. During the last 60 years, several case series and individual cases of neurologic illness occurring after receipt of pertussis immunization were reported; by 1979, more than 1000 cases of alleged neurologic damage induced by pertussis vaccine were reported.[22,36,47,49,218,252,418] Few of these reports had evidence of an adequate search for other possible causes of the neurologic disease, and in none were data available for rate calculations.

From 1967 to 1980, several attempts were made to determine the frequency of neurologic disease after receipt of pertussis immunization.[2,49,100,101,121,158,366,375] However, because controls were not included in any of the population evaluations, all rate estimates included children with temporally related events that had other causes.

A carefully designed prospective case-control study (National Childhood Encephalopathy Study [NCES]) of all hospital admissions of children aged 2 to 35 months with acute serious neurologic disorders in England, Wales, and Scotland was undertaken between 1976 and 1979.[4,21,245,270-273] The results of this study for the first time revealed an apparent statistical association between pertussis immunization and neurologic illness. Researchers found that a child who had received DTP vaccine within the previous 3 to 7 days was two to five times more likely to have neurologic disease than was a child who was not immunized during the same interval. The causal relationship between DTP immunization and neurologic illness noted in this study must be questioned, however, because both cases and controls had an equal frequency of immunization during the month preceding the index date. A more appropriate interpretation of the results is that they do not indicate cause and effect; rather, the DTP immunization calls attention to or brings out something that will occur anyway, but just moves it forward in time ("trigger effect").[50,51]

Infantile spasms, an identifiable seizure disorder of infancy, usually has its onset in the 6-month period from 2 to 7 months of age; therefore, that some cases occur after DTP immunization is not surprising. Simple calculations indicate that approximately 12 percent of all patients destined to have infantile spasms between 2 and 7 months of age will have an onset of illness within 7 days after receiving DTP immunization. The temporal association between DTP immunization and infantile spasms has led many people to assume a cause-and-effect relationship. However, controlled data from the NCES in Great Britain provide strong evidence against a causative role for pertussis vaccine in infantile spasms.[21] In another study, Melchior[266] in Denmark noted that the time of onset of infantile spasms was not altered when the time of pertussis immunization was changed from 5, 6, 7, and 15 months of age to 5 weeks, 9 weeks, and 10 months of age. In both periods, 42 percent of patients had their onset during the first 4 months of life.

Data from the NCES were reanalyzed with the exclusion of cases of infantile spasm.[271,273] From these analyses, the risk of permanent brain damage occurring from pertussis immunization has been suggested to be 1 per 330,000 vaccine doses, and the risk of any encephalopathy has been estimated to be 1 per 140,000 vaccinations. However, a review of the NCES data by other investigators indicates that both rate estimates are incorrect. Specifically, Stephenson[365] showed that the 1 per 140,000 rate for all encephalopathy is an artifact resulting from the inclusion of 9 children with febrile convulsions. Similarly, MacRae[244] noted that the increased relative risk that was observed within 7 days of receiving immunization (which was used to calculate the risk of brain damage of 1 per 330,000 immunizations) was offset by a decreased relative risk over the subsequent 3-week period. This finding, similar to the original study data and the infantile spasm data, indicates not a cause-and-effect relationship but rather a redistribution of events over time.

In the United States, the major neurologic illness that was noted in temporal association with DTP immunization was the first seizure of what turned out to be severe epilepsy. By chance alone, this association may occur 400 times a year in the United States. Four carefully performed studies that included approximately 330,000 children and 1 million immunizations have examined the possibility that pertussis immunization is a causative factor in epilepsy; no evidence of a causative role has been found.[51,126,140,356,404] More recently, two large studies in the United States and Canada found no evidence of a causal association with DTP immunization and encephalopathy.[282,327]

Similar to infantile spasms, SIDS also occurs in early life; therefore, that cases are noted to occur after administration of DTP immunization again is not surprising. Hoffman and associates[183] performed an extensive prospective case-control study of risk factors in SIDS from October 1978 through December 1979. In this study of 800 cases, investigators found that DTP immunization was not a risk factor for development of the syndrome. Other good, controlled studies have yielded similar results.[68,139] No evidence has demonstrated that DTP vaccinees have an increased risk of developing asthma later in life.[177]

Acellular Vaccines

An extensive amount of reactogenicity information has been generated in phase II and phase III studies with all licensed DTaP vaccines.[90,138,145,231,331,350,388] Because endotoxin has been removed from all DTaP vaccines, one is not surprised that all are less reactogenic than are DTP vaccines. In a double-blind study, the reactogenicity of 13 DTaP vaccines was presented and was compared with the reactogenicity of a DTP vaccine.[90] This study involved 2200 infants; 113 to 217 received an acellular product, and 370 received the whole-cell vaccine. Study participants received three doses of vaccine at 2, 4, and 6 months of age. Overall, all monitored reactions except vomiting occurred less frequently and were less severe in DTaP recipients than in DTP recipients. Specific results from this study are presented in Table 140–6. As can be seen, local redness and swelling and fever increased in frequency from the first to the third dose, whereas the complaint of drowsiness decreased.

In our efficacy trial in Germany with Acel-Immune, we monitored reactions in more than 8000 children after receipt of four doses of vaccine at 3, 4½, 6, and 15 to 18 months.[353,388] For the first three vaccine doses, the findings were similar to those noted in Table 140–6. After the fourth dose, the frequency of occurrence of local erythema and induration and fever increased considerably in comparison with their frequencies after the third dose. Ten percent of DTaP recipients had local erythema of 2.4 cm or greater, and 28 percent had temperatures of 38° C or higher. Other investigators also noted an increased frequency and severity of local reactions occurring after administration of the fourth and fifth doses of DTaP vaccines.[165,199,316,331,350] Of particular concern is the observation of extensive swelling of the thigh with booster doses of some DTaP vaccines.[331,350] Rennels and associates[331] found that this swelling occurred more commonly after immunization with DTaP vaccines containing high amounts of diphtheria toxoid. With Acel-Immune, a vaccine with a low diphtheria toxoid content, we noted that 15.4 percent of subjects had induration of more than 5 cm but less than 10 cm after receiving a fifth dose; swelling of the entire limb was not noted.[165]

In five of the 1990s efficacy trials, the occurrence of less common, more severe events (persistent crying, seizures, and hypotonic-hyporesponsive episodes) was monitored. A summary of these data is presented in Table 140–7. As can be seen, temporally related persistent crying, hypotonic-hyporesponsive episodes, and seizures were rare events after receipt of immunization with DTaP vaccines. In a large active surveillance program (IMPACT), researchers found that risks of having febrile seizures and hypotonic-hyporesponsive episodes after receiving pertussis-

TABLE 140–6 Summary of Reactogenicity Data from the Nationwide Multicenter Acellular Pertussis Trial

	DTaP			DTP		
Event	**1st Dose (%)**	**2nd Dose (%)**	**3rd Dose (%)**	**1st Dose (%)**	**2nd Dose (%)**	**3rd Dose (%)**
Local						
Redness	13.5	17.1	21.5	49.4	47.7	47.6
Swelling	8.7	12.1	13.3	39.7	34.1	35.7
Pain	3.8	2.0	2.1	27.3	18.7	15.8
Systemic						
Fever (Temp. ≥100.1° F)	4.2	11.3	15.8	27.3	34.1	37.7
Fussiness	6.6	7.7	6.7	20.1	23.5	17.3
Drowsiness	29.9	17.6	12.9	43.5	31.0	24.6
Anorexia	9.3	8.9	8.9	19.5	16.5	14.3
Vomiting	6.3	4.5	4.2	7.0	4.5	5.3
Use of antipyretic	39.3	36.7	36.3	60.5	59.8	61.4

DTaP, diphtheria-tetanus-acellular pertussis; DTP, diphtheria-tetanus-pertussis.
Data from Decker, M. D., Edwards, K. M., Steinhoff, M. C., et al.: Comparison of 13 acellular pertussis vaccines: Adverse reactions. Pediatrics 96:557-566, 1995.

TABLE 140–7 Rates* of Severe Events after Diphtheria-Tetanus-Acellular Pertussis Vaccines in the 1990s Efficacy Trials

Vaccine	Persistent Crying (≥3 hr)	Hypotonic-Hyporesponsive Episodes	Seizures
Certiva	0	0	0.4
Tripedia	0.1	0.05	0.02
SKB-2	0.8	0	0.3
Acelluvax	1.9	0.07	0
INFANRIX	1.3	0	0.07
Acel-Immune	2.0	0	0.1
Daptacel	1.5	0.1	0

**Rates per 1000 doses.*
Data from references 138, 145, 231, 385, and 388.

containing vaccines decreased significantly after the introduction of DTaP vaccines in Canada.[223]

SCHEDULES AND CONTRAINDICATIONS

Immunization schedules with whole-cell vaccines have varied throughout the world and to great measure were determined by concern relating to true and perceived reactions.[49,68] An immunization schedule involving only three doses given to infants at 2, 3, and 4 months of age has been quite effective in controlling pertussis-related morbidity and mortality in the United Kingdom.[393] However, the five-dose schedule used in the United States resulted in lower attack rates in preschool- and school-aged children.[49,68]

The recommendation for the DTaP vaccine in the United States is that it be given in the same five-dose schedule as recommended for DTP vaccines.[391] However, follow-up data from the 1990s efficacy trials, as well as reactogenicity data, suggest that administration of this "one size fits all" approach could be modified.[138,145,146,305,342,343,350,382,385] Our findings with Acel-Immune suggest that the present five-dose schedule is appropriate.[242,362] Conversely, data from other trials suggest that administration of the fourth dose of some vaccines can be postponed until the child is 4 to 6 years of age, or the third dose can be moved back to the child's second year of life.[146,305,306,342,343,382,385] These changes would not be expected to decrease efficacy but would decrease troubling local reactions with booster doses.

Acellular pertussis component quadrivalent (DTaP, IPV), pentavalent (DTaP, IPV, HBV; DTaP, IPV, Hib), and hexavalent (DTaP, IPV, HBV, Hib) vaccines have been developed and are available in many countries. In the United States, the following multicomponent vaccines are available: DTaP/Hib (TriHIBit for the fourth dose of DTaP and Hib series), DTaP/IPV/HBV (PEDIARIX), and DTaP, IPV, Hib (Pentacel). In general, multicomponent vaccine use should be encouraged to reduce the number of injections that a child receives.

In addition to providing childhood immunization, the availability of adolescent and adult Tdap vaccines allows the vaccination of adolescents and adults to be conducted.* At present, the schedule for the use of Tdap vaccines varies in different countries.[112,113,163] The most common recommendation is for universal immunization of preadolescents and adolescents and the selective immunization of adults. However, our opinion is that a universal program involving all preadolescents or adolescents and adults every 10 years will be necessary to prevent the transmission of *B. pertussis* effectively to unimmunized infants and to prevent the continued circulation of *B. pertussis* in a population.

Over the years, pertussis vaccine recommendations have undergone many changes. In particular, contraindications to vaccine are evolving continually. An important note, however, is that few scientific data support any of the present contraindications. The primary goal of national immunization programs is to vaccinate all infants and children. If excessive contraindications and their overinterpretation lead to a large number of unimmunized children, the programs will fail, and the children in greatest need of protection will contract pertussis. In the United States, the most recent recommendations of the Committee on Infectious Diseases of the American Academy of Pediatrics generally should be followed. However, individual case-by-case decisions often need to be made.

ISOLATION AND PROPHYLACTIC MEASURES

Erythromycin, azithromycin, or clarithromycin treatment in the index case shortens the duration of communicability of the organisms and thus limits spread of the disease. During the first few days of treatment, contact with susceptible persons should be avoided. In general, close contacts (household members, those in daycare centers, playmates) of the index case should be protected from infection. Such protection can be implemented by the prophylactic use of erythromycin for 14 days, azithromycin for 5

*See references 44, 45, 53, 54, 57, 59-61, 112, 113, 155, 163, 208, 257, 339.

days, or clarithromycin for 7 days.[6,43,257,364] Active immunization of all exposed persons (children, adolescents, and adults) who are not adequately vaccinated also should be conducted.

The use of prophylactic antibiotics in adolescents and adults in exposure situations such as classrooms and hospital settings frequently is recommended. This approach often involves many people and considerable expense.

In our experience, the side effects of erythromycin and other macrolides are such that adult compliance is poor. Therefore, our opinion is that erythromycin and other macrolides should not be used prophylactically but only for treatment at the first sign of respiratory illness in those exposed.

OTHER *BORDETELLA* INFECTIONS

BORDETELLA PARAPERTUSSIS

B. parapertussis infection in children can cause unrecognized infection, mild pertussis, or typical pertussis.[257] We studied 38 children with *B. parapertussis* illnesses and compared their illnesses with those occurring in 76 children with *B. pertussis* illnesses.[174] The results were as follows (*B. pertussis/B. parapertussis)*, in percentages: cough for longer than 4 weeks, 57 percent/37 percent ($p = .06$); whooping, 80 percent/59 percent ($p = .07$); whooping for longer than 2 weeks, 26 percent/18 percent ($p = .05$); paroxysms, 90 percent/83 percent; post-tussive vomiting, 47 percent/42 percent; and mean leukocyte and lymphocyte counts, 12,500 per mm^3 and 7600 per mm^3/7800 per mm^3 and 3500 per mm^3 ($p < .0001$), respectively. In another study in Italy, children with *B. parapertussis* infection had the following rates of findings: cough, 100 percent; paroxysms, 76 percent; whooping, 33 percent; post-tussive vomiting, 42 percent; apnea, 29 percent; and cyanosis, 12 percent.[254] All of these rates except cough and paroxysms were lower in children with *B. parapertussis* infections than in children with *B. pertussis* infections. Concomitant infections with *B. pertussis* and *B. parapertussis* are not rare.[188,197,267]

Before and during the early pertussis vaccine era, pertussis caused by *B. parapertussis* was considerably less common than that caused by *B. pertussis*.[257] For example, during a 16-year period in the Grand Rapids area of Michigan, 4483 cases of pertussis were caused by *B. pertussis*, and 106 cases were caused by *B. parapertussis*.[103] More recently, during the DTaP vaccine efficacy trials in Europe, the comparative rates of illness caused by *B. pertussis* or *B. parapertussis* were examined. In our trial in Germany, the rate of pertussis caused by *B. parapertussis* infection in control children was 0.9 cases per 100 person years.[362] Of 130 culture-confirmed cases, 21 percent were caused by *B. parapertussis*. The percentage of cases caused by *B. parapertussis* in five other trials varied from 2.1 to 20 percent.[254]

During the last 30 years in the United States, pertussis caused by *B. parapertussis* has been an uncommon occurrence. However, during 2004 and 2005, 493 culture-positive cases of pertussis were noted by the Wisconsin State Laboratory of Hygiene.[298] Of these cases, 14 percent were caused by *B. parapertussis*.

BORDETELLA BRONCHISEPTICA

In 1911, McGowan[260] observed that laboratory workers exposed to various animals with *B. bronchiseptica* infections on occasion had respiratory illness. In 1926, a 5-year-old girl with a pertussis-like illness was found to be infected with *B. bronchiseptica*.[35] Her illness commenced about 10 to 12 days after she had been given a rabbit with mild "snuffles." Otherwise healthy children who became infected with *B. bronchiseptica* after being exposed to farm animals or pets usually have pertussis-like illnesses.[257]

B. bronchiseptica causes respiratory infections in at least 18 different mammals.[81] Most notable are atrophic rhinitis in pigs, kennel cough (rhinotracheitis) in dogs, and bronchopneumonia in rabbits and other laboratory animals. Occasional infections in humans have been noted during the last 35 years, with the majority occurring in immunocompromised adults.[5,99,423] Most recent reports have noted *B. bronchiseptica* infections in patients with acquired immunodeficiency syndrome (AIDS). Respiratory infections have ranged from mild upper respiratory illnesses to pneumonia. In patients with AIDS, the pneumonia frequently is cavitary. Sinusitis and bronchitis also occur.

BORDETELLA HINZII

B. hinzii has been recovered from an adult patient with cystic fibrosis during pulmonary exacerbations throughout a 3-year period.[124] Bacteremia has been noted in a patient with AIDS.[80]

BORDETELLA HOLMESII

B. holmesii has been isolated from the nasopharyngeal specimens of 33 patients suspected of having pertussis.[427] Twenty-three of the cases were investigated further, and 19 (82%) of these patients were adolescents, 2 (9%) were adults, and 2 (9%) were children. All had cough: 61 percent had paroxysms, 26 percent had post-tussive vomiting, and 9 percent had whoop. *B. holmesii* also has been isolated from a 10-month-old boy with bacteremia and from patients with septicemia, endocarditis, and respiratory failure.[283,380]

BORDETELLA TREMATUM

B. trematum has been isolated from wounds and ear infections.[395]

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CHAPTER 141

CALYMMATOBACTERIUM GRANULOMATIS

Mariam R. Chacko

Granuloma inguinale is caused by *Calymmatobacterium granulomatis*. It was described first by McLeod in 1882, and *C. granulomatis* was isolated by Donovan in 1905. This disease has been known through the years by many different names, the most common being granuloma inguinale, donovanosis, and granuloma venereum.[30]

THE ORGANISM

C. granulomatis is an encapsulated gram-negative rod measuring 1.5×0.7 mm. Although some of its characteristics resemble those of *Klebsiella* and *Enterobacter*, *C. granulomatis* is considered a unique species distinct from other related organisms belonging to the subclass Proteobacteria.[21,30,31] However, the biochemical and bacteriologic characteristics of the organism have not been identified.[30,31]

Study of the organism reveals a complex cell envelope. In addition to having regular bacterial structures such as a mesosome, ribosomes, and nuclear material, the cytoplasm contains electron-dense granules; transmission electron microscopy shows an outer membrane, middle electron-opaque layer, and an inner plasma membrane. The organisms are enclosed mainly in large histiocytic cells and occasionally in polymorphonuclear cells and plasma cells. They multiply intracellularly to approximately 30 in number and eventually cause cell rupture.[7,30]

EPIDEMIOLOGY

Granuloma inguinale occurs predominantly in young and older adults and is a cause of genital ulcerative disease in tropical and subtropical regions of the world. As with all genital ulcerative diseases, granuloma inguinale has been given greater attention as a risk factor for development of human immunodeficiency virus (HIV) infection.[37] In South Africa, the prevalence of granuloma inguinale in patients with genital ulcer disease is reported to be approximately 10 percent.[34]

Granuloma inguinale is a rare occurrence in children. In the early 1950s, 4 percent of 1- to 4-year-old children in a Papua New Guinea population were found to have granuloma inguinale. Although the mode of transmission in adolescents and adults is considered to be sexual, the mode of transmission of the disease in these children was thought to be by skin-to-skin contact from sitting on the laps of infected adults.[30] More recent case reports show that otitis media, mastoiditis, neck mass, and cervical lymphadenopathy have been described as the initial manifestations of donovanosis in infants and children.[3,14,15] The modes of transmission in these cases are thought to be perinatal and by skin-to-skin contact.[3,14,15]

Granuloma inguinale has been reported since the early 1990s as endemic in South Africa, Papua New Guinea, India, and the Aboriginal community of Australia.[29] A resurgence of the disease was reported in the late 1980s in Durban, South Africa. After a rapid test for donovanosis was introduced in the early 1990s, the number of cases reported by the Durban Health Department in South Africa increased substantially.[27] As a result of the availability of the rapid test and implementation of an HIV/STI (sexually transmitted infections) initiative, the number of new cases of donovanosis in Australia decreased dramatically; five cases were reported in 2005.[2] In 1994, after 4 decades, a case of granuloma inguinale was reported in China.[13]

Granuloma inguinale is not a common occurrence in Europe or the United States today. However, the prevalence increased in developed countries in the 1990s, especially among migrant and poor people living in metropolitan areas. For example, three cases of granuloma inguinale were reported in Rome between 1993 and 2001, with most of them diagnosed in 2000 and 2001.[26] Until 1952, more than a thousand cases a year were reported to the Center for Disease Control (now the Centers for Disease Control and Prevention [CDC]). Since 1952, the prevalence of this disease declined rapidly; between 1971 and 1981, 50 to 90 cases a year were reported. Except for an isolated surge of 97 cases in 1990, fewer than 50 cases a year have been reported since 1982, and in 1993, 19 cases (15 male and 4 female) were reported. In 2000, no cases were reported in the United States.[5] Granuloma inguinale was detected in a white adolescent girl in California in 1985.[16] In 1991, nongenital granuloma inguinale was diagnosed in a man with testicular carcinoma in Texas.[25] In 1992, three unrelated cases of granuloma inguinale, seen within a period of a few weeks, were reported in Toronto; two occurred in immigrants, and one occurred in a native-born Canadian. The first patient was a recent immigrant from El Salvador, and the second patient had immigrated several years previously and had a history of sexual activity with a Jamaican who had immigrated recently. The sexual partner of the third case had come from Turkey.[18] Although the prevalence of the disease is low, this disease needs to be suspected in North America and Europe because of international travel and immigration. Granuloma inguinale usually occurs more commonly in male than in female patients. However, in adolescents, the disease can develop more commonly in girls than in boys. This ratio probably is a reflection of sexual activity between adolescent girls and adult men.[28]

The risk factors and mode of transmission of granuloma inguinale are not clear. The disease generally is considered sexually transmitted. However, in many cases, granuloma inguinale cannot be detected in the sexual partners of infected persons. Nonetheless, numerous studies have reported the disease in 12 to 50 percent of marital or steady sexual partners.[30] Anal intercourse also has been associated with rectal and penile lesions of granuloma inguinale.[22] O'Farrell and associates[35] studied the patterns of sexual behavior in men and women with genital ulcer disease and found that patients with granuloma inguinale and secondary syphilis were more likely than were patients with other

genital ulcer diseases to have had sexual intercourse despite the presence of ulcers. Studies reported from India and South Africa have noted a preponderance of granuloma inguinale cases in uncircumcised male patients with poor genital hygiene.[28]

Coexistence of granuloma inguinale with other sexually transmitted diseases occurs commonly. Syphilis has been described in as many as 23 percent of patients with granuloma inguinale. In one report, HIV-1 antibodies were found in as many as 8 percent of male patients with granuloma inguinale.[25,37]

PATHOGENESIS AND PATHOLOGY

The primary lesion in granuloma inguinale is an indurated nodule that erodes through the skin and becomes a granulomatous heaped ulcer. Adjacent lesions form and eventually coalesce, especially in the perineal area. Secondary infection of lesions may occur and may aggravate the tissue destruction and cause scarring. *C. granulomatis* organisms invade mononuclear endothelial cells. Extensive acanthosis and dense dermal infiltrates of mainly plasma cells and histiocytes have been observed in the indurated nodules. Polymorphonuclear cell infiltration also occurs, but lymphocytes are rare findings when secondary infection develops. The pathognomonic feature of granuloma inguinale is a large, infected mononuclear cell, 25 to 90 μm in diameter, that contains many intracytoplasmic cysts filled with deep-staining Donovan bodies. Metastatic spread to the bones, joints, liver, and lymphatics occasionally occurs.[30]

A possible link between human leukocyte antigen (HLA)-B57 and granuloma inguinale infection may exist; class I, class II, and DQ antigens have been detected in the genital ulcers of individuals with granuloma inguinale.[32] Circulating lymphocytes and tissue-level lymphocyte subpopulations in granuloma inguinale have been studied.[40,41] T-lymphocyte and B-lymphocyte infiltration in tissues is almost identical, without any significant difference in ulcerogranulomatous and hypertrophic variants. Both total leukocyte and absolute lymphocyte counts are increased in the ulcerogranulomatous variant of granuloma inguinale. Total T lymphocytes, CD4, CD8, and CD22 levels, and the CD4/CD8 ratio all are increased significantly in the ulcerogranulomatous variant. In contrast, the hypertrophic variant causes a significant elevation only in the CD4/CD8 ratio. This finding suggests a greater cell-mediated immune response in the ulcerogranulomatous variant of granuloma inguinale and is consistent with the paucity of Donovan bodies in smears obtained from patients with this variant.[40,41]

CLINICAL MANIFESTATIONS

The incubation period of granuloma inguinale usually is less than 2 weeks but may be as long as 3 months. The disease begins as one or more subcutaneous nodules that erode through the skin to produce clean, large, beefy-red, granulomatous ulcers that bleed easily. The lesions are sharply defined and painless, and the ulcers feel hard when palpated. The disease usually is limited to local tissue; therefore, constitutional symptoms are unlikely to be present. Autoinoculation is a common manifestation that produces "kissing" lesions. When left untreated at this early stage, the disease progresses and causes extensive mutilating lesions (see Chapter 48).[30]

The morphology of the cutaneous lesions of granuloma inguinale can vary, depending on the stage of the disease. The exuberant or hypertrophic stage appears before secondary infection develops. It consists of large, vegetating masses with overgrowth of granulation tissue, usually in the perianal region. The ulcerative stage is accompanied by secondary infection. In this stage, large, spreading, shallow necrotic ulcers with a foul odor may be noted. The cicatricial stage results after prolonged healing and is characterized by fibrosis, scarring, depigmentation, keloid formation, elephantiasis, and stenosis of the vagina, urethra, and anus. In patients in Durban, ulcerogranulomatous lesions occurred far more commonly than did hypertrophic and necrotic lesions. Although lymphadenopathy is an unusual occurrence in granuloma inguinale, pseudobuboes and pseudoelephantiasis of the genitals (massive edema) may be seen. Pseudobuboes are the result of deep, inguinal granulomata; pseudoelephantiasis is caused by cutaneous extension of lesions and inflammation.[10,25,30]

The genitalia are involved in 90 percent of cases, the inguinal region in 10 percent, the anal region in 5 to 10 percent, and extragenital sites in 1 to 5 percent. In male patients, lesions usually occur on the prepuce and also can occur on the coronal sulcus and frenulum of the penis.[30,31]

As ulcers enlarge, they can be mutilating and can lead to urethral stenosis. The most common clinical manifestation in pregnant and nonpregnant women is vulvar ulceration. Genital tract bleeding is the next most common finding in nonpregnant women. Multiple sites of genital ulceration (vulva, vagina, and cervix) have been noted only in nonpregnant women.[10,28,30] Extragenital sites, through hematogenous spread, have been reported in the mouth, chin, axillae, abdomen including the pelvic cavity, and foot.[9,11,23,30,38,39,42] These distant sites usually are associated with a primary lesion in the genital area.[30] Lesions in the oral cavity have been described after apparently successful treatment of genital lesions.[9] Donovanosis ulcers may take longer to heal in HIV-positive individuals, and greater destruction of tissue may be noted as well.[30,31]

In endemic areas, unusual manifestations of granuloma inguinale may cause confusion with a wide variety of diseases and can result in misdiagnosis. The differential diagnosis includes carcinoma, secondary syphilis, necrotic ulcerations of amebiasis, tuberculosis, and filariasis. In addition, secondary infection can confuse the diagnosis.[17,30]

DIAGNOSIS

Generally, granuloma inguinale is diagnosed on clinical grounds. The accuracy of a clinical diagnosis of granuloma inguinale can be as high as 63 percent in male patients and 83 percent in female patients. When compared with other genital ulcers, the ulcers are larger in granuloma inguinale, are painless, bleed easily to touch, and usually are not associated with inguinal lymphadenopathy.[36]

The diagnosis of granuloma inguinale can be confirmed by identification of Donovan bodies on a stained crush specimen from the lesion (see Fig. 48–22). Examination of appropriately stained specimens from active lesions remains the most reliable diagnostic test. Light-microscopic examination of biopsy specimens that have been fixed in formalin and embedded in wax is less reliable. Donovan bodies rarely are seen by this method.[30,36]

The common stain used to identify Donovan bodies is Wright-Giemsa blue-black stain. Sections that are formalin-fixed or stained with hematoxylin and eosin are less useful for detecting Donovan bodies. If donovanosis is suspected, a swab for this condition should be taken first, before swabs for other organisms, so that an adequate amount of cellular material can be obtained.[20,30,33] When *C. granulomatis* organisms are likely to be scarce or when smear or crush specimens are likely to be nondiagnostic, one should consider obtaining a biopsy of the lesion. Accordingly, a biopsy specimen preferably stained with Giemsa or silver is recommended in very early, very sclerotic, or heavily superinfected specimens.[20,30,33]

Successful isolation of *C. granulomatis* by culture on human epithelial cell lines with a modified *Chlamydia* culture technique

has been reported from South Africa and Australia.[4] Polymerase chain reaction (PCR) techniques for *C. granulomatis* also have been developed and further refined into a colorimetric detection system for use in diagnostic laboratories.

Donovan bodies have been identified on Papanicolaou smears from the cervix.[8] Serologic and skin tests for granuloma inguinale are highly sensitive but not specific. A serologic test using the indirect immunofluorescence technique has been evaluated and found to have a sensitivity of 100 percent, a specificity of 98 percent, a positive predictive value of 89 percent, and a negative predictive value of 100 percent in diagnosing granuloma inguinale. In the absence of culture methods for *C. granulomatis*, this test may prove helpful in the diagnosis of established lesions and not early ulcers.[12]

TREATMENT

Antibiotics with strong activity against *C. granulomatis* are those effective in the treatment of gram-negative bacilli or those whose lipid solubility ensures good intracellular penetration. The CDC recommends treatment of adolescents and adults with doxycycline, 100 mg orally twice a day for a minimum of 3 weeks, until lesions are healed.[5] Alternative regimens include the following: azithromycin, 1 g orally weekly for at least 3 weeks; or ciprofloxacin, 750 mg orally twice a day for at least 3 weeks; or erythromycin, 500 mg orally four times a day for at least 3 weeks; or trimethoprim-sulfamethoxazole, one double-strength tablet (160 mg/800 U) orally, twice a day for at least 3 weeks.[5] Children younger than 9 years of age can be treated with oral azithromycin suspension (10 mg/kg daily) trimethoprim-sulfamethoxazole (10 mg/kg/day trimethoprim), or erythromycin (30 to 50 mg/kg/day).[6]

Clinical response to antibiotics should be noted within a week of treatment; the lesions should become paler and less friable. After a week of treatment, the lesions become smaller, and total healing of the area takes 3 to 5 weeks. Relapse occurs in approximately 10 percent of cases, especially if use of the antibiotic is discontinued before the primary lesion has healed completely. Donovan bodies may reappear within 7 to 10 days.[30] Treatment can fail in patients with coexisting HIV infection, and more intensive and prolonged antibiotic therapy may be needed. As compared with HIV-negative patients, the mean duration to complete ulcer healing in these patients is significantly longer: 25.7 days versus 16.8 days.[19]

Merianos and associates[24] reported the possible effectiveness of ceftriaxone for chronic, recurrent granuloma inguinale. Patients in this study had been suffering from the disease for 1 to 5 years and had received 4 to 19 courses of antibiotics. A single daily intramuscular injection of 1 g of ceftriaxone diluted in 2 mL of 1 percent lidocaine was administered for 7 to 26 days. Clinical improvement was dramatic in most lesions; one third of patients recovered completely without a recurrence after receiving daily doses of ceftriaxone for 7 to 10 days. Patients with mild recurrences responded to additional ceftriaxone or short courses of oral antibiotics.[24] Vulvectomy is reserved for infections that have not responded to antibiotic treatment or for patients with severe vulvar elephantiasis.[10]

PROGNOSIS

Healing is complete in patients who seek treatment early in the course of the disease and who comply with their medication and follow-up regimens. O'Farrell[28] noted complete healing of lesions in 24 percent of patients who complied with follow-up. Complications of granuloma inguinale include pseudoelephantiasis, urethral stricture, and pelvic abscess, which may require surgery. Another complication is the acquisition of HIV infection and granuloma inguinale, especially when patients with ulcerations that are left untreated for a prolonged period have sexual contact with an HIV-positive partner.[28,30]

The severe, mutilating complications of granuloma inguinale are primarily a result of delayed treatment or poor compliance with medication. In Durban, South Africa, almost half of the male patients had ulcerations for 1 to 6 months before they sought medical care, and 16 percent had ulcerations for 1 to 3 weeks. In contrast, approximately 25 percent of female patients had ulcerations for 1 to 6 months, and 50 percent had ulcerations for 1 to 3 weeks.[28] Delayed medical attention may be related to limited education, ignorance of sexually transmitted diseases, absence of suitable medical facilities, or embarrassment in seeking treatment because of extensive genital lesions.

PREVENTION

Sexual partners of persons with the disease should be traced, examined, and treated. Treatment of granuloma inguinale when the nodule first appears is associated with a benign course. Thus, community-based eradication that targets men with granuloma inguinale in endemic areas should be implemented. Programs should be aimed at identification of lesions, provision of early treatment, and prevention of severe complications. Teaching the importance of personal genital hygiene, such as instruction on simple retraction of the foreskin in men and cleansing the penis with soap and water, also is effective.[28,30]

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CHAPTER 142

CAMPYLOBACTER JEJUNI

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Campylobacter jejuni is a frequent cause of enteritis and less often of extraintestinal infection in humans. Since it first was recognized as a common human pathogen in the 1970s, appreciation of this agent's importance as a cause of disease has been increasing steadily. *C. jejuni* is one of the most frequent bacterial causes of human enteritis in the United States and is a leading cause of bacterial foodborne diarrheal disease throughout the world.[4,10,200] Immunoreactive complications may develop after infection.[157,169]

HISTORY

The pathologic consequences of infection with members of the group of bacteria that includes *C. jejuni* were recognized first in 1909 from studies of abortions in sheep.[130] In 1947, the sheep abortion–associated organism *Vibrio fetus* was isolated from a blood culture from a pregnant woman who had an influenza-like illness and delivered a stillborn infant with a necrotic, infarcted placenta.[219] In 1957, King[112,113] hypothesized that *V. fetus*–related organisms could be associated with human enteric disease. Butzler and colleagues[31] showed that bacteria similar to *V. fetus* were present in the stools of children with diarrhea. This observation was confirmed rapidly and repeatedly.[21,33,67,189,202] Major differences in biochemical activities, growth characteristics, and DNA base nucleotide content between true vibrios and *V. fetus* led to the establishment of the new genus *Campylobacter*.[217] The genome of a representative *C. jejuni* was published in 2000.[154] Information gleaned from this and comparative analyses of additional isolates have markedly advanced knowledge of these agents.[63,85]

THE ORGANISM

C. jejuni is a gram-negative rod that may vary in width from 0.2 to 0.9 μm and in length from 0.5 to 5.0 μm.[214] The rods may be short and S-shaped or longer spirals (Fig. 142–1). The organism grows best in cultures maintained at 42° C under microaerophilic conditions (5%-10% oxygen). *C. jejuni* belongs to rRNA superfamily VI, a specialized subgroup of gram-negative bacteria that

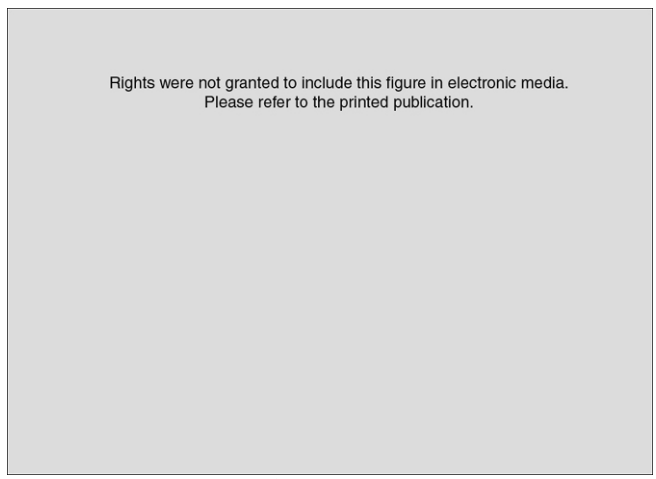

Figure 142–1 Scanning electron microscopy of *Campylobacter jejuni* strain 20-01. *(From Baqar, S., and Rice, B.: Campylobacter jejuni enteritis. Clin. Infect. Dis. 33:901-905, 2001.)*

also includes *Arcobacter* and *Helicobacter*. *C. jejuni* usually is motile, with either a single polar flagellum or two flagella, one at each end of the rod; nonmotile variants exist, and spores are not formed. Organisms obtained from stressed cultures may be coccoid or spherical. The species *C. jejuni* has two subspecies: *C. jejuni jejuni* and *C. jejuni doylei*. *C. jejuni doylei* can be differentiated from *C. jejuni jejuni* by the failure of *C. jejuni doylei* to grow at 42° C, lack of nitrate reduction, and sensitivity to cephalothin. For brevity, we refer to *C. jejuni jejuni* as *C. jejuni*. The spectrum of disease recognized as caused by *C. jejuni* is expanding.[5]

C. jejuni has a circular chromosome of 1.64 million base pairs (30.6% guanosine and cytosine).[154] Marked differences in pathogenic surrogates (motility, colonization of chicks, invasion, and translocation of cultured cells) are associated with variable chromosomal sequences within different passage sequences for a single isolate, and unique chromosomal sequences are associated with known pathogenic isolates of *C. jejuni*. Genomic analyses demonstrate that substantial differences exist among organisms classified by conventional means as *C. jejuni* and that some of these differences are associated with the capacity to cause disease in humans.[63,85,153] Genomic sequence analyses have yielded a catalogue of phase-variable surface structures (including lipo-oligosaccharide and capsule) that appear to relate to pathogenesis and immunity,[58,154] a lack of classical operon structure, repetitive DNA, and an unexpected capacity to produce polysaccharide.[154] Populations of *C. jejuni* are nonclonal, and interstrain exchange of genetic material occurs.[41]

EPIDEMIOLOGY

Human infection with *C. jejuni* occurs worldwide.[19] *C. jejuni* persists in zoonotic niches (Table 142–1), and most human infections are thought to arise from these reservoirs. Chickens and contaminated water are the main sources of campylobacteriosis in developed countries. The organism is a common commensal of the gastrointestinal tract of cattle, pigs, dogs, cats, and most birds used as human food,[25,185] and transmission from these sources occurs. In the United States, *C. jejuni* infection peaks in late summer and early fall (Fig. 142–2). Fresh-water bathing sites may yield *Campylobacter* organisms that are epidemiologically linked to human infections.[145,181,182] Dairy cows in the United Kingdom have higher concentrations of *Campylobacter* organisms in spring and autumn, but the concentration of *Campylobacter* organisms in beef cattle at slaughter does not change with season; as many as 90 percent of beef cattle may be stool-positive for *Campylobacter*.[196] In subtropical areas, the peak incidence of isolation of *C. jejuni* often is associated with the rainy season. In most tropical climates, rates of isolation are similar year-round. Studies in volunteers show an incubation period of 2 to 4 days after challenges ranging from 800 to 100 million colony-forming units.[18] Fecal shedding of *C. jejuni* by humans may last a median of 2 to 3 weeks, with a range of 3 days to several months.[33,109,149]

The distribution of *C. jejuni* infections within populations is linked to the level of industrialization. In industrialized countries, *C. jejuni* infection is found often in children and adults with enteritis and seldom in healthy individuals.[200] In less industrial-

TABLE 142–1 Illustrative Studies of Isolation of *Campylobacter jejuni* from Animal Sources

Animal	Sample	Location and Reference	Sample Size	Positive*
Chicken	Processing plants, shops	Japan[211]	156	67.9
	Flocks on farms	England[94]	49	76.0
	Flocks on farms	Russia[197]	370	31.5
	Giblets	Egypt[111]	50	23.5
	Eggs from 23 farms	United States[15]	276	0.0
	Live birds	United States[15]	10	90.0
	Processing plants, carcasses	United States[193]	325	78.5
Duck	Giblets	Egypt[111]	50	19.0
	At reservoir	United States[148]	113	73.0
Goose	At reservoir	United States[148]	94	5.0
Turkey	Giblets	Egypt[111]	50	14.5
	Feces	United Kingdom[183]	5000	100.0
Squab	Giblets	Egypt[111]	50	4.0
Crane	At reservoir	United States[148]	91	81.0
Pig	Pork at processing plants, shops	Japan[211]	94	2.1
Cow	Beef at processing plants, shops	Japan	52	0.0
	Rectal swabs	United Kingdom[93]	668	72.0
	Farms	Canada[220]	78	13.0
	Milk cows	United States[48]	78	68.0
	Milk, bulk tanks	United States[48]	108	0.9
	Housed indoors, feces	Switzerland[29]	395	38.5
	Outdoors, feces	Switzerland[29]	395	13.3
Goat	Rectal swabs	Ghana[1]	72	33.3
Sheep	Rectal swabs	Ghana[1]	13	23.0
Sheep	Liver	New Zealand[38]	272	66.2
Cat	Domestic, rectal swabs	United States[66]	430	1.0
	Zoo, rectal swab (species-positive)	United States[66]	15	6.7
	Feces	United States[83]	206	1.0
Monkey	Stool	United States[137]	50	77.0
	Stool	Indonesia[137]	50	36.0
Dog	Rectal swabs of puppies	Denmark[76]	72	22.2
	Fecal culture of puppies	United States[224]	4	100.0
Birds	Feces of migrating passerines	Sweden[151]	101	3.0
Pigeons, feral	Rectal swabs, feces	Norway[121]	200	3.0
Penguin	Feces	South Georgia Island[27]	100	3.0

**Percent positive. In instances in which studies reported ranges of percent positive, the highest rate is recorded.*

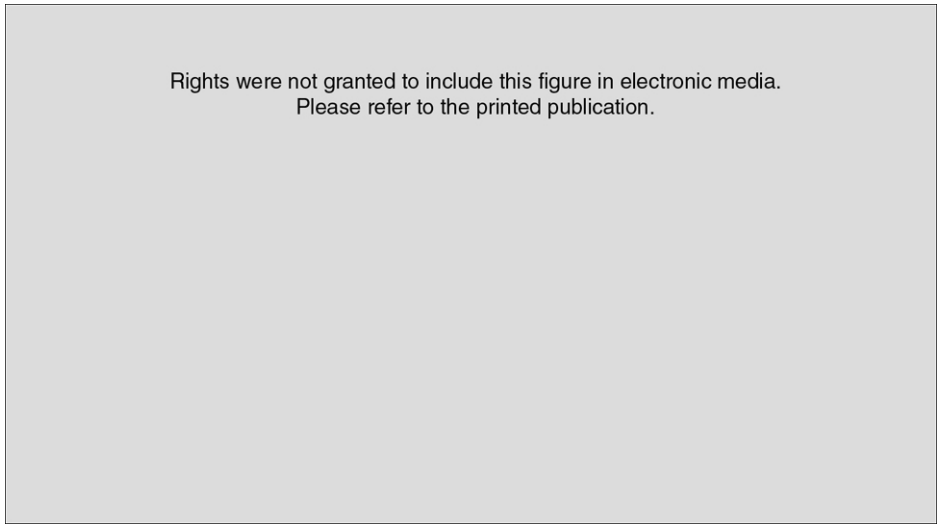

Figure 142–2 Reported human *Campylobacter* isolates by month in the United States, 1982 to 1989. *(From Tauxe, R. V.: Epidemiology of Campylobacter jejuni infections in the United States and other industrialized countries.* In *Nachamkin, I., Blaser, M. J., and Tompkins, L. S. [eds.]: Campylobacter jejuni: Current Strategy and Future Trends. Washington, D.C., American Society for Microbiology, 1992, pp. 9-19.)*

TABLE 142–2 Selected Longitudinal Studies of Frequency of *Campylobacter jejuni* Infection in Children

Location	No. of Children	Isolation of *C. jejuni* from Children*	
		With Diarrhea	Without Diarrhea
Guatemala[167]	321	12.1	8.1
Czechoslovakia[86]	5831	10.1	NR
Mexico[33]	179	0.4	1.7
Thailand[203]	411	0.4	1.1

**Results are reported as percent of stools positive.*
NR, not reported.

TABLE 142–3 Selected Cross-Sectional Studies of Frequency of *Campylobacter jejuni* Infection in Children

Location	Frequency of Isolation of *C. jejuni* from Children: Percentage of Stools with *C. jejuni* (Number of Children Studied)	
	With Diarrhea	Without Diarrhea
South Africa[26]	35.0 (78)	16.0 (63)
Zaire[43]	14.4 (416)	3.0 (200)
Rwanda[42]	9.3 (150)	0.0 (58)
Zaire[31]	8.6 (70)	0.0 (30)
Cameroon[116]	7.7 (272)	3.2 (157)
Bangladesh[78]	25.5 (102)	8.6 (93)
China[226]	18.7 (48)	8.6 (104)
China[46]	11.9 (303)	4.6 (953)
Kuwait[184]	7.0 (621)	0.0 (152)
India[141]	4.0 (607)	0.9 (529)
Saudi Arabia[37]	1.0 (7369)	0.1 (1130)
Belgium[31]	5.1 (800)	1.3 (1000)
Canada[149]	4.3 (1004)	0.0 (176)
Chile[56]	10.0 (299)	6.0 (304)

ized areas, *C. jejuni* is isolated frequently from children, even in the absence of enteritis (Tables 142–2 and 142–3).

In industrialized countries, *C. jejuni* has been isolated from between 1 and 13 percent of children with diarrhea, and the prevalence of infection in healthy individuals has been reported to be between 0 and 1.5 percent.[21,31,170,189,200] A 5-year, laboratory-based national surveillance of *Campylobacter* spp. showed an isolation rate of 5.5 per 100,000 person-years (with *C. jejuni* accounting for 99% of the *Campylobacter* isolates; Fig. 142–3).[202] Population-based isolation rates of *Campylobacter* in the United States range from 28 to 1560 per 100,000 per year.[201,202] The rate of death attributable to *Campylobacter* is estimated between 100 and 124 per year in the United States.[8,9] The case mortality rate is between 0.10[131] and 0.23 percent.[81] It is possible that the mortality rate is higher. A registry-based study demonstrated about fivefold and twofold increases in mortality rates for the first 30 days or first year, respectively, after developing *Campylobacter* infection.[81] Population-based studies in England, the United States, and Sweden showed a bimodal age distribution, with a peak of illness occurring in children younger than 5 years of age and a second peak at 15 to 29 years of age.[172,202] The highest isolation rate occurs in the first year of life (see Fig. 142–3).

In less industrialized regions, *Campylobacter* is found in association with childhood diarrhea in 8 to 45 percent of cases, but it is isolated at similar rates from healthy children.[33,39,64] The highest rates of *Campylobacter* isolation are in children younger than 5 years of age.[33] As many as 75 percent of *Campylobacter* infections occurring during the first year of life are asymptomatic.[39]

In industrialized regions, most sporadic cases occur because of handling, preparation, and consumption of contaminated raw or undercooked poultry.[21,32,44,87,184,192,202] Raw milk and contaminated water less frequently are sources (see Table 142–1).[90,96,133,204,222] Poor kitchen hygiene plays a role in transmission; the risk of acquiring infection is inversely related to the frequency of using soap to clean cutting boards.[202] Barbecues represent a special hazard because they permit easy transfer of bacteria from raw meat to hands and other food and from there to the mouth.[33] Sporadic cases of *C. jejuni* infection occur much more frequently than do outbreaks.

Campylobacter is transmitted usually by a contaminated food or water vehicle. However, direct transmission of the agent may occur, usually to individuals with direct exposure to reservoir animals or to those who process contaminated animal products.[30] Person-to-person spread may occur in areas of high contamination, such as where diapered children are present.[155] In addition, intrapartum transmission is documented.[30,218] Asymptomatic mothers may transmit the infection to their newborns.[30]

In developing countries, transmission is multifactorial. Free-roaming poultry, toddlers, unsafe water supply, and lack of adequate disposal of excreta are documented sources.[69] However, proving that *Campylobacter* cause disease in regions where the

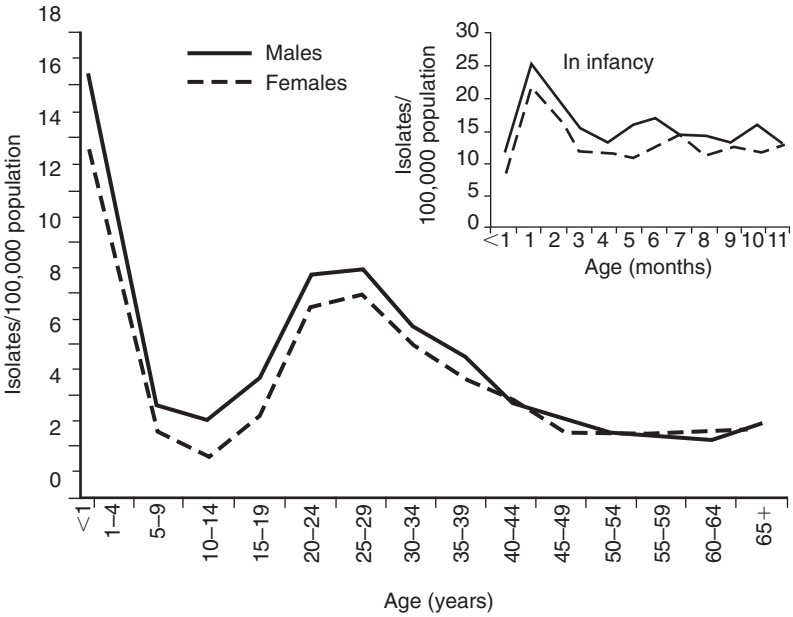

Figure 142–3 Annual isolation rates of *Campylobacter* by age and sex, United States, 1982 to 1986. *(From Tauxe, R. V., Hargrett-Bean, N., Patton, C. M., et al.: Campylobacter isolates in the United States, 1982-1986. Morb. Mortal. Wkly. Rep. CDC Surveill. Summ. 37:1-13, 1988.)*

agent is environmentally pervasive is difficult. Confounders include statistically indistinguishable rates of isolation from diarrhea cases and matched controls (see Table 142–2) and direct evidence that the presence in a household of a *Campylobacter* shared between humans and chickens is associated with protection from diarrhea.[144] Improved knowledge of the immune responses that protect from *Campylobacter* infection and disease and knowledge of the components of *Campylobacter* required to cause disease are needed to understand the agent's epidemiology better.

Because of the biochemical inactivity of *Campylobacter* organisms, discriminating species and subspecies by conventional chemotaxonomic methods is problematic. The consequences of this difficulty include ambiguities in epidemiologic investigations and low resolution in studies of mechanisms of virulence and immunity. Effort is being invested in devising genotyping schemes.[47,146,223]

PATHOLOGY

Most *C. jejuni* infections are not associated with illness or notable pathologic features. When illness does occur, watery diarrhea, invasive enteritis, or systemic infection may result. The spectrum of pathology reflects this range of manifestations. Acute watery diarrhea may occur in the absence of grossly visible pathologic features. Acute inflammation of the colon and rectum is the hallmark of *C. jejuni* invasive enteritis,[125] although hemorrhagic jejunitis and ileitis may also occur.[54,109,113,189] In patients who have undergone proctoscopy, normal mucosa is found in approximately 50 percent; in the remainder, mucosal edema, congestion, friability, and granularity are seen. The spectrum of histologic changes ranges from minimal edema with acute and chronic inflammatory cells and no vascular congestion to moderate inflammation and cryptitis to crypt abscess formation.[12] Acute appendicitis, mesenteric lymphadenitis, and ileocolitis have been reported in patients who have undergone appendectomies while they were infected with *C. jejuni*.[23] *C. jejuni* infection is at low frequency a precursor to immunoreactive complications wherein disease results from immunologic effectors generated in response to infection that damage neurons. The demonstration that, after adjustments for co-morbidities, *Campylobacter* infection is associated with increased mortality over the course of a 1-year period after infection suggests that as yet unidentified severe late disease occurs.[81] *C. jejuni* is one of a few microbes that have been hypothesized as triggering a common mechanism leading to lymphomas.[75]

PATHOGENESIS

The mechanisms by which *C. jejuni* causes diarrhea, dysentery, and, less frequently, systemic disease are not understood well. Evidence consistent with the production of a heat-labile enterotoxin has been presented.[73,100,123,174] However, this toxic activity is not universally associated with isolates from individuals who have *C. jejuni* illness. Some strains produce a cytotoxin,[101,124] but its relevance to disease has not been established. Some *C. jejuni* can invade various cultured cell lines.[32,55] Motility, surface structures, and molecular adaptations have been associated with capacity to cause disease,[13,14,60,61,74,82,107,136] but the pattern of their expression in disease-associated isolates is not uniform. Dogs,[166] rhesus,[57] rabbits,[195] mice,[57] and hamsters[92] have been evaluated as models of *Campylobacter* enteritis, but none faithfully reproduces the disease seen in humans. A mouse intranasal challenge model[16] has been developed for use in studies of *C. jejuni* invasiveness, and a ferret model[17] has been developed for studies of *C. jejuni*-induced diarrhea.

In vitro studies demonstrate a capacity to disrupt tight junctions of intestinal epithelial cell monolayers and preferentially induce proinflammatory cytokine responses. These capacities and the demonstrated microtubule-dependent eucaryotic cell evasion mechanism[114] may contribute to establishment and maintenance of *C. jejuni* in the gut.[36]

IMMUNITY

The preponderance of evidence of protective acquired immune responses to *C. jejuni* comes from studies of children in developing countries. Such children have more frequent symptomatic infections at younger ages; with increasing age, the rate of symptomatic infection decreases.* The number of *C. jejuni* organisms

*See references 20, 22, 24, 102, 103, 105, 127, 198, 203, 205.

excreted per gram of stool of infected individuals also declines with increasing age,[205] as does the duration of excretion of the organism.[203] These phenomena parallel increasing titers of *Campylobacter*-specific antibodies.[22,24,102,127,198,205,210] Additional inferential evidence includes that breast-feeding is associated with reduced frequency of *C. jejuni* diarrhea.[173,213] Adult volunteers who became ill after a first challenge were protected from illness after repeat challenge with a homologous strain,[18] and resistance related to the presence of anti *Campylobacter* antibodies. Prolonged, severe, and sometimes recurrent infections occur in immunodeficient patients.[128,132,161] Hypogammaglobulinemic patients have difficulty clearing *Campylobacter* organisms. Patients with late-stage acquired immunodeficiency syndrome (AIDS) are at increased risk of acquiring severe relapsing *Campylobacter* infection; patients with early stages of human immunodeficiency virus (HIV) infection and high CD4 counts are not at risk. Immunity to disease may not protect against asymptomatic colonization. The bacterial components against which the protective immune responses are directed are not known. The sum of this evidence forms the basis for attempts to develop *C. jejuni* vaccines.

Very little information is available on cellular immune responses to *C. jejuni*. Cellular responses may play an important role in facilitating the formation of antibody and in clearing intracellular *C. jejuni* from eukaryotic cells.

Despite the mass of evidence indicating protective roles for immune responses, the *Campylobacter* components against which protective immune responses are targeted are not known. Similarly, attempts to relate classic serotyping schemes, flagellar types, or presence or absence of virulence factors with disease-causing capacity have yielded associations of poor strength. The completion of the genome sequence of *C. jejuni*, NCTC 11168, in 2000[154] enabled studies to be pursued that are beginning to explain the relationships between bacterial components and immune responses. The *Campylobacter* glycome is remarkably plastic,[108] and *Campylobacter* spp. produce a surprising variety of carbohydrates (≈8% of the genome is dedicated to the biosynthesis of surface carbohydrates). Furthermore, multiple mechanisms exist for sequence variation for genes encoding the glycome. In a clinical trial, researchers demonstrated that passage of *C. jejuni* in volunteers can be associated with phase variation in lipo-oligosaccharide.[165]

CLINICAL MANIFESTATIONS

C. jejuni produces a spectrum of manifestations, the most common of which is enteritis. Bacteremia, other systemic manifestations, and perinatal infections occur infrequently.

ENTERITIS

Children with *Campylobacter* enteritis may have unformed stools, watery diarrhea, inflammatory diarrhea, or a combination of these symptoms.[5,21,23,33,109,149,189] Inflammatory diarrhea can be so severe that it is misdiagnosed as inflammatory bowel disease.[189] Inflammatory diarrhea is a more common occurrence in industrialized countries, and secretory watery diarrhea more typically occurs in developing areas. Patients with ciprofloxacin-resistant strains have longer duration of diarrhea than do patients infected with sensitive strains.[143]

Most cases of enteric illness subside within 7 days, although 20 to 30 percent last for 2 weeks and a few (5-10%) may persist longer, with a relapsing course lasting for weeks.[109,149] In one third to one half of patients, the initial symptoms are periumbilical cramping, intense abdominal pain, malaise, myalgia, and headache. An acute abdomen or appendicitis may be suspected at first[34] because acute abdominal pain occasionally may be the only initial symptom; pseudoappendicitis or mesenteric adenitis and terminal ileitis can be found.[158] The pain may be mild and intermittent for several weeks, and vomiting is a common occurrence. Secretory diarrhea with 10 or more profuse, watery stools per day may be present. Because this course occurs commonly in younger children, dehydration frequently (10%) is an outcome. Relapse of symptoms may occur.

The symptoms of inflammatory diarrhea are similar to those caused by *Shigella*, invasive *Escherichia coli*, and *Salmonella* and consist of generalized malaise, fever, abdominal cramps, tenesmus, bloody stools, and the presence of fecal leukocytes on light microscopy.[125] Fever without other symptoms may develop and can be associated with febrile seizures.[225] Toxic megacolon with massive bleeding may occur.[71,106,179] In neonates, blood-streaked formed stools or hematochezia may be associated with the isolation of *C. jejuni*.[71,106] The abdomen is tender, especially in the right lower quadrant. Splenomegaly occurs rarely.

EXTRAINTESTINAL INFECTIONS

Bacteremia with *C. jejuni* occurs much less commonly than does enteritis. Bacteremia was recognized first in malnourished children, patients with chronic illness or immunodeficiency, and patients at the extremes of age.[2,72,132,168] Cirrhosis, cancer, immunosuppressive therapy, and HIV infection commonly are underlying conditions in patients with bacteremia.[162] These findings led to the view that *C. jejuni* bacteremia was a disease of the relatively immunoincompetent patient. However, most *C. jejuni* blood isolates are from healthy individuals who often have histories of recent gastrointestinal disease.[190] The average incidence of *Campylobacter* bacteremia in England and Wales is 1.5 per 1000 intestinal *Campylobacter* infections. The Centers for Disease Control and Prevention (CDC) reports that only 0.4 percent of *C. jejuni* isolates in the United States are from blood cultures.[202] Most *C. jejuni* strains are susceptible to killing by serum, a finding that perhaps explains the transient nature of the bacteremia and its tendency to resolve without specific therapy. In HIV-infected patients, *Campylobacter* bacteremia occurs more frequently and with increased morbidity and higher mortality rates.[126,162,206] Fatal cases have been reported in the absence of enteric disease.[126]

The main reason for the increased recognition of extraintestinal *Campylobacter* infections appears to be the growing application of appropriate microbiologic culture methods. The incidence of *C. jejuni* bacteremia probably remains underestimated. Typically, blood cultures are not performed in individuals with the primary complaint of diarrhea. Rarely,[164] cholecystitis, urinary tract infection,[40] pancreatitis,[62] hepatitis,[115] and meningitis[49,210] can result from *Campylobacter* infection.

PERINATAL INFECTIONS

Occasionally, abortion or stillbirth, premature labor, neonatal sepsis, and meningitis caused by *C. jejuni* have been described.[156] *Campylobacter*-associated second trimester abortion generally is preceded by mild gastroenteritis.[138,187] The placenta may have areas of necrosis, infarction, microabscesses, and inflammation. The most likely route of placental/fetal infection is through the bloodstream, although a case with possible ascending spread has been reported.[45] Infected infants often are premature. Illness in neonates generally is mild or asymptomatic, but symptomatic gastroenteritis and asymptomatic bloody diarrhea caused by *C. jejuni* have been reported in newborn infants.[28,149] Bacteremia and meningitis also may occur.[68,210] The source of the organism in these cases usually has been the mother, who may be symptomatic or asymptomatic at the time of delivery.[28,186]

IMMUNOREACTIVE COMPLICATIONS

An episode of *C. jejuni* infection may be followed by immunoreactive complications such as Guillain-Barré syndrome (GBS),[79,80,104,134,171] Reiter syndrome,[99,160,163] reactive arthritis,[51,164,177] and erythema nodosum.[11,59,194] A preceding *C. jejuni* infection has been documented by serologic methods or stool culture in 12 to 60 percent of patients with GBS.[3,79,80,91,110,134] *C. jejuni* infection is the most common identified causal factor for GBS.[140,169] However, the risk of development of this syndrome after having a *C. jejuni* infection is less than 1 percent.[4] *Campylobacter*-associated GBS is associated with axonal degeneration and poorer outcome than in GBS associated with other causes.[169] During the 2 months after a symptomatic episode of *C. jejuni* infection, the likelihood of GBS is approximately 100 times (30.4 per 100,000) higher than the risk in the general population (0.3 per 100,000).[129] In a nested case-control study from 1991 to 2001 using data from the United Kingdom General Practice Research Database, 20 percent of GBS cases were attributable to *Campylobacter*.[199] GBS syndrome appears to be an age-related risk; one study found no cases in patients less than 20 years of age, 14 per 100,000 in patients aged 20 to 59 years, and 248 per 100,000 infections in those older than 60 years of age.[129] Certain serotypes of *C. jejuni* are associated more frequently with the subsequent development of GBS.[6,117]

Molecular mimicry between G_{M1} ganglioside and *C. jejuni* lipo-oligosaccharide is established as one of the causes of GBS.[227] A positive correlation of serologic evidence of *C. jejuni* and the presence of antibody to G_{M1} has been described.[70,98] Lipopolysaccharide extracted from *C. jejuni* was found to have core oligosaccharide resembling human ganglioside G_{M1}. Pure motor neuropathy with a tendency for more distal weakness and sparing of the cranial nerves has been associated with *C. jejuni* infection in patients with anti-G_{M1} antibody.[4,209] *Campylobacter* carrying the cst-II sialy transferase gene is associated with the development of GBS and Fisher syndrome and gene polymorphisms may determine which syndrome develops after having a case of *C. jejuni* enteritis.[227] Cases of Miller-Fisher syndrome, a polyneuritis variant characterized by ophthalmoplegia, areflexia, and cerebellar ataxia, also have been reported in association with *C. jejuni* infection.[97,228] Patients with Miller-Fisher syndrome often have antibodies to ganglioside G_{Q1b}. Reactive arthritis may be associated with *Campylobacter* enteritis, especially in adults with human leukocyte antigen (HLA)-B27.[51,177]

Campylobacter reactive arthritis may occur in 1 to 5 percent of patients infected.[164] The arthritis starts a few days to several weeks after the episode of diarrhea. Involvement of joints can be monarticular or multiple, as well as migratory, and both large and small joints can be affected. Synovial fluid is sterile, and fever and leukocytosis are absent; the duration ranges from 1 week to several months. The course is self-limited, and the prognosis is good.[77]

Severe, persistent, and relapsing *C. jejuni* infections have been reported in patients with immune deficiencies, including congenital and acquired hypogammaglobulinemia and malnutrition.[2,89,132] In patients with AIDS, increased frequency and severity of *C. jejuni* infection have been reported; the severity correlates inversely with the CD4 count.[120,159,161]

DIAGNOSIS

The initial characteristics of *C. jejuni* enteritis are not sufficiently unique to permit the diagnosis to be established on clinical grounds. The differential diagnosis should include *Shigella*, *Salmonella*, invasive *E. coli*, *E. coli* O157:H7, *Yersinia enterocolitica*, *Aeromonas*, and *Vibrio parahaemolyticus* infections, and amebiasis. Consideration should be given to pseudomembranous colitis caused by *Clostridium difficile* if the patient has been receiving antibiotic therapy. Fecal leukocytes are found in as many as 75 percent of cases of *Campylobacter* enteritis; gross or occult fecal blood is present in 50 percent.[18,21,125] White blood cell counts usually are normal, although a shift to the left may occur. Mild elevations in alanine aminotransferase, alkaline phosphatase, and the sedimentation rate are observed in as many as 25 percent of patients.

Methods for demonstrating *C. jejuni* include direct microscopy,[150,152] bacteriologic culture, antigen detection by electroimmunoassay (EIA) or enzyme-linked immunosorbent assay (ELISA),[84,178,212] DNA probes,[207] polymerase chain reaction (PCR),[98,118,147,216] and serology.[5] Detection of antigen by EIA is nearly as sensitive and specific as is culture. ELISA using recombinant P18 and P39 as antigens has demonstrated 91.9 percent sensitivity and 99 percent specificity.[178] PCR enables some culture-negative *Campylobacter* infections to be diagnosed.[118,119] Serologic tests appear useful for epidemiologic investigations but are not recommended for routine diagnosis.

C. jejuni can be detected by darkfield and phase-contrast examination of fresh suspensions of stool. The distinguishing characteristic of *Campylobacter* is darting motility. Gram stain of stool showing *Vibrio* forms is said to be useful in making a presumptive diagnosis.[176] Using the direct carbol-fuchsin Gram-stain method, Wang and Murdoch[221] reported 89 percent sensitivity and 99.7 percent specificity when examining stool samples of *Campylobacter*-infected patients. The indirect fluorescent antibody test can be used for identification of *Campylobacter* on smears; however, standardized reagents for this procedure are not available from commercial sources.

Establishing a definitive diagnosis of *C. jejuni* infection requires the demonstration of *C. jejuni* in stool or in a tissue sample. Unfortunately, not all laboratories culture for *C. jejuni*, despite its frequency. Culture of *C. jejuni* from stool requires special methods and special media. It can be accomplished with media that contain antibiotics[52] to which *Campylobacter* organisms are resistant. If culturing is to be done on medium free of antibiotics, diluted stool samples should be passed through a cellulose acetate membrane filter to reduce the number of other enteric microorganisms.[68] Inoculated plates should be incubated in 5 percent oxygen and 10 percent carbon dioxide at 42° C. Colony formation may not be grossly visible until 72 hours after plating. Identification of colonies as *C. jejuni/coli* is based on a Gram stain showing characteristic morphology and positive catalase and oxidase reactions. Hydrolysis of hippurate establishes an isolate as meeting the conventional inclusion criteria for *C. jejuni*. Routine media usually are adequate for isolation of *Campylobacter* from normally sterile body fluids and tissues. PCR analysis performed on fixed, routinely processed colon biopsies is an excellent diagnostic method for detecting *C. jejuni* from focal active colitis cases. An advantage of the PCR on tissue is that it can be done retrospectively from the paraffin block.[180]

TREATMENT

Most *C. jejuni* organisms are susceptible to macrolides, aminoglycosides, chloramphenicol, imipenem, and clindamycin and are resistant to cephalosporins, tetracyclines, rifampin, penicillins, trimethoprim, and vancomycin.[188,215] However, the development of resistance to macrolides is starting to emerge, with case reports worldwide.[65] Patterns of antibiotic resistance in *C. jejuni* show regional differences. In the past, quinolones were used as empiric therapy for adult traveler's diarrhea because of their good microbiologic activity against *Campylobacter*, *Shigella*, and *Salmonella* strains.[4,50,175]

However, since the late 1980s, *Campylobacter* strains have become increasingly resistant to fluoroquinolones worldwide,

TABLE 142–4 Antibiotic Resistance Pattern of *Campylobacter jejuni* Isolates

Location	Study Years	No. Tested	Percentage of Isolates Resistant to			
			Erythromycin	Fluoroquinolones	Tetracycline	Gentamicin
Netherlands[177]	1994-1997	1315	2	11-29	7-15	ND
Minnesota[172]	1994-1998	4953	ND	1.3-10.2	ND	ND
Spain, Barcelona[149]	1995-1998	909	5	81	72	1
Canada[53]	1995-1997	158	0	12.7	56	ND
Spain[158]	1997-1998	537	3.2	75	ND	0.4
Taiwan[106]	1994-1996	93	10	52	95	1
Thailand[73]	1995	57	ND	84	ND	ND

ND, no data.

except in Australia (Table 142–4). The emergence of resistance has been associated with the use of quinolones such as sarafloxacin, difloxacin, and enrofloxacin in veterinary medicine in Europe and the United States.[52,53,95,191] The association of human infection with fluoroquinolone-resistant *Campylobacter* spp. and consumption of poultry prompted the U.S. Food and Drug Administration (FDA) to withdraw enrofloxacin for use in poultry, effective September 2005.[142] Despite this measure, it will take time for fluoroquinolone-sensitive *Campylobacter* spp. to be reestablished in the environment.

In general, patients with quinolone-resistant isolates have a longer duration of diarrhea than do patients with fluoroquinolone-sensitive isolates.[191] Fluoroquinolone resistance in *C. jejuni* appears to be related to mutations in the genes encoding subunits of DNA gyrase.[88] The frequency of erythromycin-resistant *Campylobacter* isolates is low[175] (see Table 142–4); therefore, it remains the drug of choice in adults, as in children. Most patients with *C. jejuni* enteritis have mild symptoms and do not require antibiotic therapy. For these patients, oral rehydration and replacement of electrolytes are sufficient. Patients who may benefit from antibiotic therapy are those with fever, bloody stool, and symptoms lasting longer than a week.[5] Patients with HIV infection or other immunodeficiency syndromes should be treated.

Data on antibiotic treatment are controversial. A recent meta-analysis on the effects of antibiotic treatment on duration of symptoms caused by *Campylobacter* spp. looked at 11 randomized control trials. It concluded that antibiotic therapy (with erythromycin, ciprofloxacin, or norfloxacin) shortened the duration of intestinal symptoms by less than 2 days, especially if these drugs were given early in the course of disease.[208] In addition, antibiotic therapy shortened the excretion of *Campylobacter* spp. from feces.[208] All immunocompromised and bacteremic patients with *C. jejuni* infections should be treated with an appropriate antibiotic such as gentamicin, imipenem, or both drugs.[5]

PREVENTION

Tactics for prevention of *Campylobacter*iosis include breast-feeding and avoidance of raw food and food that has been cooked under conditions that permit the survival of bacteria or that has been handled in such a way that bacterial contamination may occur. Risks of foodborne illnesses including campylobacteriosis transmitted by restaurant-prepared meals may be reduced by mandating that food-service employees obtain training in food safety (e.g., thorough handwashing with soap and water after handling raw poultry or meat, cooking poultry to 180° F or until meat is no longer pink and juices run clear, separating raw poultry from other foods during preparation).[7]

A 24-month (2002 to 2004), population-based surveillance case-control study by FoodNet in the United States evaluated infants with laboratory confirmed *Campylobacter* infection and identified risk factors associated with their infection. Identified risk factors include drinking well water, eating fruits and vegetables prepared at home, having a pet with diarrhea in the home, visiting or living on a farm, riding in a shopping cart next to meat or poultry, and traveling outside the United States.[61] Prevention measures should then be targeted at the potential source. An interesting finding is that attendance at a daycare center did not pose an increased risk in this study.

Selected microorganisms may displace *Campylobacter* from its ecologic niches. This finding may prove useful in reducing *Campylobacter* contamination of food animals.[35,122,135,139] Attempts to develop vaccines against *Campylobacter*iosis continue, but no product is in advanced development.

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CHAPTER 143

Other *Campylobacter* Species

Robert J. Leggiadro

Although they are not isolated as frequently as are *Campylobacter jejuni* and *Campylobacter coli*, the "other" *Campylobacter* spp. are gaining recognition as human pathogens. *Campylobacter fetus*, a classic cause of perinatal infection, also is an infrequent cause of bacteremia in immunocompromised hosts. *Campylobacter upsaliensis*, *Campylobacter lari*, and *Campylobacter hyointestinalis* are associated primarily with diarrheal disease. Populations affected by these three species include normal as well as immunosuppressed hosts, especially persons infected with human immunodeficiency virus (HIV), with or without a history of animal exposure. The clinical spectrum of these organisms should expand as the special diagnostic tests needed to identify them become more widely available.

HISTORY

McFadyean and Stockman[59] first described the organisms now known as campylobacters in 1913. These *Vibrio*-like organisms were implicated as a cause of epizootic abortion in sheep, and a few years later, Smith[82] reported their association with bovine abortion as well and gave them the name *Vibrio fetus*. Although never confirmed microbiologically, these organisms are thought to have been *C. fetus* according to current nomenclature.[49] Vinzent and associates[94] first reported *Campylobacter* infection in humans in 1947. These investigators described a pregnant woman with *V. fetus* bacteremia who subsequently aborted at 6 months' gestation. In addition to pregnancy, gastrectomy, tooth extraction, heart disease, diabetes, and cirrhosis were predisposing conditions in King's 1957 review of 15 patients with *V. fetus* bacteremia.[51]

Many reports describing "new" *Campylobacter* spp. were published in the 1980s and early 1990s. *C. upsaliensis* was reported to be a pathogen in dogs and humans.[69,77] *C. lari*, a common isolate from healthy seagulls, was found to be a cause of gastrointestinal and extraintestinal disease in humans.[4,87] Originally identified in the intestines of swine with proliferative ileitis, *C. hyointestinalis* was reported first as a human pathogen in a homosexual man with proctitis.[26] The hydrogen-requiring campylobacters, *Campylobacter concisus*,[93] *Campylobacter rectus*,[72] and *Campylobacter curvus*, have been associated with periodontal disease. *Campylobacter sputorum* has been identified in abscesses,[64] as well as in bacteremia,[41,88] and *Campylobacter mucosalis* was reported in two children with diarrhea.[27]

Once called *Campylobacter*-like organisms, *Helicobacter cinaedi* and *Helicobacter fennelliae* now are classified in the *Helicobacter* genus.[66] These pathogens cause enteritis and proctocolitis in homosexual men and, on occasion, bacteremia.[68,90] Two former *Campylobacter* spp., *Arcobacter butzleri* and *Arcobacter cryaerophilus*, are associated with abortion and enteritis in cattle and pigs, in addition to bacteremia and diarrhea in humans.[50]

MICROBIOLOGY

Campylobacter is a Greek word meaning "curved rod." Members of this genus are gram-negative, curved, S-shaped or spiral, non–spore-forming rods that are 0.2 to 0.9 μm wide and 0.5 to 5 μm long.[64] Organisms are motile by means of a single polar flagellum, but some have a flagellum at each pole.[37] They are microaerophilic and have a respiratory type of metabolism.[64] Campylobacters are oxidase-positive and reduce nitrates but do not ferment or oxidize carbohydrates.[70] Although most grow at 37° C, *C. jejuni*, the *Campylobacter* sp. most commonly identified in humans, grows optimally at 42° C.[2]

Most *Campylobacter* spp. require a microaerobic atmosphere containing approximately 5 percent oxygen, 10 percent carbon dioxide, and 85 percent nitrogen for optimal recovery.[64] Some species such as *C. sputorum*, *C. concisus*, *C. mucosalis*, *C. curvus*, *C. rectus*, and *C. hyointestinalis* may require hydrogen for primary isolation and growth. Many different selective media for isolation of *Campylobacter* spp. have been developed, but because of species differences in antibiotic resistance patterns, no single formulation isolates all species of clinical importance.[37] For example, *C. jejuni* and *C. coli* are resistant to cephalothin, whereas *C. fetus* is susceptible. A filtration method with nonselective media may be used to complement direct culture on selective media for the detection of antibiotic-susceptible *Campylobacter* spp. Because of their small

TABLE 143–1 Growth and Biochemical Characteristics of Species of the Genus *Campylobacter*

	Growth							
Species	**25° C**	**37° C**	**42° C**	**Anaerobically**	**In CO_2 Inhibitor**	**Glycine 1%**	**Bile 1%**	**Charcoal Casein Deoxycholate**
C. lari	–	+	+	+	+	+	+	+
C. upsaliensis	–	+	+	+	+	–	+	+
C. fetus	+	+	(–)	–	+	+	–	–
C. hyointestinalis	(+)	+*	+	+	+	+	NA	NA
C. concisus	–	+	+	+	+	+	NA	NA
C. mucosalis	+	+	+	+	+	+	NA	NA
C. sputorum†	-	+	+	+	+	+	+	+

**Best at 35° C.*
†C. sputorum has three biovars with different biochemical characteristics.
+, positive; –, negative; (+), most strains positive; (–), most strains negative; NA, data not available or found; R, resistant; S, susceptible; TSI, triple sugar iron.
Adapted from Ruiz-Palacios, G., and Pickering, L. K.: Campylobacter and Helicobacter infections. In *Feigin, R. D., and Cherry, J. D. (eds.): Textbook of Pediatric Infectious Diseases. 3rd ed. Philadelphia, W. B. Saunders, 1992, pp. 1072-2084.*

size and motility, campylobacters may pass through filters with pores of 0.45 to 0.65 μm, whereas other enteric flora are retained.[2,64]

Although colonies may appear on plates within 24 to 48 hours, growth of campylobacters from stool may take as long as 72 to 96 hours. Primary isolation from blood may require 2 weeks.[2] Gram stain of young cultures reveals vibrioid forms, and longer incubation may yield spherical or coccoid bodies. *Campylobacter* spp. usually can be distinguished from one another on the basis of biochemical tests and growth characteristics (Table 143–1). Although the significant pathogens *C. jejuni*, *C. coli*, and *C. lari* cannot be discriminated reliably by the use of 16s rDNA data, these sequences provide a substantially improved basis for the identification and differentiation of *Campylobacter* spp.[36] Many investigators have applied molecular techniques to identify enteric campylobacters directly from stool.[9]

EPIDEMIOLOGY

Much needs to be learned about the epidemiology of *Campylobacter* spp. other than *C. jejuni* and *C. coli*, a group of organisms that made up only about 1 percent of *Campylobacter* spp. reported to the Centers for Disease Control and Prevention (CDC) from 1982 to 1986.[12] However, this study found that age-specific isolation rates of *C. fetus* parallel those of *C. jejuni* and *C. coli*; rates peak in infancy and increase in young adulthood, and *C. fetus* increases substantially in elderly persons. Seasonal distribution patterns of *C. jejuni*, *C. coli*, and *C. fetus* also were similar, with peaks occurring in warm months. In this surveillance study, *C. jejuni* and *C. coli* isolates were predominantly from stool, whereas 54 percent of *C. fetus* isolates with a known source were from blood.[12] The epidemiology and antimicrobial susceptibilities of 111 *C. fetus* strains isolated from 103 patients from 1983 to 2000 in Quebec, Canada, were determined.[91] The isolation site was blood in 69 percent of the patients, stools in 20 percent, and other body fluids, including aorta, bile, synovial fluid, cerebrospinal fluid, tubo-ovarian abscess, ascites, and pleural fluid, in 11 percent of these patients.[91]

C. fetus, an important cause of sporadic abortion in cattle and sheep, may be isolated from the intestines and genital tracts of these animals.[70] Contaminated food and water are the suspected sources of infection for sheep, cattle, and other animals, including goats, pigs, cats, dogs, hamsters, guinea pigs, antelopes, chickens, and turkeys.[30] A *C. fetus* strain with markers of reptile origin was isolated from the blood of a febrile patient with precursor T-cell acute lymphoblastic leukemia.[92] Although the source of *C. fetus* infection in humans generally is not apparent,[38] a 1970 review of *Vibrio fetus* infection in humans found that one third of patients had recent contact with animals or animal products and one third denied such contact; no information was available on the remaining third.[8]

C. fetus bacteremia generally occurs in immunosuppressed hosts (especially elderly men), pregnant women, and neonates.[73,89] Predisposing conditions include alcoholism or cirrhosis, diabetes mellitus, heart disease, malignancy, splenectomy, and corticosteroid or other immunosuppressive therapy.[21,45,73] *C. fetus* is not considered a major cause of gastroenteritis, perhaps because of the failure of this organism to grow in stool specimens evaluated by routine laboratory methods for *C. jejuni* and *C. coli*.[12,70]

The epidemiology of human *C. fetus* infection as a foodborne, perinatal infection was reflected in the original report by Vinzent and colleagues[94] that described a 39-year-old pregnant woman who had a history of drinking raw milk from a cow that recently had aborted a pregnancy; this woman contracted a flulike syndrome in the sixth month of pregnancy. Two blood cultures grew *C. fetus*, and after 5 weeks of illness, a stillborn infant was delivered. In addition to raw milk,[96] raw beef liver[84] and "nutritional therapy" (raw fruit, vegetable juice, and calves' liver, along with coffee enemas) have been associated with *C. fetus* infection.[13] The last report described nine patients with malignant disease and one with systemic lupus erythematosus in whom *C. fetus* sepsis was associated with such therapy. Nine patients received their "nutritional therapy" in Mexico, and one died.[13]

First associated with human disease in a homosexual man with proctitis, *C. hyointestinalis* (*hyos*, "hog"; *intestinalis*, "pertaining to the intestines") originally was isolated from the intestines of swine with proliferative ileitis.[26,70] *C. hyointestinalis* also has been isolated from the stool of persons with nonbloody, watery diarrhea.[23] Two of these patients were homosexual men, the third was an elderly woman who had been traveling in Egypt, and the fourth was an infant from a large farm family that drank raw milk. These organisms are closer to *C. fetus* by DNA hybridization than are any other catalase-positive *Campylobacter* spp. and are resistant to nalidixic acid but susceptible to cephalothin.[76]

C. lari, frequently isolated from apparently healthy seagulls, is nalidixic acid resistant and thermophilic.[81] The name is derived from *laridis*, "of a sea bird," although seagulls do not play a direct role in its epidemiology.[87] Epidemiologically and microbiologically similar to *C. jejuni*, *C. lari* has been reported to cause enteritis in patients with and without a history of animal exposure and bacteremia in two elderly patients with multiple myeloma and permanent pacemakers.[62,65,79,87] A waterborne outbreak of *C. lari*–associated gastroenteritis also has been reported.[10]

Catalase-negative or weak *Campylobacter* spp. that are hippurate-negative and thermotolerant were isolated first from dogs in 1983.[77] This *C. upsaliensis* group is associated with gastroenteritis, breast abscess, spontaneous abortion, and bacteremia in normal

						Susceptibility	
Oxidase	Catalase	Urease	Hippurate	Nitrate	H_2S (TSI)	Nalidixic Acid	Cephalothin
+	+	–	–	+	–	R	R
+	(–)	–	–	+	–	S	S
+	+	–	–	+	–	R	S
+	+	–	–	+	+	R	S
+	–	–	–	+	+	R	R
+	–	–	–	+	+	R	S
+	-	-	-	+	+	R	S

TABLE 143–2 Clinical Features Associated with Infection by "Atypical" *Campylobacter* and Related Species Implicated as Causes of Human Illness

Species	Common Clinical Features	Less Common Clinical Features	Additional Information
C. fetus	Bacteremia, sepsis, meningitis, vascular infections	Diarrhea, relapsing fevers	Not usually isolated from media containing cephalothin
C. upsaliensis	Watery diarrhea, low-grade fever, abdominal pain	Bacteremia, abscesses, abortion, hemolytic-uremic syndrome	Difficult to isolate because of cephalothin susceptibility
C. lari	Abdominal pain, diarrhea	Colitis, appendicitis, bacteremia	Seagulls frequently colonized; organism often transmitted to humans by contaminated water
C. hyointestinalis	Watery or bloody diarrhea, vomiting, abdominal pain	Bacteremia	Causes proliferative enteritis in swine
C. sputorum	Pulmonary, perianal, groin, knee, and axillary abscesses	Bacteremia	Three clinically relevant biovars: *C. sputorum* subspecies *sputorum*, *C. sputorum* subspecies *bubulus*, and *C. mucosalis*
H_2-requiring *Campylobacter**	Periodontitis	Diarrhea, osteomyelitis, bacteremia	Uncertain role as human pathogen

**Includes C. rectus, C. curvus, and C. concisus.*
Adapted from Allos, B. M., Blaser, M. J.: Campylobacter jejuni and the expanding spectrum of related infections. Clin. Infect. Dis. 20:1092-1099, 1995.

hosts, as well as opportunistic infections in immunocompromised persons.[39,42,69] Conditions predisposing to *C. upsaliensis* bacteremia include gallbladder surgery, ectopic pregnancy, kwashiorkor, and acquired immunodeficiency syndrome (AIDS).[69] Young puppies and kittens are potential transmitters of *C. upsaliensis*,[40] and an outbreak in a childcare center suggesting direct transmission between humans also has been described.[33,48] In a sample of *Campylobacter* recovered from patients with campylobacteriosis in Los Angeles County, California, in 1998, the second most frequently isolated species was *C. upsaliensis* (6 [4%] of 155 isolates).[55] Of the six patients with *C. upsaliensis* isolates, five had pets, including three dogs, a cat, and a turtle, at home.[55] Routine selective media for *Campylobacter* spp. may fail to detect this organism, which is slow growing and susceptible to cephalothin.[34,56,95] Filtration methods may improve the yield from stool cultures.[9,34,35,47,48,58,83]

PATHOGENESIS AND IMMUNITY

Information on the pathogenic and immune mechanisms involved in *Campylobacter* infections other than those caused by *C. jejuni* and *C. coli* is scarce. Much of what is known has been learned from animal, clinical, and epidemiologic data. The association of *Campylobacter* bacteremia with hypogammaglobulinemia, HIV infection, kwashiorkor, pregnancy, and malignancy indicates the importance of both humoral and cell-mediated immunity in host defense against this genus.[2,17,56,65,80,98] The predilection of *C. fetus* for endovascular surfaces in adults and the central nervous system in neonates also is well documented.[22,78]

In pregnant animals, *C. fetus* bacteremia occurs after ingestion of the organism, with subsequent development of infection of the placenta and fetus.[60,67] Examination of infected animal placentas has revealed necrosis, infarction, and microabscesses, along with disruption of the placental circulation.[18] Placental changes similar to these have been described in humans after preterm maternal bacteremia, consistent with infection as a result of hematogenous rather than ascending spread.[80] Ascending infection with premature rupture of membranes and amnionitis in the absence of maternal bacteremia has resulted in stillbirth or early-onset disease.[28,44] Contamination of the baby at the time of vaginal delivery is important in the pathogenesis of neonatal sepsis and meningitis with *C. fetus* in live-born infants.[57,97]

Bacteremia may occur more commonly with *C. fetus* than with *C. jejuni* because the former is resistant to the bactericidal effects of human serum, whereas the latter is susceptible.[6] A surface-layer protein that covers *C. fetus* functions as a capsule and appears to be an important virulence property of the organism.[5] It inhibits C3b binding, and this action explains both the serum resistance and the phagocytic resistance of *C. fetus*.[7]

CLINICAL MANIFESTATIONS

The clinical spectrum of non-*jejuni* or non-*coli Campylobacter* infections varies with the age of the patient and the individual species (Table 143–2). *C. fetus* is responsible for most reported

disease patterns, including prenatal, neonatal, bacteremic, and focal infections, caused by this "other *Campylobacter*" group of organisms.[89] Pregnancies complicated by maternal infection with *C. fetus* may result in abortion, stillbirth, and prematurity.[22,71,80] Live-born infants may suffer from sepsis and meningitis with a high case-fatality rate.

Mothers may have fever and chills with bacteremia alone or with diarrhea. Maternal blood, placenta, cervix, vaginal, and stool cultures have yielded *C. fetus* in reported perinatal cases.[22,57,80,97] Maternal outcome is excellent.

Torphy and Bond[89] reviewed eight infants 12 hours to 22 days old with reported *C. fetus* disease. The initial symptoms, including fever, cough, respiratory distress, vomiting, diarrhea, cyanosis, convulsions, and jaundice, were consistent with neonatal sepsis. Meningitis developed in all eight infants, and six died. Four were premature, and three of them had an onset of illness at 2 days of age or younger and died during the first week of life. However, a subsequent review reported three additional neonatal patients who survived *C. fetus* meningitis after contracting the disease at 1 to 3 days of age.[97] Hemorrhagic infarction and necrosis, as well as cystic degeneration of the cerebral cortex, are the cerebral lesions most commonly reported in *Campylobacter* meningitis.[97]

Descriptions of *C. fetus* infection in children outside the neonatal age group are rare.[52,96,98] One was a 2½-year-old child with *V. fetus* bacteremia who had low-grade fever for 3 weeks and a cervical mass on the day of admission and was treated successfully with penicillin.[96] Her past history included drinking raw cow's milk and untested well water. A 16-month-old girl from India whose father operated a dairy business was admitted for evaluation of fever lasting 10 days and seizures for 5 days before admission.[52] Her provisional diagnosis was encephalitis, and a blood culture grew *V. fetus*. No antibiotics were administered, and the patient recovered uneventfully. The authors of the report emphasized the undulant nature of *C. fetus* infection, similar to that of brucellosis. *C. fetus* bacteremia also was detected in a nearly 5-year-old boy with agammaglobulinemia who had a 3-week history of anorexia, lethargy, fever, and, more recently, hepatitis.[98] Blood culture grew *C. fetus*, and liver biopsy demonstrated hepatitis with multiple areas of severe focal necrosis, bridging necrosis, and Kupffer cell hyperplasia. He responded rapidly to ampicillin therapy.

Most reported patients with *C. fetus* infection are men older than 45 years of age who have bacteremia with or without focal infection.[21,73,89] Most of them have underlying conditions such as diabetes, malignant disease, and hepatorenal or cardiovascular disease.[38,45] Typically, illness begins with fever, malaise, and headache. Chills and night sweats are prominent, as is weight loss in prolonged illness. Diarrhea, nausea, vomiting, and abdominal pain occur in as many as 38 percent of cases, and hepatosplenomegaly or jaundice develops in two thirds.[38,98] Pulmonary involvement is a rare finding.[8,38]

Three patterns of invasive *C. fetus* disease have been described.[74] Clinical manifestations of the first localized infection accompanied by septicemia include meningitis,[11,57] endocarditis,[25] pericarditis,[63] thrombophlebitis,[14] mycotic aneurysm,[74] cellulitis,[29,45] gluteal abscess,[20] septic arthritis,[54] salpingitis,[11] and peritonitis.[86] The second form is transient asymptomatic bacteremia, which may be self-limited.[38,73,75] Prolonged and recurrent bacteremia, with waxing and waning symptoms as spontaneous relapses and remissions occur, is the third pattern of invasive *C. fetus* infection.[8,19,46,75,98]

The vascular tropism of *C. fetus*, especially in the presence of preexisting vessel damage, is well recognized.[14,63,98] Possible explanations for this predilection include a surface receptor on the organism with an affinity for vascular endothelium that results in endothelial damage and subsequent thrombus formation. In addition, the organism's microaerophilic growth requirements may be favored by venous oxygen tensions.[63] Previous valvular heart disease is a common finding in endocarditis.[25] *Campylobacter* infection of prosthetic devices is rare, with only four cases of *C. fetus* and one infection each of *C. lari* and *C. upsaliensis* described in the literature.[16]

A report from the CDC reviewed clinical and epidemiologic information on 12 patients with *C. upsaliensis* isolates from 1980 to 1986.[69] Eight isolates were from blood, and three were from stool. Ages of the 12 patients ranged from 6 months to 83 years. Two infants with *C. upsaliensis* bacteremia that responded to amoxicillin therapy were included. One was a 10-month-old child who had fever, leukocytosis, and a history of culture-negative diarrhea, bronchiolitis, and *Klebsiella* bacteremia 3 months previously. The second was a 6½-month-old infant with fever, respiratory distress, and erythematous tympanic membranes. A 14-month-old child who lived on a farm with a private well and several household dogs and cats had a history of pica, including dirt from ground where chickens roamed. Stool culture obtained for evaluation of febrile, watery diarrhea yielded *C. upsaliensis*, and he recovered after receiving erythromycin therapy.[69]

Underlying medical problems in adults with *C. upsaliensis* bacteremia included peptic ulcer disease and partial large-bowel resection for a benign tumor, perforated gallbladder with peritonitis, AIDS, corticosteroid therapy, ruptured ectopic pregnancy, and cirrhosis with pancreatic insufficiency and partial gastrectomy.[69] One adult with a *C. upsaliensis* stool isolate was a 35-year-old woman with relapsing acute myelogenous leukemia. She was ill with fever and blood-tinged, watery diarrhea while thrombocytopenic and neutropenic. A healthy, 20-year-old student with a history of drinking raw milk and swimming in fresh-water lakes and rivers was the second adult reported with a *C. upsaliensis* stool isolate. He had fever, severe cramping abdominal pain, and nonbloody, watery diarrhea of 3 weeks' duration that responded to oral erythromycin.[69]

Kwashiorkor and gastroenteritis were the predominant clinical features in a retrospective series of 16 pediatric patients with *C. upsaliensis* bacteremia from South Africa.[56] The age range was 2 to 36 months, with a mean age of 15.5 months. The authors suggested that *C. upsaliensis* bacteremia was secondary to intestinal infection with the same organism, but no confirmatory stool culture data were available.[56] A gastrointestinal source also was postulated for the *C. upsaliensis* isolated from a breast abscess in a previously healthy, 46-year-old woman.[31] Hemolytic-uremic syndrome was reported in a 14-year-old girl with *C. upsaliensis* gastroenteritis.[15]

C. upsaliensis was the only organism isolated from 83 patients in a large stool culture survey using a filtration system for *Campylobacter* in Belgium.[35] Ninety-two percent of patients had diarrhea, which was of acute onset in most cases. Vomiting (14%) and fever (7%) were uncommon occurrences, and symptoms generally abated in less than a week. Gross or occult blood was identified in 25 percent of cases, and neutrophils were seen on fecal smear in approximately 20 percent. Erythromycin (11 patients) or amoxicillin (2 patients) eradicated the organism, with resolution of symptoms in all 13 patients treated with antibiotics.[35] Australian workers identified *C. upsaliensis* in 19 (0.1%) of 18,516 stool specimens from August 1992 to March 1999 at the Royal Children's Hospital in Melbourne.[48] Infection with *C. upsaliensis* was associated with milder disease than was infection with *C. jejuni*; patients with *C. upsaliensis* infection had significantly less fever, diarrhea, and rectal bleeding.

Six clinical *C. lari* isolates were referred to the national *Campylobacter* reference laboratory at the CDC in 1982 and 1983.[87] Clinical illness associated with these isolates included enteritis in four patients, severe crampy abdominal pain in a 7-year-old girl, and terminal bacteremia in a 71-year-old man with multiple myeloma and chronic renal failure. The ages of the four patients

with enteritis were 8 months, 3 years, 22 years, and 39 years. Diarrhea was watery or mucoid, and fever was an unusual occurrence. Potential exposure included consuming chicken, having contact with house pets, drinking untreated surface water, and eating raw oysters. *C. lari* colitis also developed in an HIV-infected woman.[24]

C. hyointestinalis has been isolated from the stool specimens of adult and pediatric patients experiencing nonbloody, watery diarrhea[23] and from a rectal culture of a homosexual man with proctitis.[26] Over a 4-year period, 20 strains of *C. curvus* were isolated from two separate and distinct clinical settings: a hospital survey of infectious causes of bloody diarrhea and an outbreak of Brainerd (chronic) diarrhea in northern California.[1] *C. curvus* also was reported to cause polymicrobial liver and lung abscesses in two patients with cancer, respectively. *C. rectus* was reported in a polymicrobial breast abscess in a patient with lymphoma.[1] The clinical features of other *Campylobacter* spp. are presented in Table 143–2.

DIAGNOSIS

Confirmation of infection with *C. fetus* and *Campylobacter* spp. other than *C. jejuni* and *C. coli* is based on positive culture results from clinical specimens.[2] *C. fetus* has been isolated from blood, cerebrospinal fluid, joint effusions, bile, urine, and pleural and pericardial fluid in standard culture media.[38] Blood cultures generally are positive within 4 to 14 days. Isolation of *C. fetus* and "other" *Campylobacter* spp. from stool requires incubation at 37° C and media without cephalosporins. Filtration techniques also may be warranted to detect these strains in stool cultures.

TREATMENT

Gentamicin, erythromycin, and imipenem are bactericidal for *C. fetus*, as is ampicillin to a lesser extent.[25,32,43,61,91] Cefotaxime, ticarcillin, amikacin, chloramphenicol, clindamycin, tetracycline, and ciprofloxacin have variable activity against different *C. fetus* strains.[25,91] Reported synergistic antimicrobial combinations in vitro include ampicillin and gentamicin or cefazolin and imipenem with gentamicin.[25,85]

Erythromycin continues to be the drug of choice for most patients with *Campylobacter* diarrhea.[2] The newer macrolide azithromycin, which has a broader spectrum of activity than does erythromycin, is effective therapy for *Campylobacter* enteritis, as well as for diarrhea caused by *Salmonella*, *Shigella*, *Vibrio cholerae*, and *Escherichia coli*, thus rendering it a useful drug in the treatment of traveler's diarrhea.[53] Increasing *Campylobacter* resistance to quinolones related to expanded use in humans and in animals used for food, especially chickens, diminished the usefulness of quinolones such as ciprofloxacin in the treatment of *Campylobacter* gastroenteritis in adults.[2,24,53]

Gentamicin, imipenem, ampicillin, and cefotaxime are therapeutic options in treating *Campylobacter* bacteremia and other extraintestinal infections.[2,3,25,43,91,97] Synergistic combination therapy is indicated for patients with meningitis and endocarditis, in which bactericidal activity is critical.[2,61] Patients with *Campylobacter* in their stool who are being treated for an extraintestinal *Campylobacter* infection with gentamicin should be prescribed supplemental oral therapy because gentamicin is ineffective against *Campylobacter* in the gut.[2]

Prolonged antimicrobial therapy and follow-up blood cultures are warranted for patients with *C. fetus* bacteremia because of the relapsing nature of the illness.[63,75] Chloramphenicol should be used with caution in treating *C. fetus* meningitis because clinical outcome and in vitro susceptibility results for this drug have been disappointing.[57,61]

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CHAPTER 144

TULAREMIA

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Tularemia is an acute febrile illness caused by *Francisella tularensis*. Although it is primarily a disease of animals, humans also are highly susceptible hosts.

HISTORY

McCoy[103] published the first documented evidence of tularemia in 1911 when he described a plague-like disease in ground squirrels (*Citellus beecheyi*) that occurred in Tulare County, California. Within 2 years, McCoy and Chapin[104] isolated and characterized the organism *Bacterium tularense* from naturally infected ground squirrels. They also detailed the pathologic process produced in ground squirrels, defined the susceptibility of other animal species to *B. tularense*, and identified fleas as vectors for the plague-like disease in 1912.

The first description of tularemia in humans may be that by Homma Soken, a court physician in eastern Japan.[130] In 1837, he described an illness as "hare meat poisoning." Nearly a century later, Vail[162] and Wherry and Lamb[171] independently reported the first etiologically proven case of tularemia in humans in 1914. Thereafter, knowledge of the organism, susceptible hosts, modes of transmission, and clinical manifestations of disease was acquired rapidly, and retrospective information was assessed in light of this new information. Much of the current understanding of the disease in humans originated from the work of Edward Francis,[50,51] a U. S. Public Health Service surgeon. Intrigued by the new diseases, which were called "deer fly fever" by Pearse[119] in 1911 and "plaguelike disease" by McCoy, Francis relocated to Utah in 1919 and established his laboratory in an unused coal shed.[51,52,53] Soon thereafter, he recognized the singular cause of these two diseases and renamed them "tularemia" because of isolation of *B. tularense* from blood.[130] He isolated the organism from humans and jackrabbits[50] and demonstrated transmission of the organism by the deer fly.[52]

For a more complete historical review, the reader is referred to the classic paper by Dr. Edward Francis,[51] "A Summary of the Present Knowledge of Tularemia." Almost 8 decades later, this article is accurate and contains most of the knowledge essential for understanding tularemia.

ETIOLOGY

The causative agent of tularemia, after having been placed in the genera *Bacterium*, *Bacillus*, *Brucella*, and *Pasteurella*, is now named *Francisella tularensis* in honor of Dr. Edward Francis. *F. tularensis* is a small (0.2 to 1.0 μm × 1 to 3 μm), nonmotile, non–spore-forming, highly pleomorphic gram-negative coccobacillus. It is a strict aerobe that infects as a facultative intracellular bacterium.[74]

Hesselbrock and Foshay[69] described the morphology as resembling that of the pleuropneumonia group of organisms; the usual coccoid form is a spheroidal cystic structure with a delicate, transparent cell wall. The morphology and mode of reproduction (chiefly budding) suggested relationships with fungi and the pleuropneumonia group and none with the genera *Pasteurella* and *Brucella*. Subsequent electron-microscopic studies confirmed the presence of a delicate, almost transparent cell wall, which Eigelsbach and associates[32] suggested could explain the instability of lyophilized cultures.

The outstanding growth characteristic of these fastidious organisms is their requirement for cysteine or sulfhydryl compounds in amounts exceeding those usually present in nutrient media.[42] Although *F. tularensis* grows best on cysteine-glucose-blood agar and on coagulated egg yolk medium and less well in thioglycolate broth, it can be isolated in routine cultures and on enriched chocolate agar.[91,106] Hornick[72] suggested that the addition of cycloheximide and penicillin facilitates isolation of the organism from the respiratory tract or skin ulcers. In one study by Johansson and colleagues,[80] an Amies agar medium with charcoal and a Thayer-Martin medium with antibiotics were equal in preserving bacterial viability for 1 week when compared with saline or Stuart medium.

F. tularensis is killed readily by heat. Exposure to a temperature of 56° C for 10 minutes is sufficient for killing. The organisms are not destroyed by freezing and may remain viable in frozen animal carcasses for as long as 3 years. However, adequate cooking renders the meat of game birds and animals harmless. Treatment with tricresol solution (1%) for 2 minutes also kills organisms in tissue; organisms from cultures are killed in 24 hours by 0.1 percent formalin.

All strains of *F. tularensis* seem serologically identical, but individual strains may possess varying degrees of virulence. Ormsbee and associates[115] reported that the immunizing antigens seem to be concentrated in the cell wall. The purest cell wall preparations contain at least four and possibly six different antigens, but the soluble fractions did not seem to provide protection against any of these antigens when mice were immunized with these preparations.

Despite serologic homogeneity, four distinct subspecies of tularemia have been identified thus far. *F. tularensis* subspecies *tularensis* (Jellison type A) accounts for approximately 90 percent of organisms isolated in North America and has been seen in Europe as well.[65] The less virulent strain *F. tularensis* subspecies *holarctica* (Jellison type B) is found primarily in Europe and Asia. These two strains have been found to coexist in the same ecosystem.[101] *F. tularensis* subspecies *mediasiatica* has been isolated primarily in central Asian republics of the former Soviet Union,[114] whereas the subspecies *novicida*, which is isolated rarely, appears primarily to be restricted to North America. However, in 2003, *F. tularensis* subspecies *novicida* was isolated in Australia.[172] Chromosomal DNA analysis of *F. tularensis* show greater than 95 percent genetic identity among the four subspecies[11,56,83,158] Broekhuijsen and associates[11] showed that microarray analysis of chromosomal DNA supports the typical taxonomic clustering of *F. tularensis* into four separate subspecies. Worldwide analysis of chromosomal DNA reveals a highly diverse *F. tularensis* subspecies *tularensis* with a genetic affinity to the moderately virulent *F. tularensis* subspecies *mediasiatica*. Johansson and associates[82] identified two genetically distinct clades within the subspecies *tularensis*: A.I., which is genetically more diverse, and A.II.[44] In contrast, *F. tularensis* subspecies *holartica* strains have less genetic diversity within the subspecies. Increasing evidence indicates that *F. tularensis* subspecies *holartica* strains found in Japan may represent a fifth subspecies based on their genetic diversity and unique biochemical differences from strains found in Europe and Asia.[11,83,114,132,158]

The complete genome sequences of *F. tularensis* subspecies *tularensis* strain SCHU S4 and subspecies *holartica* strain OSU 18 reveal a circular chromosome of approximately 1.89 Mb with a G+C content of 32 percent and approximately 1800 to 1900 predicted coding sequences.[92,123] Of the 1804 predicted coding sequences in the SCHU S4 genome, 302 are unique to *Francisella* and 5 are unique to the SCHU S4 and are of unknown function. Much work needs to be done in identifying genes that endow the species their pathogenicity. One unique region that codes for 25 genes that have no known bacterial homologs is a 33.9-kb duplicated region known as the *Francisella* pathogenicity island (FPI).[92,123] Deletions of genes in this region have generated mutants that have difficulty with survival in macrophages.[61,63,90,107] The fastidious growth requirements of *Francisella* can be accounted for by the large number of inactivated genes. This situation results in the disruption of more than half of the predicted metabolic pathways in the SCHU S4 genome, which accounts for the requirement of 14 essential compounds for *Francisella* growth and survival. *Francisella* lacks receptors for classic iron-binding proteins but produces a ferric uptake protein, Fur. Iron is an essential nutrient in *Francisella* because genes regulated by Fur have been identified in the SCHU S4 genome.[92]

F. tularensis does not produce any exotoxin, but pharmacologic tests indicate that the organism contains a lipopolysaccharide (LPS) similar to that of gram-negative bacilli.[24] Unlike LPS of other gram-negative enteric organisms, the LPS of *F. tularensis* is 1000-fold less potent and does not activate macrophages or endothelial cells to release proinflammatory cytokines through toll-like receptors.[3,133,154] Characterization of the structure and function of LPS in different species of *F. tularensis* is in progress.[124,167] The O-antigens of the major subspecies *F. tularensis* that are pathogenic to humans are identical in their carbohydrate content and structure, a finding implying that the differences in virulence among subspecies probably is not the result of the O-antigen.[128,157,166,168]

F. tularensis also possesses a capsule composed of carbohydrates, mannose, rhamnose, and two dideoxy sugars yet to be described, along with proteins and alpha-hydroxylated 14:0 and 16:0 lipids.[70] The capsule provides protection from serum complement mediated lysis and may be differentially regulated intracelluluarly.[60,131,142]

The presence of type IV pili has been demonstrated using electron microscopy in the *F. tularensis* live vaccine strain (LVS). The expression of a type IV pili is independent of the presence of a capsule.[58] Genes for type IV pili are present in the genome of the pathogenic *F. tularensis* subspecies *tularensis* SCHU 4 strain are related closely to those found in *Neisseria* and *Pseudomonas aeruginosa* species.[92] Although the functions of type IV pili in other bacteria involve attachment to host cells, twitching motility, biofilm formation, and DNA uptake, many of these functions remain to be identified in *F. tularensis*. Forslund and associates reported that the absence of the pilin results in the decreased ability of the bacteria to spread effectively when introduced by peripheral routes.[48]

EPIDEMIOLOGY

Tularemia is ubiquitous in the Northern Hemisphere between 30 and 71 degrees of north latitude.[71] The disease has been reported throughout the United States, in East Asia, and in Europe.[54,110-112,149] *F. tularensis* has been found in Canada and Mexico but has not been reported in South America or Africa. Within the United States, the disease most commonly occurs in the south central region. From 1990 to 2000, three states (Arkansas, Missouri, and Oklahoma) accounted for approximately half of all reported cases.[16]

In the United States, the incidence of tularemia has been recorded since 1927. From 1990 to 2004, the incidence of tularemia was 0 to 0.06 per 100,000 persons.[16,17] The number of human cases declined steadily from 1939 (2291 cases[77]) until 1975 (129 cases). Since then, the number of reported cases of tularemia has increased, with a range between 90 and 310 cases annually.[17] This range probably is a gross underestimate of the actual incidence. In 1968, in a serologic survey of 1936 subjects in California, approximately 1 percent of this population had antibody against *F. tularensis*.[40] Using skin test antigens, Casper and Phillip[15] showed that 6.6 percent of 365 persons in eastern Montana had evidence of previous infection with *F. tularensis*. Of the persons in whom skin test results were positive, 80 percent had no previous history of the disease, a finding thus indicating a high number of subclinical or self-limited infections. The mortality rate for tularemia is zero to four cases per year.[17]

Persons especially at risk are hunters, trappers, meat processors, muskrat farmers,[174] cooks, lawn mowers and brush cutters,[45] sheep herders and shearers,[78] and laboratory workers.[138,163] The incidence of tularemia among American Indians/Native Alaskans (0.5 per 100,000) is significantly higher when compared with whites (0.04) and African Americans (<0.01). In all age groups, boys and men have a higher incidence of tularemia than do girls and women.[16] During 1990 to 2000, the average annual incidence of tularemia ranged from 0.06 to 0.1 per 100,000 in boys (0.03 to 0.06 in girls) 0 to 14 years of age, and the highest incidence occurred in boys aged 5 to 9 years (0.1 to 0.06 for girls). In comparison, adults more than 75 years of age have an incidence of tularemia higher than that of children.[16] Children acquire tularemia by the same routes as adults: vector bites, animal bites, and ingestion of infected, inadequately cooked meat.

Tularemia occurs year-round. However, peaks occur in the summer and winter months, depending on the region. May through August accounted for 70 percent of all cases of tularemia onset during 1990 to 2000.[16] Tularemia occurs more commonly in the central and southern states during the summer months, when ticks are more prevalent. The incidence in the northern and eastern states peaks in the winter months during the hunting season.

The most common sources of human infection are contact with infected animals or their carcasses and bites by ticks or tabanid flies (deer flies). Less commonly, people acquire the disease from the bite of a diseased animal or one in which the mouth has been contaminated by the ingestion of a diseased animal. Numerous outbreaks of human tularemia have occurred by water transmission,[8,53,79,86] in water contaminated by voles, beavers, lemmings, and muskrats. Infection also may occur by aerosolization of the organisms, especially in laboratory workers,[87,163] lawn mowers and brush cutters,[45] and occasionally farm workers (inhalation of dust and threshing material contaminated by voles and other rodents).[28,71] Person-to-person transmission has not been documented.

Hopla,[71] in describing the ecology of tularemia, states that "rarely does one encounter a zoonotic disease of such complexity." Indeed, to understand the development of epizootics and transmission to humans, one must consider the role of numerous vertebrates (humans and other animals, both wild and domestic) and many invertebrates.

Approximately 100 species of wild mammals, 9 species of domestic animals, 25 species of birds, and several species of fish and amphibians have been found to be infected naturally,[113] but probably fewer than a dozen species of mammals are important in the transmission of *F. tularensis*.[71] Lagomorphs (rabbits and hares) and some rodents (muskrats, voles) are highly susceptible to *F. tularensis*; sheep and domestic rabbits are susceptible but have low sensitivity to the organism; and cats, cattle, dogs, and horses are virtually insusceptible to infection. Vertebrate animals are called reservoirs, but they rarely are true reservoirs because

most of them become sick and either die or recover, with elimination of the organism. The varying hare (snowshoe rabbit) is less susceptible to *F. tularensis* and may serve as a reservoir because of its high natural resistance and carrier state.[49] Protozoa, which serve as reservoirs for many other intracellular pathogens, may do the same for *F. tularensis*.[1]

Many invertebrates can be infected experimentally by *F. tularensis*, but relatively few are infected naturally. In 1924, Parker and Spencer[117] first described ticks as vectors for *F. tularensis* infection in guinea pigs. However, the role of ticks in the spread of the human disease was not recognized until 1949, when Washburn and Tuohy[170] reported that 56 percent (391 of 704) of tularemia cases in Arkansas were associated with exposure to ticks. To date, ticks remain the most common vectors for *F. tularensis* in the United States and can serve as reservoirs because the organism can be transmitted from generation to generation by transovarian passage.[71]

At least 13 species of ticks have been found to be infected naturally by *F. tularensis*.[71] The species involved in transmission to humans are *Dermacentor andersoni* (wood tick), *Dermacentor variabilis* (dog tick), and *Amblyomma americanum* (Lone Star tick). A fourth tick, *Haemaphysalis leporispalustris* (rabbit tick), is important in the epidemiology of tularemia. Although this tick does not transmit tularemia directly to humans, it perpetuates the cycle of infection by acting as the sylvatic vector between rabbits.[68]

When the tick feeds on an infected animal, the organisms penetrate the gut into the hemolymph and are disseminated throughout the body, including the salivary glands. The tick then transmits the organisms by injecting them, along with saliva, when it feeds on another animal. In addition, transmission by tick fecal contamination also is likely. Ticks, biting flies (i.e., deer flies), and fleas probably are responsible for the continuing endemic disease in susceptible animals and for epizootic disease. Infrequently, mosquitoes and mites may transport *F. tularensis* mechanically from animal to animal or from animal to human.[71] Humans act as terminal hosts of *F. tularensis* because they do not transmit it to other humans or to other mammals.

A few cases of tularemia acquired by cat bite have been reported.[5,14,170] Because cats rarely are infected by *F. tularensis*, such transmission probably is the result of mechanical transmission from the contaminated teeth or claws of a cat that has come into contact with or has fed on an infected animal. Cat bites may be an important source of disease in children.

Ecogenetic analysis of *F. tularensis* in North America shows that the subspecies have specific geographic distributions across the continent.[44,144] *F. tularensis* subspecies *holartica* was found along the northwestern coast and along the tributaries of the Mississippi River. In contrast, the subspecies *tularensis* spanned the region from the east coast to Arkansas, Oklahoma, Texas, and Louisiana in the south; South Dakota in the north; and the Sierra Nevada in the west. Genetic analysis of the subspecies *tularensis* in this vast region revealed that type A.I. clusters east of the 100th meridian, whereas most type A.II. clusters to the west of this line.[144] This spatial separation of subspecies *tularensis* clades A.I. and A.II. coincides with the distribution of vectors *A. americanum* and *D. variabilis* in the east and *D. andersoni* and *Chrysops discalis* (deer fly) in the west. The spatial clustering of A.I. and A.II. also coincides with the distribution of an important host, cottontail rabbits, *Sylvilagus floridanus* and *Sylvilagus nuttallii*, respectively.[44]

PATHOGENESIS

BACTERIAL AND HOST INTERACTIONS

Much of the knowledge regarding interactions between *F. tularensis* and the host was derived from experimental studies using live attenuated strains. During World War II, live attenuated strains of *F. tularensis* were developed in the Soviet Union in an effort to produce a vaccine.[126,160] In 1956, a mixture of attenuated strains of *F. tularensis* was transferred from the Soviet Union to the United States. From this mixture, a strain of suitable virulence was selected, tested for safety and efficacy, and designated *F. tularensis* LVS.[33,35,76] This strain has been used extensively in experimental studies on the development of immunity in humans.

Capsulated *F. tularensis* organisms are thought to be opsonized by human macrophages and dendritic cells through the activation of complement, especially C3, and binding to the complement receptor 3 (CD11b/CD18).[7] The interaction of complement-coated *Francisella* with the macrophage membrane initiates an actin-dependent process then engulfs the bacteria into a unique asymmetric spacious pseudopod loop that forms the phagosome.[20,159] The bacteria resides in a moderately acidified phagosome (pH = 6.7) and accumulates the late endosomal markers (lamp-1, lamp-2, CD63). However, through as yet undefined process, phagolysosome maturation is not allowed to progress, and this prevents bacterial degradation by lysosomal hydrolases. The phagosomes are surrounded by a dense fibrillar coating that begins to disintegrate approximately 3 to 4 hours after infection, a process that allows the bacteria free access to the cytoplasm.[19] Virulence factors that facilitate this process remain to be identified. Mutation of genes in the FPI, especially *iglB* and *iglC*, have been shown to interfere with phagosome escape, macrophage growth, and virulence.[139] Approximately 24 hours after infection, replication of cytosolic *Francisella* induces apoptosis that leads to cell death and release of bacteria. Tularemia is followed by effective immunospecific protection of the host; reinfection has been documented in only nine persons.[13] Studies in which the infecting bacteria were suppressed by the use of a bacteriostatic agent such as tetracycline showed that protective immunity seems to be activated approximately 2 weeks after onset of the disease. When tetracycline treatment was initiated on the day of onset of tularemia and was given for 10 days, early relapses were common occurrences. When tetracycline treatment was administered for 14 days, relapses occurred less frequently, thus indicating that protective immunity had begun to arise.[135] Innate immunity involving interferon-γ (IFN-γ), tumor necrosis factor (TNF), and interleukin-12 (IL-12) and the cells that produce these cytokines (natural killer cells, neutrophils, macrophages) are thought to help control the initial phase of a primary infection by curtailing the replication of *F. tularensis*.[21] Approximately 2 to 3 weeks after infection, *F. tularensis* elicits both humoral and cell-mediated immune responses. Both responses reach maximal levels during the second week after infection.[18] Because *F. tularensis* is described as a facultative intracellular parasite, the general consensus is that long-term control and clearance ultimately depend on cell-mediated immunity, just as in infections with *Listeria monocytogenes* and *Mycobacterium tuberculosis*.[147,175] This finding was confirmed by several trials showing that vaccination with killed tularemia preparations, despite inducing agglutinating serum antibodies, provided poor protection.[13,76,134] Numerous studies showed that passive transfer of mononuclear leukocytes from infected animals to noninfected animals conferred resistance when the nonimmunized animals were challenged with a virulent strain.[140] Ultimately, the presence of tularemia-specific $CD4^+$ and $CD8^+$ T lymphocytes is responsible for clearance of the bacteria.[24,25,38,173] Infection with LVS disrupts the production of proinflammatory cytokines from human macrophages and endothelial cells.[47,155] This event is consistent with the lack of increase in proinflammatory cytokines, with the exception of a slight increase in IFN-γ during the early phase of the infection, in humans with ulceroglandular tularemia.[4]

The importance of the humoral immune response in tularemia is not very clear, although it is thought to be of minor significance.[37] Although the presence of immunoglobulin A (IgA) antibodies in nasal secretions correlates with resistance to infection by aerosolized organisms, earlier work by Bellanti and associates[6] showed that humoral immunity plays a minor role in resistance to tularemia. However, more recent animal studies showed that protection against tularemia LVS can be transferred by immune mouse serum and depends on host INF-γ, IL-2, and $CD4^+$ T cells.[74,129] Specific antibodies probably are involved initially in delaying the infection, thereby allowing time for the induction of cytokine production and specific T-cell–mediated immunity. This suggestion is consistent with the observation that although neutrophils have only a minor role in resistance to infection, mice depleted of $CD4^+$ T cells or $CD8^+$ T cells, or both, remained capable of controlling and partly resolving a primary sublethal *F. tularensis* infection.[24] Antibodies may function as opsonins for macrophages and neutrophils, but the level of anti-tularemia antibody does not correlate with protection.[153] Elkins and associates[36] showed that mice deficient in B cells do not succumb to sublethal doses of tularemia during a primary infection. However, in secondary infection, the presence of cytokines from B cells modulates the response of neutrophils against tularemia-infected hepatocytes and prevents the uncontrolled degranulation of neutrophils that may lead to severe tissue damage and poor clinical outcome.[10,23,27]

The role of neutrophils in the control of *Francisella* infection is thought to be minor but necessary. McCaffrey and Allen[102] recently showed that neutrophils, like macrophages and dendritic cells, rapidly opsonize *Francisella* but are prevented from mounting the respiratory burst to kill the organism. The organisms then escape to the cytosol of the neutrophils and replicate. However, studies in which neutrophils were depleted from mice showed that mice given a sublethal dose of the LVS or mice chronically infected with LVS became susceptible to infection and died.[140] Although the mechanism of action of neutrophils in these cases is unclear, it may involve direct lysis of LVS-infected hepatocytes.[23]

Persistence of immunity to *F. tularensis* depends on the T-cell response to a variety of antigens; this response may not be noted for as long as 2 weeks after exposure. Poquet and associates[127] showed that virulent strains of *F. tularensis* produce high levels of phosphoantigens that cause a proliferation in gamma-delta T cells to a greater extent than does exposure to the LVS. The reduced number of this T-cell subtype in vaccinated individuals could explain the lack of complete protection seen in vaccinated laboratory workers. As with membrane proteins stimulating an alpha-beta T-cell response, heat shock protein chaperones such as DnaK and Cpn60 also create a non-immunodominant proliferation of T cells in immunized individuals.[41] Denaturation of the protein or carbohydrate of a macromolecular antigen of *F. tularensis* has shown that T-cell reactivity is associated with only protein determinants, whereas human immune serum reacts mostly with carbohydrate determinants of the organism.[2,34,157]

INVASION AND DISEASE PRODUCTION

A single *F. tularensis* organism of a virulent strain can produce fatal infection in susceptible animals such as mice, guinea pigs, and hamsters.[176] As few as 10 organisms of a virulent strain injected intradermally or 25 organisms given by aerosol may produce systemic disease in human volunteers.[76] The organism may gain access to the human body through the skin, conjunctiva, oropharynx, respiratory tract, or gastrointestinal tract. It spreads by the lymphatics or hematogenously, and bacteremia usually develops during the first week of infection (3 to 12 days).[87] Infection commonly involves the skin, regional lymph nodes, liver, spleen, and lungs. Rarely, the gastrointestinal tract and the central nervous system also are involved.

Histopathologic examination of mammals experimentally infected with *F. tularensis* indicates that the organism disseminates and causes cellular changes in a manner typical of intracellular parasites. After the organism is introduced into a susceptible host, multiplication occurs locally, with early spread to regional lymph nodes occurring within 48 to 96 hours.[35,46] The developing cutaneous ulcer or soft tissue focus goes through a series of changes, in which polymorphonuclear leukocytes are replaced by macrophages. In time, necrosis, epithelioid cell infiltrates, giant-cell formation, and true granulomata may develop.[66,105,137] Organisms are difficult to demonstrate in tissue but occasionally are found at the periphery of lesions. In addition to the development of classic necrotic and granulomatous lesions, degeneration of the parenchyma in the liver and spleen may occur, as may marked hyperplasia of the reticuloendothelial system. These granulomata can organize to form microabscesses and abscesses. The pace of the illness depends on the virulence of the strain, as well as the inoculum size, portal of entry, and immune status of the host. When organisms enter the blood circulation, typical endotoxemia may ensue, sometimes in association with acute rhabdomyolysis.[85]

Autopsies of fatal cases of tularemia in humans have confirmed the findings in experimental animals.[62,97,121] Lymph nodes from patients with nonfatal disease have shown follicular hyperplasia with conglomerates of macrophages and caseating granulomata.[88,97,99,148] These findings are similar to those seen in miliary tuberculosis. In fact, tularemia and tuberculosis may be histopathologically distinguishable only because of the difference in timing of the development of tissue changes,[88] a difference related to the rapid replication of *F. tularensis*.

CLINICAL MANIFESTATIONS

Regardless of the portal of entry, the mode of onset of tularemia and the general features of the disease are the same. The usual incubation period of tularemia is 3 to 4 days, with a range of 1 to 21 days. The onset of symptoms is abrupt. Symptoms include fever, with a temperature usually higher than 39.4° C (103° F), chills, headache, myalgia, anorexia, vomiting, and occasionally photophobia. Fever may be continuous or biphasic, with an intermittent period of defervescence. In untreated patients, fever may persist for longer than 3 weeks. Physical findings usually include lymphadenopathy, hepatosplenomegaly, pharyngitis, and skin lesions. Temperature-pulse dissociation has been described in as many as 42 percent of cases.[43,55,118] A variety of skin rashes (e.g., maculopapular, vesicular, and pustular rashes, erythema nodosum,[122] erythema multiforme) may appear during the second week of illness and can last for a few days to several weeks.[87,151]

Laboratory studies, including a complete blood count, erythrocyte sedimentation rate, urinalysis, and *Proteus* OX2/OX19 titers, usually are not helpful in diagnosing tularemia. Sterile pyuria has been reported in patients with tularemia, 22 percent in one study[43] and 32 percent in another.[120] Sterile pyuria combined with a history of fever, dysuria, or low back pain had led to an erroneous diagnosis of urinary tract infection in 11 percent of the patients reported by Evans and associates.[43] Increased liver function test values with jaundice and atypical lymphocytosis also have been reported.[57,64] The six clinical syndromes of tularemia can be classified by the portal of entry: ulceroglandular, glandular, oculoglandular, typhoidal, oropharyngeal, and pneumonic.

ULCEROGLANDULAR TULAREMIA

Ulceroglandular tularemia, the most common form of the disease, accounts for more than 75 percent of all cases (adults and chil-

dren).[96] The organism gains access through the skin. Approximately 2 days after the onset of general symptoms, the patient complains of tender, swollen lymph nodes, most commonly in the axillary or inguinal areas. Within 24 hours, the portal of entry may become evident when a painful swollen papule develops distal to the regional node. This papule ruptures and leaves a punched-out ulcer with raised edges. The ulcer is indolent and, in untreated cases, may persist for longer than a month. The skin over the involved nodes may be inflamed. In untreated cases, approximately 50 percent of the lymph nodes suppurate and drain. In other cases, the nodes remain firm, enlarged, and tender for several months. Mild, generalized lymphadenopathy and enlargement of the liver and spleen may be present.

GLANDULAR TULAREMIA

The glandular form of tularemia is the second most common form of tularemia, and it accounts for 15 percent of all cases.[96] Glandular tularemia is almost identical to the ulceroglandular form, except the portal of entry cannot be identified.[22] The consensus is that the portal is an insignificant break in the skin. Isolated cases of cervical lymphadenopathy have been related causally to *F. tularensis* infection.

OROPHARYNGEAL TULAREMIA

The oropharyngeal form, which accounts for fewer than 5 percent of all tularemia cases,[96] resembles the ulceroglandular form. The infection is introduced into the oropharyngeal mucosa through infected, inadequately cooked meat. The organisms enter through abrasions, or aerosolization may occur during chewing. Local involvement consists of acute tonsillitis with cervical adenitis.[9] The tonsils may be covered by an exudate or membrane that extends in all directions and may resemble a diphtheritic membrane. Complaints of sore throat usually are out of proportion to the visible pathologic features, but ulcers sometimes are present. The cervical lymph nodes may suppurate. Infrequently, *F. tularensis* invades the lower portions of the gastrointestinal tract, in which case vomiting, diarrhea, and abdominal pain are prominent symptoms. Awareness of the ability of *F. tularensis* to cause oropharyngeal involvement is important for physicians caring for children.[73,95]

OCULOGLANDULAR TULAREMIA

In oculoglandular tularemia, which accounts for approximately 1 percent of all cases,[96] the portal of entry is the conjunctival sac, which may become inoculated by rubbing with contaminated fingers, having contact with infected water, splashing of infected liquids, or inhaling infected aerosols. The eyelids may become edematous and the conjunctivae inflamed and painful. Numerous small, sharply defined, yellowish nodules and ulcers may be present on the palpebral conjunctivae.[143] In some cases, corneal ulceration occurs. As seen in cat-scratch disease, the Parinaud complex of unilateral preauricular lymph node involvement and conjunctivitis can result.[143,145] The regional lymph nodes, the preauricular nodes, and the submaxillary and cervical nodes are swollen, tender, and painful.[122] In severe cases, the axillary nodes may be involved.[51]

TYPHOIDAL TULAREMIA

Typhoidal tularemia is manifested as fever of unknown cause. The symptoms are those of acute septicemia, with no localized skin lesions and often without lymphadenopathy. Patients are seriously ill, and shock may develop. The symptoms and signs of typhoidal tularemia are toxemia, continuous fever, myalgia, and severe headache. Patients may be delirious and may exhibit meningismus. Patients often complain of having severe pharyngeal pain, but pharyngeal lesions may not be evident. Diarrhea occurs in this form of tularemia in both adults and children. Sometimes, a dry cough and retrosternal pain are present. Pleuropulmonary involvement is a common finding in adults with typhoidal tularemia.[31] In children, typhoidal tularemia can be the result of ingestion of the causative agent, and necrotic lesions may be present throughout the bowel.[31] Inhalation of aerosolized organisms is a more common mode of acquisition in adults (laboratory workers, farmers).

PNEUMONIC TULAREMIA

The pneumonic form of the disease occurs most commonly in laboratory workers and is the most severe and lethal form.[76] Pneumonic tularemia may be acquired by the aerogenic route,[28,45,156] or pulmonary disease may be associated with other forms of tularemia, particularly the typhoidal type. Dienst[31] and Miller and Bates[106] published outstanding descriptions of pleuropulmonary tularemia. Miller and Bates stressed that the symptoms and signs of pleuropulmonary tularemia are nonspecific and vary with the location and degree of pulmonary involvement. The variable radiographic features may be confused with those of tuberculosis, mycotic infection, common bacterial pneumonia, lymphoma, or carcinoma of the lung. In the series of Miller and Bates[106] of 29 patients, pleural effusion developed in 6 patients. Pleural effusions have been found to contain more than 3 g of protein/100 mL and more than 1000 white blood cells/mm^3 with a lymphocytic predominance.[59] Because of the necrotizing nature of the pathologic process, the lung may heal with residual fibrosis or calcification. In other instances, the disease is so fulminant that death occurs before the pathologic features can progress fully.

ADDITIONAL CLINICAL MANIFESTATIONS

All large series of reported cases have included patients with subcutaneous nodules resembling those seen in sporotrichosis. The nodules usually are distributed on the anterior or posterior surface of the arm and may extend from the primary lesion to the regional lymph nodes. Initially, these nodules are firm and movable, but later they become fixed to the skin and may suppurate. They vary in size from less than a centimeter to more than several centimeters, if they become confluent. As many as 30 single nodules may be present. Other unusual manifestations of tularemia include pericarditis, appendicitis, peritonitis, liver abscess, cerebellitis with ataxia, meningitis, encephalitis, osteomyelitis, rhabdomyolysis, and venous thrombosis.

The incidence of these forms in children is somewhat different from that in adults. Although the pneumonic form previously was thought to be relatively rare in children,[95] more recent studies on the changing epidemiology and clinical manifestations of this disease showed that the occurrence of pneumonic tularemia is not uncommon.[75] In a 1985 study by Jacobs and associates,[75] 14 percent of tularemia cases in children were the pneumonic form. This percentage contrasts with the absence of pulmonary involvement in a series of 48 cases of tularemia in children reported by Levy and associates[95] in 1950. The distribution of these forms of tularemia also varies with the geographic location. In a report of 67 children with tularemia in Finland, 79 percent of the cases were of the ulceroglandular form, and 8 percent were glandular.[161] This distribution is significantly different from that in the United States. In the study by Jacobs and associates,[75] who

reported on 28 cases of tularemia in children, 45 percent were ulceroglandular, and 25 percent were glandular. The authors of the Finnish study attributed the higher proportion of the ulceroglandular form in their study to heightened awareness of the disease and early diagnosis. In addition, the differences also could be explained by the different strains of *F. tularensis* and different vectors in the two regions.

F. tularensis also can occur in the setting of an immunocompromised host. The pneumonic form of tularemia was reported in a patient with chronic granulomatous disease requiring lobectomy.[100] Pneumonic tularemia also was seen in a patient who previously had undergone peripheral blood stem cell transplantation for acute myelogenous leukemia (it resulted in a solitary pulmonary nodule) and in a patient infected with human immunodeficiency virus (HIV).[64,108] In addition, pneumonic tularemia was noted in a patient with a ventriculoperitoneal shunt.[125] Impairment of normal immunity could prevent adequate protection from *F. tularensis* and could necessitate more prolonged therapy.

DIAGNOSIS

The diagnosis of tularemia is established by a thorough history of possible exposure, clinical manifestations, and serial serologic tests. Obtaining a careful family history often is rewarding because several family members commonly are infected simultaneously. In some instances, however, a positive history never is elicited. Tularemia has no absolute pathognomonic features. In diagnosing tularemia, the physician must account for the endemic rate of the disease in the area, the season of the year, the clinical manifestations of the disease, the precise epidemiologic setting,[146] and the unresponsiveness of the disease to antibiotics that are not effective against tularemia.

The diagnosis is confirmed by the standard agglutination test, which is available commercially and is reliable. Unfortunately, it does not provide an early diagnosis because agglutinating antibodies usually are not detectable until the second week of illness. Occasionally, seroconversion is not confirmed until the patient has experienced 4 to 6 weeks of illness. In rare cases, agglutinating antibody may never be detected. A fourfold increase in convalescent titer confirms the diagnosis, but a presumptive diagnosis should be considered in patients with acute titers of 1:160 or greater. This titer may indicate current or past infection, but in a clinically suspicious case, it should be considered an indication for presumptive therapy. Titers of 1:1280 or greater often develop in patients with active disease as the initial manifestation of seroconversion.

The agglutination test is specific, but cross-reaction with *Brucella* and occasionally with cholera vaccine (in recent recipients) has been reported. It can easily be clarified by simultaneous testing with the individual antigens (tularemia and brucellosis). Antibiotic therapy does not prevent the development of agglutinating antibodies.

An enzyme-linked immunosorbent assay with bacterial sonicate antigen (ELISA-S) determines the presence of IgM, IgG, and IgA antibodies to *F. tularensis*.[165] This test has the advantage of confirming the diagnosis of tularemia earlier in the illness than does the agglutinating test. However, the ELISA test is not able to indicate when the onset of the disease occurred because the anti-tularemia antibody titers decrease slowly. Like other ELISA-based methods that have been developed recently, however, the ELISA-S test is not available commercially. *F. tularensis* also may be identified by direct fluorescent antibody or immunohistochemical stains of human specimens such as secretions or biopsies.[29,30]

Another method used for making the early diagnosis of tularemia is the whole-blood lymphocyte stimulation test.[89,150] During an epidemic in Finland in 1983, this technique was compared with the bacterial agglutination test in the diagnosis of 200 cases. The lymphocyte stimulation test yielded positive results in 21 percent of cases during the first week of illness and in 97 percent during the second week. In contrast, the bacterial agglutination test detected only 2 percent of the cases in the first week and 53 percent in the second week.[150] The skin test (Foshay) is an accurate method of diagnosis.[12] The skin test is positive earlier in the illness than is the agglutination test, but the skin test antigen is not available commercially.

Of the other diagnostic modalities recently evaluated, a polymerase chain reaction (PCR)–based assay holds promise for early detection of *F. tularensis* infection.[98] The PCR test has been shown to be effective in establishing the diagnosis of tularemia even when samples of a tularemic ulcer were obtained 1 to 14 days after infection.[141] When PCR and culture of the wound and blood were compared with agglutination tests, the sensitivity of PCR was 75 percent, and the sensitivity of culture was 62 percent.[80] In addition to increasing the safety for laboratory personnel, the PCR method was more sensitive and could detect infection at earlier stages. Compared with conventional PCR-based assays, real-time PCR (RT-PCR) has been shown to be more specific and 10 times more sensitive.[164] These assays are not available commercially at this time.

Based on PCR techniques, several assays have been developed to allow for the correct identification of *F. tularensis*. One such example is species level differentiation, which can be done by targeting the 16S rDNA and the *lpnA* gene of *F. tularensis*.[141] Real-time PCR analysis for a 30-bp sequence upstream of the gene for RNA helicase can differentiate between *F. tularensis* subspecies *tularensis* and subspecies *holartica*. In addition, several other high-resolution DNA analysis techniques exist mostly for the experimental purpose of species differentiation.[84]

In a Norwegian field study, a rapid immunochromatography assay proved inexpensive, time efficient, and portable when compared with PCR and ELISA, but this method was less sensitive than PCR.[8] This assay could accelerate identification and treatment decisions in clinical practice.

Gram-stained smears of patient specimens such as exudate and sputum usually do not reveal the organism. However, collecting the specimens poses no danger, and examination of direct smears helps to rule out other causative agents. Specimens should not be cultured for *F. tularensis* in the usual hospital or diagnostic laboratory because isolation of these organisms in facilities other than a level P-3 laboratory is hazardous to laboratory personnel. If confirmation by culture is indicated, physicians should notify the laboratory of the potential for the specimen to contain *F. tularensis*, so that appropriate laboratory precautions can be taken.

The differential diagnosis of tularemia depends on the clinical form of the disease. Ulceroglandular and glandular tularemia must be differentiated from disease caused by ordinary bacterial pathogens such as *Streptococcus* and *Staphylococcus*, from disease caused by *M. tuberculosis* and atypical mycobacteria such as *Mycobacterium marinum*, and from anthrax, HIV infection, and cat-scratch disease. In older patients with inguinal lymphadenopathy, lymphogranuloma venereum, granuloma inguinale, and other sexually transmitted diseases should be considered. Occasionally, sporotrichosis and infectious mononucleosis are diagnosed in these patients. Oculoglandular fever is somewhat more distinctive, but one must not rule out the possibility of disease caused by common bacterial pathogens, *L. monocytogenes* infection, herpes zoster, inclusion conjunctivitis, and keratoconjunctivitis. Oropharyngeal tularemia must be differentiated from streptococcal tonsillopharyngitis and corynebacterial disease. Typhoidal tularemia can be confused with ordinary bacteremia and must be differentiated from the more common bacterial and enteric disease, as well as from malaria, miliary tuberculosis, brucellosis,

and typhoid fever. Tularemic pneumonia must be differentiated from other bacterial as well as nonbacterial pneumonia, including tuberculosis, *Mycoplasma* infection, legionnaires' disease, psittacosis, viral pneumonia, Q fever, fungal infection, and chemical pneumonitis.

TREATMENT

Streptomycin traditionally is the drug of choice for the treatment of tularemia. The recommended dose is 30 to 40 mg/kg/day administered intramuscularly in two divided doses for 7 days. If a patient has mild symptoms initially or responds dramatically to therapy, an alternative streptomycin regimen of 30 to 40 mg/kg/day for 3 days followed by 15 to 20 mg/kg/day for 4 days may be given intramuscularly. In severe cases or if a child does not become afebrile and asymptomatic within a few days of therapy, extension of treatment beyond 7 days is indicated. Streptomycin-resistant strains of tularemia are reported, but they are rare.[116] Defervescence and alleviation of other signs and symptoms occur promptly, usually within several days. Response may be delayed if the lymph nodes have progressed to suppuration.

Because of the recent shortage of streptomycin in the United States,* alternative antibiotic regimens must be considered. Unfortunately, because of the lack of controlled clinical trials of the newer antibiotics for the treatment of tularemia, experience with alternative antibiotic regimens is limited. In a review of the various treatments of tularemia reported in the literature, Enderlin and associates[39] concluded that streptomycin remains the drug of choice, with a cure rate of 97 percent and no relapse. Gentamicin is an acceptable alternative to streptomycin, with a cure rate of 86 percent and a relapse incidence of 6 percent. These authors attributed some of the treatment failure with gentamicin to a delay in initiation of therapy and the short duration of therapy in some severe cases, as well as to other underlying medical problems.[39] The recommended dosage of gentamicin for tularemia is 5 mg/kg/day divided into two intramuscular doses.

Bacteriostatic agents such as tetracycline and chloramphenicol also have been used to treat tularemia, with cure rates of 88 percent and 77 percent, respectively. However, these agents are considered suboptimal for the treatment of tularemia because of a high incidence of relapse after therapy is stopped (12% for tetracycline and 22% for chloramphenicol).[39]

Although in vitro susceptibility testing indicates that the third-generation cephalosporins may be effective against *F. tularensis*, one report showed treatment failure in eight children given ceftriaxone.[26] Treatment with other antibiotics reported sporadically in the literature includes one successful case with imipenem and cilastatin,[94] seven successful cases with ciprofloxacin or norfloxacin in adults,[108,136,152] and four isolated cases with erythromycin.[67] An efficacy trial in Sweden of ciprofloxacin demonstrated successful outpatient management of *F. tularensis* in 10 of 12 patients 1 to 10 years of age.[81] The dosage used was 15 to 20 mg/kg/day in two divided doses for a 10- to 14-day course of therapy. Defervescence occurred in the fourth day of treatment. Despite the lack of clear data on safety in children, this treatment was reported as efficacious in a 17-year-old patient in the United States who developed tularemia after being bitten by a cat.[5]

Children receiving streptomycin should be monitored for ototoxicity. Hearing screening should be considered before initiation of streptomycin or gentamicin therapy. If the child has preexisting hearing loss, the alternative streptomycin regimen or gentamicin therapy can be considered, with close monitoring of serum levels in severe cases. In these cases, audiologic evaluation is indicated after therapy is initiated.

*As of November 1995, streptomycin is available from Pfizer Streptomycin Program, Pfizer Pharmaceuticals, New York, NY (800-254-4445).

Bed rest and supportive therapy are, of course, indicated. In a severely ill patient who shows signs of endotoxic shock, appropriate monitoring and admission to an intensive care unit are indicated, and corticosteroid therapy should be considered. Suppurative lymph nodes may require surgical drainage.

Before the advent of effective antimicrobial therapy, tularemia often was a protracted illness that lasted weeks or months. Subsequently, a long period of convalescence was necessitated by debility. Antibiotics have interrupted the natural history of the disease. When the disease is diagnosed promptly, the course generally is less than a month. As a result of administering appropriate antibiotic therapy, the mortality rate has declined from 5 to 30 percent to less than 1 percent, except in cases of fulminant pneumonic and typhoidal disease.

PREVENTION

Prevention of human tularemia depends on prevention of exposure to either the vectors or contaminated animal tissue. Children living in areas of tick endemicity should have their skin and hair checked frequently for ticks. Ticks should be removed carefully with tweezers (not with fingernails) by pulling perpendicular to the skin where the ticks have attached.[109] Care should be taken not to squeeze the ticks between the fingers. Persons living in tick-infested areas should wear clothing with tightly fitting cuffs at the wrists and ankles when staying outdoors. Tick repellents should be used with caution on children.

Children should be warned against handling sick or dead rodents or rabbits. Incineration or burial should be used to dispose of rabbits caught by household pets. Rubber gloves should be worn for preparing game animals, and the meat should be cooked thoroughly before eating. Hunters, especially rabbit hunters, should take precautions against contracting tularemia.

The only tularemia vaccine available in the United States is the *F. tularensis* LVS developed in 1960. It is unlicensed and is classified as an investigational product, but it is available to laboratory personnel. This vaccine appears to have reduced significantly the incidence of typhoidal tularemia and the severity of ulceroglandular disease in laboratory personnel. One lot of vaccine was found to induce an 80 percent response, with IgG to the ether-extracted antigen of the LPS membrane protein by day 14 and a 100 percent response by 21 days.[169] In vitro, lymphocyte responses to several antigens were noted by 14 days, in contrast to controls, in whom the ether-extracted antigen response appeared first.[169] The efficacy of this vaccine for prevention of naturally occurring disease in the United States is unknown. Vaccine strategies using bacterial proteins, such as heat shock proteins and other components, have not been successful. Further work in designing mutants that have specific gene changes to create attenuated strains may serve as a new avenue in the future.[93]

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CHAPTER 145

HAEMOPHILUS INFLUENZAE

Stephen J. Barenkamp

Relatively few species of the genus *Haemophilus* are pathogenic to humans, and nearly all that cause human disease are either encapsulated or unencapsulated strains of *Haemophilus influenzae*. These strains of *H. influenzae* are small, gram-negative pleomorphic coccobacilli that generally are considered to be normal constituents of the microbial flora of the upper respiratory tract of humans.[192,233] Strains without polysaccharide capsules often cause infections of mucosal surfaces, such as otitis media, bronchitis, conjunctivitis, sinusitis, and types of pneumonia.[256] Encapsulated strains, especially *H. influenzae* type b (Hib), cause invasive diseases such as septicemia, meningitis, septic arthritis, cellulitis, epiglottitis, pneumonia, and empyema. Before Hib vaccines became widely available, *H. influenzae* was the leading cause of bacterial meningitis in the United States and most other countries, and it also was an important cause of other bacteremic illnesses, primarily in young children.[49,418]

The organism now known as *H. influenzae* was described first in 1892 by Robert Pfeiffer,[295] who isolated it from the lungs and sputum of patients during the 1889 to 1892 pandemic of influenza. He proposed that the organism was the cause of influenza, and it initially was known as the Pfeiffer influenza bacillus. The bacteria were difficult to culture on routine media until investigators appreciated that supplementation with X (hemin) and V (nicotinamide adenine dinucleotide [NAD]) factors was required for its growth. By the early 20th century, the organism had been recovered from the blood and cerebrospinal fluid (CSF) of young children with meningitis. Although doubts remained about the etiologic role of the Pfeiffer bacillus as the cause of influenza, not until the influenza pandemic of 1918 was its etiologic role questioned seriously. In 1920, the organism was renamed *Haemophilus influenzae* to acknowledge its inappropriate historic association with influenza and to emphasize its requirement of blood factors for growth (from the Greek *haemophilus*, or "blood-loving").[420] In 1933, the viral cause of influenza was discovered, and this discovery refuted any remaining confusion about the erroneous association between *H. influenzae* and influenza virus.

Key concepts relevant to the development of treatment and prevention modalities derive from the pioneering work of Margaret Pittman[298,299] in the early 1930s. Paralleling earlier research on the pneumococcus, she defined two major categories of *H. influenzae*: encapsulated strains and unencapsulated strains. Among the encapsulated strains, Pittman characterized six distinct serotypes (designated a through f), which now are known to differ biochemically in the composition of their polysaccharide capsules (Table 145–1). She observed that Hib strains were recovered primarily from the blood and CSF of young patients with meningitis and that unencapsulated strains and other *H. influenzae* serotypes were recovered primarily from respiratory tract secretions. Furthermore, Pittman demonstrated that antibody to Hib capsule conferred type-specific protection against lethal infection in rabbits. This observation led to the use of antiserum prepared by immunization with formalin-killed Hib, initially in horses and later in rabbits, as the first treatment of disease. Before this development, Hib meningitis and other forms of invasive Hib disease almost always were fatal.[380] However, not until the late 1930s did treatment of children with meningitis with both Hib antiserum and sulfonamides substantially reduce the case-fatality rate.[7,8]

In 1933, Fothergill and Wright[125] described the age-related risk of acquiring *H. influenzae* meningitis, which affected mostly children younger than 5 years of age. Importantly, these investigators noted the correlation between the age-related risk of disease and the absence of bactericidal antibodies. Later, researchers identified antibody to Hib capsule as the major antibody contributing to the protective activity of bactericidal serum.[181,341] These observations suggested that naturally acquired type b anticapsular antibody is protective and that early stimulation of protective immunity with vaccines could be possible. Unfortunately, the advent of effective antimicrobial agents focused attention away from the need for primary prevention. Even with effective antimicrobial therapies and excellent hospital care, significant mortality rates (≈5%) and neurologic morbidity (≈20%) remained.[93,94] Ultimately, appreciation that the morbidity and mortality of the disease could not be eliminated completely by treatment gave impetus to the development of vaccines for prevention.

TABLE 145–1 Structures of *Haemophilus influenzae* Capsular Polysaccharides

Type	Structure
a	4)-β-D-Glc-(1 → 4)-D-ribitol-5-(PO4 →
b	3)-β-D-Rib*f*-(1 → 1)-D-ribitol-5-(PO4 →
c	4)-β-D-GlcNAc-(1→ 3)-α-D-Gal-1-(PO4 → 3 ↑ R = OAc | H R
d	4)-β-D-GlcNAc-(1→ 3)-β-D-ManANAc-(1 → 6 ↑ | R = L-serine | L-threonine R L-alanine
e	3)-β-D-GlcNAc-(1→ 4)-β-D-ManANAc-(1 →
e′	3)-β-D-GlcNAc-(1→ 4)-β-D-ManANAc-(1 → 3 ↑ 2 β-D-fructose
f	3)-β-D-GalNAc-(1→ 4)-α-D-GalNAc-1-(PO4 → 3 ↑ OAc

In the early 1970s, investigators purified and characterized the type b capsular polysaccharide (polyribosylribitol phosphate [PRP]) and proposed it as a potential vaccine candidate. Subsequently, the protective efficacy of a PRP vaccine against invasive Hib disease was demonstrated in older children in a 1974 field trial conducted in Finland.[288] This and other studies culminated in the licensure of PRP vaccine in the United States in 1985, thus rendering it the first vaccine available for the prevention of Hib disease.[63] Unfortunately, this vaccine induced equivocal immune responses and incomplete protection in older children and provided no protection for young infants, those at greatest risk of acquiring Hib disease.[408] Improved vaccines used polysaccharide-protein conjugate techniques, and four such vaccines were licensed for use in children. Three of these vaccines were shown to protect young infants against Hib disease,[38,330,391] and with the universal immunization of young infants, invasive Hib disease nearly has been eliminated in many populations.[4,37,289] Unfortunately, Hib disease continues to be a significant cause of morbidity and mortality among children in numerous resource-poor countries around the world where access to Hib vaccines is still limited.[9,285,362,404]

MICROBIOLOGY

MORPHOLOGIC AND CULTURAL CHARACTERISTICS

H. influenzae is a small, gram-negative coccobacillus that in clinical specimens can appear filamentous or pleomorphic, especially when specimens are obtained from patients who previously have received antibiotics. The organism is nonmotile, non–spore forming, and facultatively anaerobic, and it requires two supplemental factors for in vitro growth.[192] The X factor (hemin) is a heat-stable, iron-containing protoporphyrin essential for activity of the electron transport chain, which is important for aerobic growth. The heat-labile V factor is a coenzyme, NAD. Both factors are present within erythrocytes and are released by appropriate heating or enzyme lysis of the red blood cells that permits growth on chocolate agar. The requirement for these factors for growth remains the primary basis for the laboratory differentiation of *H. influenzae* from other *Haemophilus* spp.[22,192]

The growth of *H. influenzae* is fastidious, and clinical specimens need to be inoculated promptly onto appropriate media, such as chocolate agar.[192] The organism can be grown in most enriched liquid or solid media supplemented with X and V factors. Although not mandatory for growth, 5 to 10 percent carbon dioxide makes some strains grow better. In blood or liquid media, *H. influenzae* may not grow to sufficient quantity to result in visual turbidity; therefore, to detect positive cultures, blood and CSF cultures should be assayed for release of carbon dioxide or routinely subcultured at 24 to 48 hours. After being incubated overnight on solid media, colonies appear that are 0.5 to 1.5 mm in diameter and usually are rough or granular in appearance. Encapsulated strains generally produce slightly larger colonies that are mucoid or glistening. Fermentation reactions and other metabolic activities are variable and, therefore, not particularly useful for identification. However, a biotyping scheme based on the metabolism of indole, urea, and ornithine decarboxylase activity has been used to subtype strains.[192]

CAPSULAR POLYSACCHARIDES

Several surface structures of *H. influenzae* contribute to the organism's pathogenicity, but one of the most important is the capsular polysaccharide. Strains can express one of six unique polysaccharide capsules (a, b, c, d, e, or f; see Table 145–1) or lack capsule expression completely (nontypeable strains). The

Hib capsule is of particular clinical, pathogenic, and immunologic importance because Hib accounts for 95 percent of all strains that cause invasive disease.[49,231] The Hib polysaccharide consists of a repeating polymer of ribosyl and ribitol phosphate (PRP) that has a 1-1 linkage.[98] The genes involved in production of the Hib capsule have been cloned and consist of two repeating 17-kb DNA fragments separated by a 1-kb bridge region (*bex A*)[172,200]; 98 percent of Hib organisms tested contain this duplication.[200] Encapsulation often is unstable, with loss of capsule production associated with loss of one 17-kb repeat.[199] These strains produce type b capsule but in barely detectable amounts. Of importance is that the release of Hib capsular antigen in the body fluids of infected individuals can be detected by specific immunologic techniques (i.e., latex agglutination) that are useful for rapid diagnosis.[184,410] The other capsular serotypes are composed of hexose rather than pentose sugars,[98,99,383,384] and they also occasionally result in invasive disease.[58,66,164,278]

NONCAPSULAR CELL WALL ANTIGENS: PROTEINS

The cell envelope of *H. influenzae* is typical of other gram-negative bacteria, consisting of a cytoplasmic membrane, a peptidoglycan layer, and an outer membrane that represents the outermost layer of the envelope. The outer membrane contains an array of proteins, lipopolysaccharides (LPSs), and phospholipids, many of which serve important functional roles for the organisms. Since the late 1990s, our understanding of many of the outer-membrane components and of their contributions to the pathogenesis of disease has advanced significantly.[101,126,167,256]

Early laboratory investigations of *H. influenzae* identified and characterized numerous major and minor outer-membrane proteins (OMPs) of the organism.[31,253,255,260,272,356] However, an understanding of the functional roles of most of these proteins was lacking.[356] More recent studies have helped to clarify the roles in disease pathogenesis of an expanding number of these OMPs. Several proteins have been shown to be important for attachment of *H. influenzae* to host respiratory mucosal tissues.[53,82,251,320,427] Other surface proteins have been shown to play a central role in bacterial acquisition of iron, a phenotypic trait critical for survival of the organism in the human host.[6,179,193,343,354,424] Other studies have identified bacterial surface proteins important in resistance of *H. influenzae* to components of the host innate immune system, the first line of defense in the nonimmune host.[88,163,232] The availability for study of the completed genomes of several representative *H. influenzae* strains[121,168,254,352] is certain to increase the rate of discovery of other proteins important in disease pathogenesis during the next several years.

NONCAPSULAR CELL WALL ANTIGENS: LIPOPOLYSACCHARIDE

Early studies of *H. influenzae* LPS demonstrated that, although they differed chemically and structurally from the LPS of Enterobacteriaceae, both types of LPS shared numerous biologic properties. LPS purified from Hib produced a dermal Schwartzman reaction, was lethal to mice, caused a febrile response in rabbits, evoked polyclonal B-cell activation, and had limulus lysate activity.[123] In addition, human leukocytes incubated with *H. influenzae* LPS generated potent procoagulant activity,[247] a property that may be relevant to understanding the mechanisms responsible for intravascular coagulation in severe infection.

During the past several years, knowledge concerning the genetics, structure, biosynthesis, and role in pathogenesis of the *H. influenzae* LPS has increased greatly. The structure of *H. influenzae* LPS consists of a conserved triheptose inner core linked by a single 2-keto-3-deoxy-octulosonic acid molecule to lipid A, from which extend oligosaccharide extensions (outer core), mainly hexose sugars, that vary from one strain to another.[161,227,228] *H. influenzae* LPS lacks the repetitive side chains typical of gram-negative enteric bacteria but displays great complexity in its outer core sugars and other substituents such as phosphorylcholine[411,412] and sialic acid.[10,47] Several of these sugars and nonsugar substituents of *H. influenzae* LPS are phase variable.[159,160,412] Phase variation of many of these substituents is mediated by translational switching of genes that contain tetranucleotide repeats within the 5′ ends of the respective reading frames.[161,176,412] Phase-variable expression of several of these LPS components has been shown to contribute to the pathogenesis of disease, as further explained later.

IMMUNOGLOBULIN A PROTEASES

Immunoglobulin A (IgA) proteases are bacterial enzymes, the only known substrate of which is human IgA1. These secreted enzymes cleave the IgA heavy chain at specific sites.[196] IgA proteases are regarded as potentially important virulence factors because mucosal defense is in part IgA mediated. *H. influenzae* produces at least two distinct types of IgA protease that cleave different peptide bonds within the IgA1 hinge region.[252,300] Other work demonstrated that strains of *H. influenzae* isolated from patients with disease express higher levels of IgA protease than do strains isolated from asymptomatic carriers,[395] a finding again suggesting that IgA protease is an important virulence factor for *H. influenzae*. In more recent work, investigators identified and characterized another IgA protease gene that is present in approximately one third of *H. influenzae* strains.[115] This latter gene, designated *igaB*, is distinct from the previously characterized IgA protease gene, and its presence appears to be associated with higher levels of IgA protease expression and with a greater likelihood of causing disease.[115]

POPULATION STRUCTURE

Numerous powerful typing techniques have been developed for identifying individual *H. influenzae* strains and for defining the relationships among strains.[242,268,294] These techniques have provided valuable information for a variety of epidemiologic studies and have served to define the population structure for *H. influenzae* organisms as a whole. Encapsulated *H. influenzae* can be grouped into two distinct primary phylogenetic divisions.[268] Furthermore, the population structure of encapsulated *H. influenzae* is clonal. Most of the invasive disease worldwide is caused by serotype b strains of a limited number of clones.[200] Strains producing serotype c, e, and f capsules belong to single divisions and have no close genetic relationships with strains of other serotypes.[268] Serotype a and b strains occur in both primary phylogenetic divisions, probably as a result of transfer and recombination of serotype-specific sequences of the cap region between clonal lineages.[201,268] In contrast to the encapsulated strains, the unencapsulated or nontypeable *H. influenzae* strains demonstrate substantial genetic diversity.[267,294] Contributing to the genetic diversity of the nontypeable *H. influenzae* are apparently rather high rates of recombination among individual bacterial strains.[79,242]

ANTIBIOTIC RESISTANCE

Another important microbiologic feature of *H. influenzae* has been the development of antibiotic resistance. Resistance to a wide variety of antibiotics (sulfonamides, trimethoprim-sulfamethoxazole, erythromycin, tetracycline, penicillin) has been described, but these antibiotics are not essential for therapy.

Of greater importance is resistance to ampicillin, first noted in the mid-1970s,[13] because this drug was the primary antibiotic used for the treatment of disease. Since then, ampicillin resistance has become widespread, with between 5 and 40 percent of all isolates in various parts of the world now resistant.[57,95,183] The mechanism of resistance usually involves the production of plasmid-mediated β-lactamase enzyme, and resistant strains often are characterized by their plasmid or β-lactamase enzyme content.[183] Resistance to chloramphenicol usually is mediated by the enzyme chloramphenicol acetyltransferase.[318,400] Although chloramphenicol-resistant strains are rare findings in the United States, they are more prevalent in some areas of the world, and strains resistant to both ampicillin and chloramphenicol have been reported.[62,281,386] Currently, third-generation cephalosporins are the mainstays of therapy for invasive disease; concern, however, about the potential for increasing resistance to these highly effective agents further emphasizes the need for means to prevent disease.

PATHOGENESIS

ACQUISITION AND CARRIAGE OF ORGANISMS

Illness caused by *H. influenzae* infection results from a series of pathogenic events beginning with exposure to the organism, followed by colonization of respiratory mucosal membranes.[126] Under natural conditions, *H. influenzae* is exclusively a pathogen of humans, and it usually is transmitted asymptomatically from person to person by transfer of respiratory secretions.[279,284] The incubation period is unknown because many transmission cycles may occur before a susceptible person becomes ill. Furthermore, in some individuals, the organism can be carried in the upper respiratory tract for many months before it causes disease.[84,261,309,357]

Both typeable and nontypeable organisms may be present as part of the normal flora of the upper respiratory tract. Nearly all individuals (as many as 80%) are colonized at some point with nontypeable strains.[284,309] In the pre-vaccine era, Hib carriage rates were lowest in adults and young infants and highest in preschool-aged children.[385] In a prospective longitudinal study conducted at a daycare center in Dallas, Texas, where no invasive infections occurred, the average rate of colonization with Hib was 10 percent.[261] During the 18 months of the study, 71 percent of the children 18 to 35 months of age and 48 percent of the children 36 to 71 months of age were colonized at some time. Carriage rates were substantially higher in households or daycare centers in which a case occurred. For example, colonization prevalence rates among children in daycare centers where a case of invasive disease had occurred were as high as 58 to 91 percent.[151,407] Similarly, within families in which a case of invasive disease occurred, rates of colonization of 60 to 70 percent in siblings and 20 percent in parents were observed.[55,407] Whether the high carriage rates in these exposed semiclosed populations were the cause or the result of disease was not clear.[233,245] Close contact among exposed susceptible individuals, as occurs within families and daycare centers, facilitated the risk of transmission and acquisition of disease.

Despite a low point prevalence of Hib pharyngeal carriage (1-5%) in the pre-vaccine era, most young children became colonized with Hib during the first 2 to 5 years of life.[233,385] Consequently, specific Hib immunity developed in these colonized children.[157,341] Hib strains were demonstrated to persist in the nasopharynx for months,[245,261] and they often were not eliminated by treatment with antimicrobial agents that did not penetrate into respiratory secretions.[350,351] The relationship between carriage of Hib and the subsequent development of disease and immunity is not well understood. Factors that influence the efficiency of transmission and the ability of the organism to establish colonization also are understood poorly. Two factors that probably potentiate the risk for acquiring infection and invasive disease are the size of the bacterial inoculum[368] and the presence of a concomitant viral infection.[198]

Data derived from animal models of infection may be of some benefit in understanding the dynamic nature of the colonization process. Inoculation of Hib organisms into the nose of infant animals resulted in local infection.[249,322] In this model system, rhinorrhea was not noted, but nasopharyngeal washings revealed numerous polymorphonuclear leukocytes and organisms that reached maximal density in approximately 24 hours. Shortly thereafter, bacteremia could be detected in the animals. With colonization of humans, Hib is found on the surface of the respiratory mucosa. Rarely, by electron microscopy, an organism can be observed penetrating a nasal mucosal epithelial cell.[368] An acute inflammatory response to the submucosal bacteria occurs, but it is not marked. The exact mode of entrance of the organisms into the vascular compartment is unknown, but the assumption is that they enter through the lymphatics, probably carried by phagocytic cells, which are found in the submucosa. In support of this hypothesis, Rubin and Moxon[322] detected early transient bacteremia in rats after nasal inoculation.

PATHOGENESIS OF MUCOSAL INFECTIONS

Mucosal colonization and mucosal infections occur much more frequently than do invasive bacteremic infections, particularly in those populations in which Hib vaccine is in widespread use. These latter infections, most of which are caused by nontypeable *H. influenzae*, still cause considerable morbidity and are associated with substantial health care costs.[331,369] Mucosal infections generally involve direct movement of organisms through the nasal ostia to the sinuses, up the eustachian tubes, where they cause otitis media, and down the bronchi, where they cause bronchitis and pneumonia. Bacteremia rarely is involved, and such infections generally are not life-threatening. These infections appear to be enhanced by antecedent viral infection,[72] eustachian tube malfunction,[371] foreign bodies, or mucosal damage from smoking or other irritants.[326]

A considerable amount of recent research has focused on gaining a better understanding of the pathogenesis of mucosal surface infections caused by nontypeable *H. influenzae*. The same principles almost certainly apply to the early steps in mucosal colonization by encapsulated *H. influenzae*. The following model has been proposed to explain the early events in the colonization process.[167] Following transmission from an *H. influenzae* carrier to a new host,[84] bacteria first come in contact with the upper respiratory tract. Here they must overcome the nonspecific mucociliary defenses if colonization is to persist.[346] The major OMPs of *H. influenzae*, P2 and P5, and probably other factors, promote bacterial binding to mucus and help to establish an early foothold for the bacteria in the upper respiratory tract.[310,368] Local elaboration of LPS and other bacterial products then causes damage to ciliated cells and further impairs mucociliary function.[403] Subsequently, several adhesins, including HMW1 and HMW2, pili, Hia, Hap, and others, help to mediate direct adherence to nonciliated epithelial cells.[24,30,120,139,140,365] Cleavage of IgA1,[115,395] invasion into cells and the subepithelial space,[124,368,372] and phase and antigenic variation of surface antigens facilitate evasion of local immune mechanisms.[176,412] Binding and uptake of iron and heme allow organisms to persist on the respiratory mucosa despite the relative scarcity of these nutrients.[345,417] In the setting of a viral infection, allergic disease, or exposure to cigarette smoke, bacteria spread from the nasopharynx to other sites within the respiratory tract and produce symptomatic disease.[72,283,371]

PATHOGENESIS OF INVASIVE DISEASE

Once mucosal colonization with Hib and other potentially invasive strains has been established, dissemination may occur from the upper respiratory tract mucosa to the bloodstream and then elsewhere in the body.[322] The incidence of invasive disease is a small fraction of the carrier rate for Hib.[261] When dissemination does occur, the organism appears to invade the mucosa by separating the apical tight junctions of the columnar epithelium and moving intercellularly.[368] The resulting bacteremia initially is low in concentration but steadily increases over the course of hours.[280] The dynamics between bacterial proliferation and clearance is influenced by antibody, complement, and phagocytes, all of which have an effect on the magnitude of the bacteremia.[414] The polysaccharide capsule of Hib is antiphagocytic and a major virulence factor. In the absence of anticapsular antibody, bacteremia increases steadily over a course of hours.[158] When the bacterial concentration exceeds 10^4 organisms per milliliter, metastatic seeding occurs, especially to the meninges through the choroid plexus. Although meningitis is the most frequently recognized manifestation of invasive Hib disease, other potential metastatic sites include the lungs, joint synovium, pleura, peritoneum, and pericardium.[49,380]

The pathogenic events that lead to pneumonia, cellulitis, and epiglottitis are less well understood, even though these invasive infections are associated with bacteremia. Presumably, pneumonia occurs after the aspiration of a critical number of virulent organisms, epiglottitis involves focal infection of the epiglottis, and cellulitis occurs by secondary seeding of deep subcutaneous tissues through the bloodstream.[141] With all forms of invasive Hib disease, invasion of the bloodstream occurs as either a primary or a secondary event.

Viral interactions enhance the pathogenesis of Hib.[271,374] Influenza virus infection has been shown to reduce neutrophil chemotaxis, bacterial killing, systemic macrophage function, the number of circulating T cells, T-cell blastogenesis, and expression of delayed cutaneous hypersensitivity.[1,2,80] Reduced bacterial killing may result from a defect in phagosome-lysosome fusion, a defect that is maximal 5 to 7 days after viral infection and inoculation of Hib.

BACTEREMIA IN ANIMAL MODELS

During bacteremia, organisms are cleared continually from the vascular compartment by antibody, complement, and the reticuloendothelial system.[250,322] The balance of these processes determines the magnitude and duration of the bacteremia. If bacterial clearance is stopped by reticuloendothelial blockade, bacterial densities increase to a maximum, and death quickly ensues, presumably because of the effects of endotoxin.

Initially, bacterial concentrations are very low (≈100 organisms/mL blood). They then steadily increase in density over the course of the next 24 hours and reach a plateau value.[322,323] In young animals, this plateau level is 10^7 organisms per milliliter of blood and usually is associated with the features of human sepsis. In older animals, the plateau value is lower, approximately 10^4 organisms per milliliter of blood. These animals have low-grade fever but a relative paucity of symptoms. This primary bacteremia leads to seeding of serous surfaces, such as the peritoneum, diarthrodial joints, pleura, pericardium, and meninges. Early in the infectious process, organisms can be obtained from all these surfaces but without an observable inflammatory response; later in the process, an inflammatory response ensues. In experimental animals, the first evidence of an immune response is antibody directed against somatic antigens.

Strains of Hib vary in virulence potential. Furthermore, the other five capsular types or nonencapsulated strains result in only transient, low-level, or undetectable bacteremia, even with large inocula (>10^7 colony-forming units).[428] Complement and the spleen are critical factors for host defense in the rat. Rats depleted of C3 and splenectomized (or with iatrogenic splenic congestion caused by hemolytic anemia) have an increased incidence and magnitude of bacteremia.[69,428] These studies suggest the importance of the alternative pathway of complement (opsonic antibody) and reticuloendothelial phagocytes as determinants of intravascular clearance.

MENINGITIS

Bacteremia precedes the development of meningitis, except for rare situations in which direct extension of infection from adjacent sinuses or an ear infection occurs. Data from experimental studies in infant rats and infant monkeys support this hypothesis.[249] Both the magnitude and duration of bacteremia are probably the primary determinants of invasion of the central nervous system (CNS). After a critical bacterial concentration is exceeded in blood, Hib appears to enter the CNS through the choroid plexus.[360] This theory is supported by the following: (1) the earliest histopathologic lesion seen in the CNS is choroid plexitis, (2) the choroid plexus is one of the foci seeded from the bloodstream, and (3) bacterial density early in infection is greater in the lateral cerebral ventricles than in other CSF compartments.[360] Furthermore, pulse-chase experiments using tracer strains show that organisms enter the CSF through the choroid plexus and that inflammation of the choroid plexus is a uniform feature of meningitis. Subsequently, organisms infect the CSF and the arachnoid villi of the leptomeninges and cause blockage of CSF return, thereby increasing bacterial density and CSF pressure.[360] Generally, the magnitude of CSF bacterial density correlates with the severity of clinical illness.[114,336] Egress of CSF from the subarachnoid space is by flow through the subarachnoid villi, and bacterial density in CSF can be increased or decreased by manipulating CSF egress, which occurs in meningitis through inflammatory responses. The inflammatory response of the choroid plexus is followed by pachymeningitis, which also inhibits CSF reabsorption and increases pressure. Phlebitis of the cerebral blood vessels and thrombosis can occur. All these events contribute to decreased blood flow to the cortex. The resulting increased bacterial density, inflammation, edema, cranial nerve damage, and overall increased CSF pressure are responsible for the morbidity and mortality associated with meningitis.[93,111,360] Parenchymal invasion of the brain rarely occurs.

IMMUNOLOGY

Resistance to *H. influenzae* infection depends on successful integration of a wide variety of host defenses, including the following: (1) mucosal factors that prevent the organism from attaching and penetrating the respiratory epithelium[206,361,366]; (2) activation of the alternative and classical complement pathways that leads to killing of the organism and initiation of other inflammatory responses[367,419]; (3) induction of antibody formation[148,150]; (4) phagocytosis and killing by macrophages and polymorphonuclear cells in tissues, the circulation, and the reticuloendothelial system[34,266,421]; and (5) cell-mediated immunity.[96] Assessing the role of each of these immunologic mechanisms independently or determining which mechanisms are most important in host defense is difficult. Although antibodies are not the sole defense against bacteremia, they have been the research emphasis of vaccine development.[148] The goal has been to induce antibodies that are bactericidal, opsonophagocytic, and ultimately protective. Although usually only antibody is measured in these studies,

other immune factors are induced and probably play important roles in protection.

ANTICAPSULAR ANTIBODY

Initially, antibody activity was assessed by measuring agglutinin and bactericidal titers of serum. In 1933, Fothergill and Wright[125] suggested that bactericidal activity was responsible for immunity to Hib meningitis and that acquisition of this immunity correlated with the age of the individual. Although antibodies to several surface antigens of *H. influenzae* play roles in conferring immunity to Hib,[353] antibody to Hib capsular polysaccharide appears to be of primary importance.[317] Newborns and young infants are at low risk for the development of infection, presumably because they have maternally acquired antibody. Young children at highest risk of acquiring disease have low or undetectable levels of antibody, whereas older children at lower risk have higher antibody levels. By the time they reach 5 years of age, most children have naturally acquired anticapsular antibody that appears to provide protection,[157,341] although natural exposure also induces antibodies to OMPs, LPS, and other surface antigens of the bacteria that contribute to natural immunity. The evidence that anticapsular antibodies protect humans from the acquisition of invasive Hib disease is considerable: they activate complement,[367,419] are opsonophagocytic[273] and bactericidal,[125,273] and protect animals from lethal Hib challenge.[370] Moreover, passive prophylaxis with serum preparations containing anticapsular antibody protects agammaglobulinemic patients[317] and high-risk children from the acquisition of invasive Hib disease.[328] Furthermore, in the pre-antibiotic era, immune serum was an effective therapy for Hib disease,[7,299] However, the most compelling evidence for the protective efficacy of PRP antibody is the clinical protection achieved in older children vaccinated with purified PRP vaccine[288] and in younger infants immunized with Hib conjugate vaccines.[289] Induction of antibody to Hib polysaccharide is the immunologic basis of all Hib vaccines.

A precise minimal level of anti-PRP antibody that is protective has not been established.[148] Data from passive protection of agammaglobulinemic children, challenge experiments in infant rats, and studies of naturally acquired antibody levels in healthy individuals of various ages suggest that the minimal serum concentration of anti-PRP antibody that provides protection ranges from 0.05 μg/mL in animals[342] to 0.15 to 1.00 μg/mL in humans.[189,288] Such estimates are crude and do not take into account the different functional properties of different immunoglobulins[150,219] or the contribution of antibodies to other Hib antigens. In addition, antibody levels decline over the course of time, and a given peak level may not reflect levels at the time of exposure, which would predict long-term protection better.[17,288] In a Finnish PRP vaccine trial, an antibody level greater than 1.0 μg/mL 1 month after immunization correlated with clinical protection for a minimum of 1 year.[288] However, this antibody level may not be extrapolated readily to the immunogenicity data evaluated in different studies or with different Hib conjugate vaccines.

CLASS- AND SUBCLASS-SPECIFIC ANTIBODY

Several studies have shown variable immunoglobulin class, isotype, idiotype, and IgG subclass responses to PRP polysaccharide after natural Hib exposure, disease, and immunization.[190,342] Most individuals respond with IgG antibodies after receiving PRP immunization, although some children have predominantly IgA or IgM responses.[190] Schreiber and associates[342] showed that IgG antibody is bactericidal, opsonic for polymorphonuclear leukocytes in the presence of complement, and protective for animals. IgM antibody is equally protective and more bactericidal than is IgG in the presence of complement, but it opsonizes poorly. IgA antibody is not bactericidal, opsonic, or protective for animals. Some researchers have hypothesized that IgA-specific antibody blocks the activity of other more functional antibodies and thereby may depress immunity.[264]

Data from experiments in mice and humans suggest that polysaccharide antigens induce restricted IgG subclass responses.[315] The findings of increased susceptibility to Hib disease in IgG subclass–deficient patients (predominantly IgG2 and IgG4 deficiencies),[282,344] and the low levels of IgG2 in children younger than 2 years of age[349] suggest that differences exist in the role of subclass-specific anticapsular antibodies. In adults, natural exposure or immunization with PRP vaccine results in a predominantly IgG2 subclass response.[307] In children, IgG1 and IgG2 antibodies develop after they receive PRP immunization, but IgG1 antibodies predominate after immunization with Hib conjugate vaccines.[12,173,187] Human anti-PRP antibodies express predominantly kappa light chains,[376] and these antibodies may be grouped into a few restricted clonotypes. These clonotypes and antibody specificities have been characterized by idiotype analysis[218,221] and by amino acid sequencing of the immunoglobulin light chain.[376] Individuals of different ages produce different proportions or repertoires of antibody.[220] Some differences in binding specificity and affinity have been described with the different anti-PRP antibodies,[219,338] but whether the antibodies have different degrees of protective potency or are substantially different in proportion in individuals given different vaccines is not clear.

The role of mucosal immunity in killing Hib or inhibiting adherence or penetration of the mucosa is understood poorly, although studies of secretory IgA antibody to the Hib capsule have been conducted.[296,297] Moreover, Hib strains produce IgA proteases that can inactivate mucosal antibody.[115,252] The observation of reduced carriage of Hib in children given Hib conjugate vaccines[263,373] suggests that mucosal immunity may be important in reducing transmission of the disease.

CELLULAR IMMUNE RESPONSES

Most of our understanding of the interactions of B cells, T cells, and antigen-presenting cells (macrophages) is derived from extensive research in mice.[87] Based on T-cell involvement in antibody synthesis, antigens can be classified as T-dependent (thymus-dependent) or T-independent immunogens. Because most protein antigens induce helper T-cell regulation of antibody synthesis, they are considered T-dependent. These antigens first are recognized and processed by macrophages and then are presented to both T and B cells. The activated T cells induce proliferation and differentiation of specific antigen-reactive B-cell subpopulations. They also retain the memory necessary for subsequent booster responses.[32] Through the release of cytokines, helper T cells appear to regulate the following: (1) the magnitude of the immune response, especially in young infants; (2) the switch in immunoglobulin classes (IgM to IgG); (3) the functional activity of antibody; and (4) the capacity to elicit immunologic memory.

Polysaccharides consist of repeating oligosaccharide units and elicit weak immune responses involving minimal T-cell influences.[32] These T-independent antigens elicit antibody responses primarily by direct stimulation of B cells. In general, polysaccharide vaccines have the following T-independent immunologic characteristics: (1) delayed ontogeny of immune responsiveness in the young, (2) limited and variable quantitative immune responses, (3) restricted isotype (predominantly IgM) and IgG subclass responses, and (4) lack of a booster or anamnestic response with secondary antigenic challenge. The quest for an Hib vaccine that is immunogenic and protective in young infants has involved attempts to convert the PRP antigen from a T-

independent to a T-dependent antigen by using the carrier-hapten principles first defined by Landsteiner in the first half of the 20th century.[204] PRP can be considered a hapten that is linked covalently to a T-dependent immunogen, a carrier, to form a conjugate vaccine. The Hib conjugate vaccines, which demonstrate markedly enhanced immunogenicity, are described in a subsequent section.

GENETIC FACTORS

When compared with protein vaccines, the immune responses to most polysaccharide antigens are variable and may be influenced by genetic factors. Several studies have shown associations between the immune responses to PRP vaccine and genetically determined factors such as red blood cell antigens, human leukocyte antigen, or immunoglobulin allotypes.[11,154,348,416] However, because many factors influence immunogenicity, whether these associations have relevance is not known, and establishing controls for them is difficult. In addition, whether the antibody differences are important clinically also is not known. No single genetic relationship regulating susceptibility or the immune responses to polysaccharide antigens has been demonstrated convincingly.

COMPLEMENT

The importance of complement components in host defense against Hib is substantiated by elimination of the bactericidal activity of serum by heat, by the susceptibility of complement-depleted animals to Hib disease, and by the increased susceptibility of patients with specific congenital complement deficiencies.[83,367,419] Hib is capable of activating both the classical and the alternative complement pathways,[83,367] thereby initiating opsonophagocytosis and cell killing and eliciting other inflammatory responses. Whereas the alternative pathway probably is most important early in the course of infection in a nonimmune host, the antibody-dependent classical complement pathway is more likely to predominate as a defense mechanism at a later stage of infection.[419] Both encapsulated and unencapsulated organisms activate complement, thus underscoring the importance of noncapsular antigens in host defense.[265,266] Although the Hib capsule is a poor activator of the alternative complement pathway, antibody to the capsule activates both the classical and the alternative pathways.[367] Other cell wall antigens activate the alternative pathway, and antibody to these antigens activates the classical pathway.[83,367,419] Thus, antibodies to both capsular and noncapsular antigens activate the complement system, primarily through the classical pathway. Activation of the terminal complement components mediates the bactericidal activity of serum.

Studies have explored the contribution of LPS structure to the susceptibility of *H. influenzae* to complement-dependent serum bactericidal activity.[117,161,223,411] Phase-variable structural modifications to the core LPS structure, including additions of phosphorylcholine,[412] sialic acid,[347] and other carbohydrate moieties,[102] all have been shown to influence the susceptibility of these organisms to complement-mediated killing. Related studies in animal models of infection also have demonstrated the importance of these phase-variable LPS modifications to disease pathogenesis in vivo.[47,117,381,411]

PHAGOCYTOSIS

Opsonization leading to phagocytosis and killing of *H. influenzae* also is an important determinant of host defense.[265,266,370,421] Impairment of phagocytic function or a reduction in the number of phagocytes results in increased susceptibility to disease, as does loss of the spleen or impairment of its function (e.g., hemoglobinopathies).[70,302] The opsonic activity of serum is influenced greatly by the roles of complement and antibody. Opsonization and phagocytosis of Hib appear to be dependent on (1) IgG binding, (2) antibody activation of the classical complement pathway with deposition of C3b on the bacterial surface, and (3) direct bacterial activation of the alternative complement pathway.[275] Relatively little is known about direct cell-mediated killing of *H. influenzae*.[96]

EPIDEMIOLOGY

HAEMOPHILUS INFLUENZAE TYPE b

Humans are the only natural host for *H. influenzae*, and asymptomatic nasopharyngeal carriage, usually by unencapsulated strains, is a common finding.[108,233] In the era before widespread use of Hib conjugate vaccines, nasopharyngeal acquisition of Hib strains also was quite common and was shown to increase after infancy and to persist for weeks to months. Most children were colonized at some time during the first 5 years of life.[233] Colonization rates greater than 70 percent occurred after recent exposure in closed populations, such as among family members or daycare center contacts of a patient with disease.[261] Person-to-person transmission occurred through respiratory droplets, and fomites also were thought to play a role. As Hib conjugate vaccine administration became part of the routine infant immunization schedule in many countries, an associated and pronounced drop in the rate of nasopharyngeal carriage of Hib strains occurred.[262,373]

Before immunization was developed, invasive Hib disease was a leading infectious disease problem worldwide that affected primarily young children (Table 145–2).[401,415,418] Hib strains are responsible for more than 95 percent of invasive infections in children. According to population-based studies, an estimated 20,000 to 25,000 persons acquired invasive Hib disease annually in the United States, 85 percent of which occurred in children younger than 5 years of age.[49] The incidence of Hib meningitis and all invasive Hib disease was 40 to 69 and 67 to 130 cases per

TABLE 145–2 Worldwide Incidence of Invasive *Haemophilus influenzae* Type b Disease before the Use of Hib Vaccines in Children Younger than 5 Years

Region	Years	Hib Meningitis*	All Hib Disease*
Australia/New Zealand	1985-1987	25-53	39-92
U.S./Canada	1959-1991	40-69	67-130
Europe	1985-1990	15-26	33-60
Israel	1985-1990	18	34
Africa	1980s	36-60	NA
South America	1989-1990	15-25	21-43
Asia	1990s	1.3-1.9	1.9-2.7

Hib, Haemophilus influenzae type b; NA, not available.
**Annual incidence per 100,000 population younger than 5 years of age.*
Adapted from Vadheim, C. M., and Ward, J. I.: Epidemiology in developed countries. In *Ellis, R. W., and Granoff, D. M. (eds.): Development and Clinical Uses of Haemophilus b Conjugate Vaccines. New York, Marcel Dekker, 1994, pp. 231-245; and Bijlmer, H. A.: Epidemiology of Haemophilus influenzae invasive disease in developing countries and intervention strategies.* In *Ellis, R. W., and Granoff, D. M. (eds.): Development and Clinical Uses of Haemophilus b Conjugate Vaccines. New York, Marcel Dekker, 1994, pp. 247-264. By courtesy of Marcel Dekker, Inc.*

100,000 children younger than 5 years, respectively. Invasive Hib disease developed in an estimated 1 in every 200 children in the United States during the first 5 years of life.[77] Hib pneumonia was estimated to cause as many as 15 percent of cases of ambulatory pneumonia in children younger than 6 years old, but the true incidence was unknown because microbiologic diagnosis is difficult to establish. Overall, the incidence of Hib disease in the pre-vaccine era was similar to that of paralytic poliomyelitis during its peak epidemic years before immunization. A bimodal seasonal pattern had been observed in several studies, with one peak occurring between September and December and a second peak between March and May.[311,415] The attack rate of Hib was slightly higher in boys.[225]

The widespread use of Hib conjugate vaccines altered the epidemiology of invasive Hib disease dramatically (Fig. 145–1). The first Hib conjugate vaccine was licensed in the United States in 1987 for use in children aged 18 months or older.[64] Subsequently, decreases in the incidence of Hib disease were seen in older children and, unexpectedly, in unimmunized infants and children as well. This finding was attributed to a direct effect of vaccination on nasopharyngeal carriage, thus decreasing the environmental burden of Hib infection and, ultimately, the transmission of disease. In late 1990, Hib conjugate vaccines were approved for use in infants as young as 2 months of age, and this advance heralded a dramatic decline in the incidence of invasive Hib disease in all children.[65] In populations in which immunization rates are high, the development of invasive Hib disease has been eliminated almost completely.

Some population-based studies of the incidence of *H. influenzae* disease were conducted outside the United States, primarily in Western Europe, and they showed an incidence approximately one third to two thirds of that in the United States.[286,327,363] The incidence of Hib disease is especially high in certain ethnic groups, including the following: Aboriginal children in central Australia[166]; Navajo Native Americans; Native Alaskans; and Apache, Yakima, Athabaskan, and Canadian Native Americans.[217,246,409,426] *H. influenzae* appears to rank as a leading cause of bacterial meningitis in some developing countries as well.[9,54,362,404,425] As was seen in the United States, a substantial reduction in Hib disease occurred with the implementation of widespread vaccination programs. Studies in Asia showed a disease incidence one tenth to one thirtieth of that in the United States before the introduction of vaccine, but methodologic questions exist regarding the accuracy of these disease assessments, particularly related to antibiotic use and the validity of culture results.[425]

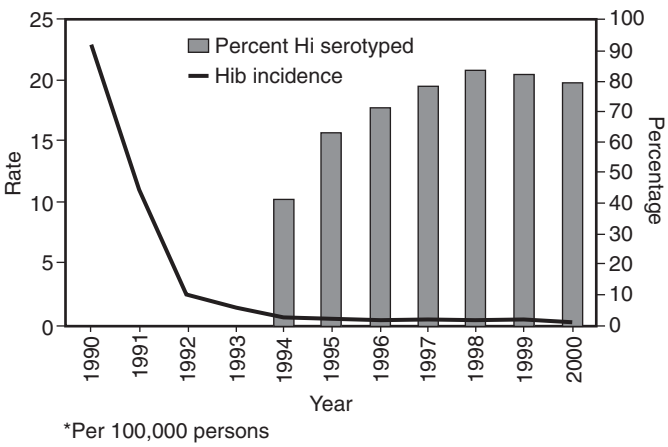

Figure 145–1 Incidence rate of *Haemophilus influenzae* type b (Hib) invasive disease per 100,000 persons and the percentage of *Haemophilus influenzae* (Hi) isolates serotyped in children younger than 5 years—United States, 1990-2000. *(From Centers for Disease Control and Prevention: Progress towards elimination of Haemophilus influenzae type b invasive disease among infants and children—United States, 1998-2000. M.M.W.R. Morb. Mortal. Wkly. Rep. 51[11]:234-237, 2002.)*

Children between the ages of 6 and 18 months are at highest risk for acquiring invasive Hib disease[225]; however, the age distribution of specific clinical syndromes of the disease varies. The peak incidence of Hib meningitis occurs in children 6 to 9 months of age and declines markedly after 2 years of age.[129,375] Hib cellulitis tended to occur during the first year of life, whereas epiglottitis generally occurred in unimmunized children older than 2 years of age. Invasive *H. influenzae* disease was seen much less frequently in adults because of the development of protective antibodies over the course of time, but it occurred more frequently in immunocompromised patients. Nonetheless, Hib caused pneumonia and meningitis in adults,[109,292] and diseases caused by other serotypes and nontypeable strains, especially pneumonia, otitis media, bronchitis, and sinusitis, are common findings in all age groups.[256] Most adults in whom invasive *H. influenzae* disease develops were not immunized as children and have an underlying condition such as chronic obstructive pulmonary disease, human immunodeficiency virus (HIV) infection, alcoholism, pregnancy, or malignancy.[50,109,292]

The development of invasive Hib disease in a given individual is the consequence of a complex interaction of a variety of factors, including the risk of exposure, the characteristics of the organism, and the host.[127] In populations that experienced a high incidence of disease, such as Native Americans or Alaskans, the age-specific incidence peaked in a younger age group (<6 months), presumably because of early or intense exposure to Hib at home or in the community.[222] Several factors that may reflect environmental exposure to the organism, such as household size,[149] crowding,[388] attendance at a daycare center,[78,177] low family income,[27,128,129] and low parental education level,[128,129] have been shown to be risk factors for the acquisition of disease, whereas breast-feeding appears to be protective.[293] Several underlying medical conditions, including HIV infection,[60,337] sickle-cell anemia,[302] asplenia or splenectomy,[70] antibody[110] and complement deficiency syndromes,[118] and malignancy,[413] are associated with an increased risk for the acquisition of Hib disease.

Although the direct contagiousness of invasive Hib disease is limited, small outbreaks and direct secondary transmission of disease can occur.[55,406,407] Numerous studies estimated the risk of developing secondary disease in household contacts in the 30 days after the onset of disease in an index case. Overall, the attack rate for contacts of all ages in the pre-vaccine era was 0.3 percent, which represents a risk approximately 600-fold higher than the age-adjusted risk in the general population.[55,406] However, attack rates varied inversely with age, with children younger than 4 years old being at greatest risk. Among household contacts, nearly two thirds of secondary cases occurred within the first week after the onset of disease in the index patient.[375] Controversy continues about the degree of risk for the development of secondary Hib disease in daycare center contacts exposed to a child with invasive Hib disease. Such risk in unimmunized children younger than 2 years of age ranged from 0 to 3.2 percent,[27,279] whereas the risk for those older than 2 years was less than 1 percent.

A cause for concern in recent years has been the reappearance or persistence of Hib disease in highly immunized populations.[23,238-240,358] The factors underlying these problems are still the subject of debate, but with the reemergence of Hib disease in the United Kingdom in the late 1990s, attention focused on the lack of a booster dosing of infants as part of the regular immunization series and the decreased immunogenicity of combination vaccines in which the Hib conjugate vaccine was com-

bined with acellular pertussis vaccine components.[238,239] The Alaskan experience uncovered other problems.[358] In this population, high Hib nasopharyngeal carriage rates in the rural population and the poor antibody response of young infants to certain Hib vaccines appeared to be responsible for the reappearance of Hib disease in Native Alaskans.[357,358]

NON–TYPE b ENCAPSULATED *HAEMOPHILUS INFLUENZAE*

H. influenzae organisms expressing one of the five non–type b capsular types are also carried in the nasopharynx of a small percentage of the population.[84,233] With the success of the Hib conjugate vaccine in preventing invasive disease caused by type b organisms, concern has been expressed that disease caused by other encapsulated strains could become more prevalent.[65] Indeed, several clusters of invasive disease caused by *H. influenzae* type a have been reported during the past few years,[5,185,292,313] as have occasional cases of disease caused by other serotypes.[278,387] However, surveillance studies for the most part have failed to document a sustained increase in the incidence of disease caused by non–type b serotypes after introduction of the Hib conjugate vaccines.[65] These data suggest that the concern about serotype replacement may be more theoretical than actual.

NONTYPEABLE *HAEMOPHILUS INFLUENZAE*

Asymptomatic colonization of the mucous membranes of the upper respiratory tract by nontypeable *H. influenzae* strains is very common, occurring in 60 to 90 percent of young children.[202] Infants often become colonized with nontypeable *H. influenzae* early during the first year of life.[106] Nasopharyngeal colonization is a dynamic process characterized by relatively rapid turnover of individual strains and, not infrequently, simultaneous carriage of several distinct clones.[84,108] With increasing age, nasopharyngeal carriage of nontypeable *H. influenzae* occurs less frequently, but it is still present in a significant percentage of the population.[202] Nontypeable *H. influenzae* organisms are associated only rarely with invasive disease,[103,276] but they are a common cause of mucosal surface infections such as otitis media, sinusitis, bronchitis, and occasionally pneumonia.[207,208,256,396]

CLINICAL MANIFESTATIONS

BACTEREMIA

Occult bacteremia (i.e., not associated with a primary focus of infection) is not a common occurrence with Hib, but bacteremia does precede essentially all invasive Hib infections. In the prevaccine era, Hib was the second leading cause, after *Streptococcus pneumoniae*, of occult bacteremia primarily affecting children 6 to 36 months of age.[16] Most children initially have fever and peripheral leukocytosis. Unlike *S. pneumoniae* bacteremia, the condition is not benign. In approximately 30 to 50 percent of children with occult Hib bacteremia, a focal infection such as meningitis, pneumonia, or cellulitis develops.[81,197]

MENINGITIS

Meningitis is the most serious manifestation of invasive Hib disease; however, no clinical feature differentiates Hib meningitis from other causes of bacterial meningitis in children.[111] The onset of disease can be fulminant,[178] but more commonly the signs and symptoms are nonspecific (particularly in young infants) and may include irritability, fever, lethargy, poor feeding, or vomiting. Children younger than 18 months old often do not have nuchal rigidity. Older children are more likely to have headache, photophobia, and meningismus. With fulminant Hib meningitis, very rapid neurologic deterioration may occur with increased intracranial pressure, seizures, coma, and respiratory arrest.[111] Ten to 20 percent of children with meningitis will have other foci of infection such as cellulitis, arthritis, or pneumonia,[26,119] and essentially all have concomitant bacteremia.

CSF examination typically reveals pleocytosis (mean, 4000 to 5000 white blood cells/μL) with a predominance of polymorphonuclear leukocytes.[93] Approximately 75 percent of patients have hypoglycorrhachia, and approximately 90 percent have an elevated CSF protein concentration. Eighty percent of meningitis cases caused by Hib will have a positive CSF Gram stain. As with other types of bacterial meningitis, however, previous antimicrobial therapy significantly decreases the concentration of Hib organisms in the CSF and diminishes the sensitivity of Gram staining.[39] Previous treatment does not affect the total blood cell count and differential, glucose, or protein substantially, thus permitting a diagnosis of meningitis to be made. In more than 90 percent of cases, capsular antigen can be detected in CSF or serum.[410] Anemia, leukocytosis, thrombocytosis, and thrombocytopenia also are observed frequently.[111]

Complications of Hib meningitis include seizures, cerebral edema, subdural effusions or empyema, inappropriate secretion of antidiuretic hormone, cortical infarction (often manifested by focal neurologic abnormalities), cerebritis, intracerebral abscess, hydrocephalus, and, rarely, cerebral herniation.[44,93,111,301] Computed tomography (CT) and magnetic resonance imaging (MRI) of the head should not be performed routinely for Hib meningitis, but these studies may be helpful if focal neurologic findings are present or if the clinical course becomes complicated. Small subdural effusions are common findings but usually do not have clinical significance.[111]

Even with prompt intensive care, the mortality rate from Hib meningitis is approximately 5 percent, and significant long-term morbidity, including sensorineural hearing loss, delay in language acquisition, developmental delay, gross motor abnormalities, vision impairment, and behavior abnormalities, may occur in 15 to 30 percent of survivors.[93,94,111,301,377] A substantial proportion of such abnormalities may resolve over time, thus emphasizing the need for long-term monitoring of these patients.[111]

PNEUMONIA

Hib pneumonia is clinically indistinguishable from other bacterial pneumonias.[142,397] Most patients have a preceding upper respiratory tract infection, fever, and cough accompanied by peripheral leukocytosis with a predominance of polymorphonuclear leukocytes. Radiologically, Hib pneumonia may be segmental, lobar, interstitial, or diffuse, and many patients have evidence of pleural or pericardial involvement on radiographic examination. Cavitation and pneumatoceles are rare occurrences but have been reported.[15,71] CT or MRI can be useful adjuncts to the evaluation of complicated disease caused by Hib.[76] Blood, pleural fluid, tracheal aspirate, and lung aspirate cultures are positive in 75 to 90 percent of cases. Detection of capsular polysaccharide in pleural fluid, serum, or urine can establish the diagnosis, particularly if antimicrobial therapy had been instituted previously. Although fever may persist for several days while the patient is receiving adequate therapy, uncomplicated Hib pneumonia rarely is associated with long-term pulmonary dysfunction. In the developing world, in addition to disease caused by Hib, pneumonia caused by nontypeable *H. influenzae* is a continuing problem[135,207] that also is associated with significant morbidity and mortality rates.

EPIGLOTTITIS

Acute upper airway obstruction caused by Hib infection of the epiglottis and supraglottic tissues occurred in the pre-vaccine era, primarily in children 2 to 7 years of age.[33,241] Its onset usually is abrupt, with high fever, sore throat, dysphagia, and sepsis. Antecedent upper respiratory tract infection with cough occurs in approximately 50 percent of patients.[48,216] The child may drool because of an inability to swallow oropharyngeal secretions, and progressive respiratory distress with tachypnea, stridor, cyanosis, and retractions may develop over a period of hours. The child usually is agitated and may sit forward with the chin extended to maintain an open airway. In children younger than 2 years old, Hib epiglottitis may be manifested in an atypical fashion, with low-grade fever and a cough suggestive of croup.[216]

The most important aspect of the management of a child with epiglottitis is maintenance of a patent airway. Nasotracheal intubation is preferable to tracheostomy because it is equally effective, is not permanent or disfiguring, and has fewer inherent risks.[33] Seventy to 90 percent of patients with epiglottitis will have positive blood cultures; tissue for culture of the inflamed epiglottis should be obtained only after the airway has been secured. A lateral neck radiograph revealing dilatation of the hypopharynx and the "thumbprint" sign (swollen epiglottis)[46] can be helpful if the clinical findings are subtle, but in most cases, diagnostic studies should not delay intubation and direct inspection of the epiglottis in controlled surroundings. The mortality rate is 5 to 10 percent, almost always related to abrupt airway obstruction.

JOINT INFECTION

Before the use of Hib conjugate vaccines became routine, Hib was the leading cause of septic arthritis in children younger than 2 years of age.[119] This condition affects a single large joint at the knee, ankle, elbow, or hip in more than 90 percent of cases. Contiguous osteomyelitis occurs in 10 to 20 percent.[119,205] No feature clearly distinguishes septic arthritis caused by Hib from that of other bacterial causes. Patients initially are febrile, and more than two thirds have decreased range of motion, local warmth, and swelling.[85,119,205] Usually, pain, swelling, and erythema of the involved joint are preceded by a nonspecific upper respiratory illness. The clinical features may be subtle, with only decreased range of motion of the joint or abnormal gait as the initial finding. Hib capsular antigen concentrations are very high in the infected joint fluid of children with septic arthritis, and rapid detection of antigen is useful diagnostically. Septic arthritis of the hip requires surgical drainage, and resolution generally is rapid, but long-term cartilage damage may result from Hib arthritis despite adequate therapy.[377]

CELLULITIS

Cellulitis is a relatively uncommon form of Hib disease and was seen in the pre-vaccine era almost exclusively in children younger than 2 years of age. Most cellulitis (74%) is located in the cheek (buccal cellulitis), the periorbital region, and the neck[85] and rarely on the extremities. Facial cellulitis occurs most commonly in infants and is manifested as acute fever and a unilateral, raised, warm, tender and indurated area that may progress to a violaceous hue, although this finding is not unique to Hib disease.[379] Aspirate cultures of the point of maximal swelling usually yield the organism, and bacteremia is a typical finding.[122,175] A secondary focus (including meningitis) may be present in 10 to 15 percent of patients.[26] Orbital cellulitis generally is a complication of ethmoid sinusitis and, consequently, can be caused by non-Hib strains. An etiologic diagnosis can be established by blood culture or aspiration of subcutaneous tissues. Antibiotics always are indicated, but the need for surgical drainage depends on the degree of involvement of the tissues within the orbit.

PERICARDITIS

Hib pericarditis usually is a complication of adjacent pneumonia and is characterized by fever, an ill appearance, respiratory distress, and tachycardia.[68,97,314] The radiographic or clinical diagnosis can be confirmed by two-dimensional echocardiography,[423] and it may be suggested by finding capsular polysaccharide in serum or pericardial fluid or in Gram-stained pericardial fluid. Cultures of pericardial fluid are positive in more than 70 percent of cases.[85] Early drainage is an important part of the management of this illness, and early pericardectomy or pericardiostomy is preferred over repeated pericardiocentesis.[248,314]

NEONATAL DISEASE

H. influenzae causes 2 to 8 percent of neonatal early-onset sepsis.[56,171,402] Most of these cases are caused by nontypeable strains, most of which are concordant with those isolated from the maternal genital tract.[402] The precise pathogenesis is unknown, but neonatal disease often is associated with prematurity, low birth weight, premature rupture of membranes, and maternal chorioamnionitis; several cases have occurred after cesarean delivery, thus suggesting in utero transmission.[324] Clinical manifestations include pneumonia, bacteremia, and conjunctivitis. More than two thirds of neonatal *H. influenzae* disease occurs on the first day of life, with an overall mortality rate of 55 percent.[132]

OTHER INVASIVE INFECTIONS

Other invasive Hib infections include endophthalmitis,[378] CSF shunt infections,[210,312] necrotizing fasciitis, pyomyositis,[270] peritonitis,[74,131] scrotal abscess,[215] brain abscess,[113] polyserositis,[243] tenosynovitis,[269] epididymitis,[156] lung abscess,[214] periappendiceal abscess,[235] and bacterial tracheitis.[59] Invasive disease also may be characterized by fever alone, fever with petechiae, or fever of unknown origin.[51,393]

DISEASES CAUSED BY NON–TYPE b *HAEMOPHILUS INFLUENZAE*

Respiratory tract infections associated with nontypeable *H. influenzae* are a major cause of morbidity and mortality today in both developed and nonindustrialized nations.[207,401] Previously, invasive disease caused by nontypeable *H. influenzae* was a rare occurrence and generally was associated with underlying medical conditions such as prematurity, malignancy, cystic fibrosis, asthma, leakage of CSF, CNS shunts, congenital heart disease, lymphoproliferative disorders, and immunoglobulin deficiency.[107,137,274] More recently, with a decline in the rate of infections caused by Hib, other *H. influenzae* serotypes such as types a, e, and f, and nontypeable strains have become relatively more common causes of both mucosal surface infection and invasive disease.[170,185,382,387]

Invasive disease caused by non–type b *H. influenzae* has been reported more frequently in recent years, although it is unclear that the absolute incidence of disease has increased.[65] Several clusters of cases of invasive disease caused by *H. influenzae* type a have been reported.[5,164,165,185,278] One such report suggested that

an IS1016-bexA deletion present in the chromosome of the recovered type a strains, a deletion similar to that present in many Hib strains,[200] could be a marker for more virulent type a clones.[5] Other serotypes also have been reported as causing cases of non–type b disease.[278] Urwin and associates[387] identified 91 cases of invasive *H. influenzae* type f disease in a multistate area over the course of a 6-year period. The incidence of invasive type f disease was 0.5 cases per 1 million population in 1989 and 1.9 cases per 1 million population in 1994. In children, pneumonia and meningitis each accounted for 40 percent of the total cases. Overall, the mortality rate from type f *H. influenzae* disease was 21 percent in children. Serotype f also has been reported as a cause of endocarditis[130] and septic arthritis[143] in healthy, normal children.

Invasive disease in normal children caused by nontypeable *H. influenzae* also has been reported occasionally.[103,274] Heath and associates[170] conducted an active prospective surveillance study of *H. influenzae* infections in the United Kingdom and Republic of Ireland. During the study period (October 1992 to December 1998), 102 cases of invasive disease caused by nontypeable *H. influenzae* were reported. Children with nontypeable *H. influenzae* infection were compared with those who had Hib disease. The former were more likely to be younger (16 vs. 22 months) and more likely to have pneumonia and bacteremia ($p < .001$) than were those with Hib disease. In 1998, the incidence of non–type b *H. influenzae* disease in all children younger than 5 years of age in the United Kingdom was 1.3 per 100,000, versus 0.6 per 100,000 population for Hib disease. Most non–type b strains isolated were nontypeable.

Mucosal Infections

Unencapsulated or non-Hib strains cause a variety of mucosal infections, including otitis media, sinusitis, conjunctivitis, and bronchitis.[195,208] *H. influenzae* is the second leading cause of acute otitis media in adults and children,[195] and it has features similar to those of other causes of otitis, namely, fever and symptoms referable to the upper respiratory tract in addition to nonspecific symptoms such as irritability, vomiting, and diarrhea.[209] Data published in 2004 suggest that nontypeable *H. influenzae* is the most common cause of persistent and recurrent middle ear disease, particularly in populations that have received the pneumococcal conjugate vaccine.[40,61,290] Sinusitis caused by nontypeable *H. influenzae* may be manifested as common cold symptoms that are persistent or more severe than usual.[396,398,399] Older children and adults are more likely to complain of headache and paranasal, dental, or facial pain. Other common symptoms are a daytime cough that may worsen at night or reactive airway disease unresponsive to therapy. Rarely, chronic otitis or sinusitis may result in the development of mastoiditis or a parameningeal abscess.[186] Conjunctivitis usually is bilateral and purulent and may occur in outbreaks.[41-43] Though common, these infections rarely are life-threatening and are not associated with bacteremia.

DIAGNOSIS

A high index of suspicion for the possibility of Hib disease must be maintained when evaluating children with appropriate clinical manifestations and findings. The primary criterion for the diagnosis of *H. influenzae* infection is Gram stain or isolation of the organism from an infected focus (e.g., CSF, pleural fluid, sputum, or blood). Because most invasive Hib disease is associated with bacteremia, blood cultures should be performed for any febrile child with potential Hib disease.[197] The specimens need to be processed immediately because the organism is fastidious. Although most commercial blood culture media support the growth of *H. influenzae*, the inoculum should be applied, when possible, directly to chocolate agar or a semi-synthetic medium containing heme and NAD.[22,134,192,291] In liquid medium, growth may not be sufficient to result in turbidity; therefore, for blood cultures, advanced detection systems or subcultures should be performed.[192] In addition, selective media that suppress the growth of gram-positive organisms may increase the recovery of *H. influenzae* from upper respiratory tract specimens.[67] Identification of an isolate as *H. influenzae* relies on its dependence on heme and NAD.[192]

Other techniques that may assist in microbiologic diagnosis include rapid antigen detection, staining techniques, and immunofluorescence.[184,230,410] Such techniques are useful in the context of a patient whose cultures are sterile because of previous antibiotic therapy, or they can confirm the clinical diagnosis before bacterial growth occurs. The three techniques most commonly used for antigen detection are latex particle agglutination (LPA), countercurrent immunoelectrophoresis (CIE), and coagglutination (CoA). False-positive results on CSF are rare by all three tests; however, false-positive reactions have occurred when testing serum and urine. False-positive reactions occur as a result of nonspecific agglutination (i.e., rheumatoid factors) and because of antigenic cross-reactivity with *Escherichia coli*, *S. pneumoniae*, staphylococci, or meningococcus. Overall, LPA appears to be more sensitive than is CoA, and LPA and CoA are more sensitive than is CIE in CSF, serum, urine, joint fluid, and pleural fluid.[184,230,237] False-positive results can occur in the urine of children with nasopharyngeal carriage of the organism or, more commonly, for several days after immunization with Hib conjugate vaccine.[364] Acridine orange is a fluorescent stain that binds to cellular nucleic acids and may be useful in situations in which smaller bacterial concentrations are present.[194] Immunofluorescent staining of purulent specimens from patients with partially treated disease also has been useful.[75]

TREATMENT

INVASIVE DISEASE

Bacteremia plays a central role in the pathogenesis of invasive Hib disease; therefore, occult invasion of the CNS always must be considered in the context of any manifestation of Hib infection.[141,197] In addition, severe Hib disease often is fatal if it is not treated adequately. Therefore, elimination of Hib bacteremia and its complications requires antimicrobial therapy that will (1) penetrate the blood-brain barrier to achieve bactericidal concentrations and (2) be of adequate duration to sterilize the primary and potential secondary foci. The choice of specific antibiotic therapy must take into account the local antibiotic susceptibility patterns of invasive isolates (Table 145–3). *H. influenzae* resistance to several antimicrobials, including ampicillin, chloramphenicol, trimethoprim-sulfamethoxazole, rifampin, and certain second-generation cephalosporins, has been increasing in several areas of the world.[95,169,183]

For proven or suspected Hib meningitis, cefotaxime or ceftriaxone is recommended until the antibiotic susceptibility of the organism is known or an alternative diagnosis is established.[111] Both antibiotics have bactericidal activity against Hib strains, including those that produce β-lactamase, and they penetrate effectively into infected CSF and are well-tolerated. Cefuroxime should not be used for the treatment of *H. influenzae* meningitis because delayed sterilization of CSF may be at least twofold more common than with ampicillin plus chloramphenicol or with the third-generation cephalosporins.[20,359] Moreover, ampicillin, formerly a mainstay of therapy for this infection, should not be used empirically to treat infections caused by Hib because as many as 50 percent of Hib isolates in the United States are resistant,

TABLE 145–3 Selected Antimicrobial Agents for Treatment of *Haemophilus influenzae* Infections

Antimicrobial Agent	Total Daily Dose (mg/kg)	Dose Frequency
Parenteral Antibiotics*		
Ampicillin†	200-400	q4-6h
Cefuroxime‡	75-150	q8h
Cefotaxime	150-200	q6-8h
Ceftriaxone	50-100	q12-24h
Oral		
Amoxicillin§	40-90	tid/bid
Amoxicillin-clavulanate	40-90	tid/bid
Erythromycin-sulfisoxazole§	40 (erythromycin)	qid
Trimethoprim-sulfamethoxazole§	8 (trimethoprim)	bid
Clarithromycin§	15	bid
Azithromycin§	10	qd
Cefuroxime axetil	30	bid
Cefprozil	30	bid
Cefpodoxime	10	bid
Cefixime	8	qd

**Other parenteral agents may be used in special circumstances, but they should not be considered first line. Such agents include ureidopenicillins, carbapenems, and fluoroquinolones.*

†*Not to be used presumptively because of the prevalence of resistant strains.*

‡*Not to be used for suspected meningitis.*

§*Do not use these oral agents if resistance is suspected or prevalent in your area.*

usually through plasmid-mediated β-lactamase production.[183] Chloramphenicol, another medication that frequently was used to treat Hib disease,[244,325] now rarely is used because safer antibiotics with greater activity are available. Although adequate bactericidal blood and CNS levels of chloramphenicol can be achieved, even with oral administration,[112,332] the use of this agent requires monitoring of drug levels. Dose-dependent, yet reversible, bone marrow toxicity may occur, particularly in neonates, in patients with liver disease, or in those who require prolonged treatment.[244] Idiosyncratic aplastic anemia, a dose-independent complication of chloramphenicol use, is an extremely rare occurrence.

Cefuroxime has good activity against *H. influenzae* and is useful for empiric treatment of nonmeningitic infections such as pneumonia, periorbital cellulitis, and septic arthritis.[392] Other parenteral agents that have activity against *H. influenzae* include meropenem, ampicillin-sulbactam, aztreonam, and other third-generation cephalosporins such as ceftazidime.[234] In general, the spectrum of activity of these agents is too broad for routine use in pediatric infections in which Hib is an important pathogen.

Children with occult Hib bacteremia need to be reevaluated carefully because focal disease may develop in 30 to 50 percent of such patients who are clinically well.[81,197] The duration of therapy is determined by the site of infection and the clinical response. Children with uncomplicated Hib meningitis can be treated for 10 days.[14,111] Children with Hib cellulitis can be switched to oral therapy after several days of parenteral therapy, provided these patients have had a satisfactory clinical response and do not have meningitis. Patients with septic arthritis should receive at least 10 to 14 days of therapy,[119] whereas children with pericarditis, empyema, or osteomyelitis may require longer courses of antibiotic treatment (3 to 6 weeks).[68,119] These patients often can be switched to oral antibiotics after susceptibility, a good therapeutic response, and adequate antimicrobial blood levels have been documented and compliance has been ensured.

Equally important in the overall management of a child with invasive Hib disease is supportive care. For Hib meningitis, several studies showed that adjunctive therapy with dexamethasone moderates the inflammatory cascade and may decrease the likelihood of hearing loss.[277] The recommended dose is 0.6 mg/kg/day given every 6 hours for 4 days, with the first dose administered just before or concurrently with the first antibiotic dose.[14,277,303] Management of a child with meningitis requires continuing careful evaluation for complications such as the development of shock, inappropriate secretion of antidiuretic hormone, seizures, subdural empyema, and secondary foci of infection.[93,94,301] Prolonged fever is a common occurrence, with approximately 10 percent of children febrile for at least 10 days.[111] Repeat lumbar puncture to document sterility of the CSF is not necessary in uncomplicated cases.

In children with epiglottitis, protection of the airway is the most important component of therapy and should be initiated even before the administration of antimicrobials.[46] Endotracheal intubation or tracheostomy is performed optimally in the operating room by personnel experienced with these procedures in children. Before establishing the airway, one should take care not to precipitate laryngospasm by attempting to examine the epiglottis or by performing procedures such as venipuncture.[33,46] Blood should be obtained for culture, and intravenous antibiotics should be initiated as soon as possible after the airway has been secured.

Patients with subdural empyema, pericarditis, or pleural empyema usually require percutaneous or surgical drainage.[68,93] Infected joint fluid should be aspirated from children with septic arthritis to confirm the diagnosis and reduce pressure.[119] Repeated aspirations or placement of a surgical drain also may be needed. Infection of the hip requires surgical incision and drainage to decompress the joint; failure to do so may result in avascular necrosis of the femoral head.[119]

NONINVASIVE DISEASE

Noninvasive *H. influenzae* infections, usually caused by nontypeable strains, include otitis media, sinusitis, conjunctivitis, bronchitis, and pneumonia. Numerous orally administered antimicrobials are available to treat these infections (see Table 145–3). Despite the increasing prevalence of β-lactamase–producing organisms, amoxicillin remains the drug of choice for empiric treatment of otitis media in most areas because of its low cost and proven safety.[14,213] Several other antimicrobials with activity against *H. influenzae* are available and include the following: amoxicillin-clavulanate; trimethoprim-sulfamethoxazole; erythromycin-sulfisoxazole; newer macrolides such as clarithromycin and azithromycin; and second- and third-generation cephalosporins such as cefuroxime axetil, cefixime, cefpodoxime, cefdinir, and cefprozil.[95] Cefaclor was used widely but has poor activity against β-lactamase–producing organisms,[229] and it causes a serum sickness–like illness in approximately 2 percent of recipients.[191,394] The quinolone antibiotics, such as ciprofloxacin, also are active but are not licensed for use in children younger than 18 years old because of quinolone-induced arthropathy seen in juvenile animals. In the context of poor clinical response or isolation of β-lactamase–producing organisms, amoxicillin-clavulanate (Augmentin), cefixime, and cefpodoxime appear to be the most useful.[14,213]

Most mucosal infections are treated presumptively without obtaining definitive cultures, and, consequently, the duration of therapy and the need for alternative antibiotics are based on assessment of the clinical response. Otitis media usually is treated for 7 to 10 days, and sinusitis should be treated for at least 1 week beyond the resolution of symptoms.[14,213,396] If resistance is suspected and leads to failure of treatment, an empiric change in antibiotics is indicated.[213]

PREVENTION

The near elimination of invasive Hib disease in the United States and other countries is a direct result of the routine use of Hib conjugate vaccines and represents a remarkable success story.[65] Prevention with hyperimmunoglobulin also has been shown to be effective in high-risk populations, but it is costly, of short-lived benefit, and not licensed for general use.[328] Antimicrobial prophylaxis is effective for the prevention of secondary Hib disease, but it represents only a minor proportion of the overall disease burden.[14,27]

ACTIVE IMMUNIZATION

The first Hib vaccine was the PRP polysaccharide vaccine, which is composed of purified Hib capsular polysaccharide PRP.[21,319] In 1985, in children older than 18 months of age, it became the first vaccine to be licensed for the prevention of Hib disease. Children younger than 18 months of age had inadequate immune responses with PRP vaccination.[18,188] PRP vaccine has immunologic properties similar to those of some other polysaccharide vaccines, which generally are considered to be T-independent immunogens.[3] As a result, antibody responses are limited and are of short duration, particularly in young children, and no booster response occurs with repeated administration of the vaccine. Furthermore, the induced antibody may have reduced functional qualities (i.e., primarily IgM, low avidity).[316,349]

Licensure of PRP vaccine was based on the findings of a large randomized clinical trial conducted in Finland in 1974[287,288] that suggested a protective efficacy of 90 percent for children who were immunized between 18 and 71 months of age. Subsequent to licensure of this vaccine in the United States, it became apparent that routine use of the vaccine resulted in efficacy less than that determined in the Finnish vaccine trial, between 0 and 88 percent.[408] This vaccine is mainly of historical significance because its role has been supplanted by development and licensure of the PRP-protein conjugate vaccines.

Hib conjugate vaccines were developed in an effort to enhance immune responses to the PRP antigen. Basic to all conjugate vaccines is the use of a covalently linked (conjugated) immunogenic protein carrier that confers on the PRP polysaccharide hapten recognition by T cells and macrophages and stimulation of T-dependent immunity.[204,408] Four Hib conjugate vaccines have been developed and evaluated in infants, and all use PRP polysaccharide or oligosaccharides derived from it as the primary immunogen (Table 145–4): PRP-D (diphtheria toxoid), HbOC (mutant diphtheria toxin), PRP-OMP (major OMP of *Neisseria meningitidis* serogroup B), and PRP-T (tetanus toxoid). The immune response after receipt of Hib conjugate vaccination has the following general characteristics: (1) it is quantitatively enhanced, particularly in younger infants; (2) repeat administration of vaccine elicits booster responses; and (3) maturation of class-specific immunity with a predominance of IgG antibody and probably enhanced functional properties occurs.

The first Hib conjugate vaccine licensed was PRP-D, but it was less immunogenic than are the conjugate vaccines developed subsequently and rarely is used in the United States. In children 15 months of age or older who received PRP-D, high antibody concentrations were achieved with a single dose.[35,173] In infants, however, antibody levels greater than 1 μg/mL developed in fewer than half, even after they received three doses.[89,187] Numerous case-control studies demonstrated that a single dose of vaccine was at least 80 percent efficacious in preventing disease in children 18 months or older. In infants in Finland who received vaccine at 3, 4, 6, and 14 to 18 months of age, the protective efficacy after three doses was 94 percent.[104] In contrast, a trial conducted in Native Alaskan infants found no evidence of protection in that high-risk population.[405]

A single dose of HbOC is highly immunogenic in children older than 18 months of age,[173,224] and after the administration of three doses in infancy, high antibody levels are achieved.[89,321] Two prospective clinical studies showed that two or three doses of HbOC administered in the first 6 months of life provide a high degree of protective efficacy. In the Kaiser Permanente northern California region population, HbOC was 100 percent efficacious after two doses administered in infancy.[38] The vaccine also was evaluated in Finland in infants who received vaccine at 4, 6, and 14 to 18 months of age, and efficacy was 95 percent after two doses.[105] Data from a third post-licensure study in infants in Los Angeles County also suggested a protective efficacy of 89 percent after two doses and 94 percent after three doses.[390]

PRP-OMP induces an immune response that is less age-dependent than is the response to the other Hib conjugate vaccines. Adults and children respond to a single vaccine dose with high antibody levels.[321] In infants as young as 6 to 8 weeks, a single dose of PRP-OMP induces a good antibody response.[52] In addition, the antibody levels achieved after two doses are higher than those achieved after two doses of any of the other conjugate vaccines,[52,89] and a third dose does not enhance the response. PRP-OMP was evaluated in a randomized, double-blind, placebo-controlled trial involving high-risk Navajo Native American infants[330] who received vaccine at 2 and 4 months of age; an overall efficacy of 95 percent was reported. Additional data from a population-based case-control study in Los Angeles County suggested a level of effectiveness similar to that seen for HbOC.[389]

PRP-T is highly immunogenic in adults and older children,[73,340] and high concentrations of antibody are achieved in infants with a three-dose immunization series at 2, 4, and 6 months of age.[89] The protective efficacy of PRP-T was evaluated in two large prospective randomized trials that were terminated prematurely because of the licensure of other Hib conjugate vaccines for infants. More than 12,000 infants were enrolled in studies in southern California and North Carolina, and no cases of invasive Hib disease occurred in the vaccinated children versus 5 cases in the control groups.[133,391] Efficacy subsequently was

TABLE 145–4 Characteristics of *Haemophilus influenzae* Type b Conjugate Vaccines

Vaccine	Polysaccharide Size	Protein Carrier	Linkage	Trade Name*
PRP-D	Medium	Diphtheria toxoid	Protein with 6-carbon spacer	ProHIBiT
HbOC	Small	CRM_{197} (mutant diphtheria toxin)	PS, no spacer	HibTITER
PRP-OMP	Medium	*Neisseria meningitidis* outer-membrane protein complex	Protein and PS with bigeneric spacer	PedvaxHIB
PRP-T	Large	Tetanus toxoid	PS with 6-carbon spacer	ActHIB

HbOC, mutant diphtheria toxin; PRP-D, diphtheria toxoid; PRP-OMP, major outer-membrane protein of N. meningitidis *serogroup B; PRP-T, tetanus toxoid; PS, polysaccharide.*
**HbOC, PRP-OMP, and PRP-T also are available in combination with diphtheria-tetanus-pertussis, diphtheria-tetanus–acellular pertussis, and hepatitis B vaccines.*

shown in a study in England; after three doses in infancy, the efficacy of PRP-T was estimated to be 100 percent.[45] In Finland, during the first 2 years of its general use, more than 100,000 infants were immunized, and only 2 cases of invasive Hib disease occurred in vaccinees, both after a single dose. In no infant who has received two or more doses of vaccine in any study has Hib disease developed.[289]

HbOC, PRP-OMP, and PRP-T are licensed for use in infants at 2, 4, 6, and 12 to 15 months of age. Any Hib conjugate vaccine can be used as the booster dose or in different sequences.[14] Several issues, however, remain to be determined regarding the use of Hib conjugate vaccines in infants. Direct comparisons of Hib conjugate vaccines need to be considered in the context of varying study designs, differences in vaccine lots, and different laboratory and statistical methodologies. Despite these difficulties, certain concepts are apparent: (1) all Hib conjugate vaccines are safe in infants; (2) PRP-D is the least immunogenic conjugate vaccine; (3) only PRP-OMP induces a good immune response after one dose in young infants, but antibody levels are lower than those induced by multiple doses of HbOC and PRP-T vaccine; and (4) PRP-OMP, HbOC, and PRP-T appear to be efficacious, but no direct comparison of the protective efficacy of these vaccines has been completed.

In addition, certain mixed sequences of Hib vaccines given to infants (i.e., PRP-OMP followed by HbOC or PRP-T) may enhance the antibody response.[146,155] Furthermore, simultaneous receipt of other non-Hib vaccines and the impact of concurrent or previous receipt of the carrier protein (carrier priming)[152,153,212] are issues that need to be explored. Combination vaccines that include Hib (Hib plus diphtheria-tetanus-pertussis [Hib-DTP], Hib plus diphtheria and tetanus toxoids and acellular pertussis [Hib-DTaP], and Hib-hepatitis B) currently are licensed, and other combinations (Hib plus inactivated poliovirus vaccine [Hib-IPV] and Hib-DTaP/IPV) are being evaluated in infants and children.[36,305,335]

Schmitt and associates[339] surveyed the impact and effectiveness of DTaP (IPV)-Hib combination vaccines in Germany. Although combination DTaP-Hib vaccines have been documented to elicit lower anti-Hib titers than separate vaccines do, the impact of this finding is unclear. Schmitt and colleagues[339] showed that combination vaccines were effective in reducing the incidence of Hib disease in Germany.[339] The study of Schmitt and associates is encouraging. More recent data from Canada also suggest that a multiple-component, Hib-containing combination vaccine is safe and effective in preventing invasive Hib disease in young children.[333,335]

The phenomenal success of the Hib vaccination strategy has been related to the effectiveness of Hib conjugate vaccines in preventing colonization. Fernandez and associates[116] reported that the anti-Hib capsular polysaccharide antibody concentration required to prevent colonization was greater than that needed for protection against invasive disease. Whether concentrations in the range that Fernandez and associates suggested was important (>5 μg/mL) can be achieved and sustained by using conjugate vaccines in all population groups remains a subject of investigation. Studies in some high-risk population groups (Native Alaskans) documented that invasive disease is prevented best by the use of PRP-OMP Hib vaccine,[357,358] at least for the first dose of vaccine, and that even the use of PRP-OMP Hib vaccine in these individuals may not eliminate carriage of the organism in the population.[91,136] Thus, the use of combination vaccines in this population group is unlikely to be as effective as is the use of PRP-OMP Hib conjugate vaccine.[358]

To date, essentially all *H. influenzae* vaccines are based on immunity to the Hib capsule. Antibodies to other components of the bacterium also have been shown to be bactericidal, opsonophagocytic, and protective in animal studies.[25,29,90,100,162,203,255,258,259,421] Vaccines containing alternative antigens in theory could provide supplemental protection against Hib, although this protection does not appear to be necessary in most individuals based on the efficacy of the available Hib conjugate vaccines. However, more importantly, such alternative vaccines could provide immunity to non-Hib strains that are ubiquitous colonizers of the upper respiratory tract of humans.[108,202] One of the basic microbiologic problems that must be overcome before such vaccines are developed is the known diversity of many of the candidate vaccine antigens of interest.[29,138] Because of the known strain-to-strain variability in many of these antigens,[92] finding an antigen relevant to all or most strains has been a challenging task.[29,257] Even so, recent human trials have reported success in preventing some cases of *H. influenzae* otitis media with a novel pneumococcal polysaccharide–*H. influenzae* protein D conjugate vaccine,[304] and many other candidate vaccine antigens remain the subject of active pre-clinical research.[28,90,203,422]

PASSIVE IMMUNIZATION

Although active immunization clearly is preferred for the control of Hib disease, passive immunization has potential utility in the following settings: (1) in selected high-risk groups with a risk of acquiring disease soon after birth and too young to respond to vaccination (i.e., Eskimos or Native Americans); (2) in functionally asplenic patients; (3) in immunocompromised patients; and (4) for prevention of secondary disease in households, daycare centers, or institutions. A human hyperimmunoglobulin from adult Hib-immunized donors called *bacterial polysaccharide immunoglobulin* has been prepared,[355] but it is not commercially available. Pharmacologic studies showed that high levels of antibody can be achieved after intramuscular injection, and significant protective efficacy against invasive Hib disease was demonstrated in Apache children who were given three doses during the first year of life.[328] The use of concurrent active immunization with bacterial polysaccharide immunoglobulin also may be an effective strategy.[211] Another possible approach in such groups would be maternal Hib immunization,[144] to induce transplacental antibody, but questions remain regarding the safety and acceptability of vaccinating pregnant women and the inability to immunize women who do not receive prenatal care.

IMPACT OF *HAEMOPHILUS INFLUENZAE* TYPE b VACCINATION

The impact of widespread vaccination with Hib conjugate vaccine has been dramatic and reproduced in several areas of the United States[4,65,263] and in many countries throughout the world.[182,289,306,334] Exclusively using HbOC vaccine,[37] the Kaiser Permanente northern California region eliminated Hib disease except for a rare case in an unimmunized child and a few cases in children with incomplete immunizations. In the Kaiser Permanente southern California region,[389] PRP-D and subsequently PRP-OMP vaccines were used in older children between 1987 and 1990. Since 1990, PRP-OMP vaccine has been administered almost exclusively. A few cases of PRP-D failure and only two cases of PRP-OMP failure occurred; disease essentially has been eliminated. Similar control of disease has been achieved with the use of PRP-OMP vaccine in Alaskan and Navajo Native American populations,[329] in Los Angeles County,[389] Minnesota, and Dallas.[263] In U.S. sites under surveillance by the Centers for Disease Control and Prevention, similar eradication of disease has been achieved, although some localized populations with increased rates of disease still remain.[65,358]

In general, the decrease in the incidence of disease has exceeded expectations, given the estimated proportion of the population completely immunized. In addition, in essentially all these populations, a significant decrease in the incidence of disease was

observed in infants before the licensure and recommended use of vaccines in that age group. These findings probably are explained by a reduction in Hib carriage as a result of vaccination[263,373] and, consequently, decreased transmission from immunized children to unimmunized young children and infants.

The immunization schedules vary in different countries to parallel immunization practices for other vaccine-preventable diseases. Moreover, the age-specific incidence of disease varies in different countries. Initially, in the United Kingdom, the occurrence of disease was such that physicians felt justified to use only a three-dose schedule at 2, 3, and 4 months of age and not to provide a booster dose in the second year of life. As alluded to earlier, this schedule, which was introduced in the United Kingdom in 1993, appeared to be related to the reappearance of Hib disease in older children observed in the late 1990s.[19,180,238,308] This experience underlies the importance of giving a fourth dose when children are between 12 and 18 months of age.

CHEMOPROPHYLAXIS

Secondary disease accounts for less than 2 percent of all cases of invasive Hib disease. Chemoprophylaxis, however, can protect susceptible persons from acquiring Hib by eliminating Hib colonization in close contacts. Children younger than 4 years old have a 600-fold increased risk of acquiring Hib disease after household contact with a case.[406] Risk also appears to be increased in daycare center settings, but this risk is defined less well.[147,279] In addition, adults and older children who are colonized can transmit Hib to susceptible children, even though they have little risk for acquiring invasive disease themselves.

Antimicrobial agents effective for chemoprophylaxis must achieve bactericidal levels intracellularly and in mucosal secretions. Rifampin, which achieves high concentrations in respiratory secretions,[236] is the most effective antimicrobial agent for eradicating Hib from the nasopharynx. Rifampin, in a dosage of 20 mg/kg once daily (maximal daily dose, 600 mg) for 4 days eradicates Hib carriage in 95 percent or more of household [27,145] or daycare center[151] contacts of a case. Cohort studies showed the effectiveness of rifampin prophylaxis in preventing secondary Hib disease in household and daycare center attendees.[145,151,226] Antimicrobials effective in the treatment of Hib disease, such as ampicillin, trimethoprim-sulfamethoxazole, erythromycin-sulfisoxazole, and cefaclor, were shown to be ineffective for antimicrobial prophylaxis.[174] They eliminated Hib carriage in fewer than 70 percent of culture-positive contacts and, therefore, are not recommended for chemoprophylaxis.

Both the U.S. Public Health Service Advisory Committee on Immunization Practices[64] and the American Academy of Pediatrics Committee on Infectious Diseases[14] recommend rifampin prophylaxis for all household contacts, including adults, and for the index patient (therapeutic antibiotics do not eradicate Hib from the nasopharynx consistently) if the household has a contact younger than 4 years old who is not fully immunized. Prophylaxis should be instituted as soon as possible because the risk of acquiring secondary disease is greatest during the few days after the onset of disease in the index patient and within 2 weeks of the onset of disease. With regard to the daycare center setting, no consensus exists concerning the need for chemoprophylaxis because of uncertainty about the magnitude of the risk of acquiring secondary Hib disease in this setting. Some authors recommend chemoprophylaxis if classroom contacts include those younger than 2 years of age, whereas others think that recommendations should be individualized.[86,279] However, virtually all experts recommend prophylaxis if two or more cases of Hib disease have occurred among attendees within 60 days.[14,64]

In all situations in which the potential for secondary disease exists, this risk should be explained to families, with an emphasis placed on the importance of seeking prompt medical attention for febrile illnesses. Clinicians should not obtain pharyngeal cultures to determine whether prophylaxis should be administered because doing so only delays the prompt administration of chemoprophylaxis.

CONCLUSION

The perspective on *Haemophilus* disease has changed dramatically in recent years. Before the availability of Hib conjugate vaccines, invasive Hib was one of the most important bacterial pathogens of children. It was the leading cause of bacterial meningitis and an important cause of other bacteremic illnesses. The spectrum of illness is broad, morbidity and mortality rates are significant, and subtleties in making an early diagnosis and instituting appropriate management exist. Currently, when one considers that most antibiotic use worldwide is for upper respiratory tract infections, including otitis media, and that *H. influenzae* causes a significant proportion of these illnesses, it still must be considered an important pediatric pathogen.

Although various aspects of disease caused by *H. influenzae* are reviewed in this chapter, what is clear is that the most important aspect has been disease prevention by routine immunization of infants with polysaccharide-protein conjugate vaccines. This achievement is the culmination of more than 100 years of research on *H. influenzae*. Although historically many technologic problems and misunderstandings about the organism and its pathogenesis have occurred, much has been accomplished in recent years. The impact of routine infant immunizations with Hib conjugate vaccines is relatively recent and very dramatic. The public health benefits parallel the eradication of poliomyelitis and the control of other vaccine-preventable childhood diseases. Widespread Hib immunization virtually has eliminated Hib disease in the United States and in many developed countries where it is used routinely. The degree of disease control exceeds all expectations and is in excess of what the known levels of immunization would have predicted. Unfortunately, Hib conjugate vaccines currently are used routinely in relatively few developing countries and only electively in some additional countries, thus leaving most of the world without the benefit of immunization. The World Health Organization has taken up the challenge to expand Hib immunization worldwide.

Progress also has been achieved at the pre-clinical level in the development of vaccines against other *H. influenzae* serotypes and nontypeable strains. Control of infection caused by these organisms will have an important public health impact, and the use of such vaccines may have a role in adolescents and adults. The technologies that led to the development of Hib conjugate vaccines served as a prototype for vaccines to prevent disease caused by other encapsulated bacteria including pneumococci, meningococci, and group B streptococci. As such, the lessons learned in the quest to eliminate Hib disease have had important implications in the control of many other bacterial diseases.

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CHAPTER 146

Other *Haemophilus* Species (*aphrophilus, ducreyi, haemolyticus, influenzae* Biogroup Aegyptius, *parahaemolyticus,* and *parainfluenzae*)

Stephen J. Barenkamp

Most serious pediatric infections caused by organisms of the *Haemophilus* genus are caused by *Haemophilus influenzae*. However, other *Haemophilus* spp. also can cause disease occasionally. Other *Haemophilus* spp. that have been well documented as causes of illness in pediatric and adolescent patients include *Haemophilus aegyptius*, *Haemophilus aphrophilus*, *Haemophilus ducreyi*, and *Haemophilus parainfluenzae*. As discussed later, *H. aegyptius* has been classified as a member of the species *Haemophilus influenzae* (*H. influenzae* biogroup aegyptius), but given the unique characteristics of this organism and the distinctive illnesses with which it is associated, it is described in this chapter. Other *Haemophilus* spp., such as *Haemophilus haemolyticus* and *Haemophilus parahaemolyticus*, rarely, if ever, cause illness in the pediatric population and are not discussed here.

Members of the *Haemophilus* genus are gram-negative coccobacillary bacteria that are facultatively anaerobic. They demon-

TABLE 146–1 Differential Characteristics of *Haemophilus* Species

Haemophilus Species	Factor Requirement		Hemolysis of Horse Blood	Fermentation of:				Presence of Catalase	CO_2 Enhancement of Growth
	X*	V		Glucose	Sucrose	Lactose	Mannose		
H. influenzae†	+	+	−	+	−	−	−	+	+
H. haemolyticus	+	+	+	+	−	−	−	+	−
H. ducreyi	+	−	−	−	−	−	−	−	−
H. parainfluenzae	−	+	−	+	+	−	+	D	D
H. parahaemolyticus	−	+	+	+	+	−	−	+	−
H. aphrophilus	−	−	−	+	+	+	+	−	+

**As determined by the porphyrin test.*
†Includes biogroup aegyptius.
D, encountered.

strate optimal growth when they are incubated in a humid atmosphere containing 5 to 10 percent carbon dioxide.[99,100,130] Most species have fastidious nutritional requirements and require special media, supplements, or both for optimal growth.[99,100] X factor (hemin), V factor (nicotinamide adenine dinucleotide), or both are required for in vitro growth.[140] Organisms with the "para-" prefix require V factor only. The specific nutritional requirements of *Haemophilus* isolates are sole characteristics used to subclassify these organisms into the different species (Table 146-1).

HAEMOPHILUS APHROPHILUS

BACTERIOLOGY

H. aphrophilus was described first by Khairat[98] in 1940 in association with a fatal case of endocarditis. He suggested the species name *aphrophilus* because the organism required relatively high concentrations of carbon dioxide for isolation on the usual media. In earlier times, a well-known manifestation of carbon dioxide was the formation of bubbles of gas in fermenting wine (i.e., froth, or *aphros*).[24,98] Although the organism originally was classified as a *Haemophilus* sp. because of the growth requirement for X factor, more recent studies suggest that it can grow independent of this factor.[99,135,177] Some authors have suggested that given this X factor independence, the organism should be placed in another genus,[99,133,177] but at present it remains in the *Haemophilus* genus.

EPIDEMIOLOGY AND PATHOGENESIS

H. aphrophilus is a component of the normal oral flora. Using a selective medium, Kraut and coworkers[103] isolated the organism from gingival scrapings and interdental material of one third of the healthy adults they examined. With respect to disease pathogenesis, dental disease or manipulation has been suggested to predispose the individual to transient bacteremia, which results in seeding of distant tissue sites where localized infection subsequently develops.[24,75,132,135,156] In addition, several case reports of patients with *H. aphrophilus* disease reported an association between human infection and contact with or bites from dogs and cats.[64,90,132,135,188] However, a causal relationship has been difficult to confirm, and the human mouth and respiratory tract probably are the portals of entry for most infections.

CLINICAL MANIFESTATIONS

H. aphrophilus is a rare cause of infection and disease in pediatric patients, with fewer than 50 cases reported in the literature.* Brain abscesses and endocarditis are the pediatric infections reported most commonly with this organism.[20,24,58,64,90,127,135] Other sites of infection in children documented in the literature include the oropharynx,[24] the abdominal cavity,[24,96] and various superficial soft tissue sites.[135,195,196] Infections with *H. aphrophilus* do not appear to be associated with any distinctive clinical features compared with infection caused by other organisms at the same sites. However, infections caused by *H. aphrophilus* frequently are associated with underlying conditions, such as congenital heart disease, trauma, and immunosuppression, thus predisposing the host to infection.[24,60,64,86,127,135,143,195]

TREATMENT

Antimicrobial susceptibility testing has not been well standardized for this organism, and disk-diffusion testing, in particular, has been found to be unreliable.[24,85,135,177] Tube or agar dilution testing generally is considered to be the preferred testing method. Older reports found that *H. aphrophilus* was susceptible uniformly to certain antibiotics, including chloramphenicol and the aminoglycosides.[24,135,177] Susceptibility to penicillins has been variable. Even so, penicillin has been used successfully for treatment of susceptible organisms,[24,75,135,188] at times in combination with aminoglycosides and other antimicrobials. Some reports document the usefulness of ampicillin or ceftriaxone for treatment of infection.[20,124,132] Numerous different antibiotics probably can be used to treat *H. aphrophilus* infections successfully. The choice for treatment should be guided by appropriate in vitro susceptibility testing.

HAEMOPHILUS DUCREYI

BACTERIOLOGY

In 1889, Ducrey[51] first identified *H. ducreyi* in purulent material recovered from genital ulcers of patients with soft chancre or

*See references 12, 20, 24, 58, 64, 89, 90, 96, 127, 195, 196.

chancroid. Although unable to culture the organism in vitro, Ducrey[51] was able to establish the specificity of the infectious agent by serial cutaneous inoculations.[128] *H. ducreyi* originally was assigned to the *Haemophilus* genus because of its requirement for hemin (X factor) and a guanosine plus cytosine content within the expected range for *Haemophilus* spp.[8,184] However, more recent studies, including genetic transformation and DNA hybridization analyses, suggest that *H. ducreyi* is not related to the true haemophili, such as *H. influenzae*, and potentially should be placed in a separate genus.[8,184]

The last few years have seen significant advances in the characterization of bacterial components that may contribute to disease pathogenesis. Two cytotoxins, a hemolysin, a diffusable cytolethal distending toxin, and two classes of serum resistance proteins have been identified, their respective genes have been cloned, and their potential contributions to disease pathogenesis have been investigated.[1,10,42,44,136,139,187] The lipo-oligosaccharide of *H. ducreyi* has been characterized in much greater detail, and relevant biosynthetic genes have been cloned and studied.[3,15,62,123,176,185,201] Genes encoding the major outer membrane proteins of the organism have been cloned and the corresponding proteins characterized,[101] and a novel class of pili expressed by *H. ducreyi* has been identified.[34] *H. ducreyi* requires heme for growth, and several of the critical molecules required for heme acquisition and utilization have been identified.[54,55,174,179] The knowledge gained from molecular characterization of these important bacterial components should enhance efforts to understand the pathogenesis of disease better.

EPIDEMIOLOGY

Chancroid is a genital ulcerative disease that is found throughout the world. It is seen frequently in Africa, Asia, and Latin America, where it may be a more common cause of genital ulcer disease than is syphilis.[8,21-23,128,181,184] Although generally considered a relatively uncommon cause of illness in the United States, chancroid continues to be diagnosed, particularly among patients, including adolescents, who present with genital ulcer disease in large urban areas.[26,80,84,125,126,163] Furthermore, data suggest that chancroid may be more common in the United States than suspected because it can be difficult to diagnose correctly using traditional clinical and laboratory means.[50,129,184] Symptomatic disease among patients in the United States has been reported most commonly among nonwhite heterosexual men.[26,65,80,128] However, symptomatic disease is not restricted to men, and female prostitutes have been implicated as important sources of infection in several of the outbreaks reported in the United States.[26,80,128]

H. ducreyi has been the subject of renewed medical and scientific interest since the early 1980s. This interest followed from epidemiologic studies, primarily from Africa, demonstrating that the presence of genital ulcer disease (much of which was chancroid) was strongly associated with an increased risk of heterosexual transmission of human immunodeficiency virus (HIV) infection.[23,92,104,105,129,144,191] Mechanisms proposed to explain this enhanced transmission have included increased shedding of HIV through the ulcers,[104,184] increased numbers of HIV-susceptible cells (e.g., CD4 T lymphocytes) in genital ulcers of a person exposed to HIV,[170,184] or increased viral load in the blood and semen of individuals co-infected with HIV and *H. ducrei*.[53]

PATHOGENESIS

Relatively little is known about the pathogenesis of *H. ducreyi* infection, although significant progress has been made coincident with renewed interest in the organism.[4,167,184] Several useful in vivo models of *H. ducreyi* infection have been developed. They include a temperature-dependent rabbit model of dermal infection,[145] a primate model of genital chancroid infection in adult pigtailed macaques,[182] a swine model of dermal infection,[87] and an experimental model of human infection.[6,7,17,18,138,167,171] Numerous putative virulence factors have been identified, and their contributions to the pathogenesis of disease are being investigated using several in vitro and in vivo model systems. Some of the molecules and characteristics studied include bacterial lipooligosaccharide,[16,36,203] pili,[3,4,19,63,74,161,168,175,185] the cytolethal distending toxin,* a hemolysin,[10,52,137,139,183,198,202] serum resistance proteins,[1,42] lectin-binding proteins,[1,91,187,190] and the ability to adhere specifically to epithelial cells of genital origin.[9,109] The contribution of these proposed virulence factors to the pathogenesis of natural infection in humans is still being defined. However, it is known that strains deficient in expression of several of these factors are attenuated in their ability to cause disease in the experimental model of human infection.[5,30,67,70,91,169] Ongoing investigations of *H. ducreyi* undoubtedly will define the virulence mechanisms of the organism more clearly and should enhance efforts to develop protective vaccines.[2,6,43,48,82]

CLINICAL MANIFESTATIONS

The incubation period of chancroid usually is between 4 and 7 days. It rarely is less than 3 days or more than 10 days.[11,128,154,155] Typically, the first lesion noted is a small inflammatory papule surrounded by a zone of erythema. Within 2 or 3 days, a pustule forms that soon ruptures, leaving a sharply circumscribed ulcer with ragged undermined edges *without* induration.[111,128,154] The base of the ulcer usually has a granular appearance and always is painful. In male patients, the most common sites of appearance of the ulcers are on the distal prepuce, on the mucosal surface of the prepuce on the frenulum, and in the coronal sulcus. In female patients, most lesions are at the entrance to the vagina.[111,128,154] Painful, tender, inguinal adenopathy is present in as many as 50 percent of patients and usually is unilateral. The involved lymph nodes rapidly may become fluctuant and rupture, with the formation of inguinal ulcers.[128,154]

The combination of a painful ulcer with tender inguinal adenopathy is suggestive of chancroid and, when accompanied by suppurative inguinal adenopathy, is almost pathognomonic.[38] However, a significant percentage of patients with *H. ducreyi* infection may have ulcers that can be confused with other genital ulcer diseases, such as herpes or syphilis.[71,111,128,154] Furthermore, as many as 10 percent of patients with chancroid may be co-infected with *Treponema pallidum* or herpes simplex virus (HSV).[38] Thus, it becomes mandatory to establish a definitive diagnosis by laboratory means if one is to be confident about the diagnosis.

DIAGNOSIS

As noted earlier, diagnosing chancroid on clinical grounds alone is difficult because the clinical presentation often is not classic, and many clinicians do not have a great deal of experience with the disease.[38,184] Definitive diagnosis of chancroid requires isolation of the organism from a genital ulcer or from involved lymph nodes. However, the organism is fastidious and is difficult to isolate, even under the best of circumstances.[128] To obtain specimens for culture, a swab should be used to obtain material from the purulent base of an ulcer, or a fluctuant inguinal lymph node should be aspirated directly. Gram stain of purulent material may reveal gram-negative rods in the characteristic "school-of-fish" pattern, but this appearance probably is more characteristic of in

*See references 45, 46, 69, 72, 76, 108, 112, 146, 147, 173, 200.

vitro propagated organisms.[127] However, even with use of the selective media now recommended for isolation of *H. ducreyi*, the sensitivity of culture has been estimated to be no higher than 80 percent.[38,111]

Given the low sensitivity of culture, alternative diagnostic tests that are not based on culture have been evaluated. Early studies examined the utility of diagnosing chancroid serologically with an enzyme-linked immunosorbent assay (ELISA) using either an outer-membrane protein preparation or a lipo-oligosaccharide preparation of *H. ducreyi*.[11] More recent studies employed slightly altered antigen preparations and serum preparation techniques meant to improve the sensitivity and specificity of the assay.[39] Although these modifications did lead to improvement in the performance characteristics of the assay, the ability of the modified assay to aid in establishing the diagnosis of acute infection remains limited because many patients do not develop a serum antibody response until several weeks after onset of infection.[39,181] More recent work has focused on the use of recombinant proteins as test antigens for a serologic test.[56] This newer assay does appear to show promise for seroprevalence studies, but its utility in the diagnosis of acute infection has yet to be demonstrated. Another newer diagnostic test relies on detection of *H. ducreyi* with monoclonal antibodies directed against the hemoglobin receptor,[141] but it has yet to undergo extensive field testing.

Nucleotide-based diagnostic methods also have been described.[93,157,184] Perhaps most promising are the polymerase chain reaction–based techniques. These assays demonstrate high sensitivity and appear to identify certain patients with chancroid from whom bacterial cultures for *H. ducreyi* are negative.[181,184] Multiplex polymerase chain reaction assays that can amplify and detect DNA from *H. ducreyi*, *T. pallidum*, and HSV from genital ulcer specimens simultaneously are undergoing field trials and have shown early promise.[117,134,184]

Even if chancroid is diagnosed definitively, patients should also be tested for HIV at the time of diagnosis. In addition, as many as 10 percent of patients with chancroid may be co-infected with *T. pallidum* or HSV.[38] Appropriate testing for these other pathogens should be considered strongly when a patient presents with any form of genital ulcer disease.

TREATMENT AND PREVENTION

Successful antimicrobial treatment of genital ulcers caused by *H. ducreyi* cures infection, resolves clinical symptoms, and prevents transmission to others. However, in cases of extensive ulcerative disease, scarring may result, despite successful antimicrobial therapy.[38] The Centers for Disease Control and Prevention (CDC) currently recommends one of four antibiotic regimens for treatment of chancroid in adolescents and adults.[38,111,162] These regimens are as follows: (1) azithromycin, 1 g orally in a single dose; (2) ceftriaxone, 250 mg intramuscularly in a single dose; (3) ciprofloxacin, 500 mg orally twice a day for 3 days; or (4) erythromycin base, 500 mg orally four times a day for 7 days.[38,162] All four regimens generally are effective for treatment of chancroid among patients without HIV infection.[162] A successful response to therapy usually is apparent within 48 to 72 hours, as shown by decreased ulcer tenderness and pain.[38,154,162] Complete healing of ulcers may take up to 28 days but often is achieved in 7 to 14 days.[154] Healing of fluctuant adenopathy is slower than that of the ulcers and may require needle aspiration through adjacent intact skin or incision and drainage to achieve a successful response to therapy.[59,162]

Patients with HIV infection must be monitored closely because they may require longer courses of antimicrobial agents than the standard regimens just outlined.[38,162] Treatment failures have been noted with several of these regimens,[29,162,186] and some suggestion exists that persons who are most immunosuppressed are at the greatest risk for failure of standard regimens.[186] Some experts recommend using the erythromycin 7-day regimen for treating all HIV-infected patients because of good experience with this regimen in the HIV-infected population and limited successful experience with the alternative regimens.[162]

It is critical to identify all sexual contacts of infected persons to prevent further spread of *H. ducreyi* disease. The CDC recommends that all persons who have had sexual contact with a patient with proven *H. ducreyi* infection within the 10 days before onset of the patient's symptoms be examined and treated.[38] Contacts should be examined and treated even in the absence of symptoms.

In the longer term, alternative strategies for control of chancroid should be examined. If feasible, vaccination for prevention of *H. ducreyi* infection would be a worthy goal. Data generated in animal models of infection are somewhat encouraging.[2,48,82] Protective immunity to both homologous and heterologous challenge has been reported after immunization of rabbits with cell-surface extracts of *H. ducreyi*.[82] In other work, a purified pilus preparation also was reported to induce immunity in that same model.[48] More recently, immunization with the hemoglobin receptor protein was reported to protect against infection in the swine model of chancroid.[2] These data suggest that prevention of chancroid by vaccination may be an achievable goal. However, data from the experimental human model of infection suggest that development of a protective vaccine for human use may not be a straightforward process.[6]

HAEMOPHILUS INFLUENZAE BIOGROUP AEGYPTIUS (*HAEMOPHILUS AEGYPTIUS*)

BACTERIOLOGY

H. influenzae biogroup aegyptius (*H. aegyptius*) was described originally by Koch[102] in 1883 in Egyptian patients with conjunctivitis. A more detailed description of the organism and the clinical characteristics of disease followed 3 years later in the work of Weeks.[193] The Koch-Weeks bacillus has continued to be an important cause of conjunctivitis since these initial reports. Because of several reportedly unique characteristics,[120] the Koch-Weeks bacillus originally was designated a unique species of the *Haemophilus* genus (*H. aegyptius*) distinct from *H. influenzae*. However, more recent phenotypic and phylogenetic studies, including DNA relatedness analyses, have raised questions about the validity of this separation.[33,178] The organism is designated currently as *H. influenzae* biogroup aegyptius, although debate continues in the literature as to the appropriateness of this designation.[114]

This organism has been the subject of intense scientific study since the 1980s as a result of its association with a newly described fulminant and often fatal disease called *Brazilian purpuric fever*.[31,32] *H. influenzae* biogroup aegyptius was isolated from 9 blood cultures and from a single hemorrhagic cerebrospinal fluid culture collected from 10 clinically ill children in Serrana, Sao Paulo State, Brazil.[32] The *H. influenzae* biogroup aegyptius strains causing Brazilian purpuric fever initially were thought to be members of a single virulent clone.[33,178] This clone was characterized by the presence of several unique features, including a 24-Md plasmid with a characteristic restriction endonuclease pattern,[33] a unique multilocus electrophoretic enzyme typing pattern,[131] one of two rRNA gene restriction fragment length polymorphisms,[33,49] a single sodium dodecyl sulfate–polyacrylamide gel electrophoresis profile for whole-cell lysates,[33] specific reactivity with monoclonal antibodies recognizing epitopes unique to Brazilian purpuric fever strains,[110,178] agglutination with antisera specific for the Brazilian purpuric fever clone,[33] and conservation of certain major outer-membrane proteins.[151] Although

most Brazilian purpuric fever-associated strains of *H. influenzae* biogroup aegyptius appear to be members of a unique clone, a few Brazilian purpuric fever-associated strains have been identified that lack some of the defining characteristics.[178,180] Furthermore, two cases of Brazilian purpuric fever reported from Australia were associated with strains clearly distinct from the Brazilian purpuric fever clone.[114,178]

EPIDEMIOLOGY

In the United States, *H. influenzae* biogroup aegyptius has remained an important cause of conjunctivitis, with disease most commonly reported from the southern states.[83] The United States has experienced only one reported case of possible Brazilian purpuric fever.[189] In Brazil, the epidemiology of Brazilian purpuric fever has been defined more clearly during the last several years.[83] The median age of patients with Brazilian purpuric fever is 2 to 3 years, with an overall range of ages of 3 months to 10 years.[31,32] Brazilian purpuric fever appears to occur with the onset of warmer temperatures and is less likely to occur during the Brazilian winter.[83] Furthermore, it appears to occur more commonly in small agricultural towns than in larger cities.[31,32,83] Case-control studies attempting to identify risk factors for development of disease identified a history of preceding conjunctivitis as associated strongly with the development of Brazilian purpuric fever[31,32,82] (although many of the controls also gave a history of conjunctivitis) and suggested that attendance at a daycare center was an additional risk factor.[32]

PATHOGENESIS

Efforts to identify virulence factors of *H. influenzae* biogroup aegyptius responsible for the fulminant nature of Brazilian purpuric fever have been ongoing since the initial descriptions of the illness. Progress to date has been limited,[37] but some genome-based studies have demonstrated clear differences between *H. influenzae* biogroup aegyptius prototype strains and other *H. influenzae*.[113,122,166] Brazilian purpuric fever clone strains express numerous novel or unique surface molecules or secreted proteins that theoretically could result in enhanced virulence.[33,37,110,114,158,197] Distinctive lipo-oligosaccharide phenotypes,[37,158] immunoglobulin A (IgA) proteases,[37,114,121] pili,[15,149,150,197] and secreted proteins[197] for Brazilian purpuric fever strains have been reported. However, none has been shown to have a specific role in bacterial virulence. One study suggested that the risk of developing Brazilian purpuric fever correlated with the lack of serum bactericidal activity against the Brazilian purpuric fever clone, but this observation needs further confirmation.[159]

Both in vitro and in vivo models have been developed for further investigating the pathogenesis of Brazilian purpuric fever.[149,158,194] In these model systems, the strains associated with Brazilian purpuric fever demonstrated increased virulence compared with control strains not associated with Brazilian purpuric fever, but, again, the specific molecular correlates of this increased pathogenicity have yet to be identified clearly.[149,158] It seems likely that during the next few years, the in vitro and in vivo models will prove useful in further defining specific virulence factors of the Brazilian purpuric fever strain.

CLINICAL MANIFESTATIONS

The clinical presentation of Brazilian purpuric fever is distinctive and dramatic.[31,32,83] The syndrome initially manifests as a purulent conjunctivitis without distinguishing characteristics. Symptoms of Brazilian purpuric fever typically appear 3 to 15 days later, after the conjunctivitis has resolved. The affected children experience the acute onset of fever, which may be associated with vomiting and abdominal pain. Death frequently ensues within 48 hours after the development of disseminated purpura, vascular collapse, and hypotensive shock. The precise pathophysiologic mechanisms responsible for progression from conjunctivitis caused by the Brazilian purpuric fever clone to full-blown Brazilian purpuric fever are unknown. The overall case-fatality rate since Brazilian purpuric fever first was recognized is estimated to be 70 percent.[83] Children may develop conjunctivitis with the Brazilian purpuric fever clone and, after recovery from the conjunctivitis, have no further problems.[83] The risk factors that predispose only some children to develop Brazilian purpuric fever are not well understood.

TREATMENT

Data from the limited number of Brazilian purpuric fever cases studied suggest that early antimicrobial therapy may improve survival.[32] One suggested regimen is high-dose ampicillin with chloramphenicol. The small number of patients treated to date does not permit a comparison of the efficacy of different antibiotic regimens.[32]

Most of the patients who developed Brazilian purpuric fever in the Brazilian studies were treated with topical antimicrobials for conjunctivitis, yet they still developed systemic disease,[32] a finding suggesting that local topical therapy is ineffective in eradicating the organism from the host. One study examined the relative efficacy of oral rifampin and topical chloramphenicol in eradicating conjunctival carriage of the Brazilian purpuric fever clone.[142] Although the number of patients who actually carried the Brazilian purpuric fever clone was small, rifampin was shown to be significantly better in eradicating carriage of the Brazilian purpuric fever clone than was topical chloramphenicol.

HAEMOPHILUS PARAINFLUENZAE

BACTERIOLOGY

H. parainfluenzae was identified as a species distinct from *H. influenzae* first by Rivers in 1922.[153] Both organisms are fastidious, gram-negative coccobacilli, but with in vitro culture, *H. parainfluenzae* can be propagated on nutrient agar plates with supplemental factor V alone (thus the "para" designation), rather than with both factor X and factor V, which are required by *H. influenzae* isolates (see Table 146-1). Testing for hemolysis on blood-containing media differentiates *H. parainfluenzae* from hemolysis-producing species, such as *H. haemolyticus* and *H. parahaemolyticus*.[99,100] Recovery of *H. parainfluenzae* organisms from blood cultures is enhanced by routine subculturing of all specimens. The organisms tend to grow as small colonies along the side walls of the blood bottles or in the red cell mass, thus leaving the broth clear.[81] Routine subculturing to supplemented chocolate agar and incubation with supplemental carbon dioxide should allow recovery of any *H. parainfluenzae* organisms that are present.[41,79,81]

EPIDEMIOLOGY AND PATHOGENESIS

H. parainfluenzae is found commonly in the oropharyngeal flora of normal children.[78,107,116] The organism can be recovered from oropharyngeal cultures of one fourth or more of healthy children. Of children who develop serious invasive disease caused by *H. parainfluenzae*, more than half give histories of identifiable preceding illnesses, such as upper respiratory tract infection, otitis

media, and dental infections.[13,25,78,88] These historical findings suggest that local inflammation in the upper respiratory tract may predispose to transient bacteremias with this organism, which allows for seeding of other sites, such as the meninges and the heart valves. No specific virulence factors of the organism have been identified to date.

CLINICAL MANIFESTATIONS

As noted previously with *H. aphrophilus*, *H. parainfluenzae* remains an uncommon cause of infection in pediatric patients. However, increasing numbers of cases have been reported since the 1970s.[25] The most commonly reported infection is meningitis.* The clinical courses of the patients described with *H. parainfluenzae* meningitis are not remarkably different from those typical of acute bacterial meningitis caused by other organisms. However, the average age of affected children is 2.2 years,[25] an age significantly greater than that of the typical pediatric patient with bacterial meningitis.

The infection next most commonly reported is endocarditis.† *H. parainfluenzae* endocarditis has numerous unique features. The reported cases of pediatric endocarditis usually occur in adolescents and involve female patients more commonly than male patients.[25] The clinical presentation often is subacute and frequently is not associated with localizing signs on physical examination (i.e., pathologic murmurs), at least initially.[41,115,116] Another unique feature of *H. parainfluenzae* endocarditis is the high incidence of major arterial occlusion secondary to release of large emboli from the heart.[25,41,73,115,172] This high incidence of embolization is thought to result from the particularly friable nature of the vegetations.[25,73] Another characteristic feature noted by several authors is the relatively slow and variable response of endocarditis caused by this organism to antimicrobial therapy.[25,41,115,116] Other *H. parainfluenzae* infections reported in pediatric patients include brain abscesses,[78,118] septic arthritis,[25] urinary tract infection,[25] peritonitis,[119] and sepsis in neonates.[40,152]

TREATMENT

H. parainfluenzae usually is susceptible in vitro to multiple antibiotics, including chloramphenicol, aminoglycosides, trimethoprim-sulfamethoxazole, and third-generation cephalosporins.[25,41,94,116] Although most isolates in the past were susceptible to penicillins, more recent studies documented an increasing incidence of β-lactamase–producing strains resistant to penicillin and ampicillin.[25,160] For β-lactamase–negative, penicillin-susceptible organisms, administration of ampicillin with an aminoglycoside has been recommended for serious *H. parainfluenzae* infections.[41,81] Individual case reports document successful treatment with a variety of other antimicrobials, including ampicillin alone, cephalosporins, chloramphenicol, and trimethoprim-sulfamethoxazole.[35,41,95,116] At present, pending results of susceptibility testing, it would be reasonable to initiate therapy for serious *H. parainfluenzae* infections with a third-generation cephalosporin, perhaps in combination with an aminoglycoside.

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CHAPTER 147

HELICOBACTER PYLORI

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The discovery of *Helicobacter pylori* in 1983 as the bacterial cause of peptic ulcer disease[161] has revolutionized the understanding, diagnosis, and treatment of acid peptic disease. *H. pylori* appears to infect approximately 50 percent of the world's population, thus rendering it one of the most common bacterial infections in humans.[87,101] An understanding of *H. pylori* is important to pediatricians because this infection usually is acquired in childhood.[87] This chapter details current consensus and understanding of this important pathogen.[39,61,87]

BACKGROUND

The presence of bacteria in the gastric mucosa has been known for more than 100 years.[146] In 1874, Böttcher[13] observed bacteria in the human stomach. Bizzozero[7] described spirochetes in the stomach of dogs in 1893, and his observations were confirmed by Solomon[150] and by Kasai and Kobayashi[88] in the dog, cat, rat, and monkey. Muhlens[121] and later Luger and Neuberger[103] reported finding spiral organisms in ulcerating carcinomas of the stomach in humans. In 1938, Doenges[33] explored the issue of gastric spirochetes and their clinical relevance. He reported a 43 percent prevalence of spiral organisms in the human stomach in an autopsy review of 242 patients without known gastrointestinal disease. Specimen autolysis, however, rendered interpretation of the pathologic significance of the gastric spirochetes impossible. Freedburg and Barron[55] verified the findings of Doenges in 1940 when they identified spirochetes in the stomachs of 37 percent of patients who had undergone partial gastric resection for carcinoma or ulcer disease. Work on the clinical significance of gastric spiral bacteria continued until a report by Palmer was published in 1954.[128] In an attempt to confirm the findings of spirochetes in the gastric mucosa, Palmer performed an exhaustive review of gastric fundus biopsies from 1000 adult patients, 80 percent of whom were being evaluated for upper gastrointestinal complaints and 20 percent were healthy control volunteers. Palmer noted, "None of the 1180 specimens was found to contain spirochetes or any structure which could reasonably be considered to be of spirochetal nature." He concluded that spirochetes are not part of the human gastric mucosa in health or illness. Palmer, a prominent researcher in gastritis, inadvertently may have curtailed further research into the role of gastric spiral bacteria.

In 1975, Steer,[151] using electron microscopy, noted curved bacteria in stomach biopsy specimens from patients with gastric ulcers. A few years later, Warren, an Australian pathologist, noted the appearance of spiral bacteria overlaying inflamed gastric mucosa. Warren noted that this organism looked like a *Campylobacter*. He and Marshall began a series of culture experiments using *Campylobacter*-specific methods. The story culminates in 1983 and 1984, respectively, with their reports of successful culture of the curved bacillus.[114,161] In an attempt to fulfill the remainder of Koch's postulates, Marshall and associates[112] and Morris and Nicholson[120] independently ingested pure cultures of *H. pylori*, and symptomatic acute gastritis developed in both. The organism subsequently was recovered from the gastric mucosa by endoscopy and successfully cultured, thus completing Koch's postulates.

MICROBIOLOGY AND PATHOPHYSIOLOGY

H. pylori initially was named *Campylobacter pyloridis* because of its resemblance to *Campylobacter* spp.[161] Later changed to *C. pylori*, it became clear that the bacterium did not belong to any known genus. An entirely new genus, *Helicobacter*, was created, with "helico" describing the spiral shape and "pylori" denoting its typical location. *H. pylori* is a spiral-shaped, gram-negative bacteria with four to seven unipolar sheathed flagella (Fig. 147–1).[9,126] It is 0.5 μm wide and 3 to 5 μm long and has a smooth surface.[1,117] It generally is S-shaped in vivo but can take on many forms, from U-shaped to cocci to rodlike.[117]

H. pylori inhabits a unique ecologic niche: the submucosa of gastric epithelium. Several attributes, including adherence, shape, microaerophilism, urease production, and motility, allow adaptation to the acidic gastric environment. *H. pylori* overlays the intercellular tight junctions of epithelial cells, beneath the mucous layer. Specific adhesion molecules appear to be tropic to gastric mucus-producing cells.[96] This adherence allows the organism to maintain colonization despite the rapid gastric cell turnover.[98] The spiral shape may allow the organism to corkscrew through the gastric mucus (see Fig. 147–1). This spiral movement has been demonstrated in vitro with methylcellulose solutions.[98] *H. pylori* is microaerophilic and grows slowly in culture media.[9,126] One-millimeter translucent colonies grow after 5 to 7 days on blood- or serum-supplemented media with a low concentration of oxygen and carbon dioxide at a temperature of 37° C, which promotes optimal growth. This microaerophilism is well suited to the low oxygen levels of the gastric submucosa.[96] *H. pylori* is the most potent urease producer of any known microbe; researchers have estimated that as much as 10 percent of its total protein production is urease, and the organism appears to surround itself in a cloud of ammonia.[1,4,9,128] The urea is converted to ammonium and bicarbonate.[8,63] Little evidence supports that the notion that the ammonium produced is cytotoxic to gastric epithelium.[79] The bicarbonate creates an alkaline environment in the gastric submucosa. *H. pylori* produces several other enzymes, including mucinase,[148] lipase,[145] catalase,[102] hemolysin, and cytopathic

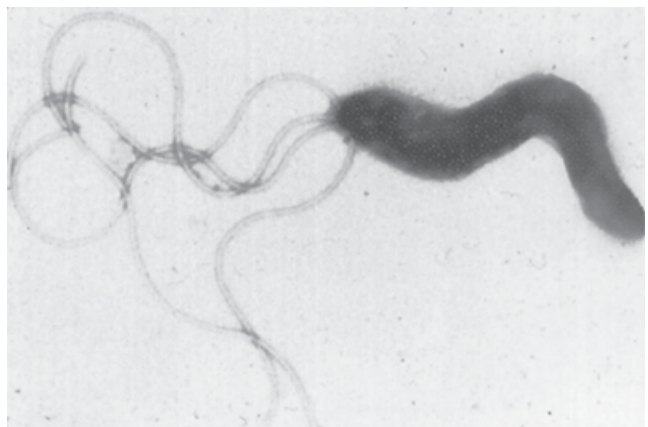

Figure 147–1 Electron microscopy of *Helicobacter pylori* demonstrating the multipolar flagella and the typical spiral shape.

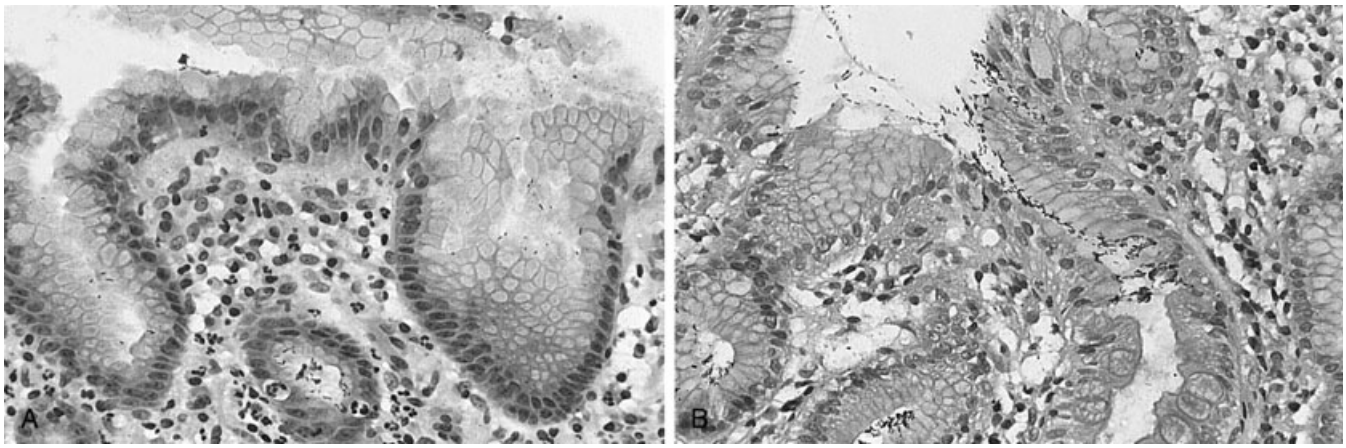

Figure 147–2 **A,** High-power light microscopy of *Helicobacter pylori* demonstrating chronic active gastritis with small numbers of *H. pylori* seen on the epithelial surface and mucus. An aggregate of *H. pylori* organisms is visible within the gastric pit (Genta stain). **B,** More *H. pylori* organisms and mild intestinal metaplasia. *(Courtesy of Dr. Robert Genta, Baylor College of Medicine.)*

toxin.[100] The multiple, unipolar flagella (see Fig. 147–1) provide motility that may enable the organism to escape the acid lumen of the stomach[98] and evade host immune responses.[96]

H. pylori may be more akin to normal gastrointestinal flora because the bacterium and the host can coexist for decades without apparent problems.[69] *H. pylori* infection produces inflammation, although in most cases the host is asymptomatic.[11] The inflammatory response is characterized by infiltration of polymorphonuclear leukocytes, monocytes, lymphocytes, and plasma cells into the lamina propria (Fig. 147–2).[102] A significant immune response, including the production of antibodies, is produced by the host, but it does not clear the organism in most cases.[100] *H. pylori* produces a chronic infection that persists for decades, possibly for life.[102] This lifelong colonization is accomplished by the same features that allow survival in the gastric mucosa, namely, high urease production, flagellar motility, spiral shape, microaerophilism, and adherence.[102]

Numerous potential virulence factors, such as urease, catalase, cytotoxin, and lipopolysaccharide, have been identified, although whether any of these substances actually contribute to symptomatic disease is unknown. The urease of *H. pylori* is a potent antigen that induces elevations in antiurease immunoglobulin G (IgG) and IgA.[102] Interestingly, the ammonia produced by the urease does not appear to have a significant role in pathogenesis.[70] Catalase, another *H. pylori* enzyme, prevents the formation of oxygen metabolites from hydrogen peroxide in neutrophils,[43] which may provide an ability to evade host destruction. A vacuolating cytotoxin has been identified in many *H. pylori* strains,[43] although whether it is more common in patients with symptomatic disease is unclear. *H. pylori* has a peculiar lipopolysaccharide outer membrane. Unlike most gram-negative bacteria, its lipopolysaccharide coat is a significantly less potent inducer of the host complement cascade, some 1000-fold less potent than that of the Enterobacteriaceae.[102] This low biologic activity of the outer membrane of *H. pylori* may be another adaptive mechanism allowing gastric colonization.[10]

EPIDEMIOLOGY

H. pylori is one of the most common bacterial infection in humans, with an estimated 1 billion people in the world being infected.[87,101] It is estimated that in developing countries more than 80 percent of the population is infected with *H. pylori*, whereas in industrialized countries the infection rate remains under 40 percent (Fig. 147–3).[68,131,136] Transmission appears to be from person to person.[97,116,154] Transmission via the fecal-oral route is the primary mode of infection.[14,45,49,110,122,153] The presence of pets may be a risk factor for *H. pylori* infection, given that the bacterium has been isolated from domestic animals.[15,16,36,80] Interestingly, breastfeeding appears to increase the risk of acquiring *H. pylori* infection, possibly secondary to close contact with infected mothers, and breast milk does not protect against infection.[35,142,163] *H. pylori* usually is acquired in childhood,[27,101] and humans appear to be a natural reservoir for infection.[72] Environmental sources of *H. pylori* infection, such as municipal water sources, have been confirmed in Peru and Mexico.[104,118]

Children are important factors in the transmission of *H. pylori* infection, which clusters within families with children.[41,109,116,125,162] Family clustering emphasizes the role of crowding and personal hygiene in the person-to-person spread of infection. The prevalence of infection increases with age. In the United States, for example, only about 20 percent of individuals younger than 30 years old are infected, as compared with 50 percent of those older than 60 years.[111] Infection in young children is a rare event in the United States and developed nations.[102] The phenomenon of increasing prevalence with age most likely reflects a cohort effect; that is, the high prevalence in older persons simply reflects a higher childhood infection rate.[102] This cohort effect may be explained best by a lower standard of living of older persons during childhood. Socioeconomic status varies inversely with the prevalence of infection, with low-income individuals having the highest rates of infection.[52] Conversely, Ozen and associates[127] found that low household population and a history of antibiotic use during the previous 6 months served as protective factors against infection. A higher prevalence of infection exists in developing countries than in developed countries (see Fig. 147–3),[116] and the prevalence of infection varies greatly around the world. In Nigeria, for example, 58 percent of children younger than 1 year old and 91 percent of children older than 10 years were infected.[82] Such differences have been attributed to socioeconomic factors, hygiene, and the number of household occupants.[78] *H. pylori* is more prevalent in blacks and Hispanics than in whites in the United States.[149]

The rate of *H. pylori* reinfection in the pediatric population ranges from 2.4 percent per year to about 20 percent per year.[89,124,139,143] Magista and colleagues[108] demonstrated that the difference in re-infection rate is dependent primarily on the socioeconomic status of the family and the local prevalence of *H. pylori*. They and others[50,51] also noted that living in an area with a high prevalence of *H. pylori* resulted in a fourfold higher risk of re-infection.

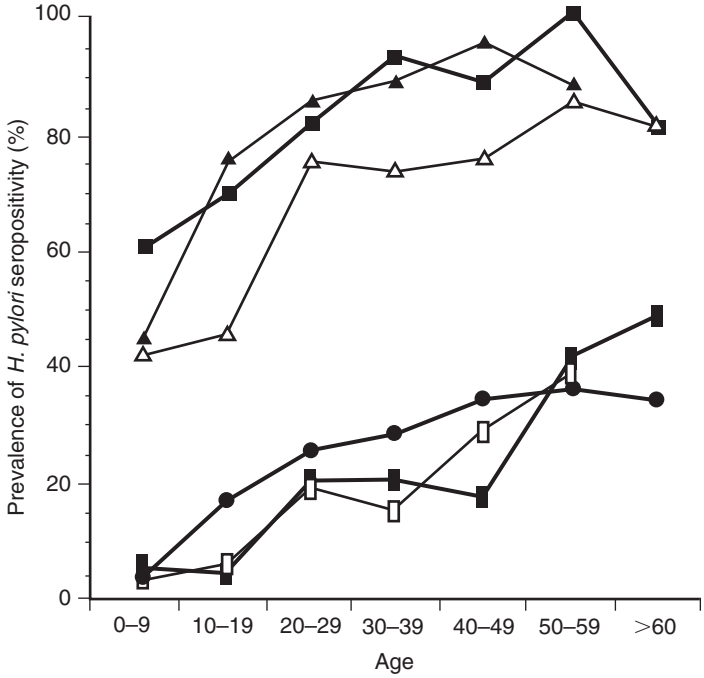

Figure 147–3 Seroepidemiology of *Helicobacter pylori* infection demonstrating the difference in disease prevalence between developing and developed countries. *(Data from Graham, D. Y., Adam, E., Reddy, G. T., et al.: Seroepidemiology of Helicobacter pylori infection in India: Comparison of developing and developed countries. Dig. Dis. Sci. 36:1084-1088, 1991.)*

Although *H. pylori* infection remains common worldwide, the seroprevalence of *H. pylori* infection in U.S. children has dropped significantly in all ethnic groups.[62]

CLINICAL MANIFESTATIONS

Gastritis develops in most *H. pylori*–infected patients, but complications associated with infection develop in only a few.[87] For example, the lifetime risk of peptic ulcer disease developing is estimated to be 10 to 15 percent, whereas gastric cancer will develop in less than 1 percent of *H. pylori*–infected persons.[87] This finding is especially true in children, in whom peptic ulcer is considerably less common than in adults. To confound matters, children have no specific symptoms of *H. pylori* infection.[60,64,65,76,106,137,140] Symptoms such as epigastric pain, nighttime awakening with abdominal pain, hematemesis, and recurrent vomiting are suggestive but in no way predictive of infection.[40,91,123] Because these clinical symptoms are typical of peptic ulcer disease, testing for *H. pylori* infection alone has no relevance but rather should be done to determine the presence of an underlying condition.[95] Such symptoms possibly are present only if *H. pylori* infection is found with a duodenal ulcer. Furthermore, the presence of *H. pylori* infection alone in both adults and children usually is asymptomatic.[31,34,38,55,66,73,134]

The association of *H. pylori* infection with recurrent abdominal pain of childhood remains a topic of ongoing debate. The critical issue is whether *H. pylori* antral gastritis is a source of abdominal pain. The issue remains unresolved.[59,105,106,124,141,154,157] A logical conclusion for the pediatrician would be that a child with active upper gastrointestinal tract complaints in whom *H. pylori* infection is suspected deserves either treatment of the infection or diagnostic upper endoscopy to determine the presence of ulcer disease. *H. pylori* infection also has been associated with protein-losing enteropathy, as well as iron deficiency anemia.[20,21,23,26,81,168]

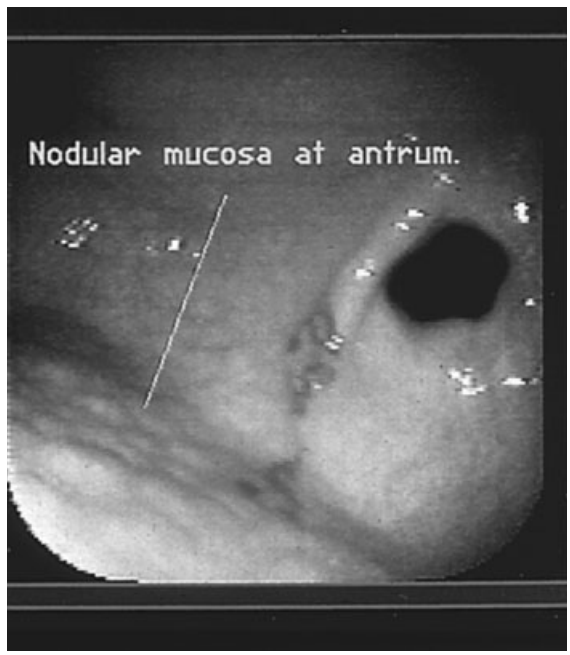

Figure 147–4 Endoscopic view of the gastric antrum demonstrating marked nodularity of the gastric surface.

DIAGNOSIS

The "gold standard" for diagnosis of *H. pylori* infection is culture of the organism from gastric biopsy samples or histologic review.[161] Upper endoscopy or other invasive means are necessary to obtain such biopsy specimens. In children, upper endoscopy can be quite helpful in that it often reveals a distinct nodularity at the antrum (Fig. 147–4), but it also may appear normal. This

nodularity is found in two thirds of infected children but only rarely in adults.[17,77,78] Histologically, prominent lymphoid follicles are seen. Routine staining with hematoxylin and eosin may be adequate but must be performed properly to see the bacteria clearly. Alternative staining techniques, such as acridine orange, silver stains, and the "triple" stain, improve visualization.[56,94,107] The triple, or Genta, stain (Steiner + hematoxylin-eosin + alcian blue) may offer some distinct advantages because it is significantly more sensitive than is hematoxylin-eosin staining and is particularly useful for the detection of small numbers of bacteria, which often is the case in children (see Fig. 147–2). Although culture and histology require the need for endoscopy, culture may be important. In cases of recurrent *H. pylori* infection in which antibiotic resistance is suspected, culture allows in vitro antibiotic sensitivities to be determined.

Noninvasive methods for detection of *H. pylori* infection are detailed in Table 147–1 and include serology, varying methods for detection of urease production, detection of salivary antibody, stool culture, stool antigen, polymerase chain reaction (PCR), and urine ammonia production.[135] The ^{13}C-urea breath test is considered the best available noninvasive diagnostic test in children.[87] Serology, on the other hand, is not useful. Measurement of *H. pylori* stool antigen can be performed by either a polyclonal or monoclonal antibody technique.[44,90] This test detects the presence of antigen, thus providing evidence of active infection for both primary diagnosis or proof of cure. It appears to be of equal accuracy to the urea breath test.

H. pylori infection induces a vigorous neutrophilic and lymphocytic (T-cell and B-cell) response that fails to clear the infection. The humoral immune response produces antibodies that can be detected in gastric secretions, as well as systemically. A number of *H. pylori* antigens have been determined with the use of bacterial cell wall sonicates, urease, or membrane extracts as the capture antigen.[24,28,30,47,147,156] Some commercial assays have been found to have inappropriate positive and negative cutoff values for use in children.[25,28] Care must be taken to ensure that the serologic tests performed have been verified for use in children. Although detection of IgA, polymeric IgA, and IgM antibodies against haptoglobin can be performed, such detection is not reproducible and thus not of any useful clinical value. Serologic diagnosis, though simple and widely available, does not indicate symptomatic disease.

H. pylori is a vigorous producer of urease. This characteristic has been used to create a variety of tests to detect the presence of urease. The urea breath test uses a labeled carbon of urea, either radioactive carbon 14 (^{14}C)[6] or a nonradioactive, stable isotope, carbon 13 (^{13}C).[71] Patients fast 4 to 8 hours and then drink the labeled carbon accompanied by a meal to delay gastric emptying; then, the amount of labeled carbon dioxide in the breath is measured. Only those with gastric *H. pylori* present (and thus gastric urease activity to degrade the labeled urea) will be identified. Urea breath testing is useful for both initial diagnosis and determination of successful treatment because the results return rapidly to normal after eradication. The radioactive ^{14}C

TABLE 147–1 Summary of Methods for Detecting *Helicobacter pylori*

Method	Sensitivity (%)	Specificity (%)	Advantages	Disadvantages
Invasive				
Histology	93-99	95-99	Widely available; detection best with special stains; can evaluate underlying mucosal damage; gold standard	Expensive; at least two biopsies required; observer error; recent antibiotics or proton pump inhibitor use can lead to false-negative results
Culture of biopsy specimens	77-92	100	In vitro antibiotic susceptibility can be determined	Expensive; organism requires special transfer and culture technique; requires up to 1 wk for results; recent antibiotics or proton pump inhibitor use can lead to false-negative results
Rapid urease test CLO test hpFast PyloriTek	89-98	93-98	Rapid results; easy to perform; less expensive than other invasive techniques	Formalin, simethicone, local anesthetic spray, recent antibiotics, bismuth, or proton pump inhibitor use can lead to false-negative results; poor technique or handling will affect results
Noninvasive				
Urea breath test ^{13}C ^{14}C	90-100	89-100	Inexpensive, represents entire mucosa (not subject to biopsy sampling bias)	Antibiotics or proton pump inhibitor use can lead to false-negative results; presence of ulcer disease not determined; can be difficult to collect in children younger than 2 yr
Serology (ELISA) HM-CAP Pylori.STAT	44-99	89-95		
Rapid serology FlexSure QuickVue				
Stool antigen testing (HpSA)	85-94	97.7	Inexpensive, more accurate than serology; may become the test of choice in children	Stool must be collected
Saliva	71-93	82-92	Easy to collect; inexpensive	Low sensitivity, especially in children younger than 2 yr

ELISA, enzyme-linked immunosorbent assay.

Data from Graham, D. Y.: Helicobacter *Today. Altrincham, Cheshire, United Kingdom, Norris Communications, 1995, p. 5, 67; Yen-Hsuan, N., Jaw-Town, L., Shui-Feng, H., et al.: Accurate diagnosis of Helicobacter pylori infection by stool antigen test and 6 other currently available tests in children. J. Pediatr.* 136:*823-827, 2000; Rothenbacher, D., Inceoglu, J., and Brenner, H.: Acquisition of Helicobacter pylori infection in a high risk population occurs within the first 2 years of life. J. Pediatr.* 136:*744-748, 2000; Gilger, M. A., Tolia, V., Johnson, A., et al.: The use of an oral fluid immunoglobulin G ELISA for the detection of Helicobacter pylori infection in children. Helicobacter* 7:*105, 2003; and Luzza, F., Oderda, G., Maletta, M., et al.: Salivary immunoglobulin G assay to diagnose Helicobacter pylori infection in children. J. Clin. Microbiol.* 35:*3358-3360, 1997.*

method does not deserve consideration for use in children. The stable isotope ^{13}C methodology currently is the simplest and most accurate noninvasive test available method for detection of *H. pylori* infection. Its usefulness remains limited, however, because of the need for specialized equipment for analysis.

The urease production of *H. pylori* can be measured directly in gastric biopsy specimens with a variety of commercial assays.[115,120] A portion of the gastric biopsy specimen is placed into a urea medium, and hydrolysis of urea leads to a color change in the medium from tan to pink. False-negative results have been noted in children because of the low numbers of organisms.[42] Such testing is useful for establishing a rapid diagnosis during endoscopy but requires gastric biopsy. Urease activity also can be detected by measurement of another nonradioactive, stable isotope, ammonium, in the urine after oral ingestion.[86] This noninvasive test has not proved to be accurate.

H. pylori has been identified in saliva and dental plaque by culture[93] and PCR.[22] Others have identified salivary antibody to *H. pylori* with an indirect immunofluorescence assay.[83] The saliva test is not accurate in young children and cannot be recommended.[58] Viable *H. pylori* organisms have been cultured from feces in Gambian children.

TREATMENT

Cure of *H. pylori* infection associated with peptic ulcer disease in both children and adults significantly reduces the likelihood of recurrence of peptic ulceration.[85,113,138,167] First-line therapy for *H. pylori* infection in children is a twice-daily, three-drug regimen that includes amoxicillin, clarithromycin, and a proton pump inhibitor given for 14 days.[19,57,87,158,165] Table 147–2 presents a variety of suggested regimens for the treatment of *H. pylori* infection in children. *H. pylori* infection is difficult to cure. Confirmation of successful treatment has been defined as absence of detectable organisms by tissue biopsy or the urea breath test at least 1 month after completion of treatment.[5] Because tissue biopsy is invasive, urea breath testing or stool antigen detection is helpful for determination of successful treatment. Serologic tests are not useful for detection of cure because of the prolonged elevation of titers, which remain elevated for 6 months to 1 year or longer after treatment.

A growing body of evidence indicates that acquisition of *H. pylori* infection in childhood is a significant risk factor for the development of gastric cancer such as adenocarcinoma (Fig. 147–5).[84,129,155] An estimated twofold to sixfold increased risk for

TABLE 147–2 Suggested Treatment Regimens for *Helicobacter pylori* Infection in Children

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From Gilger, M. A.: Treatment of Helicobacter pylori infection in children. Curr. Pharmaceut. Dis. 6:370-384, 2000.

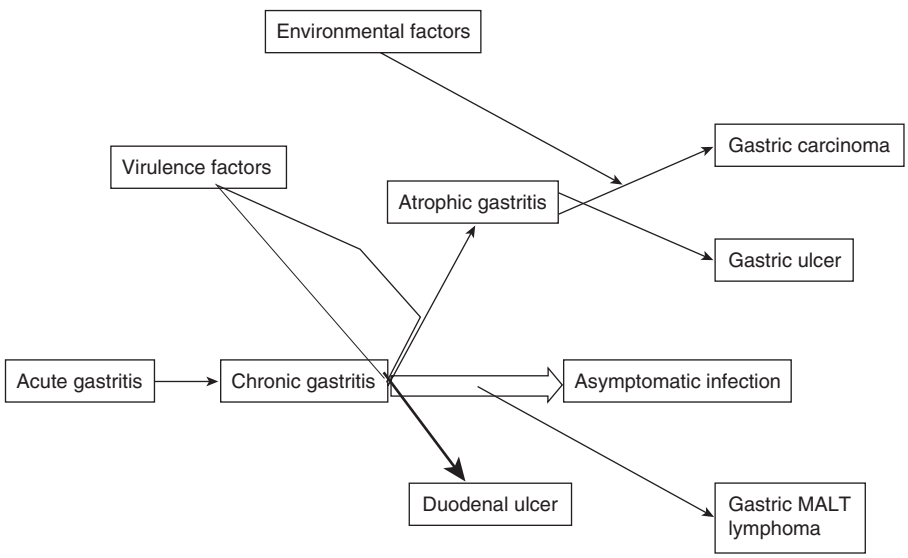

Figure 147–5 Timeline of possible outcomes of *Helicobacter pylori* infection starting from the acute acquisition of infection and possible sequelae. MALT, mucosa-associated lymphoid tissue. *(D, Graham, personal data.)*

the development of gastric cancer exists among *H. pylori*–infected patients.[32,53,159,160] A current concern is whether *H. pylori* treatment decreases the risk for development of gastric cancer.[152] Multiple studies have demonstrated that no significant difference exists between treated groups and placebo groups. Patients in whom gastric cancer develops appear to already have atrophic gastritis or metaplasia at baseline.[99,119,164] This finding argues for treatment of *H. pylori* infection in childhood, before the onset of atrophic gastritis. The location of the infection within the stomach may influence the outcome. For example, antral-predominant infection more commonly leads to increased acid secretion, which results in duodenal peptic ulcers and a low risk for gastric cancer. In contrast, fundus-predominant infection leads to decreased acid secretion, which results in atrophic gastritis and a higher risk for gastric cancer.[99] Treatment of gastric lymphoma with antibiotics directed at *H. pylori* has resulted in regression of the tumor.[2,165] Some researchers argue that the risk of developing either lymphoma or adenocarcinoma provides cause for the treatment of all children infected with *H. pylori*. Because the risk for development of cancer cannot be predicted accurately and because most children with *H. pylori* infection have no clinical symptoms, no current rationale exists for treating all children infected with *H. pylori*.

In children, the most effective anti-*Helicobacter* treatment currently available is "triple therapy" that includes amoxicillin, clarithromycin, and a proton pump inhibitor (Table 147–2).[74,87,111] Efficacy rates of 85 to 95 percent have been reported for this combination. In patients allergic to penicillin, amoxicillin may be replaced with metronidazole. Metronidazole, to which the *H. pylori* organism is highly sensitive, has a high rate of resistance because of its overuse for other infections.[46] A multicenter study in Europe found the overall resistance to be 25 percent for metronidazole, 24 percent for clarithromycin, but only 0.6 percent for amoxicillin.[92] In cases of antimicrobial resistance, the use of rifabutin, furazolidone,[29,48,75,132,133,166] and fluoroquinolones such as ciprofloxacin, along with rifampin and streptomycin, has been reported as options in treating resistant *H. pylori* infection.[37,45,54,144] These second-line regimens have no particular advantage over the first-line antibiotics and should be considered only when resistance is suspected. No single antibiotic agent given alone is effective therapy against *H. pylori*.[113,130,138] Extensive study continues to determine the optimal treatment of *H. pylori*, but to date, triple therapy remains the accepted standard.

When cure of infection has been obtained, the long-term rates of reinfection are as low as 1 percent per year in developed countries but much higher in developing nations.[12,130] However, reinfection may be more likely to occur in families, especially when small children are the first infected.[3] No conclusive evidence currently exists, however, to recommend routine treatment of the entire family.[124]

Vaccination against *H. pylori* infection appears possible. Work in a mouse model has shown that oral immunization with a sonicate of *Helicobacter fetus* plus the adjuvant cholera toxin results in protection against an oral challenge.[18] Continued animal model investigation may yield promising direction for future vaccines.

FUTURE DIRECTIONS

H. pylori is a major cause of peptic ulcer disease in children and adults, which has caused a dramatic reappraisal of many previous notions about ulcer disease. Although the dictum "No acid, no ulcer" still holds, many other previous considerations, such as genetic predisposition, must be re-examined. Figure 147-5 demonstrates a proposed *H. pylori* timeline. It emphasizes the acquisition of an acute infection in childhood, persistent chronic gastritis, and the possible progression to ulcer, atrophic gastritis, and potentially gastric carcinoma and lymphoma.

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CHAPTER 148

KINGELLA SPECIES

Thomas L. Kuhls

The genus *Kingella* consists of four species: *Kingella kingae*, *Kingella denitrificans*, *Kingella oralis*, and *Kingella potus*. *K. kingae* is being recognized increasingly as an important cause of invasive infections in children. With improved methods of recovering this fastidious member of the family Neisseriaceae and increasing awareness that the microorganism is not a culture contaminant, *K. kingae* infections are being encountered almost annually by many pediatric infectious disease specialists.[13] The assumption, however, is that most *K. kingae* infections continue to be treated empirically because the organism is difficult to isolate from clinical specimens. *K. denitrificans* rarely causes endocarditis,[4,10,26] empyema,[44] and chorioamnionitis[42] in adults; it has been isolated from a single child with endocarditis.[61] *K. potus* has been isolated from a wound caused by a bite from an arboreal mammal found normally in the rainforests of Central and South America.[38] To date, *K. oralis* has not been found to cause human infections.[17]

HISTORY

During the 1950s and early 1960s, Elizabeth O. King isolated gram-negative bacilli from clinical specimens that had phenotypic and growth characteristics identical to those of *Moraxella* spp., except that the organisms were beta-hemolytic and did not contain catalase.[28] In honor of King, the organism initially was named *Moraxella kingii* in 1968,[28] later renamed *Moraxella kingae* in 1974,[8] and finally reclassified as *Kingella kingae* in 1976.[29] The Centers for Disease Control and Prevention received 78 *K. kingae* isolates from 1953 to 1980, 75 percent of which were recovered from blood, bone, or joint specimens.[25] Most of the isolates were obtained from children younger than 6 years old. During the 1980s, increasing numbers of children with *K. kingae* infection were reported because of better awareness of the microorganism and better culturing techniques for fastidious agents. Not until the 1990s did the importance of *K. kingae* in causing invasive infections in children become apparent.

MICROBIOLOGY

Kingella spp. are small (0.6 to 1 μm × 1 to 3 μm), gram-negative rods that may resist Gram decolorization.[51] The organisms can appear coccoid and exist in pairs or short chains. The nonencapsulated bacilli are fastidious aerobes that grow best at 33° C to 37° C in both nutrient and blood agar. At least 4 days of incubation usually is required before *Kingella* spp. can be detected in clinical specimens grown on agar plates, but growth can be detected earlier with newer automated detection culturing systems.[40,80,81,88] Two types of bacterial colonies are found on agar plates: small, smooth, and nearly translucent colonies and larger spreading colonies that appear pitted because of corrosion of the agar surface. The larger colony type, however, generally is not present after the initial colonies have been subcultured, unless the organism is cultured under anaerobic conditions. Biochemical characteristics of the genus *Kingella* include negative reactions for motility, catalase, indole, and urease and a positive reaction for oxidase. All members of the genus, except *K. potus*, are able to produce trace acid from glucose.

K. kingae and *K. denitrificans* are the only members of the genus reported to cause invasive infections in children. *K. kingae* can be differentiated from *K. denitrificans*, *K. oralis*, *K. potus*, and *Neisseria* spp. by a distinct narrow zone of beta-hemolysis surrounding the colonies. Unlike *K. denitrificans*, *K. kingae* does not reduce nitrates or nitrites and produces acid from maltose. *K. kingae* can be confused easily with isolates of *Eikenella corrodens* and *Cardiobacterium hominis*, both of which can cause suppurative infections in humans. In addition, *K. kingae* can be misidentified as beta-hemolytic streptococci in Gram-stained specimens when decolorization is incomplete.

EPIDEMIOLOGY

Kingella spp. are a part of the normal oropharyngeal flora of children. In 1969, *K. kingae* was isolated from 1.1 percent of nose and throat swabs obtained from children and adults.[27] In this study, however, the cultures were incubated for only 24 hours and selective media were not used; thus, the prevalence rate of carriage most likely was much higher. More recently, throat and nasopharyngeal specimens from infants and children attending two daycare centers in Israel were cultured on selective media.[82,86] Specimens were collected every 2 weeks for a duration of 11 months. Monthly prevalence rates of *K. kingae* carriage ranged from 6 to 35 percent, with the highest rates found in the months December through April. Overall, at least one positive *K. kingae* culture was obtained in 73 percent of the children. The rate of *K. kingae* carriage in this study was similar to the previously reported carriage rates of *Streptococcus pneumoniae* in children of the same age group and much higher than the rates of *Haemophilus influenzae* type b (Hib) carriage seen in young children during the pre-vaccine era. Unlike these respiratory tract pathogens, *K.*

kingae was isolated only from the tonsillar areas of the children and not from the nasopharynx. The investigators did not isolate *K. kingae* from 2- to 4-month-old infants attending a well-care clinic but did isolate the organism from 8 percent of healthy children older than 8 years of age who were scheduled for elective surgical procedures.[82] The same investigators also evaluated more than 2000 oropharyngeal specimens in 2001 and determined that carriage was highest in children younger than 3 years of age and lowest in adults, 3.2 and 0.8 percent, respectively, and that carriage rates did not differ between sexes or during different seasons of the year.[87] High rates of oropharyngeal carriage in children attending daycare centers also have been observed in the United States and again in Israel.[22,34,83]

K. kingae isolates from the tonsils of daycare attendees have been shown by immunoblotting, pulsed-field electrophoresis, and ribotyping techniques to be more commonly type-specific than are isolates from unrelated individuals, thus suggesting that the organism is transmitted from person to person in the daycare setting.[22,34,66,83] Two outbreaks of *K. kingae* skeletal infections in daycare centers have been reported.[22,34,83] In both instances, isolates from children with invasive disease were identical to strains found in other pharyngeal-colonized children at the daycare centers.

Osteoarticular *K. kingae* infections occur most commonly in children 6 months to 4 years of age,[24,36,81,87] but *K. kingae* endocarditis occurs in all age groups, including adults.[15,24,72,77] In southern Israel, the annual incidence of *K. kingae* invasive infection has been estimated to be 22 cases per 100,000 children aged 2 years or younger.[79,87] The rate of invasive infection was a quarter that found for invasive Hib infection during a period when children were not receiving conjugated Hib vaccine in a previous study.[81] In children with suppurative arthritis, *K. kingae* has been isolated more commonly than has Hib from joint aspirates that were cultured directly in BACTEC bottles.[80] In more recent series of patients in whom suppurative arthritis developed after the advent of the routine use of conjugated Hib vaccine, *K. kingae* was the most common cause of infection in children younger than 36 months old.[41,47]

Invasive *K. kingae* infections occur more frequently in males than in females in most studies[24,36,41,79,87] and tend to occur between the months of July and December.[12,15,81,87] Upper respiratory tract symptoms, dental abnormalities, or evidence of stomatitis usually precedes the signs of invasive illness.[24,36,81,89] Infections have occurred in several children after or while experiencing a primary varicella infection or an episode of herpetic gingivostomatitis.[1,37,40,74] In Israel, 61 percent of children with an invasive infection had evidence of a respiratory tract infection or stomatitis at the time of *K. kingae* infection.[79,87]

PATHOGENESIS AND IMMUNITY

No studies have defined the mechanisms of pathogenesis of *Kingella* infection. *Kingella* spp. have type 4 pili, which may allow the organisms to adhere to oropharyngeal epithelium.[75] Because *K. kingae* normally colonizes the oropharyngeal mucosa of young children and infection frequently is associated with upper respiratory tract infections, dental abnormalities, and evidence of stomatitis, damage to the oropharyngeal mucosa by viruses or trauma probably allows *K. kingae* access to the bloodstream. After a period of transient bacteremia, *K. kingae* can seed other tissues and cause focal infection in joints, bones, disk spaces, and heart valves.

The outer-membrane proteins of *K. kingae* have been characterized, and many have been found to be immunogenic.[90] Although children convalescing from invasive infection experience increases in the amount of specific immunoglobulin G (IgG) antibodies, the responses are mild and extremely variable.[65]

CLINICAL MANIFESTATIONS

K. kingae causes various infections in children (Table 148–1), with the most common invasive infection being suppurative arthritis.[39,46,81,87] Infants and young children usually have acute monarticular joint swelling and tenderness. To date, no cases of polyarticular infection in children have been reported. No features of the illness distinguish it from other bacterial causes of suppurative arthritis. Patients often have symptoms of an upper respiratory tract illness or stomatitis shortly before or at the time of illness.[1,39,40,46,55,79,89] Eighty-six percent of children in one series had temperatures higher than 38° C at initial evaluation.[36] The knee is the joint affected most commonly.[6,16,19,21,48] However, *K. kingae* has been reported to cause infection in the hip,[7,37,48] ankle,[6,48] elbow,[6,19] wrist,[58,81] sternoclavicular joint,[15,16,73] and shoulder.[21,81] Most children do not have an underlying illness that increases their susceptibility to infection, although *K. kingae* suppurative arthritis has developed in a child undergoing therapy for acute lymphocytic leukemia.[55]

K. kingae osteomyelitis has a more insidious onset than suppurative arthritis does, with most patients having symptoms for at least 1 week before a bone infection is diagnosed. Fever is present in only half the children with *K. kingae* osteomyelitis.[36] The proximal and distal ends of the femur are the most common sites of infection,[15,18,24,64] which often occurs in an epiphysis.[7,19,36,63,81] *K. kingae* osteomyelitis of the talus[3,15,72] and calcaneus[24,37,81,84] also develops in young children, although unlike individuals with the more frequently encountered osteomyelitis associated with puncture wounds, these children have relatively mild symptoms and no history of trauma or penetrating injury. In fact, in a young child with osteomyelitis of the talus or calcaneus and no history of penetrating trauma, *K. kingae* infection should be suspected highly. Less commonly, *K. kingae* causes infection in the tibia,[24,63] clavicle,[59] ulna,[50] radius,[12] humerus,[6,81] and sternum.[18,54] In one child with *K. kingae* osteomyelitis of the neck of the femur, the histopathologic findings of the infected bone resembled those of eosinophilic granuloma.[64]

Numerous cases of *K. kingae* spondylodiskitis in children have been reported.[12,24,36,37,52,54] These children usually are younger than 5 years old and have an insidious onset of back stiffness and tenderness, eventually leading to their refusal to walk. Fever was present in two thirds of patients, but temperatures were never higher than 39° C.[41] Various disks from T11-T12 to L5-S1 have been involved, and one patient with cervical involvement has been reported.[52]

Fewer than half of the reported cases of *K. kingae* endocarditis have occurred in children,[6,22,31,74,76] and nearly three quarters of the patients have had preexisting structural cardiac defects.[24,77] The history and physical findings of *K. kingae* endocarditis do not differ from those seen in children with subacute endocarditis of other bacterial causes. At least a third of patients with *K. kingae*

TABLE 148–1 Infections in Children Caused by *Kingella kingae*

Dactylitis
Endocarditis
Endophthalmitis
Meningitis
Orbital cellulitis
Osteomyelitis
Pulmonary infection
Soft tissue abscess
Spondylodiskitis
Subglottic and epiglottic infections
Suppurative arthritis
Transient bacteremia

endocarditis have oral or pharyngeal mucosal alterations before systemic symptoms develop.[24,77] Children usually are febrile, and they often have a new or changing heart murmur, splenomegaly, and petechial rash.[9] Patients also can exhibit more acute symptoms, including evidence of septic shock and cardiac failure.[5,15,72] *K. kingae* endocarditis occasionally has occurred in immunosuppressed individuals,[15,30,77] including patients with acquired immunodeficiency syndrome (AIDS).[33,70]

Isolation of *K. kingae* from the blood of a febrile infant should not suggest necessarily that the child has endocarditis or an unidentified focal infection. Numerous children with transient *K. kingae* bacteremia during infancy in whom a focal infection did not develop have been identified.[35,48,78,79] A rash mimicking gonococcemia[58,62] or meningococcemia rarely develops in bacteremic children.[69] Rarer *K. kingae* infections include meningitis,[49,56,69,71] endophthalmitis,[11,24] pulmonary infections,[45] dactylitis,[14,81] subglottic[81] and epiglottic[32] infections, orbital cellulitis,[17] and soft tissue abscesses.[27,57] Recently, a child with *K. kingae* bacteremia and simultaneous manifestations of Henoch-Schönlein purpura was described.[23]

A 3-year-old child with vaginitis from which *K. denitrificans* was isolated and a 22-month-old with *K. denitrificans* endocarditis are the only reported examples of other members of the *Kingella* genus causing infection during childhood.[60,61]

LABORATORY FINDINGS AND DIAGNOSIS

No characteristic laboratory findings suggest the presence of an invasive *K. kingae* infection. Leukocytosis (>10,000 white blood cells/mm^3) is present in only 60 percent of children with an invasive infection. However, the erythrocyte sedimentation rate frequently is elevated (>20 mm/hr) in children with bone and joint infections.[36] Interestingly, the C-reactive protein level in *K. kingae* osteoarticular infections usually is normal, thus suggesting that this test may be less helpful than the erythrocyte sedimentation rate in diagnosing this infection.[16,41] In children with culture-proven suppurative arthritis, the leukocyte count in synovial fluid is variable (10,000 to 161,000 white blood cells/mm^3), but neutrophils usually predominate.[81] Organisms are identified by Gram stain in less than 15 percent of clinical specimens.[24,36] Plain radiographs demonstrate soft tissue swelling and joint effusions in half the cases of suppurative arthritis, but lytic lesions or disk space narrowing develops in 95 percent of cases of osteomyelitis or spondylodiskitis.[24] Technetium 99m bone scans may be of assistance in diagnosing *K. kingae* osteomyelitis during the early course of disease, when plain radiographs do not yet demonstrate abnormalities.[24] In children with *K. kingae* endocarditis, large vegetations often are demonstrated by echocardiography, especially in those who have had symptoms for a long time or when evidence of embolization is present.

K. kingae infection is diagnosed by isolating the organism from an appropriate clinical specimen. The organism is isolated from blood in only 5 percent of patients with osteoarticular *K. kingae* infections.[19] The fastidious nature of the organism, the low number of organisms found in clinical specimens, and its slow growth pattern hamper recovery of *K. kingae*. Conventional cultures should be examined at least once per week for 3 weeks to detect the organism. The recovery rate of *K. kingae* can be enhanced greatly if culture material, including purulent synovial fluid, is inoculated directly into BacT/Alert, Isolator, or BACTEC culture systems.[38,80,81,88] In one study, 91 percent of episodes of *K. kingae* suppurative arthritis would have been missed if the specimens had not been cultured in BACTEC bottles.[80] In addition, growth of the organism can be detected within 72 hours with these culturing systems; hence, prolonged incubation and extensive subculturing of blood with newer technologies is not necessary.[2,53] Once growth is detected, the clinical microbiology laboratory often has difficulty identifying it as *K. kingae*. Clinicians should suspect that a patient has a *K. kingae* infection when slow-growing, gram-negative rods that display beta-hemolysis are isolated from a normally sterile body site.

In the future, broad-spectrum polymerase chain reaction (PCR) amplification may be a routine technique used to diagnose *K. kingae* infections directly from clinical specimens.[46,68] In France, culture-negative osteoarticular specimens from children have been tested with 16S ribosomal DNA PCR, and 14 percent of samples were positive for *Kingella* DNA sequences, thus suggesting that this testing method most likely is more sensitive than are current culturing techniques.[73]

TREATMENT AND OUTCOME

K. kingae almost always is susceptible to β-lactam antibiotics.[15,31,85] However, three isolates obtained from children and an isolate from an adult patient with AIDS have contained β-lactamase.[6,67] Similarly, a *K. denitrificans* isolate obtained from the bone marrow of a 39-year-old patient with AIDS contained β-lactamase.[43] A few isolates have shown in vitro resistance to anti-staphylococcal β-lactams without the presence of β-lactamase.[6,16] Isolates of *K. kingae* universally have been susceptible to second- and third-generation cephalosporins and aminoglycosides.[15,31] Rarely, resistance to chloramphenicol and trimethoprim-sulfamethoxazole has been reported.[6,64,85] Many isolates, however, demonstrate at least partial resistance to erythromycin, clindamycin, and vancomycin.[6,15,31,85]

Children with invasive *K. kingae* infection have been treated with a variety of antibiotics, including penicillin G, ampicillin, cephalosporins, chloramphenicol, trimethoprim-sulfamethoxazole, and the anti-staphylococcal β-lactams. Occasionally, osteoarticular infections have resolved without administration of antimicrobial therapy.[39] Most infections should be treated initially with intravenous ampicillin because this antibiotic is tolerated relatively well and is inexpensive. All *K. kingae* isolates, however, should be checked for β-lactamase activity.

The duration of antibiotic treatment for *K. kingae* osteoarticular infection has ranged from 17 days to 6 months.[36] Oral therapy with an antibiotic such as amoxicillin can be considered when gastrointestinal absorption is demonstrated and compliance can be monitored. Many clinicians use normalization of the erythrocyte sedimentation rate as a guide to the duration of therapy. Surgical drainage of the bone, joint, or disk space usually is not required, except for infections in the hip and shoulder. The prognosis of patients with *K. kingae* osteoarticular infection is excellent; chronic mild complications from the initial infection have been reported only rarely. However, persistent narrowing of the intervertebral disk space commonly occurs in cases of *K. kingae* spondylodiskitis.[36]

Patients with *K. kingae* endocarditis usually respond rapidly to antibiotic therapy and become afebrile in a few days. High-dose intravenous therapy generally is continued for 4 to 6 weeks. In cases in which prosthetic valves are infected, surgical excision of the valve usually is not needed for cure unless an abscess forms. Unlike osteoarticular infections, the complication rate of *K. kingae* endocarditis is high; cerebral infarction and death have been reported after embolization of vegetations.[15,77]

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CHAPTER 149

Legionnaires' Disease, Pontiac Fever, and Related Illnesses

Paul H. Edelstein

Legionnaires' disease is an acute pneumonic illness caused by gram-negative bacilli of the genus *Legionella*. Pontiac fever is a febrile, nonpneumonic, systemic illness closely associated with, if not caused by, infection with *Legionella* spp.

HISTORY

Legionnaires' disease was recognized first as a distinct clinical entity when it caused an epidemic of pneumonia at an American Legion convention in Philadelphia in 1976; 221 people were affected, and 34 died.[61] Investigators were unable to determine the exact cause of the outbreak immediately; this mystery provoked considerable fear and widespread speculation about the cause. Approximately 6 months later, two investigators at the Centers for Disease Control and Prevention, Joseph McDade and Charles Shepard, announced that they had discovered the etiologic agent, a fastidious gram-negative bacillus.[107] Researchers subsequently determined that both the organism and the disease had been studied previously as long ago as the 1940s but had been forgotten.[34,47,142] Because of the historical association with the American Legion convention, this disease now is called *legionnaires' disease*, and the etiologic agents belong to the family Legionellaceae.

ETIOLOGIC AGENT

Legionella is the only genus in the family Legionellaceae. Fifty species of *Legionella* and more than 70 different serogroups now are recognized (Table 149–1). *Legionella pneumophila* is the species responsible for the 1976 Philadelphia epidemic, and it causes most cases of legionnaires' disease.[34,38,47,93,99,142] Serogroup 1 of *L. pneumophila* is estimated to cause approximately 70 to 90 percent of all cases of legionnaires' disease in previously healthy people.[39,103] *Legionella* spp. other than *L. pneumophila* and serogroups other than *L. pneumophila* serogroup 1 cause 25 to 40 percent of nosocomial outbreaks of legionnaires' disease; of these, *L. pneumophila* serogroups 4 and 6, *Legionella micdadei*, and *Legionella dumoffii* are the most common.[76,153] *L. pneumophila* and *Legionella longbeachae* are the only two *Legionella* spp. that normally infect nonimmunocompromised patients.

The Legionellaceae are obligately aerobic, mesophilic, motile, gram-negative bacilli with variable oxidase and catalase reactions.[46] Amino acids rather than carbohydrates are used as an energy source. One unique bacterial characteristic is a nutritional requirement for L-cysteine, a characteristic shared among gram-negative rods with only *Francisella tularensis*. In addition, both the cellular fatty acid and ubiquinone content differ from those of other mesophilic gram-negative bacilli.

Sequencing of the genomes of three different *L. pneumophila* serogroup 1 strains revealed that these bacteria harbor a number of eukaryotic-like genes, a number of secretion systems, remarkable plasticity, and the presence of mobile elements. Approximately 10 percent of the approximately 3.5-Mb genome is devoted to strain-specific genes.[21,28,29]

The antigenic relationships among different *Legionella* spp. and among serotypes of the same species are complex.[142] The serologic typing scheme is based on surface antigens (O antigens), which primarily are lipopolysaccharides. These surface antigens are shared by different species and, in rare cases, by other gram-negative bacilli, such as *Pseudomonas* spp. Thus, identifying an unknown strain with the use of serologic methods alone occasionally leads to a false or misleading identification. Cross-reactions also may be observed when testing for human antibodies to *Legionella* spp. Patients with infections caused by some *Pseudomonas* strains, *Campylobacter jejuni*, and other gram-negative bacilli may form antibodies to *Legionella* spp.[45]

TABLE 149–1 *Legionella* Species and Serogroups

Species	No. of Serogroups	Implicated in Human Disease
adelaidensis	1	No
anisa	1	Yes
beliardensis	1	No
birminghamensis	1	Yes
bozemanae	2	Yes
brunensis	1	No
busanensis	1	No
cherrii	1	No
cincinnatiensis	1	Yes
drancourtii	Unknown	No
drozanskii	1	No
dumoffii	1	Yes
erythra	2	No
fairfieldensis	1	No
falloni	1	No
feeleii	2	Yes
geestiana	1	No
gormanii	1	Yes
gratiana	1	No
gresilensis	1	No
hackeliae	2	Yes
israelensis	1	No
jamestownensis	1	No
"jeonii"	Unknown	No
jordanis	1	Yes
lansingensis	1	Yes
londiniensis	1	No
longbeachae	2	Yes
lytica	Unknown	Yes
maceachernii	1	Yes
micdadei	1	Yes
moravica	1	No
nautarum	1	No
oakridgensis	1	No
parisiensis	1	Yes
pneumophila	16	Yes
quateirensis	1	No
quinlivanii	2	No
rowbothamii	1	No
rubrilucens	1	No
sainthelensi	2	Yes
sainticrucis	1	No
shakespearei	1	No
spiritensis	2	No
steigerwaltii	1	No
taurinensis	1	No
tucsonensis	1	Yes
wadsworthii	1	Yes
waltersii	1	No
worsleiensis	1	No

Because of these complexities, definite identification of *Legionella* spp. other than *L. pneumophila* often requires the capabilities of a research laboratory. However, isolation and presumptive identification of *L. pneumophila* should be within the capabilities of most clinical laboratories.[46]

Legionella is ubiquitous in the aqueous environment and can be found, often in high concentration, in lake water, ponds, bathing water, hot-water tanks, hot-water plumbing, and air-conditioning cooling towers.[142] Its optimal temperature for growth ranges from approximately 28° C to 40° C (82° F to 104° F). The natural hosts and probable reservoirs of environmental *Legionella* bacteria probably are fresh-water amebae such as *Acanthamoeba* and *Hartmannella*.[58] Factors that promote the environmental growth of *Legionella* include the presence of other microorganisms, use of plumbing materials that promote bacterial growth (certain types of rubber gaskets), stagnation, and warm temperatures.[142] In the home, older municipal water distribution systems, use of electric rather than gas water heaters, and use of well water all appear to promote the presence of *Legionella* in water.[58,138] Most newly discovered *Legionella* spp. have been environmental isolates not associated with clinical illness.[58]

A large number of bacterial virulence factors have been described, the most important of which is the Dot/Icm complex. This complex is composed of 26 genes that form a type IV secretion system, which is required for growth of *L. pneumophila* within macrophages. *L. pneumophila* is not killed actively by macrophages, which in fact provide sustenance for the bacterium. The Dot/Icm complex allows the bacterium to evade normal phagolysomal trafficking and bacterial destruction by injecting effectors into the host macrophage within minutes of initial bacterial-macrophage contact.[2] A few effectors have been described, although more probably will be found. The end result is that the normal host processes are hijacked into providing a intracellular compartment for the bacterium that associates with the endoplasmic reticulum.[30,114,131] Other bacterial virulence factors include a variety of enzymes, iron-binding proteins, and miscellaneous proteins enhancing intracellular growth and survival or invasion.[30]

The availability of intracellular iron appears to play a key role in the growth of intracellular *L. pneumophila*. Interferon-γ, the production of which is increased during *L. pneumophila* infection of macrophages, down-regulates the number of iron-binding receptors on macrophages, which has the effect of decreasing the availability of intracellular iron and, hence, limiting intracellular growth of the bacterium.[23]

L. pneumophila is serum-resistant.[142] High-titer antibody promotes phagocytosis but may not enhance killing. Macrophage activation factors possibly are more important than is antibody in promoting phagocytosis and bacterial killing.[80]

EPIDEMIOLOGY

INCIDENCE AND FREQUENCY

The incidence of pediatric legionnaires' disease has been studied only by use of serosurveys, which also have been used for most studies involving the adult population.* Because of the less than absolute specificity of serologic testing, frequent failure to obtain appropriately timed paired serum samples, and almost uniform lack of culture confirmation of disease, these serosurveys can be used only as crude measurements of the incidence of the disease.[34,127,142] Regardless, pediatric legionnaires' disease rarely appears to be the cause of pneumonia in otherwise healthy children.† Fewer than 10 culture-confirmed cases of legionnaires' disease have been reported in otherwise normally healthy children, and fewer than 30 culture-confirmed cases have been reported in immunosuppressed children.‡

A retrospective serosurvey of 500 patients with pneumonia, 83 percent of whom did not need hospitalization, found that only 5 of the 132 patients younger than 15 years old had significant antibody titers against *L. pneumophila* and none seroconverted.[60] The estimated incidence of legionnaires' disease in the overall population was approximately 12 per 100,000 per year. In a year-long prospective study of 191 children hospitalized with pneumonia, titers rose significantly in only 1 child (0.92% of cases with paired serum samples available).[118] In another prospective study of 52 children younger than 4 years old with lower respiratory tract infections, no cases of legionnaires' disease were

*See references 4, 34, 60, 77, 112, 113, 118, 127, 129, 142.
†See references 4, 10, 25, 32, 37, 62, 78, 81, 95, 109, 123, 135, 141.
‡See references 1, 7, 19, 24, 25, 32, 35, 51, 56, 68, 69, 84, 91, 92, 94, 97, 98, 116, 121, 126, 136, 145, 147, 151.

identified.[4] Four of 211 Iowa children (1 to 19 years of age) with atypical pneumonia demonstrated seroconversion to *L. pneumophila*.[129] In a retrospective Israeli serosurvey, titers rose in two of 37 children.[113] A 2-year prospective study in France found that only two of 278 children (0.7%) hospitalized for pneumonia had legionnaires' disease based on seroconversion.[32] One of these two children was immunosuppressed. With these data, one could estimate that the mean frequency of legionnaires' disease as a cause of pneumonia requiring hospitalization in the normal pediatric population is approximately 1 percent. This rate is about the same as the 0.5 to 4 percent frequency found in the adult population.[34,103,142] Legionnaires' disease is not a major cause of pneumonia in either children or adults.

The frequency of legionnaires' disease in the immunosuppressed pediatric population is unknown. Several case reports document this disease in children being treated for acute leukemia, in children with chronic granulomatous disease, and in children after bone marrow or solid organ transplantation or after being treated with steroids for other reasons.* No large studies of this disease in the immunosuppressed pediatric population have been performed, however. One retrospective study of 55 pediatric cancer patients with atypical pneumonia found 1 patient with a rise in antibody titer to *L. pneumophila*.[132] Nosocomial or community-acquired legionnaires' disease has been reported in several "immunologically normal" infants, who may have had some degree of immune system compromise that predisposed them to this disease.[33,57,62,78,79,87,95,116,126] Neonates appear to be at risk of acquiring nosocomial, water-birthing–related, and community-acquired legionnaires' disease.[69,98] Neonates in intensive care units may be at particularly high risk for the acquisition of nosocomial legionnaires' disease because of the immaturity of their immune system and because of the humid and warm conditions of neonatal incubators, which are ideal for growth of the bacterium.

Whether atypical, mild, or asymptomatic *Legionella* infection occurs in children is unknown. Several cases of Pontiac fever in children have been reported in outbreaks of this disease.[66,82,85,100] The risk of acquiring Pontiac fever was found to be greater for younger people in one outbreak,[82] just the opposite finding in another investigation of an outbreak.[85] The true frequency of Pontiac fever in children is difficult to determine because the diagnosis is impossible to make with certainty in the sporadic form. Several of the serosurveys already cited have found asymptomatic elevations of *L. pneumophila* antibody in widely varying frequency. Andersen and colleagues[4] found that an increase in antibody titer unrelated to acute illness developed in 27 of 52 children over a period of several years; some of these children had significantly high titers. Muldoon and coworkers[112] found that 15 percent of 126 children sampled had antibody titers to *L. pneumophila* of 256 or greater, which was not significantly different from those seen in an adult control population. The striking finding was that antibody titers rose with age, doubling each year until 3 years of age, at which time the values reached a plateau; this finding held even when patients with pneumonia were excluded. In contrast, using a different and perhaps more specific antigen preparation, Mundel and colleagues[113] in Israel found a much lower prevalence of elevated antibody titers. That these findings may be geographic rather than methodologic is exemplified by the study of Orenstein and associates,[118] who obtained results similar to the Israeli study despite using the same type of antigen used by Andersen and colleagues.[4] The significance of these elevated titers in children without documented pneumonia is unclear, and these findings can be interpreted as cross-reactions to other colonizing or infecting bacteria, asymptomatic infection, or atypical disease.

*See references 35, 51, 71, 84, 91, 92, 96, 121, 122, 132, 134, 139, 143.

Several investigators have examined the question of whether atypical disease caused by *Legionella* occurs in children. Unfortunately, all these studies are based exclusively on serologic testing, which may not be entirely specific. Italian physicians found that two children with acute, reversible cerebellar ataxia exhibited significant antibody titer changes to *L. pneumophila*.[117] Another Italian study found two children with pericarditis who had significant antibody changes.[137] None of 140 British infants with sudden death had measurable antibodies to *L. pneumophila* despite suggestions that they might be linked.[149] Three studies have shown that children with cystic fibrosis have higher antibody levels to *Legionella* than normal children do, although these data are suspect because of known cross-reactions between *Legionella* and *Pseudomonas aeruginosa*.[31,49,88] Thus, despite several epidemiologic surveys, the prevalence of atypical *Legionella* infections in children is unclear.

In adults, true incidence figures for legionnaires' disease are difficult to find. Estimates based on serologic surveys range from 2 to 20 cases per 100,000 population per year.[34,127,142] A prospective study in Ohio found that the incidence of legionnaires' disease requiring hospitalization was approximately 6 per 100,000 per year, in what is thought to be a geographic region with an above-average frequency of the disease.[104] Estimates of the proportion of cases of adult pneumonia caused by legionnaires' disease range from 1 to 25 percent, with a reasonable mean of 3 percent.[103] Mild or atypical *Legionella* infection certainly occurs in adults, although the exact incidence is unknown. This approximately 3 percent prevalence is based on several outbreaks of Pontiac fever and case reports from the original Philadelphia outbreak of people with relatively mild disease.[34,61,64,65,90,142] Asymptomatic infection may occur in adults, based on the same types of serosurveys discussed for children. Neither oropharyngeal colonization nor a carrier state has been documented. A study of a cross-section of adults showed that approximately 6 percent exhibited oropharyngeal colonization with *L. pneumophila* on the basis of immunofluorescent studies, although none of the positive results could be confirmed by culture and, therefore, are suspect.[20]

DISEASE OUTBREAKS

Many epidemics of legionnaires' disease and Pontiac fever have been recognized.[19,34,66,142] In the case of legionnaires' disease, most outbreaks have occurred in residents, employees, or visitors in large buildings, such as hotels, hospitals, factories, retail stores, and office buildings. Nosocomial legionnaires' disease has been reported in hospitals throughout North America and Europe. In most cases in which thorough investigations have been performed, the reservoir has been the potable water distribution system, air-conditioning cooling towers, or both.[34,142] Outbreaks have been ended by disinfecting water and cooling tower systems by hyperchlorination or pasteurization.[34,142] In some of these outbreaks, disease occurred for many years until effective disinfection procedures were implemented. Attack rates in legionnaires' disease epidemics consistently have been less than 5 percent overall. Incubation periods ranging from 2 to 19 days have been observed.

Pontiac fever outbreaks generally have been associated with exposure to an aerosol of warm water contaminated with *Legionella*.[65,90,142] Examples include whirlpool baths, an engine assembly plant using contaminated water to cool machine lathes, and a health department building in which condensate in the air-conditioning system was contaminated. Attack rates in Pontiac fever outbreaks have been high, in the range of 95 to 100 percent. The incubation period is short, approximately 12 to 36 hours.[65]

Sporadic culture-proven cases of legionnaires' disease have been well documented. In fact, 70 to 85 percent of cases of

legionnaires' disease in the United States and elsewhere are neither nosocomial nor associated with an epidemic.[86,87,103] Some of these community-acquired cases represent undetected small case clusters, as has been shown for community-acquired cases in Glasgow, Scotland.[11] In that city, living close to a cooling tower was a risk factor for the acquisition of legionnaires' disease. Though difficult to prove, sporadic Pontiac fever probably occurs.[34,142]

Risk factors for the acquisition of legionnaires' disease can be divided logically into two main categories: those that increase exposure to contaminated water and those that suppress pulmonary defense mechanisms.[34,142] Included in the former category are occupational or residential exposure to warm or stagnant water, traveling and residence in hotels, and stays in hospitals with contaminated water distribution systems; a risk factor for residential acquisition of disease is the use of well water.[138] Included in risk factors that suppress pulmonary defense mechanisms are surgical procedures requiring general anesthesia, administration of glucocorticosteroids, cigarette smoking, chronic lung disease, and diseases (including human immunodeficiency virus [HIV] infection) or therapy that compromises the innate or cellular immune systems. Males are more than twice as likely as are females to contract the disease, perhaps as a result of the greater male prevalence of cigarette smoking and chronic lung disease. Middle-aged and older people also are at higher risk than are younger people. Other than exposure to aerosols of contaminated water, no particular predisposing factors for the development of Pontiac fever have been identified. Legionnaires' disease is not contagious, and transmission from patients to staff or other patients has never been documented.

PATHOLOGY, PATHOGENESIS, AND IMMUNITY

The exact mode of disease production in legionnaires' disease is unknown. Very good, but indirect, epidemiologic and pathologic evidence suggests that the initial infection results from inhalation of an aerosol.[34,142] The bacterial form that causes infection is unknown, with possibilities including a sporelike form, intra-amebal bacteria, bacteria enmeshed in biofilm (sessile phase), and bacteria released from biofilm (planktonic phase).[58,63] Some patients have acquired the disease after aspiration of *Legionella*-contaminated tap water rather than via aerosol inhalation.[176,83,102,146]

In guinea pigs infected by the aerosol route, multiplication of *L. pneumophila* begins within 16 hours.[36] This multiplication most likely takes place within alveolar macrophages, although some extracellular growth may occur. Cell culture studies show that multiplication occurs only intracellularly and not in the extracellular tissue culture medium.[80] This intracellular location of bacteria protects them from serum factors such as antibody and complement, as well as from the effects of antimicrobials that are not concentrated in cells. Killing of *L. pneumophila* within macrophages in cell culture is limited by failure of phagolysosomal acidification and fusion.[80]

The mechanism by which *L. pneumophila* avoids killing by macrophages is mainly by injecting phagosomal maturation-altering compounds into its host cell within minutes of initial contact, although all of the details of this process remain to be elucidated.[3,30] An additional mechanism involves shedding of bacterial flagellin into the host cytosol, which acts via the innate immune system to alter killing of the bacteria by macrophages.[128] In the late phases of bacterial multiplication in macrophages, the bacteria change phenotype to enable infection of the next susceptible macrophage; they become highly motile, quite small, and more resistant to antimicrobial agents.[8,110] The *L. pneumophila*–containing vacuole becomes huge and occupies almost the entire volume of the macrophage over a period of a few days. The bacteria effect escape from the presumably nutrient-depleted macrophage by inducing cellular apoptosis and then can infect available uninfected macrophages.[2] Innate macrophage resistance to bacterial infection may be mediated by toll-like receptors (TLRs), in particular, TLR-2 and TLR-5 and perhaps TLR-4.[72-74] Acquired macrophage resistance to infection derives from activation by interferon-γ and the resultant down-regulation of available iron stores.[22]

Polymorphonuclear leukocytes do not ingest or kill the organism effectively in vitro, although Davis and colleagues[36] suggest that they may form the bulwark of initial host defenses based on in vivo studies. Clinical evidence suggests that leukopenic hosts without concomitant macrophage dysfunction are not high-risk candidates for legionnaires' disease, although they may be at higher risk than the normal population.[26,47]

The histopathologic correlate of bacterial lung invasion is intense intra-alveolar inflammation.[142] Large airways are not affected, nor are small ones to the level of the terminal bronchioles. Both the terminal bronchioles and alveolar ducts may be involved in the inflammatory process. The interstitial spaces generally are not involved, although necrosis of alveoli may bridge the interstitial spaces. The alveoli contain a variable mixture of polymorphonuclear leukocytes, alveolar macrophages, and necrotic debris. Hemorrhage is observed, as are microabscesses. Later, fibrin formation and a predominance of histiocytes occur.

Gross lung changes evolve in the classic pattern of lobar pneumonia, with first red and then gray hepatization.[142] Lymph nodes are involved occasionally. The lung segments involved often are subpleural, a finding that sometimes suggests septic or bland infarction to the clinician. The pleural space is involved variably and seemingly is more prone to infection in immunosuppressed patients. One of the most striking pulmonary findings is the usual absence of significant intrabronchial exudate. This absence of intrabronchial exudate and lack of bronchial inflammation may mislead a bronchoscopist to conclude that pneumonia is not present.

Despite frequent signs and symptoms of extrapulmonary disease in patients with legionnaires' disease, no specific extrapulmonary pathologic findings have been identified.[142] In fatal cases, organisms often can be recovered or detected in various reticuloendothelial organs, such as the liver or spleen; however, detection of associated significant inflammation seldom occurs. Occasionally, patients have nonbacterial endocardial vegetations. Some patients have nonmassive hilar and paratracheal adenopathy, the result of bacterial adenitis. Patients may have metastatic foci, with abscesses in almost any location, including the myocardium, pericardium, peritoneum, brain, kidney, bowel wall, perirectal region, prosthetic heart valves, and hemodialysis shunts[47,142] (Table 149–2). Bacteremia occurs in some patients; it has been documented by positive blood cultures and is substantiated indirectly by the extrapulmonary foci of infection found in some patients.[47,142] Whether bacteremia accounts for some of the systemic clinical findings in legionnaires' disease is unknown. Elaboration of toxins by *Legionella* has been postulated to account for some aspects of the systemic disease, but supporting evidence is not convincing.[142]

Host cytokine production in response to *L. pneumophila* infection most likely causes most of the acute toxicity of the disease. Cell culture and animal experiments show that tumor necrosis factor, interferon-γ, and other cytokines are produced during *L. pneumophila* infection.[12,152] Humans infected with *L. pneumophila* have been shown to produce a number of cytokines during infection, as well as inflammatory markers such as procalcitonin, neopterin, and C-reactive protein.[55,124,140]

Episodes of recurrent or relapsing legionnaires' disease in patients with elevated antibody titers support experimental evidence suggesting that the humoral immune system plays a minor

TABLE 149–2 Diseases Caused by *Legionella*

Pneumonia (legionnaires' disease)
Associated with pneumonia
Bacteremia
Bowel wall abscess
Brain abscess
Colitis
Hemodialysis shunt infection
Myocarditis
Myositis, cellulitis
Pericarditis
Peritonitis
Perirectal abscess
Pleural empyema
Renal abscess
Not associated with pneumonia
Colitis
Lymphadenitis
Peritoneal dialysis–related peritonitis
Pleural empyema
Prosthetic heart valve endocarditis
Sepsis syndrome
Sinusitis
Soft tissue infections
Wound infection
Pontiac fever (? toxin-mediated)

role in this disease.[47] Also supporting this evidence is the rarity of legionnaires' disease case reports in patients with hypogammaglobulinemia or other diseases in which the humoral immune system primarily is deficient. The rise in specific antibody levels does not seem to have any clinical correlates, nor does the absolute antibody level.[47] Patients may recover from legionnaires' disease without any significant increase in antibody levels, again providing indirect evidence of the limited role of the humoral immune system.

The roles of antibody and complement in host defenses against legionnaires' disease have been explored in experimental models of infection. Several studies have shown that passive or active immunization is protective against intraperitoneal and subcutaneous chamber infection in rats, mice, and guinea pigs.[5,50,75,130] Pre-opsonization of *L. pneumophila* before intratracheal inoculation of hamsters was found to be partially protective, but several investigators have shown that active immunization fails to protect against pneumonia after intratracheal, aerosol, or intranasal inoculation of large numbers of *L. pneumophila* bacteria.[9,40,50] Protective immunity to pulmonary challenge in the guinea pig can be achieved after sublethal infection or infection with an avirulent mutant strain and by vaccination with an *L. pneumophila*–derived metalloproteinase or outer-membrane protein.[13-16,150]

Evidence for the importance of the cellular immune system in preventing infection largely is indirect and based on the greater prevalence of legionnaires' disease in immunosuppressed patients with cellular immunodeficiencies.[47,142] Curiously, legionnaires' disease in patients with acquired immunodeficiency syndrome (AIDS) has been reported infrequently, although one epidemiologic survey showed that patients with AIDS had a disease attack rate approximately 40 times greater than that in the normal population.[15,16,103,150] Development of cellular immunity after infection or vaccination can be demonstrated in vitro for both humans and animals, the clinical importance of which is unclear.[142]

Vaccination for prevention of legionnaires' disease may be feasible because it has been demonstrated to be effective in an animal model.[14-16,150] Whether vaccination in susceptible host populations would be successful is unknown, even if justified economically or on epidemiologic grounds.

The pathogenesis of Pontiac fever has not been studied. No differences have been detected between an *L. pneumophila* strain isolated from an outbreak of Pontiac fever and strains isolated from outbreaks of legionnaires' disease; these studies have included examination of virulence in an animal model, toxin production, and biochemical characteristics.[142] Detailed clinical studies of patients with Pontiac fever have not been performed, which renders creation of an experimental model of this disease difficult. Because it has a short incubation time that can be as brief as 12 hours, this disease probably does not represent widespread bacterial multiplication within the body, and more likely it represents a toxin-induced or allergic disease. In fact, the link between *Legionella* and Pontiac fever is circumstantial; entirely possible is that other microbes, or their toxins, coexisting with *Legionella* may cause the disease.[65,90] The clinical syndromes most like Pontiac fever are bath-water fever, humidifier fever, and extrinsic allergic alveolitis; these syndromes are thought to be caused either by the direct toxic activity of inhaled endotoxin or by an allergic reaction to microorganisms, most particularly amebae such as *Naegleria* spp.[48,111,133] *L. pneumophila* makes a poorly active endotoxin, thus suggesting that if inhaled endotoxin is the cause of Pontiac fever, the endotoxin comes from non-*Legionella* bacteria. Support for inhaled endotoxin as the cause of Pontiac fever is found in two studies of endotoxin present at outbreaks of the disease.[27,59]

CLINICAL FINDINGS OF LEGIONNAIRES' DISEASE

SIGNS AND SYMPTOMS

Legionnaires' disease usually is manifested as pneumonia.[47] The pneumonia is atypical in that the usual pathogenic bacteria generally are not isolated from respiratory tract secretions or blood and patients do not respond, except fortuitously, to antimicrobial agents commonly used to treat pneumonia in adults (e.g., penicillins, cephalosporins, and aminoglycosides). Beyond these findings, considerable speculation and controversy continue over whether a distinct clinical syndrome exists. Several prospective studies of both community-acquired and nosocomial legionnaires' disease have failed to demonstrate any clinical, radiographic, or nonspecific laboratory features that distinguish legionnaires' disease from other common causes of pneumonia.[41,54,70] The classic clinical findings are reviewed because many physicians believe that they are distinctive for *Legionella* pneumonia. Whether legionnaires' disease in children mimics the clinical findings in adults is unknown because of the rare instances of well-documented pediatric cases and the possibility of spectrum bias.

The onset of pneumonia may be either insidious or abrupt. Recurrent chills, abdominal pains, myalgia, headache, malaise, anorexia, and severe fatigue are common manifestations. Diarrhea consisting of loose, nonbloody stools several times a day occurs in approximately 30 to 40 percent of cases. Fever may be low-grade or absent initially. Over the course of a day to several days, these nonspecific symptoms gradually worsen, often resulting in severe debilitation. Noteworthy is the frequent absence of symptoms referable to the respiratory system. Rash, splenomegaly, adenopathy, and rhinorrhea are exceptionally uncommon findings. Physical examination early in the illness generally is remarkable for a paucity of localizing findings and the frequent impression that the patient has an influenzal or typhoidal illness.

Within a day to several days after onset, a high fever usually, but not always, develops. Pulse-temperature dissociation occurs in approximately half of epidemic-associated cases. Respiratory complaints, especially dyspnea and pleuritic chest pains, may become prominent. Cough usually is not a major complaint,

although it is a common manifestation. The sputum almost never is frankly purulent; blood-streaked sputum or frank hemoptysis is observed in 20 to 30 percent of patients. Many patients experience confusion, cerebellar ataxia, lethargy, agitation, or some other neurologic disorder. Severe abdominal or back pain may occur, sometimes with localization. Physical examination at this time reveals a "toxic" febrile patient with apparent multisystem disease. Chest examination usually discloses findings of consolidating pneumonia, with bronchial breath sounds, increased vocal fremitus, and dullness to percussion. Depending on the stage of consolidation, rales may or may not be heard. Pleural friction rubs or signs of pleural effusion can be observed. Despite frequent symptoms of abdominal pain, signs of peritoneal irritation, such as decreased bowel sounds or rebound tenderness, seldom are detected. Signs of meningeal irritation rarely occur but have been reported.

Most normal healthy patients recover without specific therapy, usually by day 7 to 10 of illness. Those who do not recover generally die of progressive respiratory failure, along with failure of other organ systems. Empyema, pulmonary cavitation, renal failure, memory loss, fatigue, and neurologic disorders all are potential complications and may persist for weeks to months after onset of the disease. A variety of extrapulmonary diseases may occur very rarely as a result of legionnaires' disease, the most common of which are pancreatitis,[108] hepatitis, and myopericarditis.[6,105] Myositis, as measured by elevated serum creatine kinase levels, occurs commonly, with severe muscle disease such as rhabdomyolysis being a rare complication.[89,148] Most of these extrapulmonary diseases improve rapidly with resolution of the pneumonia.

Focal infection without pneumonia is a very rare manifestation of *Legionella* infection, with only a handful of cases reported (see Table 149–2). Direct inoculation of bacteria into surgical or traumatic wounds is the usual mode of pathogenesis, although bacteremia and bacteremic metastatic infection have been reported.[106,125]

RADIOGRAPHIC FINDINGS

The roentgenographic hallmark of legionnaires' disease is an acinar filling pattern with consolidation.[34,47,142] There is no distinctive predilection for any lung region; pleural-based consolidation and bilateral infiltrates may occur. Nodular infiltrates may be seen, as may cavitation in the areas of original consolidation. Purely interstitial infiltrates are distinctly uncommon findings in established disease but occur rarely very early in the disease process; these interstitial infiltrates rapidly progress to consolidating ones within a day or so. Pleural effusion, with or without parenchymal infiltrates, can occur. Pleural effusion has been documented as the only chest finding in patients treated early with specific antibiotic therapy.

LABORATORY FINDINGS

General

Multiple nonspecific abnormal laboratory results can be detected in patients with legionnaires' disease.[34,47] Hematologic abnormalities may include leukocytosis or leukopenia, usually with a left shift; lymphopenia; thrombocytosis; and disseminated intravascular coagulation. Proteinuria and pyuria are common findings; myoglobinuria also may be present. Hyponatremia and hypophosphatemia occur commonly, as do elevations in aminotransferase enzymes, bilirubin, alkaline phosphatase, and creatine kinase. Severe azotemia occurs, though rarely. Elevation of cold agglutinin titers, cold agglutinin–induced hemolytic anemia, and elevation of complement fixation titers to *Mycoplasma pneumoniae* also may occur. Arterial oxygenation usually is depressed in relation to the extent of pneumonia. In patients with severe disease, severe oxygen desaturation related to either oxygen intoxication or respiratory distress syndrome also may develop. Taken together, these multiple laboratory abnormalities often suggest multisystem disease to the clinician.

Specific

Diagnosis of legionnaires' disease is accomplished best by recovery of *Legionella* from sputum or other lower respiratory tract secretions or tissues.[46] Urine antigen testing is the most useful single test but can have false-negative results. Other specific diagnostic methods are detection of *L. pneumophila* antigen by immunofluorescent microscopy and detection of *Legionella* DNA in respiratory tract specimens by polymerase chain reaction (PCR). Serologic diagnosis is of uncertain value in children but is useful in adults.

Selective media and techniques that facilitate isolation of *Legionella* from sputum are available.[46] Sputum is pretreated with an acid solution. This material, as well as a non-pretreated sample, is plated on buffered charcoal–yeast extract medium supplemented with α-ketoglutaric acid (BCYE-α) and on BCYE-α supplemented with antibiotics (BMPA or PAC and MWY or PAV media). *Legionella* organisms grow on these media 3 to 7 days after inoculation and incubation at 35° C in air. Culture diagnosis has a higher yield than does immunofluorescent microscopy or serology and does not produce any false-positive results. Sputum culture yield depends on the severity of the illness; it is close to 100 percent for legionnaires' disease in immunocompromised patients and for patients with ventilator-dependent pneumonia. However, sputum culture yield can be as low as 10 to 25 percent in mild legionnaires' disease. Blood and pleural fluid cultures have much lower yields than do respiratory tract cultures. Blood culture yield is so low that its utility is debatable; severely immunocompromised patients and those with very severe pneumonia are most likely to have positive blood cultures.

Immunofluorescent microscopy for *L. pneumophila* is a rapid and highly specific (99.9%) technique for establishing the diagnosis.[46] It can be performed in 1 to 2 hours after receipt of the specimen and, like culture, can be performed with sputum. It does have several disadvantages. The test requires considerable technical expertise in reading; if the test is performed by inexperienced technologists, its results often are erroneous. Moreover, one must take scrupulous care to avoid carryover from other samples and false-positive results caused by contaminated reagents. Cross-reacting organisms are rare findings in clinical samples; they frequently can be detected by experienced technologists on the basis of morphologic characteristics and staining pattern. Nonetheless, certain cross-reacting bacteria, such as some *Pseudomonas* strains, still cause rare false-positive results. Some strains of *Bacteroides fragilis*, *Streptococcus pneumoniae*, *Bacillus* spp., and *Candida* spp. also can cross-react with diagnostic reagents, although cross-reactions caused by these organisms rarely, if ever, result in a false-positive diagnosis of legionnaires' disease. Because of these difficulties, few laboratories perform this testing.

Serologic testing is of most value in epidemiologic studies and of least value in the acute diagnosis of sporadic cases.[45] As many as 25 percent of patients with culture-documented disease fail to undergo seroconversion against the homologous serotype; such failure is not related solely to early treatment or immunosuppression, although these factors may cause failure of antibody formation. As long as 3 months may be required for antibody levels to increase after the onset of illness; the median time is approximately 2 weeks. In addition, as with any other means of immunologic diagnosis of this disease, the multiplicity of antigenic

types renders serologic testing extremely cumbersome. Because 5 to more than 25 percent of the normal population have elevated antibody titers to *Legionella*, only a fourfold rise in titer is considered significant. Only paired samples, drawn 3 to 6 weeks apart, should be tested. Because of day-to-day variation in test results, these samples must be tested simultaneously for optimal results. For maximum yield, samples taken as long as 9 to 12 weeks after onset of the disease should be tested if earlier samples reveal no changes. As with immunofluorescent detection of bacterial antigen, a negative serologic result does not exclude disease.

The specificity of serologic diagnosis is fairly high in adults, in the range of 95 to 99 percent.[45] Cross-reactive antibodies may be found in the serum of patients with leptospirosis, melioidosis, *B. fragilis* infections, *P. aeruginosa* infections, and possibly *Haemophilus influenzae* or enteric bacterial infections. However, even 99 percent specificity is not sufficient for certainty of diagnosis of a sporadic case. If the estimated 1 percent prevalence of legionnaires' disease in children is correct, less than half of all seroconversions yield truly positive results (positive predictive accuracy of 45%). This observation, combined with the studies cited previously showing age-related elevations in anti-*Legionella* antibody in young asymptomatic children, renders serologic diagnosis of pediatric legionnaires' disease highly suspect.

Detection of soluble bacterial antigen in urine can be used to diagnose *L. pneumophila* serogroup 1 infections successfully and is the most sensitive and most rapid test for community-acquired legionnaires' disease.[44,46] An enzyme immunoassay and immunochromatographic card kit for this procedure are available commercially (Wampole, Cranbury, NJ, and Binax, South Portland, ME, respectively). The major drawback of this test is that it preferentially detects only *L. pneumophila* serogroup 1 infections. Otherwise, it has excellent sensitivity (90-99% versus culture and 60-70% in patients with community-acquired legionnaires' disease) and extraordinary specificity (>99.9%). In some cases, the urinary antigen test yields positive results when sputum culture for *L. pneumophila* serogroup 1 is negative, especially in previously treated patients and in epidemics of legionnaires' disease. The urine antigen test is less sensitive in nosocomial legionnaires' disease and in legionnaires' disease in immunocompromised patients because other serogroups and other *Legionella* spp. become likely in these groups. Thus, although the urine antigen test detects approximately 60 to 70 percent of those with community-acquired legionnaires' disease, fewer than 40 percent of nosocomial cases test positive with this test. A negative urine antigen test should not be used as the sole reason to stop therapy for legionnaires' disease because 30 to 100 percent of patients with legionnaires' disease can have a false-negative test, depending on the identity of the infecting strain.

PCR has been used to detect *L. pneumophila* in sputum, serum, and urine. Though both sensitive and specific, PCR tests have been done on a research basis and have not been validated in large well-controlled studies. A commercial PCR assay (BD ProbeTec; Becton Dickinson, NJ) has become available recently but lacks published evaluations.[115]

With the exception of the urine antigen test, none of the nonculture tests is as sensitive as is culture diagnosis under ideal circumstances. Thus, culture must be performed in every case; if desired, the other tests can be used to provide same-day answers. Because none of the *Legionella*-specific tests is 100 percent sensitive, the clinician sometimes must treat for legionnaires' disease in the absence of confirmatory laboratory tests. One or 2 days of therapy with erythromycin apparently does not affect the sensitivity of the diagnostic tests, although therapy with azithromycin or levofloxacin can affect culture yield. Regardless, therapy should not be withheld pending the results of laboratory tests.

TREATMENT OF LEGIONNAIRES' DISEASE

No adequately sized prospective clinical studies of antimicrobial therapy for legionnaires' disease have been performed. All recommendations regarding therapy are based on retrospective and small prospective clinical studies, as well as laboratory studies.

Because of the superiority of newer fluoroquinolone agents and azithromycin in experimental nonhuman studies and on the basis of uncontrolled trials of these agents in adults with legionnaires' disease, the current recommendation is that adults with severe legionnaires' disease and immunocompromised patients with the disease be given either azithromycin or fluoroquinolone therapy rather than erythromycin or clarithromycin therapy. Nonhospitalized patients with mild legionnaires' disease can be treated equally well with any of the specific therapies, including erythromycin, tetracycline, the newer fluoroquinolones, azithromycin, or clarithromycin.[42,43,101] No inherent reason other than age-specific drug toxicity precludes these guidelines for children.

Fluoroquinolone antimicrobial agents generally are contraindicated in children, although the evidence suggests that the risk for toxicity from short courses of therapy is low. The scope of this chapter does not allow discussion of the use of these drugs in children, but in some cases, the benefit of using these drugs in very severe legionnaires' disease may be greater than the potential risk for the development of drug side effects.

Erythromycin remains the drug of choice for treatment of mild legionnaires' disease in children. Generally, it is given intravenously in four daily divided doses of 15 mg/kg. Intravenous therapy can be changed to oral therapy (30 to 50 mg/kg/day in divided doses) once clinical improvement is evident. Therapy should be given for a minimum of 18 to 21 days. Relapses may occur when intravenous erythromycin therapy is changed to the oral route and after a course of therapy that is too short. Some patients with mild illness may be treated initially with oral therapy. Clarithromycin (7.5 mg/kg every 12 hours) may be used in place of erythromycin, although there is good laboratory and reasonable clinical data showing that this drug is no more effective for legionnaires' disease than erythromycin is.[18,120] Clarithromycin is not approved by the U.S. Food and Drug Administration (FDA) for the treatment of legionnaires' disease.

Azithromycin, which is approved for use in children in the United States, is as active against *Legionella* bacteria as are the newer fluoroquinolone agents, thus rendering it a candidate for the drug of choice for severe pediatric legionnaires' disease or for legionnaires' disease in immunocompromised children. The drug dose for children with this type of pneumonia has not been studied, but based on extrapolation from adults, a dose of 10 mg/kg (maximum, 500 mg) given once daily for 3 to 5 days should be sufficient in most cases, with extended duration of therapy (7 to 10 days) for severely immunocompromised patients. Azithromycin is available in both intravenous and oral forms and is approved by the FDA for use in adults with legionnaires' disease.

Newer fluoroquinolone agents, along with azithromycin, are considered the drugs of choice for the treatment of severe legionnaires' disease in adults. Levofloxacin in a dosage of 500 mg daily for 10 to 14 days is used commonly in the United States to treat this disease. No dosage guidelines exist for the treatment of pediatric legionnaires' disease because of the potential for drug toxicity in this population. Levofloxacin is the only available quinolone agent approved by the FDA for the treatment of legionnaires' disease in adults; note that neither ciprofloxacin nor moxifloxacin is approved for this indication. Ciprofloxacin appears to be effective for this indication on the basis of case reports, and moxifloxacin may be effective based on limited laboratory studies. Combining azithromycin with levofloxacin has been reported to be more effective than levofloxacin alone in a very small, inade-

quately controlled study; until this finding is confirmed in larger studies, such use probably should be avoided.[30]

Alternative drugs to erythromycin include tetracycline and possibly co-trimoxazole. Neither of these agents has been approved for this use by the FDA, although limited clinical and experimental data support their effectiveness, more so for tetracycline than for co-trimoxazole. Co-trimoxazole should be used only if there is no possibility of using a macrolide, tetracycline, or fluoroquinolone drug. Doxycycline, because of its high lipid solubility, may be more effective than is tetracycline, although whether it is remains conjecture. The dosage of doxycycline used in children is 2 to 4 mg/kg/day given in one or two doses. Use of tetracyclines is associated with a risk of dental staining in children younger than 8 years old. The daily co-trimoxazole dosage is 15 to 20 mg/kg of the trimethoprim component and 75 to 100 mg/kg of the sulfamethoxazole component in three divided doses. The duration of therapy for these drugs is the same as that for erythromycin.

Rifampin combined with erythromycin is a less favorable alternative to the use of azithromycin alone. However, if erythromycin is a preferred therapy for reasons of cost, toxicity, or availability, rifampin probably should be added in the case of severe disease or in immunocompromised patients. The dosage is 16 to 20 mg/kg/day given in two divided doses for the first 2 days of therapy. This drug rapidly diminishes bacterial counts in experimental disease and has been effective in combination with other drugs in patients in whom erythromycin therapy has failed. Use of this drug for the treatment of legionnaires' disease is not approved by the FDA. A recent small nonrandomized study has shown that adult patients treated with combination rifampin and clarithromycin had significantly longer hospital lengths of stay than did those treated with clarithromycin alone, along with a trend toward a worse outcome in other outcome measures in those given combined therapy. The best correlation with length of stay was the duration of rifampin therapy. Because the greatest effect of rifampin in experimental animals takes place in the first day or two of therapy, giving this drug for more than 2 days may do more harm than good. The best policy would be to use an alternative to erythromycin or clarithromycin in patients with severe illness and thereby avoid the question of combination therapy.[67] No good clinical or laboratory evidence indicates that combining rifampin with either azithromycin or a fluoroquinolone is of clinical benefit.

Treatment of extrapulmonary foci of infection does not appear to differ significantly from treatment of legionnaires' disease without extrapulmonary disease. The duration of therapy and indications for surgical drainage need to be assessed individually in these cases.

RESPONSE TO TREATMENT

Most patients improve dramatically within a few days after initiation of specific therapy.[34,41,47,142] Response may be as rapid as 6 hours after administration of the first dose of erythromycin therapy. Patients regain their appetite, lose symptoms of myalgia and fatigue, and feel better overall. As long as a week may be required for a patient to become completely afebrile, and rarely as long as a month is required for some severely immunosuppressed patients. The chest radiograph changes slowly and even may appear to worsen despite overall clinical improvement; progressive consolidation after 3 to 4 days of intravenous antimicrobial therapy is unusual.

The mortality rate in otherwise healthy adults who are treated promptly is approximately 5 percent, whereas in treated immunosuppressed patients, it is approximately 20 percent. Untreated fatality rates range from 15 to 20 percent in normally healthy patients and upward of 80 percent in immunosuppressed patients. Even in previously healthy patients, delayed therapy and the development of respiratory failure are exceptionally poor prognostic factors.[52,119,144]

DIFFERENTIAL DIAGNOSIS

Other causes of atypical or culture-negative pneumonia may resemble legionnaires' disease closely. *M. pneumoniae* pneumonia usually is a milder illness that does not require hospitalization. Cough is a prominent symptom in mycoplasmal pneumonia, whereas it is not in legionnaires' disease. Neither rash nor otitis is found in legionnaires' disease. Laboratory abnormalities also are seen more commonly in legionnaires' disease. Serologic testing may provide positive results for both diseases, a confusing finding that can be clarified by performing specific laboratory testing. Fortunately, treatment is the same for both diseases.

Psittacosis and Q fever also may resemble legionnaires' disease closely. A history of bird or cattle exposure may be helpful, but its absence does not exclude either of these zoonoses. An interstitial rather than an acinar-filling infiltrate on a chest radiograph would be a point against legionnaires' disease. Pathogen-specific laboratory tests help in this differential diagnosis. A tetracycline can be used successfully for all three of these diseases.

Early in their evolution some diseases, including typhoid fever, acute coccidioidomycosis, influenza, typhus or spotted fever, and leptospirosis, may resemble legionnaires' disease. Distinguishing these diseases on the basis of their clinical evolution, laboratory results, and exposure or travel history generally is easy.

Tularemia may pose a problem in the differential diagnosis because immunologic test results for legionnaires' disease can be falsely positive in patients with tularemia, and some of the growth characteristics of *Francisella tularensis* closely resemble those of *Legionella*. In regions endemic for tularemia, clinicians must work closely with the laboratory to facilitate this differential diagnosis. One case record of tularemia misdiagnosed as legionnaires' disease reported that the patient responded to erythromycin therapy.

Dual infection sometimes occurs in legionnaires' disease. Coexistence of legionnaires' disease with pneumonia caused by *Mycobacterium tuberculosis*, pneumococcus, *H. influenzae*, *Neisseria meningitidis*, *Pneumocystis carinii*, *Moraxella catarrhalis*, and various viral agents has been reported. Thus, dual infection should be suspected in patients not responding to therapy for pneumonia. Pathogen-specific laboratory tests often are useful in these cases.

CLINICAL SYNDROMES CAUSED BY OTHER *LEGIONELLA* SPECIES

Relatively few cases have been reported of disease caused by the non–*L. pneumophila Legionella* spp.[53] The ones described appear to have few differences in clinical findings, diagnostic methods, or treatment. One group contends that these infections are more difficult to treat than are *L. pneumophila* infections, but whether this difficulty represents differences in host factors or reduced susceptibility of the bacteria to antibiotic therapy is unclear.[53] Whether the lower frequency of these infections reflects decreased virulence, inadequate efforts to diagnose them, rare environmental presence, or all three is unknown. The mode of spread and the nosocomial reservoirs of these species are not defined as well as they are for *L. pneumophila*. These infections are less likely to be diagnosed by immunologic means because fewer laboratories routinely test for all possible species. As with *L. pneumophila* and even more so for the non–*L. pneumophila Legionella* spp., treat-

ment sometimes must be based solely on clinical suspicion without the benefit of confirmatory laboratory tests.

PONTIAC FEVER

CLINICAL SIGNS AND SYMPTOMS

Fever, myalgia, malaise, chills, and headache are the most common symptoms of Pontiac fever.[65] The symptoms may begin suddenly or have a more gradual onset lasting several hours. Many symptoms, such as dry nonproductive cough, chest pain, and pharyngitis, are referable to the respiratory tract. Nausea is a common manifestation, but diarrhea and vomiting occur less frequently. Neurologic symptoms, including dizziness, confusion, and poor coordination, also have been reported. Pontiac fever symptoms usually are at their worst within a day after onset of the illness and gradually resolve over a 2- to 7-day period. Physical examination shows only tachycardia and fever. Leukocytosis has been the sole laboratory abnormality reported. Chest radiographs show no abnormalities. Pulmonary function testing has not been performed in patients with Pontiac fever. Rechallenge by return to the contaminated building in the original Pontiac outbreak produced an illness that was mild in comparison to that after the first exposure; the length of time between the first and second exposure was not stated clearly.

SPECIFIC DIAGNOSIS

The diagnosis of Pontiac fever primarily is one of exclusion.[34,142] Significant rises in anti-*Legionella* antibody level, combined with characteristic symptoms, and isolation of *Legionella* from an aerosol source are the diagnostic criteria. Detailed epidemiologic and environmental studies must be performed to diagnose Pontiac fever specifically because of the nonspecificity of the symptoms and the ubiquitous presence of environmental *Legionella*. Establishing a definitive diagnosis in sporadic cases is difficult.

TREATMENT

Antimicrobial therapy does not appear to be effective for the treatment of Pontiac fever. Removal of the patient from the area of the contaminated water source appears to be the best means of management.

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CHAPTER 150

STREPTOBACILLUS MONILIFORMIS (RAT-BITE FEVER)

Carrie L. Byington ❂ Robert D. Basow

Rat-bite fever is an acute febrile illness usually acquired by humans from the bite of a rat or other rodent. It is a zoonosis with worldwide distribution.[109] *Streptobacillus moniliformis* is the leading cause of rat-bite fever in the United States, but in Asia, the illness is caused most often by a spirochete, *Spirillum minus* (see Chapter 155).[46] Infection with *S. moniliformis* is characterized by a relapsing fever, rash, and prominent arthralgia and arthritis. Infection may be acquired after the bite of an animal, by contact of skin or mucous membranes with an infected animal or contaminated cage, or by ingestion of food or water contaminated by rats. When the organism is acquired by ingestion, the resulting illness is termed "Haverhill fever" after an outbreak that occurred in Haverhill, Massachusetts, in 1926.[62,66]

HISTORY

The first description of rat-bite fever is found in the 2500-year-old Indian *Compendium of Medicine*, the *Susruta Sambita*.[22] In this text, a description of the illness is given that remains valid today: "The blood of any part of the human body coming in contact with the semen of rats or scratched with their nails or teeth is vitiated and gives rise to the appearance of nodes, swellings, eruptions of circular erythematous patches on skin, pustules, violent and acute erysipelas, breaking pain in the joints, extreme pain in the body, fever, anemia, aversion to food, shivering, and horripilation." The disease also was known in ancient Japan, where treatment consisted of the local application of herbs and dynamite to cause an "explosion in the wound."[22]

The first modern accounts of the disease are found in a lecture by professor Eli Ives at Yale University in 1831.[35] The first case report was published in 1839.[107] In 1900, Miyake[59] gave a detailed description of the disease, which he named sodoku, from the Japanese *so* (rat) and *doku* (poison). Schottmüller,[88] Blake,[12] Levaditis and colleagues[52] were the first to isolate and describe the causative agent of streptobacillary rat-bite fever in 1914, 1916, and 1925, respectively. The organism has been known as *Streptothrix muris ratti* and *Streptobacillus muris minus* but currently is referred to as *S. moniliformis*.[43]

Haverhill fever was reported first in 1926 after an outbreak of epidemic illness that was traced to contaminated milk.[66] They called the illness *erythema arthriticum epidemicum*. Shortly thereafter, the causative organism was isolated and named *Haverhillia multiformis* and was shown to be identical to *S. moniliformis.*[62]

EPIDEMIOLOGY

Approximately 200 cases of rat-bite fever have been reported in the United States.* In addition, the disease has been reported worldwide.† *S. moniliformis* is responsible for most of the cases seen in North America, whereas the spirillary form is seen more commonly in Asia. Only one case of spirillary rat-bite fever has been reported in the American literature in the past 40 years.[23] Two outbreaks of Haverhill fever are reported in the literature.[56,62]

*See references 3, 4, 7, 15, 18-20, 23, 25, 30, 44, 68, 94.
†See references 6, 8, 9, 11, 16, 29, 41, 49, 50, 56, 84, 87, 89, 96.

Rat-bite fever currently is not a reportable illness in the United States, so its true incidence is unknown. Although it is considered to be a rare occurrence, the illness probably is underdiagnosed. More than 2 million animal bites are reported annually in the United States, and rat bites account for at least 1 percent of them.[31] A review of animal bites in Maryland during a 3-year period found that approximately 4.7 percent were caused by rats or lagomorphs.[26] Among bitten patients, the risk of rat-bite fever developing is significant, with reported rates being between 4 and 11 percent.[74,106] Half of all *S. moniliformis* isolates identified at the Microbial Diseases Laboratory in Berkley, California, between 1970 and 1998 involved children younger than 9 years old.[40] Not surprisingly, children living in crowded urban centers or rural impoverished areas seem to be at greatest risk.[26,44,71,104] Recently, however, reports of rat-bite fever are emerging among individuals who keep rats as pets.[4,8,25,102] Most of the other reported cases involve laboratory personnel who handle rats.[7]

Rat-bite fever usually is transmitted through the bite of a rat, but it also may be transmitted by rat scratches.[19,25] Rat-bite fever likewise has been reported after handling of pet rats and in individuals who dwell in rat-infested homes.[34,83] Rat-bite fever was reported after varicella in a child who handled pet rats frequently while she had open skin lesions,[68] and septic arthritis caused by *S. moniliformis* was reported in a child who kissed pet rats.[42]

Between 10 and 100 percent of rats, both wild and laboratory, carry *S. moniliformis* as normal nasopharyngeal flora and excrete it in their urine.[4,97] The disease also has been transmitted to humans from mice,[72] squirrels,[58] weasels,[27] gerbils,[108] and such rat-eating carnivores as cats,[37,60] dogs,[63,75] and pigs.[105] In addition, *S. moniliformis* can cause disease in turkeys,[61] guinea pigs,[48] koalas,[82] spinifex hopping mice,[45] and nonhuman primates.[101] These animals could pose a potential source of infection for humans.

Haverhill fever is transmitted by the ingestion of food or water contaminated by rats. Previous outbreaks have involved unpasteurized milk, ice cream made from raw milk, and water.[56,66]

BACTERIOLOGY

S. moniliformis is a pleomorphic, microaerophilic, nonmotile, nonencapsulated, non–acid-fast, gram-negative bacillus. It measures 1 to 5 μm in length.[43] The organism is oxidase- and catalase-negative and will ferment glucose, maltose, fructose, galactose, and salicin.[43]

The organism is fastidious and requires special handling for isolation. Optimal growth is achieved in trypticase soy agar or broth supplemented with 20 percent horse or rabbit serum.[62] Alternatively, brain-heart infusion broth supplemented with "Panmede" (a papain digest of ox liver) also has been shown to support the growth of *S. moniliformis*.[93] Sodium polyanethol sulfonate (SPS), which is added to most aerobic blood culture bottles at a concentration of 0.05 percent, will inhibit the growth of *S. moniliformis* at concentrations as low as 0.0125 percent.[92] Blood culture bottles without SPS added should be used for primary isolation of *S. moniliformis* when rat-bite fever is suspected. SPS is not added to anaerobic blood culture bottles, and *S. moniliformis* may be isolated in standard anaerobic culture media. Cultures

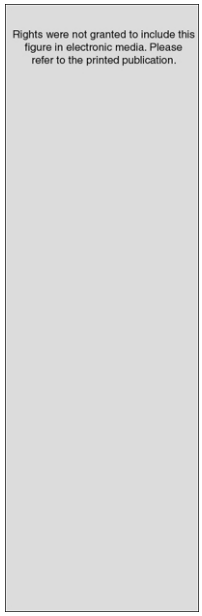

Figure 150–1 The colonies are white, soft "puffballs" 1 to 2 mm in diameter. (See companion Expert Consult web site for color version.) (*From Albedwawi, S., LeBlanc, C., Show, A., et al.: A teenager with fever, rash and arthritis. C. M. A. J. 175:354, 2006.)*

should be incubated at 35° C to 37° C in a humid environment with a partial pressure of carbon dioxide between 8 and 10 percent.

The morphologic characteristics of the bacterium are dependent on the environment.[62] In favorable media, the typical appearance is that of short rods that may grow in chains. In other conditions, the organism tends to grow in long, interwoven filaments that commonly contain beaded and fusiform swellings throughout their length. In broth culture, colonies usually appear in 2 to 10 days.[43] The colonies are white, soft "puffballs" measuring 1 to 2 mm in diameter (Fig. 150–1).[4] On blood agar plates, the colonies are round, gray, and glistening and measure 1 to 2 mm in diameter after 2 to 3 days of incubation.[43]

Stable L-forms of the organism develop spontaneously in vivo or in vitro. These cell wall–deficient forms have a "fried egg" appearance with dark centers and lacy edges when grown on solid media.[43,64] They are resistant to penicillin and other antibiotics active against the bacterial cell wall. L-phase variants may be deposited in tissues and prolong the symptoms of illness.[29]

CLINICAL MANIFESTATIONS

Streptobacillary rat-bite fever typically has an incubation period of less than 7 days, with a reported range of 1 to 22 days.[18] Young children have a predilection for shorter incubation times. The illness is characterized by the acute onset of shaking chills, fever, headache, and vomiting. There is a single case report of rat-bite fever without fever.[96]

At initial evaluation, the bite site usually is well healed, without evidence of inflammation or regional adenopathy. Within the first week of illness, arthralgia or arthritis with or without joint effusion develops in more than 50 percent of patients. The arthritis, usually involving large joints, tends to be migratory, nonsymmetric, and extremely painful.[6,34,44,89] Rat-bite fever has been mistaken for rheumatoid arthritis.[51]

Within 1 to 8 days after the onset of fever, a pink-red maculopapular rash develops in approximately 75 percent of patients and frequently involves the palms and soles (Fig. 150–2).[1,25,57,67] The rash, lasting as long as 3 weeks, may be generalized, petechial, purpuric, or pustular, and approximately 20 percent desquamate. With untreated infection, there may be persistent or recurrent episodes of fever and arthritis.[35,99] The rash generally does not recur. The untreated mortality rate of *S. moniliformis* infection is 10 percent.[18,71,77] The prognosis generally is excellent in patients treated with antibiotics, and the mortality rate is thought to be less than 1.5 percent. However, infection in infants younger than 3 months may be particularly severe or fatal, even with the administration of appropriate antibiotic therapy.[54,57,90]

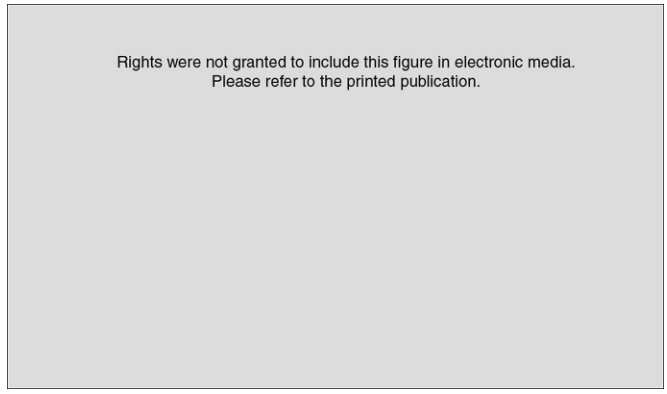

Figure 150–2 Within 1 to 8 days after the onset of fever, a pink-red maculopapular rash develops in approximately 75 percent of patients and frequently involves the palms and soles. (See companion Expert Consult web site for color version.) (*From Albedwawi, S., LeBlanc, C., Show, A., et al.: A teenager with fever, rash and arthritis. C. M. A. J. 175:354, 2006.)*

Haverhill fever is similar to streptobacillary rat-bite fever, with an abrupt onset of fever and chills (100%), rash (95%), and arthritis (97%).[2,57] Generally, the incubation period is 1 to 3 days. Upper respiratory and gastrointestinal complaints are common. Multiple recurrences of fever are found rarely, and the rash tends to be small and uniform in size.[56]

Complications of *S. moniliformis* infection include anemia,[77] cutaneous or subcutaneous abscess formation,[18,41,64,103] interstitial pneumonia,[57,90] mastoiditis,[65] meningitis,[8,90] pericardial effusion,[17] pancreatitis, prostatitis,[109] and septic arthritis.[42,53,80,84] Abscess formation in organs, including the brain,[28] the female genital tract,[64] and the spleen,[21] has been reported. Unusual and potentially devastating complications include chorioamnionitis[33] and periarteritis nodosa.[70] The most frequent serious complication is bacterial endocarditis, which was uniformly fatal in the pre-antibiotic era.[55,73,81,95] Six cases of endocarditis occurring in children younger than 18 years have been reported since 1934.[57,69,78,90,95,98] Five of the six patients died during the acute illness. Four received no antibiotic treatment. Endocarditis caused by *S. moniliformis* has been reported in an adult infected with human immunodeficiency virus.[76] The patient recovered with antibiotic therapy. Fatal endocarditis has been reported in a pet shop employee.[94]

Iron-deficiency anemia is the only complication that has been reported after Haverhill fever.[56] No fatalities caused by Haverhill fever have been reported, and the prognosis is excellent.[56]

PATHOPHYSIOLOGY AND PATHOLOGY

Factors influencing the virulence of *S. moniliformis* are not well described. The organism has an affinity for synovial tissue in both animals and humans, but the mechanisms by which *S. moniliformis* produces arthritis are unknown.[38,39,44,47,53,86,100] Studies performed in mice indicate that *S. moniliformis* is only slightly immunogenic and produces mild leukocytosis and minimal homologous antibody production.[85,86] In addition, the organism is resistant to phagocytic destruction.[85] These factors may allow chronic infection to development.

The pathologic features of streptobacillary rat-bite fever have been described in a limited number of autopsy reports. Common features include ulcerative endocarditis with secondary septic embolization in the liver and spleen, septic arthritis, and interstitial pneumonia.[12,90] Mononuclear meningitis and erythrophagocytosis also have been reported.[90] In most reports, the site of the bite reveals little histologic evidence of inflammation.[57]

DIAGNOSIS

Correct diagnosis of rat-bite fever requires a high index of suspicion on the part of the physician. The diagnosis is suggested in a febrile patient with a history of exposure to rats, but in most clinical settings, the exposure history is not elucidated until after the diagnosis is made.

Nonspecific signs include an elevation in the white blood cell count,[12] usually in the range of 10,000 to 30,000 cells/mm^3 with a left shift, mild anemia, and a false-positive serologic test for syphilis, which may occur in 25 percent of patients.[77] Direct visualization of the organism on Giemsa stain of blood or joint fluid may suggest the diagnosis. Serologic assays are not currently available for humans. An enzyme-linked immunosorbent assay has been developed to monitor infection in rodent colonies.[13] Polymerase chain reaction has been effective for detection of *S. moniliformis* from humans and animals and may offer an alternative to traditional culture methods.[5,10,14,36]

S. moniliformis has been cultured from blood, joint fluid, abscesses, pericardial fluid, meninges, and tissues obtained at autopsy.[17] The organism has strict growth requirements, and the choice of culture media and technique is of critical importance for optimal growth of the bacterium (see the section "Bacteriology"). In general, routine aerobic blood cultures are not satisfactory for isolation.

If the organism is isolated, it may be identified rapidly by gas-liquid chromatography. *S. moniliformis* has a characteristic fatty acid profile, with major peaks being palmitic, linoleic, oleic, and stearic acid.[64,79] Electrophoretic protein patterns also have been described that can distinguish Haverhill fever strains from rat-bite fever strains.[24]

The differential diagnosis for streptobacillary rat-bite fever includes illness caused by *S. minus*, which may be indistinguishable (see Chapter 155). It also includes all relapsing fevers, such as *Borrelia recurrentis*, malaria, and typhoid. Rocky Mountain spotted fever must be considered,[67] as well as other infectious entities, including leptospirosis, Lyme disease, disseminated gonococcal infection, meningococcemia, brucellosis, and syphilis. Viral infections also may be mistaken for rat-bite fever. Acute rheumatic fever should be considered as well. Noninfectious entities include drug reactions, collagen vascular disease, and Pel-Ebstein fever.

TREATMENT AND PREVENTION

In proven cases of streptobacillary rat-bite fever, the treatment of choice is penicillin G. In adults, the dosage of penicillin should be no less than 400,000 to 600,000 IU/day continued for at least 7 days.[77] If no response is seen within 2 days, the dosage should be increased to 1.2 million IU/day. Children have been treated successfully with 20,000 to 50,000 U/kg/day of intramuscular or intravenous penicillin, up to a maximum of 1.2 million IU/day.[91] In children who do not require hospitalization, oral penicillin V, 1 to 2 g/day in divided doses, may be given.[91] In penicillin-allergic adults, both streptomycin and tetracycline have been effective.[67,89] In children, streptomycin and erythromycin have been used, but treatment failures with erythromycin have been reported.[41] Clarithromycin has been used successfully to treat an adult with rat-bite fever.[102] Antibiotics, including cefuroxime, cefotaxime, gentamicin, and ciprofloxacin, have good activity against *S. moniliformis* in vitro.[32] Several reports document successful treatment with cephalosporins, including cefuroxime and ceftriaxone.[19,25,42,76]

Endocarditis secondary to *S. moniliformis* should be treated with high-dose penicillin G in combination with streptomycin or gentamicin. In children, the dosage is 160,000 to 240,000 IU/kg/day, up to the adult maximum of 20 million IU/day.[81,91] Treatment should be continued for at least 4 weeks. Antibiotic susceptibility testing of the organism should be performed, and minimum inhibitory and bactericidal concentrations for several antimicrobial agents should be determined. It is desirable to have a peak serum minimum bactericidal concentration of at least 1:8. Recently, successful treatment of endocarditis with ceftriaxone in combination with gentamicin was reported.[76]

Haverhill fever is treated in much the same way, with penicillin G being the current drug of choice. Most individuals can be treated as outpatients.

Rat-bite fever can be prevented by controlling rodents in urban areas, properly handling rodents, and avoiding unpasteurized milk products. Individuals who own rats and those who work with rodents should receive anticipatory guidance regarding the handling of these animals and the signs and symptoms of rat-bite fever. If a rat bites an individual, prophylactic antibiotics, such as amoxicillin or amoxicillin-clavulanic acid, may prevent rat-bite fever, but current data do not support the routine use of antibiotics after all rat bites. Some researchers would suggest that because of the serious nature of rat-bite fever in young infants, infants younger than 3 months who are bitten by a rat should receive antibiotic prophylaxis.[54]

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CHAPTER 151

BARTONELLA (CAT-SCRATCH DISEASE)

Joseph J. Nania ✪ Kathryn M. Edwards

Cat-scratch disease (CSD) is an acute, self-limited infection that begins as a papule or nodule at the site of a scratch or bite by a cat, followed by regional lymphadenopathy. *Bartonella henselae* is the causative agent of CSD. In a small number of patients, more serious systemic complications, including involvement of the central nervous system, liver, spleen, bone, heart, eyes, or skin, may occur. Although CSD was recognized as a clinical entity in the 1930s, the first written report, by Debre from France, was not published until 1950.

ETIOLOGY

For more than 40 years, numerous attempts were made to isolate the responsible organism from infected nodes in patients with CSD with no success. The first clue to its etiology was recognized in 1983, when Wear and colleagues[35] used Warthin-Starry silver stain to demonstrate small, pleomorphic, gram-negative bacilli in lymph nodes and skin papules from patients with CSD. Initial attempts to culture the bacillus were unsuccessful, but in 1988, an organism was isolated by English and associates[10] from the lymph nodes of patients with CSD and subsequently termed *Afipia felis*. The discovery of bacillary angiomatosis and its association with *B.* (formerly *Rochalimaea*) *henselae* encouraged further investigation of the role of this newly recognized organism in CSD.[28] In 1992, Regnery and coworkers,[27] using indirect fluorescent antibody (IFA) assays, demonstrated elevated antibodies to *B. henselae* in serum samples from patients with suspected CSD. Other researchers used enzyme-linked immunosorbent assays (ELISAs) for each agent to demonstrate that patients with CSD had significant serologic responses to *B. henselae* and *Bartonella quintana* but not to *A. felis*. These data further supported the view that the causative agent of CSD was related antigenically to the *Bartonella* genus and not to *Afipia*.[33] In a physician survey to identify cases of CSD occurring during a 13-month period in cat owners in Connecticut, Zangwill and colleagues[37] demonstrated that of 45 patients with clinical CSD, 38 had antibody titers of 1:64 or higher for *B. henselae* as compared with only 4 of 112 samples from controls ($p < 0.001$). CSD was associated strongly with owning a kitten. Finally, the use of polymerase chain reaction (PCR) helped confirm the causative agent of CSD as *B. henselae*.[2] Eventually, PCR amplification was used to detect *B. henselae* not only in purulent material aspirated from involved nodes but also in CSD skin test material.[1,16,32] Subsequently, *B. henselae* has been cultured from lymph nodes obtained from patients with CSD.[13]

B. henselae is a fastidious gram-negative bacillus that requires an incubation period of up to 5 weeks to culture.[5] It belongs to the genus *Bartonella*, along with other species such as *Bartonella bacilliformis* (the agent of Carrión disease) and *B. quintana* (the agent of trench fever). Colony morphology varies from small, dry, gray-white colonies to smooth, creamy yellow colonies.

TRANSMISSION

Domesticated house cats are healthy carriers of *B. henselae*, and cat fleas (*Ctenocephalides felis*) play a major role in cat-to-cat transmission. Approximately 90 percent of patients with CSD have a history of exposure to cats, particularly a kitten with fleas or a scratch or bite from a kitten. Although they remain asymptomatic, domestic cats serve as persistent reservoirs for *B. henselae*. Reported rates of *B. henselae* seroprevalence in cats vary considerably, with the highest rates seen in cats living in warm, humid climates. In the United States, the southeastern region, Hawaii, and coastal California have the highest *B. henselae* seroprevalence in cats (40-54.7%), whereas rates are much lower (3.7% to 6.7%) in the Rocky Mountain and midwestern regions.[20] Blood samples obtained from pet and impounded cats in the San Francisco Bay region grew *B. henselae* 41 percent of the time. *B. henselae* also was detected in fleas from infected cats by both direct culture and PCR, although no data support cat-to-human transmission via fleas. Interestingly, Chang and colleagues[8] found that *Ixodes pacificus* and *Dermacentor* ticks from five of six California counties were PCR-positive for *B. henselae*. Co-infection with *B. henselae* and *Borrelia burgdorferi* was described in a report of three patients in New Jersey.[11] *Ixodes* ticks obtained from the patients' households were PCR-positive for *B. henselae* in two of the three

cases. These findings should alert clinicians to consider the possibility of *Bartonella* infection in humans after tick bites, although the frequency of transmission by this route is unknown.

EPIDEMIOLOGY

CSD generally affects children and young adults, and, although it occurs worldwide, regional differences in disease rates exist. The Centers for Disease Control and Prevention estimates that more than 24,000 cases of CSD occur annually in the United States. Using a national inpatient database, Reynolds and coworkers[30] estimated that 437 pediatric hospitalizations for CSD (0.60 per 100,000 population) occurred in 2000. Rates were higher in children younger than 5 years and markedly higher in southern states, thus underscoring the regional differences in prevalence of *B. henselae*. Higher rates of disease generally are reported in the autumn, with 60 percent of hospitalizations in Reynolds and associates' study occurring between July and October. Zangwill and colleagues[37] suggested that the seasonality of CSD could be explained by the breeding patterns of cats and fleas, as well as increased contact with kittens, which often are kept indoors during the winter months.

PATHOLOGY

Pathologic examination of the primary inoculation lesion demonstrates dermal necrosis, with variable numbers of histiocytes and occasional multinucleated giant cells accompanied by scattered microabscesses with neutrophils, eosinophils, lymphocytes, and plasma cells. The epidermal changes are nonspecific and consist of parakeratosis, hyperkeratosis, edema, and exocytosis of inflammatory cells. Characteristic findings in the lymph nodes, similar to the primary lesion, are follicular hyperplasia, focal cortical necrosis, and necrotizing granulomata with central microabscesses and palisading histiocytes. A perivascular neutrophilic infiltrate may be present. Subsequently, the lesions progress to small cortical granulomata and "stellate microabscesses" within the granulomata. Warthin-Starry or Steiner silver impregnation stains may reveal pleomorphic bacilli in clusters or short chains within the areas of central necrosis or around small vessels. Bacteria are visualized easily in early lesions. Granulomata with microabscess formation also can be found in other sites such as the liver, spleen, and bone. Because other infections such as tularemia and fungal and mycobacterial infection may have similar histopathologic characteristics, biopsy findings must be correlated with the clinical findings, serologic studies, cultures, and PCR.

In vitro observations have identified important new properties of *B. henselae*. By inducing activation of the nuclear factor NFκB and expression of adhesion molecules, *B. henselae* infects and activates endothelial cells, an important step in the pathogenesis of CSD and bacillary angiomatosis.[8,14] Animal studies have shown that interferon-γ–mediated activation of macrophages is involved in clearing *B. henselae* infection and that microbicidal activity is mediated to a large extent by nitric oxide.[25] The importance of cell-mediated immunity in CSD is suggested by the positive skin reaction to injected material aspirated from cat-scratch nodes and the granulomatous lesions noted in biopsy specimens.

CLINICAL MANIFESTATIONS AND COURSE

The most common clinical manifestation, often referred to as *typical CSD*, is a gradually enlarging regional lymph node occurring 1 to 3 weeks after a scratch or bite from a cat. An inoculation site, which often appears as a red papule 3 to 5 mm in diameter, can be detected in two thirds of patients. Constitutional symptoms of malaise, fatigue, anorexia, emesis, and headache are common but usually are mild. Fever occurs in fewer than half the patients with typical CSD, thus rendering the common name "cat scratch fever" inexact. Physical examination generally reveals a skin papule at the inoculation site and regional lymphadenopathy (Fig. 151–1). In approximately 50 percent of cases, lymphadenopathy is the only manifestation of the disease and occurs most commonly in the upper extremity (axillary or epitrochlear lymph nodes, 46.1%), the head and neck area (cervical or submandibular, 26.1%), and the lower extremities (femoral or inguinal, 17.5%).[7] Supraclavicular lymphadenopathy, which often raises concern for malignant neoplasm, accounts for only approximately 2 percent of typical CSD. Suppuration of the involved lymph nodes occurs in about 10 percent of patients (Fig. 151–2). More unusual manifestations of CSD include neuroretinitis, subacute iritis, optic neuritis, focal chorioretinitis, and the oculoglandular syndrome of Parinaud (Fig. 151–3). Patients with

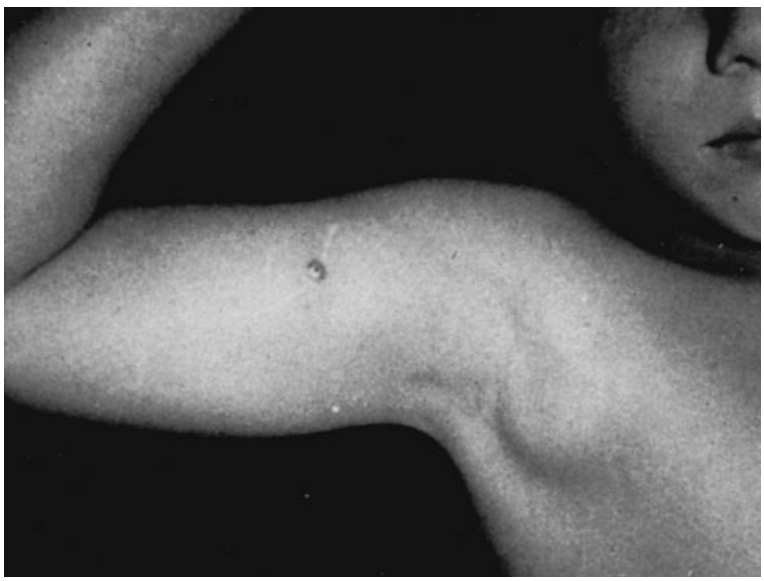

Figure 151–1 Cat-scratch disease involving an axillary lymph node with a primary granuloma of the upper part of the arm. Note that the primary lesion is within the line of a healed scratch. *(Courtesy of Hugh A. Carithers, M.D.)*

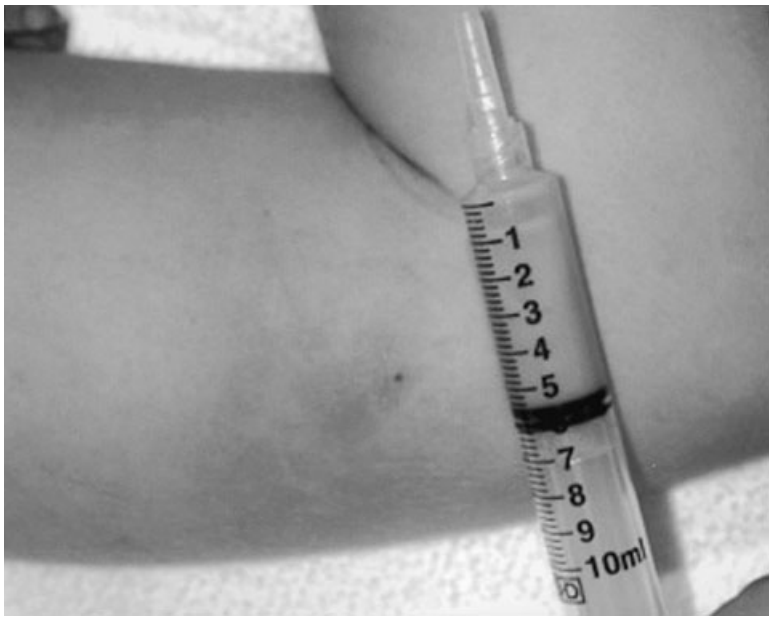

Figure 151–2 Purulent material aspirated from an epitrochlear lymph node of a child with cat-scratch disease. The primary lesion was on the index finger.

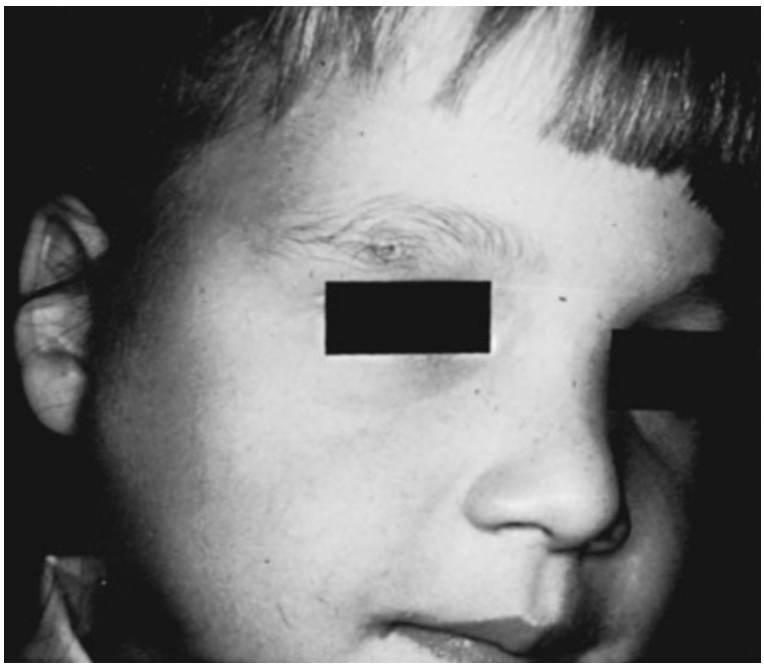

Figure 151–3 Child with the oculoglandular syndrome of Parinaud as a manifestation of cat-scratch disease. Note the parotid swelling and the primary site in the right eyebrow. *(Courtesy of James D. Cherry, M.D.)*

this syndrome have painful conjunctivitis without evidence of drainage and preauricular adenopathy. Most patients recover from Parinaud syndrome spontaneously without residua in 2 to 4 months.

Hepatosplenic CSD is an important cause of prolonged fever in children and should be included in the differential diagnosis of fever of unknown origin. Often these children will have fever for several weeks (mean, 3 weeks), abdominal pain (occurring in about two thirds of patients),[3,9] joint pain, headache, weight loss, and chills. Significant lymphadenopathy is lacking in more than 50 percent of cases, thus rendering the diagnosis more difficult to establish. Hepatic or splenic enlargement (or both) is found in half of these children, but hepatic transaminase levels usually are normal. Erythrocyte sedimentation rates invariably are elevated and frequently are greater than 100 mm/hr. Abdominal imaging usually demonstrates typical granulomatous lesions in the liver or spleen that often are diagnostic of this entity (Fig. 151–4). Although both ultrasound and computed tomography (CT) will show these lesions, a number of reports suggest that CT has lower sensitivity than ultrasound does.[9,21] As is the case with other infections causing hepatosplenic granulomata, late calcification of the lesions has been described.

Neurologic complications have been reported in approximately 2 percent of CSD cases and led to an estimated 51 pediatric hospitalizations in 2000.[30] Encephalopathy, the most common manifestation, typically occurs 1 to 6 weeks after the onset of lymphadenopathy. Fever and seizures develop abruptly, followed by confusion, disorientation, and, occasionally, combativeness. Status epilepticus is well described. Cerebrospinal fluid examination frequently is normal, but occasional mild pleocytosis

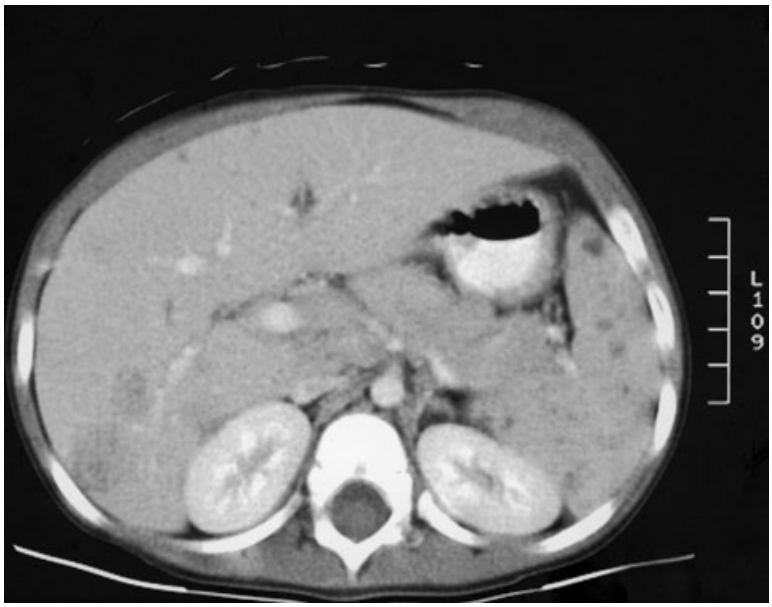

Figure 151–4 Multiple hypodense lesions in the liver and spleen on computed tomography seen in a child with prolonged fever, abdominal pain, and positive *Bartonella henselae* serology.

or elevated protein levels are seen. Electroencephalographic findings frequently are abnormal, with diffuse slowing or focal abnormalities found in most patients. CT of the head generally is normal. Resolution of seizures and normalization of mental status often occur as suddenly as does the onset. Neurologic recovery commonly occurs within weeks and almost always is complete within a year. Persistent deficits or a need for prolonged anticonvulsant therapy has been reported in only a few cases.[17,29]

Osteomyelitis caused by *B. henselae* is well described in the literature, and an early report found that it occurred in 2 of 1200 cases.[7] With improved imaging, serologic methods, and PCR for diagnosis, it is now clear that *B. henselae* osteomyelitis can occur as part of a disseminated infection that involves the liver and spleen, in the setting of otherwise typical CSD, or as a seemingly isolated manifestation in which a primary cutaneous inoculation site and regional lymphadenopathy have not been apparent.[18,34] The pathogenesis is not clear, but it probably represents hematogenous spread of the organism, although local spread from a lymph node or abscess has been reported. The clinical manifestation of osteomyelitis can be acute, but many patients have weeks or months of pain and intermittent fever before recognition of osteomyelitis. A high proportion of reported cases involve the axial skeleton, with the vertebrae and pelvis accounting for nearly 50 percent of cases. Involvement of the long bones of the limbs, the sternum, and the skull and multifocal bony lesions have been described. The most common finding on plain radiography or CT is osteolytic lesions, but bone marrow lesions without cortical destruction, seen as hyperintense foci on T2-weighted magnetic resonance imaging, also have been described.[34] Outcomes of osteomyelitis caused by *B. henselae* generally have been excellent with or without antibiotic therapy or surgical intervention.

Bartonella spp. also have been reported as the cause of endocarditis in more than 100 cases[26] and accounted for 28 percent of culture-negative endocarditis cases in a series from a large referral laboratory in France.[19] In other series, *Bartonella* endocarditis accounted for only 3 percent of all cases. Endocarditis rarely is seen in young children, but cases in several adolescents have been reported. *B. quintana* predominates and accounts for roughly 75 percent, *B. henselae* causes most of the remaining cases, and other species (*Bartonella elizabethae* and *Bartonella vinsonii*) have been isolated in single reports. The epidemiologic characteristics of patients differ considerably between the main two causative species. *B. quintana* endocarditis is highly associated with homelessness, chronic alcoholism, and body lice and is less likely to occur in the setting of cat exposure and underlying valvular heart disease. In contrast, *B. henselae* endocarditis occurs most often in patients with underlying valvulopathy (90%) and clearly is linked to exposure to cats or their fleas.[12] *Bartonella* endocarditis generally is manifested subacutely as fever, heart murmur, and frequently evidence of heart failure. The aortic valve usually is affected; right heart or prosthetic valve involvement is an unusual finding. Valvular surgery is needed in most cases, probably because of frequent delay in diagnosis and treatment. Although the mortality rate in patients with *Bartonella* endocarditis has been reported to be as high as 30 percent, recent series have shown lower rates (7%).[19]

Patients with CSD also may have skin lesions, including maculopapular rash, erythema nodosum, and thrombocytopenic purpura. Other systemic manifestations include hepatitis, splenitis, hemolytic anemia, atypical pneumonia, pulmonary nodules, an infectious mononucleosis–like syndrome, and disseminated bartonellosis. Bacillary angiomatosis (mixed neovascular and inflammatory lesions in the skin or viscera caused by either *B. henselae* or *B. quintana*), bacillary peliosis (dilated capillaries or multiple blood-filled cavernous spaces in the liver, spleen, or lymph nodes), and relapsing fever with bacteremia can develop in immunocompromised subjects.[24]

DIAGNOSIS

Until recently, clinical diagnosis of CSD required the presence of at least three of the following: a history of contact with a cat and the presence of a scratch or a primary lesion, a positive skin test using material obtained from a purulent node from a patient with CSD, regional lymphadenopathy with negative studies for other potential causes of lymphadenopathy, and characteristic histopathologic findings on a biopsy specimen. The difficulty in isolating organisms from routine cultures of lymph nodes or other tissue specimens from patients with suspected CSD has created significant problems over the years. An intradermal skin test composed of heated purulent lymph node material from patients with CSD was used for many years to aid in establishing the diagnosis. Although the skin test was 90 to 98 percent sensi-

tive and specific for the diagnosis of CSD, the material was not readily available, was not standardized, and was not licensed for routine use. In addition, the potential for transmission of other infectious agents with this test currently prohibits its use.

Specific diagnostic tests to assess serum antibodies against *B. henselae* such as IFA and ELISA and PCR amplification to identify *B. henselae* DNA sequences in tissues have reduced the need for performing skin testing and invasive surgical diagnostic procedures. Giladi and colleagues[15] developed a new enzyme immunoassay for IgM and IgG that uses *N*-lauroylsarcosine–insoluble outer-membrane antigens from agar-grown *B. henselae* and demonstrated increased sensitivity of the test. An antibody titer greater than 1 : 64 is suggestive of recent infection, although some patients maintain a titer of this magnitude for a prolonged period after infection. Some authors have suggested that a single IgG titer of greater than 1 : 256 can reliably diagnose acute infection.[24] In the setting of endocarditis, a single IgG titer of 1 : 800 or higher has a 95.5 percent predictive value for *Bartonella* as the etiology.[19] For any manifestation of CSD, detection of a significant rise in titer between acute and convalescent sera is confirmatory. Cross-reaction between antibodies directed toward *B. henselae*, *B. quintana*, and *Chlamydia* spp. can render interpreting serology difficult in some circumstances. However, the cross-reactivity can be overcome by cross-adsorption and Western immunoblotting.[19]

When tissue is obtained for diagnostic purposes, a Warthin-Starry stain and culture (ideally plated on both chocolate and rabbit blood agar and co-cultivated with eukaryotic cells) may be performed, although neither has high sensitivity. Very sensitive species- and strain-specific assays involving PCR amplification of the 16S-23S intergenic spacer regions or the highly variable regions of the 16S ribosomal RNA gene sequence of *Bartonella* can be useful in establishing a diagnosis when purulent lymph node aspirates or other infected tissue is obtained. Because PCR of blood rarely is positive, PCR of valvular tissue is the most sensitive method to diagnose endocarditis.

The differential diagnosis of CSD includes various infections such as other forms of bacterial adenitis, typical or atypical mycobacterial infections, lymphogranuloma venereum, infectious mononucleosis, tularemia, plague, sporotrichosis, blastomycosis, histoplasmosis, syphilis, human immunodeficiency virus infection, neoplasm, and sarcoidosis.

TREATMENT

In an immunocompetent host, typical CSD is self-limited and resolves spontaneously in 1 to 2 months without antibiotics. However, conflicting data exist in the literature regarding whether antibiotic therapy is ever helpful in normal hosts. Macrolides, fluoroquinolones, trimethoprim-sulfamethoxazole (TMP-SMX), doxycycline, and β-lactam agents all have in vitro activity against the organism, but gentamicin and rifampin appear to be the only bactericidal agents. Because in vitro susceptibility testing is technically challenging, lacks standards for interpretation of results, and produces minimum inhibitory concentrations that have correlated poorly with clinical efficacy, it often is not useful clinically.[31] In Margileth's[23] retrospective review of 202 patients treated with antibiotics for CSD, rifampin was more effective than ciprofloxacin, gentamicin, or TMP-SMX. Another prospective, randomized, double-blind, placebo-controlled study using oral azithromycin for 5 days was effective in reducing lymph node volume measured by three-dimensional ultrasonography.[4] Adults and children weighing more than 100 lb were given an initial single dose of 500 mg azithromycin on day 1 of treatment and 250 mg on days 2 to 5 as single daily doses. Patients weighing less received the liquid preparation of 10 mg/kg on day 1 and 5 mg/kg on days 2 to 5.

No randomized, prospective, controlled studies have evaluated therapy for the systemic manifestations of CSD. In one retrospective review, children with hepatosplenic CSD who were given rifampin (20 mg/kg/day in two doses for 14 days), either alone or in combination with another agent, experienced a decreased duration of fever.[3] In the same retrospective review, gentamicin or TMP-SMX also led to defervescence within 5 days, but penicillins, cephalosporins, tetracycline, and erythromycin had minimal or no clinical efficacy. Recently, evidence-based treatment recommendations for *Bartonella* infections have been published, and no recommendations for typical CSD, hepatosplenic CSD, or osteomyelitis were presented.[31] For endocarditis, combination therapy with gentamicin (for 14 days) and either doxycycline or ceftriaxone (for 6 weeks) is recommended. Suggested therapy for central nervous system disease includes doxycycline and rifampin; however, no data suggest that antibiotic treatment of CSD encephalopathy is beneficial. Reports of the duration of antibiotic therapy have varied from 5 days in immunocompetent patients to 6 weeks in patients with atypical CSD or immunocompromised patients.

Corticosteroid therapy has been reported in a small number of cases, including patients with typical CSD, retinal involvement, hepatosplenic disease, or encephalopathy, as well as in a single immunocompromised patient with fever and diffuse lymphadenopathy.[6,7,22,36] Because the pathogenesis of CSD with prolonged symptoms may include a postinfectious, inflammatory process,[6,31] corticosteroids appear to have some theoretical role. Despite the reported anecdotal successes, however, steroids are not recommended for routine treatment of any manifestation of CSD.

Application of moist soaks and local heat, use of analgesics, limitation of activity of the affected limb, and aspiration of fluctuant material in the nodes (in some instances, done serially) may relieve the pain and resolve the inflammation. Excisional biopsy of chronically involved lymph nodes also is performed occasionally to rule out other treatable causes.

PROGNOSIS AND PREVENTION

Typically, the prognosis of CSD is excellent without any sequelae. Although patients with atypical findings and involvement of other organs may have a prolonged course and antibiotics may be used, the prognosis is good. Reinfection occurs only rarely. Because person-to-person transmission of *B. henselae* has not been shown to occur, hospitalized patients need only standard precautions. Currently, no vaccine is available for use in animals or humans. Routine veterinary visits, control of flea and tick infestations, and declawing of young kittens are the current practical preventive measures for CSD.

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SUBSECTION 6

Treponemataceae

CHAPTER 152

BORRELIA (RELAPSING FEVER)

Kenneth M. Boyer

Relapsing fever is a vector-borne bacterial infection characterized by recurring febrile attacks separated by periods of relative well-being. Spirochetes of the genus *Borrelia* cause the disease. It may be transmitted by lice (epidemic, or louse-borne, relapsing fever) or ticks (endemic, or tick-borne, relapsing fever).

Relapsing fever was differentiated from other intermittently febrile conditions in 1868 by Obermeir,[9] who noted the presence of "myriads of living and actively motile spirilla in the blood of relapsing fever patients during the febrile attack." At the turn of the century, Mackie in India and Nicolle in Tunisia elucidated transmission of epidemic disease by the human body louse. Concurrently, in equatorial Africa, Dutton and Todd discovered that sporadic cases could be transmitted by argasid (soft) ticks.

Massive outbreaks of louse-borne relapsing fever accompanied the social and hygienic disruption in Europe and North Africa that occurred in the wake of both World Wars. At present, louse-borne disease is reported in appreciable numbers only from Ethiopia and Sudan. Tick-borne relapsing fever persists as an uncommon but widely dispersed infection in many countries, including the western portion of the United States.[23,25]

THE ORGANISM

The borreliae that cause relapsing fever differ from other pathogenic spirochetes (such as *Leptospira* and *Treponema*) in their loose-coiled morphology and ready staining by Wright or Giemsa stain. These two characteristics permit the organisms to be identified in blood smears, a diagnostic characteristic that is unique among bacterial diseases (Fig. 152–1). *Borrelia burgdorferi*, the

cause of Lyme disease, is not seen in peripheral smears, an important point in the differential diagnosis.

Borrelia microorganisms are extremely fastidious in cultivation, and taxonomic classification has depended on the specificity of strain-vector relationships.[28,67] *Borrelia recurrentis* is transmitted by the human body louse (*Pediculus humanus humanus*). The other species that cause relapsing fever are transmitted by argasid ticks of the genus *Ornithodoros* (Table 152–1).

Propagation of *B. recurrentis*, *Borrelia crocidurae*, *Borrelia hispanica*, *Borrelia hermsii*, and *Borrelia turicatae* in complex artificial media has been accomplished.[38] In common with other pathogenic spirochetes, borreliae require *N*-acetylglucosamine and long-chain fatty acids for growth. They are microaerophilic and metabolize glucose by glycolysis, with resulting accumulation of lactic acid. Further study of metabolism and DNA homology eventually may simplify their taxonomy. The guanine and cytosine content of borrelial genomic DNA is an exceptionally low 27 to 32 percent.[3] *B. burgdorferi* has 30 to 44 percent DNA homology with *B. hermsii*.[35] It clearly is a distinct species and perhaps a distinct genus. The 86 percent DNA homology between the two North American relapsing fever spirochetes *B. turicatae* and *B. hermsii*, however, suggests a close taxonomic and evolutionary relationship.[3] Analysis of DNA sequences, protein profiles, and reactivity with specific monoclonal antibodies now permits definitive speciation of isolates that have been recovered in culture.[1,64]

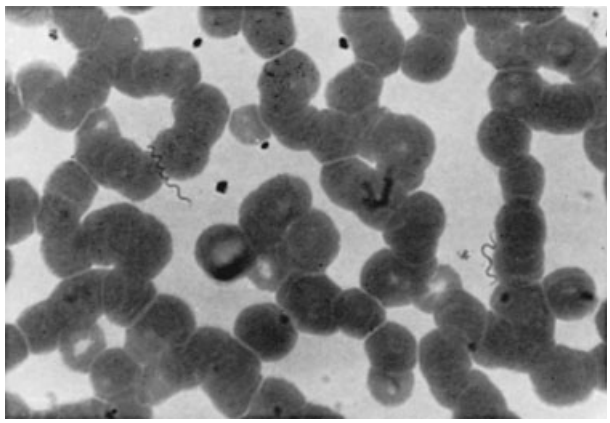

Figure 152–1 *Borrelia hermsii* spirochetes in the peripheral blood smear of a 20-year-old patient with tick-borne relapsing fever (Wright stain, ×1000). *(From Boyer, K. M., Munford, R. S., Maupin, G. O., et al.: Tick-borne relapsing fever: An interstate outbreak originating at Grand Canyon National Park. Am. J. Epidemiol.* 105*:469-479, 1977.)*

TRANSMISSION

Body lice that transmit relapsing fever become infected by feeding on a spirochetemic human. Ingested spirochetes enter the hemolymph through the gut epithelium and multiply there. Except for the central ganglion, other tissues such as the salivary glands and genital organs are not invaded. Once infected, lice remain so for their entire life but do not transmit the organism to their progeny. Transmission to other humans takes place by contamination of the bite wound with infectious hemolymph or feces when lice are crushed or wounded by scratching. Intermediate hosts for *B. recurrentis* other than humans are not known.[29]

The development of borreliae in *Ornithodoros* ticks differs from that in body lice. After the tick engorges on an infected host, spirochetes invade all tissues, including the salivary glands and, in most instances, the female genital tract. The latter phenomenon results in transovarial infection of progeny, an important survival mechanism. Once infected, ticks remain capable of transmitting infection for years via secretions of infectious saliva or coxal (analogous to urine) fluid. *Ornithodoros* ticks typically are night feeders and take blood meals lasting an average of 15 minutes, after which they detach themselves. Their bites usually are painless and are noted infrequently. With the exception of *Ornithodoros moubata*, ticks that transmit relapsing fever are maintained in nature by intermediate hosts. Rodents and other small mammals are the major reservoirs of both ticks and tick-borne borreliae.[14,28,29,68]

EPIDEMIOLOGY

The epidemiology of relapsing fever is determined by the habits of its vectors. Acquisition of louse-borne relapsing fever occurs under conditions of crowding, cold weather, and poor hygiene, which favor the spread of lice. Thus, epidemics have occurred in the setting of wars, earthquakes, famines, and floods, often in association with epidemic typhus. In Addis Ababa, Ethiopia, where an estimated 1000 cases of louse-borne relapsing fever occur each year, nomadic tribesmen, seasonal laborers, and their families, living in conditions of extreme poverty, are at risk.[12,50]

Most foci of tick-borne relapsing fever exist in nature in rather closed environments, such as rodent burrows, nests, or caves. In Africa, Asia, and South America, primitive dwellings with dirt floors, thatched roofs, and rodent nests often harbor endemic species of *Ornithodoros*. In more sophisticated societies, people become part of these ecologic systems only when "roughing it." In the western portion of the United States, for example, where *Ornithodoros hermsi* transmits most cases, a history of sleeping in

TABLE 152–1 Important Borreliae Causing Relapsing Fever, Their Vectors, and Their Current Geographic Distribution

Borrelia Species	Vector	Geographic Distribution
B. recurrentis	*Pediculus humanus humanus*	Ethiopia, Sudan
B. caucasica	*Ornithodoros verrucosus*	Iraq, southwestern former U.S.S.R.
B. crocidurae	*Ornithodoros erraticus* (small variant)	North Africa, Middle East
B. duttonii	*Ornithodoros moubata*	Eastern and Central Africa
B. hermsii	*Ornithodoros hermsi*	Western United States and Canada
B. hispanica	*Ornithodoros erraticus* (large variant)	Spain, western North Africa
B. latyschewii	*Ornithodoros tartakowskyi*	Iran, central Asia
B. mazzottii	*Ornithodoros talaje*	Mexico, Central America
B. parkeri	*Ornithodoros parkeri*	Western United States
B. persica	*Ornithodoros tholozani*	Middle East, western Asia
B. turicatae	*Ornithodoros turicata*	Southwestern United States, Mexico
B. venezuelensis	*Ornithodoros rudis*	Northern South America

Data from references 5, 13, 14, 26.

old summer cabins in forested mountain areas is the rule.[26,34,63,68] In well-studied outbreaks occurring at Browne Mountain, Spokane County, Washington,[62] Big Bear Lake, San Bernardino County, California,[19] North Rim, Grand Canyon National Park, Arizona,[10,20] and northern New Mexico,[21] cabins that were the sources of cases were found to contain large rodent nests from which infective ticks were recovered.

PATHOGENESIS AND PATHOLOGY

Borreliae undergo spontaneous antigenic variation both in vivo and in vitro.[53,60] Repeated episodes of dense spirochetemia (10^5 to 10^8 organisms/mL), each involving a different antigenic variant, account for the cyclic nature of relapsing fever in infected humans.[4,41,56] In relapsing fever *Borrelia* strains, the immunodominant surface protein is called variable major protein (VMP). In serial passage in mice, the progeny of a single organism can give rise to as many as 40 antigenically distinctive VMPs.[2,37,53] Different VMPs have some sequence homology but generally differ by considerably more than a single point mutation.[6,49] The VMP genes of *B. hermsii* are located on multiple copies of linear plasmids. Antigen variation is conferred by programmed, sequential recombination events in plasmid DNA analogous to those seen in African trypanosomiasis and falciparum malaria parasites.[3,5,39,45,49,53] With each remission of the disease, the antibodies produced against the variant strain result in immobilization, opsonization, and agglutination.[16,18,41] Agglutinated organisms are phagocytosed and cleared from the circulation. Experimental animal studies indicate that during remissions, borreliae persist in the central nervous system (CNS), bone marrow, spleen, and liver.[29]

An additional virulence factor that probably contributes to overcoming innate immunity to *B. hermsii* is its production of a surface protein that binds factor H, an important regulator of complement activation.[32]

Major pathologic findings in fatal cases include widespread petechial hemorrhaging of visceral surfaces, splenomegaly and hepatomegaly (often with multiple necrotic foci), and diffuse histiocytic interstitial myocarditis.[29,36] Other features in patients with a fatal outcome include intercurrent infections such as pneumonia, salmonellosis, or reactivated malaria; hemorrhages in the CNS; meningitis; disseminated intravascular coagulation; splenic rupture; hepatic coma; and cardiac arrhythmias.[29,36] Borreliae cross the placenta, and infection during pregnancy results in abortion or severe infection of live-born neonates.[30,59]

CLINICAL MANIFESTATIONS

After an incubation period of 5 to 11 days, relapsing fever has a sudden onset with high fever (39° C to 41° C [102.2° F to 105.8° F]), chills, headache, and myalgia.[25] An initial illness of 3 to 6 days' duration will be followed by approximately a week during which the patient is afebrile and feels weak but improved. Relapse occurs with "flulike" symptoms similar to those of the initial episode. As many as 10 febrile attacks have been recorded in untreated tick-borne cases, whereas 4 episodes is the usual maximum in louse-borne disease. Resolution of the febrile attacks, either spontaneously or after the administration of antibiotics, is by crisis. Relapses become progressively shorter and milder as the afebrile intervals lengthen (Fig. 152–2).[11]

Other clinical features are inconstant and reflect the nature of the infecting organism and the condition of the host. *B. recurrentis* and *B. duttonii* infections are uniformly severe; infections by other species tend to be somewhat milder. Splenomegaly and hepatomegaly, often with associated tenderness, are characteristic. A fleeting macular rash on the trunk, which may become generalized or petechial, is a common finding. Meningeal irritation, iridocyclitis, epistaxis, and myocarditis are more variable in their incidence but may be prominent features.[36,58] Unlike Lyme disease or tularemia, inflammation at the site of inoculation and regional lymphadenopathy are not seen. Thrombocytopenia, hyperbilirubinemia, and elevated liver enzymes are frequent laboratory abnormalities.

DIAGNOSIS AND DIFFERENTIAL DIAGNOSIS

Although an appropriate history of exposure is by far the most helpful clue to the diagnosis of relapsing fever,[30,64] physicians caring for patients who have vacationed in distant endemic areas may not consider the possibility. Often, an alert hematology technician first makes the diagnosis by recognizing loosely coiled spirochetes in a Wright-stained smear of the patient's peripheral blood. Although routine smears usually are positive while the

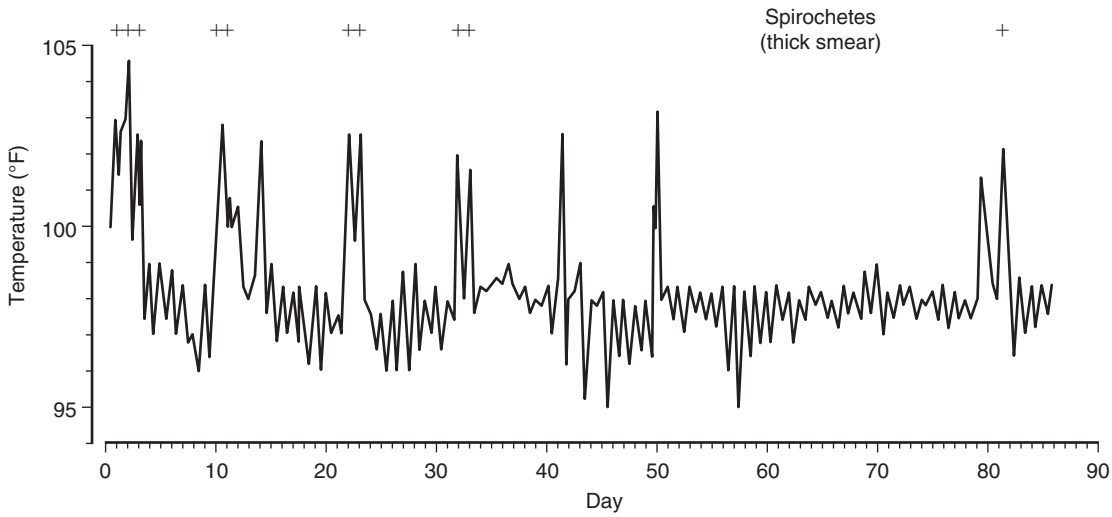

Figure 152–2 Febrile course of untreated tick-borne relapsing fever caused by *Borrelia duttonii*. Thick blood smears were examined for spirochetes on each day of illness, with positive results indicated by *plus signs*. *(Adapted from Breinl, A., Dutton, J. E., Kinghorn, A., et al.: An experimental study of the parasite of the African tick fever. Memoir XXI of the Liverpool School of Tropical Medicine. London, Williams & Norgate, 1906.)*

patient has fever, increased sensitivity is obtained by examination of dehemoglobinized thick smears or buffy coat preparations stained with Giemsa[31] or acridine orange.[57] Species-specific fluoresceinated monoclonal antibodies can enhance detection of borreliae in blood smears even further.[55] Immature laboratory mice, in which spirochetemia readily develops after intraperitoneal inoculation with infected blood, provide the most sensitive system for establishing a specific diagnosis during late relapses or remission periods.[28,67]

Because of the fastidiousness of *Borrelia*, routine blood cultures are helpful only in excluding other causes of bacteremia. A gene-specific polymerase chain reaction (PCR) developed for *B. burgdorferi*, with DNA sequence analysis of the amplicons, has been used to diagnose infection with *B. hermsii* associated with a well-documented family epidemic in Montana.[64]

Serologic diagnosis now can be achieved with indirect fluorescent antibody tests and confirmatory immunoblotting,[23] although these tests are limited by antigenic variability. False-positive results can occur in patients with Lyme disease and other spirochetal infections.[37] Conversely, enzyme-linked immunosorbent assays (ELISAs) and immunoblot serologic tests for Lyme disease may yield false-positive results in patients with relapsing fever.[23,52]

Because of its nonspecific initial symptoms and spontaneous remissions, relapsing fever may be misdiagnosed as influenza or enteroviral infection. The "saddleback" fever pattern of Colorado tick fever may resemble relapsing fever, but this condition can be recognized by its characteristic leukopenia. Other tick-transmitted illnesses, such as Lyme borreliosis, tularemia, Rocky Mountain spotted fever, and human ehrlichiosis, may be suggested by a history of exposure to ticks. In developing countries, malaria, typhoid fever, and rickettsial diseases may show similar clinical findings and are important to differentiate. The microgametocytes of *Plasmodium vivax* may look similar to borreliae in a peripheral blood smear.[24] The periodic fevers, such as familial Mediterranean fever, hyper-IgD syndrome, and the "PFAPA" syndrome (periodic fever, aphthous stomatitis, pharyngitis, and adenitis) may resemble relapsing fever but generally have longer intervals between febrile episodes.[42,46] Empiric treatment with broad-spectrum antibiotics may modify the characteristic pattern of relapsing fever or cure it before a diagnosis is made.

TREATMENT

Because of difficulties in cultivation, no in vitro data are available to compare the efficacy of antimicrobial agents against relapsing fever borreliae. Oral or parenteral tetracycline, erythromycin, and chloramphenicol are effective clinically, whereas intramuscular procaine penicillin G and oral ampicillin result in relapse rates of approximately 5 and 30 percent, respectively. Probably, ceftriaxone and amoxicillin also are effective drugs, as indicated by anecdotal experience[34] and by analogy to their efficacy in Lyme disease. The newer macrolides clarithromycin and azithromycin may have efficacy as well. Doxycycline (100 mg orally), tetracycline (250 mg intravenously or 500 mg orally), and erythromycin (250 mg intravenously or 500 mg orally) have been used successfully as single-dose regimens for the treatment of adults with louse-borne relapsing fever in Ethiopia.[17,41,42,47] Such regimens have the advantages of low cost and at least partial efficacy against other louse-borne diseases such as typhus. Prophylaxis with a single dose of doxycycline has been shown to be effective in preventing tick-borne relapsing fever in exposed military personnel in Israel.[33]

Erythromycin is considered the drug of choice for children younger than 8 years old, whereas tetracycline is considered the drug of choice for older patients. For a febrile patient, however, oral phenoxymethyl penicillin (a single low dose of 7.5 mg/kg) or intravenous penicillin G (10,000 U/kg infused over a period of 30 minutes) is recommended as initial therapy. Either should lead to gradual clearance of circulating spirochetes and defervescence. Thereafter, a 10-day course of oral erythromycin or tetracycline (40 mg/kg/day of either drug divided every 6 hours) will eradicate tissue spirochetes and prevent relapse.[48] For an afebrile child between relapses, erythromycin or tetracycline may be given alone without initial penicillin therapy.

Other than the choice of antimicrobial therapy, the major concern in treating relapsing fever is the frequent occurrence of the Jarisch-Herxheimer reaction in the first hours after therapy has been initiated. This response, an exaggeration of the crisis that normally terminates febrile attacks, is characterized by rigors and hyperthermia followed by drenching sweats, hypotension, and prostration.[12] It may be fatal in louse-borne disease but generally is less severe in tick-borne cases. However, recent reports of acute respiratory distress syndrome (ARDS) with multiple organ dysfunction occurring after treatment of tick-borne relapsing fever in Nevada, California, and Washington emphasize this potential risk.[22] Rapid clearance of spirochetes in the bloodstream initiates the process.[12,17,61,66] Release of inflammatory mediators, such as tumor necrosis factor, interleukin-6, and interleukin-8, after bacterial lysis or phagocytosis probably is the major pathophysiologic mechanism.[7,15,16,18,44,51] Purified VMPs are potent triggers of the production of tumor necrosis factor by human monocytes.[65]

Three approaches to controlling this response have been tried—supportive measures, gradual killing of spirochetes, and pharmacologic blockade. The usual supportive measures involve volume expansion and antipyretics. Penicillins result in slower elimination of circulating borreliae and yield a more prolonged but less severe reaction than tetracycline or erythromycin does.[54,66] Steroids (e.g., hydrocortisone) and pure opioid antagonists (e.g., naloxone) do not block the reaction. The opioid antagonist and partial agonist meptazinol (available in Great Britain; not licensed in the United States) effectively blocks the reaction.[61] Fekade and colleagues[27] demonstrated that pretreatment with sheep anti–tumor necrosis factor-α Fab antibody fragments suppresses Jarisch-Herxheimer reactions and reduces the associated increases in plasma concentrations of interleukin-6 and interleukin-8.

At present, the most reasonable approach to limiting Jarisch-Herxheimer reactions is to provide close supervision (either in the hospital or in an office or emergency treatment room) during the first 12 hours after therapy has been initiated. During this period, intravenous access should be in place. The use of oral penicillin as initial therapy is recommended. Positioning, volume expansion, sponging, and antipyretics should be used to control changes in blood pressure, pulse, and temperature. More aggressive measures may be necessary for full-blown reactions.

PROGNOSIS

With current therapy, case-fatality rates from relapsing fever are less than 5 percent.[51] Without treatment, louse-borne disease, in particular, carries a much higher risk of fatality. Fatal cases of tick-borne disease in North America are rare occurrences. Late relapses may occur, particularly in patients with an incompletely treated CNS "sanctuary."[43] In untreated cases, immunity persists for several years. In treated cases, the duration of immunity is unknown.

PREVENTION

Louse-borne relapsing fever is internationally notifiable to the World Health Organization. Tick-borne relapsing fever is

reportable to state health authorities in 11 western states.[21] Its occurrence in national parks and other public recreational settings renders immediate reporting desirable.[10,20]

Prevention of relapsing fever is largely a problem of avoiding its vectors. In outbreaks of louse-borne disease, time-honored measures such as environmental dusting with insecticide, cutting hair short, laundering at 49° C (120° F), and applying residual insecticides to clothing and bedding have been effective.[8] In individual cases of louse-borne disease, eradication of pediculosis with 5 percent permethrin or 1 percent lindane is an essential adjunct to specific therapy.

In endemic foci of tick-borne disease, the habits of *Ornithodoros* and humans determine the environmental and personal measures necessary.[10,19,20] In the United States, increasing use of wilderness recreational areas by the public calls for increased awareness of relapsing fever and its potential transmission. Dwellings in endemic areas should be constructed with "rodent-proof" foundations and soffits. In unsatisfactory buildings, removal of rodent nesting material and liberal spraying of walls, floors, ceilings, and crawl spaces with 1.1 percent *o*-isopropoxyphenyl *N*-methylcarbamate (Baygon) or a similar residual insecticide are proven preventive measures.[10] The use of insect repellents such as DEET has been recommended in some instances, but their effectiveness is not established.

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CHAPTER 153

Borrelia (Lyme Disease)

Barbara W. Stechenberg

Lyme disease, recognized initially in 1975, was brought to medical attention first through the concern of two mothers from Lyme, Connecticut; one contacted the Connecticut State Health Department, and the other contacted physicians at Yale about the unusual illness spreading through their community, a small town approximately 15 km north of Long Island Sound near the mouth of the Connecticut River. Their inquiries sparked an intensive clinical and epidemiologic investigation that has yielded much of the information about this disorder—its wide spectrum of clinical manifestations, etiology, pathogenesis, and treatment.

From these investigations, a distinct pattern of signs and symptoms emerged as a newly described disorder. Actually, the characteristic skin lesions soon were recognized as erythema (chronicum) migrans, a skin lesion that had been associated with a similar but more limited illness in Europe since the early 1920s.

THE ORGANISM

Because of certain epidemiologic characteristics, particularly the geographic and seasonal clustering of cases, an infectious etiology was sought, particularly one associated with an arthropod or other vector. Early speculation that Lyme disease might be caused by a virus diminished with the report of rapid resolution of erythema migrans and other symptoms if early treatment with penicillin or tetracycline was initiated.[103]

In 1982, Burgdorfer and associates[21] isolated a *Treponema*-like spirochete from the midgut of the tick *Ixodes scapularis* (also called *Ixodes dammini*). The spirochete has irregular coils ranging in size from 10 to 30 μm in length and 0.18 to 0.25 μm in diameter. Electron microscopy demonstrates their close association with the microvillar brush border of intestinal epithelium.

When infected *I. scapularis* ticks were allowed to feed on New Zealand white rabbits, no immediate adverse effect occurred. However, 10 to 12 weeks after tick engorgement occurred, small skin lesions developed that progressed into typical erythema migrans. Indirect immunofluorescence revealed that the sera of all exposed rabbits yielded high titers of antibody to the spirochetes. Sera from nine patients with clinically diagnosed Lyme disease also demonstrated positive reactions.[21]

The etiologic role of these spirochetes has been defined further. Steere and associates[115] isolated the spirochete from the blood, skin, and cerebrospinal fluid (CSF) of 3 of 56 patients with Lyme disease and from 21 of 110 *I. scapularis* ticks studied. More than 90 percent of the patients had a characteristic immunoglobulin M (IgM) and IgG antibody response; IgM antibody titers reached a peak between the third and sixth weeks after onset, and IgG antibodies rose slowly to reach a peak when arthritis was present. Benach and associates[16] isolated the same spirochete from 2 of 36 patients with Lyme disease in New York. These patients also had a similar rise in antispirochetal antibodies. Berger and his group[19] demonstrated the spirochete in 6 of 14 patients in whom the cutaneous lesions of erythema migrans were studied; four of the six positive specimens were obtained from the peripheries of lesions. Berger and colleagues[19] were able to demonstrate the organisms in secondary lesions.

Though morphologically similar to known pathogenic *Treponema* organisms, the spirochete found in the *I. scapularis* tick is distinctive because it grows on artificial media. Growth of this fastidious, microaerophilic organism occurs best at 33° C in a complex liquid medium called Barbour-Stoenner-Kelly medium.[11] The organism has an apparent slime layer, an outer membrane that is associated loosely with the underlying structures, flagella (7 to 11), a cell wall, and cytoplasmic constituents.[115] It is coiled more loosely and is longer than other spirochetes. It resembles

borreliae most closely and has been designated *Borrelia burgdorferi*.

B. burgdorferi contains many different proteins. Six outer-membrane proteins that act as surface antigens have been identified: outer-surface protein A (OspA, 30 to 32 kd), outer-surface protein B (OspB, 34 kd), outer-surface protein C (OspC, 22 to 25 kd), outer-surface protein D (OspD, 28 to 30 kd), outer-surface protein E (OspE, 19 kd), and outer-surface protein F (OspF, 26 kd). Other polypeptides include the 41-kd flagellar antigen, several heat shock proteins, and a 93-kd antigen that is part of the protoplasmic cylinder. A 49-kilobase linear plasmid contains genes that encode for the two major outer-surface proteins OspA and OspB.[13] In fact, all isolates of *B. burgdorferi* examined have had four to nine pieces of extrachromosomal plasmid DNA, both supercoiled and linear.[12] The complete genome of *B. burgdorferi* (strain B 31) has been sequenced and was found to consist of approximately 1.5 megabases.[45] The only known virulence factors of *B. burgdorferi* are the surface proteins that allow attachment to mammalian cells.

Strain differences in DNA and plasmid composition,[70] ultrastructure, and outer-surface proteins among American and European strains have been identified; the European strains generally are more diverse. Three genomic groups of the *B. burgdorferi* sensu lato complex have been identified with several methods. To date, all North American strains have belonged to group *B. burgdorferi* sensu stricto. All three groups have been found in Europe, but group 2, *Borrelia garinii*, and group 3, *Borrelia afzelii*, occur more commonly.[10,22] These differences may account for the variability in clinical expression.

EPIDEMIOLOGY

Evidence for a tick vector had accumulated early in the investigations. During 1977, the incidence of Lyme disease was 30 times greater on the eastern bank of the Connecticut River than on the western shore.[110,125] Case-control studies of patients, mainly children and young adults and their neighbors, revealed that patients did not participate in more outdoor activities but were more likely to have a cat or farm animal, a pet with ticks, or a tick bite in the year of the study. Ixodid ticks were identified as the probable vector. These ticks have a complex life cycle that spans 2 years, during which they feed once during each of their three stages. In the United States, the white-footed mouse is the preferred host for both the larval and nymph stages. The larvae feed in late summer, whereas the nymphs feed in spring or early summer. Of importance is that this animal is the host for both of these stages and that it is tolerant of infection. The host-seeking behavior of the nymphs in late May is what initiates the Lyme disease season each year. Although the prevalence of spirochetes in this stage (20-25%) is approximately half that found in the adult, nymphs are responsible for nearly 90 percent of Lyme disease cases,[44] which may be related to their smaller size, their greater abundance, and the coincidence of their peak feeding activity with human outdoor activity. The white-tailed deer is the preferred host for the adult stages and acts as the reservoir during the winter months. The adult tick feeds in the fall or winter.

Since the association of the *Ixodes* genus of ticks with a large number of cases was reported from the Lyme, Connecticut, area, Lyme disease has been identified in at least 46 states and now is the most common vector-borne disease in the United States. More than 15,000 cases have been reported each year, a 25-fold increase since national surveillance began in 1982; however, statistics from 2003 and 2004 have shown a decrease in reported cases.[26] The major geographic areas with clusters of cases include the eastern seaboard, the upper Midwest, and the West. In fact, more than 90 percent of the nationally reported cases come from 12 states: Maine, Massachusetts, New Hampshire, Rhode Island, Connecticut, New York, New Jersey, Pennsylvania, Delaware, Maryland, Wisconsin, and Minnesota. All have incidences of Lyme disease greater than the national rate (6.0 per 1000,000).[24] In the East, the tick associated with disease is the *I. scapularis* or *I. dammini* tick; in the West, it is *Ixodes pacificus*, which prefers to feed on lizards that are not susceptible to infection, thus accounting for the low frequency of disease. In the East and upper Midwest, these same ticks may be co-infected with *Babesia microti* and *Anaplasma phagocytophilum* (formerly *Ehrlichia phagocytophilia*).[124]

In Europe, cases of erythema migrans with and without meningopolyneuritis have occurred within the range of the *Ixodes ricinus* tick, although one case described outside the range of this vector has been ascribed to mosquito bites.[96] The highest frequencies of disease occur in middle Europe and Scandinavia. The infection has been documented in Russia, China, and Japan. In Asia, the vector is *Ixodes persulcatus*. Isolated cases distributed over a wide region, including Australia, where none of the vectors currently recognized are known to exist, suggest that the disease may be more widespread than first realized and may have a broader range of potential vectors. Mosquitoes and deer flies may become infected with the organism but do not appear to be able to transmit the organism to humans.[75]

B. burgdorferi is widespread in the animal kingdom. Virtually any feral or domestic animal can act as an intermediate host. Birds frequently are carriers, which may account for the unusual dispersal pattern of Lyme disease.[5] The greatest reservoir continues to be white-footed mice and deer. Wild animals are not susceptible to the development of illness; however, clinical Lyme disease may occur in domestic animals, such as dogs and horses.

As expected with this vector, the disease has a high incidence in the summer and early fall, with clustering of patients in wooded and sparsely settled areas. The age range of reported cases has been from 2 to 90 years, with a slight male preponderance. The incidence of reported disease peaks at ages 5 to 14 and 50 to 59.[26]

PATHOGENESIS

The organism must adapt to two major hosts, the ticks and mammalian hosts, to complete its enzootic cycle. In the tick midgut, OspA is expressed; when the tick feeds and a blood meal is taken, OspC is up-regulated. The spirochete is injected into the mammalian bloodstream through the saliva of the tick or deposited on the skin in fecal material.[17] Tick saliva may play a role in dissemination and survival of the spirochete.

During an incubation period of 3 to 32 days, the organism multiplies locally in the skin. It induces proinflammatory responses in the inflammatory cells of the erythema migrans lesions. Both innate and adaptive cellular elements are mobilized to fight infection. However, the organism has shown in vitro resistance to elimination by phagocytic cells, thereby increasing infectivity of the spirochete.[47] Several mechanisms may aid in dissemination of the organism to distant sites.[106] The sequences of OspC vary considerable among different strains, with a few particularly associated with dissemination. The organism binds plasminogen and its activators to its surface. Dissemination is primarily hematogenous. It has been isolated from the CSF of patients with neurologic symptoms up to 10 weeks after the initial tick bite. Success with antibiotic treatment provides evidence that the spirochete still is alive when arthritis is present.[114] The late complications probably are caused by a direct effect of infection with viable organisms and the immunologic response to them. The spirochete can adhere to many different types of mammalian cells and is a potent inducer of tumor necrosis factor, interleukin-1β, and interleukin-6.[54] In a rat model, permeability changes in the blood-brain barrier begin within 12 hours of inoculation of

the organism, and the spirochete can be cultured from CSF within 24 hours.[46]

Initially, the immune response seems to be suppressed, which may aid dissemination. However, during dissemination, adaptive T- and B-cell responses in lymph nodes lead to antibody production. The specific IgM response peaks between the third and sixth weeks of infection. It often is associated with polyclonal activation of B cells, which causes elevated total serum IgM levels, circulating immune complexes, and cryoglobulins.[57] The specific IgG response develops initially during the first 6 to 8 weeks but then matures for months with the development of a wide array of antibodies to both protein and nonprotein antigens. The unusual persistence of the IgM response in patients with severe disease suggests that such patients have a defect in their helper T-cell function needed to switch from IgM to IgG production.[115] Patients with severe and prolonged illness, particularly arthritis or neurologic disease, often have the B-cell alloantigens HLA-DR2 and HLA-DR4.[113]

Histologically, all affected tissues show infiltration of lymphocytes and plasma cells. Some degree of vascular damage may be seen in many sites, thus suggesting that the organism may have been in or around the vessels. *B. burgdorferi* seems to survive for long periods in certain areas, particularly the skin, nervous system, and joints. With the development of animal models, including hamsters, mice, dogs, and nonhuman primates, and expansion of knowledge about the immune response to this infection, new information is accumulating about the pathogenesis of this disease, which is described well in excellent reviews by Steere and colleagues.[106,111]

CLINICAL MANIFESTATIONS

The clinical findings in Lyme disease can be divided into three stages on the basis of the chronologic relationship to the original bite. In the first stage, the presence of the skin lesions is the most prominent symptom; in the second stage, cardiac and neurologic findings predominate; and in the third stage, arthritis is the most common symptom. Any of these findings may occur in isolation or recurrently. Asbrink and Hovmark[6] proposed a clinical classification analogous to that of syphilis, with early infection designated stage 1 (localized erythema migrans), followed days to weeks later by stage 2 (early disseminated infection), and then late or persistent infection designated stage 3.

The most common of the clinical manifestations is the rash erythema migrans (Fig. 153–1), seen in 70 to 80 percent of persons with Lyme disease. The rash usually begins 3 to 30 days after a tick bite, although only approximately a third of patients give a specific history of a bite, which may relate to the small size of the tick. An erythematous macule or papule forms at the site of the bite. It gradually enlarges to form a large, plaque-like, erythematous annular lesion. The median diameter is 16 cm, but it may reach 68 cm.[20] The outer border generally is erythematous and flat, although it may be indurated. The middle area may show clearing, but the center sometimes is indurated or erythematous or may have vesicles. In a study[105] of early microbiologically confirmed erythema migrans occurring within 3 days of the onset of symptoms, the lesions commonly had homogeneous or central redness associated with flu-like symptoms. The lesions often are hot to the touch and otherwise asymptomatic but may burn, prickle, or itch. They may occur on any area of the body; usual sites include the thighs, buttocks, and axillae; intertriginous areas; or places where underwear may be tight. Mucosal lesions do not occur. Multiple secondary annular lesions occur in 20 to 50 percent of patients, usually within a few days of appearance of the primary lesion. These secondary lesions are evidence of dissemination (stage 2).

The average duration of the initial skin lesion is approximately 3 weeks. It gradually resolves, sometimes with a bluish hue as it fades. Lesions can recur for up to a year or more, often coincident with subsequent attacks of arthritis. In patients treated with appropriate antibiotics, the lesions usually resolve within several days.

The skin lesions frequently are associated with systemic symptoms, most commonly malaise, fatigue, headache, stiff neck, and arthralgias; additionally, backache, myalgias, nausea, vomiting, and sore throat may be present. Fever usually is low-grade, but the patient's temperature can be as high as 40° C, with chills. Fever occurs more commonly in children than in adults.[112] Lymphadenopathy, usually regional and associated with erythema migrans but occasionally generalized, may occur. Occasionally, a malar rash, conjunctivitis, or pharyngitis is present. In

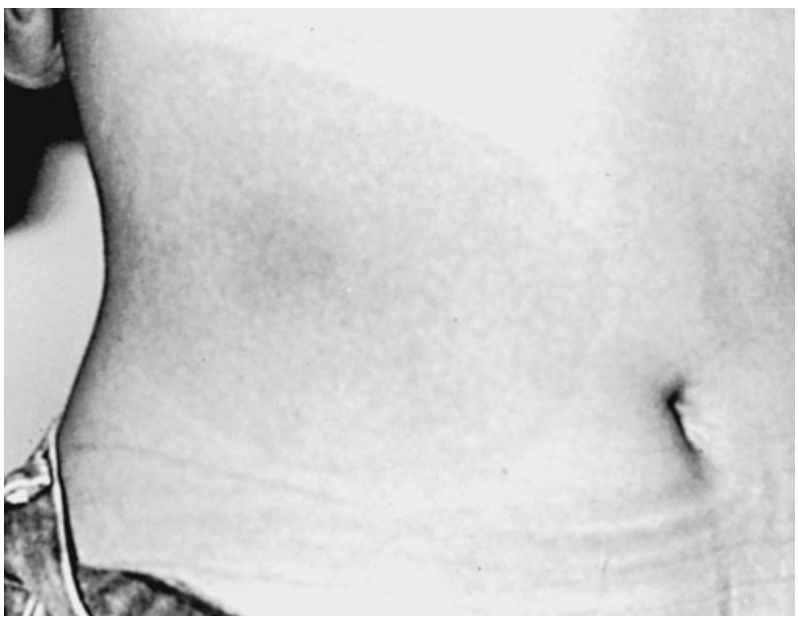

Figure 153–1 The typical skin lesion of Lyme disease, erythema (chronicum) migrans. The lesion had progressed from about 3 cm to this size within a week. *(Courtesy of Drs. Jane Grant-Kels and Nadine Wenner.)*

approximately 10 percent of patients, signs and symptoms consistent with anicteric hepatitis, including hepatomegaly and right upper quadrant tenderness are present. Most symptoms resolve over the course of a few days, but some patients experience intermittent and fluctuating symptoms for a period of several weeks. Feder and associates[42] described a group of five children with a flu-like illness but not erythema migrans that was documented serologically as Lyme disease.

Another skin lesion noted in early disseminated disease is *Borrelia* lymphocytoma. Seen in Europe in approximately 1 percent of cases of Lyme disease, it generally appears as a firm, red, red-brown, or red-purple nodule or papule, principally on the pinna of the ear in children or on the nipple or areola in adults.[76]

The late skin manifestation is acrodermatitis chronica atrophicans, a progressive dermatologic condition that develops slowly with increasing erythema and pigmentation changes of the skin of an extremity that spread over its extensor surface. After initial hyperpigmentation occurs, hypopigmented areas develop, and eventually the skin becomes frail. It is seen primarily in European cases. This condition has been associated with elevated antibodies to *B. burgdorferi* and response to antibiotic therapy.[79] Other associated lesions are fibrotic nodules, other sclerotic and atrophic lesions, and, rarely, eosinophilic fasciitis and progressive facial hemiatrophy.[52,76]

Although early Lyme disease can cause symptoms of meningeal irritation and headache, they usually are benign and self-limited. Neurologic abnormalities develop roughly within 4 weeks (range, 2 to 11 weeks, but can be up to months) after the tick bite occurs. The spectrum of involvement is wide and includes aseptic meningitis, meningoencephalitis, chorea, cerebellar ataxia, cranial neuritis, radiculopathies, mononeuritis multiplex, and myelitis.[31,55,88] The most common occurrence is aseptic meningitis, characterized by headache and stiff neck and often associated with nausea and vomiting, sensory disturbance, photophobia, and irritability. Findings in CSF are similar to those seen in patients with viral meningitis. The symptoms may occur intermittently for weeks, with mild headache persisting between attacks until spontaneous remission occurs. In a series comparing children with Lyme meningitis and children with viral meningitis, Eppes and associates[41] showed that those with Lyme meningitis were more likely to have a lower temperature, longer duration of symptoms, associated papilledema or cranial neuropathy, and milder pleocytosis. Further study by this group,[8] using a logistic regression model, showed a long duration of headache (7.5 days in Lyme meningitis versus 2.8 days in aseptic meningitis), the presence of cranial neuritis (56% vs. 3% for aseptic meningitis), and a predominance of CSF mononuclear cells as findings that differentiated Lyme meningitis from aseptic meningitis. As many as two thirds of adult patients exhibit subtle findings of parenchymal abnormality or encephalitis,[36] such as somnolence, emotional lability, memory loss, poor concentration, or behavioral changes. In a series of children with neurologic manifestations, changes in behavior that did not predate the Lyme disease occurred rarely, as did meningoradiculitis and peripheral neuropathy syndromes.[15]

The seventh cranial nerve is involved most frequently in Lyme disease; unilateral or bilateral facial palsies occur in as many as 11 percent of patients.[27] Seventh nerve palsy is seen in approximately 50 percent of patients with meningitis; it can occur alone as well. Other cranial nerves, particularly III and IV, are involved less frequently. Several children with pseudotumor cerebri in association with Lyme disease have been reported.[15,52,87] Rarely, opsoclonus-myoclonus may be seen.[83]

Neurologic involvement can develop in the third stage of illness months to years after the initial infection. These patients have neuropsychiatric symptoms, focal central nervous system disease, or, rarely, severe incapacitating fatigue.[55,82] These findings are extremely rare occurrences in children.[15,100]

Conjunctivitis is an infrequent early ophthalmologic manifestation that usually is transient.[107] A case of iritis progressing to panophthalmitis and unilateral blindness has been described.[112] Spirochetes consistent with *B. burgdorferi* were found in vitreous debris. Other eye manifestations include optic neuritis, iritis, and keratitis.[14] Rothermel and colleagues[92] described four cases of optic neuropathy in children with Lyme disease: two children had optic neuritis, one had papilledema, and one had both.

Cardiac abnormalities occur in approximately 10 percent of untreated patients, generally within several weeks after being bitten (average, 5 weeks; range, 3 to 21 weeks). Seen most commonly in young adult males, they range from fluctuating degrees of atrioventricular block to myopericarditis and left ventricular dysfunction.[78,108] Cardiac involvement usually is brief (3 days to 6 weeks). Recently, three children with a transient prolonged corrected QT interval and Lyme disease were described.[99] Patients with cardiac involvement generally have other evidence of more severe systemic disease, such as fever, rash, arthritis, or neurologic findings. Though described in children, these cardiac findings are uncommon occurrences in pediatric patients.

The second most common manifestation of Lyme disease after erythema migrans is arthritis, which occurs in approximately half of the patients without treatment.[32,38,118,129] It typically begins 4 weeks after the skin lesion (5 to 6 weeks after the bite) appears, although the time span can vary from less than 1 week to many months, and a small percentage of patients do not recall having any skin lesions. The arthritis usually is of sudden onset, monarticular or oligoarticular, and, occasionally, migratory. Large joints, often those closest to the initial rash, are affected most commonly. The knee is by far the joint involved most frequently, followed by the shoulder, elbow, temporomandibular joint, ankle, wrist, and hip.[36] They become swollen, warm, and painful but rarely red. The usual duration of the first episode is approximately 1 week, but sometimes the episode persists for several months.

Recurrent attacks of arthritis are common occurrences. Among the initial 51 patients studied, 35 (69%) had recurrent attacks.[107,118] The median number of recurrent attacks was three. During recurrences, usually more joints are involved than in the initial episode. These attacks last approximately 1 week, with intervals of 1 week to 2 years between attacks. Children experience complete remissions between attacks; however, adults often have persistent asymptomatic joint effusions or mild morning stiffness. A severe chronic erosive arthritis develops in approximately 10 percent of all patients with Lyme arthritis; it occurs approximately 1 year after the initial manifestations and often is associated with HLA-DR4.[113] A rare, unusual complication is rupture of a Baker cyst, which causes a pseudothrombophlebitis.

Other unusual manifestations of Lyme disease include recurrent hepatitis,[51] myositis,[7,59] eosinophilic lymphadenitis,[86] and adult respiratory distress syndrome.[66] Simultaneous co-infection with the agent of babesiosis or anaplasmosis may increase the severity of the initial illness or present a more confusing picture.[109] In particular, the associated fever and flu-like symptoms may be more severe.[69] The proportion of patients with co-infection varies greatly, with a range of 4 to 39 percent, depending on the population studied and the diagnostic measures used.[69,119]

Maternal-fetal transmission of *B. burgdorferi* has been documented in two infants, one with congenital heart disease[95] and the other with encephalitis.[127] Neither infant had evidence of tissue inflammation. A stillbirth that occurred after maternal Lyme disease also has been reported.[74] An analysis of 19 cases of Lyme disease occurring during pregnancy revealed five pregnancies (26%) with adverse outcomes. They occurred in all three trimesters, and no two were the same.[77] In a study of 463 infants, no association between congenital malformations and the presence of antibody to *B. burgdorferi* in cord blood or IgM antibody could be established.[128]

Strobino and associates,[122] in a prospective study of approximately 2000 pregnant women in an endemic area, found no evidence of fetal wasting, prematurity, or congenital malformations attributable to Lyme disease. In a follow-up study, they found no associated congenital heart disease.[121] Gerber and Zalneraitis[49] surveyed pediatric neurologists in a large endemic area to determine the prevalence of clinically significant nervous system disease that might be attributable to transplacental transmission; they found no cases that met their case definition.

The association of Lyme disease with adverse fetal outcomes appears to be unusual[128]; however, the importance of continued surveillance of pregnant women, as well as prompt diagnosis and treatment, should be emphasized.[126]

DIFFERENTIAL DIAGNOSIS

When the characteristic erythema migrans rash is present and recognized, identifying the etiology of subsequent symptoms should pose little problem. However, particularly when the clinical findings are atypical, the differential diagnosis is broad.

If the rash is not recognized as erythema migrans, it may be confused with streptococcal cellulitis. Erythema multiforme might be a consideration, but the lesions in that disorder often are smaller and urticarial or vesicular, are seen on mucosal surfaces, and frequently are associated with drug exposure. Erythema marginatum usually is smaller and less annular. If a necrotic or vesicular center is present in the erythema migrans lesion, it may resemble the lesion of tularemia, but the latter is not expansive and not associated with similar complications. Occasionally, a superficial reaction to a tick bite proves confusing, but generally the time course helps. A hiatus of at least 3 days should occur between the bite and development of erythema migrans.

Distinguishing Lyme disease from acute rheumatic fever is particularly important. If the skin lesion is misdiagnosed and migratory arthritis is noted in association with nonspecific electrocardiographic changes, such as a prolonged PR interval, one might assume erroneously that the modified Jones criteria have been fulfilled. Fortunately, in Lyme disease, usually no evidence of antecedent streptococcal infection is present, and the specific nature of rheumatologic involvement and cardiac involvement is different.

Other forms of arthritis that may be confused with Lyme disease include (1) pauciarticular juvenile rheumatoid arthritis; (2) psoriatic arthritis; (3) reactive arthritis associated with *Salmonella*, *Shigella*, or *Yersinia* infections; (4) Reiter syndrome; and (5) postinfectious or infectious arthritis, such as that associated with rubella, hepatitis B, or echoviruses. Several distinctive features usually allow prompt differentiation from Lyme disease.

The major neurologic manifestation of aseptic meningitis may be confused with enteroviral, leptospiral, or early tuberculous meningitis. When it becomes more chronic and relapsing, one must consider sarcoidosis, Mollaret meningitis, Behçet disease, and multiple sclerosis.

SPECIFIC DIAGNOSIS

The diagnosis of Lyme disease is made best on clinical and epidemiologic grounds and often can be established early in the course of illness from the gross appearance of erythema migrans.

Routine laboratory testing generally is nonspecific and not helpful. The sedimentation rate often is elevated. Leukocyte counts commonly are normal. Serum glutamic-oxaloacetic transaminase and serum glutamic-pyruvic transaminase levels may be elevated mildly early in the course of the disease. Complement studies may be normal, low, or high. Serum IgG and IgA typically are normal; however, IgM and cryoglobulin IgM often are elevated, particularly in patients with severe disease or in those in whom neurologic complications or arthritis develops later. Immune complexes as measured by Clq binding (or other methods) may be found in patients with Lyme disease and may be involved in the pathogenesis. They may be present at the time of diagnosis and then clear in patients without neurologic or cardiac involvement or localize to the synovium in those with arthritis.[57]

Examination of synovial fluid in patients with arthritis demonstrates leukocyte counts elevated from 500 to more than 100,000 cells/mm^3, with a predominance of polymorphonuclear leukocytes. Total protein usually is between 3 and 8 g/dL.

Patients with aseptic meningitis frequently have CSF pleocytosis, with counts ranging from 25 to 450 cells/mm^3 and lymphocytic predominance. Total protein may be elevated mildly, and glucose levels (CSF-to-blood ratio) usually are normal.

Culture of B. *burgdorferi* from specimens permits a definitive diagnosis to be established; however, the yield from culture is too low to render it practical for diagnosis. Therefore, serology currently is the only practical laboratory technique used for confirmation of the diagnosis. Although indirect immunofluorescence first was used to evaluate the immune response to *B. burgdorferi*, enzyme-linked immunosorbent assay (ELISA) appears to be more sensitive and specific.[29,93] However, because no standardization of testing has been established, interlaboratory variation in results may be marked.[58,65,68] Any serologic results must be interpreted with care. Serologic testing should be undertaken only when the clinical and epidemiologic investigations suggest Lyme disease as the diagnosis.

False-negative results are common occurrences early in the infection.[103] Only 20 to 30 percent of patients have positive responses, usually IgM, in the first few weeks of infection. However, Berardi and associates[18] have developed a sensitive capture IgM ELISA that demonstrates an IgM response in as many as 90 percent of patients with early stages of disease. Some patients treated with antibiotics early in the course of disease may not show an antibody response. In one study, 17 patients with a variety of symptoms were found to be seronegative but to have mononuclear cells with a proliferative response to *B. burgdorferi*.[33] The significance of such responses remains to be seen, but this finding should be considered extremely uncommon.

Techniques to increase sensitivity, specificity, or both include ELISA using flagellar antigen,[56] immunoblotting,[53] and polymerase chain reaction (PCR).[81] Immunoblotting, in particular, may be used to identify false-positive results.[91] Western blot or immunoblotting separates surface and subsurface proteins of *B. burgdorferi* by polyacrylamide gel electrophoresis, which then are reacted with patients' sera. This technique is more specific than is ELISA or immunofluorescent assay and is especially useful in identifying false-positive results.[48,53,91] False-positive results may be seen in patients with other spirochetal infections (e.g., syphilis, leptospirosis, relapsing fever), certain viral infections (e.g., varicella, herpes simplex virus, Epstein-Barr virus), or certain autoimmune diseases or in the presence of antibodies directed against spirochetes in normal oral flora.[4]

As a result of a national conference on serologic diagnosis of Lyme disease, the Centers for Disease Control and Prevention recommend a two-test approach for active disease and for previous infection that involves the use of a sensitive enzyme immunoassay or immunofluorescent assay followed by Western blot if the initial test is positive or the results are equivocal.[23] Negative results need not be validated. For Western blot in the first 4 weeks of disease, both IgM and IgG procedures should be performed. A positive IgM test result alone is not recommended for determining active disease in patients with illness of greater than 1 month's duration because of a high false-positive rate caused by the lower specificity of the IgM immunoblot.[130] If serologic

results in a patient with suspected early Lyme disease are negative, paired acute and convalescent specimens should be obtained. Serum samples from patients with disseminated or late-stage disease almost always have a strong IgG response to *B. burgdorferi* antigens. A recommendation is that an IgM immunoblot result be considered positive if two of the following three bands are present: 24 kd (OspC), 39 kd (BmpA), or 41 kd (Fla).[39] An IgG immunoblot result is considered positive if 5 of the following 10 bands are present: 18 kd, 21 kd (OspC), 28 kd, 30 kd, 39 kd (BmpA0), 41 kd (Fla), 45 kd, 58 kd, 66 kd, or 93 kd.[37]

In addition to having false-positive results, 5 to 10 percent of patients in the United States have asymptomatic *B. burgdorferi* infection; these serologic results may interfere with establishing the diagnosis of another significant illness. In fact, because IgM or IgG antibody responses to the organism may persist for 10 to 20 years after active disease, linking testing to appropriate clinical scenarios is important.[64]

PCR has been used to amplify and detect *B. burgdorferi* DNA in cultured spirochetes, infected animals, and patients with Lyme disease.[81,89,91] DNA sequences can be detected in blood, urine, CSF, skin, and synovial fluid.[97] *B. burgdorferi* DNA was identified in 75 of 88 synovial fluid samples from patients with Lyme disease.[81] The usefulness of PCR in clinical situations is an area of active research, but its use may be limited by problems with sensitivity and specificity.[81,89,91] Antibody response to recombinant VlsE1 antigen is promising as a sensitive and specific method of detecting an immune response.[9] The Lyme urine antigen test, immunofluorescent staining for cell wall–deficient forms of the organism, and lymphocyte transformation tests are unreliable for establishing the diagnosis of suspected Lyme disease.[25]

Diagnosing neurologic manifestations of Lyme disease is particularly difficult. Comparing intrathecal antibody assay results with serum results may be helpful.[109]

Because of the delay in specific antibody response and the possible ablation of this response in patients with localized disease who are treated early, recognition of the clinical picture—erythema migrans, a flu- or meningitis-like illness in the summer, or both—is essential for providing prompt diagnosis and treatment. Laboratory evaluation is unnecessary in early stages of the disease and should be used only to support a clinical diagnosis of Lyme disease in later stages. Overdiagnosis and overtreatment continue to be a concern in areas endemic for Lyme disease.[85]

TREATMENT

Even before the spirochete was identified as the causative agent of Lyme disease, treatment of adults with penicillin or tetracycline was associated with more rapid resolution of the rash and its associated symptoms.[117] Subsequent studies have confirmed that impression; tetracycline may be more effective in preventing late complications of the disease (meningoencephalitis, myocarditis, and arthritis).[116] In none of 88 patients treated with tetracycline did such complications develop, as compared with 7.5 percent (3 of 40) of a group of patients treated with penicillin. However, nearly half of all treated patients still had minor late symptoms such as headache, musculoskeletal pain, and lethargy. These complications correlated significantly with the initial severity of illness.

Antibiotic sensitivities to *B. burgdorferi* have been determined in vitro and in experimental animals.[60,61,63] Although the methods are not standardized, the consensus is that the organism is highly sensitive to tetracycline. It also is susceptible to ampicillin, ceftriaxone, and imipenem. Unlike *Treponema pallidum*, *B. burgdorferi* is only moderately sensitive to penicillin. Aminoglycosides and rifampin have no activity, whereas oxacillin and chloramphenicol are only moderately active. Erythromycin appears to be active in vitro but may be less so in vivo.

In fact, azithromycin and clarithromycin both are more active, although the clinical efficacy of either is controversial.[35] A trial comparing azithromycin and amoxicillin for the treatment of erythema migrans showed amoxicillin to be significantly more effective.[72] Newer oral cephalosporins, particularly cefixime and cefuroxime axetil, hold promise.[3,62] Cefuroxime axetil was comparable to doxycycline in a clinical trial in adults with early stages of Lyme disease.[73] In a similar trial comparing cefuroxime and amoxicillin in children, they were found to be equivalent.[40]

Recommendations for treatment continue to evolve. The Infectious Diseases Society of America recently presented evidence-based guidelines for treatment.[131] For early localized or disseminated Lyme disease, doxycycline, 100 mg twice a day (4 mg/kg/day to a maximum of 100 mg twice a day) for 14 to 21 days, is effective for adults and children older than 8 years. In younger children, amoxicillin, 50 mg/kg/day in three divided doses (maximum of 1.5 g/day) for the same duration, appears to be the best choice, with cefuroxime axetil, 30 mg/kg/day in two divided doses, as an alternative. For penicillin-allergic children who cannot tolerate a cephalosporin, erythromycin, 30 to 50 mg/kg/day in four divided doses for the same period, may be used, although its efficacy is less clear. The duration of treatment is based on clinical response. A recent study in adults actually demonstrated that a shorter course of 10 days may be adequate.[132] Multicenter studies of patients with early stages of disease show efficacy to be similar with doxycycline, amoxicillin, and cefuroxime axetil. Some patients may have subjective symptoms after receiving treatment, and it is important to caution families that resolution of nonspecific symptoms may continue slowly after treatment. Objective evidence of persistent infection or relapse is a very uncommon finding.

Patients with isolated seventh nerve palsy should be treated with oral regimens to prevent further complications. If the palsy is associated with other neurologic complications, parenteral therapy should be initiated.

Neurologic complications, particularly Lyme meningitis, have been treated successfully with large doses of penicillin G (20 million IU/day) in adults.[120] No large trials have been reported in children; however, penicillin, 300,000 IU/kg/day, appeared to be beneficial in two cases of pseudotumor cerebri.[87] The exquisite sensitivity of the organism to ceftriaxone renders it an attractive alternative for parenteral therapy, and it has become the drug of choice. The dose of ceftriaxone is 75 to 100 mg/kg/day, up to 2 g/day. In a study of 23 patients with late stages of Lyme disease, Dattwyler and associates[32] reported superior efficacy of ceftriaxone over penicillin. Cefotaxime is a reasonable alternative. The duration of parenteral therapy for neurologic or rheumatologic disease is not clear but should be a minimum of 14 days (as long as 4 weeks). The signs and symptoms of acute neuroborreliosis usually resolve within weeks. Even patients with late encephalopathy can be treated successfully with ceftriaxone.[71]

Patients with established Lyme arthritis also have been treated successfully with high-dose penicillin but without universal efficacy.[114] In an uncontrolled study of 33 children with arthritis, 31 were treated with oral therapy alone for 3 to 4 weeks, with elimination of synovitis and recurrent attacks.[30,38] Oral therapy with either doxycycline or amoxicillin for 4 weeks is a reasonable approach for children without neurologic involvement. Persistent or recurrent arthritis may be treated with a subsequent oral course or parenteral treatment with ceftriaxone.

The self-limited nature of the acute arthritis obviates the need for any analgesics other than acetaminophen or nonsteroidal anti-inflammatory agents. Patients with chronic arthritis generally have been treated with nonsteroidal anti-inflammatory agents.

Patients with cardiac complications usually do not require specific treatment other than an oral antibiotic regimen if they have first- or second-degree atrioventricular block. Patients with

third-degree block should be treated with parenteral therapy such as ceftriaxone, although no clinical data support this approach. Those with heart block may require temporary pacing.[108]

PROGNOSIS AND PREVENTION

Most patients with Lyme disease, particularly those treated promptly with an appropriate antibiotic, have an uncomplicated course. Series in children point to an excellent prognosis in most cases. A review of 65 children treated for erythema migrans and monitored for a mean of longer than 3 years found them all to be well and without findings of late Lyme disease.[94] Another prospective study of children with newly diagnosed disease of any stage found that all the children were cured.[100] Rose and associates[90] described 44 children with arthritis who all had an excellent prognosis. A study of cognitive sequelae in children treated for Lyme disease showed no differences between the Lyme disease and control groups.[1] In fact, a study of children with arthritis who initially were untreated for at least 4 years found few children with late or chronic problems.[123] Another study of 90 children also suggested an excellent prognosis.[50]

After receiving appropriate treatment of Lyme disease, a small percentage of patients continue to have subjective symptoms, mainly musculoskeletal pain, fatigue, and neurocognitive difficulties, sometimes called chronic Lyme disease. It is an extremely rare occurrence in children. A large study showed no difference in the frequency of pain and fatigue in adult patients who had had Lyme disease and age-matched controls.[98] In a recent study of adult patients with post-Lyme syndrome, comparison of treatment with intravenous ceftriaxone followed by doxycycline and treatment with appropriate placebos for a total of 3 months showed no significant differences between the groups.[67]

For the small percentage of patients with chronic arthritis, the course may be variable, although the illness may resolve after 12 to 16 months. With earlier treatment, this group should be held to a minimum.

Prompt recognition of this disease with its diverse manifestations should lead to early treatment and resolution. The best prevention is avoidance of contact with the tick vector.

Even in endemic areas where a large percentage of the ticks are infected, the chance of acquiring disease is not great and depends in part on the duration of attachment of the tick. Attachment for more than 24 hours is required.[84] A recent study using mice and nymphal *I. scapularis* revealed that no transmission occurred in the first 24 hours, with maximum transmission occurring between 48 and 72 hours.[34] A small study of prophylactic antibiotics for tick bites showed a low risk of acquiring disease, similar to the risk for an adverse reaction to penicillin.[28] In a study of prophylaxis after tick bites in a highly endemic area, the incidence of infection in the placebo-treated group was only 1.2 percent. The authors concluded that even in an endemic area, the use of routine prophylactic antibiotics is not indicated.[102] A recent trial demonstrated that a single 200-mg dose of doxycycline can effectively prevent Lyme disease if given within 72 hours of the tick bite.[80] One cannot generalize this finding to less endemic areas, other antibiotic regimens, or unidentified, unengorged ticks.[101] However, in certain situations, such as a highly engorged tick in a high-risk individual, prophylaxis might be considered. Data are insufficient to recommend amoxicillin prophylaxis. Analysis of ticks for spirochetal infection has poor predictive value and should be discouraged.

Important preventive measures include (1) avoiding high-risk areas, particularly wooded, grassy areas; (2) when walking in such areas, wearing light-colored, long pants tucked into socks, sneakers, and long-sleeved shirts; (3) using insect repellents such as DEET (for skin) and permethrin (for clothing); (4) most important, conducting careful "tick patrols" every day or after every potential exposure to look carefully for the ticks; and (5) removing ticks by pulling them straight out with tweezers or protected fingers.

Active immunization against *B. burgdorferi* consisting of recombinant OspA in adjuvant was available commercially in the United States for appropriate patients at least 15 years of age. In phase III efficacy and safety trials, the efficacy of the vaccine in preventing definite Lyme disease was 49 percent after two injections and 76 percent after three injections.[65] The most important factor in protection was the strength of the antibody response to the OspA epitope. The Advisory Committee on Immunization Practices of the Centers for Disease Control and Prevention advised consideration of vaccination for Lyme disease in people 15 to 70 years old who live or work in high-risk areas and who have frequent or prolonged exposure to the ticks.[2] A trial in children showed similar safety and higher geometric mean antibody titers than in adults.[104] However, this vaccine no longer is available. Studies of other vaccines are ongoing. Vigilance for tick bites will continue to be the most important preventive measure.

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CHAPTER 154

LEPTOSPIROSIS

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HISTORY

Weil[209] is credited with providing the first description of leptospirosis in 1886. Not until 1915, however, was the causal agent, "spirochaeta icterohaemorrhagiae," identified by Inada and associates.[97] Eight years earlier, Stimson[176] unknowingly had identified the same organism within sections of kidney obtained from a patient in whom yellow fever had been diagnosed incorrectly.

Noguchi[131] first recovered this organism from a Norway rat in 1917; in 1922, the first case of Weil disease in a person associated with rat exposure was identified.[204] For many years, the rat was considered the only animal host of *Leptospira icterohaemorrhagiae*. In 1944, Randall and Cooper[142] isolated this agent from a naturally infected dog, and *L. icterohaemorrhagiae* subsequently has been associated with many animal hosts, including goats, swine, cattle, and hamsters.

In 1938 and 1939, Meyer and associates[120] popularized the concept that infection with *Leptospira canicola* caused disease in dogs and humans in the United States. "Canicola fever" first was reported in Great Britain in 1946,[15] and in 1951, 40 percent of the dogs in Great Britain were noted to be seropositive.[36] Surveys have confirmed the presence of *L. canicola* infection in species other than dogs.[158,199]

In 1950, Gochenour and colleagues[83] identified *Leptospira pomona* as the agent responsible for leptospirosis in cows. Widespread *L. pomona* infection among cattle in the United States was recognized quickly, and in time this finding stimulated extensive epidemiologic investigations in livestock. Infections of cattle with *Leptospira hebdomidis* and *Leptospira grippotyphosa* were identified, and, concomitantly, infections of swine and horses with *L. pomona* were documented.[92] In Europe, *L. pomona* was identified as the agent responsible for "swineherd disease," and it was recovered from other domestic animals as well. In 1951, the first human cases of *L. pomona* infection were identified.

Many new serotypes of leptospires were recognized in the early 1950s after the establishment of serologic diagnostic services for leptospires by the Centers for Disease Control and Prevention (CDC) and the Walter Reed Army Institute of Research. Along with the identification of additional leptospiral serotypes, the spectrum of clinical disease associated with infection by leptospires was elucidated. Patients with autumnal fever (a disease in Japanese peasants and potters) and Fort Bragg fever (a febrile illness associated with pretibial eruptions described in army recruits) were shown to suffer from leptospirosis caused by *Leptospira autumnalis*.[82,154] "Mud fever," "pea-pickers disease," and "European swamp fever," terms that were used to describe a disease of undetermined etiology in eastern Germany, the Far East, and western Poland, were shown to be examples of leptospirosis caused by *L. grippotyphosa*.[42] Seven-day fever in Japan, Wycon fever and Bushy Creek fever in the United States, cane field fever in Australia, and swineherd disease in Europe were identified as examples of infection caused by *L. hebdomidis*, *L. canicola*, *L. pomona*, *Leptospira australis*, and *L. pomona*, respectively.[21,26,31,50,92]

Leptospirosis is a disease now thought to be caused most likely by a single family of organisms that has multiple serogroups and serotypes[3] and is characterized by a broad spectrum of clinical findings.[139] At least 180 strains, called serovars (serotypes), which are divided into serogroups on the basis of common antigens, have been identified.

EPIDEMIOLOGY

ANIMAL RESERVOIRS

Among mammals, rodents are the most important reservoir of leptospires, but nearly all mammals may be infected and can transmit the disease. In various parts of the world, rats, field mice, moles, gerbils, coypus, hedgehogs, shrews, dogs, foxes, jackals, mongooses, civets, skunks, raccoons, bandicoots, opossums, and cattle have been implicated as sources of human infection.* Leptospires also have been isolated in reptiles and birds, but the epidemiologic significance of these animals in terms of maintenance of the organism in nature or transmission of disease to humans is not clear. For many species, infectivity rates of 10 to 50 percent have been reported frequently.[92,145] During epizootics, the circulation of leptospires among many species of animals living within a given biocenosis has been well recognized.[56]

A biologic equilibrium whereby these organisms remain within the convoluted tubules of the kidneys of the host without producing any pathogenic effect on the tubular epithelium exists between some strains of leptospires and numerous species of animals. When this equilibrium is not established, the animal may become ill or may die.[16,69] Studies performed in skunks suggest that local resistance appears soon after infection occurs. The failure of leptospires to elicit a significant systemic antibody response in certain animal species may be due to the development of this local immunity.[182] Some animals fail to develop homologous antibody titers but harbor leptospires in their kidneys for extended periods.[182] Thus, lack of a positive titer to leptospires, as determined during the course of serologic surveys of animal populations, does not indicate absence of infection. For this reason, serologic surveys of animal populations cannot reflect the true incidence of leptospiral infection accurately.

A particular host species may serve as a reservoir for one or more serotypes of leptospires, and a particular serotype may be hosted by many different animal species. Turner[193,195] stressed that a particular animal species commonly serves as a reservoir for selected serotypes but temporarily may be infected and serve as an incidental host for other serotypes with which it usually is not infected. Two or more animal hosts for the same serotype may be present in the same geographic area. These newer insights have replaced earlier epidemiologic concepts of leptospirosis concerning a "host of election." Although any animal susceptible to infection by leptospires may become a urinary "shedder" of the organisms temporarily, only selected animal species with "biologic sympathy" for a particular strain of leptospires can become a principal or maintenance host of the serotype.[16]

TRANSMISSION OF LEPTOSPIRES TO HUMANS

Leptospires are transmitted to humans either by contact with blood, urine, tissues, or organs of infected animals or by exposure to an environment that has been contaminated by leptospires. Humans usually represent a dead end in the chain of infection because although person-to-person transmission is possible theoretically, it is rare. After direct exposure of humans to infected animals, leptospires may enter breaks in the skin or may penetrate the mucous membranes, including the conjunctiva, nasopharynx, and vagina.[21,59,153,193] Human-to-human transmission via human milk has been reported in a breast-fed infant from a lactating mother who was infected with *L. interrogans*.[30]

Indirect transmission of leptospires to humans (from soil or water) depends on the presence of an environment that favors the survival of leptospires outside the animal host. A warm climate (25° C [77° F]), the presence of moisture, and pH values of soil or surface water between 6.2 and 8.0 are optimal conditions for survival of leptospires. These conditions prevail in many tropical regions throughout the year and in temperate climates during the late spring, summer, and autumn months. Conversely, survival of leptospires outside the animal host is impeded by chemical pollution, salinity, and various absorptive properties of clay in soil.[59,193]

Smith and Self[167] demonstrated survival of leptospires in cultures of infected soil for 43 days. *L. icterohaemorrhagiae* recovered from the soil of lawns in suburban communities has been indicted in an epidemic of leptospirosis in Missouri,[43] and a case of human leptospirosis has been reported in a soil scientist who became infected after handling a soil sample that had been collected in North Queensland, Australia, and transported 1100 miles during a period of 48 hours.[188]

Fresh water, particularly that contaminated by rat urine, has been recognized as an important vehicle for transmission of leptospiral infection.[81,193] Drinking water from a fountain also has been associated with an outbreak of leptospirosis.[38] The urine of infected cows may contain as many as 100 million leptospires per milliliter. If conditions are favorable, surface water contaminated by the urine of infected cattle may remain infectious for several weeks.[39] The largest reported outbreak of leptospirosis in the United States occurred in 1998 in Springfield, Illinois, after a triathlon that was preceded by heavy rains. There were 66 laboratory-confirmed cases of leptospirosis after the event, which included a swimming portion in a lake that received runoff from

*See references 16, 59, 65, 69, 89, 154, 189, 193, 195, 197.

nearby livestock farms. Fifty-two of these cases occurred in triathlon participants, and 11 cases involved nonparticipants who came in contact with lake water near the event.[124]

Venereal transmission of leptospirosis is important in rodents and can occur in livestock. Leptospires have been recovered from the semen of bulls and have been transmitted by artificial insemination and by coitus. The possibility of seminal transmission in humans remains speculative. Transplacental infection of the fetus in utero is well documented in livestock and other animals and may occur in humans.[41,47,51,59,193,196]

The importance of occupation as related to the risk for leptospirosis was emphasized in 1965.[92] Disease appeared to occur most frequently in people with occupations that required exposure to cattle or swine or to water contaminated by rat urine. During the past several decades, the number of cases acquired during outdoor recreation has increased. In rural areas, the U.S. Department of Agriculture promoted and aided in the development of farm ponds. These ponds, along with streams and rivers, are used widely for recreation and as a water supply for livestock and wild animals.[138] That many outbreaks of human leptospirosis have been attributed to water used for dual purposes is not surprising.[26,78,146,154]

More recently, leptospirosis has become a more frequent occurrence in children, students, and housewives than in adults with occupational exposure; cases from urban and suburban communities have been reported more frequently than have cases from rural areas.[43] The dog has been incriminated increasingly as an important vector and as a reservoir of this disease. Although immunization of dogs against leptospirosis is possible, an important note to remember is that (1) immunization may not prevent the dog from having renal carriage and excreting the organism, (2) canine immunity after immunization may persist for just 1 year, and (3) immunity, when established, is effective only for serotypes present in the canine vaccine.[16] The immunized pet dog has been identified as a previously unrecognized threat to people.[16] The exact prevalence of immunized dogs in each community that excrete leptospires is unknown. In one survey of suburban and urban areas, however, between 15 and 40 percent of dogs were found to be infected.[25] These data are consistent with reports from the CDC; dogs were implicated in 58 percent of the 820 known cases of leptospirosis reported between 1962 and 1971.[43]

In a study performed in Detroit, 90 percent of rats carried *L. icterohemorrhagiae*.[62] Strain-specific tests comparing antibody titers in the sera of inner-city and suburban children were performed. Thirty-one percent of inner-city children had antibodies against *L. icterohaemorrhagiae*; 10 percent of suburban children also had antibodies to this organism.

PATHOPHYSIOLOGY

After penetrating the skin or mucous membranes, leptospires invade the bloodstream and spread throughout the body to produce the protean manifestation of the disease.[70,71,173] Stavitsky[173] has suggested that the speed with which the leptospire revolves in a corkscrew fashion enables it to bore through connective tissue. This suggestion correlates with observations in humans, which show that leptospires regularly invade the anterior chamber of the eye and the subarachnoid space without eliciting a significant inflammatory response.[88] Volland and Brede[203] detected hyaluronidase in fluid filtered from leptospiral cultures and cited it as a reason for the unusual invasive property of leptospires.

Specific factors responsible for the virulence of leptospires remain unknown. The possible role played by animal hosts in determining the virulence of leptospires for humans remains speculative. Faine[70] compared the fate of virulent and nonvirulent strains of *L. icterohaemorrhagiae* in guinea pigs. Both strains behaved similarly after intraperitoneal infection, but virulent organisms survived and multiplied, whereas avirulent strains did not. Both virulent and avirulent strains were taken up by fixed phagocytes in reticuloendothelial tissues in vivo. Phagocytosis or chemotaxis was not noted with either strain in vitro or in vivo. Virulent and avirulent strains appeared to be identical serologically. Faine[71] also showed that the severity of lesions correlated positively with the number of organisms present and that a discrete number of organisms were required to cause death. The logarithm of the dose for strains of a given virulence was related constantly to survival time after ingestion. This relationship was correlated with the growth rate in vivo. Faine[70] also suggested that the low proportion of virulent organisms within avirulent strains permits modification of the disease process by antibodies that have sufficient time to develop before the disease process becomes irreversible. He hypothesized that virulence results from the selective multiplication of virulent leptospires in vivo. This hypothesis was supported by the fact that maximum virulence can be regained after a single animal passage that follows isolation in culture. Virulence may be lost in culture by mutation to nonvirulent forms.

Nonspecific resistance to leptospirosis is not mediated by differences in phagocytosis of leptospires among animals. Specific resistance, however, apparently is mediated by antibody. Antibody increases the efficacy of clearance of leptospires from the bloodstream by enhancing opsonization and hence improving phagocytosis.[50] Wang and associates[205] demonstrated that polymorphonuclear leukocytes are not an efficient defense factor for pathogenic leptospires in nonimmune hosts. The virulence of leptospires appears to be related to their ability to resist killing both by serum and by neutrophils.

Clinical and histologic findings in human and animal leptospirosis have suggested that the pathogenicity of leptospirosis may be, in part, the result of enzyme, toxin, or other metabolites that are elaborated by or released from lysed leptospires.* Imamura and associates[96] demonstrated the presence of a thermostable dermal necrotizing toxin in extracts of a suspension of *L. icterohaemorrhagiae* by noting the necrotizing effects that occurred after intradermal injection into guinea pigs or rabbits. The skin-necrotizing effect was attributed to the presence of insoluble particles of leptospires in the sonification supernatant.[68]

The clinical and histologic findings observed in leptospirosis are similar to those noted in animals given endotoxin, which suggests that the endotoxin may be responsible in part for the pathogenic action of leptospires.[185] Arean and associates[12] were unable to demonstrate the presence of endotoxin in extracts of leptospires and concluded that *L. icterohaemorrhagiae* contained either no endotoxin or one that was labile and readily destroyed by chemical agents in the process of infection. Further study by Arean and Henry,[11] however, suggested the elaboration of some other undefined toxins that may play a role in the pathogenic action of leptospires. Inoculation of rabbits intravenously by Gourley and Low[86] with disintegrated cells or extracts of cells of *L. canicola* and *L. icterohaemorrhagiae* was followed by fever, leukopenia, thrombocytopenia, and later, leukocytosis. Their findings suggested the presence of endotoxin in these serotypes. Finco and Low,[74] using preparations of *L. canicola* organisms and several bioassay procedures, showed that *L. canicola* had little ability to elicit biologic responses characteristic of endotoxins. One thousand to 1 million more *L. canicola* organisms (on the basis of dry weight) were required to produce a febrile response in rabbits of a magnitude similar to that elicited by *Escherichia coli*. The results suggest that *L. canicola* contains material with weak endotoxin activity. Massive quantities of *L. canicola* would be required to produce endotoxin-related disease. Finco and

*See references 2, 9, 10, 12, 24, 70, 77, 85, 87, 93, 141, 186.

Low[74] concluded that (1) other factors related to leptospiral infection may increase the susceptibility of the host to endotoxin during leptospirosis or (2) endotoxin from the intestinal lumen may gain access to the bloodstream during the course of leptospirosis.

The development of hemolytic anemia and jaundice in patients with leptospirosis has suggested a role for hemolysis in the pathogenesis of this disease. Alexander and associates[5] reported the presence of a heat-labile, oxygen-stable, nondialyzable hemolysin in the supernate of leptospiral cultures; subsequently, Russell[149] noted that this hemolysin could be inhibited by leptospiral antiserum. Hemolysis may persist during leptospirosis despite the development of serum antibodies, which suggests that circulating hemolysin is adsorbed by erythrocytes early in the course of leptospirosis and that the erythrocytes lyse subsequently[24,126] despite the presence of circulating antibody. The hemolysin is thermolabile and can be inactivated by trypsin and precipitated by ammonium sulfates, which suggests that it is, in part, a protein moiety.[5,10] Lee and colleagues[110] cloned a hemolysin of *L. interrogans* serovar lai and demonstrated cell membrane pore-forming activity in vitro, in contrast to the sphingomyelinase and phospholipase activity demonstrated by other leptospiral hemolysins. The precise role of hemolysins in human disease remains unclear.

A toxic and pathogenic potential in vivo for lipid products of leptospiral metabolism has been suggested.[2] The cell wall of the leptospire is high in lipid content; component fatty acids vary among leptospiral strains. Lipids are used as a source of energy by leptospires.[2] Saprophytic leptospires invariably possess lipase activity, whereas pathogenic leptospires may be lipase-positive or lipase-negative.[2] Kasarov and Addamiano[103] investigated the lipolytic activity of leptospires on serum lipoproteins. On the basis of their ability to attack these lipoproteins, leptospires can be divided into three groups: (1) strains that degrade lecithin and sphingomyelin, (2) strains that degrade neither lecithin nor sphingomyelin, and (3) strains that degrade lecithin but not sphingomyelin. Virulent leptospires behaved as group 1 and 2 strains, whereas saprophytic leptospires behaved as a group 3 strain.

A prominent feature of experimental leptospirosis is a hemorrhagic diathesis that increases in severity before death.[9] Many investigators have attributed bleeding to depletion of serum prothrombin, to thrombocytopenia, or to both.[49,88,141] Rishavy and colleagues[147] sequenced an orthologue of vitamin K–dependent carboxylase from a pathogenic leptospiral strain and proposed that this acquired activity may result in depletion of host vitamin K. Prothrombin activity can be corrected in children and adults with leptospirosis by the administration of vitamin K; however, the overall severity of the hemorrhagic diathesis is unchanged.[9,80,176] Moreover, thrombocytopenia is not a consistent concomitant in patients who bleed during the course of leptospirosis. For these reasons, the hemorrhagic diathesis most likely reflects widespread damage to the capillary endothelium.[12,42,65,205] The precise mechanism of capillary injury is uncertain, but the damage has been suggested to be induced by toxin.[9] Generally, hemorrhage is restricted to the skin or mucosal surfaces, but rarely, death may follow the occurrence of a massive gastrointestinal hemorrhage or bleeding into a vital organ.[88]

In humans, profound derangement in hepatic function has been demonstrated. Liver cell necrosis, however, occurs infrequently, and, thus, the activity of aspartate aminotransferase and alanine aminotransferase generally is elevated only slightly.

The most striking clinical manifestation of hepatic dysfunction is jaundice. Laboratory evidence of hepatic involvement in human leptospirosis includes the following: impaired bromsulfophthalein excretion, positive cephalin flocculation reactions, reduced esterification of cholesterol, abnormal galactose tolerance test results, impaired production of the clotting factors dependent on vitamin K, decreased serum albumin, and increased serum globulins. These abnormalities have been noted in icteric and anicteric patients with leptospirosis.

Several theories attempt to explain the jaundice of leptospirosis. Early investigation suggested that hyperbilirubinemia was the result of hemolytic anemia.[23] Considerable evidence does not support the hemolytic theory; attempts to demonstrate elaboration of hemolysin by *L. icterohaemorrhagiae*, a serotype associated frequently with hyperbilirubinemia in humans, have failed repeatedly.[9] Variability in the presence of anemia, the poor temporal association between anemia (when present) and the development of icterus, the absence of hemoglobinuria and reticulocytosis, and generally normal fecal urobilinogen values provide evidence that hemolysis is not the cause of jaundice in many patients with leptospirosis.[141]

On the other hand, a significant hemolytic process can be documented in selected patients.[186] Hemoglobinuria has been documented early in the course of leptospirosis, even before the development of jaundice.[8,99] Significant anemia is a feature only of icteric cases of leptospirosis. Most likely, hemolysis occurs in selected cases of severe leptospirosis in humans, and it may contribute to the development of jaundice in some cases.[65,186]

One must conclude that the hepatic manifestations of leptospirosis, including jaundice, most likely are the result of hepatocellular injury because hemolysis is not a consistent finding and neither intrahepatic nor extrahepatic biliary stasis has been observed morphologically or clinically.[9,141,204] Although hepatocellular injury occurs, hepatocellular destruction is not significant, as reflected by complete recovery without residual hepatic dysfunction, even in survivors of severe icteric leptospirosis. Histologic changes that have been observed consistently include disorganization of liver cell plates; variations in the shape and size of parenchymal cells; large numbers of binucleated, trinucleated, and multinucleated cells with bizarre nuclei; proliferation of Kupffer cells with erythrophagocytosis; and cholestasis associated with scant infiltrates of round cells in the periportal spaces.[10,28,65] These changes also have been observed in anicteric patients.[28] Electron microscopy has demonstrated alterations in cell membranes, alteration or destruction of mitochondria, and a predominance of smooth over rough endoplasmic reticulum in hepatocytes because of altered protein turnover.[61]

Additional evidence of hepatocellular damage is provided by the histochemical demonstration of reduced activity of succinic, isocitric, glutamic, and lactate dehydrogenase concomitant with functional alteration,[11] findings suggesting that the fundamental hepatic lesion is subcellular and that critical cellular enzyme systems somehow are affected. Presumably, the hepatocellular damage is not caused by the direct action of leptospiral organisms because the most severe pathologic changes are noted at a time when leptospires are difficult to demonstrate in tissue section.[85] Moreover, leptospires rarely have been identified in sections of hepatic tissue. Elaboration of one or more toxins by leptospires or release of various products after lysis that may be injurious to hepatocytes is the most plausible explanation for hepatic injury at this time.

Renal failure is an important cause of death in patients with leptospirosis. In patients who have died during the first week of disease, renal changes included cloudy swelling or isolated tubular epithelial cell necrosis previously involving the distal convoluted tubule and the ascending loop of Henle, isolated foci of acute vasculitis, segmental thickening of the basement membrane, and isolated areas of mild interstitial edema with lymphocytic infiltrates. In patients who have died during the second week of the illness, numerous foci of tubular epithelial necrosis have been apparent. Interstitial edema and infiltrates of lymphocytes, monocytes, plasma cells, and neutrophils are more prominent. When patients die after the 12th day of illness, the inflammatory infiltrate is widespread and involves the medulla, as well as the cortex.

Foci of tubular necrosis and interstitial inflammation are large, irregular, and packed densely with plasma cells, monocytes, lymphocytes, and neutrophils. Cells lining the lumen of the renal tubules are distended and disorganized and contain hyaline, granular, epithelial, and even bile casts. The glomeruli show mesangial hyperplasia, focal fusion of the foot processes, moderate cloudy swelling of the epithelium in the Bowman capsule, and thickening of the basement membrane.[10,11,53,61,95,107,166,210] The changes seen in epithelial cells may be responsible for the protein leak observed clinically as proteinuria.[48] Leptospires have been demonstrated in the liver or renal tubules and less frequently in the interstices of the renal cortex.[10,65,107]

Hypokalemia may be noted in patients with leptospirosis and acute renal failure. Studies of Abdulkader and associates[1] suggest that these findings are a result of renal potassium wasting facilitated by increased secretion of aldosterone and cortisol.

Although some investigators[95] have emphasized that interstitial nephritis is the fundamental lesion of leptospirosis, renal failure primarily is the result of tubular damage. Interstitial nephritis occurs primarily in patients who have survived until inflammation has had an opportunity to develop. Interstitial nephritis frequently is absent in patients with fulminant disease.[10]

Hypoxia may contribute significantly to the pathogenesis of renal dysfunction in leptospirosis. The focal distribution of the lesions suggests a relationship to impaired renal blood flow. Even in relatively mild cases of leptospirosis in which glomerular function remains unaffected, tubular function as measured by the excretion of para-aminohippurate is reduced markedly.[53] In severe cases, the tubular maximum for para-aminohippurate becomes negligible, and glomerular filtration drops precipitously. On the basis of observations of this type, researchers have concluded that impaired renal blood flow is the fundamental alteration in the nephropathy of leptospirosis. Histochemical and enzymatic studies also demonstrate hypoxic damage and suggest renal ischemia.[11] Diminution of renal perfusion in leptospirosis also is suggested by the clinical occurrence of hypovolemia, hypotension, and circulatory collapse.[28,68,78,108] The reversible oliguria frequently observed during the course of leptospirosis has been attributed to reduced renal blood flow resulting from hypotension, a deficit of extracellular fluid, or both.[65] During periods of oliguria, decreased glomerular filtration rates have been noted. Renal function recovers first by restitution of glomerular function; subsequently, and more slowly, renal tubular function improves.[9]

Hypovolemia or hypotension in patients with leptospirosis may reflect dehydration secondary to vomiting, increased insensible water loss, diminished intake of fluid, and rarely, massive gastrointestinal or pulmonary hemorrhage.[14,65,75,137,211] A decrease in intravascular volume caused by a shift of fluid from the intravascular to the extracellular spaces as a result of severe endothelial injury also may occur.

Rarely during human leptospirosis, adrenal insufficiency occurs after hemorrhagic infarction of the adrenal glands.[170] The vascular collapse observed terminally in fatal cases may reflect, in part, adrenal insufficiency secondary to hemorrhage. However, it cannot be the cause of the reversible state of shock that is noted early during the course of leptospirosis.

Cardiac dysfunction also may lead to hypoperfusion in severe leptospirosis. Focal hemorrhagic myocarditis, acute coronary arteritis, pericarditis, aortitis, and cardiac arrhythmias have been documented. Rarely, sudden death results from congestive heart failure or arrhythmias.[10,60,161,170,181] Cardiac malfunction also may develop secondary to hypertension, hypovolemia, electrolyte imbalance, or uremia. Peripheral vascular collapse in leptospirosis most often occurs irrespective of any cardiac involvement, obvious dehydration, or massive hemorrhage. Regardless of the etiologic factor, shock is a common occurrence in the course of severe leptospirosis.

Pulmonary lesions in leptospirosis generally are the result of hemorrhage rather than acute inflammation. In selected cases, acute inflammation is noted but usually reflects a secondary pyogenic infection. Localized or confluent hemorrhagic pneumonitis may be noted, and petechial and ecchymotic hemorrhages are seen throughout the lungs, pleura, and tracheobronchial tree.[163] Acute hemorrhagic lobar pneumonia and massive hemoptysis have been observed in fatal cases.[13,137,211] Silverstein[163] suggested that pulmonary capillary damage was the result of a toxin because leptospires had not been demonstrated in the lung, although Yang and Hsu[211] subsequently reported a fatal case of pulmonary hemorrhage in which leptospires were visualized directly in the lung parenchyma by silver stain techniques. Alveolar septal deposition of immunoglobulin and complement may play a role in the pathogenesis of pulmonary hemorrhage.[130,211]

Central or peripheral nervous system involvement may be striking. Most investigators agree that signs of meningeal inflammation cannot be attributed to invasion of the meninges by leptospires. Leptospires frequently are isolated from cerebrospinal fluid (CSF) that otherwise is normal; thus, reaction to the presence of leptospires in the meninges appears to be minimal. The leptospires disappear rapidly after the onset of meningeal signs, usually during the second week of disease. Because meningeal reaction occurs only after the development of antibody, leptospiral meningitis has been suggested to be a reflection of an antigen-antibody reaction.[16,88] Meningitis as a result of hypersensitivity may explain the absence of pleocytosis in the early stages of meningeal involvement, the abrupt onset of meningitis at the end of the first week of leptospiral disease, and the good prognosis of patients with leptospirosis who have involvement of the central nervous system.

Pathologic examination of the meninges may reveal nothing[10] or may show thickening of the meninges, a slight increase in the number of arachnoid cells, and a predominance of mononuclear cells in the exudate.[13]

Uncommon features of leptospirosis include encephalitis, myelitis, radiculitis, and peripheral neuritis. When present, they occur during the second week of illness.[65] These neurologic findings also may be the result of hypersensitivity reactions similar to those seen in other postinfectious encephalitis syndromes.[121,122] Koppisch and Bond,[107] however, demonstrated perivascular infiltration of blood vessels in the spinal cord, basal ganglia, hippocampus, and white matter of the cerebellum and in the subcortical areas of the cerebrum; these changes are not pathologic features of postinfectious viral encephalitis.[10,84] In certain cases, neurologic manifestations have been attributed to subarachnoid, peripapillary, and subdural hemorrhages.[37,39]

The aqueous humor provides a protective environment for leptospires; despite the development of high antibody titers in serum, leptospires may remain viable in the anterior chamber of the eye for many months.[4] Persistence of leptospires in the aqueous humor may be responsible for the recurrent, chronic, or latent uveitis syndromes that have been seen in patients with leptospirosis. The acute ocular inflammatory response that occurs during the leptospiremic phase of the disease generally disappears without complications and with little or no opacification of the vitreous. Chronic ocular involvement occurs less commonly but is more significant because anterior uveal inflammation and vitreous opacification may occur. The development of hypopyon during the course of leptospirosis may be followed by loss of vision. Pathologic descriptions of ocular tissue from patients with leptospirosis are limited, which precludes a better understanding of the pathogenesis of the ocular involvement.

The myalgia reported so frequently in patients with leptospirosis most likely relates to the pathologic process that has been noted.[108] Biopsy specimens obtained early in the course of lepto-

spirosis have vacuoles within the cytoplasm of the myofibril. Subsequent focal cytoplasmic changes include fragmentation and loss of cellular detail, which results in homogeneous or irregular acidophilic masses. The polymorphonuclear infiltrates that may be seen in affected areas are minimal, even in muscle fibers that are affected severely. Infiltration by sarcoblasts, with new myofibril formation, ultimately leads to healing without significant fibrosis.[29,99] Histologic changes in the muscles of patients with mild infection generally are minimal.[65] Pathologic evidence of myopathy resolves completely and promptly in most cases; pathologic changes usually are absent in the muscles of patients dying in the second week of disease.[186]

The selective involvement of certain muscle groups in some patients with leptospirosis is not explained. Any or all muscles may be affected; generalized myalgia occurs commonly. Myalgia is an early clinical feature concurrent with leptospirosis, and these clinical findings are correlated with the timing of the histologic changes in muscle. Generally, muscle pain subsides promptly as leptospiral agglutinin titers develop and the septicemic stage of leptospirosis ends. These observations are consistent with active invasion of skeletal muscle by leptospires rather than a toxin-related effect.[108,171] Antigens of leptospires have been demonstrated by fluorescent antibody techniques in patients infected with *L. icterohemorrhagiae*.[162]

The epicardium, endocardium, and myocardium all may be involved during leptospirosis. Arean[9] described focal or diffuse epicardial hemorrhages with or without lymphocytic and monocytic infiltrates in 10 fatal cases. In four patients, mesothelial desquamation and fibrin formation in the pericardial cavity also were noted. Myocardial changes included focal or diffuse lesions characterized by interstitial edema with fragmented fibers and infiltrates of monocytes, lymphocytes, and plasma cells. Neutrophilic infiltrates also were observed in most necrotic foci. Aortic insufficiency caused by focal endocarditis involving the aortic valves likewise was noted.[10] Patchy interstitial edema, cellular infiltrates, necrosis, and focal hemorrhagic lesions have been detected in other patients.[84,186] In most cases, these findings are not mirrored by the clinical findings. Rarely, leptospires have been demonstrated in myocardium.[65]

Except for focal hemorrhages, no characteristic lesions have been noted in the adrenal glands, lymph nodes, spleen, gastrointestinal tract, pancreas, ureter, or bladder. Interstitial edema with monocytic and lymphocytic infiltrates has been found in testicular tissue associated with impaired spermatogenesis.[84] Bone involvement in leptospirosis is not a significant feature clinically or pathologically, and no explanation accounts for the apparent failure of leptospires to proliferate in bone.[98]

CLINICAL MANIFESTATIONS

Leptospirosis is an acute systemic infection characterized by extensive vasculitis. Serologic surveys in human populations indicate that a large number of subclinical infections also occur. Surveys of veterinarians and packinghouse and abattoir workers reveal positive leptospiral titers in 5 to 16 percent of people tested.[100,123,125,187]

A low index of suspicion for this disorder by physicians, coupled with the diversity and nonspecificity of its manifestations, accounts for the significant number of cases that go unrecognized. In one series of 483 proven cases, only 17 percent were diagnosed initially as leptospirosis.[26]

The incubation period generally is 7 to 12 days, but a range of 2 to 20 days has been noted.[156,173,197] The incubation period does not vary significantly among serotypes and is not of prognostic significance. Variability in incubation period may be attributed to the dose of virulent organisms to which the host is exposed and to the portal of entry of the organism.[156,173,197]

The clinical course of leptospirosis varies, but generally it is predictable: both anicteric and icteric leptospirosis follow a biphasic course (Fig. 154–1).

The first stage (septicemic phase) is characterized by acute systemic infection. The onset of symptoms is abrupt. This phase terminates after 4 to 7 days; symptomatic improvement and defervescence coincide with disappearance of leptospires from the blood, CSF, and all other tissues, with the exception of the aqueous humor and renal parenchyma. Antibody titers to leptospires develop rapidly; this immune response heralds the second, or "immune," stage of the illness.

The immune, or second, stage lasts 4 to 30 days. Leptospiruria is prevalent and continues for 1 week to 1 month; generally, it is unaffected by antibiotic therapy. Meningitis or hepatic or renal manifestations, when present, reach their peak intensity during this stage of the disease.

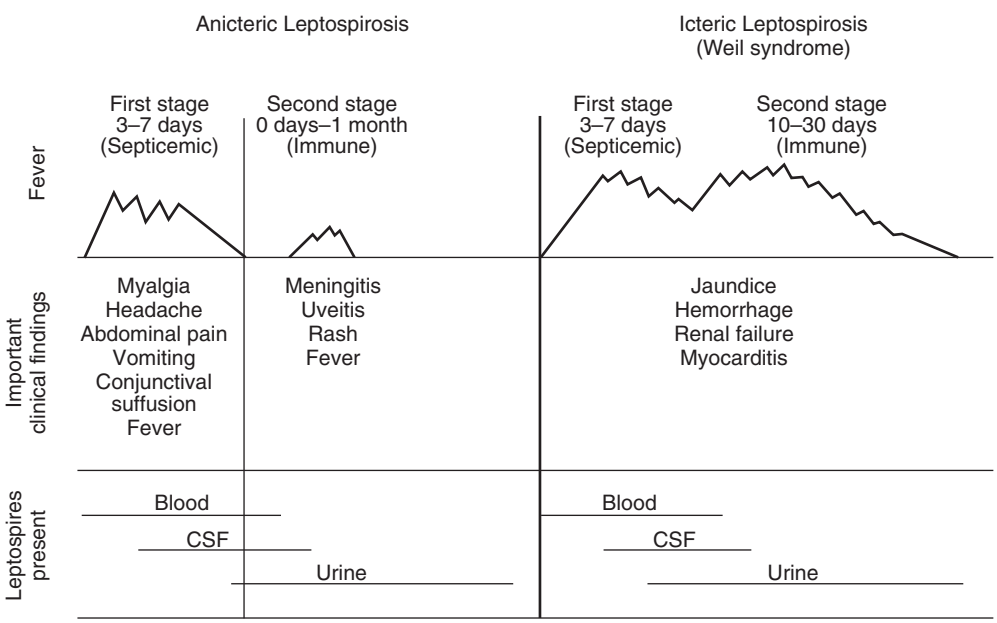

Figure 154–1 Clinical course of leptospirosis: anicteric and icteric disease.

ANICTERIC LEPTOSPIROSIS

Ninety percent or more of all patients with leptospirosis are anicteric. They frequently escape definitive diagnosis because jaundice and azotemia are absent. The onset of the septicemic phase of anicteric leptospirosis is abrupt[66] and heralded by fever, malaise, headache, myalgia, and occasionally, prostration and circulatory collapse.[116] Chills, remittent fever, headaches, severe myalgia, and abdominal pain are prominent for 4 to 7 days. Fever defervesces by lysis, and other symptoms resolve. Death is an extraordinarily rare occurrence in the first stage of anicteric illness. Some patients with anicteric leptospirosis do not experience a biphasic illness and remain asymptomatic after the first week.[66]

The second phase of anicteric disease may be characterized by fever, uveitis, rash, headache, and meningitis. If present, fever usually is of brief duration and has a lower peak than that of the septicemic phase.[65,66,92] Maximum temperatures range from 38.2° C to 40.6° C (100° F to 105° F), with one or more daily peaks. Recurrence of fever 2 or 3 weeks after leptospirosis resolves is not unusual, but no reports of the isolation of leptospires from blood document relapse on these occasions. Relapse generally occurs when the immune response of the host is peaking and at a time of maximal leptospiruria, which suggests an allergic or immune basis for the febrile episodes.[28] Headache may be intense and usually is not controlled well by analgesics. Typically, it is frontal in distribution and characterized as bitemporal or occipital. It may be associated with retrobulbar pain.[52,65,66] Persistence or recurrence of headache after termination of the septicemic phase of disease generally indicates the onset of meningitis. The factors responsible for headache in the septicemic phase of leptospirosis are unknown.

Restlessness, nocturnal confusion, mood disturbances, and mild alterations in consciousness usually occur briefly and commonly in both stages of leptospirosis.[65,92] Delirium, hallucinations, psychotic behavior, and suicidal tendencies have been reported.[65,92,160]

Anorexia, nausea, vomiting, and abdominal pain may be reported in both stages of anicteric disease. Constipation, diarrhea, and gastrointestinal hemorrhage also have been documented.[65,116,131] Generally, hemorrhagic complications are associated exclusively with icteric disease.

Physical examination during the septicemic stage may reveal dehydration, muscle tenderness, conjunctival suffusion, generalized lymphadenopathy, hepatosplenomegaly, and rashes that may be macular, maculopapular, erythematous, urticarial, petechial, purpuric, hemorrhagic, or desquamating. Skin lesions are most prominent over the trunk, but any area of the body may be affected. Pretibial eruptions have been noted in patients with infection caused by *L. autumnalis*, but other serotypes also may cause disease with pretibial eruptions. Recurrent, transient urticarial eruptions have appeared for many days after resolution of the other manifestations of leptospirosis. Pharyngitis, rales, arthritis, and nonpitting edema occur less commonly.* Tachycardia is a common occurrence, and cardiac arrhythmias are noted occasionally.[136,170] Hypotension rarely occurs in anicteric leptospirosis.[66]

Muscle pain and tenderness may be generalized, but the muscles of the calf, lumbosacral spine, and abdomen are affected most frequently. Tenderness and rigidity of the abdominal wall may suggest the possibility of an acute surgical abdomen. Tenderness of the muscles adjacent to the cervical spine often causes nuchal rigidity in patients without meningeal involvement. The muscle tenderness usually subsides with termination of the septicemic stage of the disease.

*See references 6, 10, 27, 52, 66, 92, 99, 116, 150, 191, 197.

Conjunctival suffusion, photophobia, ocular pain, and conjunctival hemorrhage are more specifically helpful diagnostic signs. Chemosis and inflammatory exudates generally are absent despite marked conjunctival infection. In anicteric disease, conjunctival infection involves primarily the bulbar conjunctiva only. It appears by the third day of illness and disappears 3 days to 3 weeks later.

Abdominal pain and tenderness, when associated with vomiting and hypoactive bowel sounds, clearly suggest the possibility of a surgical abdomen and present a challenging diagnostic problem because acute intra-abdominal catastrophes may complicate the natural history of this disease. Nonobstructive toxic dilation of the gallbladder requiring cholecystotomy has been noted repeatedly in children with leptospirosis (Fig. 154–2). Pain of this type must be differentiated from myositis, subperitoneal or subserosal hemorrhage, abdominal wall causalgia, or pancreatitis, all of which may occur in some children with anicteric or icteric disease.

Pulmonary involvement may be observed in anicteric patients, generally during the septicemic phase, and usually is manifested as a dry, hacking cough, occasionally productive of blood-stained sputum, or by the finding of infiltrates on a chest radiograph.[27,140,163] Hemoptysis, chest pain, respiratory distress, and cyanosis appear rarely during anicteric disease.[140] Hemoptysis, when present, clears in 3 to 5 days. Physical examination of the chest may reveal rales, evidence of consolidation, or a pleural or pericardial friction rub.

Chest radiographs may show (1) confluent infiltrates or massive consolidation representing larger areas of pulmonary hemorrhage; (2) small, patchy, snowflake-like lesions in the periphery of the lung fields that are restricted to a few intercostal spaces or disseminated widely; and (3) solitary, patchy lesions with ill-defined margins.[140,206] Of these radiographic appearances, the second is most common. Small pleural effusions are rare occurrences in anicteric disease,[206] and hilar adenopathy has not been described. Although the chest radiograph may help delineate the extent of pulmonary disease, it does not provide information that could be considered pathognomonic of leptospirosis.

Other signs and symptoms of the septicemic phase of anicteric leptospirosis that have been reported include parotitis,[23] orchitis,[177] epididymitis,[92] prostatitis,[92] otitis media,[27] arthralgia,[27,65,92] and monoarticular or polyarticular arthritis.[61]

The hallmark of the immune phase of anicteric leptospirosis is meningitis, and it is reflected by CSF pleocytosis with or without meningeal symptoms or signs. During the leptospiremic phase, leptospires may be found in the subarachnoid space unassociated with the presence of inflammatory cells. As an antibody titer develops, leptospires are cleared rapidly from CSF, and an inflammatory response develops.[65] If CSF is examined during the second week of illness in all patients with anicteric leptospirosis, a meningeal reaction can be demonstrated in more than 80 percent, but only 50 percent of these patients have clinical signs and symptoms of meningitis.[40,65] The severity of meningitis varies and does not correlate with the severity of other clinical manifestations of leptospirosis. Symptoms referable to the nervous system usually subside within 1 or 2 days but rarely persist for 2 or 3 weeks. The CSF pleocytosis may persist for 2 to 3 months but generally disappears within 7 to 21 days.[65] In some cases, patients are asymptomatic during the septicemic phase of leptospirosis but seek medical attention during the immune phase because of headache, vomiting, and nuchal rigidity. Papilledema rarely has been observed in patients with leptospirosis.[37]

Lumbar puncture may reveal CSF pressures varying from normal to 350 mm H_2O. Mean values generally are less than 200 mm H_2O.[94] Cell counts within CSF vary from normal to more than 500 cells/mm^3; generally, fewer than 500 cells have been reported.[65,92] Polymorphonuclear leukocytes predominate early in the immune phase, but mononuclear cells subsequently

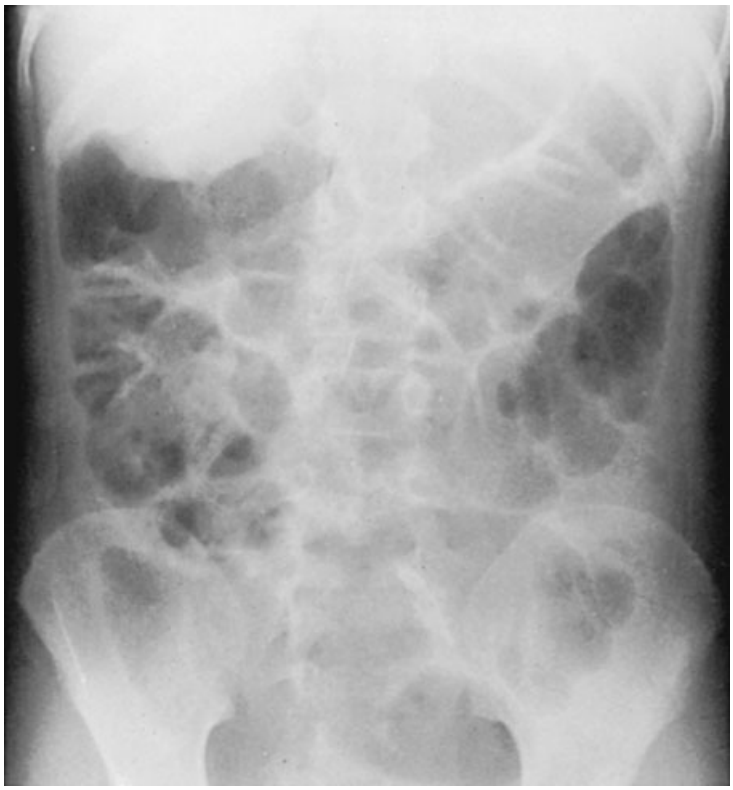

Figure 154–2 Radiograph demonstrating a dilated, opaque gallbladder protruding from the inferior margin of the liver. Nonobstructed toxic dilation of the gallbladder is present in a child with leptospirosis.

predominate. Protein concentrations within CSF range from normal to 300 mg/dL. In some cases, protein values have been elevated in the absence of pleocytosis. Abnormal values may persist for several weeks after the clinical symptoms resolve.[27,40,92,117,118] Glucose concentrations within CSF generally are normal.[40,66,117]

Encephalitis, focal weakness, spasticity, paralysis, nystagmus, peripheral neuritis, cranial nerve paralysis, seizures, radiculitis, visual disturbances, myelitis, Guillain-Barré syndrome, or acute disseminated encephalomyelitis may appear with or subsequent to the immune stage of anicteric disease.* Generally, these symptoms resolve, but complete resolution may require several weeks to months. Neurologic sequelae secondary to central nervous system hemolysis may occur.[37,39]

The anterior uveal tract may be affected as early as the third week of illness, but symptoms may be found up to 1 year after the onset of leptospirosis. Conjunctival suffusion (characteristic during the septicemic phase) is not found in the immune stage of the disease. Rather, iritis, iridocyclitis, and, occasionally, chorioretinitis are noted.[26,65,66,92] Uveal involvement may be unilateral or bilateral and may occur as a single, self-limited episode, as recurrent episodes, or as a chronic unrelenting process.[33,65] The severity of the uveitis does not correlate with the severity of other clinical manifestations. When uveitis is transient or self-limited, complete healing is the rule, but in some cases, blindness and cataract formation are noted.

The precise incidence of involvement of the uveal tract is unclear because symptoms may be minimal or may not appear until after other clinical manifestations have resolved completely. The generally benign course of uveitis may be attributable to the capacity of leptospires to survive in the aqueous humor without eliciting an intense inflammatory response.[66] Despite the presence of high titers of specific antibodies to leptospires in serum, antibodies to leptospires are absent or found in low titer in the aqueous humor.

*See references 7, 20, 44, 65, 68, 92, 117, 121, 146, 200.

Leptospiruria is the rule during the immune stage of anicteric leptospirosis, and it is not associated with impaired renal function. In contrast to many animal species, humans do not serve as a reservoir for leptospires; leptospiruria is transient. In anicteric patients, proteinuria, pyuria, microscopic hematuria, and mild to moderate azotemia may be observed.[27]

The white blood cell count may be low, normal, or elevated. Neutrophilia is the rule, regardless of the total white blood cell count. Leukocytosis generally is associated with hepatic involvement. Anemia is an inconsistent finding; when present, it may be attributable to blood loss, vascular damage, or hemolysis. In the absence of blood loss, significant anemia is not a manifestation of anicteric cases. The erythrocyte sedimentation rate consistently is elevated.

ICTERIC LEPTOSPIROSIS (WEIL SYNDROME)

The term *Weil syndrome* should be applied to define a form of leptospirosis that is distinctive in clinical expression but nonspecific with respect to serotypic etiologic agents. In addition to having the symptoms and signs of anicteric leptospirosis, Weil syndrome is set apart by the presence of impaired hepatic and renal function, vascular collapse, hemorrhage, severe alterations in consciousness, and a high mortality rate.

Weil syndrome may be heterogeneous in its manifestations, and the course may be dominated by symptoms of renal, hepatic, or vascular dysfunction. Jaundice and azotemia may be so severe that the biphasic course of illness is not observed. Fever may persist without defervescence between the septicemic and immune stages and is more prominent and of longer duration during the immune stage than in anicteric cases. The mortality rate, despite adequate supportive care, is between 5 and 10 percent.

Jaundice remains the hallmark of Weil syndrome. The intensity of jaundice varies; a maximum total serum bilirubin concentration in the range of 60 to 80 mg/dL has been reported.[117] Usually, the bilirubin concentration is less than 20 mg/dL. Both

direct- and indirect-reacting bilirubin levels increase, but an increase in the direct fraction usually accounts for most of the elevation in bilirubin.[174] Jaundice may occur as early as the third day of illness or may not appear until the second week.[13,66] The serum bilirubin concentration peaks within the first 7 days after the onset of jaundice in 85 percent of cases.[141]

Modest elevations in serum alkaline phosphatase and depressed activity of plasma prothrombin are noted occasionally.[141] Hypoprothrombinemia responds uniformly to parenteral administration of vitamin K. Serum albumin may be depressed; concentrations of 2 to 2.5 g are not uncommon.[174] Aspartate aminotransferase and alanine aminotransferase are elevated minimally; these values rarely exceed 100 and 200 units, respectively. The urine may contain bilirubin and urobilinogen.

Hepatomegaly is found in approximately 24 percent of patients, a frequency that is no greater than that in anicteric cases.[65] Transient biliary obstruction, probably intrahepatic, may occur, but no evidence that obstructive phenomena are the primary mechanism of impaired hepatic function exists. Even in severely icteric cases, acholic stools generally are not observed.[65,92] Pruritus has been reported rarely in patients with leptospirosis.[65] The presence of abnormal urinary urobilinogen values in the absence of acholic stools suggests patency of the biliary tract in most cases.[141]

In some reports of children with leptospirosis, acalculous cholecystitis has been seen in 55 percent of cases.[175] In these patients, right upper quadrant pain, tenderness, and a palpable mass were present. Abdominal radiographs confirmed the presence of a mass in the region of the gallbladder. When cholecystotomy was performed, a massively distended gallbladder containing colorless bile was noted. Routine aerobic and anaerobic cultures of bile were negative, but cultures for leptospires were positive.

Hepatic dysfunction is not an important cause of death in patients with leptospirosis. It is present, however, in most patients who die of this disease, and, conversely, a fatal outcome is extremely rare in the absence of hepatic dysfunction.[27] Renal dysfunction, cardiovascular collapse, and hemorrhagic complications occur most often in patients whose icterus is most prominent.

Renal dysfunction may be observed in all forms of leptospirosis, regardless of the severity of disease or the serotype causing infection.[27,65,92] Symptoms attributable to functional renal impairment generally are observed only during icteric leptospirosis.[13,27,65,66,197] In the leptospiremic phase, abnormal urinalysis results are noted in as many as 80 percent of cases.[27,65,175] Proteinuria is the most frequent abnormality and generally is mild. Hyaline or granular casts and cellular elements (red and white blood cells) may be found in the urinary sediment. Microscopic or gross hematuria is seen in many patients and most likely reflects the presence of a hemorrhagic diathesis rather than glomerular injury.[200]

Fatal cases of icteric leptospirosis in which urinalysis results were normal have been reported.[13] The abnormalities in urinary sediment and proteinuria may persist for weeks in patients without significant azotemia.[22]

Oliguria or anuria may be noted as early as the third day of illness but occurs more commonly after the first week. Generally, blood urea nitrogen values remain below 100 mg/dL, but values may exceed 300 mg/dL in some cases.[92,132] The height of the blood urea nitrogen value is not of prognostic value in individual cases, although in groups of patients, it correlates well with outcome.[186]

Azotemic patients with leptospirosis can be divided into two groups: (1) those with decreased renal perfusion (ratio of urine osmolality [U_{Osm}] to plasma osmolality [P_{Osm}] of about 2:1) and a good response to fluid administration and (2) those with a U_{Osm}-to-P_{Osm} ratio close to 1:1, with impaired resorption of sodium and water from the renal tubules and no response to administration of fluids. The manifestations of the second group of patients are those of acute tubular necrosis. The factors responsible for oliguria in the first group of patients (those with prerenal azotemia), including hypotension, shock, and volume depletion, if uncorrected, ultimately may progress to acute tubular necrosis as well.

Anuria is an ominous sign, whereas diuresis is a good prognostic omen.[13] The impairment in renal function may persist, and fatalities have been recorded after the onset of diuresis.[132] Hyposthenuria can persist for months in some cases.[66] Some evidence of renal disease has been demonstrated by renal function tests and renal biopsies for as long as 6 months after the onset of leptospirosis.[18] Renal failure is the principal cause of death in patients with leptospirosis, but it generally is reversible in time. Usually, renal function improves by 6 months after acute infection.[57]

Cardiac involvement is a relatively infrequent occurrence, but when present, congestive heart failure and cardiovascular collapse may occur.[66,99] Electrocardiographic changes are seen in all forms of leptospirosis.[136] In one series of patients, electrocardiograms obtained during the first week of illness were abnormal in 90 percent of patients at a time when none had signs or symptoms of congestive failure, pericarditis, or hypotension.[136] The electrocardiographic abnormalities disappeared by 10 days in most cases. The electrocardiographic changes that have been described often are nonspecific findings common to many infectious diseases or attributable to fever alone.[136,151]

Cerebrovascular accidents may occur in patients with leptospirosis.[111] In a study of 21 cases in which postmortem examination was performed, subarachnoid hemorrhage was described in 1 case, cerebral hemorrhage in 2, and recent cerebral infarction in 1.

Hyponatremia is a rather consistent finding in patients with severe icteric leptospirosis. The hyponatremia appears to be the result of (1) failure of the sodium pump, which causes sodium to move intracellularly in exchange for potassium, and (2) redistribution of fluid such that the extracellular fluid space is expanded at the expense of the intracellular space. Hyponatremia in these patients may be unresponsive to either sodium replacement or fluid restriction. It is treated best by fluid restriction, which can be continued unless systemic blood pressure falls. Clinical improvement in the patient generally follows a spontaneous increase in serum sodium, which may occur before any other evidence of clinical improvement is noted.

INDICATORS OF PROGNOSIS

Attempts have been made to identify prognostic factors associated with leptospirosis. Such studies, which aimed to evaluate the prognostic significance of certain clinical and laboratory variables at different stages of disease, have produced variable results. Hypotension, oliguria, hyperkalemia, the development of pulmonary rales, crackles, or rhonchi, and electrocardiographic repolarization abnormalities each have been associated with a poor prognosis,[58,64,114,134,184] although conflicting data exist about the implications of hypotension.[64] In a small series of 12 patients, Truccolo and colleagues[192] reported death of all patients with a leptospiral load greater than 10^4 organisms per milliliter as determined by quantitative polymerase chain reaction (PCR). Chang and colleagues,[45] in a series of 11 patients with late leptospirosis, reported mortality in all patients with hepatitis and a disproportionate aspartate transaminase level, as defined by a ratio of peak aspartate transaminase to peak alanine transaminase greater than 3. Respiratory failure, hemoptysis, metabolic acidosis, and throm-

bocytopenia also have been associated with increased mortality rates.[184]

LABORATORY DIAGNOSIS

Whenever possible, the physician should use laboratory facilities in which culture and serologic tests for leptospirosis are performed routinely. We recommend that specimens be sent to the standard reference laboratory, the National Leptospirosis Laboratory at the CDC in Atlanta. Despite proper collection and handling of specimens, obtaining laboratory confirmation of cases of leptospirosis may be difficult, even for facilities with skill in this area.

A confirmed case of leptospirosis, as defined by the U.S. Department of Health and Human Services, fulfills one of the following criteria: (1) clinical specimens that are culture-positive for leptospires or (2) clinical symptoms compatible with leptospirosis and either seroconversion or a fourfold or greater rise in the microscopic agglutination titer between acute and convalescent serum specimens obtained 2 or more weeks apart and studied at the same laboratory.

Presumptive leptospirosis is defined as the presence of clinical symptoms that are compatible with leptospirosis and a microscopic agglutination titer of 1:100 or greater, a positive macroscopic agglutination slide test reaction on a single serum specimen obtained after the onset of symptoms, or a stable microscopic agglutination titer of 1:100 or greater in two or more serum specimens obtained after the onset of symptoms.

IDENTIFICATION BY CULTURE

Leptospires can be recovered from blood or CSF obtained from patients during the septicemic stage of illness or from urine during the immune stage. Other than these body fluids, only tissue sections obtained by biopsy or at necropsy are sources from which organisms can be recovered. Rarely, organisms are isolated from intraocular fluid obtained during convalescence.[4]

Media for the cultivation of leptospires generally contain a buffered solution, with or without peptone and with or without 0.1 to 0.2 percent agar to which rabbit serum has been added to provide a final concentration in the medium of 5 to 10 percent. In addition, a pH between 7.2 and 7.8 appears to be essential. Clinical material obtained for culture frequently is contaminated; antimicrobial agents, including neomycin, vancomycin, or bacitracin, added to leptospiral media in low concentration have been found to be effective in reducing contamination and exert little if any effect on leptospires.

For routine use, Fletcher semisolid medium[76] or EMJH semisolid medium[101,173] is recommended. Stuart medium[178] has been used to prepare and maintain antigens for serologic tests. Tween 80–albumin medium (OAC) was developed not long ago and is available commercially. This medium seems to be superior for primary isolation of leptospires.

Several solid media are available but appear to be most useful for isolation and purification of leptospires from contaminated natural material such as water.[179,196] Preparation, use, and maintenance of these solid media and other media are described in other works.[119,179,196]

Multiple cultures should be obtained from patients with leptospirosis because the concentration of organisms in blood at any point in time is low.[101] Freshly drawn blood is most desirable, but leptospires may remain viable in anticoagulated blood for as long as 11 days.[178] Blood should be inoculated into several tubes of semisolid media. The number of drops of blood placed into each tube should be varied (one to four drops). Excessive amounts of blood inhibit the growth of leptospires; hence, a small inoculum yields the best results.[76] Cultures are incubated at 28° C to 30° C (82.4° F to 86° F) in the dark for 6 weeks or longer.

In semisolid media, leptospires grow in a concentrated ring about 0.5 to 1 cm below the surface. Growth may not be detected in Fletcher semisolid medium for several weeks but may occur earlier in polysorbate medium.

Contaminated specimens or suspensions of primary cultures in which contamination is suspected may be inoculated into hamsters. Upon death of any animal, phlebotomy or necropsy is performed, and sections of liver, kidney, and brain then are recultured in appropriate semisolid media.

If collected during the septicemic phase, CSF may be cultured in the same manner as blood.

Urine serves as the main source from which leptospires can be isolated during the immune and convalescent phases of leptospirosis. Clean-voided urine may be inoculated directly into an appropriate semisolid medium. Urine specimens must be diluted with sterile, buffered saline solution to ensure growth.[186] Best results are obtained by adding 0.1 mL of urine to 0.9 mL of buffered saline before inoculation into 5 mL of semisolid medium. This procedure can be continued with four additional dilutions. Other bacterial contaminants that may be present in undiluted urine cultures generally do not survive in these cultures after dilution.[179]

IDENTIFICATION BY MEANS OTHER THAN CULTURE

The morphologic appearance of all members of the genus *Leptospira* is similar. They are slender, threadlike organisms about 0.1 μm in diameter and 6 to 12 μm in length, tightly coiled on their long axis. Like other spirochetes, they cannot be seen in wet preparations by lightfield microscopy, but on darkfield examination they may be observed readily. For the detection of one leptospire per high-power field by darkfield examination, a concentration of 10,000 to 20,000 leptospires per milliliter of fluid is needed.[196] At best, darkfield examination should be considered an aid that may suggest but not establish a diagnosis of leptospirosis.

Leptospires can be stained by several silver impregnation techniques.[35,105,119,179,196] The modified method of Van Orden has been used at the CDC for demonstrating organisms in sections of liver, kidney, or other tissues. Infecting serotypes cannot be differentiated by silver impregnation techniques. Leptospiral antigen also has been detected with the use of an immunoperoxidase staining procedure.[73]

Fluorescent antibody techniques may be applied successfully to the detection of leptospires in urine or tissue.[127,128,179,196] This test is based on specific antigen-antibody reactions using fluorescence-tagged antisera. In theory, the fluorescent antibody reaction should demonstrate distorted and fragmented, as well as whole, organisms, but caution is required. Control specimens that have been treated with unlabeled antiserum before the addition of fluorescein-labeled antiserum should be used.[119] The control specimen should not fluoresce. The fluorescent antibody technique may provide the physician with useful information in the course of the disease in some patients. Positive results, however, are considered only presumptive evidence of infection.

In addition to this technique, DNA hybridization techniques or nucleic acid amplification procedures, including PCR protocols using leptospiral-specific cDNA probes or oligonucleotide primers, can be used to detect the presence of leptospires in body fluids or culture supernatants. These techniques are being developed in the laboratories of the Leptospirosis Branch at the CDC, but proof of their superiority in terms of sensitivity or specificity

in detecting leptospiral organisms in body fluids or other clinical samples has not been established yet.

SEROLOGIC TESTS

Evaluation of serologic findings to supplement clinical and epidemiologic information generally is recommended as a first step in establishing a diagnosis of leptospirosis. One of the most widely used specific serologic tests for leptospirosis has been the microscopic agglutination test (MAT), in which live antigen is used. This test is time-consuming and potentially hazardous to the technician but is considered the reference test against which all other tests are evaluated. Formalinized antigens can be used for the MAT, and they are preferred in some laboratories, but the titers obtained are lower than those obtained with live antigen, and more cross-reactions with heterologous serotypes occur. Generally, serum is used for the MAT or other agglutination tests, but CSF, urine, bile, or aqueous humor may be used as well.[119]

The CDC uses a standard panel of commonly occurring serovars representing 20 serogroups for routine performance of the MAT. However, because of geographic differences in serovar distribution, serovars are added or changed depending on the region where exposure occurred and the location from which specimens were obtained. Killed antigens remain stable for at least 12 months and are available commercially either individually or in pools. Sulzer and associates[179] have provided detailed descriptions of methods for performance of the MAT. Modifications of the MAT have been developed and include the semimicromethod and microtiter techniques.[79]

A newer serologic test involving diffusion in gel, the enzyme-linked immunosorbent assay (ELISA), has been compared with the MAT for the serologic diagnosis of leptospirosis.[56,202] The results suggest that this test is a viable alternative to the MAT because of its sensitivity, potential for standardization, and simplicity. Variations of this test have been developed; rapid serodiagnosis of leptospirosis with the use of an IgM-specific dot ELISA has proved to be as sensitive and specific as MATs.[201,207] The dot ELISAs are inexpensive and simple to perform, and they use minute volumes of leptospiral antigens.

The IgM-PK ELISA, an assay for IgM using a proteinase K–treated antigen, was compared with the Leptoteste-S macroagglutination test (CDC, Atlanta, GA) and with the MAT for the diagnosis of leptospirosis. All three tests were comparable in their ability to detect the presence of leptospirosis. Both the IgM-PK ELISA and the Leptoteste-S differed statistically from the MAT in terms of the positivity of acute-phase sera. Thirty-eight percent of patients with leptospirosis were identified earlier with either test than when the MAT was used. The IgM-PK ELISA, which had a sensitivity of 89.9 percent and a specificity of 97.4 percent, has been suggested as the test of choice for laboratories that are equipped to perform ELISA.[144]

A slide agglutination test available commercially has been compared with the ELISA IgM and has yielded equivalent results for the diagnosis of leptospirosis. The slide agglutination test is inexpensive and can be performed more quickly and more easily than ELISA, thus rendering it a useful test for laboratories that are less well equipped than those in which IgM ELISA currently is performed.[34]

Lepto Dipstick (Royal Tropical Institute, Amsterdam, The Netherlands), a dipstick assay for detection of *Leptospira*-specific IgM antibodies in serum, has been studied as a method for the diagnosis of leptospirosis in situations in which laboratory facilities may not be available.[168] Lepto Dipstick results correlated well (80% to 96.7% observed agreement, kappa value of 0.62 to 0.92) with results obtained by the IgM ELISA leptospiral antigen detection method.[19,90] A 93.2 percent agreement (kappa value of 0.66) was reported between the Lepto Dipstick and MAT, along with a high number of false-positive results as well.[19] The dipstick test is a valuable and useful tool for rapid screening for leptospirosis and may be useful in the field for detecting and monitoring outbreaks of leptospirosis.

Other tests that may be used for the serologic diagnosis of leptospirosis include a complement-fixation assay,[46] a hemolytic test,[46] an indirect immunofluorescent test,[190] an erythrocyte-sensitizing substance test,[46] countercurrent immunoelectrophoresis,[129] and flow cytometry light scatter analysis.[212] These tests are genus-specific and may yield positive results earlier in the course of leptospirosis than the agglutination tests do. Their results also revert to negative earlier; therefore, these tests are of little value for serologic surveys. They may be of value in distinguishing current from past infections when agglutination test results are equivocal.[178,179] Other works[46,55,143,190,196] provide specific details concerning the use of these techniques.

An indirect hemagglutination test offers the advantage of detecting antibodies as early as the fourth day after the onset of illness. It is genus-specific, is less time-consuming, and requires just one antigen in the test system. It has excellent sensitivity and specificity, and some investigators have suggested that it may replace the MAT as the screening test of choice.[179] Effler and colleagues,[67] however, reported discouraging results when this test was used for the diagnosis of leptospirosis in Hawaii.

Agglutination tests have been considered to be serotype-specific. Because of the antigenic complexity of leptospires, however, cross-agglutination reactions occur; serotypes that belong to the same serogroup cross-react at high titers. Early in the course of leptospirosis, heterologous reactions may be stronger than homologous reactions. Because of these paradoxical cross-reactions, one should not depend on serologic determination alone to define the infecting serotype. When agglutination tests are performed on serial specimens over the course of time, the homologous reaction becomes the dominant one in most cases. Performance of agglutination absorption studies may be necessary to define the infecting serotype in some cases. The antigen (serotype) that absorbs out agglutinin to all the serotypes in a serogroup most likely is the infecting serotype.[119,174,194]

A passive microcapsule agglutination test that uses chemically stable microcapsules instead of sheep erythrocytes has been developed.[13] When compared with the MAT, the passive microcapsule agglutination test showed a relatively greater degree of genus specificity and 4- to 32-fold higher titers. The sensitized microcapsules were stable for at least 1 year. This test is simple to perform and reproducible and can be used readily in the routine laboratory. Moreover, the test appears to be more sensitive than is the MAT in the early stages of leptospirosis.[159]

A positive leptospiral agglutination reaction generally is not found until the 6th to 12th day of illness, and maximal levels are reached between 21 and 28 days. After recovery, low titers may persist for many years. One blood sample should be obtained early in the course of illness, and a second one should be obtained at the end of 1 month. Negative reactions in serial samples do not exclude the possibility of leptospirosis because patients may be infected with a serotype not included in the battery of test antigens or with a previously unrecognized serotype. Moreover, the titer may have peaked before the acute-phase specimen was collected. Antibiotic therapy also may suppress the development of positive titers or delay their appearance.[89,119] Peak microscopic agglutination titers of 1:3000 to 1:100,000 usually are reached during the third week of illness.[65,194] An unchanging titer of 1:100 on two successive serum specimens has been defined as sufficient for making a presumptive diagnosis of leptospirosis. A fourfold increase in titer between acute and convalescent sera is indisputable evidence of active leptospirosis.

Leptospira DNA may be detected by PCR at a minimum detection limit of two to three cells per sample.[113,169] Nonradioac-

tive, arbitrarily primed PCR assays can be used to discriminate species of *Leptospira*.[148] Romero and colleagues[148] reported that PCR was more likely to facilitate the early diagnosis of leptospiral aseptic meningitis than was either the IgM ELISA or the MAT test.

PCR, IgM ELISA, latex agglutination, the Lepto Dipstick assay, and an antibody-based urine antigen assay each may be more sensitive than the MAT early in the course of disease.[104,133,152]

TREATMENT

To be of maximum therapeutic benefit, an antimicrobial agent would have to be administered before invading organisms damage the endothelium of blood vessels and various organs or tissues. One of the problems in evaluating the efficacy of treatment is the fact that generally, leptospirosis is a self-limited disease with a favorable prognosis. Even patients with severe icteric leptospirosis may recover without specific treatment.

Most claims of the beneficial value of antimicrobial agents in human leptospirosis are based on the response of individual patients rather than on controlled studies. Hall and associates[91] compared the effects of penicillin, chloramphenicol, chlortetracycline (Aureomycin), and oxytetracycline (Terramycin) with placebo in 67 confirmed cases of leptospirosis. No appreciable effect of antibiotics could be demonstrated on the duration or severity of illness or on the prevention or amelioration of central nervous system, hepatic, renal, or hemorrhagic complications of this disease. Moreover, the duration of leptospiremia and the persistence of organisms in CSF were not altered by treatment. Kocen[106] compared the effects of penicillin administered on the fourth day of illness to 28 patients with a control group of 33 who were given only supportive care and reported that the duration of fever was shorter and the incidence of jaundice, meningismus, renal involvement, and hemorrhagic manifestations was diminished in the treated group.[106] None of these controlled studies was entirely satisfactory with respect to randomization of patients.

McClain and associates[115] studied the therapeutic efficacy of doxycycline in military recruits who contracted leptospirosis while training at the Jungle Operations Training Center in Panama. Twenty-nine patients with anicteric disease were treated in a randomized, double-blinded fashion with doxycycline, 100 mg orally twice a day, or with placebo. Therapy was administered for 7 days in the hospital, after which patients were monitored for 3 weeks. The duration of illness before therapy and the severity of illness were similar in both study groups. Doxycycline shortened the duration of illness by 2 days and favorably influenced fever, malaise, headache, and myalgias. Treatment also prevented leptospiruria, and no significant adverse effects of doxycycline administration were observed.

In another randomized, double-blinded, placebo-controlled field trial at the same military training site, Takafuji and associates[183] demonstrated that doxycycline (200-mg oral dose) given weekly or at the completion of jungle training was highly effective in preventing the onset of clinical leptospirosis. Twenty cases of disease were documented in the placebo group (attack rate, 4.2%) as opposed to only one case in the treatment group (attack rate, 0.2%), findings supporting the prophylactic utility of doxycycline in this setting.

Watt and associates[208] reported the results of a trial in which a 7-day course of intravenous penicillin (6 million units per day) was compared with placebo in a randomized, double-blinded trial involving 42 patients. All the patients had severe, advanced disease. Every measurable aspect of the disease was affected favorably by penicillin. The duration of fever was shortened significantly ($p < 0.005$) in the group receiving penicillin. Penicillin therapy decreased the number of days of hospitalization and prevented the development of leptospiruria. These investigators concluded that intravenous penicillin should be given to patients with severe leptospirosis, even if therapy can be initiated only late in the course of their disease. Subsequently, Costa and associates[54] reported the contrary. Two hundred fifty-three patients with leptospirosis and longer than 4 days of symptoms were randomized to receive 6 million units of penicillin for 7 days or placebo (mean pretreatment duration of symptoms, 6.6 days and 6.5 days, respectively). Neither the number of days of hospitalization nor the in-hospital case-fatality rate differed significantly between the two groups.

Limited data exist comparing the use of alternative antimicrobial agents with penicillin. Panaphut and colleagues[135] reported no difference in time to resolution of fever, mortality, or duration of organ dysfunction between patients randomized to receive a 7-day course of penicillin or intravenous ceftriaxone. A subsequent study[180] comparing penicillin, doxycycline, and cefotaxime reported no significant differences among the three groups with regard to mortality, time to defervescence, or time to resolution of abnormal laboratory test results.

Nonetheless, we favor treatment with penicillin or tetracycline (to be avoided in children younger than 9 years) *early* in the course of disease if a diagnosis of leptospirosis is suspected. Parenteral aqueous penicillin G, 6 to 8 million U/m^2/day in six divided doses, should provide optimal blood and tissue concentrations of penicillin. For patients sensitive to penicillin, tetracycline, 10 to 20 mg/kg/day intravenously or 25 to 50 mg/kg/day orally in four divided doses for 1 week, should be provided.

Management of leptospirosis requires careful attention to supportive care. Fluid and electrolyte balance requires meticulous attention. Dehydration, cardiovascular collapse, and acute renal failure may necessitate prompt and specific treatment. In some cases, acute renal failure may be prevented by ensuring adequate renal perfusion and appropriate fluid administration early in the course of disease, when prerenal azotemia and shock may be seen.[22,164] If prerenal azotemia is suspected, diuresis should be attempted promptly with administration of a fluid or colloid load designed to expand extracellular volume and replace extracellular fluid deficits.[22] In patients who do not respond to such therapy, acute tubular necrosis may be suspected, and appropriate fluid restriction should be initiated. If azotemia is severe or prolonged, peritoneal dialysis or hemodialysis should be instituted.[72,198] The use of exchange transfusion has been suggested in patients with marked hyperbilirubinemia.[128,132,165]

The use of corticosteroids in the treatment of severe cases has not been evaluated critically, but their use in patients with impending hepatic coma has been suggested.[66] Anecdotal reports also suggest that corticosteroids may be of value in patients with profound hypotension or shock.

PREVENTION

Benches in rat-infested, fish-gutting sheds and sewers may be decontaminated. Hygienic conditions should be encouraged in slaughterhouses, farmyard buildings, and bathing pools. In addition to hygiene, prevention of leptospirosis primarily depends on immunization of animals. Immunization of workers at high risk for acquiring leptospirosis has been used successfully in mines in Japan and Poland and in rice fields in Italy and Spain.[193]

Leptospire bacterins are available commercially and have been evaluated for safety and efficacy in laboratory animals and domestic livestock.[32,102,155,172] The degree of protection attained depends largely on the antigenic potential of the immunizing agent. Requirements for the *L. pomona* vaccine used in cattle are such that not more than one eight-hundredth of the dose recommended for cattle must protect 80 percent of hamsters challenged

intraperitoneally 14 to 18 days after vaccination with a dose of 100 hamster LD_{50}s. In contrast, most dogs are immunized with a vaccine that is but one tenth of the potency of that used for cattle. Most dogs thus immunized have been protected against disease but not necessarily from carrying and excreting leptospires in their urine. Trends documenting that many cases of leptospirosis in children have been associated with contact with dogs suggest that more stringent requirements for the immunization of pet dogs are needed.

A randomized trial of doxycycline versus placebo was undertaken to assess the efficacy of doxycycline prophylaxis in the prevention of infection and disease caused by leptospires during outbreaks in North Andaman.[157] Leptospiral infection was not prevented, but the patients who received doxycycline and in whom disease subsequently developed had lower morbidity and mortality rates.

Additional resources are provided elsewhere.[17,32,63,109,112]

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CHAPTER 155

SPIRILLUM MINUS (RAT-BITE FEVER)

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Spirillum minus is one of the causative agents of rat-bite fever, or sodoku. Rat-bite fever also may be caused by *Streptobacillus moniliformis* (see Chapter 150). *S. minus* is a spirochete that was isolated first by Futaki and associates in 1917.[5] The original name for the organism was *Spirochaeta morsus muris*. *S. minus* is the most frequent cause of rat-bite fever in Asia.[9] Only one case of spirillary rat-bite fever has been reported in the American literature in the past 4 decades.[3]

BACTERIOLOGY

S. minus is a short, rigid, aerobic, gram-negative, flagellated spirochete measuring 2 to 5 μm in length. The organism is thicker than *Treponema pallidum* and usually contains two to five regular sharp turns along its length. On darkfield microscopy, the organism moves quickly with the use of a terminal flagellum.[5] In general, the thought is that *S. minus*, like other spirochetes, does not grow on artificial media and requires animal inoculation for successful isolation.[15] However, two reports in the literature describe the isolation of *S. minus* in broth,[8,14] but these cases may have represented other infectious illnesses.

EPIDEMIOLOGY AND PATHOLOGY

The epidemiology of spirillary rat-bite fever is similar to that of the streptobacillary form. Disease appears to be transmitted primarily through rat bites. Approximately 25 percent of rats are carriers of *S. minus* in the nasopharynx, and rats with conjunctivitis have been shown to have *S. minus* in the eye discharge that drains into their mouths.[11] Disease has not been reported after oral ingestion of the organism. Human-to-human transmission has not been reported but conceivably could occur during blood transfusions.

Gunning has given an excellent description of the pathologic changes associated with *S. minus* infection.[6] The infection provokes edema, mononuclear leukocyte infiltration, and necrosis at the site of inoculation. Regional lymph nodes are hyperplastic. The relapsing symptoms are associated with invasion of the blood by spirilla. Toxic, hemorrhagic, or necrotic changes may occur in the liver and kidney.

CLINICAL MANIFESTATIONS

The incubation period of spirillary rat-bite fever typically is longer than that of the streptobacillary form, with an average of 14 to 18 days and a range of 1 to 36 days.[2] The disease is heralded by the appearance of an indurated lesion at the site of the initially healed bite, which coincides with the onset of fever and chills. Chancre formation or ulceration may occur at the site, and regional lymphadenopathy is a common finding. The temperature may reach 41° C in a stepwise fashion over the course of 2 to 4 days and then fall abruptly.[6] Six to eight regularly occurring relapses of fever, separated by afebrile periods lasting 3 to 7 days, may occur. During febrile periods, the patient also may experience myalgia, headache, and vomiting. In approximately 50 percent of patients, a purple to red-brown rash develops and consists of large macules with occasional indurated erythematous plaques or urticarial lesions.[15] As opposed to streptobacillary rat-bite fever, joint manifestations are rare occurrences. In untreated cases, the illness may persist for 3 to 8 weeks, but relapses have occurred after months or years.[13]

Spontaneous cure is the general rule, but several untreated cases have persisted for more than 1 year.[15] The untreated mortality rate is reported to be 6.5 percent.[2] In protracted, untreated cases, severe complications include endocarditis,[8] meningitis,[10] myocarditis, hepatitis, and nephritis.[4] Anemia, weight loss, and severe diarrhea are common complications in infants and chil-

dren. Epididymitis, nuchal rigidity, headache, pleurisy, pleural effusion, and splenomegaly also have been reported.[12]

The differential diagnosis includes streptobacillary rat-bite fever, as well as many other infectious and noninfectious diseases (see Chapter 150 for details).

DIAGNOSIS

Definitive diagnosis requires isolation and identification of the spirochete. Although the organism rarely may be seen on a peripheral blood smear[1] or darkfield examination of ulcer exudate,[7] animal inoculation usually is required for isolation. Blood or wound aspirates are injected intraperitoneally into guinea pigs or mice. The spirochetes then may be recovered in 5 to 15 days from the animals' blood, which is examined under darkfield microscopy. This process is time-consuming and may not be available in most centers. In addition, the inoculated animals must be screened carefully for previous *Spirillum* carriage.

Nonspecific diagnostic criteria include a false-positive test for syphilis in 50 percent of patients, white blood cell count between 10,000 and 20,000 cells/mm,[3] and moderate anemia.[2,15] There are no specific serologic tests for *S. minus*. Molecular methods, such as polymerase chain reaction, may offer hope in the future for detection of *S. minus*.[4]

TREATMENT

S. minus is considerably more sensitive to penicillin than *S. moniliformis* is. In one study, a dosage as low as 24,000 U/day for 5 days was shown to be effective.[13] However, because distinguishing spirillary disease from streptobacillary disease is difficult, the two may coexist, and it is important in the acute stage to treat with dosages effective against *S. moniliformis* (see Chapter 150 for further details).

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CHAPTER 156

SYPHILIS

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Syphilis was recognized first at the end of the 15th century in Europe, where it appeared seemingly as an emerging infectious disease. It rapidly spread to reach epidemic proportions. Its European origins are a mystery that will not be solved easily, with theories ranging from its being a disease introduced from the New World by the returning crew of Columbus to its being a virulent form of the endemic yaws and bejel of African peoples that appeared later as virulent syphilis in a susceptible European population.

Syphilis initially was called the *Italian disease*, the *French disease*, and the *great pox* (as distinguished from smallpox). Its venereal transmission was not recognized until the 18th century. Delineation of characteristics of syphilis was hindered by the confusion of its symptoms with those of gonorrhea. In 1767, Hunter, a great English experimental biologist and physician, inoculated himself with urethral exudate from a patient with gonorrhea. The patient also had syphilis, and the subsequent symptoms experienced by Hunter convinced two generations of physicians of the unity of gonorrhea and syphilis. The separate natures of gonorrhea and syphilis were shown in 1838 by Ricord, who reported his observations on more than 2500 human inoculations. Recognition of the stages of syphilis followed, and in 1905, Schaudinn and Hoffman discovered the causative agent. The following year, Wassermann introduced the diagnostic blood test that bears his name.

ORGANISM

Morphologic characteristics are the primary features by which members of the family Spirochaetaceae are placed into a single taxon. Spirochetes are helix-shaped, heterotrophic bacteria. These organisms are slender, coiled, and flexible, with one or more complete turns in the helix. Spirochetes are motile, their corkscrew motility resulting from the action of axial fibrils known as *endoflagella*. The family Spirochaetaceae has five genera, of which only *Treponema*, *Borrelia*, and *Leptospira* spp. cause major human illnesses. Differentiation among genera of the family Spirochaetaceae is based primarily on the morphology of the organism.

The name *Treponema* is derived from the Greek words meaning, "turning thread." Individual organisms are 5 to 15 μm in length and 0.092 to 0.5 μm in diameter and have finely tapered ends. Whole cells appear to have a flat wave with one or more planes per cell, giving the appearance of a helical coil. Each cell has 8 to 14 evenly distributed waves. They exhibit a sluggish mobility, with drifting motion and graceful flexuous movements.[71] The internal structure of *Treponema pallidum* subsp. *pallidum* generally is similar to that of other spirochetes. An outermost thin three-layered membrane surrounds the protoplasmic cylinder of the cell. Intracytoplasmic microtubules have been described, and such structures may be specific for *Treponema* spp.

The six axial fibrils or endoflagella are long, flagella-like, intracellular organelles that originate at either end of the cell from knoblike structures and extend toward the other end. Axial fibrils vary in length but overlap one another near the middle of the cell. These fibrils are thought to determine the spiral shape of the cells and are responsible for the characteristic motility along the longitudinal axis exhibited by members of Spirochaetaceae.

The complete genome of *T. pallidum* subsp. *pallidum* (Nichols strain) was sequenced by the whole-genome rapid sequencing method.[47] The *T. pallidum* genome is a circular chromosome and is small compared with most bacteria at 1.14 Mb encoding 1041 predicted proteins. By polyacrylamide gel electrophoresis technique, the major constituent proteins of *T. pallidum* have been characterized and include integral membrane proteins with apparent molecular masses of 47 kd, 34 kd, 17 kd, and 15 kd.[98] These lipoprotein antigens are highly immunogenic in laboratory animals and humans.[7,66]

Virulent strains of *T. pallidum* are propagated by intratesticular inoculation of rabbits, and the inability to propagate the organisms in vitro has hampered study of these microorganisms. The time required for division of the organism to occur in rabbits is slow, at approximately 30 hours. Velocity sedimentation employing discontinuous gradients of radiocontrast medium (Hypaque) successfully has purified and concentrated treponemes extracted from infected rabbit testes. These organisms retain the antigens for the fluorescence test, although motility is lost.

Limited cultivation of *T. pallidum* on monolayers of baby hamster kidney cells in 7 percent carbon dioxide has been reported. Research with in vitro characteristics of these organisms also has resulted in the description of adherence of virulent *T. pallidum* to primary cell cultures of rabbit testicular cells and to an established continuous line of human epithelial cells (HEp-2). To date, however, direct in vitro culture for establishing the diagnosis of *T. pallidum* disease has not been possible. In vitro growth is hindered by the reliance of *T. pallidum* on glycolysis alone for adenosine triphosphate synthesis, its sensitivity to oxygen, and its sensitivity to growth temperature.[71]

Other pathogenic *Treponema* organisms cause characteristic clinical diseases and now are known to be genetically different from *T. pallidum* subsp. *pallidum*. These are *T. pallidum* subsp. *carateum* (pinta), *T. pallidum* subsp. *endemicum* (bejel or endemic syphilis), and *T. pallidum* subsp. *pertenue* (yaws). Several nonpathogenic treponemes also inhabit the oral cavity and intestinal tract.[18]

TRANSMISSION

ACQUIRED SYPHILIS

Syphilis is not a highly contagious disease. An individual who has had sexual contact with an infected partner has approximately a 30 percent chance of acquiring disease. The median infective dose to humans experimentally has been estimated to be 57 organisms. *T. pallidum* has the capability to invade the intact mucous membrane or microabrasions of the skin. Direct inoculation from contact with an infected individual is necessary for infection to occur because survival of the organism outside the host is very limited. The organism is killed easily by heat, drying, soap, and water. Sexual contact is the common method of transmission of acquired disease, and the site of inoculation usually is on the genital organs—the vagina or cervix in females and the penis in males. Other sites include lips, breast, tongue, and abraded areas of the skin. Examining physicians and pathologists may be infected by contact if appropriate barrier protection is not used.

CONGENITAL SYPHILIS

Congenital syphilis usually results from transplacental infection of the developing fetus from spirochetes in the maternal bloodstream. *T. pallidum* becomes widely disseminated to many tissues in an adult soon after initial infection occurs even if clinical manifestations are delayed by months. Maternal spirochetemia and placental infection are not surprising findings.[10] A newborn occasionally may be infected at delivery by contact with an infectious lesion present in the birth canal or perineum. Intrauterine transmission is supported by isolation of the organism from umbilical cord blood and amniotic fluid,[52,96,122,147] detection of spirochetes in the placenta and umbilical cord in association with typical histopathologic changes,[46,41,107] and detection of specific IgM antibody to *T. pallidum* in neonatal serum obtained at birth.[79,88,120,125] The isolation of *T. pallidum* from 74 percent of amniotic fluid specimens obtained from women with early syphilis also suggests that the organism is capable of traversing the fetal membranes, gaining access to the amniotic fluid and resulting in fetal infection.[80] Breast-feeding is not associated with transmission of syphilis, unless the mother has a chancre on her breast.

Transmission of syphilis to the fetus can occur throughout pregnancy. Occasional reports in the literature describing treponemes in fetal tissue or placentas before the fifth month of gestation were disputed for decades.[38] The thought was that the Langhans cell layer of the cytotrophoblast formed a placental barrier against treponemal invasion of the fetus. Researchers subsequently showed, however, that the layer of Langhans cells in the placenta persisted throughout pregnancy.

In 1976, Harter and Benirschke[57] visualized spirochetes by Warthin-Starry silver stain and immunofluorescence in tissue from two aborted fetuses at gestational ages of 9 and 10 weeks. The expected inflammatory response was not observed in these two fetuses, but such changes have been found in infected fetuses after the 15th week of pregnancy. These investigators noted that researchers describing syphilitic fetuses or placentas in the older literature worked with the products of spontaneous abortions. Such fetal loss caused by syphilis occurred only after 18 weeks of gestation, implying that the fetal loss was a reflection of damage incurred as a result of the host response to the organism. The observation of sequential acquisition by the fetus of the ability to respond to a variety of antigens suggests that inflammation can be present only after the fetus acquires the immunologic ability to recognize the treponeme.[132] The more recent detection of spirochetes in amniotic fluid obtained by amniocentesis from a woman with early syphilis at 14 weeks of pregnancy has shown that the fetus can be infected with *T. pallidum* in early pregnancy.[95] The rate of vertical transmission does increase with advancing gestation, however.

Vertical transmission is related directly to the maternal stage of syphilis, with early syphilis resulting in significantly higher transmission rates than late latent infection. Generally, the greater the time that has elapsed since the woman's primary or secondary infection, the less likely she is to transmit disease to the fetus. Ingraham[62] reported in 1950 that among 251 women with syphilis having a duration of 4 years or less, 41 percent of their infants were born alive and had congenital syphilis; 25 percent were stillborn; 14 percent died in the neonatal period; 21 percent had low birth weight, but no evidence of syphilis; and 18 percent were normal full-term infants. In contrast, only 2 percent of infants born to mothers with late disease had congenital syphilis. In 1952, Fiumara and colleagues[44] reported that untreated primary or secondary syphilis resulted in 50 percent of infants having congenital syphilis, whereas the other half were stillborn or premature or died in the neonatal period. With early latent infection, 40 percent of the infants had congenital syphilis, whereas only 10 percent with late latent disease developed syphi-

lis. These data are supported by a more recent study of Sheffield and colleagues,[128] in which mothers with primary, secondary, early latent, and late latent infection had transmission rates of 29, 59, 50, and 13 percent.

Syphilis is an ulcer-causing disease that is associated with increased sexual transmission of human immunodeficiency virus (HIV). In this regard, increasing numbers of newborns who are infected with congenital syphilis also are born to mothers with HIV, and vice versa. The contribution of maternal co-infection with *T. pallidum* and HIV to vertical transmission of either syphilis or HIV has not been elucidated fully. Virulent *T. pallidum* can promote the induction of HIV gene expression in macrophages, possibly resulting in increased systemic HIV levels and more rapid progression of the HIV infection.[141] A more recent study noted higher rates of congenital syphilis in infants born to co-infected mothers, but the diagnosis of congenital syphilis was based on a surveillance definition used by the Centers for Disease Control and Prevention (CDC) and not on strict diagnostic criteria.[126]

EPIDEMIOLOGY

The introduction of penicillin in 1942, and its subsequent widespread use in the 1950s, resulted in a marked decline in the occurrence of syphilis in the United States. After an increase in the number of cases occurred in the early 1960s, the rates again declined in the 1970s, only to have a dramatic resurgence from 1986 to 1991.[21,29,113] The rapid increase during these years in the rate and numbers of cases of primary and secondary syphilis in adults, specifically among women of child-bearing age, resulted in a significant increase in rates of congenital syphilis. Rates of syphilis were greatest among blacks and Hispanics residing in large urban centers. The disease was centered in populations in which substance abuse, particularly crack cocaine, was common, and where there was involvement with the sex trade industry often in exchange for drugs.[21]

Other factors that may have contributed to the increased rates of syphilis included underfunded and overwhelmed public health resources; use of spectinomycin for treatment of penicillinase-producing *Neisseria gonorrhoeae* because spectinomycin is ineffective against incubating syphilis; and failure to implement safer sexual practices, especially among adolescents and young adults. Generally, individuals who acquire syphilis characteristically are young and often have had contact with an average of five individuals during the incubation period. Because of the high rate of dual infection (8%) with *N. gonorrhoeae*, some individuals identified and treated for gonorrhea with ceftriaxone also are treated for syphilis while in the pre-primary stage of disease.

Strenuous attempts were made to control syphilis, and the lowest rate ever reported in the United States was reached in 2000, with the rate of primary and secondary syphilis declining by 90 percent between 1990 and 2000 to 2.1 per 100,000 population.[23] The reasons for this decline included (1) wider screening practices secondary to medical and public awareness of the syphilis epidemic of the late 1980s that led to identification and treatment of infected individuals; (2) increased state and local funding for syphilis control programs such as partner notification, community-based screening and presumptive treatment strategies, and risk-reduction counseling; (3) introduction of HIV prevention programs that target prevention of other sexually transmitted diseases (STDs); and (4) decrease in crack cocaine use and trading sex for drug behaviors; and possibly (5) development of acquired immunity to syphilis among high-risk populations that resulted in less reacquisition of infectious syphilis.

The concentration of cases in the United States to a few geographic areas, especially in the southeastern United States, led to an optimistic "National Plan to Eliminate Syphilis" announced by the CDC in 1999.[135] The plan had to be restated in 2006 in the face of resurgent rates.[23] A gender gap has been noted, with rates of primary and secondary syphilis in men increasing from 3 per 100,000 in 2001 to 5.7 per 100,000 in 2006, whereas a sustained increase in rates in women has been from 0.8 per 100,000 in 2004 to 1 per 100,000 in 2006. The increase in male rates has been explained partly by cases in men who have sex with men and attributed to high-risk sexual behaviors and co-infection with HIV. With the rates also increasing in heterosexuals, the rates of congenital syphilis also have rebounded.

In 1987, the reported rate of congenital syphilis was 10.5 cases per 100,000 live births. By 1991, there were 4398 cases of congenital syphilis (107 per 100,000 live births). These rates reflect a reporting case definition that was changed in 1988[21,152] and resulted in an almost fourfold increase in the number of cases reported to the CDC.[29] There also was a genuine increase, however, in the number of actual cases and case rates secondary to the increase in early syphilis among women.[68] Case rates of congenital syphilis were greatest in the Northeast (186.2 cases per 100,000) and least in the Midwest (54.8 per 100,000).[39]

In 1988, investigators recognized that prior definitions of congenital syphilis that had been used for surveillance and for treatment decisions had been difficult to apply to the clinical setting because they required a diagnosis that often could be established only over weeks or months. During that period, many children were lost to follow-up and were neither treated nor reported.[29] Because of the high incidence of congenital disease in infants born to inadequately treated mothers, current definitions of congenital syphilis for a presumptive case (which should be reported and treated) require only (1) that the infant be born to a mother with untreated or inadequately treated syphilis or (2) that the child have physical or laboratory signs of congenital syphilis. A summary of the surveillance case definition used since 1988 is provided in Table 156–1.

TABLE 156–1 Surveillance Case Definition for Congenital Syphilis

A *confirmed case* of congenital syphilis is an infant in whom *Treponema pallidum* is identified by darkfield microscopy, fluorescent antibody, or other specific stains in specimens from lesions, placenta, umbilical cord, amniotic fluid, or autopsy material

A *presumptive case* of congenital syphilis is either of the following:

A. Any infant whose mother had untreated or inadequately treated* syphilis at delivery, regardless of findings in the infant; *or*

B. Any infant or child who has a reactive treponemal test for syphilis and any one of the following:
   1. Any evidence of congenital syphilis on physical examination; *or*
   2. Any evidence of congenital syphilis on long bone radiograph; *or*
   3. Reactive cerebrospinal fluid VDRL; *or*
   4. Elevated cerebrospinal fluid cell count or protein (without other cause); *or*
   5. Quantitative non-treponemal serologic titers that are fourfold higher than the mother's (both drawn at birth); *or*
   6. Reactive test for FTA-ABS-19S-IgM antibody

A *syphilitic stillbirth* is defined as a death of a fetus weighing >500 g or having a gestational age >20 weeks in which the mother had untreated or inadequately treated syphilis at delivery

**Inadequate treatment consists of any non-penicillin therapy or penicillin given <30 days before delivery.*

FTA-ABS, fluorescent treponemal antibody absorption; VDRL, Venereal Disease Research Laboratory.

Adapted from Centers for Disease Control: Congenital syphilis, New York City, 1986-1988. M. M. W. R. Morb. Mortal. Wkly. Rep. 38:*828, 1989.*

Minor differences exist among case definitions of congenital syphilis as formulated by several agencies and experts.[118] Some experts would consider as a presumptive case a newborn who is well clinically but was born to a mother who had contact within 90 days before delivery with an individual with primary or secondary syphilis and who had not been treated or had been treated inadequately, even if the mother has nonreactive serology. Although recommendations for therapy commonly have assumed that adequate therapy given to a mother with primary or secondary syphilis during pregnancy would prevent congenital syphilis with a high degree of reliability, reasons to doubt this premise have emerged.[3,86,151] In particular, treatment failures in which the infant developed syphilis despite maternal therapy have been reported when the mother was treated first within 30 days of delivery.[12,14,84,85,117]

An infant born to a mother who was treated within 30 days of delivery is considered to have been treated inadequately, and the infant should receive appropriate therapy. Table 156–2 lists the various circumstances in which maternal therapy may be presumed to be subtherapeutic. The consequences of inadequate therapy of the mother are shown in data in Table 156–3. In that experience, 13 percent of children born to inadequately treated mothers had congenital syphilis, all of which was neurosyphilis.[116]

Substantial underreporting of infected infants did occur previously,[151] but the revised surveillance definition does not represent diagnostic criteria. Rather, it reflects the public health burden of the disease because these infants require medical and public health interventions. Notwithstanding this change in reporting guidelines, the number of cases of congenital syphilis had continued to decline from a peak of 107 per 100,000 live births in 1991, but after 14 consecutive years of decline, the rates increased 3.7 percent between 2005 and 2006 (8.2 to 8.5 per 100,000 live births).[23]

Worldwide syphilis remains a considerable public health problem. Russia reported 730 cases in 2001, an increase from 15 cases in 1991. Russia and countries of the former Soviet Union have rates of syphilis in pregnancy of 1 to 2 percent. In the Caribbean and Latin America, these rates are 2 to 7 percent, but they are even worse in Southern Africa, with 2 to 17 percent of all pregnancies occurring in women with syphilis.[143] The toll of untreated maternal syphilis on the outcomes of pregnancy is severe. An estimated one fourth of all stillbirths and 10 percent of neonatal deaths in Southern Africa are caused by congenital syphilis.[140] The World Health Organization (WHO) reckons that every year, 1 million pregnancies are complicated by syphilis, with half resulting in early pregnancy abortion or perinatal death, a quarter resulting in low birth weight or premature birth, and a final quarter resulting in overt congenital syphilis.[143]

Congenital syphilis is a disease that should be amenable almost fully to eradication if currently available prenatal health measures were implemented completely. Table 156–4 presents two studies in the United States of prenatal care of women who delivered infants with congenital syphilis and indicates the hurdles that control of the disease will have to overcome.[30,86] Lack of prenatal care is the most important one.[34,87,145] National surveillance data analyzed by the CDC in 2002 highlight the fact that cases of congenital syphilis still occur even when prenatal care is implemented if the mother's response to treatment is inadequate or if the infant's evaluation is not thorough (Fig. 156–1).[22]

Penicillin was a major reason for the decline of the treponematoses seen in the early 1950s.[41] Other factors must be considered in the attempted eradication of these infections. Nonvenereal endemic treponemal infections (endemic syphilis, bejel, yaws, pinta) have continued to flourish in underdeveloped areas of the world where hygiene is poor, despite the introduction of penicillin. Progress in control of these diseases requires that mass treatment programs be coupled with efforts to improve local living conditions. In areas where this goal could be accomplished, a resulting decline in endemic treponematoses occurred.

TABLE 156–2 Circumstances in Which Maternal Therapy for Syphilis May Be Subtherapeutic or Inadequate

Treatment with a non-penicillin regimen (including macrolide antibiotics)
History of maternal treatment was not documented fully or verifiable
Treatment during the month before delivery
Treatment in HIV-infected women
Serial post-therapy assays of maternal non-treponemal antibody titers were not performed
Serial post-therapy assays of maternal non-treponemal antibody titers did not show a fourfold decline in titers after treatment of early syphilis, not permitting assessment of adequacy of therapy, and suggesting possibility of failure to eradicate infection
Serial post-therapy assays of maternal non-treponemal antibody titers show a fourfold increase in titers, suggesting re-infection or relapse

HIV, human immunodeficiency virus.

TABLE 156–3 Clinical Findings of 148 Infants Whose Mothers Had Syphilis and Whose Treatment Was Adequate, Inadequate, or Not Provided

Rights were not granted to include this table in electronic media. Please refer to the printed publication.

From Reyes, M. P., Hunt, N., Ostrea, E. M., et al.: Maternal/congenital syphilis in a large tertiary care urban hospital. Clin. Infect. Dis. 17:*1041-1046, 1993.*

TABLE 156–4 Prenatal Care and the Occurrence* of Congenital Syphilis in Infants

	Study	
Prenatal Care	***Mascola et al., 1984[84] (n = 50)***	***Coles et al., 1995[30] (n = 318)***
No prenatal care	56	46
First prenatal test negative; testing not repeated in late pregnancy	6	14
Medical mismanagement	6	10
Failure of conventional prenatal syphilis treatment of mother	8	5
Negative maternal syphilis test at delivery	14	—
Infection late in pregnancy or no prenatal care until late in pregnancy	—	20
Mother not tested	8	3
Laboratory error	2	—

**Numbers represent percentage of total cases in study.*

Adapted from Gutman, L. T.: Congenital syphilis. In *Mandell, G. L. (ed.): Atlas of Infectious Disease. Vol. 5: Sexually Transmitted Diseases. Philadelphia, Current Medicine, 1996.*

PATHOLOGY

Syphilis often is a lifelong infection that progresses in three clear characteristic stages (Fig. 156–2). After initial invasion occurs through mucous membranes or skin, the organism undergoes rapid multiplication and is disseminated widely. Spread through the perivascular lymphatics and then through the systemic circulation probably occurs even before the clinical development of the primary lesion. Ten to 90 days later, usually within 3 to 4 weeks, the patient manifests an inflammatory response to the infection at the site of the inoculation. The resulting lesion, the chancre, is characterized by the profuse discharge of spirochetes; accumulation of mononuclear leukocytes, lymphocytes, and plasma cells; and the swelling of capillary endothelia. The regional lymph nodes are enlarged, and the cellular infiltrate resembles that of the primary lesions. Resolution of the primary lesion occurs by fibrosis.

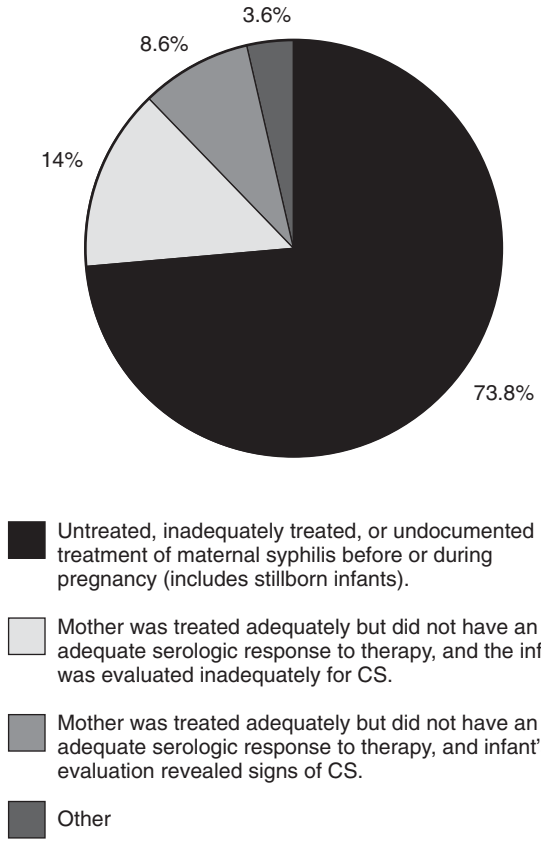

Figure 156–1 Maternal treatment history of 451 cases of congenital syphilis (CS) reported in 2002. *(From Centers for Disease Control and Prevention: Congenital syphilis—United States, 2002. M. M. W. R. Morb. Mortal. Wkly. Rep. 53:716-719, 2004.)*

Secondary lesions develop when tissues of ectodermal origin, such as skin, mucous membranes, and central nervous system (CNS), participate in an inflammatory response. Mucous patches in the mouth are caused by local vasculitis. The cellular infiltrate resembles that of the primary lesion, with the predominance of plasma cells. Little or no necrosis is present, and healing is without scarring but may include pigmentary changes.

Tertiary syphilis may involve any organ system and often is asymmetric. Gummata are lesions typified by extensive necrosis, a few giant cells, and a paucity of organisms. They commonly occur in internal organs, bone, and skin. The other major form of tertiary lesion is a diffuse chronic inflammation, with plasma cells and lymphocytes but without caseation, which may result in an aneurysm of the aorta, paralytic dementia, or tabes dorsalis. Chronic swelling of the capillary endothelium and fibrosis result in the characteristic tissue changes.

Congenital syphilis is a result of hematogenous infection and the disseminated involvement of almost all viscera. The intense inflammatory response occurs in the perivascular framework and interstitial stroma, rather than in the parenchyma.[94] Bone, liver, pancreas, intestine, kidney, and spleen are involved most reproducibly and severely. Other tissues, such as the brain, pituitary gland,[14] lymph nodes, and lungs, also may be infected. The gastrointestinal tract shows a pattern of mononuclear cell infiltration in the mucosa and submucosa, with subsequent widening of the submucosa by the ensuing fibrosis. This event is most prominent in the small bowel. In the kidney, a perivascular inflammation, particularly in the juxtamedullary region, is evident. The basic architecture of the tissue influences the ultimate pattern of involvement. The deposition of collagen around arteries of the spleen produces a typical onion-skin appearance. Periosteum and epiphyses are the most affected portions of bone, and syphilitic granulation tissue may interfere with bone forma-

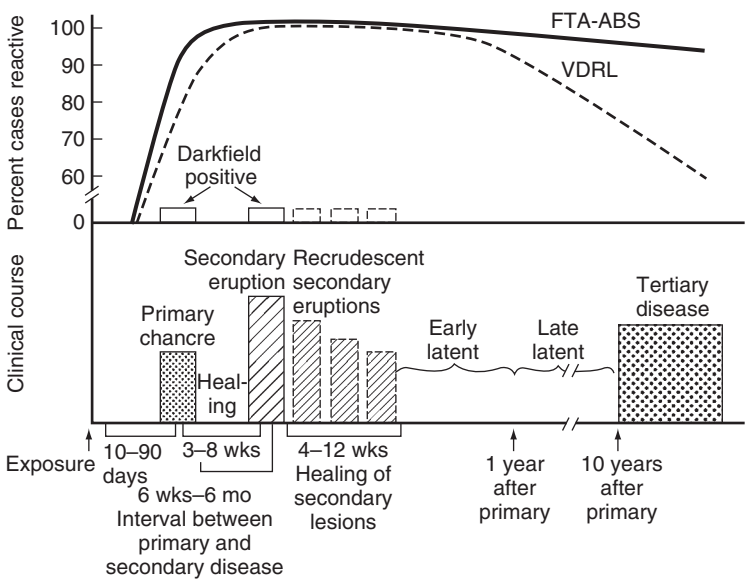

Figure 156–2 The course of untreated syphilis. FTA-ABS, fluorescent treponemal antibody absorption; VDRL, Venereal Disease Research Laboratory.

tion. Pancreatitis also is observed, with typical inflammation and fibrosis.

The fetus or newborn shows diffuse extramedullary hematopoiesis in many tissues. The placenta of infants with congenital syphilis often is large, thick, and pale. Three histopathologic features commonly are seen: enlarged and hypercellular villi, proliferative fetal vascular changes, and acute and chronic inflammation of the villi.[50,129] Erythroblastosis involving the placenta has been seen more frequently among stillborn infants with congenital syphilis than in live-born infants with or without syphilis.[129]

Spirochetes may be identified in placental tissue using conventional staining, although they may be difficult to visualize. Nucleic acid amplification methods also have been used to identify *T. pallidum* genome in involved placental specimens.[50] In addition, the umbilical cord may exhibit significant inflammation, with abscess-like foci of necrosis located within Wharton jelly and centered around the umbilical vessels, which is termed *necrotizing funisitis*.[46,129] Macroscopically, the umbilical cord resembles a "barber's pole"; the edematous portions have a spiral striped zone of red and pale blue discoloration, interspersed with streaks of chalky white. Histochemical staining may show spirochetes within the wall of the umbilical vessels. Placental and umbilical cord histopathology should be performed on every case of suspected syphilis.[129]

PATHOGENESIS AND IMMUNE RESPONSE

The host immune response to syphilis is vigorous and is responsible for many of the clinical manifestations seen in the disease. Despite this immune response, the infection may persist for life. In addition, prior infection does not confer resistance to re-infection. Researchers continue to try to explain this contradiction.[102]

TREPONEMAL VIRULENCE–ASSOCIATED FACTORS

Virulent *T. pallidum* attach to host cells during parasitism and are oriented by the proximal hook to the host cell surface. There seems to be a ligand-receptor adherence mechanism involving the treponemal outer-membrane proteins. Virulent strains attach to metabolically active mammalian cells, and treponemes are capable of multiplication only during attachment. Fetal and infant cells seem to support treponemal growth maximally, and capillary cells are the prime target of parasitism. Virulent treponemal strains produce hyaluronidase, which may facilitate the perivascular infiltration that is apparent by histopathologic study. These strains also may invade the ground substance that joins capillary endothelial cells.

Virulent *T. pallidum* is coated with fibronectin having host origin. This coating seems to protect the organism from antibody-mediated phagocytosis; allows the organism to adhere to the surface of host phagocytes with only limited ingestion of the organisms; may block complement-mediated lysis of the coated treponemes; and, finally, may allow the treponemes additionally to acquire physiologically active host proteins, such as ceruloplasmin and transferrin, on which they are dependent.[103]

HOST RESPONSE

Treponemes seem to persist in extracellular loci with little or no inflammatory response elicited. Polymorphonuclear leukocytes ingest virulent treponemes, incorporate them into phagocytic vacuoles, degranulate, and digest *T. pallidum*. In addition, phagocytosis occurs slowly and is facilitated by the presence of immune serum.[2] Large numbers of treponemes are needed to elicit this response, however, and small numbers may escape recognition.[91] Although there are few surface-exposed transmembrane proteins, one more recently described, Tpr K, seems to act as a porin.[25] It has been shown to elicit an opsonic antibody response in the rabbit model. Variable regions of the protein are explained by diversity in the encoding genes. This antigenic variation could explain the success of the treponeme in evading the host response.[24,75,89] Innate immunity through Toll-like receptor 4 is activated by lipoproteins under the outer membrane and may be important in orchestrating the overall immune response to *T. pallidum*.[16,109,119]

Alterations in host cell-mediated immunity occur during primary and secondary stages of syphilis. During early stages of disease, all aspects of cell-mediated immunity, including responses to nonspecific T-cell mitogens, are suppressed. Blastogenesis to treponemal antigens is not demonstrable until late in the course of secondary disease. Natural killer cell activity is increased during early primary syphilis and depressed in secondary and latent syphilis.[65] Some evidence supports the idea that lymphocytes from previously infected animals confer moderate protection to nonimmune animals. In human disease, $CD4^+$ T cells and macrophages predominate in primary chancres, whereas $CD8^+$ cells predominate in secondary syphilis lesions. In primary and secondary syphilis lesions, T_H1 cytokines are present.[105,119,142] Some investigators have suggested that increased apoptosis of peripheral blood lymphocytes and $CD4^+$ cells by a Fas-mediated pathway that occurs during the secondary stage of infection may explain the failure to clear the organism and set up a chronic infection.[40] Alterations in the immune environment of the mother during pregnancy may alter the balance of containment of the organism, allowing transplacental passage to the fetus.[150]

During the initial infection with *T. pallidum*, humoral IgG and IgM antibodies are detectable by the time the chancre appears. In primary syphilis, the main IgG subclass is IgG1, whereas IgG1 and IgG3 predominate in secondary syphilis.[9] If the patient is treated adequately, IgM antibody declines during the next 1 to 2 years, but IgG antibody usually persists through the lifetime of the patient. The stages of syphilis evolve, despite humoral antibody response.

Individuals with untreated syphilis have only a relative resistance to re-infection, so the development of a chancre with re-infection is unusual and probably depends on the challenge inoculum. After re-exposure, untreated individuals may develop an increased humoral antibody level.

In individuals who have been treated for syphilis, especially if treatment was provided during the secondary or earlier stages, the protective effect of prior disease is minor, and active disease frequently occurs after re-infection, regardless of whether the patient maintains a reactive non-treponemal antibody test (serofast) or is seronoreactive. Patients who have been treated for congenital syphilis also may acquire symptomatic disease.[43,101] Although active or prior syphilis modifies the response of a patient to subsequent re-infection, protection is only relative and is unreliable.

Secondary syphilis and congenital infection may be accompanied by the nephrotic syndrome. The nephrosis characteristically responds rapidly to penicillin, and light microscopy reveals membranous glomerulonephritis with glomerular mesangial cell proliferation. The subendothelial basement membrane deposits contain IgG and C3 or only globulin. Acute syphilitic glomerulonephritis seems to be an immune complex disease.[15,48,67] In infants with congenital syphilis, analysis of circulating immune complexes using immunoblotting methods showed the presence of an 83-kd *T. pallidum* antigen.[35] Findings have been similar with secondary syphilis.

CLINICAL MANIFESTATIONS

The course of untreated syphilis characteristically progresses through three or four stages over many years. Information on the natural history of untreated syphilis comes primarily from follow-up studies of almost 2000 untreated syphilitic patients who were seen initially between 1891 and 1910 in the clinic of Boeck. The subsequent studies of these patients by Bruusgaard and by other epidemiologists later provided the basis for most of our concepts of the consequences of syphilis. Clark and Danbolt[28] published a summary of some of these studies. Figure 156–2 depicts some of the characteristics of an untreated course of disease.

ACQUIRED SYPHILIS

Most recognized syphilitic disease in children is congenital. Acquired syphilis in prepubertal children seldom is reported and is assumed to resemble the clinical course of acquired syphilis in adulthood. Children with acquired syphilis should be assumed to have been infected through contact during child sexual abuse, unless another method of transmission is identified. All patients with syphilis should be tested for HIV infection.[20]

The decision to screen for syphilis during the medical evaluation of a child who is suspected to have been sexually abused should be made on an individual basis because the prevalence of STDs is low in these circumstances. Situations in which screening is recommended include the following: The perpetrator has an STD or is at high risk of having one, such as residing in a community or being of a social setting in which the prevalence of STDs is high; multiple perpetrators are involved; the child is infected with another STD, such as HIV infection, or has physical signs of sexual abuse; STDs have been diagnosed in siblings, other children, or adults in the household; the patient is postpubertal; or screening is a matter of patient or family preference.[11,70,72,130,131] A difficult aspect of the follow-up is the requirement of repeat serologic assays in 3 to 6 months because the initial test may not detect incubating syphilis.

Primary Disease

The chancre of primary syphilis typically is a single lesion, nontender and firm, with a clean surface, raised border, and reddish color. It may be overlooked by women because it frequently is situated on the cervix or vaginal wall. Systemic signs or symptoms are absent, but adjacent lymph nodes frequently are enlarged and nontender. Gutman[54] provides depictions of children with acquired primary syphilis.

Secondary Disease

The patient may experience secondary disease 2 to 10 weeks after the primary lesions manifest. Prominent findings include fever, sore throat, muscle aches, generalized lymphadenopathy, headache, and rash. The palms and soles frequently are involved, in contrast to many other dermatologic conditions. On mucous membranes, the lesions may appear as white mucous patches. Condylomata lata occur around moist areas, such as the anus and vagina. All secondary lesions of the skin and mucous membranes are highly infectious. In their review, Baughn and Musher[10] give a full description of the manifestations of secondary syphilis. Acquired syphilis in early childhood may reveal minimal dermal findings.[1]

Latent Disease

After the clinical signs of secondary disease resolve, the patient enters the stage of latent disease. *Latent syphilis* refers to infection in individuals who have reactive serologic tests for syphilis, but no clinical manifestations. The first year after infection is considered *early latent infection*, and the subsequent period is considered *late latent syphilis*. Early latent syphilis originally was classified as syphilis occurring within 4 years of the acquisition of infection, based on the time when mucocutaneous lesions may recur. More recent classification of early latent syphilis is based on the time period of highest communicability, which is in the first year after infection is acquired. Treponemes still can be present in the blood intermittently, however, and pass across the placenta to the fetus during latent syphilis in a pregnant woman.

If the duration of infection with syphilis cannot be determined, the disease is classified as syphilis of unknown duration; clinical management and treatment should be the same as that for late latent infection. If therapy for syphilis is given first during the latent stage, the patient is unlikely to show regression of nontreponemal antibody determinations. Approximately 60 percent of untreated patients in the late latent stage continue to have an asymptomatic course, whereas 30 to 40 percent develop symptoms of late or tertiary disease. Progression of disease from late latent to late symptomatic syphilis usually is prevented if appropriate antimicrobial therapy is given at this stage. See Gutman[54] for depictions of children with acquired secondary syphilis.

Tertiary Disease

Three to 10 years after the last evidence of secondary disease has occurred, the patient may develop nonprogressive, localized nodules of the dermal elements or supporting structures of the body, called *gummata*. These nodules are granulomatous and can have central necrosis. Because these lesions are relatively quiescent, the term *benign tertiary syphilis* often is used. Spirochetes are extremely sparse or absent. The gummatous reaction is primarily a pronounced immunologic reaction of the host.

Neurosyphilis

During the early stage of syphilis, approximately one third of all patients have involvement of the CNS. If untreated, only half of these patients develop late neurosyphilis. The interval between primary disease and late neurosyphilis usually is more than 5 years. Of the few children recognized to have acquired (versus congenital) late neurosyphilis, several have developed symptomatic disease at a very early age. It is possible that the disease progresses more rapidly in children than in adults.

Late neurosyphilis may be asymptomatic or, if symptomatic, may occur in a variety of ways. Classic presentations include paralytic dementia, tabes dorsalis, amyotrophic lateral sclerosis, meningovascular syphilis, seizures, optic atrophy, and gummatous changes of the cord. Neurosyphilis may resemble virtually any other neurologic disease.

Cardiovascular Syphilis

Approximately 10 to 40 years after having primary syphilis, an untreated patient may develop signs of cardiovascular involvement. Most frequently involved are the great vessels of the heart, where syphilitic aortic and pulmonary arteritis develop. One complication of this development is aortic regurgitation. The inflammatory reaction also may cause stenosis of the coronary ostia, with resulting angina, myocardial insufficiency, and death.

SYPHILIS IN PREGNANCY

Pregnancy has no known effect on the clinical course of syphilis. Untreated syphilis can affect profoundly the outcome of the

pregnancy, however, resulting in spontaneous abortion, stillbirth, nonimmune hydrops fetalis,[8,17] premature delivery, perinatal death, or two characteristic syndromes of congenital disease, early and late congenital syphilis (see later).[53,121] The outcome of untreated fetal infection varies. Intrauterine death occurs in an estimated 25 percent of infections, with abortion usually occurring after the first trimester. Perinatal death may occur in another 25 to 30 percent of untreated infected infants.[94] In a study of the perinatal outcome of congenital syphilis, the fatality rate was 464 per 1000 infected births. Of the fatalities, 27 percent were neonatal deaths, and 73 percent were stillbirths.[117] Currently, most infected infants, if not hydropic at birth, survive the neonatal period, most likely because of early identification and treatment of infected infants and improved neonatal care.

The investigations of Taber and Huber[138] illustrate the importance in pregnant women of recognition of the disease and administration of appropriate therapy. In this study, 22 women with syphilis were undiagnosed during pregnancy and did not receive prenatal therapy. Eleven infants were followed without initial therapy. At delivery, all were asymptomatic clinically, four had a reactive Venereal Disease Research Laboratory (VDRL) test, five had a nonreactive VDRL test, and two were not tested. All these infants were readmitted with obvious disease involving multisystem infection, three with proven spirochetal hepatitis, two with nephrotic syndrome, and three with CNS involvement. This experience emphasizes the importance of recognition of transmission of infection late in gestation, leading to delivery of infants who are well clinically, but whose disease emerges in the weeks after delivery if untreated.[37]

CONGENITAL SYPHILIS

At the onset of congenital syphilis, *T. pallidum* is liberated directly into the circulation of the fetus, resulting in spirochetemia with widespread dissemination. The clinical, laboratory, and radiographic abnormalities of congenital syphilis are a consequence of active infection with *T. pallidum* and the resultant inflammatory response induced in various body organs and tissues. The severity of these manifestations varies and can range from overwhelming involvement of multiple organs and body systems, as is seen in fetal hydrops, to only laboratory or radiographic abnormalities. Most of the infants born to mothers with untreated syphilis seem completely normal at birth and have no clinical or laboratory evidence of infection at birth. These infants may develop manifestations of disease several months to years later if left untreated.[27] The signs and symptoms of congenital syphilis are divided arbitrarily into early manifestations, which appear in the first 2 years of life, and late manifestations, which emerge anytime thereafter, usually near puberty.

Early Congenital Syphilis

The abnormal physical and laboratory findings in early congenital syphilis are varied and unpredictable.[110] Table 156–5 lists the major physical and laboratory findings from 310 reported cases of early congenital syphilis.[17,32,67,74,99,117,138,139] These results undoubtedly are minimal rates for each finding. The onset occurs between birth and approximately 3 months of age, with most cases occurring within the first 5 weeks of life.

SKELETAL SYSTEM

Bone involvement is the most common manifestation of congenital syphilis, occurring in 60 to 80 percent of infants with clinical signs of syphilis. In addition, it may be the only abnormality seen in infants born to mothers with untreated syphilis. Because of their frequency and early appearance, the radiographic changes in the bones, termed *osteochondritis* and *periostitis*, are of diagnostic value.[32] The femur and humerus are involved most often. Radiographically, accumulating calcified matrix is seen at the epiphyseal margin, which may be smooth or serrated. The serrated appearance is known as *Wegner sign* and represents points of calcified cartilage along the nutrient cartilage canal.

A zone of rarefaction at the metaphysis, which represents syphilitic granulation tissue containing a few scattered calcified remnants and a mass of connective tissue containing areas of perivascular infiltration of small round cells, may be seen. Irregular areas of increased density and rarefaction produce the moth-eaten appearance of the radiograph. The demineralization and osseous destruction of the upper medial tibial metaphysis are called *Wimberger sign*. Previously thought to be specific for syphilis, Wimberger sign also may occur in osteomyelitis and hyperparathyroidism. Epiphyseal separation may occur as a result of a fracture of the brittle layer of calcified cartilage. Irregular periosteal thickening also is a common finding. The changes usually are present at birth but may appear in the first few weeks of life. The bony changes are self-limited and usually are healed in the first 6 months, even in the absence of specific therapy. They may be painful lesions, and pain on motion often leads an affected infant to appear to have a limb paralysis, termed *pseudoparalysis of Parrot*.

TABLE 156–5 Findings in 310 Cases of Early Congenital Syphilis

Findings	No. Patients
Hepatomegaly	100
Skeletal abnormalities	91
Birth weight <2500 g	51
Skin lesions	45
Hyperbilirubinemia	40
Pneumonia	51
Splenomegaly	56
Severe anemia, hydrops, edema	50
Snuffles, nasal discharge	27
Painful limbs	22
Pancreatitis	14
Cerebrospinal fluid abnormalities	21
Nephritis	11
Failure to thrive	10
Testicular mass	1
Chorioretinitis	1
Hypoglobulinemia	1

Data from references 17, 32, 67, 74, 99, 117, 138, and 139.

RHINITIS

Rhinitis, coryza, or snuffles are likely to mark the onset of congenital syphilis. Usually, it appears in the first week of life and seldom later than the third month. The snuffles are more severe and persist longer than the common cold. The nasal discharge is white and often bloody, and the snuffles frequently are associated with laryngitis. Secondary bacterial superinfection may result in a purulent appearance. The nasal discharge is teeming with spirochetes and should be examined by darkfield microscopy to confirm its diagnosis.

RASH

The syphilitic rash usually appears 1 to 2 weeks after rhinitis manifests. The typical eruption is maculopapular and consists of small spots that are dark red-copper. If the rash is present at birth, it often is widely disseminated and bullous, and is called *pemphigus syphiliticus*. It is most severe on the hands and feet. The bullous

fluid contains many spirochetes. The rash erupts slowly, taking 1 to 3 weeks, and is followed by desquamation and crusting. As the rash fades, the lesions become coppery or dusky red, and pigmentation may persist. See Gutman[54] for depictions of a variety of cutaneous manifestations of early congenital syphilis.

FISSURES AND MUCOUS PATCHES

Fissures and mucous patches are not seen often but are highly characteristic features of congenital syphilis. The fissures develop around the lips, nares, and anus. They bleed readily and heal with scarring. A cluster of scars radiating around the mouth is called *rhagades* and is a characteristic of late congenital syphilis. Mucous patches may be found on any of the mucous membranes, especially in the mouth and genitalia. Condylomata are raised, moist lesions appearing on areas of the skin where there is moisture or friction. They are highly infectious because they contain many spirochetes.

HEMATOLOGIC FINDINGS

Congenital syphilis is characterized by anemia, thrombocytopenia, hemolytic processes, and leukopenia and leukocytosis.[74] The hemolytic process is Coombs test–negative and often accompanied by cryoglobulinemia, immune complex formation, and macroglobulinemia. The hemolysis, similar to the liver disease, is refractory to therapy and may persist for weeks. Paroxysmal nocturnal hemoglobinuria is a late manifestation of congenital syphilis.

CENTRAL NERVOUS SYSTEM INVOLVEMENT

In the era before the introduction of penicillin therapy, approximately 15 percent of infants with congenital syphilis developed manifestations of meningovascular disease. These findings included meningitis, meningeal irritation, bulging fontanelle, cranial nerve palsies, seizures, and hydrocephalus.[61] In addition, involvement of the pituitary gland in congenital syphilis is a common manifestation, occurring in approximately 40 percent of autopsy cases,[99] and consists of interstitial inflammation and fibrosis with formation of gumma in the anterior lobe. Clinical disease in affected infants is manifested by persistent hypoglycemia and diabetes insipidus.[33,97]

Most infants infected with *T. pallidum* are completely asymptomatic, and involvement of the CNS is inferred from abnormalities of the cerebrospinal fluid (CSF), such as reactivity on a VDRL test, pleocytosis, and elevated protein content. Because of the wide range of normal values for CSF protein, red blood cells, and white blood cells in the neonatal period, defining the proportion of infants with congenital syphilis who have abnormalities of these laboratory values has been difficult. Current consensus identifies an abnormal CSF white blood cell count in infants being evaluated for possible congenital syphilis as greater than 25 cells/mm^3 and protein as greater than 150 mg/dL (>170 mg/dL if infant is premature). A reactive CSF VDRL test is considered to be specific for neurosyphilis in older children and adults. In neonates, the significance of a reactive CSF VDRL test is suspect, however, because maternal non-treponemal IgG antibodies can pass from maternal serum to fetal and neonatal serum and then diffuse into the CSF. Children may fail to have a reactive VDRL test on initial examination and still develop later signs of neurosyphilis.

Using rabbit infectivity testing, which involves the inoculation of CSF into rabbits to determine the presence of the spirochete in the CSF specimen, Michelow and coworkers[88] found that invasion of the CNS with *T. pallidum* occurs in 41 percent of infants who have clinical, laboratory, or radiographic abnormalities of congenital syphilis. None of these infants had clinical signs of neurologic disease. Compared with isolation of spirochetes in CSF by rabbit inoculation, the sensitivity and specificity in CSF of a reactive VDRL test, elevated white blood cell count, and elevated protein were 54 percent and 90 percent, 38 percent and 88 percent, and 56 percent and 78 percent. These investigators also documented CNS infection in three infants who had normal CSF studies. Using current methods, the best indicator of CNS infection in neonates is an abnormal evaluation consisting of an abnormal physical examination, anemia, thrombocytopenia, CSF abnormalities, and abnormal bone radiographs. A normal evaluation renders CNS infection rare.

PNEUMONIA

Syphilitic pneumonia is a common occurrence in congenital syphilis, particularly in developing countries. The classic radiographic appearance is one of complete opacification of both lung fields and is termed *pneumonia alba*. More commonly today, a fluffy, diffuse infiltrate involving all lung areas is seen on the chest radiograph. At autopsy, pneumonia alba consists of a focal obliterative fibrosis with scarring and thickening of alveolar walls with loss of alveolar spaces. Follow-up evaluation of children who have recovered from congenital syphilis has shown that at least 10 percent may have chronic pulmonary disease, particularly if they were premature and required mechanical ventilation.

HEPATOSPLENOMEGALY

Hepatomegaly is the most common clinical sign in congenital syphilis. Its occurrence in the fetus has been documented by ultrasonography, and it may be a marker of inadequate treatment of the fetal infection despite maternal treatment during pregnancy.[58] Hepatosplenomegaly often is caused by extramedullary hematopoiesis. Hepatomegaly may occur in the absence of splenomegaly, but in contrast to congenital cytomegalovirus, the reverse does not. Neonatal syphilitic hepatitis is associated with visible spirochetes on biopsy specimens of liver tissue, jaundice, and cholestasis. Aspartate aminotransferase, alkaline phosphatase, and alanine aminotransferase determinations often are elevated, and direct hyperbilirubinemia is a common finding. The prothrombin time may be delayed. The liver disease often resolves slowly, even after administration of apparently adequate therapy, and may be exacerbated by penicillin therapy before improving.[127]

ECTODERMAL CHANGES

Ectodermal changes in syphilitic infants include suppuration and exfoliation of the nails, loss of hair and eyebrows, choroiditis, and iritis.

OTHER FINDINGS

Other clinical manifestations of congenital syphilis include nonimmune fetal hydrops, possibly intrauterine growth restriction, generalized lymphadenopathy, fever, failure to thrive, nephrotic syndrome, and myocarditis. Involvement of the eyes has been manifested by chorioretinitis, cataract, glaucoma, and chancre of the eyelid. Gastrointestinal presentations include rectal bleeding caused by syphilitic ileitis, necrotizing enterocolitis, malabsorption secondary to fibrosis of the gastrointestinal tract, and fetal bowel dilation seen on antenatal ultrasonography.[112,117] Some children with symptomatic congenital syphilis also may present with sepsis caused by other bacteria, including *Escherichia coli*, group B streptococci, and *Yersinia* spp. Neonates and infants with congenital syphilis may resemble infants with other illnesses peculiar to newborns, including toxoplasmosis, rubella, cytomegalovirus infection, herpes simplex virus infection, "sepsis" of the newborn, blood group incompatibilities, battered child

syndrome, "periostitis" of prematurity, neonatal hepatitis, and osteomyelitis.

Late Congenital Syphilis

Late manifestations or stigmata of congenital syphilis are the result of scarring from the early systemic disease or reactions to persistent inflammation and include involvement of the teeth, bones, eyes, and eighth cranial nerve; gummata in the viscera, skin, or mucous membranes; and neurosyphilis (Table 156–6).[45] Approximately 40 percent of surviving and untreated infected infants, as reported in the early literature, develop late manifestations of infection. Some of these changes can be prevented by treating the mother during pregnancy or the infant before the child reaches 3 months of age. In contrast, treatment of 15 children at 4 months of age or later showed 7 with dental changes.[106] Other stigmata (e.g., keratitis, saber shins) may occur or progress, despite the infants being administered appropriate therapy. Nonspecific sequelae of congenital syphilis have not been described well because follow-up studies of children with congenital syphilis are minimal. It has been the experience of one of the authors (P.J.S.), however, that infants who are diagnosed with congenital syphilis and treated appropriately in the neonatal period generally do well at least through early childhood. There is every reason to be hopeful and optimistic with the families of these children, even if they are symptomatic and have CNS involvement at the time of diagnosis.

DENTITION

Characteristic changes are found in the permanent upper central incisors, which have a notched appearance of the biting edges; x-ray study leads to diagnosis, even while deciduous teeth are in place. These are Hutchinson teeth, which also are small and hypoplastic and widely spaced with abnormal enamelization. If first molars show maldevelopment of the cusps, the finding is called *mulberry* or *moon molars*.

INTERSTITIAL KERATITIS

Interstitial keratitis is the most common late lesion. It may appear at any age between 4 and 30 years or later, but characteristically appears when the patient is close to puberty. A ground-glass appearance may develop in the cornea, accompanied by vascularization of the adjacent sclera. These changes become bilateral and usually lead to blindness. It has been seen in adolescent patients despite their having received previous and appropriate penicillin therapy during infancy. At the time of its occurrence, keratitis is not affected by penicillin therapy, but it may respond transiently to corticosteroid treatment.

TABLE 156–6 Stigmata of Late Congenital Syphilis*

Stigmata	Percent of Total Patients
Frontal boss of Parrott	87
Short maxilla	84
High palatal arch	76
Hutchinson triad	75
Higoumenakis sign	73
Relative protuberance of mandible	65
Saddle nose	63
Interstitial keratitis	39
Rhagades	26
Mulberry molars	9
Saber shin	7
Eighth nerve deafness	4
Hutchinson teeth	3
Scaphoid scapulae	0.7
Clutton joint	0.3

*An analysis of 271 patients.

Adapted from Fiumara, N. J., and Lessell, S.: Manifestations of late congenital syphilis. Arch. Dermatol. 102:78-83, 1970. Copyright 1970, American Medical Association.

CENTRAL NERVOUS SYSTEM

The same manifestations of neurosyphilis seen in acquired syphilis may occur in congenital syphilis. Paresis is seen more frequently and tabes dorsalis less frequently in the congenital form than in the acquired form of the disease. Cranial nerve palsies and optic atrophy are prominent.

EIGHTH CRANIAL NERVE DEAFNESS

Hearing loss usually is sudden and occurs when the child reaches approximately 8 to 10 years of age. It often accompanies interstitial keratitis. The constellation of eighth cranial nerve deafness, interstitial keratitis, and Hutchinson teeth is called the *Hutchinson triad*. The hearing loss usually involves the higher frequencies, and normal conversational tones become affected later. This hearing loss may respond to long-term corticosteroid treatment.

BONE AND JOINT CHANGES

Bone changes include the sclerosing lesions, saber shin and frontal bossing, which are sequelae of periostitis that involved the frontal bone and tibia. Periosteal reaction of the sternoclavicular portion of the clavicle may lead to Higouménakis sign. The gummatous or destructive lesions include saddle-nose deformity, which occurs as a sequela of rhinitis. Perforation of the hard palate is almost pathognomonic of congenital syphilis. Clutton joints are uncommon findings and represent painless arthritis of the knees and, rarely, other joints.

CUTANEOUS LESIONS

Rhagades represent scars resulting from persistent rhinitis during infancy and rarely are seen today.

DIAGNOSIS

The vagaries of the maternal history and signs or lack of signs in the infant at birth (i.e., timing of the mother's initial syphilis infection before or during pregnancy, adequacy and documentation of her treatment, an infant who is infected and symptomatic at birth, an infant who is asymptomatic at birth but may not remain so because infection has occurred, and an infant who is asymptomatic and not infected) demand a "safety first" approach to diagnosis and treatment. Efforts to diagnose infectious syphilis suffer from the lack of a method to culture the organisms on laboratory media. Methods that are used in establishing the diagnosis of syphilis include (1) direct visualization of the organism by darkfield microscopy or fluorescent antibody technique of infected fluids or lesions, (2) demonstration of the organism by special stains on histopathologic examination of tissue,[56,115] (3) animal inoculation (rabbit infectivity test), (4) demonstration of serologic reactions typical of syphilis, and (5) detection of *T. pallidum* DNA in a clinical specimen.[19]

The rabbit infectivity test is still the gold standard for the identification of viable *T. pallidum* in clinical specimens, having a sensitivity of less than 10 organisms.[81,82] It involves intratesticular inoculation of the specimen into a rabbit and awaiting serologic seroconversion and orchitis with subsequent visualization

of motile spirochetes by darkfield microscopy in testicular tissue. The rabbit infectivity test is performed only in research laboratories and may take several months for identification of the organism. *T. pallidum* DNA has been detected by polymerase chain reaction (PCR) in body fluids such as amniotic fluid and infant blood, CSF, and endotracheal aspirate.[52,88] The new genetic information about *T. pallidum* may lead to development of novel diagnostic tools.

In the clinical setting, the diagnosis of syphilis is established by visualization of the spirochete by darkfield microscopy or special staining and serology. Patients with a primary syphilis lesion (chancre) and with active secondary lesions may be diagnosed by darkfield microscopy. Because this diagnosis depends on direct visualization of motile spirochetes, the organisms must be active and viable. Prior use of many antibiotics rapidly destroys the motility of the organisms, as do many topical disinfectants. Serous fluid from the base of the lesion should be collected for darkfield examination. Syphilitic lesions of the mouth may harbor indigenous treponemes, of which the morphologic similarity to pathogenic species can confuse the interpretation of findings.

Direct darkfield examination is particularly helpful in establishing a diagnosis early in the course of the disease, before the development of seroreactivity. If a darkfield microscope is unavailable, a direct fluorescent antibody stain for *T. pallidum* may be made.[17] Exudate is collected in capillary tubes or slides and stained with specific antibody. Amniotic fluid may be examined for the presence of spirochetes using darkfield microscopy or fluorescent antitreponemal staining; the finding of spirochetes may be a marker for more severe fetal disease.[51,146] On a practical basis, however, the diagnosis is established by serologic methods.[78,149]

SEROLOGIC TESTS

The two types of serologic tests for syphilis are the nontreponemal antibody tests and the treponemal antibody tests (Table 156–7). Although the latter tests indicate experience with a treponemal infection in the past, they cross-react with the antigens of other treponemal diseases, such as the antigens causing yaws and pinta. No test is specific for syphilis, and no test is completely sensitive. Efforts to produce a more sensitive and more specific test are continuous. A promising approach is the use of recombinant clones expressing immunogenic proteins of *T. pallidum* to investigate pathogen-specific antigens.[63] Some of these products are being investigated for use as diagnostic material.[122]

TABLE 156–7 Standard Serologic Tests for Syphilis

Type	Test	Percent Reactivity During Primary Stage	Secondary Stage	Tertiary Stage
Non-treponemal				
Extracts of tissue (cardiolipin-lecithin-cholesterol)	VDRL	78	95	71
	RPR	86	98	73
Treponemal				
Treponema pallidum	MHA-TP*	76	100	94
	FTA-ABS	84	100	97

FTA-ABS, fluorescent treponemal antibody absorption; MHA-TP, microhemagglutination assay–Treponema pallidum; RPR, rapid plasma reagin; VDRL, Venereal Disease Research Laboratory.

**MHA-TP has been replaced with* Treponema pallidum *particle agglutination (TP-PA) test.*

Non-treponemal Tests

The original test for syphilis, as described by Wassermann, used syphilitic tissue as complement-fixing antigen to detect the presence of antibody (reagin) that is induced by *T. pallidum*. Extracts of other normal tissue, such as beef heart, had similar properties, and purification and standardization of these materials led to the use as antigen of a preparation containing cardiolipin and lecithin in cholesterol.

Two tests currently using cardiolipin, lecithin, and cholesterol are the VDRL and rapid plasma reagin (RPR) tests. These tests measure mostly IgG, but also IgM antibodies nondiscriminatively. The tests provide similar clinical information and have similar advantages. The RPR titer often is one to two dilutions higher than the titer obtained using the VDRL test, so caution must be exercised when making clinical decisions on the basis of results obtained from these two tests performed on the same patient. Both are inexpensive to perform and show increasing and decreasing antibody titers that often correlate with adequacy of therapy and the clinical status of a patient. A fourfold increase in RPR or VDRL titers indicates active disease, whereas a fourfold decrease suggests adequate therapy. Disadvantages include a high proportion of biologic acute and chronic false-positive reactors and an increasing proportion of false-negative reactions in the later stages of untreated syphilis. The main technical difficulty is a negative reaction owing to the prozone phenomenon, which occurs in 1 to 2 percent of individuals, usually patients with secondary syphilis, and is due to an excess amount of reagin antibody present in the patient's undiluted serum that prevents flocculation. Diluting the serum sample before testing overcomes the inhibition and results in the positive reaction. Because the RPR test generally is more sensitive than is the VDRL test, it is preferred for screening pregnant women. The VDRL test is recommended for use on CSF, however.

The RPR test is used frequently to screen pregnant women for possible infection with *T. pallidum*. Transplacental passage of IgG antibodies to the infant means that mothers with positive RPR tests usually transmit these antibodies to their infants. Taber and Huber[138] reviewed the relationship of maternal to newborn VDRL tests in their study of mothers who were undiagnosed and untreated. Although 12 of 22 mothers had VDRL test results two to four times that of their infants, 6 of these women and their infants had nonreactive serology at the time of delivery. These and other data showed that it was unusual for an infant to have a VDRL test of greater titer than the mother's, even if the infant was incubating congenital syphilis.[136] Nonetheless, the finding of a titer in the infant serum fourfold higher than that seen in the maternal serum when both are obtained at the same time is indicative of active infection in the infant. Even if the infant is asymptomatic, a 10-day course of penicillin therapy should be administered.

Treponemal Tests

The most significant development of the past 3 decades in the serologic study of syphilis was the detection of treponemal antibody by fluorescein-labeled antihuman antibody. These tests are used to confirm the validity of a positive non-treponemal test and to diagnose late stages of syphilis. The tests are sensitive and reliable.

Fluorescent treponemal antibody absorption (FTA-ABS) tests use lyophilized Nichols strain organisms as antigen and measure IgG and IgM antibodies. Antigen is fixed to a slide, and the test serum is applied, allowing reaction of antitreponemal antibody with antigen to occur. The slide is layered with fluorescein isothiocyanate-labeled, antihuman gamma-globulin, and the presence or absence of antibody is determined by fluorescent microscopy.

Test sera are preabsorbed with sorbent to eliminate group-reactive antibody. The test is rendered specific for disease with virulent treponemal species, usually *T. pallidum*. The FTA-ABS test is expensive and time-consuming, however. It is recommended not for general screening but for confirmation of positive non-treponemal tests and for establishing the diagnosis of later stages of syphilis in which the results of non-treponemal tests may be negative.

Microhemagglutination tests, and specifically the *T. pallidum* particle agglutination (TP-PA) test, depend on the passive hemagglutination of erythrocytes or latex particles that have been sensitized with Nichols strain *T. pallidum*. The test has been automated and is easy to perform technically and inexpensively. The TP-PA test has largely replaced the FTA-ABS test as the most efficient specific test for antibody to *T. pallidum*. The TP-PA test is as sensitive as is the FTA-ABS test, except in primary syphilis. The test is positive in 75 to 85 percent of patients with primary syphilis and in 100 percent of patients with secondary syphilis. Similar to the FTA-ABS test, the TP-PA test is unlikely to revert to a nonreactive state after treatment of the patient, unless treatment is given very early.

IgM Tests

In the diagnosis of congenital syphilis, having a means to differentiate between passive transplacental transfer of maternal antibody to the fetus and production by the fetus of endogenous antitreponemal antibody would be most helpful. Because antibodies of the IgM class do not cross the placenta, detection of IgM antibody in the fetal or neonatal serum would indicate antibody production by the fetus because of active fetal infection. No IgM assay currently is commercially available that is sufficiently sensitive and specific to recommend for routine use in the evaluation of infants born to mothers with syphilis. The fluorescent treponemal antibody that measures IgM antitreponemal antibodies, the IgM-FTA-ABS test, has been associated with false-positive and false-negative results. When the test was refined further by use of the IgM fraction of neonatal serum only, it had a sensitivity of 73 percent.[136] The test also is insensitive when the onset of disease is delayed. Occasional false-positive IgM-FTA-ABS tests occur because of the presence of an IgM anti-IgG antibody or rheumatoid factor. For these reasons, the CDC has recommended that the IgM-FTA-ABS test be suspended for diagnostic testing of newborns, and the test is available only as a provisional test.

Efforts to develop a sensitive and specific serologic test for congenital syphilis have led to the identification of antigenic components of *T. pallidum* that are epitopes for the immune response. A 34-kd membrane protein is a dipoprotein[137] and the target of IgM antibody formation in the sera of some congenitally infected infants.[36] The Captia Syphilis M test (Trinity Biotech, Jamestown, NY) is an enzyme-linked immunosorbent assay that uses this treponemal protein; it is commercially available but showed a sensitivity of only 88 percent among infants with clinical and laboratory findings of congenital syphilis.[76,136]

Immunoblotting has been used to characterize the specific neonatal IgM antibody responses to *T. pallidum*. Specific IgM antibodies directed against *T. pallidum* antigens with apparent molecular masses of 72 kd, 47 kd, 45 kd, 42 kd, 37 kd, 17 kd, and 15 kd have been detected in sera of fetuses[58] and infants with clinical findings of congenital syphilis, and in 20 to 40 percent of asymptomatic infants born to mothers with untreated syphilis.[36,79,88,120,122,125] IgM reactivity against the 47-kd antigen, a membrane lipoprotein of the organism, has been the most consistent finding. Similar reactivities also have been seen in CSF of infants with congenital syphilis.[79,124] A more recent study showed that a reactive serum IgM immunoblot identified all 17 infants in whom spirochetes were detected in their CSF by rabbit infectivity testing.[88] Efforts to develop rapid diagnostic tests based on these findings are in progress.[73] In addition, immunoblotting has been used to detect similar IgA reactivities among infants with congenital syphilis.[125]

Polymerase Chain Reaction

PCR has been used on neonatal blood and CSF for establishing the diagnosis of congenital syphilis.[52,88,122] Compared with isolation of the organism by rabbit infectivity testing, the sensitivity and specificity of PCR on CSF was 65 to 71 percent and 97 to 100 percent.[88,122] Among 17 infants who had spirochetes detected in their CSF by rabbit inoculation, blood PCR test was the best predictor of CNS infection with *T. pallidum*.[88] This finding supports further the concept that spirochetes gain access to the CNS by a hematogenous route.

False-Positive Reactions

All of the available serologic tests for syphilis produce occasional reactive results in patients who have no other evidence of syphilitic infection. These reactions usually are called *biologic false-positive* (BFP) reactions and are distinct from positive reactions owing to technical errors. Most BFP reactions occur with non-treponemal tests; approximately 1 percent of normal adults have a BFP reaction by non-treponemal antigen tests. These reactions probably are not more common in pregnant women than in the general population. Reaginic antibody is reactive with at least 200 antigens other than those of *T. pallidum*, and although the specific stimulus for this antibody in syphilis and other diseases is unknown, it may represent antibody to cellular lipoidal antigens of the host that are liberated during various diseases. For clinical purposes, BFP reactions may be classified as acute, in which the reactivity resolves within 6 months, or chronic, in which reactivity is persistent.

ACUTE BIOLOGIC FALSE-POSITIVE REACTIONS

Most BFP reactions are detected by non-treponemal tests and occur in patients with other acute illnesses, especially pneumonia, hepatitis, and viral exanthematous disease, or after receiving vaccinations. The prognosis for the patient's health is not affected by the finding. The titer of antibody usually is low (<1:8), and in most instances the FTS-ABS is nonreactive. Approximately two thirds of patients with BFP reactions have acute reactions, and reactivity subsides within 6 months.

CHRONIC BIOLOGIC FALSE-POSITIVE REACTIONS

Many patients with chronic BFP reactions have or develop systemic disease. Drug addiction, chronic hepatitis, old age, leprosy, and collagen vascular disease, especially systemic lupus erythematosus, are associated highly with chronic BFP reactions. A familial predisposition to this finding may exist. The antibody detected by the VDRL test in chronic BFP reactions predominantly is IgM, whereas it mainly is IgG in syphilis. Patients with chronic BFP reactions and systemic lupus erythematosus commonly also have a reactive FTA-ABS test.[77] The triosephosphate isomerase test may be helpful in the differential diagnosis in these instances. A finding of particular concern is that there seems to be a relative increase in acute and chronic BFP reactions in women who are infected with HIV.

EVALUATION AND DIAGNOSIS OF EARLY CONGENITAL SYPHILIS

The diagnosis of congenital syphilis is suggested by results of serologic tests, physical examination, laboratory tests including

CSF examination, and radiographs of long bones and is established by the observation of spirochetes in body fluids or tissue. Examination of the placenta or umbilical cord using specific fluorescent antitreponemal antibody staining is recommended.[50,107] The decision to evaluate and ultimately to treat an infant for congenital syphilis is based on clinical, serologic, and epidemiologic considerations. The evaluation includes an assessment of the mother for general risk factors for increased rates of syphilis (Table 156–8), followed by an evaluation of the mother's current known serologic status (Table 156–9). If the mother has been treated, the clinician must assess the adequacy of the therapy.

If the mother's serologic assays have been positive, the infant must be assessed for clinically apparent disease. The nontreponemal (RPR) and treponemal (TP-PA) tests measure IgG antibody and do not distinguish disease of the infant from maternally derived antibody. Many infants are born to women who have had syphilis in the past, received therapy, and remained seroreactive. Their infants also are seroreactive. Ensuring that the infant does not have congenital disease in the immediate newborn period may be impossible.

Figure 156–2 and Table 156–10 present an approach to the evaluation of infants born to mothers with reactive serologic tests for syphilis. Testing of all pregnant women with syphilis for co-infection with HIV is recommended strongly, even though infants born to co-infected mothers do not require any different evaluation for syphilis.[20] All infants born to mothers with reactive serologic tests for syphilis should have a serum quantitative nontreponemal test performed and a thorough physical examination that focuses on finding evidence of congenital syphilis. The nontreponemal test that is performed on the infant should be the same as the test done on the mother to be able to compare serologic titers. Although it is an uncommon finding, a serum quantitative non-treponemal titer that is fourfold or greater than the corresponding maternal titer is diagnostic of congenital syphilis; when it has occurred, one of the authors (P.J.S.) has isolated spirochetes from blood or CSF in all infants.

TABLE 156–8 General Maternal Risk Factors Associated with Increased Rates of Early Syphilis in Pregnancy

Infection with HIV
Adolescent or unmarried status
History of sexually transmitted disease
Substance abuse, especially cocaine
Inadequate or absent prenatal care
Prostitution or promiscuity
Localized populations or geographic areas
Treatment of gonorrhea with ciprofloxacin or spectinomycin
Poor communication among medical personnel regarding maternal/infant status

Data from references 22, 34, 116, and 145.

TABLE 156–9 Components of the Epidemiologic Evaluation of an Infant's Mother for Possible Syphilis That Aid in Evaluation and Treatment of Infant

Evaluate mother for prior history of syphilis or major risk factors for syphilis (see Table 156–8)
If mother received treatment for syphilis, evaluate course of therapy for adequacy in the treatment of congenital syphilis (see Table 156–2)
Evaluate current maternal status with a non-treponemal test (e.g., Venereal Disease Research Laboratory, rapid plasma reagin) and, if reactive, a treponemal test (e.g., fluorescent treponemal antibody absorption, *Treponema pallidum* particle agglutination test)
If mother is identified clinically or through contact tracing as having early syphilis during the 3 mo after delivery, re-evaluate the infant and consider therapy at that time
Higher maternal titers to non-treponemal tests are associated with failure of maternal therapy to prevent congenital disease[87]
Unknown duration of maternal syphilis is associated with failure of maternal therapy to prevent congenital disease[87]

The CDC recommends that the infant's serologic test be performed on serum and not umbilical cord blood because false-positive test results have been reported with the use of umbilical cord blood secondary to contamination of the specimen with maternal blood and Wharton jelly. Some clinicians continue to use umbilical cord blood, however, because it is readily available and easily collected; appropriate care should be taken during collection to minimize contamination with maternal blood. In addition, false-negative tests may occur when the maternal titer is low dilution, which argues for maternal screening rather than screening of infant serum.

Infants who have (1) an abnormal physical examination that is consistent with congenital syphilis, (2) a serum quantitative non-treponemal serologic titer that is fourfold or greater than the mother's, or (3) a positive darkfield or fluorescent antibody test of body fluid should have a complete blood cell count and platelet count performed and examination of the CSF for cell count, protein content, and VDRL test. Other tests, such as bone and chest radiographs, liver function tests, cranial ultrasound, ophthalmologic examination, and auditory brain stem response should be performed as clinically indicated. These infants are considered to have *proved or highly probable disease*; spirochetemia with invasion of the CNS occurs in approximately 40 to 50 percent of these infants.[88] Although these infants must receive a full course of penicillin therapy, which treats possible neurosyphilis, it is beneficial for follow-up purposes to establish CNS abnormalities at presentation. Nonetheless, the diagnosis of congenital neurosyphilis is difficult to establish (see section on clinical manifestations). The presence of red blood cells in the CSF as a result of a traumatic lumbar puncture can produce a false-positive serologic reaction. Examination of the CSF for *T. pallidum* DNA by PCR may prove more useful for diagnosing congenital neurosyphilis.[52,88,122]

For infants who have a normal physical examination and a serum quantitative non-treponemal test that is less than fourfold the maternal titer, further evaluation and treatment depend on the maternal treatment history and stage of infection (see Fig. 156–2 and Table 156–10). Whether to perform a complete evaluation (lumbar puncture, long bone radiographs, and complete blood cell and platelet counts) on the infant depends on the maternal treatment history and planned treatment of the infant (see section on treatment). If the mother did not receive any prior treatment for syphilis, or the treatment was inadequate, a complete evaluation must be performed and be normal if a single intramuscular dose of benzathine penicillin G therapy is administered. Although it is preferred to help establish a diagnosis of congenital syphilis, a complete evaluation is unnecessary if a full 10-day course of parenteral penicillin is provided because such therapy would treat for the possibility of congenital infection.[13,90]

The need to perform a lumbar puncture has been questioned because the yield of abnormal findings from examination of the CSF has been very low in some experiences and appreciable in others.[12,42,116] A primary benefit of obtaining CSF studies from infants who are receiving a 10-day parenteral course of aqueous or procaine penicillin therapy is identification of infants for whom follow-up of abnormal CSF results should be done. Likewise, long bone radiographs are abnormal in approximately 65 percent of infants with clinical findings of syphilis but only in a few asymptomatic infants. The finding of osteochondritis or periostitis in an infant born to a mother with reactive serologic tests for syphilis indicates congenital syphilis, and the infant requires

TABLE 156–10 Treatment Guidelines for Congenital Syphilis

Scenario	Maternal Stage/Treatment	Recommended Evaluation	Regimen
Infant with proven or highly probable disease	Any or none	CSF analysis: VDRL, cell count, protein CBC and differential; platelet count. As clinically indicated: long bone radiographs, liver function tests, cranial ultrasound, eye examination, hearing evaluation	Aqueous penicillin G 50,000 U/kg IV q12h (≤1 wk old), q8h (>1 wk old, ≤4 wk old), q6h (>4 wk old) × 10 days *or*
(a) Abnormal physical examination (b) Abnormal evaluation* (c) Serum non-treponemal titer ≥4× maternal titer (d) Visualization of spirochete in clinical specimen			Procaine penicillin G 50,000 U/kg IM × 10 days (≤4 wk old)
Infant with *normal* physical examination and serum non-treponemal titer <4× maternal titer	Any stage of infection *and*	CSF analysis: VDRL, cell count, protein; CBC and differential; platelet count; long bone radiographs	Treatment A: Benzathine penicillin G† 50,000 U/kg IM × 1
	No treatment; *or*	If *all* normal: treatment A	Treatment B: Aqueous penicillin G 50,000 U/kg IV q12h (≤1 wk old), q8h (>1 wk old, ≤4 wk old), q6h (>4 wk old) × 10 days *or*
	Inadequate or undocumented treatment; *or* Erythromycin or non-penicillin treatment; *or* Therapy ≤4 wk before delivery; *or* Adequate therapy >1 mo before delivery, but maternal non-treponemal titers have not decreased fourfold after treatment for early syphilis (primary, secondary, early latent syphilis)	If *any* abnormal or not done‡: treatment B	Procaine penicillin G 50,000 U/kg IM × 10 days (≤4 wk old)
	Adequate therapy >1 mo before delivery, and appropriate for stage of infection; *or* Maternal non-treponemal titer decreased fourfold after treatment for early syphilis; *or* Maternal non-treponemal titers remained stable and low for late syphilis; *and* No evidence of reinfection or relapse	No evaluation	Benzathine penicillin G 50,000 U/kg IM × 1§
	Adequate therapy before pregnancy and stable non-treponemal titers (VDRL ≤1:2, or RPR ≤1:4) throughout pregnancy	No evaluation	No treatment¶
Congenital syphilis in infant >28 days old	Any or none	CSF analysis: VDRL, cell count, protein CBC and differential; platelet count. As clinically indicated: long bone radiographs, liver function tests, cranial ultrasound, eye examination, hearing evaluation	Aqueous penicillin G 50,000 U/kg IV q4-6h × 10 day *and* ? Benzathine penicillin G¶ 50,000 U/kg IM × 1

**CSF examination (VDRL test, cell count, protein), bone radiographs, CBC, platelets, umbilical cord or serum VDRL/RPR (same test as performed on maternal serum).*

†*If the infant's non-treponemal test is nonreactive, no evaluation is required, but the infant should receive treatment A.*

‡*Clinical and serologic follow-up must be certain.*

§*Some experts would not treat but provide close serologic follow-up.*

¶*Benzathine penicillin G 50,000 U/kg IM × 1 if follow-up uncertain.*

¶*Some experts prefer prolonged therapy by administration of a single dose of benzathine penicillin G after the 10-day course of IV aqueous penicillin G.*

CBC, complete blood count; CSF, cerebrospinal fluid; RPR, rapid plasma reagin; VDRL, Venereal Disease Research Laboratory.

Adapted from Centers for Disease Control and Prevention: Sexually transmitted diseases treatment guidelines—2002. M. M. W. R. Morb. Mortal. Wkly. Rep. 51(No. RR-6):26-28, 2002; and American Academy of Pediatrics: Syphilis. In Pickering, L. K. (ed.) 2006 Red Book: Report of the Committee on Infectious Diseases. 27th ed. Elk Grove Village, IL, American Academy of Pediatrics, 2006, pp. 631-634.

a full course of penicillin therapy for highly probable disease. Such an infant is likely to have CNS infection, however, even in the absence of clinical signs of neurosyphilis, and close clinical and serologic follow-up would be indicated.[88]

After the newborn period, children with syphilis should have a lumbar puncture performed for evaluation of the CSF to detect asymptomatic neurosyphilis. In addition, birth and maternal records should be reviewed to assess whether such children have acquired or congenital syphilis.[27] Children with acquired syphilis should be evaluated for the possibility of sexual abuse.

TREATMENT

T. pallidum is extremely sensitive to penicillin, as defined by experimental animal work. The minimal inhibitory concentration of penicillin is approximately 0.004 U/mL (or 0.0025 μg/mL). There is no evidence of increasing resistance to penicillin by the spirochetes, but such evidence would come only from the recognition of therapeutic failures. Effective treatment of syphilis must maintain a minimal inhibitory concentration of 0.03 U/mL of penicillin in serum (or CSF) for 7 to 10 days. Therapy is designed to achieve and maintain several times the necessary inhibiting levels and to avoid penicillin-free intervals during therapy. Penicillin remains the drug of first choice because of its established efficacy and minimal toxicity.[20]

ACQUIRED SYPHILIS

Early syphilis (i.e., primary, secondary, and early latent infection) is treated with benzathine penicillin G, 50,000 U/kg up to the adult dose of 2.4 million U intramuscularly in a single dose. Late latent syphilis requires benzathine penicillin G, 150,000 U/kg up to the adult dose of 7.2 million U, administered as 50,000 U/kg up to the adult dose of 2.4 million U at 1-week intervals for 3 weeks. When the duration of infection is unknown, the patient should be treated as for late latent disease.

All patients with syphilis should be tested for HIV infection at diagnosis, and if they have early syphilis, they should be retested 3 to 6 months later. Because neurosyphilis may be asymptomatic and can be defined accurately only by examining the CSF, the CDC recommends performing a lumbar puncture in the following situations: (1) neurologic or ophthalmic signs or symptoms, (2) active tertiary syphilis, (3) treatment failure, and (4) HIV infection with late latent syphilis or syphilis of unknown duration.[20] A lumbar puncture is not recommended routinely for patients with primary or secondary syphilis. Benzathine penicillin does not produce inhibitory CSF levels of penicillin reliably.[134] Shorter acting penicillins must be employed for neurosyphilis. In evaluating a patient for neurosyphilis, a CSF specimen without contamination by peripheral blood is needed.[64]

The recommended therapy for neurosyphilis is aqueous crystalline penicillin G, 18 to 24 million U/day, administered as 3 to 4 million U intravenously every 4 hours or continuous infusion for 10 to 14 days. An alternative regimen consists of procaine penicillin, 2.4 million U intramuscularly once daily, plus probenecid, 500 mg orally four times per day, for 10 to 14 days. Because these regimens are of shorter duration than is the regimen used for late latent syphilis, some experts recommend the additional administration of benzathine penicillin G, 2.4 million U intramuscularly once per week for up to 3 weeks after completion of the 10- to 14-day course. Older children with definite acquired syphilis and a normal neurologic and CSF examination may be treated with benzathine penicillin G, 50,000 U/kg intramuscularly to a maximal dose of 2.4 million U. Children with neurosyphilis should receive aqueous penicillin G, 200,000 to 300,000 U/kg/day intravenously administered as 50,000 U/kg every 4 to 6 hours for 10 days. Similar to the procedure with adults, an additional dose of benzathine penicillin G, 50,000 U/kg intramuscularly, may be given following the 10-day intravenous penicillin treatment.

The evaluation and treatment of early syphilis in HIV-infected individuals are the same as those for individuals not infected with HIV except that they may be at increased risk for developing neurologic complications and have a higher risk of experiencing treatment failure. HIV-infected individuals who have late latent syphilis or syphilis of unknown duration should have their CSF examined for evidence of neurosyphilis before receiving treatment; further management and treatment depend on CSF findings. Close serologic follow-up of HIV-infected individuals is mandatory to detect treatment failure and the need for examination of CSF and re-treatment.

Data to support the use of alternatives to penicillin in the treatment of syphilis are limited. Any history of penicillin allergy should be documented clearly, and, whenever possible, patients should receive skin testing, desensitization, and therapy with penicillin.[20] Doxycycline (100 mg orally twice daily) and tetracycline (500 mg four times daily) have been used for 14 days for treatment of early syphilis and 28 days for late latent infection and syphilis of unknown duration. Close serologic follow-up is mandatory. Neither of these therapies is recommended for children younger than 8 years of age. Ceftriaxone (100 mg/kg/day, maximum of 1 g daily) given either intravenously or intramuscularly for 8 to 10 days may be effective for treating early syphilis, but neither the optimal dose nor duration of treatment has been defined.[59] Preliminary data also suggest that azithromycin as a single dose of 2 g may be effective in adults, but treatment failures have been described, as has emergence of resistance.[20] Because experience with these alternative antibiotics in HIV-infected individuals is limited, they should be used with caution.

Two to 12 hours after receiving treatment for syphilis, a variable proportion of patients develop an acute systemic (Jarisch-Herxheimer) reaction usually consisting of headache, malaise, fever (≥38° C), and resolution within 1 day. The reaction is observed most frequently in the early stages of syphilis, probably represents a reaction to liberated endotoxin,[49] and does not affect the course of recovery. In the later stages of syphilis, fewer than one in four patients develops the reaction. Most reactions in late syphilis clinically are insignificant, but an occasional reaction may produce damage to the CNS or cardiovascular system.

SYPHILIS IN PREGNANCY

Pregnant women with reactive serologic tests for syphilis should be considered infected unless an adequate treatment history is documented, and sequential non-treponemal antibody titers have declined. They should receive the penicillin regimen appropriate for the stage of syphilis.[20,149] For early syphilis, many experts recommend that an additional dose of benzathine penicillin G be provided 1 week after the initial dose. Management and treatment decisions may be guided further by the use of fetal ultrasonography. Evidence of fetal infection may require additional doses of benzathine penicillin G until resolution of fetal abnormalities has been achieved. The Jarisch-Herxheimer reaction may complicate treatment and result in preterm labor and fetal decelerations, although concern for its occurrence should not delay initiation of treatment.[69,93] No alternative to penicillin is available; pregnant women who have a history of penicillin allergy should undergo skin testing, desensitization, and treatment with penicillin.[148] Erythromycin is not recommended; infants have been born with congenital syphilis after maternal treatment with erythromycin. Any therapy other than penicillin is considered to be inadequate fetal therapy.[41,104]

Women who are co-infected with syphilis and HIV should receive treatment regimens corresponding to their stage of syphilis. The follow-up on treated HIV-infected women must be thorough and frequent.[55,92]

Patients who received therapy for gonorrhea with ceftriaxone have a high rate of cure of pre-primary syphilis, but failures have occurred, and efficacy in pregnancy is not well studied.[59] This regimen cannot be assumed to have provided adequate therapy for syphilis in pregnancy.

CONGENITAL SYPHILIS

Infants with Proven or Highly Probable Disease

Infants who have findings on physical examination that are consistent with congenital syphilis—a quantitative non-treponemal serologic titer that is at least fourfold greater than the mother's titer, or spirochetes visualized in body fluids—should be treated with aqueous crystalline penicillin G, 50,000 U/kg/dose intravenously every 12 hours during the first 7 days of life and every 8 hours thereafter for 10 days, or with procaine penicillin G, 50,000 U/kg/dose administered intramuscularly in a single daily dose for 10 days (see Table 156–10).[20] Although the levels of penicillin that are achieved in the CSF after procaine therapy are lower than the levels seen with intravenous penicillin treatment, the clinical significance is unclear.[6] No treatment failures have been reported after procaine penicillin therapy. If more than 1 day of penicillin therapy is missed, the entire course should be restarted.

During a penicillin shortage in the United States, ampicillin was recommended as an alternative agent, although data are insufficient for routine administration. When possible, a full 10-day course of penicillin is preferred even if ampicillin was provided initially for possible sepsis. Data also are lacking for the use of ceftriaxone in these infants. Infants born to mothers co-infected with syphilis and HIV do not require more intense or prolonged treatment for syphilis than is recommended for all infants.

Infants with a Normal Physical Examination and a Serum Quantitative Non-treponemal Serologic Titer That Is the Same or Less than Fourfold the Maternal Titer

Treatment decisions for "asymptomatic" infants are based on the maternal history of syphilis and past treatment. The following situations are associated with a high likelihood that the infant may be infected with *T. pallidum* and should receive treatment: (1) mother was not treated, was inadequately treated, or has no documentation of having received treatment[108]; (2) mother was treated with erythromycin or other non-penicillin regimen; (3) mother received treatment *less than* 4 weeks before delivery[14]; and (4) mother has early syphilis and has a non-treponemal titer that either has not decreased fourfold or has increased fourfold. If the infant's non-treponemal test is reactive, the infant should be evaluated with a complete blood cell count and differential; platelet count; long bone radiographs; and CSF analysis for VDRL test, cell count, and protein content (Fig. 156–3; see Table 156–10).[20] If the entire evaluation is normal and follow-up is certain, the infant may receive benzathine penicillin G, 50,000 U/kg as a single intramuscular dose.

If any part of the evaluation is abnormal or not done, or follow-up is uncertain, treatment should consist of aqueous penicillin G or procaine penicillin G (see earlier) for 10 days. If the infant's non-treponemal serum test is nonreactive, the evaluation may be omitted, but the infant should be treated with a single intramuscular dose of benzathine penicillin for the possibility of incubating syphilis. Some experts prefer that all infants born to mothers with untreated syphilis receive parenteral penicillin therapy for 10 days, even if the evaluation is normal, because many of these infants are likely to be infected with *T. pallidum*, especially if the mother has secondary syphilis at delivery or seroconverted during the pregnancy.[5,20]

More recent studies have re-evaluated the efficacy of treatment regimens for asymptomatic infants who are born to mothers in whom the treatment for possible syphilis was suboptimal.[100,108] Paryani and colleagues[100] randomly assigned 152 infants to receive either one injection of benzathine penicillin or a 10-day course of parenteral procaine penicillin. All study infants were asymptomatic on physical examination and had normal CSF evaluation, normal x-ray studies of long bones, and no visceral abnormalities. The results of both forms of therapy were excellent, with no treatment failures. This study indicates that single-dose therapy may have a high rate of success when an infant has negative studies and is asymptomatic.

Failure of such therapy in three infants has been reported, however, the frequency seems to be low, although it is unknown.[12,151] None of the three infants who developed clinical signs of congenital syphilis several weeks after having received a single intramuscular dose of benzathine penicillin was fully evaluated for congenital syphilis at birth. The concern has been that infected but asymptomatic infants may have CNS infection and fail therapy with benzathine penicillin because benzathine penicillin does not achieve treponemicidal concentrations in CSF.[134] Using rabbit inoculation, Michelow and colleagues[88] showed that invasion of the CNS is an infrequent occurrence among these infants with normal results on clinical, laboratory, and radiographic evaluations.

An infant does not require any evaluation in the following situations: (1) mother was treated during the pregnancy, treatment was appropriate for the stage of infection, and treatment was administered more than 4 weeks before delivery; (2) mother's non-treponemal titers decreased fourfold after administration of appropriate therapy for early syphilis or remained stable and low for late syphilis; and (3) mother has no evidence of re-infection or relapse. The CDC recommends, however, that these infants receive a single dose of benzathine penicillin G, 50,000 U/kg intramuscularly. Infection of the fetus may occur despite administration of appropriate maternal therapy during pregnancy; failure rates of maternal treatment for prevention of fetal infection have been reported to be 2 to 14 percent and are more common with maternal secondary syphilis.[4,31,95,128] Treating the infant at birth may prevent the development of clinical disease if these infants were infected in utero. Some specialists would not treat the infant, however, but provide close serologic follow-up only.

If the mother's treatment was adequate before pregnancy, and the mother's non-treponemal serologic titer remained low and stable during pregnancy and at delivery, no evaluation or treatment is recommended by the CDC. Some specialists would provide a single intramuscular dose of benzathine penicillin, however, if follow-up is uncertain for the remote possibility that the mother was re-infected. These infants are at very low risk of having congenital syphilis; for the most part, these mothers are serofast.

After the neonatal period, all children who are diagnosed with congenital syphilis should be evaluated with a complete blood cell count and differential, platelet count, and examination of the CSF. Other tests, such as auditory brain stem responses, should be performed as clinically indicated. These children and children with late congenital syphilis should be treated with 200,000 to 300,000 U/kg/day of aqueous crystalline penicillin G, given as 50,000 U/kg every 4 to 6 hours for a minimum of 10 to 14 days.

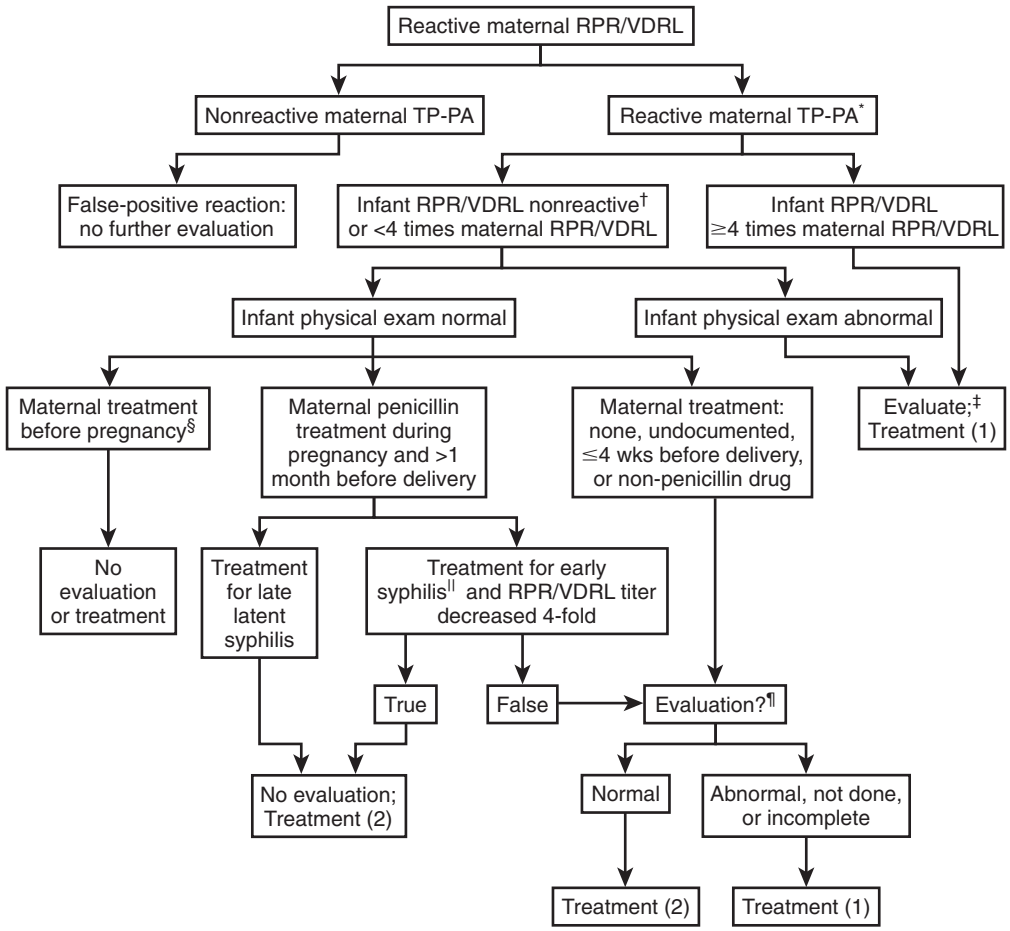

Figure 156–3 Algorithm for evaluation and treatment of infants born to mothers with reactive serologic tests for syphilis. *Test for human immunodeficiency virus (HIV) antibody. Infants of HIV antibody–positive mothers do not require different evaluation or treatment. †Infant's RPR may be nonreactive because of low maternal RPR titer or recent maternal infection. If the mother has untreated or inadequately treated syphilis, and infant's physical examination is normal, treat infant with a single intramuscular injection of benzathine penicillin (50,000 U/kg). No further evaluation is needed. ‡Evaluation consists of complete blood count; platelet count; and cerebrospinal fluid examination for cell count, protein, and quantitative VDRL. Other tests as clinically indicated include long bone x-rays, cranial ultrasound, auditory brain stem response, eye examination, chest x-ray, liver function tests, and urine or meconium toxicology. §Women who maintain a VDRL titer ≤1:2 (RPR ≤1:4) >1 year following successful treatment are considered serofast. ||Early syphilis: primary, secondary, or early latent infection. ¶Complete blood count; platelet count; cerebrospinal fluid examination for cell count, protein, and quantitative VDRL; long bone x-rays. Treatment regimens are as follows: (1) aqueous penicillin G 50,000 U/kg IV q12h (≤1 week old) or q8h (>1 week old), or procaine penicillin G 50,000 U/kg IM single daily dose ×10 days. (2) Benzathine penicillin G 50,000 U/kg IM ×1 dose. RPR/VDRL, rapid plasma reagin/Venereal Disease Research Laboratory; TP-PA, *T. pallidum* particle agglutination.

The Jarisch-Herxheimer reaction may complicate the treatment of congenital syphilis. It rarely is seen in newborns, although it occurs more commonly when syphilis is treated later in infancy. It is manifested usually by fever, but cardiovascular collapse and seizures also have been reported.

FOLLOW-UP EVALUATION

Table 156–11 summarizes recommendations concerning follow-up of infants after they have been treated for congenital syphilis and of seroreactive infants who were not treated because of the presumed adequacy of the management of the mother's serologic status.[60,61,111] Infants born to mothers with syphilis should have serial quantitative non-treponemal tests performed until the test results show nonreactivity.[56,114] Similarly, infants who are seronegative but whose mothers acquired syphilis late in gestation should be followed with serial testing after penicillin therapy is instituted.

Follow-up for infants who received appropriate penicillin therapy for possible syphilis can be incorporated into routine pediatric care at 2, 4, 6, 12, 15, and 24 months. These infants should show a fourfold decrease in non-treponemal serologic titers. In infants with congenital syphilis, non-treponemal serologic tests become nonreactive within 6 to 12 months after they have received appropriate treatment. Uninfected infants usually become seronegative by the time they reach 6 months of age.[26] Infants with persistently low, stable titers of non-treponemal tests beyond 1 year of age may require retreatment.

Untreated infants may benefit from having a non-treponemal test performed at 1 month of age. Untreated infants who are not seronegative by the time they are 6 months old should be re-evaluated clinically and treated. If the non-treponemal titer increases fourfold in any infant during follow-up, full evaluation and treatment are indicated. All infants and children should be evaluated thoroughly for the extent of disease if there is serologic evidence of failure of treatment or of recurrent disease. At a minimum, such evaluation should consist of CSF examination

TABLE 156–11 Recommended Follow-up of Infants Born to Mothers with Syphilis

1. Non-treponemal test (e.g., RPR)
   a. Treated infant: Perform at 2, 4, 6, 12, 15, and 24 months old (or post-therapy) until nonreactive. Non-treponemal test titer should decrease fourfold within 6 mo following treatment and be nonreactive by 6-12 mo. If test remains reactive ≥12 mo after treatment, consider re-evaluation and re-treatment. If non-treponemal test titer increases fourfold, full re-evaluation and re-treatment for proven disease is required
   b. Untreated infant: Perform at 1, 2, 4, 6, 12, 15, and 24 months old (or post-therapy) until nonreactive. Non-treponemal test titer should decrease fourfold within 6 mo following treatment and be nonreactive by 6 mo. If non-treponemal assay is reactive at 6 mo, re-evaluate child (including CSF examination) and treat. If non-treponemal test titer increases fourfold, full evaluation and treatment for proven disease are required
2. Treponemal test (e.g., TP-PA): Perform at 12 mo. If reactive, repeat at 18 and 24 months old*
3. Abnormal CSF examination at diagnosis: Repeat CSF examination (VDRL, cell count, protein content) at 6 months old (or post-therapy). If abnormal and no other explanation found, re-treat infant
4. Monitor yearly for neurologic, hearing, and ophthalmic disorders
5. Monitor developmental status and assess school function

**Approximately 30% of infected children maintain a reactive treponemal test beyond 18 months of age. This finding confirms the diagnosis of congenital syphilis. If child was not previously treated, treatment for proven congenital syphilis is mandatory.*

CSF, cerebrospinal fluid; RPR, rapid plasma reagin; TP-PA, T. pallidum *particle agglutination; VDRL, Venereal Disease Research Laboratory.*

Adapted from Ikeda, M. K., and Jenson, H. B.: Evaluation and treatment of congenital syphilis. J. Pediatr. 117*:843-852, 1990; Rathbun, K. C.: Congenital syphilis: A proposal for improved surveillance, diagnosis and treatment. Sex. Transm. Dis.* 10*:102-107, 1983; and Ingall, D., and Sánchez, P. J.: Syphilis.* In *Remington, J. S., and Klein, J. O. (eds.): Infectious Diseases of the Fetus and Newborn Infant. 5th ed. Philadelphia, W. B. Saunders, 2001, pp. 643-681.*

and complete blood cell count and platelet count. Other tests, such as long bone radiographs, liver function tests, hearing evaluation, and ophthalmologic evaluation, can be performed as clinically indicated.

A reactive treponemal test in an infant who is older than 18 months of age and has lost all maternal antibody confirms the diagnosis of congenital syphilis. This finding occurs, however, in only a few infants with congenital syphilis, as documented by isolation of spirochetes by rabbit inoculation of infant blood or CSF. If the child did not receive treatment previously, a full evaluation and treatment are indicated.

Infants with abnormal CSF findings should have a repeat lumbar puncture performed at 6 months after therapy. A reactive CSF VDRL test result or an abnormal protein content or cell count at that time is an indication for re-treatment.

Adults and older children with primary or secondary syphilis should be examined clinically and serologically at least at 6 and 12 months after treatment. Failure of non-treponemal titers to decrease fourfold within 6 months of treatment indicates probable treatment failure. These individuals should be re-evaluated for HIV infection, and consideration should be given to performance of a lumbar puncture for detection of unrecognized neurosyphilis and re-treatment. For latent syphilis, subsequent follow-up with quantitative serology at 6, 12, and 24 months is recommended. Failure of a fourfold decrease in initially elevated non-treponemal serologic titers (≥1:32) within 12 to 24 months of therapy is an indication for examination of CSF and re-treatment. Re-treatment for syphilis also is recommended when clinical signs or symptoms persist or recur, or if there is a sustained fourfold increase in titer of a non-treponemal serologic test. Patients with neurosyphilis require examinations and repeat CSF examinations every 6 months until the cell count is normal. If the cell count is not decreased after 6 months or the CSF is not normal after 2 years, re-treatment should be considered.

In most patients receiving appropriate therapy during the primary or secondary stages, active disease is arrested totally and permanently. Persistent seroreactivity as measured by the FTA-ABS test may be avoided if treatment is given during the pre-primary stage, but seldom thereafter. Nonetheless, progression to tertiary disease seldom, if ever, occurs. Similarly, therapy administered during early or late latent syphilis averts the development of symptomatic tertiary disease. Antimicrobial therapy for symptomatic neurosyphilis, optic neuritis, and cardiovascular syphilis may not be followed by significant clinical improvement, and established damage to vital organs may fail to resolve.

PREVENTION

Methods to control the spread of syphilis have relied extensively on treatment of case contacts. Individuals with active syphilis are interviewed to identify all sexual contacts that may have occurred (1) 3 months plus duration of symptoms for primary syphilis, (2) 6 months plus duration of symptoms for secondary syphilis, and (3) 1 year for early latent syphilis. The contacts are examined, tested serologically, and treated appropriately even if they are seronegative. Advantage is taken of the long incubation period of syphilis by preventing disease in contacts before they themselves can transmit infection.

Adverse outcomes of pregnancy and congenital infection can be prevented effectively by performing routine prenatal serologic screening of and providing penicillin treatment to infected women and their sexual partners.[133,144] All pregnant women should have a non-treponemal serologic test for syphilis at the first prenatal visit. For communities and populations in which the prevalence of syphilis is high or for patients at increased risk of contracting disease, serologic testing should be performed at 28 weeks' gestation and at delivery because some women may acquire syphilis during pregnancy.[2] In the absence of clinical symptoms or proven exposure to an active case and with a negative treponemal test, treatment may be withheld and studies repeated in 4 weeks because this probably represents a BFP result. When epidemiologic, clinical, or serologic evidence of infection is present, or the diagnosis cannot be excluded, treatment should be instituted.

Serologic screening tests should be performed on maternal serum and not on the infant's serum or umbilical cord blood.[112] An infant's serologic titer usually is one to two dilutions less than that of the mother's titer; an infant may have a nonreactive serologic test for syphilis when the maternal titer is of low dilution. Some of these infants, especially infants born to mothers with no prior treatment for syphilis, require treatment for prevention of late-onset disease. They would be missed if only infant screening is performed.

Limitations of current screening practices also exist. A falsely negative non-treponemal test can occur from a prozone phenomenon. Also, a negative maternal non-treponemal test at delivery would not exclude incubating syphilis or even primary syphilis if non-treponemal and treponemal antibodies have not yet reached detectable concentrations.[37,123] Infants born to such mothers may be infected and develop clinical disease in the ensuing 3 to 14 weeks. Repeat screening of mothers who reside in areas with a high prevalence of syphilis or engage in high-risk behaviors may be advisable at the first postpartum visit.

It is a policy statement of the CDC that no infant should leave the hospital without the serologic status of the infant's mother having been documented at least once during pregnancy and preferably again at delivery.[20,83] In this era of early and very early discharge from the hospital after deliveries, fulfilling the policy

goal may require careful planning and advocacy by the clinician.

All syphilis cases must be reported to the local public health department. This practice allows for contact investigation, appropriate follow-up, and identification of core environments and populations in which the greatest transmission of syphilis is occurring in a particular community. Despite great progress in recent years, the prevention, control, and even elimination of endemic syphilis in the United States remains an elusive goal in public health policy and on a global scale in countries where resources may be limited, and the practicalities of serologic screening and treatment in prenatal care settings are challenging.

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CHAPTER 157

Nonvenereal Treponematoses

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Pinta, yaws, and endemic syphilis (bejel) are the three chronic granulomatous diseases that constitute the pathogenic nonvenereal treponematoses of humans. These diseases are caused by morphologically and antigenically indistinguishable treponemes found almost exclusively in underdeveloped areas of the tropics and bordering arid regions. Minor genomic differences among species of treponemes have been reported by researchers, but as yet these variations cannot be used to discriminate among the causative agents of these diseases.[5-7,14,42,57,62] The pathogenic treponemes have been given subspecies designations as follows: syphilis, *Treponema pallidum* subsp. *pallidum* (*T. pallidum*); yaws, *T. pallidum* subsp. *pertenue* (*T. pertenue*); and endemic syphilis, *T. pallidum* subsp. *endemicum* (*T. pallidum*). The designation for the causative agent of pinta remains *Treponema carateum* (Table 157–1).

Although apparently neither sexually nor congenitally acquired, agents responsible for the nonvenereal treponematoses are transmitted person-to-person by means of skin or mucous membrane contact. Close contact with children or young adults, traumatized areas of skin, lack of clothing, and poor personal hygiene are important factors in the transmission of these diseases. All three nonvenereal treponematoses are potentially debilitating diseases that have enormous social and economic implications within endemic areas. Although their worldwide prevalence generally has been reduced considerably by means of seroepidemiologic and mass treatment campaigns sponsored by the World Health Organization (WHO) and the United Nations International Children's Emergency Fund (UNICEF) in the 1950s and 1960s, they remain a public health problem in several areas of the world where sporadic outbreaks continue to occur.[47,65]

Since the termination of these mass treatment campaigns, lack of attention to these diseases and diversion of resources to other public health problems, such as human immunodeficiency virus, malaria, and tuberculosis, have led to a resurgence in the nonvenereal treponematoses in some parts of the world. Because of a lack of vigilance and awareness of these diseases, they probably are underreported. Data reported by WHO in 1997 estimated that there were more than 2.5 million cases worldwide.[66] The potential effectiveness of a determined treatment and surveillance program was shown in a region of northern Ecuador where no new cases of yaws were reported from 1993 to 1998.[2] Prior to the inception of this program, there was a prevalence of 16.5 percent clinical cases in the area's population.

The history, development, and global spread of treponemal diseases have been the subject of widespread debate. Because the primary reservoirs for these organisms are humans, their history is intertwined within the economic, cultural, and social frame-

TABLE 157–1 Comparison of the Treponematoses

	Syphilis	Yaws	Endemic Syphilis	Pinta
Organism	*T. pallidum* subsp. *pallidum*	*T. pallidum* subsp. *pertenue*	*T. pallidum* subsp. *endemicum*	*T. carateum*
Distribution	Worldwide	Tropics, Africa, Asia, Pacific	Arid areas of Africa, Arabia	Tropical Central, South America
Peak ages affected	All ages	1-12 yr	<15 yr	15-20 yr
Route of entry	Mucous membranes	Skin	Oral mucosa, fomites	Skin
Incubation period	2-10 wk	3-9 wk	3 wk	2-3 wk
Experimental hosts	Rabbit, primate, guinea pig, hamster	Rabbit, hamster	Rabbit, hamster	Primate only
Sexual transmission	Yes	No	No	No
Congenital transmission	Yes	No	No	No
Skin infection	Yes	Yes	Yes	Yes
Bone involvement	5-10%	20-40%	20-40%	0
Neurologic involvement	Yes	No	Rare	No
Cardiologic involvement	Yes	No	Rare	No

work of humanity. One theory postulates that all treponemal diseases originated from pinta or yaws, first found in Central Africa. This view states that treponemes spread widely from there, and the various disease processes that we know today are a result of mutations caused by the adaptation of the organisms to environmental and development changes in their hosts. The Columbian theory suggests that treponemes began as nonvenereal pathogens in Africa that spread to the Americas via Asia thousands of years ago while mutating into endemic syphilis (bejel) as it crossed Africa and Asia. In North America, it developed into the venereally transmitted form of disease caused by *T. pallidum* that we know today as syphilis. Syphilis was carried to Europe by Columbus and his men on his return voyage from the Americas. Meanwhile, the nonvenereal forms were found in Britain, but not in Continental Europe, in the years before Columbus. Supporters of this theory point to skeletal evidence of syphilis found in the Dominican Republic before the arrival of Columbus on the island and to evidence of nonvenereal treponemal infections, but not syphilis, in Britain during the same period.[18,25,50,68]

On morphologic examination, the treponemes are thin, tightly coiled organisms that do not stain or stain poorly with the usual aniline dyes. They contain 4 to 14 spirals and are actively motile by virtue of periplasmic flagella. The organisms are quite fragile and sensitive to drying, atmospheric oxygen, and temperatures greater than 35° C. The organisms can be stored in either 15 percent glycerol at –70° C or liquid nitrogen and still retain their virulence for long periods.[58] Although limited multiplication of *T. pallidum* subsp. *pallidum* has been achieved in a tissue-culture system,[15] it has not been accomplished as yet with the nonvenereal pathogenic treponemes. No human pathogenic treponemes have been cultured in or on artificial media. Because they measure 10 to 13 μm in length but only 0.15 μm in width, they are below the resolution of light microscopy and must be identified by darkfield microscopy in infected skin or mucous membrane lesions.

Little can be done to distinguish the various species of pathogenic treponemes. Clinical and epidemiologic features of each disease state from which the treponeme is recovered constitute the primary method of differentiation. Although not practical, further differentiation is based on the response of animal models to experimental infection.[26,30,48,53,54] Each of the pathogenic treponemes stimulates cross-reactive antibodies assayed in the serologic diagnosis of the human diseases by non-treponemal and treponemal tests. Various degrees of homologous resistance and cross-immunity exist after infection occurs with any of these spirochetes.[26,27,35,36,53,59] Rabbit models have shown complete immunity against re-infection with homologous pathogens, but only incomplete or nonexistent protection against other subspecies.[58] No specific antigenic differentiation has been found among species to explain these findings. Despite differences in methods of transmission, clinical disease, geographic distribution, and rare reports of resistant cases, all human treponemes remain sensitive to penicillin.[63]

PINTA

Pinta (mal del pinto, carate, enfermedad azul) is a chronic, contagious, nonvenereally transmitted treponemal disease that normally is the mildest of these disorders. Although it affects primarily patients in the first 2 decades of life, it can affect children and adults of all age groups, producing dyschromic skin lesions without pathogenic involvement of deeper organ structures. Pinta is not a fatal disease, but a disfiguring one, which results in social ostracism and an inability to adjust to an urban society.

BIOLOGY AND IMMUNOLOGY

Pinta is caused by *T. carateum*. The organism is characterized primarily by its isolation from patients with clinically apparent pinta and by its lack of pathogenicity in small laboratory animals. Only chimpanzees have been shown to be susceptible to experimental infection.[30,31] Human studies by Medina[36] suggest that *T. carateum* induces significant protection against homologous reinfection and cross-immunity against the treponemes of yaws and syphilis; however, these two subspecies of *T. pallidum* fail to confer cross-protection against *T. carateum*.[36]

EPIDEMIOLOGY

Pinta is found primarily in tropical Central and South America among underprivileged Indians and in the Caribbean. It occurs in major river basins of Mexico, Venezuela, Colombia, Brazil, Peru, and Ecuador. Cases also have been reported in Argentina, Chile, Haiti, Guatemala, Dominican Republic, Honduras, Nicaragua, and Bolivia. Pinta rarely occurs at higher elevations in the mountain regions of these countries. In the early 1920s, approximately 500,000 cases occurred throughout Latin America; however, today, as a result of seroepidemiologic and mass treatment campaigns, few cases are being reported. The possibility of underreporting exists because a 20 percent clinical prevalence was reported in 1982 in a village in Panama.[46] A case of pinta in Austria in a visitor from Cuba was reported in 1999.[67]

The disease is found where poor hygienic and crowded conditions exist. Although endemic areas basically are rural, tribal

tradition allows very close contact among its members to occur. These tropical societies, with their absence of shoes and clothes, continually subject exposed skin to trauma and to contact with infectious lesions. Transmission of *T. carateum* occurs primarily by intimate skin-to-skin contact; a break in the skin is required for invasion of the treponeme. Pinta is not acquired congenitally or by blood transfusion. Transmission of the disease by insect vectors has been proposed but not proven[36]; insects probably initiate disease by producing the necessary breaks in the skin.[12] Pinta is distributed equally between males and females and among all races in endemic areas. It is acquired primarily by individuals aged 15 to 20 years old,[47] frequently spreading to family members because of crowded living conditions.

PATHOGENESIS AND PATHOLOGY

T. carateum penetrates skin through breaks in the epidermis, with resultant damage restricted to the dermal and epidermal tissues. The organisms multiply in these layers, eliciting cellular proliferation and plasma cell, lymphocyte, and Langerhans cell infiltration. The resulting primary lesions (one to three) may appear on any area of exposed skin but usually occur on the legs, the dorsum of the feet, the forearms, or the back of the hands.[47] The lesion enlarges as a result of continual progression and direct extension or by coalescence with adjacent primary lesions; occasionally, a regional lymphadenopathy develops. Secondary lesions, known as *pintids*, may arise years later from treponemal dissemination and exhibit a cellular proliferation and infiltration similar to that of the primary lesion. In contrast to venereal syphilis, blood vessels remain intact and do not show proliferation. The dyschromic or multicolored nature (brown, slate blue, black, or gray) of the primary and secondary lesions is more characteristic of older than of younger lesions, in which pigmentary changes usually are minimal. The variously pigmented older lesions may show hyperkeratosis or parakeratosis. Large numbers of treponemes can be found throughout the dermis and epidermis in the highly contagious primary and secondary lesions.

Late pinta is characterized by pigmentary changes, from dyschromic treponeme-containing lesions to achromic treponeme-free lesions. This depigmentation process occurs at different rates even within the same lesion, which gives rise to different degrees of hypochromia and atrophy around dyschromic and achromic lesions.[47] A concomitant lack of hair follicles and sebaceous glands may occur.[11]

CLINICAL MANIFESTATIONS

Pinta is characterized by a continuing production of early infectious lesions from either direct extension or dissemination and by the concomitant presence of lesions in various stages of dyschromia and achromia. The lesions are not well delineated, often merge, and may be accompanied by regional lymphadenopathy. Primary, secondary, and tertiary stages may or may not occur simultaneously.

The early stage of pinta includes the initial lesions, occasional regional lymphadenopathy, and secondary lesions resulting from dissemination of treponemes. A primary lesion (not always evident) develops after an incubation period of 3 days to 2 months, usually 2 to 3 weeks. The primary lesion begins as one or more small erythematous papules that may appear scaly and indurated. The papules progressively enlarge over 1 to 3 months, often coalescing, becoming pigmented, scalier and more erythematous and developing heaped-up margins. Occasionally, an accompanying regional lymphadenopathy is present. The lesion may disappear in several months or may continue to enlarge for several years, forming larger psoriasiform plaques that coalesce further.

The primary lesions of pinta generally overlap development of the secondary lesions or pintids, which appear months to years after infection occurs. During this stage, dissemination occurs, and almost any area of the skin can be involved. The lesions occur typically on exposed areas of skin and are variously pigmented. The degree of pigmentation is related to the state of development of the lesion, the age of the lesion, the degree of exposure to sun, and the host's natural pigmentation. Pintids begin as small, scaly papules that gradually enlarge and coalesce. Several colors may exist within the same lesion. Initially, pintids usually are red to violaceous; later, they become slate blue, gray, or black as a result of photosensitization. Lesions on the legs typically are yellowish brown or dark brown.

The late stage of pinta is characterized by the development of depigmented lesions. Achromia usually begins several years after the onset of the disease but may appear 3 months after onset. Areas of depigmentation spread slowly, leaving large achromic lesions, reminiscent of vitiligo, as the end result. Early achromia tends to be asymmetric, whereas later depigmented lesions are symmetric. Characteristic of the Cuban form of pinta is the development of hyperkeratosis of the palms and soles. Many chronic dermatologic entities characterized by scaly, psoriasiform lesions or by dyschromia or depigmentation can be confused with pinta in endemic areas. Maculopapulosquamous diseases, including psoriasis, parapsoriasis, and lichen planus, and dyschromic dermatologic diseases such as vitiligo, ochronosis, and argyria must be taken into consideration.

PROGNOSIS

Pinta is not a fatal disease; it produces changes related only to the skin. There is no involvement of the central nervous system, the cardiovascular system, or other internal organs. Untreated, the patient frequently develops large achromic cutaneous blemishes. The major resulting problem is one of social ostracism from the community. Infected individuals are removed from the urban society and find refuge in the rural areas. This isolation separates the patient further from the principal sources of medical therapy.

YAWS

Yaws (frambesia, pian, buba, bouba) is a communicable, nonvenereally transmitted treponematosis of the tropical zones. The disease is characterized by its early acquisition (usually before puberty) and its chronic, relapsing pattern of early benign lesions separated by periods of latency that terminate with late destructive lesions of skin, bones, and cartilaginous tissues. A very prevalent disease in the early part of the 20th century, yaws was estimated to have affected more than 50 million people. Although seroepidemiologic and mass treatment campaigns sponsored by WHO and UNICEF have reduced its overall prevalence dramatically, endemic areas of concern continue to exist.[21,47,61,65]

BIOLOGY AND IMMUNOLOGY

T. pallidum subsp. *pertenue*, the etiologic agent of yaws, is morphologically identical to other pathogenic treponemes. Although not clinically useful, animal models can be used to distinguish between syphilis and yaws. Rabbits and hamsters have been infected experimentally by both diseases, with resultant lymphadenopathy and visible lesions. Characteristic differences in the distribution, form, and number of lesions can be used to

distinguish the identity of the infectious agent. On the basis of human and experimental studies, the organism stimulates a significant degree of homologous resistance to re-infection and superinfection and cross-immunity to *T. pallidum* subsp. *pallidum* and *endemicum*.[26,29,53,58]

EPIDEMIOLOGY

Yaws is a disease of tropical countries found primarily among rural populations. It exists in the warm, moist, endemic areas of rural Africa, Southeast Asia, the Caribbean, South America, the Pacific, and central and southern Africa. As indicated, seroepidemiologic and mass treatment campaigns have resulted in a significant reduction in prevalence of the disease in most of these areas. The failure of many countries to integrate active control measures into the functions of local health services has led, however, to a gradual build-up and extension of reservoirs of yaws, with the emergence of significant numbers of new, active cases since 1974 in Ghana, Togo, Benin, Indonesia, Papua New Guinea, and the Ivory Coast.[65] In a WHO survey in the Central African Republic, Congo, and Gabon, clinical yaws was detected in more than 20 percent of the Pygmy population, and reactive serologic tests were obtained in 80 percent.[65] Health care workers in the periurban settlements of Port Moresby, Papua New Guinea, identified 494 cases between April 2000 and September 2001. A 2001 survey in the Solomon Islands showed that yaws was the sixth leading cause of morbidity, and that the prevalence was 4106 cases per 100,000 population.[56] In Latin American, cases still occur in Brazil, Colombia, Haiti, Venezuela, and Suriname.[13,37]

Poor hygiene, close crowding among children (especially in sleeping areas), and lack of protective clothing facilitate transmission of the disease by direct skin-to-skin contact, whereby *T. pallidum* subsp. *pertenue* enters traumatized exposed areas from an infected lesion. The usual portals of entry are the lower extremities, head, face, and mouth. The presence of treponemal antibodies and isolation of treponemes (most probably *T. pallidum* subsp. *pertenue* from West African monkeys[4,16]) suggest that primates in this area may act as a reservoir for spread of the disease.

Yaws usually is acquired before puberty and is most prevalent among children. Because such a large percentage of the population contracts the disease in infancy, young children represent the primary reservoir. Older children and adults generally are not infectious; therefore, congenital and sexual transmission does not occur. Boys and girls are affected equally.

PATHOGENESIS AND PATHOLOGY

T. pallidum subsp. *pertenue* enters the host through abraded skin, most frequently below the knees. The organisms multiply locally and in the regional lymph nodes. Epithelial hyperplasia and plasma cell, lymphocytic, and macrophage infiltration are elicited. The end result is the formation of a primary lesion and lymphadenopathy containing numerous treponemes. Shortly after their introduction into the host, organisms are carried to skin, bone, and cartilage through the circulation. Disseminated treponemes in these tissues multiply, elicit a chronic inflammatory response similar to that seen in the primary lesion, and produce distant papillomas, lymphadenopathy, hyperkeratosis, and bone involvement that develop uninterruptedly until they are reversed by either immune mechanisms or treatment. In contrast to those associated with venereal syphilis, vascular changes are discrete or do not occur in cutaneous yaws.

Untreated primary and secondary cutaneous lesions generally heal with only minimal scarring, unless they are complicated by secondary bacterial infection. The healing process and maintenance of latency seem to involve humoral and cellular mechanisms of immunity.[52] Relapses may occur during latency and, together with late disease, may be caused by a breakdown in the immune state, the development of antigenic variation by the treponeme, or both.

The late gummatous lesions presumably are caused by a hypersensitivity-induced mechanism similar to that postulated for the syphilitic gumma. Bone changes in late stages of yaws most often involve the long bones and manifest as hypertrophic periostitis, gummatous periostitis, osteitis, and nodular or generalized osteomyelitis.[23] Juxta-articular nodules occur near major joints and are characterized as nonspecific granulomata.[10]

CLINICAL MANIFESTATIONS

Yaws is a chronic, debilitating disease characterized by early infectious lesions; periods of latency and relapse; and late destructive lesions of cutaneous, subcutaneous, cartilaginous, and bony tissue. After an incubation period of 9 to 90 days (usually about 3 weeks), the primary lesion forms at the site of inoculation and is accompanied by regional lymphadenopathy. The lesion typically appears as a raised papule on the lower extremity that enlarges to become a hyperkeratotic papilloma measuring 2 to 5 cm in diameter, referred to as a *mother yaw*. It undergoes shallow ulceration and persists a few months to 3 years, at which time healing occurs with a resultant hypopigmented scar and surrounding dark margins.[10]

Before or weeks to months after healing of the mother yaw, crops of secondary, generalized, nondestructive papular lesions appear together with lymphadenopathy and malaise. These lesions tend to form near areas with mucous membranes, such as the nose and mouth, and may resemble condylomata lata. Some papules fade, whereas others enlarge to become papillomatous lesions referred to as *satellite secondaries* or *daughter yaws*. The multiple papillomas are circular, raised, red-yellow lesions with a granular, lobulated, and verrucous surface. They contain numerous treponemes, usually measure 1 to 3 cm in diameter, and produce a yellow discharge that dries to form a black scab. Hyperkeratotic involvement of the palms and soles occurs commonly. Painful fissuring of the soles may cause the patient to walk on the sides of the feet, producing the characteristic gait of "crab" yaws.[6] Bone pain may be severe, and nondestructive long bone lesions, including periostitis, osteitis, and osteomyelitis, may occur.[10,22]

Untreated secondary lesions may persist for longer than 6 months, at which time, owing to the development of host immune mechanisms, they usually heal without scars or residual defects, unless ulceration caused by secondary bacterial infection occurs. During the dry season, early yaws lesions often are atypical, tending to be macular and fewer in number; papillomas, which are small, scanty, dry, flat, and grayish in appearance and of short duration (about 1 month), are confined mainly to the hidden, protected, moist skin folds.[41,47,60]

Despite healing, some treponemes evade the immune process and persist in the affected tissues (latency). Latency frequently is interrupted by relapses, which tend to occur several times over a 3- to 5-year period. Fewer lesions are produced with each relapse, and they tend to be localized to the periaxillary, perianal, or circumoral area.[47] After cessation of the relapses and usually after a latent period of 3 to 10 years, tertiary lesions occur in 10 percent of patients[47]; the latent state persists in the remaining patients for their lifetime.

The tertiary lesions characteristically are solitary and destructive; they involve skin, subcutaneous tissue, bone, or cartilage, and most commonly occur after the onset of puberty. Painful hyperkeratosis of the palms and soles similar to that seen in early

yaws frequently occurs. Other lesions develop as ulcerating, subcutaneous nodules and may heal spontaneously to form scars or extend widely from their margins. The scarring results in depigmentation and sometimes contractions. Bone deformities of late-stage yaws include chronic hypertrophic periostitis, osteitis, gummatous periostitis, and osteomyelitis, each of which may ulcerate through the skin.[20] Gangosa, or rhinopharyngitis mutilans, the destructive gummatous ulceration of the skin and bones of the central face, and juxta-articular nodules, ganglions of tendon sheaths, and saber tibiae also may occur as manifestations of the late stages of the disease.[23] Gondou, a disfiguring hypertrophic osteitis of the frontal processes of the maxillae, previously often reported among western Africans with yaws, seems to have disappeared.[34] Other diseases that must be differentiated from the skin lesions of yaws include vitamin deficiencies, early stages of leprosy, venereal syphilis, tinea versicolor, molluscum contagiosum, scabies, lichen planus, plantar warts, tungiasis, psoriasis, and cutaneous leishmaniasis. Sickle-cell disease, tuberculosis, and bacterial osteomyelitis may produce clinical manifestations that mimic the bone lesions of yaws.

PROGNOSIS

Yaws is not a benign disease. If left untreated, it can produce destructive, disfiguring lesions of the face, feet, and hands and painful and disabling lesions of the fingers and long bones. Ulcers near joints may result in crippling contractures. Secondary bacterial infection of ulcers and of protruding bone lesions can result in further permanent damage to skin and bone tissues. Fractures generally do not occur.

ENDEMIC SYPHILIS

Endemic syphilis (bejel, njovera, siti, dichuchwa) is a chronic, nonvenereally transmitted disease of prepubescent children. It occurs in the warm, dry, arid regions of the world[47]; lesions are confined to the skin, bone, and cartilage. The disease, known to exist for centuries in Africa, has been recorded in epidemic proportions in areas where conditions among children allow transmission. As with yaws, although seroepidemiologic and mass treatment campaigns have reduced its overall prevalence drastically,[61] endemic areas of concern continue to exist and include the region bordering the southern Sahara Desert, Botswana, and the Arabian Peninsula.[43,47,64]

BIOLOGY AND IMMUNOLOGY

T. pallidum subsp. *endemicum*, the etiologic agent of endemic syphilis, morphologically is identical to the other pathogenic treponemes. *T. pallidum* subsp. *endemicum* exhibits similarity to syphilis and yaws by producing visible lesions and generalized lymphadenopathy in rabbits and hamsters experimentally infected by the intratesticular and intradermal routes.[26,54,58] The degree of induration of lesions, distribution and number of lesions, and the characteristic lymphadenopathy can be used to differentiate among the species. On the basis of experimental studies in inbred hamsters, *T. pallidum* subsp. *endemicum* stimulates a high degree of homologous resistance to re-infection and cross-immunity to *T. pallidum* subsp. *pallidum* and *T. pallidum* subsp. *pertenue*.[54]

EPIDEMIOLOGY

Endemic syphilis continues to persist in the warm, drier desert areas bordering the tropical belt. It is prevalent primarily among the seminomadic rural populations in the Arabian peninsula and along the southern border of the Sahara Desert in Africa known as the Sahel region[43,47]; a significant resurgence occurred in Mali, Mauritania, Niger, and the upper Volta during the 1970s.[47,65] More than 1000 cases were reported in Senegal in 1980.[37] Although scattered endemic foci did exist in central Asia, Australia, Bosnia, and India, they virtually have been eliminated from these areas by mass treatment campaigns.[43,47]

As with pinta and yaws, endemic syphilis propagates under conditions of poor hygiene, crowding, and wearing of little or no clothing. Transmission by oral mucous membranes is favored through contaminated objects, such as drinking vessels and kitchen utensils, and through contact with saliva-contaminated fingers and mouth-to-mouth contact.[9,14,28] Fomites are thought to play a role. Transmission may occur through direct oral lesion–to–skin contact.[47] Occasionally, a previously uninfected nursing mother develops a primary lesion on or near the nipple after the transfer of treponemes from her infected infant.[18,64] Congenital transmission does not seem to occur. The role of insect vectors, such as flies, has been suggested, but it may be caused by creating a portal of entry by breaks in the skin after itching. Endemic syphilis occurs predominantly among children, with onset usually occurring in children younger than 15 years and with equal sex distribution. Spread occurs most commonly within the family, and active disease can be manifest in more than one family member at any given time.

PATHOGENESIS AND PATHOLOGY

T. pallidum subsp. *endemicum* enters the host usually through the oral mucosa. The small number of treponemes introduced into a susceptible host usually precludes the local multiplication and host inflammatory response required to produce a visible primary buccal lesion.[18] When a primary lesion does occur, it appears as a papule or ulcer resulting from a chronic inflammatory response to the proliferating organisms consisting of plasma cell, lymphocytic, and macrophage infiltration. Endothelial cell swelling of small blood vessels also is evident.

The organisms are carried to the regional lymph nodes within a few hours of entry, commonly into the oral mucosa portal entry. They multiply and elicit epithelial hyperplasia and plasma cell, lymphocytic, and macrophage infiltration, with resultant lymphadenopathy. Dissemination occurs through the circulation, and the organisms are carried to the skin, bone, oral mucosa, axillae, and anogenital regions, where they multiply and elicit a chronic inflammatory response characterized by a cellular infiltration, as seen in the lymph nodes. Vascular changes and perivascular cuffing are prominent.

The untreated early lesions heal as a result of mechanisms thought to involve humoral and cellular immune responses by the host,[51] and the disease enters into a state of latency. Maintenance of latency is thought to involve similar mechanisms. The occurrence of infectious relapses during the latent period still is uncertain.

The late lesions of endemic syphilis are strikingly similar to those of late-stage yaws. Late-stage disease and relapses (if they occur) may be caused by a hypersensitivity-induced mechanism similar to that postulated for the syphilitic gumma.[17,18] Juxta-articular nodules may occur and represent a nonspecific granulomatous response occurring near major joints.[10] The rarity of cardiovascular and neurologic manifestations may be due to the slow acquisition of small numbers of organisms over a long time, which results in the immunologic protection of the heart and nervous system.[18,32]

CLINICAL MANIFESTATIONS

Endemic syphilis is a chronic, often debilitating disease characterized by early infectious secondary lesions; variable periods of latency; and late destructive lesions of cutaneous, subcutaneous, and bone tissues. As indicated, primary lesions are rare. They appear usually on the breast or nipple as a papule or shallow ulcer similar to that seen in primary venereal syphilis after an approximate 3-week incubation period[1]; they may persist for years before healing.[28]

Even without the appearance of a primary lesion, generalized infection occurs as a result of early dissemination. The onset begins after an incubation period of 3 to 6 months and is characterized by the presence of highly infectious, relatively painless, ulcerative mucous patches on the oropharyngeal mucosa, including the tongue, lips, palate, tonsils, and larynx, with accompanying regional lymphadenopathy. Involvement of the larynx usually results in hoarseness.[10] Split papules or angular stomatitis occurs at the angles of the mouth. Osteoperiostitis of the long bones of the lower extremities, similar to that seen in yaws, is a common early manifestation causing nocturnal leg pains.[10] Occasionally, axillary and anogenital secondary-type lesions result and consist of condylomata similar to those of yaws or dry papilloma annular patches, with accompanying axillary and inguinal lymphadenopathy. Disseminated papules that are indistinguishable from the papules seen in secondary venereal syphilis may occur. Other forms of cutaneous lesions can occur but are rare.

Untreated secondary lesions may persist for 6 to 9 months, at which time healing occurs because of the development of host immunity. This period of latency varies and, similar to yaws, may last for several years.

Most patients develop tertiary manifestations. Late lesions generally occur during adolescence or adult life and may resemble lesions seen in either late yaws or late-stage venereal syphilis. Gummata may affect any part of the body but commonly occur in the nasopharynx, skin, and bone, resulting in destructive, disfiguring, chronic ulcerations characteristic of gangosa or gangosa-like lesions. Late gummatous lesions can occur during childhood, possibly as a result of superinfection in an already infected host.[19] Bone involvement also is a common finding and results in painful lesions. This condition involves osteitis with gumma formation and, similar to yaws, periostitis affecting most frequently the long bones of the lower extremities. Bilateral synovitis, especially of the knees, occasionally may occur with concomitant juxta-articular nodules.[19] The cardiovascular and neurologic findings common to venereal syphilis rarely occur in endemic syphilis; when clinical manifestations do occur, they usually are atypical and very mild.[8,43]

Endemic syphilis seems to have become "clinically attenuated" in Saudi Arabia.[43] Previously florid, the classic disease seems to have been replaced by a milder form in which the number, severity, and duration of early and late lesions are reduced, and seroreactive latent infection is increased.[43] The most common late manifestation observed in this study was painful osteoperiostitis of the legs affecting mainly the tibia and fibula. Researchers have postulated that attenuation has occurred owing to improvement in hygienic conditions, better nutrition, and medical care,[43] emphasizing the importance of improvements in living conditions and the use of antibiotics in the eradication of the disease.

PROGNOSIS

The main complication of endemic syphilis is the destructive gummatous lesions of the face and bones. Severely disfiguring and disabling, these lesions prevent the patient from working effectively in the community. Many of the bone lesions are extremely painful and incapacitating.

DIAGNOSIS

In the nonvenereal treponematoses, a presumptive diagnosis is based largely on clinical presentation of the disease in an endemic area. Although darkfield examination of early lesions and lymph nodes permits visualization of treponemes, late lesions usually contain few, if any, organisms. Differentiating the treponematoses from one another solely on the basis of serologic testing is impossible because of shared antigens.[17,47] The diagnosis of yaws in nonendemic areas or during periods of latency is difficult to make. The coexistence of yaws and endemic syphilis in certain geographic locations, together with their often identical clinical manifestations, renders darkfield and serologic assays useless for differentiating these diseases in such areas.[47] Similar limitations are applicable in differentiating venereal syphilis from yaws or endemic syphilis in nonendemic areas.[47] Under these circumstances, the diagnosis can be based only on a careful history and epidemiologic data.[47]

In tropical areas, numerous other diseases may be confused with yaws.[25,47] Impetigo and chronic tropical ulcers are found frequently and may respond to penicillin therapy. Ecthyma may produce ulcers that occasionally are similar to the ulcerative papillomas of yaws.

The same serologic testing is used in the diagnosis of venereal and nonvenereal treponematoses. The rapid plasma reagin and Venereal Disease Research Laboratory tests are useful, but may be nonreactive in early lesions. They are nonspecific non-treponemal studies using cardiolipin antigens that produce similar results in the presence of each disease and cannot be used to differentiate between them. Care must be taken in the interpretation of non-treponemal tests in establishing the diagnosis because of the possibility of obtaining false-positive reactions among patients with non-treponemal diseases or conditions.[39] These tests are quantitative, however, and false-positive results usually have a titer of less than 1:4. After treatment has been given, a twofold or greater drop in titers usually signifies a successful outcome.

The treponemal tests, such as the fluorescent treponemal antibody absorption test, the *T. pallidum* immobilization test, and the *T. pallidum* hemagglutination assay, are specific but normally remain positive for life and alone may not be useful in diagnosing active cases. Combining non-treponemal and treponemal tests is useful in establishing the diagnosis where one of these diseases is suspected, whereby a false-positive non-treponemal test is likely when a treponemal test is negative. In pinta, both types of tests may not become reactive until 4 months after the development of the initial lesion, whereas in yaws and endemic syphilis, both tests become reactive within the first few weeks of illness.

Although it may be less practical than serologic testing in remote and resource-poor areas, darkfield examination of fluid obtained from the initial serous exudates from cutaneous or mucous membrane lesions generally reveals the presence of treponemes. As the lesions become more chronic, treponemes become difficult to find. Direct fluorescent antibody testing can detect treponemal species on a slide, but generally is unavailable in the field. Silver impregnation of biopsy material from skin lesions or lymph nodes may reveal the organisms, but care must be taken in differentiating treponemes from tissue artifact.

Newer techniques such as polymerase chain reaction have been used to detect the presence of treponemes. Although polymerase chain reaction is highly sensitive and specific, it cannot differentiate among species as yet and is not practical for field use in resource-poor endemic areas.[44]

TREATMENT AND PREVENTION

The treatment of choice for all treponemes is benzathine penicillin G. Patients older than 10 years of age with clinical disease, in a period of latency or in the incubatory stages, and contacts of cases should receive 1.2 million U of benzathine penicillin G intramuscularly in a single dose; children younger than 10 years of age should receive 600,000 U in a single dose by the same route.[47,65] Isolated cases of resistance have been reported in Papua New Guinea,[3] Ecuador,[2] and Guyana[55]; however, whether these cases represent true resistant strains, other concurrent infections, or problems in storage and administration of the medicine remains unclear. Penicillin continues to be considered effective for treatment and eradication. Oral penicillin was used to treat 17 active cases of yaws in Guyana in 2000 to 2001. All but one patient had complete resolution of the lesions. Although this development is promising because it would eliminate the need for refrigeration and intramuscular injections, more data on the efficacy and compliance with oral penicillin regimens are needed.[55]

Tetracycline is an alternative treatment for penicillin-allergic patients older than 8 years of age and not pregnant. The recommended dosage is 500 mg by mouth four times daily for 15 days (total dose of 30 g)[47]; children aged 8 to 15 years old may be given half the dose.[47] Although the newer tetracyclines have been used and are assumed to be equally effective, no information is available about their use. Studies to determine the efficacy of erythromycin have not been reported, but based on its use in syphilis, erythromycin is presumed to be another alternative for penicillin-allergic patients. The recommended dosage is 8 to 10 mg/kg up to the adult dosage of 250 mg four times daily.[47]

Therapy in yaws renders early lesions noninfectious in a few days, with complete healing achieved in 7 to 10 days. Recurrences after treatment may occur as a result of re-infection.[25] Late lesions heal more slowly after therapy and may require surgery. In endemic syphilis (bejel), infectious lesions disappear rapidly, and relapses usually are prevented after therapy. The clinical response to therapy is remarkably slow in pinta. Primary and early secondary lesions take 4 to 6 months to disappear, whereas late secondary lesions require 6 to 12 months for complete healing to occur.[49] Hyperchromic lesions heal without residua, and hypochromic lesions often result in depigmented areas. Old achromic lesions usually remain intact without repigmentation.

In yaws and non-treponemal bejel, test titers may revert to nonreactive if the patient is treated early in the course of the disease. The longer the patient remains untreated, however, the more slowly conversion to seronegativity occurs, and treatment during the later stages may result in the persistence of high titers.[45] Generally, the serologic response to therapy is absent or slow in pinta; non-treponemal tests may take years to decline after adequate therapy has been given.[38] In each of the treponemal diseases, treponemal tests remain reactive for life after the patient has received adequate therapy.

Improvement of living conditions and the general hygiene of the community, mass treatment of patients and contacts, and seroepidemiologic campaigns contribute significantly to the prevention of yaws. WHO recommendations state that the entire population should be treated when the prevalence exceeds 10 percent. Where prevalences of 5 to 10 percent exist, all cases, close contacts, and all children younger than 15 years are treated. Where a prevalence of less than 5 percent exists, WHO recommends that cases, close contacts, and all members of the household be treated. Where implemented, this strategy has been remarkably effective, such as seen in an eradication program in Ecuador.[2]

FUTURE

It is a tragedy that such easily curable diseases have not been eradicated. Little recent epidemiologic data are available because many governments have stopped collecting prevalence data since the presence of these diseases implies poor health conditions. Researchers expect that genomic or antigenic markers will become available and will simplify establishing the diagnosis and differentiating among species. A renewed commitment to the elimination of these diseases is imperative to prevent the suffering and social ostracism that they bring. Simple measures, such as improved hygiene, education, and better access to health care, can ameliorate greatly the severity of disease. The effective eradication campaigns of the 1950s and 1960s need to be continued, along with awareness and vigilance on the part of health care professionals and health policy makers.

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SUBSECTION 7

Anaerobic Bacteria

CHAPTER 158

Clostridial Intoxication and Infection

Gary D. Overturf

Clostridia are obligate anaerobic, endospore-forming bacilli that usually are positive on Gram stain. Of the more than 150 species of *Clostridium*, at least two dozen species are potentially pathogenic in humans, mostly by virtue of their biologically active proteins (toxins). These ubiquitous organisms frequently are found in soil, sewage, decaying tissue, and the intestinal tract of humans and other animals.[10] The genus *Clostridium* has undergone significant revisions in taxonomy since 1994, which has resulted in a proposal for definitions of several clusters based on rRNA gene sequences.[3] Human disease caused by *Clostridium tetani* is discussed in Chapter 160.

BOTULISM

Botulism is an acute descending flaccid paralysis that results when the neurotoxin of *Clostridium botulinum* blocks neuromuscular transmission. Several clinical forms of botulism have been identified and include infant (the most common), "classic" or foodborne, wound, intestinal, and occult and inadvertent. The occult or "unclassified" category was created by the Centers for Disease Control and Prevention (CDC) for adult patients who lack an apparent food, wound, or intestinal source of botulinum toxin[14]

and whose cases hypothetically may have an infant-type pathogenesis.[3] Inadvertent botulism, the form recognized most recently, is an unintended consequence of the treatment of certain movement disorders (such as dystonia) with botulinum toxin A. Recently, the potential use of botulinum toxin as a bioterrorist agent has become an important concern. Infant botulism is discussed in Chapter 159.

EPIDEMIOLOGY AND ETIOLOGY

Seven antigenically distinct types of botulinum toxins are designated by the letters A through G. Disease in humans is caused by toxin types A, B, E, F (rarely), and possibly G.[9,17,21,32,33,38,46,48] Types C and D cause botulism in animals.[21,48] From 1973 to 1996, a median of 24 cases of food-borne botulism, three cases of wound botulism, and 71 cases of infant botulism were reported annually to the CDC.[46] In the United States, approximately 50 percent of the food-borne cases are caused by toxin A and another 25 percent each by toxins E and B. Type A occurs primarily in the western states, whereas type B is seen more commonly in the eastern states. Type E outbreaks occur more frequently in Alaska and the Great Lakes region.

Botulinum toxin is considered the most potent and lethal of all naturally occurring compounds; it consists of a simple dichain composed of a "heavy" 100-kd chain joined by a disulfide bond to a 50-kd "light" chain. The toxin's light chain is a Zn^{2+}-containing endopeptidase that blocks acetylcholine-containing vesicles from fusing with the terminal membrane of the motor neuron, thereby resulting in flaccid paralysis.[33] It is heat-labile; 5 minutes of boiling destroys the toxin, and little remains after 30 minutes of exposure at 80° C. Toxin is produced by *C. botulinum* at all temperatures at which growth occurs (3° C to 48° C). Toxin also is formed at all pH values at which growth occurs (pH 4.8 to 8.5), but the toxin is unstable at pH values higher than 7. Toxin thus is produced only during active growth and released during lysis of the bacterial cells. The presence of organisms in improperly processed acidic food, however, can allow toxin production to occur.[22,48] Type E toxin may be produced quickly in small fragments of fish exposed to air and at lower pH values and cooler temperatures than noted with other toxin types.[21]

Most outbreaks of botulism in the United States are traceable to home-processed foods; however, in recent years the proportion of cases that result from restaurant-associated outbreaks has increased significantly.[46] The most important food vehicles are vegetables, fish, fruits, and condiments. Type E botulism almost always is traceable to fish and fish products, but types A and B also may be involved in outbreaks related to this type of food. Recent outbreaks have been traced to unusual foods, such as potato salad and sautéed onions served by restaurants and commercial frozen pot pies mishandled at home.

Wound botulism results from infection of traumatized tissue by *C. botulinum* type A or B and subsequent production of toxin.[38] In the United States, approximately 80 percent of cases are caused by toxin A and 20 percent by toxin B.[46] Though infrequently reported, wound infection is a disease of pediatric concern: approximately half the cases in the United States that occurred before 1991 involved children and teenagers, most of whom were boys with compound extremity fractures. In recent years, however, most new cases have occurred in users of contaminated injectable black tar heroin.[3,15,46]

PATHOPHYSIOLOGY

Botulinum toxin is absorbed from the proximal part of the intestine or an infected wound into the lymphatics and then distributed hematogenously to peripheral cholinergic nerve synapses, most notably the neuromuscular junction. The toxin does not cross the blood-brain barrier. The nerve endings take up the toxin, which then irreversibly blocks release of acetylcholine and results clinically in flaccid paralysis.[21,22,48] The cranial nerves are affected earliest and often most severely. Death occurs mainly from respiratory muscle paralysis (asphyxia) or its complications, such as cardiac arrhythmia, aspiration, and pneumonia. Recovery occurs by regeneration of terminal motor neurons and the formation of new motor end-plates. The lethal dose of botulinum toxin for humans is not known but can be estimated from primate studies; the dose of crystalline toxin lethal to a 70-kg human would be approximately 0.09 to 0.15 μg intravenously (or intramuscularly), 0.70 to 0.90 μg by inhalation, and 70 μg orally.[43]

CLINICAL MANIFESTATIONS

Because the pathogenesis of botulism involves the systemic absorption of toxin and hematogenous dissemination with subsequent involvement of the cholinergic synapses, virtually all forms of botulism have identical neurologic signs. The signs and symptoms of botulism are summarized in Table 158–1.[6] The onset of illness varies from 12 to 72 hours after the implicated food is ingested, but it has been documented to occur as late as 108 hours after ingestion in some epidemics.[6]

Food-borne illness, or classic botulism, may be preceded by abdominal cramps, nausea, vomiting, or diarrhea. However, the neurologic illness begins as a descending symmetric motor paralysis first affecting muscles supplied by the cranial nerves.[6,21] No sensory disturbance occurs, although vision may be impaired and hearing distorted because of cranial nerve involvement.

TABLE 158–1 Symptoms and Signs of Food-Borne Botulism, Types A and B

Sign or Symptoms	Percentage of Cases
Symptoms	
Dysphagia	96
Dry mouth	93
Double vision	91
Dysarthria	84
Fatigue	77
Arm weakness	73
Constipation	73
Leg weakness	69
Blurred vision	65
Nausea	64
Dyspnea	60
Vomiting	59
Dizziness	51
Sore throat	54
Abdominal cramps	42
Diarrhea	19
Paresthesia	14
Signs	
Alert mental status	90
Arm weakness	75
Ptosis	73
Leg weakness	69
Gaze paralysis	65
Facial palsy	63
Diminished gag reflex	58
Tongue weakness	58
Pupils dilated or fixed	44
Hyporeflexia or areflexia	40
Nystagmus	22
Ataxia	17

Mental processes remain clear, but anxiety and agitation may be present. Fever is absent unless a secondary bacterial infection occurs. The triad of bulbar palsies (including a sluggish or absent pupillary response to light), lucid sensorium, and absent fever always should bring botulism to mind.

Common symptoms include blurred vision, diplopia, dysarthria, and dysphagia. The degree of ocular involvement is quite variable; in severe cases, the pupils may become fixed and dilated. The mucous membranes of the mouth, tongue, and pharynx may be so dry that pain results, which may lead to the mistaken diagnosis of pharyngitis. Dizziness or vertigo may occur. Urinary retention may be seen, occasionally with stress incontinence.

Two thirds of patients have no gastrointestinal symptoms. In those who do, with type A or type B botulism the gastrointestinal manifestations are primarily abdominal pain, cramps, fullness, and diarrhea. However, after an initial period of diarrhea, constipation or obstipation may be noted and, indeed, is more typical of the disease. In contrast to those with the other types, most patients with type E botulism first have gastrointestinal symptoms, including nausea, vomiting, substernal burning or pain, abdominal distention, and decreased bowel sounds. The most common signs encountered in botulism are respiratory impairment; specific muscle weakness or paralysis; eye muscle involvement, including ptosis; dry throat, mouth, or tongue; dilated fixed pupils; and ataxia. Respiratory involvement, even aside from aspiration pneumonia, is a fairly common occurrence. Vital capacity is a more sensitive indicator of respiratory compromise than is measurement of blood gas. Postural hypotension, nystagmus, and somnolence may be noted.

The usual interval between the ingestion of food and the onset of symptoms is 18 to 36 hours, but it may be as short as 2 hours or as long as 8 days. In general, patients with shorter incubation periods are affected more severely and have a poorer prognosis. The shortness of the incubation period and the severity of illness correlate with the amount of toxin ingested. Although the syndrome is similar for each toxin type, type A toxin has been associated with more severe disease and a higher mortality rate than has either type B or type E toxin.[56]

The symptoms of wound botulism are similar to those of food botulism, but some important differences may occur.[38] Fever may or may not be present. Constipation occurs, but nausea and vomiting do not. Unilateral sensory changes in association with the trauma or infection may be noted. The wound itself may have grossly purulent drainage, but sometimes the wound shows no evidence of infection. The incubation period of wound botulism usually is 4 to 14 days.

In the event of intentional food-borne poisoning with botulism toxin, the signs and symptoms probably would resemble those of naturally occurring food-borne botulism.[6,46] If the aerosolized toxin from a bioterrorist attack were to be inhaled, the incubation period might be slightly longer, and gastrointestinal symptoms might not occur.[6,46]

DIFFERENTIAL DIAGNOSIS

The differential diagnosis of botulism includes myasthenia gravis, cerebral vascular accidents, Guillain-Barré syndrome (particularly the Miller-Fisher variant), tick paralysis, paralytic shellfish poisoning, chemical intoxication, diphtheritic polyneuritis, psychiatric disease, and the Eaton-Lambert syndrome.[21,48]

Ordinary bacterial food poisoning generally is not a problem in the differential diagnosis because of the absence of cranial nerve involvement. Chemical food poisoning may cause neurologic manifestations, but the signs almost always appear within minutes or at most hours after the consumption of contaminated food. Atropine poisoning has a very rapid onset and is distinctive because of facial flushing and hallucinations. Shellfish and fish poisonings have a rapid onset and often cause characteristic paresthesias, tremors, and other signs. Mushroom poisoning results in severe abdominal pain, violent vomiting, diarrhea, and coma.

Myasthenia gravis usually spares pupillary oculomotor function. An edrophonium (Tensilon) test should be performed. Guillain-Barré syndrome can mimic botulism but usually shows ascending peripheral paralysis and, later, cranial nerve involvement. Muscle cramps, paresthesias, and an elevated protein content in cerebrospinal fluid in the absence of cells help distinguish this disease. Electromyography may be extremely useful in differentiating botulism from atypical cases of Guillain-Barré syndrome.

The problem of identifying a case is complicated by reports of patients with features not characteristic of either botulism or the action of botulinum toxin, such as paresthesias, asymmetric weakness of the extremities, asymmetric ptosis, slightly elevated cerebrospinal fluid protein, and a "positive" response to edrophonium. Some of these symptoms may be a consequence of the high anxiety that prevails in persons who know that they have eaten a food containing botulinum toxin.

SPECIFIC DIAGNOSIS

Confirmation of the diagnosis of botulism depends primarily on detection of botulinum toxin or the organism in the patient or in the implicated food or wound.[21,48] Specimens to be examined for botulinum toxin include serum, gastric contents or vomitus, feces (at least 20 to 50 g when possible), and exudates from wounds and tissues. These specimens, particularly blood specimens, should be obtained as soon as possible and before antitoxin is given. When feasible, at least 15 to 20 mL of blood should be drawn into a large vacuum tube and sent without separation of the serum to the nearest laboratory capable of performing the mouse neutralization test and other tests for toxin. State health departments or the CDC can provide advice regarding specimen collection and handling and laboratories to which samples can be sent (see later).

Specimens should be refrigerated and examined as quickly as possible after collection. Whenever feasible, suspect food should be kept sealed in the original container. Sterile unbreakable containers should be used for other food samples. Specimens to be shipped to laboratories must be placed in leak-proof containers; packed with dry ice in a second leak-proof, insulated container; and marked "Danger, hazardous material." Extreme caution should be used in handling material that may contain botulinum toxin because even minute quantities of toxin acquired by ingestion, inhalation, or absorption through the eye or a break in the skin may cause profound intoxication and death.

Laboratory confirmation of suspected botulism should be attempted, even late in the clinical course. Detection of the organism itself may be achieved by culture or a variety of molecular methodologies.

Toxin is detected in serum or stool specimens in approximately 46 percent of clinically diagnosed cases. When stool specimens also are cultured for *C. botulinum*, confirmatory evidence is obtained in approximately 70 percent of botulism cases.[46,56] Thus, detection of *C. botulinum* toxin or organisms in the stool of a symptomatic person should be considered diagnostic.

TREATMENT

The mainstay of therapy for all forms of botulism is meticulous supportive care, with particular attention paid to the respiratory and nutritional needs of the patient and to anticipation of potential complications for the purpose of preventing them.[21,22] Symptomatic persons known to have ingested toxin-containing

food should be hospitalized, with careful monitoring of respiratory and cardiac function. Measurement of vital capacity has been a useful index of clinical status in adult patients.

In cases of food-borne botulism, if the patient is seen early, emetics and gastric lavage should be used to reduce the amount of unabsorbed toxin; in the absence of ileus, cathartic agents may be helpful. Magnesium-containing cathartic agents should be avoided because they may enhance the action of botulinum toxin. Trivalent antitoxin (types A, B, and E) or bivalent antitoxin (types A and B), both of equine origin, frequently are considered in the management of food-borne botulism in adults, although conclusive evidence of their efficacy is lacking.[21,51] The antitoxin is used to neutralize circulating botulinum toxin molecules that are not yet bound to nerve endings. The dose currently recommended in adults is one vial per patient as a single dose.[46] Hypersensitivity reactions have been reported in approximately 9 percent of persons treated with equine sera, so skin testing should be performed before administration of the antitoxin. Treatment of a single patient with suspected or proven food-borne botulism requires immediate notification of state and federal (CDC) health officials, who also are the antitoxin source, because the food responsible for the index patient's illness still may be available to other persons. The appropriate initial contact is via the state health department. If this agency cannot be reached, the CDC should be contacted immediately (CDC telephone: 404-639-2206, days, or 404-639-2888, nights). Presently, a human-derived botulinum antitoxin is available in the United States exclusively for the treatment of infantile botulism, and is discussed in Chapter 159.

In the event of a potential exposure to contaminated food, the following procedures should be implemented. Locate all persons who ate the suspect food and determine whether anyone has symptoms or signs of botulism.[21] If the patients are seen soon after the suspect meal, the use of gastric lavage, emetics, and cathartics deserves consideration. Antitoxin should be made available. Any samples of the suspect food that may remain should be collected and refrigerated. The health authorities should assist the clinician with these tasks. The fecal and serum specimens needed to establish the diagnosis should be obtained. If neurologic signs are present, one should try to identify defective neuromuscular transmission by electromyelography. If neurologic signs are absent, the patient and family should be informed of the early signs of botulinum intoxication and be instructed to return at the first manifestation of ptosis, diplopia, blurred vision, dysphonia, dysarthria, or dysphagia. Because of the serious side effects of equine botulinum antitoxin, prophylactic administration of it to asymptomatic persons who have ingested food known to contain botulinum toxin generally is not recommended.[3]

When wound botulism is suspected, exploration and débridement of the site must be undertaken, ideally after the administration of antitoxin has begun. Arrangements should be made to obtain material for anaerobic culture in the operating room and antibiotic therapy begun there. High-dose penicillin (250,000 to 400,000 U/kg/day for 10 to 14 days) is the drug of choice. Guanidine, aminopyridine, steroids, and intravenous immunoglobulin have been used to treat a small number of cases of food-borne and wound botulism, but convincing evidence of their efficacy is lacking. Metronidazole has been suggested as an alternative for penicillin, but evidence for its effectiveness is not available.

PROGNOSIS

With an emphasis on mechanical ventilation and intensive supportive care, the mortality rate from botulism has decreased steadily from 60 percent in the 1950s to 5 to 10 percent at the present time. It is lower with type B and type C disease than with type A.[9] The mortality rate is lower in individuals younger than 20 years. An important factor, as noted, is the dose of toxin ingested as reflected by the length of the incubation period. The longer the incubation period, the better the prognosis. If the index case in an outbreak can be detected early, other patients exposed to the same food will have a much better prognosis. Recovery may be prolonged, and some symptoms (e.g., fatigability) may persist for as long as 1 year. Most patients recover entirely.

PREVENTION

Local and state health authorities and the CDC should be notified immediately of all suspected cases of botulism so that appropriate investigations can be undertaken. Although commercial products still are responsible for botulism occasionally, control measures taken by the industry have done a great deal to prevent botulism from this source. Home canners still must be instructed regarding appropriate means for sterilizing containers and food preservation and for adequate cooking of food before serving.[21,48] In canning, a pressure cooker must be used to obtain temperatures well above boiling to destroy the spores of *C. botulinum* types A and B. For certain foods, a temperature of 116° C is recommended. Spores of *C. botulinum* are destroyed at 120° C after 30 minutes. Pressure cookers set at 15 lb will achieve this temperature, but correction for higher altitudes must be made. Home-canned foods should be boiled for 10 minutes before serving. Food containers that appear to bulge may contain gas produced by *C. botulinum* and should not be opened. A pentavalent botulinum toxoid vaccine has been used in military personnel to protect them from a biologic warfare assault. It is given in three subcutaneous doses and leads to detectable antibodies in 83 percent of subjects.[15]

CLOSTRIDIAL INFECTIONS

Clostridia are encountered less commonly in infections than are non–spore-forming anaerobic bacteria, but these spore formers rarely may produce devastating disease. Overall, clostridia are present in 5 to 10 percent of anaerobic or mixed (aerobic and anaerobic) infections.[9,18,21,53] Table 158–2 depicts the relative frequency of clostridial species from clinically significant infections at a single academic hospital.[3]

Clostridium perfringens is the species encountered most commonly. It may be isolated in pure culture but more commonly is part of a mixed flora involving other anaerobes and nonanaerobes as well. Other species that are important clinically or encountered commonly (or both) include *C. novyi*, *C. septicum*, *C. bifermentans*, *C. histolyticum*, and *C. sordellii* (together with *C. perfringens*, these species commonly are referred to as the "gas gangrene group"); *C. tetani* (see Chapter 160); *C. difficile*, a major pathogen in pseudomembranous colitis; and a group of clostridia important in infections other than gas gangrene or myonecrosis (wound infection, abscesses, bacteremia, etc.)—*C. perfringens*, *C. ramosum*, *C. bifermentans*, *C. sphenoides*, *C. sporogenes*, and others.

Clostridia may be involved in a wide variety of infections throughout the body. Certain of these infections have a distinctive clinical picture and are discussed in this chapter. Many other infections, including peritonitis, intra-abdominal infection, wound infection, soft tissue infection, and occasionally, pleuropulmonary infection, central nervous system infection, and urinary tract infection, are not distinctive and will not be discussed specifically here. Emphysematous cholecystitis involving *C. perfringens* has distinctive features, but it is not encountered in the pediatric age group and thus is not discussed. Bacteremia involving *C. perfringens* may or may not have characteristic fea-

TABLE 158–2 *Clostridium* Species Most Frequently Encountered in Clinical Specimens at Indiana University Hospital Anaerobe Laboratory, 1989 through 2001*

Species†	Isolates	
	No.	% of Total
C. perfringens	515	20
C. clostidioforme	421	16
C. innoculum	380	15
C. ramosum	357	14
C. difficile	287	11
C. butyricum	113	4
C. cadaveris	99	4
C. befermentans	53	2
C. sporogenes	49	2
C. glycolicum	44	2
C. septicum	42	2
C. tertium	39	2
C. sordelli, *C. subterminale*, *C. paraputrificum*, *C. symbiosum*, and *C. baratii*	15-30	1

**The 2555 isolates do not include 270 isolates that did not belong to a recognized species (total of 2825 isolates), nor do they include C. difficile from fecal specimens.*

†*Six or fewer isolates each of* C. beijernickii, C. botulinum, C. carnis, C. celatum, C. coccides, C. cochlearium, C. ghoni, C. hastiforme, C. histolyticum, C. indolis, C. limosum, C. maelenominatum, C. novyi, C. putrefaciens, C. putrificum, and C. sphenoides.

tures. The distinctive intravascular hemolysis that may occur with *C. perfringens* bacteremia is discussed in connection with female genital tract infections caused by this organism.

EPIDEMIOLOGY

The vast majority of clostridial infections are of endogenous origin. Even those secondary to trauma and contamination of a wound with foreign bodies usually involve *C. perfringens* or other clostridia from the host's flora, chiefly the intestinal tract. *Clostridium* spp. are ubiquitous in nature, with habitats in soil and the intestinal tracts of many animals, including humans and most other mammals. Clostridia even may be isolated from infant intestine as early as the first week of life, and by 6 to 20 months of age, the feces of infants contain approximately the same number of *C. perfrigens* as that of adults.[3]

PATHOPHYSIOLOGY

The principal sites of normal carriage of *C. perfringens* and many other clostridial species in humans are the colon and vagina.[3,21,22] These organisms may gain access to tissues through wounds, by perforation of abdominal viscera, or because of local disease such as tumor. The organism then may grow in tissues if the oxidation-reduction potential is low or host defense mechanisms are impaired, or both. Factors favoring anaerobic growth include the presence of abundant necrotic tissue, a poor blood supply, the presence of foreign bodies, or previous multiplication of other bacteria in the wound leading to a lowered oxidation-reduction potential.

C. perfringens produces at least a dozen different extracellular toxins or other factors that account for its pathogenicity.[21] The most important of the five major lethal toxins is alpha-toxin, a lecithinase and the main toxin responsible for destruction of tissue, hemolysis, and death. Other important factors include collagenase, hyaluronidase, leukocidin, deoxyribonuclease, and fibrinolysin. The enterotoxins produced by some strains of *C. perfringens* and *C. difficile* are important in the pathogenesis of certain gastrointestinal diseases. Gas gangrene, or clostridial myonecrosis, is characterized by profound toxicity, necrosis of muscle, edema, thrombosis of small vessels, gas bubbles in tissues, and minimal infiltration of leukocytes (probably caused by destruction of leukocytes at the site).

CLINICAL MANIFESTATIONS

GAS GANGRENE OR MYONECROSIS

Although other clostridia also are involved in gas gangrene, *C. perfringens* is the causative species in approximately 90 to 95 percent of cases. Gas gangrene usually results from contamination of open wounds with contiguous injured muscle. The tissue destruction in gas gangrene is related to the profound attenuation in blood flow as a result of activation of platelet responses by the alpha-toxin of *C. perfringens*.[10] Clostridial myonecrosis may occur in the absence of a traumatic wound, so-called *spontaneous myonecrosis*, and is caused by bacteremic seeding of muscle with either *C. perfringens* or *C. septicum*. In spontaneous myonecrosis, the source of the organism typically is the bowel, and the usual predisposing factors are mucosal tumors of the bowel or ulcerations produced by cytotoxic chemotherapy.[22] Only a handful of cases of spontaneous myonecrosis in children have been described.[39]

The typical case of clostridial myonecrosis is manifested by the sudden appearance of pain in the region of a wound.[21] The pain increases in severity but remains localized to the infected area. Subsequently, local swelling and edema occur, and a thin hemorrhagic exudate appears. The pulse is very rapid, out of proportion to the mild elevation in temperature. Initially, the skin is tense, white, somewhat colder than normal, and very tender. Bronze discoloration appears and increases with time. The process extends and becomes more severe, and the patient becomes toxemic. The skin becomes dusky or bronzed, and bullae filled with dark-red or purple fluid appear. Crepitus caused by gas may be noted, but the amount of gas produced generally is small. A peculiar sweet smell may be noted in some cases. Occasionally, a fetid odor that probably reflects the presence of a *Clostridium* organism other than *C. perfringens* may be present.[22] Toxic delirium and, later, overwhelming prostration and toxemia may develop. Some patients are alert and apprehensive, whereas others are apathetic. Later in the course of the disease, shock supervenes. At surgery, early changes in muscle consist primarily of edema and pallor, but increased reddening with mottled purple is present. The consistency of the muscle may be pasty or mucoid, and contractility disappears. Eventually, the muscle becomes diffusely gangrenous, dark greenish purple or black, friable, and even liquefied.

SOFT TISSUE INFECTION

C. perfringens and other clostridia also may be involved in less dramatic and less serious infections ranging from minor superficial infections to anaerobic cellulitis and necrotizing fasciitis.[22] The clinical picture in these various infections is no different from that noted with other types of organisms and thus is not discussed further here.

SEPTIC ABORTION AND PUERPERAL SEPSIS

C. perfringens infections of the uterus usually occur after incomplete abortions are induced under nonsterile conditions.[21] Occasionally, this type of infection will develop after spontaneous abortion, prolonged labor, ruptured membranes, or operative interference with pregnancy. Early symptoms include uterine bleeding, suprapubic and back pain, chills, and fever.[21,22] The

incubation period after the precipitating event usually is several days, but it may be less than 24 hours. In addition to vaginal bleeding, a foul-smelling, brown vaginal discharge containing necrotic tissue often is present. The uterus is tender, and the lower abdominal wall may be tense. Nausea, vomiting, and diarrhea may occur. Generalized peritonitis may complicate the picture. The most striking systemic manifestation of the disease, however, is massive intravascular hemolysis with hemoglobinemia, hemoglobinuria, and jaundice. Shock and acute renal failure may complicate the picture. Intrauterine gas formation may be detected.

NEONATAL SEPSIS AND MENINGITIS

A small number of cases of sepsis or meningitis (or both) caused by *Clostridium* spp. have been reported in newborns.[23,25,40,50] Although some of these neonatal cases have been associated with invasive procedures or devitalized tissue, others have not had an obvious source.[25,31,40] In several of these cases, the systemic infection involved both the mother and infant. The typical clinical picture in neonatal infections is one of fulminant septic shock, intravascular hemolysis, respiratory distress, and rapid death. Pneumatocephalus may occur in the presence of meningitis.

PSEUDOMEMBRANOUS COLITIS

Pseudomembranous colitis represents the most severe end of a disease spectrum that the toxins of *C. difficile* can produce. It generally is characterized by profuse watery diarrhea, abdominal cramps, fever, and small (2 to 5 mm) raised, yellowish plaques on the colonic mucosa.[22,26] At the other end of the spectrum are found asymptomatic carriage and mild diarrhea. *C. difficile* diarrhea and colitis almost invariably occur after the administration of antimicrobial drugs, the most common precipitants being ampicillin, clindamycin, cephalosporins and fluroquinolones.[22] However, almost any antibacterial agent can trigger the illness. Sporadic and community-acquired cases not associated with antibiotics have been recognized. This organism is a serious and frequent cause of nosocomially acquired diarrhea, and hospitalized adults and children become colonized with this organism.[28] *C. difficile*–associated disease (CDAD) thus has become a major cause of hospital-acquired morbidity and mortality, with mean rates of 12.1 cases per 10,000 patient-days or 7.4 per 1000 hospital admissions.[5]

CDAD is related to the action of one or both of its toxins, known as A and B. Toxin A is responsible primarily for the enterotoxic activity of *C. difficile*, whereas toxin B is much more potent as a cytotoxin than toxin A is.[30] The spectrum of *C. difficile*–associated diarrhea ranges from a trivial, self-limited disease to a severe illness simulating an intra-abdominal catastrophe. Fever to 104° C, leukocytosis to 25,000 cells/mm^3 and hypoalbuminemia occur in approximately 25 percent of patients. Stools range from 3 or 4 to 20 per day and may be loose, watery, or bloody with mucus; associated abdominal cramping is a relatively common event.[22] Occasionally, pseudomembranous colitis may be caused by *Staphylococcus aureus* or more likely, by clostridia other than *C. difficile* (e.g., *C. perfringens* type C).

Up to half of asymptomatic infants younger than 1 year old harbor *C. difficile*, with rates of carriage declining to adult rates of 2 to 5 percent by the time that the child reaches 2 years of age. As a consequence, it is not recommended that hospital laboratories routinely examine stools for clostridial toxins or report the presence of *C. difficile* or its toxin in young infants. Possibly, receptors for *C. difficile* toxin may be decreased or absent in younger children.[19] Genetic analysis of the pathogenicity locus in *C. difficile* may help distinguish pathogenic from nonpathogenic strains of *C. difficile* in the future.[16] Children's facilities and hospitals have been identified as major reservoirs of *C. difficile*. Important risk factors for the development of CDAD are situations that would decrease the protective effect of the normal intestinal flora (such as antimicrobial therapy, repeated enemas, or intestinal surgery) and thereby allow the bacteria to proliferate and elaborate toxin. Asymptomatic colonization with *C. difficile* may be seen in a proportion of pediatric oncology patients,[11] as well as in children with prolonged hospital stays.

Since the late 1990s, *C. difficile* has emerged as a significant pathogen in North American and some European hospitals.[42] The host and epidemiologic factors associated with *C. difficile* are complex and include the use of antibiotics with borderline activity against *C. difficile*, exposure to gastric acid suppressants, poor host response with low levels of serum immunoglobulins, advanced age, and the severity of disease in the underlying host (reviewed by Owens[42]). The organism responsible for the recently reported outbreaks in North America and Europe, designated BI/NAPI, is distinguishable from the previously predominant J types of strains isolated in the late 1980s and early 1990s.[44,54] The BI/NAPI strains of *C. difficile* produce greater amounts of toxins A and B; carry an additional clostridial toxin, the binary toxin; belong to toxinotype III; have increased sporulation capacity; and are fluoroquinolone-resistant.[27,37] These agents are resistant to alcohol and may have emerged in an era when alcohol hand gels were increasingly being used in place of handwashing as the mainstay of hand hygiene.

Treatment of CDAD in young children is controversial and problematic because of the high rates of carriage in asymptomatic infants.[8a] Treatment may be indicated in those with new-onset diarrhea associated with antibiotics in which toxins A or B are found in the stool, when other viral, parasitic, and bacterial pathogens have been excluded by diagnostic testing.[34a] First-line treatment has relied on orally administered metronidazole administered for 10 days.[4] Recurrence rates after first episodes of CDAD in adults treated with metronidazole have ranged from 12 to 20 percent and, after treatment of a second episode, from 50 to 60 percent.[42] Since recent studies have suggested that metronidazole is ineffective in severe disease, oral vancomycin has been suggested as the preferred agent in severe disease.[57] Severe disease has been defined as greater than 10 stools per day, a white blood count of more than 20,000/mm^3, or severe abdominal pain. For mild disease, 250 mg orally four times a day has been recommended, whereas rancomycin, 125-500 mg four times a day, is recommended for severe disease.[50a]

OTHER ENTERIC INFECTIONS

A mild and self-limited but very common form of food poisoning may be caused by *C. perfringens*, with meat and meat products being the major vehicles for such outbreaks.[21,22] Food poisoning is caused by a heat-labile enterotoxin produced by *C. perfringens* type A. The incubation period after ingestion of the contaminated food varies from 6 to 24 hours but usually is 8 to 12 hours. The major symptoms are crampy abdominal pain and diarrhea. Stools are liquid but do not contain blood or mucus. Nausea may be noted on occasion, but vomiting and fever seldom are present. The illness typically lasts less than 24 hours.

Rarely, *C. perfringens* also may produce a very severe type of enteritis known as *enteritis necroticans*. Although food poisoning is produced by *C. perfringens* type A, necrotizing enteritis involves the beta-toxin of *C. perfringens* type C.[35] Consumption of excessive amounts of rich food by people normally on a low-protein diet who have decreased levels of digestive proteases seems to be an important background factor. Additionally, proteases may be blocked by ingestion of the trypsin inhibitors found in sweet potatoes. In some cases, consumption of contaminated canned

meat is involved. In New Guinea, where this condition is known as "pigbel," enteritis necroticans in children is associated with traditional pig-feasting activities in which large quantities of pork are consumed. The disease is characterized by abdominal cramps, vomiting, shock, diarrhea (sometimes bloody), and acute inflammation of the small intestine with areas of necrosis and gangrene, particularly in the jejunum. A 12-year-old diabetic boy in whom enteritis necroticans developed after the ingestion of pig intestines (chitterlings) has been reported.[43]

MISCELLANEOUS INFECTIONS CAUSED BY CLOSTRIDIA

Fewer than 40 cases of septic arthritis attributable to *Clostridium* spp., including several pediatric patients, have been reported.[29] Anaerobic osteomyelitis caused by *Clostridium* also is a rare finding.[24] Aggressive surgical débridement and prolonged antibiotic therapy are warranted in the treatment of anaerobic musculoskeletal infections.[47]

The clinical spectrum of clostridial bacteremia ranges from an asymptomatic patient with an incidental positive blood culture to fulminant sepsis syndrome.[13] Bacteremia with *C. septicum* is considered a unique syndrome associated with malignancies, particularly leukemia and colon cancer, and it has a devastating clinical course.[36] Infections with this organism also have been linked to children with cyclic neutropenia.[3] In a recent review of 28 pediatric patients with *C. septicum* bacteremia, Caya noted that all of them had underlying cancer or gastrointestinal disease.[12] The overall mortality rate was 72 percent. Five cases of *C. septicum* infection occurring after *Escherichia coli* O157–induced hemolytic-uremic syndrome have been described.[4]

Clostridium sordelli infections have been associated with high fatality rates and most often have occurred after trauma, childbirth, and routine gynecologic procedures. They have been reported after medically induced abortions and intravenous drug injections. In one review of 45 cases, 8 (18%) occurred after normal childbirth, and 5 occurred (11%) after medically-induced abortions, with a 100 percent case-fatality rate in this group.[1] Only four cases occurred in children: a 4-year-old child with arm trauma, a 17-year-old adolescent with omphalitis, a 12-year-old child with ear infection, and an 18-year-old adolescent with a medically induced abortion. Only the 12-year-old child survived; the other three patients died of their infection.

Panophthalmitis involving *C. perfringens* or, occasionally, other clostridia is secondary to penetrating injury, usually with retention of a foreign body.[21] Pain and loss of vision occur within 12 hours after the injury. By 18 hours, evidence of fulminating panophthalmitis is present and consists of chemosis and brawny swelling of the lids, proptosis, hypopyon, increased intraocular tension, gas bubbles in the anterior chamber, and necrosis of the wound margins. Intracranial infections involving *Clostridium*, usually as a result of penetrating trauma with items such as a lawn dart or an arrow, have been described.

Pneumatosis cystoides intestinalis can be produced in animals by *C. perfringens*, and this organism has been recovered from this process in humans.[21,22] Pneumatosis cystoides intestinalis may be found in conjunction with toxic megacolon and neonatal necrotizing enterocolitis and as a complication of ileal bypass for obesity.

SPECIFIC DIAGNOSIS

The diagnosis of gas gangrene must be made on clinical grounds. The presence of a gas-forming infection and recovery of *C. perfringens* from the wound do not establish the diagnosis of gas gangrene. The key to this diagnosis is demonstration of myonecrosis. Clostridial myonecrosis must be differentiated from other gas-forming soft tissue infections, which may or may not involve *C. perfringens*, and from other causes of myonecrosis. The sudden onset, extreme toxemia, and severe pain that are noted in clostridial myonecrosis represent important differential features. Entities such as anaerobic cellulitis and streptococcal myonecrosis have a gradual onset, slight toxemia, and less pain than seen with clostridial myonecrosis. Synergistic nonclostridial anaerobic myonecrosis is a severe infection characterized by discrete areas of blue-gray necrosis of skin, along with extensive involvement of the underlying soft tissue and muscle and foul "dishwater" pus. Anaerobic cellulitis typically has much more gas and does not involve muscle.

In streptococcal myonecrosis, edema of the muscle is present initially, and then the muscle has a hemorrhagic appearance.[21] Specimens for Gram staining and culture should be obtained from the involved muscle rather than from the surface of the wound. Large gram-positive rods will be demonstrated on Gram stain in clostridial myonecrosis; no white blood cells may be demonstrable, or the white blood cells present may be distorted significantly as a result of the toxin of *C. perfringens* acting on them. Anaerobic cellulitis typically shows a mixture of organisms, which may include *C. perfringens*. Streptococcal myonecrosis reveals anaerobic streptococci, sometimes along with group A streptococci, *S. aureus*, and other organisms.[16] In synergistic nonclostridial anaerobic myonecrosis, *Bacteroides* organisms seem to be key pathogens, together with anaerobic cocci and nonanaerobic gram-negative bacilli.[22] Of importance when obtaining material for culture is to place it under anaerobic conditions promptly for transport to the laboratory. Anaerobic blood cultures also should be performed.

Uterine infection by *C. perfringens* varies in severity from secondary invasion of necrotic material into the uterus or a dead fetus to invasion of intact uterine muscle producing myonecrosis and physometra.[21] Although bacteremia is a relatively uncommon finding in gas gangrene, uterine infection with *C. perfringens* frequently is accompanied by sepsis, which leads to the dramatic picture of intravascular hemolysis described earlier. Demonstration of severe hemolytic anemia in association with uterine infection essentially is diagnostic. Anaerobic blood cultures should be performed.

Clostridia, particularly *C. perfringens*, occasionally are isolated from the blood of a patient with a clinically benign course. The usual scenario is that a hospitalized patient has a single fever spike of unclear etiology and blood cultures are performed as part of the evaluation; by the time that the culture becomes positive, no evidence of an infectious process is present. This transient and benign bacteremia probably originates from the colonic flora.[11]

C. perfringens food poisoning usually is seen in the setting of a sizable outbreak.[22] The organism grows to high counts in the responsible food and then sets up an infection in the host, with production of enterotoxin in the colon of patients. Demonstration of large numbers ($>10^5$/g) of *C. perfringens* in the implicated food and demonstration of the same serotype of *C. perfringens* in the stool of affected individuals and in the food are important in documenting the nature of the food poisoning. Enterotoxin also may be found in the stool of affected individuals, and the *C. perfringens* recovered from stool or food can be demonstrated to produce enterotoxin in vitro. Necrotizing enteritis caused by *C. perfringens* may be suspected by virtue of the dramatic clinical picture and confirmed by demonstration of *C. perfringens* type C in the stool or suspect food or demonstration of serum antibody to the beta-toxin of the organism.

C. difficile–associated diarrhea should be suspected in children with diarrhea who have received antibiotics within the previous 2 months or whose diarrhea begins 72 hours after hospitalization.[20] Typically, toxin testing of a single stool specimen will establish the diagnosis, but, occasionally, repeat testing or endoscopy may need to be performed. The finding of exudative plaques

(pseudomembranes) and a hyperemic, friable mucosa by direct endoscopic visualization suggests the diagnosis of pseudomembranous colitis. Although *C. difficile* is isolated conveniently with the use of a selective medium, distinction between toxigenic and nonpathogenic strains cannot be made. Its toxin B (cytotoxin) is identified most easily by tissue culture assay, which remains the gold standard for diagnosis of antibiotic-associated diarrhea.[22] A simple latex particle agglutination test for detection of *C. difficile* is available, although it is considered relatively insensitive and nonspecific and, for that reason, no longer is recommended.[52]

In recent years, commercially available enzyme immunoassay (EIA) kits that detect toxin B or both toxin A and B have become the preferred method of diagnosis.[52] The EIAs in general are easy to perform, relatively inexpensive, and specific. Polymerase chain reaction amplification tests for toxins A and B have been designed but are not available commercially.

Interpreting the significance of finding *C. difficile* or its toxins in very young patients with diarrhea is difficult because infants and toddlers have such a high rate of asymptomatic carriage.[34,55] Quantitation of toxin or organisms has not correlated with the presence or absence of symptoms. Consequently, once the possible presence of other diarrhea-producing pathogens (e.g., rotavirus, *Salmonella*) has been excluded, effort should be made to stop the presumptive precipitating antibacterial agent. If diarrhea with mucus or blood persists, endoscopy should be considered and specific therapy begun.

TREATMENT

The most important aspect of treating clostridial myonecrosis is immediate surgical intervention consisting of radical débridement and drainage and decompression of the fascial compartments.[21,22] The wound should not be closed after this surgery. Of crucial importance is that all bits of necrotic muscle and other tissue be removed. Polyvalent gas gangrene antitoxin was never established firmly as being beneficial in the context of modern therapy, and this product no longer is available in the United States. Hyperbaric oxygen is recommended enthusiastically by some physicians, but no definitive evidence that its use reduces mortality rates has been shown. It does facilitate demarcation of a limb with impaired vascular supply, and it appears to slow down or arrest local spread of the gangrenous infection. Clearly, however, hyperbaric oxygen must not be used as a substitute for any of the important principles of surgical management. Antimicrobial therapy also is important as an adjunct, with high-dose penicillin G (250,000 to 400,000 U/kg/day) being the drug of choice.[21,22] In individuals allergic to penicillin G, clindamycin or metronidazole may be used. In addition, chloramphenicol is routinely active against all clostridia. The combination of penicillin and clindamycin has been used widely on the basis of experimental animal models that have shown enhanced efficacy with such a combination.[49]

Clostridial cellulitis is treated by incision plus drainage and antimicrobial therapy. Radical débridement is not necessary, but it is important to lay the tissues open to effect proper drainage and permit removal of all necrotic tissue.[21,22]

Uterine curettage should be performed for diagnosis and treatment of postabortal or puerperal clostridial infections.[21] Hysterectomy may be required if the myometrium is involved and if the patient's condition is deteriorating. At times, perforation of the uterus may be present without the typical clinical findings. Exchange transfusion has been recommended for sepsis caused by *C. perfringens* when significant intravascular hemolysis is present.

Food poisoning caused by *C. perfringens* is self-limited and requires no therapy. Antitoxin to the beta-toxin produced by *C. perfringens* type C has been of considerable benefit in the treatment of necrotizing enteritis caused by this organism.

In any serious infection caused by *C. perfringens* or other clostridia, the usual supportive measures for shock, dehydration, anemia, and renal insufficiency are implemented as indicated.

Treatment of CDAD involves discontinuation of the offending antibiotic regimen when feasible. Isolates of *C. difficile* are susceptible to metronidazole and vancomycin. Oral or intravenous metronidazole (30 mg/kg/day in four divided doses) is considered the drug of choice. With the recent and rapid increase in the recovery of vancomycin-resistant enterococci, many experts[20,42] and the CDC have suggested that oral vancomycin (40 mg/kg/day in four divided doses) be used to treat *C. difficile*-induced diarrhea only in patients who are critically ill or those who do not respond to metronidazole. Intravenous vancomycin is not effective. Orally administered bacitracin also may be useful, although some experts suggest that it may be less effective than metronidazole or vancomycin. Antimicrobial agents typically are administered for 10 days, but as many as 20 percent of patients may experience a relapse requiring a second course of treatment. The use of cholestyramine or probiotics, such as *Saccharomyces* or *Lactobacillus*, in adult patients with multiple relapses has been advocated by some physicians, but such treatment has not been evaluated in children with disease caused by *C. difficile*.

PROGNOSIS

The mortality rate in patients with gas gangrene varies between 15 and 35 percent and is worse when large muscle groups, such as those of the buttock, thigh, leg, and shoulder, are involved or when areas that are difficult to débride, such as the viscera and pelvis, are involved with disease. Clostridial cellulitis has a much better prognosis, but aggressive therapy still is important to minimize mortality rates. The mortality rate in postabortal clostridial sepsis remains 50 to 85 percent. *C. perfringens* food poisoning has an excellent prognosis, but mortality rates are significant with necrotizing enteritis caused by *C. perfringens* type C.

PREVENTION

Wounds involving areas with large muscle masses are particularly prone to gas gangrene, as are compound fractures, severe crush injuries, and injuries secondary to high-velocity missiles. Extensive laceration or devitalization of muscle tissue, impairment of the main blood supply to a limb or large muscle group, and contamination by dirt and particularly by bowel contents all predispose to the development of clostridial myonecrosis. The most important aspect of prophylaxis by far is early and adequate surgical management.[21,22] All devitalized tissue must be débrided; meticulous hemostasis is very important. Primary closure and tight packing of the wound should be avoided. All aspects of the wound must be drained adequately. If a cast must be applied, it should be bivalved from the outset. Hyperbaric oxygen is not indicated prophylactically. Antimicrobial prophylaxis, however, definitely is indicated. Penicillin is the drug of choice and should be given as early as possible after the injury occurs. Of emphasis is that antimicrobial prophylaxis is strictly adjunctive and far from adequate by itself. Bathing, particularly showering, and application of a compress wet with an iodophor for 15 minutes have been shown to reduce the skin count of *C. perfringens* significantly and minimize the likelihood of postoperative gas gangrene developing. This type of decontamination, of course, also may be useful in the management of traumatic wounds.

Prevention of clostridial uterine infection involves ensuring that all products of conception are removed during abortion and that retained portions of the placenta are removed immediately after the third stage of labor. Prolonged labor should be anticipated when possible and analgesics administered judiciously. During labor, particularly with ruptured membranes, pelvic and

rectal examinations should be kept to a minimum. During delivery, trauma should be minimized and lacerations repaired according to accepted surgical principles.

Proper sanitation in food-preparing facilities and adequate refrigeration are important safeguards against the acquisition of *C. perfringens* food poisoning. In areas where necrotizing enteritis caused by *C. perfringens* type C is found with some frequency (such as in Papua New Guinea), a *C. perfringens* type C toxoid has been given with encouraging results.

Contact isolation for the duration of the illness is recommended for hospitalized children with *C. difficile*–associated diarrhea. Meticulous handwashing, the use of gloves for handling contaminated objects and fomites, environmental cleaning and disinfection, and limiting the use of antimicrobial agents have been advocated widely to control the transmission of *C. difficile* in health care facilities.[27]

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CHAPTER 159

Infant Botulism

Stephen S. Arnon

Of the three main forms of human botulism (food-borne, wound, and infant), infant botulism is the most recently recognized (1976) and the most common in the United States and some other countries. Now recognized globally,[55] infant botulism results from a unique pathogenesis. Ingested spores of *Clostridium botulinum* germinate, colonize the infant's colon, and produce botulinum neurotoxin within it. The toxin then is absorbed, binds to peripheral cholinergic synapses, and causes flaccid paralysis. Knowledge of this intestinal pathogenesis resulted in discovery of the novel pathogenic strains *Clostridium baratii* and *Clostridium butyricum*, each of which can produce a botulinum-like neurotoxin and cause the clinical picture of infant botulism. Discovery of these strains enlarged the number of organisms known to cause the "intestinal toxemias of infancy," of which infant botulism is the prototype. Parenthetically, adults and older children rarely may become susceptible to infant-type botulism after broad-spectrum antibiotic treatment, intestinal surgery, or inflammatory bowel disease[6,28,38,62] or in association with a Meckel diverticulum[36,88] or bone marrow transplantation procedures.[93]

HISTORY

Infant botulism is not a new disease; rather, it has been recognized relatively recently and is an "emerging" infectious disease. The first laboratory-proven case of human infant botulism occurred in California in 1931, although it was misdiagnosed at the time.[15] Decades later and well before the etiology of the disease was apparent, the characteristic clinical features of infant botulism had become evident to discerning observers. In 1974, Grover and associates[43] described nine patients from Pennsylvania with a neurologic syndrome of undetermined cause that from today's perspective almost certainly was infant botulism. The same idiopathic syndrome was recognized in southern California and was reported by Ramseyer and colleagues[85] in 1976 to have a characteristic electromyographic pattern. A year later, Clay and associates[29] linked their eight southern California patients to infant botulism.

The first report of frank botulism in infancy was provided by Pickett and colleagues[84] in 1976. Although the source of botulinum neurotoxin for their two patients was undetermined, the possibility of its in vivo production was suggested.[64,84] The diagnosis of botulism in these and other California patients was established by identification of *C. botulinum* toxin and organisms in the infants' feces.[64] Evidence also was obtained that ingested spores of *C. botulinum* had produced the toxin in the infants' intestinal tract.[10,64,112]

In subsequent years, the clinical spectrum of infant botulism was found to include mild outpatient cases and, in some, but not all[23] locations, sudden unexpected death indistinguishable from typical sudden infant death syndrome.[11,72,77,83] In 1985, a *C. baratii* strain that produced a type F–like botulinum neurotoxin was recognized belatedly as the true cause of a case of infant botulism that occurred in New Mexico in 1979,[44,48] and in 1986, a *C. butyricum* strain that produced a type E–like botulinum neurotoxin was recognized as the cause of two cases of infant botulism in Rome, Italy.[16] These latter two novel clostridia were discovered only because they caused human infant botulism; their existence suggests that others like them await discovery.

ETIOLOGIC AGENT

C. botulinum is a gram-positive, spore-forming, obligate anaerobe whose natural habitat worldwide is the soil. Consequently, *C. botulinum* is as ubiquitous as the dust on which it may travel, and, hence, its spores commonly are present on fresh fruits, vegetables, and other agricultural products such as honey. Members of the *C. botulinum* species are so diverse in their biochemical capabilities and nucleic acid profiles that they would not be grouped as a single species except for the similar neurotoxin molecule that each strain produces[47,97]; at present, the *C. botulinum* species is subdivided into four groups (I to IV) based on metabolic characteristics.[97] Almost all cases of infant botulism in the United States have been caused by group I proteolytic type A or type B strains. Unusual strains of *C. baratii* and *C. butyricum* that make botulinum-like toxins E and F also cause infant botulism.[16,34,44,81,102,111] The entire 3.9-megabase (Mb) chromosome and a 16.3-kilobase (kb) plasmid within a *C. botulinum* type A strain were sequenced recently and found to contain 3650 and 19 predicted genes, respectively,[92] thereby launching the era of functional genomic studies of this special bacterium.

In general, each vegetative cell of *C. botulinum* produces just one of seven serologically distinguishable toxins, which arbitrarily have been assigned the letters A to G. Antitoxin raised against one toxin type does not protect against any of the other six types. The different toxin types serve as convenient epidemiologic and clinical markers. Subtypes of several toxin types have been identified by immunologic methods,[56,106] by neutralization studies using monoclonal antibodies,[3,98] and by toxin gene nucleic acid investigations.[47] Each toxin molecule is a simple protein consisting of two polypeptide chains of approximately 100,000 (heavy chain) and 50,000 (light chain) daltons joined by a disulfide bond.

Botulinum toxin is the most poisonous substance known.[39] For this reason and because of the ease with which it may be produced, transported, and disseminated, the Centers for Disease Control and Prevention (CDC) has listed botulinum toxin as one of six "category A" (most dangerous) potential bioweapon agents.[13] By extrapolation from studies involving adult primates, the lethal dose in the bloodstream of humans is approximately 1 ng/kg body weight.[13,39] Its potency for infants may be even higher because of the narrowness of their pharyngeal airway.[109]

The basis of the phenomenal potency of the botulinum (and tetanus) toxins is enzymatic. The light chain of each neurotoxin is a Zn^{2+}-containing protease that hydrolyzes one or more of three intracellular proteins needed for vesicle fusion and release of acetylcholine into the synaptic cleft.[69,78] The specificity of the toxin for peripheral cholinergic neurons results from their expression of a lower-affinity ganglioside cell surface receptor to which the toxin attaches first, which then is followed by attachment to a second, higher-affinity protein receptor that uniquely appears from the interior of the synaptic vesicle when it fuses with the terminal membrane to release acetylcholine.[27,51,79]

PATHOGENESIS

Infant botulism is not the diminutive form of food-borne botulism, and, hence, the disease is not "infantile botulism." Rather, infant botulism results from a unique infectious disease pathway

and was so named to emphasize that fact.[10,64,112] Ingested spores of *C. botulinum* germinate, colonize the infant's colon, and produce botulinum neurotoxin within it.[10,45,66,68,112] The toxin subsequently is absorbed and carried by the bloodstream to peripheral cholinergic synapses, where it binds irreversibly. The light chain then is taken into the cytosol of the neuron, where it blocks the release of acetylcholine by enzymatic cleavage of "fusion complex" proteins.[69,78] Clinically, the most important of the peripheral cholinergic synapses is the neuromuscular junction; the toxin's action results in flaccid paralysis and hypotonia. Preganglionic cholinergic synapses in the autonomic nervous system also may be affected.[60,90]

By use of a mouse model system of intestinal colonization (in which the animals paradoxically remained symptom-free), Sugiyama and colleagues[22,68,103] have demonstrated that the intestinal microflora of adult animals ordinarily prevents colonization of the gut by *C. botulinum*. Administration of 10^6 type A spores failed to colonize the intestine of normal adult mice, whereas after treatment for $2^1/_2$ days with a combination of oral erythromycin and kanamycin, half the mice could be colonized by just 2×10^4 spores. When the antibiotic-treated mice were placed in cages with normal mice, they lost their susceptibility to intestinal colonization after 3 days.[22] (Mice normally exhibit coprophagia.) In addition, adult germ-free mice could be colonized intestinally by just 10 *C. botulinum* type A spores. When the germ-free adult animals were placed in a room with conventional mice (but not in the same cages), in 3 days the formerly germ-free animals became resistant to colonization by 10^5 spores.[68]

In contrast to the experimental work with adult mice, normal infant mice were susceptible to intestinal colonization by *C. botulinum* spores.[103] Like human infants, the normal infant mice were subject to colonization for only a limited period (7 to 13 days of age). Susceptibility of the infant mice peaked between days 8 and 11 in a pattern reminiscent of the peaking of susceptibility seen between 2 and 4 months of age in human infant botulism (Fig. 159–1).[8,103] The infective dose of spores for infant mice was much smaller than that of their antibiotic-treated adult counterparts; the 50 percent infective dose for normal infants was only 700 spores. In one experiment, just 10 spores were needed to colonize an infant mouse.[103] The minimum infective dose of *C. botulinum* spores for human infants is not known, but from exposure to spore-containing honey, it has been estimated to be as low as 10 to 100.[12]

Recognition of the central role of the host's intestinal microflora in determining susceptibility or resistance to colonization by *C. botulinum* has directed attention to factors that may influence the composition of the normal microflora. Diet may be the most important of these factors. When compared with adult-type flora, the infant flora is simpler, with fewer genera and species. The dominant members vary, depending in part on whether the infant is fed only breast milk, only formula milk, or a mixture of the two.[100,101] In addition, the composition of the intestinal flora is changed if solid foods, such as cereals, become part of the infant's diet. The normal human infant microflora contain several bacterial species, mainly *Bifidobacterium* and *Bacteroides*, that in vitro can inhibit the multiplication of *C. botulinum*.[105]

The onset of infant botulism occurs at a significantly younger age in formula-fed infants (7.6 weeks) than in breast-fed infants (13.7 weeks),[9] perhaps reflecting the earlier availability in formula-fed infants of suitable ecologic niches[9,60,100,101] and the formula-fed infants' lack of the immune factors (e.g., secretory IgA, lactoferrin) contained in human milk.[4,5,41] Moreover, introduction of solid foods may "perturb" the intestinal microflora[100] and thereby aid colonization with *C. botulinum*.[4,8,60,99]

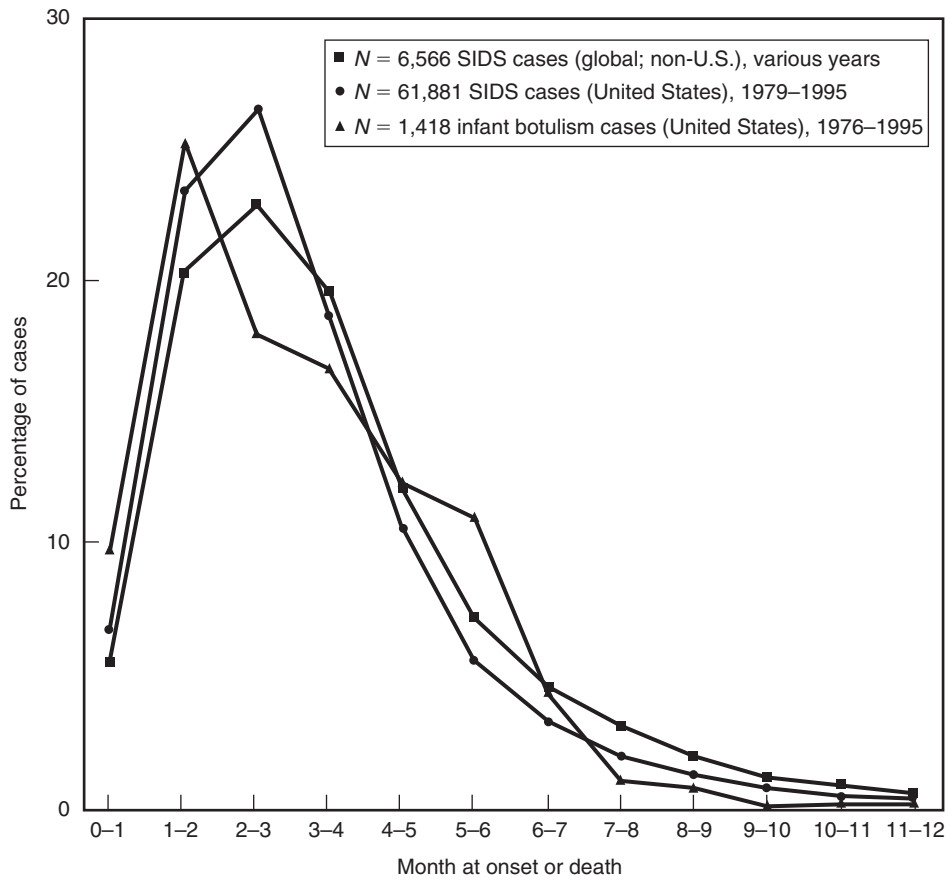

Figure 159–1 Age distribution of infant botulism and sudden infant death syndrome (SIDS).

An additional physiologic risk factor for infant botulism is slower gut motility, as measured by the frequency of defecation before the onset of illness.[91,99] Less than one bowel movement per day is a risk factor for both breast-fed and formula-fed infants, but this factor occurred in just 50 percent of cases.[91] Whether a Meckel diverticulum may predispose to the development of infant botulism caused by *C. botulinum*, as it appears to do for infant botulism caused by *C. butyricum*, is not known.[34,36,88]

EPIDEMIOLOGY

Any discussion of the epidemiology of infant botulism should be prefaced by the caveat that almost all presently available information is derived from study of only part of the clinical spectrum, namely, hospitalized patients. Accordingly, current perspectives may need to be modified as the outpatient and sudden death portions of the clinical spectrum become defined more fully. Furthermore, the perceived incidence remains more a reflection of physician awareness and access to diagnostic testing than the actual occurrence of disease. Almost half (41.6%) of U.S. cases have been reported from California, which has the largest number of births of any state. However, California does not have the highest incidence of infant botulism once adjustment is made for differences in annual births (Table 159–1). Notably, 8 of the 11 states with the highest incidence are located west of the Rocky Mountains, and 6 of the 8 are contiguous. The three eastern states with the highest incidence also are contiguous.

A unique epidemiologic feature of infant botulism is its age distribution, which, perhaps coincidentally, is virtually identical to the age distribution of sudden infant death syndrome (see Fig. 159–1).[8,11,24,99] Almost all U.S. cases (99.8%) of infant botulism reported to date have occurred in children younger than 1 year old. Some 91.2 percent of cases have occurred in the patients' first 6 months of life, 8.6 percent were distributed over the subsequent 6 months, and just 0.2 percent occurred between 53 and 72 weeks of age. The youngest known patient was just 38 hours old at onset (and had illness caused by *C. baratii* type F toxin),[19] whereas the oldest was 72 weeks old at onset. The illness has occurred in all major racial and ethnic groups and in approximately equal proportions in males and females. A national seasonality is not evident.

Infant botulism has been reported from all inhabited continents except Africa. In the United States, with 28 exceptions (1.1% of cases), all hospitalized cases known as of December 2006 were caused by either type A or type B *C. botulinum* toxin. Forty-nine of the 50 states, representing all regions of the country and including Alaska and Hawaii, now have reported infant botulism. Only Rhode Island has not. In general, the distribution of cases by type of toxin has paralleled the distribution of toxin types in U.S. soil,[96] with type B cases predominating from the great plains eastward and type A cases from the Rocky Mountains westward. The 28 exceptional cases resulted from a variety of toxin types. Two cases in Iowa and one each in California, Ohio, Oregon, New Mexico, Texas, and Wisconsin resulted from a type F–like toxin produced by *C. baratii* strains.[19,44,48,81] Fourteen cases were caused by a *C. botulinum* strain that produced mostly type B and some type A toxin (designated type Ba), and four cases resulted from Bf strains. One case caused by both *C. botulinum* type A and type B from today's perspective probably resulted from either a Ba or Ab strain. Two patients with Bf illness lived in California,[18] one lived in New Mexico, and the fourth patient had traveled there from California immediately before onset of the illness. A fifth type Bf case occurred in England.[95] The only *C. baratii* type F case reported outside the United States occurred in Hungary.[111] In Arizona in 2006, a type E case occurred, but neither *C. butyricum* nor *C. botulinum* could be isolated. All three European *C. butyricum* type E infant botulism cases were reported from Italy, and another in Asia was reported from Japan.[16,34-36]

As of December 2006, approximately a sixth (516/2932, 17.6%) of all known global infant botulism cases between 1976 and 2006 had been reported from 24 countries other than the United States.[55] Of these cases, Argentina has reported the largest number (366),[61] followed by Australia (32), Canada (27), Italy (23), and Japan (20). The remaining countries have reported only single-digit case occurrence.[55] The non-U.S. cases occurred in countries in Europe (12), Asia (3), the Middle East (3), North America (2), South America (3), and Australia. The small number of reported cases from most non-U.S. countries most likely reflects limited physician awareness of the disease and limited access to specialized diagnostic testing laboratories, as well as actual variation in disease incidence.[55]

Geographic clustering has been noted. In Pennsylvania, 43 of 53 cases in the period 1977 to 1983 occurred in four suburban counties that form an arc bordering the city of Philadelphia.[58] In Colorado, three type A cases occurred in three separate families in a small town with approximately 300 annual births. Two of the infants had used the same crib sequentially; environmental samples, including the crib, soil, and household dust, yielded *C. botulinum* type A.[50] In California, two type A cases occurred 5 years apart in the children of two families who lived one house apart. In another California family, two successive infants each acquired type A infant botulism, but the third child born in sequence did not. Soil and dust specimens from the house where all three infants lived contained *C. botulinum* type A.

The role of breast-feeding and formula-feeding as factors possibly predisposing to illness remains unsettled. All studies to date have identified an association between being breast-fed and being hospitalized for infant botulism.[5,9,58-60,70,99,108] This finding has resulted in one perspective that holds that breast-feeding predisposes to the development of illness,[58-60,99] whereas the other perspective holds that breast-feeding slows its onset sufficiently to permit hospitalization to occur.[4,5,8,9] However, among hospitalized patients in California, the mean age at onset of botulism in formula-fed infants (7.6 weeks) was significantly younger and about half that of breast-fed infants (13.8 weeks). In addition, in California the patients with fulminant-onset infant botulism who stopped breathing and died at home all were formula-fed.[9] The relative susceptibilities of formula-fed and breast-fed infants to infant botulism and the resultant severity of their disease possibly reflect differences in the availability of suitable ecologic niches in the intestinal flora for *C. botulinum*, differences in the availability of immune factors (such as lactoferrin and secretory IgA) contained in human milk but not in formula milk,[41] or other differences not identified yet.

Probably few, if any, patients with infant botulism acquire *C. botulinum* spores from infant formula, despite isolation in the

TABLE 159–1 Cases and Incidence of Infant Botulism. Top 11 States in Incidence, United States, 1977-2005

State	Cases (*N*) 1977-2005	Incidence* 1977-2005
Delaware	41	13.4
Hawaii	46	8.6
Utah	92	7.5
California	927	6.3
Pennsylvania	282	6.2
Oregon	47	3.7
Washington	74	3.5
New Jersey	106	3.4
Idaho	17	3.2
New Mexico	24	3.1
Nevada	18	2.8

**Per 100,000 live births per year.*

United Kingdom of *C. botulinum* type B from powdered infant formula consumed by a patient with type B infant botulism.[21,52] In addition, the possibility that an infant patient may have food-borne botulism needs to be kept in mind because food-borne botulism caused by home-prepared baby food has been recognized.[2]

Honey is the one dietary reservoir of *C. botulinum* spores thus far definitively linked to infant botulism by both laboratory and epidemiologic evidence.* More than 35 instances worldwide are known in which *C. botulinum* spores have been found in the actual honey fed to the affected infant before the onset of illness. In each instance, the toxin type (A or B) of the spores in the honey matched the toxin type of the *C. botulinum* that caused the infant's illness; the probability that such perfect concordance occurred by chance is less than 1 in 10 billion. Occasionally, *C. botulinum* has been isolated from honey in which the spore toxin type in the honey did not match the toxin type of the infant's illness[17,34]; in such instances, the conclusion is that the honey was not the source of the infective spores.

C. botulinum spores have been found in honey from the United States, Argentina, Australia, Canada, China (Taiwan also), Denmark, Finland, Italy, Norway, Spain, Japan, and Central America,† but not in honey from the United Kingdom.[20]

In general, only low concentrations *C. botulinum* spores have been found in honey (≤1 spore/g),[65,74] with the occasional higher concentrations (e.g., 36 to 60 spores/g[74]) thought to result from multiplication of *C. botulinum* in dead bees and bee pupae.[73] Toxin type A, B, C, and F spores all have been found in honey, with some of these toxin types linked to the geographic origin of the honey.[74] For these reasons and because honey is not nutritionally essential, all major pediatric, public health, and honey industry agencies in the United States have joined in the recommendation that honey not be fed to infants. In 2000, several brands of honey sold in the United States began to carry a warning not to feed honey to infants; an equivalent label first appeared on British honey in 1996.

Discussion of the possible role of corn syrup in infant botulism is necessitated by two reports. In 1982, the U.S. Food and Drug Administration (FDA) found *C. botulinum* type B spores in approximately 0.5 percent (5 of 961) of previously unopened retail samples of light and dark corn syrup[54]; the manufacturer then made changes in the production process. In 1989, the federal CDC reported the results of a 2-year epidemiologic study of U.S. cases from all states except California.[80,99] By subgrouping patients by age and using logistic regression modeling techniques, a statistical association was obtained among the triad of exposure to corn syrup, breast-feeding, and age at onset of 2 months or older.[80,99]

In contrast to these reports, a 1988 Canadian survey found no *C. botulinum* spores in 43 samples of corn syrup.[46] A 1991 FDA market survey of 783 syrups (354 of which were light corn syrup and 271 were dark corn syrup) concluded that none contained *C. botulinum* spores.[57] A California study (unpublished) of 103 corn syrups, 72 of which had been fed to infants who subsequently became ill with infant botulism, did not find *C. botulinum* in any sample. Moreover, a 1979 epidemiologic study that simply compared rates of exposure to corn syrup in 41 cases and 107 control infants identified feeding of corn syrup as a significant protective factor against type A infant botulism.[12] The explanation offered for the latter observation was that if a parent chose corn syrup as a sweetener for the infant, the child was unlikely to have been fed honey as a second sweetener. Thus, on the basis of the available evidence, corn syrup appears not to constitute a source of *C. botulinum* spores or a risk factor for the development of infant botulism.

In addition to honey and syrup, hundreds of traditional and nontraditional infant food items, including formula milk, have been examined and found not to contain *C. botulinum*.[63] However, a recent type B infant botulism case in the United Kingdom was traced to *C. botulinum* type B spores in powdered infant formula.[21,52] Also, in instances not associated with illness, *C. botulinum* spores have been found in raw sugar and molasses but not in refined sugar[75] and in herbal (chamomile) tea and other herbal preparations.[55,61]

Potential environmental sources of *C. botulinum* spores have been identified in many locales. The soil in Pennsylvania,[60] soil and cistern water in Australia,[71] vacuum cleaner dust in Finland,[77] and the soil and vacuum cleaner dust in California[8] obtained from case homes were found to contain *C. botulinum*, with the toxin type (A or B) in each instance matching that of the ill infant. However, despite the foregoing, it deserves emphasis that for most cases of infant botulism, no source of *C. botulinum* spores ever is identified, even circumstantially. In these cases, illness probably was acquired by swallowing spores adherent to airborne microscopic (invisible) dust.

CLINICAL MANIFESTATIONS

Like other infectious diseases, infant botulism displays a spectrum of clinical severity.[4,8,11,59,60,83,90,108] To date, almost all recognized patients have been sufficiently hypotonic and weak to need hospitalization. Consequently, the present picture of infant botulism is derived from hospitalized patients. However, outpatient cases that displayed only a few days of lethargy, poor feeding, and some decrease in frequency of bowel movement have been detected by alert physicians familiar with the more "classic" illness. At the opposite end of the clinical spectrum are patients whose "catastrophic manifestation" obscured and delayed establishment of the correct diagnosis[67] and those few cases for which the history and clinical findings were indistinguishable from typical cases of sudden infant death syndrome (crib death),[11,83,108] approximately 1 in 20 of which (in California) appears to result from fulminant infant botulism.[8,11]

The onset of infant botulism ranges from the insidious to the abrupt. At one extreme are patients who were nursing normally 6 hours before becoming so floppy that acute meningitis was the diagnosis at initial evaluation, and at the other extreme are patients who returned to their physicians four times in a week as the signs of illness gradually became apparent. Though rare, illness caused by *C. baratii* type F appears to be characterized by the triad of very young age at onset, rapid onset, and profound paralysis.[19,81] Equally rare, illness caused by *C. butyricum* type E may be manifested as a paradoxically rigid abdomen and associated bowel colonization with *Clostridium difficile*.[34,35]

In the "classic" case of infant botulism, the first sign of illness almost always is constipation (defined as 3 or more days without defecation in a previously regular infant), yet the constipation often is overlooked. A few patients (<5%) will not have a history of constipation. Usually, a mother first notices listlessness, lethargy, and poor feeding, together with breast engorgement if the infant had been nursing. The increasing weakness over the ensuing 1 to 4 days typically brings the baby to medical attention.

Botulism is manifested clinically as a symmetric, descending paralysis. Early in the course, weakness and hypotonia characterize the illness, and the remainder of the physical examination not involving the neuromuscular system is normal. The first signs of illness are found in the cranial nerves; one cannot have infant botulism without having bulbar palsies. The typical patient has an expressionless face, a feeble cry, ptosis (evident when the eyelids must work against gravity), poor head control, and generalized weakness and hypotonia (Fig. 159–2). Eye muscle

*See references 8, 12, 17, 46, 49, 54, 65, 85, 87, 88, 104.
†See references 12, 17, 46, 49, 54, 65, 74, 76, 87, 104.

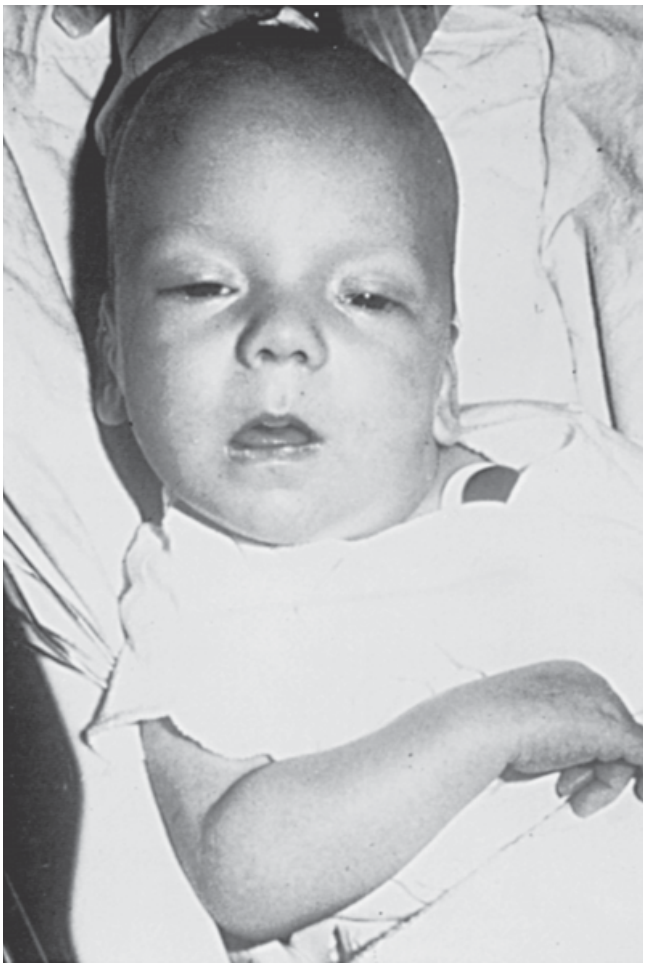

Figure 159–2 Mildly affected, 7-week-old infant with botulism. Note the minimal signs, including ptosis, mildly disconjugate gaze, expressionless face, slack jaw, and neck and arm hypotonia.

TABLE 159-2 Neurologic Signs Helpful in the Diagnosis of Infant Botulism

1. Take the patient to a dark room. Shine a bright light into the eye; note the quickness of pupillary constriction. Remove the light when the constriction is maximal; let the pupil dilate again. Then immediately repeat the light, continuing thus for 1 to 3 minutes. The initially brisk pupillary response may become sluggish and the pupil unable to constrict maximally. (Fatigability with repetitive muscle contraction is the clinical hallmark of botulism.)
2. Shine a bright light onto the fovea and keep it there for 1 to 3 minutes, even if the infant tries to deviate the eyes. Latent ophthalmoplegia may be elicited, purposeful efforts to avoid the light may diminish, or both.
3. Place a clean fifth finger in the infant's mouth while taking care to not obstruct the airway. Note the strength and duration of the reflex sucking. The suck is weak and poorly sustained. The gag reflex strength also may be quickly checked (if the infant has not been fed recently).

Adapted from Arnon, S. S.: Infant botulism. Annu. Rev. Med. 31:*541-560, 1980. Reproduced with permission from Annual Reviews, Inc.*

TABLE 159–3 Complications of Infant Botulism

Adult respiratory distress syndrome
Anemia
Aspiration
Blood pressure instability
Clostridium difficile colitis
Fracture of the femur and humerus
Inappropriate secretion of antidiuretic hormone
Misplaced or plugged endotracheal tube
Necrotizing enterocolitis
Otitis media
Pneumonia
Recurrent atelectasis
Respiratory arrest
Seizures secondary to hyponatremia
Sepsis
Subglottic stenosis
Tension pneumothorax
Tracheal granuloma
Tracheal stenosis
Tracheitis
Tracheomalacia
Transfusion reaction
Urinary tract infection

paralysis varies, and the pupils often are midposition and initially briskly reactive (Table 159–2). The gag, suck, and swallow reflexes are impaired, as is the corneal reflex if it is tested repetitively. Deep tendon reflexes frequently are normal at initial evaluation and diminish subsequently as the paralysis extends and increases. The "frog's legs" sign often is seen. Patients are afebrile unless a secondary infection (e.g., aspiration pneumonia) is present.

The results of most laboratory and clinical studies are normal. At admission, the child may have evidence of mild dehydration and fat mobilization because of diminished oral intake. Occasionally at admission, the protein concentration in cerebrospinal fluid (CSF) becomes elevated because of the mild dehydration. If infant botulism is suspected soon after the child is admitted, electroencephalography, computed tomography, and magnetic resonance imaging seldom are required, but if performed, these examinations yield nonspecific or normal results. Electromyography may offer rapid bedside confirmation of the clinical diagnosis (see "Differential Diagnosis and Diagnosis").[30,33]

Small amounts (<5 mouse LD_{50}/mL) of botulinum toxin sometimes can be identified in serum specimens if they are collected early in the course of the illness.[16,34,45,61,82,107,110] In one U.S. report, almost one patient in eight had toxin demonstrable in serum.[45] The definitively diagnostic laboratory study is examination of feces for the presence of *C. botulinum* organisms and toxin, which is the only certain way to identify the neurotoxin type (A, B, or other) responsible for the illness. Clinically suspected cases that lack an identified toxin type not are included in official tallies of infant botulism.[25,26,55]

The usual hospital course of untreated infant botulism has certain general features.[4,53,60,90] After the increasing weakness has necessitated admission, the weakness and hypotonia continue to progress and usually become generalized. The deep tendon reflexes, which may be normal at admission, may diminish or disappear temporarily. The nadir of paresis and paralysis in untreated patients usually occurs within 1 to 2 weeks after admission; such patients often remain at their nadir for as long as 1 to 3 weeks before showing signs of improvement. However, once strength and tone begin to return, the improvement continues steadily and gradually over the ensuing weeks in the absence of complications (Table 159–3). In contrast, patients treated with human botulism immune globulin have a mean hospital stay of approximately 2 weeks (see "Treatment").[14]

In the California experience, infant botulism does not have a relapsing or biphasic course, and perceived "relapses" have been

found, in retrospect, to be an indication either of the onset of a complication (see Table 159–3) or of premature discharge. However, the clinical experience elsewhere with regard to relapses has been different.[34,40,86,90] The patient is ready for discharge when gag, suck, and swallow are sufficiently strong both to protect the airway against accidental aspiration and to ensure adequacy of oral intake. Parents also may be taught to feed by gavage at home. In either situation, discharge may occur safely while head lag and constipation still are present.

DIFFERENTIAL DIAGNOSIS AND DIAGNOSIS

When initially brought to medical attention, patients with infant botulism often are so mildly weak and hypotonic that the illness is not considered. Even today, more than 30 years after the disease first was recognized, suspected sepsis remains the most common admission diagnosis for patients with infant botulism. A careful history (constipation commonly is overlooked) and physical examination (especially cranial nerve function) usually can identify patients with infant botulism correctly and render unnecessary most additional testing for the other entities typically suspected (Table 159–4). A review of entities that so closely mimicked infant botulism that botulism immune globulin was administered soon after admission identified spinal muscular atrophy type I, mitochondrial disorders, and a small number of other conditions as the actual diagnoses (Table 159–4).[37]

The diagnosis of infant botulism is established by identification of *C. botulinum* organisms in the feces of an infant with clinical signs consistent with the paralyzing action of botulinum toxin.[25,53,64] Extensive studies have demonstrated that *C. botulinum* is not part of the normal resident flora of infants or adults.[8,45,100,101] If the fecal specimen is obtained sufficiently early in the course of the illness, it also will contain botulinum toxin. Because of the patient's constipation, an enema with sterile, nonbacteriostatic water (not saline) commonly is needed to obtain a fecal specimen for diagnostic examination. The mouse neutralization test remains the most sensitive and specific assay for botulinum toxin.[26] Laboratory diagnosis that identifies the type of toxin responsible for the illness is essential for the case to be registered as infant botulism[55] and is important in determining the prognosis; mean hospital stay is significantly longer in untreated type A cases than in untreated type B cases (see "Treatment").[4,14] Physicians are reminded that in most states, botulism or suspected botulism (all types) is an immediately reportable illness.

At the bedside, electromyography sometimes can be helpful in ambiguous situations in that when a clinically weak muscle is tested, electromyography often discloses a pattern known by the acronym BSAP (brief, small, abundant motor unit potentials).[10,30,33,42,90,94] The edrophonium (Tensilon) test is unnecessary because congenital myasthenia gravis can be excluded by the history and de novo myasthenia does not occur at this age because of the immaturity of an infant's immune system. Likewise, Guillain-Barré syndrome, which is well-documented by finding a consistently elevated protein concentration in CSF, is of negligible occurrence in infancy. In infant botulism the protein concentration in CSF is normal, an occasional exception being that of a specimen collected while the child is mildly dehydrated.

TREATMENT

Specific therapy for infant botulism is now available. In California, a 5-year, randomized, double-blinded, placebo-controlled treatment trial demonstrated the safety and efficacy of human-derived botulinum antitoxin, known formally as human botulism immune globulin intravenous (BIG-IV).[7,14] Use of BIG-IV reduced mean hospital stay per case from approximately 5.5 weeks to approximately 2.5 weeks ($p < 0.001$) and reduced mean hospitalization cost per case by about $90,000 (2004 dollars; $p < 0.001$). In a 6-year, follow-on, open-label study, treatment with BIG-IV within 7 days of hospital admission reduced mean hospital stay to 2.2 weeks. Treatment with BIG-IV should be started as early in the illness as possible to maximally neutralize the toxemia and should not be delayed for laboratory confirmation of the clinical diagnosis. BIG-IV may be obtained from the California Department of Public Health as a public service orphan drug (24-hour telephone: 510-231-7600; website: *www.infantbotulism.org*).

Successful management of infant botulism also depends on meticulous supportive care and the anticipation and avoidance of potentially fatal complications (see Table 159–3). Feeding and breathing generally require the most attention. At admission, patients should receive cardiac, respiratory, and transcutaneous blood gas monitoring (especially carbon dioxide pressure) until it is clear that the paralysis no longer is progressing. An endotracheal tube often is necessary to maintain and protect the airway, even in the absence of a need for mechanical ventilation. Particular care should be taken to avoid transmission of nosocomially acquired *C. difficile* colitis.[35,89]

A third cornerstone of management is forbearance. Antibiotics should be reserved to treat the principal secondary infections (pneumonia, urinary tract infection, otitis media) because their use may result in lysis of intraintestinal *C. botulinum* with libera-

TABLE 159–4 Working Differential Diagnosis of Infant Botulism

Rights were not granted to include this table in electronic media.
Please refer to the printed publication.

From Francisco, AM. O., and Arnon, S. S.: Clinical mimics of infant botulism. Pediatrics 119:*826-828, 2007.*

tion of intracellular neurotoxin into the gut lumen and absorption. This potential problem may be avoided by prompt use of BIG-IV because its long (28-day) half-life and substantial antitoxin content enable it to neutralize all absorbed and circulating toxin.

Tracheostomy is not necessary.[1] Improved management of the airway can be accomplished by two simple positioning measures. First, for expansion of the thoracic cage and assistance in diaphragmatic function, patients should be placed in an older-style crib, the rigid bottom mattress of which can be lifted to elevate the entire body to a 30-degree angle. Second, to tip the head back and to maintain normal curvature of neck and airway, a soft cloth should be rolled to the thickness of about three fingers and placed under just the child's neck. This maneuver allows oral secretions to drain away from the trachea and into the true posterior pharynx, where they are swallowed most easily.

Intravenous feeding (hyperalimentation) is discouraged because of its potential for secondary infection and because of the success obtained with nasogastric or nasojejunal tube feeding. Mother's milk is the nutritional fluid of choice. Isolation measures or "enteric precautions" are not required, but meticulous handwashing is. Soiled diapers should be autoclaved because they can be expected to contain botulinum neurotoxin as well as viable spores and vegetative cells of *C. botulinum*. For this reason, staff with open lesions on their hands should not handle the diapers.

In the untreated (placebo) group in the 5-year, randomized clinical trial of BIG-IV, when compared with the untreated type B patients, the untreated type A patients had significantly longer mean hospital stays (6.7 versus 4.2 weeks), mean stays in the intensive care unit (ICU) (6.5 versus 3.1 weeks), and mean time on a ventilator (6.4 versus 2.2 weeks).[14] However, the distributions of the untreated type A and type B patients partially overlapped for all three parameters. Hence, untreated illness caused by type A toxin appears to be generally, but not invariably more severe than that caused by type B toxin. With use of BIG-IV, the mean duration of hospital stay has been reduced to approximately 2.2 weeks for both type A and type B patients, with comparable decreases also in the duration of stay in the ICU and time on a ventilator.[14]

OUTCOME AND PROGNOSIS

Recovery from infant botulism occurs through regeneration of the poisoned terminal unmyelinated nerve endings. The newly synthesized nerve twigs then induce the formation of new motor end-plates that are indistinguishable functionally and morphologically from the original ones.[31,32] In experimental animals and in human infants, completion of this process takes several weeks.[32] Consequently, in the absence of hypoxic cerebral complications, full and complete recovery of strength and tone is the expected outcome of infant botulism. In addition, because botulinum toxin does not cross the blood-brain barrier to any functional degree, the child's intelligence and personality remain intact. Parents often need reassurance on this latter point. Re-infection with the same or a different toxin type of *C. botulinum* has not occurred. In the United States, the case-fatality ratio of hospitalized patients is less than 1 percent, a reflection of, and tribute to, the high quality of intensive care given to these critically ill infants. In other countries, the experience has not been as fortunate.[55]

PREVENTION

At present, the one known way to prevent infant botulism is not to feed honey to infants, and all major pediatric and public health agencies have endorsed this recommendation. Breast-feeding may help moderate the rapidity of onset and the severity of illness. Persuasive evidence that links infant botulism to the ingestion of corn or other syrup is lacking. In the pre–BIG-IV era, the patient with the most protracted illness was hospitalized for 10 months in 1988 at a cost of more than $1,000,000 (2004 dollars). Mean hospital cost for the placebo-treated patients in the 1992 to 1997 randomized clinical trial of BIG-IV was $163,400, which was reduced in the BIG-IV group to $74,800, a net cost-savings of $88,600 (2004 dollars).[14] These economic facts combine with humanitarian considerations to make a compelling case for the prevention and effective treatment of infant botulism.

SUGGESTED READING

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CHAPTER 160

TETANUS

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Tetanus is caused by the anaerobic, spore-forming bacillus *Clostridium tetani*, an organism present in soil and in human and animal feces. The clinical symptoms are not caused by infection but result from a specific toxin, tetanospasmin, that is produced at the site of injury and acts primarily on the spinal cord but also on the brain, motor end-plates, and autonomic nerves. The disease may appear as a local form or as a more generalized syndrome. It is characterized by tonic spasms of skeletal muscles, little or no fever, and occasional spasms of the glottis and larynx. Bilateral trismus is the most common sign. Active immunization is highly protective. Although tetanus is controlled well today in developed countries throughout the world, it remains a major cause of death in developing countries.[26,28,118,129] In 2002, an estimated 213,000 deaths were caused by tetanus worldwide.[129] Neonatal tetanus was the cause of approximately 180,000 deaths, and maternal tetanus was responsible for 15,000 to 30,000 deaths.

HISTORY

The first description of tetanus in recorded medical history probably was written by Hippocrates.[57] A vivid picture of the clinical course of the disease was recorded by Aretaeus the Cappadocian in the second century,[6] but very little of importance was added to this knowledge during the next 18 centuries. Transmissibility of tetanus was demonstrated by Carle and Rattone[23] in 1884. They reported that when the sciatic nerves of rabbits were injected with the contents of a human "pustule," the characteristic disease developed in the animals in 2 to 3 days; inoculation of tissue obtained from their nervous systems into healthy rabbits produced a similar syndrome. The role of soil in the pathogenesis of tetanus was demonstrated by Nicolaier[83] in 1884; although he saw the organism, he was unable to recover it.

The bacterium responsible for the disease was identified first by Rosenbach,[99] who described a bacillus containing a round terminal spore in pus obtained from a human case; tetanus developed when the purulent exudate was injected into animals. *C. tetani* was isolated by Kitasato,[64] who fulfilled Koch's postulates with it in 1889. One year later, von Behring and Kitasato[121] reported the appearance of specific antitoxin in the serum of animals given injections of the tetanus toxin produced by the organism. This finding was followed, in 1926, by the development of toxoid, injections of which produced immunity.

MICROBIOLOGY

The vegetative form of *C. tetani* is a gram-positive, spore-forming, motile, anaerobic bacillus that measures 0.3 to 0.5 μm in width and 2.0 to 2.5 μm in length; in culture, long filament-like cells develop.[12,21,123,126] *C. tetani* is a strict anaerobe that grows best at 33° C to 37° C; it can be cultured in many different routine media used for anaerobic organisms, such as thioglycolate, casein hydrolysate, and cooked meat. Enhanced growth occurs in medium supplemented with reducing substances and maintained at neutral or alkaline pH. With growth, gas with a fetid odor usually is produced.

The first step in the process of spore formation is the development of a bulge at one end of the organism; this bulge contains the spore and is responsible for the characteristic drumstick or tennis racquet appearance. As sporulation progresses, the organism decreases in length and the spores are extruded. They stain poorly by the Gram method. Sporulation occurs in tissues as well as in vitro and is dependent on the composition of the medium and the temperature of the culture. Enhanced sporulation occurs in the presence of oleic acid, phosphates, 1 to 2 percent sodium

chloride, and manganese salts.[12,21,123] In vivo, sporulation is enhanced by lactic acid and other substances toxic to cells. Sporulation is inhibited by high and low temperatures (>41° C and <25° C), glucose, fatty acids, and potassium salts. The metabolic activity of *C. tetani* is limited; carbohydrates and proteins are digested poorly, and the vegetative, nonsporulated forms are killed easily by heat and numerous disinfecting agents. In contrast, the spores are resistant to boiling and phenol, cresol, 1 : 1000 bichloride of mercury, and other disinfectants; however, they are destroyed by heating at 120° C for 15 to 20 minutes. If not exposed to sunlight, the spores may survive in soil for months to years. They also may constitute part of the normal intestinal microflora of some horses, cows, guinea pigs, sheep, dogs, cats, rats, chickens, and humans.

Three nonpathogenic clostridia are present in soil and in human and animal feces: *Clostridium tetanomorphum*, *Clostridium tertium*, and *Clostridium tetanoides*. Diagnostic confusion may occur because they are morphologically similar to the organism responsible for tetanus. *C. tetani* is recovered much more commonly from cultivated than from virgin or uncultivated soil. Rural dwellers and people engaged in agricultural occupations have a higher rate of intestinal, skin, and oral carriage of the organism than city dwellers do. Dust and dirt from houses, streets, and operating rooms, as well as solutions of heroin used by injection drug users (IDUs), have been found to be contaminated with the organism.

EPIDEMIOLOGY

SOURCE OF EXPOSURE

The predominant reservoir of *C. tetani* is the soil; it is also part of the normal flora of the intestinal tract of animals, both herbivores and omnivores.[123] Intestinal spores and bacilli are shed in the feces of animals and contribute to the soil reservoir.

The worldwide morbidity and mortality attributable to tetanus is related inversely to adequate immunization with tetanus toxoid and directly to suboptimal hygiene, childbirth practices, and wound care.[123] Throughout the world, tetanus has a seasonal trend: more cases occur in the summer or in "wet" seasons. Rates of illness are highest in countries that are located near the equator and have fertile soil.

Acute wounds, including relatively minor ones, are the site of most *C. tetani* infections leading to tetanus. In addition, infection may occur after parenteral drug use and surgical procedures. In many cases the source of exposure is unknown. The source of infection in neonatal tetanus is the umbilical cord or stump as a result of unsterile delivery conditions and unhygienic cultural rituals involving the umbilical stump.[58,59,93]

INCIDENCE

The incidence of tetanus in the vaccine era is related inversely to the degree to which effective immunization programs have been implemented in a population.[123] In the early 1980s an estimated 2 million cases of tetanus occurred throughout the world, with 1 million fatal neonatal cases and 122,000 to 300,000 non-neonatal fatal cases.[50,51]

Although neonatal tetanus is of very minor importance in developed areas of the world, it has been a major cause of death in infants in developing countries.[26,27,66,72,110,118,123,129] In 1993, an estimated 515,000 deaths worldwide were caused by neonatal tetanus.[26] These deaths occurred predominantly in Southeast Asia (34.2%); Africa (28.2%); the western Pacific, including China (21.4%); and the eastern Mediterranean region (15.7%). The global mortality rate in 1993 was estimated to be 4.1 per 1000 live births. The very high frequency of neonatal tetanus in these areas probably was related to conditions surrounding the birth of infants.[49] Most babies were born in very unhygienic environments; delivery rarely took place in an adequate hospital. In addition, unclean instruments were used to sever the umbilical cord, rags often contaminated with soil or feces were used as dressings, and mud and manure were applied directly to the umbilical stump. In a study in Senegal that examined risk factors for the development of neonatal tetanus, the major source of *C. tetani* was found to be the hands of the birth attendant, and the mode of contamination of the infant was related to the method that the birth attendant and the mother used to dress the umbilical cord stump.[66] In rural Pakistan, the ritual of bundling (wrapping the baby for prolonged periods in a sheepskin cover after the application of cow dung) is a significant risk factor for the acquisition of neonatal tetanus.[10] In a more recent case-control study of risk factors for neonatal tetanus in Karachi, Pakistan, the following risks were identified: application of substances (mustard oil/ghee/surma) to the umbilical cord, home delivery, and illiterate mothers.[93]

In 2002, deaths worldwide caused by tetanus were estimated at 213,000, with approximately 180,000 occurring in neonates and 15,000 to 30,000 maternal cases.[129] Most maternal cases are the result of unclean delivery or abortion practices.

The reported incidence of tetanus and tetanus-related deaths in the United States from 1947 to 2000 is presented in Figure 160–1, and the reported cases and fatal cases and their incidence are presented by age group for 1998 to 2000 in Figure 160–2.[85] From 1947 through 1976, the incidence of tetanus fell tenfold; since 1976, the continued decrease in incidence has been less than twofold. During the same period, mortality rates fell from 91 percent in 1947 to 44 percent in 1976 to 18 percent in 1998 to 2000.

In the period 1998 to 2000, 36 percent of the cases occurred in patients 60 years or older, 55 percent in patients aged 20 to 59 years, and only 9 percent in those younger than 20 years. During this period, the vaccination status was known in only 50 of the patients (38%). Of this group, eight (16%) had received three or more doses of tetanus toxoid, with the last dose being received less than 10 years before the onset of tetanus. None of these eight patients sought medical care for the injury before the onset of tetanus, and three were younger than 20 years. The outcome of the illness was available for only 18 of the 50 patients with a known immunization history, and one death occurred—in an IDU whose last dose of tetanus toxoid was 11 years before the onset of tetanus.

During the period 1998 to 2000, 60 percent of the cases occurred in males.

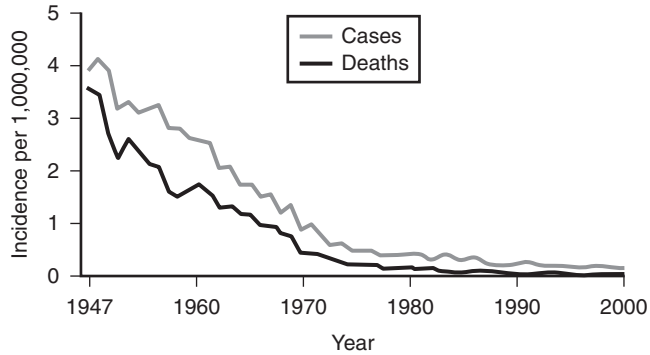

Figure 160–1 Reported incidence of tetanus and tetanus-related deaths in the United States from 1947 to 2000. *(Pascual, F. B., McGinley, E. L., Zanardi, L. R., et al.: Tetanus surveillance: United States, 1998-2000. M. M. W. R. Surveill. Summ. 52[3]:1-8, 2003.)*

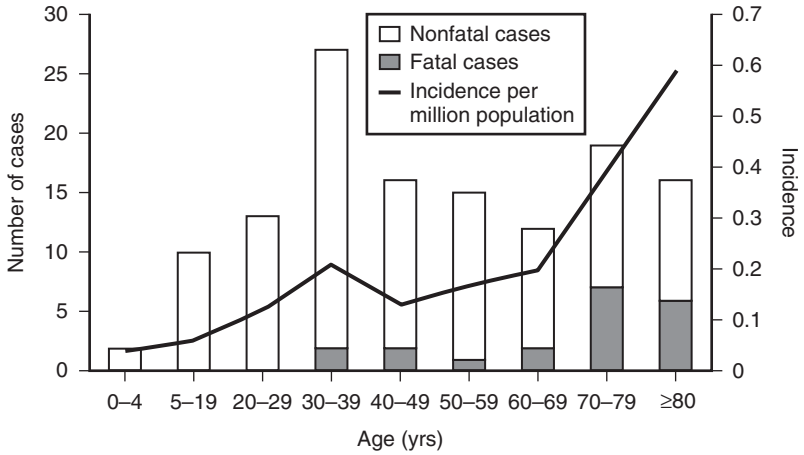

Figure 160–2 Reported tetanus cases and incidence rates by age group in the United States in 1998 to 2000. *(Pascual, F. B., McGinley, E. L., Zanardi, L. R., et al.: Tetanus surveillance: United States, 1998-2000. M. M. W. R. Surveill. Summ. 52[3]:1-8, 2003.)*

PATHOGENESIS

Tetanus is, by strictest definition, not a true infection. Spores introduced at a site of injury remain harmless until they are converted, after being stimulated by a variety of factors, to vegetative forms that multiply but do not produce injury to tissue or provoke an inflammatory response. The clinical syndrome is caused entirely by toxin elaborated in the area where the vegetative cells are growing. *C. tetani* produces two exotoxins: tetanolysin and tetanospasmin.[89] Tetanolysin induces hemolysis but plays no role in the disease. The activity of tetanospasmin is responsible for all of the clinical features of the disease. This toxin has a molecular weight of 150 kd and is activated by proteolytic cleavage into two polypeptides, a heavy (100 kd) and a light (50 kd) chain that are linked by a disulfide bond.[20] One milligram contains 6,400,000 lethal doses for mice; 0.00001 mg may kill a 20-g mouse in 2 hours. With the exception of the toxin produced by *Clostridium botulinum*, tetanospasmin currently is the most potent known poison. Humans are most susceptible to this agent and require only $^1/_{2500}$ and $^1/_{350,000}$ of the dose that is fatal for cats and chickens, respectively.

C. tetani is introduced into an area of injury as the spore, the form usually present in soil and intestinal contents. Disease does not develop until the spores are converted to the toxin-elaborating bacillus, which does not follow the simple inoculation of spores. When these spores are injected with very sharp needles into animals so that tissue is not injured but merely separated, tetanus does not develop. However, the addition of a small quantity of calcium chloride to a suspension of spores before injection leads to development of the typical disease. Such development is not caused by a specific effect of the cation on conversion of the spore to the vegetative form of the organism but is related to the necrosis of tissue produced by it, which leads to a reduction in oxidation-reduction potential and oxygen tension, factors involved in vegetation of the spore; the same effects are produced by the presence of a foreign body, trauma, or a localized suppurative process.

The pathway by which toxin, elaborated at the site at which *C. tetani* is multiplying, reaches the central nervous system (CNS) has been a matter of controversy. Researchers first thought that tetanospasmin was absorbed chiefly by motor nerve endings and then traveled along axis cylinders to the anterior horn cells.[90] Symptoms of the disease were thought to appear only after the toxin had reached the nervous system, with the initial tetanic contractions occurring first in the injured extremity, then in the opposite limb, and finally, as the toxin diffused through the spinal cord, in all the muscles. A small amount of toxin was suggested to be absorbed into the lymph and carried to the bloodstream, from which it was taken up by motor nerve endings in various parts of the body.[76]

Abel and associates[1] postulated that toxin reached the nervous system via the arterial circulation and suggested the following series of events. Some of the toxin diffuses from the local site of injury into adjacent skeletal muscle and acts on neuromuscular organs to produce a state of maintained contraction: local tetanus. Some toxin also enters the lymphatics and bloodstream, from which it is taken up by specifically reactive cells in the spinal cord, medulla, and the motor end-organs of muscle.

Friedemann and colleagues[48] noted that tetanus toxin did not penetrate the blood-brain barrier, and therefore they questioned hematogenous transmission. This finding led researchers to focus attention on transport of tetanospasmin in nerves. Wright and coworkers[130] observed that direct injection of toxin into the vagal, facial, or hypoglossal nerves of rabbits led, within 24 hours, to the development of a syndrome characteristic of involvement of the brain stem, as indicated by the appearance of strabismus, immobility of hairs in the nasal cavity, salivation, bradycardia, and torticollis. Inoculation of the vagus resulted in the successive development of these manifestations; those dependent on innervation from motor nuclei in close proximity to the nerve tended to appear early. This hypothesis is supported by observations indicating that a variety of substances, including India ink and radioactive compounds, migrate along peripheral nerves to the CNS after intraneural injection.[45] This observation, together with the demonstration by numerous investigators that toxin is present in the peripheral nerves closest to the site of inoculation, supports the concept of transport of tetanospasmin in the tissues of the nervous system.

Knowledge of the specific mechanisms involved in the absorption of tetanus toxin and its mechanisms of action on body cells is incomplete. Most of the evidence presently available indicates that some element of the peripheral nerve is involved. Whether transport occurs in the axis cylinder, the perineural space, or the lymphatics remains unsettled. Tetanus toxin is carried to the neurons of the sciatic nerve via the neurofibrillae in axis cylinders at a rate of approximately 3.35 mm/hr.[98]

PATHOPHYSIOLOGY

Tetanospasmin exerts its effects in four areas of the nervous system: (1) the motor end-plates in skeletal muscle, (2) the spinal cord, (3) the brain, and (4) the sympathetic nervous system (in some cases).*

*See references 16, 18, 19, 24, 35, 60, 62, 75, 106, 119, 132.

MOTOR END-PLATES IN SKELETAL MUSCLE

Tetanus toxin has been noted to interfere with neuromuscular transmission. Release of acetylcholine from the nerve terminals in muscle is inhibited. The presence of tetanospasmin in the transverse and terminal sacs of the longitudinal elements of the sarcotubular system of skeletal muscles suggests that it acts by interfering with contraction coupling or with the mechanisms involved in contraction and relaxation. These phenomena probably are involved in the pathogenesis of local tetanus.

SPINAL CORD

The effects of the toxin on the spinal cord are practically identical to those produced by strychnine. It does not act on reflex arcs that include only sensory and motor neurons (two-neuron or monosynaptic reflexes). However, the toxin profoundly alters the activity of the more complex polysynaptic reflexes involving interneurons, thereby leading to the inhibition of antagonists. Hyperpolarization of the membranes of neurons, a mechanism normally operating when direct inhibitory pathways are stimulated, is suppressed. The depolarization associated with excitation is not affected. Whether tetanospasmin blocks inhibitory synapses by preventing release of the inhibitory transmitter substance or by suppressing the action of this substance on the membrane of motor neurons is unknown. Selective blocking of inhibitory synapses in the CNS appears adequate to account for the primary phenomena of tetanus. Unchecked and uncoordinated excitatory impulses multiply and traverse reflex pathways to produce the characteristic tetanic spasms of muscles.

BRAIN

Some investigators have suggested that the action of tetanospasmin on the brain may be responsible for the typical seizures of tetanus.[16] This hypothesis is supported, in part, by the observation that cerebral gangliosides fix the toxin. The antidromic inhibition of evoked cortical activity is decreased. The effects of tetanospasmin on the brain are the same as those on the spinal cord, as well as those that occur after exposure to strychnine.

SYMPATHETIC NERVOUS SYSTEM

Manifestations indicating dysfunction of the sympathetic nervous system have been observed in some patients with tetanus. Signs and symptoms include profuse sweating, peripheral vasoconstriction, labile hypertension, cardiac arrhythmias, tachycardia, increased output of carbon dioxide, elevated urinary concentration of catecholamines, and hypotension late in the course of the disease.

CLINICAL MANIFESTATIONS

Although it may be as short as 1 day or as long as several months, the incubation period of tetanus usually is 3 to 21 days. A direct relationship may exist between the distance of the site of invasion by *C. tetani* from the CNS and the length of the interval between injury and the onset of disease; the greater the distance between the local area and the CNS, the longer, in general, the incubation period. Sustained or separate, repeated tonic spasms of isolated or multiple muscles are the characteristic clinical feature of the disease. Tetanus may appear in two forms: local and generalized.

LOCAL TETANUS

The occurrence of local tetanus most likely is more common than recognized. Although local tetanus is thought to occur infrequently, it probably is so because it may be uncommon as an isolated syndrome or because by the time that diffuse involvement has occurred, the spasms in the muscles in the area of the site of entry of the organism cannot be separated from the generalized spasms. Local manifestations frequently precede development of the generalized disorder. The characteristic abnormality in local tetanus is unyielding, persistent, painful rigidity of the group of muscles that lie in close proximity to the site at which *C. tetani* was introduced. Local tetanus often is present when a dose of antitoxin has been given that is adequate to inactivate circulating toxin but insufficient to neutralize what has accumulated at the site of injury. Symptoms may persist for several weeks or months and finally disappear without leaving any residua.[77,94] Local tetanus may be the only manifestation, usually is mild, and has a fatality rate of approximately 1 percent.

Cephalic tetanus is a variant of local tetanus. It generally occurs after the introduction of *C. tetani* in the course of injuries to the scalp, eye, face, ear, or neck; in conjunction with chronic otitis media; and rarely, after tonsillectomy.[7] Insect bites on the face or head, especially if secondarily infected by pyogenic organisms, also may serve as portals of entry for the organism. The incubation period of this syndrome is short, frequently no more than 1 to 2 days. The principal distinctive clinical features are palsies of cranial nerves III, IV, VII, IX, X, and XII; they may be involved singly or in any combination. Dysfunction may persist for days or many months. In general, the prognosis for survival is poor. However, if death does not intervene, complete recovery without residual neurologic dysfunction is the rule. In some, but not all, instances, generalized tetanus may develop during the course of the cephalic form of the disease.

GENERALIZED TETANUS

Generalized tetanus is the most common manifestation of the disease.[96,124] Despite the general impression that inoculation of the organism is associated most frequently with deep penetrating injury, the portal of entry in approximately 80 percent of cases is an insignificant wound.[90] Burns, injuries induced by blank cartridges, deep punctures, furunculosis, dental extraction, embedded splinters, decubitus ulcers, hypodermic injections, and compound fractures complicated by chronic active osteomyelitis are typical situations in which tetanus may develop because the environment in the tissues is optimal (decrease in oxidation-reduction potential and tension of oxygen) for conversion of spores to the toxin-producing vegetative organisms. Iatrogenic disease has occurred after the use of smallpox vaccine and surgical sutures contaminated with the spores of *C. tetani*. It also has occurred as a postoperative complication in patients exposed to the organisms present in the dust in operating rooms.[92,95,105] Very minor injuries, such as penetration by "clean" sewing needles and bites by chiggers, bees, and scorpions, have been recorded as portals of entry. Tetanus also has occurred after induced abortions, usually performed under poor asepsis.

The initial manifestation in more than 50 percent of cases of generalized tetanus is trismus; it may be unilateral early in the disease but becomes bilateral within a short time. In some cases, it may be absent during the entire course of the illness or appear only after other abnormalities have become evident. In some instances, the only symptoms and signs may be irritability, restlessness, stiffness of the neck, difficulty swallowing, and rigidity of the abdominal or thoracic muscles; these symptoms may be present singly or in various combinations. The diagnostic importance of trismus cannot be overemphasized, despite the fact that

it may be associated with numerous disorders unrelated to tetanus. Among these disorders are postmeasles encephalitis, trichinosis, suppurative and other types of parotitis, tender cervical lymphadenopathy, infected impacted upper molar teeth, and exposure to phenothiazine drugs.

As the activity of tetanospasmin persists, groups of muscles other than the masseters may become involved, including tonic spasms of the jaw, neck, back, and abdomen. Unrelenting trismus leads to the development of a characteristic facial expression, the sardonic smile (risus sardonicus). The abdominal and spinal muscles may become rigid. Intense and sustained contraction of the muscles of the chest and back results in persistent opisthotonos; in young children, this condition may be so severe that the youngster lies on one side with the soles of the feet resting on top of the head.

Generalized seizures, or tetanospasms, are unique in their appearance and peculiar to this disease. Characteristically, a sudden burst of tonic contractions of all groups of muscles occurs and leads to the development of opisthotonos, flexion and abduction of the arms, clenching of the fists on the chest, and extension of the legs. Pain in the spastic muscles usually is severe. Glottal and laryngeal spasm with the chest in the position of full inspiration may develop in some patients. During such an episode, the face becomes florid, the neck veins are distended markedly, and cyanosis develops. All the features of this syndrome are consistent with an intense and sustained Valsalva maneuver; unless the tonic contraction of the glottis and larynx subsides or a tracheostomy is performed, death results. Dysphagia and hydrophobia may develop as isolated phenomena in the absence of a generalized tetanospasm. Dysuria or urinary retention also may occur.

Autonomic nervous system dysfunction commonly occurs in tetanus.[113,122] Manifestations include labile or persistent hypertension or hypotension, persistent sinus tachycardia, tachyarrhythmia or bradycardia alternating with tachycardia, and profuse sweating.

Electroencephalographic studies of patients have indicated involvement of the brain. Of 106 patients studied by Luisto,[70] 76 percent were found to have abnormal electroencephalograms. When patients of the same age and sex who had not had tetanus were compared with 40 individuals who had contracted the disease 7 years earlier, the latter were found to have more muscle fatigue and cramps; difficulty in speech, balance, and memory; and more peripheral paresis, muscular atrophy, decreased or absent tendon reflexes, and impaired mental capacity.[63]

The generalized seizures of tetanus often are triggered by very slight external stimuli, such as a light breeze, talking, a bump on the bed, or slight touching of the patient. The intense work generated by sustained or frequently repeated tetanospasms often leads to increases in body temperature of 1° C to 3° C or more.

The intense suffering of patients with the generalized tonic seizures of tetanus and the total frustration of the physician, who in ancient times had to stand by helplessly, unable to alter the course of the disease, were described best by Aretaeus[6] in the second century BC; no better description has ever appeared:

> An inhuman calamity! An unseemly sight! A spectacle painful even to the beholder, an incurable malady! Owing to the distortion, not to be recognized by the dearest friends; and hence the prayers of the spectators, which formerly would have been reckoned not pious, now becomes good, that the patient may depart from life, or being a deliverance from the pains and unseemly evils attendant on it. But neither can the physician, though present and looking on, furnish any assistance, as regards life, relief from pain or from deformity. For if he should wish to straighten the limbs, he can do so only by cutting and breaking those of a living man. With them, then, who are overpowered by this disease, he can merely sympathize. This is the great misfortune of the physician.

Injection Drug Users

When generalized tetanus develops in IDUs, many of the clinical features differ from those present in people who do not use drugs.[67] Among such features are much higher levels of fever; absence of trismus at onset and throughout the course of the disease or its appearance some time after other manifestations have been present; marked stiffness of the neck and back, which in the absence of trismus may be confused with meningitis (the normal cerebrospinal fluid in patients with tetanus excludes this possibility); and early onset of coma. Prophylaxis is less effective in IDUs than in non-IDUs. The fatality rate approaches 100 percent.

Neonatal Tetanus

Neonatal tetanus usually begins 3 to 14 days after birth and is characterized by poor sucking and excessive crying.[49,124] Manifestations include trismus, difficulty swallowing, other tetanic spasms, and frequently, marked opisthotonos.

A study of the causes of death in patients with neonatal tetanus by Salimpour[101] indicated pulmonary disease to be most common. Bronchopneumonia or hemorrhage in the lungs (or both) was the most frequent finding at autopsy. Among the nonpulmonary disorders responsible for a fatal outcome were hepatitis, omphalitis, cerebral hemorrhage, thrombosis, and rupture of the renal vein. As has been noted not only in infants but also in older children and adults with tetanus, any gross or histologic abnormalities are very uncommon findings. The complications described by Salimpour,[101] especially those involving the lungs, probably are related to aspiration associated with the laryngoglottal spasm characteristic of the disease. Newborns who survived tetanus in rural Kenya often had evidence of brain damage.[8] Indicators of a poor prognosis for neonates include age younger than 10 days when admitted to the hospital, symptoms of less than 5 days' duration, and the presence of risus sardonicus and fever.[73]

DIAGNOSIS

DIFFERENTIAL DIAGNOSIS

The differential diagnosis depends on the major clinical manifestations of the illness.[123] Cephalic tetanus may be confused with Bell palsy, trigeminal neuritis, and encephalitis, whereas generalized tetanus can be confused with rabies, strychnine poisoning, and phenothiazine reactions. Trismus can result from dental problems, tonsillitis, peritonsillar abscess, temporomandibular joint dysfunction, and parotitis. Tetany caused by hypocalcemia or hyperventilation should be considered as well.

SPECIFIC DIAGNOSIS

The diagnosis of tetanus is established on the basis of the history of an injury, particularly one in which either soil or fecal material has been introduced. In a small number of cases, especially those in which a relatively long incubation period has occurred, the site of entry of the organism may be healed and undetectable. In a review of 130 cases that occurred in the United States between 1998 and 2000, 94 (72%) were related to an acute injury.[85] There were 47 puncture wounds, 31 lacerations, and 8 abrasions. In 34 cases not involving an acute injury, the following associations were noted: abscess, 7 cases; ulcer, 7 cases; gangrene, 2 cases; cellulitis, 1 case; gingivitis, 3 cases; and other infections, 2 cases. In 12 cases, no injury or other source location was identified. One of the 130 patients was a neonate, and 18 of the non–acute injury cases occurred in patients with a history of injection drug use. Laboratory studies are of little help. Peripheral leukocytosis may

or may not be present. The characteristic gram-positive rods, some of which may contain subterminal spores, may be seen occasionally in stained material obtained from the wound that has served as the portal of entry. Anaerobic cultures of exudate or necrotic tissue may grow typical sporulated rods and vegetative forms, but the yield of positive findings tends to be low.[31]

Low or undetectable levels of serum antitoxin at the time of onset of the illness is supportive of the diagnosis, but occasionally, serum antitoxin is present.[123] Rises in antitoxin titer occur uncommonly after infection, so the use of paired sera for retrospective diagnosis generally is not helpful; in addition, this form of serologic diagnosis is not possible when therapy included active immunization. In selected cases, electrophysiologic studies of the masseter muscle may be helpful.[9] A characteristic absence or shortening of the silent period occurs as a result of failure of Renshaw cell inhibition; exaggerated F responses indicating hyperexcitability also may be noted.

TREATMENT

Management of generalized tetanus is directed at the following goals:

1. Neutralization of toxin still present in the blood before it comes in contact with the nervous system. Neutralization is accomplished by the administration of antitoxin as soon as the possibility of the disease is suspected or confirmed.[12] However, no acceptable evidence has shown that toxin can be inactivated by antitoxin once the toxin is fixed to tissues. In fact, the effectiveness of even large doses of antitoxin in reducing the fatality rate has been questioned.
2. Surgical removal of the site of entry of the organism, when possible, to eliminate the "factory" in which tetanospasmin is being produced. Omphalectomy has been performed with good results in children with neonatal tetanus.[37] Hysterectomy has been recommended when the disease has complicated induced and often septic abortions; however, patients in whom the infected uterus is not removed occasionally may survive. When the surgical procedure may be mutilating, as is the case with lesions on the face, it should not be performed.
3. Constant and meticulous nursing care.
4. Close monitoring of fluid, electrolyte, and caloric balance because they frequently are abnormal, especially in patients with high temperature and repeated seizures, as well as in those unable to take food or liquids because of severe trismus, dysphagia, or hydrophobia.

The slightest external stimulus may precipitate potentially lethal seizures in persons with diffuse disease. For this reason, of extreme importance is that all therapeutic and other manipulations be well coordinated and carefully scheduled so that the risk of producing a tetanospasm is reduced to a minimum. All maneuvers are performed best after patients have received optimal sedation and relaxation. A quiet, darkened room in which the light is subdued and the doors padded, removed as far as possible from the mainstream of hospital traffic, is ideal, but at the same time the patient requires constant monitoring and observation in an intensive care environment.

The antitoxin of choice for the treatment of tetanus is human tetanus immune globulin (TIG) at a dose of 3000 to 6000 units intramuscularly; a second dose does not appear to be necessary. When TIG is not available, equine antitoxin (EA) may be used, but it should be avoided if at all possible. The dose of EA is 100,000 units; half is injected intramuscularly after appropriate tests to rule out sensitivity to horse serum have been performed. If EA is well tolerated, the remainder is given slowly intravenously. Patients sensitized to horse serum must be desensitized. An intramuscular injection of 80,000 units of EA produces maximal concentrations of antibody in the blood in 48 to 72 hours; very good levels may be maintained for 7 days.[53,116,117] Intravenous administration of the same dose yields concentrations of 40 or more units of antitoxin per milliliter of serum after 6 hours; it may persist for approximately 48 hours. Essentially no difference in circulating quantities of antitoxin exists 7 days after it is given by either route. Local instillation of antitoxin around the known or suspected wound may be of value if excision cannot be performed; it also has been recommended before surgical removal of the injured area.

Although reports[83] have documented that intrathecal administration of antitetanus serum did not improve the rate of survival in neonatal tetanus, the results of a study by Mongi and colleagues[80] led them to recommend this form of therapy. They found that intrathecal serotherapy may be of great value in the management of tetanus. Their experience indicated that the death rate from this disease in patients given intrathecal therapy was 45 percent; in those who did not receive this agent, it was 82 percent. However, the results of a recent meta-analysis led Abrutyn and Berlin[2] to conclude that intrathecal therapy with either EA or TIG is not of proven benefit and, therefore, should be given only during well-designed, controlled therapeutic trials.

In a controlled trial in Brazil involving 120 adolescents and adults, 58 patients received TIG intrathecally and intramuscularly and 62 received TIG only by the intramuscular route.[79] The patients who received intrathecal and intramuscular treatment had better clinical progression than did those who were treated only by the intramuscular route. Most recently, a meta-analysis that involved 942 patients in 12 trials was reported.[61] In this analysis, the overall relative risk of mortality with intrathecal (antitetanus serum or TIG) versus intramuscular therapy was 0.71 (95% confidence interval, 0.62 to 0.81). Subcategory analyses for both adults and neonates and for high- and low-dose intrathecal therapy also indicated the beneficial effect of intrathecal therapy.

Penicillin kills the vegetative forms of *C. tetani*. Parenteral administration of penicillin G, 100,000 U/kg/day intravenously every 6 hours for 10 days, has been recommended in the past in all cases of tetanus. Observations have suggested that penicillin may act as an agonist to tetanospasmin by inhibiting the release of γ-aminobutyric acid (GABA).[123] Because of this possibility and the results of a controlled study in Indonesia, metronidazole has become the antimicrobial treatment of choice in many centers.[4] The metronidazole dosage is 30 mg/kg/day intravenously every 6 hours after an initial dose of 15 mg/kg. The usual duration of therapy is 7 to 10 days.

The spasticity and seizures of tetanus are caused by exaggerated reflex responses to afferent stimuli as a result of suppression of balancing central inhibition. Several classes of drugs that act at different sites along the reflex pathway are useful for control of these manifestations. Among them are hypnotics and sedatives, which reduce sensory input and generalized excitability; general anesthetics, which produce broad depression of the CNS; centrally acting muscle relaxants or spinal depressants, which lower reflex activity and decrease motor output from the spinal cord; and neuromuscular blocking agents, which inhibit the transmission of excess motor nerve activity to effector muscles.

The ideal drug for the treatment of tetanus must control seizures and decrease spasticity without impairing respiration, voluntary movement, or consciousness. The activity of an agent in inhibiting the convulsions induced by strychnine usually has been a reliable guide for the prediction of effectiveness in the management of tetanus. However, it may not be reliable in all cases. For example, although the phenothiazines act as anticonvulsants in both naturally occurring and experimentally produced tetanus, they fail to control seizures induced by strychnine. Creech and associates[34] point out, "It may be concluded that any type of sedative or hypnotic agent, when properly administered so as to

avoid respiratory depression, has the same effect or lack of effect upon the outcome of tetanus."

Historically, many different drugs have been used in patients with tetanus. Secobarbital sodium (Seconal) and pentobarbital (Nembutal) have been favored for their relatively short half-life.[60,78] Barbiturates, however, may have deleterious effects on both the respiratory and cardiovascular systems. A patient heavily sedated with barbiturates often will have a rise in carbon dioxide pressure and a fall in oxygen pressure, as well as hypotension and a fall in cardiac output. In addition, barbiturates have a lower therapeutic index than the benzodiazepines do.[91] Chlorpromazine (Thorazine) also has been used to control the muscle spasms of tetanus, but it has some drawbacks.[32] Chlorpromazine actually may lower the seizure threshold and should be administered, if at all, only with concomitant anticonvulsant therapy. The acute dystonic reactions occasionally seen with chlorpromazine can confuse the picture markedly in a patient with tetanic spasms and rigidity. Akathisia, the need of the patient to be in constant movement, is another extrapyramidal effect occasionally seen with the phenothiazines and would be undesirable in a patient susceptible to tetanic spasms. Meprobamate historically was used to control the tonic spasms of tetanus.[88] It is relatively ineffective when given orally or intramuscularly (dissolved in propylene glycol).[78]

The barbiturates, phenothiazines, and meprobamate no longer are first-line antispasmodic drugs; benzodiazepines are the preferred drugs for the spasms and rigidity associated with tetanus.[33,84,111,120] They also have the advantage of being potent anticonvulsants, as well as sedative-hypnotic agents. Additionally, they are GABA agonists and perhaps partially overcome the effect of tetanospasmin interfering with the normally inhibitory effect of GABA.[119] Diazepam (Valium) and lorazepam (Ativan) have been the benzodiazepines most frequently used for tetanus. Lorazepam has a somewhat longer half-life and may be preferred for this reason. Very large total daily doses of diazepam (500 mg) or lorazepam (200 mg) may be required.[106] Both drugs are formulated in propylene glycol solution for intravenous administration, and the large doses required may result in significant propylene glycol toxicity, including metabolic acidosis. For this reason, an enteral preparation that is free of propylene glycol should be given enterally if at all possible.[13]

Midazolam (Versed) is a short-acting benzodiazepine that is soluble in water and thus does not include propylene glycol in its parenteral formulation. Because of its short half-life, it should be administered as a continuous infusion, and an initial infusion dose of 0.1 to 0.3 mg/kg/hr may be required. The benzodiazepines all induce tachyphylaxis and will require escalation of the dosage with time. The dosage should be titrated to prevent tetanic spasms and provide adequate sedation instead of being a specified dose. To avoid withdrawal symptoms after long-term use, the benzodiazepines should be tapered over the course of several weeks.

If the benzodiazepines are unable to control the spasms and rigidity associated with tetanus, intrathecal baclofen should be considered. Baclofen is a GABA receptor agonist that directly stimulates the postsynaptic GABA beta receptors on synapses blocked by tetanus toxin, thereby restoring physiologic inhibition of the alpha motor neuron. Numerous centers have described the efficacy of intrathecal baclofen for treating tetanus, although its safety and efficacy have not been established in children younger than 4 years.[14,40,41,103]

When the benzodiazepines and intrathecal baclofen are unable to control the spasms and rigidity associated with tetanus, neuromuscular blockade is indicated. Although succinylcholine has been used in the past,[47] newer agents have supplanted it. Additionally, theoretical reasons provide the basis to avoid the use of succinylcholine. With functional denervation of the motor endplate in neuromuscular junctions directly affected by tetanospasmin, succinylcholine may result in exaggerated release of potassium and hyperkalemia. This condition has been associated with cardiac arrhythmias and death in other denervating conditions. For this reason, nondepolarizing agents should be used. Potential agents include pancuronium, vecuronium, atracurium, cisatracurium, rocuronium, and pipecuronium—the agent of choice in a large tetanus treatment center in Vietnam.[115]

Neuromuscular blocking drugs should be used only by physicians experienced in their use in a critical care environment, typically anesthesiologists or intensivists.[65] Although neuromuscular blockers formerly were used at low doses to preserve diaphragmatic function and spontaneous respiration, current thought is that they should be administered in conjunction with endotracheal intubation and ventilatory support. Remembering that neuromuscular blocking drugs have no effect on cortical function and have no sedative effect is critical. The benzodiazepines are good sedatives and have a significant amnestic effect. They are not, however, analgesics, and if pain is present (such as from previous muscle spasms), morphine sulfate is an effective analgesic. It also may be helpful in treating sympathetic hyperactivity. If sympathetic overactivity remains problematic, a combined alpha- and beta-receptor blocker such as labetalol should be used. The use of a beta blocker such as propranolol alone should be avoided because the unopposed alpha-mediated vasoconstriction could lead to significant hypertension. Clonidine and epidural anesthesia are alternative therapies for increased sympathetic discharge. A combination of epidural bupivacaine and sufentanil has been used by some physicians to treat the sympathetic overactivity.[11]

In a recent large (256 patients) randomized, double-blinded, placebo-controlled trial, infusion of magnesium sulfate in patients with severe tetanus was compared with the administration of placebo.[115] There was no difference in need for ventilation or in survival between the two groups. However, patients randomized to receive magnesium sulfate did have better control of muscle spasms and less cardiovascular instability. Thus, magnesium sulfate infusion may have a role in the management of severe tetanus.

The management approach to a patient with tetanus spasms or rigidity should be one of escalation of therapy based on need. Benzodiazepines should be administered initially, with high doses often being required. If the benzodiazepines are not effective, intrathecal baclofen may be used.[40,41] Infusion of magnesium sulfate to decrease muscle spasms and cardiovascular instability should be considered.[115] If these therapies are inadequate or result in airway compromise or inadequate ventilation, endotracheal intubation should be performed with a nondepolarizing neuromuscular blocking agent. Intubation also should be performed if spasms result in obstruction of the airway. Some physicians have advocated that IDUs with tetanus have an airway established because of the fulminant course of the disease.[88]

Sixty-six percent of 103 children aged 1 to 12 years with severe tetanus studied by Wesley and Pathes[125] required management with total muscle paralysis and intermittent positive-pressure ventilation. The death rate in this group was 14.5 percent.

Very careful attention must be paid to care of the skin, bladder, mouth, and bowel of patients with tetanus. Adequate fluid and electrolyte balance must be maintained. Feeding by gavage in patients unable to eat because of severe trismus, dysphagia, or hydrophobia has been suggested as a means of ensuring optimal caloric intake. Gastric emptying may be impaired, and a transpyloric feeding tube will facilitate adequate nutritional support with a continuous infusion of age-appropriate enteral formula. Transpyloric placement of the feeding tube also may decrease the risk of aspiration.

The use of dantrolene with conservative treatment has been reported to reduce the fatality rate associated with tetanus significantly.[3] The need to block both the sympathetic and the parasympathetic nervous systems to stabilize hemodynamics has

been suggested. Such blockade may be produced by spinal anesthesia.[107] Patients with tetanus treated with metronidazole have been found to have a lower fatality rate, a shorter stay in the hospital, and an improved response to treatment.[4] Intravenous administration of morphine to patients with tetanus has been noted to reduce arterial blood pressure and systemic vascular resistance.[96]

Patients who have survived an episode of tetanus must be immunized actively after recovery because antitoxin usually is not detectable in serum for as long as 3 months after recovery.[116] Recurrent attacks of the disease rarely occur, however.[22]

Studies in mice have shown that adrenocortical steroids are without effect in altering the course of tetanus. The administration of cortisone after a significant delay between the injection of toxin and antitoxin or after clinical manifestations have appeared was found not only to be without benefit but also to decrease the effectiveness of the antitoxin.[30] Other studies also have indicated a lack of therapeutic effect[54,117] but have not demonstrated deleterious effects. Critically evaluated clinical experience has confirmed this finding.[68]

PROGNOSIS

The average fatality rate associated with tetanus ranges from 25 to 70 percent, but mortality rates can be reduced to 10 to 30 percent with modern intensive care.[97,123] The risk of death occurring in patients with tetanus neonatorum is particularly high; it was reported to be 99.5 percent in a group of 5794 infants in 1930.[57] With modern treatment and high-intensity supportive care, the mortality rate associated with neonatal tetanus can be reduced to approximately 25 percent.[123] IDUs are highly susceptible to the development of very severe disease and are likely to die.[63,67]

A variety of other factors play an important role in determining the outcome of tetanus.[55,114] Patients in the second and third decades of life have a higher rate of recovery than do those who are elderly. An inverse relationship exists between the length of the incubation period and risk, as first pointed out by Hippocrates. The risk of death is approximately 58 percent when the interval between injury and the onset of tetanus is 2 to 10 days. When the interval is 11 to 22 days or longer, fatality rates have been 35 to 17 percent, respectively. A relationship also appears to exist between the period elapsing from the time of appearance of the first signs of tetanus and development of the first seizure or maximal intensity of the disease; the shorter this interval, the poorer the prognosis.[31]

The clinical form of tetanus also influences the outcome.[69] Cephalic tetanus and tetanus neonatorum are associated with the highest incidence of death. In contrast, local tetanus, unless complicated by development of the generalized syndrome, has an excellent prognosis. Early administration of prophylactic antitoxin markedly increases the frequency of survival.

CAUSE OF DEATH

Because the clinical course of tetanus may be prolonged and the therapy used is complex and potentially dangerous, the cause of death often is not clear. Animals may die after the injection of toxin without any recognizable signs of the disease appearing.[46] Studies in parabiotic rats have suggested that tetanospasmin exerts a lethal effect on the respiratory center.[104] Involvement of the medulla has been observed in experimental animals and humans, in whom episodes of respiratory failure, often in the absence of seizures, have been described.[65,130] The action of the toxin on brain tissue has been postulated to be the cause of hyperpyrexia, tachycardia, hypotension, bulbar palsy, and cardiac arrest.[81] Myocardial damage also may occur, and both histologic and electrocardiographic abnormalities have been described.[87]

Death may occur during a convulsion, but the specific mechanisms involved are not clear in all cases. Laryngospasm and disturbances in electrolyte balance may play important roles. Pneumonia complicating aspiration, induced by an inability to swallow and oversedation, occurs commonly. It may be directly responsible for death or may contribute to a fatal outcome by increasing the degree of anoxia of the respiratory center.

PREVENTION

As noted in Figure 160–1, tetanus in the United States has decreased dramatically, and this decline can be attributed to routine universal use of tetanus toxoid and improved wound management, including the use of tetanus prophylaxis in emergency departments.

ACTIVE IMMUNIZATION

For complete information regarding tetanus immunization, the reader is referred to the most recent recommendations of the Advisory Committee on Immunization Practices of the U.S. Public Health Service,[25,28,29] the recommendations of the Committee on Infectious Diseases of the American Academy of Pediatrics,[5] and product information from vaccine manufacturers.

In the United States, primary immunization against tetanus is performed in conjunction with immunization against diphtheria and pertussis in the form of diphtheria and tetanus toxoids and acellular pertussis vaccine adsorbed (DTaP). The schedule involves an initial series of three doses of vaccine at 2, 4, and 6 months of age; a reinforcing dose at 12 to 18 months of age; and a booster dose at 4 to 6 years of age. After the initial series, additional booster doses of adult-type diphtheria and tetanus toxoids adsorbed (Td) are recommended at 10-year intervals.[37,39] In 2005, two adult-type diphtheria and tetanus toxoids and acellular pertussis vaccine adsorbed (Tdap) were approved for use in the United States.[28,29] Initially, these vaccines were approved for only a single dose. For adolescents, the recommendation was for Tdap to replace Td for the routine booster dose. For adults aged 19 to 64 years, the recommendation was that they receive a single dose of Tdap to replace Td for boosting. After additional study is performed, it is anticipated that a boosting schedule of Tdap will be similar to that recommended for Td (every 10 years). The minimal serum level of antitoxin needed for protection is 0.01 IU/mL.[38,71,73,108] After the initial three-dose series, the reinforcing dose, and the booster dose, levels of antitoxin 100- to 1000-fold higher than 0.01 IU/mL are attained, and levels higher than 0.01 IU/mL persist in nearly all vaccinees until the scheduled subsequent dose.

TETANUS PROPHYLAXIS IN WOUND MANAGEMENT

Antimicrobial prophylaxis against tetanus is neither practical nor useful in managing wounds.[15,131] Cleaning of the wound, débridement when indicated, and proper immunization are important factors. The need for tetanus toxoid (active immunization), with or without TIG (passive immunization), depends on both the condition of the wound and the patient's vaccination history (Table 160–1).[17,25,112] Rarely has tetanus occurred in persons with documentation of having received a primary series of toxoid injections.

A thorough attempt must be made to determine whether a patient has completed primary vaccination. Patients with unknown or uncertain previous vaccination histories should be considered to have had no previous tetanus toxoid doses. Persons

TABLE 160–1 Summary Guide to Tetanus Prophylaxis in Routine Wound Management, 1991

History of Adsorbed Tetanus Toxoid (Doses)	Clean, Minor Wounds		All Other Wounds*	
	Td†	TIG	Td†	TIG
Unknown or <3	Yes	No	Yes	Yes
≥3‡	No§	No	No‖	No

**Such as, but not limited to wounds contaminated with dirt, feces, soil, and saliva; puncture wounds; avulsions; and wounds resulting from missiles, crushing, burns, and frostbite.*

†For children younger than 7 years, diphtheria-tetanus-pertussis vaccine (diphtheria-tetanus if pertussis vaccine is contraindicated) is preferred to tetanus toxoid alone. For persons 7 years or older, Td is preferred to tetanus toxoid alone.

‡If only three doses of fluid toxoid have been received, a fourth dose of toxoid, preferably an adsorbed toxoid, should be given.

§Yes, if older than 10 years since the last dose.

‖Yes, if older than 5 years since the last dose (more frequent boosters are not needed and can accentuate side effects).

Td, adult-type diphtheria and tetanus toxoids; TIG, tetanus immune globulin.

From Centers for Disease Control and Prevention: Diphtheria, tetanus and pertussis: Recommendations for vaccine use and other preventive measures; recommendations of the Immunization Practices Advisory Committee (ACIP). M. M. W. R. Recomm. Rep. 40(RR-10):2-28, 1991.

who served in the military since 1941 can be considered to have received at least one dose. Although most people in the military since 1941 may have completed a primary series of tetanus toxoid, such protection cannot be assumed for each individual. Patients who have not completed a primary series may require tetanus toxoid and passive immunization at the time of wound cleaning and débridement (see Table 160–1).

The evidence available indicates that complete primary vaccination with tetanus toxoid provides long-lasting protection for 10 or more years in most recipients. Consequently, after complete primary tetanus vaccination, boosters—even for wound management—need to be given only every 10 years when wounds are minor and uncontaminated. For other wounds, a booster is appropriate if the patient has not received tetanus toxoid within the preceding 5 years.[86] Antitoxin antibodies develop rapidly in persons who have received at least two doses of tetanus toxoid.

Td has been the preferred preparation for active tetanus immunization in the wound management of patients aged 7 years or older. Because a large proportion of adults are susceptible, this plan enhances diphtheria protection. Thus, by taking advantage of acute health care visits, such as for wound management, some patients can be protected who otherwise would remain susceptible. For routine wound management in children younger than 7 years old who are not vaccinated adequately, DTaP should be used instead of single-antigen tetanus toxoid. Td may be used if pertussis vaccine is contraindicated. For patients of all ages who are inadequately vaccinated, completion of primary vaccination at the time of discharge or at follow-up visits should be ensured. Presently, Tdap rather than Td is recommended for wound management in adolescents and adults.[28,29]

If passive immunization is needed, human TIG is the product of choice. It provides protection longer than does antitoxin of animal origin and causes fewer adverse reactions.[74,100,109] The TIG prophylactic dose currently recommended for wounds of average severity is 250 units intramuscularly. When tetanus toxoid and TIG are given concurrently, separate syringes and separate sites should be used. The Advisory Committee on Immunization Practices recommends the use of only adsorbed toxoid in this situation.

NEONATAL TETANUS

Several approaches have been taken to reduce the incidence and fatality rate of neonatal tetanus in developing areas of the world. Among these approaches are (1) educating pregnant women concerning the danger of using contaminated materials for cutting the umbilical cord and covering the stump, (2) training midwives in the application of modern techniques of obstetric asepsis, (3) developing hospitals in which babies are born under strict asepsis, and (4) immunizing all women of child-bearing age or, if such immunization is not possible, all who are pregnant. Studies performed by the World Health Organization (WHO) have demonstrated that such immunization is practical and leads to an appreciable reduction in the incidence of neonatal tetanus.[42-44,110,118,129] Babies born to mothers who have been immunized during pregnancy not only have adequate levels of circulating antibody but also are protected against acquiring the disease. The level of protective antibody in newborns and the magnitude of the transfer rate of passive immunity to tetanus depend directly on the level of tetanus antitoxin in maternal serum. Mothers who had tetanus antitoxin levels of 1.28 IU/mL or greater could transfer protection to almost all the newborns (97-100%), irrespective of the doses of tetanus toxoid administered. Mothers who had received two doses of tetanus toxoid during pregnancy not only conferred good protection but also transferred high antitoxin levels to their newborns.[102]

In 1989, the WHO adopted a resolution to eliminate neonatal tetanus worldwide,[127] and in 1990, the World Summit for Children issued a declaration for global elimination of neonatal tetanus by the end of 1995.[26,128] In 1993, the WHO's goal was defined as elimination of neonatal tetanus as a public health problem by reducing its incidence to less than one case per 1000 live births for all health districts.[52]

From 1989 to 1993, the rate of vaccination coverage with two or more doses of tetanus toxoid administered to pregnant women in areas at risk increased from 27 to 45 percent. To achieve and maintain elimination of neonatal tetanus, 80 percent or more of infants need to be protected at birth through vaccination of their mothers with at least two doses of tetanus toxoid or through clean delivery and cord-care practices.[52] Considerable progress has been made in the reduction of maternal and neonatal tetanus, but the goal to reduce the rate of neonatal tetanus to less than one case per 1000 live births in all geographic areas has not been achieved.[118] In 2003, 57 countries still remained in which neonatal tetanus had not been eliminated in all districts.

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CHAPTER 161

ACTINOMYCOSIS

Suzanne Whitworth ✪ Richard F. Jacobs

Human actinomycosis is a clinical illness with typical histologic findings that is caused by a variety of pathogens. It often is polymicrobial, and *Actinobacillus actinomycetemcomitans* frequently is a co-pathogen. It is endogenous worldwide, with sporadic cases reported annually. Occurrence of the disease is unrelated to age, sex, season, or occupation, although it is an uncommon event in the pediatric population. These organisms have the ability to spread locally without regard to fascial planes or other anatomic barriers. Actinomycosis is characterized by localized swelling with suppuration, abscess formation, and draining sinuses. The abscesses have fibrous walls and are filled with pus and characteristic sulfur granules. The three most common types of actinomycosis are oral and cervicofacial, abdominal, and pulmonary. However, involvement of the liver, female reproductive tract, and brain has been described. Definitive diagnosis of the infection rests on isolation of the organism from pus or identification of sulfur granules on histopathologic sections of biopsy material. Adequate treatment generally consists of surgical removal of the lesion, prolonged antibiotic therapy, or both.

MICROBIOLOGY

The clinical entity of actinomycosis is caused by species of the genera *Actinomyces* and *Propionibacterium*. Human actinomycosis most often is caused by *Actinomyces israelii*, although *Actinomyces*

gerensceriae (formerly known as *A. israelii* serovar 2), *Actinomyces naeslundii*, *Actinomyces viscosus*, *Actinomyces odontolyticus*, *Actinomyces meyeri*, *Actinomyces radicidentis*, *Actinomyces neuii*, *Actinomyces radingae*, and *Actinomyces turicensis* also are causes of human illness.[46] *Arcanobacterium pyogenes* (previously in the *Actinomyces* genus) also causes human actinomycosis. *Propionibacterium propionica* is the only species in the *Propionibacterium* genus that is a cause of human actinomycosis. Some authors also include *Bifidobacterium dentium* as an etiologic agent of actinomycosis.[40]

These bacteria are irregular, non–spore-forming, non–acid-fast, nonmotile, gram-positive rods. They grow in most rich culture media and have various oxygen requirements. For example, *A. israelii* requires anaerobic conditions for growth. *A. viscosus*, however, grows in an aerobic environment with carbon dioxide. These species ferment carbohydrates as their source of energy for growth.[16] These organisms now are firmly classified as prokaryotic bacteria, although they originally were thought to be fungi because of the mycelial appearance of the organisms in sulfur granules and because of their branching morphology. However, neither genus contains chitin or glucans, which are characteristic macromolecules of fungi. In addition, they reproduce by bacillary fusion, are sensitive to antibiotics, and are resistant to antifungal agents. These organisms are members of the endogenous flora of mucous membranes. *A. israelii* always is found in the oral cavity when the appropriate anaerobic culture technique is used. It also has been found in the gastrointestinal tract, bronchi, and female genital tract.[45]

A. actinomycetemcomitans is a fastidious, non–spore-forming, nonmotile, facultatively anaerobic gram-negative rod that frequently complicates actinomycosis caused by *A. israelii*. In addition to being associated with actinomycosis, it has been implicated as a pathogen in periodontal disease and is part of the oral flora. Infection by this bacterium often is not resolved by the normal host, possibly because of inefficient phagocytosis and the weak oxidative burst response of neutrophils.[37] In addition, the bacterium has several virulence factors, including induction of apoptosis and tissue destruction.[18] *A. actinomycetemcomitans* is characterized by slow growth in culture and a requirement for incubation in an atmosphere enhanced with carbon dioxide.[29] Other bacterial species concomitantly isolated in human actinomycosis are *Eikenella corrodens*, *Fusobacterium*, *Bacteroides*, *Capnocytophaga*, *Staphylococcus*, *Streptococcus*, and members of the family Enterobacteriaceae.

PATHOGENESIS

The organisms that cause actinomycosis normally are found in the oral flora from infancy to adulthood. Actinomycetes are primary colonizers that can initiate the formation of plaque and set the stage for the development of infectious disease, as well as predispose to the formation of caries.[3,8] These bacteria adhere tenaciously to both the hard and soft tissue surfaces of the oral cavity.[61] Disruption of this mucous membrane probably is the initiating event for oral and cervicofacial disease. The organisms then invade locally and spread without regard to fascial planes. The exact mechanism of this spread is unknown but may be related to the ability of these organisms to suppress part of the host immune system. Organisms of the *Actinomyces* genus have been shown to be chemotactic, to activate lymphocyte blastogenesis, and to stimulate the release of lysosomal enzymes from polymorphonuclear leukocytes and macrophages.[16] In addition, the co-pathogens involved in this infection may reduce local oxygen tension. Dental extractions are associated with mucosal breaks and tissue necrosis and may predispose to oral or cervicofacial actinomycosis.[39] Hematogenous dissemination can occur eventually but is an uncommon development. Gastrointestinal disease probably is associated with disruption of the mucosal barrier, similar to oral and cervicofacial disease.[45] Organisms causing pulmonary actinomycosis probably reach the lungs through aspiration. Thoracic actinomycosis usually is a complication of localized pulmonary parenchymal infection. Actinomycetes produce enzymes and thus spread by extension into the lungs, pleura, and chest wall without regard to tissue planes.[53] Numerous reports in the literature associate actinomycosis with intrauterine devices (IUDs), and some question exists regarding the association of foreign bodies with actinomycosis.[5,13]

PATHOLOGY

Actinomycosis most commonly is manifested as a chronic infection with single or multiple indurated swellings. These lesions eventually soften, become fluctuant, and suppurate. The walls are fibrous and firm and often described as wooden, which frequently results in their confusion with neoplasms. Over the course of time, sinus tracts form and extend through the overlying skin or to adjacent bones or tissues. The overlying skin may have a bluish hue.[45]

Histologically, a typical lesion has a central purulent area containing neutrophils and sulfur granules, surrounded by an outer zone of granulation with collagen fibers and fibroblasts. The sulfur granules are firm, yellowish granules containing the organisms and are virtually diagnostic of actinomycosis, although they can be seen in nocardiosis and botryomycosis. In addition to neutrophils, lymphocytes and plasma cells frequently are seen in the lesions; eosinophils and multinucleated giant cells occasionally are seen.[45]

CLINICAL MANIFESTATIONS

There are three important sites of actinomycotic infection. The order of frequency of occurrence is oral/cervicofacial, abdominal/pelvic, and pulmonary. Actinomycosis resembles several other chronic inflammatory diseases and must be differentiated from mycotic infections, tuberculosis, appendicitis, *Yersinia enterocolitica* pseudoappendicitis, osteomyelitis, amebiasis, hepatic abscess, and other chronic bacterial infections, including nocardiosis.

Because oral and cervicofacial actinomycosis occurs after disruption of the mucous membranes in the mouth or oropharynx, patients who have this type of actinomycosis may have a history of oral surgery, dental procedures, or trauma to the mouth. Clinical findings may include pain, trismus, firm swelling, and fistulas with drainage that contains the characteristic sulfur granules (Figs. 161–1 and 161–2). Patients most commonly have a chronic disease course but may be seen acutely with cellulitis. Infection may spread through the sinus tracts to the cranial bones, which gives rise to meningitis. Bone is not involved early in the disease, but periostitis may develop later. The marked ability of the organisms in this disease to burrow through tissue planes and even bone differentiates actinomycosis from nocardiosis and is an important characteristic of this infection. The cervicofacial type of actinomycosis, or "lumpy jaw," has the best prognosis. With surgical débridement and excision as an adjunct to proper antibiotic therapy, the disease usually is cured. At least two cases of thyroiditis secondary to actinomycosis have been reported in pediatric patients.[19,60]

Because abdominal actinomycosis also is the result of disruption of the mucosa of the gastrointestinal tract, patients may have a history of gastrointestinal surgery, diverticulitis, or appendicitis. The patient also may have a history of trauma to the abdomen. Patients may have chills, fever, night sweats, and weight loss. The course is indolent and similar to that of tuberculous peritonitis. Because appendicitis is the most common predisposing event, on

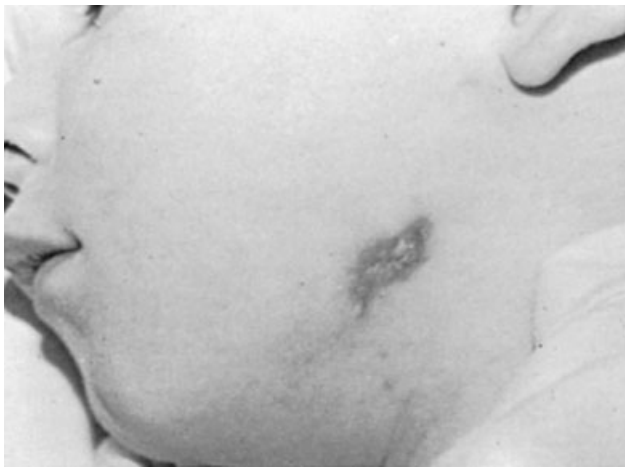

Figure 161–1 Cervicofacial disease with draining sinus tracts caused by *Actinomyces israelii*.

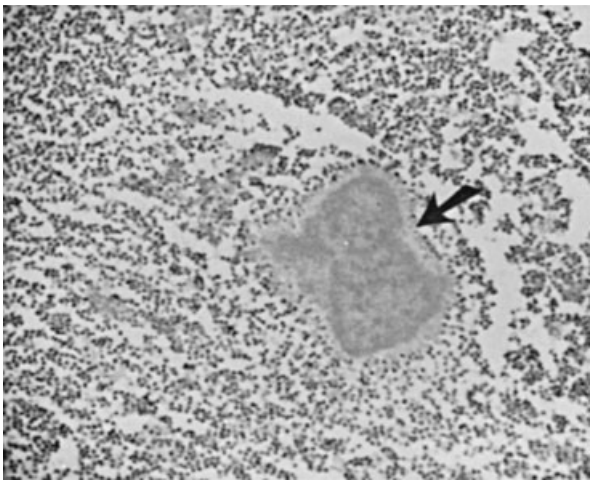

Figure 161–2 Abscess containing an actinomycotic granule surrounded by purulent exudate. The peripheral clubbing (*arrow*) is stained by eosin. (Hematoxylin and eosin; original magnification ×52.)

physical examination patients may have a hard, irregular mass in the ileocecal area that softens and then drains to the outside. Extension from such foci usually is by direct continuity (or, rarely, hematogenously) and involves any tissue or organ, including muscle, liver, spleen, kidney, fallopian tubes, ovaries, uterus, testes, bladder, esophagus, or rectum.[1,2,9,21,26,42] A delayed diagnosis of actinomycosis involving the abdomen or pelvis is typical, and it is frequently confused with malignancy.[22]

Hepatic involvement occurs in approximately 15 percent of cases of abdominal actinomycosis. Involvement of the liver can occur through direct extension from a subdiaphragmatic or subhepatic abscess. It also is a common finding in disseminated actinomycosis.[45] Occult disruption of the gastrointestinal mucosa with spread of organisms through the portal vein may provide a portal of entry for the organisms in cases of primary or isolated hepatic disease. Initial symptoms may include fever, abdominal pain, anorexia, weight loss, nausea, vomiting, shoulder pain, back pain, or diarrhea. The course usually is indolent, with 1 to 6 months of symptoms. On physical examination, the patient commonly has fever, abdominal tenderness, and hepatomegaly. A palpable abdominal mass, jaundice, or draining sinuses may be present. Disseminated intravascular coagulation has been reported to occur.[56] Hepatic actinomycosis has been found in children who have undergone appendectomies.[30] Thrombotic thrombocytopenic purpura has been described in association with hepatic actinomycosis in an adult.[42] Other causes of liver masses included in the differential diagnosis are pyogenic abscess, amebiasis, and malignancy.

Women wearing IUDs are at risk for the development of pelvic actinomycosis. These patients may have a vaginal discharge, pelvic pain, abdominal pain, menorrhagia, fever, a pelvic mass, a history of pelvic inflammatory disease, or a history of prolonged IUD use. The risk is more significant if the IUD has been in place for longer than 2 to 3 years. These devices are thought to cause an inflammatory response in the endometrium with focal necrosis. This anaerobic environment encourages the growth of *A. israelii*. In such patients, removal of the IUD plus treatment with antibiotics is necessary.[13] Pelvic actinomycosis can develop in both men and women; it can appear as a tumor-like mass and be confused with malignancy. A urachus or urachal remnant is also a risk factor.[28]

Establishing the diagnosis of pulmonary actinomycosis depends on having a high index of suspicion because neither the clinical nor the radiographic findings are specific. Patients may have a history of or risk factors for aspiration. Adults with thoracic actinomycosis usually have abnormal local defenses, such as chronic bronchitis, bronchiectasis, or emphysema; however, pediatric patients have been shown to have predisposing factors less often.[23,53] This type of actinomycosis also occurs after the introduction of a colonized foreign body. Patients with oral, cervicofacial, or abdominal actinomycotic infections are at risk for the development of pulmonary infection from direct or hematogenous spread. A history of these preexisting infections should heighten the index of suspicion. Pulmonary actinomycosis may be manifested as an endobronchial infection, a tumor-like lesion, diffuse pneumonia, or a pleural effusion.[7,10,38] The presence of chronic pleural effusion, underlying lung changes, and periosteal rib involvement is indicative of actinomycosis but seldom is reported.[58] The principal symptoms include chest pain, fever, productive cough, and weight loss. Hemoptysis has been reported.[41] The infection frequently dissects along tissue planes and may extend through the chest wall or diaphragm and produce multiple sinuses. These characteristic sinus tracts contain small abscesses and purulent drainage. Empyema necessitans has been reported in one child.[54] The differential diagnosis of pulmonary actinomycosis includes tuberculosis, lung abscess, nocardiosis, fungal infection, and botryomycosis.

Laryngeal actinomycosis rarely has been reported in older teenagers.[32] Colonization of the oropharynx with these organisms may be involved in the development of obstructive tonsillar hypertrophy.[39] Several adult cases of pericardial actinomycosis are reported in the literature.[14] Bacteremia is reported in all age groups, including neonates.[6,20,27] Septic arthritis has been reported.[24] Brain abscess has occurred in a child with complex congenital heart disease.[34] Actinomycetes have been isolated from nearly every organ in the body, including the kidneys, brain, heart, breasts, mastoids, male genitourinary tract, and eyes. Osteomyelitis is reported in children, often involving the mandible, but other bones have been infected as well.[43] *A. pyogenes* has been implicated only rarely as a cause of human infection, but cases of septicemia, endocarditis, meningitis, arthritis, empyema, pneumonia, otitis media, cystitis, mastoiditis, appendicitis, and cutaneous infection have been reported.[12,15]

Actinomycosis does not seem to be associated commonly with human immunodeficiency virus infection, perhaps because these patients frequently are in the health care system and have close monitoring. They may be treated for other infections with intermittent antibiotic therapy that also treats subclinical actinomycosis. Thoracic, esophageal, oral, and anal cases have been reported, however, and all immunocompromised hosts

should be monitored closely for this potential group of pathogens.[1,2,35,55]

*A. actinomycetemcomitan*s is a pathogen in at least 30 percent of actinomycotic infections[29] (see Chapter 137). Failure to recognize this organism and treat it adequately has resulted in clinical relapse and deterioration in patients infected with actinomycosis.[31,61] Severe forms of periodontitis, particularly localized aggressive periodontitis, also are associated with this pathogen, and studies have shown that it is related strongly to children in the 10- to 19-year-old age group.[48] It is characterized by loss of attachment of bone around the permanent incisors and permanent molars, which occurs rapidly. Treatment consists of local débridement and antibiotic therapy, usually metronidazole alone or in combination with amoxicillin. *A. actinomycetemcomitan*s also is an important pathogen in Papillon-Lefèvre syndrome, an autosomal recessive disorder characterized by prepubertal periodontitis and palmar-plantar hyperkeratosis.[44] Additionally, it is one of the HACEK (HACEK includes *Haemophilus aphrophilus*, *Cardiobacterium hominis*, *E. corrodens*, and *Kingella kingae*) organisms that have a propensity for infecting heart valves. The endocarditis caused by this organism usually is insidious, with fever occurring in fewer than 50 percent of cases.[29] This organism also has been reported to cause pericarditis, meningitis, brain abscess, parotitis, synovitis, osteomyelitis, urinary tract infection, pneumonia, and empyema.[29] Cases of endophthalmitis[4,57] and cavernous sinus infection also have been reported.[59]

DIAGNOSIS

To establish a definitive diagnosis of actinomycosis, the clinician must isolate the causative organism from tissue or pus from a normally sterile body site, such as the lungs. Isolation of the organism from the oral cavity or the female genital tract without clinical evidence of disease, therefore, is not diagnostic. Because the organisms that cause actinomycosis are exquisitely sensitive to antibiotics, clinical specimens must be obtained before initiating their use. The specimens should be processed carefully to maintain anaerobic conditions. They should undergo routine Gram stain, which will reveal gram-positive rods that are not acid-fast and appear in diphtheroidal arrangements with or without branching.[16] Gram stain is more sensitive than is culture, particularly if the patient has been given antibiotics. Immunofluorescence is available for confirmation of organisms in biopsy specimens with suggestive Gram stains. Growth on media usually appears within 5 to 7 days but may take 2 to 4 weeks.[45] 16S rDNA sequencing and 16S rDNA–based nested polymerase chain reaction have been used in some research laboratories for precise species identification.[42,49,52]

True microbiologic identification of these organisms occurs uncommonly, and the diagnosis most often rests on the clinical picture, along with identification of the characteristic sulfur granules. The sulfur granules may be found by drawing pus from a lesion, on the bandage covering the lesion, or in surgical specimens (see Fig. 161–2). Pus that is poured down the side of a glass will leave sulfur granules adhering to the sides so that the granules can be identified more easily. On hematoxylin and eosin stain, the granules are eosinophilic or variably surrounded by a radiating fringe of eosinophilic clubs. The formation of granules is a hallmark of actinomycosis and is related to a bacterial secretion that cements elements of *Actinomyces* spp. together. However, the formation of granules related to various nonfilamentous bacteria such as *Staphylococcus aureus* or *Pseudomonas* spp. is called *botryomycosis* or *bacterial pseudomycosis*, and they can look grossly similar to the typical sulfur granules of actinomycosis.[10] Washed, crushed actinomycotic granules or well-mixed pus in the absence of granules is cultured on a rich medium, such as brain-heart infusion blood agar, and incubated anaerobically and aerobically with added carbon dioxide. Plates can be examined at 24 hours and after 5 to 7 days for the characteristic colonies of *Actinomyces* spp.[16] Currently, no skin tests are available for screening purposes, and no useful serologic tests are available for actinomycosis.

Various imaging modalities have been useful in diagnosing and characterizing actinomycosis. Computed tomography has been shown to help differentiate between inflammatory masses and tumors. Additionally, the location, extension, and relationship between the mass and surrounding structures can be defined better. Ultrasonography has been shown in one report to reveal a mass with an ill-defined margin that was hypoechoic with intrinsic hyperechoic spots.[47] Magnetic resonance imaging has been used for diagnosing actinomycotic brain abscess, as well as actinomycosis of the chest, spine, soft tissues, and mandible.[23,34,43]

A. actinomycetemcomitans can be cultured on blood and chocolate agar but grows poorly on MacConkey agar. Cultures require incubation in an enhanced carbon dioxide atmosphere. Growth of the organism in a blood culture may take as long as 9 days in patients with endocarditis, and thus cultures should be held longer. On Gram stain, the organism appears coccoid to coccobacillary. Molecular techniques based on nonamplification nucleic acid probes or on polymerase chain reaction can provide rapid and accurate identification of *A. actinomycetemcomitans*.[25] Because of the frequency of co-infection with this organism in cases of actinomycosis, attempts always should be made to isolate this organism in these patients.

TREATMENT

The mainstays of therapy for actinomycosis remain surgical débridement or removal of the lesion and prolonged antimicrobial therapy. Most experts still recommend drainage of abscesses, fistulotomy, sinus tract excision, and debulking of large masses, although numerous successful outcomes with antimicrobial therapy alone are reported in the literature.[30,45] The option to treat medically and observe for clinical response seems reasonable in a stable, noncritical patient. The antibiotic of choice is penicillin. The recommended total duration of therapy ranges from 6 to 12 months. For patients who are allergic to penicillin, tetracycline or erythromycin is acceptable. Other alternatives are clindamycin, chloramphenicol, and the third-generation cephalosporins. Susceptibility breakpoints for linezolid, imipenem, meropenem, or piperacillin/tazobactam have not been established, but the minimal inhibitory concentrations appear favorable.[17,52] Most isolates are clearly resistant to ciprofloxacin and metronidazole.[17,52] Administration of daily ceftriaxone for 3 weeks followed by prolonged administration of ampicillin was effective treatment in one case.[53] Thoracic actinomycosis has been treated successfully with a total duration of therapy of only 4 months.[50] Failure to respond to adequate treatment has been noted in patients with underlying malignancy. Because the disease is an uncommon occurrence in children and no significant randomized prospective treatment trials have been performed, most authors still recommend 4 to 6 weeks of intravenous therapy followed by oral therapy for a prolonged period. The total duration of therapy is based on clinical and radiographic follow-up. Numerous cases of actinomycosis in various forms that have been treated successfully with a total duration of 3 to 6 months of therapy are reported in the literature. Osteomyelitis may require longer therapy.[43] Certainly in cases for which surgical débridement or incision and drainage have been performed, a shorter duration of therapy would be reasonable provided that close clinical follow-up can be ensured.

A. actinomycetemcomitans is susceptible to the newer cephalosporins, rifampin, trimethoprim-sulfamethoxazole, aminoglyco-

sides, quinolones, tetracycline, azithromycin, and chloramphenicol. It also is susceptible to penicillin and ampicillin in vitro, but test results do not necessarily correlate with clinical outcome. Vancomycin, erythromycin, and clindamycin have very little activity against this organism. Treatment of aggressive periodontal disease consists of local débridement and antibiotic therapy. Metronidazole and amoxicillin appear to be effective in suppressing *A. actinomycetemcomitans* to below the level of detection.[11] Endocarditis caused by this organism has been treated successfully with a combination of ampicillin and gentamicin.[36] Cefotaxime or ceftriaxone also is acceptable.

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CHAPTER 162

Other Anaerobic Infections

Steven C. Buckingham

In 1680, Antoni van Leeuwenhoek demonstrated the presence of microorganisms in an environment deprived of oxygen.[59] In hindsight, this observation seems tremendously important, but its significance must have eluded both van Leeuwenhoek and his contemporaries. It was not until 1857 that Louis Pasteur, while studying causes of beer and wine spoilage, proved that bacteria are responsible for the fermentation of sugars and that "fermentation is the consequence of life without air." Pasteur's observations brought down the theory of spontaneous generation and inaugurated the discipline of microbiology.[119]

Anaerobic bacteria constitute a significant portion of the normal flora of humans, beginning in the neonatal period. Moreover, anaerobic bacteria cause infections of many different body sites in children, although their importance as pediatric pathogens historically has been underappreciated. Many anaerobic infections in children never are diagnosed as such because clinicians fail to use appropriate methods for collection or transportation of specimens or because laboratories fail to use appropriate methods for isolation and identification of anaerobes.[38]

BACTERIOLOGY AND TAXONOMY

All bacteria can be divided into groups based on their tolerance of or requirement for free oxygen.[113]

- *Obligate aerobes* require molecular oxygen as a terminal electron acceptor and do not obtain energy through fermentation pathways.
- *Obligate anaerobes* obtain energy via fermentation and use organic compounds as terminal electron acceptors; free oxygen is toxic to these bacteria. These anaerobes may be further subdivided:
  - *Strict obligate anaerobes* cannot survive in an atmosphere containing more than 0.5 percent oxygen.
  - *Moderate obligate anaerobes* can grow in the presence of 2 to 8 percent oxygen (and constitute the majority of clinically important obligate anaerobes).
- *Facultative anaerobes* (e.g., *Staphylococcus aureus*) can grow in either the presence or absence of oxygen; they simply resort to fermentation when oxygen is not available.
- *Microaerophiles* (e.g., *Campylobacter jejuni*) require oxygen as a terminal electron acceptor but can grow only in an atmosphere containing less than 21 percent oxygen.

Why oxygen is toxic to anaerobic bacteria is not entirely clear, but in some species this toxicity is explained, at least partly, by the absence of catalase, superoxide dismutase, or peroxidase.[113]

The scope of this chapter is confined to a discussion of clinically important, obligately anaerobic bacteria that are not discussed principally in Chapters 158 to 161. Some of the most clinically important obligate anaerobes are listed in Table 162–1 and discussed in the following text.

SPORE-FORMING, GRAM-POSITIVE BACILLI

Anaerobic spore-forming, gram-positive bacilli are members of the genus *Clostridium*. These organisms and their invasive and toxin-mediated diseases are discussed in Chapters 158, 159, and 160.

NON–SPORE-FORMING, GRAM-POSITIVE BACILLI

Actinomyces spp. normally colonize the human gastrointestinal tract. Many new species have been described in recent years, and several former *Actinomyces* spp. have been reclassified in other genera.[90] These organisms and their associated diseases are discussed in Chapter 161.

Propionibacterium spp. are part of the normal flora of human skin and mucosal surfaces.[90] *Propionibacterium acnes* is the most clinically important member of the genus; others include *Propionibacterium avidum*, *Propionibacterium granulosum*, and *Propionibacterium propionicum* (formerly *Arachnia propionica*[112]). A former member of the genus, *Propionibacterium innocuum*, has been reclassified as *Propioniferax innocua*.[121] *P. acnes* is the dominant constituent of normal flora in pilosebaceous follicles[95]; is thought to play a role in the pathogenesis of acne vulgaris; and may contribute to pathogenesis of the synovitis, acne, pustulosis, hyperostosis and osteitis (SAPHO) syndrome and, more speculatively,

TABLE 162–1 Anaerobic Bacteria of Clinical Importance

Gram-Positive	
Spore-forming bacilli	*Clostridium* species
Non–spore-forming bacilli	*Actinomyces* species
	Propionibacterium species
	Eubacterium-like bacteria
	Bifidobacterium species
	Lactobacillus species
	Mobiluncus species
Cocci	*Peptococcus niger*
	Peptostreptococcus anaerobius
	Peptoniphilus species
	Streptococcus species
Gram-Negative	
Bacilli	*Bacteroides* species
	Prevotella species
	Porphyromonas species
	Fusobacterium species
	Bilophila wadsworthia
	Campylobacter species
	Sutterella species
Cocci	*Veillonella* species

the pathogenesis of sarcoidosis.[95] *P. granulosum* colonizes oily skin areas, albeit in lower concentrations than *P. acnes* does, whereas *P. avidum* is found only on moist surfaces such as the rectum, axillae, and nares.[87] *P. acnes*, *P. avidum*, and *P. granulosum* can cause infections in patients compromised by recent surgery, trauma, or implanted devices (e.g., prosthetic heart valves and cerebrospinal fluid shunts) but are isolated more commonly in the clinical laboratory as culture contaminants.[38,107] *P. propionicum* has been associated with apical periodontitis[54] and with lacrimal duct infections.[14] *Propionibacterium* spp. uniformly are resistant to metronidazole but susceptible to most other classes of antibiotics.[91,104]

Eubacterium is a genus that previously included numerous gram-positive, obligately anaerobic bacteria of questionable taxonomic position. Many of these species were reassigned recently to newly formed genera, including *Cryptobacterium*, *Mogibacterium*, *Bulleida*, *Collinsella*, *Atopobium*, *Slackia*, *Eggerthella*, *Holdemania*, *Catenibacterium*, and *Actinobaculum*; collectively, they may be termed *Eubacterium*-like genera.[90] *Eubacterium lentum*, the most frequently recovered *Eubacterium*-like organism, has been renamed *Eggerthella lenta*.[77] The *Eubacterium*-like bacteria are normal inhabitants of the mouth and intestines of humans and animals and are associated with periodontal disease in adults.[90] *Eubacterium*-like organisms have been recovered from children with abscesses or peritonitis[35]; rarely, they cause bacteremia in neonates[24] and meningitis in children.[40] In one study, only 1 of 10 *Eubacterium*-like bacterial isolates was susceptible to penicillin, and only 3 of 10 were susceptible to ceftriaxone, but all 10 isolates were susceptible to amoxicillin-clavulanate, clindamycin, meropenem, and metronidazole.[104]

Bifidobacterium spp. are predominantly commensal bacteria residing in the intestinal tracts of humans and animals. These organisms rarely are pathogenic; in fact, *Bifidobacterium longum*, *Bifidobacterium breve*, *Bifidobacterium infantis*, and *Bifidobacterium lactis* Bb12 have been included in putatively health-promoting probiotic mixes, some of which show efficacy in the prevention and treatment of infectious diarrhea and inflammatory bowel disease.[12] The pediatric infections most frequently associated with *Bifidobacterium* spp. are chronic otitis media, abscesses, and peritonitis.[35] *Bifidobacterium* spp. are susceptible to penicillin, cefoxitin, and vancomycin; isolates with resistance to tetracycline, erythromycin, clindamycin, or metronidazole have been reported.[57,86]

Lactobacillus spp. are slender, parallel-sided, gram-positive rods that do not produce catalase. As of this writing, more than 150 named species and subspecies are recognized.[60] Most clinical isolates are microaerophilic, although some are obligately anaerobic. Species-level identification of *Lactobacillus* spp. is quite difficult, and commercial identification methods can give inaccurate results.[90] These organisms normally are found in the mouth, intestinal tract, and vagina of mammals, and they can be recovered from numerous food products.[90] Lactobacilli generally possess low pathogenic potential, and, in fact, some species show promise as health-promoting probiotics. In compromised hosts, however, these organisms can, on rare occasion, cause serious disease, as illustrated by cases of probiotic-associated *Lactobacillus* bacteremia and endocarditis in adults and children.[12] In children, *Lactobacillus* spp. usually are associated with abscesses, aspiration pneumonia, and bacteremia.[35] Essentially all lactobacilli are susceptible to penicillin, chloramphenicol, and imipenem and are resistant to metronidazole; susceptibility to vancomycin, erythromycin, tetracycline, and cefoxitin varies among species.[57]

Mobiluncus spp. are found in the reproductive tract and rectum of primates and are recovered primarily from women with bacterial vaginosis.[90] *Mobiluncus* spp. have not been associated with disease in prepubertal children.

GRAM-POSITIVE COCCI

The taxonomy of the anaerobic gram-positive cocci has been revised extensively in recent years. Many species assigned to the formerly populous, if loosely defined genera *Peptococcus* and *Peptostreptococcus* have been reassigned to extant or newly formed genera, including *Anaerococcus*, *Finegoldia*, *Gallicola*, *Micromonas*, *Peptoniphilus*, and *Slackia*.[90] As of this writing, the only remaining members of the original genera are the type species *Peptococcus niger* and *Peptostreptococcus anaerobius*.[60,92] The genus *Streptococcus* includes numerous microaerophilic species, but only *Streptococcus pleomorphus* is obligately anaerobic (now that two former *Streptococcus* spp. have been reclassified as *Ruminococcus hansenii* and *Atobopium parvulum*).[60,90]

Anaerobic gram-positive cocci are normal inhabitants of the human mouth and upper respiratory tract, gastrointestinal tract, urogenital tract, and skin. They are associated with a variety of infections, including pelvic inflammatory disease, tubo-ovarian abscesses, chronic sinusitis, chronic otitis media, aspiration pneumonia, and peritonitis.[29,90] The obligately anaerobic gram-positive cocci most commonly recovered from clinical specimens are *Finegoldia magna*, *P. anaerobius*, and *Peptoniphilus asaccharolytica*.[90] Microaerophilic streptococci, though not strict anaerobes, are clinically important causes of chronic sinusitis and brain abscesses.[27]

Anaerobic gram-positive cocci usually are susceptible to penicillin, amoxicillin-clavulanate, clindamycin, metronidazole, and carbapenems.[1,78] As is true of their aerobic counterparts, some macrolide-resistant anaerobic gram-positive cocci express inducible resistance to clindamycin.[102]

GRAM-NEGATIVE BACILLI

Bacteroides spp. are the anaerobic bacteria most frequently recovered from clinical specimens. Traditionally, the most clinically important of these bacteria have been collectively classified as the *Bacteroides fragilis* group, which consists of *Bacteroides fragilis*, *Bacteroides distasonis*, *Bacteroides ovatus*, *Bacteroides thetaiotaomicron*, *Bacteroides vulgatus*, and others. Taxonomic revisions based on nucleic acid analysis, however, have thrown matters into confusion. For example, *B. distasonis*, *Bacteroides goldsteinii*, and *Bacteroides merdae* have been reclassified under the genus *Parabacteroides*, and *Bacteroides forsythus* has been reclassified as *Tannerella forsythensis*.[106] In any event, *Bacteroides* spp. and closely related taxa constitute much of the normal human colonic flora and also form part of the normal flora of the female genital tract. These organisms frequently are found in intra-abdominal infections and especially abscesses and occasionally in extra-abdominal infections such as aspiration pneumonia, chronic otitis media, and brain abscess.[65,76] Most *Bacteroides* isolates produce β-lactamases that confer resistance to penicillin and third-generation cephalosporins. Resistance to metronidazole has been reported but is an extremely rare event (Table 162–2).[76,110]

Bacteroides ureolyticus, *Campylobacter gracilis*, *Campylobacter curvus*, *Campylobacter rectus*, and *Sutterella wadsworthensis* are closely related bacteria of the family Campylobacteraceae[76,118] and are normal inhabitants of the oral cavity and intestinal tract. The anaerobic *Campylobacter* spp. are associated with oral and periodontal infections. *Sutterella* spp. are associated predominantly with intra-abdominal infections (perhaps because of their bile resistance), and *B. ureolyticus* has been associated with infections at several body sites.[76] Of the anaerobic gram-negative bacilli, *Suterella* spp. are the ones most frequently resistant to metronidazole (Table 162–2).

Prevotella spp. are pigmented (e.g., *Prevotella corporis*, *Prevotella denticola*, *Prevotella intermedia*, *Prevotella loeschii*, *Prevotella mela-*

TABLE 162–2 Percentages of Clinically Important Anaerobes Susceptible to Anti-anaerobic Drugs in Recent Reports*

Organism	Penicillin G	Amoxicillin-Clavulanate	Cefoxitin	Ceftriaxone	Clindamycin	Meropenem	Metronidazole
Bacteroides fragilis group	<20	85-95	60-93	<20	48-84	95-99	99-100
Prevotella species	<20	98-100	98-100	71-95	82-100	100	97-100
Porphyromonas species	79-95	100	95-100	95-100	90-100	100	100
Fusobacterium species	70-100	96-100	100	85-95	70-95	94-100	100
Campylobacter species		100	100	95-100	96	100	100
Sutterella species		100	100	95-100	36-69	100	70-84
Bilophila species	<50	100	64-95		85-95	100	100
Veillonella species	22-90	100	100	95-100	100	100	100
Gram-positive cocci	88-96	88-96	100	95-100	89-93	100	91-100
Clostridium species†	71-83	96-99	88-93	78	63-80	98-100	98-100
Non–spore-forming, gram-positive bacilli	55-81	100	93-100	65	82-100	100	28-50

*See references 1, 76, 78, 104, 117, 120.
†Excluding Clostridium difficile.

ninogenica, and *Prevotella nigrescens*) or nonpigmented (e.g., *Prevotella bivia*, *Prevotella disiens*, *Prevotella oris*, *Prevotella buccae*, and *Prevotella heparinolytica*) saccharolytic bacteria that once were classified under the genus *Bacteroides*. For the most part, these organisms are normal flora of the human oral cavity, and some are normally found in the urogenital and intestinal tracts. *P. bivia* and *P. disiens* are associated particularly with genital tract infections in women.[76] In children, the *Prevotella* spp. most often recovered from clinical specimens are *P. melaninogenica*, *P. intermedia*, *Prevotella oralis*, *P. oris*, and *P. buccae*, and the infections most frequently associated with these organisms are abscesses (especially peritonsillar, retropharyngeal, pilonidal, and dental), aspiration pneumonia, chronic otitis media, and peritonitis.[31] *Prevotella* spp. generally are resistant to penicillin but otherwise are more susceptible to antibiotics than *Bacteroides* spp. are (see Table 162–2).

Porphyromonas spp. are pigmented or nonpigmented gram-negative bacteria that like *Prevotella* spp. were formerly classified as *Bacteroides* spp. Unlike *Prevotella* spp., *Porphyromonas* spp. are asaccharolytic or only weekly saccharolytic. *Porphyromonas* spp. are less likely than *Prevotella* spp. to produce β-lactamase.[76] With regard to their environmental niche and spectrum of disease in children, however, *Porphyromonas* spp. are quite similar to *Prevotella* spp.[31] *Porphyromonas gingivalis* and *Porphyromonas endodontalis* are particularly associated with odontogenic infections.[65]

Fusobacterium spp. are nonmotile anaerobic gram-negative rods that produce butyric acid. The type species, *Fusobacterium nucleatum*, is the one recovered most frequently from clinical specimens.[11] This organism is part of the normal flora of the mouth and upper respiratory, genital, and gastrointestinal tracts and is associated with a range of pediatric infections similar to those of *Prevotella* and *Porphyromonas* spp.[26,76] The virulent pathogen *Fusobacterium necrophorum* is isolated frequently from peritonsillar abscesses and may cause Lemierre disease (postanginal sepsis; see later). *F. nucleatum* and *F. necrophorum* usually are susceptible to penicillin and clindamycin; resistance is more prevalent among the *Fusobacterium mortiferum/Fusobacterium varium* group.[1,76,117] Fusobacteria inherently are resistant to erythromycin, but azithromycin is active against most strains in vitro.[69,76]

Bilophila wadsworthia is the lone species of its genus. This fastidious, bile-resistant resident of the normal bowel flora often is present in intra-abdominal abscesses, including those associated with ruptured appendices.[76] More than 50 percent of isolates produce β-lactamase, but resistance to anti-anaerobic agents is a rare finding.[76,89]

Selenomonas spp. and *Leptotrichia* spp. are normal inhabitants of the oral cavity and are associated with periodontal disease in children and adults. *Leptotrichia* spp. have been isolated in blood cultures from neonates[72] and from adolescent cancer patients with chemotherapy-induced neutropenia.[100] *Leptotrichia* spp. are resistant to aminoglycosides and erythromycin.[66]

The motile bacteria of the genera *Butyrivibrio*, *Succinivibrio*, and *Desulfovibrio* are normal inhabitants of the human intestinal tract that on occasion are associated with intra-abdominal infections in compromised adults. *Desulfovibrio* spp. were isolated from an appendiceal abscess in a 3-year-old girl.[82] *Anaerobiospirillum* spp., which are normal intestinal flora of dogs and cats, rarely cause bacteremia and, possibly, diarrheal illnesses in compromised adults.[88] *Anaerobiospirillum* spp. have been isolated from the feces of several children with gastroenteritis[84,85] and from the blood of a previously healthy 11-month-old girl.[105]

GRAM-NEGATIVE COCCI

The three genera of anaerobic gram-negative cocci, *Acidaminococcus*, *Megasphaera*, and *Veillonella*, are normal fecal flora that rarely cause disease in humans. *Veillonella* spp. also are part of the normal oral flora. In children, *Veillonella* spp. occasionally have been associated with abscesses, aspiration pneumonia, and chronic sinusitis.[36]

With the exception of a single described isolate of β-lactamase–producing *Acidaminococcus fermentans*, strains of *Acidaminococcus* and *Megasphaera* are susceptible to penicillin and to cephalosporins.[67] Although *Veillonella* spp. do not produce β-lactamase, they commonly are resistant to penicillin, perhaps because of alterations in penicillin-binding proteins.[94] *Veillonella* spp. nonetheless remain susceptible to ampicillin, amoxicillin-clavulanate, cefoxitin, and third-generation cephalosporins.[101,104]

EPIDEMIOLOGY

Both aerobic and anaerobic bacteria normally colonize the skin and mucosal surfaces of humans. In the oral cavity, gastrointestinal tract, and female genital tract, anaerobes vastly outnumber aerobes; in the colon, where hundreds of different anaerobic species are found, anaerobes constitute 99.9 percent of all bacteria.[38]

The predominance of anaerobes among the normal microbial flora stands in stark contrast to the rarity of human infections caused by these organisms. However, several types of infections routinely involve anaerobic bacteria, such as odontogenic infections, human and animal bite–associated infections, aspiration pneumonia, peritonitis in the presence of a ruptured viscus,

amnionitis, endometritis, necrotizing infections of soft tissues, and abscesses of the brain, lung, tubo-ovarian tract, and perirectal area. Underlying conditions that predispose patients to anaerobic infections include trauma, foreign bodies, malignancy, recent surgery, shock, colitis, vascular disease, and current or recent infection with aerobic or facultative organisms.[38] Anaerobic infections occur less frequently in children than in adults, probably because children are less likely than are adults to have these types of infections or predisposing conditions.

PATHOGENESIS

Anaerobic infections usually arise either because mucosal surfaces become disrupted, thereby allowing anaerobic bacteria access to normally sterile tissues, or because anaerobes are directly inoculated into such tissues, as occurs with traumatic or bite wounds. Distinguishing characteristics of anaerobic infections include suppuration, abscess formation, tissue destruction, foul odor, and occasionally, formation of gas.

The pathogenesis of anaerobic infections reflects a complex interaction among bacterial virulence factors, host defense mechanisms, and synergy with other organisms. Virulence factors that have been described in various anaerobic bacteria include adherence factors, capsular polysaccharide, lipopolysaccharide, leukotoxins, immunoglobulin proteases, collagenases, trypsin-like proteases, and chemotaxis-inhibiting factors. Moreover, enterotoxin-producing *B. fragilis* isolates have been identified in livestock and humans.[74]

Anaerobic infections typically are polymicrobial and often involve multiple species of both aerobic and anaerobic flora. Experiments in mice demonstrated that although the growth of both aerobes and anaerobes is enhanced in mixed infections, enhancement of the growth of aerobes is by far more pronounced. The factors responsible for aerobic-anaerobic synergism are not fully elucidated. Some have postulated that the bacterial species involved assist one another by providing essential growth factors, interfering with phagocytic uptake and killing, secreting β-lactamase into the extracellular milieu, or altering the oxidation-reduction potential of infected tissue.[21]

CLINICAL INFECTIONS

Serious anaerobic infections are seen more frequently in compromised hosts or in newborn infants than in otherwise healthy children. Outcomes of severe anaerobic infections are tied to patients' underlying disease states and to the promptness with which appropriate antimicrobial therapy is initiated.

BACTEREMIA

Anaerobic bacteremia is a rare occurrence in children; two separate pediatric studies found that only 0.3 percent of processed blood cultures yielded anaerobic bacteria that could be implicated as pathogens.[61,114] When anaerobic bacteremia does occur, it usually arises from a primary focal anaerobic or mixed aerobic-anaerobic infection. Children at risk for developing anaerobic bacteremia are those compromised by conditions such as recent surgery (especially procedures involving the gastrointestinal tract), necrotizing enterocolitis, dental manipulation, malignancy (patients with leukemia are at particularly high risk for severe clostridial sepsis),[51] immunosuppression, neonatal status (especially if premature), asplenia (including sickle-cell disease), and infectious mononucleosis.[41] The etiologic agents vary depending on the entry portal: in the gastrointestinal tract, *Bacteroides* spp. and *Clostridium* spp. predominate; in the head or neck, anaerobic gram-positive cocci and *Fusobacterium* spp. are more likely to be found.[16] Anaerobic bacteremia in patients with lower respiratory tract infections usually is caused by *B. fragilis* group members.[16] *P. acnes* is a well-recognized cause of bacteremia in patients with indwelling vascular devices; however, it is much more likely to be a contaminant than a true pathogen when isolated from previously healthy patients.[61,114]

Anaerobic bacteremia occurs more commonly in neonates than in older children; in one study, anaerobes accounted for 29 of 1290 (2.2%) bacterial isolates from newborns.[93] Factors predisposing to the development of anaerobic bacteremia in neonates include perinatal maternal complications (e.g., premature rupture of membranes), prematurity, and necrotizing enterocolitis.[28] The anaerobes most frequently isolated from neonatal blood cultures are *Bacteroides* spp., *Clostridium* spp., and anaerobic gram-positive cocci.[28] Reported mortality rates among neonates with anaerobic bacteremia range from 4 to 38 percent,[55,61] and mortality rates are higher with *Bacteroides* spp. (34%) than with other anaerobic species (17%).[28]

HEAD AND NECK INFECTION

Anaerobes frequently are involved in odontogenic infections such as periodontitis, periapical and dental abscesses, gingivitis, and Vincent angina. They also are recovered occasionally from suppurative perioral infections such as retropharyngeal and peritonsillar abscesses, cervical lymphadenitis, deep neck abscesses, parotitis, thyroiditis, infected epidermal cysts, and wound infections after head and neck surgery.[38] The anaerobes most commonly recovered from such infections are *Prevotella* spp., *Porphyromonas* spp., *Bacteroides* spp., *Fusobacterium* spp., and gram-positive cocci. In one study of peritonsillar abscesses, anaerobic bacteria were isolated from all 16 children in whom aspiration was performed.[18] A dreaded complication of peritonsillar abscess is Lemierre disease, which is characterized by *F. necrophorum* bacteremia; jugular vein septic thrombophlebitis; metastatic abscesses of the lung, liver, or elsewhere; and a foul-smelling tonsillar pseudomembrane.[75,76]

In patients with chronic otitis media, the bacteria isolated most frequently from middle ear cultures are *S. aureus*, *Pseudomonas aeruginosa*, and anaerobes. Approximately 50 percent of cultures yield anaerobic bacteria when appropriate culture techniques are used. The anaerobic flora most often implicated in these infections are anaerobic gram-positive cocci, pigmented *Prevotella* and *Porphyromonas* spp., *Bacteroides* spp., and *Fusobacterium* spp.[38,49]

Anaerobes also may play a role in the pathogenesis of acute otitis media. In one study, anaerobes (most commonly *Propionibacterium* spp., anaerobic gram-positive cocci, or both) were isolated from the middle ear fluid of 27 percent of children undergoing tympanocentesis for this disease.[45]

Anaerobes are associated only rarely with acute bacterial rhinosinusitis (i.e., symptoms for 10 to 30 days),[109,115] but they frequently are implicated as pathogens in children with chronic sinusitis (i.e., symptoms for >30 days). In one study, sinus aspirate cultures from 37 children with chronic sinusitis yielded an average of 2.7 anaerobes and 0.6 aerobes per patient. The anaerobes associated with chronic sinusitis are similar to those found in chronic otitis media; microaerophilic streptococci also are recovered occasionally.[43]

CENTRAL NERVOUS SYSTEM

Anaerobes can be isolated from most intracranial abscesses that are associated with sinusitis, otitis media, or dental infections. In one study, cultures of brain abscess specimens from 23 children yielded 11 aerobic and 44 anaerobic isolates, and cultures of

subdural empyema fluid from 16 children yielded six aerobic and 35 anaerobic isolates. In both brain abscess and subdural empyema, the anaerobes most frequently recovered are gram-positive cocci, *Fusobacterium* spp. (especially *F. nucleatum*), *Prevotella* spp., and *Bacteroides* spp.[25] These same organisms have been isolated, alone or along with aerobes, from epidural abscesses found in patients (primarily boys aged 10 to 14 years) with frontal sinusitis and Pott puffy tumor.[6,7,64] *B. fragilis* group members are associated particularly with temporal lobe abscesses arising from chronic otitis media.[5]

Anaerobic meningitis, in the absence of an associated brain abscess, occurs rarely in children. The symptoms, signs, and laboratory characteristics of children with anaerobic meningitis are similar to those of children with meningitis caused by more common bacterial pathogens. Unlike most anaerobic infections, meningitis usually is monomicrobial. The anaerobes most frequently recovered from cerebrospinal fluid cultures are *Bacteroides* spp. (especially *B. fragilis*), *Fusobacterium* spp. (especially *F. necrophorum*), and *Clostridium* spp. (especially *Clostridium perfringens*).[40] Meningitis caused by *Bacteroides* spp. generally occurs as a complication of a preceding anaerobic infection of the oropharynx or lower respiratory tract or an underlying gastrointestinal insult (e.g., recent surgery or necrotizing enterocolitis). Meningitis caused by *F. necrophorum* in children usually is associated with an otogenic focus. Most cases of *C. perfringens* meningitis in children occur after cranial trauma or surgery; a third of such infections are fatal despite administration of appropriate antimicrobial therapy.[40] *P. acnes* has been reported to cause 1.6 percent of pediatric cerebrospinal fluid shunt infections.[108] Anaerobic gram-positive cocci, *Veillonella* spp., *Actinomyces* spp., and *Eubacterium*-like organisms very rarely cause meningitis.[40]

INTRA-ABDOMINAL INFECTIONS

Secondary peritonitis occurs when the intestinal wall is disrupted (e.g., by perforation of an inflamed appendix or surgical misadventure), and enteric bacteria consequently gain access to the peritoneal cavity. Over the course of time, the peritoneal infection may evolve and result in the formation of one or more intra-abdominal abscesses.[38] Secondary peritonitis and intra-abdominal abscesses almost always are polymicrobial processes; anaerobes are isolated in 88 percent and aerobes in 86 percent of children with these infections.[15] Dozens of species of anaerobic and aerobic bacteria have been isolated from peritoneal or abscess cultures of children with these infections, but the anaerobes recovered most frequently are *B. fragilis*, other *B. fragilis* group members, anaerobic gram-positive cocci, *B. wadsworthia*, *Fusobacterium* spp., *Lactobacillus* spp., and *Clostridium* spp. The aerobes most frequently isolated in these patients are *Escherichia coli*, viridans streptococci, *P. aeruginosa*, and *Enterococcus* spp.[9,15,80]

Traditionally, *S. aureus* has been recognized as the leading cause of liver abscesses in children. When proper anaerobic culture techniques are used, however, anaerobic isolates outnumber aerobes in liver abscess cultures, with anaerobic gram-positive cocci, *Bacteroides* spp., and *Fusobacterium* spp. being recovered most frequently.[47,46,99] Splenic abscesses are associated with a similar range of anaerobic flora.[47] In a study of retroperitoneal abscess cultures in children, the anaerobes most frequently isolated were gram-positive cocci, *Bacteroides* spp., and *Prevotella* spp; the dominant aerobic isolates were *E. coli* and *S. aureus*.[37] One case of a renal abscess caused by *Peptoniphilus asaccharolyticus* in a child has been reported.[50]

Sexually active adolescent girls are at risk for the development of pelvic inflammatory disease and its complications (e.g., tuboovarian abscess). In addition to being caused by aerobic sexually transmitted organisms (e.g., *Chlamydia trachomatis* and *Neisseria gonorrhoeae*), these infections frequently involve anaerobes such as *Prevotella* spp. (especially *P. bivia* and *P. disiens*), *Porphyromonas* spp., anaerobic gram-positive cocci, *Clostridium* spp. (especially *C. perfringens*), and *B. fragilis* group organisms.[38]

SKIN AND SOFT TISSUE INFECTIONS

Cutaneous abscesses in children can be polymicrobial and involve both aerobic and anaerobic bacteria. Aerobes (particularly *S. aureus*) predominate in infections of the hand, neck, leg, and trunk regions, whereas anaerobes are recovered more frequently in infections of the vulvovaginal, buttock, perirectal, finger, nail bed, and head regions.[19] A similar regional distribution of aerobes and anaerobes also occurs in cases of cellulitis.[33] The anaerobes most likely to be recovered from infections near the perirectal area include, not surprisingly, *B. fragilis* group and *Clostridium* spp.; infections closer to the oropharynx usually involve oral flora such as *Prevotella* spp., *Porphyromonas* spp., *Fusobacterium* spp., and anaerobic gram-positive cocci.[38] Both aerobes and anaerobes are isolated from sundry skin infections such as paronychia, infected burn wounds, gastrostomy or tracheostomy site infections, and secondary infections of scabies lesions.[17,30,34,48]

Bacteria of the normal oral flora of humans and animals figure prominently in infected bite wounds. In children with infected human bite wounds, the anaerobes isolated most frequently are anaerobic gram-positive cocci, *Prevotella* spp., and *Porphyromonas* spp.; the aerobes isolated most frequently are *S. aureus* and *Streptococcus* spp. In infected animal bites, the most common anaerobes are gram-positive cocci, *Veillonella* spp., and *Fusobacterium* spp., whereas the most frequent aerobic or facultative organisms are *S. aureus* and *Pasteurella multocida*.[23]

Anaerobes play prominent roles in complicated skin and soft tissue infections. In a study of pilonidal cysts in children, anaerobes were isolated from all children and aerobic organisms from only 32 percent.[44] Crepitant cellulitis is a superficial infection that is associated with less systemic toxicity but more noticeable crepitus than is clostridial myonecrosis; it may be caused by *C. perfringens* or by combinations of other anaerobes with facultative bacteria. Clostridial myonecrosis ("gas gangrene") is a life-threatening infection of the skeletal muscle that usually is caused by *C. perfringens* and less frequently by *Clostridium novyi* or *Clostridium septicum*; it occurs hours to days after gross contamination of deep traumatic or surgical wounds. Necrotizing fasciitis is another dreaded soft tissue infection that can be rapidly progressive. In one type of necrotizing fasciitis, anaerobes such as *Bacteroides* spp. and anaerobic gram-positive cocci frequently are recovered, often in association with various aerobic enteric flora. This type should be distinguished from necrotizing fasciitis caused by *Streptococcus pyogenes* (or, less frequently, by group B, C, or G streptococci), in which anaerobes generally are not involved.[58]

BONE AND JOINT INFECTIONS

Septic arthritis caused by anaerobic bacteria is a rare occurrence in both children and adults and, unlike most anaerobic infections, generally is not polymicrobial. Most cases result from anaerobic bacteremia with gram-negative rods (especially *Fusobacterium* spp. or the *B. fragilis* group) or anaerobic gram-positive cocci. Cases associated with penetrating trauma, however, often are caused by *Clostridium* spp., and those involving recent surgery or prosthetic joints can involve *P. acnes*. In some cases, the presence of anaerobes is suggested by foul-smelling synovial fluid, gas under pressure within the joint, negative synovial fluid cultures in the clinical picture of septic arthritis, or an anaerobic infection elsewhere in the patient.[39]

Anaerobic osteomyelitis also is quite uncommon in children. When it does occur, it results from direct extension from a contiguous focus rather than via hematogenous seeding. In a series of 26 children with culture-proven anaerobic osteomyelitis,[22] all had preexisting anaerobic infections at adjacent sites or other predisposing conditions for anaerobic infection, including chronic mastoiditis (7 patients), decubitus ulcers (5), periodontal abscesses (3), bites (3), paronychia (2), trauma (1), and perinatal scalp infection (1). Culture results reflected those of the underlying anaerobic infection or the normal flora of the nearest mucous membrane surface. All but 2 patients had polymicrobial infections; in 10 patients, aerobic flora also were recovered. In contrast to acute hematogenous osteomyelitis, these cases had an insidious onset; the mean duration of symptoms before initial evaluation was 21 days (range, 7 to 37 days). Three patients with decubitus ulcers had documented recurrences of their osteomyelitis after receiving successful initial treatment.[22]

PLEUROPULMONARY INFECTIONS

Anaerobic infections of the lower respiratory tract are rare occurrences in healthy children. They are, however, relatively common in children with tracheoesophageal malformations or central nervous system abnormalities that impair cough reflexes. Such children are predisposed toward aspirating not only food, milk, and vomitus but also their oropharyngeal secretions. Thus, aspiration pneumonia usually is a polymicrobial process involving both the aerobic and anaerobic flora of the oral cavity. The risk of pneumonia developing is further elevated in children with poor oral hygiene, gingivitis, or periodontitis. If untreated, aspiration pneumonia can progress with the development of single or multiple lung abscess or pleural empyema, or both. The predominant anaerobes recovered from children with aspiration pneumonia are anaerobic gram-positive cocci, pigmented *Prevotella* and *Porphyromonas* spp., *F. nucleatum*, the *B. fragilis* group, and other *Bacteroides* spp.; the predominant aerobic and facultative bacteria are *P. aeruginosa*, *Streptococcus pneumoniae*, *E. coli*, *Klebsiella pneumoniae*, and *S. aureus*.[43]

Anaerobes occasionally are isolated from pleural fluid cultures of patients with pleural empyema. In a study of 104 children with pleural empyema,[63] anaerobes were isolated from five adolescents, in all cases along with other aerobic or anaerobic oral flora. Predisposing conditions in these patients were pneumonia in four and alcohol intoxication in one. Anaerobes might be responsible for at least some of the roughly half of pleural empyema cases in which routine cultures are negative.[63]

Anaerobes occasionally are isolated from lower respiratory tract specimens of adults with ventilator-associated pneumonia, although their pathogenic significance in this setting continues to be debated.[103] In a study of pathogens causing ventilator-associated pneumonia in children, anaerobic bacteria associated with oral flora were recovered from 9 of 10 sputum culture specimens obtained with protective brush catheters, either alone (3 children) or as part of a mixed infection with aerobic bacteria (6 children).[32]

DIAGNOSIS

The diagnosis of anaerobic infections requires a high index of suspicion. A few anaerobic infections are diagnosed clinically (e.g., tetanus, botulism, and gas gangrene), and for these infections, culture results can be misleading. However, most anaerobic infections must be suspected on the basis of the clinical findings and then diagnosed with microbiologic methods. When anaerobic infections are suspected, specimens for culture must be collected without contamination from the endogenous flora of adjacent mucous membranes. In general, acceptable specimens for anaerobic culture include blood; aspirates of normally sterile body fluids, abscesses, or deep wounds; and surgically obtained specimens. Aspirates and tissue are preferable to swabs. Gram-stained smears of aspirated material should be examined and used to complement culture results.

Specimens submitted for anaerobic culture should be transported rapidly to the laboratory in anaerobic transport devices and promptly inoculated into appropriate media in an oxygen-free environment (e.g., anaerobic jar or glove box). Media that support the cultivation of anaerobes are supplemented with reducing substances (e.g., thioglycolate, dithiothreitol, cysteine, iron shavings, or chopped meat) and antibiotics (to suppress the growth of facultative bacteria).[113,96] Even before being inoculated, such media must be prepared and stored under anaerobic conditions to prevent the generation of superoxide or hydrogen peroxide.[96] Attention given to detail is important. In one hospital, improvements in techniques for recovery of anaerobes (i.e., media and transport conditions) and education of clinicians and microbiologists were associated with dramatic reductions in health care costs and mortality rates in patients with anaerobic infections.[8]

Bacterial isolates that grow anaerobically must be tested for aerotolerance to confirm whether they are obligate or facultative anaerobes. This process necessarily lengthens the time to reporting of culture reports to the clinician; however, the morphologic characteristics of certain anaerobes (e.g., *C. perfringens*, *B. fragilis* group, pigmented *Prevotella*, and *Porphyromonas* spp.) are sufficiently distinctive to allow preliminary reporting of presumptive results before aerotolerance testing is performed at least in experienced laboratories.[113] Definitive identification of anaerobic isolates is performed with biochemical tests, gas-liquid chromatography of fermentation reaction products, direct immunofluorescence, molecular genetic methods, or a combination of these methods.[56,79,81,113,116]

TREATMENT

Appropriate management of anaerobic infections is both surgical and medical. As a rule, abscesses should be drained (and their contents sent for routine and anaerobic culture), and débridement of necrotic tissue is critical to survival with severe infections such as clostridial myonecrosis and necrotizing fasciitis. Hyperbaric oxygen also has been used, in addition to débridement and antibiotics, in some children with clostridial myonecrosis and in others with necrotizing fasciitis, but definitive recommendations regarding its use cannot be made based on this limited experience.[38]

Patients with anaerobic infections should receive appropriate antibiotic therapy promptly. In certain infections (e.g., peritonitis with a perforated viscus, brain abscess related to chronic mastoiditis, gas gangrene), the clinician should assume that anaerobes are present and include anaerobic coverage in the antimicrobial regimen. For many anaerobic infections, there is no particular drug of choice, and many antibiotics (alone or in combination) might be reasonable for a given patient. In selecting antimicrobial therapy, the clinician must consider the aerobic and anaerobic flora that probably are involved (which may be predicted, to some extent, by the anatomic site of the infection) and the toxicity, tolerability, tissue penetration, and cost of available antibiotics.

Aminoglycosides, monobactams (e.g., aztreonam), and older fluoroquinolones are poorly active against anaerobes.[42] Other classes of antimicrobial agents vary in their activity against different groups of anaerobic bacteria (see Table 162–2 and the following sections). The duration of antimicrobial therapy for anaerobic infections generally is similar to that for analogous aerobic infections. Serious anaerobic infections should be treated

with intravenous antimicrobial agents at maximum dosages to optimize penetration into devitalized tissue and abscesses.

PENICILLINS AND PENICILLIN/β-LACTAMASE INHIBITOR COMBINATIONS

Penicillin G and ampicillin are active against most anaerobes that do not produce β-lactamase, including anaerobic gram-positive cocci, *C. perfringens*, and most non–spore-forming gram-positive bacilli (except *Eubacterium*-like bacteria). Anaerobic gram-negative bacilli, however, frequently produce β-lactamases that render penicillins ineffective (and in some cases, even third-generation cephalosporins).[42]

Combinations of penicillins with β-lactamase inhibitors achieve broad-spectrum activity against anaerobic and aerobic bacteria. These agents are particularly well suited for the treatment of intra-abdominal infections, in which β-lactamase–producing gram-negative bacilli are uniformly present. Guidelines for the treatment of complicated intra-abdominal infections in adults endorse (among other options) the use of ampicillin-sulbactam and ticarcillin-clavulanate for infections of mild to moderate severity and piperacillin-tazobactam for severe infections.[111] Unfortunately, no similar guideline exists for children. Furthermore, in the United States, piperacillin-tazobactam is not licensed for use in children, and ampicillin-sulbactam is licensed for use only in children older than 1 year.[2] Amoxicillin-clavulanate is active against most anaerobic and aerobic oropharyngeal flora and, thus, is a suitable oral antibiotic for the treatment of many anaerobic infections arising in the head, neck, and chest.

CEPHALOSPORINS

First-generation cephalosporins have anti-anaerobic activity similar to that of penicillin G, and third-generation cephalosporins are only moderately more active. The most active cephalosporins against anaerobes are the second-generation agents cefoxitin, cefotetan, and cefmetazole; of these, cefoxitin is the most active against *B. fragilis* group members.[42] Because of increasing resistance among these organisms, however, cefoxitin no longer is recommended for empiric treatment of intra-abdominal infections,[111] although it remains useful for perioperative antimicrobial prophylaxis in surgical procedures involving the abdomen.[13]

METRONIDAZOLE

Metronidazole has the best activity of any antibiotic against anaerobic gram-negative bacilli (except *Sutterella* spp.), including *B. fragilis* group members that are commonly resistant to other classes of antibiotics. Its activity against gram-positive anaerobes is less reliable, although most anaerobic gram-positive cocci are susceptible. However, *Propionibacterium* spp. and virtually all aerobic bacteria are inherently resistant to metronidazole. Because most anaerobic infections are polymicrobial, metronidazole generally is not used alone but in combination with other antibiotics active against aerobic bacteria (e.g., with a third-generation cephalosporin for the treatment of intra-abdominal infection).

Metronidazole penetrates well into many tissues, including those of the central nervous system, and can be administered orally. Although it generally is tolerated well, reported serious side effects include seizures and peripheral neuropathy. Moreover, the safety and efficacy of metronidazole in children have not been established.[2]

CLINDAMYCIN

Clindamycin is active against many anaerobes, including gram-positive cocci, gram-positive non–spore-forming bacilli, *C. perfringens*, and most anaerobic gram-negative bacilli (Table 162–2). It also is active against most gram-positive aerobes but not against *Enterococcus* spp. or gram-negative aerobes. Clindamycin resistance has increased among the *B. fragilis* group members[73]; thus, clindamycin is now less reliable than are carbapenems, penicillin/β-lactamase inhibitor combinations, or metronidazole against these organisms. As a result, clindamycin presently is better suited for the treatment of infections involving oropharyngeal anaerobes than for those involving intestinal flora.

Clindamycin achieves comparable serum levels whether administered orally or intravenously and penetrates well into most tissues and fluids, including bones, joints, and abscesses.[53] Clindamycin does not penetrate well into cerebrospinal fluid and, hence, should not be used for the treatment of central nervous system infections.[53] For treatment of polymicrobial infections, clindamycin should be combined with a β-lactam or other antibiotic active against aerobic gram-negative bacilli (e.g., *P. multocida* in animal bite wounds or *Eikenella corrodens* in human bite wounds). Although clindamycin traditionally has been associated particularly with *Clostridium difficile*–induced pseudomembranous colitis, many other antibiotics also can induce this complication.

CARBAPENEMS

The carbapenems are parenterally administered β-lactams with broad coverage against both aerobes and anaerobes, including many cephalosporin-resistant, gram-negative enterics. Because of this broad-spectrum activity, carbapenems are well suited for the treatment of intra-abdominal infections (although *Enterococcus faecium* and methicillin-resistant *S. aureus* are inherently resistant). The commercially available carbapenems approved for use in children are meropenem and imipenem-cilastatin; meropenem is the preferred agent in children because it is less likely to induce seizures. Meropenem is slightly more active than is imipenem against gram-negative bacteria; the converse is true for gram-positive bacteria.[83,97]

QUINOLONES

The quinolones most commonly prescribed in the United States are ciprofloxacin, levofloxacin, gatifloxacin, and moxifloxacin. Of these, only the first is licensed for use in children (and then only for the treatment of complicated urinary tract infections or pyelonephritis or for postexposure treatment of inhalational anthrax),[3] whereas only the last three have significant in vitro activity against anaerobes.[4,62,69] Resistance to quinolones has increased recently among *Bacteroides* spp.[10,68]; thus, these agents should not be used as monotherapy for intra-abdominal infections.[111]

Therapeutic advantages of the quinolones include their wide distribution in tissues and fluids and their good absorption after oral administration.[97] The newer quinolones may provide options for treating anaerobic infections in selected children (e.g., older adolescents). Nonetheless, the role of quinolones in pediatrics remains limited because concern lingers over their potential toxicity, because other agents (e.g., meropenem) with established safety profiles have equal or better activity against anaerobes, and because bacterial resistance to quinolones probably will continue to rise (as a result of their continued widespread use in adults).

CHLORAMPHENICOL

Chloramphenicol possesses very good activity against both gram-positive and gram-negative anaerobes, including *B. fragilis* group members. It penetrates well into tissues and is absorbed well after oral administration.[53] Unfortunately, its use is associated rarely with potentially life-threatening blood dyscrasias, so safer alternatives should be used preferentially.

OTHER ANTIBIOTICS

Macrolides (e.g., erythromycin, azithromycin, and clarithromycin) and tetracyclines are inconsistently active against anaerobic bacteria.[53,69] Vancomycin and daptomycin are active against most gram-positive anaerobes except *Lactobacillus* spp., many strains of which are resistant.[70] Linezolid showed impressively good activity against both gram-positive and gram-negative anaerobes (including *B. fragilis* group members) in a recent study; however, more experience with its clinical use for anaerobic infections is needed.[120] Generally speaking, the role of these antibiotics in the treatment of anaerobic infections is limited.

PREVENTION

Other than tetanus, which can be prevented by immunization, anaerobic infections can be avoided only by preventing the introduction of anaerobes into healthy tissues. Preventive measures include cleaning and débriding wounds properly, removing foreign bodies promptly, maintaining good dental and oral hygiene, avoiding disruption of the intestinal wall during surgery, appropriately prescribing perioperative antibiotic prophylaxis,[13] and following recommendations for the prevention of nosocomial pneumonia.[52]

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SECTION XVII

Viral Infections

CHAPTER 163

Classification and Nomenclature of Viruses

Marjorie J. Miller

Viruses originally were differentiated from other microorganisms by their small size and their filterability. Initial efforts to classify viruses were based on disease, pathogenesis, organ tropisms, and epidemiologic characteristics rather than on physicochemical properties of the virus particle. During the 1950s and 1960s, many new viruses were being discovered while evidence of virus structure and composition also was emerging, thereby prompting proposals that viruses be grouped on the basis of shared virion properties. The herpesvirus,[1] myxovirus,[2] and poxvirus[7] groups were among the first taxonomic groups delineated. As more information concerning the physicochemical characteristics of viruses accumulated, the need for a universal system of classification and nomenclature became apparent.

The International Committee on Nomenclature of Viruses (ICNV) was established in 1966 at the International Congress of Microbiology in Moscow, some 20 years after a bacterial taxonomy first was published and 75 years after the discovery of viruses. In 1973, the ICNV became the International Committee on Taxonomy of Viruses (ICTV), which operates under the auspices of the Virology Division of the International Union of Microbiological Societies. The ICTV classifies viruses isolated from vertebrates, invertebrates, plants, fungi, protozoa, bacteria, and mycoplasmas and publishes periodic reports summarizing the most recent developments in viral taxonomy.[4,6,8-10,12,16-18] Interim updates were published in *Intervirology* and more recently in *Archives of Virology*. Additional information on virus taxonomy can be accessed through the Internet at *www.ncbi.nlm.nih.gov* (TaxBrowser drop-down menu) and through a worldwide universal virus database (ICTVdB) being developed through the cooperation of the Australian National University (*http//life.anu.edu.au/viruses/welcome.htm*) in Canberra, the American Type Culture Collection, and the U.S. National Science Foundation (mirror Internet sites are the National Center for Biotechnology Information, Bethesda, MD: *www.ncbi.nlm.nih.gov/ICTVdb*; and the Integrated Approach to Crop Research, Rothamsted, UK: *www.ictvdb.rothamsted.ac.uk/*).

The hierarchic levels of virus taxonomy consist of order, family, subfamily, genus, and species and are based on the structural, physicochemical, biologic, and replicative properties of viruses.[12] Structural characteristics include size, shape, presence or absence of an envelope, and capsid symmetry. Physicochemical characterization is based on type, strandedness, and the number of segments of nucleic acid, as well as the number and size of proteins and their functional activities. Biologic properties include host range, serologic relationships, pathogenicity, and transmission; replicative properties include nucleic acid replication, transcription, and translation.

Virus orders comprise groups of families that share common characteristics (e.g., biochemical composition, viral replication strategy, particle structure, and general genomic organization) distinguishable from those of other orders and families. Virus orders are designated with the suffix *-virales*. Currently, the ICTV has approved two orders, *Mononegavirales*, which comprises the families *Paramyxoviridae*, *Rhabdoviridae*, and *Filoviridae*, and *Nidovirales*, which comprises the families *Coronaviridae* and *Arteriviridae*.

Virus families represent groups of genera that share common characteristics distinct from those of other families. Virus families are designated with the suffix *-viridae*. Most families have unique virion morphology, genome structure, or strategies of replication (Fig. 163–1). Virus subfamilies are designated with the suffix *-virinae* and have been introduced in four families: *Poxviridae* (*Chorodopoxvirinae*), *Herpesviridae* (*Alphaherpesvirinae*, *Betaherpesvirinae*, *Gammaherpesvirinae*), *Parvoviridae* (*Parvovirinae*), and *Paramyxoviridae* (*Paramyxovirinae*, *Pneumovirinae*) (Table 163–1).

Virus genera consist of groups of species that share common characteristics different from those of members of other genera. Common properties within a genus include viral replication strategy, genome size, organization, numbers of segments, sequence homologies, and vector transmission. Virus genera are designated with the suffix *-virus*.

The species taxon is the most fundamental unit in biologic classification, but the viruses have proved to be the most difficult with which to deal, and years elapsed before a definition of virus species was accepted internationally. In 1991, the ICTV accepted the definition of a virus species proposed by van Regenmortel[13]: "A virus species is defined as a polythetic class of viruses that constitutes a replicating lineage and occupies a particular ecological niche." Members of a polythetic class have several properties in common, although no single common attribute is present in all members, thereby accommodating the inherent variability of viruses. Common species properties include genome rearrangement, sequence homologies, serologic relationships, vector transmission, host range, pathogenicity, tissue tropism, and geographic distribution.[4,16] Some properties of viruses used in taxonomy are listed in Table 163–2.[12]

The ICTV also has established criteria for formal virus nomenclature[4,16] and recommended abbreviations for virus names.[4,5,16] In formal taxonomic usage, the names of orders, families, subfamilies, genera, and species are printed in italics, and the first letter of the name is capitalized. This form applies when using a species name to refer to a taxonomic category (e.g., in the Materials and Methods section of a paper when describing the particular virus used in the study). Some examples of formal taxonomic terminology are *Poliovirus*, genus *Enterovirus*, family *Picornaviridae* or *Human herpesvirus 1* (herpes simplex virus type 1) and genus *Simplexvirus*, subfamily *Alphaherpesvirinae*, family *Herpesviridae*. Thereafter, vernacular names can be used throughout the publication. In informal vernacular usage when referring to virions or virus particles or if used as an adjective, italics and capital initial letters are not required and the names are written in lowercase Roman script (e.g., "poliovirus cytopathic effect" or "picornaviruses/polioviruses were inoculated into cell

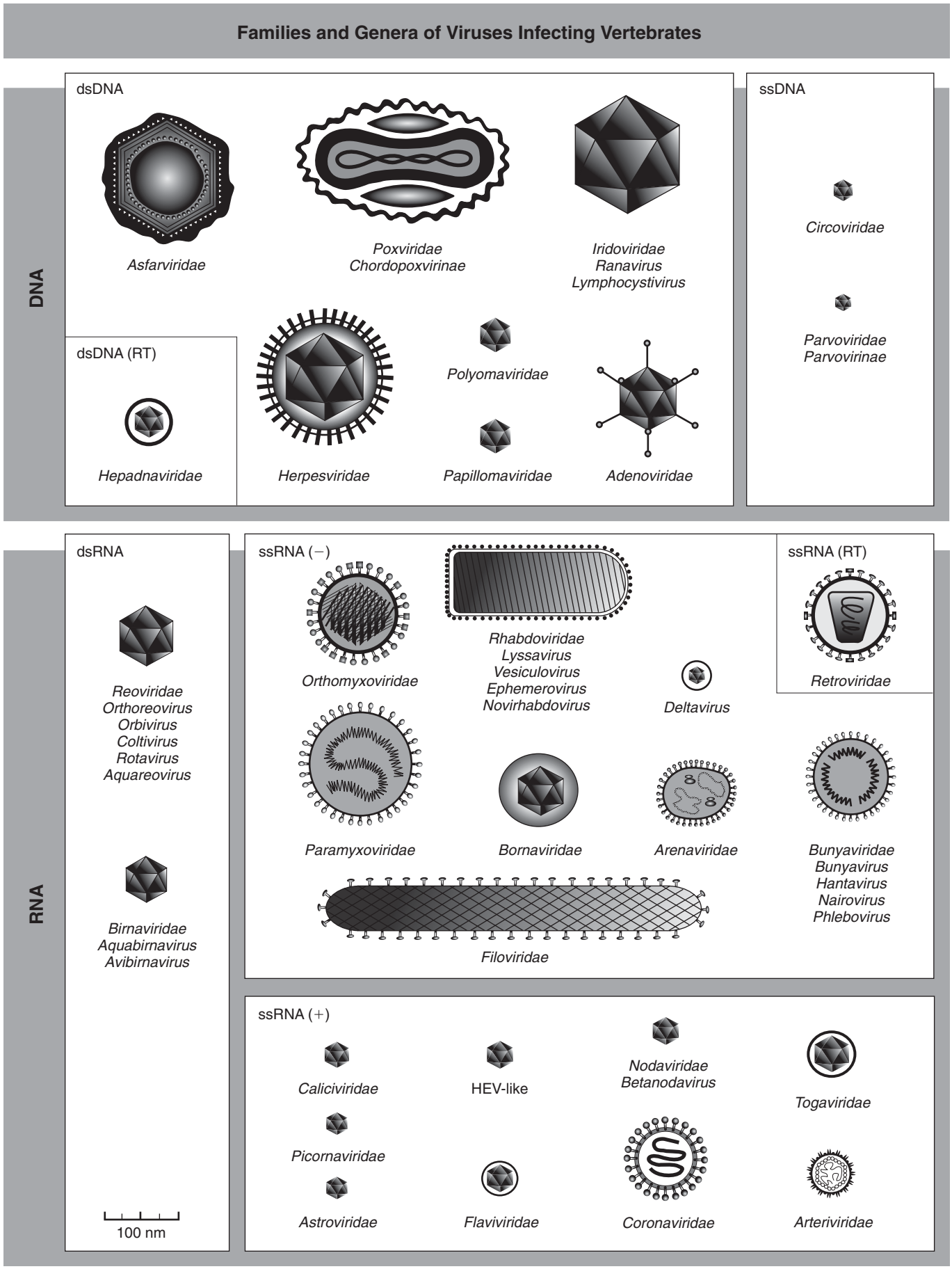

Figure 163–1 Diagram illustrating the shapes and relative sizes of animal viruses of the major families. *(From Van Regenmortel, M. H. V., Fauquet, C. M., Bishop, D. H. L., et al. [eds.]: Virus Taxonomy: Classification and Nomenclature of Viruses. Seventh Report of the International Committee on Taxonomy of Viruses. San Diego, Academic Press, 2000, p. 30.)*

TABLE 163–1 Classification and Nomenclature of Representative Viruses Affecting Humans

Family	Subfamily	Genus	Representative Species Pathogenic for Humans (Abbreviation, Disease, Synonym, or No. of Members)
DNA Viruses			
Poxviridae	*Chordopoxvirinae*	*Orthopoxvirus*	*Vaccinia virus* (VACV) *Variola virus* (VARV)
		Parapoxvirus	*Orf virus* (ORFH, contagious pustular dermatitis) *Pseudocowpox virus* (PCPV, milkers' nodule)
		Molluscipoxvirus	*Molluscum contagiosum virus* (MOCV)
		Yatapoxvirus	*Yaba monkey tumor virus*, *tanapox virus* (YMTV, TANV)
Herpesviridae	*Alphaherpesvirinae*	*Simplexvirus*	*Human herpesvirus 1* (HHV-1, herpes simplex virus type 1) *Human herpesvirus 2* (HHV-2, herpes simplex virus type 2) *Cercopithecine herpesvirus 1* (CeHV-1, monkey B virus)
		Varicellovirus	*Human herpesvirus 3* (HHV-3, varicella-zoster virus)
	Betaherpesvirinae	*Cytomegalovirus*	*Human herpesvirus 5* (HHV-5, cytomegalovirus)
		Roseolovirus	*Human herpesvirus 6* (HHV-6, human B lymphotropic virus) *Human herpesvirus 7* (HHV-7)
	Gammaherpesvirinae	*Lymphocryptovirus*	*Human herpesvirus 4* (HHV-4, Epstein-Barr virus)
		Rhadinovirus	*Human herpesvirus 8* (HHV-8, Kaposi sarcoma–associated herpesvirus)
Adenoviridae		*Mastadenovirus*	*Human adenovirus A* (HAdV-A; 12, 18, 31) *Human adenovirus B* (HAdV-B; 3, 7, 11, 14, 16, 21, 34, 35, 50) *Human adenovirus C* (HAdV-C; 1, 2, 5, 6) *Human adenovirus D* (HAdV-D; 8-10, 13, 15, 17, 19, 20, 22-30, 32, 33, 36-39, 42-49, 51) *Human adenovirus E* (HAdV-E; 4) *Human adenovirus F* (HAdV-F; 40, 41)
Papillomaviridae		*Papillomavirus*	*Human papillomavirus* (HPV, 80)
Polyomaviridae		*Polyomavirus*	*BK polyomavirus* (BKPyV, polyomavirus hominis 1) *JC polyomavirus* (JCPyV, polyomavirus hominis 2)
Parvoviridae	*Parvovirinae*	*Erythrovirus*	*B19 virus* (B19V, human parvovirus, erythema infectiosum or fifth disease)
Hepadnaviridae		*Orthohepadnavirus*	*Hepatitis B virus* (HBV)
RNA Viruses			
Reoviridae		*Orthoreovirus*	*Mammalian orthoreovirus* (MRV, 3)
		Coltivirus	*Colorado tick fever virus* (CTFV)
		Orbivirus	*Changuinola virus* (CGLV; 12); *Great Island virus* (GIV; 36)
		Rotavirus	*Rotavirus A*, *B*, and *C* (RV-A, RV-B, RV-C, numerous serotypes)
Paramyxoviridae	*Paramyxovirinae*	*Respirovirus*	*Human parainfluenza virus 1* and *3* (HPIV-1, HPIV-3)
		Morbillivirus	*Measles virus* (MeV)
		Rubulavirus	*Mumps virus* (MUV), *human parainfluenza virus 2* (HPIV-2) and *4* (HPIV-4a and b)
	Pneumovirinae	*Pneumovirus*	*Human respiratory syncytial virus* (HRSV)
		Metapneumovirus	*Human metapneumovirus* (HMPV)
Rhabdoviridae		*Vesiculovirus*	*Vesicular stomatitis Indiana virus* (VSIV)
		Lyssavirus	*Rabies virus* (RABV)
Filoviridae		Marburg-like virus	*Lake Victoria marburgvirus* (LVMARV)
		Ebola-like virus	*Zaire ebolavirus* (ZEBOV), *Reston ebolavirus* (REBOV)
Orthomyxoviridae		*Influenzavirus A*	*Influenza A virus* (FLUAV)
		Influenzavirus B	*Influenza B virus* (FLUBV)
		Influenzavirus C	*Influenza C virus* (FLUCV)
Bunyaviridae		*Bunyavirus*	*Bunyamwera virus* (BUNV, Bunyamwera serogroup), *California encephalitis virus* (CEV, California serogroup) (mosquito and culicid fly transmitted; ≈47 species, >161 serotypes)
		Hantavirus	*Hantaan virus* (HTNV, Korean hemorrhagic fever or hemorrhagic fever with renal syndrome), *Sin Nombre virus* (SNV, Four Corners hantavirus, hantavirus pulmonary syndrome), and others (rodent associated, ≈22 species)
		Nairovirus	*Crimean-Congo hemorrhagic fever virus* (CCHFV) (tick transmitted, 8 species, >34 strains)
		Phlebovirus	*Sandfly fever Naples virus* (SFNV), *Rift Valley fever virus* (RVFV) (sandfly-borne primarily); *Uukuniemi virus* (UUKV) (phlebotomine, tick, and mosquito transmitted; ≈9 species, 35 strains)
Arenaviridae		*Arenavirus*	*Lymphocytic choriomeningitis virus* (LCMV) *Lassa virus* (LASV) *Junin virus* (JUNV, Argentine hemorrhagic fever virus) *Machupo virus* (MAVC, Bolivian hemorrhagic fever virus)

Continued

TABLE 163–1 Classification and Nomenclature of Representative Viruses Affecting Humans—cont'd

Family	Subfamily	Genus	Representative Species Pathogenic for Humans (Abbreviation, Disease, Synonym, or No. of Members)
Picornaviridae		*Enterovirus*	*Poliovirus* (PV, human poliovirus 1-3)
			Human enterovirus A (HEV-A, 10 serotypes; human Coxsackie A viruses 2, 3, 5, 7, 8, 10, 12, 14, 16; human enterovirus 71)
			Human enterovirus B (HEV-B, 36 serotypes; human Coxsackie B viruses 1-6; human Coxsackie virus A9; human echovirus 1-21 and 24-33; human enterovirus 69)
			Human enterovirus C (HEV-C, 11 serotypes; human Coxsackie viruses A1, A11, A13, A15, A17-A22, A24)
			Human enterovirus D (HEV-D, 2 serotypes; human enteroviruses 68 and 70)
		Parechovirus	*Human parechovirus* (HPeV; human parechovirus 1, formerly echovirus 22; human parechovirus 2, formerly echovirus 23)
		Rhinovirus	*Human rhinovirus A* (HRV-A, 18 serotypes)
			Human rhinovirus B (HRV-B, 13 serotypes) (unassigned serotypes, 82)
		Hepatovirus	*Hepatitis A virus* (HAV, enterovirus 72)
Caliciviridae		*Norovirus*	*Norwalk virus* (NV, 7 strains)
		Sapovirus	*Sapporo virus* (SV, 6 strains)
Astroviridae		*Mamastrovirus*	*Human astrovirus* (HAstV, 8)
Coronaviridae		*Coronavirus*	*Human coronavirus* (HCoV-229E and HCoV-CC43)
Flaviviridae		*Flavivirus*	*St. Louis encephalitis virus* (SLEV), *yellow fever virus* (YFV), *dengue virus* (DENV), and *West Nile virus* (WNV) (group B arboviruses, mosquito-borne, ≈27)
			Kyasanur Forest disease virus (KFDV), *tick-borne encephalitis virus* (TBEV) (tick-borne, ≈15)
			Vector-unassociated viruses (≈17)
		Hepacivirus	*Hepatitis C virus* (HCV, parenterally transmitted non-A, non-B hepatitis)
			Hepatitis G virus (HGV-1)
Togaviridae		*Alphavirus*	*Western*, *eastern*, and *Venezuelan equine encephalitis viruses* (WEEV, EEEV, VEEV); *Ross River* and *Sindbis viruses* (RRV, SINV) (group A arboviruses, mosquito-borne, ≈27)
		Rubivirus	*Rubella virus* (RUBV)
Retroviridae	*Orthoretrovirinae*	*Deltaretrovirus*	*Primate T-cell lymphotropic virus 1* (PTLV-1, human T-lymphotropic virus 1)
			Primate T-cell lymphotrophic virus 2 (PTLV-2, human T-lymphotropic virus 2)
		Lentivirus	*Human immunodeficiency viruses 1* and *2*
Subviral Agents (Satellites, Viroids, and Agents of Spongiform Encephalopathies)			
Prions			Kuru, Creutzfeldt-Jakob disease (CJD), Gerstmann-Sträussler-Scheinker syndrome (GSS), fatal familial insomnia (FFI)
Unclassified Viruses			
		Delta virus	*Hepatitis delta virus* (HDV)
		Hepatitis E–like viruses	*Hepatitis E virus* (HEV, enterically transmitted non-A, non-B hepatitis)

culture").[4,14,15] Viruses with uncertain taxonomic status are considered "tentative" species, and names are not italicized, although the initial letter is capitalized. Though not part of the formal international code, abbreviations also have been recommended by the ICTV for every virus name to reduce the possibility of duplication when new abbreviations are proposed[4,5,16] (see Table 163–1).

Figure 163–2 is a schematic diagram showing the basic forms and composition of viruses.[3] The type of nucleic acid, capsid symmetry, presence or absence of an envelope, and peplomers (spikes) all are characteristics used in classification. Figure 163–1 is a more detailed diagram not only illustrating the relative shapes and sizes of families and genera of vertebrate viruses but also indicating the type and nature (single-stranded [ss], double-stranded [ds], sense [+/–]) of the nucleic acid, presence of reverse transcriptase (RT), and presence or absence of an envelope.

Figures 163–3 and 163–4 (adapted from Melnick[11]) show classification of DNA and RNA virus families, respectively, by some of the taxonomic characteristics listed in Table 163–2. Representative DNA and RNA viruses affecting humans are listed in Table 163–1, which uses the most recent nomenclature reported by the ICTV (for a complete listing, see Fauquet and colleagues[4]).

TABLE 163–2 Representative Properties Used In Virus Taxonomy

Virion Properties
Morphology
- Size
- Shape
- Presence, absence of envelope and peplomers
- Capsid structure, symmetry

Physical properties
- Molecular mass
- Buoyant density
- Sedimentation coefficient
- pH stability
- Thermal stability
- Solvent, detergent stability
- Cation (Mg^{2}+, Mn^{2}+) stability
- Radiation stability

Genome
- Type of nucleic acid, DNA or RNA
- Strandedness, single- or double-stranded
- Linear or circular
- Sense—positive, negative, or ambisense
- Number of segments
- Size
- Presence or absence and type of 5′ terminal cap
- Nucleotide sequence comparisons

Protein properties
- Number
- Size
- Functional activities
- Amino acid sequence comparisons

Lipids
- Presence or absence
- Nature

Carbohydrates
- Presence or absence
- Nature

Genome Organization and Replication
Organization
Strategy of replication
Characteristics of transcription
Characteristics of translation and post-translational processing
Site of accumulation of assembly, maturation, and release

Antigenic Properties
Serologic relationships
Mapping epitopes

Biologic Properties
Host range
Pathogenesis
Tissue tropisms, pathology
Mode of transmission
Vector relationships
Geographic distribution

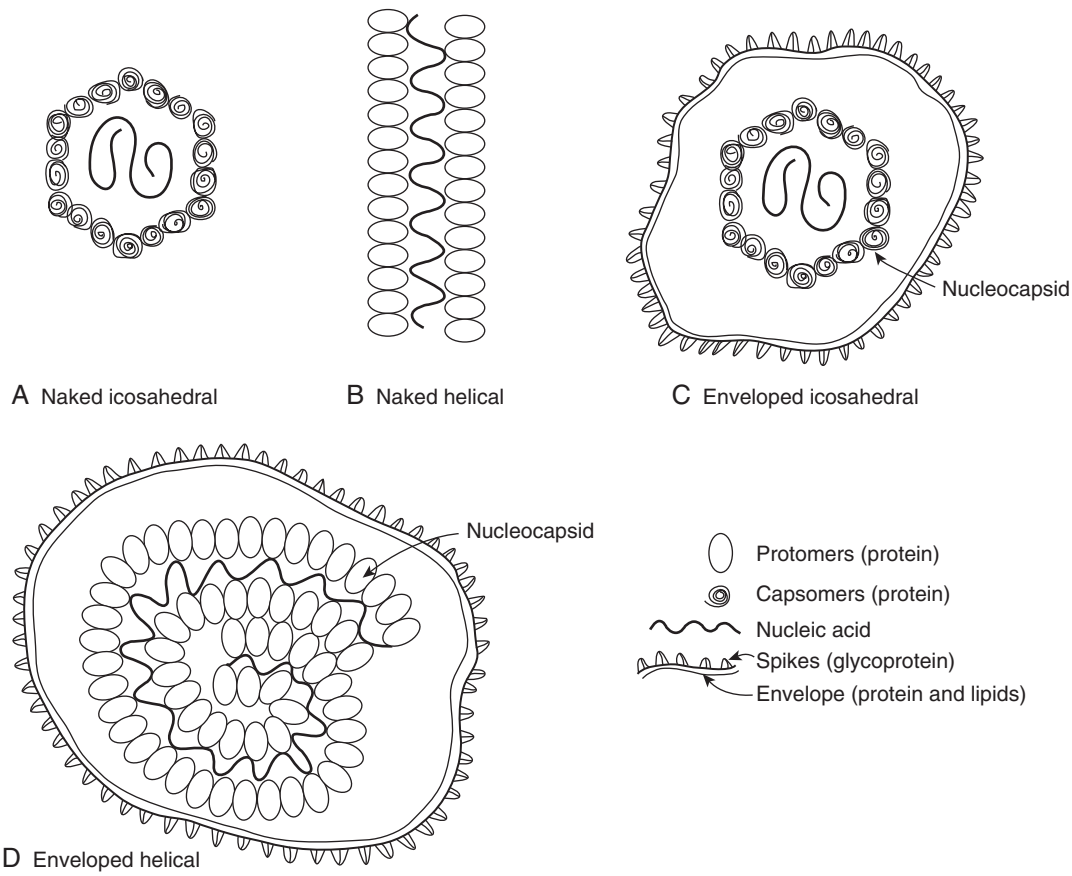

Figure 163–2 Schematic diagram of simple forms of virions and their components. The naked icosahedral virions resemble small crystals; the naked helical virions resemble rods with a fine regular helical pattern in their surface. The enveloped icosahedral virions are composed of icosahedral nucleocapsids surrounded by the envelope; the enveloped helical virions are helical nucleocapsids bent to form a coarse, often irregular, coil within the envelope. *(From Davis, B. D., Dulbecco, R., Eisen, H. N., et al.: Microbiology. 4th ed. Philadelphia, J. B. Lippincott, 1990, p. 772.)*

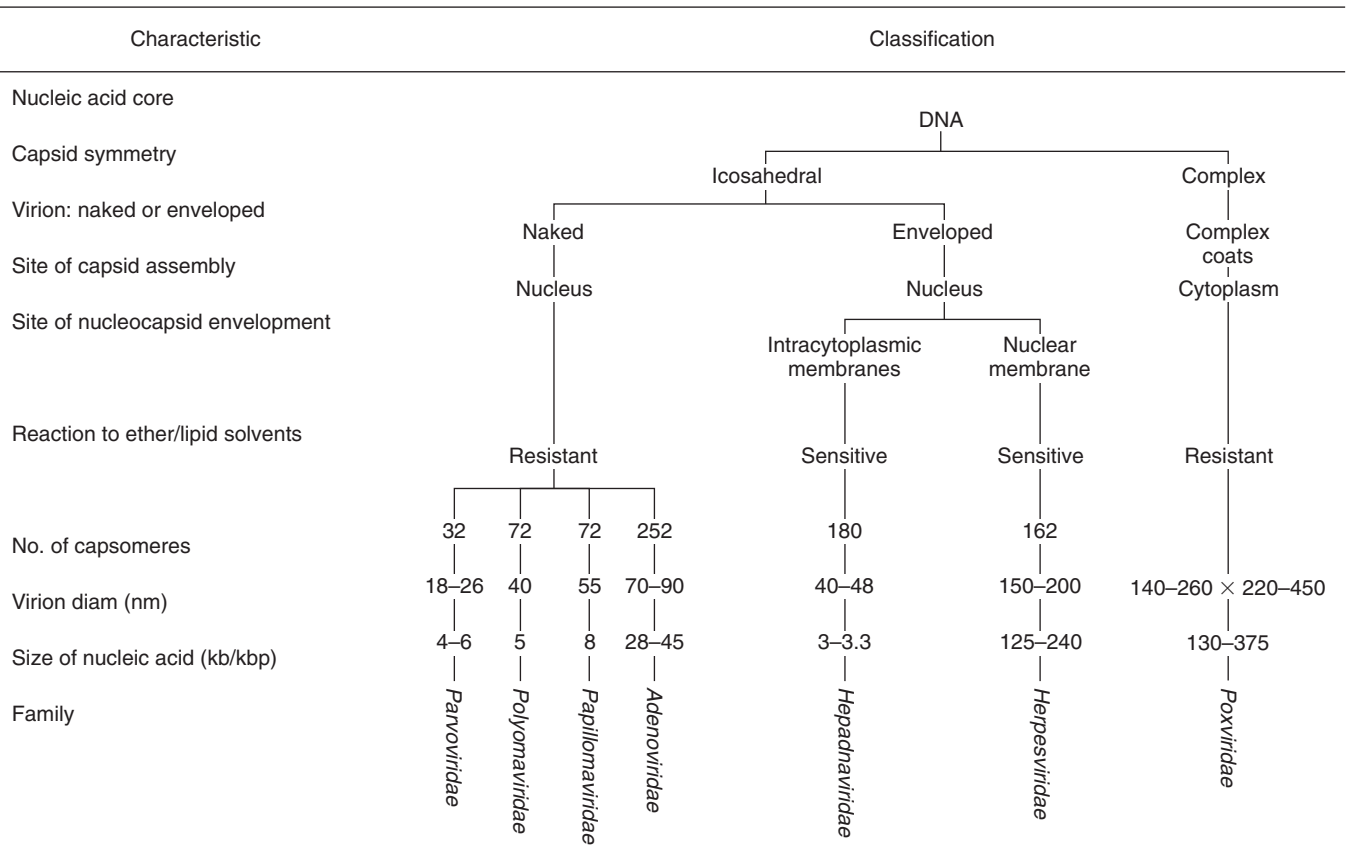

Figure 163–3 Classification of major DNA-containing viruses affecting humans. *(Adapted from Melnick, J. L.: Nomenclature and classification of viruses. In Feigin, R. D., and Cherry, J. D. [eds.]: Textbook of Pediatric Infectious Diseases. 4th ed. Philadelphia, W. B. Saunders, 1998, pp. 1603-1607.)*

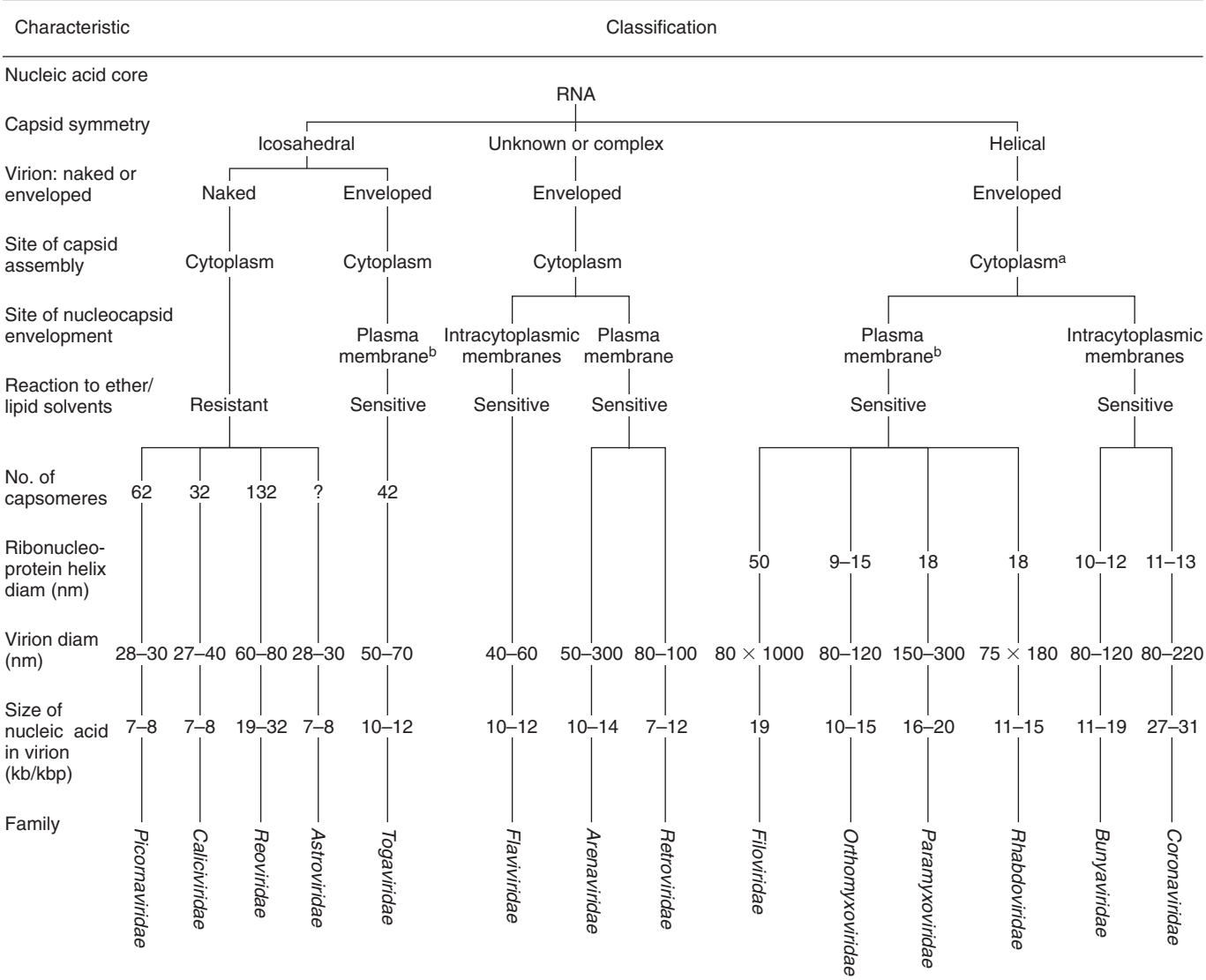

Figure 163–4 Classification of major RNA-containing viruses affecting humans. *(Adapted from Melnick, J. L.: Nomenclature and classification of viruses. In Feigin, R. D., and Cherry, J. D. [eds.]: Textbook of Pediatric Infectious Diseases. 4th ed. Philadelphia, W. B. Saunders, 1998, pp. 1603-1607.)*

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DNA VIRUSES

SUBSECTION 1

Parvoviridae

CHAPTER 164

Human Parvovirus B19

James D. Cherry ❂ Danica J. Schulte

The family *Parvoviridae* includes two subfamilies: *Parvovirinae* and *Densovirinae*.[191,313] The subfamily *Parvovirinae* has three genera: *Erythrovirus*, *Parvovirus*, and *Dependovirus*; the subfamily *Densovirinae* also has three genera: *Densovirus*, *Iteravirus*, and *Centravirus*. Densonucleosis viruses (*Densovirus* spp.) infect insect hosts and have not been found in humans or mammals.[35,353] Currently, human parvovirus B19 is the only accepted member of the genus *Erythrovirus*. Originally, it was classified in the genus *Parvovirus*. Despite this reclassification, the contemporary literature still refers to this *Erythrovirus* as parvovirus B19. This virus is autonomous and does not require the presence of a helper virus.

In 1999, *Erythrovirus* V9, a potential second member of the *Erythrovirus* genus, was found to be clinically significant and result in a spectrum of disease similar to that of B19.[165,343,399] V9 was found in the serum and bone marrow of a 6-year-old boy with transient aplastic anemia[319]; however, routine testing for B19 virus failed to identify the V9 virus.[165] V9 has greater than 11 percent variation from the published B19 genome, with nucleotide discrepancies encompassing the entire genome.[169,171] B19 typically is well conserved, with less than 2 percent sequence variability between isolates.[320] Another B19 variant, A6, has 12 percent variability from B19 DNA, and its clinical relevance is unclear.[118,488]

More recently, two additional parvoviruses have been discovered. One of these, human bocavirus, is presented in Chapter 165. The other virus, PARV4, was found in plasma samples from persons screened for acute human immunodeficiency virus (HIV) infection.[200,273]

Erythema infectiosum is the common clinical manifestation of infection with parvovirus B19. Other clinical findings include arthralgia and arthritis, aplastic crisis in patients with red blood cell defects, chronic anemia in immunocompromised patients, fetal hydrops, neurologic disease, and a variety of other significant illnesses.

HISTORY

The first parvoviruses to be discovered infecting humans were found in stool specimens by electron microscopy.[354] These fecal parvoviruses have been linked with gastrointestinal symptoms, often in outbreaks associated with the consumption of shellfish.[105,441] Their precise pathogenic role remains unclear because these viruses also may be found in the feces of asymptomatic individuals.[354]

In 1974, a second type of parvovirus was found in the serum of asymptomatic blood donors.[90,165] Discovered by chance as an agent responsible for false-positive results in the counterimmunoelectrophoresis tests then in use for detection of hepatitis B surface antigen, they were revealed by electron microscopy to be uniform icosahedral particles with a mean diameter of 23 nm.

Preliminary studies of the physiochemical nature of this agent suggested its probable identity as a member of the family *Parvoviridae*.[90] In the following years, reports concerning this virus named it variously as human parvovirus–like agent, serum parvovirus–like virus,[84] and B19[405] after one of the original isolates. After its chemical properties were elucidated in 1983,[79,424] the virus was referred to as *human parvovirus*—or *human serum parvovirus*—to denote that it is distinct from the fecal viral particles. Today, the virus most commonly is called *human parvovirus B19* or simply *B19 virus*.[10,85,206]

For many years after its discovery, infection with B19 virus appeared to be either asymptomatic or associated with a nonspecific febrile illness.[405] However, in the early 1980s, the central role of B19 virus in the etiology of aplastic crisis in patients with chronic hemolytic anemia was identified.[75,104,204,373,397] Soon thereafter came the appreciation that erythema infectiosum (fifth disease) is the common manifestation of infection with B19 virus.[17] Approximately 20 years ago, the association between B19 virus infection in pregnancy and fetal death was observed, and during the past 18 years, the clinical spectrum of B19 virus infection has broadened considerably.*

Although human parvovirus B19 was not discovered until 1974, its most common clinical disease (erythema infectiosum) was described more than 200 years ago.[44]

Tschamer's report in 1889 was considered by most reviewers to be the first description of erythema infectiosum.[437] However, Boysen[44] noted that the English dermatologist Robert Willan had reported the illness first in 1799 and then more completely in 1840.

After Tschamer's report came a succession of Austrian and German reports of the illness.[44] Tschamer thought that erythema infectiosum was a manifestation of rubella, but a few years later, Escherich[120] suggested that it was a specific disease. Stricker[422] in 1899 gave the name *erythema infectiosum* to the clinical entity.

In 1905, Shaw,[404] after having observed cases of erythema infectiosum in Austria, published the first account in the American literature. Herrick[177] was the first to document in detail an outbreak in the United States; however, cases of erythema infectiosum had been observed previously in St. Louis[494] and in Hamburg, New York.[177] During the past 75 years, numerous outbreaks and epidemics have been described in North America.† Erythema infectiosum frequently is referred to as *fifth disease* and

*See references 8, 40, 72, 165, 168, 180, 206, 207, 312, 359, 391, 396, 472, 488.

†See references 3, 21, 25, 26, 30, 46, 63, 70, 77, 81, 86, 106, 130, 135, 141, 147, 165, 216, 248, 249, 259, 294, 314, 360, 361, 375, 408, 453, 454, 467, 470, 488, 498.

in the past also was called *ring rubella*, *large-spotted disease*, and *epidemic megaloerythema*.

PROPERTIES OF THE VIRUS

Human parvovirus B19 is a naked icosahedral virus with a mean diameter of 23 nm and a mean buoyant density in cesium chloride of 1.43 g/dL.[90] The capsid is formed from two major structural proteins (VP1 and VP2) discernible by sodium dodecyl sulfate–polyacrylamide gel electrophoresis.[79] VP2 is the major structural protein, with a molecular weight of 58 kd; VP1 has a molecular weight of 84 kd. Ninety-six percent of the capsid is VP2, and 4 percent is VP1. The structure of VP2 has been determined by x-ray crystallography.[165,203] The major nonstructural protein (NS1) has a molecular weight of 77 kd. The genome consists of a single molecule of single-stranded DNA approximately 5.6 kb in length.[96] The DNA in B19 virus occurs as both plus and minus strands in approximately equal numbers.[206] When virions are disrupted with protease, the two complementary strands anneal to form a stable duplex.[424] At each end of the molecule are palindromic sequences forming "hairpin" loops. The hairpin at the 3′ end of the genome serves as a primer for DNA polymerases. The hairpin duplex at the 5′ end of the molecule consists of sequences that are neither complementary to those at the 3′ end, as is found in dependoviruses, nor as highly complementary within this 5′ end as are those at the 3′ end.[64] Folding of the VP1 and VP2 proteins creates alpha-helical loops that appear on the surface of the capsid and are available to the host immune system. The unique region of VP1 is external to the capsid; it contains linear epitopes recognized by neutralizing antibodies and elicits a more efficient immune response than the VP2 region does.[488]

Parvovirus infectivity is relatively heat-stable, tolerant of a wide range of pH, and resistant to ether.[18] Successful replication of parvoviruses can be accomplished only in a dividing cell because of the absolute requirement for the host-cell function or functions found in late S phase.[161] Human parvovirus B19 can be propagated only in human erythropoietic cells from bone marrow and in primary fetal liver culture.[52,353,416,417,429]

The cellular receptor for B19 virus infection is the blood group P antigen, which is a globoside and a neutral glycolipid.[49] The P antigen also requires a cellular co-receptor, $\alpha_5\beta_1$ integrin, for sufficient entry of parvovirus B19 into human cells.[463] P antigen is found on erythroblasts, megakaryoblasts, endothelial cells, and fetal myocytes.[49] People who lack P antigen are resistant to infection with B19 virus, and in vitro studies demonstrate that the erythroid precursors from people lacking P antigen cannot be infected, even in the presence of high concentrations of virus.[7,51,165]

Antigenically, this human erythrovirus is distinct from the parvovirus-like particles found in feces,[355] as well as from the human dependoviruses and autonomous animal parvoviruses.[90] However, the nucleotide sequence and hybridization results reported by Turton and associates[441] suggest that the viruses from gastroenteritis cases in 1977 and 1986 are similar to B19. In contrast to animal parvoviruses, no hemagglutinin has been demonstrated.

The degree to which B19 virus is related to other mammalian parvovirus types has been investigated by DNA-DNA hybridization. Although no relationship is discernible with the human dependoviruses, a distant evolutionary relationship to the genomes of the autonomous parvoviruses of rodents is apparent. Interestingly, this relationship is closer than that between B19 virus and the parvoviruses infecting domestic animals (bovine, feline, and canine parvoviruses).[91] Shackelton and Holmes[402] studied the evolutionary dynamics and phylogenetic history of the *Parvoviridae* and observed a high rate of evolutionary change, 10^{-4} nucleotide substitutions per site per year.

Many recent studies have examined genetic variability in parvovirus B19 isolates.[40,117,136,175,198,214] In general, sequence divergence usually is less than 1 to 2 percent.[169,171,320,434] Hemauer and associates[175] suggested that greater genome variability occurs in isolates from patients with persistent infection than in isolates from patients with acute infection. Erdman and colleagues[117,434] noted that geographically defined genetic lineages of parvovirus B19 existed but that no particular genotype was associated with a specific clinical manifestation.

Parvovirus B19 does not grow in routine tissue cultures. It will grow in erythroid progenitor cells from human bone marrow, fetal liver, erythroid cells from a patient with erythroleukemia, and human umbilical cord and peripheral blood.[165,206,348] It grows only in dividing cells, and, therefore, all the aforementioned tissue culture systems require the addition of erythropoietin. Parvovirus B19 also has been propagated in megakaryoblastoid cell lines.

EPIDEMIOLOGY

Outbreaks of erythema infectiosum have been observed throughout the world, but most reports have come from nontropical regions. The epidemic pattern of erythema infectiosum is surprisingly similar to that of rubella. Community epidemics are most prevalent in the winter and spring, and they usually last for 3 to 6 months. In a review of 30 well-described epidemics,* 26 had their onset in the period from December through May, and 23 of the 30 peaked in March, April, or May. When the North American literature for a 50-year period is examined, a cyclic pattern is evident, with peaks in disease activity occurring approximately every 6 years. The peak periods last for an average of 3 years. A longitudinal study of aplastic crisis in persons with sickle-cell anemia in Jamaica suggested that peaks in incidence occur every 2 to 3 years in this island population.[397]

The case-to-case interval of erythema infectiosum is reported to be between 4 and 14 days.[3,17,106,147,366,453,470] In an epidemic in an elementary school, Greenwald and Bashe[147] noted that the mean case-to-case interval was 8.7 days. Ager and associates[3] in their studies noted clustering of intervals between cases of 7 to 11 days. The data of Wilcox and Evans[470] on multiple cases in households suggest that the case-to-case interval usually is closer to 12 to 14 days. In a volunteer study in which adults were inoculated intranasally, the incubation time until onset of the rash was 17 to 18 days.[14] From this study, the case-to-case interval in the community would be expected to be between 6 and 12 days because shedding of virus occurs between days 5 and 12 of infection. This prediction accords well with intervals in the studies noted earlier, as well as intervals observed in patients with hematologic diseases and aplastic crisis.[300]

In epidemics, the attack rate is high. Lauer and colleagues[248] noted an overall attack rate of 24.3 percent in schoolchildren in grades kindergarten through the eighth grade. Similar attack rates were noted in three other school-related outbreaks.[106,143,147] In the community as a whole, the attack rate is highest in children 5 to 14 years of age,[2,3] but secondary cases occur in preschool children, teachers, and parents. In the home, secondary cases are reported more commonly in mothers than in fathers.[3] In school epidemics, the attack rate is considerably higher in girls than in boys.[3,106,248] Prevalence studies of serum IgG antibody to B19 virus have noted that 40 to 60 percent of adults and 2 to 21 percent of children younger than 11 years are seropositive.[10-12,84] The rate of transmission among household contacts is nearly 50 percent.[445]

*See references 3, 12, 21, 26, 44, 46, 70, 77, 81, 86, 106, 130, 147, 177, 216, 248, 249, 259, 314, 361, 375, 408, 419, 453, 466, 470, 498.

Nosocomial infections do occur; most often, the index case is an unrecognized, chronically infected patient.[2,236,363] During community outbreaks, the risk of acquisition by hospital workers is no greater than that in other community residents.

The role of human leukocyte antigen (HLA) class I and II alleles was investigated by Kerr and associates[217] in northwestern England. Thirty-six patients with symptomatic B19 infection and 900 controls were studied. The frequency of HLA alleles DRB1*01, DRB1*04, and DRB1*07 was significantly higher in patients with B19 infection than in control subjects.

In a cohort study of 169 children with severe anemia in Papua New Guinea, a strong association between severe anemia and B19 infection was observed; however, the authors were unable to show a combined deleterious effect of malaria and B19 infection.[467] They and others demonstrated that 60 percent of the children were infected with B19 before reaching 2 years of age and that greater than 90 percent were infected by 6 years of age.[152,352,388,469]

Although erythema infectiosum long had been postulated to be transmitted by droplet via the respiratory tract,[3] proof of this route was not obtained until a group of volunteers were infected successfully with B19 virus after intranasal inoculation. One week after inoculation, virus was excreted from the respiratory tract for 6 days.[14]

Parvovirus B19 infection also can be transmitted by blood transfusion and clotting factor concentrates.*

One case of transmission of B19 DNA via peripheral blood progenitor cell transplantation that resolved after treatment with intravenous immune globulin (IVIG) has been reported.[19] Hayakawa and colleagues[162] reported a case of parvovirus B19 infection that they thought was transmitted by IVIG. Researchers have described donor-transmitted parvovirus infection in transplant patients.[478]

PATHOGENESIS AND PATHOLOGY

The pathogenesis of erythroviral disease involves two quite separate components. The first is caused by the lytic infection of susceptible dividing cells and the second by interaction with products of the immune response.

As stated earlier, parvoviruses replicate only in dividing cells. Thus, infection of an organ or tissue in which a significant proportion of the cells are dividing may give rise to organ-specific disease. This condition is seen clearly in canine and feline parvovirus infections, in which replication of virus in the crypt cells of the intestine gives rise to a severe and often fatal enteritis.[157,268]

Parvovirus B19 is thought not to infect cells of the gastrointestinal tract; virus could not be detected in the feces of volunteers,[14] nor have viruses of this type been found in stool specimens.

After gaining entry via the respiratory tract, the virus sets up a systemic infection with copious viremia (Fig. 164–1) in which 10^{10} or 10^{11} virus particles per milliliter of blood is not an uncommon finding.[14,368]

Parvovirus infection results in profound reticulocytopenia for some 7 to 10 days, commencing during viremia.[14,488] In vitro studies on cultured bone marrow and peripheral blood have shown that B19 virus inhibits the formation of blast-forming erythroid colonies, thus suggesting that an early erythrocyte precursor cell is susceptible to infection with the virus. B19-associated damage to cells of the erythroid lineage is due to the cytotoxicity mediated by viral proteins, whereas the apoptotic features mediated by caspase 3 are induced by NS1.[74] In addition to profound erythroid hypoplasia, giant pronormoblasts typically

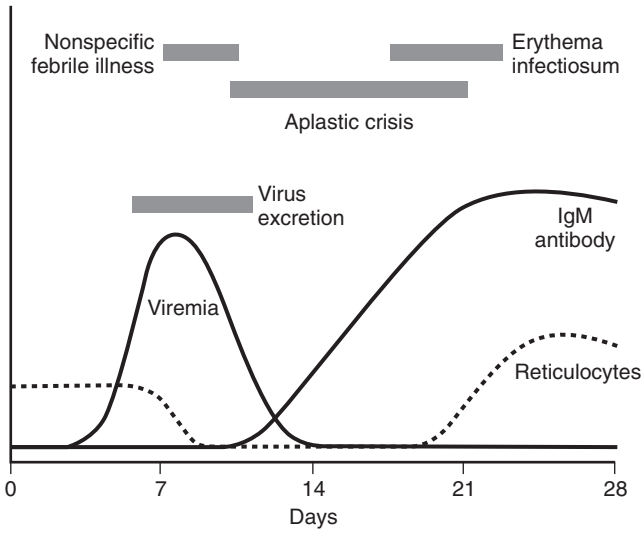

Figure 164–1 Selected virologic, immunologic, hematologic, and clinical events in parvovirus B19 virus infection.

are seen in the bone marrow at the height of transient aplastic crises, and normoblasts have characteristic intranuclear eosinophilic inclusion bodies termed *Lampion* or *Lantern cells*.[56]

Granulocytes and megakaryocytes are unaffected by virus in these in vitro systems. However, during B19 virus infection in a normal host, clinically insignificant lymphopenia and neutropenia occur, as does a drop in platelet number.[14,366] The mechanisms of loss of these cells from peripheral blood remain to be determined. Srivastava and associates[416] noted that B19 virus infection of bone marrow cells in culture results in suppression of megakaryocyte colony formation. In this study, tropism of B19 virus for cells other than erythroid progenitor cells appeared to take place, although viral DNA replication did not occur in the megakaryocyte-enriched fractions. They suggested that the virus might be toxic to cell populations that are nonpermissive of viral DNA replication.

As noted in Figure 164–1, viremia ends at the time of appearance of specific IgM antibody. In addition, researchers have found that the appearance of antibody neutralizes the inhibitory effect of B19 virus on formation of erythrocyte colonies in vitro.[489] The early IgM response is almost entirely VP2-specific. IgG antibody first appears approximately 2 weeks after exposure and later becomes the major antibody subclass; in contrast to the initial IgM response, the IgG response is directed at VP1 rather than VP2. The role of cellular immunity in recovery from infection is unclear. Most patients with persistent infection have T-cell as well as other immune defects, thus suggesting a cellular component in the host response.[40] Surprisingly, few studies of cellular immunity have been performed in patients with parvovirus B19 infection.[133,241,451] von Poblotzki and associates[451] showed that the T cells of persons with parvovirus B19 antibody had HLA class II–restricted responses against B19 structural proteins. Franssila and colleagues[133] demonstrated virus-specific helper T-cell proliferation in recently and remotely infected patients. Their data suggested that B cells that recognize the viral capsid (VP1 and VP2) receive class II–restricted help from $CD4^+$ T lymphocytes. While investigating a parvovirus B19 vaccine, this same group demonstrated that VP1 contains important B-cell epitopes required for protective B-cell immunity, and they concluded that VP2, the major structural protein of B19 virus, appears to provide the major target for B19-specific helper T cells years or decades after initial infection occurs.[131,132] A persistent, activated B19-specific $CD8^+$ T-cell repertoire with maintained effector function occurs in healthy patients who successfully

*See references 19, 102, 165, 283, 302, 365, 415, 473, 480, 491.

clear the B19 virus. Researchers have hypothesized that these activated $CD8^+$ T cells may play a prominent role in controlling acute B19 infection.[192,202,260,330] Additionally, Kerr and colleagues[208,213] reported that circulating cytokine and chemokine levels in acute B19 disease may correlate with clinical disease.

The most common result of infection with B19 virus is erythema infectiosum. Figure 164–1 shows that the symptoms of this disease begin 17 or 18 days after inoculation and that approximately 1 week after inoculation, virus can be detected in either throat swabs or blood. Skin biopsy results have been reported in three studies.[30,148,182] In three skin biopsy specimens from regions of reticulated eruption, Bard and Perry[30] noted either normal skin or very mild inflammatory changes. Hoffman[182] described edema in the epidermis and perivascular infiltration with mononuclear cells; he also noted swelling of the endothelium of superficial vessels and the presence of cleavage spaces between the epidermis and dermis. The histopathology in 10 cases was investigated by Grimmer and Joseph.[148] They reported dilation of blood vessels with lymphocytic and occasional plasma cell perivascular infiltration. However, the clinical manifestations of many of the cases in the epidemic that Grimmer and Joseph[148] studied were quite atypical.

Virus-specific IgM is present at the time of onset of the rash in erythema infectiosum, so although the mechanisms of production of the rash (and arthralgia) of erythema infectiosum remain to be elucidated, postulating an immune-mediated pathogenesis is not unreasonable.[488] The perivascular infiltrations noted by Hoffman[182] would support this suggestion. However, Schwarz and colleagues[395] have found both viral capsid proteins and viral DNA in a skin biopsy specimen of the rash from a patient with erythema infectiosum. They, therefore, suggest that the rash may be a direct effect of the virus rather than being immune complex mediated.

Intrauterine infection results in infection of the fetus and, frequently, abortion.* The main finding in infected fetuses is hydrops fetalis, which results from the anemia caused by infection of the erythrocyte precursor cells. Intranuclear inclusions are seen in nucleated red blood cells, and viral particles are identified in the same cells by electron microscopy.[67] The mechanism responsible for hydrops fetalis stems from the severe anemia, and cardiogenic failure results in generalized ascites, edema, pericardial and pleural effusions, and a thick hydropic placenta.[135] Concomitant myocarditis also occurs, which complicates the clinical picture because the blood group P antigen is expressed on fetal cardiac myocytes.[49,74] The shorter half-life of fetal erythrocytes also exacerbates the disease.[74]

In immunocompromised patients, persistent infection with B19 virus often occurs[241] as a result of failure to produce effective neutralizing antibodies.[74] Patients taking immunosuppressive calcineurin inhibitors may be unable to clear B19 virus because these medications inhibit interleukin-2 (IL-2) production and the patients are unable to mount an effective T_H1 response.[74,295]

CLINICAL MANIFESTATIONS

Infection with human parvovirus B19 results in a spectrum of clinical manifestations; classic cases of erythema infectiosum occupy a central position in this spectrum. Other major manifestations include arthritis and arthralgia, intrauterine infection and hydrops fetalis, transient aplastic crisis in patients with a variety of underlying hemolytic illnesses, and persistent infection with chronic anemia in patients with immunodeficiencies. In addition, subclinical, nonexanthematous infection occurs, especially in children.[14,366]

*See references 8, 10, 63, 67, 97, 141, 165, 221, 371, 379, 393, 472, 487, 488.

Other less common illnesses include myocarditis, vasculitis, glomerulonephritis, and neurologic disease. In addition to the established parvovirus B19 disease associations is an ever-expanding list of categories of illness in which evidence of parvovirus B19 infection has been found. These illnesses are presented in Table 164–1.

TABLE 164–1 Reported Unusual Clinical Findings in Patients with Laboratory Evidence of Parvovirus B19 Virus Infection

- **Persistent Arthritis**
  - Juvenile rheumatoid arthritis[73,223,338]
  - Rheumatoid arthritis[324,418,428]
  - Adult Still disease[261,369]
- **Neurologic Disease**
  - Encephalitis[27,126,170,209,420]
  - Meningitis[64,234,344,419,425]
  - Stroke[149,272]
  - Postinfectious neuralgic amyotropy[29,356]
  - Guillain-Barré syndrome[29,290]
  - Transverse myelitis[29]
  - Facial palsy[274]
  - Carpal tunnel syndrome[29,387]
  - Numbness and tingling of fingers[4,29,492]
  - Myofasciitis[187]
- **Myocarditis**[41,57,98,113,201,237,238,245,309,321,351,384,493]
- **Cutaneous Manifestations**
  - Papular-purpuric gloves and socks syndrome[6,9,23,37,110,155,184,286,346,409,435,446]
  - Vesiculopustular exanthema[317]
  - Erythema multiforme[139]
  - Henoch-Schönlein syndrome[76,449]
  - Petechial and purpuric rashes[1,87,103,186,254,282]
  - Pruritus without rash[267]
- **Hematologic Manifestations**
  - Thrombocytopenic purpura[5,38,127,190,253,486]
  - Pancytopenia[158,235,306]
  - Hemophagocytic syndrome[43,306,440,444,460,490]
  - Neutropenia[279]
  - Diamond-Blackfan syndrome[166,432]
  - Splenic sequestration[270]
- **Other Manifestations**
  - Sepsis[24]
  - Glomerulonephritis[189,195,234,297,468]
  - Nephrotic syndrome[340]
  - Kawasaki disease[183,323]
  - Behçet disease[222]
  - Polyarteritis nodosa[450]
  - Wegener granulomatosis[325]
  - Leukocytoclastic vasculitis[88]
  - Giant-cell arteritis[137]
  - Systemic sclerosis[124,125,343]
  - Systemic vasculitis[95]
  - Systemic lupus erythematosus[99,188,322]
  - Hepatitis[103,246,327,414,484]
  - Raynaud phenomenon[159]
  - Pseudoappendicitis or mesenteric lymphadenitis[298]
  - Necrotizing lymphadenitis[199]
  - Cervical lymphadenopathy[224,439]
  - Parotitis[274]
  - Pneumonia[299,420,459]
  - Pulmonary emboli[20]
  - Acute respiratory distress syndrome[123]
  - Chronic infection without immunosuppression but with chronic hemolytic anemia[215]
    - Persistent arthralgia[214]
    - Chronic fatigue syndrome[196,211,212,214,220,224,276,280]
    - Recurrent paresthesias[121]
    - Fibromyalgia[34]

ERYTHEMA INFECTIOSUM

Although a search of the literature related to outbreaks of erythema infectiosum reveals a conspicuous absence of prodromal symptoms in patients, infected volunteers have had febrile episodes with nonspecific symptoms of headache, chills, myalgia, and malaise accompanying the viremic phase of infection with B19 virus (see Fig. 164–1).[14] These symptoms last for 2 to 3 days and coincide with excretion of virus from the pharynx. This phase is followed by a period of 7 days during which individuals are free of symptoms before onset of the second, or exanthematous, phase of illness. Most likely, the relatively long period between symptoms in this biphasic illness prevented recognition of the link between these nonspecific prodromal symptoms and erythema infectiosum.

The exanthem in classic cases of erythema infectiosum occurs in three stages.* The first stage begins 18 days after the acquisition of infection and is characterized by a fiery red rash on the cheeks (see Fig. 64–6) ("slapped-cheek" appearance). The edges of the involved areas may be raised slightly, and a relatively circumoral pallor is present. At this stage, the appearance may be suggestive of scarlet fever, drug sensitivity or other allergic reactions, or collagen vascular disease. The facial exanthem is aggravated by the transition from outdoors to a warm room.

The second stage of the exanthem occurs 1 to 4 days after the onset of facial involvement and consists of the appearance of an erythematous, maculopapular rash on the trunk and limbs. This rash is discrete initially but spreads to involve large areas. Toward the end of this stage, central clearing of the rash from these areas gives the characteristic lacy or reticular pattern (see Fig. 64–7).

The third stage of the exanthem is highly variable in duration (i.e., lasting from 1 to 3 or more weeks) and is characterized by marked changes in the intensity of the rash, with periodic complete evanescence and recrudescence. These fluctuations are related to environmental factors such as exposure to sunlight and temperature (a hot bath may result in recrudescence in an apparently recovered child).

The rash often is pruritic, especially in adults, and it generally is more prominent on the extensor surfaces; the palms and soles rarely are affected. Slight desquamation has been noted in a small number of patients in most reviews.

Although classic cases of erythema infectiosum are easy to recognize clinically, especially during outbreaks, wide variation in the form of the exanthem can be noted—from a very faint, fleeting rash to a florid exanthem that is confluent over large areas (see Fig. 64–8). In many cases the illness may be indistinguishable from rubella.

The overwhelming majority of cases of erythema infectiosum have no enanthem. Kerr and Marsh[216] and Condon[86] noted a few children with pharyngitis.

Other symptoms and signs occur uncommonly in erythema infectiosum (Table 164–2). In general, complaints are expressed more frequently by adults than by children. Headache is reported in approximately a fifth of childhood cases and about half of afflicted adults. Joint pain and swelling and myalgia are particularly troublesome in adults.

Routine laboratory studies are of little use in erythema infectiosum. The leukocyte count usually is normal, although mild eosinophilia is noted occasionally.[25,44,454,455]

The most common complication of erythema infectiosum is joint involvement; it is relatively rare in children (<10% of cases) but is the norm in adults and occurs in 80 percent or more of cases.† A range of severity from mild arthralgia to frank arthritis is observed. The joints most commonly involved are the knees, ankles, and proximal interphalangeal joints; symptoms generally are bilateral. The joint involvement usually is transient and lasts only a few days. In some individuals, symptoms may persist for some weeks or, rarely, months. When joint involvement occurs after an exanthem, the diagnosis may be inferred, but such is not invariably the case. As with rubella, the frequency of arthralgia and arthritis is higher in women than in men.

TABLE 164–2 Frequency of Symptoms and Signs in Erythema Infectiosum

Sign or Symptom	Percent Occurrence	
	Children	Adults
Rash	100	100
Pruritus	15	50
Headache	20	50
Fever	15	25
Sore throat	15	15
Coryza	10	15
Cough	8	8
Sore eyes	10	10
Anorexia	15	22
Nausea	7	26
Vomiting	4	7
Diarrhea	4	12
Abdominal pain	10	15
Joint pain	10	70
Joint swelling	5	60
Myalgia	4	50

Data from references 3, 106, 147, 454.

OTHER EXANTHEMS

Grimmer and Joseph[148] described cases in which the rashes were morbilliform, hemorrhagic, urticarial, vesicular, and erythema multiforme–like. Their studies were conducted during an epidemic of erythema infectiosum in Berlin, Germany, in which an estimated 50,000 persons were affected. The cases with markedly unusual manifestations quite possibly were not of the same etiology as the typical cases. Other authors also have noted papular, purpuric, petechial, vesicular, urticarial, and morbilliform eruptions.*

García-Tapia and associates[139] described a 5-year-old girl with erythema multiforme bullosum but without other systemic manifestations. Her serum had B19 virus–specific IgM antibody. Additionally, a papular-purpuric or petechial "glove and sock" syndrome has been reported with acute B19 infection.† It is more commonly seen in adults but has been noted in children.

This unique rash is characterized by petechiae and purpura, with painful or pruritic edema of the hands and feet extending proximally with less severity, and it is self-limited.[6,184] A 14-year-old girl with acute parvovirus B19 infection had a petechial-purpuric pruritic rash with edema on her hands and feet, as well as severe leukopenia and mild thrombocytopenia on laboratory evaluation.[286] Henoch-Schönlein syndrome and other vasculitic rashes also have been reported.[1,76,88,103,173,343,355,391,449]

Grimmer and Joseph[148] stated that most of their patients in the Berlin epidemic had dark red spots on the pharynx, gums, soft palate, and uvula. These cases, again, must be regarded with some suspicion because the investigators also noted genital lesions and conjunctivitis in a few patients.

*See references 3, 25, 46, 70, 73, 77, 147, 404, 445, 453-455.
†See references 10, 16, 17, 25, 163, 168, 315, 376, 378, 456, 467.

*See references 70, 86, 87, 128, 186, 254, 270, 282, 317, 361, 477.
†See references 4, 6, 9, 23, 37, 110, 155, 184, 186, 407, 409, 435, 445, 446.

APLASTIC CRISIS

In individuals with chronic hemolytic anemia, the profound reticulocytopenia of human parvovirus B19 infection results in depression of hemoglobin concentrations to critical levels.* With resolution of the infection, reticulocytes reappear in the peripheral blood and hemoglobin concentrations return to the normal steady-state values for these patients. This transient arrest in production of erythrocytes is termed *aplastic crisis* and may occur in any individual whose erythrocytes have a short life span. Examples of such conditions include sickle-cell anemia,[144,397,410,497] hereditary spherocytosis,[119,158,204,333,339,465] thalassemia,[252,254,373] glucose-6-phosphate dehydrogenase deficiency,[142] and pyruvate kinase deficiency.[104] However, there are case reports of aplastic anemia secondary to B19 virus developing in immunocompetent patients without underlying diseases.[265,291,372]

Serjeant and coworkers[398] studied the epidemiology of B19 virus infection over time in 308 children with homozygous sickle-cell disease and in 239 controls with normal hemoglobin. B19 virus infection accounted for all 91 episodes of aplastic crisis that occurred. Twenty-three additional patients with sickle-cell disease had B19 virus infections; of these, 10 had mild hematologic changes and 13 had no changes. By 15 years of age, approximately 40 percent of the sickle-cell group and the control group had IgG antibody to B19 virus, thus indicating equal infection rates in the two groups. No patient or control had two infections caused by B19 virus.

Smith-Whitley and associates[410] studied a cohort of 633 patients with sickle-cell disease (including patients with various sickle-cell disease genotypes—SS-, SC-, Sβ-thalassemia—as well as those receiving chronic transfusions and hydroxyurea) for complications attributable to acute B19 infection. At study entry, 29.8 percent of the cohort had IgG antibody to B19 virus. The secondary attack rate in the group of children who had siblings with sickle-cell disease was 56 percent. Thirty-five percent of the total patient population with B19 infection did not have worsening anemia; however, transient red cell aplasia developed in 50 percent of the children with SC- or Sβ-thalassemia, genotypes characterized by less severe disease. Neutropenia developed in 18 percent of patients and thrombocytopenia in 27 percent. In only one of seven patients receiving chronic transfusions did transient red cell aplasia develop, potentially because of the lower red cell turnover in these patients.

Rao and colleagues[374] found that 70 percent of patients with transient aplastic crisis admitted to their hospitals during a 7-year period had B19 viral infections. No patients had chronic or recurrent infections. In a study of 48 patients with aplastic crises, Mallouh and Qudah[271] found 91 percent to be infected with B19 virus. In addition to the anemia, 21 percent of the patients had leukopenia, 27 percent had neutropenia, and 42 percent had thrombocytopenia. The same investigators noted acute splenic sequestration together with aplastic crisis in three patients with sickle-cell disease and B19 virus infection.[270] Lowenthal and associates[263] noted three young adults with sickle-cell acute chest syndrome associated with B19 virus infection. Yetgin and colleagues[483] reported a case of a 10-year-old child with severe, refractory aplastic anemia ultimately requiring bone marrow transplantation, which resolved the symptoms.

Interestingly, reports of exanthematous illness occurring after aplastic crisis are rare.[205,465] However, patients with aplastic crisis require transfusion with packed cells, and rashes occurring after such treatment possibly would be regarded as transfusion reactions. Joint symptoms occur fairly frequently in patients with conditions such as sickle-cell anemia, so although the symptoms may occur as a result of B19 infection, they may be diagnosed as "painful crises."

As noted earlier, human parvovirus B19 infection does not result in aplastic crisis invariably in a chronic hemolytic anemic patient. Some individuals escape this complication if they have undergone transfusion recently,[13] possibly because of a protective effect of transfused antibody (>60% of donors are immune), the substitution of longer-lived, donated erythrocytes for the patients' own fragile ones, or a combination of these two mechanisms.

In populations in which aplastic crisis does occur, the severity of the episode varies among individuals, perhaps reflecting the variation in erythrocyte life span among these patients.[13]

OTHER HEMATOLOGIC MANIFESTATIONS

In addition to pure red blood cell aplasia, numerous other hematologic manifestations, including thrombocytopenic purpura, pancytopenia, hemophagocytic syndrome, neutropenia, and Diamond-Blackfan syndrome, have been observed in association with B19 virus infection.* Thrombocytopenia has been noted as an isolated event, thus suggesting that idiopathic thrombocytopenic purpura may be associated with aplastic crisis.[5,127,190,253,312,486] Thrombocytopenia is most commonly related to a decrease in megakaryocytes in bone marrow as a result of the inhibitory effect of the virus on granulocyte-megakaryocyte colony-forming-units,[5,367,465] although acquired pure amegakaryocytic thrombocytopenic purpura with resultant absence of bone marrow megakaryocytes secondary to B19 virus also has been described.[38]

Pancytopenia has been observed in association with aplastic crisis, and hemophagocytosis often is demonstrated.† Yufu and coauthors[490] described a 15-year-old girl with hemophagocytic syndrome and lymphadenopathy resembling histiocytic necrotizing lymphadenitis (Kikuchi disease).

McClain and colleagues[279] studied 19 children with immune-mediated neutropenia and found by polymerase chain reaction (PCR) assay of bone marrow specimens that 15 had evidence of B19 virus infection.

ARTHRITIS AND ARTHRALGIA

As noted earlier, acute arthritis and arthralgia occur commonly in patients with erythema infectiosum. In most instances, the joint symptoms subside within a few weeks, but the arthralgia and arthritis persist occasionally. Researchers also have observed that the joint manifestations caused by B19 virus infection can occur without any exanthem or with an exanthem not typical of erythema infectiosum. These findings have led to investigations related to the role of B19 virus in rheumatoid arthritis (RA).‡

Nocton and associates[328] described the clinical characteristics of 22 children seen in their rheumatology clinic with joint complaints and evidence of recent B19 virus infection. Of this group, 16 were girls and 6 were boys. The youngest patient was 2 years old, and the oldest was 19 years of age. Seven of the patients had associated rashes, but none had typical erythema infectiosum. One of these children had a petechial rash on the lower extremities, and another child had a past history of urticaria. Fever, anorexia, malaise, or fatigue occurred in 11 of the patients.

*See references 10, 13, 63, 104, 142, 144, 158, 204, 205, 254, 300, 333, 339, 373, 382, 397, 410, 424, 465, 487, 489, 497.

*See references 5, 43, 127, 158, 165, 166, 168, 190, 207, 235, 251, 253, 257, 279, 306, 312, 370, 432, 440, 444, 460, 486, 492.

†See references 43, 158, 168, 207, 235, 247, 306, 312, 437, 444, 460, 490.

‡See references 65, 129, 154, 168, 206, 207, 223, 289, 293, 324, 328, 369, 413, 418, 428.

Of the 22 patients, 20 had arthritis and 2 had only arthralgia. The knee joints were most commonly involved. The following frequency of specific joint involvement was reported: knees, 82 percent; ankles and wrist, 41 percent; elbows, 32 percent; neck, small joints of the hands and feet, and hips, 27 percent; shoulders, 23 percent; and sternoclavicular joints, 9 percent. Ten of the patients had involvement of five or more joints, and in 12 patients the pattern was pauciarticular, with four or fewer joints involved. Two patients had only a single joint involved, and two patients had migratory illness.

The duration of the arthritis or arthralgia was less than 6 weeks in 50 percent of the children and less than 4 months in 64 percent. Of the 11 children with illness lasting longer than 2 months, 6 had manifestations that fulfilled the criteria for the diagnosis of juvenile rheumatoid arthritis (JRA). Selected laboratory results were as follows: the erythrocyte sedimentation rate was greater than 25 mm/hr in 32 percent of the children, antinuclear antibody titers were greater than 1:40 in 7 of 21 of the patients, and 3 of 6 children had a low total hemolytic complement (CH_{50}) value.

The relationship between B19 virus infection and JRA, RA, and adult Still disease has been studied extensively during the past 15 years, but the findings are difficult to interpret.* Clearly, B19 virus can cause illnesses that fulfill the criteria for a diagnosis of JRA[53,328,338,467] and adult Still disease.[207,261] However, three possible scenarios need to be considered: (1) B19 virus causes a similar illness, (2) B19 virus infection triggers the onset of an illness caused by another condition, and (3) B19 virus directly causes JRA, adult Still disease, and RA.

In one study involving patients with refractory RA and refractory polyarticular JRA, researchers found that both groups had a significantly higher frequency of IgG B19 virus antibody as determined by enzyme-linked immunosorbent assay (ELISA) than did aged-matched controls.[289] However, when the data in this study were examined, the prevalence of antibody in the control population clearly was less than expected. In another study, Kishore and colleagues[223] studied IgM antibody to B19 virus by ELISA and found that 28 percent of patients with JRA, 8 percent of controls, and none of the patients with RA had positive values. They interpreted their JRA findings to be either triggering B19 virus infections or perhaps intercurrent infections in patients with established JRA. In a provocative study, Takahashi and coworkers[428] found B19 virus DNA in the synovial tissue of 30 of 39 patients with RA. The data suggested that the virus identified in synovial tissue was active and led to their conclusion that B19 virus is involved in the initiation and perpetuation of RA synovitis. In contrast, Nikkari and associates[324,326] in Finland performed extensive studies and noted that chronic rheumatoid-like arthropathies triggered by B19 virus occasionally occur. However, their data do not support a general role for B19 virus in the etiology or pathogenesis of RA. B19 infection can lead to increased circulation of immune complexes, endothelial injury, inflammation, and exacerbation of underlying vasculitic disease.[56]

Tyndall and colleagues[442] described an adult woman with erosive polyarthritis associated with B19 virus infection, and Samii and coworkers[387] reported two adults with bilateral carpal tunnel syndrome associated with B19 virus infection. Berg and colleagues[34] could find no association with either IgG or IgM antibody to B19 virus and fibromyalgia in a study of 26 adult women and matched controls. Guillaume and coauthors[150] reported a case of chronic arthropathy in the lumbar facet joints after B19 infection.

Other rheumatic and vasculitic syndromes have been noted in association with parvovirus B19 infection; however, the strength of these associations is unclear. These associated syndromes include fibromyalgia,[335] systematic lupus erythematosus,[99,188,400] Kawasaki disease, leukocytoclastic vasculitis, polyarteritis nodosa, Wegener granulomatosis,[107] chronic fatigue syndrome,[211,212,280] systemic sclerosis,[124,341,342] giant-cell arteritis, transient anticardiolipin syndrome and transient antiphospholipid syndrome,[20] hemophagocytic syndrome, Henoch-Schönlein purpura,[76,173,343] uveitis with and without associated systemic disease,[174] myofasciitis,[187] and Raynaud phenomenon.*

INFECTION IN IMMUNOCOMPROMISED PATIENTS

Some immunodeficient patients suffer chronic B19 virus infections.† These patients have persistent anemia caused by continuous lysis of red cell precursors. This problem has been seen most commonly in children with acute lymphocytic leukemia.[62,92,112,122,172,210,231,242,482]

Other immune deficiency states in which chronic B19 virus was documented include Nezelof syndrome, acute myeloid leukemia, chronic myeloid leukemia, Burkitt lymphoma, lymphoblastic lymphoma, myelodysplastic syndrome, astrocytoma, Wilms tumor, HIV infection,[78,316] severe combined immunodeficiency, bone marrow transplantation,[164,218,364,423] other organ transplants,[32,69,108,239,295,347] systemic lupus erythematosus,[426] and patients receiving chemotherapy.[107,122,172,240] The immune deficiency in the aforementioned conditions can be a primary event or a secondary event related to treatment.

Eid and colleagues[109] reviewed 98 transplant recipients in whom acute parvovirus B19 infection developed after transplantation. The median time to B19 disease was 7 weeks after transplantation. Anemia developed in 99 percent, leukopenia in 38 percent, thrombocytopenia in 21 percent, and allograft loss or dysfunction in 10 percent. Three patients died of myocarditis and cardiogenic shock as a result of B19 infection.

Patients with chronic anemia have high persistent concentrations of B19 virus in their serum, although the values (10^8 particles per milliliter) are less than those seen in primary infections (10^{10} to 10^{11} particles per milliliter) in healthy individuals and in patients with hemoglobinopathies.

Viremia and anemia may, if untreated, persist for years. Fatigue and pallor are the most common clinical symptoms, and other findings of B19 virus infection, such as exanthem and arthralgia, occur rarely. Because of immunodeficiency, IgM antibody studies generally are not useful in making the diagnosis. Therefore, the diagnosis of persistent B19 virus infection depends on demonstration of specific B19 antigen or DNA in blood.[56]

In immunocompromised patients, persistent infection with B19 virus often occurs as a result of failure to produce effective neutralizing antibodies.[56,74,235] IVIG preparations have been used successfully for the treatment of B19 virus in immunocompromised patients.‡ Patients taking immunosuppressive agents, especially calcineurin inhibitors, may be unable to clear B19 virus because these medications inhibit IL-2 production and the patients are unable to mount an effective T_H1 response.[74,295] It may be necessary to reduce the dose of immunosuppressive agents in addition to IVIG treatment to successfully eradicate B19 virus.[31,258] LaMonte and associates[244] reported five children (three HIV-infected children and two otherwise normal children) with chronic infections without B19-associated anemia.

*See references 40, 53, 58, 95, 168, 206, 255, 256, 261, 266, 287, 289, 307, 308, 315, 328, 338, 358, 369, 413, 428, 443, 467.

*See references 79, 125, 137, 159, 183, 207, 220, 322, 323, 377, 490.
†See references 10, 36, 42, 54, 71, 92, 153, 168, 179, 206, 231, 232, 242, 269, 304, 310, 325, 332, 362, 390, 401, 403, 485, 487, 488.
‡See references 31, 40, 53, 100, 168, 176, 193, 206, 231, 401.

INTRAUTERINE INFECTION

Although a large epidemiologic study of an outbreak of erythema infectiosum 40 years ago failed to reveal evidence of teratogenicity, virologic and serologic studies conducted during the past 2 decades indicate that B19 virus crosses the placenta and causes infection in the fetus.* Infection in pregnancy results in fetal hydrops because of the severe anemia and leads to cardiac failure, fetal death, and miscarriage.[74,275,336] To determine the rate and outcome of fetal involvement after infection in pregnant women, a large prospective study was performed in England during a $3^1/_2$-year period from January 1985 to June 1988,[371] and more recently, from 1992 to 1995, a second study was performed.[288] These two studies included 427 pregnant women infected with B19 virus; 367 infants survived, and 129 of them had follow-up examinations at 7 to 10 years of age.[288] The excess rate of fetal loss was 9 percent, and such loss occurred only during the first 20 weeks of gestation. Seven cases of fetal hydrops occurred, and three of these fetuses survived; two of the three received intrauterine transfusions. None of the surviving infants in the two studies had abnormalities attributable to B19 virus infection, and no later effects were found at 7 to 10 years of age. Of the infants exposed in utero after 20 weeks' gestation, more than half were infected, but no clinical effects were identified. In a study by Nyman and colleagues,[337] the frequency of first-trimester fetal loss associated with B19 virus was 3 percent, with 12 percent occurring in the second trimester.

In a study in Connecticut related to a large outbreak of erythema infectiosum in which 39 infected pregnant women were monitored, two miscarriages occurred.[380] The frequency of fetal loss caused by B19 virus infection was estimated to be 5 percent. In another study in Spain, fetal loss occurred in only 1 of 60 women infected with B19 virus during pregnancy.[146] Enders and colleagues[115] studied 1018 pregnant women with acute parvovirus infection. The overall rate of fetal loss was 6.3 percent, with a rate of 11 percent if the B19 infection occurred in the first 20 weeks of gestation. The overall rate of hydrops fetalis was 3.9 percent, and the peak incidence occurred between 17 and 24 weeks' gestation.

Koch and colleagues[229] performed follow-up examinations on 19 infants born to mothers who had serologically confirmed B19 virus infection between the 4th and 38th weeks of gestation. In none of these infants did hydrops develop during pregnancy, and all were normal after birth. One child whose mother had erythema infectiosum at approximately the 20th week of gestation had a persistent asymptomatic infection for at least the first 7 months of life. Donders and coworkers[101] noted a child in whom a sonogram demonstrated fetal hydrops at 30 weeks' gestation. Cordocentesis revealed a hemoglobin concentration of 2.4 g/dL. One week later, a hydropic 1550-g infant was delivered by cesarean section. The baby's bone marrow showed an arrest in erythropoiesis with giant pronormoblasts. The baby received multiple transfusions for the first 4 months of life, and B19 viral DNA was identified in the infant's blood at 19 weeks of age. At 2 years of age, this child was found to be clinically, hematologically, and immunologically normal.

Candotti and colleagues[59] studied plasma samples from 885 pregnant women from Ghana and found that, contrary to the high rate of B19 transmission during primary viremia in pregnancy, pregnant women with B19-specific IgG and low levels of B19 DNA by PCR did not transmit virus to the fetus.

In contrast to the case just noted, Brown and associates[50] reported a baby with a similar exposure and delivery history who had persistent anemia and, despite therapy, was transfusion-dependent at 4 years of age. In addition, Brown and associates reported two other infants who had received intrauterine transfusions for fetal hydrops and had persistent anemia after birth.

At the present time, a large number of babies who were exposed to B19 virus in utero have been studied, with no convincing evidence of specific congenital malformations.[221,301,371,379,380,472] However, van Elsacker-Niele and associates[448] noted B19 virus infection in cells other than those of the erythroid series in two aborted fetuses. Ocular malformation was found in one, and evidence of extensive inflammatory reactions in all fetal and placental tissues was noted in both.

Tiesson and colleagues[433] reported an aborted fetus with a bilateral cleft lip, alveolus, and palate; micrognathia; and webbed joints. In another aborted fetus, ocular abnormalities similar to those seen in congenital rubella were observed.[464] In a newborn with anemia, blueberry muffin rash, and hepatomegaly, parvovirus B19 viral gene sequences were found in the liver and placenta.[406] Gulen and associates[151] described a premature infant born at 29 weeks' gestation with leukoerythroblastosis associated with B19-specific IgM and IgG antibody in both the mother and baby.

Yeh and colleagues[481] described severe, transient dyserythropoietic anemia in puerperal woman with pre-eclampsia and eclampsia with concomitant B19 infection.

NEUROLOGIC ILLNESS

Two children with encephalitis in association with erythema infectiosum were noted in the era before discovery of the causative role of B19 virus in erythema infectiosum.[27,156] In the present era, a number of cases of aseptic meningitis and encephalitis have been reported in association with B19 virus infection in both children and adults.* These cases have been documented by the demonstration of specific B19 virus IgM antibody or the presence of B19 viral DNA in cerebrospinal fluid (CSF). Bilge and colleagues[39] reported a 12-year-old boy with a history of renal transplantation who had recurrent episodes of encephalopathy and focal neurologic defects associated with B19 DNA in blood and bone marrow and on skin biopsy specimens. He was treated successfully with IVIG. Nolan and colleagues[329] reported an HIV-positive patient in whom parvovirus B19 encephalitis developed during immune reconstitution while receiving highly active antiretroviral therapy. Chorea-encephalopathy, with parvovirus B19 DNA in both serum and CSF, developed in a previously healthy 8-year-old girl.[126]

Other neuralgic illnesses associated with B19 virus infection[29] include postinfectious neuralgic amyotrophy,[356] Guillain-Barré syndrome,[290] acute bilateral carpal tunnel syndrome,[264,387] mononeuritis multiplex,[4] stroke,[149,272] and numbness and tingling of the fingers in seven nurses.[492] Five of these nurses had erythema infectiosum, and in the other two the only manifestation of infection was numbness and tingling. All cases in this study were diagnosed by the demonstration of specific B19 virus IgM antibody during an outbreak of erythema infectiosum in Buffalo, New York, in 1987.

MYOCARDITIS

Myocarditis in association with parvovirus B19 infection has been observed in immunocompetent children and adults.† B19-

*See references 3, 8, 10, 45, 50, 54, 63, 97, 101, 111, 140, 141, 146, 167, 168, 197, 206, 221, 225, 227, 229, 230, 275, 278, 281, 288, 292, 331, 334, 344, 359, 371, 379-381, 386, 411, 433, 447, 448, 471-476, 487, 496.

*See references 29, 39, 64, 126, 160, 168, 170, 206, 209, 233, 329, 344, 419, 420, 425, 462, 479.

†See references 57, 98, 113, 237, 245, 262, 309, 321, 349-351, 357, 384.

associated myocarditis can progress to chronic dilated cardiomyopathy.[237,238,245,262,349,350] In a study of 3345 patients who underwent cardiac biopsy for suspected myocarditis or dilated cardiomyopathy, Pankuweit and colleagues[350] found B19 DNA by PCR in 17.6 percent of patients with dilated cardiomyopathy without significant inflammation, and inflammatory heart disease, ventricular dilation, and reduced ejection fraction were noted in 33.3 percent of patients. Kühl and associates[238] showed that viral persistence in the setting of myocarditis or dilated cardiomyopathy was associated with progressive deterioration of the left ventricular ejection fraction whereas elimination of virus resulted in significant improvement in left ventricular function.

In a study by Wang and colleagues,[458] B19 virus was detected by nested PCR in cardiac tissues in 7 of 42 patients with congenital heart disease and in none of the 38 controls, thus suggesting a correlation between B19 virus and congenital heart disease.

Enders and associates[113] described two children with life-threatening myocarditis. One of these children died, and the second received a cardiac transplant. The illnesses in both these children occurred in the spring (April), and one had joint pain and urticarial lesions on the flexor surfaces of both arms, erythematous macules on the abdomen and upper part of the chest, and purpuric lesions on the back. Both children had B19 viral DNA identified in the heart and B19-specific IgM antibody.

Nigro and colleagues[321] noted three young children, aged 7, 12, and 18 months, with acute lymphocytic myocarditis and parvovirus B19 infection. Two of these children exhibited full cardiac recovery, but chronic persistent myocarditis developed in the other child. Papadogiannakis and coauthors[351] reported a healthy 11-month-old child who had severe respiratory distress and died of sudden cardiac arrest within 3 hours of admission because of parvovirus-associated lymphocytic myocarditis. Zack and associates[493] reported a 5-year-old girl who suddenly collapsed and died of acute myocarditis secondary to parvovirus B19 infection. Significant lymphocytic infiltration of the sinoatrial node and perivascular infiltration around the atrioventricular node probably resulted in conduction disturbances and death. Jonetzko and colleagues[201] reported a case of fatal parvovirus B19–associated myocarditis in a liver transplant recipient.

Parvovirus B19 infection also can mimic acute myocardial infarction[57,237] and isolated diastolic dysfunction in adults.[438]

It is hypothesized that the blood group P antigen on the endothelial cells of coronary vessels is a portal for infection and that such infection results in endothelial dysfunction, impairment of the coronary microcirculation, and inflammation of the myocardium.[29,237,245] The blood group P antigen is not present on the myocytes of children and adults; however, it is present on fetal myocytes.[49,237,245,345]

ACUTE HEPATITIS

Acute hepatitis has been noted as a manifestation of B19 virus infection. Yoto and coworkers[484] described seven children with acute hepatitis in association with B19 virus infection. They carefully studied and ruled out other common causes of acute hepatitis. Sokal and associates[414] reported fulminant hepatitis in four children younger than 5 years in association with parvovirus B19 infection. Their patients had a distinct clinical pattern consisting of low serum bilirubin concentrations and rapid recovery of liver function without transplantation. Nobili and colleagues[327] reported a case of autoimmune hepatitis followed by severe autoimmune hemolytic anemia in a 1-month-old boy with parvovirus B19 infection. Treatment with cyclosporine and prednisone resulted in resolution of his symptoms.

Hsu and associates[185] studied 54 patients with B19 virus infection in addition to hepatitis B virus (HBV) infection and 51 patients with hepatitis C virus (HCV) infection who were not taking immunosuppressive drugs. The study found that the prevalence of parvovirus B19 DNA was high in patients with HBV (85.2%) or HCV (70.6%). Co-infection with B19 and HBV or HCV did not increase the frequency of liver dysfunction in these patients with chronic hepatitis.[185,250] Additionally, in a study by Wong and associates,[475] there was no significant difference in the prevalence of parvovirus B19 DNA in liver biopsy specimens from patients with fulminant hepatitis or hepatitis-associated aplastic anemia and those with HBV or HCV infection. The role of parvovirus B19 in the development of fulminant hepatitis is currently unclear.

OTHER ILLNESSES

One adult patient had chronic red cell aplasia for a 10-year period that was treated with regular blood transfusions.[243] After diagnosis of persistent B19 virus infection, the patient was treated with IVIG, which resulted in an apparent cure. A presumably immunologically normal woman had recurrent episodes of paresthesia over the course of a 4-year period in conjunction with persistent B19 viral DNA in her blood.[121] Evidence of persistent infection in immunologically normal patients has been noted in other studies.[215,311]

Kerr and associates[214] studied 53 patients who had contracted acute B19 virus illnesses. Seven of those studied had B19 viral DNA demonstrated in serum specimens 26 to 65 months after their acute illnesses. All seven were women, and four were asymptomatic. Of the other three, one had chronic hemolytic anemia, one had persistent arthralgia of the knees, and the last had arthralgia and chronic fatigue syndrome.

Multiple case reports have described patients with chronic fatigue syndrome and persistent parvovirus B19 DNA in their serum.[196,211,212,276,280] In 2002, Kerr and colleagues[217] described three patients with chronic fatigue syndrome and chronic B19 virus infection treated with IVIG. Such treatment resulted in the clearance of B19 viremia, improvement of symptoms, and resolution of cytokine dysregulation.

Faden and associates[121] described a nurse with recurrent episodes of paresthesia who had B19 viral DNA demonstrated in her serum for almost a 4-year period. This woman also had persistent serum IgM antibody to B19 virus.

Lymphadenitis associated with parvovirus B19 infection has been described by many authors. Tsuda and coworkers[439] reported five young adults with cervical lymphadenopathy associated with B19 virus infection. All had fever, one had arthralgia, and all had leukopenia. Morinet and colleagues[298] described a 27-year-old man with pseudoappendicitis or acute mesenteric lymphadenitis and serologic evidence of B19 virus infection. A 16-month-old boy had peripheral facial palsy, parotitis, and intraparotid lymphadenitis with evidence of parvovirus B19 IgM antibody and IgG antibody seroconversion.[274] Johnson and associates[199] described an 18-year-old woman with parvovirus B19 resulting in necrotizing lymphadenitis, and Knösel and colleagues[224] reported unilateral cervical lymphadenopathy in a 16-year-old girl. In both cases, parvovirus B19 DNA was detected by PCR on lymph node biopsy specimens.

Parvovirus B19 infection also has been shown to induce proliferative glomerulonephritis, usually endocapillary or mesangioproliferative (or both), with hypocomplementemia and spontaneous recovery.[189,195,297,340,430,431,461] Ieiri and colleagues[189] studied 10 patients with acute glomerulonephritis associated with human parvovirus B19 infection. These authors found that this specific renal disease characterized by endocapillary hypercellularity was associated with hypocomplementemia, leukopenia, positive antinuclear antibody, and a female preponderance (9 of 10 patients). Ohtomo and associates[340] reported a healthy 8-year-

old boy with erythema infectiosum and glomerulonephritis with immune complex deposits associated with B19 virus diagnosed by both monoclonal antibody against human parvovirus B19 antigen and nested PCR on renal biopsy. The association between parvovirus B19 and sepsis also has been reported by multiple authors. These patients exhibited shock, severe respiratory distress, and parvovirus B19 infection.[24,123,299,351,459]

DIAGNOSIS

DIFFERENTIAL DIAGNOSIS

Because the exanthem of erythema infectiosum is unique, its diagnosis should be easy. During epidemics, no difficulties should arise, but sporadic cases can be a problem. In the differential diagnosis, rubella and scarlet fever are of most concern. Because rubella virus has been recovered from some patients with illness thought to be erythema infectiosum[27] and because an erythema infectiosum–like illness was observed in volunteers who underwent intranasal administration of a rubella virus strain recovered from a patient with erythema infectiosum–like illness,[389] this diagnostic possibility always should be considered. When the risk of development of congenital rubella is a possibility, rubella-specific diagnostic tests should be performed (see Chapter 186).

Erythema infectiosum can be differentiated from scarlet fever by the usual lack of pharyngitis in the former and a positive culture for *Streptococcus pyogenes* in the latter. Other differential diagnostic considerations are other infectious exanthems (see Chapter 64), collagen vascular diseases, drug reactions, and allergic responses to environmental substances.

Although a presumptive diagnosis of B19 virus infection may be made by exclusion of other etiologic possibilities, a definitive diagnosis can be made only by specific serologic tests or identification of B19 antigens or DNA in blood or tissue specimens.

Aplastic crisis in a patient with chronic hemolytic anemia can be diagnosed by finding a hemoglobin concentration that is 2 g/dL or more below the steady-state value for that patient, together with a reticulocyte count either less than 0.2 percent of the steady-state value or elevated above the steady-state value (indicative of hyperplasia of erythrocyte precursors in the recovery phase). Although B19 virus infection is the most common cause of aplastic crisis, moderate to severe degrees of hypoplasia may be associated with systemic bacterial infections (e.g., *Salmonella*, *Streptococcus pneumoniae*) or marrow-suppressive drugs (e.g., chloramphenicol).[284,397]

SPECIFIC DIAGNOSIS

Several tests have been developed and refined that allow a reliable serologic diagnosis of acute and past B19 virus infection to be established and B19 virus in blood and tissues to be demonstrated.* IgM and IgG antibody can be detected by enzyme immunoassay, hemadherence, radioimmunoassay, or immunofluorescence; antigen can be detected by DNA hybridization, PCR, or electron microscopy.

In a normal host, acute or recent infection is determined best by demonstration of specific IgM antibody. In immunocompromised patients with suspected acute or chronic infection, the diagnosis is made by detection of antigen in blood. Similarly, detection of antigen also can be performed early in aplastic crisis and to study aborted fetal tissues. Gallinella and colleagues[138] recommend both specific IgM and B19 DNA PCR to establish a diagnosis of B19 infection.

Past infection and immunity to B19 virus are determined by demonstration of specific serum IgG antibody.

Some caution should be observed in accepting the results of IgM serology and antigen detection in unusual clinical circumstances.[48] The sensitivity and specificity of the various serologic tests vary, and additional false-positive and false-negative results can be expected, depending on the skill of workers in individual laboratories.[55,82,436,445] Antigen-detection systems can be contaminated, and such contamination may not be discernible by conventional controls. When the results of tests on specimens from patients with unusual illnesses are positive, repeating the tests in a different laboratory is worthwhile.

Söderlund and associates[412] reported an IgG avidity assay that is highly sensitive and specific for the identification of recent primary infection with parvovirus B19. Enders and coworkers[114] also recommended VP1 IgG avidity or VP2 epitope–type specificity assays in the presence of low-level IgM or B19 DNA to confirm or refute the diagnosis of B19 infection during pregnancy. Because there is a window of 7 days after initial infection occurs until the development of IgM-specific B19 antibody and because IgM can be negative or low in the presence of overt hydrops fetalis, both serology and B19 DNA PCR are recommended on both maternal and fetal sera (or cord blood).[108]

Beersma and colleagues[33] demonstrated the superiority of B19 VP2 IgM ELISA by showing high correlation with elevated levels of B19 DNA in sera. Persistent infection may be determined by the presence of IgG antibody to NS1.[452]

If parvovirus B19 infection is suspected in pregnancy, ultrasound should be performed to assess for fetal anemia and hydrops (excess of fluid in at least two body compartments of the fetus).[108] The use of middle cerebral artery peak systolic velocity for the detection of fetal anemia may be a useful adjunct to ultrasound scanning.[89,303]

TREATMENT AND PROGNOSIS

No specific treatment of B19 virus infection exists. Symptomatic therapy for erythema infectiosum rarely is necessary, especially in children. Starch baths may be helpful in reducing pruritus. Arthralgia or arthritis may be troublesome and may be treated with analgesics.

Patients with aplastic crisis may require erythrocyte transfusions to raise their peripheral hemoglobin concentration.

The outlook in virtually all cases of erythema infectiosum is excellent. If patients with aplastic crisis receive transfusions with packed erythrocytes when necessary, the prognosis for these patients also is excellent. If B19 virus infection occurs during pregnancy, the pregnancy should be monitored carefully. At delivery, examination of cord blood or blood from the neonate for detection of virus and IgM antibody will reveal whether the virus has crossed the placenta and infected the fetus. When infection has occurred, the child should be examined carefully for any defect and monitored for some years to exclude the possibility of delayed sequelae.

Some researchers have suggested that pregnant women with symptomatic B19 virus infection be observed for fetal aplastic crisis by monitoring maternal serum for elevated levels of alpha-fetoprotein.[61] If levels are found to be elevated, serial ultrasonography can be performed to detect hydrops fetalis. Fetal hydrops can be treated by in utero transfusions.[118,145,383] However, there is a risk of infection and the potential for congenital malformations with cordocentesis and fetal transfusion. Enders and colleagues[115] reported that 84.6 percent of those with severe hydrops fetalis survived to delivery. In 2005, Matsuda and colleagues[277] successfully treated a B19-infected fetus with hydrops at 21 weeks'

*See references 10, 15, 22, 33, 47, 55, 60, 66, 68, 80, 82, 83, 93, 94, 116, 134, 138, 178, 181, 219, 226, 228, 285, 296, 318, 385, 392, 394, 427, 457, 474, 495.

gestation with two injections of B19 IgG–rich immunoglobulin injected into the fetal peritoneal cavity. The fetal ascites and pericardial effusion resolved completely 8 days after the initial injection, and a healthy baby was born at 38 weeks' gestation without evidence of cardiac dysfunction or B19 DNA detected in serum. Therapeutic abortion is not indicated for pregnant women with documented B19 virus infection.

Patients with chronic myocarditis caused by B19 virus have been treated successfully with IVIG.[421] Chronic myocarditis secondary to B19 also has been treated with immunosuppressive agents, including corticosteroids and cyclosporine.[245,309,384] Ito and associates[194] described a patient with baseline refractory autoimmune hemolytic anemia who was taking immunosuppressive medications while infected with B19 virus. The patient had a complicated course; persistent pure red cell aplasia subsequently developed but resolved after treatment with cyclosporine and IVIG.

Immunocompromised patients with chronic B19 virus infection can be treated successfully with IVIG preparations,[40,53,100,168,174,193,206,231,401] as can other patients without demonstrated immune deficiencies who have chronic infections.[121,194,196,305,421] Although the amount of specific B19 virus antibody varies among different IVIG products, all contain significant concentrations.[100] No formal treatment studies have been performed, and several different treatment programs have been used. Even though some cures have been achieved with single-dose IVIG therapy, we favor an initial 4-day course with 500 mg/kg/day. After the patient has received treatment, the viremia should cease and clinical improvement should occur. Some immunocompromised patients have required repeated treatment courses.

PREVENTION

B19 virus is spread by the oral and respiratory routes, and virus shedding during routine infection occurs when patients are not aware of their illness. Because B19 virus infections occur in outbreaks, the virus can be widespread in a community, with many infections going unrecognized.

Patients with erythema infectiosum do not need to be isolated because they have passed their period of infectivity. Although patients with aplastic crisis also usually are past the period of virus shedding, they should be isolated because some will be shedding virus at the time of initial evaluation. All patients with chronic infection should be considered contagious until treated with IVIG and demonstrated to be nonviremic.

Pregnant women are of particular concern during an outbreak.[63,141] The rate of parvovirus seropositivity is approximately 50 percent in women of child-bearing age, so no risk exists in approximately half of those who might become exposed. Determining an IgG antibody titer can allay the fear in those who are antibody-positive. Cartter and associates[63] examined occupational risk factors for B19 virus infection in pregnant women. They found the following rates of infection: school teachers, 16 percent; daycare workers, 9 percent; homemakers, 9 percent; and women working outside the home, 4 percent. In another study, Gillespie and colleagues[141] found the greatest risk for infection occurred in school and daycare personnel. These results suggest that in certain circumstances (in older women and those with past fertility problems), having women in high-risk occupations avoid the workplace during the outbreak period might be reasonable.

Technologic advances have led to the development of experimental recombinant vaccines that show considerable promise for effective prevention.[28,85,118,132,165,488] These vaccines could be useful in selected populations, such as patients with hemoglobinopathies and seronegative women of child-bearing age.

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CHAPTER 165

Human Bocaviruses

James D. Cherry

Human bocavirus was described first in 2005 by Allander and associates.[4] Since then, this virus has been associated with a significant number of respiratory illnesses in children throughout the world.*

HISTORY

Allander and coworkers[4] developed a system for molecular virus screening of clinical nasopharyngeal samples. This system was based on host DNA depletion, random polymerase chain reaction (PCR) amplification, large-scale sequencing, and bioinformatics. Using two pools of nasopharyngeal aspirate samples from patients (mainly children) with respiratory illnesses seen in November and December 2003 and March 2004, they found two agents that had an amino acid sequence that significantly matched bovine parvovirus and canine minute virus. These viruses are members of the *Parvoviridae* family, subfamily *Parvovirinae*, genus *Bocavirus*. These investigators proposed the provisional name *human bocavirus* for this new virus. During the ensuing years, this virus has been identified in respiratory samples and at other sites from patients throughout the world.†

PROPERTIES

As noted in Chapter 164, members of the family *Parvoviridae* are small, nonenveloped, single-stranded DNA viruses. The complete genome length of human bocavirus has not been determined, but at least 5299 nucleotides were identified in one of the two reference strains.[4]

The genome is thought to contain three open-reading frames; two of these frames encode the nonstructural proteins NS1 and NP-1, and the other encodes the two capsid proteins VP1 and VP2.[4,36] The function of NS1 in human bocavirus infection is not known. However, in other parvoviruses, NS1 is involved in the binding and hydrolysis of nucleoside triphosphates and has helicase activity. The function of NP-1 is unknown. The virion-associated proteins (VP1, VP2) are more immunogenic than are the nonstructural proteins, and their coding sequences have considerable variability.[36]

The cellular site of human bocavirus replication is not known.[36] The virus has not been propagated in tissue culture, and, as yet, no animal model of infection has been identified.

EPIDEMIOLOGY

Infection with human bocavirus enjoys worldwide prevalence.[36] The virus exists as a single lineage with two genotypes. The virus has been identified in nasopharyngeal samples from children hospitalized with respiratory illnesses in 1.5 to 19 percent of those studied.[2] Infection is seen most frequently in children younger than 2 years old, but infection occurs in older children as well. The prevalence of infection in adults with respiratory illness is low.

In a seroprevalence study in Japan involving sera from 204 subjects younger than 3 months to older than 20 years in which antibody to the VP1 protein was measured, the following positivity rates were found: younger than 3 months, 90.5 percent; 3 to 5 months, 40.0 percent; 6 to 8 months, 5.6 percent; 9 to 11 months, 33.3 percent; 1 year, 42.3 percent; 2 to 3 years, 83.3

*See references 1-4, 6-11, 13-15, 17-19, 21, 23-25, 27-29, 31-36, 38, 40-43.
†See references 1-4, 6, 8-11, 13-15, 17, 19-21, 23-25, 27-29, 30-36, 38, 41-43.

percent; 4 to 5 years, 89.5 percent; 6 to 19 years, 100 percent; and older than 20 years, 94.1 percent.[12] Human bocavirus is detected most frequently in nasopharyngeal specimens in children between the ages of 6 and 24 months, and the seroprevalence data from Japan[12] suggest that virtually all children have had an infection during the first 5 years of life.

Infections with human bocavirus occur throughout the year.[3,8,27] In one large study, the peak rate of infection occurred in the spring (March through May) in two successive years in San Diego, California.[6] Human bocavirus was not identified in August and September in this study. In numerous studies, respiratory illness was seen more commonly in boys.[6,8,11,18,21,25,31,32] However, in a study in Korea in which the male-to-female ratio was noted in eight different respiratory viral infections, researchers found that males predominated with all viruses except human bocavirus.[26] The ratio in bocavirus infections was 1:1.4.

Human bocavirus has been identified in stool specimens from children with diarrhea,[1,21,22,40] in the serum of children with respiratory illnesses,[3] and in nasopharyngeal specimens from well children.[15,24]

PATHOGENESIS

The method of transmission of human bocavirus is not known. However, because the virus is present in the nasopharynx in high concentrations, a reasonable conclusion is that its spread is similar to those of other respiratory viruses.[36] This spread could involve contact transmission with droplet nuclei or by aerosol, or both, as well as hand-to-hand and hand-to-surface transmission with self-inoculation.

A unique feature of human bocavirus respiratory infections is the high frequency of mixed infections with other respiratory viruses, leading to concern that bocavirus is a passenger agent and not the cause of the respiratory illness.[2,36] However, in many instances, no co-infecting virus is identified. Investigators also have noted that the severity of illness may correlate with the virus load.[16] Kantola and associates[16] demonstrated the occurrence of IgM antibody responses and titer increases of IgG antibody in children with respiratory illnesses.

In contrast with parvovirus B19 DNA, which was found in lymphoid tissue, bone marrow, and brain of autopsy specimens from persons both infected and not infected with the human immunodeficiency virus (HIV), human bocavirus was not found in any similar specimens.[29]

In one study of 512 outpatients with respiratory illness, 20 (3.9%) were found to have human bocavirus in their nasopharyngeal samples; in this same study, three (1%) controls had human bocavirus–positive nasopharyngeal samples.[15] In another study, 22 (5.3%) of 425 children with respiratory illness had bocavirus-positive nasopharyngeal samples, whereas no positive samples were found in 96 asymptomatic children.[19] In contrast with these two studies, a study in Quebec, Canada, found positive samples in 43 (43%) of 100 asymptomatic children and 31 (13.8%) of 225 symptomatic children.[24]

CLINICAL MANIFESTATIONS

RESPIRATORY ILLNESS

Human bocavirus has been found in nasopharyngeal specimens from children with both upper and lower respiratory tract illnesses. In several investigations, the signs and symptoms of illness in human bocavirus infections have been compared with those occurring in infections with other respiratory viruses.[7,10,13,15,24] In these comparative studies, the number of children by virus category is relatively small, but the overall findings in human bocavirus infections clearly are similar to those of viral infections with respiratory syncytial virus, human metapneumovirus, adenoviruses, influenza viruses, parainfluenza viruses, and rhinoviruses.

Data on routine laboratory studies in human bocavirus infections are sparse. In a U.S. study, the white blood cell count in children was 3000 to 31,000 cells/mm^3 (median, 13,300 cells/mm^3).[6] The median differential cell values were neutrophils, 40 percent; band forms, 10 percent; lymphocytes, 39 percent; and monocytes, 10 percent. The C-reactive protein level in six children ranged from 0.4 to 7.3 mg/dL (median, 0.7 mg/dL). In a German study, the white blood cell count range was 6700/mm^3 to 16,700/mm^3, and the median value was 11,300/mm^3.[42] The C-reactive protein level ranged from less than 0.3 to 114 mg/L, and the median value was 12.5 mg/L (normal value, <0.3 mg/L). In a Japanese study, the white blood cell count range was 4800 cells/mm^3 to 21,980 cells/mm^3 (median, 14,000 cells/mm^3), and the C-reactive protein level ranged from less than 0.20 to 4.48 mg/dL (median, 0.38 mg/dL).[25]

Upper Respiratory Tract Infections

In one study, 86 percent of 49 human bocavirus infections without co-infection with other agents were classified as upper respiratory illnesses.[13] In contrast, only 42 percent of 50 children in this study who had human bocavirus and a co-infecting agent were classified as having an upper respiratory illness. Pharyngitis was observed in 55 percent of the cases in which human bocavirus was the single agent identified. Of the children with human bocavirus infections without co-infections, 18 percent had acute otitis media and 12 percent had sinusitis. In another study, otitis media was reported in 61 percent of all human bocavirus infections.[24]

In a large study, Arnold and associates[7] noted the following findings at the time of presentation: fever, 68 percent; rhinorrhea, 67 percent; cough, 85 percent; conjunctivitis, 7 percent; vomiting, 30 percent; diarrhea, 21 percent; and rash, 7 percent. Of interest was that the cough was paroxysmal in 19 percent of the human bocavirus–infected children, whereas paroxysmal cough was much less common in adenovirus infections (7%) and human metapneumovirus infections (5%).

In one study in Korea, three of 36 (8.3%) children infected with human bocavirus had croup.[10] In a study in Canada, two of 58 (3%) bocavirus-infected children had croup,[8] and in a study in Japan, one of 18 (6%) had laryngotracheitis.[25]

Lower Respiratory Tract Infections

Longtin and colleagues[24] noted that 42 percent of children with human bocavirus infections had pneumonia and that 42 percent had bronchiolitis. In the large study in San Diego, California, involving 82 children with human bocavirus infections, 76 children with adenoviral infections, and 87 children with human metapneumovirus infections, the investigators were able to compare the rates of various clinical findings.[7] In this study, the following rates of events were noted in bocavirus-infected children: hospitalization, 69 percent; need for oxygen administration, 44 percent; admission to intensive care unit, 11 percent; intubation, 4 percent; clinical lower respiratory tract disease, 61 percent; hypoxia, 41percent; increased work of breathing, 59 percent; abnormal lung findings, 51 percent; "atelectasis vs. infiltrate," 11 percent; "infiltrate" or "pneumonia," 9 percent; and bronchiolitis, 46 percent. Comparatively, adenovirus-infected children had less lower respiratory tract disease, less hypoxia, and less increased work of breathing and were more likely to have normal chest radiographic findings than were children with human bocavirus and human metapneumovirus infections.

In a study in Korea, 53 percent of human bocavirus–infected children had rales noted on chest auscultation and 42 percent had wheezing.[10] The clinical diagnoses in this study were bronchiol-

itis (25%), pneumonia (56%), exacerbation of asthma (11%), and croup (8%). In a study in Germany, 32 children with lower respiratory tract disease were studied.[43] The diagnoses were bronchitis (16%), wheezing bronchitis (14%), bronchiolitis (3%), and pneumonia (18%). Ten of the 11 patients in this study with pneumonia had co-infections with other respiratory viruses. In a study in Canada involving 58 bocavirus infections, 40 percent had bronchiolitis and 22 percent had pneumonia.[8] In a study of 18 children in Japan, the lower respiratory tract diagnoses were wheezy bronchitis (33%), pneumonia (33%), bronchiolitis (11%), bronchitis (11%), and asthmatic attack (6%).[25]

GASTROINTESTINAL ILLNESS

Gastroenteritis is not an uncommon finding in human bocavirus infections.* The virus has been identified in nasopharyngeal samples and in stool samples of children with diarrhea. In one study in Hong Kong, diarrhea was noted in 11 percent of 79 patients with respiratory symptoms.[21] In the same study, human bocavirus was identified is fecal samples of 25 children with gastroenteritis. Of these children, 16 percent had blood in the stool, 8 percent had mucus in the stool, 32 percent had vomiting, and 68 percent had fever. The following respiratory findings were noted in these children with gastroenteritis: coryza (56%), acute bronchitis (16%), and pneumonia (12%). Co-pathogens were identified in 56 percent of the children: rotavirus (36%), *Salmonella* spp. (8%), *Campylobacter* spp. (4%), *Staphylococcus aureus* (4%), and *Clostridium difficile* (4%).

In a study of 962 stool samples from children with acute gastroenteritis in Seoul, Korea, a viral agent was found in 44.4 percent of the specimens.[22] The viral agents included rotavirus (25.7%), norovirus (13.7%), adenovirus (3.0%), astrovirus (1.1%), and human bocavirus (0.8%). In another study in Brazil, 2 percent of 705 diarrhea stool samples were PCR positive for human bocavirus.[1]

In studies of respiratory illness associated with human bocavirus infection, diarrhea occurs in 9 to 38 percent.[6,7,13,19,21,33,36]

INFECTIONS IN IMMUNOCOMPROMISED PATIENTS

Koskenvuo and associates[20] described three children with acute lymphoblastic leukemia who had acute febrile episodes in whom human bocavirus was detected in their respiratory secretions. In addition to fever, one of these children had cough, rhinitis, and otitis media, and another child had vomiting and diarrhea. The third child had five consecutive febrile episodes during the course of a 6-month period, and with each of these episodes, human bocavirus was found in nasal swab samples. Smuts and Hardie[39] noted eight children with HIV infections and associated human bocavirus infections but presented no details related to their illnesses.

OTHER CLINICAL FINDINGS

In addition to respiratory and gastrointestinal signs and symptoms, numerous other clinical findings and diagnoses have been observed in children with human bocavirus infections. Arnold and colleagues[6] noted four children with maculopapular erythematous rashes with prominence on the chest and trunk. Two of these children had involvement of the face. Exanthems have been noted in other studies.[11,21,32] In three studies, the rate of exanthema varied between 5 and 9 percent in the children studied. One child had an illness suggestive of roseola infantum, and another child had an illness thought to be Henoch-Schönlein purpura.[21]

In three studies, human bocavirus has been noted in respiratory or fecal samples in children clinically thought to have Kawasaki disease.[7,9,21] Other events noted in temporal association with human bocavirus infection include intussusception,[9,21] aseptic meningitis,[21] and nephritic syndrome.[21]

*See references 1, 2, 6, 7, 13, 19, 21, 22, 33, 36, 38.

DIAGNOSIS

DIFFERENTIAL DIAGNOSIS

The findings in human bocavirus respiratory infections are similar to those in respiratory syncytial virus and human metapneumovirus infections as well as adenoviral, influenza viral, parainfluenza viral, and rhinoviral infections. Similarly, gastroenteritis associated with human bocavirus infection cannot be differentiated from rotavirus infections and illness associated with other viral and bacterial agents.

SPECIFIC DIAGNOSIS

Presently, the only diagnostic method for the demonstration of human bocavirus infection is PCR. Specimens for PCR may be obtained from respiratory secretions, the stool, and blood. Primer sets used target the NP-1 region 4 and the NS1 region of the genome.[4,38]

Because recombinant human bocavirus capsid antigens have been produced and have been used experimentally to detect both IgG and IgM antibodies in the serum of patients with human bocavirus infections or in population studies, commercial serologic tests likely will become available to diagnose human bocavirus infections.[12,16,37]

TREATMENT

No specific antiviral treatment for human bocavirus infections is known. General care should be similar to that employed for other respiratory or gastrointestinal viral infections.

PROGNOSIS

Although the full spectrum of human bocavirus infections and possible sequelae are not known presently, most infections appear to be self-limited.

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SUBSECTION 2

Polyomaviridae

CHAPTER 166

Human Polyomaviruses

John A. Vanchiere

The human polyomaviruses, JC virus (JCV) and BK virus (BKV), and the simian virus 40 (SV40), which is also able to infect humans, represent a unique group of viruses that cause disease almost exclusively in immunocompromised patients. Polyomaviruses derive their name from *poly-*, meaning many, and *-oma*, which refers to their ability to induce tumors in laboratory animals. Their colorful history and critical role in the elucidation of fundamental cellular and molecular pathways have immortalized them as research tools. The finding of polyomaviruses associated with several human cancers and their ability to cause central nervous system and urinary tract disease in immune compromised patients have brought them to the foreground of clinical medicine in the past decade. The emergence of polyomavirus disease has highlighted the need to understand the natural history and fundamental host processes that control the persistence of polyomaviruses. The next decade of research undoubtedly will reveal the true nature of these fascinating viruses.

HISTORY

The recognition of progressive multifocal leukoencephalopathy (PML) as a clinical entity in 1958 marked the beginning of our knowledge of the human polyomaviruses.[10] Edward P. Richardson, Jr., a neuropathologist at Massachusetts General Hospital in Boston, and his colleagues described three cases of progressive neurologic disease in patients receiving chemotherapy for leukemia. At autopsy, they found many foci of demyelination, includ-

ing oligodendrocytes with intranuclear inclusions and astrocytes that were enlarged with bizarre nuclear changes.[10] Richardson[108] subsequently reported a larger series of PML cases and proposed a viral etiology associated with immune suppression. In 1965, papovavirus-like particles were observed by electron microscopy of brain tissue from patients with PML, and in 1971, JCV, the etiologic agent of PML, was isolated from primary human fetal glial cell cultures that had been inoculated with a brain extract from a patient with PML.[99,145] In the same year, another human polyomavirus, BKV, was cultivated from the urine of a renal transplant recipient.[44] Each virus was named with the initials of the patient from whom it was isolated.

SV40, the prototype polyomavirus, was isolated in 1960 by Sweet and Hilleman[128] as a contaminant of secondary rhesus monkey kidney cell cultures that were used to produce early poliovirus vaccines. In addition, several early adenovirus vaccines and a respiratory syncytial virus vaccine were contaminated with SV40.[83,90] Interestingly, although rhesus monkey kidney cells showed no cytopathic effect, when supernatants from vaccine cultures were used to inoculate green monkey kidney cells, a pronounced cytopathic effect was observed, thus giving rise to the original name of SV40, vacuolating virus. By that time, millions of doses of both live and killed preparations of poliovirus vaccine containing SV40 had been given to humans in the United States and Europe. Soon after SV40 was identified, researchers demonstrated that SV40-induced tumors in neonatal rodents, prompting great concern about its potential effects in humans.[48] Serologic studies showed that the formaldehyde-inactivated Salk poliovirus vaccine, but not the live-attenuated Sabin poliovirus vaccine, induced high-titer SV40-specific antibody responses.[46] Although the live-attenuated poliovirus vaccine preparations contained higher titers of infectious SV40 virus, they failed to induce virus-specific antibodies in vaccinees, despite prolonged shedding in stool.[88] A 30-year follow-up of infants possibly exposed to SV40 between 1955 and 1962 through inactivated poliovirus vaccine preparations showed no excess risk of cancer, but such epidemiologic studies have significant limitations.[21,125] Since the 1960s, the mechanism of SV40-induced tumorigenesis has been studied intensely, and as a result of SV40-related research, many facets of cell and molecular biology have been elucidated; they include transcriptional regulation, alternative splicing, eukaryotic DNA replication, tumor suppressor proteins, nuclear localization signals, and viral effects on cell cycle control.[25]

VIROLOGY

On the basis of their similar genomic structure and replication strategies, polyomaviruses and papillomaviruses are widely thought to have evolved from a common progenitor virus. Human polyomaviruses are 45 nm in diameter, with a genome approximately 5200 base pairs in length; papillomaviruses are somewhat larger, 55 nm in diameter with a genome approximately 8000 base pairs in length. The genomic organizations of the polyomaviruses and papillomaviruses differ in that polyomaviruses use both DNA strands to encode proteins, whereas papillomaviruses use just one DNA strand. Viruses in both families cause infection in immunocompetent and immunodeficient hosts and also have been associated with malignant neoplasms.

Polyomaviruses are classified in the family *Polyomaviridae*. Sixteen polyomaviruses are known, each with a limited host range in which one or several closely related species are infected.[118] On the basis of genetic comparisons, members of the family *Polyomaviridae* can be subdivided into avian, primate, and rodent subgroups. JCV and BKV infect only humans, and SV40 infects both humans and monkeys. The nucleotide sequence of JCV has 75 percent overall homology with BKV and 69 percent homology with SV40.[25] Molecular detection of two novel human polyomaviruses, KI (for Karolinska Institute) and WU (for Washington University), has been described recently in respiratory secretions from children and adults, but their clinical significance is unclear.[4,45,53,96]

The circular genome of polyomaviruses can be divided into structural, nonstructural, and regulatory regions. The structural (late) region of the viral genome encodes VP1, VP2, and VP3, the capsid proteins that envelop the viral genome. VP1 is the major capsid protein and accounts for more than 70 percent of the virion mass; it participates in host-cell recognition and stimulation of the host immune response. VP2 and VP3 are smaller, less abundant capsid proteins. The nonstructural (early) region of the polyomavirus genome encodes the large T antigen, the best studied of the polyomavirus proteins; this protein initiates viral DNA replication, stimulates cellular entry into S phase, and influences cellular and viral transcription. The small t antigen is produced by alternative splicing of the early viral mRNA and promotes G_1 cell cycle progression; it is not required for viral growth in cultured cells. The large T antigen of SV40 is responsible for the transformation of cells in vivo and in vitro. Large T antigen is a multifunctional protein that contains binding sites for the cellular pRb and p53 proteins. By binding to pRb, the large T antigen allows E2F-mediated progression of the cell cycle, thus providing the right environment for replication of viral DNA. Through its association with p53, a cellular tumor suppressor protein, the large T antigen blocks p53-induced cellular gene expression, thereby leading to genomic instability.[17] The large T antigens of JCV and BKV, similarly, can interact with a variety of cellular proteins, including the p53 and pRb family proteins.[60] The regulatory region of the polyomavirus genome contains both the origin of replication and the viral promoter. The origin is recognized by the large T antigen, in concert with host-cell factors, including cellular DNA polymerase, and viral DNA replication is initiated. Both early and late viral transcription are mediated by cellular RNA polymerase. T-antigen binding to viral DNA autoregulates the production of early mRNA and, by interaction with cellular factors, stimulates late transcription. Polyomaviruses have either an archetypal (single enhancer) or a rearranged (complex) regulatory region that may influence the ability of a particular strain to cause disease by virtue of its effect on efficiency of replication.[17,60]

EPIDEMIOLOGY

Serologic data, screening of environmental samples, and the worldwide distribution of PML cases among immune suppressed individuals suggest that human polyomaviruses are endemic throughout the world[14,41,98] (Fig. 166–1). The prevalence of antibodies against polyomaviruses increases with the patient's age, beginning in early childhood. BKV seroconversion occurs in 50 percent of children by the time they reach the age of 3 to 4 years, and JCV antibodies are acquired by 50 percent of children by the age of 10 to 14 years.[98,120] Population-based seroprevalence data suggest that more than 80 percent of adults have been infected with JCV, BKV, or both viruses.[40,71,98,101,120] BKV seroconversion has been associated with mild upper respiratory tract illnesses in a small number of children, and BK viruria has been reported in a child with acute tonsillitis.[49,50,127] To date, no distinct clinical syndrome has been associated with BKV infection of healthy children. The prevalence of antibodies in humans directed against SV40 has not been well established, primarily because of the variability in methods used by different research laboratories. Several studies have reported seroprevalences of 2 to 11 percent for SV40 with use of a plaque neutralization assay.[19,20,110] However, other studies have suggested that some SV40 reactivity may be due to cross-reactivity with BKV.[22,71,141]

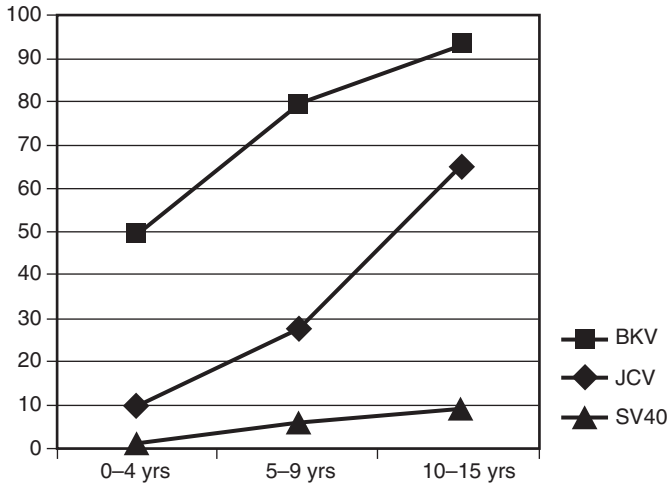

Figure 166–1 Seroprevalence estimates for JC virus (JCV), BK virus (BKV), and simian virus 40 (SV40) in childhood. *(Data from Lednicky, J. A., and Butel, J. S.: Polyomaviruses and human tumors: A brief review of current concepts and interpretations. Front. Biosci.* 4:*D153-D164, 1999.)*

Humans are the only known reservoir for JCV and BKV, whereas SV40 primarily is a simian virus that has crossed species from monkeys to humans. Polyomaviruses persist in the kidney and, to a lesser degree, in B lymphocytes and oligodendrocytes in both humans (JCV and BKV) and monkeys (SV40).[33,36,75,93] The exact mechanism of transmission of JCV and BKV is not known, but studies have shown that fecal excretion of BKV may be a common occurrence in infants, suggesting that fecal-oral transmission could explain the ubiquity of early childhood infection.[136] SV40 was introduced inadvertently into humans as a contaminant of early poliovirus vaccines; however, such direct exposure cannot account for the SV40 seropositivity in children born more recently than 1962, thus suggesting that modern human-to-human spread of the virus occurs. In the pediatric population, one SV40 seroprevalence survey found nearly 6 percent of hospitalized children with antibodies to SV40.[19] Among kidney transplant recipients in the cohort, 40 percent had evidence of SV40 infection, but whether such infection was related to immunosuppressive medications or the underlying kidney disease was not clear.[19] SV40 DNA sequences have been detected in kidney tissue from several pediatric renal transplant recipients.[18]

In immunocompetent individuals, persistence of JCV and BKV in the kidney is asymptomatic, with intermittent shedding in urine of JCV more frequently than of BKV.[2,119] Viral DNA (JCV or BKV) can be found in 30 to 50 percent of kidney samples from immunocompetent adults taken at surgery or autopsy.[24,55,134] The frequency of JCV excretion in urine increases with age, and the prevalence of BKV excretion is approximately 2 to 5 percent in healthy adults.[32,70]

JCV is lymphotropic, and the finding of JCV in plasma and peripheral blood mononuclear cells generally correlates with immune status in that it is noted less frequently in immunocompetent patients and increases in frequency in human immunodeficiency virus (HIV)–infected patients with lower CD4 counts.[31,43,72] A study of SV40 seroprevalence in HIV-infected patients found no difference between patients and age-matched HIV-uninfected controls, implying that SV40 infection may not be related to immune status.[61] Several conditions, including pregnancy and autoimmune disorders, are associated with excretion of BKV or JCV in urine.[86,126,129,130] The immunologic and virologic factors regulating initiation and maintenance of persistence are unknown.

CLINICAL MANIFESTATIONS

CENTRAL NERVOUS SYSTEM MANIFESTATIONS

PML is a clinical syndrome characterized by progressive neurologic impairment in immunocompromised patients. In PML, the host immune system fails to maintain viral latency; JCV replicates in and lyses oligodendroglia and causes destruction of cerebral white matter in a multifocal or patchy distribution. PML is the sentinel acquired immunodeficiency syndrome (AIDS)–defining illness in approximately 5 percent of adults, and its cumulative incidence in HIV-infected adults may be greater than 25 percent.[11,51] PML occurs less frequently in HIV-infected children than in adults. Other immunosuppressed individuals who are at high risk for development of PML include patients receiving chemotherapy, solid organ or hematopoietic stem cell transplant recipients, and those with primary T-cell defects.

Clinical manifestations of PML can include behavior changes; blindness, deafness, and other cranial nerve dysfunctions; motor and cognitive deficits; and lack of coordination as a result of cerebellar involvement.[12] The onset of symptoms usually is insidious, and associated systemic signs such as fever and headache are uncommon manifestations. Progression of symptoms occurs during a period of several weeks to months, and the condition is inevitably fatal. The diagnosis of PML is largely clinical and based on the history, physical examination, and characteristic findings on neuroimaging studies. Computed tomography and magnetic resonance imaging of the brain show focal demyelination and white matter edema with relative sparing of the gray matter[91] (Fig. 166–2). Cerebrospinal fluid analysis usually is normal in patients with PML, likely a result of impairment or depletion (or both) of T lymphocytes.[12] BKV also has been associated with neurologic dysfunction in patients with AIDS, although much less frequently than has JCV.[15,135] In addition, BKV encephalitis has been reported in a patient without known immunodeficiency in association with polyomavirus seroconversion.[142]

RENAL MANIFESTATIONS

Polyomaviruria is a common occurrence in immunocompetent adults and may be so in children as well.[23,63,70,112,137] Hemorrhagic cystitis related to BK viruria has been reported in several immunecompetent children, but no other nonmalignant diseases have been linked to polyomavirus infection in immunocompetent children.[114] Urinary excretion of BKV occurs in approximately 20 to 30 percent of adult and pediatric renal transplant recipients, approximately one third of whom also may have BK viremia.[56-59] Post-transplantation polyomaviruria is due predominantly to reactivation of latent infection, although whether the reactivated virus is of donor or recipient origin remains uncertain. Excretion of BKV usually begins in the first 2 to 3 months after the patient has undergone transplantation. In renal transplant recipients, BK viruria occasionally is associated with ureteral ulceration and stenosis, sometimes requiring surgical intervention to prevent or to relieve obstructive nephropathy. The prognostic significance of polyomavirus reactivation in renal transplant recipients has become a topic of great interest as management of immunosuppression administered to balance allograft rejection and infectious complications has become more sophisticated.

During the past 10 years, BKV has emerged as a significant cause of renal allograft failure. BKV-associated nephropathy (BKVAN) affects 1 to 10 percent of renal transplant recipients, resulting in allograft loss for approximately 50 percent of those affected. Initially, case reports linking polyomavirus infection and allograft failure identified BKV as a possible pathogen.[100,105] In one case series of biopsy-proven interstitial nephritis in adult

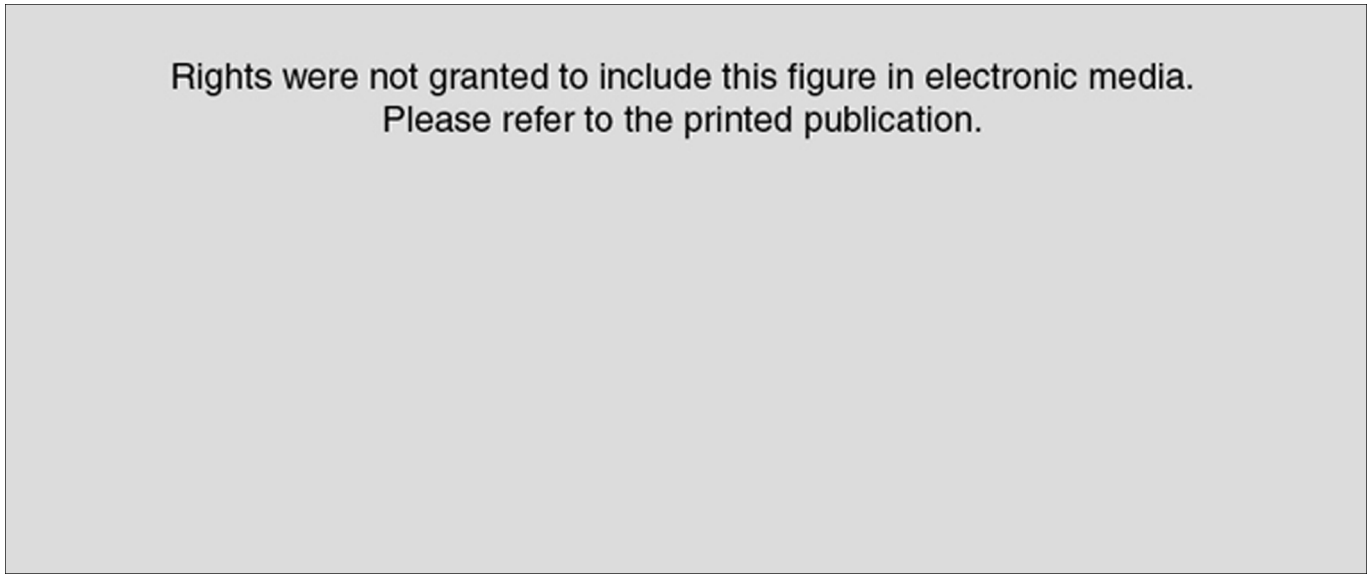

Figure 166–2 **A,** Contrast-enhanced brain computed tomogram of an HIV-infected child with progressive multifocal leukoencephalopathy showing low-density, nonenhancing, right-sided cerebellar white matter lesion without a mass effect. **B,** With T2-weighted magnetic resonance imaging, the lesion produces high signal without a mass effect or edema. **C,** The lesion gives low signal on T1-weighted magnetic resonance imaging and fails to enhance with intravenous administration of contrast material. *(From Morriss, M. C., Rutstein, R. M., Rudy, B., et al.: Progressive multifocal leukoencephalopathy in an HIV-infected child. Neuroradiology 39:142-144, 1997.)*

renal allograft recipients, 19 of 22 patients had increased levels of serum creatinine at 11.5 ± 13.0 months after transplantation.[105] Polyomavirus intranuclear viral inclusions and mononuclear inflammatory infiltrates were common histopathologic findings in allograft biopsies. Antirejection therapy was associated with graft loss in 8 of 12 patients, whereas a reduction in immunosuppression was associated with graft survival and clearance of viruria in three of six patients.[105] A smaller study in Europe had similar findings.[94] Since then, larger studies have begun to reveal the pathogenesis of BKVAN as well as the appropriate diagnostic and therapeutic strategies. Retrospective analyses have found that BKVAN is associated more frequently with the use of tacrolimus and mycophenolate, but BKVAN has been reported in patients receiving other immune suppressive agents.[1,16] Molecular characterization of BKV isolates from patients with BKVAN has not identified particular strains of BKV that are more likely to cause BKVAN.[121] The role of host factors in the pathogenesis of BKVAN is under investigation.

Polyomaviruria, especially that involving BKV, is a common finding after hematopoietic stem cell transplantation, occurring in 50 to 80 percent of patients within 2 to 8 weeks after transplantation (Fig. 166–3).[5,9,118] BK viruria is associated with hemorrhagic cystitis (BKV-HC) in 15 to 25 percent of hematopoietic stem cell transplant recipients.[8,39,78,80,81] BKV-HC is both painful and distressing for hematopoietic stem cell transplant patients, occasionally necessitating frequent erythrocyte transfusions and, rarely, surgical intervention. For the majority of patients with BKV-HC, supportive care, including intravenous hydration and erythrocyte transfusions, is adequate management, as T-cell engraftment eventually controls BKV replication and leads to resolution of symptoms. Adenovirus is an important but less common cause of hemorrhagic cystitis after hematopoietic stem cell transplantation.[3,39,81]

Interstitial nephritis caused by BKV has been described in children with various immune deficiencies, including hyper-IgM immunodeficiency and AIDS.[27,111] Renal dysfunction is a common finding in patients with AIDS, but its etiology has been linked only rarely to polyomaviruses; one study found SV40 in kidney tissue from several patients with AIDS and a collapsing variant of focal segmental glomerulosclerosis, but the pathogenesis of this disease remains uncertain.[84] Several studies of Balkan endemic nephropathy have associated this enigmatic disease in eastern Europe with polyomaviruses, but further study is needed to establish a causal link.[92,124] One pediatric study observed an association between SV40 seropositivity and renal transplantation and detected SV40 sequences in renal biopsy specimens, thus suggesting a possible (but still unproven) role for SV40 in pediatric renal disease.[18,19]

OTHER MANIFESTATIONS

Although some researchers contend that polyomaviruses can be transmitted in respiratory secretions, little evidence indicates that such transmission, if it does occur, is accompanied by clinical disease of the respiratory tract. Several case reports of BKV-associated pulmonary disease have been published, uniformly in the setting of severe immunosuppression and with poor outcomes.[27,115,135] Although interstitial pneumonitis in a patient with severe immune compromise might raise the suspicion of polyomavirus-associated disease, other viral pathogens such as cytomegalovirus and adenovirus should be considered first.

BKV-associated retinal necrosis in an AIDS-infected patient has been described, as have elevated hepatic transaminase levels in association with BK viruria and detection of BKV in normal liver specimens.[15,54,69,97] The clinical significance of these manifestations is unknown.

MALIGNANT NEOPLASMS

The polyomaviruses are named for their ability to induce tumors in laboratory animals. Their association with human tumors was noted first in 1974 when Soriano and colleagues[123] isolated SV40 from a metastatic malignant melanoma in a patient with SV40 neutralizing antibodies. SV40 T antigen and capsid antigen were detected in lung, liver, and muscle metastases of the patient but not in normal tissue.[123] Since that time, polyomaviruses have been

Figure 166–3 Cumulative percentages of bone marrow transplant recipients positive for BK virus (BKV; *n* = 26), herpes simplex virus (HSV; *n* = 22), and cytomegalovirus (CMV; *n* = 14) by the first day of detection. *(From Arthur, R. R., Shah, K. V., Charache, P., et al.: BK and JC virus infections in recipients of bone marrow transplants. J. Infect. Dis.* 158*:563-569, 1988.)*

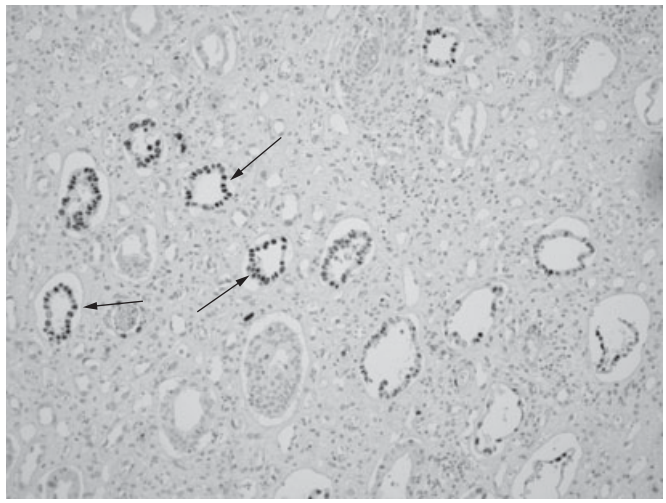

Figure 166–4 Immunohistochemical detection of the BKV T antigen in kidney tissue obtained at autopsy from a pediatric patient with disseminated BKV infection after hematopoietic stem cell transplantation. (Magnification = 40×.) Arrows indicate positively stained renal tubular epithelial cells. (See companion Expert Consult web site for color version.) *(Image courtesy of J. A. Vanchiere, M.D., Ph.D.)*

linked to a variety of neoplasms in humans, frequently analogous to the tumor types produced in rodents. SV40 has been detected in association with human cancers much more frequently than have JCV and BKV. SV40 T antigen or DNA has been detected in a significant number of meningiomas, ependymomas, choroid plexus tumors, astrocytomas, pleural mesotheliomas, and osteosarcomas.[7,62] Several studies have reported the presence of SV40 in non-Hodgkin lymphomas.[87,109,140] In the case of pleural mesotheliomas, which also have been associated with exposure to asbestos, the presence of SV40 may be a negative prognostic indicator.[103] A possible synergistic effect of SV40 and asbestos has been observed in vitro and in case-control studies of human patients with mesothelioma, suggesting that the two agents may work in concert as co-carcinogens.[13,26,73] In a study of pediatric patients, JCV DNA was detected in 11 of 23 medulloblastomas, and JCV T antigen expression was detected by immunohistochemistry in four of 16 samples tested.[74] Other investigators have not detected polyomavirus DNA or protein in medulloblastoma tissue.[68,144] JCV also has been detected in colorectal and gastric cancers and in normal tissue samples from the human gastrointestinal tract.[76,106,107] The significance of these findings is unclear at present. A study of bone marrow aspirates and white blood cells from pediatric patients with leukemia failed to find evidence of polyomaviruses.[122] BKV has been detected in both normal and neoplastic prostate tissue, suggesting a potential role for BKV in the pathogenesis of this common cancer of adulthood.[28,29] Further studies, including evaluation of host immunogenetic factors, will be required to establish whether BKV and JCV are commonly oncogenic in humans.

LABORATORY DIAGNOSIS

Although the suspicion of polyomavirus disease is primarily a clinical one, laboratory techniques are important for establishing the diagnosis in the proper clinical circumstances. Serologic tests and viral culture are of little use clinically because these tests are laborious and rarely available. Polyomaviruses in urine could be considered normal flora among immunocompetent patients, much like cytomegalovirus detected in urine after infancy. Histologic study of urinary sediment can reveal "decoy cells," which are uroepithelial cells with characteristic polyomavirus inclusions. The presence of decoy cells is a specific and moderately sensitive marker of polyomavirus-associated nephritis in renal transplant patients.[35,77] Detection of specific polyomavirus DNA from urine samples is of little significance, whereas polymerase chain reaction (PCR) detection from blood samples, especially viremia of greater than 10,000 genomes per milliliter, may correlate with the risk for development of BKVAN.[77,95,104] Immunohistochemical detection of BKV T antigen in renal biopsy material (Fig. 166–4) is the "gold standard" for establishing the diagnosis of BKVAN, although electron microscopic identification of polyomavirus-like particles in renal biopsy material also may be diagnostic.[57,85] The diagnosis of BKV-HC is based on clinical symptoms and the detection of BKV in urine, usually by PCR. Urinary viral loads in patients with BKV-HC and BKVAN can be extremely high, even 10^{12} or 10^{13} genome copies per milliliter.

Detection of JCV DNA in cerebrospinal fluid by PCR is the diagnostic test of choice for suspected PML.[52,72,116,132] In adult patients with AIDS and neurologic symptoms, PCR amplification of JCV DNA from cerebrospinal fluid is 99 percent predictive of PML, whereas brain biopsy has a slightly lower sensitivity.[30] On gross examination, tissue typical of PML has been described as "worm eaten," and microscopic examination reveals multiple foci of demyelination with enlarged oligodendroglia, giant astrocytes, and an intense phagocytic infiltration.[139] Immunohistochemical techniques and other nucleic acid detection methods also can be used to confirm the diagnosis of PML.[64] JC viremia is a general indicator of immune suppression and is a common finding in HIV-infected patients without PML, so its utility in the diagnosis of suspected PML is questionable.[72]

TREATMENT AND PREVENTION

No specific antiviral agents have been studied in randomized trials for the treatment of polyomavirus disease or polyomavirus-

associated tumors. Studies in both adult and pediatric patients have demonstrated that routine monitoring of plasma BK viral loads and preemptive reduction of immune suppression are useful for the prevention of BKVAN without significant risk of allograft rejection, and routine screening for BKV has been recommended by an international expert panel.[16,47,57] However, BKV reactivation may persist and lead to chronic renal dysfunction in some patients.[138] BKVAN should be suspected in any renal transplant recipient with otherwise unexplained allograft dysfunction. For renal transplant recipients with biopsy-proven BKVAN, reduction of immune suppression may be helpful for preservation of allograft function, especially among patients with low-grade disease.[34,42] Whether antiviral agents provide additional benefit beyond that of reducing immune suppression has not been established. Case series have reported variable results with the use of cidofovir and leflunomide in patients with BKVAN.[6,38,65-67] Despite its potential nephrotoxicity, low-dose cidofovir has been suggested by an international consensus group as a potential investigational agent for treatment of BKVAN.[57] Intravenous immune globulin has been used to treat several patients with BKVAN, but no obvious benefit has been observed.[117,143] Both leflunomide and cidofovir have been used to treat hematopoietic stem cell transplant recipients with particularly severe hemorrhagic cystitis caused by BKV, but immune restoration by T-cell engraftment is likely to be the most important factor in resolution of BKV-related cystitis. The role of fluoroquinolone antibiotics, which have some anti-polyomavirus activity, for prevention or treatment of BKV disease in immunocompromised patients is an area of intense interest, and studies of newer, investigational antiviral agents are in progress.[79,82]

Initiation of highly active antiretroviral therapy (HAART) can restore immune function and alleviate the symptoms of PML for patients with AIDS.[37,102,133] Despite administration of HAART, PML may develop in some HIV-infected patients because of incomplete immune reconstitution.[131] Cidofovir has been used successfully in conjunction with HAART in patients with AIDS and PML, but its benefit above that of HAART and in non–AIDS-infected patients with PML has not been determined.[89,113] Given the ubiquity and generally benign nature of polyomavirus infections, as well as the paucity of information about the nature of their transmission and clinical significance, specific efforts to prevent polyomavirus transmission are not indicated at this time.

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CHAPTER 167

Human Papillomaviruses

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HUMAN PAPILLOMAVIRUSES

The human papillomaviruses (HPVs) are members of the virus family *Papillomaviridae*. They are small viruses containing double-stranded DNA. They replicate in the nucleus of infected cells and have the ability to cause malignant transformation of a variety of cell types. Papillomaviruses are 55 nm in diameter, with a genome approximately 8000 base pairs in length. Papillomaviruses cause infection in immunocompetent and immunodeficient hosts and have been associated with malignancies. They also are responsible for a variety of benign, yet bothersome, cutaneous proliferations, including common skin warts. HPVs also have been associated with serious, even life-threatening illnesses, including genitourinary cancer and respiratory papillomatosis. Our current understanding of the complex role HPVs play in these diverse clinical conditions remains incomplete, and management of these diseases remains an ongoing challenge. Most recently, the licensure of two prophylactic vaccines against HPVs provides hope that the public health impact of these viruses will soon be lessened.

HISTORY

Warts or papillomas have been recognized for centuries to occur at a variety of different body sites, including the skin, genital tract, oral cavity, conjunctiva, and respiratory tract. Their infectious nature has been suspected by clinicians for many decades and has been the product of much folklore. The viral etiology of warts was discovered scientifically, however, in 1907, when human volunteers were inoculated experimentally with a cell-free extract prepared from wart tissue.[41] These early experiments also suggested that warts could be transmitted from person to person. When electron microscopy became available in the 1940s, virus particles were visualized within many of these clinical sites, initially in skin warts and subsequently in genital warts, thereby confirming the viral etiology of these lesions. However, despite the abundance of virus particles seen in some lesions such as skin warts, virologic investigation of the disease processes was hampered by the inability to propagate papillomaviruses in cell culture or laboratory animals. The limited amount of information available from the study of virus particles obtained directly from wart tissue led to speculation, in the 1960s, that HPVs were composed of a single virus type. Scientists further theorized that the specific body site and epithelium involved, rather than the virus type, were responsible for the characteristic morphology and disease process.[195] With the advent of molecular biologic techniques in the 1970s, however, more than 70 different types of HPV were recognized, and researchers quickly recognized that specific clinical diseases were associated with infection with specific HPV types. Most recently, HPV infection of the genital tract has emerged as one of the most prevalent sexually transmitted infections, and the link to some squamous cell carcinomas of the cervix has been strengthened. In addition, at least two mucosal HPV types appear to produce respiratory papillomatosis in pediatric patients. Finally, the role of some cutaneous HPV types in the evolution of squamous cell carcinoma from wart lesions in patients with epidermodysplasia verruciformis (EV) recently has been elucidated. Challenges for the future revolve around developing effective treatment strategies because current measures are palliative, at best, and most lesions recur. New antiviral chemotherapeutic agents are being developed, and immunomodulators such as interferon provide promise for the treatment of serious disease caused by HPVs.

VIROLOGY

The family *Papillomaviridae* contains the HPVs, which are small, nonenveloped viruses with a diameter of about 55 nm. They have a capsid composed of 72 capsomers arranged in icosahedral symmetry and a genome composed of double-stranded circular DNA that is approximately 8 kb in length. The complete nucleotide sequence is available for some HPVs, and partial information is available for all HPV types. The genome is divided into three regions—early, late, and regulatory—and many nucleotide sequences are shared among types. The early region is approximately 4.5 kb in length, contains eight open-reading frames (E1 to E8), and encodes genes that produce the proteins required for viral DNA replication and cellular transformation (Table 167–1). The late region is approximately 2.5 kb in length, contains two open-reading frames (L1 and L2), and codes for major and minor capsid structural proteins. The regulatory or long control region is located between the early and late regions. It is approximately 1 kb in length and contains the origin of replication and many control elements for viral transcription and replication.[178] The virus appears to replicate solely in the nucleus of the cell in association with low-molecular-weight histones. Because the HPV genome codes for only 10 proteins, it does not have a viral pro-

TABLE 167–1 Major Human Papillomavirus Genes

Gene	Protein Products and Function
Early (E) Region	
E1	Viral regulatory protein that initiates viral DNA replication
E2	Viral regulatory protein that controls replication and inhibits or activates early transcription of the viral genome
E3	Unknown
E4	A late viral protein that controls viral maturation; expressed in terminally differentiated keratinocytes
E5	Major transforming protein; causes cellular proliferation
E6	Major transforming oncoprotein; associates with the cellular target, p53, a tumor suppressor protein, and promotes its proteolytic degradation; causes cellular proliferation and perturbation of keratinocyte differentiation
E7	Major transforming protein; associates with the cellular target pRB and inactivates its cell cycle restriction function; causes cellular proliferation and perturbation of keratinocyte differentiation
E8	Unknown; ? regulates viral DNA replication
Late (L) Region	
L1	Major structural viral capsid protein
L2	Minor structural viral capsid protein

tease, DNA polymerase, or other enzymes involved in nucleotide metabolism and, therefore, requires many host-cell enzymes and cellular differentiation factors to complete its viral replicative life cycle.

The life cycle of HPVs is integrated intimately with the life cycle and maturation of epithelial cells. Initial virus infection probably occurs in the basal keratinocyte, with early transcription of the viral genome regulated by the E2 protein. Cellular proliferation and perturbation of keratinocyte differentiation then are induced by the E6 and E7 proteins. The cells are stimulated in the S phase of the cell cycle, and host enzymes for DNA synthesis are used by the virus to complete viral DNA replication. After double-stranded DNA is produced, L1 and L2, the major and minor capsid proteins, and E4, a late-associated structural protein whose gene is located in the early region of the genome, are synthesized. During the final stages of cellular keratinocyte differentiation, the final stages of virus production also occur with the assembly of complete viral particles. However, progeny viruses are not released until dead keratinocytes are sloughed from the epithelial surface.

Papillomaviruses display a high degree of species, tissue, and cellular specificity. HPVs appear to infect only humans, and most animal papillomaviruses also do not infect other species. In addition, they primarily infect the surface squamous epithelium of the skin or mucosa, and specific viral types appear to have a preference for either skin or mucosa, as well as for specific body sites.[76] However, some HPV types recently have been detected in transitional and cuboidal epithelium in the anogenital tract. More than 100 types of HPV have been recognized by DNA homology studies and sequenced,[200] and they fall naturally into two main clinical groups: cutaneous and mucosal (Table 167–2). The cutaneous HPVs contain types from a variety of benign skin warts, as well as more than 15 types recovered from a small group of patients with a rare dermatologic disorder, EV. The mucosal HPVs occur mainly in the genital tract but also infect and produce disease at other mucosal sites such as the respiratory tract, oral cavity, and conjunctiva. The mucosal HPVs also can be subgrouped into low-, high-, and intermediate- or moderate-risk types, depending on the frequency with which they are found in invasive cancers.[19,128,200,227,241]

TABLE 167–2 Human Papillomavirus (HPV) Types and Associated Clinical Conditions

Condition	Usual Location	Morphology	HPV Type
Cutaneous (Skin) Warts			
Common (verruca vulgaris)	Hands, lips, extremities	Multiple, dome shaped	2, 4
Plantar (verruca plantaris)	Bottom of feet	Single, painful	1
Flat (verruca plana)	Arms, face, knees	Multiple	3, 10, 28, 41
Filiform	Face, neck	Multiple, threadlike	2, 4
Mosaic	Feet, hands	Multiple, superficial	7
Butcher's	Hands	Multiple, dome shaped	7
Epidermodysplasia verruciformis	Face, trunk, extremities	Multiple flat warts or reddish-brown plaques	5, 8, 9, 12, 14, 15, 17, 19-25, 36-38, 47, 49
Immunosuppressed patients	Face, trunk, extremities	Dome-shaped or epidermodysplasia verruciformis-like plaques; may be persistent or progressive	1-5, 8, 10, 20, 23, 28, 49
Mucosal (Anogenital) Warts			
Subclinical	Cervix	Asymptomatic	6, 11, 13, 16, 18, 30-35, 39, 40, 42-45, 51-59
Condylomata acuminata	Cervix, vulva, urethra, anus, penis, scrotum	Multiple exophytic, pink, gray	6, 11, 16
Flat condylomata	Cervix	Asymptomatic, flat plaques	6, 11, 16, 18, 31
Giant condylomata acuminata (Buschke-Löwenstein tumors)	Perirectal area	Large, tumor-like	6, 11
Bowenoid papulosis	Penis, vulva, perirectal area	Multiple, large	16
Cervical cancer	Cervix	Asymptomatic; pigmented papillomas; erythematous or white plaques; ulcerations; mass lesion	Strong association: 16, 18, 31, 45 Moderate association: 30, 33, 35, 39, 51, 52, 56, 58, 59, 68 Weak association: 6, 11, 26, 34, 40, 43, 44, 53-55, 57, 62, 66, 74
Vulvar cancer	Vulva	Asymptomatic; pigmented papillomas; erythematous or white plaques; ulcerations; mass lesion	16
Penile cancer	Penis	Painless, ulcerative mass lesion	16
Anal cancer	Perianal area, anal canal	Asymptomatic; pigmented papillomas; erythematous or white plaques; ulcerations; mass lesion	16, 18, 31
Ovarian cancer	Ovaries	Unknown	6?, 16?, 18?
Mucosal (Other) Warts			
Respiratory papillomatosis	Larynx, trachea, bronchi, lungs	Multiple papillomas	6, 11
Nasal and paranasal papillomas	Nose, paranasal sinuses	Single or multiple papillomas	6, 11, 57, 57b
Focal epithelial hyperplasia (Heck disease)	Oral cavity	Discrete, multiple nodules	13, 32
Oral cavity papillomas	Gums, buccal mucosa, soft palate, tonsils	Single or multiple papillomas	6, 11, 16
Conjunctival papillomas	Conjunctivae	Single or multiple papillomas	6, 11, 16
Giant-cell hepatitis	Liver	Unknown	6?

Propagation of HPVs in monolayer cell culture to yield full viral particles has not been possible yet, probably because full epithelial cell differentiation and keratinization of squamous epithelial cells are required for the virus to replicate completely, and such differentiation is not achieved in conventional cell culture. Therefore, other methods have been developed to study the biology of HPVs. For example, research laboratories have propagated a few papillomaviruses successfully by inoculating virion extracts into susceptible tissue and transplanting this tissue into athymic nude mice.[117] Molecular assays of viral transformation with cloned HPV DNA have been used to define the viral genes involved in the induction of cellular proliferation and transformation. Rabbit papillomavirus animal models also have been used to study the function of E5 protein. In addition, the three-dimensional structure of the important viral proteins, such as E2 and potentially E6 and E7, have been determined by x-ray crystallography and multidimensional nuclear magnetic resonance spectroscopy.

Induction of cellular proliferation is the hallmark of infection with the papillomaviruses. Warts, for example, arise when a cell or a small group of cells in the basal cell layer of the epithelium are infected with an HPV type. The viral DNA replicates in an episomal or small circular form and stimulates the cells to proliferate and produce a self-limited tumor, also called a *papilloma*, or *wart*. In a wart, all layers of the normal epithelium are present, except with the addition of hyperplasia of the prickle cell layer, a condition called *acanthosis*. Hyperkeratosis is common in cutaneous warts but usually is absent in mucosal warts. Viral particles are produced in the differentiated, uppermost granular layers of the epithelium, where the viral cytopathic effect (koilocytosis) characteristic of HPVs is displayed. Benign lesions express both early and late genes, but if the lesion progresses to malignancy, expression of the capsid antigens and production of viral particles are inhibited, and only early-region transcripts and proteins are detectable. When invasive cancer occurs, the viral DNA usually is integrated into the cellular genome. Another unique characteristic of the HPVs is the presence of latent or persistent viral genome in apparently normal cells. Such persistence probably accounts for the recurrence of both genital and laryngeal papillomas, even after apparently successful treatment and prolonged disease-free periods.[66,226]

EPIDEMIOLOGY

The papillomaviruses are widespread in nature, and infection with HPVs occurs in people of all ages from all parts of the world. These viruses infect squamous epithelium at several body sites, and individual HPV types display marked specificity for a particular site. Infection appears to be transmitted through fomites, moisture, and minor skin trauma in the case of cutaneous warts; by sexual intercourse in the case of genital warts and condyloma; and during birth through an infected maternal birth canal in patients with juvenile-onset respiratory papillomatosis. In addition, HPV DNA has been detected in aerosolized smoke and vapor during laser and electrocautery treatment of patients with cutaneous and genital warts and laryngeal papillomatosis, and anecdotal reports of possible transmission from patient to surgeon have appeared.[1,65,78,202,203] The incubation period for most infections varies from 3 weeks to as long as 8 months, with an average of approximately 3 months. In respiratory papillomatosis, the incubation period may be 5 years or longer and, with cervical cancer, 10 years or longer. Infection with HPVs may be asymptomatic. Some may produce benign, barely noticeable warts; others may produce recurrent or growing lesions that are life-threatening and resistant to treatment; and a few infections may progress to invasive, even fatal, cancer. The outcome of an infection with HPV is influenced by several factors, including virus type, location of the lesion, immunologic status of the host, environmental and infectious cofactors, and the nature of the epithelium that has been infected.

Cutaneous warts are rare findings in children younger than 5 years old but are relatively common in older children, adolescents, and young adults. In fact, as many as 10 percent of school-aged children may have warts at some site on their bodies at any given time, and as many as 50 percent of individuals may have had cutaneous warts at some time during their life. Certain activities (e.g., use of public swimming pools and tattooing) or certain occupations (e.g., butchers; handlers of meat, poultry, and fish; workers in slaughterhouses) appear to have an increased risk of acquiring cutaneous warts.[54,105,126,197,235] Most warts regress within 2 years, presumably because the host mounts a cell-mediated immune response. Warts may increase in number and size, however, if the individual is immunocompromised, is pregnant, or has the rare familial disorder EV. The genotypes of HPV recovered from skin warts correlate, though not absolutely, with the morphology and body site of the wart. For example, HPV-1 is associated with deep plantar warts, HPV-2 with common skin warts, HPV-3 and HPV-10 with flat skin warts, and HPV-7 with butcher's warts, whereas a large number of different HPV types can be recovered from patients with EV (see Table 173–2).

Infection of the genital tract with HPVs also appears to occur commonly, but its prevalence varies widely according to the population studied and the criteria used to define infection. For example, an estimated 1 to 2 percent of sexually active individuals have external anogenital warts or *condyloma acuminatum*. However, when cytologic methods to detect subclinical disease are used, as many as 10 percent of sexually active women have been shown to have HPV-related disease of the cervix. Even higher prevalence rates have been observed when highly sensitive molecular techniques that detect both asymptomatic infection and disease are used. For instance, one study of young university women found that 46 percent were positive for at least one HPV type when extremely sensitive PCR-based methods were used.[9] Furthermore, studies suggest that as many as 75 percent of women have been infected with HPV at some time during their life.[208,232] In contrast, older women appear to have a lower prevalence of HPV infection than younger women do, even when sensitive molecular methods are used.[147,204] Not all genital infections with HPV may be transmitted sexually, however, because HPV DNA also has been detected on medical instruments and on the underwear of patients with genital HPV disease, thus suggesting that genital tract HPVs may be transmitted in certain circumstances by fomites, similar to cutaneous warts.[64,65]

Infection with HPVs is so common and so easily transmitted that having just one sexual partner often results in infection. Most infections are asymptomatic and rapidly cleared by the immune system within 12 months.[192] The persistence of high-risk HPV types 16 and 18 is associated with cervical cancer, whereas infection with low-risk HPV types 6 or 11 is associated with genital warts and other low-grade abnormalities of the genital tract.[155] The sero-incidence of new HPV 16 infections, as well as the overall seroprevalence of HPV 16 infection in both men and women, is associated with age older than 20 years and number of occasional sexual partners with whom unprotected sex occurred during the antecedent 3 months.[235a] HPV 16 infection also appears to be more common in females than in males. Most epidemiologic studies suggest that HPV infection of the genital tract is a sexually transmitted disease and that age and the number of lifetime sexual partners also are independent risk factors for infection.[16,151] Seropositivity for HPV is exceedingly rare in individuals younger than age 10 years old and peaks in those between 30 and 50 years of age in most populations.[161] Of particular concern is the high and apparently increasing prevalence of cervical HPV infection in adolescents. In fact, recent epidemiologic evidence suggests that infection with HPV is the most common

sexually transmitted disease in adolescent women. For example, in one study of more than 600 adolescents who attended three urban clinics in Colorado, 24 percent of the patients had evidence of HPV infection (15% with clinically apparent genital warts, 36% with subclinical HPV infection detected cytologically, and 49% with subclinical infection detected by the presence of HPV DNA in cervical tissue).[102] In another study of sexually active adolescents, an incidence rate of 29 percent was observed during a 13.3-month study period.[194] Whether this high prevalence of HPV infection represents persistent infection with the same HPV genotype or re-infection with a new genotype remains controversial. For example, one study in Panama showed that the proportion of women with HPV infection increased in accordance with the number of consecutive specimens tested (21 to 82% in a cohort of high-risk subjects sampled monthly for at least six visits) and suggested that persistent HPV infection with periodic viral shedding was the most likely explanation.[155,185] In contrast, other studies have shown that detection of the same HPV DNA genotype on a second examination is unusual and suggested that spontaneous regression or resolution occurred, followed by re-infection with a new type of HPV.[163,194] Male partners also represent an important source of HPV infection in their female sexual partners.[11,12,31]

Epidemiologic observations show a strong link between HPV infection and cervical cancer because they both have characteristics of a sexually transmitted disease. For instance, the number of lifetime sexual partners is a risk factor for both HPV infection and cervical cancer.[31] Furthermore, cervical carcinoma is more likely to develop in women with a history of genital warts than in women with a negative history, and it is significantly more likely to develop in women married to men in whom cancer of the penis develops.[68,84] Associations also have been found between specific genotypes of HPV infection and the presence of invasive cervical cancer. HPV types 16, 18, 31, and 45 are associated strongly with cervical cancer; HPV types 33, 35, 39, 51, 52, 56, 58, 59, and 68 have a moderate association with cervical cancer; and HPV types 6, 11, 26, 42, 43, 44, 53, 54, 55, 62, and 66 rarely have been seen in cancerous lesions of the cervix.[213] In addition, cohort studies have shown that the presence of HPV DNA precedes the development of preinvasive cervical lesions.[187] However, despite strong circumstantial evidence and compelling laboratory documentation of the role that HPV plays in cervical cancer, HPV infection alone appears to be neither sufficient nor necessary for cervical cancer to develop. The disease still may be multifactorial in etiology and involve cofactors such as demography, genetics, socioeconomic status, race and ethnicity, age, nutrition and other dietary factors, pregnancy and parity, hormonal exposure, use of oral contraceptives, smoking, immune status, and the presence of other sexually transmitted infections and diseases.[151,187,213,217] Women infected with human immunodeficiency virus (HIV) have a high prevalence of HPV infection and are infected with a broader range of HPV types and with multiple HPV types compared with HIV-negative women. These HPV types include HPV 16, 18, 31, 33, 51, 52, 53, and 61.[43]

Like cervical cancer in women, the HPVs are associated with squamous intraepithelial lesions and anal canal tumors in men. More than 80 percent of anal cancer specimens have HPV DNA detected in this tissue.[71] Although anal cancer is a rare occurrence in men, certain risk factors, including HIV infection, a homosexual or bisexual lifestyle, and the practice of receptive anal intercourse, have been identified.[68,172] More than 90 percent of HIV-positive homosexual men will have anal HPV infection, and as many as 73 percent will have infection with more than one HPV type at a single time.[172] HPV-16 is the HPV type most frequently detected, but almost all types have been identified in this clinical setting.

Another potential consequence of genital infection with HPV is perinatal transmission of the virus from mother to infant. Genital HPVs, including the high-risk genotypes 16 and 18 and the lower-risk genotypes 6 and 11, may be transmitted from mother to infant, presumably by passage through an infected birth canal, although ascending infection and postnatal acquisition also are possibilities.[33,171] HPV DNA, including HPV 16 DNA, has been detected in human breast milk, suggesting another possible mode of perinatal transmission.[201] HPV DNA has been detected in buccal and genital cells obtained from infants born to mothers infected with HPV-16 and HPV-18, and pregnant women with a high viral load of HPV DNA in cervical cells are more likely to transmit HPV infection to their newborn infants than are mothers with a low viral load.[109] Furthermore, a recent study has demonstrated that HPV DNA may persist for at least 6 months in perinatally infected infants.[33] In fact, infection of the oral mucosa appears to be a common event in healthy adults and children. DNA from HPV-6 and HPV-16 has been detected by polymerase chain reaction (PCR) in 17 and 23 percent of oral mucosa samples from adults and in 24 and 19 percent of oral samples from preschool aged children, respectively.[104] The consequences of infection of the oral mucosa with genital-type and other HPVs range from asymptomatic infection to a variety of oral, respiratory, and ocular lesions. For example, HPV types 6, 11, 16, and 18 have been seen in leukoplakia, lichen planus, oral papillomas, squamous cell carcinoma of the tongue, and verrucous carcinoma of the larynx.[28,55,133] Dysplastic and malignant lesions of the ocular conjunctiva and cornea also have been associated with HPV-16.[144] HPV-6 and HPV-11 have been linked to juvenile- and adult-onset laryngeal papillomatosis.[157] Circumstantial evidence implicating perinatal transmission of HPV as a cause of respiratory papillomatosis includes retrospective analyses that have shown an association between juvenile laryngeal papillomatosis and genital warts in the mother at the time of delivery.[17,47,183] Juvenile laryngeal papillomatosis also appears to be an uncommon finding in children delivered by cesarean section.[212] Also of note, however, is that this disease has a bimodal age distribution. Although the peak incidence of the disease occurs between birth and 5 years of age, almost half the patients with HPV-associated laryngeal papillomatosis are seen for the first time in adulthood.[45]

CLINICAL MANIFESTATIONS

Cutaneous Warts

Common cutaneous skin warts, or verrucae, have variable morphology and may appear at any location on the skin.[36,213] Common skin warts, or verrucae vulgares, usually are well-demarcated, dome-shaped papules with multiple conical projections (papillomatosis) that give the surface of the wart a rough appearance and texture. Common warts generally occur on the hands, especially the dorsum, but they also are seen frequently between the fingers, periungually, and on the palms and soles. They occasionally may be mosaic and spread superficially over the skin, or they may be filiform in morphology and appear as threadlike warts on the face and neck. They most commonly are associated with HPV-2 and HPV-4. Butcher's warts, a form of cutaneous wart seen in meat and poultry handlers who suffer repeated minor trauma to the hands, are associated with HPV-7. Plantar warts, also called *verrucae plantares*, occur most commonly in adolescents and young adults. They generally are single lesions and have a highly thickened corneal layer, or hyperkeratosis, with areas of punctate bleeding. They also are painful because they typically are found on pressure-bearing points on the plantar surface of the foot or the palms of the hand. They usually are associated with HPV-1. Flat warts, or verrucae planae, in contrast, do not have papillomatosis or hyperkeratosis, and they often are multiple. These warts are more common findings in young children and occur

most frequently on the arms, face, and knees. HPV types 3, 10, 28, and 41 have been associated with flat warts.[42,213] Although the clinical appearance of cutaneous warts almost always is diagnostic, the differential diagnosis includes other viral skin disorders, such as molluscum contagiosum, and other infectious diseases, such as actinomycosis, blastomycosis, sporotrichosis, leishmaniasis, chronic vegetating pyoderma, atypical mycobacterial infection (e.g., swimming pool granuloma), and tuberculosis verrucosa cutis (warty tuberculosis). Giant verrucae or warts also must be differentiated from squamous cell carcinoma.

Epidermodysplasia Verruciformis

EV, a rare skin disorder initially manifested in infancy or early childhood, is characterized by the inability to resolve HPV-induced, cutaneous wartlike lesions.[135] Seen worldwide, this disease is familial, and a history of parental consanguinity is noted in approximately 10 percent of reported cases, thus implying a genetic basis for the disease. Although both autosomal recessive and X-linked recessive forms of inheritance have been observed, the precise genetic defect or mode of inheritance remains elusive.[5,53,135,213] Patients with EV also have both nonspecific and HPV-specific defects in cell-mediated immunity, especially T-cell defects, and some patients will have developmental disabilities.[48,83,101,134,136,137] Although the lesions seen with EV are polymorphic, two clinical types of warts are seen primarily in these patients: flat warts and red or reddish-brown macular plaques. Both the flat warts and plaques appear first on the face, trunk, and extremities. They slowly become confluent and then appear to disseminate. Occasionally, the plaques may be achromatic with pigmented borders and resemble pityriasis versicolor.

Malignant transformation develops in 30 to 50 percent of patients with EV and occurs during adulthood, usually decades after the initial manifestation in childhood. Therefore, long-term, careful clinical follow-up of children in whom EV is diagnosed is important. The malignant transformation occurs in multiple foci in the reddish-brown plaque lesions, especially in areas that are exposed frequently to sunlight or other ultraviolet light, such as the forehead. It also is more likely to occur if the patient is infected with the highly oncogenic HPV types 5, 8, or 47. The tumors that result usually are slow growing, yet locally destructive. Histopathologically, they may appear as an in situ or invasive carcinoma.[101] They generally do not metastasize unless exposed to co-carcinogens such as x-irradiation.[169] The skin cancers in patients with EV, though a serious and challenging clinical entity, also provide an excellent example and scientific model to study host factors such as genetic defects, the infecting type of HPV, and environmental factors such as ultraviolet light in the genesis of malignant transformation and the development of cancer.[213]

Patients with EV may be infected with multiple types of HPV, including HPV-3 and HPV-10, that are associated with flat warts in healthy individuals.[135,165,213] The HPV types most commonly associated with lesions in patients with EV, however, are 5, 8, 17, and 20, types not usually seen in the general population. Other HPV types associated with this disorder are 9, 12, 14, 15, 19 to 25, 36 to 38, 47, and 49. The EV-associated HPV types that have a high oncogenic potential are 5, 8, and 47 because they appear in more than 90 percent of skin carcinomas in patients with EV, whereas HPV types 14, 20, 21, and 25 appear to have low oncogenic potential because they usually are detected only in benign skin lesions of patients with EV. It is likely that healthy individuals are infected asymptomatically with many of the HPV types seen in the lesions of patients with EV and that an immunologic defect probably allows the HPV infection to produce a chronic disease process. For example, HPV-8 antibodies have been found in healthy individuals, and HPV-5 DNA and HPV-8 DNA have been detected in refractory warts and skin carcinoma in immunocompromised patients such as renal allograft recipients.[86,130,179,225]

The appearance of EV-like skin lesions also may be seen in individuals who are immunocompromised as a result of HIV infection, as well as in transplant recipients, those receiving cancer chemotherapy, and other immunosuppressed patients.* These lesions may appear as brownish plaques, like typical EV lesions, or they may be morphologically similar to flat or common cutaneous warts. Skin warts in immunocompromised patients may be single or multiple, and they may persist or progress. These patients also have a high risk of developing squamous cell carcinoma, and the risk increases with exposure to sunlight or ultraviolet light, as well as with immunosuppression of long duration.[162,242] A variety of HPV types have been identified in patients who are immunosuppressed. For example, all the main types of HPV (1 to 4, 28) associated with skin warts in the general population have been detected in immunosuppressed patients. In addition, HPV types 5, 8, 10, 20, 23, 28, and 49, which are associated primarily with EV, have been detected, either alone or in combination, in immunosuppressed patients, especially renal transplant recipients.[135]

Infections of the Male and Female Genital Tract

Genital tract infection with the mucosal HPVs probably is the most prevalent sexually transmitted infection caused by a viral pathogen, and because of its link to cervical, penile, and anal cancer, such infection most likely imposes far greater morbidity on the general population than does cutaneous infection with HPVs. Genital tract infection with HPVs may be latent, active yet asymptomatic, manifested as genital warts or condyloma, or associated with various stages of cervical cytologic and histologic abnormalities, including low- and high-grade squamous intraepithelial lesions, carcinoma in situ, and invasive carcinoma. Pregnancy, immunosuppression, and especially HIV infection have been associated with an increased prevalence of HPV infection and disease in both men and women.[69,92]

Asymptomatic or subclinical infection occurs in as many as 10 percent of females older than 15 years, and the rates may be as high as 30 to 80 percent in some sexually active adolescent populations and more than 90 percent in homosexual HIV-positive men.[88] All genital HPVs (types 6, 11, 13, 16, 18, 30 to 35, 39, 40, 42 to 45, and 51 to 59) are involved.[172,213,249] The outcome of infection with a genital HPV is variable and includes resolution of the infection and elimination of viral DNA, viral persistence with no cytologic abnormalities, transient cytologic abnormalities that resolve completely in a few months, cytologic abnormalities that persist, and cytologic abnormalities that progress to in situ or invasive cervical cancer. Although the frequencies of these different outcomes of genital HPV infection are not known precisely, complete resolution of the infection appears to be the most frequent occurrence, whereas invasive cancer is the rarest outcome.[99,154,250] Whether re-infection with the same strain of HPV can occur is unknown. However, viral persistence appears to be likely in older women and HIV-infected individuals with cancer-related HPVs, especially types 16, 18, and 33.[98,154,213,230] Subclinical HPV infections do not cause symptoms and may be detectable only with sensitive molecular techniques that detect HPV DNA. Subclinical HPV infection also may produce subtle flat lesions that can be detected only by acetic acid treatment followed by colposcopy of the cervix.

The most common clinical manifestation of HPV infection of the genital tract is condyloma acuminatum, or genital warts.

*See references 18, 30, 61, 182, 198, 199, 219, 223, 236, 242.

These warts usually are caused by infection with HPV-6 and HPV-11 and occasionally HPV-16. They generally are multiple; exophytic; pink, purplish, or gray; and papular or pedunculated lesions composed of short or long fronds of connective tissue covered by acanthotic squamous epithelium. In females, they involve the vaginal introitus, vulva, perineum, cervix, urethra, and anus. The lesions usually are asymptomatic, but they may cause itching, burning, or pain. During pregnancy, genital warts may increase in number and size and regress after delivery. In males, genital warts occur on the penis, scrotum, perineum, and anus. They generally are asymptomatic but may cause itching, burning, and dyspareunia. Adult and adolescent women and men, as well as children, with external anogenital warts also may have HPV infection at internal cervical-vaginal or intra-anal mucosal sites.[89,100,112,113] In addition to typical papillary warts, flat condylomata may occur, especially in the cervix. These flat warts are difficult to visualize with the naked eye, and their detection may be aided by colposcopy. Flat condylomata usually are caused by HPV types 6, 11, 16, 18, and 31 and may progress to low- and high-grade cervical intraepithelial lesions.[213] Infection with HIV increases the risk for development of HPV infection and HPV-associated genital lesions and neoplasms in adult men and women, and, therefore, patients should be examined carefully for these conditions.[42,172,230,243] Whether HIV infection increases the risk for HPV infection or disease in adolescents and children also is a concern but is unclear at this time.[196,228] In addition, immunosuppression from chemotherapy and transplantation is associated with an increased risk for acquisition of HPV-associated genital infection, disease, and malignant transformation. In a renal transplant recipient, a novel HPV type 74 was discovered recently and subsequently detected in as many as 5 percent of renal transplant recipients.[127] In adolescents, anogenital warts may develop within 1 to 2 months after consensual sexual activity, as well as after sexual assault.[110] Infants and children also may have anogenital warts, which may result from sexual abuse or from perinatal transmission.[52,89,90] The risk of development of genital warts in a sexually abused child is not identified clearly; however, approximately half the cases of genital warts in children reported in the literature appear to be related to sexual abuse. In addition, molecular techniques have shown that anogenital warts in children contain HPV DNA from types 6, 11, or 16, the same types responsible for genital warts in adults, whereas HPV DNA from types 1, 2, and 4, which cause common cutaneous warts, has not been found in anogenital warts in children.[52] Anogenital warts in children are more likely to be acquired by sexual abuse than by perinatal transmission if the child is older than 2 years because this period is outside the plausible incubation period of perinatal transmission of HPV. Rapid progression of anogenital warts has been described in children with recent varicella infection. The differential diagnosis of anogenital warts includes other infectious diseases such as condyloma latum associated with secondary syphilis and molluscum contagiosum, as well as non-infectious disorders such as epithelial papillae, enlarged sebaceous glands or sebaceous cysts, seborrheic keratosis, lentigo, pigmented nevi, skin tags, hemorrhoids, Crohn disease, and carcinoma.

Giant condylomata acuminata may occur on the penis, vulva, or perirectal area. These giant tumor-like lesions also are called *Buschke-Löwenstein tumors* and *condylomatous carcinoma* and were reported first by Buschke in Germany in 1896. The growth of these lesions is indolent and rarely may cause inguinal lymphadenopathy, fistulous tracts, inflammation, fibrosis, and hemorrhage of the surrounding tissues. Histologically, these lesions are benign and appear similar to typical anogenital warts. However, progression to dysplasia and carcinoma has been documented. Buschke-Löwenstein tumors are associated with HPV-6 and HPV-11, in contrast to invasive genital carcinomas, which usually are associated with HPV-16 and HPV-18.[162]

Progression of HPV-induced anogenital condylomatous lesions to dysplasia or invasive carcinoma is well documented but unusual.[119,187,246] Most anogenital carcinomas probably arise from infection with high-risk HPV-16 and HPV-18 because more than 70 percent of human cervical cancers contain DNA from HPV-16 or HPV-18.[119,186] This epidemiologic observation complements in vitro studies that document the transforming properties of these viral types.[213,248] Other risks and cofactors for development of cervical cancer also have been identified, and progression from subclinical infection to carcinoma likely is multifactorial.[213] Invasive cervical cancer appears to evolve in a progressive cascade of cervical epithelial abnormalities that recently have been reclassified. These abnormalities currently are referred to as low-grade and high-grade squamous intraepithelial lesions. The category low-grade squamous intraepithelial lesion includes subclinical HPV infection, condyloma, and what was known formerly as grade 1 cervical intraepithelial neoplasia, or mild dysplasia. In the high-grade squamous intraepithelial lesion category, most or all of the thickness of the cervical epithelium is replaced by abnormal cells or microinvasive carcinoma. This category includes what previously was referred to as grades 2 and 3 cervical intraepithelial neoplasia, or moderate to severe dysplasia, and carcinoma in situ. If not detected and treated, high-grade squamous intraepithelial lesions may evolve into invasive carcinoma, breach the basement membrane, and metastasize to regional lymph nodes and other parts of the body. The HPV types that have a strong association with invasive cervical cancer are 16, 18, 31, and 45, whereas types 33, 35, 39, 51, 52, 56, 58, 59, and 68 are associated only moderately with cervical cancer. The remaining types (6, 11, 26, 43, 44, 53 to 55, 62, and 66) rarely have been associated with cancer. Cervical cancer usually is asymptomatic and detected by cytologic screening. However, patients with advanced invasive cervical cancer may have abnormal menstrual bleeding or pain. The lesions also may be visible by direct visual inspection and appear as pigmented papillomas, erythematous plaques, or leukoplakic lesions or, if further advanced, as ulcerations or large masses. Squamous carcinoma of the penis is a quite rare occurrence, especially in countries that practice routine newborn circumcision. However, when penile cancer does occur, it usually is manifested as a painless, slowly enlarging ulcerative mass.

Bowenoid papulosis is another manifestation of HPV infection of the anogenital tract. It typically occurs in adults younger than 40 years old and is characterized by multiple, large maculopapular lesions that are erythematous and reddish, purplish, or brownish, with a smooth, velvety surface. These lesions may regress spontaneously or persist. Bowenoid papulosis is associated with HPV-16 and histologically appears to be high-grade squamous intraepithelial lesions or squamous cell carcinoma in situ. Bowen disease, on the other hand, is seen in patients older than 40 years of age and causes single, reddish, scaling, or crusting lesions that histologically also appear to be high-grade squamous intraepithelial lesions.[246]

Traditionally, cervical dysplasia and carcinoma have been considered disorders of middle-aged and older women. However, during the past 10 to 20 years, the prevalence of HPV infection and abnormal cervical cytology in young women and adolescents has increased.[88] For example, recent studies suggest 18 to 53 percent prevalence rates for HPV infection in sexually active adolescents, with high-risk HPV-16 and HPV-18 being the most common types detected.[102] The prevalence of cervical low- and high-grade squamous intraepithelial lesions detected by Papanicolaou smear in adolescents also appears to have increased from 3 percent in the 1970s to 18 percent in the 1990s.[196] These alarming observations should alert physicians who care for adolescents and suggest that an epidemic of cervical cancer may occur in the near future.

Recurrent Respiratory Papillomatosis

Recurrent respiratory papillomatosis (RRP) is a rare, histologically benign, yet paradoxically life-threatening, condition caused by HPV-6 and HPV-11.[56,73,96a] Worldwide, it probably is the most common tumor of the larynx in children, with an incidence of 0.1 to 2.8 per 100,000 observed.[228,239] In the United States, the estimated incidence in children is 0.6 to 4.3 per 100,000 and up to 1.8 per 100,000 in adults.[56] The condition is newly diagnosed in an estimated 1500 patients each year.[56,156,239] The disease may be seen in both children and adults. Approximately two thirds of cases of RRP are seen in children, and in this population it also may be called *juvenile-onset recurrent respiratory papillomatosis* (JORRP). Approximately a fourth of the cases in children will be present before they reach 1 year of age, half by 5 years of age, and the remaining by 11 years of age. Adult-onset RRP usually is manifested in patients between 20 and 40 years of age, but occasionally it may be seen in adolescents.[73,213]

Circumstantial evidence suggests that infants and children with JORRP most likely acquire HPV perinatally during passage through an HPV-infected birth canal. For example, 30 to 60 percent of mothers of children with JORRP have a history of genital warts, as compared with less than 5 percent of mothers of children who do not have JORRP.[174,183] Furthermore, children with JORRP rarely are born by cesarean section. In fact, significant risk factors for JORRP include vaginal delivery, being firstborn, and maternal age younger than 20 years.[214] HPV-6 and HPV-11, most commonly associated with JORRP, also are responsible for most of the genital warts seen in women.[63,213] HPV DNA has been detected in the oropharynx of infants born to mothers with genital HPV infection.[213] However, RRP develops in only a small proportion of infants born to mothers with genital warts or subclinical HPV infection. The presence of HPV DNA in human breast milk renders this mode of transmission also a possibility for some infants.[201] The mode of transmission of HPV-6 and HPV-11 in adult-onset RRP is unknown. The most common initial symptom of RRP and JORRP is hoarseness or a change in voice. Infants and toddlers may have a hoarse cry, stridor, airway obstruction, respiratory distress, or difficulty in phonation. They also may have a crouplike illness. The most common site for RRP and JORRP is the true vocal cord of the larynx. Supraglottic and subglottic extension of the lesions also may occur. In addition, the disease may involve the trachea, bronchi, palate, nasopharynx, paranasal sinuses, and lungs. When the lungs are involved, pulmonary nodules, atelectasis, and secondary bacterial pneumonia may occur (Fig. 167–1). The disease also may produce permanent lung damage with bronchiectasis and cavitations (Fig. 167–2). In approximately 2 to 3 percent of patients, progression to invasive squamous papillomatosis and even malignant transformation to squamous cell carcinoma will occur, with invasion of the soft tissues of the neck, esophagus, and lung parenchyma (Fig. 167–3).[220] The incidence increases to 14 percent in patients who received the radiation therapy that commonly was used until 1970 to treat RRP.[81,138,207] Smoking and severe, recurrent disease also appear to increase the risk for development of malignant transformation in adolescents and adults. In addition, sudden and unexpected death may occur from airway obstruction if the lesions obstruct the laryngeal lumen.[221] Clinical courses of RRP and JORRP are highly variable and characterized by common and unpredictable remissions and exacerbations, even despite apparently successful removal of the lesions. Longitudinal studies on the time course of JORRP have shown infection with HPV type 11 and age younger than 3 years at initial diagnosis were associated significantly with a more aggressive tracheal disease requiring surgical débridement procedures more frequently.[184,252]

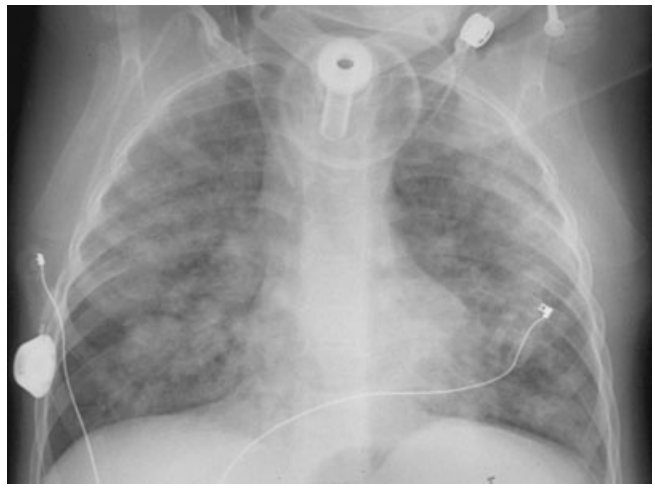

Figure 167–1 Chest radiograph of a 6-year-old girl with severe, recurrent, and progressive respiratory papillomatosis since 1 year of age.

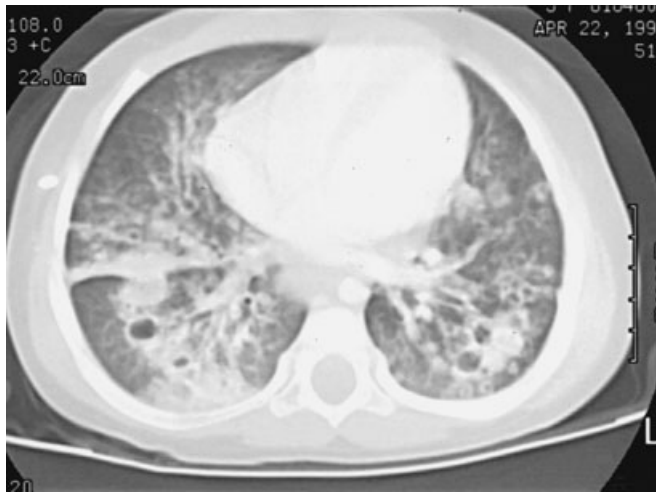

Figure 167–2 Chest computed tomography of a 6-year-old girl with severe, recurrent, and progressive respiratory papillomatosis. The tomograph shows pulmonary nodules, bronchiectasis, and cavitations.

Nasal and Paranasal Papillomas

Nasal papillomas or warts are rare tumors that may develop at any age, including childhood, and may occur as solitary lesions or in combination with papillomas elsewhere in the respiratory tract.[27,191] Histologically, they usually resemble the laryngeal papillomas seen in RRP. They most commonly are caused by HPV-6 and HPV-11, although HPV types 16, 57, and 57b also have been detected in nasal papillomas. Cocaine snorting is one risk factor for the development of nasal papillomas.[210] Papillomas also may involve the paranasal sinuses.[27,191]

Papillomas and Cancers of the Oral Cavity

Papillomas or warts in the oral cavity are heterogeneous etiologically and may be caused by cutaneous HPV types that are associated with warts on the skin, as well as by mucosal HPV types that are associated with genital warts. For example, focal epithelial hyperplasia, also called *Heck disease*, is a rare yet well-defined clinical condition of the oral mucosa that is associated with HPV-

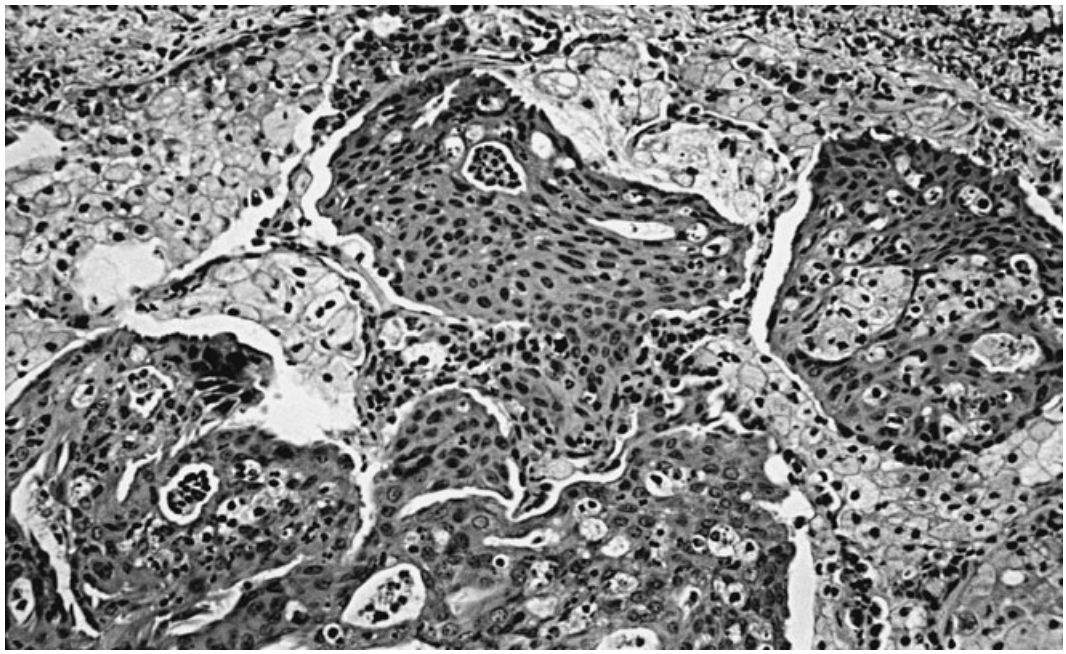

Figure 167–3 Lung biopsy tissue (hematoxylin and eosin stain, 50× original magnification, light microscopy) from a 6-year-old girl with severe, progressive, recurrent respiratory papillomatosis. This tissue demonstrates invasive squamous papillomatosis. The squamous cells are growing along preformed pulmonary structures, but no evidence of malignancy is apparent at this time because of the absence of invasion of lung tissue. *(Courtesy Dr. Claire Langston, Department of Pathology, Baylor College of Medicine and Texas Children's Hospital, Houston.)*

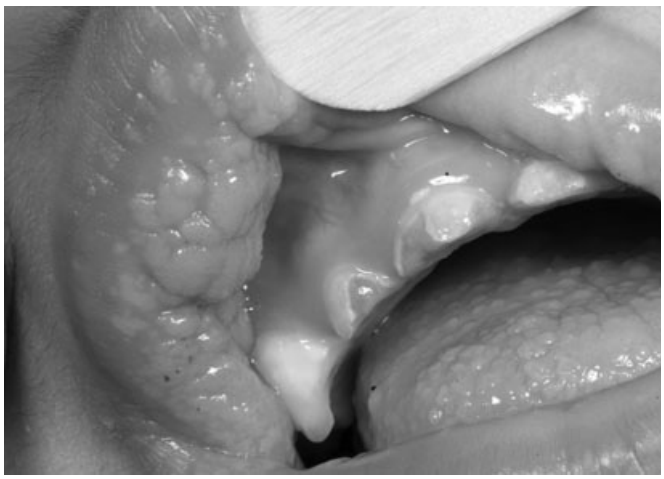

Figure 167–4 Focal epithelial hyperplasia of the oral gingival and mucosa (Heck disease). Patient was a 3-year-old Hispanic boy with a history of orthotopic liver transplantation and mild acute organ rejection, who presented with an extensive eruption of painless, rounded, soft nodules present on the tongue, buccal mucosa and gingivae. (See companion Expert Consult web site for color version.) *(Courtesy Dr. Federico Laham, Department of Pediatrics, Baylor College of Medicine. Photograph taken by Texas Children's Hospital.)*

13 and HPV-32 and infects only the oral cavity (Fig. 167–4).[213,233] It occurs worldwide but is most prevalent in Central and South America, Alaska, and Greenland. Focal epithelial hyperplasia may develop in children or adults and often clusters in families or geographic regions.[167] The lesions are discrete, multiple, elevated nodules readily visible on the oral mucosa. They may persist for years or resolve spontaneously. They do not, however, appear to undergo malignant transformation, nor do they appear to metastasize to other parts of the body. Warts caused by HPV-2, the same type associated with verrucae vulgares, or common cutaneous warts of the skin, may occur on the lips and on the mucosa of the oral cavity.[62] Oral papillomas involving the gums, buccal mucosa, soft palate, and tonsils also may be caused by HPV-6, HPV-11, and HPV-16, which primarily are associated with genital warts, or condylomata acuminata.[158,213] Recently, HPV-16 antibodies and HPV 6 and 16 DNA were found to be associated with squamous cell carcinoma of the head and neck, and HPV-16 DNA was detected in oropharyngeal, tonsillar, and tongue cancers, thus suggesting that HPV may play a role in these cancers.[44,96,150]

Conjunctival Papillomas

Conjunctival papillomas occur in all age groups but are exceedingly rare. They may be asymptomatic initially and then cause a constant foreign body sensation or chronic conjunctivitis. When large, they appear as pink mulberry- or cauliflower-like growths that may cause pain or interfere with lid closure. In most children and some adults, they appear to be caused by HPV-6 and HPV-11, which characteristically infect the genital tract and, therefore, may be transmitted during birth, similar to RRP.[120,145] HPV-16, another HPV that commonly infects the genital tract, also has been associated with conjunctival and lacrimal sac carcinoma in adults.[132,144]

Oral and Gastrointestinal Papillomas and Cancer

Tonsillar and oropharyngeal carcinomas may contain HPV DNA, and patients with head and neck cancers, including cancer of the tongue and oropharynx, may be seropositive for HPV type 16, an oncogenic HPV.[49,150] HPV 6 and 16 DNA also have been detected in papillary squamous cell carcinoma of the oropharynx and tonsillar carcinoma.[44,96] Esophageal infection with HPV may be asymptomatic or produce papillomas that cause dysphagia.[7] HPV DNA has been detected in esophageal brush specimens of

HIV-infected individuals who did not have proliferative mucosal lesions at the time of endoscopy.[237] In addition, HPVs, along with a variety of cofactors, may be involved in the genesis of esophageal carcinoma.[35] Similarly, HPV DNA has been detected in tissue samples from patients with colon cancer, but with unclear implications regarding their role.[213] The association of HPVs with anal warts, anal squamous intraepithelial lesions, and anal cancer, however, appears to be strong. Infection with HPV types 16, 18, and 31 most commonly occurs, but mere infection with these specific HPV types does not appear to be sufficient for the development of cancer. Similar to cervical cancer, cofactors such as other sexually transmitted diseases, chemical carcinogens (e.g., tobacco), and HIV infection and acquired immunodeficiency syndrome (AIDS) also may be involved in the pathogenesis of anal cancer.[73,118,162,172]

Liver Disease

Recent reports also have suggested that HPVs may play a role in liver disease. For example, HPV DNA, especially HPV-6, has been detected by sensitive molecular techniques in the liver of patients with neonatal giant-cell hepatitis, post–infantile giant-cell hepatitis, post–liver transplantation giant-cell hepatitis, and primary hepatocellular carcinoma.[58,173,211] However, the pathogenic role of HPV in these diseases, though an intriguing possibility, remains unproven at this time.

Other Cancers

The potential role of HPVs in the pathogenesis of a variety of unusual cancers has been explored by numerous investigators using different approaches, with conflicting results. Therefore, the role of HPVs in these cancers remains unproven at this time. For example, HPV DNA from types 6, 16, and 18 has been detected in tissue from some but not all patients with ovarian carcinoma.[213,238] HPVs also have been detected in tissue from patients with cancer of the endometrium, urethra, urinary bladder, and prostate, with unclear pathogenic implications.[213]

LABORATORY DIAGNOSIS

The typical appearance of verrucae vulgares on the skin and condylomata acuminata in the anogenital area in otherwise healthy individuals usually is sufficient to establish the clinical diagnosis of these HPV-associated illnesses. However, laboratory confirmation of HPV-associated lesions may be necessary for unusual manifestations in healthy individuals, for immunocompromised patients, and for patients with suspected malignant lesions. Methods commonly and traditionally used in viral diagnosis, such as cell culture, serology, and electron microscopy, have limited clinical utility in detecting HPV infection, whereas a variety of molecular methods, used alone or in combination, have proved to be quite useful in research and clinical settings.[258] Cytologic and histologic approaches also are helpful in diagnosing HPV-associated cancers.

Electron Microscopy

Virions with typical papillomavirus morphology can be detected in abundance in cutaneous warts but are difficult to detect in tissue from patients with RRP, JORRP, genital warts, or histologically diagnosed cancers.

Cell Culture

None of the papillomaviruses has been propagated in cell culture monolayer. Therefore, the routine viral cultures available in most diagnostic virology laboratories will not detect the presence of HPVs. Research laboratories have shown that HPV-1, when inoculated into skin keratinocytes or epithelial cells derived from the respiratory tract, will replicate its DNA transiently in an episomal form over several serial passages. However, viral capsid proteins and intact viral particles are not produced.[39] Other research laboratories have inoculated cervical tissue with extracts of HPV-11 virions obtained from condyloma acuminatum lesions and later transplanted the tissue beneath the renal capsule of athymic nude mice.[117] After several months, viral cytopathic effects have been seen, and viral capsid antigen and complete viral particles were produced. Clearly, these research methods need refinement before routine cultivation of HPVs becomes available to the clinician.

Serology

The inability to cultivate papillomaviruses routinely has hampered significantly the development of serologic tests to detect and study the humoral responses to infection with HPVs. Therefore, routine serologic assays to detect group- and type-specific antibody to HPVs are not available to the clinician. However, research laboratories continue to study the humoral response to HPV by a variety of methods. For example, serologic studies using purified virions of HPV-1 from plantar warts and HPV-11 obtained from mouse xenograft systems have revealed associations between seropositivity and clinical symptoms of infection with these HPV types.[24-27,38,116,180,224,244] Other studies of the serologic response to HPV have used recombinant DNA methods to clone and express late-region L1 and L2 proteins with bacterial fusion proteins.[67,82,103,254,255] Most recently, investigators have used vaccinia virus or baculovirus expression systems to produce virion-like particles composed primarily of viral capsids of HPV types 1, 6, 11, and 16.[32,85,121,159,193,231] However, serologic assays that use intact virions or virion-like particles, although technically the most successful to date, apparently still have problems with sensitivity and specificity.[170] Persistent, high-titer humoral responses are not detected in HPV infections, and in contrast to other sexually transmitted viral infections (e.g., herpes simplex virus, HIV, hepatitis B virus infections), seroconversion does not appear to be a clearly defined marker for primary HPV infection. Cross-reactivity between HPV types also appears to occur and limits the specificity of current assays. In addition to efforts to delineate the virus-specific humoral responses to HPV infection and HPV-associated cancers by measuring antibodies to HPV capsid proteins and HPV transforming proteins, studies evaluating serologic markers as predictors of invasive genital tract disease and ultimate survival also are being conducted.[231]

Colposcopy

Colposcopy is an important procedure to perform in women with abnormal Papanicolaou smears or external anogenital warts. It also may be used to diagnose asymptomatic, flat cervical warts in women whose sexual partners have external anogenital warts or to monitor women who are considered to be at high risk for acquisition of genital HPV infection for other reasons. The urethral meatus, penis, scrotum, and anus of males also may be inspected with a colposcope. Gynecologists, urologists, and family practitioners, as well as pediatricians who are specialists in adolescent medicine, usually perform the procedure. Briefly, the cervix and vulva in females or the urethral meatus, penis, scrotum, and anus in males are visualized under magnification with a colposcope to identify lesions, with special attention paid to their topography, the presence of abnormal whitening, and their vascular architecture. The area then is soaked in dilute acetic acid (3 to 5%) and visualized again for the presence of previously undetected lesions that appear as whitened plaques. Whitening

after application of acetic acid, however, is not specific for HPV infection or disease, and biopsy is required for definitive diagnosis. Colposcopic examination of the cervix not only gives a more accurate assessment of the anatomic extent of suspicious lesions or neoplasia but also allows the colposcopist to direct biopsies of suspicious areas accurately. Tissue biopsy specimens then may be sent for histologic examination and detection of HPV DNA by molecular techniques.

Cytology

Obtaining and staining exfoliated cervical cells via the Papanicolaou method is a routine procedure that detects most HPV infections and has reduced the incidence of invasive squamous cell carcinoma of the cervix dramatically. It should be performed routinely in all sexually active females, including adolescents. Cytologic analysis also has been evaluated as a screening examination of exfoliated urethral cells from males and in the urine of both men and women, but with less success and acceptance than with cervical cell screening.[159] Papanicolaou smear screening, however, is limited by the expertise of the physician who obtains the specimen and the pathologist who performs the cytologic analysis.[153] For example, because most cervical neoplasia arises at the junction of the squamous and columnar epithelium of the cervix (transformation zone), care must be taken to obtain cells from this region. Inter-observer variability also exists among pathologists who read Papanicolaou smears. Furthermore, Papanicolaou smears are not as sensitive as is colposcopy for detecting cervical cancer, and a negative Papanicolaou smear does not eliminate the diagnosis in women at high risk for cervical cancer. Therefore, women with anogenital warts and those who are immunosuppressed should undergo colposcopic examination to detect subclinical cervical lesions and not be evaluated simply with a Papanicolaou smear.

The cytologic abnormality that is specific and characteristic of HPV infection is the presence of koilocytosis (derived from the Greek word *koilos*, which means "hollow" or "cavity") or koilocytotic cells that display fat, swollen, wrinkled, or raisinoid nuclei surrounded by a halo. Other abnormalities, including dyskeratosis, parakeratosis, and hyperkeratosis, may occur but are considered secondary or nonspecific. The prevalence of cytologic abnormalities in women screened by Papanicolaou smears consistently has been estimated at 2 to 3 percent. Most abnormalities, however, resolve spontaneously in 3 to 6 months, but some persist and rarely may progress to cervical squamous cell carcinoma. If the infection progresses to disease, the koilocytosis typically diminishes, and the cells begin to display dysplastic changes and nuclear abnormalities. These abnormalities currently are graded into two categories: (1) low-grade squamous intraepithelial lesions, which include very mild dysplasia and the former grade 1 cervical intraepithelial neoplasia, and (2) high-grade squamous intraepithelial lesions, which include moderate to severe dysplasia and carcinoma in situ and the former grades 2 and 3 cervical intraepithelial neoplasia.[13-15,160]

Histology

HPV infection may occur in histologically normal tissue and be detected only by molecular methods that detect viral DNA. Benign and asymptomatic HPV infection also may cause koilocytosis, the typical histologic feature of HPV infection that has specific nuclear and cytoplasmic characteristics. Condyloma acuminatum lesions display not only koilocytosis but also other histopathologic characteristics of active HPV infection, such as hyperkeratosis, parakeratosis, acanthosis, and lengthening of the rete pegs. Atypical features, including mitotic figures above the basal layer, dysplastic cells, and single-cell keratinization, suggest dysplasia such as bowenoid papulosis. Precancerous and cancerous lesions of the cervix and penis also may be graded as low-grade or high-grade squamous intraepithelial lesions and invasive squamous cell carcinoma.

Histologic examination of tissue for the presence of HPV-associated disease may be augmented in certain circumstances by the detection of shared, genus-specific HPV capsid antigens that may be identified by immunohistochemistry with immunoperoxidase-labeled antibodies raised to bovine papillomavirus capsid antigen. This procedure usually is successful in identifying HPV antigens in low-grade squamous intraepithelial lesions but rarely detects antigen in high-grade squamous intraepithelial lesions because of the biology of HPV expression in these cancerous cells. Similarly, HPV antigens seldom are detected by this method in cutaneous or anogenital warts or other HPV-associated cancers. In addition, the utility of this method for typing the HPV infection currently is limited because unique, type-specific HPV capsid antigens are available only in research laboratories.[253]

Molecular Methods That Detect Human Papillomavirus DNA

HPV DNA may be detected in tissue by several methods, including dot-blot, slot-blot, Southern blot, and in situ hybridization assays and by amplification procedures such as PCR. HPV DNA has been detected by these methods in the majority of HPV-associated neoplasms, as well as in a significant proportion of asymptomatic individuals, including women with normal Papanicolaou smears. The variability of HPV DNA detection among studies is great and appears to be influenced by the population studied, the frequency and type of sampling, and the sensitivity and specificity of the molecular methods used. Because type-specific antisera are not available for HPVs, the diagnosis of type-specific HPV infection or disease requires molecular DNA methods.[20,256]

SOUTHERN BLOT HYBRIDIZATION

Southern blot hybridization is considered the reference standard for detection of HPV DNA in clinical samples. In this assay, nucleic acid is extracted from the sample, which usually is fresh tissue or exfoliated cervical cells, and digested or cleaved into smaller fragments by restriction enzymes. These fragments then are separated by gel electrophoresis and transferred onto special filter paper. Hybridization with radiolabeled probes directed against specific HPV DNA sequences then is performed. Advantages of Southern blot hybridization include good sensitivity (approximately 10^5 DNA copies) and specificity and the ability to distinguish HPV types easily. Disadvantages of this method include the need for special equipment and expertise. It also is technically expensive and time-consuming and requires a large sample of tissue that is destroyed during the DNA isolation procedure.

DOT- AND SLOT-BLOT HYBRIDIZATION

Dot- and slot-blot hybridization apply extracted DNA from a sample directly onto a specific area (dot or slot) of filter paper, with the gel electrophoresis and transfer steps of Southern hybridization being bypassed. The filter paper is incubated with a solution containing a complementary probe, followed by stringency washes to remove any unhybridized probe.[9,253] Detection of successful hybridization is by autoradiography if a ^{32}P-labeled probe is used or by colorimetric reaction if an enzyme-labeled probe is used. Advantages of dot-blot hybridization include commercial availability of some assays, ease of performance, rapid turnaround time, and low cost. Its sensitivity and specificity generally are slightly below those of Southern blot analysis, but the

method is sufficiently sensitive to detect HPV DNA in cytologically normal, as well as abnormal, cervical samples, and its use, in combination with Papanicolaou smear, has been advocated to improve identification of women at high risk for development of cervical cancer.[15,87] Dot-blot hybridization may be performed on tissue samples or cervical swabs collected in special sample transport medium or buffer, and its utility in screening urine for HPV DNA also has been explored.[159] Moreover, typing of HPV DNA may be performed by dot-blot hybridization methods. For example, commercial kits, by using a combination of radiolabeled probe mixtures, delineate as many as seven types of HPV by category group: types 6/11, 16/18, and 31/33/35.[87,152,245] In addition, probes to detect almost all HPV types, both high and low prevalence, have been developed in a variety of research laboratories.[200]

A novel, nonradioactive, chemiluminescent liquid hybridization assay (hybrid capture assay) is commercially available and detects as many as 14 HPV types divided into high-risk (types 16, 18, 31, 33, 35, 45, 51, 52, 56) and low-risk (types 6, 11, 42, 43, 44) groups on the basis of association with cervical cancer.[205] This DNA hybrid capture assay is relatively rapid and simple to perform and provides quantitative data that reflect viral concentration.

IN SITU HYBRIDIZATION

In situ tissue hybridization assays are performed directly on fresh or fixed tissue sections or on cytologic specimens and have the unique advantage of allowing the examiner to correlate histopathologic abnormalities with the location of HPV DNA. They may be performed with radioactive or enzyme-labeled probes. The sensitivity of many in situ hybridization assays is less than that of Southern blot assays, especially if enzyme-labeled probes are used.[34,164,234,253] Commercial reagents and kits are available.

DNA AMPLIFICATION ASSAYS

Classic PCR, as well as PCR amplification with degenerate or consensus primers that are capable of recognizing a portion of one of the late genes from a broad spectrum of papillomaviruses, are used by many laboratories. The popular MY09 (primer for the negative strand)/MY11 (primer for the positive strand) primer system amplifies a 450-base pair target region located in the HPV L1 open-reading frame; this site contains both conserved regions common to most or all papillomaviruses and divergent regions that appear to be unique for each HPV DNA type.[140] Amplification and detection by the MY9/MY11 primers in a PCR assay identifies HPV DNA, which can be confirmed by using "generic" HPV probe mixes. Typing of known HPV types then can be performed with type-specific HPV probes that are labeled oligonucleotides composed of sequences complementary to each viral type. Alternatively, restriction fragment length polymorphism or sequence analysis can be performed on the PCR product to identify new HPV types that are unable to be typed with the available type-specific probes.[21,166,176,177] In addition, commercially available real-time PCR assays have been developed to detect and quantify HPV DNA.[175]

HPV DNA detection by user-developed and commercial enzyme-labeled DNA probes and PCR-based methods can be performed on a variety of specimens, including fresh or fixed tissue, exfoliated urethral and cervical cells, and urine.[96a] Recent studies using real-time PCR assays have shown that self-collected vaginal and urine samples provide highly accurate results with excellent concordance with physician-collected cervical samples.[51,175,247]

By using traditional methods of Papanicolaou smear screening, colposcopy, and biopsy, the prevalence of cytologic, colposcopic, and histologic abnormalities of the cervix in the general population consistently has been estimated at between 2 and 3 percent.[253] However, a wide range of prevalence, between 7 and 82 percent for detection of HPV infection by molecular methods, has been reported; the prevalence varies according to the population studied and the methodology used, but PCR-based methods in high-risk groups have the highest prevalence rates.[9,112,129,185,251,257] The recognition that HPVs, especially high-risk types 16 and 18, are associated with cervical and other cancers suggests that detection of HPV DNA may be used as an adjunct to cytologic screening by Papanicolaou smear and offers the potential opportunity to identify women with cervical neoplasia who have false-negative Papanicolaou smears. On the other hand, the significance of the presence of HPV DNA, even if it is from a high-risk type, in histologically normal tissue is unclear, and the presence of HPV DNA, no matter what type, in histologically abnormal tissue does not influence management at this time. Further studies are necessary to explore and resolve the clinical role of DNA-based diagnostics for screening, diagnosis, and management of HPV infections.[88]

TREATMENT

Most cutaneous and mucosal HPV-associated warts and lesions in healthy individuals will regress spontaneously in 1 to 2 years. Treatment may be desirable if the lesions are large, multiple, or recurrent; if they cause pain or discomfort; or if they are undesirable cosmetically. Treatment is mandatory if the lesions are life-threatening, such as laryngeal papillomas that obstruct the airway, or lead to cervical cancer. A variety of treatment strategies are available, but none produces a universally effective or permanent cure. Rather, current approaches focus on reduction of the clinically apparent lesion, and most require repetitive application. Clinically significant HPV-associated lesions also usually require the additional expertise of a specialist. For example, a dermatologist should be consulted to assist in the management of a patient with severe recalcitrant cutaneous warts, a gynecologist and oncologist for patients with cervical cancer, an ophthalmologist for a patient with conjunctival papillomas, and an otolaryngologist and pulmonologist for children and adults with severe RRP. These specialists are likely to know the currently available treatment regimens most effective for each patient's HPV clinical manifestation. The role of the infectious diseases specialist in the management of HPV-associated disease is evolving and may become more prominent as HPV-specific antiviral chemotherapy becomes available for clinical trial and eventually for routine clinical use in patients. Current standard therapies focus on physical, surgical, or chemical destruction of the clinical manifestation of the HPV infection, such as the wart or papilloma.

Surgical techniques used to treat papillomas include traditional local excision by knife, cryotherapy with liquid nitrogen or dry ice, electrocautery and curettage, and ultrasonication.[8,22,23,106,142,218] Newer ablative surgical techniques that use carbon dioxide laser vaporization and flash-lamp pulsed dye laser therapy allow more precise and complete removal of visible papillomas and are becoming widely used to treat genital and laryngeal papillomas.[64,189,229] Surgical excision by knife remains the initial mainstay of many HPV diseases, however, because it provides tissue for a histopathologic diagnosis, as well as removal or debulking of large lesions. Ablation by cryotherapy with liquid nitrogen, electrocautery, or laser vaporization may be used to remove small, single, or multiple lesions or be performed in combination with surgery for large or difficult lesions.[148] These surgical therapies also may release viral antigens and produce local and systemic immunologic stimulation that may assist in eradicating the lesions. Disadvantages of these physical methods of wart and papilloma removal include pain, scarring, and disfigurement. These methods also are relatively invasive and impractical for

patients with disseminated disease. Furthermore, recurrent treatments usually are necessary. In addition, laser vapors contain HPV DNA and may be a vehicle for spreading the infection in the patient or to the treating physician or surgeon, and, thus, the vapors or smoke plume should be contained.[78] Recurrence of lesions after treatment occurs commonly and most likely is due to the presence of HPV DNA sequences in clinically and histologically normal epithelium adjacent to and beyond the treatment area.[66] The mainstay of treatment for RRP and JORRP is repeated surgical debulking of obstructing papillomata, augmented by immunomodulators and antiviral therapies.[96a] Surgical rates tend to decrease over the course of time in many patients; however, those patients infected with HPV 11 may require more frequent, more extensive, and more prolonged surgical procedures over the could of time.[184,252]

Warts and papillomas also may be disrupted physically and removed by using chemical ablatives applied topically to the lesion. Simple organic acids such as bichloracetic and trichloracetic acid or salicylic acid applied twice daily for several days have shown some success in the localized treatment of skin and genital warts.[74] They are caustic substances that produce a white slough that peels off, and they can be applied weekly until the lesion is destroyed.[74] Antimitotic agents such as the traditional podophyllin or the newer preparation podophyllotoxin can be applied twice daily for several weeks. Antimetabolites such as bleomycin, cantharidin, and 5-fluorouracil also have shown efficacy when administered locally once or twice a week because they inhibit the cellular proliferation induced by HPV infection.[115,181] These topical chemicals usually are easy to apply to skin and genital warts, but they also may cause local pain, redness, swelling, irritation, blisters, and scarring.[115] Moreover, they are not virus-specific, and recurrences occur commonly. They also are impractical for extensive lesions and may be toxic if used in certain circumstances. For example, they may damage the cornea if used to treat conjunctival papillomas, and they should not be used on pregnant women.[148] The systemically administered antitumor agent methotrexate also has been administered to individual patients with disseminated HPV disease, with variable success.

Immunomodulation is another treatment strategy for HPV-associated disease. A systemic immunologic response probably is responsible for the spontaneous regression frequently observed in cutaneous and mucosal warts. Therefore, stimulation of the immune system with immunomodulators also may produce remission in patients with HPV-associated disease. Interferons have antiviral, antiproliferative, and immunomodulating properties. Decades of clinical experience have accrued in treating HPV-associated disease with interferon administered topically, intralesionally, or systemically by subcutaneous injection. Both lymphoblastoid (a) and fibroblast (b) interferon have been used with some success to treat patients with genital warts and respiratory papillomatosis topically, locally, and systemically.[60,70,75,96a,188,209,213,] However, interferon-γ has not been shown to be beneficial. Both recombinant and natural-source interferon-α preparations have been demonstrated in placebo-controlled trials to be effective and are approved by the U.S. Food and Drug Administration for the intralesional treatment of genital warts.[60,70] A 25- to 30-gauge needle is used to inject approximately 0.1 mL (1×10^6 U) at the base of up to five warts at a time for a total dose of 5×10^6 U at each visit. This dose is repeated two to three times weekly for 3 weeks. Maximal effect usually is seen within 4 to 8 weeks. Repeat injections may be given if the warts are persistent or recurrent. Interferon therapy also may be effective if used alone or in combination with laser surgery to treat patients with RRP.[94,97,122,131] Fever, headache, chills, and myalgia frequently occur with local and systemic interferon treatment. Severe and persistent fatigue, nausea, and leukopenia are other fairly common adverse reactions, especially with systemically administered interferon. Systemic natural leukocyte interferon also causes regression of warts and reduction of the virus load in tissues in patients with EV.[4] Cimetidine, an immunomodulator that alters lymphocyte function, has been used in an attempt to treat children with recalcitrant cutaneous warts.[168]

Topical immunotherapy agents provide alternative treatment options for HPV-associated disease in some patients.[79,80,111,149,213] Imiquimod, also known as *imidazoquinoline*, is a first-generation member of a class of immune response modifiers approved for topical treatment of genital and perianal warts.[79,80,111,149] It is a synthetic compound that is a topically active Toll-like receptor (TLR) 7 agonist. It activates innate immune cells to produce interferon-α and other cytokines to enhance antigen presentation and promote antigen-specific T helper type 1 cell-mediated immune responses. Imiquimod is a 5 percent cream that is applied to lesions and appears to be effective in some patients. Resiquimod and other related, second-generation imidazoquinoline analogues also are in clinical development.

Retinoids and retinoic acid, which are analogues of vitamin A, can regulate the growth and differentiation of malignant, premalignant, and even normal cells.[3,190] Clinically, they have a documented effect against squamous cell carcinoma.[124,125] For these reasons, anecdotal reports and small series of patients have emerged in which retinoic acid was used to treat HPV-associated disease, especially RRP. In these reports, retinoids have been used with varying success, primarily as adjuvant agents with surgical therapy, to treat adult patients with severe, refractory RRP.[10,59] The combination of interferon and retinoic acid has been shown to be synergistic against breast cancer cells in vitro and potentially may be useful in refractory cases of RRP.[143]

Unique aspects of HPV infection and disease render designing specific antiviral therapies a challenge. For example, HPV disease usually is focal, the virus is involved intricately in the cell's life cycle, and HPV has great diversity with more than 70 genotypes. The inability to grow HPVs readily in cell culture also hampers our ability to study the antiviral properties and cellular toxicities of candidate compounds. Molecular assays that isolate individual viral functions, therefore, have been used to evaluate the ability of antiviral compounds to inhibit each individual step in the virus life cycle. Furthermore, animal models for HPV-associated disease are lacking. Despite these challenges, the in vitro antiviral activity and clinical efficacy of a variety of compounds have been studied. For example, ribavirin, a nucleoside analogue with a broad antiviral spectrum, has been used in clinical trials for the treatment of laryngeal papillomatosis.[146] Cidofovir, an antiviral compound effective against serious disease caused by cytomegalovirus (CMV), also appears to have activity against HPVs, and possible clinical benefit for certain HPV-associated disease has been shown. For example, repeated intralesional administration of cidofovir directly into the site of papillomata and also submucosally at the sites of resected papillomata is associated with partial or complete regression of lesions, improvement of voice quality and airway status, and decreased need for repeat surgery. Published studies report a wide variety of intralesional doses (2 to 57 mg of a 5 mg/mL cidofovir concentration solution), frequency of injections (every 2 to 8 weeks), and duration of treatment (4 months to 4 years). Rarely, cidofovir has been administered intravenously in patients with refractory, disseminated papillomatosis.[96a] The greatest benefits have most often been documented after the fourth series of injections, and benefit appears to plateau after the eighth series of injections in most patients.[37,40,57,77,93,139,215,216] However, because most published reports are single-center, uncontrolled case series with relatively small numbers of patients, controlled, multicenter trials with larger numbers of patients are necessary to accurately determine the benefits of cidofovir therapy, as well as the optimal dose, frequency, and duration of therapy for RRP and JORRP.

PREVENTION

Prevention of HPV infection and disease involves two potential approaches: behavioral strategies and vaccines. Prevention of genital HPV infection includes the use of barrier methods, such as condoms, to reduce transmission between sexual partners. Hospital infection control policies should address the potential transmissibility of HPV from patients to health care workers during laser vaporization and electrocautery therapy. Care should be taken to wear protective mask and eye wear and to use appropriate and well-functioning suction devices during these procedures.

Two prophylactic, highly immunogenic HPV vaccines, with good safety profiles, are currently licensed and recommended for prevention of HPV infection and HPV-associated disease and malignancies.[6,29,51a,72,95,107,114,141,206,222] Both vaccines contain HPV L1 virus-like particles (VLPs), generated by the expression of the major capsid protein (L1) of HPV in yeast.[123] One vaccine is quadrivalent, contains HPV types 16/18/6/11, and provides protection against the most common HPVs associated with cervical cancer and genital warts. The other vaccine is bivalent, contains HPV types 16/18, and provides protection against the highest-risk HPVs. The L1 proteins self-assemble into pentamer structures that come together to form VLPs, which are antigenically indistinguishable from HPV virions. The VLPs, however, do not contain viral DNA, and, therefore, they are noninfectious. Both vaccines appear highly effective in preventing persistent HPV infection and HPV 16/18-related high-grade intra-epithelial neoplasia (CIN 2/3), and are, therefore, likely to be effective in preventing cervical cancer. In addition, the quadrivalent vaccine is effective in preventing vulvar and vaginal HPV-associated warts and lesions. Both vaccines are recommended for females aged 11 to 12 years and are administered in a series of three injections. They likely are most effective when administered prior to sexual debut and may be given to girls as young as 9 years of age. Because males also appear to play an important role in HPV transmission, as well as suffer from HPV-associated disease, vaccination of preadolescent boys may also be investigated and considered in the near future.

Even though HPV vaccines appear to offer effective protection against HPV infection and HPV-related diseases and malignancies, they cannot completely prevent cervical cancer. Therefore, routine cervical screening should continue, even in vaccinated females, and complement HPV vaccination strategies to minimize the public health impact of HPV-related cancers and genital warts.[2,46,50,91,108,240]

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CHAPTER 168

ADENOVIRUSES

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Adenoviruses, which are responsible for a varied array of illnesses in children,* are associated most commonly with respiratory illness and gastroenteritis, but cardiac, neurologic, cutaneous, urinary, and lymphatic manifestations also occur frequently. Although many of the clinical manifestations of adenoviral infections are distinctive, the specific viral etiologic agent rarely is recognized by physicians.

Adenoviruses were noted first in explant cultures of human adenoid tissue; this finding, plus the observation of their apparent general affinity for lymphatic tissue, led to the designation of their name.[129,133,153,412]

HISTORY

The first adenoviral strains were not isolated until 1953, when tissue culture techniques became available, although epidemic disease caused by adenoviruses had been observed throughout the first half of that century. Epidemic keratoconjunctivitis first was noted and reported in Austria by several physicians in 1889.[3,157,398,423,447] Major outbreaks of epidemic keratoconjunctivitis were reported in Bombay in 1901, in Madras in 1920 and 1928, in Hawaii in 1941, and on the West Coast of the United States in 1942.[204,222,223,267,515]

Epidemics of illness such as pharyngoconjunctival fever have been observed throughout the 20th century. Béal[20] in 1907 was the first to report the syndrome; in the 1920s, epidemics associated with swimming in public pools and in lakes were noted in Germany and the United States.[16,368] Initial studies by Rowe and colleagues[412] revealed cytopathic changes in explant tissue cultures of human adenoids that had been removed surgically. Fluid from these cultures caused distinctive cytopathologic changes in other tissue cultures, and antiserum prepared in hyperimmunized rabbits neutralized the effect. In 1954, Hilleman and Werner[217] isolated similar cytopathic agents from the throat washings of military recruits with febrile acute respiratory disease. Shortly thereafter, epidemic keratoconjunctivitis and pharyngoconjunctival fever were seen to be illnesses of adenoviral etiology.[98,101-103,244]

Initially, adenoviruses were known by the following names: *adenoid degeneration* (AD) *agent* because of its recovery in human adenoid tissue explants[412]; *respiratory illness patient number 67* (RI-67) *agent*, which was recovered from a military recruit with primary atypical pneumonia during an epidemic of acute respiratory disease[216,217]; *adenoidal-pharyngeal-conjunctival (APC) agent*[233]; and *acute respiratory disease (ARD) agent*.[81] In 1956, early investigators in the field selected the term *adenovirus* for the new group of viruses. The name suggested the characteristic involvement of lymphadenoid tissue, as well as the tissue from which the organism was first isolated.[129]

Because of considerable morbidity and major economic considerations related to adenoviral epidemic respiratory disease in military recruits, the development of vaccines received attention early. Initially, inactivated vaccines were produced, and they achieved some degree of success.[186,215,289] Later, live viral preparations grown in diploid human fibroblast tissue culture became available and proved quite successful in controlling specific adenoviral infections in the military services.[119,120,176,289,407,439,465-468]

In 1975, enteric adenoviruses (adenovirus types 40 and 41) first were reported.[145,425,503] They were demonstrated by electron microscopy and subsequently were shown to be a significant cause of diarrhea in children.[45,77,275,330,389,464,475,478] Particularly important today are adenoviral infections in immunocompromised hosts, particularly transplant recipients.[271]

PROPERTIES OF THE VIRUS

CLASSIFICATION

Adenoviruses that infect humans are placed in the family *Adenoviridae* and the genus *Mastadenovirus*.[141,226,323,427] At present, 51 immunologically distinct adenoviral types have been recovered from humans.* Additional adenoviral types have been isolated from monkeys, cattle, dogs, mice, and chickens.[206,208-210] Mammalian adenoviruses have a common generic antigen that can be identified by complement fixation and enzyme-linked immunosorbent assay (ELISA).[246,378,404,505] Individual serotypes are identified by neutralization.[192,193,265]

Adenoviruses originally were subclassified on the basis of four hemagglutination patterns with rat and rhesus monkey red blood cells.[408] This subclassification is updated in Table 168–1. In 1962, researchers found that adenovirus type 12 could cause tumors in hamsters, and soon thereafter, investigators realized that adenoviruses also could be classified by their oncogenic potential in rodents.[231,470] Grouping by oncogenic potential was similar, with few exceptions, to grouping by hemagglutination properties: organisms in hemagglutination group I had moderate oncogenic potential, group II and group III organisms had low or no potential, and group IV organisms had high oncogenic potential.[168,227,238]

Adenoviruses also have been subclassified (A to F) on the basis of the percentage of guanine plus cytosine in their DNA and other biochemical and biophysical criteria[212,263,385,424,493-495] (Table 168–2). In general, subgroup A organisms are the same as hemagglutination group IV; subgroup B, the same as hemagglutination group I; subgroups C, E, and F, the same as hemagglutination group III; and subgroup D, the same as hemagglutination group II. Restriction enzyme analysis has resulted in genomic typing of specific adenoviral serologic types.[297] In particular, numerous

*See references 1, 2, 45, 47, 71, 128, 153, 220, 228, 276, 402, 416, 440, 478.

*See references 106-108, 130, 141, 207-210, 212, 213, 228, 263, 424, 494, 496, 506.

TABLE 168–1 Separation of Human Adenoviruses into Subgroups by Ability to Agglutinate Rhesus Monkey and Rat Erythrocytes

Subgroup	Characteristic	Type
I	Complete agglutination of monkey erythrocytes	3, 7, 11, 14, 16, 21, 34, 35, 50
II	Complete agglutination of rat erythrocytes	8-10, 13, 15, 17, 19, 20, 22-30, 32, 33, 36-39, 42-49, 51
III	Partial agglutination of rat erythrocytes	1, 2, 4-6, 40, 41
IV	No or little agglutination of monkey or rat erythrocytes	12, 18, 31

Data from references 108, 206, 212, 219, 222, 253, 263, 289, 356, 357, 424, 507.

TABLE 168–2 Grouping of Human Adenoviral Serotypes Based on Biochemical and Biophysical Criteria

Subgroup	Adenovirus Type(s)	Location and Manifestations of Infection
A	12, 18, 31	Gastrointestinal; may cause disease in children
B:1	3, 7, 16, 21, 50	Respiratory, eye, and gastrointestinal symptomatic infections
B:2	11, 14, 34, 35	Urinary and respiratory; symptomatic urinary tract infections (particularly in immunosuppressed patients) and symptomatic respiratory infections
C	1, 2, 5, 6	Respiratory and gastrointestinal; symptomatic respiratory infections and hepatitis in immunosuppressed patients
D	8-10, 13, 15, 17, 19, 20, 22-30, 32, 33, 36-39, 42-49, 51	Eye and gastrointestinal; symptomatic eye infections, gastrointestinal infections in patients infected with human immunodeficiency virus
E	4	Symptomatic eye and respiratory infections
F	40, 41	Symptomatic gastrointestinal infections

Data from references 108, 212, 227, 263, 289, 385, 424, 463, 494.

genotypes of adenovirus type 7 have been identified, and the severity of disease may relate to the genotype.

PHYSICAL PROPERTIES

Adenoviruses are nonenveloped DNA viruses 65 to 80 nm in diameter.[141,168,226,227,238,270,323] The virion consists of a protein capsid composed of 252 capsomeres and a nucleoprotein core that contains the DNA viral genome and two to four internal proteins.[392] The virion is roughly spherical in the form of an icosahedron; each of the 20 sides is an equilateral triangle, with the vertices of each converging in groups of five and resulting in 12 pentagonal vertices.[45,153,226] Each vertex capsomere contacts five other capsomeres and is designated a *penton*. Each penton contains a base plate and a rodlike projection, the fiber. The virion has 240 nonvertex capsomeres that occur in groups of six and are known as *hexons*.[270,357,477] Hexons contain the generic complement-fixing antigen.[153] Each capsomere has a diameter of 8 nm and a central hole that is 2 to 3 nm in diameter.[510] The capsomeres constitute 87 percent of the dry weight of the virion.

The central core of the virion, which accounts for 12 to 14 percent of its dry weight, is composed of linear double-stranded DNA (molecular mass, 23.85×10^6 daltons for adenovirus type 2) and two to four basic proteins.[158,227,510]

Adenoviruses are highly stable in general.[111,153,166,238,291] They are resistant to lipid solvents and retain activity at pH values ranging from 2 to 10.

At 24° C, maximal infectivity is maintained between pH 6 and 9.5. Adenoviruses are stable at room temperature for 2 weeks, at 36° C for 7 days, and at 4° C for at least 70 days. Infectivity is destroyed by heating to 56° C for 30 minutes. Sodium dodecyl sulfate (0.25%) inactivates virus by disruption of the capsid.[270] Adenovirus serotypes 2, 10, 40, and 41 are highly resistant to ultraviolet radiation, and, therefore, they can be used as markers of the quality of water treatment practices.[214]

ANTIGENIC COMPOSITION

The antigenic determinants of adenoviruses are contained on the protein structural subunits (hexons, pentons, and fibers).[477] The hexon antigen (alpha component) carries the generic antigenic component that is common to all mammalian adenoviruses and is measured by complement fixation or ELISA. Another hexon antigen (epsilon component) reacts with neutralizing antibodies lacking hemagglutination-inhibiting activity. The antigen related to viral fiber also induces the production of type-specific neutralizing antibody for some adenoviral types. Minor antigens are related to the pentons. One of them (the cell-detaching factor) causes rounding and clumping of tissue culture cells.

Gerna and associates,[165] using the immunoperoxidase antibody technique, found that the early antigens of all serotypes belonging to one group (see Table 168–1) react strongly with all type-specific immune sera of the same group.

TISSUE CULTURE GROWTH

Although adenoviruses can be grown in a wide variety of cells of human epithelial origin, primary or diploid cultures of human embryonic kidney are preferable for recovery of agents from clinical specimens.[291] Continuous cell lines such as HeLa and HEp-2 also are quite sensitive.[203,238,413] Adenoviruses will grow in monkey kidney tissue culture, but evolution of the cytopathic effect is considerably slower than that occurring in cells of human origin. In addition, the cytopathic effect is more variable in monkey kidney tissue culture, and many isolates will be missed.

Enteric adenoviruses have been detected in human feces by electron microscopy.* These enteric adenoviruses, identified as types 40 and 41, usually do not grow in standard tissue culture systems. Both viral types will grow in Graham 293 cells (a human embryonic kidney cell line transformed by adenovirus type 5); type 40 also will grow in tertiary monkey kidney cells, and some strains of type 41 will grow in HEp-2, Chang conjunctiva cells, and tertiary cynomolgus cells.[458,496] Grabow and associates[178] noted that the PLC/PRG/5 cell line was 100 times more sensitive to a laboratory strain of adenovirus type 41 and 10 times more sensitive to a laboratory strain of adenovirus type 40 than were Graham 293 cells.

Specimens for viral culture may be obtained from the eye, pharynx, blood, lungs, pleural and pericardial fluid, liver, stool, intestinal epithelium, lymph nodes, cerebrospinal fluid (CSF), brain, urine, and renal tissue. For best isolation rates, clinical

*See references 45, 91, 106, 246, 263, 397, 403, 475, 494, 505.

specimens should be inoculated in cell culture within 6 hours of being collected. Tissue culture tubes are incubated best at 37° C, and rolling offers no advantage over stationary incubation.[291] Although the cytopathic effect has considerable variability, the most consistent finding is marked rounding and clumping of cells, often in grapelike clusters.[392,395] A cytopathic effect may be noted as early as 1 to 2 days after inoculation, but it may be delayed as long as 4 weeks. Use of the shell viral technique, in which infected cells in a specimen are centrifuged onto HEp-2 tissue culture cells, yields positive cultures in 1 to 2 days.[131] The cytopathic effect characteristically is followed by detachment of the entire cell sheet from the glass of the tissue culture tube within 2 to 4 days.[183,204,379,410,413]

VIRUS MULTIPLICATION

Initial attachment of adenoviruses to cells is relatively slow, with up to 6 hours being required.[99,167,238] The fiber protein of the virus mediates attachment to the cellular receptor.[428] An important adenovirus receptor is the coxsackievirus and adenovirus receptor (CAR) protein. It is a high-affinity receptor for all adenoviral subgroups except subgroup B. Penetration into the host cell occurs rapidly, either by phagocytosis or by direct entry through the plasma membrane.[238,305] The eclipse period with adenoviruses varies from 11 to 21 hours, depending on the serotype.[167,238] During eclipse, viral DNA uncoating requires approximately 2 hours when determined biochemically.[238] After uncoating, the viral DNA rapidly enters the nucleus of the cell and disassociates from its internal protein.[99,168,305] After the eclipse period, viral DNA accumulates, often doubling the content of the infected cell and becoming part of the typical inclusion bodies of the infected cells.[168]

Characteristic cytopathic changes in tissue culture, as demonstrated by light microscopy of hematoxylin and eosin–stained material or by electron microscopy, appear as early as 8 to 24 hours after infection occurs.[41,43,192] Two types of intranuclear alterations may be seen. In the first, early, small, discrete eosinophilic inclusions are observed. They gradually enlarge, become more prominent, and then form a large crystalline central mass surrounded by a clear zone or halo.[41,174] This type of early cytopathic effect appears to take place in cells that contain only small amounts of infectious virus.[42] The second type of intranuclear alteration, which generally occurs later, consists of large basophilic intranuclear inclusions.[41] Occasionally, giant forms measuring greater than 14 mm across are noted.[41] These mature inclusions may expand the nucleus of the cell so greatly that the cell's DNA content is 10 times larger than that of an uninfected cell. They contain a large amount of viral antigen and infective virus. Cytopathic changes vary considerably with the different adenoviral serotypes.[43,270]

In general, virus remains within the nucleus of intact, infected cells, with less than 1 percent of the total viral content of a culture being free within the extracellular fluid at any one time.[270]

ANIMAL SUSCEPTIBILITY

Human adenoviruses usually do not produce clinical illness in laboratory animals, although infections with several different serotypes have occurred in selected animals.[238] When adenoviral strains were administered intranasally to young, "pathogen-free," colostrum-deprived pigs or 1-month-old cotton rats, pneumonia developed in the animals, and intranuclear inclusions were observed microscopically in their lungs.[238,367] An adenovirus type 5 strain was injected intravenously into a rabbit, and the virus was recovered from the animal's spleen when removed 2 months later. Many adenoviral strains will propagate in a variety of different animal tissue cultures. In addition, in some cultures, marked cytopathic effects are observed, but little infectious virus is produced.

ADENO-ASSOCIATED VIRUSES

Adeno-associated viruses are a group of small, defective, single-stranded, DNA-containing, virus-like particles that replicate in tissue culture only in the presence of adenoviruses.[35,99,153,238,261,440] Four serologic types have been identified; however, only types 2 and 3 have been recovered from humans.[153,270] These agents have been isolated from throat and anal swab specimens of children from whom adenoviral strains also were isolated.[35] Approximately 30 percent of children have complement-fixing antibody to adeno-associated virus types 2 and 3; most of these children have had evidence of adenoviral infection as well.[36] Neutralizing antibody to adeno-associated virus types 2 and 3 is noted in the sera of 60 to 80 percent of children 8 to 14 years of age.[373] The specific relevance of adeno-associated viruses is unknown.

EPIDEMIOLOGY

GENERAL PREVALENCE

Adenoviral infections account for 2 to 5 percent of all respiratory illnesses.[58,78,84,151,152,380] In children, adenoviruses are estimated to be responsible for 2 to 24 percent of respiratory viral illnesses,[47,58,64,66,225,303,313] and they are implicated in 5 to 11 percent of upper respiratory tract infections, 4 to 10 percent of cases of pharyngitis, 3 to 9 percent of cases of croup, 5 to 11 percent of cases of bronchitis, 2 to 10 percent of cases of bronchiolitis, and 4 to 10 percent of cases of pneumonia.[47,64-66,154,341,375] Enteric adenoviral infections are the cause of 5 to 15 percent of acute diarrheal illnesses in children.[77,91,295,330,389,397,475,478] Specific categories of illness caused by adenoviruses vary with respect to viral serotype, patient age, socioeconomic status, and environmental conditions.[61,85,189,202,247,519] Adenoviruses have a predilection for infants and children younger than 5 years who spend portions of their days in closed environments, such as daycare centers, orphanages, and other institutions.[25,28,61,194,304,492] Epidemics of disease caused by adenoviral infection have been associated with swimming pools, daycare centers, resident schools, certain industries, hospitals, physicians' offices, and early basic military training centers in young recruits.* Adenoviruses are recovered commonly from children in tropical countries and in situations characterized by crowding, such as lower socioeconomic settings.[61,66,79,247,519]

AGE INCIDENCE AND PREVALENCE

Although adenoviral infections occur in all age groups, the incidence of infection generally is related inversely to age.[85,270] More than 90 percent of newborn infants have transplacentally acquired complement-fixing adenoviral serum antibody.[348,448] Most infants have neutralizing antibody to one or more of the common adenoviral types, which appears to be protective during the first 6 months of life. When adenoviral infection does occur in a neonate, it often is severe and occasionally fatal.[9,138,525] By the sixth month of life, only 14 percent of infants have demonstrable adenoviral complement-fixing antibody. By the time children reach 1 year of age, complement-fixing adenoviral antibody is observed in 44 to 50 percent of sera tested.[233,351]

*See references 73, 95, 138, 180, 244, 245, 269, 272, 293, 315, 366, 376, 387, 401, 415, 417, 437, 473, 521.

The incidence of adenoviral infection peaks in infants and children between 6 months and 5 years of age.[328,380] By the time that they are 5 years of age, 70 to 80 percent of children have neutralizing antibody to adenovirus types 1 and 2, and 50 percent have antibody to adenovirus type 5.[110,151,233,241,374,380,480]

Adenoviral infections also occur commonly in grade-school and junior high school–aged children, but the incidence diminishes in high school–aged adolescents. Adenoviral infections occur in only 1 to 2 percent of college students, and they are noted infrequently in civilian adults.[132,134,135,182,450] Approximately 1 percent of adults with a respiratory infectious illness will have adenoviral infection; in hospitalized adults, the incidence is approximately 4 percent.[182] The most common adenoviral respiratory infections in children are caused by types 1, 2, 3, and 5.[25,480,490,492] Types 6 and 7 are the next most frequent isolates associated with childhood respiratory infection. Adenovirus types 40 and 41 are the most common causes of diarrhea.[77,274,295]

MILITARY RECRUITS

Epidemic adenoviral disease occurs commonly in military recruits, with virtually all illness developing during the first 8 weeks of basic training.[149,272,312,322,415,417,482,483] The attack rate has varied between 40 and 90 percent.[149,183,312,481] Adenoviruses account for 30 to 70 percent of acute respiratory disease, 67 percent of common cold-like illnesses, 62 to 77 percent of cases of acute febrile pharyngitis and tonsillitis, 67 percent of cases of bronchitis, and 24 percent of cases of pneumonia.[31,150] Some adenoviral infections are associated with minimal or no symptoms.[150,183]

A vaccine against adenovirus serotypes 4 and 7 was developed for use in military basic training centers and effectively prevented outbreaks of adenovirus. Routine vaccination began in 1971, but the sole manufacturer of the vaccine ceased its production in 1995. The first large post–vaccine era outbreak of adenovirus serotypes 3 and 7 occurred in naval recruits in Great Lakes, Illinois, in 1997.[417] A subsequent epidemic of adenovirus 4 occurred in military recruits in Fort Jackson, South Carolina, in 1998.[272]

In a commentary, Gray reported that the U.S. Department of Defense identified a new manufacturer of the adenovirus vaccine (Duramed Pharmaceuticals) in 2001. Results of phase I studies of the vaccine have shown adequate seroconversion and good safety profiles.[180]

GEOGRAPHIC DISTRIBUTION

Adenoviral infections have been noted throughout the world.* Epidemic, endemic, and sporadic infections all occur.

SEASONAL PATTERNS

Sporadic infections with adenoviruses occur throughout the year.† Epidemic adenoviral respiratory disease usually occurs in the winter, spring, and early summer.‡ Seasonal patterns depend on serotypes, population groups, and exposure. Epidemics of disease in military recruits commonly associated with adenovirus types 4 and 7 occur most frequently in the winter and spring.[37,149,150,215] Epidemics of pharyngoconjunctival fever have been noted most commonly in the summer months in school-aged children in association with summer camps or swimming pools.[315,485,510] No seasonal pattern has been identified for adenoviral gastroenteritis.[91,262,403,475]

HOST AND SOCIAL FACTORS

The overall incidence of adenoviral illnesses is higher in males.[85,204] In a study involving 3313 adenovirus isolates, the male-to-female ratio was 1.3 : 1.[85]

Susceptibility to adenoviral infection apparently does not vary by race. More severe disease has been noted in infants of native and Indian populations in New Zealand and Canada.[23,175] However, the relationship of these findings to socioeconomic conditions was not identified initially.

The incidence of adenoviral infection is greatest in lower socioeconomic population groups.[247] Spread of infection has been observed in daycare centers, schools, children's homes, hospitals, clinics, physicians' offices, and certain industrial settings.* Acute, overwhelming illness, including bronchiolitis and pneumonia with severe pulmonary residua, has been reported in neonates, small infants, and occasionally, adults.[9,202,294] Adenoviral infections are a significant problem in immunocompromised hosts; when they occur, they frequently are severe and occasionally are fatal.†

SPREAD OF INFECTION

Adenoviruses frequently are isolated from the conjunctiva, throat, and stool. Despite the ease of isolating the virus, the effectiveness of spread of infection in the general population varies considerably.[66] Adenovirus types 1, 2, 3, 5, and 7 are effective spreaders; however, they are not as highly contagious as varicella, measles, and influenza viruses.[65,66] Close contact appears to be necessary for infection to spread from one person to another.[448] Illness does not spread rapidly in the usual school setting but does spread dramatically in closed environments.[28,65,194,232] Although adenovirus type 4 spreads less effectively in the general population, spread is rapid in non-immune military recruits who live in close contact with each other.[74] In an outbreak of adenovirus type 7 in a children's home, 84 percent of the residents were shown to be infected.

Transmission of virus occurs by droplets reaching the conjunctiva, nose, or throat or, alternatively, by the fecal-oral route. In volunteer studies, adenoviruses have been shown to spread by small-droplet aerosols and, to a lesser degree, by large droplets.[11,86,87] Adenovirus type 4 has been recovered from room air and cough samples of patients with the virus in their throats.[12] A study by Russell and colleagues[415] of 341 military recruits and support personnel at the Marine Corps training center in San Diego, California, demonstrated that more than 79 percent of incoming recruits were susceptible to adenoviral infection in the postvaccine era. They identified incoming recruits with prolonged asymptomatic shedding of adenovirus and the presence of adenoviruses on multiple living quarter surfaces as potential sources of transmission. They also found evidence of extended pharyngeal shedding of adenovirus over the course of several days among recruits who had febrile respiratory illnesses.

In epidemics of pharyngoconjunctival fever, the virus appears to spread from contaminated swimming pool water to the eyes of recipients. Epidemics of keratoconjunctivitis have occurred as a result of contact with contaminated ophthalmic instruments and fingers.[18,101,216,244,245,255,269,334,442] In daycare centers, orphan-

*See references 64, 68, 94, 139, 148, 153, 156, 158, 175, 202, 211, 219, 236, 241, 243, 247, 255, 261, 268-270, 277, 282, 334, 335, 372, 429, 433, 443, 447, 448, 453, 459, 473, 479-482, 485, 510, 518, 519.
†See references 151, 155, 173, 219, 232, 303, 313, 334, 341.
‡See references 37, 44, 153, 194, 224, 241, 277, 372, 391, 480, 483.

*See references 25, 28, 30, 63, 73, 138, 189, 192, 245, 366, 376, 389, 437, 478, 521.
†See references 57, 59, 93, 100, 146, 184, 271, 306, 326, 361, 441, 525.

ages, and the military, transmission probably occurs most commonly through small-droplet aerosols in crowded quarters.[387] An alternative in children is the fecal-oral route. Enteric adenoviruses have been identified in fecal specimens for approximately 8 days after the onset of gastroenteritis.[262]

Family members may excrete adenoviruses in their feces intermittently for prolonged periods after initial infection.[47,151] In one study, 20 percent of persons excreted adenoviruses in stool for longer than 3 months. Intrahousehold spread appears to continue as long as susceptible family members are present.[151] Infants born into households in which members are adenoviral fecal excreters often become infected, presumably through the fecal-oral route. In general, 50 percent of susceptible household members will experience infection, although the rate varies inversely with age and also depends on the serotype of adenovirus. Re-infection with specific adenoviral serotypes occurs, but most are asymptomatic or associated with only minimal illness.

Nosocomial spread of both respiratory and enteric adenoviruses is a common occurrence and has been the cause of fatal illnesses in infants and immunocompromised patients.[4,112,144,273,437,525]

PATHOGENESIS AND PATHOLOGY

Adenoviral infections usually are acute and self-limited, and, therefore, opportunities to study the pathogenesis and pathologic process have been infrequent. Study of the pathologic mechanisms has been performed in human volunteers, tissue and organ culture systems, and recent murine model systems and by examination of specimens from persons dying of adenoviral disease.

VIRAL INFECTION

In general, the characteristics of adenoviral infection depend on the host and the serotype of the agent.* In most respiratory illnesses, initial viral infection occurs in the respiratory tract and involves the mucous membranes of the nose, oropharynx, and conjunctiva. Adenoviral agents have been isolated from sputum and oral secretions from 2 days before the onset of clinical illness to up to 8 days after the onset of symptoms. Deeper respiratory involvement of the trachea, pleurae, and lungs may result from initial small-particle aerosol, from progression of local infection, or perhaps as a result of viremia. Gastrointestinal infection in conjunction with respiratory infection also occurs early and probably is the result of swallowed virus. Stool isolates of respiratory viral types frequently are noted concomitantly with respiratory tract infection. However, in contrast to the upper respiratory infection, the virus may persist for a long time in the lower gastrointestinal tract. Infection with enteric adenoviral types is presumed to involve intestinal epithelial cells. In one fatal infection, adenoviral antigen was demonstrated in jejunal cells.[397,504]

In experimental studies with aerosolized adenovirus type 4, recovery of virus from throat specimens occurred on day 5 or 6, progressed to a maximum concentration on day 11 or 12, and was seen uncommonly after day 20. Maximal recovery of adenovirus type 4 from anal specimens occurred on day 13, and shedding continued for longer than 3 weeks. Adenovirus types 26 and 27 inoculated into the conjunctiva resulted in short-term isolation of virus from the eyes on days 3 to 7 and, less frequently, from the throat on day 4.[254] Isolation of virus from the rectum was common beginning at the end of the first week. Maximal fecal shedding occurred during the second and third weeks.

Viremia is a common finding in uncomplicated adenoviral respiratory infections in children. In a study involving 68 children, adenoviral DNA was noted in acute-phase serum samples from 28 (41%) of these children.[1] In the children with primary infection, 72 percent (21 of 29) had adenoviral DNA in their acute-phase serum samples. Other evidence of the presence of adenoviruses in the bloodstream early in disease is the occurrence of maculopapular, morbilliform, or petechial exanthems (or any combination of such rashes), as well as recovery of virus from multiple organs such as the brain, kidney, urinary bladder, lymphoid tissue, and liver and at postmortem examination.* The virus has been cultured from the mononuclear cells of heparinized blood.[8]

RELATIONSHIP BETWEEN ADENOVIRAL INFECTION AND OBESITY

Studies have shown that exposure to human adenovirus serotype 36 is associated with increased adiposity in chickens, mice, and nonhuman primates.[113-115] Paradoxically, reduced serum cholesterol and serum triglycerides are seen in the same animal models. Atkinson and colleagues[13,486] proposed that adenovirus serotype 36 directly affects adipocytes by increasing the number and size of preadipocytes. Vangipuram and associates[487] explored the possible mechanisms by which adenovirus types 36 and 37 increase adiposity in animal models. They performed ex vivo experiments in human adipocytes and a murine preadipocyte cell line (3T3-L1) and in vivo experiments in the rat model, which demonstrated suppression of leptin mRNA expression, decreased leptin secretion, and increased glucose uptake.

PATHOLOGY

Early pathologic changes are observed in the epithelium of the respiratory tract. The severity of adenoviral involvement varies with the different serotypes. In tracheal organ cultures, growth of adenovirus type 7 was characterized by an initial focal cytopathologic effect at 100 hours that quickly progressed to involve the whole epithelium.[89] Frequently, the cilia of inclusion-bearing cells were noted to be intact. In contrast to the findings with adenovirus type 7, a type 12 strain resulted in only a mild cytopathologic effect on the organ culture system.[89]

Microscopic examination of autopsy material from patients dying of adenoviral pneumonia revealed a loss of cilia in the tracheal epithelium, proliferation of other respiratory epithelial cells, and the presence of intranuclear inclusions.[22,30,51,67,156,508,511,513,526] In severe pneumonia, total destruction along with necrotizing bronchitis, bronchiolitis, and pneumonia is observed (Fig. 168–1). Mononuclear cellular infiltration is seen, and hyaline membranes and necrosis are present. Cilia and goblet cells are absent, and muscle fiber bundles and elastic fibers are dispersed. Frequently, epithelial cells have a characteristic appearance with adenoviral infection. These infected cells are enlarged grossly and lose their nuclear membranes; the nuclear material has migrated into the cytoplasm.[388] Blood vessels show edema, separation of filaments in their walls, and occasionally, thrombosis.[526]

On histologic examination, adenoviral inclusions are characterized by small eosinophilic and larger basophilic intranuclear bodies.[22,156,348,444]

Hepatic involvement in adenoviral infection has been reported frequently.[3,12,30,59,156,388,441] In addition to isolation of virus from the liver, focal areas of liver necrosis accompanied by character-

*See references 30, 47, 51, 80, 170-172, 348, 388, 444, 503, 514.

*See references 8, 9, 30, 38, 51, 67, 175, 185, 188, 307, 317, 430.

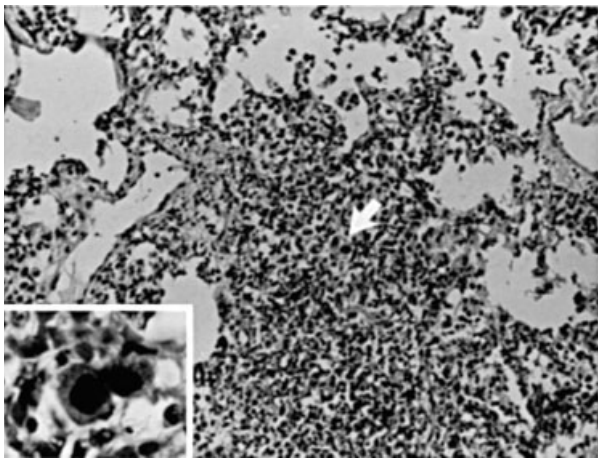

Figure 168–1 The lung of a 17-month-old infant with adenoviral pneumonia. The infant died 10 days after the onset of illness, with no other contributing disease. The adenovirus isolated was not typed. A patchy pneumonia is present, and the *arrow* indicates two cells with nuclear inclusions. *Inset*, Two similar cells with typical adenoviral nuclear inclusions at higher magnification (hematoxylin and eosin staining, ×190; inset, ×770). *(Courtesy of Dr. David D. Porter, Department of Pathology, David Geffen School of Medicine at UCLA.)*

istic hepatic intranuclear inclusions have been demonstrated.[3,59,525] Electron-microscopic examination has revealed adenoviral particles within the intranuclear inclusions.[3,30,388,508,525] Hematogenous spread to the central nervous system (CNS) has been observed.[434] Most patients with adenoviral CNS infection have had an associated pneumonia.[90,433] In CNS disease, the brain is edematous and congested. On microscopic examination, a perivascular accumulation of lymphocytes is noted, along with gigantic nuclear inclusions in the cortical neurons. Viral particles have been observed within these nuclear inclusions by electron microscopy.[76]

In epidemic keratoconjunctivitis caused by adenovirus type 8, the walls of the conjunctival vessels are damaged, and aneurysms are present. The surrounding conjunctival connective tissue is edematous. The virus has been suggested to penetrate the cornea along nerves deep into the epithelial layers in a manner similar to that occurring in herpes simplex virus (HSV) infection.[491]

Adenoviral strains have been isolated from and intranuclear inclusions have been demonstrated in renal tubular epithelium, lymph nodes, muscle, and gastrointestinal epithelium.[27,59,504,522,523,525]

IMMUNOLOGIC EVENTS

The local response in adenoviral infection depends on the site of viral inoculation, the method of transmission, the viral serotype, the concentration of inoculum, and the antibody status of the host. Three days after infection develops, when virus can be recovered from the nasopharynx, marked transudation of proteins from serum into the respiratory tract occurs, along with the production of secretory IgA antibody.[320] Approximately 7 days after the onset of illness, serum-neutralizing, hemagglutination-inhibiting, and complement-fixing antibodies appear.[99,290,390] At the same time, the nasal secretions contain specific IgA and IgG antibody.[29] In general, neutralizing antibody is the most sensitive indicator of adenoviral infection, hemagglutination-inhibiting antibody is less sensitive, and complement-fixing antibody is the least sensitive.[269,389] Antibody titers peak in 2 to 3 weeks; complement-fixing antibody declines in 2 to 3 months but may persist for up to a year.[99,390] Neutralizing antibody persists for a longer period and is measurable in many instances for periods as long as 10 years. Re-infection with the same adenoviral serotype is rare because of type-specific immunity.[26,411]

Although alterations in leukocytes are not common findings, a decrease in the number of lymphocytes has been observed before or at the onset of clinical illness and an increase in neutrophils early in the disease, followed by a decrease later. Neutropenia may develop in severe disseminated illness and is attributed to a direct toxic effect of virus on leukocytes, marrow reserves, or both.[117] The erythrocyte sedimentation rate (ESR) of children has been normal or elevated to 55 mm/hr.[447,448]

Studies by Ginsberg and associates[170-172] in murine adenoviral pneumonia model systems noted that tumor necrosis factor-α, interleukin-1, and interleukin-6 were elaborated during the first 2 to 3 days of infection. However, only tumor necrosis factor-α played an early role in the initial phase of pathogenesis. The second phase of the inflammatory response is caused by the infiltration of cytotoxic T cells. In an extensive study, Kawasaki and colleagues[256] compared numerous clinical and laboratory test results in children with adenoviral infections with those in children with influenza and respiratory syncytial virus (RSV) infections. They found that the children with adenoviral infections had higher white blood cell (WBC) counts with a greater number of neutrophils than did children with influenza or RSV infections (12,322, 7638, and 8460 WBCs/mm,[3] respectively). The children with adenoviral infections also had more atypical lymphocytes and higher C-reactive protein (CRP) and ESR values. The mean serum interleukin-6 concentration was significantly higher in the children with adenoviral infections than it was in those with influenza and RSV infections (131.0, 26.7, and 15.0 pg/mL, respectively).

Mistchenko and coworkers[329] studied cytokines and adenovirus-specific circulating immune complexes in 38 children with adenoviral infections. They placed the patients into three groups based on the severity of their respiratory illnesses: moderate illness, severe illness, and fatal illness. Serum interleukin-1 was not detected in children with moderate illness but was found in 7 of 12 with severe illness and in 13 of the 16 children who died. Tumor necrosis factor-α frequently was present in the sera of fatal cases, it was not present in the sera of moderately ill children, and it was detected in the sera of only 2 of 12 patients with severe but nonfatal illness. Interleukin-8 was noted in all sera, but values were highest in children with fatal illness. Serum immune complexes (containing IgG) were found in 7 of the 16 children who died. Finally, patients with increased concentrations of interleukin-6, interleukin-8, and tumor necrosis factor-α were those with hypoperfusion, febrile peaks, seizures, and a manifestation of septic shock. Five of 10 children with severe or fatal illness had serum autoantibodies specific for smooth muscle.

Matsubara and associates[316] noted the participation of peripheral blood $CD8^+$ T cells and HLA-DR^+, $CD8^+$ T cells in children with adenoviral infection. One child with severe adenoviral type 7 pneumonia had a marked increase in HLA-DR^+ $CD8^+$ T cells, serum levels of interferon-γ, and peripheral blood interferon-γ–producing T cells.

CLINICAL MANIFESTATIONS

Adenoviral infections are exceedingly common events, and the spectrum of disease is quite broad (Table 168–3). As noted in Table 168–3, some specific adenoviral diseases exist; however, the overall majority of infections involve a variety of anatomically associated illnesses. In many instances, similar illnesses are caused by other respiratory viruses, and the clinical spectra of the various adenoviral serotypes frequently overlap. Certain specific adenoviral types do have clinical characteristics that facilitate etiologic diagnosis, however.

TABLE 168–3 Clinical Spectrum of Adenoviral Infections

System/Organ	Illness Category	Epidemic Occurrence	Frequency	Adenoviral Type(s)
Respiratory	Common cold	No	Rare	1, 2, 3, 5, 7
	Nasopharyngitis, pharyngitis, and tonsillitis	Yes	Common	1,* 2,* 3,* 4, 5,* 7,* 7a, 14, 15, (21/H21+35)†
	Acute respiratory disease	Yes (in military recruits)	Very common	2, 3, 4,* 5, 7,* 8, 11, 14, 21
	Acute laryngotracheitis	No	Occasional	1, 2, 3, 5, 6, 7
	Acute bronchiolitis	No	Occasional	3, 7, 21
	Pneumonia (civilian population)	Yes	Common	1, 2, 3,* 4, 5, 7,* 7a, * 8, 11, 21,* (21/H21+35),† 35
	Atypical pneumonia in military recruits	Yes	Common	4,* 7,* 21
	Pertussis-like syndrome	No	Rare	1, 2, 3, 5, 12, 19
	Bronchiolitis obliterans	No	Rare	2, 3, 7, 21
	Unilateral hyperlucent lung	No	Rare	7, 21
Eye	Acute follicular conjunctivitis	Yes	Common	1, 2, 3, 4, 6, 7, 9, 10, 11, 15, 16, 17, 19, 20, 22, 34, 37
	Pharyngoconjunctival fever	Yes	Common	1, 2,* 3,* 4,* 5, 6, 7,* 7a,* 8, 14,* 37
	Epidemic keratoconjunctivitis	Yes	Occasional	2, 3, 4, 5, 7, 8,* 10, 11, 13, 14, 15, 16, 17, 19, 23, 29, 37
Skin	Morbilliform and rubelliform exanthem	Rare	Occasional	3, 4, 7, 7a
	Roseola-like	No	Occasional	1, 2
	Stevens-Johnson syndrome	No	Rare	7
	Petechial exanthem	No	Rare	7
Genitourinary	Acute hemorrhagic cystitis	No	Rare	7, 11, 21,
	Nephritis	No	Rare	3, 4, 7a
	Orchitis	No	Rare	Unknown
	Oculogenital syndrome	Yes	Rare	19, 37
Gastrointestinal	Gastroenteritis	Yes	Common	1, 2, 3,* 5, 7,* 11, 12, 15, 17, 31,* 32, 33, 40,* 41*
	Mesenteric lymphadenitis	No	Rare	1, 2, 3, 5, 7
	Intussusception	No	Rare	1, 2, 3, 5, 6, 7
	Appendicitis	No	Rare	1, 2, 7
	Hepatitis	No	Rare	1, 2, 3, 5,* 7, (11+35/H11+35)†
Heart	Myocarditis	No	Rare	7, 7a, 21
	Pericarditis	No	Rare	7
Neurologic	Encephalitis and meningitis	No	Rare	1, 2, 3, 5, 6, 7, 11, 12, 26, 32
	Transient encephalopathy	No	Rare	3
	Acute flaccid paralysis	Yes	Rare	21
Joint	Arthritis	No	Rare	7
Auditory	Deafness	No	Rare	3
Endocrine	Thyroiditis	No	Rare	Unknown

**Most common.*
†Intermediate strain.

RESPIRATORY TRACT

Common Cold

Although adenoviruses frequently receive etiologic consideration in colds, they are, in fact, associated only rarely with this illness.[248] In one report, 3 percent of common colds in children were attributed to adenovirus,[380] and in another study, 6.4 percent of children with coryza had adenoviral isolates.[128] Respiratory tract infections with adenoviruses usually are associated with fever and, frequently, with some degree of pharyngitis; therefore, when strict clinical criteria are applied, they do not qualify as colds.[232,450] Occasionally, adenoviruses, particularly types 1, 2, 3, 5, and 7, have been recovered from patients with typical colds.

Nasopharyngitis, Pharyngitis, and Tonsillitis

Adenoviral pharyngitis is an acute illness characterized by fever, sore throat, extensive exudative tonsillitis, and frequently, cervical adenopathy.[116,128,248,335,440] In two studies involving 74 children with adenoviral pharyngitis, Ruuskanen and associates[416] described the pharyngeal findings. Most commonly, only mild inflammation and redness were observed. When exudates were present, they found them to be thick and membranous, thin and follicular, or thin and spotty; most typical were follicular and spotty exudates. Associated symptoms include malaise, headache, myalgia, chills, and cough. In infants and preschool children, nasal congestion and discharge are noticeable and abdominal pain is a common complaint.[480] Children with adenoviral acute febrile pharyngitis also frequently have laryngotracheitis, bronchitis, or pneumonia.[518] The usual duration of illness varies from 5 to 7 days, although occasionally, symptoms persist well into the second week.[416]

Acute febrile pharyngitis is the most common adenoviral illness in children and is particularly important as an epidemic illness in closed environments.[28,440,448,518] For example, in an epidemic in a children's home, 63 percent of the residents were infected with adenovirus type 7a.[194] Moffet and colleagues[335] noted that adenoviruses were the etiologic agents recovered most commonly from hospitalized preschool children with febrile exudative pharyngitis; 23 percent of the children studied had adenoviral infections. In a university student health service study, 2.4 percent of illnesses with acute febrile pharyngitis were caused by adenoviruses.[346] Adenoviruses account for 37 to 75 percent of cases of nonstreptococcal pharyngitis in military recruits.

In children, 86 percent of cases of febrile pharyngitis are associated with adenovirus types 1, 2, 3, 5, and 7.[194,232,335]

On occasion, adenovirus types 7a, 9, 14, 15, and the intermediate strain 21/H21+35[213,440,448] also have been noted in association with pharyngitis. In military recruits, adenovirus types 4 and 7 are the main etiologic agents.

Acute Respiratory Disease

ARD is an epidemic disease that occurs predominantly in military recruit populations. This illness was studied extensively during World War II by the Commission on Acute Respiratory Diseases, and although individual cases were quite undifferentiated, the epidemiologic aspects of the outbreaks clearly indicated a specific etiologic agent.[81,169,440,452,473,479] The disease is an acute, febrile respiratory illness of short duration with constitutional and localized respiratory symptoms.[139] After an incubation period of 5 to 7 days, fever (mean temperature of 39.5° C), pharyngitis, laryngitis, tracheitis, and a nonproductive cough develop.[34,97,169,374] The initial dry, hacking cough may progress to paroxysms. Malaise, myalgia, chills, headache, dizziness, rhinitis, conjunctivitis, abdominal pain, and local cervical lymphadenopathy are common complaints.[97] The inflammatory process may spread to the bronchi, the bronchioles, and the parenchyma of the lungs. In epidemics, as many as 67 percent of those affected wheeze and have other evidence of small airway obstruction; pneumonia occurs in 10 to 20 percent.[215,294,338] The illness gradually resolves over the course of an 8- to 36-day period.[155] Early in the illness, the total WBC count may be slightly elevated, with a small increase in the percentage of polymorphonuclear leukocytes.

In experimental, aerosol-induced ARD, the incubation period ranged between 6 and 13 days.[87] Typical illness included fever to 39° C, rhinitis, prostration, malaise, myalgia, and headache. Pneumonia occurred occasionally. The virus was recovered from throat culture specimens 5 days after inoculation, from the nose at 6 days, and after 9 days in fecal specimens. Serum-neutralizing antibody responses occurred between the third and fourth weeks. All ill volunteers had leukopenia and an elevated ESR during the illness.

The syndrome occurs most commonly in military recruits early in basic training; illness has been documented in as many as 90 percent of new trainees within the first 8 weeks of arrival at a training site.[215] The usual etiologic agents are adenovirus types 4 and 7. Occasionally, epidemics have been associated with adenovirus types 3, 11, 14, and 21,[153] and sporadic cases have been noted in connection with adenovirus types 2, 5, and 8.[215,374] The peak seasons of illness are winter and spring.[215]

Because of the magnitude of the problem of ARD in military recruits, live attenuated adenoviral vaccines against types 4 and 7 were developed and used in all branches of the U.S. military beginning in the early 1970s.[19,181] These vaccines were used successfully until the early 1990s, when production delays occurred; subsequently, in 1995, the vaccine manufacturer stopped production. Since early 1999, no vaccine has been available, and epidemic disease has returned to military training bases.[272,414,417] Although illness may occur in civilian adults, it is an uncommon event and not epidemic. In volunteer studies, subjects with serum antibody to a particular adenoviral type are protected against disease with that type of adenovirus induced by intranasal inoculation.[26,87]

Epidemic ARD has not been described in children. However, a sporadic comparative illness, usually clinically identified as acute bronchitis, is commonly the result of adenoviral infection.[519] Adenovirus type 7 is the etiologic agent most frequently found in these cases.

Acute Laryngotracheitis

On occasion, adenoviruses have been implicated as a cause of acute laryngotracheitis. In general, the croup caused by adenoviruses is not severe and often is manifested only as a barking, brassy cough. Laryngotracheitis frequently is seen in association with febrile pharyngitis, bronchiolitis, and pneumonia.[475] Epidemics have not been observed. Adenovirus types 1, 2, 3, 5, 6, and 7 have been implicated as etiologic agents.

Acute Bronchiolitis

Adenoviruses account for approximately 5 percent of cases of bronchiolitis in infants.[511] In a recent study of 143 children with adenoviral infections, the admitting diagnosis was bronchiolitis in 24 percent.[402] The bronchiolitis caused by adenoviral infection is sporadic and usually similar to illness associated with other viral agents. Occasionally, adenoviral bronchiolitis occurring early in infancy has been fatal or has resulted in serious residual lung damage and chronic disease.[501] This severe illness has been associated with serotypes 3, 7, and 21.

Pneumonia

YOUNG CHILDREN. Adenoviruses are common isolates in children with pneumonia. The overall frequency of adenoviruses as a cause of nonbacterial pneumonia in children is less than that of RSV and parainfluenza virus type 3, but an alarming number of fatal illnesses have been noted. Severe and fatal illnesses in infants and young children have occurred in association with adenovirus types 1, 2, 3, 4, 5, 7, 7a, 7h, 7i, 8, 19, 21, 35, and the intermediate strain 21/H21+35.* The more severe cases of pneumonia have been linked to types 3, 7, and 21.[435,513] Adenoviral pneumonia has been epidemic and sporadic.[67,74]

Severe pneumonia occurs most commonly in neonates and young children aged 3 to 18 months.† The onset of illness is acute, with persistent cough and fever (>39° C). On physical examination, moderate to severe dyspnea is apparent, as is the associated tachypnea. Inspiratory and expiratory wheezes and rales are heard on auscultation. Other signs and symptoms include lethargy, diarrhea and vomiting, pharyngitis, and occasionally, conjunctivitis. Extrapulmonary complications that occur commonly are meningitis, encephalitis, seizures, splenomegaly, hepatomegaly and hepatitis, myocarditis, nephritis, bleeding tendency, and exanthems.‡ Chest radiographs reveal diffuse infiltrates, which usually are bilateral and may be bronchial, peribronchial, or interstitial.[22,282,402,509] Hyperinflation and lobar collapse occur frequently.[175] Rarely, pleural effusions or mediastinal lymphadenopathy has been described.[282] In surviving infants, symptoms persist for 2 to 4 weeks, and radiographic changes resolve slowly, frequently being present at the 3-week follow-up examination.[67,74,435] Recovery often is gradual, and exacerbations occur commonly.[240,372]

Serious sequelae often result from adenoviral lower respiratory disease, particularly in association with adenovirus types 3, 7, 7a, and 21.[15,23,60,138,175,240,282,435] Such sequelae include bronchiectasis, bronchiolitis obliterans, and unilateral hyperlucent lung.§ An estimated 14 to 60 percent of children with documented adenoviral lower respiratory tract disease have some degree of permanent pulmonary sequelae.[175,282,434,435] In a study of 27 children conducted 10 years after they had documented adenoviral type 7 pneumonia, 12 had radiographic evidence of bronchiectasis or residual pulmonary changes; 16 children had abnormal results on pulmonary function studies.[434]

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*See references 8, 22, 23, 30, 50, 51, 67, 74, 105, 112, 160, 175, 217, 240, 249, 250, 264-266, 277, 286, 307, 317, 332, 333, 344, 360, 372, 383, 384, 399, 433, 435, 444, 447, 513.

†See references 22, 23, 30, 50, 51, 60, 83, 112, 138, 175, 249, 266, 282, 317, 384, 399.

‡See references 30, 51, 67, 83, 282, 433, 435, 444, 447.

§See references 15, 23, 60, 138, 282, 310, 332, 383, 435, 511.

Macek and associates[309] have suggested that persistent adenoviral infection may be the cause of chronic obstructive airway disease in children. They noted adenoviral antigen in bronchoalveolar lavage specimens from 31 of 34 patients with chronic disease but in no bronchoalveolar specimens from a control group.

On occasion, severe and fatal adenoviral pneumonia has been related to malnutrition, environmental crowding, or a preceding severe viral disease such as measles.[15,22,23,175,277,348,435]

Severe adenoviral pneumonia associated with adenovirus types 3 and 7 also has been reported occasionally in previously healthy adults.[377,387,438] One adult had severe pneumonia caused by adenovirus type 21 that was associated with myalgia, rhabdomyolysis, and myoglobinuria.[514]

ATYPICAL PNEUMONIA IN MILITARY RECRUITS. Approximately 7 to 20 percent of cases of pneumonia in military recruits are associated with adenoviral infection.[54,150,164] Primary atypical adenoviral pneumonia commonly occurs in the winter months and generally is caused by adenovirus types 4, 7, and 21.[150] The illness is associated with fever, cough, sore throat, rhinorrhea, and chest pain. Other common symptoms include nausea, vomiting, myalgia, headache, and diarrhea. On physical examination, rales and pharyngitis are present in almost all cases. Rhinitis and generalized lymphadenopathy are observed in approximately half of those afflicted, and occasionally, conjunctivitis is noted. Chest radiographs reveal a bilaterally mottled appearance, most prominent in the lower lobes; they remain abnormal for 4 to 36 days.[54] Although serum cold agglutinins are observed, titers of 1:32 or higher are detected in only 18 percent of patients.[54,164]

Fatal pneumonia, absolute leukopenia, and disseminated disease have been reported in four previously healthy military trainees. Adenovirus type 7 was the etiologic agent in three of these cases, and the fourth illness was caused by adenovirus type 4.[122,294]

PERTUSSIS-LIKE SYNDROME. A pertussis-like illness has been noted in association with several adenoviruses, including types 1, 2, 3, 5, 12, and 19.[80,82,194,350,363] The illness occurs commonly in children younger than 36 months. The onset of illness is insidious and initially suggestive of a cold. The cough becomes progressively worse and, by 1 to 2 weeks, is paroxysmal. Severe recurrent episodes of paroxysms result in the production of mucus, post-tussive fatigue, and vomiting.[82] Approximately 50 percent of children have a typical whoop, and cyanosis occurs with the paroxysms. Peripheral leukocytosis with WBC counts ranging from 25,000 to 125,000 cells/mm^3 along with lymphocytosis and thrombocytosis is the usual finding.[80,363] The recovery time ranges from 4 to 10 weeks from the onset of illness.[82] Radiologic evidence of bronchiolitis is present in most children; interstitial pneumonia occurs occasionally. In our opinion, most and probably all of these pertussis-like illnesses are indeed *Bordetella pertussis* infections in which adenovirus is a co-infecting agent (see Chapter 140). In young infants, co-infection (adenovirus plus *B. pertussis*) may lead to more severe disease.[24,296,318]

BRONCHIOLITIS OBLITERANS. Bronchiolitis obliterans is a chronic bronchiolitis that initially was described in 1901 by Lange.[283] It has been noted to occur after measles, influenza, and pertussis, as well as after the inhalation of toxic substances.[15,23,511] Adenovirus types 2, 3, 7, and 21 have resulted in a bronchiolitis obliterans–type chronic illness.[15,23,60,332,383,501,511] These adenoviruses cause a severe necrotizing bronchiolitis that heals with fibrosis and predominantly obliterates the small airways.[19] The onset of disease is characterized by an acute febrile illness, cough, and respiratory distress. Disease may wax and wane for several weeks or months and is associated with recurrent episodes of atelectasis, pneumonia, and wheezing. Although some children recover from these episodes, the remainder have chronic pulmonary disease, including irreversible atelectasis, bronchiectasis, or hyperlucent lung syndrome.[23,501,511]

Castro-Rodriguez and colleagues[60] conducted a 5-year follow-up study of 38 children hospitalized with adenoviral pneumonia during an outbreak in Santiago, Chile, in 1998. Bronchiolitis obliterans developed in almost 50 percent of these children. Nosocomial acquisition of adenoviral infection was found to be associated more significantly with the development of bronchiolitis obliterans than community-acquired infection was.

UNILATERAL HYPERLUCENT LUNG. Unilateral hyperlucent lung is a well-defined syndrome characterized by increased translucency of all or part of one lung, along with a reduction in lung size.[92,310] The unilateral hyperlucency is associated with a decrease in the size and number of pulmonary vessels, as observed on pulmonary angiograms, and an absence of peripheral filling at bronchography.[310] Although the disease may have a number of causes, including pneumonia secondary to other viruses, it has been noted to occur after severe necrotizing bronchiolitis and pneumonia caused by adenovirus types 7 and 21.[310,511]

EYE

Acute Follicular Conjunctivitis

Acute follicular conjunctivitis is the most common and benign of the adenoviral infections of the eye. The infection in this disease is confined to the eye, generally is unilateral, and is manifested by follicular lesions on the palpebral conjunctival surface. Symptoms occur after an incubation period of 5 to 7 days and include lacrimation, itching, burning, a foreign body sensation, and conjunctival erythema.[110] Examination shows erythema and lymphoid follicular hyperplasia in the conjunctiva in association with serous drainage and increased lacrimation. Occasionally, adenopathy of the preauricular lymph nodes is seen. Symptoms resolve in 10 days to 3 weeks, with recovery usually complete.[86,110] Adenovirus types 1, 2, 3, 4, 6, 7, 9, 10, 11, 15, 16, 17, 19, 20, 22, 30, 34, and 37 have been isolated from the eyes of afflicted patients.[39,83,110,138,421,427,474]

Pharyngoconjunctival Fever

Pharyngoconjunctival fever is presented in detail in Chapter 12.

Epidemic Keratoconjunctivitis

Epidemic keratoconjunctivitis is caused most commonly by adenovirus type 8, but it also has resulted from infection with adenovirus types 2, 3, 4, 5, 7, 10, 11, 13, 14, 15, 16, 17, 19, 22, 23, 29, and 37.* Currently, adenovirus type 37 is the virus most commonly recovered from patients with epidemic keratoconjunctivitis in the United States and Europe. The most severe disease is associated with adenovirus types 5, 8, and 19.[96]

The illness occurs most commonly in adults, but a few cases have been reported in children.[195,334,459] It has no seasonal pattern. Although transmission of the viral agent from the respiratory tract to the eye occurs in sporadic cases, the usual method of viral

*See references 18, 39, 56, 63, 87, 96, 98, 101, 103, 104, 139, 189, 191, 195, 244, 245, 255, 257, 260, 334, 336, 353, 355, 421, 442, 446, 460, 518.

spread is by contaminated ophthalmic instruments and eye solutions, hand-to-eye contact by medical personnel and others, swimming pools, or hands or fomites in close-contact situations, such as in families and in industry.* The incubation period typically is 5 to 10 days but ranges from as short a period as 2 days to as long as 2 weeks.[98,269] The initial symptom generally is unilateral, acute, follicular conjunctivitis that suggests the presence of a foreign body. Photophobia, lacrimation, discharge, hyperemia, and edema of the conjunctiva are notable. Preauricular adenopathy occurs in as many as 90 percent of patients, and 50 percent of those afflicted have pharyngitis and rhinitis. Spread to the other eye may occur in 2 to 7 days. Seven to 10 days after onset of the disease, the conjunctivitis resolves, and painful, superficial, punctate epithelial opacities appear in the center of the cornea. These lesions frequently extend subepithelially and then heal, with subepithelial infiltrates left behind that may persist for months. In severe cases, hazy vision may continue for several years.[18]

An infantile form of epidemic keratoconjunctivitis has been described that usually affects children younger than 2 years old. This pseudomembranous or membranous conjunctivitis generally is accompanied by high fever, pharyngitis, otitis media, diarrhea, and vomiting. Preauricular lymphadenopathy typically is absent.[459]

Virus can be recovered from the eye for a usual period of approximately 2 weeks but has been detected for 2 to 3 years in patients with chronic papillary conjunctivitis.[96,382] In acute illness, conjunctival scrapings obtained during the first 10 days of infection reveal characteristic inclusion bodies when stained with Giemsa.[205] Virus-specific fluorescent antibody staining is diagnostic in epidemic keratoconjunctivitis.[426] Preparations of corneal and conjunctival epithelia reveal adenoviral particles when examined with the electron microscope.[103]

SKIN

Adenovirus types 1, 2, 3, 4, 7, and 7a, plus several unknown types, have been noted in connection with exanthematous disease.[70] The cutaneous manifestation most commonly associated with adenoviral infection is an erythematous maculopapular rash that appears while the child is febrile. In many instances, children with this illness have been thought to have either measles or rubella.[277] In most adenoviral infections with exanthems, other clinical findings more characteristic of adenoviruses, such as conjunctivitis, rhinitis, pharyngitis, and lymphadenopathy, also are present. In some instances, the exanthem truly is morbilliform with a characteristic confluence, but Koplik spots do not occur.

A widespread erythematous rash often is present early in the course of severe pneumonia in infants.[175] Chany and colleagues[67] noted a measles-like rash in five patients who died of adenovirus type 7a pneumonia. One report describes a child with an adenovirus type 7 infection and illness suggestive of meningococcemia.[418] This patient had fever, vomiting, diarrhea, and a petechial exanthem. Rocholl and associates[402] noted that rash occurred in 10 percent of 143 adenoviral illnesses in children.

On several occasions, illness characterized by fever and defervescence and then the appearance of a maculopapular rash suggesting the diagnosis of roseola infantum has been observed.[148,158,242,243,352] Adenoviral infections also are confused with rubella. However, the respiratory symptoms and the degree of fever associated with adenoviral infection should clarify the diagnosis. With rubella, respiratory complaints and fever are minimal. On occasion, severe disease with Stevens-Johnson syndrome has been noted. Such patients frequently have pneumonia, and the illness is quite similar to that caused by *Mycoplasma pneumoniae*.

Lähdeaho and associates[281] have noted that serum antibodies to the E1b protein–derived peptides of the enteric adenovirus type 40 are associated with dermatitis herpetiformis.

GENITOURINARY TRACT

Acute Hemorrhagic Cystitis

Acute hemorrhagic cystitis is an uncommon manifestation of adenoviral infection in immunocompetent children and is characterized by a sudden onset of dysuria and frequency, with hematuria developing 12 to 24 hours later.[287,288,339,340,342,358] Occasionally, fever, suprapubic pain, and enuresis occur. Antecedent upper respiratory tract infection is noted in some children. Symptoms persist for a few days to 2 weeks, with the usual duration being approximately 5 days. Acute hemorrhagic cystitis has been reported in both the United States and Japan. It occurs primarily in children, most often boys, and usually is associated with adenovirus type 11. Occasionally, adenovirus types 7 and 21 have been implicated. Adenoviral antigen has been identified by immunofluorescence in exfoliated bladder cells. Although no sequelae have been reported, the long-term prognosis is unknown.[339]

Nephritis

Hematuria occasionally has been reported in infants with severe pneumonia and disseminated adenoviral infection. Red blood cells and, at times, red blood cell casts also have been noted in the urine of some children with upper respiratory illnesses caused by adenoviruses and specifically in patients with pharyngoconjunctival fever associated with adenovirus types 3, 4, and 7a.[440,445,480,485] In one instance, a young boy had a maculopapular and petechial exanthem and thrombocytopenia associated with adenovirus type 7 infection. Hematuria also was present.

Orchitis

Orchitis developed in one child who had a 5-day history of pain and fever, erythema, and swelling of the right testicle.[349] The testicular involvement resolved in several days, and the illness was associated with a 16-fold rise in adenoviral complement-fixation antibody titer.

Oculogenital Syndrome

In 1977, Laverty and associates[287] reported a woman who in addition to pharyngoconjunctival fever, had cervicitis and paresthesia of the legs; a type 19 adenovirus was recovered from this woman's cervix. In Perth, Australia, adenovirus type 19 was recovered from the genital tracts of 59 men and women being examined for genital HSV infection in a clinic for sexually transmitted diseases.[236] Several of the patients also had conjunctivitis, and in two, adenovirus type 19 was isolated from conjunctival specimens. Similar oculogenital illnesses have been noted in association with adenoviral types 2, 8, and 37.[62,421,455]

Hemolytic-Uremic Syndrome

Two 2-year-old children with hemolytic-uremic syndrome in association with adenoviral infections have been described.[33]

Hemorrhagic Fever with Renal Syndrome

A 22-year-old woman with an adenovirus type 11 infection and hemorrhagic fever with renal syndrome has been reported.[476]

*See references 18, 56, 63, 96, 98, 101, 147, 244, 269, 315, 334, 336, 459, 500.

GASTROINTESTINAL TRACT

Gastroenteritis

Infantile diarrhea has been associated with epidemic and sporadic adenoviral diseases such as acute upper respiratory tract infection, severe pneumonia, and pharyngoconjunctival fever.[148,282,440,449,504] Outbreaks of diarrhea characterized by acute abdominal pain followed by diarrhea, nausea and vomiting, fever, headache, and pharyngitis have been associated with adenovirus type 3 and 7 infections.[162,448] Other symptoms occurring in patients with diarrhea include conjunctivitis, rhinitis, pharyngotonsillitis, hepatomegaly, and cervical adenitis.[449] In two patients who had diarrhea and upper respiratory illness in association with adenovirus type 15 infection, viral particles were visualized within the nuclei of mucosal cells at autopsy by electron microscopy.[123]

The widespread use of electron microscopy for the study of rotaviral diarrhea led to the finding of previously unrecognized adenoviruses that were fastidious and could not be grown in routine cell cultures.[145,425] These adenoviruses, now identified as types 40 and 41, subsequently were shown to be important causes of gastroenteritis in children.* In enteric adenoviral infection, diarrhea is the most prominent symptom.[474] In children with adenovirus type 40 infection, Uhnoo and colleagues[475] found that the mean duration was 8.6 days, as opposed to 12.2 days in those infected with adenovirus type 41. Most illnesses occur in children younger than 3 years old: the mean age for adenovirus type 40 diarrhea was 15.2 months, whereas it was 28.3 months in type 41 illnesses. Most patients had mild vomiting that lasted approximately 2 days. When compared with illnesses caused by established respiratory adenoviruses, fever occurred less commonly, was less severe, and had a shorter duration in enteric adenoviral infections. Upper respiratory symptoms and signs, such as pharyngitis, coryza, cough, and otitis media, were observed in 21 percent of children with enteric adenoviral infection. Brandt and colleagues[46] noted that dual infections with respiratory viruses (such as RSV and enteric adenoviruses) are common occurrences, so caution should be observed in attributing respiratory symptoms to enteric adenoviruses.

Yolken and Franklin[520] found that enteric adenoviruses were an important cause of nosocomial diarrhea in infants who had previously undergone gastrointestinal surgery for necrotizing enterocolitis.

Mesenteric Lymphadenitis

Adenoviral serotypes 1, 2, 3, 5, and 7 have been recovered from lymph nodes and the appendix in cases of mesenteric lymphadenitis.[27,38,268] Patients with mesenteric lymphadenitis often have abdominal pain and other symptoms similar to those of acute appendicitis. Pharyngitis is a frequently related finding. Mesenteric adenitis often is associated with concurrent or recent adenoviral illness.[83,393] Frequently, the peak incidence of mesenteric lymphadenitis occurs when adenoviral illness is common in the community.

Intussusception

The suggestion that adenoviruses could be an etiologic factor in intussusception arose because these agents frequently can be recovered from throat, stool, and mesenteric lymph node specimens obtained from children who undergo surgery for intussusception.† Most children with intussusception were younger than 2 years old; some had preceding respiratory symptoms.[409,522] Adenovirus serotypes 1, 2, 3, 5, 6, and 7 have been implicated. Typical adenoviral intranuclear inclusions have been demonstrated in cells in stool, intestinal epithelia (ileum), and the appendix by electron microscopy.[359,522,523] Mesenteric lymph nodes often are enlarged at surgery.[523] Increases in antibody titer to adenoviruses have been observed in children after the development of intussusception. Investigators have suggested that bowel wall hypermotility caused by direct viral involvement or by hypertrophy of lymphatic tissue is the lead point for the intussusception.[27,79,161,359,391,409,522,523]

Appendicitis

Adenoviruses have been associated with both acute and chronic appendicitis.[38] Right iliac fossa abdominal pain in conjunction with sore throat is a common finding. The virus has been isolated from the appendix and mesenteric lymph nodes at surgery. During acute infection, lymphoid follicles of the ileum, appendix, and mesenteric lymph nodes are infected with virus. In chronic infection, adenovirus remains in cells; on microscopic examination, slight inflammation is seen in the appendix.

Hepatitis

Hepatitis in association with adenoviral infection has been reported many times in small infants, in children with overwhelming disseminated disease, and in immunocompromised patients.[5,12,30,51,59,265,525] In one study, 27 of 30 persons thought to have sporadic infectious hepatitis were found to be infected with adenovirus type 5.[196] Adenovirus types 1, 2, and 3 were isolated from the stool specimens of 12 children younger than 3 years old in an outbreak of infectious hepatitis on a Native American reservation in Arizona.[198]

In one report, three children with severe, fatal adenovirus type 7 pneumonia had associated findings that simulated Reye syndrome: lethargy, diarrhea, seizures, elevated CSF pressure, myocarditis, hepatitis, and disseminated intravascular coagulation.[280] Edwards and colleagues[127] reported three children with Reye syndrome and adenoviral infection. They suggested that adenoviruses might be an important agent in initiating the syndrome.

HEART

Myocarditis

In children, myocarditis has been noted in association with severe pneumonia and disseminated disease caused by adenovirus types 7, 7a, and 21.[201] Similar cardiac involvement has been seen in military recruits with severe ARD.[67,74,201,440]

The use of polymerase chain reaction (PCR) assays has led to recognition of adenovirus as the cause of many cases of acute myocarditis.[40,48,187,308,314] In an extensive study of myocarditis and dilated cardiomyopathy, Bowles and coworkers[40] analyzed 773 endomyocardial biopsy samples obtained from patients of all ages. In this large group, 624 patients had myocarditis and adenoviral DNA was identified by PCR in 142 (23%) of the endomyocardial biopsy samples. There were 26 neonates, 37 infants, 20 toddlers, 25 older children, 11 adolescents, and 23 adults. The results of this study suggest that adenoviruses are the leading viral cause of myocarditis, with enteroviruses being the second most common association (14%). On histopathologic examination, only 57 (40%) of the enterovirus-positive samples were found to be acute myocarditis.

Dilated Cardiomyopathy

In the study by Bowles and associates,[40] specimens from 149 patients exhibited dilated cardiomyopathy. Of this subgroup, 18

*See references 47, 49, 77, 274, 275, 330, 362, 389, 403, 464, 475, 478.
†See references 27, 32, 79, 161, 230, 359, 387, 409, 522, 523.

(12%) had adenoviral DNA demonstrated by PCR in the endomyocardial biopsy specimen. Of these 18 samples, 2 were from neonates, 2 from infants, 2 from toddlers, 4 from older children, 2 from adolescents, and 6 from adults.

Pericarditis

Pericarditis has been associated with severe adenoviral pneumonia. In a patient with adenovirus type 7 pneumonia, electrocardiographic changes consistent with pericarditis were demonstrated, and the virus was isolated in high titer from pericardial fluid at postmortem examination.[348] In 1995, Mistchenko and coworkers[331] reported a 10-month-old boy with fatal pericarditis caused by adenovirus type 7. In serum and pericardial fluid from this child, interleukin-6, tumor necrosis factor-α, and adenovirus-specific immune complexes were identified.

NERVOUS SYSTEM

Although CNS disease in adenoviral infection is an uncommon finding, a variety of clinical manifestations have been observed. Both meningitis and encephalitis have been noted as the major manifestations of adenoviral infection or in association with severe disease at other body sites.[356,360] Adenovirus types 1, 2, 3, 5, 6, 7, 12, 26, and 32, in isolated instances, have been recovered from both brain and CSF.* Meningoencephalitis often occurs as part of disseminated adenoviral disease in immunocompromised patients, with the most commonly associated serotypes being 2, 3, 5, and 7. Adenovirus serotype 26 DNA was identified in peripheral blood, CSF, urine, and postmortem brain tissue of a 28-year-old woman undergoing radiation therapy for medulloblastoma; progressive neurologic deterioration developed, followed by her eventual death as a result of disseminated adenoviral infection.[118] In two children with respiratory and CNS symptoms, adenoviruses were recovered from spinal fluid.[140] One child was convalescing from herpes zoster and the other from varicella. Adenovirus type 7 was recovered from brain tissue cultures from an elderly patient with chronic schizophrenia.[307] In another instance, adenovirus type 32 was recovered from the brain of a man with lymphosarcoma and subacute encephalitis.[76,406] In an epidemic of adenoviral infection caused by type 7, 25 percent of the hospitalized patients had symptoms referable to the CNS.[433,435] Many of the patients died; those who survived had little residual effect. Too few cases are reported in the literature to predict the prognosis of CNS disease in children accurately. Adenovirus type 2 was isolated from a muscle biopsy specimen from a patient with inclusion body myositis.[327] Ooi and colleagues[364] reported eight children with adenovirus serotype 21–associated acute flaccid paralysis in the setting of an enterovirus 71–mediated hand-foot-and-mouth disease outbreak in Sarawak, Malaysia, in 1997. Of these eight children, four had upper limb monoparesis, two had lower limb monoparesis, and two had paraparesis. Four of the children experienced complete recovery, one was improving at hospital discharge, and three had residual flaccid weakness and wasting. Seven children with initially appearing typical adenoviral infections had transient encephalopathy.[451] All these children were infected with adenovirus type 3. On initial evaluation, five had pneumonia, one had follicular tonsillitis, and one suffered from diarrhea. Three of the children were lethargic, two were stuporous, and two were obtunded. All had elevated serum aspartate aminotransferase values (range, 39 to 401 U/L), and CSF evaluations were within normal limits. The neurologic findings resolved in all patients in about 1 week.

*See references 69, 83, 90, 118, 140, 159, 259, 370, 381, 406, 447, 453, 483.

INFECTION IN IMMUNOCOMPROMISED HOSTS

Adenovirus types 1, 2, 3, 4, 5, 6, 7, 7a, 11, 29, 31, 32, 34, and 35 have been recovered from children and adults who were immunocompromised by immunodeficiency diseases, malignancies, steroid therapy, immunosuppressive therapy, radiation therapy, and transplantation procedures.* Adenoviral infections occur more frequently in pediatric than in adult hematopoietic stem cell and solid organ transplant recipients.[17,220,229] Infection is thought to occur as a result of transmission from the donor, reactivation of latent infection, or newly acquired infection.[276] McLaughlin and colleagues[321] examined the incidence of adenovirus infection in 146 children who underwent liver and intestinal transplants. They found that adenoviral infection occurred in 4.1 percent of liver transplant recipients and 20.8 percent of intestinal transplant recipients.

In a study of eight children with liver and small bowel transplants and six with just small bowel transplants, Pinchoff and coworkers[386] noted that all had evidence of adenoviral infection of the graft. In eight of the children, histologic evidence of infection (adenoviral intranuclear inclusions) developed, and these eight had received intensive corticosteroid therapy, had virus isolated from multiple sites, and had persistent positive viral cultures. For most solid organ transplants, the source of invasive adenoviral disease often is thought to be the transplanted organ.

Kampmann and colleagues[251] conducted a prospective study on 155 pediatric stem cell transplant recipients and identified 17 percent with adenoviremia (26/155). Their study showed that early detection of adenoviremia, withdrawal of immunosuppression, and early antiviral therapy led to resolution of adenoviremia in 81 percent of the patients. de Mezerville and collaborators[109] retrospectively identified 28 solid organ transplant recipients and 27 hematopoietic stem cell transplant recipients with adenovirus infection at the Hospital for Sick Children in Toronto, Canada. Their study found a similar rate of post-transplant adenoviral infection in solid organ transplant recipients (50.9%) and hematopoietic stem cell transplant recipients (49.1%). de Mezerville's group found a much higher mortality rate in the group of hematopoietic stem cell transplant recipients (30% mortality rate versus 0% in the solid organ transplant group).

Control of adenovirus infection is thought to be T-cell–mediated, and patients receiving T-cell–suppressive regimens are thought to be at greater risk for adenovirus disease. Myers and colleagues[347] demonstrated an increased risk for development of adenoviral infection in bone marrow transplant recipients receiving alemtuzumab (Campath 1H), a monoclonal antibody used for T-cell depletion, in comparison to patients receiving antithymocyte globulin (ATG), which was composed of polyclonal antibodies directed toward T cells. Avivi and colleagues[14] demonstrated that the most important risk factors for progression of adenovirus disease included severe lymphocytopenia (absolute lymphocyte count of less than 200/mm^3) and continued immunosuppression. They also found a correlation between lack of cytomegalovirus (CMV) prophylaxis with ganciclovir and risk of progression of adenovirus disease.

Ahmad and coworkers[6] reported a case of severe adenovirus pneumonia developing after infliximab (Remicade) infused was used to treat a 35-year-old man with Crohn disease. Infliximab is a monoclonal antibody directed against tumor necrosis factor-α that is used frequently in the treatment of autoimmune and rheumatologic diseases. Ahmad's group emphasized the fact that

*See references 3, 17, 21, 57, 59, 76, 93, 100, 137, 146, 163, 184, 190, 199, 207, 237, 258, 306, 346, 354, 361, 371, 386, 406, 429-432, 436, 441, 471, 508, 525.

tumor necrosis factor-α antagonists can impair cell-mediated immunity, an essential component of recovery from adenoviral infection in immunocompromised hosts, and, therefore, can predispose patients to the development of viral pneumonia.

Other groups have demonstrated that having low lymphocyte counts at the time of development of adenovirus infection was predictive of adenovirus viremia and that clearance of infection and host survival were dependent on recovery of adenovirus-specific $CD4^+$ T-cell responses.[199,489] Lankester and colleagues[285] showed that isolation of adenovirus from multiple sites was associated with the development of adenovirus disease. Lins' group[299] demonstrated an association between isolation of adenovirus in peripheral blood and progression or dissemination (or both) of adenovirus disease. High levels of adenovirus viremia (i.e., detection of PCR product at 100-fold or greater dilution of template DNA) have been correlated with fatal outcomes in children receiving allogeneic stem cell transplants.[422,488]

Baldwin and colleagues[17] studied the outcome and clinical course of 105 adenoviral infections in 100 patients after they received bone marrow transplants. The incidence was higher in unrelated donor transplants than in matched sibling donor transplants. Diarrhea and fever were the most common initial findings. Six deaths were attributed to the adenoviral infections; five of the six patients had pneumonia, and four had associated graft-versus-host disease. Three additional patients had severe disease.

In a study involving 532 recipients of hematopoietic stem cell transplants, a 12 percent incidence of adenoviral infection was noted.[229] Forty-one patients had infections classified as "invasive," and mortality was 76 percent in this group. Recipients of allogeneic transplants were more likely to have adenoviral infections than autologous stem cell recipients were.

The most common manifestation of adenoviral infection in pediatric patients with bone marrow transplants at St. Jude Children's Research Hospital was hemorrhagic cystitis, followed by gastroenteritis, pneumonitis, and liver failure.[190] Adenovirus-induced acute hemorrhagic cystitis is a complication encountered after hematopoietic stem cell transplantation and, more rarely, after renal transplantation.[221] Gorczynska and colleagues[177] examined the incidence of virus-induced hemorrhagic cystitis in 102 children and adolescents who underwent allogeneic stem cell transplantation. Hemorrhagic cystitis occurred in 25.5 percent of these children, with 3.9 percent of the cases attributed to adenoviruses.

Shirali and coworkers[431] noted that demonstration of the adenoviral genome in endomyocardial biopsy specimens from heart transplant recipients was a predictor of graft loss in children. Scattered reports have found an association between adenoviral infection and viruria and hemorrhagic cystitis in patients with renal transplants.[143,283] A fatal case of subacute meningoencephalitis caused by an adenovirus has been described in a bone marrow transplant recipient.[100]

In many instances, a fulminant, bacterial, sepsis-like picture with high fever, cough, and lethargy is associated with adenoviral infection in compromised patients.[525] Severe pneumonia often is demonstrated, both clinically and radiologically, and hepatic involvement with disseminated intravascular coagulation also is a frequent occurrence. Fatalities are reported, and recovery often is slow.

OTHER MANIFESTATIONS

Arthritis

Arthritis has been noted in association with adenovirus type 7 infection.[369] The illness was characterized by fever, acute respiratory symptoms, erythematous macular rash, aseptic meningitis, and inflammatory arthritis of both knees.

Thyroiditis

In 1964, Swann[454] reported five patients with acute thyroiditis and thyroid enlargement in whom serologic study revealed greater than fourfold rises in titer of adenoviral complement-fixing antibody.

Deafness

Deafness of sudden onset was reported in an adult with a 2-day history of sore throat, low-grade fever, rhinorrhea, and cough. Adenovirus type 3 was isolated from the patient's throat, and a greater than fourfold rise in neutralizing antibody titer to this virus was observed.[239]

Obesity

Based on studies that adenoviruses increase adiposity in animal models, Atkinson and coworkers[13] screened 502 subjects for neutralizing antibodies to adenovirus 36. They detected antibodies to adenovirus 36 in as many as 30 percent of obese study subjects as opposed to 11 percent of nonobese subjects. Similarly, they found paradoxically decreased serum cholesterol and triglycerides in patients with antibodies to adenovirus 36. Additional studies by Whigham and colleagues[502] demonstrated that human adenovirus serotype 37 also is associated with an increase in adipocyte differentiation and reduced serum triglycerides, but increased serum cholesterol.

CONGENITAL AND NEONATAL INFECTIONS

Neonatal and congenital adenoviral infections reflect disseminated infection with the involvement of multiple organs.[2,50,75,138,266,365,447] Major manifestations include hepatosplenomegaly, progressive pneumonia, hepatitis, and thrombocytopenia. Towbin and colleagues[469] reported intrauterine adenoviral myocarditis manifested as non-immune hydrops fetalis. Illnesses frequently appear initially as an early-onset sepsis syndrome. An infant has been reported with a congenital pleural effusion from which type 3 adenovirus was recovered.[325]

A recent study by Couroucli and associates[88] has noted an association between adenoviral infection and bronchopulmonary dysplasia (BPD). They found a significant increase in the frequency of adenovirus genome in tracheal aspirates from patients with BPD versus controls.

DIAGNOSIS

DIFFERENTIAL DIAGNOSIS

The differential diagnosis of adenoviral infection must be subcategorized on the basis of the major clinical manifestations. In many instances, specific clinical symptoms such as pharyngoconjunctival fever or epidemic keratoconjunctivitis can lead to strong suspicion of the adenoviral etiology. With other, more general respiratory diseases such as pharyngitis, bronchitis, croup, bronchiolitis, and pneumonia, the adenoviral etiology cannot be established on clinical grounds. Fever in adenoviral respiratory infections tends to be higher than that occurring in parainfluenza and RSV infections but similar to that in influenza A and B viral infections.[394] High and prolonged fever occurs as commonly in adenoviral infections as it does in bacterial respiratory infections.

In pharyngitis, adenoviral infection must be differentiated both from other viral diseases, such as those caused by Epstein-Barr virus, parainfluenza and influenza viruses, and enteroviruses,

and from streptococcal disease. In a young child, follicular pharyngitis is more likely to be caused by adenoviral than by streptococcal infection.

Adenoviral pneumonia in young children frequently is not distinguishable on clinical grounds from that caused by bacteria. In older children and adolescents, in particular, adenoviral pneumonia often can be differentiated from bacterial disease by its bilateral nature. Differentiating adenoviral illness from disease caused by *M. pneumoniae* is more difficult; however, cold agglutinin titers generally are higher and more persistently positive in mycoplasmal disease.

Adenoviral eye disease must be differentiated from that caused by viruses of the herpes group, *Chlamydia* spp., and bacteria, including *Neisseria gonorrhoeae*.

Diarrhea caused by enteric adenoviruses can be differentiated from other viral diarrheas by electron microscopy, by identification with ELISA, and by specific culture of the enteric agents in Graham 293 cell culture. Enteric bacterial and parasitic agents also must be considered in the differential diagnosis.

The high, relatively prolonged fever occurring in association with lymphadenopathy, exanthem, and enanthem noted with adenoviral infection often causes confusion with Kawasaki disease.[72,402] Because Kawasaki disease must be treated early with intravenous immunoglobulin, adenoviral infections should be considered early and appropriate studies performed.

SPECIFIC DIAGNOSIS

Adenoviral infection can be diagnosed specifically by isolation of virus in an appropriate tissue culture system or by a direct antigen-detection assay. Most adenoviral types can be recovered from clinical specimens in primary or diploid cultures of human embryonic kidney.[291] The enteric viral types 40 and 41 can be grown in Graham 293 cells.[458,496] The rapidity of detection of adenoviruses by culture is enhanced by centrifugation of specimens in shell vials or plastic-welled plates.[124,131,311]

Direct identification of adenoviruses in respiratory secretions by radioimmunoassay, immunofluorescence techniques, and ELISA now is performed widely.[52,311,324,396,402,416,419,462] However, in general, the sensitivity, when compared with that of virus isolation, is relatively low; on the other hand, the specificity is high (>95%). Rocholl and colleagues[402] demonstrated the impact of rapid diagnosis of adenoviral respiratory disease with an adenoviral direct fluorescent antibody technique by noting discontinuation of empiric antibiotic use in a retrospective analysis of hospitalized patients with respiratory cultures positive for adenovirus. Rapid techniques for the identification of enteric adenoviruses in general have been sensitive and specific.[5,179,205,420,476,512] However, in newborns, false-positive results with a latex agglutination test have been reported.[235] Several techniques using DNA probes have been studied for the rapid identification of both respiratory and enteric adenoviral infection.[55,234,457] PCR assay also is useful for the rapid detection of adenoviral infection.* PCR assay is particularly useful for identifying adenoviral DNA in formalin-fixed, paraffin-embedded tissues and other biopsy and postmortem specimens.[40,187,308,314,431,472]

Several groups have demonstrated the utility of PCR detection of adenoviral DNA in acute respiratory infections in immunocompetent patients.[1,136,402,430,517] Kuypers and colleagues[279] demonstrated significantly increased sensitivity of quantitative real-time PCR assay over fluorescent antibody detection of adenoviruses. They showed that 77.7 percent of respiratory specimens that were negative for adenovirus by fluorescent antibody detection were positive by PCR detection of adenovirus DNA. Xu and Erdman[517] developed a multiplex PCR assay for rapid, type-specific identification of adenovirus serotypes 3, 7, and 21. Lin and colleagues[299] used oligonucleotide microarrays to identify adenovirus serotypes 3, 4, 6, 7, 16, and 21, which resulted in lower detection limits and rapid processing time (1.5 to 4 hours). Aberle and colleagues[1] were able to detect adenoviral DNA with a nested PCR assay in acute-phase sera collected within the first week after the onset of symptoms in 72 percent of immunocompetent children experiencing their first adenoviral infection and in 25 percent of those experiencing recurrent infection.

Other groups have demonstrated the utility of using PCR assays to diagnose and monitor adenoviral infection and disease in immunocompromised hosts.[285,422] Lion's group[300] developed a species-specific, real-time, quantitative PCR assay that allows detection and quantification of all 51 currently known human adenovirus subtypes. They showed that detection of adenovirus DNA in the peripheral blood of allogeneic bone marrow transplant recipients preceded the onset of clinical symptoms of disseminated adenoviral disease by a median of 3 weeks. Two other groups[292,498] have used real-time, quantitative PCR assays to detect adenoviral DNA load to aid in both the diagnosis and management of disseminated adenoviral infections in immunocompromised patients. Ebner and colleagues[125] recently have presented a two-stage PCR algorithm for diagnosis in immunosuppressed patients. It uses real-time quantitative PCR to broadly identify all human adenovirus serotypes without species identification. If it is positive, a species-specific PCR assay is performed with fragment length analysis and then sequencing to determine the specific adenoviral serotype. In a study of 135 pediatric stem cell transplant recipients, they detected adenoviruses in stool specimens in 39 children and the blood of 1 child. The following serotypes were identified: 1, 2, 3, 5, 6, 12, 16, 19, 31, and 49.

At present, most hospitals rely on fluorescent antibody staining, shell vial culture, and viral culture to identify adenovirus infection.

Acute infection also can be diagnosed serologically by the expression of specific serum IgM antibody.[324] Infection can be confirmed serologically by the demonstration of a rise in antibody titer in two sequential serum samples. Antibody response to the adenovirus group antigen can be detected by complement fixation or ELISA. Type-specific antibodies can be determined by neutralization, ELISA, or hemagglutination inhibition.

TREATMENT

During the febrile period of illness, adequate hydration should be maintained and excessive activity discouraged. The fever may be controlled with acetaminophen. In children with eye involvement, careful attention should be paid to the possibility of secondary bacterial infection. If local purulence develops, specimens should be procured for culture and topical antimicrobial therapy started. The use of steroid-containing ophthalmic ointments should be avoided.

A consistent, safe, and effective therapy to treat adenovirus infection has yet to be established. Whereas vidarabine, ribavirin, and cidofovir demonstrate good activity against adenoviruses in vitro, several studies have shown only limited success with the use of vidarabine, ribavirin, ganciclovir, and cidofovir in treating adenovirus disease in humans.[301,343] The potential beneficial effects must be weighed carefully against the adverse side effects of these antiviral agents, including myelosuppression, pancytopenia, and nephrotoxicity. Walls and coworkers[499] conducted a retrospective study of 26 patients undergoing hematopoietic stem cell transplantation. They were unable to demonstrate a direct correlation among adenoviral DNA load, clinical features, and severity of disease. They also identified two children who expe-

*See references 1, 7, 40, 125, 126, 136, 200, 237, 279, 285, 292, 300, 321, 337, 371, 402, 422, 430, 498, 517.

rienced low-level adenoviral viremia after undergoing hematopoietic stem cell transplantation; the children cleared the virus without treatment.

Kurosaki and colleagues[278] demonstrated a possible role for vidarabine in the treatment of adenovirus-induced hemorrhagic cystitis. Vidarabine inhibits the DNA polymerase of double-stranded DNA viruses (including human adenoviruses) and varicella-zoster virus. Vidarabine is metabolized via deamination to 9-β-D-arabinofuranosyl hypoxanthine, and both vidarabine and its metabolite are excreted in the kidney. The studies of Kurosaki and associates demonstrated dose-dependent inhibition of viral replication and adenovirus type 11 activity in vitro. Doses of 10 mg/kg given intravenously once daily for 5 days have been shown to be effective in the treatment of hemorrhagic cystitis.

Ribavirin is a guanosine nucleoside analogue used for the treatment of severe RSV infection in its inhaled form and for the treatment of chronic hepatitis C infection. In a number of instances, immunocompromised patients with disseminated adenoviral infection have been treated with ribavirin administered intravenously, and successful outcomes have been reported.[10,252,298,319,345,516] In one instance, a loading dose of 30 mg/kg/day divided into three doses was followed by maintenance therapy with 15 mg/kg/day.[319] Gavin and Katz[163] reported complete clinical recovery in two of five patients with severe adenovirus disease. A later study by Lankester and associates[284] demonstrated no effect on plasma adenoviral DNA load despite initiation of treatment with ribavirin at the first onset of clinical symptoms in four pediatric patients receiving allogeneic stem cell transplants.

Ganciclovir is another guanosine nucleoside analogue used for the prophylaxis and treatment of CMV infection. Previous studies had demonstrated weak inhibitory activity of ganciclovir in vitro against some adenoviral strains. Bruno and colleagues[53] showed that the use of ganciclovir for prophylaxis of CMV infection in patients undergoing hematopoietic stem cell transplantation had a slight protective effect against the development of adenovirus infection.

The antiviral agent with the most promise to date is cidofovir, a cytosine nucleoside analogue with efficacy against several DNA viruses, including CMV, HSV, polyomaviruses, and adenoviruses. Cidofovir was used successfully in treating a 17-year-old boy who had received a stem cell transplant and had severe adenoviral gastroenteritis.[400] Ljungman and colleagues[302] conducted a study of cidofovir for the Infectious Diseases Working Party for Blood and Marrow Transplantation (EMBT). They demonstrated successful treatment of adenovirus infections in 69 percent of allogeneic hematopoietic stem cell transplant recipients, primarily with a 5-mg/kg dose of cidofovir given intravenously one time per week for the first 3 weeks and every other week after that, along with concomitant use of probenecid and intravenous fluid hydration. Other studies have shown a similar effect with reduced dosing of cidofovir at 1 mg/kg per dose given three times a week.[197,301,343] A case report by Fanourgiakis and colleagues[137] demonstrated successful treatment of severe (grade IV) hemorrhagic cystitis in a 34-year-old man by intravesical instillation of 5 mg/kg of cidofovir in 100 mL saline twice daily. This method avoided the known nephrotoxicity associated with cidofovir. Yusuf and coworkers[524] reported complete resolution of clinical symptoms in 57 of 58 pediatric hematopoietic stem cell transplant recipients treated with cidofovir. Wallot and colleagues[497] had some success using reduced-dose cidofovir, inhaled nitric oxide, and decreased immunosuppression to treat two liver transplant recipients with disseminated adenoviral disease and acute respiratory distress syndrome.

Further studies into possible immunotherapies for the treatment of adenovirus infections also have been investigated. Feuchtinger and associates[142] were able to perform successful and safe adoptive transfer of adenovirus-specific, T-cell immunity in five of six patients with documented adenovirus infection after allogeneic hematopoietic stem cell transplantation. This effect was found to be independent of the T-cell dose. Furthermore, the adenovirus-specific, T-cell expansion was sustainable, and proliferation of the adenovirus-specific T cells was unimpaired by adenovirus viremia, in contrast to the myelosuppressive effects seen with CMV viremia.

Teuchner and colleagues[461] studied *N*-chlorotaurine (NCT), a compound produced by monocytes and granulocytes during the oxidative burst, in a double-blind investigation of adult patients with epidemic keratoconjunctivitis. Among the group of 60 patients, 33 were treated with a 1 percent aqueous solution of NCT and 27 were treated with gentamicin. They analyzed both subjective and objective scores. In the total study group, the NCT-treated subjects were subjectively better than the gentamicin-treated patients at day 8, but there were no objective differences. However, in severe infections, both the subjective and objective scores were better on days 4 and 8 in the NCT-treated patients. They concluded that NCT treatment was well tolerated and that it shortened the duration of illness. A study of the use of 1 percent cidofovir eyedrops with and without 1 percent cyclosporine eyedrops to treat adenovirus-associated keratoconjunctivitis was conducted by Hillenkamp and colleagues.[218] This study demonstrated significant local toxicity in the skin of the eyelids and conjunctiva but a decreased frequency of severe corneal opacities associated with the use of cidofovir.

Human fibroblast–derived interferon, applied topically, was used to treat epidemic adenoviral keratoconjunctivitis in a comparative trial.[405] The duration of illness was reduced in the patients treated with interferon, but many of the control patients received dexamethasone. A child with combined immunodeficiency and severe, diffuse adenovirus type 7a pneumonia improved dramatically after receiving a large dose of high-titer adenovirus type 7a immune serum globulin.[93]

PREVENTION

Serious incapacitating epidemics caused by adenoviruses in military recruits in basic training led to the development of adenoviral vaccines.[9,289] The initial vaccines were formalin-inactivated preparations of monkey kidney tissue culture–grown virus and were administered parenterally.[8,186,215] These vaccines achieved only limited success because of variable degrees of potency in different vaccine lots.[23] An inactivated adenoviral vaccine that contained types 3, 4, and 7 was prepared in monkey tissue culture for trial in children.[484] Three doses of this vaccine resulted in high levels of neutralizing antibody to the three viral types, and this antibody persisted in most infants for at least 1 year. Many of the initial lots of inactivated adenoviral vaccine were found to contain live simian adenoviruses that were capable of producing neoplasms in suckling hamsters. Because of this finding, inactivated vaccine trials were discontinued. Next, a live, attenuated adenovirus type 4 vaccine that was cultivated in human diploid tissue culture was developed and administered orally by enteric-coated capsule to volunteers.[186] Asymptomatic gastrointestinal infection occurred, and a good serum-neutralizing antibody response was elicited.[186,439,443] Most recipients of live oral vaccine excrete virus in stool for several days to a month after being vaccinated. With military use of the vaccine, shedding in stool was not associated with transmission of the virus to non-immune contacts.[338] In other studies of married couples and families with children, virus was transmitted to non-immune contacts.[338,444] Transmission usually occurred without illness.[338]

The administration of live, enteric-coated, type 4 and 7 adenovirus vaccine resulted in a significant decrease in the incidence of ARD in military recruits.[121,176,456] Unfortunately, adenoviral vaccines no longer are available, and the incidence of ARD in

military recruits again is a significant problem.[18,181,272,417] Few attempts have been made to protect children with live adenoviral vaccines. In one study, live enteric-coated adenovirus type 4 vaccine was given to children aged 5 to 11 years. Asymptomatic gastrointestinal infection resulted from administration of the vaccine.

PROGNOSIS

The overall prognosis of adenoviral infection generally is excellent. Secondary bacterial complications, if untreated, can result in prolongation of illness and permanent sequelae in some instances. The prognosis of adenoviral infection in the very young and in immunocompromised patients must be guarded.

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SUBSECTION 4

Hepatoviridae

CHAPTER 169

Hepatitis B and D Viruses

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HEPATITIS B VIRUS

Hepatitis B virus (HBV) infection occurs throughout the world, although marked geographic differences in rates of both acute and chronic infection exist. An estimated 360 to 400 million individuals (approximately 5% of the world's population) are infected worldwide.[62,105] Fifty-five to 92 million people are expected to die between 45 and 55 years of age as a result of HBV-related chronic liver disease or primary hepatocellular carcinoma (HCC).[56] Most of those with chronic HBV infection acquired the virus through vertical transmission, which can be prevented by active and passive immunization.

BIOLOGY OF THE HEPATITIS B VIRUS

HBV is the prototype of a family of DNA viruses called *hepadnaviruses* (hepatotropic DNA viruses). This group consists of enveloped, coated DNA viruses with similar structure and genome organization. All primarily infect the livers of their respective hosts and can cause acute and chronic infections. HBV is the most studied member of the family, which also includes the woodchuck hepatitis virus (WHV), the ground squirrel hepatitis virus, the duck hepatitis virus (DHV), and hepatitis viruses found in herons, Ross geese, and arctic squirrels.[157] WHV and DHV share approximately 70 percent sequence homology with HBV but do not infect humans.[161] Use of these animal models has facilitated the study of hepadnavirus structure and replication by supplementing information obtained from infected humans and cloning studies.

Three types of particles are found in the sera of HBV-infected people and are shown in Figure 169–1: the Dane particle and two subviral particles, one spherical and the other filament shaped. All three types share a common surface antigen, hepatitis B surface antigen (HBsAg), but only one, the Dane particle, contains viral DNA and is capable of replication. Neither the filament, 20 nm in width and ranging from 50 to 1000 nm in length, nor the sphere, also 20 nm in diameter, contains nucleic acid. The Dane particle, or whole virion, is 42 nm in diameter and contains a core, or nucleocapsid, enclosing the DNA. The outer shell is composed of large amounts of hepatitis B surface proteins in a lipoprotein envelope, which is derived from host cells. Inside this shell is found the nucleocapsid, an icosahedral structure composed of 240 core protein subunits with regular penetrating channels. The nucleocapsid proteins are detected serologically as hepatitis B core antigen (HBcAg). This nucleocapsid is 25 to 27 nm in diameter, and within it are contained the viral genome and polymerase. HBV e antigen (HBeAg) is a soluble antigen produced from the same open-reading frame (ORF) that HBcAg is, but unlike core antigen, HBeAg is secreted into serum, where it is thought to have a role in induction of tolerance to HBV. The viral genome is composed of double-stranded circular DNA that can be relaxed or closed, depending on the stage in the reproduction cycle.[149] This DNA, which is 3.2 kilobase pairs in size, encodes four overlapping ORFs that overlap with other regulatory *cis*-acting sequences such as promoters, enhancers, and polyadenylation and genome packaging signals.

The subviral particles (spheres and filaments) are composed of envelope proteins and host-derived lipid components.[157] These particles are abundant in the serum of infected individuals (10^6 to 10^{14} particles per milliliter), and they are highly immunogenic and stimulate the production of neutralizing antibodies. One hypothesis is that they absorb neutralizing antibodies and thereby shield the intact virion from the host immune response. Subviral particles were used to produce the first effective HBV vaccine.[180]

Figure 169–1 The three forms of HBV surface antigen are whole virions, rods, and proteins. A cutaway view of the whole virion shows the three envelope proteins: major, middle, and large. (*From Mutchnik, M. G.: Acute and chronic hepatitis B.* In *Feldman, M., and Maddrey, W. C. [eds.]: The Liver. Philadelphia, Current Medicine, 1996.*)

MOLECULAR VIROLOGY

The viral genome, as noted previously, contains four ORFs in its circular DNA. The DNA strands are not completely symmetric; the minus strand is shorter, which leads to a region of single-stranded DNA. This gap can be filled by the virus's own polymerase when required. The four genes are *S* for the surface or envelope gene; *P* for the viral polymerase gene; *C* for the core gene, which encodes nucleocapsid protein; and *X* for the X gene. The surface proteins synthesized by HBV are termed the *large*, *middle*, and *major* proteins; they are type II transmembrane proteins that can form multimers. Transcription initiated at the *pre-S1*, *pre-S2*, or *S* epitopes leads to the formation of these three proteins. Although antibodies to S protein alone will provide protection against HBV infection, epitopes of *pre-S1* also can stimulate the production of neutralizing antibody.[106] A group of antigens used for subtyping HBV strains in epidemiologic studies is found in association with S proteins. Analysis of subdeterminants of HBsAg has revealed four major serotypes, *adw*, *adr*, *ayw*, and *ayr*, thus allowing differentiation of HBV infections from different sources. The "a" determinant is found in all isolates. Antibody to hepatitis B surface antigen (anti-HBs) is directed against this determinant and provides protection against all serotypes.

Seven HBV genotypes have been identified by the use of polymerase chain reaction (PCR)–restriction fragment length polymorphism analysis of the complete viral genome.[115] They have been designated A through G, and worldwide distribution varies. Type D occurs more commonly in Mediterranean regions, whereas type F occurs more commonly in Central and Latin America.[129] A study of 187 patients with chronic HBV infection revealed the following marked regional variations in genotype: north Europeans, 63 percent A; south Europeans and Middle Easterners, 96 percent D; Africans, 53 percent A, 27 percent D, 20 percent E; and east Asians, 14 percent A, 43 percent B, and 43 percent C.[116] The clinical differences among HBV genotypes are being elucidated. Infection with genotype C was found in Taiwanese adults to be associated with more severe liver disease and HCC in those older than 50 years, whereas genotype B was associated with HCC in those younger than 50 years.[90] An association of genotype with response to interferon-α (IFN-α) treatment also has been suggested.[91] A recent study from Taiwan analyzed the responses of 58 patients with chronic HBV infection, genotypes B and C, to treatment with IFN-α.[91] The patients with genotype C had higher aminotransferase values at baseline. After a 48-week follow-up period, only 15 percent of those with genotype C were HBeAg- and HBV DNA–negative, as compared with 42 percent of those with genotype B.

HBV polymerase consists of two major domains tethered by an intervening spacer region. Elucidating the structure and biochemistry of hepadnavirus polymerases has proved difficult because they require cellular factors, which are lost during extraction from cells, for enzymatic activity.[138] The ORF that encodes the *P* gene overlaps to a large extent with the core protein ORF; indeed, both proteins are translated from the same mRNA.[28]

The core gene encodes the viral capsid proteins that combine to form the core particles. The core proteins combine first into dimers; 120 dimers unite to form a shell 36 nm in diameter.[41] HBcAg appears to be phosphorylated within cells and becomes dephosphorylated upon export.[175] The main role of core proteins is enclosure of viral DNA, but they also play a role in cytolysis of infected cells when expressed on the cell surface.[111] Mutations in the core gene, the pre-core region, and the promoter for *C* have become clinically important.

Mutations affecting all ORFs of the HBV genome have been described. Most viral genomes carry more than one mutation, and most individuals are infected with more than one variant. Some of the mutations are thought to contribute to viral latency, low-level infection, severity of liver disease, and vaccine escape. The group best studied is the pre-core mutants, which result in lack of HBeAg production, even in the presence of active viral replication, the so-called HBeAg-negative infection. In contrast to the situation in patients with wild-type virus, in whom absence of HBeAg usually signifies absent HBV replication and mild liver disease (see later), in HBeAg-negative infection, HBV DNA levels are high, antibody to HBeAg (anti-HBe) is detected, and liver disease may be severe. Sporadic cases and outbreaks of ful-

minant HBV infection have been attributed to pre-core mutants. Mutations in the *S* or *pre-S* genes, which encode envelope protein, have been reported in patients infected after vaccination and in individuals who receive monoclonal antibody to HBV after undergoing liver transplantation. This variant contains a missense mutation in the *a* determinant of the surface antigen. Subsequently, these mutants were demonstrated in chronic HBV carriers even without these immune pressures. This mutation causes an infection in which HBsAg is undetectable but in which HBeAg and HBV DNA are found, in contrast to the typical serologic pattern (see later).

The role of X protein is not understood completely as yet, but it is required for establishment of infection in vivo, although not for transfection of cells in vitro.[12,37] X protein is involved in transcriptional activation during viral replication[111] and also has been shown to transactivate the promoters of other viruses such as human immunodeficiency virus (HIV) and human T-cell lymphotrophic virus type 1.[152]

VIRAL LIFE CYCLE OVERVIEW

Like retroviruses, hepadnaviruses replicate by means of reverse transcription of an RNA intermediate[177] (i.e., the flow of genetic information is from DNA to RNA to DNA.) As shown in Figure 169–2, after binding of the virus to receptors on the cell surface, virion nucleocapsids are delivered to the cytoplasm, where they translocate to the nucleus. There, the genomic DNA is converted to the cccDNA form (a nuclear pool of viral covalently closed circular DNA that serves as a template for reverse transcription). This form of DNA is transcribed by host RNA polymerase II, and the resulting RNA is translated to originate the *P*, *C*, *S/pre-S*, and *X* gene products. The viral pol initiates encapsidation of viral pregenomic RNA into cytoplasm by binding to the 5′ encapsidation signal, epsilon, on the pregenomic RNA.[122] Through a series of complex host-viral interactions, negative-strand DNA synthesis is initiated and completed, followed by plus-strand synthesis and circularization of the genome. The progeny cores then bud into intracellular membranes and acquire glycoprotein envelopes to form nucleocapsids. Nucleocapsids are exported from the hepatocyte cytoplasm toward the cell membrane, where they become invested with surface proteins bound in the membrane bilayer, thereby resulting in budding of mature virions into the bloodstream.

Although HBV can infect numerous tissues, including the spleen, kidney, peripheral blood mononuclear cells, and pancreas, the virus replicates exclusively in the liver. This is the sole site of replication because the tissue-specific viral promoter and enhancer are found in liver cells. No efficient tissue culture technique supports HBV infection. In the cell culture systems presently available, such as primary duck hepatocytes infected with DHV, immortalized human hepatoma cell lines such as HepG2 and HuH7, and chick LMH, the viral replicative cycle is not cytopathic. The infected hepatocytes are normal morphologically and display growth rates identical to those of uninfected controls.[11,154]

Viral Binding and Cell Entry

Studies of the early phases of the viral life cycle have been conducted primarily with DHV. The binding reaction has two components: one is of low affinity and is nonsaturable, and the second is of high affinity and saturable.[95] At 37° C, cell entry and viral infection occur. After binding, the virus must fuse with the host cell membrane. This phenomenon appears to occur by a pH-independent mechanism. The binding and entry processes are slow, with maximal infection occurring after 16 hours of exposure of the cell to the inoculum.[143] The pre-S proteins appear to be involved primarily in the cell fusion interactions.[95] Monoclonal antibodies to these proteins prevent the development of infection in primary duck hepatocytes.[95] The pre-S proteins also appear to determine the narrow spectrum of the viral host determination (i.e., if the virus entry process is bypassed by transfection into heterologous cells, viral replication of heron HBV is able to proceed normally in duck cells, which usually are not susceptible to this virus).

The cellular receptors for the hepadnaviruses are unknown. The hepatotropism of HBV infection is presumed to be caused by the presence of a specific receptor, although none has been identified.[161] Many proteins, such as apolipoprotein H[23] and endonexin 2 (a phospholipid-binding protein), bind to the HBV envelope glycoproteins, particularly S protein.[76] Receptors for pre-S proteins still have not been identified, and the biologic significance of these findings is yet to be determined. The mecha-

Figure 169–2 The virus enters the cell through a receptor or receptors, and the viral genome is imported into the nucleus, where transcription occurs. Pregenomic RNA encapsidation occurs in the cytoplasm. Reverse transcription leads to negative-strand and then positive-strand synthesis. The nucleocapsid undergoes assembly with hepatitis B surface antigen, followed by budding and secretion in blood endoplasmic reticulum. (*From Loomba, R., and Liang, T. J.: Novel approaches to new therapies for hepatitis B virus infection. Antiviral Ther.* 11:*1-15, 2006.*)

nisms by which viral DNA is delivered to the nucleus after entry also are unclear.

In the nucleus, viral DNA is transformed to cccDNA, a process that entails repair of the single-stranded gap, removal of the 5′-terminal structures, and covalent ligation of the strands.[2] Host mechanisms appear to contribute greatly to this process.[98]

Genomic Replication

The first step in HBV genomic replication is encapsidation of the genomic RNA template.[178] The pregenomic RNA is encapsidated into the core particle.[52] To package this small RNA portion into the capsid, both C protein and *P* gene products are required.[77] Encapsidation of polymerase occurs by binding of the P protein to the RNA that will be packaged.[6] Single-stranded pregenomic RNA is converted to partially duplex virion DNA through reverse transcription and elongations of the plus and minus strands.

Viral Assembly and Release

The 20-nm subviral particles are assembled between the endoplasmic reticulum and the Golgi apparatus.[83] These 20-nm particles contain predominantly S proteins, which are synthesized in the endoplasmic reticulum.[71] In addition, these particles may contain M subunits but generally do not have L proteins.[73] The assembly process is encoded totally in the S domain.[109] Assembled particles are transported through the secretory pathway and traverse the Golgi complex.[92,176] The S protein carries out the entire assembly process without the involvement of other viral proteins.[2] Therefore, subviral particles containing only envelope proteins are released. In vitro studies have shown that overexpression of L proteins gives rise to filamentous viral particles in the endoplasmic reticulum.[38] The overabundance of L, M, and S aggregates apparently is cytopathic to hepatocytes in vitro. Similar cytopathic features have been seen in human infections with HBV, yet the role of envelope protein expression in hepatocyte injury remains to be determined.[2]

Assembly of the Dane particle differs from assembly of the 20-nm particles in that all three proteins, L, M, and S, are present.[73] Studies of HepG2 cell lines transfected with HBV mutants have shown that no virus budding occurs if the envelope proteins are not present (mutations in *S*) and that for formation and release of virions, both L and S proteins are needed.[21] M proteins apparently are not necessary for viral assembly.

Viral Persistence

Nuclear cccDNA appears to be crucial for viral persistence. Hepatocytes that harbor this form of viral genome are the only ones that produce virus.[2] As mentioned earlier, the replicative cycle of HBV is not cytopathic to hepatocytes, and thus infected cells multiply normally. The cytoplasmic mechanism of reverse transcription, which produces the cccDNA and consequently delivers this product to the nucleus, must therefore be passed on to progeny cells. When HBV DNA is integrated into the cell chromosome, viral DNA may be rearranged, although whether this happens before or after integration is unclear. This rearrangement may lead to disruption of viral genes, especially the core and polymerase genes, whereas the coding regions and promoters for the envelope proteins remain intact.[151]

IMMUNOPATHOGENESIS

Individuals infected with HBV show tremendous variation in clinical and immunologic responses. HBV is not directly cytopathic, and the variability in liver damage is due to host immune responses. Although HBV preferentially infects hepatocytes and replicates therein, it can infect other liver cells, such as cholangiocytes, as well as cells in extrahepatic tissue, such as peripheral blood lymphocytes, pancreatic acinar cells, and cornea, spleen, thyroid gland, kidney, adrenal gland, and smooth muscle cells.[127]

Responses to HBV infection and the likelihood of chronic infection developing are dependent on both age at the time of acquisition and immune competence. Ninety percent of infants born to mothers who are HBeAg-positive become chronically infected with HBV as compared with only 5 percent of those infected as adults. The production of antibodies to pre-S and S antigens by B cells, as well as cytotoxic T-lymphocyte (CTL) responses, mediates recovery from acute HBV infection. Individuals who clear HBV have a strong polyclonal human leukocyte antigen (HLA) class I–restricted CTL response to multiple epitopes in the HBV envelope, nucleocapsid, and polymerase regions. This CTL response can be reactivated many years after clearance of all detectable evidence of HBV infection.[116]

Infants infected perinatally are at the highest risk for development of chronic HBV infection. During childhood, those infected as neonates characteristically have high circulating levels of HBV DNA but low disease activity, with very low rates of HBeAg seroconversion, either spontaneously or after treatment with IFN.[116] In children in Taiwan with chronic HBV infection, the HBeAg positivity rate is as high as 100 percent in infancy and 76 percent at 10 to 14 years of age.[31]

Researchers have offered numerous hypotheses why this immune tolerance develops in neonates. One theory proposes that transplacental passage of HBeAg induces $CD4^+$ anergy and, therefore, no CTL responses to HBeAg occur. However, HBeAg-specific T-cell proliferation eventually is detected in those who subsequently seroconvert, thus rendering clonal deletion an unlikely mechanism.[117] Defective interleukin-2 production has been demonstrated in children who have chronic HBV, although whether it is a cause or a consequence of chronic infection is not clear.[70]

The development of chronic infection is either a failure of host immune responses or a testament to the ability of HBV to evade that response. As the virus replicates within hepatocytes, viral antigens appear on the cell surface. Subsequently, CTLs directed against HBcAg infiltrate the liver and cause hepatocyte necrosis.[45] In addition, HBV interferes with the production of cytokines of the T_H1 response, especially IFNs, that would otherwise elicit class II major histocompatibility complex (MHC) antigen expression and enhance viral clearance. For reasons not fully understood, both the HBV-specific CTL response and the $CD4^+$ helper T-cell response are weak in chronic HBV infection. The high HBV viral load may lead to depletion of peripheral T-cell responses.[57] However, HBeAg, produced in large amounts in chronic HBV infection, and HBsAg share T-cell epitopes. HBeAg may enter the thymus and induce immune tolerance to both HBsAg and HBeAg by decreasing the production of HBV-specific CTLs. However, these cells can be reactivated in patients who undergo spontaneous or IFN-induced HBeAg seroconversion.[147] Soluble HBeAg itself may contribute to persistence of HBV inasmuch as some evidence supports the fact that HBeAg favors the production of T_H2 cytokines rather than T_H1 cytokines, which have a greater role in noncytolytic control of HBV.[57] Persistence of HBV infection also may be due to infection of extrahepatic tissue such as the kidney.[57]

Fulminant hepatitis is seen in less than 1 percent of infected individuals and has a high mortality rate. It is characterized by a brisk immune response and viral clearance. Those who survive clear HBV and do not have chronic HBV infection. A higher incidence of fulminant hepatitis occurs in patients infected with HBV mutants that do not produce HBeAg, thus suggesting either an increased rate of replication or lack of immune detection of the mutant HBV.[102]

CARCINOGENESIS

HCC is a recognized sequela of chronic infection with HBV; worldwide, most cases of HCC are linked to HBV infection. In Taiwan, the incidence of HCC in chronically infected individuals is 200 to 812 cases per 100,000 person-years as compared with 10 to 30 cases per 100,000 person-years in the general population. In those with liver cirrhosis secondary to chronic HBV infection, the incidence increases to 1000 to 5000 cases per 100,000 person-years (1% to 5% per year).[35] The lifetime risk for the development of HCC in a chronically infected person is estimated at 40 to 50 percent.[9] The risk of HCC developing after treatment remains unclear; if individuals are still HBsAg-positive after receiving treatment, they remain at risk and need follow-up measurements of alpha-fetoprotein and ultrasound examination. The age at which screening for HCC should commence and the frequency with which it should be performed have not been determined.[118]

In almost 100 percent of woodchucks chronically infected with WHV, HCC develops within 3 years.[100] Detection of integrated HBV sequences in both human HCC and animal models led to numerous hypotheses regarding the mechanisms that result in HCC. Current hypotheses center on integration of viral DNA into the host genome, which may be associated with activation of oncogenes, deactivation of tumor suppressor genes, or other genetic instability. The finding that HCC cells in an infected individual contain HBV DNA in discrete rather than random sites, indicative of clonal expansion of tumor cells, supports this theory. In general terms, the integrated HBV may affect genes locally by replacing or activating genes by insertional mutagenesis, or HBV may affect more distant genes by means of gene products that can be diffused.[144] Integration of HBV occurs at random sites on host hepatocyte chromosomes rather than at one fixed site. HCC tissue itself can contain a variety of chromosomal abnormalities, such as polyploidy, allelic imbalance, and translocations.[140] More than a dozen genes have been implicated in HCC, which not only suggests heterogeneity of genetic etiologic factors but also indicates that an accumulation of genetic mutations is required for HCC to develop.

Although many mutations are found in HCC, they can be clustered into three main pathways.[179] The first is that of tumor suppressor or DNA damage repair genes typified by *p53*. A mutation in *p53* is detected in approximately 30 percent of HCC tumors, although this figure varies in different geographic regions, being more common in China and less so in Europe. The rate of *p53* mutation is increased by exposure to aflatoxin.[140] A second pathway is that of cell cycle control, the *RB1* pathway. An example is the retinoblastoma gene, which is mutated in approximately 15 percent of HCC tumors.[201] The final pathway associated with transformation of a normal hepatocyte to a malignant one is the β-catenin and adenomatosis polyposis coli (*APC*) gene system, which is involved in signal transduction. The E-adherin gene, which produces a protein that forms complexes with β-catenin, frequently is mutated in HCC.[166]

Abnormalities in hepatocyte regulatory genes may be activated by the integration of HBV X protein (HBx). HBx can function as a transcription activator, protease inhibitor, and effector of cell signaling pathways. Two other HBV proteins, middle HBV surface protein and the large HBV protein, share similar properties.[144]

The frequency and significance of integration of viral DNA into the host genome are unresolved issues in childhood HBV infection; some studies have shown early integration, whereas others describe it as a rare event.[7,30,68,160,191,195] Although HCC is detected most often after at least 20 years of chronic HBV infection, cases in children as young as 8 months old have been reported.[66,181,194] Childhood HCC associated with HBV infection have been described in both Asian[32,181] and Western[66,142] populations. Because most cases are reported retrospectively, data regarding incidence are not available. No guidelines have been developed for prospective monitoring of children with chronic HBV infection for the development of HCC, although periodic measurement of serum alpha-fetoprotein levels and hepatic ultrasound are recommended in adults.

The genotype of HBV may be an indirect marker of the risk for development of HCC. Genotype C with TCC at codon 15 of the pre-core region is associated with an increased risk for the development of HCC in a Hong Kong population with cirrhosis.[27] Prevention of HBV infection and eradication of existing infection remain the most effective measures to prevent HCC. The 5-year survival rate in children with HCC is less than 30 percent despite combined modalities of treatment.[197]

EPIDEMIOLOGY

HBV infection occurs throughout the world, although marked geographic differences in rates of both acute and chronic infection exist.

Areas of high, intermediate, and low endemicity, as reflected by the number of carriers, have been identified. Highly endemic areas are those in which more than 8 percent of the population is infected. Examples include Southeast Asia, Africa, the Commonwealth of Independent States, and China. More than 70 percent of the world's population lives in these high-endemicity areas. The Middle East, Central and South America, and parts of Eastern Europe are examples of intermediate-endemicity regions, where infection is found in 2 to 7 percent of the population. The United States, Australia, and Western Europe are areas of low endemicity, with chronic infection rates of less than 2 percent of the total population.[188] In areas of low endemicity, acute infections occur more commonly in adults, and although these infections cause significant morbidity, they are less likely to result in chronic carriage than asymptomatic infections in children are.

In the United States, the prevalence of chronic HBV infection (HBsAg-positive) in the general population, based on the National Health and Nutrition Examination Survey III (NHANES III), is estimated at 0.42 percent. NHANES III was conducted from 1988 to 1994 and revealed that 5.5 percent of the U.S. population had evidence of either past or chronic infection with HBV.[132] Rates among ethnic groups varied, with non-Hispanic blacks having the highest rate at 12.8 percent, as compared with 2.8 percent in non-Hispanic whites and 4.8 percent in Mexican Americans. The strongest predictors of HBV infection were non-Hispanic black ethnicity, increased number of sexual partners, foreign birth, cocaine use, and having less than a high-school education.[132]

HBV may be transmitted vertically, horizontally, parenterally, or sexually. Vertical transmission of HBV occurs in the perinatal setting when an infected mother transmits the infection to her child. Horizontal transmission occurs among children in groups with moderate to high endemicity[51] who were not infected at birth. This form of transmission occurs in households of an infected family member, where other members acquire HBV without sexual or overt contact with contaminated body fluids. In adolescents and adults, infection is transmitted by contaminated blood or other body fluids through percutaneous or mucous membrane routes. Examples are intravenous drug use; occupational exposure to blood; sexual contact, especially in men who have sex with men; and nosocomial infections as a result of sharing contaminated equipment.[126]

In countries where HBV infection is highly endemic, perinatal or vertical transmission occurs very commonly, whereas in regions of low endemicity, sexual transmission occurs more commonly. If a mother is seropositive for both HBeAg and HBsAg,

the risk of transmission of HBV to her child is 85 to 90 percent without immunoprophylaxis.[5] The most common risk factors for acquisition of acute HBV in adults in the United States between 1982 and 1998 were heterosexual exposure to a person with hepatitis or having multiple sexual partners (27%), intravenous drug use (18%), occupational exposure (16%), men who had sex with other men (13%), and household contact (3.6%).[67] Approximately a third of people reported no risk factors during the study period.[67]

Within the United States, the number of reported cases of acute hepatitis B decreased 67 percent between 1990 and 2002 (21,102 to 8064 reported cases).[26] The largest decline (89%) occurred in children and adolescents: from 3.0 in 1990 to 0.3 per 100,000 in 2002.[26] HBV elimination strategies such as screening of pregnant women, universal immunization of infants, and catch-up immunization of older children are credited with the reduced incidence in children. Strategies to eliminate HBV are outlined in Table 169–1. An increase in 1999 through 2002 in incidence among men older than 19 years and women older than 40 years has been noted,[26] thus reinforcing the need for targeting those engaging in high-risk behavior.

The experience from Taiwan, the first country to implement mass vaccination against HBV, is encouraging. Since 1986, all newborns have been vaccinated against HBV. The program has been extended to other children and adults, which has led to a 10-fold decrease in the chronic infection rate.[34] After approximately 10 years, a significant decrease in the incidence of HCC in children was reported.[29] The proportion of infants and children with hepatitis B as a cause of fulminant hepatic failure (FHF) in Taiwan has decreased from 82 percent before mass vaccination to 57 percent, but HBV infection remains a major cause of FHF in infants.[33]

Researchers have estimated that only 45 percent of the expected 20,000 HBsAg-positive women giving birth in the United States between 1993 and 1996 were identified.[167] This low figure may be due to numerous factors: women likely to be infected often do not receive prenatal care,[165] or women may be tested but the information not conveyed to the place of birth or to the pediatrician caring for the child.[5] Attention to this aspect of care is extremely important because the reduction in rate of transmission from mother to child with the use of hepatitis B immunoglobulin (HBIG) and hepatitis B vaccine is 90 percent.[88]

HBsAg can be found in the breast milk of HBV-infected mothers. However, in studies performed in Taiwan and England, breast-feeding by HBsAg-seropositive mothers did not seem to increase the risk for neonatal acquisition of HBV infection significantly.[141] A study of 230 babies in China demonstrated that response rates to HBV vaccination and failure rates of HBV immunoprophylaxis in breast-fed babies were similar to those of bottle-fed babies.[190] Effective immunoprophylaxis should allow safe breast-feeding.

TABLE 169–1 Strategies to Eliminate Transmission of Hepatitis B Virus Infection in the United States

Universal vaccination of infants beginning at birth
Prevention of perinatal HBV infection through
Routine screening of all pregnant women for hepatitis B surface antigen (HBsAg)
Immunoprophylaxis of infants born to HBsAg-positive women and infants born to women with unknown HBsAg status
Routine vaccination of all previously unvaccinated children and adolescents
Vaccination of previously unvaccinated adults at increased risk for infection

From Mast, E. E., Margolis, H. S., Fiore, A. E., et al.: A comprehensive immunization strategy to eliminate transmission of hepatitis B infection in the United States: recommendations of the Advisory Committee on Immunization Practices (ACIP) part 1: Immunization of infants, children, and adolescents. M. M. W. R. Recomm. Rep. 54(RR-16):1-31, 2005.

NATURAL HISTORY

ACUTE HEPATITIS B

Acute HBV infection in the United States occurs most commonly in young men older than 19 years and in women older than 40 years,[48,67] but chronic disease does not develop in most people. The clinical and serologic findings in acute hepatitis B are shown in Figure 169–3. Acute hepatitis rarely develops in children infected perinatally, but FHF can develop, particularly in infants born to HBeAg-negative mothers.[33]

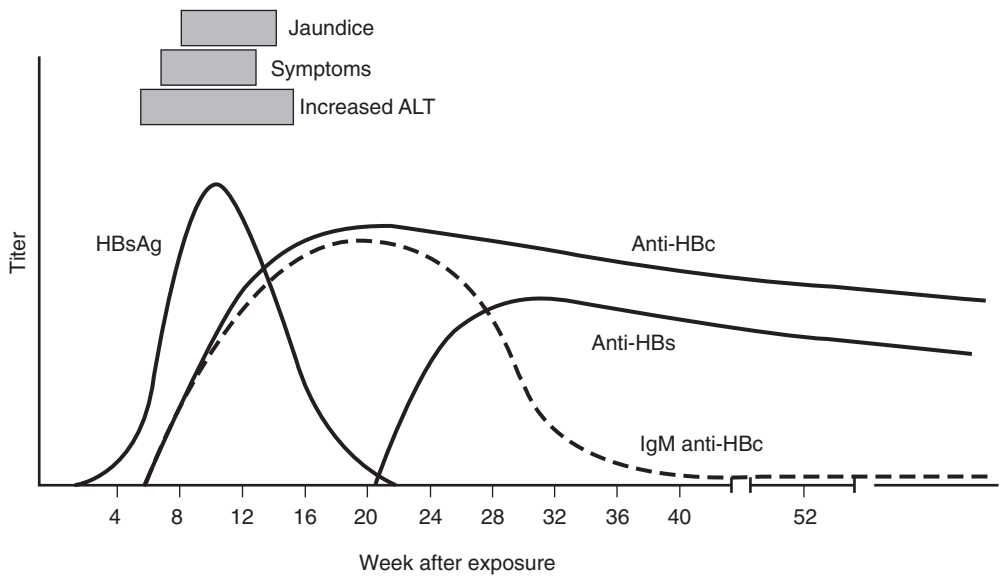

Figure 169–3 Clinical and serologic features of acute infection with hepatitis B virus. Hepatitis B surface antigen (HBsAg) is detectable within 4 weeks and reaches a peak value at 12 weeks. This coincides with the onset of jaundice. Hepatitis B e antigen (HBeAg) becomes detectable at the same time. Anti–hepatitis B core (Anti-HBc) IgM is detected from 6 to 8 weeks and peaks at 20 weeks. It then declines, but anti-HBc IgG persists. Upon resolution of acute infection, the serum contains anti-HBc, IgG type, and anti-HBs.

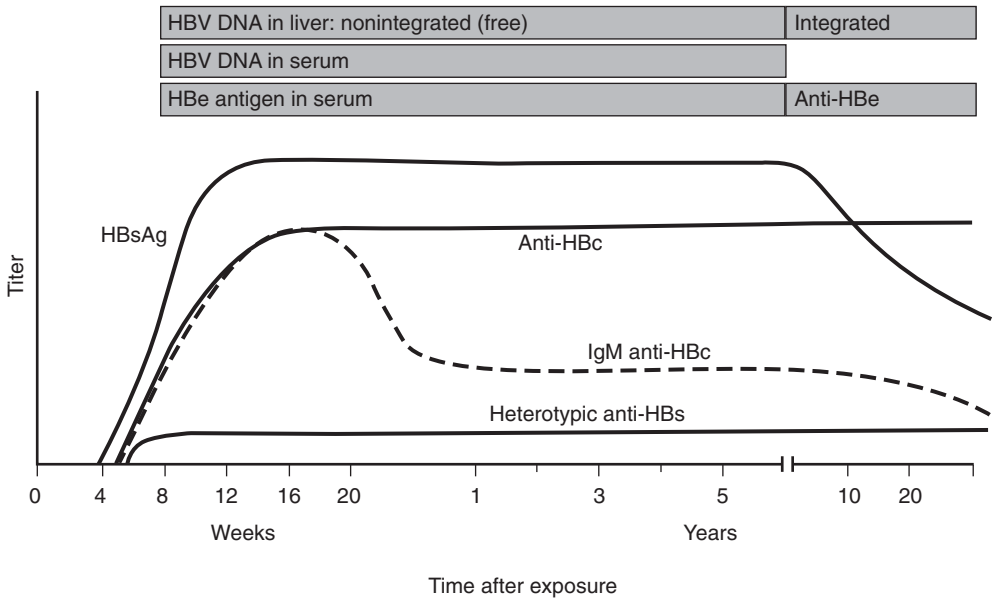

Figure 169–4 Clinical and serologic features of chronic hepatitis B virus (HBV) infection. Hepatitis B surface antigen (HBsAg), hepatitis B e antigen (HBeAg), and anti-hepatitis B core (HBc) are detectable and may persist for years. Seroconversion from HBeAg to HBe antibody is associated with very low levels of HBV DNA in serum. The diagnosis of chronic HBV infection is dependent on finding HBsAg for at least 6 months or the combination of HBsAg and anti-HBc of the IgG class.

CHRONIC HEPATITIS B

The natural history of chronic HBV infection in children has been examined in several populations. The clinical and serologic findings of chronic hepatitis B are shown in Figure 169–4. Most Chinese children, in whom chronic HBV usually is acquired perinatally, remain HBeAg-positive, with very high levels of viral replication, yet only minimal clinical liver disease.[119,120] In contrast, children in the West with chronic HBV infection frequently clear HBeAg and HBV DNA from serum during the first 2 decades of life.[18] In an extended follow-up lasting 14.5 ± 6.1 years of 89 white children in Italy after seroclearance of HBeAg, 4 experienced reactivation, 3 of whom had HBeAg-negative hepatitis.[19] Those who lose HBeAg tend to have higher alanine transaminase (ALT) levels early in life, thus indicating more active liver disease. Some of the children (15.4%) become seronegative for HBsAg as well.[19] Reactivation of viral replication is observed only rarely. Children born to Asian and Afro-Caribbean mothers in the United Kingdom who acquired HBV infection perinatally had a much lower rate (29%) of HBeAg seroconversion than did white children (80%)[20] or Italian children (98%).[19] These differences are ascribed to the older age at acquisition of HBV in the Western children, which is associated with a more efficient immune response. These observations influence the management and counseling of children with chronic HBV infection, as well as the design and interpretation of therapeutic trials.

The risk of acquiring chronic hepatitis B is related to a number of factors such as very young age, other medical conditions, and the presence of other infections. If an infected person has other medical problems, such as end-stage renal disease requiring hemodialysis, or is co-infected with HIV, the risk for development of chronic HBV infection increases. The risk for development of HCC is increased in all HBV chronic carriers, especially those with active viral replication.[9] Four of 99 Italian children had HBeAg-positive cirrhosis at the start of the study, and after 14.5 ± 6.1 years of follow-up, HCC had developed in 2, and 2 had had lost the histopathologic features of cirrhosis.[17,19]

Extrahepatic manifestations of both acute and chronic HBV infection occur more commonly in adults. As many as 25 percent of adults with acute HBV infection have extrahepatic manifestations such as arthralgia and even serum sickness–like reactions. Vasculitides can develop in patients with both acute and chronic HBV infection, and examples include polyarteritis nodosa, renal disease, and mononeuritis.[131] Membranoproliferative glomerulonephritis is an extrahepatic manifestation of chronic HBV infection in some children.[94,173] A case of epididymitis related to acute hepatitis B in a 12-year-old boy has been reported.[182]

HISTOPATHOLOGIC FEATURES

The purposes of obtaining a liver biopsy specimen from patients infected with HBV are to quantify the severity of changes, rule out other causes of liver disease, and allow a comparison of changes after treatment. In patients with typical acute viral hepatitis, obtaining a liver specimen usually is not required or indicated. If performed, the findings are liver cell degeneration and necrosis, with lobular disarray. The acute injury to hepatocytes is manifested as both ballooning and acidophilic degeneration, either of which can resolve or progress to cell death. Inflammatory cell infiltration and liver cell regeneration are also seen.[80]

The histopathologic changes of chronic HBV infection are characterized by portal tract inflammation, interface hepatitis, and fibrosis, which may progress to architectural distortion of lobules and eventually to cirrhosis.[80] Chronic HBV is characterized by "ground-glass cells" (Fig. 169–5A), or hepatocytes containing HBsAg, most easily identified by staining with orcein, Victoria blue, or aldehyde fuchsin. However, these cells also are found in other liver diseases, and the pattern of staining is important. In hepatitis B, ground-glass cells are found either singly or in clusters arranged in a haphazard pattern. "Sanded" liver nuclei are caused by accumulation of HBcAg and generally are identified by electron microscopy[85] or immunoperoxidase techniques (Fig. 169–5B).

Histopathologic staging and grading systems are used to allow standardization and meaningful comparisons in chronic hepatitis. One such system is the Knodell-Ishak score, which evaluates four histopathologic features, each scored with specific criteria.[96] This system has proved reproducible, is now in common use, and also is known as the *modified histologic activity index* (HAI). Other systems are the Batts-Ludwig system[8] and the METAVIR system.[153] Systematic classifications are useful in estimating progression of disease and response to therapy and for comparing

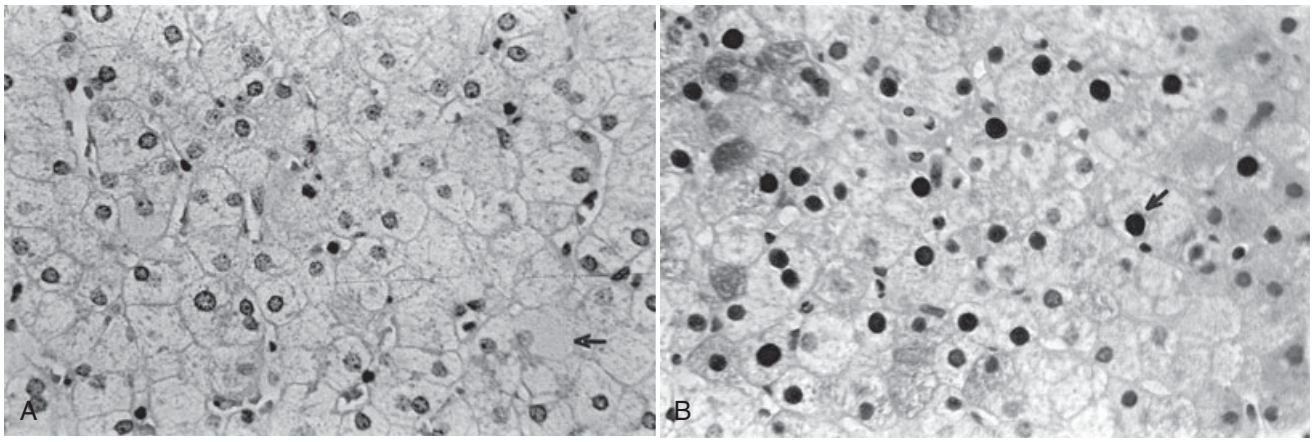

Figure 169–5 **A,** Photomicrograph of a liver biopsy specimen from a child with chronic hepatitis B virus (HBV) infection. Ground-glass hepatocytes (*arrow*) are demonstrated (hematoxylin and eosin stain; original magnification ×20). **B,** Photomicrograph of the liver of a child with chronic HBV infection. The darkly stained nuclei (*arrow*) contain hepatitis B core antigen (HBcAg) (immunoperoxidase stain for HBcAg; original magnification ×20).

outcomes of therapeutic trials. A study from Poland demonstrated that these scores were useful in comparing liver biopsy findings in children before and after they received IFN-α treatment and that inflammatory but not fibrosis scores improved after treatment.[168] However, another study demonstrated interobserver variability in grading and staging of pediatric liver biopsy specimens.[192] Clinicians must be aware of the limitations of liver biopsy reports and that description rather than scoring alone is important.[192]

Serum markers of hepatic fibrosis, such as apolipoprotein A-I, haptoglobin, and α_2-macroglobulin, have been investigated in children. They present many theoretical advantages for noninvasive monitoring of patients, but no single marker has proved useful yet in children with chronic HBV infection.[110]

TREATMENT

Treatment of acute hepatitis B is purely supportive, and most patients recover fully. The role of antiviral therapy in fulminant HBV infection has not been studied systematically. Treatment, when indicated, is directed at those with chronic HBV infection. In this setting, the long-term goals of therapy are eradication of HBV and improvement or regression of liver disease. Other goals of treatment include cessation or decrease in viral replication as indicated by loss of HBeAg and HBV DNA from serum, clearance of HBsAg, normalization of liver histopathology, and normalization of transaminase values.

To date, no agents fulfill all these goals in either adults or children. Children and adults have differences in immune tolerance and rates of progression of liver disease. Therefore, studies of treatment of chronic HBV in adults cannot be extrapolated directly to children. In addition, the timing and choice of therapeutic agents are crucial because of differences in age, maturity, stage in natural history, and co-morbid conditions.

A child being considered for treatment should have serologic evidence of HBV infection, such as HBsAg, for at least 6 months, as well as evidence of active HBV replication: HBeAg, or HBV DNA (in case of HBeAg-negative HBV infection). In addition, children with consistent elevation of ALT to at least twice the upper limit of normal are most likely to benefit from treatment. Liver biopsy should be performed before the start of therapy to provide evidence of chronic hepatitis, to stage the disease, and to rule out other processes. In most studies conducted before the high prevalence of HBeAg-negative infection, seroconversion from HBeAg to anti-HBe in serum was used as the primary outcome variable for response to therapy. Therapeutic trial results must be compared with the "background rate" of spontaneous conversion from HBeAg positivity to anti-HBe, which in children younger than 3 years old is very low (<2% per year) but increases with age such that in children older than 6 years old, yearly clearance rates vary from 14.3 to 35.3 percent.[31] Two medications are approved for the treatment of chronic HBV in children, IFN-α and lamivudine. IFN-α first was reported as a successful treatment of chronic HBV in 1976,[69] and recombinant IFN-α-2b was approved by the U.S. Food and Drug Administration in 1992 for adults and in 1998 for children with chronic HBV. Lamivudine is a nucleoside antiviral agent and was the first oral agent approved for HBV in adults and children. Adefovir dipivoxil was recently approved for children age 12 years and older.[86a] Table 169–2 summarizes published trials to date in children.

INTERFERON

The IFNs are a family of cytokines with immunomodulatory, antiproliferative, and direct antiviral actions. The three types are α, β, and γ. IFN-α has been the primary form used for the treatment of chronic HBV infection. It induces the display of HLA class I molecules on hepatocyte membranes, which in turn promotes lysis by $CD8^+$ CTLs. At the same time it directly inhibits viral protein synthesis.[111] IFN-α is available in several forms, including IFN-α-2a, IFN-α-2b, and lymphoblastoid IFN-α. Only IFN-α-2a and -2b have been licensed in the United States for use in children. Pegylated IFN-α-2a has not been studied or licensed yet for use in children with chronic HBV.

IFN-α treatment led to loss of HBV DNA or HBeAg seroconversion in 20 to 58 percent of European children as compared with 8 to 17 percent of control children.[187] Success rates are not as high for treatment of children in Asian countries, most of whom were infected perinatally; only 3 to 17 percent cleared HBV DNA or underwent HBeAg seroconversion.[104] These differences in treatment responses are thought to be related to age at the time of development of infection. However, in a large multinational, randomized control trial of IFN-α-2b, no differences were found in loss of HBsAg between children born in Asian countries (22%) and those from Europe and North America (26%) if transaminase values were elevated.[171] If only children with elevated transaminases are selected for treatment with IFN-α, children younger than 13 years with intermediate HBV DNA levels are most likely to have a good response, regardless of ethnicity.[171]

TABLE 169–2 Summary of Therapeutic Trials in Children

Author, Year	Treatment Regimens	HBeAg-Negative 6 Months after Therapy	HBV DNA–Negative 6 Months after Therapy
Barbera, 1994[4a]	Group 1 (n = 21): IFN-α-2a, 7.5 MU/m^2 Group 2 (n = 19): IFN-α-2a, 3 MU/m^2 Controls (n = 37): No treatment	Group 1: 5/21 Group 2: 4/19 Controls: 3/37 $p < 0.05$, group 1 vs 3	Group 1: 5/20 Group 2: 4/19 Controls: 7/37 NS
Sokal, 1998[171]	Treatment (n = 72): IFN-α-2b Control (n = 77): No treatment	Treatment: 25/72 Controls: 8/74 $p < 0.05$	Treatment: 27/72 Controls: 8/74 $p < 0.05$
Gurakan, 2000[71a]	Group 1 (n = 15): IFN-α-2b, 5 MU/m^2 Group 2 (n = 15): IFN-α-2b, 10 MU/m^2	Group 1: 5/15 Group 2: 9/15 $p < 0.05$	Group1: 6/15 Group 2: 9/15 $p < 0.05$
Giacchino, 1995[65]	Group 1 (n = 16): Human lymphoblastoid IFN-α preceded by 4 wk of prednisolone, 0.6 mg/kg/day Group 2 (n = 19): IFN-α preceded by placebo	Group 1: 7/16 Group 2: 0/19 NS	Group 1: 10/11 Group 2: 11/19 NS
Jonas, 2002[87]	Treatment (n = 191): Lamivudine daily × 52 wk Controls (n = 95): Placebo daily × 52 wk	Treatment: 50/191 Placebo: 14/95	Treatment: 117/191 Placebo: 15/191 $p < 0.001$
Sokal 2006[172]	Treatment (n = 213): Lamivudine daily × 52 wk, all HBeAg-positive (133 previously treated with lamivudine × 52 wk) Observation (n = 63): Previously treated with lamivudine × 52 wk, all HBeAg-negative	Treatment: 51/213 Control: 48/54 (sustained response 52 wk after end of treatment)	As before
Dikici, 2001[49a]	Group 1 (n = 30): IFN-α-2b and lamivudine Group 2 (n = 27): Same medications but for 12 months	Group 1: 11/30 Group 2: 15/27 NS	Group 1: 29/30 Group 2: 26/29 NS
D'Antiga, 2006[43]	Pilot study (n = 23) Lamivudine, 3 mg/kg daily alone × 8 wk, then lamivudine, 3 mg/kg daily × 48 wk, and IFN-α, 5 MU/m^2 3/wk × 40 wk	5/23 anti-HBe	18/23
Jonas, 2008[86a]	Group 1 (n = 113): adefovir Group 2 (n = 57): placebo	Group 1: 18/113 Group 2: 3/57	Group 1: 12/113 Group 2: 0/57

HBeAg, hepatitis B e antigen; IFN, interferon; NS, not significant.

Higher doses of IFN-α (10 MU/m^2) are not associated with higher clearance rates, although treatment for 6 months seems to improve outcome.[187] In the multinational, randomized, controlled trial referenced previously, children received 6 MU/m^2 of IFN-α-2b three times a week for 6 months.[171] However, the dose of IFN-α-2b had to be reduced because of bone marrow suppression or fever in 23 percent of children. In this study, serum HBeAg and HBV DNA became negative in 26 percent of treated children and 11 percent of controls. In children who responded to therapy, liver histology improved, and serum transaminase values normalized.[170] Prednisone priming (i.e., a course of steroids immediately before the administration of IFN-α) has been proposed to induce an acute exacerbation of hepatitis B, thereby rendering the patient more susceptible to IFN. However, a dual-center, double-blind, randomized trial of lymphoblastoid IFN-α, with or without steroid pretreatment, showed no improvement in the rate of seroconversion of HBeAg to HBeAb over that of IFN-α alone.[65]

Reported studies of IFN-α included only children older than 18 months without hepatitis C and HIV co-infection because such co-infection may alter treatment algorithms. Children with evidence of active immunologic responses to HBV (i.e., low levels of viral replication [HBV DNA] and high ALT values [greater than twice normal]) are more likely to have a response to therapy with IFN-α.[185] Children who acquired disease by vertical transmission are more likely to have established immune tolerance and may be less likely to respond to treatment. Children with very low HBV DNA levels may be in the process of spontaneous seroconversion; therefore, an expectant policy may be prudent.

Ten percent of treated adults and children lose HBsAg as compared with 1 percent of untreated patients.[101,171] Long-term (1.1 to 11.5 years) follow-up of adult responders to IFN-α showed a significantly reduced incidence of HCC in comparison to either treated nonresponders or control patients.[114] The long-term outlook for treated children is not clear. In a Turkish study, neither cirrhosis nor HCC had developed in 23 treatment nonresponders at a mean follow-up of 4.5 years.[97] A second study compared long-term (5.6 ± 3.1 years) outcome in two groups of 37 children, one group treated with IFN-α-2b and a second control group of children matched for age, sex, and baseline ALT level who received no treatment. At the time of follow-up, rates of HBeAg and HBsAg loss were 54.1 percent (20/37) and 8.1 percent (3/37), respectively, in treated children versus 35.1 percent (13/37) and 2.7 percent (1/37), respectively, in untreated children (not significant). Children with ALT levels elevated more than twice normal who were treated had higher rates of HBeAg seroconversion at follow-up than did children with elevated ALT who were not treated.[180] A study from Sweden demonstrated that pretreatment HBV DNA levels were associated with virologic response.[169] Histologic outcomes in children with chronic HBV treated with IFN-α for 20 weeks have been reported in a study of 93 children with an average age of 7.1 years in Poland. Liver biopsy specimens were obtained before treatment and 12 months after the end of the treatment and graded for fibrosis and inflammation with three different scoring systems. Thirty-five children underwent HBeAg/anti-HBe seroconversion, and 58 did not respond. Liver fibrosis did not improve regardless of responder status, but inflammatory activity was decreased after treatment in both responders and nonresponders.[192]

Side effects of IFN-α include a transient influenza-like syndrome (fever, myalgia, headache, arthralgia, and anorexia) that occurs in virtually all patients at the start of therapy. Bone marrow suppression, especially neutropenia, is a common side effect and was seen in 39 percent of children in one series.[84] Changes in personality, irritability, and temper tantrums are reported more frequently in children than in adults.[84,171] These problems resolve once treatment is withdrawn. Epistaxis not associated with

thrombocytopenia or a prolonged prothrombin time, febrile seizures, and marked elevation of transaminases have been reported. The death of a 5-year-old girl with chronic hepatitis B within hours of administration of the first dose of IFN-α-2a, which may have been related to a hypersensitivity reaction, has been reported.[93] Quality of life, as compared with their pretreatment levels, is impaired in children while they are receiving IFN therapy because of medication side effects and fear of injections. However, within 3 months of cessation of treatment, these effects are reversed.[84] Therapy in children seldom is discontinued because of side effects or inability to administer the medication. Two meta-analyses of treatment of chronic HBV in children with IFN-α have been conducted.[185,186] Each report concluded that treatment leads to a higher rate of HBeAg seroconversion than would occur spontaneously. Randomized controlled trials may be analyzed for "number to treat" (i.e., the number of children requiring treatment to have a therapeutic response in one patient). If only children with ALT values twice normal are considered, 2.5 children need to be treated for 1 to clear HBV DNA. However if all infected children are treated, 7.1 children would require therapy to clear HBV DNA from 1 child.[185] Approximately two thirds of children treated with IFN-α show no sustained response to therapy; hence, more effective and better-tolerated treatments are required.

HBV POLYMERASE INHIBITORS

This class of agents includes nucleoside and nucleotide analogues that inhibit HBV polymerase by incorporation into viral DNA, which leads to termination of the chain. They are administered orally and generally are well tolerated.[122]

Lamivudine

Lamivudine (2′,3′-dideoxycytosine), also known as 3′TC, is an oral nucleoside analogue. It is triphosphorylated intracellularly to an active intermediate that is incorporated into the growing DNA chain, thereby terminating the chain and inhibiting viral replication. Two large, randomized, controlled studies of lamivudine as initial treatment of chronic HBV infection in adults showed serologic, biochemical, and histologic evidence of benefit.[49,103]

A number of studies have demonstrated the benefit of lamivudine in children. The first was a double-blind, placebo-controlled, randomized, multicenter study of lamivudine given for 52 weeks to 191 children aged 2 to 17 years at a dose of 3 mg/kg/day (maximum dose, 100 mg) and placebo given to 97 children. All children in the study were HBeAg-positive and had detectable HBV DNA and elevated ALT values. Twenty-three percent (44/191) of those treated with lamivudine cleared HBV DNA and HBeAg as compared with 13 percent (12/97) in the placebo group ($p < 0.05$). Unfortunately, YMDD mutants developed in 19 percent (31/166). Higher response rates were observed in those with higher ALT levels, as had been noted with IFN. In an open-label study of lamivudine in 29 adolescents and young adults with maternally transmitted chronic HBV infection in Taiwan, only those with ALT greater than five times the upper limit of normal demonstrated a better response (undetectable HBV DNA, normal ALT, and HBeAg/anti-HBe seroconversion) than did control patients after 52 weeks of treatment.[134]

The optimum duration of lamivudine therapy was investigated in a multicenter, open-label study of prolonged therapy in children[172] who had participated in the previous 1-year study of lamivudine.[87] Children were enrolled into one of two groups on the basis of their virologic status at week 48 of the original study. Two hundred thirteen children were HBeAg-positive; 134 had received lamivudine and 79 placebo. All the children were offered lamivudine for a further 2 years. At the end of the study, 51 more children had achieved a virologic response; however, YMDD mutant HBV had developed in 100 of 173. The virologic response in those with YMDD mutants was only 5 percent (5/100), as opposed to 49 percent (34/70) in those with wild-type HBV. Sixty-three children who were HBeAg-negative (49 previously treated with lamivudine and 14 with placebo) at the start of the study were observed for 2 more years. At the end of the study period, 48 of 54 (89%) had a durable virologic response.

However, prolonged treatment increases the risk for emergence of mutation in the HBV polymerase gene at the YMDD locus. This mutation arises because of a methionine-to-valine or a methionine-to-isoleucine switch in the C domain of the HBV polymerase.[184] YMDD variants of HBV are associated with reemergence of active hepatitis or decompensation of chronic liver disease. Once lamivudine therapy is stopped, wild-type virus reappears.[60] Based on the observation that both therapeutic response and development of resistant mutants increase in frequency with duration of treatment, the optimal duration of lamivudine therapy has not been defined. In fact, this agent largely has been replaced in adult HBV treatment by newer drugs with more favorable resistance profiles.

Selecting children most likely to benefit from treatment with lamivudine remains a challenge. Children most likely to have a sustained virologic response to lamivudine are those with HBeAg-positive chronic hepatitis and transaminases twice the upper limit of normal. If YMDD mutants emerge, lamivudine should be discontinued and liver enzymes need to be monitored. In patients with advanced liver disease, alternative therapy may need to be provided. No serious side effects of lamivudine were reported in children.[87] Lamivudine is excreted by the kidneys, so the dose should be reduced in patients with renal failure. Because lamivudine is tolerated so well, many patients, including those with advanced liver disease, in whom IFN is contraindicated, can be considered for treatment. Researchers have suggested that the medication should be continued until HBeAg disappears from serum, anti-HBe appears, or if neither occurs, HBV DNA becomes persistently undetectable.

Upon cessation of therapy in adults, 25 percent of treated patients had serum ALT levels elevated up to three times baseline, as compared with 8 percent of placebo-treated patients. This "lamivudine withdrawal flare" may progress to jaundice and incipient liver failure, as noted in 2 of 41 patients in a Dutch study.[78] Therefore, monitoring patients after a course of lamivudine is important.

Combination of Interferon-α and Lamivudine

Preliminary studies of combination therapy with IFN-α and lamivudine have been performed in both children and adults. In adults with HBeAg-positive chronic HBV, pegylated IFN-α-2a, either alone or in combination with lamivudine for 48 weeks, was superior to lamivudine alone.[107]

A pilot study of combined IFN-α and lamivudine in children with perinatally acquired chronic hepatitis B was reported recently from the United Kingdom. It was an open-label, single-treatment arm, single-center study. IFN-α was used, and the children selected were regarded traditionally as poor responders to treatment. Twenty-three children, all infected in the first year of life, HBeAg-positive, and with normal transaminases, were treated with lamivudine alone for 8 weeks, followed by lamivudine plus IFN-α, 5 MU/m^2 three times weekly for 10 months. Five of 23 seroconverted to anti-HBe, and 4 of these 5 became HBsAg-negative and HBsAb-positive. In no child did a YMDD mutant develop, and most (78%) became HBV DNA–negative. A side effect of treatment was a decrease in weight and height increments in comparison to study entry, and the difference remained significant for height 12 months after the end of treatment.[43]

Pegylated IFN-α-2a has not been studied in children with chronic HBV.

Other agents that have been demonstrated to be effective in adults with chronic HBV infection are peginterferon, adefovir, entecavir, telbinidine, and tenofovir. A randomized placebo-controlled trial of adefovir in children with chronic HBV also has been reported.[86a]

Adefovir

Adefovir is a synthetic nucleotide analogue of adenosine monophosphate.[200] It suppresses HBV in both HBeAg-positive and HBeAg-negative adults, and drug resistance is lower and emerges later than with lamivudine. Adefovir dipivoxil has been demonstrated to decrease HBV DNA and transaminase levels with prolonged treatment (144 weeks) in adults with HBeAg-negative chronic hepatitis B without HBsAg seroconversion.[72] When treatment was withdrawn in one subgroup after 48 weeks, the virologic and biochemical benefits were lost, thus suggesting that long-term treatment is required for viral suppression in those with HBeAg-negative chronic hepatitis B. Unlike lamivudine, prolonged administration of adefovir led to infrequent development of resistance (5.9% after 144 weeks). Severe adverse events occurred infrequently, with elevation of serum creatinine being the most serious, and elevated ALT levels also were reported. The most commonly reported events were headache, abdominal pain, and pharyngitis in one study[72] but not noted in another.[200] In a double-blind randomized trial of 480 HBeAg-positive adults in China, suppression of HBV DNA was observed in those receiving adefovir, 10 mg daily for 52 weeks, even if they had wild-type or YMDD mutant infection.[200] Adefovir was recently approved for use in children.[86a]

Entecavir

Entecavir is a cyclopentyl guanosine analogue that inhibits HBV polymerase.[122] Treatment with entecavir for 48 weeks suppresses HBV DNA viral loads in both HBeAg-positive and HBeAg-negative adults at higher rates than occurs with lamivudine.[117] Entecavir is of use in adults with lamivudine-refractory, HBeAg-positive, chronic hepatitis B. A dose of 1 mg led to improvement in histology, reduction in viral load, and normalization of ALT when compared with lamivudine after 48 weeks of treatment.[163]

A number of other nucleotide and nucleoside analogues that are in trials include emtricitabine, which is active against both HIV and HBV and is in phase III trials, and tenofovir, which also is at phase III levels.[122]

HEPATITIS B IN SPECIAL POPULATIONS

HBV AND HIV CO-INFECTION

The prevalence and course of hepatitis B in adults who are co-infected with HIV has been examined in numerous studies. Of 181 HIV-infected adults in Greece, 71.8 percent of the men who have sex with men and 91.7 percent of intravenous drug users had evidence of HBV infection.[50] In a study from Australia conducted between 1985 and 1989, men with HIV infection were more likely to have chronic HBV infection than were HIV-negative men.[13] No evidence has been found of direct interaction between HBV and HIV. Individuals co-infected with HIV and HBV have higher circulating levels of HBV DNA but not worse hepatic necro-inflammation.[39] Spontaneous seroconversion of HBeAg to anti-HBe rarely occurs in this group. Special considerations for treatment and immunization are discussed in the appropriate subsections.

HEPATITIS B IN SOLID ORGAN TRANSPLANT RECIPIENTS

Chronic HBV infection became a relative contraindication to liver transplantation after initial poor results. In the 1970s and 1980s, more than 80 percent of patients became re-infected quickly, and 55 percent died within 60 days of undergoing surgery. In patients who had recurrence of HBV infection after liver transplantation, a characteristic histologic lesion, termed *fibrosing cholestatic hepatitis*, developed and eventually led to loss of the allograft.[10] The demonstration in the 1990s that HBIG is efficacious in decreasing infection of the allograft has allowed successful transplantation. Long-term use of HBIG decreases the risk of recurrence to 30 percent at 12 months and increases patient survival rates to greater than 90 percent 12 months after transplantation.[156] Lamivudine has been added to HBIG after transplantation. In a study from China of 69 patients with chronic HBV infection who received liver transplants and treatment to prevent re-infection, 10 died less than 3 months after receiving their transplants.[196] Of the remaining 59 patients, 8 were not included in follow-up because they did not receive treatment with HBIG and lamivudine, which left 51 patients in the final analysis. The mean follow-up was 14.1 months. Two of the 51 had recurrent disease; 1 died and the other cleared the infection.[196] The allograft re-infection rate is even lower if the patient does not have active HBV replication at the time of transplantation, which is why lamivudine has been added in the pre-transplant period. The cost of HBIG is a major factor, and the duration of therapy appears to be lifelong to maintain a protective HBs titer.

HBV infection is the fourth most common cause of acute liver failure in adults in the United States.[139] Rarely does a child with hepatitis B need a liver transplant for either acute or chronic liver failure.[174] Of 215 children who underwent transplantation between 1986 and 1992 at a major French center, only 4 had HBV-associated disease.[125]

The course of HBV infection in heart, lung, and kidney transplant recipients was examined in a French study. Of 874 heart transplant recipients, 69 were infected with HBV, most through nosocomial infection; chronic HBV infection had little impact on 5-year survival.[124] In a group of 120 lung transplant recipients in Israel, 11 patients who either had chronic hepatitis B or received an organ from an HBcAg-positive donor were treated with lamivudine prophylaxis. The drug was tolerated well, and resistance developed in only one patient, but it responded to a change to adefovir.[164] In a study from Taiwan, overall patient and allograft survival after kidney transplantation were not affected by HBV infection despite increased hepatic morbidity.[82] Of the 113 patients who received a kidney transplant between 1986 and 1998, 20 were HBsAg-positive and 9 were positive for both HBsAg and hepatitis C virus (HCV) antibody. Of the 20 who were infected with HBV alone, FHF developed in four, who died within 2 years of undergoing renal transplantation; cirrhosis developed in 2; and HCC developed in 2 others.[82] Overall, however, 5-year survival was not dissimilar in those infected with HBV and those not infected. Lamivudine and mycophenolate mofetil have been used in combination with some success in renal transplant recipients with chronic hepatitis B.[108]

The child most likely to be treated successfully with today's agents is one who acquired HBV beyond infancy, has evidence of immune responsiveness to HBV, and has mild to moderate inflammatory changes on liver biopsy. The child ideally has no other medical problems, and the child and family can comply with the regimen and monitoring.

Even if the child has some success with treatment but remains HBsAg-positive, the child is still at risk for the development of HCC or reactivation to HBeAg-negative hepatitis. We would suggest yearly follow-up of virologic response and ultrasound. If

the child is HBsAg-negative after treatment and HBV still can be found in the liver, a risk for development of HCC remains but is presumed to be lower than if still HBsAg-positive. Therefore, follow-up should be considered.

IMMUNOPROPHYLAXIS

A comprehensive strategy to prevent HBV infection, both acute hepatitis B and the sequelae of chronic HBV infection, must eliminate transmission that occurs during infancy and childhood, as well as during adolescence and adulthood. Transmission of HBV cannot be prevented by vaccinating only the groups at highest risk for infection. Routine visits for prenatal and well-child care can be used to target hepatitis B prevention (Table 169–3). A comprehensive immunization strategy to prevent transmission of HBV is recommended by the Centers for Disease Control and Prevention (CDC). This strategy has a number of parts as detailed in Table 169–1.

Two types of products are available for prophylaxis against HBV infection. HBIG provides temporary (3 to 6 months) protection by means of passive immunity and is indicated only in certain postexposure settings. Hepatitis B vaccine evokes active immunity against HBV and is recommended for both preexposure and postexposure prophylaxis.

HEPATITIS B IMMUNOGLOBULIN

HBIG is prepared from plasma known to contain a high titer of anti-HBs. The human plasma from which HBIG is prepared is screened for antibodies to HIV, and the process used to prepare HBIG inactivates and eliminates HIV from the final product; no evidence has been found that HIV can be transmitted by HBIG. HBIG is used for the postexposure prophylaxis of newborns of HBV-infected women and for susceptible individuals with sexual, needle-stick, or mucosal exposure. It is administered intramuscularly. The dose for infants with perinatal HBV exposure is 0.5 mL. The dose for adults is 0.06 mL/kg body weight. Efficacy ranges from 70 to 95 percent.[5] HBIG in large doses is used after liver transplantation in HBV-infected patients to prevent infection of the allograft.

HEPATITIS B VACCINE

Hepatitis B vaccines are available as a single-antigen formulation or in combination with other vaccines. The two licensed single-antigen recombinant vaccines are produced by using *Saccharomyces cerevisiae* (common baker's yeast), into which a plasmid containing the gene for HBsAg has been inserted. Purified HBsAg is obtained by lysis of the yeast cells and separation of HBsAg by biochemical and biophysical techniques. Hepatitis B vaccines are packaged to contain 10 to 40 μg of HBsAg protein per milliliter after adsorption to aluminum hydroxide (0.5 mg/mL). The two vaccines available in the United States are Recombivax HB (Merck and Company, Inc, Whitehouse Station, NJ) and Energix-B (GlaxoSmithKline Beecham Biologicals, Rixensart, Belgium). Recombinant HBsAg also is used in the three combination vaccines available in the United States: Comvax (Merck), Pediarix (GlaxoSmithKline Beecham), and Twinrix (GlaxoSmithKline Beecham). Immunogenicity is similar for single-antigen and combined hepatitis B vaccines.

The recommended series of three intramuscular doses of HBV vaccine induces a protective antibody response (anti-HBs) in more than 90 percent of healthy adults and in more than 95 percent of infants, children, and adolescents. HBV vaccine should be administered into the deltoid muscles of adults and children or the anterolateral thigh muscles of neonates and infants; immunogenicity is substantially lower when injections are administered into the buttocks. When HBV vaccine is administered to infants at the same time as other vaccines, separate sites in the anterolateral part of the thigh may be used for the multiple injections.

The vaccination schedule used most often for adults and children has been three intramuscular injections, the second and third administered 1 and 6 months, respectively, after the first. Each of the two available vaccines has been evaluated to determine the age-specific dose at which an optimal antibody response is achieved.

Perinatal transmission of HBV can be prevented effectively if the HBsAg-positive mother is identified and if her infant receives appropriate immunoprophylaxis. Administration of HBIG and the first dose of HBV vaccine within 12 hours of birth prevents the development of 90 percent of perinatal infections.[88] Serologic testing of infants who receive immunoprophylaxis to prevent perinatal infection should be performed to identify the 5 percent of infants who become HBV carriers despite these measures. Testing for anti-HBs and HBsAg in children 12 to 15 months of age determines the success of vaccination and, in the case of failure, identifies HBV carriers or infants who may require treatment or re-vaccination. The following are the current recommendations on timing of immunizations for infants in the United States.[128]

1. **Infants born to HBsAg-negative mothers** should receive the first single-antigen vaccine dose at birth or before hospital discharge. The plan thereafter depends on whether a single-antigen or combination vaccine is used. For single-antigen vaccine, the second dose should be administered at 1 to 2 months of age and the third at 6 to 18 months. If combination vaccines are used, the first dose should be of a single-antigen vaccine at birth or before hospital discharge. The second dose should be at 2 months, the third at 4 months, and a fourth dose at either 6 months or 12 to 15 months, depending on the combination of vaccines used. Testing for serologic response is not necessary and is not recommended.
2. **Infants born to HBsAg-positive mothers** should receive 0.5 mL of HBIG and the first dose of single-antigen HBV vaccine within 12 hours of birth, administered intramuscularly at separate sites. The infant then can complete the immunization series with either single-antigen or combination vaccine. If single-antigen vaccine is used, the infant should receive the second dose at 1 to 2 months and the third when the child is at least 164 days (6 months) of age. If combination vaccine is used, the child should receive single-antigen vaccine and HBIG at birth and then combination vaccine at 2 months, 4 months, and either 6 months or 12 to 15 months, depending on the combination. Premature infants of HBV-infected women should receive vaccine in the same schedule; however, those weighing less than 2000 g at birth have a decreased response to hepatitis B vaccination before they reach 1 month of age. For such infants, the dose given at birth

TABLE 169–3 Prevention of Horizontal Transmission within the Home and School

Home
Hepatitis B immunization of all household members
Universal precautions for all blood and body fluids
Coverage of all abrasions with dressings
Ban on sharing potentially contaminated items such as razors or toothbrushes
School
Hepatitis B universal immunization
Universal precautions for all blood and body fluids
Coverage of all abrasions with dressings
Full participation by child in normal school life

should not be counted as the start of the vaccine series, and three additional doses of vaccine (for a total of four doses) should be administered beginning when the infant reaches 1 month of age.[128] Testing for serologic response (HBsAg and anti-HBs) should be done when the child is 12 to 15 months of age. If the anti-HBs response is inadequate (<10 mEq/L) and the child is HBsAg-negative, the child should be re-vaccinated. If the child's anti-HBs titers are greater than 10 mU/L and the child is HBsAg-negative, that child is immune and not infected. If the child is HBsAg-positive at 12 to 18 months of age, the child should be considered potentially chronically infected and monitored appropriately.

3. **Infants born to mothers whose HBsAg status is unknown** should receive the first dose of vaccine within 12 hours of birth. Meanwhile, a maternal blood sample should be sent for HBsAg testing. If positive, HBIG should be given as soon as possible (not later than when the infant is 1 week of age). The infant then should complete the routine vaccination schedule. If the baby is preterm and weighs less than 2000 g and the mother's HBsAg is not established within 12 hours of birth, the infant should receive HBIG and single-antigen HBV vaccine and then complete four doses of HBV vaccination in total.

Others Who Should Receive Hepatitis B Vaccine

All non-immunized children and adolescents younger than 19 years should begin HBV vaccination at any health care provider visit. For older children and adolescents aged 11 to 15 years, the routine three-dose schedule may be used, but a two-dose schedule of vaccination with the Recombivax HB vaccine also has been approved. The adult dose (1.0 mL/10 μg) is administered to the adolescent, and the second dose is given 4 to 6 months later. Short-term (2-year) follow-up in groups immunized by either schedule showed similar rates of anti-HBs decline. Of emphasis is that a lower dose of vaccine is used in the three-dose schedule, and if the original dose is not known, the full three-dose schedule should be administered.[25] Immunization also should be offered to high-risk groups until universal childhood immunization has created an immune adult population.

In a three-dose schedule, increasing the interval between administration of the first and second doses of HBV vaccine has little effect on immunogenicity or final antibody titer. The third dose confers optimal protection and acts as a booster dose. Longer intervals between the last two doses (4 to 12 months) result in higher final titers of anti-HBs antibody. Larger vaccine doses, 40 μg, or an increased number of doses is required to induce protective antibody in many hemodialysis patients and other immunocompromised persons (e.g., those who take immunosuppressive drugs or who are infected with HIV). Children with end-stage renal disease respond better than adults do to HBV immunization. Ninety percent of pediatric chronic dialysis patients and 100 percent of children vaccinated before beginning hemodialysis became anti-HBs–positive in one study.[59] An immunocompromised person who previously has shown an adequate anti-HBs antibody titer that then falls to less than 10 mIU/mL should receive a booster dose.

Testing for immunity (anti-HBs titers) is advised only for patients whose subsequent clinical management depends on knowledge of their immune status (e.g., infants born to HBsAg-positive mothers, hemodialysis patients and staff, patients with HIV infection). Post-vaccination testing also should be considered for those at occupational risk who may have exposure from injuries with sharp instruments because knowledge of their antibody response helps determine appropriate postexposure prophylaxis.

When re-vaccinated, 15 to 25 percent of people who do not respond to the primary vaccine series produce an adequate antibody response after one additional dose, and 30 to 50 percent do so after receiving three additional doses.[86] Therefore, re-vaccination should be considered for people who do not respond to the initial series.

The duration of vaccine-induced immunity has been evaluated in long-term follow-up studies of both adults and children. The duration of response from neonatal or later immunization remains unclear, but studies in both Asian and European children vaccinated in infancy demonstrated that immune memory persisted at least 10 years in Italy[199] and 12 years in Hong Kong[198] but started to wane by 15 years after immunization in Taiwanese children.[123] Booster doses were administered to children in the latter study with anti-HBs titers less than 100, and 4 of 128 children demonstrated no response.[123] A previous study from the same group demonstrated excellent response in all subjects to a booster dose at 10 years of age in those who were anti-HBs–seronegative despite receiving neonatal vaccination after being born to HBsAg- and HBeAg-positive mothers.[79] The timing and necessity of a booster dose remain unclear, and hence the booster dose is not recommended on a population basis.[89]

Therapeutic vaccination, that is, immunization of those already infected in an effort to boost their HBV-specific T-cell responses, has shown some modest success in adults. However, a trial of combination immunization (pre-S2/S vaccine) with IFN-α-2b versus IFN-α-2b alone in children with chronic HBV infection did not show any benefit over IFN-α-2b alone.[74]

Vaccine Side Effects and Adverse Reactions

Hepatitis B vaccines have been shown to be safe for both adults and children. Anaphylaxis is the only serious adverse event in children and adolescents, with an observed risk of 1.1 per million vaccine doses administered reported in 2003.[14] Pain at the injection site (3-29%) and a temperature greater than 37.7° C (1-6%) have been the side effects reported most frequently, but they were reported no more frequently in vaccinees than in subjects receiving placebo.[24,180] In the United States, surveillance of adverse reactions has shown a possible association between Guillain-Barré syndrome (GBS) and receipt of the first dose of plasma-derived HBV vaccine in adults (CDC, unpublished data), and hence, plasma-derived HBV vaccine no longer is in use. An expert review did not accept or reject a causal association between hepatitis B vaccination and GBS,[24] and few cases of GBS have been reported in recent years.

Reports of multiple sclerosis (MS) developing after vaccination for HBV led to concern that the vaccine might cause MS in previously healthy subjects. This theory was refuted in a nested control study of two large cohorts of nurses in the United States in which the HBV immunization histories of each of 192 women in whom MS developed were compared with those of 5 healthy controls and 1 woman with breast cancer. No association was found.[4] In a case crossover study using the European MS database, recent vaccination against HBV, tetanus, or influenza did not appear to increase the short-term risk for relapse in MS.[40]

Until 1999, hepatitis B vaccines were prepared with thimerosal, sodium ethylmercuric thiosalicylate, to prevent bacterial and fungal contamination. This preservative has aroused great public concern regarding mercury toxicity and the amount of mercury exposure relative to body weight. No adverse outcomes have been associated clearly with thimerosal use. HBV vaccination in newborns was suspended temporarily in 1999 until thimerosal-free vaccines became available, unless the mother was infected with HBV. Two thimerosal-free HBV vaccines are available now in the United States for use in infants. Hence, parents can be reassured about the lack of exposure to mercury in HBV vaccines.

Contraindications

Only a few clinical situations exist in which hepatitis B vaccine is not recommended: those with a history of anaphylaxis to hepatitis

TABLE 169–4 Recommended Physician Visits for Children with Chronic Hepatitis B Virus Infections

Every 6 months
History taking
Physical examination, including growth
Blood work: AST/ALT, FBC, HBsAg, anti-HBs, HBeAg, anti-HBe
Review of risk factors for transmission
6 months to annual
Liver ultrasound
Alpha-fetoprotein level (optimum frequency not yet clear)

ALT, alanine transaminase; AST, aspartate transaminase; FBC, full blood count; HBeAg, hepatitis B e antigen; HBsAg, hepatitis B surface antigen.

B vaccine and those with history of hypersensitivity to yeast. Vaccination should be deferred in those with an intercurrent acute infection with or without fever. Pregnancy is not a contraindication to immunization.[128]

RECOMMENDATIONS TO PREVENT HOUSEHOLD TRANSMISSION

When a person is found to have acute or chronic HBV, immunization of household contacts is recommended. Guidance on universal precautions should be provided. As outlined in Table 169–4, parents of infected children and siblings should learn to treat body fluids, especially blood, as potentially infectious. All bloody emissions and blood-soiled items should be handled with gloves, and any blood-stained items either should be cleaned with bleach or should be disposed of carefully. Both the infected person and others in the household should cover skin abrasions with waterproof bandages. Razors and toothbrushes never should be shared. If needle sharps are in use, they should be secured and disposed of according to local guidelines. Schools, workplaces, and health care providers should practice universal precautions for all individuals, regardless of whether their HBV status is known.

FUTURE STRATEGIES AND TARGETS FOR TREATMENT

As new technologies are developed, potential treatments of hepatitis B continue to emerge. They can be classified as (1) disruption of stages of the HBV life cycle other than HBV polymerase, (2) gene therapy, and (3) immunomodulators other than IFN-α.

Disruption of formation of the nucleocapsid by members of the heteroaryldihydropyrimidine class or phenylpropenamides has shown some efficacy in cell and mouse models.[122] Direct manipulation of the HBV gene offers the possibility of viral eradication with technology such as short interfering RNA (siRNA) molecules once technical problems such as how to introduce siRNA into hepatocytes and how to prevent degradation by enzymes have been overcome. However, proof of principle already has been demonstrated in mice. Short hairpin RNA, a form of RNA interference, homologous to HBV RNA was introduced into a cell culture model and into immunocompetent and deficient mice. Substantial reductions were achieved in mouse liver HBV RNA and replicated genome, as well as a 84.5 percent reduction in secreted HBsAg in mouse serum. HBcAg expression also was reduced.[130] siRNA against different regions of the HBV gene has been shown to decrease HBsAg secretion in a human hepatoblastoma cell line.[99] Another group has shown that this effect can persist through multiple cell passages, thus suggesting integration of siRNA into the HBV genome.[148] Other members of the IFN class, γ and λ, as well as cytokines, have shown some efficacy in either cell culture or animal models but have not been tried yet in humans.[122]

Despite advances in treatment, prevention of new infections must remain at the forefront of all therapeutic endeavors. Vertical transmission of HBV to children can be prevented by proper antenatal testing, careful follow-up of results, and availability of immunization programs to infants.

HEPATITIS D

Hepatitis D virus (HDV) is a subviral particle that requires a helper virus, HBV, to cause infection. As a subviral particle, HDV, which also is known as the delta agent or virus, does not encode its own envelope protein and requires HBV to produce its envelope to become an infectious virion. HDV was described first in 1977, when a new antigen was reported in the hepatocytes of patients infected with HBV. Transmission experiments demonstrated that the antigen was indeed a new virus.[149] Infection with HDV and HBV can be contracted at the same time (coinfection), or HDV infection can be contracted as a new infection in a person previously infected with HBV (superinfection). HDV has at least seven clades (or types),[145] which have geographic variation in both incidence and genotype distribution.[162] HDV genotype 1 is found worldwide, but HDV genotypes 2 and 4 are found in the Far East.[112] HDV genotype 1 infection appears to be associated with a more fulminant hepatitis in acute infection and higher rates of cirrhosis and HCC than noted with genotype 2.[193]

VIROLOGY

HDV is a spherical particle with a diameter of 36 nm.[133] When the envelope is degraded, a nucleocapsid containing hepatitis D antigen (HDAg) and the HDV RNA genome is released. The two forms of HDAg are the small s-HDAg, which is 195 amino acids in length and is required for replication, and the larger l-HDAg, which is 214 amino acids long, is required for virion assembly, and inhibits HDV replication.[133] s-HDAg is a nuclear protein that undergoes phosphorylation by protein kinase C, and inhibitors of protein kinase C inhibit HDV replication in cell culture systems.[36] The ratio of the two forms of HDAg found in patients may vary.

The HDV RNA genome is single stranded and circular, with a length of 1679 nucleotides. During viral replication, genomic RNA serves as a template for complementary RNA, which in turn is a template for more genomic RNA. A host-derived RNA polymerase is required.[183] Each copy of the genome and antigenome contains ribozyme activity that cleaves RNA and is required for replication ofHDV.[133] Post-transcriptional modification of the RNA leads to the production of l-HDAg, in addition to the s-HDAg already transcribed.[36,183]

To leave the host cell, HDV RNA and HDAg must be packaged into virions. l-HDAg undergoes isoprenylation of a cysteine residue near its C-terminal to allow assembly of the virion. Inhibition of this isoprenylation step interferes with viral production by preventing interaction with HBsAg.[36] Once this interaction occurs, HBsAg and host-cell membrane lipids enclose HVD RNA, l-HDAg, and s-HDAg to form a virion. Non-infectious HDV particles, which contain only l-HDAg and HBsAg, can be found in serum.[133]

EPIDEMIOLOGY

The epidemiology of HDV parallels that of HBV, for obvious reasons. Modes of transmission of HDV also are similar to those

for HBV and include direct or indirect parenteral exposure to blood or body fluids and sexual and perinatal transmission. Sexual transmission of HDV is less efficient than that of HBV, as evidenced by the relatively low frequency of HDV in homosexual men.[158] Perinatal transmission can occur but is rare because HBV carrier mothers also infected with HDV usually are anti-HBe–positive and thus less infectious. Intrafamilial transmission of HDV has been demonstrated in endemic areas, such as southern Italy, by means of a combined epidemiologic and molecular study, which demonstrated that within family units, the HDV strains were nearly identical.[136] Transmission of HDV infection also has been documented in some residents of institutions for the developmentally disabled.

Geographic differences in HDV endemicity in the world are great, and four levels have been characterized. Highest endemicity (HDV found in more than 20% of asymptomatic HBV carriers, more than 60% of HBV carriers with chronic liver disease) is seen in northern South America and Africa, as well as in Romania.[202] In these populations, HDV superinfection in HBV carriers is a significant cause of chronic liver disease and has resulted in outbreaks of fulminant hepatitis. In these areas, HDV infection is seen commonly in both children and adults, and intrahousehold transmission has been implicated. Parts of the Middle East, Africa, some Pacific Islands, and parts of Asia report intermediate HDV endemicity (10%-20% of asymptomatic HBV carriers, 30%-60% of HBV carriers with chronic liver disease). Infection in these regions occurs predominantly in adults, and outbreaks are uncommon occurrences, but HDV is an important cause of chronic liver disease. Low endemicity (3-9% of asymptomatic HBV carriers, 10-25% of HBV carriers with chronic liver disease) is observed in most developed countries, including the United States, but subpopulations in these countries, such as parenteral drug abusers and prostitutes, have a high infection rate. In areas of low HBV endemicity, transmission of HDV appears to be mainly by the percutaneous route, with higher rates of HDV found in intravenous drug users and hemophiliacs than in other HBV high-risk groups. HDV is considered only a moderately important cause of chronic liver disease in developed countries.[191] Finally, for as yet unexplained reasons, certain subpopulations have a high carriage rate of HBV, but virtually no HDV infection; such groups include Native Americans, Eskimos, and residents of some Asian countries.

Within the blood donor population of the United States, the prevalence of HDV is low; only 1.4 to 8 percent of donors are infected or have evidence of previous infection.[3] Estimates from the CDC for 1990 indicate that among the approximately 250,000 cases of acute HBV infection per annum, HDV infection is acquired simultaneously in 7500. An estimated 70,000 carriers of HDV are found in the United States, where approximately 1000 deaths per year are caused by chronic HDV and 35 by fulminant HDV. In certain regions, the prevalence of HDV infection appears to be decreasing. In Italy, the prevalence of HDV infection in patients who are HBsAg carriers decreased from 23 percent in 1987 to 14 percent in 1992 and to 8.3 percent in 1997.[61,155] Corresponding data for the United States are not available.

IMMUNOPATHOGENESIS

Unlike HBV, HDAg is found only in the liver or serum.[63] The mechanism by which HDV infection leads to hepatic injury has not been elucidated. Despite some evidence that HDV can be directly cytopathic, cytopathic viruses seldom result in chronic infection. In addition, some HDV carriers have no evidence of hepatic cell damage. Both cellular and humoral components of the host immune system are involved in the response to HDV, although the relative contribution of each is controversial. Investigations into the humoral response to HDV demonstrated that HDAg contains epitopes or immunogenic domains to which sera from acutely infected humans and woodchucks react. The predominantly recognized epitopes are not exposed on the virion surface; thus, antibodies that recognize these epitopes cannot neutralize virus particles, which may explain why no association exists between the humoral response and clinical outcome.[133] Autoantibodies, especially liver-kidney microsomal type 3 antibodies and anti–basal cell layer antibody, are found occasionally in people with HDV infection. The significance of these antibodies is unclear, but they may play a role in some of the immunopathogenesis of HDV.

Cellular immune responses to HDV also have been demonstrated. $CD4^+$ and $CD8^+$ lymphocytes have been found in the liver of patients infected with HDV. These cells may either contribute to hepatitis or aid in control of HDV infection.[133] Studies have shown that patients with HDV superinfection of chronic HBV who had peripheral $CD4^+$ cells that reacted to four epitopes on HDAg were seronegative for HDV IgM, thus implying inactive disease.[136] Such $CD4^+$ T-cell clones produce IFN-γ and may be directly cytopathic. Individuals without these specific $CD4^+$ cells had active HDV disease.[137] These epitopes are in highly conserved regions of HDV, which is encouraging for the prospect of vaccine development.

An unusual mechanism of HDAg presentation by hepatocytes has been reported. Processing of HDAg peptide appears to occur outside the cell, where the peptide is cleaved into smaller fragments that bind to the class II MHC molecules of all three HLA types found on hepatocytes and mononuclear cells. This form of antigen presentation could have many possible effects on T-cell responses: amplification of the T-cell response to HDAg, stimulation of CTLs to kill uninfected hepatocytes, or exhaustion of T-cell responses to HDAg, which allows infected cells to escape detection.[133]

The relationship of HBV infection status to the pathogenesis of HDV also is unclear. In patients with active HBV replication (i.e., HBV DNA– and HBeAg-seropositive), HDV viremia is high.[149,150] Conversely, in HBV and HDV carriers without liver disease or with minimal inflammatory hepatic lesions, markers of HBV replication are absent, and HDV viremia is low.[15,150] In co-infected liver transplant recipients, HDV reinfection can occur without evidence of active HBV replication, but necroinflammatory activity does not develop until HBV infection is reactivated.[44]

DIAGNOSIS

Establishing the diagnosis of either acute or chronic HBV infection is a prerequisite for the diagnosis of HDV infection, which is made by detection of antibody to HDV (anti-HDV). HDV antigen can be measured in serum, but usually it is present only transiently during acute infection and in immunosuppressed individuals.[112] Assays for both IgM and IgG anti-HDV are available. HDV RNA can be detected by reverse transcription and real-time PCR, but these tests are not available commercially.[112]

HDV infection may occur at the same time as HBV infection does, a pattern that is termed co-infection. If it occurs and the individual has acute, resolving hepatitis B, the HDV infection also will resolve.[46] In this instance, anti-HDV is found in the IgM form during the acute illness and persists for 2 to 6 weeks. Subsequently, IgG anti-HDV is found in serum, but it also diminishes to undetectable levels when the HBV infection resolves (Fig. 169–6). In HDV superinfection of an individual chronically infected with HBV, both IgM and IgG anti-HDV are detected. Most often, superinfection with HDV leads to chronic HDV infection, which is diagnosed by persistence of both IgM and IgG anti-HDV (Fig. 169–7). Anti-HDV becomes predominantly IgG

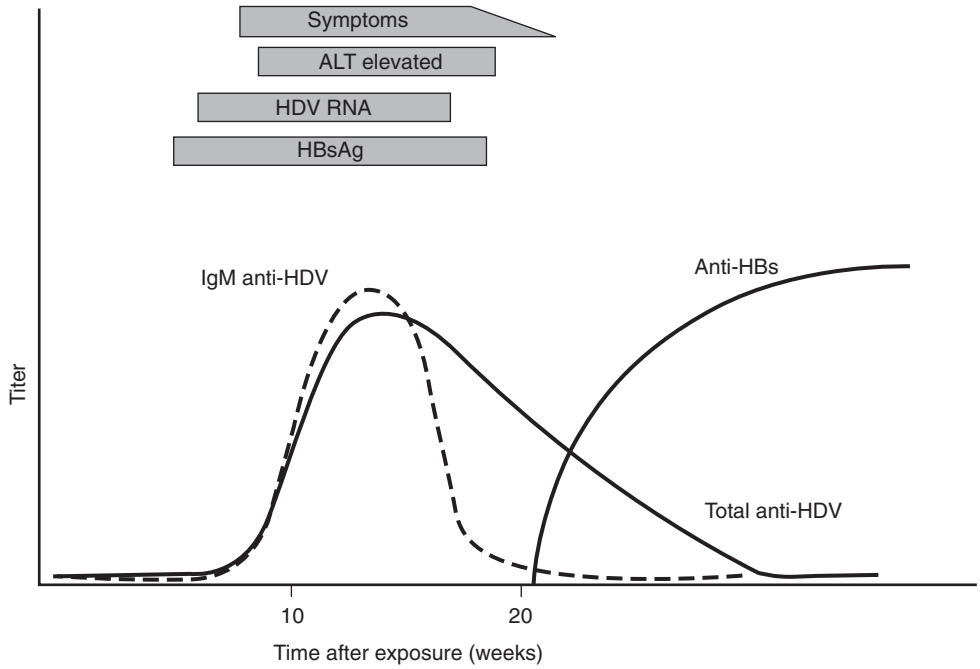

Figure 169–6 Serologic events during hepatitis B virus (HBV) and hepatitis D virus (HDV) co-infection that resolves. After exposure to both viruses, hepatitis B surface antigen (HBsAg) and HDV RNA can be detected shortly before the onset of symptoms. Serum alanine transaminase (ALT) is elevated for the duration of detectable serum HDV RNA and HBsAg. Serum IgM anti-HDV rises when symptoms are present and falls as HDV infection resolves and the anti-HBs titer becomes elevated. When both infections have resolved, anti-HBs is detectable in serum, but total anti-HDV is very low.

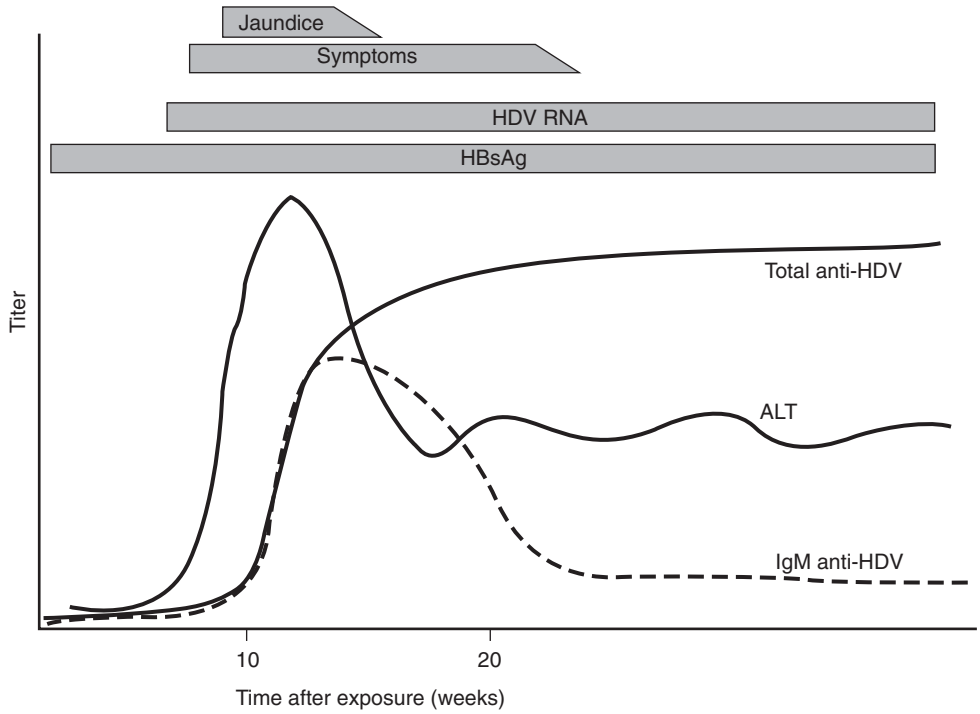

Figure 169–7 Serologic events during hepatitis B virus (HBV) and hepatitis D virus (HDV) superinfection. If an individual already infected with HBV is infected with HDV, there is a marked elevation of alanine transaminase (ALT) coincident with the onset of symptoms and the onset of jaundice. HDV RNA also can be detected at the onset of symptoms. ALT levels fluctuate over the next few months as IgM anti-HDV decreases and total anti-HDV rises. Throughout the course of HDV superinfection, hepatitis B surface antigen (HBsAg) remains detectable.

after approximately 6 weeks and persists if infection does not resolve; titers correlate well with ongoing viral replication.

The histopathologic features of HDV infection are very similar to those of isolated HBV infection (see earlier). HDAg can be identified in nuclei and, to a lesser extent, in the cytoplasm of infected hepatocytes.

CLINICAL FEATURES

In general, co-infection with HDV does not have clinical features that distinguish it from HBV infection alone. Symptoms develop 2 months after exposure, are similar to those of acute HBV infection, and usually resolve by 4 months after exposure. However, two peaks in transaminases often are noted, a pattern that is unusual in HBV infection alone. The infections resolve together.[47] Co-infection with both viruses does not increase the risk for development of chronic HBV infection.[121]

HDV superinfections in individuals with chronic HBV infection have different manifestations. HDV infection becomes persistent in most individuals (Fig. 169–8), and the predominant clinical pattern of chronic HDV infection varies by geographic location. In the United States and Europe, HDV superinfection leads to chronic liver disease in more than 90 percent of cases,

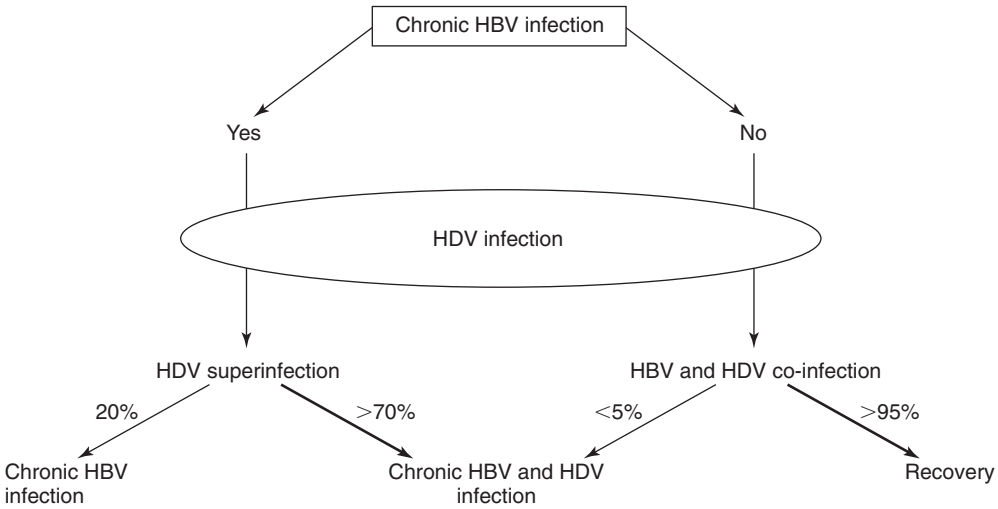

Figure 169–8 Outcomes of hepatitis B virus (HBV) and hepatitis D virus (HDV) superinfection and co-infection. When a person already infected with HBV becomes infected with HDV, there is a greater than 70 percent chance of becoming chronically infected with both HDV and HBV. In contrast, if a person acquires HBV and HDV de novo, there is less than a 5 percent chance of chronic HBV and HDV infection.

whereas in some Pacific Island and African populations, most individuals with chronic HDV are asymptomatic.[150] When chronic liver disease does occur, progression to cirrhosis occurs rapidly; in studies in both adults and children, cirrhosis has been noted to occur in as few as 2 to 10 years. The cirrhosis associated with HDV infection has two patterns: in a minority of patients, especially high-risk drug users, it progresses rapidly to hepatic failure and portal hypertension; in others, more stable cirrhosis occurs, with little inflammatory component and compatibility with prolonged survival.[18] In a small minority of those who are superinfected, a self-limited hepatitis develops, and subsequently both HBV and HDV infections are cleared. HDV infection has been demonstrated to lead to HCC at a younger age and at a higher rate than HBV infection alone does.[150] In a study of 166 patients infected with HBV and HDV from Romania, median survival after the diagnosis of HDV compensated cirrhosis was 58.3 months.[64] A multicenter European study of 200 HBsAg-positive white patients with compensated cirrhosis found that 39 (20%) were anti-HDV–positive. Those with HDV, HBV, and cirrhosis were younger than those with HBV and cirrhosis only; indeed, HDV infection may lead to the development of cirrhosis 15 years earlier.[55] In this study, the addition of HDV infection increased the risk of HCC developing threefold and risk of mortality twofold when compared with those with HBV and cirrhosis.[55] The natural history in those without cirrhosis is difficult to establish. In a study of 13 institutions for people with developmental disabilities in Illinois, 67 of 238 (28%) HBsAg carriers also had anti-HDV antibodies.[75] Long-term (20 years) follow-up information was available on 65 of 66 anti-HDV– and HBsAg-positive individuals and 166 HBsAg-positive persons. Liver-related deaths occurred in 7 of 65 (11%) anti-HDV–positive but only 1 of 166 anti-HDV–negative persons.[1] Hepatitis D infection was associated with both a higher rate of chronic liver disease and a higher liver-related mortality rate.[1]

Children with HDV infection follow a course similar to that of adults. In one European series, the subset of children chronically infected with HBV who also had HDV had more advanced liver disease, and the prevalence of HDV infection increased in parallel with the activity of hepatitis. During the study period, disease progressed more rapidly in children infected with both viruses.[54] Testing for HDV infection is recommended for any child with chronic HBV and unusually severe liver disease or an acute exacerbation of stable liver disease.

HDV infection after liver transplantation can appear without apparent HBV re-infection of either the graft or serum. However, use of PCR assay and buoyant density analysis has demonstrated that low levels of HBV infection in fact do occur in patients with recurrent HDV, and this replication of HBV is sufficient to permit replication of HDV. The HDV virion found in such infections shares the characteristics of typical HDV infection.[16]

TREATMENT

Although several antiviral agents have been studied, the only treatment that has had any beneficial effect on HDV infection is IFN-α. Trials in adults, in both Europe and the United States, have shown that treatment with either 9 million units three times weekly or 5 million units daily of IFN-α results in normalization of serum transaminase levels, decrease or disappearance of HDV RNA from serum, and improvement in hepatic inflammation in about 50 percent of cases.[54] However, most patients relapsed with hepatitis shortly after treatment was discontinued. Maintenance of response after treatment was noted primarily in individuals who became HBsAg-negative.

Pegylated IFN-α-2b as a treatment of chronic HDV has shown promise in two recently reported trials. Pegylated IFN-α-2b was well tolerated and had some efficacy. Fourteen patients were treated with pegylated IFN-α-2b, 1.5 μg/kg weekly for 12 months, and 6 (43%) had a sustained virologic response (undetectable HDV RNA) at 6 months.[22] Combination ribavirin and pegylated IFN-α-2b did not improve the virologic response over pegylated IFN-α-2b alone.[135] No factors predictive of response have been identified. Until the advent of pegylated IFN-α-2b, the prognosis in nonresponders to IFN-α and in those who relapse has been poor. The author of a recent editorial in *Hepatology* recommended continuation of IFN-α or pegylated IFN-α-2b in those with a decline in HDV RNA as long as possible or until HDV RNA and HBsAg disappear.[53]

Currently, no recommendations have been established regarding the use of IFN-α or pegylated IFN-α-2b for the treatment of chronic HDV infection in children. A trial in seven children in Greece treated with IFN-α, 3 million U/m^2 of body surface area three times a week for 1 year, had disappointing results. All remained anti-HDV IgM–positive, and four of seven had persistent HDV RNA in their serum. A significant reduction in serum transaminase values was noted after 1 year of treatment, but no significant improvement occurred in liver histology.[42] A study of eight children from Germany also found no effect of IFN-α on replication of HDV. However, serum transaminase values improved, and children underwent earlier anti-HBe seroconversion than historical controls did.[159] Lamivudine has been shown to have no effect on either HDV viremia or liver disease activity in chronic HDV infection.[146]

Some children and adults require liver transplantation for management of end-stage HDV-associated liver disease. Patients co-infected with HDV and HBV have lower rates of HBV infection of the allograft than do patients with isolated HBV infection. These data were reported in a series of 58 patients with HBV, 25 of whom also had HDV infection and underwent liver transplantation between 1984 and 1996 in Belgium. Fifty-two patients survived longer than 3 months, and median follow-up was 74 months. HDV infection improved survival rates at 5 years, 96 ± 4 percent versus 63 ± 10 percent for those infected with HBV alone.[113] As yet, no explanation has been given for the better outcome of transplantation in individuals who have both HBV and HDV infection. Administration of HBIG is required before and after transplantation, as in patients infected with HBV alone.

IMMUNOPROPHYLAXIS

Passive or active immunization specifically against HDV infection is not available. HDAg itself is highly immunogenic, but antibodies are not neutralizing. Description of a T-cell response to several HDV epitopes in individuals with less severe disease activity has stimulated speculation about possible vaccine development, but studies are preliminary. A DNA-based HDV vaccine successfully induced cellular responses in an animal model and also was successful when used with an HBV vaccine.[81] A trial of vaccine derived from B-cell epitopes of HDAg and HDAg p24 expressed in *Escherichia coli*, yeast, or baculovirus in woodchucks provoked a specific antibody response but did not protect against HDV superinfection.[58]

At the present time, individuals with chronic HBV infection, who are the same people at risk for acquiring HDV, cannot be protected from HDV except by avoidance of high-risk behavior and exposure. However, prevention of HBV infection in susceptible individuals will prevent HDV co-infection and will be the most important mechanism for decreasing the prevalence of HDV infection in a population. The current strategy aimed at decreasing susceptibility to HBV infection by universal immunization of newborns and catch-up immunization of older children and adolescents should reduce the risk for subsequent development of HDV infection substantially during young adulthood. In Italy, the decrease in HDV prevalence in those with chronic HBV infection from 23 percent in 1987 to 8.3 percent in 1997 has led to speculation that chronic HDV may be a "vanishing disease."[61]

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SUBSECTION 5

Herpesviridae

CHAPTER 170

Herpes Simplex Viruses 1 and 2

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The family of nine human herpesviruses includes herpes simplex virus (HSV) types 1 and 2; cytomegalovirus (CMV); Epstein-Barr virus; varicella-zoster virus (VZV); and human herpesviruses (HHV) 6A, 6B, 7, and 8. This chapter deals with infections caused by HSV-1 and HSV-2 acquired after the neonatal period.

Herpes derives from the word meaning "to creep" in Greek. Initially, this term referred to cutaneous, spreading lesions in general and included the classic description of fever blisters recorded by Hippocrates and Herodotus. In the 1700s, Astrus described genital herpes lesions, and by the 1800s, the term *herpes* usually was restricted to poxvirus and herpesvirus lesions. Cultivation of HSV on rabbit cornea by Gruter (1912) differentiated HSV from VZV. Subsequent work differentiated the antigenic, biologic, and epidemiologic characteristics of HSV-1 and HSV-2.[333]

HSV-1 is the prototype of the alpha-herpesvirus subfamily, which includes HSV-2 and VZV.[235,333] These viruses share the characteristic of neurotropism and establish latency in sensory ganglia; persistence is associated with periodic reactivation and reappearance of infectious virus at mucocutaneous sites. Understanding HSV-1 and HSV-2 infections requires knowing that most infected individuals do not have any clinical manifestations, either at the time of initial acquisition or during episodes of reactivation. The next step is to recognize that when HSV-1 or HSV-2 causes disease, as opposed to HSV infection, the illness may range from minor, such as "fever blisters," to life-threatening, as exemplified by HSV encephalitis. As with all herpesviruses, the immune status of the host is an important determinant of disease severity, as is whether infection is primary or recurrent. Proper management of HSV-1 and HSV-2 infection depends on clinical recognition of the common and atypical syndromes caused by these viruses and knowledge of the laboratory methods that are useful for proving the diagnosis. Attention given to the clinical and laboratory diagnosis of HSV infection is important because oral antiviral treatment is safe and often benefits healthy children and early antiviral therapy reduces the risk of severe or fatal infections developing in immunocompromised patients. When misused, some therapies, such as corticosteroids for ocular HSV infection, may worsen the outcome. HSV-1 infections in children of all ages and HSV-2 infections in adolescents are among the most common treatable viral illnesses encountered in pediatric practice.

THE VIRUSES

HSV-1 and HSV-2 virions consist of an icosahedral protein capsid enclosing a core of double-stranded DNA, surrounded by a protein tegument, and enclosed in a lipid-containing envelope.[235] The genomic DNA of HSV-1 and HSV-2 has substantial sequence homology (approximately 50%), but the viruses also have unique sequences that encode variant proteins, and they differ biologically in their patterns of replication in vitro and in vivo. Tegument proteins are located between the capsid and the viral envelope. The envelope has glycoproteins that are important targets of the humoral and cellular immune responses (gB, gC, gD, and gG). The glycoproteins gE and gI function as immunoglobulin Fc receptors. Other glycoproteins include gH and gL, which form a complex that plays a role in cell entry and spread; gK, which is required for viral exocytosis; and gM, which has a role in capsid envelopment and exocytosis. These glycoproteins are found in both HSV-1 and HSV-2 and exhibit a high degree of amino acid similarity. The glycoprotein G of HSV-2 is larger than its HSV-1 homologue and has unique sites that are recognized by virus-specific host responses. Whereas both forms of HSV can infect either oral or genital sites, HSV-1 usually is a cause of infections of oral mucocutaneous sites (above the waist), and most genital mucocutaneous infections (below the waist) are caused by HSV-2.[92,333] HSV-1 is more likely to recur at oral sites and HSV-2 reactivates more frequently in the genital area, even in persons who were infected initially at both oral and genital sites with HSV-1 or HSV-2.[171]

HSV attaches to and penetrates cells via glycoproteins B and D by using specific cell surface herpes family co-receptors. This process also involves gH. The herpes family co-receptors belong to the tumor necrosis factor family (Hve-A), the immunoglobulin superfamily of receptors (nectins), and the 3-*O*-sulfated heparin sulfates.[235] After the virion enters the cell, an orderly expression of immediate early, or alpha, genes occurs that triggers viral gene transcription and inhibits host cell function; early, or beta, viral genes that encode regulatory proteins and DNA replication enzymes, including thymidine kinase and viral DNA polymerase; and finally, late, or gamma, genes that encode structural proteins. Replication of viral DNA and accumulation of structural proteins allow assembly of the virion capsid in the cell nucleus and envelopment by modified cellular membranes that are incorporated into the virion envelope during final virion envelopment and egress. Infectious virions are released from the infected cell or can spread to adjacent cells via membrane fusion.

Human HSV replicates in tissue culture cells derived from many mammalian species, as well as in embryonated hens' eggs and various laboratory animals, including rodents, rabbits, and primates. Lack of restriction of the host range in infectivity distinguishes HSV-1 and HSV-2 from the other human herpesviruses. HSV-1 and HSV-2 infections progress rapidly in cell culture and cause relatively characteristic focal cytopathologic effects; confirmation that these effects are caused by HSV can be accomplished by staining the infected cells with specific antisera or monoclonal antibodies that differentiate HSV-1 from HSV-2 and from other viral pathogens.

HSV isolates can be analyzed further by using the technique of DNA endonuclease restriction analysis, in which viral DNA is recovered from infected cells, digested enzymatically, and analyzed in gels to determine the pattern of DNA fragments resulting from enzymatic digestion. Epidemiologically unrelated HSV isolates can be differentiated by using multiple restriction enzymes to digest viral DNA, which reveals a unique "viral fingerprint."

Precise differentiation requires genomic sequencing. These methods are useful for molecular epidemiologic studies to confirm the relatedness of viruses recovered from different sites or at different times in the same individual or to document transmission to another individual, such as mother to infant or infant to infant in nurseries; this technique has shown that apparent "outbreaks" of encephalitis were instead random, unrelated case clusters.[47,129,185]

Like other herpesviruses, HSV-1 and HSV-2 have gene products that facilitate the avoidance of immune surveillance during primary infection, latency, and reactivation. These mechanisms include elaboration of virally encoded IgG Fc receptors and complement receptors that bind host immune components; down-regulation of major histocompatibility complex (MHC) class I antigen, which prevents recognition of infected cells by $CD8^+$ T cells; interference with up-regulation of MHC class II molecules by interferon-γ, which inhibits $CD4^+$ T-cell–mediated adaptive immune responses; and inhibition of the lytic activity of natural killer cells.

TRANSMISSION

Infectious HSV-1 and HSV-2 virions are released into the oral or genital secretions of infected but asymptomatic persons who are experiencing primary or recurrent infection. This phenomenon is referred to as *viral shedding* or *excretion* and results in periodic opportunities for the virus to be transferred to susceptible contacts. Though not widely understood, most HSV-1 and HSV-2 transmission is caused by these silent infections, which are common, and transmission occurs with equivalent frequency regardless of whether the individual who is shedding the virus has ever had symptoms of oral or genital herpes.

Although HSV-1 and HSV-2 are highly infectious after inoculation of mucocutaneous sites, they are not transmitted casually from person to person. The enveloped virions are relatively unstable outside mammalian cells, and close interpersonal contact generally is required for transfer of infectious virus particles. In most circumstances, transmission requires direct apposition of infected with uninfected mucous membranes or skin during intimate contact such as kissing or sexual contact. Outbreaks associated with gingivostomatitis have occurred at daycare centers.[262] If the uninfected, exposed skin or mucous membranes are damaged, the risk of transmission appears to be enhanced. Although skin is less susceptible to direct inoculation than the mucous membranes are, transmission via inoculation of skin may be increased by local trauma. For example, affected areas are more susceptible in burned patients[105] and infants with diaper rash, and children with eczema are at risk for contracting serious disseminated HSV infections (Kaposi varicelliform eruption).[331] Transfer of HSV-1 between wrestlers (herpes gladiatorum)[267] and between rugby players (herpes rugbeiorum or "scrum-pox")[269] probably takes place after the abrasion of saliva-contaminated skin. One outbreak at a wrestling camp involved 60 of 175 participants.[33,56] Health care workers may acquire HSV infections of the paronychial region (herpetic whitlow), presumably from direct contact of ungloved hands with oropharyngeal secretions.[112,237] Medical personnel may transfer HSV to their patients and be responsible for subsequent outbreaks of gingivostomatitis. Children may acquire HSV whitlow in the course of primary HSV-1 gingivostomatitis through nail biting or thumb sucking. When herpetic whitlow develops in persons who are not health care workers, HSV-2 is the most common cause and often is associated with primary genital HSV infection.[114] The usual route of transmission to newborns is by exposure during passage through an HSV-infected birth canal. Genital and anal HSV infections are transmitted through direct contact with infected genitalia or from oral-genital or oral-anal contact.

Newly acquired HSV-1 and HSV-2 infections are associated with shedding of very high titers of infectious virus, regardless of whether the individual is symptomatic. In addition, active recurrent HSV-1 or HSV-2 lesions contain substantial titers of virus, which may increase the likelihood of transmission occurring during close contact with a susceptible individual.

The incidence of acquisition is 5 to 15 percent per year in individuals who are HSV-1– and HSV-2–seronegative or HSV-1–seropositive but HSV-2–seronegative and whose sexual partners have recurrent genital herpes.[194,195] In studies of discordant couples, transmission occurred in 10 percent of partners, with higher transmission from men (17%) than from women (4%). In women lacking antibodies to HSV-1 and HSV-2, the rate of transmission was 32 percent; if they had antibodies to type 1, the rate was 9 percent.[194] Studies of patients by HSV-2–specific serology show that many individuals remain uninfected despite having long-term contact with a person who is infected with HSV-2.[167] The extent to which HSV-1 immunity protects against HSV-2 infection is not clear, but the evidence is that symptoms of new HSV-2 infection may be prevented if the exposed individual has HSV-1 immunity, whereas subclinical infection frequently is not blocked.[40,47]

Information about whether HSV-1 or HSV-2 acquired earlier interferes with the acquisition of infection by unrelated HSV-1 or HSV-2 strains is limited, but a few individuals with multiple sexual partners have been found to shed HSV-2 viruses that are genetically distinct. HSV has been isolated from the hands of patients with oral lesions and has been shown to persist for several hours on inanimate objects or in distilled water. Nonetheless, inanimate sources have not been implicated as important reservoirs of HSV persistence and spread.[209,310] HSV transmission from transplanted organs[93] and inseminated donor sperm has been reported.[198]

When HSV-1 and HSV-2 shedding has been evaluated prospectively in seropositive individuals with or without a history of symptoms, the usual frequency of detection of virus in oral or genital secretions is approximately 1 to 2 percent.[12,333] Rates of asymptomatic shedding may be higher after recent reactivation in individuals with symptomatic recurrences. In patients with known genital herpes, silent excretion has been documented as often as 10 percent of the time when they appear to be lesion-free. Twelve percent of women with primary HSV-1 genital infection and 18 percent with primary genital HSV-2 infection subsequently shed virus asymptomatically, especially in the first 3 months after resolution of the primary infection.[156] Two percent of women attending a sexually transmitted disease clinic shed HSV-2 asymptomatically.[165] In partner studies, 70 percent of HSV-2 transmissions were associated with sexual contact during periods of asymptomatic viral shedding.[195]

Polymerase chain reaction (PCR) is considerably more sensitive than is culture for detection of HSV on mucosal surfaces.[319] The use of PCR technology has improved understanding of the extent of HSV viral shedding. PCR detects subclinical shedding of HSV-2 on 20 to 25 percent of days. Factors that influence the magnitude and frequency of shedding include the site of infection, immune status of the patient, and time of acquisition of the virus.[242] Previous studies using viral cultures rarely reported isolation of HSV-2 from the mouth, but recent quantitative PCR studies detected asymptomatic oral HSV-2 reactivation from 40 percent of men with HSV-2 genital herpes. Oral HSV-2 shedding frequently is concurrent with genital HSV-2 shedding.[151]

Investigation of HSV-1 transmission is less extensive, but the high prevalence of asymptomatic HSV-1 infection in the population and its acquisition in early childhood suggest a similar pattern of spread by exposure to virus present in oral secretions from asymptomatic individuals.

EPIDEMIOLOGY

Understanding HSV epidemiology requires distinguishing between the prevalence of infection, which is high, and the frequency of HSV-related disease, which is low. HSV-1 and HSV-2 are infectious for the human host only under usual, natural circumstances. Molecular genetic analysis indicates that the HSV genome has evolved in parallel with the evolution of *Homo sapiens* from primate ancestors. These viruses maintain their persistence in the human population through their capacity to persist and reactivate in the individual host. The prevalence of HSV-1 and HSV-2 is a consequence of efficient transmission to susceptible close contacts and the continuous presence of a large pool of infected persons in the population. Because HSV-1 and HSV-2 are not cleared after primary infection but instead establish latency and reactivate frequently in an infected host, opportunities for viral spread to new susceptible persons are extremely common. HSV-1 and HSV-2 infections are ubiquitous and global in their distribution. Seroepidemiologic studies have shown that HSV infections are found in all human populations, even in the most remote and isolated communities. HSV-1 and HSV-2 have no seasonal pattern because the mechanism of transmission is by intimate contact, which occurs year-round. Individuals who have been infected with HSV-1 or HSV-2 remain a reservoir for infectious virus throughout their lifetimes and, as intermittent shedders, are a source of virus for spread to susceptible contacts. Most transmission is from asymptomatic infection, and most acquisition is asymptomatic. For example, the rate of subclinical shedding of HSV-2 in individuals with no reported history of genital herpes was similar to that in subjects with such a history (3.0% vs. 2.7%).[325] Shedding of HSV-1 in oral secretions is equally prevalent.

When symptoms of a new HSV-1 infection do occur, the source of the infection rarely is identified because the susceptible individual usually has many infected contacts. Furthermore, the first symptomatic episodes of HSV-1 or HSV-2 often represent reactivations of earlier infections.

The average age at acquisition and the prevalence of HSV-1 and HSV-2 infection are influenced by the virus type. Although neonatal HSV infections are acquired from maternal genital infection and most often are caused by HSV-2, HSV-1 acquisition predominates in childhood. Depending on social and economic factors, 40 to 60 percent of young children are seropositive by the time they reach 5 years of age, with somewhat higher rates of acquisition in lower socioeconomic groups. Earlier acquisition of HSV-1 may be influenced particularly by whether the mother, or other major caregivers, has HSV-1 infection. Attendance at child care centers, which brings children together in close contact, may increase the likelihood of acquiring HSV-1 at an earlier age.[169,262] Acquisition of HSV-1 continues with age at an average rate of 1 to 2 percent per year throughout the childhood and adult years; by later ages, 70 to 90 percent of individuals have been infected by HSV-1.[275] Seroepidemiologic studies of higher socioeconomic groups have reported HSV-1 infection in 30 to 46 percent of university students.[111]

The prevalence of HSV-2 is highly variable and depends on the country, region, sex, age, population subgroup, and whether a population is "high risk."[273] Because transmission of HSV-2 is associated with sexual activity, the prevalence of HSV-2 begins to increase with adolescence. Now that serologic methods can be used to differentiate HSV-1 and HSV-2 infection, the seroprevalence of HSV-2 in adults has been shown to range from 20 to 80 percent. The comprehensive investigation of HSV-2 epidemiology in the United States by the National Health and Nutrition Examination Survey (NHANES) documented a 30 percent increase in prevalence from the 1970s to the 1990s, which has resulted in HSV-2 infection in approximately 20 percent of adults.[55,104] An increasing frequency of HSV-2 infection was observed at all ages above 12 years, including teenagers of all ethnicities, and its prevalence rose fivefold in white teenagers. High HSV-2 infection rates are documented in higher as well as lower socioeconomic groups.[40,165,167,297] Rates are consistently higher in women than in men, regardless of other variables, and in individuals with more sexual partners.

Changes in the epidemiology of symptomatic HSV-2 infection have been suggested by rising numbers of medical visits for genital herpes. Approximately 300,000 new cases occur each year in the United States. The extent to which the increase in symptomatic cases of HSV-2 infection reflects a true increase in the prevalence of HSV-2 or a higher likelihood that HSV-2 infection will result in symptoms is not certain; for example, a decrease in the number of young adults with HSV-1 infection acquired in childhood could be associated with loss of cross-reactive HSV immunity and an increased risk for the development of symptomatic genital herpes. A higher risk of contracting genital HSV-2 infection is associated with lower socioeconomic status, early age at first intercourse, increased numbers of sexual partners, female sex, previous marriage, urban living, black race, and incidents of trichomoniasis, bacterial vaginosis, and other sexually transmitted diseases.[104,119,146]

A retrospective analysis of genital HSV cultures obtained from a group of college students over the course of a 9-year period showed that HSV-1 is an increasingly common cause of genital herpes in some populations in the United States. In this particular population, HSV-1 accounted for more than 70 percent of positive genital cultures by the end of the study period, as compared with approximately 30 percent at the beginning.[233]

Symptomatic disease occurs in a minority of individuals after newly acquired infection at an estimated incidence of 10 to 20 percent or less. However, silent infections can give rise to symptoms that appear months or years later as a result of viral reactivation. The epidemiology of HSV-1 or HSV-2 reactivation as a cause of symptomatic illness depends on the prevalence of these infections in the population as a whole, but it also is influenced by host factors that perturb the balance between the virus and the infected individual. For example, HSV-related symptoms may be triggered by exposure to sunlight (ultraviolet), febrile illnesses, immunosuppression from illness or necessary therapies, and other variables. These variables are cofactors that affect the frequency of HSV-related disease within the infected cohort but not the epidemiologic pattern of infection.

PATHOGENESIS AND PATHOLOGY

HSV-1 and HSV-2 exhibit particular tropism for cells of ectodermal origin, including skin and neuronal cells. Initial viral replication is thought to occur at the portal of entry, usually in mucous membranes or skin. In symptomatic cases, the incubation period for primary infection appears to vary from 2 to 20 days. In contrast to varicella, HSV viremia is difficult to detect by culture in a normal host, although it may be found in some immunocompromised patients.[127,285] HSV DNA in plasma and peripheral blood mononuclear cells in HSV-infected neonates has been detected with PCR techniques.[86] If mucocutaneous lesions are induced during primary or recurrent infection, the pathologic cell changes caused by HSV-1 or HSV-2 replication include cytoplasmic enlargement and nuclear alterations; in addition, cell fusion may lead to the formation of multinucleated giant cells. The nuclei of infected cells often have eosinophilic intranuclear inclusions and marginated nuclear chromatin. As the cells manifest injury, a local inflammatory response ensues, intercellular edema develops, and vesicles form in the affected area. The vesicles become visible as they enlarge and usually are surrounded by an erythematous margin. At later stages, the vesicles become pustular and then dry and crust. HSV lesions typically are super-

ficial and do not scar. Vesicles that form on mucous membranes are transient, with rapid sloughing of the superficial layer, and they generally are seen first as shallow ulcers.

HSV-1 and HSV-2 have a characteristic ability to establish latency in neurons of the sensory ganglia by mechanisms that as yet are unidentified. The viruses persist in this latent state for various intervals; reactivation induces viral replication with the production of infectious virus at mucosal or other sites. Evidence related to a possible neural site for latent HSV was presented nearly 80 years ago when Cushing reported the appearance of herpetic vesicles in the trigeminal distribution after rhizotomy of the trigeminal nerve and destruction of the sensory ganglion.[217,333] Persons who have recurrent HSV infections frequently describe tingling sensations, itching, and burning at the site of recurrence beginning several hours before the appearance of clusters of vesicles. Persons who have recurrent genital herpes may experience severe shooting pain in their legs and even urinary retention before and in connection with recurrent genital herpes infections. Occasionally, recurrent HSV skin eruptions may occur in a zosteriform pattern and in a distribution reflecting the sensory innervation of a particular dermatome.

Replication at the portal of entry appears to result in infection of sensory nerve endings, followed by transport of the virus to the dorsal root ganglia along neuronal axons. Latency is established regardless of whether the primary infection is symptomatic, and it appears to be an invariable consequence of HSV infection with either virus type.[26,234,235] HSV-1 and HSV-2 persist in neuronal cells of the sensory ganglia, with substantial numbers of these cells harboring latent virus, as demonstrated by the detection of latency-associated transcripts. The latency-associated transcripts overlap the viral genes *ICPO* and *ICP34.5* in an antisense direction.[73,235,287] The role of these transcripts in the establishment or maintenance of latency remains to be clarified. The presence of latency-associated transcripts appears to be necessary for efficient reactivation of viral production in vivo.[219] Viral particles are not produced in latently infected cells. HSV-1 persists most predominantly in the cranial nerve ganglia, whereas HSV-2 latency occurs in the lumbosacral ganglia. Co-cultivation techniques have been used to recover HSV from the dorsal root ganglia innervating the areas of skin in which persons have experienced recurrent herpes lesions. HSV-1 has been found in trigeminal ganglia, and HSV-2 has been recovered from sacral ganglia.[28] Once latency is established in the sensory ganglia, antiviral drugs cannot eradicate the latent virus from infected neurons. Because the latent virus does not multiply, it is not susceptible to drugs that affect viral DNA synthesis, such as acyclovir. When reactivation is triggered, HSV-1 or HSV-2 is transported back down axons to mucocutaneous sites, where they replicate and release infectious virus into secretions.[26,73,234,235] Individuals with symptomatic recurrent HSV infections almost always have HSV lesions in the identical or a directly adjacent site. Reactivation is not prevented by adaptive immunity, although whether symptoms occur may be influenced by the host response.

The stimulus for reactivation of latent virus may be provided by iatrogenic or naturally occurring episodes of immunosuppression and by endocrine (such as menstruation) or exogenous factors (such as trauma, sun, acupuncture, emotional stress). Ultraviolet irradiation of persons who have a history of recurrent oral herpes reliably induces recurrence either quickly (within 48 hours) or 2 to 7 days later.[276] Skin subject to recurrent HSV infection has been transplanted elsewhere on the body and exchanged with skin taken from the site of graft placement and previously not involved in HSV infections. Subsequent recurrence of HSV infection was found to be localized to the original site, not to the original skin. These studies and others strongly suggest that latent virus does not reside in skin cells.

Primary HSV infection elicits humoral and cellular immunity, which can be detected shortly after the appearance of lesions in individuals in whom symptoms develop. Based on animal models and some human studies, the initial host response is mediated by innate, nonspecific mechanisms, followed by the acquisition of virus-specific adaptive immunity.[157] The nonspecific response consists of mobilization of polymorphonuclear leukocytes and monocytes to the site of infection, release of interferon-α and other cytokines, and activation of macrophages and natural killer cells. The innate response is followed by the production of antiviral antibodies that can be detected 2 to 12 weeks after infection occurs. HSV-specific antibodies mediate neutralization of virus, complement fixation, and cellular or complement cytotoxicity. Induction of specific cellular immunity is detected by measuring T-cell recognition of HSV proteins in proliferation and cytotoxicity assays, production of interleukin-2 and interferon-γ, and a positive delayed hypersensitivity skin test reaction. Failure of virus-specific, cell-mediated immunity to develop, as may occur in newborns and children with genetic immunodeficiencies, immunocompromised children, and other high-risk populations, can be associated with life-threatening dissemination of HSV-1 or HSV-2. If HSV disseminates, these viruses can infect and destroy cells in many organs (e.g., the lungs, liver, adrenals, and brain), with catastrophic effects.

Recently, a small group of immune-seronegative individuals with HSV-specific T-cell responses have been described. None of these individuals had clinical symptoms of oral or genital HSV. All had exposure to HSV-2–positive sexual partners, but HSV was never isolated by culture or PCR from these exposed individuals, even after extensive sampling over a prolonged period.[222] Studies to determine whether genetic immunologic determinants of disease or mutations in HSV entry receptors alter susceptibility to HSV infection are in progress.[294]

Symptomatic HSV-1 and HSV-2 recurrences are less severe than are primary lesions in immunologically intact persons. In persons previously infected with one type of virus (e.g., HSV-1), new infections with the second type (e.g., HSV-2) more often are silent, or the symptoms are less severe than in a host who has never been infected with either virus. When re-infection with unrelated strains has been identified, symptoms also have been mild and, without molecular analysis of viral DNA, would be attributed to reactivation of endogenous virus.[50] The immune response to symptomatic reactivation is not associated with a significant increase in the production of antibody, although fourfold rises and re-emergence of IgA and IgM antiviral antibody may occur. Natural killer cell activity and cytokine production increase, and relative defects in these and HSV-specific T-cell responses may predispose the individual to frequent or severe symptomatic recurrences, but definitive associations have not been established. Patients who have diseases or are being treated with agents that reduce cell-mediated immunity, such as those undergoing antitumor chemotherapy or individuals with acquired immunodeficiency syndrome (AIDS), often have frequent HSV recurrences that are longer in duration and more severe, but dissemination rarely occurs. Nonetheless, no evidence suggests a specific immunodeficiency in otherwise healthy persons who experience symptomatic HSV recurrences.

Viral encephalitis is the most severe consequence of HSV infection in an otherwise healthy host. HSV-1 is the pathogen in almost all these cases, and the pathologic mechanism is thought to be ascending infection along neuronal pathways from the cranial nerve ganglia to the brain.[79] Encephalitis may accompany or develop after primary HSV infection, but it also occurs as a consequence of reactivation of latent virus. When the virus reaches brain parenchymal cells, it replicates efficiently and induces widespread hemorrhagic necrosis and vascular compromise. The neuro-immune response to infection contributes to brain inflammation and subsequent sequelae. Activated glial cells (astrocytes and microglia) respond to HSV infection with the

production of cytokines and chemokines.[188] HSV encephalitis has been associated with an acute-phase elevation in β_2-microglobulin, neopterin, interleukin-6, and interferon-γ in cerebrospinal fluid (CSF).[20,21] During convalescence, increased levels of soluble CD8, β_2-microglobulin, neopterin, and specific anti-HSV IgG have been detected. How these markers relate to the pathogenesis of disease has not been established.

CLINICAL MANIFESTATIONS

Most HSV-1 and HSV-2 infections do not cause symptoms, but when infection is symptomatic, the clinical manifestations usually are self-limited and not severe. If symptoms occur, the disease associated with primary infection tends to be much more extensive than are the minor, localized lesions at mucocutaneous junctions caused by viral reactivation. Nonetheless, prospective studies document that new HSV infections can be as mild as symptomatic recurrences, and as a result, definitive differentiation of primary from recurrent infection is not possible with clinical criteria.

GINGIVOSTOMATITIS

Gingivostomatitis is the most common form of HSV-induced primary illness in children. A history of such symptoms has been reported in as few as 1 percent to as many as 31 percent of seropositive children, the higher percentage being from a study involving the Navajo Indians. It usually is seen in young children between 10 months and 3 years of age. In those younger than 10 months old, residual maternal antibody probably modifies or prevents the appearance of recognizable symptoms in association with primary HSV-1 infection. Although acute gingivostomatitis caused by HSV is a relatively infrequent occurrence, it is sufficiently common that most pediatricians become familiar with the condition and learn to distinguish this infection from herpangina.[214,333]

The illness begins with irritability and fever. Despite these systemic symptoms, HSV is not cultured from blood during this period.[127] More recently, investigators have detected HSV-1 DNA by PCR in whole blood or plasma early in the course of illness in approximately a third of young children with primary HSV-1 gingivostomatitis.[131] The infant usually refuses to eat and may refuse fluids. Thereafter, vesicular lesions appear around and on the lips, along the gingiva, on the anterior of the tongue, and on the anterior (hard) portion of the palate (Figs. 170–1 and 170–2). The vesicles break down rapidly, and when seen, lesions usually appear as 1- to 3-mm shallow gray ulcers on an erythematous base. The gums are erythematous, mildly swollen, and ulcerated. They may appear friable and frequently bleed on contact. The child experiences extreme discomfort, cannot or will not eat, and, if fluids are refused as well, may require hospitalization to ensure that adequate hydration is maintained. The risk of dehydration is compounded by the fever that generally accompanies this syndrome. Vesicles often extend around the lips and chin or down the neck in an immunologically intact child. The child frequently has foul-smelling breath (fetor oris). The lesions bleed easily and may become covered with a black crust. The cervical and submental nodes usually are swollen and tender. The clinical signs continue to evolve for 4 to 5 days, and the process of resolution requires at least an additional week. In an analysis of the natural history of HSV gingivostomatitis in 36 children, oral lesions persisted for an average of 12 days, most children had extraoral lesions, fever lasted for 2 to 6 days, and difficulty taking liquids was noted for 4 to 10 days. The duration of viral shedding was 7 days (range, 2 to 12 days).[5] Herpetic epiglottitis[39] and acute otitis media[60] are unusual complications.

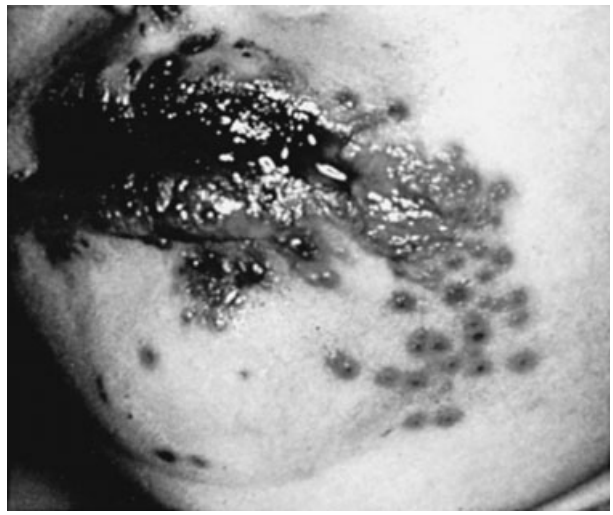

Figure 170–1 Primary HSV gingivostomatitis in a normal toddler: ulcerative-vesicular stage. *(Courtesy of Dr. Theodore Rosen, Department of Dermatology, Baylor College of Medicine, Houston.)*

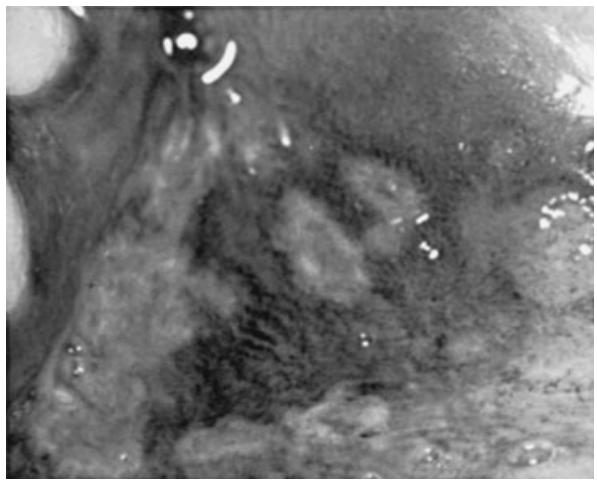

Figure 170–2 Primary HSV gingivostomatitis in a 6-year-old child, demonstrating involvement of the oral mucosa with ulcers.

HSV gingivostomatitis is differentiated from herpangina, a manifestation of enteroviral infection, by the location of ulcers in the anterior and posterior portions of the oropharynx; herpangina generally causes posterior pharyngeal ulcers. In addition, unlike HSV infection, herpangina often has a more acute onset, a shorter duration, and seasonal occurrence.[214] Whereas enterovirus-associated hand, foot, and mouth disease can be manifested as oral ulcers and a vesicular eruption on the distal portions of extremities, the bilateral distribution should differentiate it from HSV gingivostomatitis and concurrent HSV auto-inoculation of a digit. Severe Stevens-Johnson syndrome (erythema multiforme) may mimic HSV infection, but the generalized macular rash accompanied by "bull's-eye" lesions is characteristic of erythema multiforme. Rarely, recurrent HSV infection can be associated with erythema multiforme, as discussed later. Impetigo may be confused with the lesions of HSV infection, and misdiagnosis is re-enforced because colonization of skin by *Staphylococcus aureus* may be identified in bacterial cultures and be considered causative.

Parents and caregivers are familiar with "cold sores" or "fever blisters" but may not know that these lesions are caused by HSV.

Because HSV infection may be thought of as a sexually transmitted disease, the physician is advised to anticipate confusion and anxiety when making the diagnosis of HSV oral infection and to address these concerns by explaining the normal mode of acquisition of oral HSV infection by young children.

In adolescents, primary HSV-1 infection may be seen initially as a posterior, occasionally exudative pharyngitis.[193] The characteristic findings are shallow tonsillar ulcers with a gray exudate. In this setting, HSV infection must be differentiated from streptococcal and Epstein-Barr virus infection and rarely from diphtheria, acute human immunodeficiency virus (HIV) infection, and tularemia-induced pharyngitis. In one study of college students of a higher socioeconomic level, HSV was the most common etiologic agent of acute pharyngitis (24%).[113] A more recent study of 613 college students with upper respiratory complaints documented an incidence of 5.7 percent with positive HSV cultures. Twelve of the 35 students with positive cultures had vesicular lesions on their lips, throat, or gums, and 29 of the 35 had a primary diagnosis of pharyngitis that was indistinguishable from other causes of pharyngitis.[193] In some cases, acute pharyngitis is caused by HSV-2; the symptoms and clinical course appear to be similar, and viral cultures are required to identify the type of infecting virus.

VULVOVAGINITIS

Primary herpetic vulvovaginitis occurs rarely in infants and children. HSV-1 may cause this clinical syndrome, perhaps if inadvertently introduced when the genital area is touched by a caregiver with HSV on the hands. Progression of the infection may be limited to a few lesions, or it may resemble symptomatic primary genital herpes caused by HSV-2. Obtaining material for culture to identify the type of infecting virus is important because HSV-1 infection is less likely to recur and HSV-2 infection may reflect sexual abuse of the child. As is true of other potentially sexually transmitted diseases, genital HSV infection in young children warrants a careful and sensitive appraisal of the circumstances that may have led to the infection. Also useful is knowing that some children infected with HSV-2 in the newborn period have had genital lesions later in childhood.

Pediatricians are likely to encounter genital herpes in their adolescent patients. Reports on the incidence of genital herpes in which data are provided for subgroups of children and adolescents are limited, but a rate of 3 per 100,000 in the 10- to 14-year-old age group and 76 per 100,000 in the 15- to 19-year-old age group was reported in a Minnesota study of a predominantly white, middle-class population of northern European ethnicity.[61] The NHANES study reported the prevalence in 12- to 19-year-old subjects to be 5.6 percent[104]; however, recent NHANES data show a decrease in HSV-2 seroprevalence in this age group.[346] In one report of 379 adolescents aged 14 to 19 years who were treated at a sexually transmitted disease or urban community clinic, 12 percent had HSV-2 antibodies and only 22 percent of these patients had a history of genital herpes. HSV-2 seropositivity correlated with African American race or female gender but not with condom use, the number of sexual partners in the previous 2 months, or a previous history of a sexually transmitted disease, thus indicating that prevalence was related to demographic rather than behavioral variables.[295]

In contrast to earlier concepts, prospective studies have documented that many infections are subclinical and that new genital HSV-2 infections have a wide range of manifestations.[177,195] Variation is common with respect to the extent and severity of lesions; whether the lesions are bilateral or localized; the presence or absence of systemic symptoms such as malaise, myalgia, headache, and fever; dysuria; and regional lymphadenopathy. Symptoms can be mild enough to suggest recurrent infection or may be those of severe, "classic" primary genital herpes. In such cases in adults, local genital symptoms include severe pain (in 95% of men and 99% of women), itching, dysuria (44% of men and 83% of women), vaginal or urethral discharge, and tender inguinal adenopathy (80%). The lesions begin as vesicles or pustules and progress to wet ulcers and then to healing ulcers with or without crusts (Figs. 170–3 to 170–5). Crusting usually occurs only on squamous epithelium. Lesions tend to last for 2 to 3 weeks (mean, 19 days) before complete healing occurs. They may spread in a wavelike fashion from the initial site to the thighs, buttocks, and urethra. Virus shedding generally persists for 1 to 2 weeks or longer. Some primary infections are associated with atypical lesions (e.g., fissures, furuncles, excoriations), and extragenital lesions, typically on the buttocks, may be observed.[34,165]

When evaluating potential primary genital herpes in a sexually active adolescent, the history may reveal recent contact with a partner who is known to have recurrent genital herpes. When exposure can be documented, the incubation period is estimated

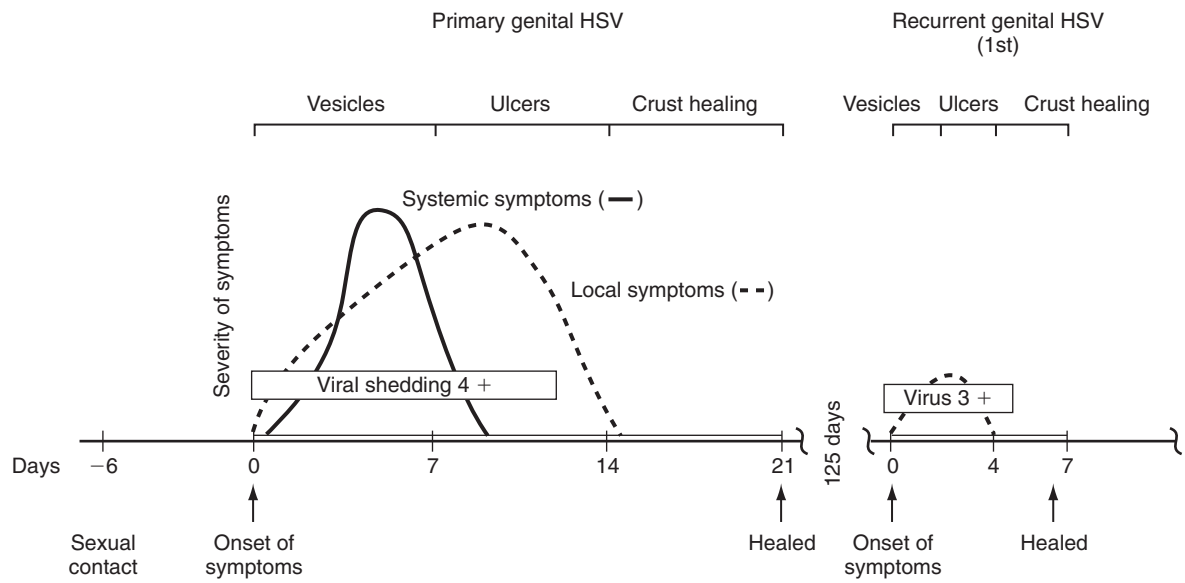

Figure 170–3 Graphic representation of the course of primary and recurrent HSV genital infection. The days illustrated are for average cases.

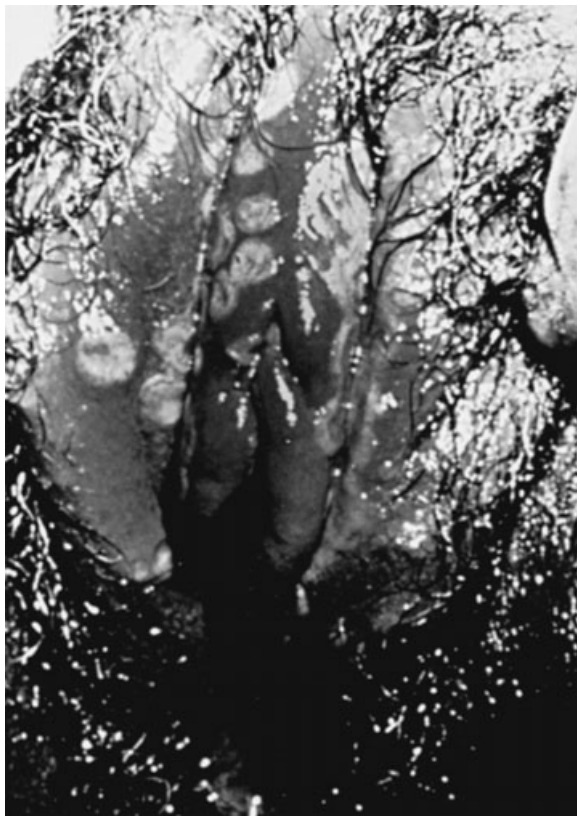

Figure 170–4 Primary genital herpes in a female. *(Courtesy of Dr. Theodore Rosen, Department of Dermatology, Baylor College of Medicine, Houston.)*

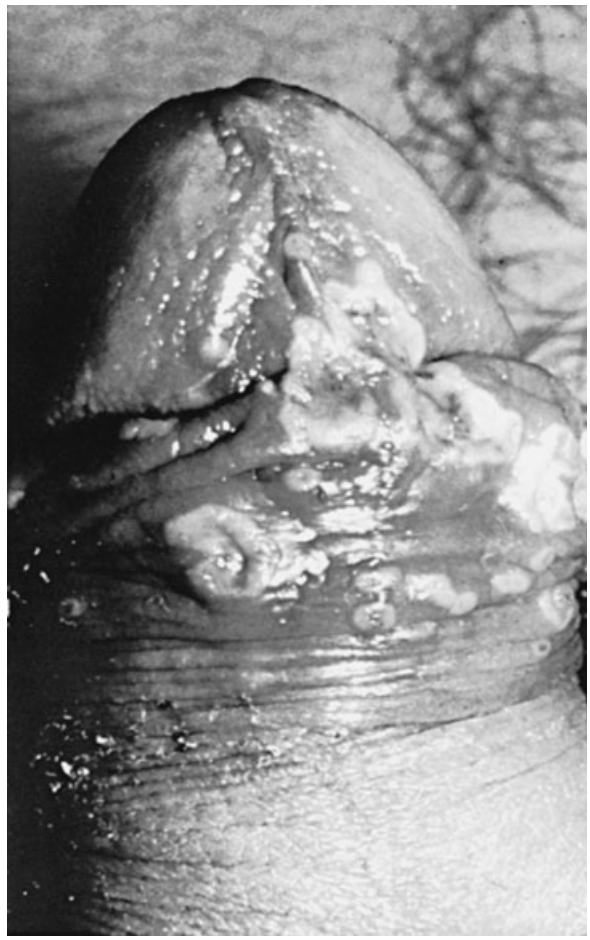

Figure 170–5 Primary genital HSV infection in a male. *(Courtesy of Dr. Theodore Rosen, Department of Dermatology, Baylor College of Medicine, Houston.)*

to be 2 to 14 days. However, most of these episodes will not be associated with a known exposure. In addition, recognizing that as many as two thirds of first-reported episodes of symptomatic genital herpes actually are caused by reactivation is important. In these cases, the exposure may have occurred months or years before the signs of HSV-2 infection appear. In some adult series, 50 to 60 percent of persons without a history of HSV infection can identify symptoms consistent with previous infection after receiving careful instruction about the typical signs, but many have not had any previous episodes.[175] HSV-1 accounts for 5 to 25 percent of primary genital HSV infections in the United States, whereas as many as 50 percent of cases diagnosed in other geographic areas, such as England and Japan, are caused by HSV-1. Indications are that HSV-1 is becoming a more common primary genital isolate in the United States. In recent years, HSV-1 has been isolated from genital cultures more often than HSV-2 has in some populations.[233] Patients with primary HSV-1 genital infection may exhibit lesions elsewhere (25%), typically on the hands and face.[34]

Primary genital HSV-2 infections may be associated with viral meningitis. This complication is manifested as fever, stiff neck, headache, and photophobia with a CSF lymphocytic cellular response and usually a normal glucose level.[134,303] HSV-2 can be isolated from CSF in the early phase of the illness. HSV-2–associated meningitis differs from HSV-1 encephalitis in that it is almost always mild, self-limited, and not associated with neurologic residua.

Other complications of primary HSV genital infection include sacral autonomic nervous dysfunction, which is manifested as poor rectal sphincter tone, constipation, sacral anesthesia, urinary retention, or impotence; extragenital lesions, which occur more commonly in women and occur on the buttocks, groin, thighs, or less commonly, the fingers or conjunctiva, generally in the second week of disease; secondary yeast infections in women; and pharyngitis, which usually is associated with fever, malaise, myalgia, headache, tender anterior cervical adenopathy, and a mildly erythematous to diffusely ulcerative or exudative posterior pharyngitis. Most of these patients have throat cultures positive for HSV. HSV has been associated uncommonly with acute salpingitis[181] and inguinal lymphadenitis.[300]

Of critical importance has been the association of previous HSV infection with an increased incidence of HIV infection.[283] A recent literature review and meta-analysis showed that serologically documented HSV-2 infection is a significant risk factor for the acquisition of HIV. The analysis suggests that the risk of acquiring HIV doubles in persons infected with HSV-2. The possibility that the risk of infection with HIV is enhanced by mucosal ulcers or that the increase in local $CD4^+$ lymphocytes resulting from HSV infection offers a greater number of target cells for HIV attachment and entry has been considered. Conversely, individuals with HIV infection reactivate HSV frequently and also have HIV present in mucosal ulcers.[322] A study from Kenya showed that HSV shedding was associated with significantly higher vaginal and cervical HIV shedding, even after controlling for plasma HIV load and $CD4^+$ count.[25] Although all patients with HSV-2 genital infection appear to be at higher risk for acquiring HIV, those with recent HSV-2 seroconversion appear to be more susceptible.[232,304]

Transmission to the newborn is a complication of primary or recurrent genital herpes in women of child-bearing age. This complication is discussed in the chapter on viral hepatitis of the fetus and newborn.

Genital herpes creates significant psychological difficulties for many patients. Whereas many people cope well with the illness and the likelihood of recurrent disease, a syndrome of profound depression, poor self-esteem, complete abstention from sexual activities, and general withdrawal develops in some. This reaction, or "leper syndrome," must be anticipated and discussed in a sensitive and caring manner. Information about the high prevalence of these infections in the population may be helpful, with emphasis placed on the fact that most infected people do not realize that they are infected and shed the virus intermittently, just like those who have had symptoms. Self-help groups of persons who have genital herpes exist in many parts of the United States and can be contacted through the American Social Health Association, Herpes Resource Center, P.O. Box 13827, Research Triangle Park, NC, 27709, *www.ashastd.org*, and the American Herpes Foundation, *www.herpes-foundation.org*.

OTHER PRIMARY SKIN INFECTIONS

Mucocutaneous junction areas are common sites of HSV infection, and damaged skin often provides a portal of entry for HSV. Vesicular lesions spread throughout the affected skin and usually crust and resolve in approximately 1 week. The illness accompanying eczema herpeticum can be severe (Fig. 170–6) and even fatal, although in most cases it resolves without specific therapy and leaves no sequelae. Increased use of topical calcineurin inhibitors has raised concern regarding possible immune suppression and an increase in cases of eczema herpeticum. One study showed the incidence density of herpesvirus skin infections to be similar in infants who received 1 percent picrolimus cream and those who received the vehicle without active drug.[215] However, of those children characterized as having eczema herpeticum (more extensive skin involvement), all infants were in the group receiving 1 percent picrolimus cream. Further studies are needed to determine the magnitude of risk, if any, of the use of topical calcineurin inhibitors in the development of eczema herpeticum. Current recommendations include discontinuing topical calcineurin inhibitor therapy and beginning appropriate antiviral therapy if eczema herpeticum develops.[215]

Widespread herpetic lesions may occur in skin altered by thermal or chemical burns. In this situation, a secondary fever may occur, usually 1 to several weeks after the initial insult. Careful inspection of the burn site or adjacent normal tissue may reveal vesicles (Fig. 170–7) or nonspecific ulcerative lesions. Without therapy, several of these patients have died of disseminated HSV infection.[105] A similar syndrome occurs rarely in children after they have incurred simple skin abrasions (Fig. 170–8). Herpetic recurrences may follow in these cases, but recurrences are localized to the area of skin that was affected initially. HSV infection can be severe as a secondary infection at the site of common diaper rash.[144]

Herpetic whitlow is a painful, erythematous, swollen lesion that occurs on the terminal phalanx, sometimes associated with a damaged cuticle.[31,112,237] The fingers (69%) and thumb (21%) are involved most frequently.[112] Less commonly, the palm may be the site of inoculation and major involvement.[256] The digit is swollen, and the painful white swelling appears to be filled with

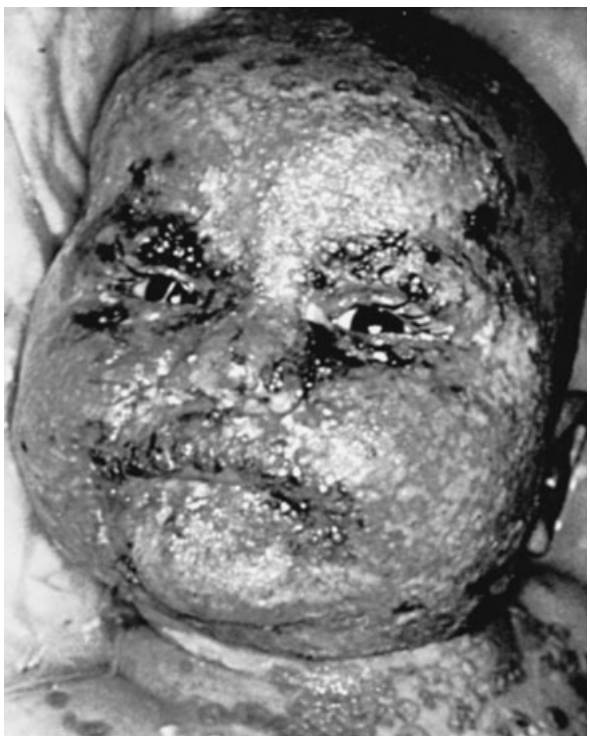

Figure 170–6 Extensive HSV infection in an infant with atopic eczema (Kaposi varicelliform eruption). *(Courtesy of Dr. Theodore Rosen, Department of Dermatology, Baylor College of Medicine, Houston.)*

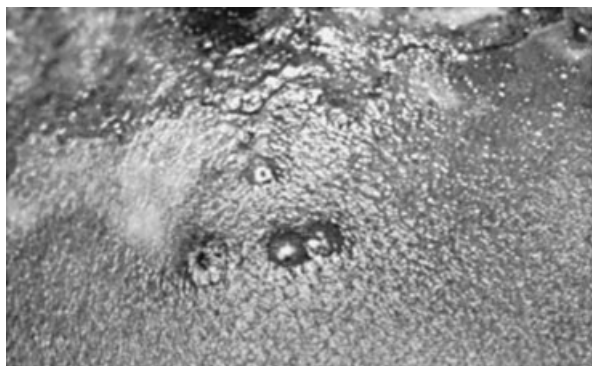

Figure 170–7 Secondary HSV infection at a burn site in a 2-year-old child. Note the vesicles at the border of the burn.

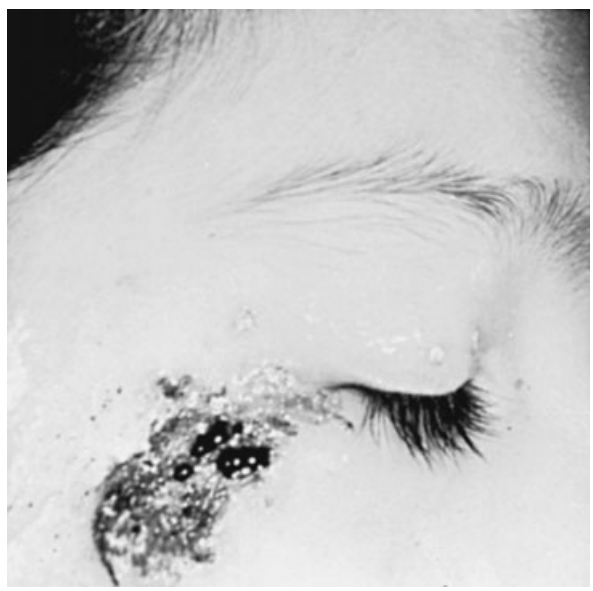

Figure 170–8 Facial HSV infection in a young girl after a mild abrasion. *(Courtesy of Dr. Johnie Frazier, Department of Pediatrics, University of Texas Medical School, Houston.)*

pus but, when opened for drainage, is found to contain little fluid and no purulent material. The white appearance is caused by the presence of necrotic epithelial cells. Occasionally, the whitlow, which may persist for 7 to 10 days, is accompanied initially by a few vesicles that may give a clue to the etiologic agent of the primary infection. Less commonly, the whitlow is associated with fever, lymphadenopathy, and lymphangitis.[251]

Whitlow is seen in four typical situations. Most commonly, infants with primary herpetic gingivostomatitis auto-inoculate their fingers (Fig. 170–9). At times, whitlow is encountered in infants without obvious oral disease, and sometimes it may be caused by adults kissing their children's fingers. In these settings, the viral isolate almost always is HSV-1.[31,112] In sexually active patients, the whitlow more commonly is a manifestation of concurrent genital disease, which should be sought by appropriate history and physical examination.[114] These infections are caused most frequently by HSV-2. In the fourth setting, persons such as dentists, respiratory therapists, nurses, and pediatricians who examine oral cavities or handle secretion-contaminated material without wearing gloves are at risk for acquiring herpetic whitlow (Fig. 170–10).[100] In addition, the same poor medical practice facilitates transfer of HSV to other patients, especially in intensive care settings.[2]

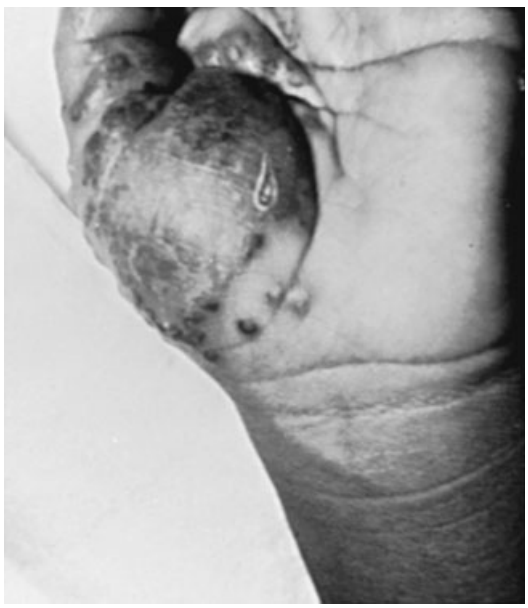

Figure 170–9 Extensive herpetic whitlow in a toddler with oral HSV infection.

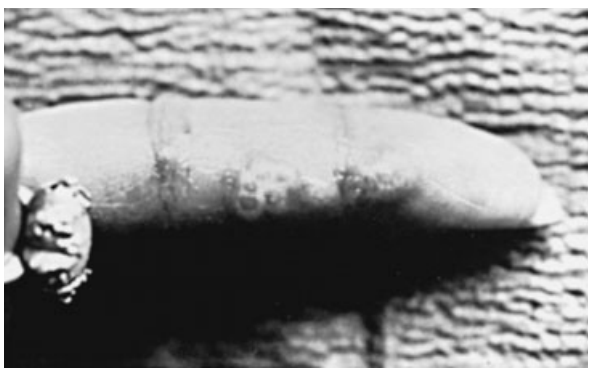

Figure 170–10 Recurrent herpetic whitlow in a pediatrician.

Herpetic whitlow frequently is confused with bacterial felon or paronychia, and such confusion may lead to the incorrect intervention of incision and drainage. This procedure is not indicated and is not beneficial in the management of herpetic whitlow, which should be treated with oral acyclovir. Needle aspiration and culture provide the diagnosis of whitlow, and initiation of antiviral therapy can be based on clinical signs.

In several sports, cutaneous (especially facial) HSV infection is a hazard, particularly in sports with close physical contact such as wrestling (herpes gladiatorum) and rugby (scrum-pox, derived from the line-up of rugby players, the "scrum" or "scrummage").[33,56,267,269,332] Frequently, the initial lesions are diagnosed as impetigo, unless HSV is considered. HSV infection develops in approximately 3 percent of wrestlers in high school, typically on the head and neck but also on the extremities and trunk and in the eyes.[56]

INFECTION OF THE EYE

Ocular herpes may occur as a result of primary infection or reactivation of virus in the trigeminal ganglion. Primary infection may be manifested as blepharitis or follicular conjunctivitis. The symptoms often are accompanied by preauricular lymphadenopathy, corneal injection, watering, discharge, itching, lid swelling, and, in a third of cases, malaise and fever. If restricted to the conjunctivae, the infection (which can be accompanied by vesicular herpetic lesions elsewhere on the face or in the nose or mouth) usually resolves without sequelae or specific features. Herpetic infection of the eye may, however, progress to involve the cornea, with far more serious potential consequences. For this reason, an ophthalmologic consultant always should examine and evaluate these cases.

Corneal involvement by HSV may be manifested initially by minute vesicles at the corneal margin. Progression of the corneal infection (best seen with the use of topical fluorescein dye) is marked by the appearance of branching lesions (a dendritic pattern) or the less diagnostic irregular (ameboid or geographic) ulcer.[32,76] The child complains of severe photophobia accompanied in some cases by blurred vision, chemosis, and lacrimation. Primary eye infection may include stromal involvement, uveitis, and rarely, retinitis.[216] Retinitis is manifested as multiple whitish yellow, punctate retinal lesions. Spontaneous healing, which generally requires 2 to 3 weeks, can be speeded by the application of topical antiviral therapy. Corticosteroids are contraindicated. The risk of visual impairment caused by direct viral damage, immunopathologic reactions, or both is enhanced greatly with recurrences. With each bout of infection, the corneal ulcers become more extensive and can result in scarring and impairment of sight. Herpetic infection of the eye recurs in approximately 32 percent of patients who have primary symptomatic infection and occurs more commonly in younger patients.[342] Recurrences may be manifested as blepharitis, follicular conjunctivitis with or without lid lesions, or ulcers and keratitis, which more often are accompanied by ulcerations deeper into the corneal stroma (diskiform or necrotizing keratitis) with resultant extensive scarring, irregular astigmatism, and even corneal perforation.[342] Children with dendritic ulcers have a better prognosis for good vision than do those with geographic or diskiform keratitis.[32]

Rarely, HSV eye infection may result in acute retinal necrosis. HSV-2 is the most common cause of acute retinal necrosis identified in childhood. Most affected children have a history of congenital or perinatal herpes infection.[174] Many patients have preexisting chorioretinal scars. Reactivation is triggered by periocular trauma or occurs with immunosuppression, such as during treatment with systemic steroids.[306] Symptoms include eye irritation and photophobia.

Very infrequently, an oculoglandular syndrome of conjunctivitis and adenopathy may be caused by HSV infection.[52]

INFECTIONS OF THE CENTRAL NERVOUS SYSTEM

HSV is the most common identifiable cause of serious or life-threatening sporadic encephalitis.[183] It accounts for 2 to 5 percent of all cases of encephalitis in the United States and for as many as 20 percent of all cases with an etiologic diagnosis (60% to 70% of cases of encephalitis have no established cause).[228] With the advent of immunization for measles, mumps, rubella, and varicella, the relative incidence of HSV in cases of encephalitis has increased, although absolute numbers remain stable in children.[162,183,228] The case-fatality rate associated with untreated HSV encephalitis is approximately 70 percent,[340] and survivors generally have severe and permanent neurologic disability. Both HSV-1 and HSV-2 have been implicated in the etiology of central nervous system (CNS) infection by HSV, but typical HSV encephalitis in patients outside the neonatal age range is caused by HSV-1.[72,255,333] Spread of HSV-1 to the CNS seems to proceed via either neurogenic pathways or hematogenous dissemination or perhaps through the cribriform plate from infected nasopharyngeal mucosa during primary infection. Recurrent infection probably results from spread via sensory neurons. HSV-2 meningitis is not associated with brain parenchymal infection; whether the infection is a consequence of hematogenous delivery or spread along neuronal pathways is not known.

Although HSV encephalitis may involve virtually any area of the brain, this infection has a striking tendency to involve the orbital region of the frontal lobes and, with particular frequency, portions of the temporal lobes. Johnson and Mims have suggested that the predilection of HSV to involve regions of the brain governing olfaction suggests that a pathogenetic pathway proceeds from the nasal-respiratory mucosa via the olfactory bulbs and along the subsequent tracts into the brain.[147] Other researchers have suggested that reactivated virus travels from the trigeminal ganglia via fifth nerve fibers to the meninges of the anterior and middle fossae.[79,80] More recent studies in children involving diagnosis by PCR assay and magnetic resonance imaging (MRI) have defined cases with more diffuse cerebral involvement.[257,333]

HSV encephalitis must be differentiated from HSV meningitis, which usually is caused by HSV-2 and is a complication of primary genital infection. In HSV meningitis, symptoms and signs of meningitis, including headache, photophobia, and stiff neck, appear before or shortly after genital lesions are noted. The signs are similar to those of other acute viral meningitides such as enteroviral meningitis. This syndrome may occur in children as well as adults.[89] HSV-2 meningitis may develop less commonly in the absence of genital lesions[259] or rarely in neonates.[258] Seizures and focal CNS findings generally are absent. Examination of CSF reveals lymphocytosis (with 300 to 2600 white blood cells) and may demonstrate low glucose levels. In cases of HSV meningitis, in contrast to HSV encephalitis, virus can be cultured from CSF,[14,72,134] and HSV PCR assay also may be diagnostic.[259] Recovery usually is complete without specific therapy, but with the availability of effective antiviral agents, HSV meningitis should be treated with acyclovir. HSV meningitis may reappear with genital recurrences. Studies using PCR DNA detection analysis have shown that HSV is the major agent responsible for benign recurrent lymphocytic meningitis, also known as *Mollaret meningitis*.[301] These adult patients had three to nine attacks of recurrent lymphocytic meningitis, with 48 to 1600 cells per liter, normal glucose, and protein concentrations of 41 to 240 mg/dL in CSF. PCR analysis detected HSV-2 and, less commonly, HSV-1. Acute viral meningitis caused by reactivation of HSV-1 also has been described in a pre-adolescent child.[67]

HSV encephalitis is a highly lethal disease caused by HSV-1 in 93 to 96 percent of cases.[25,204,333,334,336] It may be a result of primary (30%) or recurrent (70%) infection.[204,333] Although no specific data exist, researchers have suggested that HSV encephalitis is more likely to be associated with primary infection in younger persons because new infections occur more commonly in this age group. One report suggests that primary infection is more likely to be associated with fatal encephalitis. Of 113 cases of biopsy-documented HSV encephalitis, 31 percent occurred in patients younger than 20 years and 6 to 10 percent of patients were between 6 months and 10 years of age.[271,272] Unlike most other common forms of viral meningoencephalitis such as enterovirus or arbovirus infection, HSV encephalitis is not seasonal. It is an acute illness characterized by fever, headache, malaise, irritability, and nonspecific symptoms lasting 1 to 7 days, with progression to the signs and symptoms of CNS involvement in 3 to 7 days and, finally, to coma and death (Table 170–1). A biphasic illness consisting of initial improvement followed by worsening may occur. The signs of HSV encephalitis resemble those of other viral encephalitides, with initial fever and altered behavior.[132] Meningeal signs are uncommon findings. No correlation exists between isolation of HSV from sites extrinsic to the CNS (such as the oropharynx or genital tract) and the diagnosis of HSV encephalitis.[204,335] Thus, the presence of oral or genital lesions is of no help in the establishing or excluding the diagnosis of HSV encephalitis. Nonetheless, if a patient has HSV encephalitis, identical viruses have been isolated from the brain and oral secretions.[337]

The CSF generally reveals pleocytosis, with as many as 2000 white blood cells/mm^3 but usually (80% of the time) more than 50/mm^3. In 90 percent of cases, more than 60 percent of the cells are lymphocytes. Early in the course of infection, neutrophils may predominate. In 75 to 85 percent of cases, red blood cells, reflecting the hemorrhagic necrosis, are seen in the CSF. Between 5 and 25 percent of patients have hypoglycorrhachia, and 80 to 88 percent have elevated protein levels in CSF (median, 80 mg/dL), which rise to striking levels as the disease progresses. Two to 3 percent of patients with early HSV encephalitis have normal CSF.[163,204] Repeat analysis of CSF usually reveals abnormalities consistent with encephalitis. HSV almost never is cultured from lumbar spinal fluid in patients outside the neonatal age range and rarely has been grown from ventricular fluid.[106] Thus, whereas CSF examination is helpful, it is not at all diagnostic of HSV encephalitis. When the CSF of patients with HSV encephalitis

TABLE 170–1 Historical and Clinical Findings in HSV Encephalitis

Historical Finding	Initial Clinical Finding (%)
Alteration of consciousness	97
Memory loss	92
Personality changes	85
Fever	81
Dysphasia	76
Persistent seizures	71
Headache	67
Autonomic dysfunction	60
Personality change	46
Ataxia	40
Seizures	38
Focal	28
Generalized	10
Vomiting	33
Cranial nerve defects	32
Hemiparesis	24
Visual field loss	14
Papilledema	14

is compared with that of patients undergoing biopsy for suspected HSV but with another resultant diagnosis, no differentiating characteristics of CSF are found that could allow one to predict HSV infection accurately. HSV DNA can be detected by the use of PCR assay of the CSF of patients who have HSV encephalitis.[7,22,85,172,239,307]

Neurodiagnostic tests have been of limited assistance. Probably one of the most useful is the electroencephalogram (EEG) (Fig. 170–11).[44] A "typical" pattern of unilateral or bilateral (poor prognosis) periodic focal spikes against a background of slow (flattened) activity (paroxysmal lateral epileptiform discharges [PLEDs]) has been associated with HSV encephalitis. These findings are suggestive but not pathognomonic. Other findings include large-amplitude, irregular slow activity, sharp waves, and variable spikes. In 80 to 90 percent of patients, the EEG is not only abnormal but also localizing. In many cases in the pediatric and adult age groups, the EEG may be one of the earliest localizing laboratory tests.[87,120,161] Less commonly, the results of a radionuclide or computed tomography (CT) scan are abnormal and localizing (50-60% of cases).[150,182] CT results may be characteristic late in the illness and consist of low-density, contrast-enhanced lesions in the temporal area, mass effect, edema, and hemorrhage (Fig. 170–12); early in the illness, when establishing the diagnosis is critical, CT results more often are unremarkable.[120,161,200] Indeed, abnormal CT results are a poor prognostic factor.[200] Reports suggest that MRI findings are more likely to be abnormal at initial evaluation for HSV encephalitis because of its high sensitivity to changes in brain water content[264] (Fig. 170–13) and that MRI is more sensitive than is CT for detection of HSV encephalitis.[84,223] Findings include hyperintensity of the temporal areas on T2-weighted images with gadolinium enhancement. However, even MRI imaging may be normal if performed in the early stages of the disease.[3]

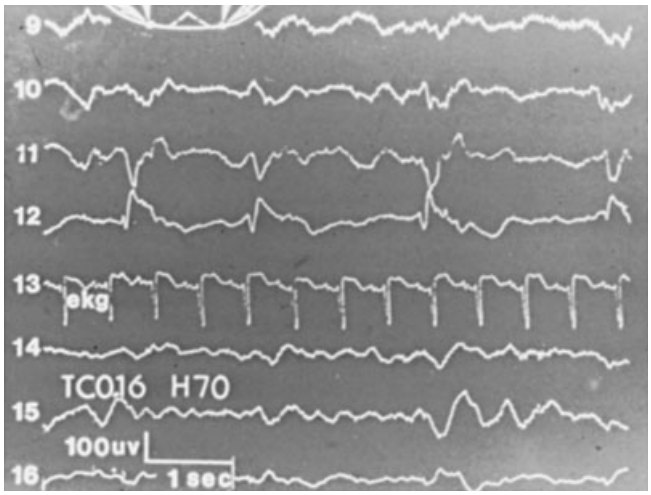

Figure 170–11 Electroencephalogram in a 9-month-old infant with HSV encephalitis. Note the paroxysmal discharges, in lead 12 especially.

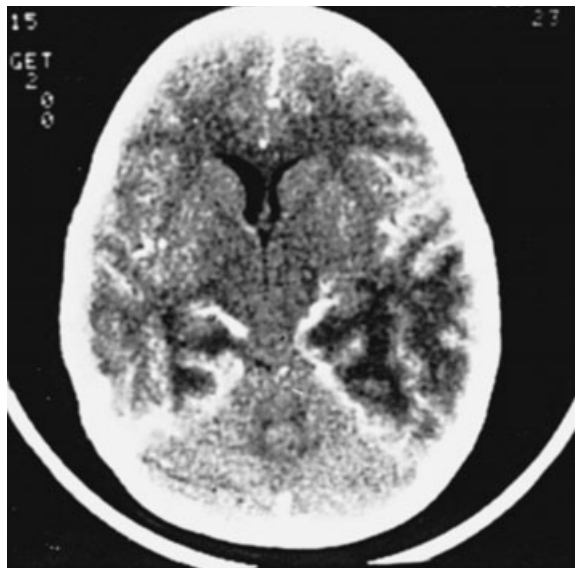

Figure 170–12 Computed tomographic scan 1 week after the onset of HSV encephalitis in a 6-year-old child. Note the bilateral temporal low-density areas with dye enhancement and the greater mass effect on the patient's left side than on the right side.

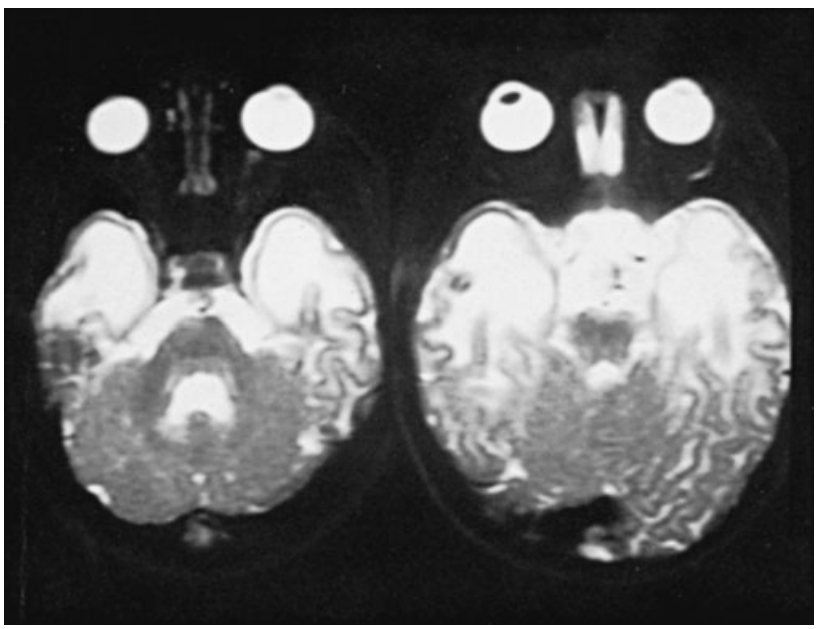

Figure 170–13 Magnetic resonance imaging in a patient with HSV encephalitis. Note the increased signal intensity bilaterally in the temporal lobes. *(From Kohl, S.: Herpes simplex virus encephalitis in children. Pediatr. Clin. North Am. 35:465-483, 1988.)*

Focal abnormalities in HSV encephalitis are significantly more likely to be observed on EEG, CT, MRI, or radionuclide brain scan than in other illnesses confused with it. All these findings are biased by the current concept of HSV encephalitis as a focal encephalitis, with very few biopsy data available on the etiology of nonfocal encephalitis. Whether a significant number of cases of HSV encephalitis are milder and nonfocal is unknown because few of them ever come to brain biopsy or even careful retrospective serologic diagnosis. Studies using MRI and PCR technology have identified cases with multifocal brain involvement[182,257]

Clinical and laboratory evaluations of patients with suspected HSV encephalitis are valuable only for increasing the index of suspicion, not for confirming the diagnosis. In one series of 24 children with HSV encephalitis diagnosed by PCR assay and compared with 38 children in whom HSV encephalitis was excluded by PCR, no significant differences were found in clinical manifestations at onset or in CSF cell counts, protein, or glucose.[142] However, more children with HSV encephalitis had localizing findings detected by CT (75% vs. 31%), whereas 36 percent of those without encephalitis had EEG abnormalities as opposed to no EEG abnormalities detected in those with HSV encephalitis. The differential diagnosis of this condition is relatively large (Table 170–2). Especially in the pediatric age range, the ability to discriminate HSV encephalitis from other etiologic agents mimicking it is poor (50% in the national collaborative series in 71 patients younger than 20 years and 42% in a smaller series of 12 patients younger than 12 years).[161] Confirmation of the specific diagnosis of HSV encephalitis remains essential, both to provide optimal aggressive therapy for that condition and, of equal importance, to achieve a diagnosis for the 50 to 60 percent of patients without HSV infection, roughly 16 percent of whom would benefit from other specific therapies.[87,161,200,335] In most cases, the diagnosis can be established by PCR analysis of CSF.[7,22,122,172,239,257,259,312] Large studies show PCR analysis to be 98 percent sensitive and 94 percent specific when compared with brain biopsy.[172] Some of the 6 percent "false-positive" results probably were due to poor handling of the biopsy tissue for culture. PCR analysis may yield positive results by 1 day after the onset of symptoms.[122] However, a negative PCR result obtained soon after the onset of symptoms does not completely exclude the diagnosis of HSV encephalitis. In one small series of patients, repeat testing of CSF 4 to 7 days after an initial negative HSV PCR assay showed positive PCR results.[329] HSV DNA persists in most specimens for a week or more, even after antiviral therapy.[334] PCR primers must be chosen to detect HSV-2, as well as HSV-1, because 4 to 6 percent of HSV encephalitis cases may be caused by HSV-2.[23]

TABLE 170–2 Differential Diagnosis of HSV Encephalitis

Infections
Fungal
Especially *Cryptococcus*
Bacterial
Abscess, cerebritis
Listeria monocytogenes meningitis
Lyme disease
Subdural, epidural empyema
Tuberculosis
Bacterial endocarditis
Meningococcal meningitis
Protozoal
Toxoplasmosis
Amebic
Rickettsial
Viral
Mumps virus
Coxsackievirus, echovirus
Arbovirus (especially St. Louis, California, and eastern and western equine encephalitis)
Postinfluenzal encephalitis
Reye syndrome
Lymphocytic choriomeningitis virus
Rabies virus
Epstein-Barr virus
Human herpesvirus 6
Rubella virus
Cytomegalovirus
Adenovirus
Tick-borne encephalitis virus
Powassan virus
Subacute sclerosing encephalitis (measles virus)
Progressive multifocal leukoencephalopathy
Non-infectious Disorders
Tumor
Vascular disease
Arteriovenous malformations
Toxins
Alcoholic encephalopathy
Leukemia
Cerebral infarction
Adrenal leukodystrophy

If PCR results are negative in a patient who has symptoms and signs of HSV encephalitis, brain biopsy may be contemplated. The risk associated with brain biopsy is low. In the national collaborative study of 432 biopsies, six complications occurred (hemorrhage in three patients and poorly controlled brain edema in three, for a 1.4% complication rate).[336] Roughly 2 to 3 percent of brain biopsies yielded false-negative results, usually because of biopsy of the wrong site.[336] Decision analysis suggests that performing a biopsy is especially critical in a patient with low CSF glucose levels.[253]

Quantitative PCR analysis may have value as a prognostic tool and as a method of assessing the response to antiviral therapy in patients with HSV encephalitis.[90,334] In 16 patients, those with high copy numbers of HSV DNA in CSF tended to have abnormal CT scans, be older, and exhibit more severe sequelae. HSV DNA levels were found to decrease during acyclovir therapy in seven patients; the exception was a patient whose copy numbers increased and who subsequently had a fatal infection.

A less acute form of HSV encephalitis in immunocompromised patients has been reported. Also less commonly, HSV has been implicated in brain stem encephalitis.[97] Relapse occurs in approximately 5 percent of treated patients.[161] Choreoathetosis may be an initial sign of relapse.[326] Reports of a post-herpetic encephalomyelitis caused by a probable autoimmune or demyelinating etiologic factor have appeared[1,158]; moreover, virus-positive recurrence of HSV encephalitis has been described months after patients have undergone apparently successful therapy.[88] These consequences can be differentiated and documented only by PCR analysis or, if necessary, brain biopsy and appropriate tissue histologic examination and culture.

INFECTION OF THE GASTROINTESTINAL TRACT IN NORMAL HOSTS

Whereas infection of visceral organs is well recognized in immunocompromised hosts, case reports of such infection in apparently healthy persons are less common. Nonetheless, HSV esophagitis has been described in these patients, including several children who were 11 months to 17 years of age.[15,29,108,199,213] HSV-1 is the usual pathogen, and the syndrome is associated with primary infection.[108] Initial symptoms include fever, severe odynophagia, retrosternal and subxiphoid pain, and an inability to eat. Skin lesions generally are absent. Esophagoscopy reveals

ulcerations and fibrinous and, at times, hemorrhagic exudate. Distal involvement may be more extensive than the proximal esophageal findings suggest. Double-contrast esophagography may be diagnostic, although endoscopy and biopsy usually are necessary for establishing a definitive diagnosis. Symptoms generally remit in 5 to 7 days after the administration of nonspecific therapy such as antacids, H_2 blockers, and hydration.[15,199]

HSV infection can be manifested as an anorectal infection in males who have sex with males.[117,225] This syndrome occasionally affects women practicing passive anal intercourse. In most series of HSV proctitis, the younger persons are adolescent males involved in passive anal intercourse. Initial symptoms include severe anorectal pain, discharge, tenesmus, hematochezia, and, in particular, fever, difficulty urinating, sacral paresthesias, constipation, and (in 50-70% of patients) ulcers or vesicles in the perianal or distal rectal area.[117] The duration of symptoms is 2 to 3 weeks. Primary HSV infection accounts for proctitis in this group in 25 to 30 percent of cases. Syphilis and infection with *Giardia lamblia*, *Entamoeba histolytica*, *Campylobacter fetus*, *Shigella* spp., other enteric bacteria, and *Neisseria gonorrhoeae* also must be considered in the differential diagnosis of this entity. Appropriate cultures and histologic analysis are crucial for establishing a specific diagnosis.

HSV is a rare cause of hepatitis in an immunocompetent host, but it has been reported in children.[27] In a review of the literature describing 35 patients who had HSV-associated hepatitis, only 14 percent had no underlying condition. The remainder had various immunocompromising conditions such as transplantation, steroid administration, pregnancy, burns, primary immunodeficiency, or cancer. These patients tended to have fulminant hepatic necrosis, extremely elevated serum transaminase levels, disseminated intravascular coagulation, and a mortality rate of 86 percent.[58] In a prospective study of healthy young adults with genital herpes (primary or recurrent), 14 percent had mild elevations in liver enzyme tests.

RECURRENT INFECTIONS

HSV-1 and HSV-2 have the characteristic capacity of herpesviruses to establish latency and undergo episodes of reactivation despite the presence of an apparently adequate immune response. Persistence is facilitated because latent infection of neurons occurs in an immunologically "privileged" site. Reactivation of HSV infection with viral shedding generally causes no lesions. HSV can be recovered from the pharynx and genital sites of asymptomatic persons. It has been found in the tears of persons with a history of recurrent ophthalmic disease, even in the absence of eye lesions or symptoms. All HSV-2–seropositive women from whom samples of vaginal secretions were obtained for more than 100 days had documented asymptomatic viral shedding, and shedding occurred on 1 percent of the days on which cultures were performed.[325]

Patterns of HSV reactivation include asymptomatic reactivation after silent primary infection, asymptomatic reactivation after symptomatic primary infection, and symptomatic recurrence after either silent or symptomatic primary infection. The risk of having symptomatic recurrences may be higher in individuals who have symptomatic primary infections. When reactivation causes disease, the clinical manifestations of recurrent HSV infection depend on the area involved. Most HSV recurrences are milder than the primary illness, although patients may have symptomatic recurrences without having had clinically apparent primary disease. Recurrent infection in a normal host also may be more severe than is the primary infection. This pattern is particularly evident in HSV infection of the eye, in which recurrent illness is associated with deep stromal damage and scarring.[221] This enhanced severity may be related to a greater extent to a more exuberant immune response than to viral damage. HSV encephalitis, which may be a manifestation of viral reactivation, is devastating.[204,333]

The most common manifestation of recurrent HSV infection is herpes labialis ("cold sores," "fever blisters"). Recurrences were observed in 25 to 50 percent of persons who had symptomatic primary HSV-1 oral infection, but in only 24 percent of those who had primary HSV-2 oral infection.[171] The mean rate of recurrence after symptomatic primary HSV-1 infection in adults is approximately 0.1 per month. These recurrences often are associated with a variety of febrile illnesses, local trauma, sun exposure, or menstruation.[24,278,281] Whether acquisition of HSV-1 in childhood is less likely to be associated with recurrent herpes labialis is not known.

Most persons with herpes labialis experience a prodrome (pain, burning, tingling, or itching) at the site that lasts a few hours to several days. Subsequently, an orderly progression ensues from papules (lasting 12 to 36 hours) to vesicles (usually gone by 48 hours) and finally to ulcers and crusting (lasting 2 to 4 days) (Fig. 170–14). The typical lesion is 35 to 80 mm in size. The majority of outbreaks are healed by 5 to 10 days (mean, 200 hours). Most pain occurs during the vesicular stage. Virus is isolated readily from vesicles (80-90% of the time) and less commonly from ulcers and crusts (34% of the time). Maximal virus titers (10^7 to 10^8) in lesions are detected in the first day or two, and virus generally is not isolated after 120 hours.[24,281] Virus also may be detected in the saliva and on the hands of persons with herpes labialis.[310]

Recurrences tend to affect the same location or closely related areas. In general, they occur on the lips, mucocutaneous junction, or other parts of the face. Recurrent lesions found inside the mouths of normal hosts rarely are caused by HSV and more likely are aphthous lesions. When HSV recurrences are within the mouth, they tend to be on tissue adjacent to bone, such as the

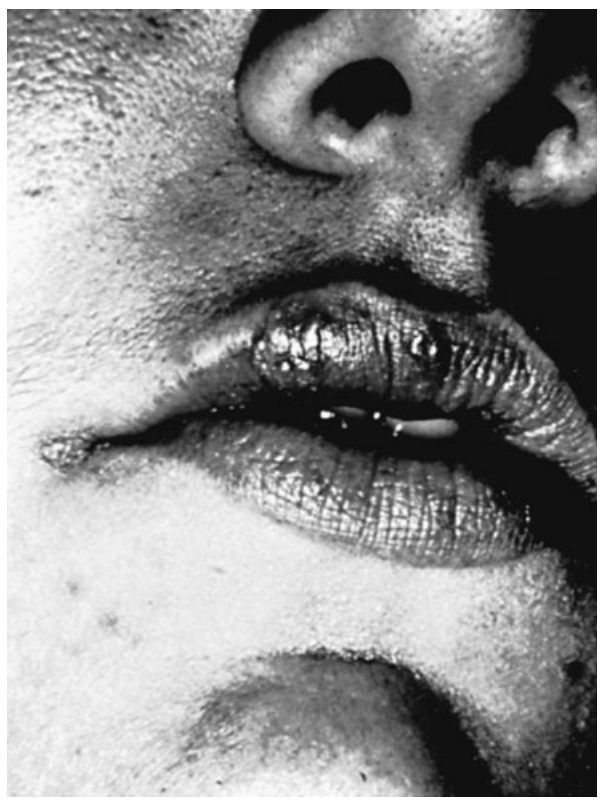

Figure 170–14 Recurrent herpes labialis (cold sore) in an adolescent.

gums or palate, and not on the lips or buccal mucosa.[276] A differential diagnosis of the condition also includes pemphigus, lichen planus, ulcers caused by cyclic neutropenia, and ulcers associated with celiac disease, ulcerative colitis, Crohn disease, pernicious anemia, and Behçet syndrome.

Recurrent genital herpes is the second most common manifestation of HSV. Studies have elucidated several factors that increase the risk for recurrent genital disease after symptomatic primary genital infection. Recurrence rates are much higher after primary HSV-2 (90%) than after primary HSV-1 (25-55%) infection.[171,229] The mean rate of recurrence is 0.02 to 0.1 per month after primary genital HSV-1 and 0.3 per month after primary HSV-2 genital infection.[171,229] Recurrences are seen more commonly in men than in women and after a recurrent lesion than after a first attack.[229]

Only 5 to 12 percent of persons with recurrent genital herpes have constitutional symptoms. Local symptoms include pain (average, 4 to 6 days), itching, dysuria (10-30%), adenopathy (20-30%), and lesions (average, 50-60 mm) lasting 4 to 5 days until crusting, with healing taking place by 9 to 11 days (range, 4-29 days) (see Fig. 170–3). Symptoms in females tend to be more severe than those in males. Virus is shed for an average of 3 to 4 days (but in some cases as long as 20 days). Virus generally is shed with titers of 10^2 to 10^4 per lesion. Vesicles are seen in dry areas, but in wet areas, the vesicles rapidly break down into ulcers. Symptoms generally are milder and of shorter duration than those in primary genital disease.[45] New crops of lesions commonly occur during the course of recurrence. The severity of recurrence is quite variable; in some cases, several discrete recurrences blend into a single, prolonged recurrence, and in rare cases, patients have almost continuous recurrences.[123] In one study of patients who were HSV-2–seropositive with no history of genital herpes, 62 percent had recognized herpetic lesions at later evaluation.[325] Recurrences in the previously asymptomatic group were shorter (3 versus 5 days) and less frequent (3.0 per year versus 8.2 per year) when the pattern of recurrence in this cohort was compared with that of subjects with a known history of genital herpes.

With endonuclease restriction analysis, researchers have clear evidence that, whereas most recurrences represent endogenous reactivation of the same latent virus, re-infection with a new homologous virus (i.e., HSV-2 and new HSV-2), as well as heterologous virus (i.e., HSV-2 and then HSV-1 or vice versa), is possible.[50] How common this occurrence is remains to be ascertained and must depend to some degree on the sexual activity and number of partners of the persons studied.[261]

Other cutaneous recurrences may develop at each anatomic site of primary infection. HSV infection may recur on the face or trunk in a typical dermatome distribution, such as that associated with VZV. Indeed, frequent repeated attacks of zosteriform-like lesions on any part of the body in a normal host suggest HSV and not VZV infection.

ERYTHEMA MULTIFORME

Erythema multiforme is thought to be an immune-mediated, "allergic" response to recurrent HSV infection.[103,139,197,212,330] It has been associated with the presence of human leukocyte DQw3 antigen.[149] In several series, approximately 15 to nearly 100 percent of patients who have erythema multiforme, especially those with recurrent erythema multiforme, gave a history of recurrent HSV infection before the skin eruption, which may be macular or urticarial.[139] In one series, 5 of 80 patients who had recurrent oral HSV infection experienced a rash (presumably erythema multiforme) that manifested 8 to 14 days after the onset of a cold sore.[281] Studies in adults and children have documented HSV antigen-antibody immune complexes and HSV DNA (detected by PCR analysis and in situ hybridization) in the skin of patients who had erythema multiforme after having HSV infection.[41,42,212] In a series of 20 children with erythema multiforme (10 who had antecedent herpes), 16 were documented to have HSV DNA at the site of the rash.[330] The mechanism of transport of viral DNA fragments is not clearly understood. Recent studies suggest that $CD34^+$ cells play a role in transport of HSV DNA fragments to lesional skin.[211]

The skin manifestations may last 14 to 21 days, and therapy generally is directed toward the allergic and not the viral component of the illness. Suppression of HSV recurrences prevents the associated episodes of erythema multiforme. Indeed, suppressive treatment of erythema multiforme with acyclovir, even in the absence of recurrent HSV infection, completely suppresses clinical manifestations.[263,299] In one series, the syndrome developed in 12 children at a mean age of 8 years within 4 days after the appearance of herpes labialis lesions, and symptoms lasted for an average of 10 days; 9 children had recurrent erythema multiforme, with an average of 2.6 episodes per year.[330] Detection of HSV by PCR assay of skin biopsy tissue was described in all cases. None of three children given acyclovir suppressive therapy had recurrences during treatment for at least 6 months, but erythema multiforme with recurrent HSV developed in one child when use of the drug was discontinued, thus supporting a causative role of HSV in the syndrome.

HSV INFECTION IN IMMUNOCOMPROMISED HOSTS

As the practice of pediatrics continues to include more patients with severe acquired immunodeficiency states brought about by increasingly intensive therapy for malignancies, expanding application of bone marrow and organ transplantation, and HIV infection, the prevalence of severe HSV infection in immunocompromised hosts is increasing. Table 170–3 lists conditions associated with unusually severe HSV infections. HSV infection in the neonatal period is discussed in another chapter on viral infections of the fetus and newborn. Aside from the several cases of HSV encephalitis in patients with agammaglobulinemia (who also had concomitant infections with enterovirus),[186] common links in these varied groups are either skin abnormalities (eczema, burns) or immunologic defects, primarily in the cell-mediated components of the immune system.[37,105,133,220,288,302] The critical defects have not been defined and may involve one or a combination of inadequate functions (e.g., of $CD4^+$ T cells, $CD8^+$ T cells, natural killer cells, macrophage antigen processing, or other factors).

The incidence of severe HSV infection in children with diseases that predispose them to these complications (see Table 170–3) is defined poorly but, in limited series, was similar to that seen in adults.[107,115,333] Most infections are caused by reactivation, as would be expected given the relative frequency of primary and recurrent infections.

In series of pediatric or adult patients who have received renal, bone marrow, or cardiac transplants, 70 to 90 percent of seropositive persons excreted HSV, usually from the oropharynx and generally at the time of peak immunosuppression (in the first month after transplantation).[205] HSV disease usually occurs within 2 to 3 weeks of solid organ or hematopoietic transplantation in patients who do not receive antiviral prophylaxis.[115] Of 68 children who underwent renal transplantation, a herpesvirus was isolated in 43 percent, with 28 percent of the isolates being HSV.[305] HSV in cardiac transplant cases causes symptomatic illness in 45 to 85 percent of seropositive patients, depending on the intensity of immunosuppression. HSV was the virus most commonly isolated in children who underwent bone marrow transplantation (23%).[327] HSV has been suggested to be one of the etiologic agents of the interstitial pneumonitis that develops

TABLE 170–3 Conditions Contributing to Unusually Severe HSV Infections

Newborn period
Malnutrition
Malignancy
Immunosuppressive therapy
Antineoplastic
Transplantation
Corticosteroids or adrenocorticotropic hormone
Primary immunodeficiency
Agammaglobulinemia
Wiskott-Aldrich syndrome
Ataxia-telangiectasia
Severe combined immunodeficiency syndrome
Nucleoside phosphorylase deficiency
Thymoma and hypogammaglobulinemia
Common variable agammaglobulinemia
Chronic mucocutaneous candidiasis
Natural killer cell defect
Acquired immunodeficiency syndrome
Pregnancy
Burns
Trauma
Skin abnormalities
Atopic eczema
Bullous impetigo
Burns
Darier disease
Ichthyosiform erythroderma
Pemphigus
Viral infection
Measles
Pertussis
Tuberculosis
Severe bacterial infection
Haemophilus meningitis
Sarcoidosis

after bone marrow transplantation.[208,227] In children with leukemia, HSV infection occurs more commonly in those with myelocytic leukemia than in those with lymphocytic leukemia, and the risk of infection developing increases with neutropenia and chemotherapy. HSV infection was the most common serious viral infection in children with leukemia. Whereas most infections occurred during periods of remission, on a per-day basis, the risk of infection developing was seven times higher during induction.[343] Of 24 patients who died of infection during remission in the St. Jude's series, 2 (8%) had HSV (1 with disseminated disease, 1 with encephalitis).

In Africa, patients suffering from underlying malnutrition and concomitant measles infection may contract fatal disseminated HSV infection; such events rarely occur in industrialized nations.[30,153,302]

Children with HIV infection and chronic mucocutaneous ulcers (persisting for more than 1 month), bronchitis, pneumonitis, and esophagitis caused by HSV are categorized as having severely symptomatic HIV disease (category C).[57] Chronic HSV mucocutaneous disease or widespread organ involvement was the AIDS-defining condition in 7 of 789 (0.9%) HIV-infected children.[309] In series of children with AIDS, 5 to 29 percent contracted HSV opportunistic infection.[107] Chronic ulcerative HSV lesions often develop in children with HIV infection, although first episodes did not result in disseminated infection.[250] Notably, many of these children also had perianal ulcers from which HSV-1 was isolated. In HIV-infected patients, illnesses caused by HSV usually involved chronic mucocutaneous ulcers or extension to the lungs, bronchi, or esophagus. Dissemination is a rare occurrence.[16] Multiple recurrences of infection are common occurrences as the immunodeficiency worsens. In patients with AIDS, HSV rarely causes a typical[128,314] or more indolent encephalitis. HSV usually does not result in mortality in HIV-infected patients, but it causes significant morbidity.[155] HSV genital ulcer disease increases the risk of acquisition of HIV.[137] Studies show that suppression of clinical and subclinical reactivation of HSV is associated with a decrease in HIV viral load.[254]

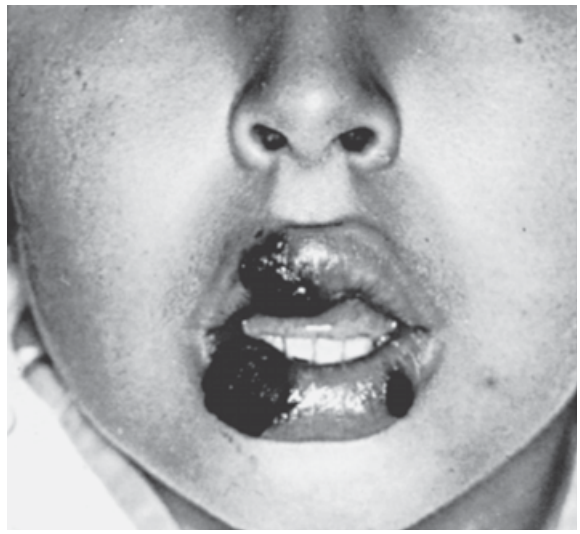

Figure 170–15 Chronic, hemorrhagic HSV infection in a girl with leukemia and a bone marrow transplant.

Several major syndromes are attributable to HSV in immunocompromised patients, with some overlap and occasionally progression from one to another. The first and most common manifestation is a local, chronic, often extensive cutaneous or mucocutaneous infection. The second form is infection involving a single organ (e.g., esophagitis or pneumonitis). The most serious illness is characterized by more widespread dissemination involving distant areas of the skin or visceral organs (e.g., the lungs, liver, adrenals) and the CNS. Although data are limited, disseminated disease probably most often represents primary infection except in the most severely immunocompromised patients. More localized syndromes may be a manifestation of either primary infection or recurrent illness.

The typical localized HSV infection begins in the mouth or about the lips, often appearing innocuously as recurrence of ordinary herpes labialis. Over the course of several days, the papules and vesicles progress to bullae, frequently with hemorrhagic fluid. The bullae or vesicles evolve into huge, chronic, bloody, coalescing, ulcerated, oozing lesions eroding into subcutaneous tissue and occasionally destroying underlying structures. The tissue is malodorous, and the lesions are painful (Fig. 170–15). The lip and palate are the sites most commonly affected. Oral lesions account for approximately 60 percent of HSV infections in children undergoing transplantation.[305] A similar syndrome, usually caused by HSV-2 infection, may be seen in the perianal or vaginal area and is one of the characteristic syndromes that occur in males who have sex with HIV-infected males (Fig. 170–16). Untreated, the lesions may lead to death because of local destruction and hemorrhage, or they may regress as the immune status of the host improves or as antiviral chemotherapy is administered. A syndrome of herpetic geometric glossitis has been reported in HIV-infected patients.[121] Affected patients have a tender tongue accompanied by dorsal longitudinal crossed and branching fissures.

Extensive HSV skin infections may occur in patients with burns, eczema, pemphigus, or abrasions, often with conversion of second-degree tissue damage to third-degree damage (see Figs. 170–6 and 170–7). Of 179 children with eczema, herpes skin

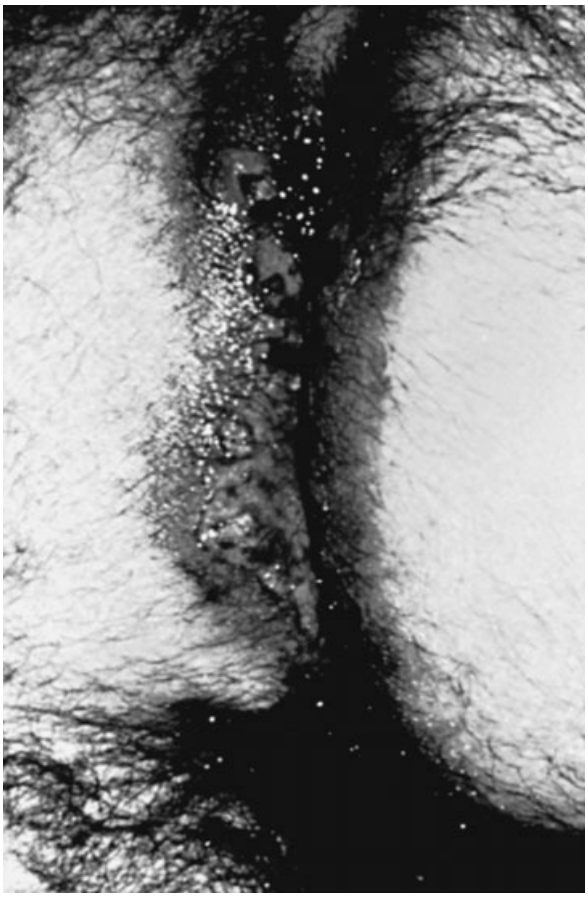

Figure 170–16 HSV proctitis and rectal infection in a homosexual male with AIDS. *(Courtesy of Dr. Victor Fainstein, Department of Internal Medicine, M. D. Anderson Cancer Center, Houston.)*

infections developed in 10, 7 of whom required hospitalization.[78] Though rare, local infection may progress to dissemination in some cases, possibly as a result of the more severe immunodeficiency occurring with several of these conditions. The widespread necrotizing lesions are known commonly as *Kaposi varicelliform eruption* or *eczema herpeticum* (see Fig. 170–6). The predisposition to severe HSV-1 infection in children with atopic eczema appears to be due to the cutaneous abnormalities because these children have T-cell responses to HSV, as well as high HSV IgG antibody titers.[118] Recent studies have looked at the role of the cathelicidin family of antimicrobial peptides in protecting children with atopic dermatitis against disseminated skin infection with HSV. Variable skin expression of cathelicidin peptide LL-37 may explain why eczema herpeticum develops in some children with atopic dermatitis and others appear to be at lower risk.[138]

Herpes skin lesions must be differentiated from bacterial infections caused by gram-positive or gram-negative organisms, chronic fungal infections (as seen with *Mucor* or *Blastomyces*), other viral infections (vaccinia, varicella), mycobacterial infection, and various non-infectious lesions such as pyoderma gangrenosum, chemotherapy-induced ulcers, or Sweet syndrome. HSV is the agent associated most commonly with oral lesions in immunocompromised patients.[107]

HSV esophagitis rarely has been reported in normal children,[29] but it is a relatively common finding in immunocompromised patients. Pathologic studies have suggested that approximately 25 percent of cases of autopsy-proven esophagitis (1% to 6% of all autopsies) are secondary to HSV infection (14 of 55 cases).[207] Underlying conditions included burns, aplastic anemia, malignancies, transplantation, a variety of other serious medical problems, and postoperative trauma induced by nasogastric tubes.[51,190,207] Twenty to 50 percent of these patients have HSV involvement elsewhere (the lungs, trachea, and less commonly, the skin). The esophagitis may be asymptomatic or associated with dysphagia, odynophagia, epigastric discomfort, and retrosternal pain. Characteristic findings in the esophagus are ulcers with raised granular margins. The ulcers often are covered with fibrinous exudate and, in advanced cases, are confluent, with progression to complete mucosal loss in large segments of the esophagus. Typically, visceral herpes infection is not suspected before the patient's death, but uncommonly, involvement of adjacent gastric tissue can be documented. Diagnostic evaluation should include a barium swallow, which may demonstrate edema, nodules, and ulceration of the esophagus; however, barium studies cannot differentiate HSV from other etiologic agents of esophagitis. Esophagoscopy accompanied by biopsy and viral culture is diagnostic and helps exclude other common causes of this syndrome, including *Candida*, CMV, and possibly other fungi and bacteria or chemotherapy-induced changes.[51,207] Although the oral cavity, esophagus, and rectum are the usual sites of HSV infection in the gastrointestinal tract of immunocompromised patients, HSV-2 colitis has been reported in a young child after combined liver and small bowel transplantation.[83]

HSV pneumonia is an unusual condition, but lung involvement has been described in neonates[35] and immunocompromised hosts. This diagnosis frequently is made post mortem almost exclusively in immunocompromised patients. In one series of 1000 consecutive autopsies,[206] HSV pneumonia was identified in 10 cases. HSV pulmonary infection occurred in 3 percent of children who had undergone renal transplantation.[305] In most adult cases, the process is a result of endogenous viral mucocutaneous reactivation and involvement of lung tissue by contiguous spread, which causes focal pneumonia (60% of the time), or involvement by hematogenous spread from an oral or genital site, which results in diffuse pneumonitis.[227] Although the largest series consisted primarily of adult patients, three of the patients were aged 7 years or younger (with Down syndrome and congestive heart failure, rhabdomyosarcoma, and pneumococcal pneumonia and a seizure disorder).[140] Patients had cough, dyspnea, fever, and hypoxia, and 50 percent had rales. Most had other concomitant pulmonary infections with bacteria, *Candida*, *Aspergillus*, and CMV. HSV pneumonia cannot be diagnosed by the association of upper airway cultures and radiographic abnormalities. It must rest on an aggressive approach involving culture and histopathologic examination of involved lung tissue obtained by either biopsy or bronchoscopy.

HSV meningoencephalitis is not a common occurrence in immunocompromised patients. It may occur as a component of widely disseminated disease, or it may be a localized condition. Several cases have been reported in patients who had agammaglobulinemia in association with concomitant enteroviral infection of the brain.[186] The meningoencephalitis may be fulminant, such as occurs in a normal host. An interesting case that followed an atypical subacute course accompanied by bilateral brain involvement has been reported in an anergic patient who had Hodgkin disease. Although HSV does not appear to have a predilection for CNS infection in immunocompromised hosts, patients with AIDS are an exception and may have ascending myelitis, acute transverse myelitis, and encephalitis.[107,110,128,314]

Hepatitis caused by HSV has been reported most commonly after solid organ or bone marrow transplantation[145,168] and during pregnancy.[154] Signs include fever, abdominal pain, and elevated liver enzyme levels. Hepatitis usually occurs during the first 3 weeks after transplantation, unless prophylactic acyclovir is given. The mortality rate is very high (67% to 100%). In at least one case, orthotopic liver transplantation resulted in survival.[268]

The most severe form of HSV in an immunocompromised host is widely disseminated disease that can involve the liver, adrenals, lungs, spleen, kidney, and often the brain. In a large series from South Africa and Kenya,[30,153,302] measles and severe malnutrition were frequent cofactors (83%) in children aged 2 to 25 months who had widely disseminated HSV infection. These illnesses represented fatal primary infections. Similar syndromes have been described; they and other underlying conditions are listed in Table 170–3. Dissemination has been reported coincident with pertussis, *Haemophilus influenzae* meningitis,[143] and other bacterial infections.[30] Disseminated HSV infection occurred in 10 percent and 25 percent of children who had HSV infection and underwent bone marrow and renal transplantation, respectively.[205,327]

The clinical manifestation of disseminated HSV usually is one of initial fever and skin or mucocutaneous involvement in 80 percent of cases, but instead of healing as expected, the infection disseminates. The cutaneous dissemination may involve a widespread vesicular eruption that looks much like varicella, or it may involve more local, large hemorrhagic vesicles and bullae. Involvement of the major target organs, as noted previously, gives rise to syndromes of hepatitis, pneumonia, shock, bleeding, disseminated intravascular coagulopathy, seizures, coma, renal failure, hypothermia, and death in days to weeks. Laboratory examination may reveal leukopenia, thrombocytopenia, elevated liver function test values, hyponatremia, azotemia, pneumonitis, hypoglycemia, CSF pleocytosis, abnormal EEG results, and electrocardiographic abnormalities. Death occurs commonly in this syndrome (90%), even after the institution of antiviral chemotherapy. Because the liver frequently is involved and biopsy may be precluded by the tenuous condition of the patient, HSV infection should be considered in all high-risk groups (see Table 170–3) with fulminant hepatitis.

Neonatal HSV infection is discussed in another chapter devoted to viral infections of the fetus and newborn.

DIAGNOSIS

Clinical findings may suggest a probable diagnosis of HSV infection, but obtaining a definitive laboratory diagnosis is necessary or useful in many circumstances.[13] Accurate laboratory diagnosis requires giving attention to obtaining the correct specimens necessary for identifying the etiology of the clinical syndrome. Appropriate interpretation of the laboratory results is the second critical factor. Evaluation of mucocutaneous lesions in high-risk patients guides the use of antiviral therapy if the lesion is herpetic. However, because HSV often is shed in immunosuppressed and otherwise healthy individuals, finding HSV in oropharyngeal secretions does not mean that the clinical condition is caused by HSV. In general, the virus must be isolated from the relevant tissue for confirmation of the diagnosis. The test selected also is important (e.g., HSV is rarely found in spinal fluid cultures of patients outside the neonatal age group with HSV encephalitis), and detection of viral DNA by PCR assay or, if necessary, by brain biopsy is required to prove the diagnosis and exclude other treatable causes. Laboratory diagnosis also is valuable when HSV infections are not life-threatening. For example, establishing that a genital lesion is caused by HSV-1 or HSV-2 facilitates making decisions about antiviral therapy and allows initiation of appropriate counseling regarding the risk of recurrences and measures that may reduce transmission of HSV to contacts.

VIRAL CULTURE

The gold standard of HSV diagnosis remains recovery of infectious virus in tissue culture. HSV grows rapidly (mean, 2 to 3 days; high-titer samples, 12 to 24 hours; low-titer samples, 5 to 7 days) and produces a typical cytopathic effect (Fig. 170–17). Human diploid cells (WI-38 or human embryonic lung cells) and primate cells (Vero) are used by most diagnostic laboratories. A tentative diagnosis can be made in 95 percent of cases by the cell types infected and the typical cytopathogenic effect. High-titer specimens of VZV or CMV occasionally cause confusion. Sensitivity can be improved by centrifugation of specimens directly onto cell monolayers; a more rapid result can be obtained by using shell vial cultures combined with antigen-detection methods.[99,141,308]

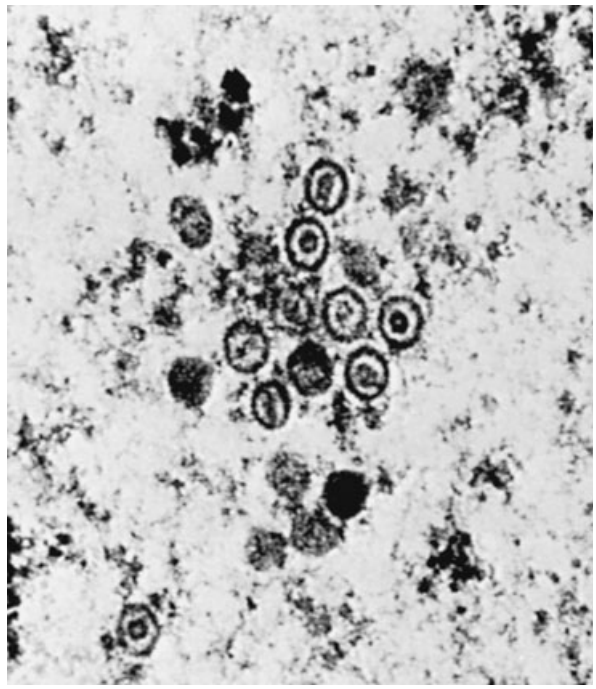

Figure 170–17 Electron-microscopic demonstration of HSV particles in a brain biopsy specimen from a 9-month-old infant with HSV encephalitis.

Definitive identification of the infecting virus is accomplished by using HSV-1– and HSV-2–specific antisera or monoclonal antibodies or by endonuclease restriction patterns. HSV-1 and HSV-2 also have biologic differences (replication in chicken embryo cells, effects on baby mice, allantoic membrane pock morphology, and sensitivity to various chemicals). Co-cultivation of ganglia tissue along with permissive cells remains the standard technique for detecting latent virus, but this technique has little application in clinical medicine.[26,28]

DIRECT DETECTION OF HSV-INFECTED CELLS

Direct detection methods allow a diagnosis of HSV infection to be established rapidly when mucocutaneous lesions are present or when tissue sections from infected organs are examined.[13,77,201,202,260] Direct immunofluorescence or immunoperoxidase staining of cells taken from mucocutaneous lesions is the procedure used most commonly. Specimens are obtained by exposing the base of the lesion, scraping to remove cells, and transferring the cells to a glass slide. The use of fluorescein-conjugated monoclonal antibodies to HSV to stain lesion scrapings yields sensitivities, when compared with viral culture isolation, in the range of 78 to 88 percent with few false-positive reactions.[210] Nonetheless, a sample for viral culture should be obtained when the lesion scraping is performed so that the direct

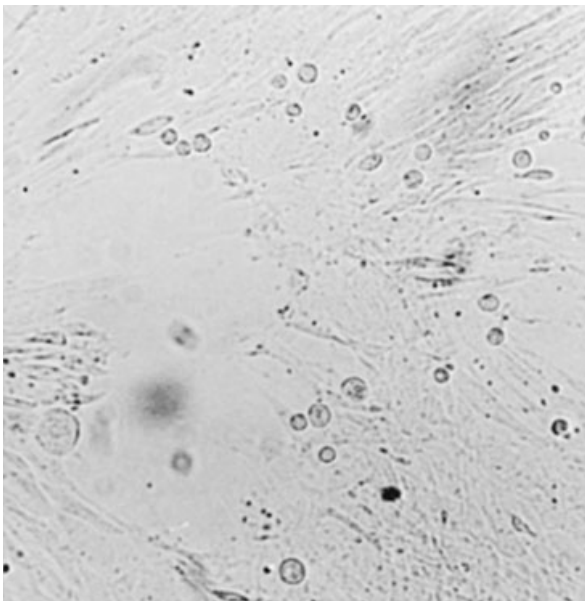

Figure 170–18 Culture of HSV on human fibroblasts demonstrating disruption of the cell monolayer and rounding and enlargement of cells to show the cytopathogenic effect.

detection result can be confirmed. Direct detection methods should not be used to test secretion specimens for asymptomatic shedding, cells from the CSF of patients with suspected encephalitis, or cells from oropharyngeal or tracheal aspirate or bronchoalveolar lavage samples. HSV antigens can be detected directly in clinical specimens by enzyme-linked immunosorbent assays (ELISAs), immunoperoxidase assays, hemagglutination assays, or avidin-biotin enzyme conjugate assays.[6,43,66,210,260] Sensitivities range from 70 to 95 percent, with specificities of 65 to 95 percent, when compared with viral culture. None of these methods is reliable for detecting asymptomatic HSV shedding.[313] Except for sporadic reports, antigen-detection tests for HSV in CSF have been unsuccessful and should not be used.[159]

Nonspecific methods such as Papanicolaou (Pap) staining or the Tzanck test have been used in the past to suggest possible HSV infection. Even though these methods may show cytologic changes in specimens obtained from suspected HSV lesions, the diagnosis of HSV should not rely on these methods in current practice because rapid, specific methods are available. Although cytologic examination of HSV lesions reveals typical giant cells and, less commonly, Cowdry type A intranuclear inclusions, these changes also are seen with CMV and VZV. Some series report finding cytologic changes in 80 percent of culture-positive specimens if the examiner is experienced, but other series find such changes in only 40 percent. Electron microscopy of vesicular fluid or tissue preparations may reveal the characteristic virus of the herpes family (Fig. 170–18). However, the microscopist cannot differentiate HSV from other herpesviruses, and this diagnostic approach rarely is available as a rapid method. Because selection of antiviral drugs for herpesviruses requires specific identification of the infecting virus (e.g., HSV must be differentiated from CMV in the brain, lungs, or liver because therapy differs), nonspecific methods have limited clinical value.

POLYMERASE CHAIN REACTION ASSAYS

PCR assays involve repeated amplification of targeted regions of HSV DNA by the design of primers that will anneal to denatured DNA and produce millions of copies of the DNA sequence.[22,239] The sensitivity of PCR methods can permit detection of fewer than 10 copies of the viral genome in a sample. PCR has been applied successfully to the diagnosis of HSV infection.[7,22,136,191,239,248] PCR clearly is more sensitive than is virus culture for detection of HSV DNA. In one study of mucosal specimens, the ratio of PCR positivity to virus culture ranged from 3.8:1 in the winter to 8.8:1 in the summer.[319] The decrease in isolation of virus by culture in the summer reflects poor viability of virus at warmer temperatures. The extreme sensitivity of PCR renders it prone to false-positive results in the event of poor technique or specimen contamination.

In clinical laboratories that have proper experience with PCR and when appropriate controls are used in the assay, PCR assay is the method of choice for detection of low copy numbers of HSV DNA and for detection of HSV DNA in CSF for the diagnosis of HSV encephalitis.[22,239,307] Sequential testing by PCR assay has rendered the use of brain biopsy unnecessary in most situations.[172] Studies consistently have demonstrated that HSV PCR has value for testing the CSF of patients with suspected herpes encephalitis.[122,152,224,239,259,271] HSV DNA was detected by PCR in the CSF of 53 (98%) of 54 patients with biopsy-proven herpes simplex encephalitis and was detected in all 18 CSF specimens obtained before brain biopsy was performed on patients with proven herpes encephalitis.[172] However, clinicians must recognize that PCR may be negative early in the course of HSV encephalitis.[329] A negative PCR result does not exclude the diagnosis of HSV because specimens obtained early in the clinical course of some infections may be negative and clinical specimens often contain inhibitors of PCR.[53,130,231] PCR also has been used to demonstrate prolonged presence of HSV DNA in genital lesions.[65] The sensitivity of the PCR method is adequate for detecting asymptomatic shedding of HSV, although a positive PCR result does not indicate the presence of infectious virus necessarily.[64,130] HSV PCR also has utility when the clinical diagnosis is difficult to make, as illustrated by detection of HSV in vitreous fluid and in unusual mucocutaneous lesions in patients with AIDS.[74,178] Primers for both HSV-1 and HSV-2 can be included in the PCR reaction to allow simultaneous detection and typing of the infecting virus.[307]

DNA hybridization with radiolabeled or biotinylated probes also can be used to detect HSV directly in pathologic specimens.[95,103] This technique may detect virus or incomplete viral DNA or RNA in latency or in sites with low levels of productive infection.

SEROLOGIC DIAGNOSIS

In contrast to methods that detect the virus (viral culture), viral DNA (PCR assay), or proteins (direct antigen detection), serologic methods historically have had limited value for the clinical diagnosis of HSV infection. With standard methods, demonstration of the presence of IgG antibodies to HSV in serum means that the individual has acquired HSV-1 or HSV-2, or both, at some time in the recent (2 to 4 weeks) or distant (many years) past. Because these viruses cause persistent infection, the presence of HSV antibodies signifies only that the individual has latent HSV-1 or HSV-2 (or both) in sensory ganglia and that the virus can be expected to reactivate and be shed in secretions intermittently. Many serologic methods can be used to assess HSV IgG immune status to document whether an individual is infected with HSV, and most are sensitive. HSV serologic testing generally has been performed by using commercial ELISA or latex agglutination procedures that use crude infected cell–protein mixtures and detect predominantly type-common antibodies.[109,318] Practitioners must understand that the extensive cross-reactivity between HSV-1 and HSV-2 antibodies renders differentiating past HSV-1 from past HSV-2 infection impossi-

ble with any of these methods.[17,18,109,297] Serologic reports that list HSV-1 and HSV-2 antibody titers separately are misleading, but they frequently are provided without the necessary explanation about the lack of sensitivity for identifying the patient's infection status.

Research laboratories use Western blot (immunoblot) analysis to demonstrate reactivity with type-specific viral proteins. For example, the fact that the gG of HSV-1 (gG_1) differs significantly from the HSV-2 homologue (gG_2) permits detection of type-specific IgG and IgM antibodies.[17,135,297] Recently, commercial laboratories have begun to use validated serologic methods that circumvent the problem of cross-reactivity between HSV-1 and HSV-2 antibodies.[318] These specialized serologic methods can be used to show whether the individual is infected with HSV-1, HSV-2, or both, but not when the infection was acquired. If paired sera are available, showing seroconversion to HSV-2 even when the patient has HSV-1 infection may be possible.[17,40,96,146,165] The newer commercial type-specific HSV tests have performed well when compared with Western Blot testing. When one of these methods was evaluated in patients with HSV-2 infection proved by viral culture and Western blot analysis, its sensitivity for detection of HSV-2 antibody was 96 percent and its specificity was 98 percent.[19] Although HSV type-specific serologic tests have become available through commercial diagnostic laboratories, practitioners must confirm that the laboratory is using such a method and not the "standard" ELISA or other tests that are commonly—and misleadingly—reported as though HSV-1 and HSV-2 antibodies have been identified accurately. The Centers for Disease Control and Prevention's Sexually Transmitted Diseases Treatment Guidelines list the type-specific glycoprotein-based assays that are validated by the U.S. Food and Drug Administration.[344] The sensitivities of these tests range from 80 to 98 percent, and specificities generally are greater than 95 percent. False-negative results are more likely to be seen early in the course of infection, and false-positive results may occur in patients with a low likelihood of having HSV infection.

If the patient is seronegative at the onset of symptoms or at initial evaluation, primary HSV infection can be documented by using any of the standard ELISA methods to show seroconversion with paired sera.[160] However, again, the serotype of HSV responsible for the seroconversion cannot be determined with most commercial serologic tests. Serologic tests also are not useful for evaluating possible recurrent HSV because recurrences often are not accompanied by a significant rise in antibody titer. Determining HSV IgG antibody titers in paired sera is not helpful because titers may vary more than fourfold in the absence of viral reactivation. Testing for IgM antibodies does not improve the serologic diagnosis; HSV IgM assays cannot be used to distinguish primary from recurrent infection because reactivation also can induce the production of IgM antibody. In addition, HSV IgM antibody assays are difficult to standardize, and false-positive results occur commonly, even when effort is made to fractionate serum IgG and IgM before testing.

Local production of HSV IgG in CSF samples can be used to document HSV encephalitis in some patients, but a 2- to 4-week interval is required to demonstrate this change,[204] and it cannot be used to guide antiviral therapy.[22,159,272,312] Testing CSF for HSV IgM antibodies has no demonstrated diagnostic value.

GENETIC ANALYSIS FOR MOLECULAR EPIDEMIOLOGY

HSV DNA from epidemiologically unrelated isolates has a specific cleavage pattern, or "fingerprint," when digested by endonuclease restriction enzymes and subjected to electrophoresis in a gel (Fig. 170–19).[49] These methods can be used to type the virus and demonstrate relatedness or differences among isolates obtained from different sites in the same person during one illness or from the same site over the course of time for the purpose of evaluating apparent "outbreaks" or nosocomial transmission.[49,50,129,185,337] This method has improved the understanding of HSV-1 and HSV-2 epidemiology, investigation of the possibility of exogenous re-infection versus recurrence, and the possibility that individuals may harbor more than one latent virus "strain." To be sure of identity, several restriction enzymes must be used to test each isolate. Thus, this method is valuable for epidemiologic purposes, but it is time-consuming and requires special expertise beyond that of most viral diagnostic laboratories. HSV genotyping by PCR and sequencing is done as a research method.

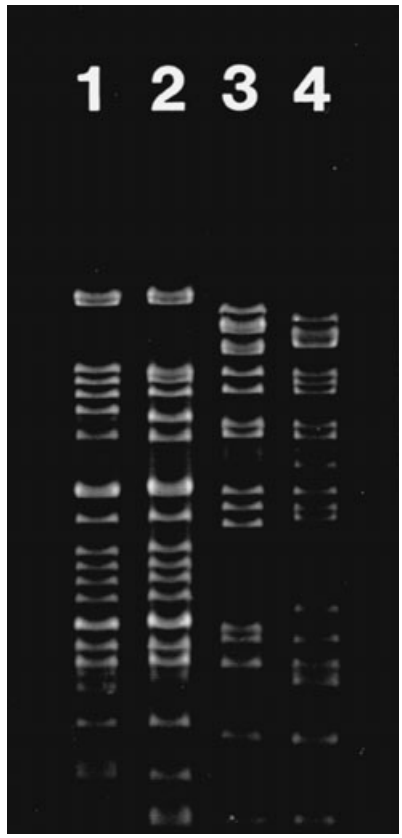

Figure 170–19 Electrophoretic separation of the DNA fragments of four HSV isolates by size, produced by restriction endonuclease enzyme digestion using the BamHI enzyme. Lanes 1 and 2 are two different HSV-2 clinical isolates, whereas lanes 3 and 4 are two different HSV-1 clinical isolates. The origin of the gel is at the top. *(Courtesy of Dr. Saul Kit, Division of Biochemical Virology, Baylor College of Medicine, Houston.)*

DRUG SUSCEPTIBILITY TESTING

Though not in general use, antiviral drug susceptibility testing of HSV isolates is becoming available in many centers. Methods for antiviral susceptibility testing include plaque reduction assay, viral cytopathic reduction assays, dye uptake methods, and DNA hybridization.[226] With the availability of specific antiviral therapy and evolving mechanisms of viral drug resistance, the clinician must rely increasingly on sensitivity tests that resemble the in vitro minimal inhibitory concentration tests used for bacteria. Evolving clinical data are providing meaningful values for "sensitive and resistant" virus, although the specific numbers vary by assay. In general, a median infectious dose (ID_{50}) of greater than 3 μg/mL for acyclovir and greater than 100 μg/mL for foscarnet

denotes a resistant isolate,[63,245] although the ID_{50} value may vary depending on the specific method used. Simpler and more rapid screening tests are being developed.[75,247] In addition, detection of HSV enzyme characteristics, such as thymidine kinase–negative HSV isolates, which therefore are resistant to acyclovir, can be useful. In an immunocompromised patient, in particular, failure to respond to antiviral therapy should alert the clinician to the need for viral susceptibility testing.

PROGNOSIS, COMPLICATIONS, AND SEQUELAE

Most HSV infections occurring beyond the fetal and neonatal periods cause minor morbidity but very rarely are life-threatening. The introduction of antiviral drugs that inhibit HSV replication has changed the management and outcome of these infections during the past 20 years. Even before these drugs were available, eczema herpeticum resolved without sequelae in most cases. Despite administration of antiviral therapy, HSV encephalitis can be fatal or be associated with serious consequences ranging from moderate to extensive and permanent neurologic disability. The case-fatality rate for untreated encephalitis may be as high as 75 percent,[340] and progressive neurologic damage may occur.[88,126,159] In a series of PCR-diagnosed cases, a Glasgow Coma Scale score lower than 11 was associated with a poor outcome, and the youngest cohort, those younger than 3 years, had higher morbidity and mortality rates.[142] Genital herpes causes significant physical and psychological morbidity. In immunocompromised patients, disseminated HSV infection can result in fatal or serious illness. HSV is one of the most common infectious causes of blindness in developed countries.

TREATMENT

HSV-1 and HSV-2 infections are treated with acyclovir or related drugs. Acyclovir is a nucleoside analogue that is a competitive inhibitor of HSV DNA polymerase, and it terminates DNA chain elongation. Acyclovir is an inactive agent; inhibition of viral DNA synthesis by blocking viral DNA polymerase and DNA chain elongation requires phosphorylation of acyclovir. HSV-1 and HSV-2 viral thymidine kinases are much more active than are mammalian cell kinases, and acyclovir becomes a specific antiviral agent in the presence of viral thymidine kinase.

Because the oral bioavailability of acyclovir is only approximately 20 percent, efficacy with oral administration requires high and frequent dosing. Concentrations of acyclovir achieved in CSF are approximately 50 percent of plasma levels. Acyclovir is excreted by glomerular filtration, so doses must be reduced in patients with impaired renal function. Acyclovir has been an exceptionally safe drug in clinical practice, but adverse reactions, including rash, nausea, vomiting, diarrhea, abdominal pain, headache, and neutropenia, can occur. Neurotoxicity may occur with an overdose, usually when the dose is not adjusted for poor renal clearance. Acyclovir also can cause acute renal insufficiency by precipitation in the renal tubules in dehydrated patients with high plasma concentrations or by rapid infusion of the drug. Valacyclovir, the L-valine ester of acyclovir, is converted to acyclovir[311] and has much higher bioavailability after oral administration, with plasma concentrations similar to those obtained after intravenous administration of acyclovir. Famciclovir is an oral prodrug of penciclovir[82]; it also is well absorbed orally and is metabolized to the active form on absorption. The major advantage of these agents is that less frequent dosing is required than with acyclovir. HSV-1 and HSV-2 seldom are resistant to acyclovir or related drugs, but resistance can occur in immunocompromised patients who receive prolonged antiviral therapy. However, these doses have not been tested in young children. HSV isolates resistant to acyclovir usually have mutations in viral thymidine kinase. Foscarnet or, in some cases, cidofovir is an alternative for the treatment of acyclovir-resistant HSV.[38] Resistance to foscarnet and, more recently, cidofovir has been reported.[345]

ORAL HSV INFECTION

Although published experience is limited, administration of oral acyclovir to children with gingivostomatitis is appropriate and can be expected to modify the duration of symptoms if it is initiated early in the clinical course. Several small placebo-controlled trials of primary herpes gingivostomatitis in young children have been reported.[4,9] In one study, the pain and hypersalivation resolved more quickly in acyclovir-treated patients.[94] In a larger study, symptoms resolved more rapidly with a 10-day course of oral acyclovir (600 mg/m^2 four times a day); differences were observed in drooling (4 vs. 8 days with placebo), gum swelling (5 vs. 7 days), speed of healing of intraoral lesions (6 vs. 8 days), appearance of new lesions (57% vs. 94%), and viral shedding in saliva (4 vs. 10 days). Therapy had no effect on the subsequent development of cold sores.[9] A comparison of oral acyclovir suspension, 15 mg/kg five times a day for 7 days, and placebo started within 72 hours documented that drug recipients had a shorter duration of oral lesions (median of 4 vs. 10 days [difference, 6 days; 95% confidence interval (CI), 4.0 to 8.0]) and earlier resolution of other symptoms, including fever (1 vs. 3 days [difference, 2 days; CI, 0.8 to 3.2]), extraoral lesions (0 vs. 5.5 days [difference, 5.5 days; CI, 1.3 to 4.7]), difficulty eating (4 vs. 7 days [difference, 3 days; CI, 1.31 to 4.69]), and difficulty drinking (3 vs. 6 days [difference, 3 days; CI, 1.1 to 4.9]).[4] Treatment also decreased the period of viral shedding (1 vs. 5 days [difference, 4 days; CI, 2.9 to 5.1]). Symptomatic therapy includes antipyretics and oral hydration. The use of oral anesthetics is not recommended and has resulted in self-injury as a result of children chewing on anesthetized oral mucosa or lips.

Although the duration of symptoms is shorter with recurrences, children with frequent or severe recurrences may benefit from treatment with oral acyclovir as soon as new lesions appear. Topical acyclovir has been licensed for the treatment of recurrent oral herpes labialis based on experience in adults. However, its benefits on clinical symptoms are minimal. If treatment in children is indicated, oral acyclovir is the best option. Studies in adult patients with herpes labialis show that patient-initiated, high-dose, short-duration treatment with valacyclovir or famciclovir initiated at the onset of symptoms significantly reduces the time to healing and hastens resolution of pain and tenderness when compared with placebo.[277,280]

Several studies have produced conflicting results regarding the use of topical acyclovir for the treatment of oral herpes recurrences. Topical acyclovir can decrease the duration of HSV shedding from 2 to 3 days with placebo treatment to 1 to 2 days,[282] and the effect on symptoms is either none or subtle (1 less day with vesicles and a 2- to 3-day acceleration of healing). Although no controlled studies have been conducted, acyclovir therapy should be used for the treatment of rare cases of HSV esophagitis in normal hosts.[108]

HSV KERATITIS

Children with HSV keratitis should be referred for immediate evaluation by an ophthalmologist who is familiar with this illness and its progressive, sight-threatening forms and with optimal antiviral therapy. The use of cycloplegic and anti-inflammatory agents, which may be necessary, requires specialized experience. Topical preparations have been shown to have a beneficial effect on HSV keratitis and probably other superficial ocular HSV

infections such as conjunctivitis and blepharitis.[184] These preparations include idoxuridine (Stoxil), vidarabine (Vira-A), trifluridine (Viroptic), and acyclovir. Orally administered acyclovir also has been used to treat dendritic keratitis, with effects that were similar to those achieved with topical acyclovir in the treatment of dendritic corneal ulceration. Several series suggest that oral acyclovir is therapeutic in patients who have stromal keratitis or kerato-uveitis.[179,265] When given in conjunction with topical antiviral therapy, the response to oral acyclovir was good, even in those who had failed topical therapy.[266] Oral therapy also is useful for prophylaxis against viral reactivation in those with stromal keratitis.[341]

HSV ENCEPHALITIS

Intravenous acyclovir is the drug of choice for HSV encephalitis.[334] For children 3 months to 12 years of age, 20 mg/kg/dose every 8 hours is recommended. For children older than 12 years of age and for adults, 10 mg/kg/dose every 8 hours is recommended. The recommended duration of therapy ranges from 14 to 21 days or longer. Two well-controlled clinical studies in Sweden and the United States that compared vidarabine with acyclovir (30 mg/kg/day in three divided doses for 10 to 12 days) provided evidence that acyclovir is significantly more effective than vidarabine in treating HSV encephalitis.[272,335] In the Swedish study, the early mortality rate was 19 percent in the acyclovir-treated group and 50 percent in the vidarabine-treated group.[272] The National Institutes of Health collaborative study[335] generated similar data. As was observed in the earlier vidarabine studies,[340] younger age and less severe neurologic signs at initiation of therapy markedly influence the outcome of HSV encephalitis treated with acyclovir. Lethargic patients have a 15 percent mortality rate, whereas comatose patients have a 40 percent mortality rate. The use of long-term therapy remains controversial.[126,159,333]

In addition to antiviral therapy, intensive care is required for optimizing the outcome of these patients. Fluid management for the prevention of overhydration is critical. Frequently, direct measurement of intracranial pressure is necessary for effective monitoring of increased pressure and treatment with diuretic agents. The use of steroids is common but remains controversial and unstudied; viral replication does not appear to be more extensive as a consequence of steroid therapy. Anticonvulsant therapy for management of the often severe and prolonged seizures, as well as ventilatory support, usually is necessary at some time during the illness.

GENITAL HSV INFECTION

Recommended antiviral therapies for genital herpes simplex infection include acyclovir, famciclovir, and valacyclovir (see Table 170–4), but acyclovir remains the best studied drug in children.[82,344] Although topical acyclovir was the first antiviral therapy used to treat genital herpes, it has been replaced by the oral agent. Some benefit was observed with the topical drug, but it is difficult to use, and its effectiveness is limited when compared with oral acyclovir; treatment decreased the mean duration of viral shedding (4.1 vs. 7.0 days with placebo) and time to crusting of lesions (7.1 vs. 10.5 days).[70] In the treatment of recurrent genital disease, topical acyclovir generally tended to have a minor effect on the duration of viral shedding (from 1-2 days in treated patients vs. 2-3 days in placebo recipients), with little or no effect on symptoms.[189,230] These results were not improved markedly with the immediate use of topical ointment. Early studies in which intravenous acyclovir was given to patients with primary genital HSV infection also were performed.[68,196] When used in a placebo-controlled, double-blind study at a dose of 5 mg/kg every 8 hours for 5 days, acyclovir decreased the duration of viral shedding (2 vs. 8-13 days in placebo-treated patients), shortened the duration of local and systemic symptoms by 2 to 5 days, and decreased the time to healing by 7 to 12 days. Complications such as extragenital lesions or urinary retention were reduced significantly.[218]

TABLE 170–4 Recommended Treatment Regimens for Adults and Adolescents with Genital HSV Infection

First Clinical Episode
Acyclovir, 400 mg orally three times a day for 7 to 10 days
or
Acyclovir, 200 mg orally 5 times a day for 7 to 10 days
or
Famciclovir, 250 mg orally three times a day for 7 to 10 days
or
Valacyclovir, 1 g orally twice a day for 7-10 days
Suppressive Therapy
Acyclovir, 400 mg orally twice a day
or
Famciclovir, 250 mg orally twice a day
or
Valacyclovir, 500 mg orally once a day
or
Valacyclovir, 1 g orally once a day
Episodic Therapy*
Acyclovir, 400 mg orally three times a day for 5 days
or
Famciclovir, 125 mg orally twice daily for 5 days
or
Valacyclovir, 500 mg orally twice a day for 3 days

**Additional dosing recommendations can be found in the guidelines referenced.*
From Centers for Disease Control and Prevention: Sexually Transmitted Disease Treatment Guidelines 2006. M. M. W. R. Recomm. Rep. 55(RR-11);1-94, 2006.

Oral acyclovir has therapeutic effects on both primary and recurrent HSV infection in adults and should be given to adolescents with these infections. The major effect of intravenous acyclovir therapy on the first episode of genital HSV infection is seen in those who have true primary disease, as opposed to those who have HSV-1 immunity and are experiencing new HSV-2 infection.[218] The intravenous route of administration has been replaced by oral therapy with acyclovir and related agents. In either case, optimal benefit requires early intervention. In a double-blind, placebo-controlled study of patients with primary genital infection, acyclovir at a dose of 200 mg five times per day for 5 to 10 days significantly reduced viral shedding (1-6 days vs. 13-15 days with placebo), lesion formation after 48 hours (0-4% vs. 43-44%), the duration of lesions (10-12 vs. 16-21 days), and the duration and severity of symptoms.[48] In similar studies of adult patients with recurrent genital HSV infection, acyclovir (200 mg five times per day for 5 days) decreased the duration of virus shedding (1-2 vs. 2-4 days), time to healing (5-6 vs. 6-7 days), and development of new lesions (2-10% vs. 19-25%), especially when administered early in the recurrence.[209a]

In these short-term studies in which patients were cautioned regarding hydration, no significant side effects were observed. Furthermore, no cytogenetic effects were noted.[62] Studies of virus from either placebo or acyclovir recipients showed that 4 to 15 percent of viruses isolated after therapy were more resistant to acyclovir, regardless of therapy.[63] Neither oral nor intravenous acyclovir reduces the rate of recurrence when used to treat either primary or recurrent genital infection. Oral acyclovir is the drug of choice for patients who have primary genital HSV infection, HSV proctitis,[236] and frequent recurrent disease. Intravenous acyclovir should be reserved for patients who have severe local or systemic symptoms or complications such as urinary retention or aseptic meningitis syndrome.[218]

In adults, valacyclovir administered two times a day was as effective as acyclovir administered five times a day in the treatment of first-episode genital herpes.[101] When compared with placebo, valacyclovir is as effective as acyclovir in suppressing HSV viral replication as measured by culture and HSV PCR.[125] Similarly, famciclovir is efficacious in the treatment of first-episode and recurrent genital HSV infection.[10,241]

Symptomatic therapy for HSV lesions should be directed toward reduction of local discomfort, promotion of healing, and prevention of auto-inoculation and superinfection. Nonspecific creams and ointments probably delay healing and increase risk for the development of maceration and infection. Keeping lesions clean and dry probably is the most important local measure. Urinating sometimes is painful and can be made less so if done in a bathtub or sitz bath. Some experts advise Burow solution sitz baths or short compress treatments. Prolonged soaking delays healing.

MUCOCUTANEOUS HSV INFECTION IN IMMUNOCOMPROMISED HOSTS

Acyclovir and related drugs given orally or intravenously are the agents of choice for serious mucocutaneous HSV-1 or HSV-2 infection in high-risk hosts. Early initiation of treatment provides the best outcome. Given the limited oral bioavailability of acyclovir, higher plasma concentrations can be achieved with valacyclovir or famciclovir, but clinical experience with these drugs is limited in children. If the infection is considered potentially life-threatening, intravenous acyclovir is the treatment of choice. Therapeutic responses to HSV infection in immunocompromised hosts have been difficult to study because of the variable nature of the disease. The experience with vidarabine demonstrated the benefits of antiviral therapy initially. In a randomized, controlled, crossover study of mucocutaneous HSV infection, vidarabine (10 mg/kg/day intravenously in a 12-hour infusion) was shown to decrease pain and induce defervescence in patients older than 40 years. In none of the 85 patients in this study did visceral dissemination of HSV develop. However, these observations are of historical interest now because the drug is not available and has been replaced by acyclovir. Acyclovir ointment appears to have some minor effects on pain, viral shedding, and time to complete healing of lesions in immunocompromised persons with mild, non–life-threatening mucocutaneous HSV infection,[338] but oral acyclovir is more effective for mild illness.

Patients with more severe illness should receive care in a hospital setting with the intravenous preparation. Intravenous acyclovir has been analyzed extensively in immunocompromised patients with mucocutaneous HSV infection.[107,333] When used early, acyclovir arrests the progression of infection. In several double-blind, placebo-controlled studies, acyclovir has been shown to be highly effective. It decreased the time to cessation of new lesions (1 vs. 3 days with placebo), time to lesion crusting (3-7 vs. 9-14 days), time to lesion healing (12-14 vs. 18-28 days), cessation of pain (4-10 vs. 7-16 days), and termination of viral shedding (3 vs. 14-17 days).[317] Acyclovir administered intravenously is the most effective agent and the drug of choice for treating HSV in an immunocompromised host. The dosage is 250 mg/m^2 or 5 to 10 mg/kg/dose every 8 hours infused over a 1-hour period. In patients with severe disease, the dose may be doubled. The major toxic effect has been renal, with reversible obstructive nephropathy and transient rises in serum creatinine levels (5-10% of patients). Adequate hydration usually prevents this problem. Dosage guidelines have been established in patients who have impaired renal function (Table 170–5). One to 5 percent of patients may experience nausea and vomiting. Less commonly (1%), reversible neurologic symptoms (lethargy, agitation, tremor, disorientation, coma, transient hemiparesthesia) and laboratory abnormalities (abnormal EEG, increased CSF myelin basic protein) have developed in bone marrow transplant recipients. These patients usually had received interferon and CNS chemoprophylaxis for leukemia.[316] Other less serious problems include phlebitis (14%) and hives (5%).

TABLE 170–5 Guidelines for Use of Acyclovir in Patients with Renal Impairment*

Creatinine Clearance (mL/min/1.7 m^2)	Dose (mg/kg)	Dosing Interval (hr)
50	5	8
25-50	5	12
10-25	5	24
0-10	2.5	24

**A dose (5 mg/kg) should be administered after each dialysis session in patients undergoing hemodialysis.*

Several studies have focused on the use of oral acyclovir in this patient population.[292] The 50 percent virus-inhibiting concentration (IC_{50}) of acyclovir generally is 0.1 to 0.5 μg/mL for HSV-1 and 0.5 to 2 μg/mL for HSV-2. Many studies use molar concentrations; in the case of acyclovir, the dose in micromoles divided by 4 equals the dose in micrograms per milliliter. Whereas peak serum levels with intravenous acyclovir vary from 8 to 15 μg/mL, the levels achieved with oral therapy are considerably lower (1-2 μg/mL). In preliminary studies of relatively small populations of immunocompromised adults, oral acyclovir at doses of 200 mg five times per day effectively promoted the healing of lesions and inhibited viral shedding.[292] Pediatric studies have used oral doses of 600 mg/m^2 given four times per day.[296] The relative efficacies of oral and intravenous acyclovir have not been compared in this setting. The choice of route of administration requires clinical judgment about the degree of immunosuppression, assessment for evidence of dissemination, and frequent re-evaluation if oral drug is given.

Preliminary pharmacokinetic information regarding valacyclovir in immunocompromised children is available.[203] Valacyclovir was administered to 28 immunocompromised children aged 5 to 12 years in an open-label, randomized, dose-ranging study. Doses of valacyclovir administered were 250 or 500 mg twice a day or 500 mg three times a day. Pharmacokinetic evaluation has estimated that the bioavailability of oral valacyclovir is twofold to fourfold greater than that of oral acyclovir. The information provided in this study provides preliminary guidance for dosing of oral valacyclovir in children; however, more studies are needed to determine efficacy and safety in children.

To date, all studies of acyclovir in immunosuppressed patients have documented the propensity of recurrent lesions to reappear when therapy is withdrawn. Whether acyclovir reduces the humoral and cellular immune responses to HSV is not certain[315]; more likely, these patients remain subject to HSV recurrence because adaptive immunity is reconstituted as a consequence of the underlying disease.

Neonatal herpes simplex infection is discussed in the chapter on viral infections of the fetus and newborn.

ACYCLOVIR-RESISTANT HSV INFECTION

Despite its widespread use, acyclovir remains an effective antiviral compound. In immunocompetent hosts, acyclovir resistance has been a rare problem, even with prolonged courses of suppressive therapy. Mutant, thymidine kinase–negative virus strains have been recovered in 3 to 10 percent of patients treated with acyclovir, but acyclovir-resistant virus also can be recovered in placebo-treated patients, thus indicating that subpopulations of

HSV are present in the lesions.[63] Thymidine kinase–negative HSV recurrences in immunocompromised patients have been documented well, with demonstration of the ability of these viruses to establish latency and recur. These viral mutants are less virulent in animal models. Patients who were culture-positive for such mutants often have thymidine kinase–positive HSV isolates recovered from lesions during their next recurrence.[270]

Acyclovir-resistant virus occasionally has been shed by immunocompetent patients before, during, or after receiving therapy, yet it usually has not been associated with treatment failure.[124,164,180,293] In pretreatment patients, 3.6 percent of isolates (31 of 870) were resistant to acyclovir (not inhibited by 3 μg/mL). A similar percentage (3.1%) of resistant isolates was recovered from 663 immunocompetent patients after administration of acyclovir therapy.[63] An immunocompetent patient was reported who had an acyclovir-resistant virus containing an altered thymidine kinase that caused multiple recurrences of genital herpes unresponsive to acyclovir therapy.[164] In another immunocompetent patient receiving prophylactic acyclovir for recurrent genital herpes and long-term prednisone therapy for chronic urticaria, recurrent genital lesions with acyclovir- and penciclovir-resistant HSV-2 developed.[166] To date, these occurrences remain rare findings in the immunocompetent patient population.

Drug resistance occasionally is a problem in the acyclovir-treated immunocompromised population. Cases of acyclovir-resistant virus causing local invasive disease and, less commonly, dissemination and acyclovir-unresponsive meningoencephalitis have been reported.[107,110,187,244] Recurrences with acyclovir-resistant HSV-1 may be encountered, as described in a child with Wiskott-Aldrich syndrome.[249] Of the three mechanisms of altered sensitivity of HSV to acyclovir (absent thymidine kinase [TK^-], altered thymidine kinase [TK^A], and altered viral DNA polymerase), the TK^- type is encountered in the vast majority of cases.[63,107,110] These viruses cannot phosphorylate acyclovir and convert it to the active triphosphate. Among bone marrow transplant recipients receiving multiple courses of acyclovir, acyclovir-resistant virus was recovered from 2 percent of patients during initial therapy and from 9 percent after treatment of a second recurrence. In a tertiary care center, acyclovir-resistant, clinically significant virus was recovered from 5 percent of immunocompromised patients (usually after receiving acyclovir), but not from any immunocompetent hosts.[98] Illness caused by acyclovir-resistant viruses was more severe in pediatric patients and occurred more commonly in very immunocompromised patients such as those with AIDS or those who had undergone bone marrow transplantation.[98] Among bone marrow transplant recipients and patients with AIDS who were tested after receiving acyclovir therapy, 18 percent (105 of 582) had isolates that were acyclovir-resistant.[63] In vitro viral susceptibility of HSV has been highly associated with clinical response to acyclovir in HIV-infected patients.[245]

Acyclovir-resistant isolates are resistant to penciclovir and ganciclovir, which also require phosphorylation. Foscarnet has been used and is associated with successful clinical response and cessation of viral shedding. The most common side effects of foscarnet are nephrotoxicity (azotemia), alterations in serum calcium and phosphorus, and neutropenia, observed in 10 to 25 percent of patients.[148,244] In an immunocompromised patient who has HSV infection unresponsive to acyclovir, foscarnet therapy is indicated. Viral susceptibility testing may aid in clinical management.[246]

More recently, in patients receiving chronic or multiple courses of foscarnet, foscarnet-resistant HSV isolates (IC_{50} > 100 μg/mL) have been recovered from lesions failing to respond to foscarnet.[245,246] Of note, these lesions often responded to acyclovir, alone or in combination with foscarnet.[246] Perhaps the mutation in DNA polymerase responsible for foscarnet resistance was in an area not related to acyclovir activity. Other experimental strategies used to treat acyclovir-resistant viruses have included high-dose, continuous-infusion acyclovir (1.5 to 2.0 mg/kg/hr) or intravenous cidofovir,[46,97a,173,274] although recent cases of cidofovir-resistant isolates have been reported.[345] In the case of acyclovir- and foscarnet-resistant viruses, topical 3-hydroxy-2-phosphonomethoxypropyl cytosine (HPMPC or cidofovir) or a combination of topical trifluorothymidine and interferon-α[36,243,249] has been used successfully in patients with chronic mucocutaneous lesions.

PREVENTION

INFECTION CONTROL

Because HSV infection is ubiquitous in the human population and intermittent asymptomatic shedding is extremely common, preventing transmission of HSV is difficult. HSV is sensitive to heat and lipid solvents, so the use of antiseptics, soap and hot water, or chlorine decreases the risk of transfer of virus in settings such as the home, spas, pools, wrestling meets, and hospitals. In addition, appropriate use of gloves by all healthcare personnel who come in contact with potentially infected body secretions or rashes should decrease the acquisition and nosocomial spread of HSV. These recommendations are part of universal body substance precaution policies. Condoms may diminish the passage of infectious HSV-2 and should be recommended routinely.[321] The use of cesarean section for preventing neonatal HSV is discussed in the chapter on viral infections of the fetus and newborn.

Health care providers should wear gloves and wash carefully before and after contact with respiratory or genital tract secretions. Parents and caretakers of infants with eczema or severe diaper rash should be especially careful to avoid making direct or indirect contact of this altered skin with an active HSV lesion. Burn patients should be protected against exposure to or direct contact with personnel or visitors who have active HSV lesions. Immunosuppressed patients with evidence of HSV infection usually are experiencing reactivation of latent virus. Primary HSV infections in immunosuppressed persons, as in neonates, may be especially severe, and protecting these susceptible patients against exposure to HSV lesions is important. Wrestlers and rugby players who have exposed skin lesions caused by HSV should be excluded from competition.

IMMUNOPROPHYLAXIS

The development of vaccines is a long-term objective that has significant potential to ameliorate the disease burden associated with HSV-1 and HSV-2.[11] However, attempts to create effective HSV vaccines have met with limited success to date.[194,238,284] In one early report, a vaccine containing the recombinant glycoprotein D of HSV-2 (gD_2) was used in patients who had established recurrent genital HSV infections, and vaccine recipients had a third fewer genital herpes recurrences than those experienced by placebo recipients during the study year.[289] However, experience with the use of recombinant vaccines to prevent primary HSV infection has yielded mixed results. Recombinant HSV-2 gD_2 and gB_2 have been demonstrated to induce high humoral and cell-mediated immune responses in seronegative and seropositive recipients.[176] Some vaccines may protect some women who have no HSV immunity at baseline.[284] An HSV-2 glycoprotein D_2 subunit vaccine with alum and monophosphoryl lipid (MPL) adjuvant was approximately 73 percent effective in preventing symptomatic genital HSV-2 disease (although it was less effective in preventing infection) in women who were seronegative for both HSV-1 and HSV-2.[286] An HSV-2 gD_2 and gB_2 subunit vaccine with M59 used as an adjuvant was ineffective in prevent-

ing disease in seronegative partners of HSV-2–infected persons or in other individuals at high risk of acquiring HSV-2 infection.[69] Although efficacy was poor overall, it appeared to be better in women than in men. The results of these vaccine studies suggest that the choice of adjuvant may be critical in inducing protective immune response to HSV antigen. These studies also illustrate that high levels of neutralizing antibody do not guarantee protection against infection. Other vaccine types in early stages of development include disabled infectious single-cycle (DISC) virus vaccines, live genetically attenuated vaccines, and naked DNA.[284,339]

No adequate controlled trials of high-dose intravenous immune globulin as an intervention to suppress frequently recurring genital herpes have been reported, and this method is not likely to be effective because these patients have high titers of HSV-specific antibodies.[192]

CHEMOPROPHYLAXIS

Intramuscular human interferon-α administered before and after surgery on the trigeminal nerve root significantly decreased the incidence of HSV shedding and clinical reactivation.[217] In similar studies, interferon had no significant effect on HSV shedding or reactivation in renal transplant patients.[59]

Intravenous acyclovir has been shown to prevent reactivation of HSV almost completely in immunosuppressed patients receiving a bone marrow transplant or antileukemic chemotherapy.[252] In patients with bone marrow transplants, intravenous therapy is begun at the onset of immunosuppression and continued through the immediate transplant period, and then therapy is switched to oral acyclovir for several months.[115] Oral acyclovir administered chronically markedly suppressed recurrence in immunodeficient patients.[298] The use of prophylactic acyclovir is indicated in HSV-seropositive, immunosuppressed pediatric patients.

Oral acyclovir (200 mg two to five times per day) administered chronically can prevent reactivation of genital HSV nearly completely in patients with frequent recurrences. Recurrences decreased by 50 to 70 percent, and the time to recurrence changed from 14 to 25 days in patients receiving placebo and from 100 to 125 days in those receiving acyclovir.[91,293] Breakthrough recurrences tended to be mild and to have less viral shedding and rarely were caused by acyclovir-resistant virus. When acyclovir was discontinued (after 12 to 15 weeks of administration), HSV recurrences reverted to the pretreatment frequency. Higher doses of acyclovir suppressed recurrences in patients experiencing a "breakthrough" with more conventional doses. In patients receiving therapy for 5 years, the number of recurrences declined from the first year (1.7) to the fifth year (0.8).[116] Twenty to 25 percent of patients are recurrence-free for 4 to 5 years.[116] After prophylactic therapy ended, suppressive therapy no longer was warranted in some patients because of longer periods between recurrences.[102,290] Patients who had resistant virus exhibited reisolation of acyclovir-sensitive virus. Thus, acyclovir suppressed recurrences without eliminating latent virus. A study has reported that acyclovir does not seem to change the rate of asymptomatic viral shedding.[291] A more recent study has demonstrated that acyclovir (400 mg twice a day) resulted in a 95 percent reduction in subclinical viral shedding when compared with placebo in women with genital herpes.[324] Side effects of chronic acyclovir therapy include an increase in mean corpuscular volume and hemoglobin concentration without anemia or megaloblastic changes, neutropenia and asthenia, and mild gastrointestinal upset.[293] Valacyclovir and famciclovir also can be used to suppress frequently recurring genital herpes effectively, and they suppress both symptomatic and asymptomatic HSV shedding.[81,125,240,323] Both drugs are more expensive than is acyclovir. The decision of whether to use episodic or suppressive therapy for individuals with recurrent genital HSV infection depends on the frequency of recurrences. Those with a recent primary infection and frequent recurrences may prefer long-term suppressive therapy. If episodic therapy is used, it should be initiated as soon as symptoms begin. Oral herpetic recurrences were prevented in skiers given a brief course of acyclovir during their ski trip.[279]

Chronic antiviral suppressive therapy with valacyclovir reduced genital HSV transmission in heterosexual HSV-2–discordant couples in one study.[71] Once-daily suppressive therapy with valacyclovir or placebo was given to the HSV-2–seropositive partner. Acquisition of HSV-2 occurred in 4 of 743 susceptible partners given valacyclovir and 16 of 741 susceptible partners given placebo (hazard ratio, 0.52). HSV was identified in genital secretions 2.9 percent of days in source partners receiving valacyclovir and 10.8 percent of days in placebo recipients. Use of condoms and abstinence during symptomatic outbreaks still are recommended. Use of HSV type-specific serologic testing to identify HSV-seropositive individuals and disclosure of HSV status, in combination with condom use, risk reduction counseling, and long-term suppressive antiviral therapy, have the potential to control the spread of HSV-2 infection.[119,320] The potential utility of antiviral therapy against herpes simplex is currently being evaluated as a strategy for prevention of HIV infection.[54]

The role of antiviral therapy during pregnancy to prevent neonatal HSV infection is still undergoing evaluation for safety and efficacy. In one randomized, blinded trial, women with recurrent genital HSV received either acyclovir, 400 mg three times a day, or placebo from 36 weeks' gestation until delivery. Genital lesions were present at delivery in 14 percent of women receiving placebo and 5 percent of women taking acyclovir ($p = 0.08$). A significant decrease in HSV culture and PCR positivity was noted in the acyclovir group near the time of delivery. No significant difference in delivery by cesarean section was found between the groups. Suppressive acyclovir treatment decreased but did not eliminate genital lesions or HSV viral detection at delivery.[328] Another randomized, placebo-controlled study evaluated the effect of daily valacyclovir suppression initiated at 36 weeks' gestation. Women receiving valacyclovir had fewer recurrences of genital HSV; however, valacyclovir suppression did not decrease the number of women who had viral shedding or lesions near delivery. A limited sample size may have contributed to failure to observe a significant difference in groups treated with drug versus placebo.[8]

Experience with acyclovir prophylaxis for reactivation of HSV in children is limited. Prophylactic oral acyclovir (30 to 60 mg/kg/day in three to five doses for 1 week) has been shown to decrease seroconversion effectively and to eradicate symptomatic cases of HSV gingivostomatitis in a nursery setting in which outbreaks of primary HSV infection were occurring.[170,223] Suppression has been effective in infants with frequent recurrences of HSV-2 after neonatal HSV-2 infection and has been safe, although prolonged use has been associated with significant neutropenia. To date, up to 10 years of suppressive therapy in adults has not resulted in serious side effects[102,116,290] or an increase in the problem of drug-resistant HSV in a healthy host.

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CHAPTER 171

Cytomegalovirus

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Cytomegalovirus (CMV) is a ubiquitous agent that commonly infects persons of all ages from all parts of the world and from all socioeconomic and cultural backgrounds. Although most CMV infections are asymptomatic, certain patient groups are at risk for acquiring serious, even life-threatening illness. Discerning which role CMV is playing in a particular patient requires a thorough understanding of the epidemiology, virology, and pathophysiology of the virus and can be difficult, even for the most experienced clinicians.

HISTORY

The recorded history of CMV probably began when Ribbert[273] described in 1881 and then reported in 1904 "protozoan-like

cells" in the organs of an infant who died of presumed congenital syphilis. In 1921, Goodpasture and Talbot[138] hypothesized that these swollen cells, or "cytomegalia," were host cells that had been injured by a virus. During the first half of the 20th century, CMV was recognizable only by the pathologic changes that it produced in infected cells, and because these cells frequently were seen in the salivary glands of animals and humans, CMV originally was called the salivary gland virus.

In 1956, human CMV was isolated in tissue culture by independent investigators, Rowe and colleagues,[282] Smith,[305] and Weller and colleagues.[355] Because several human and animal viruses subsequently were found to replicate in salivary glands, the descriptive name *cytomegalovirus* was proposed by Weller in 1960. The ability to cultivate CMV in tissue culture led to the development of serologic techniques, which in the 1960s and 1970s resulted in many important clinical and epidemiologic observations. For example, CMV was found to infect people of all ages throughout the world. By 1962, CMV was established as a significant pathogen of the fetus and newborn that was capable of producing a spectrum of clinical manifestations and neurologic sequelae.[354] In 1966, Kaariainen and colleagues[173] presented evidence supporting CMV as a cause of post-transfusion mononucleosis syndrome.

The molecular biology of the virus was explored during the 1970s and 1980s and continues to be studied by many investigators. In addition, during the 1970s and 1980s, CMV emerged as a major cause of morbidity and mortality in immunosuppressed patients, especially those who underwent organ or marrow transplantation or who had acquired immunodeficiency syndrome (AIDS). In 1976, the first clinical trial of the live attenuated CMV vaccine Towne 125 was reported; since then, more than 500 renal allograft recipients as well as healthy young men and women have received the vaccine under investigational protocols.[122,251] In addition, research on a subunit vaccine based on glycoproteins of the virus began in the 1980s; and in the 1990s and the early part of the 21st century, recombinant vaccines were developed and tested in human volunteers. However, after decades of research, a successful CMV vaccine remains elusive. In 1999, the Institute of Medicine of the National Academy of Sciences assigned a high priority for a vaccine to prevent CMV infection, an action that was quickly endorsed in 2000 by the National Vaccine Advisory Committee, based on the lifetime costs to society and the human suffering associated with CMV disease in newborns and immune compromised individuals.[18,180] Furthermore, a resurgence of awareness of CMV from public health officials and the lay community began in 2004, providing hope that prevention and treatment of CMV disease may become a priority for research, education, and public health policy.[58] Attempts by clinicians to treat CMV disease began in the late 1960s and early 1970s,[114] and now CMV infection has become a treatable disease; several specific and effective antiviral agents, including ganciclovir (licensed in 1989), foscarnet (licensed in 1991), and cidofovir (licensed in 1996), are available to clinicians to treat seriously ill patients.

VIROLOGY

CMV is a member of the *Herpesviridae* family of DNA viruses. This family contains many important human pathogens and is subdivided into three subfamilies: (1) *Alphaherpesvirinae*, fast-growing, cytolytic viruses that are latent in neurons and include herpes simplex virus (HSV) types 1 and 2 and varicella-zoster virus (VZV); (2) *Betaherpesvirinae*, slow-growing, cytomegalic viruses that contain the CMVs; and (3) *Gammaherpesvirinae*, herpesviruses that preferentially grow in lymphocytes and sometimes transform them into malignant states; this subfamily includes Epstein-Barr virus and human herpesviruses 6, 7, and 8.

CMV is the largest member of *Herpesviridae*. The genome is double-stranded DNA 240 kb in size (150×10^6 d; guanine + cytosine [G + C] content, 58%), and it has a unique long sequence and a unique short sequence, both of which are bounded by repetitive sequences that are inverted relative to each other. The genome, therefore, can assume four isomeric forms. The viral particle has a 110-nm icosahedral capsid composed of 162 capsomeres. The entire virion is enclosed by a lipid envelope, which yields a final diameter of approximately 200 nm.[324]

Replication of CMV is slow compared with that of HSV. Whereas it takes herpes simplex only 4 to 8 hours to produce infectious progeny virus, CMV requires at least 24 hours. The CMV genome is transcribed slowly in a regulated sequence; on the basis of the appearance of different classes of CMV-specific proteins, the replicative cycle can be divided into three periods: immediate-early, early, and late. The immediate-early period is defined as the first 4 hours after infection occurs. During this period, specific segments of the DNA genome undergo restricted transcription, and certain regulatory proteins are produced that allow the virus to take control of host-cell macromolecular synthesis. The early period of replication begins after the immediate-early phase and persists for almost 20 hours. This period is characterized by replication of viral DNA, synthesis of infected cell proteins, and production of progeny virus. The late period usually is considered to occur 24 hours after infection develops. During this period, the structural components of the virus are produced, and infectious virus is released from the cell. Monoclonal antibodies against the various immediate-early, early, and late proteins produced by CMV have been used as rapid viral diagnostic tools.

CMV has no distinct serotypes. However, strain relatedness or differences can be determined by molecular analysis of viral DNA. Restriction enzyme analysis of DNA extracted from CMV isolates that are linked epidemiologically (e.g., serial isolates from the same person, mother-infant pairs, or family members experiencing temporally related CMV infections) shows identical or similar DNA fragment mapping patterns. This technique or modifications of this technique have been applied to the epidemiology of CMV in a variety of patients, such as transplant recipients, congenitally infected infants, and patients whose CMV was transmitted from person to person in hospitals, daycare centers, and residences. More recent studies have used polymerase chain reaction (PCR) methodology to determine whether CMV genotypes exist by showing differences in the a sequence, major immediate-early, glycoprotein B (gB or UL55), and UL144 regions of the viral genome.[27] Four gB genotypes have been characterized, and although all genotypes cause congenital and acquired infections, studies suggest that adverse outcomes after CMV infection may be linked to infection with a specific gB genotype. Infection with the CMV gB3 genotype appears to be associated more commonly with fatal infections in bone marrow transplant recipients and hearing loss in congenitally infected newborns, and CMV gB2 and gB4 genotypes appear to be more often fatal in patients with AIDS.[27,128,262,370]

EPIDEMIOLOGY

Seroepidemiologic studies have shown that infection with CMV occurs commonly and usually is inapparent.[377] However, the burden of CMV infection and severe disease may be increasing in certain high-risk groups, such as premature and newborn infants, and older children and adults with immune deficiencies as well as transplant recipients.[296,331] The incidence of CMV infection does not appear to be seasonal. However, the prevalence of CMV IgG antibody is influenced by many factors, including the age, geographic location, cultural and socioeconomic status, race or ethnicity, and child-rearing practices of the

group. For example, in developed countries such as the United Kingdom and the United States, the prevalence of CMV antibody is 40 to 60 percent in adult populations of middle-upper socioeconomic status and more than 80 percent in lower socioeconomic status groups.[144,318,376] CMV seroprevalence in the United States also may differ by race and ethnicity, with reports of 51 percent of non-Hispanic white individuals being CMV seropositive compared with 76 percent of non-Hispanic black individuals and 82 percent of Hispanic or Mexican Americans.[79,99,321] In contrast, in developing countries, 80 percent of children acquire CMV by the time they reach 3 years of age, and almost all persons have been infected by adulthood.[12,19,350] Studies on the age-related prevalence of infection with CMV in the United States suggest three periods of increased incidence of acquisition: early childhood, adolescence, and the child-bearing years.[12,356,375]

INFANTS AND CHILDREN

Approximately 1 percent of all newborns are born congenitally infected with CMV; ranges of 0.18 to 2.5 percent are reported.[31,63,284] Maternal CMV infection that is either primary or recurrent during pregnancy can result in an infant who is infected congenitally with CMV. However, the rate of intrauterine infection with recurrent infection in the mother is less than 1 percent, whereas transmission to the fetus occurs in 32 to 50 percent of mothers who are infected primarily with CMV during pregnancy.[179,318] Although most newborns congenitally infected with CMV are asymptomatic, symptoms and sequelae are much more likely to occur in infants congenitally infected as a result of the mother's primary infection during pregnancy than in infants congenitally infected from a recurrent maternal infection.

CMV also can be transmitted perinatally from a mother to her infant. The virus can be shed in the CMV-seropositive mother's cervicovaginal secretions, urine, saliva, and breast milk. The most common and most efficient routes of perinatal transmission are ingestion or aspiration of cervicovaginal secretions at the time of delivery or ingestion of fresh breast milk after delivery. CMV-seropositive mothers, especially mothers with high levels of CMV IgG antibody, frequently shed CMV in their breast milk, and as many as 53 percent of children who are breastfed with milk that contains infectious virus or CMV DNA can become infected with CMV.[107,169,237,347] These infections usually are benign in normal term infants but may be extremely serious in preterm, very low-birth-weight neonates.[208,346] In addition, as many as 57 percent of babies whose mothers shed CMV at or around the time of delivery become infected with CMV.[272]

Children not congenitally or perinatally infected with CMV may be infected during the toddler or preschool years. The acquisition of CMV by children between 1 and 3 years of age is influenced by home exposure to the virus, by the socioeconomic status or country of origin of the family, and by group daycare exposure. Weller and Hanshaw[354] suggested that the child-rearing practices in Sweden, where group daycare was common practice, accounted for the relatively high prevalence of CMV infection in Swedish children compared with children in the United States and Great Britain, where daycare centers were not common at that time.

Pass and colleagues[241] first reported the prevalence of CMV in daycare centers in the United States in 1982. They found that 51 percent of children who attended a daycare center in Alabama excreted CMV in their saliva or urine. In this study, the prevalence of CMV excretion varied with age; 83 percent of children aged 13 to 24 months shed virus as opposed to only 9 percent of children younger than 1 year old. Pass and colleagues concluded that the high prevalence of CMV infection probably was due to horizontal spread between the children in daycare. Subsequent studies have confirmed a high prevalence of CMV excretion in children in daycare centers across the United States. Overall prevalence rates of 22 to 57 percent have been observed, with the highest prevalence of active CMV infection (29-78%) found in children 1 to 3 years of age.[1,170,223,241,243,244] Children who attend daycare centers also shed high titers of virus (up to 10^5 median tissue culture infective dose per milliliter), with a mean duration of viral shedding of 13 months in urine and 7 months in saliva.[223] This prolonged, generally asymptomatic shedding of large quantities of virus, coupled with mobility and the less than hygienic daily habits notorious in toddlers, no doubt facilitates the transmission of CMV in daycare centers.

Several studies support the idea that the high prevalence of CMV infection in young children who attend daycare centers is due to horizontal transmission; these studies used molecular fingerprinting techniques to show that infected children in contact with each other shed strains of CMV with similar or identical restriction enzyme banding patterns and that predominant strains of CMV appeared to circulate during a given period in a given daycare center.[1,2,162] Re-infection with a genetically different strain of CMV also has been observed and may be important in child-to-child transmission of CMV in the daycare center environment.[5] The importance of horizontal spread of CMV has been shown in special care centers for mentally retarded children as well.[299] CMV also has been isolated from plastic toys and from the hands of daycare center workers.[112,162,241]

CMV-infected children may transmit the virus to the daycare center workers who care for them.[2,4] In addition, children who attend daycare centers may transmit CMV to their CMV-seronegative parents. In one study of parents of children who attended one of three daycare centers in Alabama, 14 of 67 parents (21%) whose children attended daycare seroconverted, compared with none of 31 parents whose children were cared for at home.[245] Excretion of CMV by the child clearly was a key risk factor for parental seroconversion because none of the seroconversions occurred in parents whose children attended the daycare center but did not shed CMV. Moreover, this study revealed a strong trend toward a greater risk of acquiring CMV infection (seroconversion rate, 45%) in the parents of children 18 months or younger. Additional evidence implicating young children who attend daycare centers as a source of CMV infection for their parents was provided by a study in Virginia daycare centers, in which the parents of children in daycare shed CMV strains identical to the strains shed by their children.[3] Furthermore, CMV strains from daycare settings, when transmitted from toddlers to their pregnant mothers, may be the source of congenital infection.[224]

Whereas early childhood probably is the most rapid period of acquisition of CMV, annual seroconversion rates of only 3 to 6.2 percent have been observed in older children aged 3 to 10 years in the United States and other countries, thus reflecting an age-related plateau in the acquisition of CMV. One identified risk factor for the acquisition of CMV infection in a school-aged child has been recent or active CMV infection in a family member.[79,87,99,321,322,375]

ADOLESCENTS

In support of these early findings, a seroepidemiologic study of CMV infection in sexually active adolescent girls found a strong association between indicators of sexual activity and serologic evidence of CMV infection and concluded that sexual activity is an important risk factor for the acquisition of CMV infection in adolescent girls.[67,309] Early cross-sectional epidemiologic studies of CMV implied that a gradual increase in CMV antibody prevalence occurs during the teenage years, and this period of rapid acquisition was attributed to the intimate physical contact that commonly occurs during the teenage years.[12,279] However, these

studies were conducted primarily in lower socioeconomic groups. In a seroepidemiologic study of several groups in Houston, Texas, on the acquisition of CMV infection in late childhood and adolescence, the prevalence of antibody increased with age in subjects of nonwhite races, as it did according to previous studies, but the prevalence of antibody in subjects of white race did not increase with age.[356]

Vertical transmission of CMV acquired during the teenage years may result in congenital infection with CMV in infants born to teenage mothers. In 1984, Kumar and colleagues[189] studied primary CMV infection in more than 3000 pregnant adolescents in Ohio. They found that 57 percent were CMV seropositive, that 1 percent of susceptible pregnant adolescents acquired CMV, and that the risk of intrauterine transmission was 50 percent if primary CMV infection occurred during pregnancy. Information provided by the National Congenital Cytomegalovirus Disease Registry and the National Center for Health Statistics suggests that adolescents who are pregnant actually may be at higher risk than are older mothers for giving birth to an infant with congenital CMV disease because 34 percent of mothers who give birth to a baby with congenital CMV disease are younger than 20 years; however, this age group represents only 16 percent of the mothers giving birth in the United States.[88,163]

INTRAFAMILIAL TRANSMISSION

CMV also can be transmitted within the family setting. Evidence for intrafamilial transmission of CMV has been provided in the form of case reports, seroepidemiologic studies, and accounts in which molecular analysis of CMV strains was used to trace the transmission of CMV in family members or extended family members with temporally related CMV infections. In most studies, the index case was a child; when a CMV-infected child entered a household, attack rates were 47 to 53 percent.[332,337]

Three patterns of intrafamilial transmission have been observed: transmission between siblings, transmission between parents, and transmission between children and parents. Additional support for the intrafamilial transmission of CMV has been provided by published studies in which molecular analysis was performed on CMV isolates from family members or extended family members who experienced temporally related CMV infections. In each of these studies, restriction enzyme analysis of viral DNA from CMV isolates in family members showed that the strain of CMV was the same within each family.[105,246,316] New molecular techniques based on PCR amplification of a hypervariable region of the CMV genome also have corroborated the observation that genetically similar strains of CMV can be transmitted between family members over the course of time.[310]

SEXUAL TRANSMISSION

CMV also appears to be transmitted by heterosexual and homosexual contact. The evidence to support sexual transmission is anecdotal, virologic, serologic, and molecular and, taken together, suggests that sexual transmission of CMV is important in certain groups. However, because the virus is shed in saliva, cervicovaginal secretions, and semen, which form of intimate contact results in transmission between sex partners is unclear.[91,195,272]

Several observations support the idea that CMV can be transmitted sexually. For example, CMV antibody prevalence increases with age; CMV antibody is more prevalent in sexually active women than in celibate women; CMV antibody is associated with indices of sexual activity, such as recent infection with *Chlamydia trachomatis* or *Neisseria gonorrhoeae;* and a strikingly high annual incidence (37%) of primary CMV infection has been observed in young women with a recent first sexual experience.[68,78,86,126,171,309,322,362] In addition, in a longitudinal study of the site-specific shedding of CMV in human immunodeficiency virus (HIV)–seropositive homosexual and bisexual men, CMV was cultured from semen more frequently than from other body sites or fluids.[275] Moreover, molecular analysis of viral DNA has shown strains of CMV isolated from sex partners to be identical in most cases analyzed.[91,152]

Infection of the genital tract can recur by reactivation of an endogenous strain or by re-infection with an exogenous or different strain of CMV.[309] The consequences of such re-infections are unknown but may have important implications for transmission, especially if a CMV vaccine is used to control congenital CMV disease in the future. Sexual debut and recent sexual activity significantly influence CMV seroprevalence among women of child-bearing age. In addition, engaging in sexual activity within 2 years of delivery of an infant adds increased risk of delivering a congenitally infected baby. Because sexual activity appears to influence CMV seroprevalence and congenital CMV infection rates, strategies to reduce sexual transmission of CMV may be important.[126,322]

NOSOCOMIAL TRANSMISSION

CMV can be transmitted in the hospital setting by transfusion of blood products, by bone marrow and organ transplantation, and, rarely, by person-to-person transmission. Despite numerous studies that have used serologic, virologic, and molecular epidemiologic techniques, transmission of CMV from a CMV-infected patient to a health care worker has not been documented.[6,23,28,47,93,106,160] Therefore, even though health care workers are exposed to CMV-infected patients, their risk of acquiring CMV appears to be no greater than that of the general population. In addition, unlike homes and daycare centers, hospitals routinely perform rigorous infection control procedures, including universal precautions, which probably accounts for the relatively low risk of acquiring CMV as well as other infections in the hospital setting. However, infant-to-infant transmission has been shown to occur, albeit infrequently, in crowded hospital units with a high prevalence of CMV excretion in patients.[93,312] CMV also can survive on plastic surfaces and has been cultured from inanimate objects in contact with CMV-infected patients in the hospital setting.[93,312]

Blood products are a well-established source of CMV infection, and donor-to-recipient transmission of CMV has been documented by restriction enzyme analysis of viral DNA.[336] Post-transfusion CMV mononucleosis can be seen in adults who receive large volumes of fresh whole blood.[173] In addition, 15 to 17 percent of CMV-seronegative neonates who receive blood products from CMV-seropositive donors acquire CMV.[372] Post-transfusion CMV infection in newborns, especially premature infants, can cause a syndrome of shock, lymphocytosis, and pneumonitis; CMV infection also appears to hasten the progression to bronchopulmonary dysplasia in these patients.[288,372]

CMV apparently is transmitted in the residual leukocytes found in whole blood, packed red blood cells, and platelet fractions as well as by pure leukocyte transfusions. The risk for development of post-transfusion CMV infection is approximately 3 percent per unit transfused, and the risk of symptomatic infection is much higher in CMV-seronegative recipients than in CMV-seropositive recipients.

IMMUNOSUPPRESSED PATIENTS

Primary and reactivation infections as well as re-infection with CMV occur commonly in organ and marrow transplant recipi-

ents. The health care burden of severe CMV disease appears to be increasing in this special patient group.[296] Active infection with CMV occurs in almost all organ and marrow transplant recipients and usually is manifested clinically and virologically 30 to 90 days after transplantation. Rarely, CMV retinitis can occur years after a patient has undergone transplantation and generally is associated with chronic CMV viremia.[119] CMV can be transmitted to the recipient by the transplanted organ, by transfused blood products, and, theoretically, also by intimate contact with persons actively infected with CMV.

Infections with CMV are primary when they occur in CMV-seronegative recipients who receive transplants or blood products from CMV-seropositive donors. Recipients who are CMV seropositive before undergoing transplantation can experience reactivation of their own endogenous strain of CMV. In addition, CMV-seropositive transplant recipients may be superinfected by strains of CMV present in the donor organ.[71] In fact, in one study of renal transplant recipients in the United Kingdom, proven superinfection with the donor's strain of CMV occurred more frequently than did proven reactivation of the recipient's strain of virus.[148] Although reactivation, re-infection, and primary CMV infection all can produce symptoms in immunosuppressed transplant recipients, primary CMV infections are much more likely to be severe and even fatal.[256]

The type of transplant also appears to influence the type of CMV disease expressed. For example, the most severe and lethal form of CMV interstitial pneumonitis is seen in bone marrow transplant recipients, especially those experiencing a graft-versus-host reaction. CMV pneumonia also is a common occurrence after heart, lung, or renal transplantation, and severe CMV hepatitis is a special problem after liver transplantation.

The iatrogenic immunosuppression essential for graft maintenance probably is responsible for the common occurrence of reactivation infection after transplantation, as well as the increased incidence of severe and symptomatic infection seen after primary CMV infection in transplant recipients. Cytotoxic drugs such as cyclophosphamide and azathioprine, in addition to corticosteroids, have been associated with reactivation of latent CMV infection, and the addition of antilymphocyte globulin to an immunosuppressive regimen can increase the morbidity of CMV infections.[247] The use of cyclosporine as the primary immunosuppressive agent, even in conjunction with corticosteroids, does not appear to reduce the risk of acquiring CMV infection compared with a regimen of azathioprine and corticosteroids; however, it may decrease the incidence of severe, symptomatic CMV disease in some transplant recipients.[104] The use of OKT3 to treat rejection in certain transplant patients increases the risk for dissemination in those with primary CMV infection.[302]

The frequency and morbidity of CMV infection in patients with malignant neoplasms are not as high as in patients who have undergone marrow and organ transplantation; however, the use of chemotherapy, especially for leukemia, is associated with significant CMV disease, particularly pneumonitis and persistent fever with viral dissemination. CMV-seropositive patients receiving immunosuppressive therapy for connective tissue disease also can have significant reactivation infection with CMV.[100]

The progressive and profound immunosuppression in adults and children with AIDS also is associated with CMV infection and disease. Although the source of CMV infection in adults with AIDS most likely is heterosexual or homosexual contact, sources of CMV infection in young children with AIDS have not been determined. One can hypothesize, however, that many of these infections are acquired from the mother, either congenitally or perinatally. Infants infected perinatally with HIV who also acquire CMV congenitally appear to be at significant risk for experiencing rapid progression of disease in the first 18 months of life, as well as debilitating neurologic disease.[101,188,295] Patients with AIDS also may be infected with multiple strains of CMV.[103,313]

PATHOLOGY, PATHOGENESIS, AND IMMUNITY

CMV infection causes characteristic type A Cowdry intranuclear inclusions and massive enlargement of the affected cells (Fig. 171–1). It is this property of "cytomegaly" from which CMV acquired its name. The cytomegalic cells (25-40 μm in diameter) are two to four times larger than normal cells, and the nucleus usually is more than 10 μm in diameter. The intranuclear inclusion also is large (up to 10 μm in diameter) and is surrounded by an intranuclear halo and then the nuclear membrane, which gives a characteristic "owl's eye" appearance. Basophilic, granular, intracytoplasmic inclusions (2-4 μm in diameter) also may be present in cells that have intranuclear inclusions. These large cells represent productive virus infection, and both the nuclear and cytoplasmic inclusions contain viral nucleocapsids and express virus-specific antigens.[157] These cytomegalic cells frequently are associated with epithelial cells, and their presence generally indicates a productive and symptomatic infection with CMV. Cells also may be infected latently with CMV. These cells may express virus-specific antigen and contain viral nucleic acid without producing typical cytomegaly or a cytopathic effect. The significance of these cells must be considered carefully within the clinical context of the patient.

With severe, disseminated CMV disease, involvement can be seen in virtually all organ systems. The salivary glands frequently are infected in both symptomatic and asymptomatic infections, and the ductal epithelium usually is the site of pathologic involvement. The kidneys also are infected frequently in both symptomatic and asymptomatic CMV infection. In the kidneys, cytomegalic cells are most pronounced in the proximal tubules and interstitial cellular infiltrates, and even immune complex deposits can be seen in the glomeruli.

CMV infection often causes prolonged viruria, but it rarely results in significant renal dysfunction. In the lungs, cytomegalic cells are seen in alveolar and bronchial epithelium and are associated with mononuclear cell inflammation. Pulmonary alveolar macrophages also may express viral antigen and contain CMV DNA. The brain parenchyma can be involved, and a variety of pathologic processes can be observed and include sensorineural hearing loss. Other organs that can be infected or diseased with CMV include the liver, pancreas, adrenals, eyes, lymph nodes,

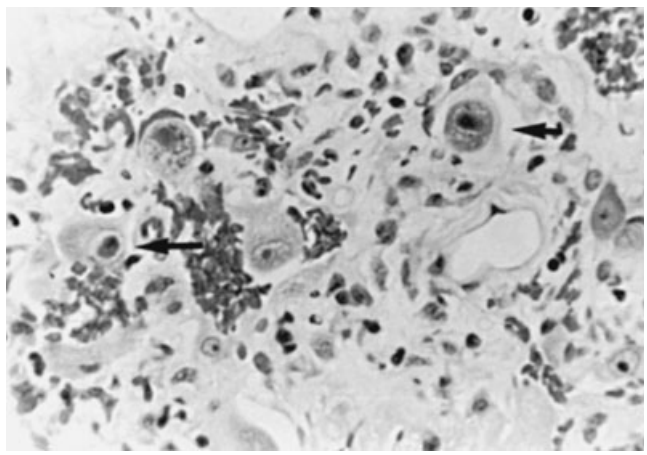

Figure 171–1 Typical cytomegalic inclusion cells seen in lung tissue obtained by open lung biopsy from a bone marrow transplant recipient with fatal cytomegalovirus pneumonitis (×640). *(Courtesy of Dr. Milton J. Finegold, Department of Pathology, Texas Children's Hospital, Houston.)*

heart, skin, bone, male and female genital tracts, esophagus, stomach, intestine, and placenta.

Infections with CMV can be latent and nonproductive, productive yet asymptomatic, or productive and symptomatic. Therefore, determination of whether a patient is "sick with CMV" or "sick from CMV" sometimes is difficult. Viral strain differences have not been shown to date to influence pathogenicity. However, immune responses, including maturity of the immune response, appear to be a major factor in virulence of the infection because CMV disease occurs more frequently in fetuses, premature neonates, transplant recipients, and patients with AIDS than in older healthy infants, children, and adults with acquired CMV infection.

The cell-mediated immune response, both specific and nonspecific, is thought to be important in host defense against CMV. The nonspecific immune mechanisms of natural killer cells and interferon production occur soon after the development of CMV infection, when early antigens are being produced and before infectious virus is released from the cell. The generation of cytotoxic T cells against CMV early antigens probably is the most important specific host immune response to CMV, and patients who are defective in this T-cell response are at high risk for acquiring serious CMV disease.[145]

Clinical and laboratory evidence also supports the concept of CMV as an immunosuppressive agent. The proliferative response of T cells to stimulation with mitogens and CMV antigen is suppressed in patients with CMV mononucleosis, and immunosuppressed patients with active CMV disease frequently have other opportunistic infections.[274] In addition, CMV pneumonitis in bone marrow transplant recipients has been hypothesized to result from host cell–mediated events produced in response to chronic viral replication.[378] Homology between certain CMV proteins and class I and class II major histocompatibility complex products has been observed. This "molecular mimicry" implies that the severe tissue destruction seen in CMV pneumonitis may be partly an autoimmune phenomenon in these patients.

Humoral immunity, on the other hand, does not appear to be a key factor in the host's defense against CMV infection. For example, the fetus can be infected by intrauterine transmission as a result of reactivation of CMV infection in a woman who is CMV seropositive before pregnancy, and infants frequently are infected perinatally from infected cervicovaginal secretions or breast milk in the presence of passive maternal antibody.[318] In addition, CMV-seropositive transplant recipients can be reinfected with a new strain of CMV from the donor organ, and viruria and viremia occur in transplant recipients despite high titers of neutralizing antibody against the specific strain of CMV.[71,72] The presence of CMV antibody, therefore, should be considered a marker of previous or current infection with the virus rather than a measure of immunity per se.

Although humoral immunity does not appear to prevent infection with CMV, it does appear to lessen the severity of associated symptoms. Infants congenitally infected with CMV as a result of reactivation of infection in their mother almost always are asymptomatic, whereas perinatally infected infants rarely have significant symptoms. Primary infections in transplant recipients are more likely to be symptomatic than is re-infection or reactivation. One hypothesis regarding how CMV eludes host humoral defenses is that the virus binds to the host protein β_2-microglobulin and masks the antigenic determinants that are important for neutralization by antibody.[211]

CLINICAL MANIFESTATIONS

FETAL AND CONGENITAL INFECTIONS

The fetus with in utero CMV infection may show ultrasonographic abnormalities, including signs of abnormal somatic or head growth, enlarged liver or spleen, echogenic bowel patterns, and hepatic calcifications, as well as biologic findings such as fetal thrombocytopenia and lymphopenia and elevated liver enzymes and elevated CMV-specific IgM antibody levels. In addition, cerebral fetal sonography may show enlarged ventricles in the fetal brain, intracranial calcifications, or microcephaly or mimic a Dandy-Walker malformation in severe cases.[21,150,203] Annually, 30,000 to 40,000 babies are born with congenital CMV infection. Of these babies, as many as 10 percent have severe, classic "cytomegalic inclusion disease" characterized by intrauterine growth retardation, jaundice, hepatosplenomegaly, thrombocytopenia with petechiae and purpura, pneumonia, and severe central nervous system (CNS) damage with microcephaly, intracerebral calcifications, chorioretinitis, and sensorineural hearing loss. Another 5 percent have atypical involvement, such as ventriculomegaly, periventricular leukomalacia, periventricular cystic malformations with or without calcifications, strabismus, optic atrophy, long bone osteitis characterized by fine vertical metaphyseal striations, isolated and transient thrombocytopenia and petechiae, cutaneous vasculitis, hemolytic anemia, ascites, chronic hepatitis, and intrahepatic cholestatic disease.[13,76,121,168] As many as 90 percent of these infants who are symptomatic at birth later have neurologic sequelae, vision loss, or progressive deafness. However, the range of severity of these sequelae appears to be broad, and severity may be predicted by head circumference and computed tomographic findings at birth.[43,231] In addition, CMV viremia and DNA in congenitally infected newborns is associated with symptomatic disease and hearing loss at birth, suggesting that the level of virus contributes to disease severity.[44,46]

The differential diagnosis of symptomatic congenital CMV disease includes congenital toxoplasmosis, congenital HSV infection, congenital syphilis, congenital rubella syndrome, congenital infection with lymphocytic choriomeningitis virus, and congenital HIV infection. In addition, non-infectious causes, such as genetic disorders, metabolic disease, and maternal exposure to drugs and toxins, should be considered.

Most infants who are infected congenitally with CMV are asymptomatic at birth. However, 10 to 17 percent of these infants later may have unilateral or bilateral deafness or differences in higher level auditory function.[80,318,361] Progressive or late-onset hearing loss also may occur in these infants, thus rendering it likely that universal newborn hearing screening programs will miss rather than detect many of these children.[124,232] Small retinal lesions may occur as well.[76] Earlier studies suggested that developmental problems may occur in asymptomatically infected children, but more recent studies suggest that cognitive outcome appears to be normal compared with uninfected children.[177]

Very low-birth-weight infants who are infected congenitally with CMV may experience pulmonary and systemic deterioration temporally associated with steroid therapy.[339]

PERINATAL INFECTIONS

Perinatally acquired infections in healthy infants usually are manifested when the child is between 4 and 16 weeks of age, but most such infections are asymptomatic. However, as many as one third of infants exposed to CMV perinatally may have signs and symptoms of disease associated with CMV infection, most often self-limited lymphadenopathy, hepatosplenomegaly, hepatitis, or pneumonitis.[191] Perinatally acquired infection with CMV also can cause a viral, sepsis-like syndrome or severe, protracted pneumonitis that has been associated with the development of bronchopulmonary dysplasia in premature infants.[208,288] A limited number of studies suggests that these infections, however, do not appear to cause neurodevelopmental sequelae or deafness.[192] The differential diagnosis includes other perinatally acquired infections, such as *Chlamydia* pneumonitis, hepatitis B virus infection, and

infection with HIV, as well as postnatally acquired infections with enteroviruses, adenovirus, and a variety of bacterial pathogens.

MONONUCLEOSIS SYNDROME

CMV-induced mononucleosis occurs as a primary infection in both immunocompetent and immunosuppressed persons and, occasionally, as a reactivation infection in immunosuppressed patients. It can result from person-to-person transmission of the virus as well as from transmission by blood products or by organ or marrow transplantation. Although originally described in adults and most often occurring in patients between 20 and 40 years of age, it also can be seen in adolescents, children, and even infants.[206,240] Typical CMV-induced mononucleosis is characterized by fever and strikingly severe malaise of approximately 1 to 4 weeks' duration, peripheral lymphocytosis with atypical lymphocytes, and mildly elevated liver enzymes. In some patients, headache, myalgias, and abdominal pain with diarrhea are prominent symptoms. In premature infants with transfusion-acquired CMV mononucleosis, prominent manifestations include shock, hepatosplenomegaly, pneumonitis, thrombocytopenia, and renal failure.[312] In contrast to Epstein-Barr virus–induced mononucleosis, CMV-induced mononucleosis rarely causes pharyngitis, tonsillitis, or significant splenomegaly, and it does not result in production of heterophil antibodies.[77,161] However, like Epstein-Barr virus–induced mononucleosis, it can be associated with morbilliform rash after administration of ampicillin, elevated erythrocyte sedimentation rate, polyclonal hypergammaglobulinemia, and production of other antibodies such as rheumatoid factor, cold agglutinins, and antinuclear antibodies. Complications rarely occur but include interstitial pneumonitis, myocarditis, pericarditis, hemolytic anemia, thrombocytopenia with or without petechiae or purpura, hemophagocytic syndrome, arthralgias and arthritis, maculopapular rashes, adrenal insufficiency, splenic infarction, ulcerative colitis and proctitis, Guillain-Barré syndrome, and meningoencephalitis.[66,77,83,118,161,186,187,200] Severe, icteric hepatitis as well as granulomatous hepatitis also can occur, but hepatic necrosis and liver failure caused by CMV have not been documented convincingly in normal hosts.[41,77,161,187,269]

The differential diagnosis of CMV-induced mononucleosis includes mononucleosis induced by other viruses such as Epstein-Barr virus, hepatitis A or B virus, and HIV. In addition, acquired toxoplasmosis can produce a mononucleosis syndrome in healthy persons.

INTERSTITIAL PNEUMONITIS

CMV is a major cause of interstitial pneumonia in both adults and children who are immunosuppressed because of congenital immunodeficiency, AIDS, organ or marrow transplantation, or malignant disease. In recipients of bone marrow transplants, CMV accounts for 17 to 70 percent of cases of interstitial pneumonitis in adult patients but only 10 percent of cases in patients younger than 21 years.[353] Pneumonia also can occur in apparently immunocompetent young infants with perinatally acquired CMV infection and in healthy adults with CMV-induced mononucleosis.[181,191,317,357] Whereas the pneumonia in immunocompetent hosts almost always is benign and self-limited, CMV pneumonia in immunosuppressed patients is a serious, often fatal illness, especially in bone marrow transplant recipients, who have a mortality rate of up to 90 percent. It also can be particularly troublesome after pediatric heart and lung transplantation.[230] CMV pneumonitis usually occurs 1 to 3 months after the patient has undergone transplantation and begins with symptoms of fever and a dry, nonproductive cough. It then can progress during the course of 1 to 2 weeks to dyspnea, retractions, wheezing, and hypoxia, which require ventilatory support. It may occur as the only disease manifestation or be part of a disseminated CMV infection. The radiographic appearance of CMV pneumonia usually is diffuse, interstitial infiltrates, but peribronchial infiltrates with hyperinflation and nodular pulmonary infiltrates also have been described (Fig. 171–2).[181,334] Co-infection with other pathogens, especially gram-negative enteric bacteria and fungal pathogens in transplant recipients and in patients with *Pneumocystis carinii* infection and AIDS, can occur.[349]

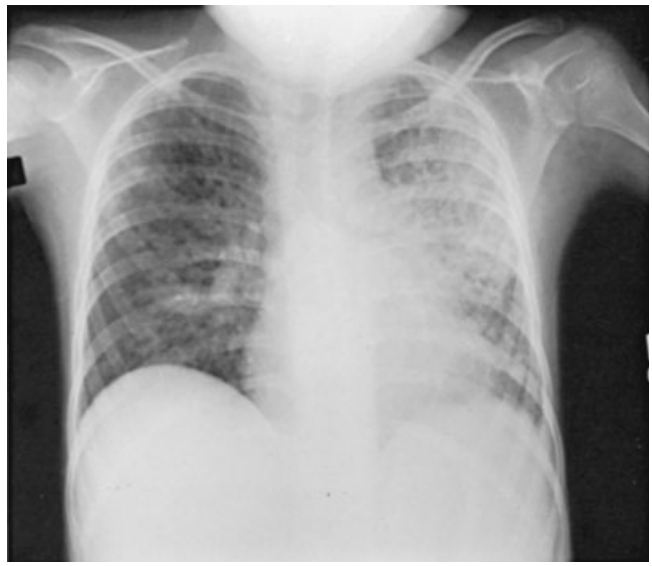

Figure 171–2 Chest radiograph of a bone marrow transplant recipient with rapidly fatal cytomegalovirus pneumonitis. An open lung biopsy specimen showed numerous cytomegalic inclusion cells, exhibited cytomegalovirus early antigens by immunoperoxidase staining, and grew cytomegalovirus after inoculation into tissue culture.

Congenitally infected infants also may be born with CMV pneumonitis, which often is sufficiently severe to require ventilatory support and usually is part of a multisystem CMV disease process. Very low-birth-weight infants who are infected congenitally with CMV may experience CMV pneumonitis in temporal association with steroid therapy.[339]

The differential diagnosis of CMV pneumonitis in immunocompromised patients, including neonates, is extensive. It includes infection with other viruses, such as HSV, VZV, measles virus, respiratory syncytial virus, influenza A and B viruses, parainfluenza viruses, and adenoviruses; bacterial pneumonia caused by a variety of gram-positive and gram-negative organisms; infection with protozoa, such as *P. carinii* and *Toxoplasma gondii; Chlamydia* and *Mycoplasma;* and fungal pneumonia, caused especially by *Candida* and *Aspergillus.* Non-infectious causes of pneumonitis, such as pulmonary hemorrhage, aspiration pneumonia, rejection, and pulmonary damage from chemotherapeutic agents, also should be considered.

RETINITIS AND OTHER EYE ABNORMALITIES

Chorioretinitis as well as optic atrophy, cortical blindness, and strabismus occurs in 17 to 41 percent of newborns with symptomatic congenital CMV infection and rarely in children born with asymptomatic congenital CMV infection.[42,76,284] Although most retinal lesions in congenitally infected infants appear to be inactive at birth, some observations suggest that progression of preexisting lesions and late-onset new lesions resulting in vision

loss may occur rarely in both symptomatic and asymptomatic congenitally infected infants.[42] Retinitis does not seem to be a prominent part of perinatally acquired infection.[192] CMV retinitis once was a rare manifestation of CMV disease in solid organ transplant recipients undergoing chronic immunosuppression for more than a year and in patients receiving chemotherapy for malignant disease.[119] In the 1980s, CMV retinitis emerged as a frequent manifestation of CMV disease in patients with severe immunosuppression, especially bone marrow transplant recipients and patients with AIDS. It probably is a result of hematogenous spread of the virus to the retina, with continued local viral replication. Despite the common occurrence of CMV retinitis in adults with AIDS, however, it rarely has been reported in children with AIDS.[201]

CMV produces characteristic white, perivascular infiltrates and hemorrhage, with a necrotic, rapidly progressive retinitis. It descriptively has been called *cottage cheese retinitis* and *ketchup* or *brushfire retinitis*.[40] Early, peripheral retinitis can be asymptomatic, or the complaints may be minimal and nonspecific; it is especially difficult to ascertain in infants and young children. It does not cause eye pain, photophobia, or conjunctivitis. Once the retinitis has progressed, it can cause blurred vision, decreased visual acuity, visual field defects, and blindness. Young children and infants who have suffered visual loss as a result of CMV retinitis may exhibit strabismus or failure to fix and follow objects within their visual field. CMV retinitis also can progress rapidly to total blindness if the macula is involved. Immunosuppressed children with CMV disease should receive regular expert ophthalmologic examinations to monitor for the development of sight-threatening retinitis. Establishing the diagnosis early may allow prompt institution of antiviral therapy, which may be sight saving.[201]

The ophthalmoscopic appearance of CMV retinitis usually is characteristic. However, in patients in whom the appearance of the retina is not typical or in whom the retinitis has progressed despite specific antiviral treatment, other causes of retinal lesions that should be considered include cotton-wool spots associated with hypertension, diabetes, connective tissue disease, anemia and leukemia, ocular toxoplasmosis, and candidal infection of the retina as well as syphilis, HSV infection, lymphocytic choriomeningitis virus infection, and VZV infection. Detection of the virus by culture or its DNA by a PCR-based method in vitreous fluid may help establish the diagnosis in difficult or atypical patients.[116] CMV antibodies also have been detected in the tears of patients with active ocular infections, including retinitis.[283]

CMV also has been associated in some studies with other unusual eye abnormalities. In coincidentally congenitally infected infants, microphthalmos, anophthalmia, optic nerve hypoplasia and coloboma, optic nerve atrophy, Peter anomaly, and irregular retinal pigment have been observed.[129] However, the role that CMV is playing in these anomalies is unclear and unsubstantiated by prospective studies.[76] CMV also does not appear to cause congenital cataracts.[301] CMV also has been isolated from tears and has been associated with conjunctivitis in patients with CMV mononucleosis and AIDS as well as with corneal epithelial keratitis and disk neovascularization.[198]

HEPATITIS

CMV hepatitis in bone marrow, heart, and lung transplant recipients, in patients with cancer or AIDS, and even in healthy persons experiencing a primary CMV infection usually is manifested as mild hepatomegaly and mildly elevated serum hepatic enzyme levels. It commonly occurs in conjunction with fever, thrombocytopenia, and lymphopenia or lymphocytosis. Jaundice and hyperbilirubinemia usually do not occur, severe hepatitis or cirrhosis is exceedingly rare, and hepatic necrosis and liver failure caused by CMV hepatitis have not been documented convincingly in these patients. CMV infection also has been associated with granulomatous hepatitis.[41,269] In addition, CMV hepatitis is a unique and prominent problem in children who have undergone liver transplantation.[49] Most CMV hepatitis occurs 1 to 2 months after the patient has undergone transplantation, but it may be noted as early as 2 weeks or as long as 4 months afterward.[51] It is more common and more severe after a primary CMV infection and is associated with liver transplantation from a CMV-seropositive donor and the use of OKT3 antibodies for severe rejection.[303] CMV hepatitis in liver transplant recipients is characterized by prolonged fever, leukopenia, thrombocytopenia, elevated liver enzymes, hyperbilirubinemia, and liver failure. Distinguishing between CMV hepatitis and acute rejection often is difficult, even with a liver biopsy, and the two commonly coexist. CMV infection also has been associated with ascending cholangitis, chronic rejection, and the vanishing bile duct syndrome in liver transplant recipients.[51,233]

Infants with congenital CMV disease also may have hepatitis. The liver usually is smooth and nontender and commonly extends 3 to 5 cm below the right costal margin. Ascites may be present prenatally and may persist postnatally for 1 to 2 weeks. The hepatomegaly usually resolves by the time the infant is 3 months of age, and persistence beyond 1 year is highly unusual. Mild hepatitis generally is present, and transaminase levels in neonatal hepatitis caused by CMV rarely exceed 500 IU. Hyperbilirubinemia is present at birth in approximately a third of newborns with congenital CMV and may be striking, with conjugated (direct) bilirubin levels up to 30 mg/dL.[163] The abnormal results of liver function tests gradually resolve during the course of the first few weeks of life, and chronic hepatitis as a result of congenital infection with CMV is an unusual occurrence. Congenital CMV disease also has been associated with intrahepatic and extrahepatic biliary atresia in some studies, but the direct role of CMV in neonatal cholestatic disease is unclear.[13,121,168]

The differential diagnosis of CMV hepatitis includes other causes of viral hepatitis, such as hepatitis A virus, hepatitis B virus, hepatitis C virus, Epstein-Barr virus, HSV, enterovirus, and adenovirus as well as toxoplasmosis; other infections, such as bacterial ascending cholangitis; and non-infectious causes, such as ischemic injury, vascular thrombosis, hemolysis, rejection, and hepatitis induced by drugs or toxins. In newborns with a significant and persistent direct hyperbilirubinemia, the diagnosis of biliary atresia should be considered as well.

GASTROINTESTINAL DISEASE

Serious gastrointestinal disease causing esophagitis, gastritis, gastric outlet obstruction, gastroenteritis, pyloric and small bowel obstruction, duodenitis, colitis, proctitis, pancreatitis, hemorrhage, and acalculous cholecystitis has been associated with CMV infection in immunocompromised persons, especially patients with AIDS or neoplastic disorders and those who have undergone bone marrow, kidney, intestinal, or liver transplantation.* Rarely, self-limited CMV gastroenteritis, colitis, and proctitis have been associated with CMV mononucleosis syndrome in apparently normal individuals.[77,161,259,260] Characteristic signs and symptoms in infants and children include nausea, vomiting, dysphagia, epigastric pain and tenderness, delayed gastric emptying, watery guaiac-positive stools or gastrointestinal hemorrhage, and disaccharide and monosaccharide intolerance. Severe disease may cause dehydration and failure to thrive. Endoscopy with biopsy is required to establish a definitive diagnosis and usually shows linear, localized, or punctate ulcers. Hemorrhagic lesions or diffuse erosion can occur in severe disease. Characteristic cyto-

*See references 11, 94-96, 204, 219, 236, 249, 258, 267, 342.

megalic inclusion cells can be seen in the gastrointestinal endothelium, epithelium, and glandular tissue; CMV may be cultured from stool or biopsy specimens; or CMV DNA may be detected by PCR-based methods.[137] In addition, these patients often are viremic and occasionally have evidence of disseminated CMV infection with involvement of the lungs and retina.

The differential diagnosis of CMV colitis includes infection with other viruses, especially HSV and adenovirus, and infection with bacteria, particularly *Salmonella*, *Shigella*, *Campylobacter*, and *Yersinia*, as well as *Clostridium difficile* and *Mycobacterium avium-intracellulare*. Parasitic infection with *Cryptosporidium*, *Giardia*, and amebae also should be excluded. The differential diagnosis of CMV esophagitis and gastritis includes HSV infection, *Candida* esophagitis, reflux esophagitis, and peptic ulcer disease.

MENINGOENCEPHALITIS AND OTHER NEUROLOGIC DISORDERS

CNS involvement is well described and occurs relatively frequently in infants with symptomatic congenital CMV infection. Although the severity of damage to the CNS during congenital infection varies greatly, postmortem examination of severely affected infants has demonstrated necrotizing encephalitis, especially in the deep periventricular structures, and scattered areas of necrosis and inclusion-bearing cells. Although direct viral infection of neural structures probably plays a major role in CNS disease in congenital CMV infection, infectious vasculitis also may occur. In addition, because congenital CMV disease can be associated with marked thrombocytopenia, intracranial hemorrhage can contribute to CMV-related CNS injury.[26,57,153,345] Clinical manifestations of this disease process include microcephaly, cerebral palsy, intracerebral calcifications, seizures, hemiparesis, developmental delay, ventriculomegaly, paraventricular cysts, intraventricular strands, periventricular leukomalacia, lissencephaly-pachygyria, porencephaly, multifocal deep white matter lesions, meningoencephalitis, and sensorineural deafness (Fig. 171–3).[193,330,341] Remarkably, despite the well-documented neuropathologic process in congenital disease, isolation of CMV from the cerebrospinal fluid (CSF) of a congenitally infected child is an extremely unusual finding.[167] CMV DNA has been detected in the CSF of congenitally infected infants, and its presence at birth appeared to identify infants at risk for a poor neurodevelopmental outcome.[20]

The differential diagnosis of symptomatic congenital CMV infection with neurologic disease includes congenital toxoplasmosis, congenital HSV infection, congenital rubella syndrome, congenital infection with lymphocytic choriomeningitis virus, brain tumors such as craniopharyngioma, and calcified hematoma. In addition, congenital CMV disease involving the CNS also may be mimicked by genetic disorders such as tuberous sclerosis, Sturge-Weber syndrome, and Aicardi syndrome; metabolic conditions such as hyperthyroidism; α_1-antitrypsin deficiency; galactosemia; peroxisome disorders such as Zellweger syndrome, neonatal adrenoleukodystrophy, and infantile Refsum disease; urea cycle deficiencies; organic acidemias; and liposomal storage disorders.[25] Maternal exposure to drugs and toxins, especially isotretinoin, cocaine, and alcohol, also is included in the differential diagnosis. The presence or absence of intracerebral calcification as well as the pattern of calcifications when they are present may be helpful in distinguishing among these disorders. In addition, the appropriate microbiologic studies, chromosome analysis, metabolic studies, and drug screens should be performed.

In postnatal life, CMV meningoencephalitis appears to be rare yet well documented.[24] It may occur as a complication of CMV mononucleosis, as an isolated manifestation of primary CMV infection in a normal host, or as a primary or recurrent infection in an immunocompromised patient.[260] Symptoms include headache, photophobia, nuchal rigidity, memory deficits, and inability to concentrate. CSF findings include mild mononuclear pleocytosis and slightly elevated protein. Although the virus is isolated from the CSF and brain parenchyma exceedingly rarely, neuropathologic findings of intranuclear inclusions and microglial nodules are characteristic.

CMV encephalitis may complicate adult immunocompromised transplant recipients, and recognition of CMV encephalitis in patients with AIDS is growing.[30] As many as 50 percent of patients with AIDS may have evidence of CMV infection of the CNS at postmortem examination.[226] CMV has been reported to cause a subacute, occasionally progressive encephalitis in patients with AIDS and has been implicated as a cofactor in the pathogenesis of the AIDS dementia complex seen in both adults and children.[82,156,220,306,360] In this disease, CMV may be isolated from CSF, or CMV DNA may be detected in the fluid or brain by PCR-based methods.[140] In children, this syndrome is characterized by weakness, confusion, and loss of developmental milestones.

The differential diagnosis of CMV encephalitis and meningoencephalitis in a normal host includes primarily other neurotropic viruses, such as HSV, Epstein-Barr virus, VZV, enterovirus,

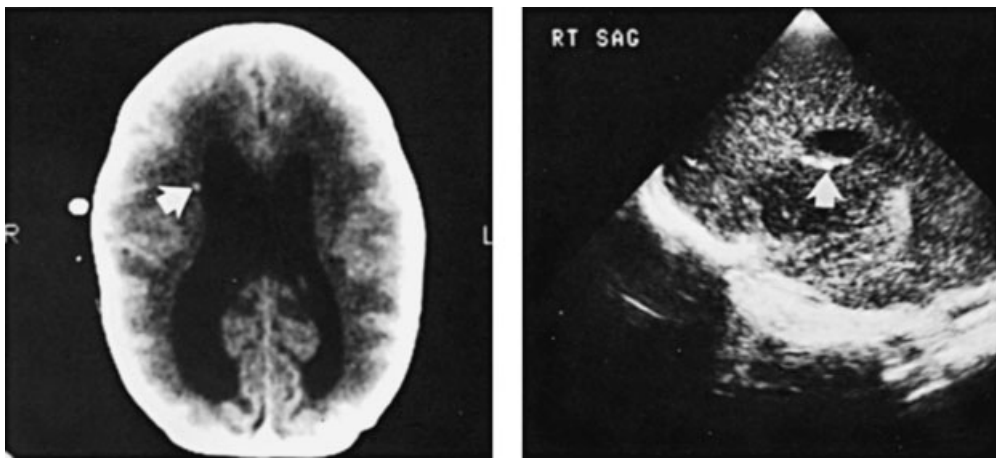

Figure 171–3 Computed tomographic scan *(left)* and ultrasound examination *(right)* of the head of an infant with congenital cytomegalovirus disease. Both tests showed moderate asymmetric enlargement of the lateral ventricles with punctate periventricular calcifications.

and arboviruses. Neurosyphilis and tuberculous meningitis also should be considered. In immunocompromised patients, especially those with AIDS, the following should be added to the differential diagnosis of CNS infection: progressive multifocal leukoencephalopathy caused by papovavirus; HIV encephalitis; fungal CNS infection caused by *Cryptococcus neoformans*, *Candida* spp., *Aspergillus*, or *Histoplasma*; protozoal infections with *T. gondii* and, rarely, *P. carinii* and *Strongyloides stercoralis;* bacterial infections with *M. avium-intracellulare* and *Nocardia asteroides;* non-infectious diseases such as primary cerebral lymphoma and lymphomatoid granulomatosis; and vascular complications such as hemorrhage and infarction.[14] In immunosuppressed patients, more than one of these conditions can coexist in the CNS.

CMV also can invade the peripheral nervous system and cause a painful peripheral neuropathy in patients with AIDS.[130,278,344] Ascending paralysis caused by myelitis, with or without vasculitis or necrosis, also can occur and may appear similar to Guillain-Barré syndrome.[344] In addition, CMV polyradiculopathy has been described in adult patients with AIDS and may occur in older children. This disease usually begins with leg pain and sacral paresthesias and may progress to weakness and flaccid paralysis. The CSF characteristically has a polymorphonuclear pleocytosis and moderately elevated protein.[182] The association of CMV infection with infantile spasms, Guillain-Barré syndrome, Charcot-Marie-Tooth disease, Huntington disease, Alzheimer disease, myasthenia gravis, and neuropsychiatric diseases (e.g., schizophrenia) has been reported, but a definite causal relationship between CMV and these diseases remains to be proved.[24]

DEAFNESS AND OTHER EAR DISORDERS

Hearing loss is present at birth in approximately 25 to 50 percent of infants with symptomatic congenital CMV infection and in approximately 15 percent of infants with asymptomatic congenital CMV infection.[163] Given that congenital CMV infection affects 30,000 to 40,000 infants annually in the United States, this congenital infection probably is the most common cause of nonhereditary sensorineural deafness. Investigators have estimated that congenital CMV infection may account for as many as 40 percent of cases of congenital hearing loss.[32,97,147] Progression or fluctuation of the hearing loss occurs in at least two thirds of these children through the preschool years, and continued progression may occur through the school-age and adolescent years.[125,361] Although CMV has been detected in the endolabyrinth of infants who died of congenital CMV disease, whether this progressive deafness is caused by continued viral replication in the inner ear, re-infection with a new strain of virus, or a complex cascade of immunopathologic events remains unclear.[85] Mondini dysplasia of the temporal bones has been seen in infants with congenital CMV infection, but the importance of this observation relative to the pathogenesis of the progressive hearing loss commonly seen in congenitally infected infants is unknown at this time.[36] Some studies suggest that higher levels of CMV in urine and blood are present in newborns who develop hearing loss from congenital CMV infection,[17,194,277,348] whereas other studies speculate that genetic mutations or inflammatory genes may play a role in CMV-associated hearing loss.[232,234,281,294]

CMV has been isolated from the middle ear effusions of healthy and immunocompromised children with otitis media.[70,109] In addition, CMV infection, defined serologically, has been associated with sudden-onset deafness, acute labyrinthitis, and Meniere syndrome.[326,363] Older children who were born with asymptomatic congenital CMV infection also may exhibit differences in higher auditory function, even though they do not have sensorineural or conductive hearing loss.[80]

MYOCARDITIS AND OTHER CARDIOVASCULAR DISORDERS

Myocarditis has been described as a rare complication of severe congenital CMV disease and CMV mononucleosis in presumably healthy adults and children.[335,351,364] CMV myocarditis also has been seen in renal and heart transplant recipients, usually as part of a disseminated CMV infection and associated with graft rejection treated with high-dose immunosuppressive therapy.[136,257,297] Patients can have heart failure, cardiomegaly, electrocardiographic abnormalities, and poor left ventricular function on echocardiography; cytomegalic inclusion cells and the presence of CMV DNA can be documented by myocardial biopsy.

The association of CMV infection with other cardiac disorders such as congenital heart block, structural cardiac anomalies, and pericarditis is anecdotal, and a cause-and-effect relationship is not well documented.[176] CMV coronary endotheliitis with superimposed thrombosis and myocardial infarction has been described in adult heart transplant recipients and was reported in an infant who died of disseminated CMV disease with an apical ventricular aneurysm.[218,286] CMV infection also has been postulated to play a role in the pathogenesis of atherosclerosis and coronary artery disease in both normal persons and heart transplant recipients.[142,210,213]

ENDOCRINE SYSTEM

Histopathologic evidence of involvement of the organs of the endocrine system is described well in both congenital and postnatally acquired disseminated CMV infections.[157] Endocrine disorders such as Graves disease and diabetes insipidus have been associated with congenital CMV infection, but these reports may represent coincidental findings.[214,285] Longitudinal studies are required to determine whether the autoimmune endocrinopathies in children with congenital rubella parallel the findings in children with congenital CMV infection. CMV infection also has been associated with autoimmune type 1 diabetes, although a specific cause-and-effect relationship has not been established.[239] In addition, immunosuppressed patients, especially persons with AIDS, may manifest clinical endocrinopathies caused by CMV infection, such as adrenal insufficiency and adrenal necrosis.[143] CMV inclusions also have been found in the pituitary gland of patients with AIDS, all of whom showed evidence of CMV encephalitis or disseminated infection elsewhere in the body.[117] Moreover, involvement of the thyroid and parathyroid glands with CMV has been reported in adults with AIDS.[127]

GENITOURINARY SYSTEM

Disease of the male and female genitourinary system as a result of CMV has been reported. Patients with AIDS may have symptomatic epididymitis and cystitis caused by CMV.[38,261,328] Lower urinary tract infections and hemorrhagic cystitis caused by CMV have been diagnosed in patients after they have undergone stem cell and solid organ transplantation.[238] CMV also commonly yet asymptomatically infects the cervicovaginal secretions and semen of both healthy and immunosuppressed adults.[171,195]

SKIN

Cutaneous manifestations of CMV infection are described well and can occur with both congenital and acquired CMV infection.[200] Infants with symptomatic congenital infection may have nonpalpable petechiae, purpura, or bruises, usually as a result of thrombocytopenia. Violaceous or dark, magenta infiltrative papules or nodules, called *blueberry muffin lesions*, also may occur,

but these lesions are more characteristic of congenital rubella syndrome. The skin lesions associated with acquired CMV infection usually are localized cutaneous ulcers or a widespread, exanthematous, maculopapular eruption, although vesiculobullous lesions also have been described.[248] CMV mononucleosis syndrome in adults and children may be accompanied by a maculopapular, rubelliform rash that may be pruritic. In addition, ampicillin-associated rashes may occur with CMV mononucleosis. CMV also may cause a cutaneous, leukoblastic vasculitis.[287] Well-demarcated, ulcerated lesions that show histopathologic evidence of CMV infection may be seen in immunocompromised patients who have undergone transplantation or in those who suffer from AIDS. Finally, CMV may play a role in the neoplastic process in Kaposi sarcoma, but definitive proof of a causal relationship remains to be shown.

UNUSUAL ASSOCIATIONS

CMV infection, either congenital or acquired, has been detected in association with a wide variety of conditions, including defects in tooth structure and the formation of enamel, portal vein thrombosis associated with protein S and protein C deficiency, unexplained fevers in burn patients, bacterial sepsis in burn patients, congenital eventration of the diaphragm, inguinal hernia, and fatal *Staphylococcus epidermidis* infection in very low-birth-weight infants.[16,37,174,190,205,319] However, given the common occurrence of CMV infection, these associations may be coincidental rather than part of a cause-and-effect relationship.

LABORATORY DIAGNOSIS

DETECTION OF THE INFECTIOUS AGENT

CMV can be isolated in tissue culture with fibroblast cell lines such as human foreskin fibroblasts and human embryonic lung fibroblasts.[293] Specimens that contain a high titer of virus, such as those from congenitally infected infants, may show growth in 24 hours. Some specimens, such as those from persons with acquired asymptomatic infection, may require as long as 6 weeks for detectable growth, but most cultures grow in 1 to 2 weeks. CMV has been isolated from a variety of specimens, including urine, saliva, nasopharyngeal and sinus washings, conjunctiva, tears, middle ear fluid, breast milk, semen, cervicovaginal secretions, stool, CSF, white blood cells, amniotic fluid, bronchial lavage samples, and biopsy or autopsy specimens. All samples for isolation of virues (except blood, which should be at room temperature) should be held at 4° C (on wet ice or in a refrigerator) until processed in the virology laboratory. Specimens for isolation of virus should be inoculated within hours of collection for an optimal isolation rate. Although isolation of CMV proves that a productive infection is present, it does not confirm an etiologic relationship with the disease process and requires careful interpretation within the patient's clinical context.

An adaptation of tissue culture is a low-speed centrifugation enhancement, monoclonal antibody culture technique, also called *shell vial assay*. In this test, inoculated tissue culture cells in small vials are stained with a fluorescein-conjugated monoclonal antibody to either an early or a late CMV antigen (or both). Cells infected with CMV exhibit nuclear and membrane fluorescence 18 to 72 hours after inoculation. This rapid viral diagnostic technique is especially reliable with urine and bronchoalveolar lavage specimens and has been applied with variable results to blood and tissue specimens.[135] However, maximal sensitivity and specificity are obtained when shell vials are used as an adjunct to and not in place of routine tissue culture. CMV-infected cells also can be detected by direct immunofluorescence assay on exfoliated cells in bronchoalveolar lavage specimens or in frozen tissue specimens.[151] These procedures, however, require a laboratory experienced in direct immunofluorescence assay technique.

Traditional DNA-DNA hybridization methods and, more recently, traditional and real-time PCR-based methods are available in many laboratories for detection of CMV DNA. These very sensitive tests may be used to detect and to quantify CMV DNA in a variety of samples and are useful for diagnosis or prediction of CMV disease and for monitoring, as well as for monitoring response to antiviral treatment.[54,62,84,90,134,306,309,315]

Detection of CMV DNA in the CSF of patients with AIDS seems to be a reliable diagnostic method for detection of CMV infection of the CNS, and detection of CMV DNA in the CSF of newborns with congenital CMV disease correlates with poor neurodevelopmental outcome.[20,75,140,366] Similarly, detection of CMV DNA by PCR in vitreous fluid provides persuasive evidence that a patient's retinitis is CMV related.[104] CMV DNA also may be detected in the white blood cells of patients with CMV infection, and detection of CMV DNA in the plasma or serum of selected groups of patients, such as newborns and immunocompromised patients, appears to correlate with severity of disease and viral dissemination.[225,300,314,315] Detection of CMV DNA in amniotic fluid can provide prenatal diagnosis of congenital CMV infection and disease.[203] PCR assays also have detected CMV DNA in dried blood spots or Guthrie cards collected as part of newborn screening programs and used to diagnose congenital CMV infection and disease.[32,33,348]

Another use for PCR-based diagnosis of CMV infection is the very early diagnosis of CMV infection in high-risk patients, such as transplant recipients, before the development of potentially fatal CMV disease, such as pneumonitis. This approach may allow preemptive antiviral therapy to be initiated when CMV infection appears active but before overt disease is detected.[53,367] The role of CMV DNA detection by PCR assay in monitoring a patient's response to antiviral therapy, however, appears to be limited because CMV DNA persists for long periods after the resolution of CMV-related clinical symptoms in many patients.[131]

CMV pp65 antigen also may be detected in the white blood cells of patients with CMV infection and disease, and this CMV antigenemia test is used by many clinical laboratories as a rapid screen for CMV viremia, providing results the same day. It is relatively easy to perform, and the degree of antigenemia may be quantitated to monitor response to antiviral therapy.[131]

Exfoliated cells in urine or bronchoalveolar lavage specimens or cells in tissue obtained by biopsy can be examined for histologic evidence of CMV infection. Cells that are infected productively with CMV are enlarged, have type A Cowdry intranuclear inclusions, and occasionally have perinuclear inclusions. The appearance of these cells is characteristic and has been likened to owl's eyes. Immunohistochemical staining can be used to augment the detection of these typical cells. The presence of these cells correlates with the presence of active CMV disease and may be useful clinically.

SEROLOGY

Standard serologic techniques also can be applied to the diagnosis of CMV infections. CMV IgG antibody can be determined in serum by several different methods, including complement fixation, hemagglutination inhibition, indirect fluorescent antibody assay, anticomplement immunofluorescence assay, enzyme-linked immunosorbent assay (ELISA), and latex agglutination and neutralization tests. ELISA and the indirect fluorescent antibody assay are used most commonly in clinical virology laboratories. The presence of CMV IgG antibody in a single serum specimen implies that the patient at some time has been infected

with CMV. On the other hand, a negative IgG antibody determination is good evidence against current or past CMV infection because CMV antibody usually is present at the time of infection and persists for life. Severely immunocompromised patients, especially bone marrow transplant recipients, however, can lose their ability to make IgG antibody and become CMV seronegative, even though they are infected actively with CMV. This occurrence has a poor prognosis and usually is a terminal event. Primary infection with CMV is documented best by clear seroconversion from negative to positive CMV IgG antibody. A fourfold rise in CMV IgG antibody titer is not diagnostic of a primary infection because reactivation infection also can cause titers to fluctuate. In addition, the height of the titer or ELISA index in a single serum specimen is not diagnostic.

CMV IgM antibody can be determined in serum by radioimmunoassay, indirect fluorescent antibody assay, or ELISA. Both the indirect fluorescent antibody assay and ELISA are used commonly in clinical laboratories, although some indirect fluorescent antibody assays have a considerable false-positive rate. Accurate interpretation of CMV IgM antibody results requires knowledge of the methods used and careful consideration of the clinical context to exclude diseases that produce cross-reacting antibody or polyclonal responses. Test methods also should remove rheumatoid factor from the test serum, a common cause of false-positive IgM reactions. If the test is performed properly, the presence of CMV IgM antibody implies a current or recent primary CMV infection. In healthy adults, CMV IgM antibody usually persists for 6 weeks and may be present for as long as 3 to 12 months, rarely even longer, after the primary infection.[92] Western blot assays using viral structural proteins separated from purified viral particles or from recombinant viral proteins appear to be sensitive and specific for detection of CMV IgM. Currently, these tests are available only in research or reference laboratories.[197] The CMV IgG avidity index, another research test, also may be used to time a suspected primary infection with CMV.[196,270] A low avidity index (30%) suggests a recent primary infection, usually within 3 months, whereas a high avidity index (above 60%) suggests a past or recurrent CMV infection.[141] In immunocompromised adults experiencing clinically significant reactivation infection with CMV, CMV IgM antibody may be detected for prolonged periods.[227] The sensitivity and specificity of CMV IgM antibody determination in diagnosis of acquired, primary CMV infection or clinically significant reactivation infection in infants and children have not been studied systematically, although clinicians frequently use this test for this purpose.

LABORATORY DIAGNOSIS OF SPECIFIC CLINICAL SYNDROMES

Congenital Infection

Viral culture is the diagnostic test of choice in considering congenital infection with CMV.[88] The diagnosis is established by isolation of the virus from urine or saliva in the infant's first 1 to 2 weeks of life. Urine cultures obtained after 3 weeks of life must be interpreted cautiously because perinatally acquired and transfusion-acquired infections with CMV may be manifested as early as 3 weeks of age.[288] Detection of nuclear inclusion-bearing renal epithelial cells in urinary sediment collected in the first 2 weeks of life also implies the presence of congenital infection. This technique is insensitive, however, compared with tissue culture and is important only historically. Detection of CMV DNA in the urine, saliva, serum, or CSF of newborns by DNA hybridization or DNA PCR amplification techniques also correlates with congenital infection and disease.[20,52,90,225] Detection of CMV DNA in dried blood spots or Guthrie cards collected for newborn metabolic screens also has been used for the retrospective diagnosis of congenital CMV infection and disease. However, this method does not detect all congenitally infected newborns, especially those newborns with silent or asymptomatic infection, because the level of CMV in blood is lower in asymptomatic congenital CMV infection.[33-35,308,341,368,369] Newborns with high CMV DNA levels by quantitative PCR methods appear to be at highest risk for hearing loss and, therefore, are potential candidates for CMV antiviral therapy.[194,348]

The diagnosis of fetal intrauterine CMV disease in single, twin, and multiple births also may be established by isolating the virus from amniotic fluid or detection of CMV DNA by PCR amplification in amniotic fluid obtained at least 2 weeks after the suspected time of maternal primary infection.[196,373] Fetal condition also can be determined by serial fetal ultrasound examinations.[98,159,270] Fetal blood samples that show the presence of CMV IgM antibody, thrombocytopenia, or leukopenia or elevated liver function test results also provide supportive evidence of CMV-associated fetal disease and may predict severe disease. Prenatal screening of pregnant women and prenatal diagnosis of congenital CMV infection should be accompanied by prenatal counseling by personnel knowledgeable about all the possible outcomes that can occur and all the options that are available to the parents and physician.[110,270]

Standard serologic tests also can be applied to diagnose congenital infection with CMV, but this approach is cumbersome and retrospective. The absence of CMV IgG antibody in cord blood rules out congenital infection, whereas its presence may imply passive transfer from the mother or indicate a congenital infection. Serial serologic specimens also can be obtained when the infant is 1, 3, and 6 months of age. If CMV IgG antibody levels disappear during the infant's first months of life, congenital infection is ruled out. However, if CMV IgG antibody persists, the infant either was infected congenitally or acquired CMV infection perinatally or postnatally. The presence of CMV IgM antibody in cord or infant blood collected in the first 3 weeks of the infant's life suggests the diagnosis of congenital CMV infection.[146,320] However, CMV IgM antibody may be insensitive (22%) compared with urine CMV culture for the diagnosis of congenital infection, according to one study.[225]

Perinatal and Postnatal Infection

The diagnosis of perinatal infection with CMV is difficult to establish but is documented best by a negative CMV viral culture and CMV IgM antibody level at birth, a positive viral culture and CMV IgM antibody at 8 to 16 weeks of age, and persistence of CMV IgG antibody. Postnatal primary CMV infection is diagnosed by CMV IgG seroconversion, the presence of CMV IgM antibody, and viral shedding in saliva, urine, and other body fluids.

Cytomegalovirus Syndromes in Immunocompromised Hosts

In immunocompromised patients, determination of whether serologic or virologic evidence of active CMV infection correlates with disease is difficult because CMV commonly is shed from saliva, urine, and respiratory tract secretions in these patients without clear evidence of a disease process. Therefore, detection of a productive virus infection in the organ system suspected to be involved usually is necessary to establish the diagnosis of CMV disease in an immunocompromised patient. For example, interstitial pneumonitis caused by CMV is documented best by an open lung biopsy specimen that shows characteristic CMV histopathologic features and positive viral culture. Detection of CMV in bronchoalveolar lavage specimens also correlates with lung biopsy results.[134] Similarly, the diagnosis of CMV hepatitis or colitis is documented best by the presence of cytomegalic inclusion cells, isolation of CMV, or detection of CMV by DNA

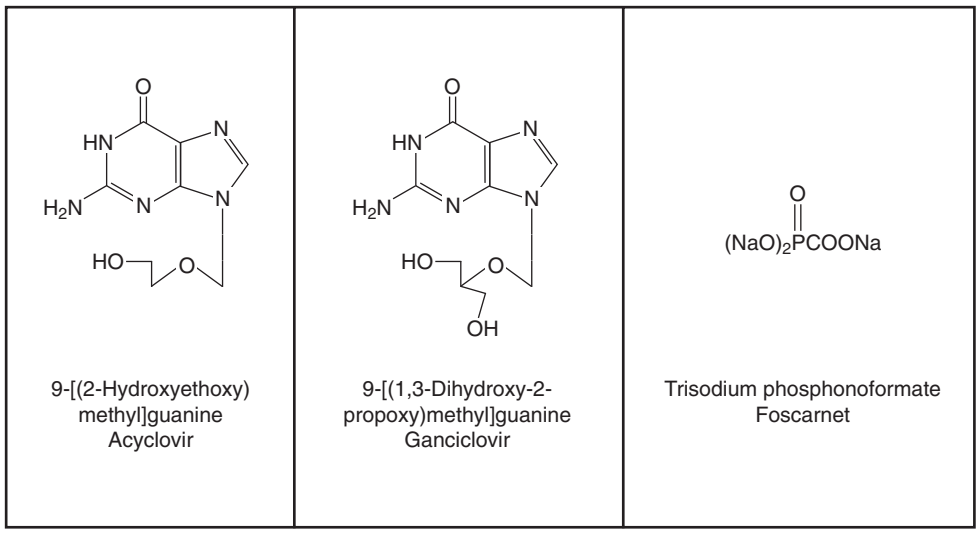

Figure 171–4 Structures of acyclovir, ganciclovir, and foscarnet.

PCR or immunohistochemical stains from biopsy specimens. In addition, viremia often precedes or accompanies serious disease, such as CMV pneumonitis in bone marrow transplant recipients or CMV retinitis or colitis in patients with AIDS.[315,343]

Prospective monitoring of high-risk adult patients by serial CMV cultures of urine or blood or bronchial lavage samples, serial blood samples to test for evidence of CMV antigenemia or DNAemia, and collection of serum to detect seroconversion by serologic testing usually are indicated because they may allow early administration of preemptive therapy with a CMV-specific antiviral agent, such as ganciclovir, when CMV infection is active but before overt disease develops.[292,334]

TREATMENT

Antiviral agents with activity against CMV include derivatives of acyclovir, such as ganciclovir and valganciclovir, as well as foscarnet and cidofovir (Fig. 171–4).[10,56,58,175,199,276,327] Acyclovir and valacyclovir also may have limited activity against CMV under certain conditions. In addition, biologics such as immune globulin and CMV hyperimmune globulin may benefit selected patients, and novel approaches such as antisense and non-nucleosis inhibitors and cell-based adoptive immunotherapy also are being investigated.[15,39] Treatment is beneficial for adult and pediatric immunocompromised hosts as well as for newborns with serious CMV disease but currently is not indicated routinely for normal hosts with asymptomatic or mildly symptomatic CMV infection.[183-186,235] However, normal hosts with severe CMV disease may benefit from antiviral therapy.[260]

Treatment of CMV-associated disease, such as retinitis, pneumonitis, hepatitis, colitis, esophagitis, or encephalitis, in immunocompromised hosts usually involves a 2- to 3-week period of induction therapy with an intravenous antiviral medication, usually ganciclovir.* In special circumstances, such as suspected drug resistance, foscarnet or cidofovir may be indicated.[111,329] The induction period should be accompanied by clinical improvement and a virologic response. If the host is expected to remain severely immunocompromised after receiving successful induction therapy, maintenance therapy at a reduced dosage schedule, three to five times a week, administered intravenously or orally, is indicated through the expected period of immune suppression. In patients with AIDS, oral or intravenous maintenance therapy usually is continued indefinitely.[102] In adult patients with AIDS who have refractory CMV retinitis, treatment with a ganciclovir implant may augment systemic therapy, but published experience evaluating this approach in children is limited.[60,338] One study showed that valganciclovir, an orally bioavailable prodrug of ganciclovir, appears to be as effective as is intravenous ganciclovir for induction therapy for CMV retinitis.[207] If, despite administration of antiviral therapy, CMV disease persists or progresses or if a virologic response does not occur, drug resistance should be considered. Resistance to ganciclovir has been documented most commonly in patients with AIDS, but it also can occur in patients with malignant neoplasms and in transplant recipients.[73,164] Most ganciclovir-resistant CMV strains have a mutation in the UL97 phosphotransferase gene, but specific mutations in the UL54 DNA polymerase gene also may occur alone or in combination with UL97 mutations.[64] Most ganciclovir-resistant CMV strains are susceptible to foscarnet but may exhibit cross-resistance to cidofovir, and strains simultaneously resistant to ganciclovir, cidofovir, and foscarnet also rarely may occur.[74,329]

The administration of ganciclovir along with intravenous immune globulin or CMV hyperimmune globulin to bone marrow transplant recipients with CMV pneumonitis has been shown to increase survival over that of historical controls who were treated with a variety of other antiviral regimens, including ganciclovir alone, immune globulin alone, acyclovir, vidarabine, and interferon. However, differences in the method used to diagnose CMV pneumonitis, duration of illness, and treatment regimens between study subjects and historical controls obscure interpretation of these studies.[108,264] Nonetheless, CMV-associated interstitial pneumonitis in bone marrow transplant recipients may be an immunopathologic process, and combination therapy may prevent active virus replication (ganciclovir, foscarnet, cidofovir) while blunting the immune response to viral antigens already expressed on CMV-infected cells (immune globulin).[298] In contrast, a small pilot study showed that the addition of CMV hyperimmune globulin did not appear to enhance the efficacy of ganciclovir treatment in patients with AIDS-associated CMV retinitis.[166]

Standard immune globulin or CMV hyperimmune globulin should not be used alone for the treatment of established CMV infections in immunocompromised patients.[212] The combination of high-dose corticosteroids with ganciclovir does not appear to improve survival in bone marrow transplant recipients with biopsy-proven CMV pneumonitis.[265] On the other hand, a

*See references 55, 81, 94, 115, 123, 149, 165, 178, 202, 204, 209, 311, 352.

regimen combining ganciclovir with hematopoietic growth factor may allow ganciclovir to be continued in selected patients with mild marrow toxicity.

Treatment of infants with symptomatic congenital CMV infection was reported first in 1969 and 1971,[114] and more recent reports suggest that antiviral agents, such as ganciclovir, may benefit selected infants.[217,290] Anecdotal reports have shown clinical and virologic improvement in congenitally infected newborns treated with ganciclovir.[158,228,268,333,339] A multicenter phase I/II study of the pharmacokinetics, antiviral effects, and safety of intravenous ganciclovir was conducted in 47 newborns with symptomatic congenital CMV disease and neurologic involvement.[337,358,379] In this study, a 6-mg/kg dose of ganciclovir administered by a 1-hour infusion every 12 hours for a period of 6 weeks produced a significant reduction in the quantity of urinary virus excretion; however, viral shedding recurred when ganciclovir treatment was discontinued. Neutropenia, thrombocytopenia, and elevated liver enzymes also were observed. Limited clinical follow-up in 30 of the original 47 infants showed that hearing loss improved or stabilized in 16 percent of the treated infants. Subsequently, a phase III, multicenter, randomized clinical trial enrolled 100 infants and was completed in 1999.[183] In this study, ganciclovir treatment in the newborn period appeared to have an impact on hearing loss by slightly improving hearing, maintaining normal hearing, or preventing hearing deterioration, as measured when the infants reached 6 and 12 months of age. Because only 44 percent of the enrolled infants were assessed in follow-up, a long-term follow-up study of these infants is ongoing to determine whether the beneficial effect lasts through childhood and adolescence. In addition to an effect on hearing loss, treatment provided more rapid resolution of hepatitis, when present, and improved short-term growth in weight and head circumference and developmental milestones. Currently, the decision to administer antiviral therapy to a newborn with congenital CMV disease remains at the discretion of the clinician.[359] Short-term antiviral therapy is likely to benefit infants who have multisystem disease with viremia, pneumonia, severe or persistent thrombocytopenia or hepatitis, or active sight-threatening retinitis. In these clinical circumstances, a reduction in viral load should improve the infant's clinical condition and resolve end-organ disease. However, the sustained effect of short-term treatment on long-term sequelae, such as hearing loss and neurodevelopmental disabilities, is not clear at this time. A longer duration of therapy, perhaps with an oral antiviral agent such as valganciclovir, possibly will improve or prevent the development of long-term sequelae in these infants.[359] Anecdotal reports and phase I pharmacokinetic and pharmacodynamic studies have demonstrated that valganciclovir oral solution provides plasma concentrations and antiviral effects similar to those levels achievable with use of intravenously administered ganciclovir.[184,222] Clinical trials evaluating the long-term benefits of prolonged treatment with oral valganciclovir in congenitally infected infants are under way. In addition to specific antiviral therapy, management of congenitally infected infants includes supportive care and control of seizures. If thrombocytopenia is severe and persistent, platelet transfusions and immune globulin also may be of benefit. Long-term management includes careful attention to nutrition to ensure adequate growth, serial hearing tests to detect progressive or late-onset hearing loss, developmental assessments to evaluate for cognitive and motor disabilities, and ophthalmologic follow-up of abnormalities present in the newborn period.[359,361]

PREVENTION

Prevention of CMV disease is important because it causes significant morbidity and mortality in a variety of patients. In immunocompromised hosts, it also is associated with the development of other complications, such as opportunistic co-infections and graft rejection in transplant recipients, and it increases resource use in transplant programs. In premature newborns, potentially fatal viral sepsis syndromes can occur. Furthermore, congenital infection is a leading cause of deafness and developmental disabilities in children. Approaches to the prevention of CMV disease include the use of CMV-seronegative blood products, selection of CMV-seronegative donors for transplant recipients, passive immunoprophylaxis with immune globulin, prophylactic or preemptive use of antiviral agents, active immunization with a CMV vaccine, and behavioral strategies to reduce exposures to CMV-infected secretions.

BLOOD PRODUCT, HUMAN MILK, AND TRANSPLANT DONOR SELECTION

Transplant recipients who are CMV seronegative and receive solid organ or bone marrow transplants from CMV-seropositive donors are at significant risk for acquiring symptomatic primary CMV infection. Therefore, whenever possible, CMV-seronegative recipients should receive transplants from CMV-seronegative donors, and all blood product transfusions should be from CMV-seronegative donors.

Infection with CMV in seriously ill CMV-seronegative neonates can be prevented by use of blood products from CMV-seronegative donors or by use of frozen deglycerolized red blood cells.[48,372] Saline-washed red blood cells also reduce but do not always prevent the acquisition of CMV infection in neonates, even though as many as 90 percent of the leukocytes can be removed by this method.[89] An alternative method of preparing leukocyte-depleted blood, filtration through a cotton-wool filter, appears to prevent post-transfusion acquisition of CMV infection in neonates.[133] Many institutions now provide CMV-seronegative or leukocyte-depleted blood products routinely to all neonates or to all low-birth-weight neonates, regardless of the CMV serostatus of the mother. Ingestion of CMV-positive breast milk by premature infants has been associated with illness, and donor selection or pretreatment of human milk administered to extremely premature infants may reduce the risk of acquisition of CMV-associated disease in these infants, although clinical trials to prove this hypothesis have not been published yet.[169,208,237,346,347]

PASSIVE IMMUNOPROPHYLAXIS

Although immune globulin or CMV hyperimmune globulin should not be used alone for the treatment of established CMV disease in immunocompromised patients, these preparations may be used to prevent the acquisition of serious CMV disease in selected immunocompromised patients. Passive immunization remains controversial, however, partly because studies have used different dosages (100 to 200 mg/kg) administered at varying intervals (1 week before transplantation and every 1 to 3 weeks after transplantation) for varying times (60 to 120 days). CMV immune globulin has been shown to decrease the incidence of symptomatic CMV disease from 60 to 21 percent in CMV-seronegative renal transplant recipients who received a kidney from a CMV-seropositive donor.[307] CMV immunoprophylaxis also has decreased the incidence of CMV pneumonitis in CMV-seronegative bone marrow transplant recipients who did not receive granulocyte transfusions.[45,215,263,365] The use of CMV hyperimmune globulin in pregnant women with primary CMV infections to prevent or to ameliorate CMV infection in the fetus has been reported, but definitive recommendations await results of randomized clinical trials.[221,229]

PROPHYLAXIS AND EARLY PREEMPTIVE THERAPY WITH ANTIVIRAL AGENTS

The prophylactic use of antiviral agents in transplant recipients has been evaluated and in some studies appears to reduce the incidence of serious CMV disease. However, this approach remains controversial because no regimen has been shown to completely prevent the acquisition of CMV infection or disease.[249] For example, acyclovir is used by some clinicians as prophylaxis for CMV disease in organ transplant recipients, despite evidence that acyclovir is inactive against most strains of CMV and CMV disease occurs despite such prophylaxis.[303] In one study, intravenous administration of acyclovir, 500 mg/m^2 of body surface area per dose every 8 hours for 5 days before and 30 days after transplantation, to CMV-seropositive bone marrow transplant recipients appeared to reduce the incidence of CMV disease.[216] High-dose oral acyclovir administered 1 day before and for 12 weeks after transplantation also has been shown to reduce the incidence of CMV disease and infection in renal transplant recipients.[29]

The prophylactic administration of human leukocyte interferon-α has been shown to reduce the incidence of severe CMV disease in renal transplant recipients, but it has no apparent benefit in bone marrow transplant recipients, and it is not used routinely in any patient population.[69,155,266] Many clinicians currently favor the prophylactic use of ganciclovir in transplant recipients at high risk for acquiring serious CMV disease. Prophylaxis treatment regimens vary, however, and it is difficult to make recommendations about which regimen is best. Most solid organ transplant recipients appear to benefit from intravenous ganciclovir, 5 to 10 mg/kg/day administered once or twice daily for 2 to 6 weeks after transplantation, usually followed by continuing antiviral prophylaxis with a reduced dose of ganciclovir.[22,340] Prophylactic ganciclovir does not appear, however, to benefit adult lung transplant recipients in most published studies. Similarly, administration of a short 2-week course of ganciclovir near the time of transplantation, without continuing antiviral prophylaxis, does not appear to reduce significantly the incidence of CMV disease in most solid organ transplant recipients studied.[22,340] The impact of intravenous immune globulin or CMV hyperimmune globulin, when it is given with a prophylactic antiviral agent, on the incidence of CMV disease in solid organ recipients is unclear. However, many clinicians administer it concomitantly with an antiviral agent in attempting to prevent CMV disease in certain high-risk transplant recipients. In bone marrow transplant recipients, the administration of ganciclovir prophylaxis after marrow engraftment reduced CMV disease but did not seem to reduce the overall mortality rate significantly. In addition, ganciclovir-treated bone marrow transplant recipients experienced prolonged neutropenia.[139]

The strategy of preemptive antiviral therapy has certain advantages over strategies that either treat only patients with overt clinical disease or administer prophylactic antiviral agents to many patients at risk, only a few of whom appear to benefit.[132] In addition, preemptive or very early antiviral treatment strategies include viral surveillance of blood, urine, and respiratory samples. Viral surveillance of these samples can be by standard viral culture, viral antigen detection, viral nucleic acid detection by qualitative or quantitative PCR assay, or a combination of these tests. Detection of CMV viremia and culture-positive bronchoalveolar lavage samples have correlated with the development of serious CMV disease in transplant recipients.[53] At least two studies of bone marrow transplant recipients now have documented the efficacy of early intervention with intravenous ganciclovir therapy at the time that positive CMV surveillance cultures were obtained but before clinical disease developed.[139,292] This strategy also appears to be efficacious in solid organ transplant recipients.[303] Another approach to preemptive therapy that does not entail viral surveillance techniques is to administer an antiviral agent, such as ganciclovir, during times of rejection, when CMV reactivation is likely to occur. This approach has been modestly successful in high-risk renal transplant recipients.[154] Simultaneous virologic and immunologic follow-up is the best approach for monitoring CMV infection in transplant recipients. Lack of immune reconstitution or presence of graft rejection or graft-versus-host disease usually heralds repeated episodes of recurrent or persistent CMV infection or disease, requiring multiple or prolonged courses of antiviral therapy.[132] Antiviral resistance to single or multiple agents may occur in these high-risk patients and has been increasingly recognized as a significant problem.[64]

ACTIVE IMMUNIZATION

Prevention of acquisition of CMV disease through active immunization should be a priority for the 21st century.[289,325] Pregnant women and their fetuses as well as transplant recipients would benefit greatly if a safe, effective CMV vaccine became widely available.[18,289,291] The ideal CMV vaccine should be safe, effective, immunogenic, and cost-effective. It should prevent the development of primary CMV infection without causing chronic persistent infection. The vaccine also should not be capable of infecting the fetus, and it should not be oncogenic.

In 1975, Plotkin and associates[253] characterized and reported a candidate CMV vaccine strain, Towne 125, that was isolated originally from the urine of a congenitally infected infant named Towne. Since then, more than 500 subjects, including renal transplant recipients and healthy adult male and female volunteers, have received the investigational Towne 125 vaccine.[61,122,172,251-253,255,323] Studies of these subjects showed that Towne 125 is attenuated and relatively safe and that it induces humoral and cellular immunity in both healthy and immunosuppressed subjects.

The vaccine also appeared to be protective in a randomized, placebo-controlled study of 91 immunosuppressed renal transplant recipients.[252] In this study, 30 CMV-seronegative vaccine recipients received a kidney from a CMV-seropositive donor, and the incidence of severe CMV disease was significantly lower in the vaccine group than in the placebo group. However, the CMV infection rate did not differ significantly between the groups, and members of both groups experienced mild to moderate CMV disease. Subsequent studies have confirmed that CMV-seronegative renal transplant recipients who receive a live attenuated CMV vaccine are more resistant to serious CMV disease.[254] Whether a CMV vaccine given to CMV-seronegative women of child-bearing age before pregnancy will protect the fetus from intrauterine infection or disease is not known, but studies that may answer this question are in progress.

Another vaccine strategy investigated was the development of mutant hybrid strains of CMV that combine the safety of the Towne strain with Toledo, another CMV strain, to produce enhanced immunogenicity and possibly, however, also enhanced virulence.[8,250] Other investigators have evaluated subunit vaccines for CMV that contain purified glycoprotein B (gB) complexed with a powerful adjuvant.[50,113,242] Most recently, the cellular immune system has been targeted by "DNA vaccines" that encode gB and appear to induce protective cellular immunity against CMV. Finally, adoptive immunotherapy with sensitized T cells is available to patients enrolled in clinical trials.

BEHAVIORAL STRATEGIES TO PREVENT PRIMARY CYTOMEGALOVIRUS INFECTION

With the current complexities associated with the development of a CMV vaccine and the challenges of administering effective

antiviral therapy, an alternative practical option for prevention of CMV infection during pregnancy and other high-risk times of life is education to increase public awareness about CMV and its modes of transmission.[65,167a,271,280,374] Educational materials are available from clinicians and from the Web sites www.cdc.gov/cmv and www.bcm.edu/pedi/infect/cmv and provide factual information and guidelines for reducing the risk of CMV transmission to pregnant women, women of child-bearing age, and other susceptible individuals. Reliable, relatively inexpensive serologic evaluation is available, so all women contemplating pregnancy should know their CMV serologic status. In addition, because epidemiologic studies have shown a major source of CMV infection to be close contact with young children, women who are CMV seronegative should be aware that a high percentage of young children are infected actively with CMV and that while pregnant, they should exercise good hygienic practices when in close contact with young children, especially those who attend daycare centers or are known to have an active CMV infection.[59,243] In fact, some studies have shown that the incidence of child-to-parent transmission of CMV may be reduced by interventions that identify susceptible pregnant women and educate them about increasing protective behaviors, such as handwashing after diaper changes and wiping tears and nasopharyngeal mucus, and decreasing risky behavior for acquiring CMV, such as kissing on the mouth and sharing eating utensils.[7,9,120] In addition, CMV can be transmitted from husband to wife, and if the spouse or partner experiences a CMV mononucleosis syndrome, a CMV-seronegative woman may wish to consider avoiding pregnancy for an individualized period.[91]

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CHAPTER 172

Epstein-Barr Virus

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Epstein-Barr virus (EBV) is an extremely common herpesvirus that is the etiologic agent of classic infectious mononucleosis (IM). Evidence that the virus also plays a principal role in the development of certain lymphoproliferative diseases and epithelial malignancies continues to mount. Its ability to remain in a dormant (latent) state after infection allows reactivation and recurrence of disease, especially under immunosuppressive conditions. Unfortunately, antiviral agents have little effect on EBV-associated diseases, but progress is being made toward improved treatment for EBV-associated malignancies. Eventually, vaccination may provide protection for those persons at highest risk for development of severe disease manifestations.

This chapter reviews progress in understanding the immunobiology of EBV and its disease associations. Enhanced understanding of EBV is leading to newer diagnostic methods and improved management of EBV-associated diseases.

HISTORY

One can identify four distinct periods of history related to IM and its etiologic agent EBV[124,445]: (1) early descriptions of IM, (2) identification of the heterophile antibody test associated with IM, (3) discovery of EBV in Burkitt lymphoma (BL) cells, and (4) the association of acute EBV infection with heterophile-positive IM.

Credit for the original description of a disease consistent with what is now called *infectious mononucleosis* commonly is given to a German physician named Pfeiffer,[394] who, in 1889, described relatively mild illness characterized by fever and lymphadenopathy; later, his report was translated into English.[327] The illness came to be known as *Drüsenfieber*, or *glandular fever*. Although some cases of glandular fever were typical of what is now recognized as IM, certain characteristics of this illness, including its predominance in children and familial clustering, did not fit. In England, glandular fever and IM have become synonymous, yet many experts consider glandular fever to be a different illness.

In the early 1900s, numerous case descriptions of illnesses epidemiologically and clinically compatible with IM appeared.[55,183,233] However, because the illness now recognized as IM had not yet been described and these illnesses were suggestive of leukemia, most of them were reported erroneously as acute leukemia with spontaneous cure. Although the term *infectious mononucleosis* was used first in one of these case reports,[233] the illness was not distinguished from leukemia until Sprunt and Evans published their landmark paper in 1920.[466] These authors described a series of patients with clinical syndromes consistent with typical IM, including classic hematologic findings. A subsequent report by Downey and McKinlay provided a more detailed description of the hematologic manifestations.[107]

Although most cases of IM could be identified by hematologic and clinical findings, not until a specific serologic test was identified in 1932 could classic IM be better defined. In the course of investigating heterophile antibodies in serum from patients with rheumatic fever and other diseases, Paul and Bunnell[386] noted high titers of heterophile antibodies in serum from a control patient. Further investigation revealed that the control patient was a medical student with IM. Evaluation of sera from three other patients confirmed the presence of high titers of heterophile antibodies during acute IM.

In 1958, Dennis Burkitt, an English surgeon, described 38 cases of "round-cell sarcoma" developing in children and adolescents living in one region of Uganda, Africa.[53] Eventually, the tumors were determined to represent lymphomas, not sarcomas. Although a new mosquito-borne infectious disease was suspected as the culprit initially, Epstein and colleagues[115] eventually were able to cultivate and evaluate tumor cells by electron microscopy; they noted particles with ultrastructural characteristics similar to those of herpes simplex virus (HSV), which had been described a few years earlier. Further work established this virus as the fourth human herpesvirus (after herpes simplex 1, herpes simplex 2, and cytomegalovirus [CMV]).

The association between this new herpesvirus and IM was noted serendipitously in 1968 when an IM-like illness developed in a technician working in the laboratory of the Henles.[197] Stored, pre-illness serum from this technician contained neither heterophile nor EBV antibodies, but both heterophile and EBV antibodies appeared 6 days after the onset of symptoms. In addition, virus was cultivated from the technician's peripheral blood cells. Subsequent testing of serial specimens from other patients with

IM also demonstrated seroconversion to EBV in all cases. This work, as well as studies from Yale University,[123,365] among others, firmly established EBV as the cause of IM. Investigators subsequently showed that EBV has the capacity to transform ("immortalize") human B-lymphocytes in vitro, which allows the derivation of EBV-transformed B-lymphoblastoid cell lines capable of being propagated indefinitely in tissue culture.[199,397]

VIROLOGY

EBV is in the family *Herpesviridae*, subfamily *Gammaherpesvirinae*, genus *Lymphocryptovirus*.[96] Its closest relative, human herpesvirus 8 (also known as *Kaposi sarcoma associated herpesvirus*) is also a gammaherpesvirus. Although its taxonomic name *(human herpesvirus type 4)* is used by most bibliographic search services, most scientists and physicians use its historic name.

STRUCTURE AND GENOME

The structure of the EBV is typical for a member of the herpesvirus family: It displays an inner core of DNA surrounded by a nucleocapsid, tegument, and an envelope.[116] Within the inner core, which measures approximately 45 nm, exist approximately 184 kilobase pairs (kbp) of double-stranded DNA coding for nearly 100 proteins. The surrounding nucleocapsid displays icosahedral symmetry (162 capsomeres) and measures approximately 100 to 110 nm in diameter. Outside the capsid lies the tegument, which consists of loosely packed, amorphous proteinaceous material, and the tegument in turn is surrounded by the viral envelope containing glycoprotein spikes. The complete virion measures approximately 150 to 220 nm.

The entire EBV genome has been sequenced.[28] The structure of EBV is characteristic of all the herpesviruses, with short and long sections of "unique" sequences (U_s and U_L, respectively) separated by a major internal repeat region (IR_1) generally consisting of 6 to 12 direct repeats of a 3 kbp sequence. The virus also has numerous other regions of repeated nucleotide sequences, including direct repeat of a 0.5 kbp sequence at its termini.

MOLECULAR BIOLOGY

Much of the early information regarding the molecular biology of EBV comes from laboratory strains derived from BL tumor cell lines (e.g., HR-1)[208] and marmoset lymphoid cell lines transformed by viral strains infecting human beings (e.g., B95-8).[346]

REPLICATION

EBV infection of cells is initiated by attachment of EBV glycoprotein gp350 to a cell-surface receptor (CR2, CD21) found primarily on B-lymphocytes and nasopharyngeal epithelial cells[459,543] and less commonly on other cells.[254,336,538] This receptor is the same as the surface moiety that functions as the receptor for the third component of serum complement (the C3d receptor).[133] Penetration of the cell requires a complex consisting of viral glycoproteins gH, gL, and gp42 (for B-cell infection), whereas only gH and gL are necessary for infection of epithelial cells[515]; gp42 binds with human leukocyte antigen (HLA) class protein found in B cells but not present in epithelial cells. After entering the B lymphocyte, the virus is transported through the cytoplasm and is stripped of its envelope in endocytic vesicles.[362] Subsequently, the viral genome circularizes and is maintained in the cell nucleus as a multicopy plasmid (or episome).[318] The initial and predominantly replicative (lytic) EBV infection probably occurs in epithelial cells of the oropharynx and adjacent structures,[457,532] with virus then transmitted to circulating B cells passing through these lymphoepithelial tissues. Although lytic genes may be found in EBV-associated tumors (e.g., post-transplant lymphoproliferative disease [PTLD]),[349] the overall proportion of replicating cells is far surpassed by latently infected B cells. In contrast, after infection of epithelial cells occurs, active replication occurs and leads to lysis and death of the cell. Alternating replication of viral glycoproteins in B cells and epithelial cells appears to modify the viral tropism, allowing virus from replicating B cells to infect epithelial cells more efficiently, and vice versa.[45] Recent data also suggest that monocytes may play an important role as a reservoir for EBV and could provide an important link for the transfer of virus from B cells to the oral epithelium.[504] Proteins synthesized during viral replication are important for viral gene regulation, replication of the genome, production of mature particles, and modulation of the immune system.

LATENCY

Latently infected B cells are the primary reservoir of EBV in the body. In a typical healthy EBV-seropositive adult, from 1 to 50 per million B cells are infected with EBV, and this condition remains relatively constant over many years.[25] Although more than 80 gene products may be expressed during active viral replication, only a limited number are expressed during viral latency. This is one method by which the virus limits cytotoxic T-cell recognition of EBV-infected cells. The EBV gene products that may be expressed during latency include six nuclear proteins (EBV nuclear antigens [EBNAs] 1, 2, 3A, 3B, 3C, and a leader protein [EBNA-LP]); three membrane proteins (latent membrane proteins [LMPs] 1, 2A, and 2B); two small, nonpolyadenylated (noncoding) RNA molecules (EBV-encoded RNAs [EBERs] 1 and 2); and the Bam H1A rightward transcripts (BARTs).

Based on in vitro genetic and somatic cell hybrid experiments, three different patterns of latent gene expression can exist in EBV-infected cells from patients with EBV-associated disease.[282,417,483,496,539,545] Type I latency, exemplified by cells derived from BL, exhibits a limited repertoire of genes consisting of EBNA-1, EBERs, and BARTs. Type II latency typically is observed in cells derived from Hodgkin disease (HD) and nasopharyngeal carcinoma (NPC). Besides EBNA-1, EBERs, and BARTs, these cells also express latent membrane proteins (LMP-1, LMP-2A, LMP-2B). Lymphoblastoid cell lines, as well as tissues from patients with IM, PTLD, and acquired immunodeficiency syndrome (AIDS)-associated lymphomas, display the broadest pattern of latent gene expression (Type III), wherein all genes associated with latency are expressed.[282,441,494,542] A "Type 0" latency also may exist within circulating EBV-infected memory B cells from healthy persons, wherein only EBERs and BARTs are expressed.[417] Although these latency expression pattern categories may be useful for histopathologic characterization, it is important to note that significant heterogeneity among histopathologically similar tumors can occur.

A brief description of latency-associated EBV genes follows:

EBNA. EBNA-1 is required for replication and maintenance of EBV episomal DNA in latently infected cells.[282] It also interacts with various viral promoters in regulating transcription of the EBNAs (including itself), as well as LMP-1. As mentioned previously, amino acid repeat structures in the EBNA-1 protein protect it from proteosomal degradation, thereby stabilizing protein levels and possibly preventing presentation by MHC Class I molecules.[307] A role for EBNA-1 in B-cell transformation and oncogenesis remains somewhat debatable.[483] EBNA-2 clearly is a viral oncogene that is essential for cellular transformation. It

transcriptionally activates several viral genes (e.g., LMP-1, -2A) and cellular genes (e.g., CD21 and CD23) via its interaction with the DNA-binding protein RBP-Jκ (Jκ recombination-binding protein).[195] All three EBNA-3 proteins (A, B, and C) are transcriptional regulators of key cellular and viral promoters, but only 3A and 3C have proven to be indispensable for in vitro B-cell transformation.[501] Like EBNA-2, the primary regulatory functions of EBNA-3 proteins occur via interactions with RBPJκ.[421] Moreover, EBNA-3C is able to overcome the arrest of the retinoblastoma (Rb) gene cell cycle and increases expression of LMP-1.[316] The exact mechanistic contribution of EBNA-LP (also called EBNA-5) to B-cell immortalization is not well understood. Although EBNA-LP is not required for transformation of B cells, the absence of EBNA-LP drastically reduces the efficiency of transformation.[331] EBNA-LP possibly contributes to immortalization by enhancing EBNA-2-mediated transcriptional activation of the LMP-1 gene.[391] Also, previous studies have demonstrated that EBNA-LP cooperates with EBNA-2 in driving cells into the G1 cell cycle phase by binding and inactivating the tumor suppressors Rb and p53.[485]

Latent Membrane Proteins. Like EBNA-2, LMP-1 (a viral oncogene) is essential for EBV transformation of B cells and appears to be the main transforming protein of EBV. LMP-1 displays pleiotropic effects when expressed in EBV-infected cells. Most prominently, LMP-1 mimics the CD40 B-cell receptor, which plays a key role in the activation and differentiation of B cells.[271] The protein also induces expression of anti-apoptosis genes (e.g., BCL-2 and A20), as well as activation and adhesion of B cells.[545] LMP-2 consists of two transmembrane proteins: LMP-2A contains two intracytoplasmic domains (at the amino and carboxy termini) and 12 integral membrane domains; LMP-2B differs by the lack of the amino terminal cytoplasmic domain. LMP-2 generally is recognized as a modifier of normal B-cell development, promoting maintenance of viral latency.[545] LMP-2A mimics the B-cell receptor and alters normal B-cell signal transduction by inducing a range of genes involved in cell cycle induction, inhibition of apoptosis, and suppression of cell-mediated immunity. Functions of LMP-2B are less well described, but a recent report indicates a key role in regulating LMP-2A activity.[432]

EBERs. These two small nonpolyadenylated (non-coding) RNAs are the most abundant viral transcripts found in latently infected EBV cells (approximately 10^7 copies per cell) and, indeed, serve as important diagnostic tools through the use of in situ hybridization (see "Diagnosis—EBV Nucleic Acid" later). Recent studies indicate that EBERs may play important roles in viral persistence and oncogenesis for some tumors. For example, EBERs bind to double-stranded, RNA-activated protein kinase (PKR), which serves to inhibit interferon-inducible apoptosis.[360] Moreover, EBERs play a key role in the maintenance of malignant phenotypes of BL cells.[487]

BARTs. These consist of a family of differentially spliced RNAs, first identified in NPC and later found in other EBV-associated malignancies.[460] Although noted abundantly in all forms of latency, protein products of their open reading frames have not been clearly identified.

TRANSFORMATION

In vitro, resting human B cells infected with EBV develop into lymphoblastoid cell lines. These cells are described as immortalized or transformed because instead of following one of two normal pathways (differentiation or apoptosis [programmed cell death]), they proliferate continuously. Current evidence suggests that EBV proteins coded by six latency-associated genes (described previously) are important for this cell transformation process: EBNA-2 and LMP-1 are absolutely essential; EBNA-1, EBNA 3A, EBNA-3C, and EBNA-LP also play critical roles.[417] The remaining latency-associated viral gene products, although not essential for transformation, are involved in the initiation and maintenance of cell transformation.

EBV generally transforms relatively mature B lymphocytes, secreting a complete immunoglobulin product (heavy chain plus light chain).[49,370] EBV also is capable of infecting and transforming B cells in earlier stages of development (e.g., pre-B cells [producing only μ chains] and lymphoid precursors lacking immunoglobulin gene rearrangement)[143,144,261]; mature plasma cells cannot be infected by EBV. Tumor cells and cell lines from BLs are B cells that generally express small amounts of immunoglobulin, which may be detected as surface immunoglobulin molecules, cytoplasmic/secreted molecules, or both. The major heavy-chain isotype expressed by Burkitt tumors appears to be μ chain (IgM), although other isotypes can be observed.[370] Similarly, the EBV-infected cells observed in the blood of persons with mononucleosis and the tumor cells found in immunodeficient hosts with EBV-associated lymphoproliferative diseases and lymphoma are B lymphocytes; these EBV-infected cell populations may include cells producing any of the major classes of human immunoglobulin (IgG, IgA, or IgM).[49,370,423,424] EBV-related malignant lymphoproliferations in immunosuppressed patients may be polyclonal, oligoclonal, or monoclonal; the lymphoproliferations probably are polyclonal at first but eventually progress to monoclonality.[100] BLs, on the other hand, always appear to be monoclonal at initial evaluation.[34,370]

EBV GENOTYPES

Two genotypes of EBV (EBV-1 and EBV-2) were identified in the 1980s on the basis of marked genomic differences in EBNA LP, 2, 3A, 3B, and 3C genes.[433,439] Using this methodology, seroepidemiologic and virologic studies suggested geographic differences in the prevalence of these two genotypes, with both types found widely in central Africa and elsewhere, but type 1 predominating in Western countries. Most EBV-infected persons appear to harbor one genotype, yet immunocompromised persons may be co-infected with both type 1 and type 2 genotypes. Recently, more detailed genetic analyses have been conducted, revealing more subtle differences in the genome and the presence of multiple EBV "strains" in healthy patients.[456,499,514] Multiple strains probably are acquired at the time that the initial EBV infection occurs,[499] yet it is possible that superinfections with other EBV strains occur following primary infection with one strain. No substantial evidence supports the view that any one genotype (or strain) is responsible for specific lymphoproliferative diseases.[404]

IMMUNOPATHOGENESIS

INFECTIOUS MONONUCLEOSIS

EBV elicits a wide range of immunologic responses in humans, partly because of its propensity to infect B lymphocytes. In a normal host, the immunologic effects probably are responsible for most EBV-associated disease manifestations. Both cellular and humoral immunity develop in response to EBV infection. Detection of humoral antibodies directed against viral capsid and nuclear proteins is important for establishing the diagnosis of acute infection. However, the responsibility for effective control of EBV infection lies primarily with the cellular immune elements. Two to 7 weeks after exposure, up to 20 percent of circulating B lymphocytes become infected during primary EBV infection, although 1 percent is typical.[422] A brisk cellular immune

response ensues. In the acute stage, proliferating EBV-infected B cells are controlled principally by natural killer (NK) T cells, helper ($CD4^+$) T cells, and cytotoxic-suppressor ($CD8^+$) T cells.[416] In some rare EBV syndromes, these T cells can themselves become infected and directly cause disease.[242,274] In adults the proliferation of $CD8^+$ T cells causes a temporary inversion of the normal $CD4^+/CD8^+$ T-cell ratio; however, $CD8^+$ T-lymphocyte expansion is less marked in children.[518] After this T-cell response, the number of EBV-infected B cells falls dramatically during the next 4 to 6 weeks to concentrations of approximately 1 per million circulating B cells.[425] During convalescence, HLA-restricted cytotoxic T lymphocytes keep EBV in check, primarily by targeting the latently expressed EBNA-3 protein.[489]

During the acute stages of IM, despite a brisk immune system response to EBV, widespread and extensive impairment in general cell-mediated and humoral immunity also exists. For example, low or absent delayed-type hypersensitivity reactions develop in response to tuberculin and other antigens.[180,330] In addition, T cells from patients with IM display unusually weak proliferative responses when exposed to recall antigens (e.g., *Candida albicans*, tetanus toxoid).[350,493] Although EBV-specific cytotoxic and suppressor T cells appear during acute infection, the T-cell response is primarily nonspecific and HLA unrestricted.[275,276,383,412] Furthermore, a polyclonal humoral response occurs during acute infection with EBV.[431] Each B cell committed to one isotype continues to produce that isotype after transformation of EBV, thereby leading to secretion of all classes of immunoglobulins, though primarily IgM.[482,533]

Once primary infection occurs, EBV, like other herpesviruses, is able to persist in a latent state in a human host throughout that person's lifetime. This ability indicates that EBV exerts some influence on the immune response to prevent its complete eradication. With its large genome, this process of immune system evasion is carried out primarily through the coding of functional homologues of many cellular factors that are involved in cell cycle regulation, signal transduction, and inhibition of programmed cell death (apoptosis). For example, the EBNA-1 protein contains a spacer region consisting of multiple Gly-Ala repeats that inhibit protein processing and MHC class I recognition of cells expressing EBNA-1.[308] Another viral protein (BARF1) blocks the receptor for colony-stimulating factor type 1, which normally stimulates secretion of antiviral factors such as IFN-α through interaction with monocytes.[79] In addition, one crucial EBV protein, LMP-1, up-regulates certain anti-apoptosis factors, allowing for prolonged survival of cells.[544]

EPSTEIN-BARR VIRUS–ASSOCIATED TUMORS

In normal hosts, cellular immune responses are adequate for control and sequestration of EBV-infected cells. However, cellular immune deficiency may allow uncontrolled proliferation of EBV-infected B cells to occur during primary or reactivated (recrudescent) EBV infection. Such an excessive EBV-associated production of B cells may be histologically pleomorphic (such as B cell lymphoproliferative diseases) or relatively uniform (monomorphic, such as B cell lymphomas). In organ transplant recipients, no consistent chromosomal translocations are associated with the lymphomas. Many of these lesions regress once immunosuppressive therapy initiated after transplantation is withdrawn.[542] In rare, severe cases, B cells harboring EBV DNA and expressing EBNA may become disseminated throughout the body as plasmacytoid cells visible on peripheral blood smears and as B-lymphoid cells potentially invading all organs of the body. Life-threatening EBV infections also may be correlated with an overly strong virus-induced T-cell proliferation that might cause autoaggressive activity, producing hypogammaglobulinemia or other major organ dysfunctions.[33]

The pathogenesis of EBV-associated malignant disorders in some settings may be multifactorial and involve a mixture of virologic, genetic, and environmental factors. NPC, which occurs predominantly in a specific geographic locale, has been linked etiologically with EBV.[201,209] The identification of epithelial cells as potential targets for EBV infection, thereby allowing a lytic (productive) infection, has strengthened this relationship.[457] Further studies have revealed that NPC is a monoclonal proliferation that develops subsequent to EBV infection, with EBV gene products required for tumor growth.[384] However, NPC also clearly has a genetic association, inasmuch as strong linkage to a specific HLA type exists.[310] Furthermore, early EBV gene expression can affect profoundly the growth of infected cells, with full malignant potential reached when mutations occur on chromosomes 3 or 9 (presumably affecting tumor suppressor genes).[223,224] These mutations possibly result from adverse environmental conditions (e.g., exposure to chemical carcinogens such as the volatile nitrosamines and polycyclic hydrocarbons in salted fish).[247]

The EBV genome and expression of EBNA-1 are present in more than 95 percent of BL cells derived from African patients with BL (endemic BL), with the genome existing as circular episomes. In contrast, only 15 to 30 percent of cases of nonendemic (or "sporadic") BL occurring in Europe and the United States contain EBV DNA.[227,553] On the other hand, EBV is present in more than 95 percent of primary lymphomas of the central nervous system (CNS) in patients with AIDS.[324] BL cells, but not the lymphoid cells found in IM, exhibit characteristic chromosomal alterations associated with enhanced malignant potential. Approximately 90 percent of BL tumors exhibit a reciprocal translocation involving the long arms of chromosomes 8 and 14, t(8;14); most of the remainder have t(8;2) and t(8;22).[278,434] These translocations place the c-*myc* oncogene under the control of an immunoglobulin promoter,[93,492] which results in transcriptional deregulation and overexpression of the c-*myc* oncogene.[88,297] Malaria is well known to cause chronic B cell stimulation, providing enhanced opportunities for c-*myc* translocations within germinal centers of lymphatic tissue. Healthy children living in areas with holoendemic malaria may have high viral loads of EBV in peripheral blood, with acute malaria further augmenting the viral load[541] and anti-malaria treatment subsequently reducing the EBV load in the peripheral blood.[106] Therefore, the evolution of endemic BL appears to be a multistep process involving EBV infection, malaria-induced B cell proliferation, c-*myc* gene rearrangement and activation, and probably other elements.[97,98,277,369,451]

PTLD may develop in organ and bone marrow transplant recipients during the intense immunosuppression that occurs after placement of the graft. PTLDs span a spectrum of lymphoproliferation from polyclonal disease to monoclonal, monomorphic disease that often is difficult to distinguish from typical malignant lymphomas developing in patients with intact immune systems. No proof exists that an orderly progression leading to malignant lymphoma occurs; however, one model for the pathogenesis of PTLD proposes that defective immune surveillance after transplantation allows polyclonal proliferation of EBV-infected cells displaying a type III latency pattern (similar to that observed in IM).[80] Subsequently, genetic mutations at various loci (e.g., c-*myc*, p53) may allow progression to full malignant lymphoma to occur.[99]

HISTOPATHOLOGY

INFECTIOUS MONONUCLEOSIS[90,162,500]

Because IM typically is benign and the diagnosis is based primarily on clinical and serologic findings, histopathologic examination

of tissues seldom is required. However, histopathologic information is available on some tissues (e.g., lymph nodes, tonsils, spleen) from unusual cases and from patients requiring surgery. For patients in whom lymphoproliferative disease and other serious manifestations of EBV infection develop, a larger amount of information is available from a broader range of tissues.

Lymph nodes are enlarged diffusely during acute IM. Histologically, active lymphoid follicles are noted, with lymphoid proliferation extending to the sinuses, blood vessels, trabecula, and capsule. The capsule remains intact despite substantial hyperplasia. The lymphoid response consists primarily of T and B immunoblasts, typically with a pleomorphic pattern and frequent mitoses indicative of rapid cell turnover. Other cells identifiable in lymph node tissues include a substantial number of small lymphocytes, large atypical lymphocytes, plasma cells, histiocytes, and eosinophils. Micronecrosis may be present, although it is less extensive than is that observed with herpes lymphadenitis. Tonsillar tissue also contains an active lymphoproliferative response, but with more prominent follicles and extensive necrosis.

The spleen is enlarged two to three times its normal weight during acute IM, primarily as a result of hyperplasia of the red pulp. Like lymph nodes, the cellularity is principally from immunoblasts with substantial pleomorphism. Hemorrhage, primarily subcapsular in location, is identified commonly. The liver typically shows minimal disease, with infiltration by lymphocytes and monocytes occurring principally in the portal areas and possibly minor degenerative changes taking place in hepatocytes. Bone marrow may appear relatively normal during IM, but some series have reported hypercellularity and small granulomata. From the relatively few cases of CNS disease associated with EBV infection, histopathology reveals lymphocytic infiltration of the meninges.[9] Less common findings include demyelination, degeneration, focal hemorrhage, congestion, and edema.

EPSTEIN-BARR VIRUS–ASSOCIATED MALIGNANCIES

The most common form of non-Hodgkin lymphoma (NHL) is BL, which consists of sheets of small noncleaved cells that are histologically uniform. Most arise from a single infected cell (monoclonality). The majority of CNS NHL tumors display large-cell morphology, with virtually all containing monoclonal EBV DNA. In PTLD, a wide range of histologic findings may be noted. At one extreme, one finds benign lymphoid hyperplasia with normal tissue architecture and a pleomorphic response arising from numerous infected cells (polyclonality). At the other end is observed frank malignant lymphoma.[86]

HD is somewhat unique among cancers, as less than 1 percent of cells within the tumor are actually neoplastic.[268] HD tissues are characterized by the presence of diffuse inflammatory cell infiltrates admixed with a low level of neoplastic cells: classic Reed-Sternberg cells and mononuclear Hodgkin cells (referred to collectively as *H-RS cells*). Reed-Sternberg cells are large (15-45 μm), multinucleated cells demonstrated to derive from germinal center–derived B cells.[282] These cells exhibit strongly stained nucleoli surrounded by a characteristic clear area resembling a halo. The World Health Organization (WHO) classification system for HD recently has been revised,[238] and two main subgroups now are defined: nodular lymphocytic-predominant HD and classic HD. Within classic HD are four distinctive histologic categories, based on the predominant cell type and characteristics of the background cellularity: (1) lymphocyte-rich, (2) nodular sclerosis, (3) mixed cellularity, and (4) lymphocyte depleted. In Western countries, EBV can be identified in approximately 40 percent of HD tissue specimens; mixed cellularity and lymphocyte-depleted types are associated most strongly with EBV. Children most commonly develop the nodular sclerosing variety.

Of the three histopathologic categories of epithelial tumors in the nasopharynx, the most common form in children is the undifferentiated variety, which is associated most strongly with EBV.[24] Examination of these tumors reveals undifferentiated squamous cells, with substantial infiltration by lymphocytes. Malignant cells consistently contain multiple copies of monoclonal EBV DNA within episomes, and several EBV proteins are expressed.

OTHER EPSTEIN-BARR VIRUS–ASSOCIATED DISEASES

The tissues involved in hemophagocytic lymphohistiocytosis (HLH) typically include the bone marrow, spleen, liver, and lymph nodes, but the skin or brain may be affected as well.[134] In the bone marrow, hypocellularity often is noted. Activated macrophages (or "histiocytes") appearing "stuffed" are observed engulfing all bone marrow cellular elements or their precursors or fragments, including erythrocytes, leukocytes, and platelets.[134,420,529]

Tissues affected by oral hairy leukoplakia (predominantly in adult patients with AIDS)[114] reveal keratin or parakeratin projections, mild hyperparakeratosis and acanthosis, ballooning and hyperplasia of the prickle-cell layer, and only a sparse inflammatory cell infiltrate in the subepithelial connective tissue.[166] The principal histologic findings in pulmonary tissue from children infected with human immunodeficiency virus (HIV) and having lymphocytic interstitial pneumonitis are lymphoid nodules and diffuse infiltration of the alveolar septa and peribronchiolar regions by lymphocytic cells, including lymphocytes, plasma cells, plasmacytoid lymphocytes, and immunoblasts.[17] Some lymphoid nodules may be observed as well. The infiltrating lymphocytes consist of B cells and T cells.[256] Necrosis is not observed, and blood vessels are not affected.

EPIDEMIOLOGY

SEROPREVALENCE

Epidemiologic studies of EBV were stimulated by development of reagents for detection of specific anti-EBV antibodies in serum in the mid-1960s. Specifically, tests detecting antibodies to VCA (long-lasting, early in infection) and EA (short duration, early in infection) were used. Henle and Henle,[196] using an immunofluorescence method, first noted a high prevalence of EBV VCA antibodies (100%) in patients with BL, but they also discovered a relatively high prevalence (85%) in normal adults. Subsequent studies in American[152,198,399,473] and British[393] populations have established that 80 to 95 percent of adults have serologic evidence of past EBV infection, with most infections occurring during infancy and childhood.

The age at initial (primary) infection varies markedly in different cultural and socioeconomic settings, a fact that has great pertinence to manifestations of disease associated with primary infection. In developing countries, EBV infection generally occurs at an early age, with 80 to 100 percent of children becoming infected by the time they reach 3 to 6 years of age. In these settings, most children with primary EBV infection have clinically silent or mild disease.[38,135,476] In privileged communities and in industrialized countries, primary infection with EBV commonly occurs later in life. In these settings and for reasons that are unclear, primary infection in older age groups, for example, individuals between 10 and 30 years of age, is more likely to induce clinical symptoms, most often a mononucleosis syndrome. An IM case rate of 50 to 75 percent associated with primary EBV infection was documented in U.S. college students.[366] An unexpectedly high rate of IM also has been noted with eventual

development of primary EBV infection in siblings of a pediatric case of IM.[480]

INCIDENCE

The incidence of IM from population-based studies ranges between 50 and 100 cases per 100,000 population.[123,192,194] These studies indicate that the highest incidence rates for IM occur in the age group 15 to 19 years. Although two reports have described a modest seasonal change for IM,[173,192] most studies have indicated no seasonal predilection. IM consistently has developed at a higher rate in persons of white race than in other ethnic groups.[192] In one study, IM developed in males at a rate slightly higher than that in females.[194]

VIRAL SHEDDING

The initial indication that EBV was shed in oropharyngeal secretions came from Chang and Golden[64] in 1971 when they identified a "leukocyte transforming agent" in throat washings from four of eight adults with IM. Although EBV was suspected, specific laboratory tests could not confirm it. Other investigators using more specific identification methods clearly established EBV as the leukocyte transforming agent.[154,319,347] Subsequent studies in healthy populations have indicated the following:* (1) most children and adults with acute IM shed EBV in their oropharynx, (2) between 6 and 20 percent of the general population are shedding EBV in the oropharynx at any given point of time, (3) oropharyngeal shedding may be intermittent or continuous, and (4) high concentrations of EBV in oropharyngeal secretions are associated with high concentrations of EBV in B-lymphocytes in the peripheral blood but not with concentrations of EBV-specific serum antibodies.

In addition to the oropharynx, EBV DNA may be found in the urine of persons during and for several months after an episode of IM.[288] Shedding of EBV also has been reported in the uterine cervix,[458,495] the male reproductive tract,[235,495] and breast milk.[257]

TRANSMISSION

Common Modes of Transmission

Reports in the first half of the twentieth century, before widespread acceptance and use of the heterophile antibody test, suggested the occurrence of epidemics of IM.[182,371,498] However, researchers generally thought that the criteria used in most of those reports for establishing the diagnosis of IM were inadequate to convince one that the outbreaks were indeed associated with EBV.[212] Although some success was reported for transmission of human EBV infections to certain primate species,[250] attempts to experimentally infect humans with EBV collected from the oropharyngeal secretions, blood, urine, and feces of patients with IM generally failed.[120,121,364,463] These experiments, conducted prior to development of specific EBV antibody tests, presumably were unsuccessful because most experimental subjects were immune as a result of having had a previous EBV infection.

Intimate sharing of oral secretions is thought to play a major role in the transmission of EBV.[122,212] The fact that EBV infection is extremely common worldwide, especially in underdeveloped regions, suggests that the virus is spread relatively efficiently in the general population. Surprisingly, outbreaks of EBV infection are uncommon events. However, transmission of EBV, even among close contacts of a person with acute EBV infection, may occur slowly. Studies of families of index cases of IM have indicated that EBV is not transmitted efficiently.[66,138,194] Furthermore, Chang[67] reported a lack of EBV transmission from a man with IM to his EBV-seronegative wife, even after 16 months of follow-up. In addition, data from another family study indicated that after the index IM episode, EBV antibodies (seroconversion) developed in only 35 percent of the non-immune sibling contacts after an average observation period of 5.6 contact months.[480] Nonetheless, this same study also noted that the eventual seroconversion event in siblings was more likely to be associated with an IM episode than would be expected in the general population.[480] The complexity of EBV transmission and induction of IM is suggested further by the finding of multiple strains and variability of strains in distinct body compartments of individuals with primary EBV infections.[456]

Evidence has shown that EBV replicates in oropharyngeal epithelial cells,[457] with subsequent infection of B cells possibly occurring as a result of contact with these epithelial cells.[4] Further studies indicate that latent and productive EBV infection occurs in lymphoid cells on the surface of the tonsillar epithelium and within the crypt, but not in epithelial cells.[10] Thus, transmission of EBV from person to person may be due to transfer of virus from persistently infected EBV lymphoid cells; EBV-infected epithelial cells may not be required for this phenomenon.[368] The incubation period for typical EBV-associated IM is imprecise but is estimated at 30 to 50 days.[138,212]

Transmission via Blood Products or Transplanted Organs

Transmission of EBV via blood transfusion has been reported,[2,153,200,212,342,491] but it occurs much less commonly than does transmission of CMV. High levels of immunity, transfer of neutralizing antibodies during transfusion, and limited survival of B cells may account for the rarity of this occurrence.[203] In transplant recipients, EBV-positive donor organs may transmit EBV to the recipient.[62,189]

Intrauterine and Perinatal Transmission

Because most females reaching child-bearing age already have been infected with EBV, primary infections during pregnancy occur infrequently.[137,229,294] Therefore, the risk of fetal transmission is difficult to study. One prospective study of primary infections occurring during pregnancy (silent or symptomatic) indicated no adverse fetal outcomes and, when studied, no viral transmission to the fetus.[136] Moreover, Chang and colleagues[65,69] could find only one EBV-infected specimen among 2696 samples of cord blood lymphocytes. On the other hand, case reports suggest that rare instances of intrauterine transmission may occur.[50,159,253] The significance of one report noting EBV DNA in breast milk of approximately 50 percent of healthy women is unclear.[257]

Sexual Transmission

Detection of EBV in genital ulcers,[401] the uterine cervix,[458] and the male reproductive tract[235] raises the possibility of venereal transmission. Additional epidemiologic findings suggest that EBV transmission and onset of IM can be correlated with sexual intercourse or closely associated behaviors.[87]

*See references 65, 68, 102, 130, 160, 347, 367, 474, 477, 479, 480, 537.

NONMALIGNANT CLINICAL SYNDROMES ASSOCIATED WITH EBV INFECTION

SILENT, NONSPECIFIC INFECTIONS

Similar to other lymphotropic human herpesviruses, primary EBV infection in children typically is silent but may result in mild clinical symptoms. Nonspecific clinical symptoms may include prolonged low-grade fever with or without lymphadenopathy, cough, rhinorrhea, and pharyngitis.

INFECTIOUS MONONUCLEOSIS

The signs and symptoms of classic IM[70,110,132,213,478] develop in most adolescents and adults, as well as some young children, when they experience a primary (initial) EBV infection. Factors determining an asymptomatic versus symptomatic response to EBV infection are largely unknown. Adults developing IM may have an exaggerated, pathologic T-cell response to primary EBV infection. The degree of viremia does not appear to be related to symptomatology, with EBV viral loads in blood from symptomatic subjects being comparable to those undergoing silent EBV infection.[453]

Prodrome

In most persons with IM, a prodrome of 2 to 5 days develops during the end of the incubation period. Prodromal symptoms typically are mild and may consist of malaise, fatigue, and possibly fever. At this stage, differentiating IM from other viral infections is difficult.

Acute Phase

The prodrome is followed by the classic clinical features of IM (i.e., fever, sore throat, malaise, and fatigue), and physical examination reveals lymphadenopathy and tonsillopharyngitis (Table 172–1). The fever usually begins rather abruptly, ranges from 38° C to 40.5° C (usually <39° C), is more prominent in the afternoon and evening, and persists for 1 to 2 weeks. Occasionally, the fever may last 4 to 5 weeks. Lymphadenopathy occurs in more than 90 percent of children and adults and typically involves the anterior and posterior cervical lymph nodes (Fig. 172–1). Affected nodes are moderately enlarged in a symmetric manner. However, generalized lymphadenopathy also is characteristic and involves the occipital, supraclavicular, axillary, epitrochlear, and inguinal chains. Like other causes of viral lymphadenitis, the lymph nodes in patients with IM may be slightly tender, but they generally are nontender, nonerythematous, and discrete. Lymphadenopathy is most prominent during weeks 2 to 4 of the illness. Tonsillopharyngitis develops during the first week of illness and usually resolves rather abruptly the next week. Although mild symptoms occur in some patients, most have substantial symptomatology. Exudative pharyngitis, similar to that found with *Streptococcus pyogenes* pharyngitis, occurs in approximately half of patients (Fig. 172–2). *S. pyogenes* is present in the posterior pharyngeal region of 5 percent of patients with IM and probably represents carriage rather than simultaneous bacterial tonsillopharyngitis.

Splenomegaly occurs in approximately 50 percent of patients with IM, but more frequently in younger children. Usually only the tip of the spleen is palpable, with maximal size developing by the end of the second week of illness. The splenomegaly typically resolves by the third or fourth week. Hepatomegaly occurs in approximately 60 percent of young children with IM and less commonly in older age groups. However, silent inflammation of the liver is a common finding.[221] Cough and rhinitis are noted frequently in young children with IM, perhaps as a result of other concurrent viral infections. Rash (unassociated with antibiotic administration) develops in less than 15 percent of adults with IM but occurs more commonly in children and adolescents (18-34%).[478] The strong correlation in young adults between the administration of ampicillin and the subsequent development of a rash is not observed in children with IM, possibly overshadowed by the overall increased incidence of cutaneous manifestations in children with IM (see "Complications of Infectious Mononucleosis" for a detailed discussion of rashes associated with IM). Children are more likely than are adults to have abdominal pain, failure to thrive, otitis media, and recurrent tonsillopharyngitis preceding or following IM.[478] A palatal enanthem is present in approximately a third of adults, but it is a nonspecific finding. Eyelid edema also is described in up to a third of adults but occurs less commonly (15%) in children.[478]

TABLE 172–1 Clinical Manifestations of Infectious Mononucleosis in Children and Adults

	Frequency (%)		
Sign or Symptom	**Age <4 yr***	**Age 4-16 yr***	**Adults (Range)***
Lymphadenopathy	94	95	93-100
Fever	92	100	63-100
Sore throat or tonsillopharyngitis	67	75	70-91
Exudative tonsillopharyngitis	45	59	40-74
Splenomegaly	82	53	32-51
Hepatomegaly	63	30	6-24
Cough or rhinitis	51	15	5-31
Rash	34	17	0-15
Abdominal pain or discomfort	17	0	2-14
Eyelid edema	14	14	5-34

**From references 110, 122, 215, 334, 438.*

Figure 172–1 Anterior cervical lymphadenopathy in an 8-year-old child with infectious mononucleosis. *(Courtesy Dr. James Brien, Scott and White Hospital, Temple, Tex.)*

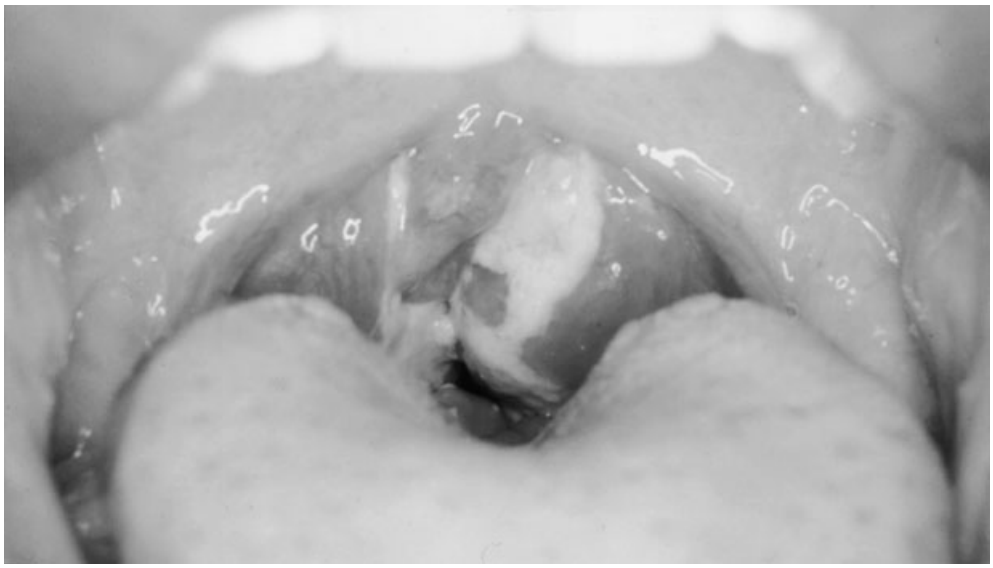

Figure 172–2 Exudative pharyngitis in an 8-year-old child with infectious mononucleosis. *(Courtesy Dr. James Brien, Scott and White Hospital, Temple, Tex.)*

Resolution Phase

The acute phase of IM lasts several days to 3 to 4 weeks, with gradual and uneventful resolution. Occasionally, biphasic illness is observed, with recrudescence of acute symptoms after significant improvement. Organomegaly may persist for 1 to 3 months. The severe fatigue usually resolves within 3 to 4 weeks, but several months may be required for persons with IM to fully resume their pre-illness activity levels. There is evidence, albeit minimal, suggesting that subsequent fatigue-like syndromes in patients with IM may be related to (or predicted by) the presence of preexisting lower physical fitness or prolonged bed rest during convalescence, whereas mood disorders are more related to premorbid psychiatric history.[524]

DISSEMINATED EBV INFECTION IN X-LINKED LYMPHOPROLIFERATIVE DISEASE

Overwhelming primary EBV infection, seen rarely in immunocompetent patients, occurs more commonly in children with certain primary immunodeficiency disorders, most notably, boys with x-linked lymphoproliferative disease (XLP).[406,472] These patients typically first present with features consistent with IM, but fulminant disease leading to death subsequently develops. Widespread, uncontrolled lymphoproliferation is found at autopsy, and causes of death include acute hemorrhage, meningoencephalitis, secondary bacterial infection, and liver failure.[355] Survivors usually have hypogammaglobulinemia, and B-cell lymphomas may develop later. XLP is associated with the deletion or mutation of a specific gene (SLAM-associated protein) located in the chromosomal Xq25 region, which is involved in key cellular signaling pathways for immune responses[78,363,443]; absence of this functional protein permits overwhelming and usually fatal infection from a primary EBV infection.

HEMOPHAGOCYTIC LYMPHOHISTIOCYTOSIS

Hemophagocytosis, first described in 1939, is a pathologic condition demonstrating engulfment of bone marrow cellular elements (erythrocytes, leukocytes, platelets, and their precursors) by activated macrophages (Fig. 172–3) Hemophagocytic lymphohistiocytosis (HLH, also called *hemophagocytic syndrome*) is characterized primarily by fever, pancytopenia, splenomegaly, and hemophagocytosis in lymphoreticular tissue (bone marrow, spleen, and lymph nodes). Since the initial description of its association with viral infections in 1979,[420] numerous viral infections, including EBV, have been linked with HLH.[379,413] Hereditary forms of HLH exist, most prominently those occurring in children with XLP (see previous section).[355] A disseminated form of EBV infection with features of HLH may develop in these patients; most other cases are sporadic. In contrast to IM, which involves infection of B cells, primary infection in patients with sporadic HLH results in an EBV-driven T-cell proliferation with proinflammatory cytokines playing a central pathogenetic role.[242] Untreated EBV-associated HLH carries a high mortality rate.

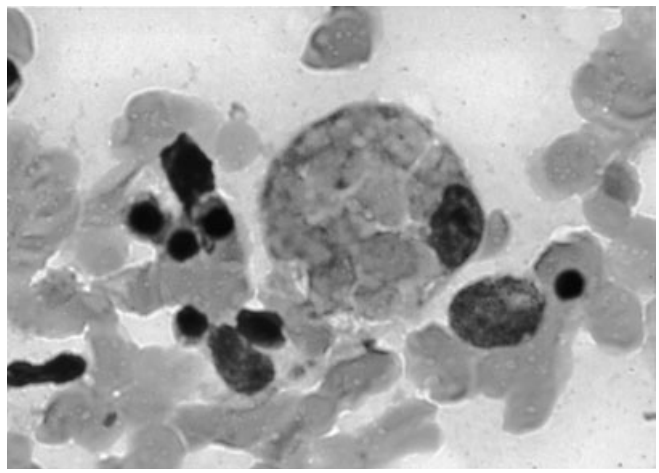

Figure 172–3 Bone marrow from an 18-year-old woman with Epstein-Barr virus–associated hemophagocytic lymphohistiocytosis revealing a macrophage (center) filled with phagocytosed red blood cells. *(From Blood 93:1991, 1999. Photo courtesy of Lindsey Badenm M.D., and Frank Evangelista, M.D., Beth Israel Deaconess Medical Center, Departments of Medicine and Pathology, 330 Brookline Ave., SL-435, Boston.)*

CHRONIC ACTIVE DISEASE

Although first described in 1978,[511] chronic active Epstein-Barr virus (CAEBV) infection is not as well-characterized as are other

EBV-associated diseases. It shares with HLH the central theme of infection and proliferation of non-B cells. CAEBV occurs more commonly in Japan,[273] with children affected more frequently than adults. Two forms of the disease have been described, one involving EBV-infected T cells and the other affecting NK cells.[274] Patients with the more severe form can be considered as having a chronic version of HLH, with similar symptoms and a uniformly poor prognosis. After having a typical IM-like illness, these patients develop persistent infection of their peripheral T cells ($CD4^+$, $CD8^+$, or both) and do not recover fully. Continuous or intermittent signs and symptoms, persisting for 3 months or longer, typically include fever, hepatosplenomegaly, lymphadenopathy, headache, malaise, and fatigue.[450,526,527] More severe cases display neurologic disease (e.g., encephalitis), malignancy (e.g., lymphoma or leukemia), hepatic failure, hematologic disease (e.g., hemophagocytosis), and disease localized to other organs (e.g., myocarditis, pneumonitis).[273] One distinctive characteristic of these patients is the extremely high antibody titers against EBV replicative antigens, including VCA and EA, and low or absent responses to EBNA. Poor prognostic factors include older age and high EBV viral loads.[273] Some cases may be related to mutations in the perforin gene.[262] Clonal expansion of EBV-infected T cells probably plays a central role in disease pathogenesis.[273] A second form of the disease, described principally in Japanese patients, is characterized by milder IM symptoms, less extreme EBV serologic abnormalities, granular lymphocytosis, and high IgE levels.[274] Interestingly, these patients also typically develop pronounced skin reactions in response to mosquito bites. Large expansions of EBV-infected, CD56-positive NK cells are observed in peripheral blood and in the skin reactions. CAEBV infection should not be confused with chronic fatigue disorder, which has no clear association with EBV.

OTHER DISEASES

EBV has been linked with various autoimmune disorders in children and adults, based primarily on seroepidemiologic information. These disorders include rheumatoid arthritis,[56] systemic lupus erythematosus,[240,340] and multiple sclerosis.[5] Further careful studies must be conducted in order to substantiate these early reports of an etiologic relationship with EBV.

COMPLICATIONS OF INFECTIOUS MONONUCLEOSIS

Acute EBV infection in a healthy child or adolescent, regardless of whether it is associated with typical IM, rarely is complicated by serious illness. The most frequent complications, exanthems and mild hepatitis, more appropriately are considered part of the normal spectrum of disease. More serious complications involving major organs have been reported regularly since IM first was described as a distinct clinical entity, but they remain rare (for review, see elsewhere).[6,245,392,478] Most complications, including the more severe ones, are transient.

In one prospective study,[478] significant complications involving mainly the pulmonary, neurologic, and hematologic systems were noted in approximately 20 percent of children with IM. Thrombocytopenia with hemorrhagic manifestations, severe airway obstruction, and neurologic complications developed in children with IM more frequently than in adults; jaundice occurred less frequently.

EXANTHEMS

Ampicillin Rash. Historically, patients with IM were treated frequently with antibiotics, primarily for suspected streptococcal pharyngitis or bacterial lymphadenitis. Soon after the release of ampicillin, reports linked the antibiotic with the occurrence of exanthems in patients with IM. Two large case series have indicated that administration of ampicillin (or amoxicillin) precipitates an exanthem in most (95-100%) adolescents and adults with EBV-associated IM[382,405]; it is less common in children with IM.[478] This phenomenon has become known as the "ampicillin rash" associated with IM. The rash usually develops 5 to 10 days after treatment is initiated and resolves within a few days of discontinuation. Characteristically, the cutaneous lesions are maculopapular and pruritic and involve the trunk, face, and extremities (Fig. 172–4).[382] A rash also may develop in patients with IM

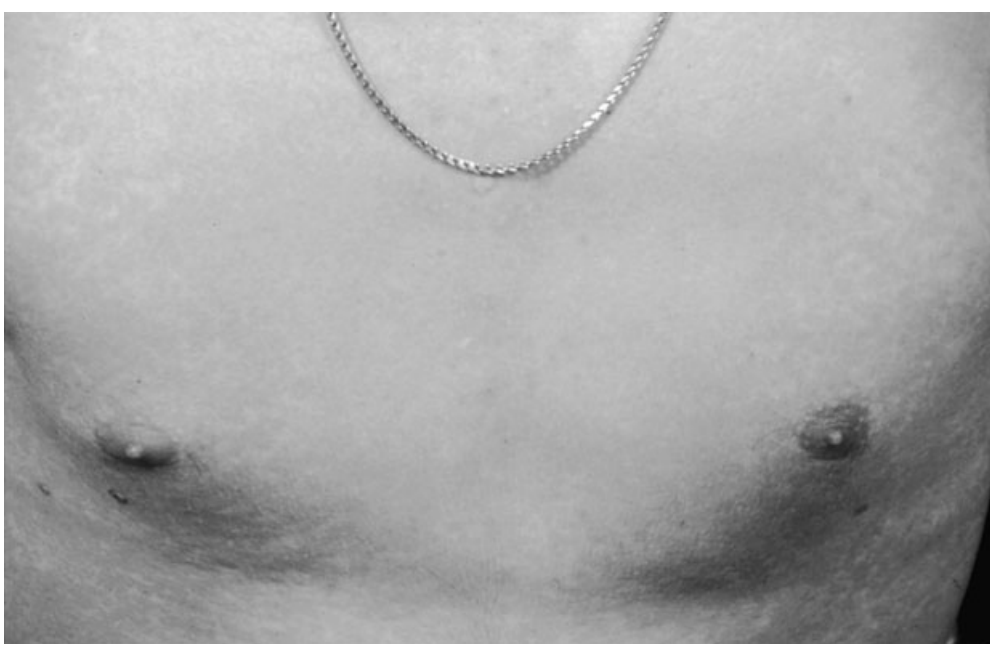

Figure 172–4 "Ampicillin rash" occurring in an adolescent with infectious mononucleosis after the administration of amoxicillin. *(Courtesy Dr. James Brien, Scott and White Hospital, Temple, Tex.)*

treated with other β-lactam antibiotics, but such rashes do not represent hypersensitivity reactions.

Other Exanthems. An exanthem will develop in approximately 3 to 15 percent of persons with IM not treated with β-lactam antibiotics.[27,213,251,334,410,478] These rashes are maculopapular but can be urticarial, scarlatiniform, or erythema multiforme–like. Erythema nodosum also has been noted.[43] Although most commonly associated with hepatitis B, some cases of Gianotti-Crosti syndrome (papular acrodermatitis of childhood) also have been related to primary EBV infection.[217]

CARDIAC

Mild electrocardiographic abnormalities (mostly nonspecific ST and T wave changes) have been observed in 6 to 16 percent of adults with IM.[110,214] Abnormalities usually are noted during the second or third week of illness and disappear by the fourth week, but they may persist longer. A case of myocarditis heralding typical IM has been noted in a young adult.[513] Serious cardiac complications occur extremely rarely.[142]

HEMATOLOGIC

Hemolytic Anemia. Hemolytic anemia occurs in 1 to 3 percent of patients with IM,[215,478] and many cases have been reported in children with previously unrecognized hemoglobinopathies. The anemia typically develops during the second or third week of illness and resolves in 1 to 2 weeks. Corticosteroid therapy may hasten resolution.[151]

Aplastic Anemia. This anemia is a rare complication of IM, with only scattered case reports in the literature.[169,293,507] Steroids have been beneficial,[169] but bone marrow transplantation has been necessary in some cases.[309] Fatalities have been recorded, usually in association with pancytopenia.[1,507]

Thrombocytopenia. Thrombocytopenia occurs in 25 to 50 percent of patients with IM[58,59] and probably is immune-mediated. Platelet counts usually return to normal levels 4 to 6 weeks after the onset of illness. Severe thrombocytopenia (platelet counts $<20 \times 10^3/mm^3$) occurs rarely, with fewer than 40 cases identified in one review.[395] These authors noted a complication rate of 27 percent and a mortality rate of 5 percent associated with severe thrombocytopenia. Case reports have suggested beneficial effects of corticosteroids[313,488] or intravenous immunoglobulin[109] for severe, life-threatening thrombocytopenia.

Neutropenia. Mild to moderate neutropenia occurs frequently during IM.[57,60] Neutrophil counts typically reach their lowest values during the third and fourth weeks of illness but may persist for another month or more. Although severe neutropenia (absolute neutrophil count <200) and agranulocytosis are rare events, with patients recovering uneventfully, death from overwhelming bacterial infection has been reported.[361] Antineutrophil antibodies elicited during EBV-induced polyclonal antibody stimulation probably are the source.[444]

Pancytopenia. Pancytopenia has been reported to develop in a small number of children and adults with IM.[293] Fatalities attributable to overwhelming bacterial infection have occurred.[239,507]

SPLEEN

Detectable splenomegaly develops in up to 50 percent of patients with IM, with one study demonstrating splenic enlargement by ultrasound in 100 percent of patients hospitalized with IM.[105] Patients with a palpable spleen are at risk for having spontaneous rupture. In the United States, spontaneous rupture of the spleen occurs in an estimated one in every 1000 adults with IM[127,328]; the rate of splenic rupture in children is unknown but probably is lower. Rupture typically occurs during the first to third week of illness and is heralded by abdominal pain. Signs of hypovolemia occur frequently and include orthostasis, tachycardia, and syncope. Pain in the left shoulder (Kehr sign),[322] caused by diaphragmatic irritation from blood, is observed in approximately 50 percent of patients with splenic rupture; right shoulder and subscapular pain may develop as well.[440] Splenic abscess has been described in a child with IM.[73]

To reduce the risk of splenic rupture, physicians customarily caution patients against engaging in strenuous physical activity and contact sports during recovery from the acute illness and for the period of significant organomegaly.[436,516] Recommendations for reduced activity vary between 3 weeks and 6 months. In a recent review, the lack of evidence-based information regarding the timing of return to activity for athletes is acknowledged, and physicians are discouraged from making decisions on the basis of a single parameter.[516] Instead, individualized decisions must be made. In general, if the patient is asymptomatic without a palpable liver or spleen, a gradual return to pre-IM levels of activity probably is safe. Imaging procedures (e.g., computed tomography [CT] or ultrasound) generally are not cost effective or accurate in determining the timing for normal activity, but they may be considered for athletes desiring to return to play early and for those with ambivalent examination findings.

Most cases of splenic rupture require emergency splenectomy.[127] However, in an effort to maintain the patient's hematologic and immunologic competence, practical management of splenic rupture is evolving. At some institutions, selected patients with splenic rupture who meet certain requirements, including hemodynamic stability with low transfusion requirements and a normal level of consciousness, may be managed with medical therapy,[22] or endovascular interventional methodologies,[47] instead of traditional splenectomy.

GASTROINTESTINAL TRACT

Liver. Mild to moderate, subclinical inflammation of the liver occurs in more than 90 percent of patients with IM.[221] Rarely, more serious cases of hepatic disease, including children with fulminant hepatic failure, have been described.[101,145,191,281,321,325,332] Chronic hepatitis also has been reported.[490]

Other. Other gastrointestinal diseases purportedly associated with IM include pancreatitis, gastritis, typhlitis, and cholecystitis.[193,281,312,452,548]

NEUROLOGIC

Acute neurologic disorders[83,454] develop in 1 to 5 percent of persons with IM.[36,213,454] Neurologic complications usually occur at the height of the typical manifestations of IM, but they also may develop during the resolution phase. In addition, some reports document neurologic disease as a heralding event, before the development of typical IM, or even as the sole manifestation of EBV infection, especially in children.[44,104,118,354] The most common neurologic complications include those occurring in the CNS, such as aseptic meningitis, meningoencephalitis, and encephalitis; however, other central and peripheral neurologic complications have been described. The pathophysiology of CNS manifestations is not well established. Possible mechanisms

include direct viral invasion, immunologic mechanisms, and inflammation.

Encephalitis and Aseptic Meningitis. EBV frequently affects the CNS, causing cerebrospinal fluid (CSF) abnormalities and electroencephalographic abnormalities, yet most patients exhibit no symptoms.[390] In two large series of patients with encephalitis, EBV was responsible for 2 to 5 percent of cases,[266,279] and EBV caused 8 percent of cases of focal encephalitis.[525] Clinical findings in EBV encephalitis include fever, headache, vomiting, seizures, alteration in mental status, irritability, disorientation, lethargy, and, occasionally, a comatose state. Symptoms suggestive of schizophrenia, such as delusions, hallucinations, and extreme agitation, are seen occasionally. Patients with aseptic meningitis typically complain of a stiff neck, headache, fever, and vomiting. Symptoms of IM occur uncommonly in children developing EBV encephalitis. Typical CSF findings in children with EBV encephalitis include mild to moderate pleocytosis (lymphocytic predominance) in 50 percent, mildly elevated protein levels in half of cases, and normal glucose.[104] EBV antibodies, EBV DNA, and even EBV itself have been identified in the CSF or brain tissue of persons with EBV-associated neurologic complications.[185,230,252,388] Some reports suggest that the immune dysregulation caused by primary EBV infection may act as a triggering mechanism for the development of measles-associated subacute sclerosing panencephalitis.[129,216] Because typical IM symptoms seldom occur in children with EBV encephalitis, and clinical findings are not specific, EBV should be considered a possible etiology for any child with acute encephalitis.

Other CNS Manifestations. EBV-associated cerebellitis is a rare occurrence that develops primarily in children and young adults.[76,83,118,157] Full recovery occurs within weeks to months. Cranial nerve palsies, singly or in combination, also occur rarely; the facial nerve is involved most commonly.[344,546] Other manifestations of cranial nerve involvement include optic neuritis,[140] deafness,[118,528] and ophthalmoplegia.[438] Some cases of brain stem encephalitis[372,449] and recurrent aseptic (Mollaret) meningitis[163] have been reported.

Non-CNS Neurologic Complications. Outside the CNS, Guillain-Barré syndrome (ascending polyradiculoneuritis) is the most common complication of EBV infection.[170,171,415] Other non-CNS neurologic complications of IM include peripheral neuropathy,[112] autonomic dysfunction,[536] hemiplegia,[29] and transverse myelitis.[503]

RENAL

Silent abnormalities of the urine sediment, including proteinuria and microscopic hematuria, occur commonly in patients with IM.[213,299] However, clinically significant renal disease is an extremely rare event. Tubulointerstitial disease and nephrotic syndrome have been observed most commonly.[42,510] Acute renal failure with and without rhabdomyolysis has been reported.[141,302,510] Some children and adolescents have developed hemolytic uremic syndrome related to acute EBV infection.[447,455]

RESPIRATORY TRACT

Airway Obstruction. Severe airway obstruction occurs in 1 to 5 percent of children and adults with IM. Alpert and Fleisher[6] observed severe airway obstruction in a fourth of children admitted for complications of IM. Patients typically have severe tonsillopharyngitis with progressive symptoms of airway obstruction, usually in association with dysphagia and odynophagia. Airway obstruction may occur at any of several levels, including inflammation and hypertrophy in the Waldeyer ring, edema of the epiglottis and pharynx, and development of pseudomembranes in large airways.[156,178] Management of airway obstruction has included systemic corticosteroids, tonsillectomy, tracheostomy, and nasopharyngeal airway placement, as well as general supportive measures such as humidification and elevation of the head of the bed.[462,530] Nonsurgical management, including the administration of systemic steroids, usually is beneficial, although tonsillectomy may be necessary in refractory cases.[462,469,530] With the widespread availability of intensive care and mechanical ventilation, mortality from this complication is much lower than previously observed.

Neck Abscesses. Cervical and peritonsillar abscesses have been described in association with IM.[54,187,249,522] This uncommon complication follows severe tonsillopharyngitis in patients with IM and typically is caused by bacteria found in the oral cavity, including alpha-hemolytic streptococci and anaerobes. Appropriate management includes administration of antibiotics and surgical drainage. Some concern exists that corticosteroid therapy of incipient airway obstruction may predispose to the development of an abscess.[54,187] On the other hand, abscesses may develop in the absence of previous corticosteroid therapy.[149,400]

Pulmonary Disease. Radiographic evidence of pulmonary infiltrates occurs in 0 to 5 percent of patients with IM,[110,289] yet significant symptomatology rarely is present.[110,213,289] Some cases of IM with pulmonary disease may represent co-infection with other etiologic agents.[15,131] However, several immunocompetent adults (but few children) with acute EBV infection have been described with pulmonary disease seemingly unrelated to other organisms.[77,184,373] Typically, unilateral or bilateral interstitial infiltrates are observed, and pleural effusions may be present.[113,280] In some cases, EBV has been detected in lung tissues.[467,508] Recovery is the rule, although mechanical ventilation may be necessary.

PSYCHIATRIC

Chronic Fatigue. In the early 1980s, a chronic debilitating disorder (now designated *chronic fatigue syndrome*) began receiving increased publicity and purportedly was linked etiologically to EBV.[255,471] The syndrome occurs more commonly in middle-aged women and is characterized primarily by chronic debilitating fatigue, as well as low-grade fever, mild lymphadenopathy, pharyngitis, neuropsychological problems, and other symptoms. The association with EBV was drawn mainly from epidemiologic features and some abnormal EBV-specific immune responses. Variable general immunologic abnormalities have been described in some cases. However, no consistency exists in these findings, and a plausible pathogenetic association has not been demonstrated. Most experts today do not regard EBV as a major etiologic agent in the development of this syndrome, although it may play a contributory role along with other viruses (e.g., human herpesvirus 6, CMV).

"Alice-in-Wonderland" Syndrome. This unusual syndrome, technically termed *metamorphopsia*, is a visual illusion manifested as a distortion in size, form, movement, or color, and it is associated most commonly with migraine headaches, epilepsy, and hallucinogenic drugs. Copperman[85] first described its association with IM in 1977, and other similar cases also have been described.[74,119,286,287] Onset may occur during or soon after resolution of the clinical IM symptoms, and it may resolve within 4 to

6 weeks. Visual-evoked potentials suggest diminished cerebral perfusion in affected cases.[287]

MISCELLANEOUS COMPLICATIONS

Other complications purportedly associated with IM or primary EBV infection include recurrent tonsillitis,[509] sinusitis,[475] periorbital cellulitis,[475] ocular disease,[335] rhabdomyolysis,[377] genital ulcers,[401] and orchitis.[409]

MALIGNANT DISEASES ASSOCIATED WITH EPSTEIN-BARR VIRUS

BURKITT LYMPHOMA

Endemic BL is a rapidly progressive and fatal tumor that predominantly affects young children in central Africa and Papua New Guinea (Fig. 172–5).[53] Approximately 60 percent of African patients with BL present with jaw masses, with abdominal masses being second most common.[550] Less common sites include the CNS and the eye. Non-African patients with BL are most likely to have abdominal masses initially. In some patients with BL and some with congenital or acquired immunodeficiencies (including AIDS), CNS involvement is present or eventually develops and is characterized by intracranial mass lesions of lymphomatous cells or a CSF pleocytosis consisting of malignant cells, or both.[14,550,551]

HODGKIN DISEASE

The association of EBV with HD is based primarily on serologic studies[306,356] and identification of EBV DNA in H-RS cells (the malignant cells characteristic of HD).[267,520] The differential incidence distribution of HD in children throughout the world corresponds with the age at first EBV infection: It increases in adolescence in developed countries and increases in early childhood in developing countries.[177,248] Virtually all cases of HD in developing countries are associated with the presence of EBV, whereas the virus is present in approximately 40 percent of patients with HD in developed countries.[268] However, in developed countries, EBV is strongly associated with HD in children younger than 10 years old and in tumors displaying the mixed cellularity variant.[20,205,380] HD rarely occurs in children younger than 5 years old in Western countries, but it is seen more commonly at younger ages in developing countries. The precise pathogenetic mechanisms for EBV-associated HD are being unraveled, but evidence indicates that HD is primarily a result of chronic inflammation, with EBV contributing to the overproduction of various cytokines and chemokines by H-RS cells and the surrounding inflammatory cells.[268] Patients typically have cervical or supraclavicular lymphadenopathy, as well as mediastinal adenopathy (occasionally with obstructive symptoms).[225] Constitutional symptoms such as fever, nightsweats, and weight loss develop in approximately a fourth of patients with HD, and these symptoms worsen the prognosis.

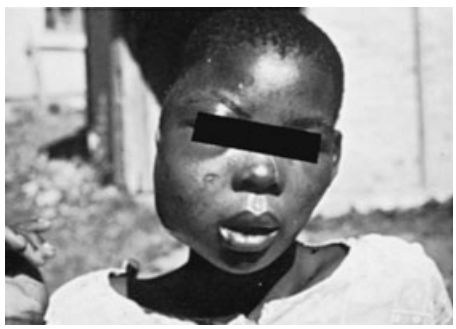

Figure 172–5 African boy with Burkitt lymphoma involving the jaw. *(Courtesy Dr. George Miller, Yale University.)*

NASOPHARYNGEAL CARCINOMA

NPC occurs primarily among adults in southern China, but it may be seen in Greenland, Alaska, and the Mediterranean region as well; the tumor is extremely rare in Western countries.[125] In endemic regions, children younger than 16 years old account for only 1 to 2 percent of all cases of NPC, whereas this proportion is higher (10%) in the United States.[232] Children with NPC most commonly present with a painless unilateral neck mass, nasal symptoms (congestion, epistaxis, discharge), auditory symptoms (otalgia, otitis media, hearing loss), and cranial nerve abnormalities.[24,247] At initial evaluation, children with NPC are more likely than are adults to have locoregional disease.[24,232]

POST-TRANSPLANT LYMPHOPROLIFERATIVE DISEASE

Children immunosuppressed for organ transplantation have an increased risk for developing EBV-related lymphoproliferative syndromes and B-cell lymphomas (collectively termed *PTLD*).[211,283,464] Ho and colleagues[211] reported a greater frequency of lymphoproliferative lesions in pediatric transplant recipients (4%) than in adult transplant recipients (0.8%). Pediatric organ transplant recipients are thought to have an increased risk for development of EBV-related PTLDs because, when compared with adult transplant recipients, they are more likely to be seronegative for EBV at transplantation, and primary infection is more likely to progress to PTLD and other severe EBV-associated disorders.[210,211] Some variability exists in the incidence of PTLD according to the specific organ transplanted: The incidence is highest in children receiving intestinal transplants (31%),[470] probably attributable to more profound immunosuppressive regimens and greater loads of lymphatic tissue in the grafts; generally lower PTLD incidences are noted in other pediatric organ transplant populations (liver: 7-13%; thoracic organs: 4-12%; kidney: 1-9%).[470] After human stem cell transplantation, the 10-year cumulative incidence rate for PTLD is approximately 1 percent overall.[89] Rates of PTLD are substantially higher if benign, hyperplastic cases of PTLD are included.[547]

The clinical history, physical examination findings, and confirmatory virologic laboratory testing are required in order to establish a diagnosis of PTLD. Symptoms may be quite nonspecific, especially early in the course, and include fever, malaise, weight loss, abdominal symptoms (bleeding, nausea, vomiting), sore throat, swollen lymph nodes, headache, focal neurologic symptoms, and indications of graft dysfunction.[165] On examination, signs may include lymphadenopathy, large tonsils, hepatosplenomegaly, pallor, subcutaneous nodules, and focal neurologic findings.[165]

Subsequent EBV-associated, smooth-muscle tumors (e.g., leiomyosarcomas) have been found to develop in some adolescent and young adult patients undergoing organ transplantation.[298,426]

LYMPHOPROLIFERATIVE DISEASE IN OTHER IMMUNODEFICIENT PATIENTS

Patients with a wide variety of congenital immunodeficiencies (e.g., severe combined immunodeficiency syndrome, Wiskott-

Aldrich syndrome, ataxia telangiectasia, Chédiak-Higashi syndrome, common variable immunodeficiency), as well as iatrogenically immunosuppressed patients (e.g., patients receiving cancer chemotherapy or antitumor necrosis factor alpha monoclonal antibodies), also are at risk for developing various EBV-associated lymphoproliferative diseases and severe atypical EBV infections. One should keep in mind, however, that IM and other forms of EBV infections in immunocompromised children and adults usually run a course similar to that in immunocompetent persons; actually, even in immunosuppressed patients (and post-transplantation subjects), the majority of EBV infections are clinically silent.[202,211]

OTHER MALIGNANCIES

Other rare lymphoproliferative diseases (B cell and T cell), as well as carcinomas, have putative associations with EBV. The strength of these associations is quite variable. Pyothorax-associated lymphomas, occurring at sites of long-standing inflammation (e.g., tuberculosis), are quite strongly associated with EBV, with EBV identified in tumor cells in 70 to 100 percent of cases.[147] Lymphomatoid granulomatosis, seen predominantly in immunosuppressed patients, has the distinctive histopathologic finding of malignant cells surrounding blood vessels (angiocentricity). This malignancy is considered to have a pathogenesis similar to that of PTLD, with diminished T-cell surveillance probably allowing proliferation of EBV-transformed B cells.[414] Lymphomatoid granulomatosis occurs primarily in the lung but may be found at other sites including lymph nodes of children.[108] Plasmablastic lymphomas (extraordinarily rare in children) are unusual tumors typically found in the oral cavity of adult patients with AIDS, but they also sometimes are found in immunocompetent patients.[61] They have a strong association with EBV.

Several T-cell lymphomas are associated with EBV.[414] They include (1) angioblastic lymphadenopathy (or AILT)[521]; (2) enteropathy-type T-cell lymphoma (often associated with celiac disease)[535]; (3) extranodal nasal T/NK-cell lymphoma, an aggressive tumor involving the nasal or midline facial area, or other non-nasal, extranodal sites[237]; and (4) aggressive NK-cell leukemia, a rapidly fatal disease heralded by an acute symptom complex that includes fever and multiorgan system failure.[237]

Gastric carcinomas are more common in Asia, but they are extremely rare in children. In adults, two types of gastric tumors are noted to be associated with EBV. Gastric adenocarcinoma is a more common tumor, but EBV is found in only approximately 10 percent of tissues. In contrast, EBV can be found in the majority of tissues from patients with gastric tumors displaying an undifferentiated (lymphoepithelioma-like) histopathology.[270] Also in Asian populations, EBV is detectable within most lymphoepithelioma-like salivary gland tumors[448] and could play an etiologic role. Considerable ongoing debate continues regarding the etiologic relationship of EBV with breast cancer.[292]

EPSTEIN-BARR VIRUS AND HUMAN IMMUNODEFICIENCY VIRUS

Several studies have indicated that underlying HIV infection is responsible for abnormal responses to EBV infections. These findings probably are attributable to the relative ineffectiveness of T cells from patients with AIDS in controlling EBV-infected lymphocytes.[41] In HIV-infected adults, these unusual reactions include exaggerated antibody responses to certain EBV antigens, including VCA and EA,[408] and enhanced oropharyngeal shedding of EBV.[7,243] Oropharyngeal shedding of EBV occurs more commonly in younger HIV-infected children, and children whose HIV disease progressed more rapidly had a substantially higher rate of excretion.[243] In another study, EBV infection developed in HIV-infected children at an earlier age and was more likely to involve hepatosplenomegaly.[389] Whether EBV contributes directly to the progression of immune deficiency in these patients continues to be a matter of conjecture, although in vitro studies do indicate that some EBV proteins (e.g., LMP, BZLF1) are able to transactivate HIV-1.[186,329]

Studies clearly have revealed that certain lymphoproliferative and other diseases develop in HIV-infected patients at rates higher than those in healthy persons. These diseases include NHL, HD, body-cavity lymphoma, smooth muscle tumors, lymphocytic interstitial pneumonitis (LIP), and oral hairy leukoplakia.

NHL has remained the most common malignancy occurring in children with AIDS[39] and is an AIDS-defining condition.[84] In the present era of highly active antiretroviral therapy (HAART), NHL also has surpassed Kaposi sarcoma as the most common malignancy in adults.[114] In one pediatric study, approximately one third of NHL tumors contained EBV DNA.[339] Primary CNS lymphomas are extremely rare occurrences in healthy children, but they have accounted for 8 percent[339] to 23 percent[39] of NHLs in children with AIDS. Primary CNS lymphomas are linked very strongly to EBV, with the virus present in virtually every case.[324] NHL invariably occurs in patients with AIDS who have profound immune suppression (e.g., $CD4^+$ concentration <200 cells/μL). Pediatric patients with AIDS and NHL typically have systemic symptoms of fever and weight loss. Signs of extranodal disease include hepatosplenomegaly, abdominal distention, jaundice, bone marrow involvement, and, rarely, CNS symptoms.[338]

Although not an AIDS-defining condition, HD in adults with AIDS historically has occurred at rates 5 to 15 times higher than the general population. HD is an uncommon occurrence in children with AIDS, but it has been described.[339,403] HIV-infected patients, including children,[403] appear more likely than non–HIV-infected patients to present with disseminated disease, sometimes without obvious nodal involvement on physical examination.[396] In adults with AIDS, the incidence of HD has increased significantly in the HAART era.[114] Interestingly, HD incidence rates are higher in patients with AIDS and moderate compared with severe immune suppression, and the increased incidence is postulated possibly to be related to the inability of EBV-infected H-RS cells to recruit sufficient lymphocytes and histiocytes for their survival.[40]

Body cavity lymphoma (or primary effusion lymphoma) is a rare malignancy that occurs almost exclusively in adult patients with AIDS, but it has been reported in children.[296] Patients typically have immunoblastic lymphomatous effusions without solid tumors in body cavities such as the peritoneum or pleura.[358] More than 75 percent of these nonsolid tumors are associated with human herpesvirus 8; however, EBV also is identified frequently in tumor cells. EBV may play a cofactorial role in the pathogenesis of these unusual tumors.[414]

Leiomyosarcoma is an extremely rare occurrence in healthy patients,[540] but it is the second most common malignancy in children with AIDS[337] and is an AIDS-defining condition (Category B).[84] It also has developed in solid organ transplant recipients[298] and in patients with other types of immunodeficiency.[345] EBV may be responsible for the unusually high incidence of these smooth-muscle tumors found in children with AIDS.[244,339] Leiomyosarcoma tissues from patients with AIDS have high concentrations of EBV (CD21) receptors, contain clonal (or oligoclonal) EBV DNA, and express EBERs and EBNA-1.[244,339] Leiomyosarcoma may develop in any smooth muscle tissue in patients with AIDS, but more typically it is noted first in the gastrointestinal tract and lungs.

LIP is a lymphoproliferative disease that occurs primarily in children with AIDS.[16] In the era prior to HAART, the incidence of LIP was 12 percent among children enrolled in a large longi-

tudinal natural history study[161]; in the HAART era, this incidence has dropped to less than one case of LIP per 1000 person-years. Previous serologic and virologic evidence pointed to an association with EBV,[16,264,411,435] but the exact role that EBV plays in this lymphoid proliferation is unclear at present. LIP is associated with an improved prognosis for survival.[435] Radiographically, diffuse interstitial infiltrates are noted. The course of disease is variable: Many infections in infants are asymptomatic, and whereas some children remain stable for long periods, others may exhibit a slow, but relentless progression to chronic lung disease typified by tachypnea, cough, wheezing, and hypoxemia. Advanced disease is characterized by clubbing, wasting, cor pulmonale, and bronchiectasis.

Oral hairy leukoplakia is a nonmalignant squamous cell proliferation that develops in the oropharynx of patients with AIDS. Although a common occurrence in adults with AIDS, it occurs in less than 5 percent of HIV-infected children.[326] EBV DNA and virus replication can be detected within epithelial cells,[167,461] with defective variants possible.[148] Oral hairy leukoplakia lesions are manifested as nonremovable gray or white plaques on the lateral surface of the tongue; they often have vertical corrugations and a shaggy or "hairy" appearance when dry.[446]

DIAGNOSIS OF INFECTIOUS MONONUCLEOSIS

GENERAL LABORATORY FINDINGS

Patients with IM typically have an absolute lymphocytosis (>50% lymphocytes, with total leukocytes >5000/μL), prominent atypical lymphocytes (usually >10% of total leukocytes), and a positive test result for Paul-Bunnell heterophile antibodies (positive "differential heterophile"). The atypical lymphocytes observed in the blood of patients with mononucleosis (Fig. 172–6) predominantly consist of activated T lymphocytes responding to the B-cell infection.[385] Although often considered a hallmark of IM, atypical lymphocytes also may be observed in association with CMV infection, toxoplasmosis, measles, mumps, roseola, rubella, drug reactions, and other conditions.[72,534]

For a typical uncomplicated case of IM, establishing a specific diagnosis of EBV infection is unnecessary, with a complete blood count, differential, and heterophile antibody test being sufficient for diagnostic testing. These tests are uncommonly normal early in the course of disease and thus may need to be repeated during the first 3 to 4 weeks before diagnostic results are achieved. The clinician should be aware that very young (<4 years) patients with primary EBV infection frequently have a negative heterophile test.[477] Some recent studies suggest that a higher ratio (>0.35) of lymphocytes to white blood cell counts,[531] or the detection of lymphatic tissue with fibrinous membranes observed via nasopharyngeal endoscopy,[517] may distinguish between IM and acute bacterial tonsillitis.

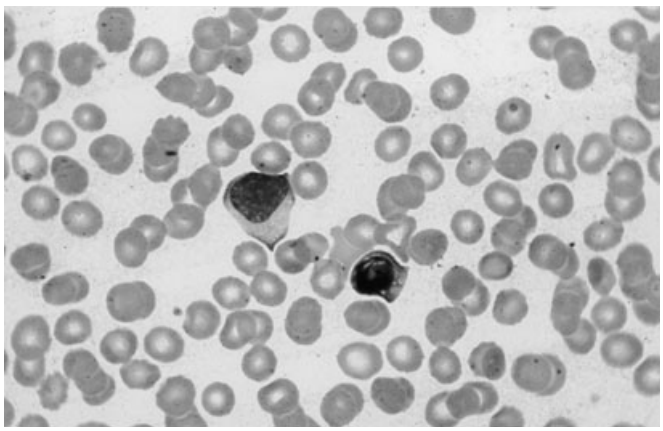

Figure 172–6 Atypical lymphocytes in the peripheral blood of a patient with infectious mononucleosis. *(Courtesy Dr. Margaret Gulley, University of North Carolina, Chapel Hill, N.C.)*

Heterophile antibodies classically were measured as sheep erythrocyte agglutinins.[386] Beef, ox, and horse red blood cells (RBCs) also are agglutinated by the heterophile antibodies found in the serum of patients with IM, but these heterophile antibodies do not bind to guinea pig kidney antigen extracts. These properties of mononucleosis heterophile antibodies distinguish them from the naturally occurring Forssmann heterophile antibodies and from the heterophile antibodies found in serum sickness and other conditions. Thus, traditional tests for Paul-Bunnell heterophile antibodies involve absorption of the test serum with beef or ox RBCs (which remove Paul-Bunnell heterophile antibodies) and guinea pig extract (which does not remove them). Tests in current use (e.g., Mono-Test, Mono-Diff, Mono-Spot) typically use horse RBCs, which provide a more sensitive assay than do sheep erythrocytes. However, when horse erythrocyte agglutination tests are performed, the specificity of a positive result always should be confirmed by absorption of the serum with at least guinea pig kidney extract or, preferably, with both guinea pig kidney extract and beef (or ox) RBCs. Materials for the absorption steps are included in many, but not all, of the kits available commercially. Another heterophile antibody test, the beef (or ox) erythrocyte hemolysin assay, does not require absorption of the test sera but is somewhat less sensitive than is horse erythrocyte agglutination testing. Other tests[139,181,303,428] using purified forms of heterophile antigen do not seem to offer any significant advantage over the rapid slide test using horse erythrocyte agglutination.

Other laboratory tests in patients with severe mononucleosis syndromes or in those with atypical clinical manifestations may indicate involvement of major organs, but such involvement rarely is associated with severe complications (see the previous section "Complications of Infectious Mononucleosis"). These laboratory abnormalities include elevated transaminase levels, mild hemolytic anemia, and neutropenia.

EBV-specific laboratory testing should be reserved for patients with an EBV-suspected disease (1) lacking a heterophile antibody response; (2) exhibiting atypical, severe manifestations; or (3) associated with significant lymphoproliferative, oncogenic, or chronic findings. In most cases of EBV-associated IM episodes in immunocompetent patients, EBV-specific serology (i.e., antibody determination) is sufficient to affirm the viral-specific diagnosis. Isolation of virus from body secretions, fluids, and tissues (see "Virus Isolation," later) is labor intensive and not widely available. Quantitation or semiquantitation of EBV-specific viral load provides some measure of activity or putative influence of the virus in a variety of EBV-associated diseases. Other laboratory methods demonstrating high concentrations of actively replicating virus or latent antigens (such as immunohistochemical methods for antigen testing or quantitative PCR) may be more useful in associating EBV infection with disease, particularly in immunocompromised patients (Table 172–2).

EPSTEIN-BARR VIRUS ANTIBODIES

The viral-specific diagnosis of EBV-associated primary infections as IM in immunocompetent individuals (and commonly in immunocompromised patients) usually requires three types of antibody analysis on acute serum: IgG to EB VCA (VCA IgG), IgM to EB VCA (VCA IgM), and IgG to EBNA (EBNA IgG). (The EBNA complex is composed of six nuclear antigen or proteins, principally EBNA-1; the antibody response to the complex will herein

TABLE 172–2 Summary of Laboratory Tests for Epstein-Barr Virus (EBV)–Associated Diseases

Detection of	Test	Purpose
Antibodies	Heterophile antibody	Detect heterophile antibodies indicating infectious mononucleosis (more reliable in patients older than 4 yr of age)
	EBV antibodies	Measure antibody response to viral proteins in serum samples; distinguish acute from remote infection
EBV DNA and RNA	Southern blot	Assess clonality of lesions with respect to EBV DNA structure; distinguish latent from replicative infection
	In situ hybridization	
	RNA (EBERs)	Identify EBER transcripts in specific cell types within histologic lesions
	DNA	Identify EBV DNA in specific cell types within histologic lesions
	PCR	Detect and quantitate EBV DNA in blood or CSF to diagnose and monitor disease
EBV proteins	Immunohistochemistry	Identify EBV protein expression in specific cell types within histologic lesions; distinguish latent from replicative infection on the basis of expression profiles
EBV	Virus culture	Detect infectious virions or latently infected B cells; impractical for routine clinical use
	Electron microscopy	Identify whole virions representing replicative infection; impractical for routine clinical use

CSF, cerebrospinal fluid; EBER, EBV-encoded RNA.
Modified from Gulley, M. L.: Molecular diagnosis of Epstein-Barr virus–related diseases. J. Mol. Diagn. 3:1-10, 2001.

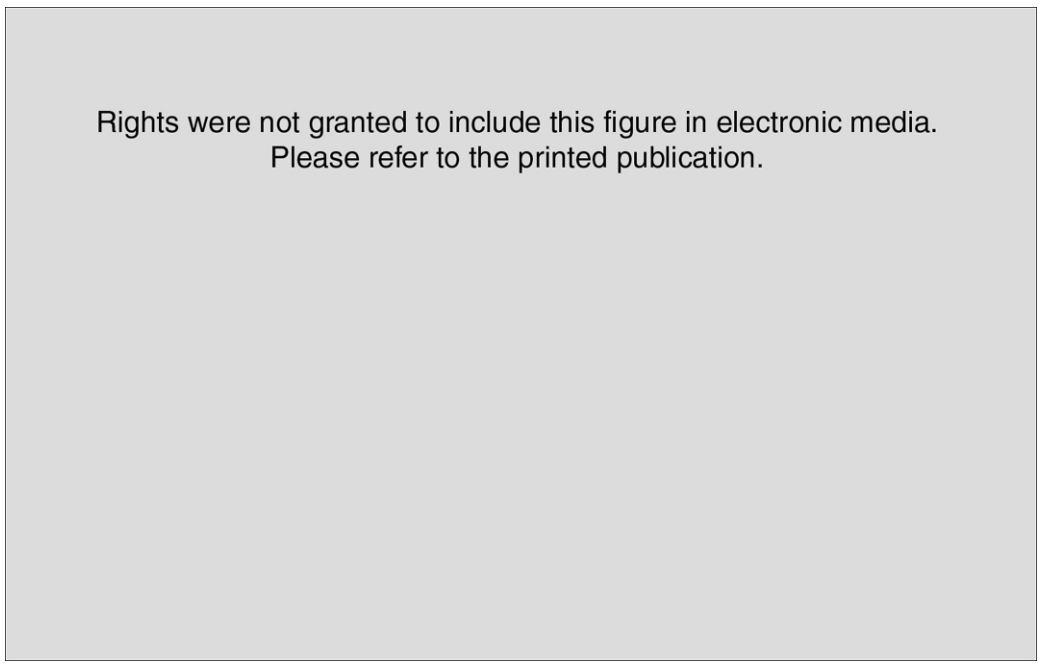

Figure 172–7 Schematic representation of the evolution of antibodies to various Epstein-Barr virus antigens in patients with infectious mononucleosis. The titers are geometric mean values expressed as reciprocals of the serum dilution. The minimal titer tested for viral capsid antigen (VCA) and early antigen (EA) antibodies was 1:10, and for Epstein-Barr nuclear antigen (EBNA) it was 1:2.5. The IgM response to capsid antigen was divided because of the significant differences noted according to age. *(From Jenson, H.B., Ench, Y., and Symaya, C.V.: Epstein-Barr virus.* In *Rose, N.R., de Macario, E.C., Folds, J.D., et al. [eds.]: Manual of Clinical Laboratory Immunology. 5th ed. Washington, D.C., American Society for Microbiology, 1997, pp. 634-643.)*

be referred to as IgG to EBNA, or EBNA IgG.) The three EBV antibodies can be detected by commercially available immunofluorescent techniques or by various enzyme immunoassays. The need to add the determination of IgG to EB early antigen, a serologic response commonly found early in the primary infection, usually is not necessary for IM-like episodes.

Evolution of the serologic response to EBV antigens after a prototypic EBV-associated IM episode is depicted in Figure 172–7. By the time clinical evaluation for IM and probably other forms of primary EBV infection is performed, an appreciable antibody response to EBV VCA has developed in most persons.[201,477] In the case of primary infections (e.g., IM), the initial serum sample often contains both IgG and IgM antibodies to VCA. Most children also have (or they will develop shortly) antibodies to the EA complex, which can be measured separately as antibodies to the restricted (R) or diffuse (D) components.[135,477] During the early phase of primary infection, most persons do not have detectable IgG antibodies to EBNA; a few have marginally detectable anti-EBNA titers. Thus, the serologic diagnosis of a recent or current primary EBV infection typically consists of a positive IgG and IgM anti-VCA response and negative anti-EBNA, with positive results in the anti-EA assay, if performed.

Following the acute primary EBV infection (reflected in IM), IgM antibodies to VCA typically disappear within 2 to 3 months, and IgG antibodies to EA usually disappear within 6 to 12 months after infection (see Fig. 172–7).[477] IgG anti-VCA and anti-EBNA

antibodies persist for life and are indicative of a typical, chronic virus-carrying state.[477] Some studies also have shown the persistence of anti-EA antibodies for several years after an acute EBV infection and the occasional appearance of anti-EA antibodies in healthy seropositive persons; however, the clinical or virologic importance of this finding is not clear. Heterophile antibodies may persist for months and sometimes for a year or more in persons who have recovered from IM; they remain detectable longer (usually more than a year) when the more sensitive horse erythrocyte agglutination and a quantitative method for determining the antibody response are used.

The antibody profile for an acute primary EBV infection, particularly that associated with an IM episode, is relatively clear in comparison with the variability of serologic or antibody profiles found in persons with other EBV-related disorders (Table 172–3). When a disease is not a consequence of primary infection but may be related to persistent or reactivated EBV infection (e.g., BL, NPC, some lymphomas of immunocompromised hosts, some chronic or atypical illnesses), the following serologic profile may be observed: positive IgG anti-VCA, often in high titer; usually negative IgM anti-VCA; often elevated anti-EA titer; and a positive anti-EBNA assay (though sometimes at low titer and occasionally undetectable).[187,202,203,246,406,472] Of note is that males with XLP frequently have no or low levels of EBV antibodies in response to EBV infection.[437]

Some studies[333,418,429] suggest that monitoring for decreases in anti-EBNA antibodies, or identification of increased immunologic mediators as IL-4 and soluble CD23 and increased circulating EBV (see later, "EBV Antigens"), may be useful in providing early serologic signs of EBV-associated tumor development in organ and bone marrow transplant recipients. Further, testing for IgA antibody responses to EBV antigens may be helpful in establishing a diagnosis and evaluating the response to therapy for nasopharyngeal carcinoma.[258]

Molecular cloning of subregions of the EBV genome into high-expression plasmid vectors can generate large amounts of pure proteins containing EBV-encoded polypeptide sequences for use in immunoblot and enzyme-linked immunosorbent assay (ELISA)-based tests.[52,63,126,207,333,418,429] These methods have extended the availability and utilization of EBV laboratory diagnosis and follow-up. There is a growing number of commercially available EBV serologic tests, particularly enzyme immunoassays.[51,206] Yet after several decades of use, immunofluorescent techniques still remain the "gold standard" in EBV-specific serologic testing because of their overall excellent specificity and sensitivity. However, they require more expensive equipment, time, effort, and experience in interpretation. ELISAs are important alternates for serologic testing because of their ease in performance and enhanced sensitivity. However, their specificity may be inferior to that of immunofluorescent techniques.[305] It is noteworthy that there is insufficient standardization of antigen preparation, antigen and substrate selection, and cross-comparability of serologic techniques and manufacturers involved with commercially available EBV serologic tests. Therefore, interpretations should be drawn carefully, particularly with serum of young children, an age group that has been less well evaluated.[304]

Improvements in standardization, as well as specificity and sensitivity, of commercially available serologic testing would benefit the laboratory diagnosis of primary EBV infections. Current EBV-specific serologic testing for nonprimary infections, particularly in immunocompromised patients, provides more limited diagnostic interpretations. The expanding capacity in the detection and quantitation of EBV viral load in body compartments and lesions (see next section) is of growing diagnostic importance in the latter circumstances.

EPSTEIN-BARR VIRUS NUCLEIC ACID (DNA OR RNA)

In young patients, EBV serologic testing may reveal false-positive results owing to retained maternal antibodies from the placenta, or from excessive blood product infusions, or from asymptomatic seroconversion. In patients treated with immunosuppressants, a false-negative serologic response to EBV antigens may occur. In such situations, demonstrating EBV nucleic acid in tissue sections or in blood may be necessary for diagnostic purposes.[8] Methods include Southern blotting, in situ hybridization, and detection of EBV DNA by PCR. Besides their utility as diagnostic aids, these tests also are useful for epidemiologic and pathogenetic purposes relevant to EBV infections.

Southern blot hybridization has been very useful in demonstrating the association of EBV with disease.[14,519] Although it is not as sensitive as PCR, Southern blotting has the advantage of providing additional information regarding the monoclonality of EBV-associated lesions, detection of lytic EBV processes, and recognition of viral integration into the genome.[166,175,179,407]

Because of their ubiquity in EBV-infected cells (up to 10^7 copies per cell), detection of EBERs in tissues by in situ RNA

TABLE 172–3 Correlation of Clinical Status and Characteristic Serologic Responses to Epstein-Barr Virus (EBV) Infection*

Clinical Status	Heterophile Antibodies (Quantiatitive Test)	Response EBV-Specific Antibodies				
		IgM-VCA	IgG-VCA	EA-D	EA-R	EBNA
Susceptible	—	—	—	—	—	—
Infectious mononucleosis						
Acute primary infection	++[†]	++/+++	+/+++	+/+++	–[‡]	–/+
Recent primary infection	+/–	–/+	++++	+++	–[‡]	—
Remote infection	—	—	++	–[§]	–/+	+/++
Reactivation immunosuppressed/immunocompromised	—	—	+++/++++	–[§]	++/+++	–/+++
Burkitt lymphoma	—	—	+++/++++	–[§]	++/+++	+/++
Nasopharyngeal carcinoma	—	—	+++/++++	++/+++	–[¶]	++/+++

**Antibody response scale is none or negative (–), low (+), average (++), above average (+++), and very high (++++). A scale is used for application to various antibody tests and techniques. This table is modified from numerous studies. Individual responses outside the characteristic range may occur.*

[†]Children younger than 4 years of age may have weak or undetectable heterophile antibody responses as determined by qualitative rapid slide tests.

[‡]In some young children and in adults with asymptomatic seroconversion, the anti-EA response may be mainly to the EA-R component.

[§]A minority of individuals will have the anti-EA response mainly to the EA-D component.

[¶]A minority of individuals will have the anti-EA response mainly to the EA-R component.

hybridization has become a standard method for detecting and localizing latent EBV in tissues.[32,176,324] Probes for detection of EBERs ("riboprobes") are labeled conveniently by immunoperoxidase methods. Detection of EBV DNA by in situ hybridization also can be performed,[48] although DNA detection is less sensitive because of the lower copy numbers per cell in comparison with EBERs.

Amplification of EBV DNA by PCR has emerged as an important test for establishing the diagnosis and treatment of several EBV-associated diseases. In adult patients with AIDS and primary CNS lymphomas, detection of EBV DNA in CSF has high sensitivity and specificity in most studies.[18,75,91,324] PCR for EBV in CSF, possibly in combination with certain imaging studies,[18] may be sufficient for establishing the diagnosis of CNS lymphoma and could preclude performing brain biopsy, which can be associated with complications. On the other hand, lumbar puncture in patients with cerebral lesions also must be considered carefully because of the risk for cerebral herniation.

In stem cell and solid organ transplant recipients, quantitative PCR for EBV DNA in peripheral blood serves as a useful tool for the diagnosis of PTLD and as a marker during follow-up.[165,300,512,539] Levels of EBV DNA in blood typically are low in healthy control subjects, high in those with PTLD or other EBV-associated diseases, and intermediate in asymptomatic organ transplant patients. A high EBV load in the blood has a clear relationship with development of PTLD,[164] and with successful management, EBV DNA concentrations typically diminish to baseline levels.[164,222,427] Although negative predictive values for these tests are higher than 90 percent, positive predictive values are substantially lower. Healthy subjects with IM, asymptomatic seroconvertors, and transplant recipients with PTLD may have comparably high levels of EBV detected in the blood, thus making it difficult to separate out true PTLD from increases unassociated with PTLD. Detection of EBV within cellular specimens such as whole blood or PBMCs appears to be more reproducible and accurate than are tests measuring cell-free EBV in plasma or serum. Further studies clearly are necessary to establish quantitative laboratory standards for diagnosing and monitoring EBV-associated PTLD. Histopathology of a suspicious tumor site is much more informative and less controversial regarding diagnostic utility.

Quantitative PCR also may be useful for diagnosing and monitoring other EBV-associated malignancies, including NPC,[295] HD,[301] and NHL.[468] Larger studies are necessary to define better the role of EBV viral load testing in these diseases.

EPSTEIN-BARR VIRUS PROTEINS

Immunofluorescent techniques may be used to detect the presence of cells carrying EBV proteins in touch imprints of biopsy material or in frozen sections of cryopreserved tissue.[166,324,398,542] Most commonly, tissues are examined for VCA, EBNA-1, and LMP-1 proteins with the use of mouse monoclonal antibodies.

VIRUS ISOLATION

EBV from blood or oropharyngeal specimens (rarely, other specimens) can be isolated in tissue culture with a transformation assay.[246] For oropharyngeal specimens, the test requires incubation with fresh cord blood mononuclear cells for as long as 6 weeks. Transforming virus can be identified in peripheral blood by incubating mononuclear cells in the presence of cyclosporine (for inhibition of cytotoxic T cells, which can suppress the growth of EBV-positive B cells). An end-point dilution assay may be used to quantitate the amount of EBV within blood specimens.[425] Virus isolation tests are used primarily for research purposes and are not widely available.

ELECTRON MICROSCOPY

EBV virions also may be detected by electron microscopy, which has been used in patients with oral hairy leukoplakia,[236] NPC,[21] and other EBV-associated lesions. The ultrastructural features of EBV virions, however, are indistinguishable from those of other herpesviruses, and confirmatory tests usually are necessary. Electron microscopy generally is available only at larger research centers and, therefore, is impractical for routine diagnostic testing.

IMAGING STUDIES

Imaging studies seldom are necessary in typical patients with IM. In one study of patients hospitalized with IM, the most common findings on chest radiography were splenomegaly (47%), hilar adenopathy (13%), and interstitial infiltrates (5%).[289] Imaging studies are used more commonly for evaluation of EBV-associated neurologic disease, especially in immunocompromised patients. Children with EBV-associated encephalitis usually have normal neuroimaging results.[104] However, some patients may display diffuse swelling or patchy low-density lesions by CT, and magnetic resonance imaging (MRI) may reveal increased signal on T2-weighted images, as well as atrophy.[104,449] Contrast CT scans of primary cerebral lymphoma in patients with AIDS typically reveal large (2-6 mm) lesions occurring anywhere in the cerebrum with diffuse and homogeneous enhancement[486]; however, some overlap exists with findings observed in other diseases such as cerebral toxoplasmosis. Thallium 201 single-photon emission CT (SPECT), in conjunction with MRI and PCR testing of CSF for EBV DNA, may be useful in distinguishing CNS lymphomas from toxoplasmosis in adult patients with AIDS.[18]

DIFFERENTIAL DIAGNOSIS

Infectious Mononucleosis. EBV causes an estimated 80 to 95 percent of IM syndromes.[481] Numerous agents have been associated with heterophile-negative IM. CMV is responsible for most cases of heterophile-negative IM with typical clinical and hematologic findings.[220] Certain features of CMV mononucleosis may help distinguish it from EBV (e.g., less common sore throat and lymphadenopathy, less intense atypical lymphocytosis, and prominent splenomegaly), but these characteristics have considerable overlap with EBV-associated IM. Serologic testing consistent with acute CMV infection in the absence of characteristic EBV serologic findings confirms the diagnosis. The remainder of cases may be attributable to infection with *Toxoplasma gondii*, adenoviruses, rubella virus, and hepatitis A virus. Acute HIV syndrome also may mimic IM. However, in children with non-EBV IM, the etiology frequently remains elusive. In heterophile-negative children with mononucleosis and both the clinical and hematologic characteristics of the syndrome, the most likely known agents are EBV and CMV.

Other EBV-Associated Disorders. EBV-associated HLH may be confused with IM, systemic connective tissue disorders, septicemia, and certain hematologic malignancies.[231] EBV–associated CNS lymphomas in adults with AIDS are difficult to distinguish from cerebral toxoplasmosis on the basis of clinical findings and imaging studies. Historically, a negative *Toxoplasma gondii* serum antibody test eliminates toxoplasmosis, whereas a

positive test may necessitate a trial of antitoxoplasmic therapy. Response within a week suggests toxoplasmosis, but a nonresponse indicates the need for stereotactic brain biopsy to establish a diagnosis. Identification of EBV DNA in CSF by PCR may help confirm the diagnosis of lymphoma. Because PTLDs may affect the graft, a high EBV viral load in conjunction with graft abnormalities (e.g., elevated liver enzyme values in a liver transplant recipient) may be confused with graft rejection; histologic evaluation of biopsy tissue from the graft should differentiate PTLD from acute graft rejection.

TREATMENT: NONMALIGNANT EPSTEIN-BARR VIRUS–ASSOCIATED DISEASES

INFECTIOUS MONONUCLEOSIS

Supportive Care. Support, including rest, fluids, and antipyretics, is all that is required for uncomplicated IM. Although clinical trials and case reports dating back to the 1950s generally have demonstrated a beneficial effect of corticosteroids in reducing the symptoms associated with IM (e.g., fever, pharyngitis, lymphadenitis), current guidelines suggest that physicians use corticosteroids cautiously, if at all.[341] This decision is based partly on the unknown long-term consequences of such therapy, including a potential deleterious effect on the immune system. Nonetheless, small series and anecdotal reports suggest that some severely symptomatic patients (e.g., those with high fever, severe pharyngitis) may benefit from short-term courses of corticosteroids.[13,35,46] Though little controversy exists regarding the use of corticosteroids for patients with complicated illnesses (e.g., with stridor from massively enlarged tonsils or paratracheal adenopathy, or for those with hematologic or neurologic complications) (see "Complications of Infectious Mononucleosis" earlier), personal experience and clinical judgment largely dictate the use of corticosteroids in practice.[103,497] For example, in a recent report of patients with IM evaluated as outpatients and inpatients, 45 percent received systemic corticosteroids, with only a small proportion (less than 10%) of these patients having airway concerns.[497]

Antiviral Treatment. Numerous antiviral agents inhibit replication of EBV in vitro. Such agents include acyclovir, famciclovir, penciclovir, ganciclovir, and other nucleoside analogues.[26,81,94,158,317] None of these agents is capable of eliminating latent EBV present as episomes in infected cells. Five randomized, placebo-controlled trials of acyclovir (with or without corticosteroids) in adolescents and adults with IM demonstrated no beneficial effect in resolving the clinical signs and symptoms associated with illness, as well as no reduced rate of complications.[11,12,378,505,506] Moreover, a meta-analysis substantiated these findings.[502] Acyclovir did significantly reduce the rate of EBV shedding in the oropharynx, albeit temporarily.[502]

EPSTEIN-BARR VIRUS–ASSOCIATED MALIGNANCIES

Providing details regarding treatment of EBV-associated lymphoproliferative diseases, including lymphomas, is beyond the scope of this chapter. Pediatric oncologists must be involved in the care of children with these malignancies. A brief overview of treatment of the principal diseases is provided.

Post-transplant Lymphoproliferative Disease

The most effective therapy for PTLD in transplant recipients is reduction (or complete elimination)[228] of immunosuppression to allow more effective control of rampant EBV infection by the cell-mediated immune system. This measure halts progression of PTLD in approximately two thirds of pediatric solid organ transplant recipients developing polymorphic PTLD in the first year after undergoing transplantation.[165] Decisions concerning reduction of immunosuppression must be weighed against the risk of graft rejection, and careful follow-up with frequent EBV viral load measurements must be assured. If no improvement occurs after a trial of reduced immune suppression, then other therapeutic modalities must be considered singly or in combination.

Although antiviral agents (acyclovir, ganciclovir, valganciclovir) are used frequently for PTLD, based largely on an anecdotal report published in 1982,[188] no clinical trials have confirmed their efficacy. Because these agents treat lytic EBV infections, yet PTLD primarily involves latent virus, the consensus has been that these agents should have minimal impact on PTLD. However, this view has been challenged by recent reports in experimental animals, suggesting that the small proportion of lytically infected cells within these tumors may contribute to the growth of EBV-associated malignancies by enhancing angiogenesis and inducing paracrine B cell growth factors.[218,219]

There is now considerable experience with the use of rituximab, a chimeric monoclonal antibody directed at CD20, against PTLD.[155] Although no randomized controlled clinical trials have been conducted to date in children, data from cohort studies and case reports indicate that rituximab is safe and effective.[155] Most adverse events are mild or moderate and usually are related to the first infusion of the antibody. Despite a profound reduction of circulating B cells in peripheral blood, hypogammaglobulinemia is an uncommon occurrence with rituximab treatment (although more likely in young children), and patients are not at higher risk for development of infectious complications. Most centers now use rituximab as a primary therapeutic agent for PTLD, if reduction of immune suppression is unsuccessful.

IFN-α has induced complete remissions in some studies,[95,311] but relapses and graft rejections have occurred. Conventional NHL chemotherapy (CHOP regimen) consisting of cyclophosphamide, daunorubicin, vincristine, and prednisone may be associated with increased toxicity and mortality associated with infection when standard doses are used,[359,484] thus leading to investigation of low-dose CHOP regimens consisting of cyclophosphamide and prednisone.[30,172]

Experimental therapy using EBV-specific cytotoxic T-lymphocytes (CTLs) has been successful for PTLD in human stem cell transplant recipients,[430] but it is more complicated for solid organ transplant recipients with PTLD. In contrast to stem cell transplant recipients, whose EBV-associated PTLD tumors are derived from the donor, tumors in solid organ transplant recipients are invariably derived from the recipient. Therefore, EBV-specific CTLs must be prepared by ex vivo EBV transformation of either the recipient's B cells[269] or at least partially HLA-matched banked B cells.[190] Surgery and radiation therapy generally are reserved for local complications of tumors, including compression of an airway or gastrointestinal bleeding,[165] but they have been used more widely at some institutions.[82] A multifaceted approach, such as that used by Comoli and colleagues,[82] ultimately may yield the highest response rates against the most aggressive forms of PTLD.

B-Cell Lymphoma

Endemic (African) BLs, which are highly associated with EBV,[284] are treated primarily with cyclophosphamide-based chemotherapy.[285,549] Favorable responses were observed by Labrecque and coworkers[285] in patients with tumors expressing lytic genes. Treatment of sporadic BL also is based primarily on multiagent chemotherapy that includes cyclophosphamide.[314] Case reports have indicated positive responses to rituximab, and Children's

Oncology Group protocols now include rituximab as first-line treatment for BL.[155]

B-cell lymphomas in patients with AIDS are extremely difficult to treat, primarily because of the underlying severe host immune deficiency. Early regimens using standard chemotherapeutic regimens were largely failures, with survival rates lower than those of untreated patients; most patients died of opportunistic infections, drug toxicity, and resistant tumors. Alternative therapeutic strategies have improved outcomes for this malignancy, yet mortality rates remain substantial.[320] These strategies have included lower doses of standard drugs,[259] continuous infusion of chemotherapy,[465] hematopoietic growth factors,[291] novel immunotherapies (e.g., IL-2),[37] and adoptive immunotherapy,[523] as well as improved management of opportunistic infections and HIV. Recent in vitro data indicate the effectiveness of anti-NFκB treatments, including bortezomib[552] and certain statin drugs,[263] and the hope is that human studies will follow. An exciting new area of therapy is termed *lytic induction* and involves treatment of EBV-positive lymphoma cells with certain agents that induce a transition from latent to lytic infection, thus rendering them susceptible to antiviral therapy (e.g., ganciclovir).[92]

AIDS-related primary CNS lymphomas are extremely difficult to treat. Historically, palliative whole brain radiation therapy in conjunction with corticosteroids was used for treatment, but with disappointing survival rates of only a few months. Combined-modality therapy with whole brain radiation and chemotherapy has been investigated but generally also produces poor outcomes.[260] However, most studies were conducted prior to wide availability of HAART. A minority of healthier patients with AIDS may respond better to combined-modality regimens.[260] For patients not already on HAART, initiation of an appropriate regimen is recommended because case reports have described improvement.

Hodgkin Disease

Historically, children with HD were treated with high-dose radiation. However, high rates of skeletal growth inhibition and secondary malignancies were noted in survivors, so new trials using lower involved-field radiation in conjunction with multiagent chemotherapy were conducted. These trials form the basis for combination treatment regimens used currently in growing children.[226,402] Although the presence of EBV in tumor (more likely in mixed-cellularity types) currently plays no role in pretherapy staging, some studies suggest improved outcomes for patients with EBV-positive tumors.[351,357]

Nasopharyngeal Carcinoma

NPCs are sensitive to radiotherapy, as well as chemotherapy. Surgery usually is not an option because of the location of the primary tumor, although it may be necessary for persistent or recurrent lymph node disease. For early localized disease, radiotherapy generally is indicated, whereas combined-modality treatment (radiation and chemotherapy) may be considered for more advanced disease.[3] Children failing treatment have a high rate of distant metastases, principally to bone.[23,232]

NONMALIGNANT EPSTEIN-BARR VIRUS–ASSOCIATED DISEASES

XLP. In an effort to prevent fulminant IM, intravenous immunoglobulin is recommended for patients with XLP who display hypogammaglobulinemia, although such therapy is not fully effective.[150] Traditional therapy with antivirals and IFN-α has been very disappointing for fulminant IM, but recently reported success with rituximab (in combination with corticosteroids and other agents) has provided some enthusiasm.[348] Males with XLP also can develop lymphomas, which may respond to traditional chemotherapy regimens. Presently, the only definitive cure for XLP is allogeneic hematopoietic stem cell transplantation. Fifteen such transplants were reported between 1986 and 2005, with 10 survivors.[290] Younger age appears to be associated with improved survival in this limited population. Administration of rituximab may be considered as a bridge between the diagnosis of XLP and a transplantation.[290] Now that the gene defect in XLP has been identified, consideration may be given to a gene therapeutic approach.

HLH. Early studies using non–etoposide-containing regimens were largely unsuccessful in significantly reducing the high mortality rate associated with HLH.[19] Recently, chemotherapeutic regimens including etoposide (toxic to macrophages) have reduced the mortality rate significantly. Results of a standardized protocol (HLH-94) developed by the Histiocyte Society were published recently.[204] Agents in this protocol included etoposide and corticosteroids for initial therapy, followed by etoposide, corticosteroids, and cyclosporine A (continuation therapy), leading to bone marrow transplantation. A similar standardized protocol (HLH-2004), but including cyclosporine A at initiation, currently is being evaluated.[242] Little data are available on salvage therapy for those children unresponsive to the HLH-94 protocol, but Janka[242] has summarized anecdotal successes with standard NHL or HD treatment protocols, some monoclonal antibodies (e.g., anti-CD25 and anti-CD52), and one child treated with fludarabine (an antiviral agent) who survived until bone marrow transplantation could be performed.

CAEBV. Treatment modalities for chronic active EBV infection have varied widely because the rarity of this disease precludes performing proper clinical trials. Resolution of symptoms has been reported in case reports using antiviral drugs (e.g., acyclovir, ganciclovir),[234] etoposide,[375] immunomodulatory agents (e.g., corticosteroids, intravenous immune globulin, cytokines),[146,265] adoptive transfer of cytotoxic T cells,[442] or combinations thereof. For the most severe forms of disease, stem cell transplantation may be considered.[374]

HIV-Associated Diseases. Oral hairy leukoplakia generally does not require therapy because patients typically are asymptomatic and the lesions have no malignant potential. However, therapy may be required for troubling symptoms or cosmetic or psychological reasons. Small lesions may be treated topically,[352] but large lesions require systemic oral antiherpesviral agents.[381] Treatment strategies for LIP are not defined well. Supportive care, including supplemental oxygen, may be necessary. Suppression of HIV with effective antiretroviral agents also is logical to improve immune function and minimize the potential direct effect of HIV. Although no controlled studies have documented the value of antiretrovirals, benefit is suggested by the declining prevalence of LIP in children during the HAART era.[161] Corticosteroids also may be of benefit.[17,343] No controlled studies have evaluated antiherpesviral drugs (e.g., acyclovir) for LIP.

PROGNOSIS

The prognosis for healthy persons with IM is extremely good, with mortality considered a rare occurrence. In a series of 30 fatal cases of IM from the 1950s, Lukes and Cox[323] found splenic rupture, Guillain-Barré syndrome, hemorrhage, and secondary infection to be the most common causes of death. In Penman's[392] later study of 20 documented deaths, predominantly of young adults, the causes of death (in order of frequency) were neurologic complications, splenic rupture, secondary infection, hepatic

failure, and myocarditis. EBV-induced aplastic anemia, a rare but dreaded hematologic complication, now is thought to have a better prognosis for eventual recovery if the patient can be supported successfully through the acute stages.[31,293]

The prognosis for patients with EBV-associated lymphoproliferative diseases is variable and depends on the immunologic status of the patient and the type of lymphoproliferation. A registry of 161 males with XLP indicated a mortality rate of 70 percent by the age of 10 years, and 100 percent by the age of 40.[168] HLH has a greater than 90 percent fatality rate without treatment; most deaths are caused by hemorrhage, overwhelming infection, disseminated intravascular coagulation, or multiorgan failure.[71,241] Etoposide-based treatment has improved outcomes considerably.[242] The largest case series of CAEBV (from Japan), which includes 21 children age younger than 18 years old, reports a mortality rate of approximately 30 percent despite various forms of therapy.[272] Historically, PTLD that develops after solid organ transplantation has carried a relatively high mortality rate (50-80%), with higher mortality rates in bone marrow transplant recipients.[128] Presently, the mortality rate is probably much lower, but further time is necessary to assess the impact of newer therapeutic modalities. The prognosis for healthy children with NHL generally is good, with 5-year relative survival rates of 80 percent.[419] However, the prognosis for patients with AIDS and NHL is poor. In adults with AIDS, median survival is 2 to 4 months for CNS lymphomas (virtually all of which are EBV-associated), with only slightly longer survival noted in patients on HAART.[260] Childhood HD generally has a very good prognosis, with 5-year survival rates of approximately 95 percent.[419] Using combined modality therapy, children with NPC have long-term survival rates in the range of 70 to 90 percent.[24]

PREVENTION

EBV is shed regularly in saliva, and, therefore, infection control policies are difficult to develop. In addition, mechanisms for EBV transmission are not well understood, and EBV outbreaks occur rarely, if at all. For these reasons, no more than standard infection control precautions are recommended for patients hospitalized with EBV infections, including IM.[387] Although transfusion-associated EBV infection occurs rarely, persons with recent EBV infection of mononucleosis-like illnesses are urged to refrain from donating blood or organs.[387]

VACCINE

Efforts continue in the development of an EBV vaccine. Because of concern over the administration of a vaccine with unknown long-term consequences, including malignancy, most efforts have been focused on development of subunit EBV vaccines. A subunit vaccine containing the EBV gp350 protein has proved effective in preventing EBV infection in an experimental animal model.[117] A vaccinia virus-based gp350 vaccine has been evaluated in a small number of Chinese patients, eliciting good antibody responses and providing some protection from EBV infection;[174] however, these findings were not corroborated, and this vaccine was not developed further. A recombinant gp350 vaccine with adjuvant has been evaluated in Belgium, with phase I and phase II data published recently.[353,464a] The vaccine was immunogenic, safe, and well tolerated. Although vaccine recipients were not protected from EBV infection, symptoms of IM were reduced compared with controls.[417,464a] An effective vaccine could be considered for persons at high risk for developing EBV-associated lymphoproliferative disease and cancer, such as patients with AIDS, those with XLP, and organ transplant recipients. Before such a vaccine is approved, further research must be conducted to address issues such as protection against exogenous superinfection from wild type EBV strains.[514] Therapeutic vaccines, aimed primarily at latent antigens expressed in EBV-associated malignancies such as NPC, also are under investigation.[111,315]

PREVENTION OF POST-TRANSPLANT LYMPHOPROLIFERATIVE DISEASE

Strategies for preventing PTLD reflect many of those used for treatment of established PTLD (see earlier section on Treatment of PTLD). Children at high risk for developing PTLD (e.g., EBV seronegative children and infants age younger than 12 months) typically are given prophylactic anti-CMV IG and an antiviral following transplantation. A recent retrospective analysis of a large renal transplant database covering Europe and North America (96% adults) has suggested a beneficial effect of anti-CMV IG (e.g., Cytogam) in the prevention of B-cell lymphomas.[376]

After undergoing transplantation, these children should have frequent EBV viral load testing. If EBV viral loads persist above a significant cutoff value established by the laboratory, preemptive measures should be considered. Although real-time PCR tests are quite sensitive in predicting development of PTLD, they are not highly specific and lack standardization. Cutoff values for "significant" concentrations of EBV DNA vary from laboratory to laboratory. Once a decision is made that preemptive therapy is warranted, reduction or elimination of immunosuppressive medication(s) should be performed.

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CHAPTER 173

Human Herpesviruses 6, 7, and 8

Charles Grose

Before 1986, five human herpesviruses (HHVs) were known. They were herpes simplex virus (HSV) type 1 (oral) and type 2 (genital), cytomegalovirus (CMV), varicella-zoster virus (VZV), and Epstein-Barr virus (EBV). The herpesviruses are important pathogens causing a variety of childhood diseases that are described in other chapters of this textbook. An inherent characteristic of herpesviruses is their ability to form a latent infection, in which the viral genome continues to reside within the host. When the virus reactivates, it often causes further symptoms and signs. Thus, herpesviruses cause a

spectrum of illnesses during the lifetime of the infected human host.

During the last decades of the 20th century, three novel herpesviruses were discovered. Two of them have been designated HHV-6 and HHV-7, whereas the newest member of the family has been called either Kaposi sarcoma–associated herpesvirus or HHV-8. These three viruses and the diseases they cause are described in this chapter.

HUMAN HERPESVIRUSES 6 AND 7

One unexpected consequence of the epidemic of acquired immunodeficiency syndrome (AIDS) was the discovery of a new human DNA virus. The virus was isolated first from the white blood cells of six patients with lymphoproliferative disorders, two of whom had AIDS.[27] Further electron-microscopic characterization of the virus demonstrated properties of a herpesvirus, including (1) an icosahedral capsid composed of 162 capsomers covered by a lipid membrane and (2) an enveloped particle with a diameter of 200 nm (Fig. 173–1). Because the virus was isolated originally from B lymphocytes, the agent was designated human B-lymphotropic virus. However, this apparent tropism for B lymphocytes was not confirmed by other investigators, who found that the virus preferentially infected $CD4^+$ T lymphocytes and not B lymphocytes.[20,30] Thus, the initial designation of human B-lymphotropic virus was changed to HHV-6.

The DNA sequence and the deduced amino acid sequence of the HHV-6 genome have been published.[6] Calculation of the percentage of amino acid identity shared by HHV-6 proteins with those in other herpesviruses revealed that HHV-6 proteins most closely resembled those of human CMV. The strains of HHV-6 have been divided into group A and group B. The HHV-6 isolates related to prototype strain U1102 have been called group A, whereas those related to strain Z29 have been designated group B. In 1990, while searching for additional strains of HHV-6, Frenkel and coworkers[14] isolated a new T-lymphotropic herpesvirus that was designated HHV-7. HHV-7 is related closely at a genetic level to HHV-6 and to a lesser degree to CMV.[6] All three of these agents are subclassified as beta-herpesviruses.

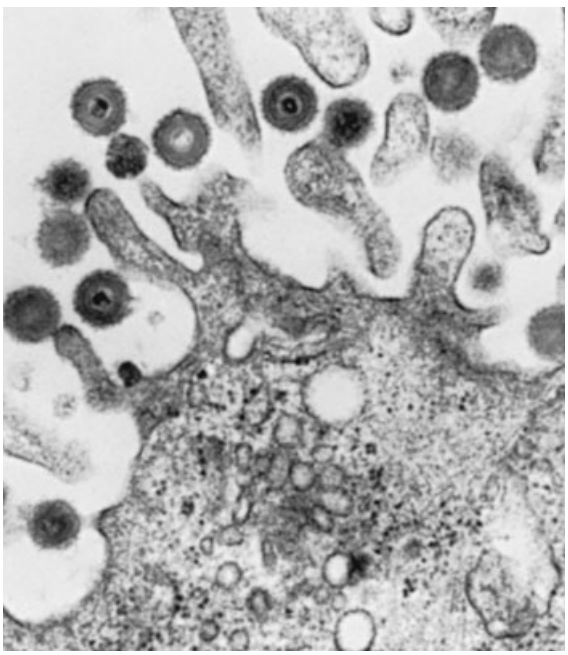

Figure 173–1 Electron micrograph of cultured mononuclear cells infected with HHV-6. Several enveloped viral particles are visible in the extracellular area. *(Courtesy of Dr. Y. Asano.)*

Most persons contract their primary HHV-6 infection before reaching the age of 5 years.[3] In the United States, primary HHV-6 infection occurred in 40 percent of children by the time they reached 12 months of age and in 77 percent by the age of 24 months.[41] In these young children, group B strains of HHV-6 appear to be a major cause of the disease roseola (exanthem subitum). Roseola is discussed at greater length in Chapter 65. Children also may contract a primary HHV-6 infection without manifesting a rash. After acute infection, HHV-6 forms a latent infection, probably in T lymphocytes. Endogenous virus reactivates in adults with immunosuppressive disorders, such as AIDS, although reactivation has not been associated with a specific disease entity. Seronegative recipients of organ transplants may acquire a primary HHV-6 infection from latent virus in the donor organ. In a small number of infants, acute HHV-7 infection appears to cause a roseola illness much like that of acute HHV-6 infection.[17]

DISEASES CAUSED BY HHV-6 AND HHV-7

Studies in Japan first delineated the nature of the illness associated with acute HHV-6 infection in young children.[37] HHV-6 was cultured from the peripheral blood leukocytes of four infants with roseola (exanthem subitum); each subject was 6 months old and had an acute febrile illness followed by a concurrent fall in temperature and the onset of a rash. The blood samples were collected during the febrile stage of the disease. In a subsequent paper by the same group, two infants (6 and 7 months of age) were described who developed HHV-6 infection *without* a rash.[29] Both infants had been seen by physicians because of a 2- to 3-day history of high temperature (38.5° C to 39.5° C). The only abnormal clinical finding was congestion of the throat. In both cases, the temperature rapidly returned to normal around day 3 of illness, but no rash was ever observed. Cultures of peripheral blood cells from both infants were positive for HHV-6.[29] Further seroprevalence studies performed in Japan showed that most Japanese children (86%) contracted HHV-6 infection by the time they were 24 months of age.[38] Thereafter, the increase in seropositivity among the childhood populations was small. These statistics suggest that HHV-6 infection goes unrecognized in most children.

Primary HHV-6 infection has been implicated in a few cases of severe hepatitis. The first case was that of a 21-year-old patient with cystic fibrosis who received a liver transplant.[34] The recipient lacked antibodies to HHV-6, and the organ donor was seropositive. Two weeks after undergoing transplantation, the recipient developed fever and grand mal seizures; her hepatic function deteriorated. HHV-6 was cultured from her peripheral blood cells, and herpesviral particles were seen by electron-microscopic examination of a liver biopsy specimen. The patient also developed antibodies to HHV-6 by day 16 after transplantation. The apparent source of infection was the donor liver, from which HHV-6 presumably was reactivated after transplantation. She gradually recovered her hepatic function during the course of several weeks.

A fatal case of fulminant HHV-6 hepatitis has been reported in a 3-month-old boy.[4] The infant was admitted to a hospital because of fever, jaundice, convulsions, and loss of consciousness. His serum bilirubin concentration, liver transaminases, and blood ammonia level were elevated markedly. Within 7 days, he became comatose and died. As part of the diagnostic evaluation, HHV-6 was cultured from his mononuclear cells. Furthermore, HHV-6 DNA was detected in biopsy samples of liver and brain, which were obtained immediately after death. A serum sample drawn

TABLE 173–1 Selected Features in Roseola Associated with Infection in Children

Clinical Findings	%
Temperature above 37.5° C	98
Rash	98
Diarrhea	68
Nagayama spots	65
Cervical adenopathy	31
Edematous eyelids	30
Bulging fontanelle	26
Prodromal symptoms	14
Convulsions	8
Cough	0

Data modified from Asano, Y., and Grose, C.: Human herpesvirus type 6 infections. In *Glaser, R., and Jones, J. (eds.): Herpesvirus Infections. New York, Marcel Dekker, 1994, pp. 227-244.*

before death showed reactivity to HHV-6. On the other hand, the child had no serologic evidence of acute infection with hepatitis A, B, or C virus or with any other HHV.

In a large retrospective review of roseola in association with acute HHV-6 infection, the signs and symptoms were tabulated.[3] The study population included 94 boys and 82 girls, who ranged in age from 3 weeks to 18 months (Table 173–1). As would be predicted, the two most common clinical findings were fever and rash. The temperature often rose to 39° C, and fever persisted for 2 to 4 days. The rash usually appeared when the fever lessened; the rash was papular in 54 percent, macular in 40 percent, and maculopapular in the remainder of the children. The exanthem typically persisted for 3 to 4 days and was not followed by desquamation. Diarrhea was a surprisingly frequent occurrence, but it was not severe. An enanthem called *Nagayama spots* in Japan consisted of papules on the mucosa of the soft palate and uvula. Of the total study group, 8 percent developed febrile seizures. More severe central nervous system (CNS) complications have been documented in children not enrolled in this study. A few children with HHV-6 encephalitis have had abnormal electroencephalographic recordings. Some children with encephalitis have had permanent neurologic sequelae. In the United States in particular, acute HHV-6 infection also appears to be associated with concurrent acute otitis media.[24]

Reactivation of previous HHV-6 infection has been demonstrated in some healthy children and adults who contracted a second herpes-type infection, such as primary EBV infection (infectious mononucleosis) or primary CMV infection.[3] A similar serologic survey was performed in 10 renal transplant recipients who initially were HHV-6 seropositive.[3] After transplantation, all 10 showed greater than fourfold rises in antibody to HHV-6. Only two of the ten developed a febrile illness, and both of them also had primary CMV infection. More recent evidence suggests that HHV-6 infection in combination with CMV infection may worsen post-transplantation pneumonitis previously thought to be associated solely with CMV infection. HHV-6 does not appear to be related causally to Kawasaki disease,[22] nor does HHV-6 infection appear to alter the course of human immunodeficiency virus (HIV) infection.[28]

Whether HHV-7 causes a distinct illness has been the subject of many medical investigations since 1990. In general, HHV-7 antibody is acquired by mid-childhood, but there appears to be no corresponding sentinel illness. However, in two infants with roseola, both isolation of HHV-7 and seroconversion to HHV-7 were documented; in addition, another five children with roseola were found to have undergone seroconversion to HHV-7.[32] HHV-7 also has been isolated from one infant with an acute febrile illness.[25] Therefore, in a small number of young children, acute HHV-7 infection may be associated with fever and sometimes a roseola-like rash.

Finally, congenital infections also can occur in pregnant women already infected with HHV-6. In a survey of 5638 cord blood samples, HHV-6 DNA was detected in 57 samples (1%), but the congenital infections did not cause an identifiable disease in the newborn.[16] In contrast to HHV-6, HHV-7 DNA was not detected in any cord blood sample.

NEUROLOGIC COMPLICATIONS

In the preceding paragraphs, CNS symptoms were mentioned briefly. In a large series of clinical studies performed in Japan, seizures were a common feature of acute HHV-6 infection.[3,40] In one series of 105 young children with acute febrile convulsions, 21 had evidence of primary HHV-6 infection, as assayed by either isolation of virus from blood or seroconversion of HHV-6 antibodies. When the age of the patient was assessed, HHV-6 infection was found in 13 of 23 seizure patients younger than 1 year; thus, the seizure group with HHV-6 infection was significantly younger than the seizure group without HHV-6 infection. In addition, the frequency of clustering seizures, long-lasting seizures, partial seizures, and postictal paralysis in children having their first febrile convulsion episode was significantly higher in those with primary HHV-6 infection than in those without HHV-6 infection. One child with fatal acute necrotizing encephalitis and concurrent HHV-6 infection also has been described.[21]

In studies from Japan, assays for HHV-6 and HHV-7 DNA were performed on cerebrospinal fluid samples from 43 children with CNS symptoms.[39] All children had symptoms compatible with aseptic meningitis. HHV-6 DNA was detected in the peripheral blood cells of 15 and in the cerebrospinal fluid of 7 children; all were HHV-6 variant B. HHV-7 DNA was detected in the peripheral blood cells of 28 and in the cerebrospinal fluid of 6 patients. Thus, the clinical study demonstrated that both HHV-6 and HHV-7 can invade the CNS or, alternatively, frequently reactivate within the CNS during childhood neurologic disease. The results from Japan have been confirmed in a similar large analysis of young children with seizures in Britain and Ireland.[36] Among 156 children hospitalized with febrile seizures, 26 (17%) were associated with acute HHV-6 or HHV-7 infection. The median age for hospitalization in the HHV-6 group was 53 weeks, and the median age for the HHV-7 group was 60 weeks. In one study in the United States, however, an association between acute HHV-6 infection and seizures was not found.[41]

PATHOGENESIS OF HHV-6 AND HHV-7 INFECTION

The pathogenesis of HHV-6 infection certainly includes a viremia while the child is asymptomatic; the total duration of the incubation period is not well defined but may be approximately 10 days. The prodrome, which signals the end of the incubation period, includes 2 to 4 days of fever, which precedes the onset of the rash.[3,26] During this period, virtually 100 percent of peripheral blood cell cultures are positive for HHV-6. By day 3 or 4, when the rash first appears, the viremia is abating. Between 5 and 7 days after the onset of the fever (or 2 to 4 days after appearance of exanthem), viremia persists in less than 20 percent of the children. Viremia is absent later in convalescence, when the antibody response is detectable. HHV-6 occasionally reactivates from latency throughout the lifetime of an infected human, but usually no apparent illness is present.[7]

The mothers of infants with HHV-6 infection have been studied to determine whether they are the source of the infectious agent.[16] Because most mothers are seropositive, the possibility

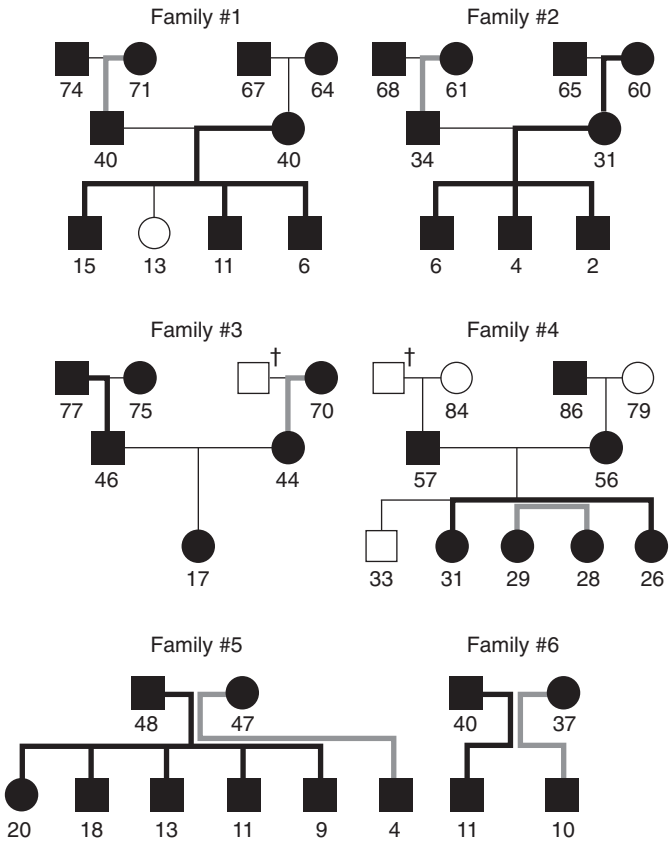

Figure 173–2 Pedigrees of six families with HHV-7 infection. All families resided in Okayama, Japan. Males and females are indicated as boxes and circles, respectively. Persons from whom HHV-7 was isolated are shaded. A person who was already deceased is marked†. Members who have similar HHV-7 DNA restriction patterns are connected with bold lines. Numbers under the boxes and circles indicate ages. *(From Takahashi, Y., Yamada, M., Nakamura, J., et al.: Transmission of human herpesvirus 7 through multigenerational families in the same household. Pediatr. Infect. Dis. J. 16:975-978, 1997.)*

exists that HHV-6 infection could reactivate in the mother, who would then transmit the infection to her child, possibly through exchange of saliva. Cultures of peripheral blood mononuclear cells and saliva specimens of 14 mothers of infected infants failed to yield conclusive results. However, in another study, HHV-6 DNA was detected in the vaginal secretions of young adult women, so perinatal HHV-6 transmission remains a possibility. Older siblings are another possible source of infection within a household.[41]

In contrast with HHV-6, HHV-7 seropositivity occurs in children but often after the first 2 years of life. HHV-7 transmission has been investigated in large multigenerational Japanese families living in the same household.[31] The results indicated that HHV-7 is transmitted gradually from older generation to younger generation (Fig. 173–2). Transmission often occurs in children before the child enters a traditional primary school at the age of 6 years. Either parent or presumably an older sibling can transmit the virus. A reasonable explanation for this pattern of transmission is repeated exchange of infectious saliva among family members living in the same household during the course of many years.

DIAGNOSIS

Infection with HHV-6 can be diagnosed by several means, including (1) measurement of antibody, (2) isolation of virus, (3) detection of viral antigen, and (4) detection of viral DNA. The first method (i.e., the traditional approach for diagnosis of virus infection) usually requires acute and convalescent serum samples for determination of a fourfold or greater rise in titer of virus-specific IgG antibody. The finding of IgM specific for HHV-6 in a single serum sample also would indicate an acute infection in a young child. The titer of IgG antibody rises during reactivation of the virus in adults, but whether IgM antibody to HHV-6 appears regularly at the same time is not known. HHV-6 antibodies usually have been measured by an indirect immunofluorescence method, although neutralization tests also have been performed.[3,4,35]

Isolation of HHV-6 was the original technique for identification of this herpesvirus.[27] The method is more difficult to perform than are those methods commonly used for most herpesviruses (e.g., HSV, CMV, VZV) but is similar to that required for isolation of another herpesvirus, EBV. HHV-6 usually is isolated in a cell substrate consisting of mononuclear cells obtained from cord blood of a newborn infant. The source of virus in the patient also is the peripheral blood mononuclear cell population.[35]

Viral genome can be detected in infected tissues by DNA hybridization techniques.[35] Viral DNA also can be detected in tissue samples by polymerase chain reaction (PCR). In one fatal case of HHV-6 infection,[4] DNA was extracted from postmortem liver and brain samples and amplified by the PCR test. On direct gel electrophoresis, HHV-6–specific DNA was detected in both the liver and the brain specimens. These studies indicate that HHV-6 infection can be diagnosed by both traditional and newer molecular techniques. Likewise, HHV-7 infection usually is diagnosed by isolation of virus or detection of viral DNA by PCR testing in samples from patients.

TREATMENT OF HHV-6 INFECTION

In most instances of HHV-6 infection in healthy children, treatment with an antiviral medication is not indicated. Recovery is complete within a few days after onset of the rash, and sequelae rarely occur. However, HHV-6 disease in immunocompromised persons may be more persistent or severe (e.g., encephalitis). Several groups have analyzed the in vitro sensitivity of HHV-6 to four antiviral drugs: acyclovir, ganciclovir, phosphonoformic acid, and cidofovir.[1,3] All four compounds have been used in treatment of other herpesvirus infections. Multiplication of HHV-6 in cell culture is inhibited readily by ganciclovir at a concentration of 2 to 10 μg/mL, easily achievable levels in humans. This effect is of interest because ganciclovir inhibits human CMV to a similar degree; as mentioned earlier, CMV and HHV-6 are related closely at a genomic level. Phosphonoformic acid (foscarnet) at a concentration of 6 μg/mL also is effective against HHV-6 infection. On the other hand, HHV-6 is affected by acyclovir only at concentrations of 50 μg/mL, considerably higher levels than those required for treatment of HSV-1, HSV-2, and VZV infections and probably toxic in humans. Likewise, cidofovir is more toxic than is ganciclovir and would not be recommended for HHV-6 treatment. At present, none of these antiviral drugs has been approved specifically for treatment of HHV-6 infections.

KAPOSI SARCOMA HERPESVIRUS (HUMAN HERPESVIRUS 8)

Yet another consequence of the HIV epidemic in the 1980s was the appearance of Kaposi sarcoma in many people with AIDS. The increase in Kaposi sarcoma was especially puzzling because the tumor occurred more frequently in people who acquired HIV by sexual transmission rather than by infusion of infected blood

products, such as factor VIII in hemophiliac patients. The question often arose whether a second infectious agent was involved in the etiology of Kaposi sarcoma. The answer to that question was provided by a report published in late 1994. The authors announced the identification of a previously unknown herpesvirus-like DNA sequence collected from patients with AIDS in New York.[9] The viral DNA was called *Kaposi sarcoma–associated herpesvirus* (KSHV). Other virologists prefer that the agent be called *human herpesvirus 8* (HHV-8).

DISEASES CAUSED BY KAPOSI SARCOMA–ASSOCIATED HERPESVIRUS

The authors of the original KSHV report were investigating patients with Kaposi sarcoma. They discovered the herpesvirus-like DNA sequences by using a combination of genetic techniques, including amplification of DNA by PCR and subsequent representational difference analysis.[9] Thereby, they were able to identify and to characterize unique DNA sequences in Kaposi sarcoma that were absent from nonmalignant tissue from the same patient. One such sequence showed close homology to regions of the genome of *Herpesvirus saimiri*, a simian herpesvirus, and to a lesser degree to EBV, the agent that causes infectious mononucleosis. A second DNA fragment was homologous to another region in the *H. saimiri* genome. In their study, the authors located one or both of these herpesvirus-like DNA sequences in 20 of 27 different samples of Kaposi sarcoma tissue. The investigators concluded that they had discovered DNA of a previously unknown herpesvirus within Kaposi sarcoma tissues.

Their results were confirmed quickly by other groups. In one study from California, investigators detected identical viral sequences in 13 of 13 biopsy specimens of Kaposi sarcoma from patients with AIDS.[2] This study also found the same sequence in the peripheral blood cells collected from 10 of the 13 patients but not in the blood samples of 20 patients with no history of Kaposi sarcoma. A third study from France found the herpesvirus-like DNA in biopsy specimens from five patients with Mediterranean-type Kaposi sarcoma; all five patients were HIV seronegative.[11] In subsequent studies, HHV-8 DNA has been detected in most Kaposi sarcomas (classic) in HIV-seronegative individuals, often men residing in the Mediterranean area.

The authors of the original KSHV report subsequently found the herpesvirus-like DNA sequences in body cavity–based lymphomas from patients with AIDS.[8] HHV-8 DNA also has been found in the B-cell lymphoproliferative disorder known as *multicentric Castleman disease*.[5] Of equal importance, KSHV sequences were not found in several other lymphomas or leukemias (e.g., small-lymphocyte lymphoma, monocytoid B-cell lymphoma, follicular lymphoma, diffuse large-cell lymphoma, Burkitt lymphoma, lymphoblastic lymphoma, anaplastic large-cell lymphoma, multiple myeloma, hairy-cell leukemia, acute lymphoblastic leukemia, or cutaneous T-cell lymphoma).

TRANSMISSION OF HHV-8 AMONG CHILDREN

One of the most perplexing aspects of HHV-8 epidemiology is the mode of transmission. That the seroprevalence is high among a population of adult men who have sex with men is well known.[23] Yet, the question remains whether larger segments of the population are infected on a worldwide basis and, in particular, whether children are infected. To answer this question, several HHV-8 seroepidemiology studies were surveyed. Two studies provide partial answers to the question of childhood infection.

The first study was performed in a village in French Guiana, South America, among 1337 individuals of African origin.[24] They ranged in age from 2 to 91 years. The serologic data indicated that HHV-8 seropositivity was strongly age dependent. Among 14 children aged 2 to 14 years, 3 of 146 were positive; aged 5 to 9 years, 14 of 278 were positive; aged 10 to 14 years, 31 of 232 were positive; aged 15 to 19 years, 24 of 149 were positive; aged 20 to 29 years, 38 of 236 were positive; aged 30 to 39 years, 16 of 120 were positive; aged 40 to 49 years, 19 of 70 were positive; and older than 50 years, 32 of 106 were positive. These seroprevalence data clearly show a gradual acquisition of infection during early childhood, with a stepwise increment from approximately 5 to 12 percent at age 10 years.

HIV infection did not play a role in the likelihood of transmission because HIV infection is not a common occurrence in this village. Instead, extensive analyses of intrafamilial relationships demonstrated a highly significant familial correlation in HHV-8 seropositivity between mother and child (especially when children were younger than 10 years) and between siblings. The correlation was highest when the siblings had an age difference of less than 5 years. Of interest, a similarity in transmission appears to exist between HHV-8 and HHV-7, when HHV-7 was studied in multigenerational Japanese households (see Fig. 173–2).

A second large HHV-8 seroepidemiology analysis was performed in Israel.[10] The incidence of classic Kaposi sarcoma in Israel is among the highest in the developed world. Because of this statistic, the investigators undertook a study to ascertain the HHV-8 seroprevalence in Israel and also to investigate HHV-8 intrafamilial transmission. The study population included 1648 healthy blood donors determined to be positive for hepatitis B antigen and 2403 family members.

The seroprevalence data showed that 9 percent of children aged 2 to 14 years and 9 percent of adolescents and young adults aged 15 to 24 years were HHV-8 seropositive. Seroprevalence in older adults ranged from 12 to 18 percent. HHV-8 positivity was more likely to occur in children when at least one of the parents was positive compared with children with neither parent positive. The most important predictor of a child's HHV-8 seropositivity status was maternal seropositivity.

These two large seroepidemiologic studies demonstrate that HHV-8 is spread by nonsexual means in children in many countries around the world. In these same countries, HHV-8 seropositivity rises throughout adolescence and adulthood, regardless of HIV infection status.[33] However, in North America and western Europe, the seropositivity rate among HIV-seronegative adults is considered to be low, approximately 3 to 5 percent. The reasons for these differences between continents are not resolved. Nonetheless, the most reasonable hypothesis for transmission is exchange of saliva within family groups. Studies have proved that the virus is present in saliva, in particular in epithelial cells contained within saliva.[12] An alternative mode of infection, described next, is transmission by blood transfusion.

HEMOPHAGOCYTIC LYMPHOHISTIOCYTOSIS

Hemophagocytic lymphohistiocytosis (HLH) is a rare and sometimes fatal disorder of immune regulation, often associated with mutations in the perforin gene. Even carriers of the mutated gene may require an acute infection to trigger HLH. HHV-8 is one of the triggers. HLH in association with HHV-8 infection has occurred in adults with severe HIV infection or with a recent kidney transplant.[13,19]

HLH has been diagnosed in two identical siblings from Iowa during the first 6 months of life.[15] The siblings were born prematurely and spent several months in intensive care. Both siblings had mutations in the perforin gene. Both siblings had HHV-8 infection. Because the mother of the two was negative for HHV-8 infection, one possible route of transmission was a blood transfusion given while they were in the nursery. In fact, in a large

study in East Africa, blood transfusions were shown to transmit HHV-8 infection.[18] Therefore, children who have had multiple blood transfusions may be at risk for acquiring HHV-8 infection.[11]

DIAGNOSIS AND TREATMENT OF HHV-8 INFECTION

Diagnosis of prior HHV-8 infection can be made by serology. Diagnosis of acute HHV-8 infection requires a scraping of cells from the mouth for testing by PCR amplification of HHV-8–specific DNA.[9,11,12] Mononuclear blood cells also may be tested by HHV-8–specific PCR. These PCR protocols are becoming more widely available.

Treatment is not necessary in most healthy hosts because the virus quickly enters latency after the primary infection. No disease has been associated with a latent infection in children, except those with defects in the perforin gene. However, acute HHV-8 infection in recent transplant recipients or HIV-seropositive children could be an indication for treatment.[13,19] Some of the same antiviral drugs described for HHV-6 treatment also inhibit HHV-8 replication (e.g., ganciclovir), but currently there are too few clinical data on which to base a standard regimen, so treatment would need to be individualized for each infected patient.

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CHAPTER 174

Varicella-Zoster Virus

Anne A. Gershon

Varicella-zoster virus (VZV) causes two diseases, varicella (chickenpox) and zoster (shingles). Varicella, the primary infection, usually occurs in childhood and is manifested as a pruritic rash accompanied by fever and other systemic signs and symptoms that usually are of a mild to moderate nature. Zoster is primarily a disease of adults, although it can occur in children. It develops in a setting of low cell-mediated immunity to VZV, such as occurs with normal aging or after disease or the use of various therapies, such as steroids, cancer chemotherapy, organ transplantation, and irradiation. During varicella, VZV establishes a latent infection in sensory nerve ganglia; zoster results when latent VZV reactivates and returns from the ganglion to infect the skin and produce a unilateral dermatomal eruption.

The origin of the nomenclature "chickenpox" is uncertain, but it may have come from the French *pois chiche* (chickpea) or from the domestic fowl (in Old English, *cicen*, and in Middle High German, *kuchen*).[39] *Herpes* is the Greek word meaning "to creep," and *zoster* comes from the Greek and Latin word meaning "girdle" or "belt."

THE ORGANISM

VZV is an alpha-herpesvirus. It has a DNA core surrounded by a nucleocapsid composed of 162 hexagonal capsomers that form an icosahedron with a diameter of approximately 100 nm. A tegument surrounds this structure, which in turn is surrounded by a lipid-containing envelope. Enveloped virions have a diameter of approximately 200 nm.[46]

The genome of VZV has been sequenced; it contains 71 open-reading frames.[47] The linear, double-stranded DNA consists of a long unique segment, U_L, and a short unique sequence, U_S, flanked by internal and terminal repeats.[48,51,173] The genome of the Oka vaccine strain also has been sequenced, and the molecular basis for attenuation of the strain is being investigated.[4,79,124]

At least six open-reading frames (genes 4, 21, 29, 62, 63, and 66) have been detected in human sensory ganglia during latent VZV infection. VZV is synthesized by an orderly cascade of gene expression consisting of immediate-early (IE), early (E), and, finally, late (L) lytic genes that encode structural proteins. The genes detectable during latent infection are IE and E genes; L genes are not expressed. One hypothesis is that cellular immunity to VZV normally can control early replication of the virus so that productive or lytic infection does not occur.[34,41-43,92,106,107,131,132,133]

During productive infection, VZV synthesizes[41-44] more than 30 polypeptides; the function of most of these polypeptides remains unknown, but some are structural and others are nonstructural and presumably have regulatory action. VZV synthesizes at least seven glycoproteins (g) termed gB, gC, gE, gH, gI, gK, and gL.[45,82,109] These glycoproteins correspond somewhat to those of herpes simplex virus (HSV), but unlike HSV, VZV has no equivalent of gD, which is the major glycoprotein of HSV. The major glycoprotein of VZV is gE. The VZV glycoproteins not only provide structures for the virion but also play roles in infectivity, such as mediating adhesion and promoting entry of VZV from an infected cell into another uninfected cell. The glycoproteins also are antigenic, as is the tegument, and immune responses are directed toward these structures.

Just one antigenic type of VZV has been identified. Three major genotypes of wild-type VZV have been described, usually referred to as European (E), Japanese (J, for strains homologous to the Oka parental vaccine strain), and Mosaic (M, for strains displaying a combination of European and Japanese genotype-like mutations).[17,128,162,182] In 2001-2002, an analysis of 130 clinical specimens from varicella cases in the United States showed that 81.5, 3, and 15.5 percent were genotypes E, J, and M, respectively.[162]

Wild-type VZV DNA can be distinguished from that of vaccine-type virus by restriction enzyme analysis of DNA from cultured virus or by polymerase chain reaction (PCR).[4,79,116,127,135,140]

VZV replicates in the nuclei of infected cells, where the DNA core and capsid are synthesized. The capsid is enveloped by a complex process, after which it traverses the cell in cytoplasmic vesicles.[70] In vitro, VZV grows rather slowly and is highly cell associated; its synthetic pathway entraps it in endosomic vesicles, where it is inactivated before it can be released from cells in infectious form. In vivo, however, infectious VZV is released from cells of the superficial epidermis and is highly contagious; VZV avoids the endosomal route in this particular tissue because of the loss of its receptor and thereby exits cells by the secretory pathway.[36]

VZV has an extremely limited host range and infects mostly primates. Hairless guinea pigs may be infected with VZV; the illness produced is extremely mild, and latent infection occurs only infrequently, but specific immune responses can be demonstrated.[141] Rodent models of latent VZV infection have been described.[95,107,137,195] An in vitro model of latency and reactivation of VZV in intestinal neurons from guinea pigs also has been developed.[35]

TRANSMISSION

VZV is spread by the airborne route[119] and requires face-to-face contact with an infected individual for transmission to occur. In varicella, VZV is transmitted mainly from the skin[35]; the respiratory tract cannot be excluded but is not likely to be the usual method of spread. VZV is isolated readily from skin lesions, but isolation of the virus from the respiratory tract is extremely difficult.[78,145,176] Spread of VZV from varicella patients before the onset of rash in closed communities has been reported, however, thus implicating respiratory spread.[80] VZV DNA has been detected by PCR in the nasopharynx of children during the preeruptive and early stages of varicella.[108,146,160] Investigations of leukemic recipients of live attenuated varicella vaccine have implicated spread of VZV from skin. No transmission to siblings from vaccinees who did not have a rash was found, but a 14 percent transmission rate occurred when siblings were exposed to a recently immunized child who had a vaccine-associated rash.[177] The chance of transmission occurring was directly proportional to the number of skin lesions present.[177] Recent observations in children with wild-type varicella despite their having been immunized indicated that higher transmission rates are associated with greater numbers of skin lesions.[164] Finally, an attack rate of more than 20 percent occurred after medical students were exposed to VZV during an autopsy of a patient with disseminated varicella; clearly, this transmission could not have occurred by the respiratory route.[147] Taken together, these observations suggest that VZV spreads mainly from skin lesions rather

than from the respiratory tract. Epidemiologic studies suggest that transmission is most likely to take place in the early stages of varicella.[139]

Zoster is not transmissible per se, but the vesicular lesions of zoster contain infectious VZV and can transmit varicella to other individuals.[26,113] Zoster is less contagious than is varicella,[161] and whether VZV is spread from the respiratory tract of patients with zoster is unknown.

EPIDEMIOLOGY

VZV infections occur worldwide, and both sexes are affected equally. The virus spreads less efficiently in countries with tropical climates than in those with temperate climates,[110] thereby resulting in a high rate of susceptibility in adults reared in tropical countries. In the pre-vaccine era, approximately 3.8 million cases of chickenpox, an entire birth cohort, occurred annually in the United States. In the United States in the pre-vaccine era, 8 to 9 percent of children between the ages of 1 and 9 years contracted the illness annually.[153] These data were collected before the time that many children began to attend daycare facilities; exposure to VZV in the daycare setting may lead to earlier acquisition of disease.

The link between varicella and zoster was recognized first about 100 years ago when Bokay[22] appreciated that cases of varicella often occurred after exposure to a patient with zoster. Early in the 20th century, medical investigators inoculated vesicular fluid from zoster patients into varicella-susceptible children, who then contracted chickenpox.[26,113] Weller[186] first successfully isolated VZV in vitro, gave the virus its name, and demonstrated that the viruses isolated from patients with varicella and zoster are identical. Garland[58] proposed that zoster is the result of reactivation of latent VZV. Hope-Simpson[96] presciently recognized the importance of the immune system in preventing reactivation of VZV; he postulated that zoster results when immunity to VZV wanes with age. Declining cell-mediated immunity to VZV has been identified as contributing to the development of zoster.[9,90] With the use of molecular techniques for DNA analysis, researchers have established that the DNA of the viruses causing varicella and zoster is the same, and autopsies have revealed that latent VZV is detectable in neurons of the sensory ganglia of individuals with a past history of varicella.[44,130,133] Zoster is not acquired by contact with patients who have zoster or chickenpox.[63] Zoster develops in approximately 20 percent of the varicella-immune population during their lifetimes.[96]

Varicella is highly contagious; clinical infection develops in 80 to 90 percent of susceptible individuals exposed in a household.[156] Secondary varicella cases in a family usually are more severe than are the primary cases, probably because of intensity of exposure.[150,156] Approximately 75 percent of American adults with no past history of varicella have detectable antibodies to VZV,[118] thus indicating that subclinical varicella can occur. One epidemiologic study suggests that its incidence is approximately 5 percent.[156]

Subclinical zoster also may occur, and zoster with dermatomal pain and no rash has been described (zoster sine herpete). Increases in VZV immune responses and episodes of viremia shown by PCR in asymptomatic individuals suggest that silent reactivation occurs.[66,83,193] Asymptomatic shedding of VZV is not known to occur.

Second attacks of varicella are uncommon but may occur more frequently in the immunocompromised than in immunologically normal individuals.[71,103] Immunologic boosting to VZV occurs commonly on re-exposure to the virus.[7,71] Whether boosting is necessary for long-term maintenance of immunity to VZV is not known for certain.

Adults and children older than 2 years with zoster usually have a history of a previous attack of varicella.[96] Zoster is an unusual event in children, but the incidence is increased in young children who had varicella either in utero or before reaching their second birthday.[50] Chickenpox in the first year of life increases the risk for development of childhood zoster by a relative factor between roughly 3 and 21,[86] possibly because of immaturity of the immune response to VZV in young infants. Infants with the congenital varicella syndrome are at greatly increased risk, as high as 18 percent in the first few years of life, for the development of zoster.[59]

The incidence of zoster in a population begins to increase sharply at approximately 50 years of age.[96] The loss of cell-mediated immunity to VZV that occurs naturally with normal aging contributes to the increased incidence of zoster,[18,27] and investigators recently have shown that zoster can be prevented by immunization. In a double-blind, placebo-controlled study involving more than 38,000 healthy individuals older than 60 years, immunization with a live attenuated zoster vaccine (Oka strain) reduced the burden of illness of zoster by 61 percent.[144] Zoster also develops with increased frequency in patients with neoplasms and organ transplants[126]; in severely immunocompromised patients with zoster, disseminated infection with viremia also may occur.[53] Spinal trauma, irradiation, and corticosteroid therapy may be additional precipitating factors. Children who are infected with human immunodeficiency virus (HIV) and have $CD4^+$ levels less than 15 percent when varicella develops are at great risk for the development of zoster; in one published series, the incidence was 70 percent.[64]

On occasion, however, zoster may develop in healthy children or young adults who are not immunocompromised, presumably the result of a transient fall in cell-mediated immunity to VZV caused by a stimulus such as another viral infection or stress. Zoster does not develop in all immunocompromised persons; the deficiency of cell-mediated immunity to VZV is a necessary but not sufficient requirement for this illness. The distribution of lesions in chickenpox, which primarily involves the trunk and head, is reflected in a proportionately greater representation of these regions in the dermatomal lesions of zoster.[171] Zoster may recur, in either the same or a different dermatome; the chance of developing recurrent zoster is similar to the chance of having a first attack for a particular age group and is reported as occurring in 2 to 5 percent of adults.[96,154]

Varicella occurs most commonly in the winter and early spring. In contrast, zoster occurs at equal rates during all seasons of the year.

PATHOGENESIS

The incubation period of varicella ranges from 10 to 23 days (average, 14 days).[80] During the incubation period, VZV is thought to spread to regional lymph nodes, undergo multiplication, and cause a primary low-grade viremic phase after approximately 5 days that spreads the virus to the viscera, where further multiplication of VZV occurs. This process results in the second and greater viremic phase, which delivers the virus to the skin, where it causes the characteristic rash.[84] VZV can be isolated from blood cultures either a few days before the onset of rash or within 1 to 2 days after onset in immunocompetent children.[11] An alternative pathogenic mechanism, recently proposed, is that VZV reaches keratinocytes soon after infection occurs, by virus-infected $CD4^+$ memory T cells. These memory cells, when uninfected, normally circulate through the skin. In this model, overcoming of innate immunity in skin accounts for the 2- to 3-week incubation period that follows infection.[111,112]

The skin lesions of VZV infection begin as macules and progress rapidly to papules, vesicles, pustules, and scabs. Histologic changes in the skin lesions are similar for chickenpox and zoster. The hallmarks of each disease are multinucleated giant cells and intranuclear inclusions. In varicella, they are localized primarily in the dermis and epidermis, where ballooning degeneration of cells in the deeper layers is accompanied by intercellular edema. As the edema progresses, the cornified layers and basal layers separate to form a thin-roofed vesicle. An exudate of mononuclear cells is seen in the dermis.[2,186] In zoster, in addition to skin lesions that resemble those of varicella, a mononuclear inflammatory infiltrate is present in the dorsal root ganglion of the affected dermatome. Hemorrhagic necrosis of ganglion cells and demyelination of the corresponding axon also may be present.[52,93,130]

Both humoral and cell-mediated immune responses to VZV develop within a few days after the onset of varicella. Peak antibody levels are attained 4 to 8 weeks after onset, remain high for approximately 6 months, and then decline. IgG VZV antibody titers are detectable in healthy adults for decades after recovery from varicella.[74,170,192] After active immunization against varicella develops, antibody titers are lower than after natural infection occurs but persist for as long as 20 years in healthy vaccinees immunized as children.[12,187] Serum IgG, IgA, and IgM develop after both varicella and zoster. Zoster occurs despite high levels of specific antibodies, but significantly higher titers develop during convalescence and are indicative of an anamnestic response to VZV in this secondary infection.[24,179]

Cell-mediated immune responses are thought to play the major role in host defense against the virus. Cell-mediated immunity to VZV can be demonstrated in vitro by stimulation of lymphocytes with VZV antigens,[89,196] by an intradermal skin test,[118] by specific lysis of histocompatible target cells by cytotoxic T cells,[8] and by an enzyme-linked immunosorbent spot assay.[123] Natural killer cell and antibody-dependent cellular toxicity to VZV also has been described.[99] Immune responses of normal subjects with remote clinical evidence of varicella are characterized by occasional high activity in the absence of symptoms, thus suggesting either exposure to VZV with boosting of the immune response or subclinical reactivation of the virus. Cell-mediated immune reactions remain positive for years after a case of varicella, although this response may wane in many individuals older than 50 years.[18,27] The predominant cell in vesicular lesions is the polymorphonuclear leukocyte. Polymorphonuclear leukocytes may play a role in generating interferon in vesicular lesions, which may be a factor in recovery.[172]

Exactly how immunity to varicella and zoster is mediated is unclear. Because patients with isolated agammaglobulinemia do not experience either severe or recurrent varicella, researchers long presumed that cell-mediated immunity is more important in host defense than is humoral immunity. We now recognize that T-cell cytotoxicity is crucial in recovery from VZV infection.[6] The response or responses that prevent clinical illness after reinfection with VZV are presumed to be those of cytotoxic T lymphocytes, but antibodies also may play a role. Elderly individuals with poor cell-mediated immune responses to VZV are not subject to recurrent chickenpox. In addition, specific antibodies must have some importance because passive immunization is used successfully to prevent or to modify varicella in exposed susceptible persons, perhaps by neutralization of cell-free VZV early in the infection. However, the issues are not straightforward. Varicella may develop in young infants after exposure despite detectable transplacental antibody titers,[14] and modified cases of breakthrough varicella have developed in vaccinated leukemic children despite the presence of humoral or cell-mediated immune responses at exposure to VZV.[72,75] Still, clinical illness is far less likely to develop in individuals with detectable antibody titers or positive cell-mediated immune responses (or both) at exposure to VZV than in those without positive immune responses. Thus, some forms of cell-mediated immunity and specific antibodies each probably play roles in host defense against VZV, and there may be some redundancy in the system.

NOSOCOMIAL VARICELLA

Although it is probably less of a problem since licensure of varicella vaccine, nosocomial varicella is a potentially serious and expensive problem in hospitals, where both patients and employees may be susceptible to chickenpox.[81,88,184] Because varicella-susceptible hospital employees may serve as vectors for the spread of VZV to susceptible patients, serologic testing of employees for immunity to chickenpox if they have no past history of having had clinical varicella and offering vaccine to those who are susceptible are now standard measures in many hospitals.

The risk of horizontal transmission in maternity wards or the newborn nursery after hospital exposure to an adult or child is surprisingly low.[59] A few episodes of nursery transmission of varicella have been reported,[56,65,87] but the low incidence of such transmission may be, at least partly, a result of the fact that many infants are in isolettes. Furthermore, most hospital employees and most mothers and their newborn infants have antibodies to VZV and are at low risk for the development of clinical illness. Even in low-birth-weight infants, antibodies to VZV may be detectable.[136,155]

CLINICAL MANIFESTATIONS

VARICELLA

Varicella is a highly contagious, usually self-limited systemic infection characterized by fever and a generalized pruritic rash lasting approximately 5 days. A prodromal phase in children is unusual, but malaise and fever for 1 to 2 days before the onset of rash is a common manifestation in adults.[39] The rash is more intense on the trunk and head than on the extremities, and it typically evolves as a series of "crops" during the course of 1 to 2 days in normal hosts. Most children with varicella have 250 to 500 superficial skin lesions, many of which are vesicular (Fig. 174–1).[156] Not uncommonly, a few lesions may develop in the mouth, conjunctiva, or other mucosal sites. Residual scarring is exceptional but can occur, and depigmented areas of skin may occur in dark-skinned patients. A self-limited increase in hepatic transaminase levels without jaundice is not an uncommon occurrence during varicella.[149] Rarely, thrombocytopenia and neutropenia may transiently occur. Severe infections are more likely to develop in adults than in children, presumably because of less robust cell-mediated immune responses to VZV in adults than in children.[60,142] Newborn infants who acquire varicella from their mothers in the few days before delivery also are at risk for acquisition of severe varicella because of immaturity of the cell-mediated immune response.[59]

Complications of Varicella

The most frequent complication of varicella in normal hosts is bacterial superinfection of the skin, lungs, or bones, most often by *Staphylococcus aureus* or group A beta-hemolytic streptococci.[25,194] Central nervous system complications, which may precede or follow varicella, include transient cerebellar ataxia, severe cerebral encephalitis, aseptic meningitis, and transverse myelitis.[101,102] Encephalopathy as a sequela of Reye syndrome has become a rare complication because aspirin no longer is recom-

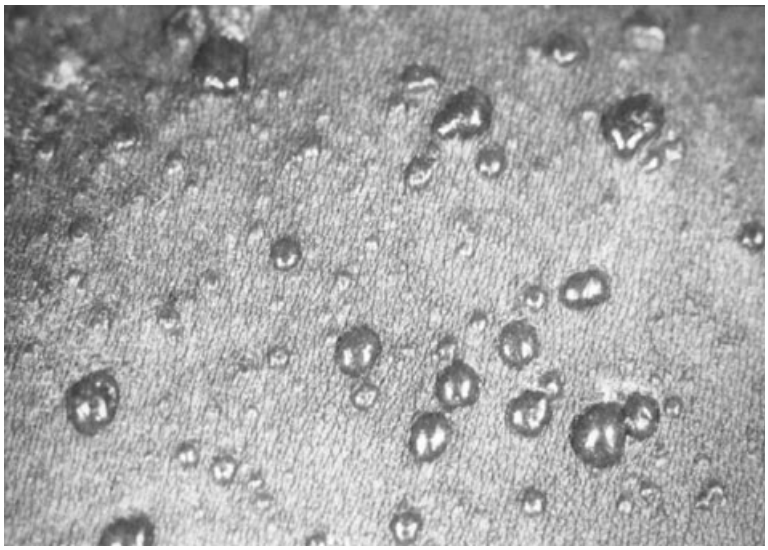

Figure 174–1 Typical skin lesions of varicella.

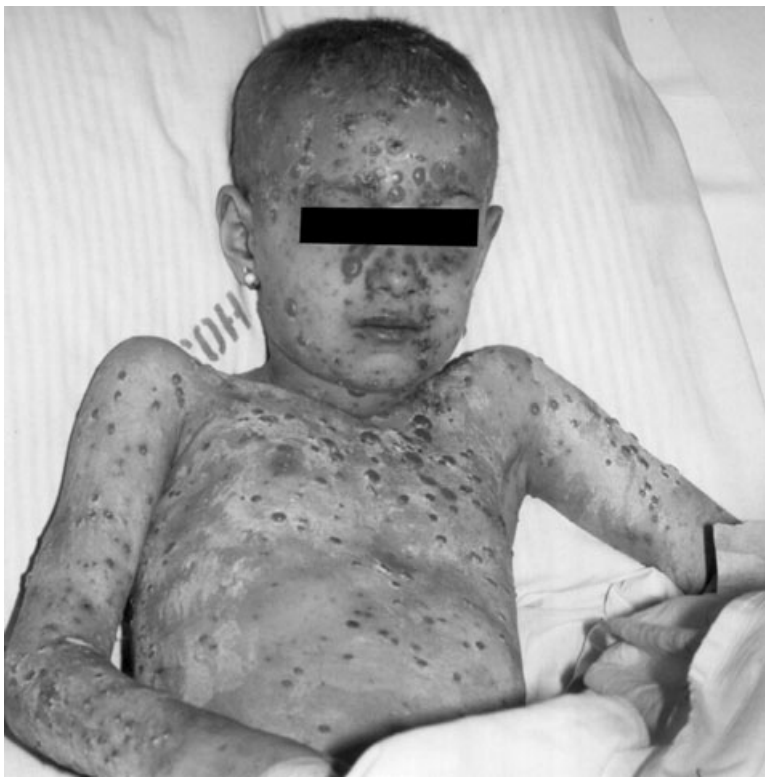

Figure 174–2 Progressive varicella in a 9-year-old child with underlying leukemia.

mended for children with varicella. Other complications of chickenpox encountered less commonly include arthritis, glomerulonephritis, myocarditis, and purpura fulminans.[186]

Varicella may be severe and even fatal in immunocompromised patients, including those with an underlying malignant neoplasm or congenital or acquired deficits in cell-mediated immunity, such as patients who have undergone organ transplantation or have underlying HIV infection, and in children receiving high doses of corticosteroids for any reason (Fig. 174–2).[55] These patients may be susceptible to progressive varicella, with continuing fever and the development of new vesicular lesions for 2 weeks or longer. Their skin lesions characteristically are large, umbilicated, and hemorrhagic, and primary varicella pneumonia is a frequent complication. Alternatively, in some immunocompromised patients, an acute form of varicella with disseminated intravascular coagulation develops and is rapidly fatal, at times before antiviral therapy can be instituted. A 30 percent rate of dissemination with a 7 percent fatality rate was reported in leukemic children in whom chickenpox developed in the pre–antiviral drug era.[54] Severe varicella has been observed in children with underlying HIV infection, especially those classified as having acquired immunodeficiency syndrome (AIDS).[104] In most HIV-infected children, however, mild to moderate forms of varicella develop, although the illness generally is more severe than that in otherwise healthy children.[64] Varicella does not seem to be a cofactor for clinical progression of HIV infection to

AIDS, but in approximately 5 percent of these children, chronic wartlike hyperkeratotic VZV lesions may develop, presumably being a chronic form of zoster in which the infection is low grade but persistent.[5]

Primary varicella pneumonia accounts for many of the fatalities ascribed to varicella, particularly in immunocompromised patients, adults, and neonates.[148] In male military recruits with varicella, radiographic evidence of pneumonia was found in 16 percent.[185] Symptoms include fever, cough, and dyspnea. Other common symptoms and signs are cyanosis, rales, hemoptysis, and chest pain. The chest radiograph typically reveals a diffuse nodular or miliary pattern, most pronounced in the perihilar region.[175] Blood gas analyses and pulmonary function tests indicate a diffusion defect that may persist in some cases for months after recovery.[21] The availability of antiviral chemotherapy has improved greatly the outcome of this complication.

Congenital Varicella Syndrome

LaForet and Lynch[115] in 1947 were the first to describe an infant with multiple congenital anomalies after contraction of maternal chickenpox. The infant had hypoplasia of the right lower extremity, clubfoot, absent deep tendon reflexes on the right, cerebral cortical atrophy, cerebellar aplasia, chorioretinitis, torticollis, insufficiency of the anal and vesical sphincters, and cicatricial cutaneous lesions on the left lower extremity, which are now recognized to be typical. In 1974, Srabstein and colleagues[169] rediscovered this syndrome. Their report of another case and review of the literature concluded that although the virus could not be isolated from affected infants, the congenital syndrome consisted of a typical constellation of birth defects as originally described by LaForet and Lynch. This syndrome occurs after maternal VZV infection develops in the first or second trimester of pregnancy; approximately 2 percent of the offspring of pregnancies complicated by varicella are affected. Approximately 100 affected infants have been described in the literature; 95 percent occurred after maternal varicella and 5 percent after maternal zoster. In the pre-vaccine era, approximately 40 affected infants were thought to be born annually in the United States.[59]

Cicatricial scars of the skin, the most prominent stigmata, are reported in more than 60 percent of cases.[59] Other frequent abnormalities include chorioretinitis, microphthalmos, Horner syndrome, cataract, nystagmus, hypoplastic limbs, cortical atrophy or mental retardation (or both), and early death. Many of these children develop zoster (in which VZV can be identified) in the early months of life.

ZOSTER

Zoster usually begins as a localized unilateral vesicular skin eruption involving one to three dermatomal segments (Fig. 174–3). The skin lesions resemble those of varicella but tend more toward confluence and are likely to be painful or pruritic, especially in adults. Zoster generally is a milder disease in children than in adults.

Complications of Zoster

Between 25 and 50 percent of persons older than 50 years in whom zoster develops may experience protracted pain, or postherpetic neuralgia, after healing of the rash. Postherpetic

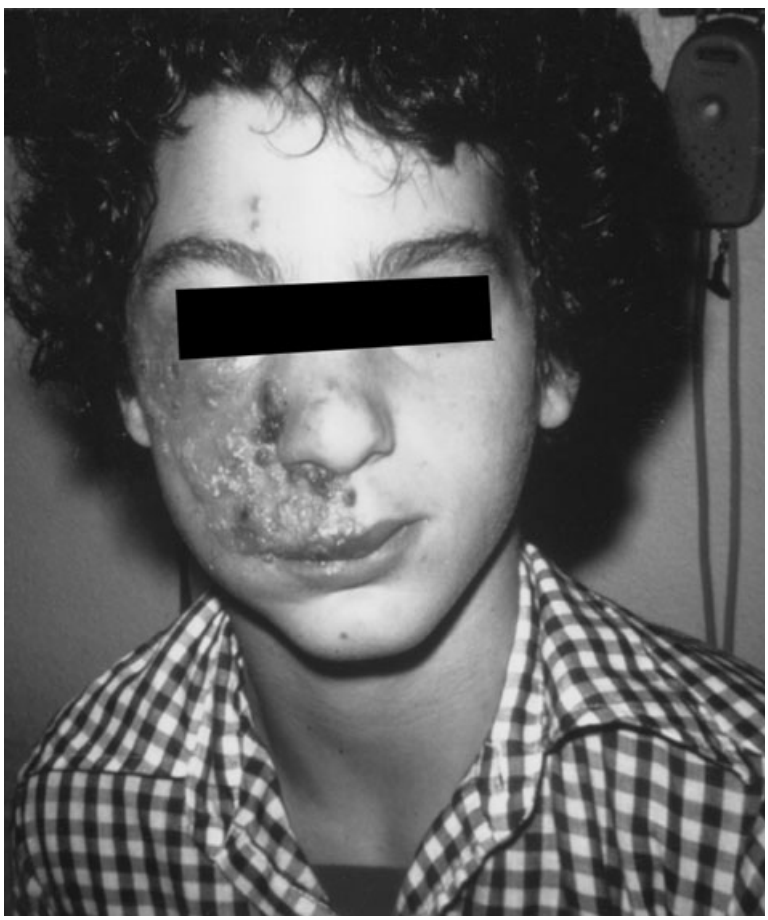

Figure 174–3 Zoster in an otherwise healthy 10-year-old boy.

neuralgia also develops frequently in immunocompromised patients. Pain may persist for months to years and is described as aching, jabbing, or boring. The cause of postherpetic neuralgia is unknown. Children rarely experience postherpetic neuralgia; both the incidence and duration are related directly to increasing age.[76,77] Zoster may be particularly severe and common after bone marrow transplantation; in the first year after undergoing transplantation, 20 to 70 percent of recipients develop zoster.[61]

DIAGNOSIS

CLINICAL DIAGNOSIS OF VARICELLA AND ZOSTER

It usually is not difficult to establish a clinical diagnosis of VZV infection because the vesicular rash is so characteristic. In questionable cases of varicella, epidemiologic information, such as a history of recent exposure to varicella or zoster and subsequent transmission of varicella to another person, may be useful. The differential diagnosis of varicella includes generalized HSV infection, rickettsialpox, impetigo, allergic reactions including Stevens-Johnson syndrome and poison ivy, and insect bites.

In one study of zoster, 13 percent of clinically diagnosed cases were proved by culture to be caused by HSV infection.[105] The unilateral rash of zoster most frequently appears on the trunk or face. The trigeminal nerve, most commonly the ophthalmic branch, is an especially significant site because the eye may be involved.

LABORATORY DIAGNOSIS

Laboratory diagnosis of VZV infection is facilitated by the presence of VZV in superficial skin lesions, where it is easily accessible for testing. The diagnosis is made best by the demonstration of specific viral antigens in skin scrapings by immunofluorescence with a commercial monoclonal antibody to VZV that is conjugated to fluorescein.[62] This diagnostic method is highly sensitive and rapid and can be completed within approximately an hour.

The diagnosis also may be made by isolation of virus from skin lesions. Vesicular fluid for culture should be obtained as early in the course of illness as possible for successful virus isolation. Within several days after the onset of varicella, vesicular fluid no longer is likely to be infectious, although viable VZV may be present in zoster lesions for a longer period, especially in immunocompromised patients. VZV cannot be isolated from skin lesions that have become pustular or dry. Isolation of VZV is a slow method because approximately 48 hours is required before the first signs of a viral cytopathic effect are seen. Virus isolation is less sensitive than is immunofluorescence staining because infectious virus persists for a shorter time in vesicles and is more labile than are viral antigens. VZV rarely is isolated from infected cerebrospinal fluid and respiratory secretions. Isolation of VZV or demonstration of viral antigen in material obtained from skin or other lesions or autopsy tissue is diagnostic of a current infection with VZV because, unlike other herpesviruses, no known carrier state or shedding of VZV by asymptomatic individuals exists.

PCR testing of skin scrapings, vesicular fluid, respiratory secretions, and cerebrospinal fluid has been used successfully to establish the diagnosis of VZV infection.[62] In many virology laboratories, PCR is supplanting culture for diagnosis of VZV infection because it is both more rapid and sensitive. PCR also may be used to distinguish between vaccine (Oka strain) and wild-type VZV infections.[62]

Numerous serologic tests, including the fluorescent antibody to membrane antigen method, latex agglutination, and enzyme-linked immunosorbent assay, are useful for measuring antibodies to VZV.[62] Antibody to VZV develops within a few days after the onset of varicella, persists for many years, and is present before the onset of zoster. VZV infections may be documented by a fourfold or greater rise in VZV antibody titer in acute and convalescent serum specimens. Specific IgM in one serum specimen suggests recent VZV infection. Persistence of VZV antibody beyond 8 months of age is highly suggestive of intrauterine varicella. Immunity to varicella is very likely to be present if an antibody titer to VZV is demonstrated on a single serum sample. However, serologic methods, particularly commercial enzyme-linked immunosorbent assays, may fail to identify individuals who have been immunized.[62,170]

The value of serologic procedures for the diagnosis of zoster is limited. Heterologous increases in antibody titer against VZV in patients with HSV infection who previously had varicella may occur and has been ascribed to antigens common to the two viruses.[62]

TREATMENT

Traditionally, nonspecific measures, such as frequent bathing to discourage bacterial skin infection, antihistamines given orally, calamine lotion applied locally, oatmeal baths to decrease itching, and cutting fingernails short to discourage scratching, have been used to treat varicella. Fever is controlled best with acetaminophen rather than aspirin, which may predispose to Reye syndrome.[91] The issue of whether treatment with ibuprofen is associated with group A streptococcal superinfection in varicella has not been resolved, and, therefore, avoidance of its use for symptomatic treatment of this disease seems to be the best approach.[28,38,121,198]

Useful specific antiviral therapy for VZV became available in the mid-1980s with the introduction of acyclovir, an inhibitor of DNA polymerase and a DNA chain terminator. The antiviral effect of acyclovir depends on its being phosphorylated in the body by virus-induced thymidine kinases, which accounts for its relative lack of toxicity.[190]

Patients with severe or potentially severe VZV infections should be treated with intravenous acyclovir (30 mg/kg/day for adults and adolescents and 1500 mg/m^2/day for children, both given in three divided doses). Orally administered acyclovir is less reliable for immunocompromised patients because only approximately 20 percent of this formulation is absorbed from the gastrointestinal tract, and no data on its efficacy in high-risk patients have been published. Because acyclovir is excreted by the kidneys, patients with a creatinine clearance of less than 50 mL/min/1.73 m^2 should receive one half to one third of this dosage. Intravenous acyclovir is infused for at least 1 hour, with maintenance fluids given both before and during the infusion. Providing adequate hydration is important to prevent renal damage from precipitation of the drug in the renal tubules. Other adverse effects of acyclovir include phlebitis, rash, nausea, and neurologic manifestations such as headache and tremor. In general, however, acyclovir is extremely well tolerated.[190]

Early intravenous therapy should be instituted for patients at high risk for development of severe VZV infection, such as leukemic children and those who have undergone organ or bone marrow transplantation, to prevent the dissemination of VZV.[166,189] Not only may this therapy be potentially lifesaving in immunocompromised patients, but it also prevents considerable morbidity from VZV infection. Children thought to be somewhat less immunocompromised, such as those with HIV infection but without AIDS, may be given a treatment trial with oral acyclovir under close medical supervision. For such patients who are not doing well, intravenous therapy should be started promptly. In zoster patients, the use of intravenous acyclovir is

associated with more rapid healing of skin lesions and resolution of acute pain than if no specific treatment is given.[191]

Considerable controversy has ensued about the role of orally administered acyclovir for the treatment of varicella and zoster in otherwise healthy children because most of these infections are self-limited. Customarily, however, adults, who are at greater risk for development of severe infection, are treated. Oral dosages used are 80 mg/kg/day (in four divided doses) for children and 4 g/day (in five divided doses) for adults. Double-blind, placebo-controlled studies in healthy children given oral acyclovir at 80 mg/kg/day for 5 days or placebo beginning within 24 hours of the onset of varicella rash have revealed that the number of chickenpox skin lesions is reduced significantly by acyclovir. A modest benefit was derived from acyclovir; children who received it had fever for approximately 1 day less, but they did not return to school any more rapidly, nor did they have fewer complications of chickenpox.[16,49]

Some evidence indicates that early administration of oral acyclovir may decrease the acute pain associated with zoster.[98] However, the need to administer specific therapy for zoster in otherwise healthy children for whom pain is not a particular problem rarely occurs.

A newer drug, penciclovir, has an action similar to that of acyclovir.[94] It is administered as famciclovir, a prodrug that when given orally is converted rapidly to penciclovir in the body. A major advantage of famciclovir is that it is administered only three times a day (1500 mg/day for an adult), whereas acyclovir is given four or five times daily. Penciclovir has an antiviral action similar to that of acyclovir. One study suggests that famciclovir given to elderly patients with zoster early in the course of infection decreases the duration of postherpetic neuralgia, although not its incidence.[178] No data regarding whether varicella may be treated successfully with famciclovir have been published, nor have any data on the use of famciclovir in immunocompromised patients or in children.

The prodrug of acyclovir, valacyclovir, also is given orally, reaches blood levels that are approximately three to four times higher than those of acyclovir, and has been shown in one study to be superior to acyclovir for treatment of zoster.[19] Neither valacyclovir nor famciclovir is licensed in the United States for use in children.

Of concern about the potential widespread use of acyclovir is that drug resistance may develop. At present, resistance is less a problem with VZV than with HSV, but VZV resistant to acyclovir has been reported in a few patients with underlying AIDS.[100] A vaccinated child with neuroblastoma and zoster caused by the Oka strain that became resistant to acyclovir after prolonged treatment has been described.[122] Foscarnet was licensed by the Food and Drug Administration for the treatment of VZV infections that are resistant to acyclovir and famciclovir. Foscarnet inhibits the synthesis of VZV DNA polymerase.[157,158,168] Intravenous foscarnet is given at a dosage of 180 mg/kg/day in two divided doses, adjusted according to renal function. The main toxicity of foscarnet is renal damage and electrolyte imbalance.[61]

PROGNOSIS

The prognosis of varicella and zoster is excellent in children without underlying health problems. The outlook for immunocompromised patients receiving antiviral chemotherapy also is good, especially if treatment is begun at an early stage of the illness. In general, zoster carries a better prognosis than varicella does, possibly because it is a secondary infection. If the disease is diagnosed promptly, complications such as bacterial superinfections usually can be treated successfully with antimicrobials. In the pre-vaccine era, despite the availability of antiviral therapy and passive immunization, the Centers for Disease Control and Prevention (CDC) estimated that approximately 100 deaths from varicella occurred annually in the United States, mostly in children with no previous health problems. An epidemiologic study of more than 250,000 health maintenance organization records indicated that the rates of varicella in adolescents and young adults and the complication and hospitalization rates had increased in recent years by a factor of approximately 5.[37]

The widespread use of varicella vaccine in the United States has decreased the morbidity and mortality rates from chickenpox sharply. By 2000, in the three sentinel communities in the United States, vaccine coverage among children 19 to 35 months was more than 80 percent, and reported varicella cases had declined roughly from 70 to 80 percent, compared with the pre-vaccine era. Hospitalizations for varicella also declined by approximately 80 percent. The greatest declines occurred in young children.[143]

PREVENTION

VZV is such an infectious agent that general measures are not useful for prevention of varicella in susceptible individuals. Some protection, however, can be achieved by isolation of hospitalized patients, particularly in rooms with negative-pressure ventilation. Hospitalized patients with active VZV infection should be admitted to a private room, and hospital personnel and visitors should wash their hands before and after entering the room and wear masks, gowns, and gloves while in it. The CDC now recommends postexposure vaccination for healthy varicella-susceptible exposed individuals.[31]

PASSIVE IMMUNIZATION AGAINST VARICELLA

Varicella-susceptible children at high risk for development of severe chickenpox should be passively immunized if they are closely exposed to VZV. Passive immunization may be lifesaving; it may modify varicella or prevent it.

Passive immunization is indicated for varicella-susceptible individuals who have been closely exposed to varicella or zoster and are at high risk for development of severe chickenpox. This population includes immunocompromised children and adults, pregnant women, newborn infants whose mothers have active varicella at the time of delivery, and premature infants of less than 28 weeks' gestation or who weighed less than 1000 g at birth. Children at high risk should be considered to be susceptible to varicella if they have no history of having had chickenpox. False-positive antibody test results may occur in immunocompromised individuals, and, therefore, susceptible children may be identified serologically as immune; hence, a history of illness is a preferred indication of these patients' immune status. Passive immunization usually is reserved for VZV-exposed adults only if they have been proved serologically to be susceptible to chickenpox because most adults with a negative history of varicella raised in the continental United States are immune.

Patients with HIV infection, especially those with AIDS, have some increased risk for development of severe varicella, and their management should be similar to that of immunocompromised children with regard to passive immunization. Even children who are receiving intravenous globulin for treatment of HIV infection should receive passive immunization if they have no past history of varicella and close exposure has occurred.

Infants whose mothers have an onset of chickenpox 5 days or less before delivery or within 48 hours after delivery should be given passive immunization as soon as possible after birth.[59] The transplacental route of infection and the immaturity of the immune system probably account for the severity of varicella in

these infants.[59] Attack rates for varicella as high as 50 percent in infants exposed to mothers who have varicella have been reported, despite their having passive immunization.[59,152] Passively immunized infants should be observed closely, but usually they can be managed as outpatients. Intravenous acyclovir should be reserved for the rare, passively immunized infant with varicella in whom an extensive rash (more than 200 vesicles) or possible pneumonia develops.[15]

Passive immunization need not be given to full-term infants who are exposed to VZV after they are 48 hours old. Passive immunization is optional for newborn infants (<1 week old) if their siblings at home have active varicella. Infants exposed to VZV after birth almost always have mild varicella. Although the reported mortality rate from varicella in children younger than 1 year is four times that in older children, both rates are exceedingly low, 8 per 100,000 cases and 2 per 100,000 cases, respectively.[151] The mortality rate for adults and for leukemic children receiving chemotherapy, in contrast, is 20 and 1000 times higher, respectively.[153] Passive immunization is not useful to treat or to prevent zoster, and whether it is useful for pregnant varicella-susceptible women who have been exposed to VZV to protect the fetus from congenital varicella syndrome is not known.

Until recently, varicella-zoster immune globulin (VZIG), distributed by the Massachusetts Public Health Biological Laboratory and the American Red Cross, was used for passive immunization. Because of limited requests for VZIG since licensure of varicella vaccine in the United States, however, VZIG no longer is being produced. A new product, varicella immune globulin (human), VariZIG, manufactured in Canada, is now available under an investigational new drug application expanded-access protocol.[29] VariZIG can be obtained in the United States, but central institutional review board approval is required, and local institutional review board approval also may be necessary. The sole distributor of VariZIG is FFF Enterprises (800-843-7477), in Temecula, California. An alternative to VariZIG is intravenous immune globulin, 400 mg/kg.

Although passive immunization has been shown to be effective when it is given within 4 days and perhaps as long as 5 days, it should be administered as soon as possible after an exposure to VZV.[29] The dose is 1 vial or 125 units for every 10 kg of body weight, with a maximal dosage of 5 vials or 625 units, intramuscularly. VariZIG should be readministered to high-risk susceptible individuals who are closely re-exposed 3 weeks after a first exposure.[3]

ACTIVE IMMUNIZATION AGAINST VARICELLA

Live attenuated varicella vaccine was developed in Japan more than 30 years ago. It was licensed in the United States in 1995 for universal immunization of healthy children and adults who are susceptible to varicella[32] and has proved to be extremely safe and well tolerated.[20,165] The most frequent adverse reaction is a mild rash that develops several weeks after vaccination in approximately 5 percent of healthy children.[73,74,165,187] These rashes can be serious in immunocompromised children who are vaccinated inadvertently[68,165] but usually respond to antiviral therapy. When healthy vaccinees develop rash from the Oka strain, the potential for transmission of the vaccine virus exists, but transmission is rare, with fewer than 10 known instances from healthy vaccinated individuals after more than 10 years of routine immunization of as many as 40 million American children.[25,85,117,165] In contrast, the rate of transmission of the Oka strain was 14 percent after household exposure from vaccinees with underlying leukemia who had rash and exposed their susceptible healthy siblings.[177] Serious neurologic events have not been causally demonstrated to be connected to varicella vaccine.

Live attenuated varicella vaccine is highly effective in healthy children and adults, but not all vaccinees are completely protected. Approximately 10 to 20 percent of children given one dose may develop a modified breakthrough illness after intimate exposure to VZV. Varicella vaccine has, however, been 97 percent effective in preventing severe varicella.[180,181] The vaccine is also highly effective in adults; after two doses, more than 75 percent of adults are completely protected from varicella after household exposure to the virus.[1,69,73,159] Severe wild-type varicella in vaccinated adults is rare.

Originally, it was observed that approximately 85 percent of healthy children are protected after only one dose of vaccine,[114,180,181,187,188] and there was little concern about breakthrough varicella because it is almost always a modified illness. Loss of VZV antibodies, furthermore, occurs rarely in healthy vaccinated children, some of whom have been monitored for as long as 20 years after immunization.[10,12,40,114,167,183,197]

Beginning in approximately 2000, however, breakthrough varicella became a growing concern in the United States, and investigators suspected that primary or secondary immune failure occurred in some vaccinees. The incidence of varicella in sentinel geographic areas where active surveillance was being carried out revealed that although the incidence of disease fell after 1995, it leveled off at a low rate in about 2000.[163,164] Moreover, the number of reports of outbreaks of varicella among vaccinated children was increasing; some of these studies indicated vaccine effectiveness as low as 44 and 56 percent, although most showed 80 to 85 percent effectiveness.[33,57,120] Also recognized was that children with breakthrough infections could spread wild-type VZV to contacts almost as efficiently as could unvaccinated children.[164] Attempts at outbreak control with second doses of varicella vaccine proved to be expensive and complicated.[134] Finally, one study suggested that the seroconversion rate could be as low as 76 percent, indicating an unexpectedly high rate of primary vaccine failure after one dose of vaccine.[138] The fear was that without a second routine dose, a gradual accumulation of young adult susceptibles would develop as a result of primary vaccine failure and would be at risk to develop severe chickenpox.

In the light of all these problems, a second dose of varicella vaccine was recommended for all children, not just those older than 12 years, by the CDC in June 2006. Early studies exploring immune responses to two doses had indicated the safety of a second dose and a marked boost in immunity and the seroconversion rate afterward. Interestingly, this marked booster effect (by a factor of 10) was observed for the varicella component of measles-mumps-rubella-varicella (MMRV) only. MMRV was approved by the Food and Drug Administration in 2005 for use in children; thus, today, many infants and children are immunized with two doses of MMRV, depends on its availability. Catch-up immunization also is recommended for children who received only one dose of varicella vaccine.

Because the risk from wild-type VZV is greater than is the risk from vaccine-type VZV, immunization is recommended for health care workers and persons whose varicella-susceptible family members are immunocompromised or pregnant.[31] Varicella vaccine is recommended only for healthy persons. Vaccinees in whom an extensive VZV rash develops within 2 to 3 weeks after immunization are likely to have wild-type infection.[165] In such situations, PCR and restriction fragment length polymorphism analysis can be used to differentiate between wild-type and vaccine-type VZV.[79,116,129] Passive immunization could be considered for immunocompromised individuals inadvertently exposed to a vaccinee with rash resembling chickenpox that might be caused by wild-type VZV.

Most states now require varicella vaccination for children to attend daycare or school. In geographic areas where vaccine use in children younger than 3 years is 70 percent or more, the incidence of varicella has decreased sharply, not only in the

vaccinated but in all age groups, a process indicative of herd immunity.[163] Zoster appears to be less of a problem after immunization than after natural infection in vaccinated immunocompromised individuals,[63,67] which suggests that it also will be less of a problem in healthy vaccinees.

DRUG PROPHYLAXIS

Prophylaxis of varicella in exposed persons may be achieved by the administration of acyclovir.[13,97,125,174] This approach is not recommended often, however, because the optimal dosage and timing of administration have not been studied in large groups of children, nor is it known whether long-term maintenance of immunity exists in all children. In the United States, prevention of varicella by vaccination is a preferable approach. Long-term acyclovir therapy may be used to prevent the development of zoster in patients who have undergone bone marrow transplantation; however, its value is questionable because zoster commonly develops after administration of acyclovir is stopped.[30]

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CHAPTER 175

SMALLPOX (VARIOLA VIRUS)

James D. Cherry ✪ Samantha Johnston

Smallpox was a dreaded febrile exanthematous disease caused by the orthopoxvirus variola virus.[4,6,8,23,25,37] After an extensive decade-long World Health Organization (WHO) program to eradicate smallpox, the world was certified free of smallpox in 1979.[16,104] Increased concern relating to biologic terrorism occurred during the 1990s, and the events of September 11, 2001, and immediately thereafter brought home the potential reality of this threat for the present and future.[3,23,52,53,56,73,77,94,104]

HISTORY*

Evidence suggests that endemic smallpox was occurring before 1500 BCE. Three mummies dating from the 18th to the 20th dynasties in Egypt had pustular lesions all over their bodies.[37] Reliable written accounts of smallpox first appeared during the fourth century. Smallpox was differentiated from measles in 340 CE by Ko Hung in China. At that time in China, smallpox was an established endemic disease that had come from the west 300 years earlier. During the period from 340 to 1000 CE, the disease was described in Egypt, India, Korea, Japan, southern Europe, and North Africa. A major contributor to the spread of smallpox was the great Islamic expansion across North Africa and into Spain in the seventh and eighth centuries.

By the year 1000 CE, smallpox probably was endemic in populated areas of Europe, Asia, and the African Mediterranean countries. Smallpox was established further in northern Europe by the population movements related to the Crusades. By the 16th century, smallpox was a serious disease in Europe, as indicated by death statistics in Geneva, London, and Sweden. In London during the 17th century, approximately 10 percent of the yearly deaths were attributed to smallpox.

The disease was introduced into the American colonies in 1507 and into Mexico soon thereafter. Epidemics of smallpox posed serious problems for the colonists, as well as for the Native Americans. These epidemics also may have been important in certain stages of the American Revolution.[6,39] Smallpox persisted in the United States and Mexico until the 1940s and 1950s, respectively, despite concerted efforts to eliminate the problem.

Soon after this disease was recognized, the Chinese are reported to have made efforts to prevent it. The technique used presumably was variolation, the intentional intranasal or intracutaneous inoculation of susceptible persons with vesicular fluid or crusts from patients with smallpox. This technique apparently originated in China and subsequently was used in many countries, including the United States. As recently as 1968 and 1969, isolated instances of the use of variolation were noted in remote areas of Africa and Asia.[39,58]

After making his observations in 1796 on the immunity against smallpox that was conferred by inoculation with material obtained from cowpox lesions, Jenner extended his research to include intentional and deliberate exposure of some of those persons immunized with cowpox. This strategy provided convincing evidence of solid protection against smallpox.[62] Encouraged by the results, Jenner predicted in 1801 the ultimate eradication of smallpox, a remarkable prediction indeed.[39,51]

The Intensified Smallpox Eradication Program of the WHO, established in 1967, initiated remarkable progress toward total global eradication of this disease.[55] Several factors responsible for this rapid progress included the following: emphasis on surveillance of disease, with containment rather than routine vaccination; improved vaccines and vaccination technology; and sound administrative and fiscal support.[39,40,54,55,68] Of the more than 30 countries in 1967 in which smallpox was endemic, the disease persisted in only 5 in 1975 and in 2 in 1977. The world's last case of endemic smallpox occurred on October 31, 1977, in Merca, Somalia.[29] A year later, in 1978, a photographer working at the University of Birmingham in England was infected with a laboratory strain of smallpox virus and died.[100]

After the worldwide eradication of smallpox was achieved, the World Health Assembly in 1980 recommended that all countries cease vaccination.[104] Subsequently, a WHO expert committee recommended that all laboratories throughout the world destroy their stocks of variola virus or transfer them into either of two WHO reference laboratories.[56] The laboratories were the Russian State Research Center on Virology and Biotechnology, Koltsovo, Novosibirsk Region, and the Centers for Disease Control and Prevention (CDC) in Atlanta, Georgia.[5] Reportedly, all countries complied with this recommendation.

The WHO committee at a later date recommended that all variola virus stocks be destroyed by 1999, and the World Health Assembly concurred with this recommendation.[8] In 1998, a committee of the Institute of Medicine reviewed the possible importance of retaining variola virus for research purposes.[61] In May 1999, the World Health Assembly agreed to postpone the destruction of all variola stocks until 2002.[42] No further date has been set for the destruction of variola stocks.[33,93] At the present time, research using the virus is being conducted in the United States and Russia. This research is related to the development of safer vaccines and to antiviral agents.

In the early 1990s, evidence revealed that during the previous 10 years, the Soviet government apparently had developed a successful program to produce variola virus for use as a biologic weapon.[56,90] In the late 1990s, concern increased with regard to bioterrorism and, in particular, with the possibility that Russian expertise and equipment could have fallen into non-Russian hands because financial support for laboratories and workers had declined.

Smallpox has the dubious distinction of being used as a biologic weapon almost 250 years ago.[30] In the French and Indian Wars (1754 to 1767), British forces sent smallpox-contaminated blankets and handkerchiefs to the Native Americans surrounding Fort Pitt in the summer of 1763.

ETIOLOGY

Smallpox is caused by variola virus, an orthopoxvirus.[38,55,80] The virus particles are brick shaped and measure 250 to 300 × 200 to

*See references 25, 30, 34, 37, 38, 48, 55, 58, 59, 88, 89, 102.

250 × 100 nm; the size and shape are similar for all orthopoxviruses. The distinctive appearance is helpful in rapidly identifying members of this virus group by electron microscopy of vesicular fluid or crusts. The virus is stable when dried and may remain viable for long periods of time in dried crusts and indefinitely under freeze-drying techniques in the laboratory. It is propagated readily on the chorioallantoic membrane of embryonated eggs and in a variety of mammalian cell cultures.

EPIDEMIOLOGY

In infected patients, variola virus was found in respiratory secretions and in vesicular fluid from skin lesions. Transmission usually occurred after close personal contact, but airborne spread also could occur.[7,55,56,101] Patients with smallpox were most infectious from the onset through the first 7 to 10 days of rash.

All persons were susceptible to smallpox unless they previously had been infected with variola virus itself or with cowpox or vaccinia virus. Thus, the occurrence of smallpox depended entirely on the presence of a source of infection and effective exposure of susceptible persons. The age distribution of cases varied in individual countries, depending largely on vaccination practices. Preschool-aged children often were not vaccinated and thus had the greatest prevalence of disease.

The seasonal influence was substantial and perhaps is shown best by the spread of disease after the winter and spring introduction of smallpox into Europe; spread of infection was more than 30 times as great after the introduction of infection into European nations between December and May, in contrast to substantially less frequent spread after introduction between June and November.[60]

Substantial contrast in case-fatality rates was recognized in different geographic areas; these differences did not seem to depend on the supportive care available and did not appear to be related to ethnic or specific resistance factors. The only logical explanation for these different rates was substantial differences in the virulence of the virus prevalent in the regions. The mildest form of smallpox, described as *variola minor*, or *alastrim*, was prevalent in Brazil, Ethiopia, and adjacent countries. Case-fatality rates of 1 percent or less were customary, and permanent residual scarring and blindness were most unusual occurrences. In other areas of Africa and Indonesia, the disease appeared to be of intermediate severity, whereas on the Indian subcontinent, case-fatality rates were as high as 30 percent, with residual permanent scarring, blindness, and other sequelae occurring frequently in those who survived the acute illness.[39]

PATHOLOGY

The characteristic pathologic lesions affected the skin and mucous membranes. They involved the deeper layers of the skin and progressed through macular, papular, vesicular, and pustular stages, with the subsequent formation of crusts. Lesions similar to those on the skin also were found in the lower respiratory and gastrointestinal tracts.

Secondary bacterial infection occurred frequently and often affected the skin and lungs. Hemorrhagic complications (hemorrhagic smallpox) were not uncommon events.

CLINICAL MANIFESTATIONS[34,55,67,79]

After an incubation period of between 7 and 17 days, the disease began abruptly with fever (temperatures between 38.9° C and 40.5° C [102° F and 105° F]), headache, and marked malaise.[9] Backache and muscle pain were prominent symptoms. Nausea, vomiting, and abdominal pain also were present.

After these prodromal signs and symptoms were present for 2 to 4 days, the fever usually decreased, and the characteristic cutaneous eruption appeared. The rash was most extensive on the face and extremities, and the individual lesions passed through the stages of macules and papules; by the third or fourth day, they clearly were vesicular. Lesions in a single area of the body were characteristically at the same stage of development.

By the sixth day, the vesicular fluid usually was cloudy, and the individual pustules frequently were umbilicated. Pustules often converged and become confluent. By the 10th day, the individual lesions began to dry and formed characteristic crusts that remained intact for several days before they were shed. The patient's temperature customarily fell during the early appearance of the rash, although the fever usually returned. Significant fever that persisted after the 10th or 12th day of disease suggested the presence of bacterial superinfection.

Although most cases were similar to this description, which was characteristic of the ordinary type of smallpox, other clinical variations were described. The hemorrhagic type was the most severe form, with a case-fatality rate that approached 100 percent. In this form, hemorrhagic manifestations appeared during the prodromal stage, with extensive cutaneous extravasation of blood and bleeding from the various body orifices. Death usually occurred within the first week of illness, and frequently, few typical diagnostic lesions appeared on the skin surfaces before death ensued. Fortunately, this form of disease occurred in only 2 or 3 percent of the total cases of variola major in Asia, and it rarely was seen elsewhere.

A flat variety had been reported in approximately 6 percent of cases observed in India. In this variety, the cutaneous lesions remained flat and soft to the touch, in contrast to the ordinary variety; these lesions characteristically resolved without pustulation. This form was associated with case-fatality rates of 75 to 96 percent.[39]

A modified form of disease occurred almost exclusively in previously vaccinated persons. Although the prodromal illness often was severe, skin lesions were few, evolved rapidly, and were more superficial. The prognosis was excellent.

The mildest form of the disease, alastrim or variola minor, was caused by a specific variola virus having less pathogenicity in humans. Serious forms of the illness, as with variola major, were unusual occurrences. The skin lesions tended to be superficial, and the clinical course resembled that of varicella, with the exception of the distribution of cutaneous lesions. These lesions involved the face and extremities, in contrast to the characteristic central body distribution of varicella. Residual scarring, if it occurred at all, usually reflected secondary bacterial infection of individual lesions.

In recently vaccinated persons, asymptomatic infection with variola virus after exposure had been demonstrated by increases in antibody titer against the virus when acute and convalescent sera were tested. This uncommon event was of neither clinical nor epidemiologic significance.

Complications included hemorrhagic events and various secondary bacterial infections, including impetigo, pneumonia, empyema, and otitis media. Nephritis and arthritis with permanent joint changes were described.

DIFFERENTIAL DIAGNOSIS

The typical course, the characteristic cutaneous lesions, and the presence of other cases after contact 7 to 17 days earlier left little doubt about the etiologic agent. Varicella presented the greatest problem in differential diagnosis, particularly from the variola minor or alastrim form of the disease, but the distribution of

cutaneous lesions for each condition was characteristic. Generalized vaccinia or eczema vaccinatum was distinguished by a history of exposure to vaccinia virus, previous skin lesions, and the distribution of the rash. Impetigo (especially the bullous variety caused by staphylococcal infection), scabies, secondary syphilis, and yaws were other considerations. Usually, little difficulty was encountered clinically in distinguishing among these infections.

Monkeypox can be clinically similar to smallpox, and, therefore, specific laboratory procedures are required for diagnosis (see Chapter 176). Other conditions included in the differential diagnosis were erythema multiforme, pityriasis rosea, measles, rickettsialpox, disseminated herpes simplex infection, syphilis, enteroviral exanthems, and bacterial, viral, and rickettsial petechial and purpuric rashes.

SPECIFIC DIAGNOSIS

Several laboratory procedures are available to provide specific and accurate diagnosis. Vesicular fluid, crusts, or scrapings from skin lesions reveal the characteristic brick-shaped viral particles of the variola-vaccinia virus group when examined by electron microscopy. These orthopoxviruses differ in appearance from the herpesviruses, which in the past were the agents most frequently confused with smallpox. Electron microscopy is precise and rapid and is preferred when facilities for this examination are available.[26] Smallpox virus DNA also can be identified rapidly by polymerase chain reaction (PCR).[34,35,66,84] PCR has an advantage over electron microscopy in that it can specifically distinguish smallpox virus from the orthopoxviruses.

Orthopoxviruses may be recovered and propagated on the chorioallantoic membrane of embryonated eggs or in tissue culture.[34] Recovery of the virus requires more time (3 to 7 days) than the direct antigen-detection tests do, but it provides the specific active virus required for differentiation among the various orthopoxvirus types.[81,83] Infection also can be determined serologically by using acute-phase and convalescent-phase sera in enzyme-linked immunosorbent assays (ELISA), Western blotting, or virus neutralization assays.[34]

The specific diagnosis of a disease that does not currently exist anywhere in the world presents unique challenges. Recognizing these challenges, the CDC during the last few years developed an overall *Smallpox Response Plan and Guidelines*, available at www.bt.cdc.gov/agent/smallpox/response-plan. Presented in Figure 175–1 is an algorithm that lists the symptoms associated with acute, generalized vesicular or pustular rash illness and categorizes the risk of smallpox according to the patient's signs and symptoms. Figure 175–2 presents a flow chart for laboratory testing of specimens from patients presenting with acute generalized vesicular or pustular rash illness. A two-armed algorithm is presented to reduce the time to receive results and to ensure that testing of high-risk specimens is confined to laboratories with appropriate biosafety levels and expertise. The two arms of the testing algorithm are for (1) specimens from individuals with low- and moderate-risk symptoms and (2) specimens from individuals with high-risk symptoms.

TREATMENT

Therapy primarily was supportive and symptomatic. The skin was kept clean, the bed linen was changed at regular intervals, and local or systemic therapy was provided for the frequent bacterial complications. Attention given to appropriate fluid and nutritional support was required.

Methisazone, convalescent smallpox serum, and vaccinia immune globulin (VIG) were effective in preventing the disease after exposure, but no evidence showed that these agents altered the course of the disease once symptoms occurred.[63] Idoxuridine was used for corneal lesions.[6]

At the present time, methisazone is not available. Stockpiling of VIG is under way in the United States, and intravenous formulations of VIG are being manufactured.[103] Studies with the antiviral cidofovir indicate that this agent may be useful for the treatment of smallpox.[23,79]

PREVENTION

ACTIVE IMMUNIZATION

In the 20th century, many strains of vaccinia virus were used in the effective prophylaxis of smallpox. In 1967, at least 15 strains were in use in various countries.[1] In the late 1960s and 1970s, most vaccinations were performed with the New York City Board of Health (NYCBOH) strain or the Lister (Elstree) strain, which were similar. They were prepared by the freeze-drying (lyophilization) process, and inoculation was performed best with use of a bifurcated needle, with 5 (for primary immunization) to 15 (for re-vaccination) punctures within a small (5-mm diameter) area of the skin of the upper left deltoid region. The vaccination site was not to be covered tightly, although a loose, dry dressing was applied during the height of the reaction for decreasing the possibility of transferring the vaccinia virus by fingers to the eye or to skin lesions, such as insect bites or impetigo lesions. A small but definite risk of developing complications, both local and neurologic, was associated with vaccination.[69,82]

In the late 1990s, concerns arose that smallpox could be used as a weapon of bioterrorism. The events of September 11, 2001 and immediately thereafter highlighted the potential reality of this threat.[3,23,52,53,56,77,94] Estimates were that probably less than 20 percent of the U.S. population had any immunity.[53] These concerns led to recommendations by the Advisory Committee on Immunization Practices (ACIP) for the use of smallpox vaccine to protect persons who may need to work with orthopoxviruses and to prepare for a possible bioterrorism attack.[15] The government took steps to prepare the country in the event of a bioterrorist attack involving smallpox. These measures included building an adequate supply of vaccine and bifurcated needles, enhancing the laboratory capacity for diagnosis of variola and vaccinia virus infection, and training health care and public health personnel.[45]

The current stockpile of vaccine is enough to immunize the entire U.S. population. Studies have shown that the current supply of vaccine is viable and in good titer, although dilution to titers of less than 10^7 may result in a reduced rate of successful vaccination.[43,44]

The primary strategy used to control an outbreak of smallpox and to interdict the transmission of virus has been known as a surveillance and containment (ring vaccination) strategy. This strategy identifies infected persons through intensive surveillance and subsequently both isolates the individuals with the disease and vaccinates household and close contacts of the infected patient. Vaccination also would be recommended under these conditions for contacts of the primary contact with the patient (so-called secondary contacts). This strategy was instrumental in the eradication of smallpox as a naturally occurring disease. Depending on the size of any smallpox outbreak and the availability of resources for rapid and thorough contact tracing, ring vaccination was supplemented previously in areas with identified smallpox cases to include voluntary vaccination of all those individuals without vaccine contraindications. This strategy was pursued to expand the ring of immune individuals within an outbreak area and to reduce further the chance of any secondary transmission of smallpox to patients before they could be identified and isolated.

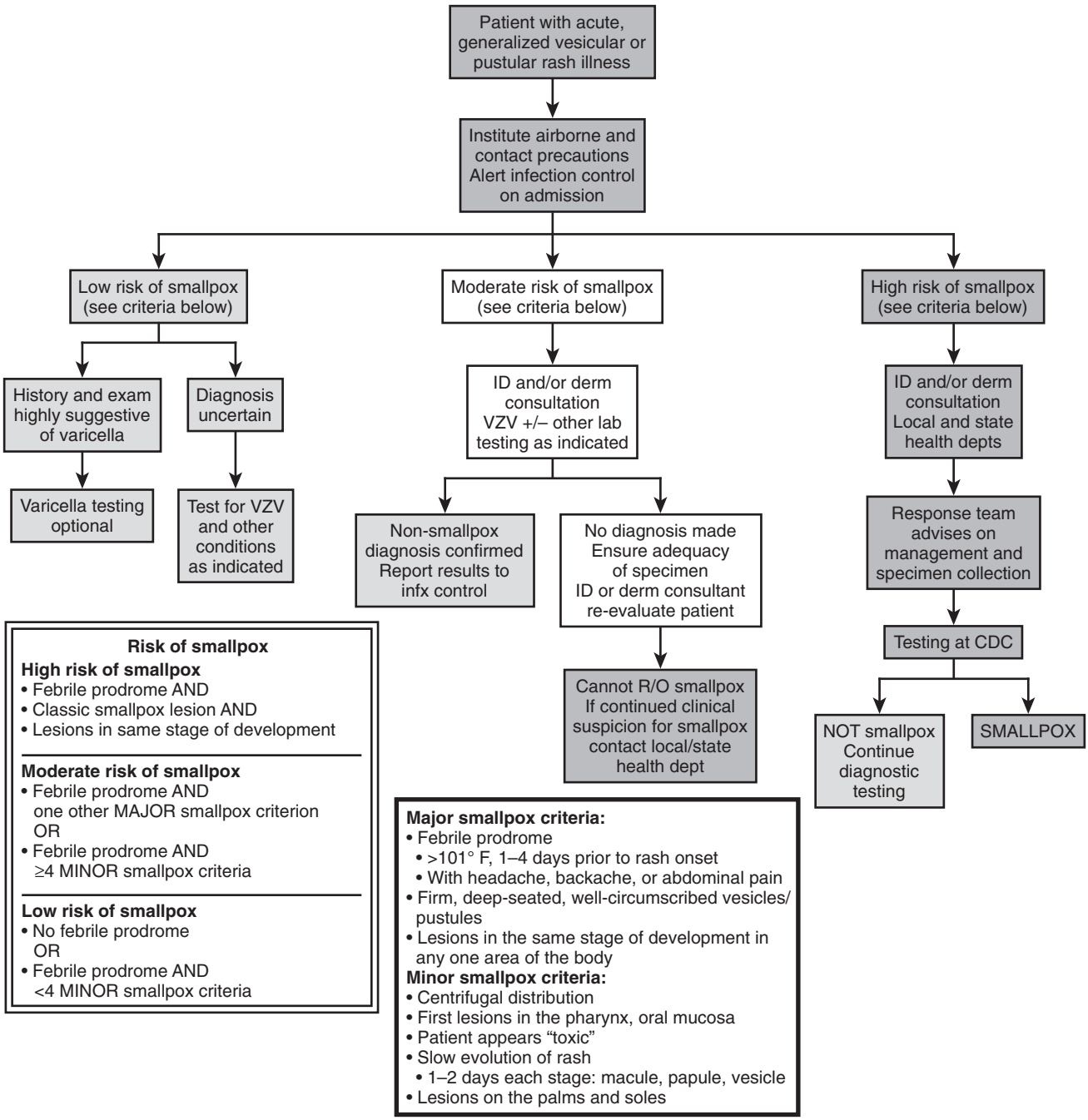

Figure 175–1 Acute, generalized vesicular or pustular rash illness protocol. CDC, Centers for Disease Control and Prevention; Derm, dermatologic; ID, infectious disease; Infx, infection; R/O, rule out; VZV, varicella-zoster virus. *(From Centers for Disease Control and Prevention: CDC Protocol for Evaluating Patients for Smallpox. Available at http://www.bt.cdc.gov/agent/smallpox/diagnosis/rashtestingprotocol.asp.)*

Most recently, the ACIP reconsidered its recommendations for the use of smallpox vaccine. In so doing, the ACIP considered the following factors: the level of disease risk or threat, the expected severe adverse reactions to vaccination, the supply of VIG that may be available, the supply of vaccine, the deployment of vaccine, and the vaccination capacity at state and local levels. In addition to these factors, the following additional assumptions were made: (1) vaccine currently is available only under an investigational new drug protocol and requires appropriate informed consent, patient follow-up, and oversight administratively by federal, state, and local public health officials; (2) based on the information available at the time of the ACIP meeting, the risk of smallpox as a result of deliberate release by terrorists was considered low but not zero; (3) the epidemiology of person-to-person transmission after bioterrorism release of smallpox would be consistent with prior experience; (4) vaccinia vaccine and vaccine immune globulin would be available for use in sufficient supply and would be handled and administered appropriately; and (5) appropriate screening for contraindications to vaccination would be implemented and would include both vaccinated persons and their contacts, and recommended precautions would be taken to minimize the risks of adverse events among vaccinees as well as among their close contacts; (6) healthcare workers and others would be afforded protection from infection through

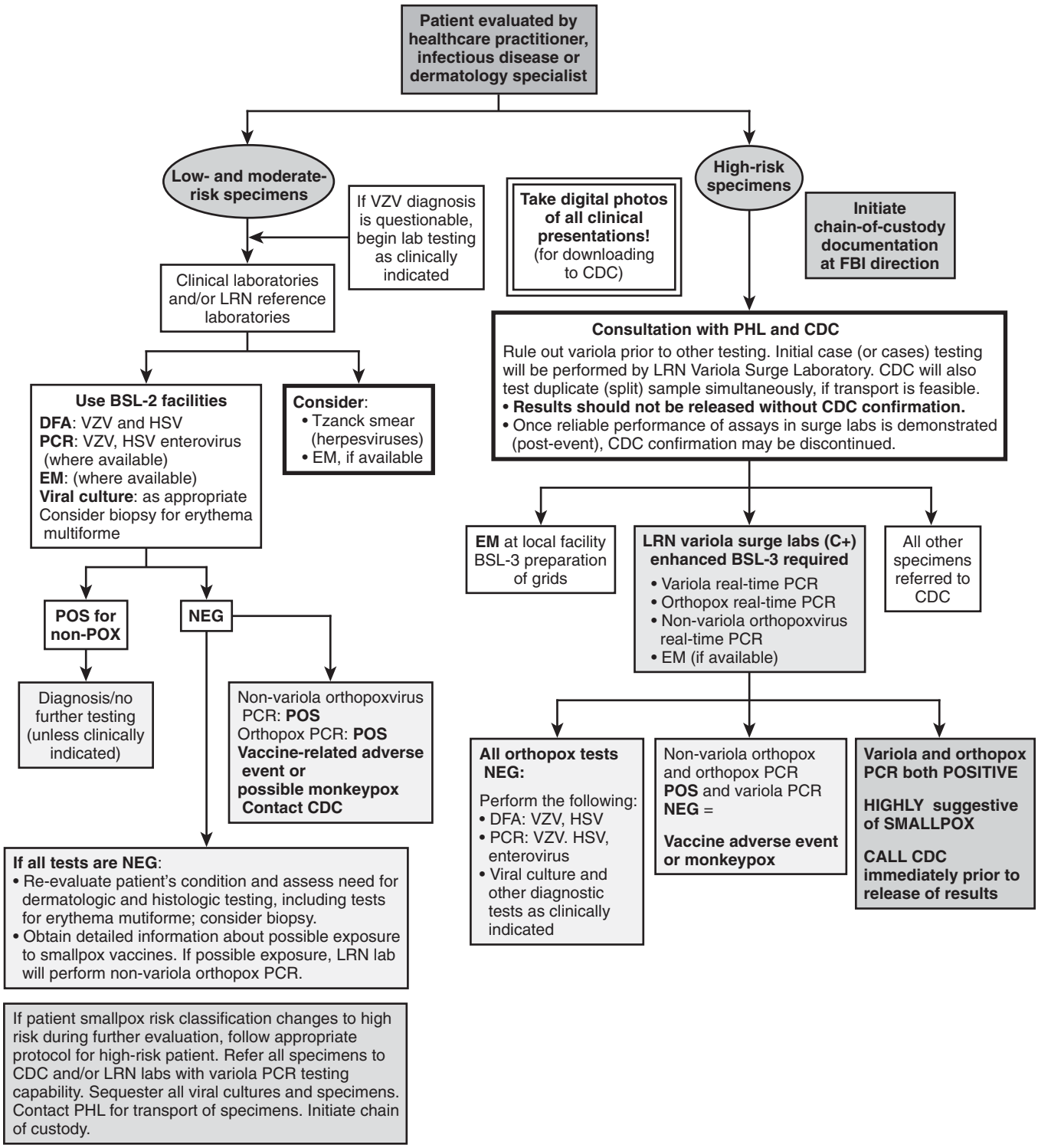

Figure 175–2 Laboratory testing for acute, generalized vesicular or pustular rash illness in the United States. BSL-2, biosafety level 3; CDC, Centers for Disease Control and Prevention; DFA, direct fluorescent assay; EM, electron microscopy; FBI, Federal Bureau of Investigation; HSV, herpes simplex virus; LRN, Laboratory Response Network; NEG, negative; PCR, polymerase chain reaction; PHL, Public Health Laboratory; POS, positive; VZV, varicella-zoster virus. *(From Centers for Disease Control and Prevention: CDC Protocol for Evaluating Patients for Smallpox. Available at http://www.bt.cdc.gov/agent/smallpox/diagnosis/rashtestingprotocol.asp.)*

implementation of appropriate infection control measures, including use of appropriate personal protective equipment; and (7) surveillance and containment would be considered the strategy for control and containment of smallpox, and, furthermore, state and local health departments were presumed to have the capacity to vaccinate the entire population of their areas within a period of several weeks if so indicated.

The CDC developed protocols to permit rapid, simultaneous delivery of smallpox vaccine to every state in U.S. territory within 12 to 24 hours. Smallpox response planning at the federal level

has been conveyed to state and local health departments to address the rapid distribution of the vaccine if vaccination programs are suggested.

The CDC currently recommends that cases of febrile rash illnesses for which smallpox is considered in the differential diagnosis be reported immediately to local or state health departments, or both. After evaluation is performed by local or state health departments, if smallpox laboratory diagnostics are considered necessary, the CDC Rash Illness Evaluation Team should be consulted at 770-488-7100 or 404-639-2888. At this time, laboratory confirmation for smallpox is available routinely only at the CDC. Both a clinical consultation and a preliminary laboratory diagnosis can be completed within a period of 8 to 24 hours.

To assist public health and other medical personnel in evaluating the possibility of smallpox in patients who present with febrile rash illnesses, the CDC developed a rash illness assessment algorithm. Copies of this algorithm are available from state health departments and may be viewed on the CDC Web site at http://www.cdc.gov/agent/smallpox/diagnosis/nskalgorithm. Updated additional information can be found on the CDC interim smallpox response plan and guidelines at the following Web site: http://www.bt.cdc.gov/agent/smallpox.

Medical evaluation of the current risks of acquiring smallpox under current conditions and in the absence of a case of smallpox or a confirmed smallpox bioterrorism threat compared with the potential risks of developing vaccine complications was such that the ACIP concluded that vaccination of the general population was not recommended because the potential benefits of vaccination did not outweigh the risks of vaccine complications. In 2001, the ACIP recommended smallpox vaccine for laboratory workers who directly handle (1) cultures or (2) animals contaminated or infected with vaccinia, recombinant vaccinia viruses, or other orthopoxviruses that infect humans (e.g., monkeypox, cowpox).[15] Other health care workers (e.g., physicians, nurses) whose contact with these viruses is limited to contaminated material (e.g., dressings) but who adhere to appropriate infection control measures are at lower risk of inadvertently acquiring infection than are laboratory workers. However, because a theoretical risk of developing infection does exist, vaccination may be considered for this group. Because of the low risk of developing infection, vaccination is not recommended for persons who do not handle virus cultures or materials directly or who do not work with animals contaminated or infected with these viruses. According to available data on the persistence of neutralizing antibody after vaccination, persons working with vaccinia, recombinant vaccinia viruses, or other non-variola orthopoxviruses should be re-vaccinated every 10 years.

In 2003, the ACIP and the Healthcare Infection Control Practices Advisory Committee (HICPAC) published supplemental recommendations for using smallpox vaccine in a pre-event vaccination program.[17] A summary of these recommendations is presented here:

To facilitate preparedness and response, smallpox vaccination is recommended for persons designated by public health authorities to conduct investigation and follow-up of initial smallpox cases that could necessitate direct patient contact. The ACIP recommends that each state and territory establish and maintain more than one smallpox response team. The ACIP and the HICPAC recommend that each acute-care hospital identify health care workers who can be vaccinated and trained to provide direct medical care for the first smallpox patients requiring hospital admission and to evaluate and manage patients who are suspected as having smallpox. When feasible, the first-stage vaccination program should include previously vaccinated health care personnel, to decrease the potential for adverse events. Additionally, persons administering smallpox vaccine in this pre-event vaccination program should be vaccinated.

Smallpox vaccine is administered by using the multiple-puncture technique with a bifurcated needle, packaged with the vaccine and diluents. According to the product labeling, two to three punctures are recommended for primary vaccination and 15 punctures for re-vaccination. A trace of blood should appear at the vaccination site after 15 to 20 seconds; if no trace of blood is visible, an additional three insertions should be made by using the same bifurcated needle without reinserting the needle into the vaccine vial. If no evidence of vaccine take is apparent after 7 days, the person can be vaccinated again.

Optimal infection control practices and appropriate site care should prevent transmission of vaccinia virus from vaccinated health care workers to patients. Health care personnel providing direct patient care should keep their vaccination sites covered with gauze in combination with a semipermeable membrane dressing to absorb exudates and to provide a barrier for containment of vaccinia virus to minimize the risk of transmission; the dressing should also be covered by a layer of clothing. Dressings used to cover the site should be changed frequently to prevent accumulation of exudates and consequent maceration. The most critical measure in preventing contact transmission is consistent hand hygiene. Hospitals should designate staff to assess dressings for all vaccinated health care workers. When feasible, staff responsible for dressing changes for smallpox health care teams should be vaccinated; all persons handling dressings should observe contact precautions. Administrative leave is not required routinely for newly vaccinated health care personnel, unless they are physically unable to work as a result of systemic signs and symptoms of illness, have extensive skin lesions that cannot be adequately covered, or are unable to adhere to the recommended infection control precautions. Persons outside the patient care setting can keep their vaccination sites covered with a porous dressing; hand hygiene remains key to preventing inadvertent inoculation.

The U.S. Food and Drug Administration recommended that recipients of smallpox vaccine be deferred from donating blood for 21 days or until the scab has separated. Contacts of vaccinees, who have inadvertently contracted vaccinia, also should be deferred from donating blood for 14 days after complete resolution of their complication.

CURRENT SMALLPOX VACCINE

The smallpox vaccine currently licensed and used in the recent vaccination efforts in the United States is a lyophilized preparation of infectious vaccinia virus produced in the 1970s (Dryvax; Wyeth Laboratories; available from the CDC). The vaccine was prepared from calf lymph with a seed virus derived from the NYCBOH strain of vaccinia; it has a concentration of 10^8 pock-forming units (pfu) per milliliter. Vaccine is administered by using the multiple-puncture technique with a bifurcated needle.[17] The CDC is holding three other formulations in reserve: another previously manufactured vaccine from Sanofi Pasteur, as well as two newly developed vaccines: ACAM1000, which is grown in human embryonic lung cell culture (MRC-5), and ACAM2000, which is grown in African green monkey cells (VERO).[18]

After percutaneous administration of a standard dose of smallpox vaccine, neutralizing or hemagglutination-inhibition antibody develops in more than 95 percent of primary vaccines (i.e., persons receiving their first dose of vaccine) at a titer of 1:10 or higher.[21] Neutralizing antibody titers of 1:10 or higher are found in 75 percent of persons for 10 years after receipt of two doses and for up to 30 years after receipt of three doses of vaccine.[32,74] The level of antibody required for protection against vaccinia infection is not known. However, when the response to re-vaccination is used as an indication of immunity, fewer than 10 percent of persons with neutralizing titers of 1:10 or higher exhibit a primary-type response at re-vaccination as compared

with more than 30 percent of persons with titers of less than 1:10.[76]

In the present era with concerns relating to smallpox as a bioterrorism agent, more precise knowledge about the duration of immunity in previously vaccinated persons is critical.[22] In this regard, a study performed in Liverpool, England, in 1902 to 1903 is of interest. In this study, researchers found that the smallpox case-fatality rate in adults older than 50 years who had been vaccinated in infancy was 5.5 percent, whereas it was 50 percent in adults of similar age who had not been vaccinated. Similar, but better, protection was noted in younger adults, and in general, adults who had been vaccinated as infants had less severe disease than did those who had not been vaccinated.

Since concerns were first raised over the country's preparedness in the event of a bioterrorism attack involving smallpox, interest has developed in producing smallpox vaccines that have fewer complications than those associated with Dryvax, that can be generated in tissue culture, and that can be used in patients who are immune-compromised and/or have atopic dermatitis.

Since October, 2002, tissue culture cell vaccines are being prepared by Acambis/Baxter Laboratories. Vero monkey kidney cells and a human fibroblast cell line (MRC5) are used to prepare the vaccine. The NYCBOH strain is the seed virus for both vaccines. Phase II studies of ACAM2000 (prepared in VERO cells) have shown non-inferiority of the 6.8×10^7 pfu/mL concentration when compared with Dryvax in previously unimmunized subjects.[2,78] No significant difference in adverse events between Dryvax and ACAM2000 has been reported. If cell culture vaccines are successful in clinical trials, one will supplant the calf-lymph vaccine.

Alternatives to NYCBOH strains also have received attention. German scientists in the 1950s developed the modified vaccinia Ankara (MVA) strain that had been used in vaccine studies in the 1970s. At that time, attenuated vaccine with MVA was studied with children, adult, and elderly subjects, as well as in subjects with skin conditions, and it demonstrated decreased neurologic virulence as compared with Dryvax and no serious adverse events.[75] Although MVA was never tested in field conditions with naturally occurring smallpox, experts suggested exploring the use of MVA as an alternative to vaccines based on NYCBOH strain because of its decreased virulence in older studies. More recently, phase I trials using an attenuated clone derived from MVA strain 571 (IMVAMUNE) at different titers demonstrated good antibody response as well as few side effects, although the sample size of 86 subjects was small.[97]

Studies also are under way to evaluate recombinant envelope protein subunit vaccines. Several groups[36,41,47,57,85] explored vaccination with recombinant variola virus proteins (intracellular mature virion L1R and A27L and extracellular enveloped virions A33R and B5R). These groups achieved protection against lethal doses of various orthopoxviruses in mice and rhesus macaques.

Response to Vaccination (Fig. 175–3)

A papule develops at the site of vaccination 2 to 5 days after percutaneous administration of vaccinia vaccine to a nonimmune person (i.e., primary vaccination).[18,45] The papule becomes vesicular, then pustular, and reaches its maximum size in 8 to 10 days. The pustule dries and forms a scab that separates within 14 to 21 days after vaccination, and then it leaves a typical scar. Primary vaccination can produce swelling and tenderness of regional lymph nodes, beginning 3 to10 days after vaccination and persisting for 2 to 4 weeks after the skin lesion has healed. Maximum viral shedding occurs 4 to14 days after vaccination, but vaccinia can be recovered from the site of vaccination until the scab separates from the skin.[68,104]

Formation by day 6 to 8 of a papule, vesicle, ulcer, or crusted lesion, surrounded by an area of induration, is referred to as a *major reaction* or a *take*, and it signifies a response to vaccination. It usually results in formation of a scar. Before the eradication of smallpox, persons who had scar formation had lower attack rates when they were exposed to smallpox than did those who had no scar formation. A "take," then, has become a surrogate of smallpox immunity. All other reactions are considered equivocal and raise concern for lack of immunity to smallpox. On the other end of the spectrum of vaccine reactions, 10 percent of first-time vaccinees experience large reactions (>10 cm). These reactions generally do not signify an adverse event and rarely result in secondary bacterial infection.[18]

Side Effects and Adverse Events

The risk of adverse events resulting from smallpox vaccine is presumed potentially to be at least as great, in terms of severity and frequency of events, as during the era when universal vaccinia vaccination was performed[18,46] (Figs. 175–4 through 175–7). One

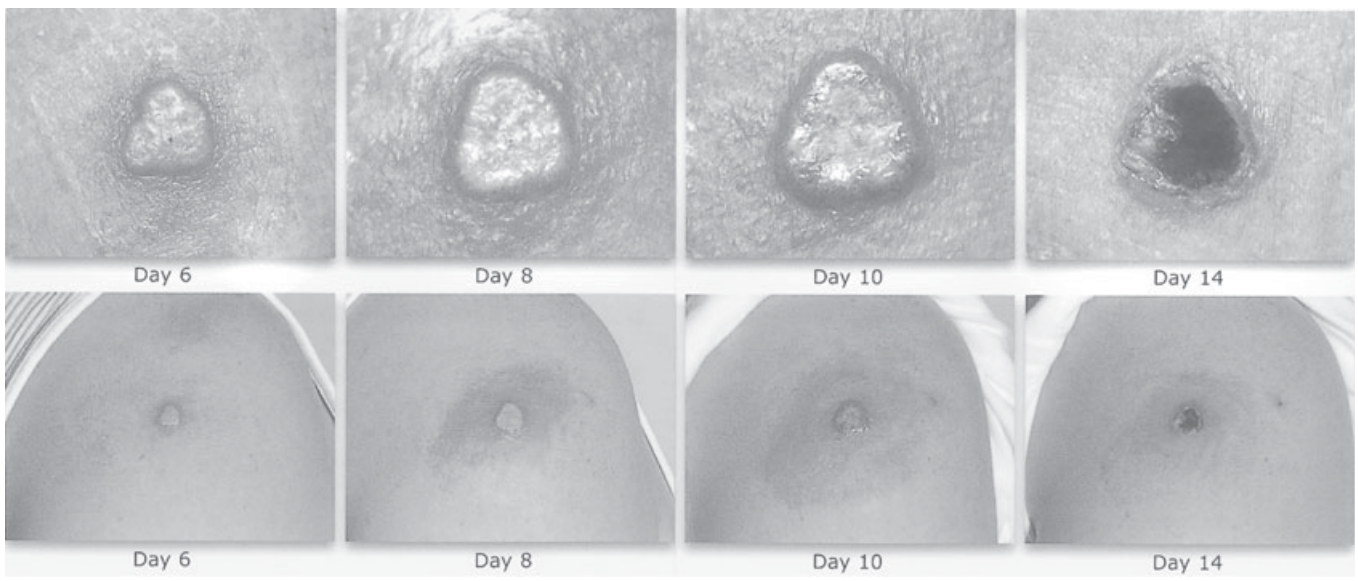

Figure 175–3 **A** to **D**, Typical sequential appearance of the response ("take") following primary smallpox vaccination in a child.

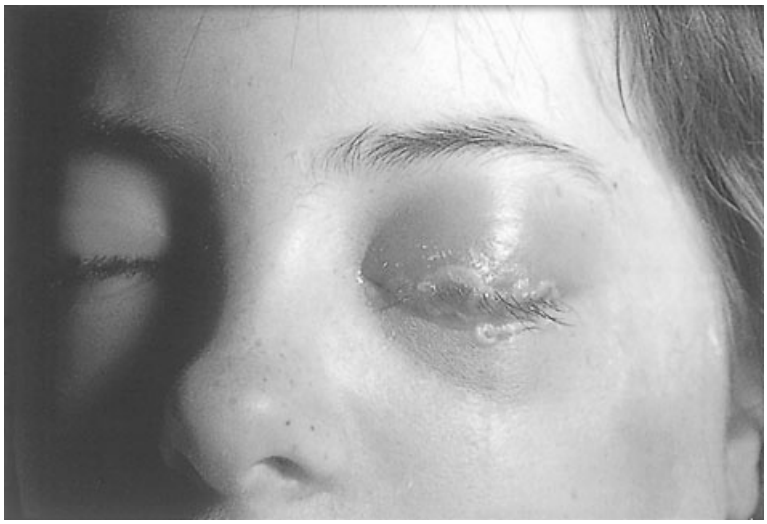

Figure 175–4 Accidental implantation of vaccinia virus, after primary vaccination, by autoinoculation or contact transfer.

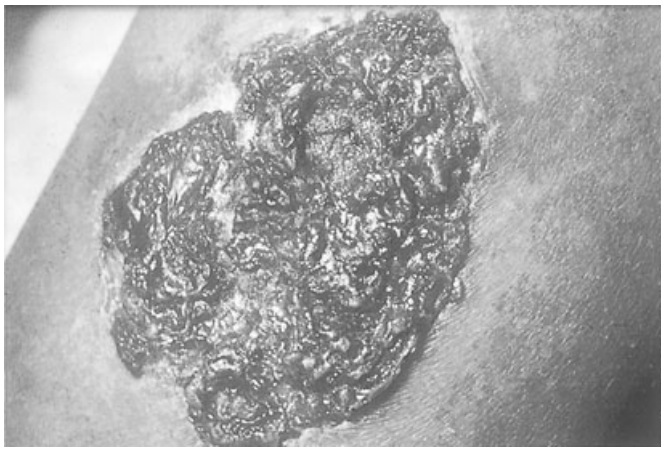

Figure 175–5 Secondary bacterial infection at the site of primary vaccination.

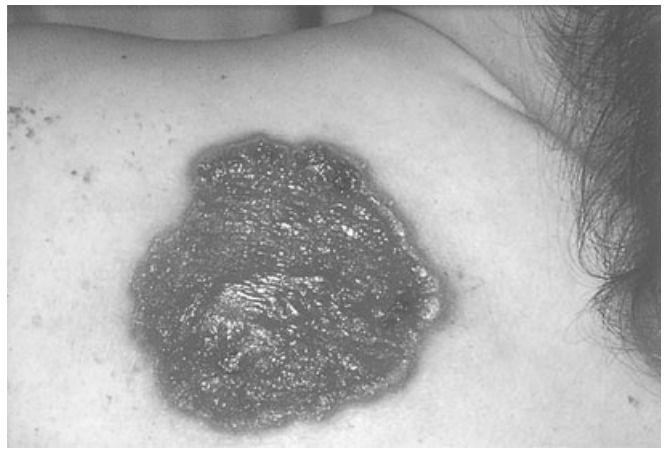

Figure 175–7 Progressive vaccinia in an immunocompromised person who was inadvertently vaccinated.

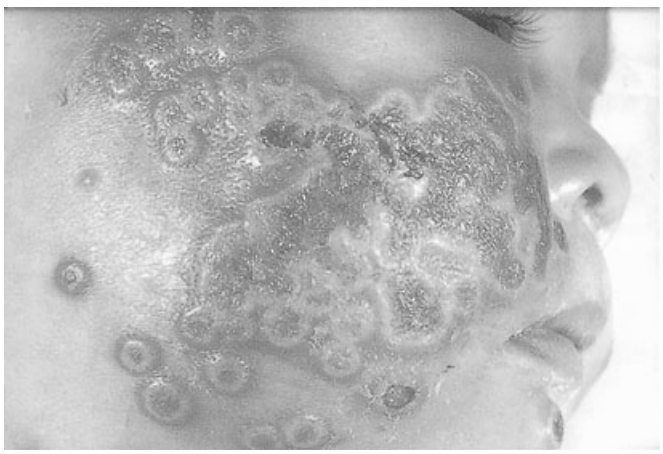

Figure 175–6 Eczema vaccinatum. Accidental implantation of vaccinia virus in a child with eczema.

can suspect that the number of adverse reactions may be increased in frequency in the current era compared with the 1960s and 1970s because a larger proportion of the population of the United States and the world may have an inherited or acquired a form of immunodeficiency or may be receiving a variety of agents that suppress the immune system. Factors thought to predispose to development of adverse events include pregnancy or breast-feeding, extensive skin eruptions, atopic dermatitis, T-cell immune defect, immunosuppressive therapy, inflammatory or disruptive diseases of the cornea, and age younger than 1 year.[18]

Fever occurs commonly after smallpox vaccination. As many as 70 percent of children have 1 or more days with a temperature of 37.8° C (100° F) or higher 4 to 14 days after receiving primary vaccination,[21,76] and 15 to 20 percent have temperatures of 38.9° C (102° F) or higher. After receiving re-vaccination, 35 percent of children have temperatures of 37.8° C (100° F) or higher, and 5 percent have temperatures of 38.9° C (102° F) or higher.[76] Fever after vaccination or re-vaccination occurs less commonly in adults than in children.

An erythematous or urticarial rash may occur approximately 10 days after receipt of primary vaccination. The vaccinee usually is afebrile, and the rash resolves spontaneously within 2 to 4 days. Rarely, bullous erythema multiforme (Stevens-Johnson syndrome) occurs.[67]

Inadvertent inoculation at other sites, the most frequent complication of vaccinia vaccination, accounts for approximately half of all complications of primary vaccination and re-vaccination. Inadvertent inoculation usually results from autoinoculation of

TABLE 175–1 Rates of Reported Complications Associated with Vaccinia Vaccination (Cases/Million Vaccinations)

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Please refer to the printed publication.

From Lane, J. M., Ruben, F. L., Neff, J. M., and Millar, J. D.: Complications of smallpox vaccination, 1968: Results of 10 statewide surveys. J. Infect. Dis. 122:*303-309, 1970.*

vaccine virus transferred from the site of vaccination. The most common sites involved are the face, eyelid, nose, mouth, genitalia, and rectum. Most lesions heal without specific therapy, but VIG may be useful for cases of ocular implantation (see "Treatment of Complications of Smallpox Vaccine").

Generalized vaccinia in persons who have no underlying illness is characterized by a vesicular rash of varying extent. The rash generally is self-limited and requires little or no therapy, except in patients whose conditions appear to be toxic or in those who have a serious underlying illness.

Other expected systemic symptoms associated with smallpox vaccination include malaise, myalgia, headache, chills, nausea, and fatigue (0.3-37%).[43,44] Soreness at the site of the vaccination is a very common development, as are local lymphadenopathy and erythema surrounding the vaccination site.[45]

Expected local events occurring relatively infrequently require symptomatic treatment. They include satellite lesions within 2.5 cm of the primary lesion, viral lymphangitis, local edema, and intense inflammation surrounding the papule.[43-45]

More severe complications of vaccinia vaccination include eczema vaccinatum, progressive vaccinia, myopericarditis, and post-vaccinial encephalitis. These complications, with the exception of myopericarditis, occur at least 10 times more often among primary vaccinees than among re-vaccinees and more frequently among infants than among older children and adults.[70-72]

The number of adverse events per million doses of primary smallpox vaccination and re-vaccination is reported in Table 175–1. The rates described are based on surveys of vaccination-associated complications that occurred in the late 1960s in the United States. Whether the growth of vaccine in tissue culture rather than on calf skin will change the neurotropism of vaccinia virus is not known. The high prevalence of immunosuppression in the United States today as compared with the late 1960s may render the rates of vaccinia necrosum higher than in 1968. The rate of post-vaccinial encephalitis in adults was difficult to document in the 1960s because very few adults were primary vaccinees. Studies performed in Europe showed higher rates of post-vaccinial encephalitis in adults than in young children. Studies performed since 2002, when efforts were made to vaccinate members of the military and civilians, have shown similar rates of adverse reactions, although cardiac side effects have been more notable (Table 175–2).

Eczema vaccinatum is a localized or systemic dissemination of vaccinia virus in persons who have eczema or a history of eczema and other chronic or exfoliative skin conditions (e.g., atopic dermatitis). The illness often is mild and self-limited but may be severe and occasionally fatal. The most serious cases in vaccine recipients occur in primary vaccinees and appear to be independent of the activity of the underlying eczema.[98] Severe cases also have been observed after contact infection has occurred.

TABLE 175–2 Number of Reported Events Associated with Smallpox Vaccination in the Military: 2002 to 2007

Event Type	Department of Defense Experience*	Grabenstein and Winkenwerder†
Inadvertent inoculation	61	Self: 48 Transfer to contact: 21
Generalized vaccinia	43	36
Eczema vaccinatum	1	0
Progressive vaccinia	0	0
Myopericarditis	140	37
Ischemic heart disease	16	N/A
Encephalitis	N/A	1
Treated with VIG	6	N/A
Death‡	8	0

**Department of Defense experience: December, 2002 to May, 2007, 1,200,000 million vaccinees.[28]*
†Experience of U.S. military: December, 2002 to May, 2003, 450,293 vaccinees.[50]
‡After review, one death was the result of an acute lupus-like illness that may have been caused by the vaccine, and others were thought to result from other causes.[28]
NA, not applicable; VIG, vaccinia immune globulin.

Progressive vaccinia (vaccinia necrosum) is a severe, potentially fatal illness characterized by progressive necrosis in the area of vaccination, often with metastatic lesions. It occurs almost exclusively in persons with cellular immunodeficiency.

More recent experience with vaccination highlights how rare the incidence of eczema vaccinatum and progressive vaccinia are when a population is screened for susceptible individuals. Among more than 1,200,000 persons vaccinated through the U.S. Department of Defense, only a single case of eczema vaccinatum occurred, and no cases of progressive vaccinia were reported.[28,50,96]

In the 1960s, cardiac complications were reported but were not considered significant. Myopericarditis now is thought to be a true adverse event related to smallpox vaccine, although most cases resolve without further complications.[10,31] Cases of ischemic heart disease occurring after receipt of smallpox vaccination also have been reported. Although this association has not been shown to be causal, the ACIP recommended excluding persons with

cardiac disease from participation in current smallpox vaccination programs.[17] The incidence of cardiac events in children is not known because the recent vaccination program has targeted adults.

The most serious complication is post-vaccinial encephalitis. Usually, it affects primary vaccinees younger than 1 year of age. Fifteen to 25 percent of affected vaccinees with this complication die, and 25 percent have permanent neurologic sequelae.[49,71,72]

Death rarely occurs after receipt of vaccinia vaccination; reports note approximately one to two deaths per million primary vaccinations and 0.1 death per million revaccinations. Death most often is the result of post-vaccinial encephalitis or progressive vaccinia.

Vaccinia may be transmitted when a recently vaccinated person has contact with a susceptible person. In the CDC's 10-state survey of complications of smallpox vaccination, the risk of transmission to contacts was 27 infections per million total vaccinations; 44 percent of these contact cases occurred in children 5 years of age or younger.[72] Since 1980, several cases of contact transmission of vaccinia from vaccinated military recruits have been reported and include six cases transmitted by a single vaccine recipient.[11,13,14]

Since 2001, among 1,200,000 vaccinated military personnel, 61 suspected instances of transfer of vaccinia occurred, 36 of which were laboratory confirmed.[28] This number included two cases of tertiary transfer, one from a service member to his wife and then to their breast-feeding baby, and another among male sports partners.[19,45] Fetal vaccinia occurs rarely (<40 reported cases), but usually it is fatal.[46]

More than 60 percent of cases of contact transmission result in uncomplicated inadvertent inoculation. Approximately 30 percent of these cases result in eczema vaccinatum, which may be fatal.[72] Eczema vaccinatum may be more severe in contacts than in vaccinated persons, possibly because of simultaneous multiple inoculation at several sites.[24,71] Contact transmission rarely results in post-vaccinial encephalitis or vaccinia necrosum.

Precautions and Contraindications

Before administering smallpox vaccine, the physician should obtain a careful history to document the absence of contraindications to vaccination among both vaccinees and household contacts of vaccinees. Special efforts should be made to identify vaccinees and household contacts who have eczema, a history of eczema, or immunodeficiencies. Vaccinia vaccine should not be administered if these conditions are present in a possible recipient or, in most instances, if they are present in household contacts.

Specific precautions and contraindications include a history or the presence of eczema, pregnancy, altered immunocompetence, infection with human immunodeficiency virus (HIV), allergies to vaccine components, underlying heart disease, or three or more major known cardiac risk factors. The reader is referred to the CDC for more complete information on contraindications.[17,18]

Prevention of Contact Transmission of Vaccinia

Vaccinia virus may be cultured from the site of primary vaccination beginning at the time that a papule develops (2 to 5 days after vaccination) until the scab separates from the skin lesion (14 to 21 days after vaccination). During this time, care must be taken to prevent spread of the virus to another area of the body or to another person. Present recommendations state that the vaccination site should be covered at all times with a porous bandage until the scab has separated and the underlying skin has healed.[15] An occlusive bandage should not be used. The vaccination site should be kept dry. When the vaccinee bathes, the site should be covered with an impermeable bandage. Vaccinated health care workers may continue to have contact with patients, including those with immunodeficiencies, as long as the vaccination site is covered and good handwashing technique is maintained.

Semipermeable polyurethane dressings (e.g., Op-Site) are effective barriers to vaccinia and recombinant vaccinia viruses.[42] However, exudate may accumulate beneath the dressing, so care must be taken to prevent viral contamination when the dressing is removed. In addition, accumulation of fluid beneath the dressing may increase the maceration of the vaccination site. Accumulation of exudate may be decreased by first covering the vaccination with dry gauze, then applying the dressing over the gauze.

Studies have shown variable results with respect to vaccinia recovery from dressings. A 2004 study found no difference in the percentage of positive PCR results from bandage site among self-adhesive dressing, gauze with tape, or gauze with porous dressing.[99] However, a similar study by the same researchers found higher rates of vaccinia recovery by culture from gauze dressings versus occlusive semipermeable bandage or foam hydrocellular bandage, although foam dressing resulted in more maceration of the site.[91] One case of autoinoculation occurred in a patient with gauze dressings. Another analysis of vaccination sites covered with adsorbent base with an overlying semipermeable layer saw no cases of secondary vaccinia or autoinoculation, a finding suggesting that this combination may decrease the rates of these events.[92]

Of interest is that the aforementioned recommendations differ considerably from recommendations made before the discontinuation of routine smallpox vaccination.[67] Specifically, dressings were not recommended for the reason that secondary infections were more likely to occur when dressings were used because the vaccine was not sterile. My opinion is that the present dressing recommendations prolong the duration of the vaccination lesion and, therefore, prolong the period of contagion. At present, care should be taken not to dress vaccination sites excessively, and sites should be examined daily for signs of maceration and possible secondary bacterial infections. The most important measure to prevent inadvertent implantation and contact transmission from vaccinia vaccination is thorough handwashing after changing the bandage or after any other contact with the vaccination site.

Treatment of Complications of Smallpox Vaccine

The only product currently available for the treatment of complications of smallpox vaccination is VIG. Cidofovir is being studied as a potential therapy.[18] VIG is an isotonic sterile solution of the immunoglobulin fraction of plasma from persons vaccinated with vaccinia vaccine. It is effective for the treatment of eczema vaccinatum and for some cases of progressive vaccinia, and it may be useful in the treatment of ocular vaccinia resulting from inadvertent implantation. However, VIG is not recommended for treating isolated keratitis. VIG also is recommended for treatment of severe generalized vaccinia if the patient has a toxic condition or a serious underlying disease. VIG is of no benefit in the treatment of post-vaccinial encephalitis and probably is of no benefit in vaccine-induced myocarditis.[10]

VIG is available as an intramuscular (VIGIM) and an intravenous (VIGIV) preparation.[18] It should be administered as early as possible after the onset of symptoms. Doses may be repeated, usually at intervals of 2 to 3 days, until recovery begins (e.g., no new lesions appear).[15]

The CDC is the only source of VIG for civilians. An adult patient with progressive vaccinia is reported to have benefited with the administration of ribavirin and VIG.[65]

Ocular vaccinia should be treated in conjunction with an ophthalmologist who performs serial slit-lamp examinations. Topical off-label use of ophthalmic trifluoride or vidarabine has been recommended by some ophthalmologists.

Cidofovir also may prove useful for the treatment of vaccinia complications. Cidofovir has been shown to be effective in treating other poxvirus infections in both humans and animal models.[27] At this time, use of this drug in treating complications of smallpox vaccination is recommended only under a research protocol sponsored by the CDC. Under this protocol, cidofovir may be released for civilian use by the CDC and for military use by the Department of Defense if (1) a patient fails to respond to VIG, (2) a patient is near death, or (3) all inventories of VIG have been exhausted.[18]

The drawbacks of cidofovir, including its poor oral bioavailability and nephrotoxicity, have encouraged research into other antiviral agents that could be used to treat orthopoxvirus infections, including vaccinia. Currently, promising research in mouse models shows effectiveness of experimental antiviral compounds that may one day be tested and used in humans.[86,87,105]

Misuse of Vaccinia Vaccine

Smallpox vaccine never should be used therapeutically for any reason. No evidence exists that it has any value in the treatment or prevention of recurrent herpes simplex virus infection, warts, or any disease other than that caused by human orthopoxviruses.[64] Misuse of vaccinia vaccine to treat herpesvirus infections has been associated with severe complications.[12,95]

Smallpox Vaccine Availability

The CDC is the only source of smallpox vaccine and VIG for civilians.

PASSIVE IMMUNIZATION AND ANTIVIRAL PROTECTION

Transient protection after exposure was provided by VIG when available.[63] Because this material often was in short supply, the use of methisazone was considered, although this drug was not licensed and was available only on special request. When used prophylactically, methisazone provided some measure of protection. Vomiting was a frequent side effect, and dosing presented problems in some persons.

CONTROL OF SOURCES OF INFECTION

All known contacts of persons with cases needed to be vaccinated or re-vaccinated promptly and kept under surveillance until at least 17 days transpired after the last contact with a known case. When fever developed in such persons, prompt isolation was required until the nature of the illness was determined. All personnel in contact with an index case or involved in the surveillance of contacts needed to be vaccinated or re-vaccinated.

Environmental isolation and disinfection precautions also were required because the virus persisted in crusts for many months. Proper double wrapping and disinfection of all articles leaving the patient's room were necessary. Precautions also were required to prevent transfer of crusts by the shoes or clothing of personnel to areas outside the isolation unit. Isolation precautions for the patient were necessary until all crusts had been shed. After recovery or death of the patient, terminal disinfection of the room was required.

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CHAPTER 176

Monkeypox and Other Poxviruses

James D. Cherry ❂ Samantha Johnston

Smallpox, which is caused by variola virus (see Chapter 175), at one time was the most important human poxvirus disease, but this disease has been eradicated from the world. Today, only one human poxvirus, molluscum contagiosum virus, causes specific human illness. In addition, nine other poxviruses can cause human infections. Eight of these viruses are acquired zoonotically, and the other, vaccinia, is acquired by active immunization, or inadvertently by spread from a vaccinated person or laboratory accident.

PROPERTIES OF THE VIRUSES

CLASSIFICATION

The family *Poxviridae* has two subfamilies: *Chordopoxvirinae* (vertebrate poxviruses) and *Entomopoxvirinae* (insect poxviruses).[39,75] Eight genera are included in the *Chordopoxvirinae* subfamily, and four of these genera contain species that infect humans (Table 176–1).

STRUCTURE

Poxviruses are the largest animal viruses and are discernible by light microscopy.[8,75] In general, by electron microscopy, orthopoxviruses appear brick shaped, with a length of 350 nm and a width of 270 nm. They contain double-stranded DNA genomes that vary from 130 to 300 kbp, depending on the particular species. A 30-nm lipoprotein bilayer (envelope) surrounds the virus core. The envelope contains seven or more distinct glycoproteins. Parapoxvirus organisms have a different structure than do *Orthopoxvirus*, *Yatapoxvirus*, and *Molluscipoxvirus* organisms. They are ovoid and vary from 260 to 160 nm for orf virus to 300 to 190 nm for pseudocowpox virus.

TABLE 176–1 Poxviruses That Can Cause Human Illness

Genus	Species
Orthopoxvirus	Variola virus
	Monkeypox virus
	Vaccinia virus
	Cowpox virus
Parapoxvirus	Orf virus
	Bovine papular stomatitis virus
	Pseudocowpox virus
Yatapoxvirus	Tanapoxvirus
	Yabapoxvirus
Molluscipoxvirus	Molluscum contagiosum virus

SPECIFIC VIRUSES AND THEIR ILLNESSES

MONKEYPOX VIRUS*

Monkeypox virus was isolated first from sick laboratory primates in Copenhagen in 1958.[102] Human disease occurs in a large geographic area from Sierra Leone in the west to the Democratic Republic of Congo in the east.[58] The disease was not recognized as being distinct from smallpox until 1970, when, after smallpox had been eliminated, a similar illness continued to circulate in the Democratic Republic of Congo (formerly Zaire).[35,51,58,77] To understand the illness better, the World Health Organization (WHO) launched a surveillance campaign from 1981 to 1986 in that country. During this period and in the preceding 11 years, 338 cases, with an animal source of infection suspected in 245 and secondary transmission in 93 instances, were identified. Most cases were found in children (mean age, 4.4 years).[58,74,77] Since the WHO's initial efforts in monkeypox surveillance were initiated, several isolated outbreaks, mostly in the Democratic Republic of Congo, have been reported in the literature.

Epidemiology

Epidemiologic studies conducted since 1970 identified several outbreaks of human monkeypox, with most cases arising in the Democratic Republic of Congo. As immunity to smallpox waned, concerns were raised that person-to-person transmission could possibly allow monkeypox to replace a niche previously occupied by smallpox.[50] Between 1970 and 1979, 47 cases of human monkeypox were identified, 38 of which occurred in the Democratic Republic of Congo. Seven of these cases were fatal. Of the 47 reported cases, secondary transmission occurred in 4, for secondary attack rates of 3.3 percent among susceptible contacts.[7,50] From 1981 to 1986, Jezek and associates[63] studied 338 patients and found that among patients with primary cases, 50 percent were younger than 4 years of age, and 93 percent were younger than 14 years of age.[61] Ninety-six percent of these children were not vaccinated against smallpox.[6] Animal sources were suspected in 245 of 338 cases, and human-to-human transmission occurred in the remaining 93 cases. Analysis of unvaccinated contacts of

*See references 6, 7, 17-24, 35, 51, 53, 58, 62, 63, 65, 74, 76, 77, 86.

the patients showed a secondary attack rate of 9.3 percent.[6] The longest chain of infection was four generations.[61]

Reporting decreased in the late 1980s and early 1990s because of political instability in the region; from 1996 to 1998, 511 cases of human monkeypox were identified, again mostly in the Democratic Republic of Congo.[16,50] Analysis of 320 possible and probable cases showed attack rates of 18 percent in susceptible individuals, 3 percent if vaccinated and 26 percent if unvaccinated.[50] Eighty-five percent of cases occurred in patients younger than 16 years of age. The case-fatality rate was 1.5 percent, lower than the previously reported rate of 10 percent.[16] The definition of a case as fever and vesiculopustular rash possibly included numerous infections with varicella, thus artificially increasing the rates of reported secondary transmission as well as artificially decreasing the case-fatality rates.[35]

Meyer and colleagues[74] used viral isolation and amplification by polymerase chain reaction (PCR) to detect monkeypox after a 2001 outbreak in the Democratic Republic of Congo. The group found that co-infection and co-transmission of varicella could have misrepresented actual cases of monkeypox. Of the seven small outbreaks, two were proven to be the result of monkeypox (16 cases, 4 deaths); two showed evidence of co-circulating monkeypox and varicella (7 cases, 1 death); two outbreaks were the result of varicella-zoster virus (6 cases, 0 deaths); and neither virus was isolated in the final outbreak. Children were the principal victims.

Disease surveillance continues in the Democratic Republic of Congo, and reports indicate that many more cases occurred than those reported in the literature, with some 1265 cases noted between January 1998 and December 2002. Specimens were collected in 215 cases and showed evidence of human monkeypox infection in 88 cases. The ages of patients ranged from 10 months to 38 years, with mean age of 16.5 years.[77]

Of concern in the United States is that, in May of 2003, the Centers for Disease Control and Prevention (CDC) reported the first outbreak of monkeypox in humans in the Western Hemisphere among 72 patients, 37 of whom had laboratory-confirmed cases.[17-21,26] No case fatalities and no cases of secondary transmission occurred. Two severe cases in children required hospitalization: one girl developed encephalitis that resolved over the course of a 14-day hospitalization, and a second girl developed diffuse oral and pharyngeal lesions that impaired swallowing; she also recovered.[19] The outbreak was linked to the distribution of pet North American prairie dogs that had become ill after being housed with an infected rope squirrel and an infected giant pouched rat shipped from Ghana. Patients were identified in Illinois, Indiana, Kansas, Missouri, Ohio, and Wisconsin.[17-21,52,86] PCR analysis of tissue obtained from the prairie dogs revealed 100 percent similarity with the monkeypox virus strain Zaire 96-I-16 that had been isolated previously from the Democratic Republic of Congo.[67]

The virus is thought to spread to humans by handling of infected animals or by direct contact with an infected animal's body fluids or lesions. Person-to-person spread occurs by large respiratory droplets, although it is much less efficient than that for smallpox.[23,77,88] Risk factors for secondary transmission include close contact with a primary case. Evidence of previous vaccination is thought to be protective.[33] Mortality rates have varied between 0 and 17 percent among outbreaks.[1,6,35,42,53,58,60,62,74]

Modeling of attack rates with waning immunity from smallpox vaccination predicted a worst-case scenario of 147 cases causing 290 to 661 secondary outbreaks that could last as long as 14 generations. The estimated attack rates modeled were far less than for measles or chickenpox. Researchers suggested that 78 percent of primary cases would not result in secondary cases.[106] In light of this information, continued vaccination with vaccinia vaccine to prevent monkeypox has not been recommended, particularly considering the increasing prevalence of human immunodeficiency virus (HIV), which can result in serious adverse events on immunization with currently available vaccinia vaccine.[50]

Serologic evidence suggests that many animals, including rodents and nonhuman primates, are infected with monkeypox virus.[35,52] Epidemiologic studies from the Democratic Republic of Congo have implicated squirrels living in agricultural areas as primary candidates to sustain transmission among people in nearby settlements, although no definitive host has been identified. Concerns that monkeypox virus could spread through North American rodent populations and could serve as a vector for continued zoonotic exposure have not played out.[35]

Clinical Features

The clinical features of human monkeypox virus infection are similar to those of smallpox, but the overall illness tends to be less severe. The characterization of symptoms during outbreaks in Africa has been limited by poor access to medical care, retrospective identification, and civil unrest in areas of outbreaks. The outbreak reported in the midwestern United States allowed researches to describe clinical and laboratory parameters of disease.[51] Vaccinia vaccination markedly lessens the severity of the illness.

After a 10- to 14-day period of incubation (range, 1 to 31 days), a 2-day prodrome of fever, malaise, and lymphadenopathy precedes the manifestation of rash.[7,35,58] Lymphadenopathy occurs in 90 percent of unvaccinated individuals and is a key feature distinguishing monkeypox from smallpox because lymphadenopathy generally does not occur in smallpox. Affected lymph nodes may be unilateral or bilateral and include submandibular, cervical, postauricular, axillary, inguinal, or any combination of these locations.[6] Other signs and symptoms include chills, sweats, headache, backache, sore throat, cough, and shortness of breath.

The rash usually appears first on the face. As in smallpox, lesions develop and progress together in the same body region through the stages of macules, papules, vesicles, and pustules. Over the course of a 2- to 4-week period, the lesions progress until they finally scab over and desquamate. Most patients have discrete lesions, 23 percent have semiconfluent lesions, and 7 percent have confluent lesions. Mucous membrane involvement and conjunctivitis are common manifestations.[63,74] Lesions usually are found on the extremities, head, and trunk, as well as on the palms and soles.[51] During the first week of the rash, patients are considered to be infectious.[62]

Complications include the following: secondary bacterial infection of the skin; pneumonia; vomiting, diarrhea, and dehydration; keratitis and corneal ulceration; septicemia; and encephalitis. The death rate in non-vaccinated patients was 11 percent in the study reported by Jezek and associates,[63] and all deaths occurred in children aged 8 years or younger. Case-fatality rates reported in the 1996 to 1997 outbreak in the Democratic Republic of Congo were 1.5 percent, but this outbreak likely represented both chickenpox and monkeypox, and case-fatality rates probably were falsely low.[16] No fatalities were associated with the U.S. outbreak.

One case of congenital monkeypox infection has been reported.[58] This child's mother had typical illness on August 12, 1983, and the child was born prematurely on September 23, 1983. At birth, the child had generalized skin lesions and died 6 weeks later of malnutrition. No data are available on the clinical course of monkeypox in patients with HIV/AIDS (acquired immunodeficiency syndrome) or other immune compromised states.

Unlike African patients, most of the patients in the United States had a mild, self-limited febrile rash illness.[21] The majority of patients had localized lesions associated with direct contact with infected animals, and only one patient, a child, had a gen-

eralized rash. Differences in the severity of disease may result from differences in virulence among genetically distinct groups of monkeypox viruses: the West African monkeypox virus group and the Congo basin isolates. Isolates from the Congo basin are thought to be more virulent, based on epidemiologic and genetic sequence analyses.[27,39,58,71] The viral isolates involved in the U.S. outbreak were West African in origin.

Laboratory alterations noted in patients of the midwestern U.S. outbreak included elevated transaminases in 50 percent of patients, low blood urea nitrogen (61%), hypoalbuminemia (50%), leukocytosis (45%), and thrombocytopenia (35%).[51] The CDC defined guidelines for case definitions in 2004 (Table 176–2).

Diagnosis

All cases should be reported immediately to the local health department. Laboratory confirmation is required for establishing a definitive diagnosis. The CDC case definitions can help to guide clinicians.[24] Samples that may be used for diagnosis include blood and cutaneous tissue from at least two scabs that have been unroofed and collected in a sterile fashion. Once lesions have been unroofed, the base of the vesicle should be swabbed with sterile cotton or polyester swab and the material applied to a clean microscope slide and air-dried. The material should be sent to the CDC or national reference laboratory for diagnostic testing. Other specimens that can be analyzed include skin biopsy specimens, which may be indistinguishable from those of smallpox lesions, with necrosis of the stratum basale, adjacent dermal papillae, and stratum spinosum. Electron microscopy of the lesions shows abundant large, brick-shaped orthopoxvirus particles in the cytoplasm of infected epidermal cells.[94]

Blood samples may be analyzed for paired acute-phase and convalescent-phase serum samples; however, serology is limited because of the close antigenic relationship that exists among orthopoxviruses. More specific methods used by the CDC include cell culture or chick chorioallantoic membrane isolation along with PCR and sequencing to differentiate monkeypox virus from other orthopoxviruses.[32,70]

Treatment and Prevention

In 1968, investigators reported that monkeys could be immunized against monkeypox virus using smallpox vaccine.[72] In outbreaks in the Democratic Republic of Congo described by Jezek and colleagues,[63] prior smallpox inoculation was found to be protective and to result in a milder form of disease, often difficult to distinguish from chickenpox. However, the smallpox vaccination status did not alter either the severity of illness or hospitalization rates in the midwestern U.S. outbreak.[51] Hammarlund and associates[47] described protective immunity to monkeypox virus in persons previously vaccinated against smallpox. The CDC recommended vaccination with smallpox vaccine for healthcare workers and household contacts of patients with confirmed monkeypox cases. Whenever possible, care for patients with suspected or confirmed monkeypox should be given by vaccinated health care workers and those without contraindications to vaccination. Pre-exposure vaccination is preferred, but vaccination can be administered after laboratory confirmation of an infection is obtained and when vaccine is available. Vaccination should take place within 4 days but may be considered up to 14 days after exposure.[24]

Other researchers looked at the potential development of live virus vaccine against monkeypox and reported improved survival rates among vaccinated macaques. In light of the potential side effects of the current smallpox vaccine, perhaps developing safer vaccines for monkeypox would be useful.[49] Because of the high incidence of complications resulting from the vaccinia virus

TABLE 176–2 Centers for Disease Control and Prevention Case Definition for Human Cases of Monkeypox (January 2004)

Human Monkeypox Case Classification

Suspect Case

The patient meets one of the epidemiologic criteria and has fever or unexplained rash and two or more other signs or symptoms with onset of first sign or symptom within 21 days after the last exposure.

Probable Case

The patient meets one of the epidemiologic criteria and has fever and vesicular-pustular rash with onset of the first sign or symptom less than 21 days after the last exposure meeting epidemiologic criteria; or if rash is present but type is not described, the patient demonstrates elevated levels of IgM antibodies reactive with orthopoxvirus between at least 7 and 56 days after the rash onset.

Confirmed Case

The patient meets one of the laboratory criteria.

Clinical Criteria

Rash (macular, papular, vesicular, pustular, generalized or localized, discrete or confluent)
Fever (subjective or measured, ≥37.4° C)
Other signs and symptoms (chills and/or sweats, headache, backache, lymphadenopathy, sore throat, cough, shortness of breath)

Epidemiologic Criteria

Exposure (includes living in a household, petting or handling, or visiting a pet holding facility such as a pet store or veterinary clinic) to an exotic or wild mammalian pet (including prairie dogs, Gambian giant rats, and rope squirrels, among others to be considered on a case-by-case basis) obtained on or after April 15, 2003, with clinical signs of illness (e.g., conjunctivitis, respiratory symptoms, and/or rash)
Exposure (as described earlier) to an exotic or wild mammalian pet (as described earlier) with or without clinical signs of illness that has been in contact with either a mammalian pet (living in a household or originating from the same pet holding facility as another animal with monkeypox) or a human
Exposure (skin-to-skin or face-to-face contact) to a suspect, probable, or confirmed human case

Laboratory Criteria

Isolation of monkeypox virus in culture
Demonstration of monkeypox virus DNA by PCR testing of a clinical specimen
Demonstration of virus morphologically consistent with an orthopoxvirus by electron microscopy in the absence of exposure to another orthopoxvirus
Demonstration of presence of orthopoxvirus in tissue using immunohistochemical testing methods in the absence of exposure to another orthopoxvirus

Exclusion Criteria

An alternative diagnosis can explain the illness.
OR
The case was reported on the basis of primary or secondary exposure to an exotic or wild mammalian pet (as described earlier) or a human subsequently determined not to have monkeypox, provided other possible epidemiologic exposure criteria are not present.
OR
A patient without a rash does not develop a rash within 10 days of onset of clinical symptoms consistent with monkeypox.
The case is determined to be negative for non-variola generic orthopoxvirus by PCR testing of a well-sampled rash lesion by the approved protocol.
The case is determined to have undetectable levels of IgM antibody 7 to 56 days after rash onset.

IgM, immunoglobulin M; PCR, polymerase chain reaction.

Adapted from Centers for Disease Control and Prevention: Updated interim case definition for human monkeypox, January 2004. Available at http://www.cdc.gov/ncidod/monkeypox/casedefinition.htm; and from Centers for Disease Control and Prevention: Update: Multistate outbreak of monkeypox: Illinois, Indiana, Kansas, Missouri, Ohio, and Wisconsin—2003. M. M. W. R. Morb. Mortal. Wkly. Rep. 52:561-564, 2003.

vaccine for smallpox, Stittelaar and colleagues[96] described the use of a modified vaccinia virus strain, Ankara, that protected macaques against lethal respiratory challenge with monkeypox virus, as a possible candidate for safer vaccine against smallpox. This vaccine candidate, MVA-BN, is undergoing phase I and II clinical trials in humans.

As a potentially preventive measure, in the Midwest outbreak, 28 residents (26 adults and 2 children) of the outbreak area received smallpox vaccine to try to prevent further transmission of monkeypox.[20] No data exist on the usefulness of vaccinia immune globulin, but this treatment can be considered in severe cases of monkeypox and for prophylaxis in exposed persons who are severely immunocompromised.[35]

Although cidofovir has in vitro activity against the virus,[33] no data have shown its effectiveness for treating human monkeypox virus. The CDC recommends that cidofovir be considered in severe monkeypox virus infections and in immunocompromised hosts, although the drug should not be used for prophylaxis because of its significant toxicities.[22] Studies in mouse models of oral cidofovir are ongoing,[83,84] and they may show some promise. ST-246, a compound with activity against orthopoxviruses, has shown promise in treating severe monkeypox virus infection in ground squirrels and may one day be available for use in people.[89]

Among hospitalized patients, isolation precautions should include standard, droplet, and contact, as well as airborne, precautions because the virus probably is transmitted by direct contact or by large respiratory droplets.[23] Isolation can be discontinued for patients with vesiculopustular rash after all the lesions have crusted; however, contact with immunocompromised persons should be limited until all the crusts have separated. For patients without rash, isolation should be continued for 7 days after the onset of fever. Affected individuals should not donate any body fluids or organs while they are ill.[24] Asymptomatic contacts should remain under symptom surveillance for 21 days after the last exposure, and temperature should be monitored twice daily.

Bioterrorism Concerns

Concern over use of monkeypox as an agent of bioterrorism was raised, but no evidence suggests that human monkeypox becomes more severe or that the virus becomes more virulent or easily transmissible after one or more passages through human hosts.[61] Furthermore, evidence that monkeypox evolved independently from smallpox alleviated some fears that the virus could "evolve back" into smallpox. The consensus is that monkeypox does not represent a serious bioterrorist threat because of low rates of primary infection, limited transmissibility, and low case fatality.[5] Some concerns remain about the potential of molecular biologic techniques to manipulate the virus genetically into a more lethal form.[55,90]

COWPOX VIRUS

In Europe, a disease with ulcers on the teats of cows (cowpox disease) has been recognized for hundreds of years.[39] Milkers who milked cows that were infected often developed similar ulcers on their hands, and milkmaids who had contracted cowpox were known to be immune to smallpox. This observation led Edward Jenner, in May of 1796, to inoculate James Phipps, an 8-year-old boy, with cowpox material obtained from a lesion on a local dairy maid.[4,56-58] After experimental challenge with material from a smallpox lesion, smallpox did not develop in Phipps.

Studies conducted since the 1970s indicate that bovine cowpox is not a common illness and cows are not the natural reservoir of the virus in nature.[2,4,8] The virus is distributed geographically throughout Western Europe, and the reservoir hosts are wild rodents.

Baxby[2] reported 12 human illnesses caused by cowpox virus. Five of the patients were exposed to infected cows, and the other 7 had no direct contact with cattle. All the patients, however, lived in or had visited a rural area before experiencing the onset of illness. Of 10 patients in whom lesions were described, 6 had lesions on the hand only, 3 had lesions on the chin or face only, and a single patient had involvement of both the face and hand. Most patients had local edema, lymphadenitis, and fever. The lesions were confused with anthrax in two instances.

Twelve cases of cowpox causing cutaneous infection, one with lethal outcome, were reported from various European countries. Most of the cases resolved between 3 weeks to 6 months, although the authors reported a 7-year-old girl with persistent lymph node involvement 2 years after the initial presentation. Infection developed after exposure to ill cats in nine cases, to ill dogs in two cases, and to a wild rat in one case.[79] The lethal outcome occurred in an immunocompromised 18-year-old man, who was thought to have acquired the infection from a cat with a skin lesion on the anterior paw.[30] Schupp and others[90] reported a case of cowpox virus causing necrotic ulcers on a 12-year-old boy in Germany. The virus was detected by PCR, and probably was transmitted from the patient's cat.

VACCINIA VIRUS

Vaccinia virus is the live immunizing antigen successfully used in the global program to eradicate smallpox. This orthopoxvirus is different from cowpox virus, the agent that Jenner and others used for vaccination in the early 19th century. Vaccinia virus has been used for vaccination for more than 100 years. Restriction endonuclease studies indicated that strains of vaccinia virus from different parts of the world are similar to each other and distinctly different from cowpox virus.[39] The origin of vaccinia virus is unknown.[8] Four hypotheses regarding its origin are that (1) it evolved from variola virus through continual passage in the skin of cows or humans, (2) it evolved from cowpox virus through continual passage in the skin of animals, (3) it is a hybrid between cowpox virus and variola virus, and (4) it is a virus from an animal (the natural host) that is now extinct.

Vaccinia virus causes outbreaks of disease in buffaloes in India.[8,39] However, the animal infection is thought to have resulted originally from contact of buffaloes with vaccinated humans during smallpox eradication programs, rather than from the virus' being a primary buffalo pathogen. More recently, in several Brazilian provinces, skin lesions on dairy cows and their milkers that resemble cowpox were caused by a variety of vaccinia virus strains belonging to two groups of vaccinia virus. All these cases were thought to have derived from repeated introduction of vaccinia virus during the 18th and 19th centuries.[98]

Because of the original success of the smallpox eradication program, routine vaccinia vaccination in the United States was discontinued in 1971.[10,15] In 1976, the recommendation for routine vaccination of health care workers also was discontinued.[11] In 1982, the only active licensed producer of vaccinia vaccine in the United States ceased production for general use, and distribution to civilian populations was discontinued in 1983.[14]

For several years, all military personnel continued to be vaccinated routinely. Although more recently only selected groups of military personnel were vaccinated against smallpox, with the initiation of action in Iraq and the concurrent threat of biologic warfare, all troops designated for military action in that area were

given smallpox vaccinations. Since January of 1982, smallpox vaccination has not been required for international travelers, and International Certificates of Vaccination no longer include smallpox vaccination.[107]

In 1980, the Advisory Committee on Immunization Practices (ACIP) recommended the use of vaccinia vaccine to protect laboratory workers from possible infection while working with nonvariola orthopoxviruses (e.g., vaccinia, monkeypox).[12] In 1984, these recommendations were included in guidelines for biosafety in microbiologic and biomedical laboratories.[99] These guidelines expanded the recommendation to include persons working in animal care areas where studies with orthopoxviruses were being conducted and recommended that these workers have documented evidence of satisfactory smallpox vaccination within the preceding 3 years. The CDC has provided vaccinia vaccine for these laboratory workers since 1983.[13] Because studies of recombinant vaccinia virus vaccines have advanced to the stage of clinical trials, health care workers (e.g., physicians, nurses) and veterinarians now may be exposed to vaccinia and recombinant vaccinia viruses and should be considered for vaccinia vaccination.[43,48]

Recombinant Vaccinia Virus

Vaccinia virus is the prototype of the genus *Orthopoxvirus*. It is a double-stranded DNA virus that has a broad host range under experimental conditions and rarely is isolated from animals outside the laboratory.[8,31,39,41,46,108] Many strains of vaccinia virus exist and have different levels of virulence for humans and animals. For example, the Temple of Heaven and Copenhagen vaccinia strains are highly pathogenic in animals, whereas the NYCBOH strain, from which the Wyeth vaccine was derived, has relatively low pathogenicity.[40]

Vaccinia virus can be engineered genetically to contain and express foreign DNA without impairing the ability of the virus to replicate. Such foreign DNA can encode protein antigens that induce protection against one or more infectious agents. Recombinant vaccinia viruses have been engineered to express the immunizing antigens of many viruses, bacteria, parasites, and tumors of veterinary and medical importance.[29,43,48,53,66,92,93,106]

Recombinant vaccinia viruses have been created from several strains of vaccinia virus. In the United States, recombinant viruses have been made from the NYCBOH strain or a mouse neuroadapted derivative, the WR strain. Some recombinant viruses have been made from the Copenhagen and Lister vaccinia strains, which are more pathogenic in animals than the NYCBOH strain is. More recently, studies using recombinant vaccinia to vaccinate against prostate cancer, malaria, tuberculosis, and HIV have used the vaccinia virus Ankara strain.[3,54,82] Animal studies generally suggest that recombinant viruses may be no more pathogenic than the parent strain of vaccinia virus is. However, no consistently reliable laboratory marker or animal test predicts the attenuation of vaccinia virus or a particular recombinant virus for humans.[85] Laboratory-acquired infections with vaccinia or recombinant viruses have been reported.[64,81,91] However, because no surveillance system has been established to monitor laboratory workers, the risk of acquiring infection in persons who handle virus cultures or materials contaminated with these viruses is not known.

With the initiation of human and veterinary trials of recombinant vaccines, physicians, nurses, veterinarians, and other personnel who are exposed to recipients of these vaccines could be exposed to both vaccinia and recombinant agents. This exposure could occur from contact with dressings contaminated with the virus or through exposure to the vaccine. The risk of transmission of recombinant viruses to exposed health care workers is unknown. To date, no reports of transmission to health care personnel from vaccine recipients have been published. If appropriate infection control precautions are observed, health care workers probably are at less risk of acquiring infection than are laboratory workers because of the smaller volume and lower titer of virus in clinical specimens than in laboratory material.[45,105] However, because of the potential for transmission of vaccinia or recombinant vaccinia viruses to such persons, the ACIP suggested that health care personnel who have direct contact with contaminated dressings or other infectious material from volunteers in clinical studies be considered for vaccination. Laboratory and other health care personnel who work with viral cultures or other infective material always should observe appropriate biosafety guidelines and should adhere to published infection control procedures.[45,100,105]

ORF VIRUS

Orf virus is a member of the genus *Parapoxvirus* (see Table 64–1 in Chapter 64). Infection with orf virus causes the disease by the same name (orf), which also is called *ecthyma contagiosum*.[39] The reservoir hosts of orf virus are sheep and goats, and the virus has worldwide distribution. Orf is an occupationally acquired disease, and most cases occur in adults.[44,68] Human disease is characterized by single or multiple lesions that most often are located on the hands. The lesions last approximately 35 to 40 days and progress through six stages[68]: maculopapular, 1 to 7 days; target, 7 to 14 days; acute, 14 to 21 days; regenerative, 21 to 28 days; papillomatous, 28 to 35 days; and regressive, 35 to 40 or more days. Patients may have low-grade fever, but regional lymphadenitis is an uncommon finding. Two patients with a widespread papulovesicular eruption, fever, malaise, and lymphadenopathy were described.[104] In addition, a giant orf granuloma developed at the site of a rope burn in a 12-year-old boy who lived on a farm.[80]

The CDC reported four unrelated cases of cutaneous, self-resolving orf virus infections in people exposed to sheep or goats that had either been vaccinated recently with live orf virus vaccine or exposed to animals with oral lesions. These cases occurred in New York, Illinois, California, and Tennessee. Patients' ages ranged from 11 to 51 years.[25] Uzel and colleagues[101] reported nine cases of orf virus infection in Turkey related to feasts of sacrifice. These cases occurred in adults involved in sacrifice or preparation of the animals for consumption.

Until recently, diagnosis of orf virus depended on electron microscopy or serology. In 2001, Torfason and Guonadottir[97] described a method of PCR to detect orf virus that helped to make identification of the virus more accessible.[78]

Orf virus has been shown to have antiviral activity against hepatitis B and herpes simplex virus in mice. By modulating the host innate immune system inducing phagocytosis, natural killer cell activity and release of interferon-α, tumor necrosis factor-α, interleukin-2, and granulocyte-macrophage colony-stimulating factor, it appears that orf virus protects mice from lethal herpes simplex virus type 1 infection, as well as guinea pigs from recurrent genital herpes disease. This finding may have implications for immune therapy for patients suffering serious infection from these viruses.[103] Treatment with imiquimod of complicated cases in immunocompetent hosts[38] and in immunocompromised patients has been reported.[69]

OTHER PARAPOXVIRUSES

Human infections with pseudocowpox virus and bovine papular stomatitis virus are, like orf, occupational diseases. Pseudocowpox infections occur on the hands of milkers, and infections with

bovine papular stomatitis virus become evident on the hands of veterinarians and others with close contact.[9,39,73] The lesions of milker's nodule are relatively painless, but they may be pruritic; they are red initially and then become purple. They are firm, do not ulcerate, and last 4 to 6 weeks. The lesions caused by bovine papular stomatitis virus are wartlike and last 3 to 4 weeks.

Humans also have been infected with the parapoxviruses of camels and seals.[87] One report noted human infection with sealpox virus resulting in an orflike lesion that resolved after biopsy specimens were obtained.[28]

YATAPOXVIRUSES

Tanapox virus, yaba-like disease virus (YLDV), and yaba monkey tumor virus (YMTV) are three monkey viruses that can cause human infection.[32,36,37,39,59] Tanapox in humans first was observed along the Tana River in Kenya in 1957, and outbreaks also were studied in the Democratic Republic of Congo.[39] DNA maps of tanapox virus and YLDV are extremely similar, a finding indicating that they may be the same agent. YMTV was isolated from tumors in monkeys in Nigeria. This virus has caused illness in animal handlers in centers for primates in the United States, but human infections have not been identified in the field in Africa.

Outbreaks of tanapox in Africa have involved both children and adults.[59] The illness associated with tanapox and YLDV starts with fever, headache, backache, and mild prostration. Skin lesions occur 2 to 4 days after the onset of illness. Individual lesions start with itching, followed by the development of a pocklike lesion. At 7 days, the lesion is approximately 10 mm in diameter with surrounding erythema. Lymphadenitis is both local and regional.

The lesions ulcerate during the second week of illness and last approximately 6 weeks. Most patients have a single lesion, but some have 2 to 10 lesions. The prognosis usually is good, although secondary bacterial infection can occur. YMTV produces epidermal histiocytomas, tumor-like masses of histiocytic polygonal mononuclear cell infiltrates that may advance to suppurative inflammation.[32]

Two cases of tanapox have been reported in the United States. A 51-year-old man developed papular lesions with general malaise after visiting Tanzania and being exposed to a cat.[95] A second case occurred in a 21-year-old female student from New Hampshire, who developed fever, headaches, malaise, and several papules after being exposed to chimpanzees in the Democratic Republic of Congo.[34] The symptoms of both patients were diagnosed using PCR techniques and resolved spontaneously. With increasing international travel, more case reports of such poxvirus illnesses may surface. PCR techniques to diagnose such infections quickly have been developed,[34,95,109] and these methods will assist in rapid diagnosis of poxvirus illnesses that may be confused with more serious illnesses as smallpox and monkeypox.

MOLLUSCUM CONTAGIOSUM VIRUS

In contrast to the other poxviruses discussed in this chapter, which are zoonotic agents, molluscum contagiosum virus is a human virus that is a common cause of human skin lesions[39] (see Chapter 67 and Table 64–1 in Chapter 64). The virus has not been grown in cell culture as yet, and it does not cause infection in experimental animals.

Although the virus has not been cultivated in the laboratory, large amounts of virus can be extracted from human lesions. Analysis of viral DNA from lesions in patients from different parts of the world indicates that two major subtypes and a third, rare subtype exist. The clinical aspects of molluscum contagiosum virus infection are presented in Chapter 67.

DIAGNOSIS AND DIFFERENTIAL DIAGNOSIS

Because all but two viruses presented in this chapter are zoonotic agents, careful attention must be given to geographic location and animal exposure. All these diseases are characterized by local lesions, so virus is readily available for direct identification and culture. All poxviruses can be identified by examination of material from lesions by electron microscopy and by PCR. All viruses except molluscum contagiosum virus grow in one or more tissue culture systems, the chorioallantoic membrane of embryonated eggs, or both. Unusual agents should be referred to specific reference laboratories for species identification.

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CHAPTER 177

MIMIVIRUSES

Ramya Ramraj

In 1992, while investigating the source of an outbreak of community-acquired pneumonia in northern England, investigators discovered a bizarre microorganism.[5,6] The microorganism, resembling a small gram-positive coccus, was found inside a free-living ameba in a water cooling tower. Researchers initially thought it was a bacterium, but subsequent analysis demonstrated a double-stranded DNA virus. Originally named *Mimivirus* because it resembled bacteria in Gram-stained smears, it now is designated officially as *Acanthamoeba polyphaga Mimivirus*.[7]

STRUCTURE AND PROPERTIES

A. polyphaga Mimivirus is a giant, double-stranded, icosahedral DNA virus.[18] With a diameter of approximately 650 nm, *Mimivirus* is the largest virus known to date. Morphologically, *Mimivirus* resembles nucleocytoplasmic large DNA viruses (NCLDVs) such as the *Iridovirus*, *Asfarvirus*, and *Phycodnavirus*.[3,17] However, evolutionary dissimilarities define a new family, the *Mimiviridae*.[5] The *Mimivirus* comprises a central dense core that is surrounded by two lipid membrane layers inside a capsid protein shell covered by fibrils. The 1.2-Mb genome sequence[10,12] is complex and possesses genes that are shared by the NCLDV families, as well as several that have not been identified previously in viruses. The origins and the various processes[1] involved in shaping gene content of the *Mimivirus* are not entirely clear at this time.[10,15]

VIRAL REPLICATION

In *A. polyphaga*, the replication cycle of the *Mimivirus* starts with entry of the virus by phagocytosis, followed by a 4-hour eclipse phase inside the nucleus,[5] then the formation of "virus factories"(VF) in the cytoplasmic space,[16] and ending with cell lysis and virus release 24 hours after infection (Fig. 177–1). The VF,[11] where virus replication and assembly occur, is the major site of production of *Mimivirus* DNA and induces profound alteration of the infected cell structure, such as by recruitment of organelles and organization of cellular compartments. Very specific mechanisms and complex interactions between viral and cellular factors that are not entirely clear at this time must be involved to build this remarkably large and efficient VF, which can generate this sophisticated microorganism rapidly.[16]

ANIMAL SUSCEPTIBILITY

An experimental model of infection[4] was developed to establish a possible role of *Mimivirus* as a human pathogen. Autopsy specimens of laboratory mice that were inoculated intracardially with infecting units of *Mimivirus* revealed histopathologic evidence of pneumonia and were re-isolated from samples from the lung. Inoculation of human macrophages with *Mimiviruses* showed evidence of infection, although no evidence of a lytic cycle was observed.[13] Efforts to induce infection in other cell lines were futile, and the target cell in mice remains to be identified.

MIMIVIRUS INFECTION IN HUMANS

In December 2004, a laboratory technician in France[14] developed subacute pneumonia with dry cough, fever, and chest pain. This technician was in charge of performing *Mimivirus* serologic testing and Western blot analysis and thus had handled relatively large amounts of *Mimivirus* antigens. Radiography of his chest revealed bilateral basilar infiltrates (Fig. 177–2). The illness did not respond to amoxicillin-clavulanate treatment prescribed on day 15 after the onset of symptoms, but after an additional 2

Figure 177–1 Schematic representation of the *Acanthamoeba polyphaga Mimivirus* replication cycle. *1*, *Mimivirus* entry through a phagocytic vacuole. *2*, Fusion of phagocytic vacuoles and delivery of *Mimivirus* genetic material into the cell cytoplasm *(3)*. *3*, *Mimivirus* DNA entry into the host nucleus, where the first round of DNA replication could begin *(4)*. *5*, At 3 hours after infection, *Mimivirus* DNA came from the host nucleus to form the virus factories (VF) replication center. *6*, At 5 hours after infection, the VF size showed a 50 percent increase, and viral proteins began to be detected; proviral capsid assembly and viral capsids budding from the VF central core could be observed. 7, Empty or DNA-filled capsids accumulated near the central core, resulting in a growing VF with viral particles free in the cytoplasm. *8*, Complete viral capsids surrounded by fibrils may be released through cell lysis. *(From Suzan-Monti, M., La Scola, B., Barrassi, L., et al.: Ultrastructural characterization of the giant volcano-like virus factory of Acanthamoeba polyphaga Mimivirus. PloS ONE 2:e328, 2007.)*

Figure 177–2 Chest radiograph of a laboratory technician who was infected with *Mimivirus*, with bilateral basilar infiltrates evident. *(From Raoult, D., La Scola, B., Birtles, R., et al.: The discovery and characterization of Mimivirus, the largest known virus and putative pneumonia agent. Clin. Infect. Dis. 45:95-102, 2007.)*

weeks, the illness resolved, and the technician made a full recovery. Serum samples screened against pneumonia agents showed seroconversion (<1 : 50 to 1 : 3200) only for *Mimivirus*, which was confirmed by testing using two-dimensional Western blotting against 23 identified proteins of *Mimivirus*.

PREVALENCE OF ANTIBODIES TO *MIMIVIRUS* IN PATIENTS WITH PNEUMONIA

Three studies investigated the prevalence of antibodies to *Mimivirus* in specific human populations.[13] The first study tested serum samples obtained from 376 Canadian patients with community-acquired pneumonia and from 511 healthy subjects. A total of 9.66 percent of patients with pneumonia had antibodies to *Mimivirus*, compared with 2.3 percent of control subjects.[9] Patients with pneumonia who had antibodies to *Mimivirus* were more likely to be hospitalized from a nursing home and to be rehospitalized after discharge.[8] The second study included 26 patients from Marseille, France, who acquired pneumonia while in an intensive care unit, and 50 control serum samples (from blood donors). Antibodies to *Mimivirus* were detected in samples obtained from 5 patients but in none of the control samples.[8] The third serosurvey involved 157 patients in intensive care units who had pneumonia. Serum specimens obtained from these patients were tested against a panel of antigens from "conventional" pneumonia agents, as well as ameba-associated microorganisms, including *Mimivirus*.[2] Evidence of infection with "conventional" pathogens was found in 28 cases and with ameba-associated pathogens in 18 cases. In the group infected by ameba-associated pathogens, more patients had seroconversion to *Mimivirus* than to any other pathogen (5 cases), and seroconversion was more common among patients with ventilator-associated pneumonia than among those with community-acquired pneumonia (31.6% and 10.5% of patients, respectively). Taken

together, these three studies reveal a significant rate of seroconversion in patients with either community-acquired pneumonia (especially among patients who have been hospitalized from a nursing home) or nosocomial pneumonia.[13]

DIAGNOSTIC METHODS

Diagnostic strategies include collection of bronchoalveolar lavage (BAL) fluid and serologic samples. *Mimivirus* can be cultivated by co-culture with ameba or DNA extracted (QIAMP tissue kit, Qiagen, Hilden, Germany) from BAL samples for polymerase chain reaction (PCR) assay for enhanced detection.[2] Serologic testing includes immunofluorescence assay of samples of acute-phase and convalescent-phase serum for antibodies to proteins specific for *Mimivirus* (Becton Dickinson, Rutherford, NJ).[2] Strong evidence of infection includes positive BAL culture, positive PCR assays, or a fourfold increase in antibody titer between acute-phase and convalescent-phase serum samples or significant seroconversion for *Mimivirus*.[2]

MIMIVIRUS AS AN EMERGING PATHOGEN

Evidence indicates that *Mimivirus* is a new human pathogen that causes pneumonia. However, *Mimivirus* does not replicate efficiently in co-culture with any of the mammalian cells tested to date, and serologic cross-reactions among pathogens are observed commonly. Isolates of the virus from patients with pneumonia have not been obtained.[13] Prevalence data on infection with children with *Mimivirus* are unavailable as of this publication date.

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RNA VIRUSES

SUBSECTION 1

Picornaviridae

CHAPTER 178

ENTEROVIRUSES AND PARECHOVIRUSES

James D. Cherry ✪ Paul Krogstad

Enteroviruses (EVs) and parechoviruses comprise two genera of the family *Picornaviridae*. These small RNA viruses are responsible for frequent and often significant human illnesses with protean manifestations.* To bring order to the growing list of poliovirus-like agents, coxsackieviruses, echoviruses, and polioviruses first were categorized together and named in 1957 by a committee sponsored by the National Foundation for Infantile Paralysis.[666] These viruses were grouped together because of similarities in their biophysical properties, shared features in their epidemiology and pathogenesis, and the many disease syndromes that they caused. The human alimentary tract was thought to be the natural habitat of these agents, and the term *enterovirus* was coined. Newer agents later assigned to the genus *Enterovirus* were simply numbered sequentially, and more than 100 distinct human EV (HEV) types now are recognized. The two viruses originally classified as echoviruses 22 and 23 were reclassified into a new

*See references 166, 168, 169, 233, 378, 525, 604, 658, 662, 864, 991-995.

genus *(Parechovirus)* within the *Picornaviridae* family because of features of their structure and replication that distinguish them from the EVs.[893]

HISTORY

Poliomyelitis, the first enteroviral disease to be recognized and the most important one, has a long history.[761] The earliest record is an Egyptian stele of the 18th dynasty (1403 BCE to 1365 CE), which shows a young man with a withered, shortened leg, the characteristic deformity of paralytic poliomyelitis.[431,659] Michael Underwood,[949] a London pediatrician, published the first medical description in 1789 in *A Treatise on Diseases of Children.* During the 19th century, many reports appeared in Europe and the United States describing small clusters of cases of "infantile paralysis." The authors were puzzled about the nature of the affliction; not until the 1860s and 1870s was the spinal cord firmly established as the seat of the pathologic process. The contagious nature of poliomyelitis was not appreciated until the late 19th century. Medin, a Swedish pediatrician, was the first to describe the epidemic nature of poliomyelitis (1890), and his pupil Wickman worked out the basic principles of the epidemiology.[998]

The virus was isolated first in monkeys by Landsteiner and Popper[567] in 1908. The availability of a laboratory animal assay system opened many avenues of research that in the ensuing decades led to the demonstration that an unrecognized intestinal infection was the usual manifestation and the paralytic disease was a relatively uncommon event.

Coxsackieviruses and echoviruses have had a shorter history. Epidemic pleurodynia was described clinically in 1735 by Hannaeus[392] more than 200 years before the coxsackieviral origin of this disease was discovered. In 1948, Dalldorf and Sickles[234] first reported the isolation of a coxsackievirus after inoculation of suckling mice.

In 1949, Enders and associates[276] described the growth of poliovirus 2 in tissue culture, and their techniques paved the way for recovery of a large number of other cytopathic viruses. Most of these "new" viruses failed to produce illness in laboratory animals. Because the relationships of many of these newly recovered agents with human disease were unknown, these agents were called *orphan viruses*.[658] Later, several agents were grouped together and were called *e*nteric *c*ytopathogenic *h*uman *o*rphan viruses, or echoviruses.

Live-attenuated poliovirus vaccines became available in the early 1960s, and the most notable advance since the late 1980s has been a dramatic reduction in worldwide poliomyelitis as a result of the global immunization initiative.[207,450,752,924] The last case of confirmed paralytic poliomyelitis in the Western Hemisphere caused by a wild-type virus strain occurred in 1991.[812]

THE VIRUSES

CLASSIFICATION[293,457,662-665,751,789,833,894]

EVs and parechoviruses are RNA viruses belonging to the family *Picornaviridae* (*pico*, "small"). These viruses are grouped together because they share certain physical, biochemical, and molecular properties. In electron micrographs, the viruses are seen as 30-nm particles that consist of naked (nonenveloped) protein capsids, constituting approximately 70 to 75 percent of the mass of particles, and dense central cores (nucleoid) of the genomic RNA.

The original classification of HEVs is shown in Table 178–1. The EVs originally were distributed into four groups based on their different effects in tissue culture and pattern of disease in experimentally infected animals: polioviruses (causal agents of poliomyelitis in humans and nonhuman primates), coxsackieviruses A (associated with herpangina, human central nervous system [CNS] disease, and flaccid paralysis in suckling mice), coxsackieviruses B (human CNS and cardiac disease, spastic paralysis in mice), and the echoviruses (nonpathogenic in mice and not linked to human disease at first).

Although this scheme was useful initially, many strains were isolated subsequently that do not conform to such rigid specificities. For example, several coxsackievirus A strains replicate and have a cytopathic effect in monkey kidney tissue cultures, and some echovirus strains cause paralysis in mice. For this reason, and to simplify the nomenclature, EVs subsequently were assigned sequential numbers. Following this convention, the prototype EV strains Fermon, Toluca-1, J 670/71, and BrCr (identified between 1959 and 1973) were designated EV 68 through EV 71, respectively. Additional EVs continued to be identified that could not be using antisera specific for the classic serotypes. More than 30 additional such EV types have been assigned provisionally, although many have not been linked to human disease.

Complicating matters somewhat, studies of echoviruses 22 and 23 found that these agents exhibited genomic and proteomic differences from other EVs, and hence they were reclassified in the new genus *Parechovirus* as parechovirus types 1 and 2.[789,893,895] Similarly, hepatitis A virus initially was assigned the designation EV 72, but it was reclassified as the sole member of the hepatovirus genus within the picornavirus family because of marked genetic and biologic distinctions from the EVs.

TABLE 178–1 Original Classification of Human Enteroviruses: Animal and Tissue Culture Spectrum*

Virus	Antigenic Types†	Cytopathic Effect		Illness and Pathology	
		Monkey Kidney Tissue Culture	Human Tissue Culture	Suckling Mouse	Monkey
Polioviruses	1-3	+	+	−	+
Coxsackieviruses A	1-24‡	−	−	+	−
Coxsackieviruses B	1-6	+	+	+	−
Echoviruses	1-34§	+	±	−	−

**Many enteroviral strains have been isolated that do not conform to these categories, leading to the revised classification scheme shown in Table 178–2.*
†New types, beginning with type 68, were assigned enterovirus type numbers instead of coxsackievirus or echovirus numbers. Types 68 through 71 were identified.
‡Type 23 was found to be the same as echovirus 9.
§Echovirus 10 was reclassified as a reovirus: echoviruses 22 and 23 were made the first members of the Parechovirus genus of Picornaviridae, and echovirus 28 was reclassified as a Rhinovirus.
+, present; −, absent; ±, variably present.
Modified from Cherry, J. D.: Enteroviruses. In *Remington, J. S., and Klein, J. O. (eds.): Infectious Diseases of the Fetus and Newborn Infant. 3rd ed. Philadelphia, W. B. Saunders, 1990, pp. 325-366.*

More recently, genetic, biologic, and molecular properties were used to revise picornavirus taxonomy and led to a reorganization of the HEVs into five groups: the polioviruses and four alphabetically designated HEV species (HEV-A, B, C, and D) (Table 178–2). Determining the nucleotide sequence encoding the viral VP1 capsid protein plays a major role in the approach to taxonomy and predictably identifies viruses originally classified by serologic means, thus leading to the term *molecular serotyping*.[734,735,778,785] This approach will, no doubt, dominate future phylogenetic studies. Additional reclassification of the EVs is likely. For example, some researchers proposed that the polioviruses be included as members of the HEV-C species because the three polioviruses appear not to have any poliovirus-specific nucleotides, amino acid sequence, or motif that allows them to be distinguished from current HEV-C members.[101] The application of molecular phylogenetic approaches also revealed that recombination between circulating EVs is a frequent event and is likely to increase their genetic diversity.[148,181,611-613,736,739,882] This propensity for recombination has played a role in more recent outbreaks of paralytic diseases involving vaccine-derived stains.[522]

MORPHOLOGY AND REPLICATION

The genome of EVs and parechoviruses is a single-stranded, positive-sense RNA molecule approximately 7.4 kb in length.[780] It consists of a 5′ noncoding region followed by a single long open-reading frame, a short 3′ noncoding region, and a polyA tail. The 5′ noncoding region contains an internal ribosome entry site, which is essential for the initiation of translation. The 3′ noncoding region folds into highly conserved secondary and tertiary structures that are thought to play a role in the initiation of the replication of the viral genome. This genome is packaged into naked capsids that exhibit icosahedral symmetry with 20 triangular faces and 12 vertices

TABLE 178–2 Genomic Classification of Enteroviruses

Species Designation	Original Enterovirus
Poliovirus	Poliovirus types 1-3
Human enterovirus A (HEV-A)	Coxsackievirus A types 2-8, 10, 12, 14, 16 Enterovirus types 71, 76, 89, 90, 91, 92
Human enterovirus B (HEV-B)	Coxsackievirus A9 Coxsackievirus B types 1-6 Echovirus types 1-9, 11-21, 24-27, 29-33 Enterovirus types 69, 73-75, 77-88, 93, 97, 98, 100, 101
Human enterovirus C (HEV-C)	Coxsackievirus A types 1, 11, 13, 17, 19, 20-22, 24 Enterovirus types 95, 96, 99, 102
Human enterovirus D (HEV-D)	Enterovirus types 68, 70, 94

Coxsackievirus (CV) A15 has been reclassified as a strain of CV A11, and CV A18 as a strain of CVA13.

From Fauquet, C. M., Mayo, M. A., Maniloff, J., et al. (eds.): Virus Taxonomy: VIIIth Report of the International Committee on Taxonomy of Viruses. London, Elsevier Academic Press, 2005.

Replication of EVs begins with the adsorption of virions to cell surface receptors, which, for the most part, are integrins or immunoglobulin-like proteins (Table 178–3). The virions penetrate the surface of cell and uncoat, and the viral genome functions as messenger RNA for the viral polyprotein. This polypeptide contains three domains, P1 to P3, which are cleaved into three to four proteins each. The P1 region is liberated from the polyprotein by the viral 2A protein, a chymotrypsin-like protease. P1 initially is split into three proteins, VP0, VP1, and VP3, by the viral 3C protease. VP0 then is processed further into two smaller proteins, VP4 and VP2. Portions of VP1, VP2, and VP3 are exposed at the surface of the virion, whereas VP4 is entirely internal. VP1, VP2, and VP3 have no sequence homology but share the same topology.[789] Specifically, they form an eight-stranded antiparallel β-barrel that is wedge shaped and is composed of two antiparallel β-sheets. The amino acid sequences in the loops that connect the β-strands and the N- and C-terminal sequences that extend from the β-barrel domain of VP1, VP2, and VP3 give each EV its distinct antigenicity. In contrast to the EVs, the parechovirus 2A protein does not function as a protease; viral capsids are composed of three proteins: VP2, VP3, and an uncleaved VP0 protein. Whereas parechoviruses appear structurally similar to other picornaviruses in electron micrographs, the structural arrangement of the three capsid proteins has not been established.

The replication of EVs typically occurs in the cytoplasm in membrane-associated replication complexes and is completed rapidly (5 to 10 hours). Studies of polioviruses and coxsackieviruses have shown that enteroviral replication is associated with disruption of cellular protein secretion, and host-cell protein synthesis is suppressed because of cleavage of eIF4G (human eukaryotic translation initiation factor 4G) by the enteroviral 2A protein. The coxsackievirus 2A protein also cleaves dystrophin, a cytoskeletal protein; this activity has been hypothesized to play a role in damage to the myocardium.[43,250]

The parechoviruses replicate in a similar fashion.[553] Integrins ($\alpha_v\beta_3$ and perhaps $\alpha_v\beta_{1)}$ are used as receptors, and replication occurs in cytoplasmic structures. However, as noted earlier, the P1 portion of the viral polyprotein is processed into only three capsid proteins, and only one protease has been identified in the parechoviruses. In addition, parechovirus replication occurs in small, discrete foci in the cytoplasm, rather than in large accumulations of membranous vesicles like the EVs. Moreover, transcription and translation do not appear to be disrupted by the parechoviruses, thus perhaps explaining their relatively mild and delayed cytopathic effect when they are grown in tissue culture.[893]

TABLE 178–3 Cellular Receptors and Cofactors for Infection of Representative Enteroviruses

Virus	HEV Species	Receptor	Cofactor for Infection*
Polioviruses 1-3	Poliovirus	CD155; Pvr	
Coxsackieviruses B1-6	HEV-B	CAR	Coxsackieviruses 1, 3, 5 may use CD55 (DAF)
Coxsackievirus A9 Echovirus 9	HEV-B	$\alpha_v\beta_3$ integrin (vitronectin receptor)	MAP-70
Echoviruses 1, 8	HEV-B	VLA-2 ($\alpha_2\beta_1$ integrin)	Heparan sulfate
Coxsackieviruses A13, 17, 20, 21, 24	HEV-C	ICAM-1	
Enterovirus 70	HEV-D	CD55 (DAF)	

**The cofactors generally facilitate adhesion to cells, but their sole expression is insufficient to permit infection to occur.*

CAR, coxsackie and adenovirus receptor; DAF, decay accelerating factor; HEV, human enterovirus; ICAM 1, intercellular adhesion molecule 1; MAP-70, microtubule-associated protein 70; Pvr, poliovirus receptor; VLA-2, a human integrin.

REPLICATION CHARACTERISTICS AND HOST SYSTEMS[662,664,751,819]

EVs and parechoviruses are relatively stable viruses in that they retain activity for several days at room temperature and can be stored indefinitely at ordinary freezer temperatures (–20° C). They are inactivated quickly by heat (>56° C), formaldehyde, chlorination, and ultraviolet light, but they are refractory to ether, ethanol, and isopropanol.

Enteroviral strains grow rapidly when adapted to susceptible host systems and cause cytopathologic features in 2 to 7 days. The typical tissue culture cytopathic effect is shown in Figure 178–1; characteristic pathologic findings in mice are shown in Figures 178–2 and 178–3. Final titers of virus recovered in the laboratory vary markedly among different viral strains and the host system used; typically, concentrations of 10^3 to 10^7 infectious doses per 0.1 mL of tissue culture fluid or tissue homogenate are obtained. Unadapted viral strains frequently require long periods of incubation. In both tissue culture and suckling mice, evidence of growth usually is visible. Blind passage occasionally is necessary for the cytopathologic features to become apparent.

Although many different primary and secondary tissue culture systems support the growth of various EVs, primary rhesus monkey kidney cultures generally are accepted to have the most inclusive spectrum.[105] Other simian kidney tissue cultures, however, also have the same broad spectrum. Tissue cultures of human origin have a more limited spectrum, but several echovirus types have shown more consistent primary isolation in human than in monkey kidney culture. A satisfactory system for the primary recovery of EVs from clinical specimens would include the following: primary rhesus, cynomolgus, or African green monkey kidney; a diploid, human embryonic lung fibroblast cell strain; rhabdomyosarcoma cell line tissue cultures; and intraperitoneal and intracerebral inoculation of suckling mice younger than 24 hours.[62,399,412,662,664,819]

ANTIGENIC CHARACTERISTICS

Although some minor cross-reactions exist among several coxsackievirus and echovirus types, common group antigens of diagnostic importance have not been defined well.[659,661,662,664,751] Heat treatment of virions and the use of synthetic peptides have produced antigens with broad enteroviral reactivity.[926,927] These antigens have been used in enzyme-linked immunosorbent assay (ELISA) and complement-fixation (CF) tests to determine immunoglobulin G (IgG) and IgM enteroviral antibodies and for antigen detection. In one study, Terletskaia-Ladwig and colleagues[927] reported the identification of patients infected with EVs by the use of an IgM enzyme immunoassay (EIA). This test used heat-treated coxsackievirus B5 and echovirus 9 as antigens, and it identified patients infected with echoviruses 4, 11, and 30. The sensitivity of the test was 35 percent. In another study involving heat-treated virus or synthetic peptides, the respective sensitivities were 67 and 62 percent.[926] However, both tests lacked specificity. Intratypic strain differences are common findings, and some strains (prime strains) are neutralized poorly by antisera to prototype viruses. In animals, these prime strains induce antibodies that neutralize the specific prototype viruses, however.

The identification of polioviral, coxsackieviral, and echoviral types by neutralization in suckling mice or tissue culture with antiserum pools is relatively well defined. Neutralization is induced by the epitopes on structural proteins VP1, VP2, and VP3; in particular, several epitopes are clustered on VP1. Prime strains do cause diagnostic difficulty because frequently they are not neutralized by the reference antisera, a particular problem with echoviruses 4, 9, and 11 and EV 71. If these types are suspected, in some instances this problem can be overcome by using antisera in less diluted concentrations or antisera prepared against several different strains of problem viruses. Kubo and associates[555] were able to type enteroviral isolates not identified through

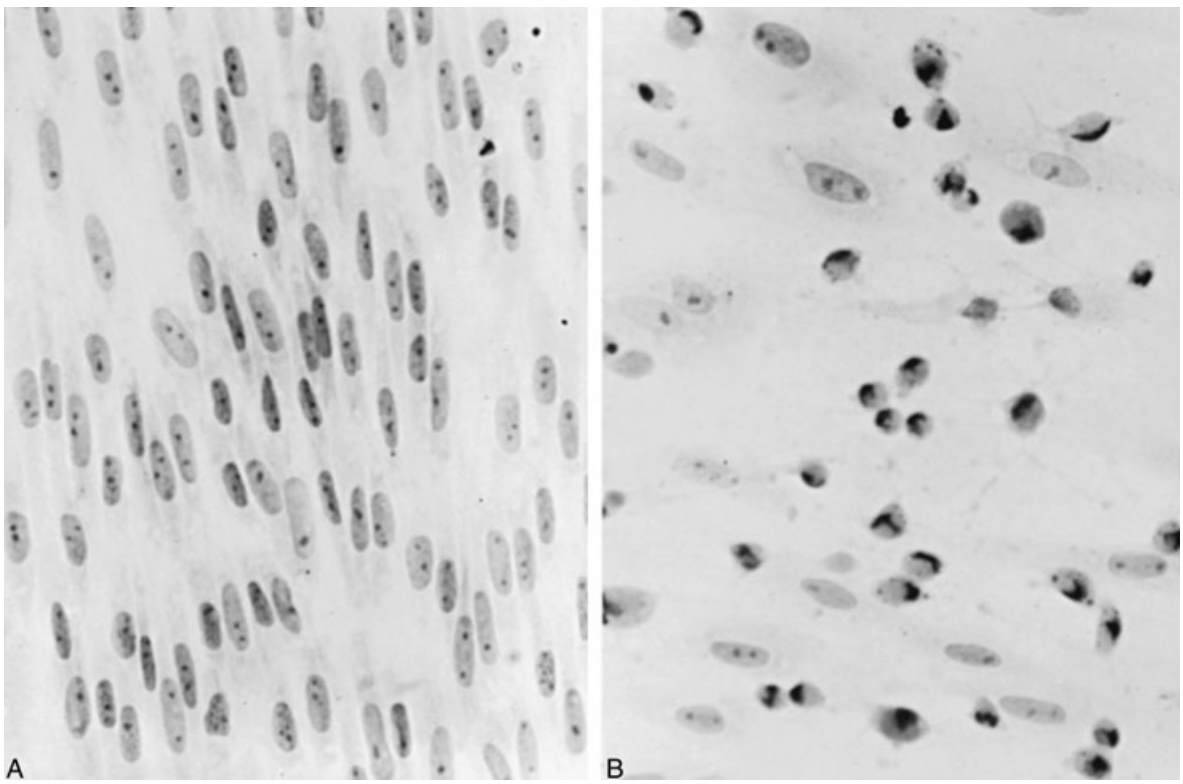

Figure 178–1 Fetal rhesus monkey kidney tissue culture (HL-8). **A,** Uninoculated tissue culture. **B,** Echovirus 11 cytopathic effect. *(From Cherry, J. D.: Enteroviruses.* In *Remington, J. S., and Klein, J. O. [eds.]: Infectious Diseases of the Fetus and Newborn Infant. Philadelphia, W. B. Saunders, 1976.)*

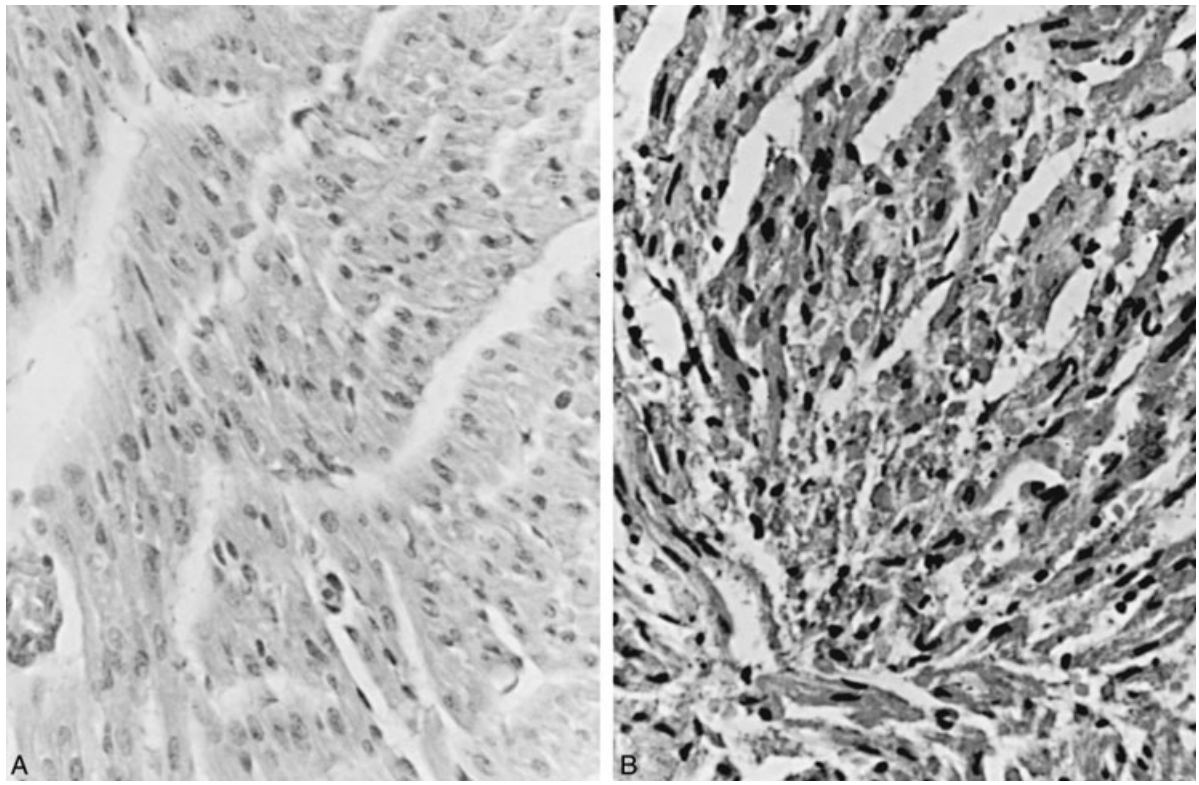

Figure 178–2 Suckling mouse myocardium. **A**, Normal suckling mouse myocardium. **B**, Myocardium of a suckling mouse infected with coxsackievirus B1. *(From Cherry, J. D.: Enteroviruses.* In *Remington, J. S., and Klein, J. O. [eds.]: Infectious Diseases of the Fetus and Newborn Infant. Philadelphia, W. B. Saunders, 1976.)*

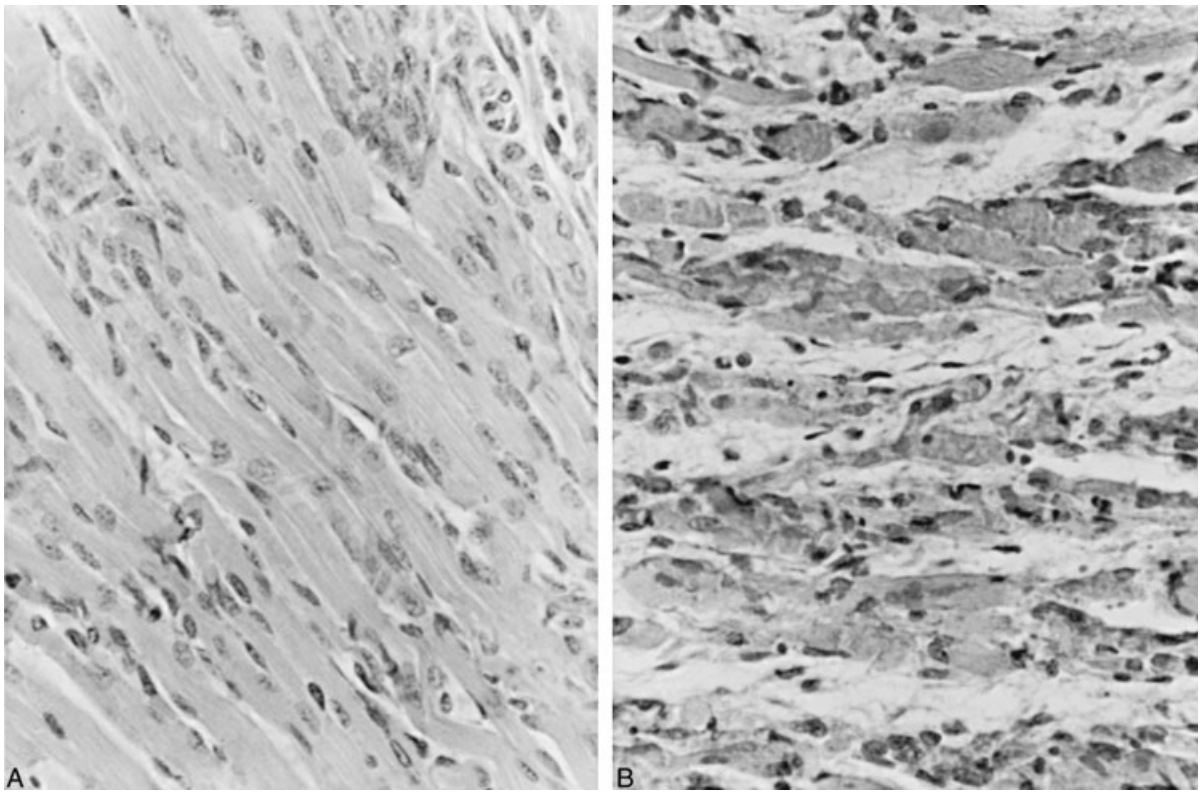

Figure 178–3 Suckling mouse skeletal muscle. **A,** Normal suckling mouse skeletal muscle. **B,** Skeletal muscle of a mouse infected with coxsackievirus A16. *(From Cherry, J. D.: Enteroviruses.* In *Remington, J. S., and Klein, J. O. [eds.]: Infectious Diseases of the Fetus and Newborn Infant. Philadelphia, W. B. Saunders, 1976.)*

neutralization by nucleotide sequence analysis of the VP4 gene. They specifically identified prime strains of echovirus 18 and EV 71. Sequence analysis of the VP1 gene also is useful for typing enteroviral prime strains not identified by neutralization.[729]

HOST RANGE

Humans are the only natural hosts of polioviruses, coxsackieviruses, and echoviruses.[233,339,525,658,718,864,992,994] However, EVs have been recovered in nature from sewage, flies, swine, dogs, a calf, a budgerigar, a fox, mussels, and oysters.[168] In addition, serologic evidence of infection with EVs similar to human strains has been noted in chimpanzees, cattle, rabbits, a fox, a chipmunk, and a marmot.[168] Infection of these animals probably resulted from direct contact with an infected human or human excreta. Eighteen genetically distinct EVs have been isolated from nonhuman primates.[779] Of 10 of these strains, 7 were related closely genetically to HEVs, whereas the other 3 were related only distantly. Contamination of shellfish also is interesting because in addition to their possible role in human infection, shellfish offer a source of enteroviral storage during periods of cold weather.[168] Contaminated food is another possible source of human infection.[168]

EPIDEMIOLOGY

TRANSMISSION

The spread of HEVs and parechoviruses is from person to person by the fecal-oral and possibly the oral-oral (respiratory spread) route. Swimming and wading pools may serve as a means of spread of EVs during the summer.[518] Oral-oral transmission by way of the contaminated hands of health care personnel and transmission by fomites were documented in a chronic care pediatric ward.[482] EVs have been recovered from trapped flies, and such carriage probably contributes to the spread of human infection, particularly in lower socioeconomic populations with poor sanitary facilities.[168]

Children are the main susceptible cohort; they are immunologically susceptible, and their unhygienic habits facilitate the spread of infection. Spread is from child to child (from feces to skin to mouth) and then within family groups. Recovery of EVs is related inversely to age, but the prevalence of specific antibodies is related directly to age. The incidence of infection and the prevalence of antibodies do not differ between boys and girls.

GEOGRAPHIC DISTRIBUTION AND SEASON

EVs and parechoviruses have worldwide distribution.[168,304,339] Neutralizing antibodies for specific viral types have been noted in serologic surveys throughout the world, and most strains have been recovered in global isolation studies. In any given area, frequent fluctuations occur in predominant types. Epidemics probably depend on newly susceptible individuals in the population rather than on re-infection. Epidemics may be localized and sporadic, and they may vary in origin from place to place in the same year. Pandemic waves of infection also occur.

In temperate climates, enteroviral infections occur primarily in the summer and fall, but in the tropics, they are prevalent all year.[168,339,661] A basic concept in understanding the epidemiology of these illnesses concerns the far greater frequency of unrecognized infection than clinical disease, as illustrated by poliomyelitis, which remained an epidemiologic mystery until researchers appreciated that unrecognized infections were the main source of contagion. Serologic surveys were instrumental in elucidating the problem: in populations living in conditions of poor sanitation and hygiene, epidemics did not occur, but wide dissemination of polioviruses was confirmed by demonstrating the presence of specific antibodies to all three types in almost 100 percent of children by the time they reached 5 years of age.

Epidemics of poliomyelitis first began to appear in Europe and the United States during the latter part of the 19th century; they continued with increasing frequency in economically advanced countries until the introduction of effective vaccines in the 1950s and 1960s.[88,198,431,761] The evolution from endemic to epidemic follows a characteristic pattern beginning with collections of a few cases, then endemic rates that are higher than usual, followed by severe epidemics with high attack rates. The age group attacked in endemic areas and in early epidemics is the youngest; more than 90 percent of paralytic cases begin in children younger than 5 years of age. Once this pattern of epidemicity begins, it is irreversible unless preventive vaccination is performed.

As epidemics recur over a period of years, a shift in age incidence occurs whereby relatively fewer cases are seen in the youngest children; the peak often is in the 5- to 14-year-old group, and increasing percentages of cases develop in young adults. These changes are correlated with socioeconomic factors and improved standards of hygiene: when children are protected from immunizing infections in the first few years of life, the pool of susceptible persons builds up, and introduction of a virulent strain often is followed by an epidemic.[431] The extensive use of vaccines since the late 1950s resulted in the elimination of paralytic poliomyelitis from large geographic areas, but the disease remains endemic in various parts of the world. Although seasonal periodicity is distinct in temperate climates, some viral activity does take place during the winter months.[168] Infection and the acquisition of post-infection immunity occur with greater intensity and at earlier ages in crowded, economically deprived populations with less efficient sanitation.

Molecular techniques have allowed researchers to study the genotypes of specific viral types in populations over the course of time.[256,463,598,698] For example, Mulders and colleagues[698] studied the molecular epidemiology of wild poliovirus 1 in Europe, the Middle East, and the Indian subcontinent. These investigators found that four major genotypes were circulating. Two genotypes were found predominantly in eastern Europe, a third genotype was circulating mainly in Egypt, and the fourth genotype was dispersed widely. All four genotypes were present in Pakistan.

PREVALENCE OF DIFFERENT TYPES

The epidemiologic behavior of coxsackieviruses and echoviruses parallels that of polioviruses, in which unrecognized infections far outnumber infections with distinctive symptoms. The agents are disseminated widely throughout the world, and outbreaks caused by one or another type occur regularly. These outbreaks tend to be localized, with different agents prevalent in different years. In the late 1950s, however, echovirus 9 had a far wider circulation; it swept through a large part of the world and infected not only children but also young adults. This behavior has been repeated occasionally with other EVs: after a long absence, a particular agent returns and circulates among susceptible persons of different ages who have been born since the last epidemic. Other agents remain endemic in a given area and surface as sporadic cases and occasionally in small outbreaks. Multiple types frequently are active at the same time, although one agent commonly is predominant in a given locality.

Listed in Table 178–4 are the five most prevalent nonpolio enteroviral isolations per year in the United States from 1961

TABLE 178–4 Predominant Types of Nonpolio Enteroviral Isolations in the United States: 1961 to 2005*

	Five Most Common Viral Types Per Year				
	First	**Second**	**Third**	**Fourth**	**Fifth**
1961	Coxsackievirus B5	Coxsackievirus B2	Coxsackievirus B4	Echovirus 11	Echovirus 9
1962	Coxsackievirus B3	Echovirus 9	Coxsackievirus B2	Echovirus 4	Coxsackievirus B5
1963	Coxsackievirus B1	Coxsackievirus A9	Echovirus 9	Echovirus 4	Coxsackievirus B4
1964	Coxsackievirus B4	Coxsackievirus B2	Coxsackievirus A9	Echovirus 4	Echovirus 6, coxsackievirus B1
1965	Echovirus 9	Echovirus 6	Coxsackievirus B2	Coxsackievirus B5	Coxsackievirus B4
1966	Echovirus 9	Coxsackievirus B2	Echovirus 6	Coxsackievirus B5	Coxsackievirus A9, A16
1967	Coxsackievirus B5	Echovirus 9	Coxsackievirus A9	Echovirus 6	Coxsackievirus B2
1968	Echovirus 9	Echovirus 30	Coxsackievirus A16	Coxsackievirus B3	Coxsackievirus B4
1969	Echovirus 30	Echovirus 9	Echovirus 18	Echovirus 6	Coxsackievirus B4
1970	Echovirus 3	Echovirus 9	Echovirus 6	Echovirus 4	Coxsackievirus B4
1971	Echovirus 4	Echovirus 9	Echovirus 6	Coxsackievirus B4	Coxsackievirus B2
1972	Coxsackievirus B5	Echovirus 4	Echovirus 6	Echovirus 9	Coxsackievirus B3
1973	Coxsackievirus A9	Echovirus 9	Echovirus 6	Coxsackievirus B2	Coxsackievirus B5, echovirus 5
1974	Echovirus 11	Echovirus 4	Echovirus 6	Echovirus 9	Echovirus 18
1975	Echovirus 9	Echovirus 4	Echovirus 6	Coxsackievirus A9	Coxsackievirus B4
1976	Coxsackievirus B2	Echovirus 4	Coxsackievirus B4	Coxsackievirus A9	Coxsackievirus B3, echovirus 6
1977	Echovirus 6	Coxsackievirus B1	Coxsackievirus B3	Echovirus 9	Coxsackievirus A9
1978	Echovirus 9	Echovirus 4	Coxsackievirus A9	Echovirus 30	Coxsackievirus B4
1979	Echovirus 11	Echovirus 7	Echovirus 30	Coxsackievirus B2	Coxsackievirus B4
1980	Echovirus 11	Coxsackievirus B3	Echovirus 30	Coxsackievirus B2	Coxsackievirus A9
1981	Echovirus 30	Echovirus 9	Echovirus 11	Echovirus 3	Coxsackievirus A9, echovirus 5
1982	Echovirus 11	Echovirus 30	Echovirus 5	Echovirus 9	Coxsackievirus B5
1983	Coxsackievirus B5	Echovirus 30	Echovirus 20	Echovirus 11	Echovirus 24
1984	Echovirus 9	Echovirus 11	Coxsackievirus B5	Echovirus 30	Coxsackievirus B2, A9
1985	Echovirus 11	Echovirus 21	Echovirus 6, 7†		Coxsackievirus B2
1986	Echovirus 11	Echovirus 4	Echovirus 7	Echovirus 18	Coxsackievirus B5
1987	Echovirus 6	Echovirus 18	Echovirus 11	Coxsackievirus A9	Coxsackievirus B2
1988	Echovirus 11	Echovirus 9	Coxsackievirus B4	Coxsackievirus B2	Echovirus 6
1989	Coxsackievirus B5	Echovirus 9	Echovirus 11	Coxsackievirus B2	Echovirus 6
1990	Echovirus 30	Echovirus 6	Coxsackievirus B2	Coxsackievirus A9	Echovirus 11
1991	Echovirus 30	Echovirus 11	Coxsackievirus B1	Coxsackievirus B2	Echovirus 7
1992	Echovirus 11	Echovirus 30	Echovirus 9	Coxsackievirus B1	Coxsackievirus A9
1993	Echovirus 30	Coxsackievirus B5	Coxsackievirus A9	Echovirus 7	Coxsackievirus B3
1994	Coxsackievirus B2	Coxsackievirus B3	Echovirus 6	Echovirus 30	Enterovirus 71
1995	Echovirus 9	Echovirus 11	Coxsackievirus A9	Coxsackievirus B2	Echovirus 30, coxsackievirus B5
1996	Coxsackievirus B5	Echovirus 17	Echovirus 6	Coxsackievirus A9	Coxsackievirus B4
1997	Echovirus 30	Echovirus 6	Echovirus 7	Echovirus 11	Echovirus 18
1998	Echovirus 30	Echovirus 9	Echovirus 11	Coxsackievirus B3	Echovirus 6
1999	Echovirus 11	Echovirus 16	Echovirus 9	Echovirus 14	Echovirus 25
2000	Coxsackievirus B5	Echovirus 6	Coxsackievirus A9	Coxsackievirus B4	Echovirus 11
2001	Echovirus 18	Echovirus 13	Coxsackievirus B2	Echovirus 6	Echovirus 4
2002	Echovirus 7	Echovirus 9	Coxsackievirus B1	Echovirus 11	Coxsackievirus B5
2003	Echovirus 9	Echovirus 30	Coxsackievirus B1	Coxsackievirus B4	Coxsackievirus A9
2004	Echovirus 30	Echovirus 9	Coxsackievirus A9	Coxsackievirus B5	Coxsackievirus B4
2005	Coxsackievirus B5	Echovirus 6	Echovirus 30	Echovirus 18	Coxsackievirus B3

**The majority of patients from whom viruses were isolated had neurologic illnesses.*
†*Third and fourth place tie.*
Data from references 12, 129, 133, 134, 136, 139, 168, 329, and 905 and from personal communication from A. LaMonte-Fowlkes, Epidemiology Branch, Division of Viral Diseases, National Center for Immunization and Respiratory Diseases, Centers for Disease Control and Prevention, Atlanta, for the 2005 data.

through 2005.[12,129,136,139,158,159,168,329,905*] Most patients from whom viruses were isolated had neurologic illnesses. Other EVs also possibly were prevalent but did not cause clinical disease sufficiently severe enough to induce physicians to submit specimens for study. In addition, probably many coxsackievirus A infections, even in epidemic situations, have gone undiagnosed because inoculation of suckling mice was not performed. Although more than 62 nonpolio enteroviral types and 4 parechovirus types have been identified, of interest is that in the 45 years covered in Table 178–4, only 24 different virus types are noted. Khetsuriani and associates[138] at the CDC presented an extensive report on enteroviral surveillance in the United States for the period from 1970 to 2005. During this period, the most common enteroviral isolates, in order, were echoviruses 9, 11, and 30, coxsackievirus B5, and echovirus 6. During the period from 2000 to 2005, the most common isolates, in order, were echoviruses 9, 30, 18, 13 and coxsackievirus B5.

Similar data for the most common enteroviral isolates in Spain from 1988 to 1997 and in Belgium from 1980 to 1994 are available.[259,941] The EV isolated most frequently in both countries was echovirus 30. In 1997 and 1998, major epidemic disease caused by EV 71 occurred in Taiwan, Malaysia, Australia, and Japan.[100,146,423,547,650]

Even though the use of live polioviral vaccine has eliminated epidemic poliomyelitis in the United States, determining the effect that polio vaccine viruses has had on enteroviral ecology

*Also personal communication from A. LaMonte-Fowlkes, Epidemiology Branch, Division of Viral Diseases, National Center for Immunization and Respiratory Diseases, Centers for Disease Control and Prevention (CDC).

has been difficult. In 1970, polioviruses accounted for only 6 percent of the total number of enteroviral isolations from patients with neurologic illnesses.[168] Although the numbers are not directly comparable, more than one third of the enteroviral isolations in 1962 from similar patients were polioviruses.[168] However, Horstmann and colleagues[432] studied specimens from sewage and from asymptomatic children during the early vaccine era and noted that the number of yearly polioviral isolations (presumably vaccine strains) was greater than the number of nonpolio EVs. The prevalence of vaccine viruses apparently did not affect the seasonal epidemiology of other EVs.

PATHOGENESIS AND PATHOLOGY

EVENTS DURING PATHOGENESIS[88,168,169,662,751,837]

Figure 178–4 diagrams the events in pathogenesis. After initial acquisition of virus by the oral or respiratory route, implantation occurs in the pharynx and the lower alimentary tract. Within 1 day, the infection extends to the regional lymph nodes. On approximately the third day, minor viremia develops and results in the involvement of many secondary infection sites. In congenital infections, infection is initiated during the minor viremia phase. Multiplication of virus in secondary infection sites coincides with the onset of clinical symptoms. Illness can vary from minor infection to fatal disease. Major viremia occurs during the period of multiplication of virus in secondary infection sites; this period usually lasts from the third to the seventh day of infection. In many echovirus and coxsackievirus infections, involvement of the CNS apparently occurs at the same time as does other secondary organ involvement. Occasionally, the CNS symptoms of enteroviral infections are delayed, thus suggesting that seeding occurred later in association with the major viremic phase.

Cessation of viremia correlates with the appearance of serum antibody. The viral concentration in secondary infection sites begins to diminish on approximately the seventh day. However, infection continues in the lower intestinal tract for prolonged periods.

In Figure 178–5, clinical and subclinical events in polioviral infections are presented. By 3 to 5 days after exposure, virus can be recovered from blood, the throat, and feces. This finding may be accompanied by symptoms of the "minor illness," or the infection may be unrecognized clinically. The end of the period of viremia coincides with the appearance of antibodies and the onset of clinical signs of CNS involvement. The available evidence favors blood as the main pathway of CNS invasion in natural disease, but experimental infections in monkeys indicated that the virus can travel along the axons of peripheral nerves. Possibly, when tonsillectomy is performed on a child with an inapparent poliovirus infection, the virus enters the nerve fibers exposed during surgery and spreads to the cranial nerve nuclei in the brain, thereby resulting in bulbar paralysis.

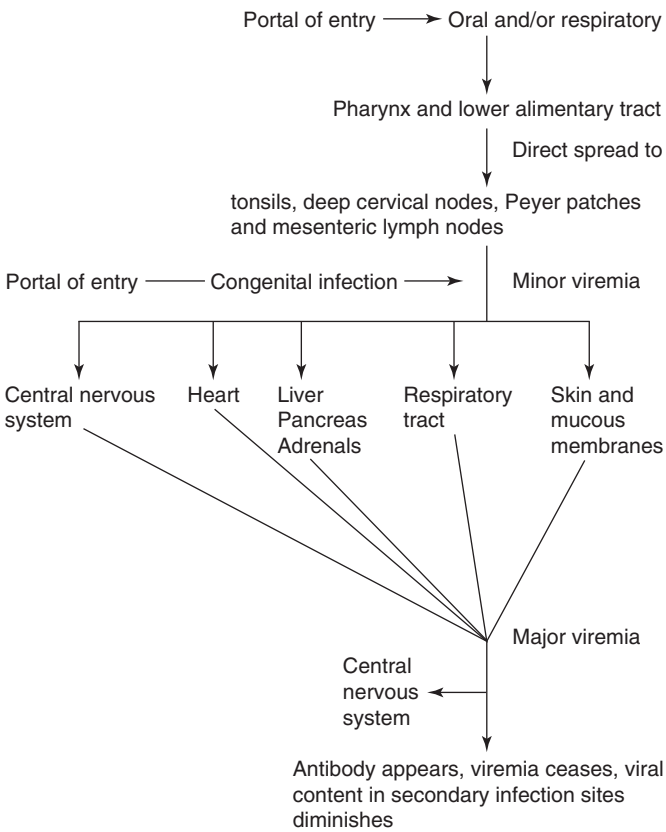

Figure 178–4 Pathogenesis of enteroviral infections. *(Modified from Cherry, J. D.: Enteroviruses.* In *Remington, J. S., and Klein, J. O. [eds.]: Infectious Diseases of the Fetus and Newborn Infant. Philadelphia, W. B. Saunders, 1976.)*

FACTORS THAT AFFECT PATHOGENESIS

The pathogenesis and pathology of enteroviral and parechoviral infections depend on the virulence, tropism, and inoculum concentration of virus, as well as on many specific host factors. EVs obviously have marked differences in both tropism and virulence. Although some generalizations can be made with regard to tropism, marked differences occur even among strains of specific viral types. Differences in the virulence of specific enteroviral types may be the result of recombination among EVs or point mutations.[795,810,849]

Van Eden and associates[955] studied 17 families during an outbreak of poliomyelitis caused by type 1 virus in the Netherlands. The findings suggested that human leukocyte antigen (HLA)–related genetic factors were important in the occurrence of paralytic disease.

Enteroviral infections in the fetus and neonate generally are thought to be more severe than are similar infections in older persons. This situation undoubtedly is the case with coxsackievirus B infection and probably also with coxsackievirus A and echovirus infections. Although the reasons for this increased severity are unknown, several aspects of neonatal immune mechanisms have been suggested. In addition, the similarity of coxsackievirus B infections in suckling mice to those in human neonates provided a useful animal model system. Heinberg and associates[406] compared coxsackievirus B1 infections in 24-hour-old suckling mice with similar infections in older mice. These investigators noted that adult mice produced interferon in all infected tissues, whereas suckling mice produced only small amounts of interferon in the liver. These researchers thought that the difference in outcome of coxsackievirus B1 infection in suckling and older mice could be explained by the inability of cells of the immature animals to elaborate interferon. Additional studies of abnormalities of innate immunity in neonates may enhance our understanding of the severity of enteroviral infections in newborns.[591]

Other researchers suggested that the increased susceptibility of suckling mice to severe coxsackievirus infections is related to the transplacentally acquired, increased concentrations of adrenocortical hormones.[60,89] Kunin[558] suggested that the difference in age-specific susceptibility could be explained at the cellular level. He showed that various tissues of newborn mice bind coxsackievirus B3, whereas tissues of adult mice are virtually inactive in this regard.[557,558] The progressive loss of receptor-containing

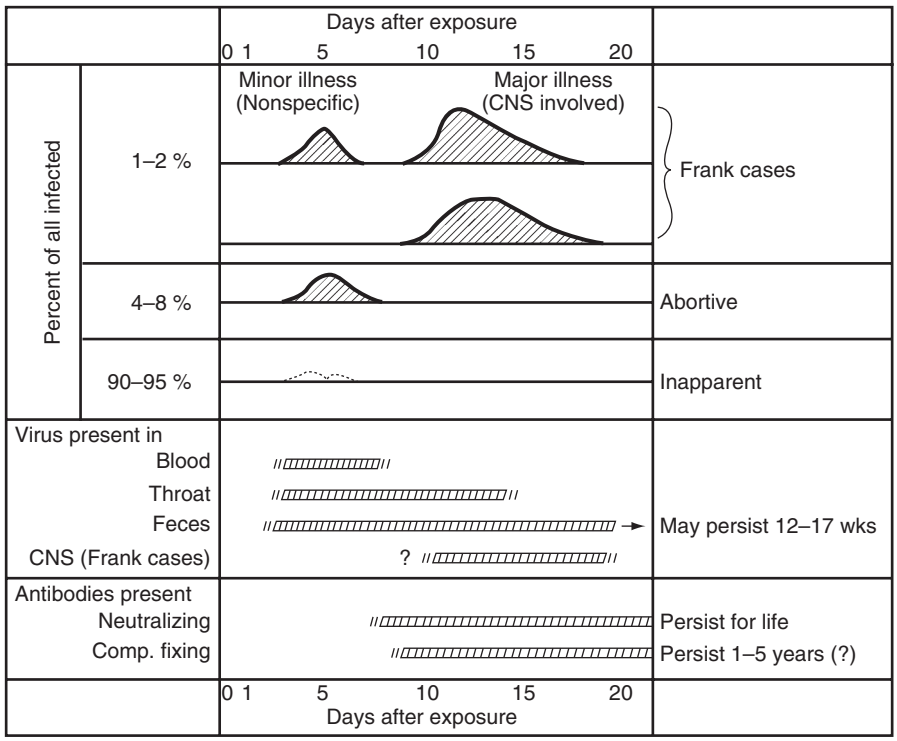

Figure 178–5 The course of clinical and subclinical forms of poliovirus infection in relation to the presence of virus and the development of antibodies. CNS, central nervous system. *(From Bodian, D., and Horstmann, D. M.: Poliomyelitis.* In *Horsfall, F. L., and Tamm, I. [eds.]: Viral and Rickettsial Infections of Man. 4th ed. Philadelphia, J. B. Lippincott, 1965, pp. 430-473.)*

cells with increasing age may be the mechanism that accounts for less severe infections in older animals. Supporting this suggestion, Ito and colleagues[466] showed that expression of coxsackievirus and adenovirus receptor (CAR) decreases with increasing age in rats. In the past, researchers assumed that specific disease in various organs and tissues in enteroviral infections was caused by the direct cytopathic effect and tropism of a particular virus. In more recent years, however, large numbers of studies using murine model systems suggested that host immune responses contribute to the pathologic process.* These studies suggested that T-cell–mediated processes and virus-induced autoimmunity cause both acute and chronic tissue damage. In contrast, other studies suggested that the primary viral cytopathic effect is responsible for the tissue damage and that the various T-cell responses are a reaction to the damage and not its cause.[648]

Since the early 1960s, the clinical manifestations caused by several enteroviral serotypes have changed. For example, echovirus 11 infection initially was noted in association with an outbreak of upper respiratory infection in a daycare center in 1958.[768] Then in the 1960s, this infection was found to be related to exanthem and aseptic meningitis.[172,177] Following this event and occurring at present is the association of echovirus 11 infection and severe sepsis-like illness with hepatitis in neonates.[90,168,169,386,491,675,693]

Another example relates to EV 71 infections. Initially, this virus was noted in association with aseptic meningitis, and only a few patients also had exanthem.[516,858] Since the late 1990s, severe epidemic disease with EV 71 has occurred in Taiwan, Singapore, Australia, Malaysia, and Japan. In these epidemics, hand, foot, and mouth syndrome is a major finding, and the neurologic disease is more severe than in the past.†

These phenotypic changes could be the result either of point mutations or of recombination among EVs.[148,181,520,611,612,736,849,882] Chan and AbuBakar[148] presented evidence indicating that a recombination event occurred between EV 71 and coxsackievirus A16.

PATHOLOGY

The clinical signs of enteroviral infection vary widely, so great variations in pathology also exist. Because pathologic material generally is available only from patients with fatal illness, this section discusses only the more severe manifestations. Worth emphasizing, however, is that these fatal infections account for just a small portion of all enteroviral infections. The pathologic findings in children with milder infections, such as nonspecific febrile illness, have not been described.

Coxsackieviruses A

Records of severe illness associated with coxsackieviruses A are rare. Gold and colleagues,[356] in a study of sudden unexpected death in infants, recovered coxsackievirus A4 from the brains of three children. In none of these patients were histologic abnormalities noted in the brain or spinal cord. An adult with a fatal coxsackievirus A7 infection had diffuse pancarditis and organized pneumonitis.[45]

Coxsackieviruses B

Of the nonpolio EVs, coxsackieviruses B have been associated most frequently with severe and catastrophic disease. The most common findings in these cases have been myocarditis, meningoencephalitis, or both. Involvement of the adrenals, pancreas, liver, and lungs also has been noted.

*See references 28, 160, 331, 334, 410, 413, 425, 494, 568, 751, 754, 787, 796, 818, 868, 1010.

†See references 118, 145, 146, 150, 153-155, 164, 189, 317, 423, 436, 441-443, 464, 500, 547, 593, 599-602, 610, 634, 649, 650, 728, 744, 781, 972-974, 1024.

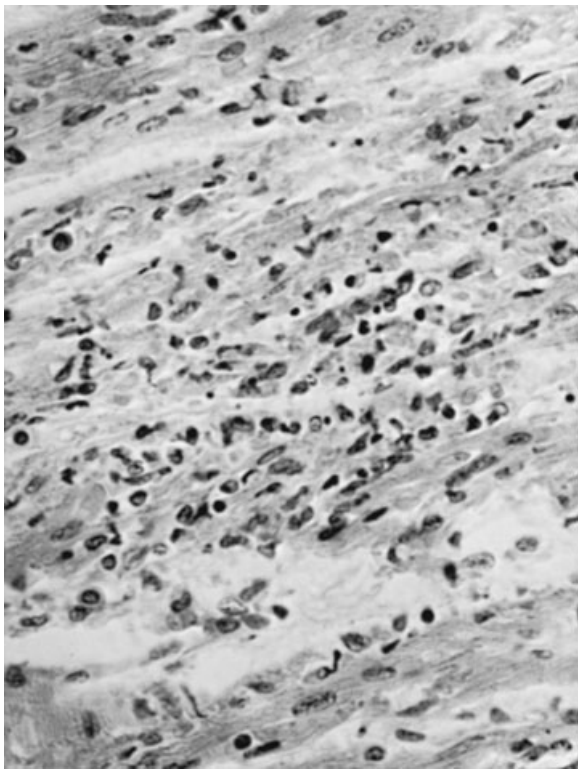

Figure 178–6 Coxsackievirus B4 myocarditis in a 9-day-old infant. Note the myocardial necrosis and mononuclear cellular infiltration. *(From Cherry, J. D.: Enteroviruses.* In *Remington, J. S., and Klein, J. O. [eds.]: Infectious Diseases of the Fetus and Newborn Infant. Philadelphia, W. B. Saunders, 1976.)*

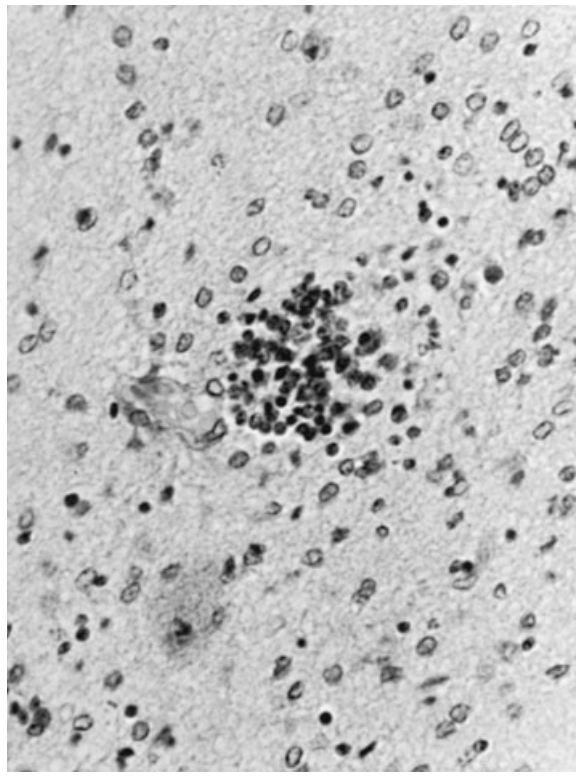

Figure 178–7 Coxsackievirus B4 encephalitis in a 9-day-old infant with focal infiltration of mononuclear and glial cells. *(From Cherry, J. D.: Enteroviruses.* In *Remington, J. S., and Klein, J. O. [eds.]: Infectious Diseases of the Fetus and Newborn Infant. Philadelphia, W. B. Saunders, 1976.)*

HEART[168,288,338,1011]

Grossly, the heart usually is enlarged, with dilation of the chambers and flabby musculature. Microscopically, the pericardium frequently contains some inflammatory cells along with thickening and edema, and the endocardium may have focal infiltrations of inflammatory cells. The myocardium (Fig. 178–6) is congested and contains infiltrations of inflammatory cells (lymphocytes, mononuclear cells, reticulum cells, histiocytes, plasma cells, and polymorphonuclear and eosinophil leukocytes). Involvement of the myocardium often is patchy and focal but occasionally diffuse. The muscle shows loss of striation as well as edema and eosinophilic degeneration. Muscle necrosis without extensive cellular infiltration is a common finding.

BRAIN AND SPINAL CORD[168,288,684,1011]

The meninges are congested, edematous, and occasionally mildly infiltrated with inflammatory cells. Lesions in the brain and spinal cord are focal rather than diffuse, but they frequently involve many different areas. The lesions consist of areas of eosinophilic degeneration of cortical cells, clusters of mononuclear and glial cells (Fig. 178–7), and perivascular cuffing. Two children with fatal coxsackieviral B infection (types 2 and 4) had, in addition to typical inflammatory encephalitic lesions, widespread multifocal areas of liquefaction necrosis without inflammation.[279]

OTHER ORGANS[11,101,168,336,684]

The lungs frequently have areas of mild focal pneumonitis with peribronchiolar mononuclear cellular infiltration. The liver often is engorged and occasionally contains isolated foci of liver cell necrosis and mononuclear infiltration. In the pancreas, occasional focal degeneration of islet cells occurs. Congestion has been observed in the adrenal glands, along with mild to severe cortical necrosis; inflammatory cells are present.

Echoviruses

Although frequently responsible for illness, echoviruses rarely were associated with fatal infection until relatively recently. In several different reports with eight different echovirus types, hepatic necrosis was a major pathologic finding.[83,168,341,386,675,693,1017] Massive hepatic necrosis has been seen with echoviruses 3, 6, 7, 9, 11, 14, 19, and 21. At autopsy, one infant with echovirus 6 infection had cloudy and thickened leptomeninges, liver necrosis, adrenal and renal hemorrhage, and mild interstitial pneumonitis.[168] One infant with echovirus 9 infection had an enlarged and congested liver with marked central necrosis,[168] and another with this virus had interstitial pneumonitis without liver involvement.[168] Three infants with echovirus 11 infection had renal and adrenal hemorrhage and small vessel thrombi in the renal medulla and in both the medulla and inner cortex of the adrenal glands.[702] These patients' livers were normal. Two infants, one with echovirus 6 and the other with echovirus 31 infection, had only extensive pneumonia.[97,168]

Enteroviruses

The brain of a child who died of acute encephalitis caused by EV 71 infection was examined.[1022] Grossly, the brain appeared normal, but microscopically, it revealed typical features of acute

encephalitis consisting of perivascular cuffing with mixed inflammatory cells, neuronophagia, and inflammatory nodules.

Polioviruses[88,198]

The neuropathy of poliomyelitis usually is pathognomonic; only certain cells and areas of the neuraxis are susceptible to the virus. Neuronal damage is caused directly by virus multiplication, but not all affected neurons are killed. The injury may be reversible, and function may be restored within 3 to 4 weeks after onset. Little histologic evidence of meningeal reaction exists. Perivascular cuffing and some interstitial glial infiltration are present. Histologic sections generally reveal more widespread lesions than would be estimated from the clinical findings. Scattered neurons may undergo considerable destruction without causing clinical disability.

Regions in which neuronal lesions occur are (1) the spinal cord (anterior horn cells chiefly and, to a lesser degree, the intermediate and dorsal horn and dorsal root ganglia), (2) the medulla (vestibular nuclei, cranial nerve nuclei, and the reticular formation that contains the vital centers), (3) the cerebellum (nuclei in the roof and vermis only), (4) the midbrain (chiefly the gray matter but also the substantia nigra and occasionally the red nucleus), (5) the thalamus and hypothalamus, (6) the pallidum, and (7) the cerebral cortex (motor cortex). The viruses spare the following areas: (1) the entire cerebral cortex except the motor area, (2) the cerebellum except the vermis and deep midline nuclei, and (3) the white matter of the spinal cord. This distribution of lesions permits the physician to establish a histologic diagnosis of poliomyelitis.

Extraneural disease usually is a secondary phenomenon. Bronchopulmonary changes such as aspiration pneumonia, atelectasis, and purulent bronchitis may occur because of impaired coughing and decreased thoracic movement. Cardiovascular changes may result in hypertension, cardiac failure, and pulmonary edema. Prolonged immobilization leads to negative nitrogen and calcium balance, along with urinary lithiasis, renal failure, hypertension with encephalopathy, and convulsions. Treatment itself may cause untoward complications, such as urinary tract infection (after catheterization), decubitus ulcers, and psychotic disturbances. Ulcerations in the alimentary tract may result in serious bleeding and occasional perforation. Respiratory failure culminates in respiratory acidosis and anoxic changes.

Parechoviruses

Although parechoviral infections have a broad spectrum of clinical manifestations, including encephalitis and sepsis-like neonatal disease, pathologic data are not available.[1,71,92,575,981]

CLINICAL MANIFESTATIONS: NONPOLIO ENTEROVIRUSES AND PARECHOVIRUSES

Nonpolio enteroviral and parechoviral infections are exceedingly common, and the spectrum of disease is protean (Tables 178–5 through 178–18). Many of the clinical-virologic associations listed in Tables 178–5 through 178–18 are based on a limited number of cases. Because EVs and parechoviruses frequently are carried asymptomatically in the gastrointestinal tract for relatively long periods of time, some of the observed illnesses and the viruses concomitantly recovered may not have a cause-and-effect relationship. However, repeated observations since the 1960s have supported many virus-illness associations, even though their occurrence has been sporadic.

TABLE 178–5 Approximate Frequencies of Asymptomatic Infection with Selected Coxsackieviruses and Echoviruses

	Asymptomatic Infection Frequency (%)
Coxsackieviruses	
A	50
A16	50
B	20
B2	11-50
B3	25-96
B4	30-70
B5	5-40
Echoviruses	50
4	Uncommon-60
6	Rare
9	15-60
18	Rare-20
20	33
25	30
30	50

TABLE 178–6 Enteroviruses and Parechoviruses Noted in Association with Nonspecific Febrile Illness and Respiratory Disease

	Virus Types		
Clinical Categories	**Coxsackieviruses A**	**Coxsackieviruses B**	**Enteroviruses and Parechoviruses**
Nonspecific febrile illness	All types	All types	All types
Common cold	Mainly 21, 24; rarely other types	Mainly 1-5; rarely 6	Mainly 2, 20; rarely other types
Pharyngitis (pharyngitis, tonsillitis, tonsillopharyngitis, and nasopharyngitis)	Probably all types; mainly 9	Probably all types; mainly 1-5	Probably all types; mainly 2, 4, 6, 9, 11, 16, 19, 25, 30, 71, PE 3, 6
Herpangina	1-10, 16, 22	1-5	6, 9, 11, 16, 17, 25, PE 1
Lymphonodular pharyngitis	10		
Stomatitis and other lesions in the anterior of the mouth	5, 9, 10, 16	2, 5	9, 11, 20, 71
Parotitis	Coxsackievirus A not typed	3, 4	70
Croup	9	4, 5	4, 11, 21
Bronchitis		1,4	8, 12-14, PE 1
Bronchiolitis and infectious asthma	Many types	Many types	Many types
Pneumonia	9, 16	1-6	6, 7, 9, 11, 12, 19, 20, 30, 68, 74, 78, PE 1
Pleurodynia	1, 2, 4, 6, 9, 16	1-6	1-3, 6-9, 11, 12, 14, 16-19, 24, 25, 30, PE 2

PE, parechoviruses.

TABLE 178–7 Enteroviruses and Parechoviruses Noted in Association with Gastrointestinal Complaints

	Virus Types		
Clinical Categories	**Coxsackieviruses A**	**Coxsackieviruses B**	**Enteroviruses and Parechoviruses**
Gastrointestinal, not specified	2, 4, 5, 6, 7, 9, 10, 14, 16	1-5	1-9, 11, 12, 14, 16-21, 24, 25, 30, 71, PE 1, 2, 3, 6
Nausea and vomiting	9, 16	2-5	2, 4, 6, 9, 11, 16, 18-20, 30, PE 1
Diarrhea	1, 9, 16	2-5	3, 4, 6, 7, 9, 11-14, 16-21, 25, 30, PE 1
Constipation	9	3-5	4, 6, 9, 11
Abdominal pain	9, 16	2-5	4, 6, 9, 11, 18, 19, 30
Pseudoappendicitis			1, 8, 14
Peritonitis		1	
Mesenteric adenitis		5	7, 9, 11
Appendicitis		2, 5	
Intussusception		3	7, 9
Hepatitis	4, 9, 10, 20, 24	1-5	1, 3, 4, 6, 7, 9, 11, 14, 20, 21, 30
Reye syndrome	2	4	14, PE 1
Pancreatitis	9	3-5	
Diabetes mellitus		1-5	

PE, parechoviruses.

TABLE 178–8 Frequency of Vomiting in Outbreaks of Illness Caused by Coxsackieviruses and Echoviruses

Virus Type	Age Group (yr)	Vomiting (%)	Main Characteristics of Outbreak	References
Coxsackievirus A9	Mainly children	60-73	Meningitis	168
	Children	14-20	Rash	168, 587
Coxsackievirus A16	Children and adults	3-15	Hand, foot, and mouth syndrome	9, 813
Coxsackievirus B2	Mainly children	50	Meningitis	168
	Mainly children	18-66	Febrile illness, respiratory illness	168
Coxsackievirus B3	Adults and children	33	Pleurodynia	168
	Children	9	Febrile illness, respiratory illness	168
	Mainly children	25	Fever, diarrhea	290
Coxsackievirus B4	Mainly children	27	Nonspecific fever	292
Coxsackievirus B5	Adults and children	31-37	Pleurodynia	362, 880
	Adults and children	50-95	Meningitis	168, 186, 381, 832
	Mainly children	25-33	Nonspecific febrile illness	186, 832, 1006
	Children	45	Febrile illness, respiratory illness	848
	Mainly children	100	Hepatosplenic syndrome	880
	Children	40	Rash	176
Echovirus 2	Children	12	Respiratory illness, rash	168
Echovirus 4	Mainly children	70-90	Meningitis	506, 799
	Mainly children	9-50	Epidemic disease including meningitis, minor illness	168, 726
Echovirus 6	Mainly children	55-98	Meningitis	394, 505, 528, 562, 986
	Mainly children	50-75	Epidemic disease including meningitis	168, 1006
Echovirus 9	0-4	64-71	Rash, meningitis	168, 839
	5-9	81-83		
	10-19	61-92		
	>20	20-38		
	Mainly children	39-95	Rash, meningitis	168, 777, 986, 997
	Mainly children	26	Pharyngitis	168
	Mainly children	71	Epidemic disease including meningitis	168
Echovirus 11	Not specified	100	Febrile illness, respiratory illness	168
Echovirus 16	Children	30	Rash	387
Echovirus 19	<0.5	25	Meningitis, upper respiratory illness, nonspecific febrile illness	168
	0.5-2	31		
	3-5	37		
	6-12	68		
	13-18	61		
	19-25	53		
	26+	43		
Echovirus 20	Children	67	Febrile illness, respiratory illness	218
Echovirus 30	Mainly children	15-91	Meningitis	366, 502, 771, 935
	<5	50	Febrile illness, respiratory illness	168
	>5	70		

TABLE 178–9 Frequency of Diarrhea in Outbreaks of Illness Caused by Coxsackieviruses and Echoviruses

Virus Type	Age Group (yr)	Diarrhea (%)	Main Characteristics of Outbreak	References
Coxsackievirus A9	Adults and children	7-100	Fever, rash	168, 586
Coxsackievirus A16	Mainly children	4-33	Hand, foot, and mouth syndrome	9, 171, 584, 817
Coxsackievirus B2	Mainly children	9-56	Febrile illness, respiratory illness	168
	Mainly children	12	Meningitis	626
Coxsackievirus B3	Mainly children	2-5	Pleurodynia	168
	Children	11	Meningitis	216
	Mainly children	54	Nonspecific febrile illness	290
Coxsackievirus B4	Children	9	Meningitis	217
	Mainly children	8	Febrile illness, respiratory illness	291
Coxsackievirus B5	<1	21	Meningitis and fever, respiratory illness	775
	1-9	15		
	10-29	3		
	Mainly children	5-9	Meningitis	168, 216, 1006
Echovirus 4	Mainly children	5-75	Meningitis	506, 577, 836
	Mainly children	65	Nonspecific febrile illness	577
Echovirus 6	Mainly children	6-12	Meningitis	
Echovirus 9	0-4	14	Meningitis, rash	168, 394, 505, 836
	5-9	10		
	10-19	8		
	<20	0		
	Mainly children	3-40	Meningitis	168, 219, 836
	Children	15	Rash, febrile illness	258
	Mainly children	5-15	Respiratory illness	168
Echovirus 11	Adults	33-100	Gastrointestinal illness	106, 538
	Mainly infants	40	Nonspecific febrile illness	78
Echovirus 13	Children	11	Respiratory illness	168
Echovirus 16	Children	20	Rash	387
Echovirus 17	<1-4	100	Fever, diarrhea	168
Echovirus 18	Infants	100	Diarrhea	270
Echovirus 19	<2	30	Meningitis	168
	2-4	7		
	5-11	7		
	12-17	0		
	>18	9		
	Mainly children	3	Meningitis	358
	Mainly children	10	Respiratory illness	217, 358
	Mainly children	9	Diarrhea	369
Echovirus 20	Children	100	Respiratory, enteric illness	218
Echovirus 30	<5	12	Meningitis	168
	>5	5		
	Adults and children	7-10	Meningitis	388, 771, 935

TABLE 178–10 Frequency of Abdominal Pain in Outbreaks of Illness Caused by Coxsackieviruses and Echoviruses

Virus Type	Age Group (yr)	Abdominal Pain (%)	Main Characteristics of Outbreak	References
Coxsackievirus A9	Mainly children	7	Meningitis	168
	Children	20	Rash	175
Coxsackievirus A16	Mainly children	12	Hand, foot, and mouth syndrome	587, 813
Coxsackievirus B2	Mainly children	22	Meningitis	626
Coxsackievirus B3	Children and adults	25-90	Pleurodynia	168
Coxsackievirus B4	Children and adults	10	Meningitis	168
	Mainly children	36	Febrile illness, respiratory illness	291
Coxsackievirus B5	Mainly children	10	Meningitis	168
	Children	13	Febrile illness, respiratory illness	848
	Children and adults	23-67	Pleurodynia	362, 835, 880
Echovirus 4	Mainly children	28-50	Meningitis	168, 506
	Mainly children	13	Febrile illness, respiratory illness	726
Echovirus 6	Mainly children	17-43	Meningitis	168, 394, 505, 528
Echovirus 9	0-4	28	Meningitis, rash	168, 186, 584, 839
	5-9	30		
	10-19	25		
	≥20	0		
Echovirus 11	Adults	38	Gastrointestinal illness	106
Echovirus 16	Children	20	Rash	387
Echovirus 19	<0.5	3	Meningitis	168
	0.5-2	3		
	3-5	27		
	6-12	38		
	13-18	13		
	19-25	9		
	26+	3		
Echovirus 30	Mainly children	17	Meningitis	502, 935

TABLE 178–11 Frequency of Conjunctivitis in Outbreaks of Illness Caused by Coxsackieviruses and Echoviruses

Virus Type	Age Group	Conjunctivitis (%)	Main Characteristics of Illness	References
Coxsackievirus A9	Children	20	Pharyngitis, rash	587
Coxsackievirus A16	Mainly children	0-18	Hand, foot, and mouth syndrome	157, 619, 813
Coxsackievirus B2	Mainly children	2	Nonspecific febrile illness, respiratory illness	168
Coxsackievirus B3	Children	2-42	Upper respiratory illness	168
Coxsackievirus B4	Children	25	Upper respiratory illness	168
Coxsackievirus B5	Mainly children	7-50	Rash, meningitis, hepatosplenic illness, respiratory illness, pleurodynia	177, 216, 835, 848
Echovirus 2	Children	25	Rhinorrhea	168
Echovirus 4	Children	Rare	Meningitis	624
Echovirus 9	Mainly children	13-30	Rash, meningitis	127, 168, 839
Echovirus 11	Children	15	Rash	177
Echovirus 16	Children	6-10	Rash	387, 717
Echovirus 30	Mainly children	10-60	Meninigitis, nonspecific febrile illness	168, 388, 935

TABLE 178–12 Nonpolio Enteroviruses and Parechoviruses Associated with Pericarditis and Myocarditis

Virus Type		Age Group or Age of Individual Patients	Heart Involvement				Other Aspects of Illness	References
			Etiologic Importance*	Pericarditis	Myocarditis	Unspecified		
Coxsackievirus	A1	Infants and adults	±	+	+			168, 373
	A2	Adults	±			+		34, 168
	A4	Mainly children	+	+	+		Sudden infant death	34, 356, 374, 983
	A5	—	±	+				956
	A7	Adults	±	+	+		Hand, foot, and mouth syndrome	45
	A8	—	±			+		373
	A9	Mainly infants	+		+		High fatality rate	374
	A10	—	±			+		34
	A16	Infants and child	±		+		High fatality rate	168, 971, 1018
Coxsackievirus	B1	Infants, children, and adults	++	+	+		Rare hepatitis	10, 31, 34, 109, 168, 244, 373, 525, 647, 712, 817, 859, 992
	B2	Infants, children, and adults	+++	+	+		Occasional pneumonia	31, 34, 108, 109, 168, 244, 294, 378, 435, 525, 549, 647, 712, 817, 859, 864, 983, 992
	B3	Infants, children, and adults	+++	+	+		Rare hepatitis	34, 40, 66, 108, 109, 168, 244, 378, 436, 525, 549, 592, 712, 817, 864, 909, 978, 979, 983, 992
	B4	Infants, children, and adults	+++	+	+		Occasional pneumonia; myocardial calcifications; rare hepatitis, orchitis, nephritis, hemolytic uremic syndrome	31, 34, 40, 52, 99, 107-109, 168, 244, 294, 378, 436, 525, 549, 712, 800, 817, 911, 945, 948, 983
	B5	Infants, children, and adults	+++	+	+		Occasional pneumonia, pleurodynia, and meningitis; rare orchitis	31, 33, 44, 91, 109, 168, 344, 362, 404, 409, 549, 606, 647, 689, 712, 733, 773, 775, 783, 859, 864, 983, 988, 992, 1006

TABLE 178–12 Nonpolio Enteroviruses and Parechoviruses Associated with Pericarditis and Myocarditis—cont'd

Virus Type		Age Group or Age of Individual Patients	Heart Involvement				Other Aspects of Illness	References
			Etiologic Importance*	Pericarditis	Myocarditis	Unspecified		
Echovirus	1	Adolescents	±	+	+			34, 856, 992
	4	—	±			+		34, 655
	6	Children and adults	++	+				34, 63, 168, 992
	7	Children and adults	±			+		34, 168, 682
	8	Adolescents	±	+				168, 486, 499
	9	Children and adults	+	+	+			34, 168, 174, 592, 655, 680, 983
	11	Children and adults	+			+		34, 63, 168, 817
	14	—	±			+		682, 956
	17	—	±			+		34
	19	Children and adults	±	+	+			63, 168
	25	Children	±		+			63, 65
	30	—	±			+		34, 592
Enterovirus	71			±		+	Encephalitis	423
Parechovirus	1	Children	±		+			625, 834

*++++ = *most common*; ± = *rare*.

Few specific EV or parechovirus diseases exist, but, rather, various interrelated syndromes and anatomically associated illnesses are reported. Many illnesses and syndromes can be caused by different viral types, and most types are capable of inducing a variety of clinical syndromes. Conversely, certain specific coxsackieviral and echoviral types have clinical characteristics that facilitate an etiologic diagnosis.

Since the late 1980s, few careful studies have been conducted in which specific clinical findings were correlated with individual enteroviral types. This paucity was fostered by the expense of serotyping and the lack of using inoculation of suckling mice for isolation of virus. The increased use of polymerase chain reaction (PCR) also has contributed to the lack of identification of manifestations of disease by specific viral type. This situation is unfortunate because the clinical manifestations caused by specific viral types are not constant, and disease severity varies by specific enteroviral type.

ASYMPTOMATIC INFECTION

Because researchers have known that 90 to 95 percent of poliovirus infections are not recognized clinically, they have assumed that most infections with EVs and parechoviruses are asymptomatic. This opinion is strengthened by the finding that these viruses can be recovered frequently from the stools of healthy children. However, relatively few data are available on the rate of asymptomatic infection with nonpolio EVs and parechoviruses. All too frequently, isolation of EVs and parechoviruses from stool is equated with asymptomatic infection. This assumption is an error because illness, if it takes place, occurs shortly after the acquisition of virus and is short-lived; a particular infection may have been associated with a nonspecific illness 2 or 3 months before a stool specimen is obtained in a surveillance program. Unfortunately, in several studies involving controlled population groups in which accurate clinical expression rates could have been determined, clinical observations apparently were of secondary importance to the investigators.[168,339,340,342,546] The data available suggest differences in clinical expression among coxsackieviral and echoviral types.

Table 178–5 lists the approximate frequency of asymptomatic infections with selected coxsackieviruses and echoviruses. As can be seen, the rates vary among the group as a whole and even within specific types. Overall, approximately half, and perhaps more, of all nonpolio enteroviral infections appear to be associated with clinical manifestations. In general, the more carefully clinical symptoms are examined, the smaller is the percentage of truly asymptomatic infections. Clinical expression also is related inversely to age. With coxsackievirus A16, asymptomatic infection occurs in only approximately 10 percent of children younger than 5 years old, whereas rates are higher in older children and adults.[9,168] Sabin[836] reported no asymptomatic infections in infants with echovirus 18, and Nishmi and Yodfat[726] noted fewer than 20 percent of infections without illness in children younger than 8 years during an echovirus 4 epidemic. At the other extreme, Clemmer and associates[206] reported that 96 percent of infections with coxsackievirus B3 were asymptomatic. In other studies, the illness rate of coxsackievirus B3 was 60 to 75 percent.

NONSPECIFIC FEBRILE ILLNESS

Nonspecific febrile illness is the most common manifestation of coxsackieviral and echoviral infection (see Table 178–6). All viral types cause this clinical finding, but its frequency varies considerably among the individual viruses.

The onset of illness usually is abrupt, without a prodrome. In young children, the initial finding is fever and associated malaise. In older children, headache generally is noted. The temperature ranges between 38.3° C and 40° C (101° F and 104° F) and has a mean duration of 3 days. In some instances, the fever is biphasic: it occurs for 1 day, is absent for 2 to 3 days, and then recurs for an additional 2 to 4 days. In many young children, the only manifestation of illness is fever, and its presence is discovered by chance by a parent.

Malaise and anorexia often are related to the degree of temperature elevation, as is headache in older patients. Sore throat is a common complaint, but an inflamed pharynx is not seen on examination. Nausea and vomiting occasionally occur at the onset of illness, as does mild abdominal discomfort. One or two

TABLE 178–13 Frequency of Exanthem in Outbreaks of Illness Caused by Enteroviruses and Parechoviruses

Virus Type		Age Group	Occurrence of Rash	Characteristics of Rash	Associated Manifestations	References
Coxsackievirus	A2	Children	Rare	Maculopapular	Fever	34, 836
	A4	Children	Rare	Maculopapular, vesicular	Fever, herpangina, hepatitis	34, 172, 302, 691, 1036
	A5	Mainly children	Occasional	Hand, foot, and mouth syndrome	Fever	34, 221, 298, 302
	A7	Children and adults	Rare	Morbilliform; hand, foot, and mouth syndrome, pancarditis	Meningitis, pneumonia	45, 372
	A9	Mainly children	4%	Maculopapular, vesicular, urticarial, petechial; hand, foot, and mouth syndrome	Fever, meningitis, pneumonia	34, 168, 172, 175, 429, 449, 487, 490, 530, 587, 686, 732, 1001
	A10	Mainly children	Occasional	Hand, foot, and mouth syndrome	Fever	34, 110, 168, 203, 260, 590
	A16	Children and adults	88%, <5 yr 38%, 5-12 yr 11%, adults	Hand, foot, and mouth syndrome	Fever	9, 15, 26, 31, 34, 103, 168, 171, 172, 281, 302, 315, 337, 354, 355, 418-420, 590, 619, 620, 652, 687, 703, 809, 813, 932
	B1	Children	Occasional	Maculopapular, vesicular	Fever, meningitis	34, 168, 170, 172, 239, 616
	B2	Children	Rare	Maculopapular, vesicular, petechial	Fever, herpangina, meningitis	31, 34, 302
	B3	Mainly children	Occasional	Maculopapular, vesicular, petechial	Fever, hepatosplenomegaly	31, 34, 115, 168, 212, 290, 395, 447, 586
	B4	Mainly children	Occasional	Maculopapular, vesicular, urticarial	Fever, respiratory illness	168, 291, 506, 527
	B5	Mainly children	10%	Maculopapular, petechial, urticarial	Fever, meningitis	34, 91, 168, 176, 212, 244, 381, 403, 712, 727, 773, 775, 848, 880, 902, 906, 912, 1006, 1020
	B6	Children and adults	20%	Morbilliform	Pneumonia	357
Echovirus	1	Children	Rare	Maculopapular	Conjunctivitis	638
	2	Children	Rare	Macular, maculopapular	Fever, pharyngitis	168, 839, 896
	3	Children	Rare	Petechial	Fever, meningitis	34, 400, 430
	4	Mainly children	10%-20%	Macular, maculopapular, petechial	Fever, meningitis	168, 170, 268, 324, 506, 577, 624, 726, 799, 892
	5	Infants and adults	Occasional	Macular	Fever	342, 870
	6	Mainly children	Rare	Maculopapular, macular, papulopustular, vesicular	Fever, meningitis	34, 505, 632, 646, 651, 963, 966, 1006
	7	Children	Occasional	Maculopapular	Fever, meningitis	34, 475, 638
	9	Children and adults 41%, 5-9 yr 6%, >10 yr	57%, <5 yr	Maculopapular, petechial, vesicular	Fever, meningitis	34, 75, 168, 202, 210, 219, 237, 286, 299, 325, 328, 401, 429, 473, 487, 496, 525, 527, 540, 561, 581, 618, 645, 686, 712, 723, 777, 784, 797, 831, 839, 889, 903, 916, 997
	11	Mainly children	Occasional	Maculopapular, vesicular, urticarial	Fever, meningitis	34, 106, 168, 177, 246, 864
	13	Children	Occasional	Maculopapular		426, 890
	14	Mainly children	Rare	Maculopapular, scarlatiniform	Fever, meningitis	34, 839
	16	Children	Occasional	Roseola-like	Fever, herpangina	166, 213, 387, 714-717
	17	Children	Occasional	Macular, maculopapular, papulovesicular	Fever, diarrhea, herpangina, meningitis	34, 103, 170, 178
	18	Children and adults	Occasional, epidemic	Rubelliform	Fever, meningitis	517, 654, 686, 712
	19	Children and adults	Occasional	Maculopapular	Fever, meningitis, upper respiratory illness	209, 217, 358
	25	Children	Occasional	Maculopapular, hemangioma-like	Fever, pharyngitis	65, 690, 708
	30	Children and adults	Occasional	Macular, maculopapular	Fever, meningitis	34, 168, 502, 935, 1021
	32	Children	Rare	Hemangioma-like	Fever	170
	33	—	Rare	—	Meningitis	504, 514
Enterovirus	71	Children	Occasional	Macular, maculopapular, vesicular; hand, foot, and mouth syndrome	Fever, meningitis, encephalitis, paralytic disease	13, 343, 464, 516, 660, 674
Parechovirus	1	Infants	Rare	Morbilliform	Respiratory illness	79
	3	Infants	Rare	—	—	981
	6	Infants	Rare	—	—	981

TABLE 178–14 Symptoms and Signs of Coxsackievirus A16 Illness (Hand, Foot, and Mouth Syndrome)*

Symptoms and Signs	Percentage of Cases (%)
Enanthem	90
Buccal	61
Tongue	44
Palate, uvula, anterior pillars	36
Gums	15
Exanthem	64
Hands	52
Feet	31
Buttocks	31
Legs	13
Arms	10
Face	5
Sore mouth or throat	67
Malaise	61
Anorexia	52
Fever	42
Submandibular and/or cervical adenitis	22
Coryza	11
Cough	11
Diarrhea	10
Nausea and vomiting	3

**Data from references 15, 173, 302, 315, 619, 652, 809, 813.*
From Cherry, J. D.: Newer viral exanthems. Adv. Pediatr. 16:233-286, 1969.

loose stools may be noted. Generalized myalgia also is observed, and children complain of a scratchy feeling in the throat.

Physical examination usually yields benign findings. Minimal conjunctivitis, infection of the pharynx, and cervical lymphadenitis may be present. The duration of illness varies from 24 hours to approximately 6 days, with an average of 3 to 4 days. The white blood cell count (WBC) count is normal. EVs, particularly coxsackieviruses A, are significant causes of febrile convulsions in young children.[437]

RESPIRATORY MANIFESTATIONS

Common Cold

Although numerous coxsackieviruses and echoviruses have been recovered from children with mild upper respiratory infections, only rarely do the illnesses qualify as common colds (see Table 178–6). (The common cold is an acute illness with nasal stuffiness, rhinitis, no objective evidence of pharyngitis, and no or minimal fever.) In most instances, significant fever (temperature >38.3° C [>101° F]) is associated with enteroviral infections and usually some degree of pharyngitis.

Coxsackievirus A21 is the only EV that clearly qualifies as a common cold virus.[168,484] This agent has produced epidemics of mild respiratory illness in military populations. In adult volunteers, instillation of this virus in the nose has resulted in the common cold syndrome. Epidemic disease has not been observed in children. Other viruses that have been associated with the common cold syndrome include echoviruses 2 and 20 and coxsackieviruses B1, B2, B3, B4, B5, and A24.[168]

Pharyngitis (Pharyngitis, Tonsillitis, Tonsillopharyngitis, and Nasopharyngitis)

Pharyngitis is a common clinical manifestation of coxsackieviral and echoviral infection. Probably all EVs on occasion cause mild pharyngitis. The most common coxsackieviruses and echoviruses associated with pharyngitis are as follows: coxsackieviruses A9, B1, B2, B3, B4, and B5; echoviruses 2, 4, 6, 9, 11, 16, 19, 25, and 30; and EV 71.*

TABLE 178–15 Signs and Symptoms in Echovirus 9 Disease*

Sign or Symptom	Percentage of Cases (%)
Fever	92
Headache	85
Nuchal rigidity	83
Nausea and vomiting	71
Pain (neck, back, trunk)	44
Exanthem	35
Nonexudative pharyngitis	28
Cough	24
Sore throat	20
Cervical lymphadenopathy	18
Coryza	18
Abdominal pain	17
Photophobia	16

**Modified from references 75, 237, 285, 299, 325, 473, 561, 581, 831, 889, 997.*
From Cherry, J. D.: Newer viral exanthems. Adv. Pediatr. 16:233-286, 1969.

Pharyngitis in coxsackievirus and echovirus infections frequently is associated with other clinical findings such as meningitis, pleurodynia, and exanthem. These other manifestations become more important than pharyngitis in individual cases in the minds of parents as well as clinicians.

Pharyngitis caused by coxsackieviruses and echoviruses usually is abrupt in onset, without a prodrome. Although pharyngeal involvement is present at the onset of disease, the initial complaint usually is fever. The temperature usually ranges between 38.3° C and 40° C (101° F and 104° F), but higher temperatures are not unusual symptoms. In general, fever tends to be more pronounced in younger patients. Young children have malaise and anorexia. School-age children complain of headache and myalgia. Sore throat, coryza and vomiting, diarrhea, or a combination thereof also may be noted.

Examination of the tonsils and pharynx shows varying degrees of erythema. In some cases, only infection is noted, whereas in others, severe pharyngitis with patches of exudate is seen. The usual duration of uncomplicated coxsackieviral or echoviral pharyngitis is 3 to 6 days. Routine laboratory study is of minimal value in enteroviral pharyngitis; the total WBC count may be normal or slightly elevated with a normal differential determination. Throat culture rules out disease caused by group A streptococci.

Other Intraoral Manifestations

HERPANGINA (See Chapter 11)

ACUTE LYMPHONODULAR PHARYNGITIS

In 1962, Steigman and colleagues[898] reported a unique enanthem associated with coxsackievirus A10 infection. The lesions had the typical distribution of herpangina; they were papular, discrete, 3 mm in diameter, and surrounded by a zone of erythema. The lesions were white to yellow and persisted for 6 to 10 days. This entity has not been reported again, although coxsackievirus A10 has been noted in association with hand, foot, and mouth syndrome.[168,865]

STOMATITIS AND OTHER LESIONS IN THE ANTERIOR OF THE MOUTH

The main enteroviral cause of stomatitis and ulcerative lesions in the anterior of the mouth is coxsackievirus A16, and the clinical

*See references 168, 175-177, 218, 282, 387, 402, 505, 506, 515, 525, 584-586, 691, 835, 839, 880.

TABLE 178–16 Clinical Exanthematous Manifestations of Enteroviruses

Clinical Manifestations	Associated Viral Agents and Prevalence of Manifestations			
	Virus Subgroup	Common	Occasional	Rare
Macular rash	Coxsackievirus A			
	Coxsackievirus B		1, 2, 5	
	Echovirus and enterovirus		2, 4, 5, 13, 14, 17, 19, 30	18, 71
Maculopapular rash	Coxsackievirus A	9	2, 4, 5, 10, 16	6, 7
	Coxsackievirus B		1-5	
	Echovirus and enterovirus	4, 9	2, 5-7, 11, 16-19, 25, 30, 71	1, 3, 13, 14, 27, 33
Vesicular rash	Coxsackievirus A	5, 16	8-10	4, 7
	Coxsackievirus B			1-3, 5
	Echovirus and enterovirus		11	6, 9, 17, 71
Petechial or purpuric rash	Coxsackievirus A	9	4	
	Coxsackievirus B		2-5	
	Echovirus	9	4, 7	3
Urticarial rash	Coxsackievirus A	9	16	
	Coxsackievirus B		4, 5	
	Echovirus		11	
Erythema multiforme or Stevens-Johnson syndrome	Coxsackievirus A		9	10, 16
	Coxsackievirus B			4, 5
	Echovirus			6, 11
Exanthem and meningitis	Coxsackievirus A		2, 9	7
	Coxsackievirus B		1, 2, 4, 5	
	Echovirus and enterovirus	4, 9	6, 11, 17, 18, 25, 30	3, 14, 33, 71
Exanthem and pneumonia	Coxsackievirus A		9	7
	Coxsackievirus B		6	1
	Echovirus			9, 11
Hand, foot, and mouth syndrome	Coxsackievirus A	16	5, 10	7, 9
	Coxsackievirus B			1, 3, 5
	Echovirus and enterovirus	71		
Hemangioma-like lesions	Coxsackievirus A			
	Coxsackievirus B			
	Echovirus			25, 32
Herpangina and exanthem	Coxsackievirus A		4	9
	Coxsackievirus B			2
	Echovirus		16, 17	
Roseola-like illness	Coxsackievirus A			6, 9
	Coxsackievirus B		5	1, 2, 4
	Echovirus		16, 25	9, 11, 27, 30
Anaphylactoid purpura	Coxsackievirus A			4
	Coxsackievirus B			
	Echovirus			9, 18
Zoster-like rash	Coxsackievirus A			
	Coxsackievirus B			
	Echovirus			5, 6
Pityriasis-like rash	Coxsackievirus A			
	Coxsackievirus B			6
	Echovirus			
Chronic or recurrent rash	Coxsackievirus A	16		
	Coxsackievirus B			
	Echovirus			11

entity is the hand, foot, and mouth syndrome. This condition is presented in detail later in this chapter. Worth mentioning here, however, is that occasionally, enanthem occurs without the exanthem, and this enanthem also has been associated with other coxsackieviruses (A5, A9, A10, B2, B5), echovirus 33, and EV 71.[168,239,260,298,603,865]

In echovirus 9 infection, Tyrrell and colleagues[948] reported six children with a unique enanthem: painless whitish dots, ulcers, or vesicles on the buccal surfaces near the Stensen duct. These investigators also noted the occasional occurrence of similar lesions under the tongue.

Deseda-Tous and colleagues[246] reported an adolescent with hemorrhagic vesicular lesions on the pharynx, mucosal surfaces, and tongue in association with an echovirus 11 infection. Clarke and Stott[201] observed reddish macules on the buccal mucosa of a patient with echovirus 20 infection, and Cherry and Jahn[171] noted a child with hand, foot, and mouth syndrome in whom the buccal lesions suggested Koplik spots, although the lesions were larger and yellower in this patient.

Parotitis

Parotitis in association with herpangina and coxsackievirus A infection was reported in 1957 by Howlett and associates.[440] In 1960, Kraus[552] described two additional cases, and Bertaggia and associates[84] and Winsser and Altieri[1009] noted parotitis in association with coxsackieviruses B3 and B4, respectively. Three patients with acute hemorrhagic conjunctivitis and parotitis caused by EV 70 also have been reported.[854]

TABLE 178–17 Neurologic Manifestations of Nonpolio Enteroviruses and Parechoviruses

	Associated Viral Agents and Prevalence of Manifestations				
Clinical Manifestations	**Virus Subgroup**	**Common**	**Occasional**	**Rare**	**References**
Aseptic meningitis	Coxsackievirus A	9	7	1-6, 8, 10, 11-14, 16-18, 21, 22, 24	13, 14, 37, 71, 75, 81, 124, 135, 146, 150, 168, 237, 266, 275, 286, 287, 299, 324, 325, 359, 378, 383, 391, 408, 423, 473, 475, 479, 487, 512, 513, 525, 536, 539, 543, 547, 561, 578, 579, 584, 641, 646, 650, 660, 661, 682, 688, 730, 808, 820, 825, 831, 836, 864, 871, 875, 889, 890, 940, 952, 965, 981, 997, 1001, 1021
	Coxsackievirus B	2, 4, 5	1, 3	6	
	Echovirus	4, 6, 9, 13, 30, 33	3, 11, 12, 14, 16, 18, 19, 25, 31	1, 2, 5, 7, 8, 15, 17, 21, 24, 26, 27, 29, 32	
	Enterovirus	71	75	77	
	Parechovirus			1, 2, 3	
Encephalitis	Coxsackievirus A		9	2, 4-7, 10, 16	13, 118, 145, 146, 150, 153-155, 164, 168, 189, 317, 378, 403, 423, 436, 441-443, 464, 500, 525, 547, 551, 565, 575, 593, 599-602, 610, 614, 634, 649, 650, 677, 682, 728, 744, 745, 781, 825, 858, 972-974, 1024
	Coxsackievirus B	5	1, 2, 4	3	
	Echovirus	4, 6, 9, 11, 30	3, 25	1, 2, 5, 7, 8, 12, 13, 14, 15, 17-21, 24, 27, 31, 33	
	Enterovirus	71			
	Parechovirus			1, 2	
Paralysis (lower motor neuron involvement)	Coxsackievirus A		4, 7, 9	2, 5, 6, 10, 11, 14, 21, 24	3, 4, 7, 11, 13, 15, 21, 34, 146, 150, 158, 168, 192, 235, 263, 295, 300, 343, 351, 371, 375, 377, 378, 402, 423, 464, 525, 547, 550, 619-621, 650, 660, 737, 772, 798, 845, 864, 897, 967, 969, 1027, 1035
	Coxsackievirus B		2, 3	1, 4-6	
	Echovirus		9, 11, 30	1-4, 6-8, 12, 14, 16-19, 25, 27, 31, 33	
	Enterovirus	70, 71		74, 75	
	Parechovirus			1, 6	
Guillain-Barré syndrome and transverse myelitis	Coxsackievirus A		9	2, 4, 5, 6, 19	13, 49, 64, 67, 85, 244, 295, 337, 364, 367, 469, 481, 524, 525, 582, 650, 671, 845, 915, 950
	Coxsackievirus B			1, 4	
	Echovirus		6	5, 7, 19	
	Enterovirus		70		
	Parechovirus			1	
Cerebellar ataxia	Coxsackievirus A		9	4, 7	73, 290, 378, 387, 464, 525, 632, 640, 706, 1036
	Coxsackievirus B			3, 4	
	Echovirus		6, 9	16	
	Enterovirus		71		
Peripheral neuritis	Coxsackievirus A				864
	Coxsackievirus B				
	Echovirus		9		
Neurologic sequelae and other neurologic illness	Coxsackievirus A		3	9	141, 146, 150, 193, 423, 525, 547, 650, 764, 816, 864, 869, 970, 975, 1016
	Coxsackievirus B		3	2, 4	
	Echovirus		9	19, 25, 30, 33	
	Enterovirus	71			

TABLE 178–18 Coxsackieviruses and Echoviruses Associated with Epidemic Aseptic Meningitis

Virus Type		Age Group	Common Non-neurologic Findings	References
Coxsackievirus	A7	Children and adults	Fever	372
	A9	Mainly children	Fever, rash, pharyngitis	168, 587
	B1	Mainly children	Fever, pharyngitis	168
	B2	Children and adults	Fever, pharyngitis, rhinitis, abdominal pain, diarrhea	168, 626
	B3	Children and adults	Fever, pharyngitis, conjunctivitis	168
	B4	Children and adults	Fever, pharyngitis, rash, conjunctivitis	168, 291
	B5	Mainly children	Fever, pharyngitis, rash, pleurodynia, abdominal pain, diarrhea, rhinitis, myocarditis	44, 168, 176, 186, 381, 643, 775, 832, 906, 912, 914
Echovirus	3	Children	Fever, rash	400
	4	Mainly children	Fever, pharyngitis, abdominal pain, rash, conjunctivitis	168, 301, 324, 501, 506
	6	Children and adults	Fever, pharyngitis, abdominal pain, pleurodynia, cardiac involvement	168, 394, 505, 528, 562
		Children	Fever, rash	475
	9	Mainly children	Fever, rash, abdominal pain, pharyngitis	168, 237, 299, 316, 359, 401, 473, 496, 561, 618, 725, 784, 831, 839, 889, 903, 936, 986, 994
	11	Mainly children	Fever, upper respiratory illness, pneumonia	658
	13	Mainly children	Fever	135
	16	Children	Fever, rash	387
	18	Children and adults	Fever, rash	517
	19	Mainly children	Fever, upper respiratory illness, rash	209, 358
	25	Children and adults	Fever, rash	65
	30	Children and adults	Fever, pharyngitis, rhinitis, conjunctivitis, rash, abdominal pain	168, 366, 388, 502, 771, 1021
	31	Children and adults	Fever	578
	33	Children and adults	Fever, rash	504, 514
Enterovirus	71	Mainly children	Fever, rash	146, 150, 423, 516, 547, 650

Croup

In large studies of respiratory illness in young children, croup is associated sporadically with coxsackieviral and echoviral infection.[156,168,308,347,996] In general, these illnesses are mild when compared with croup caused by parainfluenza and influenza viruses.

An outbreak of croup associated with echovirus 11 in a daycare center was reported.[768] In this instance, 17 of 53 ill children were found to be infected with the U strain of echovirus 11. A subsequent study noted the same virus in 4 children with croup.[769] Croup also was reported in outbreaks of coxsackievirus B5 infection.[42,216] Other specific agents associated with croup include coxsackieviruses A9 and B4 and echoviruses 4 and 21.[168]

Bronchitis

An acute febrile illness with cough, rhonchi, and referred breath sounds occasionally is a sporadic manifestation of enteroviral infection. A specific association of coxsackieviruses B1 and B4, echoviruses 8, 12, 13, and 14, and parechovirus 1 has been found.[156,168,216,267,269]

Bronchiolitis and Infectious Asthma

Coxsackieviruses and echoviruses have been associated sporadically with bronchiolitis, infectious asthma, and the precipitation of asthmatic attacks in atopic children.[156,168,267,308,347,369,427,996] Epidemic disease has not been observed, and the illnesses usually have been mild.

Pneumonia

In general studies of respiratory infections in children, sporadic coxsackieviruses and echoviruses have been noted in 1 to 7 percent of patients with pneumonia and positive viral cultures.[156,168,308,347] Specific virus types include the following: coxsackieviruses A9, A16, B1, B2, B3, B4, B5, and B6; echoviruses 6, 7, 9, 11, 12, 19, 20, and 30; and EV 71.*

In only three instances can an outbreak of pneumonia caused by specific viruses be suggested to have occurred. During the summer of 1959, Lerner and colleagues[587] noted that 3 of 15 children infected with coxsackievirus A9 had pneumonia. The patients with pneumonia had a vesicular rash, and one of these children died.

Eckert and associates[267] observed 6 children with coxsackievirus B1 infection and pneumonia during the summer of 1963. These illnesses were not described further, although the mean WBC count in 12 children who had lower respiratory infections with coxsackieviruses B was reported as 11,383 with a normal differential. All children were hospitalized.

Goldwater[357] reported a coxsackievirus B6 outbreak in south Australia during the summer of 1992 to 1993. Twenty-seven patients had pneumonia associated with high fever and severe cough that lasted several weeks. In addition to the fatal case of coxsackievirus A9 infection described by Lerner and colleagues,[587] deaths have resulted from infection with coxsackieviruses B1, B5, and A16.[297,471,1018]

Pleurodynia (Bornholm Disease)

Epidemic pleurodynia[44,168,233,362,378,525,682,913,980] is an illness that was noted first in 1735 by Hannaeus, a Danish physician.[392] Not until the late 19th century, however, did the illness receive further attention in the medical literature. At this time, several epidemics in Scandinavian countries were described.[168,225,296] Although Finsen referred to the disease as *pleurodynia*, others who designated outbreaks used geographic names, such as Skien disease, Bamle disease, Drangedral disease, and Bornholm disease, or

*See references 168, 218, 267, 297, 343, 471, 505, 587, 683, 855, 880, 993.

descriptive names such as epidemic myalgia, devil's grippe, epidemic diaphragmatic spasm, or epidemic benign pleurisy.[362] In 1933, Sylvest[913] published the classic monograph on the subject, and from this publication the name Bornholm disease (from Bornholm Island, a Danish island in the Baltic Sea) came to be associated with the illness. The enteroviral origin of epidemic pleurodynia was established in 1949.[224,989]

Historically, pleurodynia is an epidemic disease, but sporadic cases do occur. A characteristic incubation period of approximately 4 days is followed by the sudden onset of fever and pain. The pain typically is located in the chest or upper part of the abdomen and is muscular in origin and of variable intensity. Occasionally, the pain occurs in other areas of the body. Frequently, the pain is excruciatingly severe and sudden and is associated with profuse sweating, so the patient may appear pale and as though in shock. The pain is spasmodic, with durations varying from a few minutes to several hours. Most commonly, the spasmodic periods last approximately 15 to 30 minutes. During spasms, respirations usually are rapid, shallow, and grunting, suggestive of pneumonia or pleural inflammation. Coughing, sneezing, or deep breathing makes the pain worse. Older children and adults describe the pain as stabbing or knifelike. An older person often fears that a heart attack is occurring.

When pain localizes in the abdomen, it frequently is crampy and suggests colic in a younger child. The child may double over and refuse to walk or move. Occasionally, the abdominal pain in association with a pale, sweaty, shocklike appearance suggests acute intestinal obstruction. Splinting and guarding of the abdomen also lead to consideration of appendicitis and peritonitis.

The fever and pain usually last 1 to 2 days. Frequently, however, the illness is biphasic; after the initial febrile period, the patient is asymptomatic for several days, and then the pain and fever recur. Rarely, patients have several recurrent episodes over the course of several weeks. In these patients, fever is less prominent.

Some degree of tenderness is present in the areas of pain, but frank myositis with muscle swelling is not observed. Pleural friction rubs may be noted on auscultation, and they may appear and disappear with the coming and going of episodes of pain.

In epidemics, both children and adults are afflicted, but most cases occur in persons younger than 30 years. Most children have other symptoms of enteroviral infection, such as anorexia, nausea, vomiting, headache, and sore throat. Routine laboratory study is not very helpful. The WBC count varies considerably, but increased percentages of polymorphonuclear neutrophils and band forms are frequent findings. The erythrocyte sedimentation rate also is inconsistent; normal to extremely high values may be observed. The chest radiograph usually is normal.

Complications in pleurodynia seldom develop. Aseptic meningitis has been noted in some patients, and men have experienced orchitis. Cardiac involvement, in the form of myocarditis and pericarditis, also may complicate pleurodynia.

The major etiologic agents in epidemic pleurodynia are coxsackieviruses B3 and B5.[44,168,447,835,861,880] Other viruses associated with epidemic disease include coxsackieviruses B1 and B2 and echoviruses 1 and 6.[31,168,262] Agents associated with sporadic occurrences of pleurodynia include the following: coxsackieviruses A1, A2, A4, A6, A9, A16, B1, B2, B3, B4, B5, and B6; echoviruses 1, 2, 3, 6, 7, 8, 9, 11, 12, 14, 16, 17, 18, 19, 24, 25, and 30; and parechovirus 2.*

Pleurodynia rarely is reported today. Because the EVs that cause pleurodynia still circulate, cases probably are overlooked or misdiagnosed. An outbreak caused by coxsackievirus B1 in football players at a public high school was reported.[458] Unfortunately, no clinical data were presented.

*See references 63, 168, 216, 262, 388, 499, 530, 647, 682, 911.

GASTROINTESTINAL MANIFESTATIONS

Gastrointestinal manifestations occur commonly in coxsackieviral and echoviral infections.* Clinical manifestations in addition to vomiting and diarrhea are varied (see Table 178–7).

Early studies paid particular attention to diarrheal disease in children. However, when specific studies of infantile diarrhea were undertaken, the enteroviral association was far from clear.[61,168,361,757,891,1032] The correlation of infection and disease in these studies was compromised by the problem that the main source of culture in study patients and controls was from stool; persistent infection in the bowel rendered separation of controls and ill patients impossible.

Infections with all coxsackieviruses and echoviruses frequently have one or more gastrointestinal symptoms as part of the general illness. The intensity and spectrum do vary among the specific agents, however, and also among strains of particular viral types. In a review of World Health Organization (WHO) virus reports covering a 4-year period, Assaad and Cockburn[34] found that the main clinical sign or symptom was gastrointestinal in 12 percent of coxsackieviral infections and in 6.8 percent of echoviral infections. In another analysis, Assaad and Borecka[33] noted 16 deaths during the period 1967 to 1975 in patients with coxsackievirus and echovirus infections in whom the principal clinical association was gastrointestinal.

In a 20-year survey in Wisconsin, Nelson and colleagues[712] noted that gastrointestinal symptoms occurred in approximately one third of all patients from whom these investigators recovered nonpolio EVs. Morens and colleagues[686] reported that in 4 percent of patients from whom nonpolio EVs were recovered during the 1971 to 1975 period, gastrointestinal disease was the major diagnosis. Horn and coworkers,[427] studying respiratory viral infections, noted that 21.2 percent of subjects from whom EVs were recovered had gastrointestinal complaints.

Vomiting

Vomiting is a common manifestation of infection with many coxsackieviral and echoviral types, but it rarely is the major complaint of the patient or the parent.† The frequency of vomiting during outbreaks of illness caused by coxsackieviruses and echoviruses depends on the specific types of virus and on the major manifestation during a particular outbreak. Table 178–8 presents the frequency of vomiting by viral type. In 14 different enteroviral types, vomiting has been noted as a significant aspect of the illness during disease outbreaks. Except for coxsackievirus A16 infections (hand, foot, and mouth syndrome), in which it is an uncommon complaint, vomiting occurs in approximately 50 percent of all patients with epidemic enteroviral disease. Vomiting is noted most commonly in meningitis and least commonly in pleurodynia and uncomplicated exanthematous disease.

Diarrhea

Diarrhea occurs commonly in coxsackieviral and echoviral infections, but it usually is just one of many manifestations of systemic

*See references 33, 34, 168, 192, 233, 378, 427, 525, 570, 636, 639, 644, 647, 658, 682, 685, 686, 718, 792, 864, 891, 991, 992, 994.

†See references 9, 32, 168, 175, 176, 186, 218, 219, 244, 291, 292, 362, 381, 387, 388, 394, 502, 505, 506, 515, 528, 538, 584, 626, 629, 726, 771, 777, 799, 813, 832, 836, 839, 848, 880, 899, 935, 980, 986, 997, 1005, 1006.

illness.* Specific studies of diarrheal disease in infants and children have had varied results; some studies indicated an enteroviral origin, whereas others revealed that coxsackieviruses and echoviruses were recovered from well children at the same prevalence.

Ramos-Alvarez and Olarte[793] carried out an extensive study of diarrheal disease in Mexico City and noted that echoviruses were recovered eight times more frequently from children with diarrhea than from control children without diarrhea. These investigators found that echoviruses 6 and 19 predominated, but types 3, 7, 9, 12, 14, 18, and 21 also were recovered. Pelon and colleagues[762] noted an association between coxsackieviruses B4 and B5 and acute diarrhea; they observed a 42 percent coxsackievirus B isolation rate in children with acute diarrhea and a corresponding rate of only 12 percent in those without diarrhea. Goodwin and associates[361] noted echoviruses in the stools of children with diarrhea at twice the rate observed in healthy children. In a study by Yow and colleagues,[1032,1033] no association was found between EVs and infantile diarrhea. Echoviruses were recovered twice as frequently from children ill with diarrhea as from children without gastrointestinal illness.[1033] In Canada, McLean and colleagues[644] could find no association between enteroviral infection and gastroenteritis. In a large study of diarrhea in India, coxsackieviruses A9, B3, and B6 and echoviruses 12 and 21 were recovered more commonly from ill patients than from children without diarrhea.[704]

In several studies of specific diarrhea outbreaks, afflicted persons were noted to be actively infected with a particular virus type. Goldwater and Laws[358] observed three children younger than 6 months who were infected with echovirus 19 and who had gastroenteritis without other signs or symptoms. Klein and colleagues[538] recovered echovirus 11 from the blood of two laboratory workers with acute gastroenteritis. Diarrhea occurred in three of nine volunteers given echovirus 11.[106] Eichenwald and associates[271] and Cramblett and colleagues[216] reported epidemic diarrhea caused by echovirus 18 in neonates. An outbreak of diarrhea associated with coxsackievirus A1 was noted in 7 of 14 bone marrow transplant recipients during a 3-week period.[937]

Table 178–9 presents the frequency of diarrhea in outbreaks of specific coxsackieviral and echoviral illnesses. Diarrhea varied with regard to the viruses represented and in different studies with the same agents. Diarrhea in enteroviral disease rarely is severe. In most instances, loose stools occur for a 2- to 4-day period. The stools rarely are watery, never are bloody, and at most number six to eight per day.

Constipation

Some degree of constipation is a rather frequent occurrence in many acute infectious illnesses, but evaluation is rendered difficult by the subjective nature of the complaint. In coxsackieviral and echoviral diseases, a short period of constipation may be associated with fever, vomiting, and anorexia early in the course of the illness. This period frequently is followed by mild diarrhea.[106] As noted in Table 178–7, constipation has been reported specifically as a symptom with four coxsackieviral and four echoviral types. Constipation is a particularly common event in children with enteroviral meningitis; it occurs in 10 to 40 percent of cases.[168,291,292,505,506,561]

*See references 9, 31, 32, 78, 103, 106, 168, 171, 216-219, 244, 291, 292, 358, 362, 378, 387, 388, 394, 505, 506, 515, 525, 538, 563, 577, 584, 626, 636, 639, 644, 704, 758, 762, 771, 775, 782, 792-794, 813, 835, 836, 864, 891, 892, 922, 935, 992, 994, 1006, 1032, 1033.

Abdominal Pain

Abdominal pain is a common complaint in many coxsackieviral and echoviral infections. Table 178–10 lists the frequency of occurrence of abdominal pain and the main characteristic of the illness by virus type. Approximately 10 percent of patients with coxsackievirus A16 hand, foot, and mouth syndrome complain of abdominal pain. In approximately one fourth of patients, many coxsackieviruses and echoviruses associated with meningitis cause abdominal pain.

The magnitude of abdominal pain as a clinical complaint in coxsackieviral and echoviral infections varies considerably. For example, in aseptic meningitis, headache and other neurologic complaints overshadow the abdominal symptoms. In other situations, fever and abdominal pain are diagnostically troublesome because of the possible presence of a surgical abdomen (discussed further in the next paragraph). The pain most frequently is periumbilical; it may be either constant or colicky. The fever is most often higher than 38.3° C (101° F).

Peritonitis, Pseudoperitonitis, Appendicitis, Pseudo-obstruction, Mesenteric Adenitis, and Intussusception

Occasionally, coxsackieviruses and echoviruses are associated with illnesses that suggest severe abdominal involvement. Liebman and St. Geme[595] described two children with abdominal findings suggestive of acute appendicitis (semirigid, tender abdomen, rebound tenderness, rectal tenderness) who had associated infections with echoviruses 1 and 14. McLean[643] described a surgical abdomen in one child with coxsackievirus B1 infection. In this case, virus was recovered from the peritoneal exudate; at surgery, this boy was found to have peritonitis, but his appendix was normal. Thomas[928] reported a 5-year-old boy with coxsackievirus B5 infection who at surgery was found to have excessive clear peritoneal fluid and markedly enlarged mesenteric lymph nodes. A pregnant woman with acute abdominal pain and rebound tenderness associated with echovirus 8 infection was described.[760]

In immunofluorescence studies, Tobe[933] showed the presence of coxsackievirus B2 and B5 antigens in the mucous membranes and mesenteric lymph nodes of patients with appendicitis more often than in similar studies in control subjects. He suggested that the viral infection acts as a trigger for appendicitis. Bell and Steyn[68] recovered echoviruses 7 and 9 from the mesenteric lymph nodes of children with intussusception.

Hepatitis*

Marked liver involvement is not a rare finding in disseminated coxsackieviral and echoviral infections in neonates.[169] The association of hepatitis and enteroviral infection in older children is defined less clearly but probably occurs more commonly than generally realized, based on the number of individual cases reported. Caution must be exercised in accepting EVs as the exclusive etiologic agent of hepatitis, however, because hepatitis A virus infection has been ruled out in only a few of the available studies.

Morris and associates[691] described an illness suggestive of a coxsackieviral or echoviral infection in an 18-month-old child who had hyperbilirubinemia and abnormal liver function test results. From this child, coxsackievirus A4 was recovered from blood during the acute illness, and a rise in neutralizing antibody titer to the isolated virus was demonstrated. Chang and Wein-

*See references 10, 142, 151, 233, 435, 525, 570, 576, 629, 685, 686, 691, 748, 855, 862, 864, 880, 909, 962, 977.

stein[151] reported a 3-year-old boy with pharyngitis, urinary abnormalities, and neurologic symptoms who had elevated liver enzyme values (aspartate transaminase value of 1600 and alanine transaminase value of 1180). Coxsackievirus A9 was recovered from this child's cerebrospinal fluid (CSF), and the child's antibody response to this agent was significant. Coxsackievirus A20 has been associated with clinical hepatitis, and simultaneous infections with coxsackievirus A24 and hepatitis A virus have been demonstrated.[862]

An adult with a coxsackievirus B1 infection had both myocarditis and liver involvement.[10] A similar illness caused by coxsackievirus B3 in a 19-year-old woman was described.[909] An 11-month-old boy had a Reye-like syndrome in conjunction with coxsackievirus B2 infection.[509] Reye syndrome has been associated with echovirus 3 infection.[400] Siegel and colleagues[880] described a hepatosplenic syndrome in which 15 patients had hepatomegaly and coxsackievirus B5 infection. These patients, most of whom were children, had otherwise typical enteroviral illnesses. Liver function studies were performed in only three instances, and the results were normal.

Echoviruses 1, 3, 4, 6, 7, 9, 11, 14, 20, 21, and 30 have been associated with hepatitis.[168,448,525,624,682,855] Hepatomegaly occurs commonly in enteroviral infections.[168,171,290,563,587] During the period from 1971 through 1975, more than 7000 cases of nonpolio enteroviral infection were reported to the Viral Diseases Division at the CDC.[686] In this group were 13 cases of hepatitis and 6 of Reye syndrome; coxsackieviruses A2 and B4 and echoviruses 14 and 22 were associated with Reye syndrome.

Pancreatitis

One of the effects of coxsackievirus B in suckling mice is extensive infection in the pancreas. As with other similarities of infection between suckling mice and human neonates, generalized coxsackievirus B infection in neonates also is accompanied frequently by extensive pancreatic damage. In contrast, pancreatic involvement in older children and adults is not a common occurrence.[117,700,705,951] Coxsackieviruses B3, B4, B5, and A9 have been noted.

Diabetes Mellitus

A possible relationship between juvenile diabetes mellitus and the seasonal occurrence of coxsackievirus B4 was suggested in 1969 by Gamble and Taylor.[326] In a second study, Gamble and associates[327] noted that titers of coxsackievirus B4 antibodies in patients with insulin-dependent diabetes mellitus (IDDM) within 3 months of the onset of disease were higher than those in physiologically normal subjects or in patients with chronic diabetes.

Since the 1960s, many studies in animal models and children have looked at the relationship between juvenile-onset, type 1 IDDM and coxsackieviruses B.* Between 1973 and 1984, 10 case-control studies examined the prevalence of antibody to various coxsackieviruses B in patients with IDDM and controls. In 8 of these studies, the prevalence was greater in the patients with IDDM. In 3 of these studies, IgM antibody to the coxsackieviruses B was examined, and the prevalence in each was greater in the patients with IDDM than in controls. A Finnish study noted that patients with IDDM were more likely to have IgA antibodies to coxsackievirus B4 than were controls.[454]

Helfand and associates[407] performed a well-done case-control study of IDDM. These investigators found that new-onset cases of IDDM in patients 13 to 18 years of age were more likely than were controls to be IgM antibody-positive for 9 of 14 EV serotypes. The serotypes were as follows: coxsackieviruses B2, B3, B4, B5, and B6; coxsackievirus A9; and echoviruses 9, 30, and 34. Green and associates[368] performed a systematic review of published case-control studies relating to coxsackievirus B serology and IDDM. In 13 studies that looked at antibody to all coxsackievirus B types, 7 (54%) had significantly more positive antibody values in the cases than in the controls. The findings were less impressive when rates of antibody by specific coxsackievirus B serotypes were compared. However, with coxsackievirus B4, the odds ratio was higher than 1 in 9 of 17 (53%) comparisons, and this difference was significant in 6 (35%) of the studies.

In an extensive study, Skarsvik and colleagues[886] noted that children with IDDM had an impaired type 1 T-cell response against coxsackievirus B4 compared with the response in healthy children. The findings led these investigators to suggest that the defect in T-cell function delayed clearance of coxsackievirus B4 and made damage to beta cells more likely. In another study involving adults, Varela-Calvino and coworkers[958] noted that T-cell proliferative responses against the VP2 protein of coxsackievirus B4 was significantly reduced in patients with IDDM compared with the responses in control subjects.

Cudworth and colleagues[222] noted a correlation between HLA type BW15 and coxsackieviruses B1, B2, B3, and B4 antibodies in patients with IDDM. In 1979, Yoon and colleagues[1029] reported the recovery of coxsackievirus B4 from the pancreas of a previously healthy 10-year-old boy who died after being in a diabetic coma. Maria and coworkers[627] noted the simultaneous onset of type 1 IDDM in a mother and her 10 year-old son coincident with a coxsackievirus B5 infection.

Clements and coworkers[204] noted that 9 of 14 serum samples from children with new-onset IDDM were positive for EV RNA by PCR. In contrast, only 4 percent of serum samples from control children had evidence of EV RNA.

In a murine model, See and Tilles[866] found that a diabetogenic strain of coxsackievirus B4 infection resulted in persistent detection of viral RNA in the pancreases of most infected mice. This persistence of antigen was associated with chronic islet cell inflammation and elevated islet cell antibody levels. Stimulated peritoneal macrophages caused lysis of islet cells either directly or by an antibody-dependent mechanism. A study in prediabetic children found that the presence of EV RNA in serum was a risk factor for development of beta-cell autoimmunity and IDDM.[609] Glutamic acid decarboxylase (GAD_{65}) is one of the major beta-cell target antigens in the autoimmune beta-cell damaging process that leads to the development of IDDM.[607] Antibody and cellular immunity cross-reactivity exists between GAD_{65} and the 2C protein of coxsackievirus B4.[35,607] The results of several studies suggest that enteroviral infections are associated with the development of beta-cell autoimmunity and the eventual destruction of islet cells.[113,421,608] Time periods from enteroviral infection to the development of beta-cell autoimmunity and the eventual development of IDDM from beta-cell destruction have a great range that perhaps explains the lack of a more specific seasonally related onset of IDDM. A case of neonatal IDDM associated with a maternal echovirus 6 infection has been reported.[749]

EYE FINDINGS

Acute Hemorrhagic Conjunctivitis*

Although conjunctivitis has been a frequent finding in nonpolio enteroviral illnesses since the 1950s, its occurrence as a dominant complaint has been observed only since the 1960s. In June of

*See references 22, 35, 47, 48, 143, 204, 222, 249, 313, 314, 368, 397, 407, 416, 421, 434, 495, 498, 533, 607-609, 627, 672, 749, 860, 866, 886, 931, 953, 958, 1004.

*See references 82, 124, 130, 137, 168, 353, 367, 378, 417, 548, 756, 852, 923.

1969, Chatterjee and associates[157] noted an epidemic of acute hemorrhagic conjunctivitis in Accra, Ghana. This disease was nicknamed Apollo 11 disease because it coincided with the time of the Apollo 11 moon landing. Since 1969, many epidemics of acute hemorrhagic conjunctivitis have been described. In most epidemics, EV 70 has been the etiologic agent, but similar epidemics have been caused by a variant of coxsackievirus A24.[199] More recently, this virus has been the cause of more epidemics than has EV 70.[130,137,205,353,756,799,923] Most epidemics have occurred in tropical and semitropical countries; however, outbreaks have been observed in Minnesota, as well as in Moscow, London, and other European cities.[560,1027] In the continental United States, epidemic disease has occurred in Florida and North Carolina.[128] During epidemics, all age groups are affected, but the highest attack rate is in school-age children.[982]

Acute hemorrhagic conjunctivitis has a sudden onset, with severe eye pain and associated photophobia, blurred vision, lacrimation, erythema, and congestion of the eye, as well as edema and chemosis of the lids.[417] Subconjunctival hemorrhages of varying size, and frequently transient punctate epithelial keratitis, conjunctival follicles, and preauricular lymphadenopathy are present. The eye discharge initially is serous but becomes mucopurulent with secondary bacterial infection. Systemic symptoms, including fever, are rare manifestations. Within 2 to 3 days after the onset of illness, patients note some improvement, and recovery is usually complete in 7 to 12 days. In a study in American Samoa, researchers found that illness caused by coxsackievirus A24 was somewhat different from that caused by EV 70.[852] In cases caused by coxsackievirus A24, conjunctival hemorrhage was less severe, and upper respiratory and systemic symptoms occurred more frequently. In a study in American Samoa of disease caused by EV 70, researchers found that children 2 to 10 years of age had the highest attack rate, and antibody from previous infection gave only partial protection against symptomatic re-infection.[82]

Occasionally, findings suggestive of pharyngoconjunctival fever have been noted. A few patients have had a poliomyelitis-like illness or polyradiculomyeloneuropathy after EV 70 acute hemorrhagic conjunctivitis.[367,969] Epidemics are explosive, with spread mainly by the eye-hand-fomite-eye route.

Conjunctivitis Associated with Other Enteroviral Illness

Conjunctivitis is a common minor manifestation of enteroviral illness with several specific agents. Table 178–11 presents the frequency of occurrence of conjunctivitis in selected outbreaks of disease caused by coxsackieviruses and echoviruses. Conjunctivitis was most prevalent in coxsackievirus B3 and B5 and echovirus 9 and 30 infections. In addition to the outbreaks reported in Table 178–11, conjunctivitis has been noted in isolated illnesses associated with echoviruses 1, 6, and 20.[525]

Photophobia

As would be expected, photophobia occurs commonly in aseptic meningitis caused by coxsackieviruses and echoviruses. During epidemic meningitis, researchers have noted it with the following viruses: coxsackievirus A9 and echoviruses 3, 4, 6, 7, 9, 19, and 30.* It is a most common occurrence with echovirus 9 and 30 infections, but the incidence of this complaint varies greatly in different reports. In one echovirus 30 outbreak, 80 percent of the patients studied had photophobia.[935] On average, 20 percent of patients with meningitis have remarkable photophobia. Photophobia also is associated with pleurodynia caused by coxsackievirus B infections.[835,980]

*See references 67, 168, 362, 369, 501, 502, 505, 506, 528, 584, 624, 771, 935.

Other Eye Findings

Nodular lesions on the palpebral conjunctiva were observed in some patients with coxsackievirus A10 infection and lymphonodular pharyngitis.[898] A corneal ulcer occurred in one patient with hand, foot, and mouth syndrome.[619] Optic neuritis was described in a boy with pleurodynia, although the enteroviral origin of the illness was not confirmed in the laboratory.[946] Keratoconjunctivitis was noted in two boys with echovirus 13 infections.[532] Periorbital edema was observed in one patient with echovirus 9 meningitis. A woman with panuveitis associated with coxsackievirus B3 infection was described.[303] One report detailed monofocal outer retinitis in a 36-year-old man with hand, foot, and mouth syndrome.[382] Five extensive outbreaks of uveitis caused by echoviruses 11 and 19 in hospitalized young children were noted in three Siberian cities.[959] These cases occurred predominately in infants hospitalized for other illnesses such as bronchitis, pneumonia, gastroenteritis, sepsis, dystrophy, and premature birth. Sore eyes and other unspecified eye complaints also have been noted frequently in patients with coxsackieviral and echoviral infections.[168,286,301,381,577,618,714,850,856]

CARDIOVASCULAR MANIFESTATIONS

Pericarditis, Myocarditis, and Dilated Cardiomyopathy

Pericarditis or myocarditis or both have been noted in association with 27 different nonpolio EVs. The relative importance of the different serotypes is presented in Table 178–12. The coxsackieviruses B have been implicated most frequently in heart disease. Coxsackievirus B5 has been the most common causative agent, but types 2, 3, and 4 also have been reported frequently. Of the echoviruses, type 6 has been associated most often with cardiac involvement, but the clinical findings with this agent have been described in only a few cases.

In patients with coxsackievirus B cardiac disease, hepatitis, pneumonia, nephritis, meningitis, and orchitis have been occasional associated findings. Sometimes, arrhythmias are the only clinical manifestations of myocarditis.[174,945] Constrictive pericarditis occurs occasionally.[637] The mortality rate for acute coxsackieviral and echoviral heart disease is unknown, but it is significant. Unfortunately, proper virologic study rarely is performed in nonfatal disease. In the only published follow-up study, researchers found that patients who survived acute coxsackievirus myocarditis usually recovered completely, without any residual disability.[907]

The early descriptions of coxsackievirus B infection with myocarditis in the 1950s and early 1960s indicated acute, usually fulminant illnesses in which a coxsackievirus B serotype could be recovered from multiple sites such as the throat, stool, and CSF, as well as from the heart and other organs at autopsy.[338,376,524,526,954] Coxsackieviruses B originally were isolated from suckling mice, and the infection in these mice caused acute, overwhelming fatal infections involving multiple organs, including the heart.[233,488] Coxsackievirus B infections in mice were studied in mouse model systems in the 1950s and 1960s, and researchers found that infections in older mice were affected markedly by the virus strain, mouse genetics, and drugs.[89,439,531] Since the 1970s, studies in mouse model systems have become more sophisticated, but they still depend on specific coxsackievirus B strains and specific mouse strains.*

*See references 20, 112, 116, 195, 331, 332, 334, 335, 338, 350, 410, 413, 424, 445, 446, 497, 523, 529, 537, 568, 617, 635, 648, 713, 740, 754, 787, 802, 805, 806, 814, 867, 868, 939, 942, 1010.

More recent studies in mouse model systems noted the progression of acute coxsackievirus B infection to chronic infection and cardiomyopathy.[20,424,497,635,787,802] Since the mid-1980s, acute, subacute, and chronic cardiomyopathy in patients has been studied by antigen-detection techniques and newer serologic methods.*

Bowles and colleagues[96] found coxsackievirus B nucleic acid sequences in myocardial biopsy samples from several patients with cardiomyopathy. Muir and colleagues[697] detected EV-specific IgM antibodies in the sera of nine patients with chronic relapsing pericarditis. Fujioka and coworkers[319] reported positive enteroviral PCR results from endomyocardial biopsy specimens in 6 of 31 patients (19%) with dilated cardiomyopathy (DCM). Andréoletti and collaborators[23] noted EV RNA in 18 of 25 samples from the heart tissue of patients with DCM or ischemic cardiomyopathy. However, in two other well-controlled studies, enteroviral RNA could not be identified in endomyocardial biopsy samples or in heart tissue from patients undergoing heart transplantation.[208,596] In 1994, Muir and Archard[694] and Melchers and associates[656] reviewed the evidence for persistent enteroviral infection in chronic medical conditions, including idiopathic DCM. Muir and Archard concluded that persistent enteroviral infection is associated causally with chronic medical conditions, whereas Melchers and colleagues found no clear evidence for enteroviral persistence and the subsequent development of chronic medical conditions.

Since the mid-1990s, additional studies in humans have been performed, and they again have had mixed results with regard to the role of coxsackievirus B in the origin of chronic cardiomyopathy. Bowles and associates[95] studied cardiac samples for PCR analysis from 624 patients of all ages with myocarditis and 149 patients with DCM. Enteroviral genome was found in the cardiac samples of 85 patients with myocarditis and in the samples from 12 patients with DCM. The histopathologic findings in 9 of these 12 patients demonstrated borderline or mild findings of inflammatory infiltrates. Another study by investigators in the same group examined 80 explanted hearts from children with end-stage heart disease, and an enteroviral genome was not detected.[309] In another study, Zhang and coworkers[1037] found evidence of enteroviral capsid protein VP1 by immunohistochemical staining in 47 of 89 patients with DCM. In a study performed in Japan, Fujioka and associates[318] detected EV RNA in 7 of 30 American patients (23%) and in 15 of 47 Japanese patients (32%) with DCM. In our opinion, the data in these studies suggest that EVs have a role in some patients with DCM.

The general theme in most contemporary mouse model studies is that damage to the myocardium is not caused by the direct cytopathic effect of the virus, but rather is caused by the cellular immune response of the host. Unfortunately, the mouse model data and the less than definitive data from human studies have led cardiologists and others to think that the model systems are representative of human disease.[289,605] No adequate follow-up studies of survivors of acute enteroviral myocarditis or pericarditis have been conducted. Levi and colleagues[589] compared follow-up cardiac data on 10 adults who had acute myocarditis associated with coxsackieviral infections 42 to 68 months previously with similar cardiac data from normal age- and sex-matched subjects. No statistically significant differences in the two groups were found in the seven tests evaluated.

Orinius[747] performed cardiac examinations on 53 adults who had coxsackievirus B infections 2 to 11 years previously. The initial diagnoses in these 53 patients were meningitis in 33 cases, encephalitis in 3 cases, pleurodynia in 6 cases, pericarditis in 2 cases, and nonspecific complaints in 9 cases. Of this group, the "possibility of cardiomyopathy was established" in 2 (4%) cases. Two patients who had had pericarditis were normal at follow-up.

Sainani and associates[840] performed follow-up studies on 20 adults and 2 teenagers who had coxsackievirus B myocarditis or pericarditis and found that 5 (23%) patients had chronic heart failure. Unfortunately, the duration of time since the primary illness of the patients was not reported.

Bergström and colleagues[77] performed follow-up examinations on five patients (two teenagers and three adults) who had had enteroviral myopericarditis and found that none had significant complaints or physical signs. No follow-up data are available for neonates, infants, and children who have had enteroviral myocarditis.

In 1954, Stürup[907] reported a follow-up study of patients admitted to the hospital in 1930 to 1932 with pleurodynia. Of nine patients evaluated, including four who had a history of acute pericarditis, none had constructive pericarditis.

Other Cardiac Manifestations

Several investigators have studied the possible association of coxsackievirus B infection and myocardial infarction.[370,376,572-574,679,719,724,774,1013,1015] In many instances, patients with infarction have been demonstrated to have concomitant coxsackievirus B infection.* In controlled studies, the results have varied. Nikoskelainen and associates[724] noted that 9 of 59 patients with acute myocardial infarction had coxsackievirus B infection, whereas in the control group of 38 patients without infarction, only 1 patient had evidence of infection. Similarly, Nicholls and Thomas[719] found that 26 percent of patients had infections, but no infections were found in the control subjects.

In contrast, five other studies failed to show an increased rate of infection in patients with myocardial infarction when compared with controls.[370,376,742,774,1013] However, two of these studies were performed during a nonenteroviral season,[774,1013] and in a third study, little coxsackievirus B activity occurred in the community during the study period.[370] Analysis of the available studies indicates that coxsackievirus B infection would appear to have a role in myocardial infarction. In one coxsackievirus B5 infection, inferolateral wall myocardial necrosis occurred, but coronary arteriography did not demonstrate obstruction.[245]

Chandy and associates[149] presented data in which antibody to group B coxsackieviruses was related to rheumatic-like valvular heart disease, and Burch and associates[109] demonstrated coxsackievirus B antigens in rheumatic lesions of the heart. Limson and colleagues[597] could find no association between group B coxsackieviruses and rheumatic fever. Soboleva and associates[888] noted an association between coxsackievirus A13 and rheumatic fever; they reported seven children with concomitant streptococcal and coxsackievirus A13 infection. Children with fulminant EV 71 infection have been noted to have acute left ventricular dysfunction.[146,150,442]

GENITOURINARY MANIFESTATIONS

Orchitis and Epididymitis

Group B coxsackieviruses are second only to mumps as causative agents of orchitis.† Coxsackievirus B5 is the virus associated most commonly with this disease, although coxsackieviruses B2 and B4 also have been implicated on many occasions. In almost all instances, orchitis is a secondary event in enteroviral infections.

*See references 19, 21, 23, 29, 95, 208, 241, 309, 318, 319, 405, 594, 596, 656, 694, 695, 697, 1037.

*See references 370, 376, 572, 573, 679, 719, 724, 742, 1013, 1015.
†See references 31, 168, 214, 311, 378, 525, 686, 864, 911, 992.

The most common association is with pleurodynia. The illness frequently is biphasic: fever and pleurodynia develop initially, and then apparent recovery is followed by orchitis approximately 2 weeks after onset. Many patients also have epididymitis. In epidemics of disease caused by group B coxsackieviruses, the occurrence of testicular involvement varies considerably. Generally, orchitis occurs infrequently, but in one outbreak of coxsackievirus B2, 17 percent of postpubertal male patients had orchitis, and 7 percent also had epididymitis.[168] Orchitis also is associated frequently with pericarditis and myocarditis. In one instance, coxsackievirus B5 was recovered from a testicular biopsy specimen.[214]

In virtually all instances, testicular involvement has occurred in postpubertal patients, mostly in young adults. In addition to coxsackieviruses B2, B4, and B5, other EVs have been implicated: coxsackieviruses B1 and B3[168,525] and echoviruses 6, 9, and 11.[525,990] Meningitis and exanthem have been associated with orchitis.

Nephritis

Scattered cases of nephritis associated with nonpolio enteroviral infections have been reported. Bayatpour and colleagues[58] noted acute glomerulonephritis in a 9-year-old boy with an extensive coxsackievirus B4 infection. This child had a concomitant rise in anti-streptolysin O titer. Yuceoglu and associates[1034] reported twins with echovirus 9 infection and acute glomerulonephritis; Burch and Colcolough[107] observed a patient with progressive fatal pancarditis and nephritis in whom coxsackievirus B4 antigen was found in the kidneys. Mesangiolytic glomerulonephritis associated with an echovirus 6 infection was described in an infant with immune deficiency.[444]

Other Genitourinary Findings

Hemolytic-uremic syndrome has been associated with virologic or serologic evidence of infection with coxsackieviruses A2, 4, 9, 10, 16, 21, and B1 to B6, echoviruses 4, 6, and 7, and parechovirus 1.[36,242,346,743,800,801] De Petris and colleagues[242] demonstrated titer rises to various EVs in patients with hemolytic-uremic syndrome who were both positive or negative for infections with verocytotoxin-producing *Escherichia coli*. Other abnormal renal or urinary findings in nonpolio enteroviral infections include the following: acute oliguric renal failure with coxsackievirus B5[30]; pyuria, hematuria, or proteinuria with echoviruses 1, 6, and 9 and coxsackievirus B5[168,505,584,839,856]; and hemorrhagic cystitis.[686] A 7-year-old girl with coxsackievirus A10 infection had vaginal ulcerative lesions,[673] and Wassermann test results have been falsely positive in patients with echovirus 9 and coxsackievirus B5 infections.[786]

HEMATOLOGIC FINDINGS

In 1968, Horwitz and Moore[433] described an outbreak of infectious lymphocytosis during which 27 mentally retarded children were studied. The mean WBC count of the study group patients was 57,200; lymphocytes accounted for at least 50 percent of the total in all cases. Approximately half the patients had low-grade fever, and 15 of 27 had moderate diarrhea. A nontypeable EV suggestive of coxsackievirus group A was recovered from the ill patients, and neutralizing antibody to a strain (EVU-16) of the isolated virus developed in 31 percent of the patients in whom serologic studies were performed.[379,433]

Letsas and colleagues[588] reported a 17-year-old-boy with fulminant myocarditis and hemophagocytic syndrome. Over the course of a 3-day period, his hematocrit decreased from 40.9 to 31.7 percent, and his platelet count went from 90,000 to 38,000/mm^3. Examination of the bone marrow revealed many mature histiocytes with active hemophagocytosis. The patient improved dramatically with inotropic and intravenous immunoglobulin (IVIG) treatment.

MUSCLE AND JOINT MANIFESTATIONS

Arthritis

During the period from 1971 to 1975, during which EVs were recovered from 7075 persons in the United States, 9 instances of rheumatic disease were reported[686]; although not clearly specified, 3 patients apparently had arthritis. Blotzer and Myers[86] noted an adult with echovirus 9 infection and the concomitant onset of arthritis that persisted for 3 months. Echovirus 9 was recovered from the synovial fluid of a man with acute monocytic arthritis,[556] and one adult and one adolescent had serologic evidence of coxsackievirus B4 infection in association with the onset of rheumatoid arthritis–like illness.[453]

Myositis

Because group A coxsackieviruses routinely cause myositis in suckling mice, a reasonable approach is to suspect a similar clinical manifestation in people. Myalgia also is a common complaint in illnesses caused by many coxsackieviruses and echoviruses.[31,168,311,388,562,647] However, almost no direct (demonstration of virus in muscle) or indirect (elevations in muscle enzymes) evidence of muscle involvement in routine enteroviral illnesses exists. Of the nine patients with rheumatic disease described by Morens and colleagues,[686] six apparently had myositis; in one instance, coxsackievirus A2 was implicated, and in another patient with polymyositis, coxsackievirus A9 was the related agent. Three patients with echovirus 18 infection and polymyositis have been reported.[517] Acute rhabdomyolysis in one adult and myositis, myoglobinemia, and myoglobinuria in another adult have been associated with echovirus 9 infection.[477,492] Christensen and associates[197] noted that children with dermatomyositis were more likely to have serum antibody to one or more coxsackieviruses B than were control children. Chou and Gutmann[193] noted EV-like particles in the muscles of two patients with fatal dermatomyositis; Tang and colleagues[920] demonstrated coxsackievirus A9 in the muscles of an 11-year-old girl with chronic myopathy. Using both PCR and dot-blot hybridization assays, Fox and colleagues[307] could not find evidence of persistent enteroviral infection in 32 adults with inflammatory muscle disease (1 patient with dermatomyositis and 31 patients with polymyositis).

Yousef and coworkers[1031] reported EV RNA in biopsy specimens from 6 of 13 patients (46%) with idiopathic polymyositis or dermatomyositis but in no samples from 13 patients with other muscle disorders. Behan and associates[59] looked for picornavirus RNA in biopsy specimens from 41 patients with inflammatory myopathy, but all results were negative.

Dekel and associates[240] reported a 4-year-old girl with localized thigh swelling caused by coxsackievirus A21. On muscle biopsy, perivascular infiltrates were noted, but the muscle fibers were not affected. Widespread edema was found in the muscle.

SKIN MANIFESTATIONS

Nonpolio EVs as a group are a common cause of a variety of skin manifestations.[166,993,997] In the summer and fall, these viruses are the leading cause of exanthems. Variation in the clinical expression rate of exanthem and in the age of the host is marked among the various viral types. In general, dermatologic expression is

related inversely to the age of the infected patient. The frequency of exanthem and common associated illnesses by viral type are presented in Table 178–13.

Coxsackievirus A2

Rash associated with coxsackievirus A2 has occurred rarely. Febrile illness with exanthem in conjunction with coxsackievirus A2 infection was observed in Cincinnati in 1957; no details of this outbreak were presented.[836] Assaad and Cockburn[34] reviewed approximately 15,000 enteroviral illnesses for the period from 1967 to 1970 that were reported to the WHO and noted that in 45 instances, skin or mucous membrane lesions associated with coxsackievirus A2 infection were the main clinical manifestations.

Coxsackievirus A3

One case was reported, but no details are available.[682]

Coxsackievirus A4

Exanthem has been noted infrequently with coxsackievirus A4 infection.[34,172,302,691,1036] However, many cases may be missed because this virus grows poorly in tissue culture, and suckling mouse inoculation rarely is used in diagnostic laboratories. Six children were observed in one outbreak.[302] All patients initially had herpangina, and then the exanthem appeared concurrently with or after defervescence. It initially was erythematous, maculopapular, and discrete. In some children, the rash resolved within 4 days, but in others it progressed and became vesicular. The vesicular lesions occurred in crops, spread to the extremities but not the palms and soles, and were yellowish, opaque, and 5 to 10 mm in size. They persisted for 1 to 2 weeks and regressed, with a brownish discoloration. The lesions easily were confused with resolving bug bites. Other manifestations of coxsackievirus A4 infection include an exanthem suggesting combined scarlet fever and rubella,[337] a maculopapular rash that clears with desquamation,[691] and an anaphylactoid, purpura-like rash (see Fig. 64–15).[172]

Coxsackievirus A5

During the 4-year period studied by Assaad and Cockburn,[34] coxsackievirus A5 was second only to coxsackievirus A16 in the number of instances for which exanthem or mucous membrane involvement was the main clinical manifestation. Even with this apparent frequency, most instances of exanthem have been sporadic rather than part of an outbreak of exanthematous disease. The most common finding is the hand, foot, and mouth syndrome, which is not clinically discernible from that caused by coxsackievirus A16.[221,298,302] In one patient, the virus was recovered from vesicular fluid.[298]

Coxsackievirus A7

Coxsackievirus A7 was recovered from one child with a morbilliform rash and aseptic meningitis.[372] An adult with a fatal coxsackievirus 7 infection had pancarditis, pneumonia, and hand, foot, and mouth syndrome.[45]

Coxsackievirus A9

Coxsackievirus A9 is a common cause of exanthem (see Figs. 64–12 and 64–14).* In contrast to coxsackievirus A16 and echovirus 9, in which rates of clinical exanthem expression are high, the rate of skin manifestations in coxsackievirus A9 infection is low. A review of 259 coxsackievirus A9 infections in 1970 revealed an exanthem rate of 4 percent.[168] Most cases of coxsackievirus A9 exanthem occur sporadically, although a few outbreaks have been described.[175,586,732]

Skin manifestations in coxsackievirus A9 infection have been interesting and varied. The most common rash illness is characterized by fever and an erythematous, maculopapular rash that starts on the face and neck and spreads to the trunk and extremities. Aseptic meningitis is a common associated finding.

In 1960, Lerner and colleagues[587] reported on 11 children with exanthem associated with coxsackievirus A9 infection. Six had vesicular lesions, and 2 patients had associated viral pneumonia. Since 1960, vesicular exanthem has been noted on several occasions.[168,175,449] Most commonly, illnesses have been described as hand, foot, and mouth syndrome, but when the reports were analyzed, the rashes were vesicular but not always in a peripheral pattern.

Papular urticaria and large urticarial lesions have been an occasional manifestation of coxsackievirus A9 infection (see Fig. 64–12).[172,175] Other manifestations include Stevens-Johnson syndrome and a severe illness simulating meningococcemia (see Fig. 64–14).[172,686]

Coxsackievirus A10

The most common exanthematous illness associated with coxsackievirus A10 infection is the hand, foot, and mouth syndrome.[110,168,203,260,465,590] Stevens-Johnson syndrome was associated with coxsackievirus A10 infection in one instance,[168] and a child with ulcerative genital lesions also was reported.[673]

Coxsackievirus A16

In the WHO review of enteroviral infections for the 4-year period from 1967 through 1970, coxsackievirus A16 was associated with almost half of all skin or mucous membrane diseases. Coxsackievirus A16 is the major cause of hand, foot, and mouth syndrome (see Figs. 64–9 through 64–11 and 64–16). Of historical interest is that hand, foot, and mouth syndrome apparently was a new clinical entity in 1956.[731] It was noted in sporadic outbreaks until approximately 1963, and since that time it has been a regularly recurring disease throughout the world.* Serologic data suggest that coxsackievirus A16 was not in wide circulation until approximately 1963.[171]

The symptoms and signs of coxsackievirus A16 hand, foot, and mouth syndrome are recorded in Table 178–14. All illnesses have a typically enteroviral pattern with a short incubation period (4 to 6 days) and a summer and fall seasonal pattern. The clinical expression rate of the enanthem-exanthem complex is high: close to 100 percent in young children, 38 percent in schoolchildren, and 11 percent in adults.[687] Exanthem occurs more commonly in children, but in adults, the rash occurs more frequently with coxsackievirus A16 than with any of the other enteroviral agents.

Illness is ushered in with a mild prodromal fever, anorexia, malaise, and frequently a sore mouth. Enanthem occurs 1 to 2 days after the onset of fever, and the exanthem appears shortly thereafter. Of the cutaneous lesions, oral lesions are present more consistently than lesions of the skin. Because of this prevalence, illness, particularly in adults, is identified mistakenly as aphthous stomatitis (canker sores) or herpes simplex virus (HSV) infection. The intraoral lesions are ulcerative and average approximately 4

*See references 34, 168, 172, 175, 429, 449, 487, 490, 530, 587, 686, 732, 1003.

*See references 9, 15, 26, 31, 103, 168, 171, 196, 281, 302, 315, 337, 352, 354, 355, 418, 420, 590, 619, 620, 652, 687, 710, 809, 813, 932.

to 8 mm in size. The tongue and buccal mucosa are involved most frequently.

As noted in Table 178–14, the hands are involved more commonly than are the feet. Buttock lesions also occur frequently, but they usually do not progress to vesiculation. The lesions on the hands and feet generally are vesicular and vary in size from 3 to 7 mm; they typically occur more commonly on the dorsal surfaces but frequently on the palms and soles as well. The vesicles contain virus, but cytologic examination usually does not reveal diagnostic findings. These lesions clear by absorption of the fluid in approximately 1 week.

Of considerable interest is the frequent association of coxsackievirus A16 with subacute, chronic, and recurring skin lesions.[281,710] Evans and Waddington[281] described an 84-year-old woman with chronic recurring skin lesions of more than 2 years' duration. Nankervis and associates[710] noted both subacute and recurring lesions in children. Higgins and Crow[418] reported a 31-year-old woman with Darier disease who had a Kaposi varicelliform eruption caused by coxsackievirus A16, and a similar illness was noted in a 1-year-old boy with eczema.[703] One child with a Gianotti-Crosti–like eruption (papular acrodermatitis of childhood) associated with coxsackievirus A16 infection was described.[472]

Coxsackievirus B1

Exanthem occasionally occurs in coxsackievirus B1 infections.[34,168,170,172,239,616] The most common cutaneous finding is an erythematous maculopapular eruption that is discrete. Two children with illnesses suggestive of hand, foot, and mouth syndrome have been observed.[172]

Coxsackievirus B2

Coxsackievirus B2–associated exanthem is a rare occurrence. Maculopapular, vesicular, and petechial lesions have been noted.[31,34,302]

Coxsackievirus B3

Exanthem occasionally is reported as a sporadic event in coxsackievirus B3 infections, and a small outbreak occurred in one instance.[31,34,115,212,292,395,447,586,1035] Erythematous maculopapular eruptions occur most commonly, but petechial rash illnesses suggestive of meningococcemia also have been observed. Hand, foot, and mouth syndrome was reported in one child,[586] and another child had a more generalized vesicular eruption. An adult with recurrent hand, foot, and mouth syndrome had a rise in neutralizing antibody titer to coxsackievirus B3.[212]

Coxsackievirus B4

Although cutaneous manifestations with coxsackievirus B4 were noted as frequently as were those associated with coxsackieviruses B1, B2, and B3,[34,168] few clinical descriptions exist.[506,527] Morbilliform, petechial, and urticarial rashes have been described.

Coxsackievirus B5

Of the group B coxsackieviruses, type B5 is noted most frequently to have skin manifestations.* The rash usually is maculopapular; petechial lesions are observed occasionally, and one child with urticaria was reported.[176] Several children studied by Cherry and colleagues[176] had a roseola-like illness pattern. During outbreaks of coxsackievirus B5 illness, approximately 10 percent of young children have an exanthem. Many patients have concomitant aseptic meningitis. One adult patient with recurrent hand, foot, and mouth syndrome had serologic evidence of coxsackievirus B5 infection.

Coxsackievirus B6

Goldwater[357] identified 97 children and adults with coxsackievirus B6 infection. The most prominent finding in these cases was cough and pneumonia. Twenty patients had exanthems; the rash was morbilliform and was associated with high fever and cough.

Echovirus 1

Exanthem and conjunctivitis have been associated with echovirus 1 infection in several children.[638]

Echovirus 2

Exanthem occasionally occurs with echovirus 2 infection.[168,839,896] The rash has been erythematous macular in some instances but usually is maculopapular and discrete. Most children have had associated fever and pharyngitis.

Echovirus 3

Exanthem was noted in 2 of 29 children with echovirus 3 infection.[400] In both instances, the rashes were petechial; most patients in this outbreak had aseptic meningitis. Details of other echovirus 3 infections and exanthems are lacking.[34,430]

Echovirus 4

Echovirus 4 is a common cause of epidemic aseptic meningitis, and exanthem is an associated finding in 10 to 20 percent of pediatric cases.[172,300,324,506,577,624,726,799,892] The rash usually is macular or maculopapular, has its onset 1 to 3 days after the initial fever, and lasts 1 to 2 days. A child with a petechial rash was noted.[506]

Echovirus 5

One major outbreak of echovirus 5 infection with exanthem was described.[342] This outbreak, which involved a maternity unit, resulted in a macular rash in 36 percent of the infants and in 14 percent of the mothers. The rash was most prominent on the limbs and buttocks, appeared 24 to 36 hours after the onset of fever, and lasted 2 days. Selwyn and Howitt[870] reported a child who had a macular rash with a zoster distribution. A neonate with sepsis-like illness and exanthem was described.[384]

Echovirus 6

Although echovirus 6 has been one of the more prevalent EVs since the late 1970s, it has been associated only sporadically with exanthematous disease. The following manifestations have been observed: morbilliform,[963] maculopapular,[646,1006] macular,[632] and papulopustular exanthems[505]; Stevens-Johnson syndrome[966]; and pityriasis rosea in an adult and a child.[505] Meade and Chang[651] reported an interesting zoster-like eruption in a 7-year-old boy in whom echovirus 6 was recovered from several bullae.

Echovirus 7

Echovirus 7 has been associated occasionally with exanthem.[34,226,475,682] In one outbreak of aseptic meningitis, 5 of 13

*See references 34, 91, 168, 176, 212, 244, 381, 403, 727, 773, 775, 848, 880, 902, 906, 912, 1006.

patients had erythematous maculopapular rashes that occurred during fever.[475] One child with a discrete maculopapular rash and thrombocytopenia was described.[638] The case of a 37-year-old woman with echovirus 7 infection, leukocytoclastic vasculitis, and palpable purpura was reported.[184]

Echovirus 9

Since the 1960s, echovirus 9 has been the most prevalent nonpolio EV, and exanthem is a common clinical manifestation (Table 178–15; see Fig. 64–13).* Nonspecific febrile illness and aseptic meningitis are the usual major manifestations of echovirus 9 infection. Exanthem occurs in approximately one third of cases. The prevalence of exanthem is related inversely to age; 57 percent of children younger than 5 years of age have a rash, whereas only 6 percent of those older than 10 years have similar cutaneous findings.[584] The rash usually is rubelliform, but in addition or as the sole manifestation, petechiae are common findings. Rash and fever generally appear at approximately the same time, and frequently the illness closely mimics meningococcemia. The rash usually lasts approximately 3 to 5 days. In one instance, the rash progressed to a vesicular stage.[581]

Echovirus 11

Exanthem occasionally occurs with echovirus 11 infection, and it varies considerably in appearance.[34,106,168,177,246,864] The most notable lesions have been bug bite–like vesicles and urticaria.[177] A child with subacute recurrent vesicular lesions[177] and a woman with a disseminated vesicular eruption were reported.[246]

Echovirus 13

One child with a maculopapular eruption was described.[426] During an outbreak of echovirus 13 aseptic meningitis in Israel, 15 patients had a concomitant exanthem.[890]

Echovirus 14

Echovirus 14 is a rare cause of exanthem, and few details are available.[34,839] One child with a scarlatiniform eruption and aseptic meningitis was reported.[839]

Echovirus 16

The first of the enteroviral exanthematous diseases to be described was that caused by echovirus 16. It initially was studied by Neva and Enders[715] and, later, Enders and colleagues,[717] and the illness was called *Boston exanthem*. Two outbreaks of echovirus 16 infection with exanthem were documented by Neva[714] in 1951 and by Neva and Zuffante[716] in 1954, and another occurred in Paris in 1960.[213] Since then, Boston exanthem has been reported only once. Seven cases were observed in 1974.[387]

The exanthem associated with echovirus 16 infection is erythematous, maculopapular, and discrete and is similar to that of other enteroviral infections. What has been unique with echovirus 16 infection is the relationship of rash with fever. Frequently, the illness resembles roseola in that the rash occurs at the time of or after defervescence.[166,387] Ulcerative lesions on the soft palate and tonsillar pillars (herpangina) sometimes have been observed.

*See references 34, 75, 168, 202, 210, 219, 237, 286, 299, 316, 325, 328, 359, 401, 429, 473, 487, 496, 525, 527, 540, 561, 581, 618, 645, 686, 723, 777, 784, 797, 831, 839, 889, 903, 916, 997.

Echovirus 17

Rash is an occasional occurrence in echovirus 17 infection.[34,103,172,178] In one outbreak, transient erythematous rashes were noted.[103] In cases that I (J. D. C.) studied, papular, maculopapular, and papulovesicular lesions were noted; two patients had herpangitic enanthems, and one had aseptic meningitis.[172,178]

Echovirus 18

Kennett and associates[517] described an extensive epidemic of echovirus 18 infection in which aseptic meningitis with or without exanthem was the major finding. In 15 patients, exanthem was the chief complaint. The rash was described as rubelliform. Most patients were children, but adults also had exanthem.

Echovirus 19

In one extensive epidemic of echovirus 19 infection, exanthem was a common occurrence.[209] Fifty percent of children younger than 6 months old had exanthem, and many of these infants presented a picture of septicemia with peripheral circulatory failure. Rash occurred in adults and older children, but the percentage decreased with age. The rash usually was erythematous, maculopapular, and discrete. It started on the face and upper part of the trunk and spread to the extremities. Fever occurred in all cases, and meningitis and signs of upper respiratory tract involvement were noted frequently. In another outbreak of echovirus 19 infection, 33 percent of those infected had exanthem,[358] whereas in an outbreak studied by Cramblett and colleagues,[217] exanthem was a rare finding.

Echovirus 21

A 19-day-old infant with aseptic meningitis and rash was reported.[191]

Echovirus 24

A 4-month-old infant had rash and aseptic meningitis as a result of echovirus 24 infection.[191]

Echovirus 25

Echovirus 25 has been associated with an array of different skin manifestations.[64,173,380,690,708] In one epidemic of febrile pharyngitis, approximately one third of the patients had exanthem.[690] The rash usually was maculopapular and discrete, but occasionally it displayed a morbilliform confluence. The rash most frequently occurred during the period of defervescence.

Of considerable interest are two children with acute hemangioma-like lesions.[173] The lesions were erythematous and papular and surrounded by a 1- to 4-mm-wide halo of blanched-appearing skin. The center of each lesion had a bright red dot that suggested a dilated capillary or terminal arteriole. The whole lesion blanched with pinpoint pressure in its middle.

Echovirus 30

Echovirus 30 is a common cause of epidemic aseptic meningitis, and exanthem occasionally is noted concomitantly. The rash is macular or maculopapular.[34,168,460,502,579,935,1021]

Echovirus 32

Two children with echovirus 32 infection and hemangioma-like lesions were described by Cherry and colleagues.[173] Some lesions

seemed to be composed of many dilated capillaries; they easily blanched with pressure.

Echovirus 33

Four patients with aseptic meningitis and echovirus 33 infection also had exanthem.[504,514] Two adults had vesicular lesions that suggested HSV infection.[239]

Enterovirus 71

Kennett and associates[516] studied 49 patients with EV 71 infection and noted exanthem in 11. Six patients had aseptic meningitis, and rash was the predominant complaint in 5 patients. The exanthems varied: some were erythematous maculopapular, some vesicular, and some a combination of lesions. Two children had hand, foot, and mouth syndrome, and a single child had a florid, diffuse erythematous rash. Outbreaks of hand, foot, and mouth syndrome caused by EV 71 have been observed in Japan, Sweden, Australia, the United States, Taiwan, and Malaysia.* In many cases, varied neurologic manifestations also occurred.

Parechovirus 1

A morbilliform rash was noted in three infants with respiratory disease and parechovirus 1 infection.[79]

Clinical Exanthematous Manifestations and Syndromes

The major clinical exanthematous manifestations and syndromes of coxsackieviruses and echoviruses are presented in Table 178–16. Unusual findings include hemangioma-like lesions with echoviruses 25 and 32, anaphylactoid purpura with coxsackievirus A4 and echoviruses 9 and 18, zoster-like rash with echoviruses 5 and 6, pityriasis-like rash with echovirus 6, and chronic or recurrent rash with coxsackievirus A16 and echovirus 11.

NEUROLOGIC MANIFESTATIONS

Neurologic illness is a frequent manifestation of infection with most EVs and parechoviruses. The most common illness is aseptic meningitis, but encephalitis and other manifestations also occur. The prevalence of nonpolio EVs and parechoviruses in the various clinical syndromes is presented in Table 178–17.

Aseptic Meningitis

Aseptic meningitis caused by EVs occurs both in epidemics and as isolated cases. The etiologic agents most often associated with epidemic disease are presented in Table 178–18. Epidemic disease has occurred most commonly with coxsackievirus B5 and echoviruses 4, 6, 9, 13, and 30. In general, illness occurs more frequently in children, but if a specific outbreak is large, adults also are involved. Virtually all patients have fever and pharyngitis; other respiratory manifestations occur commonly. Rash is a frequent occurrence but varies with the specific viral agents. Between one third and one half of all patients with echovirus 9 meningitis have exanthem. Abdominal pain is a common complaint in patients with epidemic enteroviral aseptic meningitis.

Except for rash, herpangina, pleurodynia, or myocarditis, little else occurs clinically to help identify the origin in a sporadic case of aseptic meningitis. Initial symptoms include fever, headache, malaise, nausea, and vomiting. The headache usually is frontal or generalized; adolescents and adults frequently note retrobulbar pain. Pain in the neck, back, and legs occurs commonly. Abdominal pain is noted in approximately one fifth of patients, but this symptom varies with the specific etiologic viral type. Photophobia is a common occurrence.

Physical examination reveals a temperature in the range of 38° C to 40° C (100.4° F to 104° F). Skin rash occurs often and usually is erythematous, maculopapular, and discrete. Frequently, particularly with echovirus 9 infection, the rash is petechial and suggests meningococcemia. Hand, foot, and mouth syndrome is a common event in cases of aseptic meningitis caused by EV 71 infection.[146,150,423,547,650] Pharyngitis occurs frequently. Generalized muscle stiffness or spasm usually is observed, although the degree varies considerably; the Kernig and Brudzinski signs are positive in fewer than half the cases. Deep tendon reflexes usually are normal. In one study, 9 percent of children with enteroviral meningitis had the syndrome of inappropriate secretion of antidiuretic hormone.[161] The onset of this syndrome was noted 36 hours after admission to the hospital, and it usually lasted less than 2 days.

Examination of CSF reveals considerable variation among patients and in the same patient on repeated examination. CSF leukocyte counts vary from a few cells to a few thousand per cubic millimeter; the median is in the range of 100 to 500 cells/mm^3. The percentage of neutrophils also varies greatly. Initial examinations frequently reveal a predominance of neutrophils, but rarely more than 90 percent, as seen in bacterial disease. Usually, a range between 30 and 60 percent neutrophils is found on initial examination. Repeated examinations of CSF demonstrate an increasing percentage of mononuclear cells. Dagan and colleagues[227] observed that the rate of isolation of virus from the CSF in enteroviral meningitis was directly proportional to the number of leukocytes in the fluid. Dalal and associates[232] found that children with echovirus 4 meningitis had elevated values of interleukin 6 and interferon in the CSF and that the interleukin 6 value correlated with the CSF leukocyte values.

CSF protein levels usually are elevated mildly, and glucose concentrations usually are normal; hypoglycorrhachia rarely occurs.* Occasionally, the CSF findings suggest tuberculosis meningitis with mononuclear pleocytosis, hypoglycorrhachia, and elevated protein levels.[623] A child with coxsackievirus B4 meningitis had eosinophils in the CSF.[179] The results of other routine laboratory studies such as the WBC count occasionally are abnormal but are not helpful diagnostically.

The duration of illness varies significantly. In most patients, the temperature returns to normal within 4 to 6 days, and disability as a result of neurologic involvement lasts 1 to 2 weeks. Occasionally, the pattern of illness is biphasic: an initial period with fever, headache, nausea, vomiting, and muscle aches and pains of a few days' duration is followed by general recovery and then a return to the same symptoms in addition to more pronounced neurologic involvement.

Wilfert and associates[1000] performed a longitudinal assessment of children who had enteroviral meningitis early in life and found that receptive language functioning was significantly worse than that of children in a control group without meningitis. In another study, Sells and colleagues[869] examined the long-term effects of CNS enteroviral infection in 19 children. Of this group, 11 were free of detectable abnormalities, 5 had possible defects, and 3 had definite neurologic sequelae. In more recent studies, patients who have had meningitis have

*See references 118, 145, 146, 150, 153-155, 164, 189, 317, 343, 423, 436, 441-443, 464, 500, 547, 593, 599-602, 610, 634, 650, 660, 728, 744, 745, 748, 781, 972-974, 1022, 1024.

*See references 38, 180, 272, 563, 583, 628, 871, 881, 884, 908.

performed as well as controls in follow-up developmental evaluations.[76,820,825]

Encephalitis

In the United States, approximately 2500 cases of encephalitis per year, on average, are reported to the CDC.[127] Of this group, only approximately 2 percent of these patients demonstrate an enteroviral origin. However, the seasonal pattern of disease and the absence of arboviral activity in many geographic locations suggest that 500 to 1000 cases of enteroviral encephalitis actually occur each year in the United States. The prevalence of coxsackieviruses and echoviruses as etiologic agents in encephalitis is presented in Table 178–17. Echovirus 9 is the most frequent cause of enteroviral encephalitis. Other enteroviral types commonly associated are echoviruses 4, 6, 11, and 30 and coxsackievirus B5. Echoviruses 4, 6, and 11 have been noted most frequently over a long period.

In general, the prognosis in encephalitis caused by enteroviral infection is good, but fatalities do occur. The viral types that have been isolated from the brain or CSF in fatal cases are as follows: coxsackieviruses B3 and B6; echoviruses 2, 9, 17, and 25; and EV 71.[378,525] Since the mid-1980s, outbreaks of disease caused by EV 71 have occurred in Southeast Asia.* These outbreaks have been characterized by hand, foot, and mouth syndrome, herpangina, and a spectrum of neurologic diseases including severe, often fatal, encephalitis.

In an outbreak in Taiwan in 1998, 30 of 78 (38%) patients with severe illness had encephalitis, and 25 of these patients with encephalitis also had pulmonary edema or hemorrhage, 2 had myocarditis, and a single patient had acute flaccid paralysis.[423] In another study, 30 of 34 children with CNS involvement had brain stem encephalitis, and 9 of this group (30%) died.[976] Children who recovered from EV 71 encephalitis associated with cardiopulmonary failure often had neurologic sequelae with delayed neurodevelopment and reduced cognitive function.[155,781]

Paralysis

Paralysis caused by anterior horn cell disease occasionally results from infection with nonpolio EVs. In contrast to the prevalence of poliovirus, which in the pre-vaccine era resulted in epidemic paralytic disease, paralysis caused by nonpolio EVs usually is a sporadic event. Coxsackievirus A7 has been associated with outbreaks of paralytic disease on three occasions.[375,377,967] Many cases of illness similar to poliomyelitis have occurred during outbreaks and epidemics of illness caused by EV 71.[13,235,343,402,464,660,845] Paralytic disease also has been noted during epidemics of acute hemorrhagic conjunctivitis caused by EV 70.[969,1027]

Guillain-Barré Syndrome and Transverse Myelitis

As Table 178–17 shows, many coxsackieviruses and echoviruses apparently have been associated with Guillain-Barré syndrome. In general, no specific viral types appear to cause the disease. Rather, the disease occurs sporadically in association with the prevalent enteroviral types.

Other Neurologic Illnesses

Cerebellar ataxia has been associated with coxsackieviruses A4, A7, A9, B3, and B4 and echoviruses 6, 9, and 16 (see Table 178–17). Scott[864] specifically commented on peripheral neuritis with echovirus 9 infection. Coxsackievirus A9 has been associated with focal encephalitis and acute hemiplegia on two occasions,[141,168] and echovirus 25 infection was noted in a 5-year-old boy with focal encephalitis and subacute hemichorea.[764] Coxsackievirus B4 was isolated from the CSF of a 22-year-old woman with intracranial hypertension,[1016] and postencephalitis Parkinson syndrome occurring after an episode of coxsackievirus B2 meningoencephalitis was described.[970] Two children with coxsackievirus B3 infections had a syndrome of opsoclonus-myoclonus.[554] The case of a 4-year-old boy with "Alice in Wonderland" syndrome (complex symptoms of perceptual distortion) associated with coxsackievirus B1 infection was described.[975]

Phillips and colleagues[770] and Barrett and associates[51] noted the simultaneous occurrence of enteroviral and St. Louis encephalitis viral infection in the same community. In six instances, dual infections occurred, and the afflicted children tended to have more serious illnesses. Ribai and colleagues[807] noted an 18 month-old boy with transient cerebral arteriopathy and an enteroviral infection. The child had aphasia, right hemiplegia, and seizures, which occurred intermittently over a 3-day period.

Ergul and associates[277] described a 7-year-old boy with vestibular neuritis from whom enteroviral RNA was found in both the CSF and a nasopharyngeal sample. Multiple attacks of enteroviral aseptic meningitis in the same individuals have been noted occasionally.[541,707]

Woodall and associates[1014] found conserved enteroviral sequences in spinal cords from subjects with sporadic motor neuron disease and from one patient with possible familial motor neuron disease. Berger and associates[74] noted EV RNA sequences in spinal cord specimens from 13 of 17 patients with amyotrophic lateral sclerosis, but Walker and colleagues[776] could not duplicate these findings in a study of 20 spinal cord specimens from similar patients. Simonsen and coworkers[883] noted an outbreak of vertigo in Wyoming in August of 1992 in which IgM antibody studies suggested an enteroviral origin.

CHRONIC FATIGUE SYNDROME/FIBROMYALGIA

From molecular techniques and antibody prevalence data, chronic enteroviral infections have been suggested to play a role in the chronic fatigue and postviral fatigue syndromes.[25,114,205,694,1030] However, these findings have not been confirmed by other investigators.[656,670,910] More recently, Douche-Aourik and associates[253] found enteroviral RNA in muscle biopsies from 4 of 30 (13%) patients with fibromyalgia/chronic fatigue syndrome but in no biopsy samples from 29 healthy subjects. However, in none of the 4 positive samples could the presence of VP1 enteroviral capsid protein be demonstrated using a specific monoclonal antibody. In another study, Lane and colleagues[569] found enteroviral RNA in muscle biopsy samples of 10 of 48 patients (20.8%) with chronic fatigue syndrome and in no samples from 29 controls. These investigators also found that 9 of the 10 subjects with positive muscle biopsies had abnormal lactate responses to exercise in the subanaerobic threshold exercise test (SATET). In an editorial relating to these data, Dalakas[229] warned that similar findings in the past were "epiphenomenal" because EVs are ubiquitous in humans and technical flaws inherently connected to contamination in laboratories working with these viruses are inevitable.

The biggest proponents for a causal role of EV in chronic fatigue syndrome are Chia and Chia.[183,185] Although they have extensive experience with chronic fatigue syndrome and have conducted several studies, none of their data has been controlled or subjected to peer review.[545]

*See references 118, 145, 146, 150, 153-155, 164, 189, 317, 423, 436, 441-443, 464, 500, 547, 593, 599-602, 610, 634, 649, 650, 728, 744, 745, 781, 972-974, 1024.

SUDDEN INFANT DEATH

Balduzzi and Greendyke[46] recovered coxsackievirus A5 from the stool of a 1-month-old child who experienced sudden infant death. In a similar investigation of sudden infant death, Gold and associates[356] recovered coxsackievirus A4 from the brains of three babies. Coxsackievirus A8 also was recovered from the stool of a child in whom anorexia was noted on the day before death. Coxsackievirus B3 was recovered at the autopsy of an infant who died suddenly on the eighth day of life.[46] Morens and colleagues[686] noted sudden infant death eight times in association with enteroviral infection; parechovirus was found on two occasions. In a subgroup of infants with sudden unexplained death in whom the "clinical, biologic, and histologic" findings suggested viral infection, evidence of enteroviral infection was found more frequently than in infants without findings of viral infection.[365] Specifically, enteroviral RNA was detected in the respiratory tract in 54 percent of the viral infection group and in none of the group without findings suggestive of viral infection. These results were supported by IgM antibodies to coxsackieviruses B in 56 percent of the first group and in none of the second group. Four infants with suspected sudden infant death syndrome were found to have coxsackievirus B3 myocarditis with minimal histopathologic changes.[248] In five instances of crib death in one study, echovirus 11 was isolated from the lungs in two cases, from the myocardium in one case, and from the nose or feces in the other two cases.[83]

CHRONIC ENTEROVIRAL INFECTIONS IN IMMUNOCOMPROMISED PATIENTS

Patients with cell-mediated and combined immunodeficiencies are susceptible to chronic and often fatal infections with many viruses.[321] Patients with agammaglobulinemia and normal cell-mediated function generally survive infections with these same viruses. EVs are the exception, however, in that chronic, unusual infections with a variety of EVs have been reported.* The most common illness is meningoencephalitis, but arthritis and polymyositis are other frequent findings. Echovirus 11 has been the most common cause of chronic infection, but the following other EVs also have been causative: echoviruses 2, 3, 5, 6, 7, 9, 13, 14, 15, 17, 18, 19, 21, 24, 25, 26, 29, 30, and 33; coxsackieviruses A11, A15, B1, B2, and B3; and parechovirus 1.[642] EVs have been recovered from many other body sites, such as the liver, heart, lung, pancreas, lymph nodes, bone marrow, muscle, throat, and stool, in addition to the CSF.

Several patients with X-linked agammaglobulinemia have had polymyositis-like or dermatomyositis-like syndromes caused by echovirus infections; the following echoviruses have been implicated: types 2, 3, 5, 9, 11, 17, 19, 24, 25, 30, and 33.[50,220,653] The case of a 15-year-old boy with X-linked agammaglobulinemia and chronic arthritis caused by echovirus 11 was reported.[8] Three children with X-linked hyper-IgM syndrome and persistent enteroviral meningoencephalitis were described.[223] Persistent enteroviral infections of the CNS were reported in pediatric and adult patients infected with human immunodeficiency virus (HIV).[265,753] In addition, enteroviral infections caused deaths in bone marrow transplant recipients.[24,322]

CONGENITAL INFECTIONS

Abortion

Landsman and associates[566] studied 2631 pregnancies during an epidemic of echovirus 9 and could find no difference in antibody to echovirus 9 in women who aborted and in those who delivered term infants. A similar study in Finland revealed no increase in the abortion rate in women infected in early pregnancy with echovirus 9.[797] Although coxsackieviral infections occur commonly, epidemics with specific viral types involving large populations have not been studied.

Two women with coxsackievirus A16 hand, foot, and mouth syndrome had spontaneous abortions.[741] In one instance, coxsackievirus A16 was recovered from the products of conception. Frisk and Diderholm[312] found that 33 percent of women with abortions had IgM antibody to coxsackieviruses B, whereas only 8 percent of controls had similar antibody. In a second, larger study, the same research group confirmed their original findings.[39]

Congenital Malformations

In a large prospective study, Brown and Karunas[102] made a serologic search for selected maternal enteroviral infections in association with congenital malformations. Sera from 630 mothers of infants with anomalies and from 1164 mothers of children without defects were studied carefully. Specifically, serologic evidence of infection with coxsackieviruses B1, B2, B3, B4, B5, and A9 and with echoviruses 6 and 9 was sought during the first trimester and during the last 6 months of pregnancy. In this study, infants were examined for 113 specific abnormalities; these anomalies were grouped into 12 categories for analysis. The investigators demonstrated a positive correlation between maternal infection and infant anomaly with coxsackieviruses B2, B3, B4, and A9. The overall anomaly rate associated with first-trimester infection with coxsackievirus B4 was significantly higher than that in controls. Maternal coxsackievirus B2 infection throughout pregnancy, coxsackievirus B4 infection during the first trimester of pregnancy, and infection with at least one of the five group B coxsackieviruses during pregnancy all were associated with urogenital anomalies when compared with controls. Coxsackievirus A9 infection was associated with digestive anomalies and coxsackieviruses B3 and B4 with cardiovascular defects. When coxsackieviruses B were analyzed as a group (B1 to B5), an overall association with congenital heart disease was found; the likelihood of having cardiovascular anomalies was increased when maternal infection with two or more coxsackieviruses B occurred.

Gauntt and colleagues[333] found that ventricular fluid from 4 of 28 babies with severe anatomic defects contained neutralizing antibody to one or more coxsackievirus B types. In one case, specific IgM antibody to coxsackievirus B6 was demonstrated.

In a serologic study in Scotland, Ross and colleagues[821] found no association between maternal coxsackievirus B infection and fetal developmental anomalies. Elizan and associates[273] were unable to find any relationship between maternal infection with coxsackieviruses B and congenital CNS malformations. In three studies, no association between maternal echovirus 9 infection and congenital malformation was noted.[540,566,797]

Prematurity and Stillbirth

Bates[55] reported an 8-month-old stillborn fetus with calcific pancarditis and hydrops fetalis at autopsy. Fluorescent antibody study revealed coxsackievirus B3 antigen in the myocardium. Burch and colleagues[108] reported three stillborn infants who had fluorescent antibody evidence of coxsackievirus B myocarditis, one each with coxsackieviruses B2, B3, and B4. These investigators also described a premature boy who had histologic and immunofluorescent evidence of cardiac infection with coxsackieviruses B2, B3, and B4; he lived only 24 hours.

Freedman[310] reported the occurrence of a full-term stillbirth in a woman infected with echovirus 11. Because the baby had no

*See references 8, 83, 87, 211, 220, 223, 261, 264, 278, 385, 389, 414, 507, 642, 828, 957, 985, 987, 999.

pathologic or virologic evidence of infection, Freedman attributed the event to a secondary consequence of maternal infection caused by fever and dehydration rather than primary transplacental infection. In another stillbirth in which echovirus 11 was recovered from amniotic fluid, the fetus was found to have evidence of focal encephalitis, massive adrenal hemorrhage, and diffuse subarachnoid hemorrhage.[887] Echovirus 27 was recovered from amniotic fluid in an intrauterine fetal death at 28 weeks' gestation.[719] A 26-week, 1300-g stillborn fetus with hydrocephalus, fibrotic peritonitis, and hepatosplenomegaly was found by PCR and immunohistochemical study to have an EV 71 infection.[194] A baby of 26 weeks' gestation with non-immune hydrops fetalis with an intrauterine infection with coxsackievirus B3 was reported by Ouellet and coworkers.[750]

NEONATAL INFECTIONS

Epidemiology and Pathogenesis

Neonatal infection with coxsackieviruses and echoviruses can result from transplacental viral transmission, contact infection during birth, and human-to-human contact after birth. Transplacental passage of coxsackieviruses and echoviruses at term has been noted on many occasions. Benirschke[69] studied the placentas in three cases of congenital coxsackievirus B disease and could find no histologic evidence of infection. In 1956, Kibrick and Benirschke[526] reported the first case of intrauterine infection with coxsackievirus B3. In this instance, the infant was delivered by cesarean section and became symptomatic several hours after birth. Brightman and associates[98] recovered coxsackievirus B5 from the placenta and rectum of a premature infant. No histologic abnormalities of the placenta were noted.

Berkovich and Smithwick[80] noted an asymptomatic neonate who had specific IgM parechovirus 1 antibody in cord blood, thus suggesting intrauterine infection with this virus. Hughes and colleagues[448] reported a newborn infant with echovirus 14 infection who had markedly elevated IgM (190 mg/dL) on the sixth day of life. This child probably also was infected in utero. In addition, echovirus 19 has been noted in a transplacentally acquired infection.[767] Other evidence of intrauterine infection has been presented for coxsackieviruses A4, B1, B2, B3, B4, and B5 and echoviruses 9, 11, and 19.[55,70,90,168,178,280,395,491,503,622,750,767,925]

Little definitive evidence exists for either ascending infection or contact infection with coxsackieviruses or echoviruses during birth. However, transmission of infection during the birth process seems probable.[165,178,503] The fecal carriage rate of EVs in asymptomatic adult patients varies between 0 and 6 percent or higher in different population groups.[168] Cherry and colleagues[178] reported that in 2 of 55 mothers (4%), EVs were present in feces shortly after delivery.

Coxsackievirus B5 was recovered from the cervices of four women with febrile illnesses during the third trimester of pregnancy.[804] Echovirus 11 was isolated from the cervix of a mother whose baby became ill on the third day of life with fatal echovirus 11 necrotizing hepatitis.[803] Chang and colleagues[152] detected coxsackievirus B3 in breast milk of two symptomatic mothers, and their babies both suffered severe illnesses with hepatic necrosis and meningitis caused by coxsackievirus B3.

Several epidemics with coxsackieviruses and echoviruses in newborn nurseries have been studied.[98,119,667,702] Brightman and associates[98] observed an epidemic of coxsackievirus B5 in a premature nursery. Their data suggested that the virus was introduced into this nursery by an asymptomatic infant who had been infected in utero. Secondary infections occurred in 12 babies and 2 nurses. The timing of the secondary cases suggested that three generations had occurred and that the nurses had been infected during the second generation. The investigators suggested that the infection had spread from infant to infant and from infant to nurse.

Javett and associates[476] reported an acute epidemic of myocarditis associated with coxsackievirus B3 infection in a Johannesburg maternity home. Unfortunately, no epidemiologic investigation or search for asymptomatic infected infants was performed. However, in analyzing the dates of onset of the illnesses, single infections apparently occurred for five generations, and then five children became ill within a 3-day period.

Kipps and colleagues[535] carried out epidemiologic investigations in two coxsackievirus B3 nursery epidemics. In the first epidemic, the initial infection probably was transmitted from a mother to her baby; this baby then was the source of five secondary cases in newborn infants and one illness in a nurse. Four of the five secondary cases were located on one side of the nursery, but only one crib was close to that of the index baby, and this crib did not adjoin the cribs of the three other contact cases. In the second outbreak, a baby who also was infected by his mother probably introduced the virus into the nursery. The three secondary cases were geographically far removed from the primary case.

Cramblett and colleagues[215] reported an outbreak of echovirus 11 disease in four infants in an intensive care nursery. All infants were in enclosed incubators, and three patients became ill within 24 hours; the fourth child became ill 4 days later. Echovirus 11 was recovered from two members of the nursery staff. These data suggest that transmission from personnel to infants occurred because of inadequate washing of hands.

In another outbreak in an intensive care unit, the initial patient was transferred to the nursery because of severe echovirus 11 disease.[702] After transfer, the senior house officer and a psychologist in the unit were infected. The investigators inferred that these infected personnel spread the disease by respiratory droplet to nine other babies. In another nursery outbreak of echovirus 11, Mertens and colleagues[667] found that the infection spread through close contact between infected newborns and the nurses in the unit. Spread of infection was interrupted with the installation of vigorous hygienic and isolation measures. In an outbreak of echovirus 11 in an intermediate care unit, Kinney and colleagues[534] found that gavage feeding, mouth care, and being a twin were risk factors for acquiring illness.

Many other instances of isolated nursery infections and small outbreaks with coxsackieviruses and echoviruses have been reported. The most consistent source of original nursery infection seems to be transmission from a mother to her baby,* but virus can be introduced into the nursery by hospital personnel.[461,571,912]

In a longitudinal study of neonatal enteroviral infections carried out during the summer and fall of 1981, Jenista and associates[478] found that the nonpolio enteroviral infection rate was 12.8 percent. Lower socioeconomic status and lack of breastfeeding were found to be risk factors for acquiring infection. Nonpolio enteroviral infections were determined to be a significant cause for re-admission of the cohort neonates to the hospital. During a community outbreak of echovirus 11 disease, Modlin and colleagues[678] found that passive transplacental passage of antibody to neonates prevented the development of severe disease but not mucosal infection.

Clinical Manifestations

Coxsackieviral and echoviral infections in neonates result in a wide variety of clinical manifestations ranging from asymptomatic infection to fatal encephalitis and myocarditis.[169] Unfortunately, in more recent years, enteroviral illnesses have not been examined by specific viral type, but rather have been evaluated in a more generic fashion.[5,6,857] An overview by illness category and prevalence is presented in Table 178–19.

*See references 28, 53, 159, 163, 168, 169, 180, 209, 236, 272, 284, 387, 462, 563, 604, 628, 676, 702, 788, 912, 919.

TABLE 178–19 Major Manifestations of Neonatal Nonpolio Enteroviral and Parechoviral Infections

Specific Involvement	Common	Rare	References
Inapparent infection		Cox A9, B1, B2, B4, B5	80, 168, 178, 270, 283, 342, 467, 692, 709
	Echo 22	Echo 3, 5, 9, 11, 14, 20, 30, 31	
Mild, nonspecific, febrile illness	Cox B5	Cox B1-B4, A9, A16	26, 53, 72, 78, 93, 168, 178, 236, 272, 342, 398, 470, 504, 604, 681, 702, 878, 947
	Echo 5, 11, 33	Echo 4, 7, 9, 17, 30 Parechovirus 4	
Sepsis-like illness	Cox B2-B5	Cox B1, A9	6, 27, 71, 83, 90, 92, 162, 168, 169, 187, 209, 215, 236, 285, 356, 386, 491, 503, 563, 604, 628, 667, 675, 693, 702, 767, 803, 847, 851, 900, 912, 960, 1018
	Echo 5, 11, 16	Echo 2-4, 6, 9, 14,19, 21 Parechoviruses 1, 2, 3	
Respiratory illness (general)		Cox B1, B4, B5, A9	178, 411, 448, 467
	Echo 11 Parechoviruses 1, 3	Echo 9, 17	
Herpangina		Cox A5	159
Coryza		Cox A9	80, 217, 411, 709, 858
		Echo 11, 17, 19 Parechovirus 1	
Pharyngitis		Cox B4	78, 411, 654, 847, 878
		Echo 11, 17, 18	
Laryngotracheitis or bronchitis		Cox B1, B4	267, 411, 684
		Echo 11	
Pneumonia		Cox B4, A9	80, 168, 187, 236, 285, 411, 467, 668, 934, 1002, 1009
		Echo 9, 11, 17, 31 Parechovirus 1	
Cloud baby		Echo 20	270
Gastrointestinal			
Vomiting or diarrhea		Cox B1, B2, B5	78, 168, 209, 270, 271, 285, 342, 396, 411, 467, 604, 668, 792, 847, 912
	Echo 5, 17, 18	Echo 4, 6, 8, 9, 11, 16, 19, 21 Parechovirus 1	
Hepatitis		Cox A9, B1, B3, B4	152, 168, 169, 436, 491, 525, 675, 693, 1018, 1025
	Echo 11, 19	Echo 6, 9, 14, 21	
Pancreatitis		Cox B3, B4, B5	544, 1009
Necrotizing enterocolitis		Cox B2, B3	563
Cardiovascular			
Myocarditis and pericarditis	Cox B1-B4	Cox B5, A9	52, 108, 168, 270, 286, 337, 386, 435, 452, 462, 467, 476, 503, 524, 526
		Echo 11, 19	527, 535, 681, 684, 917, 947, 1018
Skin	Cox B5	Cox B1, 3	80, 93, 168, 172, 176, 215, 226, 342, 387, 503, 604, 654, 668, 727, 847, 925
	Echo 5, 17 Parechoviruses 1, 3	Echo 4, 7, 9, 11, 16, 18	
Neurologic			
Aseptic meningitis	Cox B2-B5	Cox A9, A14, B1	80, 93, 168, 169, 176, 215, 251, 270, 288, 386, 395, 400, 401, 487, 503, 628, 667, 668, 727, 857, 858, 863, 900, 912
	Echo 3, 9, 11, 17	Echo 1, 14, 21, 30 Enterovirus 71	
Encephalitis	Cox B1-B4	Cox B5 Echo 9 Parechovirus 2, 3	71, 180, 283, 284, 401, 851, 863, 1018
Paralysis		Cox B2	487
Sudden infant death		Cox B3, A4, A5, A8 Parechovirus 1	46, 356, 686

Cox, coxsackievirus; echo, echovirus.

INAPPARENT INFECTION

Although inapparent infection probably occurs occasionally with many different EVs, little documentation of this assumption exists. Cherry and colleagues[178] studied 590 normal neonates during a 6-month period and noted only a single asymptomatic infection. This child was infected in utero or immediately thereafter with coxsackievirus B2. The mother had an upper respiratory illness 10 days before delivery.

During a survey of perinatal viral infections, 44 babies were found to be infected with parechovirus 1 in the study period May to December of 1966.[468] The prevalence of virus and the incidence of new infections during this period were fairly uniform. No illness was attributed to parechovirus 1 infection, and the virus disappeared from the nursery in mid-December. Asymptomatic infections with parechovirus 1 were noted on two other occasions.[79,709] Infections without evidence of illness also have been noted with coxsackieviruses A9, B1, B4, and B5 and echoviruses 3, 5, 9, 11, 13, 14, 20, 30, and 31.[168,169,270,283,342,467,478,692]

MILD, NONSPECIFIC FEBRILE ILLNESS

In a review of 338 enteroviral infections in early infancy, 9 percent were classified as nonspecific febrile illnesses.[685] Illness may be sporadic or part of an outbreak with a specific viral type. When the illness is part of an outbreak, the clinical manifestations vary, depending on the viral type: some infants have aseptic meningitis and other signs and symptoms, whereas others simply have nonspecific fever. Specific viruses related to nonspecific fever are listed in Table 178–19. Although by definition illness in this category is mild, awareness that viral infection may be extensive is important. When sought, virus may be isolated from the blood, urine, and CSF of infants with mild illnesses.[53,470] In an outbreak in six neonates of relatively benign illness caused by echovirus 7, all the infants were found to have high C-reactive protein values.[226]

SEPSIS-LIKE ILLNESS

The main diagnostic problem in neonatal enteroviral infections is differentiation of bacterial from viral disease. Even in an infant with mild, nonspecific fever, bacterial disease must be considered strongly. The sepsis-like illness described here always is alarming. Illness is characterized by fever, poor feeding, abdominal distention, irritability, rash, lethargy, and hypotonia.[387,563] Other findings include diarrhea, vomiting, seizures, and apnea. In severe, frequently fatal illnesses, most often caused by echovirus 11 infection, jaundice, hepatitis, disseminated intravascular coagulation (DIC), thrombocytopenia, and hypotension occur.[491,675,693,803]

Sepsis-like illness is a common occurrence. Morens[685] noted its presence in one fifth of 338 enteroviral infections in infants. In an attempt to differentiate bacterial from viral disease, Lake and associates[563] studied 27 infants with enteroviral infection. WBC counts were not helpful because the total count, the number of neutrophils, and the number of band-form neutrophils were elevated in most cases. Of most importance were historical data. Most mothers had suffered a recent, febrile, viral-like illness. In addition, other factors often associated with bacterial sepsis, such as prolonged rupture of membranes, prematurity, and low Apgar scores, were unusual findings in the enteroviral infection group. Bone marrow failure developed in a newborn baby boy with a sepsis-like illness caused by echovirus 11 infection.[921] The neutropenia resolved spontaneously, and the thrombocytopenia normalized after treatment with IVIG.

Abzug[4] reviewed the prognosis in 16 neonates who had a sepsis-like illness with hepatitis and coagulopathy. The case-fatality rate was 31 percent. In addition to having hepatitis and coagulopathy, the 5 patients with fatal cases had myocarditis, and 3 patients had encephalitis. The follow-up of 6 survivors noted normalization of liver function and platelet counts and the absence of subsequent significant medical problems.

RESPIRATORY ILLNESS

Respiratory complaints generally are overshadowed by other manifestations of neonatal enteroviral disease. Only 7 percent of 338 enteroviral infections in early infancy were classified as respiratory illness in one study.[685] Herpangina has been observed and photographed only once; Chawareewong and associates[159] noted several infants with herpangina and coxsackievirus A5 infection.

Hercík and colleagues[411] reported an epidemic of respiratory illness in 22 neonates associated with echovirus 11 infection. All these infants had rhinitis and pharyngitis, 50 percent had laryngitis, and 32 percent had interstitial pneumonitis. Berkovich and Pangan[79] studied respiratory illnesses in premature infants and reported 64 babies with illness, 18 of whom had virologic or serologic evidence of parechovirus 1 infection. In addition, many had high but constant levels of serum antibody to parechovirus 1. Some of the infants with high antibody levels probably also were infected with parechovirus 1. The children with proven parechovirus 1 infection could not be differentiated clinically from those without evidence of such infection. Ninety percent of the infants had coryza, and 39 percent had radiographic evidence of pneumonia.

Except for echoviruses 11 and parechovirus 1, respiratory illness associated with EVs has occurred sporadically. The following other viruses have been noted: coxsackieviruses A5, A9, B1, B4, and B5 and echoviruses 9, 17, 18, 19, 20, 22, and 31. In the review by Morens,[685] only 7 of 338 enteroviral infections of infancy were classified as pneumonia. A newborn with an echovirus 11 infection developed meningitis, DIC, and persistent pulmonary hypertension.[1002] Postmortem examination revealed pneumonia, hyaline membranes, pulmonary interstitial emphysema, meningitis, and DIC.

Eichenwald and Kostevalov[271] recovered echovirus 20 from four full-term infants younger than 8 days old. Although these infants were asymptomatic, they were found to be colonized extensively with staphylococci, and they disseminated these organisms into the air around them. Because of this ability to disseminate staphylococci, they were called *cloud babies*. The investigators thought that these cloud babies contributed to the epidemic spread of staphylococci in the nursery. Because active staphylococcal dissemination occurred only when echovirus 20 could be recovered from the nasopharynx, viral-bacterial synergistic activity was thought to be present.

GASTROINTESTINAL MANIFESTATIONS

Significant gastrointestinal illness occurs in approximately 7 percent of enteroviral infections of infancy.[685] Vomiting and diarrhea occur commonly but usually are only part of the overall illness complex and not the major manifestations. In 1958, Eichenwald and associates[270] described epidemic diarrhea associated with echovirus 18 infection. In a nursery unit of premature infants, 12 of 21 babies were mildly ill. Neither temperature elevation nor hypothermia occurred. Six infants were lethargic and listless, and moderate abdominal distention developed in 2 infants. The diarrhea lasted from 1 to 5 days; these infants had five or six watery, greenish stools per day, occasionally expelled explosively. Two infants had a small amount of blood, but no mucus or pus cells, noted in their stools. Five other babies in another nursery also had similar diarrheal illness. Echovirus 18 was recovered from all ill infants.

In 22 infants with epidemic respiratory disease caused by echovirus 11, all had vomiting as a manifestation of the illness.[411] Linnemann and colleagues[604] reported vomiting in 36 percent and diarrhea in 7 percent of neonates with echoviral infection. In another study, Lake and associates[563] found diarrhea in 81 percent and vomiting in 33 percent of neonates with nonpolio enteroviral infections.

Hepatitis is an important neonatal nonpolio enteroviral illness. Morens[685] reported that 2 percent of neonates with clinically severe enteroviral disease had hepatitis. Lake and colleagues[563] observed that hepatomegaly was present in 37 percent of neonates with enteroviral infection, and hepatosplenomegaly was observed by Hercík and associates[411] in 12 of 22 newborns with echovirus 11 respiratory illness.

Severe hepatitis, frequently with hepatic necrosis, has been noted with echoviruses 6, 9, 11, 14, 19, and 21.* Echovirus 11 most often has been associated with severe and usually fatal hepatitis; findings include DIC and thrombocytopenia, as well as apnea, lethargy, poor feeding, and jaundice.

Philip and Larson[767] reported three catastrophic neonatal echovirus 19 infections that resulted in hepatic necrosis and

*See references 78, 90, 168, 169, 386, 448, 491, 675, 693, 767.

massive terminal hemorrhage. One infant, infected in utero, was symptomatic at birth. The Apgar score was 3, and multiple petechiae were observed. Generalized ecchymoses and apneic episodes occurred, and the infant died at 3.5 hours of age. Thrombocytopenia was noted, and echovirus 19 was isolated from the brain, liver, spleen, and lymph nodes. The other two infants who died of echovirus 19 infection were twins. They were normal during the first 3 days of life but then became mildly cyanotic and lethargic. Shortly thereafter, apneic episodes occurred, and jaundice and petechiae developed. Both twins became oliguric, and they died on the eighth and ninth days of life with severe, terminal gastrointestinal bleeding. Both twins were thrombocytopenic, and virus was recovered from systemic sites in both.

Pancreatitis was found in three of four newborns with coxsackievirus B5 meningitis[544] and in a coxsackievirus B4 infection at autopsy.[1009] In other fatal coxsackievirus B infections, pancreatic involvement has been detected, but clinical manifestations rarely have been observed.

Lake and associates[563] reported three infants with necrotizing enterocolitis. Coxsackievirus B3 was recovered from two of these infants, and coxsackievirus B2 was recovered from the third.

CARDIOVASCULAR MANIFESTATIONS

In contrast to enteroviral cardiac disease in children and adults, in which pericarditis is a common finding, neonatal disease almost always involves the heart muscle. Most cases of neonatal myocarditis are caused by coxsackievirus B infection, and nursery outbreaks have occurred on several occasions. In 1961, Kibrick[524] reviewed the clinical findings in 45 cases of neonatal myocarditis; his findings are summarized in Table 178–20. Of interest is that many of the early experiences, particularly in South Africa, involved catastrophic nursery epidemics. Since the observation in 1972 of five newborns with echovirus 11 infection and myocarditis, no other nursery epidemics have been reported.[257]

The illness caused by coxsackieviruses B most commonly was abrupt in onset, with symptoms of listlessness, anorexia, and fever. A biphasic pattern was noted in approximately one third of the patients. Progression was rapid, and signs of circulatory failure appeared in a 2-day period. If death did not occur, recovery occasionally was rapid but usually took place gradually over an extended period. Most patients had cardiac findings such as tachycardia, cardiomegaly, electrocardiographic changes, and transitory systolic murmurs. Many patients showed signs of respiratory distress and cyanosis. Approximately one third of the infants had signs suggesting neurologic involvement. Of the 45 cases analyzed by Kibrick,[524] only 12 patients survived.

TABLE 178–20 Signs and Symptoms of Neonatal Coxsackievirus B Myocarditis

Category	Frequency (%)
Feeding difficulty	84
Listlessness	81
Cardiac signs	81
Respiratory distress	75
Cyanosis	72
Fever	70
Pharyngitis	64
Hepatosplenomegaly	53
Biphasic course	35
Central nervous system signs	27
Hemorrhage	13
Jaundice	13
Diarrhea	8

Modified from Kibrick, S.: Viral infections of the fetus and newborn. Perspect. Virol. 2:140-159, 1961.

In an echovirus 11 nursery outbreak reported by Drew,[257] 5 of 10 babies had tachycardia out of proportion to their fever. Three of these babies had electrocardiograms; supraventricular tachycardia was noted in all, and ST-segment depression was observed in 2 patients' records. Supraventricular tachycardia also has occurred with coxsackievirus B infection.[467] Echovirus 19 has been associated with myocarditis, and coxsackievirus A9 was noted in a child with pericarditis.[217,917]

In the 1970s, neonatal myocarditis caused by EVs was seen less commonly than it was in the 1950s and early 1960s. In his review, Morens[685] noted only 2 instances among 248 severe neonatal enteroviral illnesses. Chan and Lun[147] reported a neonate with coxsackievirus B4 myocarditis who developed a ventricular aneurysm.

EXANTHEM

Exanthem as a manifestation of neonatal enteroviral infection has been reported with coxsackieviruses B1, B3, and B5, with echoviruses 4, 5, 7, 9, 11, 16, 17, and 18, and with parechovirus 1. In most instances, rash is just a minor manifestation of severe neonatal disease. In 27 infants studied by Lake and colleagues,[563] 41 percent had exanthem. Cutaneous manifestations generally commence between the third and fifth days of illness. The rash usually is macular or maculopapular. Petechial lesions are noted occasionally. Surprisingly, vesicular lesions have not been described, nor has any rash illness in neonates been associated with coxsackievirus A16. Hall and associates[387] reported two neonates with echovirus 16 infections in which the illnesses resembled roseola. These patients had fever for 2 and 3 days, defervescence, and then the appearance of a maculopapular rash.

A newborn with a vesiculopapular-crusted rash on the face, trunk, and extremities caused by coxsackievirus B3 was reported by Theodoridou and associates.[925] It was a congenital infection in which new lesions appeared over the course of a 5-day period, and the total duration of the rash was 10 days.

NEUROLOGIC MANIFESTATIONS

As noted in Table 178–19, neurologic illness has been associated with coxsackieviruses B1, B2, B3, B4, and B5 and with many echoviruses as well. In neonates, differentiating meningitis from meningoencephalitis usually is difficult. Meningoencephalitis occurs commonly in infants with sepsis-like illness, and postmortem studies revealed many infants with disseminated viral disease (heart, liver, adrenal glands) in addition to CNS involvement. In Morens' review,[685] 50 percent of the patients with enteroviral infection who were analyzed had encephalitis or meningitis.

The initial clinical findings in neonatal meningitis or meningoencephalitis are similar to those in nonspecific febrile illness or sepsis-like illness. Most often, the child is normal and then becomes febrile, anorectic, and lethargic. Jaundice frequently is noted in newborns, and vomiting occurs in neonates of all ages. Less common findings include apnea, tremulousness, and general increased tonicity. Seizures occur occasionally.

Examination of CSF reveals considerable variation in protein, glucose, and cellular values. In seven newborns with meningitis caused by coxsackievirus B5 studied by Swender and colleagues,[912] the mean CSF protein value was 244 mg/dL, and the highest value was 480 mg/dL. The mean CSF glucose value was 57 mg/dL, and one of the seven infants had pronounced hypoglycorrhachia (a value of 12 mg/dL). The mean CSF leukocyte count in the seven babies was 1069 cells/mm^3, with 67 percent polymorphonuclear cells. The highest cell count was 4526 cells/mm^3, with 85 percent polymorphonuclear cells. In another study involving 28 children younger than 2 months in which coxsackievirus B5 was the implicated pathogen, 36 percent of the infants had CSF leukocyte counts of 500 cells/mm^3 or greater.[628] In this

same study, only 13 percent of the infants had CSF protein values of 120 mg/dL or greater; 12 percent of the infants had glucose values lower than 40 mg/dL.

In summary, the CSF findings in neonatal nonpolio enteroviral infections frequently are similar to those in bacterial disease. In particular, the most consistent finding in bacterial disease, hypoglycorrhachia, is noted in approximately 10 percent of newborns with enteroviral meningitis.[180,272,563,628,912]

Johnson and associates[487] reported a 1-month-old boy with right-sided facial paralysis and loss of abdominal reflexes. The facial paralysis persisted through convalescence; the reflexes returned to normal within 2 weeks. The boy was infected with coxsackievirus B2.

Euscher and associates[280] detected coxsackievirus RNA in placental tissue from six of seven newborn infants with respiratory difficulties and other manifestations at birth. Of these infants, one died shortly after birth, and the other six suffered neurodevelopmental delays.

CLINICAL MANIFESTATIONS: POLIOVIRUSES[16,88,167,169,198,438]

When a susceptible person has had effective contact with a poliovirus, one of several responses may occur, in the following order of frequency: (1) inapparent infection, (2) minor illness (abortive poliomyelitis), (3) nonparalytic poliomyelitis (aseptic meningitis), and (4) paralytic poliomyelitis. Paralytic poliomyelitis is the most dramatic expression of the infection and the only one clinically recognizable as caused by a poliovirus; it accounts for not more than 1 to 2 percent of infections during epidemics and considerably less under endemic conditions (see Fig. 178–5). The aseptic meningitis syndrome is similarly infrequent; nonspecific "minor illness" is estimated to occur in 4 to 8 percent, and 90 to 95 percent of those infected have inapparent infections. Factors that determine the type of clinical response are poorly understood, but the degree of virulence of the virus and certain host characteristics are important.

Age has a significant effect on patterns of infection; older patients are more likely to have severe paralytic disease and a higher mortality rate. Pregnancy increases the risk, probably primarily because of hormonal factors but also because pregnant women may be exposed more to young children, who are the main sources of contagion. Tonsillectomy in the presence of inapparent infection can precipitate bulbar poliomyelitis; evidence also suggests that tonsillectomy at any time in the past results in enhanced susceptibility to the bulbar form of the disease. Recent diphtheria-tetanus-pertussis vaccination increases the likelihood of development of paralysis; the site of injection and the site of paralysis appear to be correlated. Physical exertion and trauma around the time of onset also increase the risk of severe paralysis, especially in adults.

MINOR ILLNESS (ABORTIVE POLIOMYELITIS)

The minor illness is mild and nonspecific, with low-grade fever, malaise, anorexia, and sore throat. Physical examination reveals no significant abnormalities, CSF is normal, and recovery occurs within 24 to 72 hours. The illness often is so mild that it goes unrecognized, and patients rarely are seen by a physician.

NONPARALYTIC POLIOMYELITIS (ASEPTIC MENINGITIS)

The onset of nonparalytic poliomyelitis is associated with vague malaise followed by fever, headache, aching of the muscles, and sometimes hyperesthesia and paresthesia. Anorexia, nausea, vomiting, constipation, or diarrhea also may be present. The temperature rises to 37.8° C to 39.5° C (101° F to 103° F); stiffness of the neck, back, and hamstrings soon appears.

Approximately two thirds of affected children have a short, symptom-free interlude between the first phase (minor illness) and the second phase (CNS or major illness). This two-phase course occurs less commonly in adults, in whom the evolution of symptoms is more insidious. Nuchal and spinal rigidity is necessary for establishing the diagnosis of nonparalytic poliomyelitis during the second phase.

Physical examination reveals nuchal-spinal signs and changes in superficial and deep reflexes. With cooperative patients, the nuchal-spinal signs are sought first by active tests. The child is asked to sit up unassisted. If doing so causes undue effort, if the knees flex upward, and if the patient writhes a bit from side to side while sitting up and uses the hands on the bed for the tripod supporting position, unmistakable spinal rigidity is present. Still sitting, the patient is asked to flex chin to chest and is observed for nuchal rigidity. Alternatively, from the supine position with the knees held down gently, the patient is asked to sit up and kiss the knees. If the knees draw up sharply or if the maneuver cannot be completed adequately, the patient has stiffness of the spine caused by muscle spasm. If the diagnosis still is uncertain, attempts should be made to elicit the Kernig and Brudzinski signs. Gentle forward flexion of the occiput and neck elicits nuchal rigidity, which may precede spinal rigidity. Head drop may be demonstrated by placing the hands under the patient's shoulders and raising the trunk. Normally, the head follows the plane of the trunk, but in poliomyelitis, it often falls backward limply. The frequency of the head-drop sign, even in nonparalytic poliomyelitis, with no subsequent residuals indicates that it is not caused by true paresis of the neck flexors. In struggling infants, distinguishing voluntary resistance from clinically important involuntary nuchal rigidity may be difficult. One may place the infant's shoulders flush with the edge of the table, support the weight of the occiput in the hand, and then flex the head anteriorly. Nuchal rigidity that persists during this maneuver may be interpreted as involuntary. When not closed, the anterior fontanelle also may be tense or bulging.

In the early stages, the reflexes are normally active and remain so unless paralysis supervenes. Changes in reflexes, either increased or depressed, may precede the onset of weakness by 12 to 24 hours; hence, detecting such changes is important, especially in nonparalytic patients managed at home. The superficial reflexes (i.e., cremasteric and abdominal and the reflexes of the spinal and gluteal muscles) are usually the first to be diminished. The spinal and gluteal reflexes are elicited by tapping segmentally downward on each side of the spine and buttocks. These reflexes may disappear before the abdominal and cremasteric ones do. Changes in the deep tendon reflexes, whether exaggerated or depressed, generally occur 8 to 24 hours after depression of the superficial reflexes and indicate impending paresis of the extremities.

Laboratory findings consist of a normal or slightly elevated WBC count and the characteristic CSF changes of aseptic meningitis: approximately 20 to 300 cells, predominantly lymphocytes, a normal glucose level, and a normal or slightly elevated protein level. If a spinal tap is performed in the first few hours after onset, a predominance of polymorphonuclear leukocytes may be seen, but it shifts in 6 to 12 hours to more than 90 percent lymphocytes. If no further progression of clinical signs occurs, the disease remains nonparalytic, the temperature falls to normal, and signs of meningeal irritation gradually disappear. Recovery ensues in 3 to 10 days, depending on the severity of the illness.

PARALYTIC POLIOMYELITIS

The manifestations of paralytic poliomyelitis are those enumerated earlier for nonparalytic poliomyelitis in addition to weakness in one or more muscle groups, either skeletal or cranial. Patients in whom paralysis is destined to develop often wear an anxious expression; they are extremely alert, restless, and flushed and appear acutely ill. The fever is higher than that in abortive disease,

and the patient may have intense muscle pain. Shortly before actual muscle weakness is detected, the superficial and deep reflexes often diminish or disappear on the affected side. Frequently, a symptom-free interlude of several days occurs between the initial illness phase and the recurrence of symptoms that culminate in paralysis.

The onset of paralysis may be extraordinarily sudden and may progress in a few hours to complete loss of motion in one or more extremities. Asymmetric involvement is typical in milder cases. More gradual spread of weakness also occurs and may continue over a period of 3 to 5 days. Bladder paralysis of 1 to 3 days' duration develops in approximately 20 percent of patients, and bowel atony frequently is noted, occasionally to the point of paralytic ileus. In general, when the fever subsides, no further paralysis is likely to occur. The lower limbs are affected more commonly than are the upper, but in severe cases, quadriplegia and loss of function of the intercostal, abdominal, and trunk muscles with resultant respiratory difficulty may ensue. The superficial and deep reflexes in the affected limbs are lost; twitching of the muscles and diffuse fasciculations may be seen transiently. Sensory abnormalities are rare occurrences.

Flaccid paralysis is the most obvious clinical expression of the neuronal changes. The ensuing muscular atrophy is caused by denervation in addition to the atrophy of disuse. The pain, spasticity, nuchal and spinal rigidity, and hypertonia early in the illness probably are caused by lesions in the brain stem, spinal ganglia, and posterior columns. Respiratory and cardiac arrhythmias, blood pressure and vasomotor changes, and similar manifestations reflect damage to vital centers in the medulla.

On physical examination, the distribution of paralysis characteristically is spotty. To detect mild muscular weakness, one often must apply gentle resistance in opposition to the muscle group being tested. The spinal form has weakness of some of the muscles of the neck, abdomen, trunk, diaphragm, thorax, or extremities. The bulbar form is characterized by weakness in the motor distribution of one or more cranial nerves, with or without dysfunction of the vital centers of respiration and circulation. Patients with bulbar disease often are extremely agitated, even delirious, or they may become stuporous. The 10th cranial nerve nuclei are involved most commonly and result in paralysis of the pharynx, soft palate, and vocal cords. Facial paralysis occurs less commonly; it usually is asymmetric and involves only selected muscle groups. Ocular palsies are unusual findings.

Components of both the bulbar and spinal forms occur together in bulbospinal poliomyelitis. In the encephalitic form of the disease, irritability, disorientation, drowsiness, and coarse tremors not explained by inadequate ventilation are noted. Even during epidemics of poliomyelitis, this form can be recognized only if some peripheral or cranial nerve paralysis coexists or ensues. Hypoxia and hypercapnia caused by inadequate ventilation from respiratory insufficiency may produce disorientation without true encephalitis.

Numerous components acting together may result in insufficiency in ventilation (Table 178–21). The most serious consequences are hypoxia and hypercapnia, which may produce profound effects on many other systems. Respiratory insufficiency should be detected early to diminish its widespread effects, and because the situation may shift rapidly, continued clinical evaluation is essential. Despite weakness of the respiratory muscles, the patient may respond with so much respiratory effort that normal alveolar ventilation is maintained. In fact, the increased effort (associated with anxiety and fear) actually may produce overventilation at the outset and may result in respiratory alkalosis. Such effort is fatiguing and soon leads to respiratory failure.

For clarity, certain terms characterizing patterns of disease need definition. First, *pure spinal poliomyelitis with respiratory insufficiency* refers to tightness, weakness, or paralysis of the respiratory muscles (chiefly the diaphragm and intercostals) without discernible clinical involvement of the cranial nerves or vital centers. The cervical and thoracic spinal cord segments chiefly are involved. Second, *pure bulbar poliomyelitis* refers to paralysis of the motor cranial nerve nuclei with or without involvement of the vital centers that control respiration, circulation, and body temperature. Involvement of the 9th, 10th, and 12th cranial nerves is most important because it results in paralysis of the pharynx, tongue, and larynx, with consequent airway obstruction. Third, *bulbospinal poliomyelitis with respiratory insufficiency* refers to involvement of the respiratory muscles with coexisting bulbar paralysis.

TABLE 178–21 Common Sources of Hypoxia and Hypercapnia in Poliomyelitis

1. Cranial nerves IX to XII involved with
   a. Pharyngeal paralysis and pooling of secretions
   b. Laryngeal involvement, either spasm of laryngeal muscles or paralysis of vocal cords
   c. Lingual paralysis
   d. Tracheal accumulation of secretions from inability to cough
   e. Aspiration of vomitus
2. Vital center involvement with
   a. Inefficient, irregular respiration
   b. Cardiovascular disturbance
   c. Hyperpyrexia causing increased oxygen consumption
3. Cervical and spinal cord involvement causing paresis of the primary and accessory muscles of respiration
4. Pulmonary complications (e.g., pneumonia, atelectasis, and edema)
5. Contributory factors
   a. Panic
   b. Gastric dilation
   c. Sedation
   d. Inadequate equipment (e.g., small-bore tracheostomy tubes, unsuitable respirator settings)

From Cherry, J. D.: Enteroviruses. In *Behrman, R. E., and Vaughan, V. C. (eds.): Nelson Textbook of Pediatrics. 12th ed. Philadelphia, W. B. Saunders, 1983, pp. 791-804.*

The clinical findings resulting from involvement of the respiratory muscles are as follows: (1) an anxious expression; (2) inability to speak without frequent pauses, a situation resulting in short, jerky, "breathless" sentences that can be demonstrated by asking the child to count numbers serially; (3) increased respiratory rate; (4) movement of the alae nasi and the accessory muscles of respiration; (5) inability to cough or sniff with full depth; (6) paradoxical abdominal movements caused by diaphragmatic immobility from spasm or weakness of one or both leaves; and (7) relative immobility of the intercostal spaces, whether segmental, unilateral, or bilateral. When the arms are weak and especially when deltoid paralysis occurs, one should beware of impending respiratory paralysis because the phrenic nerve nuclei are in adjacent areas of the spinal cord. Observing the patient's capacity for thoracic breathing while the abdominal muscles are splinted manually can be performed to assess minor degrees of paresis. Light manual splinting of the thoracic cage helps in evaluating the effectiveness of diaphragmatic movement.

The clinical findings of bulbar poliomyelitis with respiratory difficulty (other than paralysis of the extraocular, facial, and masticatory muscles) include the following: (1) a nasal twang to the voice or cry as a result of palatal and pharyngeal weakness (hard consonant words such as "cookie" or "candy" bring out this condition best); (2) an inability to swallow smoothly that results in an accumulation of saliva in the pharynx and indicates partial immobility (holding the larynx lightly and asking the patient to swallow confirms the immobility); (3) accumulated pharyngeal secretions, which may cause irregular respiration because each inspiration must be "planned" and cannot be "subconscious" in view of the risk of aspirating; the respirations thus may appear interrupted and abnormal even to the point of falsely simulating intercostal or diaphragmatic weakness; (4) the impossibility of

effective coughing, with resultant constant and fatiguing efforts to clear the throat; (5) nasal regurgitation of saliva and fluids caused by palatal paralysis, with an inability to separate the oropharynx from the nasopharynx during swallowing; (6) deviation of the palate, uvula, or tongue; (7) involvement of vital centers, as manifested by an irregularity in the rate, depth, and rhythm of respiration; by cardiovascular alterations that include changes in blood pressure (especially increased), alternate flushing and mottling of the skin, and cardiac arrhythmias; and by rapid changes in body temperature; (8) paralysis of one or both vocal cords that causes hoarseness, aphonia, and, ultimately, asphyxia unless recognized by laryngoscopy and managed by immediate tracheostomy; and (9) the "rope sign," an acute angulation between the chin and larynx caused by weakness of the hyoid muscles (the hyoid bone is pulled posteriorly, thus narrowing the hypopharyngeal inlet). Myocardial failure sometimes develops secondary to pulmonary complications or as a result of acute myocarditis. The initial manifestation of poliovirus infection on occasion can resemble that of Guillain-Barré syndrome.[1028]

Congenital Infections

ABORTION

Poliomyelitis is associated with an increased incidence of abortion. Horn[428] noted 43 abortions in 325 pregnancies complicated by maternal poliomyelitis. Abortion was related directly to the severity of the maternal illness, including the degree of fever during the acute phase of illness. However, abortion also has occurred in association with mild nonparalytic poliomyelitis. Siegel and Greenberg[879] noted that fetal death occurred in 14 of 30 instances (46.7%) of maternal poliomyelitis during the first trimester. Kaye and associates[510] reviewed the literature in 1953 and recorded 19 abortions in 101 cases of poliomyelitis in pregnancy. In a small study in Evanston Hospital in Illinois, the abortion rate in maternal poliomyelitis was little different from the expected rate.[94]

CONGENITAL MALFORMATIONS

Although isolated instances of congenital malformation and maternal poliomyelitis have been noted, little statistical evidence supports the suggestion that polioviruses are teratogens. In their review of the literature, Kaye and colleagues[510] noted 6 anomalies in 101 infants born to mothers with poliomyelitis during pregnancy. In the reviews of Horn,[428] Bates,[56] and Siegel and Greenberg,[879] no evidence of maternal polioviral infection–induced anomalies was found. Similarly, no evidence suggests that infection with poliovirus vaccine during pregnancy causes congenital malformations.[396]

PREMATURITY AND STILLBIRTH

In Horn's study[428] of 325 pregnancies, 9 infants died in utero. In each instance, the mother was critically ill with poliomyelitis. Horn[428] also noted that 45 infants weighed less than 6 lb, and 17 of them had a birth weight less than 5 lb. These low-birth-weight infants were born predominantly to mothers who had poliomyelitis early in pregnancy. In New York City, Siegel and Greenberg[879] also noted an increase in prematurity after maternal poliomyelitis infection. It was related specifically to maternal paralytic poliomyelitis.

Neonatal Infections

GENERAL

In the excellent review by Bates[56] in 1955, 58 cases of poliomyelitis in infants younger than 1 month were described. Although complete data were not available on many of the cases, 51 had paralysis, died of their disease, or both. Of the total number of infants on whom these investigators had clinical data, only one infant had nonparalytic disease. More than half the cases were secondary to maternal disease. Because other investigators have noted congenital infection without symptomatic maternal infection, infection in the mother probably was the source for an even greater percentage of the neonatal illnesses. The incubation period of neonatal poliomyelitis has not been established, and, therefore, determining how many of the babies were infected in utero is difficult. Probably, most illnesses that occurred within the first 5 days of life were congenital. Most of the neonates had symptoms of fever, anorexia or dysphagia, and listlessness. Almost half the infants noted in this review died, and of those surviving, 48 percent had residual paralysis.

INFECTION ACQUIRED IN UTERO

Elliott and associates[274] described an infant girl in whom "complete flaccidity" was noted at birth. This child's mother had had mild paralytic poliomyelitis, with the onset of minor illness occurring 19 days before the infant's birth. Fetal movements had ceased 6 days before delivery, a finding suggesting that paralysis had occurred at this time. On examination, the baby was severely atonic; when supported under the back, she was passively opisthotonic. Respiratory efforts were abortive and were confined to the accessory muscles; laryngoscopy revealed complete flaccidity in the larynx.

Johnson and Stimson[483] reported a case in which the mother's probable abortive infection occurred 6 weeks before the birth of the baby. The baby initially was thought to be normal but apparently underwent no medical examination until the fourth day of life. At this time, the physician noted right hemiplegia. On the following day, a more complete examination revealed lateral bulging of the right side of the abdomen accompanied by crying and maintenance of the lower extremities in a frog leg position. Adduction and flexion at the hips were weak, and the knee and ankle jerks were absent. Laboratory studies were unremarkable except for examination of CSF. It revealed 20 lymphocytes and a protein level of 169 mg/dL. During a 6-month period, this child's paralysis gradually improved and resulted in only residual weakness of the left lower extremity.

Paresis of the left arm was noted shortly after birth in another child with apparent transplacentally acquired poliomyelitis.[615] At 2 days of age, the baby was quadriplegic, but patellar reflexes were present, and the child had no respiratory or swallowing difficulties. This child had pneumonia at 3 weeks of age, but otherwise, general neurologic improvement occurred. Examination at 8 weeks of age revealed bilateral atrophy of the shoulder girdle muscles. The CSF from this patient had 63 leukocytes/mm^3, 29 percent of them polymorphonuclear cells, and a protein concentration of 128 mg/dL.

All three of the infants just discussed apparently were infected in utero several days before birth. Their symptoms were exclusively neurologic; fever, irritability, and vomiting did not occur.

POSTNATALLY ACQUIRED INFECTION

In contrast to infections acquired in utero, those acquired postnatally are more typical of classic poliomyelitis. Shelokov and Weinstein[876] described a child who was asymptomatic at birth. The onset of minor symptoms in the mother occurred 3 weeks before delivery, and the onset of major symptoms occurred 1 day before delivery. On the sixth day of life, the infant suddenly became ill with watery diarrhea. He looked grayish and pale. On the following day, he was irritable, lethargic, and limp and had a temperature of 38° C. Mild opisthotonos and weakness of both lower extremities developed. He was responsive to sound, light, and touch. The CSF had an elevated protein level and an increased

number of leukocytes. His condition worsened during a total period of 3 days, and then gradual improvement began. At 1 year of age, he had severe residual paralysis of the right leg and moderate weakness in the left leg.

Baskin and associates[54] described two infants with neonatal poliomyelitis. The first child, whose mother had severe poliomyelitis at the time of delivery, was well for 3 days and then had a temperature of 38.3° C. On the fifth day of life, the boy became listless and cyanotic. Examination of CSF revealed a protein level of 300 mg/dL and 108 leukocytes/mm^3. His condition worsened, and extreme flaccidity, irregular respiration, and progressive cyanosis developed; he died on the seventh day of life. The second infant was a boy who was well until he was 8 days old but then became listless, with a temperature of 38.38° C. During the next 5 days, flaccid quadriplegia developed, as did irregular, rapid, and shallow respirations and an inability to swallow. The child died on the 14th day of life. Acute poliomyelitis had developed in his mother 6 days before the onset of his symptoms.

Abramson and colleagues[3] reported four children with neonatal poliomyelitis, two of whom died. In three of the children, the illnesses were typical of acute poliomyelitis in older children. The other child died at 13 days of age with generalized paralysis. The onset of his illness was difficult to define, and he was never febrile. Bates[56] described infants with acute poliomyelitis and clinical illnesses similar to those that occur in older persons.

DIAGNOSIS AND DIFFERENTIAL DIAGNOSIS

CLINICAL DIAGNOSIS

Clinical differentiation of enteroviral disease frequently is thought to be impossible. Although treatable bacterial illnesses always should be considered and treated first, also true is that when all the circumstances of a particular illness are considered, enteroviral diseases can be suspected on clinical grounds. The most important factors in clinical diagnosis are the season of the year, geographic location, exposure, incubation period, and clinical symptoms.

In temperate climates, prevalence of EVs is distinctly seasonal, so disease usually is seen in the summer and fall. Enteroviral disease is less likely to occur in the winter. In the tropics, EVs are prevalent throughout the year, and the season, therefore, is not diagnostically helpful.

As with all infectious illnesses, knowledge of exposure and incubation time is important. A careful history of maternal illness is critical in neonatal disease. For example, nonspecific, mild febrile illness in a mother that occurs in the summer and fall should warn of the possibility of severe neonatal illness. Specific findings (i.e., aseptic meningitis, pleurodynia, herpangina, pericarditis, myocarditis) should alert the clinician to enteroviral illnesses. The short incubation period of enteroviral infections should be considered.

LABORATORY DIAGNOSIS

Virus Isolation and Detection Techniques

Most viral diagnostic laboratories have facilities for the recovery of most EVs that cause illness. A three-tissue culture system that includes primary monkey kidney, a diploid, human embryonic lung fibroblast cell strain, and the RD cell line will allow the isolation of virtually all coxsackieviruses B and echoviruses and some coxsackieviruses A (e.g., coxsackieviruses A9 and A16). In a study in which Buffalo green monkey kidney cells and subpassage of primary human embryonic kidney cells were used in addition to primary monkey kidney and human diploid fibroblast (MRC-5) cells, the recovery rate of EVs was increased 11 percent.[190] For a complete diagnostic isolation spectrum, suckling mouse inoculation also should be performed.

Proper selection and handling of specimens are most important in the isolation of viruses. Enteroviral infections tend to be generalized, so collection of material from multiple sites is important; specimens should be collected from any or all of the following: nasopharynx, throat, stool, blood, urine, CSF, and any other body fluids that are available. Swabs from the nose, throat, and rectum should be placed in a carrying medium containing a small amount of protein. Hanks balanced salt solution with 2 percent agamma calf serum and antibiotics is satisfactory. Fluid specimens should be collected in sterile vials; specimens of postmortem material are collected best in vials that contain carrying medium. In general, specimens should be refrigerated immediately after collection and during transportation to the laboratory. The specimens must not be exposed to sunlight during transportation. If an extended period will elapse before a specimen is processed in the laboratory, shipping and storing it frozen are advisable.

Contrary to popular belief, tissue culture evidence of enteroviral growth takes only a few days in many cases and less than a week in most.[366] The use of spin amplification, the shell vial technique, and monoclonal antibodies has been shown to reduce the time of detection in enteroviral cultures significantly.[542,763,874,938] After an EV has been isolated, type identification is performed conventionally by neutralization, and this process, unfortunately, frequently takes a long time.

Nucleic acid techniques with cDNA and RNA probes have been shown to be useful for direct identification of EVs.[120,238,455,765,823,824,827] Most important, however, has been the development of numerous PCR techniques. Since 1990, many reports have described enteroviral PCR methods and their use in identifying EV RNA in clinical specimens.* PCR has proved most useful for the direct identification of EVs in the CSF of patients with meningitis.[830,853,918,929,1026] When compared with culture of CSF specimens, PCR is faster and more sensitive, and the specificity is equal.

PCR also has proved useful in the identification of EVs in blood, urine, and throat specimens.[7,18,111,720,721,873] Particularly impressive are the findings of Byington and associates.[111,811] Using PCR on specimens of blood and CSF, these investigators found that more than 25 percent of infants admitted to the hospital for suspected sepsis in 1997 had nonpolio enteroviral infections. Based on this study and the work of Andréoletti and coworkers,[18] we consider that the general work-up for febrile children hospitalized for possible sepsis should include PCR for EVs in both blood and CSF, if available. A shortcoming of PCR is that EV RNA is identified, but a specific enteroviral type is not. Because of this shortcoming, we recommend that conventional culture be performed in addition to PCR.

EV RNA also has been identified in numerous tissue specimens from patients with chronic medical conditions such as idiopathic DCM. However, as discussed earlier ("Cardiovascular Manifestations"), the possibility of a lack of specificity (false-positive results) is a concern. Polioviruses can be separated from other EVs, and poliovirus vaccine strains can be identified rapidly by PCR.[2,182,268,1023]

Serology

Except in special circumstances, the use of serologic techniques for establishing the primary diagnosis of suspected enteroviral

*See references 2, 7, 18, 23, 41, 111, 182, 204, 205, 208, 268, 307, 319, 363, 390, 456, 559, 564, 596, 631, 656, 694, 720, 738, 790, 811, 815, 822, 825, 830, 850, 857, 872, 873, 877, 885, 901, 910, 918, 929, 943, 961, 964, 968, 1012, 1023, 1026.

infection is impractical. Standard serologic study depends on the demonstration of a rise in antibody titer to a specific virus as an indication of infection with that agent. Although ELISA, hemagglutination inhibition, and CF take only a short time to perform, these tests can be carried out only after a second, convalescent-phase blood specimen has been collected. These tests also are impractical in searching for the cause of a specific illness in a child because of the existence of so many antigenically different EVs. As noted previously ("Antigenic Characteristics"), group antigens can be produced that allow one to establish a serologic diagnosis by IgM EIA and CF, but these tests lack specificity.[846,926,927]

In the evaluation of a patient with a suspected enteroviral infection, serum should be collected as soon as possible after the onset of illness and then again 2 to 4 weeks later. This serum should be stored frozen. In most clinical situations, performing serologic tests on the collected serum is not necessary because demonstration of a rise in antibody titer in the serum of an infant from whom a specific virus has been isolated from a body fluid obviously is superfluous. However, collected serum can be useful diagnostically if the prevalence of specific EVs in a community is known. In this situation, looking for antibody titer changes to a selected number of viral types is relatively easy. Faster diagnosis can be made with a single serum sample if a search for specific IgM enteroviral antibody is made.[144,188,330,348,349,360,878,944,1038]

Unfortunately, no EV IgM antibody tests are commercially available in the United States. Commercial laboratories do offer enteroviral CF antibody panels. However, these tests are expensive, and their results in the clinical setting almost always are meaningless unless acute-phase and convalescent-phase sera are analyzed.

Histology

Enteroviral infections have no specific histologic findings such as those seen in cytomegalovirus or HSV infection. However, tissues can be examined for specific enteroviral antigens by immunofluorescence and for RNA by PCR.[108,142,630]

DIFFERENTIAL DIAGNOSIS

The differential diagnosis of enteroviral infection depends on the clinical manifestations. In general, the most important considerations relate to bacterial diseases such as those commonly associated with pharyngitis, pneumonia, pericarditis, meningitis, and septicemia. Other viruses must be considered with upper respiratory illnesses, gastrointestinal infections, rashes, encephalitis, and neonatal illness.

Paralytic poliomyelitis usually presents no diagnostic problem in the presence of an outbreak, but sporadic cases are another matter, especially in countries such as the United States, where the disease (except for the vaccine-associated form) has disappeared and many pediatricians have never seen a case. Rarely, other EVs have been shown to cause paralytic syndromes that are indistinguishable from poliomyelitis.

Several other diseases must be considered in the differential diagnosis of sporadic cases of paralytic illness. Guillain-Barré syndrome is the most common and difficult differential diagnostic problem. Fever, headache, and meningeal signs usually occur less commonly in Guillain-Barré syndrome; the paralysis characteristically is symmetric, and sensory changes are common findings. Also in Guillain-Barré syndrome, the CSF contains few cells but a significant elevation in protein concentration. Other illnesses confused with paralytic poliomyelitis include peripheral neuritis (post-injection, toxic, herpes zoster), arboviral infection, rabies, tetanus, botulism, and tick paralysis.

TREATMENT

SPECIFIC THERAPY

No specific therapy for any enteroviral infection is approved for use in the United States. In severe, catastrophic, and generalized neonatal infection, in all probability the baby received no specific antibody for the particular virus from the mother. In this situation, administering immunoglobulin to the infant probably is advisable because high titers of neutralizing antibody to many EVs frequently are present in immunoglobulin.[194,228] Little evidence supports the claim that this therapy is beneficial; however, it can be expected to stop further organ seeding secondary to continued viremia. Intravenous or intraventricular injection of immunoglobulin with IVIG and administration of hyperimmune plasma have been useful on some occasions and not others in the treatment of enteroviral infection in patients with agammaglobulinemia.*

Abzug and associates[5] performed a small but controlled study in which nine EV-infected neonates received IVIG and seven similarly infected infants received supportive care. In this study, no significant difference was found in clinical scores, antibody values, or the magnitude of viremia and viruria in those treated versus control infants. However, five infants received IVIG with a high neutralizing antibody titer (>1:800) to their individual viral isolates, and they experienced faster cessation of viremia and viruria. A neonate with disseminated echovirus 11 infection and hepatitis, pneumonitis, meningitis, DIC, decreased renal function, and anemia who survived after receiving a large dose of IVIG and supportive care was described.[489] Administering IVIG to older children with life-threatening enteroviral infection also seems reasonable. A specific recommended dosage is unknown, but 400 mg/kg/day for 4 days or 2 g/kg in one dose has been used.

Jantausch and associates[474] reported an infant with a disseminated echovirus 11 infection who survived after receiving maternal plasma transfusions. A neonate with fulminant echovirus 11 infection survived after undergoing orthotopic liver transplantation.[200]

The antiviral drug pleconaril offers promise for the treatment of enteroviral infections.[57,104,247,511,766,826,829,984] This drug is a novel compound that integrates into the capsid of EVs. It prevents the virus from attachment to cellular receptors and the uncoating and subsequent release of viral RNA into the host cell. In a double-blinded, placebo-controlled study of 39 patients with enteroviral meningitis, a statistically significant shortening of duration of disease was noted: from 9.5 days in controls to 4.0 days in drug recipients.[829] Pleconaril also has been used on a compassionate-release basis for the treatment of patients with life-threatening infection.[57,104,826] The following categories of enteroviral illness have been treated: chronic meningoencephalitis in patients with agammaglobulinemia or hypogammaglobulinemia, neonatal sepsis, myocarditis, poliomyelitis (wild type or vaccine associated), and encephalitis, as well as in bone marrow transplant recipients. Although these treatment studies have not had control arms, favorable clinical responses have been observed in 26 of 50 treated patients, including 12 of 18 patients with chronic meningoencephalitis. Pleconaril is not available in the United States.

In severe illnesses such as neonatal myocarditis or encephalitis, one frequently is tempted to administer corticosteroids. Although some workers have thought that this therapy is beneficial in treating coxsackieviral myocarditis, we recommend that corticosteroids should not be given during acute enteroviral infection. The deleterious effects of these agents in coxsackieviral

*See references 8, 87, 220, 264, 278, 323, 385, 414, 642, 653, 657, 957, 987.

infection of mice are particularly persuasive factors in this opinion.[89,531] Immunosuppressive therapy for myocarditis of unknown origin with prednisone and cyclosporine or azathioprine was evaluated in a controlled trial of 111 adults, but no beneficial effect was observed.[633]

Because the possibility of bacterial sepsis cannot be ruled out in many instances of enteroviral infection, antibiotics frequently should be administered for the most likely potential pathogens. Care in the selection and administration of antibiotics is urged so that drug toxicity is not added to the problems of the patient.

NONSPECIFIC THERAPY

Mild, Nonspecific Febrile Illness

In patients in whom fever is the only symptom, careful observation is important. Many patients who eventually become severely ill initially have 2 to 3 days of fever without other localized findings. Care should be taken to administer adequate fluids to febrile infants, and excessive elevation of temperature should be prevented if possible.

Myocarditis

Myocarditis has no specific therapy. However, congestive heart failure and arrhythmias occur, and they should be treated by the usual methods. In administering digitalis to patients with enteroviral myocarditis, paying careful attention to the initial dosage is most important because the heart often is extremely sensitive; frequently, only small amounts of digoxin are necessary.

Meningoencephalitis

In patients with meningoencephalitis, convulsions, cerebral edema, and disturbances in fluid and electrolyte balance all occur frequently and respond to treatment. Seizures are treated best with phenobarbital, phenytoin, or lorazepam. Cerebral edema can be treated with urea, mannitol, or large doses of corticosteroids. As noted, the use of corticosteroids in patients with active enteroviral infection seems unwise because the local benefit may be outweighed by the overall deleterious effects. Fluids should be monitored closely, and serum electrolyte levels should be determined frequently because inappropriate antidiuretic hormone secretion is a common occurrence.

Poliomyelitis

The broad principles of management are to allay fear, to minimize the ensuing skeletal deformities, to anticipate and meet complications in addition to the neuromusculoskeletal ones, and to prepare the child and family for the prolonged treatment that may be required and for permanent disability when it seems likely. Patients with the nonparalytic and mildly paralytic forms of the disease may be treated at home.

Most patients with paralytic poliomyelitis require hospitalization. A calm atmosphere is desired. Suitable body alignment is necessary to avoid excessive skeletal deformity. A neutral position with the feet at a right angle, knees slightly flexed, and hips and spine straight is achieved by the use of boards, sandbags, and occasionally, light splint shells. Active and passive motion is indicated as soon as the pain has disappeared. The orthopedist and physiatrist should see these patients as early in the illness as possible and should assume responsibility before fixed deformities develop.

Management of pure bulbar poliomyelitis consists essentially of maintaining the airway and avoiding all risks of inhalation of saliva, food, or vomitus. Gravity drainage of accumulated secretions is favored by the head-low (foot of the bed elevated 20 to 25 degrees), prone position with the face to one side. Aspirators with rigid or semirigid tips are preferred for direct oral and pharyngeal use, and soft flexible catheters may be used for nasopharyngeal aspiration. Fluid and electrolyte balance is maintained best by intravenous infusion because tube or oral feeding in the first few days may incite vomiting. After the initial few days, sips of sterile water may be given from a spoon, with increments as indicated by the child's ability to swallow. In addition to close observation for respiratory insufficiency, blood pressure should be recorded at least twice daily. Hypertension is not an uncommon occurrence and occasionally leads to hypertensive encephalopathy. Patients with pure bulbar poliomyelitis may require tracheostomy because of paralysis of the vocal cords or constriction of the hypopharynx. Most patients with pure bulbar poliomyelitis who recover have little residual impairment; some patients exhibit mild dysphagia and occasional vocal fatigue with slurring of speech.

Impaired ventilation must be recognized early; mounting anxiety, restlessness, and fatigue are early indications for prompt intervention. Tracheostomy is indicated for some patients with pure bulbar poliomyelitis, spinal respiratory muscle paralysis, and bulbospinal paralysis. Unlike other patients on whom tracheostomy is performed, these patients generally are unable to cough, sometimes for many months. Frequent and swift endotracheal aspiration under aseptic conditions is necessary. Mechanical ventilation often is needed. Patients are fully conscious and aware; terrifying procedures are performed best with an outward atmosphere of calm. Explaining the procedure and having the parents on hand may be helpful. A reduction in thoracic compliance occurs early, and higher than expected pressure gradients may be required to achieve adequate ventilation. Weaning a patient from dependency on respiratory assistance is a torturous process, as is total musculoskeletal rehabilitation. Motivation of the patient and the team of personnel is paramount.

PROGNOSIS

The prognosis for nonpolio enteroviral infections is excellent in most instances. Virtually all morbidity and mortality are related to cardiac and neurologic disease in older children and to these diseases in addition to general disseminated infection with hepatitis in neonates.

The prognosis for poliomyelitis varies with the degree of muscle involvement. In patients with mild muscle weakness, complete recovery is the rule. If paralysis is present, recovery of muscle function continues for a period of approximately 18 months to 2 years. By 3 months, approximately 60 percent of the ultimate improvement has been achieved, and by 6 months, 80 percent. The final result depends on the extent and localization of nerve cell damage.

Respiratory failure is responsible for most of the deaths in paralytic poliomyelitis. With the many improvements in techniques for handling this complication, the overall mortality rate has been reduced to approximately 4 percent; with the bulbar form and in adults, it still may be as high as 10 percent.

Occasionally, new neuromuscular symptoms develop later in life in patients who have had paralytic poliomyelitis.[121,230,231,485,791,930,1007,1008] Although the cause of this late-onset weakness and muscle atrophy (post-polio syndrome) is not understood completely, it most likely is the result of routine attrition of anterior horn cells associated with aging rather than persistent neural infection with polioviruses. However, specific immunopathologic mechanisms possibly play a role in some instances.[345]

Leparc-Goffart and associates[580] presented data suggesting the presence of poliovirus-specific genomic sequences in the CSF of

patients with post-polio syndrome. However, Muir and colleagues,[696] who performed similar studies, found no association of chronic neurologic disease with the presence of enteroviral RNA in CSF.

PREVENTION

NONPOLIO ENTEROVIRAL INFECTIONS

Attenuated and inactivated viral vaccines for EVs other than polioviruses are not available. However, if a virulent enteroviral type were to emerge, a specific vaccine for active immunization probably could be developed.

Passive protection with IVIG may be useful in preventing disease. In practice, however, it would seem worthwhile only for sudden and virulent nursery outbreaks. For example, if several cases of myocarditis occurred in a nursery, administering IVIG to all babies in the nursery would seem reasonable. Pooled human immunoglobulin in most instances can be expected to contain antibodies against coxsackieviruses B1, B2, B3, B4, and B5, as well as several coxsackieviruses A and echoviruses.[228] Therefore, this procedure would offer protection to infants without transplacentally acquired specific antibody who had not become infected yet. Immune serum was useful in the management of three nursery enteroviral outbreaks.[119,701,759]

POLIOVIRAL INFECTIONS

In the United States, the total annual number of paralytic cases fell from an average of 16,000 in the 5 years before the introduction of vaccine to approximately 10 cases per year between 1980 and 1984. The experience in 1979, however, when 26 paralytic cases were reported, served as a reminder that virulent polioviruses still could surface in susceptible persons.[125,126] Most of the 1979 cases occurred in Pennsylvania and several other states in Amish population groups who had not been immunized. A similar epidemic in Connecticut in 1972 involved a pocket of unimmunized students in a Christian Science school.[122] These outbreaks reflected the reality that poliomyelitis was still occurring in many parts of the world. The possibility of the introduction of virulent strains was ever present, and only through continued and extensive immunization programs could the disease be prevented from reappearing in epidemic form.[431]

The remarkable overall record of the decline in paralytic poliomyelitis in the United States was a result of the development and use of two effective vaccines.[431] Inactivated poliovirus vaccine, the first to be licensed, was used extensively beginning in 1955 and considerably reduced the incidence of the disease, although epidemics continued to occur. Live-attenuated oral poliovirus vaccine, licensed in 1961 and 1962, subsequently was recommended as the method of choice in the United States based on its superiority in terms of immunogenic capacity, ability to induce local IgA antibody in the oropharynx and intestinal tract and thus provide greater resistance to re-infection, and ease of administration. Oral poliovirus vaccine gradually supplanted inactivated poliovirus vaccine, and between 1973 and 1978, it was the only vaccine available. Its extraordinary effectiveness at that time, despite reaching only 65 percent of children younger than 5 years of age with the recommended three doses, suggested that the capacity of the attenuated strains to spread contributed to a much higher immunization rate than was indicated by vaccination statistics. The potential impact of such spread on the immunity of the population was illustrated by the observations of Fox and Hall,[306] who conducted long-term virologic surveillance of middle-income families in Seattle. Polioviruses (vaccine strains) accounted for 50 percent of the 2937 viral isolates from healthy children, their parents, and others in the community. In an analysis of 611 of the poliovirus isolates, researchers found that 75.6 percent were from vaccinees, 10.5 percent were from vaccinee contacts, and 14 percent were from persons without recent known contact with vaccine or a vaccinee. These findings provided a vivid picture of the pervasiveness of the attenuated strains and their continuous circulation in the population. This feature also was supported by the almost invariable recovery of polioviruses from weekly samples of sewage that were collected throughout the year, in the early vaccine era, in urban communities.[432]

Despite its striking success, the oral vaccine had some problems. One was greatly reduced seroconversion rates when the vaccine was given to children living in the tropics: as few as 50 percent had satisfactory responses, in contrast to the more than 95 percent response rate in the United States and similar countries.[252,661] Viral interference from other EVs played some role, and the presence of an inhibitory substance in the oropharynx that prevented significant multiplication of the vaccine strains also was involved. The seroconversion problem was lessened by using pulse immunization programs.[480,838] For example, the strategy of national annual vaccination days twice a year, 2 months apart, has been successful in developing countries.

Another problem with oral poliovirus vaccine—and the major problem in the United States—was the occurrence of a small number of vaccine-associated cases of poliomyelitis.[431,661] The immunogenic effectiveness of the vaccine depends on multiplication of the attenuated strains in the intestinal tract. Because no poliovirus strain is completely stable, the progeny of vaccine strains underwent a certain degree of mutation, which rarely resulted in increased virulence and vaccine-associated cases in recipients and their contacts, most often their parents.

Since 1980, no indigenous cases of wild poliovirus disease have occurred in the United States.[132,904] From 1980 to 1989, 80 cases of vaccine-associated paralytic poliomyelitis and 5 cases of imported disease were reported. The overall rate of vaccine-associated paralytic poliomyelitis was 1 case per 2.5 million doses of distributed vaccine; the risk for recipients was 1 case per 6.8 million doses, and for household contacts it was 1 case per 6.4 million doses. Of the 80 cases, 30 occurred in vaccinees, 32 occurred in household contacts, 4 were community acquired, and 14 occurred in immunologically abnormal persons.

Further analysis revealed that the risk associated with the first dose of vaccine was 1 case per 700,000 doses, but it was only 1 case per 6.9 million for subsequent doses; for vaccine recipients, the calculated risks were 1 case per 1.4 million initial doses and 1 case per 41.5 million subsequent doses. The calculated risks for contact cases were 1 case per 1.9 million initial doses of vaccine and 1 case per 13.8 million subsequent doses.

Immunodeficient children are at particular risk of acquiring vaccine-associated paralytic poliomyelitis.[123,904] From 1969 through 1976, 11 percent of vaccine-associated cases occurred in immunodeficient patients; 10 of 11 of these patients were children younger than 1 year of age. From 1980 to 1989, 18 percent of vaccine-associated paralytic poliomyelitis cases occurred in immunodeficient patients.[904]

Although the risks mentioned earlier were considered acceptable in view of the benefits provided, the question raised repeatedly since the 1970s was whether the United States should return to the use of inactivated poliovirus vaccine, which does not carry a risk of acquiring paralytic disease and has been highly successful in several small European countries in which more than 95 percent of the population is immunized.[305,320,422,493,508,699,755,841-844,904]

The problem was reviewed in detail by a committee of the Institute of Medicine (IOM) of the National Academy of Sciences, which reported its recommendations in April of 1977.[722] The conclusion at the time was that given the situation in the

United States, in which not more than 65 percent of susceptible children were vaccinated, oral poliovirus vaccine should continue to be the principal vaccine for routine immunization. Inactivated poliovirus vaccine, conversely, should be provided for two groups: immunodeficient persons, because of their greatly enhanced risk of acquiring vaccine-associated disease after receiving the oral vaccine; and adults receiving primary immunization, because of their greater susceptibility to paralytic disease. Also suggested was that the inactivated vaccine be available as an alternative for those who prefer it. In addition, a single dose of trivalent oral poliovirus vaccine was suggested for all entrants into the seventh grade of school as a means of added protection for later years when they became parents.

In January of 1988, a panel appointed by the IOM again reviewed policy options for vaccination against poliomyelitis in the United States.[459] The IOM panel concluded that no change in policy should be recommended at that time. However, they did recommend that a new enhanced inactivated poliovirus vaccine replace the old vaccine when inactivated vaccine was indicated. They also suggested that when a new enhanced diphtheria-tetanus-pertussis inactivated poliovirus vaccine became available, a regimen of two or more doses of it followed by the oral vaccine be considered.

In 1996, the U.S. poliovirus vaccine immunization program was evaluated extensively by both the Advisory Committee on Immunization Practices (ACIP) and the Committee on Infectious Diseases of the American Academy of Pediatrics (AAP). The ACIP recommended that sequential administration of inactivated poliovirus vaccine followed by oral poliovirus vaccine be the schedule of choice in the United States.[746] The schedule consisted of two doses of the inactivated vaccine at 2 and 4 months of age and two doses of the oral vaccine at 12 to 18 months and 4 to 6 years of age. Both committees indicated that schedules that include all the doses of each vaccine also were acceptable.[393,746] At the present time, an all-inactivated poliovirus vaccination schedule is recommended in the United States.[17,669] It is a four-dose schedule with doses at 2 and 4 months, 6 to 18 months, and 4 to 6 years. The reader is advised to consult the recommendations of the ACIP and the AAP, as well as the manufacturers' literature, for full consideration of the contraindications and indications for poliovirus vaccines.

Routine primary poliovirus vaccination of adults older than 18 years is not conducted in the United States. However, adults at risk of exposure to wild polioviruses (laboratory workers, international travelers, health care workers) should be immunized. For the vaccination of adults, inactivated poliovirus vaccine is recommended. Patients with immunodeficiency diseases should not be given oral poliovirus vaccine; live virus also should not be used in households in which an immunodeficient person resides.

GLOBAL ERADICATION OF POLIOMYELITIS

The WHO established the Expanded Program on Immunization (EPI) in 1974.[451,979,1019] Subsequently, the use of oral poliovirus vaccine in developing countries vastly increased. In 1980 in Brazil, researchers demonstrated that mass administration with the oral vaccine on National Immunization Days (NIDs) led to a dramatic reduction in the incidence of poliomyelitis.[243] This demonstration led in 1985 to the targeted eradication of poliomyelitis from the Western Hemisphere by 1990. This campaign was successful in that the last confirmed case of paralytic poliomyelitis caused by wild poliovirus occurred in 1991 in Peru.[126] In September 1994, an international commission convened by the Pan American Health Organization certified that indigenous transmission of wild poliovirus had been interrupted in the Americas.[131]

In 1988, the World Health Assembly established the objective of global eradication of polio by 2000.[451] This program was based on four strategies recommended by the WHO: (1) maintenance of high vaccination coverage levels among children with at least three doses of oral poliovirus vaccine; (2) development of sensitive systems of epidemiologic and laboratory surveillance, including use of the standard WHO case definition (a confirmed case of poliomyelitis is defined as acute flaccid paralysis and at least one of the following: laboratory-confirmed wild poliovirus infection, residual paralysis of 60 days, death, or no follow-up investigation at 60 days); (3) administration of supplementary doses of oral poliovirus vaccine to all young children (usually those <5 years old) during NIDs to interrupt rapidly the transmission of poliovirus; and (4) "mopping up" vaccination campaigns, which are localized campaigns targeted at high-risk areas where wild poliovirus transmission was most likely to persist at low levels. NIDs are mass campaigns conducted over the course of a short period (days to weeks) during which two doses of oral poliovirus vaccine are administered to all children in the target age group regardless of previous vaccination history, with an interval of 4 to 6 weeks between doses.

From 1985 through 1990, worldwide routine vaccination coverage levels increased from 47 to 85 percent and stabilized at 80 to 81 percent from 1991 to 1994.[132] From 1985 through 1994, the number of cases reported annually decreased 84 percent, from 39,361 to 6241. The number of countries reporting polio cases decreased steadily from 1985 (99 of 196 [51%]) to 1988 (88 of 196 [45%]) and 1994 (51 of 214 [24%]). In addition, the number of countries reporting zero polio cases increased from 1985 (84 [43%]) to 1988 (104 [53%]) and 1994 (145 [68%]). The number of countries with endemic polio that conducted NIDs each year increased from 15 in 1988 to 37 in April of 1995; 24 additional countries scheduled their first NIDs for later in 1995.

At the beginning of the 21st century, NIDs had been conducted in every country in the world with endemic polio. In 1999, 7141 cases of poliomyelitis were reported worldwide. These cases occurred in 30 countries, mainly in south Asia and central Africa. By the end of 2000, fewer than 3500 cases occurred in endemic areas throughout the world, and wild-type 2 poliovirus has not been detected since October 1999.[924] At the close of 2001, the area of endemic poliomyelitis had been reduced to 10 countries, with fewer than 1000 cases reported.[711]

The global eradication program suffered a major setback in 2004 when oral poliovirus vaccine immunizations in northern Nigeria were suspended because of misinformation regarding vaccine safety.[415] Following this suspension, epidemic disease caused by type 1 virus occurred throughout Nigeria and also spread directly or indirectly to 21 previously polio-free countries.[140] This epidemic, which had extensive spread, had a major negative impact on the Global Polio Eradication Initiative. During 2005, 1856 cases were reported globally, and 1000 of these cases occurred in countries with outbreaks caused by importation.

Much attention is being paid to the end-game strategy for global polio eradication.[254,255,519] Of particular concern for the future following world eradication of wild poliovirus strains is vaccine-associated paralytic poliomyelitis.[521,522] Other concerns are the long-term excretion of highly evolved vaccine-derived polioviruses in persons with primary immunodeficiencies and polio outbreaks associated with circulating vaccine-derived polioviruses in areas with low rates of oral poliovirus vaccine coverage. Ideally, an end-game strategy would transition to the use of inactivated poliovirus vaccine, but this strategy is not realistic in much of the developing world.

Despite the substantial progress that has been made toward global eradication of polio, several challenges remain, including the following: (1) increasing vaccination levels in unvaccinated

subpopulations; (2) preventing the re-introduction of wild poliovirus into polio-free areas by eliminating reservoirs in polio-endemic countries (particularly in the Indian subcontinent); (3) increasing the awareness of donor agencies and governments in industrialized countries of the substantial financial and humanitarian benefits of global eradication of polio, thus engendering support from unaffected countries beyond that already provided by organizations such as Rotary International; (4) encouraging all countries that remain polio endemic to make eradication of polio a high priority, including the implementation of NIDs and the initiation of acute flaccid paralysis surveillance; and (5) providing support to vaccination program managers for training to develop managerial skills for implementing and maintaining effective vaccination and surveillance programs in all countries.[132] The success of the Polio Eradication Initiative will depend on finding solutions to these financial, managerial, political, and technical challenges.

The latest goal for global eradication of poliomyelitis is the year 2008. World events and new knowledge relating to reversion of poliovirus vaccine strains and recombination of poliovirus vaccine strains with nonpolio EVs led to the realization that decisions related to discontinuation of immunization after world eradication are exceedingly complex.[520,711,924,1012]

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CHAPTER 179

RHINOVIRUSES

Robert L. Atmar

HISTORY

In 1954, the first recognized human rhinovirus (HRV) (type 1A) was isolated in monkey kidney cell culture by Pelon and associates[226] from a recruit at Great Lakes Naval Training Center in Chicago during an outbreak of afebrile common colds in his training company. Independently, Price and colleagues[234] reported the isolation of an antigenically identical virus from nurses and children with colds. In 1963, the HRVs were so named because of their association with illnesses of the nasal passages.[282]

Before these actual virus isolations, evidence suggested that viruses cause the common cold. Despite indications that some coldlike illnesses could be complicated by bacterial infections,[77,292] bacteria-free nasal filtrates from persons with apparent symptomatic respiratory infections clearly were able to initiate these illnesses. Kruse,[179] in 1914, first demonstrated transmission with apparently sterile filtrates, and, in the late 1920s, his results were confirmed in a series of experiments in humans and chimpanzees by Dochez and associates.[77] In the 1930s, workers from this latter laboratory reported growth of the agent in tissue culture and embryonated eggs, but their results were not confirmed.[6] In the 1940s and 1950s, Andrewes and colleagues[7] at the Common Cold Unit in Salisbury, England, and Dingle and the members of the U.S. Armed Forces Commission on Acute Respiratory Diseases were able to transmit colds from person to person with apparently sterile filtrates.[54] Jackson and colleagues[158] performed similar experiments in Chicago and also demonstrated immunity. In 1953, the Salisbury researchers reported isolation of an agent, DC, in serially passaged filtrates of tubed tissue cultures of human embryonic lungs; the virus was detected by its ability to produce colds in humans. Although these results could not be substantiated at the time, a virus *was* present; in 1968, the DC filtrates were found to contain HRV-9.[6,56]

The next major advance in growing common cold viruses took place in the late 1950s in Salisbury. Tyrrell and Parsons[283] inoculated human embryonic kidney cells with nasal filtrates and incubated the cultures under conditions simulating those of the nasal passages (e.g., 33° C, neutral pH, and in a roller drum for aeration). Six distinct types of HRVs (types 1B to 6)[61] were isolated by observing cytopathic effects.[270] Shortly thereafter, Hamparian and coworkers[61,130,173] isolated 18 HRVs (types 7 to 12 and 18 to 29) by using a semi-continuous diploid cell strain obtained by Hayflick and Moorhead[138] from human fetal lung cultures. The

use of Hayflick's cell lines greatly accelerated the isolation and characterization of "new" HRVs, and 100 serotypes (or 101 if HRV-1A and HRV-1B are counted as 2 serologic entities) officially have been identified.[129] More remain untyped, but the number of additional serotypes seems limited.[129,170,213]

Although the HRVs are associated primarily with mild upper respiratory tract disease, they also may be involved in bronchitis, sinusitis, and, on occasion, pneumonia in all age groups. These viruses seem to be precipitants of "infectious asthma" attacks. HRVs cause approximately 30 to 50 percent of all acute respiratory illnesses.[61,97]

THE ORGANISM

GENERAL DESCRIPTION

Rhinovirus is one of five genera of human pathogens in the family *Picornaviridae*.[91] *Enterovirus* includes polioviruses, coxsackieviruses, and echoviruses; *Parechovirus* includes two serotypes formerly identified as echovirus types 22 and 23; Aichi virus is the single member of *Kobuvirus*; and hepatitis A virus is the single member of *Hepatovirus*. Like the other picornaviruses, the HRVs are small (30 nm), nonenveloped (therefore resistant to lipid solvents such as ether and chloroform), and icosahedral (20-sided, hexagonal in cross section), with a genome consisting of single-stranded RNA (molecular weight, 2.5×10^6) approximately 7200 nucleotides long.[91] A picornavirus can be thought of as an RNA genome surrounded by a 12-sided protein coat (the capsid) (Fig. 179–1). The RNA alone is infectious and can serve as a messenger.

HRVs are distinguished from enteroviruses by being rendered noninfectious at an acidity lower than pH 5 and by their higher buoyant density in cesium chloride.[120] HRVs can be subdivided based on various virus characteristics, as follows: (1) into three species (HRV A, HRV B, and HRV C) based on genomic sequence analysis; (2) into three groups based on receptor usage (see later); (3) into two groups based on susceptibility to capsid-binding antiviral agents; and (4) and into 100 or more serotypes, as described earlier.[254]

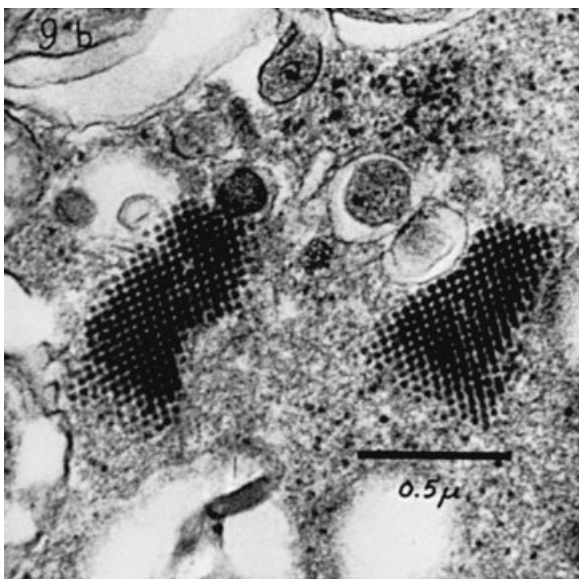

Figure 179–1 Rhinovirus type 2 in human fetal lung cells. Note the hexagonal virus crystals closely packed in a rectangular lattice (×40,000). *(From Kawana, R., and Matsumoto, I.: Electron microscopic study of rhinovirus replication in human fetal lung cells. Jpn. J. Microbiol. 15:207-217, 1971.)*

The genomes of several HRVs, including HRV-1B, HRV-2, HRV-14, HRV-16, HRV-39, and HRV-89, have been sequenced completely.[87,152,183,259,264] Partial sequences are available for the remaining recognized serotypes. Within an HRV species, a 61 to 98 percent pairwise nucleotide sequence identity of the VP1 gene exists among the different serotypes, whereas between HRV serotypes, the pairwise nucleotide sequence identity for this gene is 46 to 55 percent.[180] HRV-87 has greater sequence homology to the *Enterovirus D* species (EV-68 and EV-70) than to other HRVs, and some clinical isolates identified serologically as EV-68 lose infectivity in acid (a finding suggesting that these viruses should be classified as HRVs).[27,218] The interrelationships of viruses in these two genera will become clearer as more sequence data are obtained.

STRUCTURE OF THE VIRION

The virion is composed of 60 copies of each of 4 structural proteins: VP1, VP2, VP3 and VP4. Knowledge of the structure of the HRVs is based on the determination of the capsid structures in atomic detail for 5 serotypes, HRV-1A, HRV-2, HRV-3, HRV-14, and HRV-16.[10,175,246,247,288,303] The thin (5-nm) protein capsid has an undulating exterior marked by 12 vertices (the icosahedral fivefold axis) equally spaced over the surface. Surrounding these vertices is a steep (2.5-nm deep), narrow canyon. VP1, VP2, and VP3 are exposed on the surface of the virion, whereas VP4 is buried within the capsid.

A pocket of unknown function whose walls are lined by 17 amino acids that vary with each HRV type has been found at the base of the canyon.[8] Various organic molecules have been determined to bind in this pocket; when these molecules are bound, either the virus is prevented from uncoating (necessary to release viral RNA for its translation to viral protein) or the cell receptor is prevented from docking in the canyon.[98,229] Numerous drugs made by several different drug companies in the United States, Europe, and Japan have been developed to exploit the antipicornavirus potentialities of this pocket, and the sensitivity of the HRVs to these drugs has been used as an additional means of classifying the HRVs (into inhibitor groups A and B).[8,9] One group of these drugs, the WIN compounds synthesized by Sterling-Winthrop, has had wide scientific exposure; however, clinical trials have been discouraging.[272,275] One disadvantage of these compounds is that the various HRV serotypes vary widely in sensitivity to these organic molecules.[8] Another problem has been failure to attain effective concentrations of drug at the site of infection.

VIRUS LIFE CYCLE

The first step in the virus life cycle is binding to its cellular receptor. Only 3 different receptor binding sites exist for the 101 recognized HRV serotypes: 90 serotypes bind to intercellular adhesion molecule-1 (ICAM-1, the major receptor group), 10 other serotypes bind to members of the low-density lipoprotein receptor family (LDL-R, the minor receptor group), and 1 serotype (HRV-87) binds to a sialoprotein that may be a decay-accelerating factor (DAF, the recognized receptor for EV-70).[1,27,113,285]

ICAM-1 binds to the base of the canyon of HRVs in the major receptor group.[246,248,285] Virus-neutralizing antibody prevents virus attachment to cells by adhering to serospecific sites and blocking the cell receptor from access to its binding site at the canyon base. These antibody sites may directly overlap ICAM-1–binding sites, or they may be separate from the ICAM-1–binding sites but at a point sufficiently close to allow the binding site to be blocked by steric hindrance.[42,261] Binding of the major

receptor group HRVs to ICAM-1 leads to destabilization of the virus capsid and uncoating of the viral RNA.[114]

The LDL-R family binds to a different site for the minor receptor group of HRVs than that recognized by ICAM-1. The binding site for these HRVs is the small, star-shaped dome above the canyon on the icosahedral fivefold axis. Attachment of minor receptor group viruses to LDL-R does not lead to virus uncoating, in contrast to what is seen with binding of major receptor group HRVs to ICAM-1. Instead, internalization into acidic endosomal compartments is required for uncoating of minor receptor group HRVs.[145]

Replication of the HRVs is similar to that of the enteroviruses.[240] After attachment to the cellular receptor and endocytosis of the virus, RNA is released into the cytoplasm. Genomic viral RNA functions as messenger RNA and attaches to ribosomes, which stimulates its translation by host enzymes into a single long polyprotein. This polyprotein is cleaved by viral proteases to yield viral RNA polymerase, capsid proteins, proteases, and proteins to halt the synthesis of host proteins and other products. Under laboratory conditions, infectious virus first is formed after approximately 2 hours and reaches a maximum of approximately 1000 infectious particles per cell at approximately 7 hours. Infectious virus, however, comprises a minority of the virus-like particles formed; only approximately 1 in 200 is a complete virus capable of replicating in cell culture. All viral replication occurs in the cytoplasm, and viruses are released by cell lysis in a process involving apoptosis.[68,240]

HOST RANGE

Animals

Rhinoviruses have been isolated from natural infections in only cattle, chimpanzees, and humans.[91,192] Just two bovine rhinovirus serotypes have been reported, and they may cause infections ranging from subclinical to overt respiratory disease in epizootics; no evidence of human infection has been reported.[245] Chimpanzees and humans both are infected with HRVs; however, chimpanzee infections are subclinical.[71,258] A natural, subclinical outbreak of HRV-31 in chimpanzees was reported in a primate center.[70,71]

Many attempts have been made to infect a wide variety of animals with HRVs, but other than the chimpanzee, only the gibbon has been susceptible, and it is not reliably so.[71] An HRV-2 strain was adapted to grow in a mouse cell line (L cells), and the adapted virus was shown to grow in a mouse model.[301] Adaptation involves changes in the sequence of two nonstructural viral proteins that bind to intracellular host proteins to promote replication.[133] HRV-16, which uses ICAM-1 as its receptor, can replicate in mouse cells, but infection is blocked by the absence of ICAM-1 on the surface of mouse cells. The block can be overcome by engineering mouse cells to express ICAM-1, a finding that recently led to the development of a transgenic mouse model for HRV infection.[23,132,279] Equine "rhinoviruses" have been described, but these viruses belong to different picornavirus genera.[91,216]

Cell and Tissue Cultures

The spectrum of tissue culture cells infected by human HRVs also is narrow.[57,181] For initial isolation, human embryonic diploid cells generally are used (Fig. 179–2), although for some HRV types, an especially sensitive strain[55] of a continuous cell line, HeLa, serves as well or better.[57,181] Some HRVs grow only in human organ cultures and, perhaps, some only in living human beings.[181]

The first HRV (HRV-1A) was propagated in primary cell culture from primate kidneys (rhesus monkey cell cultures are used most commonly); surprisingly, chimpanzee kidney cell cultures do not propagate HRVs.[69] However, most HRVs propagate on original isolation only in cells of human origin, and they are called H strains; others also can be isolated in monkey kidney cell cultures and are called M strains. After laboratory propagation, all HRVs seem adaptable to the HRV-sensitive HeLa cell strain.[55] HeLa-grown HRVs attain much higher titers than those in the primary human diploid cells used for initial isolation.

ANTIGENIC PROPERTIES

An outstanding characteristic of HRV is its great antigenic diversity; at least 101 serotypes exist.[129] Evidence has shown, however, that the number of additional serotypes may be limited.[97,129,170,213] Certain serotypes cross-react, but little evidence indicates that this cross-reaction could be exploited in vaccination.[58,95,207]

HRVs often are poor antigens. Significant (fourfold or greater) increases in serum antibody may not develop in as many as 50 percent of patients from whom HRVs are isolated, and the levels attained often are low.[95-97]

HRVs may be undergoing continuous antigenic change.[207] Sufficiently marked antigenic variation has been found for HRV-22 and HRV-51 to interfere with their typing; however, this drift does not seem to predict a continuing proliferation of HRV types.[97,213,256,265]

EPIDEMIOLOGY AND GENERAL TRANSMISSION CONSIDERATIONS

SEASONAL DISTRIBUTION

During the usual September through May "cold season," HRV infections often are predominant at both ends, early fall and middle to late spring (Fig. 179–3). They also are important causes of summer colds. This seasonal pattern seems general because similar findings have been reported from families in Charlottesville, Virginia, Seattle, and elsewhere.[18,96,97,120,143,194] In the Southern Hemisphere, a similar seasonal pattern occurs, but during opposing months.[164] The general spring-summer-fall seasonal predominance of the HRVs does not mean that HRVs are absent during the remainder of the year; they are found in varying degrees year-round.[61,96,97,194,213,243]

CYCLING AND CIRCULATION OF INDIVIDUAL RHINOVIRUS TYPES

Several HRV serotypes usually circulate simultaneously within a community,[131] frequently coincident with other respiratory viruses.[72,149,209] For example, in a study of 24 neighboring families in Madison, Wisconsin (Eagle Heights Village), 14 different HRV types in addition to several nontypeable HRVs were found during the 2 academic years 1963 to 1965. Only a single type (HRV-15) was found both years. The other common respiratory viruses also were present. In virus surveillance studies performed in families in Seattle and Tecumseh, Michigan, a few "common" serotypes were found to be more prevalent in a given respiratory season and to be identified in subsequent respiratory seasons.[97,213] However, most individual HRV types do not repeat within a population from year to year.

CIRCULATION WITHIN SCHOOLROOMS

The mechanism of respiratory virus dissemination throughout the community may be the schoolroom or similar environments.

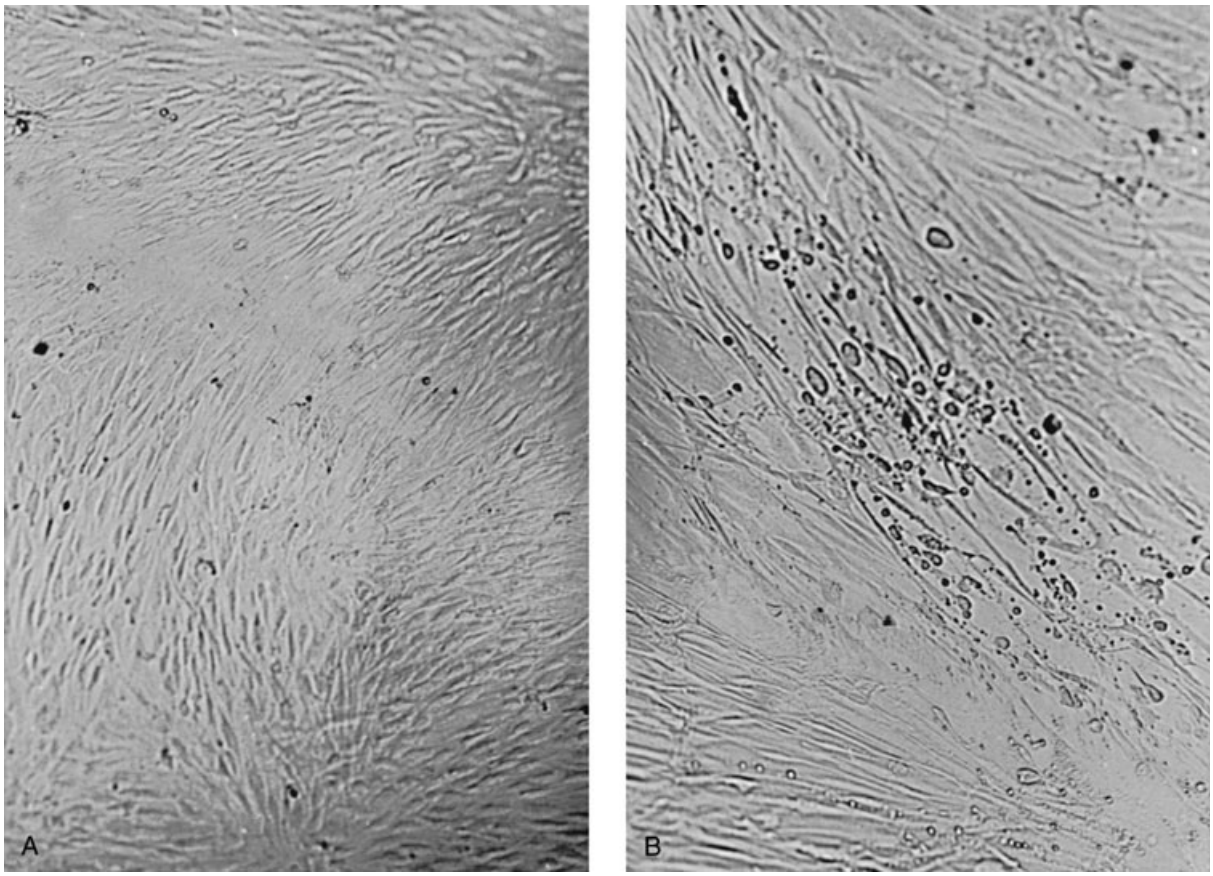

Figure 179–2 Rhinovirus type 16 infection. **A**, Normal human diploid (fetal tonsil) cell sheet. **B**, Cell sheet infected for 2 days with rhinovirus type 16. The rounded and misshapen cells are characteristic. *(Courtesy of Dr. David M. Warshauer, University of Wisconsin, Madison, WI.)*

In a Chicago nursery population, Beem[24] observed that some HRV types spread extensively and involved as many as 77 percent of the 22 children studied. He observed similar widespread infection with respiratory syncytial virus (RSV) but not with parainfluenza and influenza viruses. Similar results were observed in an upper middle-income Madison second-grade schoolroom (Fig. 179–4). The HRV types studied infected 18 to 55 percent of susceptible children; however, influenza B virus was the agent that circulated most widely.

Although several HRV types usually circulate concurrently, all types present are not equal in prevalence. In the aforementioned Chicago nursery school study,[24] 14 different serotypes were isolated during the academic year 1962 to 1963, but 10 of the serotypes did not spread at all. Only 3 types disseminated widely, and they infected more than 40 percent of the children. Similarly, in the 1963 to 1965 Madison Eagle Heights Village study, 14 HRV types were isolated, but only 3 types (HRV-43, HRV-51, and HRV-55) were "spreaders." Similar patterns of serologic prevalence were reported from laboratories in Tecumseh,[209] New York City, Seattle,[96,97] and Charlottesville.[143]

PREDOMINATING RHINOVIRUS TYPES

Although the pattern of predominating HRV types within a circumscribed population and a defined time frame seem to be well established, a predominance of serotypes over large geographic areas or over many years does not seem to occur. This phenomenon was studied exhaustively in widely separated locations and over many years: the Gulf South from 1962 to 1970, Tecumseh from 1966 to 1971 and from 1976 to 1981, and Seattle from 1965 to 1969 and from 1975 to 1979.[96,97,207,210,213] Although "common types" (usually the isolation of at least five to eight strains of a serotype during the period studied) occurred during each period and at each place, different types were "common" findings in different places and times. For example, at Tecumseh, Monto and colleagues,[210,213] during two 6-year study periods, obtained 475 HRV isolates covering 70 serotypes (out of a possible 89 at that time),[169] but only HRV-1B, HRV-10, HRV-28, and HRV-58 were isolated in both periods. However, these viruses were not particularly common because only 16, 22, 15, and 18 isolates, respectively, of these 4 types were found during the 12 years. In the neighboring state of Wisconsin from 1963 to 1965,[72] the common types were HRV-43, HRV-51, and HRV-55, completely different from those in Michigan. Finally, the late John Fox sifted data from family populations surveyed by him and others in New York City, Seattle, and Tecumseh.[96,97] He found only 4 common serotypes (HRV-1B, HRV-12, HRV-15, and HRV-38) from among 802 isolates. Dominant HRV serotypes often appear to occur locally over relatively short periods but do not extend for decades or over the nation, at least in the United States.

PERSON-TO-PERSON TRANSMISSION

EPIDEMIOLOGIC OBSERVATIONS

Individual HRV serotypes often disseminate with surprising difficulty, as noted previously in studies of Eagle Heights Village,[72]

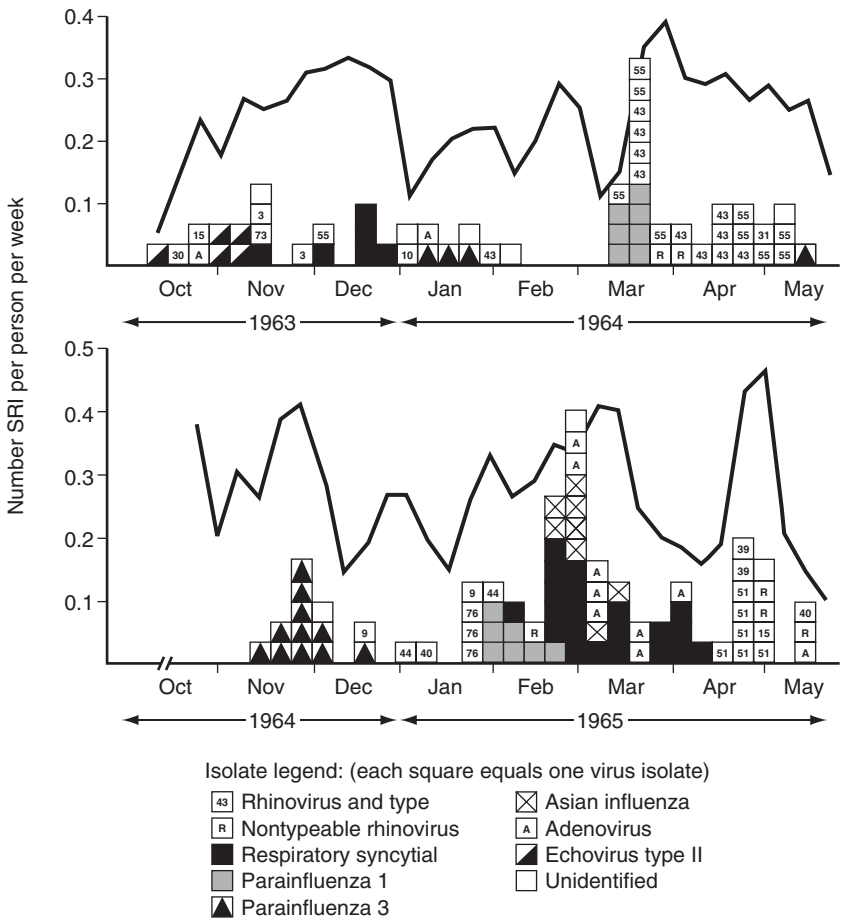

Figure 179–3 Rhinoviruses and other respiratory viruses associated with symptomatic respiratory infection. This study included three neighboring apartment buildings in Eagle Heights, a University of Wisconsin housing village in Madison, Wisconsin (24 to 26 families, ≈100 persons), from 1963 to 1965.

	Viral agent	Number of susceptible students*	Number infected (%)
1968	Nontypeable 'M' Rhinovirus	19	7 (37%)
	Rhinovirus 19	17	3 (18%)
	Rhinovirus 36	11	6 (55%)
	Parainfluenza 3	2	2 (100%)
	Respiratory syncytial	N.D.	5
1968–69	Rhinovirus 6	24	9 (37%)
	Influenza B	0	13
	Respiratory syncytial	3	8

*Number with neutralizing antibody titers ≤1:8 at the onset of study

Figure 179–4 Attack rates of several respiratory viruses within a Madison, Wisconsin, second-grade classroom of 26 students, three semesters, from 1968 to 1969.

a Madison elementary school (see Fig. 179-4), and a Chicago nursery school.[24] At least within family populations, the most common finding was that a specific HRV serotype did not spread from the index case. Hendley and associates,[143] in a study of 19 families in Charlottesville, found that HRVs from 10 of 22 index cases did not spread at all, and in only 7 families did dissemination to at least another person take place. In only 4 of the 19 families did further sequential spread occur. Fox and associates,[96,97] in a surveillance of more than 200 Seattle families, found intrafamily secondary attack rates in susceptible individuals (no homotypic antibody) to be 44 percent from 1965 to 1969 and 28 percent from 1975 to 1979. In both periods, children younger than 5 years had the highest secondary attack rate, 60 percent and 30 percent, respectively. Monto and Johnson and others[208,211] reported similar findings in 48 Panamanian families: with 5 HRV isolates used as antigens, the secondary attack rate varied from 10.5 to 56 percent.

In Madison, Wisconsin, interfamily and intrafamily dissemination of the 3 "spreading" HRV types, HRV-43, HRV-51, and HRV-55, was examined in the 24 to 26 neighboring families living in fourplex apartments in Eagle Heights Village (Fig. 179–5). These 3 serotypes were the only ones of 14 to spread beyond the index family. HRV-51 attacked 23 percent of susceptible individuals, and many family members and close neighbors remained uninfected. HRV-43 infected 34 percent of susceptible individuals; in one building, only the families in a single-end fourplex became infected. Conversely, HRV-55 caused a mini-

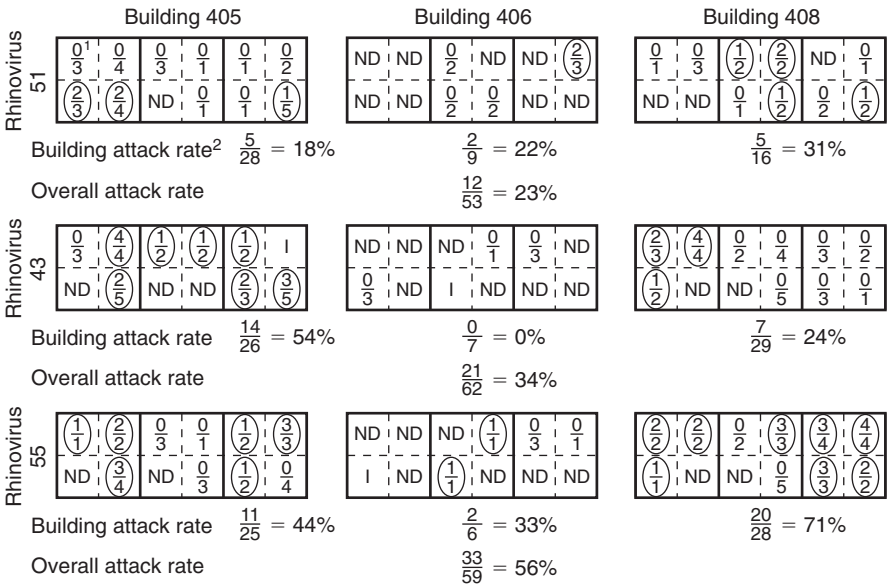

Figure 179–5 Attack rates of three "spreading" rhinoviruses in three neighboring Eagle Heights apartment buildings, Madison, Wisconsin. I, all members immune; ND, not done; O, family with one or more infected members.

[1]No. diagnosed cases/no. susceptibles (no detectable antibody)
[2]As diagnosed by either virus isolation and/or a fourfold or greater serologic response

epidemic in two buildings; several families had all members infected. HRV dissemination seemed to focus on the fourplex, probably because in the winter months the children played in the hallways and stairs connecting the fourplex apartments. Nonetheless, even the spreading HRVs left many susceptible individuals untouched among next-door fourplex neighbors and within many families.

The erratic spreading patterns of these viruses in Eagle Heights Village were perplexing. Determination of the reason for such erratic spreading patterns can be sought in well-controlled chain-of-infection experiments with human volunteers. HRV transmission experiments from several laboratories are described next.

PERSON-TO-PERSON TRANSMISSION TO HUMAN VOLUNTEERS

Experiments with Nasal Secretions from Persons with Colds of Unknown Origin

Experimentation with common colds in human volunteers began at England's Common Cold Unit in Salisbury shortly after World War II.[280,281] However, most of the transmission trials at this unit were performed with nasal secretions before discovery of most of the respiratory viruses, and the results are difficult to interpret (adenoviruses were cultivated first about 1953, followed by parainfluenza viruses, RSVs, HRVs [1956], and coronaviruses [1965]). Nonetheless, the general findings of these early experiments were correct; the usual common cold often is surprisingly difficult to transmit.[280]

Early Experiments with Rhinovirus Colds

Even in pure culture, the HRVs retained their uncertainty of transmission. Couch and colleagues,[59,63] trying to repeat a successful coxsackievirus A21 aerosol transmission experiment with HRV-15, attained no transmission after housing 15 donors (persons infected intranasally with laboratory-grown virus) with 12 recipients (persons without antibody to the donor's virus) for 26 days. At approximately the same time, the Madison laboratory performed transmission trials with HRV-55 and HRV-16 in a series of experiments ranging in duration from a 1- to 1.5-minute kiss to a 3-day weekend in a dormitory room. In 26 donors and 33 recipients, only 2 transmissions occurred, one from a 1.5-minute kiss and the other from a weekend in a dormitory.[64] Only when young married student couples were used was a substantial rate attained; among 24 couples, 9 donor spouses infected their mates, for a transmission rate of 38 percent.[65]

Median Human Infectious Dose for Rhinovirus

Judging from HRV challenge experiments with laboratory-grown virus administered by nosedrops or aerosol, less than one median tissue culture infective dose ($TCID_{50}$) can initiate infection.[64,78] Much more (2000 times) is required when placed on the tongue or dried just outside the anterior nares (10,000 times).[64] How accurately these conditions approach the natural state is not known.[141]

Characteristics of a "Good" Rhinovirus Transmitter

In the experiments with married couples, "successful" donors shed enough virus to contaminate their environment, exhibited signs and symptoms of a moderate to severe cold, and spent many hours at home with their spouses.[65] As an example of the effect of high virus shedding, intracouple transmission rates were 71 percent for donors with nasopharyngeal HRV titers of 5000 to more than 80,000 $TCID_{50}$, 33 percent for those with 1000 to 5000 $TCID_{50}$, and only 18 percent for those with less ($p = .025$). Illustrative of both shedding and production of sufficient nasal secretions was contamination of the hands with HRV: donors whose hands were assayed for virus and who transmitted infections to their spouses all had HRV on their hands, whereas none of the nontransmitters did ($p = .03$). The amount of time spent together seemed important in transmission, although many infected spouses who spent hours of direct contact with their partners did not transmit the infection.

Some Early Conclusions

The preceding findings both in epidemiologic studies and in human volunteers suggest that, in general, attending a concert or a motion picture with persons who have even obvious signs of colds is unlikely to result in infection. (Conversely, students who spend day after day in classrooms may have a higher chance of acquiring infection.) In addition, a relatively short stay (a few hours) among friends or relatives with HRV-caused colds is not likely to cause infection, even with brief embracing. Moreover, evidence from a group practice in Massachusetts showed that pediatricians' waiting rooms may *not* be places where respiratory viruses commonly are transferred.[188] These conclusions come with a caveat, however, in that direct experience is with only HRVs. Respiratory illnesses in which cough is a large component, such as influenza and measles, can result in substantial dissemination, even in a pediatric clinic.[43,112]

Route-of-Transmission Experiments

Because HRVs and perhaps other respiratory viruses often seem to spread with relative difficulty under many normal circumstances, the route of transmission becomes important because controlling the transmission of respiratory viruses in various habitats such as schoolrooms and families may be feasible by blocking transmission routes. Since the early 1970s, human volunteer experimental infection models have been used to explore HRV transmission routes. These studies have been performed primarily at the University of Virginia, England's Common Cold Unit, and the laboratory at the University of Wisconsin.

Gwaltney and Hendley[121] at the University of Virginia focused on the possibility that HRV colds were disseminated by direct or indirect hand contact. Interest in this approach had been provoked by the inadvertent transmission of HRVs from infected to non-infected persons through the vehicle of an ethanol-sterilized nasal speculum. Subsequently, the plausibility of direct hand transmission of HRVs was supported by the observation that nose picking and eye rubbing were common among Department of Medicine Grand Rounds participants and Sunday school attendees[144] (infection through conjunctival swabs had been reported previously).[33] These investigators then showed that HRV-39 retained infectivity for several hours on various surfaces and could be found on the hands of persons infected with HRV-39. The authors concluded that transmission of HRV could occur through self-inoculation by environmentally contaminated hands and demonstrated, in human volunteers, HRV-39 self-inoculation from an environmental source.[144]

The University of Virginia team in Charlottesville subsequently conducted a series of three experiments that identified direct inoculation as a route of virus transmission.[123] In two of the experiments, groups of recipients were exposed to HRV-infected donors (the HRV used was an untypeable strain, HH) in circumstances wherein they were exposed naturally in separate closed rooms to large-particle (12 recipients) or small-particle (10 recipients) aerosols produced by donors during periods of 45 minutes (large particles) or 3 days (small particles). One of the large-particle aerosol recipients was infected, whereas no one exposed to small-particle aerosols was. These essentially negative transmission rates by aerosol exposure were not surprising[121] because, as noted previously, Couch[60] had been unsuccessful in transmitting infection through the airborne route over a period of 26 days, and the Madison laboratory attained only a single HRV transmission among 11 recipients after 3 days' exposure in a dormitory.[64] The third group of Charlottesville recipients was exposed to HRV by inoculating themselves with the donor's nasal mucus. The donors blew their noses into their hands. The recipients then stroked the donors' contaminated hands for 10 seconds and then inoculated themselves by deliberately placing their fingers, two or three times, on their nasal and conjunctival mucosa. This inoculation procedure was repeated on 3 successive days. Eleven of the 15 hand-contact recipients (73%) were infected with the donors' HRV. The authors concluded that transmission by the hand-contact/self-inoculation route was much more efficient than the aerosol route was and "may be an important natural route of HRV transmission."[123]

One deficiency of this experiment was that donors and recipients shared the same air space over a measured, often brief, period during which fresh nasal mucus was transferred from the donor to the recipient. Nonetheless, Gwaltney did achieve a high rate of HRV transmission with nasal secretions, something that had not been accomplished readily in more "natural" experiments.[59,64] Also in support of hand transmission was the observation in the aforementioned married couple experiments that the ability to transmit HRV was correlated significantly with the presence of the virus on the donors' hands.[65] As a result of these various pieces of evidence and lack of compelling evidence to the contrary, hand-to-hand or fomite transmission became the accepted route for HRV contagion.[36,60] In later experiments, the Charlottesville group demonstrated approximately 50 percent transmission through fomites by using the same hand-contact/self-inoculation method described earlier, except either a coffee cup handle or a plastic tile was interposed between donors' and recipients' hands.[122]

At England's Common Cold Unit, Reed[241] investigated the likelihood of natural hand-contact transmission among residents (roommates) of several housing units, 38 of whom had been infected with HRV-2. Reed[241] found that 16 of the donors had HRV on their fingers, but none of the virus was transmitted to the fingers of their 18 roommates, even though virus could be recovered from some (6 of 40) objects recently handled by HRV-2–infected donors. Reed also found that none of 29 virus transfers from virus dried on the fingers could pass through a fomite to a recipient; the conclusion was that "spread of colds is unlikely to occur via objects contaminated by the hands of the virus shedder. . . ."

Laboratory workers at the University of Wisconsin continued their attempts to devise a natural transmission model to examine routes of transmission and, potentially, methods for interruption of transmission. Although the results of the transmission experiment with married couples were illuminating, the system itself had one great deficiency as an experimental model: the participants could not be observed during their interaction periods.[65] A model was needed in which donors and recipients could be monitored at all times. Ultimately, the scheme for such a system was suggested by two virologic-epidemiologic events in Antarctica, one from a few summer seasons (1975 to 1980) in which Dick and associates studied respiratory virus dissemination in an isolated Antarctic population and the other from a human volunteer experiment conducted in an Antarctic field party from England's Common Cold Unit.[291] Both experiences suggested that high natural transmission rates after just a few days of exposure could be achieved only in an environment with a high donor-to-recipient ratio and in a population density such as could be found in the classrooms and dormitories of a boarding school. Accordingly, a model was devised whereby donors (with the qualities of a "good" transmitter as described earlier) and recipients interacted during waking hours by playing cards and board and video games and engaging in communal studying. During the night, donors and recipients bunked together in the same or an adjoining room. With such an arrangement, graduate student monitors recorded activities and all clinical signs 24 hours a day. These artifices were successful; with 8 donors and 12 to 16 recipients, a 50 percent recipient attack rate usually was attained over a 12-hour period and a 100 percent rate over the course of a week.[76,202] Informally, this model, a fully observed monotypic HRV mini-epidemic, was named "Antarctic Hut," from its origins.[142]

Route-of-Transmission "Blocking" Experiments

Using the Antarctic Hut model, a series of 4 HRV-16 route-of-transmission blocking experiments was conducted to determine whether the virus was spread by hand contact, aerosol, or both.[76] The first 3 experiments lasted 12 hours, with 8 donors and 12 recipients; the donor-recipient interaction was through playing variants of stud and draw poker (Fig. 179–6). This interaction mode facilitated both aerosol and fomite/hand-contact transmission. Half the recipients were blocked completely from any hand contact/self-inoculation by wearing arm restraints (see Fig. 179-6). Evidently, little transmission resulted from hand or fomite contact because the HRV-16 infection rate in the restrained recipients was only slightly lower than in the unrestrained recipients, 56 versus 67 percent, an insignificant reduction ($p = .494$).

The fourth experiment, involving aerosol blocking, commenced immediately after the third. The poker game continued in the original room with eight donors alone, and all the equipment from the third experiment, including the cards (which literally were gummy from the previous 12-hour game), poker chips, pencils, and so forth, was moved to an identical room across the hall. The continuing eight-donor poker game in the room used for the third experiment was refurnished with poker implements. Subsequently, 12 new recipients entered the second room, began playing poker with the now heavily used cards and other paraphernalia, and made exaggerated hand-to-nose self-inoculation movements. Aerosol transmission was blocked effectively by the two brick walls between the rooms. Once each hour, the cards, poker chips, and other portables were exchanged between the donor and recipient rooms to maintain a freshly contaminated supply of fomites in the recipient room. The result was extremely surprising; not 1 of the 12 "fomite recipients" caught HRV-16. Judging from the results of these Antarctic Hut experiments in which fomite/hand-contact and aerosol transmission were blocked alternately, aerosol transmission clearly seemed the predominant route, at least for HRV-16 among student poker players at the University of Wisconsin. A few months after the 1987 publication of these results, an editorial appeared in *The Lancet* titled "Splints Don't Stop Colds: Surprising!"[76,263] It concluded that ". . . it looks as though coughs and sneezes *do* spread these diseases more than sticky fingers."

Subsequently, a set of experiments was conducted to determine how HRV-16 had disappeared along the five-step fomite transmission chain from nose to hand to fomite to hand to nose.[163] The experiments were a more elaborate version of Reed's previous studies,[241] and the same results were noted. The virus seemed to disappear precipitously at each step in the chain; 10^6 $TCID_{50}$ of HRV-16 in the donor's nose was reduced to 0 to 64 $TCID_{50}$ on the card-playing implements, and then the virus nearly disappeared on reaching the recipients' hands.

Additional studies examined other methods to interrupt transmission. The University of Virginia team performed a blocking experiment in the field in which mothers of families attempted to prevent hand contact/self-inoculation by dipping their fingers in iodine. The results of this 1979 to 1982 study were reported as part of a general review.[142] The illness rate during the 4 years was 40 percent lower in the mothers with iodinated fingers than in the placebo mothers, and the difference was significant ($p = .047$). However, the authors noted the difficulty of conducting and interpreting these investigations.[142] In another set of experimental human infection studies, the application of organic acids present in hand cleansers (e.g., salicylic acid, pyroglutamic acid) to the hands of volunteers decreased the likelihood of rhinovirus infection when volunteers subsequently touched their nasal or conjunctival mucosa 10 minutes after rhinovirus had been placed on their hands.[273]

The Madison group also examined transmission interruption methods to prevent spread from person to person. As part of a general investigation of the epidemiology of respiratory viruses in an isolated (≈200 persons) Antarctic population at McMurdo

Figure 179–6 A typical "Antarctic Hut" transmission experiment in progress using stud and draw poker as the interaction promoter. At each of four tables are two "donors" (infected with rhinovirus type 16) and three "recipients" (no antibody to rhinovirus type 16) (wearing surgical scrub suits). This particular experiment is one in which hand-contact/self-inoculation transmission is blocked in 6 of the 12 recipients by wearing Thatcher collars. The collars block hand-contact transmission by preventing touching of the face by the hands; therefore, virus can pass from donors to recipients only by aerosol.

TABLE 179–1 Apparent Complete Interruption by Virucidal Tissues of Rhinovirus Type 16 Transmission*

Study Group	Cotton Handkerchiefs Experiment A	Virucidal Tissues		Cotton Handkerchiefs Experiment D
		Experiment B	Experiment C	
Recipients who "caught" HRV-16 colds/total (%)†	5/12 (42)	0/12	0/12	9/12 (75)

**In each of 4 experiments, 8 volunteers with HRV-16 colds played poker for 12 hours with 12 other volunteers (without HRV-16 antibody) and for nasal sanitation used either ordinary cotton handkerchiefs (experiments A and D) or virucidal tissues (B and C). Three-ply Kleenex tissues were treated, per 100 g, with 9.1 g citric acid, 4.5 g malic acid, and 1.8 g sodium lauryl sulfate. Cotton or paper handkerchiefs were used to clear nasal passages, to smother coughs and sneezes, and to wipe surfaces. Virucidal tissues were used carefully and copiously.*

†Diagnosed by at least one isolation of HRV-16 or a fourfold rise in antibody to HRV-16 in the convalescent serum sample, or both.

HRV-16, human rhinovirus type 16.

Adapted from Dick, E. C., Hossain, S. U., Mink, K. A., et al.: Interruption of transmission of rhinovirus colds among human volunteers using virucidal handkerchiefs. J. Infect. Dis. 153:352-356, 1986.

Station in 1979, use of an iodinated facial tissue developed by the S. C. Johnson Company (Johnson's Wax) of Racine, Wisconsin, was successful in interrupting transmission.[73,291] Three days after all personnel began copious use of these tissues, the incidence of respiratory illness dropped rapidly and significantly ($p < .01$); in fact, respiratory illness nearly disappeared from the base.[75] Although this effect was striking, the trial could be controlled only historically. However, the apparent success was sufficient to interest the Kimberly-Clark Corporation, makers of Kleenex tissues, in applying virucidal facial tissue technology to interrupt HRV-16 transmission in the Antarctic Hut model. Kimberly-Clark soon made available a nontoxic, highly virucidal facial tissue. The tissues completely stopped transmission of HRV-16, in comparison to transmission rates of 42 and 75 percent in control recipients using ordinary cotton handkerchiefs ($p < .001$) (Table 179–1).[74] Kimberly-Clark test-marketed these tissues under the brand name "Avert," but their sales were lower than expected. Subsequently, two field trials of Avert in families were conducted in Charlottesville and Tecumseh, with reductions in viral transmission of 5 to 39 percent, respectively.[90,189] These results were disappointing but not unexpected. Changing established habits of personal nasal sanitation was not easy in the highly supervised, well-motivated adults in Antarctica; it may be impossible in unsupervised children.

Despite all the experimental studies to identify the principal route of virus transmission, no single route appears to be predominant. What is clear from these studies is that rhinovirus illnesses may be transmitted by both the hand-contact and aerosol routes. Certainly, the evidence presented in favor of the aerosol route as predominant should *not* discourage anyone from careful handwashing, especially around small children, in whom transmission by virus in wet mucus indeed could occur.

PATHOGENESIS AND HOST FACTORS

GENERAL COURSE OF INFECTION

Presumably, infection usually occurs through the respiratory route, although infection by the conjunctival route has been demonstrated.[33,144] To infect by the respiratory tract, a mucus blanket approximately 200 times the width of the HRV and propelled forward by continuously beating cilia must be penetrated by as yet unknown means.[141] Possibly, it is a formidable barrier and may account for the difficulty of HRV transmission. Nonetheless, when the virus is given intranasally by pipette, only one $TCID_{50}$ is needed to initiate infection.[64,78] The incubation period for a cold normally is 2 to 3 days, but a period of up to 7 days has been reported.[81,143] Ciliary dysfunction can predispose to respiratory infection.[28,35] HRVs replicate well in the upper respiratory tract (see later), and substantial evidence indicates that they replicate in the lower respiratory tract as well.[106,127,223,224]

RVs may be shed in large amounts (1000 to 1,000,000 infectious particles per milliliter of nasal washing) during the first 2 to 3 days of a cold and may be produced for 2 to 3 weeks thereafter.[76,78,81,202] However, the effect on cells of the nasopharyngeal cavity seems benign despite much local reaction to the virus, which results in the usual signs and symptoms of a cold. Systematic studies of HRV-inoculated human volunteers at the University of Virginia demonstrated that damage to the respiratory epithelium was slight, although a few sloughed ciliated epithelial cells did contain HRV antigen.[276,295] HRV was recovered only at focal sites in the nose and nasopharynx at the time of maximal symptoms, and in situ hybridization studies demonstrated evidence of HRV infection in only a minority of epithelial cells.[16,22,297] Furthermore, using primary monolayer cultures of ciliated and nonciliated epithelial cells, Winther and associates[296] demonstrated that HRV, coronavirus, influenza A virus, and adenovirus all grew well in these cell cultures; however, HRV and coronavirus produced no discernible cytopathic effect, whereas influenza virus and adenovirus nearly destroyed the cell sheet by 96 hours.

INNATE IMMUNE RESPONSE

Innate immunity is the initial host response to infection with rhinoviruses. Toll-like receptor 3 (TLR3) is activated by double-stranded RNA that is generated during virus replication, and TLR3 is up-regulated during HRV infection and is associated with decreased viral replication.[146] TLR activation leads to the production of type 1 interferons, which may be the mechanism by which viral replication is inhibited. Impaired induction of interferon-β leads to increased viral replication and is a defect that has been identified in the respiratory epithelium of asthmatic patients.[290] Numerous other components of innate immunity are also induced by rhinovirus infection, including beta-defensins, nitric oxide, and a variety of proinflammatory cytokines and chemokines (e.g., interleukin-1 [IL-1], IL-6, IL-8, RANTES [regulated on activation, normal T cell expressed and secreted]).[238,252]

Because the HRVs seem to cause only mild pathologic changes in cells of the respiratory tract, attention has turned to various immunologic or inflammatory substances as possible causes of symptomatic illness.[271] Researchers have long known that peripheral blood neutrophils increase in the first 2 to 3 days of illness in HRV-infected volunteers.[37,271] Phagocytes (neutrophils and monocytes) also have been demonstrated in large numbers in the nasal secretions of HRV-infected symptomatic human volunteers.[187] Although the microbe-killing ability of phagocytes is well known, these cells in themselves also can cause cold symptoms through the release of various toxic products, such as superoxide and hydrogen peroxide, during the respiratory burst.[31,268] In fact, increasing numbers of leukocytes in the peripheral blood

correlate with increased symptoms, and in infected human volunteers who are not ill, the white blood cell count does not increase.[271] An association has been noted between the nasal levels of two proinflammatory mediators involved in granulocyte regulation, granulocyte colony stimulating factor (G-CSF) and IL-8, and blood and nasal neutrophilia have been identified.[107,220] Nasal IL-8 levels also correlate with the severity of clinical symptoms.[115,278]

Increased nasal levels of other inflammatory mediators (e.g., IL-1β, IL-6, RANTES, kinins) have been associated with more severe clinical symptoms.[215,220,235,236,305] Furthermore, topical nasal administration of IL-6 or bradykinin leads to development of some of the symptoms of the common cold.[102,237] At least some of these inflammatory responses appear to depend on an activation pathway regulated by nuclear factor kB (NF-kB).[304,305] Resolution of clinical symptoms may be caused by the elaboration of inhibitors of inflammation, such as IL-1 receptor antagonist.[302] These inflammatory mediators may serve as a target for the development of drugs to relieve the symptoms of HRV infection, although the use of a specific kinin inhibitor did not lead to clinical improvement.[118,148]

IMMUNITY CAUSED BY SERUM ANTIBODY

Serum antibody to the various HRV types develops with age (presumably from repeated infections), and by adulthood, antibody may be detected against approximately 50 percent of HRV types tested.[119] The presence of serum antibody correlates positively with immunity, and resistance to infection and the degree of disease expression are related to the amount of antibody present.[3,4,32] As examples, in the Eagle Heights families, 21 of 75 persons (28%) without antibody were infected with either HRV-43 or HRV-55, whereas only 5 of 35 (14%) with homologous antibody to either of these agents were infected.[72] No one was infected who had homologous serum antibody levels above 16. Similar results were observed in the Charlottesville and Seattle families.[96,97,143] However, large doses can overwhelm antibody. At the clinical center of the National Institute of Allergy and Infectious Diseases, where human volunteers were used, 1000 $TCID_{50}$ was found to infect persons with serum antibody titers up to 256.[38] Judging from the paucity of reports of natural infections in persons with serum antibody titers greater than 16, virus inocula in naturally contracted cases must be low.

IMMUNITY CAUSED BY ANTIBODY IN NASAL SECRETIONS

At the National Institutes of Health, researchers found that intranasal administration of HRV vaccine produced both serum and nasal antibody, whereas two intramuscular injections of this vaccine produced little, if any, nasal antibody.[39] When the volunteers given the vaccine intranasally were challenged with approximately 100 $TCID_{50}$ of homologous HRV, they were protected significantly against both clinical illness and infection, whereas those administered the same vaccine intramuscularly remained susceptible.[227]

Conversely, other investigators who used the same vaccine preparation but gave it subcutaneously in 3 injections found nasal antibody responses in 21 of 46 (45%) volunteers.[80] These investigators discovered that when results were compared with those in unimmunized, antibody-free controls, challenge of these volunteers with 3 $TCID_{50}$ of virus produced significantly less virus shedding and reduced the duration and severity of illness. Possibly, this lower infectious dose more closely approximates a natural situation. This study also showed that protection against infection with the low virus challenge correlated with the magnitude of serum antibody and not with the presence of secretory antibody. The relative roles of serum and secretory antibody in HRV infections are still unsettled.

ANTIBODY APPEARANCE OVER THE COURSE OF INFECTION

A general pattern of the development of humoral (secretory and serum) antibody emerged from several studies.[32,83] Approximately 24 hours after infection, a sharp increase in nasal immunoglobulin A (IgA) secretion occurs. When symptoms begin approximately 48 hours after infection, rhinorrhea commences, and the transudate is composed of significant amounts of IgG. After approximately 1 week and after the actual episode of illness, virus-specific antibody, predominantly IgA, appears in the nasal passages, tears, and the parotid saliva. Serum antibody (usually IgG but occasionally IgM) also begins to be formed at 1 week and rises to peak levels at 1 month. Both serum and secretory antibodies appear sooner and rise more rapidly in persons with detectable neutralizing antibody in their pre-infection serum specimens. Antibody has been detected in nasal secretions and serum approximately 1 year after infection and, judging from the high proportion of the adult population with antibody to many HRV serotypes, probably lasts much longer.[30,119]

CELL-MEDIATED IMMUNITY

As noted previously, evidence that neutrophils increase both in the peripheral blood and in the nasopharynx during the first few days of an HRV cold is substantial.[37,294] Evidence that peripheral blood lymphocytes actually decrease in the first 2 to 3 days of an HRV cold and that migration of lymphocytes into the nasal secretions increases at this time also exists.[37,187] As noted earlier, specific humoral antibody usually is not present in the serum or nasopharynx this early in the illness, a finding suggesting that these in situ nasopharyngeal lymphocytes, through cell-mediated immunity, play an important role in controlling HRV proliferation. Hsia and colleagues[151] examined the ability of peripheral blood lymphocytes to liberate various cytokines (IL-2 and interferon-γ) and participate in other cellular immune processes (cytotoxicity and antigen-stimulated blastogenesis) during the early days of HRV colds in experimentally infected human volunteers. These investigators found that during infection, all these cell-mediated immunity activities were increased significantly when compared with pre-infection levels. Of special interest was their observation that the cellular ability to liberate higher levels of IL-2 correlated inversely and significantly ($p < .02$) with virus shedding and nasal mucus production. Cross-reactive T-cell epitopes are shared by HRVs, and T cells isolated from peripheral blood and from tonsillar tissue proliferate and secrete a variety of cytokines (interferon-γ, IL-2, IL-4, IL-5) after exposure to HRV antigens.[105,134,293] Thus, activation of T cells may contribute not only to virus clearance but also to airway inflammation.[105]

INTERFERENCE AMONG RHINOVIRUSES

Interference in infection with heterotypic HRV serotypes, which lasts between 5 and 16 weeks, has been reported in human volunteers.[92] Complete resistance to infection was observed after inoculation with as much as 2000 $TCID_{50}$ of HRV-16 (administered by nasopharyngeal spray) in a subject with an unsuspected "wild" HRV (not typed) infection present at the time of inoculation.[185] However, epidemiologic studies in a nursery school population,[24] a military population,[244] and a family population[206] showed sequential heterotypic HRV infections occurring frequently, sometimes at intervals as short as 2 days. In the family population, one subject was infected by three different HRVs

within a 30-day period. The factor or factors responsible for the interference observed in the experimental human infection model have not been identified. Simultaneous infection with an HRV and other respiratory viruses, including influenza A and B viruses, coronaviruses, parainfluenza type 1, RSV, adenoviruses, various enteroviruses, or other HRV serotypes, also has been reported.[86,97,184]

INFLUENCE OF A COLD ENVIRONMENT ON THE COURSE OF INFECTION

Exposure to cold temperatures is thought either to initiate or to exacerbate respiratory infections. In fact, chilling animals was shown to increase the severity and frequency of viral infection.[289] However, investigation of the effects of chilling on humans infected with "common cold viruses" and with HRV-15 did not demonstrate significant effects.[82] In the HRV-15 experiments, the conditions were realistic. The subjects (men) were cooled sufficiently in air to cause shivering for approximately an hour or were immersed in water long enough to cause a decline in rectal temperature.

EFFECT OF AGE AND SEX

A much higher prevalence of HRV infection is found in infants and young children than in older persons. In Seattle,[96] the HRV infection rate in children 0 to 5 years of age was nearly twice that of older children and adults (0.77 versus 0.41 infections per person per year, respectively); in Tecumseh,[210] the isolation rate in the 1- to 4-year-old group was far higher than that in any other age bracket. The high attack rates in these young children were not unexpected because fewer than 10 percent of them had antibody to any of the 56 HRV types,[121] and children ordinarily are subjected continuously to the family-like environment so conducive to transmission of HRVs. In especially crowded populations, attack rates may be high. In an Alaskan Eskimo village, 70 percent of 395 children were infected during a spring outbreak of HRV-16 infection.[299]

The frequency of infection with HRV generally declines throughout life. Beginning at the age of school attendance, the number of HRV infections declines gradually from one to two per year to 0.25 per year in the age group older than 60 years.[119,212] Results from most laboratories have found that persons 20 to 30 years of age account for an exception to the general decline in incidence with age[96,143,209,212]; HRV infections as well as infections with other respiratory pathogens increase during this period of life. Because the increase is especially marked in mothers,[96,143] it probably is the result of the transfer of respiratory illness in small children to parents.

In Tecumseh, the number of respiratory illnesses per year was consistently higher for female patients than for male patients (3 to >60 years of age).[212] If one assumes that HRVs account for a similar proportion of disease in each gender, this finding would indicate that girls and women generally have more HRV infections. As noted earlier, women with young children clearly seem to have more HRV infections than do others in their age group. In Seattle,[96] mothers had 1.5 times more HRV infections than fathers did, and in Charlottesville, women of child-bearing age had approximately 1.2 times more HRV illness than male study subjects did.[119]

EFFECT OF PSYCHOSOCIAL FACTORS

Numerous psychosocial factors have been shown to influence susceptibility to HRV infection, primarily in the experimental human infection model. Increased psychological stress, as measured by numerous stress indices, was associated with an increased susceptibility to infection and an increased likelihood of developing illness.[52,53] In contrast, having a more diverse societal network (increased number of social ties) and having a positive emotional style (i.e., sense of well-being, vigor, and calmness) were associated with resistance to infection and illness.[51,85] Antibody-negative (serum antibody levels < 1:2) subjects were equally susceptible to infection regardless of the degree of stress or number of social ties, although these factors did influence the likelihood of clinical illness.[51,52] Increased stress in working adults also was associated with an increased risk of having a natural common cold.[267]

CLINICAL MANIFESTATIONS

Consonant with their benign cytopathology in the respiratory tract, HRV infections in any age group usually cause only mild upper respiratory tract illness, that is, common colds.[60] Investigators estimate that HRVs cause 30 to 50 percent of common colds, at least in adults.[191] In healthy adults, HRV infections usually are so innocuous that human volunteers can be infected safely with these agents to study their epidemiology, pathogenesis, and control with experimental drugs.[60,142,162,202,281] However, even in adults, development of serious illness is possible: HRV-associated, radiograph-positive, atypical pneumonia has been described in military trainees[103] and in immunocompromised adults,[108,157] graft dysfunction has occurred in lung transplant recipients,[168] and exacerbation of underlying lung disease has been seen in adults with asthma and chronic obstructive pulmonary disease.[20,111,217,257] Severe respiratory disease, pneumonia, and death also have occurred in outbreaks of rhinovirus infection in geriatric patients residing in nursing homes.[147,190]

Nonetheless, children, particularly the very young, are the ones most likely to be subject to serious, sometimes fatal HRV-caused illness. As an example of a fatal outcome, a report from Los Angeles described an 11-month-old infant with mild asthma who died, totally unexpectedly, during sleep of apparent acute asthma and interstitial pneumonitis caused by HRV-47 (isolated from lung and blood specimens).[182] The authors wrote a special plea to other physicians to be alert for similar exigencies in their own patients. In this respect, two deaths of infants from possible rhinoviremia, one of which occurred during a cold, and six "cot" deaths, diagnosed as bronchiolitis/pneumonia and from which HRVs were isolated, may suggest that HRV involvement in fatal illnesses in infants is more common than realized.[243,286]

Viremia has been noted to occur in a minority of children with HRV infection and respiratory disease.[300] At least in theory, generalized illness following viremia seems possible with HRV infections; the HRVs are a division of the larger picornavirus group, which contains many viruses capable of generalized and fatal infection and whose genomes have considerable homology with the HRVs (see "Structure of the Virion," earlier).[222]

SPECTRUM OF RESPIRATORY DISEASE

Early Studies: Severe Disease in Young Children

Investigators have known since the discovery of the first HRV serotypes that these viruses cause considerably more severe illness in children than in adults. In 1959 and 1960, Hamparian, Hilleman, and their associates[131,242] conducted a clinical and virologic investigation of 15 children (<8 years old) and 20 adults from the Philadelphia area who had acute respiratory disease and from whom HRVs (then called coryzaviruses) had been isolated (Table 179–2). The 20 adults all had typical upper respiratory illness,

some with low-grade fever (peak of 37.3° C [99.2° F]). In contrast, 60 percent of the children had temperatures higher than 37.7° C (100° F), 20 percent had otitis, and 53 percent (eight children) exhibited one or more signs of lower respiratory tract involvement. One had laryngotracheitis (croup); one, bronchitis; two, asthmatic bronchitis; and one, bronchopneumonia. The last infant was 2 months old, and crepitant rales were heard over the right side of the chest anteriorly and posteriorly; the child's chest cleared in 2 days.

Shortly thereafter, in 1963, the Eagle Heights Village study of young student families began.[72] The epidemiologic aspects of this investigation were described previously (see "Epidemiology and General Transmission Considerations," earlier). Daily home clinical surveillance was performed, and, although the illnesses generally were so mild that the participants did not see a physician, the same differential severity between adults and children was noted (Table 179–3). With all viruses, including HRVs, children were much more likely to be febrile, and their symptoms were much more likely to last longer. Overall, however, HRV-caused illnesses were milder than those caused by other viruses.

A thorough investigation of infants and young children hospitalized with lower respiratory disease at Madison General Hospital was conducted at the same time (Table 179–4).[45] A virus was cultured from 38 percent of these patients, and HRVs predominated (11 of 27 isolates). These children (average age, < 1 year) with HRV infections were seriously ill: the mean temperature was 39.4° C (103° F), and eight had bronchopneumonia. Six of the virus-infected children yielded a bacterial pathogen in predominance in throat cultures. Five of these pathogens—beta-hemolytic *Streptococcus* in one, *Streptococcus pneumoniae* in two, and *Haemophilus influenzae* in two—were present coincidentally with the HRV isolates. All the patients with HRV-associated cases had antecedent milder respiratory symptoms, especially coryza.

TABLE 179–2 Signs and Symptoms of Respiratory Illness in 20 Adults and 15 Children (2 Months to 8 Years of Age) with Rhinovirus Infections in Philadelphia and New Jersey: 1959 to 1960*

Sign or Symptom	Adults	Children
Fever†	4 (20%)	9 (60%)
Eye, ear, nose, and throat		
Rhinorrhea	18 (90%)	10 (67%)
Purulent nasal discharge	4 (20%)	1 (7%)
Pharyngitis	10 (50%)	5 (33%)
Conjunctival infection	2 (10%)	1 (7%)
Anterior cervical lymphadenopathy	1 (5%)	8 (53%)
Hoarseness	4 (20%)	0
Croup	0	1 (7%)
Infection of the tympanic membrane	0	3 (20%)
Chest		
Cough	8 (40%)	15 (100%)
Dyspnea	0	4 (27%)
Refractions	0	3 (20%)
Rhonchi	0	7 (47%)
Rales	0	2 (13%)
Wheezing	0	4 (27%)

**The children were from the outpatient clinics and wards of the Children's Hospital of Philadelphia, and the adults were employees of Merck and Company, Inc., Rahway, NJ.*

†In adults, 37.2° C (99° F) or higher by mouth: The peak was 37.3° C (99.2° F). In children, 37.7° C (100° F) or higher by rectum; range, 37° C (98.6° F) to 39.1° C (102.4° F); mean, 37.9° C (100.4° F).

Adapted from Reilly, C. M., Hoch, S. M., Stokes, J., Jr., et al.: Clinical and laboratory findings in cases of respiratory illness caused by coryzaviruses. Ann. Intern. Med. 57:515-525, 1962.

Rhinovirus Preeminence in the Respiratory Disease of Larger Populations

Two large populations, one a general medical practice in Roehampton in England, near London,[149] and the other a group of families in Tecumseh, a small town in the United States near Detroit,[209] were assayed for the various respiratory viruses in illnesses of differing severity. Each had a pediatric population base of 900 or more. HRVs easily were the most common cause of respiratory illness in either population (Table 179–5): 26.3 percent of isolates in England and 38.1 percent in Michigan.

In both populations, HRVs frequently were associated with lower respiratory tract illness (Table 179–6); the proportion was much higher in the Roehampton clinic. The major HRV diagnosis in lower respiratory tract[149] disease was wheezy bronchitis: 42 percent of all HRV isolates in Roehampton and 15 percent in Tecumseh.[213] Otherwise, the severity of disease associated with HRV infection often was milder; in Roehampton, none of the HRV isolates were from children with bronchiolitis or pneumonia, whereas 8.4 percent of the RSV isolates were associated with one of these diagnoses. In Tecumseh, restriction of activity was

TABLE 179–3 Comparison of the Clinical Illness Attributable to Rhinoviruses with That Attributable to Other Respiratory Viruses Obtained from 24 Families (89 Persons) in the University of Wisconsin's Eagle Heights Village: 1963 to 1965*

Virus	Age Group	Number of Patients	Average Duration of Illness (Days)	Percentage with				
				Fever (° C)†		Cough	Nasal Discharge	Sore Throat
				37.7-38.3	<38.3			
Rhinovirus	Children	26	11	15	4	73	92	15
	Adults	25	9	8	4	68	96	56
Respiratory syncytial	Children	21	10	37	20	90	95	9
	Adults	1	8	0	0	100	100	0
Parainfluenza 1	Children	7	9	27	27	57	100	29
	Adults	6	8	17	0	33	83	67
Parainfluenza 3	Children	10	6	30	60	70	90	40
	Adults	1	6	0	0	0	100	100
Influenza	Children	2	10	100	100	100	100	50
	Adults	3	8	33	66	100	67	67

**The 42 children surveyed varied evenly in age from younger than 1 to 7 years; only 3 children were older than 7 years.*

†The temperature range 37.7° C to 38.3° C is 100° F to 101° F; less than 38.3° C is less than 101° F.

Adapted from Dick, E. C., Blumer, C. R., and Evans, A. S.: Epidemiology of infections with rhinovirus types 43 and 55 in a group of University of Wisconsin student families. Am. J. Epidemiol. 86:386-400, 1967.

TABLE 179–4 Clinical Findings in 11 Children Hospitalized with Severe Pulmonary Disease in Madison, Wisconsin, from Whom Rhinoviruses Were Isolated, from January Through May 15, 1964

	Patients										
Clinical Findings	**A**	**B**	**C**	**D**	**E**	**F**	**G**	**H**	**I**	**J***	**K**
Age (yr)	6/12	6/12	7	4-6/12	1	5	8/12	5/12	5/12	2/12	2
Sex	M	M	M	M	F	F	M	M	F	M	F
History											
Cough	+	+					+		+		+
Coryza	+				+	+	+	+	+	+	+
Respiratory distress	+	+		+		+	+	+		+	
Antibiotics before study	+					+	+	+		+	
Physical findings											
Highest											
Temp (° C)	39.7	40	38.2	39.4	40.5	38.7	40.1	39.3	40.2	38.1	38.3
Temp (° F)	103.6	104	100.8	103	105	101.8	104.2	102.8	104.4	100.6	101
Tonsillitis or pharyngitis				+		+					
Hoarseness or croup											+
Tachypnea	+	+	+					+		+	+
Chest refractions	+							+		+	
Rhonchi							+	+		+	+
Rales			+				+				
Wheezing	+	+	+					+			
Laboratory studies											
Initial leukocyte count ($1000 \times mm^3$)	10	15.5	8.7	11.4	19.8	10	10.7	7.3	30.5	13	11.2
Neutrophils (%)	42	55	93	77	74	79	49	69	72	73	75
Pneumonitis on chest radiographs	+	+		+		+	+	+	+	+	
Throat culture†	Pn	NF	HI	St	NF	NF	HI	NF	NF	Pn	NF
Diagnosis	BP Br	BP Br	Bron	BP Tons	URI UTI	BP Tons OM	BP	BP	BP	BP Atel	Cr

**Hospitalized since birth with choanal atresia. Right upper lobe pneumonia and atelectasis were present for 1 month before the study.*
†Only the predominant organism is recorded.
Atel, atelectasis; BP, bronchopneumonia; Br, bronchiolitis; Bron, bronchitis; Cr, croup; HI, Haemophilus influenzae; NF, normal flora; OM, otitis media; Pn, Streptococcus pneumoniae: St, beta-hemolytic Streptococcus; Tons, tonsillitis; URI, upper respiratory infection; UTI, urinary tract infection.
Adapted from Cherry, J. D., Diddams, J. A., and Dick, E. C.: Rhinovirus infections in hospitalised children. Arch. Environ. Health 14:390-396, 1967.

TABLE 179–5 Rhinoviruses and Other Respiratory Viruses Isolated from Some English and American Children Younger than 15 Years with Symptomatic Respiratory Infections

Agent	**Roehampton, England*†**	**Tecumseh, Michigan‡§**
Rhinoviruses	162 (26.3)¶	82 (38.1)
Parainfluenza	111 (18.0)	56 (26.0)
Influenza A and B	89 (14.5)	34 (15.8)
Respiratory syncytial virus	56 (9.1)	19 (8.8)
Adenoviruses	45 (7.3)	9 (4.1)
Enteroviruses	66 (10.7)	15 (6.9)
Other agents	58‡¶ (9.4)	—
Double isolates	27** (4.4)	—
Total isolates	614	215

**Results from a general practice clinic (919 children) during 1968 to 1972. Roehampton is a residential suburb of London.*
†Adapted from Horn, M. E. C., Brain, E., Gregg, I., et al.: Respiratory viral infection in childhood: A survey in general practice, Roehampton 1967-1972. J. Hyg. (Camb.) 74:157-168, 1975.
‡Results from surveillance (472 children) during 1966 to 1969. Tecumseh is a city of about 10,000, located approximately 50 miles southwest of Detroit.
§Adapted from Monto, A. S., and Cavalloro, J. J.: The Tecumseh study of respiratory illness. II. Patterns of occurrence of infection with respiratory pathogens, 1965-1969. Am. J. Epidemiol. 94:280-289, 1971.
¶Percentage of total isolates.
¶Mumps 3; herpes simplex, 17: Mycoplasma pneumoniae, *37: psittacosis (serologic diagnosis), 1.*
***Includes nine rhinoviruses.*

an important differential etiologic marker in that only 24.3 percent of those patients with HRV illness curtailed their normal activities, whereas the percentages often were double that for the other respiratory viruses, varying from 42 percent for RSV to 63 percent for influenza B virus.

TABLE 179–6 Association of Rhinoviruses with Upper and Lower Respiratory Infection in Some English Children (Roehampton) and American Families (Tecumseh, Michigan)*

	Percentage of Rhinovirus Isolates	
Infection	**Roehampton**	**Tecumseh†**
Upper respiratory infection	47	71
Lower respiratory infection	53	21

**See Table 179–5 for virus isolation data and population description. Roehampton data from Hamory, B. H., Sande, M. A., Sydnor, A., Jr., et al.: Etiology and antimicrobial therapy of acute maxillary sinusitis. J. Infect. Dis. 139:197-202, 1979; Tecumseh data from Lefkowitz, L. B., Jr., and Jackson, G. G.: Dual respiratory infection with parainfluenza and rhinovirus: The pathogenesis of transmitted infection in volunteers. Am. Rev. Respir. Dis. 93:519-528, 1966.*
†Includes rhinovirus isolates from both children and adults (82 isolates from children and 58 isolates from adults).

Rhinovirus Infections in Hospitalized Children

In Bristol, England, in 1971, 377 infants hospitalized for respiratory disease yielded 199 (53%) viral diagnoses.[160] RSV was predominant and accounted for 79 percent of the diagnoses, but HRVs were second at 12 percent (23 patients). Half of the HRV diagnoses were in infants with bronchiolitis or pneumonia. HRV illnesses were comparable in severity to those caused by RSV. One 4-month-old child with HRV-associated bronchopneumonia died. Except for the absence of deaths, similar results were found in 102 hospitalized children in Colorado, where the etiologic diagnosis rate was 85 percent.[221]

Serious HRV infections in young children have been reported from several locations, and some of these outbreaks have allowed

TABLE 179–7 Physical Findings in Young Children* with Respiratory Illness at the Time of Their Admission to St. Anna Children's Hospital, Vienna, from 1984 to 1986: Comparison between Rhinovirus and Respiratory Syncytial Virus Infections

Physical Findings	All Patients† (*n* = 519)	RSV (*n* = 119)	RV (*n* = 60)
Stridor during expiration	67 (13%)	15 (13%)	10 (16%)
Cyanosis	18 (3%)	4 (3%)	2 (3%)
Swollen cervical glands	87 (17%)	17 (14%)	9 (15%)
Red throat	344 (66%)	75 (63%)	41 (68%)
Nasal flaring	75 (14%)	29 (16%)	11 (18%)
Crepitations	20 (4%)	7 (6%)	2 (3%)
Chest radiograph (positive)	260 (50%)	79 (66%)	35 (58%)
Wheezing	113 (22%)	30 (25%)	13 (22%)
Moist rales	174 (34%)	43 (36%)	22 (36%)
Dry rales	49 (9%)	13 (11%)	5 (8%)
Respiratory rate/min			
Median (range)	43 (10-96)	40 (10-84)	36 (10-64)
Body temperature			
Median (range)	38.4° C (36.4° C-41.8° C)	39.9° C (36.8° C-41.0° C)	38.5° C (36.9° C-41.0° C)
Duration of illness			
Median (range)	11.6 days (2-45)	10.5 days (4-12)	12.8 days (3-31)

**Ten days to 3 years of age (median age, 6.6 months).*
†One hundred twelve (21%) children had upper respiratory tract, and 342 (66%) had lower respiratory tract illnesses: 471 (91%) were inpatients, and 48 (9%) were outpatients.
RSV, respiratory syncytial virus; RV, rhinovirus.
Adapted from Kellner, G., Popow-Kraupp, T., Kundi, M., et al.: Clinical manifestations of respiratory tract infections due to respiratory syncytial virus and rhinoviruses in hospitalized children. Acta Paediatr. Scand. 78:390-394, 1989.

TABLE 179–8 Clinical Diagnosis of Respiratory Illnesses of Young Children* at St. Anna Children's Hospital, Vienna, from 1984 to 1986: Comparison Between Rhinovirus and Respiratory Syncytial Virus Infections

Clinical Diagnosis	All Patients (*n* = 519)	RSV (*n* = 119)	HRV (*n* = 60)
Upper Respiratory Tract Infection			
Rhinitis	65 (12%)	4 (3%)	5 (9%)
Otitis media	10 (2%)	0	2 (3%)
Epiglottitis	6 (1%)	1 (1%)	2 (3%)
Pharyngitis	23 (4%)	3 (2%)	4 (6%)
Laryngitis	8 (2%)	1 (1%)	2 (3%)
Other diseases	25 (5%)	0	1 (2%)
Total	137 (26%)	9 (7%)	16 (26%)†
Lower Respiratory Tract Infection			
Croup	14 (3%)	4 (3%)	4 (6%)
Tracheobronchitis	100 (19%)	46 (39%)	16 (27%)
Bronchitis	24 (5%)	3 (2%)	0
Obstructive bronchitis‡	78 (15%)	28 (24%)	8 (14%)
Pneumonia	126 (24%)	29 (25%)	15 (25%)
Other diseases	40 (8%)	0	1 (2%)
Total	382 (74%)	110 (93%)	44 (74%)

**See footnotes to Table 179–7.*
†RVs were more likely to cause upper respiratory tract infection ($p < .01$).
‡This diagnosis included children with expiratory wheeze and evidence of air trapping. In a later article, this same definition was used for wheezy bronchitis.
RSV, respiratory syncytial virus; RV, rhinovirus.
Adapted from Kellner, G., Popow-Kraupp, T., Kundi, M., et. al.: Clinical manifestations of respiratory tract infections due to respiratory syncytial virus and rhinoviruses in hospitalized children. Acta Paediatr. Scand. 78:390-394, 1989.

direct comparisons between HRV and RSV infection. In the intensive care nursery of Strong Memorial Hospital in Rochester, New York, eight infants aged 2 weeks to 6 months became nosocomially infected, four with HRV and four with RSV, in early February of 1980.[287] One of the HRV isolates was from an asymptomatic baby. All seven symptomatic babies had a dramatic and sudden onset of respiratory illness that included cyanosis, apnea, labored respirations, increased nasal or tracheal secretions, tachypnea, and lethargy. Wheezing, tachycardia, irritability, and cardiac arrest each occurred in a single infant. None of the signs and symptoms differentiated between HRV and RSV illnesses.

At St. Anna Children's Hospital in Vienna from 1984 to 1986, Kellner and colleagues[172] compared the clinical features of HRV and RSV infection in 519 children aged 10 days to 3 years (median age, 6.6 months). Viral pathogens were detected in 227 (44%) of the children, and of these, 119 (23%) were RSV and 60 (12%) were HRV. The physical findings (Table 179–7) and the clinical diagnosis (Table 179–8) of the children with HRV or RSV infection were the same, except HRV infections were more likely than were RSV infections to be associated with upper respiratory tract infection.

Several retrospective reviews of clinical and laboratory records also implicated HRVs as probable causes of lower respiratory illness in infants. In 1982 and 1983, Krilov and associates[178] found 32 HRV-infected children who had significant signs of pulmonary disease in Boston hospitals; half of these children had radiologic evidence of new focal infiltrates. From 1984 to 1988, Abzug and associates[2] examined all virus-positive cultures from pneumonias in neonates younger than 30 days in Denver hospitals. The definition of pneumonia was strict and included new infiltrates on chest radiographs in addition to several typical physical signs. Forty patients were found; RSV was isolated from slightly more than half the cases, and HRVs and enteroviruses, at 6 isolates each, were the second most frequent. McMillan and colleagues[199] identified 48 pediatric patients who had HRV infections and were admitted ($n = 40$) or treated in the emergency center ($n = 8$) at their institution in Syracuse, New York, between 1985 and 1989. Most of these patients ($n = 41$) were younger than 1 year old and almost half ($n = 20$) had a clinical picture of bronchiolitis. Suspected sepsis ($n = 9$) was the next most common diagnosis. Jacques and colleagues evaluated 193 French children younger than 3 years of age hospitalized with bronchiolitis. HRV was the second most common cause of bronchiolitis (23% of cases), after RSV (30%).[161]

Kim and Hodinka[174] identified 93 pediatric patients with HRV infection who were evaluated in the emergency center ($n = 5$) or admitted ($n = 88$) to their hospital in Philadelphia between 1990 and 1996. Most of these children ($n = 67$) were younger than 1 year old. An acute respiratory illness was the most common clinical finding ($n = 78$, 84%), with fever and suspected sepsis the next most common ($n = 13$, 14%). A second viral or bacterial pathogen was identified in 8 (9%) of the subjects. Many of the subjects ($n = 62$, 67%) had an underlying condition such as prematurity or reactive airway disease. These investigators concluded that HRV infection was associated with severe lower respiratory illness and the need for hospitalization and could be a complicating factor in patients with underlying conditions.[174] Chidekel and colleagues[46] examined 40 patients with bronchopulmonary dysplasia and noted 8 episodes of worsening lung disease in 6 infants associated with HRV infection. The findings and clinical course of these infants were similar to those noted in other infants infected with RSV, although the need for mechanical ventilation was greater in association with RSV infection.

Glezen and colleagues[109] performed a prospective study to evaluate the occurrence of respiratory virus infection in subjects with respiratory or cardiac disorders admitted to three hospitals in Houston, Texas, between 1991 and 1995. HRV infections were identified in 51 subjects from a total of 1198 evaluated ill-

TABLE 179–9 Quantity of Virus Found in 87 Throat Swab Squeezings from Children Aged 3 to 11 Years: Madison, Wisconsin, from 1971 to 1972*

	Per 0.1 mL
≤1 $TCID_{50}$	22
>1 to ≤50 $TCID_{50}$	46
≥50 to <50 $TCID_{50}$	14
>50 to <500 $TCID_{50}$	5

**Fourteen rhinovirus types are represented.*
$TCID_{50}$, median tissue culture infective dose.

nesses; 6 of them were associated with a second respiratory virus infection.[89] In children younger than 5 years of age, exacerbation of asthma, bronchiolitis, and suspected sepsis were the most common clinical findings, whereas in older children and young adults, almost all subjects had an exacerbation of asthma. In older adults, a complication of an underlying disease (asthma, chronic obstructive lung disease, congestive heart failure) or pneumonia was diagnosed. In this prospective study, HRV infections were an important cause of lower respiratory tract illness in all age groups.[89]

Despite the foregoing evidence that HRVs can be an important cause of severe lower respiratory tract disease, large studies in Chapel Hill, North Carolina,[67] Huntington, West Virginia,[25] and Tucson, Arizona[298] by experienced investigators yielded the usual respiratory viruses in appropriate numbers. However, only 1 to 3 percent of these viruses were HRVs. These studies probably underrepresent the importance of HRV-caused disease in these populations because negative cultures do not necessarily mean the absence of HRVs (see "Diagnosis of Infection," later). These viruses are difficult to grow, even in supposedly sensitive cell cultures, and often only a few organisms are present in the specimen (Table 179–9).

In summary, evidence is accumulating that HRVs can cause serious lower respiratory illness, especially in young children. In some populations, HRVs may be second to RSV in importance and may cause comparably severe signs and symptoms.

Asthma

A specific lower respiratory illness in which the HRVs clearly appear important, perhaps most important, is "wheezy bronchitis" or "infectious asthma." The association between HRVs and these illnesses first was noted by Horn, Gregg, and their associates[149,150] as part of their previously described 1967 to 1972 Roehampton study of ill children (see Table 179-5). Forty-two percent of the 162 HRV isolates at Roehampton were from children with attacks of wheezy bronchitis, approximately twice the percentage of other viruses causing this syndrome. Many of these children were known to be subject to recurrent episodes.

During the later years (1971 to 1972) of this period, the Madison laboratory performed a longitudinal clinical and microbial study (children were sampled at least twice weekly for viral and once monthly for bacterial culture) of 16 nonatopic children with "infectious asthma" and 15 of their unaffected siblings. A clear temporal relationship was found between (1) the onset of symptomatic respiratory infection, (2) an asthmatic episode, and (3) the presence of viruses, predominantly HRVs, in the pharynx (Fig. 179–7).[204,205] Precipitation of an asthmatic episode occurred much more frequently during severe symptomatic respiratory infections than during mild ones. HRVs caused an asthma attack in 14 of 15 severe symptomatic respiratory infections but in only 1 of 6 mild ones. Subclinical infections never precipitated an attack. Except for one instance in which *H. influenzae* may have caused asthma, bacterial infections were not associated with asthma attacks, even when they were accompanied by severe symptomatic respiratory infection. As shown in Figure 179-7, the severe group A streptococcal infections in subjects CC and P did not precipitate asthma, whereas severe HRV-49 infections in these two children caused more than three asthma attacks that were sufficiently incapacitating for the child to stay home from school.[205] The asthmatic siblings seemed especially susceptible to symptomatic infection; total infections of probable viral origin ($p < .02$), known viral infections ($p < .01$), and HRV-caused symptomatic respiratory infections ($p < .01$) all were significantly greater in the asthmatic than in the nonasthmatic siblings.[203]

A somewhat similar year-long prospective investigation was conducted in England[156] in 30 preschool children with histories of recurrent respiratory infection often accompanied by wheezing, as well as in their unaffected siblings. The children with recurring infections had approximately twice the number of illnesses and viruses isolated than the controls did. Their illnesses also were much more severe, and they often involved the lower respiratory tract. HRVs were heavily preponderant, at 57 percent of the isolates, and were associated with wheezy bronchitis four times more often than were RSVs. Atopic children, as measured by positive skin testing and raised serum IgE levels, did not seem especially subject to development of recurrent infection.

At the University of Colorado, McIntosh and associates[197,198] demonstrated in 1973 the importance of other respiratory viruses in asthma; later, these investigators suggested that RSV is the major agent associated with asthma in children younger than 4 years and that HRVs are preeminent in older children and adults.[197,198] Subsequent reports demonstrated the importance of HRVs in older children, as well as in many younger children. As examples of HRVs in younger children, in patients with recurrent asthma who were seen at the pediatric allergy unit in Oslo from 1981 to 1983, most cases of acute bronchial asthma were HRV associated (45%), with RSV being second (19%).[34] Twenty-nine percent of those patients in the virus-infected group were between 2 and 3 years of age, and the incidence gradually decreased to 1 percent in adolescents 15 years of age. Also illustrative of HRV in very young children is the prospective study of the 30 English preschool children with recurrent wheezing infections described earlier, in which HRVs were most important in children whose mean age was 2.2 years.[156] Mertsola and colleagues[201] in Turku, Finland, examined children with wheezy bronchitis (mean age, 3.2 years) and found HRVs and coronaviruses to be most important. The comparative importance of RSV and HRV in asthma in infants (median age, 6.6 months) with probable wheezy bronchitis (see "Obstructive bronchitis" in Table 179-8) was examined from 1984 to 1986 by Viennese investigators; 28 isolates yielded RSV, and only 8 yielded HRV.[171] In a later article from this group covering the period from 1986 to 1990, 179 RSV isolates and 49 HRVs were recovered from these hospitalized infants; most of the HRVs were recovered from infants with some wheezing.[170] Only 3 patients had pneumonia without wheezing.

A cohort of 9- to 11-year-old children with asthma was evaluated from April of 1989 to May of 1990.[165,167] Nucleic acid detection methods (reverse transcriptase–polymerase chain reaction [RT-PCR]) were used in addition to traditional culture methods to identify HRV infections. Respiratory viruses were detected in 80 to 85 percent of illness episodes (the rate depended on the definition of illness), and HRVs were present in approximately half the illnesses. The occurrence of respiratory viral infections in this cohort correlated strongly with hospital admissions for both children and adults in the same geographic area.[166] HRVs also have been associated with exacerbations of asthma in adults, although at a somewhat lower frequency (as many as 33% of episodes).[20,217]

Viral infection is an important risk factor for wheezing in children of all ages who are receiving emergency care.[88,186] Having either an RSV or an HRV infection that results in a wheezing

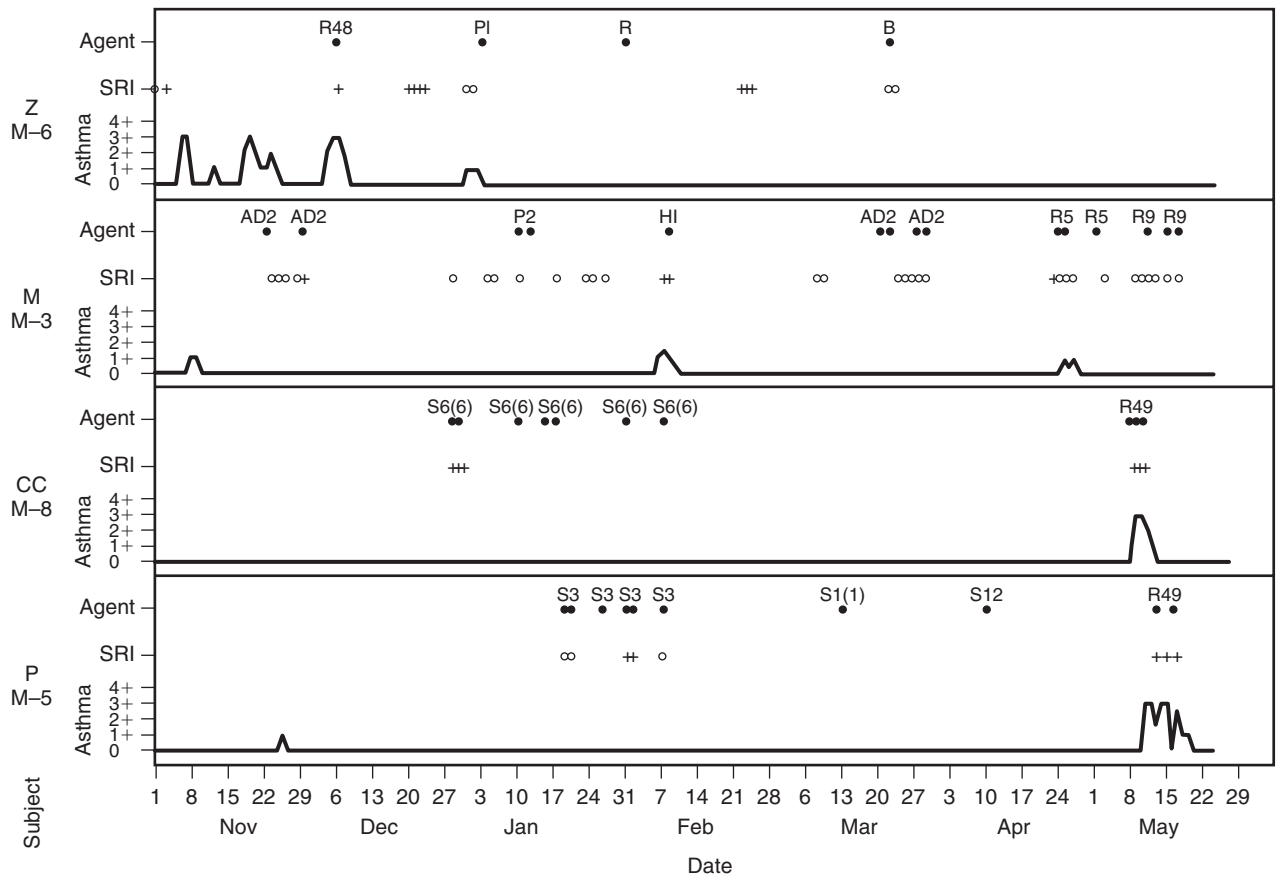

Figure 179–7 Temporal relationship among asthma, symptomatic respiratory infection (SRI), and infectious agent in four children with "infectious" asthma. AD, adenovirus; B, influenza B virus; HI, *Haemophilus influenzae*; P, parainfluenza virus; R, rhinovirus; S, group A *Streptococcus*; *open circle*, mild; +, severe (fever or more than one sign or symptom). Numbers after R and P indicate type; numbers after S indicate T type (M type, if typeable, is in parentheses). *(Adapted from Minor, T. D., Dick, E. C., DeMeo, A. N., et al.: Viruses as precipitants of asthmatic attacks in children. J. A. M. A. 227:292-298, 1974.)*

illness during the first year of life is an independent risk factor for wheezing during the third year of life.[186] RSV infection and passive exposure to tobacco smoke were associated most frequently with wheezing illness in young children (<2 years).[88] Thirty-one percent of children older than 2 years had an accompanying viral infection, usually an HRV infection, and were more likely to have IgE antibody to inhalant allergens than were the younger children. This study illustrates the dichotomy of causes for wheezing illness in the pediatric age group: RSV infection is the predominant cause in infants, and atopy combined with viral infection, usually HRV infection, is the main cause in older children.

Two investigative groups serotyped the HRVs isolated from children with wheezy bronchitis and found no types to be particularly "asthmagenic"; the Viennese group identified 12 serotypes, and the Madison group reported 21.[170,203-205] Only 3 serotypes overlapped: HRV-1B, HRV-20, and HRV-32. Therefore, a total of 30 different HRV serotypes precipitated asthmatic attacks.

The mechanism of bronchospasm in virus-infected patients with wheezy bronchitis has generated much speculation and research. HRVs can replicate in the lower respiratory tract and have been found in the lower airways.[106,127,154,223,224] Whether the virus-associated bronchospasm is caused by viral replication in the lower airways or by induction of inflammatory mediators resulting from their growth in the upper respiratory tract has been a subject of debate, although accumulating evidence favors the former possibility.[104] HRV infection clearly induces the production of numerous proinflammatory cytokines, and an increased number of inflammatory cells has been demonstrated in the lower respiratory tract in association with HRV infection.[100,104] The presence of activated cytotoxic T cells in the lower airways has been suggested to contribute to asthma-associated mortality.[219] Host factors also play a role, in that bronchial epithelial cells from asthmatic individuals produce less interferon-β and have less apoptosis and greater viral replication following infection with HRV than do cells from nonasthmatic subjects.[290] Animal models also suggest the possibility that reflex neurogenic pathways may be activated in association with respiratory viral infection and may lead to bronchoconstriction. The pathogenesis of respiratory virus-induced bronchospasm is an area of active research, with the hope that preventive or therapeutic intervention strategies can be developed.

Otitis Media

Although acute otitis media is chiefly a bacterial disease, with approximately two thirds of middle ear fluid specimens yielding *S. pneumoniae*, *H. influenzae*, or both, and *Moraxella catarrhalis* and other bacteria playing lesser roles, otitic involvement often is preceded or accompanied by a putative viral upper respiratory tract illness.[11,13,176,250] Most bacterial pathogens found in middle ear fluid are colonizers of the nasopharynx, and bacterial infection of the middle ear is considered to be a direct extension from that area.[176] Clear and comprehensive evidence of viral extension from the nasopharynx to the middle ear has been established.

Sarkkinen and coworkers,[253] using antigen-detection techniques on middle ear fluid and nasopharyngeal secretions, found RSV, adenovirus, and parainfluenza viruses in either the middle ear fluid or the nasopharyngeal secretions of 58 of 131 children (44%) with acute otitis media. Twenty-four of these children had viruses in middle ear fluid, and only 1 of the 24 did not have the same viral antigen in both the middle ear fluid and nasopharyngeal secretions. Most of the sampling was conducted during a local epidemic of RSV infection, and the curve of RSV detection in the middle ear fluid and nasopharyngeal secretions followed the pattern of the RSV epidemic curve. Other investigators have found similar relationships between the viruses in middle ear fluid and nasopharyngeal secretions.[47,177]

Gwaltney[117] isolated one HRV from culture of 16 middle ear fluid specimens in 1971. In 1986, Chonmaitree and colleagues[47] reported 3 HRV isolates in 84 middle ear fluid specimens; then Arola performed a thorough investigation of the role of HRVs in this illness.[11-14,250] In 1987 to 1988, nasopharyngeal secretions were taken from 363 patients (mean age, 2.5 years) with acute otitis media, and viruses were detected in 154 (42%); surprisingly, HRV predominated over RSV, 24 versus 13 percent.[11] HRVs were isolated all through the year, but the usual fall and spring peaks were observed. Patients with RSV infection generally were sicker, with higher fever ($p < .05$) and more severe cough ($p < .01$), but the appearance of the tympanic membrane was similar in both patients with HRV and RSV infections.

Arola and collaborators[13] attempted to detect virus in both the nasopharyngeal secretions (116 cases) and middle ear fluid (143 cases) of patients with acute otitis media (mean age, 1.5 years) and were successful in 33 (28%) and 16 (11%), respectively. HRVs were the predominant isolate, with 21 (18%) in nasopharyngeal secretions and 11 (8%) in middle ear fluid. Fifty-three percent of the patients harbored a bacterial pathogen in middle ear fluid. In the HRV-positive group in whom both viral and bacterial cultures were performed, 6 of 9 middle ear fluid specimens yielded no bacterial pathogens; that is, HRV was the only pathogen found. Unfortunately, these investigators were not able to serotype the HRVs to determine whether the same serotype was present in both the nasopharyngeal secretions and the middle ear fluid.

More recent studies have found evidence of HRVs in middle ear effusion with RT-PCR methods. Pitkaranta and colleagues[231] isolated respiratory viruses from the middle ear effusions of 30 percent of 100 children who had otitis media with effusion. HRVs constituted approximately two thirds of the respiratory viruses identified. Bacterial pathogens were identified in 35 percent of the samples, and both bacteria and viruses were found in 11 percent. A criterion for selection was the absence of an upper respiratory tract illness within the preceding week, so whether the presence of HRV RNA represented evidence of ongoing or past infection is unclear. Blomqvist and colleagues[26] found HRV RNA in middle ear effusions from 41 percent of children with otitis media in the first 2 years of life, and Chantzi and colleagues[41] found a similar prevalence of HRV RNA in otitis media effusions.

Although in general, the presence of both bacterial and viral pathogens in middle ear fluid does not interfere with the effectiveness of antibacterial therapy, in those specific patients whose acute otitis media is refractory to treatment, it may.[14] When measured against a comparison group of 66 "normal" acute otitis media cases (controls), 22 patients with refractory cases harbored significantly more viral pathogens than did controls (68% versus 41%, respectively; $p < .05$). In addition, when only the middle ear fluid of these groups was examined for the presence of viruses, the patients with refractory cases had virus in 32 percent of samples versus 15 percent in controls. HRVs were the dominant viruses in both groups. Bacterial pathogens were grown from the middle ear fluid of 4 of the 22 in the poor-responder group, and all harbored concomitant respiratory viruses, 2 of which were HRVs; only 1 of the 4 bacteria was resistant to the antibacterial agent used in therapy. These investigators also examined patients in the 66-patient control group who were refractory to treatment and found significantly more ($p < .05$) viruses in the middle ear fluid of this group than in those with a good response to therapy. These authors concluded that the presence of viruses in the middle ear fluid of children with acute otitis media can delay response to antibacterial therapy.

Chonmaitree and associates[48] in Galveston, Texas, also investigated the role of viruses in middle ear fluid as agents that may interfere with antibacterial therapy. In the initial report by these investigators, viruses were cultured from the middle ear fluid of 11 of 58 children (19%); RSV and HRV were the most frequent viruses isolated, at 3 each. Of the patients in whom therapy failed, significantly more ($p < .05$) harbored viruses as well, and of those whose bacteria were susceptible to the treatment antibiotic, significantly more had combined viral-bacterial infections.

In two subsequent reports, the highest rate of poor bacteriologic outcome occurred when HRV was isolated from middle ear fluid. Seven of nine instances of HRV infection resulted in bacteriologic treatment failure.[266]

These studies from Finland and Texas suggest that viruses, including HRVs, may play a role in acute otitis media, especially in prolonging the response to antimicrobial treatment. Perhaps pertinently, McBride and associates[196] at the University of Pittsburgh and the University of Virginia used HRV-infected human volunteers and found that the eustachian tube became occluded in 50 percent of the volunteers and that abnormal middle ear pressure was present for as long as 10 days. These studies were extended by Buchman and colleagues,[29] who inoculated 60 volunteers with HRV-39. Middle ear pressures of less than –100 mm H_2O were noted in 22 subjects (37%). In 2 of the 3 patients who had pressures less than –100 mm H_2O, upper respiratory illness with middle ear effusion developed. In this study, the otologic manifestations of experimental HRV infection were extended to include otitis media.

Arola and colleagues[12] examined the role of viruses in 61 children (mean age, 3.2 years) with subacute or chronic asymptomatic otitis media with effusion. Five HRVs and one adenovirus were found in the middle ear fluid. In addition, bacterial pathogens were isolated from the patient with adenovirus infection and from two of the patients with HRV infection. None of these patients with otitis media and effusion were ill with an upper respiratory tract infection at the time the specimen was obtained, and the effusion had endured 30 to 60 days before myringotomy was performed.

Sinusitis

RVs can cause a clinical syndrome of rhinosinusitis that appears similar to that caused by bacterial infection. Gwaltney and colleagues[124] performed computed tomography of the sinuses on adults with acute common colds (<4 days' duration). HRV infection was documented by culture in 27 percent. Radiographic abnormalities included mucosal thickening and air-fluid levels. The signs and symptoms resolved without antibiotic therapy, a finding thus suggesting that the radiographic signs were caused by an acute viral infection. Few attempts have been made to recover viruses directly from the sinuses. Two reports from Virginia noted that 140 aspirates obtained by direct puncture of the maxillary sinuses yielded 86 positive specimens, only 12 of which contained viruses. Seven of these viruses were HRVs, and the remainder were influenza A or parainfluenza viruses.[125,128] Five of the viruses were isolated in conjunction with bacterial pathogens. In a more recent study, Pitkaranta and associates[230,232] used RT-PCR and in situ hybridization to identify HRVs in 50 percent of adults with acute community-acquired sinusitis. Because the

signs and symptoms of viral rhinosinusitis and bacterial sinusitis can be difficult to distinguish, the American Academy of Pediatrics developed clinical practice guidelines for the diagnosis and treatment of bacterial sinusitis.[50]

Rhinovirus Infections in Nonindustrialized Populations

Not many HRV surveillance studies have been conducted in nonindustrialized populations, but those that have reveal some unique findings. In the spring of 1967, Wulff and associates[299] observed an outbreak of HRV-16 and HRV-29 infection in a 93-family (429 children) Eskimo population in Bethel, Alaska. The larger, HRV-16 outbreak was analyzed thoroughly. The investigators found 37 HRV-16 isolates, but only 19 of them were from ill children; therefore, nearly half, 18 children, had subclinical infections. The ill children often were only mildly so; 12 had common colds, and only 1 child had bronchitis and 1 child had pneumonia. HRV-16 spread widely in the population under study; 70 percent of antibody-free children demonstrated a fourfold or greater serologic response. Eighty-five of the 93 families were infected with HRV-16, and the 8 families that escaped infection had only 1 to 3 children each. The amount of HRV dissemination was not especially surprising because it was not much higher than that of HRV-55 in Eagle Heights Village, but, unexpectedly, half the HRV infections were subclinical.

Much subclinical HRV infection also was reported in a surveillance of 136 preschool children in 2 small villages (combined population of 1750 in 1982) on an island in the Melanesian nation of Vanuatu, a group of 80 islands located in the South Pacific approximately midway between Australia and Fiji.[269] HRVs by far were the predominant viruses (21 isolates), all were type 16, and all caused subclinical infection. In addition, pneumococci often were found in conjunction with these symptomless HRV-16 cases and rarely otherwise.

These two examples of a high rate of subclinical HRV infection are unusual judging by experience in more industrialized societies. Surveillance of a year-long (1971 to 1972), twice-weekly sampling of 33 children aged 3 to 11 years in Madison produced much different results. As depicted in Figure 179-7 and Table 179–10, only 11 (0.8%) cases of asymptomatic shedding occurred all year. Conversely, all these children were members of at least middle-income families, and some continental U.S. populations may be analogous to those in underdeveloped nations. Gwaltney[119*] noted that the rate of overall inapparent HRV infection in the continental United States may approach 25 percent.

However, as emphatically described in reviews by Chretien and associates[49] and by Graham,[110] asymptomatic infection with respiratory viruses is not the major problem of families in communities and nations struggling to raise their children in healthy environments. Graham[110] estimated that worldwide, 98 to 99 percent of deaths from acute respiratory disease occur in developing nations. A study of children aged 5 years or younger was conducted in such a nation, Kenya.[139] Eight hundred twenty-two children with severe acute respiratory disease were examined etiologically by modern cell culture techniques. Fifty-four percent of the children yielded viruses (444 isolates), with enterovirus by far being the most common (162 isolates), followed by RSV (98 isolates) and the HRVs (54 isolates). Half the HRV isolates also were culture-positive for possible bacterial pathogens, and 11 HRV isolates were from infants younger than 3 months. The authors concluded that HRVs may be significant respiratory pathogens in Kenya.

HRVs were found to be prevalent in a 29-month household-based study in an impoverished urban population in Fortaleza, Brazil; 175 children younger than 5 weeks in 63 families were surveyed for clinical illness and respiratory viruses.[66] The study yielded two major findings: (1) the burden of respiratory illness in these children was so continuous that assigning beginning and ending dates was impossible, and (2) HRVs, by far the most common virus isolated, accounted for 46 percent of all viruses obtained. HRV was dominant in all age groups, but overwhelmingly so in children 0 to 6 months of age. In this age group, only HRV and parainfluenza virus were isolated, in a ratio of approximately seven HRVs to one parainfluenza virus. The role of HRVs in nonindustrialized nations must be assessed carefully. For example, what proportion of HRVs in this study in Brazil were just "innocent bystanders," as found in the subclinical infections in Bethel and Vanuatu (see previous discussion)?

TABLE 179–10 Rhinoviruses and Other Viral and Bacterial Pathogens Isolated during Periods of Respiratory Illness and When Free of Illness from 33 Middle-Income Children Aged 3 to 11 Years (Average Age, 6 Years): Madison, Wisconsin, from 1971 to 1972*

Pathogens	1971-1972 (Nov-Feb)	1972 (Mar-May)	Total
Illness-Free	753	566	1319
Agents isolated			
Rhinoviruses	4 (0.5)†	7 (1.2)	11 (0.8)
Other viruses‡	10 (1.3)	6 (1.1)	16 (1.2)
Bacterial pathogens§	14 (1.9)	4 (0.7)	18 (1.4)
Total	28 (3.7)	17 (3.0)	45 (3.4)
Respiratory Illness	305	195	500
Agents isolated			
Rhinoviruses	13 (4.3)	57 (29.2)	70 (14)
Other viruses	44 (14.4)	14 (7.2)	58 (11.6)
Bacterial pathogens	35 (11.5)	10 (5.1)	45 (9.0)
Total	92 (30.2)	81 (41.5)	173 (34.6)

**Throat swabs were taken twice weekly and inoculated into three cell lines and a sheep blood agar plate.[203,205]*

†Percent isolation rate (i.e., 4/753, 0.5%).

‡Adenoviruses, influenza viruses A and B, parainfluenza virus, herpes simplex virus, and enteroviruses. Coronaviruses would not have been isolated; the fact that they often are midwinter viruses may account for the relatively low isolation rate for 1971 to 1972.

§Chiefly group A streptococci in more than 100 colonies per pour plate.

DIAGNOSIS OF INFECTION

HRV infections cannot be diagnosed solely on the basis of clinical grounds because they cause such a wide spectrum of respiratory illness, particularly in infants and children. However, because of the characteristic spring-summer-fall seasonal pattern of the HRVs, tentatively assigning an HRV origin in patients with mild to moderate respiratory illness during these months is not unreasonable.

Obtaining a culture of the etiologic agent and interpreting the meaning of a positive specimen can be difficult with HRVs. These viruses often propagate unpredictably, even in relatively sensitive diploid cell cultures such as WI-38 and FT cells or in Ohio strain HeLa cells,[17,55,57,61] and often these cells are highly variable in their susceptibility.[119] Isolation of HRV from children is hampered by the common practice of relying on a throat swab. Inocula from many throat swabs contain one tissue culture infectious virus particle or less and seldom contain infectious particles by the hundreds (see Table 179-9). Hendley and colleagues[143] found nasal specimens to be superior for HRV studies, and diagnostic rhinovirologists from many nations successfully use nasal aspirates.[150,171,201] Specimens should be placed in cell culture as soon as possible and without previous freezing and thawing (however, refrigeration [4° C] overnight results in little loss). If freezing is necessary and dry ice is used, great care must be taken to avoid contamination of the specimen with sublimed carbon

dioxide because the resultant carbonic acid rapidly kills the virus. Cell cultures should be incubated at 33° C and slowly rolled. Evidence of virus growth usually is seen between 2 days and 2 weeks. Once a cytopathic effect is evident, standard techniques for identification are used.[62] Because of the technical difficulties in isolating HRVs, before initiating etiologic studies of viral respiratory illness, specimen collection and culture procedures should be developed carefully in collaboration with an experienced respiratory virologist.

HRVs are known to be found in the absence of symptoms, but infrequently, at least in industrialized nations. Cherry[44] compiled shedding data from well children who were part of nine independent studies (1798 specimens) and found the total subclinical shedding rate to be 3 percent. HRV shedding from well children was 5 percent or less in 8 of these studies but was 11 percent in the remaining survey of a Chicago nursery school population.[24] Only 11 (0.8%) of 1319 specimens from healthy Madison children aged 3 to 11 years yielded an HRV by culture (see Table 179-10). However, the rate of subclinical HRV shedding is known to be high, up to 50 percent, in some populations in developing countries, so healthy control specimens should be obtained when possible to measure background HRV carriage rates.[269,299]

As described early in this chapter, HRVs have many base sequences in common among the RNA genomes of the various serotypes, especially in the noncoding region at the 5′ end. Several laboratories have taken advantage of this observation by designing RT-PCR assays for the detection of HRVs. Some assays do not distinguish HRVs from enteroviruses, whereas others are HRV-specific.[101,126,155] The superiority of RT-PCR techniques compared with cell culture methods first became evident in a clinical study of children with acute respiratory illnesses. Johnston and colleagues[167] detected HRV by PCR in 146 of 292 samples (50%) that yielded HRV in only 47 (16%) by standard culture. PCR was thus three times more sensitive than was culture for detecting HRV infection. Many subsequent studies confirmed the superiority of RT-PCR assays over culture methods for the detection of HRVs.[5,18,20,153] The development of sensitive real-time RT-PCR assays raised the possibility that clinical virology laboratories would become able to identify HRV infection rapidly.[255] RT-PCR assays also can be used to classify picornavirus isolates as enteroviruses or HRVs; Atmar and Georghiou[19] found 100 percent concordance between PCR results and acid lability testing.

PREVENTION AND TREATMENT

Prevention and treatment are addressed thoroughly in Chapter 8 and elsewhere, and only measures specific to the HRVs are added here. Although some protection after the use of inactivated vaccines has been demonstrated,[80,228] the existence of at least 101 serotypes limits the prospect of vaccine development. However, based on the observation that cross-reacting antigenic groupings exist among the HRVs, developing a vaccine that targets these epitopes may be possible.[58]

The most effective HRV cold-preventive agent described to date that has been demonstrated to be effective for natural HRV colds in Australia and the United States is interferon-α, a protein produced as part of the host's natural antiviral defense and now produced for experimentation by genetic recombinant methods.[84,135] Investigators administered this agent by nasal spray to other members of a family after symptoms appeared in the index case; 80 percent of the secondary HRV colds were prevented. However, interferon given in this fashion does not seem to be effective against other respiratory viruses; for example, in a follow-up study in Seattle, interferon-α did not reduce the incidence of colds when administered in a protocol identical to that used in the previous family studies, chiefly because HRV infections were in the minority.[79,84,99,135] HRVs often have a decided seasonality, so rapid diagnosis of the index case by PCR would be helpful to determine when interferon-α could be used effectively to stop intrafamily spread. A combination of rapid diagnosis and family prophylaxis with interferon-α could be most helpful in families such as those with asthmatic children, in whom HRV infection may be serious (see "Clinical Manifestations"). The disadvantage of interferon prophylaxis is that prolonged intranasal administration (>7 days) or repeated treatment produces an inflammatory response consisting of nasal stuffiness, ulceration, and blood-tinged discharge.[251]

Numerous antiviral agents that have in vitro activity against HRVs have been developed. The earlier section in this chapter on structure of the virion describes the development of some of these agents, development made possible by taking advantage of current detailed knowledge of HRV structure and specific cell receptors. Several of these preparations, including capsid-binding agents, soluble receptor (soluble ICAM-1), and antibody to the receptor (ICAM-1), have been tested in clinical trials, but development of most of these agents has not been pursued for a variety of reasons,[21,277] as reviewed by Arruda and Hayden.[15] Another capsid-binding agent, pleconaril, has in vitro antiviral activity against many enteroviruses and HRVs, and phase III clinical trials on naturally acquired colds demonstrated some reduction in the severity and duration of several respiratory symptoms.[136] However, pleconaril induces the cyp3A4 enzyme that can lead to menstrual irregularities in women taking oral contraceptives.[93] This concern, along with the drug's modest efficacy, prevented its use from being approved by the U.S. Food and Drug Administration; an intranasal form is still under development.[225] Another anti-RV drug, rupintrivir (formerly AG7088), is a potent, irreversible inhibitor of viral 3C protease that has reached clinical trial.[137,195] Further studies of both these agents, especially in children, will be needed to determine their potential benefits.

Colds usually are treated symptomatically with mild nonsteroidal anti-inflammatory drugs, sympathomimetics (e.g., phenylpropanolamine), and antihistamines; these agents are discussed in Chapter 8.[262] Anticholinergic agents (e.g., topical ipratropium bromide) and first-generation antihistamines reduce severity of rhinorrhea, whereas second-generation ("nonsedating") antihistamines have no effect. The efficacy of the first-generation antihistamines may result from the ability of these agents to block muscarinic as well as histaminic receptors and to cross the blood-brain barrier.[214] Sympathomimetics decrease nasal obstruction, although topical administration may lead to rebound nasal obstruction. Nonsteroidal anti-inflammatory agents decrease the severity of some of the systemic symptoms associated with colds (e.g., malaise, headache).[272] A combination of antiviral and antimediator preparations may provide effective therapy.[120] Various other treatments, including vitamin C,[40,140] *Echinacea*[200,274] intranasal humidified air,[94,284] and zinc lozenges,[159,193,233] have been evaluated, and some studies have reported a clinical benefit, although other studies have failed to confirm such findings.[272] Almost all clinical evaluations of symptomatic and alternative therapies have been performed in adults; very few data exist on the utility of such therapy in children.[193,260]

The use of oral or inhaled corticosteroids appears to enhance the shedding of viable virus.[116,239] One potential consequence of corticosteroid use, seen in a study of pediatric patients with a common cold syndrome, is a significant increase in the risk of developing acute otitis media in HRV-infected subjects.[249] Thus, steroids do not have a role in the treatment of HRV infections. Similarly, no role exists for the use of antibiotic therapy in uncomplicated HRV infection.

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CHAPTER 180

HEPATITIS A VIRUS

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HISTORY

Hepatitis A has been recognized as a clinical entity for centuries, and large epidemics of jaundice occurred during military campaigns in ancient and modern times. During the past several decades, epidemiologic and clinical studies defined the infectious nature of the disease, which led to the differentiation of "infectious hepatitis," now designated hepatitis A.[177] Before 1970, attempts to isolate a virus associated with hepatitis A were uniformly unsuccessful, and information concerning the clinical course of infection, the fecal-oral route of transmission, and the efficacy of immunoglobulin in preventing disease was obtained from studies conducted in humans.[177,179,363] In 1973, hepatitis A virus (HAV) was identified by immune electron microscopy in stool samples of patients with hepatitis A.[113] This discovery led to the development of serologic tests that differentiate acute and resolved infections, characterization of the virus, definition of pathogenetic events during infection, and further definition of the epidemiology of HAV infection. HAV has been propagated in cell culture,[74,261,349] which has led to the development and licensure of vaccines shown to be highly efficacious in preventing infection and disease in immunized individuals.[46,156,347]

PROPERTIES

CLASSIFICATION

HAV is a 27-nm, nonenveloped, positive-sense RNA virus belonging to the family *Picornaviridae*. Although HAV initially was classified in the genus *Enterovirus*, nucleotide analysis indicates that HAV is distinct from all other picornaviruses.[193,358] Compared with other enteroviruses, HAV has essentially no nucleotide or amino acid homology, does not have an intestinal replication phase, replicates slowly in cell culture and rarely produces a cytopathic effect, and is relatively resistant to inactivation by heating.[71] For these reasons, HAV has been reclassified in a separate genus designated *Hepatovirus*.[223,358]

GENOMIC ORGANIZATION AND GENETIC VARIATION

The HAV genome is composed of single-stranded, positive-sense RNA containing approximately 7500 nucleotides. HAV genomic organization and replication are similar to those of poliovirus and other picornaviruses: (1) the 5′ end is not translated, contains an internal ribosomal entry site, and has a covalently linked protein (VPg); (2) sequences for structural proteins are located toward the 5′ end, followed by sequences encoding nonstructural proteins, including proteases and RNA polymerases; and (3) the viral RNA encodes a single polyprotein from which functional structural and nonstructural proteins are cleaved proteolytically.[193,350,358] Each capsid structural motif is composed of three major polypeptides (VP1, VP2, and VP3) of 22,000 to 33,000 d that form an outer shell with icosahedral symmetry. On the basis of nucleotide sequence, a fourth polypeptide, VP4 (2500 d), should be encoded but has not been identified in mature virions.[358]

HAV exists as a single serotype. The neutralization site appears to be conformational and derived from epitopes located on VP1 and VP3 as identified by neutralizing monoclonal antibodies.[232,255] A high degree of nucleotide conservation exists among geographically diverse human HAV isolates. However, nucleotide variation in VP1 and VP3 has been used to define four human HAV genotypes and several subgenotypes,[230,270] and this genetic variation has proved useful in identifying clusters and linking apparently sporadic cases.[6,84,151,231,268] Genotype does not appear to influence the clinical presentation or outcome of infection.[124] In addition, HAVs that appear to be restricted in their primary replication to Old World monkeys have been isolated.[233,320]

HAV replicates more slowly in cell culture than do other picornaviruses; wild-type virus requires many weeks of adaptation before infectious foci or HAV antigen is detected.[29,197,290] HAV produces a high ratio of defective to complete (infectious) virus both in cell culture and during infection.[37,358] Cell culture adaptation is associated with mutations in nonstructural proteins and the 5′ nontranslated region.[102,103] Mutations in the VP1/2A and 2C genes are associated with attenuation, and virulence can be restored if genes from the wild-type virus are re-introduced.[101] Cell culture–adapted HAV rarely produces a cytopathic effect, although cytopathic strains have been isolated and serve as a useful model for laboratory studies.[72,235] Adaptation also results in loss of virulence (attenuation) when it is evaluated in the chimpanzee model of infectivity.[164] Cell culture systems that are more permissive to replication of virulent wild-type virus have been reported.[111,174]

The HAV virion appears to be extremely stable, although the molecular determinants of this characteristic are not known. HAV is stable in the environment, with only a 100-fold decline in infectivity when it is stored for longer than 4 weeks at room temperature.[71,213,214,253] The virus retains infectivity when it is treated with nonionic detergents, organic solvents, and low pH at 38° C for 90 minutes.[71] HAV is more resistant than is poliovirus to heat in that it is inactivated only partially at 60° C for 1 hour.[71] Temperatures of 85° C to 95° C for 1 minute are required for complete inactivation of HAV in foods such as shellfish.[71,222] HAV is inactivated completely by formalin (0.02% at 37° C for 72 hours) but appears to be relatively resistant to free chlorine, especially when the virus is associated with organic matter.[205,288] For general-purpose disinfection, a 1:100 dilution of 5 percent sodium hypochlorite (i.e., household bleach) in tap water inactivates HAV in most situations.[46,109]

EPIDEMIOLOGY

ROUTES OF TRANSMISSION

Routes of HAV transmission are determined by the timing and location of virus replication, circulation, and excretion during infection. HAV replicates in the liver, is excreted in bile, and is found in highest concentration in stool (up to 10^8 infectious particles per milliliter).[311] The highest concentration in stool occurs during the 2-week period before jaundice develops or liver enzymes become elevated, followed by a rapid decline after jaundice appears (Fig. 180–1).[113,294,311] Children and infants may shed HAV for longer periods than adults do. Through the use of

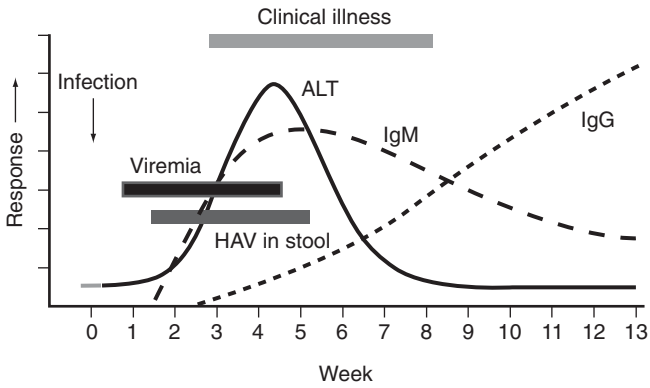

Figure 180–1 Immunologic, virologic, and biochemical events during the course of a typical hepatitis A virus (HAV) infection. ALT, alanine aminotransferase.

polymerase chain reaction (PCR) to amplify viral nucleic acid, HAV RNA has been detected in the stool of infected neonates for as long as 6 months after infection, and some studies have shown excretion in older children and adults 1 to 3 months after the onset of clinical illness.[156,268,271,361] Chronic shedding of HAV does not occur; however, virus may be present in stool during relapsing illness (see the section on relapsing hepatitis A).[293] Viremia occurs during the prodromal stage of infection and extends through the period of liver enzyme elevation (see Fig. 180–1), with virus concentrations several orders of magnitude lower than those in stool.[36,64,178,191] In experimentally infected animals, HAV can be detected in saliva during periods of peak excretion in stool,[64] but transmission by saliva has not been demonstrated.

Detection of HAV antigen in stool by enzyme immunoassay or detection of HAV RNA in serum or stool by PCR does not demonstrate that an infected person is infectious because these assays may detect defective as well as infectious viral particles. Nucleic acid amplification by immunocapture PCR requires the presence of intact virus,[39,164] and HAV RNA may be detected in stool for months with immunocapture PCR. However, the period of infectivity appears to be shorter than the period when HAV RNA can be detected in stool. Data from epidemiologic studies suggest that peak infectivity occurs during the 2 weeks before the onset of symptoms. For practical purposes, both children and adults with hepatitis A can be assumed to be non-infectious 1 week after jaundice appears.

Because of the high concentration of virus in the stool of infected persons, HAV transmission occurs primarily by the fecal-oral route, usually by person-to-person transmission in households and extended-family settings and between sexual contacts.[299] Person-to-person transmission results in high rates of infection in young children in developing countries and has been the predominant mode of transmission in the United States, particularly during community-wide outbreaks, as well as in outbreaks in childcare centers.[22,32] HAV can remain infectious in the environment,[214] and fecal contamination of food or water can result in common-source outbreaks. HAV has been transmitted by transfusion, but such transmission occurs rarely because the blood donation must occur during the early prodromal stage of the disease or from an asymptomatic person who is viremic.[191] In the United States, nucleic acid amplification tests such as PCR now are applied to screening of source plasma used for the manufacture of plasma-derived products. These assays are sufficiently sensitive to remove most units that have HAV, and serosurveillance for HAV infections among clotting factor recipients in the United States indicates that HAV infections are now rare in this risk group.[49]

Two published case reports have described intrauterine transmission of HAV during the first trimester that resulted in fetal meconium peritonitis.[190,215] After delivery, both infants were found to have a perforated ileum. The risk of transmission to newborns by pregnant women in whom hepatitis A develops in the third trimester of pregnancy appears to be low.[317] However, newborns who acquire HAV infection in this manner or from a transfusion usually are asymptomatic, and the infection is detected by the development of hepatitis A in hospital staff or other persons having contact with the infant.[271,342]

PATTERNS OF DISEASE WORLDWIDE

Worldwide, the endemicity of HAV infection differs markedly among and within countries (Fig. 180–2). Patterns of HAV infection can be differentiated, each being characterized by distinct age-specific profiles of prevalence of antibody to HAV (anti-HAV), incidence of hepatitis A, and prevailing environmental (hygienic and sanitary) and socioeconomic conditions (Fig. 180–3).[18,22,140,160]

In areas with a high endemic pattern of infection, represented by the least developed countries (i.e., parts of Africa, Asia, and Central and South America), poor socioeconomic conditions allow HAV to spread readily (see Fig. 180–2). Most persons are infected as young children, and essentially the entire population is infected before reaching adolescence, as demonstrated by the age-specific prevalence of anti-HAV (see Fig. 180–3).[16,73,160,321] Because virtually all HAV infections occur in age groups in which asymptomatic infection predominates, reported disease rates may be low and outbreaks rare.

In areas of intermediate endemicity, HAV is not transmitted as readily because of better sanitary and living conditions, and the predominant age at infection is older than that in highly endemic areas (see Figs. 180–2 and 180–3).[60,160] Paradoxically, the overall incidence and average age of reported cases often increase because high levels of virus circulate in a population that includes many susceptible older children, adolescents, and young adults, in whom symptoms are likely to develop with HAV infection.[137] Large common-source outbreaks also can occur because of the relatively high rate of virus transmission and the large number of susceptible persons, especially among those of higher socioeconomic levels. Such an outbreak occurred in Shanghai in 1988, with more than 300,000 cases associated with the consumption of contaminated clams.[143] Nonetheless, person-to-person transmission in community-wide epidemics continues to account for much of the disease in these countries.[334]

Shifts in age-specific prevalence patterns indicating a transition from high to intermediate endemicity are occurring in many parts of the world (see Fig. 180–3). As this transition occurs, marked variations in hepatitis A epidemiology are seen among countries and within countries and cities, with some areas displaying a pattern typical of high endemicity and others of intermediate endemicity (see Fig. 180–2).* Considerable hepatitis A–related morbidity and associated costs can occur, even in developing countries. Hepatitis A is reported to account for 50 to 60 percent of all acute viral hepatitis cases among children in Pakistan, and 232 children with fulminant hepatic failure secondary to hepatitis A were admitted to one tertiary care referral hospital in Karachi during a 9-year period.[280] Hepatitis A was the etiology of the fulminant hepatitis of two thirds of the children treated at two hospitals in Argentina during a 15-year period; and in one of these hospitals performing liver transplantation, a third of the liver transplantations in children were required because of

*See references 17, 22, 79, 118, 126, 137, 155, 181, 182, 256, 257, 310, 322, 340.

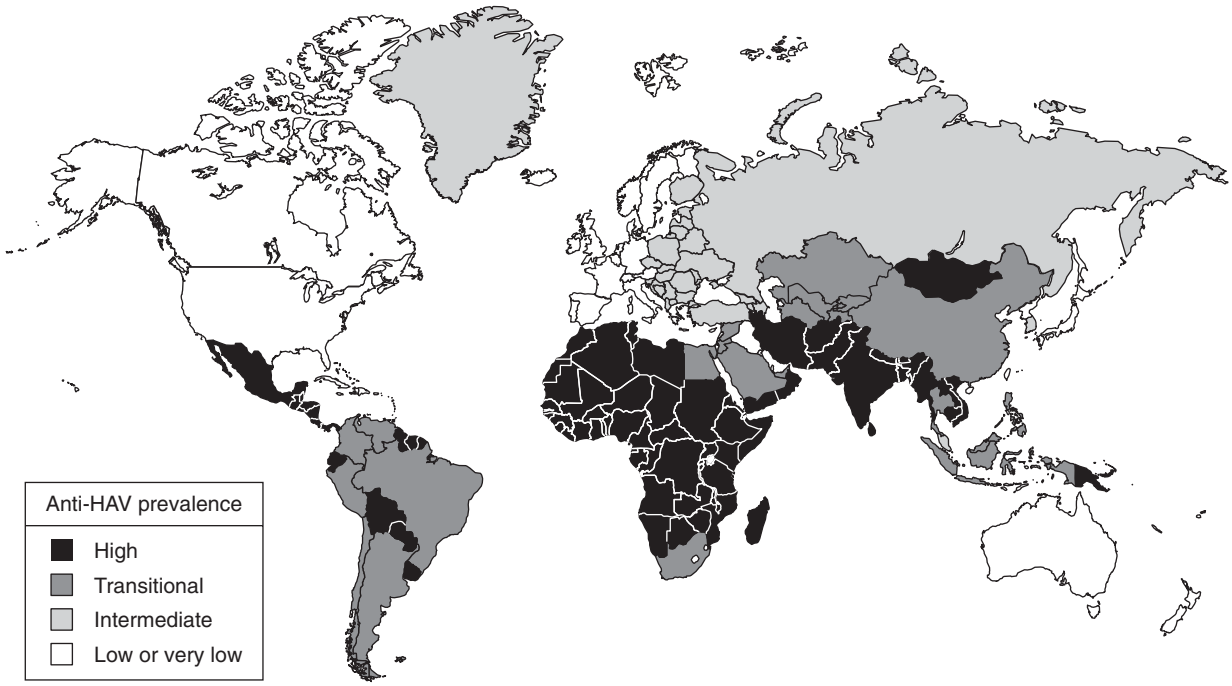

Figure 180–2 Geographic distribution of patterns of endemicity of hepatitis A virus (HAV) infection. The distribution was generalized from the best available data.

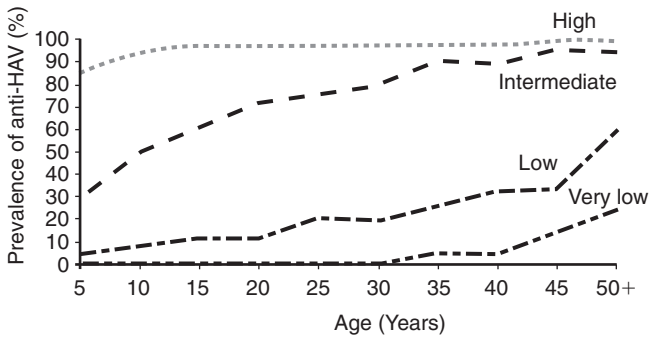

Figure 180–3 Patterns of hepatitis A virus (HAV) infection worldwide.

fulminant hepatitis A.[61] Another small series from Turkey indicated that HAV was the most common identifiable cause of acute liver failure among children, accounting for 26 percent of cases.[13]

In most areas of North America and western Europe, sanitary and hygienic conditions are such that the endemicity of HAV infection is low (see Figs. 180–2 and 180–3). Relatively fewer children are infected, and disease often occurs in the context of community-wide and childcare center outbreaks and occasionally as common-source outbreaks.[24,129,259,281,313] In some regions (e.g., Scandinavia), the endemicity of HAV infection is very low and disease occurs almost exclusively in defined risk groups, such as travelers returning from areas where HAV infection is endemic or illicit drug users.[34]

PATTERNS OF DISEASE IN THE UNITED STATES

Although the epidemiology of hepatitis A in the United States has been altered profoundly by the introduction of hepatitis A vaccines, hepatitis A remains a frequently reported vaccine-preventable infectious disease (Fig. 180–4). Rates have declined sharply since the mid-1990s. In 2004, 5683 cases were reported to the Centers for Disease Control and Prevention (CDC), representing an 82 percent decline compared with rates from 1990 to 1997 and significantly lower than previous incidence nadirs.[45,341] However, national surveillance systems collect data on symptomatic cases, and incidence models indicate that most infections are not symptomatic. One such analysis estimated that an average of 271,000 infections occurred per year during 1980 to 1999, 10.4 times the reported number of symptomatic cases.[8] With use of similar modeling methodology, an estimated 56,000 infections occurred in 2004.[45]

Variation by Age and Race or Ethnicity

Historically, the highest hepatitis A rates were reported among children 5 to 14 years of age, with approximately one third of cases occurring among children younger than 15 years.[51] Because many young children have unrecognized or asymptomatic infection, they also are likely to represent a major reservoir for HAV transmission. Incidence models indicated that during the 1980s and 1990s, more than half of HAV infections occurred among children younger than 10 years, most of whom were younger than 4 years.[8] Since the mid-1990s, decreases in rates among children have been greater than among adults, and the proportion of cases among children dropped from 35 percent in the pre-vaccine era to 19 percent in 2003.[341]

Among racial and ethnic groups, before the use of hepatitis A vaccine, rates among Native Americans and Alaska Natives were more than 10 times the rate in other racial and ethnic groups, and rates among Hispanics were approximately three times higher than among non-Hispanics. These disparities have lessened or disappeared in the era of routine childhood vaccination, with rates among Hispanics in 2003 approximately twice those among non-Hispanics (86% decline compared with 1990-1997); rates among Native Americans and Alaska Natives are now the same as or lower than those among other ethnic groups (Fig. 180–5).[341]

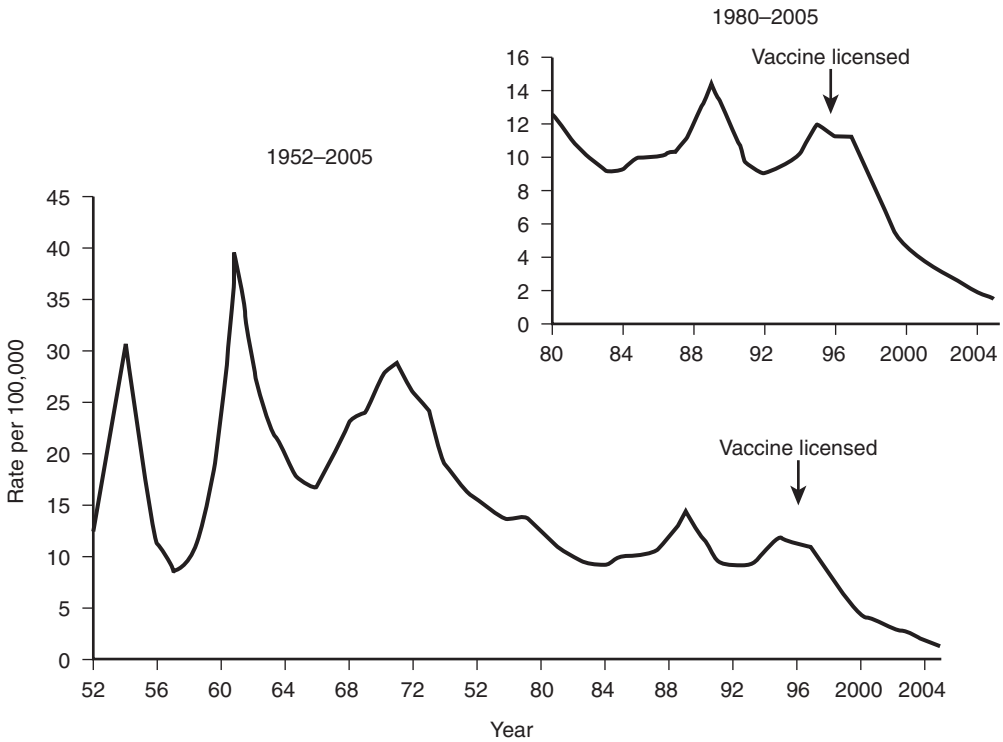

Figure 180–4 Hepatitis A incidence rates (per 100,000 population) based on cases reported to the Centers for Disease Control and Prevention (1966-2005).

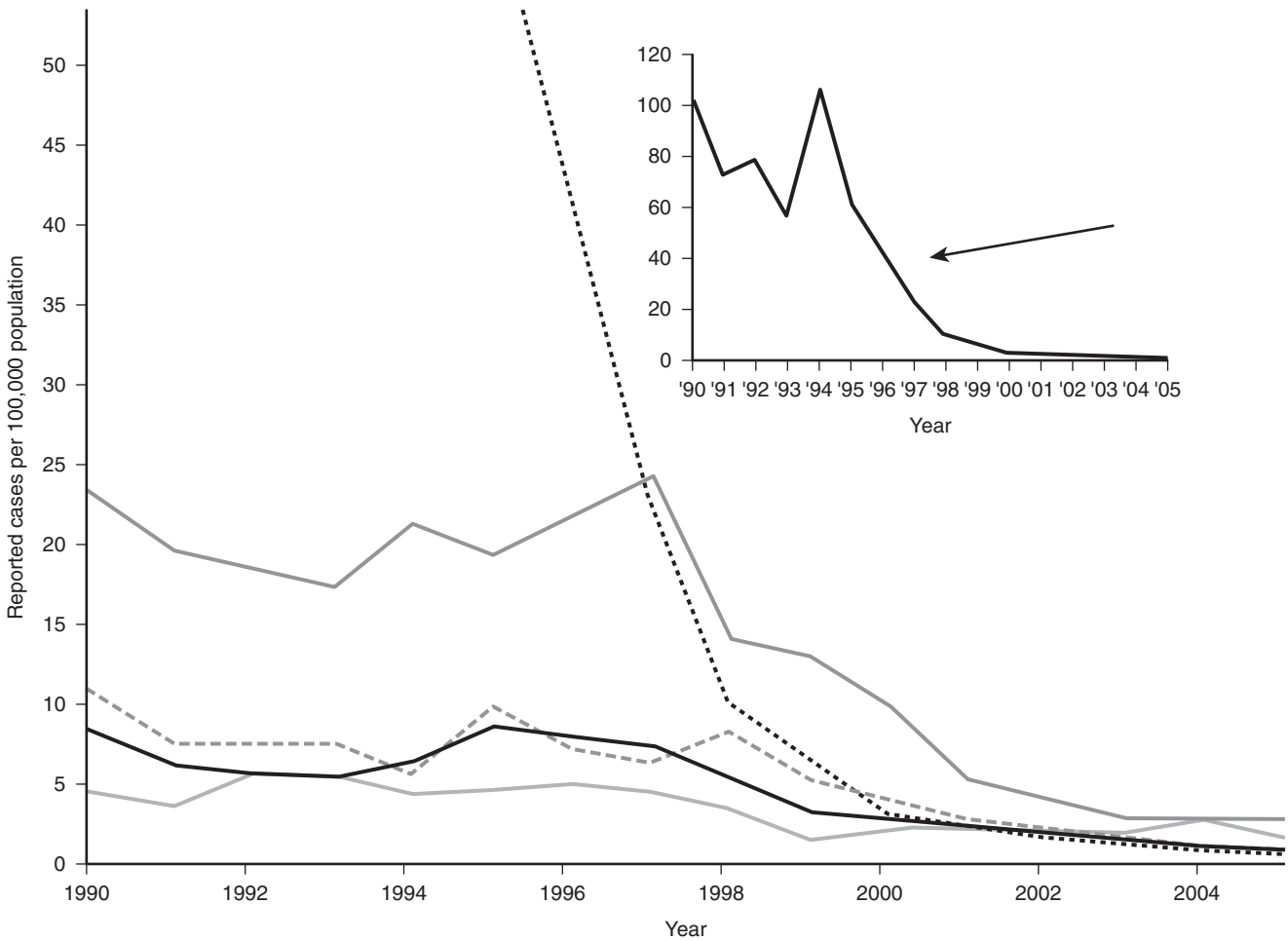

Figure 180–5 Hepatitis A incidence, United States, by race and ethnicity, 1990 through 2005. The black dotted line represents the rate among Native Americans and Alaska Natives; the dark gray line, the rate among Hispanics; the gray dashed line, the rate among blacks; the black line, the rate among whites; and the light gray line, the rate among Asian/Pacific Islanders. The inset depicts the much higher rates among American Indians/Alaska Natives separately, allowing an appropriate y-axis scale to be used. *(Data from the Centers for Disease Control and Prevention, National Notifiable Diseases Surveillance System, Atlanta, GA.)*

As determined by the Third National Health and Nutrition Examination Survey conducted during 1988 to 1994, approximately one third of the U.S. population had serologic evidence of previous HAV infection.[21] Anti-HAV prevalence was related directly to age (ranging from 9 percent in children 6 to 11 years of age to 75 percent in persons older than 70 years), and it was related inversely to income. The age-adjusted prevalence of anti-HAV was higher among foreign-born (68%) than U.S.-born (25%) participants and higher among Mexican Americans (70%) than non-Hispanic blacks (39%) and whites (23%).[21]

Geographic Variation

Analyses of national surveillance data collected during the 1980s and 1990s showed striking regional variation in the incidence of hepatitis A. The national average incidence was approximately 10 cases per 100,000 persons per year during 1987 to 1997, with the highest rates and most cases consistently occurring in a limited number of states and counties concentrated in the western and southwestern United States.[304] Approximately two thirds of cases were reported from the 17 states with the highest rates, even though only one third of U.S. residents lived in these states. With the implementation of routine vaccination of children in these states in 1999, rates equalized across the country,[341] with significantly greater reductions in incidence in areas where vaccination was recommended (Fig. 180–6).[275,341]

Potential Sources of Infection

The distribution of potential HAV exposures that are identified among reported cases also has changed in the vaccination era. The proportion of cases attributable to household or sexual contact with a person who has hepatitis A, the most commonly reported potential source of infection, has declined as routine hepatitis A vaccination of children has increased, from 15 to 25 percent to 12 percent of reported cases in 2005.[45,47] The number of international travel–associated cases has remained approximately the same; but as overall incidence declined, the proportion of cases attributable to this exposure rose to an average of 13 percent during 2002 to 2004. Almost 40 percent of cases among children report recent international travel.[45] Recognized foodborne outbreaks account for a small proportion of cases in most years (3% to 5%), but large outbreaks such as those associated with contaminated green onions in 2003 have increased the proportion of cases associated with food in some years to as high as 16 percent.[6,45,47,348] Cyclic outbreaks occur among men who have sex with men and users of injecting and non-injecting drugs; during outbreak years, this exposure can account for 10 to 15 percent of nationally reported cases.[45,47,67,123,144,150,276] Nearly 50 percent of patients with hepatitis A do not have a recognized source of infection[45,47] but might be contacts of persons, especially children, with asymptomatic infection.

Community-Wide Epidemics

Historically, most cases of hepatitis A in the United States occurred in the context of community-wide epidemics, during which infection is transmitted from person to person in households and extended-family settings.[24] Once initiated, these epidemics often persisted for several years and proved difficult to control,[249,286] even when attempts were made to vaccinate some portion of the population rapidly.[11,69] Children played an important role in HAV transmission during these epidemics. In communities with historically the highest hepatitis A rates, as exemplified by Native American and Alaska Native communities, the pattern of transmission was similar to that found in countries with an intermediate endemicity of HAV infection (see the section on patterns of disease worldwide).[42,83,186,285,355] The best explanation for these observations is that asymptomatic transmission was occurring in susceptible young children at low levels during interepidemic periods until a cohort of susceptible children became large enough to sustain a community-wide outbreak.[285,295] Asymptomatic transmission among children also has been important in sustaining outbreaks in areas with consistently elevated rates. During community-wide outbreaks, serologic studies of members of households with an adult case and no identified source have found that 25 to 40 percent of contacts younger than 6 years had serologic evidence of having had recent HAV infection (CDC, unpublished data).[268,299] In one of these studies, 52 percent of households of adults without an identified source of infection included a child younger than 6 years, and the presence of a young child was associated with household trans-

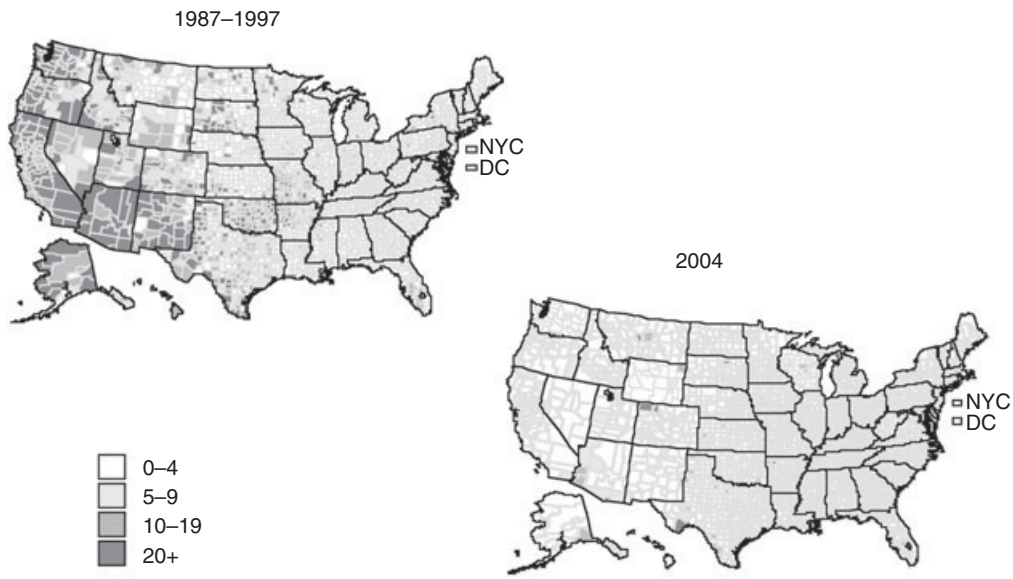

Figure 180–6 Hepatitis A incidence rates by county, United States, 1987 to 1997 *(top)* and 2004 *(bottom)*. (See companion Expert Consult web site for color version.) *(Data from the Centers for Disease Control and Prevention, National Notifiable Diseases Surveillance System, Atlanta, GA.)*

mission of HAV.[299] In this study, transmission chains were identified involving as many as six generations and more than 20 cases. With the advent of routine vaccination of children, community-wide outbreaks have largely ceased. Community-wide outbreaks that have been sustained by transmission among adults in high-risk groups continue to be identified, and novel strategies to provide vaccination in settings such as jails have had some success.[47,231,241,314,338]

EPIDEMIOLOGY OF HEPATITIS A IN SPECIFIC SETTINGS

CHILDCARE CENTERS

The role of children with asymptomatic infection has been recognized in outbreaks in childcare centers since the 1970s.[282,283] Because infection in children usually is mild or asymptomatic, these outbreaks often are not recognized until adult contacts (usually parents) become ill.[142] Outbreaks rarely occur in centers that do not have children in diapers and occur more commonly in larger centers.[142] Both poor hygiene in these children and the need for staff to handle and change diapers contribute to spread. Despite the occurrence of outbreaks when HAV is introduced into a childcare center, studies of childcare center employees do not show a significantly increased prevalence of HAV infection in comparison to control populations.[120,159] On occasion, outbreaks in childcare centers can be the source of more extensive transmission within a community.[86,141,330] However, most disease within childcare centers probably reflects transmission from the community. Hepatitis A outbreaks within childcare centers have become unusual events in recent years and are likely to be eliminated as routine vaccination of all young children is implemented.

OTHER GROUPS AND SETTINGS

Hepatitis A cases in schoolchildren usually reflect disease that has been acquired in the community. However, multiple cases among children within a school may indicate a common-source outbreak.[151] Historically, HAV infection was endemic in institutions for the developmentally disabled; but with smaller facilities and improved conditions, the incidence and prevalence of infection have decreased, and outbreaks rarely are reported in the United States.[305]

During the past two decades, outbreaks have been reported with increasing frequency in illicit drug users in North America, Australia, and Europe.[56,144,150,152,241,276,284,314,338] In the United States, these outbreaks often have occurred in the context of a large community-wide outbreak; and in the past decade, they frequently have involved users of injected and non-injected methamphetamine, who may account for as many as 30 percent of reported cases in these communities during outbreaks.[24,150,152,231,338] Cross-sectional serologic surveys have demonstrated that injecting drug users have a higher prevalence of anti-HAV than the general U.S. population does.[157,336] Transmission among injecting drug users probably occurs through both percutaneous and fecal-oral routes.[152]

Hepatitis A outbreaks in men who have sex with men have been reported frequently, most recently in urban areas in the United States, Canada, England, and Australia.[54,67,123,303] These outbreaks may occur in the context of an outbreak in the larger community.[24] Some studies conducted during outbreaks and seroprevalence surveys among men who have sex with men have identified specific sex practices associated with illness, whereas others have not demonstrated such associations.[67,68,146,165,336]

Transfusion-related hepatitis A rarely occurs because HAV does not result in chronic infection and blood donors are screened for elevated aminotransferase levels. Currently, nucleic acid amplification tests also are used to screen source plasma used in the manufacture of plasma-derived products. However, during the mid-1990s, outbreaks were reported in Europe and the United States in patients who received factor VIII and factor IX concentrates prepared by solvent-detergent treatment to inactivate lipid-containing viruses.[207,297] HAV is resistant to solvent-detergent treatment, and contamination presumably occurred from plasma donors with hepatitis A who donated during the incubation period. The risk of acquiring infection in patients with hemophilia is not known, although data from one serologic survey of hemophiliac patients suggested that they might be at increased risk for acquisition of HAV infection.[204] However, no HAV infections attributed to blood products were identified in an analysis of serosurveillance data collected from 140 hemophilia treatment centers in the United States between 1998 and 2002, suggesting that improved viral inactivation procedures, donor screening, and increased hepatitis A vaccine coverage among clotting factor recipients can reduce the risk of transmission of HAV to recipients of clotting factors.[49] Transmission related to blood transfusions also has resulted in nosocomial outbreaks in neonatal intensive care units.[170,240,271] Hepatitis A has been reported in adult cancer patients treated with lymphocytes that apparently were incubated in serum from a donor with HAV infection, although the patient source was not identified.[345]

Nosocomial transmission from adult patients to health care workers usually has been associated with fecal incontinence of the patient.[135,247] Such transmission is a rare event, however, because most patients with hepatitis A are hospitalized after the onset of jaundice, when infectivity is low.[110,294] Health care workers have not been found to have an increased prevalence of anti-HAV in comparison to control populations in serologic surveys conducted in the United States.[128]

Persons from developed countries who travel to developing countries with a high, transitional, or intermediate endemicity of HAV infection are at substantial risk of acquiring hepatitis A (see Fig. 180–2).[301] A more recent study of Swiss travelers estimated the risk to be 6 to 30 cases per 100,000 months of stay in developing countries among persons who did not receive immune globulin or vaccine before departure.[228] The risk is higher among travelers staying in areas with poor hygienic conditions,[183] it varies according to the region and the length of stay, and it appears to be increased even among travelers who reported staying in urban areas or luxury hotels.[301] In some European countries, returning international travelers with hepatitis A account for a substantial proportion of reported cases (16% to 40%).[59,351] In the United States, the proportion of cases attributed to recent international travel increased from 5 to 7 percent in the 1990s to 18 percent in 2004; children accounted for nearly 50 percent of cases attributed to international travel in 2004.[45] Children from immigrant communities who are visiting friends and relatives in their parents' countries of origin have been identified as an important source of cases. In one large study among San Diego children during 1998-2000, the number of cases increased 1 to 2 months after times when travel was most often reported (Fig. 180–7).[344]

Food-borne hepatitis A outbreaks are recognized relatively infrequently in the United States and are associated most commonly with contamination of food during preparation by a food handler with HAV infection. Implicated foods include those not cooked after handling, such as sandwiches and salads, glazed pastries, ice, and cold drinks, as well as partially cooked foods.* Control of these outbreaks usually requires intensive public

*See references 43, 55, 77, 115, 139, 184, 203, 211, 225, 229, 248, 346.

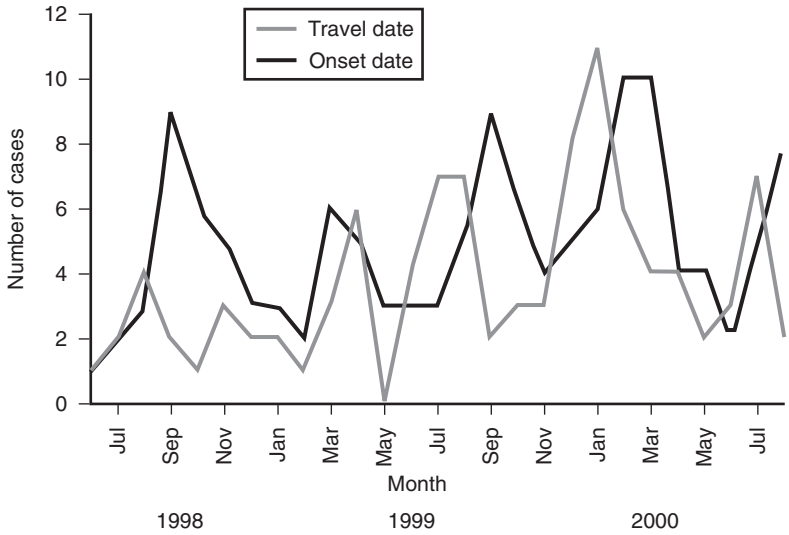

Figure 180–7 Number of hepatitis A cases among children, San Diego County, June 1998–August 2000, by date of onset and date of international travel.[344]

health effort.[77] However, persons who work as food handlers are not at increased risk for acquiring hepatitis A because of their occupation. Food contaminated before retail distribution, such as lettuce or fruits contaminated at the growing or processing stage, has been recognized increasingly as the source of hepatitis A outbreaks.[6,84,151,239,265,272,348] Outbreaks related to contaminated shellfish, commonly noted in the past,[85,90,244,258] have become increasingly uncommon but occasionally are identified.[27] No water-borne hepatitis A outbreaks in the United States have been reported since the 1980s; earlier outbreaks were linked to sewage contamination or inadequate treatment of water.[26,31,35,40,87,122,205]

PATHOGENESIS AND PATHOLOGY

PATHOLOGY

The light microscopic findings in acute hepatitis A, which include inflammatory cell infiltration, hepatocellular necrosis, and liver cell regeneration, are common to all forms of acute viral hepatitis. These histologic findings vary with the stage and severity of hepatitis. Early biopsy specimens generally show portal infiltration by lymphocytes, plasma cells, and periodic acid–Schiff positive macrophages.[91,312] Spotty or focal necrosis, as evidenced by balloon degeneration, shrinkage, and fragmentation of hepatocytes, is seen commonly.[91] HAV antigen is found primarily in the cytoplasm of hepatocytes, but it also can be found in liver macrophages. However, because HAV infection is self-limited and does not result in chronic liver disease, liver biopsy rarely is indicated (see the section on treatment).

Differences have been noted in the light microscopic findings of hepatitis A and other forms of viral hepatitis, particularly hepatitis B. In addition to degeneration of hepatocytes in the perivenular area, periportal inflammation and destruction of hepatocytes adjacent to the portal area may be more pronounced than in hepatitis B.[1,245,312] Findings in some patients, including extension of the inflammatory infiltration from the periportal area into the hepatic parenchyma and disruption of the limiting plate, may be difficult to distinguish from chronic hepatitis.[1,91] Cholestasis may be more prominent than in hepatitis B.[277]

The histologic findings in fulminant hepatitis A are indistinguishable from those in other forms of fulminant viral hepatitis. Examination of pathologic specimens shows massive hepatic necrosis, abnormal architecture of surviving hepatocytes, and a diffuse inflammatory response.[172] Viral antigen can be found in pathologic specimens.

PATHOGENESIS

The mechanism by which HAV crosses gastrointestinal epithelium and infects liver cells has not been established. HAV is able to bind to human cells through a specific cellular receptor inserted into the plasma membrane, and DNA that encodes this receptor (huhavcr-1) has been identified in many human tissues.[112] This cellular receptor, also known as TIM-1, appears to regulate the size and effector functions of T cells and also might play a role in the development of atopy.[216] Other researchers have shown that HAV-IgA complex can translocate across epithelial cells and that HAV-IgA complexes are taken up by hepatocytes through the asialoglycoprotein receptor.[95,96] In this model of HAV infection, virus would first infect gastrointestinal cells and then be transported into the enterohepatic circulation as part of an HAV-IgA complex with subsequent selective uptake by hepatocytes.

CELLULAR IMMUNE RESPONSE

Unlike other picornaviruses, infection of cultured cells with wild-type HAV has no cytopathic effect. The general assumption is that HAV infection also is noncytopathic in vivo and therefore the cytopathic changes in the liver associated with hepatitis A are immune mediated. HAV is able to suppress interferon-β gene expression in the initial stages of infection, which might facilitate prolonged viral production and infection of neighboring cells.[114] Symptoms and biochemical evidence of liver injury do not occur at the time of maximum virus replication and fecal shedding (during the late incubation period). Rather, liver injury is associated closely with viral clearance. $CD8^+$, class 1–dependent, cytotoxic, and virus-specific T cells that are capable of producing interferon-γ are present in the circulation and the liver.[119,324,325] Additional inflammatory cells, recruited to the site of infection by interferon-γ and other cytokines secreted by $CD8^+$ cells, may be responsible for much of the liver injury. Complement has been shown to bind to HAV capsid proteins, and serum complement levels drop during infection, but whether complement-mediated cellular injury occurs is unclear.[210] The mechanism by which infection is resolved remains uncertain.

HUMORAL IMMUNE RESPONSE

Antibodies directed against conformational epitopes displayed on intact virions as well as empty viral capsids are produced during the later stages of infection (see the section on genomic organization and genetic variation). Virus-specific IgM and IgG and IgA antibodies are present in serum; IgM anti-HAV generally can be detected at the onset of symptoms (see Fig. 180–1).

CLINICAL MANIFESTATIONS

Similar to other forms of viral hepatitis, the clinical manifestations of HAV infection are variable and range from asymptomatic anicteric infection to symptoms of acute hepatitis, including fever, malaise, anorexia, nausea, vomiting, right upper quadrant pain, and jaundice. The likelihood of having symptomatic HAV infection and the severity of the illness are related to the age of the patient. In early childhood, infection usually is asymptomatic, whereas infection in adulthood generally is accompanied by symptoms. The diagnosis of hepatitis A must be confirmed by serologic testing because no constellation of symptoms is pathognomonic of the disease.

INCUBATION PERIOD

The average incubation period is 28 to 30 days but can range from 15 to 50 days.[176] The average incubation period has been reported to be shorter in patients who acquired HAV infection by parenteral transmission from contaminated blood products and in chimpanzees infected parenterally than in those infected orogastrically.[210,287]

SPECTRUM OF ILLNESS

HAV infection, confirmed by the detection of IgM anti-HAV in serum, can be inapparent (asymptomatic, with no elevation in serum aminotransferase levels), subclinical (asymptomatic, with elevation of serum aminotransferase levels), or clinically evident (with symptoms). Specific symptoms of liver dysfunction include jaundice and dark urine caused by hyperbilirubinemia. However, symptomatic hepatitis A without jaundice (anicteric) does occur. Nonspecific symptoms of acute hepatitis A can include fever, myalgia, anorexia, nausea, right upper quadrant pain or discomfort, diarrhea, and pruritus.

Many acute HAV infections, particularly inapparent and subclinical infection and anicteric hepatitis A, are not recognized as cases of viral hepatitis.[299,360] The frequency of symptoms with acute infection is influenced strongly by age. Children are less likely to have symptomatic infection than adults are, and jaundice rarely occurs in children younger than 6 years.[131] In one report describing outbreaks in several daycare centers, the proportion of infected children without symptoms was 84 percent in children younger than 3 years, 50 percent in children 3 to 4 years of age, and 20 percent in children 5 years or older.[142] Symptoms develop in most adults with acute infection. In a study of two outbreaks among young adult U.S. military personnel, symptoms developed in 76 to 97 percent of infected persons, and approximately 55 percent were icteric.[187]

CLINICAL SIGNS AND SYMPTOMS

In an individual patient, the clinical symptoms of acute hepatitis A are indistinguishable from those caused by other forms of viral hepatitis. Particularly in older children and adults, the onset of illness often is quite abrupt and may consist of fever, myalgia, anorexia, malaise, nausea, intermittent dull abdominal pain, and vomiting. Fever (temperature rarely higher than 102° F) and headache occur more frequently than in other forms of acute viral hepatitis.[306] Pediatric patients may have diarrhea or, less commonly, upper respiratory symptoms such as cough, sore throat, and runny nose, and the diagnosis of hepatitis A might not be considered in children with predominantly respiratory or gastrointestinal symptoms and transient fever without the typical malaise, fatigue, and anorexia.[117,195] Dark urine followed by jaundice and light-colored stool, if present, will appear within a few days to a week after onset of the prodromal symptoms.[25,138,187,195] When this icteric phase begins, symptoms often resolve and appetite returns in young children, but older children and adult patients may experience a transient worsening in the prodromal symptoms of anorexia, malaise, and weakness.[172,227]

In addition to jaundice and scleral icterus, physical findings may include mild hepatomegaly and tenderness, but severe tenderness suggests other diagnoses. The spleen may be palpable in 10 to 20 percent of patients, and posterior cervical adenopathy may be present.[63,187,318] Pleural effusions have been reported to occur, do not appear to be associated with more severe disease, and resolve spontaneously.[3] Ascites, peripheral edema, and findings indicative of hepatic encephalopathy suggest the presence of a more severe form of hepatitis (see the section on atypical clinical manifestations and complications of hepatitis A). Ultrasonographic findings in children with uncomplicated hepatitis A have included edema or thickening of the gallbladder wall, abdominal lymphadenopathy, and, less commonly, transient ascites and pancreatic abnormalities.[63,169]

The symptoms of hepatitis A last for several weeks on average and usually not longer than 2 months.[173] Prolonged or relapsing hepatitis A can occur (see the section on relapsing hepatitis A) and, in the case of prolonged hepatitis A, may be associated with genetic markers for autoimmune hepatitis.[107]

LABORATORY ABNORMALITIES

As in other forms of viral hepatitis, during HAV infection, inflammation of the liver is accompanied by abnormalities in serum hepatic enzymes, with increases in serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase, and gamma-glutamyltranspeptidase (GGTP) levels. Elevations of serum ALT and AST occur most consistently and may precede the appearance of symptoms by a week or more (see Fig. 180–1). Peak levels generally occur 3 to 10 days after the onset of symptoms and are between 200 and 5000 IU but can reach as high as 20,000 IU. The level of ALT usually is higher than that of AST because the inflammatory response is destructive, particularly to the plasma membrane, in acute viral hepatitis. ALT is found in the cytosol of the plasma membrane, whereas AST is located mainly in cell mitochondria.[138]

Serum bilirubin levels, although frequently elevated, usually remain below 10 mg/dL and peak 1 to 2 weeks after illness begins. Higher levels can be seen in some patients, especially when HAV infection is complicated by cholestasis (see the section on cholestatic hepatitis A) or hemolysis secondary to an underlying glucose-6-phosphate dehydrogenase (G6PD) deficiency state.[58] In patients with G6PD deficiency, indirect bilirubin may account for more than 50 percent of the total bilirubin. Alkaline phosphatase concentration and 5′-nucleotidase activity usually are elevated only mildly, rarely reaching more than 2 or 3 times the normal level. GGTP levels generally are 3 to 10 times the upper limit of normal.

Serum immunoglobulin levels often are elevated, and IgM levels frequently are higher than those in acute hepatitis B and

non-A, non-B hepatitis.[362] In patients without underlying liver disease, the prothrombin time usually is normal. A prolonged prothrombin time, generally associated with severe liver damage, is a prognostic indicator for the development of fulminant hepatitis.[356]

Patients with acute HAV infection usually have a mild lymphocytosis with occasional atypical mononuclear cells.[227] Except for patients who have hemolysis associated with G6PD deficiency, the hematocrit generally is normal.[58,227]

Apart from patients who have relapsing or cholestatic hepatitis A (see the section on atypical clinical manifestations and complications of hepatitis A), serum bilirubin and aminotransferase levels usually return to normal by 2 to 3 months after the onset of illness in most patients.[173]

DIAGNOSTIC TESTS

Because the pattern and magnitude of symptoms and the hepatic enzyme abnormalities of hepatitis A are not distinctive for hepatitis A, the diagnosis requires serologic detection of IgM anti-HAV along with demonstration of an acute onset of signs and symptoms of hepatitis and jaundice or elevated aminotransferase levels. Persons with signs and symptoms of hepatitis and an epidemiologic link to other confirmed cases are likely to have hepatitis A, even if no laboratory confirmation is available. Sensitive and specific radioimmunoassays or enzyme immunoassays show that virtually all patients have detectable IgM anti-HAV during the acute or early convalescent phase of HAV infection (see Fig. 180–1).[200] A small proportion (3%) of patients tested within 3 days of the onset of symptoms may be IgM anti-HAV negative but become IgM anti-HAV positive within the initial 2 weeks of illness.[200] During the first 4 to 8 weeks after the onset of symptoms, the titer of IgM anti-HAV in serum is high.[145] Antibody generally disappears within 6 months, although rarely it can be detected for 2 years or longer.[93,145,171,200] False-positive IgM anti-HAV test results among persons who have no other evidence of recent infection have been reported, suggesting a low positive predictive value when it is used to test asymptomatic persons with no known recent HAV exposures.[44,48] IgM anti-HAV may be detectable for a longer time in patients with symptomatic illness than in those with asymptomatic infection.[145,163,296]

IgG anti-HAV is present in low titer at or shortly after the onset of acute HAV infection, and the titer rises during the course of several weeks as the IgM anti-HAV titer falls (see Fig. 180–1). IgG anti-HAV remains detectable in serum for the lifetime of the individual and confers lasting protection against disease. Secretory IgA antibodies are detected in a minority of humans or primates with acute HAV infection but are unlikely to provide any significant protection against HAV infection.[300] IgG anti-HAV is transferred passively across the placenta and declines to undetectable levels in most infants by the time they reach 12 to 15 months of age.[20,201,202]

Commercially available enzyme immunoassays either detect total (IgG and IgM) antibody against HAV capsid proteins by using a competitive inhibition (blocking) format or detect IgM antibody to capsid proteins by using an IgM capture format.[194] These assays do not measure the neutralizing antibodies responsible for biologic activity against HAV, but detection of total anti-HAV by conventional assays is correlated with the appearance of neutralizing antibodies.[175,194] Neutralizing antibody can be detected by assays that measure inhibition of HAV in cell culture (i.e., radioimmunofocus inhibition test or plaque assay).[7,72,194,196] Neutralizing antibodies elicited against one strain of HAV have been shown to have biologic activity against other HAV strains.[196]

When tested in parallel with a World Health Organization anti-HAV reference reagent, the lower limit of detection of most commercially available assays is approximately 100 mIU/mL of anti-HAV.[127,194] Administration of immune globulin provides a high level of protection against hepatitis A, although the antibody concentrations achieved by passive immunization with immune globulin or active immunization with vaccine are 10- to 100-fold lower than those produced after natural infection.[194] Antibody concentrations achieved by passive or active immunization and known to provide protection against HAV infection both in vivo and in vitro may be below the level of detection of most commercial immunoassays.

The lower limit of antibody necessary to provide protection against HAV infection is unknown. In vitro studies with cell culture–derived virus suggest that low levels of antibody (e.g., <20 mIU/mL) are neutralizing.[196] Clinical trials that evaluated vaccine efficacy have not provided an estimate of the minimum protective antibody level because vaccine-induced levels of antibody have been very high and few infections have occurred in vaccinees. Experimental studies in chimpanzees indicate that very low levels of passively transferred antibody (<10 mIU/mL) obtained from immunized individuals do not protect against infection but prevent clinical hepatitis and shedding of virus.[262]

PCR assays can be used to detect HAV RNA in stools and sera of persons with HAV infection, although these tests are available only in a few research laboratories. PCR techniques using serum specimens have been useful in some clinical, epidemiologic, or environmental studies. Although HAV concentrations in stool decline quickly after illness onset, HAV RNA can be detected in most serum specimens that are collected within 4 to 6 weeks after onset of illness.[36]

ATYPICAL CLINICAL MANIFESTATIONS AND COMPLICATIONS OF HEPATITIS A (Table 180–1)

RELAPSING HEPATITIS A

Relapsing hepatitis is a relatively common manifestation of hepatitis A that occurs in approximately 10 percent of patients.[278] One to 4 months after having the initial episode of acute hepatitis, these patients have a second episode; more than one relapse rarely occurs.[133,278,316] Patients with relapsing hepatitis A have no distinctive clinical features of their first disease episode. After the first episode, most patients experience a significant improvement in symptoms and biochemical abnormalities. However, the frequency with which serum aminotransferase levels completely normalize during this period has been variable; in one report, normalization occurred in only one of seven patients with relaps-

TABLE 180–1 Atypical Clinical Manifestations and Complications of Hepatitis A Virus Infection

Relapsing hepatitis A[133,278,316]
Fulminant hepatitis A[212,242,264,315]
Cholestatic hepatitis A[136,316]
Hepatitis A triggering autoimmune hepatitis[316,332]
Extrahepatic manifestations
Transient rash or arthralgias[130,316]
Papular acrodermatitis of childhood[273]
Cutaneous vasculitis[78,153,154]
Cryoglobulinemia[153,154,278]
Guillain-Barré acute syndrome[306]
Other neurologic syndromes (e.g., myeloradiculopathy, mononeuritis, vertigo, meningoencephalitis)[14,33,251,307]
Renal syndromes (acute renal failure, nephritic syndrome, acute glomerulonephritis)[12,82,161]
Pancreatitis[224]
Aplastic anemia and thrombocytopenia[104,206]

ing hepatitis A.[316] The relapse episode of hepatitis rarely is more severe than the initial episode and is accompanied by elevated serum aminotransferase levels (typically to > 1000 U/L) and persistence of IgM anti-HAV. Molecular studies have demonstrated the presence of HAV in stool and HAV RNA in serum during relapse, but whether patients are infectious is unknown.[133] The illness usually lasts a total of 16 to 40 weeks and results in full recovery.[133,278] Although the pathogenesis of relapsing hepatitis is unknown, it probably is immunologically mediated.[278] Persistent HAV infection with a relapsing clinical course has been reported in patients after they have undergone liver transplantation for fulminant hepatitis A; HAV-specific genomic sequences have been identified in the grafts of these patients.[106,356]

FULMINANT HEPATITIS A

In the United States, a relatively small proportion of all fulminant hepatitis is caused by hepatitis A.[212,246,264,279,315] A prospective multicenter study conducted among 348 children with acute liver failure in the United States, Canada, and the United Kingdom found that only three cases (1%) could be attributed to hepatitis A.[298] However, 26 to 60 percent of children with acute liver failure had hepatitis A in tertiary care center–based studies conducted in Turkey, India, Argentina, and Pakistan.[10,13,280] Among patients hospitalized with hepatitis A, the case-fatality rate has been estimated to be 0.14 percent.[219] In the 1988 Shanghai epidemic that involved primarily adolescents and young adults, 47 deaths (0.015%) were recorded among the 310,746 diagnosed cases.[66] The case-fatality rate among cases reported through national surveillance in the United States from 2001 to 2005 was 0.5 percent but varied by age, from 0 percent among those younger than 5 years to 1.4 percent of cases older than 60 years.[45-47] However, based on all cases of hepatitis A reported in the United States, the case-fatality rate from fulminant hepatitis A is approximately 0.4 percent.[45] Host factors reported to be associated with an increased risk for development of fulminant hepatitis include older age[38,180,219,337,359] and underlying chronic liver disease.[2,80,167,192,331,337,357,359] Reported findings among persons with fulminant disease include lower viral load and nucleotide or amino acid substitutions in the 5′ untranslated region,[125] P2 region, and P3 region of the HAV genome.[230]

Fulminant hepatitis A has no distinctive clinical features that distinguish it from fulminant hepatic failure of other causes. Within approximately 8 weeks of the onset of illness, symptoms of hepatic encephalopathy and marked prolongation of the prothrombin time are noted in patients with no history of previous liver disease.[243] Complications can include cerebral edema, sepsis, gastrointestinal bleeding, and hypoglycemia. The prognosis of fulminant hepatitis A without transplantation is better than that of fulminant disease related to other viral causes, and 40 to 70 percent of patients can be expected to recover.[130,242,279,315] In one hospital series, more rapid onset to liver failure after onset of jaundice was associated with improved outcome, and higher bilirubin concentration and more prolonged prothrombin time were associated with poor outcomes.[81]

EXTRAHEPATIC MANIFESTATIONS

During acute hepatitis A, transient rash and arthralgias occur in as many as 14 and 19 percent of patients, respectively, particularly during the prodromal period.[130,316] Urticaria has been reported but occurs less frequently than in acute hepatitis B.[92] Papular acrodermatitis of childhood, the Gianotti-Crosti syndrome, rarely occurs in the United States but has been reported elsewhere in association with HAV infection.[273] The cutaneous lesions, which consist of nonpruritic, symmetric flat papules on the face, extremities, and buttocks, may persist for several weeks before spontaneously resolving.[97]

Other extrahepatic manifestations that occur chiefly in association with cholestatic or relapsing hepatitis A include cutaneous vasculitis and cryoglobulinemia.[78,153,154] The vasculitis, manifested as erythematous maculopapular lesions often affecting the lower extremities and buttocks and typically associated with purpura, appears as leukocytoclastic vasculitis and granular deposits of IgM anti-HAV and complement in blood vessel walls in skin biopsy specimens. Cryoglobulinemia includes cryoglobulins composed of IgG and IgM and IgM anti-HAV antibodies.[78,153,154,278] These manifestations resolve spontaneously with resolution of the hepatitis.

In the absence of fulminant disease, neurologic syndromes have been observed only rarely in association with hepatitis A. Guillain-Barré syndrome has been reported to occur 3 days to 2 weeks after the onset of hepatitis A, as have myeloradiculopathy, vertigo, mononeuritis (cranial or peripheral nerve), meningoencephalitis, and exacerbation of multiple sclerosis.[14,33,251,307] Renal complications, including acute renal failure, nephrotic syndrome, and acute glomerulonephritis, also rarely have been reported in children who did not have fulminant disease.[12,82,161] Self-limited, mild pancreatitis likewise appears to occur.[224] Reported hematologic complications include aplastic anemia and severe thrombocytopenia.[104,206] Gestational complications, including premature rupture of membranes and placental separation, have been reported among pregnant women with acute hepatitis A, but infants born to these women were healthy otherwise.[99]

CHOLESTATIC HEPATITIS A

Cholestatic hepatitis occurs in a small percentage of patients with hepatitis A. These patients are deeply icteric and may have pruritus, fatigue, fever, loose stools, anorexia, dark urine, and weight loss. In two reports of 10 patients with cholestatic hepatitis A, peak serum bilirubin levels generally were higher than 10 mg/dL, with some as high as 38 mg/dL, and remained elevated for 12 to 16 weeks.[136,316] Serum aminotransferase levels declined but remained elevated during the period of cholestasis. In one of these reports, five patients had prolonged prothrombin times that normalized with the administration of vitamin K.[136]

Cholestatic hepatitis A can be distinguished from obstructive jaundice by normal abdominal ultrasound findings. Conducting further invasive diagnostic procedures, such as liver biopsy or direct forms of cholangiography, is not necessary in most cases.[278] Although patients will recover completely without therapy, a course of corticosteroids with a gradual taper during a span of at least 4 weeks has been reported to hasten relief of symptoms and resolution of cholestasis.[278]

HEPATITIS A TRIGGERING AUTOIMMUNE HEPATITIS

Several reports have described patients in whom hepatitis A is followed by type 1 autoimmune chronic hepatitis.[263,316,332] Laboratory studies demonstrated a T-cell defect in these patients, thus suggesting a genetic predisposition to the development of autoimmune hepatitis that is "triggered" by HAV infection. These patients have required corticosteroid therapy, sometimes for long periods.

TREATMENT

Hepatitis A has no specific therapy, and because HAV infection is self-limited and does not result in chronic infection or chronic liver disease, treatment generally is supportive. Hospitalization

may be necessary for patients who are dehydrated from nausea and vomiting or who have fulminant hepatic failure. Because no conclusive data indicate that bed rest or inactivity influences the course of illness, no restriction of activity is necessary. Similarly, no specific diet is indicated, although many patients may have an intolerance to fatty foods during their illness. Medications, particularly those that have the potential to cause hepatic damage and those that are metabolized by the liver, including acetaminophen, should be used with caution. The half-life of these medications may be prolonged.

A specific therapy for fulminant hepatic failure caused by hepatitis A has not been established. Small uncontrolled trials conducted among adults in the 1980s suggested that some patients benefited from prostaglandin E or interferon, but no additional data to indicate that these therapies are efficacious have been published. No evidence exists that exchange transfusions, plasmapheresis, or corticosteroids are effective.[105,138] Adults with fulminant hepatitis A who received prostaglandin E or interferon have been reported to show improvement in small, uncontrolled trials.[199,291] Amantadine and ribavirin have shown activity in vitro against HAV.[70] Liver transplantation is successful in some patients.[315,356] Persistent HAV infection has been demonstrated in some transplant recipients, but whether it affects survival is unknown.[98,106] Because survival rates of adult and pediatric patients are relatively high without transplantation and no single factor is predictive of a poor outcome, establishment of criteria for choosing candidates for transplantation has been difficult.[130,243,315,329] Reported survival rates after transplantation in patients with fulminant hepatitis from all viral causes range from 40 to 89 percent, depending on the severity of liver failure and other factors.[188,315,356]

PREVENTION

In addition to general measures of good personal hygiene, particularly handwashing, provision of safe drinking water, and proper disposal of sanitary waste, pre-exposure or postexposure immunization primarily with hepatitis A vaccine or with immune globulin can prevent the acquisition of hepatitis A.

IMMUNE GLOBULIN

Immune globulin is a sterile solution of antibodies prepared by a serial cold ethanol precipitation procedure from pooled human plasma that has tested negative for hepatitis B surface antigen, antibody to human immunodeficiency virus (HIV), and antibody to hepatitis C virus.[65] This precipitation procedure has been shown to inactivate hepatitis B virus and HIV.[57] Since 1995, immune globulin prepared in the United States has been required to be negative for hepatitis C virus RNA by PCR amplification or to be produced by a method that ensures additional virus inactivation. When it is administered before exposure or within 2 weeks after exposure, immune globulin is more than 85 percent effective in preventing hepatitis A by passive transfer of anti-HAV.[171,226,302] Whether immune globulin completely prevents infection or leads to asymptomatic infection and the development of persistent anti-HAV (passive-active immunity) probably is related to the amount of time that has elapsed between exposure and administration of immune globulin.[195,302] Although in recent years immune globulin lots have had slightly lower titers of anti-HAV, probably because of a decreasing prevalence of previous HAV infection in plasma donors, no clinical or epidemiologic evidence of decreased efficacy has been reported.[309]

Previously unvaccinated household and sexual contacts of patients with hepatitis A should receive hepatitis A vaccine or immune globulin as soon as possible but no later than 2 weeks after exposure.[44a] Aggressive use of immune globulin or vaccine is indicated to control hepatitis A outbreaks in childcare centers in which hepatitis A is diagnosed in a child or employee[44a,46,283] and in other settings (e.g., hospitals, facilities for developmentally disabled persons) when outbreaks occur.[449] When a food handler is identified with hepatitis A, postexposure prophylaxis should be administered to other food handlers at the establishment and under limited circumstances to patrons.[43,44a,115] Once cases are identified that are associated with a food service establishment, it generally is too late to administer postexposure prophylaxis to patrons because the 2-week postexposure period during which prophylaxis is effective will have passed.

Immune globulin also may be given to persons who are traveling to countries with high, transitional, or intermediate endemicity of HAV infection (see Fig. 180–2) instead of or in addition to hepatitis A vaccine.[44a] Immune globulin should be given to children younger than 12 months who are traveling to such countries because hepatitis A vaccine is not licensed in the United States for children in this age group (see the section on vaccines).[44a] Although hepatitis A often is asymptomatic in infants and young children, pre-exposure prophylaxis is indicated to prevent the rare severe cases and transmission to others after return from abroad.

For postexposure prophylaxis, 0.02 mL/kg body weight of immune globulin is administered intramuscularly. For infants and young children, the injection can be administered in the anterolateral aspect of the thigh or the deltoid muscles; for older children and adolescents, the injection should be administered in the deltoid or gluteus muscles, into which a large volume of immune globulin can be injected.[50] If the immune globulin is administered in the gluteus, the injection should be given in the superior-lateral aspect to avoid injury to the sciatic nerve.[50]

For pre-exposure prophylaxis of travelers, the dose of immune globulin is 0.02 mL/kg body weight if travel will be for less than 3 months. Because of the decay of passive immunity during the course of time, a dose of 0.06 mL/kg is necessary for persons who will be abroad for 3 to 5 months, and re-administration every 5 months is necessary for extended trips. Hepatitis A vaccine, if it is not contraindicated, is a better choice for such persons.

Serious adverse events from immune globulin rarely occur. Because anaphylaxis has been reported after repeated administration to persons with IgA deficiency, these persons should not receive immune globulin.[100] Pregnancy or lactation is not a contraindication to the administration of immune globulin. For infants and pregnant women, a preparation that does not include thimerosal is preferable.

Immune globulin does not interfere with the immune response to oral poliovirus vaccine, to yellow fever vaccine, or, in general, to inactivated vaccines. However, immune globulin can interfere with the immune response to some live attenuated vaccines (e.g., measles-mumps-rubella vaccine [MMR], varicella vaccine). Administration of MMR and varicella vaccines should be delayed for at least 3 months and at least 5 months, respectively, after the administration of immune globulin. Immune globulin should not be given within 2 weeks after the administration of MMR or within 3 weeks of the administration of varicella vaccine, unless the benefits of immune globulin administration are greater than the benefits of vaccination.[50] For travelers younger than 1 year in whom the use of immune globulin may interfere with the administration of other needed vaccines (e.g., MMR, varicella), the use of inactivated hepatitis A vaccine could be considered (see the section on inactivated vaccines).

HEPATITIS A VACCINE

The ability to propagate HAV in cell culture allowed the development of hepatitis A vaccines. Both inactivated and live attenu-

ated hepatitis A vaccines have been developed by use of defined isolates from infected cell lines.[88,208,209,221,260,292] However, only inactivated vaccines have been evaluated for efficacy in controlled clinical trials and licensed in the United States, and the live attenuated vaccines evaluated to date do not appear to offer any distinct advantage over inactivated vaccines.[156,347]

Vaccine Preparation and Performance

Inactivated hepatitis A vaccines are prepared by a method similar to that used to prepare inactivated poliovirus vaccine, by propagation of cell culture–adapted virus in human fibroblasts, purification by ultrafiltration or other methods, formalin inactivation, and adsorption to an aluminum hydroxide adjuvant.[9,62] Inactivated vaccines using the HM175 strain and the CR326F′ strain have been licensed in pediatric and adult formulations for intramuscular administration and are available in the United States for persons 12 months of age and older (Table 180–2). One of these vaccines (HM175 strain) is formulated with 2-phenoxyethanol as a preservative, whereas the other is formulated without a preservative (see Table 180–2).[250] The antigen content of one vaccine (CR326F′ strain) is expressed as units of HAV antigen as defined by a standard; the antigen content of the other vaccine (HM175 strain) is determined by reactivity in a quantitative immunoassay for HAV antigen and is expressed as enzyme-linked immunosorbent assay units (ELU; see Table 180–2).

Two other inactivated hepatitis A vaccines are manufactured and available in Europe and other parts of the world.[154,185,252,335] In addition, a combination inactivated hepatitis A and recombinant hepatitis B vaccine is available in the United States for persons 18 years and older[46]; a pediatric formulation is available in Europe, Canada, and other parts of the world.[89]

In extensive studies in children and adults, the inactivated hepatitis A vaccines available in the United States have been found to be highly immunogenic. In general, after one dose of vaccine, 95 to 100 percent of children 1 year or older and adults respond with concentrations of antibody considered to be protective; a second dose given 6 to 18 months later results in a boost in antibody concentration and probably is important for long-term protection.[15,20,62,148,218,234] Persons whose second dose is delayed as long as 3 to 8 years after the first dose have responses to vaccination similar to the responses of those who receive the vaccine on licensed schedules.[19,158,354] Studies conducted among infants and children younger than 18 months have demonstrated that simultaneous administration of hepatitis A vaccine with diphtheria–tetanus–acellular pertussis (DTaP), *Haemophilus influenzae* type b (Hib), hepatitis B, MMR, and inactivated poliovirus vaccines does not affect the immunogenicity and reactogenicity of these vaccines.[20,76,132,220,323] IgM anti-HAV occasionally can be detected by standard assays, primarily if it is measured soon (i.e., 2 to 3 weeks) after vaccination (CDC, unpublished data).[289,333]

Conditions that may result in reduced immunogenicity include HIV infection, chronic liver disease, and older age. Among adults with HIV infection, 61 to 87 percent had protective antibody concentrations after completing the vaccination series.[147,168,237,339] Higher $CD4^+$ T-lymphocyte count at baseline was associated with response to vaccination.[147,168,267,339] However, HIV-infected persons with normal or near-normal $CD4^+$ T-lymphocyte counts have response rates that exceed 95 percent.[343] Among persons with chronic liver disease, seroprotection rates were similar to those observed among healthy adults, but the final antibody concentrations were substantially lower.[166,189]

Studies in children younger than 1 year indicate that the vaccine is safe and immunogenic for those who do not have passively transferred antibody from previous maternal HAV infection.[20,198,254,319] In studies of infants who received hepatitis A vaccine according to several different schedules, those with passively transferred maternal antibody at the time of vaccination responded, but final antibody concentrations were approximately one third to one tenth those of infants who did not have passively transferred antibody and were vaccinated according to the same schedule.[20,76,198,201,254,319] The clinical significance, if any, of these lower antibody concentrations is unknown. One study found that all infants vaccinated in the presence of passively transferred maternal antibody responded to a booster dose given 6 months later with an anamnestic response, suggesting that they had been primed by the primary series.[76] However, in another small study, two of six children who had lost detectable antibody did not have an anamnestic response to a booster dose administered approximately 6 years after they had received the primary vaccine series in infancy in the presence of passively transferred antibody.[116] Most infants born to anti-HAV–positive mothers have lost detectable antibody by the time they reach 12 to 15 months of age, and both pediatric hepatitis A vaccines are now licensed for use in children as young as 12 months.[121,202]

Inactivated hepatitis A vaccine has been shown to be highly efficacious in preventing clinically apparent disease. In a study of approximately 40,000 Thai children 1 to 16 years of age, the efficacy of inactivated vaccine (HM175 strain) was 94 percent (95% confidence interval, 79% to 99%) after two doses (360 ELU per dose) administered 1 month apart.[156] In a study of another inactivated vaccine (CR326F strain) involving approximately 1000 children aged 2 to 16 years in a New York community with high hepatitis A rates, efficacy was 100 percent (lower bound of the 95% confidence interval, 87%) starting 17 days after the administration of one dose (25 U).[347]

Since hepatitis A vaccines became available in the United States in 1995, studies, demonstration projects, and surveillance data have evaluated their effectiveness in controlling and preventing the development of hepatitis A in communities. In areas with the highest hepatitis A rates, such as Native American and Alaska Native communities, vaccination of the majority of children—and in some cases adolescents and young adults—resulted in a rapid decline in the incidence of disease, and with ongoing routine vaccination of children, the reduction in incidence of disease has been sustained.[28,52,217] In larger, more heterogeneous

TABLE 180–2 Recommended Doses and Schedules for Inactivated Hepatitis A Vaccines Available in the United States[46]

Age (yr)	Vaccine*	Dose†	Volume (mL)	No. of Doses	Schedule‡ (mo)
1-18	HAVRIX	720 ELU	0.5	2	0, 6-12
	VAQTA	25 U	0.5	2	0, 6-18
≥19	HAVRIX	1440 ELU	1.0	2	0, 6-12
	VAQTA	50 U	1.0	2	0, 6-18
>18	TWINRIX	720 ELU	1.0	3	0, 1, 6§

**HAVRIX is manufactured from HAV strain HM175 by GlaxoSmithKline; VAQTA is manufactured from HAV strain CR326F′ by Merck & Co, Inc.; TWINRIX is a combined hepatitis A and hepatitis B vaccine that also contains 20 μg per dose recombinant hepatitis B surface antigen protein.*

†ELU, enzyme-linked immunosorbent assay units.

‡Zero months indicates initial dose; subsequent numbers represent months after the initial dose.

§An alternative 4-dose schedule, given on days 0, 7, 21-30, followed by a dose at month 12, also is licensed in the United States.

communities with lower but consistently elevated hepatitis A rates, interruption of ongoing community-wide epidemics by vaccination of children proved more difficult.[69] In contrast, sustained routine vaccination of children can reduce hepatitis A incidence markedly during the course of time (see vaccine recommendations and use).[11,75,94,341]

Hepatitis A vaccine also is effective when it is used to prevent infection after exposure. Hepatitis A vaccine administered soon after exposure prevented infection in a chimpanzee model.[269] Hepatitis A vaccine was found to be 79 percent efficacious in preventing infection compared with no treatment in a small randomized trial conducted in Italy.[274] However, the confidence interval was wide (7% to 95%), and the study did not include a comparison group that received passive postexposure prophylaxis with immune globulin.[23] In a randomized controlled trial conducted in Kazakhstan, among persons aged 2 to 40 years who were contacts of hepatitis A cases, the efficacy of hepatitis A vaccine administered within 14 days after exposure to HAV was shown to be similar to that of immune globulin in healthy children and adults less than 40 years of age.[333] Based on their results and because the vaccine offers a number of advantages over immune globulin, the Advisory Committee on Immunization Practices (ACIP) of the U.S. Public Health Service currently recommends the hepatitis A vaccine in preference to immune globulin for postexposure prophylaxis of healthy persons from the ages of 12 months to 40 years.[44a] Advisory groups or expert panels in some other countries also have recommended that hepatitis A vaccine be used as the primary means of preventing hepatitis A after exposure.[236,308]

Experience to date indicates that the incidence of adverse events after vaccination is comparable to that after the administration of other widely used vaccines. In prelicensure clinical studies in children, the side effects reported most frequently included soreness, tenderness, warmth, or induration at the injection site (4% to 19%); feeding problems (8%); and headache (4%).[46,132,220] Through 2005, more than 188 million doses have been sold worldwide, including more than 50 million doses in the United States.[46] No serious adverse events in children or adults have been identified that could be definitively ascribed to hepatitis A vaccine.[30,46] The Vaccine Adverse Events Reporting System (maintained by the U.S. Food and Drug Administration and the CDC) has received approximately 6000 reports of adverse events occurring after receipt of hepatitis A vaccine. The most common events were minor and of brief duration, such as fever, injection-site reactions, rash, and headache. The 871 serious adverse events included Guillain-Barré syndrome, transaminitis, idiopathic thrombocytopenic purpura, and seizures among children.[46,238] Rare adverse events reported postmarketing include syncope, jaundice, erythema multiforme, anaphylaxis, brachial plexus neuropathy, transverse myelitis, encephalopathy, and others. For events for which incidence rates are available, such as Guillain-Barré syndrome, reported rates were not higher than reported background rates.[46]

Anti-HAV persists at protective levels in vaccinated adults for at least 10 to 12 years after vaccination[327,328] and in children for at least 5 to 6 years after vaccination.[108,326] In one follow-up study, two thirds of infants who did not have passively transferred maternal antibody at the time of vaccination had detectable anti-HAV 6 years later, and all who had lost antibody had an anamnestic response to a booster dose.[116] Estimates based on kinetic models of decline in antibody suggest that the duration of protection could be 15 to 25 years or longer.[326,352,353]

In some settings, performing pre-vaccination serologic testing may be considered in an attempt to reduce cost by not vaccinating persons with previous immunity.[41] Testing of children is not indicated because of their expected low prevalence of infection and the lower cost of vaccine for this age group. Testing may be considered for older adolescents in certain population groups with a high prevalence of infection (e.g., Native Americans, Alaska Natives), but the cost of testing, the vaccine cost, and the likelihood that the person will return for vaccination should be taken into account. Post-vaccination testing is not indicated because of the high rate of response to the vaccine. Furthermore, most anti-HAV testing methods licensed for use in the United States cannot reliably detect the low anti-HAV concentrations generated by immunization.

Vaccine Recommendations and Use

Recommendations for the use of hepatitis A vaccine were issued first by the ACIP (Table 180–3), the American Academy of Pediatrics, and other groups in 1996 and updated in 1999 and 2006.[4,46,51,53] As part of an incremental strategy aimed at achieving widespread routine vaccination, the 1999 recommendations called for routine vaccination of children living in areas where rates of hepatitis A consistently had been elevated (see Table 180–3). Various vaccination strategies that were suggested included vaccinating one or more single-age cohorts of children or adolescents, vaccinating children in selected settings (e.g., daycare), and vaccinating children and adolescents over a wide range of ages in a variety of settings, such as when they seek health care for other purposes. The final step in this incremental strategy, routine vaccination of children nationwide, was recommended by the ACIP in 2006.[46]

The impact of routine vaccination of children initially was shown in areas that historically have had the highest hepatitis A rates (e.g., Native American communities), where this strategy had been recommended since 1996. Surveys conducted in 1999 to 2000 indicated vaccination coverage of 50 to 80 percent of preschool- and school-age Native American and Alaska Native children, thus suggesting that the recommendation for routine vaccination was being implemented.[28] By 2000, the incidence of

TABLE 180–3 ACIP Recommendations for Routine Preexposure Use of Hepatitis A Vaccine

Group	Comments
All children at age 12-23 months*	Integrate into routine childhood vaccination schedule; children who are not vaccinated by age 2 years can be vaccinated at subsequent visits
Children aged 2-18 years	Maintain existing program†; can be considered in areas without existing programs
International travelers	Except persons traveling to Canada, western Europe, Japan, Australia, or New Zealand, who are at no greater risk than in the United States
Men who have sex with men	Includes adolescents
Illicit drug users	Includes adolescents
Persons with chronic liver disease	Increased risk of fulminant hepatitis A with HAV infection
Persons receiving clotting factor concentrates	
Persons who work with HAV in research settings	
Anyone wishing to obtain immunity	

**Hepatitis A vaccine is not licensed for children younger than 12 months.*

†States covered by 1999 ACIP recommendations (Alabama, Alaska, Arizona, California, Colorado, Idaho, Minnesota, Missouri, Nevada, New Mexico, Oklahoma, Oregon, South Dakota, Texas, Utah, Washington, Wyoming, and selected areas in other states).[51]

From Centers for Disease Control and Prevention: Prevention of hepatitis A through active or passive immunization. Recommendations of the Advisory Committee on Immunization Practices (ACIP). M. M. W. R. Recomm. Rep. 55(RR-7):1-23, 2006.

hepatitis A in Native Americans and Alaska Natives had declined by 97 percent compared with the beginning of the preceding decade and was the same as the overall U.S. rate.[28]

National surveillance data indicate that routine vaccination of children living in areas with consistently elevated rates, which was recommended in 1999, has had an impact on the overall incidence of hepatitis A in the United States. Since 2001, incidence rates in each successive year have been historic lows, with the 2004 rate of 1.9 cases per 100,000 population demonstrating a 79 percent decline compared with previous lows observed in the early 1990s (see Fig. 180–4). Rates declined most dramatically among children in parts of the country where routine vaccination of children was recommended and where the estimated coverage was highest.[341]

Vaccine coverage data are limited but indicate that declines in incidence have occurred with modest levels of coverage, suggesting a strong herd immunity effect.[5,75,275] Modeling studies suggested that 39 percent of potential cases were averted in 2001 because of the direct effects of immunization and herd immunity.[275] Similar observations have been made in Israel and Spain. The incidence of hepatitis A declined by 95 percent in Israel within 3 years after initiation of an immunization program among children 18 to 24 months old, even though no catch-up vaccination of other age groups was attempted.[75] Routine vaccination of 12-year-old children in Catalonia, Spain, was followed by a 58 percent reduction in overall incidence.[94] Hepatitis A incidence is cyclic, and data from additional years are needed to verify that low rates are sustained and attributable to vaccination and to provide a definitive determination of the overall impact of this strategy of routine childhood vaccination. However, the consistency and strength of these data render alternative explanations unlikely.

The data indicating that vaccination of children could have a powerful impact on incidence rates in all ages and the demonstration of vaccine safety and immunogenicity for children as young as 1 year were key factors supporting the decision to expand routine vaccination to all children aged 12 to 23 months in the United States. Economic analyses also were considered. One study estimated that routine vaccination given when children reached age 1 year in the United States would result in 183,806 fewer infections and 32 fewer deaths in a single U.S. birth cohort, with a cost-effectiveness ratio of $173,000 per life year gained and $24,000 per quality-adjusted life year gained.[266]

Vaccination of persons at increased risk of acquiring hepatitis A, including travelers to countries where hepatitis A is endemic, adolescent and adult men who have sex with men, persons who use illegal drugs, those who work with HAV in research settings, and persons who have clotting factor disorders, also is recommended.[46] Persons with chronic liver disease who acquire hepatitis A have been reported to have a high case-fatality rate and are recommended for vaccination also.[46]

Vaccination of successive cohorts of children eventually should result in a sustained reduction in the incidence of disease nationwide and thus provide the opportunity to eliminate HAV transmission. To achieve this goal, implementation of recent ACIP recommendations for vaccination of young children nationwide will be needed. Pediatric combination vaccines that include hepatitis A vaccine would facilitate this effort.

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SUBSECTION 2

Caliciviridae

CHAPTER 181

Caliciviruses

David O. Matson

Caliciviruses currently include four genera that differ in genomic organization and phylogenetic comparisons of genomic sequence identity. Recent studies have blurred distinctions among the genera in the host of origin and ecologic relationships.

Caliciviruses were recognized to be distinct pathogens in 1932, when outbreaks of a vesicular exanthem restricted to domestic pigs occurred in California (vesicular exanthem of swine virus [VESV] infection, prototype of the *Vesivirus* genus of *Caliciviridae*).[12] The illness initially was thought to be foot-and-mouth disease of cattle, and the potential for confusion between foot-and-mouth disease and VESV in an era when rapid and definitive diagnostic assays were unavailable led to the designation of VESV as a foreign animal disease and to extensive programs to eradicate the agent. These programs continued for decades and had a major impact on the agricultural industry of the West Coast of the United States. VESV strains, when later visualized by electron microscopy, had distinctive and unique features that led to the name *calicivirus* (from "chalice," for the virion's surface cups). A program of sterilization of feed and restriction of feed types finally stopped the outbreaks in the 1950s. In the 1970s, the source of the outbreaks was suggested to be marine animals fed to pigs, especially fish meal.[243] The hypothesized existence of a close relationship between caliciviruses isolated from marine mammals and VESVs was proved by genome sequencing, which established that these caliciviruses have marine and terrestrial reservoirs.[203]

Caliciviruses in humans were recognized first in 1972, when viral particles were linked to an outbreak of gastroenteritis in schoolchildren, teachers, and their household contacts in Norwalk, Ohio.[4,137] The Norwalk agent (prototype of the *Norovirus* genus) was round and had a rough particle surface when visualized by electron microscopy, but these features did not permit any definitive classification to be made. Many similar small, round-structured viruses (SRSVs) were described subsequently from outbreaks of gastroenteritis around the world. A few experiments with these outbreak agents suggested that at least some of them had physical properties similar to those of the known animal caliciviruses.[136]

In the 1970s, investigators using electron microscopy to survey diarrhea stool specimens from children visualized particles that did not have the appearance of SRSVs but instead were similar to the animal caliciviruses previously identified.[6,165,185] These "typical" caliciviruses occurred in a small percentage of sporadic diarrhea stool specimens and in a few outbreaks of gastroenteritis, including an outbreak in an orphanage in Sapporo, Japan (prototype of the *Sapovirus* genus). Genomic sequencing has confirmed that the SRSVs and human viruses with typical calicivirus morphologic features are caliciviruses.[131,160,181]

In 1984, a highly fatal, highly contagious hepatitis was observed in European rabbits bred in China.[275] This syndrome spread some 7000 miles across Asia into Europe and reached Spain

within 4 years, a dispersal rate of approximately 5 miles (8 kilometers) per day in a sedentary host. European rabbit populations experienced mortality rates exceeding 90 percent within 3 days of exposure. Typical calicivirus particles were visualized in infected rabbit livers, and this calicivirus, the rabbit hemorrhagic disease virus (RHDV; prototype of the *Lagovirus* genus), eventually was linked to the syndrome.[209,215]

These summaries illustrate the diversity of the ecologic relationships of the caliciviruses, highlight the broad spectrum of illness (from mild gastroenteritis to fatal hepatitis) associated with members of the family, and indicate how recently many of these widespread caliciviruses have been discovered.

PROPERTIES OF CALICIVIRUSES

Evidence of human infection has been detected for each of the four calicivirus genera. Each genus is discussed, with special emphasis on human infection.

STRUCTURAL FEATURES OF THE VIRION

The *Caliciviridae* is a family of nonenveloped RNA viruses classified until 1978 within the *Picornaviridae*.[40] The caliciviral genome is a positive-sense, single-stranded RNA molecule of approximately 8000 nucleotides. Caliciviruses have a single structural polypeptide with a molecular weight of approximately 60,000 d. Typical calicivirus virion particles are 40.5 nm in diameter and have an icosahedral symmetry with 32 cup-shaped surface depressions (Fig. 181–1).[221] Distinguishing the identifying features of a calicivirus in a stool specimen is not always easy (see Fig. 181–1B). Staining of particles, particle integrity, and component debris vary among clinical samples. In one orientation, the surface cups combine to generate a Star of David image under the electron microscope (see Fig. 181–1C). In another orientation, the depressions are responsible for spikelike projections from the surface (see Fig. 181–1D).

Cryoelectron microscopy and x-ray crystallography have resolved the three-dimensional structure of typical and SRSV morphologic types.[35,219-222] Typical virion particles have 90 true arches protruding from the surface of a shell that has a diameter of 27 nm. When such particles are visualized by negative-stain electron microscopy, as in a clinical laboratory, the particles are smaller (30 to 35 nm) than the true 40.5 nm, presumably from desiccation. The arches form surface spikes in some particle orientations, and when the particles are rotated, the compressed two-dimensional image of the three-dimensional particle transforms the walls of the cup-shaped depressions into the distinctive Star of David appearance. SRSVs differ from typical caliciviruses in that they have shorter arches; otherwise, the structures of the two forms are similar.

TAXONOMIC RELATIONSHIPS AMONG THE CALICIVIRUSES

Recent decisions made by the International Committee on Taxonomy of Viruses include the separation of caliciviruses from picornaviruses in 1978,[40] inclusion of hepatitis E virus in the family in 1995,[46] recognition and naming of distinct calicivirus genera, separation of hepatitis E virus from the family in 1999,[15,83,85] and renaming of the Norwalk-like virus (now *Norovirus*) and Sapporo-like virus (now *Sapovirus*) genera in 2002.[182]

Phylogenetic evaluation of sequence identity among calicivirus strains sorts them according to the same four groups as does sorting by genomic organization (see later).[15] The most informative genomic region for classification of a strain within the family and within a genus is the capsid region, whereas the RNA polymerase gene is conserved sufficiently among the genera to blur distinctions. The strains fall into four genera; each genus also includes multiple clades of uncertain biologic significance (Fig. 181–2).

PROTOTYPE STRAINS AND HOSTS OF ORIGIN

The four calicivirus genera differ in genomic organization and degree of shared sequence identity (Table 181–1).[15,173] Strains commonly recovered from natural human gastroenteritis illness fall into two genera: *Norovirus*, a virtual synonym for SRSVs,

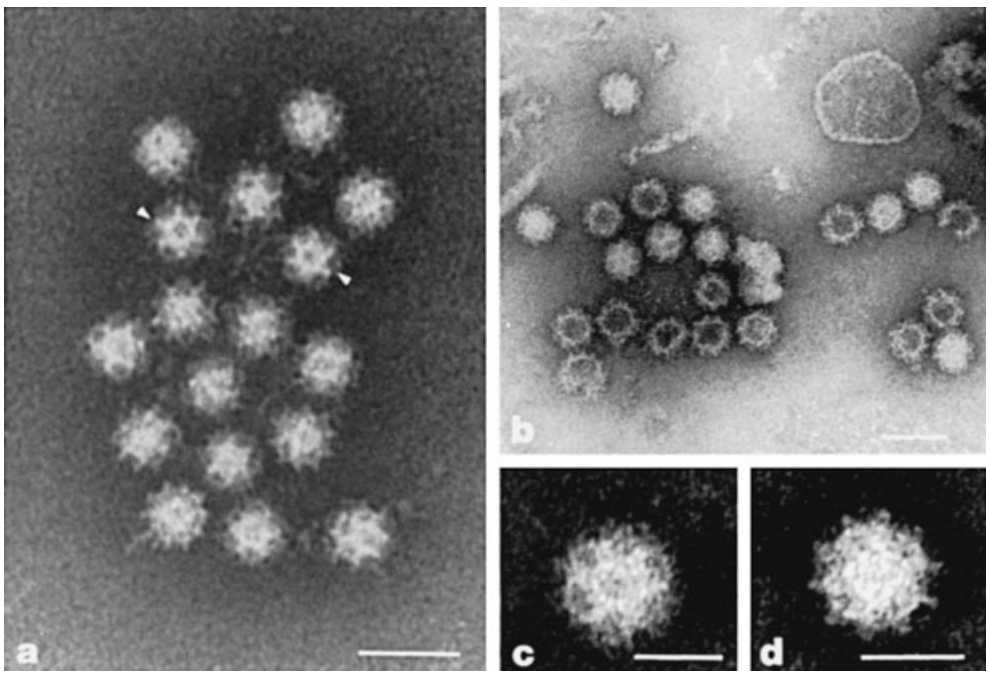

Figure 181–1 Human calicivirus particles visualized by electron microscopy. Particles in a preparation of a purified Sapporo/1982/Japan prototype strain show distinct surface cups, and the Star of David pattern is visible on some particles (**a,** *arrowheads).* Particle staining, particle type, and debris in clinical specimens are variable, which frustrates the recognition of distinct structural features. **b,** Specimen from a child from Houston, Texas, infected with an antigenically distinct *Sapovirus.* Two particles at high magnification show the Star of David pattern (**c**) and the 10 surface projections (**d**) characteristic of calicivirus (specimen from a symptomatic child attending a daycare center in Phoenix, Arizona). Bar in **a** and **b** = 50 nm; bar in **c** and **d** = 25 nm.

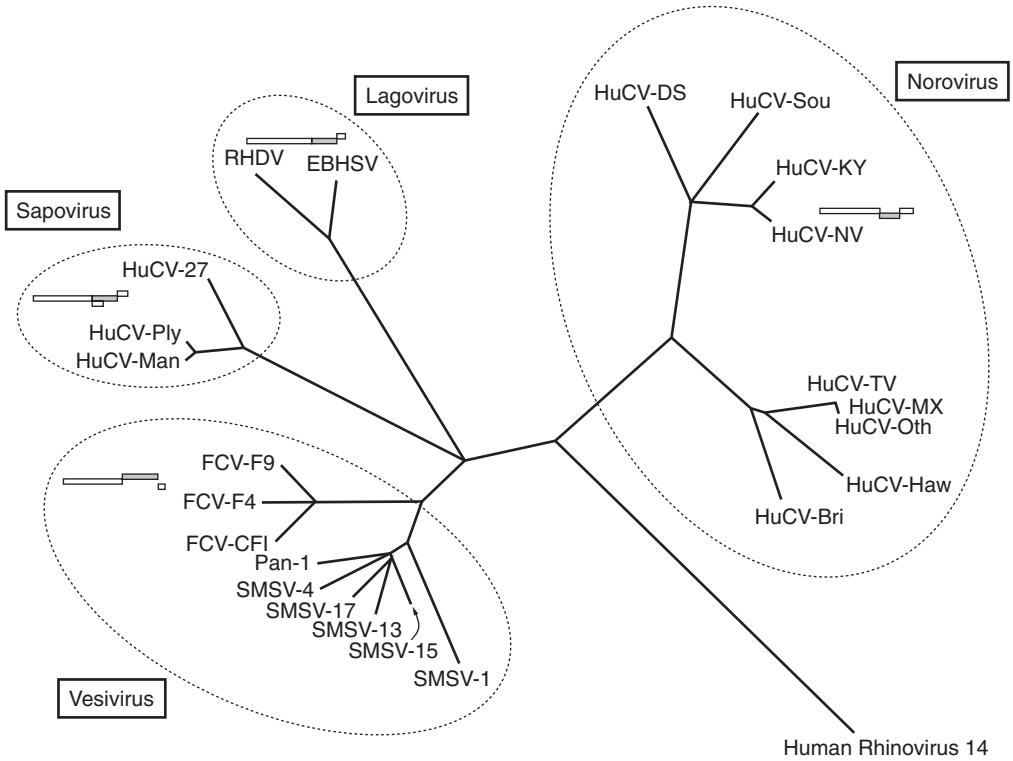

Figure 181–2 Distance tree (Fitch-Margoliash method) constructed from the capsid-region nucleic acid sequences of selected caliciviruses by use of 1000 bootstrapped dataset replications and the computer program FITCH of PHYLIP 3.5c. The branch points indicated are those appearing in more than 95 percent of the bootstrapped trees. Ovals indicate strains with a common genomic organization. Rhinovirus 14 is included as an outgroup. *(From Berke, T., Golding, B., Jiang, X., et al.: Phylogenetic analysis of the Caliciviruses. J. Med. Virol. 52:419-424, 1997.)*

TABLE 181–1 Genera of Human and Animal Caliciviruses

Genus	Known Hosts	Examples
*Norovirus**	Human, cow, pig	Norwalk and Snow Mountain viruses; Jena virus (bovine)
*Sapovirus**	Human, pig	Sapporo caliciviruses 82/Japan, 86/Houston, 90/Houston; Manchester and Plymouth viruses; porcine enteric calicivirus
Lagovirus	Rabbit, hare? pig? dog? human? fox? mouse? bird?	Rabbit hemorrhagic disease virus of Europe, Asia, Australia, New Zealand; European brown hare syndrome virus
Vesivirus	Pig, cat, chimpanzee, sea lion, dolphin, mussel, sea otter, raccoon, Aruba Island rattlesnake, human	A48; feline caliciviruses F4, CFl, F65, F9; Pan-1, Tur-1; San Miguel sea lion virus types 1 to 17; SMSV-5 Hom-1

**Previous names of these genera were Norwalk-like and Sapporo-like viruses; synonyms for* Norovirus *and* Sapovirus *were small, round-structured viruses and typical caliciviruses, respectively.*
Data from references 15, 83, 242, 244.

which include the Norwalk virus and Snow Mountain virus prototypes, and *Sapovirus*, a virtual synonym for typical human caliciviruses. The genera of common animal caliciviruses include *Lagovirus* and *Vesivirus*. *Lagovirus* prototypes include RHDV and European brown hare syndrome virus. *Vesivirus* prototypes include VESV; feline calicivirus, a cause of hemorrhagic pneumonia in cats; and other strains of marine and terrestrial animal origin. Additional genera may exist because many clinical specimens contain virus particles with structural features of caliciviruses that have been resistant to genomic characterization.

The perceived restriction of *Norovirus* and *Sapovirus* infections to humans has been broken by the finding of Newbury agent and Jena virus, noroviruses of cows, and porcine enteric calicivirus (a *Sapovirus* of pigs).[53,57,68,95,161] In addition, evidence of *Vesivirus* infections in humans is conclusive, although the spectrum of illness remains uncertain. The *Vesivirus* San Miguel sea lion virus serotype 5 (SMSV-5) produced blisters on the extremities of a laboratory worker, an illness similar to that observed in the original sea lion host.[244] Similar, less conclusive cases of *Vesivirus* illness also have occurred in field biologists working with marine mammals, anti-*Vesivirus* antibody has been detected in association with non-A-G hepatitis in humans, and *Vesivirus* genome has been detected in the blood of asymptomatic donors.[245] Other *Vesivirus* strains have caused illness in several primate species.[251] Evidence of *Lagovirus* infection in humans is suggestive. Antibody developed in one person exposed to the *Lagovirus* RHDV in a Mexican outbreak.[87] A case-control study of exposure and prevalence of antibody to RHDV in Australia after continent-wide escape of the virus from Australian Animal Health Laboratory facilities suggested that subclinical human infection was a common occurrence in persons with certain rabbit exposure,[173] although this conclusion is controversial.[23,24,186,249]

GENOMIC ORGANIZATION

The genomic organizations among calicivirus genera differ in the number and size of open-reading frames (ORFs), the presence of certain genes, and the need for post-translational cleavage for gene product function (Fig. 181–3).[25,59,131,160,188,226] The genome

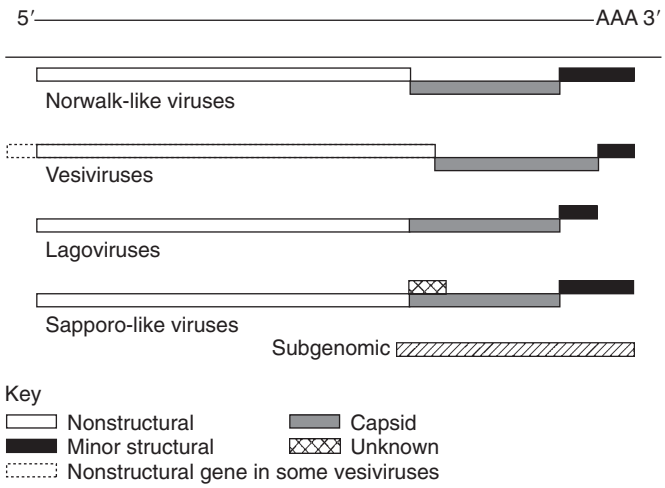

Figure 181–3 Genomic organization of calicivirus genera. The genome of *Norovirus* has three open-reading frames (ORFs) that in the 5′ to 3′ direction include a nonstructural polypeptide, the virion capsid gene, and a minor structural protein. The genomes of the other three genera differ from that of noroviruses in the length of the ORFs, including a unique gene at the 5′ end of ORF1 in some vesiviruses and a post-translationally cleaved N terminus of the capsid protein in vesiviruses and sapoviruses. A subgenomic RNA beginning just 5′ of ORF2 and continuing to the poly-A tail is synthesized during replication. Some sequence comparisons suggest that the longer ORF3 of *Norovirus* (and the comparable gene of *Sapovirus*) arose by intragenic recombination.

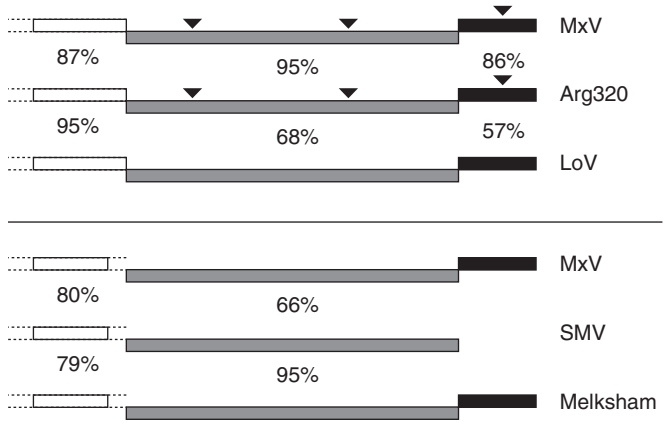

Figure 181–4 Sequence comparisons of Argentine calicivirus strain 320 (Arg320; *top*) and Snow Mountain virus (SMV; *bottom*), each with two other noroviruses. Arg320 is significantly closer to Lordsdale virus (LoV) than to Mexico virus (MxV) in the RNA polymerase genome region and significantly closer to MxV than LoV in the capsid and ORF3 genes. Deletions or insertions in the capsid and ORF3 genes of Arg320 are shared with MxV but not with LoV. SMSV is equally close to MxV and Melksham virus in the known polymerase region but much closer to Melksham than MxV in the capsid region. The ORF3 sequence is not available for SMV. Box shadings are as in Figure 181–3. *(Data from references 104 and 125.)*

of noroviruses has three ORFs. ORF1 encodes a polyprotein cleaved during replication into a set of nonstructural proteins, ORF2 encodes the capsid protein, and ORF3 encodes a protein that appears to be a minor structural protein.[253] Vesiviruses differ from noroviruses in that a longer genome is present in some vesiviruses (e.g., Pan-1)[226] but not in others (e.g., feline calicivirus)[25] and a longer ORF1 with an additional predicted protein is found at the N terminus. The ORF2 of vesiviruses is longer than that of noroviruses, with the extra nucleotides at the 5′ end of ORF2.[226] This extra sequence encodes a protein fragment that must be post-translationally cleaved to agree with experimental data of *Vesivirus* virion structure.[221] The ORF3 of vesiviruses (~120 amino acids) is approximately half the size of that of noroviruses (250 to 275 amino acids). In lagoviruses and sapoviruses, the genes found in the ORF1 and ORF2 of noroviruses and vesiviruses are fused into one longer ORF1. A gene comparable to that of ORF3 of noroviruses also is present. An ORF in another frame at the 5′ end of the capsid gene occurs in sapoviruses but not in all *Sapovirus* strains. ORF2 and ORF3 genes are included in a subgenomic RNA that is conterminal with the genomic RNA. Consensus amino acid motifs encoded within the proteins are markers for enzymatic and structural functions (2C, 3C, 3D, and PPG) shared with picornaviruses and other viruses with a single-stranded RNA genome.

Each completely sequenced *Norovirus* genome exhibits conservation of the 5′ end nucleotide sequence of the genome and the 5′ end of the capsid gene. This sequence conservation is high within the same strain and among strains in the same genus.[149,174] The conserved sequence also probably favors recombination of RNA among calicivirus strains. The conserved sequence surrounding the consensus amino acid motifs is a target for primers to initiate reverse transcriptase in reverse transcription–polymerase chain reaction (RT-PCR) detection assays that rely on amplification of the viral RNA.[130]

RECOMBINANTS

EVIDENCE FOR RECOMBINATION WITHIN CALICIVIRUSES. With the description of statistically distinct phylogenetic clades within calicivirus genera, data were available to recognize strains that might be natural recombinants within caliciviruses. Two examples are the well-characterized Argentine strain 320 (Arg320) and the prototype Snow Mountain virus, recognized to be recombinants when the RNA polymerase and capsid regions of these strains were characterized (Fig. 181–4).[104,125] In both strains, the change in relative sequence identity took place at the ORF1/ORF2 junction, where sequence conservation at the 5′ end of the genome also occurs (see earlier), thus indicating that the recombination site occurred there. This site also was suggested (see later) to be the break-and-rejoin site for recombination between caliciviruses and picornaviruses. For Arg320, the ORF1 sequence was closest to the Lordsdale virus, a contemporaneously circulating strain, and the capsid and ORF3 sequences were closest to Mexico virus, also prevalent when Arg320 was collected. A similar change in relative sequence identity also occurred with Snow Mountain virus, which was detected when partial polymerase and capsid sequences were compared with reference Mexico and Melksham viruses, again strains circulating at the same time.

Except for the recombinant genomic structure, the recombinants otherwise are unremarkable, and the strains were recognized only because they initially were characterized in two genomic regions. Recombination may be a mechanism by which caliciviruses quickly escape host immunity, analogous to antigenic shifts in influenza viruses but by a different molecular mechanism. The recombination event could have occurred recently or remotely in the past.

POTENTIAL ORIGINS OF RECOMBINANTS WITHIN CALICIVIRUSES. Generation of recombinants within caliciviruses requires certain biologic and molecular attributes. Infection of single cells simultaneously by two caliciviruses implies an absence of immune or molecular interference. Outbreaks caused by multiple *Norovirus* strains and co-infection with different

Norovirus strains do occur.[70,74,80,103,179,256] Calicivirus RNA also must have attributes that permit or favor recombination, such as a site where errors in procession of RNA polymerase can occur. The subgenomic RNA is the molecule most likely to participate in recombination because of its relatively high copy number in infected cells and because it would have an accessible end. The highly conserved 5′ end sequence of the genome and the 5′ end of the capsid gene in noroviruses is an obvious common target for calicivirus RNA polymerase to switch from one RNA molecule to another. The recognized recombinants may be a subset, those viable, among a larger set of recombinant molecules produced during replication.

EVIDENCE FOR RECOMBINATION OF CALICIVIRUSES WITH OTHER VIRUS FAMILIES. After sequencing a portion of feline calicivirus strain F9, Neill[202] observed that ORF1 contained significant sequence identity with picornaviruses. This significant identity was concentrated around certain amino acid motifs within ORF1 that are homologous to those within the nonstructural region of picornaviruses and encode, in order, the 2C, 3C, and 3D genes. The order of these motifs and the approximate number of nucleotides between them were the same in both virus families (Fig. 181–5). The capsid gene of caliciviruses also is homologous to the VP1 to VP4 capsid proteins of picornaviruses to the extent of a shared PPG amino acid motif in a relatively conserved 5′ portion of the capsid gene or genes, formation of capsomeres having polypeptide β-pleated sheets as a core structural element, and formation of a spherical virion capsid by the protein or proteins.[219] The reversal of order of these major domains in the genomes of the two virus families led to the hypothesis that at some point in time, the caliciviruses and picornaviruses were "recombination partners."[60]

ANTIGENIC PROPERTIES OF THE VIRION

The presence of a single calicivirus capsid protein ought to limit the antigenic complexity of the virion. Despite this limitation, circulating caliciviruses are highly diverse (e.g., see Hohdatsu and colleagues[108]). Assays to detect viral antigen and antiviral antibody have been developed for prototype strains of the genera and for distinct genetic clades within them.[97,123,132,197] Within the limits of testing, analysis of convalescent and hyperimmune antisera does not detect strains across generic boundaries, thus suggesting that different genera represent distinct antigenic groups. Furthermore, epitopes not shared across phylogenetic clades within a genus have been identified.[100,129,276,277]

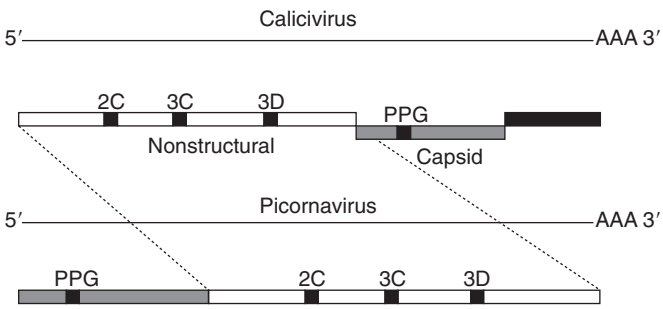

Figure 181–5 Genomic organization of caliciviruses and picornaviruses showing the switch in order of nonstructural and structural genes. In caliciviruses, the nonstructural genes are encoded in ORF1; several of the genes were recognized because of the presence of significant sequence identity and consensus motifs like those known for picornaviruses (2C, 3C, and 3D). The capsid gene of caliciviruses is encoded in ORF2 (noroviruses and vesiviruses; see Fig. 181–3), which lies 3′ to the nonstructural genes, and is marked by a PPG motif that signals a relatively conserved region between the families. In picornaviruses, this order of nonstructural-structural "gene cassettes" is reversed, with the order and approximate distance of motifs within the nonstructural peptide the same. Box shadings are as in Figure 181–3.

Many vesiviruses can be cultivated readily in cell culture, whereas cultivation of viruses in the other genera has been successful for only a few strains.[68,242,247,251] The vesiviruses include a large number (>40) of serotypes (neutralization types).[242] Similarly, characterization of lagoviruses suggests the existence of serotypes.[205,272] Determination of whether the other genera have similar diversity has not been possible because of the lack of ability to test strains for their neutralization characteristics. Antigenic characterization of caliciviruses is at a primitive level of development.

NOROVIRUS AND *SAPOVIRUS* HUMAN CALICIVIRUSES

Noroviruses and sapoviruses share a number of properties. To avoid repetition, I discuss features common to the genera under each of the following headings and then those distinct to each genus.

EPIDEMIOLOGY

General Prevalence

Serologic studies indicate that *Norovirus* and *Sapovirus* infections occur commonly, with virtually all individuals having antibody by the time they reach the second decade of life, wherever studied. Noroviruses and sapoviruses have been found everywhere that they have been sought and probably have a worldwide distribution. Strains from multiple genetic clades and antigenically distinct strains within each genus co-circulate wherever it has been studied.[124,279] In the same way, regional differences exist in the prevalence of antibody to specific types and in the number and distribution of the genogroups and sub-genogroup clades.[71,72,81,110,166,206,208,260]

Noroviruses are known best for causing outbreaks of gastroenteritis in adults, commonly associated with contaminated water and food, particularly shellfish. Noroviruses account for approximately 60 percent of such outbreaks in North America and Europe for which no bacterial or parasitic cause can be found.[63,86,107,120,147,148,191] Studies suggest that large changes in the relative prevalence of *Norovirus* clades have occurred during the past 30 years. For example, prototype Norwalk virus and closely related strains appear to have been the predominant noroviruses in the 1970s in several regions studied.[86] Thereafter, Snow Mountain virus and related strains (in a separate genetic clade within *Norovirus*) have been predominant, and strains within the clade that contains the prototype Norwalk virus are found infrequently (<5%).[82,128,129,172,260,268] In addition, some *Norovirus* strains appear to prevail within a community for as short a time as 1 year.[121,166] The causes of such temporal variation are uncertain, but they at least are related to widespread distribution of contaminated foodstuffs or water (or both) in a pattern like that observed for *Salmonella*.[102,180] This epidemiologic pattern also suggests the existence of type-common neutralizing epitopes; for individual antigenic types, each causes only a small percentage of illness episodes in a region at any time. Contamination events, in which a single type is delivered across a large geographic region in a short period with an inoculum large enough to overcome (mild) host resistance, would thus be needed for any one antigenic type to be predominant. Such predominance would be of short duration.

Recombinant norovirus Arg320 and Snow Mountain virus first were recovered from persons with gastroenteritis in Argen-

tina and the United States, respectively.[125,136] Arg320 also was recovered from outbreaks of gastroenteritis in the United States[132] and the Netherlands.[146] Snow Mountain–like strains occur worldwide. The widespread occurrence of these recombinants in symptomatic persons suggests their ready infectivity, easy transmissibility, sustained virulence after multiple passages in humans, and genetic and ecologic stability.

Unlike the noroviruses, sapoviruses are known primarily from sporadic cases of diarrhea in children, although outbreaks in closed populations do occur.[36,37,45,124,132,200] The proportion of outbreaks traced to sapoviruses probably will increase as improved methods for detection of them are applied. Typical caliciviruses visualized in stool specimens by electron microscopy but not yet further characterized probably fall into this genus.[43-45,50,185,210]

Morbidity and Mortality

Cross-sectional surveys of diarrhea stool specimens from children or adults yield *Norovirus* in 0.5 to 16 percent of samples, with the higher values reported by recent studies and achieved by applying more sensitive detection methods.[55,56,143,167,207,212-214,224,279] Attack rates in outbreaks frequently are high: 20 to 90 percent. These observed attack rates for outbreaks are similar to those from volunteer studies involving a single (low) inoculum of the Norwalk virus 8FIIa prototype, in which 82 percent of volunteers were infected and 56 percent were ill.[79] Secondary attack rates in common-source outbreaks range from 5 to 30 percent.[78] A general tendency is that noroviruses are detected more frequently in cross-sectional studies of subclinical or moderately severe illness than in episodes resulting in hospitalization; this trend is the reverse of that observed for rotaviruses, which are detected more frequently in more severe illness.[213,279] Death from *Norovirus* illness rarely occurs. Cross-sectional studies in adults show noroviruses to be comparably prevalent, although studies from severely ill adults are conspicuously absent.[56,144,169,229] In adults, the severity of *Norovirus* infections often is greater, and this trend is in part a consequence of an unusual pattern of protective immunity observed for these strains (see later).

Like noroviruses, sapoviruses are identified more frequently in less severe gastroenteritis episodes than in cases resulting in hospitalization. Cross-sectional surveys of gastroenteritis in nonhospitalized children have found sapoviruses in as many as 20 percent of cases.[172,201,207,228,267] Similar studies of hospitalized children have found sapoviruses in as many as 5 percent of acute gastroenteritis episodes.[279] Death from *Sapovirus* infection is rare but does occur.[67]

Age Incidence and Prevalence

Norovirus infections in developed countries generally have been thought to occur in individuals aged 4 years or older. This conclusion was derived from comparative serologic studies that revealed large differences in patterns of acquisition of age-specific antibody between developing and developed countries. For example, in Bangladesh, the prevalence of antibody in 4-year-old children was 100 percent, as opposed to a prevalence of 19 percent in children of the same age in the United States.[16,138] In addition, studies of outbreaks suggested that young children in developed countries were spared.

More recent studies using more sensitive assays have changed these conclusions. For example, among 154 infants aged 23 months who participated in a vaccine trial in Finland from 1987 to 1989, 73 percent had anti–Norwalk virus antibody.[157] In England, the prevalence of antibody to Norwalk virus in 1991 and 1992 was 48 percent in 1-year-old toddlers overall, with the prevalence in that age group ranging from 12 to 60 percent in different regions.[81] In South Africa, antibody prevalence in 1992 was 57 percent in 1-year-old children.[260] These results contrast with those from Hokkaido, Japan, where the prevalence of antibody to Norwalk virus in children aged 1 to 3 years was approximately 6 percent.[206] In England and Japan, but not in South Africa, a significant increase in the prevalence of antibody to Norwalk virus was noted in school-age children, in whom it reached 80 percent in England and 70 percent in Japan by the time they reached the third decade of life.

Children are likely to be infected with multiple *Norovirus* types.[47,179] Testing of a large population-based collection of sera from two cities in Chile for antibody to prototype Norwalk virus and Mexico virus (a Snow Mountain–like *Norovirus*) suggested that these two viruses had different modes of transmission and that exposure to the two types differed in that population.[208] For example, the overall prevalence of antibody to Norwalk virus was 83 percent and that to Mexico virus was 91 percent in this age-stratified sample of persons living in Santiago, whereas it was 67 percent and 90 percent, respectively, in Punta Arenas ($p < .001$ for antibody to Norwalk virus). Consumption of uncooked vegetables was an independent risk factor for the acquisition of antibody to both viruses in Santiago but only for Mexico virus in Punta Arenas. Consumption of seafood and attendance at childcare centers were independent risk factors for the acquisition of antibody to both viruses in Punta Arenas but not in Santiago. Results of this kind, to which attributes of the person providing serum, other than just age and location, are matched to the results of antibody testing, are needed for a more complete understanding of the modes of transmission and ecologic differences among calicivirus genera. The large differences in the prevalence of age-specific antibody among and within countries suggest that "herd immunity" to neutralizing epitopes specific to such clades can develop in populations. Such differences also suggest that the acquisition of antibody is the result of selected, variable, and cumulative exposure, such as from multistate and multicontinent movement of strains in contaminated food.[5,133,153,231]

Serologic studies confirm that children acquire *Sapovirus* infection soon after birth and that virtually all are infected by the time they reach 4 years of age.[48,198,233] Children are infected with multiple *Sapovirus* types during this period.[48,177,178] Outbreaks have been described in infants, young children, and adults.[107] The prevalence of antibody to sapoviruses exceeds 70 percent in adults studied in Asia, Australia, Africa, Europe, and North America.[48,198,214]

Norovirus and *Sapovirus* outbreaks tend to be recognized in closed populations where common exposure occurs, such as nurseries, childcare centers, schools, hospitals, camps, hotels, and ships.* Outbreaks in young adults in military camps and on deployment, especially navy ships, are a special example because of the often increased severity of *Norovirus* illness in young adults.† Vehicles of infection in outbreaks include water from regulated and unregulated delivery systems and foods commonly washed, such as salad and fruit.‡ Shellfish are a frequent source of infection. Person-to-person spread also occurs. New infections can occur during the course of several weeks, with the longest outbreak lasting 3 months. Such prolonged or repeated outbreaks have been associated with persistent asymptomatic excretion in food handlers, continual surface contamination at the site, and re-exposure to the same strain from contaminated foods.[33,36,49,52,76,258,273]

Seasonal Patterns

Both sporadic and outbreak-associated *Norovirus* infections have a winter seasonality (Fig. 181–6).[84,195,279] This trend

*See references 33, 36, 83, 170, 178, 179, 199, 224, 240, 266.
†See references 1, 2, 8, 9, 17, 32, 38, 41, 89, 119, 167, 175, 184, 211, 234, 240, 241, 262.
‡See references 3, 6, 19, 20, 42, 43, 65, 88, 92, 154, 164, 196, 227, 270.

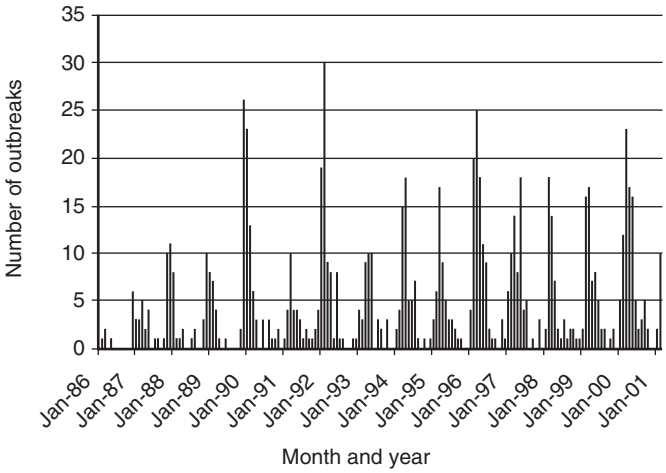

Figure 181–6 Seasonality of 840 presumed viral outbreaks in nursing homes for the elderly in Maryland, 1986 to 2000. Detailed analyses of 20 outbreaks found noroviruses in 18. *(Data extracted from Figure 1 in Green, K. Y., Belliot, G., Taylor, J. L., et al.: A predominant role for Norwalk-like viruses as agents of epidemic gastroenteritis in Maryland nursing homes for the elderly. J. Infect. Dis.* 185*:133-146, 2002.)*

has exceptions, however. Infections related to certain foodstuffs will occur when such foodstuffs are harvested and eaten fresh (fruit, salads, shellfish) or processed, shipped, and consumed later (frozen fruits, ice). Infection from exposure related to certain types of recreation, such as swimming, should have a seasonal predominance. In addition, contamination of water supplies should occur more frequently when flooding results in mixing of water and sewage and when shellfish are harvested.[98,183,187,217,236]

A distinct seasonal prevalence of *Sapovirus*-associated illness is not as apparent, but a winter predominance is suggested from several studies.[177,214,279]

PATHOGENESIS AND PATHOLOGY

Viral Infection

The incubation period after exposure to *Norovirus* ranges from 10 to 51 hours, with a mean of 24 hours.[37,79,139,178,261,274] Illness usually lasts 2 to 3 days.[79] Excretion begins as early as 15 hours after the development of infection, peaks at 25 to 72 hours after infection occurs, and persists at least 7 days. Transmission of infection occurs by fecal-oral spread. Noroviruses also have been detected in vomitus, thus suggesting the possibility of spread by aerosol or dispersion of vomitus particles.[27,28,141,235]

Some volunteers administered Norwalk virus 8FIIa prototype had no response, neither illness nor evidence of infection, to the inoculum. These nonresponders have B blood type.* Results from binding assays support the role of complex sugar structures that determine blood-type specificity in *Norovirus* virion attachment. The absence of these cell surface structures prevents illness because the cell is not infected. In at least one instance, this resistance has been traced to a single amino acid change in a vital protein.[263] These findings complement those suggesting that certain ethnic groups are inherently resistant to infection.[62]

Similar information about sapoviruses is lacking.

*See references 66, 105, 106, 111, 112, 114, 115, 117, 118, 126, 127, 155, 159, 168, 192, 194, 230, 259.

Pathology

The pathogenic features of *Norovirus* infection have not been determined. The following information is based on studies of porcine and calf enteric caliciviruses.[30,34,69,96,101,230] Enteric animal caliciviruses cause lesions in the proximal portion of the small bowel. The first day after inoculation, enterocytes swell along the sides of the microvilli at the base. Damaged enterocytes subsequently slough, with stunted villi left behind. The adjacent villi fuse, which helps maintain the integrity of the intestinal mucosa. Neutrophils and mononuclear cells infiltrate the epithelium. The peak of pathologic abnormalities is 3 to 7 days after inoculation occurs, at which time gradual healing begins, with normal villus architecture being restored approximately 10 days after inoculation. Mucosal injury is accompanied by deficiencies of β-galactosidase activity and D-xylose absorption. The capacity for D-xylose absorption recovers by the time the normal histologic appearance of the villi is restored, but the β-galactosidase deficiency is delayed for an unknown period thereafter. Individual responses to inoculation vary in both the distribution and severity of lesions.

Prototype Norwalk virus causes similar histologic abnormalities, also located in the proximal part of the small bowel.[237,238,271] However, the abrupt onset of illness in adults and the consistent finding that individuals with antibody are more likely to be ill than are individuals lacking antibody suggest that an immune mechanism plays a role in illness.[145,158]

The pathogenic features of *Sapovirus* calicivirus infection in humans have not been determined and are presumed to be the same as those for *Norovirus* caliciviruses.

Immunologic Events

The humoral immune responses to *Norovirus* and *Sapovirus* infection are like those for infection with other virus. Individuals lacking antibodies show a rapid rise in the titer of serum and fecal antibody that persists for months, and individuals with high serum antibody levels are unlikely to mount an antibody response when they are re-exposed to the virus. Children with anti-*Norovirus* or anti-*Sapovirus* antibody appear to be protected against subsequent infection, at least in the short term.[49,64,157,158,200] A striking and consistent observation is that adult volunteers inoculated with noroviruses who have higher levels of preexisting serum or fecal antibody are more likely to excrete virus or to be ill than are individuals who have low or no preexisting antibody.[79,216] The mechanism for this apparent conflict is unknown, but the usual observation is that serum or fecal antibody is a marker for protection against infection. Likely, the assays used to assess protective status measure predominantly cross-reacting, non-neutralizing antibody induced by multiple exposures, rather than the specific, neutralizing antibody needed for protection.

When the symptom profile of 50 Norwalk virus volunteers was assessed, the increased risk of being symptomatic with higher preexisting antibody titers was statistically significant for ill subjects who had vomiting as the predominant clinical feature.[79] Because vomiting in the gastroenteritis syndrome may result from a process different from that causing diarrhea, this observation suggests an alternative pathogenic mechanism peculiar to the noroviruses. The existence of such an alternative pathogenic mechanism would explain the unusual observation that age-specific morbidity tends to be greatest in adults. One issue unresolved from these studies is whether the antibody assays used in these volunteer studies measured neutralizing or non-neutralizing anti–Norwalk virus antibody. Moreover, asymptomatic infection apparently can occur independent of the absence of attachment structures related to blood type.[79,116]

CLINICAL MANIFESTATIONS

The clinical manifestations of *Norovirus* and *Sapovirus* are those of acute gastroenteritis.[79,136,232,264] In 50 adult volunteers inoculated with Norwalk virus, diarrhea was noted in 59 percent, nausea in 66 percent, cramps in 66 percent, headache or body ache in 66 percent, vomiting in 39 percent, chills in 24 percent, and fever in 22 percent.[79] This constellation of common symptoms is indistinguishable from those associated with other gastroenteritis pathogens. As is the case for most other viral pathogens, most *Norovirus* and *Sapovirus* infections are likely to be asymptomatic. Extraintestinal infection caused by noroviruses and sapoviruses is unknown. Protracted illness can occur in individuals with immune compromise.[73]

In 154 hospitalized children with calicivirus infection, vomiting was present in 120 (78%) patients, diarrhea in 102 (66%), and fever in 82 (53%).[279] The prevalence of these symptoms was similar in *Norovirus*- and *Sapovirus*-associated illness. Fever was noted more commonly (76%) in patients younger than 3 months than in older children (45%, $p < .001$). Similarly, vomiting occurred more commonly (86%) in older patients than in patients younger than 3 months (56%, $p < .001$). The median duration of symptoms was 1 day for vomiting (median number of episodes, 6 per day; range, 1 to 35 per day), 2 days for diarrhea (median of 6 stools per day; range, 1 to 50), and 1 day for fever. Conditions associated with hospitalization in these 154 children included dehydration ($n = 42$, 27%), respiratory symptoms ($n = 15$, 10%), and seizures ($n = 6$, 4%). Metabolic acidosis ($n = 2$), hypoglycemia ($n = 1$), and hypocalcemia ($n = 1$) were rare findings. The median cost for an episode of calicivirus-associated illness in these children was $3574 (1999 dollars), more than 90 percent of which was for the cost of hospitalization. These 154 hospitalized children had 191 visits to a physician's office and 45 visits to an emergency department, in addition to the hospital stay.

In direct comparison to rotavirus infection, *Norovirus*- and *Sapovirus*-associated illness was a mean of 2 points, of a 20-point score, less severe than rotavirus-associated illness.[214,279] Younger children (<3 months) had less severe illness than older children did ($p = .006$). The combination of vomiting, diarrhea, and fever occurred more frequently (58% of cases) in rotavirus-associated illness than in calicivirus-associated illness (23%, $p < .001$). Coinfections with rotavirus increased the severity of calicivirus-associated illness. Children hospitalized for rotavirus illness were older (median age, 408 days) than those hospitalized for calicivirus illness (median age, 257 days; $p < .001$).

Sapovirus illnesses also usually are indistinguishable from those caused by other gastroenteritis pathogens. A few children who were studied intensively after they were hospitalized for *Sapovirus* illness had prolonged malabsorption, frank rectal bleeding, severe dehydration and acidosis, and transient leukopenia. Three children with combined immunodeficiency and *Sapovirus* infection early in life that resulted in chronic diarrhea and death have been described.[18,43,73] Sapoviruses have been the only identified pathogen in the stool specimens of a few children who died of gastroenteritis, but little information describing the illness or the pathologic findings in these children has been reported.[43,67]

DIAGNOSIS

Differential Diagnosis

Recognized outbreaks of calicivirus infection have tended to be associated with a rapid onset of symptoms and a broad age range of affected persons, especially if they are associated with common exposure to water or food. The pattern of symptoms is a fairly good marker for calicivirus-associated outbreaks: a brief duration of illness, 2 to 3 days; vomiting as a prominent symptom in more than 50 percent of the outbreak cases; an incubation period of 24 to 48 hours; a high (30%, usually > 50%) secondary attack rate; and cultures of freshly processed stool specimens negative for bacterial, parasitic, or fungal pathogens.[99,140,162] When outbreaks have such characteristics, noroviruses will be detected in more than 80 percent of them. Sapoviruses have been detected with current diagnostic techniques in approximately 3 to 5 percent of such outbreaks. Stool samples should be submitted to a facility skilled in using individual assays for these pathogens. Caliciviruses are destroyed by repeated freezing and thawing.[45,113] Therefore, bulk stool samples stored at 4° C and not frozen should be collected early after the development of infection and referred promptly. Consultation with the reference laboratory should precede sample referral.[156]

Specific Diagnosis

Detection methods relying on target nucleic acid amplification are needed for noroviruses and sapoviruses because virus is excreted in stool in low concentration, rarely exceeding 10^6 particles per gram of stool. In addition, assays for antigen detection are relatively specific; they apparently do not detect group-specific epitopes on the viruses, and the sensitivity of commercial assays is approximately a third that of RT-PCR for noroviruses in outbreak or cross-sectional collections.[26,54,58,77] Only a few laboratories routinely perform calicivirus detection assays, and some strains are not detected by the best designed consensus primer oligonucleotides because of genomic sequence diversity among strains.* RT-PCR amplicons from the viral RNA polymerase genomic region are produced most reliably, but conservation among strains within and between genera in this region is sufficiently strong to warrant caution in assigning causality of an outbreak of illness to a particular strain based on the sequence of the (frequently small) amplicons from this region.[14,15] Paired serum samples may be required to detect and to confirm the presence of an etiologic agent.

VESIVIRUS CALICIVIRUSES

EPIDEMIOLOGY

General Prevalence

Vesiviruses include a large number of serotypes isolated from many different animal hosts, including pigs, cows, cats, dogs, sea lions, sea otters, walruses, whales, several snakes, pygmy chimpanzees, and gorillas, as well as a few isolates from humans.[13,61,75,171,242-244,252] These viruses probably have a worldwide distribution in terrestrial and marine environments, although studies of San Miguel calicivirus–like strains are lacking outside North America. Vesiviruses include at least 40 neutralization types (serotypes). The prevalence of these serotypes in different regions has had little study.

Morbidity and Mortality

Vesiviruses cause a variety of severe illnesses in animals. The morbidity and mortality rates of individual strains differ from species to species in experimental infections. One laboratory worker was infected with SMSV-5 and experienced an influenza-like illness, followed during the next several days by viremia and vesicular exanthem similar to that observed in the original pin-

*See references 29, 93, 94, 109, 123, 129, 130, 135, 163, 190, 223, 225, 269, 278, 279.

niped host.[244] SMSV-5 was visualized in vesicular fluid by electron microscopy and was cultured from the lesions; the genome was amplified and sequenced. The worker also mounted a greater than fourfold serum neutralizing antibody response to SMSV-5. Biologists working with marine mammals had similar illnesses but were studied less intensively than was the laboratory worker.[12,244,248] Anti-vesivirus antibodies have been detected in 12 percent of normal blood donors, at higher prevalence (21%) among otherwise normal donors with elevated serum levels of liver transaminases, and at highest prevalence (47%) among patients with hepatitis associated with blood exposures.[245] Some persons in the groups with normal and elevated transaminase levels also had *Vesivirus* RNA circulating in the blood.

Host and Social Factors

Although vesiviruses cause a variety of diseases, only a few cause gastroenteritis, a feature common to *Norovirus* and *Sapovirus* caliciviruses.[51] Among the vesiviruses are a number that exhibit a broad range of hosts. For example, SMSV-5 has been found to infect sea lions, seals, opaleye fish, pigs, cattle, and laboratory workers.[242,250]

PATHOGENESIS AND PATHOLOGY

Viral Infection

Vesiviruses cause infection on mucosal surfaces (pneumonia, aphthous ulcers) and disease after viremia (generalized vesicular eruption, encephalitis, hepatitis, spontaneous abortion). The incubation period after exposure is short: 1 to 4 days.[255] Excretion varies among species. For three cultivatable vesiviruses, asymptomatic and persistent excretion of virus was detected for months after primary infection.[134,247,251]

Pathology

The pathogenic features of *Vesivirus* infection vary and depend on the syndrome of the affected host.

CLINICAL MANIFESTATIONS

The proven clinical manifestations of *Vesivirus* calicivirus infection in humans are limited to a few cases. Because vesiviruses cause a variety of illnesses in animals and these viruses occur naturally in primates, the potential spectrum of illness in humans is broad. Groups at greatest risk of acquiring infection probably would be zoo workers, indigenous populations handling marine mammals, veterinarians, and laboratory workers, but studies of these groups have been limited.[249] The clinical implications of the finding of anti-*Vesivirus* antibody and RNA in serum are uncertain. The RNA in serum was found in persons judged well enough to donate blood.[245]

DIAGNOSIS

Many vesiviruses can be cultivated in cell culture, and many are found in sufficiently high concentration in clinical samples to permit the use of electron microscopy and classic virologic methods for detection of infecting viruses. Other strains have resisted cultivation, and molecular techniques have been developed for them.[176,204,239] Vesiviruses are heat labile, and clinical samples should be submitted promptly and unfrozen.

LAGOVIRUS CALICIVIRUSES

EPIDEMIOLOGY

General Prevalence

The prototype *Lagovirus* is RHDV. Lagoviruses include the genetically distinct European brown hare syndrome virus and multiple genetically distinct strains recovered from asymptomatic rabbits.[22,23,150-152,272] Rabbits also are infected with *Vesivirus* strains, which may lead to confusion.[171] Antigenic diversity occurs in the *Lagovirus* genus, but the number of serotypes in the genus is unknown, for like noroviruses and sapoviruses, lagoviruses cannot be cultivated in the laboratory.[272] RHDV has been epidemic in Asia and Europe since it was first recognized in China in 1984. Rabbits in more than 40 countries have been affected. Disease spreads rapidly and appears to "leap" large distances in a short time. The ability of the virus to spread rapidly across large bodies of water (e.g., the English Channel) suggests that arthropods or other flying vectors have a role in transmission.[31] One outbreak in Mexico was traced to a shipment of rabbit meat from Asia; extensive slaughter of rabbits in Mexico was required to eradicate the agent.[87] Three outbreaks have occurred in North America and Cuba.[21] The source of these outbreaks is unknown, although deliberate importation and release has been suspected in one instance.

RHDV was released in 1995 in controlled field studies off the coast of mainland Australia but escaped from the study site the same year and spread onto the continent.[122] Subsequently, the virus was introduced illegally into the South Island of New Zealand. Clandestine, deliberate spread in both countries to control exotic European rabbits led to wide dispersal of the agent. These releases occurred despite conflicting evidence of RHDV infection in other animal species and, subsequently, in humans.[24,173]

Morbidity and Mortality

RHDV infections are highly fatal (>80%) in wild and domesticated European rabbits. Subclinical infections occur in young rabbits, and immunity from early infection appears to attenuate later exposure. In the food-associated outbreak in Mexico, antibody developed in one human. Zoo workers, rabbit breeders, and foresters would have the largest occupational risk of RHDV exposure. The human infection in the Mexican outbreak apparently was subclinical.[87] Ostensibly subclinical infection also occurred in some persons exposed to rabbits in Australia after escape of the virus there.[173]

Host and Social Factors

The range of hosts of lagoviruses and the host of RHDV before it was recognized in rabbits in China are unknown. In Australian studies, numerous Australian animal species challenged with a subimmunogenic dose of RHDV had no antibody response. On the other hand, antibodies did develop in kiwis, mice, dogs, and foxes; in the case of foxes, the serum antibody response was attributable to oral feeding.[39] Natural spread in rabbits is enhanced by their colony breeding behavior.

CLINICAL MANIFESTATIONS AND PATHOGENESIS

The incubation period after exposure is 1 to 3 days.[265] Lagoviruses cause a fatal hepatitis in rabbits and hares that results in disseminated intravascular coagulation and diffuse hemorrhage. Death is rapid: 6 to 12 hours after the onset of illness. RHDV and European brown hare syndrome virus appear to be sufficiently distinct to preclude cross-protection.

DIAGNOSIS

Because the infections in the Mexican worker exposed during the RHDV outbreak and in the workers in Australia who tested antibody positive apparently were subclinical, the spectrum of potential infection in humans is unknown. Samples from persons with suspected infection should be submitted to reference laboratories after consultation. Diagnosis can be established with immunologic and molecular techniques.

TREATMENT AND PREVENTION

SPECIFIC MEASURES

No specific treatment of calicivirus infection has been reported. The existence of at least partially effective vaccines for feline calicivirus and RHDV indicates that this prevention method would work for other caliciviruses. A norovirus vaccine incorporating an immunogen-expressed capsid protein that self-assembles into virion-like particles is being developed.[10,11,90,257] Feline interferon and ribavirin inhibit the replication of feline calicivirus (a *Vesivirus*) in feline cell lines.[189,218] Morpholino antisense oligomers blocked production of virion by three *Vesivirus* strains in multiple cell lines[17a,254] These antisense compounds have potential for prophylaxis and treatment of illness.[246]

NONSPECIFIC MEASURES

Children with diarrhea are more likely than are children without diarrhea to be excreting enteric viruses, which reflects an association between the incidence of diarrhea and the cleanliness of the environment. This principle is likely to hold for caliciviruses as well. For children in care settings with other children, a few infection control measures are likely to reduce the spread of infection. Such measures include trained personnel, cleanliness of surfaces and food preparation, exclusion of ill or carrier care providers, adequate handwashing, and exclusion or cohorting of ill children.[51,91] Laboratory workers exposed to caliciviruses should be aware of the risk of acquiring infection. Infection with noroviruses requires a very small inoculum, on the order of 100 particles.[261,274]

PROGNOSIS

Understanding of the features of calicivirus infection has increased rapidly since 1990, when molecular tools for studying the viruses became available. The availability of refined diagnostic reagents will permit an accurate assessment of the extent and variety of calicivirus-associated illness and of the food and water sources of infection to be made. Rapid progress is occurring in improvement of detection methods for noroviruses in various types of food contamination[164] as well as of methods to kill them on the food before consumption.[142] Specific preventive measures are being developed, but their potential is clouded by uncertainty about the antigenic and genetic diversity of strains and the strength of cross-protection induced by exposure to one or more strains.

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CHAPTER 182

Hepatitis E Virus

Ravi Jhaveri

Hepatitis E virus (HEV) is a virus that was characterized only recently but probably has been infecting humans for hundreds of years. New information about the animal reservoirs, epidemiology, and molecular biology is constantly becoming available. Recent natural disasters and regional warfare have highlighted HEV as an agent that causes epidemics in areas where sanitation and public health systems have broken down. This chapter is a summary of the discoveries so far with emphasis on the impact of children infected with HEV.

HISTORY AND DISCOVERY OF VIRUS

As methods to detect hepatitis A and B viruses as the major causes of fecal-oral and blood-borne hepatitis, respectively, became more reliable in the 1970s, more cases were being classified as non-A, non-B hepatitis. Investigations in regions of south Asia looked at epidemic and endemic cases of acute hepatitis.[40,67,147] Case patients all had a clinical course that was very similar to hepatitis A virus (HAV) infection, despite having a remote history of HAV infection, and the result of testing for other causes of viral hepatitis was negative. Many researchers considered that a distinct enteric viral agent was the cause of these cases.

The first evidence of this came when Balayan and colleagues[18] used stool extracts from infected humans orally administered to a human volunteer known to be HAV immune. Classic hepatitis symptoms ensued, and stool from the infected volunteer was used to re-infect cynomolgus macaques. Electron microscopy of stool samples from the asymptomatic and early symptomatic phases showed virus-like particles (VLP) approximately 30 nm in diameter that banded at 1.35 g/cm^3 on a cesium chloride gradient.[24,25] VLPs showed no cytopathic effect when passaged in tissue culture, and mice inoculated with the VLPs showed no signs of disease.[18]

The field experienced a major advance after reverse transcription–polymerase chain reaction (RT-PCR) was used to identify hepatitis C virus in 1989.[30] Similar methods were used to determine the nature of the non-A, non-B enteric virus.[111] Reyes and colleagues[111] constructed cDNA clones from infected bile that subsequently hybridized with RNA from infected animal and human liver as well as isolates from five distinct geographic outbreaks. Sequence analysis revealed the presence of an RNA-dependent RNA polymerase, a common feature of plus-stranded RNA viruses. Northern blot hybridization identified a 7.4-kb molecule present only in homogenates from infected liver samples. The agent was pronounced *hepatitis E virus*, and the same group cloned and sequenced HEV during the subsequent year.[127]

MICROBIOLOGY AND GENOME ORGANIZATION

HEV is a plus-sense RNA virus with a genome size of approximately 7200 base pairs.[127,148] Initial cloning of the Burma strain revealed the presence of three overlapping open-reading frames (ORFs).[127,137] ORF1 is approximately 5000 base pairs in length and encodes the nonstructural proteins.[73,127] ORF2 begins 37 base pairs downstream from the ORF1 stop codon and codes for the viral capsid.[127] ORF3 is only 369 base pairs and overlaps both ORF1 and ORF2. The viral RNA contains a short 5′ untranslated region of approximately 27 base pairs and a 3′ untranslated region of approximately 65 base pairs.[127] The genome has a 7-methyl cap at the 5′ untranslated region and a poly-A tail of approximately 150 to 200 bases.[62] The organization of the genome is represented in Figure 182–1.

Initial comparative genomic analysis deduced the likely functions of the ORF1 nonstructural regions.[73] They include the methyltransferase "capping" motif (~amino acids 56-240), the papain-like cysteine protease (~amino acids 433-592), the RNA helicase (~amino acids 960-1204), and the RNA-dependent RNA polymerase (~amino acids 1207-1693). Details of ORF1 processing are limited, but recent data examining ORF1 with use of a baculovirus system showed extensive cysteine protease processing.[9,103,116] The enzymatic activities of the methyltransferase and RNA-dependent RNA polymerase have been demonstrated in independent studies.[7,83]

ORF2 codes for the viral capsid protein.[127] Specific studies of the ORF2 have shown a membrane export signal sequence (amino acids 1-36) at its extreme N terminal, several glycosylation sites, and multiple smaller processed forms.[58,112,127,151] ORF2 has dimerization sites and can assemble into VLPs when the first 110 amino acids are deleted.[78,79,149]

The specific role of the ORF3 has yet to be determined, although many studies have revealed varied effects, which include interaction with the MAPK/ERK pathway, ORF2 binding, and self association.[66,74,142,143]

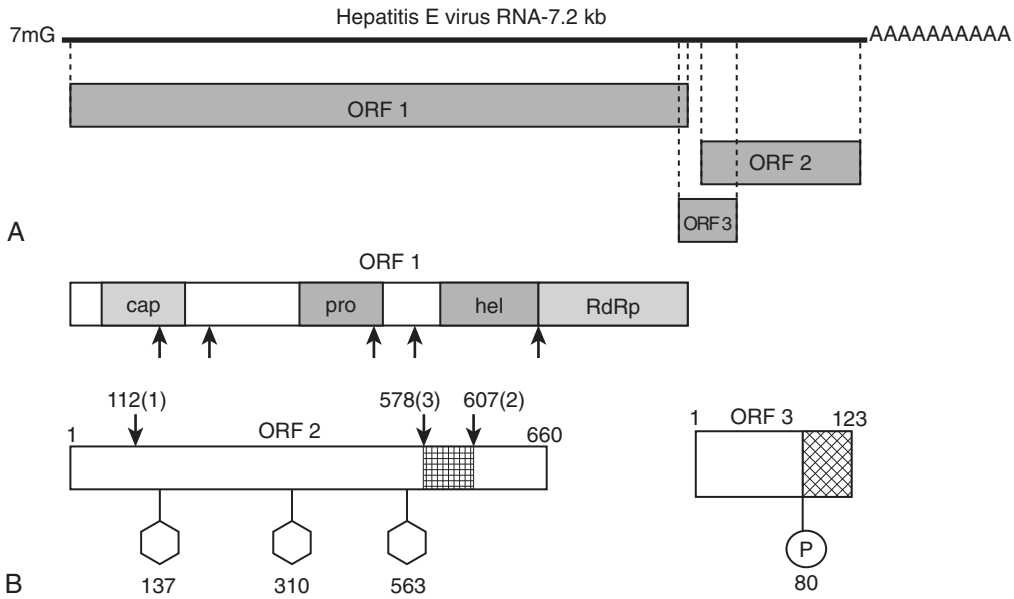

Figure 182–1 Representation of HEV genome and open-reading frames. **A,** HEV genome overview. The HEV RNA is single stranded and features three overlapping reading frames: ORF1, ORF2, and ORF3. **B,** Individual features of the HEV ORFs. ORF1 contains the nonstructural protein domains *(shaded)*, which include the methyltransferase (cap), cysteine protease (pro), helicase (hel), and RNA-dependent RNA polymerase (RdRp). Proposed cleavage sites are illustrated with the bold arrows at their approximate locations. ORF2 is the structural capsid protein, which is 660 amino acids in length. Cleavage sites are indicated by the bold arrows at their determined amino acid positions. Glycosylation sites are represented with polygons at their determined amino acid positions. The domain of ORF2 that has been determined to be the neutralization epitope is represented by the crosshatched box. ORF3 is the smallest protein, and its phosphorylation site is represented by the circle labeled P. The domain responsible for dimerization is represented by the diagonal hatched box.

VIRAL STRUCTURE AND STABILITY

Electron micrographs from fecal samples of patients and animals infected with HEV show the virus to be approximately 27 to 30 nm in diameter.[18,24,25]

Ultrastructural studies of HEV have involved ORF2 expression, and the empty VLPs produced are slightly smaller than the infectious HEV.[79,149] The VLPs assemble from 30 dimers to form a spiky hollow sphere.[149] Although the empty VLP shares much similarity with the native virion in appearance, further confirmation is lacking until a tissue culture system is available.

Several strains of HEV are almost completely heat inactivated by 60° C, compared with 66° C for HAV[39] with some interstrain variability. Virus-containing stocks have been stable at extreme cold temperatures with several freeze-thaw cycles.[39] Based on persistent HEV infections during an outbreak from water containing chlorine concentrations of 0.3 to 0.6 mg/L, HEV may be resistant to chlorine inactivation.[50]

VIRAL LIFE CYCLE

An important note is that HEV does not grow in tissue culture conditions, so detailed information about viral structure and life cycle is limited.[40] Limited studies have shown evidence of HEV replication in primary macaque hepatocyte cultures.[128,129] When in vitro–transcribed RNA from a full-length cDNA clone was transfected into liver cell lines, viral proteins could be detected and the culture supernatant could be used to infect a rhesus monkey.[103]

Currently, no information is available about the viral receptor for HEV. After the virus gains access to the cell, it uncoats and translates its RNA to produce a sufficient number of nonstructural proteins to begin the transcription-replication process.[103] A genomic and 2-kb subgenomic RNA are produced, as is a negative-stranded replication template.[46,129] With the accumulation of transcripts, structural proteins as well as more plus-sense daughter RNA begin to accumulate. ORF2 gets cleaved, dimerizes, and associates with the HEV RNA to form new virions.[124] These accumulated virions subsequently are released by an unknown mechanism.

VIRAL GENETICS

Four main genotypes of HEV have been identified.[148] Genotype 1 is distributed throughout the world from south Asia to northern Africa. Genotype 2 is seen most commonly in Mexico but has been isolated in certain countries in Africa. Genotypes 3 and 4 are the zoonotic strains of HEV that are found in swine and humans from developed Western countries and China, respectively. Genotypes 1 and 2 are approximately 70 to 80 percent homologous, whereas genotype 3 strains are more removed from genotype 2 strains (25-50% homology).[93] In general, genotypes 1 and 2 are closer to each other[82] and are responsible for endemic and epidemic diseases, whereas genotypes 3 and 4 are more closely related[82] and are responsible for zoonotic disease and sporadic human cases.

Only one serotype of HEV has been identified.[90,91,115,148] Some evidence indicates that HEV develops quasispecies during epidemics that allow adaptation within individual hosts.[47]

TAXONOMY AND CLASSIFICATION

Before its characterization, HEV was thought to be closely aligned with the calicivirus family on the basis of physical characteristics.[18,127] After the full cloning of the virus and the identification of the three ORFs were achieved, the virus was classified formally with the caliciviruses.

Subsequent work showed that HEV shared several characteristics with rubella virus and other "alpha-like" viruses.[73] An analy-

sis performed comparing the helicase and polymerase regions of HEV with members from the *Caliciviridae*, *Picornaviridae*, and *Togaviridae* families showed significant divergence.[20] The International Committee on Taxonomy of Viruses decided to separate HEV from the *Caliciviridae* family on the basis of these findings and leave it unclassified, where it still remains.[48]

EPIDEMIOLOGY

ENDEMIC HEPATITIS E

Hepatitis E is endemic in areas of the developing world where sanitation and clean water are not readily available. South and Southeast Asia and north Africa experience endemic disease, as do regions of Mexico and South America. Large epidemics of hepatitis E occur in these endemic countries and are related to widespread contamination of water after natural disasters. Cases of hepatitis E occurring outside these areas inevitably were traced back to travel to one of these areas.

Unlike with HAV, most of the seroprevalence studies performed in endemic countries have shown that children are not universally infected with HEV early in life. Children in the first decade have anti-HEV–positive rates of approximately 3 to 4 percent,[10,15] and the peak of HEV seropositivity occurs in the second and third decades of life. However, studies from regions of Egypt and northern regions of India demonstrate a pattern that is similar to HAV, with 60 to 70 percent of children younger than 10 years being anti-HEV positive.[6,42,89] Egypt has much higher prevalence rates than does neighboring Israel, whereas southern and central India have anti-HEV rates much lower than those of the northern regions previously described.[10,65,96] A study of children from Moscow showed HEV seroprevalence rates that fell between those of most other studies.[1] Explanations for these regional differences are lacking. Table 182–1 is a summary of HEV seroprevalence studies performed in children from different parts of the world.*

One of the Egypt seroprevalence studies performed follow-up to examine seroconversion and estimated the incidence of HEV infection.[123] Of the 919 villagers who were anti-HEV at the beginning of the study, 34 seroconverted in 10 months' time for an incidence of 41.6/1000 person-years for this group.

Studies of sporadic acute hepatitis in endemic areas indicate the burden of HEV disease in those countries. Studies examining children presenting with acute hepatitis symptoms in Egypt and Sudan show that HEV is either the leading or second leading cause in children.[38,55,56] A similar study from India showed that, although HEV was the second leading cause after HAV of acute hepatitis requiring admission to a children's hospital, it caused a slightly higher percentage of acute liver failure.[106] One study found that HAV and HEV infections led to worse outcomes in acute liver failure, but other studies have not substantiated those findings.[16,85]

Several studies also from endemic areas have been designed specifically to examine the differences in HAV and HEV infections.[27,114] The results of these studies have shown that HAV disease peaks earlier in life than does HEV disease and that although the clinical symptoms of acute hepatitis are very similar, the hepatic dysfunction from HEV tends to peak earlier in the illness and may take longer to resolve. Because the peaks of HAV and HEV seroprevalence and disease in endemic countries do not overlap, some researchers have suggested that HAV disease either somehow protects against or interferes with HEV infection.[125] No specific proof of this hypothesis is available currently.

*See references 1, 6, 8, 10, 19, 21, 32, 35, 42, 57, 65, 80, 88, 89, 107, 109, 118, 133.

Although the reservoir for HEV is not known, the detection of HEV from various sewage samples combined with results of studies of patients with prolonged viremia indicates that the virus probably can exist in these environments to sustain its transmission cycle.[100,104,144]

EPIDEMIC HEPATITIS E

Hepatitis E epidemics often are related to events that cause disruption of sanitary conditions (war, earthquakes, flooding). Recent war-associated epidemics among the refugees from the Darfur region of Sudan and Iraq in 2004 illustrate the nature of HEV epidemic circulation.[22,44,50,145]

In a refugee camp in western Sudan, more than 2600 cases of HEV infection were reported among the estimated 78,000 residents in the second half of 2004.[22,50] Of the total cases, almost 10 percent required hospitalization, and of the hospitalized cases, 45 (17.8%) died.[22] Most of the deaths involved pregnant women, 19 of 45 (42.2%), and in fact the sentinel cases for the hepatitis E epidemic were two pregnant women who died in July 2004. The overall mortality rate for the epidemic was 1.7 percent. A total of 1133 pregnant women were in the refugee camp; 220 reported jaundice (19.4% attack rate), and the mortality rate was 8.2 percent.

The main source of water for the camp was unchlorinated groundwater pumped in through pipelines or surface water with chlorine added that refugees could access.[50] In the analysis of those patients with symptomatic HEV infection and those with asymptomatic infection, patients aged 15 to 45 years had a higher attack rate and more symptomatic infections (4.3% and 71%, respectively) compared with the group aged younger than 15 years (2.4% and 22%, respectively). Intrafamilial spread of HEV does not appear to play a significant role during epidemic conditions.[5,12]

ZOONOTIC HEPATITIS E

As more widespread serologic studies were performed to assess rates of anti-HEV seropositivity, cases with no identifiable travel risks were discovered in developed countries with no known endemic HEV activity.[43,88,134] Many of the cases had heavy animal exposures, most commonly to swine. This finding led researchers to investigate the possibility that HEV was, in fact, a zoonosis and that either direct animal contact or ingestion of raw or undercooked meat could produce HEV infection. Meng and colleagues[94] conducted a multiphase study to determine if swine were infected with HEV and to isolate the virus. They performed enzyme-linked immunosorbent assays (ELISAs) on multiple swine herds and found that the majority were anti-HEV positive and that the seroprevalence increased with age. They observed newborn piglets, monitored for seroconversion, and demonstrated concurrent histologic hepatitis and viremia by RT-PCR. In a follow-up study, serum from the infected piglets was used to infect pathogen-free experimental pigs.[92] Pigs could not be infected with human strains of HEV. Rhesus monkeys and chimpanzees, a human surrogate, were successfully infected with the swine HEV.[93] An isolate of HEV from the United States that was genetically similar to the swine HEV successfully infected pathogen-free pigs, thus proving that swine HEV could infect both pigs and humans and was the likely cause of sporadic cases of hepatitis E in the United States and other developed countries. HEV genotypes 3 and 4 now have been established as being the strains most frequently isolated from animals that are nonendemic HEV cases from industrialized countries.[33]

Many animal species likely are infected with HEV. Among those described are swine, deer, chickens, rodents, and cats.[51-53,61,101] Of

TABLE 182–1 Seroprevalence Studies of Hepatitis E in Children

Country and Region	Age Group	Percentage Positive	No. Positive/Tested	Reference
India, south, Chennai	0-2 yr	5.3	1/19	Mohanavalli, 2003
	2-4 yr	9.0	2/22	
	4-6 yr	7.3	4/55	
	6-8 yr	7.9	3/37	
	8-10 yr	16.7	3/15	
	10-12 yr	9.0	3/33	
India, south, Vellore	1-5 yr	0.5	1/200	Daniel, 2004
	6-15 yr	1.0	2/200	
	16-40 yr	8.0	8/100	
India, central, Pune	6-10 yr	3.9	50/1302	Arankalle, 2001
	11-15 yr	6.3	83/1349	
India, north, New Delhi	6-12 mo	11.0	23/210	Mathur, 2001
	13-24 mo	9.3	19/203	
	25-48 mo	23.7	96/405	
	49-72 mo	29.5	126/426	
	73-96 mo	31.3	129/412	
	97-120 mo	35.8	152/424	
India, north, Lucknow	0-5 yr	64	18/28	Aggarwal, 1997
	6-10 yr	59	13/22	
	11-18 yr	64	16/25	
Nepal, Kathmandu	12-19 yr	16	10/64	Clayson, 1997
Japan, Nagoya	1-11 yr	2.6	8/309	Goto, 2006
Taiwan, central	3 yr	3.1	6/196	Lin, 2004
	4 yr	3.4	22/652	
	5 yr	3.4	42/1247	
	6 yr	3.4	15/443	
Russia, Moscow	0-3 yr	11.8	2/17	Abe, 2004
	4-6 yr	7.7	3/39	
	7-9 yr	23.2	19/82	
	10-12 yr	20	18/90	
	12-15 yr	17.7	20/113	
Turkey, Istanbul	0.5-4 yr	3.7	12/321	Sidal, 2001
	5-9 yr	2.1	7/318	
	10-16 yr	0.3	1/270	
Turkey, varied	<19 yr	0	0/105	Thomas, 1993
Morocco, Melilla	Mean 9.2 yr (±4.03)	0	0/321	Bernal, 1995
Israel, varied	1-5 yr	1.1	1/93	Karetnyi, 1995
	6-10 yr	0	0/30	
	11-15 yr	1.6	2/124	
	16-20 yr	3.8	4/105	
Egypt, Nile delta and Upper	0-4 yr	36.2	130/359	Fix, 2000
	5-9 yr	64.7	1600/2473	
	10-14 yr	75.6	1944/2571	
	15-19 yr	75.5	1438/1905	
United States, northern California	<20 yr	0.4-0.7	Not stated	Mast, 1997
Cuba, Havana	5-15 yr	0	0/22	Quintana, 2005
Mexico, national sample	1-4 yr	1.1	1/91	Alvarez-Munoz, 1999
	5-14 yr	4.4	27/619	
	15-19 yr	9.6	109/1138	
Chile, Valdivia	Not stated	36	60/166	Ibarra, 1994
Bolivia, Chaco region	1-5 yr	3.9	2/51	Bartoloni, 1999
	6-10 yr	3.8	4/105	
	11-20 yr	5.5	5/90	
Venezuela, rural villages	4 yr	1.3	1/75	Pujol, 1994
	5-9 yr	2.7	2/75	
	10-15 yr	5.8	4/69	

note, avian HEV, which has been demonstrated as the cause of hepatosplenomegaly syndrome in chickens, shares only 50 to 60 percent homology with human and swine HEV and has not been shown to be able to infect nonhuman primates.[52,54]

VERTICAL AND BREAST MILK TRANSMISSION

Studies of pregnant women with HEV infection also have shown that vertical transmission does occur. The small studies performed show that HEV is likely to be transmitted at high rates, that fetal demise can occur before birth, and that infants can be severely affected.[70,119]

Limited studies of breast milk transmission have been performed. HEV RNA and anti-HEV antibody have been detected in colostrum components, but at significantly lower titers compared with serum.[29] No cases of HEV transmission in the infants were linked definitively to breast milk; however, the mothers with advanced liver disease were not allowed or able to breast-feed in this study. In short, no recommendations exist to contraindicate breast-feeding in this population aside from those mothers with severe disease.

OTHER MODES OF HEPATITIS E VIRUS TRANSMISSION

Cases have been reported of HEV linked with transfusion of contaminated blood. Donors were in the presymptomatic viremic phase, and recipients of those blood products developed symptoms of acute hepatitis with a shorter incubation period than that of patients infected through the usual fecal-oral route.[13,14,23,71]

Consumption of undercooked wild game meat has been implicated in HEV infections. Several cases have been reported of acute HEV infection occurring after consumption of uncooked or undercooked deer and wild boar meat.[76,131,132]

PATHOGENESIS AND IMMUNITY

PATHOGENESIS

The details of pathogenesis in HEV infection are largely unknown. How the virus infects enterocytes and spreads from the intestine to the liver after ingestion of HEV-contaminated water or food remains unknown. Studies in swine show replicative HEV RNA present in colon, small intestine, lymph nodes, tonsils, and liver as early as 3 days after infection.[146]

Evidence of HEV replication within the liver as detected by HEV antigen is apparent by 7 days after infection. At the peak, HEV antigens can be detected in 70 to 90 percent of hepatocytes before the onset of symptoms, and then the prevalence drops off rapidly.[4] Viremia is detectable in serum, stool, and bile before the onset of symptoms.[28,81,136] Anti-HEV appears at the same time as do the histologic signs of inflammation.[31] Similar to HAV infection, disease likely is caused by the immune response to the virus and not by the virus itself.[4]

Most of the information about progression and pathogenesis comes from studies of inoculation of HEV into animals.[18,81,136,138] These studies usually involved intravenous administration, with an incubation period from administration to development of viremia of approximately 3 weeks, approximately 1 week less on average than occurs in humans. HEV RNA can be detected in serum, stool, and bile 1 week before clinical symptoms become apparent.

In the HEV volunteer studies, the patients developed constitutional symptoms at approximately 4 weeks after administration.[18,28] Specific symptoms and the biochemical abnormalities of hepatitis developed 1 week later. Early studies showed that viral particles appeared in stool with the onset of symptoms and continued through the peak period of hepatitis symptoms; antibody was present at the only time they tested, 1 week after the peak hepatitis symptoms occurred.[18] Subsequent studies using RT-PCR showed that HEV RNA was present in serum before stool samples and was present from 1 week before to 2 weeks after symptoms had begun.[28] Peak alanine aminotransferase elevations corresponded with peak symptoms, and symptoms lasted between 30 and 80 days. HEV IgG persisted well beyond the study end-point.[28]

The presence of HEV RNA in stool and serum can vary significantly from patient to patient.[3] In 20 patients diagnosed with sporadic HEV, HEV RNA appeared in serum at days 4 to 7 after the onset of symptoms in 89 percent of patients tested and persisted beyond day 22 in 21 percent. HEV RNA in stool samples was present in 73 percent of patients at days 8 to 14 after symptoms, peaking slightly later than did serum HEV RNA, and it persisted beyond day 22 in 13 percent. A subset of patients will have HEV RNA in serum and stool for up to 120 days.[100] Patients with prolonged shedding possibly serve as a reservoir for spread of HEV to the environment.

A summary of the clinical progression of HEV infection is graphically represented in Figure 182–2.

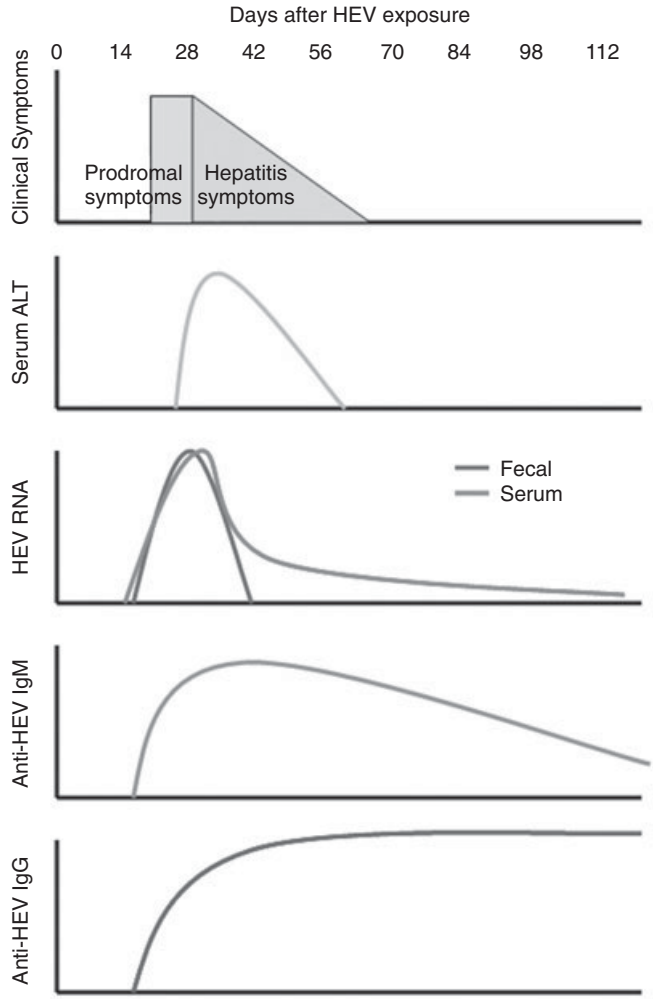

Figure 182–2 Representation of the course of HEV infection with approximate times after HEV exposure. In volunteer and animal experiments, prodromal symptoms will appear approximately 3 weeks after infection, which precedes the symptoms of hepatitis by about 1 week. The hepatitis symptoms can last 4 to 6 weeks on average, with significant variation. Serum alanine aminotransferase peaks with the onset of hepatitis symptoms. HEV RNA can be detected in either stool or serum approximately 3 weeks after infection with the onset of the prodrome and be detected for 1 to 3 weeks in duration, with some patients experiencing prolonged viremia. HEV IgM and IgG appear in concert with the onset of symptoms and after the peak of HEV RNA. IgM peaks slightly earlier than IgG and can taper quickly, with some disappearing by 3 months. IgG peaks later and lasts several months after infection. (See companion Expert Consult web site for color version.)

IMMUNITY

HEV antibody appears with the onset of symptoms and after the peak of viremia. IgM appears just ahead of IgG. HEV IgA appears at the same time as does IgM and appears to persist slightly (about 1 month) longer after infection than IgM does.[126,135] ORF2 is the dominant immunogen across all types of HEV, although antibodies to ORF3 can be detected in patients.[49,55,56,87,150] A more detailed discussion of neutralizing antibodies can be found in the vaccine subsection.

Some studies have shown that HEV IgG responses wane quickly after infection, whereas others show a more durable antibody response.[45] The waning of IgG levels is the basis for the theory that accumulation of susceptible adults forms the vulnerable population during HEV outbreaks.

Although evidence of cellular immunity to HEV exists, its role in eradication of infection and persistence of viral immunity is unknown.[99]

CLINICAL MANIFESTATIONS

The signs and symptoms of acute HEV infection are indistinguishable from those seen with HAV infection.[4,27,56,114] A prodromal illness consisting of malaise, anorexia, nausea, abdominal pain, fever, myalgia, and headache precedes the findings of jaundice, hepatomegaly, and serum transaminase elevations. No cases of chronic infection with HEV have been reported.

One study examining children after a common-source exposure provides insight into the manifestations of HEV in this group. Twenty students from a private school in urban north India were exposed to HEV while on a camping trip.[17] The exact source was never identified, but investigators were able to monitor the children for signs and symptoms of HEV infection as well as for serologic and virologic confirmation of infection. The clinical symptoms are summarized in Figure 182–3. Of the 20 children, 17 developed clinical illness (85%) but only 10 developed symptoms of acute hepatitis. The other seven children remained anicteric, with symptoms of malaise, nausea, abdominal pain, and headache. The children with jaundice had a different incubation period (25-38 days; median, 33 days) compared with the children without jaundice (22-95 days; median, 27 days). HEV IgM was found in all patients. HEV RNA was found in 11 children, including one of the children who never developed any symptoms. Because this study is one of the few to have a point source of exposure, determination of whether the very high rate of infection is due to the younger age of the patients, the strain of the virus, or the nature of the exposure is difficult.

Rates of fulminant hepatic failure and mortality from HEV disease are estimated to be approximately 1 to 4 percent. Studies from endemic areas suggest that the rate may be higher in children, but those data have not been widely substantiated.[105] The rate is closer to 15 to 50 percent in patients with preexisting liver diseases and compensated cirrhosis, whether caused by chronic hepatitis B or C or by other metabolic liver disease.[110]

Pregnant women are highly vulnerable to HEV infection.[34,69] Before HEV infection was fully characterized, infected pregnant women were recognized as having high rates of fulminant liver failure and mortality rates of 20 percent or higher.[72] This feature of HEV infection is not restricted to a particular region or genotype, as illustrated by the Darfur outbreaks. The mechanism for severe disease in these women is unknown, although one study showed an increased T_H2 cytokine bias in peripheral blood mononuclear cells from pregnant HEV-infected patients that could allow a dysregulated inflammatory response.[22,102]

Extrahepatic manifestations of HEV infection have been reported, but they are uncommon findings. Pancreatitis can occur shortly after the onset of acute hepatitis symptoms.[59,84,95] Several reports have described patients with significant hemolysis with glucose-6-phosphate dehydrogenase deficiency associated with acute HEV infection.[2,152] Neurologic manifestations described include transverse myelitis, Bell palsy, and Guillain-Barré syndrome.[36,37,63,86,120] All of these manifestations resolve spontaneously in conjunction with the hepatitis. Whether they are a result of direct viral effects or are an immune-mediated phenomenon remains unclear. An interesting note is that most of the extrahepatic manifestations have been reported in children.

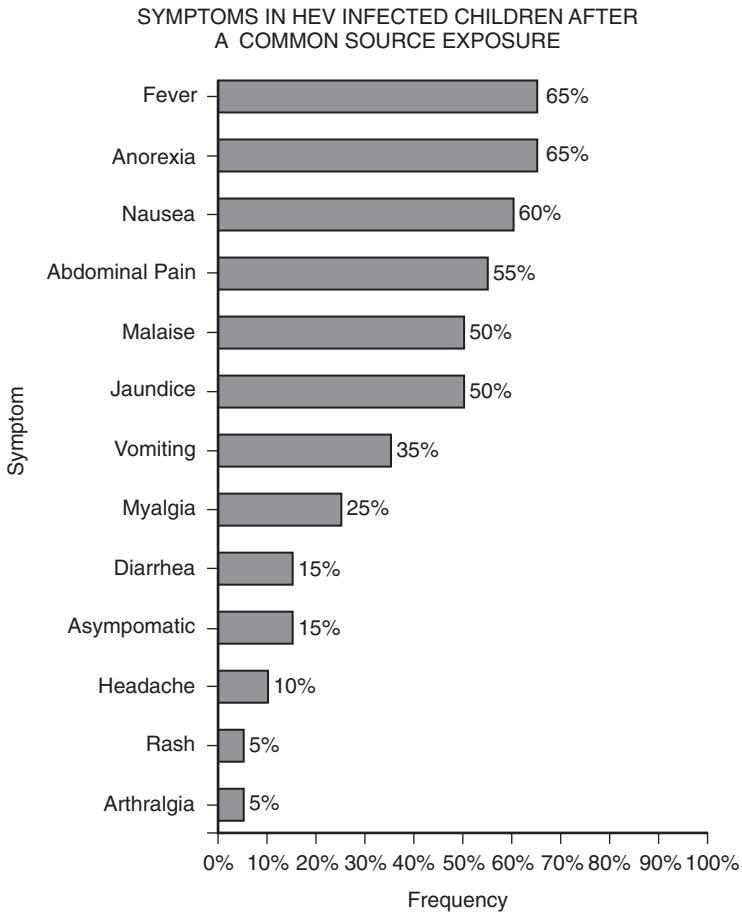

Figure 182–3 Symptoms observed in children after a common-source exposure to HEV. Jaundice was seen in only 50 percent of patients overall, and 15 percent were completely asymptomatic. *(From Arora, N. K., Panda, S. K., Nanda, S. K., et al.: Hepatitis E infection in children: Study of an outbreak. J. Gastroenterol. Hepatol.* 14*:572-577, 1999.)*

DIAGNOSTIC TESTING

SEROLOGY

Antibody confirmation is the most widely available method used to diagnose HEV infection. ELISA for both IgG and IgM can be obtained at many reference laboratories. The ORF2 capsid protein is the most common antigen used to detect antibody.[141] Some studies have shown that inclusion of the other ORFs can increase the specificity and positive predictive value of the test. A few studies have shown that HEV IgA may perform better than IgM-based testing does.[126,135] This finding needs to be confirmed in larger studies.

A comparison of different HEV ELISA kits during an epidemic in Indonesia found that the sensitivity of the tests was much better in symptomatic patients compared with asymptomatic ones (72-91% versus 39-51%, respectively).[97] Also, the Walter Reed kits for IgG and IgM performed better than did the GeneLabs and Abbott IgG tests. Testing of normal healthy controls who were HEV negative by PCR showed that the serologic testing had high specificity, again with the Walter Reed tests performing best. The caveat of the study is that the GeneLabs IgM kit was an older version of the test, and the limitations of the assay may not apply to the newer kit.

A new rapid immunochromatographic assay showed promising results when it was evaluated for use in resource-poor countries to diagnose acute HEV infection.[98] With use of 25 μL of serum or 35 μL of whole blood applied to the test strip and a 15-minute incubation period, the kit had a sensitivity of 93 percent and a specificity of 99.7 percent.

POLYMERASE CHAIN REACTION ANALYSIS

RT-PCR for HEV RNA can be performed within the first month of illness on either serum or stool.[3] The testing is less available commercially and likely to be conducted in research or select public health laboratories. Studies have shown that real-time PCR testing may allow more rapid and sensitive detection of HEV RNA, with the additional benefits of detecting multiple HEV genotypes and the ability to test environmental samples in addition to clinical samples.[41,60]

ELECTRON MICROSCOPY

HEV in stool samples from patients also has been visualized by electron microscopy.[18,121] In general, these methods incorporate HEV antibody to adsorb intact virus to increase the likelihood of detection. Again, this testing is not generally available.

DISEASE PREVENTION AND VACCINE CANDIDATES

AVOIDANCE OF DISEASE

Practical measures can be taken to reduce the risk of acquiring HEV infection when traveling to endemic regions.[26] Consuming only boiled or bottled water, avoiding ice, eating fruits that can be peeled, and eating only well-cooked foods are recommended measures.

Pregnant women and patients with chronic liver disease should avoid travel to areas endemic for HEV or regions experiencing HEV disease outbreaks.

ANTIBODY PREPARATIONS

There are no available antibody preparations that can be used as preexposure or postexposure prophylaxis for HEV infection. Small studies have shown successful passive immunization in animals.[139] Studies with immune serum globulin in endemic areas have shown mixed effects.[11,68] All the studies were small, and the antibody preparations were of varying amounts given at varying times, so definitive conclusions about the role of antibody cannot be made.

THERAPIES

Few studies of therapeutic options for acute HEV infection have been performed. One uncontrolled study used glycyrrhizin in patients with significant acute hepatitis from HEV, but no meaningful conclusions can be drawn from its results.[130] A hammerhead ribozyme targeted to the 3′ untranslated region revealed decreases in the in vitro activity of an HEV reporter.[122] Development of therapies for HEV infection is not likely to be effective for the simple reason that the peak of viral activity usually is before and along with the onset of symptoms. The patients with prolonged viremia are not necessarily the sickest patients; indeed, they often are asymptomatic.

VACCINE CANDIDATES AND FUTURE THERAPIES

Early vaccine studies used cynomolgus monkeys actively immunized with a recombinant ORF2 protein. Subjects were protected after challenge with homologous virus compared with passively immunized or control animals.[139]

Work to identify neutralizing antibody showed that the epitopes recognized were in the terminal region of ORF2, amino acids 578 to 607.[116] These antibodies were protective of HEV infection when incubated with HEV before administration to monkeys. This epitope is present within the 56-kd form of the capsid protein.

A recombinant 56-kd ORF2 vaccine protected rhesus monkeys challenged with HEV 1 month after a two-dose series was completed.[108,140] Repeated challenge at 6 months and 1 year showed a rate of protection against hepatitis of 75 percent, which increased to 100 percent in animals given a third dose of vaccine 1 month before challenge.[153] Postexposure vaccination did not prevent development of HEV infection.

Subsequent phase I testing in humans induced anti-HEV titers of 40 U/mL or greater in 88 percent of vaccinees after receipt of three doses.[113] Only a few minor local reactions and no significant adverse events occurred. On the basis of these early trials, a large human field trial was completed in Nepal in 2001. Two thousand seronegative soldiers were randomized to receive three doses during 6 months of the recombinant HEV vaccine or placebo.[117] The subjects were observed for signs and symptoms of acute hepatitis for 2½ years. There were 69 total cases of acute HEV, 66 in the placebo group and 3 in the vaccine group, an efficacy of 95.5 percent in subjects receiving three doses of vaccine. The intent-to-treat analysis showed an efficacy of 88.5 percent. The vaccine was well tolerated, with local pain at the injection site being the most frequent complaint.

In an alternative vaccine strategy that is being investigated, VLPs are given orally to induce local and systemic immunity. As described previously, the HEV ORF2 forms dimers in vitro that assemble into VLPs.[79] These VLPs are very immunogenic when administered to guinea pigs directly and also were used to detect anti-HEV antibody from infected macaques. Mice immunized with HEV VLPs in increasing amounts developed serum IgG

and IgM responses at 2 to 4 weeks after immunization.[75] Mice given the higher inoculums (50 and 100 μg) also developed fecal IgA responses. Subsequent challenge experiments in cynomolgus macaques showed that oral immunization with VLPs not only produced serum and fecal antibody but protected the animals from intravenous challenge with native HEV strains.[77] These studies provide promise that an oral vaccine for HEV is a possibility.

DNA vaccines have shown encouraging initial results as well. Macaques given a DNA plasmid encoding the HEV ORF2 by the gene gun technique developed significant anti-HEV IgG within 4 months of vaccination.[64] The macaques then were challenged with intravenous HEV, and the vaccinated macaques were negative for HEV RNA in serum and stool and showed no increase in the titers of anti-HEV throughout the test period, whereas the unvaccinated group had detectable HEV RNA, one had alanine aminotransferase elevations, and all had a significant increase in their anti-HEV titers.

SUMMARY

Despite the lack of a tissue culture system for HEV, much progress has been made toward development of a vaccine that is effective. Fundamental questions of virus entry and pathogenesis still linger, as well as why certain patients are highly susceptible to severe HEV disease, whereas others are not. The nature of the differences in the epidemiology and clinical manifestations of HEV infection in children also needs to be studied further. When a vaccine is available, studies on the most effective use in endemic and epidemic settings will also need to be undertaken.

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SUBSECTION 3

Reoviridae

CHAPTER 183

Reoviruses

James D. Cherry

Reoviruses are ubiquitous in nature, but their role in human disease is vague. After reoviruses were classified in 1959, numerous reports noted their association with human disease.[36,64,76] Since that time, these viruses have been evaluated extensively in laboratory animal studies, but few cases of human disease have been reported during the last 4 decades.

HISTORY

On the basis of its cytopathic effect in monkey kidney tissue culture and its recovery from stool specimens at a time of enterovirus surveillance, the first recovered reovirus was designated ECHO virus type 10.[9,10] This virus and four similar strains were recovered in 1954 from the stools of healthy children in Cincinnati and Mexico.[59,60] By 1959, researchers were aware that ECHO 10 viral strains appeared to have many characteristics that were different from those of enteroviruses, such as large size and unique cytopathic effect, and they were withdrawn from this grouping. The term *reovirus* was chosen to stress the association of these agents with both the respiratory *(r)* and enteric *(e)* tracts. The *o* for orphan was retained in the designation. In the early 1960s, reoviral infections were noted in association with human illness.[15,26,30,36,68,69,102]

PROPERTIES[8,51,52,63,64,93-95]

Reoviruses are members of the family *Reoviridae*.[17,52] This family has numerous double-stranded RNA viruses that infect mammals, birds, reptiles, fish, mollusks, insects, plants, and arthropods.[52] There are nine genera in the family, and they can be subdivided by the presence or absence of a turret-like protein that projects from the innermost capsid layer. Of the turreted *Reoviridae*, only orthoreoviruses infect humans and are addressed in this chapter. Orthoreoviruses commonly are called simply reoviruses. Nonturreted *Reoviridae* include the following viruses that cause disease in humans: rotaviruses (Chapter 185) and orbiviruses and coltiviruses (Chapter 184). Three serotypes of orthoreoviruses cause infection in humans. All mammalian reoviruses are related by a common group-specific, complement-fixing antigen.[72,94] Three distinct human serotypes can be identified by neutralization or hemagglutination inhibition.[70]

Reovirus particles are composed of an inner protein shell (core) with a diameter of 60 nm that is surrounded by an outer protein shell (outer capsid) measuring 81 nm in diameter.[52] The outer capsid has icosahedral symmetry and is composed of between 92 and 180 hexagonal and pentagonal subunits (capsomers). The genome of orthoreoviruses has a total size of approximately 23,500 base pairs with 10 gene segments: three large (L1, L2, L3), three medium (M1, M2, M3), and four small (S1, S2, S3, S4). These genes encode 12 proteins. The outer capsid is composed of four proteins: sigma-1, sigma-3, lambda-2, and mu-1. Sigma-1 is the reovirus cell-attachment protein against which serotype-specific neutralizing antibodies are developed. This protein also is the hemagglutinin. The reovirus receptor is the junctional adhesion molecule 1 (JAM1).[18,87] Sigma-3 protein binds double-stranded RNA and is sensitive to protease degradation. It is a zinc metalloprotein that has effects on translation. Lambda-2 protein is important in particle assembly and mu-1 in cell penetration.

The inner capsid is composed of four proteins: lambda-3, lambda-1, mu-2, and sigma-2. Lambda-3 is an RNA-dependent RNA polymerase. Lambda-1 is a zinc metalloprotein that binds RNA. Mu-2 binds RNA, and sigma-2 binds double-stranded RNA.

The four nonstructural proteins are mu-NS, mu-NSC, sigma-1s, and sigma-NS. Mu-NS is a core-binding protein, and sigma-NS binds single-stranded RNA. The roles of mu-NSC and sigma-1s are unknown.

Reoviruses are moderately heat-stable. The half-life at 56° C of a type 3 strain is 1.6 minutes; at 37° C, it is approximately 2.5 hours. Reoviruses are inactivated by visible light in the presence of heterocyclic dyes. They are inactivated by ultraviolet light but are more resistant to this treatment than are RNA viruses with single-stranded nucleic acid. Reoviruses are stable through a wide pH range and also are stable as aerosols, particularly when the relative humidity is high. They are relatively resistant to 3 percent formaldehyde solution, 1 percent hydrogen peroxide, and 1 percent phenol but are inactivated completely by 70 percent ethyl alcohol at room temperature for 1 hour. Brief exposure to 70 percent ethanol is ineffective in disinfecting reoviruses, but brief exposure to 95 percent ethanol or sodium hypochlorite is effective.

Reoviruses replicate with a cytopathic effect in a large number of tissue culture systems of both primate and other animal origins. For recovery from clinical material, monkey kidney tissue culture is satisfactory. Cytopathic effect is enhanced in rolled compared with stationary cultures.[37] Infected cells develop characteristic cytoplasmic inclusions that contain double-stranded RNA, virus-specific proteins, and complete and incomplete viral particles. All three serotypes agglutinate human erythrocytes, and this virus-cell interaction is stable in a wide temperature and pH range.

Of all viruses that naturally infect humans, reoviruses have the broadest host range. They have been recovered from natural infections in cattle, chimpanzees, monkeys, mice, dogs, turkeys, sheep, pigs, chickens, and cats.[11,22,24,42,44,45,65,73,98,99] In addition, hemagglutination-inhibiting and neutralizing antibodies to one or more of the three reoviral serotypes have been found in the serum of rabbits, horses, trout, guinea pigs, antelopes, zebras, warthogs, bats, wallabies, quokkas, kangaroos, and several genera of New World monkeys.[50,67,70,72,82,83]

Because of their widespread prevalence in nature and the ease of causing infection in laboratory animals, reoviruses have been used widely in pathogenicity studies. The following unique illnesses have been observed: diabetes mellitus in mice; hydrocephalus in hamsters, ferrets, rats, and mice; encephalitis in mice; chronic infection with runting in mice; myocarditis in mice;

TABLE 183–1 Hemagglutination-Inhibiting (H1) Antibodies to Reovirus Type 2 in 253 Serum Specimens Collected in Boston, Massachusetts, from 1959 to 1962

Age Group	Percentage with H1 Titer ≥1:20
Premature newborn	68
0-6 months	25
7-12 months	9
13-24 months	27
2-5 years	37
6-10 years	52
11-20 years	54
21-40 years	34
41-60 years	83
>60 years	73

From Lerner, A. M., Cherry, J. D., Klein, J. O., et al.: Infections with reoviruses. N. Engl. J. Med. 267:947-952, 1962. Reprinted with permission from The New England Journal of Medicine.

chronic obstructive jaundice associated with choledochal obliteration in mice; and lymphomas in mice with chronic infection.*

EPIDEMIOLOGY

The serologic data presented in the preceding section of this chapter demonstrate that reoviruses are prevalent infectious agents in the animal world. Similarly, surveys of sera collected from humans indicate worldwide human infection.† The occurrence in nature of identical viruses in many different animals and humans leads to consideration of possible transmission from species to species. At present, this transmission has not been demonstrated.

Reoviruses are recovered frequently from sewage.[16,29,33,39,43,53] In San Diego, reoviruses have been recovered more consistently than has any other virus throughout the year from sewage.[16] This finding suggests that reoviral infection is endemic in humans in the San Diego area.

The type 2 hemagglutination-inhibiting antibody prevalence pattern by age in sera collected in Boston from 1959 to 1962 is presented in Table 183–1. Antibody is transmitted transplacentally to the newborn. During the first year of life, this acquired antibody wanes, after which antibody prevalence increases with increasing age. Approximately 50 percent of school-aged children have hemagglutination-inhibiting antibody titers to type 2 reovirus greater than or equal to 1:20; approximately 80 percent of adults have similar titers. Two more recent studies have shown a similar prevalence of antibody to reovirus type 3.[77,88]

The method of transmission of reoviruses is unknown. However, because they are recovered most frequently from the feces, the primary spread probably is by the fecal-oral route, similar to that of enteroviruses. Because the reoviruses are stable in aerosols and because respiratory illness has been associated with reovirus infections, the respiratory route is an additional possibility.

CLINICAL MANIFESTATIONS

The role of reoviral infections in human disease is far from clear. In most instances, virologic or serologic evidence of reoviral infection has been a sporadic finding in human illness, so that cause and effect are difficult to establish. However, in volunteer studies in young adults, infection resulted in clinical illness.[26,66]

The prevalence of antibody to all three reoviral types in humans and the frequency of reovirus isolation from human sewage indicate that human infection is a common occurrence, but the relative paucity of virus recovered during studies of community disease suggests that most infections are inapparent or associated with trivial illness.

UPPER RESPIRATORY ILLNESS

In the winter of 1957, Rosen and colleagues[68] noted an outbreak of infection with reovirus type 1 in nursery children in a welfare institution. Illness was noted in 16 of 22 infected children and was characterized by low-grade fever (rectal temperatures from 38.1° C to 38.6° C [100.6° F to 101.5° F]), rhinorrhea, and pharyngitis. The average duration of fever was 2.2 days; in nine children, the duration was only 1 day. Three children had diarrhea, and three had mild otitis media. In another study conducted at the same institution during the winter of 1955 to 1956, four children with reovirus type 3 infection and illness were noted.[69] One child had a temperature of 38.9° C (102° F), coryza, and tonsillitis; another child had fever (temperature of 38.2° C [100.8° F]), cough, and diarrhea; and two children had only coryza. During another reovirus type 3 outbreak in the fall of 1957, all six infected infants had symptoms. Five children had mild fever, five had coryza, and four had diarrhea. Pharyngitis was not observed in any of the infected children.

Other sporadic instances of similar mild upper respiratory illnesses have been described.[12,23,26,80,84] In volunteer trials in young adults, reovirus type 1 infection was associated with malaise, rhinorrhea, cough, sneezing, pharyngitis, and headache in some subjects in one study,[66] and coldlike illness was observed in 37 percent of subjects in another trial.[26] In both volunteer studies and natural infection, mild diarrhea occurred with the upper respiratory illness.

PNEUMONIA

Tillotson and Lerner[91] described a 5-year-old girl who had extensive pneumonia and died after 15 days of illness. This child initially had fever, cough, rhinorrhea, and a generalized maculopapular rash. When admitted to the hospital on the 10th day of illness, the child was cyanotic and in marked respiratory distress; rash was present no longer, but mild pharyngitis and conjunctivitis were. A chest radiograph revealed a diffuse confluent pneumonia, and reovirus type 3 was recovered from the lungs, adrenals, liver, spleen, kidney, a lymph node, heart, brain, and blood.

Joske and associates[30] described a 10-month-old girl who died after having a respiratory illness of 4 days' duration. A reovirus type 1 was recovered from the stool and brain of this child, and postmortem study revealed interstitial pneumonia, myocarditis, hepatitis, and encephalitis. El-Rai and Evans[15] reported the case of an 18-year-old man who had fever (temperature of 39.4° C [103° F]), nausea, vomiting, cough, and patchy pneumonia. He had serologic evidence of infection with reovirus type 1. Pneumonia has been noted in another child with reovirus type 3 infection.[84]

GASTROINTESTINAL MANIFESTATIONS

Mild diarrhea has been noted both in association with upper respiratory illness and as an isolated event.[58,66,68,69,73] Because reovirus type 3 consistently produces steatorrhea in mice, this clinical manifestation has been sought in illnesses of children and noted in six.[72,84] Three patients with hepatitis and encephalitis

*See references 27, 32, 40, 41, 54, 55, 57, 78, 81, 85, 94.
†See references 4-6, 19, 31, 35, 36, 38, 56, 71, 75, 89, 92.

have been described.[30] Zalan and associates[102] have noted two patients in whom abdominal pain and cramps were prominent.

In 1980, Bangaru and associates[1] reported the similarity of induced hepatobiliary injury caused by reovirus type 3 infection in mice and biliary atresia in human infants. Subsequent to this observation, Morecki and colleagues[20,48,49] looked for an association between reovirus type 3 infection and biliary atresia in humans. In their first report, they found that 17 of 25 patients (68%) with biliary atresia had antibodies (indirect immunofluorescent antibody technique) to reovirus type 3, whereas similar antibodies were recovered in only 3 of 37 control sera.[48] In a second study, they found that 62 percent of babies with extrahepatic biliary atresia and 52 percent of infants with idiopathic neonatal hepatitis had antibodies to reovirus type 3; only 12 percent of control children had similar antibodies.[20] In an ultrastructural and immunocytochemical study, they found evidence of reovirus type 3 in the porta hepatis of an infant with extrahepatic biliary atresia.[49]

Using similar serologic techniques, Dussaix and associates[13] were unable to find any relationship between reovirus type 3 antibody and either biliary atresia or neonatal hepatitis. They found reovirus type 3 antibody in sera from 45 percent of infants with biliary atresia, 50 percent of infants with neonatal hepatitis, and 50 percent of control infants. Minuk and colleagues[47] found no association between reovirus type 3 infection and idiopathic cholestatic liver disease in adults. Brown and colleagues[7] reported a relatively large study of reovirus type 3 infection and extrahepatic biliary atresia and neonatal hepatitis. They interpreted their data as demonstrating no correlation between the virus and the illnesses studied. However, the geometric mean antibody value in the combined biliary atresia and neonatal hepatitis groups was significantly higher than that of the control group. Richardson and associates[62] reported a study in which they examined the percentage of IgG, IgA, and IgM serum antibodies to reovirus type 3 in 40 infants with extrahepatic biliary atresia, 59 infants with neonatal hepatitis, 61 infants with cholestatic liver disease with causes other than extrahepatic biliary atresia or neonatal hepatitis, and 138 control infants with no liver disease. They found no difference in the prevalence of IgG and IgA antibodies between the groups with liver disease and the control subjects. They did, however, note a greater prevalence of IgM antibody in each of the groups with liver disease compared with the rate in the control group. However, this increased prevalence of IgM antibody could be the result of false-positive titers due to the liver disease rather than evidence of recent or ongoing infection. Steele and associates,[86] using a reverse transcriptase–mediated polymerase chain reaction (RT-PCR), found no evidence of reovirus type 3 in preserved tissues from infants with cholestatic liver disease. Recently, Saito and associates,[74] using RT-PCR, could find no amplification product in tissue or stool specimens from patients with biliary atresia, infantile obstructive cholangiopathy, or congenital dilation of the bile duct.

EXANTHEM

Exanthem has been a common manifestation of clinically apparent reoviral infections.[15,30,36,91] Lerner and associates[36] noted exanthem in six of seven children infected with reovirus type 2. Predominant symptoms in these patients included fever, malaise, anorexia, and pharyngitis. Two children had adenopathy, and one child had diarrhea. The rash was maculopapular in five patients and vesicular in one child. One child had a measles-like illness with photophobia, conjunctivitis, cervical lymphadenopathy, and a confluent maculopapular rash that lasted approximately 1 week.

Exanthem has been noted in a 5-year-old girl with pneumonia and type 3 reovirus infection, a 28-month-old girl with encephalitis and type 2 infection, and an 18-year-old adolescent male with pharyngitis and cervical and posterior occipital lymph node enlargement and type 2 infection.[15,30,91]

NEUROLOGIC DISEASE

Joske and colleagues[30] described three cases of hepatitis-encephalitis syndrome with reoviral infections. All these cases had abnormal liver function test results and clinical and laboratory evidence of meningeal and cerebral involvement. One child died, one had mild neurologic residua at the 6-week follow-up, and one recovered without difficulty. Two patients were infected with reovirus type 2 and one with reovirus type 3.

El-Rai and Evans[15] found serologic evidence of reovirus type 2 infection in two children with aseptic meningitis, and Zalan and colleagues[102] described two children with reovirus type 2 infections associated with leg weakness and pain. Johansson and colleagues[28] described a 3-month-old girl with meningitis, diarrhea, vomiting, and fever. Reovirus type 1 was isolated from this child's cerebrospinal fluid, and the child had a fourfold rise in neutralizing antibody to the isolated virus. Krainer and Aronson[34] described a 29-year-old woman who died of disseminated demyelinating encephalomyelitis in which a reovirus was recovered from the cerebrospinal fluid and brain. Recently, Tyler and associates[96] isolated a type 3 reovirus from the cerebrospinal fluid of a 6.5-week-old child with meningitis. This child recovered without obvious neurologic sequelae.

OTHER MANIFESTATIONS

A 25-year-old man with Hodgkin disease had persistent reovirus type 1 viruria for a 5-week period, but no associated clinical illness was demonstrated.[14] Reoviruses have been recovered from biopsy material from patients with Burkitt lymphoma.[2,3] One child with hemorrhagic bullous myringitis was infected with reovirus type 2.[102] Terheggen and colleagues[90] noted a 28-year-old man with mild myocarditis from whom a reovirus was isolated from the stool sample.

REOVIRUSES AS POTENTIAL ANTI-CANCER AGENTS

Studies during the present decade indicate the potential of reoviruses to selectively destroy many different types of neoplastic cells.[21,25,46,79,97,100,101] Many malignant cells have an activated Ras pathway that results in a deficient ability to mount an anti-reovirus response that is mediated by the cellular protein PKR. Organ culture and animal model systems have shown promising results with reovirus type 3 in numerous different malignant neoplasms. At present, many phase I and phase II trials are in progress.[61,97]

DIAGNOSIS

Because no specific clinical features suggest reoviral infection, virologic and serologic studies are necessary for establishing the diagnosis. Reoviruses can be recovered from clinical material in primary monkey kidney tissue culture.[63] Care must be taken in interpreting results, however, because reoviruses can be contaminants of monkey tissue cultures. Cytopathic effect in tissue culture is enhanced by rolling during incubation.[37] Identification of virus is made by neutralization; the distinctive cytopathic effect should be helpful in selecting strains for study with reovirus antisera. In research laboratories, reoviruses also can be detected

by molecular techniques such as in situ and dot-blot hybridization and PCR.[94] Paired serum specimens can be examined for antibody titer rise to reoviruses by neutralization, indirect immunofluorescent antibody technique, enzyme-linked immunosorbent assay, or hemagglutination inhibition. Recent infection can also be identified by the demonstration of specific IgM antibody by enzyme-linked immunosorbent assay.

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CHAPTER 184

Orbiviruses, Coltiviruses, and Seadornaviruses

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Viruses in the genera *Orbivirus*, *Coltivirus*, and *Seadornavirus* differ from others in the family *Reoviridae* structurally, in physicochemical properties, and by their arthropod mode of transmission.[2,4,44] More than 100 orbiviruses are classified into 14 serogroups defined by relationships in complement fixation, agar gel precipitation, and immunofluorescent assays. Additional serocomplex relationships are recognized, whereas individual viruses are differentiated by neutralization tests. Orbiviruses are principally animal pathogens (e.g., the bluetongue viruses); only viruses in the Kemerovo, Orungo, Lebombo, and Changuinola serogroups have been shown to cause human illness. Coltiviruses recognized to be human pathogens include Colorado tick fever (CTF) virus and Eyach virus; Banna virus is the only seadornavirus recognized to cause human illness.[2,4]

The orbiviruses are spherical nonenveloped viruses with two protein shells: an outer shell (capsid) approximately 86 nm in diameter and an inner shell (core) 69 nm in diameter. The inner core VP7 protein, arranged in trimers, contains group-reactive antigens, and the outer capsid VP2 or VP5 proteins bear serotype-specific antigens. The viruses are acid labile, and some exhibit sensitivity to lipid solvents. The double-stranded RNA viral genome is composed of 10 segments associated with viral structural and nonstructural proteins. Their complete nucleotide sequences and coding assignments have been determined for a bluetongue virus, and partial information is available for others. In general, reassortments of genomic segments among viruses within serogroups are viable, but not between groups, thus validating the broad serogroup classification. The genetic relation-

ships (e.g., RNA hybridization) among viruses within serogroups diverge from the antigenic relationships in some instances, which provides an additional basis for taxonomic classification.

The coltiviruses and seadornaviruses resemble the orbiviruses in size and in having two capsids but possess genomes organized into 12 RNA segments.[2,44] Seadornaviruses' morphologic features and structural protein similarities with rotaviruses suggest an evolutionary relationship.

COLORADO TICK FEVER VIRUS

CTF is an acute tick-borne febrile illness caused by the eponymous coltivirus.[5,12,17,18]

Eyach virus isolated from *Ixodes* ticks in France and Germany, S6-1403 isolated in California from a gray squirrel, and mosquito strains from Indonesia are related to but distinct from CTF virus and have not been associated with human disease.[4,27] RNA hybridization studies of CTF viral strains from a 33-year interval found minor heterogeneity, thus suggesting that a single gene pool has been maintained by mixing of viral strains and by constraints on viral replication within tick vectors and vertebrate hosts. A wide variety of small and large mammals are infected naturally with the virus, but clinical illness develops only in humans. Experimental infection of rhesus monkeys, hamsters, and mice produces hematologic changes similar to those occurring in human infections.[20]

EPIDEMIOLOGY

Cases occur principally in association with the habitats and activity patterns of the wood tick *Dermacentor andersoni*. Most cases occur in May and June, when adult ticks are most active, but infections from March to November have been reported. Infections are acquired principally in the western part of the United States and Canada in the known geographic distribution of the vector (Fig. 184–1).[7,10-12] CTF viral antibodies have been found in serosurveys in South Korea, but no viral isolates have been recovered (unpublished observations, C. H. Calisher).

In rare cases, CTF has occurred in persons who had not traveled to areas of known risk, such as those exposed to ticks brought home on the clothing of family members and, in one case, by transfusion.[33,38,42,43]

CTF virus is maintained in a 2- to 3-year cycle among small mammals, principally rodents, and *D. andersoni* ticks (Fig. 184–2).[5,12,18] Once infected in the larval stage, ticks remain infected through the nymphal and adult stages (trans-stadial transmission). Larvae are infected by viremic rodents. After molting, they carry the virus through the winter, and as nymphs, they infect other rodents and renew the cycle of transmission the following spring. Humans become infected incidentally by the bite of infected adult ticks.

The least chipmunk *(Eutamias minimus)*, the golden-mantled ground squirrel *(Spermophilus lateralis)*, and the porcupine *(Erethizon dorsatum)* appear to be the primary hosts for larval, nymphal, and adult *D. andersoni;* secondary hosts include rock mice *(Peromyscus maniculatus)*, meadow voles *(Microtus pennsylvanicus)*, and pine squirrels *(Tamiasciurus hudsonicus)*.[6]

D. andersoni is found exclusively in the high plains and in mountainous terrain between 4000 and 10,000 feet in altitude in the geographic distribution previously mentioned. The specific microhabitats where infected ticks are most prevalent are south-facing slopes with open stands of ponderosa pine, moderate shrubs, and rocky surfaces that provide favorable habitats for the intermediate rodent hosts.[6,32] Both male and female adult ticks can transmit infection to humans, and the period of attachment required for transmission of the virus may be very brief.

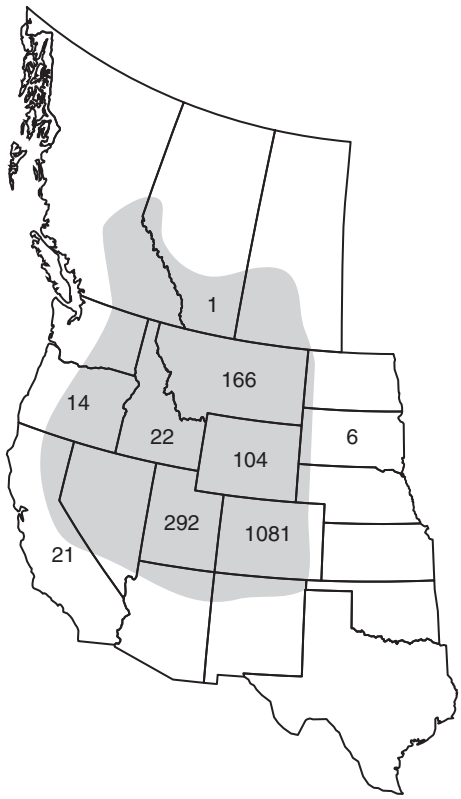

Figure 184–1 Geographic distribution of *Dermacentor andersoni* ticks and reported cases of Colorado tick fever, 1980 to 1991.

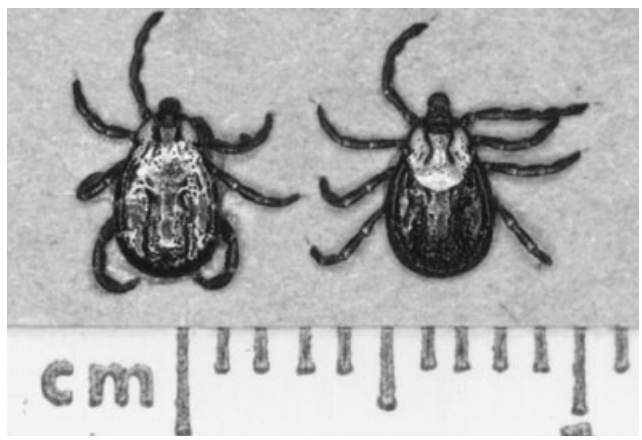

Figure 184–2 Adult male *(left)* and female *(right) Dermacentor andersoni* ticks. Both can transmit Colorado tick fever virus.

CLINICAL MANIFESTATIONS

A history of a tick bite or tick exposure is given by more than 90 percent of patients.[21] The incubation period usually is 3 to 4 days, with a range of 0 to 14 days after known exposure to a tick.[21] The onset typically is abrupt, with fever, chills, malaise, headache, retro-orbital pain, myalgia, lumbar pain, and hyperesthesia. Nausea, vomiting, and abdominal pain occur less frequently. Upper respiratory symptoms usually are absent, although conjunctival injection may develop. Lymphadenopathy and hepatosplenomegaly are found in some patients. Maculopapular and petechial eruptions have been observed in 5 to 12 percent of cases.[41]

The disease classically is diphasic; an initial attack lasting 2 to 3 days is followed by an equal interval of defervescence and a second and rarely a third recurrence. However, this saddleback pattern is absent in more than 50 percent of cases. Symptoms resolve several days after the second bout of fever, but prolonged asthenia lasting for several weeks is a typical finding, especially in adults.[21]

Leukopenia is a hallmark of the illness. The mean initial leukocyte count is 3900/mm^3, which diminishes to a nadir 5 to 6 days after the onset of illness, often during the period of remission. A left shift with an absolute neutropenia and relative lymphocytosis is a usual finding. Examination of bone marrow aspirates shows maturational arrest in the granulocytic series, with absent mature forms and numerous metamyelocytes and myelocytes. Megakaryocytes are depleted, and a reduced peripheral platelet count (<150,000/m^3) is found in most patients; however, thrombocytopenia rarely reaches a level of clinical significance.[25,31]

Uncomplicated recovery is the rule. Epididymo-orchitis, pneumonitis, hepatitis, and pericarditis have been reported as complications, but the most serious complications are encephalitis and hemorrhage,[15,19,21,23,30] which have been reported nearly exclusively in children younger than 10 years.[10,21,39,41] Three deaths have been reported, all in children who exhibited signs of generalized bleeding accompanied by a reduced platelet count.[9,21] Examination of the bone marrow in one child disclosed a generalized depression of myeloid and erythroid elements and a marked reduction in megakaryocytes. Central nervous system signs consistent with meningitis and encephalitis have been reported in both children and adults. Examination of cerebrospinal fluid disclosed elevated protein and mononuclear cell pleocytosis in the few cases that have been described.

D. andersoni is a vector for both CTF and Rocky Mountain spotted fever (RMSF).[4] Although dual isolation of CTF virus and *Rickettsia rickettsii* from the same tick has not been reported, a concurrent rickettsial infection could not be discounted in all of the previously mentioned fatal cases attributed to CTF. Furthermore, a dual infection could develop in an individual exposed to more than one tick.

CTF virus is teratogenic in experimentally infected mice, but few clinical data are available on the teratogenic potential of the virus in humans.[22] One pregnant woman aborted 2 weeks after having an illness; and in an infant delivered 6 days after the onset of CTF in the mother, a febrile illness with leukopenia developed at 3 days of age, thus indicating a possible vertically acquired infection. In a single reported instance, a second attack of CTF has been documented, which indicates that immunity may not be permanent.[21]

The combined historical elements of tick exposure and outdoor activity in an enzootic area should suggest the diagnosis in patients with a spring-summer grippe-like illness. In a controlled study, abdominal pain, pharyngitis, and rash were less common symptoms in patients with CTF than in patients with other acute illnesses in the same season. Although RMSF occurs rarely in the states in which CTF is prevalent, the possibility of this potentially fatal disease should be considered in the differential diagnosis. Helpful differentiating features include the rarity of a petechial rash in CTF and its biphasic course. Tick-borne relapsing fever also follows a remitting course, but it has a more acute and toxic onset; splenomegaly occurs commonly, and remission is by crisis. The geographic distribution of the diseases overlaps; however, human encounters with the argasid (soft) tick vectors of relapsing fever (*Ornithodoros* ticks) are likely to occur in cabins and other protected areas.

Eyach virus has been implicated in central nervous system infections and polyradiculitis in Europe. However, its tick-borne transmission from *Ixodes ricinus* that also transmits *Borrelia*, *Anaplasma*, and Kemerovo group viruses has prevented establishment of a clear interpretation.

PATHOPHYSIOLOGY

Experimental infection of rhesus monkeys, rodents, and human marrow cells in vitro has shown that CTF virus infects the erythropoietic elements (erythroblasts to reticulocytes) at an early stage of infection.[13,14,20,26,36,38] After the infected cells mature and are released into the peripheral circulation, the virus persists intracellularly, where it has been identified by electron microscopy.[14,36] CTF virus has been cultured from circulating erythrocytes for up to 120 days after the onset of illness.[24] Red cell survival evidently is not shortened, and infected cells circulate in the presence of neutralizing antibody. Prolonged viremia has not been associated with either a protracted or a more severe course of illness. Infected CD34$^+$ progenitor cells and impaired mononuclear cell production of colony-stimulating factors may contribute to the acute aregenerative cytopenia.[37] High levels of serum interferon-γ correlate with fever during the acute phase of illness.[1]

LABORATORY DIAGNOSIS

Direct immunofluorescent examination of blood smears for intraerythrocytic viral antigen is an accessible and rapid approach to laboratory diagnosis.[13] However, polymerase chain reaction analysis and viral culture of blood samples are more sensitive.[3,26] Ninety-six percent of all cases diagnosed by seroconversion are identified by isolation of virus from acute or convalescent blood samples in baby mice or Vero or BHK cells.[8,13] Freezing and thawing of the clot should be avoided. Virus has been isolated from a blood clot that had been refrigerated for 14 months. Serologic diagnosis by demonstration of fourfold rises in neutralizing, complement-fixation, immunofluorescent, or enzyme-linked immunosorbent assay antibody is confirmatory.[3] Enzyme-linked immunosorbent assays that distinguish between CTF and Eyach virus antibodies are available.[34] Neutralizing antibody rises slowly; only a third of patients had detectable neutralizing antibody 10 days after onset, but fourfold rises appeared within 30 days of onset in 92 percent.

TREATMENT

No specific therapy is available. Because thrombocytopenia may occur and hemorrhage is a reported complication in children, antipyretics that interfere with coagulation should not be used.

PREVENTION

Repellents containing permethrin should be sprayed on clothing, and repellents containing diethyltoluamide (DEET) or picaridin should be applied to exposed skin (see Table 187–2 in the chapter on western equine encephalitis). Long pants should be tucked into socks, and shirts should be worn tucked into slacks. Clothing, gear, and skin should be inspected frequently for attached ticks. Light-colored clothing facilitates such inspections. One case of human-to-human transmission by transfusion of infected blood has been reported.[42] Persons with documented CTF should be prohibited from donating blood until the often prolonged viremia has cleared.[38]

BANNA AND WX-3 VIRUSES

Banna and WX-3 viruses have been associated with febrile illness and encephalitis in China. Both have 12 RNA segments and are antigenically distinct from the CTF, S6-1403, and Eyach viruses (coltiviruses from the Rocky Mountain region, California, and Germany). Banna virus has been classified taxonomically as a seadornavirus (Southeast Asian dodeca RNA viruses).[2,4] Banna virus was isolated from encephalitis patients in Yunnan Province in southern China and later from 98 patients with fever, headache, and arthralgias from Xinjiang Province in western China.[28] WX-3 and nine related strains that segregate into four distinct RNA electropherotypes were isolated from *Culex tritaeniorhychnus* mosquitoes in Gansu Province and the suburbs of Beijing in 1991.[40] Serologic evidence of recent infection with the strains was found in 50 percent of patients with encephalitis in Henan Province and in 17 percent of patients in Jiangsu Province, suggesting that these coltiviruses may be a leading cause of summer viral encephalitis after Japanese encephalitis. Details of the clinical illness and epidemiology of infection have not been reported. A real-time polymerase chain reaction assay for Banna virus nucleic acid has been developed.[45] Related seadornaviruses from China and Southeast Asia are orphan viruses not yet associated with human illness.

KEMEROVO AND RELATED VIRUSES

The Kemerovo serogroup contains more than 50 chiefly tick-borne viruses divided into four serocomplexes. Only Kemerovo, Tribec, and Lipovnik viruses have been associated with human illness. Kemerovo virus was isolated from the cerebrospinal fluid of two patients with encephalitis and from ticks in the Kemerovo region of Russia; seroconversion was demonstrated in 10 other patients with meningoencephalitis with tick bites and in whom Russian spring-summer encephalitis was excluded.[7,29] The virus is transmitted in an *Ixodes persulcatus*–rodent cycle. Lipovnik virus has been reported in neurologic infections in the former Czechoslovakia, where flaviviral tick-borne encephalitis is endemic; one study showed seroconversion to Lipovnik virus in half the patients with encephalitis who were studied, including some with suspected dual infection with tick-borne encephalitis virus.[29] This finding seems plausible because both viruses are transmitted by *I. ricinus* ticks, and multiple exposure to singly infected ticks or infection with a dually infected tick may be possible. In addition, serologic evidence of Lipovnik or Tribec infection was reported in patients with chronic polyradiculoneuritis; however, spirochetal infection was not ruled out in these cases. The increasing geographic range of *I. ricinus* ticks in Europe to more northerly latitudes and to higher elevations, attributed to global warming, could lead to increased transmission of these orbiviruses, as has been observed with tick-borne encephalitis virus.

On the basis of serologic rises in patients with acute febrile illness diagnosed clinically as RMSF, a Kemerovo-related virus is suspected to occur in the southwestern region of the United States. Patients with a history of a tick bite or tick exposure, whose sera were negative for *R. rickettsii*, demonstrated fourfold or greater changes in immunofluorescent titers to Lipovnik and Six Gun City viruses (Kemerovo group). Rises in immunofluorescent antibody titers to 128 to 512 suggested recent infection with a Kemerovo-related virus, possibly a novel agent or related to rabbit syncytium virus, which is enzootic in the United States. The patients had acute febrile illnesses with myalgia, vomiting, and severe abdominal pain, along with leukopenia, thrombocytopenia, and anemia, similar to RMSF. No agent has been isolated, but neither was the possibility of *Ehrlichia* infection excluded. Serologic evidence of infection was found in a cotton rat in the vicinity of one case. The syndrome tentatively is called Oklahoma tick fever.

ORUNGO VIRUS

Orungo virus is unrelated antigenically to the other orbiviruses. Infection is prevalent in western, central, and eastern Africa and apparently is transmitted in a sylvatic monkey–*Aedes* mosquito cycle similar to that of yellow fever. Human-to-human transmission by *Anopheles* mosquitoes is speculated to occur. The virus has been isolated from patients with fever and headache, and serologic evidence of infection has been reported in outbreaks of illness characterized by fever, headache, myalgia, nausea, and vomiting. Seroconversion has been observed in patients studied during yellow fever outbreaks, presumably because of concurrent transmission of the viruses. The virus also was isolated from the blood of a child with convulsions and flaccid paralysis.[16,35]

LEBOMBO VIRUS

Lebombo virus is not grouped antigenically. The virus was isolated first from *Aedes circumluteolus* mosquitoes in South Africa. Subsequently, the virus was recovered from a Nigerian child with nonspecific febrile illness; the virus also was isolated from rodents and mosquitoes in Nigeria.[35]

CHANGUINOLA VIRUS

Changuinola virus belongs to an antigenic complex of 12 principally phlebotomine-borne orbiviruses. The virus is transmitted in Panama among forest mammals and *Phlebotomus* flies. Only one human case has been reported—a nonspecific febrile illness in a mosquito catcher.

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CHAPTER 185

Rotaviruses

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Rotaviruses are recognized as the single most important cause of severe infantile gastroenteritis worldwide.[124,183,212,262,412] In the United States, these viruses are estimated to cause between 24,000 and 110,000 hospitalizations in young children annually[182,183,226,227,323,331,466] and 20 to 60 deaths.[182,183,226,227] Direct medical costs associated with rotavirus disease in the United States have been estimated to be between $100 and $400 million annually.[10,331,545] Indirect costs are significant, accounting for an additional $500 million each year,[296,545] leaving the total rotavirus costs at $1 billion per year in the United States.[545] On a world scale, rotaviruses are estimated to be responsible for more than 600,000 deaths annually.[183,244,262,410] For these reasons, rotaviruses have received a high priority as a target for vaccine development.[244,455]

Transmission of rotaviruses occurs by the fecal-oral route, providing a highly efficient mechanism for universal exposure that has circumvented differences in regional and national cultural practices and public health standards. The symptoms associated with rotavirus disease typically are diarrhea and vomiting accompanied by fever, nausea, anorexia, cramping, and malaise that can be mild and of short duration or produce severe dehydration.[261,265,282,435,465,477,511,515,549] Severe disease occurs primarily in young children, most commonly between 6 and 24 months of age. Approximately 90 percent of children in both developed and developing countries experience a rotavirus infection by the time they reach 3 years of age.[261,262] Rotavirus infection normally provides short-term protection and immunity against subsequent severe illnesses but does not provide lifelong immunity; furthermore, numerous cases of sequential illnesses have been reported.* Neonates also can experience rotavirus infections, and they occur endemically in some settings but typically are asymptomatic.[29,32,203,213,364,424] These neonatal infections have been reported to reduce the morbidity associated with a subsequent rotavirus infection.[29,32] Rotavirus illnesses also occur in adults[125,136,161,237,275,468,473,599] and the elderly,[111,326,327,514] but as with other sequential rotavirus infections, the symptoms usually are mild. Recently, however, a series of reports have described a unique association between severe gastrointestinal disease in adults and serotype G2P[4] rotaviruses, the properties of which are described later in this chapter.[65,202,242,373,537,608]

Because of the frequent occurrence of rotavirus infections and the reduced severity of illness typically associated with sequential infections, a realistic goal for a rotavirus vaccine may be to protect against severe disease. Several vaccine candidates have been developed and evaluated in infants, with promising results.†

*See references 32, 35, 76, 95, 123, 166, 300, 330, 388, 456, 564, 607.
†See references 22, 25, 28, 30, 82, 85, 159, 289, 356, 475, 566, 567, 574.

Incorporation of an effective rotavirus vaccine into the infant immunization schedule in developed countries could reduce the number of hospitalizations due to dehydrating diarrhea in young children by 40 to 60 percent.[455] More important, worldwide use of such a vaccine could decrease by approximately 10 to 20 percent the total number of deaths caused by diarrhea.[124,244,455] Once effective rotavirus vaccines become universally available, control of rotavirus disease no longer will be limited to the nonspecific methods, such as rehydration therapy for replacement of body fluids and electrolytes, presently being used.

HISTORY

Viruses with morphologic features later associated with rotaviruses were observed first by electron microscopy in 1963 in intestinal tissues and rectal swab specimens from mice and monkeys.[4,324] These agents, called epizootic diarrhea of infant mice virus and simian agent 11, respectively, were described as 70-nm particles that had a wheel-like appearance. Hence, they were later designated "rota" viruses from the Latin word for wheel.[153,606] In 1969, Mebus and colleagues[350] demonstrated the presence of these particles in stools of calves with diarrhea, thus associating these viruses with a diarrheal disease in cattle. The correlation between these viruses and human diarrheal disease was reported first in 1973 by Bishop and colleagues,[34] who used electron microscopy to examine biopsy specimens of duodenal mucosa from children with acute gastroenteritis. Within a short time, these and other investigators confirmed the association between the presence of rotavirus in feces and acute gastroenteritis.[33,59,119,152,266] In addition to their distinctive morphologic features, these human viruses along with their animal rotavirus counterparts were later shown to share a group antigen[263,602] and have been classified as members of the *Rotavirus* genus within the *Reoviridae* family.[336] In 1980, particles that were indistinguishable morphologically from established rotavirus strains but lacked the common group antigen were discovered in pigs.[43,481] This finding subsequently led to the identification of rotaviruses belonging to six additional groups (B to G) based on common group antigens, with the original rotavirus strains classified as group A.[482] Only groups A to C have been associated with human diseases, and most known cases of rotavirus gastroenteritis have been caused by group A strains. However, non–group A rotaviruses have been associated with large outbreaks in China, particularly among adults,[241] and Japan,[288,334] which suggests that they could become major pathogens in the future. This suggestion is supported by seroepidemiology data showing high prevalence of group C rotavirus antibody in different countries.[249,381,462,544]

PROPERTIES

Visualization of the rotavirus particle by conventional electron microscopy revealed a double-shelled structure with icosahedral symmetry.[139,228,328] The outer shell is composed of two structural proteins, VP4 and VP7, which form capsomers that radiate from the inner capsid composed of the major structural protein VP6.[142,262] This inner shell surrounds a core containing the segmented, double-stranded RNA viral genome and three additional structural proteins, VP1, VP2, and VP3.

Much greater structural definition was obtained by cryoelectron microscopy (Fig. 185–1). This technique showed that the rotavirus core is surrounded by a third protein shell composed of VP2,[440,500] which can self-assemble into corelike particles when it is expressed in insect cells by a baculovirus recombinant.[108,621] Thus, the mature rotavirus particle contains three protein layers with radii of 21 to 26.5 nm (inner layer), 26.5 to 35 nm (intermediate layer), and 35 to 38 nm (outer layer). Detailed analysis of the outer layer suggests that it is composed primarily of the VP7 glycoprotein (780 molecules/virus), which contains 132 aqueous channels that are positioned over 132 channels within the perforated VP6 intermediate shell, also composed of 780 molecules/virus.[442,610] Sixty dimers or trimers of the VP4 protein, 20 nm in length, are anchored to the VP6 layer and form spike-like projections as they extend through and 11 to 12 nm beyond the VP7 layer.[439,440,500,609,611] Thus, the full diameter of the mature rotavirus particle, including the VP4 spikes, is approximately 100 nm.

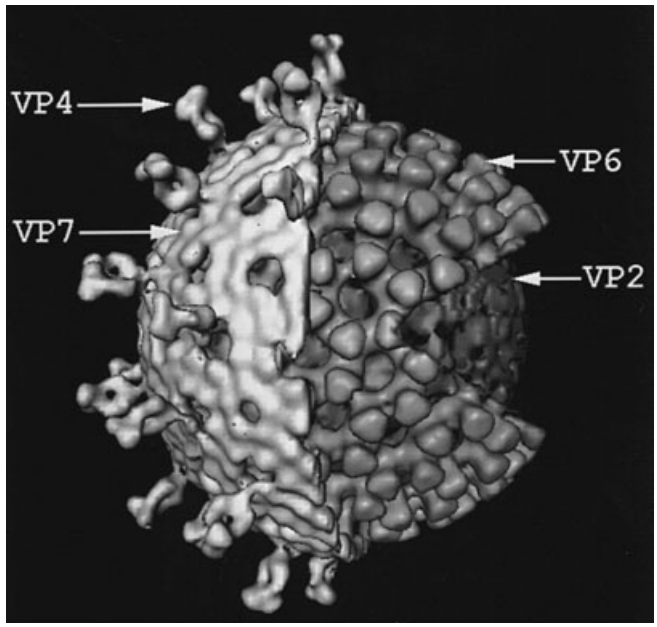

Figure 185–1 Computer-generated image of the triple-shelled rotavirus particle obtained by cryoelectron microscopy. The cutaway diagram shows the outer capsid composed of VP4 spikes and VP7 shell, intermediate VP6 shell, and inner VP2 shell surrounding the core containing the 11 double-stranded RNA segments and VP1 and VP3 proteins. *(Courtesy of Dr. B. V. V. Prasad, Baylor College of Medicine, Houston, TX.)*

Further examination of the rotavirus structure by cryoelectron microscopy has indicated that the inner layer composed of VP2 molecules not only encloses the viral genome but interacts with it as well.[441] This interaction has been found to cause significant conformational changes in the VP2 structure. Furthermore, VP1 and VP3 have been proposed to form a complex below the VP2 layer that interacts with ordered portions of the genome.[441] This interaction appears to be a prerequisite for transcription and capping of new mRNAs by VP1 and VP3, respectively, on initiation of the rotavirus replication cycle in an infected cell.[291] Finally, the amino terminus of VP2 has been reported to be necessary for the encapsidation of VP1 and VP3.[293,621] The genome of rotavirus is composed of 11 segments of double-stranded RNA that encode the six structural proteins—VP1 to VP4, VP6, and VP7—and six nonstructural proteins designated NSP1 to NSP6.[142] Each segment encodes one known rotavirus protein, except segment 11, which encodes both NSP5 and NSP6 using alternative open-reading frames.[338] The function of each of these proteins is now at least partially understood.* The genome segments range in size from approximately 660 to 3300 base pairs, and their encoded proteins, the known functions of which are briefly described in Table 185–1, have molecular weights of approximately 12,000 to 125,000.

*See references 16, 40, 64, 142, 145, 239, 269, 305, 308, 325, 414, 436, 558, 562, 565.

TABLE 185–1 Sizes of Rotavirus Gene Segments and Properties of Encoded Proteins

RNA Segment	No. of Base Pairs	Encoded Protein	Molecular Weight of Protein ($\times 10^{-4}$)	Properties of Protein
1	3300	VP1	12.5	Inner core protein RNA binding RNA transcriptase
2	2700	VP2	10.2	Inner capsid protein RNA binding
3	2600	VP3	9.8	Inner core protein Guanylyltransferase Methyltransferase
4	2360	VP4	8.7	Outer capsid protein Hemagglutinin Neutralization protein Receptor binding Fusogenic protein
5	1600	NSP1	5.9	Nonstructural protein RNA binding IRF regulatory protein
6	1360	VP6	4.5	Intermediate capsid Group and subgroup antigen
7	1100	NSP3	3.5	Nonstructural protein RNA binding Translational control
8	1060	NSP2	3.7	Nonstructural protein RNA and NSP5 binding Virosome formation
9	1060	VP7	3.7	Outer capsid glycoprotein Neutralization protein
10	750	NSP4	2.0	Nonstructural glycoprotein Transmembrane protein Enterotoxin
11	660	NSP5	2.2	Nonstructural protein Phosphorylated NSP2 and NSP6 binding
		NSP6	1.2	Nonstructural protein NSP5 binding

The double-stranded RNA genome segments of rotavirus can be extracted from viral particles and separated by polyacrylamide gel electrophoresis into 11 distinct bands visualized by ethidium bromide or silver staining (Fig. 185–2). Each rotavirus strain has a characteristic RNA profile or electropherotype, a property that has been used extensively in epidemiologic studies of these viruses.* The characteristic RNA electrophoretic pattern of group A rotaviruses consists of four size classes containing segments 1 to 4, 5 and 6, 7 to 9, and 10 and 11. RNA segments of strains belonging to less well characterized rotavirus groups (i.e., groups B to G) also can be separated into four size classes, but the distribution of segments within these classes differs from group to group.[44,421,480,482]

REPLICATION

Rotaviruses are activated by cleavage of the outer capsid VP4 protein by trypsin-like proteases into proteins VP5* and VP8,† which remain virus associated.[9,91,140,143,306] After attachment to receptors on the cytoplasmic membrane‡ through association with protein VP8,§ the activated virion either passes directly through this membrane or is taken within a vesicle into the cytoplasm.[141,168,258,303,523,524] Either during membrane penetration[110,524] or soon thereafter, the outer capsid proteins are removed, thus stimulating the RNA-dependent RNA polymerase (i.e., the VP1 transcriptase) associated with the inner shell to synthesize the 11 viral mRNAs that are capped by VP3, extruded from the virus cores through channels in the VP2 and VP6 protein layers at the 12 vertices of the viral particles, and subsequently translated into viral proteins.[138,157,221,292,329,414,417,436,565] Once viral proteins accumulate within the cytoplasm, large inclusions or viroplasms are formed in which the assembly of virion precursors is initiated.[5,8,145] Particle assembly may be initiated by the formation of complexes within the viroplasm that contain plus-stranded RNAs from the 11 genome segments along with VP1 and VP3 and RNA-binding nonstructural proteins NSP2, NSP5, and NSP6.[413,529] Although the mechanism is unknown, the virus faithfully assembles and packages one of each of the plus-stranded RNAs within individual precursor viral complexes. These complexes eventually lose their nonstructural proteins, evolve into double-layered viral particles with the sequential addition of VP2 and VP6, and convert their single-stranded RNAs into double-stranded genome segments.[73,169,325,414-416,418,600] The double-layered particles then bud into the rough endoplasmic reticulum after their transient association with the NSP4 transmembrane glycoprotein.[70,318,354,385,437,531] VP4 is added either before entry into the endoplasmic reticulum or sometime thereafter. The other rotavirus glycoprotein VP7, which becomes sequestered within the rough endoplasmic reticulum, then is added to complete the formation of mature viral particles.[93,317,318,438,520] These mature viruses accumulate within the lumen of the rough endoplasmic

*See references 255, 374, 386, 434, 463, 464, 496, 526, 552, 585.
†See references 17, 81, 167, 209, 210, 222, 253, 307, 351, 353, 522, 619.
‡See references 17, 81, 107, 167, 194, 208-210, 222, 253, 307, 351, 353, 522, 618, 619.
§See references 17, 81, 127, 167, 209, 210, 222, 253, 307, 312, 313, 351-353, 474, 522, 619, 620.

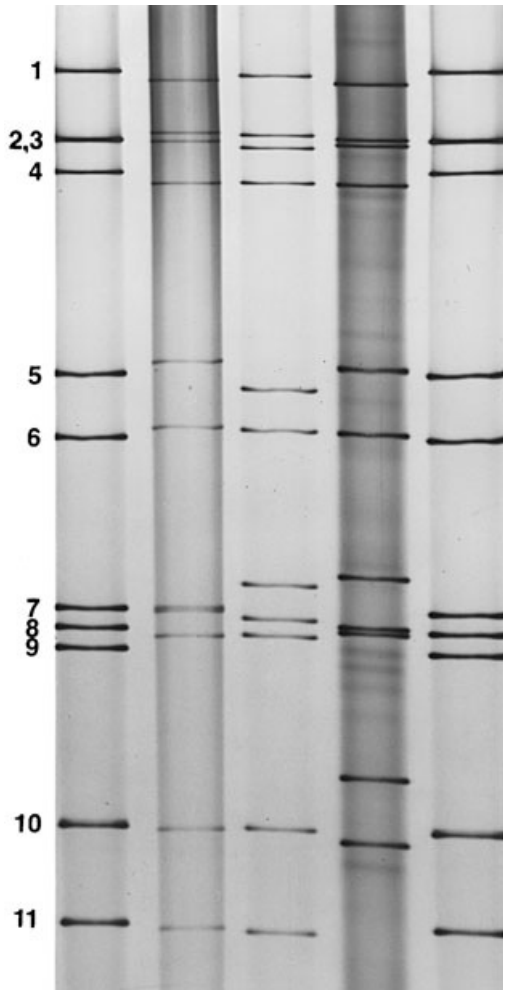

Figure 185–2 Polyacrylamide gel electrophoretic patterns of genomic RNAs obtained from group A human rotaviruses and visualized by silver staining. The patterns demonstrate the characteristic four size classes of RNA separated into groups of 4, 2, 3, and 2 segments each. Human rotavirus strains included (from left to right) are as follows: lane 1, Wa; lane 2, 248 strain; lane 3, 456 strain; lane 4, DS-1; lane 5, Wa.

reticulum until cell lysis occurs, as a result of either accidental cell death (oncosis)[428] or programmed cell death (apoptosis).[521] In cell culture, maximum production of infectious rotaviruses is found at approximately 12 hours after infection is initiated.[90]

CLASSIFICATION OF ROTAVIRUS

ELECTROPHEROTYPES AND GENOGROUPS

A variety of classification schemes have been used to characterize rotaviruses for epidemiologic purposes. Each scheme, however, is intertwined with a unique property of viruses with segmented genomes (i.e., the ability to form reassortants). During the rotavirus replication cycle, newly formed plus-sense viral mRNAs are free within the viroplasm before incorporation into replication intermediates in the first stages of virion assembly.[447] From these genomic precursors, the appropriate number and combination of segments are selected for assembly of progeny virions. Co-infection of cells with more than one virion permits reassortment of the mRNAs from both parents. If co-infection is with different strains of virus, reassortment of mRNAs results in progeny that are genetic mosaics of the co-infecting strains (Fig. 185–3). These new strains, or reassortants, are identified by their specific array of genome segments, usually through their electrophoretic mobilities during polyacrylamide gel electrophoresis (i.e., electropherotypes). The properties of the new virus strains depend on which segments are inherited from which parent and the functional behavior of each particular combination of segments and their protein products.

Rotavirus reassortants form readily in cell culture[170,192,447,554,586,587,589] in co-infected experimental animals[53,184] and in humans,[246,339,598] which is responsible, at least partially, for the variety of rotavirus strains found in nature.[188,298,541,594,617] Reassortant formation between rotavirus strains is not a universal phenomenon, however. For example, no evidence that reassortants form between strains belonging to different rotavirus groups has been found.[612] Even within group A rotaviruses, severe limitations exist within strain combinations that are capable of forming stable reassortants, limitations that appear to be related directly to the degree of genetic variation between strains.[447,586]

One outcome of restricted reassortant formation between rotavirus strains is the concept of genetic families[160] or genogroups.[371] A genogroup is composed of rotavirus strains with gene segments that form interstrain RNA-RNA hybrids of sufficient stability to migrate as defined bands during polyacrylamide gel electrophoresis.[371] Thus, members of a genogroup share a high degree of genetic relatedness and have significantly less genetic homology with members of other genogroups. Because rotavirus genogroups appear to be species specific,[154,370,371] interspecies transmission of rotaviruses should be detectable readily by genogroup analyses. Almost all human rotaviruses belong to the Wa or DS-1 genogroup,[160,372,597] a designation developed from these prototype strains. The concept of genogroup has been used extensively to determine the origin of rotaviruses causing human infections and disease, particularly to detect viruses or reassortants with gene segments of animal origin.[55,113,117,118,134,361,368,369,553]

SEROTYPES

Both outer capsid proteins of rotavirus, VP4 and VP7, contain neutralization epitopes, and thereby both are involved in determination of serotype.[198,200,235,257,391] Serotyping originally was based solely on differences in the VP7 protein because animals hyperimmunized with rotaviruses develop most neutralizing antibody to this protein. Cross-neutralization studies conducted with these hyperimmune sera readily separated the strains into VP7 serotypes.[236,604] When researchers later found that VP4 could, in some cases, be the dominant neutralization protein,[86,423,591,594] a dual serotyping scheme was required. Rotavirus classification based on VP4 and VP7 designates P type and G type to describe the protease sensitivity and glycosylated structure of these two proteins, respectively.[142] Although VP7 serotypes could be determined readily by cross-neutralization studies, serotype determination was more difficult to make for VP4.[186,319,407,507,527] Therefore, two numeric systems were devised to classify the VP4 protein in rotavirus strains. One is based on comparative nucleic hybridization and sequence analyses (genotypes),[142,171,190] and the second is based on neutralization (serotypes) with use of antisera against baculovirus-expressed VP4 proteins[186] or reassortants with specific VP4 genes.[507] The P serotype usually is indicated by an open number, whereas the P genotype is noted with a bracketed number. For example, the most common P type worldwide belongs to serotype P1A and genotype 8, thus designated P1A[8].

Until recently, 15 G types[142,232] and 20 P types[173] had been identified. However, several new P types have been identified on the basis of sequence analyses within the past few years, bringing

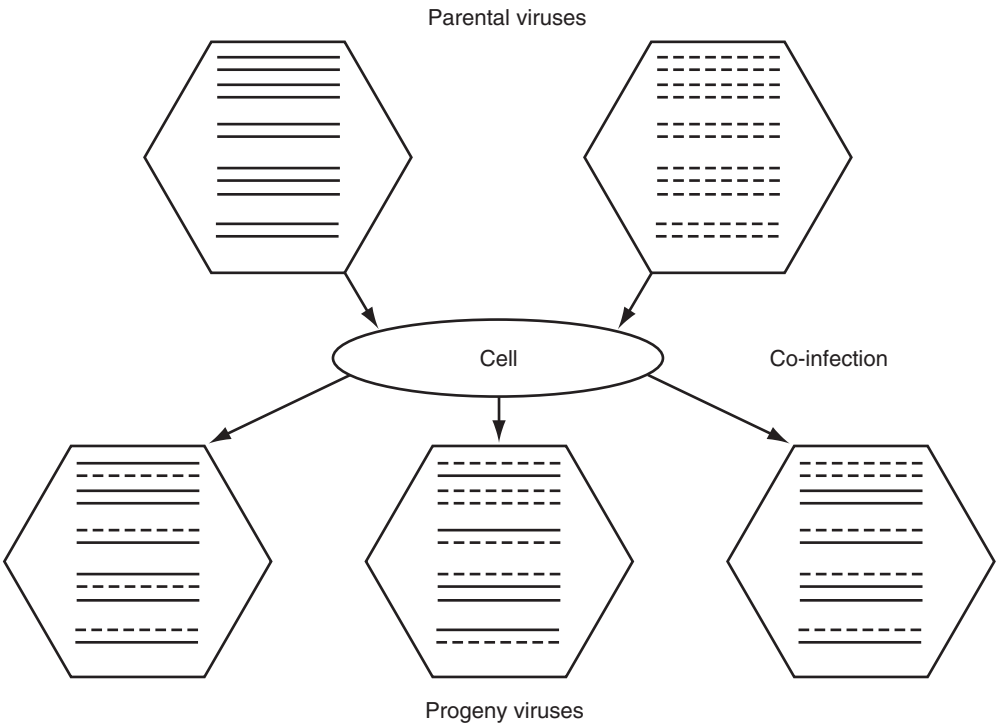

Figure 185–3 Diagram of the formation of reassortant progeny rotaviruses after co-infection of a cell with two different (parental) strains of rotavirus.

the total to 25.[232,302,344,444,448] Human rotaviruses belonging to 11 G serotypes have been isolated,[176] but most have been identified as G1, G2, G3, or G4, and strains belonging to these G types commonly have been designated serotype 1, 2, 3, or 4, respectively.[262] The severity of illness caused by viruses belonging to these four serotypes has varied little if at all.[15,20,450] Likewise, 7 P genotypes have been found in humans, but almost all illnesses have been associated with P genotypes 4, 6, and 8.[172,262] However, other G and P types commonly have been isolated in some settings; particularly, serotype G9 strains have been found worldwide, sometimes representing a large fraction of the isolates.[112,113,173,362,445,489,518,538] A Latin American study also suggested that the severity of illness may be greater with G9 strains.[301]

GENETIC LINKAGE

If the G and P types of rotaviruses found in humans could associate freely because of reassortant formation, the combinations of types for these proteins should be generated randomly; however, this clearly is not the case. For example, G1P[8] and G2P[4] rotaviruses, similar to the prototype Wa and DS-1 strains, respectively, frequently are isolated but belong to two distinct genogroups of human rotaviruses.[371] Therefore, they rarely should form stable reassortants, an assumption that has been substantiated through analyses of numerous rotavirus strains.

Other associations among gene segments also have been found. The VP6 protein or group antigen can be divided into two subgroups (I and II) on the basis of antigenic differences within this protein.[199,528] Almost all G2 and G8 human rotaviruses belong to subgroup I, whereas G1, G3, G4, and G9 human rotaviruses belong almost solely to subgroup II.[87,174,236,463,526] G3 also is a common serotype in animal strains; but in contrast to results found with G3 human strains, almost all G3 animal rotaviruses belong to subgroup I. In addition, subgroup I human but not animal strains have been found to have a characteristic "short" electropherotype associated with an inversion in the migration order of segments 10 and 11.[256,286] Thus, distinct genetic linkages have been found by serotype, genotype, subtype, and electropherotype analysis as well as by genogroup determination.

CROSS-SPECIES ROTAVIRUS INFECTIONS

Rotaviruses have an extremely wide host range, but natural cross-species infections, particularly those between animals and humans, may be rare occurrences. However, numerous human isolates appear to be animal strains or animal-human rotavirus reassortants, as determined by genogroup and sequence analyses.[55,113,117,118,134,337,361,368,369,553] The importance of these strains in human disease may be limited. Researchers have suggested, however, that once they are adapted to replication in humans, such strains may become important human pathogens.[370]

The property of host restriction has been used extensively to develop rotavirus vaccines for humans from naturally attenuated bovine, ovine, and simian rotaviruses. Oral immunization of infants with these experimental live virus vaccines has resulted in low to moderate levels of intestinal replication and partial protection against human rotavirus illnesses.[22,82,85,159,289,356,566,567] Thus, the barrier of host restriction can be bypassed sufficiently under these controlled conditions to permit the development of protective immune responses in a heterologous host. Experimental studies in animals have shown that intestinal replication of rotaviruses in heterologous species generally is limited, and if shedding of progeny viruses is detectable, it often occurs only when animals are inoculated with high doses of the heterologous viruses.[53,61,100,148,342,396,595,596]

The basis for host-range restriction is unknown and probably involves the collective properties of at least several genes. When reassortants between a murine and a simian rotavirus were used in a mouse model, however, a significant linkage to host-range restriction was associated with gene 5 encoding NSP1,[53] the primary function of which may be to block development of innate immune responses to the virus by its interaction with interferon regulatory factor 3.[16,191] Other studies also report nonrandom selection of gene 5 in progeny after co-infection of cells in

culture[192] and in mice,[184] suggesting a possible growth advantage associated with this gene. The gene encoding NSP1 also shows a high amount of sequence divergence among rotaviruses of different species,[143] which supports its possible role in host restriction. Of note, however, is that in a study in which the NSP1 gene from a bovine rotavirus that produces an abortive infection in pigs was substituted in a porcine rotavirus that replicates productively in pigs, the new reassortant still demonstrated productive replication in piglets.[47] Thus, NSP1 is not the only determinant of host range.

EPIDEMIOLOGY OF ROTAVIRUS

AGE-DEPENDENT SUSCEPTIBILITY TO ROTAVIRUS DISEASE

In addition to restrictions in interspecies transmission of rotaviruses, age restrictions are associated with rotavirus disease. In animals, rotavirus illness appears to be limited to the first days or weeks of life. Mice are susceptible to rotavirus disease only for their first 15 days of life but can experience a rotavirus infection for their entire lifetime.[347] Similarly, piglets and calves are most susceptible to rotavirus diarrhea during their first days of life.[45,276] In contrast, severe human rotavirus disease occurs most commonly in infants between 6 and 24 months of age (Fig. 185–4),[262,467,585] but milder rotavirus illnesses occur throughout our lifetimes.

Causes for the reduced severity of rotavirus disease before 6 months and after the first years of life continue to be subjects of intense investigation. Possibly, nonimmunologic, age-dependent changes that occur within the intestine, including an observed decrease in virus-specific receptors on enterocytes between suckling and adult mice, could account for this reduced severity.[461] A similar suggestion has been made for calves.[561] This suggestion also may help explain why human infants are more susceptible to rotavirus illnesses than are older children or adults. Decreased concentrations of proteases needed to cleave the VP4 protein in intestinal secretions of newborns relative to older infants also could help explain the resistance of neonates to rotavirus disease.[295]

Neonatal rotavirus infections are common occurrences and appear to be endemic in some newborn nurseries.[32,424,463] On the basis of sequence analyses, researchers proposed that neonatal strains possess unique VP4 genes, which have been classified as genotype P[6].[142,156,185] Because neonatal rotavirus infections also typically are asymptomatic, rotaviruses containing P[6] VP4 genes were designated neonatal or asymptomatic strains. Further epidemiologic studies have shown that these descriptions are not accurate; that is, asymptomatic neonatal infections sometimes are caused by non-P[6] strains,[116,118] and many symptomatic infections of older infants are caused by P[6] strains.[112-114,173,362,445,517,538] In fact, P[6] rotaviruses are probably the third most common P genotype associated with rotavirus illnesses worldwide today. Therefore, why most neonatal rotavirus infections are caused by P[6] strains and whether the P[6] genotype is in any way responsible for the asymptomatic phenotype of these infections remain unclear. However, the virulence of P[6] strains found in African neonates was associated with genetic differences in the genes encoding not only VP4 but also VP7 and NSP4, suggesting a possible linkage between multiple rotavirus genes and the virulence of neonatal strains.[408]

The onset of rotavirus disease in infants has been reported to coincide with the decline of maternal IgG antibody titers to low concentrations.[624] Furthermore, excellent correlations have been observed between responsiveness to the live rotavirus vaccine Rotarix and transplacental neutralizing antibody titers to the vaccine strains (unpublished results). Therefore, the commonly asymptomatic nature of neonatal rotavirus infections may be due, at least partially, to protection from transplacental antibody that may persist for the first months of life.[27] Mechanisms by which transplacental maternal antibody might protect against intestinal infection are unclear. Passive transfer of neutralizing antibody to the intestine of both humans and animals is associated with protection,[45,122,135,224,483,495,506,509] but circulating anti-rotavirus IgG

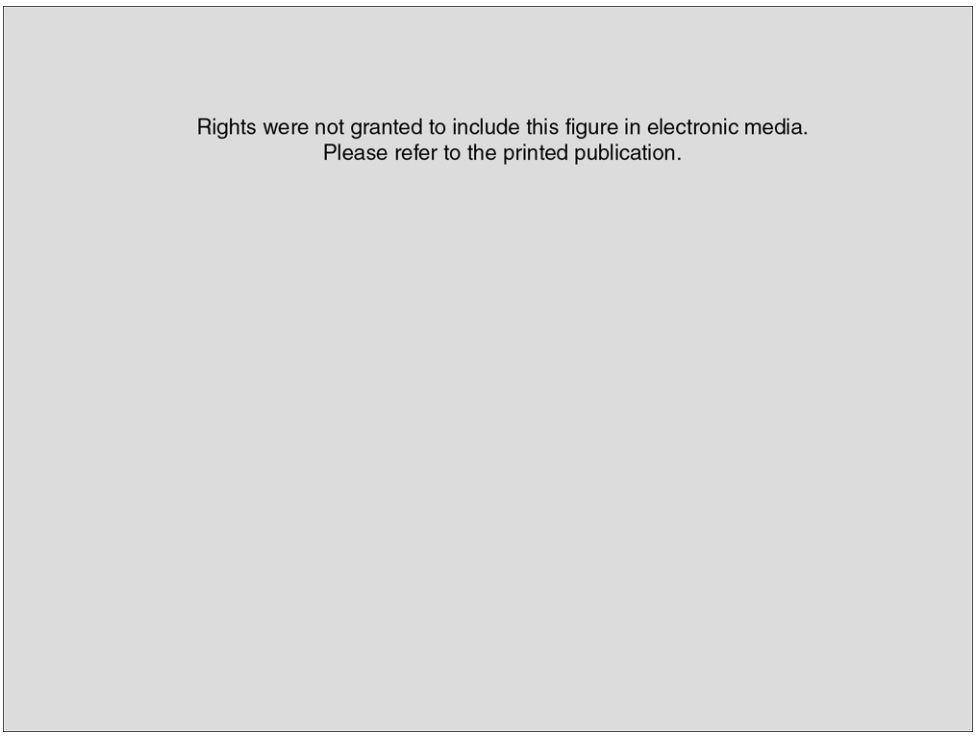

Figure 185–4 Age-related incidence of clinically significant rotavirus episodes in the Matlab region of Bangladesh for residents under surveillance in 1985 and 1986. *(From Ward, R. L., Clemens, J. D., Sack, D. A., et al.: Culture adaptation and characterization of group A rotaviruses causing diarrheal illnesses in Bangladesh from 1985 to 1986. J. Clin. Microbiol. 29:1915-1923, 1991.)*

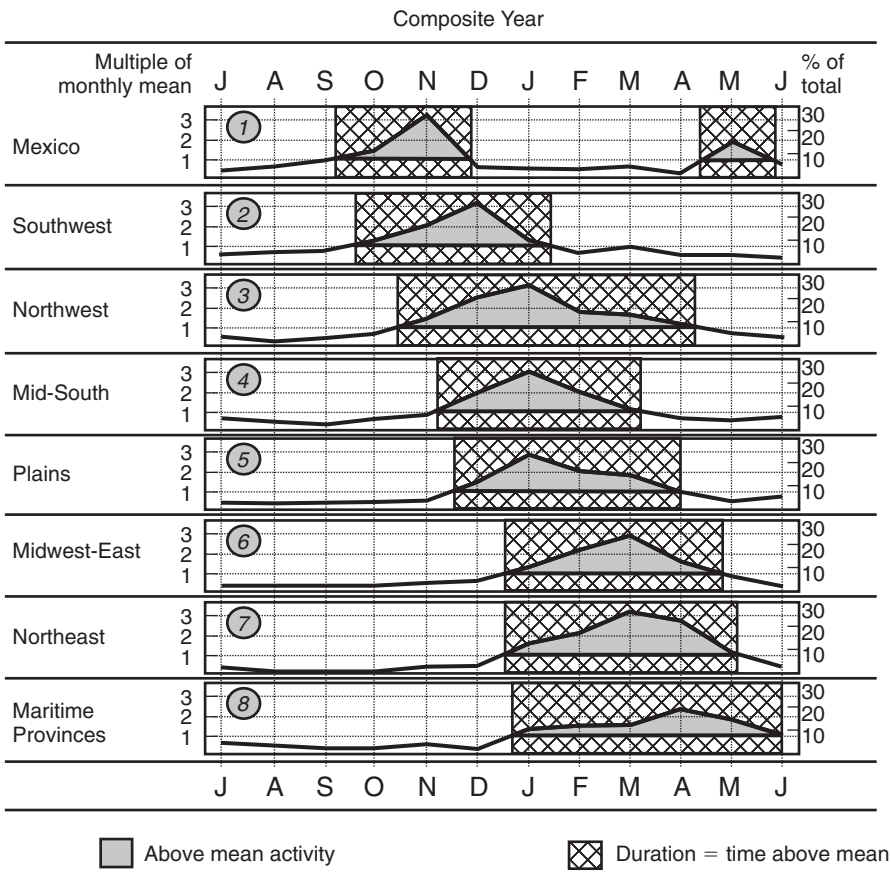

Figure 185–5 West to east movement of annual rotavirus epidemics in North America based on a monthly average of rotavirus illnesses between 1984 and 1988. Results are from 88 centers in Canada, Mexico, and the United States. *(From LeBaron, C. W., Lew, J., Glass, R. I., et al.: Annual rotavirus epidemic patterns in North America. Results of a 5-year retrospective survey of 88 centers in Canada, Mexico, and the United States. Rotavirus Study Group. J. A. M. A. 264:983-988, 1990.)*

appears to confer little if any protection in animals.[394,508,509] Possibly, maternal IgG in humans is taken into the intestine, where it neutralizes rotaviruses before infection. Regardless of why rotavirus infection of neonates typically is asymptomatic, these infections have been found to reduce the severity of rotavirus illnesses in older infants.[29,32] For these reasons, two rotavirus strains obtained from neonates were developed initially as vaccine candidates,[14,356] and two others obtained from India[117,118] have been evaluated recently in a safety and immunogenicity study.[30]

The reduced severity of rotavirus disease in older children and adults probably is due primarily to immune responses stimulated by previous rotavirus infections. In developed as well as in developing countries, almost all humans experience at least one rotavirus infection by the time they reach 3 years of age, and circulating rotavirus antibody remains detectable indefinitely.[261,262] Protection against rotavirus infection and disease in children and adults has been correlated with titers of both circulating and intestinal rotavirus antibody.* Although these antibodies have not been established as the effectors of protection, their presence indicates a natural infection that has elicited a protective immune response.

ROTAVIRUS SEASONALITY AND SOURCES OF EPIDEMIC STRAINS

As with other respiratory and enteric viruses, distinct seasonality is associated with rotavirus disease.[104,212,266,281,546] It is particularly evident in temperate climates, where rotaviruses probably are responsible for the large increase in diarrheal deaths that occur during the winter season.[182,226,227,294] The seasonality of rotavirus disease is less apparent in tropical climates but is still more prevalent in the drier, cooler months.[213] The cause for the seasonality of rotavirus disease is a topic of considerable interest but remains unknown.

The transmission of rotavirus infections is thought to be fecal-oral, with little evidence of airborne transmission. Yet a unique pattern of rotavirus infections that follows the general direction of the prevailing winds is observed annually in North America.[294] These infections begin in Mexico and the southwestern United States in mid to late fall and travel systematically across the continent, ending in the northeastern United States and Maritime Provinces of Canada in the spring (Fig. 185–5). Until recently, this was the only description of a repetitive geographic sequence for the seasonal epidemic activity of a viral agent. However, a similar pattern of rotavirus spread was reported for western Europe, where the seasonal peak started in Spain in January and ended in the more northern countries in March.[281] These annual events, including wind movements, have no satisfactory explanations. The phenomenon appears to be independent of latitude, which argues against temperature-dependent associations and humidity. Furthermore, the electropherotypes and serotypes of isolates found in different geographic locations can vary,[581] a counterindication for a gradual, physical transmission of rotavirus infections as a wave to the north and east.

Because rotavirus illnesses occur with seasonal regularity and decrease to almost undetectable levels during the off-season, the virus must be retained in a less active state during most of each year. Retention of human rotavirus in animal reservoirs between seasons is unlikely because of the low interspecies transmissibility associated with this virus, as already discussed. Therefore, the virus may continue to replicate at low levels in humans until conditions are favorable for the annual epidemic. The occasional

*See references 35, 76, 95, 105, 195, 268, 332, 389, 563, 564, 582, 583.

rotavirus illnesses that occur in the off-season support the suggestion that humans are a reservoir. It is also possible that the virus survives in the environment, which provides continuous exposure throughout the year but results in sustained rotavirus illnesses only during seasonal epidemics. Rotaviruses are shed in extremely high concentrations (i.e., approximately 10^{11} particles/g of human feces),[590] retain their infectivity for many months at ambient temperatures,[144,272,358] and are detectable readily on environmental surfaces.[63] Therefore, the environment could be a reservoir for human rotavirus and a possible source for the initiation of seasonal epidemics.

To provide clues about the origin of rotavirus strains responsible for epidemics, many extensive studies have been performed to characterize the circulating viruses, primarily using electropherotypes, genotypes, and serotypes. From these studies, investigators have determined that rotavirus strains in a specific locale can vary little over sequential seasons or change dramatically, even within a single season.[434,463,496,526,585] Furthermore, multiple strains often are present within a region at any period during an epidemic. Because gene reassortment can be extensive after coinfection with rotavirus,[114,188,298,301,339,416,447,598] identification of the source of new strains within a defined geographic area is difficult. They could be derived from outside sources, they could be obtained from local reservoirs, or they could arise by gene reassortment of circulating strains. Clearly, if the source of virus responsible for initiating annual rotavirus epidemics could be identified, much would be learned about the epidemiology of rotavirus.

PATHOLOGY AND PATHOGENESIS

HISTOLOGIC AND STRUCTURAL CHANGES IN INTESTINAL VILLI

After fecal-oral transmission of rotavirus, infection is initiated in the upper intestine and typically leads to a series of histologic and physiologic changes. These changes have been examined extensively, particularly through experimental infections of animals (Fig. 185–6). Studies in calves revealed that rotavirus infection caused the villus epithelium to change from columnar to cuboidal, which resulted in shortening and stunting of the villi.[348,349,420] The cells at the villus tips became denuded; in the underlying lamina propria, the numbers of reticulum-like cells increased and mononuclear cell infiltration was observed. The infection started at the proximal end of the small intestine and advanced distally. The most pronounced changes usually, but not always,[539] were associated with the proximal small intestine. When bovine rotaviruses of different virulence were compared, a low-virulence strain infected the proximal small intestine poorly but infected more villus enterocytes in the mid and distal intestine than did the high-virulence strain.[48,216] Although the low-virulence strain replicated in these cells and caused cytopathic effects, it did not damage the intestinal structure or affect function. Similar observations were made after piglets were infected with rotavirus.[98,197,419,532]

The pathology of murine rotavirus infection also has been examined in several studies, and the results are similar to those found in larger animals.[3,13,19,96,285,304,405,513] Many of these studies have been conducted with heterologous rotavirus strains that require infection with much greater quantities of virus because of the restricted replication of these viruses in mice.[19,53,148,201,396,596] The histologic changes induced by these heterologous strains are similar to those found after murine rotavirus infection, even though viral replication is limited after oral inoculation with these viruses. Mice are susceptible to rotavirus diarrhea during their first 2 weeks of life, and recently a series of strikingly clear results on murine rotavirus infection in neonatal mice were reported.[39] They included time-dependent histologic changes in the intestine, kinetics of rotavirus replication, shortening of intestinal villi, induction of apoptosis, and alterations in cell migration kinetics.

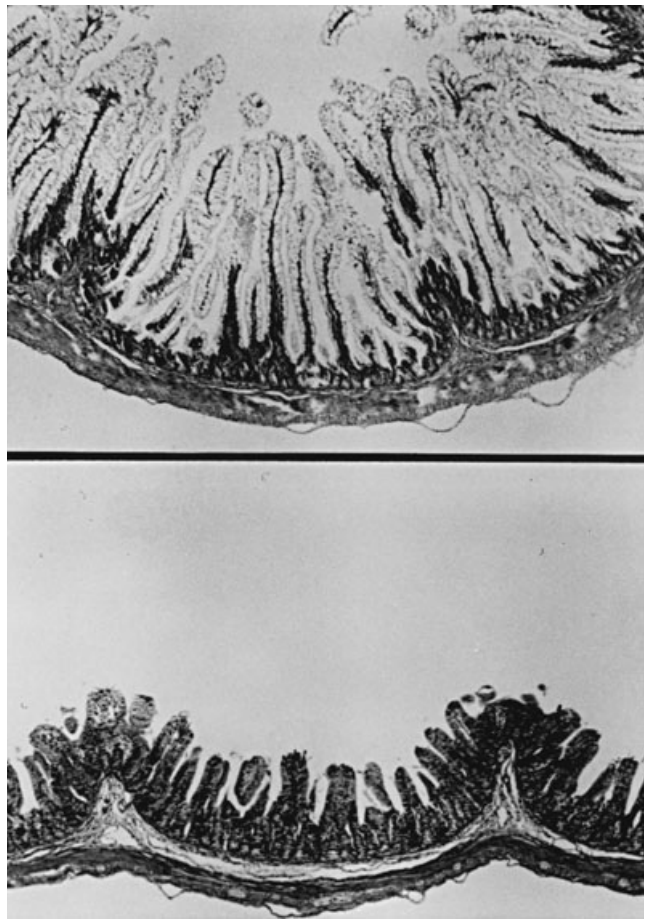

Figure 185–6 *Top*, Normal histologic appearance of ileum from an 8-day-old gnotobiotic pig. Normal mature vacuolate absorptive cells cover the villi. (Hematoxylin and eosin stain.) *Bottom*, Ileum from an 8-day-old gnotobiotic pig after oral inoculation with virulent human rotavirus (Wa strain). Severe villous atrophy and early crypt hyperplasia are evident. (Hematoxylin and eosin stain.) *(Courtesy of Dr. L. A. Ward, Ohio Agricultural Research and Development Center, The Ohio State University, Wooster.)*

Studies with the mouse model also have revealed a potential hazard associated with the possible use of heterologous viruses as vaccine candidates. Although replication of the homologous strains appeared to be restricted to the intestine, oral inoculation of either mice with severe combined immune deficiency (SCID) or normal mice with a simian rotavirus resulted in its spread to the liver and induction of hepatitis.[550,551] Because of evidence that shows abnormal liver function occurs during natural rotavirus infection in humans,[204,206,283] this observation caused concern about the use of animal strains as vaccine candidates. However, no significant alteration of liver function has been associated with any rotavirus vaccine candidate after extensive investigations, even though most candidates have been derived from animal rotavirus strains. As discussed in detail in a later section, however, use of the same simian rotavirus strain in combination with three monoreassortants of this virus as a vaccine in infants was found to increase the incidence of intussusception in the week after administration of the first dose of vaccine.[404] This occurrence caused the removal of the vaccine from the market.

A few studies have examined the pathologic changes in the intestines of humans, but the results appeared to be similar to

those found in animals.[228,525] Tissue tropism for rotavirus infection in humans also appeared to be restricted normally to villi of the small intestine. Sporadic instances of nongastrointestinal rotavirus-associated disease, including the association with abnormal liver function mentioned before, as well as respiratory and nervous system involvement have been reported.* However, because no consistent evidence of extraintestinal replication of rotavirus had been forthcoming, the general assumption was that rotavirus disease is strictly intestinal. This assumption, however, changed dramatically in 2003 when both antigenemia (presence of rotavirus protein in the blood) and viremia (presence of live rotavirus in the blood) were found during rotavirus infection in several strains of animals and in humans.[38] Since that time, this observation has been confirmed with a number of studies that included several in humans.[11,37,75,109,147,151,373] On the basis of these observations, the possibility that rotaviruses are a common cause of a variety of nonintestinal diseases has gained impetus and will be a subject of intense investigation in coming years.

As with host-range restriction, the molecular basis for pathogenicity has not been established. Offit and coworkers[392] reported that the virulence of reassortants generated between heterologous rotaviruses and tested in a mouse model correlated with the presence of the VP4 protein from the more virulent virus. Neither rotavirus strain used in this study (a simian and a bovine strain) replicated efficiently in mice, however, suggesting that the observation may have limited applicability. A later study with murine-simian rotavirus reassortants revealed no association between the VP4 protein and virulence.[53] In that study, the strongest association between virulence and a gene product was with NSP1, a nonstructural protein.

Associations between virulence and specific gene segments also were examined in piglets. Virulence variants that appeared to differ only in their VP4 genes were isolated from the feces of an infected pig.[46,60] To eliminate the possibility that virulence was determined by other gene products, the VP4 gene of the virulent strain was transferred into the avirulent strain by reassortment.[530] This transfer caused the avirulent strain to become virulent.[51] In another study with reassortants between a virulent porcine virus and a human strain attenuated for piglets, investigators found that the porcine rotavirus genes encoding VP3, VP4, VP7, and NSP4 were required for virulence in piglets.[233] Whether either of these observations has general applicability or pertains only to a limited combination of rotavirus strains because of specific interactions among their proteins remains to be determined. Passage of a porcine rotavirus in piglets was reported to increase its virulence dramatically,[46] whereas passage of a virulent porcine rotavirus in cell culture attenuated this virus.[548] Thus, the associations between specific rotavirus genes and virulence can be altered readily by natural selection through mutation. Until very recently, one could not make specific nucleotide changes within rotavirus genes and re-incorporate the modified gene into new infectious rotavirus particles. However, a system of reverse genetics for rotavirus has now been reported, at least for the rotavirus gene segment encoding VP4.[279] Expansion of this system of reverse genetics to the other rotavirus genes should permit investigators to identify specific amino acid and protein structural changes that are responsible for rotavirus virulence and attenuation.

MECHANISMS OF DIARRHEA

Although rotaviruses cause severe diarrhea in numerous species, including humans, the mechanisms responsible have not been determined and may be due to multiple factors. An early study in piglets indicated that net sodium and calcium fluxes were not different between control and infected animals, but glucose-mediated sodium absorption was diminished by rotavirus infection.[120] On the basis of this and other physiologic changes, the authors concluded that retarded differentiation of uninfected enterocytes that migrated at an accelerated rate from the crypts after the virus had invaded villus cells was responsible for absorptive abnormalities. Another study with piglets led to the conclusion that destruction of the villus tip cells causes carbohydrate malabsorption and osmotic diarrhea.[193] In mice, researchers reported that carbohydrate malabsorption did not occur as in piglets and, therefore, crypt cell secretions may be the cause of fluid loss.[97] Intestinal prostaglandin E_2 concentrations were reported to be elevated after rotavirus infection of piglets, leading to the suggestion that prostaglandin E may be a secretory component of diarrhea in this animal model.[625] Additional studies in animals and humans concerning changes in the absorption of macromolecules across the intestinal surface after an episode of rotavirus infection have revealed no general pattern.[197,223,243,384] Uptake of some molecules, such as horseradish peroxidase and 2-rhamnose, is increased; uptake of other molecules, such as lactulose and D-xylose, is decreased. Therefore, the relationship between the absorptive properties of intestinal mucosa induced by rotavirus infection and development of diarrhea remains unclear.

The importance of virus replication for induction of rotavirus diarrhea also has been challenged. Inoculation of mice with heterologous rotaviruses had been observed to produce diarrhea only when mice were inoculated with large quantities of these viruses, and in these cases, diarrhea occurred despite a lack of efficient viral replication.[53,396,596] Subsequently, inoculation with a large number of inactivated particles from a heterologous rotavirus was reported also to result in diarrhea.[502] The authors suggested that rotavirus attachment or entry into cells was sufficient to induce diarrhea in this model and that the mechanism of rotavirus-induced diarrhea was consistent with a viral toxin-like effect exerted during virus-cell contact.

Diarrhea also has been induced in infant mice and rats by intraperitoneal inoculation with the rotavirus NSP4 protein as well as with a 22–amino acid peptide derived from this protein.[12,360] Investigators observed that this protein and its peptide caused an increase in calcium concentration in insect cells when added exogenously.[535] In subsequent experiments, NSP4 and its peptide were found to increase the levels of intracellular calcium[536] by activating a calcium-dependent signal transduction pathway that mobilizes transport of this ion from the endoplasmic reticulum.[133,535] Further reports suggest that NSP4 possesses membrane destabilization activity[54,534] that may result from increased intracellular calcium concentrations, resulting in cytoskeleton disorganization and cell death.[56,57,428,429] Thus, binding of NSP4 to intestinal epithelium after its release from infected cells may contribute to altered ion transport and diarrhea. Another possible target for secreted NSP4 is the enteric nervous system, which lies under the villus epithelium. Rotavirus infection has been shown to activate this system in mice, and drugs that block nerve activity attenuate rotavirus-induced fluid secretion in vitro and attenuate diarrhea in vivo.[314] Whether this is a major mechanism of diarrhea occurring after rotavirus infection remains to be determined. Some additional studies in mice support a role for NSP4 as a cause of diarrhea,[231,622] whereas others indicate that mutations in NSP4 are not responsible for attenuation of rotavirus in either mice or humans,[7,297,593] thus questioning its importance as a cause of diarrhea in nature.

IMMUNITY

Much has been learned about the immune response to rotavirus, but the key question of what provides protection from infection

*See references 220, 229, 230, 254, 270, 280, 299, 322, 335, 363, 378, 380, 383, 409, 486, 550, 551, 555, 557, 601, 613, 615, 623.

or disease remains unanswered. Rotavirus infections clearly induce a humoral immune response beginning with production of IgM antibodies and later including IgA and IgG antibody.[121,205,340,458] Infection also induces local, intestinal antibodies that are predominantly IgA but also include IgG and IgM initially.[24,106,121,205,332,340,458] After infection develops in mice, as many as 50 percent of all IgA-producing B cells in the lamina propria of the intestine can be, for at least a brief time, rotavirus-specific.[503] Cell-mediated immunity, including lymphoproliferative responses, also can be detected transiently after infection develops in adult humans,[247,321,400,401,469,540] and a brisk cytolytic T-cell response ($CD8^+$) has been found in the intestines and spleens of infected mice.[397,398]

The most immunogenic protein appears to be VP6. Thus, antibodies measured by enzyme-linked immunosorbent assay (ELISA) are directed mainly at this protein, but they are not neutralizing antibodies.[501] Some evidence suggests, however, that IgA antibodies directed at VP6 can be protective by mechanisms that are not completely understood but may involve intracellular inhibition of rotavirus replication within infected enterocytes during polymeric antibody transport to the intestinal lumen.[62,149,498] Antibodies directed at either the VP4 or the VP7 proteins can neutralize virus and provide protection when they are given passively to animals.[333,402] Although numerous problems are associated with measurement of the VP4- and VP7-specific responses in humans,[390] the preponderance of information suggests that after infection occurs, the predominant response appears to be to the VP4 protein[58,591,594]; VP7 antibodies were noted more commonly after vaccination with a poorly replicating vaccine, WC3.[23,588] Both VP4 and VP7 proteins can induce type-specific and cross-reactive serotype responses, although most of the VP7 responses are type specific, whereas those directed at VP4, especially the VP5 region,* are more likely to be cross-reactive.[186,196,319,359,527] These findings have important implications for vaccine development, as will be discussed.

Rotavirus-specific cytotoxic T lymphocytes recognize epitopes on several rotavirus proteins that generally are not serotype specific, including at least VP7, VP4, VP6, and VP2.[164,393,398] Adoptive transfer of splenic lymphocytes from mice infected with homologous or heterologous rotavirus strains can protect suckling mice.[399] Protection was major histocompatibility complex restricted and appeared to depend on the presence of $CD8^+$ lymphocytes. Similarly, $CD8^+$ splenic or intraepithelial lymphocytes obtained from the intestine of rotavirus-infected mice can eliminate the chronic rotavirus shedding seen in SCID mice.[132] Adoptive transfer of $CD8^+$ cells from mice immunized with baculovirus recombinants expressing VP1, VP4, VP6, or VP7 also can terminate the chronic shedding in SCID mice.[131] Studies revealed that adoptive transfer of splenic $CD4^+$ cells from either naive or rotavirus (VP6)–immunized mice resolved shedding in chronically infected, immunodeficient Rag-2 mice.[346] Therefore, either $CD8^+$ or $CD4^+$ T cells are capable of resolving rotavirus infections. Possible roles of these cells in resolution or prevention of human rotavirus infections remain to be determined.

An obvious place to begin to understand rotavirus immunity is to determine the effectiveness of previous rotavirus infections in prevention of subsequent infections and disease. The important questions relate not only to the degree of protection but also to whether protection is serotype specific. As discussed earlier in this chapter, multiple serotypes of human rotavirus are based on neutralization epitopes on the VP4 (P serotypes) and VP7 (G serotypes) outer capsid proteins. Thus, protection may be limited to those strains that share neutralization epitopes, or it may be associated with the development of other B- or T-cell immune responses to shared epitopes.

Many investigators have reported that natural rotavirus infections produce incomplete protection, but little doubt exists that previous infections protect against severe disease associated with re-infection.* In a large study, protection from both rotavirus re-infection and rotavirus diarrhea increased with each new infection.[563] Sequential infections even with the same serotype have been reported, however. In the initial study reporting rotavirus disease with re-infection by the same serotype, the investigators noted that protection of young children in a Japanese orphanage lasted 6 months then declined after 1 year.[76] This study noted a close correlation between titers of serotype-specific antibody and protection. Some animal studies also support a role for serotype-specific infection. Thus, in a study with piglets, immunization with reassortant viruses containing either the VP4 or the VP7 protein of the same serotype was protective, whereas immunization with reassortants containing heterotypic genes for these proteins was not protective.[234] In other studies using mice, this association was not as clear,[596] and protection was better correlated to serum and intestinal levels of IgA.[148,342,347]

Although, as discussed earlier, re-infections with rotavirus appear to be common occurrences, other studies have shown protection that lasts at least 1 year.[26,32,581] Neonates infected within the first 2 weeks of life were protected against severe disease but not against re-infection in one study.[32] In another, infants who developed a symptomatic or an asymptomatic rotavirus infection during the first year of study were protected against contraction of a subsequent rotavirus illness or even an asymptomatic re-infection during the following year.[26] Similarly, when the placebo recipients of a large vaccine trial were observed, a natural rotavirus infection in the first year was found to be 93 percent protective against a symptomatic re-infection in the second year.[581] This protection occurred even though the G1 strains that circulated during the first year were responsible for only 66 percent of rotavirus disease in the second year. Other studies conducted in less developed countries and in daycare centers have not shown the same degree of protection.[35,95,330,456,563] Differences in these studies may be due to the variation in circulating strains, the dose of exposure, or the duration of protection.

Protection has been correlated to both serum and stool antibody titers produced after natural rotavirus infection.[35,76,95,105,106,332,389,433,563,564,583] In one study, serum antibody levels, especially IgA, were found to be a marker for protection.[564] Reports of a correlation with serotype-specific neutralizing antibody and protection[76,389] have not been supported in other larger studies.[225,584,624] In the largest study, which was conducted in Bangladesh during a 2-year period when four major G serotypes circulated, the titers of both homologous and heterologous neutralizing antibody were significantly lower in patients with acute rotavirus disease than in matched control subjects.[584] However, further analysis could not find a correlation with serotype-specific neutralizing antibody. Thus, protection seemed to be correlated better with the magnitude of the response rather than with specific neutralizing responses. Similarly, animal studies in both calves and mice have shown that protection can occur in the absence of serotype-specific neutralizing antibodies in the serum, feces, or intestinal washes.[49,50,342,345,596,603]

Some results from rotavirus vaccine trials also fail to support a role for serum neutralizing antibody and protection. Thus, immunization with heterologous animal rotavirus vaccines has provided protection in some studies without inducing serum neutralizing antibody to human serotypes.[84,569] In other studies that failed to demonstrate overall efficacy, protection was seen in those who developed the highest antibody titers to the heterologous vaccine,[27,592] again implicating the magnitude of the response

*See references 26, 32, 35, 76, 95, 123, 166, 330, 388, 456, 476, 563, 564, 581, 607.

*See references 26, 32, 35, 76, 95, 123, 166, 330, 388, 456, 476, 563, 564, 581, 607.

rather than specific neutralizing antibody titers. It is possible, however, that serum antibodies are merely markers for the true protective responses in the intestine.

Studies evaluating the protective role of previous infections and the mechanism of protection have used adults in challenge studies. Although essentially all adults have been infected previously with rotavirus, they are susceptible to re-infection and mild disease on natural exposure.[237,599] Initial challenge studies revealed an association between the pre-inoculation titer of serotype-specific neutralizing serum antibody and protection.[268] These studies later were extended to show a correlation between VP7 antibody and protection but failed to establish a relationship between intestinal antibodies and protection.[195] When similar studies were conducted with a larger group of adults, the correlation between both serum and intestinal antibody became clearer.[582,583] The most significant correlations were found to be between serum rotavirus IgG and shedding and between intestinal neutralizing antibody and illness. However, some subjects with high titers of antibody became infected and ill, whereas some subjects with low titers appeared to be protected.

As already noted, animal models also have proved useful for examining protective immune responses. Initially, large animals, such as piglets and calves, were used, whereas mouse models were limited to the study of passive protection because mice are susceptible to rotavirus diarrhea only for the first 15 days of life. Use of mice was extended to studies on protection with the advent of the adult infection model in which protection against fecal shedding of rotavirus antigen, rather than protection against diarrhea, was used as the indicator of active immunity.[596]

Initial studies of passive immunization, including cross-fostering studies in mice, found that gastrointestinal but not circulating antibodies were protective and that secretory IgA was more effective than was IgG at providing protection.[45,311,394,483,495,506] Animals can be protected with antibodies directed at either VP4 or VP7.[395,402] Similarly, active immunization against both homotypic and heterotypic challenge has been demonstrated in mice, calves, pigs, and rabbits.[50,99,101,234,345,596,602] Protection has been seen after both oral and parenteral immunization, although what provides protection is not clear. In studies of mice, although no correlation could be found between protection and either serum or intestinal neutralizing antibody, levels of serum or fecal rotavirus IgA did correlate with protection.[148,342]

The use of these models has been extended to gene knockout mice to distinguish the role of $CD8^+$ cells and antibody in protection and have particular relevance to vaccines. These studies indicated that $CD8^+$ cytolytic cells are important for resolution of an infection, but only antibody could provide protection from a subsequent challenge.[162,163,165,341] Other reports have indicated that antibody may be important for complete resolution of a rotavirus infection as well as for protection against subsequent infection.[343,559] A study in mice revealed that oral immunization of mice with a rotavirus that was fully heterotypic to the challenge strain provided nearly complete protection against fecal rotavirus shedding, but this protection was dependent on the ability of antibody to be transported through intestinal epithelial cells.[560] This result supports the earlier suggestion that heterotypic protection after live virus immunization may be due to intracellular inhibition of virus replication, at least in mice.[149,498,560] Taken together, these results indicate that the levels, location, and targets of protective antibody as well as the proteins against which it is directed are of immediate importance, provided the results found in mice are relevant to larger animals and humans.

Information on protective immune responses continues to become available and should prove useful in the development of rotavirus vaccines. The absence, to date, of a reliable immunologic marker of protection, however, continues to render vaccine trials more difficult.

VACCINES

The development of rotavirus vaccines has been a high priority for public health institutions.[244,455] Cost-effectiveness analyses have shown that a rotavirus immunization program would be cost-effective from the perspective of society and the health care system.[545] Because natural rotavirus infections induce excellent protection, at least against severe rotavirus disease, vaccine efforts have been directed mostly at the development of live attenuated rotavirus vaccines.[102,155,180] Most of these efforts have concentrated on the use of animal rotavirus strains, labeled the Jennerian approach[264,389] because it relies on the natural attenuation of animal viruses in humans for safety and largely heterotypic immune responses for protection. The initial efforts with animal rotavirus vaccines yielded inconsistent results. Therefore, in an attempt to make the vaccines more closely related to human strains, human rotavirus genes coding for the proteins that induce neutralizing antibody were introduced into these animal strains by creating reassortant viruses as described earlier. This approach has been labeled the modified Jennerian approach.[155] Table 185–2 lists some of the major efficacy trials conducted to date.

Just 10 years after the identification of rotavirus as an agent of severe diarrhea, the first vaccine trials were performed.[569] The initial study used RIT 4237, a bovine rotavirus. It was soon followed by evaluations of other animal rotavirus vaccines, including a rhesus monkey rotavirus (RRV or MMU 18006) and another bovine rotavirus (WC3). Next, animal-human reassortants using RRV and WC3 and a human strain (89-12) attenuated by multiple tissue culture passages were evaluated. These three vaccines went on to become licensed products (discussed later). Less extensive studies also have been performed with neonatal human viruses M37 and RV3[14,158,578] that are thought to be naturally attenuated as well as cold-adapted vaccines.[88,155] Most recently, two other promising vaccines have entered clinical trials: a bovine human (UK) reassortant[267,574] and naturally reassorted human neonatal strains isolated in India.[30,179]

Jennerian Vaccines

The initial studies of RIT (bovine) vaccine produced variable results. The vaccine was safe and effective in Finland, providing a protective efficacy of approximately 50 percent against all disease and a greater than 80 percent protection against severe disease.[566-568] However, later studies in developing countries were disappointing,[126,218,289] showing little or no efficacy. Similarly, the vaccines failed to provide protection in a study performed on the Navajo reservation in Arizona.[492]

The WC3 rotavirus vaccine similarly is of bovine origin and also appears to be free of side effects. It replicates poorly in humans, but infants develop a neutralizing antibody response to WC3 and both rotavirus IgA and IgG antibody.[27] The initial studies conducted in Philadelphia appeared promising,[84] but later trials in Cincinnati[27] and in less developed countries[175] did not show significant protection. WC3 was, therefore, used to make reassortants containing rotavirus genes encoding the VP7 and VP4 proteins of human serotypes, which became the vaccine approved in the United States (RotaTeq) and discussed later.[88]

RRV replicates better in humans than do the bovine vaccine strains, perhaps because it is a G3 strain and shares serotype specificity with human G3 strains, but it also produces mild side effects, including low-grade fever and mild diarrhea, especially when it is given to older children who have lost maternal antibodies.[310,426,570] RRV induces serum rotavirus IgG and IgA, intestinal rotavirus IgA, and RRV-neutralizing antibody but no neutralizing antibody against non-G3 human rotavirus.[79,311,426] Protection with this vaccine was inconsistent, ranging from greater than 50 percent, even in developing countries,[159,320,425,452] to moderate (20-50%),[453,576] to nonexistent.[79,492] Some evidence suggests that pro-

TABLE 185–2 Selected Vaccine Studies

Vaccine	Country	No. of Subjects	No. of Doses	Protection (Overall/ Severe Disease)	Reference
RIT 4237	Finland	328	2	58/82	568
	Rwanda	245	3	0/0	126
	Gambia	185	3	0/37	218
	Peru	391	3	40/75	289
WC3	United States (Philadelphia, PA)	104	1	43/89	84
	United States (Cincinnati, OH)	206	1	17/41	27
	Central African Republic	472	2	0/36	175
RRV	United States (Rochester, NY)	176	1	0/0	79
	Venezuela	247	1	68/100	159
	Finland	200	1	38/67	576
	United States (Indian reservation)	321	1	0/ND	492
RRV reassortants					
RRV G1	Finland	359	1	67/ND	577
RRV G2	Finland			66/ND	
RRV G1	United States	898	3	69/73	22
RRV TV				64/82	
RRV G1	United States	1187	3	54/69	451
RRV TV				49/80	
RRV TV	Venezuela	2207	3	48/88	427
WC3 reassortants					
WC3 TV	United States	417	3	75/100	83
WC3 pentavalent	United States and Finland	57,134	3	74/98	575
Human rota 89-12					
89-12	United States	215	2	89/100	25
RIX 4414	Finland	405	2	73/90	573
RIX 4414	Finland and Latin America	20,169	2	ND/85	475
RIX 4414	European Union	4274	2	87/96	571

tection is more effective in G3 than in G1 outbreaks,[102,155] but the evidence is not conclusive.

Reassortant (Modified Jennerian) Vaccines

Because of the consensus that homotypic immunity might increase the protection seen with rotavirus vaccines and because of a lack of consistent protection with the animal strains, the next efforts were directed toward creating reassortant vaccines that contain the VP7 or the VP7 and VP4 encoding genes of human rotavirus strains, with the remainder of the genes from an animal strain. As discussed earlier, these proteins were chosen because they induce neutralizing antibody. The segmented genome of rotavirus renders production of these reassortant rotaviruses relatively easy. One goal of this strategy is to create a multivalent vaccine containing viruses with human rotavirus genes representing the main human serotypes. Reassortant vaccines were developed with RRV[355,357] and WC3[25,85,542] and later UK[267,574] as the animal strain.

The RRV-human VP7 rotavirus reassortants were developed into Rotashield. This vaccine contains RRV and reassortants with the VP7 genes of G1, G2, or G4 serotype and the 10 other genes from RRV (Fig. 185–7). The G3 serotype virus of this vaccine is RRV. Thus, four of the common VP7 serotypes were incorporated into the vaccine. Vaccination produced a rotavirus IgG and IgA serum antibody response, but neutralizing antibodies are produced predominantly to RRV rather than to the human serotypes.[22,423,451] This finding appears to be consistent with the experiments showing that VP4, rather than VP7, is more immunogenic after natural infection.[591,594]

Extensive evaluations of the tetravalent RRV reassortant were completed before licensure.[21,252,427,451] In two large trials conducted at centers across the United States,[21,451] the tetravalent vaccine was found to be safe, with the subjects having a slight increase in temperature after the first dose. The efficacy of the vaccine against rotavirus disease for the first year was 49 to 64 percent, whereas during a 2-year follow-up, it was 57 percent. Protection increased with severity scores to a level of about 80 percent for severe disease. Vaccination also significantly decreased the number of medical office visits for rotavirus gastroenteritis or dehydration. Vaccination appeared to provide protection against both G1 and G3 serotypes. Similar results were reported from Finnish studies, except that fever occurred somewhat more commonly and efficacy was somewhat enhanced.[252] Protection similar to that seen in the U.S. studies also was provided by the vaccine when it was used in a less developed country, Venezuela.[430]

For these reasons, the vaccine Rotashield was licensed in the United States in 1998 and recommended for general use in all infants.[6] However, less than 1 year after licensing, 15 cases of intussusception that occurred shortly after vaccination was administered were reported to the Vaccine Adverse Events Reporting System.[67] Subsequent analysis led the American Academy of Pediatrics Advisory Committee on Immunization Practices to conclude that an association existed between receipt of the vaccine and development of intussusception and to withdraw the recommendation for use.[1,68] The vaccine was, therefore, withdrawn by the manufacturer.

The initial publication describing the association reported an increased risk for development of intussusception 3 to 14 days after receipt of the first dose of vaccine (adjusted odds ratio, 21.7).[366] A smaller risk also existed after receipt of the second dose of vaccine. Researchers estimated that one case of intussusception attributable to vaccination with RRV would occur for every 4670 to 9474 infants vaccinated. Ecologic studies that followed reported a lower vaccine-attributable risk.[365,504] The exact excess burden of intussusception during the life of the child has yet to be defined, but the best estimates are 1 per 10,000 to 12,000.[278,284,430,504] Further analysis also has revealed that the risk is age related and substantially increased in those older than 90 days.[472,505] The

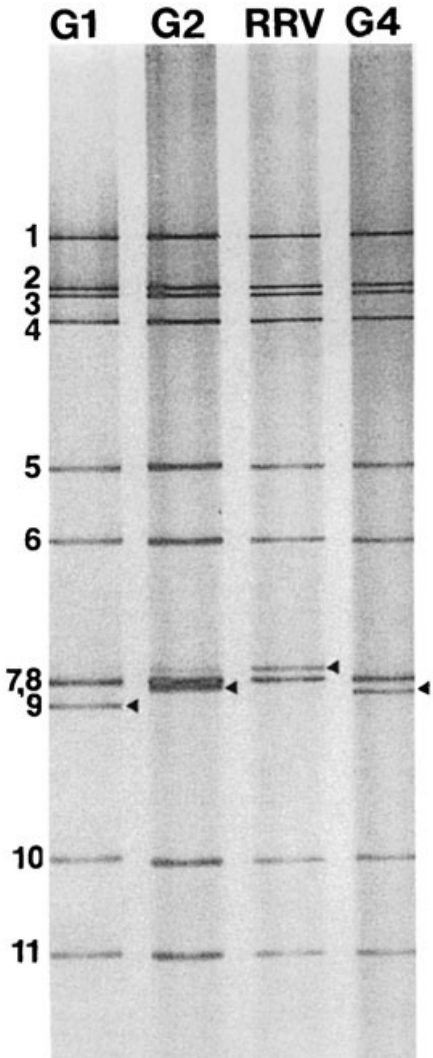

Figure 185–7 Polyacrylamide gel electrophoretic patterns of the genome segments from RRV and the G1, G2, and G4 reassortant strains that compose the tetravalent RRV-based vaccine. The strains all contain 10 RRV genes and differ only in the gene segment encoding the VP7 protein, which migrates in the seventh (RRV) or ninth (reassortants) position, as designated by arrowheads.

pathogenic mechanism for this association has yet to be defined.[316] The relationship, if any, between wild-type rotavirus infection and intussusception is discussed in the clinical section.

Further evidence for the effectiveness of this approach can be found in a large post-licensure vaccine effectiveness study. In this study, Rotashield was 100 percent (95% confidence interval: 75%, 100%) effective in preventing hospitalization due to rotavirus[512] in children receiving three doses of vaccine. When two doses of vaccine were given, the vaccine also was 100 percent effective (95% confidence interval: 62%, 100%), and one dose was 89 percent (49%, 97%) effective.

In 2006, a pentavalent WC3-based reassortant vaccine, RotaTeq, was licensed, first in the United States and subsequently in the European Union, Canada, and Mexico. After the WC3 vaccine was shown to be safe but not consistently effective,[27,84,175] a monovalent vaccine containing the VP7 protein of a human G1 rotavirus was developed. This vaccine was reported to be effective, especially against more severe disease, during a predominantly serotype G1 outbreak in Rochester.[564] Next, a WC3-based reassortant quadrivalent vaccine including both VP7 and VP4 human rotavirus gene substitutions with G1, G2, G3, or P[8] substitutions and most other segments from WC3 was evaluated. In studies conducted at multiple centers in the United States, it was shown to be safe and effective against all cases of rotavirus gastroenteritis (75%) and especially against severe cases (100%).[83] An important note is that in the multiple trials of WC3 and WC3 reassortant vaccines, the viruses replicate less well than does RRV and do not induce fever as was noted with RRV and RRV reassortants.

The pentavalent RotaTeq vaccine contains the VP7 and VP4 reassortants plus a VP7 G4 reassortant.[89] The vaccine is suspended in a liquid sodium citrate and phosphate buffer at a minimum titer of approximately 1.2×10^7 infectious units per dose. The vaccine is given in a three-dose schedule in infants between 6 and 32 weeks of age, with a first dose administered between 6 and 12 weeks of age.

Because of the association of Rotashield with intussusception, the next rotavirus vaccines were required to undergo very large safety trials. The large pivotal trial of RotaTeq of more than 70,000 infants showed that the vaccine was safe and, unlike Rotashield, did not induce fever.[575] Most important, no association with intussusception was found. Intussusception occurred in 12 vaccine recipients and 15 placebo recipients within 1 year after the first dose of vaccine was administered; these figures include six vaccine recipients and five placebo recipients who developed intussusception within 42 days after receiving any dose (relative risk, 1.6; 95% confidence interval, 0.4 to 6.4). The vaccine also was highly effective, reducing all G1-G4 rotavirus gastroenteritis by 74.0 percent, severe gastroenteritis by 98.0 percent, and hospitalizations and emergency department visits by 94.5 percent.

Attenuated Human Vaccine

The other new vaccine, available first in Mexico in 2005 and then in the European Union and throughout much of the world including developed, less developed, and developing nations, is Rotarix. It is a live oral vaccine, based on the attenuated human strain 89-12. Strain 89-12 is a G1P[8] strain, the most common strain worldwide, initially obtained from an infant with rotavirus gastroenteritis in Cincinnati, Ohio.[28] The isolate was attenuated by multiple passages in tissue culture and then evaluated as a vaccine. Studies using 89-12 showed that it was safe, although similar to Rotashield, the 89-12 vaccine induced a low-grade fever in 19 percent of recipients. Results of a multicenter efficacy trial showed that two doses of this vaccine provided 89 percent protection against any rotavirus disease and 100 percent protection from more serious disease.[25]

The 89-12 strain was then purified by limiting dilution and further passaged in tissue culture. The final product, initially called RIX 4414 and later marketed as Rotarix, was evaluated in several studies. Initial safety testing[572] revealed that the vaccine was safe and did not induce the fever that followed the parent 89-12 vaccine. Subsequent reports from Singapore,[432] Finland,[573] Latin America,[475,485] and the United States[129] confirmed the safety, verified that the vaccine was not associated with fever, and reported that the vaccine remained highly immunogenic. Thus, the additional passages or limiting dilution purification resulted in a vaccine strain that was not reactogenic. The vaccine also did not interfere with the immune responses to the other concomitantly used vaccines.[129,485] In the initial efficacy trial of RIX 4414 conducted in Finland during the course of two rotavirus seasons, the vaccine was found to be 73 percent protective against all rotavirus gastroenteritis and 90 percent protective against severe gastroenteritis, despite the relatively low dose of vaccine used.[573]

As with RotaTeq, this vaccine was then evaluated in a very large safety trial.[475] In a study of more than 63,000 infants conducted primarily in several countries in Latin America, the

vaccine proved to be safe and did not induce fever and, most important, was not associated with intussusception. Thus, during the 31-day window after each dose, six vaccine recipients and seven placebo recipients had definite intussusception with no evidence of clustering (difference in risk, –0.32 per 10,000 infants; 95% confidence interval, –2.91 to 2.18; $p = .78$). For the duration of the study, 16 cases of intussusception occurred in the placebo group compared with nine in the vaccine group.

In this large study, efficacy was 85 percent against severe rotavirus diarrhea and hospitalizations and reached 100 percent against more severe gastroenteritis. Of note, efficacy was high (>86%) against severe rotavirus diarrhea caused not only by G1P[8] strains but also by the VP4-related G3P[8], G4P[8], and G9P[8] strains. Efficacy against G2P[4] strains in the few subjects infected with these viruses was less, 41.0 percent. However, in several meta-analyses, the efficacy was 67 to 71 percent, indicating that the vaccine will be efficacious against strains that do share VP4 or VP7 proteins.[475,571] An important note is that direct comparison of the two new vaccines is difficult to make because each used different scoring systems and the studies were conducted in populations with different risks for developing severe gastroenteritis. In the most recent trial of more than 4000 infants conducted in six European countries, a population more similar to that used for evaluation of RotaTeq, protection was 87 percent against any rotavirus gastroenteritis, 96 percent against severe disease, and 100 percent against hospitalization due to rotavirus.[571] In this study, efficacies against G3, G4, and G9 rotaviruses were similar to the efficacy against G1 and exceeded 95 percent, and efficacy against G2 strains was 75 percent. Of importance, efficacy against hospitalization due to gastroenteritis of any cause was 75 percent. The vaccine is reconstituted in a calcium carbonate buffer and given in a two-dose schedule at approximately 2 and 4 months of age.

Non-Live Vaccines

Preclinical studies have included the use of virus-like particles[103,387] that appeared to be protective in mice but not in pigs[616]; DNA vaccines[74,78]; and subunit vaccines, including VP7, VP4, and VP6.[77,580] The excellent protection in mice, particularly with VLPs or VP6 protein, remains to be verified in human studies.

CLINICAL MANIFESTATIONS

Rotavirus is the most common cause of dehydrating diarrhea in children in the United States and worldwide and infects nearly every child in the first few years of life.[42,115,124,213,259,261,262,412] In the United States, more than 600,000 cases of severe diarrhea are caused annually by rotavirus, resulting in 20 to 60 deaths.[181-183,226,227,274] An estimated 1 in 80 children younger than 5 years is hospitalized, and 1 in 7 requires an outpatient or emergency department visit because of rotavirus diarrhea.[183] A worldwide estimated 610,000 deaths are associated with rotavirus infection.[124,244,262,410] The incidence of rotavirus infections is highest in children between the ages of 6 and 24 months,[467] but the age may be lower in less developed countries.[215,450] Adults, including elderly patients, also are susceptible to re-infection, which can cause mild and sometimes severe disease.[111,136,237,275,326,468,599]

Initial and subsequent rotavirus infections often are asymptomatic. In cohort studies in which infants were observed from birth, as many as 50 percent of initial rotavirus infections were asymptomatic.[211,450,467,563,624] In one report, a carrier state was described.[69] Reports of neonatal infections that are asymptomatic in selected settings are common.[29,32,203,213,364,424]

The incubation period for rotavirus is approximately 1 to 3 days.[268] In children with symptoms, the onset often is abrupt, with fever and vomiting followed by explosive, watery diarrhea. Vomiting may precede the diarrhea in approximately half the cases.[214] Stools are non-bloody and generally lack fecal leukocytes, but mucus may be found in 20 percent.[240,433] Fever occurs commonly during rotavirus illness, with estimates of between 45 and 84 percent of patients.[282,465,519,549] The disease usually is self-limited, lasting 4 to 8 days, although the duration of symptoms ranged between 2 and 22 days in a Guatemalan study.[607] In one cohort study in children with symptomatic disease, 62 percent had mild disease, 35 percent had moderate disease, and 3 percent had severe disease; 7 percent of children required hospitalization.[450] In another study, 64 percent of children with rotavirus diarrhea had vomiting, 64 percent had fever, 14 percent were dehydrated, and 18 percent were hospitalized.[427] In children hospitalized with rotavirus infection, 63 percent had fever, vomiting, and diarrhea at presentation; children also presented with any two or only one of these symptoms.[511] When hospitalization is required, the stay usually is brief, with an average of 4 days and a range of 2 to 14 days.[465] Recovery generally is complete, but persistent diarrhea associated with lactose intolerance has been described.[18,273]

In general, rotavirus infections are more severe than are those caused by other viral gastrointestinal agents.[36,465] In developing countries, 20 to 40 percent of hospitalizations for diarrhea in children younger than 5 years are due to rotavirus infections,[124] whereas in the United States, rotavirus is estimated to account for between one third and two thirds of admissions for diarrhea in children.[41,466] Vomiting, dehydration, and hospitalization occur significantly more often in patients infected with rotavirus than in those with other causes of diarrhea.[465,549] In one study,[465] vomiting and diarrhea also lasted longer in patients infected with rotavirus (2.6 versus 0.9 days and 5.0 versus 2.6 days, respectively). In another study, the severity score for rotavirus diarrhea was almost twice that of disease having other causes.[476]

In children with dehydration caused by rotavirus, dehydration was more likely to be isotonic than was dehydration caused by other agents.[465] However, in another study, one fourth of the patients infected with rotavirus presented with hypernatremic dehydration.[282] Other reported findings in children with rotavirus include irritability and pharyngeal or tympanic membrane erythema. Numerous reports associate respiratory symptoms (e.g., cough, pharyngitis, otitis media, and pneumonia) with rotavirus infections, but the relationship of these symptoms to rotavirus is unclear, and the ability to isolate rotavirus from respiratory secretions has varied among studies.[214,299,465,493,623] Laboratory findings are related mostly to the extent of dehydration and can include elevated blood urea nitrogen and evidence of mild metabolic acidosis.

The peak viral shedding in stools occurs on approximately day 3 of illness.[119,579] When it was evaluated by polymerase chain reaction (PCR), the duration of rotavirus shedding ranged from 4 to 57 days in hospitalized patients.[457] Approximately half of the children shed for fewer than 10 days and 70 percent for fewer than 20 days. In Guatemala, prolonged viral shedding also has been reported for as long as 5 weeks and was associated with immunodeficiency.[330,494]

Other clinical manifestations associated either etiologically or incidentally with rotavirus infection have been described and include encephalitis and meningitis*; various upper and lower respiratory infections, including otitis media, laryngitis, pharyngitis, and pneumonia[299,379,465,493,623]; Kawasaki syndrome[335]; sudden infant death syndrome[613]; hepatic abscess[206]; and pancreatitis.[378] Elevated liver function test results also have been reported during rotavirus infection.[204,206,283] Neonatal necrotizing enterocolitis also has been linked with rotavirus.[271,471] However, the difficulty of false-positive reactions obtained with some ELISA tests in neonates must be considered.[80,449,543] Rotavirus infections can be

*See references 230, 254, 270, 315, 322, 380, 486, 557, 601, 615.

more severe in immunosuppressed persons, including bone marrow transplant recipients, patients infected with human immunodeficiency virus, and those who are malnourished,[137,178,250,260,614] and they may spread to the liver and kidney.[178] Of interest, rotavirus antigen recently has been reported to be found also in the blood of immunocompetent children with confirmed rotavirus gastroenteritis.[38,75,151] The role that circulating rotavirus may have in these extraintestinal manifestations is intriguing but unknown.[446]

In the past, central nervous system involvement associated with rotavirus infections,[486] including aseptic meningitis[555,601] and encephalopathy,[270] was determined by electron microscopy and antigen detection through immunologic methods. However, more recently, cases have been reported by identification of rotavirus RNA in the cerebrospinal fluid with use of reverse transcription–polymerase chain reaction (RT-PCR) analysis. Rotavirus RNA has been found in the cerebrospinal fluid in rotavirus-infected children with seizures,[315,383,409] encephalitis,[230,315,380,615] and encephalopathy.[322] Of the 20 reported cases, 15 were reported to have full recovery, but three had significant neurologic complications and two children died. Each of these reports suggests that rotavirus may have a spectrum of organ involvement and disease that extends beyond the gastrointestinal tract.

The relationship between intussusception and natural rotavirus infection is of great interest because of the reported link between the tetravalent RRV vaccine and intussusception (discussed in the section on vaccines). Several studies have investigated the possible infectious etiology of intussusception.[31,92,238,280,363,377] Given the rarity of intussusception, these studies have had a small sample size, and several different pathogens have been identified in these case series. Adenoviruses have been recovered most often.[31,92,238,377] Although several studies have argued that the lack of seasonal variation for intussusception suggests that wild-type rotavirus does not cause intussusception,[71,72,411,454] the possibility remains that many intestinal pathogens, including rotavirus, may cause intussusception, and multiple agents may account for the lack of a seasonal peak.

LABORATORY DIAGNOSIS

Rotavirus infection cannot be diagnosed on the basis of clinical presentation, even in combination with stool examination and nonspecific laboratory tests. Therefore, specific tests have been developed. Findings suggestive of rotavirus infection include a mildly febrile illness with vomiting and watery diarrhea that is occurring in the winter months in temperate climates, especially in patients 6 to 24 months of age. The presence of more severe dehydration also is suggestive. In the initial epidemiologic studies, electron microscopy was used for identification because of the large number of particles present in stools ($>10^{10}$/g) and the characteristic appearance of rotavirus. This technique largely has been replaced by enzyme immunoassay-based and latex agglutination tests, for which kits are available commercially. Both tests have good sensitivity compared with electron microscopy.[130,137,177,278,533] One problem noted in the past was false-positive results in neonatal stools with certain ELISA kits.[4,80,449,543] Inclusion of a rotavirus-negative capture antibody as a control in these kits could eliminate these false-positive reactions if it were used. However, this is rarely done.

Rotavirus also can be grown in tissue culture,[219,590,605] although the methods used for routine viral cultures do not detect rotavirus. However, live virus can be detected readily, even in stool specimens, by use of focus assays.[583] The serotype of cultured strains and virus in stools can be identified by use of monoclonal antibodies.[265] DNA probes[290,516] and, particularly, RT-PCR have been used effectively to identify genotypes as surrogates for serotypes.[171,189] Electrophoresis of extracted RNA also can identify rotavirus by its characteristic 11 segments and is used to define electropherotypes.[255,464,496] All these methods have proved useful as epidemiologic tools and in vaccine studies. Identification of electropherotypes is especially useful for epidemiologic studies because it allows identification of specific strains.

Rotavirus infection, both symptomatic and asymptomatic, also can be identified by changes in rotavirus antibody. ELISA assays are used most commonly to measure serum IgM, IgA, and IgG levels as well as stool and intestinal antibodies. Specific neutralizing titers also can be measured for each serotype of rotavirus by plaque reduction[236,604] or focus reduction assays.[24,52] One ELISA-based antigen reduction neutralization assay has been found to be better suited for the analyses of large numbers of specimens.[277] Serologic detection of infection is more difficult in the first months of life because of the presence of maternal antibodies. Detection of rotavirus IgA, which does not cross the placenta, has been used as a marker for previous infection in the first months of life.

PROTECTIVE FACTORS

Several studies have shown that breast-feeding protects against symptomatic rotavirus infections.[94,367,376] In one study, exclusive breast-feeding was found to be protective against severe rotavirus diarrhea, but no overall protection was present during the first 2 years of life, suggesting that breast-feeding simply postpones acquisition of rotavirus disease to an older age.[94] Similarly, cohort studies found that the highest incidence of rotavirus-associated diarrhea was in children aged 4 to 6 months, which coincided with the age when weaning from breast-feeding began.[406,450,493] A study also found that breast-feeding was protective against hospitalization for acute gastroenteritis caused by rotavirus in infants younger than 6 months.[128]

Although breast-feeding alone could be the protective factor, other factors, such as presence of maternal antibody and lack of opportunity for exposure, also could account for this period of protection. Supporting the role of human milk in protection against rotavirus is a study demonstrating that infants who received human milk with higher concentrations of the glycoprotein lactadherin were more likely to have asymptomatic rotavirus infections compared with those receiving milk with lower concentrations.[376]

TREATMENT

Treatment of rotavirus gastroenteritis is aimed largely at restoring the proper fluid balance in dehydrated patients. Emphasis has shifted from intravenous rehydration to oral rehydration with glucose-electrolyte solutions. The glucose in the solution enhances sodium and, thereby, water absorption in the intestine.[375] Several studies have shown the utility of this approach for treatment of rotavirus gastroenteritis.[479,490,491] The solution accepted by the World Health Organization contains 30 mEq/L of sodium, 30 mEq/L of potassium, and 30 mEq/L of bicarbonate, which is similar to other oral rehydrating solutions available commercially. Should oral rehydration efforts fail or in cases of severe dehydration and shock, fluid should be administered intravenously.

Other experimental approaches to treatment are based on the success of passive oral therapy in animals. Chronic rotavirus shedding has been treated successfully with human milk that contained rotavirus antibody.[494] Treatment and prophylaxis of normal children with immune colostrum, immunoglobulin, or milk from rotavirus-immunized cows also have been evaluated

with some success.[122,224,309,547] The treatment of normal children with one dose of human serum immunoglobulin given orally was reported to be effective in reducing the mean duration of diarrhea, viral excretion, and hospital stay,[207] and prophylaxis with formula supplemented with bovine antibody decreased the number of days with rotavirus diarrhea.[547] Trials of bismuth subsalicylate also have been conducted with some reported success,[150,510] as have trials with probiotics.[478]

Racecadotril (acetorphan), an enkephalinase inhibitor with antisecretory and antidiarrheal properties that decreases intestinal hypersecretion but not motility by preventing the breakdown of endogenous enkephalinase in the gastrointestinal tract, has been shown to be effective in the treatment of diarrhea in adults and children.[217,484] In one study, oral treatment with racecadotril decreased stool output by 46 percent in boys 3 to 35 months of age with watery diarrhea. A similar decrease was seen in boys with rotavirus infection.[484]

Nitazoxanide, a drug licensed to treat *Cryptosporidium parvum* and *Giardia lamblia* infections, was found to be effective in decreasing the time to resolution of rotavirus illness in children hospitalized with severe rotavirus diarrhea to an average of 31 hours for the nitazoxanide group compared with 75 hours in the placebo group.[470] In addition, tizoxanide, the active metabolite of nitazoxanide, inhibited replication of rotavirus in cell culture. For this study, only 50 children in Egypt were investigated; larger studies are planned.

NON–GROUP A ROTAVIRUSES

As described earlier, rotaviruses can be classified into seven groups (A to G). All seven groups are associated with diarrhea in animals, although only groups A, B, and C have been associated with disease in humans.[482] Of these, group A causes most illness. However, major epidemics of group B rotavirus have been reported in China, particularly in adults.[84,85,146,241] Seroprevalence studies have identified group B rotavirus infections in other areas such as Hong Kong, Myanmar, Thailand, Australia, Canada, England, Sweden, Africa, and the United States, although the prevalence is lower than that in the epidemic regions of China.[482] Group C infections have been reported largely from Japan,[288,334,403,431,556] but they also have been detected in the United States and elsewhere.[251,381,422,443,482,517,544] Unlike group A rotavirus, group B and C rotaviruses appear to cause disease predominantly in children older than 3 years and in adults.[146,288,334,382,403] Group B and group C rotavirus infections may be distributed widely, although the exact extent of infection has been limited by the poor assays that were available. If infection is suspected, the diagnosis is suggested by electron-microscopic detection of the typical rotavirus particles with a negative test result for rotavirus by the routine assays that detect only group A rotaviruses. The detection of typical RNA migration patterns by polyacrylamide gel electrophoresis of RNA also is suggestive. PCR and ELISA analyses have been reported.[187,248,382,499,544] Evidence of infection has been reported in Asia, Australia, Europe, Central and South America, Africa,* and the United States.[251] Serologic evidence has shown infection rates of 30 to 43 percent in England and the United States.[245,249,462]

Outbreaks caused by water-borne or food-borne spread of group B rotavirus in China[241] and group C rotavirus in Japan have been reported, although person-to-person spread also has been implicated.[146] As discussed before, outbreaks usually involve older children and adults,[146,288,382,403] but infants apparently can be infected.[251] Group C rotaviruses have been associated with extrahepatic biliary atresia.[459,497] Most recently, group C rotavirus RNA was detected in liver specimens from 10 of 20 patients with biliary atresia but in no controls.[460]

*See references 1, 2, 66, 287, 381, 422, 482, 487, 488, 517, 544.

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CHAPTER 186

Rubella Virus

James D. Cherry

Rubella (German measles) is a generally mild, exanthematous, infectious illness in which morbidity and mortality in children usually are minimal. However, infection in pregnancy may result in fetal infection, which usually is associated with considerable adversity for the developing infant. The rubella virus has only one known type.

HISTORY

In ancient history, rubella as a disease is lost among the other prominent exanthematous diseases (e.g., scarlet fever, measles, and smallpox). In an extensive review, Griffith[207] suggested that rubella was known to the early Arabian physicians under the name *al-hamikah*; they considered rubella a form of measles, however. Two German physicians, de Bergen in 1752 and Orlow in 1758, usually are credited with providing the first clinical descriptions of rubella as a specific entity.[207,557] In early writings, rubella generally was called *Röteln*.[164,557] However, because of the great interest of German physicians in the disease during the period from the mid-18th to the mid-19th centuries, the name *German measles* frequently was used in other countries.

In 1866, a Scottish physician named Veale[533] described 30 cases of German measles. In his paper, he gave the illness its present name, *rubella*. His opinion was that the German name Röteln was too harsh and foreign and that other possible names—*rubeola notha* and *rosalia idiopathica*—were too long for general use and could be confused with measles.[164] Other historical synonyms of rubella include rubeola, rubeola sine catarrho, rubeola epidemica, rubeola morbillosa, rubeola scarlatinosa, rosania, roseola, roseola epidemica, rosalia, scarlatina morbillosa, scarlatina hybrida, morbilli scarlatinosi, feuer masern, roséole, roséole idiopathique, rubéole, rougéole fausse, French measles, false measles, bastard measles, hybrid measles, and bastard scarlatina.[207]

In 1881 at the International Congress of Medicine in London, a consensus was reached that rubella is a distinct disease. Rubella was thought to be similar in some respects but not identical to measles or scarlatina. By the beginning of the 20th century, the clinical description of rubella was complete, except that joint manifestations had received curiously little notation.[15,116,164,207,336,438,517]

Rubella gained its present-day importance in 1941 when Gregg,[206] an Australian ophthalmologist, reported congenital defects in babies of mothers who had rubella during early pregnancy. In spite of considerable skepticism, Gregg's observations were confirmed quickly by Swan and colleagues[498,499] in Australia and other investigators in the United States and the United Kingdom.[149,414,421,557] By 1947, 28 communications describing 500 children with severe congenital defects associated with maternal rubella had appeared in the literature.[557]

In 1938, Hiro and Tasaka[244] demonstrated that rubella is a disease of viral etiology by transmission of disease to humans through the subcutaneous injection of filtered nasal washings. In 1942, Habel[214] was able to infect monkeys with nasal washings and blood from human cases. In 1962, rubella virus first was propagated in the laboratory; two investigative teams, Weller and Neva[556] and Parkman and colleagues,[379] using different techniques, reported the growth of rubella virus in tissue culture.

The isolation of rubella virus in 1962 paved the way for definitive study of the 1964 rubella pandemic. The results of extensive virologic, serologic, and epidemiologic investigation were presented at a Rubella Symposium in May 1965.[282] After the rubella virus was isolated in tissue culture, an intensive worldwide effort to develop vaccines was mounted. The accumulation of these experiences resulted in an extensive body of knowledge related to rubella and rubella immunization that was presented at the International Conference on Rubella Immunization in February 1969.[283] Live attenuated rubella virus vaccines were licensed for use in mid-1969 in the United States.[284,335] In the 38-year period since vaccine licensure and its universal use in the United States occurred, the yearly occurrence of rubella fell from 40,000 cases to 11 cases in 2005.[68,70,73,74,76,77,401,413,563]

At present, sustained transmission of rubella no longer occurs in the United States.[69,412]

PROPERTIES

CLASSIFICATION

Rubella virus is placed in the *Rubivirus* genus of the family *Togaviridae*.[158,447,568] At present, it is the only species in this genus. The virus is similar physiochemically to the other member of its family (alphavirus) but is unrelated serologically. Rubella virus has no invertebrate host (a characteristic of all alphaviruses), and humans are the only known vertebrate host.

PHYSICAL PROPERTIES

The rubella virion is spherical, with a diameter of 60 to 70 nm, and consists of a capsid protein (C) and two glycoproteins (E1 and E2).[84,301,318,388,447,568] E1 (relative molecular weight, 58,000) and E2 (relative molecular weight, 42,000 to 47,000) are glycosylated and are located on the viral surface membrane. E1 is the viral hemagglutinin that is found on 5- to 6-nm surface projections.[247,388] The nucleocapsid has a diameter of 30 to 40 nm and is composed of a polypeptide (C protein) and the genomic RNA. The nucleic acid of rubella virus is single-stranded RNA with a molecular weight of 3.2 to 3.8 $\times$ 10^7.[436] The outer coat of the virus (envelope) is lipoprotein in nature with host-cell lipid and virus-specified polypeptides.

Rubella virus is relatively sensitive to heat; it generally has been found to lose infectivity within 30 minutes at 56° C.[155,380,393] However, Kistler and Sapatino[276] observed that some infectivity persists even after heating for 60 minutes at 70° C. At 37° C in the presence of 2 percent serum, 90 percent is inactivated in 3

hours.[380] At 4° C, with protein stabilization, viral titers are maintained for 7 or more days. The virus is stable indefinitely at –60° C and below but labile at normal (–10° to –20° C) refrigeration temperatures. When it is stabilized with protein, the virus can survive several rapid freeze-thaw cycles without significant loss of titer.[441]

Rubella virus is sensitive to ultraviolet light. In 1 hour, a high-titer cell-free virus suspension was inactivated by an intensity of 1350 μW/cm^2; on the other hand, a tissue culture suspension of virus was not inactivated completely when it was exposed to a similar intensity of radiation.[276] Rubella virus is sensitive to visible light, and this photosensitivity can be potentiated by the basic dye proflavine.[46]

The virus also is sensitive to pH extremes of less than 6.8 and greater than 8.1.[81] The following chemicals rapidly inactivate rubella virus: ether, acetone, chloroform, deoxycholate, formalin, β-propiolactone, ethylene oxide, free chlorine, and 70 percent alcohol.[393] It is resistant to thimerosal.

ANTIGENIC COMPOSITION

Rubella virus infection of tissue culture cells results in the production of infectious virus that can be neutralized by specific antiserum. Specific viral antibodies can be identified by hemagglutination inhibition (HI), complement fixation (CF), precipitation in gel, platelet aggregation, passive hemagglutination, single radial hemolysis, latex agglutination, enzyme-linked immunosorbent assay (ELISA), and immunofluorescence.* Neutralization and HI identify antibodies that inhibit specific biologic functions of the virus, whereas the other assays identify only the formation of antigen-antibody complexes. The E1 glycoprotein is the predominant erythrocyte-binding and neutralization site of the virus.[519,551] Weak neuraminidase activity also has been associated with purified rubella virus.[24]

In 1967, Stewart and associates[490] reported that tissue culture–grown rubella virus produced hemagglutination of erythrocytes from chickens that were younger than 1 day old and from one goose and one lamb, but no hemagglutination was observed with adult chicken, guinea pig, and other red cell preparations commonly used. Subsequently, techniques involving careful control of test system diluents have revealed that red cells from many different animals are agglutinated by rubella virus.[306,449] Viral hemagglutinin is stable at –20° C for months and at 4° C for several weeks but is destroyed rapidly by heat.[182,217]

Sever and colleagues[464] first demonstrated that supernatant fluid from primary African green monkey kidney (AGMK) and RK-13 rabbit kidney tissue cultures contained useful complement-fixing antigens. Two distinct rubella complement-fixing antigens exist.[451] One of the antigens is similar in size and weight to both the hemagglutinin and infectious virus; the other "soluble" antigen is smaller, with a buoyant density of 1.08 g/mL. The soluble antigen is noninfectious and does not contain nucleic acid.[393] Rubella complement-fixing antigens retain their antigenicity after either treatment.

Two major small-particle antigens have been identified in the medium of tissue culture–infected cells by immunodiffusion.[298,299,452] These two soluble antigens are structural components of the virion, and natural infection with rubella virus results in the formation of serum precipitating antibodies. These antigens have been designated *theta* and *iota*. Their importance lies in the fact that antibody to the iota antigen rarely is noted in the serum of recipients of some rubella vaccines; therefore, they may be of value in studying vaccine-induced immunity.[64,300]

The E1 glycoprotein is the dominant surface molecule of rubella virus.[85] It is the main target of the humoral antibody response for both detection and elimination of the virus. HI and neutralization sites have been localized to a small segment of the E1 glycoprotein ($E1_{245}$ to $E1_{285}$).

Seventeen T-cell epitopes have been identified with the lymphoproliferation assay.[85,347] They involve the capsid protein and E1 and E2 glycoproteins.

Molecular analysis of rubella virus epidemiology from 1961 to 1997 in North America, Europe, and Asia found no major antigenic variations.[175] Further phylogenetic analysis of a collection of 103 E1 gene sequences from rubella viruses isolated from 17 countries from 1961 to 2000 found the existence of at least two genotypes.[577] Rubella genotype 1 isolates were predominant in Europe, Japan, and the Western Hemisphere, whereas genotype 2 isolates were limited to Asia and Europe.

TISSUE CULTURE GROWTH

Rubella virus grows in many different tissue cultures, including cell strains, cell lines, and primary cells.[123,321,322,380,393,567,568] Cell sources include mature and embryonic tissue from humans and other primates, rabbits, swine, dogs, birds, hamsters, and cattle. In tissue culture, growth of rubella virus can be identified by either its cytopathic effect or its ability to interfere with the growth of another tissue culture–susceptible virus.

The method used most commonly for primary isolation of rubella virus from clinical material is the interference technique with primary AGMK cells.[379] In this system, nonadapted rubella virus grows readily but does not produce a cytopathic effect. Infection is demonstrated in AGMK tissue culture by the failure of a typical enterovirus cytopathic effect to occur after the culture is challenged with echovirus 11 or another suitable enterovirus. A common alternative to the AGMK–echovirus 11 interference system for primary isolation of rubella virus is use of the RK-13 rabbit kidney cell line, in which infection can be identified by cytopathic effect. For laboratory study and determination of neutralizing antibody, many different cell lines (e.g., RK-13, BHK-21, and LLC-MK2) can be used. The highest titers of rubella virus are produced in the BHK-21 and Vero cell lines.

Kinetic studies in tissue culture indicate that virus adsorption is complete within 90 minutes and that the eclipse period lasts approximately 12 hours. The first new virus noted is cell associated; it is followed in 2 to 4 hours by extracellular virus. In primary cell culture, titers of both cell-associated and free virus reach 10^3 $TCID_{50}$/mL by the fourth day; peak titers are not attained until approximately the 17th day. In all cell systems, chronic infection occurs but is limited in some cultures by the cytopathic effect. Rubella virus induces the formation of plaques in several cell lines.

ANIMAL SUSCEPTIBILITY

Although natural infection is known to occur only in humans, several other primates have been infected experimentally.[321,380,393] In addition to primates, rabbits, hamsters, ferrets, guinea pigs, and suckling mice all have been infected with rubella virus.

EPIDEMIOLOGY

In contrast to measles and other diseases having clearly apparent dramatic cycles, knowledge of rubella epidemiology has been acquired primarily during the last 75 years. Major events during this period that stimulated interest in its epidemiology were the observation of teratogenicity in 1941,[206] the isolation of the virus

*See references 33, 39, 83, 86, 87, 147, 148, 160, 172, 240, 299, 300, 319, 384, 393, 434, 435, 530, 568, 578.

in the early 1960s,[379,556] and the pandemic of 1964. Unfortunately, rubella was not a reportable disease in the United States until 1966, so considerable gaps exist in the available information. At present, we are in a new epidemiologic era because of the widespread use of rubella vaccine. Predicting the incidence of rubella today must take into account the extent and method of vaccine use in the population under surveillance.

INCIDENCE AND PREVALENCE

Epidemic Behavior

The epidemic pattern of rubella in selected areas of the United States in the pre-vaccine and early vaccine eras is presented in Figure 186–1. The number of reported cases of rubella and congenital rubella syndrome by year in the vaccine era is presented in Figure 186–2. The rubella epidemic cycle usually is described as one of 6- to 9-year intervals, with each cycle consisting of a build-up and fall in incidence during the course of a 3- to 4-year period.[253,254] However, a close look at Figure 186–1 suggests that the basic pattern in the pre-vaccine era was a 3.6-year cycle. Of the 11 peaks from 1928 to 1968, all but two occurred in a 2- to 4-year span, with a median of 3 years. Over and above the 3-year cycle is the better known 6- to 9-year cycle of major disease. Pandemics occurred in the periods 1941 to 1944 and 1963 to 1965.

Since the introduction and widespread use of rubella vaccine in the United States, epidemic rubella has occurred only once on a national scale (see Fig. 186–2).[70,72,411] In 1991, 1401 and 47 cases of rubella and congenital rubella syndrome, respectively, were reported. The number of cases of rubella and congenital rubella syndrome by year from 1998 to 2005 is presented in Figure 186–3.[68] At present, rubella virus no longer is circulating in the United States, and the cases that occurred in the more recent years were importations.[69,412] In countries in which effective universal childhood immunization had not been achieved, epidemics continued to occur periodically. Epidemic rubella was documented in the former Czechoslovakia in 1972; in Australia in 1969 to 1970, 1975 to 1976, 1993 to 1996, and 2003; in Israel in

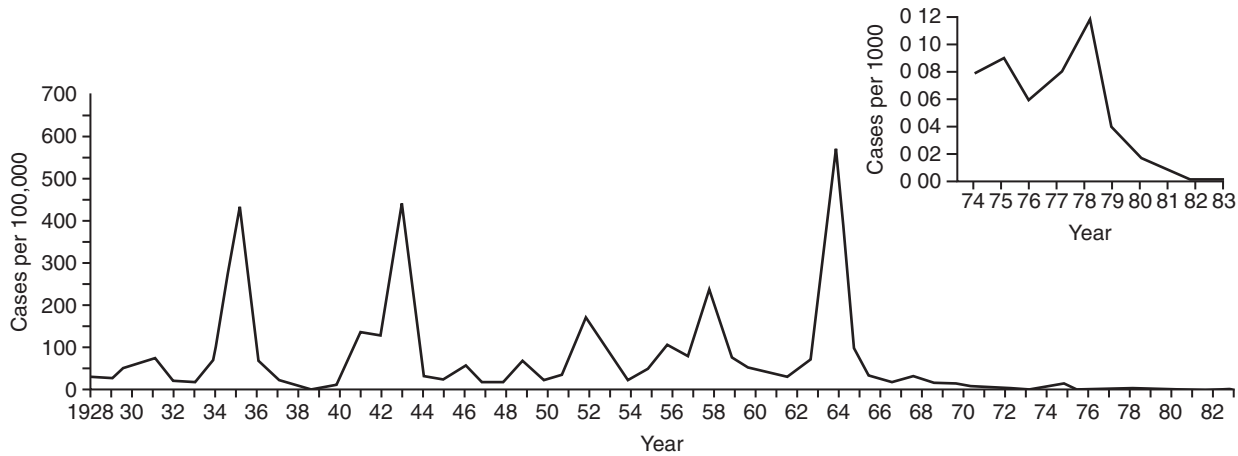

Figure 186–1 Rubella incidence in 10 selected areas (Maine, Rhode Island, Connecticut, New York City, Ohio, Illinois, Wisconsin, Maryland, Washington, and Massachusetts) of the United States, 1928 to 1983. *(From Williams, N. M., and Preblud, S. R.: Rubella and congenital rubella surveillance, 1983. M. M. W. R. CDC Surveill. Summ. 33:1SS-10SS, 1984.)*

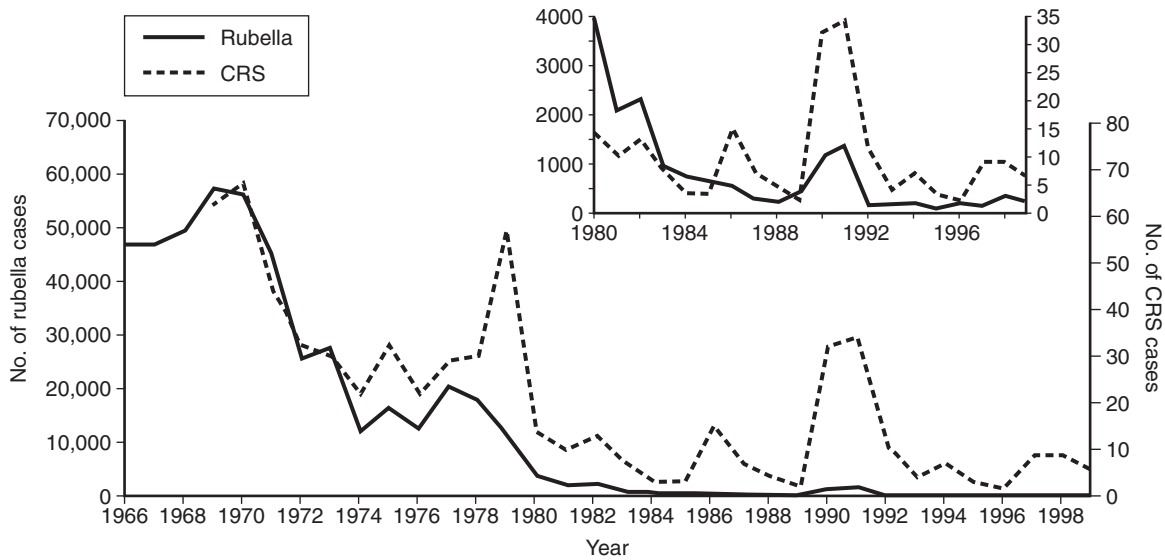

Figure 186–2 Number of reported cases of rubella and congenital rubella syndrome (CRS) in the United States from 1966 to 1998. *(From Reef, S. E., Frey, T. K., Theal, K., et al.: The changing epidemiology of rubella in the 1990s: On the verge of elimination and new challenges for control and prevention. J. A. M. A. 287:464-472, 2002. Copyright 2002, American Medical Association.)*

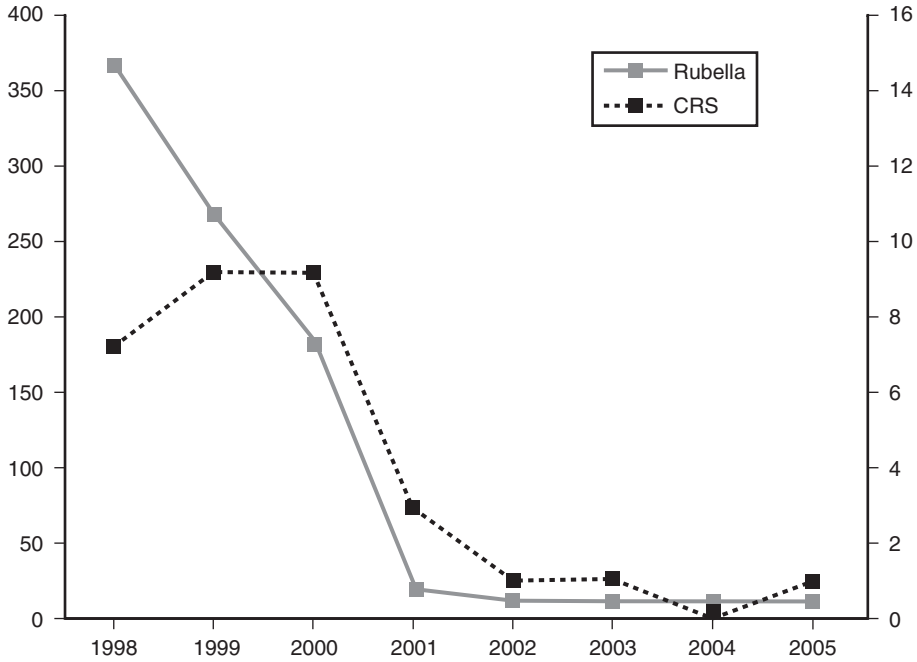

Figure 186–3 Number of reported cases of rubella and congenital rubella syndrome (CRS) in the United States from 1998 to 2005.[68]

1972, 1979, and 1983; in Japan in 1975 to 1977 and 1982 to 1998; in Brazil in 1981 and 1997 to 2001; in the United Kingdom in 1971 to 1973, 1978, and 1983; and recently in a number of European countries.*

The incidence of rubella varies with the epidemic cycle, the number of susceptible people within a population group, and the intrapersonal contact within the group. In closed populations such as military training centers and institutions for the mentally handicapped, the attack rate after the disease is introduced approaches 100 percent in susceptible individuals.[256,257,302] Introduction of disease in the family also affects virtually all susceptible persons.[183,184] In community epidemics, attack rates in susceptible people are estimated to range from 50 to 90 percent.

Age Groups

The age distribution of reported rubella cases and estimated incidence rates in Illinois, Massachusetts, and New York City for 1966 through 1968 and the entire United States for 1985 through 1987[76] are presented in Table 186–1. In the period immediately before the vaccine was introduced (1966 to 1968), the attack rate was highest in the 5- to 9-year-old age group, and the incidence was high in preschool-aged children. The overall reduction in the rate of rubella from the pre-vaccine era to 1987 was 99.2 percent. However, 50.2 percent of the cases reported in the period 1985 through 1988 were in persons older than 19 years; in the pre-vaccine period (1966 through 1968), the percentage in this age group was 10.2. In 1999, 75 percent of the reported cases occurred in persons older than 19 years.[413] During the period 1980 to 2002, outbreaks of rubella have occurred in prisons,[79] in colleges and universities,[78] in hospitals,[80,400,492] at work sites,[77,198,413] in communities with high foreign-born populations,[413] and among the Amish in six areas of the United States.[74]

Because rubella was not a reportable disease in the United States until 1966, few age-specific incidence or prevalence data relating to epidemic disease are available. In Table 186–2, age-specific attack rates are presented for two communities during epidemic rubella in 1964.[104] The attack rate curves during epidemic rubella in 1964 are similar to the curve for the pre-vaccine nonepidemic period from 1966 to 1968 seen in Table 186–1. The overall attack rate during the 1964 epidemic in the two communities was 23 percent. Eighty-six percent of the cases occurred in children younger than 15 years. In 1999, 272 cases of rubella were reported in the United States, and of the 269 with known ages, only 14 percent occurred in children younger than 15 years.[413]

Antibody prevalence by age group in the pre-vaccine era in the St. Louis area is presented in Figure 186–4.[91] As can be seen, rubella HI antibody prevalence went from less than 10 percent in children younger than 3 years of age to almost 80 percent in

TABLE 186–1 Age Distribution of Reported Rubella Cases and Estimated Incidence Rates*—Illinois, Massachusetts, and New York City, 1966-1968,† and Total United States, 1985-1987†

Age Group (yr)	1966-1968 Average‡ %	1966-1968 Average‡ Rate	1985-1987 Average§ %	1985-1987 Average§ Rate	Rate Change¶ (%) 1966-1987
<5	21.6	63.3	24.8	0.6	–99.1
5-9	38.5	101.3	11.8	0.3	–99.7
10-14	17.0	44.0	5.2	0.1	–99.7
15-19	12.7	35.7	8.0	0.2	–99.5
≥20	10.2	3.7	50.2	0.1	–96.5
Total	100.0	24.3	100.0	0.2	–99.2

**Reported cases per 100,000 population. Patients with unknown age are excluded.*
†Average annual figures during a 3-year period.
‡Represents pre-vaccine years. National age data were not available before 1975 and were not reported consistently (i.e., >75% of cases) until 1980.
§Total United States data (1986 population projections) are used for 1985-1987; because the overall number of reported rubella cases currently is small, fluctuations (such as the epidemic in New York City in 1985) in only these three reporting areas skewed the data for this period.
¶Based on actual rates.
From Centers for Disease Control: Rubella and congenital rubella syndrome—United States 1985-1988. M. M. W. R. Morb. Mortal. Wkly. Rep. 38:173-178, 1989.

*See references 156, 163, 168, 186, 197, 215, 225, 252, 270, 291, 292, 330, 415, 439, 479, 500, 501.

TABLE 186–2 Age-Specific Attack Rates in Two Communities During Epidemic Rubella in 1964

	Doraville, Georgia			Kingston, Tennessee		
Age Group (yr)	Total Population	Cases	Attack Rate (%)	Total Population	Cases	Attack Rate (%)
0-4	87	32	36.8	69	30	43.5
5-9	206	104	50.5	127	90	70.9
10-14	208	59	28.4	127	68	53.5
15-19	78	9	11.5	90	25	27.8
20+	427	11	2.6	487	19	3.9
Unknown	8	—	—			
Total	1014	215	21.2	900	232	25.8

From Communicable Disease Center: Morbidity and Mortality Weekly Report 13*:349-360, 1964.*

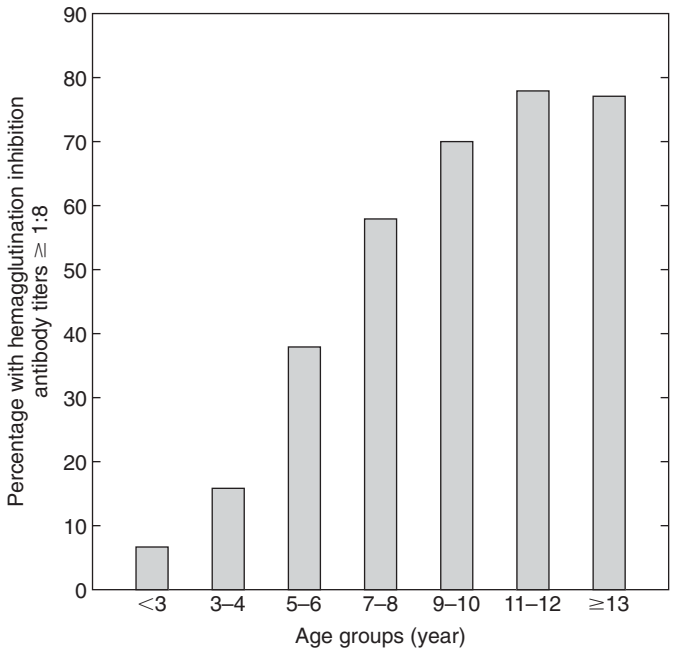

Figure 186–4 Percentage of children with rubella hemagglutination-inhibition antibody titers of 1:8 or greater in a St. Louis study in 1969. *(From Cherry, J. D.: Rubella: Past, present, and future. Volta Rev. 76:461-465, 1974. Reprinted with permission from The Volta Review. Copyright 1974 by the Alexander Graham Bell Association for the Deaf, 3417 Volta Place, NW, Washington, DC 20007.)*

pre-adolescents. Surveys of adolescents and young women of child-bearing age conducted before 1969 generally have indicated an immunity rate (HI antibody titer ≥1:8) of approximately 75 to 85 percent.[105]

Immunity to rubella as indicated by the curve in Figure 186–4 obviously is affected by epidemic periods. With epidemic disease, the curve itself most probably maintains the same slope, but it moves to the left; prevalence of antibody in each age group of children increases significantly (perhaps 10-30%). However, studies in the pre-vaccine era on sera from young adults indicate that the percentage of susceptible persons in this age group is affected only slightly by epidemic disease.[105,163,461,467,567] In a survey of sera from 600 pregnant women in 1962, Sever and associates[467] noted that 17.5 percent had no detectable antibody. In a similar study in 1966 in which the mean age was slightly less (23.6 versus 25.6 years), the percentage without detectable antibody was 7.8. Other surveys of rubella antibody in the sera of young adults acquired after the 1964 pandemic indicate a susceptibility rate in the 15- to 20-percent range, similar to pre-1964 data.[105,459,467]

Effect of Vaccination

Rubella vaccine was licensed for use in the United States and many other countries in 1969, and it has been used extensively for more than 35 years.[75,401,413,549] The immunization effort in the United States initially focused on children.[128,280,284] A secondary goal was to immunize seronegative postpubertal girls and women of child-bearing age, but little effort was extended in this area until 1978. The overall effect of the immunization effort in the United States on the young adult population is difficult to interpret. As noted in Table 186–1 and subsequent data, the reported number of cases and the incidence of rubella decreased significantly from the pre-vaccine period until the present.[68,70,413,549] A marked reduction in the number of reported cases of congenital rubella also has occurred since 1969 (see Figs. 186–2 and 186–3). However, the actual number of cases and the incidence of rubella in adolescents and young adults did not decrease until 1981, and 58.2 percent of all patients in the period 1985 through 1987 were 15 years of age (see Table 186–1). Also alarming was the marked upswing in the number of congenital rubella cases in 1990 and 1991.[73,413] Antibody survey data reported between 1981 and 1993 indicate that 5 to 25 percent of the adolescent and young adult populations in the United States were susceptible to rubella.*

In four studies in which prevalence of rubella antibody was analyzed by the vaccination status of the participants, differences were significant.[294,341,372,420] Between 87 and 96 percent of vaccinated persons had rubella antibody, whereas only 70 to 80 percent of nonvaccinated persons had antibody.

U.S. serosurvey data for the period 1988 to 1994 found the following seropositive rates by age group: 6 to 11 years, 91.8 percent; 12 to 19 years, 82.6 percent; 20 to 29 years, 84.6 percent; 30 to 39 years, 88.7 percent; 40 to 49 years, 92.5 percent; 50 to 59 years, 93.7 percent; and 60 years and older, 95.7 percent.[145] In this survey, persons born from 1970 to 1974 were found to have the lowest rate of seropositivity (78%). For the period 1999 to 2004, the overall U.S. seropositivity level was 91.3 percent. Selected age-related rates in children aged 6 to 11 years and adolescents aged 12 to 19 years were 96.2 percent and 93.7 percent, respectively.[260] Seropositivity was 91.5 percent among women and 88.0 percent among men.

In contrast to the immunization program in the United States, which focused on children and elimination of epidemic rubella, immunization efforts in the United Kingdom and many other countries initially were aimed at girls aged 11 to 14 years, with selective immunization of women of child-bearing age. This approach would not be expected to disrupt the epidemic pattern of rubella but only to decrease disease in young adult women. Serologic surveys in the United Kingdom indicated a significant

*See references 124, 140, 172, 272, 294, 372, 373, 420, 454, 488, 493, 541.

reduction in the number of seronegative persons in the target population.[99,218,341-343,425,514] In one study in which 10,000 serum samples were analyzed, 93 to 96 percent of females born after 1956 (who would have been offered rubella vaccine in school) were found to have antibody, whereas only 80 to 89 percent of those born before 1954 were found to have antibody.[99] In spite of the high level of antibody prevalence in women of child-bearing age, rubella infection in pregnancy and congenital rubella continued to be a major problem in the United Kingdom.[343,514] From 1971 to 1982, 625 cases of congenital rubella were reported, and from 1974 to 1981, 3273 women had their pregnancies terminated because they were infected with rubella or had been in contact with a person with rubella in England and Wales.[514]

In Finland, where initial immunization in 1975 involved 11- to 13-year-old girls and after 1982 involved a two-dose program for all children, the susceptibility rate in 1992 for 16- to 19-year-old girls was 3 to 5 percent, whereas for boys it was 30 percent.[527]

In Sweden, the initial immunization program, which began in 1973, targeted school girls, susceptible women after pregnancy, and women at special risk; their second program, which began in 1982, was a universal two-dose schedule at 18 months and 12 years.[49] The rate of susceptibility in pregnant women in Sweden decreased from 12 percent in 1975 to below 2 percent in 1994.

Recent serosurveys from around the world have noted varied rates of seropositivity in adults.[102,127,138,264,520] In Jordan, 90.9 percent of women of child-bearing age were immune, but only 83 percent of younger women aged 15 to 19 years were immune.[264] In Catalonia, Spain, 98.1 percent of adults had antibody to rubella[138]; in Israel, approximately 95 percent of women of child-bearing age were immune to rubella, whereas only approximately 85 percent of men of similar age were immune.[102] In this serosurvey, as many as 40 percent of adolescent men were rubella nonimmune, but only approximately 5 percent of similarly aged women were nonimmune. In Taiwan, prevalence of rubella antibody in women varied markedly by birth cohort.[520] Rubella antibody was undetectable in 29.2 percent, 7.3 percent, and 8.3 percent of cohorts born before 1971, between 1971 and 1976, and after 1976, respectively. In Argentina in 2002, 91.2 percent of women of child-bearing age had antibody to rubella.[127]

Congenital Rubella

Congenital rubella was not a reportable disease in the United States until 1966, so good data on incidence and prevalence during epidemics of rubella are not available. In the 1964 to 1965 rubella epidemic in the United States, 20,000 cases of congenital rubella occurred, 5000 therapeutic abortions were performed, fetal wastage in excess of 6250 was reported, and more than 2100 neonatal deaths occurred.[105] Estimates of the risk of acquiring congenital rubella after maternal infection vary considerably in different studies. In general, studies performed before 1964, which included nonepidemic periods, tended to underestimate the risk, whereas early retrospective studies after epidemics resulted in high incidence values.[164] Clearly, however, the individual risk of acquiring congenital rubella depends on the month of pregnancy in which maternal infection occurs. Sallomi[433] analyzed eight published studies that met his rigid criteria and noted the following rates of anomalies by gestational age when maternal infection occurred: weeks 1 to 4, 61 percent; weeks 5 to 8, 26 percent; and weeks 9 to 12, 8 percent. In pregnancies complicated by rubella in weeks 1 to 8, only 36 percent ended in normal live births, 39 percent ended in abortion or stillbirth, and 25 percent produced gross fetal anomalies. Peckham[383] noted that 85 percent of infants born to mothers infected during the first 8 weeks of pregnancy had detectable defects during the first 4 years of life. Infection at other times during pregnancy revealed the following rates of detectable defects: 9 to 12 weeks, 52 percent; 13 to 20 weeks, 16 percent; and after 20 weeks, no defect. Other studies indicate a risk of malformation of 3 percent and a risk of abortion and stillbirth of 4 percent when intervals from conception to acquisition of rubella are greater than 12 weeks.[101]

Infection with rubella virus confers lifelong immunity against clinical illness, but asymptomatic re-infection does occur. Asymptomatic re-infection is noted frequently in pregnant women, but it generally has not been considered a risk to the fetus. However, in rare instances, re-infection has resulted in severely damaged babies.[1,23,26,60,125,419,552]

TRANSMISSION

Rubella infection generally is thought to be spread by the respiratory route. Although definitive evidence supporting this assumption is not available, data from volunteer projects and studies of natural disease strongly support this view.[204,238,445] Infected persons regularly shed large concentrations of virus in the nose and throat, and droplets of secretions are released into the environment, which allows respiratory-to-respiratory transmission. Also possible is that the initial hosts may contaminate their own hands and then transmit the infectious agent to environmental surfaces or directly to contacts. In this circumstance, new hosts can acquire infection through the fomite-hand-respiratory or hand-hand-respiratory route.

In experimental transmission studies, Green and colleagues[204] noted that efficient transmission of infection to susceptible persons required prolonged, repeated contact; after a brief, single contact, only one in five children acquired disease, whereas all but one of 17 subjects with prolonged, repeated contact were infected. Although the period of communicability has never been determined accurately, almost 100 years ago the period of infectivity was noted to precede the eruptive phase of illness.[207] Volunteer studies indicate that virus is present in nasopharyngeal secretions from 7 days before to 14 days after the onset of rash.[204,238] Maximal shedding and presumably maximal transmissibility occur for an 11-day period of 5 days before to 6 days after the appearance of rash. Infants with congenital rubella shed virus from the nose and throat for many months and have been responsible for the spread of virus to susceptible contacts.[255,443]

SEASONAL PATTERNS

Rubella is a disease of winter and spring, with the largest number of cases in the United States occurring in March, April, and May.[104,105] This seasonal pattern occurs in years with both a high and a low incidence of rubella. Presumably, some transmission and sporadic illness occur throughout the year in large urban areas.

GEOGRAPHIC DISTRIBUTION

Although clinical rubella had gone unrecognized in many countries in Africa, Asia, and South and Central America, serologic surveillance indicates that it was present throughout the world.[101] In remote islands, rubella may not be endemic, so large segments of the population may be susceptible.[53,242] In these locales, the introduction of rubella results in epidemic infection that involves approximately 90 percent of the susceptible population. In populated areas of the world in the pre-vaccine era, rubella was both endemic and epidemic, and between 80 and 90 percent of the adult population had serum rubella antibody.[101] An interesting note is that in the pre-vaccine era in several well-populated large islands that were not remote (Jamaica, Taiwan, Barbados, Trini-

dad, Hawaii, and Japan), a smaller percentage of the total population had antibody, and rubella was not endemic.[101,154,184,202]

OTHER FACTORS

Sex

The incidence of clinical rubella is similar in boys and girls.[104,105] In adults, more cases of rubella are reported in women.[105] This finding possibly is the result of interest and concern related to congenital rubella rather than a true difference on the basis of sex. Of interest is that in rubella vaccine trials, girls have been noted to have geometric mean convalescent-phase antibody titers higher than those of boys.[339,480]

Mitchell and associates[349] studied the IgG, IgM, and IgA antibody responses in men and women to rubella virus structural proteins (E1, E2, and C). IgA E2 antibodies did not develop in men, but they did in women. Men had lower IgG antibody to E2, an earlier onset of E1-specific IgG and IgM antibodies, and a greater proportion of total antibody against E1 than women did. In another study, Mitchell[346] reported that men had a more rapid cell-mediated immune response to whole, inactivated rubella virus and a panel of rubella virus peptides after reimmunization than women did.

Genetics

Hattis and associates[234] showed that individuals differ in their ability to transmit rubella. During a rubella epidemic, they noted a small number of persons who had high potential for transmitting virus to susceptible persons ("spreaders"). Most persons demonstrated only minimal virus transmission ("nonspreaders"). Honeyman and colleagues[248,250] suggested that the ability to spread rubella virus is favored by the cell surface antigen HL-A1 or the combination of HL-A1 and HL-A8. In a rubella vaccine trial, Spencer and associates[480] noted that 44 percent of persons with high rubella HI antibody titer (≥1:512) responses had HL-A28. In this study, a high convalescent-phase geometric mean antibody titer also was noted to occur in subjects with the AB blood type.

Ovsyannikova and associates[376] recently studied human leukocyte antigen haplotypes in the genetic control of immune response to measles-mumps-rubella (MMR) vaccine. They found that DRB1*04-DQB1*03-DPB1*03 was associated with high lymphoproliferative responses to rubella vaccine virus. The same haplotype was associated with lower rubella virus IgG antibody levels.

PATHOLOGY AND PATHOGENESIS

VIRAL INFECTION

The sequence of events in uncomplicated, postnatally acquired rubella is presented in Table 186–3. Although much is known about rubella infection in humans, considerable gaps regarding specific events exist.[204,236-238,445] Estimates for the timing of events in rubella infection (see Table 186–3) have come from volunteer inoculation studies. In many instances, artificial inoculation has resulted in a reduction in the length of the incubation period of clinical disease. This finding suggests that the size of inoculum is important in the initial generation of human infection. It also helps explain the rather wide boundaries of the incubation period noted in many clinical studies.[207,336,438]

The primary site of infection is the respiratory epithelium of the nasopharynx. Initial infection of the respiratory epithelium apparently is minor; a more important event is early spread of virus to the regional lymphatics. In volunteers given 100 $TCID_{50}$ of rubella virus intranasally, viral multiplication at the respiratory site was noted on the third day.[445] After viremia develops, extensive nasopharyngeal infection occurs. In persons who have received either attenuated or unattenuated virus by the subcutaneous route, nasopharyngeal shedding in varying concentrations always occurs.[92,93,204,445] Concentrations of virus generally are greater in specimens collected from the nose than in those from the throat.

Viremia peaks just before the onset of exanthem and disappears shortly thereafter. In contrast, virus continues to be present consistently in the nasopharynx for a 6-day period after the onset of rash and occasionally for an additional week thereafter.[204] In addition to the blood and nasopharynx, other sites from which rubella virus has been recovered are the lymph nodes,[181] urine,[445] cerebrospinal fluid,[484] conjunctival sac,[237] breast milk,[57] synovial fluid,[241] and lungs.[469] In patients with rubella, the virus was recovered from the skin at sites both with rash and without rash.[236,237]

TABLE 186–3 The Sequence of Rubella Viral Infection in Uncomplicated Primary Disease

Day	Event
0	Rubella virus from the respiratory secretions of an infected person comes in contact with the epithelial surface of the nasopharynx of a susceptible person. Localized infection in the respiratory epithelium is established, and virus spreads through lymphatics and possibly by transient viremia to regional lymph nodes
1-22	Viral replication in localized areas of the nasopharynx and regional lymph nodes
3-8	First evidence of nasopharyngeal viral shedding
6-20	Viremia (virus free in serum and associated with leukocytes)
8-14	Establishment of infection in the skin and other viremic sites, including generalized nasopharyngeal involvement
10-17	Maximal viremia and viruria
10-24	Maximal nasopharyngeal viral shedding
17-19	Viremia decreases and then ceases. Viral content at viremic sites rapidly diminishes

From references 204, 236-238, 445.

IMMUNOLOGIC EVENTS

Antibody

After having natural or vaccine rubella viral infection, patients regularly have an antibody response. Serum antibodies to different rubella viral antigens can be measured by HI, CF, neutralization, immunofluorescence, precipitation, radioimmunoassay, ELISA, single radial hemolysis, passive hemagglutination, latex agglutination, and platelet aggregation.*

In natural postnatal rubella infection, HI and neutralizing antibodies appear 14 to 18 days after exposure, at the time of the rash. HI antibody titers usually peak approximately 2 weeks after the onset of clinical illness, stay at a high level for several weeks, decrease about fourfold during the course of a year's time, and then generally persist for life. Immunofluorescence, ELISA, radioimmunoassay, and latex agglutination reveal antibody patterns similar to those determined by HI.[240] Antibody detected by

*See references 14, 39, 114, 135, 159, 191, 200, 219, 240, 246, 300, 304, 319, 333, 334, 345, 361, 384, 393, 462, 464, 513, 534, 538, 564, 578.

passive hemagglutination does not appear until 3 to 4 weeks after the onset of illness, and that detected by single radial hemolysis is delayed until 1 to 2 weeks after onset. In an Amazon Indian tribe, the geometric mean rubella HI antibody titer 12 years after infection, with no intercurrent rubella exposure, was 1:33.[40] The pattern of the neutralizing antibody response is similar to the HI antibody response, except that the peak is delayed slightly. Brody and colleagues[53] noted that all but 10 percent of an island population had rubella neutralizing antibody 22 years after the time of epidemic illness.

CF antibody first appears approximately a week after HI and neutralizing antibody, peaks approximately 1 month after illness, and in general does not persist as long as does either HI or neutralizing antibody. On occasion, the CF antibody response is delayed and appears 1 month after the exanthem, with peak titers occurring 2 to 5 months later. Sever and colleagues[465] noted CF antibodies in only 44 percent of persons with neutralizing antibody who were studied 10 to 20 years after illness. The use of an antigen prepared by alkaline extraction has increased the sensitivity of CF, but low levels of antibody still are best identified by HI, ELISA, or neutralization.[393,450]

After natural infection occurs, precipitating antibodies develop to both theta and iota antigen.[299] Antibody to theta antigen appears early, parallels HI antibody, and is persistent. In contrast, the response to iota antigen is delayed, with a slow rise in concentration during a 2- to 3-month period. Five years after infection has occurred, anti-iota antibody cannot be detected.

After immunization has been administered, the antibody response pattern varies according to the type of vaccine used.[41,300,369,395,396,444,544] With RA 27/3 vaccine, the serum antibody response is similar to that after natural disease, except that the peak HI and neutralizing antibody titers attained usually are lower. Serum antibody responses noted after HPV-77 and Cendehill vaccine viral infection are different from those found after natural infection in that CF and anti-iota antibodies are noted only irregularly and then in minimal concentration.

Primary rubella virus infection, either naturally acquired or vaccine induced, is characterized by the initial appearance of antibody in the IgM and IgG serum components.[29,38,118,134,213,381,382,485] In general, the IgM-specific response is short-lived and not detectable more than 8 weeks after the onset of infection. On occasion, it has been detected in the serum for extended periods.

IgA nasal HI, ELISA, and neutralizing antibodies also occur regularly after natural viral infection. After immunization, the nasal antibody response varies with the type of vaccine and the route of administration.[5,9,117,120,369,393] After subcutaneous immunization with HPV-77 vaccine is given, rubella-specific nasal IgA antibody is a rare finding; it occurs in most subjects who receive RA 27/3 vaccine administered intranasally and in approximately half of those given this vaccine by the subcutaneous route.

Specific Cell-Mediated Responses

Rubella-specific, cell-mediated lymphocyte responses regularly occur after infection with rubella virus.*

Steele and associates,[487] using an in vitro lymphocyte-mediated cytotoxicity assay, first noted that lymphocytes from persons who previously had rubella caused cell destruction in a tissue culture chronically infected with rubella virus. Rubella antigen–specific, cell-mediated immunity also has been demonstrated in lymphocyte cultures by blast transformation, production of migration-inhibition factor, and production of interferon.[59,249,357,478,508,536,537] With vaccination, rubella-specific, cell-mediated immunity first was noted to occur 7 days after immunization, with peak responses at 3 weeks.[536,537] Honeyman and associates[249] noted that the rubella antigen–specific, cell-mediated response commenced 1 week before the humoral immune response occurred in both natural and vaccine-induced rubella viral infection. They also noted that the cell-mediated response was of greater magnitude and duration after natural disease than after immunization. Rossier and associates[427] studied cloistered nuns and noted that specific cell-mediated immunity to rubella virus persisted until they reached 79 years of age in the probable absence of re-infection.

Morag and colleagues[357] demonstrated the specific appearance of cell-mediated immunity in tonsillar lymphoid tissue after natural infection or intranasal immunization with rubella vaccine. This responsiveness was conspicuously low after vaccination was administered by the subcutaneous route. In most instances, the presence of cell-mediated responsiveness correlates with the presence of antibody; specific rubella lymphocyte transformation has been noted in the absence of antibody, however.[478] The magnitude of the rubella-specific, cell-mediated response is suppressed during pregnancy.[508]

McCarthy and colleagues[323] identified potential determinants of human cellular immunity to rubella virus by using synthetic peptides representing well-defined sequences of rubella virus structural proteins. They used the following peptide subsequences: two capsid domains (C_1 to C_{29} and C_{64} to C_{97}), a glycoprotein E1 domain ($E1_{202}$ to $E1_{283}$), and a glycoprotein E2 domain ($E2_{31}$ to $E2_{105}$). All but the C_{64} to C_{97} subsequences stimulated specific lymphoproliferative responses in peripheral blood mononuclear cells in 25 to 50 percent of immune subjects. The immunodominant T-proliferative epitope (C_{14} to C_{29}) was recognized by only 50 percent of the peripheral blood mononuclear cells of the study population. Relatively immunodominant T-cell epitopes vary among different persons.

Using a lymphocyte proliferation assay, Mitchell and associates[348] reported positive cell-mediated immune responses to 16 peptides, including six that contained antibody neutralization domains after revaccination.

Nonspecific Responses

A large number of nonspecific, immunologically related responses can be demonstrated during rubella virus infections. Niwa and Kanoh[364] performed a comprehensive study of these responses in 85 children and adults during an epidemic of rubella. They noted a decreased number of total leukocytes, neutrophils, and T cells initially, which returned to normal values within 1 week. Some patients had slightly elevated levels of serum IgM, and total hemolytic complement was elevated in 12 of 30 patients. Marked increases in C4 and C9 also were present. In addition, they noted a marked insensitivity to dinitrochlorobenzene and purified protein derivatives in many patients. Atypical lymphocytes, autoantibodies, and reduced blastogenesis as measured by phytohemagglutinin (PHA) stimulation were noted in some patients. Other studies consistently have demonstrated a reduction in the lymphocyte response to PHA.[56,63,185,262,289,314,325] In general, this reduction lasts less than 1 month, and infections with attenuated strains of rubella vaccine virus are less immunosuppressive than are infections with unattenuated rubella virus.

Hyypiä and colleagues[262] noted that during rubella virus infection, the proportion of suppressor-cytotoxic T cells was increased and the proportion of helper-inducer T cells was decreased. Polyclonal activation of B cells was associated with these findings.[263]

Zaknun and coworkers[574] noted a marked increase in urine neopterin levels in two children with acute rubella. Their levels increased dramatically 4 days before the onset of exanthem.

*See references 85, 239, 249, 269, 311, 314, 323, 346-349, 357, 376, 375, 426, 427, 478, 487, 536, 537.

FETAL EVENTS

Viral Infection

A considerable amount of information about fetal infection became available from extensive studies during the 1964 epidemic of rubella,* and further information has been obtained more recently from both natural and vaccine viral infections.[43,136,146,293,390,458,531,572] In spite of the number and extent of investigations performed to date, we know little about transmission of the virus to the fetus in maternal infections in the latter half of pregnancy.

With maternal infection during the first trimester, placental infection regularly occurs and often persists throughout the remainder of the pregnancy. In the therapeutic abortion studies of Alford and associates,[8] fetal infection occurred in approximately 50 percent of placental infections. However, other studies have revealed almost identical isolation rates from both placental and fetal tissue.[410,507] Persistent infection is the usual outcome of first-trimester fetal infection. This fetal infection usually involves multiple organs, and virus can be isolated at birth regularly from the throat, rectum, and urine.[255,391]

Little is known about events in second- and third-trimester maternal rubella infection. Most probably, placental infection is a regular occurrence, and transmission of virus to the infant in utero also may occur regularly. Because few infants have defects when they are born after maternal rubella infection in the second and third trimesters, a careful search for rubella infection in these infants by virologic or serologic methods rarely has been conducted. Random studies seem to indicate that rubella virus often infects the fetus after the first trimester, and occasionally the infection becomes persistent.[119,136,227,255,353,539,560] Other studies have failed to show virologic or serologic evidence of infection in infants in whom maternal rubella occurred in the second and third trimesters.[94,354,491]

With maternal rubella infection, the cervix also is involved, so fetal infection could occur by the ascending route as well as by primary placental infection.[458,531] In addition, fetal infection has resulted from maternal disease that occurred before conception.[162,561,572]

Rubella virus can be recovered regularly after birth from infants with congenital rubella. The percentage of infants with persistent infection decreases during the course of the first year of life; by their first birthdays, between 10 and 20 percent of children still shed virus in nasopharyngeal secretions.[110,410] Rawls and colleagues[410] were unable to isolate virus from the throats of 15 congenitally infected infants after they reached 18 months of age, and Sever and Monif[466] and Cooper and Krugman[110] were unable to demonstrate persistence of nasopharyngeal virus in older children. A 4.5-year-old boy with congenital rubella was found on one occasion to be shedding rubella virus in the throat.[470]

Immunologic Findings

SPECIFIC ANTIBODY. Humoral antibody in a congenitally infected fetus is acquired transplacentally from the mother and is produced actively by the fetus. In a normal maternal-fetal relationship, transport of antibody to the fetus is minimal until the midpoint of the second trimester (16 to 20 weeks).[7,8] With first-trimester maternal infection (transplacentally acquired), rubella antibody titers in serum amount to only approximately 5 percent of maternal values. The fetal immune system becomes functional during the second trimester,[295] and small amounts of specific rubella fetal IgM antibody can be detected. From the midpoint of pregnancy, antibody levels in the developing fetus rise so that at birth, the maternal and infant values are similar. Although the values of total antibody are similar, the composition is different. Maternal antibody at the time of delivery usually is composed entirely of IgG. In contrast, the infant titer consists of fetal IgM, presumably fetal IgG, and occasionally fetal IgA and transplacentally derived maternal IgG.

Long-term rubella antibody patterns in congenitally infected infants after birth are different from those of their mothers or from those of a group of children with acquired disease.[108,226,274,522] Cooper and colleagues[108] monitored a group of 223 mothers of children with congenital rubella and noted that at the end of 5 years, all still had detectable HI antibody and the geometric mean titer for the group had undergone a fourfold reduction. In contrast, 5-year follow-up of the congenitally infected infants revealed a 16-fold decline in geometric mean titer; 8 of 29 infants had serum HI antibody titers less than 1:8 when they were examined at 5 years of age. Other investigators have observed similar declines in rubella antibody titers of congenitally infected infants.[226,274,522]

Another unique aspect of rubella antibody in congenitally infected infants is the persistence of specific IgM. Cradock-Watson and colleagues[121] studied 40 infants with congenital rubella and noted that IgM antibody persisted for approximately 6 months in most cases and for up to 2 years in a few children. de Mazancourt and colleagues[129] studied the antibody response to rubella virus structural proteins in infants with congenital rubella syndrome and found that the immunoprecipitation patterns were different from those in sera from postnatally infected adults. The sera from congenitally infected infants had little or no C-specific antibody, occasionally only antibody to E1 was precipitated, E1 protein was precipitated in relative excess of E2 protein, and the relative amount of E2 antibody was greater than antibody to E1.

SPECIFIC CELL-MEDIATED IMMUNITY. Rubella-specific, cell-mediated immune responses have been studied in children with congenital rubella by the following assays: lymphocyte-mediated cytotoxicity, lymphocyte transformation, lymphocyte interferon production, and leukocyte migration-inhibition factor production.[58,180] By all methods of study, infants with congenital rubella have decreased rubella-specific, cell-mediated responses compared with persons who previously had acquired rubella postnatally. Buimovici-Klein and associates[58] noted that the degree of suppression correlated with the time of in utero infection: the earlier in pregnancy the maternal infection, the greater the depression of specific cell-mediated responses. In the study of an infant with late-onset congenital rubella syndrome, Verder and associates[535] noted decreased activity of killer and natural killer cells and alloreactive direct cytotoxic cells. Their data indicated that defective cytotoxic effector cell function was the primary cause for failure to eliminate virus in the illness.

NONSPECIFIC RESPONSES. Desmyter and colleagues[132] noted that infants with congenital rubella produced normal amounts of interferon after receiving measles immunization. They also found that the clinical response and antibody development in these measles-vaccinated children were similar to those in normal children. Lebon and associates[296] noted that sera collected from rubella-infected fetuses and infants with congenital rubella contain an acid-labile interferon. Michaels[337] observed that infants with congenital rubella who still were shedding virus in their throat or urine had depressed antibody responses to diphtheria and tetanus toxoids. White and colleagues[559] reported decreased in vitro lymphocyte blast transformation responses to vaccinia and diphtheria toxoid antigens in children with congenital rubella in comparison to normal children. They also noted

*See references 8, 66, 94, 110, 111, 142, 224, 227-230, 255, 351-355, 391, 410, 443, 446, 491, 561.

depressed skin reactivity to intradermal *Candida* antigen in the congenital rubella group. Buimovici-Klein and associates[58] observed a marked reduction in lymphocyte transformation after PHA stimulation in their congenital rubella group. The most marked defect was seen in children in whom maternal rubella occurred during the first 8 weeks of pregnancy. Pukhalsky and associates[403] noted that rubella immunization resulted in defective lymphocyte response to a mitogen (PHA). After immunization, serum interferon-α was slightly increased at day 7 and then fell significantly so that it was not measurable at day 30 in several subjects. The interferon-α/interleukin-10 ratio significantly decreased after immunization.

PATHOLOGY

Postnatally Acquired Disease

Almost no data on the histologic findings in uncomplicated rubella are available, but occasionally, postmortem tissue has been studied from patients with encephalitis. Giuliani and associates[195] studied lymph nodes from patients with rubella and noted edema, reticulum cell hyperplasia, and loss of the usual follicular morphologic features. Sherman and associates[469] reported six cases of rubella encephalitis and noted the autopsy findings in three cases. They specifically searched all organs for inclusion bodies, syncytial giant cells, focal cellular necrosis, and unusual proliferative changes, but none of these was found. Only mild, nonspecific, follicular hyperplasia in the spleen and lymph nodes was seen. Histologic examination of the brain of a 7-year-old girl who died of encephalitis revealed diffuse swelling, nonspecific degeneration, and a sparse, mononuclear perivascular and meningeal exudate.

A synovial biopsy specimen in a woman with rubella arthritis revealed scattered areas of fibrinopurulent exudate and synovial cell hyperplasia; inflammatory cell infiltration composed mainly of lymphocytic cells was present, and vascularity was increased.[573]

Congenital Infection

In contrast to postnatal rubella, the pathologic process of congenital infection has been studied extensively.*

Table 186–4 summarizes the main pathologic findings by anatomic location or system in congenital rubella. As noted, defects in congenital rubella result from both specific cell damage and cellular deficiency. Although specific cellular necrosis is important in certain early lesions such as in the inner ear, of greater overall importance are the secondary effects of generalized vascular damage. Also of presumed major importance is the noncytolytic cellular infection characteristic of rubella virus. It results in mitotic arrest and a reduction in the total number of cells in many organs.

CLINICAL MANIFESTATIONS

POSTNATAL ILLNESS†

Although clinical rubella is a distinctive exanthematous disease, its features are not as clearly discernible as are those of measles or chickenpox. The exanthematous illnesses caused by enteroviruses, adenoviruses, and other common respiratory viruses often are clinically similar or identical to those of rubella (see Chapter 64). Because of these other viral illnesses that simulate rubella, descriptions of clinical rubella made before modern virologic diagnostic techniques became available are not always accurate, particularly when rubella in infants and young children is described because exanthems caused by other viruses occur most commonly in these age groups. Unfortunately, in spite of the availability of a vast amount of clinical material collected during the rubella epidemic of 1964, most clinical knowledge relating to postnatally acquired rubella was formulated before the present virologic era.

Incubation Period

Although prodromal complaints and lymphadenopathy frequently precede the development of exanthem in rubella, the incubation period in most studies has been calculated as being from the time of exposure to the onset of rash. Almost a century ago, Michael[336] reviewed the incubation periods in 59 different reports and noted a variation of 5 days to 4 weeks. However, in most reports, the minimal incubation time was 14 days or more and the maximum was 17 to 21 days. The mean incubation period from modern reviews is considered to be 18 ± 3 days.

In carefully controlled studies, Green and colleagues[204] noted an incubation time of 13 to 15 days to the onset of rash after the intramuscular inoculation of serum from rubella-infected patients and a longer incubation time (16 to 21 days) in cases acquired by contact with ill patients. In similar volunteer studies in young adults, Schiff and colleagues[445] noted that the onset of rash occurred 11 to 12 days after the administration of 100 $TCID_{50}$ of tissue culture–grown rubella virus. The investigators attributed this shorter incubation period to a larger inoculum than that occurring in natural transmission.

Prodromal Period

Complaints before the onset of rash in rubella vary with age. In young children, the first evidence of disease usually is the appearance of rash. On occasion, mild coryza and diarrhea precede the development of exanthem in younger patients. In contrast to the lack of prodrome in children, adolescents and adults usually have symptoms before the onset of rash.[88,161,209] In one study, 94 percent of college students with rubella had prodromal complaints. In decreasing order of frequency, the reported symptoms were eye pain, sore throat, headache, swollen glands, fever, aches, chills, anorexia, and nausea.[161] Gross and associates[209] reported prodromal upper respiratory complaints, including malaise, cough, sore throat, red eyes, and runny nose, in 65 percent of an infected adolescent study group.

Prodromal symptoms usually precede the onset of rash by 1 to 5 days. In the studies of Green and associates[204] involving volunteers, the onset of lymphadenopathy commonly was noted 5 to 7 days before the onset of rash. In contrast, Schiff and colleagues[445] observed the appearance of lymphadenopathy only 1 day before the rash appeared; fever was noted 1 to 4 days before the onset of rash, and most of the volunteers also had malaise and sore throat. Pain on lateral and upward eye movement occurs frequently and occasionally is distressing.[88,161]

Exanthem Period

The rubella exanthem appears first on the face. Spread of the rash is centrifugal from the head toward the hands and feet. The progression, extent, and duration of the exanthem vary considerably. In a typical case, the rash involves the entire body during the first 24 hours, begins to fade on the face during the second

*See references 4, 6, 30, 45, 54, 65, 98, 111, 141, 150-153, 170, 176, 177, 210, 222-224, 271, 277-279, 308, 352, 355, 362, 374, 389, 397, 398, 408, 411, 417, 422, 423, 429, 457, 474, 475, 481, 489, 494, 509, 516, 547, 551, 558, 571.

†See references 88, 89, 161, 164, 204, 207, 283, 290, 336, 438, 445, 521, 557.

TABLE 186–4 Pathologic Findings in Congenital Rubella

Anatomic Location or System	Gross and Microscopic Findings	References
Placenta	Perivascular mononuclear cellular infiltration in the deciduas	374
	Edema, fibrosis, and necrosis of villi; cytoplasm inclusion bodies noted in swollen Hofbauer cells in villous stroma	
Generalized growth retardation	Subnormal number of cells in many organs	362
Nervous system	Chronic meningitis with infiltrates of large mononuclear cells, lymphocytes, and plasma cells in the leptomeninges	422, 423, 474, 509
	Vascular degeneration, ischemic lesions, and retardation of myelinization throughout brain	
Eye	Lens: cataract, cortical liquefaction, and spherophakia	45
	Iris and ciliary body: necrosis of ciliary body, iridocyclitis, iris atrophy, and pigmentation defects	
	Retina: posterior pigmentary disturbances	
	Cornea: usually normal; occasional endothelial degeneration	
	Optic nerve: posterior bowing	
Ear	Hemorrhage in fetal cochlea resulting in epithelial necrosis	176, 177, 547
	Inflammatory cells in stria vascularis	
	Adhesions between Reissner membrane and tectorial membrane	
	Sacculocochlear degeneration of Scheibe (strial atrophy, collapse of Reissner membrane, atrophy of organ of Corti, rolled-up tectorial membranes, and collapse and degeneration of sacculus) noted after birth	
Cardiovascular	Common heart defects in order of frequency: patent ductus arteriosus, pulmonary artery stenosis, ventricular septal defect, and atrial septal defect (These rubella-induced lesions do not differ from similar non–rubella-induced lesions.)	4, 150, 170, 278, 279, 551
	Myocarditis with swelling of muscle fibers and loss of striations; necrosis	
	Intimal proliferation of major arteries	
Pulmonary	Chronic interstitial pneumonia with large mononuclear cells, lymphocytes, and plasma cells within interstitial spaces and alveoli	278, 389, 474
Liver	Hyalinization and swelling of hepatocytes, hematopoiesis, and multinucleated giant cells	152, 153, 489, 494
Skin	Purpuric lesion: focal areas of erythropoiesis in dermis and upper subcutaneous adipose tissue	65, 277
	Chronic reticulated rash: acute and chronic inflammatory cells and histiocytes in dermis	
	Edema in dermal papillae	
Bone	Thinning of metaphyseal trabeculae and decrease in number of osteoblasts and osteoclasts	411, 429, 457, 558
	Many plasma cells in metaphyses and cartilaginous epiphyses and around vessels	
	Occasional giant cells with cytoplasmic inclusions	
	Thinning of cartilage	
Muscle	Focal abnormalities: very small fibers with darkly staining nuclei and muscle bundles containing empty connective tissue tubes	480
Teeth	Necrosis of enamel-forming epithelial cells	210, 516
Hematologic	Transient thrombocytopenia with decreased megakaryocytes in bone marrow; increased platelet adhesiveness and platelet agglutinins	30, 98, 398, 408
	Lymph node consistent with histiocytosis; unorganized cell mass made up of mononuclear cells with dense round nuclei and irregularly shaped cytoplasm	
Immunologic	Spleen: fibrosis	98, 221, 397, 417
	Loss of normal architecture and absence of germinal centers in spleen and lymph nodes	
	Dysgammaglobulinemia usually with decreased IgG and IgA and elevated IgM	

day, and has disappeared from the body by the end of the third day. The characteristic rash is erythematous, maculopapular, and discrete (see Fig. 64–3). Its appearance on an adolescent's face occasionally is confused initially with an exacerbation of acne. Frequently, the rash is only macular with a scarlatiniform appearance. In some patients, the rash is present for less than a day, although sometimes it persists for 5 days or longer. Particularly in adults, the exanthem frequently is pruritic. This complaint is troublesome because it often leads the patient as well as the physician to attribute the rash to an allergic cause rather than rubella virus infection.

The exanthem occasionally progresses to confluence with a morbilliform appearance. In these cases, the rash usually is less coppery and pinker than that in measles and heals without desquamation or brownish discoloration. The typical picture of erythema infectiosum (slapped-cheek appearance and reticular rash) has been observed in rubella-infected patients. Balfour and associates[21] described eight children with erythema infectiosum from whom rubella virus was recovered concurrently and two additional children with serologic evidence of rubella infection. The preliminary results of volunteer studies with a virus recovered from one of the patients in this study produced a slapped-cheek appearance but a nonreticulated rash in four of five men. One 3-year-old child from whom the author recovered rubella virus had typical erythema infectiosum.[88] A 14-month-old girl had a roseola-like illness and arthritis.[241]

In the volunteer studies of Schiff and associates,[445] the rash was noted to be pink-red and maculopapular. It appeared initially on the face, chest, upper part of the arms, and shoulders and then spread rapidly over the abdomen, back, and thighs. It developed

into an erythematous blush on the face and abdomen. The median duration was 3 days, with extremes of 2 and 5 days. No pruritus was noted.

Rubella infection without rash is a rather common occurrence. In some patients, the infection is without symptoms; in other persons, careful questioning reveals prodromal symptoms, and lymphadenopathy is found on examination. Green and associates[204] noted that approximately 25 percent of exposed children who became infected had subclinical infection. In an intensive study of 46 susceptible children and adults, all but one subject had clinical symptoms with infection[460]; 60 percent of the group had both rash and characteristic posterior auricular or suboccipital lymphadenopathy, and 40 percent had lymphadenopathy without rash. In another study of rubella in an institution for retarded children, Horstmann and associates[257] noted that only approximately half the children who became infected had a rash. Of nine children without rash, significant posterior auricular lymph node enlargement developed in five. Buescher[55] reported a subclinical-to-clinical infection ratio in a military recruit population of 6.5:1.

Lymphadenopathy is a major clinical manifestation of rubella. The most characteristic enlargement occurs in the suboccipital and posterior auricular nodes, but generalized involvement occurs as well. In the volunteer studies of Schiff and colleagues,[445] the lymph node enlargement usually lasted between 5 and 8 days. In two outbreak studies involving adolescents and young adults, posterior auricular and suboccipital lymphadenopathy was noted in all patients with rash.[161,209] In contrast to these findings, Landrigan and associates[290] noted that only 47 percent of children and 58 percent of adolescents had similar lymph node enlargement during epidemic rubella illness. Although a frequent suggestion is that the finding of exanthem and suboccipital lymphadenopathy is pathognomonic for rubella, this suggestion is incorrect. In young children, similar involvement is seen frequently with enteroviral and adenoviral infections. In adolescents and young adults, the association more strongly indicates rubella, but infectious mononucleosis, *Mycoplasma pneumoniae* infection, acquired toxoplasmosis, and other possibilities also must be considered.

The occurrence of fever in rubella varies; when it does develop, the temperature usually is elevated only minimally. In children with experimentally induced rubella, Krugman and Ward[285] noted that 5 of 13 had temperatures of 38° C (100.4° F) or higher. Two children had maximal temperatures of 38.5° C (101.6° F). Schiff and colleagues[445] noted that all nine infected volunteers had fever with a median duration of 5 days. Landrigan and associates[290] found fever in 74 percent of children and only 47 percent of adolescents; Gross and coworkers[209] observed fever in only 6 of 17 adolescents. On occasion, children with apparent rubella have been noted to have markedly elevated temperatures. Few such cases have undergone virologic study, so some doubt must be raised regarding whether the illnesses were induced by rubella virus or were caused by other viral agents more commonly associated with marked febrile responses, such as enteroviruses and adenoviruses. I have seen an 8-year-old boy with virologically and serologically confirmed rubella with a temperature of 40° C (104° F) on the day before the appearance of his rash. The 14-month-old girl with arthritis described by Hildebrandt and Maassab[241] had a temperature as high as 40.5° C (105° F).

In 1898, Forchheimer[165] described what he thought was the enanthem of German measles. He described pinhead-sized macular lesions with a rose-red color on the soft palate and uvula that appeared at approximately the time of the exanthem and lasted less than 24 hours. This exanthem has not been identified in children I have examined; however, petechial lesions on the soft palate and uvula have been seen occasionally. Mild pharyngitis is not an uncommon occurrence. Other symptoms and signs in rubella include mild conjunctivitis, sore throat, coryza, cough, and headache.

The duration of illness in uncomplicated rubella varies considerably. Most patients would continue normal activity if the rash were not present. In general, full return to normal activity occurs within 3 days. A small number of adults are bothered by persistent headache, eye pain, and pruritus for 7 to 10 days.

The white blood cell count in rubella tends to be low. Schiff and colleagues[445] found leukopenia in all nine volunteers. In these subjects, leukopenia paralleled the pattern of fever, with onset occurring 24 hours before the rash was manifested and persistence lasting for 4 to 5 days. Before rubella could be confirmed by both specific serologic and virologic methods, many experts thought that rubella could be confirmed accurately by characteristics of the white blood cell count.[243,261] Leukopenia was found at the onset of disease; the total count rose to a high-normal value during a 10-day period. Relative neutropenia was noted by Hynes[261] in many patients; one patient had a neutrophil count of 868 cells/mm^3 on the first day of illness. Plasma cells, Türk cells, or both were noted in acute rubella in all cases studied by Hynes[261] and Hillenbrand.[243] A Türk cell is a developing plasma cell that is 25 to 40 μm in diameter and contains a 15- to 30-μm nucleus. The nucleus has two to five prominent nucleoli and a well-defined, light reticulum. The cytoplasm often is vacuolated. Twenty-five percent of the patients studied by Hynes[261] had elevated erythrocyte sedimentation rates during the first week of illness.

Complications

JOINT INVOLVEMENT. The incidences of reported cases of arthritis and arthralgia vary considerably in different studies.[35,82,187,209,268,290,473,526,557] In general, both arthralgia and arthritis occur more commonly in adults than in prepubertal children. Women are afflicted more often than are men. In a large outbreak in Bermuda in 1971, 42 percent of 125 patients studied complained of joint pain or discomfort.[268] Three patients had swelling of the joints. The prevalence of joint symptoms increased from 18 percent in the 0- to 9-year-old age group by approximately 20 percent increments per decade; 73 percent of those older than 30 years had symptoms. Joint complaints generally were more common in females than in males older than 10 years. This difference was most marked in the 10- to 20-year-old age group. Landrigan and associates[290] studied the location of joint symptoms in adolescents and found that the fingers were involved most often; the knees and wrists also were implicated commonly.

Yanez and associates[573] studied 11 patients with rubella arthritis. In all instances, multiple joints were involved. The onset of arthritis occurred 1 to 6 days after the beginning of the exanthem and lasted 3 to 28 days (mean, 9 days). The erythrocyte sedimentation rate was elevated in three of seven cases, and one patient had markedly positive latex test results. The white blood cell count was below 5000 cells/mm^3 in five of seven patients. One woman had bilateral carpal tunnel syndrome. Four children with transient carpal tunnel syndrome accompanying rubella virus infection have been described.[42] Panush[378] noted serum hypocomplementemia with rubella arthritis in a 25-year-old woman.

The possibility that rubella viral infection is related to rheumatoid arthritis has been studied on several occasions.[48,128,317,370,576] Martenis and colleagues[317] described a 21-year-old woman in whom rheumatoid arthritis developed after typical rubella with arthritis. Deinard and associates[128] found that all serum specimens from 80 patients with rheumatoid arthritis contained rubella HI antibody. In contrast, only 86 percent of an equal number of nonarthritic controls and a group of persons with other forms of arthritis had measurable rubella antibody titers. Ogra and associates[370] noted that patients with juvenile rheuma-

toid arthritis had IgM and IgG serum rubella antibody levels that were four to six times higher than those observed in controls during rubella infection. They also noted specific staining for rubella virus antigen in the synovial fluid of 33 percent of these patients with juvenile rheumatoid arthritis. Grahame and associates[199] repeatedly recovered rubella virus from the synovial fluid of six patients with inflammatory oligoarthritis or polyarthritis during a 2-year period.

NEUROLOGIC MANIFESTATIONS. Encephalitis is a rare complication of rubella.* Sherman and colleagues[469] noted 6 cases of encephalitis in an epidemic during the spring of 1964 that involved approximately 30,000 children. This rate of encephalitis (1 per 5000 cases) is similar to the rate of 1 per 6000 noted in Detroit in 1942.[316] Rubella encephalitis is clinically similar to encephalitis from measles virus infection but is thought to be less severe. Mortality and morbidity rates have varied considerably. Sherman and colleagues[469] noted that three of six children studied in Pittsburgh died of this complication during the spring of 1964, whereas in Atlanta during the same epidemic period, six patients recovered uneventfully.

The onset of encephalitis usually occurs 2 to 4 days after the rash appears, but occasionally, rash and neurologic symptoms occur at the same time; in other instances, the appearance of encephalitis is delayed as much as 1 week after the onset of illness. Examination of cerebrospinal fluid usually reveals mild pleocytosis (20 to 100 cells/mm^3), with most cells being lymphocytes. The protein content is normal or slightly elevated, and the sugar concentration is normal.

Kenny and associates[273] studied seven survivors of rubella encephalitis 1 year after their illnesses and could find no significant loss of intellectual function. Five of the seven had abnormalities on electroencephalography, and two patients had minor neurologic abnormalities. Gibbs and colleagues[190] found abnormal electroencephalographic tracings in 6 of 45 children with uncomplicated rubella.

Other neurologic complications associated with rubella include progressive panencephalitis, carotid artery thrombosis, myelitis, optic neuritis, Guillain-Barré syndrome, and peripheral neuritis.†

Of particular interest is the common occurrence of numbness, tingling, and other symptoms consistent with neuritis during rubella infection. Cuetter and John[122] studied 20 patients with complaints of neuritis accompanying rubella and could find no objective sensory deficits or nerve conduction abnormalities.

Wolinsky and colleagues,[569] Lebon and Lyon,[297] and others have described a slowly progressive and fatal nervous system disorder with rubella that was similar to subacute sclerosing panencephalitis. This illness has occurred as a late manifestation of congenital rubella and also has occurred in children who acquired their initial infection in childhood.[51]

THROMBOCYTOPENIA. Thrombocytopenic purpura occurs in rubella at an incidence of 1 per 3000 cases.[30] Children are afflicted more frequently than are adults, and girls are affected more often than are boys.[21,212,265,310,358,377,486,548] The median interval between the onset of exanthem and the occurrence of purpura is approximately 4 days. On occasion, rash and purpura develop simultaneously; often, however, the hemorrhagic manifestations do not become apparent until 2 weeks after the exanthem develops. The illness usually is self-limited, but its duration varies from a few days to several months; although deaths caused by hemorrhagic complications have occurred, recovery is the general rule.

OTHER COMPLICATIONS. Myocarditis and pericarditis are rare complications of rubella.[181] A 30-year-old woman was noted to have erythema multiforme exudativum and arthritis with apparent clinical rubella.[178] In an outbreak that involved 46 military recruits, testicular pain was a complaint in 25 percent.[448]

During a rubella epidemic in Japan in 1976 in which 79 patients were studied, 71 percent were noted to have mild catarrhal or follicular conjunctivitis.[225] Six patients had epithelial keratitis that persisted for 2 to 7 days. Seventeen patients had preauricular lymph node swelling in association with their eye findings. During the same epidemic, 13 cases of hemolytic anemia (including 2 cases of hemolytic-uremic syndrome) were noted after the patients had infection with rubella virus.[525] During an epidemic of rubella in Japan, Sugaya and colleagues[496] found that 7.5 percent of 241 patients had liver involvement.

CONGENITAL RUBELLA

From Gregg's original observation in 1941 of congenital defects in babies born to mothers who had rubella during early pregnancy until the pandemic of 1964, congenital rubella syndrome was considered to include only some combination of abnormalities involving the eyes, ears, brain, and heart. However, observations in 1964, supported by new virologic and serologic techniques, revealed a far more complex congenital rubella syndrome picture: rubella syndrome was expanded to include many new anatomic findings and to acknowledge the reality of chronic persistent infection.

Congenital rubella is the result of in utero fetal infection, which usually occurs during the first 12 weeks of pregnancy. The fetal infection generally is subacute or chronic and may result in abortion, stillbirth, congenital malformations, active processes at birth (such as thrombocytopenia, encephalitis, or hepatitis), and, rarely, infected infants without defects. Table 186–5 summarizes the clinical findings in congenital rubella, an estimation of their frequency, and their main characteristics.

General: Infant Death and Growth Retardation*

The most common manifestation of congenital rubella, readily apparent at birth, is generalized retardation of growth. Between 50 and 85 percent of all babies weigh less than 2500 g although gestational age is normal. Virtually all babies with intrauterine growth retardation have one or more other stigmata of congenital rubella. After birth, babies with intrauterine growth retardation often demonstrate continued growth retardation. In some instances, the failure to thrive is severe. Others show a normal growth pattern, but the child is proportionally small. The mortality rate of children with congenital rubella is high during the first year of life, with death specifically related to congenital pneumonia, heart defects and myocarditis, hepatitis, thrombocytopenia, encephalitis, immune deficiency, and failure to thrive.

Eye Findings†

Approximately a third of all babies with congenital rubella have cataracts. Cataracts may be bilateral or unilateral and are either central in location with a surrounding clear zone or diffuse. In most instances, cataracts are present at birth, but occasionally they are not observed until later in infancy. Retinopathy

*See references 3, 28, 50, 97, 106, 126, 144, 316, 332, 357, 424, 469, 484, 543, 565.

†See references 3, 19, 62, 82, 106, 179, 231, 235, 245, 432, 515, 566, 569, 570.

*See references 17, 112, 171, 203, 226, 255, 279, 303, 312, 328, 338, 381, 392, 430, 431, 433, 446, 463, 475, 509.

†See references 17, 32, 45, 103, 107, 112, 151, 171, 189, 196, 203, 226-228, 255, 278-281, 326, 360, 371, 428-430, 440, 446, 455, 463, 475, 509, 516, 521, 523.

TABLE 186–5 Frequency and Main Characteristics of Clinical Findings in Congenital Rubella Virus Infection

Clinical Findings	Frequency (%)	Main Characteristics	Selected References
General			
In utero death	10-30	Spontaneous abortion; stillbirth	203, 226, 433
Intrauterine growth retardation	50-85	Generalized effect	96, 170, 171, 226, 228, 255, 279, 303, 312, 328, 398, 429, 430, 446, 475, 509
Extrauterine growth retardation	10	Failure to thrive	228, 303, 328, 338, 381, 509
Neonatal and infant deaths	10	Due to pneumonia, heart disease, hepatitis, thrombocytopenia, failure to thrive, immune deficiency, encephalitis	112, 203, 226, 279, 430, 446, 509
Eye			
Cataracts	35	Present at birth	17, 32, 45, 112, 171, 189, 203, 226, 228, 255, 278, 279, 328, 360, 430, 440, 446, 474, 509, 521, 523
Retinopathy	35	Present at birth; usually does not cause problems with vision	45, 103, 111, 189, 203, 226, 255, 280, 281, 328, 521, 523
Microphthalmos	5	Usually associated with cataract	45, 151, 189, 255, 279, 430, 455, 509
Glaucoma	5	Usually present at birth	45, 111, 226, 228, 255, 279, 430, 455, 463
Cloudy cornea	Rare	Usually present at birth; resolves spontaneously	45, 228
Severe myopia	Rare	Usually present at birth; defect may progress	107
Hypoplasia of the iris	Rare	Present at birth	45, 428
Strabismus	5	Associated with other eye defects	227, 328, 371
Iridocyclitis	Rare	Transient; associated with other eye defects	474
Auditory			
Nerve deafness	80-90	May be bilateral or unilateral; moderate or severe; often not recognized early	17, 47, 130, 151, 171, 203, 211, 226, 228, 288, 328, 344, 387, 392, 446, 463, 521, 523, 528
Central deafness	5	Often associated with other central nervous system defects	224
Middle ear damage	5	Usually associated with nerve deafness	418
Intraoral, Nasal, and Facial			
Cleft palate or lip	Rare		151, 203, 446
Dental abnormalities	Rare		65, 210, 328
Micrognathia	Rare		253, 446
Chronic rhinitis	Rare	Transitory finding	392
High-arched palate	Rare		226
Neurologic			
Motor defects	10	Associated with mental and other neurologic defects	328, 463, 575
Hyperirritability (tremors)	Rare	Transitory finding	423
Microcephaly	Rare		17, 228, 255, 328, 463, 509, 546, 562
Mental retardation	10-20	Associated with other stigmata	107, 203, 224, 226, 326, 463, 495
Full anterior fontanelle	10	Transitory finding related to meningoencephalitis	109, 430
Meningoencephalitis	10-20	Transitory finding but may last for 1 year	131, 228, 278, 279, 392, 430, 474, 509
Spastic diplegia and quadriparesis	Rare	Associated with other stigmata	109, 131
Seizures	Rare	Frequently transitory and related to meningoencephalitis	131, 229, 328, 392
Hypotonia	Rare	Transitory defect	130, 131
Brain calcification	Rare		387, 389, 474, 509
Cerebral arterial stenosis	Rare		224
Anencephaly	Rare		446
Encephalocele	Rare		446
Meningomyelocele	Rare		495
Behavior disorders	10-20	Frequently related to deafness	107, 130
Central language disorders	5		157, 203, 555
Autism	5		107, 157, 224
Aqueductal occlusion or hydrocephalus	Rare		203, 436
Poor balance	Rare		130, 575
Progressive panencephalitis	Very rare	Has onset during adolescence	518, 554

TABLE 186–5 Frequency and Main Characteristics of Clinical Findings in Congenital Rubella Virus Infection—cont'd

Clinical Findings	Frequency (%)	Main Characteristics	Selected References
Cardiovascular			
Patent ductus arteriosus	30	Frequently associated with other defects	17, 32, 151, 171, 226, 228, 233, 266, 278, 353, 463, 474, 509, 523, 551
Pulmonary arterial hypoplasia, supravalvular stenosis, valvular stenosis, and peripheral branch stenosis	25	Frequently associated with other defects	171, 226, 228, 233, 266, 463, 472, 523, 551
Aortic stenosis	2-5		170, 233, 471, 540, 551
Ventricular and atrial septal defects	2-5		32, 463, 523, 551
Tetralogy of Fallot	2-5		151, 228
Myocarditis and myocardial necrosis	10		4, 151, 278, 279, 353, 509, 551
Intimal fibromuscular proliferation of many arteries	5		150
Ventricular aneurysm	Rare		532
Pulmonary			
Interstitial pneumonitis	5-10	May be acute, subacute, or chronic	44, 151, 228, 278, 279, 353, 387, 389, 392, 446, 474, 509, 562
Tracheoesophageal fistula	Rare		446
Respiratory distress	Rare	Secondary to acute pneumonia	328
Gastrointestinal			
Esophageal atresia	Rare		446
Hepatitis	5-10	Associated with other evidence of disseminated disease	17, 151, 153, 228, 351, 352, 489, 509
Obstructive jaundice	5		278, 279, 353, 423, 474
Chronic diarrhea	Rare	Related to failure to thrive and immune deficiency	392, 509
Pancreatitis	Rare	May lead to diabetes in later life	61, 139
Duodenal stenosis	Rare		133
Jejunal or rectal atresia	Rare		151
Genitourinary			
Undescended testicle	Rare	Cause-and-effect relationship with rubella infection in doubt	328, 474
Polycystic kidney, ectopic kidney, renal agenesis, or bilobed kidney	Rare	Cause-and-effect relationship with rubella infection in doubt	150, 151, 331
Hypospadias	Rare	Cause-and-effect relationship with rubella infection in doubt	45, 171, 474
Duplication of ureter	Rare	Cause-and-effect relationship with rubella infection in doubt	32
Renal artery stenosis	Rare		327
Hydroureter and hydronephrosis	Rare	Cause-and-effect relationship with rubella in doubt	229, 387, 463, 474
Inguinal hernia	Rare	Cause-and-effect relationship with rubella in doubt	229, 387, 463, 474
Nephritis and nephrocalcinosis	Rare		474, 509
Testicular agenesis	Rare	Cause-and-effect relationship with rubella in doubt	151
Orthopedic			
Bone radiolucencies	10-20	Radiolucencies in metaphyses of long bones	228, 328, 387, 398, 407, 411, 429, 430, 457, 546, 558, 562
Pathologic fractures	Rare		429
Bone deformities	Rare		88, 203, 309
Clubfoot	Rare		446
Myositis	Rare	Transitory defect	481
Skin			
Dermal erythropoiesis (blueberry muffin syndrome)	5	Transitory defect; usually associated with severe disease	277
Chronic rash	Rare		65, 222
Dermatoglyphic abnormalities	5		2, 10, 404
Dimples	Rare		220
Endocrine			
Diabetes mellitus	Rare		169, 267, 329
Thyroid disorder	Rare		107
Precocious puberty	Rare		107
Growth hormone deficiency	Rare		402

Continued

TABLE 186–5 Frequency and Main Characteristics of Clinical Findings in Congenital Rubella Virus Infection—cont'd

Clinical Findings	Frequency (%)	Main Characteristics	Selected References
Hematologic			
Thrombocytopenic purpura	5-10	Usually associated with severe disease with high death rate; transitory	17, 30, 32, 36, 111, 171, 228, 255, 279, 287, 328, 387, 392, 398, 407, 408, 429, 430, 446, 463, 542
Hemophagocytic syndrome	Rare		31
Hemolytic anemia	Rare	Transitory	350, 387, 408
Hypoplastic anemia	Rare	Transitory	109, 287
Extramedullary hematopoiesis	5-10	Usually associated with severe disease	509
Immunologic			
Thymic hypoplasia	Rare		224
Dysgammaglobulinemia	Rare		101, 221, 397, 417, 475
Asplenia	Rare		254
Reticuloendothelial			
Generalized lymphadenopathy	10		109, 171, 222, 509
Hepatosplenomegaly	10-20	Usually associated with severe disease; transitory	32, 279, 398, 430, 446, 463
Genetic			
Chromosomal abnormalities	Rare	Cause-and-effect relationship with rubella not established	13, 367

consisting of pigmentary defects occurs commonly in congenital rubella and is useful diagnostically, but it rarely adversely affects visual acuity. Microphthalmos occurs relatively frequently and usually is unilateral. Cataracts frequently are associated with microphthalmos.

Congenital glaucoma occurs in approximately 5 percent of congenitally infected infants. This defect usually is present at birth, but it often is overlooked. Diagnosis must be established early if sight is to be preserved.

Auditory Defects*

Sensorineural deafness is the most common manifestation of congenital rubella; almost all patients have some degree of hearing impairment. The hearing loss usually is bilateral but may be unilateral. Frequently, the only manifestation of congenital infection is deafness. An important note is that deafness is overlooked frequently in infancy, and children incorrectly are considered to be mentally retarded. All children born to mothers who had rubella during the first half of pregnancy should undergo evaluation of their hearing several times during the first 5 years of life, regardless of whether they have other manifestations of congenital infection.

Neurologic Findings†

Between 10 and 20 percent of all infants with congenital rubella have active meningoencephalitis at birth. Manifestations of this infection include one or more of the following: a full anterior fontanelle, irritability, hypotonia, seizures, lethargy, and head retraction and arching of the back. Examination of the cerebrospinal fluid reveals elevated protein and mild pleocytosis. Later neurologic disease, such as mental and motor retardation, can be related to the severity and persistence of the initial meningoencephalitis. Active central nervous system infection has been demonstrated for a year or more.

Behavior disorders occur commonly in children with deafness and often cannot be associated with apparent meningoencephalitis. Congenital rubella children with generalized retardation of growth and a proportionally small head often have normal intelligence. In contrast, the prognosis for mental development in a child with true microcephaly is poor.

Chronic progressive panencephalitis has developed in a small number of adolescents with congenital rubella, similar to measles-related subacute sclerosing panencephalitis.

Cardiovascular Findings*

In severe congenital rubella with multisystem involvement, myocarditis occurs and often is a cause of death. Of the structural defects of the heart, patent ductus arteriosus is the most common. It may be the only lesion noted, but two thirds of patients have other lesions as well. Pulmonary artery stenosis is the next most common defect. It may involve the main pulmonary artery or its branches. Pulmonary valvular stenosis is the third most frequent defect. Pulmonic valvular or arterial stenosis and patent ductus arteriosus commonly occur together.

Other Manifestations†

The other manifestations of congenital rubella can be separated into three categories: manifestations related to active persistent infection, structural defects, and delayed manifestations of congenital rubella.

*See references 17, 47, 130, 151, 171, 203, 211, 224, 226, 228, 288, 328, 344, 387, 392, 418, 446, 463, 521, 523, 528.
†See references 17, 19, 107, 109, 130, 131, 157, 203, 224, 226, 228, 255, 278, 279, 328, 387, 389, 391, 423, 424, 428, 430, 433, 436, 446, 463, 474, 495, 509, 518, 546, 554, 555, 562, 565.

*See references 4, 17, 150, 151, 170, 171, 226, 228, 233, 256, 266, 278, 279, 353, 463, 471, 472, 474, 509, 523, 540, 551.
†See references 4, 10, 13, 17, 30, 32, 36, 44, 45, 61, 65, 88, 100, 101, 107, 109, 112, 139, 150, 151, 153, 169, 171, 193, 203, 220-222, 224, 228, 229, 255, 267, 277-279, 287, 309, 329, 350-353, 367, 387, 389, 392, 397, 398, 402, 408, 411, 417, 423, 429-431, 446, 457, 463, 468, 474, 475, 481, 489, 494, 509, 542, 546, 558, 562.

MANIFESTATIONS RELATED TO ACTIVE PERSISTENT INFECTION. This category encompasses a broad constellation of clinical events that largely were unknown before the pandemic of 1964. Collectively, they frequently are called the expanded congenital rubella syndrome and include interstitial pneumonitis, hepatitis, nephritis, bone radiolucencies, myositis, dermal erythropoiesis, chronic rash, thrombocytopenic purpura, hemolytic and hypoplastic anemia, immunologic deficiency, generalized lymphadenopathy, hepatosplenomegaly, meningoencephalitis, and myocarditis. Most infants with the expanded rubella syndrome clinically have low birth weight, exanthem caused by thrombocytopenia or dermal erythropoiesis or both, hepatosplenomegaly, and jaundice. Radiographs usually reveal long bone radiolucencies. Respiratory distress caused by both diffuse pulmonary disease and myocarditis occurs commonly, and meningoencephalitis usually is evident. The duration of chronic infection in these babies varies. Approximately 20 percent of survivors still are shedding virus at 1 year of age. Between 10 and 20 percent of babies with hepatosplenomegaly and thrombocytopenia die during the first year of life.

STRUCTURAL DEFECTS. Other than deafness, eye defects, and cardiac anomalies, which have been discussed previously, the association of other malformations with congenital rubella infection is less well established. Malformations such as tracheoesophageal fistula, jejunal atresia, inguinal hernia, and others recorded in Table 186–5 occur frequently without evidence of in utero infection. Because they are noted only sporadically in babies born after maternal rubella, they possibly are chance associations rather than cause-and-effect relationships.

DELAYED MANIFESTATIONS. The following delayed manifestations of congenital rubella that were not present in early life have been noted: endocrinopathies, deafness, ocular damage, vascular effects, and progressive rubella panencephalitis.[468] Of particular importance is the association between endocrine abnormalities and autoimmunity.[100] In one study of 201 deaf adolescents with congenital rubella, 23.3 percent had positive thyroid microsomal or thyroglobulin antibodies, and of these patients, 19.6 percent had thyroid gland dysfunction. Patients with congenital rubella have an increased incidence of insulin-dependent diabetes mellitus.[193] In a 40-year observation study of 280 Japanese patients with congenital rubella syndrome, only three of the patients developed insulin-dependent diabetes.[503]

DIAGNOSIS

DIFFERENTIAL DIAGNOSIS

Postnatally Acquired Disease (see also Chapter 64)

Because no pathognomonic finding in rubella exists, the clinical diagnosis in an individual case often is difficult to establish. However, as with other exanthematous diseases, the key to establishing a diagnosis is careful elicitation of historical data. Rubella is an epidemic disease with a high clinical rate of expression of exanthem. Therefore, it is unusual, when proper investigation is performed, not to find the contact case or other cases in the community. Season also is an important consideration. Rubella generally occurs in the winter and spring, whereas enteroviral exanthems, which are the greatest masqueraders in young children, occur mainly in the summer and fall.

The incubation period also is important in separating rubella from exanthems caused by common enteroviruses or respiratory viruses. In rubella, the incubation period is long (18 ± 3 days), whereas the period in the other illnesses usually is much shorter (3 to 7 days). Age also is important. Today, rubella mainly is an illness of adolescents and young adults, and enteroviral exanthems are uncommon findings in patients at these ages.

The nature of fever also is useful in establishing the diagnosis of rubella. Temperature higher than 38.5° C (101.5° F) is an unusual occurrence in rubella but common with enteroviral exanthems, measles, and *M. pneumoniae* infection. In general, a past history of rubella infection is not particularly reliable. However, if a past illness can be documented by year, season, and symptoms, accurate information may be obtained. Useful characteristics of the rubella exanthem are its mild, erythematous, maculopapular, and discrete nature; marked pruritus in adolescents and adults; and an acneiform appearance on the face in adolescents.

Although suboccipital and posterior auricular lymphadenopathy often is thought by some investigators to be pathognomonic, its presence in nonrubella exanthems often leads to undue concern. In general, in a young child, suboccipital and posterior auricular lymphadenopathy occurs as commonly with enteroviral illnesses as with rubella. In young adults, however, this lymphadenopathy is much more useful because the enteroviral differential consideration is less of a problem. Similar lymphadenopathy does occur with acquired toxoplasmosis, infectious mononucleosis, and *M. pneumoniae* infection. A major problem in the differential diagnosis in adults is allergy. However, fever (even low grade), lymphadenopathy, headache, and eye pain, which are common events in rubella, should occur rarely in contact or other simple allergies.

Congenital Rubella

Establishing the diagnosis of congenital rubella in infants with known maternal exposure generally is not difficult. However, examining an apparently normal child at periodic intervals during the first few years of life is important so that deafness and subtle neurologic defects are not missed. The diagnosis of congenital rubella after an uneventful pregnancy is more difficult to make. All babies with evidence of intrauterine retardation of growth or stigmata suggestive of congenital infection should undergo virologic and serologic study for rubella as well as for other infectious agents. Determination of the amount of serum IgM also can be useful in the study of babies with intrauterine retardation of growth or babies born to mothers in whom rubella or other infection was suspected to have occurred during pregnancy.[491] Values greater than 21 mg/dL during the first week of life strongly indicate congenital infection; normal values do not rule out congenital infection, however.

SPECIFIC DIAGNOSIS

Postnatally Acquired Disease

Rubella viral infection can be diagnosed specifically by isolation of virus from nasal or throat specimens in AGMK or other sensitive tissue culture systems or by polymerase chain reaction (PCR)[114,115]; by the observation of a significant change in value of HI, ELISA, immunofluorescence, CF, or neutralizing antibody in two sequential serum samples; or by the demonstration of specific rubella IgM antibody in a single serum sample. Most often today, the diagnosis of rubella is attempted by the use of a single serum test for identifying rubella IgM antibody.* A number of commercial rubella IgM immunoassays are now available, and in general their sensitivity and specificity ranges vary from 75 to 95 percent and from 85 to 100 percent, respectively.[135,513] Determination of rubella-specific IgM antibody in saliva 7 to 42 days

*See references 12, 22, 33, 34, 67, 83, 87, 117, 135, 148, 160, 167, 194, 251, 315, 334, 359, 382, 453, 476, 513, 534.

after the onset of illness also has been shown to be both sensitive and specific.[406] Although these tests are practical, one should realize that the specificity and sensitivity of all routinely used tests are not 100 percent accurate. False-positive results occur all too frequently and commonly lead to unnecessary interventions. When the diagnosis is critical, such as with suspected rubella in pregnancy, a wise approach is to study IgG antibody in paired sera collected 1 to 2 weeks apart in addition to determining IgM antibody.

Unfortunately, a rise in IgG antibody titer as well as an IgM positive test result can occur in re-infection as well as in primary infection. Because re-infection in a pregnant woman is very unlikely to lead to damage to the in utero fetus, distinguishing primary infection from re-infection is important and can be done by measuring the avidity of rubella IgG antibody.[219,246,405] In primary infection, the avidity is low, whereas it is high in re-infections.

Congenital Rubella

The best method for establishing a definitive diagnosis of congenital rubella is viral isolation or PCR. Specimens for viral culture or PCR should be obtained from the nose, throat, urine, buffy coat of blood, and cerebrospinal fluid. Because of transplacental passage of maternal IgG, establishment of the diagnosis of congenital rubella in the neonatal period by serologic methods is fraught with difficulty. Usually, specific rubella IgM antibody can be demonstrated with presently available techniques. In questionable cases, follow-up studies comparing infant and maternal antibody values often establish the diagnosis. If the infant's value is the result solely of transplacentally acquired antibody, it should drop fourfold to eightfold by the time the infant reaches 3 months of age and continue to fall to nondetectable values by 6 to 8 months of age. However, the antibody value in some congenital infections also may fall, so disappearance of antibody in serum does not rule out in utero infection completely. Also in questionable cases, the study of IgG rubella antibody avidity may be useful.[239,405]

The retrospective diagnosis of congenital rubella in late infancy and the second year of life has been difficult to make. However, researchers have shown that affected children have low avidity of specific IgG antibody, and, therefore, a retrospective diagnosis of congenital rubella can be made by specific avidity assays.[239] Also useful for the serologic diagnosis of congenital rubella during the prenatal and newborn periods are rubella IgG peptide-based enzyme immunoassay and rubella immunoblot assay.[326] Newborns who were infected during the first 12 weeks of gestation have reduced levels of antibodies directed at both the linear E1 epitope (SP15) and the topographic E2 epitope.

Fetal rubella can be diagnosed in amniotic fluid samples by reverse transcription–PCR.[416,504]

Qualitative Demonstration of Rubella Antibody

The original screening method for rubella antibody was HI. Today, HI has been replaced by more rapid and easier tests involving enzyme immunoassay, erythrocyte agglutination, and latex agglutination. In general, all are both highly sensitive and specific.[86,159]

TREATMENT

POSTNATALLY ACQUIRED DISEASE

Uncomplicated Rubella

No specific therapy is necessary or indicated for uncomplicated rubella. Starch baths may be useful in adults with troublesome pruritus. Of importance is that affected patients understand that they are contagious and that transmission of infection to a pregnant woman could have serious consequences.

Complications of Rubella

On occasion, arthritis can be severe in adults. When weight-bearing joints are affected, rest is encouraged. Symptoms readily respond to aspirin therapy; corticosteroids are not indicated. In rubella encephalitis, care is supportive, with adequate maintenance of fluids and electrolytes.

Thrombocytopenia usually is self-limited; however, severe bleeding has occurred on occasion. Splenectomy is not indicated. Corticosteroid therapy often is used, but with little evidence of specific benefit in rubella-infected patients. In patients who do not recover rapidly and in those with severe bleeding, treatment with intravenous immunoglobulin should be considered.

MANAGEMENT OF EXPOSED PREGNANT WOMEN

Ideally, all pregnant women should have received rubella vaccine previously or been shown to have rubella antibody by an appropriate serologic test. If a pregnant woman is exposed to a person with rubella and the history of previous immunization or antibody presence is unknown, an immediate blood specimen should be obtained and a rubella antibody test performed. If antibody to rubella is demonstrated, no action is necessary. Susceptible rubella-exposed women should undergo careful clinical observation for fever, lymphadenopathy, or exanthem for a 4-week period. If illness occurs, a nasal specimen should be cultured for rubella virus and serum should be examined for rubella IgM antibody. A second serum specimen should be submitted for rubella antibody examination. If illness occurs, a specimen should be collected 1 to 2 weeks later; if the woman has no illness, the second serum specimen should be collected 6 to 8 weeks after the exposure. If rubella antibody seroconversion is noted or specific IgM antibody is demonstrated, the risk of fetal infection and malformation is considerable. Because false-positive rubella IgM antibody test results are not rare occurrences, all tests that yield positive results should be repeated for confirmation (by another assay and in another laboratory if possible). Because IgM antibody can occur in re-infections and fetal risk is then minimal, determining the avidity of IgG antibody also may be useful.[219,246,405] The patient should be so advised, and therapeutic abortion should be discussed.

In situations of known exposure of a susceptible pregnant woman in which therapeutic abortion is not possible and exposure can be documented to have taken place within 72 hours, my opinion is that 20 mL of immunoglobulin should be administered immediately. The use of immunoglobulin is controversial, but in certain controlled situations, it has been effective in preventing disease.[53,340,442,529]

MANAGEMENT OF PREGNANT WOMEN WITH AN EXANTHEM THOUGHT TO BE RUBELLA

In this circumstance, if previous rubella serologic study results are available, they are extremely useful. If previous serum antibody has been noted, the mother should be reassured that the present illness is not likely to be rubella. However, because false-positive rubella screening results do occur, a wise approach is to carry out rubella serologic study and, when possible, also viral culture. An acute-phase serum should be examined for rubella-specific IgM antibody. A second serum specimen should be collected 1 to 2 weeks after the disappearance of the rash. If rubella antibody has risen significantly or IgM antibody is demonstrated,

it is highly likely that congenital infection has occurred and that anomalies may result. Again, a wise approach is to confirm IgM positive test results and, if available, do IgG rubella antibody avidity study. In this circumstance, the woman should be counseled about therapeutic abortion.

If a previous serum rubella antibody value is not available, a serum sample should be collected immediately and another collected 2 to 3 weeks later. These sera should be examined as paired specimens for rubella antibody and analyzed for specific rubella IgM antibody. If a rubella antibody rise is demonstrated or the presence of rubella IgM is noted, one must assume that rubella viral infection has occurred, and the patient should be advised about the risk of congenital infection and the possibility of therapeutic abortion.

MANAGEMENT OF CHILDREN WITH CONGENITAL RUBELLA

Isolation Procedures

Most babies with congenital rubella remain actively infected at the time of birth, are contagious, and therefore should be placed in isolation. Room isolation and urine precautions are the major necessities. The isolated baby should be cared for only by persons known to be seropositive for rubella. Because rubella viral shedding has been known to occur for a year or more in some babies, isolation of infants with congenital rubella should be continued for this duration unless repeated viral cultures have proved negative.

After the child is discharged from the hospital, no special precautions are necessary in the household setting. However, the parents should be advised of the potential risk to pregnant visitors.

Neonatal Period

As noted, the clinical manifestations of congenital rubella are varied, and in many infants, no symptoms are manifested during the first few months of life. In these apparently asymptomatic infants, no particular management problems are encountered. In other neonates, symptoms of continued viral infection are readily apparent and frequently are severe. In these infants, the following findings are important: pneumonia, thrombocytopenia, eye findings, heart defects, hyperbilirubinemia, and hepatosplenomegaly.

Although purpura and petechiae secondary to thrombocytopenia may be impressively severe in these infants, true hemorrhagic difficulties have not been a major problem. Corticosteroid therapy does not seem to be indicated, but considering treatment with intravenous immunoglobulin might be worthwhile. Careful evaluation of the eyes is important. Of immediate concern is the search for corneal clouding because its presence probably indicates infantile glaucoma. Cataracts and retinopathy also should be sought carefully. Infants with glaucoma should be referred immediately for ophthalmologic evaluation and therapy. Children with cataracts or retinopathy also should be referred, but therapy for cataracts is best delayed until the child reaches a later age.

Respiratory distress secondary to extensive viral involvement should be managed similar to other neonatal respiratory disease: assisted ventilation and careful attention to arterial blood gas values and pH. Although jaundice secondary to congenital rubella infection rarely is severe, standard criteria for the treatment of hyperbilirubinemia should be followed. Hepatosplenomegaly may be marked in some instances but is of no therapeutic concern.

Cardiac evaluation should be the same as in affected infants without rubella. Specifically, congestive cardiac failure should be treated vigorously; in malignant conditions (patent ductus arteriosus, coarctation of the aorta), lifesaving surgery should be contemplated.

Long-term Problems

DEAFNESS. Hearing disability is the most frequent abnormality after infection with congenital rubella; more than 80 percent of infected infants have some degree of hearing disability. In many instances, deafness is the only clinical manifestation of congenital rubella, and because the establishment of this diagnosis in early infancy is difficult, the diagnosis frequently is delayed. However, early diagnosis of deafness and institution of proper educational programs are the most productive measures in the long-term management of children with congenital rubella. All too frequently, poor medical advice has been responsible for the delay in making an appropriate diagnosis and providing therapy. Any time that a mother suspects that her child is deaf, specific audiometric testing should be performed. Many general practitioners, pediatricians, and even otolaryngologists think that hearing cannot be tested in infants. This concept must be discouraged vigorously; at proper centers, a severely deaf child can be recognized in virtually all instances.

If deafness is diagnosed, the child should be referred immediately to a training program. Information about training programs can be obtained from the Alexander Graham Bell Association for the Deaf, 3417 Volta Place, NW, Washington, DC 20007, *http://www.agbell.org*; and the John Tracy Clinic, 806 West Adams Boulevard, Los Angeles, CA 90007, *http://www.johntracyclinic.org*. In virtually all instances, severely deaf children should be enrolled in an education program before or during the second year of life, and the child should be fitted with a proper auditory amplification device. Although deafness in congenital rubella is sensorineural, a surprising finding is that conduction defects also are noted in many older children. For these children, other aspects of otolaryngologic care may be indicated.

EYE PROBLEMS. All children with eye problems (cloudy cornea, glaucoma, cataracts, retinitis, strabismus) should be referred at an early age for ophthalmologic evaluation. Glaucoma needs immediate attention. Decisions about cataract surgery should be left to the discretion of the ophthalmologist but, in general, are well deferred until after the end of the first year. Retinopathy, although frequently impressive on ophthalmoscopic examination, rarely causes much visual defect. Strabismus is managed as it is in children without rubella. Advice on eye problems in congenital rubella can be obtained from the American Foundation for the Blind, 11 Penn Plaza, Suite 300, New York, NY 10001, *http://www.afb.org*.

HEART PROBLEMS. Congenital heart disease secondary to in utero rubella infection should be managed as heart disease is in children without rubella. Of importance is that the children be referred to cardiac centers where sophisticated diagnostic techniques and cardiac surgery facilities are available for correctable lesions.

MUSCULOSKELETAL PROBLEMS. Isolated musculoskeletal defects are relatively uncommon findings in congenital rubella. However, when the symptoms indicate, referral to a cerebral palsy clinic is useful, both for specific therapeutic modalities and for the camaraderie of group therapy for the children as well as the parents.

CENTRAL NERVOUS SYSTEM PROBLEMS. Careful analysis of the data available suggests that many infants who have been labeled retarded actually are children with auditory or visual defects who have not had proper diagnosis and training for their handicaps. No child with congenital rubella should be labeled

mentally subnormal until extensive audiologic and ophthalmologic investigations and perhaps specific therapy have been performed. Probably only approximately 10 percent of all congenitally infected rubella children have a central nervous system defect that precludes normal development.

IMMUNOLOGIC DEFECTS. A small number of children with congenital rubella have been noted to have specifically low levels of serum IgG. These infants have systemic continued viral infection and in general do poorly. Although outcome studies are not available, administration of immune serum globulin (intramuscular or intravenous immunoglobulin) to these infants periodically seems prudent.

MULTIPLE HANDICAPS. All too frequently, children infected in utero with rubella virus suffer from one or more of the handicaps mentioned. Care of these infants and children requires many different resources and modalities of therapy. Frequently, the physician is the one who must coordinate both the diagnostic and the long-term educational efforts that are necessary for optimal progress of an affected child. In addition to the Alexander Graham Bell Association for the Deaf, the John Tracy Clinic, and the American Foundation for the Blind, already mentioned, the following agencies may be helpful to physicians or the parents of congenital rubella children: United Cerebral Palsy Research and Educational Foundation, 1600 L Street, NW, Suite 700, Washington, DC 20036-5602, *http://www.ucpa.org*; Easter Seal Research Foundation, 230 West Monroe Street, Suite 1800, Chicago, IL 60606, *http://www.easter-seals.org*; The Arc of the United States, 1010 Wayne Avenue, Suite 650, Silver Spring, MD 20910, *http://www.thearc.org*; and Maternal and Child Health Division, Department of Health and Human Services, Parklawn Building, 5600 Fishers Lane, Rockville, MD 20852, *http://www.hrsa.gov*.

PREVENTION

ACTIVE IMMUNIZATION: LIVE ATTENUATED RUBELLA VIRUS VACCINE

At present, one attenuated rubella virus vaccine is available for use in the United States (RA 27/3 strain grown in WI38 human embryonic lung tissue culture). Vaccination can be expected to produce antibodies in more than 95 percent of those immunized.[20,307,394,444,480,544,549,553] Antibody titers after RA 27/3 vaccination are slightly lower than those after natural infection, but they have been demonstrated to persist for an 11- to 15-year period with a pattern similar to that occurring after natural infection, even in the absence of re-exposure to rubella virus.[41,258,394]

Because of universal immunization, indigenous rubella has been eliminated.[69,412] Finland's vigorous two-dose immunization program also resulted in the elimination of indigenous rubella.[385,386]

Recommendations for Use

For complete information about rubella immunization, the reader is referred to the most recent recommendations of the Immunization Practices Advisory Committee of the U.S. Public Health Service,[70,75] the recommendations of the Committee on Infectious Diseases of the American Academy of Pediatrics,[11] and the vaccine manufacturer's product information.

Rubella vaccine is recommended for all children 12 months of age or older, adolescents, and adults, particularly women, unless otherwise contraindicated. Vaccination of children protects them from rubella and thus prevents them from subsequently spreading it. Vaccination of susceptible postpubertal women confers individual protection from rubella-induced fetal injury. Vaccination of adolescents or adults in population groups such as those in colleges, places of employment, or military bases protects them from rubella and reduces the chance of epidemics occurring in partially immune groups.

Rubella vaccine should not be administered to infants younger than 1 year of age because persisting maternal antibodies may interfere with seroconversion. When rubella vaccine is part of a combination vaccine that includes measles antigen, it should be administered to children approximately 12 to 15 months of age. A second dose of MMR vaccine is recommended at school entry. Children who have not received rubella vaccine at the optimal age should be vaccinated promptly. Because a history of rubella is not a reliable indicator of immunity, all children for whom vaccine is not contraindicated should be vaccinated.

Vaccination of all unimmunized prepubertal children and adolescents as well as of adult women in the child-bearing age group must be emphasized. Because of the theoretical risk to the fetus, women of child-bearing age should receive vaccine only if they are not pregnant and understand that they should not become pregnant for 3 months after receiving the vaccination.

Educational and training institutions such as colleges, universities, and military bases should seek proof of rubella immunity (a positive serologic test result or documentation of previous rubella vaccination) from all students and employees in the child-bearing age. Nonpregnant women who lack proof of immunity should be vaccinated unless contraindications exist.

For the protection of susceptible female patients and female employees, persons working in hospitals and clinics who might contract rubella from infected patients or who, if infected, might transmit rubella to pregnant patients either should have serologically demonstrated immunity to rubella or should receive the vaccine.

Routine premarital serologic testing for rubella immunity would enhance efforts to identify susceptible women before pregnancy. Prenatal or antepartum screening for rubella susceptibility should be undertaken and vaccine administered in the immediate postpartum period—before hospital discharge. Previous administration of anti-Rh_0(D) immunoglobulin (human) or blood products is not a contraindication to vaccination; however, 6- to 8-week postvaccination serologic testing should be performed for confirmation of seroconversion in those few who have received the globulin or blood products. Obtaining laboratory evidence of seroconversion in other vaccinees is not necessary.

No evidence has shown that live rubella virus vaccine given after exposure prevents illness or that vaccination of a person incubating rubella is harmful. However, because a single exposure may not result in infection and postexposure vaccination would protect a person in the event of future exposure, vaccination is recommended unless it is otherwise contraindicated.

Adverse Reactions

Vaccination in young children rarely is associated with any symptoms. On occasion, rash, lymphadenopathy, mild fever, and upper respiratory symptoms have been observed.

More severe reactions have been noted rarely in children but have been an occasional problem in adults. Most of these complications were reported in association with the previously available vaccines, but complications with the RA 27/3 vaccine also have been noted.[20,143] Particularly troublesome are arthralgia and arthritis.*

*See references 16, 27, 95, 113, 201, 305, 356, 368, 399, 483, 502, 505, 506, 510-512, 545.

These complaints occur most commonly in adults. Arthralgia develops in approximately 25 percent of susceptible postpubertal females after they have received the RA 27/3 vaccination, and approximately 10 percent have been reported to have arthritis-like signs and symptoms.[37,208,399] Infrequently, chronic or recurrent arthralgia, sometimes with arthritis or neurologic symptoms, including paresthesias, carpal tunnel syndrome, and blurred vision, reportedly have developed in susceptible vaccinees, primarily adult women.[75,259] One group of investigators has reported the frequency of chronic joint symptoms and signs in adult women to be as high as 5 to 11 percent[510-512]; however, other data from the United States and other countries suggest that such complications caused by RA 27/3 vaccine are rare or perhaps nonexistent.[113,166,174,232,366,477,482]

Other complications, mainly with vaccines other than RA 27/3, include polyneuropathies (catcher's crouch, carpal tunnel syndrome, neuritis, and myeloradiculoneuritis),[90,95,192,216,275,365,437] marked lymphadenopathy,[88] and vasculitis and myositis.[223]

Geiger and associates[188] noted a persistent rubella virus infection in a 16-year-old boy with acute lymphoblastic leukemia in remission who was vaccinated. The virus was identified in the patient's peripheral blood mononuclear cells 8 months after immunization. In contrast, persistent infection could not be demonstrated in 10 children with symptomatic human immunodeficiency virus type 1 infection.[173]

Contraindications

Administration of live rubella virus vaccine is contraindicated in women who are pregnant; in individuals with altered immune states, such as immunodeficiency, leukemia, lymphoma, and generalized malignant disease; or in patients treated with steroids, alkylating drugs, antimetabolites, and radiation. In addition, rubella vaccination should not be performed during febrile illnesses or when viral interference from another agent might preclude a "take" from the rubella immunization. Rubella immunization also, in most instances, should be deferred after the administration of blood products, including immune serum globulin (for duration, see table in reference 11, p. 445).

Inadvertent Rubella Immunization in Pregnancy

From January 1971 to April 1989, the Centers for Disease Control and Prevention observed to term 321 known rubella-susceptible pregnant women who had been vaccinated with rubella vaccine within 3 months before or 3 months after conception.[75] Ninety-four women received HPV-77 or Cendehill vaccine, 1 received a vaccine of unknown strain, and 226 received RA 27/3 vaccine. None of the 324 infants born to these women had malformations compatible with congenital rubella, but five of the infants had serologic evidence of subclinical infection.

In conjunction with a nationwide measles-rubella vaccination campaign in 2001, the fetal risk associated with rubella vaccination during pregnancy was studied in Costa Rica.[18] The study involved 1191 mother and child pairs, and no adverse pregnancy outcomes or cases of congenital rubella syndrome occurred.

PASSIVE IMMUNIZATION

The use of immunoglobulin for the prevention of rubella has been controversial for many years.* However, my opinion is that its use is indicated in certain circumstances. The specific indication for immune serum globulin is for the prevention of rubella in a woman thought to be susceptible to rubella who is in the first 20 weeks of pregnancy. If the exposure can be documented clearly as one to a specific, single person with rubella and immunoglobulin is given within 72 hours of that exposure, both maternal disease and congenital infection likely can be prevented. On the other hand, if the exposure was more general in nature (e.g., a schoolteacher exposed to a child or children in the school setting), the woman probably was exposed for a considerable time before she realized the exposure. Therefore, because immunoglobulin probably will be administered too late (well into the incubation period of her disease and after viremia), congenital infection is not likely to be prevented. The dose of immunoglobulin for the prevention of rubella during pregnancy is 20 mL intramuscularly.

*See references 25, 52, 137, 205, 313, 320, 324, 340, 442, 529.

QUARANTINE AND DISEASE CONTAINMENT

Patients with rubella should not have contact with susceptible persons until the rash has disappeared. Containment of rubella is a vital part of the prevention policy in the United States today. Rubella is a reportable disease, and compliance is the obligation of all physicians and other health care professionals. Reporting cases of rubella enables public health workers to organize vaccination programs so that small outbreaks of disease can be prevented from developing into major epidemics.

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CHAPTER 187

ALPHAVIRUSES

CHAPTER 187a

EASTERN EQUINE ENCEPHALITIS

Theodore F. Tsai

Eastern equine encephalitis (EEE) is an arthropod-borne viral infection of humans, horses, and other vertebrates that occurs in North and South America. In North America, infections recur in highly focal, primarily coastal locations in association with the habitat of *Culiseta melanura*, the enzootic vector. Birds are the amplifying hosts, and humans and horses are infected incidentally.

ETIOLOGIC AGENT

EEE virus is an antigenically distinct member of the *Alphavirus* genus in the *Togaviridae* family. On the basis of nucleotide differences, it has been inferred that EEE and Venezuelan equine encephalitis diverged 1000 to 2000 years ago, with a subsequent division of EEE virus into North American and South American varieties.[7,43,45] South American strains can be differentiated by monoclonal antibodies and by short-incubation hemagglutination-inhibition tests.

North American strains collected over a broad geographic and temporal span demonstrate remarkable genetic stability in one or two major lineages, with minor local divergences occurring within isolated geographic loci. South American strains are genetically heterogeneous. They have been isolated in northward-migrating birds captured in the United States, but they have never been shown to become established.

ECOLOGY

Equine cases have been reported as far north as Quebec, Ontario, and Alberta provinces; in South America, EEE viral activity has been reported from the Caribbean, Mexico, Guatemala, Honduras, Panama, Colombia, Venezuela, Peru, Guyana, and Brazil and as far south as Argentina.[4,8,23,49] The viral transmission cycles in the Caribbean and South America are not well characterized but apparently involve small mammals, birds, and *Culex (Melanoconion)* mosquitoes.[23]

In North America, the distribution of virus activity closely follows the distribution of freshwater swamps on the Eastern Seaboard, Gulf Coast, and other inland areas and corresponds to the distribution of the principal enzootic vector, *C. melanura*.[10,19,23] *C. melanura* feeds nearly exclusively on birds, so various epizootic (bridging) vectors are responsible for infecting humans and horses.[20,27,31,33,47] Numerous species may be involved, including *Coquillettidia perturbans*, *Aedes sollicitans*, *Aedes vexans*, and *Aedes canadensis*.

Infection and viremia are subclinical in most native birds, whereas whooping cranes and exotic birds such as emus, house sparrows, ring-necked pheasants, Pekin ducks, and chukar partridges may become ill and die of infection.[3,12,13,48,52] Outbreaks resulting in thousands of deaths have occurred in commercial pheasant flocks, in some instances perpetuated by cannibalistic pecking or preening of persistently infected quills.[3] Illness and deaths in pigs, goats, calves, rodents, and other mammals also have been reported.[24,39]

C. melanura is found in and near freshwater swamps, where larval stages breed in acidic waters associated with mucky peat soils. These foci (from north to south) are found in upland red maple, coastal white cedar, and southern loblolly bay biotypes.[10,23]

The viral overwintering mechanism has not been elucidated, but the remarkable permanence of endemic foci is a strong argument for overwintering in local reservoirs.[10,30,31,33,39]

EPIDEMIOLOGY

EEE is a rare sporadic infection; a median of three cases occurs annually in the United States[35] (Fig. 187–1). The states with the highest rates of infection show an average annual incidence of less than 1 per 10 million (Fig. 187–2). However, these estimates obscure the remarkably consistent and focal distribution of cases on the Atlantic and Gulf Coasts, from Ontario to Texas, and in isolated pockets of activity inland.[4,8] For example, until 1995, all human cases in Massachusetts had occurred east of Highway 495 in Essex, Norfolk, Plymouth, Bristol, and Middlesex counties; the six southernmost counties account for most of the epizootic activity in New Jersey; and foci of EEE viral activity recur in upstate New York counties near Syracuse, southwestern Michigan, northeastern Indiana, and northeastern Florida.[11,31] Outbreaks of human cases caused by North American viral strains have been reported from Jamaica, Trinidad, and the Dominican Republic.[5,16] Isolated epizootics with sporadic human cases have occurred in South America.[49] The unpredictability of risk is underscored by the occurrence of a case in a United Kingdom tourist who had a brief itinerary in Massachusetts.

Viral transmission, reflected in equine cases, occurs all year in Florida, although the peak incidence is from May to September.[6] Human cases have appeared as early as February and as late as December in Florida, with a peak occurring from June to August.

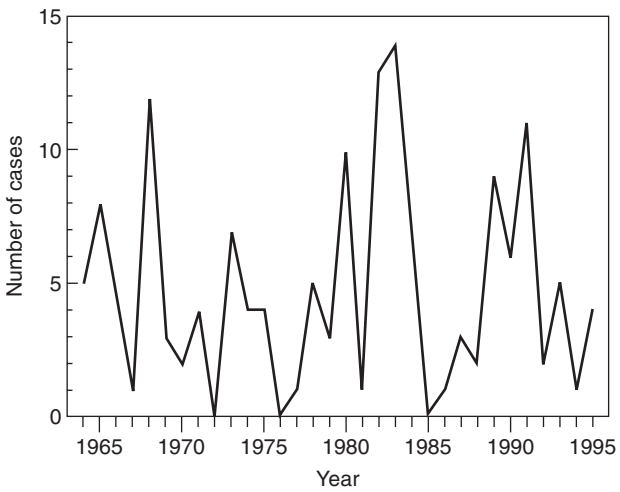

Figure 187–1 Reported cases of eastern equine encephalitis by year, 1964 to 1995.

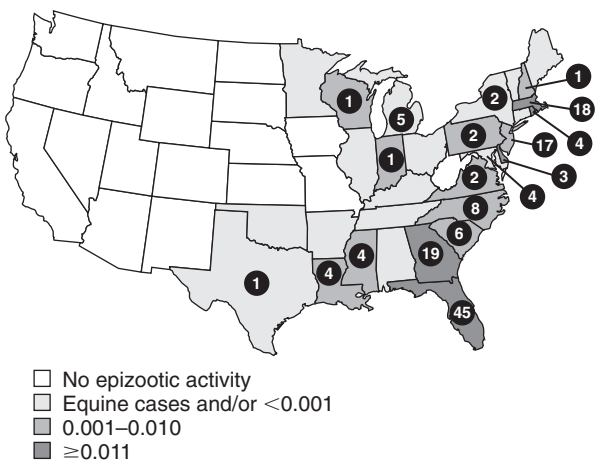

Figure 187–2 Reported cases and average annual incidence (per 100,000) of eastern equine encephalitis by state, 1964 to 1995.

In the Northeast, cases usually appear in late summer, from August to September, and as late as the third week in October.[35]

Cases occur chiefly at the extremes of age. However, serologic studies performed during a New Jersey outbreak disclosed that infection occurs with equal frequency in all age groups, thus indicating that biologic responses to infection rather than factors associated with exposure were responsible for the lower attack rates in young and middle-aged adults.[25] The ratio of inapparent infections to cases was highest in the middle years of life (29:1) and lowest in children younger than 4 years (8:1) and adults older than 55 years (16:1).[25]

Family clusters were observed in this epidemic and in the 1947 Louisiana outbreak.[29] In the New Jersey study, family members of cases had twice the rate of inapparent infections as the general population did, and in the southern Louisiana outbreak, two fatal cases and two seropositive members were observed in the same family.[25,29] Clusters of equine cases on a single premise are reported frequently.

Asymptomatic infections are uncommon occurrences, and long-term residence in an enzootic area leads to only a slight increase in population immunity. For example, even in an unusually active focus in southern New Jersey, only 7 percent of persons who had resided there 45 years or more had neutralizing antibody.[25] In serosurveys of endemic foci in Massachusetts, evidence of past infections was found in 0.5 to 0.7 percent of residents,[44] and a post-epizootic serosurvey of at-risk Connecticut pheasant farmers showed no evidence of infection.[37]

Specific behavioral risk factors have not been described; however, residence or outdoor activity near swampy habitats has been reported anecdotally as a possible contributing factor.

CLINICAL MANIFESTATIONS

EEE is a fulminant encephalitis with rapid progression to coma and death in one third of patients.* In infants and children, an abrupt onset of fever, irritability, and headache is followed closely by lethargy, confusion, seizures, and coma. The often desperately ill infants may have a bulging fontanelle, meningismus, high temperature, and generalized flaccid or spastic paralysis.[1,17,22,51,53] Some patients are evaluated initially for status epilepticus. The prodrome in adults and older children usually is brief, with nonspecific symptoms of fever, headache, and dizziness followed by clouding of the sensorium and rapid deterioration to coma. Remarkably, some patients have a prolonged prodrome lasting longer than a week, with a waxing and waning course of nonspecific symptoms, which may be associated with a better prognosis.[42,44,51] Various neurologic deficits have been described; in some cases, unilateral seizures, hemiparesis, hemiplegia, and aphasia have indicated focal areas of involvement.[15,40,42]

The peripheral leukocyte count usually is elevated with a shift to the left. White blood cell counts of 20,000/mm^3 are typical, ranging as high as 60,000 with 55 to 89 percent neutrophils.[44] Cerebrospinal fluid (CSF) usually shows a polymorphonuclear pleocytosis with a total leukocyte count ranging from 500 to 2000/mm^3. The initial neutrophilic pleocytosis of 60 to 100 percent may persist into the second week before shifting to a predominance of mononuclear cells.[45] CSF protein level is elevated and glucose concentration is reduced in most cases. Erythrocyte counts higher than 500/mm^3 are found in some cases.

Imaging studies disclose only cerebral edema in three quarters of patients. However, focal rim-enhancing lesions and areas of low alteration with mass effect have been observed on computed tomographic scans in the frontal cortex, thalami, midbrain, and

*See references 1, 2, 11, 15, 17, 18, 21, 26, 36, 40, 42, 44, 51, 53.

lentiform nuclei.[15,40,42] Isointense diffusion-weighted images and hyperintensity on apparent diffusion coefficient images early in the course of illness in one case suggested vasogenic edema in the T2-weighted hyperintense areas.[26] Electroencephalographic tracings with focal or background slowing have been associated with a favorable outcome, whereas disorganized background activity, a burst-suppression pattern, and high-voltage delta slowing have been associated with a poor prognosis.[44]

Mild nonencephalitic illnesses usually are not diagnosed. However, in the 1959 outbreak in New Jersey, fever, headache, nausea, vomiting, and sore throat were common symptoms in 19 patients who had serologic evidence of infection. A third of the patients had illnesses sufficiently severe to motivate them to consult their physicians.[25] Other patients with bladder dysfunction, dysesthesias, weakness, and signs of myelitis have been described.[9,34] One case of infection during pregnancy has been reported. The third-trimester infection was severe, but the woman recovered from encephalitis and coma and delivered an apparently normal baby. Serologic studies of the neonate's blood were not performed.

PATHOLOGY

Pathologic changes in the brain are characterized by lesions in the cortical and deep gray matter, with varying degrees of neuronal loss from mild to extensive focal necrosis.[14,41] Viral antigen is found predominantly in neurons and only occasionally in astrocytes.[21] Rare viral particles have been identified by electron microscopy in principally extracellular locations.[2,32] Neutrophils predominate in cellular infiltrates in the meninges, vascular cuffs, and foci of tissue damage in the cortex and brain nuclei of patients dying acutely; at later stages, neutrophilic infiltrates are replaced by mononuclear cells.[2,14,21,41] Immunohistochemical examination of one patient disclosed a predominance of helper T cells in perivascular infiltrates, with some B lymphocytes. The most intense inflammatory reaction occurred in areas where antigen-positive neurons were absent, presumably where cell lysis already had occurred. Perivascular macrophages contained cleared viral antigen, but antigen could not be demonstrated in vascular endothelial cells.[21]

PATHOGENESIS

After peripheral inoculation of experimental animals, local viral replication occurs at a low level or may be undetectable. Viremia develops after this eclipse period, with disseminated infection of the spleen, liver, and kidneys noted in monkeys and guinea pigs and infection of the spleen, heart, and lungs observed in mice. Infectivity can be demonstrated in the brain only after viremia and infection in the viscera are established, thus suggesting that invasion of the central nervous system occurs by hematogenous spread.[41] Neurologic injury is associated with vasogenic edema in the earliest stages and subsequently with virus-induced cytolysis and host inflammatory responses.[26]

The immune response in humans presumably is similar to that observed in experimental alphavirus infections in mice, in which host resistance depends on rapid elaboration of a humoral immune response.

PROGNOSIS AND SEQUELAE

The case-fatality ratio is 33 percent among reported cases and is highest in the elderly; outcome is best in young adults aged 20 to 59 years, in whom the case-fatality rate is 24 percent.[35] Patients with a long prodromal illness (>4 days) have a better prognosis, which is consistent with the protective effect of a peripheral antibody response in the pre-neuroinvasive phase.[44]

Residual neurologic damage is observed more often in young children. In a Florida series, serious sequelae were seen in four of seven survivors younger than 5 years and in one of ten survivors in other age groups.[6] Similar findings were reported in a follow-up of epidemic cases in Massachusetts: seven of eight survivors younger than 3 years of age had neurologic sequelae, and only one of four surviving adults had residua.[1,19] Neurologic impairment ranged from mild unilateral spasticity to profound mental retardation, seizure disorders, and quadriplegia.

DIAGNOSIS

A specific laboratory diagnosis usually is made from serologic studies. Isolation of virus from CSF is unusual. The virus should be sought from brain biopsy tissue or autopsy material. EEE virus grows rapidly in a variety of cell lines, including Vero A549 and MRC-5 cells, and causes widespread cytopathic effect in several days.[46] Specific identification of viral isolates can be accomplished rapidly by immunofluorescent or immunoperoxidase techniques. EEE virus has been identified by immunofluorescence and by electron microscopy in brain tissue.[2,21,32] Viral antigen in viremic bird blood, infected mosquito pools, and infected equine brains can be detected directly by antigen capture enzyme-linked immunosorbent assay. Polymerase chain reaction analysis of CSF has been reported but has not been evaluated extensively.[45]

Serologic testing is available in many state laboratories. Virus-specific IgM usually can be detected in acute serum and CSF samples.[38] Indirect immunofluorescence, neutralization, and hemagglutination inhibition also are sensitive procedures. Antibodies often are present in the first week of illness. Neutralizing antibody appears 3 to 4 days after the onset of illness, and hemagglutination-inhibition (HI) antibody appears with almost equal rapidity.[25] Both HI and neutralizing antibodies appear to be long-lived.[25] Complement-fixing (CF) antibody is slower to rise and can be noted 11 days after onset, with diagnostic fourfold rises often appearing only in the third week after onset.[25] The peaks of both HI and CF titers are observed 3 to 4 weeks after onset.[26] CF antibody declines more rapidly. Measurable CF antibodies were found in approximately 50 percent of persons infected 8 years earlier in one study, although the effects of re-exposure could not be ruled out in this endemic area.[25]

The low prevalence of EEE antibody in the general population suggests that detection of EEE viral antibody in the acute serum of a patient with encephalitis indicates a high probability of that diagnosis. Applying the Bayes theorem, if the "rate" of EEE is 1 in every 2000 cases of encephalitis, if HI antibody to EEE is present in 100 percent of cases in the first week of illness, and if the prevalence of HI antibody in the general population is 0.05 percent, the presence of EEE in a patient with encephalitis who has demonstrable EEE antibody in a single serum specimen is a certainty. Thus, the probability of the diagnosis is high when specific antibody is found in any (i.e., acute) serum specimen.

DIFFERENTIAL DIAGNOSIS

The fulminant clinical course of EEE and the laboratory findings of neutrophilic leukocytosis and polymorphonuclear pleocytosis in the CSF may suggest bacterial cerebritis or meningitis. Because no specific therapy is available, EEE should be a diagnosis of exclusion after effort has been made to diagnose and to treat empirically against bacterial and herpes viral infections.

TREATMENT

Specific treatment is unavailable. Therapy aimed at supporting cardiorespiratory function, homeostasis of fluid, electrolyte balance, and control of cerebral edema and convulsions may be lifesaving. In a single case, intravenous immunoglobulin and glucocorticoids, given for their potential immunomodulatory effects, were associated with survival in an elderly patient.[26]

PREVENTION

An effective killed vaccine is licensed for horses, but no human vaccine is licensed. An investigational killed vaccine, available under investigatory permit, is used to protect laboratory personnel. Vaccination of the general public is not feasible as a public health measure because of the low incidence of disease.

Climatologic studies have shown a correlation between outbreaks and heavy rainfall in the summer of an epidemic year and in the preceding fall.[30,33,35] Although such predictors would have considerable utility in guiding control measures, outbreaks of EEE have been too few for their validity to be tested. Isolation of Highlands J virus, which shares a common enzootic cycle with EEE virus, often peaks 2 to 3 weeks before the appearance of EEE virus in *C. melanura*.

Surveillance and public health interventions to prevent EEE have been shown to be economic when they are balanced against the direct and indirect costs of even one human case.[50] Infection rates in *C. melanura* of 0.39/1000 have been highly predictive of risk for human cases in areas of Massachusetts surveyed during a 26-year interval.[28] Larviciding of swampland to control *C. melanura* is difficult because of the large areas involved, the potential toxic effects in fish and other wildlife, and the relative inaccessibility of the larvae. Emergency application of adulticides to control epizootic vectors is indicated when viral, mosquito, and animal surveillance suggests a risk for epizootic transmission. Public health advisories to avoid outdoor activity near enzootic foci and closure of campgrounds and parks in these locations may be necessary when viral transmission indices suggest a high level of risk. The use of repellents and avoidance of outdoor activity 1 to 2 hours after sunset, when many mosquitoes are most active, may reduce the risk of exposure; however, some vector *Aedes* spp. are daytime biters.

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CHAPTER **187b**

Western Equine Encephalitis

Theodore F. Tsai

Western equine encephalitis (WEE) is an endemic and enzootic acute central nervous system (CNS) infection of humans and horses in the western part of the United States, Canada, Mexico, and parts of South America. In North America, *Culex tarsalis*, the principal mosquito vector, also maintains the virus in an avian enzootic cycle.

ETIOLOGIC AGENT

WEE and other alphaviruses (group A arboviruses) form a genus of principally mosquito-borne viruses in the family *Togaviridae*.[43,61] Three of the eight viruses constituting the WEE antigenic complex are found in North America: Highlands J, Fort Morgan, and WEE viruses; among them, only WEE virus is a human pathogen. Much of what is known about the molecular biology of alphaviruses has been inferred from studies of the Sindbis (the type species and a member of the WEE complex) and Semliki Forest viruses.

Alphaviruses are small, enveloped, positive-stranded RNA viruses. Virions are spherical and 69 nm in diameter (including the length of their glycoprotein spikes), with a lipid bilayer enveloping a nucleocapsid core containing the 11.7-kb RNA viral genome. Glycoprotein spikes embedded in the viral envelope bind to cell membrane receptors and initiate infection by endocytotic fusion. The viral and lysosomal membranes fuse in a pH-dependent step, and the viral nucleocapsid is released into the cell cytoplasm, where RNA and protein synthesis occurs. The 5′ terminal two thirds of the RNA genome encodes four nonstructural proteins, and the 3′ terminal third of the RNA genome encodes the three structural proteins. The 30-kd nucleocapsid and two 50-kd glycoproteins—E1 and E2—are translated as a polyprotein from subgenomic 26S RNA. The envelope proteins—E1 and E2—are assembled in trimers of a single E2 protein and an E1 dimer. Eighty such trimer spikes are arranged in an icosahedral lattice on the virion surface.[9] Capsid proteins assemble with the viral genome in the cytoplasm and then bud through the virally modified cell membrane and acquire an envelope.

The E1 glycoprotein possesses a group-reactive hemagglutinin and group-reactive epitopes linked to cross-protection and cell-mediated cytotoxic effects. E1 also mediates viral cellular membrane fusion. Epitopes on E2 are linked to cell receptor recognition, viral neutralization and clearance, and neurovirulence and cellular apoptosis.[65]

Molecular genetic studies indicate that WEE virus arose more than 1000 years ago as a recombinant of eastern equine virus and an ancestral, now extinct, Sindbis-like virus, with the recombinant having acquired the neurotropic potential of eastern equine encephalitis virus while retaining the antigenic characteristics of the non-neurotropic Sindbis virus.[19,43,55,62,63] Alphaviruses are thought to have originated in the New World with separate introductions, presumably by birds, to the Old World, resulting in establishment of the present-day Sindbis-like viruses and Semliki Forest–related viruses. A slow rate of evolution, circa 10^{-4} nucleotide changes per year (versus 10^{-2}/year for other RNA viruses), probably reflects the natural pressure on viruses constrained to replicate in both insect and vertebrate cells.[63]

ECOLOGY

WEE virus is transmitted in an enzootic cycle to mosquitoes, birds, and other vertebrate hosts.[46,47,51] Horses and humans are dead-end hosts, but severe CNS infection may develop. Although WEE has been a public health and veterinary problem primarily in the western United States and Canada, the geographic range of the virus includes Mexico, Guyana, Brazil, Uruguay, and Argentina. Epizootics in horses, accompanied by small numbers of human cases, were reported in 1972 and 1983 in Argentina, where the virus apparently is transmitted by *Aedes albifasciatus* to introduced European hares and possibly birds. In addition, a distinct sylvatic subtype of WEE virus is transmitted in the subtropical Chaco province.[3,6]

In the United States, the geographic distribution of the virus and *C. tarsalis*, its principal vector, includes the western and central United States, southern Canada, and Mexico. A related virus—Highlands J virus, isolated from *Culiseta melanura* in the eastern United States—overlaps in its range in the east central part of the United States. Highlands J virus causes encephalitis in horses and possibly in humans.[35]

In the western region of the United States, *C. tarsalis* breeds in ground pools found on pasture lands; in irrigation wastewater; and at the margins of lakes, ponds, marshes, and flooded riversides. Some of these aquatic habitats are shared by birds that participate in the amplification of virus in nature.[46,47,51]

The female mosquito becomes infected after feeding on a viremic bird (or mammal). After an extrinsic incubation period of 7 to 9 days, when the virus propagates in the mosquito and the salivary glands become infected, the mosquito can transmit virus to other birds or mammals (amplifying hosts) or to humans and horses (dead-end hosts).

Passerine (perching) birds, especially sparrows and finches, have proved to be particularly important in amplification of the virus. In midsummer, the mosquito shifts its host-seeking activity to mammals. The shift may be influenced by an increase in the defensive behavior of nestling birds, which are its preferred host. The shift corresponds temporally to the appearance of cases in horses and people and appears to be a critical element enabling *C. tarsalis* to function as both an enzootic and an epizootic vector. In California, an auxiliary *Aedes melanimon*–jackrabbit *(Lepus californicus)* cycle has been demonstrated.

The overwintering mechanism for WEE virus has not been elucidated. However, virus has been recovered from adult *Aedes dorsalis* mosquitoes collected as larvae from a coastal area of California, thus indicating a possible role for vertical transmission of the virus in mosquitoes in some locations. Arguments also have been advanced for viral overwintering in adult mosquitoes and in persistently infected mammals, birds, and poikilotherms (snakes, frogs, and turtles). In Canada, *Aedes* spp. and *Culiseta inorata*, which emerge in the spring before *C. tarsalis* does, have been proposed as early amplifying vectors.[34]

Numerous climatologic and biologic indices with various degrees of predictive value have been shown to correlate with the occurrence of WEE outbreaks; these indices include vector population size, mosquito infection rates, and virus transmission rates to sentinel chickens or wild-caught sparrows. Such transmission indices are monitored in surveillance activities by public health agencies; however, their sensitivity, specificity, and predictive value for forecasting epidemics have been difficult to evaluate because of the sporadic nature of outbreaks.[64] A retrospective analysis of a 21-year experience in California showed correlation between average daily numbers of *C. tarsalis* females and the occurrence of human encephalitis.[41] In a Texas study, house sparrow infection and antibody rates were the best predictors of human disease, and *C. tarsalis* light trap indices were of borderline significance.[25]

Physical measures associated with the incidence of disease in humans or transmission of virus to sentinel species include the ambient air temperature, the snow pack in mountains providing runoff water, the river flow rate, the soil temperature inversion date (the date that the surface soil temperature exceeds the subsurface soil temperature), and the date when 50 or more days of 70° F (21° C) temperature have accumulated. Snow pack and river flow rate are associated with an abundance of irrigation water and flooding, which in turn are associated with the availability of breeding habitats and mosquito population size.[7,24,46,47]

Longitudinal and intraseasonal observations show that high temperatures are associated with a reduced risk of human disease. High ambient air temperature (>89.6° F [32° C]) decreases mosquito survival, adversely affects the competence of *C. tarsalis* to become infected with and to transmit WEE virus, and limits host-seeking activity.[50] In a model of global warming, higher temperatures are predicted to move the range of WEE viral transmission northward.[47]

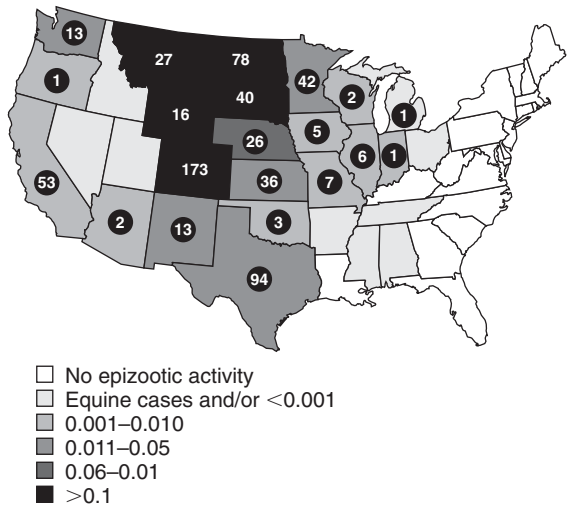

Figure 187–3 Reported cases and average annual incidence (per 100,000) of western equine encephalitis by state, 1964 to 1995.

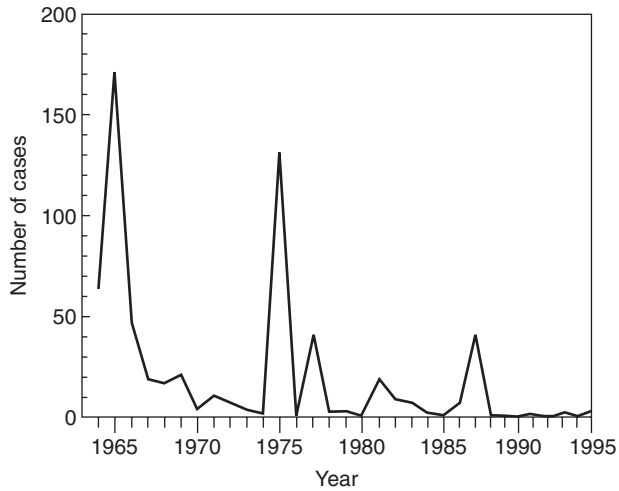

Figure 187–4 Reported cases of western equine encephalitis by year, 1964 to 1995.

EPIDEMIOLOGY

WEE occurs sporadically and in epidemic form, principally in Canadian provinces and states west of the Mississippi River (Figs. 187–3 and 187–4). Infections occur mainly in rural areas, where water impoundments, irrigated farmland, and naturally flooded sites provide breeding habitats for *C. tarsalis*; however, the increasingly rare interface between vector mosquitoes and humans has resulted in a point seroprevalence of 1 to 2 percent even in locations where the virus is transmitted in an enzootic cycle.[49] The annual median number of cases between 1964 and 1995 was only four, and only four cases were reported between 1988 and 1997, a lower incidence than that for eastern equine encephalitis. Historically, however, periodic outbreaks have led to scores or hundreds of cases (see Fig. 187–4).

Recurrent endemic and epidemic transmission was recorded in the Yakima Valley, Washington (1939 to 1942); California's Central Valley (1939 to 1952); the north central states and Canadian provinces, including Minnesota, North and South Dakota, Alberta, Manitoba, and Saskatchewan (1941 and 1975); and the high plains panhandle of Texas (1963 to 1966).[13,20,26,45] The largest outbreak on record, in 1941, resulted in more than 3400 human cases in Minnesota, North and South Dakota, Nebraska, Montana, Alberta, Manitoba, and Saskatchewan; equine cases were estimated to number in the hundreds of thousands. The outbreak centered in North Dakota, where the attack rate for the state was 167 cases per 100,000.[13,29] In 1952, an outbreak in California's Central Valley led to 348 reported cases, an incidence of 36 cases per 100,000 residents.[26] More recent outbreaks resulted in 277 cases in the central United States and Manitoba in 1975 and 40 cases in the central and mountain states in 1987.[11,30,34]

Most cases occur between June and September (Fig. 187–5), often preceded by cases occurring in horses several weeks earlier. Surveillance of equine cases is a widely used approach to assess the risk for epidemic transmission. However, the low frequency of laboratory-confirmed diagnoses, vaccination, and underreporting limit the precision of equine surveillance as a predictive marker.[42]

Several risk factors for acquiring WEE have been identified:

1. Attack rates usually are highest at the extremes of age[2] (Fig. 187–6). The experience from the 1952 California outbreak showed that a third of cases occurred in infants younger than 1

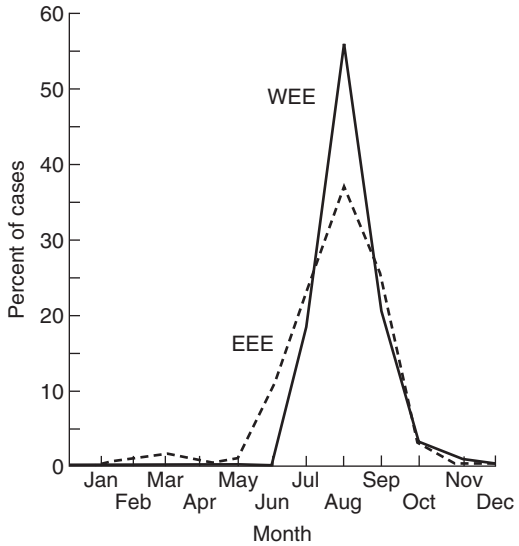

Figure 187–5 Reported cases of western and eastern equine encephalitis by month, 1972 to 1989.

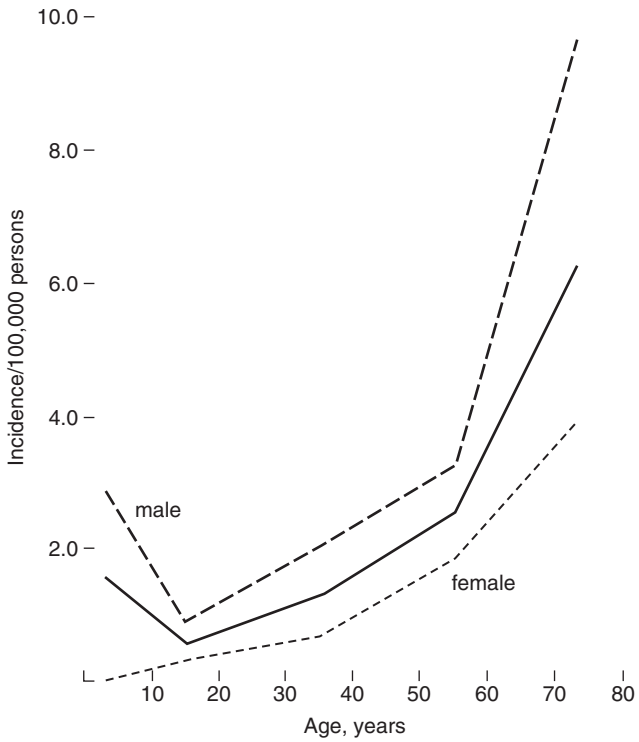

Figure 187–6 Age- and sex-specific incidence of western equine encephalitis, Colorado, 1987. – – –, male; —, total; - - -, female.

year.[26,47] Other reports confirm the bimodal pattern of an elevated risk in infants, a declining risk in children and young adults, and a gradual increase in risk in the elderly.

2. Attack rates in males are twofold higher than those in females in every age group[2] (see Fig. 187–6). Biologic differences in susceptibility to infection may account for the observed disparity in infancy, and greater occupational and recreational exposure outdoors might be responsible for the differences in adults. In the 1981 outbreak in Manitoba, 21 of 25 cases were in males; the four affected women were widows who maintained their premises alone.[11]

3. Rural residence is associated with attack rates 1.5 to 5 times higher than those for urban residence (Table 187–1). Counties lying in major river drainage areas and with more irrigated acreage have had the highest incidence of equine and human disease[2] (Fig. 187–7).

4. Agricultural occupation has been suggested as a risk factor in several studies.

5. Length of residence in areas where WEE is endemic is associated inversely with a risk of developing illness. Acquired immunity, through asymptomatic or mild infection, accumulates with length of residence; in endemic areas, the point prevalence of specific antibody previously approached 20 percent by adulthood.[46,47]

CLINICAL MANIFESTATIONS

The clinical illness ranges from a nonspecific syndrome of headache and fever to aseptic meningitis, meningoencephalitis, and frank encephalitis. The estimated case-infection ratio is 1:58 in children 1 to 4 years of age, but it declines to 1:1150 in adults.[16,47,51]

The onset of illness typically is abrupt, with sudden fever, headache, malaise, chills, and nausea and vomiting,[4,13,23,45] occasionally preceded by signs of an upper respiratory infection. Signs of CNS infection gradually become evident as dizziness, drowsiness, increasing headache, stiff neck, and disorientation develop during the course of hours or days. Infants typically have a sudden cessation in feeding, fussiness, fever, and protracted vomiting. The prodromal interval is abbreviated, and convulsions and a lethargic unresponsive state develop rapidly.

On examination, patients appear somnolent and may have signs of meningeal irritation. The sensorium is depressed, and patients may alternate between agitation and somnolence. Generalized muscle weakness is present, and deep tendon reflexes are diminished. Focal neurologic signs suggesting herpes encephalitis have been reported in anecdotal cases[1,5]; however, in the 1941 Minnesota outbreak, only 3 of 226 cases reported had unilateral weakness.[13]

In infants, the fontanelle may be tense or bulging, often accompanied by spastic paresis and generalized convulsions. The frequency of seizures is related inversely to age; they occur with greatest frequency in infants younger than 3 months (75-80% of cases), whereas in 2- to 4-year-old children, seizures have been reported in 15 percent of cases.[33]

The peripheral leukocyte count is unremarkable. Cerebrospinal fluid (CSF) obtained at an early stage in the illness generally

TABLE 187–1 Western Equine Encephalitis Epidemic Attack Rates by Population Density*

	Minnesota, 1941	Kern County, CA, 1952	Hale County, TX, 1963-1966	Manitoba, 1975
Rural	15.8-22.0	149.2	2.62	10.8
Small town	2.3-5.6			2.4
Urban		28.5	1.0	0.9

**Per 100,000.*

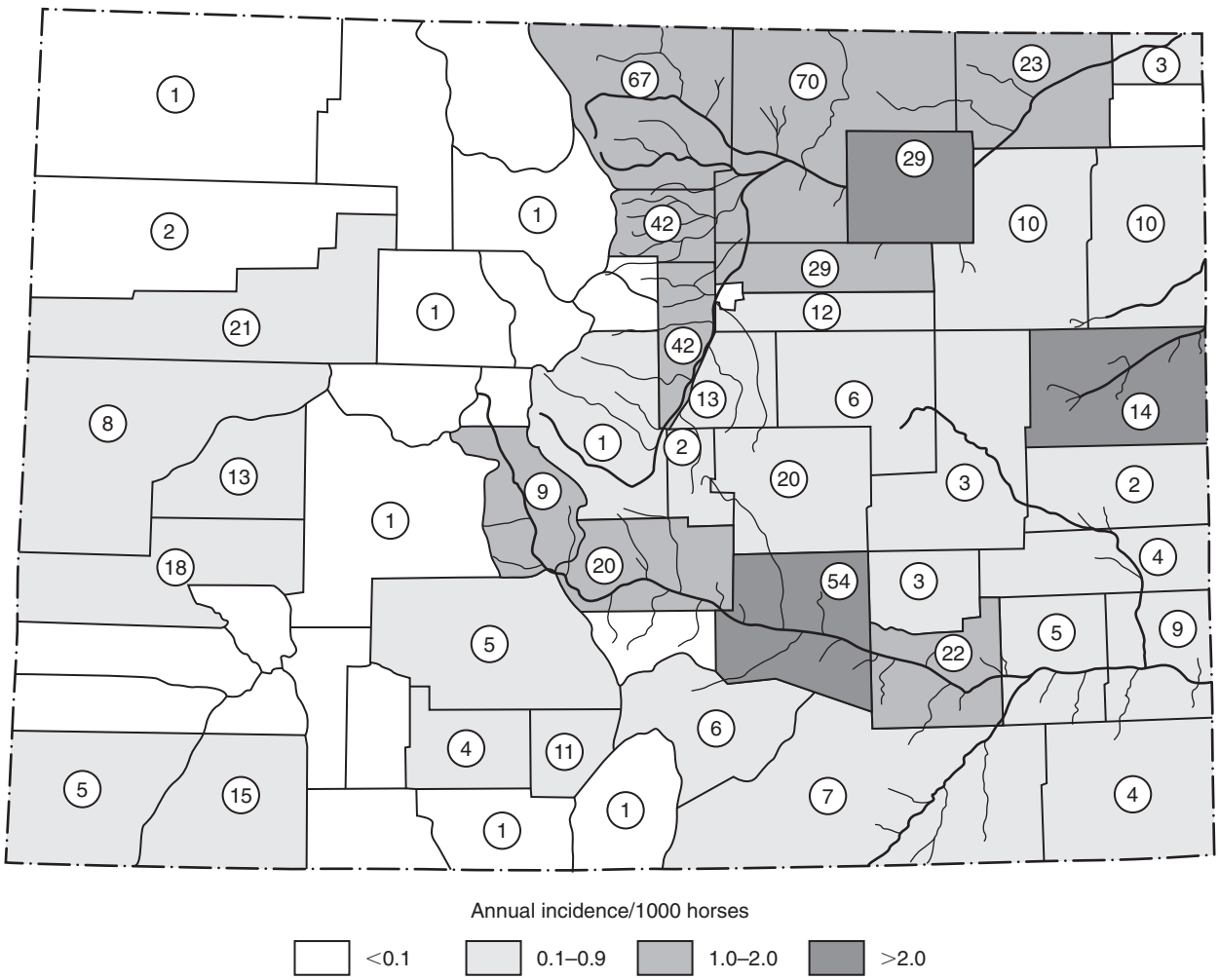

Figure 187–7 Reported equine cases and incidence of western equine encephalitis by county, Colorado, 1975 to 1988.

exhibits normal glucose concentration, elevated protein level, and a leukocyte count between 10 and 300/mm^3.[30] Mononuclear cells usually predominate.

Five instances of late third-trimester infection in pregnant women have been reported to result in perinatal illness or encephalitis.[10,33] The women's illnesses had an onset 0 to 10 days before delivery, and the infants became ill on the fifth and sixth postpartum days. Teratogenic effects of infection occurring earlier in gestation have not been reported for WEE virus but are suspected for other group A arboviruses.[36]

PATHOLOGY

Specific pathologic changes are confined to the CNS.[4,30,39] On gross examination, the brain appears normal or may be congested and swollen, with minimal changes observed in the meninges. Microscopic lesions affecting gray and white matter appear throughout the brain but predominate in the basal ganglia. Disseminated small focal abscesses infiltrated with neutrophils are a distinctive feature. The vessels appear congested, and small focal hemorrhages or diffuse extravasations of erythrocytes are present along with neutrophilic infiltration of the vascular wall and endarteritis. Vascular lumina may be occluded by endothelial proliferation and swelling.

Extensive patchy areas of demyelination are found throughout the brain. Older lesions appear as sharply circumscribed, punched-out plaques. Secondary microglial reaction in demyelinated areas is minimal.

The spinal cord is affected in the same fashion, with focal, perivascular, and diffuse lesions attracting both polymorphonuclear and mononuclear cells. Lesions predominate in the central gray matter.

PATHOGENESIS

Peripheral inoculation of experimental animals with WEE virus is followed by viral replication in various extraneural sites before invasion of the CNS (including the peripheral site of inoculation, viscera, muscle, and perhaps vascular endothelial cells) occurs.[27,31,40,67]

Host resistance and recovery from infection depend chiefly on an effective antibody response. Antibody contributes to recovery by a variety of means, including viral neutralization and antibody-mediated restriction of viral gene expression.[18,65] Interferon may contribute to containment of local viral spread in the CNS. Antibodies are protective and may be cross-protective to other alphaviruses. Antibodies to the E2 glycoprotein typically are virus specific and associated with neutralization and protection. Antibodies to E1 exhibit greater cross-reactivity with other alphaviruses.[61] Immune serum given to monkeys prophylactically protects them from challenge with WEE virus, and passive protection has been demonstrated in monkeys inoculated with WEE virus and treated with immune serum within 24 hours.[67] However, immu-

notherapy in monkeys is uniformly unsuccessful in preventing death once signs of CNS infection have appeared. In guinea pigs peripherally inoculated with WEE virus, immunotherapy given within 24 to 48 hours of inoculation leads to survival of some animals and delayed death in others.[40] In one human case, passive immunization led to a delayed antibody response and was followed by the development of parkinsonism.[17]

The age-dependent virulence of specific Sindbis virus strains has been linked to the immaturity of the suckling mouse T- and B-cell repertoire. Fibroblasts from newborn but not weanling mice are susceptible to Sindbis virus infection, and viral infection of immature mouse neurons leads to apoptotic cell death. Mature neurons are protected against apoptosis by induction of the *bcl-2* oncogene, whose products convert the infection to a persistent nonlytic infection. Viral persistence in the brain of recovered mice is modulated by antibody.[18]

PROGNOSIS

Major neurologic sequelae of WEE, including quadriplegia, hemiplegia, spasticity, intracerebral calcifications, developmental delay in children, and epilepsy, have been reported in approximately 13 percent of cases.[12,14,23,68] The risk for development of serious neurologic sequelae in infants is triple that of other age groups, and 30 percent of recovered infants younger than 1 year remain seriously impaired.[14,16,60] In infants younger than 1 year, convulsions during the acute phase of illness were associated with a greater risk of poor long-term outcome, including continued seizures. Multiple intracranial calcifications were observed in one recovered infant with persistent seizures.[59] Minor psychiatric or neurologic deficits remained in approximately one fourth of children and adults monitored for 18 months after infection.[14] Central apnea has been reported as a complication of encephalitis in two adult cases.[66]

Parkinson syndrome has been a residual effect in at least 15 cases of adults surviving WEE.[17,38,57,58] Its onset may be immediate or delayed for several years after recovery from the illness. Two retrospective investigations of patients with idiopathic Parkinson disease found no difference between cases and controls in seroprevalence to several arboviruses, including WEE virus.

The case-fatality rate in reported cases in the United States from 1955 to 1978 was 3 to 4 percent. In outbreaks in the central portion of the United States and Canada in 1941, 1975, and 1981, approximately 7 to 9 percent of patients died, with higher fatality rates in the elderly.

DIAGNOSIS

Virus rarely can be recovered from blood or CSF, but it can be isolated readily from the brain of fatal cases. In a few attempts, WEE virus has been isolated from brain biopsy material; in principle, rapid diagnosis can be made by immunofluorescent identification of WEE viral antigen in brain biopsy tissue. The virus can be isolated readily in Vero cell culture or in suckling mice.[58] Nucleic acid–based sequence amplification of CSF appears to be sensitive and specific.

IgM capture enzyme-linked immunosorbent assay (ELISA) is the preferred serologic procedure.[32] IgM antibody usually is present in acute serum within a week after the onset of illness, and its presence is presumptive evidence of recent infection. IgM often can be detected in CSF before it can be identified in serum, and its presence, which indicates intrathecal production of antibody, confirms a recent CNS infection.[32] Identification of virus-specific IgM in serum and CSF by immunofluorescence also is possible; however, this approach is somewhat less sensitive than is IgM capture ELISA and may be confounded by the effects of rheumatoid factor.

Hemagglutination-inhibition and neutralizing antibodies are elevated in the first week of illness in most cases, and a diagnostic fourfold rise in titer usually is observed in the second week. Complement-fixing antibody is slower to rise, with fourfold changes delayed until the third to fifth week after onset. The complement-fixation response may be blunted in older patients.[60]

Neutralizing and hemagglutination-inhibition antibody titers have considerable longevity, with minimal decay noted 30 months after onset. In contrast, complement-fixing antibody is relatively short-lived, and its presence is a useful indicator of recent infection, particularly in endemic areas where long-term residents may have neutralizing and hemagglutination-inhibition antibody from previous exposure. Only two thirds of patients are estimated to show complement-fixing antibody 2 years after onset, and less than 15 percent have residual complement-fixing antibody after 5 years.[60]

DIFFERENTIAL DIAGNOSIS

The clinical findings in patients with encephalitis seldom are sufficiently characteristic that a specific diagnosis can be made on clinical grounds alone. Fever, vomiting, signs of meningeal irritation, and confusion are nonspecific features of encephalitis and cerebral edema. Early symptoms in infants, such as fever, lethargy, and vomiting, may be even less specific.[28]

Furthermore, the peak occurrence of arboviral infection in midsummer to late summer temporally overlaps the seasonal occurrence of other CNS infections by enteroviruses (e.g., echovirus 13, which recently has become epidemic in the United States), leptospires, free-living amebae, and mosquito-borne West Nile and St. Louis encephalitis. CNS signs may be prominent in other summertime diseases, such as Rocky Mountain spotted fever, heat stroke, shigellosis, and lead encephalopathy. High priority should be given to establishing a specific diagnosis of treatable conditions, such as herpes and enteroviral infection, while bearing in mind that dual arboviral and enteroviral infection has been reported.[5]

Other causes of CNS infection without an established summertime seasonality that also should be considered in the differential diagnosis include fungal, mycobacterial, and partially treated bacterial meningitis; brain abscess and infected subdural collections; bacterial endocarditis; toxoplasmosis; cat-scratch disease; and infections with rabies virus, mumps virus, herpes simplex virus, Epstein-Barr virus, human herpesvirus 6, adenovirus, lymphocytic choriomeningitis virus, and human immunodeficiency virus. Parainfectious disorders such as postinfectious viral encephalitis, acute cerebellar ataxia of childhood, mycoplasmal infection, neuroblastoma with opsoclonus, and Reye syndrome also may enter into the differential diagnosis. Vascular disorders formed the largest single group of disorders mimicking herpes encephalitis in several studies. Drugs (such as trimethoprim-sulfamethoxazole, penicillin, isoniazid, phenazopyridine, ibuprofen, tolmetin, sulindac, and carbamazepine), high doses of bismuth, OKT3 antibody, and vidarabine also may cause aseptic meningitis or encephalopathy.

Because of the potential for their overlapping circumstances of exposure, diethyltoluamide (DEET) encephalopathy should be excluded. DEET-containing insect repellents have been implicated as a cause of seizures and encephalopathy after brief cutaneous exposure (see later).

Valuable clues to the diagnosis of an arbovirus infection are gleaned from a history of travel, residence, or occupational and recreational activities within the appropriate incubation period. Suspected cases should be reported to public health officials.

TREATMENT

Specific antiviral therapy is not available. Supportive treatment of acutely ill patients includes monitoring of cardiorespiratory function, fluid and electrolyte balance, and intracranial pressure. A case of laboratory infection treated with immune equine serum resulted in recovery; however, acute Parkinson syndrome developed in this patient and coincided temporally with serum sickness resulting from immunotherapy.[17]

PREVENTION

Killed vaccine prepared in chick embryo fibroblast culture is available in the United States under an investigatory permit for laboratory and field personnel who are at risk for occupational exposure. Effective killed vaccines are available for horses and usually are administered in bivalent or multivalent formulations (with eastern equine encephalitis, Venezuelan equine encephalitis, and influenza viruses). Vaccination of the general public, even in endemic areas, is not a practical consideration because of the low risk of disease. Personal protective measures include avoidance of outdoor activity during the hours of peak mosquito activity at dusk and use of repellents.

The Centers for Disease Control and Prevention (CDC) recommends repellents containing DEET or picaridin (KBR3023) as active ingredients.[7,8,15,21,44] DEET is absorbed readily through the skin, and its absorption and absorption of oxybenzone, a common sunscreen preparation, are increased when they are used in combination. Some authorities recommend that sunscreens be applied first and more liberally, followed by insect repellent applied sparingly because of concerns about the potential neurotoxicity of DEET.[7,53,54] Three cases were fatal. In one recovered child, encephalopathy and seizures developed after only two applications. In an additional child, seizures and encephalopathy occurred after the child had cutaneous and respiratory exposure to DEET in an automobile with closed windows. Additional cases of seizures in patients who used small quantities of DEET have been reported, but whether these events were coincidental in populations in which the prevalence of DEET use is high is unclear.[8] DEET may have an effect on ammonia metabolism; in one case, encephalopathy occurred in a patient with partial ornithine carbamoyltransferase deficiency.[22] DEET is a proven neurotoxin when it is ingested; however, the incidence of neurologic side effects after cutaneous exposure is unknown. Nonetheless, avoiding formulations containing DEET in high concentration is prudent; when it is applied simultaneously with sunscreen, DEET repellency is unhampered but ultraviolet protection may be reduced. Precautions for the prudent use of repellents as recommended by the CDC and U.S. Environmental Protection Agency (EPA) are listed in Table 187-2.

Repellents containing oil of lemon eucalyptus (but not "pure" oil of lemon eucalyptus) and registered with the EPA as repellents also may provide a similar level of protection.

Permethrin, a synthetic pyrethroid available as a 0.5 percent aerosol (Permanone), is both insecticidal and a repellent.[8,15,56] Permethrin is extremely effective in reducing the incidence of bites of mosquitoes and ticks when it is sprayed on clothing and shoes, and it also can be applied to tents, mosquito nets, and other gear.[15,55] Its use on the skin is not approved, except as treatment of scabies and head lice.

Prevention is focused primarily on interruption of the transmission of virus from mosquitoes to humans. In many localities where WEE is endemic, mosquito abatement districts monitor mosquito populations, virus infection rates in the vector population, or evidence of transmission of virus to wild or sentinel birds and chickens to provide a basis for predicting epidemic transmission and for emergency mosquito control.[37,52]

TABLE 187–2 Precautions to Reduce Potential Adverse Reactions from Repellents

DEET is approved for use on children older than 2 months. Read and follow label instructions.
Repellents should be applied only to exposed skin and clothing (as directed on the product label). Do not use under clothing.
Never use repellents over cuts, wounds, or irritated skin.
Avoid mucosal contact—do not apply to the eyes and mouth, and apply sparingly around the ears. When using sprays, do not spray directly onto the face; spray on the hands first and then apply to the face.
Do not allow children to handle this product, and do not apply to children's hands. When using on children, apply to your own hands and then put it on the child.
Do not spray in enclosed areas. Avoid breathing a repellent spray. Avoid ingestion, and do not use it near food.
Use just enough repellent to cover exposed skin and clothing. Heavy application and saturation are unnecessary for effectiveness; if biting insects do not respond to a thin film of repellent, apply a bit more.
After returning indoors, wash treated skin with soap and water or bathe. This precaution is particularly important when repellents are used repeatedly in a day or on consecutive days. Also, wash treated clothing before wearing it again.
Pregnant and nursing women should minimize the use of repellents.
If you suspect that you or your child is reacting to an insect repellent, discontinue use, wash treated skin, and then call your local poison control center. If you go to a physician, take the repellent with you.
Specific medical information about the active ingredients in repellents and other pesticides is available at the National Pesticide Information Center at 1-800-858-7378.

Modified from U.S. Environmental Protection Agency recommendations. Available at http://www.epa.gov/pesticides/factsheets/chemicals/deet/htm.

As a general rule, *C. tarsalis* densities exceeding 10 to 15 females per trap night and mosquito infection rates greater than 5 to 10 per 1000 signal a risk for epizootic transmission. Evidence of seroconversion in sentinel chickens and observations of equine cases are further indications that human cases may occur.

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CHAPTER **187c**

Venezuelan Equine Encephalitis

Theodore F. Tsai

Mosquito-borne Venezuelan equine encephalitis (VEE) arguably is the most important viral zoonosis in Latin America. The

disease, known locally as *peste loca*, has occurred in combined epizootics and epidemics at regular intervals since the 1920s, sometimes leading to hundreds of thousands of human and equine cases. Between 1935 and 1961, 11 outbreaks were reported, and from 1962 to 1973, epizootics recurred in every year except 1965.[14,35] The virus was isolated from horse brain in 1938 during an outbreak in Venezuela. Although outbreaks have arisen principally in northern South America, especially from Colombia and Venezuela, in a remarkable period between 1969 and 1971, epizootics and epidemics were reported from Colombia, Venezuela, Ecuador, Peru, all the countries of Central America (except Panama), Mexico, and the state of Texas.[11,21,35] Subsequently, no major epizootics or epidemics were recognized until a large outbreak emerged in Venezuela and Colombia in 1995.[24,31,32,38] Molecular phylogenetic analyses performed in the wake of that outbreak suggested that epizootic strains arise spontaneously from sylvatic viral strains circulating silently in nature.[2,5,19,38]

ETIOLOGIC AGENT

VEE virus is antigenically distinct from other alphaviruses but is itself a complex of antigenically and ecologically distinct viral subtypes[23,28,36,37,40] (Table 187–3). Epizootic subtypes IAB and IC are so named because they have been associated with major outbreaks in horses. Sylvatic viral subtypes circulate in silent cycles of rodent- or bird-mosquito transmission and in general do not cause encephalitis in equines. Both epizootic and sylvatic viral strains cause human illness.

VEE virus is thought to have evolved approximately 1400 years ago from a now extinct ancestral alphavirus in one of two extant lineages of New World alphaviruses represented by eastern equine encephalomyelitis and VEE viruses.[23] Because epizootic VEE viral strains had never been isolated from nature except during epizootics, their reservoirs and the mechanisms by which they emerge to cause outbreaks had remained a puzzle. However, recent molecular phylogenetic analyses have found a close genetic relationship between the epizootic IC and the sylvatic ID strains, thus suggesting that these epizootic strains might arise spontaneously by mutation from naturally circulating ID virus.[2,5,37] Other outbreaks have had an iatrogenic source from improperly inactivated equine vaccine.[15,16,23,25,37,38]

Comparisons of the attenuated TC-83 vaccine strain of VEE virus and the epizootic IAB parent virus and mutations of virulent infectious clones have shown that changes associated with attenuation occur principally in genes encoding the E2 and E1 glycoproteins but also in the 5′ nontranslated region.[2,5,15,28] Attenuation is associated with reduced neuroinvasiveness (faster clearance from blood and lower viremia levels) as well as reduced neurovirulence (minimal histopathologic changes after intracerebral inoculation of horses).

VEE virus rapidly produces cytopathic effects in a variety of cell cultures, including Vero, LLC-MK2, and BHK-21, and in primary chicken and duck embryo cells. Epizootic strains cause lethal infections in horses, donkeys, mules, rabbits, and dogs. In certain guinea pig strains, pathogenicity is correlated with equine virulence.

EPIDEMIOLOGY AND ECOLOGY

Epizootics and concurrent epidemics of VEE caused by the IAB and IC strains typically have led to thousands and, on at least one occasion, hundreds of thousands of cases in humans and equines. Most such outbreaks have occurred in Venezuela and Colombia and have been caused by IC viruses (see earlier) (Fig. 187–8).[24,27,37,38] The circumstances leading to the emergence of outbreaks are poorly understood, but outbreaks often have occurred in arid areas during years of heavy rainfall and flooding, especially during the dry season.[35] The importance of a nonimmune horse population to amplify the virus has been underscored in the most recent outbreak in 1995, which occurred in areas of Colombia and Venezuela where equine immunizations had lapsed.[24,31,32,38] Equines are the most important vertebrate amplifying host because high levels of viremia develop and are sustained in equines, and they provide a large surface area for biting mosquitoes. Numerous species of mosquitoes and other blood-feeding insects, among them *Aedes taeniorhynchus*, a salt marsh mosquito, and *Psorophora confinnis*, found in ground pools, can transmit the virus from horse to horse and from horse to human.[30]

Infections are transmitted rapidly among animals and to people such that outbreaks can disseminate at rates of several miles per day. The epidemic curve of human cases usually follows the equine epizootic by several weeks, and epidemic transmission ceases when the number of susceptible horses has been exhausted by immunization or natural infection.[12,35]

The role of other animals, including humans, in sustaining epidemic viral transmission has been investigated in urban outbreaks, during which household clustering of cases has been observed, and in recent outbreaks, during which few horses were

TABLE 187–3 Viruses in the Venezuelan Equine Encephalomyelitis Complex

Subtype	Variety	Pattern of Transmission	Location	Transmission Cycle
I	AB	Epizootic	South, Central, and North America	Various mosquitoes and biting insects–equines
	C	Epizootic	Northern South America	Various mosquitoes and biting insects–equines
	D	Enzootic	Ecuador, Panama, Colombia, and Venezuela	*Culex (Melanoconion) ocossa* and *panocossa*–rodents, aquatic birds
	E	Enzootic	Central America	*Culex (Melanoconion) taeniopus*–rodents
	F	Enzootic	Brazil	Unknown
II (Everglades)		Enzootic	Southern Florida	*Culex (Melanoconion) cedecei*–rodents
III	A			
	Mucambo	Enzootic	South America	*Culex (Melanoconion) portesi*–rodents
	B			
	Tonate	Enzootic	South America	Unknown
	Bijou Bridge	Unknown	Western North America	*Oeciacus vicarius*–birds
	C	Enzootic	Peru	Unknown
	D	Enzootic	Peru	*Culex (Melanoconion)–Proechimys* spp.
IV (Pixuna)		Enzootic	Brazil	Unknown
V (Cabassou)		Enzootic	French Guiana	Unknown
VI		Enzootic	Argentina	Unknown

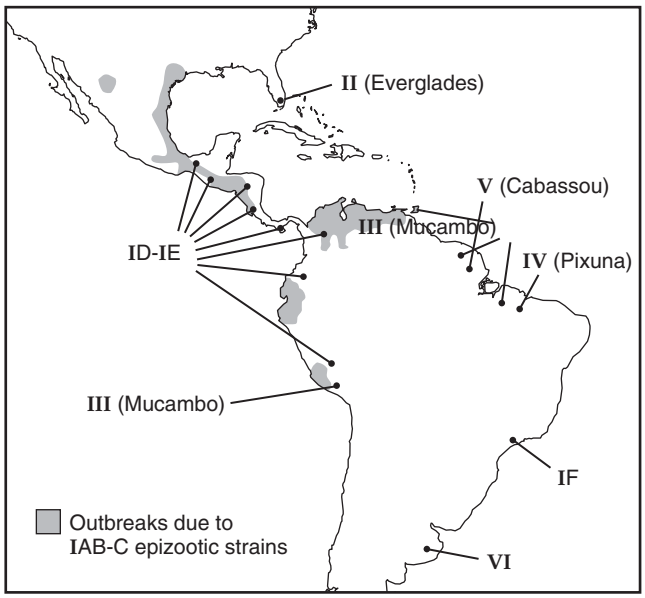

Figure 187–8 Locations of Venezuelan equine encephalitis (VEE) outbreaks caused by type IAB-IC epizootic strains and locations where VEE sylvatic subtypes have been recognized.

kept in the community. VEE virus levels in human blood are sufficiently high to infect mosquitoes, and virus also has been isolated from the pharynx of ill persons, indicating the possibility of person-to-person transmission by mosquitoes such as *Aedes aegypti* or by direct close contact.[2,29] Although such transmission mechanisms may account for some cases, a household survey found that rates of apparent secondary transmission were no higher than the underlying community attack rate.[24,31] Community attack rates of 20 to 50 percent have been recorded, and the brief course of epidemics in intervals as short as 1 month underscores the considerable force of epidemic transmission.

The series of epizootics beginning in Guatemala in 1969 and reaching Texas in 1971 was caused by an IAB virus that is almost genetically identical to the 1943 Trinidad donkey (epizootic IAB) virus used in inactivated vaccines, thus leading to the conclusion that these outbreaks were iatrogenic and caused by the use of inadequately inactivated equine vaccine.[16,37] Another outbreak in Argentina was traced to a similar cause.

In contrast to the epizootic strains, sylvatic VEE viruses are avirulent in horses and cause a low level of viremia after infection, subclinical or mild illness, and minimal inflammatory change after direct intracerebral inoculation.[34] However, outbreaks of enzootic IE-like viruses rarely have caused outbreaks and deaths in horses (e.g., in Chiapas, Mexico, in 1993). The viruses are maintained in mosquitoes, principally *Culex (Melanoconion) taeniopus* in marshy coastal lowlands, in rodents, and in aquatic birds. In coastal lagoons in Panama, ibises *(Endocinus albus)* and spoonbilled ducks *(Cochlearius cochlearius)* appear to serve as intermediate hosts.[1] VEE subtype II virus, the Everglades virus, is enzootic among cotton rats and other small mammals in the Everglades swamp and probably over a much larger area of Florida but rarely has caused human illness.[6,9] The other VEE virus found in the United States, Bijou Bridge virus, is a nonpathogenic virus thought to have evolved from South American subtype III virus transferred as recently as 40 years ago, probably by a migrating bird.

VEE infections caused by sylvatic strains can be considered "diseases of place" that occur sporadically in persons who enter sylvatic habitats where the viruses are maintained.[1,8] Sporadic cases and rare outbreaks among soldiers in field bivouacs have been reported, but seroprevalence rates in local populations range as high as 75 percent in some locations (e.g., Chiapas, Mexico).[13,26] Many infections are undoubtedly undiagnosed. Bridge vectors, mosquitoes that feed on mammals in the enzootic transmission cycle and subsequently on humans, usually are responsible for these infections.

Numerous cases and outbreaks of VEE have occurred in laboratories when infective aerosols were generated in laboratory procedures.[17] Laboratory manipulations should be undertaken only in BL-3 laboratories and by immunized personnel.

CLINICAL MANIFESTATIONS

The incubation period is brief—2 to 5 days—and in many accounts, the onset of illness was so sudden that it was timed to an exact hour.[17] Fever, chills, headache, myalgia, and malaise are the earliest symptoms, and illness quickly leads to prostration. Photophobia, neck stiffness, backache, conjunctivitis, and sore throat are also common symptoms that occur in approximately one quarter or more of cases. Gastrointestinal complaints, especially nausea and vomiting, and, to a lesser degree, loose stools or diarrhea are reported frequently.[4]

Physical examination discloses severe prostration and few specific findings. The face may appear hyperemic; inflammation of the pharynx is common; and occasionally, tonsillitis, palatal ulcers, or petechiae are observed. The cervical lymph nodes may be enlarged and tender. Conjunctivitis and conjunctival suffusion are noted frequently. Nuchal rigidity can be elicited in 10 percent of cases, more often in children. Despite the disease's name, confusion, agitation, and mild disturbances in consciousness suggesting encephalitis are present in only 5 to 10 percent of cases, and patients with significant neurologic findings, such as cranial nerve palsy, motor weakness and paralysis, seizures, and coma, usually account for less than 5 percent of all cases.[4,24,25] In epidemics, neurologic findings and encephalitis occurred more commonly in children; however, sporadic cases, including encephalitis caused by VEE subtype II (Everglades) and III (Tonate) viruses, have occurred in middle-aged or elderly adults.[9,13] The fatality rate among patients with encephalitis is 10 to 25 percent, or about 0.5 percent of all cases.[24,25]

In many patients, the illness has an apparently biphasic course; seizures, projectile vomiting, and ataxia occur several days after the onset of fever, followed rapidly by a complete resolution of symptoms. Sequelae such as nervousness, forgetfulness, recurrent headache, and easy fatigability are common occurrences and may persist for months or even up to 1 year. Motor abnormalities usually resolve without residual deficit; however, rarely, sensory and motor abnormalities may persist. Long-term effects on psychometric examination have been reported.[18]

Experimental observations in animals suggest that congenital infection may lead to central nervous system anomalies such as porencephaly, micrencephaly, and hydrocephalus.[19] A similar pattern of structural brain abnormalities has been observed in virus culture–positive fetuses aborted during outbreaks.[38,39] Pancreatic beta-cell infection occurs in experimentally infected animals and has led to speculation that VEE may be followed by diabetes, but epidemiologic studies have failed to find an association.[18]

The principal clinical laboratory finding is leukopenia (<4500/mm^3), the nadir of which occurs on the fourth day of illness.[4,8] After the onset of illness, the absolute neutrophil count declines from normal values to 500 to 2000; total lymphocytes are depressed at the onset of illness and gradually recover after 1 week. The platelet count may be diminished below 100,000 mm^3, and lactate dehydrogenase and hepatic transaminases may be elevated moderately. Cerebrospinal fluid shows a lymphocytic

pleocytosis of up to several hundred cells, moderately elevated protein level, and normal or slightly depressed glucose concentration.

PATHOLOGY AND PATHOGENESIS

VEE virus is both lymphotropic and neurotropic.[7,33] Pathologic changes are observed consistently in the lymph nodes, spleen, lungs, liver, gastrointestinal lymphoid tissue, and brain, with inflammatory infiltrates of mononuclear and polymorphonuclear cells. The lymph nodes and spleen show pronounced lymphoid depletion and necrosis of the germinal centers, along with neutrophilic infiltration and lymphophagocytosis. The selective depletion of lymphoid follicles, with sparing of the paracortical areas and the thymus, suggests that VEE virus principally destroys B cells. These histopathologic changes are reflected in the early lymphopenia seen in the peripheral blood. The liver shows patchy hepatocellular degeneration typical of viral hepatitis. A diffuse interstitial pneumonia with a mixed intraseptal inflammatory cell infiltrate is a consistent finding, and some cases also exhibit intraalveolar hemorrhage, secondary bronchopneumonia, or both. The brain shows only congestion and edema in most cases. Mild, often focal meningitis and changes associated with encephalitis, perivascular inflammatory infiltrates, and neuronal degeneration are found in a large number of cases.[7] The consistent presence of congestion and edema in various organs and the necrotizing vasculitis seen in some cases suggest that vascular endothelial cells may be a target of infection. This speculation is supported by the observation of VEE viral antigen in the vascular endothelial cells of experimentally infected animals. Secondary immune-mediated destruction of infected vascular endothelia and lymphoid cells may account for delayed clinical manifestations.

LABORATORY DIAGNOSIS

VEE virus can be recovered from blood during the first 3 days of illness in at least 75 percent of cases, although isolates have been made after 6 days.[3] Virus also has been recovered from throat swabs in approximately 25 percent of cases.[21] Specimens should be inoculated onto Vero or other susceptible cell cultures, but only in a laboratory with BL-3 level containment because of the risk of laboratory aerosol-associated infection. As an alternative to viral isolation, genomic sequences in acute viremic blood can be detected rapidly and with high sensitivity by polymerase chain reaction. Establishing the subtype of VEE viral isolates is vital because subtypes IAB and IC have a potential for epidemic spread. Isolates can be identified rapidly with subtype-specific monoclonal antibodies.

IgM capture enzyme-linked immunosorbent assay is the recommended serologic procedure. Both serum and cerebrospinal fluid specimens can be tested by this means. Elevated virus-specific IgM in a single serum or cerebrospinal fluid specimen is diagnostic and often obviates the need for a second paired serum. An epitope-blocking assay can differentiate antibody responses among serotypes.[36]

DIFFERENTIAL DIAGNOSIS

The self-limited acute febrile illness that characterizes most VEE cases resembles infection caused by dengue, Oropouche, Mayaro, group C bunyaviruses, and various other arboviruses. The epidemic occurrence of cases may suggest dengue or Oropouche fever; however, the absence of rash and hemorrhagic manifestations and an association with equine deaths strongly indicate VEE until it is disproved. Eastern and western equine encephalitis overlap with VEE in some areas of Latin America, but only sporadic human cases have been recognized. Clinical recognition of sporadic VEE caused by sylvatic viruses is difficult; the diagnosis should be entertained in patients with central nervous system infection and an appropriate exposure history.

TREATMENT AND PREVENTION

No specific treatment is available. Symptomatic treatment with antipyretics and fluids alone is sufficient in most cases. In patients with encephalitis, anticonvulsants may be needed, and intensive supportive care, especially early recognition and treatment of secondary pneumonia, may improve the outcome. Combinations of short interfering RNAs and passive immunization with humanized monoclonal antibodies are under investigation as therapeutic interventions.

Immunization with experimental live attenuated TC-83 vaccine is indicated for laboratory and field personnel with a high risk of exposure to VEE virus.[22] However, the vaccine is immunogenic in only 85 percent of vaccinees, and significant side effects consisting of fever, stiff neck, malaise, and myalgia develop in approximately 20 percent of vaccinees.[10] Previous vaccination with alphaviral vaccines may interfere with response to TC-83, but after immunization with experimental inactivated TC-84 vaccine, antibody will develop in approximately 75 percent of persons who had failed to respond to TC-83.[10,20] Safer candidate vaccines that may provide better protection against respiratory infection are being investigated.

Immunization of equines with TC-83 vaccine is the best approach to preventing the emergence of outbreaks. Inadequately inactivated vaccine prepared from epizootic IAB strains poses a danger of producing iatrogenic outbreaks and should be used only if appropriate safety and quality assurance standards of vaccine production have been met. Because the live vaccine provides rapid immunity after a single dose, it is the most effective approach in combating outbreaks. Attenuated vaccine is available commercially in certain Latin American countries, but in the United States, only inactivated vaccine formulated with western and eastern equine encephalitis and equine influenza and tetanus antigens is licensed. An improved candidate vaccine, V3526, has been shown to be efficacious in horses.[10]

Mosquito control with a combination of larvicides and adulticides may be indicated to mitigate large outbreaks. Taking precautions against mosquito bites, such as using repellents, staying in well-screened or air-conditioned areas when possible, and wearing long-sleeved shirts and long pants, is advised for persons who cannot avoid traveling to areas experiencing epidemics.

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CHAPTER **187d**

CHIKUNGUNYA

Scott B. Halstead

Chikungunya is a benign, dengue-like syndrome characterized by the abrupt onset of fever, arthralgia, maculopapular rash, and leukopenia. The term in Makonde means "that which bends up" and refers to the characteristic symptom of arthralgia.[33] In historical times, the terms *knokkel koorts*, *abu rokab*, *mal de genoux*, *dengue*, *dyenga*, and *3-day fever* have been given to epidemics probably caused by chikungunya virus.[10]

The classic account widely cited as being the initial description of epidemic dengue fever is that of David Bylon,[7] who was *staads chirurgyn* to the City of Batavia (Jakarta) in 1779. Dr. Bylon, who himself contracted the illness, wrote the following:

> It was last May 25, in the afternoon at 5:00 when I noted while talking with two good friends of mine, a growing pain in my right hand, and the joints of the lower arm, which step by step proceeded upward to the shoulder and then continued onto all my limbs; so much so that at 9:00 that same evening I was already in my bed with a high fever. . . .
>
> It's now been three weeks since I . . . was stricken by the illness, and because of that had to stay home for 5 days; but even until today I have continuously pain and stiffness in the joints of both feet, with swelling of both ankles; so much so, that when I get up in the morning, or have sat up for a while and start to move again, I can not do so very well and going up and down stairs is very painful.

This account of a febrile illness of acute onset with involvement of the joints clearly suggests that the disease was chikungunya (see the section on clinical manifestations).

In the same year in Cairo and Alexandria, Egypt, another outbreak of disease occurred that bears a close resemblance to chikungunya.[10,52]

An important pandemic of chikungunya occurred in the years 1870 to 1873; it appeared first on the East African coast and then on the Arabian coast and in Port Said, Egypt. From there, it was carried to Mumbai and Calcutta, India, and Java. The 1870 outbreak led to the discovery that the Swahili word for this disease is *ki-dinga pepo*.[15] Further, the term *denga* or *dyenga* had been used to designate the disease in Africa in an earlier outbreak in 1823; this pandemic of "dengue" had spread with the slave trade to the Caribbean, where in 1827 and 1828 an extensive outbreak occurred in the West Indies. In Cuba, the Spanish word *dengue* was used first. No further outbreaks of chikungunya occurred in the American hemisphere, and during the course of time, the term *dengue* was applied to the disease caused by dengue viruses. Written or serologic evidence exists of chikungunya pandemics in India in 1824 and 1825, 1871 and 1872, 1923, 1964 to 1965, and 2006.[10]

Chikungunya virus was isolated first by inoculation of blood into suckling mice from an explosive dengue-like epidemic in Tanganyika in 1952.[48]

ETIOLOGIC AGENT

CLASSIFICATION

According to epidemiologic criteria, chikungunya virus is arthropod-borne because it is transmitted by several species of mosquito. With the use of antigenic relationships demonstrated by hemagglutination inhibition and complement fixation, chikungunya is placed in the *Alphavirus* genus of the family *Togaviridae*.[30] Antisera prepared to chikungunya virus show strong cross-reactions with o'nyong-nyong by complement fixation and virus-dilution neutralization. Mayaro and Semliki Forest viruses, however, demonstrate only weak cross-reactions by hemagglutination inhibition and no reactions by complement fixation with chikungunya antigen. Cross-comparisons by plaque-reduction neutralization tests have shown little relatedness among alphaviruses.[54] Similar results have been obtained by fluorescent antibody technique.

Phylogenetic analysis of the E1 envelope glycoprotein from 18 strains divides chikungunya virus into three distinct genotypes corresponding to their geographic origin.[45] One chikungunya clade consists of viruses from Senegal and Nigeria and represents the West African genotype. These viruses had only 78 to 85 percent nucleotide sequence identity to viruses of the Central/East African genotype and the closely related Asian genotype. The phyletic grouping is consistent with the introduction of East African strains into Asia within a period 50 to 310 years ago.[45] The African and Asian strains of chikungunya virus are not antigenically separable with the use of mouse immune sera.[41] Although differences in plaque size and heat stability have been described between these strains, these differences might be attributable to the high number of mouse passages by the prototype African strain. Wild-type strains from all genotypes share the property of autointerference and production of hemorrhagic enteritis (Halstead, S. B., unpublished data, 1965).[20]

MORPHOLOGY

Chikungunya virions are spherical particles approximately 42 nm in diameter.[26] They possess a lipid-containing envelope with fine projections. The central core, approximately 25 to 30 nm in diameter, is roughly hexagonal in cross section and contains a nucleocapsid of uncertain symmetry. Together with other alphaviruses, the genome is a positive-sense, single-stranded RNA of 12 kb that is messenger active and specifies the viral structural and nonstructural protein, including the polymerase. After an initial round of RNA replication, a subgenomic RNA of approximately 4 kb (26S RNA) is produced. This RNA encodes the viral structural proteins, which include two envelope and capsid proteins.[27] The gene is translated from the 5′ end, where genes for nonstructural proteins are located. Assembly of virus particles at the cell surface occurs by a budding process involving incorporation of the "core" virus precursor into virus particles.[26] Host-cell membranes are modified during infection and contain viral antigen when incorporated into viral envelopes. The protein hemagglutinin spikes are mounted on a phospholipid envelope.

GROWTH

Intracerebral inoculation of chikungunya virus produces death in infant mice, rats, and hamsters. Serial passage of the virus in mice has resulted in selection of a strain with a short incubation period that is lethal to weanling mice.[48] Virus grows to titers of 10^8 to 10^9 infectious doses per milliliter. Low-passage material is highly infectious for humans during routine laboratory handling, and therefore appropriate precautions should be taken by laboratory workers. It is difficult to distinguish chikungunya from o'nyong-nyong virus serologically, but suckling mice that are susceptible to chikungunya are hardly susceptible to o'nyong-nyong virus.

If a mouse brain seed suspension of low-passage virus is prepared and inoculated into other mice at a 1:5 or 1:10 final concentration, death may be delayed significantly, may be sporadic, or may not occur at all. This difference is due to autointerference.[20] Low-passage strains recovered in suckling mice characteristically demonstrate autointerference on inoculation at dilutions below 1:100.

Chikungunya virus produces a cytopathic effect in primary hamster kidney cells and in BHK-21, BSC-1, Vero, FL, HeLa, and rhesus kidney cells.[30] Virus multiplies in *Aedes aegypti*, *Aedes vittatus*, *Aedes albopictus*, *Anopheles stephensi*, and *Culex fatigans* continuous cell lines and in a cell line derived from *Drosophila*. Plaque assays have been described in LLC-MK2, Vero, and BHK-21 cells and in duck and chick embryos.[30]

TRANSMISSION

Chikungunya virus has been recovered from wild *A. aegypti* in Tanzania, Nigeria, India, and Thailand; from *Aedes africanus* in Uganda and Bangui; from *Aedes furcifer-taylori* in South Africa and Senegal; and from *Aedes luteocephalus* and *Aedes dalzieli* in Senegal.[2,16,18,29,37,39] Occasional isolates have been recovered from *Mansonia fuscopennata* in Uganda and from *C. fatigans* in Thailand and Tanzania.

Transmission to humans has been demonstrated with the *A. furcifer-taylori* group; transmission to monkeys or mice has been demonstrated with *A. aegypti*, *A. albopictus*, *Aedes calceatus*, *Aedes triseriatus*, *Aedes togoi*, *Aedes pseudoscutellaris*, *Aedes polynesiensis*, *Anopheles albinanus*, *Mansonia africana*, *Erethmapodites chrysogaster*, and *Aedes apicoargenteus*.[30,36,44,51]

Tesh and colleagues[55] examined *A. albopictus* strains from 13 geographic locations from Hawaii to Africa and found considerable variation in susceptibility to infection by oral feeding. The 50 percent oral infective dose (ID_{50}) of a Hawaiian *A. albopictus* strain for a wild-type virus from India was $10^{5.4}$ plaque-forming units per milliliter. The amount of virus replicated by different mosquito strains varied between $10^{4.6}$ and $10^{7.4}$ plaque-forming units per mosquito. These observations, plus a mathematical model of chikungunya virus transmission developed by deMoor and Steffens,[17] suggest that major factors in determining the endemicity of chikungunya may be arthropod related. Tesh and associates[55] suggested that susceptibility to oral infection and the amount of virus replicated may be under genetic control, whereas deMoor and Steffens[17] found mosquito longevity to be the most important determinant in epidemic transmission of chikungunya.

Serologic studies have demonstrated repeatedly the presence of antibodies in humans throughout the moist forests and semiarid savannas of East and West Africa.[1,18,39,49] Most recorded chikungunya infections in people occur in areas infested with *A. aegypti*. The epidemiology in these areas is similar to that of other *A. aegypti*–borne diseases and parallels the distribution of the vector. When *A. aegypti* mosquitoes are abundant in occupied dwellings, infection rates can be expected to be highest in women and children who are at home during daylight hours. When *A. aegypti* is found in public buildings, schools, and hospitals, outbreaks may involve persons in occupational patterns. Characteristically, chikungunya pandemics are explosive. Studying *A. aegypti* in laboratory mice, Rao and colleagues[46] documented mechanical transmission. Viremia in humans may be as high as 10^8 infectious doses per milliliter.[46] Because the extrinsic incubation period in *A. aegypti* is relatively long, the explosive nature of chikungunya outbreaks is explained best by mechanical transmission.

The large chikungunya epidemic on Réunion Island was transmitted by *Aedes albopictus*. This outbreak was unusual because of its large size. However, as is characteristic of *A. albopictus* outbreaks, the epidemic proceeded at a deliberate pace for a period of many months. Because *A. albopictus* breeds outside houses, the outbreak did not respond to anti-mosquito efforts such as space spraying that would have effectively interrupted virus transmission by *A. aegypti*.[47,53]

EPIDEMIOLOGY

HOST RANGE

Chikungunya virus has the ability to replicate in a broad spectrum of vertebrate species. Newborn mice, hamsters, rats, rabbits, guinea pigs, and kittens all can be infected by subcutaneous inoculation of field strains of chikungunya virus,[12] which produces viremia, sickness, and, in most instances, death. Adult rabbits, mice, rats, and chickens inoculated peripherally have an asymptomatic viremia followed by an antibody response. Neutralizing or hemagglutination-inhibition antibodies to chikungunya have been recovered occasionally from sera obtained from ungulates. Attempts to infect cattle, goats, sheep, or horses experimentally failed to produce either viremia or an antibody response.[37] Vervet monkeys and baboons are infected readily. Rhesus monkeys were infected by intravenous and intramuscular inoculation and had viremia titers in excess of 10^7 mouse LD_{50}. *A. aegypti* transmitted virus to rhesus monkeys and could be infected by biting viremic animals.

Chikungunya antibodies have been found in vervet monkeys, baboons, chimpanzees, and red-tailed monkeys in Zimbabwe, South Africa, and Uganda.[35] The zoonotic status of chikungunya virus in Asia has not been studied carefully.

In Africa, zoonotic transmission to subhuman primates is surmised to take place in a wide variety of habitats, with transmission occurring in the forest canopy, at ground level, or both.[36] Chikungunya appears to be enzootic throughout much of eastern, central, southern, and western Africa.[30] Subhuman primate populations are affected in epizootics, which involve critical numbers of the susceptible population, followed by disappearance of the virus. Thus, chikungunya may maintain itself in wildlife populations by constantly moving epizootic activity, in much the same fashion as respiratory and enteric virus infections are maintained in humans. To expect that intercurrent and epidemic human infections in Africa are related to epizootic activity seems reasonable. Many putative or identified chikungunya vectors feed on people as well as on subhuman primates.

GEOGRAPHIC DISTRIBUTION

When susceptible human and *A. aegypti* populations are above the threshold level required for transmission, a person-mosquito-person cycle is established. This cycle probably is responsible for most of the large urban outbreaks of chikungunya studied during the past 40 years. In Africa, chikungunya outbreaks have been reported from Uganda, Tanzania, Zimbabwe, South Africa, Angola, Zaire, Nigeria, and Senegal.[30] This distribution best fits the present location of virus research laboratories. A reasonable assumption is that chikungunya is endemic throughout sub-Saharan Africa.

In February 2005, chikungunya cases appeared on Réunion Island. The epidemic continued for more than 18 months, and by July 2006, there had been nearly 300,000 cases, with 219 deaths attributed to the disease. Cases spread from Réunion to nearby islands of Mauritius, Madagascar, and the Seychelles.[53] Tourists on Indian Ocean islands who acquired chikungunya disease brought infections to European countries, creating a scare that epidemics would soon occur.[31]

According to historical evidence, chikungunya has spread from the African enzootic focus and caused large pandemics throughout both the Asian and American tropics.[10,15,52] Pandemics have swept India in 1824, 1871, 1902, 1923, 1964 to 1965, and 2006, with Sri Lanka (formerly Ceylon) involved in 1965 and 2006.[10] In the 19th century, chikungunya epidemics were reported in the Indonesian archipelago. An extensive serologic survey using the plaque-reduction neutralization test has suggested chikungunya activity possibly during World War II in Kalimantan and Sulawesi, Indonesia. During the late 1950s and early 1960s, chikungunya appears to have established itself endemically in Southeast Asia and was transmitted continuously in urban populations in Thailand, Cambodia, and South Vietnam, possibly into the 1970s.[13,22,59] Involvement of urban populations in Burma appears to have been intermittent, with outbreaks recorded in 1963 and from 1970 to 1973.[38] A large chikungunya epidemic affected much of Indonesia in 1983 and 1984 (Slemons, R. D., personal communication) and Burma in 1984 and 1985.[57] Chikungunya virus was isolated in Australia in 1989.[23] Serologic evidence of chikungunya virus infection has been found throughout the Philippines, possibly during World War II; since then, localized outbreaks have occurred in Manila, Philippines, in 1967 and Negros, Philippines, in 1968[3,9,34] and as recently as 1986.[14] From 1990 to 1995, chikungunya remained endemic at low levels in Thailand, Myanmar (formerly Burma), and Indonesia.[56] Little or no chikungunya virus infection has been reported in the 20th century in Papua New Guinea, the Solomon Islands, Vanuatu (formerly New Hebrides), the Caroline Islands, the Pacific Islands, or any of the American tropics.[54]

A pertinent question to ask is why chikungunya and dengue viruses do not have an identical geographic distribution although both are transmitted by *A. aegypti*. The question may be answered partially by differences in the transmission of chikungunya and dengue. The threshold for infection of chikungunya virus in *A. aegypti* is relatively high: approximately $10^{5.6}$ mouse infectious doses are required to infect 50 percent of adult females.[55] The infection threshold of female *A. aegypti* for dengue virus is similar.[19] However, *A. aegypti* mosquitoes infected with chikungunya virus transmit virus to vertebrates poorly, whereas dengue-infected mosquitoes transmit with great regularity.[19] Thus, transmission of chikungunya virus would be expected to occur only in areas with high human susceptibility rates and consistently high densities of *A. aegypti* and possibly by mechanical transmission.

CLINICAL MANIFESTATIONS

The incubation period of chikungunya fever usually is 2 to 4 days. In infants, the disease typically begins with the abrupt onset of fever, followed by flushing of the skin. Febrile convulsions may occur in as many as one third of patients. After 3 to 5 days of fever, a generalized maculopapular rash and lymphadenopathy are noted. Conjunctival injection, swelling of the eyelids, pharyngitis, and symptoms and signs of upper respiratory tract disease are common manifestations. No enanthem occurs. Some infants have a biphasic fever curve, and arthralgia may be severe, although it is not seen frequently.[5,11,21,28,43]

In older children, fever develops acutely and is accompanied by headache, myalgia, and arthralgia involving various joints. Residual arthralgia is an uncommon occurrence. An early macular blush and a maculopapular rash accompany or immediately precede defervescence. At the same time, marked lymphadenopathy occurs. Febrile convulsions are observed commonly. Hemorrhagic findings, including a positive tourniquet test result, are rare events.[5,11,21,28,43]

TABLE 187–4 Chikungunya and Dengue in Children*: Comparison of Onset of Illness

	Hospitalized				Outpatient			
	Chikungunya (32 cases)		Dengue (523 cases)†		Chikungunya (17 cases)		Primary Dengue (29 cases)	
Day of Illness	*Number*	*Percent*	*Number*	*Percent*	*Number*	*Percent*	*Number*	*Percent*
0	8	25	2	0.4	0	0	0	0
1	15	47	44	8	11	65	8	27
2	5	16	67	13	1	6	5	17
3	2	6	145	28	2	12	3	10
4	1	3	148	28	0	0	7	24
5	1	3	84	16	3	17	4	14
6			20	4			2	
7			11	2			7	
8 or more			3	0.6				

**Patients with simultaneous dengue and chikungunya are excluded from analysis.*
†Includes primary and secondary infections.
Data from Nimmannitya, S., Halstead, S. B., Cohen, S. N., et al.: Dengue and chikungunya virus infection in man in Thailand, 1962-1964. I. Observations on hospitalized patients with hemorrhagic fever. Am. J. Trop. Med. Hyg. 18:*954-971, 1969; and Halstead, S. B., Nimmannitya, S., and Margiota, M. R.: Dengue and chikungunya virus infection in man in Thailand, 1962-1964. II. Observations on disease in outpatients. Am. J. Trop. Med. Hyg.* 18:*972-983, 1969.*

TABLE 187–5 Chikungunya and Dengue in Children: Comparison of Duration of Illness

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Arthralgia or arthritis is the most conspicuous feature of chikungunya in adults. Usually, patients can identify the precise time of onset and the joint first affected in a chikungunya illness. Swelling and redness of joints and even the pinnae of the ear may occur.[11]

Although dengue and chikungunya illnesses are similar, important distinguishing features are summarized in Tables 187–4 through 187–8 from clinical data obtained from children in Thailand.[21,43]

Table 187–4 shows the abrupt onset and early severity of chikungunya versus dengue illnesses, many of which came to medical attention only several days after the onset of fever.

Chikungunya virus infections are shorter in duration than are dengue virus infections (Table 187–5). Almost half of children with chikungunya had a fever that ended within 72 hours after onset, whereas the median duration of dengue fever was 2 days longer.

Many constitutional signs and symptoms occur with similar frequency in chikungunya and dengue viral infections and cannot be used to differentiate the illnesses clinically (Table 187–6). However, a terminal maculopapular rash, arthralgia or arthritis, and conjunctival injection were more common symptoms in chikungunya than in dengue (Table 187–7). Shock has been reported infrequently in chikungunya.[11,50,58] It was not observed in Thai cases.[43] Changes in taste perception, post-illness bradycardia, and post-illness depression or asthenia are found rarely in chikungunya; these manifestations are distinctive findings in patients with dengue.

Hemorrhagic phenomena rarely occur with chikungunya virus infection. The frequencies of hemorrhagic findings in chikungunya and primary and secondary dengue viral infections in Thai children are compared in Table 187–8. The frequency of minor hemorrhagic manifestations in outpatient and inpatient dengue did not differ significantly from that in chikungunya cases. However, petechial rash and spontaneous hematemesis or melena developed only in hospitalized dengue cases.

Brighton and Simson[5] divided chikungunya cases into four groups: (1) patients who experienced joint pain but after days, weeks, or months fully recovered, (2) patients who experienced little spontaneous joint pain but found it was exacerbated by exercise, (3) patients with residual joint stiffness, and (4) patients with residual joint pain and swelling. Approximately 8 percent of all patients fell into the last two groups. Most of these studies are composed of adult patients. Little is published describing chikungunya arthritis in children.

PATHOGENESIS AND PATHOLOGY

The pathologic process of fatal human chikungunya illness has not been studied extensively.[58] Wild-type chikungunya strains produce a hemorrhagic enteritis in mice and hamsters.[20]

DIAGNOSIS

The differential diagnosis includes the viral causes of dengue fever syndrome. In Australia and the western Pacific area, Ross River fever is a frequent cause of epidemic, arthropod-borne, viral arthralgia.

By classic test methods, the diagnosis depends on demonstration of a significant increase in antibody after an illness. With use of a single serum sample collected 5 or more days after onset, detection of antibodies of the IgM class is possible with an IgM capture enzyme-linked immunosorbent assay (ELISA).[8,57] Ordinarily, a serum sample collected within 5 days of the onset of fever will not contain hemagglutination-inhibition, complement-fixation, or neutralizing antibodies.[11,21] Neutralizing and hemagglutination-inhibition antibodies generally are present

TABLE 187–6 Chikungunya and Dengue in Children: Comparison of Frequency of Clinical Findings

	Hospitalized				Outpatient			
	Chikungunya (32 cases)		Dengue (142 cases)*		Chikungunya (17 cases)		Primary Dengue (27 cases)*	
Findings	Number†	Percent	Number†	Percent	Number	Percent	Number	Percent
Headache	13/19	68	37/83	45	2	12	4	15
Injected pharynx	28/31	90	121/125	97	12	71	27	100
Enanthem	3/27	11	7/84	8	0	24	0	22
Rhinitis	3/31	6	6/47	13	4	6	6	41
Cough	7/30	22	17/79	22	1	35	11	56
Vomiting	19/32	59	73/126	58	6	6	15	15
Constipation	12/30	40	16/30	53	0	18	4	4
Diarrhea	5/32	16	5/78	6	1		1	7
Abdominal pain	6/19	32	38/76	50	3		2	
Lymphadenopathy	8/26	31	32/79	41				
Restlessness	10/30	33	17/79	22				

**Includes primary and secondary dengue infections.*
†Number with finding/number with observations recorded.
Modified from Nimmannitya, S., Halstead, S. B., Cohen, S. N., et al.: Dengue and chikungunya virus infection in man in Thailand, 1962-1964. I. Observations on hospitalized patients with hemorrhagic fever. Am. J. Trop. Med. Hyg. 18:*954-971, 1969; and Halstead, S. B., Nimmannitya, S., and Margiota, M. R.: Dengue and chikungunya virus infection in man in Thailand, 1962-1964. II. Observations on disease in outpatients. Am. J. Trop. Med. Hyg.* 18:*972-983, 1969.*

TABLE 187–7 Chikungunya and Dengue in Children: Clinical Findings Occurring with Different Frequency

From Nimmannitya, S., Halstead, S. B., Cohen, S. N., et al.: Dengue and chikungunya virus infection in man in Thailand, 1962-1964. I. Observations on hospitalized patients with hemorrhagic fever. Am. J. Trop. Med. Hyg. 18:*954-971, 1969.*

TABLE 187–8 Chikungunya and Dengue in Children: Comparison of Hemorrhagic Manifestations

Modified from Nimmannitya, S., Halstead, S. B., Cohen, S. N., et al.: Dengue and chikungunya virus infection in man in Thailand, 1962-1964. I. Observations on hospitalized patients with hemorrhagic fever. Am. J. Trop. Med. Hyg. 18:*954-971, 1969; and Halstead, S. B., Nimmannitya, S., and Margiota, M. R.: Dengue and chikungunya virus infection in man in Thailand, 1962-1964. II. Observations on disease in outpatients. Am. J. Trop. Med. Hyg.* 18:*972-983, 1969.*

in samples collected 2 weeks or more after the onset of fever. Neutralizing antibody can be measured by the virus-dilution method in suckling mice (or weanling mice with use of the Ross high-mouse passage strain) or by the serum-dilution method in any of a variety of tissue cultures or plaque assay techniques. The detection of IgM-containing ELISA antibodies or a fourfold or greater rise in antibodies in paired sera is diagnostic of a recent infection.

Virus may be identified by use of polymerase chain reaction or by isolation of the agent by inoculation of acute-phase serum or other suspect materials intracerebrally in 1- to 2-day-old mice or in tissue cultures. On initial passage, death may occur within 2 to 5 days after inoculation. An autointerference phenomenon is noted if low-dilution mouse-to-mouse passages of infected mouse brain are performed. Passage at a 10^{-3} dilution or higher avoids this effect.

Vero cells and suckling mice are equally effective for primary isolation.

TREATMENT

Treatment is supportive. Chikungunya symptoms are refractory to aspirin or other nonsteroidal anti-inflammatory drugs. However, one report demonstrated that chloroquine phosphate (250 mg/day) provides prompt relief from chronic arthralgia in a high proportion of sufferers.[4] Anecdotal reports from India during the 2006 epidemic describe similar efficacy, including relief of acute-phase symptoms. In cell cultures in vitro, chloroquine has some antiviral activity against chikungunya. Controlled trials are in progress.

Bed rest is advised during the febrile period. Antipyretics or cold sponging should be used to keep the body temperature below 40° C (104° F).

Analgesics or mild sedation may be required to control pain. Post-illness arthritis may require continued treatment with anti-inflammatory agents and graduated physiotherapy.

Salicylates, because of their hemorrhagic potential, are contraindicated.

Febrile convulsions are treated with phenobarbital given intravenously or orally and continued until the temperature is normal. Severe or intractable convulsions may respond to intravenous diazepam.

Children who have lost excessive fluid because of vomiting, fasting, or thirsting and who cannot take oral fluids may require intravenous rehydration. Individuals with severe hemorrhagic phenomena should be studied for underlying hemostatic disorders.

PROGNOSIS

In a few instances, isolation or serologic evidence of recent infection has been obtained in persons with severe hemorrhagic findings or in individuals dying during an acute febrile illness.[11,28,40,50,58] In addition to hemorrhage, neurologic and myocardial involvement has been reported during chikungunya infection in adults.[11,14] In adults, arthralgia may persist for weeks, and exercise may prolong this symptom. Typically, pain shifts from joint to joint and is worse in the morning and on first use of the joint. Swelling of ankles, wrists, and fingers occurs frequently. In older patients, the sequelae may resemble rheumatoid arthritis. A post-illness destructive arthropathy has been reported.[6] Chikungunya virus infection might coincide with other pathologic processes and result in death of the individual.[40] Carefully studied, virologically documented cases have shown neither thrombocytopenia nor severe neutropenia.[28] Until more is known of the pathogenesis of chikungunya virus infection, estimation of the frequency with which death can be attributed directly to chikungunya fever will be difficult.

Infants with chikungunya may experience residual neurologic deficits after febrile convulsions.

PREVENTION

Formalin-treated chikungunya virus (Ross strain) grown in African green monkey kidney cells produces a satisfactory immune response and resistance to challenge when it is administered in three divided doses in monkeys.[24] A vaccine prepared under similar conditions produced hemagglutination-inhibition, complement-fixation, and neutralizing antibody responses in susceptible human volunteers.[25] A comparative study from the same laboratory was made of formalin-inactivated chikungunya vaccines prepared from the virus propagated in African green monkey kidney monolayers and concentrated chick embryo suspension cultures.[60] The latter vaccine was significantly more protective to mice against live homologous virus challenge and stimulated the production of four to five times more circulating antibodies than did the vaccine prepared with virus grown in African green monkey kidney cultures. Nakao and Hotta,[42] studying chikungunya grown in BHK-21 cells, found that ultraviolet-inactivated preparations were significantly more immunogenic than were formalin-treated virus. Tween-ether–extracted virus preparations also have been found to be immunogenic. A live attenuated chikungunya vaccine has been developed.[32] After the pandemic of 2006, the disability and deaths attributed to chikungunya renewed interest in development and licensing of chikungunya vaccines.

At present, prevention consists of avoiding mosquito bites. For urban outbreaks in most of the Asian and African tropics, the regimen for individual protection and for chronic and emergency control is the same as has been described for dengue. When other vectors are involved, measures designed to combat *A. aegypti* may fail. In such outbreaks, expert entomologic advice will be needed to design appropriate preventive measures.

For control of mosquitoes, epidemic measures, and health education, see Chapter 188d.

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CHAPTER **187e**

Ross River Virus Arthritis

John G. Aaskov

"Epidemics" of a benign disease causing polyarthralgia and rash were first described in Australia in 1927.[55] Following the recovery of the causative agent and the advent of serologic tests able to diagnose Ross River virus infection, epidemic polyarthritis has been recognized as endemic in Australia and has occurred as epidemics in numerous Pacific nations. Approximately 4000 cases of epidemic polyarthritis are reported in Australia each year, with a peak of 7800 cases in 1996.

Some confusion has been generated recently by use of the term *Ross River fever* to describe clinical Ross River virus infections because fever does not develop in more than half of those with clinical disease.[52] Additional confusion has been generated by efforts to describe any polyarthritis caused by an Australian arbovirus as epidemic polyarthritis. The term *epidemic polyarthritis* should be used to describe only clinical disease caused by Ross River virus.

ETIOLOGIC AGENT

Investigations of an epidemic of polyarthritis in southern Australia in 1956[10,67] suggested that an alphavirus was the causative agent. This agent was confirmed when Ross River virus was isolated from *Aedes vigilax* mosquitoes collected beside Ross River, near Townsville in northern Australia, and shown, on serologic grounds, to be the causative agent of epidemic polyarthritis.[22,25] Although the first isolation of virus from a human was from a febrile child without arthritis,[21] subsequent use of mosquito cell lines enabled numerous isolations to be made of Ross River virus

from epidemic polyarthritis patients in Australia and the Pacific Islands.[8,26,60,70]

Ross River virus is related serologically to Getah and Bebaru viruses in the Semliki Forest virus subgroup.[19] Nucleotide sequencing has confirmed this close relationship among Ross River, Getah, and Semliki Forest viruses.[59] Phylogenetic analysis of a short (505 nucleotide) region of the E2 and E3 genes of Ross River virus suggested the existence of three or four genotypes of this virus.[62] No association has been observed between any particular genotype and disease in humans.

Cryoelectron microscopic studies[15,58,72] suggest that the nucleocapsid of Ross River virus is approximately 400 Å in diameter and has a T = 4 quaternary structure. It is surrounded by a membrane bilayer that is penetrated by 80 spikes, also arranged in a T = 4 lattice. Each spike is a trimer of heterodimers of the envelope glycoproteins E1 and E2. The E2 protein makes contact with heparin on the surface of host cells, and antibodies that combine with this protein neutralize infection in vitro.[68,71]

The virus can be adapted to grow to high titer in the muscle and brain of day-old mice, in which it causes paralysis and death.[50,53] Ross River virus also grows to high titer in vertebrate and mosquito cell lines.[8]

TRANSMISSION AND EPIDEMIOLOGY

Ross River virus is endemic in all mainland states of Australia and in Papua New Guinea. No transmission of Ross River virus had been reported from the Pacific island states involved in the 1979 to 1980 epidemic (Fiji, Samoa, Tonga, Cook Islands)[5,26,45,60,70] until 2003, when cases of epidemic polyarthritis began to be detected in travelers who had visited Fiji.[46] Sporadic cases, without local virus transmission, have occurred in Europe and the United States in tourists and military personnel returning from Australia.

Isolation of Ross River virus from more than 20 species of mosquitoes,[61] in particular *A. vigilax*[25,37] and *Culex annulirostris*,[24,37] and the presence of antibody to the virus in almost as many species of animals, particularly mammals,[22,23] suggested a mammal-mosquito cycle with humans as an incidental host. However, improved laboratory diagnostic services have shown clinical infection in humans occurs year-round, although most cases are seen in the late summer and in autumn, and most patients are city dwellers.[7,52] Taken together with the explosive spread of disease during the 1979 to 1980 epidemic of Ross River virus infection in the Pacific,[5,26,60,70] this virus appears to be maintained in either of two cycles, mammal-mosquito-mammal or human-mosquito-human, with movement of virus between the two. Evidence also has been obtained of transovarial transmission of Ross River virus in *Aedes* mosquitoes.[44,47]

Patients may be viremic for up to 7 days after the onset of symptoms.[8,60] The incubation time from infection to the onset of symptoms may vary from 1 to 27 days, 7 to 9 days being the usual interval in endemic areas.[30]

In endemic areas, the ratio of subclinical to clinical infection may be as high as 50:1,[7] but during outbreaks, the ratio can be reduced to 4:1 or less.[5,39] Approximately 1.5 percent of humans living in northern Australia are infected with Ross River virus each year,[7] but the rates are much higher in some areas.[17,18]

Infection rates are the same in both sexes, and early reports that clinical disease was more common in females than in males[20,52] are not supported by Australian national data for the past decade. An association between HLA-DR7 and clinical disease also has been observed.[36]

Ross River virus has been shown to cross the placenta in mice[3] and humans.[6] In mice, the result is extensive postpartum mortality,[3] but in humans, no evidence of morbidity or mortality in children infected in utero has been found.

CLINICAL MANIFESTATIONS[10,20,22,27,28,38,52,54]

Epidemic polyarthritis occurs as a mild to severe illness characterized by joint pain, particularly in the knees and the small joints of the hands and feet. Frequently, the joint pain is accompanied by a maculopapular or vesicular rash on the trunk and limbs and sometimes by fever or chills or both. Sore throat, lymphadenopathy, paresthesia and tenderness of the palms and soles, exanthems, and, more rarely, petechiae have been observed. Most patients experience several weeks of painful arthritis followed by a slow decrease in the severity of symptoms during the 30 to 40 weeks required by most for recovery.[2] Infrequently, symptoms may persist for a year or more,[28] and some patients may experience episodes of severe arthritis during convalescence. One study has linked Ross River virus infection to a chronic fatigue syndrome.[41]

In a small proportion of patients (<0.1%), clinical disease may develop without arthritis.[21] Rare cases of glomerulonephritis,[33] hematuria,[11] and central nervous system symptoms[1,48,57,63] accompanying Ross River virus infection in humans also have been reported.

The severity and duration of symptoms are age related. Most clinical disease occurs in adults, who also have the most severe and prolonged symptoms. Teenagers may be incapacitated for only a few days and asymptomatic after 1 to 2 weeks.

The only other species known to develop clinical disease (musculoskeletal symptoms) after a natural infection with Ross River virus is equines.[13]

PATHOLOGY

The pathogenesis of Ross River virus disease in humans is not understood. The literature describing the pathologic process of Ross River virus infection in mice is extensive,[50,53,64] but with one exception,[51] very little similarity between the patterns of disease in humans and in mice exists.

Synovial tissue from patients with epidemic polyarthritis may show a marked mononuclear leukocyte infiltrate with small amounts of fibrin deposition and synovial-cell hyperplasia.[40,69] No virus or viral antigen has been detected in synovial tissue from patients with epidemic polyarthritis, but a 250-nucleotide fragment of the viral genome was detected by reverse transcription–polymerase chain reaction (RT-PCR) in synovium from 2 of 12 patients 5 weeks after the onset of symptoms.[69] Ross River virus has been shown to replicate for a similar period in human synovial cells maintained at 35° C in vitro.[42] Whether virus persists in joints as long as symptoms are present remains unclear, and ethical and technical constraints prevent this issue's being explored in a systematic manner.

Fluid from affected joints consists almost entirely of mononuclear leukocytes at all stages of disease.[16,29,31] Human synovial fibroblasts infected with Ross River virus in vitro produce elevated levels of mRNA coding for chemoattractants and other mediators, such as monocyte chemoattractant protein 1, interleukin-8, granulocyte-macrophage colony-stimulating factor, and tumor necrosis factor–α, but elevated levels of the proteins for which they code have not been demonstrated.[49]

No evidence of immune complexes or complement activation in arthritic joints has been found, and viral antigen can be detected (in macrophages) for only 5 to 7 days after the onset of symptoms, despite the persistence of arthritis for 30 to 40 weeks.[32] No significant levels of anticollagen antibodies could be detected in serum from epidemic polyarthritis patients.[35] Neither anti–Ross River virus antibody levels nor a primary, virus-specific, cell-mediated immune response to Ross River virus infection correlated with the presence or absence of arthritis in human infections.[4] In patients with epidemic polyarthritis, a nonspecific

immunologic response (natural killer cells) correlated well with the presence or absence of arthritic symptoms.[2,4] Functional natural killer cells have been recovered from the knee of a patient with epidemic polyarthritis,[40] and natural killer cells have been found to kill autologous synovial tissue in vitro.[2]

A rash develops in approximately 30 percent of patients with epidemic polyarthritis.[52] The dermis underlying these lesions contains a perivascular infiltrate of CD8+ T cells and some monocytes and macrophages.[34] No immunoglobulin or complement deposition has been observed in these lesions, although Ross River virus antigen was detected in basal epidermal and eccrine duct epithelial cells.[34]

DIAGNOSIS

The signs and symptoms of a Ross River virus infection (see earlier) are too nonspecific to permit establishment of a diagnosis on clinical grounds alone. Most clinical diagnoses of epidemic polyarthritis are confirmed by use of an indirect enzyme-linked immunosorbent assay (ELISA)[56] to detect Ross River virus–specific IgM antibodies in serum collected 7 to 10 days after the onset of symptoms. Various forms of this assay are available now from commercial suppliers. IgM can be detected for approximately 3 months after the onset of disease.[7]

In rare cases (perhaps less than 1 in 5000), anti–Ross River virus IgM antibody production may persist for years. Assays for serum anti–Ross River virus IgA might be an alternative to IgM assays because the duration of IgA production is shorter than for IgM.[14] However, such IgA assays have not been adopted by routine diagnostic laboratories. More recently, indirect ELISA assays that measure the avidity of anti–Ross River virus antibody have been shown to be able to discriminate between recent and old infections with this virus.[43]

Although virus sometimes can be isolated from seronegative, acute-phase sera,[5,8,26,60,70] this procedure is not performed in most routine diagnostic laboratories. A number of mostly unpublished RT-PCR protocols[66] have been developed for the detection of Ross River virus RNA, but they are less sensitive than is virus isolation for the detection of virus and are not used routinely for the diagnosis of human disease.

Because epidemic polyarthritis is an arthritic disease, care must be taken to avoid false-positive results caused by the presence of rheumatoid factor when indirect ELISA is used to detect anti–Ross River virus IgM antibodies. Such precautions include removal of IgG or rheumatoid factor before testing sera or performing tests for rheumatoid factor in parallel with ELISA.

Another source of false-positive diagnoses is the production of anti–Ross River virus IgM antibody caused by polyclonal B-cell activation after infection of a Ross River virus immune host with Epstein-Barr virus, cytomegalovirus, *Coxiella burnetii*, or *Plasmodium* spp.

Other viral infections to be considered when a patient is suspected of having epidemic polyarthritis include infections with Barmah Forest, Sindbis, Kunjin, or rubella viruses.

TREATMENT AND PROGNOSIS

No specific antiviral therapy is available, although experimental evidence suggests that interferon-γ may ameliorate acute disease.[65] Nonsteroidal anti-inflammatory medication or steroids[12] have been used to treat patients with severe disease or prolonged symptoms, but the efficacy of such treatment has not been evaluated in any systematic manner. Rest, while maintaining mobility and muscle tone, appears to provide significant relief.

Epidemic polyarthritis has not been shown to progress to chronic joint disease.

PREVENTION

A killed virus vaccine is being developed.[9] In urban areas, public health mosquito control programs reduce mosquito numbers, but elimination of mosquito exposure for those with outdoor occupations and pastimes is impossible. Personal protection (insect screening of houses, wearing of protective clothing, and application of insect repellents) is the only reliable way to avoid being infected.

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CHAPTER **187f**

Other Alphaviral Infections

Theodore F. Tsai

O'NYONG-NYONG

The virus name is derived from the Acholi term meaning "very painful and weak," which describes the acute constitutional symptoms and polyarthritis associated with the disease of the same name.[13,23] The virus is related closely to chikungunya virus, but unlike most arboviruses, it is transmitted by anopheline mosquitoes in an interhuman cycle analogous to that of malaria in rural Africa. Apart from the human-to-human epidemic cycle, the natural transmission cycle and involvement of other vertebrate hosts are largely unknown. The disease first came to attention in, arguably, the largest arboviral epidemic ever recorded. After emerging in Uganda in 1959, the epidemic spread to South and West Africa and produced an estimated 2 million cases before the outbreak died out spontaneously 3 years later. The disease apparently had disappeared from East Africa, although the virus was isolated sporadically from mosquitoes until 1996, when only the second ever recognized outbreak appeared, in Uganda.[13] In retrospect, the virus had been circulating at low levels in the area several years before this epidemic occurred. Human and animal infections have been documented serologically in Nigeria, Sierra Leone, Ghana, and Chad. Factors underlying emergence of the virus, its natural reservoir, and the reasons for its disappearance can only be surmised, but exhaustion of susceptible human hosts is a likely explanation for the cessation of epidemic transmission. Clinically, the disease is similar to chikungunya, although lymphadenopathy is more pronounced and mimics, in some cases, the enlarged cervical lymph nodes of sleeping sickness.[2] Virus can be isolated from acute-phase blood samples, or the diagnosis can be confirmed serologically by detection of specific IgM. Personal protective measures against malaria (e.g., use of bed nets) should be effective against acquiring infection.

IGBO-ORA FEVER

Igbo-Ora virus has been determined genetically to be a subtype of o'nyong-nyong virus and, like it, is transmitted by anopheline mosquitoes and (without adaptation) is nonpathogenic in suckling mice.[19] The viral transmission cycle has not been defined. Sporadic cases and small outbreaks have been reported from Igbo-Ora and Ibadan, Nigeria, the Ivory Coast, and the Central African Republic. Based on a single case description, the illness consists of fever, polyarthritis, and pharyngitis.

BARMAH FOREST FEVER

Barmah Forest fever occurs only in Australia, where it has been a sporadic and occasionally hyperendemic infection leading to a polyarthropathy indistinguishable from that of Ross River fever.[6,21,23] The viral transmission cycle seems to share features with that of Ross River virus; however, independent outbreaks have occurred in locations where both viruses are enzootic, thus indicating differences in their transmission cycles or human susceptibility. Seroprevalence to Barmah Forest virus is generally lower. Anecdotal cases complicated by central nervous system signs and glomerulonephritis have been reported.[12,23] Serologic diagnosis is straightforward in most cases, with little cross-reaction between the viruses.

SINDBIS FEVER

Sindbis virus was named after a district north of Cairo, Egypt, where it first was isolated. It is the prototype of an antigenic complex that also includes western equine encephalitis virus and several Sindbis viral subtypes, including Ockelbo and related strains isolated from Scandinavia and the Babanki strain from West Africa. Two genotypes consisting of the South African/Scandinavian strains and the Australian/Asian strains have been defined. The close genetic relationship of the South African and Scandinavian strains indicates a recent introduction to Europe, possibly by a migrating bird.

The virus is distributed widely over four continents, although only sporadic cases have been reported from Asia and Australia. Transmission is endemic and occasionally hyperendemic in Africa and Scandinavia. The virus is transmitted to birds by various species of *Culex* mosquitoes, and humans are infected when virus transmission "spills out" of the enzootic cycle by the intervention of bridging vectors that feed on viremic birds and later on humans.

Endemic infections occur with varying intensity in areas of Africa.[10,17] Seroprevalence rates range from a few percent to 20 percent in some areas. Outbreaks producing hundreds to thousands of cases have been described. Concurrent transmission with West Nile virus, transmitted in the same avian cycle, is common. In South Africa, transmission occurs during the austral summer from December to April. Infections rarely may be acquired during travel.[4]

An endemic focus in Scandinavia (between 60 and 63 degrees latitude in Sweden) was recognized after a series of outbreaks in 1981 led to several hundred cases in Sweden, Norway, Finland, and adjacent areas of western Russia called, locally, Ockelbo fever, Pogosta disease, and Karelian fever.[5,15,18] Seroprevalence is low, around 5 percent. By estimation from seroprevalence rates, 600 to 1200 clinical cases may occur annually in Sweden alone. Most cases occur from July to September in middle-aged adults exposed to forested areas while picking berries or mushrooms or during other recreational activities. Outbreaks seem to peak at 7-year intervals.

Acute arthralgia and rash are the principal features of the illness. They may be preceded by mild fever, headache, and myalgia, but patients may have arthralgia alone, and children may exhibit fever and nonspecific symptoms alone. The joints, especially the ankles, wrists, knees, fingers, and toes and less often the hips, shoulders, elbows, neck, and back, are involved symmetrically. The joints appear swollen, and movement is limited. The Achilles and wrist tendons may be inflamed as well, sometimes causing nerve entrapment and paresthesias. Some patients are confined to bed and unable to walk, but typically, the joint pain and stiffness lead to a lesser degree of discomfort and compromised function. A fine papular and sometimes pruritic rash appears on the trunk and extremities (including the palms and soles) but usually spares the face and head, and after a few days the rash develops a stained appearance and disappears. Lesions on the hands and feet may vesiculate. Joint symptoms resolve within 3 to 4 months in approximately 60 percent of cases; however, in the remainder, symptoms may persist for 3 to 4 years.[11,23] Serologic evidence of infection in patients with central nervous system signs has been reported from China.[7]

The illness is not differentiated easily from West Nile fever, which is transmitted under similar epidemiologic circumstances, and has been mistaken for rubella. The diagnosis is confirmed serologically by detection of specific IgM in acute-phase serum samples. IgM is detectable for months in some patients. The virus can be isolated, but not reliably, from acute-phase capillary blood and from skin lesions. Viral genomic products can be detected in skin biopsy samples.[9]

Treatment is symptomatic. Persons who choose to enter sylvatic transmission foci in the summer should apply mosquito repellents and dress appropriately.

MAYARO FEVER

Infections are highly prevalent in the forested areas of South and Central America, where Mayaro virus is transmitted among *Haemagogus* mosquitoes, marsupials, and small mammals, somewhat analogous to the jungle cycle of yellow fever. Seroprevalence rates increase with age to higher than 50 percent in native populations in some locations. The virus was isolated first from sporadic fever cases on Trinidad and named after the island's Mayaro district.[1] Outbreaks have occurred in Bolivia, Pará (a state in Brazil), and Surinam, principally in men occupationally exposed to forested sites and in residents of adjoining villages, as well as in travelers returning from Central America.[3,20,22,24] Urban epidemic transmission is a hypothetical risk if the virus were introduced to *Aedes aegypti*–infested cities. The virus is placed in the Semliki Forest antigenic complex; its Una subtype causes arthritis in horses.

The illness consists of acute polyarthritis and rash.[23] Joint swelling, pain, and stiffness principally involve the hands, wrists, ankles, toes, elbows, and knees. The hands may be so swollen that they cannot be closed, and joints of the lower extremities may be so painful that patients limp or are unable to walk. Milder cases may occur, however. The morbilliform rash is difficult to distinguish from rubella, with individual or coalescent papular lesions occurring on the trunk and extremities and usually sparing the face. Rash occurs more often in children than in adults. Mild fever, pharyngitis, conjunctivitis, headache, and lymphadenitis occur in some patients. The constitutional symptoms resolve rapidly, but the joint symptoms may wax and wane for several weeks or months. Pneumonitis and renal dysfunction that have been described in some cases may have been caused by concurrent infections. Transient leukopenia usually develops; some patients have had mild elevations in liver enzyme activities and bilirubin concentration. A fatal case of yellow fever was reported in a patient recovering from Mayaro fever.

The illness is differentiated from other tropical febrile illnesses with rash and musculoskeletal pain by the specific involve-

ment of the joints and the sylvatic setting of exposure. Some patients have features closely resembling rubella. Virus can be isolated from acute-phase blood specimens, but laboratory confirmation by IgM serology is sensitive, specific, and more practical. Symptoms are treated with nonsteroidal anti-inflammatory drugs and rest. Repellents should be used when sylvatic-enzootic foci cannot be avoided.

SEMLIKI FOREST VIRAL INFECTION

Febrile illnesses with severe persistent headache have been reported from Central and West Africa, where Semliki Forest virus is transmitted principally by forest *Aedes* mosquitoes.[16] The virus appears to be a common cause of infection in horses as well. The MeTri subtype has been implicated serologically in encephalitis cases in Vietnam; its transmission cycle has not been described. A fatal case of encephalitis in a laboratory worker has been reported.[8]

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SUBSECTION 5

Flaviviridae

CHAPTER 188

FLAVIVIRUSES

CHAPTER 188a

ST. LOUIS ENCEPHALITIS

Theodore F. Tsai

Until the recent introduction of West Nile virus, St. Louis encephalitis was the most important arboviral infection in the United States because of its leading role as a cause of widespread epidemics. Encephalitis is the principal clinical manifestation, although milder central nervous system (CNS) syndromes do occur, especially in children. The virus is transmitted between birds and *Culex* mosquitoes; humans are incidental hosts.

ETIOLOGIC AGENT

The St. Louis encephalitis (SLE) virus is a member of the family *Flaviviridae* (group B arboviruses) within the antigenic complex that also includes Japanese encephalitis, Murray Valley encephalitis, and West Nile viruses.

Analysis of the E glycoprotein sequences of strains from the United States and Central and South America has identified three

genotypes corresponding to origins in the eastern and western United States and South America. Strains from a more than 60-year interval diverge by less than 10 percent in the total E gene nucleotide sequence, thus indicating a highly constrained viral adaptation to vector and vertebrate hosts. Strains from the three principal genotypes differ in biologic characteristics; epidemic-associated strains from the Ohio and Mississippi river basins and Florida display greater virulence in mice than do strains from the western United States associated with an endemic pattern of transmission.[13,40] Multiple viral genotypes with distinct biologic characteristics may be transmitted in relatively delimited areas within a region as small as a county in some years and more widely in others.[13,28]

ECOLOGY

SLE virus is transmitted in an enzootic cycle among birds and *Culex* mosquitoes in three distinct cycles associated with its four principal vector species: *Culex tarsalis* in the western and central United States, *Culex pipiens* and *Culex quinquefasciatus* in the east central and Atlantic states, and *Culex nigripalpus* in Florida. *Culex salinarius* and *Culex restuans* may have accessory roles in viral amplification and transmission (Fig. 188–1).[31,34,39,64]

In the western United States, *C. tarsalis*, which also is the vector of western equine encephalitis (WEE), serves as both an enzootic and epizootic vector. This species feeds chiefly on birds early in the summer and switches to mammalian hosts, including humans, in midsummer. The transmission cycles of West Nile and SLE viruses overlap in the eastern United States and also with WEE viruses in the West. Sparrows, finches, and other small birds, and especially their nestlings, are the principal amplifying hosts.[37] In the West, human infections result from encounters with the vector along natural and artificial waterways, irrigated farmland, and other breeding sites found chiefly in rural agricultural areas. The recent introduction of West Nile virus into an overlapping transmission cycle may have an effect of modulating SLE virus transmission because West Nile virus–infected birds are more refractory to development of infection and viremia with SLE virus than the reverse.[22]

In the Ohio and Mississippi valleys and on the Gulf Coast, infections are acquired in the peridomestic environment where *C. pipiens* and *C. quinquefasciatus* are, respectively, the enzootic vectors in northern and southern states. The mechanism by which the virus overwinters is not known; however, several observations suggest that the virus could persist locally in a resident vertebrate host (e.g., birds, bats), with resumption of viremia contributing to the reinitiation of local transmission in the spring.[2] Other data suggest that the virus could overwinter by transovarial (vertical) transmission in *C. pipiens* complex mosquitoes or in overwintering adult mosquitoes.[5,42]

The intermittent occurrence of urban SLE outbreaks has been associated with climatic factors, such as an antecedent mild winter, wet spring, and dry hot summer.[39] Snowpack and the resultant availability of irrigation water and an area supportive of mosquito breeding appear to be highly predictive of viral transmission in southern California.[68] In a model of global warming, SLE transmission is predicted to move to northern latitudes and, in existing endemic areas, to become seasonal in the spring and fall.[52] Periods of drought followed by a briefer wet interval have been associated with viral transmission in Florida.[17,59]

EPIDEMIOLOGY

The epidemiologic patterns of SLE virus transmission reflect human interactions with reservoirs of the virus and its principal mosquito vectors.[30,31,39,64] In western states (Fig. 188–2), perennial transmission of WEE and SLE virus leads to an endemic pattern of transmission, but epidemics are limited by a high level of immunity in the population.[53,66] Infections occur chiefly in rural areas in association with the habitat of the vector. Combined WEE-SLE outbreaks have taken place typically in rural agricultural areas and their small towns.[29] Studies in Texas indicated that an overlap of urban and rural cycles contributed to an urban outbreak in Dallas after introduction of the virus to the urban *C. quinquefasciatus* cycle from rural *C. tarsalis*.[25,30,31]

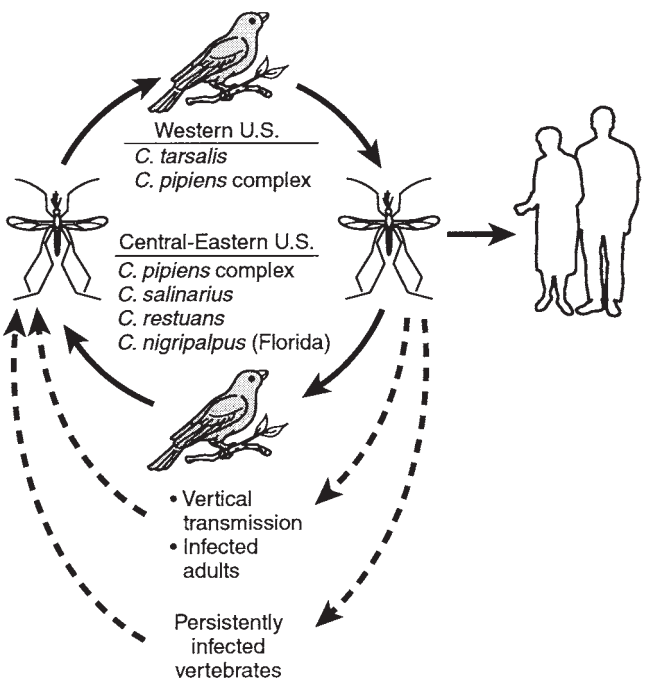

Figure 188–1 Transmission cycles of St. Louis encephalitis virus in North America. *Culex* mosquito vectors differ geographically: *C. tarsalis* in the West, *C. pipiens* and *C. quinquefasciatus* in the Ohio and Mississippi valleys, and *C. nigripalpus* in Florida. Epidemics occur when intense viral transmission in the enzootic cycle "spills over" and results in human infections. The viral overwintering mechanism, possibly in mosquitoes or persistently infected vertebrates, has not been proved *(broken lines)*.

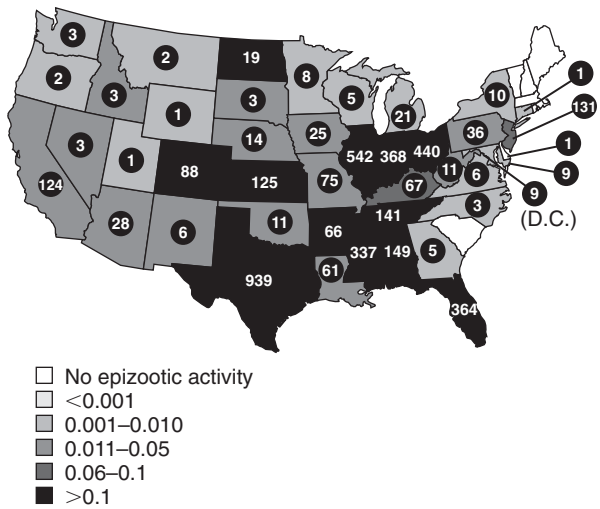

Figure 188–2 Reported cases and crude incidence of St. Louis encephalitis per 10,000 by state, 1964 to 1994.

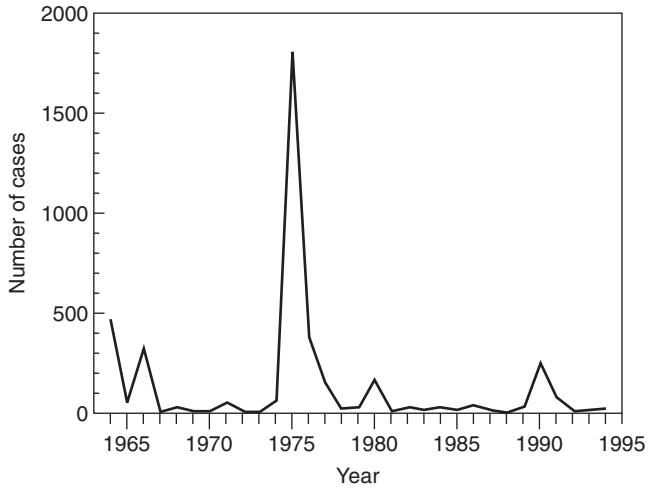

Figure 188–3 Reported St. Louis encephalitis cases by year, United States, 1964 to 1994. A nationwide epidemic in 1975 produced more than 2000 cases.

Epidemics frequently have occurred in urban locations or their peripheries. In 1975, a nationwide epidemic led to outbreaks in Houston, Chicago, Memphis, and Detroit as well as smaller outbreaks in rural towns throughout the South and Midwest (Fig. 188–3). Outbreaks in large urban centers generally have been associated with attack rates of fewer than 40 cases per 100,000 residents and often have clustered in low socioeconomic status areas, the most recent example occurring in Louisiana in 2003.[15,25,27,39,50,64,65,70] In recent urban outbreaks, homelessness and infection with human immunodeficiency virus (HIV) have been the principal risk factors for acquiring SLE.[47,67] However, in the 1933 St. Louis outbreak, attack rates were highest in wealthy areas where open sewers, streams, ponds, and weeds were prevalent; and in other outbreaks, lush vegetation around houses, which provides shelter for *C. nigripalpus*, and closed sewer systems clogged with grass clippings were factors associated with high attack rates in upper socioeconomic areas.[17,38]

In *C. pipiens*– and *C. quinquefasciatus*–borne outbreaks, epidemic attack rates are 1.2 to 3 times higher in females than in males, possibly reflecting increased exposure of females to these peridomestic vectors,[35,39,65] whereas in western *C. tarsalis*–borne outbreaks, attack rates are usually higher in males.[29,53] A similar observation has been made in Florida, where SLE attack rates are highest in working-age males.[18,38,45] *C. nigripalpus* and *C. tarsalis* are most active in twilight hours, and outdoor activity during these periods is associated with a greater risk of exposure.

Most cases occur in late summer or early fall (Fig. 188–4). However, in Florida, epidemics have continued through mid-December.[38,58] In the 1975 nationwide epidemic, outbreaks appeared first in the southeastern states in June, followed by an appearance in northern foci later in the summer and fall.[39]

The most important risk factor for acquiring neuroinvasive SLE is advanced age.[32,39] Age-specific attack rates are lowest in children and rise steadily in adulthood, with attack rates 5 to 40 times higher in persons older than 60 years than in those younger than 10 years. During outbreaks, infections occur uniformly in all age groups, with as many as 300 asymptomatic infections for each clinically apparent case. Therefore, the higher clinical attack rates in the elderly are a function of susceptibility and not exposure.[32,39] The clinical expression of illness is more severe and the case-fatality rate is also highest in the elderly. The biologic basis for this age-related risk probably reflects aging of the immune system as well as comorbidities compromising cerebrovascular integrity.[11] In addition, a secondary peak of increased risk is seen in infants.[65]

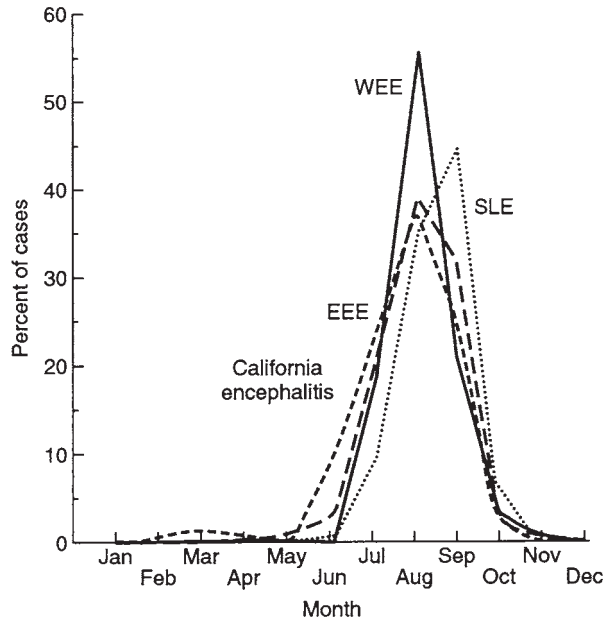

Figure 188–4 Reported arboviral encephalitis by etiology and month of onset, United States, 1972 to 1989.

In the West, where SLE infections are endemic, an increasing level of immunity associated with length of residence leads to an adult population with lower susceptibility (11% in the most recent serosurvey[53]). Consequently, cases often are seen in children and young adults. In a Florida outbreak, immunity to other flaviviruses (mainly from previous dengue infection) also was shown to protect against the acquisition of SLE.[8]

Sporadic SLE cases acquired in tropical America (Jamaica, Surinam, French Guiana, northern Argentina, and possibly Brazil) principally have resulted in mild febrile illnesses. However, an increasing seroprevalence rate with age indicates a higher frequency of endemic infection than is clinically apparent.[1,15,18,61,62]

CLINICAL MANIFESTATIONS

Clinical manifestations range from a mild influenza-like illness to fatal encephalitis.[9,51,56,70] A case definition that stratifies cases into clinical syndromes of encephalitis, aseptic meningitis, and febrile headache[9] (Table 188–1) has proved useful in surveillance (Fig. 188–5). Although children as a group exhibit milder symptoms, encephalitis develops in more than half of confirmed and presumptive pediatric cases, and 95 percent have objective clinical signs of CNS infection.[6,7,70]

SLE cannot be differentiated easily from other viral CNS infections on the basis of clinical features. Photophobia, headache, fever, nausea and vomiting, malaise, and neck stiffness are typical early symptoms. In approximately half of reported cases, an abrupt onset of weakness, incoordination, disturbed sensorium, or other neurologic signs may occur; but equally often, patients have nonspecific symptoms with subtle changes in coordination or mentation during a prodromal phase lasting several days to more than a week. Convulsions have been reported in a third of adult cases.[67]

In addition to fever and signs of meningeal irritation, patients nearly uniformly exhibit alterations in state of consciousness, such as restlessness, confusion, lethargy, delirium, or coma. Neurologic examination usually reveals general weakness, hyperreflexia, and tremulousness, but focal deficits are unusual findings.

TABLE 188–1 Definitions of Clinical Syndromes Caused by St. Louis Encephalitis

Encephalitis* (including meningoencephalitis and encephalomyelitis)
Acute febrile illness (oral temperature ≥37.8° C (≥100° F)
One or more signs in either of the following categories:
Altered level of consciousness (confusion, disorientation, delirium, lethargy, stupor, coma)
Objective signs of neurologic dysfunction (convulsion, cranial nerve palsy, dysarthria, rigidity, paresis, paralysis, abnormal reflexes, tremor)
Aseptic meningitis*
Acute febrile illness
Signs of meningeal irritation (stiff neck with or without Kernig or Brudzinski sign)
No objective signs of neurologic dysfunction
Febrile headache*
Acute febrile illness
Headache (also may have other systemic symptoms, such as nausea or vomiting)
No signs of meningeal irritation or neurologic dysfunction

**Cerebrospinal fluid pleocytosis is present in patients with encephalitis and aseptic meningitis; it also may be found in patients with the syndrome of febrile headache. Modified from Brinker, K. R., and Monath, T. P.: The acute disease.* In *Monath, T. P. (ed.): St. Louis Encephalitis. Washington, DC, American Public Health Association, 1980, pp. 503-534.*

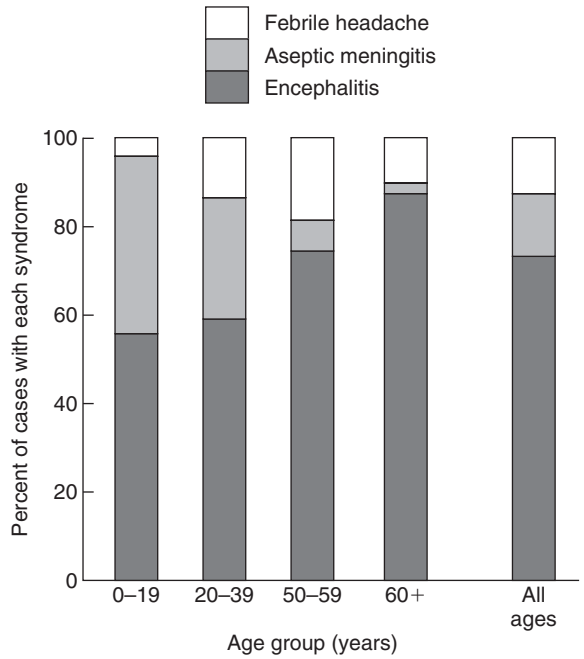

Figure 188–5 Distribution of St. Louis encephalitis cases by clinical syndrome and age. Although mortality rates and disease severity increase with age, most cases in children are clinical encephalitis. *(Data from Zweighaft, R. M., Rasmussen, C., Brolnitsky, O., et al.: St. Louis encephalitis: The Chicago experience. Am. J. Trop. Med. Hyg. 28:114-118, 1979.)*

Cranial nerve palsies, especially those involving nerves VII, IX, and X, occur in 10 to 25 percent of cases.[9] Clinical signs of increased intracranial pressure have been reported in a few patients. Unusual localizing neurologic signs have been described in patients with involvement of the midbrain, thalamus, or brain stem. In a 4-year-old girl, paralysis of upward gaze was associated with a brain stem infection.[28] Ataxia has been observed in a fourth of the cases in children, and opsoclonus has been reported in several cases in young adults.[20,21,67] In a series of 26 children with SLE, convulsions were observed in 8, 6 of whom had focal seizures; however, enteroviruses were isolated concurrently in 5 of the 8 patients.[6,49] A confirmed case has been reported in an infant as young as 19 days.[69] In most cases, the fever abates 4 to 7 days after onset, and clinical improvement is evident early in the second week of illness. Polyradiculopathy and transverse myelitis have been reported.[9,57]

The peripheral leukocyte count is elevated modestly, with a shift to the left in most patients. Cerebrospinal fluid (CSF) usually contains fewer than 100 leukocytes/mm^3, with a median value between 100 and 200. However, in some cases, fewer than 10 cells and occasionally no cells are discovered in the CSF. An increasing ratio of mononuclear to polymorphonuclear cells is observed on successive examinations. CSF protein rarely exceeds 200 mg/dL, and hypoglycorrhachia is unusual.[9]

In one series, elevated serum aldolase levels suggested a myopathic process, and muscle biopsy and electromyography disclosed lower motor neuron dysfunction, possibly from spinal root involvement. Moderate transaminase elevations have been reported in 19 to 48 percent of adult patients. Decreased serum osmolality attributed to inappropriate secretion of antidiuretic hormone was observed in a fourth to a third of cases in two series.[9,10]

Urinary incontinence, frequency, or retention and pyuria or proteinuria have been reported in adult patients, and viral antigen was detected on cells in the urinary sediment by immunofluorescence in one report.[33] Lower motor neuron lesions may have been the mechanism for bladder symptoms in some patients.

Electroencephalographic abnormalities have included focal discharges, including periodic lateralized epileptiform discharges, but more consistently show diffuse generalized slowing.[6,10,67] Computed tomographic scans may be normal, but magnetic resonance images have disclosed T2-weighted hyperintense abnormalities in the substantia nigra, consistent with edema.[12]

PATHOLOGY

Pathologic changes are found predominantly in the midbrain, thalamus, and brain stem but also in the cerebral cortex and cerebellum. Lesions are observed in the spinal cord in some cases.[55,60,63] The leptomeninges are affected to a variable degree with edema, small hemorrhages, and round-cell infiltration.

Perivascular and parenchymal inflammation is prevalent in the brain nuclei and scattered in the white matter of the brain stem and spinal cord and subcortical and deep cerebral areas. Nodular collections of inflammatory cells composed of lymphocytes, microglial cells, and monocytes are found in proximity to or surrounding degenerating neurons characterized by eccentric nuclear displacement, nuclear pyknosis, and cellular contraction. Vascular infiltrations and thrombosis have not been described, as they have in WEE and eastern equine encephalomyelitis. Focal demyelination, which is observed in WEE, has not been characteristic of SLE.

Transmission electron microscopy may provide a pathologic diagnosis on tiny tissue fragments.[16]

PATHOGENESIS

After infection, viral replication occurs locally and in regional lymph nodes. After this eclipse phase, blood-borne (viremic) dissemination ensues, with secondary sites of replication occurring in muscle, endocrine, lymphoreticular, and other tissues and, in less than 1 percent of infections, in the CNS. Vascular endothelial cells may have a role in actively transporting virus from the

capillary lumen to the brain and in supporting secondary viral replication.[19] Conditions that compromise cerebrovascular integrity and disrupt the blood-brain barrier may predispose to neuroinvasion; for example, concurrent CNS cysticercosis may be a risk factor for acquiring Japanese encephalitis, a related flaviviral infection. Although some experimental studies suggest that CNS invasion occurs through the olfactory epithelium, pathologic studies of Japanese encephalitis cases indicate widespread involvement of the brain stem, deep nuclei, and cortex, most consistent with hematogenous infection.

Recovery from flaviviral encephalitis depends on early intrathecal synthesis of antibodies and viral clearance by macrophages. The inflammatory response in related CNS infections consists of helper-inducer T cells and, to a lesser degree, B lymphocytes infiltrating the perivascular space and parenchyma from the blood. Macrophages and activated microglial cells in the perivascular space and parenchyma, respectively, are responsible for viral clearance. Outcome is determined by the comparative rates of viral spread and neuronal infection, the migration of inflammatory cells into the CNS, and the rapidity of the antibody response. Production of interferon is elicited in the brain of human SLE patients, but its role in limiting the spread of virus in the CNS is unclear.[34] The clinical course of SLE in HIV-infected persons has not been significantly different from that of other cases; however, few patients have been studied.

Resistance and susceptibility to some flaviviral infections in mice have been linked to genes associated with permissiveness to infection and the immune response. Environmental factors that influence susceptibility to flaviviral infection in experimental models include stress from cold or isolation, reticuloendothelial cell blockade, and heavy metal intoxication.

DIAGNOSIS

A laboratory diagnosis of SLE usually is made serologically, although virus rarely has been recovered in the acute phase of illness from blood.[30] Virus can be sought from brain and other organs obtained by biopsy or at autopsy by inoculation of Vero cell cultures and suckling mice intracerebrally.[14] However, nucleic acid sequence–based amplification to detect virus in CSF and brain may be more sensitive. Viral antigen has been demonstrated in brain sections and in urinary cell sediment.[33,54]

IgM capture enzyme-linked immunosorbent assay (ELISA) is the preferred serologic screening procedure.[41] Intrathecal production of virus-specific IgM reflects recent infection because virus-specific IgM can be detected in serum or CSF in 75 percent or more of specimens obtained within 4 days of onset. In most patients, IgM levels decline in the convalescent sample and disappear approximately 4 months after onset, although IgM persisted at high titer in a few patients for 6 months.[66] In areas where SLE is endemic, virus-specific IgM carried over from the previous transmission season potentially could lead to an erroneous diagnosis. The indirect immunofluorescent antibody procedure offers a similar capacity for rapid diagnosis; however, it is less sensitive than ELISA, and IgM rheumatoid factor can lead to a false-positive result. Differentiation of antibody responses to West Nile virus, particularly during simultaneous outbreaks of the infections, has been aided by development of a duplex assay.[26,35]

Hemagglutination-inhibition, complement-fixing, and neutralizing antibody assays are used only in reference laboratories but may provide clues to the timing of infection.

The serologic response in a primary flavivirus infection is usually type specific, but repeated infections lead to broad heterologous responses that are often difficult to interpret. Human infections with indigenous flaviviruses (e.g., Rio Bravo, Powassan) other than SLE are rare occurrences; however, in patients with antecedent yellow fever immunization or infection with dengue virus acquired in areas of the United States where dengue was previously endemic (e.g., Texas, Florida) or acquired during travel or residence abroad, a broadly reactive flavivirus antibody response may obfuscate the diagnosis. Recent immigrants with preexisting dengue antibodies are the most common source of flaviviral antibodies in most areas of the United States.

DIFFERENTIAL DIAGNOSIS

In an endemic setting, clinicians should consider SLE principally in the differential diagnosis of patients with aseptic meningitis or acute encephalitis. However, in the context of an outbreak, the diagnostic threshold should be lowered to include patients with less specific findings, especially acute febrile illnesses with headache, and patients who exhibit confusion or are encephalopathic without fever. Increased suspicion of SLE in mildly ill patients should be directed particularly at children and young adults, in whom the infection often is manifested without meningoencephalitis.

The clinical findings of SLE cannot be distinguished easily from those of other CNS infections. However, focal neurologic deficits are less characteristic of SLE and should suggest other diagnoses.[43] The combination of global confusion and tremulousness in SLE may suggest a metabolic encephalopathy, and in elderly patients, the initial manifestations of SLE can overlap the signs and symptoms of a cerebrovascular accident.

West Nile encephalitis and SLE are virtually identical in their clinical findings, their predilection for the elderly, and other epidemiologic features, including late summer seasonality, urban locus of epidemic transmission, and similar *Culex*-bird transmission cycles, such that individual cases cannot be differentiated except by specific laboratory testing.[26,35,36]

The progression of symptoms and localizing signs associated with herpes encephalitis contrasts sharply with the clinical findings in SLE, in which localizing signs are less usual. Enteroviral infections have an overlapping summer seasonality but often are associated with clustering of illnesses in families and other epidemiologic clues of person-to-person spread. Skin eruptions, respiratory symptoms, pericarditis, myocarditis, and conjunctivitis are helpful distinguishing characteristics. Concurrent enterovirus infection and SLE may be associated with an increased risk of convulsions during the acute phase of illness.[6,49] Enteroviral infections can be confirmed by detecting viral genomic sequences in CSF by polymerase chain reaction, by IgM capture ELISA, or by recovery of virus from stool or CSF. Primary HIV, human herpesvirus 6, and mumps virus infections are other common infections that should be entertained in the differential diagnosis. A third of patients with mumps encephalitis do not have associated parotid gland swelling.

Adenoviral encephalitis is a rare infection in children, more severe than SLE, and sometimes associated with hepatic and other extraneural sites of infection. The presence of lymphoreticular involvement and typical hematologic features should suggest the clinical diagnosis of Epstein-Barr virus infection.

Partially treated bacterial meningitis and parameningeal pyogenic infections are the principal bacterial causes to exclude. Cat-scratch fever can be manifested as fever and encephalopathy mimicking arboviral encephalitis.[46] Convulsions associated with *Shigella* enteritis and ataxia associated with typhoid fever potentially could be interpreted as a primary CNS infection. Tuberculous meningitis is typically a subacute illness; evidence of active extraneural sites of infection should be sought. A low CSF glucose level is a clue to the diagnosis.

In urban areas or where parental occupational exposure may occur, lead intoxication remains an important potential cause of encephalopathy in summer. Hyperthermia associated with sus-

tained exposure to elevated environmental temperature likewise may lead to encephalopathy with convulsions, signs of raised intracranial pressure, and evidence of hepatic dysfunction. This constellation of symptoms also may suggest Reye syndrome. However, Reye syndrome typically occurs in the winter and spring after a respiratory viral infection and is characterized by hypoglycemia and an elevated blood ammonia level. Patients with salicylism exhibit similar clinical findings, but a history of salicylate ingestion, an elevated anion gap early in the course of illness, and an elevated blood salicylate level should suggest that diagnosis.

TREATMENT

Supportive therapy, as outlined in the earlier sections, should be aimed at maintaining cardiorespiratory function and fluid and electrolyte balance, controlling convulsions, and monitoring and maintaining normal intracranial pressure.[36] No specific antiviral therapy is available.

PROGNOSIS

The principal risk factor for a fatal outcome is advanced age (see Fig. 188–5).[9,30,32,39,65,66] Among all cases reported to the Centers for Disease Control and Prevention from 1955 to 1971, 8 percent died, but the age-specific mortality rate in persons 60 years or older was 19.5 percent. Fatality rates as high as 38 to 80 percent were recorded in the 1933 St. Louis outbreak in patients who were 60 to 89 years of age. Mortality rates in children have ranged from 2 to 5 percent, with the highest risk in children younger than 5 years. The overall case-fatality rate in an outbreak in Hermosillo, Sonora (involving principally children), was 20 percent.[15] The high fatality rate in this instance is anomalous because fatality rates are usually lower in *C. tarsalis*–borne SLE outbreaks in the West. The role of neurocysticercosis as a risk factor for acquiring SLE should be examined in view of its potential involvement as a risk factor for Japanese encephalitis (see the subchapter Japanese Encephalitis).

In adults, coma, a low CSF leukocyte count (<100 cells/mm^3), and underlying hypertensive vascular disease have been factors associated with a fatal outcome.[11] Risk factors for mortality in children have not been described.

Recovery from SLE usually is complete or associated with soft sequelae such as emotional disturbances, dizziness, headache, memory impairment, and tremor.[4,23,24,44] In one study of 193 cases, 25 percent of children who were 1 to 4 years of age at the time of infection had serious neurologic sequelae, the highest rate of any age group.[48] The incidence of sequelae was 10 percent in children aged 5 to 9 years. Children who were younger than 1 year at the time of infection appeared to be spared any serious sequelae. Deficits in motor and intellectual function were reported in some cases. Convulsions were not a significant residual abnormality. The same cohort monitored at intervals of 6 months to 14 years had no perceptible residual differences in intelligence quotient compared with the normal population. In case reports, persistent ataxia of the extremities and trunk and dysarthria were reported as residual findings in a 4-year-old patient and a case of postinfectious encephalomyelitis after a mild illness.[26,58]

No deleterious effects of SLE on the outcome of pregnancy have been reported. However, infection with Japanese encephalitis virus in the second trimester resulted in transplacental viral transmission and abortion, whereas infection in the third trimester was not associated with fetal damage. In experimentally infected mice, vertical transmission led to learning deficits in congenitally infected pups.[3]

PREVENTION

Preventive public health efforts have focused on surveillance of viral activity in the enzootic cycle to predict epidemic activity. In many east central states, weekly serologic surveys of captured wild birds and sentinel chickens are conducted to detect rising seroprevalence rates that reflect viral amplification[42] and an increased risk for human infection. The abundance of female vector mosquitoes and increased viral infection rates in vectors also have been correlated with a risk of epidemic transmission.[68]

When viral activity is elevated, ground and aerial application of insecticides is aimed at reducing the infected adult vector population. If insecticides are applied early enough, viral amplification potentially can be attenuated and epidemic transmission aborted.

Avoidance of outdoor activity during the twilight and evening hours, use of repellents appropriately, and repair of screens or installation of air conditioners in residences are simple but effective measures that reduce exposure to adult mosquitoes. Improvement of drainage and removal of containers that could serve as mosquito breeding sites are important steps in preventing the disease.

A vaccine is not available, nor is vaccination of the general public a realistic preventive measure because of low attack rates and the intermittent and focal nature of outbreaks. However, if a vaccine or chemoprophylactic were available, selective administration to a targeted population, particularly the elderly, might be appropriate in an outbreak. A West Nile virus vaccine under development potentially could provide cross-protection against SLE. Passive immunotherapy and short interfering RNA molecules are under investigation as therapeutic modalities.

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CHAPTER **188b**

West Nile Virus

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HISTORICAL BACKGROUND

West Nile virus (WNV) was isolated first from the blood of a 37-year-old African woman in December 1937 in Omongo, West Nile district, in the Northern Province of Uganda while researchers were attempting to isolate yellow fever virus.[171] In the same report, these investigators demonstrated that sera from patients recovering from Japanese encephalitis virus could neutralize WNV. Shortly thereafter, this finding was confirmed and expanded to document that WNV was antigenically related not

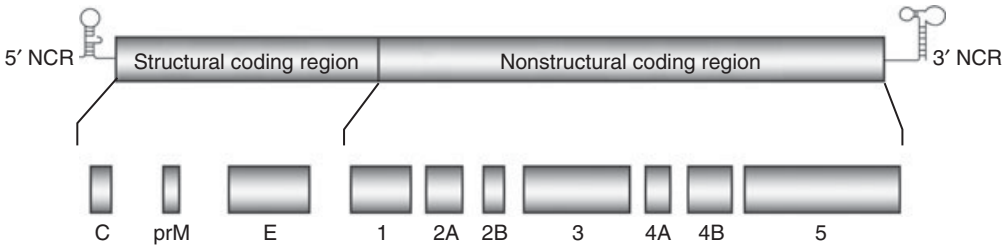

Figure 188–6 Organization and protein products of the West Nile genome.

only to Japanese encephalitis virus but also to St. Louis encephalitis virus.[172]

In 1950, Melnick and coworkers[118] isolated WNV from the blood of three healthy children in Egypt, thereby demonstrating that WNV was not confined to a single geographic location. Before 1999, WNV was known to be endemic to Africa, the Middle East, Europe, west and central Asia, and Oceania. With its incursion into the United States in 1999[6,131] and subsequent spread throughout North America, Central America, and the Caribbean,[45,97,151] WNV has established a worldwide distribution.

The first reports of WNV as a cause of outbreaks of febrile illness came from Israel in the early 1950s and ultimately led to its being associated with a clinical entity coined West Nile fever.[13,63] Studies performed by Taylor and colleagues[180] at the U.S. Naval Medical Research Unit in Egypt in the early 1950s provided information about the epidemiology of WNV infection and clinical picture of West Nile fever in children. This information was augmented by the seminal report by Marberg and colleagues[113] describing the clinical characteristics of serologically and virologically confirmed cases of West Nile fever among adolescent and young adult military personnel during a 1953 epidemic in Israel. Sporadic reports of central nervous system (CNS) complications associated with WNV infection followed closely after the early clinical descriptions of West Nile fever and pointed to the neuropathogenic potential of WNV.[12,72,150,175,176,178] Further characterization of West Nile neuroinvasive disease followed as additional outbreaks of WNV were reported.

During the past half century, outbreaks of WNV have occurred worldwide. Notable human outbreaks include Israel, 1951-1954, 1957, and 2000; France, 1962; South Africa, 1974; Algeria, 1994; Tunisia, 1997; Romania, 1996; and Russia, 1999.* Two of the largest epidemics recorded occurred in South Africa in 1974 and the United States in 2003. During the former, an estimated 18,000 cases of West Nile fever occurred.[45,72]

After the introduction of WNV into the North American continent in 1999, cases of West Nile fever and neuroinvasive disease remained at relatively low levels through 2001 and exploded in 2002 and 2003 when 4156 and 9862 cases, respectively, of WNV infection were reported to the Centers for Disease Control and Prevention (CDC).[75] The 2002 epidemic of WNV in the United States resulted in the largest epidemic of neuroinvasive West Nile disease ever recorded, with nearly 3000 cases reported.[137] Currently, WNV has superseded all autochthonous North American arboviruses as the single most important cause of arboviral disease in the United States.[36] The magnitude of the number of WNV infections is exemplified by an estimate, based on 2003 national blood donor screening data for WNV infection, that 735,000 cases of WNV infection occurred that year.[20]

*See references 13, 39, 45, 48, 54, 63, 72, 105, 141, 146, 181.

VIROLOGY

West Nile virus is a member of the *Flaviviridae* family of RNA viruses.[84,109] The viral family is composed of three genera: *Flavivirus*, *Pestivirus*, and *Hepacivirus*. WNV is a member of the *Flavivirus* genus and falls within the Japanese encephalitis virus serocomplex along with Cacipacore, Japanese encephalitis, Kunjin, Murray Valley encephalitis, St. Louis encephalitis, Usutu, and Yaoundé viruses. Kunjin virus, which is endemic to Australia and Asia, is a subtype of WNV. Members within the Japanese encephalitis virus serogroup are antigenically related to each other.

WNV is a spherical, enveloped virus measuring approximately 50 nm in diameter. The viral envelope is derived from the lipid bilayer of the host cell and surrounds the approximately 25-nm nucleocapsid core of the virion. The host cell–derived envelope is modified by the insertion of two viral proteins: E (envelop protein) and M (membrane protein).

The genome of WNV is a single-stranded, positive-sense (messenger sense) RNA molecule measuring 11,029 nucleotides in length (Fig. 188–6).[103,109] Two noncoding regions (5′ and 3′) flank the 10,302-nucleotide viral single open-reading frame and measure 96 and 631 nucleotides, respectively. The open-reading frame is organized as 5′-C-prM-E-NS1-NS2A-NS3-NS4A-NS4B-NS5-3′ and is transcribed into a single polyprotein of 3434 amino acids.[103,109] The N-terminal portion of the polyprotein consists of viral structural proteins, whereas the remainder of the protein contains the nonstructural (NS) proteins. The viral polyprotein is co- and post-translationally processed by host proteases and the viral protease NS2B-NS3 to yield three structural (E, prM, C) and seven NS (1, 2A, 2B, 3, 4A, 4B, and 5) proteins.

Viral attachment, membrane fusion, and viral assembly are mediated by the E protein. The search for the cellular receptors of WNV has identified the integrin $\alpha_v\beta_3$ and the lectins DC-SIGNR and DC-SIGN as capable of binding WNV in vitro.[40,48] However, whether they are physiologically relevant in vivo remains to be documented. Once bound to its receptor, WNV gains entry into the cell through endocytosis; after a pH-dependent change occurs in the conformation of the E protein, the viral and host endosomal membranes fuse. The viral nucleocapsid then is released into the cytoplasm of the cell. Viral replication is thought to occur on the membrane of the rough endoplasmic reticulum. The C or capsid protein binds to the viral RNA genome and forms a ribonucleoprotein complex required for packaging of the genome.[127] The immature virions are assembled, accumulate in vesicles, and bud into the endoplasmic reticulum. The immature virions are processed as they traverse the secretory pathway. Immature viral particles contain a premembrane (prM) protein that blocks premature viral fusion by the E protein. The prM protein is cleaved to the M protein by the trans-Golgi protein furin during the egress of the mature virion from the secretory pathway. Virions are transported to the cell membrane in vesicles and ultimately released through exocytosis.

The NS proteins regulate viral transcription and translation as well as attenuate host immune responses to WNV.[109,159] The NS1 protein serves as a cofactor for the viral replicase. Host interferon responses are inhibited by NS2A. This protein also may play a role in viral assembly. The NS3 protein is a protease and requires NS2B as a cofactor for activity. It also possesses NTPase and helicase activities. NS proteins 4A and B modulate interferon signaling. The NS5 functions as the RNA-dependent RNA polymerase and a methyltransferase.

Phylogenetic analysis of WNV isolates from around the world has demonstrated the existence of two lineages.[14,103,160] Strains that comprise lineage 2 are found exclusively in sub-Saharan Africa and Madagascar. In contrast, lineage 1 strains are found in geographically dispersed regions around the world and can be subdivided into three clades.[104] WNV strains from Africa, Europe, the United States, the Middle East, and Russia cluster within clade 1a. Kunjin virus and India WNV strains form clades 1b and 1c, respectively.

A correlation appears to exist between genotype/lineage and virulence phenotype.[104] Viruses from lineage 1 have been associated with outbreaks of West Nile neuroinvasive disease, whereas those from lineage 2 appear to cause only asymptomatic infection or West Nile fever. Neurovirulence data by use of a murine model corroborate this finding, indicating that lineage 1 strains of WNV are more neuroinvasive than are those of lineage 2.[10]

WNV strains isolated from birds and a human during the 1999 outbreak in the United States and a WNV strain isolated in 1998 from a dead goose in Israel have been shown to be closely related.[103] Phylogenetic analysis of the prM protein and E glycoprotein has demonstrated that they share greater than 99.8 percent nucleotide homology, indicating that the current North American strain of WNV almost certainly originated in the Middle East.

EPIDEMIOLOGY

In temperate climates, WNV infections occur seasonally during the summer months, coinciding with periods of increased activity of its mosquito vectors. The peak period of activity for transmission of WNV to humans in the United States, Europe, and the Mediterranean basin generally is from July through October.[137,192] However, as WNV has spread southward in the United States, the period of reported transmissions has increased, with human cases being reported as early as April and as late as December.[75] Year-round transmission may be possible in tropical climates.[145]

Infections of humans (i.e., epizootic infection) by WNV are incidental to its enzootic life cycle and, with rare exception, result in a "dead-end" infection without subsequent human-to-human transmission. Epizootic transmission of WNV occurs when sufficient numbers of mosquitoes that bite both birds and humans become infected to allow transmission to humans. Important risk factors for the acquisition of human WNV infection are the amount of time spent outdoors when mosquitoes are biting, the presence of dead birds in the neighborhood, the exposure to mosquitoes, and the vegetation cover suitable for harboring potentially infected adult mosquitoes.[19,71,125]

The enzootic cycle of the WNV requires replicative phases in its mosquito vectors and avian (or other) hosts. After WNV-infected blood meal has been ingested, the virus penetrates the mosquito's gut.[62] Viral replication then takes place in the salivary glands and nervous system of the mosquito. In mosquito tissues, WNV is nonlytic and results in persistent infection for the lifetime of the vector.

Mosquitoes play an important role in trans-generational and trans-seasonal maintenance of the WNV enzootic cycle. Female *Culex pipiens* mosquitoes have been documented to transmit WNV vertically to F1 progeny.[51] In addition, WNV can survive the winter by overwintering in hibernating female mosquitoes, as documented with *C. pipiens*, which thereby serve as a source of WNV to initiate the enzootic cycle the following spring.[130]

Although numerous mosquito species have been found to be infected with WNV, ornithophilic members in the *Culex* genus are the principal vectors for the transmission of WNV around the world. These include *C. pipens* and *Culex quinquefasciatus* in Europe, *Culex perexiguus* in North Africa and the Middle East, *Culex univittatus* in sub-Saharan Africa, *Culex tritaeniorhynchus* in south Asia, *Culex annulirostris* in Australia, and species of the *Culex vishnu* complex in India.[96]

Although WNV has been found in 59 of the 173 North American species of mosquitoes, only about a half-dozen are considered to be important vectors for transmission to humans.[75] As in other regions of the world, *Culex* spp. of mosquitoes are important vectors[75]; *C. pipens* (the northern house mosquito) and *C. quinquefasciatus* (the southern house mosquito) are considered to be the predominant vectors in the northeast and southern/southwestern United States, respectively. *Culex tarsalis* is a predominant vector west of the Mississippi River. *Culex nigripalpus* and *Culex salinarius* may be important vectors in regions where they are abundant. Last, *Culex restuans* also has been found to be a significant contributor to WNV-positive mosquito pools. Although arthropods can be infected with WNV, they are not thought to be important in transmission of WNV.

Birds are thought to be the principal amplifying hosts for WNV. Nearly 200 species of North American birds are susceptible to infection by WNV.[96] However, only birds capable of sustaining high-titer viremia for significant periods are capable of infecting the mosquitoes that feed on them. In vivo experiments using *C. tarsalis* have shown that 74 to 100 percent of mosquitoes consuming blood meals containing $10^{7.1}$ plaque-forming units (PFU) per milliliter become infected. In contrast, 36 percent or fewer mosquitoes become infected after the consumption of blood containing $10^{4.9}$ PFU/mL.[65] As a point of comparison, on the basis of blood tested of viremic blood donors, the maximum viral load in human blood is approximately $10^{3.2}$ PFU/mL.[75] This finding would indicate that humans fail to produce blood viral titers of sufficient magnitude to infect mosquitoes. Most mammals also fail to have sufficient levels of viremia to permit transmission to mosquitoes.

Song birds (Passeriformes), shorebirds (Charadriiformes), owls (Stringiformes), and hawks (Falconiformes) develop adequate viremia to infect mosquitoes feeding on them (pigeons and ducks do not). House finches, house sparrows, common grackles, and, in particular, corvids such as crows, magpies, and jays have been shown to be highly infectious to mosquitoes.[75,96] In addition, they have a high mortality rate after acquiring infection with WNV. Die-offs of avian species serve as epidemiologic markers for WNV activity in the environment.[67,126]

Unlike mammals, some amphibians appear to be capable of sustaining levels of viremia that potentially could be sufficient to infect mosquitoes and may serve as competent reservoirs for their infection. In the United States, alligators *(Alligator mississippiensis)* infected with WNV produced sufficient viral loads to infect mosquitoes.[95] In Russia, the lake frog *(Rana ridibunda)* may be a host for WNV.[75,98]

In areas where WNV traditionally has been endemic, seroprevalence studies document that most individuals acquire WNV infection during childhood. Such early acquisition of WNV infection results in large portions of the adult population with serologic evidence of immunity to WNV and may attenuate the occurrence of large outbreaks of WNV infection. Studies conducted in the 1950s in Egypt documented that a rapid rise in the presence of antibodies occurred in children aged 1 to 4 years such that by the time children were 4 years of age, 50 to 80 percent

had neutralizing or complement-fixing antibodies to WNV.[118,180] Similar patterns of acquisition of WNV-specific antibodies have been documented in the Sudan, Central Africa, and Nigeria.[131,173,180] Interestingly, more recent studies conducted in Egypt indicate that the seroprevalence rates in children and young adults, although still high, may be decreasing.[42,44] One can speculate that mosquito abatement programs and, possibly, changes in lifestyle have resulted in decreasing numbers of infections in children.

Seroepidemiologic studies conducted in areas where recently introduced WNV has resulted in large outbreaks of West Nile fever and neuroinvasive disease document low seroprevalence rates among the general population.[112,125,128,181] Investigation of the seroprevalence of WNV infection after an outbreak of WNV in Romania following the first major WNV epidemic in Europe found it to be 4.6 percent.[181] After the initial North American outbreak of West Nile encephalitis in New York in 1999, the seroprevalence at the outbreak's epicenter was found to be approximately 2.6 percent.[125] Overall, the reported post-epidemic seroprevalence, ranging from 2.6 to 14 percent in regions where WNV has been introduced recently, is too low to protect against future large-scale outbreaks of WNV disease.[45,128]

Unfortunately, detailed seroepidemiologic information about rates of WNV infection specific to children in the United States is scarce. After an outbreak of WNV in northeast Ohio, the county-wide seroprevalence rate was found to be 1.9 percent. However, among children younger than 17 years, the seroprevalence rate was nearly 3.5 times higher: 6.4 percent.[112]

Multiple novel, non–vector-borne modes of WNV transmission were identified during the North American epidemics. Transfusion-associated transmission of WNV was reported for the first time in 2002, when 23 cases were confirmed.[121,144] Four of the cases occurred in children younger than 18 years. Three of the children had immunocompromising conditions (acute myeloid leukemia, rhabdomyosarcoma, stem-cell transplant). The three children received platelet infusions and developed West Nile meningoencephalitis; all survived. The sole adolescent was a victim of a motor vehicle accident and received a transfusion of red blood cells. This patient subsequently became an organ donor and was implicated in four cases of transplantation-associated transmission of WNV.[27,85]

As a result of transfusion-associated WNV transmission, national screening of blood donations for the presence of WNV by nucleic acid amplification–based testing was initiated in June of 2003. From 2003 to 2005, a total of 1425 presumptive viremic donors from 41 states were reported to the CDC and their donations removed from the donor pool. Although active screening was performed, six probable or confirmed cases of transfusion-acquired WNV infection occurred in 2003, one in 2004, and two in 2006; they most likely were attributable to donations containing very low levels of virus.[34,37,121]

In addition to transmission that occurs through transfusion of blood products, seven cases of WNV transmission through organ transplantation have been documented.[27,35,85] The risk of acquiring West Nile neuroinvasive disease is approximately 40 times greater in organ transplant recipients than in the general population.[99] Unlike blood donations, organ donations currently are not required to be screened by nucleic acid amplification for WNV infection, nor is it performed routinely.

Confirmed or suspected maternal-fetal and maternal-infant vertical transmission of WNV has been reported.[3,29,30,74,136] In the most rigorously documented case,[3,30,74] confirmed maternal WNV infection occurred during the 27th week of gestation. The infant, delivered at 38 weeks of gestation, was found to have bilateral chorioretinitis, severe bilateral white matter loss in the temporal and occipital lobes, and tissue destruction in a temporal lobe. The presence of WNV-specific IgM was confirmed in cord blood, infant serum, and cerebrospinal fluid (CSF), although the CSF contained red blood cells. Maternal serum also contained WNV-specific IgM at the time of birth. The WNV genome was detected in the umbilical cord tissue and placenta by reverse transcription–polymerase chain reaction (RT-PCR). No WNV genome was detected in the infant's CSF.

Evaluation of 71 pregnancies reported to the CDC's WNV pregnancy registry failed to identify any confirmed cases of congenital WNV infection.[136] However, three infants who lacked evidence of anti-WNV IgM in cord blood or infant serum at the time of delivery subsequently were found to have serologic evidence of infection that may have been acquired congenitally. A breast-fed term infant, whose mother had onset of West Nile neuroinvasive disease 6 days before delivery, developed West Nile meningitis at 10 days of age. Another breast-fed infant, born to a woman with acute West Nile fever at the time of delivery, was noted to have a transient rash at birth. No serum or cord blood was available for testing. However, at 1 month of age, the infant was found to have WNV-specific IgM antibodies. In the final case, the infant's mother had a febrile illness 3 weeks before delivery. The infant appeared to be healthy at birth but at 7 days of age developed seizures. Evaluation of the infant at 17 days of age found lissencephaly and West Nile encephalitis with WNV-specific IgM antibodies detectable in the CSF. Because two of the infants were breast-fed, the possibility exists that WNV was transmitted in that manner. In addition, in none of the cases could the possibility of mosquito-borne transmission of WNV be excluded conclusively. This report also failed to link WNV infection during pregnancy with an increased frequency of spontaneous abortion, premature birth, or low birth weight.

WNV has been detected in the breast milk of infected women.[29,136] At least one case of possible transmission of WNV associated with breast-feeding has been reported.[29,73,136] The mother received packed red blood cell transfusions on the first and second postpartum days. Ten days post partum, the mother developed headache followed by fever. On evaluation, she was found to have CSF pleocytosis and the presence of WNV-specific IgM in the CSF. The infant was breast-fed for the first 17 days of life, and although the infant was clinically asymptomatic, a serum sample obtained at 25 days of age was found to have WNV-specific IgM. At this time, the risk of WNV transmission from mother to infant through breast-feeding is unknown. The current state of knowledge about transmission of WNV through breast milk does not warrant a change in breast-feeding recommendations.

Additional nontraditional modes of WNV transmission continue to be identified. Confirmed or possible transmission of WNV has been reported through laboratory exposure, percutaneous injury, aerosol inhalation, and dialysis.[31-33,70]

With the exceptions of Alaska and Hawaii, every state in the United States has reported human, mosquito, avian, or animal WNV activity (Fig. 188–7). From 1999 to 2005, 19,706 cases of WNV infection were reported to the CDC (Table 188–2). Neuroinvasive syndromes accounted for 8384 cases (42.5%), WNV fever was reported in 10,876 cases (55.2%), and 443 cases of unspecified disease accounted for the remainder. During the same period, 785 deaths (4%) were reported.

Epidemiologic and clinical data about WNV infections specific to children are beginning to emerge.[73-75] For the same time period, 1177 cases (6%) of WNV infection were reported in children and adolescents younger than 19 years.[73] Three deaths were reported in children aged 1 month, 14 years, and 15 years. Two of the children who died of neuroinvasive disease had underlying medical conditions: one had an immunodeficiency and the other lissencephaly.[75,135]

In adults, encephalitis and meningoencephalitis account for approximately 60 percent of West Nile neuroinvasive syndromes.[6,22,39,94,131,136,165,180,188] Meningitis has been reported to occur in approximately 15 to 40 percent of those with West Nile

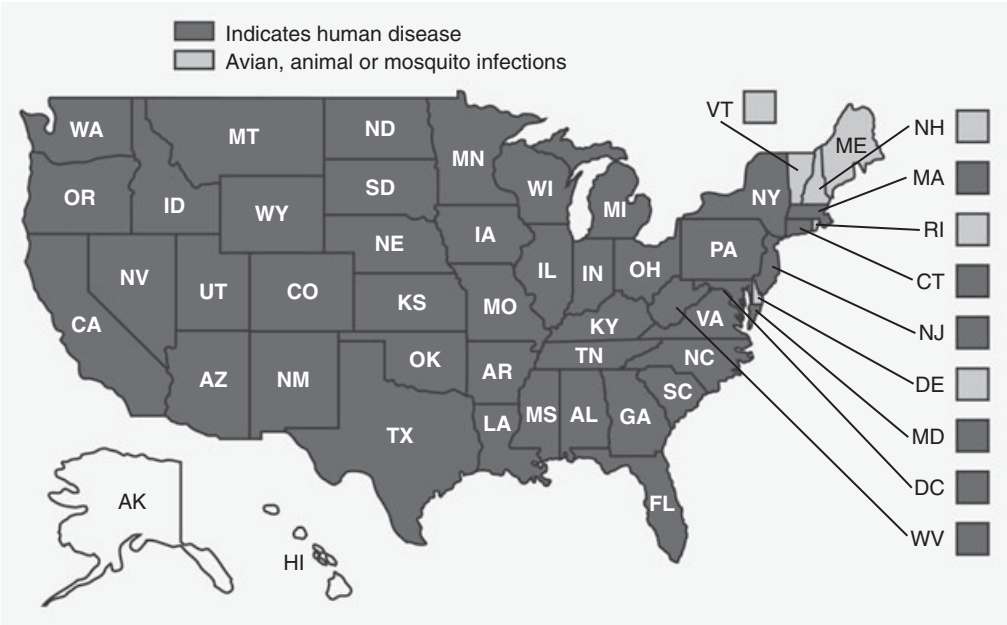

Figure 188–7 West Nile virus activity in the United States in 2006.

TABLE 188–2 West Nile Virus Infections in the United States, 1999-2005

Year	Deaths	Total Cases	Meningitis-Encephalitis	Fever	Unspecified
1999	7	62	59	3	0
2000	2	21	19	2	0
2001	9	66	64	2	0
2002	284	4,156	2,946	1,163	50
2003	264	9,862	2,860	6,830	166
2004	100	2,539	1,142	1,269	128
2005	119	3,000	1,294	1,607	99
Total	785 (4%)*	19,706	8,384 (43%)	10,876 (55%)	443 (2%)

**Percentage of total cases.*

neuroinvasive disease. WNV also has been linked to the development of acute flaccid paralysis syndrome, which occurs in approximately 10 percent of cases of neuroinvasive disease.[28,86,94,107,131,162-164]

The distribution of WNV syndromes among children has been difficult to define because of underreporting of pediatric cases of West Nile infection.[73] From 2002 through 2004, 1051 cases of WNV infections in children younger than 19 years were reported to the CDC.[75] WNV neuroinvasive disease was reported in 30.2 percent (317) of cases. Approximately a third of these incidents occurred in children younger than 10 years. The distribution of disease caused by WNV was defined in a subset of 150 cases of patients younger than 19 years reported in 2002.[74] Twenty-seven percent of cases were classified as West Nile fever, 70 percent had neuroinvasive disease, and 3 percent were classified as unknown illness. Meningitis, encephalitis, and unspecified neuroinvasive disease accounted for 38, 28, and 34.3 percent, respectively, of the definable cases of CNS disease. Thirty-seven percent (55 cases) of children in this cohort were younger than 10 years.[74] More recently, the reported disease distribution among 261 cases of WNV infections in children during 2004 and 2005 was 50 cases of encephalitis (19%), 54 cases of meningitis (21%), 136 cases of West Nile fever (52%), and 21 cases (8%) of unknown or other clinical presentations.[73] Flaccid paralysis was reported in five children of this cohort.

Taken together, the available information about the distribution of West Nile neuroinvasive disease in children indicates that in individuals younger than 19 years, the predominant West Nile neuroinvasive syndrome appears to be meningitis.[166] In addition, acute flaccid paralysis developing as a result of WNV infection appears to occur less frequently in children than in adults.[173]

PATHOGENESIS[160]

The pathogenesis of West Nile encephalitis has been explored in animal model systems.[50,53,153,189] Subcutaneous inoculation of WNV is thought to result in WNV infection and replication in Langerhans dendritic cells.[21] Under control of interleukin-1β, activated Langerhans cells migrate to regional lymph nodes and result in a primary viremia that develops after the virus reaches the systemic circulation through the thoracic duct. In mice, peak viremia occurs by the second day after infection and clears by the sixth day. Virus subsequently can be detected in spleen, brain, and spinal cord. Because of the simultaneous appearance of virus in the brain and the spinal cord, a hematogenous route for the CNS infection has been postulated. Similar to human infections, CNS infection in mice and hamsters results in infection of the neurons of the brain stem and the spinal cord, resulting in meningoencephalitis and an acute flaccid paralysis–like syndrome similar to that seen in humans. Several mechanisms have been postulated for the neuronal injury seen in WNV infection[168]: viral injury to infected neurons through apoptosis; targeting of infected

neurons by cytotoxic T lymphocytes; and neuronal death as a result of bystander injury.

The mechanism by which WNV gains access to the CNS is not fully understood. Recently, WNV binding to Toll-like 3 receptor has been shown to result in Toll-like 3 receptor–dependent inflammatory response with resultant production of tumor necrosis factor–α and interleukin-6.[184] Tumor necrosis factor–α may modulate the ability of WNV to invade the CNS, inducing breakdown of the blood-brain barrier. Additional postulated mechanisms through which WNV may breach the blood-brain barrier include infection or passive transport through the choroid plexus epithelial cells or the endothelium, infection of olfactory neurons, transport within infected immune cells that traffic into the CNS (Trojan horse mechanism), and retrograde axonal transport in infected peripheral neurons.[159]

Cessation of the viremia occurs by the sixth day in mice and is coincident with the appearance of type-specific antibody response. Viral clearance from all tissues is complete by 2 to 3 weeks after infection. Humoral immunity is essential to the recovery from WNV infection as demonstrated by the uniform death of B cell–deficient mice.[50] B cell–deficient mice could be protected from lethal infection by the passive transfer of serum from infected and immune wild-type mice. γδ T cells that secrete interferon-γ may control viral replication through direct antiviral mechanisms and contribute to adaptive immune responses.[159]

Although clearance of WNV from the CNS and other tissues depends primarily on humoral immunity, cytotoxic T cells also play a role.[169,185] Increased mortality rates in association with higher and sustained WNV loads in the CNS and spleen were observed in mice deficient in $CD8^+$ T cells or class I major histocompatibility complex molecules. In addition, control of WNV infection in the CNS requires interferon-α/β. Interferon-α/β also may help increase the survival of neurons. Last, chemokines such as CXCL10 and CCL5 and their ligands CXCR3 and CCR5 aid in the recruitment of $CD4^+$ and $CD8^+$ T cells and monocytes to the CNS.[159]

CLINICAL MANIFESTATIONS

Most WNV infections in children and adults result in subclinical disease. In general, WNV infection in children more frequently results in asymptomatic infection or milder disease than that in adults.[39,63,131,166,179] A recent report documented that children were 4.5 times more likely to become infected with WNV during an epidemic but 110 times less likely to develop neuroinvasive disease.[112] In another report, the estimated incidence of neuroinvasive disease was 1.4/100,000 in children aged 5 to 17 years compared with 14.3/100,000 in individuals aged 18 years or older.[101] In adults, multiple reports have identified increasing age as the single most significant risk factor for the development of neuroinvasive disease as well as adverse outcome.[131,145] The incidence of severe neurologic disease is 10 times higher in individuals aged 50 to 59 years and 43 times higher in persons aged 80 years or older compared with those 20 years of age or younger. Chronic illnesses, such as hypertension, diabetes mellitus, cardiovascular disease, and alcohol abuse, have also been identified as risk factors for severe disease.[15,129,131,145] These factors have not been linked to an increased risk for the development of severe disease in children.

In the early 1950s, therapeutic inoculation of WNV into patients with malignant neoplasms in advanced stages resulted in a high incidence of development of encephalitis among recipients and pointed to the role of immunosuppression as a risk factor for West Nile neuroinvasive disease.[176] This finding subsequently was substantiated by the recognition that transplant recipients were 40 times more likely to develop West Nile neuroinvasive disease.[85,99,100,154] Reports of West Nile neuroinvasive disease in immunocompromised children are consistent with immunosuppression as a risk factor in this population also.[4,56,79,154,177] Reports also have indicated that the use of immunomodulating agents such as infliximab, rituximab, methotrexate, and fludarabine may predispose to the development of severe WNV infection.[9,38,116]

Approximately 20 percent of infected adults develop West Nile fever. Because of the fever's mild nature, only about half of individuals with West Nile fever ever seek medical attention. WNV neuroinvasive syndromes (encephalitis, meningitis, meningoencephalitis, acute flaccid paralysis) develop in less than 1 percent (1 in 140 to 320) of adults infected with WNV.[20,125,131,145,181,182] The occurrence of West Nile neuroinvasive disease in children is significantly less.[101,112] In one study, it was calculated to occur in approximately 1 in 4200 infected children.[112]

The incubation period of WNV infection ranges from 2 to 14 days but usually is 2 to 6 days.[22,63,96,113] Viremia may be present from approximately 2 days before to approximately 4 days after the onset of the illness.[64] In immunosuppressed individuals, incubation periods up to 3 weeks have been reported and may exhibit prolonged periods of viremia.[85,144]

In children[41,56,63,74,82,113,191] and adults, West Nile fever is an influenza-like febrile illness characterized by an abrupt onset of fever that typically ranges from 38° C to 40° C. It is accompanied by fatigue, malaise, anorexia, headache, myalgias, and weakness.[22,63,113,145,187] Ocular pain on eye movement has been reported.[64,114] A diffuse nonpruritic, macular, papular, or morbilliform exanthem and diffuse lymphadenopathy also may be seen. On occasion, it has been described as petechial.[17,41] The rash spares the palms and soles. The exanthem may be present more frequently in children than in adults, regardless of the WNV syndrome.[18,41] Neck pain and photosensitivity often are reported. Gastrointestinal complaints such as nausea, vomiting, abdominal pain, and diarrhea may occur. Sore throat and cough also have been reported. The illness generally is short-lived, lasting only 3 to 6 days.[63,82,113] Convalescence, however, may take many weeks and is characterized by fatigue.[63,113,186]

Other non-neurologic WNV syndromes that have been reported include cardiac dysrhythmias,[76] myocarditis, pancreatitis, hepatitis, rhabdomyolysis,[117,121] myositis, and orchitis.[6,17,79,116,131,191]

Although cases of West Nile neuroinvasive disease have been reported in children,* most of the information about these clinical syndromes has been derived from reports that have involved predominantly adults.† However, on the basis of a limited number of pediatric cases and series, the clinical presentation in children appears to parallel that of adults.[166] Although discussion of each of the neuroinvasive syndromes individually (i.e., meningitis, encephalitis, acute flaccid paralysis) is convenient, one should recognize that patients may have components of each in their clinical presentation. Last, the clinical findings in West Nile neuroinvasive disease in children and adults are not sufficiently distinct to distinguish them from other viral meningitides or encephalitides.

The onset of West Nile meningitis tends to be abrupt with fever, headache, and neck stiffness and meningeal signs (Kernig and Brudzinski signs).‡ Photophobia and phonophobia may be present. The headache pain may be severe enough to warrant the use of narcotic analgesics. Weakness frequently is reported.[41,56] Additional associated symptoms may include those seen with

*See references 4, 17, 24, 41, 48, 49, 55, 56, 61, 72, 77, 79, 80, 93, 101, 123, 133, 135, 144, 150, 154, 155, 177, 183, 189, 191.

†See references 6, 15, 22, 25, 38, 39, 86, 94, 107, 131, 145, 157, 162-165, 180, 188.

‡See references 2, 15, 22, 39, 41, 46, 56, 73, 74, 80, 94, 101, 131, 145, 165, 166, 181, 188, 189, 191.

West Nile fever: gastrointestinal and respiratory symptoms (i.e., nausea, vomiting, diarrhea, sore throat, cough), rash, and lymphadenopathy. Dyskinesias in the form of tremors, myoclonus, or parkinsonism may also occur.

The reported clinical findings of West Nile encephalitis in children* are similar to those described in adults.[15,22,39,46,131,145,166,181] The onset of frank encephalitis may be preceded by a brief period or "prodrome" of fever, headache, rash, and malaise consistent with West Nile fever. In some cases, the appearance of fever is coincident with the onset of mental status changes. The mental status changes may range from a mild confusional state to severe encephalopathy and coma. Generalized weakness is a common component of the clinical spectrum of encephalitis. Paresis or palsies may be present. Ataxia, dysmetria, tremors, myoclonus, and parkinsonism may be seen.[41,123,133] Signs of bulbar dysfunction, such as dysarthria, dysphagia, and respiratory failure, have been reported. If a component of meningitis is present (i.e., meningoencephalitis), signs and symptoms described before for meningitis may also be present. Seizures have been reported in children[4,24,56,92,135,155,177] but occur in less than 10 percent of adult cases of West Nile encephalitis.[15,18,94,131] Focal seizures and neurologic signs may occur and mimic that of herpes simplex virus encephalitis.[155,183]

Acute flaccid paralysis in association with WNV infection was reported first in 1979[58] and may occur in the absence of meningitis or encephalitis. Several reports of acute flaccid paralysis, alone or in association with encephalitis, developing after the occurrence of WNV infection in children have been published.[17,56,77,157] The onset of the paralysis occurs early in the illness, usually within the first 72 hours.[162] Pain in the affected limb preceding the onset of weakness is reported in most cases.[162] Patients exhibit asymmetric weakness, hypotonia, and absent or diminished deep tendon reflexes. Pain or sensory function in the affected limbs remains intact.[6,86,94,107,157,162] However, at least one report has documented electrophysiologic evidence of sensory fiber loss in association with severe spinal cord involvement.[52] It is postulated that this finding could be explained by damage to neurons in the dorsal root ganglia. Tremor, myoclonus, and parkinsonism are common findings.[163,165] In severe cases, changes in bowel and bladder function or quadriplegia may occur. Involvement of the brain stem motor nuclei of the vagus and glossopharyngeal nerves can result in dysphagia, dysarthria, and acute respiratory failure requiring assisted mechanical ventilation.[17] Patients with dysphagia and dysarthria are at greater risk for the development of respiratory failure.[162]

Ocular manifestations of WNV infection include chorioretinitis, vitritis, and uveitis.[7,166] Chorioretinitis has been described in congenital and postnatal WNV infections[3,7,166] and may be more common than is realized.[76] Postnatal cases of West Nile chorioretinitis have been reported solely in adults. The lesions are reported to cluster in the temporal and nasal regions of the peripheral retina and have been described as multifocal and "target-like." One suggestion is that the distribution and appearance of the lesions are sufficiently distinct to be considered characteristic for WNV infection.[78] In the sole reported case of congenital WNV infection, the infant had a large chorioretinal scar involving the retina of the right eye. Mild chorioretinal scarring was observed in the far temporal periphery.[3,7] Vitritis also has been reported in children and can lead to blurring and loss of vision.[191]

Other rarely reported neurologic manifestations of West Nile neuroinvasive disease in adults include optic neuritis, myelitis, Guillain-Barré syndrome,[1] and polyradiculitis.[143]

*See references 4, 17, 24, 41, 48, 49, 56, 73, 74, 79, 92, 123, 135, 154, 155, 177, 183.

OUTCOME

Although complete recovery from West Nile fever is expected, it may take several weeks before children and adults return to pre-illness levels of health.[63,143] In their report, Watson and colleagues[187] found that the median time to complete recovery in adults was 60 days. Post-illness fatigue was reported in 98 percent, and the median duration of fatigue was 36 days. Sixty-one percent of patients reported muscle weakness of a median duration of 28 days. Other symptoms that persisted for longer than 7 days were reported in more than half of the subjects and included headache, muscle pain and aches, difficulty concentrating, joint pain and aches, and sensitivity to light. Of those who worked or attended school, nearly 60 percent reported absenteeism of more than 7 days.

Neurologic outcome of children after West Nile neuroinvasive disease has not been assessed systematically. The published case reports of West Nile neuroinvasive disease in children are most likely biased by the disease severity or outcome and limited by a lack of long-term follow-up of patients. As such, reliable information about the outcome of West Nile encephalitis and acute flaccid paralysis is lacking. However, on the basis of the review of 45 reported cases of West Nile neuroinvasive disease in children and adolescents for whom clinical and outcome information was provided,* recovery from meningitis appears to be complete in children ($N = 20$). Among cases of encephalitis, meningoencephalitis, and acute flaccid paralysis ($N = 25$), approximately 50 percent of children were reported to have neurologic sequelae at the time of last medical encounter or at the time the report was submitted. The reported sequelae among children include seizures, weakness, paralysis, gait disturbances, motor difficulties, language and speech deficits, dysphagia, behavior problems, cognitive deficits, mental retardation, difficulty concentrating, difficulties with memory and comprehension, facial palsy, hypertonicity, and persistent vegetative state.†

The reported sequelae of West Nile neuroinvasive disease in adults include fatigue, fever, weakness, difficulty concentrating, memory loss, depression, paresis, tremor, myoclonus, and parkinsonism.[22,25,93,140,165,188] Between 50 and 65 percent of individuals will have evidence of neuropsychiatric dysfunction at the time of discharge.[188] Sejvar and colleagues[165] documented that the neuropsychiatric outcome at 8 months for patients with meningitis and encephalitis was generally favorable. All patients with meningitis had returned to work and reported having normal or near-normal function. In cases of severe encephalitis, achievement of premorbid function levels without evidence of residual disability was observed in most of the patients. Patients recovering from West Nile acute flaccid paralysis fared variably. Although the degree of initial paralysis was not correlated with long-term outcome in an early report,[23] a more recent publication indicates that the degree of initial paralysis may be an important indicator of long-term outcome.[163] The greatest recovery in strength occurred during the first 4 months after paralysis.[163]

Although reports of death in children as a result of WNV infection are rare,[26,61,73,79,111] WNV outbreaks associated with significant pediatric mortality rates (12.9%) have been documented.[49,105] Case-fatality rates in adults have ranged from 4 to 18 percent.[15,22,39,57,94,131,145,165,188] Hospitalized persons 75 years or older are nine times more likely to die than are younger patients. Other reported independent risk factors for death have been change in level of consciousness and anemia at presentation.[39]

*See references 4, 24, 17, 40, 48, 56, 77, 79, 80, 101, 135, 177, 183, 189, 191.

†See references 4, 24, 17, 56, 77, 80, 135, 177, 183, 189, 191.

LABORATORY FINDINGS

As is the case with the clinical findings of WNV infections, routine laboratory studies are nonspecific and do not provide conclusive evidence about the etiologic agent. Similar to those in adults, hematologic findings in children include leukocytosis, leukopenia, normal total white cell count, and lymphopenia.[4,41,48,101,155,177,183,189,191] Pediatric and adult cases of encephalitis hyponatremia have been reported.[17,39,188] Elevations of transaminases and creatine kinase may be seen in children with hepatitis and myositis.[17,191]

Cytochemical evaluation of the CSF generally reveals an abnormal white cell count and protein concentration.* A predominantly lymphocytic pleocytosis with, generally, fewer than 200 cells/mm^3 is a frequent finding. Cell counts of more than 2000 cells/mm^3 have been reported. Early in the infection, the CSF may show a polymorphonuclear cellular predominance that later shifts to a lymphocytic pleocytosis.[43] A mildly increased protein concentration of less than 100 mg/dL generally is seen. However, considerable variation in CSF protein concentration has been reported in patients with West Nile encephalitis. A normal CSF glucose concentration generally is observed.

ELECTRODIAGNOSTIC STUDIES

Electrodiagnostic study findings are abnormal in most patients with West Nile encephalitis.† Electroencephalographic abnormalities have included diffuse irregular slow waves, focal sharp waves, and subclinical electrographic seizures. An anterior preponderance of slow waves has been suggested as characteristic of West Nile encephalitis.[59] Reports of focal electroencephalographic findings suggestive of herpes simplex virus encephalitis have been reported in children.[155,183]

In patients with acute flaccid paralysis, electromyography and nerve conduction studies document normal nerve conduction velocities, normal or reduced compound muscle action potentials, and normal sensory nerve action potentials consistent with anterior horn cell disease.‡ Electrophysiologic documentation of severe loss of sensory fibers exists.[52]

NEUROIMAGING

Magnetic resonance imaging is more sensitive than is computed tomography in identifying CNS abnormalities in patients with West Nile neuroinvasive disease. However, even in cases of severe encephalopathy, magnetic resonance imaging may fail to find any abnormalities.[18] Reported magnetic resonance imaging findings in cases of West Nile neuroinvasive disease appear as hyperintense signal on T2-weighted and fluid-attenuated inversion recovery images and are nonenhancing and of normal intensity on T1 weighting.[86,147] In children and adults, lesions tend to be seen more frequently in the deep gray matter of the brain and, in particular, the basal ganglia, posterior thalami, and substantia nigra.§ Leptomeningeal and paraventricular enhancement may also be seen.[17,154] Focal abnormalities occasionally may be seen, as noted in two cases of children with encephalitis in whom focal enhancement of the right temporal lobe was noted.[48,57,183]

In cases of acute flaccid paralysis, abnormal T2-weighted signals may be seen in the spinal cord gray matter.[2,77,86,107] In a case of a child with acute flaccid paralysis, magnetic resonance imaging of the spine documented edema of the anterior horns of the cervical spinal cord.[77]

VIRAL CULTURE AND NUCLEIC ACID AMPLIFICATION DETECTION OF WEST NILE VIRUS

Multiple reports have documented that WNV can be detected from the blood and brain of infected individuals by use of mouse inoculation or cell culture.[13,61,63,64,70,81,118,171,176] However, this approach to establishing the diagnosis of WNV infections is of limited utility because of lack of sensitivity due to the brevity of the viremia, which begins approximately 2 days before and persists until approximately 4 days after the onset of the illness.[64,145,176] In addition, because of the potential risk to laboratory personnel, attempts to isolate WNV from human specimens should be undertaken only in an appropriate biocontainment environment (i.e., biosafety level 3 containment).

RT-PCR, using a fluorescent probe in a 3′ exonuclease assay (TaqMan), and real-time accelerated reverse transcription loop-mediated isothermal amplification assays for the detection of WNV have been developed.[102,142] Both have been shown to be more sensitive than conventional PCR for the detection of WNV genome. Diagnosis of acute WNV infections by nucleic acid amplification–based detection of WNV genome from blood and CSF clinical specimens also has limitations due to the short period of viremia.[102,145] Although genome could be detected in all brain tissue specimens tested, only 57 percent of CSF specimens and 14 percent of sera from patients with serologically confirmed infection were positive by a real-time RT-PCR assay.[102] Nucleic acid amplification–based detection of WNV has not been proven to be useful for the clinical diagnosis of WNV infections, but it has been extremely valuable in the screening and detection of asymptomatic viremia in blood donors.[122]

DETECTION OF WEST NILE VIRUS–SPECIFIC ANTIBODIES

Given the limitations of virologic and nucleic acid amplification–based diagnostic assays, detection of virus-specific antibodies forms the mainstay for establishing the diagnosis of WNV infections. In cases of neuroinvasive disease, the presence of WNV-specific IgM in serum and CSF increases by 10 percent per day the first week of illness such that it is usually detectable in 70 to 80 percent of patients by the eighth day after onset of illness.[76,179] Interestingly, WNV-specific IgM may persist for long periods in serum and CSF of acutely infected individuals.[156] Roehrig and colleagues[156] documented that 58 percent of samples obtained approximately 500 or more days from onset of West Nile encephalitis were in the positive or equivocal range for WNV-specific IgM.[156] Similarly, WNV-specific IgM has been detected in the CSF for up to 199 days after onset of illness.[90]

The inter-flavivirus cross-reactivity of anti-flaviviral IgG antibodies is well recognized. Traditionally, confirmation of anti-flaviviral antibody specificity requires the use of a functional antibody assay such as the plaque reduction neutralization test.[110] Although multiple reports have documented greater specificity of anti-flaviviral IgM antibodies and, in particular, those of WNV-specific IgM antibodies,[114,115,179] cross-reactions may still occur. On the basis of cross-reactivity with related flaviviruses (i.e., Japanese encephalitis virus and St. Louis encephalitis virus), an algorithm for the use of CDC-developed IgM capture enzyme-linked immunosorbent assay (MAC-ELISA) has been developed to differentiate human WNV and St. Louis encephalitis virus

*See references 4, 6, 18, 22, 24, 41, 43, 48, 56, 77, 79, 83, 94, 101, 131, 133, 135, 145, 154, 155, 164, 165, 170, 177, 183, 188, 189, 191.
†See references 2, 4, 18, 28, 48, 77, 79, 86, 94, 106, 107, 131, 135, 155, 164, 165, 177, 183.
‡See references 2, 28, 52, 77, 94, 106, 107, 131, 164, 165.
§See references 4, 16, 18, 56, 72, 131, 145, 165, 174, 188.

infections.[115] The algorithm compares the positive-to-negative ratios of WNV-specific and St. Louis encephalitis virus–specific IgM determined in a CDC-standardized MAC-ELISA and has a positive predictive value of 97.8 percent for detection of WNV in the midst of an epidemic. This algorithm has not been validated for commercial MAC-ELISA kits. In the absence of use of the CDC MAC-ELISA, confirmation of WNV infection may require documentation of presence of WNV functional (neutralizing or hemagglutination inhibition) antibodies.

The diagnosis of WNV infection currently relies on the detection of WNV-specific antibodies in CSF or paired serum samples.[1,76,166] The MAC-ELISA is the most sensitive and commonly used diagnostic assay. It is capable of detecting IgM antibodies in CSF 3 to 5 days into the clinical illness and 3 or more days earlier than detectable serum antibody.[179] The finding of WNV-specific IgM in the CSF of a child with a clinically compatible CNS syndrome generally is considered diagnostic of acute neuroinvasive infection. However, as discussed previously, persistence of WNV-specific IgM antibodies for as long as 199 days in the CSF has been reported.[90]

Detection of WNV-specific IgM antibodies in serum from a single specimen may not be diagnostic of WNV infection because of its prolonged persistence in serum.[156,166] When only serum is used for establishing a diagnosis, a second serum sample should be obtained at least 2 weeks later to document a fourfold increase in specific antibody titers with use of a functional assay such as neutralization or hemagglutination inhibition.[166] Serologic cross-reactions among St. Louis encephalitis virus, WNV, and Powassan virus may occur[182] and can confound the diagnosis in those regions where multiple viruses are endemic. Serologic testing ideally should include a battery of region-specific arboviral antigens. Confirmatory assays, such as viral neutralization with the plaque reduction neutralization test, are available at many local and state public health laboratories or from the CDC's Division of Vector-Borne Infectious Diseases. The sensitivities and specificities of commercially available tests for the detection of WNV-specific antibodies may vary. An attenuated immune response may occur in immunocompromised hosts and result in a delayed antibody response.[144] As such, serologic assays should be interpreted with caution in this population, and adjuncts such as nucleic acid amplification should be used to establish the diagnosis.

NEUROPATHOLOGY

Gross examination of the brain, spinal cord, and meninges generally is unremarkable but may demonstrate evidence of edema. In severe cases, the leptomeninges may show a perivascular monocytic infiltrate on microscopic examination.[181] Although the parenchymal findings of West Nile encephalitis can be seen distributed in nearly all areas of the CNS, the deep nuclei (thalamus, caudate, lentiform nuclei), the substantia nigra, and other regions of the brain stem (in particular the pons and medulla), cerebellum, and spinal cord[22,66,91,131,139,158,181] may be more severely involved.

The CNS pathologic changes are the result of direct viral replication in neuronal and, less so, glial cells as well as the cytotoxic immune response to infected cells.[22] WNV demonstrates a penchant for infecting neurons. Viral antigen is detectable in neurons of fatal cases.[6,22,66,177] Neuronal pyknosis or neuronophagia is seen. Features in fatal cases of West Nile encephalitis include perivascular mononuclear inflammatory infiltrates composed predominantly of B lymphocytes, $CD8^+$ T lymphocyte–dominant microglial nodules in the brain parenchyma, and focal mononuclear inflammation along the cranial nerve roots of the medulla.[6,16,57,66,131,158] In the cerebellum, the Purkinje cell layer demonstrates inflammation, neuronal loss, and gliosis.

In cases of acute flaccid paralysis, WNV demonstrates a penchant for infection of the anterior motor neurons. The anterior horns of cervical and lumbar regions are the most severely involved areas.[16,57,66] Evidence of neuronophagia, neuronal loss, and perivascular monocytic infiltrates have been found.[16] Loss of ganglionic neurons, nodules of Nageotte, and perivascular lymphocytic aggregates have been seen in the dorsal roots and sympathetic ganglia.[16,57]

TREATMENT

Treatment of West Nile fever and neuroinvasive syndromes is primarily supportive. In the case of West Nile encephalitis and meningoencephalitis, close monitoring of respiratory status is essential. Patients with severe muscle weakness, paralysis, dysphagia, or dysarthria may require mechanical ventilation.

No effective antiviral therapy currently is available for the treatment of WNV syndromes. Ribavirin, a broadly active antiviral, has been shown to be active against WNV in vitro and may have as its mechanism of action error-prone replication.[47,88] In the sole pediatric case report, ribavirin was administered orally to a 14-year-old with Hodgkin lymphoma and West Nile meningoencephalitis who recovered completely.[177] Although encouraging, a report of ribavirin in an unblinded, uncontrolled manner for the treatment of WNV infection in 37 patients during an outbreak in Israel in 2000[39] associated its use with a higher mortality rate. Bivariate analysis indicated that its use was associated with a greater likelihood of death (odds ratio, 6.7; 95% confidence interval, 3.0 to 15.2). Because this was an uncontrolled trial, selection bias for its use in more severely ill patients cannot be excluded. However, in a hamster model of WNV infection, ribavirin treatment alone increased the mortality rate.[124] These reports indicate that the use of ribavirin for the treatment of WNV infections appears to be unwarranted and unsupported at this time.

In cell culture and animal models, interferon alfa has been shown to be beneficial against WNV.[124] Although anecdotal reports[89,161] in humans suggest that the use of interferon alfa-2b may improve recovery from West Nile encephalitis, an open label, nonblinded trial did not indicate benefit.[166]

Work in animal models[11] and anecdotal reports[17,68,69,79,167] of the use of an intravenous immunoglobulin preparation containing a high neutralizing titer of anti-WNV antibodies (Omr-IgG-am) for the treatment of West Nile neuroinvasive disease in adults[68,69,167] and children[17,79] have been published. The limited information suggests a benefit from its use. A phase I/II blinded, clinical trial of this preparation in patients aged 18 years or older with West Nile encephalitis was completed recently in the United States.

PREVENTION

Prevention of WNV infections requires both community-based public health programs and personal measures. Mosquito abatement through elimination of mosquito breeding sites, use of larvicides to breeding areas, and application of pesticides targeted to adult mosquitoes are approaches used to reduce the abundance of mosquitoes in the environment.

The use and maintenance of door and window screens as physical barriers to prevent mosquitoes from gaining access to homes can limit exposure to mosquitoes. Similarly, during engagement in outdoor activities where mosquitoes are prevalent, the use of mosquito netting in sleeping quarters also may decrease exposure to mosquito bites. Individuals should use insect repellent on skin and clothes when exposure to mosquitoes is probable or possible. Repellents such as DEET, picaridin, and

lemon eucalyptus oil have been shown to be effective and provide long-lasting protection.[8] Soybean oil also has been shown to be effective as a mosquito repellent. Permethrin and DEET have been shown to be effective protection against mosquitoes when they are applied to clothing.

Last, avoidance of environments where mosquitoes are found to be in high concentrations as well as avoidance of outdoor activities during dusk and dawn, when mosquito activity is the highest, should be encouraged. Efforts to prevent transmission of WNV through blood transfusions has led to the use of nucleic acid amplification–based tests for the screening of donated blood. At this time, screening of organ donors for the possible presence of West Nile infection has not been recommended.[92]

Killed vaccines and recombinant canarypoxvirus vaccines for the prevention of equine WNV disease are available for use in the United States.[119,132] Live attenuated recombinant, recombinant subunit, and DNA vaccines for immunization against WNV disease in humans are in developmental stages.[5,108,120,134,149] A recombinant WNV–yellow fever virus vaccine and a WNV DNA vaccine have begun clinical testing.[120,134] Results of the double-blind clinical trial of the recombinant vaccine have been published.[120] An infectious clone of yellow fever 17D virus was used as the background for the homologous exchange and insertion of the WNV prM and E genes. The WNV E gene was mutated at three sites to reduce neurovirulence.[5] The chimeric WNV–yellow fever virus vaccine was well tolerated, and the incidences of adverse events were similar in both the vaccine and the placebo groups. A transient viremia was observed in 93 percent of recipients of chimeric vaccine. Within 14 to 28 days after a single dose of the WNV–yellow fever chimeric vaccine was administered, high levels of neutralizing antibodies were detected in 100 percent of the recipients by day 21 after vaccination. $CD8^+$ responses were induced in 93 to 100 percent of vaccine recipients.

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CHAPTER **188c**

YELLOW FEVER

Duane J. Gubler

Yellow fever is a mosquito-borne viral disease of humans and lower primates that occurs naturally in tropical Africa and the Americas (Fig. 188–8). Epidemics, which can occur in both urban and rural areas, often are associated with severe hemorrhagic disease and high fatality rates.

HISTORY

Yellow fever was described as a disease entity first in 1648 in the Yucatan, Mexico. It apparently was part of a larger regional epidemic that affected the Caribbean islands and Central America from Barbados to Mexico from 1647 to 1649.[11] Although it was first described in the Americas, yellow fever virus, along with its principal urban epidemic mosquito vector, *Aedes aegypti*, most likely originated in Africa and was introduced to the New World by the slave trade. During the 17th, 18th, 19th, and early 20th centuries, epidemic yellow fever was a major public health problem in the Western Hemisphere. Large epidemics occurred in tropical America as well as in the United States (as far north as Boston) and Europe (as far north as England).[15] Epidemics

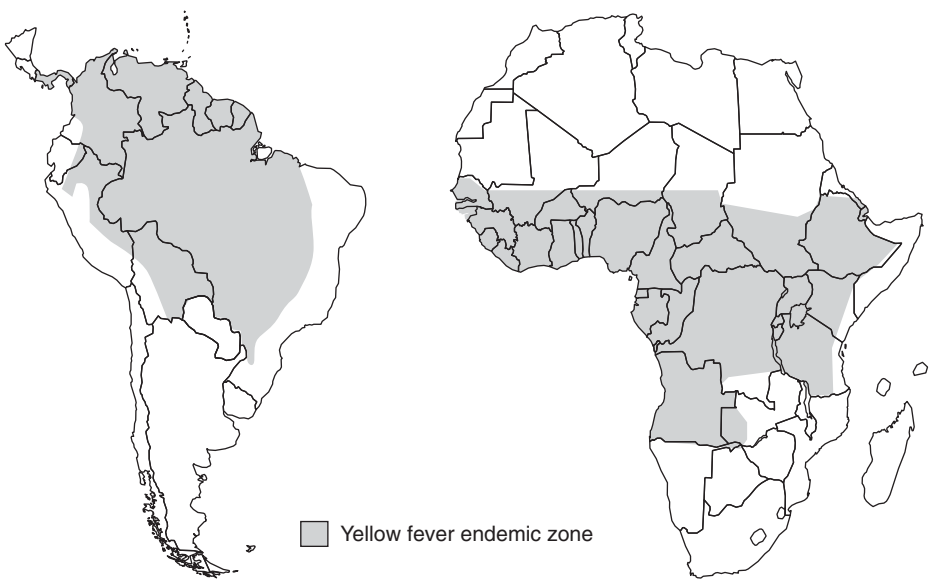

Figure 188–8 Geographic distribution of yellow fever in Africa and the Americas.

occurred primarily in port cities as a result of spread by sailing vessels and commerce.

After transmission by mosquitoes was documented by Reed and the Yellow Fever Commission in Cuba,[14] major efforts were undertaken to keep the disease in check by mosquito control. The first yellow fever virus was isolated in 1927. By 1938, an effective live attenuated virus vaccine had been developed. Yellow fever was controlled in the Americas by eradication of the mosquito vector of urban disease, *A. aegypti*, from most Central and South American countries. In Francophone countries of West Africa, yellow fever was controlled by mass vaccination programs. The result was the disappearance of major urban epidemics of yellow fever in both Africa and the Americas during the 1950s, 1960s, and 1970s. In the mid-1980s, however, the urban form of disease re-emerged in West Africa, with major epidemics in Nigeria and increased transmission in other countries.[9] Kenya experienced its first epidemic in history in 1993. In the Americas, *A. aegypti* has reinfested most Central and South American countries from which it had been eradicated, and urban centers of the American tropics are at their highest risk for epidemic urban yellow fever in more than 60 years.[2] Thus, the disease continues to be an important public health problem in both Africa and the Americas. Yellow fever transmission has never been recognized in Asia or the Pacific.

ETIOLOGIC AGENT

Yellow fever is caused by yellow fever virus, the prototype of the genus *Flavivirus*, family *Flaviviridae*. The genus *Flavivirus* contains 53 viruses, which are small (40 to 50 nm in diameter), spherical, and enveloped, with single-stranded RNA about 11 kb in length.[5] Many of the flaviviruses are very closely related antigenically, which has resulted in extensive cross-reactivity in most serologic tests, so a laboratory diagnosis is difficult to make. Yellow fever virus is part of an antigenic complex that is unique from other members of the family *Flaviviridae*.[3]

EPIDEMIOLOGY

Yellow fever viruses are maintained in natural zoonotic cycles involving lower primates and canopy-dwelling mosquitoes that breed in tree holes in the rain forests of both Africa and the Americas, called the jungle or sylvatic cycle of yellow fever.[9,11,15] Humans become involved accidentally when they encroach on this forest cycle. Humans thus infected may transport the virus back to a village or city during the incubation period of 2 to 14 days. If infected persons are fed on by urban *A. aegypti*, the virus then can be transmitted to other humans by mosquito bite after an extrinsic incubation of 8 to 10 days. Major urban epidemics are transmitted by this highly domesticated mosquito, which lives in close association with humans in most tropical cities of the world.[2]

Yellow fever occurs throughout much of sub-Saharan Africa and tropical America in the sylvatic cycle (see Fig. 188–8). In Africa, cercopithecoid and celobid monkeys are the main vertebrate hosts; infection rarely causes illness and death in these species. Year-round enzootic transmission by *Aedes africanus* occurs in the rain forests. In wet savanna areas bordering the rain forests of western and central Africa, transmission increases during the rainy season and decreases during the dry season. *Aedes furcifer*, *A. africanus*, and *Aedes leuteocephalus* are the main mosquito vectors in this "zone of emergence," and the virus is transmitted from monkey to monkey, monkey to human, and human to human. In the dry savanna zones, yellow fever activity is intermittent and takes place mainly during the rainy season, but it also occurs in major epidemics in urban areas, where stored water provides the ideal larval habitat for *A. aegypti*. In East Africa, *Aedes simpsoni (Aedes bromeliae)* provides the link between the *A. africanus* sylvatic cycle and humans in areas bordering gallery forests.

In tropical America, howler, spider, squirrel, owl, capuchin, and wooly monkeys all act as vertebrate hosts for yellow fever virus. Mosquitoes of the genus *Haemagogus* are the principal vectors in tropical American rain forests, where they feed in the canopy as well as at ground level. Other mosquito species, such as *Sabethes chloropterus*, *Aedes leucocelaenus*, and *Aedes fulvus*, may play secondary roles. Although most cases of yellow fever have been reported from Bolivia, Brazil, Venezuela, and Peru in recent years, the enzootic zone probably includes the rain forests of at least 10 countries (Colombia, Ecuador, Peru, Bolivia, Brazil, Venezuela, Guyana, Suriname, French Guiana, and Trinidad[17]) and involves "wandering" enzootic transmission among the monkey populations. The humans involved are mainly adult men who work in the forest.

Vertical transmission of yellow fever virus from an infected female mosquito through the eggs to her offspring plays an important role in survival and maintenance of yellow fever virus in enzootic cycles and has been demonstrated in nature in West Africa by isolation of the virus from male *A. furcifer* mosquitoes. Experimentally, *Haemagogus* and *A. aegypti* mosquitoes have been shown to be capable of vertical transmission. This mechanism is thought to be of major importance in survival of the virus during prolonged dry periods in both enzootic regions.

Urban epidemics of yellow fever have reappeared in West Africa in the past 17 years.[13] Unfortunately, surveillance is very poor, and the actual number of cases reported is thought to be grossly underestimated. For example, in Nigeria in 1986 and 1987, the number of reported cases and deaths during epidemics was 2612 and 973, respectively. Seroepidemiologic studies, however, estimated that the actual number of cases and deaths was 130,000 and 29,000, respectively.[13]

In the Americas, the last urban yellow fever epidemic occurred in 1942. Reinvasion of American tropical urban centers by *A. aegypti*, however, has placed more than 300 million susceptible individuals at risk. As might be expected, urban transmission has been reported recently. In 1998, urban transmission of yellow fever was documented in Santa Cruz, Bolivia.[16] Although not comprising a large number of cases, this outbreak underscores the high risk that many tropical urban centers have at the beginning of the 21st century. In addition, six fatal cases of yellow fever in travelers who visited South America (four cases) or Africa (two cases) in recent years have been confirmed. Three of these cases were U.S. citizens, and three were European. Why contemporary yellow fever epidemics have not occurred in the Americas, despite the high risk, is not known. The dramatic increase in transmission of dengue in urban centers of the American tropics in recent years possibly has resulted in a high prevalence of heterotypic flavivirus antibody that protects against severe yellow fever clinical illness. Another possibility is that the enzootic virus strains are not well adapted to urban transmission by *A. aegypti*.

Epidemic yellow fever has never been reported in Asia or the Pacific. The reason is not known, but both variation in mosquito vector competence and partial protection by heterotypic flavivirus antibody have been suggested as reasons that this virus has never become established in that part of the world. If urban epidemics do occur in the American tropics, however, the virus is expected to move very quickly to this and other permissive areas where urban *A. aegypti* mosquitoes are found; this movement undoubtedly would cause a major international public health emergency.

CLINICAL MANIFESTATIONS

Infection with yellow fever virus causes a spectrum of illness ranging from inapparent infection to severe yellow fever with the classic triad of jaundice, hemorrhage, and albuminuria, which is associated with a high case-fatality rate. Most yellow fever infections are manifested clinically as a mild to severe viral syndrome without symptoms of intoxication; 10 to 20 percent of infections may result in classic yellow fever.[8,11,12]

The incubation period may be as long as 13 days but generally is 3 to 6 days.[11] The onset of illness is abrupt, with fever, headache, backache, myalgia, nausea, and other nonspecific signs and symptoms. In mild cases, the illness will last for several days, after which recovery is uneventful and complete. In severe cases, prostration occurs commonly, and examination reveals congestion of the skin, conjunctivae, and mucous membranes. The pulse rate usually increases early in the illness, and blood pressure is normal. Leukopenia is a frequent finding, and mild albuminuria may be noted. The temperature generally ranges between 38.5° C and 40° C. Nausea and vomiting are common manifestations. Minor hemorrhagic manifestations such as epistaxis and bleeding gums may be observed. This period of infection may last for approximately 3 days, at which time the congestion declines and a relative bradycardia may occur despite the elevated temperature (Faget sign). The temperature falls to or below normal, and the patient feels better.

In most patients, this period of remission signals the beginning of convalescence; but in severe cases, it may last only a few hours, after which the patient enters a period of intoxication characterized by venous congestion and extreme bradycardia, despite a secondary rise in temperature. Nausea and vomiting are severe and associated with epigastric pain. Prostration, jaundice, marked albuminuria, and anuria are present. Hemorrhagic manifestations include hematemesis and melena.

The jaundice in some patients is not striking; it is difficult to detect in early disease and often is not detected until after death. The severity of hemorrhagic manifestations also varies greatly, but some hemorrhage can be found in most cases. As indicated earlier, minor hemorrhagic manifestations may be observed in the early stage of illness; severe hemorrhage usually develops late in the illness, although it may occur in fulminant cases as early as the second or third day. Hemorrhage may be so severe that it causes shock and death from blood loss. Albuminuria is one of the most common findings in yellow fever, and it occurs in all but very mild cases. It is present early in the illness and may increase rapidly. The albuminuria probably is related to renal involvement during the period of infection. Anuria, on the other hand, appears to be related to hepatic involvement and never is seen in the absence of other signs of liver infection.

Death may ensue as early as 2 to 4 days after onset but usually occurs after 7 to 10 days of illness in 20 to 50 percent of severe cases. In patients in whom death occurs later, autopsy generally reveals a cause other than yellow fever. Patients with severe infection often have lowered resistance to secondary infection, which may develop at the time of convalescence. Other complications include kidney abscess, pneumonia, suppurative parotitis, and skin infection. Convalescence usually is rapid and complete except for a general weakness that may last for several weeks. Permanent damage to the liver or kidneys is not apparent.

PATHOLOGY

The gross pathology in fatal cases of yellow fever is not striking.[12] The skin, sclerae, serosa, some internal organs, and subcutaneous fat usually have moderate icterus. Serous effusions, edema, and hemorrhages, including petechiae and purpuric lesions on the skin, conjunctivae, mucous membranes, stomach, duodenum, and bladder, often are present. Gastrointestinal hemorrhage may be prominent.

The most characteristic lesions caused by yellow fever virus are seen in the liver, although liver failure is not generally the cause of death. The liver may have a yellowish color and be enlarged and fatty in consistency. In typical cases, marked necrosis of the midzone of the lobule is present. The necrosis extends both centrally and peripherally and on average involves 80 percent of the lobule in fatal cases.[8,12] The cells bordering the central vein and portal areas usually are spared. Councilman and Torres bodies can be observed in hepatocytes. Little or no inflammatory response occurs, and the reticulin framework is preserved.

The kidneys generally are tense and swollen. Glomerular changes are minor, but acute tubular necrosis and fatty metamorphosis may be significant. Cloudy swelling, degeneration, and fatty infiltration may occur in the myocardial fibers. The spleen and lymph nodes are depleted of lymphocytes, and mononucleocytes or histiocytes accumulate in the follicles of the spleen. Edema and petechiae may be observed in the brain.

LABORATORY FINDINGS

Leukopenia and albuminuria are common findings in early stages of the disease. In severe cases, prolonged prothrombin and partial thromboplastin times, thrombocytopenia, fibrin split products, and elevated liver enzymes are observed. Total and conjugated serum bilirubin levels are elevated. Albumin levels in urine usually are less than 5 g/L, but in rare cases they may reach 40 g/L. The urine contains bile. Cerebrospinal fluid usually is normal but may be under increased pressure.

DIFFERENTIAL DIAGNOSIS

Clinically, yellow fever is difficult to differentiate from many other viral, bacterial, and parasitic infections, including other viral hemorrhagic fevers such as Lassa, Ebola, Marburg, and Rift Valley in Africa; the illnesses due to arenaviruses in the Americas; and dengue hemorrhagic fever in both continents. Other diseases that cause fever and jaundice, such as viral hepatitis, leptospirosis, falciparum malaria, tick-borne relapsing fever, typhus, typhoid, and Q fever, also should be considered in the differential diagnosis. A definitive diagnosis of yellow fever can be made only by use of the appropriate laboratory test.

LABORATORY DIAGNOSIS

Specific laboratory diagnosis requires isolation of the virus or serologic, nucleic acid amplification, or immunohistochemical tests. Virus can be isolated most easily from acute-phase serum taken during the first 4 days of illness, but it has been isolated as long as 14 days after the onset of illness as well as from the liver after death.[12] The most sensitive method of virus isolation is inoculation of mosquitoes followed by AP-61 cell culture from *Aedes pseudoscutellaris* mosquitoes. Vero cells and inoculation of suckling mice also can be used but are less sensitive. Polymerase chain reaction is highly sensitive, and yellow fever RNA can be amplified from these same tissues with a sensitivity higher than that of virus isolation.[6] Viral antigen can be demonstrated in the liver by immunohistochemical methods.[4] Either fresh or formalin-fixed tissue can be used with these techniques, which may be performed to establish a virologic diagnosis after virus has been cleared from the blood.

Serologic diagnosis depends on the collection of properly timed acute- and convalescent-phase serum samples to demonstrate a rise in specific antibody. Serologic tests commonly used to diagnose yellow fever include hemagglutination inhibition, complement fixation, and the plaque reduction neutralization test (PRNT) as well as newer tests, such as enzyme-linked immunosorbent assay (ELISA) for both IgG and IgM antibodies.[3] The immunofluorescent assay is used in some laboratories.

Antibodies detected by hemagglutination inhibition, PRNT, immunofluorescent assay, and IgM capture ELISA appear within 5 to 7 days after the onset of illness, whereas complement-fixation antibodies appear later, usually after 10 to 14 days. Hemagglutination-inhibition and PRNT antibodies persist at detectable levels for many years (>50) in most patients, whereas the duration of complement-fixation and IgM antibodies is uncertain, but they probably wane to undetectable levels after 12 to 18 months.

PRNT is the most sensitive and specific of the serologic tests. In patients who have had no previous flavivirus infection, this test can be used to make a specific diagnosis of yellow fever, as can IgM capture ELISA. Cross-reaction between yellow fever antigen and antibodies to other related flaviviruses complicates the serodiagnosis of this and other flavivirus diseases. Hemagglutination inhibition, immunofluorescent assay, and IgG ELISA are nonspecific tests in which considerable cross-reactivity with heterologous flavivirus antibodies occurs.

The use of yellow fever 17D vaccine in disease-endemic areas also may complicate serologic diagnosis. Vaccination induces low-titer (1:10 to 1:40) hemagglutination-inhibition and neutralizing antibodies but no detectable immunofluorescent or complement-fixation antibodies. Vaccination also induces IgM antibody, which may remain at detectable levels for as long as 18 months. Vaccination of individuals who have had a previous flavivirus infection induces an anamnestic response of heterotypic flavivirus antibodies at high titer (≥1:1280).

TREATMENT

Treatment of yellow fever is supportive because no specific therapy exists.[8,10] Currently, no antiviral drugs are available. Patients with severe disease requiring hospitalization should have complete bed rest with good nursing care and close monitoring of vital organ functions. Salicylates should be avoided, but mild sedatives may be helpful. Maintenance of fluid and electrolyte balance is critical. Guidelines for intensive care of severe yellow fever cases have not been established. Secondary bacterial infections may occur and should be treated with appropriate antibiotics.

PROGNOSIS

Mortality in all yellow fever infections is low (<5%), but in severe cases requiring hospitalization, it may be 20 to 50 percent.[8,12] The prognosis is poor for patients who enter a period of intoxication with rapidly increasing albuminuria, jaundice, fever, and severe hemorrhage. Patients in the terminal stage of illness usually are somnolent, have below-normal temperatures, and may have intractable hiccups.

PREVENTION AND CONTROL

The most practical and cost-effective method of preventing yellow fever is vaccination. A single dose of 17D vaccine provides effective, long-term (10 years) protection and should be used in the World Health Organization Expanded Program of Immunization in enzootic countries of Africa and the Americas.

The live attenuated 17D vaccine is prepared from infected chicken embryos and produces effective immunity in more than 95 percent of recipients.[12] Adverse reactions rarely occur, but in recent years, such events in elderly persons have been reported increasingly.[1] Infants younger than 4 months have a high risk for development of encephalitis and should not be vaccinated with the live attenuated 17D vaccine until they are 9 months of age. Pregnant women also should avoid being vaccinated because vaccine virus may infect the developing fetus, although the risk of adverse events associated with congenital infection is unknown. Finally, the live attenuated 17D vaccine should not be given to persons who are allergic to eggs or to those who are immunodeficient or receiving immunosuppressive drugs.

The other method of preventing yellow fever is mosquito control, especially during epidemic activity. The principal mosquito vector of urban yellow fever, *A. aegypti*, is a highly domesticated species that lives in and around the houses of humans.[2] It breeds primarily in artificial containers that collect rainwater or in domestic water storage containers. The most sustainable and effective prevention, therefore, is to control, discard, or chemically treat larval habitats in the domestic environment.[2] This process is labor-intensive but can be done with the help of the citizens in the community.

Some authorities recommend adult mosquito control with insecticide space sprays, primarily ultra-low-volume sprays. Recent field trials, however, have shown this approach to be ineffective unless portable sprayers are used to treat the inside of each dwelling. Because of the excessive cost and lack of efficacy of ultra-low-volume application of insecticides, this method is not recommended.

Patients suspected of having yellow fever should be protected from mosquitoes. The most effective protection is to use a mosquito net on the bed during the acute febrile period of illness. Alternatively, patients can be kept in screened rooms. Effective repellents are available to treat clothing and exposed skin.

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CHAPTER **188d**

Dengue and Dengue Hemorrhagic Fever

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Dengue fever is an acute febrile illness syndrome caused by several arthropod-borne viruses and characterized by biphasic fever, myalgia or arthralgia, rash, leukopenia, and lymphadenopathy. Synonyms are dengue and breakbone fever. Dengue hemorrhagic fever, a febrile disease caused by dengue viruses, is characterized by abnormalities in hemostasis and by leakage of fluid and protein from capillaries, which in severe cases results in shock (dengue shock syndrome). It is thought to have an immunopathologic basis. Synonyms are hemorrhagic dengue; acute infectious thrombocytopenic purpura; and Philippine, Thai, and Singapore hemorrhagic fever.

The dengue subgroup is composed of four antigenically distinct members.[21,48,56,96] From 1956, according to reports received by the World Health Organization (WHO), dengue viruses were thought to be responsible for more than 5,500,000 hospital admissions and 74,000 deaths in Southeast Asia, southern China, India, Sri Lanka, Pakistan, Cuba, Venezuela, Colombia, Guyana, Brazil, Puerto Rico, and Central America, mostly in vital, healthy children. In tropical Asian countries, dengue is among the 10 leading causes of death in children ranging in age from 1 to 15 years.[46]

The first outbreak that resembled a disease now recognized as dengue fever was described by Benjamin Rush in Philadelphia, Pennsylvania, in 1780.[11,103] Epidemics probably caused by dengue were common from the 18th to the 20th centuries in inhabitants of the Atlantic coastal areas of the United States and South America, the Caribbean islands, and the Mississippi basin.[103] Dengue viruses almost certainly were the cause of the 5- and 7-day fevers that occurred in European colonists in tropical Asia.[11] Similar epidemics occurred commonly in settlers in tropical Australia, where in 1905 *Aedes aegypti* was identified as a dengue vector by Bancroft.[72] Ashburn and Craig found the etiologic agent in human blood and showed that it could pass through a diatomaceous earth filter.[103] An intrinsic incubation period of 3 to 8 days in humans, an extrinsic incubation period of 8 to 11 days in mosquitoes, immunity in people and monkeys, and the nonsusceptibility of most domestic animals were demonstrated in the classic studies of Siler and Simmons and their coworkers[103,104] between 1924 and 1930. When dengue viruses were isolated in laboratory mice in 1943 and 1944, the modern era of dengue research began.[57,98] Two strains from Hawaii and Papua New Guinea failed to cross-protect humans. From this experiment, researchers recognized the existence of at least two different dengue viruses; they were named dengue virus type 1 and type 2.[97,98]

During most of the pre-virologic era, dengue viruses were thought to be the cause of a generally benign, self-limited febrile exanthem. However, death, shock, and severe hemorrhagic manifestations accompanied the classic dengue fever outbreaks in Australia in 1897 and thereafter for 15 years. Similar phenomena were recorded in Greece in 1928 and in Formosa in 1931.[35] This "new" syndrome was recognized again in Manila in 1954. It was called Philippine hemorrhagic fever because of a resemblance to the epidemic hemorrhagic fever then occurring in United Nations troops in the Korean Peninsula.[48] In 1956, Philippine hemorrhagic fever was associated with dengue when types 3 and 4 were recovered.[48] It now has become endemic throughout tropical Asia.[35,43,46] Since 1967, the terms dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS) have come into general use.[18]

In 1981, Cuba reported a severe outbreak involving more than 116,000 patients hospitalized within 3 months, 10,000 of whom had DHF/DSS.[63] In 1986, an epidemic of DHF/DSS occurred on Hainan Island, China[80]; in 1988, the Maldive Islands, Sri Lanka, and India were involved.[105,114] From about 1987, DHF/DSS outbreaks have occurred in Guyana, Venezuela, Brazil, Colombia, Central America, and, on a lesser scale, Puerto Rico.[46,83]

By epidemiologic criteria, dengue viruses are arthropod-borne (arboviruses) because they are transmitted biologically by various members of the genus *Stegomyia*.[72,75,103,104] Gene structure, replicative strategy, and antigenic relatedness place the dengue viruses in the family *Flaviviridae*.[56,115] At present, 68 members of the flavivirus family have been identified; 29 of them are established as human pathogens.[56] Cross-comparisons by plaque reduction neutralization tests have shown dengue viruses to be an antigenic subgroup with little relationship to other flaviviruses.[21] In addition to their antigenic relatedness and their ability to be transmitted by *Stegomyia*, each type of dengue virus produces a closely similar clinical syndrome in susceptible human beings.[36,97,103,104]

Dengue virions are spherical particles approximately 50 nm in diameter. The central core, approximately 25 nm in diameter, has icosahedral symmetry and contains a single plus strand of RNA. Dengue RNA consists of approximately 11,000 nucleotides coding from the 5′ end for core, premembrane, envelope, and five nonstructural proteins.[34] Large amounts of nonstructural protein 1 (NS1) are released from infected cells.[69] The envelope, which is studded with poorly resolved projections, is composed of many replicates of the envelope protein (54 kd) embedded in a lipid bilayer. When it is assembled on the virion, the envelope protein bears some epitopes unique to serotypes. Antibodies to these epitopes neutralize by hindering viral attachment or entry into cells. Other epitopes are shared between dengue viruses (dengue subgroup antigens) and other flaviviruses (group antigens).

Four clearly defined types exist, as determined by plaque reduction neutralization tests using antibodies raised by infection of monkeys or fluorescent antibody tests using monoclonal antibodies raised in mice.[21,94] Presumably, the cell attachment receptor differs for each dengue serotype and is blocked by neutralizing antibody.[49] The four types have distinctive genetic structures.[68,69,86,87,115,118] Phylogenetic studies suggest that human dengue viruses diverged from four zoonotic dengue types relatively recently, whereas the four zoonotic types evolved from a common ancestor in the more remote past.[115] Different dengue strains cluster in groups that differ genetically (genotypes).[15,16,68,69,86,110] Genotypes consist of viruses of similar genetic structure that usually circulate within one geographic area.[16,69,110] Genotyping can be used to trace the movement of dengue viruses between the continents. Of particular interest, a strain of dengue type 2 isolated in Jamaica in 1981 and the dengue 2 viruses recovered from 1981 Cuban DHF patients belong to a Southeast Asian genotype.[86,100] The most sharply divergent genetic differences are among human viruses and strains from Asian and African zoonotic cycles.[86,115]

Dengue virus can be grown in 1- to 2-day-old mice or hamsters by intracerebral inoculation or in various mosquitoes by oral

or parenteral inoculation. High mouse-passaged virus grows and produces deaths in weanling mice. Various tissue cultures of vertebrate and invertebrate origin support dengue virus growth in vitro, as reviewed in the following section.

TRANSMISSION

A. aegypti, a crepuscular daytime-biting mosquito, is the principal vector. All four virus types have been recovered from naturally infected *A. aegypti*.[35,42] In most tropical areas, *A. aegypti* is highly domesticated and breeds in water stored for drinking, washing, or bathing or in any container collecting fresh water. Dengue viruses also have been recovered from naturally infected *Aedes albopictus*, which breeds outdoors in vegetation.[29,35,43,75] Outbreaks in the Pacific area have been attributed to *Aedes scutellaris* and *Aedes polynesiensis*.

In urban areas, transmission of dengue may be explosive and involve as much as 70 to 80 percent of the population.[103] Because *A. aegypti* has a limited flight range, spread of virus is mainly by mobile viremic human beings.

Dengue viruses replicate in the gut, brain, and salivary glands of infected mosquitoes without apparent harm to adult mosquitoes.[90] Mosquitoes are infectious for a lifetime and as long as 70 days in experimental circumstances.[104] Because female mosquitoes take repeated blood meals, long-lived female mosquitoes have great potency as vectors. Several species of *Stegomyia* and *Toxorhynchites* are infected readily by intrathoracic inoculation, although the threshold of infection by oral feeding is higher.[89,90] *A. aegypti* and *Culex quinquefasciatus* can transmit dengue mechanically by interrupted feeding.[46,104] The contribution of mechanical feeding to the spread of dengue virus during epidemics has never been measured. Because of the "skittishness" of *A. aegypti* and its habit of feeding during the day when its intended victim is awake and often moving, interrupted feeding with simultaneous transmission to multiple hosts within a household must be a common occurrence.

A. aegypti preferentially feeds on people and hence is most abundant in and around human habitations. The mosquito breeds in clean water. Biting activity is reduced at temperatures below a wet bulb temperature of 14°.[23] Transmission of dengue in temperate countries is interrupted during winter weather, and dengue has not established itself endemically at latitudes above 25 degrees north or south. Breeding sites may be provided by humans through living habits, as in Thailand, where water is stored in and around homes in large earthenware jars.[35,42] In contrast, *A. aegypti* is not abundant in some parts of India because only small amounts of water are brought to homes from village wells for immediate use. Water in flower vases, household offerings, ant traps, coconut husks, tin cans, and rubber tires may supply breeding sites for *A. aegypti*.[29,42]

In the tropics, outbreaks of dengue generally coincide with the monsoon season. Eggs, which resist desiccation, are deposited inside water containers above the water line.[42] With the beginning of monsoon rains, a large number of eggs laid outdoors are hatched. Indoor populations do not show seasonal change. Temperature is important in controlling viral transmission. Evidence indicates that the extrinsic incubation period shortens with increasing mean temperatures; mosquito biting rates increase with increased temperature and relative humidity.[102]

In sylvan settings, dengue virus has been isolated from three subgenera of *Aedes*, namely, *Stegomyia*, *Diceromyia*, and *Finlaya*, some in circumstances suggesting the occurrence of transovarial transmission. This phenomenon has been demonstrated experimentally, but its contribution to maintenance of virus in a habitat is unknown.[91]

EPIDEMIOLOGY

HOST RANGE

Inoculation of strains of dengue with known human pathogenicity does not produce demonstrable infection in adult chickens, lizards, guinea pigs, rabbits, hamsters, or cotton rats.[101,104]

Subhuman primates generally are susceptible to infection by dengue viruses. Numerous species belonging to *Macaca*, *Cercopithecus*, *Cercocebus*, *Papio*, *Hylobates*, and *Pan* can be infected by the bite of virus-infected mosquitoes or by injection of infectious virus preparations.[38,101,104] Infection is essentially asymptomatic. Viremia occurs at levels sufficient to infect mosquitoes. Simmons and colleagues[104] were the first to note that wild-caught *Macaca philippinensis* resisted dengue infection whereas *Macaca fuscatus* (Japanese macaque) was susceptible. Work by Rudnick[93] in Malaysia has revealed a jungle cycle of dengue transmission involving canopy-feeding monkeys and *Aedes niveus*, a species that feeds on both monkeys and humans. Although the existence of a jungle dengue cycle in the Malaysian rain forest has been documented, the full geographic range of the subhuman primate zoonotic reservoir is not known. In the 1980s and 1990s, extensive epizootics of dengue virus type 2 involved subhuman primates over wide areas of West Africa.[88] Genetic and epidemiologic studies have shown that urban human dengue and jungle monkey dengue are relatively compartmentalized.[86,115] Urban dengue is vectored by anthropophilic mosquitoes, and the virus travels along routes of transportation. *A. aegypti* and susceptible humans are so abundant and so widespread that should dengue viruses be exchanged between humans and monkeys, detection would be extremely difficult. If, in the future, urban dengue is eliminated but vector mosquito populations are unabated, dengue viruses may once again be introduced from jungle cycles.

GEOGRAPHIC DISTRIBUTION

Outbreaks of dengue fever have been documented on every continent except Antarctica.[35] Evidence suggests that human dengue may have originated from enzootic or endemic foci in tropical Asia.[37,75] The probable spread of *A. aegypti* during historical times from Africa throughout the world provided an ecologic niche quickly occupied by several human viral pathogens: yellow fever, chikungunya, and the dengue viruses. During the 18th and 19th centuries, epidemics occurred in newly settled lands, largely because of the necessity for storage of domestic water in frontier areas. Isolated shipboard or garrison outbreaks often confined to nonindigenous settlers or visitors were reported in Africa, the Indian subcontinent, and Southeast Asia.[11,72,103] During World War II, dengue virus infections occurred commonly in combatants of the Pacific War and spread to staging areas not normally infected: Japan, Hawaii, and Polynesia.[38,46,97] DHF-like disease was described clinically in Thailand beginning in 1950 and in the Philippines from 1953; cases were confirmed etiologically as dengue in 1958 and 1956, respectively.[48] DHF first was described in Singapore and Malaysia in 1962, Vietnam in 1963, India in 1963, Ceylon (Sri Lanka) in 1965, Indonesia in 1969, Burma (Myanmar) in 1970, China in 1985, and Kampuchea and Laos from about 1985; major outbreaks have occurred in Sri Lanka and India since 1988, in French Polynesia since 1990, in Pakistan since 1998, and in Bangladesh since 1999.[1,26,37,38,80,105,114,119] DHF has occurred at consistently high endemicity in Thailand, Burma, Vietnam, and Indonesia.[43] In Thailand, it is the third ranking cause of hospitalization and death in children. Intermittent epidemics have involved Malaysia and the Philippines. The largest epidemic in history occurred in Southeast Asia in 1998, when

more than 490,000 hospitalizations and 4000 deaths were reported to the WHO.

During the past 20 years, major epidemics of all four dengue serotypes have occurred on several Pacific islands.[29,37,38,46]

American genotype dengue 2 virus circulated in the American hemisphere for hundreds of years and was the only virus to survive an intensive period of control of *A. aegypti* designed to eradicate urban yellow fever.[29] In 1963, an Asian strain dengue 3 was introduced into the Caribbean, dying out in the 1980s. Dengue viruses became fully established in the hemisphere after the introduction of a Southeast Asian dengue 1 in 1977.[22,83,86] Dengue 1 spread rapidly and now is endemic on the larger Caribbean islands and in Mexico, coastal Central America, and the tropical areas of Guyana, Venezuela, Colombia, Ecuador, Peru, and Brazil.[37,46,116] A sharp dengue virus type 2 epidemic in Cuba in 1981 involving 116,000 hospitalizations in a 3-month period led to island-wide control of *A. aegypti* and apparent eradication of the virus.[63,82] In 1981, an Asian dengue 4 was introduced into the Caribbean, spread widely, and now is endemic throughout the Caribbean basin. In 1986 and 1987, dengue virus type 1 spread through most of coastal Brazil and from there to Paraguay and to Peru and Ecuador.[22,29,83] In 1990, more than 9000 dengue cases were reported from Venezuela; 2600 of them were classified as DHF, and 74 deaths were associated with the epidemic.[83] Dengue virus types 1, 2, and 4 were isolated.[83] Shortly thereafter, DHF/DSS caused by dengue type 2 was reported from Brazil and French Guiana.[79,84] In 1994, dengue virus type 3 was introduced into the region.[53] In 1997, dengue virus type 2 with a Southeast Asian genotype was introduced into Santiago de Cuba and caused a sharp DHF/DSS outbreak observed only in individuals 20 years and older.[33]

Dengue virus types 1 and 2 have been recovered from humans with mild clinical illness in Nigeria in the absence of epidemic disease.[37] In 1983, dengue virus type 3 was isolated in Mozambique.[43,46] DHF/DSS has not been reported, and even dengue fever outbreaks are rare events. In this respect, Africa resembles the situation in Haiti, where multiple dengue serotypes are transmitted at high rates among a predominantly black population but severe disease is not recognized.[47]

CLINICAL MANIFESTATIONS

DENGUE FEVER

In classic dengue fever (seen most frequently in adults), after an incubation period of 2 to 7 days, patients experience a sudden onset of fever, which rapidly rises to 39.5° C to 41.4° C (103° F to 106° F) and usually is accompanied by frontal or retro-orbital headache. On occasion, back pain precedes the fever. A transient, macular, generalized rash that blanches under pressure may be seen during the first 24 to 48 hours of fever. The pulse rate may be slow in proportion to the degree of fever. Myalgia or bone pain occurs soon after onset and increases in severity. During the second to sixth day of fever, nausea and vomiting are likely to occur, and generalized lymphadenopathy, cutaneous hyperesthesia or hyperalgesia, aberrations in taste, and pronounced anorexia may develop.

One or 2 days after defervescence, a generalized, morbilliform, maculopapular rash appears, with sparing of the palms and soles. It disappears in 1 to 5 days. In some cases, edema of the palms and soles may be noted, and desquamation may occur. About the time of appearance of this second rash, the body temperature, which has fallen to normal, may become elevated slightly and establish the biphasic temperature curve.

Epistaxis, petechiae, and purpuric lesions are uncommon manifestations but may occur at any stage of the disease. Swallowed blood from epistaxis may be passed per rectum or be vomited and could be interpreted as bleeding of gastrointestinal origin. Gastrointestinal bleeding, menorrhagia, and bleeding from other organs have been observed in some dengue fever outbreaks.[85,111,117] Very clear evidence argues that peptic ulcer predisposes to gastrointestinal hemorrhage; in some cases, patients may exsanguinate during an otherwise normal dengue fever.[111] This syndrome is confused with DSS (see later) and contributes to a misunderstanding of the pathogenesis of severe dengue disease. The mechanism of the hemorrhagic diathesis that commonly occurs with dengue virus infection is not known, but speculation centers on platelet abnormalities. The DHF syndrome is differentiated from dengue fever by its association with thrombocytopenia and capillary leakage.[123]

After the febrile stage, prolonged asthenia, mental depression, bradycardia, and ventricular extrasystoles are noted commonly in adults.[72]

Primary infections with dengue virus types 2 and 4 are thought to be largely inapparent, particularly in children.[33,55,112,113] Primary infections with dengue virus types 1 and 3 in adults produce biphasic fever and rash as the most characteristic features of the dengue fever syndrome.[10,20,36,72,97,104] Manifestations vary with age and among patients. In infants and young children, the disease may be undifferentiated or characterized by a 1- to 5-day fever, pharyngeal inflammation, rhinitis, and mild cough. A distinctive mean incubation period, duration of illness, or spectrum of clinical findings could characterize disease with different dengue types, although these factors have not been studied carefully. Differences in mild dengue syndromes, hospitalized dengue cases (predominantly during secondary dengue virus infections), and chikungunya illnesses are illustrated in the section on chikungunya (see Tables 187–4 to 187–8).

Chikungunya virus infections produce a dengue fever syndrome. Because chikungunya and the dengue viruses are transmitted by the same vector mosquitoes, the diseases frequently are observed in the same patient populations. Chikungunya illnesses begin more abruptly than does dengue and are of shorter duration. Maculopapular rash, conjunctival injection, and myalgia or arthralgia occur more frequently in chikungunya than in dengue illnesses, but other features associated with both viruses are remarkably similar.[11,77]

DENGUE HEMORRHAGIC FEVER/DENGUE SHOCK SYNDROME

DHF/DSS is an acute vascular permeability syndrome accompanied by abnormal hemostasis. The incubation period of DHF/DSS is unknown but is presumed to be the same as that of dengue fever. In children, progression of the illness is characteristic.[18,76,77,123] A relatively mild first phase with an abrupt onset of fever, malaise, vomiting, headache, anorexia, and cough may be followed after 2 to 5 days by rapid deterioration and physical collapse. In Thailand, the median day of admission to the hospital after the onset of fever is day 4. In this second phase, the patient usually has cold and clammy extremities, a warm trunk, a flushed face, and diaphoresis. Patients are restless and irritable and complain of midepigastric pain. Frequently, scattered petechiae appear on the forehead and extremities, spontaneous ecchymoses may develop, and easy bruisability and bleeding at sites of venipuncture are common findings. Circumoral and peripheral cyanosis may occur. Respirations are rapid and often labored. The pulse is weak, rapid, and thready, and the heart sounds are faint. The pulse pressure frequently is narrow (≤20 mm Hg); systolic and diastolic pressure may be low or unobtainable. The liver may become palpable two or three fingerbreadths below the costal margin and usually is firm and nontender. Chest radiographs show unilateral (right) or bilateral pleural effusions. Approxi-

mately 10 percent of patients have gross ecchymosis or gastrointestinal bleeding. After a 24- or 36-hour period of crisis, convalescence is fairly rapid in children who recover. The temperature may return to normal before or during the stage of shock.

PATHOGENESIS AND PATHOLOGY

In rhesus monkeys experimentally infected with dengue virus by subcutaneous inoculation, virus replicates initially in the skin. Virus disseminates rapidly to regional lymph nodes and then to lymphatic tissue throughout the body.[73] Early in the viremic period, virus can be recovered only from the skin inoculation site and lymph nodes, whereas 2 to 3 days later, evidence of general dissemination to skin and other tissues is found. Virus is recovered from the skin, lymph nodes, and several leukocyte-rich tissues for up to 3 days after termination of viremia. Virus can be recovered from circulating leukocytes and from the skin only at the end of the viremic period. The number of sites from which virus can be recovered increases as the infection progresses. Intracellular infection is terminated abruptly 2 to 3 days after viremia ceases.

In humans, dengue viruses infect and replicate efficiently in intracutaneous Langerhans cells in vitro and in tissue explants.[124] Virus ultimately targets liver parenchymal cells, where infection produces apoptosis, but such cells may not serve as replicative hosts.[51] Late in infection, viral antigen is found associated with circulating B lymphocytes.[58] Fluorescent antibody, virus isolation, electron-microscopic, and in situ hybridization studies suggest mononuclear phagocytes as major infection hosts.[5,37-41,43,53a,78]

Animals infected initially with dengue virus type 1, 3, or 4 and then with dengue type 2 virus had higher viremia than when the same strain was inoculated into susceptible animals.[38,43] This phenomenon, in vivo antibody-dependent enhancement of dengue virus infection, provides an explanatory hypothesis of the immunopathogenesis of dengue in humans. Epidemiologic, clinical, and virologic studies of DHF/DSS in humans have shown a significant association between severe illness and infection in the presence of circulating dengue antibody, whether it is passively acquired from the mother or actively acquired from previous infection.[9,31,32,44,59,60,95,99] This circulating antibody has two biologic activities: neutralization of virus and enhancement of infection.[39,41] In Thailand, DHF/DSS developed in infants during dengue virus type 2 infection only when maternal neutralizing antibody had catabolized to low titer and infection-enhancing antibodies were left in circulation.[59] Similarly, in a prospective study of dengue virus infection in Thai children, DHF/DSS occurred in children who had circulating enhancing antibodies from a previous single dengue virus infection, but it did not occur in children whose first infection left them with low levels of cross-reactive dengue virus type 2 neutralizing antibody at the time of the second dengue virus infection.[60] A similar mechanism explains the failure of secondary infections to produce DHF/DSS with the American genotype dengue type 2.[116] American genotype dengue 2 viruses are significantly neutralized by human anti–dengue 1, whereas Southeast Asian dengue 2 viruses are not.[62] The full-length sequences of the American and Southeast Asian dengue 2 genomes reveal limited amino acid differences.[68]

In vitro studies of dengue virus type 2 demonstrated enhanced growth in cultures of human mononuclear phagocytes that were supplemented with very small quantities of dengue antibodies.[45] Investigators have proposed that the number of infected mononuclear phagocytes in humans with naturally or passively acquired antibody may exceed that in nonimmune individuals.[38,39,43] In serial blood samples taken early in the illness in children experiencing secondary dengue infections, enhanced viremia levels or enhanced levels of dengue NS1 predicted severe disease.[69,113] Vascular permeability is thought to result when infected cells are attacked by activated T lymphocytes, with the subsequent release of vasoactive cytokines.[3,27,28,38-41,64,74,92] Cytokine production should be quantitatively related to the number of infected target cells. The reduced risk for DHF/DSS in protein-calorie malnourished children[109] and the increased risk for DHF/DSS in girls versus boys are consistent with the hypothesis that a competent immune elimination system must be available to generate the cytokines that produce DHF/DSS.[38,40,46]

Evidence indicates the existence of a human dengue resistance gene. Epidemiologic studies of the 1981 Cuban outbreak demonstrated a higher risk for DHF/DSS in white than in black individuals.[31,63] A search for DHF/DSS in black children in Haiti revealed no cases, despite the presence of high dengue type 1, 2, and 4 infection rates and circulation of the Southeast Asian genotype dengue 2 viruses.[47] Several HLA antigens have shown differing frequencies in DHF/DSS cases and controls.[13] Early in the acute stage of secondary dengue virus infection, rapid activation of the complement system occurs.[8,81] During shock, blood levels of C1q, C3, C4, C5, C6, C7, C8, and C3 proactivator are depressed and C3 catabolic rates are elevated. The blood clotting and fibrinolytic systems are activated.[40,120] As yet, neither the mediator of vascular permeability nor the complete mechanism of bleeding has been identified unequivocally. The kinin system apparently is not involved. Recent studies suggest a role for tumor necrosis factor, interleukin-2, and interferon-γ.[64,92] Capillary damage allows fluid, electrolytes, protein, and, in some instances, red blood cells to leak into intravascular spaces.[121] This internal redistribution of fluid, together with deficits caused by fasting, thirsting, and vomiting, results in hemoconcentration, hypovolemia, increased cardiac work, tissue hypoxia, metabolic acidosis, and hyponatremia. A mild degree of disseminated intravascular coagulation, plus liver damage and thrombocytopenia, could contribute additively to produce hemorrhage.[120]

If tissue cultures or suckling mice are used for recovery of virus, dengue virus usually is absent in tissues at the time of death.[78] If patients experienced a second dengue infection, their tissue suspensions contain large quantities of dengue-neutralizing antibodies. The use of mosquito inoculation techniques improves viral isolation rates.[89,90,107] Genetic probes increase viral detection sensitivity still further.[51]

On pathologic examination, usually no gross or microscopic lesions are found that might account for death.[5] In rare instances, death may be caused by gastrointestinal or intracranial hemorrhage. Minimal to moderate hemorrhage is seen in the upper gastrointestinal tract, and petechial hemorrhage occurs frequently in the intraventricular septum of the heart, on the pericardium, and on the subserosal surfaces of major viscera. Focal hemorrhaging is seen occasionally in the lungs, liver, adrenals, and subarachnoid space. The liver usually is enlarged, often with fatty changes. Yellow, watery, at times blood-tinged effusions are present in serous cavities in approximately three fourths of patients. Retroperitoneal tissues are markedly edematous.

On microscopic examination, perivascular edema in soft tissues and widespread diapedesis of red blood cells can be seen. Maturational arrest of megakaryocytes may be noted in the bone marrow,[66] and increased numbers of such megakaryocytes are seen in the capillaries of the lungs, in the renal glomeruli, and in the sinusoids of the liver and spleen. Proliferation of lymphocytoid and plasmacytoid cells, lymphocytolysis, and lymphophagocytosis occur in the spleen and lymph nodes.[5] In the spleen, malpighian corpuscle germinal centers are necrotic. Depletion of lymphocytes occurs in the thymus. In the liver, varying degrees of fatty metamorphosis, focal midzonal necrosis, and hyperplasia of Kupffer cells are present.[5] Non-nucleated cells with vacuolated acidophilic cytoplasm resembling Councilman bodies (apoptotic hepatocytes[51]) are seen in the sinusoids. A mild, proliferative glomerulonephritis is present. Biopsy specimens of the rash

reveal swelling and minimal necrosis of endothelial cells, subcutaneous deposits of fibrinogen, and, in a few cases, dengue antigen in extravascular mononuclear cells and on blood vessel walls.[40,43]

DIAGNOSIS

DENGUE FEVER

A clinical diagnosis can be made by having a high index of suspicion and knowledge of the geographic distribution and ecology of dengue viruses. Activities of the patient during the period preceding the onset of illness may give important clues to the possibility of infection.

The differential diagnosis includes many viral, respiratory, and influenza-like diseases and the early stages of malaria, typhoid fever, scrub typhus, hepatitis, and leptospirosis. Abortive forms of these diseases may never evolve beyond a dengue-like stage. Four arbovirus diseases are dengue-like: chikungunya and o'nyong-nyong fever (togaviruses), West Nile fever (flavivirus), and Oropouche (bunyavirus). Four other diseases are dengue-like but without rash: Colorado tick fever, sandfly fever, Ross River fever, and the mild form of Rift Valley fever. Because of the variation in clinical findings and the multiplicity of possible causative agents, the descriptive term *dengue-like disease* should be used until a specific etiologic diagnosis is provided by the laboratory.

DENGUE HEMORRHAGIC FEVER/DENGUE SHOCK SYNDROME

According to WHO criteria, DHF is a dengue illness accompanied by thrombocytopenia (<100,000/mm^3) and hemoconcentration (hematocrit >20% of the recovery value). Early detection of vascular permeability remains a diagnostic problem; however, the use of strain-gauge plethysmography documents up to 50 percent higher microvascular permeability in DHF/DSS patients than in controls.[4] Pleural or peritoneal effusions observed by ultrasonography or radiography are virtually pathognomonic. DSS is diagnosed when these manifestations are accompanied by hypotension or narrow pulse pressure (≤20 mm Hg). In areas endemic for dengue, hemorrhagic fever should be suspected in children with a febrile illness who exhibit shock and hemoconcentration with thrombocytopenia. Hypoproteinemia, hemorrhagic manifestations, and hepatic enlargement are frequent accompanying findings. Because many rickettsial diseases, meningococcemia, and other severe illnesses caused by a variety of agents may produce a similar clinical picture, the diagnosis should be made only when epidemiologic or serologic evidence suggests the possibility of dengue. Hemorrhagic manifestations have been described in other diseases of viral origin, including the arenavirus hemorrhagic fevers of Argentina, Bolivia, and West Africa (Lassa fever); the tick-borne hemorrhagic fevers of India and the former Soviet Union; hemorrhagic fever with renal syndrome, which occurs across northern Eurasia, specifically from Scandinavia to Korea; and Marburg and Ebola virus infections in central Africa.[54]

LABORATORY STUDIES

An etiologic diagnosis can be made by serologic study of properly collected serum samples, by isolation of the virus, and by identification of viral RNA or the nonstructural protein NS1 in acute-phase sera.[2,52,123] The acute-phase serum or plasma collected for isolation of virus should be stored optimally at –65° C or colder. Serologic diagnosis depends on a fourfold or greater increase in antibody titer by hemagglutination inhibition, complement fixation, radioimmunoassay, enzyme-linked immunosorbent assay (ELISA), or neutralization. IgM capture ELISA has revolutionized dengue serology, and commercial kits are available.[19,52] Primary and sequential (secondary) dengue virus infections result in the production of dengue-reactive IgM antibodies, which appear during the acute phase and disappear within 60 days of infection.[52] Secondary or primary dengue virus infections can be confirmed in a single serum specimen by quantitating IgM-IgG antibody ratios. IgG antibody concentrations are abundant in secondary but minimal in primary dengue virus infection. Dengue NS1 proteins can be detected in blood during the acute illness phase by dengue group–specific antibodies, and these have been formatted into commercial ELISA or rapid immunochromatographic tests.[2a,25a] By combining IgM-capture ELISA and NS1 detection, point-of-care tests may soon be available to diagnose acute dengue infections.

Numerous techniques are available for the recovery and identification of dengue viruses.[101,123] Recommendations for general use have been made by a WHO expert committee.[123] Acute-phase serum, mosquito suspensions, or other materials thought to contain dengue virus may be inoculated into suckling mice, which may be examined for sickness or subtle neurologic signs or challenged at 14 days with a neurovirulent dengue virus. Repeated subpassage markedly increases the neurovirulence of dengue virus. Alternatively, materials may be inoculated into any of several tissue cultures of mammalian or mosquito origin and examined for plaques under agar or methylcellulose overlay, for cytopathic effect or resistance to a challenge cytopathic virus by use of a fluid overlay, or for fluorescence or other markers with the use of an appropriate detection system. Intrathoracic inoculation of *A. albopictus*, *A. aegypti*, or *Toxorhynchites* spp. is a highly sensitive dengue virus recovery system.[89,90]

TREATMENT

DENGUE FEVER

Treatment is supportive. Bed rest is advised during the febrile period. Antipyretics or cold sponging should be used to keep the body temperature below 40° C (104° F). Paracetamol (10-15 mg/kg every 4-6 hours) is the preferred antipyretic agent. Analgesics or mild sedation may be required to control pain. Fluid and electrolyte replacement therapy is required when deficits caused by sweating, fasting, thirsting, vomiting, or diarrhea are present. Because of the risk of Reye syndrome and the dengue hemorrhagic diathesis, aspirin should not be given to reduce fever or to control pain.

DENGUE HEMORRHAGIC FEVER/DENGUE SHOCK SYNDROME

Explicit recommendations for management of DSS have been made by a WHO expert committee.[120] These and earlier recommendations by Cohen and Halstead[18] and recent studies by Dung and colleagues[25] and Wills and associates[122] are the basis of this section.

No specific antiviral treatment exists, but in DHF/DSS, symptomatic and supportive measures are effective.

The major pathophysiologic abnormality seen in DHF/DSS is an acute increase in vascular permeability that leads to leakage of plasma. Plasma volume studies revealed a reduction of more than 20 percent in severe cases. Supporting evidence of plasma leakage (and consequent hypovolemia) includes a rapid, weak pulse; diaphoresis; cool, pale skin of the extremities; decreased urine output; and direct measurement by strain-gauge plethysmography,[4] pleural effusion on chest radiography or ultrasonography, hemoconcentration, and hypoproteinemia. Pleural effusion may not be evident until after fluid resuscitation is started.

In the absence of increased vascular permeability, clinically significant hemoconcentration may result from thirst, dehydration, fever, anorexia, and vomiting. Fluid intake by mouth should be as ample as tolerated. Electrolyte and dextrose solution (as used in diarrheal disease), fruit juice, or both are preferable to plain water. With high fever, a risk of convulsions exists, so antipyretic drugs may be indicated. Salicylates should be avoided because they are known to cause bleeding and acidosis. Acetaminophen is preferable at the following doses: younger than 1 year, 60 mg per dose; 1 to 3 years of age, 60 to 120 mg per dose; 3 to 6 years of age, 120 mg per dose; and 6 to 12 years of age, 240 mg per dose.

Children should be observed closely for early signs of shock. The critical period is the transition from the febrile to the afebrile phase. Frequent hematocrit determinations are essential because they reflect the degree of plasma leakage and the need for administration of intravenous fluid. Hemoconcentration usually precedes changes in blood pressure and pulse. The hematocrit should be determined daily from the third day until the temperature becomes normal for 1 or 2 days.

Oral or parenteral fluid therapy can be administered in an outpatient rehydration unit for correction of dehydration or acidosis or when signs of hemoconcentration are present. The volume of fluid and its composition are similar to the fluids used for the treatment of diarrhea with moderate dehydration. The fluids should consist of the following:

- One third to half of the total fluid as physiologic saline solution.
- Half to two thirds of the remainder as 5 percent glucose in water.
- For acidosis: one fourth of the total fluids should be one-sixth molar sodium bicarbonate.
- Solution for fluid therapy in DHF: lactated Ringer solution, 5 percent glucose in one half normal physiologic saline solution, 5 percent glucose in one half lactated Ringer solution, 5 percent glucose in one third physiologic saline solution.
- Fluids as listed are calculated to be given during a 24-hour period. If the child seems severely dehydrated, half the calculated fluid is given in the first 8 hours and the second half in the next 16 hours. During rapid administration of fluids, watching for signs of cardiac failure is especially important.

Written orders should be explicit about the type of solution and the rate of administration. A rough estimate of flow may be derived from the formula

$$\text{mL/hr} = \text{drops/min} \times 3$$

SHOCK

Patients should be hospitalized and immediately treated when they have any of the following signs and symptoms of shock: restlessness or lethargy, cold extremities and circumoral cyanosis, rapid and feeble pulses, narrowing of pulse pressure (≤20 mm Hg) or hypotension, and sudden rise in hematocrit or continuously elevated hematocrit despite the administration of intravenous fluid.

Shock is a medical emergency. Immediate administration of intravenous fluid to expand plasma volume is essential. In children, shock may develop or subside during the course of a 48-hour period, so close observation 24 hours a day is imperative. Patients with similar degrees of severity should be grouped together. Those with shock require intensive 24-hour care by nurses and physicians. Paramedical workers or parents can assist in provision of oral fluid therapy or in surveillance of the rate of intravenous fluid administration and general status of the patient.

Initial fluid therapy with lactated Ringer or isotonic saline solution (20 mL/kg intravenously) infused as rapidly as possible may be required. Positive pressure may be necessary. In continued or profound shock, plasma expanders (6% dextran 70 or 6% hydroxyethyl starch [MW 200,000]) may be given to replace the initial fluid and administered at a rate of 10 to 15 mL/kg/hr or more until improvement in vital signs is apparent. In most cases, not more than 20 to 30 mL/kg of plasma is needed.

Intravenous fluids (5% dextrose, half-normal lactated Ringer, or half-normal saline solution) are continued, even after improvement in vital signs and a declining hematocrit. The rate of fluid replacement should be adjusted as judged by the rate of plasma loss. Plasma loss may continue for 24 to 48 hours. Microhematocrit determination is a simple and reliable index for estimating plasma leakage. Monitoring of central venous pressure may be necessary in the management of severe cases of shock that are not easily reversible.

Administration of intravenous fluids should be discontinued when the hematocrit drops to approximately 40 percent and the patient's appetite improves. Good urine flow indicates sufficient circulating volume. In general, fluid therapy is not needed beyond 48 hours after termination of the shock. Extravasated plasma is reabsorbed and causes a further drop in hematocrit after the administration of intravenous fluid is stopped; if more fluid is given, hypervolemia, pulmonary edema, or heart failure may result. Of importance is that a drop in hematocrit at this stage is not viewed as a sign of internal hemorrhage. A strong pulse and blood pressure along with a wide pulse pressure and diuresis are good vital signs at this resorption phase. They rule out the likelihood of gastrointestinal hemorrhage, which occurs most frequently during the shock stage.

Hyponatremia and, commonly, metabolic acidosis occur. Electrolyte and blood gas determinations should be performed periodically in severely ill patients as well as in those who do not seem to respond as promptly as expected. These determinations will provide an estimate of the sodium deficit and help determine the presence and degree of acidosis. Acidosis, in particular, may lead to disseminated intravascular coagulation if it is uncorrected. Heparin may be indicated in some of these patients, but extreme caution should be exercised in its use. In general, early volume replacement and early correction of acidosis with sodium bicarbonate result in a favorable outcome, and heparin is not required. Heparin should be reserved for patients with laboratory evidence of consumptive coagulopathy (disseminated intravascular coagulation) or intractable bleeding.

Sedatives are needed in some cases because of marked agitation. Hepatotoxic drugs should be avoided. Chloral hydrate administered orally or rectally is recommended in a dose of 30 to 50 mg/kg as a single hypnotic dose (maximal dose, 1 g). In patients without pulmonary complications, paraldehyde, 0.1 mL/kg intramuscularly (maximal dose, 10 mL), also may be used.

Oxygen therapy should be given to all patients in shock, but an oxygen mask or tent may increase apprehension.

Blood transfusion is indicated only in patients with severe bleeding (e.g., gastrointestinal bleeding, hematemesis, melena). Fresh whole blood is preferable. Blood grouping and matching for prompt treatment should be carried out as a routine precaution for every patient in shock.

In general, steroids do not shorten the duration of disease or improve the prognosis in children receiving careful supportive therapy.[108]

Frequent recording of vital signs and determination of hematocrit are important in evaluating the results of treatment. If patients show any signs of shock, vigorous antishock therapy should be instituted promptly. Patients should be monitored con-

stantly until it is reasonably certain that the danger has passed. In practice, the following should be carried out:

1. Pulse, blood pressure, respiratory rate, and temperature should be taken every 15 to 30 minutes or more often, until the shock resolves.
2. Hematocrit or hemoglobin studies should be performed every 2 hours for the first 6 hours and then every 4 hours thereafter until the patient is stable.
3. An accurate record of intake and output, including the type of fluid given, should be made. The frequency and volume of urine output should be recorded.

Having a pro forma sheet for recording symptoms, signs, and treatment of DHF and DSS cases is useful.

A blinded comparison of four intravenous fluids by Dung and associates[25] provided evidence that the relatively more expensive lactated Ringer solution provides no greater benefit than 0.9 percent saline does. Dextran could contribute to altered hemostasis.[46] Patients with DHF/DSS are resuscitated as though they have diarrhea. A more apt therapeutic analogy may be burn injury or hypovolemia from "third-space" loss in surgery. Of interest would be a trial of small-volume hypertonic saline with or without colloid.[46] The widespread and unstudied use of blood products to treat hemorrhage or simple thrombocytopenia[14] suggests a need for many more careful studies of DHF/DSS resuscitation.[46] In this regard, placebo-controlled or blinded studies are the ideal.[25,108]

EPIDEMIC DENGUE HEMORRHAGIC FEVER

During epidemics, outpatient and inpatient facilities may be overwhelmed. Under these conditions, only children requiring hospital care should be admitted. A recently elevated body temperature and positive tourniquet test result are sufficient to suggest DHF; when possible, a microhematocrit and platelet count should be performed in the outpatient department. Patients with thrombocytopenia and an elevated hematocrit should be sent to a rehydration ward or, if the hematocrit does not fall or rises in the face of fluid therapy, admitted to a hospital. If a patient lives a long distance from the hospital and nearby accommodations are not available, admission for observation may be necessary. Triage can be performed by properly instructed paramedical workers. Competent laboratory assistance is an essential factor.

Cool extremities, skin congestion, circumoral cyanosis, and rapid pulse are signs that suggest the need for hospitalization. Patients should be hospitalized until 2 days after the fever terminates.

REGULATORY MEASURES

Dengue diseases are not subject to international quarantine or surveillance regulations. An intensive and effective voluntary reporting system has been devised by the regional offices of the WHO.

PROGNOSIS

Not all patients suspected of having DHF need to be hospitalized because circulatory failure and shock may develop in only approximately a third of patients. Mild and moderate cases may be treated on an outpatient basis. For the purpose of early recognition of shock, parents should be advised to bring the patient back if evidence of clinical deterioration is noted or such warning signs as restlessness with or without lethargy, severe abdominal pain, cold extremities, and skin congestion occur on or after the third day following the onset of fever.

In most cases, early and effective replacement of lost plasma with plasma, plasma expanders, or fluid and electrolyte solutions (or any combination of these products) results in a favorable outcome. The acute onset of shock and the rapid, often dramatic clinical recovery, together with the fact that no destructive or inflammatory vascular lesions are observed, suggest that the disease is produced by transient functional vascular changes caused by short-acting pharmacologic mediators.

Sequelae in dengue or in DHF have not been studied systematically. Common sequelae of mild and uncomplicated dengue virus infection include bradycardia and ventricular extrasystoles during the convalescent stage, often persisting for several weeks. Profound asthenia with or without mental depression has been described. In patients with DHF/DSS, great care must be taken to reduce use of invasive procedures for managing shock. Nosocomial infections such as gram-negative sepsis can masquerade as DHF/DSS. Overhydration during the shock resuscitation phase may lead to heart failure and a complicated, stormy post-shock stage. Infrequently, residual brain damage occurs, apparently as a result of either prolonged shock or, occasionally, intracranial hemorrhage. Children in whom profound shock develops rapidly with no detectable diastolic pressure or with unobtainable blood pressure, children in shock with delayed admission to the hospital, or children in shock with gastrointestinal hemorrhage have a poor prognosis. Mortality rates may exceed 50 percent in these groups.

PREVENTION

Tissue culture–based vaccines for dengue virus types 1, 2, 3, and 4 are immunogenic but not yet licensed for use.[6,7] Numerous multivalent dengue vaccines using a variety of approaches are in various stages of development.[30,50,61,65] At present, prophylaxis depends on the use of insecticides, repellents, protective body clothing, and screens on houses to avoid mosquito bites. Destruction of *A. aegypti* breeding sites also is effective.[16] If water storage is mandatory, a tight-fitting lid or a thin layer of oil may prevent eggs from being deposited or hatching. A larvicide such as temefos (Abate), which is available as a 1 percent sand granule formulation and effective at a concentration of 1 ppm, may be added safely to drinking water.

EPIDEMIC MEASURES

The WHO recommendations are as follows. On the basis of epidemiologic and entomologic information, the size of the area that requires adult mosquito abatement should be determined. With technical-grade malathion or fenitrothion at 438 mL/hectare, two adulticide treatments at a 10-day interval should be made with the use of a vehicle-mounted or portable ultra-low-volume aerosol generator or mist blower.[71,123] Cities of moderate size should stockpile at least one vehicle-mounted aerosol generator, five mist blowers, 10 swing fog machines, and 1000 L of ultra-low-volume insecticide to be prepared to perform adulticide operations over a 20-km^2 area rapidly. With limited funds, such equipment and insecticides can be stockpiled centrally for rapid transportation when required. Priority areas for launching ground applications are those that have a concentration of cases. Special attention should be focused on areas where people congregate during daylight hours, such as hospitals and schools.

During the early stages of epidemics, an ultra-low-volume spray of 4 percent malathion in diesel oil or kerosene may be used to spray all houses within a 100-m radius of the residence of patients with DHF.

ERADICATION AND CONTROL

A. aegypti was eradicated successfully from countries and whole continents with use of the techniques pioneered by the Rockefeller Foundation in its worldwide program to control urban yellow fever.[106] With time, the species successfully re-established itself in much of its former range. An eradication campaign in the United States was abandoned and replaced by a program of disease surveillance and containment of introduced virus.

Mosquito control or eradication programs require the simultaneous use of two approaches: a reduction in breeding sites and the application of larvicides. Alternatively, a significant reduction in population may be effected by closely spaced applications of adulticide.[82]

Source reduction requires public support either by legal sanctions or by voluntary actions (see the following section). Source reduction campaigns should be well organized, supervised, and evaluated. Proper disposal of discarded cans, bottles, tires, and other potential breeding sites not used for storage of drinking or bathing water should be performed. Sides of water storage containers should be scrubbed to remove eggs when the water level is low. Water storage containers for drinking and bathing and flower vases should be emptied completely once weekly. Water containers that cannot be emptied should be treated with Abate 1 percent sand granules at a dosage of 1 ppm (e.g., 10 g of sand to 100 L of water). Treatments should be repeated at intervals of 2 to 3 months.

Vehicle-mounted or portable ultra-low-volume aerosol generators or mist blowers can be used to apply technical-grade malathion or fenitrothion at 438 mL/hectare. Three applications made at 1-week intervals can suppress *A. aegypti* populations for approximately 2 months.

In dengue-endemic countries, little effort has been made to adopt building codes or waste collection methods to reduce the number of mosquito breeding sites.[67] Furthermore, almost no way has been found to use the private sector to implement vector control despite ample evidence of long-term successful programs in the United States.[12,17]

HEALTH EDUCATION

Control of *A. aegypti* has been maintained effectively in some tropical areas through the simple expedient of emptying water containers once a week. During the yellow fever campaigns, strong sanitary laws made the breeding of mosquitoes on premises a crime punishable by fine or jail.[106] In the modern era, Singapore and Cuba have adopted these measures successfully. Health education through mass media or through the schools has been attempted in Burma, Thailand, Malaysia, and Indonesia, but without spectacular success.[24] The goals of health education and community participation approaches are to make the population aware of the identity of the vector of DHF, to describe its biting habits (daytime feeding) and its breeding habits (containers holding clean water), and to motivate people to reduce breeding sources by emptying water from containers on a regular basis.[42] The use of piped water rather than water storage should be encouraged. Studies in Malaysia after the 1973 epidemic of DHF indicated a very low level of functional knowledge among the inhabitants of Kuala Lumpur, Malaysia, about the vector of DHF.[24] Discouragingly, persons who were informed correctly, in most instances, took no action to protect themselves against mosquito breeding in their homes. Extensive effort is being made to apply social science methods to gain the voluntary participation of the population in sustained mosquito control programs.[70]

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CHAPTER **188e**

Japanese Encephalitis

Theodore F. Tsai

Japanese encephalitis (JE), a mosquito-borne flaviviral infection, is the leading cause of childhood viral encephalitis in Asia.

HISTORY

JE first was recognized after an outbreak in Japan in 1924 led to 6125 cases of clinical encephalitis. In retrospect, similar summer-autumn outbreaks were recognized as early as 1871 to 1873. In 1933, Hayashi recovered the virus from monkeys and the brain of a patient, and in 1938, Mitamura confirmed its mosquito-borne mode of transmission by isolating the virus from *Culex tritaeniorhychnus*.[29] Inactivated vaccine prepared from infected mouse brain was licensed in Japan in 1956, and its use has led to control of the disease in developed Asian countries.[58] As poliomyelitis has been brought under control in Asia, JE has become the leading viral central nervous system infection on that continent. New cell culture–derived vaccines are now displacing the 50-year-old mouse brain–derived product.

ETIOLOGIC AGENT

JE virus is the prototypic member of an antigenic complex that includes St. Louis, Murray Valley, Kunjin encephalitis, and West Nile viruses. Molecular taxonomic studies based on nucleotide sequences of the E protein gene or the pre-M region have segregated JE strains into five genotypes that can be mapped roughly to regions of epidemic and endemic transmission in temperate Asia and India or Southeast Asia (four types, including one limited to Indonesia). However, the virus exists as a single serotype, and correspondence of genotype to human virulence has not been demonstrated epidemiologically. Sequence analysis has been helpful in tracing the potential origins of epidemic strains and has suggested the emergence of ancestral JE virus within the last 300 years.

Strains isolated from nature exhibit a range of virulence in mice. With repeated cell culture passage, biologically derived neuroattenuated strains have been produced for use as vaccines. Combinations of mutations in genes of the E glycoprotein, nonstructural proteins, and noncoding regions have been identified with attenuation of the live vaccine strain SA 14-14-2.[55] Comparisons of neurovirulent and attenuated viruses and manipulations of infectious clones have identified specific codon changes (resulting in amino acid changes principally in the E protein) that make fully replicative viruses avirulent.

As with other flaviviruses, important biologic functions such as viral neutralization are associated with the E glycoprotein. Canarypox, yellow fever 17D, and vaccinia recombinant viruses containing various JE viral sequences protect mice from challenge, thus demonstrating the biologic importance of specific genes and their mutations.[7,50,55] Some of the recombinants have utility as synthetic antigens in diagnostic assays or as human or animal vaccines.

ECOLOGY

In its basic transmission cycle, JE virus is transmitted between birds, especially certain egrets and herons, and *Culex* mosqui-

toes.[2,3,72] However, pigs, when present, are the most important source of viral amplification (Fig. 188–9). Pigs maintain a high sustained viremia and may be hosts to thousands of mosquitoes in a single night, thereby providing an abundant source of infected vectors that can transmit the infection further. In the typical setting in rural Asia, the onset of human cases each summer occurs shortly after pigs become infected.[60,72] The importance of pigs to epidemic transmission can be seen in countries such as Bangladesh, Malaysia, and Indonesia, where JE occurs principally in the non-Moslem population, which does not eschew pigs. However, outbreaks of JE have occurred in areas devoid of pigs, where birds, including ducklings, pigeons, sparrows, and possibly other small birds found near human residences, have been the principal amplifying hosts; furthermore, other sylvatic cycles have been proposed.[17,70,72]

C. tritaeniorhychnus is the major mosquito vector in most areas of Asia, although in various regions, related species *(Culex pseudovishnui*, *Culex vishnui*, *Culex gelidus*, *Culex fuscocephalus*, and *Culex bitaeniorhychnus)* are important locally.[2,3] *Culex annulirostris* is the principal vector in northern Australia and the Pacific. Certain anopheline mosquitoes may contribute to the transmission of JE in northeastern India. The primary *Culex* vectors use ground pools and especially rice paddies in their pre-adult stages. Immense numbers are produced from the flooded rice paddies that frequently surround individual residences and villages. With the custom of keeping pigs near or inside houses, all elements of the viral transmission cycle are found close to human activity. The mosquito *C. tritaeniorhychnus* is most active in the evening and night and feeds outdoors. The vector is zoophilic and prefers to feed on large animals rather than on humans.

Domestic animals such as dogs and cattle can be infected, but sufficient viremia does not develop to support further transmission. Because they are attractive to JE vectors, their presence may divert mosquitoes from humans (zooprophylaxis). Clinical illness develops in horses after infection and results in periodic outbreaks and economically significant loss of prize horses. Equine vaccines are administered in China, Mongolia, and Japan to prevent disease. Adult pigs remain asymptomatic after infection; however, sows infected during pregnancy abort or deliver piglets with lethal congenital malformations. Pig vaccines are widely used to prevent these congenital infections.

The overwintering mechanism for JE virus has not been defined clearly, although considerable evidence suggests a carryover of virus in mosquito eggs, with re-establishment of the transmission cycle by vertically infected mosquitoes.[15,72] Viral persistence in local mammalian reservoirs such as bats and reintroduction from external sources by migrating birds or windblown mosquitoes also have been proposed.

EPIDEMIOLOGY

The disease occurs mainly in rural areas, where high levels of virus transmission lead to infection at an early age. Nearly all cases occur in children younger than 10 years, with a slight preponderance in boys.[18] More than 99 percent of infections are subclinical, and cumulative exposure with age leads to seroprevalence rates of 80 percent or more by adulthood.[1,18] In Japan and other developed Asian countries, where children are protected by mass vaccination, adult cases occur principally in adults and especially in the elderly. Waning immunity or other biologic factors associated with aging have been speculated to be risk factors.[35]

Transmission is seasonal: late summer and early fall in temperate regions (July to September) and a longer interval in southern China and Southeast Asia (April to November). The seasonality is more complex in tropical areas, where mosquitoes proliferate after monsoon rains, and two epidemic seasons or year-round transmission is possible in some tropical locations. Although an abundance of mosquitoes usually corresponds to the rainy season, in many locations, vector populations now follow irrigation-controlled schedules of rice field flooding.[57]

JE occurs in nearly every country in Asia (Fig. 188–10), with 30,000 to 50,000 cases reported annually from the region. Trans-

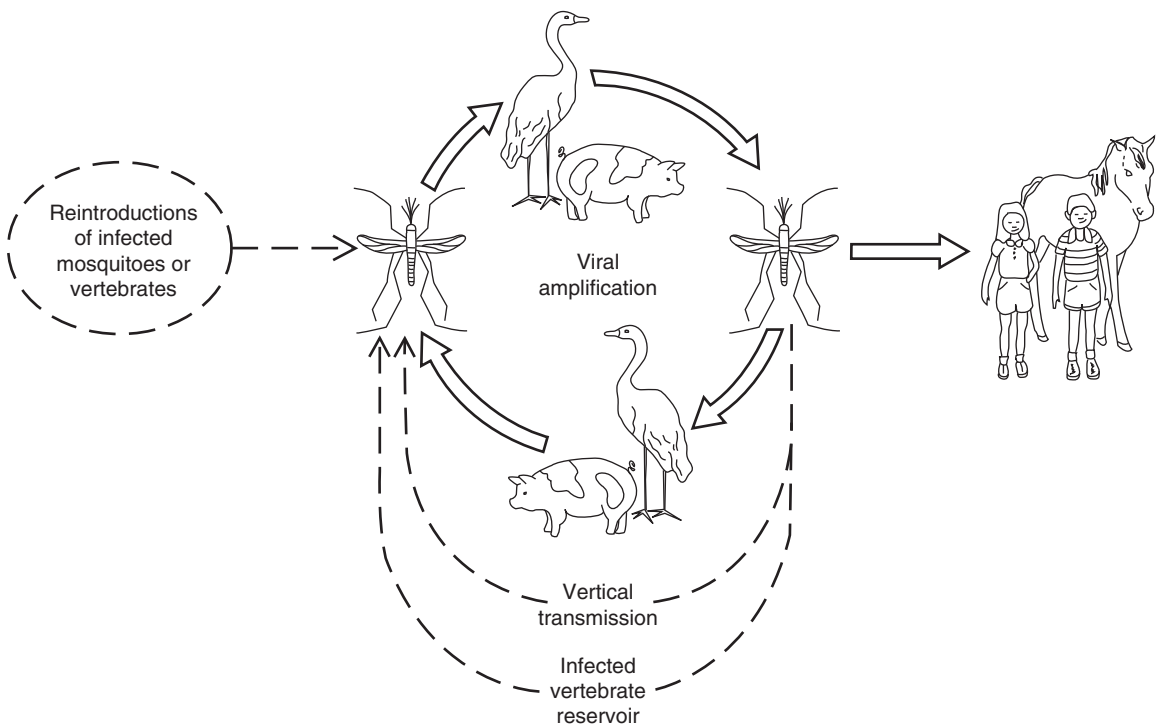

Figure 188–9 Transmission cycle of Japanese encephalitis virus. Speculative portions of the cycle are shown in broken lines.

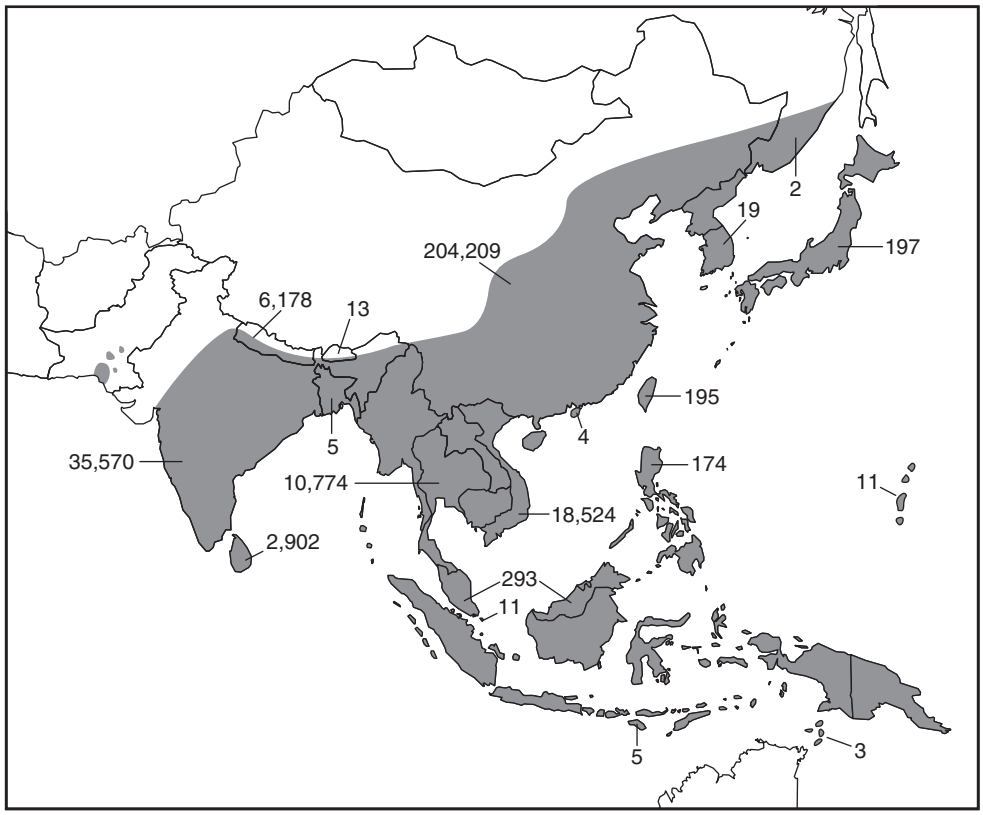

Figure 188–10 Geographic distribution of Japanese encephalitis and reported cases from 1985 to 1994. Torres Strait cases are from 1995.

mission is endemic; annual incidence rates of 1 to 10 per 100,000 population are observed in China and most areas of Southeast Asia, but epidemic attack rates as high as 100 per 100,000 population have been described. Only sporadic cases are reported from Indonesia, Malaysia, and the Philippines, whereas periodic, often sizable outbreaks typify transmission in India. Rare outbreaks have occurred in Oceania, on Guam and Saipan in 1949 and 1989, respectively, and in far northern Australia in 1995 and 1998.[22,59] These outbreaks appeared to have occurred after introduction of the virus by migrating birds or other mechanisms, coupled with unusual conditions of human activity or weather patterns. Establishment of JE virus on the Australian mainland appears to have been limited by the zooprophylactic intervention of alternate hosts, as well as by immunity in the vertebrate host population as a result of infection by other flaviviruses.[85] Few cases are reported from countries where vaccination rates are high (e.g., Japan, Korea, Taiwan) and where development through urbanization, decreased land under cultivation, and improved standard of living have reduced human exposure. The use of agricultural pesticides and centralized pig rearing also may have contributed to a decline in infected vectors in these countries. The significant reduction in human cases belies the persistence of enzootic viral transmission in rural areas of these countries, however.[1,41]

Although economic development has paralleled a decline in JE in some countries, in other areas, development in the form of deforestation, construction of dams, and irrigation schemes has led to increases in transmission of JE virus or its emergence in areas where the disease had not occurred previously. Examples include development projects in the Terai in southern Nepal and the Mahaweli Valley in Sri Lanka, where JE and malaria have become hyperendemic after large-scale programs of deforestation and agricultural development.[60]

Travelers to areas where JE is endemic may be at risk for acquiring the illness.[78] However, risk in the general traveling public is low; approximately 50 cases have been reported in travelers and expatriots from North America, Europe, and Australia in the past 20 years, many of them in military personnel and their family members. The risk has been estimated to be in the range of 1 per 15,000 to 1 per 150,000 person-months of exposure. This low rate can be understood by factoring the probability of development of an illness after a single mosquito bite: only certain vector species transmit the virus, typically less than 3 percent of vector mosquitoes are infected, and only one in several hundred infections leads to clinical illness. Because the principal vector species are found in rural areas and they feed mainly outdoors and in the evening and night, the risk is low in the great majority of travelers who can avoid these circumstances of exposure.

CLINICAL MANIFESTATIONS

Only one in several hundred infections leads to clinical illness, and the overwhelming majority of infections are inapparent or manifested as mild self-limited illnesses. Patients who come to medical attention may have aseptic meningitis or encephalitis, and 5 to 25 percent die. After an incubation period of 4 to 14 days, the earliest symptoms are lethargy, nausea or abdominal pain, headache, and feverishness (Fig. 188–11). During a period of 2 to 3 days, lethargy increases and the child may exhibit uncharacteristic patterns of behavior and motor abnormalities.[34,40,43,69,80] In other cases, a long prodrome of a week or more may occur, with periods of confusion or agitation and unsteadiness. A sudden convulsion is frequently the initial symptom. Unusual manifestations, such as acute psychosis and Guillain-Barré syndrome, also have been reported; the latter may lead to

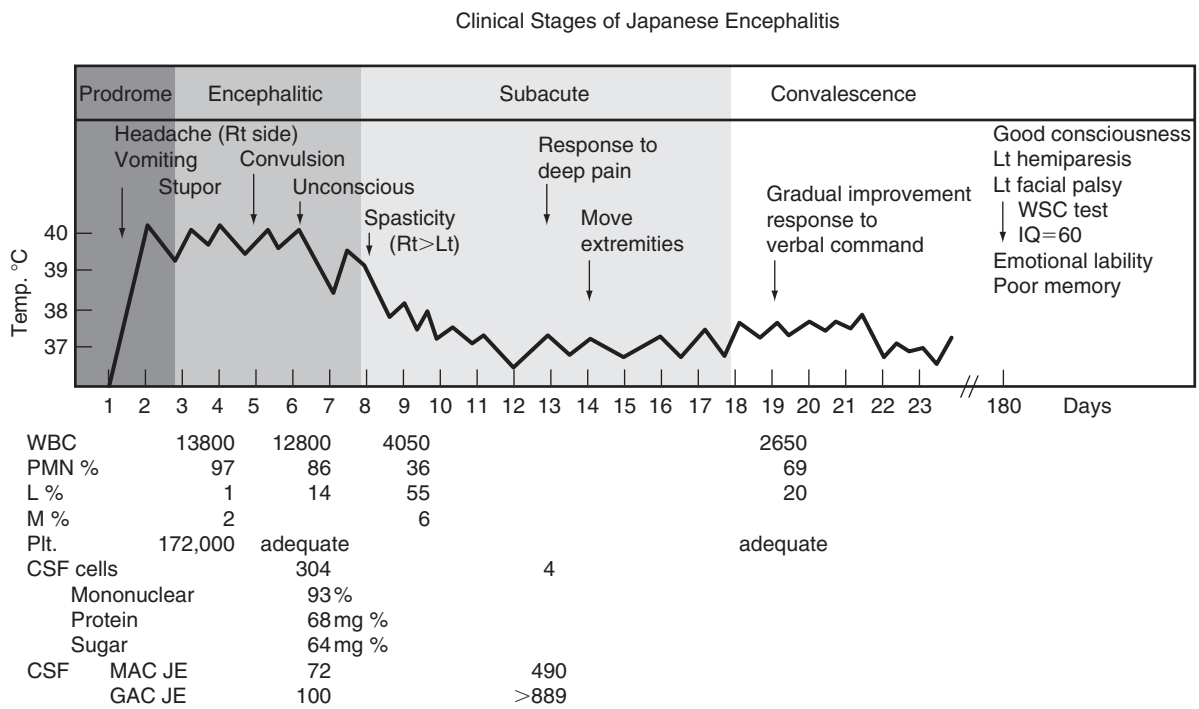

Figure 188–11 Clinical stages of a typical Japanese encephalitis case.

a misdiagnosis of acute poliomyelitis in areas of Asia where that disease has not been eradicated.[68]

The principal physical findings are high fever and obvious alterations in consciousness ranging from mild mental clouding, frank disorientation and delirium, to coma. Some children exhibit bizarre behavior, including shouting, spitting, and other personality changes. Mutism is an initial feature in some cases. Signs of meningeal irritation can be elicited in a third to two thirds of cases. Cranial nerve palsies, mainly disconjugate gaze and central facial paralysis, are observed in a third of cases. Muscle weakness, either flaccid or spastic, is a usual symptom. Weakness may be generalized or in many cases is asymmetric, with hemiparesis or an unusual distribution of flaccid and spastic paralysis. Muscle tone generally is increased, with hyperreflexia and ankle clonus; the Babinski sign and other abnormal reflexes are variable. Some patients exhibit erratic flailing movements. Tremor, rigidity, expressionless facies, or thick slurred speech may be initial features, but more frequently, choreoathetosis and other extrapyramidal signs become evident in the second week of illness.[33,64] Focal or generalized convulsions develop in 50 to 75 percent of patients.[48] Papilledema is seen in 10 percent of cases, and patients occasionally may be hypertensive. Patients with fulminant infections often die during the first 5 days of illness.

In most other cases, fever abates during the next week, and neurologic function gradually improves during the course of several weeks. Further recovery of motor function occurs during the next several months to years, although in other cases neurologic abnormalities may not appear until months after hospital discharge. In more than a third of patients, coma and respiratory failure necessitate institution of ventilatory support. During this prolonged period of recovery from coma and paralysis, stasis ulcers, urinary tract infections, pneumonia, and bacteremia are frequent complications and may be secondary causes of death. A biphasic pattern of illness has been described in anecdotal cases, with a recurrence of motor and behavioral abnormalities 2 to 5 weeks after initial improvement accompanied by larger and more numerous associated magnetic resonance imaging lesions.[63]

Routine laboratory studies initially show a peripheral leukocytosis, often with a left shift and a total leukocyte count as high as 30,000/mm^3. Opening pressures on lumbar puncture usually are normal or slightly elevated. Cerebrospinal fluid (CSF) pleocytosis ranges from fewer than 10 cells to several thousand, with a median of several hundred per cubic millimeter. Lymphocytic pleocytosis is a typical finding, but some patients have an initial predominance of polymorphonuclear cells. CSF glucose and protein levels are generally normal (80% of cases); when elevated, protein levels rarely exceed 100 mg/dL.

Electroencephalograms typically show diffuse theta to delta wave slowing, whereas spike and seizure discharges are relatively uncommon findings. Computed tomograms show diffuse white matter edema and nonenhancing low-density areas, mainly in the thalamus, basal ganglia, and pons. Temporal lobe involvement affects principally the hippocampus, aiding in the differential diagnosis of herpes from encephalitis.[21] Thalamic lesions frequently are associated with unilateral or bilateral hemorrhage. A thalamic location of involvement is consistent with the electroencephalogram's pattern of slowing. Magnetic resonance imaging, particularly diffusion-weighted imaging, shows a similar distribution of abnormalities on T2 studies, with lesions of high or mixed signal intensity in the thalamus, midbrain, basal ganglia, substantia nigra, cerebellum, pons, and spinal cord.[21,33,34,65] JE-associated abnormalities have been reported to appear more frequently in the ipsilateral hemisphere affected by neurocysticercosis-related changes.[79] Electromyograms show a neurogenic pattern consistent with anterior horn cell involvement. Central motor conduction times are prolonged, indicative of diffuse subcortical damage.

PATHOLOGY

Pathologic changes are found in the lungs and viscera in addition to the brain.[20,25,31,32,42,76] On gross examination, the brain appears swollen, and the meninges may be congested. Punctate hemorrhages may be visible macroscopically. Microscopic examination discloses a moderate inflammatory response in the meninges. Foci of neuronal degeneration with parenchymal and perivascular inflammatory responses are found principally in the thalamus

and brain stem as well as in the hippocampus, temporal cortex, cerebellum, and spinal cord. Areas of neuronophagia may be surrounded by microglial nodules, but sharply defined round areas of softening or necrolysis without an inflammatory response also are seen. Purkinje cells and cerebellar glial shrubs also may be lost.

PATHOPHYSIOLOGY

After virus is introduced by a mosquito bite, replication locally and within regional lymphatic tissue leads to a secondary amplified viremia and infection of various organs and the brain. Neuroinvasion is thought to occur through cerebral capillaries; infection crosses from the vascular side of the endothelial cell to the perivascular space, with subsequent neuronal infection.[20] Neurons show evidence of viral antigen in the cell body, axons, and dendrites, suggesting a mechanism of viral spread within the brain from cell to cell.[32] CSF levels of catecholamines and their metabolites are acutely lowered.[33] Infiltrating T cells elicit a broad inflammatory response, with B and T cells and macrophages found in perivascular cuffs and macrophages and T cells in the parenchyma.[9,20,33,87] Neuronophagia proceeds with the formation of microglial nodules and the eventual disappearance of neurons, leaving ghost-like remnants with antigen accumulated within macrophages.

The rapidity of the neutralizing antibody response is thought to be a principal determinant of outcome.[4,32,54,56,58] Most fatal cases occurring within approximately 5 days after the onset of illness have no detectable CSF antibody response while virus is recoverable from the CSF, a finding indicative of unimpeded viral replication. In experimentally infected animals, passive immunization reduces mortality, even when it is given 4 to 5 days after inoculation.[54] However, other studies have found that antigen persists in neurons for extensive periods in the presence of intrathecal antibody and immune complexes, thus suggesting a failure of antibody-mediated viral clearance.[14] A role for immunopathologic mechanisms, including the development of antineurofilament antibodies, has been proposed as an alternative correlate of outcome.[13] CSF interleukin-8 levels remain elevated for a longer interval in patients with severe prolonged illness.

Why most JE virus infections are subclinical or lead to no signs of infection in the central nervous system is unclear. Epidemiologic observations indicate an elevated risk for JE in the elderly and increased severity in young children, but the biologic basis for this increased susceptibility has not been defined. Cross-reactive flaviviral immunity as a result of previous dengue virus infection may modulate the severity of JE in some cases.[16] Pathologic observations have shown a higher prevalence of neurocysticercosis in fatal JE cases than in deaths from other causes, which suggests that physical or physiologic disruption of the brain architecture by infection or other mechanisms could facilitate neuroinvasion (see earlier).[11,33,42,44,45] Experimental dual infection of animals with JE virus and other agents supports this hypothesis. In experimental animals, other host factors associated with a risk of acquiring illness and having a poor outcome include a specific gene defining resistance, age, levels of sex hormones, and cold and stress responses.

COMPLICATIONS

The principal complication is secondary bacterial infection occurring in the acute and subacute phases of illness. Stress-induced gastrointestinal hemorrhage and hyponatremia caused by inappropriate antidiuretic hormone secretion also can occur. Concurrent malaria and other parasitic or bacterial infections may complicate management. Although JE occurs in areas of Asia where human immunodeficiency virus (HIV) infection may be prevalent, reports of their interaction have been limited, with no conclusion on differences in outcome.

Cases of clinical relapse with seizures, coma, and weakness, several occurring 6 to 9 months after recovery from the acute illness, have been reported. These patients and other asymptomatic recovered patients had evidence of persistent JE virus infection in peripheral blood mononuclear cells.[77] A study of 253 patients found laboratory evidence of subacute central nervous system infection in 5 percent of cases, with persistent intrathecal production of JE virus–specific IgM beyond 50 to 180 days or CSF containing JE virus antigen or virus more than 3 weeks after recovery.[67] Further studies are needed to confirm and to characterize the persistence of JE virus and its clinical significance.

JE acquired during the first two trimesters of pregnancy may lead to fetal infection and miscarriage. JE virus has been isolated from products of conception in a few cases. Infections acquired during the third trimester have not been associated with adverse outcomes to the pregnancy.[8,47] Whether congenital JE virus infections are associated with sublethal malformations in humans or whether congenital infections follow subclinical JE virus infection remains unknown. The virus is an important abortifacient in pigs and produces central nervous system and other lethal in utero malformations.

LABORATORY DIAGNOSIS

A specific diagnosis can be confirmed serologically by identifying JE virus–specific IgM antibody in serum or CSF by enzyme-linked immunosorbent assay (ELISA) or by demonstrating fourfold titer changes in neutralization, hemagglutination-inhibition, complement-fixation, or immunofluorescent antibodies between acute- and convalescent-phase serum samples. Serologic cross-reactions with dengue virus and other flaviviruses are a common problem that sometimes can be resolved with cross-neutralization. Some laboratories have established empiric ELISA absorbance cutoffs that can differentiate dengue and JE virus infection.[30] IgM can be detected in serum or CSF (or in both) in nearly all cases by 1 week after the onset of illness. ELISA kits as well as rapid, point-of-care immunoblot assays are available commercially.[66] Patients who are moribund or severely ill on admission may be seronegative and are most likely to yield viral isolates from CSF.[5] Real-time polymerase chain reaction and T7 promoter–based assays have proved to be sensitive in related West Nile and St. Louis encephalitis cases and can distinguish the two infections in areas of South Asia, where the viruses circulate.[78] Immunofluorescent staining of CSF mononuclear cells can provide a specific diagnosis within several hours after a lumbar puncture is performed, but this procedure has a reported sensitivity of only 60 percent.[46]

JE virus occasionally can be isolated from the blood of patients in the pre-neuroinvasive phase of illness, usually no later than 6 to 7 days after onset. Virus can be recovered from brain biopsy and autopsy material by intracerebral inoculation of baby mice and various cell cultures, such as primary chick or duck embryo cells, and in Vero, LLCMK-2, C6-36, and AP-61 cell lines.

DIFFERENTIAL DIAGNOSIS

In rural Asia, the principal considerations include tuberculous and pyogenic meningitis; typhoid fever manifested as tremors and ataxia; cerebral malaria; dengue virus infection with encephalopathy; and herpes simplex, measles, enterovirus (especially EV71), HIV, and other causes of viral encephalitis.[10,80] West Nile virus (and its Kunjin subtype) and Murray Valley encephalitis virus overlap JE virus in their distributions in Asia and Australia,

have similar clinical findings, and because of their antigenic relatedness pose a laboratory diagnostic challenge as well. Nipah virus, a newly described bat-associated paramyxovirus that led to large outbreaks of encephalitis in Malaysia in persons exposed to infected pigs, occurs in the same rural circumstances as does JE virus and may have similar clinical features. The absence of thalamic and basal ganglia involvement and multiple white matter lesions on T2-weighted magnetic resonance imaging scans are important differentiating features. Outbreaks of encephalitis caused by Chandipura virus, a sandfly- and mosquito-borne rhabdovirus, have been confused with JE in South Asia, where both viruses circulate.[6] In areas of Asia where poliomyelitis still has not been eradicated, JE should be included among the possible causes of acute flaccid paralysis. In a series from Lucknow, India, in which 394 children 6 months to 12 years of age with an acute encephalopathic illness underwent virologic studies, 23 percent had JE. Similarly, JE and dengue accounted for 35 percent of defined viral encephalitis cases in Thailand.[10] Meningitis or encephalitis develops in some scrub typhus patients; rash, adenopathy, and an eschar, if present, are helpful diagnostic signs. Acute encephalitis with convulsions is encountered in two thirds of patients with neurocysticercosis, which may be detected by brain imaging. Neurocysticercosis itself may increase the risk for acquiring JE (see the section on pathophysiology). In some developing countries, because aspirin continues to be used in febrile children, individual cases and even outbreaks of Reye syndrome have been mistaken initially as JE.

Noninfectious causes of acute encephalopathy to be considered include heat stroke, vascular occlusion and intracranial hemorrhage, acute electrolyte disturbances, lead encephalopathy and other poisonings (especially caused by insect repellents), and inherited metabolic disorders.

TREATMENT

No specific antiviral therapy is available. A few patients have been treated with interferon alfa, but its efficacy has not been evaluated in wider trials.[23,24] Supportive care and control of intracranial pressure are critical for a good outcome. Mannitol is used routinely in many areas of Asia, but early high-dose dexamethasone therapy was shown to have no clinical efficacy in a prospective controlled clinical trial.[27] Corticosteroids also have been given, without apparent benefit, in the late stages of illness as empiric therapy for late neurologic changes that were presumed to have an immunopathologic basis.[16] Other supportive measures, including control of fever and convulsions, attention to fluid balance, respiratory support, and prevention and treatment of secondary infections, have contributed to increased survival and improved outcomes. Small interfering and hairpin RNA molecules are being investigated as therapeutic agents.[39]

PROGNOSIS

The case-fatality ratio varies from 10 to 35 percent, depending on the accessibility and quality of supportive care. Younger children (<10 years) are more likely to die of the infection and to have more serious neurologic complications acutely and as sequelae. Gross neurologic impairment, such as paralysis, weakness, abnormal muscle tone, seizures, ataxia, and extrapyramidal movement disorders, are found in approximately a third to a half of recovered patients several months to a year after onset.[37,38,51,75] Electroencephalographic abnormalities have been detected in more than 50 percent of surviving children 1 year after recovery. Behavioral disorders and subnormal performance on psychological testing may be found in as many as 75 percent of surviving patients 5 years after onset. Thus, in areas where the disease is prevalent, JE may account for substantial disability in the resident population.

PREVENTION

In areas of Asia where JE is endemic, universal childhood immunization is recommended. Three JE vaccines are used, the most widely distributed of which is an inactivated vaccine produced from infected mouse brain. Vaccine effectiveness of 98.5 percent after administration of three doses has been reported, with partial protection provided after two doses.[26,58,88] The others, a killed primary hamster kidney cell–derived vaccine and a live vaccine made from the attenuated SA 14-14-2 strain, are produced exclusively in the People's Republic of China.[55]

Inactivated JE vaccine derived from mouse brains had been the only product distributed in the United States and internationally for use in travelers.[12,19,26,36,39] However, production by the principal Japanese manufacturer has been discontinued, and supplies in the United States are expected to be depleted in 2009. A novel inactivated cell culture–derived vaccine is now available, however, only for adults 17 years of age and older. Pediatric clinical trials are in progress, and an expanded indication for children from 1 year of age and older is expected by 2011. Two doses of the Vero cell–derived vaccine provides protective neutralizing antibody levels in more than 95% of subjects and higher antibody titers than three doses of the mouse brain–derived vaccine. Neutralizing antibodies persist at protective levels for at least 1 year, but the booster interval has not yet been defined. The vaccine is associated with significantly fewer local adverse reactions than the mouse brain vaccine.

Until the pediatric indication is approved, and while supplies are available, children younger than 17 years of age must receive the mouse brain vaccine. The dose for children 1 to 3 years of age is 0.5 mL, given on days 0, 7, and 30 or 14; the vaccine is not approved for use in children younger than 1 year. Children vertically infected with HIV in Asia have demonstrated a diminished response to vaccination.[71]

Local reactions and mild systemic reactions such as fever, headache, and myalgia occur in 10 to 25 percent of vaccinees who receive the mouse brain vaccine.[28] Allergic reactions consisting of generalized urticaria and facial and peripheral angioedema have been a cause for concern because of the potential for respiratory obstruction and anaphylaxis and because the onset of reactions may be delayed for 12 to 72 hours after vaccine administration.[62] Among Japanese vaccinees, immediate reactions (within 1 hour of vaccination) have been associated strongly with IgE specifically reactive to gelatin (a vaccine stabilizer), whereas children with delayed reactions exhibited gelatin-specific IgG.[73,74] Gelatin hypersensitivity may not underlie all cases with these adverse events. However, an allergic history, including atopy, is a risk factor.[53,62] In addition to antihistamines, parenteral corticosteroid therapy often has been needed. Allergic side effects are estimated to occur in 0.5 percent of vaccinees, a sufficiently high rate of a potentially serious adverse event that the vaccine has not been recommended as a routine immunization for travel to Asia.[28,62] In addition, anecdotal cases of temporally associated acute disseminated encephalomyelitis have been reported, with a frequency as high as 1 in 50 to 1 in 75,000 vaccinees.[61]

Current recommendations specify that the vaccine be reserved for expatriates, for persons spending 30 days or more in an endemic area during the transmission season, and for persons with briefer itineraries if they have a high risk of exposure (Table 188–3). The course of immunization should be completed 7 to 10 days before the onset of travel.[28] Production of this mouse brain–derived vaccine was discontinued in 2006, and vaccination of U.S. travelers now relies on a limited stockpile. An inactivated Vero cell culture–derived vaccine that is as immunogenic after

TABLE 188—3 Risk of Japanese Encephalitis by Country, Region, and Season

Country	Affected Areas or Jurisdictions	Transmission Season	Comments
Bangladesh	Few data, probably widespread	Possibly July-December, as in northern India	Outbreak reported from Tangail district, Dacca division; sporadic cases in Rajshahi division
Bhutan	No data	No data, presumed to be similar to Nepal	Not applicable
Brunei	Presumed to be enzootic as in Malaysia	Presumed year-round transmission	Muslim population does not raise pigs
Cambodia	Endemic—hyperendemic countrywide	Presumed to be May-October	Highly prevalent in rural areas near Phnom Penh; confirmed cases in large epidemics October-December, 1993-1998
Democratic Republic of Korea	Presumed to be countrywide in rural areas <800 m	July-October	Epidemics in 1970s; few recent data
Hong Kong	Rare cases in new territories	April-October	Vaccine not routinely recommended India
India	Reported cases from all states except Arunachal, Dadra, Daman, Diu, Gujarat, Himachal, Jammu, Kashmir, Lakshadweep, Meghalaya, Nagar Haveli, Orissa, Punjab, Rajasthan, and Sikkim	*South India:* May-October in Goa; October-January in Tamil Nadu; August-December in Karnataka; second peak April-June in Mandya district *Andhra Pradesh*: September-December *North India*: July-December	Outbreaks in West Bengal, Bihar, Karnataka, Tamil Nadu, Andhra Pradesh, Kerala, Assam, Uttar Pradesh, Manipure, and Goa Urban cases reported, e.g., Lucknow
Indonesia	Kalimantan, Bali, Nusa Tenggara, Sulawesi, Mollucas, West Irian Java, and Lombok	Probably year-round risk; varies by island; peak risks associated with rainfall, rice cultivation, and presence of pigs Peak periods of risk: November-March; June-July in some years	Endemic on Bali; sporadic cases recognized elsewhere Vaccine not recommended if travel to urban areas only
Japan*	Rare—sporadic cases on all islands except Hokkaido	June-September, except Ryukyu Islands (Okinawa): April-October	Vaccine not routinely recommended for travel to Tokyo and other major cities; enzootic transmission without human cases observed on Hokkaido
Laos	Presumed to be endemic—hyperendemic countrywide	Presumed to be May-October	No data available
Malaysia	Sporadic—endemic in all states of Peninsula, Sarawak, and probably Sabah	November-January peak on Peninsula	Most cases from Penang, Perak, Salangor, Johore, and Sarawak; differentiate from Nipah encephalitis
Myanmar	Presumed to be endemic—hyperendemic countrywide	Presumed to be May-October	Repeated outbreaks in Shan State in Chiang Mai Valley
Nepal	Hyperendemic in southern lowlands (Terai); sporadic cases in Katmandu valley	July-December	Vaccine not recommended for travelers to high-altitude areas only
Pakistan	May be transmitted in central deltas	Presumed to be June-January	Cases reported near Karachi; endemic areas overlap those for West Nile virus
Papua New Guinea	Sporadic cases from d'Entrecasteaux islands, Gulf, Milne Bay, South Highland, West Sepik, and Western provinces	Unknown	Vaccine not routinely recommended
People's Republic of China	Cases in all provinces except Xizang (Tibet), Xinjiang, and Qinghai	Northern China: May-September Southern China: April-October (Guangshi, Gwangdong, Southern Fujian, Yunnan, Szechuan, Guizhou, Hunan, and Jiangsi provinces)	Vaccine not routinely recommended for travelers to urban areas only
Philippines	Presumed to be endemic on all islands	Uncertain, speculations based on locations and agro-ecosystems: West Luzon, Mindoro, Negro Palawan: April-November Elsewhere: year-round; greatest risk April-January	Outbreaks described in Nueva Ecija, Luzon, and Manila
Republic of Korea	Rare sporadic cases	July-October	Last major outbreaks in 1982-1983
Russia	Far eastern maritime areas south of Khabarovsk	Peak period July-September	Sporadic transmission in both rural and sylvatic cycles
Singapore	Rare cases; last in 1992	Year-round transmission no longer detected	Vaccine not routinely recommended Local transmission on adjacent islands
Sri Lanka	Endemic in all but mountainous areas; periodically epidemic in northern and central provinces	October-January; secondary peak of enzootic transmission May-June	Outbreaks in central (Anuradhapura) and northwestern provinces
Taiwan*	Endemic, sporadic cases; island-wide	April-October; June peak	Cases reported in and around Taipei
Thailand	Hyperendemic in north; sporadic—endemic in south Reduced incidence due to vaccination	May-October	Annual outbreaks in Chiang Valley; sporadic cases in Bangkok suburbs
Vietnam	Endemic, hyperendemic in all provinces	May-October	Highest rates in and near Hanoi
Western Pacific and Australia	Epidemics reported in Guam, Saipan (northern Mariana Islands), and Torres Strait Islands and Cape York (Australia)	September-January in the Pacific; February-April in northern Australia	Enzootic cycle may not be sustainable; epidemics only follow introduction of virus

**Local Japanese encephalitis incidence rates may not reflect risks to nonimmune visitors accurately because of high immunization rates in local populations. Humans are incidental to the transmission cycle. High levels of viral transmission may occur in the absence of human disease.*

Note: *Assessments are based on publications, surveillance reports, and personal correspondence. Extrapolations have been made from available data. Transmission patterns may change. From Tsai, T. F., and Yu, Y. X.: Japanese vaccines.* In *Plotkin, S., and Orenstein, W. (eds.): Vaccines, 3rd ed. Philadelphia, W. B. Saunders, 1999, p. 700.*

just two doses is being evaluated and should be available in the United States and in Europe before the mouse brain vaccine is exhausted.

The live attenuated SA 14-14-2 vaccine now is used in certain states in India and in epidemic zones of Sri Lanka and Nepal. Although the recommended schedule of immunization is three doses given at 1, 2, and 6 years of age, studies of case-control effectiveness suggest that two doses could have the protective effect of 98 percent.[55] The effectiveness of a single dose is uncertain; one case-control study disclosed an effectiveness of 85 percent, but with wide confidence intervals. The methodology of two other case-control studies has been questioned. The acute and long-term safety of the live vaccine has been a concern.

Inactivated equine vaccines are used widely in some Asian countries, and a DNA equine vaccine recently has been shown to be highly efficacious.[7] A recombinant live attenuated vaccine for human use is being developed.[50]

Avoidance of outdoor activities during the evening hours, staying in screened or air-conditioned quarters, and sleeping under a bed net will reduce the risk of exposure to vector mosquitoes. Wearing long-sleeved shirts and long pants and using mosquito repellents on clothing and exposed skin are recommended for outdoor activities.

The production of vector mosquitoes in rice fields has been controlled by scheduled changes in water levels, application of larvicides and larval predators, and nontargeted effects of agricultural pesticides.

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CHAPTER **188f**

MURRAY VALLEY ENCEPHALITIS

John G. Aaskov

Outbreaks of an acute, severe encephalitic illness, clinically similar to Japanese and St. Louis encephalitis, occurred in rural areas of southeastern Australia in 1917, 1918, 1922, 1925, 1951, and 1974[1,9,16,17] and in north and northwestern Australia in 1981, 1993, and 2000.[8,13,37] They are thought to represent a single entity for which various names (Australian X disease, Murray Valley encephalitis, Australian encephalitis) have been used. Approximately 420 cases were reported in these 9 outbreaks, and sporadic cases have occurred in most years since 1978 in which outbreaks have not occurred.[37]

Case-fatality rates, as high as 70 percent in the early years,[9,11] declined to 20 percent in the 1974 outbreak and have remained at about this level since then.[5,10,13] However, significant residual neurologic disability occurs in as many as 50 percent of survivors.[10,13]

The presence of this disease in Papua New Guinea was confirmed in 1956.[22] The causative virus was transmitted to experimental animals as early as 1918,[6,11] although those strains could not be maintained. The definitive isolation and characterization of Murray Valley encephalitis virus in 1951[21] led to epidemiologic studies that suggested its survival in bird-mosquito cycles in northern Australia but not in the area of epidemic occurrence in southern Australia.[1]

Murray Valley encephalitis is caused by Murray Valley encephalitis virus. In an effort to dissociate a disease from a specific locality, the term *Australian encephalitis* was proposed by residents of Murray Valley for the disease caused by Murray Valley encephalitis virus. Some researchers subsequently have attempted to expand the term *Australian encephalitis* to include encephalitis caused by any Australian arbovirus. Because the term *Australian encephalitis* has no scientific validity and is ambiguous, it should not be used.

ETIOLOGIC AGENT

Murray Valley encephalitis virus was isolated post mortem from the brains of patients in 1951[21] and 1974[26] and shown to be related antigenically to Japanese encephalitis virus.[21,26]

A partial nucleotide sequence of Murray Valley encephalitis virus[14] confirmed the previous classification of this virus, on serologic grounds, as being a member of the Japanese encephalitis, St. Louis encephalitis, West Nile fever subgroup of flaviviruses.[34] Distinguishing Australian strains of Murray Valley encephalitis virus from those isolated in Papua New Guinea has been possible on the basis of limited nucleotide sequencing.[12,27]

TRANSMISSION AND EPIDEMIOLOGY

The mosquito *Culex annulirostris* is thought to be the major vector of Murray Valley encephalitis virus,[17,18,29] although other mosquitoes, such as *Aedes normanensis*, *Aedes tremulus*, and *Culex quinquefasciatus*, also may play a significant role in transmission of the virus.[7]

Murray Valley encephalitis virus is thought to survive in cycles of infection between birds and mosquitoes in northern Australia and Papua New Guinea,[1] where regular infection of humans and other animals is indicated by seroconversion in the summer-autumn "wet" season[15] as well as by clinical disease.[7,37] Recent studies also suggest that the virus might survive, after vertical transmission, in desiccation-resistant eggs of *A. tremulus* mosquitoes.[7] Epidemics that occurred in more populous areas of southeastern Australia are thought to have followed the introduction of virus when abnormal spring rainfall allowed chains of bird-mosquito transmission to occur through northern Australia.[2] However, several studies also have suggested that interepidemic survival of virus occurs in southern Australia.[23]

Although disease occurs most commonly in children, clinical infection occurs in individuals of all ages.[1,4,8,10,13,17,28] Data are insufficient to determine whether the lower incidence of disease in adults is due to immunity acquired from previous subclinical infections.

CLINICAL MANIFESTATIONS[5,9,10,13,36]

An initial period of nonspecific prodromal symptoms and signs, such as fever, headache, nausea, vomiting, muscle pain, and photophobia, is followed within 2 to 5 days by drowsiness, mental obtundation, confusion, disorientation, incongruous behavior, ataxia, speech disturbance, or convulsions, sometimes with a grand mal character. Neurologic signs were present in most patients on admission to the hospital, and additional signs appeared as the disease progressed. In some cases, the signs fluctuated from hour to hour. Bennett[5] recognized three groups of patients according to eventual clinical outcome:

1. Patients with mild disease commonly had disturbed mentation short of coma, incoherent or slurred speech, aphasia, speech perseveration, incontinence, neck stiffness, intention tremor, and limb hypertonicity but rarely required assistance for respiration. The neurologic changes stabilized in 5 to 10 days, and the patients' clinical conditions improved.
2. Patients with severe disease showed more profound central nervous system (CNS) involvement consisting of impairment of consciousness to coma, more marked signs of upper motor neuron involvement, and pharyngeal or respiratory paralysis requiring artificial respiration.
3. In fatal cases, patients had either spastic quadriplegia progressing to almost complete loss of nervous function or severe disease with superimposed infection.

The differential diagnosis of Murray Valley encephalitis may be assisted by attention given to signs attributable to spinal cord involvement and to cranial nerve palsies, tremor, and frequency of seizures (seizures rarely develop in adults with Murray Valley encephalitis, whereas they may occur in up to 90% of children). Although computed tomographic scans in these patients may be normal, and electroencephalograms may not show focal features,[10] magnetic resonance imaging reveals changes similar to those reported in the brains of patients with Japanese encephalitis[24]—thalamic edema, hypointensity on T1-weighted images, and hyperintensity on T2-weighted images.[19,25] Changes also were noted in the reticular formation, the substantia nigra, and the cervical spinal cord.[19] In the absence of serology, the magnetic resonance images might be regarded as diagnostic of an infection with herpes simplex virus rather than with Murray Valley encephalitis virus.[39]

PATHOLOGY

A period of viremia probably precedes infection of the CNS, during the 1- to 3-week incubation period,[1] but it has not been demonstrated. Pathologic changes in fatal cases were restricted to the CNS and included extensive perivascular cuffing (especially in the cortex), lymphocytic infiltration of the meninges, neuron degeneration and neuronophagia in the cerebellum and spinal cord, and thalamic necrosis. Evidence of repair, including calcification, was described in patients who died late in the course of the disease.[6,10,11,33,35] These pathologic changes do not distinguish Murray Valley encephalitis from other arthropod-borne encephalitides.

In rodents infected peripherally with Murray Valley encephalitis virus, the virus enters the CNS through the olfactory pathway after replicating in regional lymph nodes. From the olfactory lobe, it spreads through interconnected neural circuits to the cortex, hippocampus, thalamus, cerebellum, medulla oblongata, and spinal cord. Extensive neuronal necrosis was observed in the olfactory bulb and hippocampus. The severity of the subsequent encephalitis correlated with the magnitude of mononuclear and polymorphonuclear cell infiltrates. Infiltration of neutrophils was preceded by increased expression of tumor necrosis factor–α and the chemokine N51/KC in the CNS. Previous depletion of neutrophils or inhibition of inducible nitric oxide synthetase resulted in prolonged survival and decreased incidence of mortality in mice infected with Murray Valley encephalitis virus.[3,30] However, the pronounced mononuclear cell content of cerebrospinal fluid in some patients with Murray Valley encephalitis[10,19] contrasts with the picture in rodents.[30]

DIAGNOSIS

Clinical and epidemiologic features may suggest the diagnosis of Murray Valley encephalitis, especially during recognized epidemics, but individual cases may be difficult to distinguish from cases of encephalitis or encephalopathy of other cause (e.g., herpesvirus or Japanese encephalitis or Kunjin viruses).[5,32,39] Establishment of a specific diagnosis depends on serologic evidence of infection concurrent with disease. Detection of IgM antibody that reacts with Murray Valley encephalitis virus in hemagglutination-inhibition[20,38] or in enzyme-linked immunosorbent assays is the most useful indication of recent infection. Other flaviviruses, especially Kunjin virus, may cause encephalitis, subclinical infection, or minor illness during epidemics of Murray Valley encephalitis,[37] and interpretation of serologic cross-reactions between Kunjin and Murray Valley encephalitis viruses may require specific tests (e.g., neutralization) with several viruses.[17] Serologic tests for the laboratory diagnosis of Murray

Valley encephalitis are not available commercially and so are performed only at larger regional laboratories. Isolation of virus has not been successful for antemortem diagnosis. However, some reports[19,31] document the detection of Murray Valley encephalitis viral RNA in serum or cerebrospinal fluid from patients by use of the reverse transcription–polymerase chain reaction (RT-PCR). Subsequent serology confirmed these RT-PCR data. Given the need for rapid clinical intervention in this disease, more extensive use of RT-PCR in a diagnostic setting is warranted.

TREATMENT AND PROGNOSIS

No specific antiviral therapy is available. Administration of corticosteroids has been recommended during the acute phase of the illness to reduce brain edema. Artificial respiration has been lifesaving, and all patients should be transported to base hospitals with facilities for the management of patients with respiratory paralysis.[5] Most patients also require parenteral nutrition or nasogastric feeding.[10]

The early epidemics left some patients with neurologic and psychiatric sequelae, but the lower case-fatality rate in recent outbreaks, presumably because of use of modern intensive care techniques, has been associated with a high rate of residual disability.[10,13] Bennett[5] observed 18 patients up to 16 months after they experienced infection. Four of 11 patients with mild disease had emotional problems and mild degrees of impaired motor coordination and mental acuity. All 7 patients with severe disease had serious defects, including paraplegia or quadriplegia and mental disturbance.

This pattern of high residual disability has continued to the present.[10,13]

PREVENTION

Because of the irregularity of outbreaks and the large areas over which they occur, institution of measures to prevent Murray Valley encephalitis is difficult. However, both government and personal programs for restriction of exposure of humans to the common mosquito vector *C. annulirostris* are likely to minimize the incidence of disease.

No vaccine is available, but there may be value in assessing the capacity of the next generation of live attenuated Japanese encephalitis virus vaccines to provide cross-protection against infection with Murray Valley encephalitis virus.

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CHAPTER 188g

Tick-Borne Encephalitis

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Tick-borne encephalitis (TBE) refers here to the neurotropic tick-transmitted flaviviral infections that occur across the Eurasian land mass from the Far East to western Europe. The Far Eastern form of the disease frequently is called Russian spring-summer encephalitis (RSSE); in Europe, where the disease is distinctly milder and often biphasic, it is called tick-borne encephalitis, spring-summer meningoencephalitis, central European encephalitis, or, because sometimes it is transmitted by raw infected milk, biphasic milk fever.[23,31,66,100]

HISTORY

After an outbreak of encephalitis in the Far Eastern region of Russia in 1932, Zilber isolated the virus from viremic humans and from *Ixodes persulcatus* ticks. A milder form of the disease with a similar seasonality had been described previously in Sweden and Austria, but its etiology was not defined until 1948, when the virus was isolated in the Czech Republic and Slovakia. Milk-borne transmission of TBE virus (TBEV) from infected livestock animals first was recognized in an outbreak in 1951 and 1952.

ETIOLOGIC AGENT

TBEV and the closely related viruses of RSSE and Powassan encephalitis are flaviviruses placed antigenically within a complex of tick-borne flaviviruses that also includes the agents of Kyasanur Forest disease, Omsk hemorrhagic fever, and an encephalomyelitis syndrome in sheep variously called louping ill in the British Isles and Spanish, Greek, or Turkish sheep encephalomyelitis in their respective countries.[39] Molecular taxonomic studies based on nucleotide sequence differences in the E glycoprotein gene of the virus show the early divergence of a mammal-associated clade from seabird-associated agents. Viruses in the mammal-associated clade exhibit a continuous east-to-west cline consistent with an evolutionary origin of TBEV in the Far East and dispersion westward to Europe and the British Isles (Fig. 188–12).[27]

ECOLOGY

The viruses of RSSE and TBE are transmitted principally by hard ticks in the *Ixodes ricinus* complex: *I. ricinus* in Europe and *I. persulcatus* in the Far East.[23,31,66,100] Other tick vectors include *Ixodes arboricola*, *Ixodes hexagonus*, *Haemaphysalis punctata*, *Haemaphysalis concinna*, *Dermacentor marginatus*, and *Dermacentor reticulatus*. Viral circulation is maintained by continuous horizontal infection between ticks and animals and through the winter by vertical transmission in vector ticks and by latent infection in hibernating animals. The viruses are transmitted trans-stadially from larval to nymphal to adult tick stages and transovarially. All stages of the tick and both male and female ticks transmit infection to animals and humans. Ixodid ticks feed on three hosts, one for each of the stages, during the typical 3-year life cycle. Larval and nymphal ticks feed preferentially on birds and small mammals, such as wild mice, voles, and dormice; adult ticks feed on larger mammals, such as roe deer, hedgehogs, foxes, hares, badgers, deer, domestic livestock (pigs, goats, sheep, and cows), dogs, cats, and humans. RNA sequence heterogeneity of the 5′ noncoding region in different TBE virus isolates recovered from the same biotope suggest that virus populations consist of a number of subtypes with specificities for individual vertebrate hosts.[13] Infections in animals, except occasionally in dogs, are asymptomatic, and the viremia is of brief duration. Therefore, a large population

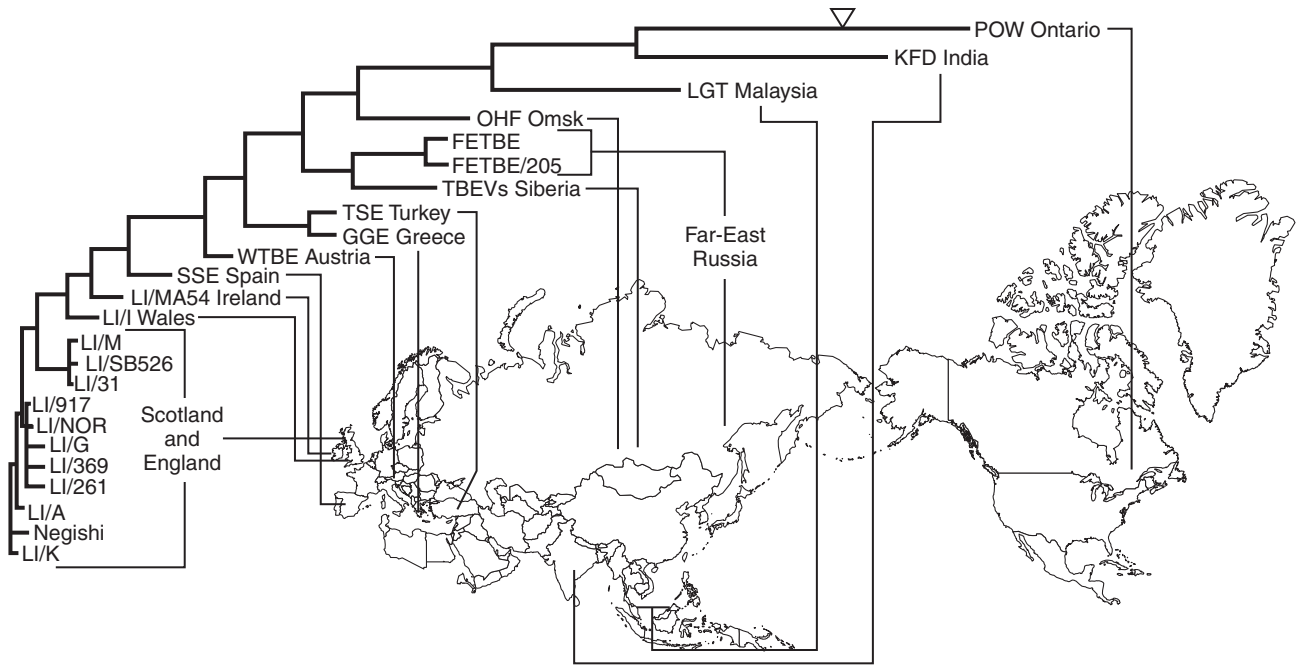

Figure 188–12 Cladogram of tick-borne encephalitis complex viruses based on E gene sequences presented from west to east with respect to louping ill (LI) virus. FETBE, Far Eastern tick-borne encephalitis; GGE, Greek goat encephalitis; KFD, Kyasanur Forest disease; LGT, Langat; OHF, Omsk hemorrhagic fever; POW, Powassan encephalitis; SSE, Spanish sheep encephalitis; TSE, Turkish sheep encephalitis; WTBE, western tick-borne encephalitis. The phylogenetics of these and other mosquito-borne flaviviruses are rooted in the Powassan branch indicated by the triangle. *(From Zanotto, P. M., Gao, G. F., Gritsun, T., et al.: An arbovirus cline across the Northern Hemisphere. Virology* 210*:152-159, 1995.)*

of susceptible vertebrate hosts is needed to maintain viral transmission. Human infections are incidental to the natural cycle of transmission. Birds and large mammals contribute to the spread of vector ticks and viral foci.

Ticks in the *I. ricinus* complex require high soil and ambient humidity (>80-90% relative humidity) and moderate temperature, within the 8° C isotherm. They typically are found in the transitional vegetation zone from the forest edge to fields or meadows or in areas where dense brush or ground vegetation provides a sheltered microenvironment. Vector ticks are absent from mountainous areas with an elevation above 1000 to 1500 m. Foci of TBE transmission are restricted geographically and ecologically to these biotopes and have tended to be highly stable from year to year. However, global warming has been speculated to underlie the changes, both expansion and contraction of transmission foci, in northern and central-southern Europe, respectively.[75,100] New data also suggest a shift of tick activity to higher altitudes in recent years, potentially allowing vertical expansions of TBE foci.[20]

Tick activity varies with seasonal temperature and humidity. In central Europe, activity begins in March and April, reaches a peak in May, and declines during the summer in July and August. With the return of cooler temperatures, a second peak of activity occurs in September. In temperate regions, tick activity begins later and is greatest in the summer months. In Mediterranean climates, ticks are most active from November to January.

In foci with hyperendemic transmission, tick density may exceed one per square meter. Viral infection rates in ticks generally are in the range of 0.1 to 5 percent. These rates typically are 10-fold lower than *Borrelia burgdorferi* (sensu lato) infection rates in *I. ricinus* in the same areas, although *I. persulcatus* infection rates greater than 10 percent have been reported.[58] The mechanisms underlying this difference are unclear but may include the variable infectiousness of the agents and their interactions with modulatory factors in tick saliva; the relatively brief duration of TBE viremia in animals, which lasts only a few days and thus results in a reduced chance of transmission of virus to feeding ticks, as opposed to the persistent *B. burgdorferi* infections in rodents, with tick feeding more likely to result in infection; and potential differences in the principal reservoir hosts for the respective infectious agents, with a more limited and focal distribution of important hosts for TBEV, such as goats.[93] The two agents evidently do not interfere with each other in their infection of *I. persulcatus* ticks; dually infected ticks and singly TBE-infected and *Borrelia*-infected ticks stand in a ratio of approximately 1:4:14.[58] *I. ricinus* also can be infected with and transmit *Francisella tularensis* and *Anaplasma phagocytophilum*, the agent of human granulocytic ehrlichiosis.[131]

The geographic distributions of RSSE and European TBE correspond to the ranges of their principal tick vectors; however, transmission is highly focal within this range because of the locations of biotopes that support viral circulation (Fig. 188–13). New foci are reported periodically as a result of better recognition of the disease, its natural spread, human modifications of the landscape, and environmental and climate changes (e.g., global warming).[26,49,75,100]

EPIDEMIOLOGY

TBE has been recognized in all countries of Europe except Portugal and the Benelux countries, but endemic transmission is most intense in central Europe.[66,100] The incidence of TBE previously ranged as high as 50 per 100,000 population in Austria, Poland, Hungary, Russia, the Czech Republic, Slovakia, and former Yugoslavia; in certain areas, similar levels of transmission still may prevail. Vaccination has reduced the incidence of disease regionally, especially in Austria, where a national program of immunization in effect since 1981 has reduced the incidence to less than 1 per 100,000 population.[135] Currently, isolated cases are reported from Norway, Denmark,[121] France,[35] Greece, and Liechtenstein, and fewer than 200 cases are reported annually in Sweden, Germany, and Italy. Switzerland recently reported a threefold increase in human TBE cases between 2004 and 2006,[136] which resulted both from more infections in endemic areas and from infections in newly recognized foci. In the Far East, cases of RSSE occur principally in forest workers. The disease is recognized in Russia and China, and the first cases acquired in Japan (transmitted by *Ixodes ovatus*) were reported recently.

Within each country, the distribution of cases is highly focal in certain areas. Local seroprevalence may exceed 20 percent, but the general seroprevalence usually is less than 1 per 100,000 population. Frequent exposure during long-term residence leads to a general trend of increasing seroprevalence with age. Seroprevalence rates as high as 50 percent have been observed in groups at high risk for exposure, such as farmers and forestry workers. Although, in general, rates are lower (1 to 5%) and in certain groups are similar to the seroprevalence of hantaviruses, lymphocytic choriomeningitis virus, and *Anaplasma* spp., *Borrelia burgdorferi* seroprevalence rates are 10-fold higher or more.[85,91] The frequency of clinical cases of TBE, borreliosis, and dual infection closely approximates the relative frequency of tick infection, singly and dually. Adults 20 to 50 years of age characteristically accounted for most of the cases. In some studies, cases in males (adults and children) predominate by a ratio of 2:1.[24,36,74]

Cases have occurred in children as young as 3 weeks,[46,50] but generally, risk in children increases with age as a result of their increased mobility and activity in the sylvatic environment.[74] These epidemiologic patterns are changing in areas with high immunization coverage.[135] Vaccination effort has focused principally on hyperendemic areas and on high-risk occupational groups such as forestry workers. The low number of cases currently reported from areas where vaccination coverage is high belies the continued transmission of virus in these locations.

Cases may be acquired during outdoor activities, such as berry picking and mushroom gathering, and infection occasionally has been acquired from ticks brought from endemic areas on Christmas trees and other objects. One study found that the seroprevalence in Swedish orienteers (1%) was not substantially different from the general seroprevalence in residents of Stockholm County (5%), an area where TBE is endemic. The absence of a higher risk of acquiring TBE in persons with occasional sylvatic exposure reflects the low infection rate of ticks and the generally low risk to persons with sporadic or short-term exposure. Neither of two studies of American soldiers stationed in central Europe found a clinical case, although one seroconversion in 3297 person-months of exposure, an infection rate of 0.9 per 1000 person-months, and four seroconversions in 959 persons (0.4%) were detected.[83,110] With the dissolution of the former Soviet Union and increasing commerce with eastern Europe, interest in the risk of TBE's being acquired by travelers to Europe and Russia has increased. The available data suggest that the risk is low for most travelers and that vaccination is not indicated except for unusual circumstances of prolonged stay in an endemic area.

The seasonal distribution of cases lags roughly 1 month behind that of tick activity and extends from April until November. The peak incidence in Sweden is in August; in Austria, the peak occurs in June and July, with a secondary rise in October.

Milk-borne TBE previously accounted for 10 to 20 percent of all cases in central Europe. Infections frequently were acquired from consuming unpasteurized milk or cheese from infected goats, sheep, and cows, and outbreaks resulting in thousands of cases have been reported. Transmission from infected milk now

Figure 188–13 The geographic distributions of Russian spring-summer encephalitis and European tick-borne encephalitis correspond to the ranges of their principal tick vectors; however, transmission is highly focal within this range because of the locations of biotopes that support viral circulation.

is a rare occurrence, but as recently as 1994, an outbreak in Slovakia led to 7 cases in a group that regularly drank raw milk from a family goat.[58] Also, an outbreak of 21 human cases caused by contaminated cheese produced from unpasteurized sheep milk has been reported.[132] Contact infection, acquired during slaughter of an infected goat, also has been observed.

CLINICAL MANIFESTATIONS

Seroepidemiologic studies indicate that as many as 90 percent of human infections with TBEV remain asymptomatic or result in a nonspecific illness.[2,6,34,54] The classic manifestation of the European form of TBE is an acute febrile illness characterized by a biphasic course consisting of a nonspecific prodromal syndrome followed by central nervous system (CNS) disease (Fig. 188–14).[30,129] Infection with TBEV may occur solely as the primary, nonspecific phase without the secondary CNS phase.[23,78] Infection with Far Eastern strains of TBEV results in a more severe, monophasic illness that progresses directly to neurologic involvement with a poorer prognosis for survival and full recovery. TBE has been observed in all age groups. The median age in pediatric case series is 8 to 10 years (range, 0 to 17 years).[16,36,74,76,127] The youngest patient with serologically and virologically documented TBE described in the literature was a 17-day-old infant.[50] Congenital infection has not been reported.

The clinical features of TBE in children are summarized in Table 188–4. The diagnosis of TBE should be considered in all acutely ill patients with fever, CNS abnormalities, and a history of potential tick exposure or ingestion of raw milk in an area

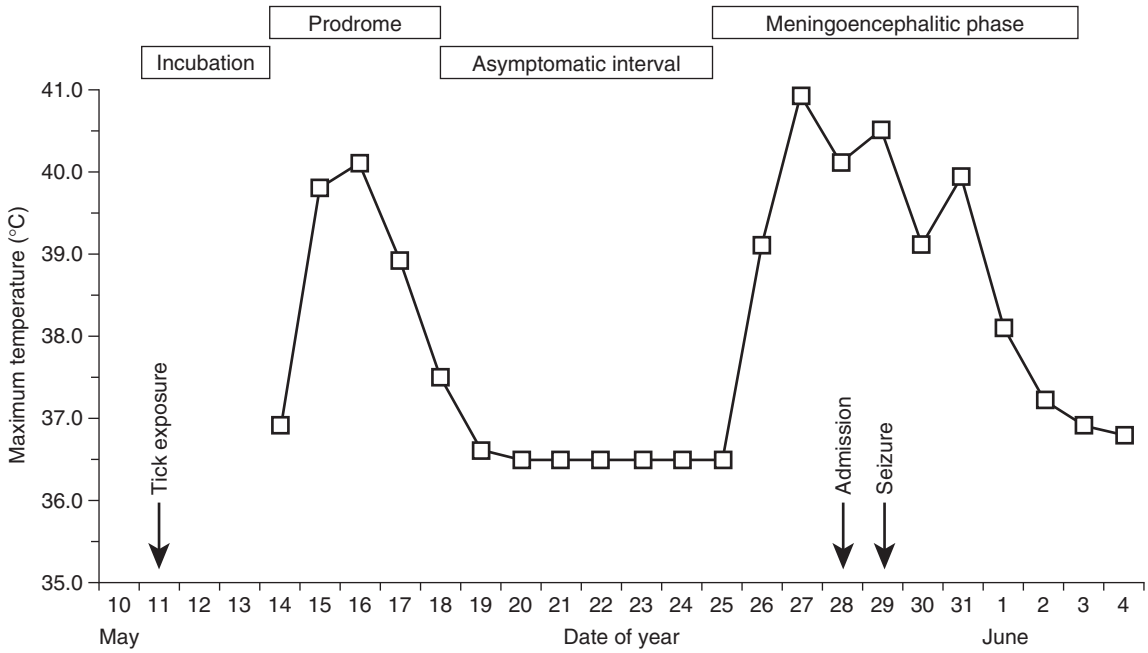

Figure 188–14 Clinical course of tick-borne encephalitis (TBE) in a 5-year-old boy exposed to a tick bite in a known endemic area for TBE in the pre-alpine region of central Switzerland. Lumbar puncture on admission revealed mild cerebrospinal fluid (CSF) pleocytosis (14×10^6/L, 50% polymorphonuclear cells). Serum anti-TBE virus IgM and IgG were present; serum and CSF antibodies against *Borrelia burgdorferi* were absent. CSF enteroviral polymerase chain reaction was negative. Recovery was uneventful.

TABLE 188–4 Clinical Features of Serologically Documented Tick-Borne Encephalitis in 5 Pediatric Case Series (333 children)

	Rate (%)
History	
Tick exposure	47-78
Biphasic illness	77-90
Major Symptoms at Presentation	
Fever >38.0° C	100
Headache	90-100
Vomiting	60-90
Central Nervous System Signs	
Nuchal rigidity	74-90
Photophobia	10-25
Impaired consciousness	9-11
Ataxia or tremor	5-26
Seizures	0-5
Paresis	0-5
Extent of Central Nervous System Involvement	
Meningitis alone	49-89
Meningoencephalitis	9-48
Meningoencephalomyelitis	0-5
Fatal outcome	0
Cerebrospinal Fluid Parameters	
Pleocytosis >15 × 10^6/L	98-100
Glucose normal	100
Protein elevated	9-55
Abnormal electroencephalogram	80-87

Data from references 16, 36, 52, 74, 76, 127.

endemic for TBE. A high degree of diagnostic awareness is required because the clinical presentation of TBE itself is nonspecific. After an incubation period of 2 to 28 days, in most cases 7 to 14 days,[24,36,74,76,129] the patient may have a prodromal illness consisting of fever, malaise, nausea and vomiting, headache, myalgia, and, occasionally, upper respiratory tract symptoms.[24,36,60,129] Defervescence occurs after 2 to 7 days, and the patient subsequently remains asymptomatic for 1 to 20 days, usually 2 to 10 days.[24,36,60,74,127] This prodromal illness may be absent. In various case series, a biphasic course was reported in 30 to 90 percent of children with TBE.[36,52,74,76,127] An abrupt onset of fever, headache, emesis, and symptoms of meningeal irritation heralds the beginning of the second phase of disease.[76] In adults, no association exists between the length of the incubation period and the severity of clinical illness.[52] The typical evolution of fever in a child with TBE is shown in Figure 188–14.

In this second phase of illness, most affected children (50 to 90%) have meningitis without clinical evidence of parenchymal CNS involvement.[36,59,64,76,88,105,127] In adult patients, by contrast, meningitis without encephalitis or myelitis occurs in 20 to 60 percent of cases.[2,52,60,101,120] On physical examination, fever, signs of meningeal irritation, and photophobia are the most common features.[76,127] In uncomplicated cases, patients defervesce within 5 to 10 days.[36,74,76,127] Meningoencephalitis or meningoencephalomyelitis (or both) manifested by impaired consciousness, seizures, and focal neurologic signs, including limb paresis, occurs in 0 to 34 percent of children.[16,36,52,74,76,127] In a large series of 139 pediatric patients with TBE meningoencephalitis, Lesnicar and coworkers[74] observed tremor in all patients, impaired consciousness in 27 (7%), ataxia in 18 (5%), behavioral changes in 16 (4%), cranial nerve paresis in 14 (4%), and limb paresis in 11 (3%). In a series of 13 children, Harasek[36] observed ataxia in 10 patients, somnolence in 4, paresthesias and seizures in 2 each, and central facial palsy and nystagmus in 1 each. In 2 of these patients, transient unilateral shoulder girdle weakness that occurred during the second week of CNS disease suggested involvement of the cervical anterior horn or radiculitis. Overall, limb or cranial nerve pareses occur in 0 to 5 percent of children with TBE (see Table 188–4). In a series of 133 children with TBE, Cizman and colleagues[16] reported transient pareses in 5 patients and irreversible hemiparesis in 1.

In large case series of predominantly adult patients, a paralytic course secondary to bulbar, spinal, or radicular injury was

observed in 5 to 25 percent of cases.[52,56,83,120,124,129] Unilateral, flaccid paresis of an upper extremity is the most common manifestation of lower motor neuron disease complicating adult TBE.[83,124,129] Involvement of the cranial nerves occurs somewhat less frequently and is revealed most commonly by external ocular muscle paralysis (usually cranial nerve VI), peripheral facial palsy (VII), otovestibular manifestations (VIII), or involvement of the pharyngeal muscles (IX, X, XI).[24,52,83,122] Lower extremity weakness and, occasionally, autonomic nervous system affliction manifested as bladder dysfunction also may occur.[24,53,122] Whereas most manifestations of parenchymal CNS involvement evolve during the acute stage of TBE, paralysis resulting from radiculitis may develop up to 14 days after the onset of CNS disease.[36,66]

As many as 5 percent of children with TBE experience seizures during the acute stage of TBE.[16,36,127] Because most patients are older than 6 years and thus are unlikely to suffer from febrile seizures, these episodes probably reflect encephalitis. In adults, seizures have been observed in less than 2 percent of patients.[24,52]

Extracerebral manifestations of TBE seldom are reported and are of minor clinical relevance. Mild hepatitis[41,52] and electrocardiographic abnormalities have been described in adults.[125] A single case of myopericarditis in a child with TBE has been reported.[25]

The peripheral white blood cell count is not altered in a characteristic way. Although it is usually in the normal range, both leukopenia and moderate leukocytosis may be found.[36,76,122] In adult patients, several investigators observed leukopenia during the viremic prodrome and normal or moderately elevated white blood cell counts during the second phase of illness.[52,60,76,80] In a report describing hematologic values during the prodromal phase of TBE, 23 of 28 (82%) patients had mild to moderate thrombocytopenia (60 to 130×10^9/L), with values returning to normal during the second phase of illness.[80] Cerebrospinal fluid (CSF) analysis in children with serologically proven TBE reveals predominantly mononuclear pleocytosis in all patients. However, as with enteroviral meningitis, neutrophils may predominate during the first 1 to 3 days of illness.[48] Typically, the CSF white blood cell count is 100 to 1000×10^6/L.[36,63,74,76,122,127] On occasion, lower values occur, as reported by Krausler and associates,[59] who found less than 100×10^6/L in 24 of 75 (32%) pediatric cases. Wahlberg and colleagues[129] reported an absence of pleocytosis in 18 percent of 94 adult patients examined. Harasek[36] could not find any correlation between the CSF leukocyte count and the severity of clinical disease. In contrast, some investigators[52,53,56] reported that adult patients with a high CSF white blood cell count (>300×10^6/L) were more likely to experience a severe course of TBE and persistent neurologic sequelae. CSF pleocytosis disappears within 4 to 5 weeks of the onset of acute CNS disease. The CSF glucose concentration is normal,[76] and the protein concentration is normal or moderately elevated (<1000 mg/L),[36,60,76,122] with evidence of dysfunction of the blood-brain barrier in most patients.[53]

Electroencephalographic examination findings during acute TBE usually are abnormal and characterized by a nonspecific reduction in rhythmic background activity, bilateral periodic slowing, and, rarely, focal abnormalities.[36,51,52] Attenuation of background activity with periodic delta groups has been shown to correlate with parenchymal CNS involvement in adult TBE patients.[51] Reorganization of electroencephalographic activity commonly lags behind clinical improvement, and abnormalities may persist for months to years.[36,51,127]

The limited information available on magnetic resonance imaging in children with severe TBE indicates that parenchymal lesions characteristically are located in the thalamus (Fig. 188–15).[16,52,55,130] The diagnostic and prognostic value of neuroimaging studies in pediatric TBE has not been established. In adults, magnetic resonance imaging studies may detect abnormality in approximately 20 percent of patients.[52] Focal lesions are more likely to be found in those with severe neurologic abnormalities[52] and are confined mainly to the thalamus.[5,9,52,77,98,128,130] Less frequently, lesions are located in the basal ganglia,[5,52,77,98] cerebel-

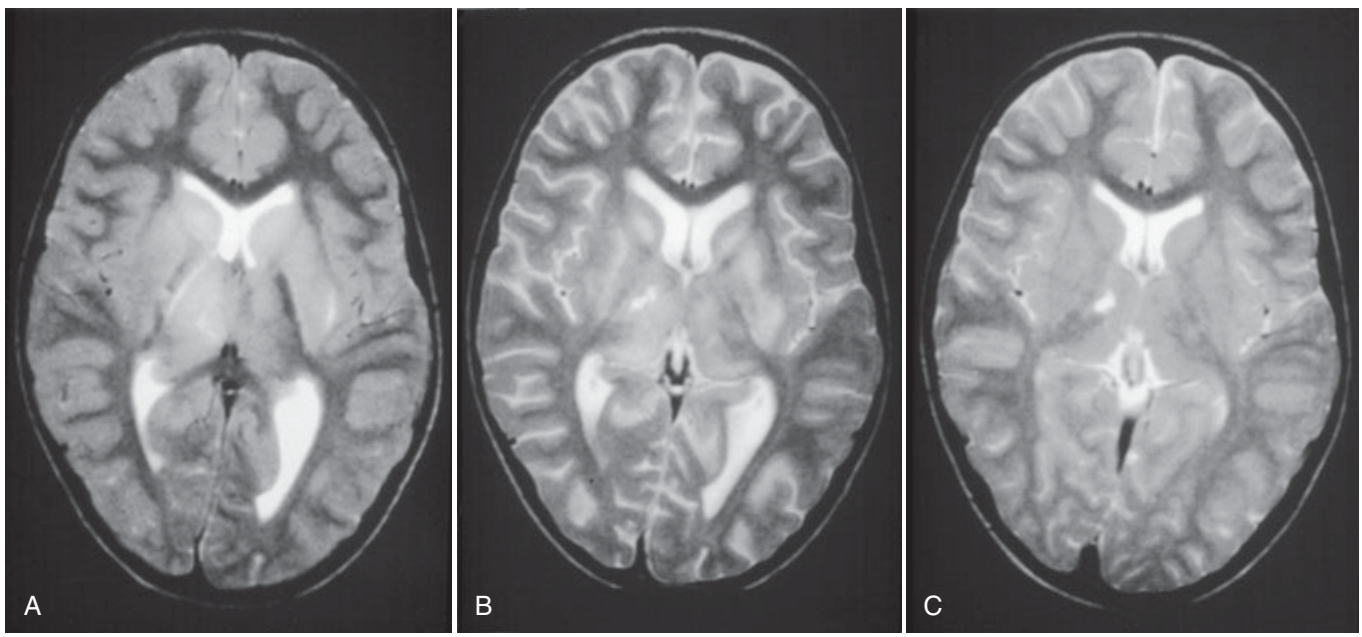

Figure 188–15 T2-weighted magnetic resonance imaging series of a 5-year-old girl with a severe course of tick-borne encephalitis. **A,** Acute phase. Note the T2 hyperintensity in the right side of the thalamus, basal ganglia, and diencephalon. **B,** One month later, partial recovery of T2 hyperintensity and enlargement of cerebrospinal fluid spaces can be seen. **C,** Three months later, normal T2 intensity and a cerebrospinal fluid–filled cavity in the right side of the thalamus are apparent. *(Courtesy of Professor David Nadal, M.D., University Children's Hospital, Zurich, Switzerland.)*

lum,[9,52] brain stem,[10,16,52] and anterior portions of the cervical spine.[9,16]

PATHOLOGY

CNS findings are mainly those of acute meningeal inflammation and focal gray matter encephalomyelitis. The white matter rarely is involved. Macroscopic findings include congestion of the leptomeninges and swelling and hyperemia of the cerebral parenchyma, particularly in the brain stem and cervical region of the spinal cord.[31,90] Petechial hemorrhages are seen in the brain stem, the anterior horns of the spinal cord, and, less consistently, the cerebellum and the anterior central region of the cortex. Histologic changes are dominated by infiltration and ganglion cell damage in the gray matter of these same areas. Changes are particularly pronounced in the anterior horns of the cervical spine (consistent with the poliomyelitis-like manifestations of paralytic courses of TBE),[114] the medulla oblongata, the cranial nerve nuclei of the pons, and the Purkinje cell layer of the cerebellum.[31] Inflammatory foci are characterized by lymphocytic perivascular infiltration and various stages of degenerative changes in neuronal cells. Areas of neuronal necrosis are characterized by perifocal edema, lymphocytic and neutrophilic infiltration, and, at a later stage, nodular microglial proliferation at sites of complete neuronophagia. The presence of granzyme, B-expressing, cytotoxic T cells in close contact with TBE-expressing neurons indicates that immune mechanisms contribute to tissue injury in these areas.[28] Rarely, the spinal nerve roots, spinal ganglia, and peripheral nerves are involved. Spongiform changes, particularly after protracted illness, appear as sharply defined areas of softening with minimal inflammatory reaction. In the Far Eastern form of the disease, extensive poliomyelitis of the spinal cord with destruction of anterior horn cells, particularly in the upper cervical and lower lumbar areas, and poliomyelitis of the brain stem are noted.

PATHOGENESIS

Tick-mediated inoculation of TBEV and viral replication within local dermal cells are followed by lymphatic spread to regional lymph nodes, where further replication occurs.[53] Subsequently, viremia leads to generalized infection, especially of reticuloendothelial cells, followed by secondary rounds of viremia that result in neuroinvasion. Viral penetration of the CNS occurs through capillary endothelia and by TBEV-infected, infiltrating mononuclear cells. Envelope glycoprotein E, the immunodominant TBEV-encoded surface protein, mediates attachment to and fusion with the host cellular membrane.[82] The molecular mechanisms of neural invasion by TBEV have not been elucidated in detail. Current knowledge of the molecular pathogenesis of TBEV has been reviewed elsewhere.[37-39]

LABORATORY DIAGNOSIS

Because neither clinical nor CSF findings differentiate TBE from other causes of meningitis or meningoencephalitis, the diagnosis of TBE rests on demonstration of specific antibody by serologic testing. The presence of specific serum IgM or a significant rise in titer of specific IgG antibody in paired sera (or both) is diagnostic of TBE[44,45,53] unless the patient previously received TBE vaccine doses. Enzyme-linked immunosorbent assay (ELISA) has replaced complement-fixation, hemagglutination-inhibition, and neutralization assays in the routine laboratory diagnosis of TBE.[43,45] ELISA technology offers increased sensitivity, reliably differentiates between IgM and IgG antibodies, and, in contrast to neutralization, uses nonviable viral antigens.[40,105] For the detection of specific anti-TBEV IgM, an IgM capture ELISA system has proved to be more sensitive and specific than is the conventional three-layer ELISA system because high titers of specific IgG and rheumatoid factor do not interfere with IgM binding.[40,105] With this method, specific IgM virtually always can be demonstrated during the acute illness and thereafter may persist for as long as 9 months.[44,53,105] The highly sensitive capture ELISA format allows a determination of anti-TBEV IgM, even in serum samples obtained late during the acute illness, when high titers of specific IgG already are present. A potential problem of TBEV IgG ELISA systems is cross-reactivity with other flaviviruses, notably yellow fever virus, dengue virus, and Japanese encephalitis virus.[21,22,92] This limitation should be considered in the interpretation of seroprevalence studies and in patients with a relevant travel or immunization history.

Specific IgM and IgG also can be measured in CSF.[40,105] Detection of TBEV-specific intrathecal antibody production by ELISA is highly specific for the diagnosis of TBE but somewhat less sensitive than is determination of serum IgM during the first several days of CNS disease.[45,53] Intrathecal TBEV antibody assays are less widely used in clinical practice, but they may be useful if serum antibody titers fail to differentiate between infection- and vaccine-induced immunity.

Detection of virus or viral RNA is technically feasible, but it is not used routinely in clinical practice. TBEV can be recovered from blood by viral culture, but because detectable viremia occurs during the prodromal stage (when the diagnosis of TBE seldom is considered), culture is not useful in clinical practice. TBEV may be recovered from the brain tissue of patients who died at an early stage of disease.[17] Amplification of TBEV-specific RNA by reverse transcription–polymerase chain reaction (RT-PCR) has been established for detection of TBEV in ticks[99] and in humans.[119] Primers that amplify the highly conserved 5′ noncoding region of the TBEV genome have been shown to be sensitive and specific.[118] Successful amplification of TBEV-specific RNA from blood, CSF, or brain tissue has been reported.[18,119,126,130] However, like culture, RT-PCR usually is positive only during the prodromal stage and thus generally is not useful in clinical practice.[50]

DIFFERENTIAL DIAGNOSIS

The clinical course of TBE is nonspecific in most cases, and a history of exposure to ticks in an endemic area can be elicited in 40 to 75 percent of pediatric patients.[36,52,74,76,88,117,127] Because serologic confirmation of the diagnosis is not available immediately in most cases, the differential diagnosis includes a wide spectrum of diseases causing fever and CNS manifestations.

Enteroviruses are the most common cause of symptomatic CNS infection in children during the warm seasons in regions where TBE is prevalent. Fever in enteroviral infection may occasionally be biphasic,[86] although the duration of both the first phase and the asymptomatic interval usually is shorter than that noted in TBE.[14] Routine tests of CSF do not differentiate between the two entities. Skin and mucosal manifestations are indicative of enteroviral infection rather than of TBE, whereas encephalitis and myelitis can occur in both. Enteroviral meningitis is diagnosed most readily by PCR of CSF[111] or by culture of virus from CSF and mucosal surfaces. Mumps meningitis is to be considered in the differential diagnosis in cases without parotid enlargement, particularly in areas with low rates of mumps immunization.[123] The diagnosis of mumps meningitis is made by viral culture of CSF or by detection of specific serum IgM antibody. Although it is a rare occurrence in childhood, herpes simplex virus (HSV) encephalitis always should be considered in the differential diagnosis. Because the case-fatality rate of HSV encephalitis is greater

than 90 percent if it is left untreated, therapy with intravenous acyclovir should be initiated without delay, if that diagnosis is thought possible. The combination of fever, which may be biphasic,[106] and localizing signs observed by neurologic examination, electroencephalographic studies, or magnetic resonance imaging[47] suggests HSV encephalitis and rarely occurs in pediatric TBE, particularly if the temporal lobe or the orbital portion of the frontal lobe is affected. Meningeal irritation usually is absent in HSV encephalitis. The diagnosis of HSV encephalitis is confirmed by PCR of CSF.[8,72] Epstein-Barr virus encephalitis can occur in immunocompetent children, and Epstein-Barr virus shares with TBEV a preference for causing thalamic and basal ganglia lesions.[52,95] Other viral causes include common respiratory tract viruses such as influenza, parainfluenza, and adenovirus.

Among bacterial infections, partially treated pyogenic meningitis, encephalitis in association with *Mycoplasma pneumoniae* infection,[108] and encephalopathy caused by *Bartonella henselae* may resemble TBE. Tuberculous meningitis runs a subacute course, and although near-normal CSF chemistry may be recorded very early in the disease, hypoglycorrhachia invariably develops and CSF protein rises to high levels. CNS infection by *B. burgdorferi* is particularly important in the differential diagnosis because in Europe, this pathogen and TBEV are transmitted by the same tick vectors, *I. ricinus* and *I. persulcatus*, and because one of the most prevalent European genospecies, *Borrelia garinii*, is more neurotropic than is *B. burgdorferi* sensu stricto.[107] In contrast to TBE, the incubation period for early neuroborreliosis in children generally is 4 to 10 weeks, high-grade fever is unusual, and most patients have cranial nerve paresis. However, several cases of concomitant infection with TBEV and *B. burgdorferi* have been reported in the literature,[1,15,62,94] and coexistence of TBEV and *B. burgdorferi* in ticks has been documented.[58] In some cases of TBE, persistent or late-appearing limb paralysis has been suggested to be attributed to concomitant, but undiagnosed, borrelial radiculoneuritis rather than TBE-related myelitis with anterior horn involvement.[62] In this situation, diagnostic evidence of Lyme borreliosis should be sought because this condition requires antimicrobial therapy. Seroepidemiologic and clinical evidence indicates that concomitant infection with TBEV and *Anaplasma phagocytophilum*, the agent of human granulocytic ehrlichiosis, also may occur.[58,79]

TREATMENT

No specific antiviral therapy is available to treat TBE. Specific hyperimmune globulin is indicated exclusively for passive immunization within 96 hours of exposure to ticks. Anecdotal evidence suggests that the administration of TBE hyperimmune globulin thereafter may have a detrimental effect on the course of disease.[3,70] In severe cases, supportive therapy is aimed at preventing the development of sequelae related to increased intracranial pressure, seizures, and bulbar dysfunction. After the acute stage, neurorehabilitation may be necessary for patients with motor, cognitive, or emotional disturbances. Because TBE rarely causes a chronic seizure disorder, prolonged anticonvulsive therapy seldom is indicated in patients who had convulsions during acute disease.

PROGNOSIS

The main risk factor for neurologic residua and a fatal outcome of TBE is advanced age.[2,52,120] Substantial sequelae appear to be infrequent in children,[36,52,64,76,115,127] although no prospective long-term follow-up studies of pediatric TBE patients have been performed to date. Individual cases of children with persistent neurologic damage, including hemiparesis,[16,50,55,130] unilateral arm paresis,[129] epilepsy,[50,55,104] and extrapyramidal movement disorder, have been reported.[55] Recent evidence, however, indicates that subtle abnormalities in attention and psychomotor skills may persist in many children for prolonged periods.[115] In adult patients, long-term neurologic defects are observed in 2 to 10 percent of patients with clinical evidence of parenchymal CNS involvement during the acute illness.[2,30,52,56,129] Risk factors for persistent sequelae have been assessed by Kaiser[52] and, by univariate analysis, include impaired consciousness (i.e., Glasgow Coma Scale score <7), ataxia, paresis, abnormal findings on magnetic resonance imaging, CSF pleocytosis greater than 300×10^6/L, and CSF protein concentration of more than 600 mg/L. Gunther and colleagues[33] reported that encephalitic symptoms were associated with low levels of intrathecal IgM in early stages of TBE. Paresis of the extremities and ataxia are the most common persistent findings on neurologic examination.[2,24,52,56,81,120] Much more common, however, are ill-defined manifestations such as chronic fatigue, headache, sleep disorders, memory dysfunction, and emotional disturbances, which are reported by most adults recovering from TBE and persist for months to years.[24,73,120] In some patients, electroencephalographic findings may remain abnormal for prolonged periods, with seizure activity occasionally demonstrated.[36,115] Most of these patients are asymptomatic, although epilepsy secondary to TBE has been described infrequently.[36,89] The reported case-fatality rate in adult TBE patients is approximately 1 percent in large series,[2,24,30,52,56,81] with most deaths being attributed to severe bulbar encephalitis or related to underlying cardiovascular disease in elderly patients.[24] Fatal outcome of TBE in children is exceedingly rare.[84]

PREVENTION

General preventive principles include avoidance of known endemic areas of TBE, use of protective clothing, and rapid removal of ticks attached to the skin. The topical repellent DEET (*N,N*-diethyl-*m*-toluamide) has a definite albeit moderate effect against ticks.[109] Because of its potential neurotoxicity, the content of DEET in products used for children should not exceed 15 percent.[12,96] These measures are effective in reducing tick exposure and transmission of TBEV to some degree. Pasteurization of raw milk prevents enteric transmission of TBEV.[4,57]

Reliable protection, however, requires active immunization against TBE. Inactivated whole-virus vaccines are produced in Austria (Baxter), Germany (Chiron), and Russia. The vaccines are made from different central European TBEV strains and are produced by concentrating and purifying cell culture fluid from infected chick embryo cells. Adjuvants (aluminum hydroxide) are added to the formalin-inactivated cell culture fluid. The Russian vaccine is an inactivated, unconcentrated cell culture fluid from infected African green monkey kidney cells. TBE vaccine is available in the United States under investigational drug exemption to military personnel deployed in endemic areas.[11,19]

Currently approved TBE vaccines are approved for children 12 months of age and older. Basic immunization consists of three doses given at 0, 1 to 3, and 5 to 12 months. Rapid immunization schedules also are available. The vaccines are immunogenic and safe.[29,30,32,97,116] Seroconversion rates for neutralizing antibodies after the first, second, and third dose are 70, 95, and 99 percent, respectively.[65,69] Reliable protection is achieved 2 weeks after administration of the second dose.[134] Data on the kinetics of vaccine-induced serum antibodies led to the recommendation for booster doses every 3 years.[65,67] Recent data on the decay of neutralizing antibodies, however, indicate that protection may last considerably longer.[102,103] Minor adverse events (fever and local reactions) are reported by 4 to 15 percent of vaccinees after receiving the first dose and less frequently after receiving subse-

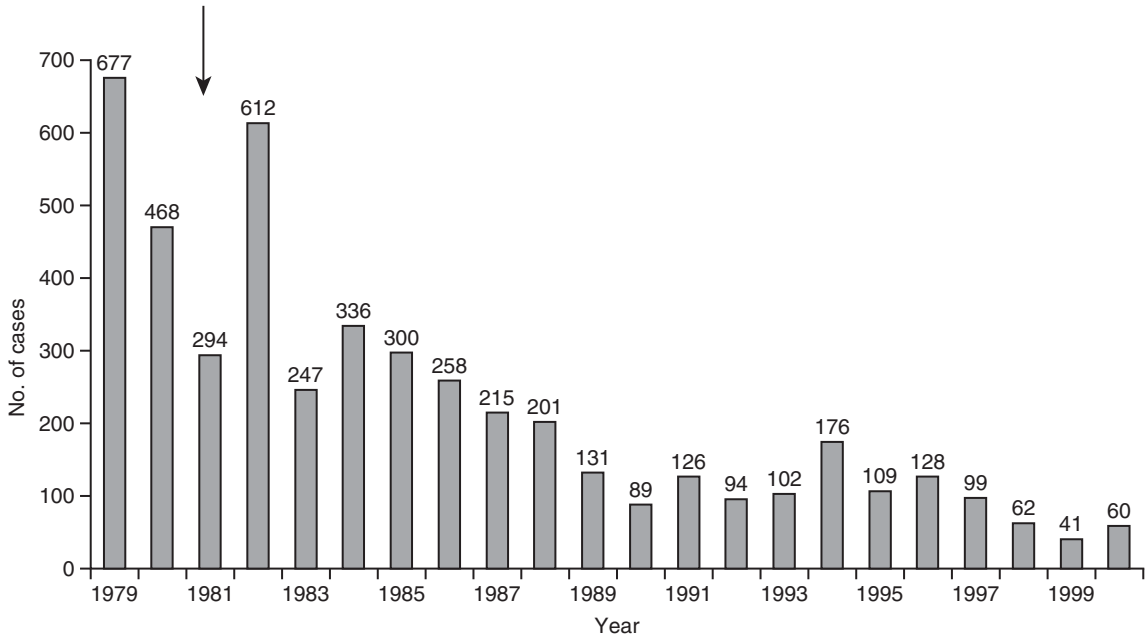

Figure 188–16 Annual number of cases of tick-borne encephalitis in Austria before and after the introduction of mass immunization in 1981 *(arrow). (Data obtained from the International Scientific Working Group on Tick-Borne-Encephalitis, www.tbe-info.com/report.)*

quent doses.[67,97,133] Mostly transient neurologic adverse events temporally associated with the administration of TBE vaccine have been reported rarely.[29,30,112,113,116] Their true incidence has not been established. Based on passive notification of adverse events, an incidence of one neurologic illness in 1 million doses of TBE vaccine has been calculated.[42] Placebo-controlled trials of vaccine efficacy have not been conducted. Observational studies in Austria using historical controls indicate that the effectiveness of the vaccine is greater than 90 percent.[68,71]

In Austria, the TBE vaccine is recommended for mass immunization of all potentially exposed individuals. Since the beginning of the vaccination campaign in 1981, a dramatic reduction in nationally notified cases has been observed (Fig. 188–16).[71,135] Mass immunization is the probable cause of this decrease because neighboring countries (e.g., Switzerland, southern Germany, Slovenia) reported stable or increasing numbers of cases of TBE during the same time. In these and other endemic countries of central and eastern Europe, authorities recommend immunization of school-aged children and adults who live in endemic areas and have a risk of exposure to ticks.[16,91] In younger children, TBE vaccination is not recommended routinely because neurologic complications are exceedingly rare.[16,74]

A commercial TBE hyperimmune globulin for pre-exposure and postexposure immunoprophylaxis is available in Europe.[3] In several countries, however, this product is not approved for use in children younger than 14 years because numerous breakthrough TBEV infections have been observed in recipients of postexposure prophylaxis.[52,55,77,128] Among these recipients, some unusually severe cases raised concern about a causative role of TBE immunoglobulin in enhancement of disease.[7,77,130] Antibody-dependent enhancement, a mechanism of pathogenicity proposed in dengue hemorrhagic fever,[87] was discussed as a possible explanation,[55] but experimental data in support of this hypothesis in TBE are lacking.[61]

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CHAPTER 188h

Other Flaviviral Infections

Theodore F. Tsai

POWASSAN ENCEPHALITIS

Powassan virus was isolated from a fatally infected patient and named after the patient's Ontario town of residence. The virus is classified within the antigenic complex of tick-borne flaviviruses. Two viral lineages in North America may represent genotypes circulating in separate enzootic cycles.[11] More than half of surviving cases suffer from residual neurologic deficits, frequently associated with upper cervical damage leading to upper extremity muscle weakness and wasting.

EPIDEMIOLOGY AND ECOLOGY

More than 30 cases of naturally acquired Powassan encephalitis have been reported from North America and others from Russia. Two cases of laboratory-acquired Powassan infection have been reported as well. Approximately one third of the cases from North America have occurred in children younger than 15 years, and the preponderance of cases have been in males. With one exception, infections have occurred in the summer or early fall; one patient had an onset in December.[16,21,31]

The probable sites of exposure of cases have been confined to the eastern states and Canadian provinces, principally Maine, Vermont, New York, Pennsylvania, and Massachusetts and Ontario and Quebec.* However, two cases have been reported from upper Wisconsin and Michigan, and the known and suspected geographic distribution of the virus is even wider, with isolation of virus recorded from Connecticut, Massachusetts, West Virginia, Colorado, South Dakota, and California, and serologic evidence of infection in humans or animals reported from Wyoming, North Dakota, British Columbia and Alberta, and Sonora, Mexico.[22] Viral isolates also have been recovered from ticks, mosquitoes, and birds from southeastern Russia, and evidence of viral transmission in China and Southeast Asia has been reported.[19-21]

In North America, the virus is transmitted by *Ixodes cookei*, *Ixodes marxi*, *Ixodes spinipalpus*, and *Dermacentor andersoni* to small mammals (ground hogs, *Marmota monax*; red squirrels, *Tamiasciurus hudsonicus*; weasels, *Mustela*; skunks, *Mephitis*; foxes, *Vulpes*; chipmunks, *Tamias straiatus*; mice, *Peromyscus*; snowshoe hares, *Lepus americanus*; voles, *Microtus*; and gray squirrels, *Scurius carolensis*). In Russia, the virus is transmitted by *Ixodes persulcatus* and various *Haemaphysalis* ticks; *Apodemus* mice and *Microtus* voles are the principal vertebrate hosts. Powassan virus is transmitted trans-stadially in *D. andersoni* and *Ixodes pacificus*, and transovarial transmission has been shown in other species. Powassan virus infection in humans probably is rare because the implicated ixodid ticks are encountered infrequently and usually confine themselves to host animal burrows and people. Human infections are associated with outdoor activities and subsequent exposure to infected ticks. In one case involving a 13-month-old infant, however, the infecting tick was brought into the home by a domestic cat.[38] Animal serosurveys indicate that infections occur in dogs, and exposure to ticks on domestic animals may be an alternative source of infection. In a human serosurvey from Ontario, 3 percent had Powassan antibody; however, in other areas, the prevalence of antibody has been less than 1 percent. Experimental studies have shown that *Ixodes scapularis*, the vector of Lyme disease, can transmit Powassan virus, and a field isolate has been reported.[8] The absence of a Powassan epidemic paralleling that of Lyme disease suggests differences in the agents' transmission cycles.

Although no Powassan cases have been attributed to milk-borne transmission, experimental studies have shown that domestic goats can be infected with Powassan virus and can shed virus into milk. In a survey of New York goats, 2 percent had serologic evidence of past Powassan virus infection, which indicates the possibility of milk-borne Powassan virus infection in the United States.[40]

Clinical and pathologic signs of encephalitis have been produced in experimentally infected horses. Unlike Lyme disease and some rickettsial infections, Powassan virus infection can be transmitted after a brief period of tick attachment.[12]

CLINICAL MANIFESTATIONS

The incubation period may be several weeks after known exposure to a tick. Fever, headache, lethargy, retro-orbital pain, and photophobia are early symptoms that may be followed abruptly by changes in sensorium, generalized or focal seizures, paresis, and paralysis. Focal neurologic signs have been observed in most patients in whom clinical descriptions have been reported. In one patient, olfactory hallucinations, focal seizures, and localizing electroencephalographic irregularities were concordant and thus suggested a temporal lobe focus.[13,16]

Three deaths have been reported, and significant neurologic sequelae (hemiplegia, quadriplegia, aphasia), including residual shoulder girdle atrophy and weakness analogous to sequelae that occur after European and Far Eastern tick-borne encephalitis, are common occurrences. In another patient, wasting and weakness of a leg consistent with lumbosacral poliomyelitis were reported.[7,23]

Clinical and experimental observations of tick-borne encephalitis suggest that chronic central nervous system infection characterized by a convulsive disorder (epilepsia partialis continua), weakness, and dementia may occur after recovery from the acute phase of illness. A retrospective study of 22 Canadian patients with a similar clinical syndrome and in whom histologic changes in the brain resembled those of chronic tick-borne encephalitis showed no evidence of Powassan virus infection. However, none of these patients had a history of acute encephalitis.

LABORATORY DIAGNOSIS

Virus has been recovered from the brains of patients with fatal cases of the disease. A serologic diagnosis can be achieved more rapidly by detecting specific IgM antibody in acute-phase serum or spinal fluid. A serologic diagnosis by hemagglutination inhibition and complement fixation is specific in most instances, but heterologous antibodies from other flavivirus infections (e.g., dengue, St. Louis encephalitis) or vaccinations (yellow fever) may obscure some results.

DIFFERENTIAL DIAGNOSIS

The clinical findings of encephalitis in a patient with a history of a tick bite acquired in an endemic area should suggest the possibility of Powassan encephalitis. However, because ixodid tick bites may be inconspicuous, a negative history does not exclude the diagnosis. Other viral agents of encephalitis, especially eastern

*See references 5, 7, 10, 13, 16, 18, 23, 31, 35, 36, 38, and 40.

equine encephalitis virus and California group viruses, which are prevalent in New York State and eastern Canada, should be considered in the differential diagnosis. Lyme disease, because of its known geographic distribution and association with a tick vector, also may be associated with neurologic complications; however, a history of having a typical rash and arthritis should differentiate the conditions. In a series of 145 clinical encephalitis cases at a Canadian tertiary pediatric hospital, one was attributed to Powassan virus.[24] An imported case of tick-borne encephalitis, encountered in Ohio but acquired in Austria, underscores the value of obtaining a travel history.

TREATMENT AND PREVENTION

No specific therapy for Powassan encephalitis is available. Personal protective measures to avoid tick bites are advised. Consumption of unpasteurized goat milk should be avoided because of the theoretical risk of contracting Powassan virus infection and the well-documented risk of acquiring other infections associated with raw milk. Inactivated tick-borne encephalitis vaccines licensed in Europe do not cross-protect against Powassan virus infection.

ROCIO ENCEPHALITIS

Rocio encephalitis virus was isolated from the brain of a fatal case of encephalitis in a patient during an outbreak in 1975.[15] The virus is related peripherally to viruses in the Japanese encephalitis viral antigenic complex. The disease has occurred exclusively in the coastal São Paulo State and adjacent Paraná State, Brazil, principally in the Ribiera Valley and Santista lowlands. More than 1000 cases were documented in outbreaks between 1975 and 1977; only one symptomatic case (in an infant) has been recognized subsequently, although recent infections (IgM in asymptomatic individuals) were documented in later serosurveys in northeastern and southeastern Brazil. Thus, the virus may be transmitted undetected in an area beyond the Ribiera Valley. The viral transmission cycle has not been elucidated; however, field and laboratory observations suggest transmission between birds and *Psorophora* or *Aedes* mosquitoes. Humans are dead-end hosts. Human cases have occurred principally in men with outdoor occupations, especially fishermen.

The incubation period is estimated to be 7 to 14 days. Prodromal symptoms of fever, headache, malaise, vomiting, and conjunctivitis are followed by mental status changes, meningismus, and motor impairment. Cerebellar signs occur commonly. Signs of bulbar involvement also are seen. Coma and a fatal outcome occur most commonly in children (30%) and in the elderly; overall, 10 percent of cases are fatal and 20 percent of patients have neuropsychiatric sequelae. Pathologic findings of encephalitis principally involve the thalamus, cerebellar dentate nucleus, brain nuclei, brain stem, and spinal cord.

The diagnosis should be suspected in patients with acute encephalitis and a consistent history of exposure. Virus has been isolated from autopsy brain specimens. The presence of virus-specific IgM in cerebrospinal fluid or a fourfold change in serum antibody titer confirms a case. IgM in serum is presumptive evidence of recent infection. Treatment is supportive. Emergency application of adulticides and larvicides has been used to control epidemics.

LOUPING ILL VIRUS

Louping ill virus derives its name from an old Scottish term describing the leaping motions of encephalitic sheep. Historical accounts of the disease in sheep date from 1795, and the virus was isolated from ill sheep in 1931. Louping ill virus, a member of the antigenic complex of tick-borne flaviviruses, is transmitted by *Ixodes ricinus* to sheep, deer, mountain hares, and grouse.[19-21] Grouse may become infected by ingesting infected ticks.[17] The disease is enzootic in pasturelands of Scotland, England, Wales, and Ireland. Naturally acquired human infections have occurred mainly in sheep farmers, veterinarians, and abattoir workers or butchers who had direct contact with animals. Antibody prevalence is 10 percent in abattoir workers in enzootic areas. Laboratory-acquired infections are common and account for half of all reported human cases. These observations suggest that infections are transmitted easily by direct mucous membrane or respiratory infection. Tick-transmitted cases also have been reported. Hospital surveillance in an enzootic area found louping ill virus to be a rare occurrence that was responsible for less than 0.5 percent of encephalitis cases. Related tick-borne viruses in Spain, Greece, Norway, and Turkey have not been associated with human disease. The virus is shed in sheep and goat milk, but unlike tick-borne encephalitis, milk-transmitted cases have not been reported.

The incubation period can be as short as 3 days. Three clinical syndromes have been described.[9] Approximately a third of patients have a self-limited influenza-like illness with fever, headache, dizziness, and myalgias. The febrile illness is followed by clinical improvement and a second encephalitic phase in more than half the cases. Neurologic symptoms include meningismus, severe headache, vomiting, drowsiness, and tremor; one fatal case was reported. A poliomyelitis syndrome with muscle weakness or paralysis has been described in a few cases. Hemorrhagic fever also was reported in one atypical laboratory-acquired case.

The diagnosis should be suspected in febrile patients with occupational or other exposure, especially if central nervous system symptoms are present. The diagnosis is confirmed serologically by demonstrating virus-specific IgM in cerebrospinal fluid or serum or by a fourfold rise in antibody titer or real-time polymerase chain reaction.[29] Treatment is symptomatic. The use of unpasteurized milk products should be avoided. Persons with outdoor exposure in enzootic areas are advised to use repellents and other protective measures against tick bites.

KYASANUR FOREST DISEASE

Kyasanur Forest virus was isolated in 1957 after an outbreak of hemorrhagic fever, initially suspected to be the first outbreak of yellow fever in Asia, appeared in India in the Kyasanur Forest of Mysore (now Karnataka).[2,32] The virus, including the Alkhurma subtype, is a member of the tick-borne flaviviral antigenic complex.[19-21,28] It is transmitted by *Haemaphysalis spinigera* (among numerous other ixodid ticks) to forest rodents, insectivores, and possibly bats; cattle and other large animals are important tick hosts but do not appear to amplify the virus. Langur monkeys sicken in epizootics and die of the infection. Human cases occur in dry-season epidemics, chiefly in persons who have contact with forests. The disease has spread contiguously as villagers clear forests for pastureland. Between 1982 and 1988, 1847 cases were reported, 254 of which were fatal. Hyperendemic transmission in Karnataka state continues despite the introduction of mass vaccination.[32]

After an incubation period of 3 to 8 days, illness begins abruptly with fever, headache, myalgias, chills, and gastrointestinal symptoms.[33,34] Facial hyperemia, conjunctival suffusion, lymphadenopathy, hepatomegaly, papulovesicular enanthem, and petechiae are the principal physical findings. Bradycardia and hypotension are present and may progress to become life-threatening. Epistaxis, hemoptysis, and gastrointestinal bleeding may be prominent. Bronchopneumonia and hemorrhagic pulmonary edema complicate the illness in 40 percent of cases. Renal failure may develop. After the resolution of symptoms and an afebrile interval of 1 to 3 weeks, fever, recurrence of symptoms,

TABLE 188–5 Less Commonly Recognized Flaviviral Infections

Virus	Clinical Syndrome	Geographic Distribution	Transmission Cycle	Mode of Transmission
Alkhurma	Hemorrhagic fever encephalitis	Saudi Arabia	Unknown	Z, DC, ?V
Alma-Arasan	Febrile illness, meningitis	Kazakhstan	*Ixodes persulcatus*–?	V
Apoi	Encephalitis	Japan	Rodent–?	L
Banzi	Nonspecific febrile illness	South, East Africa	*Culex rubinotus*–rodent	V
Bussuquara	Fever, arthralgias	Brazil, Colombia, Panama	*Culex melaconion*–rodent	V
Edge Hill	Fever, polyarthritis	Australia	*Aedes vigilax*–marsupial	V
Ilheus	Fever, myalgia, encephalitis	Argentina, Brazil, Colombia, Guatemala, Panama, Trinidad	*Psorophora ferox*–bird	V, E
Karshi	Nonspecific febrile illness	Uzbekistan	Various ticks–rodent	V
Kokobera	Fever, polyarthralgia	Australia, Papua New Guinea	*Culex annulirostris*–? marsupial	V
Koutango	Fever, rash, arthralgia	Western and central Africa	Tick–rodent	L
Langat	Fever, encephalitis	Malaysia, Thailand, Russia	*Ixodes* tick–rodent	L
Modoc	Aseptic meningitis	Western United States, Canada	Rodent–rodent	Z
Negishi	Encephalitis	Japan, China, Russia	Tick–unknown	L, V
Rio Bravo	Nonspecific febrile illness, meningitis	Western United States, Canada	Bat–bat	Z, L
Sepik	Nonspecific febrile illness	Papua New Guinea	*Mansonia* species–?	V
Spondweni	Fever, arthralgia, rash	Southern and western Africa	*Aedes* species–?	L, V
Usutu	Fever, rash	Southern and central Africa	*Culex* species–bird	V
Wesselsbron	Fever, arthralgia, rash, encephalitis	Sub-Saharan Africa, Thailand	*Aedes* species–?	V, L, DC?
Zika	Fever, rash, arthralgia, conjunctivitis	Western, eastern, and central Africa; Indonesia, Malaysia, Pacific Islands	*Aedes* species–monkey	V

DC, direct contact with infected sheep; E, experimental infection; L, laboratory-acquired infection; V, vector-borne infection; Z, zoonotic infection.

and meningoencephalitis develop in 15 to 50 percent of cases, as occurs in tick-borne encephalitis. Leukopenia with a left shift, thrombocytopenia, and an elevated hematocrit reflecting hemoconcentration are seen. Elevations in liver enzymes occur commonly. The case-fatality rate is 3 to 15 percent; keratitis and iritis occur as sequelae. The virus frequently can be isolated from acute-phase blood specimens (<12 days after onset), or the diagnosis can be confirmed serologically. A formalin-inactivated chick embryo cell culture vaccine is produced locally in India and is distributed in epidemic areas.

OMSK HEMORRHAGIC FEVER

Omsk hemorrhagic fever virus was isolated from a viremic human during a series of outbreaks in the Omsk region of western Siberia from 1945 to 1949.[37] Two subtypes have been differentiated.[32] Between 1945 and 1958, approximately 1500 cases were reported in the forest-steppe zones within the Omsk, Novosibirsk, Kurgan, and Tjumen regions. The virus is related antigenically and genetically to other tick-borne flaviviruses. It circulates among microtine rodents and *Dermacentor* ticks, which results in a spring–early summer peak of infected ticks with a smaller peak in early autumn. The disease epidemiology changed after muskrats were introduced into the region; extensive muskrat epizootics occurred and led to an increase in human cases and geographic spread of the disease. Muskrat trappers, who may be infected by direct contact with infected tissue or blood, continue to account for most cases, but tick-borne infections also occur in other local residents, including children. Seroprevalence rates range to higher than 30 percent in some locations. Laboratory-associated cases frequently have occurred in workers not immunized with tick-borne encephalitis vaccine.

The incubation period may be as brief as 2 to 4 days. The illness is similar to Kyasanur Forest disease, but with an earlier onset of hemorrhagic phenomena (e.g., epistaxis). Hemorrhages tend to be less severe, neurologic complications are less frequent, and the overall mortality rate is lower (less than 3%). Neuropsychiatric sequelae, however, occur commonly. Uncomplicated cases usually resolve within 7 to 10 days. The diagnosis is made serologically. Vaccine produced against tick-borne encephalitis virus is reported to provide some degree of cross-protection.

OTHER FLAVIVIRAL INFECTIONS

Flaviviral infections of lesser public health importance or that are recognized less frequently are listed in Table 188–5. Several of the viruses are known to cause human illness only after laboratory exposure. The clinical manifestations of these infections may differ from naturally acquired infection because of their mode of transmission by respiratory, mucosal, or other routes of infection. Other viruses that cause nonspecific syndromes of febrile illness were discovered through fever surveys; although few cases may have been reported, their prevalence may be underestimated because few cases are recognized. Because immune responses to repeated flaviviral infections lead to broadly cross-reactive responses, serologic diagnosis may be misleading unless extensive cross-neutralization assays are undertaken. An outbreak of Zika virus infection in Yap, causing fever and arrhythmias, could easily have been misdiagnosed as dengue had PCR and cross-neutralization assays not been undertaken.[25] Of the zoonotic flaviviruses (transmitted from animal to animal without an arthropod vector), only Rio Bravo and Modoc viruses are known to cause human illness.

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CHAPTER 189

Hepatitis C Virus

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Since 1990, when hepatitis C virus (HCV) was recognized as the primary cause of non-A, non-B hepatitis, this infection has assumed a prominent role in the field of hepatology and infectious disease. Substantial progress has been made in understanding the virology, epidemiology, and natural history of HCV infection. An estimated 4 million people in the United States have been infected with this virus, of whom 3.2 million are chronically infected. HCV has become the most common cause of hepatitis, cirrhosis, and end-stage liver disease in this country. However, HCV does not always cause significant morbidity, at least in the first 2 or 3 decades after infection, a finding that raises specific issues for treatment of pediatric patients. HCV remains the subject of numerous studies around the world, in laboratory and clinical settings, and significant advances in defining the molecular virology, viral-host interactions, and effective therapy are being accomplished.

VIROLOGY

Before the discovery of HCV, the agent responsible for most transfusion-associated non-A, non-B hepatitis was presumed to be a small, enveloped virus. This conclusion was based on the ability to pass the infectious agent through an 80-nm filter and to inactivate it with organic solvents. In 1989, a group at the Chiron Corporation[41] identified a cDNA derived from human plasma that shares sequence similarities with flaviviruses and pestiviruses. Intrahepatic inoculation of the full-length RNA transcribed from this clone subsequently was shown to induce hepatitis in chimpanzees.[109,202] Until recently, research in the molecular virology of HCV was hampered by the lack of a cell culture system in which to propagate the virus. In 2005, a critical technologic breakthrough was achieved in the establishment of such a cell culture system.[124,194] Although much remains to be learned about the molecular virology of HCV, henceforth progress should be rapid.

HEPATITIS C VIRUS VIRION

HCV is the prototype virus within the new *Hepacivirus* genus of the family *Flaviviridae*. The HCV virion particle is approximately 30 to 60 nm in diameter, based on filtration studies[2,3] and electron microscopy.[18,175] The capsid is thought to be enveloped by a lipid bilayer because infectivity is inactivated by chloroform.[78] The envelope contains two viral glycoproteins, E1 and E2, and the nucleocapsid contained within is composed of core protein and the viral RNA genome.

HEPATITIS C VIRUS GENOME

The HCV genome is a 9.6-kb positive, single-stranded RNA (Fig. 189–1). A single open-reading frame (ORF) encodes a 3000-

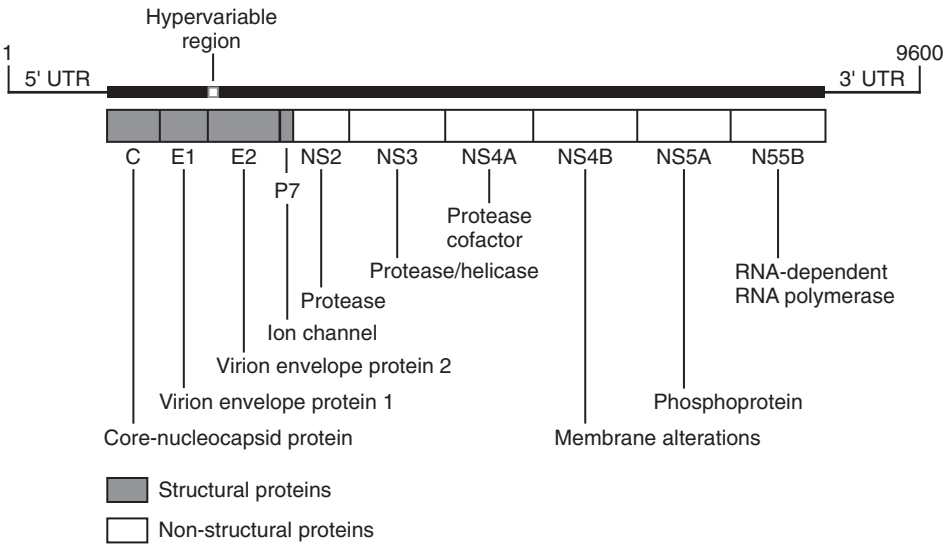

Figure 189–1 Schematic of the hepatitis C virus genome and encoded proteins.

amino acid polyprotein that undergoes proteolytic processing to yield at least 10 individual gene products. Structural proteins (core and envelope) are encoded in the 5′ quarter of the genome. Downstream is a small integral membrane protein, p7, which appears to function as an ion channel.[74] Nonstructural proteins (proteases, helicase, and RNA polymerase) comprise most of the remaining portion. The flanking 5′ and 3′ nontranslated regions (NTRs) contain conserved sequences that regulate both genome replication and ORF translation.

The HCV 5′ NTR is approximately 340 nucleotides long and forms a structure similar to that of the 5′ NTRs of the related pestiviruses and GB virus B (GBVB).[33] Computer modeling and cleavage protection studies of the HCV 5′ NTR predict the formation of a similar internal ribosome entry site (IRES)–like structure that includes the canonical four stem loops, suggesting a similar 5′ cap-independent mechanism of translation.[189] Furthermore, in vitro expression[165] and mutational analysis of the HCV 5′ NTR support this conclusion. Deletion analysis reveals both positive and negative regulatory elements in the 5′ NTR. Deletion of stem loop I increases translation efficiency, whereas domains II and III are essential for IRES activity. The base of domain III and the initiation codon are particularly important for several interactions subsequent to binding of the 40S ribosomal subunit, leading to initiation complex formation.[152] Stem loop IV contains the initiator AUG, and its destabilization leads to more efficient translation initiation.[82,83,86] Both viral and host-derived factors can bind to the IRES region and alter its activity in vitro,[3,66,77] but in vivo roles of the 5′ NTR remain to be defined. The 5′ NTR also contains sequences that are essential for HCV replication.[64]

The 3′ NTR contains sequences required for viral replication and has a three-part structure composed of (1) a variable region immediately downstream of the stop codon of the polyprotein, (2) a polypyrimidine tract of variable length, and (3) a highly conserved 98-nt-long RNA element designated the X-tail.[64,110,185] The X-tail forms three stem loops, one of which (SL2) forms long-range base pairs with sequences located in the coding sequence of NS5B that are essential for viral replication.[63]

Translation of the single ORF results in a single polyprotein precursor that is cleaved by both host and viral proteases to produce 10 viral proteins.[123] The structural proteins (core, E1 and E2) are processed by host signal peptidase, and the nonstructural proteins subsequently are cleaved by virally encoded NS2-NS3 and NS-3 proteases. The core protein is at the polyprotein N-terminus, and, together with the viral RNA genome, it comprises the HCV nucleocapsid. The core protein is highly conserved[34] and may be involved in other processes such as apoptosis,[84] intracellular signaling,[104] transcription,[177] and modulation of the host immune response.[106] The envelope proteins, E1 and E2, are *N*-glycosylated with hydrophobic C-termini that anchor the proteins to the lipid envelope. E2 binds specifically to the host protein CD81,[156,157] a finding suggesting that it mediates viral entry into the cell. Unlike the core protein, E1 and E2 demonstrate considerable sequence heterogeneity from different isolates. In particular, the N-terminal of E2 contains a hypervariable region (HVR1) that is an important viral neutralization determinant.[176] HVR1 is also a T-cell determinant, able to activate helper T-cell responses during HCV infection. The sequence variability of E2 may account, at least in part, for the ability of HCV to elude the host immune system and establish persistent infection.

The 3′ region of the genome encodes seven nonstructural proteins that participate in post-translational proteolytic processing and replication of HCV genetic material. The HCV polyprotein undergoes processing through a sequence of successive cleavage steps.[201] Initially, host protease generates the NS2-NS3 fusion protein precursor. This intermediate species autoproteolyzes to liberate the individual NS2 and NS3 proteins. NS3, which encodes a multifunctional serine protease, then cleaves the remaining junctions of the HCV polyprotein. NS4A complexes with NS3 and serves as a cofactor for the cleavage of the NS3/4A and NS4B/5A junctions. Whereas the N-terminal domain of NS3 contains the proteolytic domain, its C-terminal contains an RNA helicase that may function during viral replication. NS4B is an integral membrane protein that alters membrane structure and contains a guanosine triphosphate–binding domain essential for HCV replication.[55] Its function may be related to the observation that HCV replicates in a subcellular structure termed the *membranous web*. NS5A is a multifunctional protein with key roles in modulating viral replication and altering the intracellular milieu in response to viral infection.[130] NS5A is localized to the membranous web of infected cells, and mutations in NS5A are found in culture-adapted strains. NS5A is found in single- and multiple-phosphorylated forms. Adaptive mutations that favor increased viral replication in vitro are associated with a decrease in hyperphosphorylated NS5A. Despite these observations, the exact biochemical function of NS5A remains unknown.

Some investigators have designated amino acid residues 2209 to 2248 of the NS5A protein the "interferon sensitivity determining region" (ISDR) because mutations within the region are

thought to be associated with variations in responsiveness to interferon (IFN) therapy.[56,143,196,200] However, this designation remains somewhat controversial.[148,170] Additional variables such as virus genotype (see later) and synergistic mutations (in the NS2 gene) may play a role in modulating IFN responsiveness.

The NS5B protein is the RNA-dependent RNA polymerase thought to be the replicative polymerase for HCV.[121] The enzyme lacks proofreading activity, and this characteristic may account for the high error rate and consequent sequence divergence.[58,149] NS5B activity requires a primer for initiation of polymerization, which is thought to occur in vivo by self priming at the 3′ terminal NTR hairpin.[17] The crystal structure of the NS5B apoenzyme reveals a globular shape unique among polymerases and implicates new structural features important for binding the RNA template and cognate ribonucleotide substrates.[128] These crystallographic results also provide a structure-based framework for biochemical analyses and drug design efforts. In vitro inhibitors of HCV RNA-dependent RNA polymerase have been reported.[2] Yet to be elucidated are the exact steps by which the polymerase replicates the HCV genome, such as in vivo primer requirements, replicative intermediates, and post-replication processing, all of which may provide targets for antiviral agents.[123]

HCV exhibits extensive genetic heterogeneity. Isolates from around the world have been divided into 6 major genotypes, designated 1 through 6, and more than 100 subtypes. The genomes of the most divergent HCV isolates differ by up to 35 percent. The impact of HCV genotypes on clinical factors, such as viremia level and severity of liver disease, remains controversial. However, clear differences in response to antiviral therapy have been demonstrated, with improved response to treatment correlating with genotypes 2 and 3 versus genotype 1 or genotype 4.[76,80,102,132,137,193] Within infected individuals, HCV circulates as quasispecies, a mixture of closely related but distinct genomes. Viral genomes of quasispecies typically differ by 1 to 2 percent. In an infected person, quasispecies may either be present from the onset, the product of simultaneous transmission, or develop over the course of time as a result of accumulations of mutations. Such mutations may enable more efficient HCV replication or evasion of host immune responses. Certain regions of the genome are hypervariable and are responsible for most, but not all, of the genomic differences in quasispecies. HVR1 is found at the N-terminus of the envelope E2 protein, at amino acids 384 to 410. This site probably is on the surface of the folded envelope protein and represents a neutralization epitope for humoral immunity. Although the mutation rate at this site is rapid, it may be even further accelerated in patients subjected to immunostimulation. Conversely, the rate is decreased in individuals with agammaglobulinemia. Appearance of antibodies against HVR1 in infected subjects is followed by emergence of new variants in the region. For these reasons, HVR1 is thought to play a role in HCV persistence and chronic infection. HVR2 is a second hypervariable region in the E2 protein, identified in genotype 1b isolates.

REPLICATION CYCLE

Hepatocytes are the primary site of viral replication. HCV entry into the cell is mediated by a specific interaction between viral envelope proteins and a host-cell surface receptor, and this interaction may underlie host specificity and cellular tropism. The HCV envelope protein E2 has been shown to bind the host-cell surface protein CD81,[157] and using an in vitro cell fusion assay, researchers demonstrated that both E1 and E2 proteins are required for fusion.[183] Additional host proteins other than CD81, such as the low-density lipoprotein receptor, may be required for viral entry.[174] Evidence from studies of other *Flaviviruses* support a model in which entry through receptor-mediated endocytosis is followed by envelope fusion with the endosomal membrane to release the nucleocapsid into the cytoplasm. There, ribosome binding to the viral genome enables translation of the encoded polyprotein, with the formation of a replicative ribonucleoprotein complex. The resulting negative-strand intermediate then serves as a template for the production of positive-strand RNA. Virion assembly begins with interaction between the noncoding region of the RNA genome and the capsid proteins to assemble the nucleocapsid. As with other *Flaviviruses*, budding of virus likely occurs into intracellular vesicles, which release free virus from the cell by exocytosis. How HCV acquires its envelope or specifically excludes cellular proteins and RNAs during virion assembly is not known.

IMMUNOPATHOGENESIS

HCV persists in approximately two thirds of those it infects, and the outcome (clearance versus persistent infection) is determined within 6 months of exposure to the virus. Which path an infection takes depends on the effectiveness of the adaptive immune response. As described later, cell-mediated immunity seems to play a critical role in resolving the infection, whereas the role of humoral immunity is less well understood. Antibodies to several HCV proteins can be detected by 7 to 30 weeks after infection is established. However, the extraordinarily high rates of chronic disease and persistent viremia observed in humans suggest that HCV fails to induce an effective neutralizing antibody response. After a primary HCV infection, repeat challenge of convalescent chimpanzees with the same or different strains of HCV results in the reappearance of viremia, caused by infection with the subsequent challenge virus.[60] This reappearance of viremia is associated with mild elevations of alanine aminotransferase (ALT) and histopathologic signs of acute hepatitis. Conversely, resolution of HCV can occur in the absence of anti-HCV antibodies in chimpanzees.[47] Although antibodies to the E2 region of the HCV genome have been identified as potential neutralizing antibodies, their effectiveness is extremely limited, and their role in protection has not been demonstrated.[61,176] Individuals with acute, self-limited HCV infection have early, vigorous responses from both T-helper lymphocytes ($CD4^+$) and cytotoxic T cells ($CD8^+$).[47,119] The sequences that are recognized by HCV-specific T-helper cells are immunodominant (the NS3 protein in particular) and conserved among HCV genotypes.[52] Individuals with various major histocompatibility class (MHC) haplotypes demonstrate efficient presentation and recognition of a set of common viral epitopes.[114] These findings suggest that these antigens may be important factors in development of immune reactivity and possibly may be considered in vaccine development. HCV-specific T-helper ($CD4^+$) and T-suppressor lymphocytes become detectable in blood 3 to 4 weeks after infection occurs. T-cell infiltration of the liver correlates with increase in ALT activity because cytotoxic lymphocytes lyse HCV-infected cells. Effector functions of the $CD4^+$ and $CD8^+$ cells include synthesis of a series of proinflammatory and anti-inflammatory cytokines. After a patient recovers from HCV infection, circulating HCV-specific T-helper and cytotoxic lymphocytes may be present for decades, even when the humoral response declines and HCV antibodies become undetectable.[182]

Although the exact mechanism for viral persistence and the frequent development of chronic infection is not known, HCV-specific cytotoxic lymphocytes are found at only very low levels in the blood of chronically infected individuals.[79] In contrast, in one report, patients with resolved infection had cytotoxic lymphocytes directed against a broader distribution of epitopes, and the cells exhibited stronger responses to stimulation.[118] Consistently, patients with stronger polyclonal cytotoxic lymphocyte responses in blood and liver have lower levels of HCV viremia.[79] Both HCV-specific and nonspecific $CD8^+$ T cells are found in

the liver of infected persons; immune-mediated liver disease is thought to be initiated by the HCV-specific cells but amplified by the nonspecific cytotoxic cells.

Several mechanisms have been postulated to explain HCV persistence.[30] They include escape of innate immune response by up-regulation of MHC expression on infected cells, viral sequence variations and mutations that eliminate humoral and cellular target epitopes, and lack of susceptibility of HCV to T-cell cytokines. Viral variant sequences are seen more commonly in the presence of cytotoxic lymphocytes than in the absence of these cells.[39] HCV may actively interfere with the host immune response; inhibition of activation of the IFN-inducible protein kinase has been demonstrated by sequences in the HCV E2 and NS5A proteins.[111,186] The cellular immune defect is HCV-specific and does not seem to affect the response to other infectious agents.

EPIDEMIOLOGY

An estimated 3 percent (170 million) of the world's population has been infected with HCV.[10] It is the most common cause of non-A, non-B hepatitis. In a report from the United States, the prevalence of anti-HCV positivity was 1.6 percent (4.1 million persons), and of these, approximately 80 percent tested positive for HCV RNA. This percentage translates to 3.2 million persons chronically infected with HCV.[14] Approximately 28,000 new infections occur each year.[4] This figure represents a substantial decline in incidence starting in the 1990s[13] (Fig. 189–2), when the first-generation anti-HCV test was used for screening donated blood.

Young adults are at highest risk for HCV acquisition because they are most likely to engage in high-risk behaviors. As risk of HCV from transfusion has diminished, the proportion of cases associated with intravenous drug abuse has increased rapidly, up to more than 40 percent. Heterosexual contact is reported in 6 percent of infected individuals, household contact in 3 percent, and occupational exposure in only 2 percent. In those with repeated percutaneous exposures, such as injecting drug users, the prevalence is 60 to 90 percent, whereas in health care workers, it is 1 percent. Volunteer blood donors, who represent the lowest-risk group, have a seroprevalence of less than 0.5 percent. In several early studies, the proportion of cases of HCV infection with no identifiable risk factor had been consistently 35 to 40 percent.[5] However, although high-risk behavior may not be documented within months of establishing an HCV diagnosis, a history of more remote risk factors can be obtained in most of these cases. Thus, more than 90 percent of new cases can be attributed to parenteral or sexual behaviors.[4]

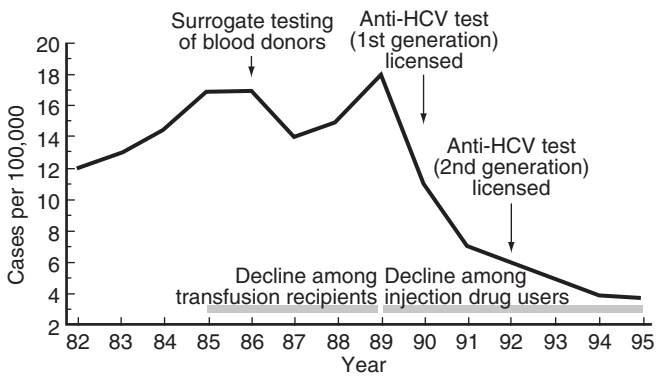

Figure 189–2 Estimated incidence of acute hepatitis C in the United States from 1982 to 1995. The incidence did not change substantially from 1995 to 1999. HCV, hepatitis C virus.

In the United States, a wide variation in seroprevalence exists among different subgroups.[14] The male population is twice as likely to have anti-HCV antibody as is the female (2.1% versus 1.1% prevalence). Among persons with a history of injection drug use, the seroprevalence is 57.5 percent. A positive correlation exists between HCV exposure and lifetime number of sexual partners, ranging from 0.5 to 12 percent seroprevalence in those having no sexual partner or a single sexual partner versus more than 50 partners, respectively. The peak prevalence with regard to age remains among those born between 1945 and 1964.

The distribution of the different genotypes of HCV varies geographically.[53,140] Genotypes 1 and 2 are distributed widely throughout the world. Genotype 1 is predominant in North and South America, Europe, and Asia, although the relative frequencies of the different subtypes vary. Genotypes 1a and 1b both are common in the first three continents, but only 1b is predominant in Asia. In the United States, genotypes 1a and 1b each account for more than 40 percent of isolates. Type 3 also is distributed widely, and subtype 3a has been seen with increasing frequency among persons who acquired their infection recently. However, subtype 3b has been identified only in Japan, Nepal, Thailand, India, Bangladesh, and Indonesia. Type 4 is predominant in northern and central African countries, and type 5 occurs in southern Africa. Type 6 has been found in 20 to 30 percent of isolates in Hong Kong and Vietnam.

INCIDENCE AND PREVALENCE IN CHILDREN

Infection with HCV occurs throughout the world, in children as well as in adults. The prevalence in pediatric groups varies both by risk factors and geographic location. No large seroprevalence studies have been done in the general pediatric population in the United States, but one survey in an adolescent population revealed very low seroprevalence.[98] Data from other countries disclose widely varying frequencies of HCV infection. A Japanese study of 1442 healthy children found a prevalence of 0 percent anti-HCV positivity.[184] At the other end of the spectrum, 14.5 percent of 696 randomly sampled children in Cameroon were anti-HCV seropositive.[146] Studies looking at household contacts of known HCV-infected individuals found intermediate seroprevalence rates, which increased proportionally to the age of the contact,[1,159,166] a finding indicating that duration of contact was an important risk factor. Mechanism of transmission in these settings has not been defined clearly, but the association with low socioeconomic status indicates the role of crowding and hygiene.

In the United States, the HCV antibody seroprevalence is 0.2 percent in children younger than 12 years old and 0.4 percent in those aged 12 to 19 years.[7] Children at risk for acquiring HCV infection are listed in Table 189–1. Before 1990, the predominant risk for acquisition of HCV infection by children was by blood or blood product transfusion. Although this mode of transmission is responsible for many current cases of pediatric HCV infection, new infections in children are caused primarily by perinatal (vertical) transmission, as was demonstrated in Italy.[26] The incidence of new infections in children through this mechanism is not known, but it could be estimated from the prevalence of HCV infection in women of child-bearing age and the risk of transmission with each pregnancy.

TRANSFUSION-ASSOCIATED INFECTION IN CHILDREN

Until screening became routine in 1992, receipt of blood or blood products had been the major mode of transmission of HCV to children.[24,147] In addition to erythrocyte and platelet transfusions, implicated products included clotting factors,[21,103] plasma,

TABLE 189–1 Children at Risk for Hepatitis C Virus Infection

Children Repeatedly Transfused with Blood or Blood Products for
Thalassemia
Sickle cell anemia
Other congenital anemias
Hemophilia
Hemodialysis
Hypogammaglobulinemia or other immunodeficiency (intravenous immunoglobulin)
Children with a History of Transfusion before 1992 for
Childhood malignant disease, especially leukemia
Major surgery: cardiac, orthopedic
Prematurity, neonatal intensive care
Conditions requiring extracorporeal membrane oxygenation
Adolescents with the Following High-Risk Behaviors
Intravenous drug use
Intranasal drug use
Body piercing or tattooing
Infants of Hepatitis C Virus–Infected Mothers

and intravenous immunoglobulin.[32] Transmission to children by transplanted organs or tissues also has been demonstrated.[73,155] In general, as described for adults, the risk of acquisition of HCV increases with the number or units of blood or blood products received.[99,105,145] Children from all parts of the world who have been transfused multiple times with either blood or acellular blood products, such as patients with thalassemia[113,164] or hemophilia,[21] have infection rates from 50 to 95 percent. Children with moderate but not ongoing exposure to transfusions, such as those who have been treated for childhood malignant diseases,[125,168] those who have been treated with hemodialysis[73,99] or extracorporeal membrane oxygenation,[145] or those who have undergone surgery for congenital heart disease,[147] have intermediate seroprevalence rates of 10 to 20 percent. Screening of donated blood for anti-HCV, the use of recombinant and heat-inactivated clotting factors, and the addition of virus-inactivating physicochemical processes in the production of immunoglobulin have reduced the incidence of HCV transmission drastically. However, these advances are relatively recent; many children infected with HCV through blood products are followed in clinical practices, and others are yet to be identified.

VERTICAL TRANSMISSION

Vertical transmission of HCV currently is the principal route of HCV infection in children. Both intrauterine and perinatal routes appear to be important. Since the mid-1990s, numerous studies have sought to identify the factors that could correlate with the rate of HCV transmission. The most important principle to emerge from these studies is that vertical transmission occurs almost exclusively when mothers have detectable HCV RNA. In the absence of HIV co-infection, the likelihood of HCV transmission increases with higher levels of maternal HCV viremia. In several studies, no women with fewer than 10^6 copies/mL of serum HCV RNA transmitted the infection to their newborns,[69,72,122,150] although such was not the case in one report from Italy.[163] Studies in the United States and Europe suggested a 4 to 6 percent perinatal transmission rate from viremic mothers.[46,57,70,135] This rate compares to a frequency of 40 to 90 percent for the perinatal transmission of hepatitis B virus. The frequency of anti-HCV seropositivity in newborns of HCV-infected women was shown to be 14 to 100 percent; in most instances, this antibody was present only transiently, a finding indicating that it had been acquired passively through placental transfer.[62,69,162,188]

Maternal co-infection with HIV increases the rate of perinatal HCV transmission four- to fivefold,[167] even without concomitant transmission of HIV. However, aggressive treatment of HIV-infected pregnant women that results in low or undetectable HIV levels at delivery may mitigate this increase in transmission of HCV.[46]

The question remains open as to when vertical transmission of HCV occurs. The possibility of in utero transmission in at least some cases was suggested by the detection of viremia in six infants on the day of birth.[163] However, in a prospective study, infection documented at birth by detection of HCV RNA in cord blood often was transient and not always predictive of eventual newborn infection.[46] A European study tested 54 HCV-infected children within 3 days of birth and noted that 17 (31%) of those infants infected with HCV were positive for HCV RNA, a finding suggesting in utero transmission.[142] If infection is not detected at birth, as is most often the case,[46,70] transmission around the time of delivery may be more important. The role of peripartum factors, such as prolonged rupture of amniotic membranes (≥6 hours) and the use of scalp monitors, has been examined, and there appears to be a correlation between these variables and increased risk of HCV transmission to neonates.[70,72,135,136] Cases of HCV transmission after cesarean births have been reported.[153] In one report, transmission frequency after vaginal delivery was 4 percent, and after cesarean delivery it was 6 percent.[163] When this topic was examined by taking into account the duration of ruptured membranes and comparing emergency with elective cesarean deliveries, once again the likelihood of transmission seemed to correlate with this factor rather than with the mode of delivery.[70] HCV transmission has been documented in women who have acute infection during the last trimester of pregnancy.[131] As noted earlier, in some instances, viremia in the neonates was transient and was not associated with the development of liver disease.[69]

Despite the detection of HCV in breast milk and colostrum,[142,169] breast-feeding does not appear to affect the rate of vertical transmission of HCV.[78] However, breast-fed babies of mothers co-infected with HIV have an increased risk of HCV transmission.[57] For example, none of 17 breast-fed infants born to HCV-positive, human immunodeficiency virus (HIV)–negative mothers was infected after follow-up of 12 to 27 months.[133] In another study, the perinatal transmission of HCV in breast-fed infants was 7 percent compared with 4 percent in formula-fed infants, but this difference was not statistically significant.[163] None of 76 breast milk samples from 73 anti-HCV positive German women contained HCV RNA, even though 62 of these women were HCV RNA seropositive.[158] Only 1 child had evidence of HCV infection detected 1 month after birth. Based on these data, currently there is no recommendation to avoid breast-feeding by HCV-infected women *unless* co-infection with HIV is present.

INTRAFAMILIAL AND OTHER HORIZONTAL TRANSMISSION

Horizontal transmission refers to transmission to children after the perinatal period or to transmission from infected children to others, such as family members or schoolmates. Studies of household transmission from Europe, South America, and Asia have been accomplished by screening household contacts of index cases for anti-HCV positivity; in some instances, further testing of seropositive contacts with polymerase chain reaction (PCR) for HCV RNA and even comparative genotype analysis[85] was done. In these studies, prevalence of anti-HCV seropositivity in household contacts varied from 0 to 14.8 percent.[1,12,35,40,49,144,159,181] Non-spouse seroprevalence rates were 0 to 6.5 percent. Risk of anti-HCV positivity increased with age or duration of exposure, or both.[40,159] Rates were particularly low in households of HCV-

infected patients with hemophilia.[31] The risk to children in the households of chronically HCV-infected individuals separate from perinatal risk was difficult to ascertain, but prevalence rates were very low (approaching zero) in the youngest children and increased steadily with increasing age.[1,50,144] Because the mechanism of nonsexual, nonperinatal infection of children in these families is not known, counseling to prevent this transmission is limited to avoidance of sharing household items such as razors, toothbrushes, and fingernail clippers. No justification exists at this time to have family members avoid sharing of eating utensils or bathrooms. HCV-infected adults should be educated about the extremely low likelihood of spreading HCV to their children by routine family contact, including kissing and day-to-day care.

Few data exist regarding the transmission of HCV from infected children to others. In a Spanish study, 80 household contacts (without independent risk factors for infection) of 27 HCV-infected children were tested. None of the parents was found to be infected, but an infected sibling (1/32) was identified.[36] In an Italian study of 44 index children, 1 parent was infected through an accidental needle stick, and no transmission to other children was demonstrated.[191] Thus, it appears that horizontal transmission of HCV between children is quite rare. There is no need to restrict school or daycare attendance, or participation in any routine activity including contact sports, for HCV-infected children.[9] In addition, notification of school personnel of a child's HCV infection is not required because routine universal precautions already recommended are adequate for children with HCV.[8]

OTHER MODES OF TRANSMISSION IN CHILDREN

The most frequent mode of transmission of HCV in adults is sharing of needles for the purpose of intravenous illicit drug injection. To the extent that older children and teenagers participate in this activity, they are at risk for acquiring infection. In fact, HCV infection occurs within 6 to 12 months after beginning injection drug use in most individuals.[67] Other percutaneous exposures that may be more common than is intravenous drug use in children and adolescents, such as body piercing and tattooing, have been implicated as risk factors in Italy,[139] but not in the United States.[45] Studies to evaluate these risks in the United States are ongoing. Intranasal administration of cocaine is considered a risk factor.[7] Sexual transmission of HCV is thought to occur by both homosexual and heterosexual activity,[6,187] but its importance to the overall prevalence of HCV infection is controversial.[151] The prevalence and causes of sporadic or community-acquired HCV infection in children, in the absence of a risk factor, are not known.

DIAGNOSIS

The use and significance of methods for diagnosing hepatitis C infection are summarized in Table 189–2 and Figure 189–3. Direct tests for the viral antigens of HCV in serum are not available. Assays for antibodies directed against several viral antigens have been facilitated by the genomic sequencing of HCV. Currently, the third-generation enzyme-linked immunosorbent assay (ELISA)-3 is the most widely used. It is based on several antigens from both the core (c22-3 antigen) and nonstructural (c200 antigen) regions, the latter including the c100-3 antigen used previously. Seropositivity for anti-HCV by ELISA indicates current or past infection with HCV. A nonreactive test result does not completely exclude either current or past infection because levels of anti-HCV positivity may be undetectable in early infection or may remain undetectable in individuals with altered immunity.

TABLE 189–2 Serologic Diagnosis of Hepatitis C Virus Infection

	Acute HCV	Resolved HCV	Chronic HCV
Anti-HCV ELISA-3	±	±	+*†
Anti-HCV IgM (experimental)	+	–	–
HCV RNA by PCR	+	–	+

**Should be confirmed with another test.*
†May be negative in immunosuppressed individuals.
ELISA, enzyme-linked immunosorbent assay; HCV, hepatitis C virus; IgM, immunoglobulin M; PCR, polymerase chain reaction.

Early problems with sensitivity and specificity of ELISA led to the development of a recombinant immunoblot assay (RIBA) for antibodies to the c100-3 antigen, as well as another viral antigen, 5-1-1, and superoxide dismutase.[54] Further refinement of this test led to the development of the RIBA-2, which incorporates recombinant antigens c33-C and c22-3.[190] RIBA has been replaced largely by PCR-based tests (see later).

Detection of HCV genomes in serum and tissue through the use of PCR currently is the principal method used to detect active infection. HCV RNA can be quantified using a variety of methods that differ in sensitivity, linear range, precision, and reproducibility. The most widely used quantitative PCR test can detect levels of virus down to 100 copies/mL of serum. A less cumbersome assay that uses branched DNA and alkaline phosphatase–labeled probes to detect and quantify viral nucleic acid has been applied to the detection and quantification of HCV RNA[117] (Quantiplex HCV-RNA Assay, Chiron Diagnostics, Emeryville, CA). The Quantiplex bDNA 2.0 test is as sensitive as are some quantitative PCR tests, and it has great accuracy at high viremia levels.[91] Real-time PCR based on the TaqMan chemistry system (Roche Molecular Diagnostic Systems, Indianapolis, IN) has increased sensitivity to less than 100 copies/mL, as well as high precision, good reproducibility, and a broad linear range. At this point, caution must be used because the values obtained by the different tests are not interchangeable. However, changes in viral load over the course of time using the same assay are meaningful. The adoption of the standardized measure of international units (IUs) has mitigated interassay variability.

In summary, the diagnosis of HCV infection in an individual with chronic hepatitis, defined as infection persisting for more than 6 months, includes the detection of anti-HCV positivity in serum; confirmation with a determination of HCV RNA should be determined by a qualitative PCR assay. The diagnosis of acute HCV infection is more problematic because anti-HCV positivity may not appear in serum for up to 2 months. In addition, immunocompromised individuals, including patients with hypogammaglobulinemia and immunosuppressed transplant recipients, may not mount an anti-HCV response. For these latter situations, the diagnosis is made by detection of HCV RNA in serum by either the PCR or branched DNA amplification technique. Quantitative HCV RNA testing is reserved for determination of therapeutic response.

CLINICAL FEATURES

In adults, acute HCV infection typically is mild and most often subclinical. There are no reports of the clinical manifestations of acute HCV infection in children infected either perinatally or through blood transfusion. Acute, severe hepatitis, with atypically high ALT values, jaundice, malaise, anorexia, and hepatomegaly, was seen in some of the children who were infected during the outbreak associated with intravenous immunoglobulin.[96] The reasons for this unusually severe presentation are not clear, but possibilities include greater amounts of virus inoculated, repeated

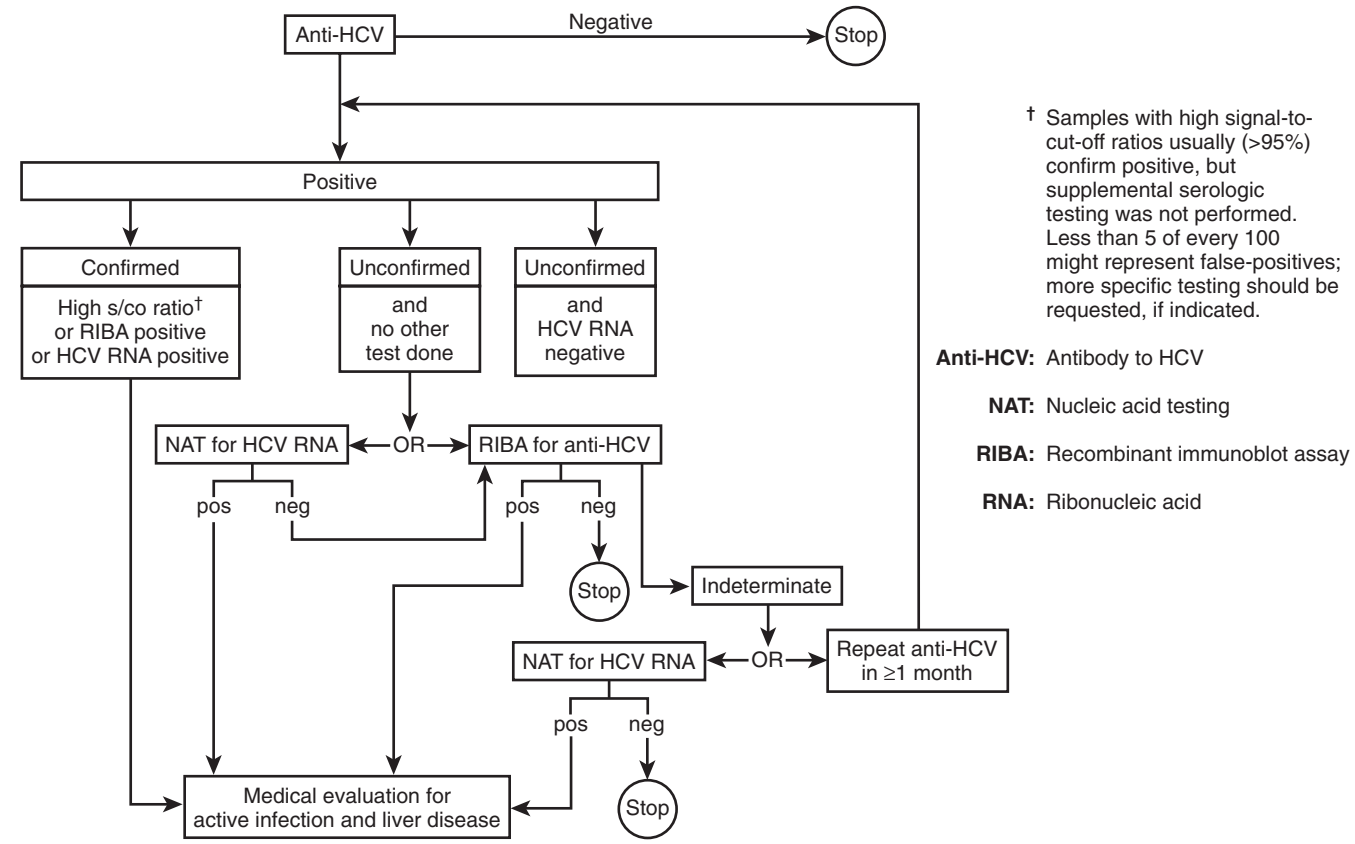

Figure 189–3 General algorithm for diagnosing chronic hepatitis C in children who are more than 1 year of age and in adults. ALT, alanine aminotransferase; EIA, enzyme immunoassay; HCV, hepatitis C virus; RIBA, recombinant immunoblot assay. *(From the Centers for Disease Control and Prevention: Available at http://www.cdc.gov/hepatitis)*

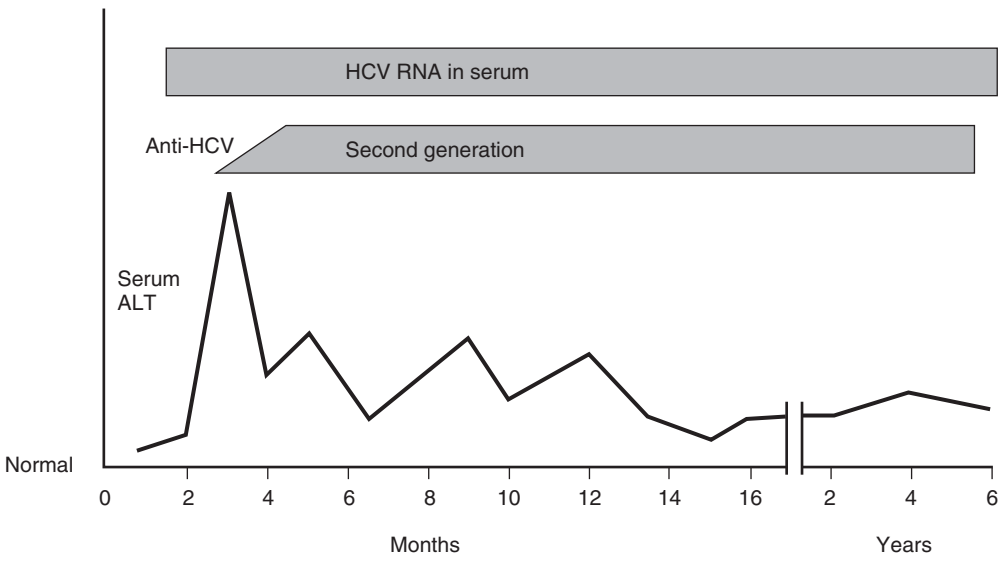

Figure 189–4 Integrated time course showing natural history of serum alanine aminotransferase (ALT), hepatitis C virus (HCV) antibody, and HCV RNA in chronic infection.

inoculations, or the underlying immunodeficiencies in the hosts. Individuals who have clinically apparent acute hepatitis C with symptoms and jaundice have a higher likelihood of having acute, self-limited infection. No reports focus specifically on clinical features of chronic HCV infection in children. Studies of epidemiology, natural history, and treatment often describe the children as being asymptomatic or having nonspecific fatigue or abdominal pain, or both.[24,25,28,29,88,97,125,147] Most cases are identified by testing asymptomatic children who are recognized to have a risk factor for acquisition of HCV infection, such as receipt of transfusion before 1992 or an HCV-infected mother. Once infection is identified and serial ALT values are followed, most children are found to have modest elevations in the first years of infection, with normal or near-normal values for many subsequent years (Fig. 189–4). Non–organ-specific autoanti-bodies sometimes are found: antinuclear antibodies have been described in 7.5 to 11 percent, smooth muscle antibodies in 9 to 17.5 percent, and type 1 liver-kidney microsomal antibodies in 10 to 13 percent of European cases.[27,116] However, clinically apparent autoimmune manifestations are rare. One of us cared for a 13-year-old girl with HCV-associated cirrhosis who also had membranoproliferative glomerulonephritis with nephrotic syndrome,

a 6-year-old boy with chronic HCV and lichen planus, and a 17-year-old girl with HCV infection and cutaneous vasculitis that disappeared with antiviral therapy (unpublished data). HCV-associated cryoglobulinemia and porphyria cutanea tarda have not been reported in children. This low frequency of clinical signs or symptoms and the fact that "routine" serum ALT determinations are not done as part of pediatric medical care indicate that chronic HCV infection in children probably is underrecognized.

NATURAL HISTORY

Studies of the natural history of HCV infection in children are fewer and less definitive than are those in adults. Factors that influence the natural history of HCV infection in adults include age at infection, mode of acquisition, co-morbid disease, ethanol ingestion, and obesity. In children, age and mode of acquisition often are difficult to separate because most infections acquired in infancy are perinatal. Children treated for leukemia typically receive many blood transfusions, and such patients treated before 1990 have a very high rate of HCV infection.[11] In one cohort, prolonged follow-up (13 to 27 years) did not reveal serious liver disease.[126]

In contrast, an American study of individuals treated for childhood cancer revealed one death from liver disease and two deaths from hepatocellular carcinoma in the decades following HCV acquisition.[180] The same report described three (9%) cases of cirrhosis that developed 9 to 27 years after the diagnosis of the primary malignancy was established. Clearly, some cases of HCV infection acquired in childhood by transfusion are associated with serious liver disease in the decades following infection.

Children with thalassemia requiring chronic transfusions have a very high prevalence of HCV infection. Many individuals with hemophilia contracted HCV during childhood, before the availability of heat-inactivated and recombinant clotting factors. The natural history of transfusion-associated HCV infection may differ according to the underlying disease for which the transfusion is required. Secondary hemochromatosis in thalassemic patients may contribute to the hepatic injury as well as affect the response to therapy for HCV infection.[43,113]

Two studies addressed the natural history of HCV infection acquired early in life at the time of surgery for congenital heart disease. In a Japanese study, 29 children were followed for at least 4 years, and only 50 percent had persistent viremia. Although these children had histologic chronic hepatitis, none had cirrhosis in this time period.[138] A more recent study from Germany reported similar findings after a longer follow-up period. In that study, 14.6 percent of children who were transfused at the time of cardiac surgery were positive for anti-HCV 20 years later, but only 55 percent of these subjects were viremic at follow-up.[192] Only one of these HCV RNA–positive patients had an abnormal ALT value, which possibly was explained by the fact that the patient also was in congestive heart failure. Only 17 of the children underwent liver biopsy, and 1 child (5.9%), who had been co-infected previously with hepatitis B, had cirrhosis. Overall, the clinical course of these children seemed more benign than would be expected if they had been infected as adults. The frequency of viral persistence is lower than that reported in adults who were infected through transfusion. The reason for this relatively high spontaneous clearance rate is not clear. Conversely, few of the patients underwent liver biopsy, so the frequency of occult chronic liver disease is not known.

Understanding the natural history of perinatally acquired HCV infection has become increasingly important given that perinatal acquisition has become the major route of infection for pediatric patients. Because the chronic liver damage is thought to result largely from the immune response to the infection rather than to direct cytotoxicity, the immaturity of the host immune system at the time of infection conceivably could alter the course of the disease as compared with adults. Initially, several small reports from Japan and Italy were published regarding this issue.[25,154,171] More recently, larger patient series provided a broader perspective on chronic, perinatally acquired HCV. Infection acquired vertically frequently is associated with biochemical evidence of hepatic injury early in life. Spontaneous resolution of infection in some of these infants, even those with active hepatitis as suggested by abnormal ALT values, can occur, but in most children, perinatally acquired HCV follows a persistent course. In most cases, it causes only mild chronic hepatitis in the first 1 to 2 decades, and the degree of fibrosis correlates with the duration of infection. Cirrhosis can occur within a decade of acquisition of infection, but this outcome is much less common. A report of HCV 35 years after the initial acquisition of perinatal infection[38] described a more benign course than the other reports referenced earlier, with a higher spontaneous resolution rate and milder liver disease. A conclusion to be drawn from these studies is that the long-term management of chronic HCV in children will need to take into account the cumulative effects of the infection over the course of decades of life. Although the incremental progression of the disease may be slow and insidious, the long-term progression of fibrosis ultimately may lead to end-stage liver disease in a substantial number of middle-aged individuals infected when they were neonates. Balancing treatment side effects (see later) against the possibility of progression of disease thus can be difficult because of the high variability among individuals. The aggregate cost of medical care associated with HCV in children has been addressed.[94] Using epidemiologic data, estimates of treatment efficacy (sustained virologic response [SVR]), and severe complications (end-stage liver disease requiring transplant), the authors of this article projected the costs directly stemming from pediatric HCV infection during the next decade to approximately 250 to 350 million U.S. dollars. Approximately two thirds of this cost consists of monitoring, and one third consists of treatment costs. Childhood HCV may, therefore, exact a significant financial burden in the coming years.

HISTOPATHOLOGIC FEATURES OF THE LIVER IN HEPATITIS C VIRUS INFECTION

Histologic features of the liver associated with HCV infection in adults have been described in several series.[19,108,120,172] The major features are portal inflammation with lymphoid aggregates, varying degrees of steatosis, and bile duct injury (Fig. 189–5). In HCV-infected adults, severity of histologic abnormalities does not correlate with biochemical parameters of hepatic dysfunction, such as ALT levels. The implication is that patients may have progressive liver disease in the absence of clinical signs. Thus, histopathologic examination of the liver is an important tool in the understanding of childhood HCV infection.

Although histologic features in small numbers of HCV-infected children were described in several reports,[24,90,113,138] most cases were not systematically examined and scored by conventionally accepted scoring systems.[19,107,172] Varying degrees of necroinflammatory activity were noted, but fibrosis was not described uniformly; cirrhosis was reported in 0 to 11 percent of cases. Three articles focused primarily on histologic findings, with scoring, in large series of children.[15,75,100] In all series, the characteristic histopathologic lesions of HCV infection (see Fig. 189–5), including portal lymphoid aggregates or follicles, steatosis, sinusoidal lymphocytes, and steatosis, were seen with approximately the same frequency as in adults. In 109 Japanese children primarily infected through transfusion,[100] the average histologic activity was 3.8 by the Scheuer system.[172] No cases of cirrhosis were encountered, and only 3.6 percent of the children had bridging fibrosis with architectural distortion. Viral genotypes

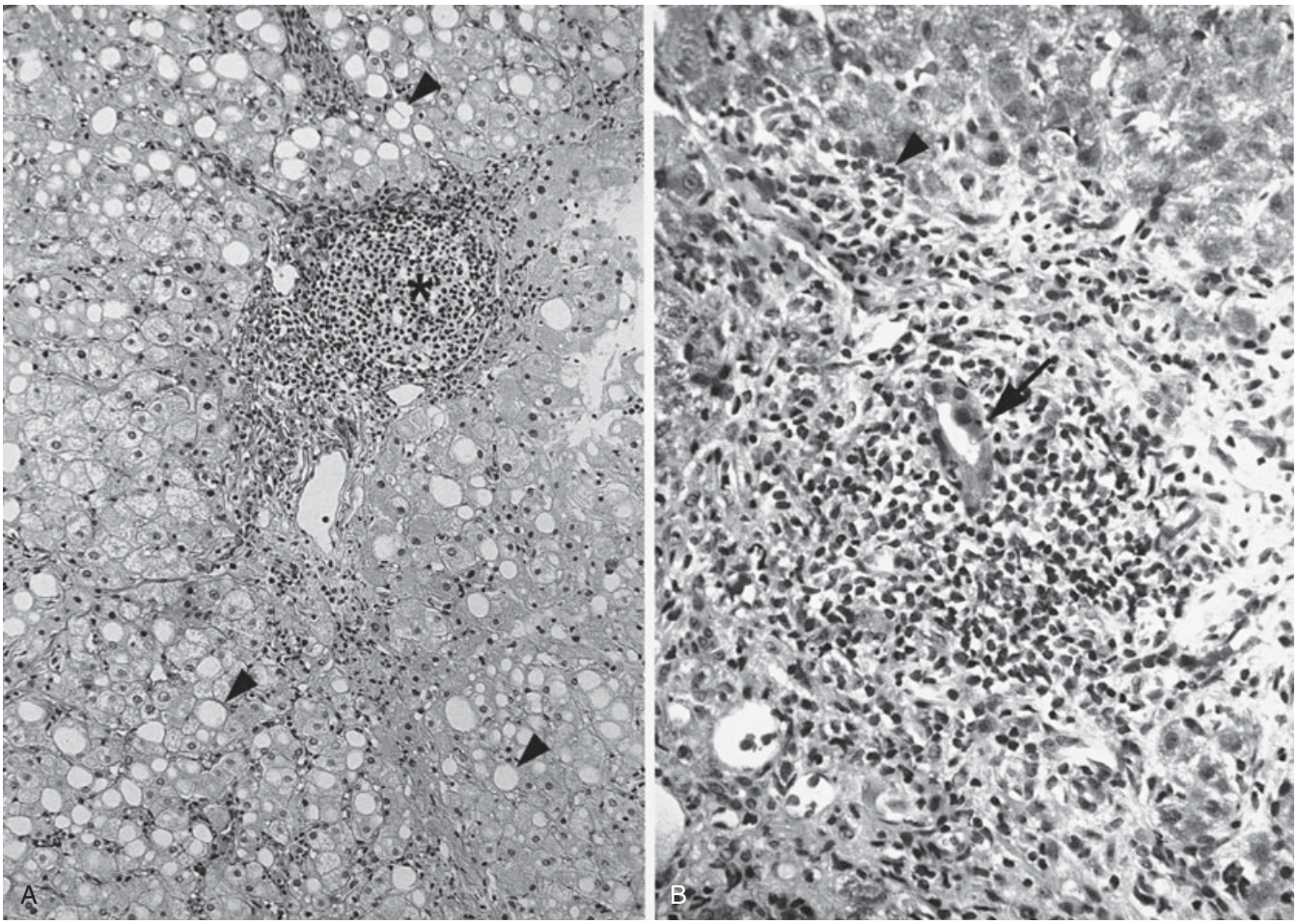

Figure 189–5 Photomicrograph of the liver in pediatric patients with chronic hepatitis C. **A** demonstrates portal lymphoid aggregate *(asterisk)* and moderate steatosis *(arrowheads)* (hematoxylin and eosin stain). **B** depicts bile duct injury *(arrow)* and interface hepatitis *(arrowhead)* (trichrome stain). These features are typical of chronic hepatitis C virus infection in children and adults.

were not reported, and the mean duration of infection was only 2.6 years. In contrast, although histologic activity (Scheuer[172] and METAVIR[19] schemes) generally was mild in a series of children in the United States, portal fibrosis was much more frequent, seen in 78 percent of specimens from 40 children.[15] Fibrosis was mild in 26 percent, moderate in 22 percent, and severe in 22 percent, and cirrhosis was found in 8 percent. Two of the children with cirrhosis were young adolescents who had acquired HCV infection perinatally. A newly described finding was pericellular fibrosis, typically around the central veins, in 52 percent of specimens; whether this abnormality is unique to pediatric HCV infection has yet to be determined, and this fibrosis was not included in the histologic grading. In this series, 60 percent of children had HCV of genotype 1a, and 32 percent had genotype 1b. The mean duration of infection, in those children in whom it could be accurately determined, was 6.8 ± 5.3 years. In a series of 80 children from Italy and Spain,[75] most of whom were infected with HCV genotype 1a or 1b, with a mean duration of infection of 3.5 ± 4.3 years, inflammatory scores generally were quite low. The frequency and severity of the bile duct damage and lymphoid follicles increased with the age of the patients. Fibrosis was present in 72.5 percent of cases and increased with duration of disease and age, just as in the American series. Only one (1.25%) child had cirrhosis.

These studies demonstrate that histologic features of chronic HCV infection in children are quite similar to those reported in adults. Necrosis and inflammation usually are mild, but fibrosis is seen commonly and, most importantly, progresses with increasing age and duration of infection. Thus, the natural history of HCV infection acquired in childhood may in some instances be associated with significant morbidity as the children progress into young adulthood.

TABLE 189–3 Guidelines for Prevention of Perinatal Hepatitis C Virus Infection

Recommended (Supported by Current Data)
Targeted testing of pregnant women who have risk factors for HCV
Aggressive treatment of HIV in co-infected pregnant women
Testing of infants for anti-HCV when they are 12 to 15 months of age
Considered (Suggested by Current Data)
Avoidance of internal fetal scalp monitoring during labor
Delivery of infant within 6 hours of rupture of membranes
Not Recommended (No Current Data to Support)
Universal testing of pregnant women
Elective cesarean delivery
Avoidance of breast-feeding
Immunoglobulin administration to newborns

HCV, hepatitis C virus; HIV, human immunodeficiency virus.

MANAGEMENT

Current recommendations regarding perinatal HCV transmission are listed in Table 189–3. Because the rate of perinatal

transmission of HCV is low, the risk factors that increase this rate are not fully defined, and because intervention for the neonate does not exist at this time, routine screening of all pregnant women is not warranted. Pregnant women, or those considering pregnancy, who have risk factors such as intravenous drug use, blood transfusions before 1992, or unexplained ALT elevations, should be offered screening for anti-HCV. However, in a study that screened more than 4000 pregnant women for HCV, most (16/22) newly identified cases were without prior identifiable risk factors.[195] Pregnant women found to be anti-HCV seropositive should undergo testing with PCR to confirm the presence of current infection. At the present time, no specific recommendations are made to physicians caring for HCV-infected pregnant women in attempts to reduce the frequency of perinatal transmission.[89] Based on preliminary data, prolonged amniotic membrane rupture and fetal scalp monitors should be avoided if possible. Post-exposure prophylaxis with immune globulin is not effective in preventing transmission of HCV infection and is not recommended for infants born to HCV-infected women. Infants born to mothers infected with HCV during pregnancy or at delivery should be tested for anti-HCV positivity after they reach 12 to 18 months of age; a positive test at that time is likely to correlate with true infection because maternal antibody will have disappeared by that age. If anti-HCV is positive, testing for HCV RNA at 12 to 18 months of age will determine whether the infection has resolved spontaneously or has become persistent. The algorithm for identification of women at risk, as well as for detecting perinatally acquired infection, is depicted in Figure 189–6.

Acute infection, which may be acquired from a transfusion, other parenteral exposure, or at the time of birth, rarely causes symptoms recognizable as acute hepatitis. Fulminant hepatitis attributed to HCV is an extremely rare event in children, as it is in adults. Whereas no reports exist of treatment of acute HCV infection in children, successful treatment of adults with acute HCV, using IFN monotherapy, has been reported.[37] In an open-label study, Jaekel and colleagues reported SVR of 98 percent.[93] More recently, an 8-week course of pegylated IFN was shown to induce SVR in 95 percent, 92 percent, and 76 percent of acutely infected patients at week 8, 12, or 20 after infection, respectively.[101] Similar results were reported by Wiegand and colleagues.[197] Fulminant hepatitis attributed to HCV is an extremely rare occurrence in children, as it is in adults.

When chronic HCV infection is diagnosed in children, the duration of infection is likely to be shorter than that in many adult patients who present for treatment. Liver disease typically is mild, with low necroinflammatory and fibrosis scores. Children are less likely to have serious co-morbid conditions, such as HIV infection, chronic alcohol use, and autoimmune disorders, although some will have secondary iron overload secondary to chronic transfusion therapy, and some will be obese and have varying degrees of fatty liver. However, overall, the lower frequency of co-morbid conditions may confer a higher likelihood of therapeutic response. Children have a longer life expectancy, with the expectation that over the course of time, progressive hepatic injury and scarring will develop. These considerations provide some compelling reasons to examine the role of treatment in children.

IFN monotherapy trials in children with chronic HCV infection yielded SVRs ranging from 33 to 45 percent.[92,95] In general, biochemical responses paralleled virologic responses, and histologic findings in liver biopsy specimens improved in treated patients irrespective of complete virologic response, as noted in adult studies.

This rate is significantly higher than the SVR rates reported in large trials of IFN monotherapy in adult patients. Unfortunately, even with these higher response rates, most children remain infected after undergoing treatment. In addition, this medication is associated with important adverse side effects. The flu-like illness described in adults is seen commonly in children but typically resolves after one or two doses. Dosage reductions frequently are required because of development of neutropenia, although serious infectious complications have not been described. Although coexisting autoimmune diseases are seen less commonly in children, type II autoimmune hepatitis, associated with liver/kidney microsomal antibody, can be mistaken for or coexist with HCV infection, and it may be exacerbated with administration of immunoreactive fibronectin IFN.[27,75] A frequent side effect of IFN is weight loss with or without anorexia. Children may lose up to 10 percent of their body weight, with serious implications for growth if the condition is not addressed promptly.[97] A study in children treated for chronic HBV infection suggested that this weight loss and accompanying decrease in linear growth velocity may be transient and improves rapidly once the IFN is discontinued.[44] Significant depression was described in adults receiving IFN for chronic hepatitis; although serious mental illness was not reported in children treated with IFN-α, irritability, decreased school performance, and other behavioral disturbances may be precipitated by this medication. One child developed seizures while receiving IFN-α for HCV infection.[141] Infants treated with IFN-α for life-threatening hemangiomas were noted to have an increased incidence of spastic diplegia.[16] Judicious use of this drug and careful monitoring are

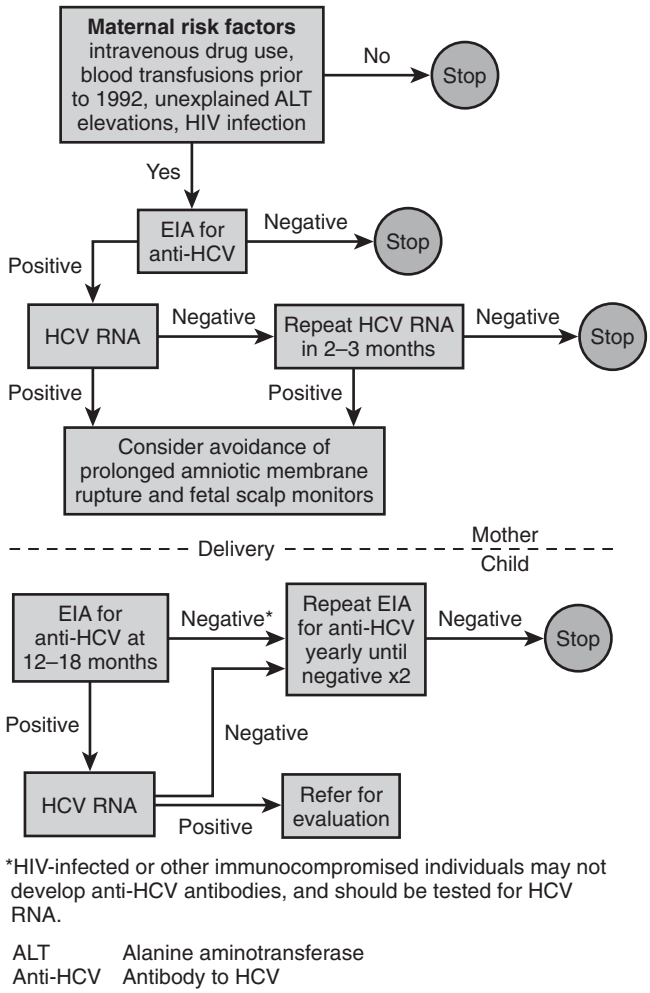

Figure 189–6 Algorithm for identification of women at risk, as well as detecting perinatally acquired infection. ALT, alanine aminotransferase; EIA, enzyme immunoassay; HCV, hepatitis C virus; RIBA, recombinant immunoblot assay; RT-PCR, reverse transcription polymerase chain reaction.

required in young patients. These concerns and others that include the expense of IFN-α, the potential for serious side effects, and the necessity for parenteral administration have led some clinicians to conclude that treatment with IFN is not warranted in children. No cost-to-benefit analysis has been performed in this patient group (see the earlier discussion of financial burden, however).

Combination therapy with IFN-α-2b and ribavirin is the treatment approved by the U.S. Food and Drug Administration (FDA) for chronic HCV in children aged 3 years and older. However, ribavirin has the potential for serious toxicity, including hemolytic anemia, mutagenicity, and teratogenicity. Clinical trials during the past few years have shown the efficacy of this regimen to be similar in children and adults.[65,71,198] In an earlier trial, 41 children were treated with either 3 or 5 MU/m^2, resulting in an SVR of 61 percent. The side effects of treatment were fever, flu-like symptoms, anorexia, and thyroid function abnormalities, but only one patient dropped out because of side effects. In a more recent study, 118 children aged 5 to 17 years were treated with IFN-α-2b and ribavirin (8, 12, or 15 mg/kg/day).[71] The SVR was 46 percent overall, but several notable subgroups were identified. Among patients with genotype 1, those with viral levels of 2 million or fewer copies versus 2 million or more copies/mL had SVR rates of 48 percent versus 26 percent, respectively. As observed in adults, patients infected with HCV genotypes 2 or 3 had substantially higher SVR (84%, 21/25) than those with genotype 1 (36%). In the phase II trial included in the same report, the pharmacokinetic properties of the drugs were similar to those in adults. Although ribavirin was associated with dose-dependent anemia, the anemia was less severe than in adults, and the higher dose (15 mg/kg) was associated with a higher rate of SVR. Severe adverse events, the most common of which was neutropenia, occurred in 19 percent (23/118) of the subjects. Depression was reported in 13 percent and was mild to moderate in severity. Based on these data, the recommended dose of IFN-α-2b is 3 MU/m^2 three times weekly, to a maximum of 5 MU per dose. Ribavirin is given at 15 mg/kg/day in two divided doses. A liquid preparation of ribavirin (40 mg/mL) is available and has bioavailability similar to that of the capsules.[71]

Pegylated IFN is a modified form of IFN in which an attached polyethylene glycol moiety prolongs the clearance of the drug. This confers the advantage of weekly dosing, but it may affect drug toxicity and efficacy. In adults, pegylated IFN in combination with ribavirin has become the standard treatment and has resulted in SVR rates approximately 10 percent higher than those of combination treatment with standard IFN.[65] The safety and efficacy of pegylated IFN in children has been investigated. One open-label trial[199] showed an overall SVR rate of 59 percent. Among the children infected with genotype 1, the SVR rate was 48 percent (22/46). In those infected with genotype 2 or 3, the SVR was 100 percent (13/13). The side effects were similar to those reported in trials of standard IFN.

Important differences exist between HCV infection in adults and in children that may have a significant impact on therapy. These differences are listed in Table 189–4. As transfusion-associated infection declines, the most prevalent means of HCV acquisition in children will be perinatal transmission. If, as it seems from small preliminary studies, more of these infections are likely to resolve without treatment or cause less severe liver disease, then treatment of the youngest children may not be necessary. Conversely, young adolescents with cirrhosis and end-stage liver disease caused by vertically acquired HCV have been encountered. More data are needed about the factors that cause rapid evolution of this disease in pediatric patients, so that cogent decisions regarding patient selection and timing of treatment can be made.

TABLE 189–4 Special Considerations Regarding Treatment of Children with Hepatitis C Virus infection

Differences in Natural History
Mode of acquisition: perinatal
Shorter duration of infection
Co-morbid diseases
Longer anticipated life expectancy
Differences in Liver Disease
Milder grades of necroinflammation
Less frequent severe fibrosis or cirrhosis
Differences in Response to Interferon-α Treatment
Higher frequency of response*
Lower frequency of relapse*
Less correlation of ultimate response with HCV RNA at 12 weeks*
Fewer instances of drug discontinuation resulting from side effects
Unknown long-term side effects
No cost-to-benefit data

**Studies leading to these conclusions include relatively small numbers of patients.*
HCV, hepatitis C virus.

CO-INFECTION WITH HUMAN IMMUNODEFICIENCY VIRUS

Because HCV and HIV share the route of acquisition and risk factors for infection, co-infection with both viruses is not an uncommon finding. The prevalence of HCV infection in HIV-infected adults is 33 percent.[179] In the highest-risk groups, patients with hemophilia and injection drug users, 66 to 100 percent of HIV-infected patients also are infected with HCV.[22] Although sexual transmission of HCV is an uncommon occurrence, the rate is substantially higher in the presence of HIV co-infection.[51] The same is true for perinatal HCV transmission.[153,203,204]

HCV infection has a more aggressive course in the presence of HIV co-infection.[173] The progression of chronic liver disease is accelerated in co-infection; advanced fibrosis and disease activity correlate with a low CD4 count.[20] A significantly higher fraction of HCV/HIV–co-infected patients develop liver failure and hepatocellular carcinoma.[23,59,134] The presence of HCV infection does not seem to exacerbate HIV infection.[161]

The pathogenic mechanism of this accelerated HCV liver disease is not known, but it is thought to stem from direct cytopathic effects of the virus on hepatocytes, perhaps more so than in immunocompetent individuals. Lower CD4 counts are associated with HCV RNA levels in the blood 10 to 100 times higher than those in controls, but no correlation exists between HCV RNA and ALT levels or disease severity.[68] Additional mechanisms, such as drug toxicity, likely play roles in hepatic injury in HIV-infected patients.

Antibody-based tests such as enzyme immunoassay (EIA) and RIBA have a higher incidence of false-negative results in immunosuppressed individuals, including those with HIV infection.[129] If anti-HCV testing is negative, direct testing for HCV RNA using PCR should be performed to exclude HCV infection in the HIV-infected patient.

The treatment of HCV/HIV–co-infected patients has been studied only in adults. In a study of 90 HCV/HIV–co-infected patients, the response rate to IFN-α therapy was similar to that of the non–HIV-infected group (29% versus 34%), but the relapse rate was higher in the HIV-infected group (31% versus 12.5%).[178] One study of 20 HIV/HCV–co-infected patients reported the use of ribavirin in combination with IFN and showed viral clearance rates comparable to those of singly infected patients (50%).[115] However, the toxic side effects of drugs, such as hemolysis induced by ribavirin and myelosuppression by IFN, may be more severe in combination with the antiretroviral medications used to treat HIV,[112] and these patients should be monitored closely.[42]

FUTURE TREATMENTS AND PREVENTION OF HEPATITIS C

Vaccine development for prevention and therapy of HCV infection is an active focus of research.[87] Numerous strategies, including injecting recombinant envelope proteins, plasmid DNA encoding viral components, and recombinant attenuated viruses containing HCV antigens, have been employed. These approaches and others provide hope that HCV ultimately will be a preventable disease. Obstacles include extensive heterogeneity of the HCV envelope proteins and the apparent lack of immunity following a resolved infection. However, neutralizing antibodies do exist; they most likely target one or both of the envelope proteins. Conversely, neutralization may be a short-lived phenomenon, given the rapid development of HCV quasispecies in infected individuals. In both humans and chimpanzees, acute self-limited HCV infection does not confer protection against either heterologous or homologous strains of the virus.[60,160] Autonomous replication of subgenomic HCV RNA has been demonstrated in a human hepatoma cell line after transfection of constructs composed of nonstructural HCV genes and the neomycin phosphotransferase gene.[127] In addition, purified HCV-like particles have been synthesized in insect cells from a recombinant baculovirus expressing C, E1, and E2 proteins.[18] These particles are being evaluated as potential immunogens. Synthetic vaccines that include peptides corresponding to cytotoxic T lymphocyte and helper T-cell epitopes of the HCV core are being used experimentally to induce cytotoxic T lymphocyte responses and memory in an attempt to stimulate cell-mediated immunity,[81] which may be the more important protective mechanism.

The incidence of new HCV infections in the United States has decreased markedly.[7,14] The cause of this decrease is not entirely clear because prevention of transfusion-associated infections is responsible for only a small proportion of this dramatic change in incidence. However, as yet no clear indication exists that the incidence of new infections in children is decreasing, especially because many currently infected individuals are women of child-bearing age. Although pediatric cases represent a minority of HCV infections and may not commonly progress to serious liver disease during the childhood years, certainly instances of significant morbidity and even mortality from this disease have occurred.

Available therapy is cumbersome, uncomfortable, not without risk, and ineffective in many cases. For these reasons, consideration must be given to measures that may be instituted to prevent new pediatric HCV infections. With recognition of the predominant role of perinatal transmission as the source of most new pediatric infections, this process is the logical target for development of prevention strategies. Research to develop better treatments for HCV has focused mostly on inhibiting the virally encoded enzymes that are required for virus propagation. They include the NS3-NS4A protease and the NS5B polymerase. In addition, synthetic nucleic acid–based agents and novel immunomodulatory drugs are in development phases. The ability of the virus to mutate rapidly allows resistance to single agents to develop rapidly. In all likelihood, a cocktail of drugs likely will be required for maintenance of adequate viral suppression and, possibly, cure.[48]

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SUBSECTION 6

Orthomyxoviridae

CHAPTER 190

Influenza Viruses

W. Paul Glezen

Influenza is an acute respiratory infection caused by strains of the orthomyxoviruses. The first of the human respiratory viruses to be isolated and characterized,[278] influenza viruses also have been studied the most extensively and are the best understood of these agents from biologic, epidemiologic, and clinical standpoints.[100,161] Yet despite great sophistication in our understanding of it as a disease, influenza remains "the last great plague of man."[161]

Despite the improvements that have been made in living standards and the introduction of antibiotics, the overall impact of influenza on mortality rates has not diminished. In fact, the average number of excess deaths has increased since 1984 to 1985 to at least 51,000 per year,[290] and the number of hospitalizations attributable to influenza is approximately 385,000 annually.[291] Population dynamics—increasing population density and aging of the world's population—dictates that these numbers will continue to increase.[96] The specter of a recurrence of the catastrophic 1918 to 1919 epidemic, in which an estimated 550,000 deaths occurred in the United States alone,[50] has re-emerged with the avian influenza epizootics.[303] Annual epidemics have continued unabated. Each year, the peak of influenza virus activity coincides with the peak of health care visits and hospitalizations for acute respiratory tract disease.[47] Despite a decline in mortality rates from other causes, an increasing number of deaths is attributed to influenza each year.[273-275,290]

As other agents capable of causing respiratory tract infection in children have been identified, influenza has received relatively less attention. Yet the morbidity and mortality rates of influenza in children can be considerable, and the spectrum of clinical manifestations resulting from influenza viral infections is broad.[17,244]

HISTORY

Although the authenticity of influenza in medical antiquity is difficult to establish, the disease has existed for more than 2000 years. The epidemic in 412 BCE described by Hippocrates and Livy probably was influenza.[289] Epidemic influenza-like disease occurred in Europe in the 6th and 10th centuries, but the first generally accepted influenza epidemic occurred in December 1173.[134] Hirsch[134] noted 299 epidemics of influenza between 1173 and 1875. The first pandemic involving Europe, Asia, and North Africa occurred in 1580, and the first epidemic recorded in the Western Hemisphere occurred in 1647. From 1580 until 1918, at least eight pandemics of influenza occurred.

In more recent times, pandemics of influenza caused by different influenza A subtypes occurred in 1874, 1889, 1900, 1918, 1957, 1968, and 1977. The most noteworthy of all pandemics of influenza occurred in 1918. This event has the dubious distinction of producing the greatest morbidity and mortality rates of all time: more than 40 million deaths occurred in the world.[6,168]

The term *influenza* may have come from the Latin word *influo*, "to flow in," perhaps indicating its airborne transmission. It may be Italian, relating to an "influence," such as the weather or mystical astrologic causes.[168]

PROPERTIES OF THE VIRUS

CLASSIFICATION

Classified taxonomically as orthomyxoviruses, the influenza viruses are negative-sense, single-stranded RNA viruses of three major types—A, B, and C—and with multiple subtypes of influenza A viruses.[161] Influenza A and B viruses are the most important in human disease and have been studied far more extensively than have influenza C viruses. All these viruses have the property of hemagglutination and, with the exception of influenza C, possess the enzyme neuraminidase. For types A and B, these properties reside on a pair of surface glycoproteins, hemagglutinin (HA) and neuraminidase (NA), but influenza C has a single glycoprotein with HA, esterase, and fusion activity.[172] The current World Health Organization (WHO) system of nomenclature for influenza virus strains specifies type, host (for strains of animal origin), geographic source, strain number, and year of isolation, to which code designations of HA and NA subtypes are appended.[212] Thus, the "Russian" influenza A strain was designated A/USSR/90/77 (H1N1); the "Philippines" influenza A strain was designated A/Philippines/2/82 (H3N2). Strains are characterized and named at the WHO influenza reference centers in Atlanta (Centers for Disease Control and Prevention [CDC]), London, Melbourne, and Tokyo.

PHYSICAL PROPERTIES

In electron-microscopic preparations, influenza viruses are irregular, spherical particles 80 to 120 nm in diameter that also may exhibit filamentous or icosahedral structures (Fig. 190–1).[212] Numerous HA and NA "spikes" bristle from their surfaces. The virion proteins all are specified by the segmented viral genome, but the lipid bilayer and the carbohydrate constituents of glycoproteins and glycolipids in the viral envelope are derived from the host cell (Fig. 190–2). Besides the HA and NA, eight other virus-coded proteins have been characterized (Table 190–1). Matrix or membrane protein (M1) is the most abundant protein and the major structural component of the viral envelope. The M2 protein of influenza A virus is a smaller tetrameric protein that acts as an ion channel extending through the viral envelope. M2 has an important role in the penetration and release of viral RNA into the host cell.[284] The function of the comparable polypeptide of influenza B virus, BM2, is similar even though the structure of BM2 differs from the M2 of A viruses.[172] Nucleoprotein is associated with the RNA genome of the virus in ribonucleoprotein complexes in which multiple nucleoprotein molecules are associated with eight segments of the single-stranded RNA. Proteins PB2, PB1, and PA, the largest proteins of the virus, are

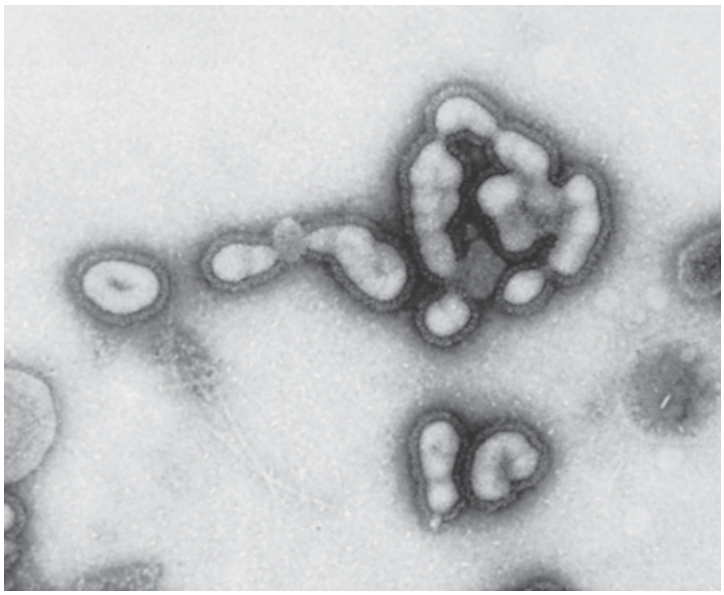

Figure 190–1 Influenza A/USSR/90/77 (H1N1). Note the hemagglutinin and neuraminidase "spikes" and occasional filamentous forms. *(Courtesy of G. R. Noble, M.D., Centers for Disease Control and Prevention, Atlanta.)*

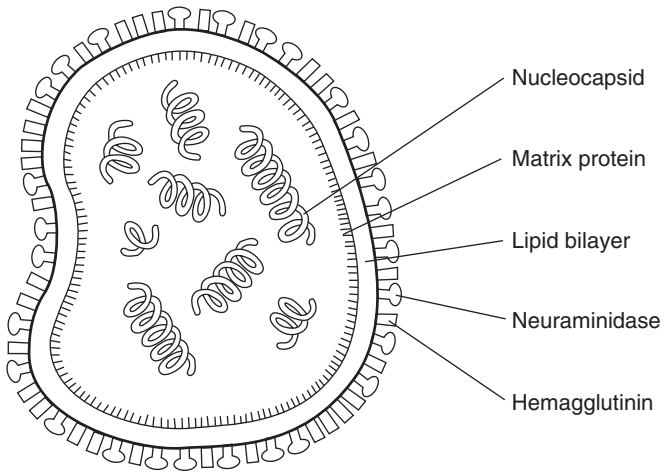

Figure 190–2 An influenza virion. Nucleocapsid structures consist of segmented RNA complexed with nucleoprotein and P proteins.

TABLE 190–1 Virus-Coded Proteins of Influenza Virus

Gene Segment	Protein Designation	Function	Antigenicity
1	PB2	RNA synthesis	?
2	PB1	RNA synthesis	?
3	PA	RNA synthesis	?
4	HA	Hemagglutinin	Subtype specific
5	NA	Neuraminidase	Subtype specific
6	NP	RNA synthesis	Type specific
7	M1, M2	Matrix	Type specific
8	NS1, NS2	Nonstructural	?

so designated because two are basic proteins and one is an acidic protein.[140] They are involved with the synthesis of three different kinds of virus-specific RNAs. Evidence suggests that the PB2 protein is the cap-recognizing protein and that PB1 and PA are involved in chain initiation or in chain elongation.[20,21,171,234,294] Nonstructural proteins, although not incorporated into viral progeny, are virus-coded proteins, NS1 and NS2. NS1 has an anti-interferon effect,[87] and NS2 promotes nuclear export of the viral ribonucleoprotein.[227]

The biologic and antigenic diversity of influenza viruses is attributable in part to their unique, segmented RNA genome. Interchange of corresponding segments of a linear genome, in the traditional sense of genetic recombination, did not account for this extraordinarily high frequency. Hirst,[135] in 1962, proposed the hypothesis that the influenza genome consists of subgenomic pieces capable of semiautonomous replication and random "reassortment" during the process of assembly. This theory accounted for observed recombination frequencies and subsequently was supported by the finding that the RNA of influenza virions was indeed segmented on analysis by polyacrylamide gel electrophoresis.[247] More refined electrophoretic studies in urea-polyacrylamide gels established that the influenza A genome consists of eight segments (Fig. 190–3). Reassortment in nature has been documented for the 1957 and 1968 pandemic viruses as well as for other A viruses.[212] The 1918 pandemic virus probably was an avian virus that mutated in a manner that allowed facile transmission in human populations.[293]

ANTIGENIC COMPOSITION

Of the protein constituents of influenza viruses, four are known antigens. The "internal" nucleoprotein and matrix protein are antigenically type-specific and stable. Nucleoprotein is the antigenic basis for typing strains as A, B, or C and is the predominant constituent of the "soluble" antigen employed for complement-fixation serologic testing. Antibodies to the nucleoprotein are used in tests for direct detection of virus in clinical specimens. Although antibody against nucleoprotein is formed regularly after natural infection occurs and antibody against matrix protein has been detected after severe illness, these antibodies are short-lived and appear to have limited protective value.

In contrast, HA and NA antigens are subtype-specific and variable. HA is required for attachment of infecting virus to host-cell membranes. HA-inhibiting antibodies neutralize viral infectivity and are the most important index of immunity against influenza in humans.[136] NA appears to be required for release of virus from infected cells. Specific anti-NA antibodies reduce the size of the plaque, mitigate the pathogenic effects of

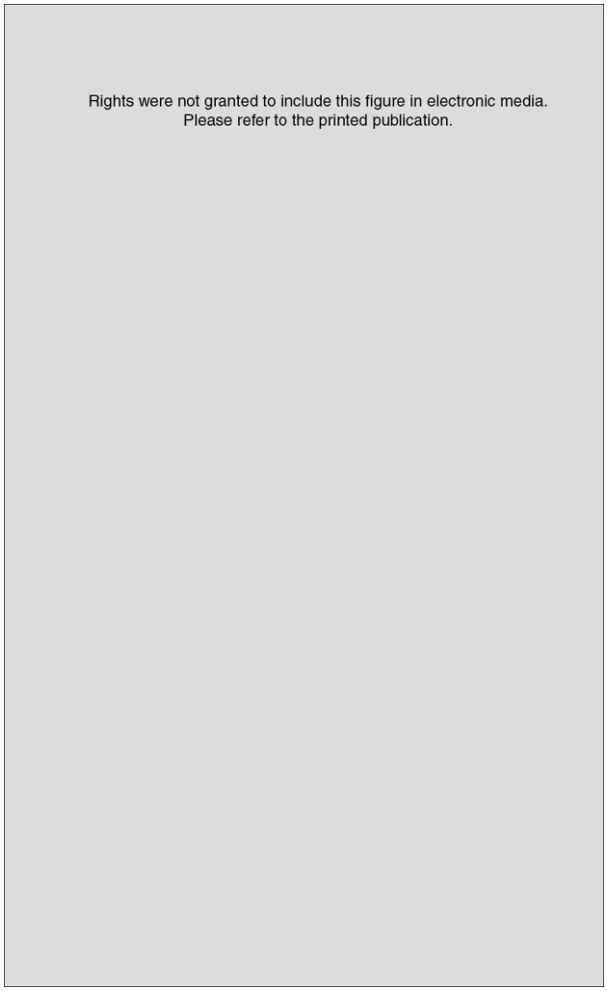

Figure 190–3 Polyacrylamide-urea gel electrophoretic maps of the RNA genome of influenza A/PR/8/34 (H0N1) and A/HK/68 (H3N2). *(From Ritchey, M. B., Palese, P., and Schulman, J. L.: Mapping of the influenza virus genome. III. Identification of genes coding for nucleoprotein, membrane protein, and nonstructural protein. J. Virol. 20:307-313, 1976.)*

influenza in experimentally infected mice, and correlate inversely with viral shedding and severity of illness.[214] Thus far, three HAs, H1, H2, and H3, and two NAs, N1 and N2, have been recognized in influenza A viruses that spread readily in human populations.

Variation in HA and NA specificity is the basis for antigenic *drift* and *shift* in prevalent viruses. *Drift* implies a minor change in either antigen, without a change in subtype; *shift* implies a major change in either or both antigens, with a change in subtype. Antigenic drift occurs in influenza A and B viruses; antigenic shift occurs only in influenza A.

Antigenic drift is the result of point mutations. Selective pressure in an immune population results in selection of mutant viruses with altered antigenic determinants that allow a growth advantage in the presence of prevalent antibody. Supporting this concept are the in vitro studies of Laver and Webster,[175] in which antigenic mutants isolated by serial egg passage in the presence of low-avidity antiserum developed changes in the peptide makeup of their HA subunits.

Antigenic shift occurs when an influenza A virus acquires HA or NA components that differ from antecedent strains by a quantum jump. The phenomenon has been well studied in influenza A viruses. The H3 HA, by chromatographic analysis of peptide composition, is sufficiently distinctive to make highly improbable its emergence by point mutation from the H2 HA of a preceding Asian strain.[176]

Considerable evidence indicates that antigenic shift strains arise by reassortment of gene segments between human and animal influenza viruses during chance simultaneous infection. The 1957 Asian H2N2 virus derived the HA, NA, and PB1 gene segments from an avian virus and the remaining five gene segments from the circulating A (H1N1) strain. The so-called Hong Kong influenza that appeared in 1968, A (H3N2), had avian HA and PB1 gene segments combined with six gene segments from the preceding A (H2N2) virus.[212]

An analogous event initially was suspected as the origin of the swine-like A/New Jersey/8/76 (H1N1) virus recovered from infections in Fort Dix military recruits in 1976. However, the RNA genome of A/NJ was shown by polyacrylamide gel chromatography to be virtually identical to contemporary strains of influenza virus isolated from swine.[229] It shows no RNA homology to human strains. The spread of virulent avian viruses with the H5 HA to humans beginning in Hong Kong in 1997 is another alarming event that supports the possibility of the emergence of pandemic strains by direct infection of humans with an avian virus possessing surface antigens not previously prevalent in the human population.[139] As this virulent avian virus, A(H5N1), continues to spread in Southeast Asia, eastern Europe, and Africa, the possibility that mutations would allow human-to-human transmission increases. Furthermore, examination of the viral nucleic acids recovered from persons who died in the 1918 pandemic indicates that this A(H1N1) virus was entirely of avian origin. Thus, the emergence from animal reservoirs of influenza strains with sufficient virulence to cause widespread human epidemics remains a valid hypothesis.

TISSUE CULTURE AND CHICKEN EMBRYO GROWTH

Influenza viruses grow well in a variety of culture systems, although embryonated chicken eggs and the Madin-Darby canine kidney cell line are used most widely. Primary rhesus monkey kidney tissue culture and other monkey cell lines are alternative choices for isolation of influenza virus. Intra-amniotic and intra-allantoic inoculation of 10- to 11-day-old eggs is followed by incubation for 3 to 4 days at 33° C. Fluid samples are harvested and tested for the presence of hemagglutinating virus by addition of guinea pig or chicken red blood cells. Monkey kidney cells have maximum sensitivity to influenza viruses when they are maintained after inoculation in a serum-free medium. Subtle cytopathic effects may appear but are variable. Detection of virus is carried out in the Madin-Darby canine kidney cells or with use of the rhesus kidney cell line, then hemadsorbing with guinea pig red blood cells. Influenza C viruses grow best in eggs; Madin-Darby canine kidney and monkey kidney cells generally give higher yields of influenza A and B viruses. Although parainfluenza viruses also may be isolated in these tissue culture lines, they can be distinguished from influenza viruses by their characteristic syncytial cytopathogenicity and their poor growth in embryonated eggs.[79,80] Identification of respiratory viruses can be expedited by use of "shell vial" cultures with a mixed cell monolayer culture and low-speed centrifugation.[280] Definitive identification of virus isolates is performed by indirect immunofluorescence or hemagglutination inhibition with use of specific antisera. Antigen detection and identification of influenza viruses by use of enzyme-linked immunosorbent assays (ELISAs) have been valuable additions to the diagnostic laboratory.[16,59,126,262,301] Reverse transcription–polymerase chain reaction (RT-PCR) techniques have the possibility of revolutionizing the diagnosis of respiratory virus infections.[4,68]

ANIMAL SUSCEPTIBILITY

Influenza virus types replicate and produce disease in the ferret.[161] This animal commonly is used for experimental studies. Influenza viruses also can be adapted to grow in mice for research purposes. Other animals, including hamsters, guinea pigs, monkeys, squirrels, chipmunks, chinchillas, and mink, have varying degrees of susceptibility to influenza viruses.

Horses, swine, and birds are infected naturally by type A influenza viruses. In most instances, these animal strains are different from those that spread readily in humans. Avian strains are the most important reservoir for viruses with pandemic potential.[212] Influenza A(H5N1) avian virus has produced a prolonged epizootic in Southeast Asia and Africa. Infection with this virus is usually benign for migrating wild aquatic birds, but mutations have produced strains that are extremely virulent when introduced into domestic fowl—chickens, turkeys and quail.[303]

EPIDEMIOLOGY

INCIDENCE AND PREVALENCE

Influenza is the most important cause of acute respiratory illnesses that lead patients to seek medical care.[91,93,209] Population dynamics that includes increasing population and increased population density resulting from urbanization throughout the world facilitates the spread of these viruses.[96] Rapid movement of persons throughout the world allows new variants or subtypes to spread readily in a short time. Transmission by small-particle aerosol, as well as by direct contact, facilitates dissemination.[288] Unless new control measures are added, the impact of epidemics will increase steadily as the population ages.[273,290,291] Studies have demonstrated that the hospitalization rates for preschool-age children equal those of elderly persons.[148,209,220,244] Furthermore, influenza viruses are the most important causes of acute respiratory tract illnesses leading to hospitalization of schoolchildren.[102,104,235]

Although local outbreaks and individual cases of influenza are reportable optionally in all states, information about disease activity as it varies from year to year derives mainly from ongoing surveillance in public health departments and university medical centers.[184] Longitudinal studies of viral respiratory tract illnesses in families,[73,119,150,287] in hospitalized children,[104,107] in private pediatric practices,[103,106,116] and in public clinics[99] have provided useful data about the community impact of influenza infections.[156] Schoolchildren have the highest attack rates for infection each year, ranging from 30 to 50 per 100 children. Detection of new influenza variants and their spread is facilitated by reporting of virus isolations and serologic responses in specimens submitted to an international network of WHO laboratories.[33]

Weekly tabulations of deaths from "pneumonia" and "influenza" from larger cities in the United States provide a valuable index of mortality from influenza.[274] Several methods have been used to estimate the expected number of deaths in the absence of influenza so that excess mortality can be derived from the observed number during epidemics. Reported deaths in excess of an epidemic threshold usually correlate well with the occurrence of widespread influenza activity (Fig. 190–4).[108,173,274] The CDC now requests reporting of all laboratory-confirmed deaths in children. A total of 153 deaths were reported during the first year, 2003 to 2004.[17] These deaths comprise only a fraction of the projected total, but the sample provides some insight into the problem. Many of the deaths were unexpected; the children died at home or in the emergency room; 29 percent died within 3 days after onset of symptoms, and 31 percent died outside the hospital setting. Thirty-three percent had an underlying condition recognized to increase the risk of developing influenza-related complications, but 20 percent had other chronic conditions; 47 percent had been previously healthy. The highest mortality rate was calculated for infants younger than 6 months of age, at 0.88 per 100,000 children.

Influenza spreads first among children in school, which allows introduction into the household and community spread to adult populations.[36,93,99,100] Although deaths caused by influenza occur most frequently at both extremes of age, increases in pneumonia

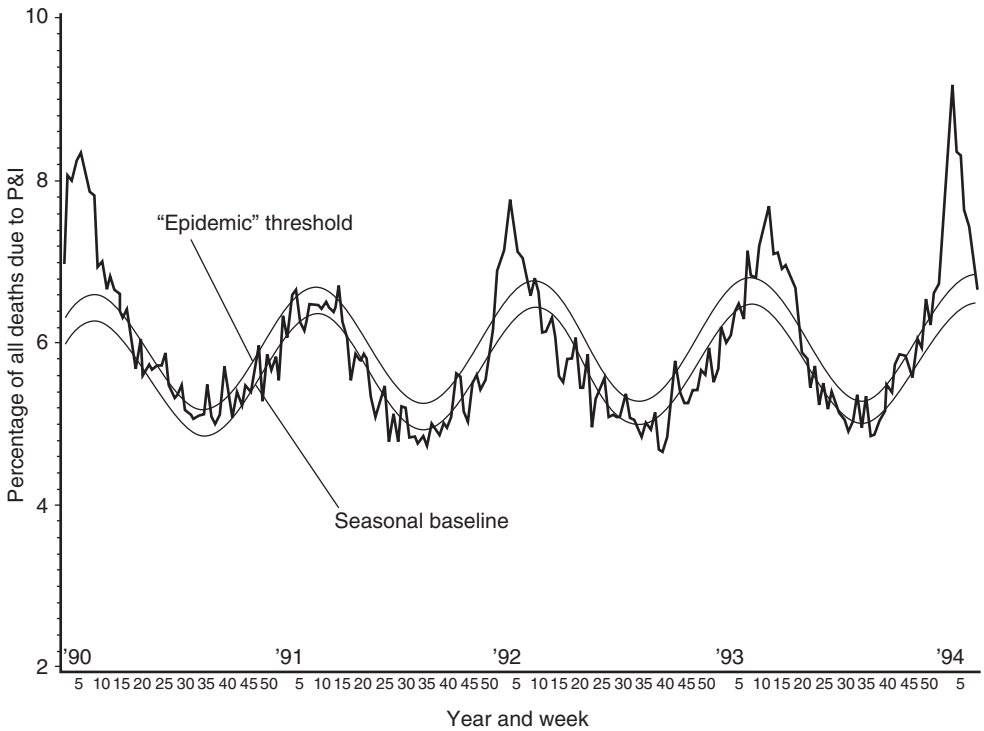

Figure 190–4 Percentage of deaths attributed to pneumonia and influenza (P & I) in 121 cities, United States, 1990 to 1994. *(From Centers for Disease Control and Prevention: United States Influenza Surveillance, March 4, 1994.)*

and influenza deaths in recent years have been greatest among elderly persons.[74,273-275,290] Regardless of age, influenza is fatal more frequently in persons with preexisting heart disease, chronic pulmonary disorders, diabetes mellitus, chronic renal disease, neuromuscular disorders, and neoplasms.

ECOLOGY AND RECAPITULATION

A remarkable ecologic feature of influenza viruses has been the tendency of one particular virus subtype to achieve worldwide distribution at the same time its predecessor disappears from human circulation.[161] Since their first isolation in the 1930s, three major subtypes of influenza A viruses have circulated widely. At the transition years of these "influenza eras," a major antigenic drift or shift has occurred. The prototypic strains for these time intervals are as follows: 1933 to 1957, influenza A/Puerto Rico/8/34 (H1N1); 1957 to 1967, influenza A/Japan/305/57 (H2N2); and 1968 to 1977, influenza A/Hong Kong/8/68 (H3N2). In 1977, influenza A/USSR/90/77 (H1N1) made its appearance; since then, both influenza A(H1N1) and A(H3N2) strains have been prevalent.

The antigenic make-up of viruses prevalent before the 1930s has been inferred by seroepidemiologic studies. By this means, investigators have determined that persons born before 1924 have a high prevalence of antibodies against the swine-like H1 HA that appeared in 1918.[272] Similarly, in serum specimens collected before the emergence of H2N2 and H3N2 strains, antibodies against the H3 HA were found in persons born before 1889.[191] Thus, the number of influenza A viruses that circulate among human populations may be finite, and the three major subtypes may recycle periodically. This concept is supported by the re-emergence of A/USSR/90/77 (H1N1). Antigenically and genetically, this strain of influenza A was found to be identical to influenza A/Fort Warren/1/50 (H1N1), the H1N1 variant prevalent between 1950 and 1953.[105,217,322]

SEASONAL AND GEOGRAPHIC PATTERNS

Influenza infections generally have sharp seasonality.[100] In temperate zones, epidemics usually occur in winter months. Beginning in 2000, an alarming tendency for earlier arrival of epidemics was noted; epidemic disease was confirmed as early as November in 2000, 2002, and 2003.[96] Winter circulation in the Southern Hemisphere sustains influenza viruses during the summer months of the Northern Hemisphere. Occurrence of influenza is less predictable in tropical areas. Some regions may have two annual outbreaks, whereas others may have outbreaks in the rainy season. Geographic variations in the incidence of influenza also may reflect global patterns of spread of new virus strains that is facilitated by increased international travel. Isolated populations may escape the dispersion of new viruses; when outbreaks do occur in such highly susceptible populations, explosive spread and high attack rates in all age groups have been observed.[28,93]

TRANSMISSION

Microdroplet spread, with inhalation of airborne particles produced by coughing and sneezing, generally is accepted as the most common mode of natural influenza transmission.[288] Spread also may occur by direct contact and large-particle aerosols. Small-particle aerosol, by which virus particles are deposited directly into the lower respiratory tract, is the most efficient means of inducing influenza in volunteer studies. In one such study, a human infectious $dose_{50}$ (HID_{50}) of influenza A/Bethesda/10/63 (H2N2) by aerosol was equivalent to 0.6 to 3.0 tissue culture infectious doses ($TCID_{50}$).[1] In contrast, studies in which influenza A/Aichi/2/68 (H3N2) was given by direct instillation or coarse spray into the nose revealed a range of HID_{50} of 127 to 320 $TCID_{50}$.[45,51] The contribution of small-particle aerosol to transmission of influenza under natural conditions is controversial,[288] but it may be important in the pathogenesis of primary influenza pneumonia.[100]

Once infection is established, virus shedding generally follows the development of clinical symptoms. Virus may be recovered 1 day before the onset of symptoms with influenza B and up to 6 days in the case of influenza A. Virus shedding is detected for a variable period in children but usually persists for 1 week for influenza A and for up to 2 weeks after influenza B infection.[83] At the height of illness, respiratory tract secretions may contain 10^6 or more infectious viral particles per milliliter.[211]

The incubation period of influenza ranges from 1 to 7 days but commonly is 2 to 3 days. This brief incubation period, coupled with the quantity of infectious virus in secretions and the relatively small dose necessary for infection to occur in susceptible contacts—particularly by small-particle aerosol—accounts for the intensity of outbreaks of influenza. Spread occurs most rapidly in closed populations, such as schools, colleges, military barracks, and nursing homes. In community outbreaks, school-age children usually have the highest attack rates, with secondary spread to their parents and younger siblings (Fig. 190–5).[36,53,91,100,109] Surveillance cultures from patients presenting with acute respiratory illness show that the incidence in school-age children predominates in the early stages of an influenza epidemic. As the epidemic progresses, the proportions of adults and preschool children increase, whereas the proportion of school-age children decreases. Several studies have taken advantage of this mode of spread, and several field trials have demonstrated protection of adults and younger children by immunization of schoolchildren. Herd protection by immunizing the main spreaders of influenza is a promising strategy for control of epidemic influenza.[98]

Nosocomial infection may occur during community epidemics of influenza and has been documented in hospitalized adults,[22] infants,[115] and premature infants.[196,209] In hospital settings, separating highly susceptible patients from other patients and personnel with acute respiratory tract illness is a reasonable measure. It can be accomplished by performing rapid antigen detection at the time of admission to the hospital to identify those infected with influenza viruses. Prompt treatment with specific antiviral drugs will reduce not only the risk of complications but also the concentration of virus in respiratory secretions and thereby the risk of transmission to contacts in the hospital.

PATHOGENESIS AND IMMUNITY

VIRAL INFECTION AND PATHOGENESIS

To establish infection, influenza viruses must penetrate the mucous blanket lining the respiratory tract and escape inactivation by nonspecific inhibitors as well as specific local antibodies. The major site of infection is the ciliated columnar epithelial cell.[100,133] The receptor on the viral HA attaches to a sialic acid moiety on the cell membrane. The virus is endocytosed and creates an acid environment by the ion channel activity of the M2 protein, which exposes the fusion activity of the HA to allow fusion of the lipid coat with the cell membrane. This process allows entry of the viral RNA into the cytoplasm. Necrosis of ciliated epithelial cells occurs as early as the first day after the onset of the symptoms. Many of the symptoms may be attributed to the release of proinflammatory cytokines and chemokines from the infected epithelial cells.[128,277] The levels of cytokines and chemokines in nasal secretions coincide with the intensity of symptoms.[84] Local edema and cellular infiltration by lympho-

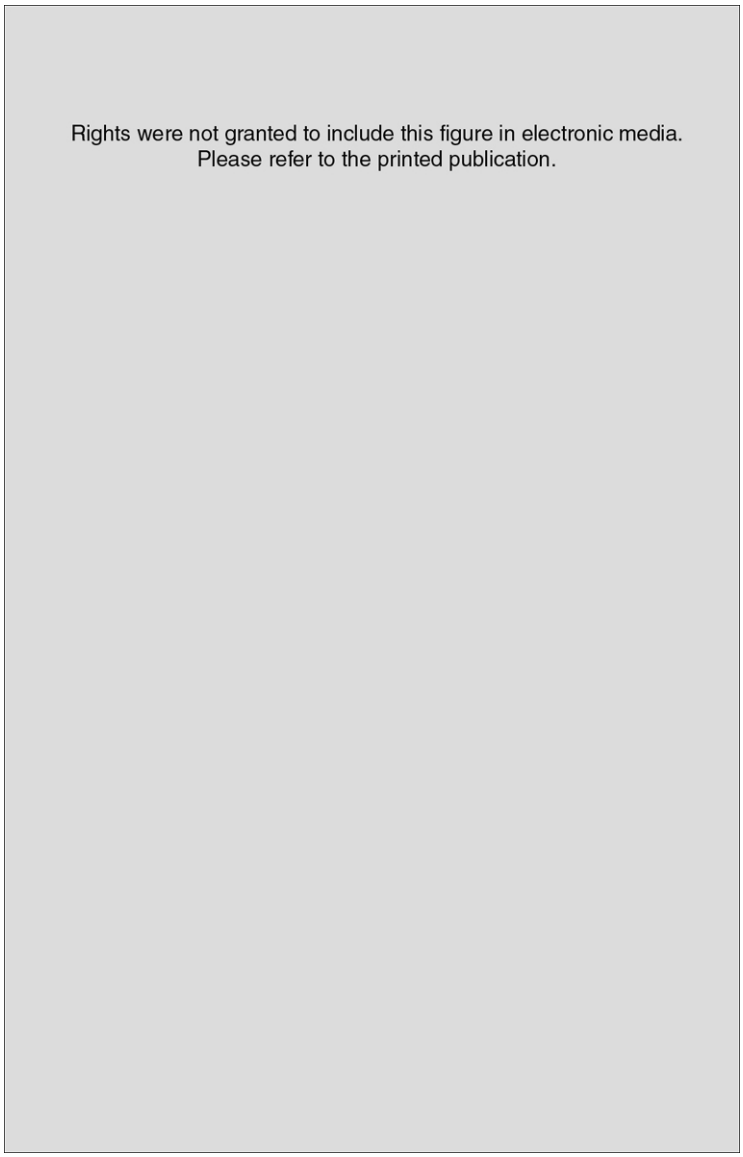

Figure 190–5 Epidemic curve of infections by influenza B in Hazleton, Iowa, 1961 to 1962. Note that the epidemic wave in school-age children precedes the epidemic wave in household contacts.[36] *(From Chin, T. D. Y., Mosley, W. H., Poland, J. D., et al.: Epidemiologic studies of type B influenza in 1961-1962. Am. J. Public Health 53:1068-1074, 1963.)*

cytes, histiocytes, plasma cells, and polymorphonuclear cells follow. Repair of the epithelium begins between the third and fifth days, as indicated by mitoses in the surviving basal cells. A pseudometaplastic response of undifferentiated epithelium reaches its maximum 9 to 15 days after the onset of the infection. After 15 days, cilia and mucus production reappear. With secondary bacterial infection, more extensive inflammatory cell infiltration and destruction of the basal cell layer and basement membrane are seen, with consequent delay in regeneration of the ciliated epithelium.

Pneumonia associated with influenza virus infection may result from primary viral infection, bacterial superinfection, or combined bacterial-viral infection.[185] Fatal primary influenza pneumonia, fortunately a relatively rare occurrence in children, is characterized at autopsy by diffuse hemorrhagic alveolar exudates, necrosis of bronchiolar epithelium, peribronchial lymphocytic infiltration, and marked lymphocytic infiltration of the alveolar walls and interstitial lung tissue.[208]

Although the major pathologic process in influenza occurs in the respiratory tract, focal and diffuse myocarditis, mediastinal lymph node disorganization and necrosis, and diffuse cerebral edema also have been noted in fatal cases. The pathologic entity of diffuse encephalopathy and fatty degeneration of the liver (Reye syndrome) has been established as a complication of influenza, particularly type B, in children.[178] Reye syndrome is associated with administration of salicylate-containing products to children with influenza or varicella and has declined in incidence as the use of salicylates has decreased.[143] Encephalopathy and encephalitis associated with influenza have been reported frequently in Japan, where an estimated 100 children die each year.[271] Recognition of this complication is increasing in the United States and warrants consideration for universal immunization of children.[189] The hypotheses for pathogenesis include direct viral invasion of the central nervous system and inflammation secondary to high levels of proinflammatory cytokines.[147]

IMMUNOLOGIC EVENTS

Immunity against influenza results from a complex interplay of humoral, secretory, and cell-mediated mechanisms. Because of the brief incubation period of the disease, anamnestic stimulation of antibody affords little protection. Thus, some degree of preexisting antibody appears to be essential to prevent infection. The

sequential point mutations of the HA and NA gene segments result in antigenic changes that allow the virus to evade immunity generated by previous infections. The major antigenic changes that accompany antigenic shifts render total populations susceptible and account for pandemic spread.

After natural influenza infection occurs, both mucosal and humoral antibodies are elicited against HA, NA, nucleocapsid, and matrix protein antigens. Antibodies to the HA are critical for neutralization of virus. Antibodies against NA are associated with diminished severity of illness and reduced rates of person-to-person transmission. Antibodies against nucleocapsid and matrix proteins do not appear to provide protection or to modify transmission. Because influenza is a respiratory epithelial surface infection rather than a systemic infection, some uncertainty exists about the relative degree of protection afforded by mucosal and humoral antibodies. Studies in human volunteers demonstrated good protection against experimental challenge by influenza A/HK/1/68 in subjects who possessed serum antibodies but lacked detectable mucosal antibodies.[46] Subjects challenged by influenza B/Eng/65 also demonstrated a better correlation of protection with serum antibody levels than with nasal antibodies.[63] Moreover, in a large experience with volunteers that was summarized by Hobson and associates,[136] an impressive linear correlation between serum hemagglutination-inhibiting antibody titers and protection was evident. Thus, although an important role for local antibodies as a "first line of defense" against influenza is accepted, serum antibodies clearly contribute to resistance.

Waldman and colleagues[299] demonstrated that the predominant influenza-neutralizing antibody in nasal secretions is secretory immunoglobulin A (IgA), whereas the predominant neutralizing antibody in tracheobronchial secretions is IgG. A synthesis of the available data suggests that local secretory IgA in nasal secretions may be important in the prevention of infection that is transmitted by droplet spread and that originates in the upper respiratory tract. Serum and local IgG antibodies appear to be of greater importance in neutralizing infection transmitted directly to the lower respiratory tract by aerosol or in preventing extension of upper respiratory tract infection to the lungs.[320]

Cell-mediated immune mechanisms of several varieties have been demonstrated in influenza infection and vaccination. A T-cell "helper" function was demonstrated in strain-specific humoral antibody response against HA.[298] Both nonspecific and specific mechanisms of lymphocyte cytotoxicity were found in model systems,[67] but only type-specific cytotoxic T cells were found in humans.[18,24,65] Cytotoxic T lymphocytes are important for recovery from infection in susceptible hosts. Antibody-dependent cell-mediated cytotoxicity was shown to correlate with the titer of serum anti-HA antibody.[111] This mechanism also may play a role in recovery.[57,267]

The immunologic imprint made by the first influenza infection of childhood has lasting effects. According to the "doctrine of original antigenic sin," developed by Francis and colleagues[77] from seroepidemiologic data, hemagglutination-inhibiting antibody against the strain of first infection is recapitulated with each subsequent infection by an antigenically distinctive strain of influenza A. Since 1957, however, researchers have found that infections or immunizations with H2N2 or H3N2 strains do not recall antibody against earlier strains in persons primed by H1N1 viruses.[188] Therefore, the doctrine holds only within groups of influenza A strains possessing some degree of homology of antigens. Virelizier and associates[297] analyzed the basis for the phenomenon using irradiated mice immunologically reconstituted with bone marrow from immune donors. These studies indicated that antigenic "original sin" derives from cross-stimulation of a population of committed memory B lymphocytes that persist after primary infection.

After natural infection occurs, the duration of immunity and the degree of protection against challenge by heterologous variants appear to be variable. On the basis of clinical experience in a Yorkshire general practice, Pickles and associates[236] concluded that natural immunity against influenza A strains lasted at least 4 years. Conversely, serologic reinfection rates of 2 and 12 percent against influenza A and influenza B, respectively, were noted by Hall and colleagues[119] during a 3-year observation period in children in Seattle. Some protection against the re-emerged influenza A (H1N1) virus of 1977 was noted for persons who had been infected with a similar virus in the 1950s.[105] The cross-immunity was lost gradually as new H1N1 variants appeared since 1977, a finding demonstrating a divergence in antigenic properties of variants that appeared after 1950 and 1977.

Frank and colleagues[78,81] reported that variations in reinfection among young children may be multifactorial but, most important, may be related to the age at first infection and the antigenic differences of virus variants in subsequent challenges. Thus, protection against clinical disease appears to persist for many years after influenza infection occurs in older children but may be of much shorter duration in infants and very young children; subclinical reinfection probably occurs after much shorter intervals. A gradual increase in duration and breadth of immunity against related virus strains probably occurs over a period of years. Frank and associates[82] found that, unlike the findings for influenza A (H3N2) infections, infection with influenza B virus provided consistent protection for most persons against infection with the next influenza B variant. The protection decreased somewhat for subsequent variants and longer intervals. Beginning in 1987, two antigenically distinct lineages of influenza B have been prevalent.[33] These lineages are identified as the B/Yamagata/16/88 lineage and the B/Victoria/2/87 lineage. Generally, infections with viruses of one lineage fail to provide good cross-protection against infection with the other, especially for children with limited prior experience.

CLINICAL MANIFESTATIONS

Disease caused by epidemic influenza A virus may occur in persons of all ages and results in a febrile respiratory illness. In contrast, although other respiratory tract viral agents (respiratory syncytial virus [RSV], rhinoviruses, parainfluenza viruses) also may cause community epidemics that involve both children and adults, the illness is different in the two age groups; young children with primary viral infections with non-influenzal agents have febrile illnesses, whereas older children and adults with similar infections most commonly have common colds and other upper respiratory tract involvement with little or no fever.[90,94]

The symptoms and signs in children and adults of type A influenza caused by the Asian subtype (H2N2) are compared in Table 190–2.[150] The following findings have occurred significantly more frequently in children: sudden onset, anorexia, abdominal pain, vomiting, nausea, cervical adenopathy, and temperature higher than 38.9° C. Influenza C viruses cause illnesses similar to influenza A infection, but the severity of disease usually is less and the duration shorter. In addition, because antigenic changes in influenza B viruses are less frequent, illnesses may be milder in adults. Influenza B may cause an epidemic in which children will have typical influenza with fever, but many adults in the population will have only upper respiratory tract[101] illnesses without significant fever. In other outbreaks caused by antigenically variant influenza B virus, significant illness has been identified in adults.[102,121]

OLDER CHILDREN AND ADOLESCENTS (CLASSIC INFLUENZA)

School-age children have the highest attack rates during influenza epidemics. School absentee rates and visit rates for health

TABLE 190–2 Frequency of Symptoms and Signs of Proven Influenza A (H2N2) in Children (0-14 Years of Age) and Adults*

	Percentage with Symptoms or Signs	
	Children (N = 95)	Adults (N = 30)
Symptoms		
Sudden onset	66*	46
Systemic symptoms		
Feverishness	93	71
Headache	81	72
Anorexia	69*	37
Malaise	68	67
Chilliness	37	64†
Myalgia	33	62†
Respiratory symptoms		
Cough	86	90
Nasal discharge	67	82
Sore throat	62	62
Nasal obstruction	54	52
Sneezing	38	67†
Hoarseness	22	37
Sputum production	19	41†
Other symptoms		
Abdominal pain	31*	0
Vomiting	26*	7
Nausea	23*	4
Diarrhea	2	0
Signs		
Maximum temperature		
≤37.7° C	11	13
37.8° C to 38.8° C	29	58†
≤38.9° C	60*	29
Conjunctival abnormalities	61	56
Pharyngeal injection	60	68
Nasal injection/edema	50	64
Nasal discharge	38	20
Cervical adenopathy	38*	8
Rhonchi or rales	2	0
Pharyngeal exudates	1	0

**Significantly more frequent in children* ($p < .05$, *Fisher exact test).*
†Significantly more frequent in adults ($p < .05$, *Fisher exact test).*
Data from Jordan, W. S., Denny, F. W., Badger, G. F., et al.: A study of illness in a group of Cleveland families. XVII. The occurrence of Asian influenza. Am. J. Hyg. 68:190-212, 1958.

TABLE 190–3 Relative Frequency of Symptoms and Signs during Classic Influenza in Older Children and Adolescents

	Occurrence*
Symptoms	
Chilly sensation	++++
Cough	+++
Headache	+++
Sore throat	+++
Prostration	++
Nasal stuffiness	++
Dizziness	+
Eye irritation or pain	+
Vomiting	+
Myalgia	+
Signs	
Fever	++++
Pharyngitis	+++
Conjunctivitis (mild)	++
Rhinitis	++
Cervical adenitis	+
Pulmonary rales; wheezes or rhonchi	+

**++++, 76 to 100 percent; +++, 51 to 75 percent; ++, 26 to 50 percent; and +, 1 to 25 percent.*
From Cherry, J. D.: Influenza viral infections. In *Vaughan, V., McKay, R., and Behrman, R. (eds.): Nelson Textbook of Pediatrics. 14th ed. Philadelphia W. B. Saunders, 1987, pp. 675-678.*

care are high. Even uncomplicated illnesses significantly disrupt family activities.[218] The symptoms and signs of classic influenza in older children and adolescents are presented in Table 190–3. The onset of illness is abrupt, with fever and associated flushed face, chills, headache, myalgia, and malaise.[30,243,252,268] The temperature ranges between 39° C and 41° C (102° F and 106° F), with a general inverse correlation with age. The systemic symptoms are reported to be more severe in older children, probably because of the children's ability to describe them. Although a dry cough and coryza also are early manifestations of influenza, these symptoms may be overlooked in the early stages because of the predominance of the systemic manifestations. Sore throat occurs in more than half of the cases and usually is associated with nonexudative pharyngitis. Ocular symptoms include tearing, photophobia, burning, and pain with eye movement.

In uncomplicated illness, the fever usually persists for 2 to 3 days but may last as long as 5 days. A biphasic temperature pattern may occur, even without apparent secondary bacterial complications. By the second to the fourth day, respiratory tract symptoms become more prominent, and the systemic complaints begin to subside. The cough is dry and hacking and usually persists for 4 to 7 days; cough, in association with some degree of general malaise, occasionally persists for 1 or 2 weeks after the rest of the illness has subsided. Illness caused by influenza B virus generally is associated with more prominent nasal and eye complaints and fewer systemic findings, such as dizziness and prostration, than is influenza A illness. In a study in which both influenza A (H1N1) and influenza C were noted in a population of young adults, the illnesses could not be differentiated by clinical findings.[64]

In uncomplicated classic influenza, the leukocyte count most often is normal, but leukopenia (<4500 cells/mm^3) has been noted in approximately 25 percent of cases. The differential cell count is of no diagnostic value because approximately one third of patients will have normal values, one third will have relative lymphopenia, and one third will have relative neutropenia. The relative proportion of each white blood cell type may depend on the time during the course of infection when the differential count is determined.[167] Approximately 10 percent of older children and adolescents have clinical signs and radiographic evidence of pulmonary involvement.

YOUNGER CHILDREN

General Considerations

Clinical expression of influenza in younger children and infants has been studied intensively, a reflection of increasingly sensitive and specific respiratory tract viral diagnosis. With some exceptions,* most studies have contained disproportionate numbers of hospitalized patients† and thus may tend to exaggerate the more severe end of the influenza spectrum.

In younger children, the manifestations of influenza viral infections frequently are similar to those resulting from other respiratory tract viruses (parainfluenza, RSV, rhinovirus, and adenovirus) (Table 190–4). Laryngotracheitis, bronchitis, bronchiolitis, pneumonia, and upper respiratory tract illness all occur.[160]

*See references 73, 75-78, 102, 141, 166, 182, 264.
†See references 27, 32, 90, 138, 181, 186, 207, 222, 231, 246, 295, 317.

TABLE 190–4 Relative Frequency of Clinical Manifestations of Influenza Viral Infections in Children Younger Than 5 Years

	Occurrence*
Major Clinical Category	
Upper respiratory tract illness	++++
Laryngotracheitis	+
Bronchitis	+
Bronchiolitis	+
Pneumonia	+
Symptoms	
Cough	++++
Anorexia	++
Coryza	++
Vomiting	++
Diarrhea	+
Sore throat	+
Signs	
Fever	++++
Pharyngitis	+++
Cervical adenitis	++
Otitis media	++
Convulsions	+
Exanthem	+
Generalized adenitis	+

*++++, *76 to 100 percent;* +++, *51 to 75 percent;* ++, *26 to 50 percent; and* +, *1 to 25 percent.*

From Cherry, J. D.: Influenza viral infections. In *Vaughan, V., McKay, R., and Behrman, R. (eds.): Nelson Textbook of Pediatrics. 14th ed. Philadelphia, W. B. Saunders, 1987, pp. 675-678.*

Clinical descriptions of these illnesses are presented in other chapters. The overall rate of hospitalization of children with lower respiratory tract involvement is the same for influenza virus infections as for other viruses, but influenza tends to affect older children.[92]

Primary infection with influenza A in these age categories typically is seen as an undifferentiated febrile upper respiratory tract illness.[314] Infants younger than 2 months old frequently are hospitalized with fever to rule out bacterial sepsis, particularly when influenza A (H3N2) viruses are epidemic.[107] Fever tends to be high, and temperature exceeds 39.5° C in most patients. Affected children appear moderately toxic, with clear nasal discharge, cough, and irritability as almost constant findings. Pharyngitis usually is present, with diffuse erythema and boggy, enlarged tonsillar tissue. Acute otitis media is a frequent complication and has been found in 30 percent of those patients with laboratory confirmation of disease.[95] Between 5 and 10 percent of those infected have some degree of pulmonary involvement; in hospitalized children, this percentage may be as high as 50 percent. Fleeting erythematous, macular, or maculopapular discrete rashes have been observed.

Because of the explosive nature of influenza epidemics, clinics that provide urgent care may be severely taxed during outbreaks.[209,220,244] Preschool children present to health care facilities in large numbers, with resulting long waiting periods for care. Universal vaccine recommendations may reduce this burden, but these recommendations should be combined with rapid antigen detection tests and early intervention with antiviral treatments to reduce morbidity and reduce spread of influenza to contacts.[23,223,245]

Gastrointestinal Symptoms

In contrast to illness patterns in older children and adults, gastrointestinal symptoms have been noted in several studies of influenza infection in young children. Among 68 children admitted to the hospital during a community epidemic of influenza B/Hong Kong/72 in 1974, Kerr and colleagues[159] encountered acute abdominal pain, with minimal associated respiratory tract symptoms, as the presenting complaint in 37 patients. This symptom was noted most frequently in children 4 to 10 years of age and led to performing unnecessary laparotomies in two patients. In infants, infection by influenza A virus may elicit diarrhea and vomiting. Of 18 infants aged 6 months or younger with proven influenza A/HK/68 infections, Price and associates[248] noted prominent anorexia, diarrhea, or vomiting in 13 (72%), and only 5 (23%) had respiratory tract symptoms alone. Five of the infants with gastrointestinal disturbance had moderate to severe dehydration. Similarly, among 53 hospitalized infants younger than 1 year old with infections of influenza A, Paisley and associates[228] found diarrhea to be a prominent symptom in 18 patients (34%). Thus, unlike adults with influenza, infants and young children indeed may display "gastric flu."

Febrile Convulsions

Febrile convulsions precipitated by fever of abrupt onset have been cited as common presenting complaints in several studies of hospitalized children with influenza. Among 75 children with infections by influenza A/Hong Kong/68 (H3N2), 26 (35%) of the patients described by Price and associates[248] presented with a febrile convulsion. Of the 77 hospitalized children described by Brocklebank and colleagues,[27] 31 (40%) had convulsions at onset; 27 of the children in this series were 3 years of age or younger, a distribution consistent with the usual age-specific susceptibility pattern of febrile seizures. In a large series from Hong Kong, almost 20 percent of children admitted with influenza virus infection had febrile seizures.[35]

Croup

Acute laryngotracheobronchitis (croup) has been noted as a prominent feature of influenza A in young children during the H3N2 era. Illness tends to be more severe than is the rule for the croup syndrome induced by parainfluenza viruses, and tenacious tracheal secretions may necessitate tracheostomy or endotracheal intubation in a higher proportion of hospitalized patients. During the peak month of a composite of 13 consecutive influenza A outbreaks, influenza A virus was demonstrated in 68 percent of patients with croup who were hospitalized in Washington, D.C.[150] Severe croup caused by influenza virus infection may occur in children at an older age than that of those usually infected with parainfluenza viruses.[66,142,246]

NEONATES

In neonates, influenza infection may suggest bacterial sepsis: lethargy, poor feeding, petechiae, poor peripheral circulation with mottling of the skin, and apneic spells. Nosocomial influenza A outbreaks have occurred in neonatal nurseries in association with symptomatic illnesses among nursery staff and parents.[8,196,210] Six of the eight infants in one study had apneic spells.[196] Two infants required mechanical ventilation for frequent apneic spells for a period of 6 to 8 days.

HIGH-RISK CHILDREN

Other host characteristics generally considered to influence the clinical expression of influenza include preexisting chronic pulmonary, cardiac, and neuromuscular disease. Most deaths occur in these vulnerable patients.[292] The basis for this doctrine, however, is derived mainly from pathologic and epidemiologic analysis of influenza in adult patients,[132] but several studies

addressed the vulnerability of children with underlying conditions. Among the 77 hospitalized children with influenza A described by Brocklebank and colleagues,[27] 13 of 23 (56%) with chronic diseases or congenital malformations developed lower respiratory tract infection, compared with 10 of 54 (19%) children without preexisting chronic conditions. Kempe and colleagues[158] studied influenza virus infection in children with malignant neoplasms and found that affected children had both more frequent and more severe influenza-related illnesses than did their healthy contacts or age-matched controls. Neuzil and colleagues found influenza-attributable hospitalization rates of 19.2, 7.6, and 2.3 per 1000 for children younger than 1, 1 to 2, and 3 to 14 years of age, respectively, over a period of 19 years.[221] The rates for influenza-related outpatient visits were 122, 202, and 126 per 1000 children and for antibiotic courses were 65, 141, and 125 per 1000 children, respectively, for the same age groups. Asthma was the most common underlying condition among these children.

The few comprehensive virologic studies conducted in asthmatic children yielded conflicting results. In a longitudinal study of young children (<3 years) hospitalized for prolonged periods because of severe extrinsic asthma, McIntosh and associates[194] serologically documented 11 episodes of infection by influenza A/Hong Kong/68, all of which were mild and none of which was associated with exacerbations of wheezing. Studies of pediatric outpatients who were more than 3 years old and who had intrinsic asthma found that influenza virus infections played important roles in triggering asthma attacks. A population-based study in Houston found that influenza was the most common infection triggering hospitalization of school-age children with asthma; 21 percent of all hospitalizations during a 4-year period were associated with influenza virus infection.[104] Other investigators documented influenza virus infections with exacerbations of asthma in children.[200,201,258] A retrospective cohort study showed that influenza vaccine protects against severe exacerbations in asthmatic children after adjustment for asthma severity.[170] A controlled trial in the Netherlands found no reduction of laboratory-proven asthma exacerbations by the use of the inactivated vaccine.[29] In contrast, a direct comparison of live attenuated influenza vaccine (LAIV) and inactivated vaccine showed superior protection with the live vaccine in school-age asthmatic children.[72] Therefore, the preponderance of evidence from clinical studies incriminates influenza as an important instigator of asthma attacks in children.[97]

DIAGNOSIS

Infection with influenza virus often can be deduced more accurately from epidemiologic features than from clinical presentation. Epidemics occur each winter and usually begin with a sudden increase in presentation to primary care facilities of school-age children with febrile respiratory tract illnesses.[91] Routine laboratory studies provide little help in the differentiation of influenza from other viral respiratory tract diseases. Serial monitoring of induced infections in adults has revealed a characteristic moderate increase in total white blood cell count, with relative lymphopenia, during the height of symptoms and low serum iron values.[58,60,69] In children, however, hematologic manifestations are variable, with marked leukocytosis frequently observed in infants.[115] Chest radiographs are useful primarily for determining the presence of complicating interstitial or lobar pneumonia. Transient alterations in pulmonary function tests have been documented in a high percentage of normal adults with uncomplicated influenza.[120] Thus, oxygen saturation determinations may be useful in children with influenza and clinical evidence of lower respiratory tract involvement, even if chest radiographs do not show infiltrates.

Definite diagnosis of influenza depends on detection of antigen or isolation of virus from respiratory tract secretions or a significant rise in serum antibody during convalescence. In contrast to shedding of adenoviruses or herpes simplex virus from the respiratory tract, asymptomatic carriage of influenza viruses is a rare occurrence. Thus, detection of virus alone is considered conclusive evidence of the cause of an illness. Hemagglutinating agents often can be detected in embryonated eggs, Madin-Darby canine kidney, or primary monkey kidney tissue culture within 72 hours of inoculation. However, longer incubation and serial passage are required before cultures can be regarded as negative.[61] Rapid detection of influenza antigens in nasopharyngeal epithelial cells with specific fluorescent antibody conjugates has been successful in many studies.[180,226] A combined approach employs short-term incubation of clinical specimens in tissue culture, followed by rapid identification of hemadsorbing agents with fluorescent antibody.[9] Shell vial techniques that include low-speed centrifugation of the clinical specimen onto a tissue culture monolayer and detection of virus with fluorescent antibodies after a short (<72 hours) incubation period provide rapid confirmation of influenza infection. Enzyme immunoassay can be used for early detection of influenza A antigen.[59,301] When this information is available to the clinician in a timely fashion, antibiotic use is curtailed, and the use of specific antiviral treatment is enhanced.[215] Tests that detect both influenza A and B are now available in primary care settings.[223-225] Other studies confirmed the utility of rapid identification of influenza virus infections.[23,245,270] Usually, clinicians are not taking advantage of the opportunity to apply specific treatment.[244] Further advances in diagnosis of influenza result from incorporation of techniques for RT-PCR. PCR provides greater sensitivity (95%) than do other antigen detection methods (usually 60% to 75%).[4] Multiplex quantitative RT-PCR may allow identification of all the important respiratory viruses of children: influenza, parainfluenza, and RSV.[68,131]

Serologic diagnosis may be accomplished by use of complement-fixation or hemagglutination-inhibition techniques.[61] The complement-fixation test detects antibody against the "soluble" nucleoprotein antigens that are common to all strains of influenza A or influenza B. Reagents are commercially available, and the test is provided by most clinical laboratories. Complement-fixing antibodies are of relatively brief duration; titers wane within 6 months of infection. Hemagglutination-inhibiting antibodies, which are subtype-specific, provide more definitive evidence of infection. However, subtype-specific reagents are required and are less widely available in clinical laboratories. Titers persist for years and are boosted by infection with related strains. The hemagglutination-inhibition test has the added advantage of greater sensitivity. Neutralizing antibodies correlate best with protection against reinfection and are more sensitive indicators; the microneutralization test is preferred for evaluation of vaccine effectiveness.[80] Rises in NA-inhibiting antibody also may be detected after infection, but such studies are technically cumbersome and are restricted to specialized applications. A versatile method for measuring antibodies against influenza antigens employs ELISA.[19,215] This system is technically simple and has the advantage of permitting specific identification of IgA and IgM antibodies as well as IgG subclasses. It is particularly useful for measuring antibodies in respiratory secretions that reflect mucosal immunity.

COMPLICATIONS

BACTERIAL INFECTIONS

The most frequent complications of influenza are bacterial infections of the respiratory tract, particularly pneumonia, otitis media, and sinusitis.[70,130,179,185,202,228,269,282] Characteristically, these

complications arise in early convalescence, with bacterial invasion of portions of the respiratory tract resulting in denuded ciliated epithelium and defective mucociliary transport. In 37 young infants experiencing their first infections by influenza A viruses, Wright and colleagues[314] noted otitis media in 10 and pneumonia in 7. Hall and Douglas[115] documented complicating bacterial pneumonia in 5 of 12 patients who developed nosocomial infection by influenza A on an infant ward; most of these patients had underlying chronic cardiorespiratory disease. The incidence of complicating bacterial infections in community studies, including children of all ages, is approximately 10 percent, and otitis media is the most frequent finding.[150] A 14-year longitudinal study of young children demonstrated a 28 percent incidence of otitis media after influenza A and B virus infections and an increased risk of recurrent disease.[130] Studies of vaccine effectiveness support reports of influenza-associated acute otitis media of 30 to 40 percent for children in daycare during the respiratory disease season.[95]

Although most cases of bacterial pneumonia complicating influenza are pneumococcal,[269] the two most feared pulmonary complications are progressive primary viral pneumonia and staphylococcal pneumonia.[185] Progressive primary viral pneumonia has been observed most frequently in adult patients with preexisting rheumatic heart disease, but it may occur in previously healthy children as well.[148,151,220,228] It is characterized radiographically by diffuse bronchopneumonic infiltrates and clinically by intense dyspnea and a relentless downhill course, despite administration of antimicrobial and supportive therapy. Staphylococcal pneumonia may occur as a postinfluenzal lobar pneumonia progressing to pneumatoceles and empyema. More characteristically, staphylococcal pneumonia in association with influenza occurs as a fulminant, synergistic, viral-bacterial process with diffuse involvement on radiographs, leukopenia, intense dyspnea, blood-tinged sputum, and rapid death. Necrotizing pneumonitis with microabscesses and positive lung cultures for both influenza A virus and *Staphylococcus aureus* are characteristic.[185]

ACUTE MYOSITIS

Acute myositis occurs in the setting of early convalescence from a typical influenzal illness.[7,55,199] Severe pain and tenderness in the calves of both legs come on suddenly, and the patient often refuses to walk. Other muscle groups may be involved as well, but the gastrocnemius and soleus muscles are affected in virtually all cases. Elevated levels of serum creatine kinase and aspartate aminotransferase are characteristic. Influenza B virus was isolated in 20 of 26 such cases by Middleton and associates[199] and in 11 of 17 cases by Dietzman and colleagues.[56] Infection by influenza A was documented in one case of the former series. The condition generally is self-limited, but rhabdomyolysis with myoglobinuria and acute renal failure has been described in severe cases occurring in association with influenza A.[37,55,157]

ENCEPHALOPATHY AND REYE SYNDROME

Encephalitis or encephalopathy is a common complication of influenza in children.[10,54,193] Alarming reports of the occurrence of encephalitis and encephalopathy in young children emerged from Japan beginning in 1995.[271,283] This timing coincided with the discontinuation of the influenza vaccine program in Japanese schoolchildren.[254] More than 200 cases per year have been reported, and more than 100 cases have been fatal or associated with permanent neurologic sequelae.[154] Evidence of influenza virus infection has come from isolation of virus or PCR detection in the cerebrospinal fluid (CSF).[85,147] The usual presentation is sudden onset of fever with convulsions followed by progression to a comatose state. In older children, the onset may be more subtle, with headache and disorientation. Since the outbreak associated with influenza B virus infection was reported in Chicago in 1971,[137] the reports in the United States have become more frequent.[187,242] The pathogenesis of these illnesses is unknown but may result from direct viral invasion of the central nervous system or from high levels of proinflammatory cytokines that breach the blood-brain barrier.[147,205]

Reye syndrome is a condition of obscure pathogenesis characterized by fatty degeneration of the liver and diffuse cerebral edema.[255] Although the condition has been recognized under varying nomenclatures since its partial description by Brain and colleagues[25] in 1929, only more recently was influenza infection implicated as an inciting factor. Of 85 patients treated at Cincinnati Children's Hospital, 74 (87%) had a respiratory tract prodrome clinically indistinguishable from influenza, and 11 patients (13%) had varicella.[232] During the 1974 outbreak in Cincinnati, influenza B/HK/8/73 was isolated from the nasopharyngeal secretions of 9 of 23 affected children, and an additional 3 children had serologic evidence of influenza B.[178] Numerous other reports demonstrated similar associations of influenza A (H1N1 and H3N2) as well as influenza B with subsequent outbreaks of Reye syndrome.[43,125,146,285,310] Surveillance data indicate a significant decline in both the incidence and the mortality ratio.[2,5] In a survivor of Reye syndrome, who was studied intensively by Partin and colleagues,[232] influenza A/Vic/3/75 (H3N2) virus was recovered from nasotracheal secretions, CSF, liver, and skeletal (gastrocnemius) muscle, findings that led the investigators to postulate visceral dissemination of virus as an element in the pathogenesis of this condition. Clinically, Reye syndrome, most common in white, male, school-age children, is marked by nausea, vomiting, and stupor during convalescence from a viral illness, most commonly characterized by respiratory tract symptoms.[2,5] In this setting, the finding of elevated serum transaminase and blood ammonia levels, with unremarkable CSF, is sufficient for establishing the diagnosis. During the 1974 outbreak, Reye syndrome was estimated to have occurred at a rate of 31 to 58 cases per 100,000 infections with influenza B in children,[43] although a lower incidence was reported among Colorado children in association with the H1N1 outbreak of 1978 to 1979.[125] The case-fatality ratio is 26 percent.

An ever-increasing body of data reveals a strong association between the use of salicylates or salicylate-containing medications and Reye syndrome, and as a result, strict warnings have been issued against the use of salicylates in children with influenza.[124,143,281,300,319] The decreased use of aspirin for children with influenza and varicella has paralleled the decline in the number of reported cases of Reye syndrome.[2]

OTHER COMPLICATIONS

Neurologic Disease

Apart from encephalopathy or Reye syndrome, other severe neurologic illnesses have been reported rarely in association with influenza viral infections.[197,263] As noted earlier, the most common manifestation is encephalitis in association with a respiratory tract illness. In most instances, no evidence of concomitant meningitis is present. Guillain-Barré syndrome and transverse myelitis also have been associated with influenza.[304]

Cardiac Disease

Pericarditis and myocarditis have been noted rarely in association with influenza viral infections.[42,48,202,282] Cardiac involvement has occurred in normal children and adults as well as in those with preexisting heart disease.

Sudden Deaths

During influenza epidemics, sudden, unexpected deaths are observed occasionally. They occur in persons of all ages, and postmortem examination most frequently indicates respiratory tract involvement. Cases of sudden infant death syndrome have been associated with influenza viral infection.[62,286]

Other Observations

Glomerulonephritis and renal failure have been associated with influenza viral infections.[306,308] In two instances, acute parotitis occurred in patients with influenza A infection.[22] Although evidence of the transplacental passage of influenza virus to a 30-week-old male fetus was reported,[318] no epidemiologic evidence of influenza virus teratogenicity is known.[187,309]

TREATMENT

SPECIFIC THERAPY

Specific treatment has not been used routinely for children in the United States. With the development of rapid tests for influenza diagnosis, treatment should be considered. Early treatment has the potential not only to shorten the course and reduce complications, but also to reduce spread of the virus to contacts.

The antiviral agents amantadine and rimantadine are active in vitro against influenza A viruses and have been shown to provide prophylactic and therapeutic benefit in both adults and children.[40,44,49,118] Amantadine acts by blocking the function of the M2 protein, the ion channel that functions to facilitate fusion of viral and cell membranes and permits entry of viral RNA into the host cell.[302] Amantadine lacks activity against influenza B viruses. Numerous pediatric studies also have documented the prophylactic effect of amantadine or its analogue, rimantadine, against community-acquired influenza A.[40,49,233,259,260] Few pediatric treatment trials have been performed.[71,165] The use of rimantadine, administered to young children with limited prior experience with the infecting subtype, resulted in the emergence of resistant viruses.[15,118] Amantadine-rimantadine resistance of influenza A virus results from point mutations of the M2 protein.[11,15] In some instances, the resistant viruses have been noted to infect susceptible contacts.[127] Until 2005, fewer than 1 percent of field isolates of influenza A were found to be resistant to rimantadine.[323] A dramatic change occurred in 2005 when the CDC reported that approximately 90 percent of influenza A viruses were resistant to amantadine; use of the M2 inhibitors was discouraged for the 2005 to 2006 season. The mutability of influenza viruses dictates sustained surveillance of sensitivity of circulating influenza A viruses because of the regular introduction of new strains. In any case, precautions should be taken to limit contact between young patients being treated with M2 inhibitors and subjects receiving prophylaxis.[203] This situation allows transmission of resistant viruses.

The armamentarium of antiviral drugs for influenza has been supplemented by the addition of NA inhibitors. The inhaled inhibitor zanamivir is approved for use in children 5 years of age and older.[129] Early treatment with zanamivir twice daily shortened the course of illness by 1.25 days compared with placebo. Zanamivir-treated children resumed normal activities 1 day sooner than did placebo recipients and used less relief medication than did placebo recipients. Adverse events occurred with comparable frequency in treated patients and controls. The drug is effective against both influenza A and B, and no resistant viruses were detected with treatment. The inhaled route of administration results in delivery of drug to the site of infection, but this route limits utility in young children unable to use the inhaler and may not be tolerated by persons with reactive airway disease (RAD). If this drug is used for patients with RAD, short-term bronchodilators should be available.

An oral NA inhibitor, oseltamivir, is efficacious for treatment of both influenza A and B in children as young as 1 year of age.[307] In children given a dose of 2 mg/kg twice daily for 5 days starting early in the illness, the resolution of influenza was accelerated by 1.5 days compared with controls. The duration of viral shedding and the quantity of virus in respiratory secretions were significantly decreased in treated children. The incidence of acute otitis media was reduced by 44 percent in the treated children, a medically significant benefit that was reflected in decreased use of antibiotics. The frequency of vomiting in treated children was 5 percent in excess of that in placebo recipients but usually did not result in cessation of therapy. Nausea was reduced by administration with food. Viral isolates with higher than pretreatment values for 50 percent inhibitory concentration for NA inhibition were detected in 5.5 percent of oseltamivir-treated children with influenza A infection; no increased resistance was found for influenza B isolates. In Japan, where oseltamivir has been used frequently to treat children, influenza B infections have been less responsive to treatment with oseltamivir.[155] The clinical significance of this observation is unknown; resistant strains generally have been less virulent and less transmissible in laboratory studies.

The broad-spectrum antiviral agent ribavirin was used successfully in the treatment of both influenza A and B in adult patients when it was administered as an aerosol.[167,169,192,311] Experience with this treatment modality in children is limited mainly to other viruses, but use of this drug has resulted in no significant adverse reactions.[88,117,195,257] Combination therapy has been recommended by some investigators who have shown increased benefit by simultaneous treatment with both M2 and NA inhibitors.[110] This approach may be especially important if a new pandemic strain emerges; if so, the possibility of combining ribavirin with one of the other drugs may be an important option for treatment of patients hospitalized with severe infections.

PROGNOSIS

The prognosis for clinical recovery from uncomplicated influenza generally is considered excellent. Of the complications of influenza, primary influenza pneumonia, staphylococcal pneumonia, encephalitis, and Reye syndrome have a guarded prognosis. However, a bewildering array of chronic pulmonary conditions has been noted to begin with undifferentiated childhood respiratory tract infections, among which "influenza" often is cited but infrequently proved.[174,265] These conditions include the following: lobar atelectasis; localized and generalized bronchiectasis; and such clinicopathologic entities as Swyer-James syndrome (unilateral hyperlucent lung), bronchiolitis obliterans, Hamman-Rich syndrome (diffuse interstitial pneumonia), and desquamative interstitial pneumonitis. Only precise virologic diagnosis of acute respiratory tract disease, especially viral pneumonia, will delineate the long-term complications that may occur in a small proportion of cases. Universal immunization in healthy children will also assist in sorting out rare outcomes by reducing disease occurrence.

PREVENTION

Immunization offers the best hope for prevention of influenza. Influenza is an uncontrolled epidemic disease that is vaccine-preventable. Despite the availability of effective vaccines, excess mortality and hospitalizations attributable to influenza have continued to rise. To be effective, vaccines must contain antigens

similar to those of the prevalent influenza viruses. Predicting the epidemic potential of new influenza virus variants is difficult. In years when a new variant arises and causes widespread outbreaks, the available vaccine may contain the previous variant with only modest heterologous immunizing potential. Conversely, in years in which new variants do not arise, vaccines may be formulated ideally, but the epidemic potential of virus strains that have already circulated may be diminished, although this is not always true. The A/Sydney (H3N2) variant that predominated from 1997 to 2000 produced severe epidemics for three consecutive seasons. Thus, prevention of epidemics through the use of inactivated vaccines has been limited. Currently, two types of influenza vaccines are licensed in the United States—the traditional inactivated vaccine given by intramuscular injection and the LAIV administered by nasal spray.

INACTIVATED INFLUENZA VACCINE

Many improvements have been made in the formalin-inactivated influenza vaccines since their introduction in the late 1930s.[198] These innovations have included enhanced vaccine production by use of high-yield reassortant viruses that grow rapidly in eggs,[230] exclusion of host antigens and other toxic impurities by zonal ultracentrifugation (current "whole-virus" vaccines),[206] disruption of viral particles with ether or detergents (current "split-product" vaccines),[51,113] and, most recently, physical purification of HA and NA (HANA or "subunit" vaccines).[177]

The antigenicity and reactogenicity of whole-virus and split-product vaccines were studied extensively in children during the trials of A/New Jersey/76 (H1N1) monovalent and A/New Jersey/76 (H1N1)-A/Victoria/75 (H3N2) bivalent vaccines in 1976.[316] Whole-virus vaccines were more immunogenic and, at the same time, more reactogenic than were split-product vaccines because they contained a greater concentration of antigens. In two-dose regimens, the split-product vaccines produced adequate antibody levels without acute reactions. The A/Victoria/75 component of the bivalent vaccines had significantly greater immunogenicity than did the A/New Jersey/76 component in children, a finding that reflects previous priming by H3N2 viral strains but a lack of previous exposure to H1N1 viruses. The consensus of these studies was that split-product vaccines, by virtue of their minimal reactogenicity, should be preferred for vaccination of children. Additional large-scale trials in 1978 evaluating monovalent A/USSR/77 (H1N1) and trivalent A/USSR/77 (H1N1), A/Texas/77 (H3N2), and B/Hong Kong/72 inactivated influenza virus vaccines were in agreement with previous results.[312] Protective efficacy, which with whole-virus vaccines had ranged from 50 to 95 percent after homologous challenge,[162] was comparable for split-product vaccines,[45,112,237,250] even against antigenic drift virus strains.[45,237] Gruber and associates[114] reported a protective rate of 60 percent with B/USSR/83 vaccine against the next variant, B/Ann Arbor/86, in children 3 to 18 years of age. A double-blind, placebo-controlled trial in 6- to 18-year-old asthmatic children failed to show any protection against culture-positive asthma exacerbations over a 2-year period from 1999 to 2001.[29]

Inactivated vaccines generally are less immunogenic for younger children, but the incidence of acute otitis media could be reduced by 30 to 40 percent in children in daycare.[87] During the 1996 to 1997 epidemic, children in daycare received two doses of inactivated vaccine in a controlled trial; protection was 45 percent against influenza B infection and 31 percent against influenza A (H3N2) infection.[144] Children with preexisting antibodies had better responses than did those without, a finding indicating that priming by natural infection improved antibody response to the inactivated antigens. A single dose of inactivated antigen is sufficient for primed children.[219] Two doses of inactivated vaccine are necessary for toddlers aged 6 to 23 months, and protection is not evident until 2 weeks after the second dose is administered. Vaccine effectiveness in fully vaccinated children in this age group was 25 percent against influenza-like illness in 2003 to 2004.[256]

The side effects of vaccination with inactivated vaccines in children deserve further comment. Reactions to whole-virus vaccines, when they occur, include fever, "flu-like" symptoms of malaise and myalgia, and local tenderness at the site of inoculation.[316] In the past, febrile convulsions were cited as a particular risk of vaccination in the very young.[190,315] In the large trials cited, however, 813 children aged 6 months to 5 years received varying doses of whole-virus and split-product vaccines.[112,316] None of these children had febrile convulsions. During the 1978 trials with subunit vaccines, standardized for HA content, local and systemic reactions were minimal in high-risk children.[312] Current subunit inactivated influenza vaccines are minimally reactogenic and are well tolerated.

Guillain-Barré syndrome occurred in roughly 1 in 100,000 older adult recipients of the A/New Jersey/76 vaccine in the National Influenza Immunization Program.[261,266] The incidence of this complication among pediatric vaccine recipients, as well as among members of the military services who were younger than 25 years of age, was not increased. Surveillance of subsequent years revealed no excess occurrence of Guillain-Barré syndrome among pediatric influenza vaccine recipients.[33,145,152,153] The risk of developing Guillain-Barré syndrome after acquiring natural influenza virus infection is unknown; although sporadic cases have been reported, influenza infection is not an important triggering agent for this syndrome.[304]

LIVE ATTENUATED INFLUENZA VACCINE

The LAIV was developed originally by Maassab, who selected temperature-sensitive, attenuated strains by serial passage at gradually lower incubation temperatures down to 25° C ("cold-adapted").[52] Live vaccines, administered intranasally, correspond most closely to natural infection in their capacity to produce both secretory and humoral immunity. LAIV was licensed in the United States after the pivotal efficacy trial was performed in children 15 to 71 months of age.[13,14] These vaccines have been administered to adults and children; both local and systemic antibody responses resulted, with no significant side effects.[31,39,216,313,320] These vaccines also have been shown to be safe and nontransmissible in both these populations and result in protection against challenge with wild influenza virus.[31,34,38,213,313] LAIV is genetically stable, with no reversions to virulence after human passage.[296] Jefferson and colleagues assessed the efficacy and effectiveness of influenza vaccines in healthy children older than 2 years of age in 2005; these investigators reported overall efficacy of 79 percent for LAIV and 65 percent for inactivated vaccines.[149] They found no evidence of benefit of inactivated vaccines for children younger than 2 years of age, but they did not include all the trials cited earlier.

Live attenuated, cold-adapted vaccines administered by nasal spray appear to be the most promising new approach to influenza control.[183] In the pivotal trial, LAIV administered to children aged 15 months to 6 years gave 93 percent protection against culture-positive illness.[14] In the follow-up year, the A/Wuhan (H3N2) vaccine strain provided 86 percent protection against the new variant, A/Sydney (H3N2).[13] The vaccine is well tolerated and accepted by children. Attributable reactions were limited to mild rhinitis in 9 percent and low-grade fever in 5 percent on day 2 for first-time recipients. No reactions were discernible with subsequent doses.[241] Comparable protection against influenza A (H1N1) and a variant B strain was demonstrated in the Central Texas Field Trial. A single dose was sufficient to provide 92

percent protection against A (H1N1), and the protection persisted to a second season.[86,122] Direct comparisons of LAIV and inactivated vaccines were carried out in Europe and Israel. One study showed superior relative efficacy of LAIV in young children aged 6 to 71 months.[3] The other study found that LAIV was well tolerated by asthmatic children aged 5 to 19 years and provided significantly greater relative efficacy of 34.7 percent against culture-confirmed influenza than did the inactivated vaccine.[72] LAIV also was found to be superior to inactivated vaccine in a large head-to-head comparison that enrolled more than 8000 children aged 6 to 59 months[12]; 54.9 percent fewer culture-confirmed illnesses occurred among LAIV recipients. Another advantage of LAIV is the observation of almost immediate protection after intranasal administration, as demonstrated during the Central Texas Field Trial in 2003 to 2004.[123,239]

Vaccination of normal children aged 6 months to 18 years now is recommended routinely in the United States.[33] The risk of hospitalization for children younger than 3 years of age is as high as that for elderly persons,[148,220] and unlike in the elderly, fewer than 20 percent of young hospitalized children have a chronic underlying condition.[235] Because children are the most important population group in the propagation of epidemics, a vaccine strategy aimed at epidemic prevention would necessarily be focused on the pediatric age group, particularly children in school and daycare.[93,305] Supporting this concept, mass vaccination of schoolchildren had a measurable effect on the overall incidence of influenza A/HK/68-associated illness in Tecumseh, Michigan, during the 1968 pandemic.[204] The protective effect was most evident in adults 20 to 30 years of age, a finding suggesting that immunization of children lowered the incidence of influenza in their parents. Even more impressive was the large trial in Moscow, where vaccine was offered only to children in a defined geographic area including more than 400,000 people.[89] Rates of influenza-related illness were compared with a similar district where vaccine was not offered to children. Vaccine was not given to any elderly or high-risk adults; however, significant protection of unvaccinated elderly persons was found for those living in the area where the children were immunized. Rates for medical encounters in elderly persons were the same for both populations during the summer months, a finding establishing the comparability of the two areas.

Proof of the concept of indirect protection afforded by childhood immunization is the Japanese program for universal vaccination of schoolchildren.[254] Japanese schoolchildren were required to have two doses of inactivated influenza vaccine for school attendance for many years, particularly during the decade between 1977 and 1987. As many as 80 percent of children aged 9 to 15 years were immunized annually. The program declined when the requirement was relaxed in 1987, and the program was stopped in 1994. Subsequently, total excess mortality has increased remarkably. In retrospect, definite flattening of the mortality curve occurred during the program, in contrast to sharp seasonal peaks of mortality rates coinciding with influenza epidemics before and after the program. The only exception was seen in 1975 to 1976, when a new variant, A/Victoria (H3N2), appeared unexpectedly and produced a severe epidemic; the previous variant, A/Port Chalmers (H3N2), provided no cross-protection against A/Victoria. The immunization of schoolchildren in Japan is estimated to have saved 37,000 to 45,000 lives each year. Vaccine was not recommended for elderly or high-risk persons whose lives were spared; the reduction in mortality rates resulted from the indirect effect of immunizing schoolchildren. The sparing of elderly and high-risk individuals could not be attributed to the economic recovery of Japan following World War II; the summertime baseline mortality rate was unrelated to the wintertime excess mortality.[253]

Universal immunization of children in school or daycare would reduce significantly the serious morbidity in this age group and the social disruption created by school absence, necessity for health care visits, and parents' work absence.[218] The school-based program designed by King and colleagues showed significant amelioration of the morbidity and social consequences by offering LAIV to schoolchildren.[164] School-based programs also would have the potential for dampening epidemics and reducing risk of exposure to virus for vulnerable high-risk patients,[93] as demonstrated by Piedra and colleagues.[238] The cold-adapted, attenuated vaccine developed by Maassab has broader and longer-lasting immunity for children younger than 10 years.[41,86,122,237] The administration by nasal spray is accepted better by children and is easier to administer than is the inactivated vaccine given by injection.

Universal immunization of children in the United States would not replace but rather would supplement the current vaccination strategy, focused on the prevention of complicated illness in population groups at highest risk of dying or requiring hospitalization during epidemics. The current list of those given priority for influenza vaccine includes more than 250 million persons.[33] Also given priority for vaccine are pregnant women as well as health care providers and household contacts (including children) of high-risk patients and children younger than 5 years of age.[33] Vaccinating pregnant women has the potential to protect two persons at a vulnerable period in their lives—both the pregnant woman and her infant, who will benefit from the passive immunity provided during the first months of life.[249] Maternal immunization not only will reduce pregnant women's risk of needing hospitalization for pneumonia but will boost the amount of protective antibodies transmitted to their newborn infants and reduce the occurrence of serious infections in the first 6 months of life.[109,249,321] Vaccination programs must be supplemented by better surveillance and viral diagnosis. Early specific treatment of influenza-infected children with appropriate antiviral drugs will not only shorten the course of illness but also reduce virus in respiratory secretions.[307] This effect should allow reduction in transmission of virus to contacts, another effort to contain this venerable foe.

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SUBSECTION 7

Paramyxoviridae

CHAPTER 191

Parainfluenza Viruses

Caroline Breese Hall

> Physical ills are the taxes laid upon this wretched life;
> some are taxed higher, and some lower,
> but all pay something.
>
> *Philip Stanhope, Lord Chesterfield*
> *Letter to the Bishop of Waterford, 1757*

The human parainfluenza viruses (hPIVs) are ubiquitous agents with well-earned recognition as being among the most important viral respiratory pathogens of humans. The three major hPIVs (1-3) have been estimated as being among the three most frequent causes of hospitalizations for young children with respiratory tract illnesses.[66,67,73] The impact of these three hPIVs on our health care resources is well illustrated in the estimates of their causing among children under 5 years of age approximately 16,000 to 100,000 hospitalizations each year.[29] Hospitalizations from hPIV-3 alone are estimated to range from about 9,000 to 52,000 each year. The rates of outpatient visits for hPIV infections among young children are even higher, estimated to be tenfold the hospitalization rates.[40a] The taxes imposed by the burden of all the hPIVs, types 1 to 4, and at all ages are unestimated.

Respiratory tract illnesses from the hPIVs may occur and recur throughout life, and they are subordinate only to respiratory syncytial virus (RSV) in the morbidity caused by acute lower respiratory tract disease in infants and young children. Among the agents of acute laryngotracheobronchitis, the hPIVs are second to none. The frequently epidemic nature of hPIV types 1 and 2, the prolonged seasonal occurrence of type 3, and the ability of all types to cause repeated infections indicate why their import has not been diminished, despite the technologic advances available toward control of viral diseases.

HISTORY

The historical delineation of the parainfluenza viral family is intertwined with the discovery of the related animal viruses and marked by the colorful names descriptive of their origin (Table 191–1). The first strain of PIV was discovered in Japan and named *Sendai*, or the hemagglutinating virus of Japan (HVJ).[88] This agent was recovered from mice that had been inoculated with postmortem lung tissue of infants with pneumonia. The first PIV from human sources was recovered several years later by Chanock[19] from infants with croup, and thus it became known as the croup-associated (CA) virus. Subsequently, three additional hPIVs that were distinct antigenically were isolated from children with acute respiratory illness.[22,77] In contrast to the CA virus, which was recognized by its ability to produce syncytia in tissue culture, the strains of the next two types of PIVs produced few or no cytopathic effects but were recognized by their ability to cause hemadsorption of guinea pig erythrocytes to infected tissue culture. These viruses thus were called hemadsorption type 1 (HA-1) and hemadsorption type 2 (HA-2) viruses.

Once the similarity of the familial characteristics of the CA virus with those of the other three viruses was recognized, the viruses were renamed the hPIVs, type 1 (HA-2), type 2 (CA), and type 3 (HA-1).[4] The fourth hPIV, discovered subsequent to this reclassification, is without a sobriquet and simply is termed *hPIV-4*, with two subtypes, A and B. The first type 4 strain was recovered in monkey kidney cell culture from a college student afflicted with a common cold.[77]

Several animal species appear to be natural hosts to the PIVs (see Table 191–1), with the exception of hPIV-4, which has been found only in humans. In animals, PIVs may be important pathogens. Bovine PIV (bPIV), similar to hPIV-3, often in combination with infection by *Pasteurella* organisms, causes an illness of considerable morbidity and cost in cattle called *shipping fever*. Naturally acquired antibodies to the PIVs are found not only in cows but also in rodents, monkeys, rabbits, and other mammals. Various rodents, along with ferrets, dogs, and lambs, have been used for experimental infection. The virus previously called *equine Morbillivirus* has been demonstrated to be in the Para-

TABLE 191–1 Parainfluenza Viruses

Parainfluenza Virus Type/ Related Animal Virus	Natural Host	Experimental Infection	Preferred Tissue Culture for Initial Isolation	Cytopathic Effect	
				Initial Isolation	Passage
Parainfluenza type 1, hemadsorption type 2 (HA-2)	Human, guinea pig, rabbit, monkey, marmoset	Hamster, ferret	Monkey kidney, human diploid, human embryonic kidney	±	Rounding, cell destruction
Sendai, hemagglutinating virus of Japan (HVJ)	Mouse, pig				
Parainfluenza type 2	Human, monkey, rabbit, guinea pig	Hamster, dog	Monkey kidney, human embryonic kidney	+ (syncytial on monkey and human cells)	Syncytial, "Swiss cheese"
Simian virus 5 (SV 5)	Monkey				
Simian virus 41 (SV 41)	Monkey				
Parainfluenza type 3, hemadsorption type 1 (HA-1)	Human, guinea pig, monkey	Hamster, ferret, cotton rat, mouse, lamb	Monkey kidney, human embryonic kidney	±	In monkey cells: elongated, detachment of cell sheet In human cells: syncytial
Shipping fever (SF-4)	Cow	Hamster, guinea pig	Primary kidney	±	Rounding granular, vacuolated
Parainfluenza type 4	Human				

myxoviridae family and named *Hendra virus*. This virus, which causes lethal illness in horses, was recognized to cause illness in humans also. Individuals in close contact with the horses in Australia contracted the virus, and two died.[124] Subsequently, in 1998, an outbreak of severe, often fatal encephalitis occurred in Malaysia and Singapore in individuals who had close contact with pigs.[25,49] The agent of this outbreak, called *Nipah virus*, is phenotypically and antigenically similar to Hendra virus (80% genomic homology).

CHARACTERIZATION OF PARAINFLUENZA VIRUSES

CLASSIFICATION AND STRUCTURE

The five hPIVs, hPIV types 1, 2, 3, 4A, and 4B, are members of the Paramyxoviridae family, belonging to the *Respirovirus* and *Rubulavirus* genera (Fig. 191–1). When discovered in the late 1950s, the PIVs were distinguished quickly from the orthomyxoviruses (influenza viruses) in size, nucleocapsid structure (non-segmented), antigenic composition, and laboratory growth characteristics.

hPIVs are single-stranded, negative-sense, enveloped RNA viruses appearing as pleomorphic particles of medium size, with an average diameter of 150 to 200 nm.[21,81a,147] The genomes of all the hPIVs, consisting of an average of approximately 15,000 nucleotides, code for at least 6 structural proteins (3′-N-P-M-F-HN-L-5′) and one or more nonstructural proteins (C, V, D) (Fig. 191–2). Enclosed within the spherical virion is the helical nucleocapsid, with its herringbone core tightly wrapped by the nucleocapsid protein (NP). It is surrounded by the envelope, derived from the host cell, which is studded with spikes of surface glycoproteins, the hemagglutinin-neuraminidase (HN) and fusion (F) proteins, which are the major protective antigens. The large (L) polymerase protein and the phosphorylated nucleocapsid-associated protein also form clusters within the nucleocapsid structure. The sixth structural protein is the nonglycosylated matrix (M) protein, located between the nucleocapsid and the envelope.

Replication and assembly of the virus occur solely in the cytoplasm of the host cell.[21,81a,147] The NP, along with the P and L proteins, is responsible for the primary transcription that occurs sequentially, producing predominately nonoverlapping subgenomic messenger RNAs from which the viral proteins are translated. The genome also is replicated from the formation of a full-length antigenome with positive-sense RNA. The NP and the genomic RNA comprise the helical structure, and with the subsequent addition of the P and L protein complexes, the nucleocapsid is formed. The envelope proteins are assembled at the cell surface. The M protein is the smallest of the major structural proteins and is the most abundant of the virion's proteins. The M protein facilitates the interaction between the nucleocapsid and the surface glycoprotein by the attachment and the insertion of the surface glycoproteins to the host cell and recruitment of the completed NP to the budding site of new virions, which are released from the cell surface by evagination of the plasma membrane.

The HN protein is a dimer of two polypeptide portions held together by a disulfide link in the hydrophilic region and at the bases by hydrophobic bonds. The HN is the major immunogenic and type-specific determinant. It is important in multiple viral functions, including mediating the attachment of the virus to the host cell by binding to sialic acid–containing mucoprotein receptors present on the cell. The hemagglutination component of the HN protein mediates the hemagglutination of mammalian erythrocytes. The neuraminidase component of the HN protein functions during late infection to release progeny virions by dislodging the cellular bond by cleaving the sialic acid residues and prevents reattachment of the viral particles to the cell surface.

The F protein effects entry of virus by fusing the host and viral cellular membranes. Once adsorption of the virus occurs, the F protein precursor, F0, is cleaved by host proteases into the active F1 and F2 protein fragments, which mediate penetration of the virus into the cell, with subsequent fusion of the viral and cell membranes and hemolysis. The N-terminal residues of the F1 protein (the fusion peptide) promote the penetration and fusion of the virus with the cellular membrane. In contrast to the foregoing proteins, the nonstructural C, V, and D proteins are not encoded by all the hPIVs but are produced variably by the different hPIV types and appear to have roles in transcription, replication, production, and pathogenicity of the virions.[21,64]

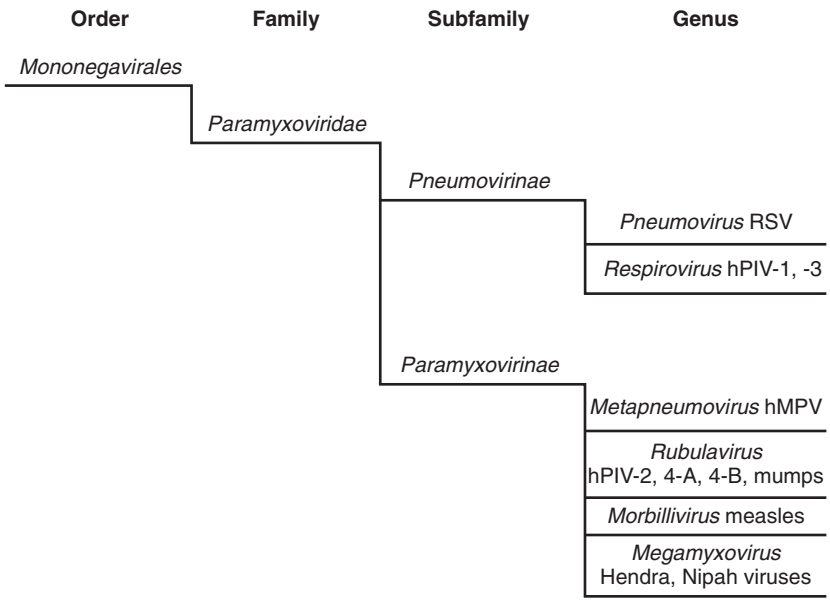

Figure 191–1 Classification of parainfluenza viruses that cause human infection.

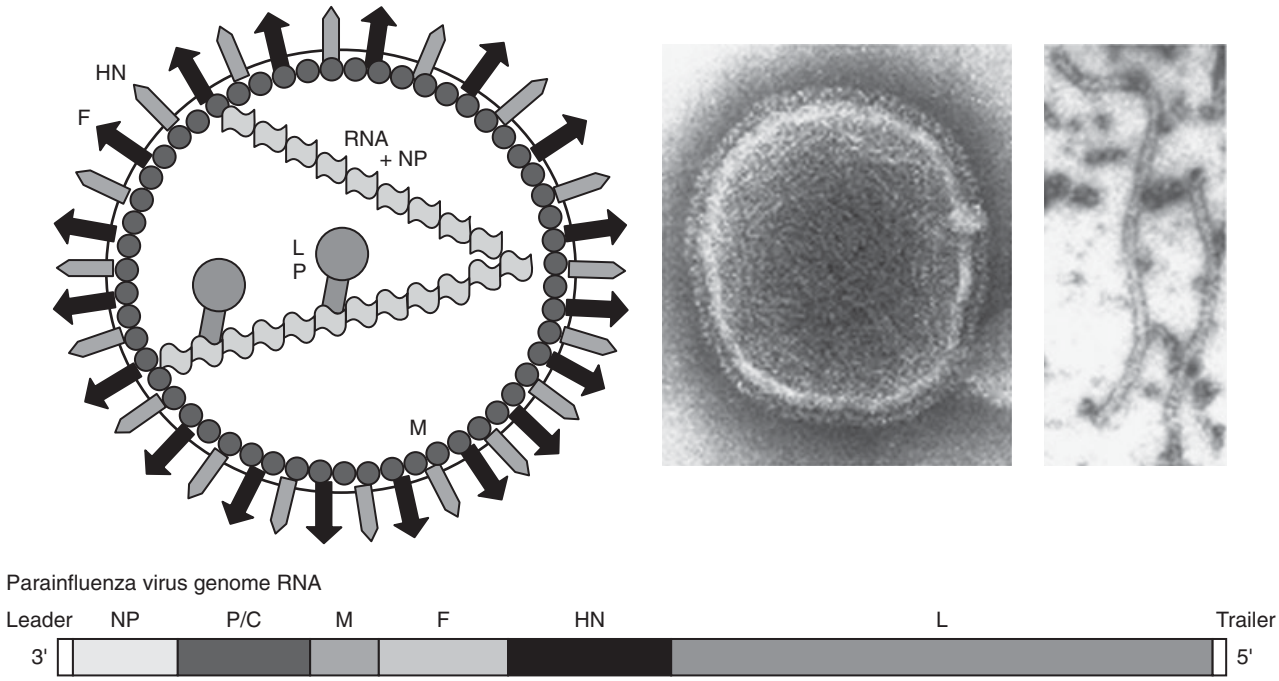

Figure 191–2 Structure of parainfluenza virus (Sendai virus, *top left*) and its genomic RNA *(bottom)*. Electron micrograph of Sendai virus *(top middle)* and its helical nucleocapsid *(top right)*. *(From Takimoto T: Parainfluenza viruses.* In Harper, D. (ed.): *Encyclopedia of Life Sciences. Chichester, U. K., John Wiley and Sons, pp. 678-684, 2006.)*

hPIVs share common antigens but also may be differentiated into two antigenic groups. hPIV-1 and hPIV-3 are within the *Paramyxovirinae* genus *Respirovirus*, and hPIV-2 and hPIV-4A and -4B are within the *Paramyxovirinae* genus *Rubulavirus*[21] (see Fig. 191–1). Their common antigenicity is illustrated by serologic heterotypic antibody responses to infection with hPIV-1, hPIV-2, and hPIV-3 and mumps virus. Although the hPIVs do not undergo major antigenic alterations in their surface antigens similar to influenza virus, genetic and antigenic variations within all four types have been detected, and their evolution can be determined by analyses of strains obtained over time.[21,64,81a,167]

ISOLATION AND IDENTIFICATION

The hPIVs are inactivated rapidly by acid at a pH of 3.0 and by exposure to lipid solvents, such as ether and chloroform, which destroy the envelope of the virus. They also are relatively labile to temperatures higher than 37° C.[32,33] Media containing protein, however, tend to protect against loss of infectivity when the virus is exposed to heat and when it is frozen at –70° C.[32,72]

Isolation of the hPIVs occurs in permissive cell cultures after an incubation period of 3 to 7 days at 35° C to 37° C. The preferred cell cultures for the isolation of the hPIVs are primary or continuous monkey kidney cells, such as LLC-MK and human embryonic kidney cells, along with a continuous line of mucoepidermoid human lung carcinoma cells, NCI-H292.[21,41,62,64] In general, the growth of hPIVs is best in primary monkey kidney cells, although the growth of hPIV-2 may be variable in monkey kidney cells. hPIV-1 also grows well in human embryonic lung and less well in diploid fibroblastic lines. In other types of kidney tissue, the growth of the hPIVs is more variable. hPIV-3 replicates in bovine and canine kidney cells, and hPIV-4 replicates in bovine and hamster kidney cells. After passage, hPIVs can be propagated in a variety of other cell lines. Persistent infection of some cell lines has been observed with hPIV-1, hPIV-2, and hPIV-3.[42,108] Cells persistently infected with hPIV-3 have little or no cytopathic effect, which is related to the lack of cell fusion, despite the production of large amounts of the cleaved active form of the F1 protein.[157] The growth of the hPIVs in embryonated eggs is poor or inconsistent according to the type and strain. hPIV-1, hPIV-2, and hPIV-3, but not hPIV-4, have been adapted to grow in the amniotic cavity of embryonated chicken eggs, although some strains appear resistant to such adaptation.

The production and characteristic type of cytopathic effect in tissue culture vary according to the hPIV strain (see Table 191–1).[127] hPIV-1 and hPIV-3 on initial isolation are unlikely to demonstrate distinctive cytopathic effects, but, depending on the cell line, they may on passage. hPIV-4 is the most difficult hPIV to grow and identify in tissue culture because the cytopathic effect is not evident, and 2 to 4 weeks may be required before identification by hemadsorption is possible. Identification of hPIVs in tissue culture traditionally has relied on the ability of the hPIVs to hemadsorb erythrocytes, most commonly guinea pig, human group O, or chicken erythrocytes, at 4° C or 25° C. However, the sensitivity of this method is variable, especially for the rubulaviruses hPIV2 and hPIV4.[64] Isolation and identification of the hPIVs may be accelerated by use of shell viral cultures and subsequent identification by immunofluorescence assay (IFA) techniques.[127]

Rapid antigen identification methods, such as direct visualization of exfoliated cells by IFA and enzyme-linked immunosorbent assays (ELISA), have generally had highly variable sensitivity, and antigen assays for the hPIVs have not been widely available.[52] Reverse transcriptase-polymerase chain reaction (RT-PCR) with differential hybridization techniques, such as hybridization with enzyme immunoassays, has markedly increased the detection and specific identification of the hPIVs in patient specimens.[17,38,64-66,133,158] RT-PCR assays can detect a single respiratory virus or multiple viruses simultaneously, and, although previously confined to research laboratories, these assays are becoming more widely available commercially.

EPIDEMIOLOGY

GEOGRAPHIC DISTRIBUTION

The ubiquitous nature of the hPIVs has been illustrated by many serologic and viral isolation studies in varied populations, in many parts of the world, and in differing climates.[45,101,106,118,144,158] Despite the wide variations in climate and geography, experience with the hPIVs is similar in most countries.

PREVALENCE AND AGE AT INFECTION

The frequency and impact of hPIV infections are greatest in preschool-aged children and are estimated to account for approximately one third of the lower respiratory tract infections in this age group.[45,64,67,87,132] Among the hPIVs, the type most commonly isolated is hPIV3, which usually is acquired first, followed by hPIV-1 and hPIV-2 (Fig. 191–3).

Like RSV infection, hPIV-3 infection is a common occurrence during the first few months of life. One half to two thirds of infants have acquired infection by the time they reach 12 months of age.[45,106,111] Experience with hPIV-1 and hPIV-2 usually occurs later, most commonly during the preschool years.[45,46,106,132]

The frequency of hPIV infection in the young child has been well illustrated by the Houston Family Studies, in which infections were detected by both viral isolation and serology among children observed from birth.[45,46] By the time they reached 2 years of age, 92 percent of the children had experienced at least one infection with hPIV-3, and 32 percent had been infected more than once. hPIV-3 is the most frequent cause among hospitalizations associated with hPIV infections. hPIV-1 and hPIV-2 are associated primarily with hospitalizations of children aged 2 to 6 years. The age distribution of children with hPIV-1 and hPIV-3 viral infections seen in private pediatric practice, however, may overlap substantially (see Fig. 191–3). In outpatients in Rochester, New York, approximately half of the hPIV-3 viral infections occurred in children younger than 24 months, compared with approximately one third of the hPIV-1 infections.[87] Among children aged 2 to 5 years, the reverse was true, with half of the hPIV-1 infections and approximately one third of the hPIV-3 infections occurring in this older age group.

The epidemiology of hPIV-4 is less well described. In part, the reason may be that clinical disease is infrequent or mild, thus rendering identifying hPIV-4 infection difficult and the prevalence underestimated.[1,12,89,134] Some serologic studies, however, suggest that infection with hPIV-4 is a common occurrence during the preschool years, and between 50 and 90 percent of 5-year-old children possess antibodies.[44,85,90]

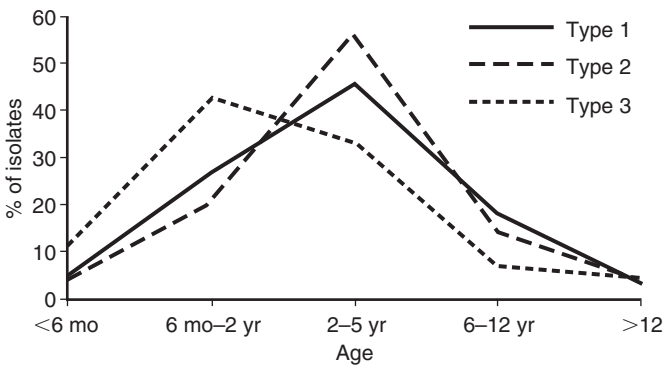

Figure 191–3 Age distribution of infections with human parainfluenza virus types 1, 2, and 3 in outpatient children in Rochester, New York, from 1976 to 1992. *(From Knott, A., Long, C. E., and Hall, C. B.: Parainfluenza viral infections in pediatric outpatients: Seasonal patterns and clinical characteristics. Pediatr. Infect. Dis. J. 13:269-273, 1994.)*

SEASONAL OCCURRENCE

The seasonal patterns of the hPIVs may vary according to location and year, but they remain distinctive in their predictable and repetitive behavior. Before the early 1960s, the hPIVs were mostly endemic.[19] Since the mid-1960s, however, hPIV-1 began changing its profile by preferentially appearing in the fall and settling into its most common current pattern of causing outbreaks every other year in the autumn.[45,87,132]

The behavior of hPIV-2 has been more erratic.[45,64,87,132] During 20 years of surveillance in Rochester, New York, hPIV-2 appeared sporadically in low numbers, usually at the end of the fall outbreaks of hPIV-1 in the odd-numbered years (Fig. 191–4).[56,87] hPIV-3 may be present in a community in low numbers for many months, but it does produce swells of more epidemic activity in the spring to fall.[45,56,87] During long-term surveillance, approximately 75 percent of hPIV-3 isolates were recovered in the spring and summer, and most of the remainder were obtained in the autumns of the even-numbered years, when hPIV-1 was absent.[87]

PATHOGENESIS

TRANSMISSION

Clinical and experimental observations indicate that the hPIVs are able to spread readily and effectively.[5,28,59,81,111] Close contact with an infected individual or their secretions appears necessary, to allow spread of infection to occur through large droplets or by fomites followed by self-inoculation. Contagiousness, therefore, depends on the following: (1) the quantities of virus contained in the nasal secretions[16,59]; (2) how effectively the infected secretions are propelled into the environment, such as through sneezing and coughing; and (3) how well the infectious virus can survive in the environment.[16]

Children with primary infection caused by hPIV-1 have considerable quantities of infectious virus in their nasal secretions, an average of approximately 1000 tissue culture infective doses ($TCID_{50}$) per milliliter.[59] With the tendency of hPIVs to cause illnesses associated with frequent sneezing, coughing, and profuse nasal discharge, contamination of the environment from infected individuals, especially young children, is inevitable. Thus, spread of infection occurs readily by fomites and by the other putative major mode of spread, large-particle aerosols of secretions from an infected individual. For large particles (>10 μm in diameter) to be inhaled, close person-to-person contact is required. Although the routes of inoculation that occur naturally have not been studied adequately, adults have been infected experimentally by intranasal and oropharyngeal inoculation. The eye as a site of inoculation has not been examined similarly, but it may be an important portal of infection from self-inoculation with contaminated hands.

Experimental data suggest that hPIV secretions survive well on hard, nonporous surfaces, such as those found in hospitals, workplaces, and homes. The hPIVs remain infectious on nonporous surfaces for as long as 10 hours and on porous surfaces for as long as 4 hours, but survival may be diminished by drying.[16] hPIVs may remain infectious after being transferred to hands from environmental surfaces, a finding further suggesting that fomites are an important mode of dissemination. A curious conundrum is the survival and persistence of hPIV-1 and hPIV-3

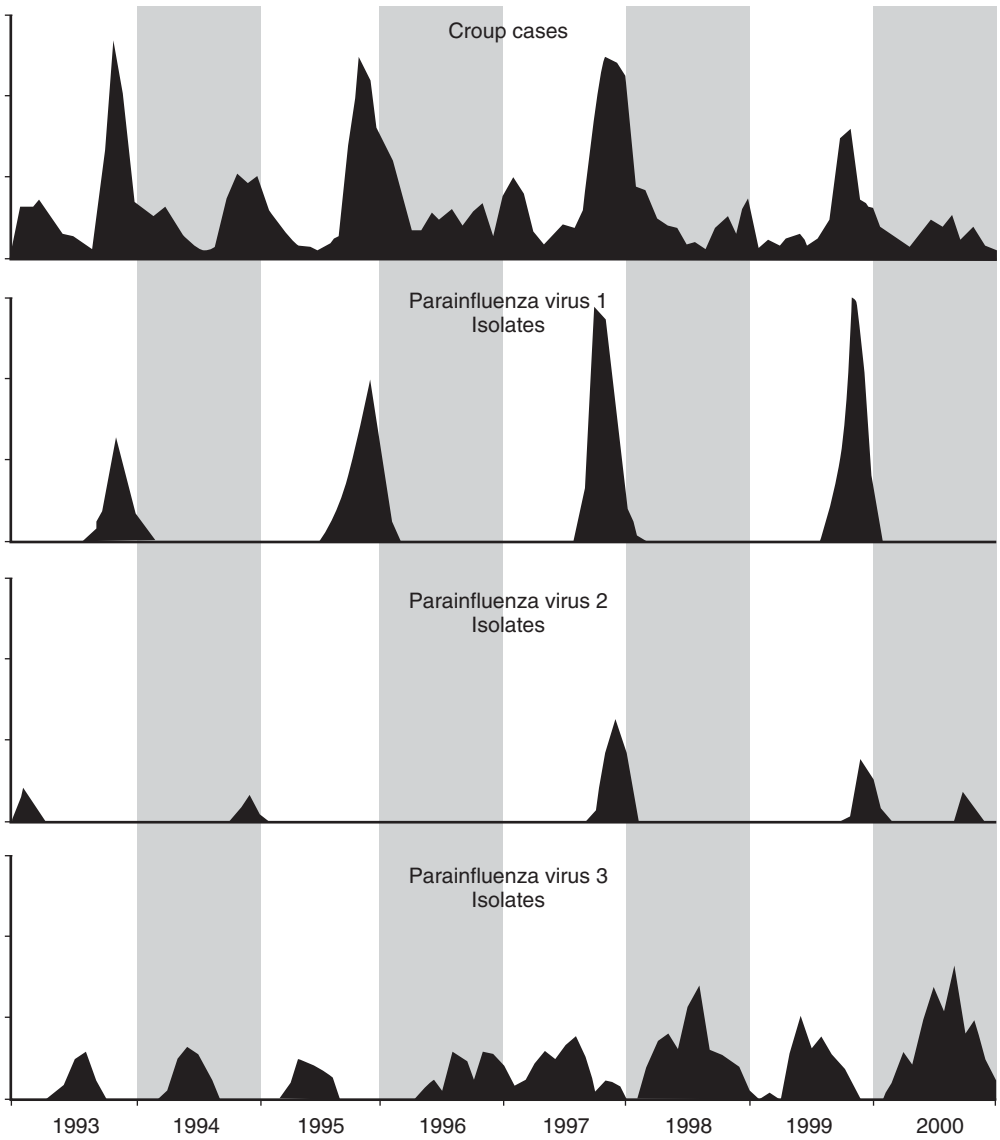

Figure 191–4 Number of cases of croup reported by the primary care physicians in Rochester and Monroe County, New York, from 1993 through 2000, to the Community Infectious Disease Surveillance Program of the University of Rochester School of Medicine, Rochester, New York. Numbers of cases are shown in relation to the periods of isolation of the parainfluenza viruses.

infections in a group of individuals isolated in the frigid environs of the South Pole for 8.5 months.[110,122,123] Despite no outside contacts, infection with hPIV-1 and hPIV-3 occurred repetitively in the quarantined individuals. This phenomenon raised the possibility that persistent infection may be engendered not only in tissue culture but also in humans under such circumstances as extreme environmental conditions.

PATHOLOGY

Inoculation of the hPIVs into the upper respiratory tract results in infection of the nasal epithelium and nasopharynx and the appearance of clinical signs after an incubation period of 2 to 4 days. The hPIVs appear to replicate preferentially in the superficial ciliated epithelial cells lining the airways of the upper and lower respiratory tract. The factors determining the tropism and extent of the viral infection are incompletely understood. Such factors may include host immunity, the protease activity of various tissues, and virus-specific determinants, such as the cleavage phenotype of the F0 protein.[21,107]

The importance of factors that affect the degree of viral replication is indicated by the clinical and experimental correlation of pathogenicity with the level of productive infection.[15,54,59] The development of live attenuated PIV vaccines by reverse genetics confirmed this correlation by the correlation of precise viral mutations with clinical attenuation.[10,34,80,116]

Pathologic studies of children with hPIV infections are limited. The few cases of confirmed hPIV infection examined have shown that in the young child with primary infection, inflammation, marked primarily by necrosis of the epithelium, is evident throughout the respiratory tract. The subglottic tissues in croup appear particularly involved, but with primary infection, the conducting airways at all levels and the alveoli may be affected.[20,168]

Viral shedding is most abundant and prolonged in young children with primary and severe disease.[59] Children with hPIV-1 infections may shed the virus for an average of 4 to 7 days but for as long as 12 days. With hPIV-3 infections, shedding tends to be longer, occasionally for 2 to 3 weeks, and in adults with chronic lung disease, shedding may be both prolonged and intermittent.[53,64]

Experimental infection of animals with PIVs provided a model for the study of the pathophysiology of these infections.[21,121,129] Inoculation of rodents with hPIV-3 produced histologically evident, but usually asymptomatic, interstitial pneumonitis or bronchiolitis. Ferrets also can be silently infected, but in newborn ferrets, PIV infection may be progressive and fatal.[104] Chimpanzees and several species of monkeys may be infected, but clinical manifestations generally are lacking, except with hPIV-3 infection of chimpanzees and African green monkeys. Inoculation of hPIV-2 into the respiratory tract of a canine model produced clinical signs of cough and rhinitis accompanied by histologic changes in the airways.[155]

IMMUNE RESPONSE: ROLE IN PATHOGENESIS AND PROTECTION

Innate immunity's role in controlling PIV infection and how it is elicited are of increasing interest but currently of limited clarity. Better understanding of the pathogenesis of the innate response and its effect on the transition to adaptive immunity may lead to novel and better means of controlling the ubiquitous hPIV infections that occur during infancy and thereafter.

The innate antiviral responses to infection of viruses of the *Paramyxoviridae* family have been studied in experimental models. The response is elicited within the respiratory tract once the viral genome enters and replicates in the cytoplasm of the host cell and is characterized by the production of a cascade of immunomodulatory mediators, chemokines, and proinflammatory cytokines.[30,119,120] Toll-like receptors (TLRs), a class of pattern-recognition receptors, appear to be important early in the innate antiviral defense.[30,102] The products of viral replication trigger and activate specific TLRs that allow the binding of the virus to the host cell and the expression of the genes associated with the host immune response. TLRs may help mediate the immune response, both by their interaction with infected respiratory epithelial cells and by the induction of dendritic cell maturation that is integral in the subsequent development of adaptive immunity with the production of effector and memory T cell and B cells.[94,137] Although TLRs appear to have a pivotal place in innate antiviral defense, experimental evidence suggests that replicating infection of paramyxoviruses and other respiratory viruses may induce and enhance the innate immune response by other pathways that are not dependent on TLR-mediated signaling.[91,94,96]

How representative the findings of these experimental studies are to the pathogenesis of the innate immune response in children with hPIV infection remains unclear because the number of studies in young children is limited. Experimental PIV infection can induce interferon-α and interferon-β, and interferon production has been demonstrated during natural parainfluenza infection in young children. [21,50,58,166] However, some PIVs may be able to evade this protective response by inhibiting interferon production through accessory proteins (C and V) expressed from the P gene.[21,50,84]

The major components of the adaptive immune response to hPIV infection and reinfection are both humoral and cellular. The protective effect engendered by serum and mucosal antibodies to hPIV is variable according to age, past infection, and hPIV serotype.

In contrast to infection with hPIV-1 and hPIV-2, the most serious illness caused by hPIV-3 infection usually occurs during the first year of life, when infants possess maternally derived specific antibody. This finding has raised the question whether interactions of maternal antibody and virus may be detrimental.[101] In animal models, however, passively administered PIV antibody and monoclonal antibodies against the F and HN proteins appeared protective. Furthermore, the trials with an inactivated trivalent hPIV vaccine in the 1960s did not suggest a pathogenic role for antibody.[43] Although not protective, the vaccine was immunogenic and produced serum antibody in the vaccinees. Subsequently, the vaccinees did not experience augmented disease, as observed with the simultaneously tested formalin-inactivated RSV vaccine.

Clinical observations also show that the more severe infections involving the lower respiratory tract occur during initial infection in young, seronegative children, but recurrent infections usually are mild. Naturally acquired immunity, therefore, although not complete and of variable durability, does provide protection against more severe disease.[21,46] Antigenic variation in the hPIVs is not sufficient to explain the lack of complete or durable immunity. More likely is the waning of both humoral and cellular immune responses that occurs during the intervals between repeated exposure to one of the hPIVs.

Serum hemagglutination-inhibition antibody and neutralizing antibody are detectable within 1 to 2 weeks.[82] Neutralizing antibody is directed primarily to epitopes on the HN and F proteins. Both of these proteins are important in the immunologic and protective responses, but in experimental animals, some evidence exists that antibody against the HN protein is more protective than is antibody formed against the F protein.[131,146] Infants with primary hPIV infection usually produce a greater antibody response toward HN than F. With repeated infections, an anamnestic and broader humoral antibody response occurs, producing antibodies with cross-reactivity against the different hPIV types. In young children with primary hPIV-3 infection, the antibody response to the HN protein has been shown to be directed at multiple neutralizing antigenic sites.[150] The response to the F protein, whether with primary or recurrent infection, appears to be more variable in magnitude and restricted in terms of antigenic sites. The presence of maternal antibody, however, inhibits the infant's ability to produce specific humoral antibody.

The normal mucosal immune response is characterized predominantly by secretory immunoglobulin A (IgA), but IgG and IgM also are produced.[21] Primary and secondary infections with a heterotypic strain result in low and transient levels of secretory IgA antibody, whereas homotypic reinfection produces an enhanced response.[160] The presence of specific mucosal antibodies was shown to be pivotal to protection against PIV infection in experimental models. Passive administration or induction of mucosal IgA antibody was demonstrated to be more protective than serum IgG antibodies.[143,149] In adult experimental infection, resistance to hPIV infection correlated better with the neutralizing activity in the secretions than in the serum. The titer of IgA antibody, however, did not correlate with the neutralizing activity or with the severity of illness. The production of IgE antibody in the secretions of infants, in contrast, was correlated with more severe respiratory tract disease.[161]

The cell-mediated immune response to hPIV infection is not well defined. The increased severity and prolonged period of viral shedding in immunocompromised hosts, such as those receiving organ transplants, indicate the importance of intact cellular immunity in the control of disease and recovery from hPIV infection.[37,92,152] In animal models, CD8 cytotoxic T lymphocytes were demonstrated to be central to recovery and clearance of PIV infections.[71,83] In the peripheral blood lymphocytes of healthy adults, both CD8 and CD4 cytotoxic T lymphocytes that responded in vitro to hPIV antigens were detected.[31,141] Cell-mediated responses to hPIV infections in children have been suggested as important in the pathogenesis of more severe hPIV disease. Children who have croup from hPIV infection compared with those children experiencing milder hPIV infections have been shown to have increased lymphoproliferative responses and decreased histamine-induced suppression of lymphocyte transformation responses to hPIV.[159]

CLINICAL MANIFESTATIONS

PRIMARY INFECTION

The types of illnesses caused by hPIV infections have characteristic associations with the age of the child, the season, and the hPIV serotype (see Fig. 191–3; Figs. 191–5 and 191–6).[46,48,73,87,90,132,158,165] Most primary infections are symptomatic, and many affect the tracheobronchial and lower respiratory tract. Of the primary infections that involve only the upper respiratory tract, fever and laryngeal or tracheal signs are common occurrences. Reinfections occur frequently in persons of all ages and usually are mild and limited to the upper respiratory tract, or sometimes they are asymptomatic.[46]

In a general practice in Britain, the attack rates of hPIV infections per 1000 population were 43.2 for those 0 to 4 years of age, 12 for those 5 to 14 years of age, and 3.7 for those 40 years of age or older.[8] Among 20- to 50-year-old patients in the Tecumseh study, 16 to 28 percent were infected with hPIVs each year, and the hPIVs ranked second only to the rhinoviruses as the most frequently identified agents of upper respiratory tract infections.[105]

The hPIVs cause a greater proportion of acute respiratory illnesses in outpatients than of the respiratory illnesses in hospitalized children.[87,90] Of all viral respiratory illnesses examined in a pediatric practice, the hPIVs caused approximately two thirds of the croup cases, one fourth of the tracheobronchitis cases, and approximately one half of the upper respiratory tract illnesses, including colds, laryngitis, pharyngitis, and otitis media.

The proportion of children with hPIV infection who require hospitalization, mainly those with croup, has markedly diminished in recent years, primarily because of better outpatient management and therapy of croup (see "Management and Therapy").[138,165] Currently, most children hospitalized with hPIV infections are infants with pneumonia and bronchiolitis from hPIV-3, and they are most likely to be admitted during the spring.

The onset of the typical primary hPIV infection generally is acute and associated with mild fever and upper respiratory signs of the common cold, such as rhinitis, sore throat, and cough. Laryngeal involvement manifesting as hoarseness is apt to be more prominent than with other viruses that commonly produce colds in young children. After 3 to 4 days, these upper respiratory tract symptoms may progress, primarily in infants with hPIV-3 infection, to involve the lower respiratory tract. The characteristic manifestations of pneumonia or bronchiolitis, including dyspnea, crackles, wheezing, and hyperaeration, follow. Children with hPIV-1 or hPIV-2 infection, who usually are aged 2 years or older, also have acute onset of fever and upper respiratory tract signs, but if the infection progresses in this age group, it is more likely to evolve into croup. Children with hPIV-1 infections who were seen in a private pediatric practice during a community outbreak of hPIV-1 predominantly had fever, upper respiratory tract signs, and tracheobronchial or tracheolaryngeal signs of croup.[59] Fever, which frequently was high, occurred in 75 percent. Only 12 percent had an afebrile upper respiratory tract illness.

In children with croup, upper respiratory tract signs are followed by a harsh, barking cough (the "seal's bark"), which heralds the classic manifestations of stridor, retractions of the chest wall, and dyspnea. The course of croup characteristically is variable, with unpredictable waxing and waning in the intensity of the stridor and dyspnea. The acute symptoms usually last for 3 to 4 days. In more severely ill children, hypoxemia may occur from viral involvement of the lung parenchyma, which, although important in management, may not be recognized because of the focus on the apparent major site of inflammation, the subglottic area (see Chapter 22).

Otitis media may complicate the upper respiratory tract manifestations in young infants and older children with hPIV infection.[24,63,87,109,130] Of the otitis media cases associated with hPIV, the serotype most frequently identified is hPIV-1, followed by hPIV-3. hPIVs have been detected in middle ear aspirates as the sole agent or with a bacterial agent. In some children with hPIV upper respiratory tract infections, only bacterial agents are recovered from the middle ear aspirates. hPIV infection, therefore, appears to play both primary and secondary roles in predisposing these patients to middle ear infection.

The association of acute hPIV infection with a nonrespiratory illness or the isolation of hPIV from sites other than the respiratory tract occurs rarely. Case reports have associated hPIV infections with a variety of diseases, including adult respiratory distress syndrome, parotitis, myopericarditis, and diseases of the central nervous system.[6,70,74,153,164,169] Anecdotal reports also have associated hPIV infection with collagen vascular diseases and sporadic severe hepatitis, characterized histologically by syncytial giant hepatocytes.[125,126]

The clinical manifestations associated with hPIV-4 are less well described, which may be explained partially by the technical difficulty of isolating hPIV-4.[1,12,18,68,89,93] With the use of multiplex RT-PCR, hPIV-4 has been identified in specimens from patients with respiratory disease at rates varying from about 3 to 15 percent.[1,18] Most hPIV-4 isolates have been associated with a

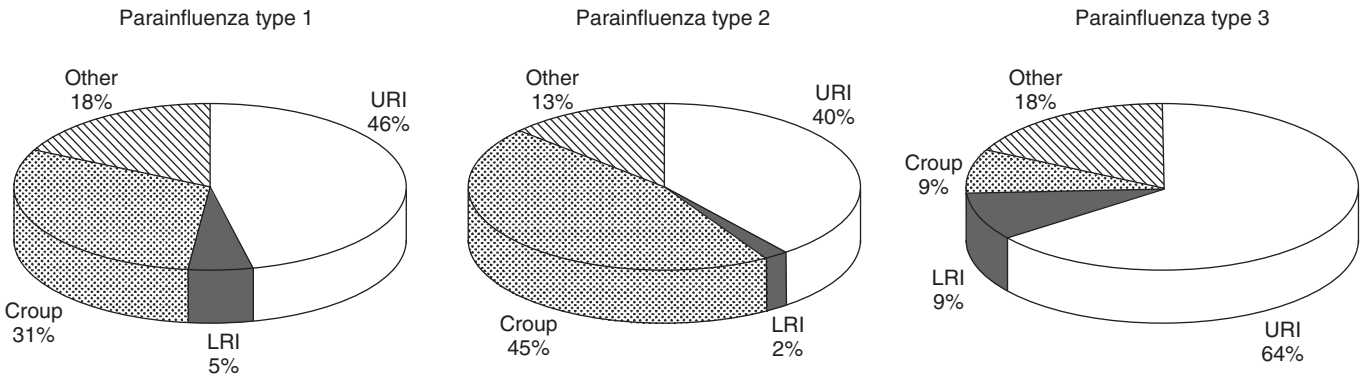

Figure 191–5 Clinical manifestations of parainfluenza virus infections according to serotype in pediatric outpatients in Rochester, New York (1976 to 1992). LRI, lower respiratory tract infection other than croup; URI, upper respiratory tract infection (including otitis, colds, pharyngitis). *(From Knott, A., Long, C. E., and Hall, C. B.: Parainfluenza viral infections in pediatric outpatients: Seasonal patterns and clinical characteristics. Pediatr. Infect. Dis. J. 13:269-273, 1994.)*

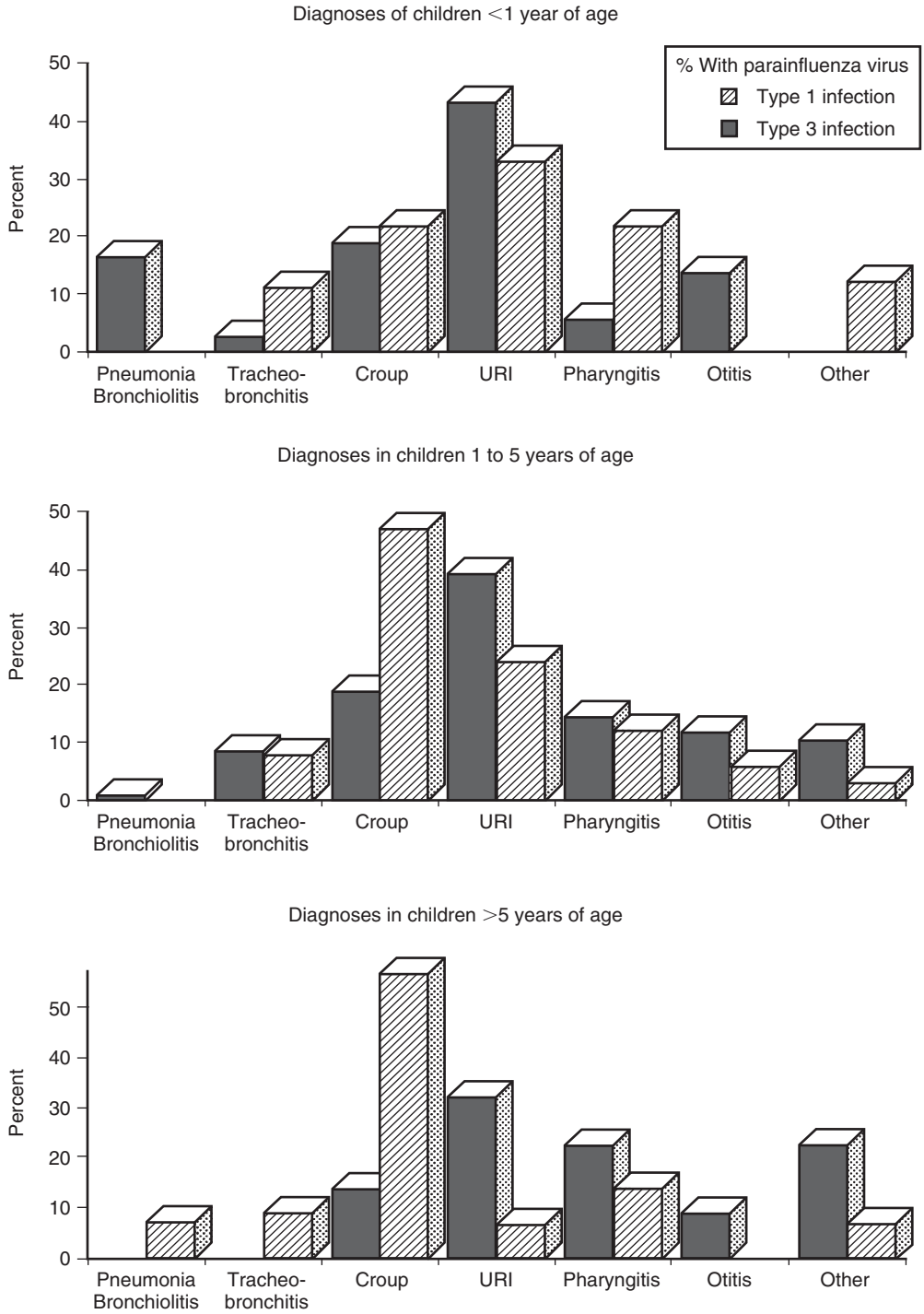

Figure 191–6 Type of illness with human parainfluenza virus type 1 compared with human parainfluenza virus type 3 according to age in outpatients from private pediatric practices who presented with acute respiratory or febrile illnesses. URI, upper respiratory tract infection. *(Data obtained 1984 through 1988 from the Community Infectious Disease Surveillance Program of the University of Rochester School of Medicine, Rochester, New York.)*

mild, usually afebrile, upper respiratory tract infection. However, lower respiratory tract illness associated with hPIV-4 has been reported.[93]

REINFECTION

The common reinfections that occur with hPIV infections usually are mild, with symptoms of the common cold. In adults, hPIV-1, hPIV-2, and hPIV-3 constitute approximately 2 to 5 percent of the upper respiratory tract infections, but one fifth or more of these infections may be asymptomatic.[14,68] Of persons with clinical illness, approximately 30 to 92 percent develop fever, which is commonly associated with malaise, sore throat, and cough. Rhinorrhea, which occurs in less than 25 percent of the adult infections, is not seen as frequently as in children.

hPIV infection may cause more severe disease in elderly persons and also may exacerbate symptoms in both children and adults with chronic lung disease, including chronic bronchitis, asthma, and cystic fibrosis.[27,47,78,115,148] On occasion, pneumonia has been described with hPIV infections in previously healthy adults, such as among military recruits, but is a rare development in normal, healthy populations of adults and school-aged children.[39,163]

INFECTION IN IMMUNOCOMPROMISED PATIENTS

As demonstrated by multiple reports of outbreaks of hPIV infections occurring on transplant units, children and adults with compromised immunity, especially those with deficient cellular immunity, are at high risk for development of severe, and sometimes fatal, hPIV disease.[13,37,92,99,100,136,151,152,162] Among patients who have received bone marrow transplantation, close to half of hPIV infections involve the lower respiratory tract, and approximately 15 to 40 percent of these infections are fatal.[92,136,162] In approximately one third of these bone marrow recipients with hPIV lower respiratory tract disease, the virus may be recovered from the upper respiratory tract.[162] Frequently, the period of viral shedding is prolonged with hPIV infection, a feature that increases the risk of an outbreak of infection among patients and personnel on transplant units. Furthermore, hPIV infection often is not considered among the opportunistic infections that plague these patients, and diagnosis is confounded by the lack of sensitivity of detecting the virus in specimens from the upper respiratory tract.

The incidence of hPIV infections among lung transplant recipients has been estimated to be 5.3 per 100 patients and is most associated frequently with hPIV-3.[152] Infection in these patients often manifests initially by nondistinctive upper respiratory tract symptoms. Progression to involvement of the lower respiratory tract follows in approximately 10 to 70 percent of cases, depending on multiple host factors, including the degree of immunosuppression and the type of transplantation procedure. The risk of developing lower airway disease in transplant recipients is further augmented because of mechanical factors present in transplanted lungs and because of altered or absent mucosal immunity.[152] The bronchus-associated lymphoid tissue (BALT) in the lung, which is integral to the protective immune response against pulmonary infections, is injured during transplantation, thus resulting in diminished control and clearance of the viral infection.

Therefore, the high rate of symptomatic infection (95%) with hPIV infections among lung transplant recipients is not surprising.[152] Cough has been reported as present in 70 percent, dyspnea in 66 percent, fever in 16 percent, and lower respiratory tract disease, which is diagnosed as viral pneumonia, in 16 percent. Of growing recognition and concern is the observation that subsequent to the transplantation, hPIV and other viral infections may lead not only to graft rejection, but also to severe long-term complications. Thirty-two percent of lung transplant recipients have developed acute bronchiolitis obliterans, which has occurred an average of 6 months (range, 1 to 14 months) after their hPIV infection.[37,152]

Among children with immunodeficiency syndromes who have diminished cellular immunity, especially children with severe combined immunodeficiency syndrome, hPIV has been notably severe and frequently fatal.[75] Complicated disease and lower respiratory tract involvement are observed less frequently with hPIV infection among children with less severely compromised cellular immunity, such as patients with human immunodeficiency virus (HIV) infection.[79,97,103] The clinical severity of hPIV infection among patients with HIV infection is variable, but even in those with mild disease, the shedding of hPIV usually is prolonged and may persist beyond the period of clinical symptoms.

DIAGNOSIS

The diagnosis of hPIV infections often may be surmised on clinical and epidemiologic grounds, such as the patient's age, the type of illness, and the seasonal patterns of hPIV in the community. Specific laboratory diagnosis may be made during the acute phase of the illness by isolation of the virus or by identification of the viral antigen in specimens from the patient and during the patient's convalescence by serologic assays (see "Isolation and Identification," earlier).

Serologic diagnosis has been accomplished by numerous assays, including complement fixation, hemagglutination inhibition, hemadsorption inhibition, neutralizing, enzyme immunoassay, and immunofluorescence.[40,65] However, serologic diagnosis is confounded by heterotypic antibody responses. During reinfection, both homotypic and heterotypic antibody rises may occur, or sometimes little antibody response is detected, despite shedding of the virus from the nasopharynx.[21,123] Less is known about the antibody response to hPIV-4 infection, but in primary infection, a homotypic response is usual.[85] Detection of specific IgM antibodies, which usually persist for 2 to 11 weeks after infection, has been of limited success as a diagnostic technique.[154]

DIFFERENTIAL DIAGNOSIS

Most of the other major respiratory viral pathogens can mimic the illnesses produced by hPIV infections. Clinical differentiation rarely is possible. More helpful are such epidemiologic clues as the differing seasonal patterns and age-related attack rates of the common viral agents of respiratory illnesses in children.[57]

Croup from hPIVs or any other virus must be differentiated from bacterial tracheitis and epiglottitis, which have rapidly progressive courses and a potentially fatal outcome. With the widespread use of the *Haemophilus influenzae* type b vaccine, epiglottitis now rarely occurs. However, bacterial tracheitis has been increasingly observed.[69] Croup associated with hPIV usually has a more gradual onset, with a preceding upper respiratory tract infection. Its fluctuating course and prominent "seal's bark" cough without drooling help differentiate it from epiglottitis and bacterial tracheitis. Children with croup associated with hPIV infection usually have peripheral white blood cell counts that are normal or initially slightly elevated with a mild left shift. In contrast, among children with stridor from epiglottitis or bacterial tracheitis, the left shift usually is marked.

In countries that continue to have diphtheria, laryngeal diphtheria may be a consideration in an unimmunized child. However, laryngeal diphtheria usually is gradual in onset and manifests with characteristic membranous pharyngitis. Stridor and other signs of croup also may be present in children with other congenital or acquired conditions that obstruct the laryngeal airflow, such as aspiration of a foreign body, tracheomalacia, and acute angioneurotic edema.

MANAGEMENT AND THERAPY

Most hPIV infections are self-limited and require no treatment. Specific therapy currently is not available for hPIV infections. Ribavirin inhibits hPIV replication in vitro and has appeared in a few studies to provide some benefit in controlling hPIV infections among a limited number of immunocompromised patients.[26,35,36,114,145] Controlled trials, however, are lacking. Among potential therapies being explored are sialic acid inhibitors, such as those used in treating influenza, which inhibit hPIV F and HN activities.[2,51,113,128] Other therapeutic approaches include interference with viral attachment and replication with the use of inhibitors of protein and nucleic acid synthesis, synthetic peptides, and antivirals inhibiting *S*-adenosyl-L-homocysteine hydrolase.[11,21,64,147]

Management of croup in the home is primarily supportive.[165] The armamentarium used during the past century has been varied and usually anecdotal, ranging from cold night air to steam to

antiemetics. No such home therapies have proved beneficial. Their long tenure as helpful modes for treating croup may result from the characteristic fluctuating course of croup and its usual self-limited course. Most children with croup improve spontaneously within a few days.

Children who are more severely affected require outpatient evaluation, usually in an emergency department. With the current management offered in emergency departments, few children's symptoms progress such that hospitalization is required.[29,138,165] The current consensus and recommendations for the management of acute viral laryngotracheobronchitis for the child who is hospitalized or being evaluated in the emergency department have evolved since the 1980s and have resulted in a more consistent beneficial outcome.[3,165] Administration of aerosolized epinephrine has been demonstrated to provide rapid relief to the airway obstruction. Nebulized racemic epinephrine initially was preferred over L-epinephrine because nebulized racemic epinephrine generally was thought to be accompanied by fewer side effects. However, this consensus subsequently proved to be erroneous.[156] The relief afforded by nebulized epinephrine is transient, usually lasting no longer than 2 hours. Furthermore, hypoxemia, if present, and the outcome are not affected by treatment with nebulized epinephrine.[140,165]

Currently, administration of corticosteroids has become the cornerstone of management for the more severely affected child who fails to improve with the usual supportive care. Corticosteroids administered intramuscularly, intravenously, orally, and by nebulization have been shown to provide significant and lasting benefit on the clinical course and outcome, compared with placebo and to other therapies.[3,135] Parenteral dexamethasone in high doses (>0.3 mg/kg or its equivalent) or oral dexamethasone (0.15 to 0.6 mg/kg) has been shown to diminish the severity of disease and need for hospitalization in children with moderate to severe disease.[3,76] The recommended dose most commonly used is a single dose of 0.6 mg/kg of dexamethasone intramuscularly or intravenously.[165] For more mildly affected children treated as outpatients, a lower dose of oral dexamethasone (a single dose of 0.15 mg/kg) has been shown to be effective.[3,165] The treatment of croup is discussed more thoroughly in Chapter 22. Antibiotic therapy should be reserved for documented episodes of secondary bacterial infection, which infrequently are associated with hPIV infections.

PROGNOSIS

Most previously healthy children recover from hPIV infections completely and without complication. Follow-up studies of normal children with hPIV infections support the generally good prognosis. Some children who have had croup have pulmonary function abnormalities detected subsequently, but proof that these abnormalities were caused by their hPIV infection is lacking.[55,95]

The potential for development of acute complications, morbidity, and mortality is related to the presence of a co-morbid condition such as an immunodeficiency and diseases that affect cardiopulmonary function, including congenital malformations and acquired conditions obstructing the airway.[165] Of children hospitalized with hPIV infections who were studied during a 4-year period, 35 percent had preexisting pulmonary or cardiac abnormalities, prematurity, or asthma.[61] Compared with the children who previously were normal, the children with underlying conditions had significantly more severe disease and more complicated courses requiring longer hospitalization. Immunodeficient patients, as described previously, have particular difficulty in controlling and clearing hPIV infections, and, thus, these children have complicated, prolonged, and recurrent respiratory infections. Prolonged or persistent infection with hPIVs, especially with hPIV-3, has been noted to occur in some patients with chronic bronchitis, possibly related to the lack of a sufficient specific antibody response in the sputum.[53] The possibility that PIV-like viruses may be latent in the human central nervous system also has been suggested by the occasional detection of such viruses in the central nervous tissue of patients with chronic neurologic conditions, such as multiple sclerosis, and by the recognition that PIVs are capable of establishing persistent infection.[108,117,123,157]

PREVENTION

For decades, attempts to prevent hPIV infections focused on the development of effective vaccines. The current lack of a licensed vaccine for hPIV infections attests to the problematic nature of infection with hPIVs and of the response of the human host at different ages. Many of the hurdles encountered in the development of an effective vaccine for hPIV are similar to those for RSV. The vaccine, at least for hPIV-3, would have to be administered during the first few months of life when the infant's immunologic system is not fully developed and when the potential inhibitory effect of passive maternal antibody exists. Furthermore, to elicit durable protection, the vaccine would have to produce an immunologic response superior to that derived from natural infection because recurrent infections occur throughout life.

Hence, the goals of immunization for hPIVs, like those for RSV, currently may need to be modified toward prevention of more serious disease in targeted high-risk groups, which may require multiple vaccines tailored for these individual groups. Because the greatest clinical and economic burdens have been estimated to occur with hPIV-3 infection among infants, compromised patients, and elderly individuals, many of the candidate vaccines have been aimed at providing protection against hPIV-3 infection.[21,34,112,147]

Initially formalin-inactivated, parenteral hPIV vaccines, along with formalin-inactivated vaccines for RSV, were developed and evaluated in clinical trials.[23,43,86] In general, these vaccines, whether monovalent, trivalent, or combined with other respiratory vaccines, were immunogenic, inducing a variable humoral antibody response, but they were not protective.[23,43] Subsequent natural hPIV infection in the vaccinees, however, did not result in more severe disease, as occurred following administration of the alum-precipitated RSV vaccine.[23,43]

Development of candidate vaccines currently focuses primarily on live-attenuated and subunit vaccines. Live-attenuated vaccines compared with inactivated or subunit vaccines have the potential advantages of inducing immunity more closely mimicking natural infection by eliciting both humoral and mucosal immunity and a longer duration of protection. Furthermore, because significant yearly antigenic change, as observed with the influenza viruses, does not occur with hPIVs, a single monovalent or polyvalent vaccine may suffice, although later booster doses may be required. The potential disadvantages of a live vaccine are more adverse or symptomatic reactions, the possible transmission of the vaccine virus, and insufficient genetic stability to prevent reversion to the more virulent virus from which it was derived.

Cold-passage hPIV-3 mutant strains derived from a wild-type hPIV-3 strain produced in cell culture were demonstrated to be stable and to grow well at the lower temperatures of the nasal passages, but not at the higher temperatures of the lower respiratory tract, and they were attenuated clinically.[34] The *cp* 45 mutant strain was developed into a candidate hPIV-3 vaccine containing 20 point mutations with the desired characteristics of cold adaptation *(ca)*, temperature sensitivity *(ts)*, attenuation *(att)*, and genetic stability.[9,10,139] A second candidate hPIV-3 vaccine was

developed from a closely related bPIV-3. The protective surface glycoproteins, HN and F, of bPIV-3 and hPIV-3 share amino acid identities of 75 and 78 percent, respectively. The bovine virus has restricted replication in humans and, thus, is attenuated. Nonetheless, immunization with bPIV-3 induced sufficient immunity to resist challenge with hPIV-3.

Both these vaccines were evaluated in phase I and II clinical trials in adults, seropositive children, and seronegative infants as young as 1 month of age. In seropositive children and adults, the vaccines were not sufficiently immunogenic or protective. In some trials of seronegative infants, the hPIV3-*cp*45 vaccine was too reactogenic and was associated with otitis media.[34] In a subsequent trial of hPIV3-*cp*45 vaccine involving seronegative children 6 to 18 months old, the vaccine was immunogenic and was not associated with the occurrence of otitis media.[10] Clinical evaluation of both bPIV-3 and hPIV3-*cp*45 candidate vaccines are on going. Other candidate intranasal vaccines also are being developed from unmanipulated bPIV-3 and, for immunization against hPIV-1, Sendai virus, the murine PIV-1.[34,142,147]

The use of reverse genetics is particularly applicable and beneficial in advancing the development of PIV vaccines because the molecular virology of the PIVs has been relatively well deciphered.[112] This technology has allowed the development of such PIV candidate vaccines as those produced by recovering infectious hPIV and bPIV from cDNA. The systematic introduction of mutations into the viral genome resulted in potential recombinant and chimeric vaccines with more precise and promising profiles. Among them are chimeric human-bovine vaccines for PIV-3, in which the HN and F glycoproteins of bPIV-3 are replaced by those of hPIV-3, or the NP of hPIV-3 replaced by that of bPIV-3.[7,60] Potential vaccines for hPIV-1 and hPIV-2, some of which contain the internal genes of hPIV-3 with the surface glycoproteins of hPIV-1 or hPIV-2, also have been produced by recombinant technology. Multiple other approaches to developing candidate hPIV vaccines through recombinant DNA technology are being explored and include chimeric PIV vaccines with heterologous paramyxoviruses and with viruses for which concurrent immunization would be desirable, such as RSV and influenza.[34,98,112,116]

Subunit vaccines containing HN and F proteins from hPIV-1, hPIV-2, and hPIV-3 are being investigated concurrently as single- or multiple-valent vaccines. These glycoproteins have been produced through viral purification procedures and by expression of recombinant viruses with the use of vectors such as vaccinia and baculovirus. Neutralizing antibodies are produced by vaccination with either the HN or the F protein, but protection against infection may require immunization with both glycoproteins. On the basis of the encouraging studies with these subunit vaccines in rodents and in primates, some vaccines have reached initial clinical trial evaluation.[21,147] Multiple unique approaches combined with these creative techniques, such as novel adjuvants, immunomodulators, and vehicles for incorporation and administration of the vaccine, are evolving and may enhance further the protective immune response from PIV immunization.

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CHAPTER 192

Measles Virus

James D. Cherry

Measles virus is a singular agent that causes a relatively distinct, exanthematous disease characterized by fever, cough, coryza, conjunctivitis, an erythematous maculopapular confluent rash, and a pathognomonic enanthem. Clinical measles is an epidemic disease, the incidence of which in the United States was reduced by the use of attenuated vaccines from 315 reported cases per 100,000 persons in the pre-vaccine era to less than 1 per 100,000 in 1992. At present, measles no longer is an endemic disease in the United States. However, measles is still a major problem because of its worldwide prevalence, associated morbidity and mortality, and changing epidemiologic patterns in countries where vaccine use has been less than optimal.

HISTORY

In antiquity, measles and smallpox frequently were confused with each other, as well as with other exanthematous diseases.[26,332] Major epidemics of both measles and smallpox occurred 1800 years ago in the Roman Empire and China.[44,288] The first written record of measles generally is credited to Rhazes, a 10th century Persian physician.[26,44,227,455] However, Rhazes quoted writers, including El Yahudi, a famous Hebrew physician, who lived 300 years earlier.[44] Rhazes identified measles as an entity distinct from smallpox.

During the Middle Ages in Europe, smallpox and measles continued to be confused. By the beginning of the 17th century, however, differentiation between the two diseases was relatively clear; death reports by London parish clerks in 1629 listed measles and smallpox separately.[455] Repeated epidemics of measles were described in the English medical literature during the 17th and 18th centuries.[227]

The first account of measles in America was given by John Hall, who described epidemic disease in Boston in the fall of 1657.[67] The next epidemic in colonial America was reported in 1687. During the next 150 years, the interval between epidemics in Boston gradually decreased from 30 years to approximately 3 years. Epidemics during the 17th and 18th centuries involved persons of all ages, including neonates; coincident with a reduction in the interval between epidemics was a reduction in the age-specific incidence of measles. The reduction in time between epidemics can be attributed to the increased incidence of importation of measles that occurred as more and faster ships crossed the Atlantic and the population density in North America gradually increased. By the turn of the 19th century, both Boston and Philadelphia had sufficient populations for measles to propagate itself.

The first recognition of the contagiousness of measles is unclear. Shakespeare in *Coriolanus* was aware of its human-to-human transmission.[455] Home[201] in 1758 attempted to immunize against measles by applying a technique similar to the variolation used in smallpox.[362] In 1911, Goldberger and Anderson[165] produced clinical measles in monkeys by injecting filtered material from acute cases of human disease.

The classic epidemiologic study of measles is the account described by Panum[350] of the 1846 epidemic in the Faroe Islands. In this study, Panum confirmed that spread occurred solely through human-to-human contagion by the respiratory route, the incubation period was 14 days, and infection conveyed lifelong immunity.

The enanthem of measles, which is pathognomonic, was described carefully and presented by Koplik[242-244] in 1896, 1898, and 1899.[38] However, Koplik spots clearly were recognized specifically about a century earlier by John Quier, a physician in Jamaica,[38,163] as well as by Richard Hazeltine, a general practitioner in rural Maine.[67]

Although Plotz[363] reported cultivation of measles virus in 1938 and Rake and Shaffer[368] noted similar findings in 1940, reliable tissue culture methods were not available until approximately 10 years later. In 1954, Enders and Peebles[127] isolated eight agents (from cases of measles) in human or simian renal cell cultures. These investigators also demonstrated the ability of convalescent-phase serum from a patient with measles to neutralize the viral cytopathogenic effect. The stage for vaccine development was set by recovery of tissue culture of the virus,[127] adaptation of

viral growth in chicken embryos,[295] and, finally, cultivation of the virus in chicken embryo tissue culture cells.[229]

After extensive trials were conducted from 1958 through 1962, tissue culture–grown, inactivated ("killed"), and attenuated ("live") measles viral vaccines became available for general use in 1963.[90,226,247] In the United States, a nationwide immunization effort instituted in 1965 and 1966 led to a dramatic reduction in the incidence of epidemic measles for several years. Epidemic measles recurred in 1971, 1977, and 1989, but at overall levels less than those in the pre-vaccine era.[22,68,69,76,90,248] After initiation of the Childhood Immunization Initiative in 1977 and the Measles Elimination Program in 1978, the incidence of measles in the United States fell in 1981 to fewer than 1.5 cases per 100,000 population and remained at this low level until 1986.[22,74,75] In 1990, the rate was 11.2 cases per 100,000 population, which was the highest it had been since 1977. After 1990, a substantial decline in the incidence of measles occurred in the United States, to a low of 86 confirmed cases in 2000.[77] Currently, endemic transmission of measles no longer is occurring in the United States.[345] However, approximately 1 million children worldwide die of measles each year.[160,344]

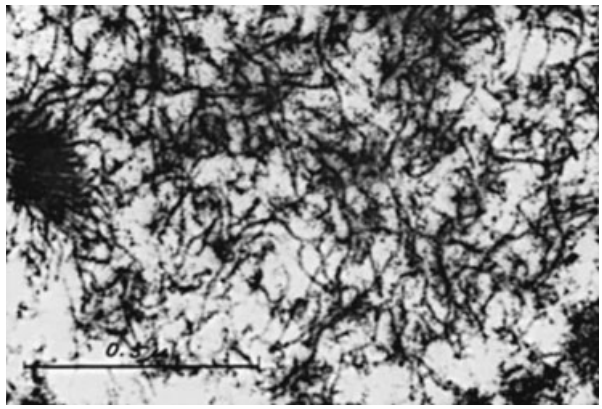

Figure 192–1 Nucleocapsid fragments. Electron micrograph. *(Courtesy of Dr. John M. Adams.)*

PROPERTIES

CLASSIFICATION

Measles virus is a relatively large virus with helical capsid symmetry and an RNA genome.[35,133,256] It is a singular virus, but antigenically it is related closely to canine distemper. These agents, as well as peste des petits ruminants virus, dolphin morbillivirus, phocine distemper virus, and rinderpest virus, currently are included in the genus *Morbillivirus*; they are members of the family *Paramyxoviridae*. Measles virus differs from the other paramyxoviruses in that it does not possess specific neuraminidase activity and does not adsorb to neuraminic acid–containing cellular receptors.[307,327,359,444] Measles virus hemagglutinates, whereas the other members of its genus do not.

PHYSICAL PROPERTIES

Measles virus is a roughly spherical but pleomorphic virus that ranges from 100 to 300 nm in diameter.[35,133,177,319,332,444,445] The virion is composed of an outer lipoprotein envelope and an internal helical nucleocapsid. The virion contains eight proteins. The outer viral envelope is 10 to 22 nm thick, has short surface projections (peplomers), and contains three virus-coded proteins (F, H, and M).[92,307,332] F protein is a dumbbell-shaped peplomer that causes membrane fusion of the virus and host cell and enables penetration of the virus into the host cell. H protein is the hemagglutinin and is a conical peplomer. In infection, H protein reacts with a host cellular receptor. CD46 appears to be the cellular receptor for attenuated measles vaccine strains but not wild-type viral strains.[54,119,272,273,321,342] More recent data suggest that the signaling lymphocyte activation molecule (SLAM) is the cellular receptor for wild-type virus.[117,342] M (matrix) protein is nonglycosylated and associated with the inner lipid bilayer of the envelope. It plays an important role in the maturation of the virus. The nucleocapsid (N) protein (Fig. 192–1) is a coiled rod with a diameter of 18 nm and a length of 1 μm that contains the viral genomic RNA.[330-332,442,444] N protein has a molecular weight of approximately 60 kd. Approximately 5 percent of the nucleocapsid is RNA.[188,431,443] The other internal proteins of the virus are the L (large), P (phospho), C, and V proteins.

The virus genome has a molecular weight of 4.5×10^6 d. It is a linear, single strand of RNA that contains approximately 15,900 nucleotides.

Measles virus is labile.[227,228,314] It is inactivated rapidly by heat, ultraviolet light, lipid solvents such as ether and chloroform, and extreme degrees of acidity and alkalinity (i.e., pH < 5 and >10). Longevity is prolonged when protein is present in the viral suspending medium and when the virus is lyophilized with a protein stabilizer. Protein specifically protects against the adverse effects of heat and light. Protein-stabilized measles virus can be stored at –70° C for 5 or more years without significant loss of infectivity. At room temperature, a 60 percent loss in titer occurs in 3 to 5 days; at 56° C, the virus is inactivated within 30 minutes.[41,314]

ANTIGENIC COMPOSITION

Clinical and epidemiologic data and early laboratory study suggested antigenic homogeneity of all measles strains.[228] More recent nucleotide sequence analysis identified distinct lineages among wild-type measles virus isolates.[35,314,383,423] The following properties have been associated with measles virus: a hemagglutinin (for simian cells), complement-fixing antigens, hemolytic activity, and a giant-cell–inducing factor.[56,189,227,228,328,329,336,359,393] Human measles virus infection results in serum antibodies that are capable of neutralizing viral infectivity, fixing complement with viral antigen, and inhibiting viral hemagglutination and hemolysis.

Cross-seroreactivity occurs among members of the genus *Morbillivirus* but not with other members of the family *Paramyxoviridae*.[47,62,210] Measles virus serum antibody in humans reacts with distemper virus, but canine serum after a case of distemper does not react with measles virus. A two-way cross between measles and rinderpest viruses has been demonstrated.

TISSUE CULTURE GROWTH

Measles virus can be propagated in many different primary and cell line tissue cultures.[47,228,282] However, for isolation of virus from patient specimens, primary human and monkey kidney cultures have been most successful over the years. In one study, an Epstein-Barr virus–transformed marmoset lymphocytic line (B95-8) was found to be superior to primary monkey kidney cell culture for isolation of virus from nasopharyngeal specimens.[238] In tissue culture, measles virus has two distinct cytopathogenic effects. With initial isolation, syncytial formation occurs as a result of cell fusion, and the resulting giant cells may contain 10

to 50 or more nuclei (Fig. 192–2). On stained preparations, both the nuclei and cytoplasm contain eosinophilic inclusions. The second type of cytopathogenic effect is characterized by the alteration of single cells into spindle shapes or stellate forms. In general, tissue culture-adapted measles viral strains are more likely to cause this cytopathogenic effect than is giant-cell formation. In most cultures, both forms of cytopathogenic effect are evident, and changes in medium composition render one or the other type predominant.

Infection in tissue culture is associated with an attachment-adsorption phase lasting approximately 1 hour and an eclipse period of 6 to 12 hours.[282,307] Antigen first is noted in the perinuclear cytoplasm by 12 hours; by 24 hours, it is distributed throughout the cytoplasm. By 30 hours, most antigen is detected at the cell surface. In mature cultures, more cell-associated virus is present than is found free within the medium.

ANIMAL SUSCEPTIBILITY

Humans are the natural hosts of measles virus, but with human contact, monkeys also are infected easily.[292] Laboratory strains of measles virus have been adapted to suckling mice and hamsters.[55,61,211,439]

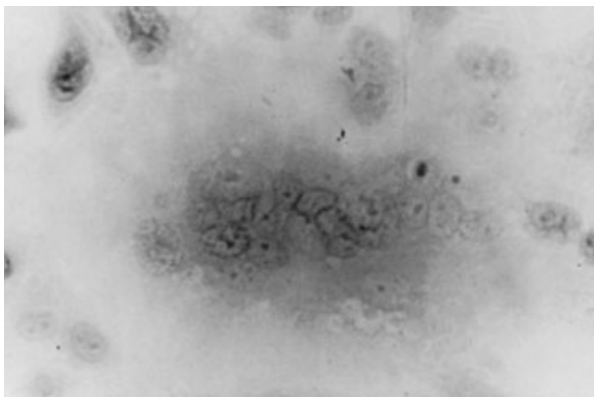

Figure 192–2 Measles virus cytopathogenic effect in monkey kidney tissue culture. Giant cell with approximately 20 nuclei.

EPIDEMIOLOGY

PREVALENCE

The prevalence of measles throughout history has been affected markedly by population density and, since the 1960s, by the use of measles vaccine. Reported cases of measles in the United States from 1963 to 1998, analyzed by year, are presented in Figure 192–3. After the universal use of measles vaccine began in 1965, the number of cases in the United States fell to 22,231 in 1968.[76] In 1971 to 1972 and 1976 to 1977, modest epidemics occurred; then, after a national commitment to measles immunization, the number of cases of measles fell to an all-time low of 1497 cases reported in 1983. Beginning in 1986, the number of measles cases again increased, with 27,786 cases reported in 1990. After 1990, measles in the United States declined, with only 100 cases reported in 1998.[77] Since 1998, the endemic transmission of measles in the United States has been eliminated.[345] In 2005, 66 cases were reported in the United States, and 95 percent of these were linked to importations.[78] Half of all the cases in 2005 were traced to one unvaccinated traveler who was infected in Europe. All but two of the contact cases in this outbreak were unvaccinated because their parents had declined immunization. In the 20th century, before the widespread use of measles vaccine, between 200,000 and 600,000 cases of measles were reported annually in the United States.[99,258] Careful survey suggested that in the past, reported cases accounted for only approximately 15 percent of the actual number of cases of measles.[90]

In the pre-vaccine era in the United States and other concentrated populations throughout the world, measles epidemics occurred regularly. In the United States, urban-centered measles epidemics took place every 2 to 5 years, with each epidemic lasting 3 to 4 months.[26,44,186,195,266,450,463] In general, the larger the community, the shorter the interval between epidemics. In the vaccine era, the epidemic pattern has been changed. As noted in Figure 192–3, the total number of cases has been reduced, and the cycle between peaks has lengthened.

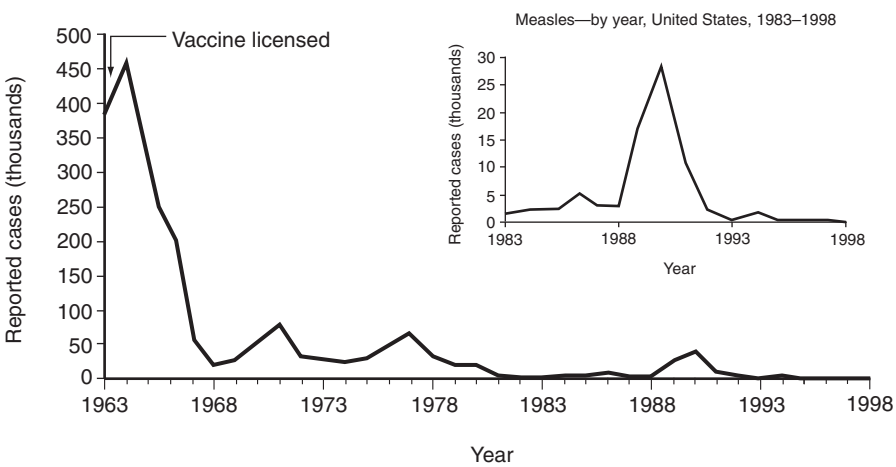

Figure 192–3 Reported measles cases by year in the United States, 1963 to 1998. *(From Summary of notifiable diseases—United States, 1998. M. M. W. R. Morb. Mortal. Wkly. Rep. 47:48, 1999).*

AGE INCIDENCE AND PREVALENCE

In modern times in populous areas, measles has been a disease of children. The age-specific incidence in the United States for selected years is presented in Table 192–1. In the pre-vaccine era during the 20th century, the highest measles attack rate occurred in children aged 5 to 9 years.[26,44,195,454] In the period 1960 to 1964, data from five reporting areas showed that more than half of all measles cases occurred in this age group.[191] Before the present vaccine era, infections and epidemic loci centered in elementary schools. Younger children acquired measles as secondary cases from their older siblings who were attending school. In rural areas, the interval between measles epidemics tended to be greater; therefore, a broader spread in ages occurred in the percentage of measles cases. In a nationwide serum survey of U.S. military recruits in 1962, Black[43] found that 99 percent had measles antibody.

As noted in Table 192–1, the age-related incidence and percentage of measles cases by age group have changed markedly since 1964. In 1991, more than 40 percent of measles cases occurred in persons older than 10 years; in the period from 1960 to 1964, fewer than 10 percent of the patients were older than 10 years. Approximately a third of the cases in 1991 were in adolescents and young adults. Evidence also indicates high primary measles attack rates in newborns to 4-year-old children in areas of suboptimal vaccine utilization.[22,77,90,146,193] In 2005, 66 measles cases were reported.[78] Seven cases (10.6%) were in infants, 4 (6.1%) in children aged 1 to 4 years, 33 (50%) in persons aged 5 to 19 years, 7 (10.6%) in persons aged 20 to 34 years, and 15 (22.7%) in adults aged 35 years or older. Most measles cases that have occurred in the United States since 1994 have been imported or importation-associated.[77,382,384] Measles in heavily populated but developing countries has its greatest incidence in children younger than 2 years.[96,139,194,308,337]

GEOGRAPHIC DISTRIBUTION

In the 20th century, measles occurred regularly throughout the world in all but the most remote areas.[26,44] In island and other isolated populations, intervals between epidemics can be 10 years or more, and the occurrence of disease depends on its introduction from outside the area.

SEASONAL PATTERNS

Epidemic measles is a winter-spring disease in temperate climates, with peak activity in the Northern Hemisphere occurring in March or April. In equatorial regions, epidemics of measles are less marked but tend to occur in the hot, dry seasons.[26,310,377,420]

HOST AND SOCIAL FACTORS

No difference is found in the incidence of measles in the male and female population. One suggestion, however, is that complication rates are higher in boys or men than in girls or women. Tidstrom[425] found that acute laryngitis occurred more than twice as frequently in male patients as compared with female patients. In other studies, otitis media, pneumonia, and death occurred slightly more often in male patients.[26,338] Miller,[294] in a large survey in England in 1963, found no difference in the incidence of complications in male and female patients. Christensen and associates,[93] in studies of an epidemic in Greenland, noted a greater number of complications in female patients older than 15 years, attributable primarily to an increased incidence of pneumonia. In a review of 375 confirmed cases of subacute sclerosing panencephalitis (SSPE), the male-to-female ratio was 2.4:1.[303] According to Black,[44] antibody titers are slightly higher in women than in men. Our studies involving young adults failed to reveal a similar sex-associated difference in antibody levels, however.[406] Green and associates[172] noted that a group of women had a higher postimmunization geometric mean antibody titer than did vaccinated men.

Although the severity of disease in certain populations appears to suggest differences based on race, this difference may be the result of nutritional and other environmental factors. Deseda-Tous and associates[113] were unable to demonstrate any differences in measles antibody by human leukocyte antigen (HLA) or by ABO blood types. In a more recent study, the findings of Ovsyannikova and coworkers[347] suggested that HLA alleles relate to antibody responses after immunization. The following alleles were positively associated with hyperseropositivity: HLA-B*7, -DQA1*0104, and -DPA1*0202. In contrast, HLA-B*44, -DRB1*01, -DRB1*08, -DQB1*0301, and -DPD1*0401 alleles were negatively associated with hyperseropositivity. In another study, the same group looked for associations between HLA homozygosity and measles antibody levels.[415] They found a negative correlation between homozygosity (=1 loci) and antibody value.

In the pre-vaccine era, the age of patients at the time of acquiring measles infection was related inversely to the number of siblings in the family.[26,44,459] In general, measles occurred at an earlier age in city dwellers and those of lower socioeconomic classes than in rural families and well-educated, upper-income groups.

In the present era of antibiotics and other medical modalities, the greatest factors in measles-related morbidity and mortality are age and nutritional status. The mortality rates for measles are highest in children younger than 2 years and in adults.[26,44] The severity of measles and rates of mortality correlate in general with the severity of malnutrition.[44,337] However, extensive investigations by Aaby and others[3,4,159] since the 1980s suggest that overcrowding and intensive exposure may be more important

TABLE 192–1 Incidence and Percent Distribution of Reported Measles Cases by Age Group in Selected Years, United States

	1960-1964*			1974			1979			1991		
Age Group	**Cases**	**%**	**Incidence†**	**Cases**	**%**	**Incidence†**	**Cases**	**%**	**Incidence†**	**Cases**	**%**	**Incidence†**
<1-4	93,653	37.2	766	5,899	26.7	36	2,331	20.7	18.0	4,756	49.3	24.7
5-9	132,956	52.8	1,237	5,391	24.4	30	2,473	21.9	18.1	991	10.2	5.5
10-14	16,403	6.5	169	7,799	35.3	38	3,054	27.1	20.4	905	9.4	5.3
15-19	8,635	3.4	10	2,475	11.2	12	2,633	23.3	15.2	1,102	11.4	6.2
20+				552	2.5	>1	786	7.0	0.6	1,890	19.6	1.8
Totals	**251,647**			**22,094**			**11,277**			**9,643**		

**Data from four reporting areas: Washington, D.C., New York City, Illinois (including Chicago), and Massachusetts.*[22,68,72,191]
†Incidence, cases per 100,000 population, extrapolated from the age distribution of known cases.

determinants of measles-related mortality than is nutritional status alone.

Studies of measles conducted early in the 20th century and in developing countries indicated that secondary cases in households were likely to be more severe than were primary cases.[2-4,159] However, more recent studies in the United States found no difference in severity between primary and secondary cases in families.[58,417]

SPREAD OF INFECTION

Measles is a highly contagious disease in nonimmune persons. It is spread from an infected ill person to a new host by the respiratory route.[26,44,374,375,378] Although monkeys can acquire measles from humans, practically speaking, no animal reservoir exists.[292] Available evidence suggests that infection is spread by persons ill with measles. Asymptomatic contagious carriers are unknown, and persons with acute asymptomatic infection probably are not contagious or only minimally so. The period of greatest contagion occurs during the prodromal period.[165]

Transmission of measles is thought to occur mainly by aerosolized droplets of respiratory secretions. Acquisition of infection by a new host is by the nose and possibly the conjunctivae.[351] Infection can be initiated by small-droplet nuclei, which stay suspended in air for considerable periods of time, or by direct hits of large droplets at close range.[48,372] Also possible is that spread involves close person-to-person contact in young children, with large virus-containing droplets of nasal secretions being picked up on the hands of the future host and then applied to the nose.

PATHOGENESIS AND PATHOLOGY

VIRAL INFECTION

The sequence of viral events in uncomplicated measles is presented in Table 192–2. Although much is known about measles virus infection in humans, considerable gaps exist regarding specific events. Experimental studies in other primates have been performed in an attempt to fill in the gaps and thereby provide a more complete picture.[224,283,326,341,396,461] The primary site of infection appears to be the respiratory epithelium of the nasopharynx. Measles vaccine virus instilled into the nose or by aerosol results in infection.[45,246] Papp[351] reported studies suggesting that infection resulted from conjunctival contact and proposed that this means was the primary portal of entry. However, experiments with vaccine virus generally have been unsuccessful when conjunctival inoculation has been performed.[45] Initial infection of the respiratory epithelium appears to be minimal; a more important event is the early spread of virus to regional lymphatics. A presumption based on data derived from Fenner's ectromelia-mouse experimental model[132] is that after such spread, primary viremia occurs, followed by extensive multiplication of virus in the reticuloendothelial system at both regional and distant sites. Multiplication of virus also continues at the site of initial infection.

During the fifth to seventh days of infection, extensive secondary viremia takes place and results in the establishment of generalized measles viral infection. The skin, conjunctivae, and respiratory tract are obvious sites of infection, but other organs may be involved as well. From the 11th to the 14th days, the viral content of the blood, respiratory tract, and other organs peaks and then rapidly diminishes over the ensuing 2- to 3-day period.

In immunologically compromised patients with defects in cell-mediated factors, measles virus is not cleared from the secondary infection sites, and progressive, frequently fatal illnesses occur.[8,128,297,299,311] During infection, measles virus replicates in endothelial cells, epithelial cells, and monocytes and macrophages.[180,304]

TABLE 192–2 Sequence of Measles Virus Infection in Uncomplicated Primary Disease

Day	Event
0	Measles virus in droplet nuclei or large droplet comes in contact with the epithelial surface of the nasopharynx or possibly the conjunctiva
	Infection of epithelial cells and virus multiplication
1-2	Extension of infection to regional lymphoid tissue
2-3	Primary viremia
3-5	Multiplication of measles virus in respiratory epithelium at the site of initial infection and in the reticuloendothelial system regionally and at distant sites
5-7	Secondary viremia
7-11	Establishment of infection in the skin and other viremic sites, including the respiratory tract
11-14	Virus in blood, respiratory tract, skin, and other organs
15-17	Viremia decreases and then ceases
	Viral content in organs rapidly diminishes

Data from references 175-177, 180, 184, 224, 230, 235, 262, 326, 341, 358, 378, 386, 396, and 461.

PATHOLOGY

The characteristic pathologic feature of measles is the widespread distribution of multinucleated giant cells, which are the result of cell fusion.[47,224,227,262,282,326,341,379,396,461] Two main types of giant cells occur in measles: (1) Warthin-Finkeldey cells, which are found in the reticuloendothelium; and (2) epithelial giant cells (Fig. 192–4), which occur principally in the respiratory epithelium but also on other epithelial surfaces.[262]

Warthin-Finkeldey giant cells are found throughout the reticuloendothelial system in the adenoids, tonsils, Peyer patches, appendix, lymph nodes, spleen, and thymus. They vary in size and contain as many as 100 or more nuclei. The cells contain both cytoplasmic and intranuclear eosinophilic inclusions, with cytoplasmic inclusions more common than intranuclear lesions. During the prodromal stage of measles, epithelial giant cells regularly are present on respiratory surfaces and frequently are sloughed free (see Fig. 192–4).

Measles Exanthem

Hematoxylin and eosin–stained sections of skin biopsy specimens have revealed typical epithelial syncytial giant cells with nuclear and cytoplasmic inclusions.[235,416] The giant cells contain 3 to 26 nuclei. Other findings include the following: focal parakeratosis, dyskeratosis, spongiosis, and intracellular edema. The superficial blood vessels are dilated, with a sparse, surrounding lymphohistiocytic infiltrate.

Koplik Spots

Suringa and colleagues[416] observed that the histopathologic features of Koplik spots were similar to those of the rash. These investigators noted more giant cells with more nuclei, a greater degree of edema, and a lessened inflammatory response in the enanthem biopsy sample, however.

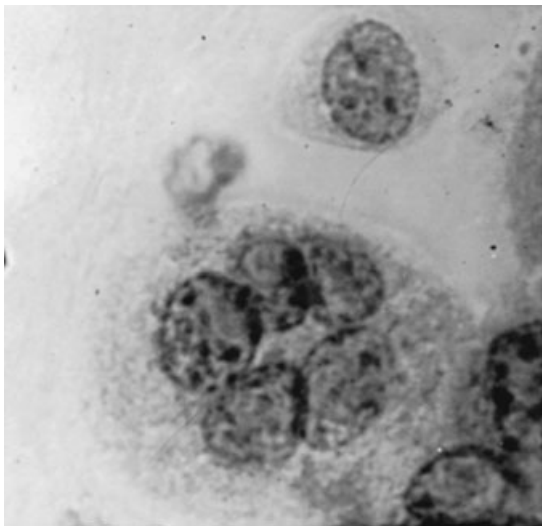

Figure 192–4 Epithelial giant cell with five nuclei. Pharyngeal smear. *(Courtesy of Dr. John M. Adams.)*

Respiratory Tract

The extent of respiratory involvement in uncomplicated measles is not known. However, clinical symptoms and the extensive radiographic studies of Kohn and Koiransky[240] suggested that pharyngitis, tracheitis, bronchitis, and pulmonary infiltration are the rule rather than the exception. Unfortunately, the human pathologic data available have been obtained mainly from complicated cases, so the findings cannot be considered representative.[111,379] In a study in monkeys, Nii and colleagues[326] noted giant cells in the mucosal epithelium of the trachea, bronchi, and bronchioli. The lumina of these airways contained sloughed syncytial cells. Warthin-Finkeldey cells were found in the adjacent lymphatics.

In the lungs of experimentally infected monkeys, interstitial pneumonitis was observed with giant-cell formation.[326] Infiltration of neutrophils, eosinophils, and mononuclear cells also was noted.

IMMUNOLOGIC EVENTS

After natural or attenuated measles virus infection develops, large numbers of specific and nonspecific immunologic responses occur. Many questions relating to immunity in measles remain unanswered, although a considerable body of knowledge has been gathered since the 1950s.

Antibody

After measles viral infection develops, an antibody response regularly occurs. Serum antibodies to the N, F, H, and M proteins of measles virus can be demonstrated by hemagglutination-inhibition (HI), complement-fixation (CF), neutralization, immune precipitation, hemolysin inhibition, enzyme-linked immunosorbent assay (ELISA), and fluorescent antibody (FA) methods.[105,156,170,236,249,322,328,333,433] Antibody-dependent cellular cytotoxicity and antibody-dependent complement-mediated lysis also have been demonstrated.[144,145,233] In natural infection, HI and neutralizing antibodies appear at approximately the 14th day, peak at around 4 to 6 weeks, and decrease approximately fourfold from the peak over the course of a year. Most naturally infected persons have demonstrable HI and neutralizing antibodies for life. Krugman[248] noted an average 16-fold reduction in HI antibody titer 15 years after natural infection in a group of children who had no measles exposure during the observation period.

After immunization has been administered, both HI and neutralizing antibodies are present by the 14th day.[156,249] CF antibody appears slightly later than does HI antibody and in general does not persist as long as does either HI or neutralizing antibody. Primary infection is characterized by the initial appearance of antibody in the immunoglobulin M (IgM) and IgG serum components.[142,196,394,395,428] The IgM-specific response is short-lived, and rarely can measles antibody in this fraction be demonstrated more than 9 weeks after infection. IgA secretory antibody also occurs regularly after vaccine viral infection and after natural infection.[33,151]

IgG antibody appearing after infection is primarily subclasses IgG1 and IgG4.[280] Antibody detected by neutralization and by HI is mainly to H protein and correlates with clinical protection against illness.[114,180,328] Antibody to N protein is the main antibody detected by CF.[328] Antibody to F protein is demonstrated by inhibition of hemolysis of monkey erythrocytes by measles virus or by immunoprecipitation.[329,333] Antibody to F protein may contribute to neutralization by disrupting the fusion of virus membrane with host-cell membranes. Only small amounts of antibody to M protein are elicited after infection with wild virus or vaccine virus.[170]

Specific Cell-Mediated Responses

Investigators recognized in the 1960s that patients with defective cellular resistance factors frequently died of progressive measles virus infections.[318] In the 1970s, techniques became available that demonstrated measles-specific lymphocyte sensitization.[158,171,173,245,255,385] Graziano and colleagues[171] found that lymphocytes from persons who previously had measles had a blastogenic response in vitro when these cells were incubated with measles antigen. Labowskie and associates,[255] using an in vitro lymphocyte-mediated cytotoxicity assay, noted that lymphocytes from persons who previously had measles caused cell destruction in a tissue culture chronically infected with measles virus. Ruckdeschel and colleagues[385] observed that two physicians without detectable measles antibody who had experienced repeated recent exposure to measles without developing illness had strong in vitro cellular responsiveness to measles virus. Krause and associates[245] demonstrated excessive measles-specific lymphocyte blastogenic responses in some persons previously immunized with killed measles vaccine.

Today, T cells are appreciated to be important both in B-cell antiviral antibody responses and as effector cells for the clearance of virus-infected cells from tissues.[180] Both helper ($CD4^+$) and suppressor ($CD8^+$) cells participate in the cellular response.[441] During infection, $CD8^+$ T cells eliminate virus-infected cells by major histocompatibility complex class I–restricted cytotoxic mechanisms.[180] $CD4^+$ T cells respond to measles virus infection by the secretion of cytokines. After initial exposure to virus occurs, $CD4^+$ T cells mount a T-helper cell (T_H1) response. Before the rash develops, levels of plasma interferon-γ (IFN-γ) are increased, and with the rash, interleukin-2 (IL-2) appears. With clinical recovery, plasma levels of IL-4 increase (a T_H2 response) and stay elevated for several weeks, whereas the initial T_H1 response subsides.

On re-exposure, T_H1 and T_H2 responses occur as indicated by the production of INF-γ, IL-2, and tumor necrosis factor-β (TNF-β), IL-4, IL-5, and IL-10. The T_H1 cytokine response is important for macrophage activation (through the action of INF-γ), lymphocyte proliferation (through IL-2), and major histocompatibility complex class II–restricted cytotoxicity (through TNF-β). The T_H2 cytokine response is important for macrophage deactivation (through the action of IL-4 and IL-10) and B-cell help (through IL-4, IL-5, and IL-10). In studies in rhesus

monkeys, researchers found that humoral immunity had only a limited role in the control of measles virus replication in primary infection.[360]

Ovsyannikova and colleagues[348] found a predominant T_H1 cytokine pattern following measles vaccine viral infection. This pattern occurred after both primary immunization and revaccination. In this study, no relationship was found between any specific cytokine level and serum measles antibody values. In another study, the same research group found a subset of vaccinees in whom the cytokine responses were skewed towards the T_H2 type.[118] These investigators suggested that this response in this subset may not be sufficient for long-term immunity.

Other Responses

Listed in Table 192–3 are nonspecific, immunologically related responses that can be demonstrated during natural or vaccine measles virus infection. Anderson and colleagues[16] demonstrated a temporary defect in neutrophil motility during acute measles that resolved by the 11th day after the onset of rash. Leukopenia has been observed in natural measles[36] and after immunization.[46] With immunization, the numbers of both neutrophils and lymphocytes are reduced; this reduction lasts approximately 1 week, with an onset occurring approximately 7 days after vaccination. Coovadia and colleagues[100,102] showed that the numbers of T, B, and null cells in the lymphocyte population are reduced. With a reduction in the number of T lymphocytes, no change occurs in the ratio of helper and suppressor-cytotoxic cell phenotypes.[10,19,218]

Thrombocytopenia occasionally has been associated with natural measles[207] and vaccination.[11,452] Oski and Naiman[346] noted a mild reduction in the peripheral platelet count during routine measles immunization. Transitory complement defects vary in measles; Charlesworth and associates[81] found evidence of pathologic complement activation in 20 of 50 patents studied. Coovadia and colleagues[102] noted slight but significant reductions in serum IgA levels and elevated IgM values in acute measles virus infections. These investigators found the IgG concentrations to be normal. Increased levels of serum or plasma IgE were noted in two studies.[178,397] Delayed hypersensitivity responses in skin are suppressed in both natural and vaccine measles virus infection.[136,407] Similarly, in vitro lymphocyte blastogenic responses to common antigens are suppressed.[451]

MECHANISMS IN RECOVERY FROM MEASLES VIRAL INFECTION

Acute clinical measles is characterized by viral multiplication in many organs of the body and then rapid subsidence of the infection by the 17th day. As noted earlier, measles virus infection is associated with both serum and secretory antibody responses and specific cell-mediated responses that coincide with clinical recovery. All three factors would seem to be important in recovery from acute measles virus infection, but confusing information clouds the issue. For example, researchers have noted that children with simple agammaglobulinemia in whom measurable measles antibodies do not develop after measles virus infection recover from the disease normally.[167] This finding suggests that development of measles antibody is not important for recovery from acute infection. However, by using a sensitive plaque-reduction measles neutralizing antibody technique, Black[42] found small amounts of antibody in the sera of three agammaglobulinemic patients.

In contrast to antibody data, data on patients with defects in the cell-mediated immune system show that they clearly do poorly with measles virus infection and frequently succumb to progressive infection despite the administration of large doses of measles antibody-containing immunoglobulin. T cells (both $CD4^+$ and $CD8^+$) clear virus-infected cells from tissues by cytotoxicity. INF-α, which is released by a variety of cells, including T cells, inhibits the spread of virus infection.

In studies in a transgenic mouse model system, Tishon and associates[427] showed that CD4, CD8, or B cells alone controlled measles virus infection. However, combinations of CD4 T cells and B cells or CD4 and CD8 T cells were essential in clearing infection. In contrast, the combination of CD5 T cells and B cells was ineffective.

MECHANISMS IN PREVENTION OF REPEAT ILLNESS IN PERSONS PREVIOUSLY INFECTED WITH MEASLES VIRUS

In contrast to mechanisms of recovery in acute measles, the role of serum antibody in protection against recurrent disease is solid. Before the present vaccine era, researchers repeatedly demonstrated that the administration of measles antibody-containing immunoglobulin could prevent clinical measles.[214,413] Similarly,

TABLE 192–3 Nonspecific, Immunologically Related Responses during Measles Virus Infection

Category	Findings	Reference
Leukocytes	Defective neutrophil motility	16
	Leukopenia (both lymphocytes and neutrophils)	36, 44
	Decreased T, B, and null lymphocytes	10, 19, 100, 102, 218
	Decreased natural killer cell activity	183
	Decreased helper T cells	106, 234
	Prolonged suppression of interleukin-12 production	21
	Prolonged depression of virus-specific interferon-α production	320
Interferon-α	Elevated plasma levels	182
Neopterin	Elevated plasma levels	182
Interleukin-2 receptor (soluble)	Elevated plasma levels	181
Platelets	Reduction in peripheral count	346
Complement	Frequent pathologic activation of the complement system; reduction of C1q, C4, C3, and C5	81
Serum immunoglobulins	IgA reduced and IgE and IgM elevated	102, 178, 397
In vitro lymphocyte response to phytohemagglutinin	Suppressed in presence of autologous serum; normal in presence of calf serum	451
In vitro lymphocyte response to *Candida*	Suppressed	451
Cutaneous delayed hypersensitivity	Depressed	136
C-reactive protein	Elevated at onset of rash	179
Circulating immune complexes	Noted in 25% of patients 7 to 13 days after rash onset	467

Ig, immunoglobulin.

infants with transplacentally acquired measles antibody are immune.[249] Whether other factors also are important in protection against reinfection is not clear. Often cited is that patients with agammaglobulinemia do not have repeated measles virus infections, so other factors must be involved.[167]

Of interest is that patients have been described in whom antibody did not prevent acquisition of atypical measles after immunization with killed measles vaccine.[264] The data of Krause and associates[245] suggested that persons in whom the capacity for exaggerated measles-specific lymphocyte activity persists (some previous killed vaccine recipients) may be subject to illness in spite of the presence of antibody. However, the antibody produced after receipt of killed measles vaccine was incomplete; it lacked specific antibody to F protein.[92] Black[42] noted one child with a low measles neutralizing antibody titer, presumably from natural measles virus infection, in whom measles later developed. Chen and coinvestigators[83] found that some students with measurable but low neutralizing antibody titers were not protected from clinical measles on exposure.

CLINICAL MANIFESTATIONS

Before the present vaccine era, measles was an inevitable disease of childhood that was recognized readily by parents and other laypersons, as well as by physicians. Despite the occasional confusion with other exanthematous diseases,[85] the epidemic character of measles usually resulted in an accurate diagnosis. Currently in North America, few of the new generation of parents, physicians, and other medical personnel have seen measles, and therefore the illness can be misdiagnosed.

TYPICAL ILLNESS[26,86,90,227,252,378,412]

Incubation Period

The incubation period of measles is approximately 10 days (range, 8-12 days). Although extensive virologic and immunologic events occur during this period, the individual has virtually no outward sign of illness. Goodall[168] suggested (and Partington and Quinton[353] presented supporting data) that some patients have mild transient respiratory symptoms and fever shortly after initial acquisition of the virus.

Prodromal Period

The prodrome of measles lasts approximately 3 days (range, 2-4 days). Initial symptoms are respiratory and suggest the possibility of a cold, except fever is an early sign. In fact, in situations of close observation, slight temperature elevation has occurred and then subsided for a day or so before the appearance of typical respiratory symptoms.[412]

The onset of clinical measles is characterized by general malaise, fever, coryza, conjunctivitis, and cough. These symptoms worsen during a 2- to 4-day period. Early in the prodromal phase, a transitory rash occasionally has been observed. It has been urticarial or macular, has occurred with the initial onset of fever, and has disappeared before onset of the typical exanthem.

During the prodromal period, the temperature increases gradually to a value of 39.5° ± 1.1° C (103° ± 2° F) over the course of a 4-day period. The nasal symptoms resemble those of other respiratory viral infections and are similar to those of the common cold or acute nasopharyngitis. Sneezing, rhinitis, and congestion are common symptoms. The degree of prodromal conjunctivitis varies considerably. Initially, the conjunctival infection is divided by a transverse marginal line across the lower lids.[411] The conjunctivitis is associated with considerable lacrimation, and older patients in particular are bothered by photophobia, which frequently is severe. Slit-lamp examination reveals both corneal and conjunctival lesions.[25,138]

The cough in prodromal measles frequently is troublesome. It worsens throughout the period and often has a brassy quality suggesting laryngeal and tracheal involvement. On approximately day 10 ± 1, Koplik spots, the pathognomonic enanthem of measles, first appear (see Fig. 64–1). Koplik[242-244] originally described the lesions as bluish-white specks on a bright red mucosal surface. In my experience, the lesions always have appeared white, and a blue component has not been observed. Koplik spots first arise on the buccal mucosa opposite the lower molars but usually spread quickly to involve most of the buccal and lower labial mucosa. The lesions are approximately 1 mm in size at first but occasionally seem to coalesce into larger lesions. Initially, only a few lesions appear, but within 12 hours, the number usually is uncountable. Of equal importance in establishing the diagnosis is the appearance of the background mucosal surface, which is always bright red and granular. Frequently, 1-mm lesions (Fordyce aphthae), which commonly occur normally in adolescents and adults, are confused with Koplik spots.[84] These lesions, however, can be differentiated easily because they appear on a normal pale mucosal surface rather than the bright red background of measles.

During the prodromal period, erythematous maculopapular lesions also are observed occasionally on the palate. At the end of the prodromal period, the posterior pharyngeal wall usually is erythematous and infected, and the patient may complain of a sore throat.

Exanthem Period

In typical measles, the exanthem appears on approximately the 14th day after exposure. The exanthem occurs at approximately the peak of the respiratory symptoms and when the temperature usually is approximately 39.5° C (103° F). At this time, the manifestation of Koplik spots has peaked, and during the next 3 days they disappear. However, after the specific white spots have disappeared, the red, sandpapery mucosal background remains present for a day or so.

The measles exanthem first appears behind the ears and on the forehead at the hair line. Spread of the rash is centrifugal from the head to the feet. By the third day, the rash has involved the face, neck, trunk, upper extremities, buttocks, and lower extremities sequentially. The rash initially is erythematous and maculopapular but progresses to confluence in the same centrifugal manner as it is spread. Confluence always is more prominent on the face; frequently, the lesions on the lower extremities remain discrete. At the height of the rash, the appearance suggests microvesicles on top of a generalized erythematous confluent base (see Fig. 64–2).

The exanthem begins to clear on the third to fourth day, again following the centrifugal course of progression. During the initial stages of the rash, its color is red, and it readily blanches on pressure. As the rash fades, it takes on a coppery appearance, after which a brownish discoloration is seen that does not clear with pressure. With healing, a fine desquamation frequently occurs in confluent areas with brownish discoloration. The duration of the exanthem usually is 6 to 7 days.

During the exanthem period, the fever generally peaks on approximately the second or third day of the rash and then falls by lysis during a 24-hour period. Fever that persists after the third or fourth day of exanthem usually is an indication of a complication. Conjunctivitis and nasal symptoms generally subside at about the time of defervescence. Continued nasal dis-

charge, whether purulent or not, suggests bacterial secondary infection. With the appearance of the rash, the cough loosens up, and in older persons it frequently becomes productive. The cough may persist for 10 days or more.

Pharyngitis is a common development during the exanthem period, as is enlargement of cervical lymph nodes. Generalized lymphadenopathy with suboccipital and postauricular involvement is not an uncommon finding, nor is splenomegaly. Young children occasionally have diarrhea, vomiting, laryngitis, and croup. Abdominal pain also can be troublesome.

Laboratory Findings

Laboratory studies rarely are indicated for acute uncomplicated typical measles because the diagnosis can be established on a clinical and epidemiologic basis, and the results of studies rarely affect management of the patient. During the periods of prodrome and rash, the total leukocyte count is low. Numbers of neutrophils and lymphocytes are reduced, but the most marked reduction, when absolute counts are considered, is in the number of lymphocytes.[36] In difficult cases, such as the first apparent case in a particular locality, a specific diagnosis can be made by serologic study, most easily by measles antibody studies performed by ELISA. If an acute serum sample is obtained during the prodrome and a second serum sample is obtained 7 to 10 days later, a significant rise in IgG antibody titer usually is demonstrated. At the time that the rash is manifest, the most useful test for identification of measles antibody is the IgM fraction.

MODIFIED ILLNESS*

Modified measles is an infection that occurs in a partially immune person. It is characterized by a generally mild illness that usually follows the regular sequence of events in measles. The prodromal period is shorter; cough, coryza, and fever are minimal. Koplik spots are few and transient, and they frequently do not occur. The exanthem follows the progression pattern of regular measles, but confluence of the lesions does not occur. Because serologic studies reveal some children with measles antibody who have never had clinical disease suggestive of measles, some modified infections probably occur without exanthem and perhaps without overt symptoms at all.[42,250]

Modified measles develops under a variety of circumstances, the most important of which historically was the result of intentional alteration of disease by the administration of immune serum globulin to an exposed susceptible child. Naturally occurring modified measles is seen occasionally in infants younger than 9 months old because of the presence of transplacentally acquired maternal measles antibody.

Although the magnitude of the problem is unknown, modified measles also occurs as an occasional manifestation of failure of live vaccine. In these instances, patients have had modified illness but demonstrated a secondary measles serum antibody response (only IgG antibody).[90,91,404] With increasing time from immunization, this response possibly will occur more frequently.[112] However, few data currently support this possibility.

Recurrent measles also rarely results in modified illness. The frequency of recurrent measles is unknown. In general, most authorities have discounted the existence of recurrent measles and suggested that the recorded experiences were the result of confusion with infection by other exanthem-producing agents.[85,247] However, Cherry and colleagues,[90] Schaffner and associates,[391] and Schluederberg[395] have described children with modified illnesses, secondary immunologic responses, and well-documented instances of previous cases of measles.

ATYPICAL MEASLES

Atypical measles is a clearly defined clinical syndrome that occurs in some previously immunized persons after they have been exposed to natural measles. Most cases have occurred in persons who initially received inactivated (killed) measles viral vaccines, but some cases also have been noted in children who received only live measles vaccines.[89,264,323]

Historical Aspects

The initial studies with inactivated measles vaccines in the early 1960s demonstrated that multiple doses were necessary to stimulate an antibody response and that measurable serum antibody levels were short-lived.[155,185,225,249,456] In the initial trials, it soon became apparent that some study participants who had received killed vaccine were still susceptible to measles. On being exposed to natural measles, some children developed typical measles and others a mild, modified illness.[155] As a result, regimens were developed in which children were immunized with two or three doses of killed vaccine at monthly intervals, followed in a month or more by a single dose of Edmonston B live measles vaccine (KKL or KKKL). Studies at the time demonstrated that good antibody levels were achieved after administration of both regimens. After measles vaccine was licensed in 1963, killed-live measles vaccine regimens enjoyed modest popularity in the United States (as well as in other countries) because live Edmonston B strain vaccine frequently was associated with alarming febrile responses and occasional febrile convulsions.

In 1965, Rauh and Schmidt[370] reported an unusual illness after exposure to natural measles in some children who had received killed vaccine 2 years previously. The significance of their findings became more apparent during the following 2 years when Fulginiti and colleagues[157] and Nader and coworkers[316] noted many instances of "atypical measles" in previous recipients of KKK and KKL vaccination regimens. In these two instances, original immunization had taken place 4 to 6 years before the occurrence of atypical illness.

In 1968, killed measles vaccine was taken off the market in the United States after the distribution of approximately 1.8 million doses in the period from 1963 to 1968.[343] During the 12-year period from 1968 to 1980, atypical measles was reported frequently.* I am unaware of reports since 1980 of further cases of atypical measles. However, I observed one 26-year-old physician and a 28-year-old nurse with the syndrome in the 1980s. Sporadic cases in adults may still be occurring but are misdiagnosed because physicians caring for adults are unaware of the syndrome; therefore, the history of killed measles vaccine is not uncovered, and specific antibody studies are not performed.

In 1971, during study of an extensive measles epidemic, Cherry and colleagues[89] observed six children who had relatively mild, atypical measles-like illnesses but had received only live measles vaccine. Linnemann and colleagues[264] similarly noted two children with atypical measles-like illnesses who had received only live measles vaccine. Nichols[323] and St. Geme and associates[410] also have reported bizarre measles illnesses in patients who formerly received live vaccine.

*See references 26, 30, 83, 90, 91, 125, 155, 198, 214, 252, 355, 373, 391.

*See references 18, 82, 89, 154, 164, 187, 199, 205, 259, 286, 296, 323, 325, 335, 448, 449, 464, 465.

Clinical Characteristics*

Atypical measles was a common illness from 1967 to 1978. Because killed measles vaccines have not been available in the United States since 1968, two obvious facts need to be mentioned: with each passing year, potential patients and actual patients with atypical measles will be 1 year older and also 1 year farther from the time of receiving the primary killed measles vaccine immunization series. Both these aspects raise concern about whether the clinical manifestations of the syndrome will remain the same today as when the illness first was described. It was my opinion in 1981 that the syndrome had changed slightly but still was recognizable from original descriptions of the illness.[86]

The incubation period of atypical measles is similar to that of typical measles—between 7 and 14 days in duration. The prodromal period is characterized by the sudden onset of high fever (39.5° C-40.6° C, 103° F-105° F) and usually headache. Abdominal pain and myalgia also are common complaints. Dry, nonproductive cough is noted in most patients and vomiting in approximately one third of those afflicted. Pleuritic chest pain and weakness also are common complaints. Although few reports to the contrary exist, Koplik spots appear to be rare in atypical measles.

Two to 3 days after onset of the illness, the rash appears. It is unique in that it first develops on the distal ends of the extremities and progresses in a cephalad direction. Usually, the rash initially is erythematous and maculopapular. I have been impressed by a slight yellowish hue of the exanthem when compared with that of typical measles. The rash is particularly prominent on the wrists and ankles; it involves the palms and soles. Spread of the rash varies considerably. In some patients, only the wrists and ankles are involved, whereas in others, the entire extremities as well as the lower part of the trunk are affected. In a peculiar fashion, the rash frequently seems to end its cephalad progression in a line at the level of the nipples. Occasionally, a few erythematous, maculopapular but discrete lesions are found on the face. In some cases, the rash becomes vesicular, with the lesions approximately 2 to 3 mm in diameter; they do not proceed to scab formation as in varicella, but occasionally pruritus is a problem, and excoriation occurs from scratching. The exanthem often has a petechial or purpuric component, and urticaria also occurs frequently. Edema of the extremities has been a common finding.

Although coryza has been noted in a few reports, it is not a prominent feature, nor is conjunctivitis. Respiratory distress with dyspnea and rales occurs commonly, and radiographic examination reveals pulmonary involvement in virtually all cases. Most patients have hilar adenopathy and pneumonia. Pleural effusion also occurs frequently. The pneumonia in atypical measles usually is lobular or segmental, and the lesions often appear nodular (Fig. 192–5). Although initial descriptions of the syndrome suggested an illness of approximately 1 week's duration, later observations indicated illnesses of 2 weeks or more. In one case, fatigue and other symptoms persisted for more than 1.5 years.

In the original description by Rauh and Schmidt,[370] one patient had an exanthem that was biphasic; initially, a transitory rash suggested modified measles. Two weeks later, the more characteristic atypical exanthem developed. The same observers also noted a second case with a biphasic exanthem in which the first lesions were vesicular and occurred when the child had little fever. The second exanthem was maculopapular and associated with a febrile response. Zahradnik and colleagues[465] also noted a similar sequence in two young adults. Two patients who had received killed measles vaccine in the past had radiographic evidence of characteristic pulmonary findings, but the typical exanthem did not develop.[335,464]

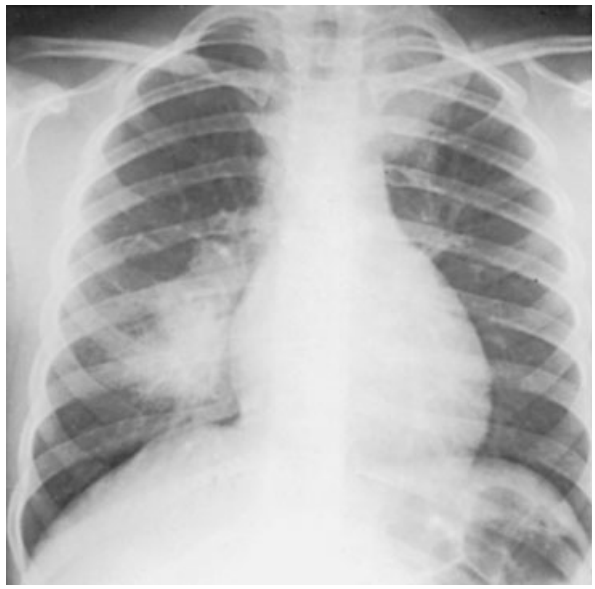

Figure 192–5 Nodular pulmonary infiltrates in a child with atypical measles.

Other findings in atypical measles include marked hepatosplenomegaly,[370] marked hyperesthesia,[316] weakness,[259] and numbness and paresthesia.[286] Personal observations of cases in adolescents and young adults suggest that the exanthem is less prominent than in past cases, and the fever and overall morbidity are of greater duration.[86] Follow-up radiographic studies demonstrated the persistence of nodular pulmonary lesions for longer than 1 year in several patients and up to 6 years in one patient.[259,296,464]

Measles antibody studies in atypical measles are remarkably diagnostic. If an initial serum sample is obtained before or at onset of the exanthem, CF and HI titers usually are less than 1:5. By the 10th day of illness, both titers are elevated markedly, and most are 1:1280 or greater. In contrast, in typical natural measles at the 10th day of illness, the titer rarely is greater than 1:160.

Measles virus was not recovered from a patient with atypical measles, but only a few adequately performed studies were performed. The epidemiologic data currently available suggest that patients with atypical measles are not contagious. Other laboratory studies are not particularly useful in atypical measles. The erythrocyte sedimentation rate is elevated. When serial blood counts have been performed, slight early leukopenia and late eosinophilia have been noted.[157]

The pathogenesis of atypical measles was studied by several investigators, and several possible mechanisms were suggested,[364] including a generalized Arthus reaction, induction of abnormal measles virus–specific, delayed-type hypersensitivity, and an imbalance in antibody responses to H and F proteins caused by denaturation of F protein during formalin inactivation of the vaccine virus. In a study in monkeys, Polack and associates[364] found that atypical measles resulted from previous priming for a nonproductive type 2 $CD4^+$ T-cell response and not from the lack of functional antibody against F protein. In revaccination studies performed by our group in the 1970s, we found that vaccinees with severe local reactions had marked lymphocyte reactivity to inactivated measles virus and absent or minimal HI antibody.[245]

*See references 53, 82, 86, 89, 154, 157, 164, 187, 199, 205, 259, 286, 296, 316, 323, 325, 334, 335, 370, 448, 449, 464, 465.

UNUSUAL MANIFESTATIONS AND COMPLICATIONS OF MEASLES

In addition to typical measles, modified measles, and atypical measles, many other clinical manifestations and complications occur at a broad range of frequency. By definition, unusual manifestations are a direct result of the primary viral infection, whereas complications are a result of damage by a secondary infection with another microorganism. In many instances, determining whether a particular manifestation is just viral or involves a second agent is difficult. Combinations of infections are common occurrences.

In general, complications resulting from secondary infection are not as common today as they were before the antibiotic era; however, no evidence suggests that unusual manifestations of illness occur less frequently today than formerly. To demonstrate the magnitude of the problem today, the findings in a 1970 to 1971 hospital survey in St. Louis are revealing.[88,90] In this period, an extensive epidemic occurred, with 10,000 cases of measles. In 8 area hospitals, 130 children (1.3%) were admitted; 66 cases of pneumonia and 6 fatalities occurred, and 6 children had encephalitis.

The records of measles patients in 3 hospitals were reviewed carefully. Of this group of 71 patients, 53 had pneumonia; 37 of the patients with pneumonia had either previous cardiorespiratory or other chronic systemic disorders. Two children had mediastinal and subcutaneous emphysema. All 6 deaths were caused by fulminant pneumonia. Of the 6 children with encephalitis, severe residual neurologic damage developed in 3. One child had acute measles appendicitis with perforation and peritonitis, and another patient had mesenteric lymphadenitis.

Pneumonia

Pulmonary involvement in measles, as a result of the viral infection, is probably the rule rather than the exception. Kohn and Koiransky[240] performed careful radiographic studies in 130 children with measles and noted that 55 percent had pneumonic infiltration and 74 percent had hilar adenopathy. In most instances, the pneumonia was observed early in the course of the illness, a finding that suggested primary viral involvement rather than secondary bacterial infection. In the 1970 to 1971 St. Louis measles epidemic, approximately 1 in every 150 patients with measles was hospitalized because of pneumonia.

Pneumonia in measles has varied radiographic manifestations.[6,88,174,240,265,271,279,338,418] Clearly, viral pneumonia is characterized by bilateral hyperinflation with diffuse fluffy infiltrates that are more confluent at the hilum. Unilateral, segmental, and lobar pneumonias also are observed. Gremillion and Crawford[174] reviewed 106 cases of pneumonia that occurred in 3220 Air Force recruits with measles between 1976 and 1979. Illnesses were severe, but no deaths occurred. Bacterial superinfection was documented in 30.3 percent of the cases; bronchospasm occurred in 17 percent of the recruits with pneumonia. In one study, seven children with massive and bilateral lung consolidation had clinical findings consistent with adult respiratory distress syndrome.[6]

Clinically, young infants have a picture of bronchiolitis with expiratory distress. In severe cases at all ages, a marked ventilation-perfusion deficit is noted.[265,354] Patients with defects in the cell-mediated immune system are particularly prone to progressive fatal bilateral infection.[128,239,297,299,402,405]

Secondary bacterial pneumonia is the result of common respiratory pathogens, particularly *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Streptococcus pyogenes*, and *Staphylococcus aureus*. Co-infection with other viruses also was noted in an extensive study of measles-associated pneumonia in the Philippines.[367] In this study, parainfluenza virus and adenoviruses were isolated most frequently.

Other Respiratory Manifestations

Otitis media is the most common complication and is age-related. In the immediate pre-vaccine era in the United States, otitis media developed in approximately 5 to 15 percent of patients with measles. It now is less a problem because of the change in the age-related incidence of measles. The bacterial pathogens in otitis media associated with measles are similar to those in otitis media in children without measles. Mastoiditis was a common complication of measles in the era before antibiotics, but fortunately, today it is rare.

Laryngitis and mild laryngotracheitis occur commonly. Occasionally, frank, severe laryngotracheobronchitis occurs and may require tracheotomy.[380] Measles-associated bacterial tracheitis is not an uncommon finding.[97] Secondary bacterial infection of the cervical lymph nodes and secondary bacterial pharyngitis also are rather frequent complications of measles. Field[134] attributed 3.2 percent of cases of childhood bronchiectasis to former infection with measles virus.[134] Measles also has a deleterious effect on the course of tuberculosis.[408]

Cardiac Manifestations

Myocarditis and pericarditis occasionally occur in cases of measles.[111,135] Nonspecific, transient electrocardiographic abnormalities were noted in more than half of 71 children with measles in one study.[381] In another study, 19 percent of patients had transient but clear-cut abnormalities, including T-wave changes, atrioventricular conduction defects, and premature auricular contractions.[166] Although cardiac involvement appears to occur frequently in measles, clinical consequences from such involvement are rare developments.

Neurologic Manifestations

Neurologic involvement is not an uncommon finding in patients with measles.* Gibbs and associates[162] noted that 51 percent of 680 patients with measles who had no clinical evidence of encephalitis had abnormal electroencephalographic results during acute or immediate postacute illness. Although the incidence varies, clinically evident encephalitis occurs in approximately 0.5 to 1 of every 1000 measles cases.[26,70,90,126,226] From 1962 to 1979, the average measles encephalitis-to-case ratio was 0.73:1000.[70] Both mortality and the incidence of sequelae have varied in the reports available. LaBoccetta and Tornay[254] noted a mortality rate of 32 percent in 50 patients in a group seen before 1947 and a rate of 11.5 percent in a group seen between 1947 and 1957. Ziegra[466] reported only 2 deaths in a group of 38 cases. Long-term morbidity data also vary considerably. In general, between 20 and 40 percent of patients who recover from measles encephalitis have manifestations of brain damage. Douglas[120] could find no evidence of later subnormal school performance in a group of children who had uncomplicated measles.

Symptoms of encephalitis usually develop during the period of measles exanthem and within 8 days of the onset of illness.[5,254,379] Occasionally, the onset of central nervous system (CNS) signs and symptoms occurs during the prodromal period. LaBoccetta and Tornay[254] noted the following frequencies of signs and symptoms at the onset of measles encephalitis: convulsions, 56 percent; lethargy, 46 percent; coma, 28 percent; and irritability, 26 percent. Patients with encephalitis frequently have multiple findings:

*See references 5, 26, 37, 126, 129, 162, 169, 254, 268, 285, 349, 365, 430, 466.

headache, abnormalities in respiratory rate and rhythm, twitching and other involuntary movements, and disorientation. Cerebellar ataxia, myelitis, retrobulbar neuritis, transient mental disorders, and hemiplegia are findings noted during the subacute stages of illness. Long-term sequelae include various degrees of retardation and selective brain damage, recurrent seizures, deafness, and hemiplegia and paraplegia. An 8-year-old girl developed pseudotumor cerebri 3 weeks after having a case of measles.[421]

Examination of cerebrospinal fluid (CSF) in measles encephalitis usually reveals mild pleocytosis with a predominance of mononuclear cells, mildly elevated protein values, and a normal glucose level.[254,285,339,430] In one study, 15 percent of the cases did not have CSF pleocytosis.[254]

Considerable controversy relates to the mechanisms in measles encephalitis.* Some investigators failed to isolate measles virus or to demonstrate measles virus RNA or other viral antigens in the brains of affected patients.[161,222,231,304] These findings led to the widespread consensus that the illness is autoimmune (acute disseminated encephalomyelitis) and that viral invasion of the CSF is unnecessary. However, other investigators have recovered measles virus from the CSF and brain of affected patients, a finding indicating that the virus is involved directly in the process.[129,141,285,291] In the hamster model of measles virus encephalitis, virus can be cultured directly from the brain.[352]

Other Manifestations

Measles has been associated with many other manifestations and complications. Of historical interest was the occurrence of a severe, often fatal, form of measles called *black measles* that was characterized by a confluent hemorrhagic skin eruption.[252] Patients with this illness had signs of both encephalitis or encephalopathy and pneumonia. Extensive bleeding from the mouth, nose, and bowel frequently occurred. Severe hemorrhagic measles rarely is seen today, and little is known about its pathogenesis. Disseminated intravascular coagulation would appear to play a role.

Another complication of measles involving bleeding is thrombocytopenic purpura.[6,207] It is a post-infectious illness and different from hemorrhagic measles. Although bleeding is extensive on occasion, the ultimate prognosis usually is good. Stevens-Johnson syndrome has been noted occasionally in measles.[274] Other manifestations include pneumomediastinum, subcutaneous emphysema, hepatitis, appendicitis, ileocolitis, mesenteric lymphadenitis, cervicitis, acute glomerulonephritis, corneal ulceration, and gangrene of the extremities.†

Measles in pregnancy results in significant maternal and fetal morbidity and mortality.[24,124,217,311,409] Jespersen and associates[217] retrospectively reviewed 10 epidemics of measles in Greenland; these investigators obtained adequate data on 327 women infected during pregnancy, and they also were able to examine 252 of the offspring. Thirty-two percent of women infected during the first trimester had spontaneous abortions, and 9 percent of these pregnancies that continued to term resulted in stillbirths. Congenital malformations occurred in eight of 300 live-born infants.[217] Pneumonia is a frequent maternal complication of measles during pregnancy.[24,124]

SUBACUTE SCLEROSING PANENCEPHALITIS

SSPE is an uncommon, slowly progressive disease of the CNS, with an almost invariably fatal outcome. The disease was recognized by a number of investigators early in the 20th century.[50,109,387] Several early accounts, especially those published by Dawson in 1933,[109] suggested a viral origin of this disease; however, not until the mid 1960s was measles virus clearly demonstrated to be the causative agent.[41,81,98,203,356,423]

Epidemiology

SSPE is a rare disease that in the pre-vaccine era had an annual incidence of approximately 1 case per 1,000,000 population in most of the developed world, including the United States. The estimated incidence after wild-type measles virus infection in the pre-vaccine era was approximately 1 case per 100,000 population. The incidence of SSPE in the pre-vaccine era in some developing nations greatly exceeded that of developed nations. In southern India, the incidence of SSPE was as high as 21 per 100,000 population,[388] and in Pakistan, the rate was estimated to be 100 per 100,000.[241] A report from Papua New Guinea suggested a similarly high incidence of SSPE.[267] Several characteristics of natural measles virus infection in developing countries in the pre-vaccine era were associated with the development of SSPE and included the high incidence of measles and the frequent occurrence of measles in children younger than 2 years of age. Both these risk factors for SSPE are modifiable, if not preventable, by vaccination.

The disease has been reported in all countries of the world, and, although no outbreaks have been reported, clustering of cases prompted suggestions that environmental factors or unique strains of viruses may contribute to the development of SSPE after measles. The epidemiologic study of SSPE in the Netherlands described 4 cases of SSPE that occurred in 1 year in one city of 120,000 people, a number that calculated to 4 cases of SSPE per 6000 cases of measles, a rate 10-fold greater than that for the rest of the country.[32]

The incidence of SSPE in the pre-vaccine era was approximately five times higher in the southeastern United States than that in other regions of the country,[190,213,302] also suggesting the possibility of environmental cofactors. In addition, in the pre-vaccine era, a preponderance of cases of SSPE occurred in the United States, as well as in other developed and developing countries, in children from rural areas, independent of the incidence of measles in these areas.[59,60,190,270,301] SSPE appears to have no racial predilection, although whites represent the largest number of cases.[213,302] The incidence of SSPE consistently is two to three times more common in male than female patients.[32,213] No relationship between any human leukocyte antigen genotype and the development of SSPE has been demonstrated, and cases of SSPE in only one member of identical twins have been reported.[94,116,206] The usual age of presentation in the pre-vaccine era was between 6 and 10 years, with ranges of 2 to 35 years reported.[32,152,153,190] The youngest case reported was a 10-month-old child, and the oldest was a 52-year-old man with depressed cellular immunity.[39,419] In most cases, symptoms attributable to SSPE begin some 4 to 8 years after measles virus infection; however, in some series, older patients (>10 years of age) appeared to have experienced a prolonged interval between measles virus infection and the development of SSPE.[32,213,302]

Although several risk factors for the development of SSPE have been demonstrated repeatedly, the most consistent is the acquisition of measles before reaching the second birthday.[32,115,190,302,388] An estimated minimum of 50 percent of those with SSPE have had early measles.[190] In India, an estimated 60 percent of measles infections occurred before the patients reached 2 years of age, a factor possibly contributing to the high incidence of SSPE in this country.[388] Other risk factors have included exposure to animals.[190]

The decreased incidence of SSPE after institution of an effective measles vaccine program was demonstrated in several coun-

*See references 7, 137, 141, 161, 180-182, 220-222, 231, 285, 291, 304, 317, 356, 361.

†See references 147, 197, 226, 232, 252, 263, 287, 305, 324, 401, 426, 460.

tries.[32,49,73,293,340,446] The incidence of SSPE in the Netherlands fell 10-fold after universal vaccination was initiated.[32] The incidence in Japan fell at least 10-fold and possibly more.[340] Development of SSPE after receipt of measles vaccination has been noted to occur. In a case-control study of patients with SSPE in the United States, 17 of 52 (32.7%) patients with SSPE had received measles vaccine before they developed disease.[190] Similarly, SSPE occurring after measles immunization was noted in the Netherlands, but the incidence of vaccine-related SSPE was less than 1 case per 2.5 million immunizations.[32] In Japan, the estimated incidence of measles vaccine-related SSPE was 0.9 cases per million doses of vaccine.[340] An inherent problem common to all of these studies was the inability to identify patients with subclinical, wild-type measles virus infection before they received vaccination. Subclinical measles virus infections are thought to be common in developing countries, as illustrated by studies from India that suggested that as many as 20 to 40 percent of patients with measles may have had a subclinical infection.[388]

In the United States, the success of measles immunization clearly resulted in a profound reduction in the incidence of SSPE.[49,73,446] In the earlier years of the immunization program in the United States, rare cases of SSPE developed in vaccinees. Some of these children and perhaps all of them had unrecognized measles infection before they were vaccinated, and the SSPE was caused by the natural measles virus infection and not the vaccine virus.[446] I am unaware of any cases of SSPE in the United States since the year 2000 that can be attributed to measles vaccine virus.

Bellini and associates[34] studied brain samples from 11 patients with SSPE during the period 1992 to 2003. Nine of the patients in this group had a history of measles immunization. Nonetheless, in all 11 cases, the measles virus genotype identified was that of wild measles and was not consistent with vaccine virus. These researchers attributed the measles infections to the epidemic that occurred in the United States from 1989 through 1991.

In the period 1989 through 1997, 55,622 measles cases were reported. From these data, a rate of 22 cases of SSPE per 100,000 reported cases of measles was calculated. This rate is approximately 10 times higher than previous estimates in the United States and similar to those noted in developing countries in the pre-vaccine era.[241,267,388]

Pathogenesis and Pathology

Autopsy specimens from patients who died of SSPE reveal mild to striking changes in the gross appearance of the cerebral cortex, with ventriculomegaly in some cases. Histopathologic changes include minimal meningeal cellular infiltrate, with a perivascular accumulation of lymphocytes and plasma cells. Within involved areas of the brain, dense infiltrates of T lymphocytes and marked cellular expression of class II major histocompatibility antigens have been described.[52] A prominent microglial and astrocytic hyperplasia often is present. One of the characteristics of SSPE is the presence of intranuclear and cytoplasmic inclusion bodies.

The study of these structures by electron microscopy in 1965 led to the finding of paramyxovirus-like particles in autopsy material.[51,423] Subsequently, Connolly and colleagues[98] described the presence of measles virus antibody in the CSF of patients with SSPE, as well as the presence of measles virus antigens in brain tissue from autopsy specimens of patients who had SSPE. Shortly thereafter, several laboratories reported the recovery of defective measles virus in specimens of brain from patients with SSPE.[52,202,203] Virus was isolated only by co-cultivation and not as cell-free material. Together, these findings suggested that SSPE was caused by a persistent infection of the brain by measles virus.

Several mechanisms, such as an abnormal host immune response to a common infection or a mutant virus, have elicited the most interest. Available data suggest that it may be a combination of these two possibilities. Several observations of the natural history of SSPE are consistent with an abnormal host immune response to a primary measles infection. They include the following: (1) the chronicity of measles virus infection in children with SSPE indicated a failure to eliminate the agent; (2) the importance of early acquisition of measles virus in the development of SSPE suggested that the immature immune system may predispose the host to a persistent infection; and (3) the onset of clinical disease long after a primary infection also suggested a failure of a previously protective immune response. Early on, the consensus was that patients with SSPE had subtle defects in cellular immune responses as measured by decreased cutaneous reactivity to common skin test antigens, decreased lymphocyte proliferative responses to mitogens and measles virus antigens, and reduced production of cytokines. Subsequent studies failed to confirm these conclusions, and most investigators now suggest that patients with SSPE can generate vigorous cellular immune responses after exposure to mitogen and measles-specific antigens.[52] Studies demonstrated the presence of several cytokines, including IL-1b, IL-2, IL-6, TNF, heat-labile toxin, and IFN-γ, as well as other markers of immune activation, in brain lesions from patients with SSPE.[52] Still, some debate continues about the immunocompetence of patients with SSPE. Finally, measles virus is associated with chronic progressive encephalitis in immunocompromised patients (measles inclusion body encephalitis); however, the clinical course and histologic findings of this disease clearly are different from those of SSPE.

In contrast to the questions that surround the cellular response to measles virus in patients with SSPE, antibody responses to measles virus–encoded proteins are well preserved. Antibodies of all isotypes are produced peripherally and within the CNS. Antibodies against all the proteins encoded by measles, including the M (matrix) protein, have been detected in the sera from patients with SSPE.[52] The paradox of elevated levels of circulating anti-measles antibody as well as CSF anti-measles antibody in the presence of persistent infection suggested a possible immune-mediated origin of this disease. Evidence was presented that anti-measles antibody could interfere with spread of the virus and syncytial formation. Fujinami and Oldstone[152] demonstrated that anti-measles antibody reversibly could modulate the intracellular expression of measles virus–encoded proteins, thereby providing a mechanism by which antiviral antibody could convert an acute productive infection into a chronic persistent infection. This decrease in expression of measles antigen also could shelter virus-infected cells from immunologic recognition. This hypothesis also is supported by observations suggesting that early acquisition of measles predisposes the individual to the development of SSPE because the limited quantity of passively acquired maternal anti-measles antibody may be insufficient to prevent development of infection and dissemination of measles virus but adequate to modulate expression of measles virus. This series of events then would predispose the patient to the development of a chronic persistent infection. Animal models consistent with this disease mechanism have been described.[52]

The second general mechanism for the development of SSPE is the generation of viral mutants during acute infection that then can establish a persistent infection. Persistence may result from antigenic variability or a viral mutation leading to decreased production of viral proteins below a level recognizable by the immune system or an extension of cell tropism. That measles viruses from the CNS of patients with SSPE are replication-defective is well documented. Analysis of these isolates does not define a virulence feature of SSPE strains but does suggest several mechanisms that could account for the generation of viral mutants. Much interest has been focused on the decreased expression of the M protein in explants of brain tissue from patients

with SSPE.[52] This viral phenotype also was consistent with the decreased production of anti-M antibody in patients with SSPE, a finding suggesting the possibility of subthreshold production of antigen in these patients. Although the decreased expression of M protein was proposed originally as the mechanism for the persistence of mutant measles virus in patients with SSPE, subsequent studies showed that this is only one of many mutant phenotypes in strains of measles virus associated with SSPE.

Further studies of the replication of measles virus revealed several mechanisms that favor the production of mutant viruses. The RNA polymerase of measles viruses, like that of other RNA viruses, does not have proofreading functions and therefore frequently misincorporates nucleotides.[64] Thus, mutant progeny virus arises regularly and can be selected either by host immune responses or because of its extended host-cell tropism. In addition to this general mechanism of genetic diversity in RNA viruses, measles virus also exhibits what has been described originally as biased hypermutation and more recently as A/I hypermutation.[65,66] This mutational event results in clusters of U (uridine) to C (cytidine) or A (adenosine) to G (guanosine) transitions, possibly as the result of novel host-derived enzymatic activity referred to as *double-stranded RNA unwindase*.[52] This activity results in the replacement of A residues with inosines in duplex RNA molecules formed between genomic RNA and mRNA. Replication of the inosine-modified genomic strand then results in the replacement of U by C. Evidence of this proposed mutational event has been found in the genomes of measles viruses from the brains of patients with SSPE, in which up to 132 of a possible 266 U residues in the M coding sequence were converted to C.[64]

Studies clearly demonstrated that the M gene is the most heavily mutated of measles virus genes, often with early stop codons. Researchers stressed that this mechanism of genetic change is operative during lytic as well as persistent infection, and mutants that persist must do so as the result of growth advantage, such as evasion of the host immune response. The frequent mutations found in the M gene suggest that the measles virus can tolerate extensive genetic change in the M protein yet still replicate within cells of the CNS. The finding of clonal spread of a hypermutated measles virus genome within the brain of a patient with SSPE is consistent with this hypothesis.[28] Other genes of the measles virus also exhibit mutations that alter function, including the gene encoding the hemagglutinin (H) protein. Mutations of this protein were shown to limit cell surface expression but not the function of hemagglutinin in the replicative cycle of measles virus.[40] Cells infected with this phenotype likely are poorly recognized by the immune system because of the limited cell-surface expression of the H protein, yet enough functional activity remains to allow spread of the mutant virus within the CNS by a mechanism of cell-to-cell fusion. Thus, the generation of measles viruses that can induce disease and establish persistent infections results from viral strategies of genetic diversity coupled with selective pressure of the host immune response.

Clinical Manifestations

The initial stage of the disease can be described best as a period of progressive psychointellectual disturbances.[376] These disturbances can include lability of mood, deterioration of school performance, hyperactivity or lethargy, depression, and occasionally altered states of consciousness. Although retrospective analysis often allows precise definition of the onset of SSPE to be established, many of these disturbances are so subtle that they escape parental or physician detection. Physical findings vary and often are nonspecific. A peculiar pigmented retinopathy has been observed in a small number of patients.[62] The duration in stage I varies, depending on the clinical staging system, but in larger series, this stage is relatively short, usually lasting fewer than 6 months.[376] The duration in stage I appeared to be prolonged in older patients, compared with patients younger than 10 years of age, in the series from the Netherlands.[32] Accelerated progression to stage II has been reported.[376]

Stage II is characterized best by a variety of convulsive and motor disorders. The motor disorders are striking, ranging from akinetic drop attacks to violent myoclonic jerks. The motor disturbances have been described consistently as stereotypic and rhythmic. Rigidity or spasticity may be present in the later part of this stage.[52] Extrapyramidal findings, such as choreoathetotic and ballismic movements as well as parkinsonism with abnormalities in the basal ganglia, have been reported. Intellectual functions continue to deteriorate, but patients may retain receptive function during this stage. In one series of patients, as many as 50 percent with stage II disease had abnormal retinal findings, including optic atrophy. The duration of this stage is quite protracted; as many as 50 percent of patients remain in this stage for longer than 6 months and as many as 20 percent remain so for more than 1 year.

Progression to stage III is suggested by increased frequency of myoclonic jerks, development of spasticity or rigidity, and decerebrate and decorticate posturing. Hypothalamic dysfunction becomes prominent, with hyperpyrexia, diaphoresis, and periods of pallor and flushing. Cortical activity rapidly decreases, and patients usually become comatose in this preterminal stage of disease. Duration of this stage is relatively short, lasting fewer than 6 months in most cases. Death usually is associated with complications that accompany the vegetative state, although destruction of essential structures within the brain stem or hypothalamus may result in death.

The previous descriptions account for most patients with SSPE; however, a significant number may exhibit a less predictable course. Some 5 to 10 percent of patients can be expected to have a prolonged survival measured in years. These patients may progress to stage II or III and then remain static without relapse, or they can experience a periodic and sometimes fatal relapse of disease. Conversely, some 10 percent of patients may have a fulminant, rapidly progressive course lasting less than 3 months.[379]

Laboratory Findings

The definitive diagnosis of SSPE relies on a combination of laboratory findings and a compatible clinical course. Before the association between measles and SSPE was made, the electroencephalogram (EEG) was extremely helpful in establishing the diagnosis.[149,275,376] The classic pattern of the EEG in SSPE is described as periodic, synchronous, bilateral discharges with a frequency of 3 to 20 seconds.[95] The discharge contains high-amplitude polyphasic slow-wave complexes, often consisting of two or more delta waves.[275] Frequently, the background of the EEG is suppressed, thus creating the familiar burst suppression pattern. The EEG provides little help in predicting progression of SSPE. Also of note is that the absence of the classic findings as well as other abnormalities on the EEG has been reported in patients with SSPE.[149,209,219] Alternatively, the finding of periodic generalized bursts of fast waves should prompt consideration of SSPE.[278]

More recent advances in imaging technology have aided greatly in the diagnosis of SSPE. Computed tomographic (CT) scans are abnormal in more than 50 percent of patients with SSPE, but the findings often are nonspecific.[52,100,110,121,312] In the later stages of the disease, cortical atrophy often is a prominent finding on CT scan. Magnetic resonance imaging (MRI) of patients with SSPE has revealed the presence of focal lesions consistent with inflammation, often in the white matter, in multiple areas of the brain.[110] In some cases, the location of abnormalities detected by MRI closely reflects the clinical symptoms of the patient, whereas in others, the MRI findings do not cor-

relate with clinical findings.[422] Finally, brain biopsy has been used as an important means of establishing the diagnosis in the past and still is of considerable value in atypical cases.

Perhaps the most useful laboratory examination of the patient with suspected SSPE is the examination of the CSF. Normal to slightly increased levels of protein with an absolute increase in the gamma-globulin fraction are a consistent finding in patients with SSPE.[219] Furthermore, the increase in the gamma-globulin fraction is caused almost exclusively by elevation of immunoglobulin.[403,440] Additional studies showed that 20 to 40 percent of the CSF IgG was oligoclonal and that the oligoclonal fraction contained anti-measles antibodies.[434,435] This finding almost is pathognomonic for SSPE in patients with compatible clinical presentations. All isotypes of antibodies have been found in the CSF, although the finding of immunoglobulin M (IgM) anti-measles antibody remains controversial.[52,128,204,442] Because the levels of anti-measles antibodies within the CSF are elevated as well as oligoclonal, most investigators contend that these antibodies are produced locally within the CNS, apparently in response to ongoing measles virus replication. Other CSF findings include normal levels of glucose and slight pleocytosis consisting primarily of lymphocytes. Patients with SSPE have normal to elevated levels of circulating anti-measles antibody; in some cases, this antibody response also appears clonally restricted.[52]

Treatment

In the past, 5-bromo-2-deoxyuridine, transfer factor, ribavirin, and amantadine proved to be of little value.[52] In the 1970s, an antiviral agent, isoprinosine (Inosiplex), was introduced and was thought to have antiviral activity in vivo and in vitro, although this claim remains controversial. Some studies suggested that isoprinosine treatment may result in stabilization, prolongation of survival, or actual clinical improvement in as many as 50 to 60 percent of patients. In a large study, 98 patients with SSPE were treated continuously, and their actuarial survival was compared with that of 500 historical controls.[223] The results of this study showed that median survival for the treated patients was 3.2 years, compared with 1.2 years for the historical controls. The use of historical controls, however, raised a number of questions about the validity of the results. The results of this trial also suggested a greater benefit of treatment with isoprinosine in patients with more slowly progressive SSPE. Other studies did not demonstrate any efficacy of isoprinosine.[52] Inosiplex and intrathecal INF-α seem to elicit transit improvement in some patients.[122,123,253,269,300] INF-β combined with inosiplex also may offer benefit in patients with SSPE.[17]

MEASLES IN DEVELOPING COUNTRIES

Measles has been and continues to be a staggering problem in developing countries. The mortality rate in much of Africa is approximately 10 percent,[337] and a reasonable assumption is that the rates are similar in some regions of Central and South America and Asia.[306,377,420] In developing countries, measles is a disease of young children. For example, in Kenya in the pre-vaccine era, 25 to 30 percent of children contracted measles before reaching their first birthday and 55 to 60 percent before they were 2 years of age; virtually all children had measles by the time that they were 4 years old.[194] In Kenyan children, mortality peaked in the 17- to 20-month-old age group, and the median age for hospital admission was 14 months.

Although many factors, such as the age at infection, suboptimal medical facilities, and failure to seek medical care, contribute to the excessive morbidity and mortality in children of developing countries, the single overriding factor generally has been thought to be the nutritional status of the infected children.[309,310,337] The data of O'Donovan[337] clearly indicate a direct relationship between malnutrition and hospital admissions for measles and deaths. Studies by Coovadia and colleagues[101] and by Carney and associates[63] indicated both humoral and cell-mediated defects in protein-calorie malnutrition. The clinical picture of measles in children with malnutrition is frequently similar to that in patients with known defects in cell-mediated functions.

Low serum retinol concentrations nearly always are present in children with measles in developing countries.[29,103,208,212,276,371,436] Low retinol levels correlate directly with measles mortality, and treatment with vitamin A reduces this mortality rate.[29,208,276] Studies performed since the 1980s years indicate that the intensive exposure that occurs in children in developing countries is a major factor in measles mortality.[1,3,159]

Clinical Manifestations

Measles in children in developing countries is characterized by two different types of severe disease. One type of illness is a fulminant, toxic illness without apparent localizing complications. The other is a more prolonged illness with obvious complications; the complications may be caused by infection with secondary bacterial or other infectious agents, persistent measles virus infection, or a combination of both.

In a group of 507 hospitalized children, O'Donovan[337] noted that 301 had pneumonia, 96 had gastroenteritis, 36 had croup, 11 had convulsions, 67 had two or more complications, and 140 had nonlocalized systemic toxic effects. The measles rash in malnourished children tends to result in greater confluence and progresses to dark red and then violet.[310] Desquamation is marked and occurs in large scales.[392] After desquamation, patchy depigmentation lasts for some weeks. Other common problems are stomatitis and the resultant sore mouth, which leads to further loss of nutritional intake. Acute corneal ulceration, which occurs after measles in malnourished children, is a common cause of blindness.[389] Multiple skin abscesses and noma (cancrum oris) are rare secondary infectious problems.[226]

MEASLES IN IMMUNOCOMPROMISED HOSTS

Today, because of the extensive use of immunosuppressive therapeutic modalities and greater duration of survival in certain rapidly fatal diseases, a sizable population of children and adults is immunologically compromised. Measles virus infection in a patient with disease-induced or iatrogenically caused immune deficiency usually is severe and protracted and frequently fatal.

The most common severe measles virus infection in an immunocompromised host is giant-cell pneumonia.[128,239,289,297,298,369,402] The mode of manifestation of this illness varies. Some patients initially have severe but otherwise typical measles after a normal incubation period. Clinical findings at the time of the exanthem indicate pulmonary involvement and respiratory distress, and radiographic findings become rapidly worse over a period of approximately a week or less. Other patients initially have rather vague illness, frequently without rash. In these cases, the pulmonary process may progress over the course of a month or longer. Siegel and associates[402] reported a child with leukemia who recovered from typical measles and then died the following year of diffuse interstitial pneumonia in which characteristic measles giant cells were seen.

A unique form of measles encephalitis also is manifested in immunosuppressed patients.[8,148,192,290,312,315,366,429,457] Although the symptoms in different described cases varied, the illness appears to be intermediate between the acute encephalitis occurring in patients without known immune defects and the chronic picture of SSPE. The incubation period has varied between 5 weeks and 6 months.[8] Convulsions frequently are the initial symptom, and

they are a prominent aspect of the illness. The seizures have been focal, unilateral, or permanent localized twitching. Other findings include hemiplegia, stupor, coma, hypertonia, and slurred speech. Most cases have been fatal, and the duration of illness has been from 1 week to 2 months.

DIAGNOSIS

DIFFERENTIAL DIAGNOSIS

The differential diagnosis of typical measles must include all illnesses in which an erythematous maculopapular rash occurs (see also Chapter 64). The following are most important in establishing the diagnosis of measles: a consideration of possible exposure; the duration of the incubation period; the presence of Koplik spots; the presence of the typical febrile prodrome with cough, coryza, and conjunctivitis; and progression of the rash in a caudal direction. The brown discoloration and the intensity of the measles rash are such that the illness usually should not be confused with rubella, erythema infectiosum, roseola infantum, or enteroviral infection. Of greatest differential difficulty are the exanthems of infectious mononucleosis, *Mycoplasma pneumoniae* infection, and drug eruptions.

In the past, atypical measles was extremely difficult to diagnose. Today, this disease, if it occurs at all, would occur only in adults aged 40 years or older. The key to diagnosing this illness is careful elicitation of an accurate vaccination history. Even if it is not known whether the vaccination that the patient received as a child was live or killed vaccine, it usually can be determined by the number of doses given; if a child received more than one dose of vaccine in a short interval, killed vaccine almost certainly was administered. Differential considerations in atypical measles include Rocky Mountain spotted fever, anaphylactoid purpura, *M. pneumoniae* infection, and drug eruptions.

SPECIFIC DIAGNOSIS

Measles virus infection can be diagnosed specifically by the following methods: isolation of virus in an appropriate tissue culture system; demonstration of measles antigen in exfoliated cells and tissues by FA techniques or polymerase chain reaction (PCR)[281]; or the demonstration of a rise in HI, CF, ELISA, FA, or neutralizing antibody titer in two sequential serum samples or specific measles IgM antibody in a single serum sample (see Chapter 264). For practical purposes, most measles cases can be diagnosed by the demonstration of specific IgM antibody in an acute-phase serum specimen. False-positive IgM ELISA results may occur.[216,432] Acute measles also can be diagnosed by PCR using throat-swab specimens.[432]

TREATMENT

UNCOMPLICATED MEASLES

No specific therapy for uncomplicated measles exists. During the febrile period of illness, activity should be discouraged, and fluid status should be maintained by the liberal provision of soft drinks and ice. Fever may be controlled with acetaminophen. Cough frequently is distressing and can be managed by the judicious use of common antitussives. Room humidification also is useful in controlling the cough and generally can be expected to make the patient more comfortable. As the fever disappears, a gradual return to normal activity is indicated. However, measles virus infection is associated with considerable damage to the ciliated epithelium of the respiratory tract; therefore, resumption of normal activities too soon and exposure to other children and their bacterial pathogens can be associated with severe secondary infection.

Children in developing countries frequently have vitamin A deficiency. Measles morbidity correlates with this deficiency, and treatment with vitamin A is beneficial.[29,103,208,212,276,371,436] Studies in the United States indicate that vitamin A levels are low in a substantial number of measles cases and that morbidity is increased in these deficient children.[13,20,57,150] Vitamin A supplementation also has been shown to enhance IgG antibody levels and total lymphocyte numbers.[104] In 1993, the Committee on Infectious Diseases of the American Academy of Pediatrics recommended providing vitamin A supplementation for children with measles in selected circumstances.[13] Vitamin A was recommended for children aged 6 months to 2 years who require hospitalization and all patients 6 months or older with immune deficiencies or possible vitamin A deficiency. The dose of vitamin A is 100,000 IU for children aged 6 months to 1 year and 200,000 IU for children aged 1 year or older. The dose should be repeated 24 hours and 4 weeks after the first dose in children with ophthalmologic evidence of vitamin A deficiency.

ATYPICAL MEASLES

The most important aspect of therapy for atypical measles is proper diagnosis. In patients with atypical measles, Rocky Mountain spotted fever, other septic conditions, lymphoma, or collagen vascular disease frequently is diagnosed erroneously, and their work-up is associated with extensive blood cultures, other diagnostic procedures, and vigorous antibiotic therapy. Careful attention given to a history of previous administration of killed measles vaccine should clarify the diagnosis and preclude the unnecessary trauma associated with extensive diagnostic and therapeutic procedures.

In atypical measles, chest radiographs always should be obtained because the pneumonia that usually develops in these patients frequently is much more extensive than the clinical findings would indicate. Activity should be discouraged in acutely ill patients, and follow-up chest radiographs should be used as a guide to resumption of normal activity. In some patients, pulmonary abnormalities have persisted for a considerable period.

COMPLICATIONS OF MEASLES

Otitis Media

Otitis media is the most frequent complication of measles. The infectious agent of otitis media in measles is no different from that in other children without measles of comparable age, so conventional antibiotic therapy is all that is necessary (see Chapter 19).

Laryngotracheitis

Management of the laryngotracheitis caused by measles virus infection is similar to that in other patients with croup caused by other viral etiologic agents. The mainstays of therapy are the administration of humidified air and a concerted effort to relieve the apprehension of the patient. Administration of corticosteroids is contraindicated in measles, and antibiotics are indicated only in patients with laboratory or clinical evidence of secondary bacterial infection (see Chapter 22).

Pneumonia

Pneumonia is a common complication of measles, and it is the leading cause of death. Pneumonia may be a manifestation of

primary viral infection, or it may result from a superimposed bacterial infection. The differential diagnosis between primary viral and superimposed bacterial disease cannot be made with certainty. Because the diagnosis of viral pneumonia often is uncertain, most patients should be treated with antibiotics (see Chapters 26 and 27). In primary viral pneumonia, treatment with aerosolized ribavirin should be considered.

In one uncontrolled study in adult patients, intravenous ribavirin was found to be well tolerated, and its use was associated with clinical improvement.[143] In a study in pregnant women, Atmar and associates[24] were unable to demonstrate clear clinical benefit with the administration of aerosolized ribavirin.

Encephalitis

The course of measles encephalitis is unpredictable, and treatment is symptomatic and supportive. Trained nursing care is essential. Careful attention given to fluid and electrolyte balance is necessary. In prolonged states of coma, parenteral hyperalimentation is indicated. Status epilepticus should be treated vigorously with the use of a structured protocol for ensuring optimal control (see Chapter 42).

Numerous review articles indicate that measles encephalitis is a post-infectious encephalitis and is predominantly a disease of white matter (acute disseminated encephalomyelitis [ADEM]).[177,220,221] This concept resulted from a study of 19 patients with measles encephalitis that was published in 1984 by Johnson and associates.[222] I am aware of only two patients subsequent to this article who had ADEM associated with measles virus infection. Clinical evidence supports the finding that ADEM not related to measles virus infection is responsive to corticosteroid therapy.[140,284]

Considerable evidence indicates that measles encephalitis usually is not an ADEM and that active direct CNS infection is involved in the process.[129,141,285,291,357] A controlled trial of 32 children with measles encephalitis failed to find benefit in the steroid-treated group.[466] These data suggest to me that corticosteroids should not be used to treat measles encephalitis unless evidence of ADEM is clear and active measles virus infection has subsided. In patients with severe intractable seizures or other evidence of cerebral edema, the use of intravenous mannitol therapy (0.25 to 1 g/kg of a 20% solution administered over the course of a 30- to 60-minute period) is indicated. In occasional circumstances, respiratory arrest is a problem, and artificial ventilators should be used to tide patients over until respiration becomes normal. Mustafa and coworkers[315] noted improvement in an immunocompromised child with subacute measles encephalitis who was treated with intravenously administered ribavirin.

Appendicitis

Acute abdominal pain occurs occasionally in primary measles, and it can be caused by generalized mesenteric adenitis secondary to measles virus appendicitis. In appendicitis, evidence of measles virus involvement of the appendix is present. However, therapy should be similar to that in other cases of appendicitis; removal of the appendix is indicated because measles appendicitis perforates with a frequency equal to that in non–measles virus infection.

PROPHYLACTIC ANTIBIOTICS

In the developing world, secondary bacterial infections in measles are a major cause of mortality. Accordingly, interest has arisen in the use of antibiotics prophylactically.[79,398] A meta-analysis in 1997 of studies involving the use of prophylactic antibiotics noted that the available data were poor and provided only weak evidence for giving antibiotics to all children with measles.[398] The recommendation from this study was that antibiotics should be given only if a child has clinical signs of pneumonia or other evidence of sepsis. Chalmers[79] pointed out the necessity and urgency for performing controlled prophylactic trials.

PREVENTION

ACTIVE IMMUNIZATION: LIVE ATTENUATED MEASLES VIRUS VACCINE

Attenuated measles vaccines are prepared in chicken embryo tissue cultures. Vaccination produces a mild or inapparent noncommunicable infection that induces active immunity in more than 95 percent of recipients. Vaccine-induced antibodies persist for many years, and although reinfection with illness has been noted on occasion in apparently successfully immunized children, waning immunity does not appear to be of significant epidemiologic importance.[15,90,91,112,248] Symptoms associated with measles immunization are minimal and are limited to fever, mild malaise, and occasionally a faint rash occurring approximately 1 week after immunization. For complete information and recommendations related to measles immunization, the reader is referred to the most recent recommendations of the Advisory Committee on Immunization Practices,[23,74,446] the Committee on Infectious Diseases of the American Academy of Pediatrics,[12] and the manufacturer's package insert. Only a summary of recommendations is presented here.

Recommendations for Use

The widespread epidemics of measles in the United States in 1989 to 1991 were the result of a failure to immunize children at the appropriate age and the increased number of susceptible older children and adults because of vaccine failure.[22,74,75,260,277] Therefore, continued eradication of measles in the United States depends on ongoing programs that (1) enroll all children of initial vaccination age and (2) allow re-vaccination of all persons whose primary vaccine failed. Because routine immunity testing is not a viable public health option, re-vaccination of primary vaccine failures can be accomplished only by universal re-immunization.

Currently, live measles vaccine is recommended in a two-dose schedule.[12,74,446] In general, live measles vaccine should be administered at 12 to 15 months of age and the second dose at entry into school.[9,12,23,71,251,399,446,453,462] However, the second dose can be given after any interval longer than 1 month after administration of the first dose.[23] Children who have not received vaccine during infancy may be immunized at any age, and adults who have not had natural measles also should be immunized. When measles is endemic or epidemic in a community, all children aged 6 months or older should be immunized. In children who initially were vaccinated before they were 12 months of age, a second vaccination should be administered at 12 to 15 months of age, and a third dose at entry into school is necessary to complete the schedule.

Precautions

Measles vaccination should be deferred at times of febrile illness or when interference from another viral infection could cause failure of measles vaccine. Measles immunization also should be postponed for 3 to 11 months in persons who have received whole blood, blood plasma, or immunoglobulin because these products may contain sufficient measles antibody to neutralize the vaccine virus. The duration of postponement depends on the

product administered and the dose.[12,23] Children treated with intravenous immunoglobulin for Kawasaki disease should not receive measles vaccine until 11 months after receiving the immunoglobulin, whereas 3 months' time is adequate for children given immunoglobulin for hepatitis A prophylaxis.

Contraindications

Live measles vaccine should not be administered to pregnant women or to some persons with diseases or therapeutic programs associated with impaired cell-mediated immunity. These conditions in general include the following: leukemia, lymphoma, or other generalized malignant diseases; primary and secondary immunologic disorders; and therapy with steroids, radiation, antimetabolites, or alkylating agents. Measles immunization is recommended for children and adults with asymptomatic human immunodeficiency infection and for symptomatic patients who are not severely immunocompromised.[12,14]

Complications

Serious complications associated with administration of measles vaccine are exceedingly rare. Serious neurologic disease (encephalitis, Reye syndrome, cranial nerve palsy, cerebellar ataxia, and Guillain-Barré syndrome) within 30 days of immunization occurs at a rate of approximately one case per million doses of vaccine administered.[68,257] This rate is lower than the rate of occurrence of encephalitis of unknown cause in children for any 30-day period. However, the clustering of cases on days 8 and 9 after immunization noted from claims submitted to the National Vaccine Injury Compensation Program suggests that a causal relationship between measles vaccine and encephalopathy may be a rare complication of immunization.[447] In addition, recovery of measles virus from the CSF of a vaccinated child with encephalitis also suggests that rare vaccine-induced neurologic disease may occur.[141] In a large population-based study in Denmark between 1991 and 1998 it was found that measles-mumps-rubella (MMR) immunization was associated with a transient increased rate of febrile seizures.[437] However, the long-term rate of epilepsy was not increased in children who had febrile seizures after receiving vaccination compared with children who had febrile seizures related to other causative events. Thrombocytopenic purpura, anaphylaxis, hearing loss, and toxic epidermal necrolysis also have been associated with measles immunization.[11,27,31,215,390,400,414,452]

In developing countries, high-titer measles vaccines were used to induce seroconversion at a young age.[458] However, follow-up studies in some countries noted that the mortality rate was increased in female recipients of these high-titer vaccines over the course of a 3-year period when compared with children who received conventional doses of vaccine.[200,237] Nonetheless, follow-up studies in other countries showed no increased mortality rates in recipients of high-titer measles vaccine.[3,261]

Studies by Wakefield and colleagues[424,438] suggested that MMR vaccine may have a causal role in inflammatory bowel disease and autism. However, subsequent epidemiologic studies did not support an association between receipt of MMR vaccine and development of either inflammatory bowel disease or autism.[107,108,130,131]

QUARANTINE AND DISEASE CONTAINMENT

Before the use of measles vaccines became widespread, quarantine measures were practiced widely but were largely ineffective in preventing the spread of measles. However, containment of disease is practical today because the widespread use of measles vaccine has reduced the general number of susceptible young children and thus has decreased the rapidity of epidemic development. Epidemic measles now generally involves a greater age range of the population (cases in adolescents and young adults occur frequently), and progression of disease from one age group to another is slower than in epidemics that involve one uniform, largely susceptible population.

Containment is a vital part of the measles prevention policy in the United States. Measles is a reportable disease throughout the United States, and compliance is the obligation of all physicians. After receiving early reports of sporadic measles, health department workers can organize local immunization clinics so that the disease often can be contained in a small geographic area rather than developing into a widespread epidemic.

PASSIVE IMMUNIZATION: IMMUNOGLOBULIN

In the present vaccine era, little need for passive immunization exists. However, when a known susceptible child has had definite exposure to measles, immunoglobulin should be administered in a dose of 0.25 mg/kg (maximal dose, 15 mL). If this treatment is administered within 5 days of exposure, prevention of infection and disease can be expected. Administration of immunoglobulin later in the incubation period may modify the illness but does not prevent it. The use of immunoglobulin is particularly important in children who have not been immunized because of the contraindications to vaccination mentioned previously. In these children, immunoglobulin (0.5 mL/kg; maximal dose, 15 mL) should be administered when measles is epidemic in the community in which they reside. The dosage should be repeated every 4 weeks until the epidemic subsides. Intravenous immunoglobulin may be used (400 mg/kg).

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CHAPTER 193

Mumps Virus

James D. Cherry

Mumps (epidemic parotitis) is an acute communicable disease caused by the mumps virus, a member of the genus *Rubulavirus*. As a result of universal immunization, mumps is an uncommon disease in children in the United States today.

HISTORY[79,145]

In the fifth century BCE, Hippocrates described an outbreak on the island of Thasus.[79] He noted that most patients had bilateral swelling near the ears and that the others had unilateral swelling. He also noted that some patients had bilateral or unilateral pain and swelling of the testicles.

The origin of the name *mumps* is not known. It may be from the English noun *mump*, which means "a lump," or the English verb *mump*, one of whose definitions is "mumble." This latter possible origin is based on the mumbling speech that patients with significant parotitis may have.

In 1790, Robert Hamilton[59,60] presented an extensive study of mumps in which he noted orchitis, associated the illness with

neurologic involvement, and described the neuropathology of a fatal case. In 1886, Hirsh[68] noted that mumps occurred throughout the world and that it was a major cause of morbidity in Confederate troops during the American Civil War. In the first half of the 20th century, investigators recognized that mumps virus infection involved multiple organs, and the causative agent was shown by Johnson and Goodpasture in 1934[40,73] to be a filterable virus.

The growth of mumps virus in embryonated eggs was reported in 1945, and 10 years later its propagation in tissue culture was noted.[57,63] This latter development led to the development and licensure of a live attenuated mumps vaccine in 1967.

PROPERTIES

CLASSIFICATION

Mumps virus is a member of the genus *Rubulavirus*, subfamily *Paramyxovirinae*, and family *Paramyxoviridae*.[91] It contains a single-stranded, nonsegmented, negative-sense RNA genome that is surrounded by a helical nucleocapsid and a surface envelope.

PHYSICAL PROPERTIES[15,82,91]

The virus generally is spherical, but marked pleomorphism occurs. Its size varies from 100 to 600 nm. Seven major proteins exist: a nucleocapsid-associated protein (NP), a phosphoprotein (P), a membrane or matrix protein (M), a fusion protein (F), a hydrophobic membrane-associated protein (SH), a hemagglutinin-neuraminidase (HN), and a polymerase protein (L). The genes for these proteins have been sequenced, and the gene order is 3-NP-P-M-F-SH-HN-L-5. The viral envelope is studded with 12- to 15-nm projections that contain either of the two structural glycoproteins (HN or F).

The P structural protein is associated with the nucleocapsid, and an RNA-dependent RNA polymerase is located within the nucleocapsid structure. The envelope has a high lipid content, and it contains the M protein.

The SH protein gene is the most variable region of the genome and, therefore, can be used to differentiate viral strains.[1,15,86,103,125] Analysis of the variations in SH gene nucleotide sequences has been used to study outbreaks, identify vaccine viruses, study vaccine adverse events, and identify new viral strains.

Mumps virus infectivity is destroyed by heat (56° C for 20 minutes), and its infectivity is reduced by ultraviolet light, Tween 80, ether, and formalin. The virus is stable at 4° C for several days, and when placed in a buffered salt solution (such as Hanks) with 1 to 2 percent inactivated fetal calf serum, it can be stored indefinitely at –70° C.

ANTIGENIC COMPOSITION

The three major antigenic components of mumps virus are the two glycoproteins (HN or V antigen and F protein) and the nucleocapsid protein (NP or S antigen).[82] The glycoproteins that project from the viral surface are the antigenic target for specific antibodies. Host antibodies to HN and F proteins confer protective immunity against the virus. Mumps viral particles agglutinate erythrocytes of several mammalian and avian species (human, avian, rodent, and simian); at 37° C, the virus causes partial hemolysis of susceptible erythrocytes when it is attached to cellular surface receptors. Specific antibody blocks hemagglutination, hemadsorption, and hemolysis.

Mumps virus is considered to have a single immunotype. However, polyclonal antibodies to parainfluenza and Newcastle disease viral antigens cross-react with antibodies to mumps virus in complement-fixation and hemagglutination-inhibition assays. With monoclonal antibodies, an antigenic relationship between the NH and NP proteins of Sendai virus (a murine parainfluenza type 1 virus) and mumps virus has been demonstrated.[104]

Although mumps is a monotypic virus, genetic variation between strains exists. A standardized nomenclature and an analysis protocol have been proposed for the genetic characterization of mumps strains to facilitate the expansion of molecular epidemiologic studies.[71] This nomenclature includes 12 genotypes (A to L) based on the nucleotide sequence of the hydrophobic (SH) gene.

TISSUE CULTURE GROWTH AND ANIMAL SUSCEPTIBILITY

Mumps virus can be propagated in many different primary and cell-line tissue cultures.[82] To isolate the virus, primary monkey kidney cells generally are used. Its cytopathic effect is similar to that of other paramyxoviruses. When stained, multinucleated giant cells and cytoplasmic eosinophilic inclusions may be observed. In culture, the addition of erythrocytes results in hemadsorption to surface virus.

Mumps virus infects monkeys, rabbits, dogs, cats, and rodents. The virus is isolated readily after inoculation of the amniotic sac of 7- to 8-day-old chicken embryos.

EPIDEMIOLOGY

INCIDENCE

In the United States, mumps was a reportable disease from 1922 to 1950 and has been again since 1967.[22] Between 1950 and 1967, incidence data were gathered from voluntary reporting by cooperating states. Incidence data from 1922 to 1982 are presented in Figure 193–1, and vaccine-era data are presented in Figure 193–2.[16,27] In the pre-vaccine era, mumps was a yearly disease, with epidemic peaks occurring approximately every 4 years. The peak epidemic year was 1944, when the rate was 250 per 100,000 population.

In the pre-vaccine era, mumps was a disease predominantly of young children.[22] However, outbreaks of mumps were a significant problem in young adults in the military.[52,95] The age distribution of mumps in the United States during selected years is presented in Table 193–1. After the mumps vaccine was licensed in 1967, mumps remained a disease of predominantly young children from 1967 to 1971. However, by 1981, most reported patients were 10 years or older. In 1987, 76 percent of the cases occurred in persons 10 years or older (see Table 193–1). In 1994, 1537 cases were reported, and of those with age noted, 21.8 percent were in patients 20 years or older. The increase in reported cases that occurred in 1987 (see Fig. 193–2) was the result of a marked increase in cases in nonimmunized persons aged 10 to 19 years. This group had been protected for a time by herd immunity because of the rapidly declining incidence of mumps as a result of routine vaccine use beginning in 1977.[33] In 1998, only 666 cases of mumps were reported in the United States.[23] Nineteen percent of the patients were younger than 5 years old, and 36 percent were 15 years or older. In 2005, 59.6 percent of the reported cases were in persons 15 years of age or older (see Table 193–1).[24]

In the pre-vaccine era, the peak incidence of mumps occurred in the winter and spring months, and this peak continued well into the vaccine era.[22] Since 1989, little seasonal variation in cases has been noted.[21,135] In the period from 2001 through 2004, an

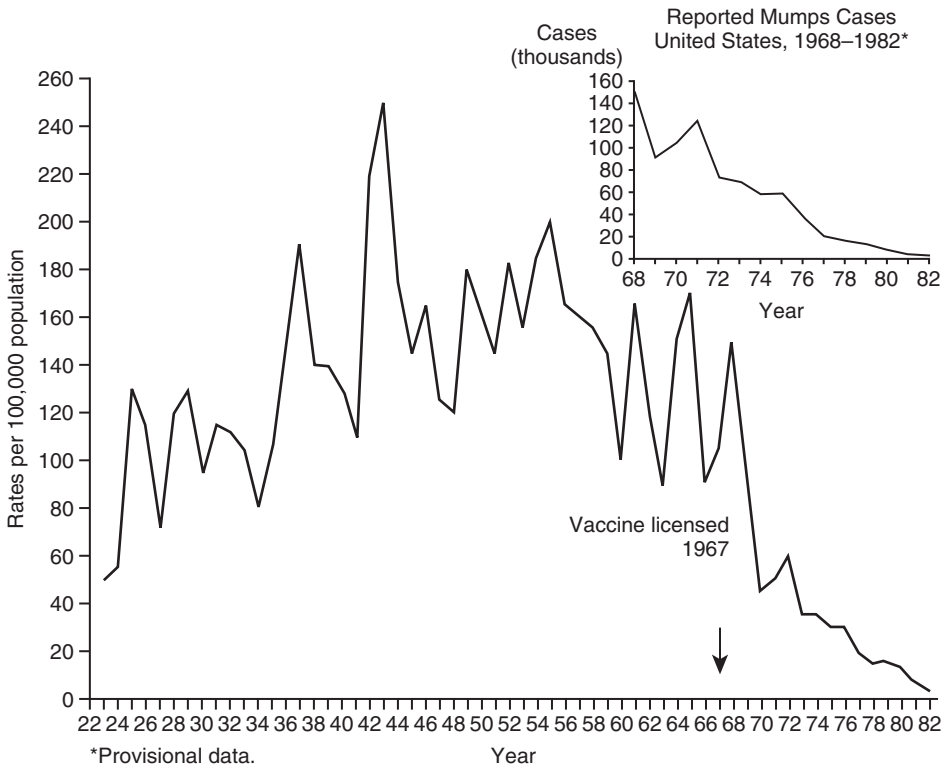
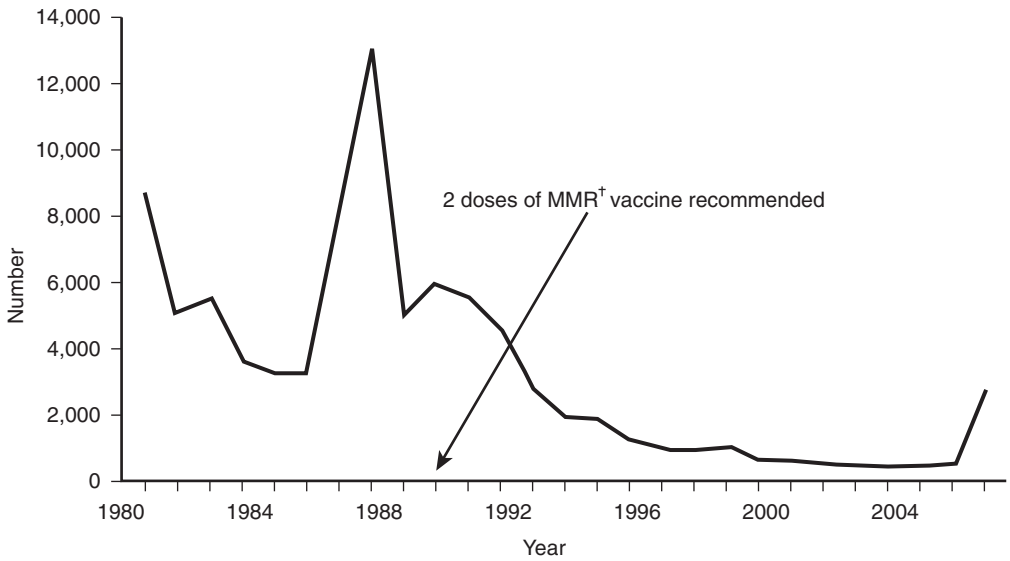


Figure 193–1 Incidence of reported mumps in the United States from 1922 to 1982 and the number of reported cases from 1968 to 1982. *(From Centers for Disease Control: Mumps Surveillance, January 1977-December 1982. Atlanta, Centers for Disease Control, 1984.)*



*Data for 2005 and 2006 are provisional.
†Measles, mumps, and rubella.

Figure 193–2 Number of reported mumps cases, by year in the United States, 1980 to 2006. *(From Centers for Disease Control and Prevention: Update: Multistate Outbreak of Mumps—United States, January 1-May 2, 2006. M. M. W. R. Morb. Mortal. Wkly. Rep. 55:559-563, 2006.)*

average of 265 mumps cases were reported each year.[25] However, in 2006, by March 28 a total of 219 mumps cases were reported in Iowa alone; this was a 44-fold increase in cases from the preceding decade. Of the 219 cases, the median patient age was 21 years (range, 3 to 85 years), with 48 percent of the patients aged 17 to 25 years old; 30 percent of the patients were known to be college students. This outbreak in Iowa in December 2005 was found to involve 10 additional states by May 2, 2006.[27] A total of 2597 cases was reported. For the period January 1 to October 7, 2006, 5783 cases of confirmed or probable mumps were reported from 45 states and the District of Columbia.[28] Eighty-four percent of the cases were reported in six states: Iowa (1968), Kansas (904), Wisconsin (750), Illinois (591), Nebraska (357), and South Dakota (288). The median age was 22 years, and 63 percent were female. Vaccination status data from Iowa found that 7 percent were unvaccinated; 14 percent had received one dose of measles-mumps-rubella (MMR) vaccine; and 49 percent had received two or more doses of MMR vaccine. The vaccination status of 30 percent of patients, most of whom were adults, was unknown.

In 2004 to 2005, a mumps epidemic occurred in the United Kingdom.[26] There were 56,390 reported cases. In 2004, 79.1 percent of the confirmed cases were in persons aged 15 to 24 years, and two thirds of all cases occurred in persons who had not been vaccinated. In many respects, this epidemic is similar to

TABLE 193–1 Age Distribution of Mumps Cases in the United States during Selected Years

	1967-1971*		1987		1994		2005		
Age Group (yr)	Cases	%	Cases	%	Cases	%	Age Group (yr)	Cases	%
<5	2,932	17.1	804	6.5	250	17.4	<5	51	16.4
5-9	10,413	60.8	2,196	17.9	473	33.0	5-14	75	24.0
10-14	2,372	13.8	4,567	37.3	271	18.9	15-24	67	21.5
15-19	1,418†	8.3	3,455	28.2	128	8.9	≥25	119	38.1
≥20			1,235	10.1	312	20.8			

**Average annual reported cases for California, Massachusetts, and New York City.*
†Includes all reported cases in patients 15 years or older.
Data from references 20-22, 24, and 135.

the one in the United States in 1987; herd immunity had protected this adolescent and young adult cohort for a number of years.

MORBIDITY AND MORTALITY

The most common clinical manifestations of mumps virus infection are fever and parotitis. However, approximately 30 percent of infected persons do not have parotitis and therefore are not recognized.[85] Epididymo-orchitis occurs in 20 to 30 percent of clinical cases in postpubertal male patients. Approximately 60 percent of clinical mumps cases involve cerebrospinal fluid (CSF) pleocytosis, but only one sixth of these patients have meningeal symptoms.[6] In 1966, 628 cases of encephalitis (0.5%) were reported, and of these, 10 (1.6%) had a fatal outcome.[16] Encephalitis occurs more frequently in male patients (61%), and the rate of occurrence is greatest in adults. Deafness after a case of mumps has been estimated to occur in 0.5 to 5.0 per 100,000 cases.[42,137]

From 1966 to 1975, 200 mumps-associated deaths were reported in the United States.[16] Of these, 44 (22%) were in patients with encephalitis; in the others, the causes of death were not identified. Forty-one percent of the deaths occurred in adults 40 years or older. During the 10-year period from 1988 through 1997, only 8 deaths caused by mumps were reported.[23]

SPREAD OF INFECTION

Mumps virus is contagious in nonimmune persons. It is spread from an infected person to a new host by the respiratory route. The virus can be isolated from the saliva of infected patients from 7 days before the onset of parotitis to 9 days after onset.[82] Transmission is greatest during a 7-day period beginning 2 days before the onset of parotitis. Asymptomatically infected persons also can transmit the virus. The finding that outbreaks of mumps occurred in young adult populations in the pre-vaccine era suggests that mumps virus is less contagious than is measles; a significant number of persons passed through childhood without being infected with mumps virus. A serologic study reported by Black[9] in 1964 noted that 24 percent of army recruits lacked hemagglutination-inhibition antibody to mumps, whereas only 1 percent lacked measles antibody. The incubation period usually is 16 to 18 days, although it can vary from 12 to 25 days.[82]

PATHOGENESIS AND PATHOLOGY

VIRAL INFECTION[43,82,145]

After respiratory or perhaps oral acquisition of the virus occurs, primary viral replication occurs in the upper respiratory mucosal epithelium. Virus multiplies and is spread by drainage to local lymph nodes.[43] Subsequently, viremia develops.[76,105,133] As a result of viremia, infection occurs in multiple secondary infection sites. Most prominent is infection of the salivary glands that results in inflammation and swelling. This infection causes virus shedding for 1 to 2 weeks. Other secondary sites of infection include the inner ear (cochlea), pancreas, heart, nervous system (meninges and brain), joints, kidneys, liver, gonads, and thyroid.

PATHOLOGY[145]

In the salivary glands, virus infects the ductal epithelium and causes periductal interstitial edema and a local inflammatory reaction involving lymphocytes and macrophages.[143] Tissue damage ensues, and the involved cells desquamate. Virus enters the central nervous system (CNS) by the choroid plexus through infected mononuclear cells. Virus multiplies in choroid and ependymal cells on the ventricular surfaces, and these cells desquamate into the CSF and result in meningitis. In encephalitis, perivascular infiltration with mononuclear cells, scattered foci of neuronophagia, and microglial rod-cell proliferation occur.[12] Periventricular demyelination also takes place. In the male gonad, the primary site of viral replication is the seminiferous tubules, where infection results in lymphocytic infiltration and edema of interstitial tissue.

IMMUNOLOGIC EVENTS

Infection gives rise to serum antibodies to the HN glycoprotein (V antigen), the F antigen, and the NP protein (S antigen).[82] Antibody to NP protein develops first, 3 to 7 days after the onset of symptoms. Antibodies to NP protein are short-lived and usually are absent after 6 months; they cross-react with parainfluenza viruses. Antibodies to HN glycoprotein develop 2 to 4 weeks after the onset of illness and persist for long periods after infection.

Immunoglobulin G (IgG) and IgM antibody responses (determined by enzyme-linked immunosorbent assay [ELISA]) regularly occur after infection.[132] IgG antibody levels measured by ELISA correlate best with those derived by complement-fixation and hemolysis-in-gel assays. ELISA results are less specific than are those achieved with the plaque-reduction neutralization assay because of the high rate of cross-reacting antibodies against parainfluenza viruses detected by ELISA.[15,93,110] IgM antibodies develop early in the course of infection (second day of illness), peak within the first week of illness, and usually are undetectable 3 months after the onset of illness; occasionally, mumps-specific IgM antibody persists for 5 to 6 months.[82,132] Mumps-specific IgG antibody appears at the end of the first week of illness, peaks 3

weeks later, and persists throughout life. The IgG response is mainly the IgG1 subclass; a minor IgG3 subclass response also occurs.[15,115] Salivary IgA antibodies to mumps virus regularly appear after infection.[47]

During infection with vaccine virus, the cell-mediated response to tuberculin is diminished for up to 4 weeks.[87] During the same period, a mumps-specific, cell-mediated immune response develops.[82,145] This response has been demonstrated by skin test hypersensitivity, in vitro lymphocyte proliferative responses, and cytotoxic T-lymphocyte studies.[11,13,84]

In general, immunity against recurrent disease has been thought to be lifelong. However, Gut and associates[56] noted 26 patients with mumps who had a previous history of mumps and antibody response patterns suggestive of reinfection and not primary infection. Specifically, these patients had IgG but not IgM titer rises.

CLINICAL MANIFESTATIONS

In epidemics of mumps, cases can be separated into five groups: (1) those with a short course whose signs and symptoms are nonspecific, (2) those in whom the disease is full-blown with salivary swelling but no complications, (3) those with severe mumps and complications (epididymo-orchitis or meningoencephalitis, or both, or other complications), (4) those with no apparent symptoms but with typical antibody responses, and (5) those with meningoencephalitis or orchitis but without involvement of the salivary glands.[124] Approximately 75 percent of all cases of apparent mumps in children belong to the full-blown type without complications. Involvement of the gonads rarely occurs before puberty.

TYPICAL MUMPS WITHOUT COMPLICATIONS[124]

The average case in children has a prodromal period of 1 to 2 days that consists of fever, anorexia, headache, vomiting, and generalized aches and pains. The headache often is particularly marked and probably is caused by mild meningoencephalitis.[45] The temperature usually rises slowly to 102° F or 103° F (38.9° C or 39.4° C) as the disease becomes full blown, but at times fever is only slight or absent.

After the prodromal period, one or both parotid glands begin to enlarge (Fig. 193–3). Mumps is bilateral in approximately 70 to 80 percent of cases. A few days to a week or more may intervene between the swelling of the two sides. A distinctive "puckering" sensation is experienced at the angle of the jaw in the early stage, and it may be increased by the application of sour liquids, such as lemon juice or vinegar, to the tongue. This sign, when present, may be useful for early establishment of the diagnosis. The swelling of the gland also is distinctive in that a brawny type of edema occurs about the parotid gland, the borders of which are not discrete, in contrast to the discrete swelling typical of lymphadenitis, in which the node generally is outlined easily. The lobe of the ear is in the center of the swelling, which usually cannot be separated by palpation from the angle of the mandible. Pressure is painful, and opening the jaw often is difficult.

The swelling of an individual gland reaches its maximum in approximately 3 days, remains at its peak for approximately 2 days, and then slowly recedes. The extent of the swelling varies considerably but at times is sufficient to distort the outline of the face and head completely. The submaxillary and sublingual glands may be involved separately or with the parotids in any combination.

During the prodromal phase, slight redness of the orifices of the Stensen or Wharton ducts, when present, has diagnostic significance. The amount of saliva usually is unchanged, although the mouth may be dry or salivation may be extreme. Gellis and Peters[51] described a few cases with edema over the upper part of the sternum, apparently caused by pressure on the lymphatics in the neck.

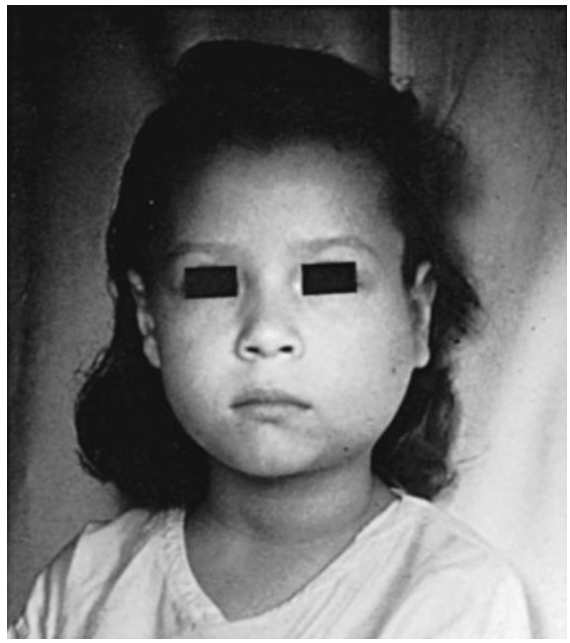


Figure 193–3 Note the swelling on the left side of the face related to parotitis secondary to mumps virus infection. The left ear protrudes from the side of the head.

In uncomplicated mumps, the white blood cell count usually is low, with a slight relative lymphocytosis. Serum amylase typically is elevated.

MENINGITIS, MENINGOENCEPHALITIS, AND ENCEPHALITIS

Meningitis and mild meningoencephalitis are the most frequent complications of mumps in children. Azimi and Cramblett[5] reviewed 51 children with mumps meningoencephalitis who were admitted to Columbus Children's Hospital between July 1964 and December 1967. Of this group of patients, fever occurred in 94 percent, vomiting in 84 percent, nuchal rigidity in 71 percent, lethargy in 69 percent, parotid swelling in 47 percent, headache in 47 percent, convulsions in 18 percent, abdominal pain in 14 percent, sore throat in 8 percent, diarrhea in 8 percent, and delirium in 6 percent.

Clinical findings in neurologic illness caused by mumps virus infection differ according to the age of the patient. Meningeal signs are recognized more readily in older children, adolescents, and adults, whereas nonspecific findings such as drowsiness and lethargy occur more frequently in young children.[145] Although seizures develop in 20 to 30 percent of hospitalized patients, electroencephalographic results usually are normal. Even in patients with severe obtundation, the electroencephalogram reveals only diffuse slowing with increased voltage. Focal findings are rare. The outlook in mumps meningoencephalitis generally is good and usually is better than in encephalitis of other viral causes (see Chapter 42). Even patients with profound obtundation generally recover without having residual damage. Rarely, deaths do occur, however.[23]

In the typical case, CSF has normal glucose and elevated protein levels and pleocytosis. Glucose levels are slightly low in approximately 20 percent of cases. At the onset of symptoms,

modest mononuclear pleocytosis is present. The CSF cell count peaks on the third day of illness; counts average 250/mm^3, but counts greater than 1000/mm^3 are not uncommon. Mumps meningitis usually develops in patients with parotitis approximately 5 days after the onset of illness, but CNS findings can precede the parotid findings and can develop without any salivary gland involvement.

Herndon and colleagues[65] noted that ependymitis regularly occurs in cases of mumps meningitis. A rare complication of mumps appears to be acquired aqueductal stenosis, and researchers have suggested that it may be caused by the preceding ependymitis.[65,101,123,130] In one reported fatal case, hydrocephalus developed within 5 days of onset of disease.[102] More recently, acute hydrocephalus associated with mumps meningoencephalitis was noted in an 8.5-year-old boy.[2] This child received a ventriculoperitoneal shunt and at follow-up had no neurologic deficit.

A 3-year-old boy with mumps cerebellitis, nystagmus, and focal localization of brain lesions noted by electroencephalography was described.[92] Also reported were facial palsy in a 3-year-old Japanese boy and transverse myelitis in an adult.[41,136] A girl aged 4 years and 3 months who had brain stem encephalitis and acute disseminated encephalomyelitis following mumps was described.[122] This child responded rather dramatically to treatment with glucocorticoids and intravenous immunoglobulin. Haginoya and associates[58] reported the case of a 14-year-old Japanese girl who had chronic progressive mumps virus encephalitis. This child had an illness similar to subacute sclerosing panencephalitis, and her antibody titers suggested that the infecting virus may have had a defect in the HN protein.

GONADAL INFECTION (EPIDIDYMO-ORCHITIS AND OOPHORITIS)

Epididymo-orchitis and oophoritis almost never occur before puberty.[124] However, in adolescent boys and adult men, epididymo-orchitis is second only to parotitis as a manifestation of mumps virus infection.[85] Cases of orchitis have been reported in children as young as 3 years old.[113] In postpubertal male patients, orchitis develops in 30 to 38 percent with mumps.[7,109] The rate of orchitis is highest in those 15 to 29 years of age. The greatest number of cases occur during the second, third, and fourth decades of life. Approximately 80 percent of cases of epididymo-orchitis appear during the first 8 days of involvement of the salivary gland, but a few cases occur a considerable time after the parotitis has subsided.[124]

The onset of testicular involvement usually is manifested as chills, recurrence of fever, and swelling of the testes. Pain over the renal area or in the lower part of the abdomen, bilateral or unilateral, may precede or accompany the orchitis. Occasionally, this pain, if on the right side, may suggest appendicitis.

The orchitis usually is unilateral, but bilateral involvement has been reported in 17 to 38 percent of cases.[7,109] Although atrophy may develop after orchitis, in those with unilateral involvement, sterility is not a concern. Sterility has resulted after some cases of bilateral orchitis, however. A 16-year-old adolescent with mumps epididymo-orchitis was found to have persistent mumps virus RNA in his semen for 40 days.[72] In follow-up studies, he was found to have antisperm antibodies. The development of malignant disease in affected testes also has been reported.[7,75]

Oophoritis occurs in approximately 7 percent of postpubertal female patients. Pelvic pain and tenderness are noted.[116]

PANCREATITIS

In a retrospective survey of 2482 hospitalized patients with mumps, pancreatitis was noted in 75 (3%).[4] Cases occurred in children and adults, and pancreatitis was 1.6 times more common in male than in female patients. Severe involvement of the pancreas is a rare occurrence, but mild or subclinical infection may occur more frequently than recognized.[116] It may be unassociated with salivary gland manifestations and be misdiagnosed as gastroenteritis. Epigastric pain and tenderness are suggestive; they may be accompanied by fever, chills, vomiting, and prostration. A child with acute hemorrhagic pancreatitis and a pseudocyst caused by mumps virus infection has been reported.[44]

DIABETES MELLITUS

Diabetes mellitus long has been suspected to be associated with antecedent mumps.[16,61] In experimental animals, mumps virus infection has been linked to hyperglycemia and histologic lesions of the pancreatic islets. Mumps virus can invade the human pancreas and can infect and destroy human and rhesus beta cells in vitro,[112] but pancreatic damage has not been documented in reported cases of diabetes that developed after mumps or mumps vaccination.[121]

In humans, many cases of temporal association have been described both in individuals and in siblings,[36,37,81,97,100] and outbreaks of diabetes mellitus a few months or years after outbreaks of mumps have been reported.[55,96,128] Although evidence has not established a causal association in these cases, a study in Surrey, England, suggested that a small proportion, if any, of diabetes cases that start in childhood (only 15 of the 1663 patients in the study, or <1%) may have resulted from a recent mumps virus infection.[49] Antibody studies have shown fewer positive titers for mumps in diabetic patients than in normal subjects, even in children.[114] Infection may contribute to the development of diabetes either by specifically damaging islet cells or by precipitating diabetes in patients whose disease is latent. Teng and colleagues[129] reported the case of an 11-year-old girl with mumps infection complicated by transient hyperinsulinemic hypoglycemia.

NEPHRITIS

Viruria is a common occurrence in uncomplicated mumps, and mild abnormalities in renal function occur.[133] Severe and fatal nephritis was reported as a rare complication of mumps occurring 10 to 14 days after parotitis.[116] Fujieda and associates[48] reported the demonstration of mumps virus genomic RNA by polymerase chain reaction (PCR) in a renal biopsy specimen from a 5-year-old girl with IgA nephropathy.

DEAFNESS

Deafness is an important but rare complication of mumps virus infection.[22] Its incidence has been estimated at 0.5 to 5.0 per 100,000 cases of mumps.[33,115] However, the incidence rate of minor degrees of hearing impairment, such as high-tone hearing loss, is probably much higher.[31]

Mumps-associated deafness occurs with or without meningoencephalitis and may develop after asymptomatic infection.[16,42,99] The deafness usually is unilateral and often permanent. Twenty-two of 103 cases (21%) reviewed by Everberg[42] were bilateral. Mumps virus has been isolated from perilymph fluid in a case of sudden-onset, unilateral, complete deafness that began 2 days after the onset of mumps.[14,144] Vertigo also is noted occasionally in patients with mumps; it occurs most commonly in those in whom deafness develops.[70]

MUMPS AND PREGNANCY[23]

The incidence of mumps during pregnancy was estimated at 0.8 to 10 cases per 10,000 pregnancies[117] before vaccine was licensed in the United States. No vaccine-era data are available for comparison. Maternal complications such as mastitis,[109] aseptic meningitis,[10] and fatal glomerulonephritis[39] have been reported. Mumps virus has been isolated from human breast milk.[77]

Increased rates of fetal mortality were reported in women who contracted mumps during the first trimester. In a large prospective case-control study, a 27.3 percent rate of fetal wastage was noted in women with mumps during the first trimester versus a 13.0 percent rate in matched, non-ill controls during the first trimester.[120] No significant differences in birth weight were noted among the live births.[119] Because fetal loss usually occurs in such cases within 2 weeks of development of maternal infection, investigators postulated that factors related to maternal gonadal infection with resulting hormonal changes may be responsible. A histopathologic study of the products of conception in mothers with gestational mumps revealed severe proliferative necrotic villitis and vasculitis in the placenta and viral inclusions, as seen in mumps infection, in fetal tissues.[50] Mumps virus was isolated from a spontaneously aborted 10-week-old human fetus.[89]

No evidence has shown that gestational mumps in humans increases the risk of development of fetal malformations,[118] although a few cases of various congenital malformations with no consistent pattern have been reported.[69]

OTHER MANIFESTATIONS

Other rare clinical manifestations include exanthem and enanthem,[30] arthritis,[53] myocarditis,[8] thrombocytopenia,[88,90,111] keratouveitus,[78] lower respiratory tract infection,[46] and other glandular involvement (thyroiditis, mastitis, dacryoadenitis, and bartholinitis).[85,107]

DIAGNOSIS

DIFFERENTIAL DIAGNOSIS

Not all patients with mumps have parotid swelling, and mumps virus is not the only cause of parotitis. Mumps virus infection must be considered in all children with aseptic meningitis, meningoencephalitis, and encephalitis (see Chapters 41 and 42). In addition to mumps virus infection, many other infectious agents and noninfectious conditions are associated with parotitis or parotid swelling (see Chapter 16). Other viruses that can cause parotitis include Epstein-Barr virus, coxsackieviruses and echoviruses, influenza A virus, parainfluenza viruses, cytomegalovirus, human herpesvirus-6, and lymphocytic choriomeningitis virus.[14,38] Purulent parotitis can be differentiated from mumps by the exquisite tenderness of the region, an elevated white blood cell count, and the observation of pus coming from the Stensen duct. Other viral causes of parotitis can be differentiated by the respective epidemiologic and clinical characteristics of specific agents and appropriate culture, serologic study, or both.

Enlargement of lymph nodes in proximity to the parotid gland must be differentiated from parotid enlargement (see Chapter 15). The cervical lymph nodes are below the ramus of the mandible. The preparotid nodes generally are anterior to the parotid, and their enlargement usually is associated with conjunctivitis. Occasionally, an enlarged lymph node within the parotid gland may cause some confusion. Lesions of the ramus of the mandible, such as osteomyelitis, occasionally have been mistaken for parotid enlargement. In this case, the enlargement usually is persistent.

SPECIFIC DIAGNOSIS

In an epidemic situation, the diagnosis of mumps is clinically straightforward, and performing laboratory tests is unnecessary. The critical points are a history of exposure, an incubation period of 2 to 3 weeks, and a typical clinical picture consisting of fever and parotitis. In a sporadic case or in a previously vaccinated child, confirming the cause by laboratory study is important. Mumps virus, as well as most other viruses that cause parotitis, can be isolated readily from saliva, throat swabs, or mouthwashings during acute illness. In patients with meningoencephalitis, virus also can be recovered from CSF. Virus is isolated in primary monkey kidney tissue culture.

Mumps virus infection also can be confirmed by demonstrating a significant rise in antibody titer in paired serum specimens by complement fixation, hemagglutination inhibition, or ELISA. However, because mumps cross-reacts with parainfluenza viruses, these methods are not ideal.[110] Mumps-specific IgM antibody determined by ELISA is the usual test performed; the presence of this antibody indicates a recent infection. Mumps-specific IgM antibodies determined by enzyme immunoassay in oral fluid also are useful for establishing the diagnosis.[138] Mumps virus RNA also can be detected directly from clinical samples by real-time PCR.[131] In unusual cases in which the source of facial swelling is obscure, determination of a serum amylase level may be helpful; a high value indicates parotid involvement.

TREATMENT

Conservative therapy is indicated in the treatment of mumps. Adequate attention given to hydration and alimentation of patients is important. Patients may have difficulty with acidic foods such as orange juice. In addition, orange juice may cause vomiting in an already nauseated patient. The diet should be light, with a generous offering of fluids.

Occasionally, analgesics are necessary to treat severe headache or discomfort caused by parotitis. Stronger analgesics such as codeine or meperidine (Demerol) rarely are required for headache but may be useful for orchitis. Vomiting seldom is sufficiently severe to require intravenous fluids. In these instances, however, electrolytes lost by vomiting should be replaced.

Although lumbar puncture seldom is necessary for establishing a diagnosis in patients with meningoencephalitis accompanying mumps, patients often indicate that they have experienced relief of headache after undergoing this procedure. No antiviral agent is appropriate or indicated for the treatment of mumps, which is a self-limited illness.

PROGNOSIS

The overall prognosis in uncomplicated mumps is excellent. The outlook in meningoencephalitis also generally is favorable, but death and neurologic damage can occur. Deafness and sterility are rare complications.

PREVENTION

IMMUNIZATION

A summary of the recommendations of the Advisory Committee on Immunization Practices (ACIP) and the Committee on Infectious Diseases of the American Academy of Pediatrics for the use of mumps vaccine follows.[3,18,28a] For more complete information,

the reader should consult the most recent ACIP statement or the Report of the Committee on Infectious Diseases of the American Academy of Pediatrics.

The mumps virus vaccine available in the United States (official name: mumps virus vaccine, live) is the Jeryl Lynn strain and is prepared in chick embryo cell culture. The vaccine produces a subclinical, noncommunicable infection with few side effects. Mumps vaccine is available in both a monovalent (mumps only) form and combinations: mumps-rubella, MMR, and measles-mumps-rubella-varicella (MMRV) vaccines.

In initial trials, mumps vaccine was approximately 95 percent efficacious in preventing mumps disease,[67,80,126] and after vaccination, measurable antibody developed in more than 97 percent of persons known to be susceptible to mumps.[142] Vaccine-induced antibody was protective and long-lasting,[140,141] although it was of considerably lower titer than antibody resulting from natural infection.[142] The duration of vaccine-induced immunity is unknown, but serologic and epidemiologic data collected during 35 years of use of live vaccine suggested both persistence of antibody and continuing protection against infection. The epidemic occurrence of mumps in 2006 in the United States as well as in other countries resulted in further evaluations of vaccine efficacy and waning immunity.[11,28a,34,62,74,106,134] In a study in England, the effectiveness of vaccine after one dose declined from 96 percent in 2-year-old children to 66 percent in children aged 11 to 12 years old.[34] In those children who had received two doses, the effectiveness declined from 99 percent in children aged 5 to 6 years old to 86 percent in children aged 11- to 12-years old. These data suggest that waning immunity contributes to outbreaks of mumps in older, previously vaccinated populations. Studies in Belgium and Korea also demonstrated waning immunity as contributing to outbreaks of mumps.[29]

In Finland, a two-dose MMR vaccination program with the Jeryl Lynn mumps vaccine strain was launched in 1982.[108] This program was highly successful, and Finland was the first country documented to be free of indigenous mumps, as well as rubella and measles. In a study in Finland that was published in 2007, researchers found that previous vaccinees who were found to be seronegative nonetheless had mumps antigen-specific lymphoproliferative responses.[74]

General Recommendations

Susceptible children, adolescents, and adults should be vaccinated against mumps unless vaccination is contraindicated. Mumps vaccine is of particular value for children approaching puberty and for adolescents and adults who have not had mumps. MMR is the vaccine of choice for routine administration and should be used in all situations in which recipients also are likely to be susceptible to measles, rubella, or both. The favorable benefit-to-cost ratio for routine mumps immunization is more marked when vaccine is administered as MMR.[83,139] Persons should be considered susceptible to mumps unless they have documentation of (1) physician-diagnosed mumps, (2) adequate immunization with live mumps virus vaccine on or after their first birthday, or (3) laboratory evidence of immunity.

Persons who are unsure of their history of mumps disease or mumps vaccination should be vaccinated. No evidence has shown that persons who previously either received mumps vaccine or had mumps are at any increased risk for developing local or systemic reactions from receiving live mumps vaccine. Testing for susceptibility before administering vaccination, especially in adolescents and young adults, is not necessary. In addition to the expense, some tests (e.g., mumps skin test, complement-fixation antibody test) may be unreliable, and tests with established reliability (e.g., neutralization, enzyme immunoassay, radial hemolysis antibody test) are not readily available.

Dosage

Two doses of MMR vaccine separated by at least 1 month in the volume specified by the manufacturer should be administered subcutaneously.

Age

Live mumps virus vaccine is recommended at any age on or after the first birthday for all susceptible persons, unless a contraindication exists. In routine circumstances, mumps vaccine should be given in combination with measles and rubella vaccines as MMR, and the currently recommended schedule for administration of measles vaccine should be followed. It should not be administered to infants younger than 12 months old because persisting maternal antibody may interfere with seroconversion. To ensure that the patient has developed immunity, all persons vaccinated before their first birthday should be revaccinated on or after their first birthday.

PERSONS EXPOSED TO MUMPS

Use of Vaccine

When given after exposure to mumps, live mumps virus vaccine may not provide protection. However, if the exposure did not result in infection, vaccine should induce protection against infection from subsequent exposure. No evidence has indicated that the risk of vaccine-associated adverse events increases if vaccine is administered to persons incubating disease.

Use of Immunoglobulin

Immunoglobulin has not been demonstrated to be of established value in postexposure prophylaxis and is not recommended.

Adverse Effects of Vaccine Use

In field trials before vaccine was licensed, illnesses did not occur more often in vaccinees than in unvaccinated controls.[66] Reports of illnesses occurring after receipt of mumps vaccination have been episodes mainly of parotitis and low-grade fever. Allergic reactions, including rash, pruritus, and purpura, have been associated temporally with mumps vaccination but are uncommon and usually mild and of brief duration. The reported development of encephalitis within 30 days of receiving a mumps-containing vaccine in the United States (0.4 per million doses) is not greater than the observed background incidence rate of CNS dysfunction in the normal population. Other manifestations of CNS involvement in the United States, such as febrile seizures and deafness, also have been reported infrequently. Complete recovery usually occurs. Reports of nervous system illness occurring after receipt of mumps vaccination do not necessarily denote an etiologic relationship between the illness and the vaccine. In parts of Europe, Canada, and Japan, where different mumps vaccines (Leningrad 3 strain and Urabe Am 9 strain) have been used, rates of vaccine-induced aseptic meningitis have been high.[32,35,94,98,127]

CONTRAINDICATIONS TO VACCINE USE

Pregnancy

Although mumps vaccine virus has been shown to infect the placenta and fetus,[146] no evidence has indicated that it causes congenital malformations in humans. However, because of the theoretic risk of fetal damage, a prudent approach is to avoid

giving live virus vaccine to pregnant women. Women should avoid becoming pregnant for 3 months after receiving vaccination. Routine precautions for vaccinating postpubertal women include asking whether they are or may be pregnant, excluding those who say that they are, and explaining the theoretic risk to those who plan to receive the vaccine. Being vaccinated during pregnancy should not be considered an indication for termination of pregnancy. However, the final decision about interruption of pregnancy must rest with the individual patient and her physician.

Severe Febrile Illness

Administration of vaccine should not be postponed because of minor or intercurrent febrile illnesses such as mild upper respiratory infections. However, vaccination of persons with severe febrile illnesses generally should be deferred until they have recovered.

Allergies

Because live mumps vaccine is produced in chick embryo cell culture, persons with a history of anaphylactic reactions (e.g., hives, swelling of the mouth and throat, difficulty breathing, hypotension, shock) after ingesting eggs should be vaccinated only with caution and according to published protocols.[54,64] Children known to be allergic should not leave the vaccination site for 20 minutes after being vaccinated. Evidence indicates that persons are not at increased risk if they have egg allergies that are not anaphylactic. Such persons may be vaccinated in the usual manner. No evidence has demonstrated that persons with allergies to chickens or feathers are at increased risk of having a reaction to the vaccine.

Because mumps vaccine contains trace amounts of neomycin (25 μg), persons who have experienced anaphylactic reactions to topically or systemically administered neomycin should not receive mumps vaccine. Most often, allergy to neomycin is manifested as contact dermatitis, which is a delayed-type (cell-mediated) immune response rather than anaphylaxis. In such persons, the adverse reaction, if any, to 25 μg of neomycin in the vaccine would be an erythematous, pruritic nodule or papule at 48 to 96 hours. A history of contact dermatitis to neomycin is not a contraindication to receiving mumps vaccine. Live mumps virus vaccine does not contain penicillin.

Recent Immunoglobulin Injection

Passively acquired antibody can interfere with the response to live attenuated virus vaccines. Therefore, mumps vaccine should be given at least 2 weeks before the administration of immunoglobulin or be deferred for 3 to 11 months after the administration of immunoglobulin. The duration of deferral depends on the dose of immunoglobulin administered.[139]

Altered Immunity

In theory, replication of the mumps vaccine virus may be potentiated in patients with immune deficiency disease and by the suppressed immune responses that occur with leukemia, lymphoma, or generalized malignancy or with therapy with corticosteroids, alkylating drugs, antimetabolites, or radiation. In general, patients with such conditions should not be given live mumps virus vaccine. Because vaccinated persons do not transmit mumps vaccine virus, the risk of exposure to mumps in these patients may be reduced by vaccinating their close susceptible contacts.

An exception to these general recommendations is in children infected with human immunodeficiency virus (HIV): all asymptomatic HIV-infected children should receive MMR vaccine at 12 months of age.[17,139] If measles vaccine is administered to symptomatic HIV-infected children, the combination MMR vaccine generally is preferred.[18]

Patients with leukemia in remission whose chemotherapy has been terminated for at least 3 months also may receive live mumps virus vaccine. Short-term (less than 2 weeks' duration) corticosteroid therapy, topical steroid therapy (e.g., nasal, skin), and intra-articular, bursal, or tendon injection with corticosteroids are not contraindications to the administration of mumps vaccine. However, mumps vaccine should be avoided if systemic immunosuppressive levels are reached by prolonged, extensive, topical application.

CONTAINMENT OF DISEASE

Containment is important in prevention of mumps in the United States. Mumps is a reportable disease, and compliance is the obligation of all physicians. After early reports of sporadic mumps cases, health department workers can organize local immunization clinics and exclude susceptible students from school so that disease can be contained in a small geographic area.

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CHAPTER 194

Respiratory Syncytial Virus

Caroline Breese Hall ❂ Edward E. Walsh

As by one bow on varied strings
the tune is played,
By both the microbe and the host,
disease is made.

—*CBH*

Respiratory syncytial virus (RSV) is the most important respiratory pathogen of infancy and early childhood and the major cause of hospitalization for bronchiolitis and pneumonia in infants.[37,123,124,125,199,220,318,323] According to 1986 estimates of the Institute of Medicine, each year approximately 91,000 infants were hospitalized with RSV infection in the United States at a cost of $300 million.[179,197] More recent studies suggest that these figures underestimate the current number of hospitalizations associated with RSV infections. Significant rises in the number of admissions for RSV bronchiolitis have been documented in both the United States and Canada.[237,364] In the United States, 62,500 to 100,000 hospitalizations for RSV bronchiolitis alone are estimated to occur annually in children younger than 5 years old.

The mien of RSV is marked by its contagiousness and by its propensity to cause the most severe disease during the first few months of life, despite the uniform presence of specific maternal antibody. RSV causes sizable outbreaks each year, with the result that most children become infected in their first 2 years of life.[127,182] Nearly all these first infections are symptomatic, and many involve the lower respiratory tract. Reinfections occur throughout life and usually are sufficiently symptomatic to cause absence from work or school.[146,149,182]

HISTORY

In 1956, Morris and associates[284] noted a cropping of colds with coryza in a colony of chimpanzees that had been under observation for the previous 3 to 24 weeks. A new virus was recovered from 1 of the 14 afflicted chimpanzees, and it was appropriately named *chimpanzee coryza agent* (CCA). Specific antibody to the CCA agent developed in the remaining 13 animals during convalescence; thus, the attack rate was 100 percent. An upper respiratory tract infection and a serologic antibody response to CCA also developed in a person working with these chimpanzees, but the virus was not isolated from his secretions. However, further evidence of the pathogenicity of CCA was obtained subsequently when the CCA virus isolated from one chimpanzee was inoculated into susceptible chimpanzees and a coryzal illness developed after 3 days.

The suspected human origin of the chimpanzee coryza agent was confirmed a year later when Chanock and Finberg[57] recovered two agents indistinguishable from the CCA virus from the throat swabs of an infant with bronchopneumonia (Long strain) and from a child with laryngotracheobronchitis (Snyder strain). These investigators also observed an antibody response to these viruses in patients with respiratory disease and noted that by the time they reached 4 years of age, 80 percent of children had neutralizing antibody against the Long strain. Nonetheless, these investigators could not determine a definite etiologic association between the virus and lower respiratory tract disease in their young patients. They proposed to call this group of viruses (Long, Snyder, and CCA) *respiratory syncytial virus* because of their similar clinical and tissue culture manifestations. Confirmation of RSV as a major agent in respiratory disease soon accumulated from studies throughout the United States, and investigators from many countries subsequently documented and further delineated the importance of RSV.[28,37,56,101,106,268,302]

PROPERTIES

A tiny thistle—
of coiled spine
and outer quill

CLASSIFICATION

The original classification of RSV with the Newcastle disease and parainfluenza group of viruses was based on their similar internal particle structure, eosinophilic inclusions, and syncytial appearance in tissue culture. However, RSV is antigenically distinct and does not hemagglutinate erythrocytes. Subsequently, the diameter of the nucleocapsid of RSV was determined to be between those of the larger paramyxoviruses and those of the smaller influenza viruses. Further study of the structure of RSV resulted in its current classification in the order Mononegavirales, family *Paramyxoviridae*, and subfamily *Pneumovirinae*.[69,335] RSV belongs, along with pneumonia virus of mice, bovine RSV, ovine RSV, caprine RSV, and turkey rhinotracheitis virus, to the subfamily *Pneumovirinae*, which consists of two genera, *Pneumovirus* and *Metapneumovirus* (MPV). The second genus, *Metapneumovirus*, contains human metapneumovirus (hMPV) first described in 2001 by van den Hoogan and colleagues[387] in the Netherlands.

STRUCTURAL AND ANTIGENIC PROPERTIES

The virion of RSV consists of a nucleocapsid enclosed within an envelope consisting of a bilipid layer derived from the plasma membrane of host cells. Transmembrane glycoprotein spikes on its surface give the virus the appearance of a thistle on electron microscopy. The genome of RSV (strain A2) has been completely sequenced and is composed of 15,222 nucleotides. It is a nonsegmented, single-stranded, negative-sense RNA genome that encodes 11 proteins. The major messenger RNAs (mRNA) (Fig. 194–1) each encodes for one of the major proteins, except for M2 mRNA, which possesses two overlapping open-reading frames that encode for two separate proteins (M2-1 and M2-2).[69,413] Eight of these, including the seven largest (L, G, F, N, P, M, and SH), are structural proteins; two (NS1 and NS2) are nonstructural proteins; and M2-2 is a regulatory protein.

Of the structural proteins, three form the viral capsid: N (nucleoprotein), P (phosphoprotein), and L (polymerase). Two are matrix membrane–associated proteins: the nonglycosylated M and M2 (the transcriptional anti-terminator protein or transcriptase processivity factor, M2-1). The other three structural proteins, the glycosylated F (fusion) and G (attachment) proteins and the small, nonglycosylated hydrophobic SH protein, are transmembrane surface proteins. The F and G proteins are integral to RSV's infectivity and pathogenicity. The primary function of G, the largest glycoprotein, appears to be in the initial attach-

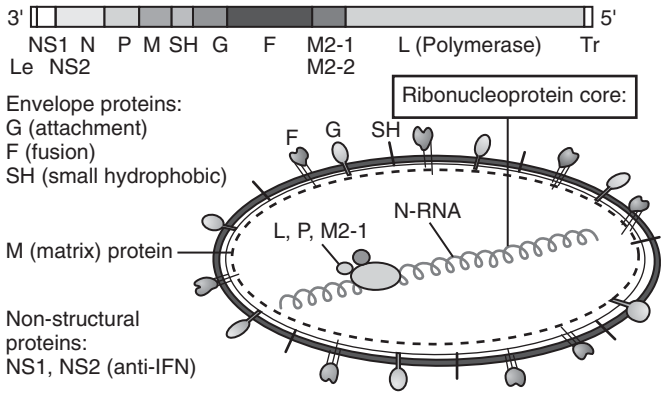

Figure 194–1 The genome of respiratory syncytial virus (RSV) and a schematic diagram of an RSV virus particle. Le, leader; Tr, trailer.

ment of the virus to the host cell and is unusual compared with the attachment proteins of other paramyxoviruses because it has no hemagglutinin or neuraminidase. Initial penetration of the virus then is mediated by the F protein by fusing the viral and host cellular membranes, a process that allows cell-to-cell spread of the virus and results in the syncytia characteristic of RSV. However, all three of the surface glycoproteins, F, G, and SH, appear to act in concert to enhance the fusion process.

Significant strain variations among RSV isolates cause strains to be divided into two major groups.[20,413] These two major groups, A and B, have intergroup and intragroup variations in several proteins, including F, G, P, and N. The primary differences reside in the largest surface glycoprotein, the G protein. The amino acid sequence of the G protein varies by approximately 50 percent between the RSV A and RSV B strain groups. Intragroup strain variation also occurs mainly within the G protein. In contrast, within F protein, the amino acid sequence is conserved relatively well, and thus, neutralizing antibody to F protein is cross-reactive between the two strain groups.[69] The F proteins of prototype strains from groups A and B have greater than 90 percent amino acid homology and a high degree of antigenic relatedness.

LABORATORY GROWTH

RSV is relatively labile and is destroyed rapidly at 55° C. At 37° C, infectivity does not diminish for 1 hour, but by 24 hours, only 10 percent remains. When placed at 4° C for 1 week, 1 percent remains.[170]

The viability of RSV depends in part on the salt and protein content of the media. At 4° C, the addition of 1 mol/L of magnesium sulfate maintains viral stability for 5 weeks.[102] The virus withstands freezing and thawing poorly.[170] Preservation is enhanced by rapid freezing in a dry ice and alcohol bath and by the addition of sucrose or glycerin to the storage media. Infectivity also is influenced by the pH of the medium; at values less than 5, infectivity rapidly diminishes. The optimal pH for preservation is 7.5.[170] RSV is inactivated rapidly by detergents such as 0.1 percent sodium deoxycholate, sodium dodecyl sulfate, and Triton X-100, as well as by chloroform and ether.

RSV can grow in a variety of human and animal cell cultures. Human heteroploid cells such as HEp-2, HeLa, and A549 cells are used most frequently for primary isolation. Additional cell cultures that may be used, but generally are less sensitive, include diploid fibroblastic, monkey kidney, human kidney, and amnion cells. However, the sensitivity of these cell lines is variable, especially with passage, and must be monitored constantly. The characteristic cytopathic effect of RSV in continuous cell lines is syncytial formation with eosinophilic cytoplasmic inclusions. The syncytia usually are evident 2 to 7 days after inoculation and progress to complete degeneration within about 4 days.[150] The cytopathic effect, however, depends on the strain of virus, the medium, the sensitivity and thickness of the cell cultures, and the number of passages. Syncytia tend to be less evident in fibroblast cell lines, and in some primary cell cultures, RSV may produce rounded, refractile cells.

The growth cycle of RSV has been shown to consist of a period of adsorption, in which 50 percent of the inoculum is adsorbed in 2 hours, followed by an eclipse period of 12 hours. New virus appears shortly thereafter and enters a log phase of replication lasting for approximately 10 hours. Viral antigen can be documented by fluorescent antibody staining 7 to 10 hours after inoculation. Shortly thereafter, cell-free virus may be demonstrated in the culture medium, but 50 to 90 percent of the virus remains associated with the cell surface at the time maximal titers are reached. Most of the cell-associated virus consists of incomplete noninfectious virions that may be released by agitation and sonication. With the laboratory Long strain (group A), peak titers usually are reached in 48 hours. For each infected cell, approximately 10 plaque-forming units generally result. Titers of virus are enhanced by inoculation of cell monolayers that are not yet confluent.

ANIMAL SUSCEPTIBILITY

Susceptible animals that could serve as a model for the lower respiratory tract disease of infants have been sought for some time. Each has advantages and disadvantages in replicating primary RSV disease in infants, but none is ideal.[84] Although RSV grows in the lung of a variety of animals, direct inoculation into their respiratory tract generally produces infection that is clinically and pathologically silent.

The natural hosts for symptomatic RSV infection are primarily humans and chimpanzees, and cows for bovine RSV.[42,84] RSV also has been recovered from asymptomatic goats and sheep. Other domestic animals, such as dogs and cats, have been found to possess antibody to RSV, the significance of which is unclear.

Chimpanzees most closely resemble humans in their clinical response to RSV. However, lung disorders and some degree of clinical disease have been induced in many other primates.[42,84,345] Infection in ferrets is age-dependent, with limited histopathologic features of the nasal turbinates and trachea developing in adults. In infant ferrets, however, the virus replicates in the lung.[330] The use of the cotton rat model resulted in major progress in understanding the pathogenesis of RSV infection, as demonstrated by the seminal studies of Prince and colleagues.[331,333,334] With intranasal inoculation, viral titers peak in the lung and nasal turbinates after 4 to 5 days. Histologic changes in the lung are present but may be inconsistent, and immunologic reagents are limited in comparison with those for mice. Active replication of RSV in other rodents generally is limited. BALB/c mice are among the most susceptible, and marked pulmonary disease may occur with high-titer, large-volume inocula, which in older mice is accompanied by evidence of clinical illness.[131,135]

A lamb model also was developed, which after challenge with ovine, bovine, or human RSV resulted in pathologic changes in the lung, fever, and tachypnea.[234] Bovine RSV in cattle has been an appealing natural model because of its clinical, epidemiologic, and histologic similarities to human RSV infection in infants.[235] Calves, despite possessing maternal antibody, can develop bronchiolitis. The disease can be severe and aggravated by bacterial superinfection, which occurs infrequently in infants. Despite this

appreciable morbidity that naturally occurs with bovine RSV, experimental infection in cattle has sometimes been difficult and inconsistent. The natural pathogen of mice, pneumonia virus of mice (PVM), a pneumovirus similar to RSV, also has been used successfully as a model for studies of pathogenesis.[84] PVM offers the advantages of good viral replication followed by severe respiratory disease and histologic changes.

EPIDEMIOLOGY

What occult power pries loose the lid
to give you winter flight,
But with the lengthening light of spring
gives cloak and leaden wing?

—CBH

GEOGRAPHIC DISTRIBUTION

Experience with RSV infection is ecumenical, as is its predominant pathogenicity for the very young and for those with underlying high-risk conditions. The timing, length, and intensity of RSV outbreaks, however, vary geographically. Outbreaks in warm and tropical climates tend to be more prolonged, with less distinctive peaks of activity.

SEASONAL PATTERNS

The distinctive epidemiologic character stems from the singular consistency and size of the outbreaks of infection it produces each year and the health care burden it leaves in its wake.[48,104,105,148] In the United States, data from the National Respiratory and Enteric Virus Surveillance System between 1990 and 2000 indicated that RSV activity usually lasts for 20 or more weeks, from November to May, and may completely encompass the outbreak of influenza.[168,199,286] The peak of activity of RSV in the United States occurs in January or February (Table 194–1). Epidemiologic patterns tend to be similar in other countries with similar temperate climates, whereas in countries with warmer climates, RSV activity may correlate with the rainy seasons or may be present throughout the year.

Outbreaks of RSV infection are associated with increased numbers of young children hospitalized with acute lower respiratory tract disease. These consistent ramifications are barometers of the onset and severity of RSV activity in a community. The peak periods each year of both admissions for children with lower respiratory tract disease and of outpatient medical visits for bronchiolitis are associated primarily with RSV activity (Fig. 194–2).[106,122,148,151,220]

STRAIN VARIATION

Variation in circulating strains of RSV has been suggested as partly accounting for the fluctuating clinical impact of RSV epidemics.[20,315,324,357] The two major strain groups, A and B, usually circulate simultaneously during an outbreak, but the proportion of strains from each group may vary by season and geography, as does predominance of the subgroups of A and B strains.[20,112,263,319,324,357] In most areas, the annual dominant strains appear to be group A. In Rochester during a 20-year period, group A strains predominated in 11 of the years, group A and B strains were relatively equal in another 5 years, and in 4 seasons group B strains accounted for more than 75 percent of the year's isolates.[167] A study of strains from 14 cities across the United States demonstrated that the variability of strains occurs both seasonally and geographically.[20] Several distinct but varying genotypes within the A and B strain groups predominate each year in a community,

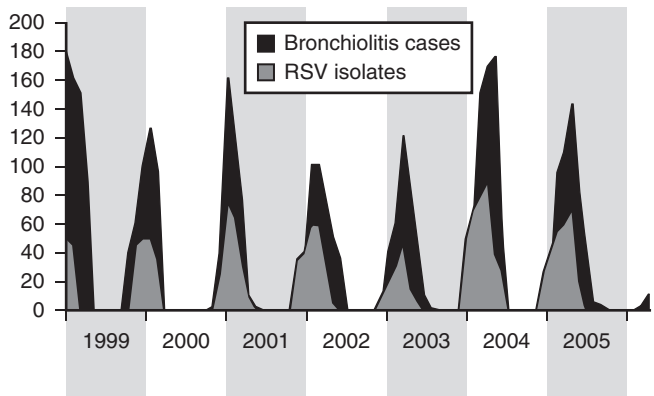

Figure 194–2 The number of cases of bronchiolitis and the number of isolates of respiratory syncytial virus from children less than 5 years of age who were evaluated in private offices and outpatient facilities from 1998 to 2005 identified by a community surveillance program in Monroe County, New York.

TABLE 194–1 Summary of Median Values of Respiratory Syncytial Virus Season Characteristics for Entire Nation and U.S. Census Regions from National Respiratory and Enteric Virus Surveillance System Data, 1990 to 2000

	Median Weeks of Year for RSVs			
Location	**Onset**	**Peak**	**Offset**	**Duration**
Nation	51 (late December)*	5 (early February)	13 (end of March)	15
West	52 (late December)	6 (mid-February)	13 (end of March)	14
Midwest	1† (early January)	7 (late February)	13 (end of March)	13†
South	47‡ (late November)	1 (early January)	10 (mid-March)	16†
Northeast	49 (early December)	4 (end of January)	12 (late March)	15

**Numbers in parentheses, time of year.*
†Statistically different from the rest of the nation, $p < .05$.
‡Statistically different from the rest of the nation, $p < .01$.
RSV, respiratory syncytial virus.
From Mullins, J. A., LaMonte, A. C., Bresee, J. S., and Anderson, L. J.: Substantial variability in community respiratory syncytial virus season timing. Pediatr. Infect. Dis. J. 22:857-862, 2003.

a finding suggesting that local preexisting immunity to previously circulating RSV strains may engender a selective advantage for strains with genotypes most diverse from those recently present in a community.[81,263,319,324,413] The relationship between the circulating strain group or subgroup and the size or clinical severity of an RSV outbreak, thus far, has not appeared to be strong or consistent.[36,81,315,362] Further understanding of these relationships and the molecular mechanisms, pathogenesis, and clinical importance of differing genotypes may be integral to the development of effective vaccines and the control of RSV.[413]

ACQUISITION AND RAMIFICATIONS OF INFECTION

The major burden that RSV inflicts on the health care of children is illustrated by its ubiquity and the proportion of lower respiratory tract disease it causes in early childhood, especially in young infants. RSV accounts for 30 to 90 percent of bronchiolitis cases, up to 50 percent of pneumonia admissions during infancy, and 10 to 30 percent of cases of pediatric bronchitis.[37,181,199,220,308] In contrast, only relatively small proportions, less than 10 percent, of croup cases have been associated with RSV infection. RSV is isolated rarely from patients (<1%) without respiratory disease.[58,220]

During the first year of life, approximately 50 to 70 percent of infants acquire RSV infection, despite the presence of maternal specific neutralizing antibody in the sera of all newborns.[126,181,318] The level of antibody in a term newborn is similar to the maternal level, which gradually declines during the first 6 months of life. In patients older than 7 months, detectable specific serum antibody usually is the result of natural infection. Essentially all children by 3 years of age possess specific serum antibody.

The impact of RSV infections on communities around the world has been examined in numerous studies and varies depending on the geographic location, the population, and the methods of assessment. Nonetheless, several generalizations are evident: (1) RSV is a highly contagious agent resulting in ubiquitous infection by the time one reaches 2 to 3 years of age; (2) in this age group, RSV is the major cause of lower respiratory disease, predominantly bronchiolitis and pneumonia; (3) RSV infection of the lower respiratory tract in normal children almost always is the result of primary infection; (4) reinfection occurs throughout life, and subsequent infections are milder and usually are upper respiratory tract infections; (5) the severity of illness from reinfections remains appreciable at any age in persons with co-morbid conditions; and (6) medical visits by outpatients with RSV infection comprise an appreciable portion of the burden to health care.

Infants with underlying chronic conditions are at high risk of requiring hospitalization for RSV illness, but numerous environmental and other host factors have been reported to augment the risk of a previously healthy infant's developing more severe RSV infection.[123,126,342,356] These factors include becoming infected within the first few months of life, living in crowded and lower socioeconomic circumstances, geographic location, and gender. Although the rate of infection among girls and boys is equal, boys are hospitalized 1.5 to 2 times more frequently than are girls, a finding suggesting that boys are more likely to develop severe infection.

As many as 120,000 children are hospitalized each year with RSV infection and close to 150,000 children are hospitalized with bronchiolitis.[126,191,323,364,365] The annual rate of hospitalizations caused by RSV in comparison with that from influenza or the parainfluenza viruses has been approximately three times greater for children aged 5 years and younger and six to eight times greater for children younger than 1 year of age.[168,199,300]

In the United Kingdom for more than 11 winter respiratory seasons, RSV was associated with more deaths than was influenza in young children, especially in the first year of life.[105] In the United States, the number of deaths associated with RSV infection each year among children younger than 5 years of age was estimated in 1985 to be 4500.[197] During 1979 to1997, this number was reported to have declined to an estimated 510 or less each year in children younger than 5 years of age.[237,364,365] The rates of hospitalization associated with RSV, however, have not declined but appear to be increasing from the estimate of 110,000 in 1997 in children younger than 5 years of age.[191,364] The rate of admissions attributable to RSV is markedly higher among infants within the first year of life than among children aged 1 to 4 years. In infants, the estimated rates have varied from 5 to 41 per 1000 each year, depending on the area and the methods used.[34,236,257]

Population-based studies reported annual hospitalization rates attributable to RSV in the United States of 3 to 3.5 per 1000 children younger than 5 years of age.[168,199] These rates were approximately six times greater than that associated with the influenza viruses and three times greater than that associated with the parainfluenza viruses. The rates of hospitalization for RSV among infants were even greater, 12.9 per 1000 children younger than 1 year of age, compared with 1.7 for influenza and 3.2 for the parainfluenza viruses. The youngest infants, those younger than 6 months old, had the greatest risk of requiring admission for their RSV infection, with a yearly rate of 17 per 1000 children.[168]

In other countries, population-based studies demonstrated similarly high rates of hospitalizations related to RSV among infants and young children.[94,104,106,300,403] Annual population-based hospitalization rates attributable to RSV from European countries were reported per 1000 children to be 2.5 for those younger than 4 years of age, 11 for children younger than 3 years of age, and 4 to 7 for those younger than 2 years of age. Among children within the first 12 months of life, 19 to 22 per 1000 children were admitted each year with RSV infection.

The magnitude of ambulatory visits among children resulting from RSV infection has not been well quantified and generally has received less attention. Surveillance conducted in Nashville, Tennessee, and Rochester, New York, for acute respiratory illness occurring among children aged 0 to 59 months showed that RSV was the agent most frequently identified among children evaluated for respiratory illness in outpatient practices and emergency departments.[168] Approximately one fifth of these children had laboratory-confirmed RSV infection, and an estimate of the health care burden from these RSV outpatient visits was appreciably greater than that from RSV-associated hospitalizations. The potentially greater impact of RSV outpatients visits on health care resources is further supported by a German study in which the annual incidence rate for RSV illness in outpatients was notably high at 77 per 1000 children younger than 3 years of age.[106]

Current estimates of the economic and clinical costs related to RSV infections in young outpatient children are likely to be too low because they reflect only a portion of the impact RSV imposes each year. Not included are the medical visits among older children and among healthy and elderly adults in whom illness from RSV infection occurs frequently and can be severe.[100,146,149,395]

SPREAD OF INFECTION

> Each breath's toll is virus spread and shed.[144]
>
> *—CBH*

RSV spreads effectively through exposed families, and introduction of the virus into the family appears to occur most commonly through a school-aged child.[128,129,160,282] Serious disease in infancy

TABLE 194–2 Attack Rate of Respiratory Syncytial Virus in Families According to Age

	Attack Rate*					
	Crude Rate		*In RSV-Positive Families*		*Secondary Rate*	
Age (yr)	No.†	%	No.†	%	No.†	%
<1	10/34	29.4	10/16	62.5	5/11	45.4
1-<2	2/7	28.6	2/5	40.0	0/3	0.0
2-<5	9/34	26.4	9/19	47.0	2/12	16.6
5-<17	9/48	18.7	9/24	38.0	4/19	21.0
17-45	9/55	16.8	9/21	43.0	6/18	33.3
Total	39/178	21.9	39/85	45.9	17/63	27.0

**The crude attack rate according to age is shown for all family members studied and for members of RSV-positive families. The secondary attack rate is also shown for members of RSV-positive families, excluding all primary and co-primary cases.*

†Number of persons infected with RSV/total number of persons exposed.

Reprinted, by permission, from Hall, C. B., Geiman, J. M., Biggar, R., et al.: Respiratory syncytial virus infection within families. N. Engl. J. Med. 294:414-419, 1976.

is likely to follow a mild "cold" in an older sibling.[160] In a prospective study of families with an infant and one or more older siblings, 44 percent of the families became infected with RSV during a 3-month epidemic. In almost all these families, older siblings (2-16 years of age) introduced the virus into the family, and the infants became secondarily infected (Table 194–2). Intrafamilial spread of the virus, according to the Tecumseh study, also was related to the number of family members.[282] Families with six members had approximately three times the rate of infection observed in families with three members.

How this labile virus can spread so effectively has remained mostly a conundrum. Transmission primarily by small-particle aerosol seems unlikely according to clinical and experimental observations.[152] Small-particle aerosols of RSV are unstable at the low relative humidity of 20 to 30 percent usually encountered indoors during the winter months.[340] At 30 and 80 percent relative humidity, RSV in aerosol was maximally inactivated. Maximal stability occurred at 60 percent relative humidity.

Spread may occur, however, through large droplets of secretions or through contact with contaminated secretions.[129,152] Infants with RSV infection have high viral loads of RSV in their secretions of both the upper and lower respiratory tract.[82,155,156,325,420] The duration of shedding also tends to be prolonged in young children. RSV in the nasal secretions of infants with acute infection remains infectious on countertops for longer than 6 hours and on cloth and tissue paper for approximately 30 minutes.[161] These nasal secretions can remain infectious after transfer from objects or hands to the hands of another person, thus indicating that contact with clothing, furniture, or tissues contaminated by the secretions of infected children can result in spread.[129,161]

This mode of spread has been supported by studies demonstrating that infection occurs in volunteers who touch surfaces contaminated by secretions and then their eyes or nasal mucosa.[152] In contrast, no infections developed in volunteers exposed to infected infants at a distance of greater than 6 feet, thus suggesting that small-particle aerosol spread of RSV was not a major mode of transmission. RSV infection, therefore, appears to require close contact with infected individuals or their secretions.

PATHOLOGY AND PATHOGENESIS

The newly born
from mother shorn,

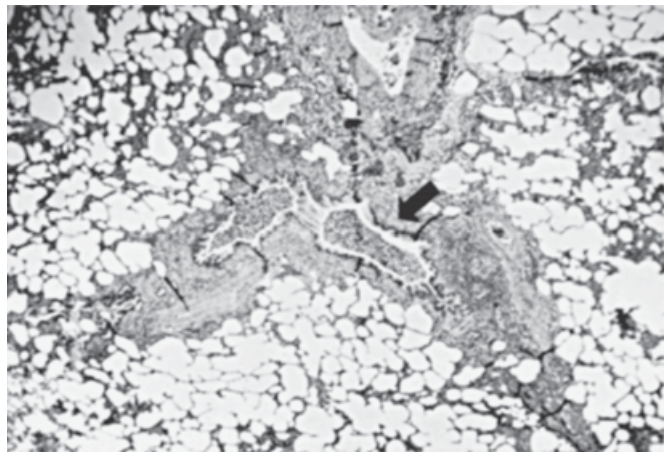

Figure 194–3 Histologic examination of an infant dying of respiratory syncytial virus bronchiolitis. The arrow denotes a bronchiole filled with inflammatory exudate and comparatively normal alveoli.

A seed just sown,
its growth unknown.

—CBH

The incubation period of illness from RSV has been reported variably as being between 2 and 8 days, most frequently as 4 to 6 days. Experimental infection in adult volunteers produced an incubation period of 3 to 6 days, with an average of 5 days.[157,202] With primary infection, the incubation period may be shorter.[157,166] Inoculation is through the upper respiratory tract, followed by infection of the respiratory epithelium. Both the eye and the nose appear to be equally sensitive routes of inoculation.[157] In contrast, inoculation by mouth results in infection much less frequently. Spread along the respiratory tract occurs mainly by cell-to-cell transfer of the virus along intracytoplasmic bridges and may involve the conducting airways at all levels.

The major pathologic findings shown in infants dying of RSV bronchiolitis are (1) peribronchiolar mononuclear infiltration, (2) necrosis of the epithelium of the small airways, (3) plugging of the lumina, and (4) hyperinflation and atelectasis (Fig. 194–3).[4,9,147,390] The initial lesions in bronchiolitis occur in the small airways (75 to 300 μm).[9] Lymphocytic peribronchiolar infiltration develops along with edema of the walls, the submucosa, and adventitial tissue. Subsequently, the epithelium of the bronchioles undergoes striking necrosis, sometimes with proliferation of the epithelium into the lumen. Sloughing of necrotic material into the lumina of the small airways impedes the flow of air, which is aggravated by increased mucus secretion.

Peripheral to the sites of partial occlusion, air trapping occurs, similar to a ball-and-valve mechanism. During the negative intrapleural pressure of inspiration, air can flow past the site of partial obstruction, but with the positive pressure of expiration the lumen narrows, resulting in more complete obstruction and the hyperinflation characteristic of bronchiolitis. The trapped air may be absorbed and result in multiple areas of focal atelectasis. Functionally, these pathologic changes translate into an increased lung volume and higher expiratory resistance in the infant. Pneumonia may be present concurrently and is marked by interstitial infiltration of mononuclear cells. Lymphocytic infiltration of the bronchiolar walls also may be observed. The lung parenchyma appears edematous, with areas of necrosis leading to alveolar filling, consolidation, and collapse.

Recovery from acute bronchiolitis may be evident in 3 to 4 days, with regeneration of the bronchiolar epithelium, but cili-

ated cells rarely are present before 2 weeks.[9] Complete histologic and functional recovery may require another 4 to 8 weeks.[169]

IMMUNE RESPONSE: IMMUNITY AND DISEASE PATHOGENESIS

The immune response to RSV is complex, governed by factors such as age at the time of infection, genetic variation of the host, and virus-specific attributes.[276,306,322] Each affects various aspects of innate and adaptive T- and B-cell immune responses that determine the outcome of infection and the level of protection from subsequent infection. Much of our knowledge regarding immune responses to RSV is derived from in vitro studies or in vivo animal experiments, supplemented by inconsistent human data. Nonetheless, a relatively consistent theme has emerged, suggesting that virus-induced chemokine and cytokine patterns and the balance between T-helper (T_H1) and T_H2 T-cell responses determine such disease characteristics as the clinical manifestations, the severity, and possibly the ultimate development of asthma (Fig. 194–4).[276] The role of the immune response in the pathogenesis of RSV disease is supported further by the observation that RSV is only moderately cytopathic in vivo. Immune mechanisms most likely also accounted for the enhanced disease that occurred after immunization with the formalin-inactivated RSV vaccine in the 1960s.[221]

MATERNALLY ACQUIRED ANTIBODY

Transplacentally acquired immunoglobulin G (IgG) provides the first line of defense to the newborn infant encountering RSV for the first time.[126,188,303] Glezen and coworkers found that the age at the time of hospitalization for infants with primary RSV infection was directly correlated with the level of cord blood neutralizing antibody titer.[126] With a half-life of approximately 21 days, the protective effect rapidly wanes, thus accounting for a relatively brief period of protection.[303] These data, coupled with studies in experimental animals, provided impetus for the development and use of high-titered RSV intravenous immunoglobulin (RSV-IGIV) and subsequently of the monoclonal antibody, palivizumab, for RSV prophylaxis in high-risk infants.[139,196,332]

INNATE IMMUNITY

Infection of respiratory epithelial cells and antigen-processing cells (macrophages and dendritic cells) triggers early innate immune responses characterized by synthesis of chemokines and proinflammatory cytokines and the recruitment of inflammatory cells, neutrophils, eosinophils, and interferon-γ (IFN-γ)–secreting natural killer (NK) cells, to the infected pulmonary tissues.[203,358,381] More recent work has emphasized the importance of Toll-like receptors [TLRs] during RSV infection in mediating and defining the inflammatory cytokine and chemokine production. TLRs may effect the polarization of the cytokine responses toward a T_H1 or T_H2 profile and potentially lead to the sensitization and subsequent hyperreactivity of the airway epithelium.

The interaction of RSV with TLRs (TLR4, TLR3, TLR7, TLR9) on mononuclear cells and alveolar macrophages results in production of antiviral and immunomodulatory mediators, including type I and III interferons, IFN-α, IFN-β, and IFN-γ, chemokines CXCL8 (interleukin-8 [IL-8]) and CCL5 (RANTES), and cytokines IL-6 and tumor necrosis factor-α (TNF-α).[140,142,174,231,262,314,351,354,360] TLR-mediated immune responses have been shown to be TLR-specific. Thus, the function and effectiveness of the components of the elicited immune response will vary according to the interaction of RSV with specific TLRs. For example, TLR4-deficient mice have impaired viral clearance, whereas TLR3 deficient mice develop T_H2-polarized responses but continue to eliminate virus with normal kinetics.[174,352]

RSV infection of respiratory epithelial cells in vitro stimulates synthesis of CC chemokines (CCL5, CCL3 [macrophage inflammatory protein 1α; MIP-1α], CCL4 [MIP-1β] and CCL2 [monocyte chemoattractant protein; MCP]), CXC chemokines (CXCL1, CXCL2, and CXCL3 [GRO-α, -β, -γ]), as well as the CX3C chemokine fractalkine.[280,423] CXCL8, CCL3, CCL5, and CCL11 (eotaxin) are chemoattractants for polymorphonuclear cells and eosinophils, whereas fractalkine induces pulmonary migration of T cells bearing the CX3CR1 receptor.[77,173,200,201,264] These cells further contribute to the local inflammatory response by secreting additional cytokines. Many of these immunomodulators are detectable in secretions of RSV-infected infants, and increased levels of CCL3, CCL5, and CXCL8 have been correlated with more severe disease, with the presentation of bronchiolitis, or with wheezing.[114,190,241,272,301,366,408]

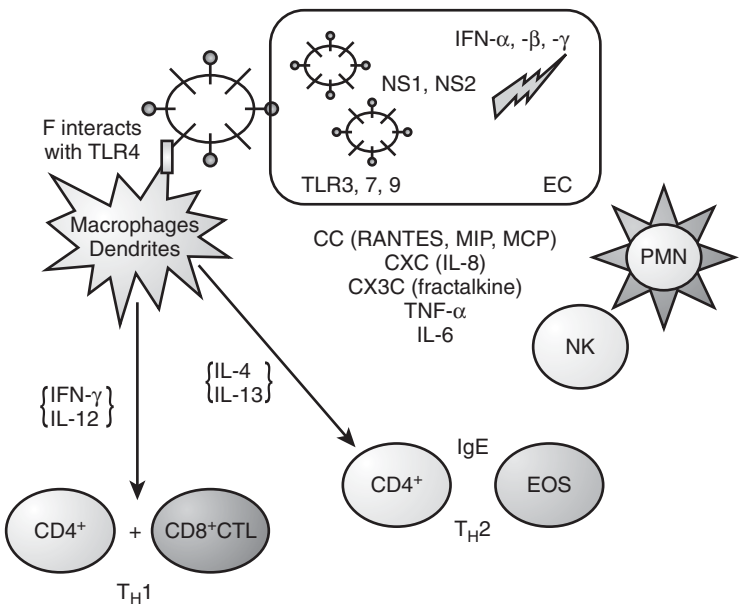

Figure 194–4 Schematic of the early innate and adaptive immune responses to respiratory syncytial virus (RSV). RSV's envelope glycoprotein G attaches to the respiratory tract epithelial cells (ECs) via glucosamine glycans expressed on the cell surface, and F interacts with antigen presenting cells (macrophages, dendritic cells) via Toll-like receptor 4 (TLR4) protein. This triggers the production and release of antiviral interferons (IFN-α, IFN-β, IFN-γ) and cascade of proinflammatory cytokines and chemokines. Two early nonstructural RSV gene products (NS1, NS2) antagonize IFN production. The chemokines recruit polymorphonuclear neutrophils (PMN), natural killer (NK) cells, $CD4^+$ and $CD8^+$ T cells. A T_H1-type cellular response becomes dominant under the influence of IFNγ and IL-12, whereas under the influence of IL-4 and IL-13 the cellular response is skewed toward T_H2 with the production of IgE and eosinophils (EOS).

Like other viruses, RSV has evolved mechanisms to avoid the antiviral effects of innate IFNs. Transcription of IFN-α mRNA is suppressed in macrophages exposed to RSV in vitro, and IFN-α is absent in nasal secretions of infected infants.[158,159,247,267,373] Studies indicate that NS1 and NS2, two early RSV gene products, effectively block induction and function of IFN-α and IFN-β in vitro, an effect that also may favor T_H2 polarization of the T-cell response.[204]

B-CELL RESPONSES

The humoral and mucosal immune responses to RSV infection in infants have been well characterized.[289,290,392,401,410] After acquiring infection, infants develop antibodies to many of the 11 RSV-specified proteins, the most important of which are antibodies to F and G proteins as they have viral-neutralizing activity. Serum-neutralizing and F and G protein-specific antibodies are observed in the majority of infected infants, regardless of the severity of their illness.[289,410] However, neutralizing and F antibody responses are blunted in those younger than 6 months of age, and diminished responses to the G protein are associated with high levels of preexisting maternal antibody.[289,383] No evidence exists that preexisting antibody suppresses RSV-specific, cell-mediated responses.[75] The antibody response to the F protein is broadly cross-reactive with both A and B strains because neutralizing epitopes are conserved between the two groups. This situation is in marked distinction to the group- and genotype-specific response to the highly variable G protein.[43,184,285] Overall, the serum IgG response to RSV is relatively weak. Titers often dissipate within a year after infection, thus requiring several additional infections before levels approximate those found in adults.[392] Nonetheless, levels of serum antibody are directly correlated to the degree of protection against natural reinfection in infants and adults, a finding confirmed in experimental challenge studies in adults.[166,239,270,394] Nonetheless, solid protection from reinfection is of brief duration, as a substantial proportion of adults can be reinfected within several months of a naturally occurring infection.[166]

During primary RSV infection, RSV-specific IgA antibody is detectable in nasal secretions and coincides with the cessation of viral shedding.[166,270,386] Using an adult challenge model, some investigators correlated high levels of RSV-specific IgA to protection.[166] In animal models, the durability of mucosal IgA antibody protection was relatively brief when compared with the lower tract protection afforded by serum IgG antibody.[331,372,412] Some investigators noted, in addition to IgA antibody, the appearance of RSV-specific IgE in nasal secretions during RSV infection, a finding correlated to an increased risk of the development of wheezing both during acute disease and later in childhood.[407,409,411] Elevated levels of cysteinyl leukotrienes in nasal secretions also have been associated with wheezing.[388]

T-CELL RESPONSES

The basis for much of our knowledge about cell-mediated immunity to RSV is derived principally from studies in the Balb/c mouse model and limited human data. A unifying theme has emerged in which generation of T_H1-dominant responses characterized by induction of IFN-γ–secreting $CD4^+$ and $CD8^+$ cytotoxic T lymphocytes (CTLs), neutralizing serum antibody and mucosal IgA antibody results in viral clearance with limited disease severity.[276,306,322] Conversely, a T_H2-polarized response, described by IL-4–secreting $CD4^+$ cells, eosinophils, and IgE production, is associated with airway hyperreactivity and more severe disease.

RSV long has been recognized to have an inhibitory influence on lymphocyte proliferative responses in vitro.[328,348,355] In addition, RSV-infected dendritic cells have diminished capacity to activate $CD4^+$ T cells.[60,78,141] Despite these effects, RSV infection induces a full range of T-cell responses, including T_H1 $CD4^+$ and $CD8^+$ CTLs as well as T_H2 $CD4^+$ cells.[61,194,198,266] T_H1 $CD4^+$ cells are characterized by secretion of IL-2, IFN-γ, and TNF-α, whereas T_H2 $CD4^+$ cells produce IL-4, IL-5, and IL-13.[306] Human and murine $CD8^+$ CTLs recognize F, M2, M, and NS2 epitopes in the context of class I antigens.[35,55,59,176,206,229,297,353] Each cell type and its associated cytokines have been correlated to the degree of severity of illness, the presence of wheezing, and the nature of the inflammatory response.[11,12,44,132,133,253] Both $CD4^+$ and $CD8^+$ T cells can clear virus from the lungs of infected animals, with CTLs most efficient.[12,44] IFN-γ $CD4^+$ cells (T_H1 pattern) eradicate the virus while inducing minimal disease, whereas IL-4–secreting $CD4^+$ cells (T_H2 pattern) are associated with pulmonary eosinophil infiltration and airway hyperreactivity.[11,132] Although RSV-specific $CD8^+$ CTLs clear virus rapidly, excessive numbers of CTLs can be detrimental.[12]

The balance between T_H1 and T_H2 induction depends on multiple factors, including host genetics, the local cytokine environment, and the antigen.[13,41,63,118,180,192,379,419] Replicating antigens, such as live RSV, generally induce a T_H1 response with CTLs, whereas inactivated virus (i.e., the formalin-inactivated vaccine used in the 1960s) or subunit vaccines induce T_H2-dominant responses.[133,205,374,399] The F antigen is associated with a T_H1 response, whereas the G protein tends to evoke a T_H2-type response. The mechanism by which the RSV G protein induces a T_H2 polarized T-cell response is not clear, but a highly conserved central cysteine-rich region with structural analogy to the CX3C chemokine fractalkine may be involved. The CX3C motif on the G protein competes with fractalkine binding on T cells and appears to inhibit pulmonary trafficking by CX3CR-positive CTLs.[173] Additional evidence from studies in both mice and humans suggests that RSV is a poor inducer of central memory T-cell responses that are protective, a finding that may account in part for the failure of long-term immunity.[54,346]

Peripheral blood mononuclear cells from infants with primary RSV infection secrete both IFN-γ (T_H1) and IL-4 (T_H2).[21,384] The presence of a T_H2-dominant cytokine pattern in respiratory secretions during RSV infection, or a T_H2 response induced by in vitro stimulation of lymphocytes, has been linked to disease that is more severe or associated with wheezing.[2,30,241,359] Not all studies, however, measured RSV-specific responses, and not all investigators noted this relationship between T_H2 polarization and more severe illness.[21] The patient's age at the time of infection also may influence the T-cell response. Infants younger than 3 months of age were found to have higher levels of T_H2 cytokines in nasal secretions compared with older infants, who were more likely to have a T_H1 cytokine pattern.[228] Information regarding cellular immune responses and protection from subsequent infection is sparse. In one report, RSV-specific lymphoproliferative responses were relatively short-lived after primary infection and did not boost after reinfection.[31]

In summary, the complexity of the immune response to RSV is yet to be unraveled fully. Certain basic tenets, however, appear to be supported by available animal and human data. Both humoral and mucosal antibodies provide a modest degree of protection from infection, and virus provoked innate and adaptive T-cell responses play a defining role in disease pathogenesis and in viral clearance. An environment conducive to induction of T_H1 responses, either during primary infection or with infection following prior immunization, may minimize pathologic changes, airway hyperreactivity, and disease severity.

CLINICAL MANIFESTATIONS

We view their chests ballooning, with the fears
that they're "pink puffers" of more tender years.[144]

PRIMARY INFECTION

An infant's first encounter with RSV almost always is apparent, but the symptoms may range from those of a mild cold to severe bronchiolitis or pneumonia. Rarely is a child's first encounter with RSV entirely asymptomatic. Among infants examined over the course of a 10-year period with known primary RSV infection, who initially were younger than 6 months of age, 45 percent had the clinical manifestations of bronchiolitis or pneumonia, compared with 5 percent of children aged 2 to 5 years who had experienced two or more outbreaks of RSV infection (Fig. 194–5). Higher rates of lower respiratory tract disease have been documented to occur in children with underlying cardiopulmonary disease, those in closed populations such as daycare centers, and those from certain geographic areas or with particular ethnic backgrounds.[182,252,273,342,405]

In the longitudinal Houston family studies, 69 percent of children in their first year of life acquired RSV infection, and one third of these infections were lower respiratory tract illnesses[127] (Table 194–3). By the time they were 24 months of age, essentially all children had been infected at least once, and about half had experienced two infections, such that the rate of infections during the second year of life was 83 percent. The proportion of RSV infections involving the lower respiratory tract remained appreciable during the second, third, and fourth years of life, but the severity decreased. In general, lower respiratory tract illnesses associated with RSV after the second year of life most frequently are tracheobronchitis and reactive airway disease (see Fig. 194–4).[127]

Pneumonia and bronchiolitis, the most common forms of RSV lower respiratory tract disease in infants, frequently coexist and may be difficult to differentiate clinically or radiographically. Children with hyperinflation and wheezing, the hallmarks of bronchiolitis, may have densities on a chest film that may appear similar to infiltrates from pneumonia (Fig. 194–6). However, in bronchiolitis, these shadows most likely result from atelectasis rather than from the interstitial inflammation and alveolar filling of pneumonia.

Upper respiratory tract symptoms usually precede lower respiratory tract involvement by several days. Low-grade fever is

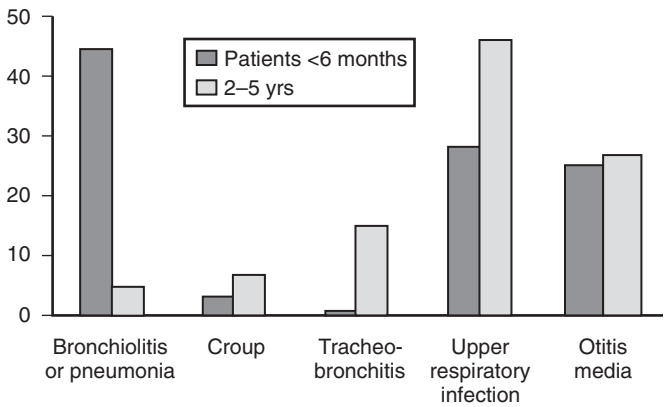

Figure 194–5 The clinical diagnoses of children with respiratory syncytial virus (RSV) infection according to age. The proportion of children presenting with each respiratory syndrome who were younger than 6 months of age and had primary RSV infection is shown in comparison with those children aged 2 to 5 years who had a repeated RSV infection. Data were obtained from 415 outpatient infants and children evaluated in Rochester, New York. *(From Hall, C. B., unpublished data.)*

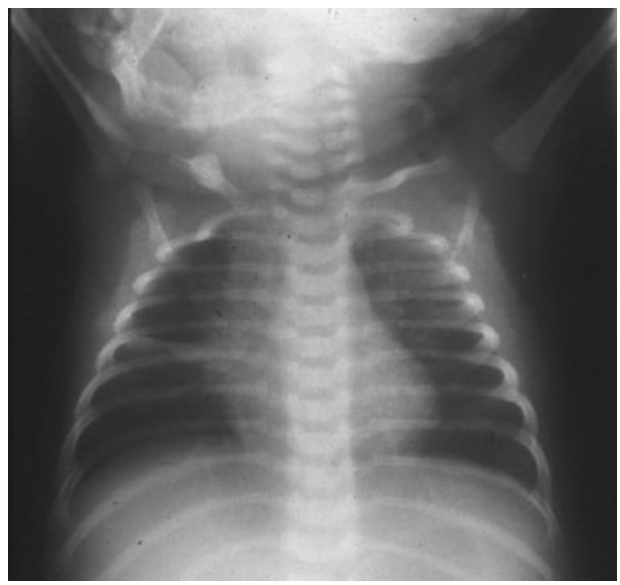

Figure 194–6 Bronchiolitis from respiratory syncytial virus (RSV) infection. This chest roentgenogram of a 3-month-old infant with bronchiolitis from RSV infection shows hyperaeration of the lung and area of atelectasis in the right middle lobe.

TABLE 194–3 Frequency of Respiratory Syncytial Virus Infection among Children Studied from Birth*

Age (mo)	No. of Child-Years	No. with Respiratory Syncytial Virus				
		Primary	Reinfection	Total (Rate/100 Child-Years)	LRD (Rate/100 Child-Years)	LRD (Rate/100 Infections
0-12	125	85	1	86 (68.8)	28 (22.4)	32.6
13-24	92	33	43	76 (82.6)	12 (13.0)	15.8
25-36	65	1	29	30 (46.2)	7 (10.8)	23.3
37-48	39	0	13	13 (33.3)	3 (7.7)	23.1
49-60	24	0	12	12 (50.0)	0 (0)	—
Total	345	119	98	217 (62.9)	50 (14.5)	23.0

**Houston Family Study, 1975 through 1980.*
LRD, lower respiratory tract disease; RSV, respiratory syncytial virus.
Reprinted with permission from Glezen, W. P., Taber, L. H., Frank, A. L., et al.: Risk of primary infection and reinfection with respiratory syncytial virus. Am. J. Dis. Child. 140:543-546, 1986. Copyright 1986, American Medical Association.

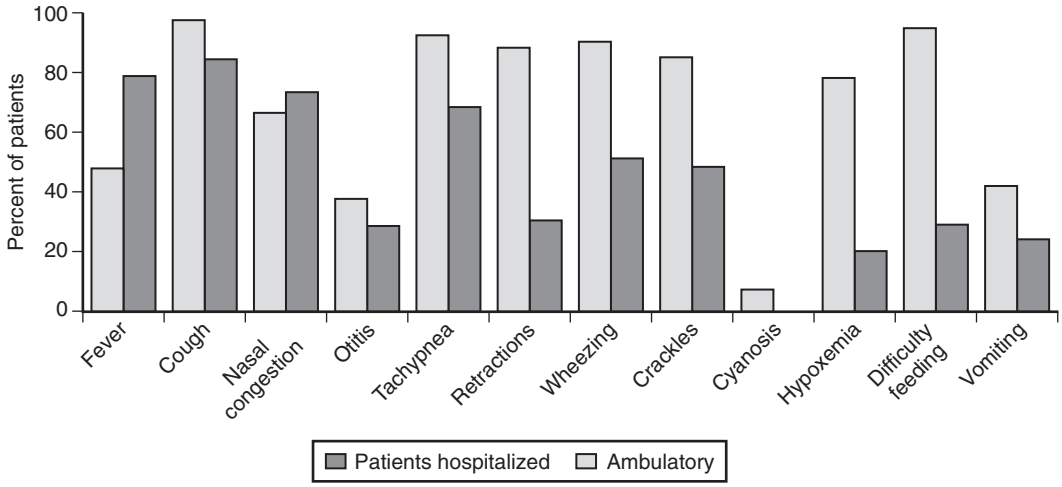

Figure 194–7 Clinical manifestations in children with respiratory syncytial viral infection who were evaluated in Rochester, New York. Shown are the proportions of 1132 hospitalized children in comparison with the proportions of 489 outpatient children with each clinical sign.

a common manifestation during this initial phase of the illness, but it may have disappeared by the time of hospitalization (Fig. 194–7). Among 565 children hospitalized with RSV infection, fewer than half had a temperature higher than 38° C at the time of admission.[165] Although fever occurs more commonly in primary infections, 20 to 40 percent of children with their second, third, or fourth infections are febrile.[182]

Increased coughing commonly heralds involvement of the lower respiratory tract, which subsequently may be indicated by an increased respiratory rate, dyspnea, and retractions of the chest wall (see Fig. 194–7). Variability of the child's clinical findings within minutes to hours is characteristic of RSV lower respiratory tract disease. Auscultatory findings of crackles may be present on initial examination but absent on reexamination shortly thereafter. Ill children who elicit concern because they are lethargic and short of breath may have minimal auscultatory signs. The correlation between the findings on auscultation, or on the chest radiograph, with severity of disease or outcome is not consistent.

RADIOGRAPHIC FINDINGS

The radiographic findings in infants hospitalized with RSV lower respiratory tract disease may be variable, but in bronchiolitis, hyperinflation with hyperluceny of the lung, flattened diaphragms, and peribronchiolar thickening usually are evident.[108,309,344,416] Patchy areas of atelectasis also may be present, most often in the right middle and upper lobes. A little less frequent are interstitial infiltrates of pneumonitis, which tend to be present in more than one lobe (see Fig. 194–6). The radiographic findings usually persist beyond the clinical illness.

With tracheobronchitis and upper respiratory tract infections, the accompanying signs and symptoms tend to be more severe in RSV infection than in infections with other common respiratory agents. Cough often is predominant and prolonged, and fever may be present. Otitis media frequently is associated with the initial RSV infection in young children, but it also occurs in older children with recurrent infection.[19,177,326] Among children with RSV infection, the virus has been detected in 30 to 74 percent of middle ear aspirates. Among children presenting with otitis media, RSV is the viral pathogen most frequently identified and has been associated with prolonged symptoms and apparent failure of therapy.[23] RSV may be detected as the sole pathogen or simultaneously with a bacterial agent, thus suggesting that RSV may play both a primary and a secondary role in the pathogenesis of otitis media.

RSV infection has been associated occasionally with a variety of neurologic, cardiac, and other conditions.[25,68,121,215,296] However, in most of these reports, the causal role of RSV is tenuous.

INFECTION IN NEONATES

Infection with RSV in the first 2 to 4 weeks of life appears to occur relatively infrequently.[318] In Washington, D.C., the incidence of RSV infection during the first month of life was noted to be only one third of that during the second month of life. The protected environment of newborns with diminished exposure to others, as well as the high levels of maternal antibody or other early immune factors, may explain in part the generally lower incidence of illness in the newborn period. RSV infection in neonates may be variable and atypical; nonspecific findings and minimal respiratory signs may result in an unsuspected or delayed diagnosis.[163,275,349]

In full-term neonates, RSV infection may be primarily an upper respiratory tract illness and usually mild.[83,113] However, in premature infants, the morbidity may be pronounced.[143,163] During a community outbreak of RSV in Rochester, New York, 25 percent of infants kept in a neonatal intensive care unit for more than 6 days acquired RSV infection.[163] Infants older than 3 weeks tended to have lower respiratory tract disease characteristic of RSV and apnea, whereas infants in the first 3 weeks of life were more likely to have nonspecific findings, without clinical evidence of lower respiratory tract disease. Fewer than half had upper respiratory tract signs; nonetheless, four (17%) of the infants died.

ACUTE COMPLICATIONS

Apnea, a major complication of acute RSV infection in young infants, occurs in approximately 10 to 20 percent of infants hospitalized with RSV infection.[17,40,65,162,225] Apnea is most likely to occur in infants born prematurely, in those with a young postnatal age, especially infants who have not yet reached a postconceptional age of 44 weeks, and in infants with a history of apnea of prematurity.[65] The apnea usually develops at the beginning of the RSV infection, often as the first manifestation of illness, and is short-lived and nonobstructive.[17,65] Most infants do not have apnea subsequently, even with later respiratory infections.

Simultaneous or secondary bacterial infection appears to be a relatively frequent complication of RSV infection in some countries in the developing world.[249,268] In the United States and other developed countries, it is an unusual complication, and most clinical and pathologic studies indicate that RSV infection rarely predisposes one to acquisition of a bacterial infection.[137,165,230,277,336,337,382]

In a 9-year prospective study of 565 children hospitalized with RSV lower respiratory tract disease, the rate of subsequent bacterial infection was 1.2 percent in the total group of children infected with RSV and 0.6 percent in the 352 children who

received no antibiotics.[165] If a bacterial infection is present simultaneously, it most frequently involves the urinary tract and is detected by a positive urine culture.[244,277,336] Whether RSV infection has any direct relationship with concurrent urinary tract infection is unclear. Other epidemiologic factors may explain the coexistence of these infections; for example, both RSV and urinary tract infection frequently are recognized in this age group, and both are more common in boys. Serious concurrent bacterial infections are unusual enough that evaluations for sepsis and meningitis are not recommended routinely.[16,337] Controlled studies with randomized antibiotic treatment of lower respiratory tract disease caused mostly by RSV have shown no difference in the severity or duration of illness or in the child's outcome.[103,107]

More frequently, co-infection with other respiratory viruses occurs.[106,110,123,242,279,300,313] Studies examining whether coexistent viral infections in normal children augment the severity of infection showed mixed results. In part, this finding may result from the studies' differing methods of assessment and the difficulty of determining the onset of infection, especially with viruses that have prolonged shedding. In addition, other viruses that infect predominantly this same young age circulate during the same months as does RSV.

Infants hospitalized with RSV infection also have been noted to be at increased risk for aspiration and subsequently for developing hyperreactive airways.[87,217-219] One study reported that during a follow-up period of 1 year, 83 percent of the infants hospitalized with RSV bronchiolitis who received no therapy for aspiration or ribavirin developed reactive airway disease, compared with 45 percent of those patients given thickened feedings and early ribavirin therapy.[87,218] Furthermore, the reduction in the episodes of reactive airway disease was greater with the combined therapy than with either ribavirin or thickened feedings alone.

PROGNOSIS

> Thus, if infected at this tender stage
> will they become "blue bloaters" as they age?[144]

PREDICTION AT TIME OF INITIAL EVALUATION

Determining whether an infant with RSV infection who initially presents as an outpatient is likely to require subsequent hospitalization has been problematic. Numerous clinical findings have been evaluated, but most have relatively poor predictive value for individual patients.[16] Among the more consistent and helpful findings at the time of evaluation are oxygen saturation and global assessment of the degree of illness. Fever does not correlate with the severity of disease.[39,271,363] Some studies have associated an increased respiratory rate with the risk of developing more severe illness, whereas others have not.[16,347] However, the lack of tachypnea (≥70/minute) has been correlated with less chance of development of lower respiratory tract infection in infants.[256,259] Although repetitive assessments may help, the rapid fluctuations in clinical manifestations, characteristic of RSV lower respiratory disease, render arriving at an accurate evaluation difficult.

Clinical scoring systems commonly are used, but their use is limited by variable design, rendering comparison and standardization impossible.[16] The Respiratory Distress Assessment Instrument has been standardized, but it is not validated in predicting the severity or outcome of children with bronchiolitis.[6,251]

Infants hospitalized with RSV bronchiolitis or pneumonia usually show clinical improvement after 3 to 4 days, and most previously normal infants are discharged within 3 to 7 days, with an average of 3 to 4 days.[323,337,338] However, children with underlying conditions that put them at risk for development of more complicated illness tend to require 2 to 7 days more of hospitalization.[338]

PATIENTS AT RISK FOR COMPLICATED RSV INFECTION

Infants most likely to develop severe or fatal RSV infections are those who were premature or have an underlying condition that compromises pulmonary function.[34,45,66,94,104,164,168,188,304,342,397] The risk of requiring rehospitalization for infants correlates inversely with birth weight and gestational age. The risk of needing hospitalization with RSV has been reported as 3.7 times greater for infants of less than 36 weeks' gestation than for those of 36 weeks' gestation or longer.[15,255,273,376] In an Australian study, the highest risk of developing an RSV infection severe enough to require hospitalization occurred among infants born weighing less than 2500 g and who had older siblings.[342]

The highest risk of development of severe and fatal RSV infection occurs in premature infants of very low birth weight, 1500 g or less, and of gestational ages of 28 weeks or less.[189] Their high rate of severe RSV infection related to their compromised cardiopulmonary status is augmented further by the lack of maternal neutralizing antibody to RSV in infants of very low birth weight.[80]

Infants with hemodynamically compromising congenital heart disease, such as conditions associated with pulmonary hypertension, have been shown to have a markedly elevated risk of dying.[254] The mortality rate of children with functionally important cardiac conditions has declined from greater than 30 to approximately 3 percent.[10,254,281,294] The improved outcome of infants with cardiac disease likely is attributable to advances in techniques for surgical correction and medical management, as well as recognition of the risk associated with RSV infection in these patients.

Innate host factors that predispose a child to development of more severe RSV infection have long been assumed but remain mostly undefined. More recent studies, however, show a correlation of genetic polymorphisms with primary RSV disease and severity. Variations within a number of genetic loci potentially associated with controlling immune function, such as the expression of IL-4 and other T-lymphocyte cytokines and chemokines associated with the production of IgE antibody and hyperactive airways, have been shown to be overrepresented in children with RSV lower respiratory tract disease and with disease that is more severe (see "Immune Response: Immunity and Disease Pathogenesis").[64,118,187,192,193,233,248,306,379]

IMMUNOCOMPROMISED PATIENTS

Recognition of the risk of complicated RSV infection in immunocompromised patients has been heightened with the concurrently increasing numbers of highly immunosuppressed patients, such as recipients of bone marrow and solid organ transplants. A close correlation generally exists between the degree of immunosuppression and the clinical severity of the RSV infection.[51,73,164,246,261,299,414] This correlation is illustrated well by the severe and potentially fatal outcome of RSV infection among children with congenital immunodeficiency syndromes who have both defective B-cell and T-cell immunity. Limited information exists about the severity of disease in children with isolated humoral or partial humoral and cellular deficiencies.

RSV infection has been reported as occurring in approximately 50 percent of bone marrow transplant recipients and often is acquired nosocomially.[1,278,339] The morbidity and clinical manifestations associated with RSV in these patients are highly variable.[1,29,79,339] Risk factors for development of more severe disease in transplant recipients include not only the degree of immunosuppression, but also allogenic transplantation, acquisition of the

RSV infection within 2 months of undergoing transplantation but before engraftment, and the presence of graft versus host disease. The associated mortality rate among transplant recipients with lower respiratory tract disease has been reported as 70 to 100 percent, but more recently it has declined to closer to 50 to 60 percent. Upper respiratory tract symptoms, sometimes with fever, may be the sole signs of the infection, or they may herald the progression to lower respiratory tract involvement.[29] Findings on the chest radiograph include focal interstitial infiltrates, sometimes associated with lobar consolidation or with hyperinflation, or more severe involvement of generalized alveolar and interstitial infiltration, and marked hypoxemia. The major findings with high-resolution computed tomography (CT) are small centrilobular nodules, airspace consolidation, ground-glass opacities, and bronchial wall thickening. These abnormalities frequently are present in both the periphery and central areas of the lung, are bilateral, and they are asymmetric in distribution.[117]

In this setting, RSV infection often is not recognized or considered because it may occur at times when RSV is not circulating in the community, and the clinical picture may mimic that of other opportunistic agents that commonly infect immunocompromised patients. Furthermore, establishing the diagnosis is problematic because the most frequently available assays, rapid antigen assays applied to swabs of upper respiratory tract secretions, often are inadequate and are relatively insensitive in these patients. A higher yield is obtained with bronchopulmonary lavage specimens.[92,414]

PATIENTS WITH HUMAN IMMUNODEFICIENCY VIRUS INFECTION

The effects of co-infection of human immunodeficiency virus (HIV) infection with RSV infection are unclear. Some, but not all, studies suggest that increased morbidity occurs with RSV infection among HIV-infected patients. In a cohort of South African children followed for 5 years, the incidence of hospitalization from RSV lower respiratory tract disease in children with HIV infection was more than two times greater than that in those not infected with HIV (45 versus 19.4 per 1000 children).[255]

A retrospective Spanish study examined viral respiratory infections in children with HIV infection compared with children with cancer found that RSV was the most common (43%) of the viral agents identified and that 38 percent of the viral infections were acquired nosocomially.[278] The RSV infection involved the lower respiratory tract disease in half of the children with cancer and also in half of those with HIV infection, and hospitalization was required for 12 and 20 percent, respectively. However, the RSV infection was fatal for 21 percent of the hospitalized children with cancer, whereas all the HIV-infected patients survived.

Other studies suggested that adverse outcomes in HIV-infected patients are unlikely to occur or are limited to greater or more prolonged viral shedding.[53,70,223,255] The reported variability in the clinical course in HIV-infected patients who also are infected with RSV likely results from the different ages, degree of immune compromise, location, and therapy of the HIV-infected populations studied. Nonetheless, the risk of developing severe disease from RSV for most HIV-infected patients appears to be less than that observed among immunosuppressed transplant recipients.

PULMONARY SEQUELAE

Clearly, many infants with RSV bronchiolitis are destined to develop recurrent wheezing during childhood.[183,213,260,307,380] Much interest and investigation have been devoted to exploring whether a link exists between RSV lower respiratory tract disease in young children and the later development of asthma or pulmonary structural and functional changes, but a definitive answer remains unclear. Both epidemiologic and immunologic evidence supported a connection between subsequent wheezing and the occurrence of bronchiolitis in young children. The great preponderance of these studies examined children who were hospitalized and, therefore, had more severe bronchiolitis. Among this population, follow-up studies, accumulated over the course of decades, indicated that 30 to 50 percent of children with previous RSV bronchiolitis subsequently will have wheezing.[183,213,260,369,375]

The wheezing episodes tend to be most frequent in the first several years after the bronchiolitis and then diminish or disappear over a period of time that has been reported as ranging from 3 years to adolescence.[195,260,321,369,370,380] Even with this clinical quiescence, these children may be at increased risk of developing asthma as adults. Atopy does not appear to play a major role in the development of wheezing subsequent to RSV infection. However, whether RSV has a direct causal role in later occurrence of hyperreactive airways is controversial. In the Tucson study of respiratory illness in children, RSV lower respiratory tract disease was found to be an independent risk factor for recurrent wheezing.[380] Similar subsequent episodes of wheezing, however, also were shown to develop after lower respiratory tract disease from other common respiratory viruses prevalent in childhood.[186,195,243]

Additional epidemiologic evidence suggested as supporting a link between RSV and asthma is the observation that in the United States and in many other countries, the incidence of children with asthma and of hospitalization for asthma has increased markedly recently.[8,88] In the United States, the prevalence of asthma among children rose from 3.6 percent in 1980 to 5.8 percent in 2003, and greater increases have been noted in other countries such as Australia and Scotland. Certain hypotheses have been suggested to explain this phenomenon. Among these is the "hygiene hypothesis," which proposes that recent technology and knowledge have resulted in an environment that is increasingly clean and devoid of the antigenic exposure early in life necessary to stimulate the development of a normal immune response. Support for this theory comes from clinical studies showing that children with early exposure to multiple infections and environmental allergens have less risk of developing asthma or subsequent wheezing.[26,88,210,311,343,380,391]

The similarities of the immune response associated with RSV infection and asthma have been hypothesized as the mechanism for their epidemiologic and phenotypic link.[95,260,304,305,307,406] The immunologic response associated with RSV infection has been characterized variably as being a mixed T_H1 and T_H2 cytokine phenotype (see "Immune Response: Immunity and Disease Pathogenesis").[86,227,385] A predominantly T_H2 response has been correlated with lower respiratory tract disease and more severe RSV disease. This finding suggests that RSV infection tips the immune response toward a T_H2 pattern that results in the release of the regulatory proinflammatory cytokines and chemokines by the epithelium of the airways with subsequent increased production of IgE in respiratory secretions, and of neuropeptides, and of other mediators implicated in the pathogenesis of wheezing and asthma.[305,385,406]

Alternative hypotheses have proposed that RSV infection in infancy alters the subsequent normal growth and development of the lung or that the virus acts as a trigger to a hyperreactive response of the airways among inherently predisposed individuals.[119,260] The development of wheezing most likely results from the multiple genetic and environmental factors, which vary among individuals, thus rendering the contribution of single factors, such as RSV infection, impossible to assess. Only intervention studies with an effective vaccine or other preventive and therapeutic measures are likely to determine whether RSV has a

direct, indirect, or no link to the development of recurrent wheezing and asthma among these young wheezers.

REPEATED INFECTION

The most striking proof of RSV's incomplete, inconsistent, and impermanent immune response is the burden of illness levied by the repeated infections it causes. RSV has no respect for age. Recurrent infections occur across the age span from toddlers to elderly persons, whether healthy or infirm. Reinfection occurs most frequently among those in close contact with young or school-age children, as in daycare facilities and primary schools, or among those in confined settings, including military recruits.[127,146,148,149,160,182,250,310] Most of these infections are upper respiratory tract illnesses, which often are more severe than is the average cold and often are complicated by sinusitis and otitis media. Recurrent infections involving the lower respiratory tract usually are manifest as tracheobronchitis or as exacerbations of wheezing. Even in young, healthy adults without asthma or other chronic pulmonary conditions, acute RSV infection may produce hyperreactivity of the airways that does not resolve for as long as 8 weeks.[169]

Recurrent Infections in Children

Among longitudinally followed children, up to three fourths have been shown to be reinfected each year. The intervals between infections often are no longer than the period between successive outbreaks of RSV infection or even less, especially among children in daycare, in whom the attack rates have been reported for the first, second, and third infections to be as high as 98, 75, and 65 percent, respectively.[182] Of note is the finding that immunity resulting from a single infection appeared to have no ameliorating effect on illness associated with reinfection 1 year later. Not until the third infection was severity reduced appreciably. Among preschool children, 20 to 50 percent of the repeated RSV infections involved the lower respiratory tract, but the illness tended to be mild.[127] Most children had lower respiratory tract illness only once, and those with repetitive lower respiratory tract infections tended to have reactive airway disease.

Infections in Adults

Healthy adults frequently are exposed to RSV in their workplace and as parents or members of families with preschool and elementary school-age children. Despite these repetitive exposures, most of them remain susceptible to RSV infection during the subsequent outbreaks and, if infected, are symptomatic.[85,99,149,161,310] Recent infection, however, may result in diminished clinical findings and virus shedding, as demonstrated in a study of 15 young healthy adults who became infected with a RSV A strain during a community outbreak and developed upper respiratory tract illnesses (Fig. 194–8).[166] These individuals subsequently were inoculated intranasally with RSV A strain at repetitive intervals over the course of 2 to 26 months. The number reinfected and the proportion manifesting any symptoms continually declined during the 26 months. Concurrently, the quantity and duration of virus shedding also diminished.

Among 211 working adults followed over the course of multiple seasons, RSV re-infection was symptomatic in 84 percent and manifested as an upper respiratory tract illness in 74 percent, two thirds of whom had accompanying fever.[149] The other fourth of the reinfections involved the lower respiratory tract, with the diagnosis of tracheobronchitis or wheezing. Some individuals became infected with RSV and, at a separate time, with both RSV and influenza virus. Comparison of the two illnesses occurring in the same individual revealed that influenza and RSV were clinically similar (Fig. 194–9). Influenza was accompanied more frequently by fever and absence from work, whereas RSV infection was associated significantly more often with sinusitis, otitis, and a longer duration of illness.

The clinical similarity of illness from RSV and from influenza among young adults and the difficulty of differentiating the two also were shown in university students and in military recruits.[250,310] In the close military environment of basic training, new military recruits frequently acquired acute respiratory tract infections, especially from RSV.[310] RSV infections in these recruits were very similar to those from influenza, except wheezing was associated more frequently with RSV infection. The proportion of patients requiring confinement to a ward was the same for those with RSV infection as for those with influenza.

The importance of RSV infection as a cause of morbidity and mortality in older adults, both those previously healthy and those with underlying conditions, has been recognized increasingly.[85,97,98,100,394,417] Between 2 and 9 percent of all lower respiratory tract hospitalizations in elderly persons have been estimated to be caused by RSV, with a cost of $150 million to $680 million annually.[18,172] Not included in these estimates are the additional hospitalizations and costs associated with exacerbations of underlying chronic conditions.

The impact of RSV infection in elderly individuals was well illustrated in a prospective study of 608 healthy community-dwelling elderly patients and 504 adults with high-risk condi-

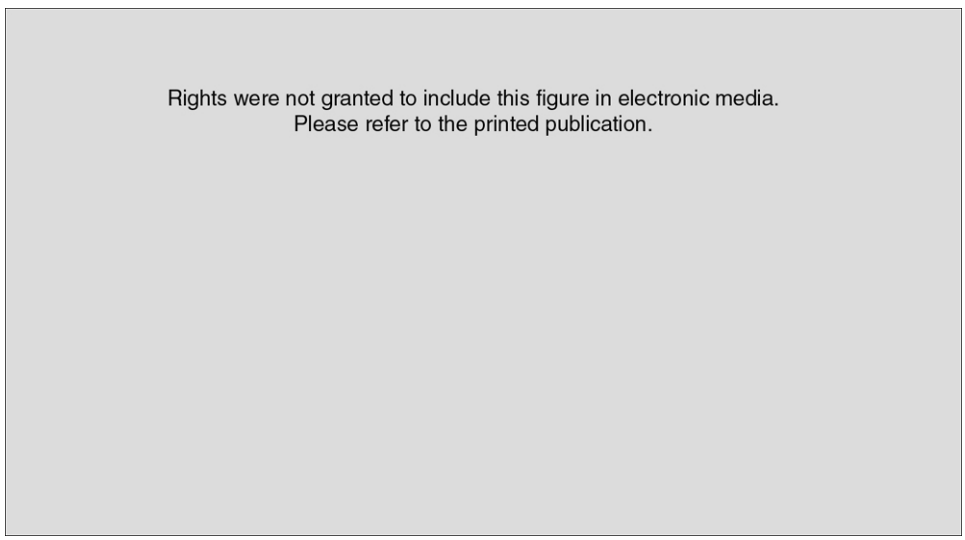

Figure 194–8 Re-infection with respiratory syncytial virus (RSV). Proportion of young adults with natural RSV A infection who become reinfected, shed virus, and were symptomatic when they were subsequently rechallenged intranasally with RSV A after intervals of 2, 4, 8, 12, 20, and 26 months from time of their natural infection. *(From Hall, C. B., Walsh, E. E., Long, C. E., and Schnabel, K. C.: Immunity to and frequency of reinfection with respiratory syncytial virus. J. Infect. Dis.* 163*:693-698, 1991.)*

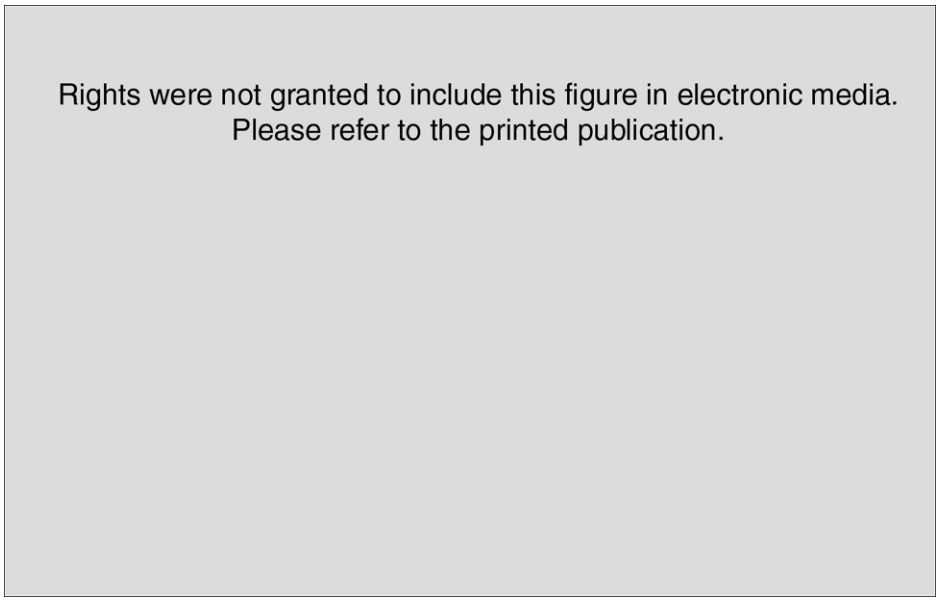

Figure 194–9 Respiratory syncytial virus (RSV) infection and influenza. Comparison of clinical findings observed during RSV infection compared with influenza infection among 211 prospectively followed previously healthy adults who acquired both infections, RSV infection at one time and influenza infection at a separate time. *(From Hall, C. B.: Nosocomial respiratory syncytial virus infections: The "cold war" has not ended. Clin. Infect. Dis. 31:590-596, 2000.)*

tions. An additional 1388 individuals who were hospitalized with acute cardiopulmonary conditions also were studied.[100] The mean age of the individuals studied ranged from 70 to 75 years. During the four consecutive winter seasons of viral surveillance, using viral isolation, reverse transcriptase–polymerase chain reaction (RT-PCR), and serology, RSV infection was identified in 102 (10%) of the patients in the two prospectively studied cohorts and in 142 (10%) of the hospitalized patients. Influenza A infection was identified in 44 (4%) of those in the prospectively followed cohorts and in 154 (11%) of the hospitalized patients. The proportion of individuals developing RSV infection each year ranged in the healthy group from 3 to 7 percent and in the high-risk group of adults from 4 to 10 percent. In comparison, influenza A infection was detected annually in 2 to 4 percent of the healthy elderly cohort and in less than 1 to 5 percent of the high-risk group. Noteworthy is that the illnesses from RSV and influenza A could not be distinguished clinically. Among the hospitalized patients, intensive care was required by 15 percent of those with RSV and by 12 percent of those with influenza A infection, and 8 percent of the RSV and 7 percent of the influenza A infections were fatal. Although RSV infection generated fewer office visits than did influenza among the healthy, older age group, those with high-risk conditions used health care services similarly if they were infected with RSV or influenza. The impact on health care services and costs by RSV is underscored by the proportion of hospitalizations caused by RSV for pneumonia (10.6%), chronic obstructive pulmonary disease (11.4%), congestive heart failure (5.4%), and asthma (7.2%). The role of RSV in elderly patients with these diagnoses frequently is unrecognized and not diagnosed.

DIAGNOSIS

Too minuscule
for human eyes,
It leaves its scent
and prints precise,
A hidden hare
for hunt and snare . . .

—CBH

Because RSV has a repetitive and distinctive behavior, the diagnosis often is made on the basis of the age of the child, the clinical syndrome, especially bronchiolitis, and the usual period of peak RSV seasonal activity which, if not monitored locally, is available nationally through the Centers for Disease Control and Prevention Surveillance.[48] An increase in the number of outpatient visits and admissions for bronchiolitis and respiratory illness in children younger than 2 years of age usually is a local indication that RSV is active within the community (see Fig. 194–2).[151,286] Other respiratory pathogens of young children, particularly parainfluenza virus type 3, hMPV, and bocavirus, may produce similar clinical illnesses, such as bronchiolitis. However, these viruses are less common causes of lower respiratory tract disease in infants and are not as closely associated with the peaks of bronchiolitis and hospital admissions for respiratory illness in infants.[168,199,207,216,226,258,269,418] The seasonal occurrence of parainfluenza virus type 3 is much broader with its peak in the spring. hMPV and bocavirus appear to have their primary activity from the fall through spring months, although it is more variable and with a less distinctive peak of activity than observed with RSV.[418] Although the parainfluenza viruses, hMPV, and bocavirus may cause bronchiolitis in children younger than 2 years of age, generally the mean age is a few months older than that for RSV. Nonetheless, for each infant with the findings of bronchiolitis or viral pneumonia who presents during a community outbreak of RSV infection, these major alternative causes should be considered.

A specific diagnosis of RSV infection may be made by isolation of the virus, or by using standard cell culture or shell vials, direct and indirect immunofluorescent assays, enzyme immunoassays (EIAs), and methods using nucleic acid amplification PCR. The specimens employed usually are upper respiratory secretions from a nasopharyngeal swab or, less frequently, nasal aspirate. A nasal wash or tracheal aspirate is more sensitive than is a nasopharyngeal swab specimen for the recovery of RSV.[150,178] Nasal wash specimens may be obtained with a suction apparatus or, more simply in young children, by the use of a tapered rubber bulb (Fig. 194–10).

In the past, tissue culture isolation of the virus was the standard technique used for establishing the diagnosis of RSV. However, culture techniques now are used less frequently. The disadvantages of viral isolation include the expense, the number of days required (an average of 3 to 5 days), the need for considerable technical expertise and time, and the need for reliably sensitive tissue culture.[98,185] Viral isolation techniques, however, do have the advantage of being able to detect co-infecting viral agents.

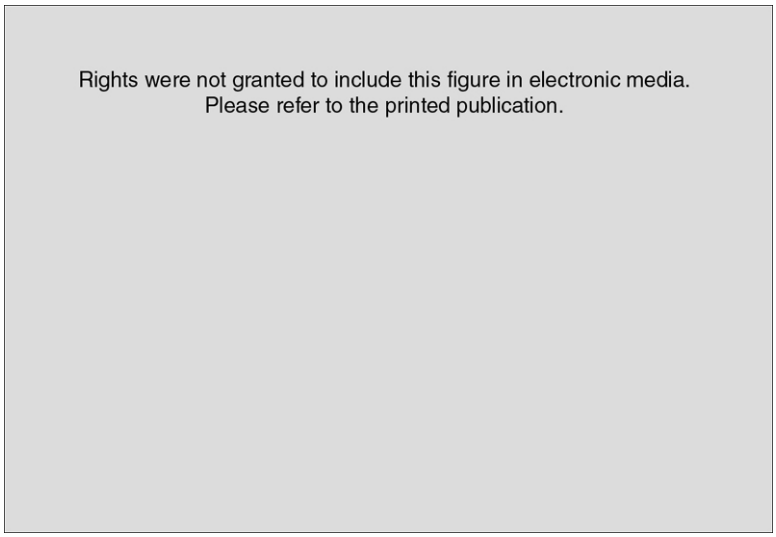

Figure 194–10 Simple method for obtaining nasal wash specimens from young children. A 1-oz tapered rubber bulb is used to inject and collect 5 to 10 mL of saline with one squeeze. *(From Hall, C. B., and Douglas, R. G., Jr.: Clinically useful method for the isolation of respiratory syncytial virus. J. Infect. Dis.* 131*:1-5, 1975.)*

Indirect and direct immunofluorescence assays applied to nasopharyngeal secretions from children have an average sensitivity and specificity of approximately 95 percent when these tests are performed by experienced personnel.[185] However, the ranges of sensitivity and specificity are wide and depend on the laboratory's expertise and on their suitability for certain populations, such as elderly persons, in whom these tests are less sensitive.[98]

Rapid antigen-based assays, primarily EIAs, and optical immunoassays (OIAs), currently are the assays most frequently used to diagnose RSV infection. Their major advantages are their rapidity, low cost, and simplicity. Nonetheless, they are screening assays, and their sensitivity and specificity vary highly, depending on the prevalence of activity of RSV in the community. During times of low prevalence (i.e., non-RSV seasons or at either end of the RSV season when RSV activity is low), their sensitivity, specificity, and positive predictive value plummet. In addition, for certain patients, such as those who are immunocompromised or elderly, these tests tend to be less sensitive.[98,185]

PCR techniques are the most sensitive and specific of current methods. Although they generally have been used primarily in research laboratories, these assays are becoming more widely available. Their sensitivity derives from the amplification of very small amounts of viral RNA in specimens that may be obtained from a variety of sources. Nested PCR assays add sensitivity, allow multiple viruses to be identified simultaneously, and help to differentiate RSV group A versus B strains. RT-PCR assays have the advantages of rapidity, from a diminished number of procedural steps, and of a decreased chance of contamination, but most are limited in the number of viruses that may be detected concurrently. Quantitative RT-PCR has the further advantage of determining the viral load, which has been well correlated with viral quantitation by other methods. PCR techniques have been shown consistently to be the optimal means for detecting viral infections and for establishing diagnoses in populations of children and adults; these tests detect approximately 30 percent or more infections than do viral isolation techniques.[99,168,250,404] However, the timing and duration of viral isolation have been shown to correlate with the timing and duration of clinical illness. This correlation has not been demonstrated for PCR techniques. These molecular techniques are constantly being improved in terms of rapidity, suitability of application to multiple viruses, and availability.[38]

Serologic diagnosis usually is reserved for research purposes because it generally is not helpful in the acute diagnosis and management of patients. Furthermore, serologic assays may be falsely negative in 10 to 30 percent of patients.[100,185] Serologic assays available include complement-fixation assays, neutralization assays, and EIAs, using purified F and G proteins of RSV for IgM and IgG antibodies. The complement-fixation assay tends to be the least sensitive. Serologic diagnosis appears to be more sensitive among elderly than younger subjects.[393] Faster diagnosis by the use of a single high titer has been tried, but it has had variable results and is not well standardized.

TREATMENT

For most children with RSV lower respiratory tract disease, supportive care is sufficient. Ensuring that the child has adequate fluid intake and, when necessary, clearing the nasal passages by gentle bulb suctioning are important in making the infant comfortable.

Supportive care also is integral to the management of the more severely ill infant. Increased production of mucus and secretions in the nasal and lower airways and respiratory rates of more than 60 to 70 per minute are apt to compromise the infant's ability to take fluids and sleep, thus resulting in an increased risk for requiring intravenous hydration and assisted ventilation.[16,67] Deep pharyngeal or tracheal suctioning and methods aimed at loosening secretions, such as chest percussion and vibration, have not been shown to be of benefit in children hospitalized with bronchiolitis.[16,298,402]

The criteria used for determining the need for administration of supplemental oxygen to previously healthy infants are controversial, in part because the usual fluctuating respiratory findings characteristic of RSV lower respiratory tract disease confound the evaluation of the oxyhemoglobin saturation (SpO_2) level at which supplemental oxygen administration provides measurable clinical improvement. SpO_2 levels on room air of less than 95 to 90 percent have been used, but current recommendations suggest that persistent measurements of SpO_2 of less than 90 percent indicate the need for initiating supplemental oxygen.[16]

The use of bronchodilating agents, corticosteroids, and antibiotics for the management of infants with RSV lower respiratory tract illness has been highly variable among centers in the United States and other countries.[27,396] Bronchodilators have been administered to an average 75 to 80 percent of infants hospitalized with RSV infection. Corticosteroid therapy has been used in 10 to 40 percent and intravenous antibiotics in 15 to 40 percent of infants admitted with documented RSV infection.[27]

Trials evaluating alpha-adrenergic or beta-adrenergic bronchodilating agents have given inconsistent results.[6,16,67,214] These trials have differed in the agents and outcomes measured. The patients studied have varied in age, presence of underlying conditions, and history of previous episodes of wheezing, rendering comparisons difficult to make.

A Cochrane systematic review of bronchodilator therapy concluded that a limited transient improvement in assessment scores may be observed in one of every seven children treated, but the clinical benefit was questionable.[214] After a comprehensive review of the clinical trials, the American Academy of Pediatrics Subcommittee on Diagnosis and Management of Bronchiolitis[16] recommended that for management of infants with a first episode of wheezing, bronchodilators should not be used routinely. The guidelines note that a carefully monitored trial of inhaled alpha-adrenergic and beta-adrenergic agents was a possible option in some cases. However, bronchodilator administration should be continued only if benefit was documented by objective means of evaluation. Some evidence suggests for trials conducted in emergency departments and hospitals, epinephrine may be the preferred nebulized agent. In the home and outpatient settings, however, racemic epinephrine would not be advisable because of its potential adverse effects and its short duration of action. Anticholinergic medications alone or in addition to bronchodilatory agents have not been shown to improve the course of viral bronchiolitis.[16,398]

Several reviews analyzing the randomized and controlled trials of administering systemic glucocorticoids in the management of acute viral bronchiolitis concluded that the evidence was not sufficient to support or recommend the use of systemic glucocorticoids on a routine basis.[6,16,115,320] The studies that were included varied in methods and in the type of patients included. Nonetheless, most of them showed no benefit in the clinical scores, the rate of hospitalization following glucocorticoid use during the initial medical visit, the length of stay in those hospitalized, or the rates of readmission or return outpatient visits. One meta-analysis showed a decrease of 0.43 days in hospitalization, but when the population was limited to only first-time wheezers, no benefit existed.[115]

Two placebo-controlled studies that examined the use of oral glucocorticoid therapy administered in the emergency department to preclude the need for hospitalization showed some benefit of glucocorticoid therapy over placebo.[76,361] More recently, however, a large study involving 20 emergency departments during the course of 3 RSV seasons examined the outcomes of 608 children aged 2 to 12 months who presented with a first episode of bronchiolitis in a double-blind, placebo controlled trial that administered 1 dose of oral dexamethasone (1 mg/kg).[71] The rate of hospitalization and the Respiratory Assessment Change Score (RACS) of these groups did not differ significantly. Furthermore, no benefit was observed among the subgroup of children with asthma or with a family history of asthma. Thus, the 2006 American Academy of Pediatrics guidelines[16] concluded that corticosteroid medication should not be used routinely in the management of bronchiolitis, and that current data do not support a recommendation for using corticosteroid therapy to prevent hospitalization. However, some evidence exists that certain subsets of children, including older children and those with prior episodes of wheezing and with asthma, may potentially benefit from the administration of systemic glucocorticoid therapy.[7,16,320]

Antibiotics should not be administered prophylactically to children with RSV infection or as therapy unless specific indications of the coexistence of a bacterial infection are present.[6,16] As noted previously, concurrent or secondary respiratory bacterial infection is an unusual occurrence, except for otitis media, among children with RSV lower respiratory tract disease, and prolonged administration of intravenous broad-spectrum antibiotics may augment the risk of development of secondary bacterial infection.[165]

The only specific treatment currently approved for children hospitalized with RSV infection is ribavirin (1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamide). This synthetic nucleoside has been administered as a small-particle aerosol for 12 to 22 hours per day until clinical improvement is evident, usually about 3 days. Much shorter, intermittent courses of higher doses also appear to be an acceptable mode of administration.[91] An analysis of the use of ribavirin for RSV lower respiratory tract disease included 11 randomized trials.[16,224] Seven of these studies showed benefit in the clinical outcome, and 4 did not. Long-term studies examining subsequent development of pulmonary function abnormalities or recurrent wheezing have had mixed results. However, the total number of patients in these controlled studies is small, the design of the trials is heterogeneous, and the degree of benefit in clinical outcome is unclear. For these reasons, plus its high cost, the American Academy of Pediatrics recommends that ribavirin should not be used routinely in the management of RSV lower respiratory tract disease.[15] Its use should be reserved primarily for infants with or at high risk for developing severe disease, on an individual basis.[16]

PREVENTION

With perseverance and
the dreamer's scanning scope,
reality is made of distant hope . . .

—CBH

BREAST-FEEDING

The number of infants who have been breast-fed has increased in recent years because of the growing recognition of the correlation of breast-feeding with both general and specific beneficial effects on the health of the infant.[49,52,116] Most studies evaluating the protective effect of breast-feeding against RSV infection show a diminished likelihood of acquiring more severe infection.[16,24,116,317] Although colostrum and breast milk contain IgG and IgA antibodies and neutralizing activity against RSV, the mechanism of the protective effect is complex and may relate more to many other multifunctional components in human milk, which during digestion inhibit infection by viral, bacterial, and other infectious agents.[295] The degree and duration of breast-feeding necessary to provide against RSV infection, however, remain undefined.[232,293]

Numerous studies performed over the course of several decades have indicated that breast-fed infants are less likely to be hospitalized with RSV and other respiratory infections than are those children who are not being breast-fed.[16,24,50,317] Breast-feeding and the risk of hospitalization for lower respiratory tract disease in healthy, full-term infants were examined in a meta-analysis of 33 studies,[24] all of which indicated a protective association. However, in this meta-analysis, only 9 studies met all the inclusion criteria. Infants who were not breast-fed were almost three times (risk ratio, 0.28) more likely to be hospitalized for lower respiratory tract disease in comparison with those exclusively breast-fed for 4 months. One study estimated that 30 percent of hospitalizations of infants for infection would have been avoided for each additional month of full breast-feeding beyond 3 months.[317] The authors thus estimated that if all 4-month-old infants had been exclusively breast-feed, 56 percent of hospital admissions for infants in the first year of life would have been prevented.

INFECTION CONTROL AND NOSOCOMIAL INFECTION

Preventing RSV infection from being acquired by normal infants and young children, as well as avoiding the RSV infections that frequently occur subsequently, is difficult, if not impossible, considering the prevalence and often covert presence of RSV during the annual outbreaks. Infection control procedures in the home should focus on interrupting the spread of RSV by using effective hand hygiene practices, including frequent handwashing and the use of hand sanitizers.[33,129,240] If the hands are not visibly soiled, alcohol-based sanitizers are preferentially recommended.[33] Their use generally has been more effective, has been better accepted by both children and adults, and has shown significant decreases in the occurrence of respiratory and diarrhea illnesses in daycare facilities and schools.[33,145,171,208,415] Objects contaminated with respiratory secretions, which act as fomites, should be cleaned, with used tissues carefully disposed.

Within medical settings, infection control procedures are the mainstay of controlling nosocomial infections from RSV.[33,47] RSV infection acquired nosocomially is recognized increasingly as a problem of magnitude, cost, and concern, considering the current number of patients with high-risk conditions.[130,145]

More than half a century ago, in 1941, Adams[3] described an epidemic of pneumonitis that occurred in January through February in the nurseries of two Minneapolis hospitals. Thirty-two infants, mostly in the second and third months of life, were affected, and 29 percent died. Cytoplasmic inclusions in the bronchial epithelium were observed in all fatal cases. The distinctive epidemiology, clinical syndrome, and pathology of these cases led Adams to propose a viral origin. Twenty years later, he and his colleagues described a markedly similar epidemic of respiratory illness in infants in whom they identified RSV as the cause.[4,5] RSV was thus indicted as the agent of the earlier, first-described nosocomial outbreak.

During community outbreaks of RSV, the risk of the nosocomial spread of RSV infection is high and, if not recognized, results in increased morbidity and prolonged hospitalization. On infant wards and neonatal units, nosocomial RSV infection has been especially hazardous and sometimes fatal for the more compromised infants.[143,145,154,175] The risk of acquiring nosocomial infection is related to the child's age, underlying disease, length of hospitalization, and, clearly, adherence to proper infection control procedures.

Among immunocompromised children and adults, nosocomial RSV infections often are serious and difficult to control.[32,72,90,93,145,245,273,378,414] Outbreaks in transplant units in particular have been associated with a high mortality rate and prolonged and intermittent transmission (see "Immunocompromised Patients").

RSV infection frequently is introduced to the ward by hospital staff, families, and visitors who may have only a mild cold. Genetic analysis has demonstrated that nosocomial outbreaks may be caused by one or multiple strains of RSV during a single outbreak or season.[22,143,265,357,377] Infection rates may be high among staff members, who often require absence from work.[145] However, even uninfected staff may spread RSV infection by fomites, through contact with infectious secretions contaminating environmental surfaces.

Various infection control procedures have been employed to prevent the nosocomial spread of RSV (Table 194–4). Because the acquisition of RSV occurs primarily by close contact with an infected person or with surfaces contaminated by secretions, rather than by small-particle aerosols that may transverse great distances, most important is careful hand hygiene practiced by all persons entering and leaving a patient's room.[145,152] Integral to this precaution is the education of the staff, including frequent reminders to ensure compliance throughout the RSV season.[33,145,238] The efficacy of additional barrier precautions such as gowns, gloves, and masks has varied in different settings. In two controlled studies, the routine use of gowns and masks did not add further benefit to conscientious handwashing and other infection control procedures.[153,292] Masks that cover the nose and mouth are of limited benefit because touching or rubbing the eyes or nose are the major modes of self-inoculation.[111,152,157] Thus, eye-nose goggles or goggles in addition to a mask appear to be more effective in diminishing nosocomial infection in both staff and infants.[111]

As facilities allow, infants with respiratory signs of unknown origin should be admitted to single rooms.[47] The use of rapid diagnostic tests as a basis for cohorting has been controversial, in part because of their varying sensitivity and specificity, especially if these tests are used other than during the period of peak RSV activity.[16]

TABLE 194–4 Infection Control Procedures, Both Standard Precautions and Contact Precautions for Prevention of Respiratory Syncytial Virus Infection, Recommended by Centers for Disease Control and Prevention

Recommendation Category or Procedure	Comment(s)
Category I-B Recommendations*	
Handwashing	Water with soap or antibacterial agent or waterless antiseptic hand rub
Wearing gloves	Combined with handwashing before and after each glove change; may diminish self-inoculation
Wearing gowns	When direct contact with patient or patient secretions is likely
Wearing masks and eye protection	Eyes and nose are major sites for inoculation
Housing patient in private room or in a cohort isolated from other patients	Patients with documented infection can be grouped and isolated from other patients; beds should be separated by >0.9 m
Use of dedicated patient-care equipment sometimes recommended with less or no supporting evidence	Equipment, including toys, assigned to specific patients
Staff assigned according to patient's RSV status	Specific staff care only for patients with RSV infection
Visitor restrictions during RSV season	Some qualify by restricting young children only
Screening visitors for illness during RSV season	Visitor assessed by trained personnel or advised by use of an educational patient information list

**Centers for Disease Control and Prevention I-B recommendations based on "strong rationale and suggestive evidence" and strongly recommended for all hospitals and "reviewed as effective by experts in the field."*

RSV, respiratory syncytial virus.

Modified from Ref. 145.

PROPHYLAXIS

Currently, prophylaxis is centered on prevention of hospitalization and diminishing the severity of RSV infection in high-risk infants with the use of passively administered humanized monoclonal antibodies against RSV.[15,350,389] Prophylaxis with passive anti-RSV antibodies was initiated by the licensure in 1996 of high-titered intravenous polyclonal RSV immunoglobulin. The subsequent licensed RSV humanized monoclonal antibody, palivizumab, was 50 to 100 times more effective than was the polyclonal immunoglobulin and circumvented many of the difficulties and adverse effects of administering a large intravenous dose to small infants.[312] Palivizumab was developed from a mouse monoclonal antibody that recognizes a protective epitope of the surface glycoprotein, the F protein. Only the antigen recognition site from the mouse monoclonal antibody was inserted into the spine of a human antibody, thus diminishing the chance of having allergic reactions to the mouse-raised antibody.[14,367] Multiple studies have evaluated prophylaxis with palivizumab and the risk of hospitalization with RSV infection in high-risk infants.[15,16,46,389] The two blind, randomized, placebo-controlled trials pivotal for the licensure of palivizumab involved 2789 infants and young children who were premature or who had underlying chronic lung disease or congenital heart disease.[196,329] The rate of hospitalization for RSV illness was reduced more than 50 percent in the infants given prophylaxis in comparison with the placebo group. In different subgroups, the reduction of RSV hospitalization rates ranged from 39 to 78 percent, with the lower rates of protection observed among infants with chronic lung disease and congenital heart disease.[46] Post-licensure studies suggested that reduction rates in the necessity for hospitalization with RSV infection may be even greater.[350]

The American Academy of Pediatrics recommended that palivizumab be administered to selected children younger than 2 years of age with chronic lung disease, of gestational age of less than 35 weeks, and with functionally important congenital heart disease.[15] Palivizumab should be administered in five doses every 30 days to cover the 5 months of major RSV circulation, which for most of the United States would mean starting in early November.[15,274,286] Prophylaxis is recommended for subgroups of these high-risk children depending on their gestational and postnatal ages, the severity of their underlying conditions, their need for recent medical therapy, and the presence of other risk factors. Prophylaxis with palivizumab should be considered for children with chronic lung disease who have required therapy within 6 months of the start of the RSV season, for infants born at 28 weeks' or less gestation during their first RSV season, for infants born at 29 to 32 weeks' gestation if younger than 6 months of age at the start of the RSV season, and for infants born at 32 to 35 weeks' gestation who have two or more risk factors for acquiring more severe RSV disease and are younger than 6 months of age at the start of the RSV season. Prophylaxis also is recommended for children with congenital heart disease if it is hemodynamically significant. Once a child has qualified at the beginning of the season for the administration of prophylaxis, it should be continued throughout the season, but with no more than the recommended five monthly doses.[15] Palivizumab has not been shown to be effective in the treatment of RSV disease.

The use of palivizumab, nonetheless, remains controversial. Economic analyses, in general, have not demonstrated an overall savings in health care costs if all high-risk children receive prophylaxis.[89,341] Cost-to-benefit analyses have been difficult to determine because of considerable geographic variation in the population of infants and hospitalization costs for such infants. Palivizumab immunoprophylaxis has not been correlated with a significantly diminished rate of mortality for infants with RSV infection who receive the prophylaxis, but the mortality rate from RSV currently is very low. The effect of palivizumab prophylaxis on subsequent sequelae, such as recurrent wheezing and pulmonary function abnormalities, is unknown.

Other products for prophylaxis that are being investigated include a humanized recombinant IgG1 monoclonal antibody that is derived from palivizumab. It also is directed against the F glycoprotein of RSV, but its greater potency has been suggested by animal experimental data showing a binding affinity that is 70 times greater than that of palvizumab.[422] In subsequent phase III trials in high-risk infants, this monoclonal antibody has been shown in comparison with palivizumab to provide significantly greater reductions in the number of hospitalizations and outpatient visits for RSV infection.[45a]

VACCINES

Control of RSV has long lured but eluded investigators. Although the outcome of infants with RSV infection has improved greatly in recent years, marked by a decrease in the mortality rate, the burden on health care that RSV imposes yearly remains notable among both children and adults.[100,120] Only effective immunization is likely to alleviate this burden. Certain characteristics of RSV infection, however, have posed barriers to the successful development of a vaccine during the 4 decades of effort.

The shroud of past experience with the formalin-inactivated vaccine s conferred caution and concern regarding the development of candidate vaccines. The unfortunate outcomes of trials with the formalin-inactivated vaccine nonetheless stimulated research that has divulged information integral to the evolution of future vaccines.[62,109,209,221] The information derived from rodent models of the exaggerated disease and lung disorder illustrated the importance of the type of immunizing agent in determining whether a beneficial or detrimental humoral and cellular immune response results; these models suggested that a successful vaccine should produce a balanced immune response with both $CD4^+$ and CTL cells.[134,305]

Furthermore, the specific antigens to be included in a vaccine potentially will alter the immune response (see "Immune Response: Immunity and Disease Pathogenesis"). Experiments immunizing mice with recombinant vaccine viruses expressing the F, G, and the transcription anti-terminator (M2) proteins illustrate the mutable immune response in animal models.[305] Immunization with the G protein, which primes T_H2 cells, tends to produce eosinophilic lung disease, whereas immunization with the F protein is associated with results in CTL priming, T_H1 response, and lung histology characterized by polymorphonuclear cell infiltration. Subsequent to immunization with M2, the observed RSV disease is dominated by cytotoxic lymphocytes. Despite the wealth of information derived from these animal experiments, the lack of a model to test candidate vaccines that is reliable, adequate, and predictive of the effect in humans remains a significant barrier.

An RSV vaccine also should reflect the immune response of natural infection yet improve its inconsistent and nondurable protection. In addition, it would need to be administered in the first few weeks of life, when the most severe disease usually occurs. Little experience exists regarding immunization with live viruses within the neonatal period, when the immature and variably developed immune system of the neonate and the presence of maternal antibody are likely to exert unpredictable hindrances.[305] Furthermore, the effect of currently growing numbers of antigens from other vaccines administered early in the first few months of life must be considered.

The initial RSV vaccine trials, with the alum-precipitated, formalin-inactivated vaccine (FI-RSV), were conducted in 1966 to 1967 concurrently with administration of an inactivated and trivalent parainfluenza vaccine. Infants aged 2 months or older and older children previously infected with RSV were given FI-

RSV in three doses. With exposure to RSV during the subsequent RSV outbreak, the FI-RSV vaccinees were more protected against infection than were the controls who had received the parainfluenza vaccine. However, among those RSV vaccinees who did become infected, the severity of the RSV disease was significantly greater among the FI-RSV vaccinees, and the severity was inversely correlated with age.[62,109,209,221] Two children died of pneumonia, and their lungs showed marked inflammation with eosinophilia, lymphocytic, and neutrophilic infiltration. Subsequent investigation indicated that the FI-RSV evoked abnormal and unbalanced cellular and humoral immune responses with antibody deficient in neutralizing and fusion-inhibiting activity.[222,288,291]

Vaccine development subsequently has been cautious and has taken mainly two approaches, production of live-attenuated or subunit candidate vaccines. Live-attenuated vaccines have the advantage over subunit vaccines of producing an immune response that more closely mimics the natural route of inoculation into the upper respiratory tract, thus eliciting more balanced local and systemic humoral and cellular responses and offering potentially broader and more durable protection.

The initial live attenuated candidate vaccines were derived from cold-passaged *mutants* propagated from wild-type RSV (wt RSV) strains grown at progressively lower temperatures, and strains were selected by growth at low temperatures, similar to that of the upper respiratory tract. Subsequent passage in the presence of chemical mutagenic agents produced candidate strains that were temperature-sensitive *(ts)* and unable to replicate at the higher body temperatures of the lower respiratory tract.[69] These candidate (*cp* and *ts*) vaccines generally produced promising results in adult volunteers and seropositive children but proved to be unsuitable for infants and young, seronegative children. These vaccines were associated with unacceptable degrees of illness, were overattenuated and not protective, or were genetically unstable; some were accompanied by prolonged shedding of wild-type virus, which most frequently was observed in infants and resulted in transmission of the shed virus to 20 to 25 percent of the unvaccinated contacts.[211]

More recent vaccine candidates have been derived from previously combined *cp* and *ts (cpts)* mutants, which contain mutations generated by reverse genetics that allow the precise desired enhancements of stability, attenuation, and immunogenicity. For example, one candidate, *cpts 248* strain, was further mutated and attenuated into *cpts 248/404*. Each of its mutations was introduced alone or in combination into wt RSV for evaluation in experimental animals of the mutations' immunogenic and attenuating effects. This candidate *cpts 248/404* vaccine was immunogenic in seropositive and seronegative children older than 6 months, but upper respiratory tract illness with appreciable nasal congestion developed in younger infants 1 and 2 months of age.[421]

Reverse genetics has produced a lineage of further "designer gene" candidate vaccines with one or combined deletions of NS1, NS2, M2-2, and SH genes. Two recombinant candidate vaccines, *cpts 248/404/ΔSH* and *cpts 248/404/1030ΔSH*, illustrate this technique's ability to correlate a vaccine's attenuating and protective effect to specific mutations but not to predict the host's mitigating factors such as age. Both appeared highly attenuated, immunogenic, and stable in initial clinical evaluations of adults and children 6 months of age and older.[212] *Cpts 248/404/1030ΔSH* produced more restricted replication in seronegative children, but only 44 percent of infants aged 1 to 2 months developed IgA antibody responses. However, despite the lack of a measurable antibody response, the infants demonstrated some protection against a second dose of the RSV vaccine, a finding suggesting that immunity not reflected in the measured humoral response was evoked. The two vaccines, *cpts 248/404/ΔSH* and *cpts 248/404/1030ΔSH*, differed only in a missense mutation (1030) in the polymerase gene, and this indicated that the 1030 mutation conferred attenuation.

Many other mutations have been evaluated in experimental animals, not only for their immunogenic, attenuating, and stability characteristics but also for properties required for augmenting the safety, efficacy, and acceptance of potential vaccines, such as increasing the degree and breadth of antigenic expression and the compatibility with different viral antigens that would allow combination vaccines of RSV with parainfluenza and other respiratory viruses.[69,283]

Candidate subunit vaccines have used primarily the two major surface glycoproteins, F and G, which are the major targets for the production of neutralizing antibodies.[74,367] Clinical investigation has focused on vaccines of the F protein, which has greater cross-reactivity between RSV A and RSV B strains. Three generations of purified F protein vaccines, PFP-1, PFP-2, and PFP-3, that consist of immunoaffinity-purified, alum-absorbed F glycoprotein, have been evaluated in clinical trials. The initial PFP-1 vaccine contained small amounts of the G protein, which did induce an antibody response. PFP-1 and PFP-2 vaccines have been administered to normal adults, institutionalized adults, normal children, and those with chronic underlying conditions including chronic lung disease, cystic fibrosis, and asthma.[74,96,136,138,316] The PFP vaccines generally have been safe and immunogenic, producing good levels of neutralizing antibody, especially in seropositive individuals and in those receiving vaccine that also contained small amounts of the G protein.

The PFP-2 vaccine, administered during the third trimester in a study of maternal immunization, showed no adverse effects on the infants, but it was poorly immunogenic in the mothers.[287] At delivery, only low levels of maternal neutralizing antibody were present. However, increased levels of IgG, but not IgA, antibody to the F protein did occur in breast milk. The protective effect of this approach for breast-fed infants requires further study.

A meta-analysis of the PFP vaccine trials concluded that a significant relative risk of 0.55 (95% confidence interval, 0.35 to 0.88) in the overall number of RSV infections existed, but the heterogeneity of the studies cast doubt on the validity of this conclusion.[371] Vaccination with PFP did not show any effect on reducing the incidence of RSV lower respiratory tract disease.

Additional candidate vaccines that have been evaluated include a peptide vaccine, BBG2Na, containing a conserved region of the G protein fused to the albumin-binding domain of the streptococcal G protein, and chimeric vaccines of the F and G proteins.[327,368,400] More immunogenic vaccines are being investigated by using recombinant vectors and plasmids that contain complementary DNA of the F and G genes.

Novel adjuvants also are being developed, some of which can shift the immune response evoked by subunit vaccines from a T_H2- to a T_H1-type response in experimental animals. Enhanced immunogenicity appears possible with enterically active adjuvants and carriers, such as liposomes and biodegradable microspheres, and with adjuvants in vesicles of immunostimulating complexes.[74]

The novel development of individual vaccines and approaches is reducing the number of hurdles and detours on the road toward successful control of RSV. In view of the number of different populations from infants to elders who would benefit from protection against RSV infections, multiple approaches and vaccines may be required to achieve effective control of RSV infection and diminish its associated human and financial cost.

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CHAPTER 195

Human Metapneumovirus

James D. Cherry

Human metapneumovirus (hMPV) is a respiratory tract pathogen first described in 2001 by van den Hoogen and associates.[88] It is the first known mammalian metapneumovirus associated with disease.[87,88] Avian pneumovirus (APV) is the only other known metapneumovirus.[91] hMPV is considered ubiquitous in humans on the basis of detection of the virus by polymerase chain reaction (PCR) in respiratory specimens of symptomatic children and adults* and the presence of a high rate of hMPV-specific antibodies in diverse populations throughout the world.[47,58,88] The features of hMPV infection in children and adults have been reasonably well characterized during the last 7 years. In children, the manifestations frequently are clinically indistinguishable from those of human respiratory syncytial virus (RSV).†

HISTORY

hMPV was isolated initially from specimens collected during a 20-year period from a group of 28 epidemiologically unrelated patients with respiratory tract disease in the Netherlands.[88] All viral isolates were from nasopharyngeal specimens collected in the winter months. The unidentified isolates replicated predominantly in tertiary monkey kidney (tMK) cells. The cytopathic effects of replicating virus were difficult to distinguish from those caused by RSV, except that the onset of features was delayed by several days. Electron microscopy revealed typical paramyxovirus-like pleomorphic particles and short envelope projections, with nucleocapsids rarely being observed. Reports of hMPV infection from Australia, Canada, England and Wales, Finland, and the United States soon followed.* The virus had been circulating within the Dutch population since at least 1958 based on the universal presence of antibodies to hMPV in sera collected from subjects who were aged 8 to 99 years old at that time.[88] Researchers have speculated that because APV is the closest relative of hMPV, a possible zoonotic event may have occurred before this time.[88] The late recognition of hMPV as a respiratory pathogen was the result of its unique growth requirements and kinetics in vitro.[88]

PROPERTIES

CLASSIFICATION

The complete sequence of the hMPV has been determined. The virus is a member of the *Metapneumovirus* genus in the subfamily

*See references 1-4, 6-12, 14, 16, 17, 19, 20, 22-31, 35, 38, 43, 45, 47-50, 53, 54, 57-68, 70-72, 74, 76-78, 80, 81, 83, 86, 91, 93-102.
†See references 1, 8, 16, 25, 61, 63, 64, 67, 88, 95-98, 102.

*See references 6, 9, 42, 45, 46, 58, 65, 70-72, 82, 99.

Pneumovirinae and family *Paramyxoviridae*.[46,87] It is genetically similar to the avian pneumovirus.

STRUCTURAL AND ANTIGENIC PROPERTIES

The hMPV virion, like all paramyxoviruses, contains a nucleocapsid of single-stranded, negative-sense RNA within a lipid bilayer envelope derived from the plasma membrane of the host cell.[52] The virion, however, does not contain hemagglutinin or neuraminidase, which distinguishes it as a member of the *Pneumovirinae* subfamily.

Ultrastructural analysis of the hMPV virion by electron microscopy also is typical of paramyxoviruses and demonstrates spherical, pleomorphic, and filamentous particles with short envelope projections.[71,88] The spherical particles vary from 150 to 600 nm in diameter, with a mean of 209 nm,[71,88] whereas the envelope projections are 13 to 17 nm in size.[88] The nucleocapsid's diameter is 17 nm with lengths from less than 200 to more than 1000 nm.[71] Filamentous particles average 282 by 62 nm in size.[71]

The hMPV genome is approximately 13.4 kb in length and consists of eight identifiable open-reading frames that are transcribed into eight viral proteins.[87,88] All are distinct structural proteins and have been identified as transmembrane surface glycoproteins (fusion [F], attachment [G], and small nonglycosylated hydrophobic [SH]), matrix proteins (M and M2), and nucleocapsid-associated proteins (nucleocapsid [N], phosphoprotein [P], and polymerase [L]). Two structural features principally determine the distinction between the genera *Metapneumovirus* and *Pneumovirus* (Fig. 195–1). Metapneumoviruses lack the nonstructural (NS) proteins NS1 and NS2, and the gene order differs between the two genera. The genetic alignment of the M and L open-reading frames of metapneumoviruses (3′-N-P-M-F-M2-SH-G-L-5′) is different from that of pneumoviruses (3′-NS1-NS2-N-P-M-SH-G-F-M2-L-5′). However, the location of hMPV viral structural proteins within the virion is similar to that described for RSV.

Sequence analysis of the hMPV genome reveals the highest degree of identity with APV.[88] Of the four known serotypes of APV, hMPV most closely resembles APV serotype C, the type most commonly described in birds in the United States.[13,88] Sequence information on APV serotype D for comparison with hMPV is limited.[88]

Similar to RSV, significant variation also exists in genomic sequences or strains for hMPV isolates. Phylogenetic analysis of isolates has confirmed the existence of at least two distinct clusters or lineages and four sublineages of hMPV worldwide based on limited sequence analysis of the N, M, F, and L open-reading frames.[5,6,55,56,58,82,88,90] In separate studies, overall nucleotide comparison reveals 80 to 88 percent genetic similarity between the two clusters and 93 to 100 percent within each cluster. At the amino acid level, the differences are less distinct, with 94 to 97 percent similarity existing between groups and 97 to 100 percent shared identity found within each group. hMPV genetic strains from both clusters have been noted to co-circulate in the same year.[6] Strains from different years also have been identified in the same subcluster.[6,55,88] On the basis of differences in virus neutralization titers, the two distinct genomic lineages (genotypes A and B) also can be considered two serotypes.[90]

LABORATORY GROWTH

The use of tMK or LLC-MK2 (Rhesus monkey kidney) cells has shown the greatest success in culture of hMPV from clinical respiratory samples obtained by nasopharyngeal aspiration, nasopharyngeal swabs, endotracheal aspiration, or bronchoalveolar lavage.[6,71,75,88] The virus replicates poorly in Vero or A549 cells, with no growth detected in chick embryo fibroblast (CEF), Madin-Darby canine kidney (MDCK), or human lung mucoepidermoid carcinoma (NCI-H292) cells.[6,58,71,88] The characteristic cytopathic effect in cell culture is focal small round, granular, and refringent cells, without large syncytial formation in most cases.[6,71] The appearance of the cytopathic effect ranges from 3 to 23 days with a mean of 17.3 days.[6,88]

Other biologic properties of the *Paramyxoviridae* are detected with hMPV culture. Standard chloroform treatment results in a significant reduction in the median tissue culture infective dose ($TCID_{50}$) for tMK cells.[88] Virus-infected cell culture supernatants do not adsorb the erythrocytes (negative hemagglutinating activity) of chickens, guinea pigs, or turkeys.[71,88] Viral replication in vitro is dependent on the addition of trypsin to the cell culture medium.[88] This last feature of hMPV may have been what contributed to a delay in recognizing the virus as a respiratory pathogen because seminal research performed to identify respiratory agents failed to use trypsin as a culture supplement.[88] Other practices that also would have contributed to this delay include the poor replication of hMPV in the continuous cell culture lines routinely used in diagnostic virology laboratories for viral isolation, the slow viral replication in vitro leading to discarding of potential positive isolates before detection, an apparent lack of serologic cross-reactivity to other paramyxoviruses (e.g., RSV, influenza and parainfluenza viruses), and finally, the low nucleotide sequence homology to other known viral pathogens.[88]

ANIMAL SUSCEPTIBILITY

hMPV reproduces efficiently in the respiratory tract of cynomolgus macaques *(Macaca fascicularis)* and causes clinical infection.[88] Virus replication appears to peak between 2 and 8 days after inoculation into either the respiratory tract or conjunctivae.[88] The clinical features of primate infection are consistent with those of mild upper respiratory tract disease and suppurative rhinitis on histologic examination.[88] A similar attempt at experimental infection of turkeys and chickens has been unsuccessful, thus providing further supportive evidence that hMPV is a

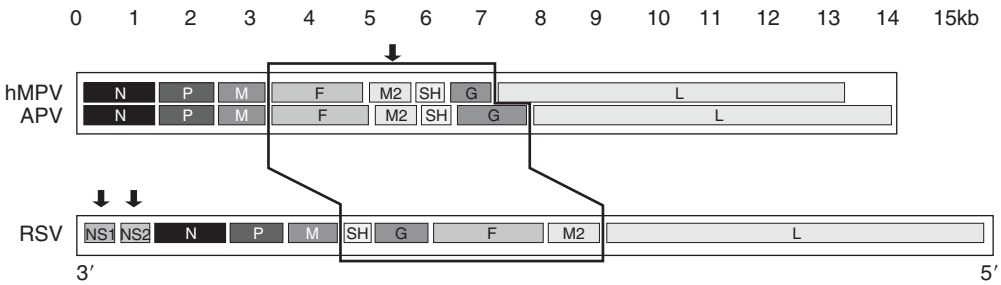

Figure 195–1 Genomic map of human metapneumovirus (hMPV) compared with avian metapneumovirus (APV) and human respiratory syncytial virus (RSV). The putative open-reading frames and the approximate nucleotide positions within the genome are indicated. The arrows signify areas of difference in gene constellation among hMPV, APV, and RSV.

primate pathogen associated with respiratory tract disease.[88] Intranasal inoculation of guinea pigs and ferrets does lead to the production of virus-specific antisera but no clinically obvious infection.[88] Mouse and cotton rat models have been developed for the study of hMPV infections.[78]

EPIDEMIOLOGY

GEOGRAPHIC DISTRIBUTION

hMPV has worldwide prevalence involving all continents but Antarctica and has universal seroprevalence in both the Northern and Southern Hemispheres.*

SEASONAL PATTERNS

hMPV has been recovered from children with respiratory illnesses throughout the year.[27,46,65,69,90,99] In temperate climates, hMPV infections peak in late winter and spring. This peak activity coincides with or occurs shortly after the peak of RSV respiratory infections.

STRAIN VARIATION

In contrast to influenza virus, of which a small number of strains spread around the world each year, outbreaks of hMPV appear to be local phenomena.[46] An outbreak in one community in one season might involve genotype A, whereas the major genotype in another community might be B. In a given year, viruses of both genotypes and their respective subgroups can circulate. Vicente and associates[93] looked for differences in clinical severity in 55 children infected with genotype A and 14 children with genotype B infections. Pneumonia occurred more frequently in genotype A infections (27.3%) than in genotype B illnesses (0%) ($p = .03$). Children with genotype A infections also were more likely to have oxygen saturation of less than 90 percent (21.8% vs. 0%), to be hospitalized (63.6% vs. 42.8%), and to be admitted to an intensive care unit (9.1% vs. 0%).

INCIDENCE AND PREVALENCE

Prospective observation for hMPV disease in the Netherlands and Finland during the winter of 2000 detected the virus in 9 and 10 percent, respectively, of respiratory samples from children with unexplained respiratory tract infection or acute wheezing.[45,88] In the United States, hMPV was detected retrospectively in 4 percent of hospitalized children younger than 5 years old with an acute respiratory illness in which a viral agent could not be identified.[65] In contrast, a community-based retrospective analysis of children in Tennessee during a 25-year period confirmed the presence of hMPV in 20 percent of nasal wash specimens collected from children with an undiagnosed lower respiratory tract illness.[99] In Australia, the annual detection rate of hMPV in respiratory specimens from children was 4.5 percent, although the incidence increased to 13 percent during the months of peak disease activity.[58]

hMPV infection in children, like RSV infection, inflicts its burden on children younger than 2 years old, particularly those younger than 12 months.[65,88] In children hospitalized for acute respiratory infection in the United States, hMPV affected predominantly children 0 to 12 months of age (50%), followed by those aged 1 to 2 years (42%).[65] Of these infants, infection occurred more commonly in the 7- to 12-month age group (31%) than in those aged 0 to 6 months (19%).[65] Children of white descent were overrepresented compared with children of all other races (65% versus 35%) in this series of patients.[65]

In numerous studies, the percentages of hMPV-caused respiratory illnesses in children has been compared with similar illnesses caused by RSV and other respiratory viruses as well.* In general, in any single year, RSV infections occur two to three times more frequently than do hMPV virus infections. Overall, rhinoviral and RSV infections occur most frequently, whereas adenoviral, parainfluenza virus 1, parainfluenza virus 3, and human bocavirus infections have a frequency similar to that of hMPV infections.

hMPV is assumed to be widely prevalent on the basis of the high level of hMPV-specific serum antibodies in the community. In the Netherlands, 70 percent of children demonstrated hMPV antibodies on immunofluorescent antigen assay by the time they reached 5 years of age; by the age of 10 years, 100 percent had these antibodies.[88] Similar work in Australia has confirmed the ubiquitous presence of seropositivity to hMPV, with 90 percent of the population positive at 5 years of age and 98 percent positive by 10 years.[58] Both groups have observed that total antibody titers are higher for those older than 2 years,[58,88] which may be due to serologic boosting and secondary infection in the second year of life after a primary infection when the child was an infant.

Ebihara and colleagues[25] compared the seroprevalence of antibodies to RSV and hMPV by age group. In the group younger than 4 months, the percentage positives were similar (hMPV 67% versus RSV 78%). In contrast, in the group aged 4 months to 1 year, only 11 percent of the infants had antibody to hMPV, whereas 48 percent had antibody to RSV. In the groups aged 1 to 2 years and 2 to 5 years, no significant differences in percentage positives were found between the two viruses.

Where stated, most reports of hMPV infection in children treated at hospitals have found a preponderance of affected males to females (1.2 to 2.3:1).† hMPV infections in older children have been noted, particularly in those who are immunocompromised or have a preexisting chronic lung condition.[6,26,29,70] hMPV has been touted as a potentially significant pathogen in immunocompromised transplant recipients, similar to RSV.[17,44,60] No information is available on the impact of differing socioeconomic environments on the age at initial infection with hMPV or severity of disease.

The spread of infection to nonimmune children also has yet to be determined, although it probably follows a pattern similar to that described for RSV, for which infection is introduced by an older family member.[34]

PATHOGENESIS AND PATHOLOGY

The method of transmission of hMPV is not known. However, because the virus is present in the nasopharynx in high concentrations, a reasonable conclusion is that its spread is similar to that of other respiratory viruses,[33,39] which could include contact transmission with large-particle respiratory secretions as well as hand-to-hand and hand-to-surface transmission, with self-inoculation. The virus in nasal secretions is mainly non–cell associated,[79] which suggests the possibility of small-particle aerosol transmission as well. In ill children, hMPV is shed in nasal

*See references 1, 3, 4, 6, 10-12, 16, 20, 22, 24, 27, 28, 30, 38, 42, 43, 46, 49, 50, 53, 58, 60, 62, 64, 65, 67, 68, 72, 74, 76, 81, 82, 88, 96-99, 101, 102.

*See references 1, 2, 4, 6, 11, 12, 16, 22, 25, 28, 30, 43, 49, 53, 61, 62, 67, 68, 74, 102.

†See references 1, 4, 11, 16, 30, 49, 62, 65, 88, 102.

secretions for a median of 5 days, a duration shorter than with RSV infections.[95] The case-to-case interval within households has not been well studied. In one small study, 82 percent of family members of children with hMPV infections had symptoms of upper respiratory tract infection.[95] These symptomatic illnesses occurred from 1 week before to 3 weeks after the onset of the child's illness. An older sibling was the apparent source of the illness in four of the seven young children. In addition to the presence of hMPV RNA in nasopharyngeal secretions, the viral RNA also was detected in perspiration of two of seven children and in the saliva of one of the seven children.[95]

In experimental hMPV infections in cynomolgus macaques, virus replication occurred in ciliated epithelial cells and pneumocytes, with associated lesions found throughout the respiratory tract.[46] Minimal to mild, multifocal erosive and inflammatory changes throughout the airways and an increased number of macrophages in the alveoli were noted. Vargas and associates[92] studied bronchoalveolar lavage specimens from six children with acute hMPV infections; all of these children had underlying or intercurrent diseases or both. They found epithelial degenerative changes and eosinophilic cytoplasmic inclusions within epithelial cells, multinucleate giant cells, and histiocytes.

Primary infection with hMPV results in a humoral antibody response that has been detected by neutralization in tissue culture and indirect immunofluorescence and enzyme-linked immunosorbent assay.[25,35,89]

van den Hoogen and colleagues[89] studied primary infections in cynomolgus macaques. They found that infection induced neutralizing antibody responses. The infected primates had higher antibody titer responses to homologous compared with heterologous viral strains. Postinfection antibody protected completely from homologous re-infection for 6 weeks. Eleven months after primary infection, protection had waned so that robust re-infection occurred.

With RSV and other respiratory viruses, a common hypothesis suggests that innate inflammatory responses contribute to pathogenesis.[51] Laham and coworkers[51] compared the levels of interleukin (IL)–12, tumor necrosis factor–α, IL-6, IL-1β, and IL-8 in the nasal washes of infants infected with hMPV with similar cytokine levels in infants with RSV and influenza infections. The IL-12, tumor necrosis factor–α, IL-6, IL-1β, and IL-8 levels in hMPV-infected infants were significantly lower than in those in infants with RSV infections. The IL-10 levels were low in both hMPV and RSV viral infections compared with the response in children with influenza viral infections. The authors concluded that their findings suggested that hMPV and RSV either cause disease by different mechanisms or share a common mechanism, which, however, is not the result of immune activation. Because the clinical manifestations are so similar, the latter suggestion seems more likely.

In a study using peripheral blood mononuclear cells from adults, Douville and associates[21] found that exposure to hMPV resulted in a stronger IL-6 and lower frequency, weaker intensity interferon-γ, IL-10, and CCL5 cytokine production than occurred with exposure to RSV.

Children who have a positive family history of asthma may be at increased risk for the development of postinfectious bronchial hyperreactivity or asthma.[72] Finnish children with acute wheezing episodes associated with hMPV infection had high levels of IL-8 and low levels of RANTES (regulated by activation, normal T cell expressed and secreted).[45] This finding is in contrast to that noted for RSV infections in vitro, in which RANTES concentrations (chemotactic factor for eosinophils) are high and IL-8 concentrations (chemotactic factor for neutrophils) are variable.[77] Whether the agent or the resultant disease leads to asthma or chronic lung disease is debatable, with conflicting reports in two small series of patients infected with hMPV.[45,72]

CLINICAL MANIFESTATIONS

hMPV has been found in nasopharyngeal specimens from children with both upper and lower respiratory illnesses. In numerous investigations, the clinical characteristics of hMPV infections have been compared with the clinical characteristics of RSV infections and less commonly with those occurring in other respiratory viral infections. In these comparative studies, the overall findings in hMPV infections clearly are similar to those in RSV infections. Overlapping findings also occur with human bocavirus, adenovirus, influenza virus, parainfluenza virus, and rhinovirus infections.

hMPV does not appear to be associated with asymptomatic carriage in the nasopharynx. van den Hoogen and colleagues[88] noted that no hMPV was found in respiratory samples taken from 400 asymptomatic children younger than 2 years.

UPPER RESPIRATORY TRACT MANIFESTATIONS

In a study of 41 children with hMPV infections, six were found to have upper respiratory illnesses and three had laryngotracheobronchitis.[76] Of the six with upper respiratory illness, only two were evaluated initially for respiratory symptoms. In a 20-year experience in Nashville, Tennessee, 118 children with upper respiratory illnesses were reported.[101] In this group, the following findings were noted: fever, 64 percent; coryza, 82 percent; cough, 66 percent; hoarseness, 8 percent; otalgia, 31 percent; rhinitis, 79 percent; conjunctivitis, 3 percent; pharyngitis, 44 percent; and abnormal tympanic membrane, 63 percent.

In a relatively large study in Switzerland of hMPV infections in outpatients, 5 percent had conjunctivitis, 73 percent had rhinitis, 40 percent had pharyngitis, 18 percent had otitis media, and 5 percent had croup.[4] Croup has been noted in 5 to 10 percent of cases in three other large studies.[1,11,12] In other studies, upper respiratory illness and common cold have been noted in association with hMPV infections.[1,12,78]

Acute otitis media is a common complication of hMPV infection.[38,78,100,101] In a study in Finland, 41 percent of 39 children with hMPV infections had acute otitis media.[38] The frequency of this complication was 61 percent in children aged 1 to 2 years old, 27 percent in those aged 2 to 4 years old, and 17 percent in those aged 5 to 9 years old. In a 25-year cohort of children with hMPV lower respiratory infection, one third of the children had acute otitis media.[100] In eight children with acute otitis media and hMPV infection, six had bacterial pathogens identified in middle ear fluid.[100] In one of these cases, hMPV was also identified in the middle ear fluid.

In another study of acute otitis media, 13 percent of the cases occurred in hMPV-infected children.[78] In a study in Japan in which eight children had acute otitis media in association with hMPV infection, three had hMPV identified in middle ear fluid.[83] Two of these children also had *Streptococcus pneumoniae* identified in the middle ear fluid. In other large studies, acute otitis media has been noted in 8 to 26 percent of hMPV infections.[1,4,81,98,102]

LOWER RESPIRATORY TRACT MANIFESTATIONS

The clinical manifestations of hMPV infections have been itemized in numerous studies, many of which have compared the findings with those occurring in RSV infections.* Because the frequency of clinical manifestations varies in different studies, defining meaningful differences between the clinical manifesta-

*See references 1, 3, 4, 6, 11, 12, 16, 24, 27, 29, 30, 43, 49, 64, 65, 67, 71, 72, 74, 81, 82, 86, 88, 97-99, 102.

TABLE 195–1 Symptoms and Signs in Young Children with Human Metapneumovirus Lower Respiratory Tract Infections*

Clinical Feature	Frequency Ranges (%)
Fever	50-90
Cough	50-90
Coryza	50-90
Respiratory distress	40-80
Crackles, rales	8-60
Wheezing	30-50
Tachypnea	60-90
Anorexia	40-60
Vomiting	10-20
Diarrhea	20-40
Pharyngitis	40-60
Irritability	40-60
Conjunctivitis	7-10
Rash	5-10
Otitis media	10-26

**References 1, 10, 11, 16, 27, 30, 49, 64, 65, 67, 74, 81, 97, 99, 102.*

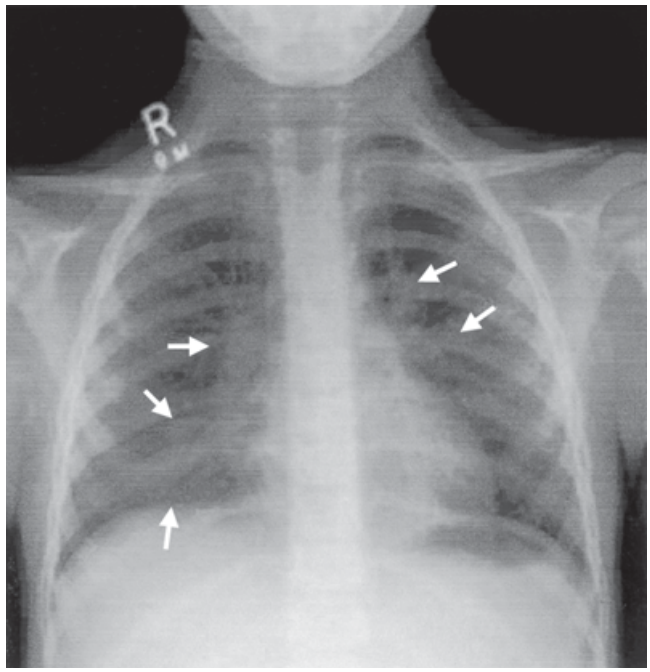

Figure 195–2 Chest radiograph of a child infected with human metapneumovirus demonstrating bilateral pneumonic infiltrates *(arrows)* and hyperinflated lung fields.

tions of hMPV infections and those of RSV infections is difficult. Bronchiolitis appears to occur more frequently in RSV infections and pneumonia more frequently in hMPV infections.[11,20] In a study in Norway, 48 percent of the hMPV infections were diagnosed as bronchiolitis, whereas 83 percent of the RSV infections had the same diagnosis ($p = .005$).[20] In the same study, pneumonia was diagnosed in 34 percent of the hMPV infections but in only 11 percent of the RSV infections. In this same study, children with hMPV lower respiratory tract infections appeared to be generally older than RSV-infected children with similar illnesses. Seventy-five percent of the RSV illnesses occurred in children who were younger than 2 years old, whereas only 50 percent of the hMPV illnesses occurred in the same time period.

Frequency ranges of symptoms and signs in hMPV infections in young children are presented in Table 195–1. As can be seen, the ranges vary considerably by feature in the studies reviewed. Presented in Figure 195–2 is the chest radiograph of a child with hMPV pneumonia.

In a study in Australia conducted between 2001 and 2004, 273 infections with hMPV were analyzed.[81] Of this total, 76 percent had an illness requiring hospitalization for 3 days or longer. Severe disease occurred in 10.6 percent of the children. Robinson and associates[76] noted 41 children with hMPV infections. Of this group, the majority were hospitalized, and 68 percent had an underlying medical condition. One child with multiple congenital anomalies including heart defects died. This child had pneumonia with a plural effusion. In another study, 35.2 percent of children with hMPV infections had associated underlying medical problems, such as a history of premature birth, chronic lung disease, complex congenital heart disease, and compromised immunity.[27] In a study in South Africa, 31 percent of the hMPV-infected children had underlying problems, including congenital heart disease in six, human immunodeficiency virus (HIV) infection in five, premature birth in two, chronic lung disease in two, acute lymphoblastic leukemia in one, gastroenteritis in four, renal failure in one, and gastroesophageal reflux in four.[64]

hMPV infection commonly is associated with recurrent wheezing and infectious asthma.[24,30,46,99] As might be expected, febrile convulsions are not uncommon occurrences in hMPV lower respiratory tract illnesses.[3,24,98]

Co-infections with hMPV and RSV were found to be more severe in several studies.[47,63,80] In a study in England, children with dual infections (hMPV and RSV) were more likely to be admitted to an intensive care unit for mechanical ventilation than were children with single-agent infections.[80] Madhi and coworkers[59] found that children hospitalized with hMPV infections frequently had pneumococcal co-infections.

INFECTIONS IN IMMUNOCOMPROMISED PERSONS

In a recent review, Kahn[46] noted that hMPV illnesses in immunocompromised patients often were prolonged and serious and occasionally were associated with death. However, most studies have involved adults and not children.[17,19,26] The most experience with hMPV infections and the immunocompromised state has occurred in children with HIV infections.[59,60,64] In one study, hMPV infections in 45 HIV-infected children were compared with hMPV infections in 154 children without HIV infections.[60] In this study, the HIV-infected children had a lower mean oxygen saturation, were less likely to have bronchiolitis, were more likely to have pneumonia, were more likely to have a longer hospitalization, and were more likely to have a positive bacterial blood culture than were the children who did not have HIV infections. In addition, two of the HIV-infected children died, whereas none of the non–HIV-infected children died. In another study, an HIV-infected child died with a nosocomially acquired hMPV infection.[64]

In cystic fibrosis, hospitalization for pulmonary exacerbations was associated with hMPV infection.[29]

ENCEPHALITIS AND ENCEPHALOPATHY

Glaser and associates[32] analyzed 1570 patients with encephalitis; in this study, there were four hMPV infections temporally related to the encephalitis. Before that study, Schildgen and coworkers[78] noted a similar association in a single patient, and, most recently, Hata and colleagues[37] described a fatal encephalopathy in a 6-month-old girl with hMPV infection. Computed tomographic scan of the brain showed low-density areas in the white matter.

DIAGNOSIS

DIFFERENTIAL DIAGNOSIS

The findings in hMPV respiratory infections are similar to those found in RSV infections as well as in human bocavirus, adenoviral, influenza viral, parainfluenza viral, and rhinoviral infections. With pneumonia, primary and secondary bacterial infections should be considered.

SPECIFIC DIAGNOSIS

Presently, reverse transcriptase (RT)-PCR is the most common method used to detect hMPV.[63,66,84] Specimens for PCR may be obtained by nasopharyngeal aspiration, nasopharyngeal swab, nasal wash, or bronchoalveolar lavage. Primer pairs for RT-PCR have used conserved genomic regions of the M, N, F, and L open-reading frames of the hMPV genome.[6,45,46,66,71,72,82,84,99] Real-time RT-PCR also has been developed, and with selected primers, both subgroups of both genotypes as well as other respiratory viruses can be detected.

hMPV grows poorly in cell culture, and growth requires supplementation of the medium with trypsin.[46] In general, cell culture is not clinically useful for diagnosis. However, the use of LLC-MK2 cells in shell vials offers promise.[75]

hMPV can also be detected in respiratory secretions by immunofluorescence.[46,66] Antibody to hMPV can be detected by virus neutralization by plaque reduction in tissue culture.[18] At present, no commercial tests are available to detect IgG or IgM antibodies to hMPV.

TREATMENT

Treatment that has been supportive in hospitalized cases has included regular nasal suctioning, intravenous fluids, oxygen therapy, and mechanical ventilation when necessary.[62,88]

Ribavirin has been shown to have antiviral activity against hMPV in vitro and in mouse models.[36,63,66,84] An adult patient with severe hMPV pneumonia after lung transplantation recovered after receiving treatment with intravenous ribavirin for 15 days.[73] This patient initially did not improve with inhaled ribavirin for 4 days. The sulfated sialyl lipid (NMSO3), heparin, and a low-molecular-weight benzimidazole derivative all have been shown to have in vitro activity against hMPV.[46,84,103] In a cotton rat system, a recombinant human monoclonal antibody to hMPV fusion protein (FabDS7) had a treatment effect when administered intranasally 3 days after infection occurred.[104]

PROGNOSIS

The full spectrum of hMPV infections and possible sequelae are presently not known. Most infections are self-limited. However, severe infections requiring mechanical ventilation occasionally occur in previously normal children, and fatal illnesses have occurred in immunocompromised patients.

PREVENTION

Good infection control practices in health care environments are the only preventive measures for hMPV infection. At present, no vaccine or immune globulin or monoclonal antibody is available for the active or passive immunization against hMPV. However, in animal model systems, both active and passive biologic agents have shown promise.[15,40,41,46,85] Similar to the past experience with formalin-inactivated RSV vaccines, enhanced pulmonary disease has occurred after challenge in cotton rats immunized with a formalin-inactivated hMPV vaccine.[98]

Ulbrandt and associates[85] have shown that two different monoclonal antibodies that bind to the hMPV F protein were protective in hamsters. Their studies suggest that this approach could be useful in preventing respiratory illness caused by hMPV in high-risk young children.

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SUBSECTION 8

Rhabdoviridae

CHAPTER 196

Rabies Virus

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Rabies is an acute, progressive viral infection of the central nervous system (CNS) transmitted from animals to people. Introduction of the agent by bite, scratch, or aerosol enables it to attach to and travel up the nerves to the brain. The encephalitis so caused is characterized by hydrophobia and almost always is fatal.

HISTORY[114,205]

Rabies has a long and colorful history. The Greeks called the disease *lyssa*. Democritus is thought to have made the first description of rabies in the dog in 500 BCE. The ultimate derivation of the English word *rabies* is from the Sanskrit word *rabhas*, which means "to do violence." Early writers believed that rabies followed the rising of the star Sirius, and the disease became associated with the dog days of summer.

Celsus, the Roman physician, writing in 100 CE, displayed an accurate understanding of the disease by attributing infection to a "virus" (i.e., a poison) in the saliva. He advocated cauterization of the wounds produced by rabid animals. Galen, writing a century later, advised surgical resection as the method for preventing rabies.

Rabies was well known in Europe during medieval times. The great Mohammedan physicians also mention it in their writings. Epizootics, however, were not recorded until 1271, when rabid wolves attacked people in Franconia. Epizootics associated with wolves then occurred frequently in various areas of Europe until early in the 18th century, when outbreaks of rabies in domestic dogs began in cities. Canine rabies probably was transmitted from Europe to the New World, where it became a common occurrence in North America and the West Indies by the 18th century and spread to South America early in the 19th century. However, the existence of rabies in New World bats probably predates that importation. The history of the disease in Asia is not as well known, but rabies clearly has been present since ancient times in China and India, although the current variants may be from a relatively recent crossover.[102]

The scientific study of rabies began with the demonstration by Zinke in 1803 that saliva transmitted the disease. The modern prevention of rabies was made possible by the work of Louis Pasteur in the 1880s. He showed that rabies could be transmitted by intracerebral inoculation of a preparation made from the brain of a rabid dog into uninfected rabbits or dogs and then serially transmitted by the same route. He thus demonstrated that rabies is a disease of the CNS. Among Pasteur's other discoveries were

the differentiation of furious and dumb rabies, the production of rabies by the intravenous route, and the attenuation of rabies virus in the dried spinal cords of infected rabbits. The famous episode of Joseph Meister, the French boy who received rabies vaccine in 1885, demonstrated that serial inoculations of dried infected rabbit spinal cord, proceeding from those containing most attenuated virus to least attenuated, in principle could protect against rabies.

Vaccination with nerve tissue vaccines quickly became standard, with important modifications being introduced by Roux, Fermi, and Semple. Semple vaccines, composed of infected sheep or goat brain tissue suspensions in which virus is completely inactivated by phenol, became the standard vaccine in the 1920s. Fuenzalida and Palacios introduced the use of suckling mouse brain vaccines in the mid-1900s to reduce the adverse events associated with myelin in brain-derived vaccines. In the latter 20th century, nerve tissue vaccines were replaced gradually by production of cell culture in most developed nations.[92]

THE VIRUS

The rabies virus apparently can infect all species of mammals, although wide variations in the sensitivity of different species have been observed. Laboratory propagation may be accomplished readily in mice or other standard laboratory animals; in vitro in neuroblastoma or certain hamster cell cultures; or, after adaptation, in certain cell lines of human or other mammalian origin.[167]

Electron-microscopic studies reveal that rabies is a bullet-shaped virus typically maturing at cytoplasmic plasma membranes and intracellular membranes of the endoplasmic reticulum in infected cultured cells (Fig. 196–1).[104] Standard virions are approximately 75 nm in diameter and 160 to 180 nm in length. Regular arrays of standard-sized virions maturing from plasma membranes are observed in infected salivary glands. Budding of virus from plasma membrane is much less pronounced in neurons of the CNS than in cell culture or salivary gland cells. However, meticulous electron-microscopic examination of experimentally and naturally infected brains has revealed that budding from membranes of perikarya and dendrites, as well as presence of virus in intracellular spaces of brain (especially at synaptic junctions), occurs regularly.[105] In addition, CNS neurons often exhibit both typical and bizarre morphologic forms of virus maturing in the cytoplasm. Cytoplasmic forms often develop in proximity to nucleocapsid matrix inclusions (Negri bodies).[144]

The gross morphologic characteristics and biochemical composition of rabies virus place it within the family *Rhabdoviridae*, genus *Lyssavirus*. Lyssaviruses of Old World or Australian origin isolated more recently exhibit antigenic and genetic relationships to rabies, predominantly on the basis of similarities of the nucleoprotein detected by fluorescent antibody and complement-fixation tests, reactions with monoclonal antibodies, and sequencing studies of the N protein gene. Thus, seven species (often designated genotypes) are now recognized, and more may exist.[87,132,199,242]

Rabies virus is lyssavirus type 1 and continues to account for the vast preponderance of isolates. Type 2 (Lagos bat virus), type 3 (Mokola virus), and type 4 (Duvenhage) were isolated exclusively in Africa, where they have predominantly bat reservoirs, but only Mokola and Duvenhage viruses have been associated with rare human infections. Lyssaviruses associated with European bat reservoirs have been separated into lyssavirus type 5 (European bat lyssavirus 1) and type 6 (European bat lyssavirus 2, rarely associated with human disease). Lyssavirus type 7 has been identified in Australia (Australian bat lyssavirus) and has a primary reservoir in fruit-eating bats but has caused fatal human infection, thereby ending Australia's previous rabies-free status.[192] Cats in South Africa have been infected frequently with Mokola virus and have died of the infection despite having received prior vaccination.[229] Indeed, types 2 and 3 are genetically more distant from canonical rabies virus, and vaccination may not protect against them.[163] The rabies virion now is recognized to be of a molecular composition similar to that of the rhabdovirus prototype virus, vesicular stomatitis virus. It is composed of a helical nucleocapsid core, which contains the RNA genome coupled to

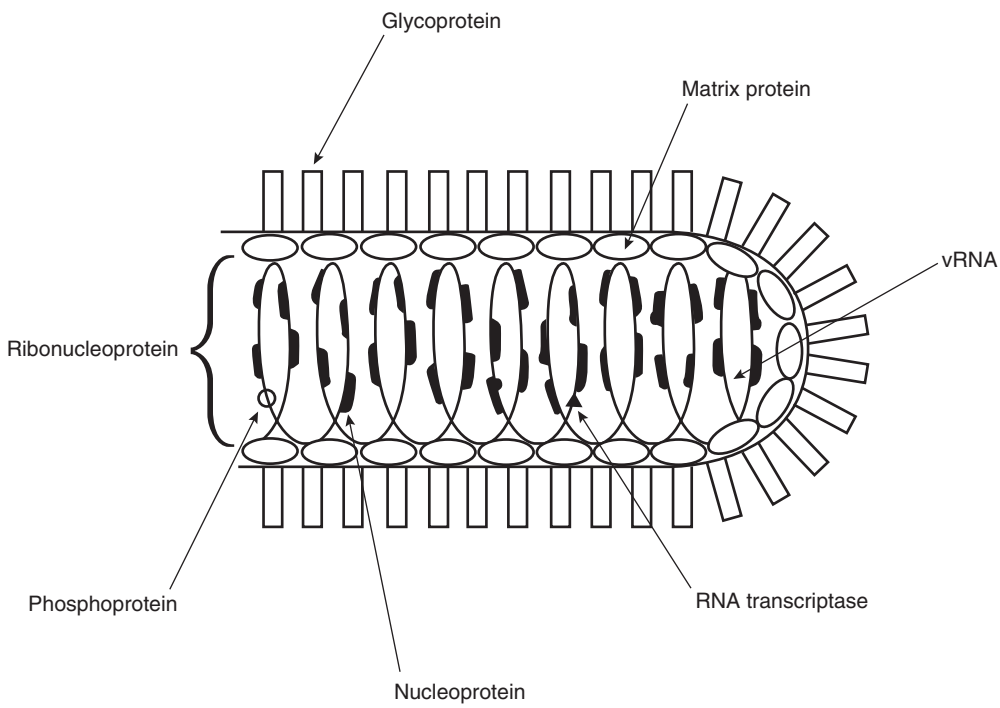

Figure 196–1 Drawing of the rabies virus. *(Courtesy of Dr. William Wunner, Wistar Institute, Philadelphia.)*

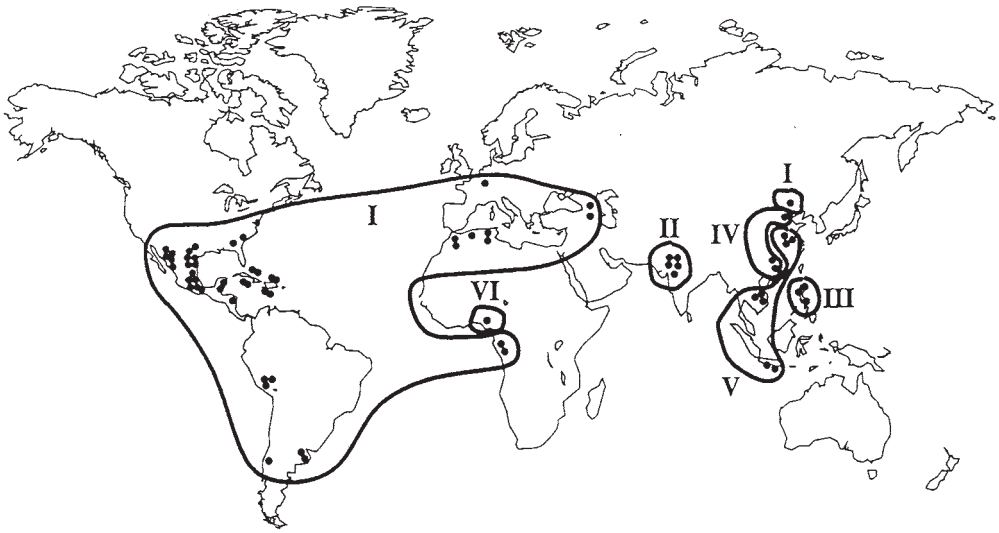

Figure 196–2 Distribution of genotypes of rabies virus. *(From Smith, J. S., Orciari, L. A., Yager, P. A., et al.: Epidemiologic and historical relationships among 87 rabies virus isolates are determined by limited sequence analysis. J. Infect. Dis. 166:296-307, 1992. Courtesy of Dr. J. S. Smith, Centers for Disease Control and Prevention, Atlanta.)*

nucleoprotein, the phosphoprotein, and a large protein presumably representing the RNA transcriptase replicase.[51] The core is surrounded by a lipoprotein envelope, which contains a matrix protein, a single glycoprotein, and lipids (primarily phospholipids and cholesterol).[29,203] The genome is a single-stranded RNA molecule containing approximately 11,932 nucleotides with a molecular mass of approximately 4.0×10^6 daltons.[28] It is a negative-stranded genome, that is, it must be transcribed to produce messenger RNA necessary for replication. Defective virions containing characteristic (for a given strain) sizes of incomplete RNA molecules have been demonstrated in cell culture-propagated virus populations, but their occurrence or role in natural infections has not been determined.

Important surface antigenic properties are associated primarily with the single glycoprotein,[203] a polypeptide containing three major oligosaccharide side chains.[60] The glycoprotein is the sole antigen responsible for induction of and reaction with virus-neutralizing antibody. Antibody to nucleoprotein administered passively has no virus-neutralizing or protective capacity but is the essential antibody for the diagnostic detection of intracellular rabies virus antigens (including Negri bodies) by immunofluorescent staining.[242] Active immunization with rabies nucleocapsid may contribute to immune protection, especially to heterotropic lyssaviruses, apparently by induction of a lyssavirus-specific T-cell immune response.[40,67]

Historically, only a single antigenic type of the rabies type 1 lyssavirus had been identified. However, strain specificity can be recognized on the basis of genomic sequence analysis[219] and strain differentiation by monoclonal antibodies. By these means, animal-specific strains and geographic distribution of strains can be discerned, and strains from human cases can be traced to the animal and place of origin.[201]

Researchers have demonstrated that rabies virus, like some other RNA virus populations, occurs as a quasi-species. The presence of viral subpopulations permits rapid adaptation to new hosts.[124,156]

Antigenic differences between both strains of fixed virus and different field isolates of diverse origin have been detected with the use of monoclonal antibodies[244] directed against glycoprotein or nucleoprotein antigens of the virus.[65] The use of monoclonal antibodies also allows easy distinction of rabies from rabies-related lyssaviruses.[187] Studies with monoclonal antibodies have revealed that worldwide, clustering of certain rabies virus antigenic subtypes is geographic and species specific.[210] As shown in Figure 196–2, at least six genetic groups of rabies viruses with relatively specific geographic localizations exist.[201] Within those areas, the virus may be transmitted by different species, such as dogs and foxes. Strains recovered from urban dogs fall into at least six variants according to the location of the city. In the United States, readily distinguishable subtypes of rabies virus have been determined to circulate in each of the major terrestrial wild mammal vector species (primarily skunks and raccoons) and in bats. Bat viruses rarely occur in terrestrial animals in North America, whereas in South America, viruses isolated from cows frequently originate from vampire bats.[65] In all well-studied rabies enzootic areas, researchers have determined that rabies circulates predominantly in a limited number of mammalian species characteristic of that area, and a single antigenic phenotype is characteristic of that transmission cycle.[65] However, distinct bat-adapted rabies virus strains may circulate independently in these areas or in areas where terrestrial animals are free of rabies. Different geographic subtypes of street rabies virus share varying proportions of the immunologically critically important glycoprotein epitopes with the vaccine strain (Pasteur) virus. Rabies viruses isolated from human cases of rabies in the United States have been shown to differ quantitatively in their glycoprotein antigenic composition from vaccine strain virus.[245] Despite intense investigation, however, no evidence supports the contention that cross-protection between the Pasteur strain rabies virus vaccine and antigenically distinguishable street strains is reduced to a degree justifying inclusion of new virus strains in human vaccines. Physicians have every reason to believe that present vaccine strains protect against all feral strains of lyssavirus type 1, provided the vaccine is sufficiently potent. The same cross-reactivity does not exist for all other lyssaviruses.

TRANSMISSION AND EPIDEMIOLOGY[41,42,221]

Infection with rabies may be induced readily in experimental animals through the oral, the respiratory, or a variety of parenteral routes. Epidemiologic evidence suggests that the vast preponderance of natural infections of humans and animals is caused by physical inoculation into subcutaneous and muscle tissue by a bite wound and rarely by exposure of mucosal surfaces; saliva is the usual body fluid that serves as the vehicle for virus to infect other animals or humans. Exceptional infections through the respiratory route have been noted in animals exposed to certain

caves heavily infested with bats and in persons exposed to virus aerosols experimentally produced in the laboratory.[115]

Domestic animals constitute the largest source of human exposure to rabies in most parts of the world. Dogs and cats are the principal transmitters of rabies to people in Asian countries, such as India, Indonesia, China, and the Philippines. The magnitude of the problem is illustrated by the fact that approximately 12,000 to 20,000 deaths are attributed to rabies annually in India alone,[240] and as many as 50,000 to 100,000 may occur throughout the world.[147] The incidence in India of two to four rabies deaths per 100,000 population may be exceeded only by that of Ethiopia, where the rate is 18 per 100,000.[92] The total number of people given postexposure vaccination probably is 4 million or more annually. On the other hand, many areas, including Japan, Italy, Switzerland, France, Spain, Scandinavia, and others, are considered to be rabies free.[30]

In a single year of an urban epizootic of dog rabies in one city in Mexico, an estimated 2.5 percent of the population received dog bites and 2.7 percent were given postexposure prophylaxis for rabies.[72] In Africa, the dog still is the most important vector, although in the south, jackals and mongooses also are common sources of rabies exposures.[27,53] In much of Africa, the incidence of rabies in people and dogs has increased in recent years because of social and political disruptions.[240]

Rabies in wildlife, predominantly foxes, now is enzootic in much of eastern continental Europe, having spread from Poland during the last 60 years.[30] This outbreak gradually has moved to involve most of Europe, excluding Great Britain and Scandinavia. However, vaccination of wildlife has reduced sharply the prevalence in the West.[113,169] The raccoon, dog, and arctic fox are important vectors in eastern Europe and Siberia.[31,123] Bat rabies exists throughout continental Europe, especially in Denmark, the Netherlands, and coastal areas.[159]

Rabies in Central and South America is related predominantly to dogs, but vampire bats also have considerable importance. Although humans sometimes are bitten, often they also are exposed to cattle infected by bat bites. However, despite common exposure, transmission of rabies from cattle to humans is an extremely rare event.[58]

In the United States and Canada, the skunk once was the rabid wild animal that most commonly attacked humans. In recent years, the raccoon has replaced the skunk as the most important potential source of infection among terrestrial mammals because of a rapid extension of raccoon rabies northward and southward from Virginia, in a range now continuous from Canada to Florida and west to Ohio.[39,68] This outbreak stems from introduction of raccoons into Virginia from a long-standing nidus of raccoon infection previously restricted to Florida and southeastern Georgia. Despite massive spread of raccoon rabies engendering great costs in terms of postexposure treatment and efforts at wildlife control, actual human cases of rabies attributable to raccoons have been rare.[5] The significance of the bat to rabies epidemiology varies. In the United States, a redoubtable reservoir of rabies in insectivorous bats is present in every state except Hawaii. This bat reservoir probably is responsible for a high proportion of all U.S. cases, including those that have no antecedent history of animal bite.[152,200] Between 1981 and 1998, an average of two human rabies cases occurred per year; 12 imported cases resulted from dog bites. However, between 1990 and 2005, 31 of 34 domestic cases (excluding four transplant-transmitted infections)[204] were caused by bat strains of rabies, predominantly strains from the silver-haired and Eastern pipistrelle bats.[3,37,81,123,167] The same trend has been observed in Canada.[11]

Strains isolated from the silver-haired bats are better able to grow in non-neuronal cells, and although prophylactic vaccination is effective, the infectiousness of these strains apparently is high.[158] In Colorado, 15 percent of tested bats were positive for rabies,[167] which may be a relatively silent infection in that mammal.[182]

Bats may bite sleeping humans but leave little trace behind.[2,129,235] Therefore, vaccination always is indicated if a confirmed rabid bat is found in the chamber of a sleeping person, and it may be indicated if a bat entered the chamber but escaped if there is a reasonable probability of bite exposure. In Europe, bats have been responsible for transmission of rabies-related lyssaviruses. Despite the rarity of this event, bats and vampirism are linked inextricably in legend with Dracula, the 15th-century despot who lived in current-day Romania.[94] In contrast, true vampire bats in Central and South America are responsible for considerable losses of domestic animals as well as human cases.[15]

On the average, only one or two cases of human rabies are reported in the United States each year, but thousands of animals still are infected. The distribution of infection among animals is shown in Table 196–1.[128] The cat now is a more important host of domestic rabies than is the dog.

True canine rabies has been eliminated in the United States. Moreover, vaccination has curtailed the prevalence of rabies in cats in the United States so greatly that many cities and other areas may be considered free of rabies in terrestrial animals, although the new spread of raccoon rabies has reversed this trend in the Middle Atlantic states. The importance of these facts is discussed under Postexposure Prevention.

As a result of the rarity of human rabies in the United States, many cases have not been diagnosed until autopsy. Transmission of rabies by corneal transplant to a new host from fatal cases of undiagnosed encephalitis has been reported.[104,204]

TABLE 196–1 Cases of Animal Rabies in the United States, 2004

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Please refer to the printed publication.

From Krebs, J. W., Mandel, E. J., Swerdlow, D. L., and Rupprecht, C. E.: Rabies surveillance in the United States during 2004. J. Am. Vet. Med. Assoc. 227:1912-1925, 2005.

PATHOGENESIS[66,106]

The initial stages of the infectious process are the least understood. Disease certainly depends on the entry of the virus into peripheral nerves, after which centripetal spread occurs rapidly. Replication of virus in muscle cells at a peripheral inoculation site has been demonstrated experimentally, and the virus has been shown to persist in myocytes at least 60 days after inoculation[45] but has not been proved to occur in nonexperimental situa-

tions.[46,160] Nonetheless, replication at a low level in myocytes, followed by subsequent infection of nerve cells, would explain the occasionally extended incubation periods of disease. Entry of virus into the peripheral nerve endings is mediated by attachment to the nicotinic acetylcholine receptor and other ganglioside receptors such as neural cell adhesion molecule (NCAM).[133] Once the virus is in the nervous tissue, spread can occur by cell-to-cell contact.[44,220] In any case, virus is thought to enter peripheral nerves at an extremely variable time after exposure, presumably at the site of neuromuscular or neurotendinal spindles, at motor end-plates, or (in the case of aerosol exposure) at olfactory end-organs.[161] Viremia apparently is an inconstant occurrence and of no import in the dissemination of infection.

The fact that virus transits along nervous pathways has been demonstrated by repeated experimental observations that the progress of disease may be interrupted by surgical excision or chemical destruction of nerves at sites central to the inoculation site.[19] Central progression of virus along neuronal pathways also has been demonstrated by electron-microscopic, immunofluorescent, and serial infectivity studies. Virus appears to be sequestered within neuronal cells during transmission (by axoplasmic flow) through peripheral nerves, and the virus can, therefore, be used experimentally to trace synaptic circuits.[119] Intra-axonal retrograde transport appears to be aided by interaction of the rabies virus P protein with the cytoplasmic protein dynein light chains.[109,181] Minimal numbers of virions are observed within neurons, and connective tissue elements of nerve sheaths never are infected. Because little maturation and release of virus from nerve plasmalemmal membranes occur during transit, little or no virus is presented to the immune system, which explains the absence of detectable humoral antibody until late in the disease that is characteristic of even prolonged incubation periods with rabies. Nonetheless, neutralizing antibody can enter the neuronal cytosol by endocytosis and block viral transcription.[64]

Virus enters the CNS at the spinal cord and thereafter travels rapidly to the brain. Early selective infection of the limbic system in the brain may cause disease characterized by extreme excitability and agitation (furious rabies), whereas the encephalitic depression symptoms are associated with early, widespread infection in the brain.[151] In either case, infection rapidly spreads to infect nearly all brain neurons within a few days of the onset of brain infection and CNS symptoms. Brain infection leads rapidly to death by mechanisms that are poorly understood. Fatal cases of encephalitis reveal little damage of neuronal cells, despite the ubiquitous neuronal infection.[82] On the other hand, persons kept alive by vigorous supportive therapy for lengthy periods of time after onset of disease may develop severe histopathologic encephalitic lesions. Studies in mice have shown that apoptotic mechanisms may be induced by rabies virus[107,224] and that more virulent rabies virus strains induce less apoptosis by expressing less G protein in neurons[157] or, alternatively, that the rabies G protein expression contribute to pathogenesis by down-regulating apoptosis.[157] Although ascending infection is specific to neurons, once the brain is infected, rabies virus spreads centrifugally to peripheral nerve plexuses, salivary glands, muscle fibers, and hair follicles.[108]

The entire manner of progression of the infecting virus, particularly those points in its transit vulnerable to immune intervention, remains to be determined fully. Clearly, preexisting humoral antibody protects, apparently by inactivating virus before it gains entry into the nervous system. After an immunized person is exposed to rabies, disease can be delayed or prevented in some cases by passive antibody, interferon treatment, or both,[112] but protection becomes efficient only when active immunization with potent vaccine accompanies such treatment.

That neutralizing antibodies are key to protection against rabies has been obvious for many years. However, studies have demonstrated the complexity of the immune response.[162] Although some experimental evidence argues for the importance of cytotoxic T lymphocytes in protection against rabies, Hooper and colleagues[103] infected mice that were unable to mount a cellular response and found no difference in disease with respect to normal mice. Nonetheless, early inflammatory responses, as well as rapid production of antibody, correlated with recovery from rabies. Thus, interferon and other mediators may supplement antibody in the prevention of rabies. Interestingly, rabies-specific cytotoxic T lymphocytes do not develop during the disease, and wild rabies virus may act as an immunosuppressor. Moreover, chemical immunosuppression enhances the development of experimental rabies. Interferon[20] and interleukin-2,[166] both important immunomodulators produced by cells, enhance protection against rabies. More sophisticated understanding of rabies pathogenesis is required before ideal combinations of immunotherapeutic procedures, possibly including interferon, finally can be formulated.

The reverse side of the coin is shown by the "early death" phenomenon, in which exposed animals or humans developed earlier onset of rabies if previously they had been immunized only partly. Sugamata and associates[207] have shown that early death depends on the presence of T cells, indicating that it is mediated by an immunopathologic cellular response. In addition, some authors have argued that cytotoxic T lymphocytes actually may contribute to rabies neuritic paralysis.[207,239]

CLINICAL MANIFESTATIONS

IN ANIMALS

Rabies encephalitis in animals is expressed in either a paralytic (dumb) or furious syndrome. Typical infections are characterized by behavioral changes and a rapid clinical course leading to coma and death, but rarely encephalitis can be nonfatal.[17] Because literally millions of animal bites of humans occur annually in the United States, the identification of aberrant behavior is a critical factor in many decisions to administer prophylaxis for rabies.

The prodromal stage of disease is marked by nonspecific signs, such as restlessness and malaise. Subsequently, placid dogs, cats, cattle, or horses may become vicious. Fear of humans and of areas frequented by them may be lost by wild animals. Thus, rabid foxes, normally nocturnal, may be seen wandering abroad in daylight, even in populous areas. Similarly, rabid bats often have been encountered flying in daytime hours. Early behavioral changes are not accompanied by paralysis.

The clinical course progresses rapidly to either dumb or furious disease. Dumb rabies typically is a depressed encephalitic disease. In addition to lethargy, selectively severe paralysis of throat muscles may be observed, which causes drooling of saliva because of difficulty in swallowing. Hydrophobia is not noted in animals. Furious rabies is characterized by an unusual state of alertness in which any visual or sound stimulation may incite an attack. Animals may roam indiscriminately, frequently feeding on stones, twigs, and other inanimate objects. Pets alternatively may exhibit unusually affectionate and playful behavior and then viciously bite those playing with them. Biting behavior may be noted by herbivores such as horses, mules, and cattle as well as by normally carnivorous pets and wild animals.

Both dumb and furious rabies have a rapid clinical course in domestic and wild animals. The period between onset of prodromal signs and death caused by respiratory paralysis rarely exceeds 7 to 10 days.[214] Although 10 days usually is given as the limit of virus excretion in the saliva of dogs before death supervenes, rare exceptions involving longer excretion have been reported experimentally and in animals observed in both Africa and Asia.[74,226]

The diagnosis of rabies in an animal depends on the demonstration of rabies antigens in the brain by fluorescent antibody

stains or of rabies RNA by reverse transcriptase–polymerase chain reaction (RT-PCR).

Control of Animal Rabies

Aside from large-scale removal of potentially rabid animal species, such as stray dogs, the most potent control method is vaccination. In domestic animals, immunization is accomplished readily by a variety of veterinary vaccines, although boosters must be given annually or after other stated intervals, depending on the vaccine.[4]

In wild animals, primarily raccoons in the United States and foxes in Europe, oral vaccination with use of baits containing vaccinia-vectored rabies glycoprotein or attenuated live virus has been successful.[68,169]

IN HUMANS[150,232,234,253]

The incubation period of rabies in some cases may be so long as to qualify it as a slow virus disease. Well-documented cases have occurred as long as 6 years after the bite.[232] However, most cases occur within 20 to 90 days after exposure. The shortest incubation period appears to be 7 to 10 days.

That incubation periods are longer after bites on the legs than after bites on the face is well known. The reason for this duration appears to be related not to the length of the nerve that the virus must traverse, because it travels rapidly even from the farthest site, but to the extent of innervation of different parts of the body. Bites on the tips of the fingers or on the genitalia have relatively short incubation periods for this reason. Children in general tend to have shorter incubation periods. Curiously, cases of vaccination failure tend to have shorter incubation periods, a fact that also is observed in experimental trials in animals. This early death effect is discussed under "Pathogenesis."

Hemachudha[96] has published a comprehensive review of human rabies. The first symptoms of rabies usually are vague and insidious. The patient simply may feel unwell or have anxiety or depression. Some fever or nausea may be present. The most striking prodromal symptom is itching, pain, or tingling at the site of the bite. This paresthesia is not present always and may take various forms, but its localization is a definite harbinger of rabies. The prodrome lasts 2 to 10 days, when the acute neurologic phase begins. The symptoms of this second phase are divided into furious versus paralytic rabies, most cases being in the furious category.

In furious rabies, the emphasis is on agitation, hyperactivity, fluctuating consciousness, bizarre behavior, and perhaps nuchal rigidity. Sore throat and hypersalivation are prominent complaints, and laryngospasm may cause hoarseness.

The legendary but all too real pathognomonic sign of furious rabies is hydrophobia. Initial attempts to swallow liquid result in painful spasms of the pharyngeal and laryngeal muscles, with aspiration of the liquid into the trachea. A conditioned response appears to be created, in which fear exacerbates the actual spasms. Warrell and associates[233] have hypothesized that brain stem encephalitis leads to destruction of inhibitors of inspiratory motor neurons. Respiratory tract instant reflexes are exaggerated, leading to inspiratory spasms. The hydrophobia has an important psychological element. In an extreme case, spasms occur if the patient merely is approached with water. Also frequently present is aerophobia, in which spasms occur when a current of air is fanned across the face. Priapism and increased libido also have been reported.

The neurologic examination findings in rabies are not uniform. Meningismus is a commonly occurring abnormality. Even more common occurrences are cranial nerve signs, particularly paralysis of the palate and vocal cords. The voice may develop a hoarse, barking quality. The reflexes may vary from hyperactive to absent, and involuntary movements are prominent. Magnetic resonance imaging shows involvement of gray matter in the hippocampus, hypothalamus, and brain stem.[143]

A distinct pattern of neurologic involvement is shown by approximately 20 to 30 percent of patients with rabies, particularly those bitten by vampire bats. Flaccid paralysis may start in the limb that was bitten originally and spread to other limbs. The cranial nerves become involved, and the face, rather than showing agitation, becomes expressionless. Hydrophobia is not a feature. Paralytic rabies is confused easily with Guillain-Barré syndrome. Hemachudha and colleagues[97,98] emphasized the importance of fever, intact sensation, urinary incontinence, and percussion myoedema in paralytic rabies.

The cerebrospinal fluid (CSF) is abnormal in a minority of patients, particularly those in whom meningismus is present clinically. When abnormal, the CSF shows a mild pleocytosis, mainly mononuclear. The peripheral white blood cell count shows increased polymorphonuclear cells.

The acute neurologic phase lasts 2 to 10 days, with eventual deterioration of the patient's mental status into coma. The patient may survive in this state for 2 weeks, particularly in dumb rabies. Before the final deterioration, the patient may have alternating periods of wild agitation and alert cooperation. During the alert state, patients may be able to discuss their illnesses and express fear of impending death. However, most often, death rapidly follows onset of coma unless intubation and ventilatory assistance are offered, in which case survival may be prolonged for months. During the comatose state, a variety of problems may be present, including cerebral edema, inappropriate antidiuretic hormone secretion, diabetes insipidus, and other manifestations of hypothalamic dysfunction; hypotension or arrhythmia; and pneumonia. Death caused by rabies in the acute stage occurs because of cardiac or respiratory problems. Cardiac arrhythmias with circulatory collapse commonly occur, and virus may be recovered at autopsy from the heart, which shows pathologic evidence of myocarditis. Respiration becomes increasingly labored, and death may occur during a laryngeal spasm or aspiration.

However, survival has been documented in a small number of individuals who had been vaccinated[7,38,91,178] and in a 15-year-old girl who was treated with coma induction, midazolam, ribavirin, ketamine, and amantadine.[252] The last two drugs may have rabies receptor blocking ability.

DIAGNOSIS

The diagnosis of rabies in humans begins with its diagnosis in the animal. The technique now most commonly used is to stain the brain of the previously apprehended animal with a fluorescein-labeled antibody to rabies. More recently, enzyme-coupled antibodies have been introduced, allowing the colored product to be seen under the light microscope. Rabies RNA also can be detected by dot hybridization or by RT-PCR amplification.[117] Virus isolation is used for confirmation, either by the classic procedure of intracerebral inoculation in mice or by inoculation of neuroblastoma cells followed by fluorescent antibody staining for rabies virus antigen.

Although the clinical expression of the disease in humans often is characteristic and establishment of the diagnosis is simplified by having a history of known exposure to an animal bite, encephalitic illness may occur, in which rabies virus involvement cannot be diagnosed with certainty without laboratory assistance.

When a patient has a history of being bitten by an animal, paresthesia at the wound site, and hydrophobia, determination of a clinical diagnosis of rabies is not difficult. Other diseases in which encephalitis occurs, such as those caused by arboviruses,

TABLE 196–2 Antemortem Diagnostic Test Results for 20 Human Patients with Rabies in the United States, 1980-1996

Test	No. of Patients Positive for Rabies Virus/Total No. Tested (%)	Earliest Positive (Day of Test Illness)
RT-PCR of saliva for rabies virus RNA	10/10 (100)	5
Brain biopsy for rabies virus antigen	3/3 (100)	8
Nuchal skin biopsy for rabies virus antigen	10/15* (67)	5
Virus isolation from saliva	9/15† (60)	5
Antibody to rabies virus in serum	10/18 (56)	5‡
Rabies virus antigen in touch impression from cornea	2/8 (25)	14
Antibody to rabies virus in cerebrospinal fluid	2/13 (15)	15§

**Two patients had earlier skin biopsy findings that were negative but became positive on subsequent biopsy.*
†One patient had an earlier test result that was negative.
‡Latest negative, 24 days; median to positive, 10 days.
§Latest negative, 24 days.
RT-PCR, reverse transcriptase–polymerase chain reaction.
Data from Noah, D. L., Drenzek, C. L., Smith, J. S., et al.: Epidemiology of human rabies in the United States, 1980 to 1996. Ann. Intern. Med. 128:922-930, *1998.*

enteroviruses, and herpes simplex virus, occasionally may cause confusion. However, if one finds signs of brain stem involvement in a patient whose sensorium basically is clear and who has no signs of a space-occupying lesion, the other diagnoses usually can be set aside.

Paralytic rabies may be misdiagnosed as Guillain-Barré syndrome, poliomyelitis, or post–rabies vaccine encephalomyelitis. Careful neurologic examination and analysis of the CSF often help rule out these diagnoses.

The spasms of tetanus can cause momentary confusion, but trismus is not part of rabies, and hydrophobia is not part of tetanus. Botulism (wound or ingestion) will cause paralysis, but the absence of sensory changes should exclude rabies.

Postvaccination encephalitis after nerve tissue vaccine poses a diagnostic problem in countries in which it still is used. It may require the performance of laboratory tests.

Perhaps the most confusing differential problem is hysteria in a person who thinks he or she has rabies. Normal blood gas analyses and the absence of variation in bizarre behavior suggest pseudorabies. Psychiatric and drug-induced reactions also may cause transient diagnostic problems.

Laboratory diagnosis now is possible before death. The virus may be demonstrated by fluorescent antibody stain of smears of corneal epithelial cells[126,195] or sections of skin from the neck at the hairline.[34,202] These test results are positive because virus migrates down the nerves from the brain, and both the cornea and hair follicles are richly innervated.

Serologic diagnosis also can be obtained if the patient survives beyond the acute period. In persons not given postexposure prophylaxis, only low levels of antibodies appear.[97] In contrast, patients who have received vaccine show a rapid rise in titer of virus-neutralizing antibodies between 6 and 10 days after the onset of symptoms.[89] Such antibodies are detected most rapidly by an in vitro rapid fluorescent focus inhibition test or by mouse neutralization tests. Rabies may be diagnosed in immunized persons by a rise in titer after the onset of clinical symptoms and is suggested by any antibody titer equal to or greater than 1:5000, a level not usually achieved by vaccines. High antibody levels in CSF are characteristic late in the course of rabies encephalitis; CSF antibody is not induced efficiently by vaccination.[89,234]

Rabies virus has been isolated from human saliva between days 4 and 24 after onset of disease.[89] Virus also may be isolated in some cases from CSF, brain tissue, or concentrated urine sediment during the first 2 weeks of illness. In persons surviving longer than 2 weeks, isolation of virus from body tissues or fluids (or from postmortem brain) may be impossible, presumably because of virus neutralization by humoral antibody.

Postmortem diagnosis can be confirmed by the presence of pathognomonic cytoplasmic inclusions (Negri bodies) in brain tissue, but they are present in less than 80 percent of cases. Rabies virus antigen may be detected by fluorescent antibody examination, with higher frequency in brain tissues of persons dying after a brief, acute course of disease. In postmortem tissues, histochemical staining with monoclonal antibody to rabies virus ribonucleoprotein especially may be useful because ribonucleoprotein possesses epitopes resistant to the formalin fixation and the paraffin-embedding process.[148] In studies of paraffin-embedded brain tissues (samples up to 40 years old), digestion of sections with proteinase K followed by immunofluorescence or RT-PCR assay gave 100 percent (300 of 300) positive results.[231] However, as in the case of virus isolation attempts, identification of virus antigen in brains of persons kept alive for prolonged periods after onset of disease may be extremely difficult.

Noah and coworkers[165] have summarized the results of attempts to diagnose rabies in humans pre mortem. Their data are summarized in Table 196–2,[165,172] which shows that RT-PCR analysis of saliva and brain biopsy are the most accurate methods of diagnosis, although even they may not be positive until the fifth day of illness.

Crepin and associates[56] had 100 percent success (9 of 9) in diagnosing rabies by use of RT-PCR assay of saliva accompanied by immunofluorescent examination of skin biopsy specimens. Most of these patients were diagnosed with specimens collected less than 5 days after onset of symptoms.

Rabies now has been recognized post mortem in patients whose history included transplantation of corneas from donors dying of encephalitis. All cases of undiagnosed fatal encephalitis, therefore, should be studied for rabies, and tissue from such patients should not be used for transplantation.[174]

POSTEXPOSURE PREVENTION[173]

LOCAL TREATMENT

Removal of saliva containing rabies virus is a crucial part of treatment and should be undertaken urgently. The wound should be flushed copiously with soap and water. Some authors, including us, would follow flushing with a local application of povidone-iodine or ethyl alcohol in whatever form is available immediately. The concentration of ethanol is important; 43 percent (86 proof) or higher gives the best results.[243]

Experimental data suggest that regardless of the solution used, adequate flushing is important, particularly in puncture wounds. Catheters should be inserted into puncture wounds and fluid

instilled by means of an attached syringe.[57,197] If this procedure proves to be too painful, the area can be anesthetized safely with local procaine-type anesthetics.[118,243] Suturing should be done only in line with good surgical practice and avoided when unnecessary.

EQUINE RABIES IMMUNE GLOBULIN

An experimental basis for the desirability of combining antiserum with vaccine was established in 1954.[127] A field test of combined vaccine-serum protection was made possible by a natural disaster, the attack of a rabid wolf on 29 Iranian villagers, 18 of whom were bitten on the head and neck.[23] Rabies developed in three of five persons who were treated with nerve tissue vaccine only but in only one of 13 persons who were given antiserum with nerve tissue vaccine.

Animal rabies serum is no longer available in the United States. However, an immune globulin from the serum of rabies-immunized horses (equine rabies immune globulin) is used extensively outside of the United States because of its lower price and greater availability (compared with human rabies immune globulin).[249] To reduce allergic reactions, pepsin digestion is used to convert the intact equine globulin to a $F(ab')_2$ preparation. The product is dispensed at a concentration of 200 IU/mL, and the dose that should be administered is 40 IU/kg, as much as possible infiltrated into the wound. Administration of higher doses may dampen the active immune response. The manufacturers recommend an intradermal skin test with equine products, but the sensitivity and specificity of the test for prediction of subsequent allergic reactions are poor, and abandonment of the skin test has been proposed.[250] At least one preparation of equine rabies immune globulin was purified sufficiently so that the allergic reaction rate was less than 1 percent,[248] whereas another yielded a 6 percent reaction rate.[247] Anaphylaxis with current equine rabies immune globulin rarely occurs.[193] A new equine rabies immune globulin is now available, heat treated to reduce the risk of viral contamination.[135] Because of shorter half-lives of fractional $F(ab')_2$ preparations, the World Health Organization (WHO) recommends human rabies immune globulin for severe bite exposure.

HUMAN RABIES IMMUNE GLOBULIN

To avoid possible reactions to animal proteins, gamma globulin was prepared from the plasma of volunteers hyperimmunized with rabies vaccine. Although expensive, human rabies immune globulin is preferable to equine rabies immune globulin for use in postexposure prophylaxis in the United States. Because the gamma globulin is homologous in humans, human rabies immune globulin persists longer in the circulation of inoculated persons but, for this reason, may have an even greater dampening effect on active immunization. Thus, Hattwick and associates[90] found that 23 doses of the old duck embryo vaccine were needed to overcome the suppressive effect on antibody production of 15 to 40 units of human rabies immune globulin per kilogram.

Pharmacokinetic measurements[95,142,146,186] resulted in a recommendation to give 20 IU of human rabies immune globulin per kilogram immediately, with as much as possible being injected locally. Local injection is important because serum levels of antibody after intramuscular injection are not high.[47] Although intravenous administration of human rabies immune globulin produces higher serum titers,[12] local injection still is preferred. No further dose is necessary or desirable because excessive antibody diminishes the active response to vaccine. Two equivalent preparations are available in the United States, one produced by Sanofi Pasteur and the other by Talecris.

If neither equine nor human rabies immune globulin is available, vaccination should be started immediately, followed by administration of human rabies immune globulin if it arrives within a week.[121] Because of the expense and occasional difficulty in obtaining adequate supplies of human rabies immune globulin as well as substantial variability of immunoglobin products tested in an animal model,[88] the WHO now is exploring the possibility of using instead "cocktail" mixtures of neutralizing monoclonal antibodies to rabies virus prepared in either murine[196] or human[62] hybridoma cell cultures. Such cocktails have shown effectiveness in animal studies.[22,86,179]

NERVE TISSUE VACCINE

Although used for 100 years following the introduction of the original Pasteur vaccine, nerve tissue vaccines are being replaced increasingly by cell culture vaccines in developing countries. Vaccines made in sheep brain or goat brain were widely used throughout Asia and Africa but were associated with a definite incidence of postvaccinal encephalitis.[14] The susceptibility to rabies vaccine–induced encephalomyelitis has been suggested to vary according to the genetic pattern of major histocompatibility class II alleles in the vaccinated host.[171] Suckling mouse brain vaccines are still used to some degree in Latin America to decrease sensitization to myelinated nerve tissue, which is thought to be the cause of autoimmune allergic encephalitis.[83]

However, a study from Tunisia found no evidence for humoral response to myelin in patients with encephalitis after receiving nerve tissue vaccine. Instead, antibodies to GM_1 and GD_{1a} gangliosides present on human cells were demonstrated.[138] Thus, the mechanism of encephalitis remains uncertain, beyond the conclusion that it results from non-rabies (virus) antigens in the nervous systems of animals, including sheep, lambs, and mice.

The efficacy of nerve tissue vaccine in humans never was evaluated by controlled studies. The Pasteur Institute of Southern India analyzed the incidence of disease in vaccinated and unvaccinated people bitten by animals that were proved rabid by the transmission of rabies to other animals or humans. Fifty-six percent of untreated persons developed rabies, compared with only 7 percent of vaccinated persons, for an effectiveness of approximately 88 percent.[225]

On the other hand, vaccination with nerve tissue vaccine of persons bitten on the head or neck by rabid wolves in Iran still was followed by a 40 percent overall mortality rate. In comparison, 15 of 32 villagers (47%) who failed to seek treatment after being attacked by a rabid wolf developed rabies.[101]

DUCK EMBRYO VACCINE

Duck embryo vaccine was developed to overcome the problem of encephalitic reactions to nerve tissue vaccine. In this respect, it was successful; the incidence of neuroparalytic reactions fell from 1 in 1000 with nerve tissue vaccine to 1 in 25,000 with duck embryo vaccine.[173,185,194] Unfortunately, a high price was paid in immunogenicity. In postexposure treatment of Americans, Corey and associates[54] found that 8 percent of those given courses of duck embryo vaccine failed to respond with the production of measurable antibody. Even more disturbing was the fact that 23 percent of recipients of duck embryo vaccine plus rabies antiserum did not develop antibody. Duck embryo vaccine contained enough duck protein to produce a significant number of allergic reactions.[185] Several human rabies cases were reported despite the use of duck embryo vaccine combined with serum.

CELL CULTURE VACCINES[175]

The ideal solution to the problems of immunogenicity and the safety of rabies vaccine clearly lay in the development of vaccines prepared from rabies virus grown in cell culture free of neural tissue. To avoid inclusion of foreign host proteins in the vaccine, the optimal cell cultures would be derived from humans.

The basic ingredients for the production of human diploid-cell vaccine (HDCV), the first cell culture vaccine widely used, were the development of the WI-38 normal human fibroblast cell line by Hayflick and Moorhead[93] and the adaptation of the Pitman-Moore strain of rabies virus to growth in WI-38 by Wiktor and associates.[241] Virus grown in human fibroblasts was concentrated by ultrafiltration to increase antigen content and then inactivated by beta-propiolactone.

After various schedules of HDCV were tried, researchers found that three properly spaced intramuscular doses invariably produced an immune response. A similar schedule of duck embryo vaccine resulted in titers 10 to 20 times lower. The excellent immunogenicity of HDCV has been confirmed amply,[3,13,35,55,85,177,222,246] and it became the "gold standard" against which other vaccines were measured.

Table 196–3 shows the serologic data of three persons who received postexposure prophylaxis.[176]

The crucial test of a rabies vaccine is protection of those actually exposed to the virus. In Europe, the HDCV has been used to treat thousands of people exposed to possible rabies. However, those situations in which rabies was confirmed in the biting animal are particularly important to consider. Kuwert and associates,[130,131] in Essen, vaccinated 68 persons after exposure to dogs, cats, cows, or wild animals with laboratory-confirmed rabies virus infection or exposed as a result of a laboratory accident. The schedule used was 1 mL intramuscularly on days 0, 3, 7, 14, 30, and 90. They had no failures of protection, no significant reactions, and excellent neutralizing and complement-fixing antibody responses.

Bahmanyar and associates[21] in Iran conducted another test of HDCV. Forty-five persons who were bitten by rabid wolves or dogs were given rabies antiserum, followed by the same schedule of vaccine as that used in Germany. Once again, no rabies was seen in vaccinees, despite a 40 percent risk (estimated from previous experience) if they had remained unvaccinated. Antibody measurements showed mean titers as follows: 7 days, 1.1 IU; 14 days, 10.7 IU; 30 days, 49 IU; and 100 days, 312 IU.

The Centers for Disease Control and Prevention (CDC) distributed HDCV of American manufacture after exposure to persons whose exposure to rabies was established.[8] No vaccine failure occurred, and all who received the full schedule of five 1-mL doses intramuscularly responded with antibodies to date.

Although a small number of vaccine failures have been reported in other countries after administration of the HDCV, ancillary prophylaxis recommendations were not followed scrupulously in each case.[10] The failure rate is estimated to be at most 1 in 12,000 courses of vaccination given in countries with high risk for rabies, such as Thailand.[164] General anesthetics given while the wound is repaired may increase the risk of vaccine failure.[76]

An effort has been made to produce vaccines with the desirable properties of HDCV at a lower cost that would make them broadly available in the developing nations, where the risk of human rabies is most severe.

Purified Vero cell rabies vaccine (PVRV) is the Pasteur strain of rabies (as used in HDCV) grown in the Vero cell line in industrial fermenters. The virus is concentrated, inactivated, and purified. PVRV has an immunogenic potency similar to that of HDCV. Successful postexposure experience has been reported in Tunisia,[43] China,[230] and Thailand, even after severe exposure.[110,209,234] The Vero cell vaccine has been purified further by chromatography.[136]

Purified chick embryo-cell (PCEC) rabies vaccine is produced in primary chick embryo-cell culture with use of the Flury low egg passage (LEP) strain of rabies virus. It is inactivated, concentrated, and purified, yielding a vaccine preparation of antigenic potency similar to that of PVRV and HDCV.[24] PCEC rabies vaccine was uniformly successful in preventing rabies in postexposure trials in the former Yugoslavia, including subjects who were exposed by wolf bite.[52,227] PCEC rabies vaccine also was shown to be equivalent to HDCV for postexposure immunization,[180] for boosting previously immunized subjects,[32] and for protection of mice against bat strains of rabies.[63]

Purified duck embryo-cell rabies vaccine is produced in embryonated duck eggs. The Pitman-Moore virus harvested from the eggs is purified, concentrated, and inactivated.[120]

Primary hamster cell kidney vaccine has been produced in China and was demonstrated to be immunogenic and to provide postexposure protection.[73] It now has completely replaced Semple vaccine in China.[73]

An argument has been made that a potent rabies virus might be made at low cost in continuous BHK-21 cells, and if it were inactivated with beta-propiolactone, the beta-propiolactone would so damage the cell DNA that the theoretical danger posed by cell DNA in vaccine would be eliminated totally.[155]

TABLE 196–3 Antibody Response in Three Children to Postexposure Rabies Vaccination with Human Diploid-Cell Vaccine

	Inoculation Schedule and Antibody Titers (IU) by Rapid Fluorescent Focus Inhibition Test					
Time after Vaccination	**Case 1**		**Case 2**		**Case 3**	
0 day*	V (+1 day)†	<0.5	V (+3 days)†	<0.4	V (+5 days)†	<0.3
3 days	V				V	
7 days	V	<0.1	V	0.6	V	<0.3
14 days	V	13.5	V	1.1	V	0.4
21 days			V			
28 days				17	V	27
30 days	V	20				
38 days		30				
42 days				90		30
3 months		35				
4 months				22		7
7 months						
18 months				2.4		

**Day of first vaccination is day 0.*
†Number of days after bite on which human diploid-cell vaccine first was administered.

Three types of rabies vaccine are licensed in the United States: HDCV; PCEC rabies vaccine; and a tissue culture vaccine made in fetal rhesus diploid cells by the Michigan State Department of Health, called *rabies vaccine adsorbed* (RVA), but which is not now distributed.[25] HDCV or PCEC rabies vaccine is recommended for postexposure prophylaxis when it is given by the intramuscular route according to the regimens listed in Table 196–4. The dose should not be reduced for children.[12] Decreased immune responses have been noted in vaccinees given injections in the gluteal area,[79] and therefore all intramuscular injections of rabies vaccine should be given in the deltoids. In the event of re-exposure of persons with a prior history of vaccination, two intramuscular booster doses should be given, as also stated in Table 196–4. Virtually 100 percent of these patients will have an anamnestic response.[211]

Numerous regimens have been developed for rapid immunization of exposed patients in poor countries who may seek medical help late or who may not return for subsequent doses.[208,228,234] To reduce costs, advantage is taken of the smaller volume needed for intradermal administration. However, no intradermal vaccine is licensed in the United States (see later). These regimens are listed in Table 196–4 and have the advantage of being less expensive. Note that if the 2-1-1 regimen is chosen, immune globulins will interfere with the response to the vaccine.[6]

The effectiveness of current rabies vaccines to protect against most rabies-related lyssaviruses appears to be good.[87,159]

DECISIONS TO VACCINATE (Table 196–5)

The physician must take into account numerous human and zoologic factors in deciding when to vaccinate, although reluctance to use vaccine now is based more on cost than on the pain of injections. A study performed in the United States[154] revealed that administration of rabies prophylaxis was inappropriate in 40 percent of cases in which it was given. However, the study also showed that 6 percent of those not receiving prophylaxis should have received it. Thus, the judgment of physicians needs improvement, not only for medical reasons but also because some incidents of exposure to rabies result in vaccination of many persons, with high cost.[184] Among the factors to be considered are the following:

Pregnancy

Pregnant women tolerate rabies vaccination without problem.[49,206]

Geography

Terrestrial animal bites in most large urban areas in the developed world are unlikely to be from rabid animals, although Philadelphia, Baltimore, New York, and Washington, DC, have become part of the raccoon epizootic, with transfer of disease to urban cats.

TABLE 196–4 Regimens for Pre-exposure and Postexposure Vaccination with Rabies Vaccines

Vaccination	Route	Days on Which Doses Are Given	Remarks
Pre-exposure	IM†	0, 7, 21, or 28	Standard U.S. 3-dose regimen
	ID‡	0, 7, 21, or 28	Economical, but not to be used in those taking antimalarial medications; unlicensed route in U.S.
Postexposure*	IM†	0, 3, 7, 14, 28	U.S. and WHO 3-dose recommendation
	IM†	0 (2 doses), 7, 21	Used in some countries when RIG is not indicated; so-called 2-1-1 schedule
	ID‡	0, 3, 7 (2 doses each), 28	Used in Thailand with PVRV, PCECV; so-called 2-2-2-0-1 schedule
	ID‡§	0 (8 doses), 7 (4 doses), 28, 90	Used in developing countries with HDCV, PCECV, or PVRV cell culture vaccines; so-called 8-0-4-0-1-1
Booster (for re-exposure)	IM†	0, 3	Only after documented vaccination with cell culture vaccine‖
	ID‡	0, 3	Only after documented vaccination with cell culture vaccine‖

**Together with rabies immune globulin.*
†Give 0.5 mL (PVRV) or 1.0 mL, depending on the vaccine, into the deltoid.
‡Give 0.1 mL, over the deltoids.
§Give 0.1 mL at multiple sites (see text).
‖Or demonstrated presence of virus-neutralizing antibodies after other vaccines.
ID, intradermal; IM, intramuscular; HDCV, human diploid-cell vaccine; PCECV, purified chick embryo-cell vaccine; PVRV, purified Vero cell rabies vaccine; RIG, rabies immune globulin.

TABLE 196–5 Rabies Postexposure Prophylaxis Guide—United States[34]

Animal Type	Evaluation and Disposition of Animal	Postexposure Prophylaxis Recommendations
Dogs, cats, and ferrets	Healthy and available for 10 days of observation	Persons should not begin prophylaxis unless animal develops clinical signs of rabies*
	Rabid or suspected rabid	Immediately vaccinate
	Unknown (e.g., escaped)	Consult public health officials
Skunks, raccoons, foxes, and most other carnivores; bats	Regarded as rabid unless animal proven negative by laboratory tests†	Consider immediate vaccination
Livestock, small rodents, lagomorphs (rabbits and hares), large rodents (woodchucks and beavers), and other mammals	Consider individually	Consult public health officials Bites of squirrels, hamsters, guinea pigs, gerbils, chipmunks, rats, mice, other small rodents, rabbits, and hares almost never require antirabies postexposure prophylaxis.

**During the 10-day observation period, begin postexposure prophylaxis at the first sign of rabies in a dog, cat, or ferret that has bitten someone. If the animal exhibits clinical signs of rabies, it should be euthanized immediately and tested.*
†The animal should be euthanized and tested as soon as possible. Holding for observation is not recommended. Discontinue vaccine if immunofluorescence test results of the animal are negative.

Type of Animal

Cats always are suspect if they go out of their way to bite. Dogs on the Mexican border are more suspect than are those farther north. Skunks, foxes, and raccoons involved in biting incidents must be considered rabid until proven otherwise. With the exception of woodchucks, rodents such as squirrels are unlikely to be rabid.

Bat Exposures

As stated before, rabies transmission from bats has become a threat to humans in the United States owing to an apparent increase in exposures to silver-haired and Eastern pipistrelle bats. The in vitro characteristics of the bat rabies strains, which may allow them to replicate better in non-neural tissues, plus the small and sometimes imperceptible bites these species inflict, led to the recommendation that vaccination may be appropriate when bats are handled without gloves and when sleeping individuals awake to find a bat in the room.[3]

Circumstances of Bite

The attempt to feed an undomesticated animal always must be considered provocative behavior. Invasion of an animal's territory may result in an attack, which is less suggestive of rabies than is an attack by an animal that invades human environments. However, judgment as to whether a bite was provoked is poorly predictive of rabies in enzootic areas.[127] If an animal appears clinically rabid, it should be euthanized immediately for confirmation of rabies by examination of the brain. If a dog, cat, or ferret appears normal, it may be kept for 10 days to see if it develops rabies.[150] Meanwhile, exposed humans should or should not be started on prophylaxis, depending on the known prevalence of rabies in the particular region.

Animal Vaccination

Rabies is an extremely rare occurrence in properly vaccinated animals.

Table 196–5 summarizes recommendations by the Advisory Committee on Immunization Practices on vaccination against rabies.[3] Advice can be sought from state and local health departments (particularly with regard to the occurrence of rabies in animals) and from the CDC directly.

FAILURE OF RABIES PROPHYLAXIS

The most common cause of vaccine failure is that immune globulin was not used simultaneously.[59,84,251] Active immunization will not regularly produce antibodies until 7 to 14 days after the first dose is given. Lack of local infiltration of immune globulins and the immunosuppressive effect of chloroquine may account for other failures. The presence of B-cell immunodeficiency in a patient requires measurement of antibodies after vaccination. Patients infected with human immunodeficiency virus (HIV) are likely to respond if their $CD4^+$ lymphocytes are higher than 300/μL. If not, their postvaccination antibodies also should be measured.[110] In HIV-infected children, a standard postexposure course of HDCV had no effect on the level of $CD4^+$ lymphocytes or the HIV viral load.[215] However, even with correct prophylaxis, failures may occur, perhaps because in severe exposures, virus is deposited directly onto nerve endings[100] or because of inadequate monitoring of vaccine and rabies immune globulin potency in developing countries.[18]

PRE-EXPOSURE IMMUNIZATION (Table 196–6)

The development of modern vaccines has made effective immunization of persons at risk of coming into contact with rabies virus

TABLE 196–6 Rabies Pre-exposure Prophylaxis Guide—United States[34]

Risk Category	Nature of Risk	Typical Populations	Pre-exposure Recommendations
Continuous	Virus present continuously, often in high concentrations	Rabies research laboratory workers*	Primary course
	Specific exposure likely to go unrecognized Bite, nonbite, or aerosol exposure	Rabies biologics production workers	Serologic testing every 6 months; booster vaccination if antibody titer is below acceptable level†
Frequent	Exposure usually episodic with source recognized, but exposure also might be unrecognized	Rabies diagnostic laboratory workers*	Primary course
	Bite, nonbite, or aerosol exposure Veterinarians and staff Animal-control and wildlife workers in rabies-enzootic areas	Spelunkers	Serologic testing every 2 years; booster vaccination if antibody titer is below acceptable level†
Infrequent (greater than in population at large)	Exposure nearly always episodic with source recognized	Veterinarians and animal-control and wildlife workers in areas with low rabies rates	Primary course
	Bite or nonbite exposure	Veterinary students Travelers visiting areas where rabies is enzootic and immediate access to appropriate medical care including biologics is limited	No serologic testing or booster vaccination
Rare (population at large)	Exposure always episodic with source recognized Bite or nonbite exposure	U.S. population at large, including persons in rabies-epizootic areas	No vaccination necessary

**Judgment of relative risk and extra monitoring of vaccination status of laboratory workers are responsibilities of the laboratory supervisor.*

†Minimum acceptable antibody level is complete virus neutralization at a 1:5 serum dilution by the rapid fluorescent focus inhibition test. A booster dose should be administered if the titer falls below this level.

possible before the actual exposure occurrs. Veterinarians, animal handlers, laboratory workers, and spelunkers need pre-exposure immunization. Large numbers of veterinary students have been vaccinated under a three-dose schedule at 0, 7, and 21 or 28 days. Rabies virus antibody titers were determined on the serum of each veterinary student. Nearly 100 percent developed antibodies, with geometric mean titers of 10 IU or greater, which is equivalent to a neutralizing antibody titer of at least 1:200.[183] The three-dose regimen listed in Table 196–4 induces antibodies in 100 percent of recipients. Pre-exposure immunization may be given with use of HDCV, PCEC rabies vaccine, or RVA by intramuscular administration. Outside the United States, any one of the alternative cell culture vaccines can be used on the same schedule.[191] Alternatively, and more economically, immunization may be performed intradermally according to the same schedule.

Because rabies in developing countries is primarily a disease of children, exploratory studies have been performed to determine if pre-exposure vaccination could be incorporated into routine pediatric schedules. Preliminary results from Vietnam show that routine rabies vaccination in infancy is feasible[137] and could even be performed by low-dose intradermal administration.[71] In areas enzootic for canine rabies, universal vaccination of children can be cost-effective if the incidence of dog bite is high.[48] Children infected with HIV can be immunized successfully if their $CD4^+$ cells exceed 15 percent of lymphocytes. Those with fewer $CD4^+$ cells will need at least double doses of rabies vaccine.[215]

Missionaries and Peace Corps personnel operating in rabies-endemic countries should receive pre-exposure vaccination.[16]

INTRADERMAL VACCINATION

For pre-exposure use, the intradermal route is considered an acceptable alternative to intramuscular injection, with the important proviso that persons receiving antimalarial or other immunosuppressive agents and perhaps older persons should have their titers checked after vaccination or receive the injections by the intramuscular route.

The success of intradermal vaccination depends on a technique that ensures intradermal rather than subcutaneous injection, but a margin of error exists.[26,78]

Today, essentially all intradermal vaccinations are done with PVRV and PCDC rabies vaccine. To reduce the costs of vaccination, intradermal vaccination for postexposure use has become popular in developing countries, but it no longer is approved in the United States. Although single-dose preparations are not available for the intradermal administration of the 0.1-mL dose, extensive experience in Thailand and elsewhere has validated the successful prevention of rabies with vaccine extracted from vials intended for intramuscular use.[71,223,236,237] However, attention must be paid to the correct administration of the dose into the skin, the sterility of unused portions of the vial, the volume of vaccine in the ampule, and the antigenic content of the vaccine used. The antigenic content should be at least 0.25 IU per 0.1 mL. The most popular intradermal schedule is the one developed by the Thai Red Cross, consisting of inoculations on days 0, 3, 7, and 28 days, with double doses given in the first three administrations. A former recommendation for a 90-day dose has been abandoned.[122] Poor responses to intradermal vaccine have been noted in those concurrently receiving chloroquine or immunosuppressives, such as corticosteroids.[3,168] Therefore, persons who must be vaccinated while they are taking chloroquine or related antimalarials should be given injections into the deltoid muscle, and postvaccination rabies serologic studies should be obtained on those patients and others who are immunosuppressed.

ALTERNATIVE SCHEDULES

Although the WHO schedules are firmly established for the induction of optimal immune responses, other schedules have been tested extensively to reduce the number of vaccination visits, particularly in the developing world. The most popular of these are the 2-1-1 schedule, in which a double dose is given intramuscularly at day 0, followed by single doses on days 7 and 21,[50,149] and the regimen developed by Warrell and associates,[236] consisting of eight intradermal doses on day 0, four intradermal doses on day 7, and single doses on days 28 and 91.

The 2-1-1 schedule may not be reliable if immune globulins also are administered.[134,136]

BOOSTER DOSES

Even with administration of cell culture vaccine, antibodies fall off rapidly after initial immunization, although most cell culture vaccine recipients have some detectable antibody at 2 years after initial vaccination. Nonetheless, once an immune response to rabies vaccine has developed, revaccination is almost certain to evoke a rapid response.[211] One booster of cell culture vaccine given to previously vaccinated persons results in a dramatic anamnestic response, with titers in one study rising from 2.8 IU to 94 IU at 14 days and more than 100 IU in 35 days.[175] As mentioned, with exposure to rabies in a previously vaccinated person, two intramuscular booster doses are recommended to provide a margin of safety. Single intramuscular or intradermal boosters are given to maintain immunity in individuals chronically exposed to rabies, according to the recommendations made by the CDC in Table 196–6 if serology suggests a waning titer.

In persons who have received pre-exposure immunization to rabies, the necessity of boosters is an important issue. Persons who definitely were exposed to a rabid animal should receive two booster doses of vaccine by the intramuscular route. Individuals who are likely to be exposed, such as rabies laboratory workers, are boosted to maintain their antibody titers above 0.5 IU (or complete neutralization at a dilution of 1/5). A follow-up study by Briggs and Schwenke[33] is interesting. They found maintenance of an adequate titer (>0.5 IU) at 1.5 to 2 years after vaccination in 99 percent of subjects who received vaccine by the intramuscular route and in 93 percent who received vaccine by the intradermal route. In Thailand, researchers demonstrated that persons who received pre-exposure rabies vaccination by the intradermal route mounted a slow response to boosters, and the authors suggested that in severe exposures, rabies immune globulin should be given despite the prior immunization.[110] However, the intradermal route can be used successfully to boost prior immunity.[213] Thraenhart and associates[217] observed 100 percent positive antibodies in 18 subjects studied between 2 and 14 years after they received vaccination. Thus, if a person is properly vaccinated with modern cell culture vaccines, subsequent boosting with rabies immune globulin is not indicated in postexposure prophylaxis.

ADVERSE REACTIONS

The available tissue culture vaccines are well tolerated. In more than 1770 human volunteers receiving pre-exposure immunization with HDCV administered intramuscularly, sore arm was noted in approximately 20 percent, headache in about 8 percent, malaise in 5 percent, and allergic edema in 0.1 percent.[175] During incidents involving mass postexposure prophylaxis, pain, swelling, and other local symptoms occurred in 30 to 74 percent of individuals.[3] Pregnancy is not a contraindication to receiving modern rabies vaccines.[49] Guillain-Barré syndrome and other

TABLE 196–7 Some Rabies Vaccines Developed for Humans

Vaccine Types	Remarks
Pasteur: dried rabbit spinal cord	Residual live virus
Fermi: phenolized sheep or goat brain	Residual live virus
Semple: phenol-inactivated sheep or goat brain	Contains nerve tissue
Fuenzalida: phenol-inactivated suckling mouse brain	Contains less myelin
Duck embryo: BPL inactivated	Allergy to duck proteins
Human diploid cell (HDCV): BPL-inactivated fetal human cell culture vaccine	Current standard; booster allergic reactions
Rhesus diploid cell (RVA): fetal rhesus cell culture, BPL inactivated	Fewer allergic reactions
Vero cell (PVRV): BPL-inactivated virus	Purified by density gradient centrifugation; grown in Vero monkey kidney cell line
Chick embryo cell (PCEC rabies vaccine): inactivated virus grown in chick embryo cells	Purified like PVRV
Vaccine recombinant glycoprotein (VRG): genetic construct expressing rabies	Probably will be used only in animals (see text)

BPL, beta-propiolactone.

neurologic problems have been rare occurrences, and their relationship to HDCV is uncertain.[125] Guillain-Barré syndrome that occurs after administration of nerve tissue vaccine is associated with antibodies to myelin basic protein.[99]

In contrast, booster vaccinations with HDCV have been associated with allergic reactions in approximately 6 percent of subjects.[1] These reactions are caused by the presence in the vaccine of human albumin that has been altered by the beta-propiolactone used to inactivate the virus.[9,212,238] The reactions are of the immune complex type (type III), with urticaria, edema, joint manifestations, fever, and malaise. CDC data suggest that when primary vaccination is given intramuscularly and booster intradermally or vice versa, reactions are more common than if all vaccination is by the same route.[80] Because the reaction is associated with the particular formulation of HDCV rather than with the rabies virus antigen itself, additional boosters may be given if necessary with PCEC rabies vaccine, PVRV, or HDCV manufactured in Canada (Sanofi Pasteur).[77] Reactions to PVRV and PCEC cell culture vaccines generally have been mild.[69] A comparative study of PVRV with HDCV showed lower or equivalent rates of local and systemic reactions, with no serious adverse events.[116] Because PCEC rabies vaccine is manufactured in chicken cells, egg allergy is a possible problem, and anaphylactic events as well as some rare and perhaps unrelated neurologic complications have been reported.

FUTURE DEVELOPMENTS

The world of rabies is far from static. Table 196–7 shows the progress of rabies vaccine development for humans. Perhaps the most dramatic future prospect is the development of vaccines in which the gene for rabies glycoprotein has been inserted into a viral vector, such as vaccinia, or the use of rabies virus itself as a cloning and expression vector system by reverse genetics.[36,190] These vaccines already have been used extensively in a large variety of animal species, in which they have been found to be safe and highly immunogenic.[188] In field studies, raccoons and other wild animals have been immunized successfully by the oral route with baits containing rabies recombinant vaccine. Such vaccination has eliminated fox rabies from large areas of western Europe[30] and reduced raccoon rabies in the eastern United States.[189,198] Other avenues being pursued include the use of potent adjuvants to enhance the immunogenicity of subunit vaccine,[75] the addition of the internal ribonucleoprotein to enhance protection through induction of cellular immune responses and higher antibody responses,[218] synthetic peptides constructed from epitopes of the single glycoprotein and nucleoprotein,[61] and plasmid vectors containing cDNA of the glycoprotein gene.[255]

Particular effort has been directed toward the development of DNA vaccines. They are alleged to be potentially inexpensive and particularly thermostable, but to date most have required an unsatisfactory interval to produce virus-neutralizing antibodies or to have induced virus-neutralizing antibodies of inadequate titer.[139-141] Dogs given two doses of Pasteur strain G protein DNA, administered intramuscularly, were shown to develop virus-neutralizing antibodies to rabies and to European bat lyssaviruses 1 and 2 and to have protection against virus challenge.[170] Jallet and colleagues[111] produced chimeric G DNAs of rabies and EBL1, or rabies and Mokola virus, to generate vaccines designed to induce virus-neutralizing antibodies in dogs to either all European lyssaviruses or all African lyssaviruses, respectively. In primates, rabies G DNA induced virus-neutralizing antibodies only if it was administered by gene gun.[141]

Other approaches have included efforts to express rabies G protein in yeast cells, which failed,[216] and in baculovirus in insect cells, which was successful. Although it is less glycosylated than is the virion G protein, the baculovirus-expressed G protein induced virus-neutralizing antibodies efficiently in mice; its immunogenicity was not improved by adding baculovirus-expressed rabies N protein.[70] In approaches using the rabies G protein cloned into adenovirus, researchers have shown that a replication-defective recombinant elicited enzyme-linked immunosorbent assay antibodies in mice inoculated onto mucosal surfaces[254] and that an adenovirus incorporating a special promoted-intron expression cassette efficiently induced rabies G protein in cell culture that induced virus-neutralizing antibodies in intraperitoneally inoculated mice.[145] The rabies G protein gene was cloned into canine herpesvirus; this recombinant induced virus-neutralizing antibodies efficiently in dogs inoculated by the intranasal route, suggesting that it might be useful as an oral product to control canine rabies.[256] Similar hopes are offered by a new recombinant canine adenovirus vaccine.

Molecular biologic approaches have not yet produced an ideal vaccine, especially for human use. In the near future, the most cost-effective and protective vaccines may be virion products propagated to high titer in an easily managed cell culture system, for example, the BHK-21 continuous cell line or transgenic plants.[153] Also, monoclonal antibodies may offer an effective alternative to human rabies immune globulin. Regardless of developments in human medicine, the key to future progress is a focus on elimination of canine rabies.

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SUBSECTION 9

Arenaviridae and *Filoviridae*

CHAPTER 197

Lymphocytic Choriomeningitis Virus Infection

Rémi N. Charrel ❂ Xavier de Lamballerie

Lymphocytic choriomeningitis virus (LCMV) belongs to the genus *Arenavirus*, family *Arenaviridae*. *Mus musculus* (the common house mouse) constitutes the reservoir of LCMV in nature, but hamsters can also carry the virus. Humans usually become infected through direct contact with infected rodents or by inhaling infectious rodent excreta or secreta during occupational exposure (laboratory workers, rodent sellers) or when caring for rodents as pets. Although infection with LCMV usually is asymptomatic or mild and self-limiting, it can be severe and manifest as meningitis and encephalitis. Infection during pregnancy may cause abortion or congenital malformations.

HISTORY

In the 1930s, LCMV was discovered, at about the same time and independently, in three different localities in the United States. Armstrong and Lillie encountered the agent in a monkey when they passaged a recent isolate of St. Louis encephalitis virus.[3,6] In 1935, Rivers and Scott[47] described the first two cases of human infection with LCMV. Both these patients developed febrile illnesses characterized by headache, vomiting, and stiff neck; lumbar puncture revealed 720 and 1700 cells/mm^3, nearly all of which were lymphocytes. These investigators subsequently isolated five strains from patients with meningitis.[47] Traub[52] revealed the virus in a colony of albino mice. Mice were incriminated in these first isolations, and later observations proved beyond a doubt that *M. musculus* is the principal reservoir of LCMV in nature. However, not until 2007 was definitive evidence presented that LCMV-infected rodents caused disease in humans, as established by genetic comparative analysis.[24] For some time after its discovery, LCMV was regarded as the sole etiologic agent of Wallgren acute aseptic meningitis. However, it became clear that Wallgren syndrome had a multitude of viral causes, among which LCMV was of little relevance. In the years after other etiologic agents of acute aseptic meningitis, such as enteroviruses, mumps, or herpesviruses, were identified, LCMV progressively disappeared from the sight of virologists and general practitioners. This situation is exemplified by the low numbers of human cases or outbreaks of LCMV infection reported in the medical literature (<50 articles). Meanwhile, LCMV was used as an excellent model for the study of a variety of phenomena of biologic and medical relevance, such as immunologic tolerance, viral immunopathology, slow viral diseases, and latent viral infections, the last demonstrated by the large number of scientific articles on that topic (>2500 references in the PubMed bibliographic database found using "lymphocytic choriomeningitis" as the search criterion).

EPIDEMIOLOGY

LCMV belongs to the genus *Arenavirus*, family *Arenaviridae*. *M. musculus* (the common house mouse) constitutes the reservoir of LCMV in nature, but hamsters also can carry the virus. Humans usually become infected through direct contact with infected rodents or by inhaling infectious rodent excreta or secreta during occupational exposure (laboratory workers, rodent sellers) or household exposure (pet owners). The epidemiologic features vary when animals other than *M. musculus* are involved. Thus, LCMV transmitted from Syrian hamsters has occurred in areas where infected mice are not found. Altogether, 47 human cases have been traced to these pets and may have caused family outbreaks but no epidemics.

Human infection with LCMV is a rare occurrence. Specific tests conducted with diagnostic materials from several large hospitals revealed an incidence seldom exceeding one case per year.[48] Because *M. musculus* is the reservoir from which most human infections can be traced, efforts were made to clarify the geographic distribution of LCMV-carrying mice. Of 1795 house mice trapped in Germany, 65 carried the virus.[1] In 44 of 376 trapping areas, infected mice were found; these 44 positive sites were unevenly distributed, with a majority located in northern and northwestern Germany. In a later serologic survey, the proportion of people with LCMV-specific antibodies was significantly higher in rural areas where infected mice were known to live as compared with zoonosis-free districts. In areas where LCMV-carrier mice are frequently found, 9.1 percent of the rural population had neutralizing antibodies. In contrast, only 1.2 percent of the people residing in southern Germany, which is essentially free of LCMV-carrying mice, had neutralizing antibodies. Extrapolating from these statistics, approximately 1000 persons are newly infected each year in Germany. Thus, researchers estimate that in western Germany, at least 72,000 persons must have had an infection, based on an average life expectancy of 70 years and an approximate rate of new cases of 1000 per year. If this estimation is correct, most infections are not diagnosed, and many remain inapparent.

The occurrence of LCMV infection in persons from homes where mice have been proved to be carriers of the virus is well known.[6] Researchers have suggested that small, persistent zoonotic foci of infection exist, on the basis of the known natural history of the disease in mice and the circumscribed range of activity of mice. The percentage of infected house mice apparently varies in different communities, and it has been recorded at 21.5 percent in Washington, D.C.[5] and at 4 percent in Boston and New York.[33,56] However, because infected mouse colonies are dispersed unevenly, the proportion of infected animals in a given infected colony can be high, up to 95 percent.[24,27]

Many human cases have been reported in the context of occupational exposure to rodent colonies.[7,12,14,15,21,23,29-31,36,40,53] Outbreaks and isolated cases of LCMV infections were reported in laboratory personnel having contact with hamsters, not mice.[12,37,38]

Until 2005, no case of person-to-person transmission had been documented, and the general consensus was that LCMV could infect humans only after direct or indirect contact with an infected rodent. The more recent evidence that LCMV can be transmitted through organ donation should lead physicians to evoke LCMV as a possible cause of infection in the absence of established close contact with mice or hamsters.[16,26] In a report of LCMV infection developing after organ transplantation, the fatality rate was much higher (seven deaths of eight cases) than previously noted, perhaps a reflection of the immunosuppressive regimens required by these transplant recipients.[26] Another, similar episode occurred in Australia, where three patients died after receiving an organ transplant from the same donor. Metagenomic approaches provided clues about possible involvement of an arenavirus related to LCMV.[35,45]

Serologic studies conducted in healthy populations of large cities either by immunofluorescence assay (IFA) or enzyme-linked immunosorbent assay (ELISA) found immunoglobulin G (IgG) antibody prevalence varying between 0.3 and 4.7 percent: 0.3 percent in Marseille, France[20]; 1.7 percent in Spain by IFA[39]; 3.3 percent in Argentina[46]; 4.7 percent in Baltimore.[19] A study conducted in a region where murine typhus is endemic in Croatia reported an unexpectedly high prevalence, 36 percent, through IFA assay.[22]

CLINICAL FORMS

Several possible clinical forms of LCMV infection have been identified. In most cases, LCMV infection is minimally symptomatic or asymptomatic and consists of an influenza-like illness with fever, headache, myalgia, fatigue, general malaise. These symptoms are self-limited and resolve after a few days. These mild illnesses, which usually do not motivate patients to see a physician, most likely represent most LCMV infections, but the exact proportion is unknown. Historical articles reported that respiratory symptoms occur in LCMV infections. In a certain (but unknown) proportion of cases, a second phase is observed during which neurologic manifestations, such as meningitis, encephalitis, or even acquired hydrocephalus, can be recorded.

In 1942, Farmer and Janeway[25] proposed the following classification of LCM into four clinical categories:

1. Inapparent and subclinical infections: Immunologic studies suggested that these forms are quite common: 11 percent of 2000 sera tested contained neutralizing antibodies against LCMV.[4]

2. Nonmeningeal form, non–central nervous system (CNS) infection, systemic infection, influenza-like infection: This form consists of two stages: systemic illness, then convalescence. The incidence probably is underestimated because of the lack of laboratory documentation and the absence of specific or suggestive signs. Only vague assumptions can be made concerning the number of underdiagnosed cases. The diagnosis of LCMV usually is not suspected unless attention is directed to signs and symptoms suggestive of meningoencephalitis, although researchers have shown that generalized systemic infection may occur without any evidence of involvement of the CNS.[50] How often the disease manifests in this form or how often apparently asymptomatic infection occurs is not known. The demonstration of neutralizing antibodies in the blood of as many as 10 percent of the adult population suggests that infection with LCMV may occur more frequently than is suspected.[54]

3. Lymphocytic choriomeningitis, meningeal form: This form usually consists of three stages: systemic illness, meningitis (possibly delayed up to 2 to 3 weeks), and convalescence.[8]

4. Lymphocytic choriomeningitis, encephalomyelitic form: This form has three stages: systemic illness, encephalitis (possibly delayed), and convalescence.

Other CNS manifestations such as transverse myelitis, Guillain-Barré–type syndrome, and transient or permanent acquired hydrocephalus can be associated with LCMV infection.[10] LCMV-associated acquired hydrocephalus has been described only five times since the virus was discovered.[9,17,34,51] In all cases, the clinical picture was severe, and patients required ventriculostomy.

A fifth category (congenital LCM) should be added to the previous classification, corresponding to fetal consequences of LCMV infection during pregnancy. LCMV is a fetal teratogen. It can cause hydrocephalus, microcephaly or macrocephaly, intracranial calcifications, chorioretinitis, and nonimmune hydrops. More than 50 cases of congenital LCMV infection have been documented since 1955, with more than two thirds of these cases diagnosed since 1993. Chorioretinitis and hydrocephalus are the predominant characteristics among children diagnosed with congenital LCMV infection.[32] LCMV was isolated for the first time in the cerebrospinal fluid (CSF) of a congenitally infected infant, who had congenital hydrocephalus caused by LCMV infection with severe neurologic sequelae, including, in addition to hydrocephalus, chorioretinitis, blindness, and developmental delay.[49] The differential diagnosis of congenital LCMV infection includes toxoplasmosis, rubella, cytomegalovirus infection, herpes simplex, enteroviral infection, human parvovirus B19 infection, and syphilis.[11,43,55]

DIAGNOSIS

As in other viral diseases, direct diagnosis can be established by virus isolation using cell culture; Vero and L929 cells have proved effective for LCMV isolation, but other mammalian cells also can be used. Usually, either no effect or a mild cytopathic effect is noted. Several molecular techniques using polymerase chain reaction (PCR) technology have been described.[13,18,24,42,44] The main problem with all these techniques is that the genetic diversity of LCMV is poorly understood. Sequence data acquired from newer LCMV strains indicate that the genetic heterogeneity could be greater than 20 percent between two strains.[24] Therefore, one must keep in mind that a diagnostic assay able to amplify recognized LCMV strains may provide false-negative results with a new, uncharacterized strain. Further genetic analysis of newly discovered strains therefore will be key to developing better diagnostic tools in the future.

Several techniques have been described for establishing the diagnosis of LCMV infection in patients. Among these techniques, the one that was used commonly in the past, complement fixation assay, has been demonstrated to be poorly sensitive.[37] IFA and ELISA techniques are equivalent in terms of specificity and sensitivity. However, because of broad cross-reactivity among members of the genus *Arenavirus*, confirmation should rely on

neutralization assays to discriminate between LCMV and other arenaviruses.

Serologic study can be performed with serum or CSF samples. As in other viral diseases, a fourfold difference in titers indicates a recent infection. In a single sample, detection of specific IgM suggests a recent infection. Molecular detection of LCMV genomic RNA, as well as virus isolation, can be performed from CSF during CNS manifestations and possibly from serum or plasma.

TREATMENT

No treatment exists for LCMV infection. Ribavirin has been shown to be efficient for Lassa fever,[41] a viral hemorrhagic fever caused by another arenavirus related to LCMV. Ribavirin has never been used for treating a patient with LCMV infection.

CONCLUSIONS

Since 2000, several reports have shown that LCMV has not disappeared and that it still can cause severe or fatal cases. Renewed attention was drawn to LCMV after seven recipients of solid organ transplants died of LCMV infection.[26] Historically, this virus was a major cause of aseptic meningitis in the United States; LCMV ranked first during the World War II era and second during 1947 to 1952 period.[2] At that time, other major causes were mumps, herpes simplex, and leptospirosis. The focus on LCMV declined over the intervening decades, even though the rate of unrecognized causes of CNS infections still remains high, at more than 50 percent.[28] The reason for the diminishing interest in LCMV is not clear, but it may be related to the historical prevalence of the disease in rural rather than urban populations of developed countries. However, the growing proportion of urban people living below the poverty level may lead to conditions compatible with contact with mice and, therefore, may boost the incidence of rodent-associated diseases.

In addition, the capacity to diagnose LCMV has declined dramatically since the 1980s. Hence, the decreasing number of reported cases may reflect the decrease in clinical interest and the lack of diagnostic tests, rather than a genuine modification of the epidemiology of the disease. Standard techniques, such as IFA or virus isolation, have been virtually abandoned in clinical microbiology laboratories. In Europe, the situation has been critical since 2000; barely a handful of laboratories are capable of performing recognized serologic or molecular diagnostic tests. LCMV infection still exists, and cases are reported when physicians include LCMV in the differential diagnosis and when appropriate diagnostic tests are conducted. Efforts are essential to determine the epidemiologic landscape of the disease and to implement etiologic investigations of newborns with congenital malformations who present with CNS infections. The combination of poor diagnostic testing capacity and a lack of awareness of the virus among physicians may contribute directly to an underestimation of the current role of LCMV in medicine.

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CHAPTER 198

Arenaviral Hemorrhagic Fevers

Rémi N. Charrel ✪ Xavier de Lamballerie

HISTORY

In the 1950s, a new disease emerged in the Buenos Aires Province of Argentina, a rich farming region. The causative agent of the Argentine hemorrhagic fever (AHF), named Junín virus, was isolated in 1958.[85] Junín virus is hosted by rodents widely distributed in the region, and human infection occurs through contact with infected excreta, usually by a respiratory pathway. AHF is a severe disease that is fatal in approximately 20 percent of cases in the absence of specific treatment. Intensive deforestation and extensive agricultural practices in this region have increased considerably the number of contacts between humans and rodents and thereby fueled the emergence of severe annual outbreaks. The cases were expanding progressively into north-central Argentina until they finally were controlled by the availability of a live-attenuated vaccine developed in early 1980s. Junín virus is estimated to have caused approximately 30,000 cases of symptomatic disease since its discovery.

Bolivian hemorrhagic fever (BHF) was described first in 1959 in the El Beni Department of eastern Bolivia. The causative agent, named *Machupo virus*, was isolated in 1963 and was found to be antigenically and genetically distinct from but related to Junín virus.[56] Machupo virus was responsible for large outbreaks with high incidence (up to 21% of the population) and a case-fatality rate approximately 20 percent during the 1960s. Effective rodent-control efforts interrupted these epidemics, and, after the mid-1970s, no cases of BHF were reported. After 20 years of silence, however, a total of 19 additional cases were reported in the same region from 1993 to 1999.[5]

An outbreak of severe hemorrhagic fever began in the state of Portuguesa, located in the central plains of Venezuela, in 1989. Initially thought to be dengue hemorrhagic fever, the causative agent was isolated in 1990, identified as a new arenavirus species, and named *Guanarito* after the municipality where the first epidemic occurred.[92] Venezuelan hemorrhagic fever (VHF) presents a seasonal occurrence clearly related to intense agricultural activity and to human contact with the soil and the rodents hosting Guanarito virus.

Little is known about Sabiá virus, which was responsible for one fatal case of hemorrhagic fever near São Paulo (Brazil) in 1994.[22] Subsequently, two other cases were reported in laboratory workers. The epidemiology and the natural reservoir of Sabiá virus remain unknown.

In 1999 and 2000, three fatal cases of illness were reported in female patients aged 14, 30, and 52 years; two resided in northern California and one in southern California.[17] These cases were associated with Whitewater Arroyo virus infections, a recently described arenavirus indigenous to southwestern United States and hosted by *Neotoma* rodents.[37]

In the Old World, Lassa fever was described initially in Nigeria in 1969, and the causative virus was identified during that same year.[10] The disease occurs also in Sierra Leone, Guinea, Liberia, and more sparsely in other countries of West Africa.

ETIOLOGIC AGENTS

Arenaviruses are enveloped single-stranded RNA viruses, with a genome consisting of two RNA segments, designated large (L)

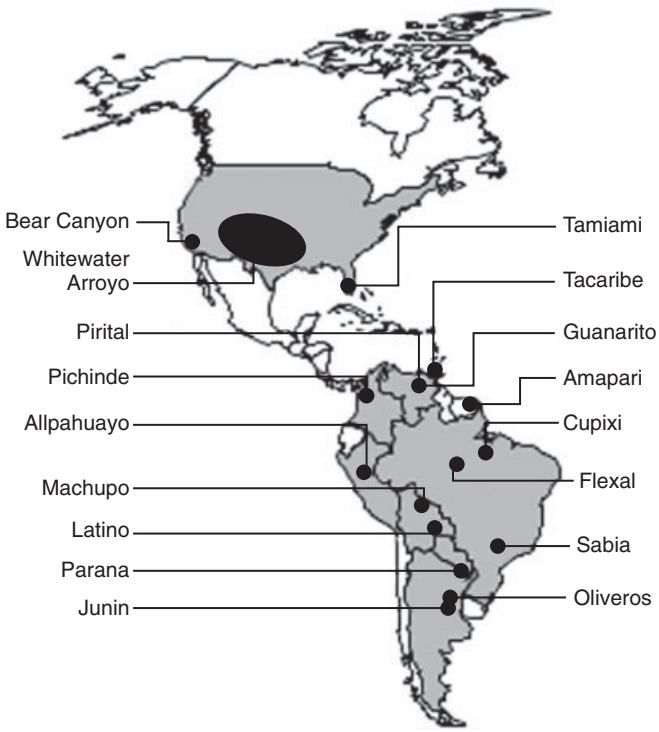

Figure 198–1 Geographic distribution of New World arenaviruses. Lymphocytic choriomeningitis virus is distributed worldwide and is not presented specifically on the map.

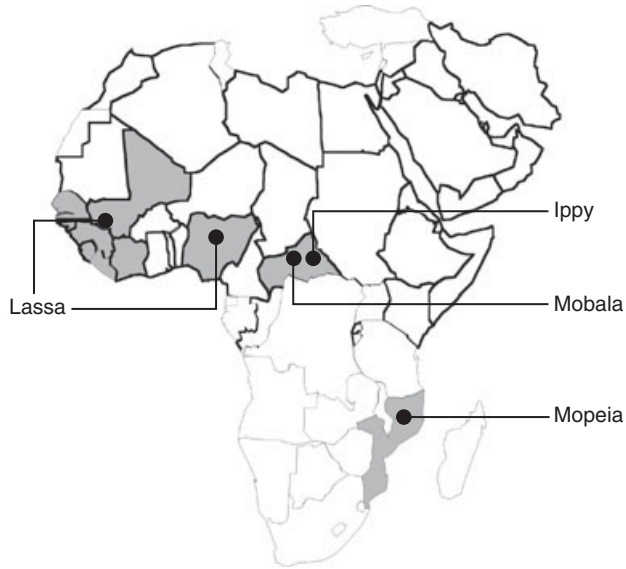

Figure 198–2 Geographic distribution of Old World arenaviruses. Lymphocytic choriomeningitis virus is distributed worldwide and is not presented specifically on the map.

and small (S). The L genomic segment (~7.2 kb) encodes the viral RNA-dependent RNA polymerase and a zinc-binding protein. The S genomic segment (~3.5 kb) encodes the nucleocapsid protein (N) and glycoprotein precursor (GPC; secondarily cleaved into the G1 and G2 envelope glycoproteins) in non-overlapping open-reading frames of opposite polarities.[96] Nucleocapsid antigens are shared by most arenaviruses, and quantitative relationships show a basic split between viruses of Africa and viruses of the Western Hemisphere. Individual viruses immunologically are distinct by neutralization test, which depends on the specificity of epitopes contained in the envelope glycoproteins.[88]

At the time this chapter was written, 22 arenaviruses were recognized. They have been classified according to their immunologic characteristics. Two groups are recognized: the Tacaribe serocomplex, including the New World viruses (Fig. 198–1), and the Lassa lymphocytic choriomeningitis (LCM) serocomplex, including the ubiquitous LCM virus and all recognized Old World viruses (Fig. 198–2). Genetic studies are congruent with serologic analyses. They also indicate that the 22 arenaviruses represent four phylogenetic lineages. The Old World (Lassa-LCM serocomplex) lineage comprises five viruses (LCM, Lassa, Mopeia, Mobala, and Ippy) and is deeply rooted in the three New World (Tacaribe serocomplex) lineages, designated A, B, and C (Fig. 198–3). The lineage A includes three North American viruses (Whitewater Arroyo, Tamiami, and Bear Canyon) and five South American viruses (Pirital, Pichindé, Flexal, Paraná, and Allpahuayo). The lineage B includes seven South American viruses (Sabiá, Junín, Machupo, Guanarito, Amapari, Tacaribe, and Cupixi). The lineage C comprises three South American viruses (Oliveros, Latino, and Pampa). Phylogenies reconstructed from each of the four genes independently demonstrated that the three North American arenaviruses (Whitewater Arroyo, Tamiami, and Bear Canyon) possessed a chimeric genome with the glycoprotein gene inherited from a clade B ancestor and the nucleoprotein gene inherited from a clade A ancestor; this mechanism could not be identified within the large genomic segment with both polymerase and matrix genes more closely related to those of clade A viruses.[18-20]

Lassa virus is the only Old World arenavirus that causes human hemorrhagic fever, whereas four New World members of the lineage B (Junín, Machupo, Guanarito, and Sabiá) have been associated with hemorrhagic fever in South America. Whitewater Arroyo virus has been associated recently with fatal human cases in North America.

EPIDEMIOLOGY

Specific rodents (usually one or two closely related species) are the principal hosts of the arenaviruses for which natural host relationships have been characterized (Table 198–1); the only exception is Tacaribe virus, associated with bats. The consensus is that the diversity of arenaviruses is the result of a long-term, shared evolutionary relationship (termed *co-evolution* or *co-speciation*) between the virus members of the family *Arenaviridae* and the rodents of the family *Muridae*.[7] Chronic infections of the host appear to be crucial to the long-term persistence of arenaviruses in nature. The infection in rodents is accompanied by a chronic viremia or viruria. Because of this specific association, the geographic area where each arenaviral disease is diagnosed is limited by the geographic distribution of the corresponding rodent host.

The rodent host of Junín virus is *Calomys musculinus*, a small field rodent. Since it was first isolated in 1959, the endemic area has expanded from a region of 16,000 km^2 in the north of Buenos Aires Province to a region that is now 150,000 km^2 (reaching north of Buenos Aires, south of Santa Fé, southeast of Córdoba, and northeast of La Pampa provinces). The human population at risk now is estimated to be almost 5 million.[32] AHF typically is a seasonal disease, with a peak frequency in the corn harvesting season from March to June; during this period, 75 percent of the infected people are male agricultural workers who are contaminated by inhalation of infected aerosols produced from rodent

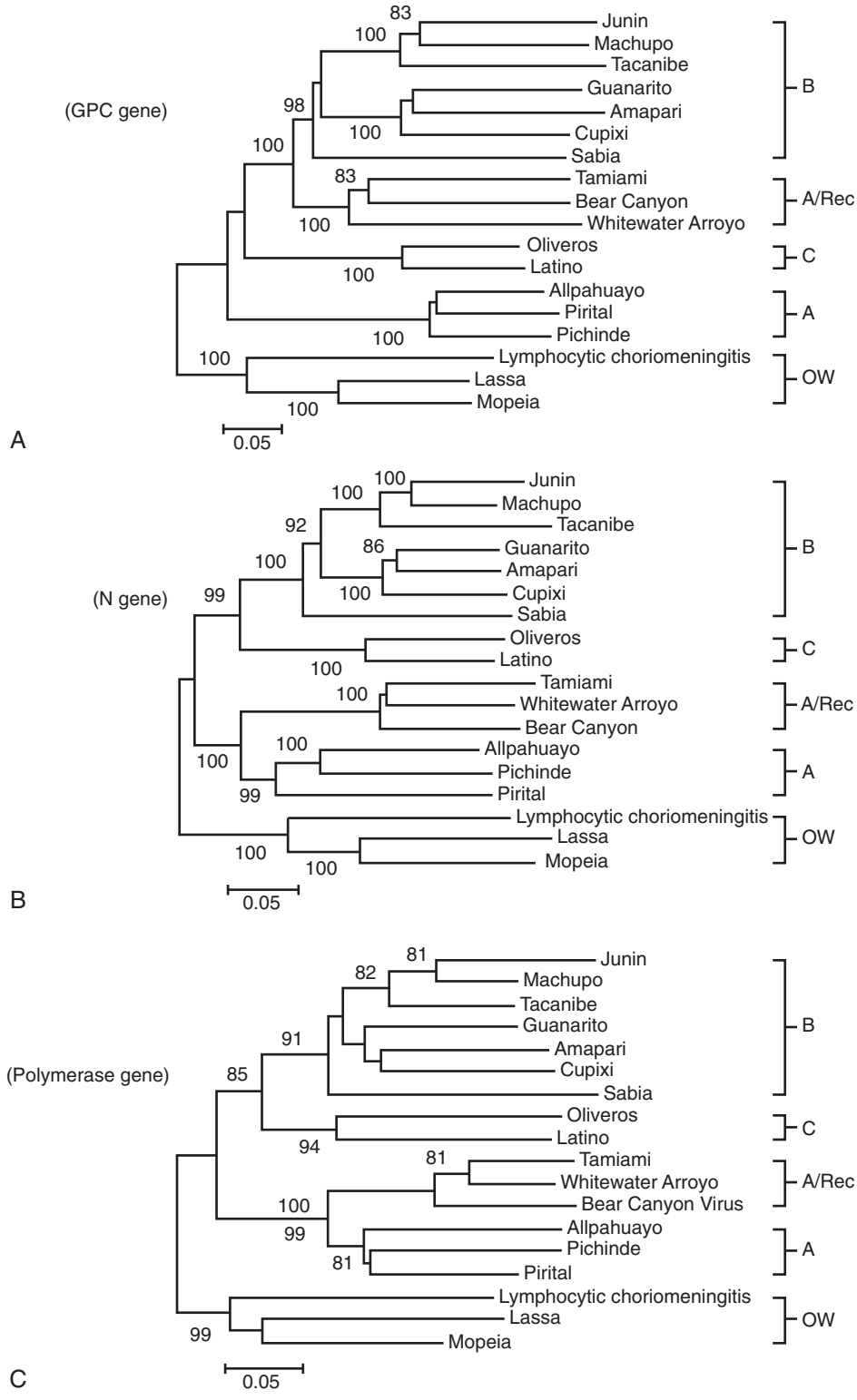

Figure 198–3 Phylogeny of arenaviruses based on the analysis of complete sequences of the GPC gene (**A**) and the N gene (**B**) and partial sequences of the polymerase gene (**C**). NW, New World arenaviruses; OW, Old World arenaviruses; A, B, and C show the three evolutionary lineages of New World arenaviruses. A/Rec denotes the recombinant lineage including the three North American viruses. Bootstrap values above 75% are indicated and correspond to 500 replications.

excreta or from rodents caught in mechanical harvesters.[70] Since 1958, cases have been recorded annually, with a variation ranging from several hundreds to 3500. The mortality rate for patients with confirmed AHF was 14 to 17 percent before immune plasma was used routinely.[71] Evidence indicates that the number of human cases reflects the proportion of infected *C. musculinus* in a particular area.[81]

Machupo virus, the causative agent of BHF, is hosted by *Calomys laucha* rodents. As for Junín virus, the dynamics of the rodent population determine the epidemiologic features in humans.[79] By contrast to *Calomys callosus*, *C. laucha* invades houses during inundation at the rainy season; this invasion results in human cases occurring with an attack rate identical in men, women, and children. The disease has been recorded only from

TABLE 198–1 Some Epidemiologic Features of Arenaviral Hemorrhagic Fevers

Fever	Virus	Case, Season, Place, Pattern	Annual Incidence	Geographic Distribution	Ecology	Rodent Reservoir	Comment
Argentine hemorrhagic fever	Junín	Males; corn harvest; March-June	20-200	North-central Argentina	Temperate pampa	*Calomys musculinus*	3- to 4-year rodent-disease cycle
Bolivian hemorrhagic fever	Machupo	All ages, both sexes; villages; February-July	<10	Northeast Bolivia	Tropical savanna	*Calomys callosus*	Rodent control successful
Venezuelan hemorrhagic fever	Guanarito	All ages, both sexes equally; house, gardens; no seasonality	0-100	Central Venezuela	Tropical mixed savanna	*Zygodontomys brevicauda* *Sigmodon alstoni*	Recently described
Lassa fever	Lassa	All ages, both sexes; villages; no seasonality	10,000	West Africa	Tropical forest savanna	*Mastomys natalensis*	No long-term cycle; nosocomial infections

a sparsely populated region in northeast Bolivia, the El Beni Province. From 1962 to 1964, a series of outbreaks involved more than 1000 patients, 180 of whom died. Nosocomial transmission of Machupo virus was clearly demonstrated,[87] although most of the recorded infections were acquired by direct contact with *C. laucha* or by aerosol contact through infected excreta.

In 1990, new cases of hemorrhagic fever were investigated in Venezuela; the culprit was found to be a new arenavirus, designated Guanarito virus after the region where the outbreak occurred.[92] Natural and experimental data suggest that two different rodent species are involved in the transmission cycle of Guanarito virus in nature: the cane rat *Zygodontomys brevicauda* and the cotton rat *Sigmodon alstoni*.[38,39,100] Since its discovery, Guanarito virus has been responsible for at least 200 cases of VHF. For unknown reasons, the number of reported human cases has dropped spontaneously since 1992, although rodent infection can still be demonstrated easily inside and even outside the original endemic zone.[106]

In California, three fatal cases recently were associated with Whitewater Arroyo virus, initially isolated from a wood rat (*Neotoma* spp.).[17,37] The present-day geographic range of rodents of the genus *Neotoma* extends from western Canada southward to Guatemala, Honduras, and Nicaragua. Of the 20 recognized *Neotoma* spp., nine occur in the United States, and evidence of arenavirus infection has been reported for five of them. Recent field studies have provided strong evidence that Whitewater Arroyo virus circulates in the states of New Mexico, Utah, Texas, Oklahoma, Arizona, and Colorado.[11,40] The abundance and habits of wood rats suggest that potential contact between *Neotoma* rodents and humans is limited.

Lassa virus is associated with rodents belonging to the *Mastomys* genus, widely distributed in sub-Saharan Africa. Documented cases of Lassa fever have been reported in different countries of West Africa, such as Nigeria, Liberia, Sierra Leone, Burkina Faso, Guinea, Ivory Coast, Ghana, Senegal, Gambia, and Mali.[12,42,43,52,77,93,101] Between 1985 and 1987, three of 5000 (0.06%) randomly selected persons living in six central African countries (Cameroon, Central African Republic, Chad, Congo, Equatorial Guinea, and Gabon) tested for serologic evidence of Lassa virus infection were found to have antibody.[46] In contrast, a second study conducted with individuals living in different parts of Nigeria found that 21.3 percent of the 1677 tested antibody positive.[101] Lassa fever is a disease of major public health importance in West Africa because it causes an estimated 5000 deaths and as many as 300,000 infections annually. The first epidemic was described in 1969 in Nigeria,[10] and soon was followed by several other epidemics in nearby countries. Among hospitalized patients, the mortality rate is estimated at 15 to 20 percent.[107] In hospitals located within the endemic area, 10 to 16 percent of the admissions are caused by Lassa fever. However, serologic surveys suggest that subclinical cases occur and result in a lower overall mortality (<1%).[77] The overall incidence of Lassa fever in West Africa may be tens of thousands to hundreds of thousands annually. The main characteristic of Lassa fever is that it is highly transmissible from human to human through direct contact with blood or secretions from infected patients and by aerosol exposure.[60] Many nosocomial outbreaks have been described. Another characteristic of Lassa fever is that a number of cases are acquired by local residents who seek out rodents for consumption.[98] Because of the prevalence of virus transmission, Lassa fever is a prominent threat of imported hemorrhagic fever cases; such cases have been reported in England,[23] Germany,[48] Japan,[53] the Netherlands,[104] Israel,[95] and the United States.[54] A recent comprehensive report of imported Lassa fever recorded 24 cases occurring from 1969 to 2004, with a fatality rate at 29 percent.[68]

PERSON-TO-PERSON TRANSMISSION

Although human-to-human transmission of BHF has been seen only rarely and then only after intimate contact, Machupo virus clearly was responsible for severe nosocomial outbreaks in which all cases were associated with a single index case who had returned from the endemic region. The only recognized hospital-based outbreak resulted in four secondary cases followed by a tertiary case acquired from a necropsy incident; all but one died. An epidemic recently was reported in which seven members of the same family were infected, with a fatal outcome for six.[15]

Nosocomial transmission is a common feature of Lassa fever, and many nosocomial outbreaks have been described.[33,60] However, it is apparent that this aspect of Lassa fever has been overestimated in reports based on infections in hospitals. The additional risk to hospital workers within the endemic zone is not great as judged by serosurveys, provided basic hygiene measures are respected in hospitals in dealing with suspected cases.[51] Good barrier nursing and protection of personnel by use of gloves, gowns, and eye shields have reduced the incidence of such infection in recent years. Broader surveys of community infections have revealed Lassa fever to be an important community-acquired disease, with a wide spectrum of clinical manifestations. Because Lassa virus has been isolated from milk, clearly a risk to nursing children exists.

In conclusion, the data demonstrating nosocomial and human-to-human transmission of several arenaviral diseases highlight the necessity for implementing biocontainment precautions in dealing with arenavirus-infected patients and materials.

TABLE 198–2 Some Laboratory Findings in Arenaviral Hemorrhagic Fevers

Disease	Viremia	RBC Increase	WBC	Urine Protein	AST/ALT
Argentine hemorrhagic fever	+	++	↓↓	+	N-200
Bolivian hemorrhagic fever	+/–	++	↓↓	+	N-200
Venezuelan hemorrhagic fever	++	++	↓↓	+	??
Lassa fever	++++	++	N-↑	++	100-1500

ALT, alanine aminotransferase; AST, aspartate aminotransferase; RBC, red blood cell; WBC, white blood cell count.

CLINICAL MANIFESTATIONS

The clinical picture of the South American arenaviral hemorrhagic fevers is almost identical, regardless of the virus responsible for the disease. The incubation period usually is 7 to 14 days, with an extreme extending from 5 to 21 days. Infection secondary to high-load inoculum may result in the reduction of the incubation period to 2 days. The onset is gradual with fever and malaise, secondarily joined by myalgia, back pain, headache, and dizziness. Hyperesthesia of the skin is a common manifestation. Petechiae of the skin and hemorrhages from the gums, vagina, and gastrointestinal tract beginning about the fourth day of illness herald the advent of hypovolemic clinical shock. Blood loss usually is minor, so the hematocrit generally increases as the capillary leak syndrome, the hallmark of the disease, becomes more severe (Table 198–2). Bleeding and prothrombin time may be prolonged, and reductions of factors II and VII of the coagulation cascade have been noted. Renal function generally is preserved until shock occurs, but urinary protein concentration may be high. Neurologic manifestations are prominent; intention tremor of the hands and inability to swallow or to speak clearly may develop, and these symptoms can progress to grand mal convulsions, coma, and death in the absence of significant capillary leak or hemorrhagic signs. Death usually occurs 7 to 12 days after onset of disease. Those who survive generally recover completely without permanent sequelae, although transient loss of scalp hair and Beau lines in digital nails are a common consequence of the high and sustained fever.

Symptoms that appear to be more specifically associated with one or another of the viruses have been reported.[103] Although the frequency of clinical and laboratory findings is identical for Junín and Machupo virus infections, clear differences exist with Guanarito virus infections. Pharyngitis, vomiting, and diarrhea are observed more frequently with Guanarito virus; in contrast, petechiae, erythema, facial edema, hyperesthesia of the skin, and shock are observed more frequently in the case of Junín or Machupo infection.

Fatal outcome of Junín virus infection is observed more frequently in pregnant women in the last trimester, and a high fetal mortality is associated with Junín and Machupo virus infection.[8,27,57]

The patients infected with Whitewater Arroyo virus were healthy before acquiring the virus infection. In one case, the virus probably was acquired by aerosol pathway while cleaning rodent droppings at home. In all cases, no history of travel outside California was found during the 4 weeks preceding the illness. The onset was characterized by nonspecific febrile symptoms including fever, headache, and myalgia. All patients presented with acute respiratory distress syndrome, and two developed liver failure and hemorrhagic manifestations. Death occurred within 8 weeks after the onset.[17]

Concerning Lassa fever, the clinical description is based on signs observed in hospitalized patients.[41,59,88] After having an incubation period that ranges from 7 to 14 days, patients suffer an insidious onset characterized by progressive fever, malaise, and generalized myalgia. Retrosternal pain frequently is associated with this phase. These symptoms increase in severity during the following week and are accompanied by nausea, vomiting, diarrhea, chest pain, abdominal pain, headache, sore throat, cough, and dizziness. Patients complain of sore throat, pharyngeal inflammation, and chest pain. As the disease progresses, vomiting, diarrhea, hemorrhagic manifestations, encephalopathy, and evidence of vascular permeability may be noted. During the second week, recovery may begin or the disease progressively worsens until harbingers of a fatal outcome are evident: severe edema, mucosal hemorrhages, pulmonary involvement, encephalopathy (with seizures and coma), or shock; at this stage, pleural and pericardial effusions and ascites are not rare findings. There frequently is liver involvement, but icterus is not usual. Death occurs in 15 to 20 percent of the hospitalized patients, usually in the second or third week of the course, and is associated with sudden cardiovascular collapse caused by hepatic, pulmonary, and myocardial damage. Few patients develop severe central nervous system (CNS) signs, and disturbances in consciousness and convulsions are markers of a poor prognosis. Deafness is an important sequela of Lassa fever; it may be unilateral or bilateral and is a consequence of the eighth cranial nerve dysfunction; it can be observed in as many as 30 percent of the patients.

Clinical disease among children generally is similar to that in adults. Systematic data are available for only Lassa fever patients.[83,107] Fever, vomiting, diarrhea, and cough are common presenting symptoms. Pulmonary signs, including rales and pleural effusion, are seen more commonly in children than in adults. Among very young children, especially those infected ante partum, an unusual condition called *swollen baby syndrome* has been described. It is marked by abdominal distention, widespread edema, and spontaneous bleeding.

Lassa virus replicates at a very high level in the placenta of pregnant women, which might account for the high mortality rate observed in these patients. The risk of death in pregnant women is estimated to vary from 7 percent in the first two trimesters to 30 percent in the third trimester, compared with the 13 percent mortality in nonpregnant women. A high fetal mortality rate varying from 92 percent in early pregnancy to 75 percent in the last trimester has been observed.[80,82,89]

Very little is known about the health consequences of infection with the other arenaviruses. Aerosol infections are common in workers of laboratories where arenaviruses are manipulated. Pichindé virus has resulted in several seroconversions without any notable clinical significance.[11] Flexal virus has resulted in two severe laboratory infections and should be regarded as potentially dangerous (F. Pinheiro, personal data). Tacaribe virus has resulted in a single case of febrile disease with mild CNS symptoms (J. Casals, personal data).

PATHOGENESIS AND PATHOLOGY

Human and nonhuman primate infection initially involves virus replication at the site of infection, usually in the lung after infected aerosol deposition. Replication occurs in the hilar lymph nodes, and despite interstitial infiltration, pneumonic foci usually

are not observed. Regardless of the route of infection, the macrophage is a site of important viral replication. With the exception of the hepatitis peculiar to Lassa fever, the pathologic process of arenaviral hemorrhagic fever is notable for the general lack of parenchymal histologic damage. Edema of tissues and focal hemorrhages in mucosal surfaces and fascia of many organs are common events. Rarely are blood clots found that are large enough to be of clinical significance. Thus, the crucial and life-threatening lesion in these diseases appears to be the capillary leak. How this lesion, in which plasma protein and fluid escape the circulation at a much higher rate than erythrocytes do, is produced still is not clear.[88]

Hemorrhages are common findings in New World arenaviruses causing hemorrhagic fever. The prominent thrombocytopenia has a central origin possibly linked to high levels of interferon observed in these patients.[62] Minor foci of necrosis with acidophilic inclusions (Councilman bodies) have been reported in patients with BHF,[21] and erythroid hypoplasia of bone marrow as well as lymphoid depletion of nodes and spleen may be present. Histologic evidence of disseminated intravascular coagulation generally is absent. Inflammatory lesions of the CNS are lacking. Mild inflammation of the myocardium has been noted in patients with BHF. The Argentine and Bolivian fevers may be marked by encephalopathy. Virus is not recovered from cerebrospinal fluid of patients presenting such CNS symptoms with the South American agents. Macrophages and lymphocytes or their precursors are targets for infection, and an activation of the complex cytokine cascade occurs, which can cause endothelial cells of small capillaries to lose the tight continuity that keeps protein and red cells inside the circulation. As part of this cascade, levels of interferon are elevated. Analysis of the serum levels of hematopoietic growth factors suggested a link between granulocyte colony-stimulating factor serum levels and the severity of the illness in humans.[74] Similarly, interleukin-8 is thought to play an essential role in neutrophil activation.[73] Specific antibodies may not appear until 3 to 4 weeks after the onset of the disease. However, New World arenaviruses causing hemorrhagic fever are neutralized more readily, and immune serum–based therapy has been successful for humans. Preliminary studies of VHF suggest that this disease resembles that caused by other South American relatives.[25,92] Genetic analysis of Junín and Guanarito virus sequences amplified from human cases in Argentina and Venezuela, respectively, failed to identify any genetic marker correlating with the human pathogenicity or with the severity of clinical forms.[44,106]

Although only three cases have been reported to date, Whitewater Arroyo virus infection seems to be associated with profound lymphopenia and thrombocytopenia.[17] To date, no studies have been performed to investigate the pathogenesis of Whitewater Arroyo virus infection. An interesting note is that Whitewater Arroyo virus belongs to the lineage A (based on genetic analysis performed in the nucleocapsid gene), in which no other members have been recognized so far as human pathogens. However, the small genomic segment of Whitewater Arroyo virus has been shown recently to have a dual origin: the gene encoding the nucleocapsid is inherited from an ancestor belonging to the lineage A, whereas the glycoprotein precursor gene is inherited from an ancestor belonging to the lineage B.[18] Further investigations are needed to clarify whether the lineage B–inherited GPC gene is responsible for the putative human pathogenicity of Whitewater Arroyo virus.

The hepatitis of Lassa fever is represented by different stages successively characterized by hepatocellular injury, necrosis, and regeneration, any of which may be present at death. In no instance is the degree of hepatic damage sufficient to be responsible for hepatic failure; consequently, the hepatitis of Lassa fever is not the primary cause of death.[58,76] For the cases characterized by encephalopathy, correlation could not be established with the presence of Lassa virus in the cerebrospinal fluid or with virus antibodies in cerebrospinal fluid or serum; no evidence shows that the encephalopathy observed in Lassa fever is the consequence of either direct cytopathic or immune-mediated mechanisms.[24] Direct damage to circulating leukocytes is not demonstrated, and profound thrombocytopenia does not occur.

In human cases of Lassa fever, no correlation exists between antibody levels and outcome of the disease.[35] More than one third of patients have antibodies to the nucleocapsid of the virus on admission to the hospital. These antibodies do not neutralize the virus, which is found in blood in concentrations much higher in severe infection than in any of the other arenaviral diseases. During convalescence, viral neutralizing antibodies evolve for months and rarely reach levels even one tenth of those that develop after infection with the other viruses in this group. Recovery from Lassa virus infection usually precedes the appearance of neutralizing antibodies, indicating that cellular immunity plays a primary role in viral clearance. Thus, it seems reasonable to speculate that compromise of cell-mediated immunity is responsible for uncontrolled virus replication and functional capillary collapse. Primates challenged with Lassa virus or naturally infected patients who recovered from acute Lassa fever usually do not exhibit a measurable neutralizing antibody response. Individuals who are seropositive exhibit a strong memory $CD4^+$ T-cell response against the nucleocapsid protein of Lassa virus.[99] Experiments based on inactivated vaccines have shown that in spite of the resulting synthesis of high titers of antibodies, vaccination did not prevent virus replication and death in nonhuman primates.[35] It, therefore, seems obvious that control of human Lassa fever involves mostly T cells through a T_H1-type immune response. In addition, immune response requires epitopes located on both GP1 and GP2 glycoproteins to protect efficiently primates challenged with Lassa virus.[35]

Several animals have provided reasonable models for the clinical disease seen in humans. Junín virus causes fatal hemorrhagic disease in guinea pigs; rhesus monkeys are good models for Lassa[55] and Machupo virus infections; and marmosets *(Callithrix jacchus)* reproduce the pathogenesis of Junín virus. Indeed, rhesus monkeys were used to predict the effectiveness of the antiviral drug ribavirin to treat Lassa fever, and guinea pigs were instrumental in the development of an attenuated vaccine for AHF. Recently, the paired Syrian golden hamster and Pirital virus (a nonpathogenic clade A arenavirus) has been shown to be a model to mimic clinical virologic and pathologic changes observed with Lassa fever in humans.[94]

Alpha-dystroglycan has been identified as a major receptor for LCM and Lassa viruses. In New World arenaviruses, only clade C viruses (Oliveros and Latino) use alpha-dystroglycan as a major receptor, whereas clade A and clade B arenaviruses use distinct and widely expressed receptors.[90,97] Functional receptors for South American hemorrhagic fever arenaviruses (Guanarito, Junín, and Machupo) were found on most human cell types and cells derived from nonhuman primates and rodents.[91]

DIAGNOSIS AND DIFFERENTIAL DIAGNOSIS

DIRECT DIAGNOSIS

Serum or heparinized plasma should be collected during the acute febrile stages of the disease; the samples need to be frozen on dry ice or in liquid nitrogen. Storage at temperatures higher than –40° C will result in the progressive loss of infectivity. Classically achieved by virus isolation, direct diagnosis now may be performed by reverse transcription–polymerase chain reaction (RT-PCR) assay. Isolation of the virus is achieved readily in cell culture, and Vero cells are a sensitive substrate for such direct

diagnosis. Because cytopathic effect is not always observed, arenavirus-infected cells are detected by immunofluorescence test.

For South American arenaviruses causing hemorrhagic fevers, the delay for isolation on Vero cells is 1 to 5 days, much faster than animal inoculation that requires 7 to 20 days before illness develops. Serum and throat washings collected 3 to 10 days after onset usually yield virus. Virus is isolated less frequently from urine. Specifically, Machupo virus is recovered from only 20 percent of acute-phase sera and even less frequently from throat washings or urine. When serum or throat samples are collected within 2 weeks of clinical onset, the success of isolating Lassa virus is very high. Virus is isolated less frequently from urine.

Viral RNA can be purified from serum, plasma, urine, throat washings, and tissues and can be used as a target for cDNA synthesis, with use of a reverse transcriptase. This cDNA can be amplified by a PCR assay with consensus primers or specific primers. The amplified region finally is sequenced for diagnostic confirmation. RT-PCR–based diagnosis offers the advantage of reducing the delay of response and of possessing a greater sensitivity compared with cell culture. This technique has been applied successfully to Lassa fever diagnosis. Specific primers designed to amplify certain arenaviruses have been proposed but still are not used for diagnostic purposes in epidemic conditions. Molecular tests based on the RT-PCR methodology are being developed increasingly for the diagnosis of arenaviral infections.[67] For Junín and Machupo virus infection specifically, this technique is the only one to be early and sensitive enough to detect low viremia encountered during the period in which immune plasma therapy can be used effectively.[64] At the time of admission to the hospital, RT-PCR detected Lassa virus RNA in 79 percent of the patients; for comparison, in these patients, immunofluorescence assay detected antibodies in only 21 percent. By the third day of admission, 100 percent of the patients tested positive by RT-PCR analysis, whereas only 52 percent tested positive by the immunofluorescence assay.[26] The drawbacks of molecular methods consist mostly of the need for sophisticated equipment to be fully applicable in the field conditions when an outbreak occurs.

Additional techniques for direct detection of viral presence in tissues are being actively developed; they include in situ nucleic acid hybridization techniques. Antigen-detection enzyme-linked immunosorbent assay (ELISA) has been a sensitive tool in a number of patients.

More recently, molecular assays taking advantage of the real-time PCR technology have been reported for the detection of the four South American arenaviruses causing VHF. Apart from assays specific for one virus, a universal clade B PCR assay proved able to detect 5 to 500 $TCID_{50}$ per reaction for Junín, Machupo, Guanarito, and Sabiá viruses and also for Tacaribe, Cupixi, and Amapari viruses.[105] These tests have application to laboratory diagnosis on human specimens but also can be used to detect arenaviruses in rodent tissues.

The first quality assurance study on the rapid detection of Lassa virus RNA included 24 participant laboratories from 17 countries; tenfold dilutions of Lassa virus–inactivated genetic material were detected with success varying from 21 to 85 percent, depending on the concentration in the sample.[84] Of interest is that specimens containing high concentration of viral RNA may lead to false-negative results by the inhibition of enzymatic reaction, suggesting that clinical specimens should be either spiked with internal control to monitor enzymatic reaction efficacy or tested after dilution.[28,84]

INDIRECT DIAGNOSIS

Serologic diagnosis is made by demonstration of a fourfold rise in the titer of specific antibody. A high immunoglobulin G (IgG) antibody titer or the presence of specific IgM is indicative of a probable case. Nucleocapsid antigens are shared by most of these viruses, and quantitative relationships show the basic split between the viruses of Africa and the Western Hemisphere. Individual viruses immunologically are distinct by neutralization test, which depends on the specificity of epitopes contained in the envelope glycoproteins G1 and G2. Samples collected in view of serologic diagnosis can be kept at –20° C. Blood obtained in the early convalescence may be infectious despite the presence of antibodies and therefore should be handled accordingly. Antibodies often are detected by indirect immunofluorescence because it is an inexpensive, rapid, and sensitive test. An alternative is the ELISA, the specificity of which is greater than, and the sensitivity of which is on the level of, the neutralization test. ELISA is therefore the test of choice for differentiating viral strains of arenaviruses, for confirmation of unexpected results, and for detection of infection from the distant past. All these tests have been tested in the conditions of an outbreak and revealed effective.

Antibodies specific to South American arenaviruses appear 12 to 30 days after the onset, which often correlates with clinical improvement.[86]

In the case of Lassa fever, antibodies detectable by immunofluorescence appear early and often are present during the acute illness with no apparent relation to viremia or clinical status; 50 percent of patients with Lassa fever develop IgM or IgG antibody during the first week, and more than 66 percent do so during the next week. Antigen-detection ELISA in conjunction with IgM-capture ELISA has shown promise for rapid and sensitive diagnosis of Lassa fever. Neutralizing antibodies usually develop only after 4 to 6 months of convalescence.

PREDICTIVE VALUE

In West Africa, the association of fever, pharyngitis, retrosternal pain, and proteinuria presented a predictive value of 81 percent. In the endemic area of Argentina, the constellation of asthenia and dizziness accompanied by petechiae and conjunctival congestion has been shown to be indicative for the diagnosis of AHF; the presence of leukopenia, thrombocytopenia, and proteinuria further reinforces the diagnostic suspicion based on clinical findings. The association of a platelet count of less than 100,000/mm^3 and a white blood cell count of less than 2500/mm^3 or less than 4000/mm^3 was reported to have a sensitivity at 87 percent and 100 percent, respectively, and a specificity at 88 percent and 71 percent, respectively.[49]

DIFFERENTIAL DIAGNOSIS

Differential diagnosis of Lassa fever should include malaria, typhoid, shigellosis, dengue, and yellow fever. For South American viruses causing hemorrhagic fever, the differential diagnosis includes yellow fever, dengue hemorrhagic fever, hepatitis, leptospirosis, hemorrhagic fever with renal syndrome caused by hantaviruses, rickettsial diseases, typhoid, sepsis with disseminated intravascular coagulation, and, in the case of CNS involvement, viral encephalitis.

Because of biosafety reasons, suspected cases must be reported to the national laboratories having the expertise and capacity for molecular diagnostics of viral hemorrhagic fevers together with a procedure adapted to handling and transportation of specimens. Isolation of virus must be attempted in biosafety level 4 (BSL-4) laboratory facilities.

PROGNOSIS AND TREATMENT

PROGNOSIS

The case-fatality ratio of AHF, which is greater than 15 percent without specific treatment, drops spectacularly to less than 1 percent when specific treatment is available.[49] Granulocyte colony-stimulating factor serum level seems to be a marker of severity of the illness.[74]

For Lassa fever, no correlation exists between antibody levels and outcome. Viremia levels in Lassa fever are important in terms of prognostic value; basically, the higher the viremia, the higher the mortality rate. The serum aspartate aminotransferase level also is an accurate prognostic marker; values above 150 U/mL are associated with a 55 percent mortality rate, whereas the overall mortality rate is approximately 15 percent.[58]

TREATMENT

Arenaviral hemorrhagic fever should be managed by monitoring and correction of fluid, electrolyte, and osmotic imbalances and of metabolic acidosis. Hydration should be done cautiously because of the generalized capillary leak and the possibility of precipitating pulmonary edema. If possible, bleeding should be treated with clotting factor and platelet replacement as guided by laboratory test results.

For Junín virus infection, immune serum therapy is effective in reducing the incidence of mortality when it is given within the first 8 days of illness.[30,71] Experimental evidence suggests that the plasma may work through attacking infected cells as well as viral neutralization. An important note is that 10 percent of the patients who received this treatment secondarily developed cerebellar signs, such as headaches and tremors. These clinical signs are transient; they are thought to be related to immunopathologic mechanisms induced by the treatment with convalescent plasma.

Despite the recognized efficacy of convalescent plasma for treatment of Machupo virus infection, the paucity of survivors and the lack of programs for collection and storage of BHF immune plasma anticipate the problem of shortage in case of a new outbreak. In recent cases, ribavirin was offered to two patients presenting with life-threatening infection; both recovered without sequelae. These promising results suggest the need for performing more extensive clinical studies to assess the usefulness of ribavirin for treatment of BHF.[61]

Junín and Guanarito viruses showed very high in vitro sensitivity to ribavirin and also showed antiviral effect in patients.[31] One of the laboratory-acquired Sabiá virus infections was treated successfully with ribavirin; the treatment was started early after the onset, and there was no production of neutralizing antibodies.

The late evolution and low titers of Lassa virus neutralizing antibodies render immune serum–based therapy less efficient than in treatment of South American arenaviral fevers.[75] In contrast, clinical studies have shown ribavirin to be of benefit in severe cases and that it therefore should be used as early as possible in the course of the disease. The intravenous regimen that is recommended is as follows:

- a loading dose of 30 mg/kg, then
- 15 mg/kg every 6 hours for 4 days, then
- 7.5 mg/kg every 8 hours for 6 days.

A recognized side-effect of ribavirin is hemolytic anemia, reversible when drug administration is interrupted.

THE FUTURE OF ANTIVIRALS IN THE DOMAIN OF ARENAVIRUSES

In the past 5 years, basic research has used reverse genetics to develop replicons and infectious clones that are used to investigate virus replication mechanisms, to test antivirals in vitro, and to better understand arenavirus cycles. The development of reverse genetic systems allows conduction of a detailed molecular characterization of the viral *cis*-acting signals and *trans*-acting factors that control each of the steps of the virus cycle, including RNA synthesis, packaging, and budding. Likewise, the ability to generate predetermined specific mutations in the genome and to analyze their phenotypic expression will contribute to elucidation of the arenavirus-host interactions as well as the mechanisms leading to severe hemorrhagic disease in humans. The development of a mini-replicon system for Lassa virus will help the investigation of replication and transcription and may facilitate the testing of antivirals outside BSL-4 laboratories.[50]

Infectious LCM virus has been recovered entirely from cDNA. Intracellular transcription from polymerase I–driven vectors and co-expression of the minimal viral transacting factors NP and L from polymerase II–driven plasmids resulted in the efficient formation of infectious virus with genetic tags in both genome segments; this cDNA-derived virus behaves identically to wild-type virus in both cell culture and infected mice.[36]

High throughput screening of molecules for their antiviral effects is being performed increasingly by both public laboratories and private companies. From 2003 to 2006, six articles reporting testing of molecules with antiviral efficacy on arenaviruses were published.[1,3,5,12,47,102]

PREVENTION AND CONTROL

Prevention of arenavirus disease consists of preventing transmission from rodents to humans, from humans to humans, and from infected specimens to laboratory personnel. Strategies for avoiding contact between rodents and humans have been effective in BHF.[69] Simple trapping of *C. callosus* in towns was successful in reducing human contact and thus reducing the disease to essentially zero. This is more difficult in AHF because conditions under which human exposure occurs are different from those in BHF. *C. musculinus* (reservoir of Junín virus) distribution is much wider than that of *C. callosus* (reservoir of Machupo virus), and Argentine agricultural practices continue to place workers at risk of exposure to reservoir hosts.

A collaborative effort conducted by the United States and Argentine governments led to the production of a live attenuated Junín virus vaccine named Candid 1, which has passed safety and immunogenicity tests in U.S. volunteers. Its efficacy was established in a double-blind trial in 15,000 agricultural workers at risk for natural infection in Argentina. Subsequently, more than 100,000 persons were immunized with Junín virus vaccine in Argentina. Recent animal protection studies suggest that the Junín vaccine could be protective against Machupo virus infections as well. A prospective study conducted during two epidemic seasons among 6500 male agricultural workers in Argentina showed that Candid 1 vaccine efficacy was 84 percent or higher and that no serious adverse effects could be expected.[72]

Neutralizing antibody titers to Candid 1 vaccine against AHF were studied for 2 years after vaccination in 330 volunteers. Of a total of 160 volunteers who received Candid 1, 54 had no detectable preinfection with arenaviruses, 55 had preexisting antibodies to Junín virus, and 51 had preexisting antibodies to LCM virus; the remaining 170 individuals received placebo. Levels of anti–Junín virus antibodies displayed a trend in which

titers increased with the virulence of the infecting strain, from Candid 1 through subclinical Junín virus infection, vaccination after subclinical infection, and Junín virus symptomatic infection. The study also demonstrated that the mean titer and neutralizing antibodies to Candid 1 did not vary significantly during the 2 years, was significantly lower than that elicited by wild strains of Junín virus, significantly increased the titers of preexisting anti-Junín antibodies, and was not modified by preexisting anti-LCM virus antibodies.[2]

Comparative sequence analysis of the L RNA segment of Candid 1 strain and the more virulent ancestors (XJ 44 and XJ 13) revealed 12 point mutations in the L polypeptide that are unique to the vaccine strain[45]; whether these changes are associated with the attenuated phenotype remains to be investigated by the use of the reverse genetic systems.

For VHF, no preventive measures have been developed. Attenuated Junín virus strains do not protect experimental animals against challenge with Guanarito virus. In VHF, the evidence suggests that transmission occurs around houses and in fields as in BHF, so measures to avoid contact between rodents and humans should be effective, as shown for BHF.

For Lassa fever, reducing the contact with *Mastomys natalensis* is a formidable task in West Africa, and this option does not seem to have a promising future. Because person-to-person transmission has been reported in Lassa fever, precautions should be taken to place patients in single rooms. The health care team should be small and adequately trained; they should wear gloves, gowns, and filter masks.[14,16] All secretions should be decontaminated.[63] Laboratory procedures should be done with care by use of inactivating methods of heat, chemicals, or irradiation.

In rhesus monkeys *(Cercopithecus aethiops)*, the humoral antibody response measured after they were challenged with purified inactivated Lassa virus, although as high as in humans who recovered from Lassa fever, was insufficient to protect the animals from a fatal outcome.[78] A naturally attenuated strain (Mopeia virus, from Mozambique) protects rhesus monkeys against challenge with Lassa virus, but field studies are required to establish the extent and nature of natural human infection with this virus before it can be considered seriously as a candidate for human vaccine development. Alternative approaches, including the use of vaccinia virus vectors bearing the Lassa virus GPC or N gene, are being investigated actively and show promising preliminary results.[34,35] Recently, two peptides encoded by the glycoprotein precursor were shown to display high-affinity binding to human leukocyte antigen (HLA)-A*0201 transgenic mice; mice immunized with these peptides were protected against challenge with a recombinant vaccinia virus that expressed Lassa virus glycoproteins. These two epitopes represent candidates for inclusion in epitope-based vaccine constructs.[6]

THE FUTURE OF VACCINE RESEARCH

Tremendous advances have been made during the past 5 years for vaccine development. Many different approaches were used. Reverse genetic exchange of the viral glycoprotein for foreign glycoproteins created attenuated vaccine strains that remained viable although unable to cause disease in infected mice; this phenotype remained stable even after extensive propagation in immunodeficient hosts. The engineered viruses induced T cell–mediated immunity, protecting against systemic infection and severe liver disease on wild-type virus challenge.[4] The yellow fever vaccine 17D has been used as a vector for the Lassa virus glycoprotein precursor, resulting in construction of recombinant virus, which was replication competent and processed Lassa virus glycoprotein in cell culture. The recombinant virus replicated poorly in guinea pigs but still elicited specific antibodies against Lassa and yellow fever antigens, and single subcutaneous injection protected guinea pigs against fatal Lassa fever.[66] Clone ML29 is a reassortant virus that encodes the nucleocapsid protein and glycoproteins from Lassa virus and the RNA polymerase and matrix protein of Mopeia virus; replication of ML29 was attenuated in guinea pigs and nonhuman primates. Guinea pigs vaccinated with ML29 survived after challenge with Lassa virus without developing either signs of disease or histologic lesions. Rhesus macaques inoculated with clone ML29 developed primary virus-specific T cells capable of secreting interferon in response to homologous and heterologous challenge (the latter with Mopeia virus and LCM virus). Vaccinated monkeys did not present any histologic lesions or signs of disease.[65]

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CHAPTER 199

Filoviral Hemorrhagic Fever: Marburg and Ebola Virus Fevers

Eric Leroy

Ebolavirus and *Marburgvirus* are the only genera of the family *Filoviridae*, which belongs to the order Mononegavirales.[13] Ebola and Marburg viruses are among the most virulent pathogens for humans and great apes. Human outbreaks of acute hemorrhagic fever are associated with case-fatality rates of up to 88 percent, and Ebola virus also causes concomitant devastating outbreaks in Central African great apes. No specific treatment or vaccine is available.

Ebola and Marburg viruses are biohazard level 4 and biothreat category A agents and obviously must be manipulated in maximum security facilities.

ETIOLOGIC AGENTS

Members of the *Filoviridae* all have similar morphologic features. The viral particles are filamentary and often form bizarre configurations, such as 6-shaped forms and hairpins (Fig. 199–1), and may be up to 14,000 nm in length.[13,62] In contrast, the filaments are always 80 nm wide. The capsid contains a single negatively stranded RNA genome that encodes seven structural proteins and one nonstructural protein, the soluble glycoprotein.[63,76,77] Filoviruses replicate readily in Vero, MA-104, and SW-13 cells, although specific species seem to prefer particular primate cell lines. These viruses also are highly pathogenic for macaques, producing a hemorrhagic disease and killing more than 75 percent of infected animals.[15,17] The *Filoviridae* comprise two genera. The only member species of the "Marburg-like viruses" genus is Marburg virus itself. The "Ebola-like viruses" genus comprises four antigenically and genetically related species, named *Zaire ebolavirus*, *Sudan ebolavirus*, *Ivory Coast ebolavirus*, and *Reston ebolavirus*. With the possible exception of *Reston ebolavirus*, filoviruses are highly pathogenic organisms that must be handled in special laboratories (biosafety level 4) with maximal biologic containment. Guidelines on the management of patients and specimens suspected of harboring these viruses are available from the Centers for Disease Control and Prevention in Atlanta *(http://www.cdc.gov/ncidod/dvrd/spb)*. There are virtually no antigenic relationships between Marburg virus and Ebola virus. The four Ebola virus species exhibit extensive cross-reactivity in immunofluorescence methods and enzyme-linked immunosorbent assays (ELISAs). Virus neutralization, in contrast, has been virtually impossible to measure for any of the filoviruses, which has hindered epidemiologic and ecologic studies of these agents. Unlike the other three Ebola species, which are confined to Africa, *Reston ebolavirus* is found only in the Philippines among nonhuman primates.[30] Only the three African Ebola virus species appear to be pathogenic for humans.

HISTORY AND EPIDEMIOLOGY

MARBURG VIRUS

In 1967, simultaneous outbreaks of a previously unknown hemorrhagic fever occurred in Marburg and Frankfurt (Germany) and

in Belgrade (Serbia, former Yugoslavia) (Table 199–1).[50,51,65] The source was traced to African green monkeys *(Cercopithecus aethiops)* from a primate export facility in Entebbe, Uganda. Thirty-one cases occurred, including six by secondary transmission, and seven deaths occurred among primary cases. An unknown infectious agent was recovered from the blood and organs of these persons and was named Marburg virus, after the German city where the first cases occurred. Sporadic cases since then have been diagnosed in the south part of Africa in 1975[21] and in Kenya in 1982.[67] A lengthy outbreak occurred between 1998 and 2000 in Durba (eastern Democratic Republic of the Congo), affecting 154 people and killing 83 percent of victims (Fig. 199–2).[7] Most patients (94%) worked in underground gold mines. Because of the civil war raging in 1997, the cause of the outbreak was not identified until 1999, a year after the first cases occurred. The Durba outbreak was characterized by the circulation of multiple viral strains, pointing to multiple independent introductions from the unknown natural reservoir.[6,7] The largest recorded Marburg outbreak occurred recently, in Uige Province of northern Angola. It was the first recorded outbreak in the west side of Africa and lasted from October 2004 to July 2005, causing 252 cases and 227 deaths (fatality rate, 90%).[10] Complete genomic characterization showed that the culprit strain, named *Angola marburgvirus*, was closely related to previous East African isolates.[73-82]

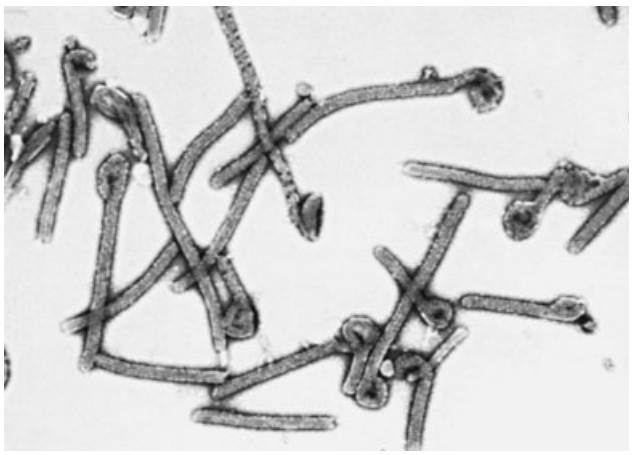

Figure 199–1 Electron micrograph of Ebola virus (×38,750). *(Courtesy of T. W. Geisbert.)*

EBOLA VIRUS

1976-1979: The First Recorded Ebola Outbreaks

Ebola first emerged in the form of two nearly simultaneous outbreaks in 1976, one due to *Sudan ebolavirus* and the other due to *Zaire ebolavirus*.

The first outbreak was due to *Sudan ebolavirus* and occurred in Sudan, near the border with the Democratic Republic of the Congo, mainly affecting the towns of Nzara and Maridi.[68] There were 284 cases and the mortality rate was 53 percent, a proportion characteristic of *Sudan ebolavirus* infection. The outbreak due to *Zaire ebolavirus* occurred in the Democratic Republic of the Congo, near the border with Sudan, 2 months later.[36] The epicenter was Yambuku, about 800 km from Nzara. This previously unknown disease was named for the river Ebola, which flows past Yambuku. There were 318 cases and the mortality rate was 89 percent, characteristic of *Zaire ebolavirus* infection. Later, an unconfirmed lethal case involving a 9-year-old girl living in Tandala, Democratic Republic of Congo, was reported,[31] followed by another *Sudan ebolavirus* outbreak in 1979, again in Nzara, with 34 cases and 22 deaths.[5]

1994-1997: Ebola Resurgence

After a 15-year period in which no further cases were recorded, Ebola re-emerged in 1994 for a 3-year period. This new phase

TABLE 199–1 Year and Location of Filoviral Hemorrhagic Fever Cases and Outbreaks

Year	Virus Species	Country	No. of Human Cases	Fatality Rate (%)
1967	*Marburg*	Germany and Serbia	37	19
1975	*Marburg*	South Africa	3	33
1976	*Zaire Ebola*	Zaire	318	88
1976	*Sudan Ebola*	Sudan	284	53
1976	*Sudan Ebola*	England	1	0
1979	*Sudan Ebola*	Sudan	34	65
1980	*Marburg*	Kenya	2	50
1987	*Marburg*	Kenya	1	100
1989	*Reston Ebola**	United States	0	0
1990	*Reston Ebola**	United States	0	0
1992	*Reston Ebola**	Italy	0	0
1994	*Marburg*	Zaire	11	Unknown
1994	*Zaire Ebola*	Gabon	44	63
1994	*Côte d'Ivoire Ebola*	Ivory Coast	1	0
1995	*Zaire Ebola*	Zaire	315	81
1996	*Zaire Ebola*	Gabon	37	57
1996	*Zaire Ebola*	Gabon	60	75
1996	*Zaire Ebola*	South Africa	2	50
1996	*Reston Ebola**	United States	0	0
1996	*Reston Ebola**	Philippines	0	0
1998-2000	*Marburg*	DRC†	130	71
2000	*Sudan Ebola*	Uganda	425	53

**Symptomatic infections and fatal cases were observed in nonhuman primates only.*
†*Democratic Republic of Congo (DRC), formerly was known as Zaire.*

Figure 199–2 Geographic distribution of human outbreaks of Marburg virus fever in Africa.

was marked by the identification of a new species, *Ivory Coast*, and by four *Zaire ebolavirus* outbreaks. The only case caused by *Ivory Coast ebolavirus* occurred in 1994, when an ethnologist sickened a few days after performing an autopsy on a chimpanzee found dead in Tai National Park in Ivory Coast.[18,42,43] A large outbreak then occurred in 1995, in and around the town of Kikwit, south of the Democratic Republic of Congo,[56] with 315 cases and a mortality rate of 81 percent. Despite the deployment of more sophisticated scientific and medical resources than were available in 1976, this outbreak was as large as the 1976 one, probably because it affected a town of several hundred thousand inhabitants and where person-to-person transmission occurred mainly in two hospitals. Three other outbreaks, all due to *Zaire ebolavirus*, struck northeast Gabon between 1996 and 1997.[28] The first occurred in northeastern Gabon, close to the border with Cameroon,[2] in three gold-digger camps located in the heart of the forest. Some of the victims left the camps for the nearest hospital, located in Makokou, where they infected other patients and caused a second wave of virus dissemination. In total, 49 clinical cases and 29 deaths were recorded. The second outbreak hit the village of Mayibout, located south of Mekouka.[28] The first victims were 18 children who had helped to carry and butcher a chimpanzee carcass found in the forest. These 18 children infected their families and friends, who in turn transmitted the disease to neighboring villages. In total, this outbreak involved 31 people and caused 21 deaths. The third outbreak in this region occurred between October 1996 and March 1997.[28] It started among hunters, who infected a traditional healer, his assistant, and some of his patients, who in turn transmitted the disease to several towns and villages in Gabon. Fifteen cases and 11 deaths were recorded in Libreville, and a South African nurse was infected by a Gabonese physician who had traveled to Johannesburg. This outbreak, with 60 cases and 45 deaths occurring in a 6-month period, was noteworthy for its wide geographic range.

2000-2004: A Geographic Pattern of *Zaire Ebolavirus* and *Sudan Ebolavirus* Resurgence

ZAIRE EBOLAVIRUS OUTBREAKS

The first outbreak during this period occurred in northeast Gabon and northwest Democratic Republic of Congo between October 2001 and May 2002.[47] It spread along the main road between Mekambo (Gabon) and Mbomo (Democratic Republic of Congo) and, in fact, involved several independent epidemic chains of human-human transmission with different source animals.[46] All these epidemic chains started when local hunters found and manipulated the carcasses of an antelope, chimpanzees, and gorillas. Simultaneously, a second outbreak occurred in the Democratic Republic of Congo, in villages close to the Gabonese border, 200 km south of Mbomo. It then spread to the town of Kelle, about 65 km away. The origin of this outbreak is unknown. The third outbreak also affected the region of Mbomo, between 2002 and 2003.[20] It had two independent sources resulting in two independent chains, one in Mbomo and the other in Kelle. This outbreak involved 143 cases and caused 128 deaths. The fourth outbreak again affected the region of Mbomo, in late 2003; the first cases occurred in Mbanza, a village located about 30 km north of Mbomo. Thirty-five cases and 29 deaths were recorded.[80] The last outbreak occurred in 2005 in Etoumbi, 60 km south of Mbomo, where 12 cases and 9 deaths were reported. Simultaneously with the human outbreaks, *Zaire ebolavirus* also infected animals belonging to several species, including gorillas, chimpanzees, and duikers, probably accounting for the sharp declines in animal populations observed in these regions between 2001 and 2005.[32,46,79]

SUDAN EBOLAVIRUS OUTBREAKS

Two outbreaks of *Sudan ebolavirus* also occurred during this period (Fig. 199–3). One hit Uganda in 2000, causing 173 deaths

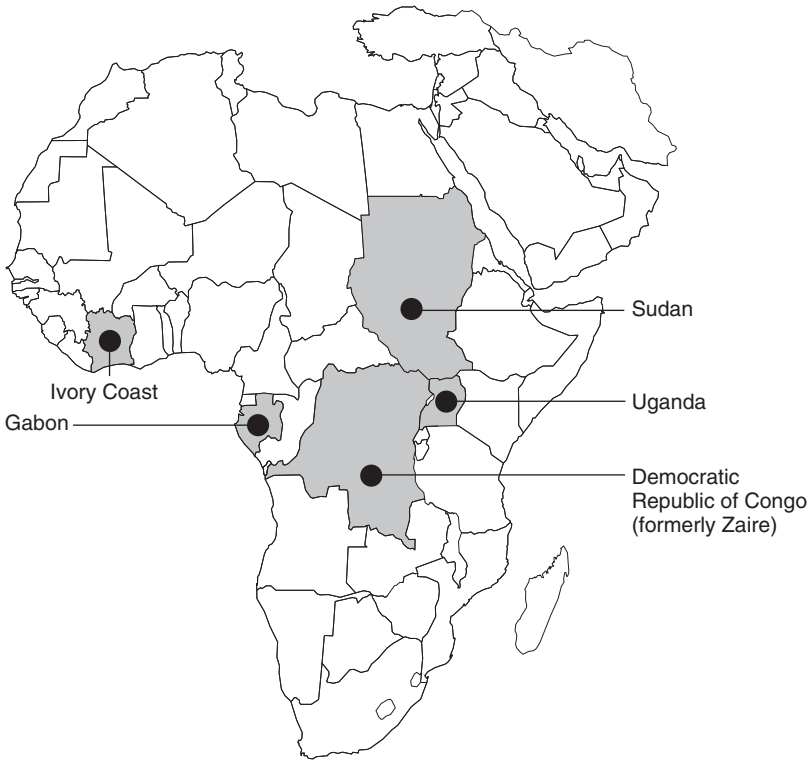

Figure 199–3 Geographic distribution of human outbreaks of Ebola virus fever in Africa.

among its 425 victims (mortality rate, 40.7%).[83] It was the largest of all recorded outbreaks and comprised three foci, one in the immediate area of Gulu, one in the town of Masindi, and one in Mbarara; the last outbreak was small, and the index patient was a soldier. A second *Sudan ebolavirus* outbreak occurred in Sudan in 2004 in the town of Yambio, located only a few dozen kilometers from Nzara and Maridi, which had been struck in 1976 and 1979. There were 17 cases and 7 deaths.[81]

THE PARTICULAR CASE OF *RESTON EBOLAVIRUS*

In 1989, it became apparent that Ebola virus is not restricted to Africa. A group of cynomolgus monkeys *(Macaca fascicularis)* from the Philippines, quarantined in a laboratory in Reston, Virginia, developed a lethal hemorrhagic disease.[34] Virions antigenically similar to Ebola virions were detected in tissues, and Ebola virus infection was confirmed by culture in Vero cells. In the following weeks, the introduction of new monkeys and the spread of the infection to several animal rooms meant that the entire cohort of more than 400 animals had to be destroyed. The building was fumigated and abandoned. The virus was recovered from another macaque in Philadelphia and later in a laboratory in Italy.[57] Although no humans fell ill during these episodes, several monkey handlers in the United States were shown to have seroconverted. In 1996, a cynomolgus monkey imported from the Philippines died in an animal facility in Texas, where two additional cases subsequently occurred, all caused by *Reston ebolavirus*.[61]

EBOLA VIRUS ECOLOGY AND BREAKTHROUGH TO HUMANS

The sources of most human outbreaks of viral hemorrhagic fever, including most Marburg outbreaks, the *Zaire ebolavirus* outbreaks that occurred between 1976 and 1997, and all outbreaks of *Sudan ebolavirus*, have not been identified.

In contrast, several outbreaks have been firmly linked to infected animal carcasses. As mentioned before, an ethnologist became infected by *Ivory Coast ebolavirus* in 1994 while performing an autopsy on a chimpanzee.[43] Similarly, the 1996 Mayibout outbreak in Gabon started among children who found and butchered a chimpanzee carcass in the forest.[28] The 1967 outbreak of Marburg virus infection in Marburg and Belgrade was linked to the handling of organs and tissues from vervet monkeys imported from Uganda.[50,65] Likewise, the sources of the *Zaire ebolavirus* outbreaks that occurred in the border region of Gabon and Democratic Republic of Congo between 2001 and 2003 are well documented; they all occurred after people handled animal carcasses they had found in the forest (mainly gorillas, chimpanzees, and duikers).[46]

Since 1976, 13 outbreaks and two isolated cases of Ebola, totaling about 1850 human cases and 1300 deaths, have struck countries spanning the equatorial forest regions of Africa.[80]

Ebola hemorrhagic fever is thus a zoonotic disease that often is transmitted to humans by direct contact with infected animal carcasses, which are themselves probably infected from the reservoir animal species that harbor the virus, symptomatically or asymptomatically. Since the first recorded human outbreak occurred, many field and laboratory studies have been conducted in attempts to identify Ebola virus reservoir species. Wild animals (vertebrates and invertebrates) were captured in the field, and blood and tissues were used to inoculate Ebola virus–permissive Vero cells. Six field studies, conducted from 1976 to 1995, examined a total of 7000 vertebrates and 30,000 invertebrates, but none was found to harbor Ebola virus.[36-54] Although small Ebola virus nucleotide sequences and Ebola virus–like nucleocapsids were detected in organs of six mice (*Mus musculus* and *Praomys* sp.) and a shrew *(Sylvisorex ollula)* caught in Central African Republic in 1999,[54] no conclusions could be drawn as to their reservoir status.

All attempts at experimental inoculation of rodents, bats, birds, reptiles, mollusks, arthropods, and plants have failed, although some bat species belonging to the genera *Epomophorus* and *Tadarida* developed transient viremia lasting nearly 4 weeks.[72,75] These results did not provide conclusive evidence that bats serve as an Ebola virus reservoir.

Other field collections were conducted during 2002 and 2003 in Gabon and Republic of Congo, in areas hit by the different outbreaks. A total of 1030 animals were captured, autopsied, and analyzed. The captures and laboratory analyses spanned a period of 4 years. The results showed that three species of fruit bat (*Hypsignathus monstrosus*, *Epomops franqueti*, and *Myonycteris torquata*) were asymptomatically infected by Ebola virus.[45] Indeed, anti–Ebola virus IgG was detected in the serum of 16 bats (four *Hypsignathus*, eight *Epomops*, and four *Myonycteris*), but no other animal species, including other bat species, were positive. Likewise, Ebola virus nucleotide sequences were detected in the organs of 13 bats (three *Hypsignathus*, five *Epomops*, and five *Myonycteris*). Sequencing of the amplified fragments confirmed they were specific to Ebola virus. Phylogenetic analysis of the sequences by the Bayesian and maximum parsimony methods showed they belonged to the species *zaire*. Although the virus itself was not isolated, this work provided the first biologic evidence that some fruit bat species harbor Ebola virus. In addition, these findings were in keeping with certain epidemiologic clues from previous outbreaks and with the transient viremia observed in some bat species after experimental challenge. The distribution of the bat species also matched that of the outbreaks.

CLINICAL MANIFESTATIONS

Marburg virus and the four Ebola virus species all cause severe hemorrhagic fever, but they differ in terms of the incubation period, clinical severity, and case-fatality rate.[49] After an incubation period ranging from 2 to 21 days (mostly 4 to 9 days), symptoms of Ebola virus fever occur abruptly. Most patients develop high fever, headache, muscle pain, stomach pain, fatigue, and diarrhea; some patients also have sore throat, hiccup, rash, and red and itchy eyes.[9,49] Bloody diarrhea and hematemesis also can occur. After about a week, probable fatal outcome is signaled by the onset of chest pain and shock, together with blindness and bleeding in some cases. The incubation period of Marburg virus fever is almost identical to that of Ebola virus fever; clinical onset also is abrupt, starting with fever, chills, headache, and muscle pain. A week later, some patients develop a maculopapular rash, nausea, vomiting, chest pain, abdominal pain, diarrhea, and sore throat. Clinical status deteriorates gradually, with jaundice, delirium, shock, liver failure, massive hemorrhaging, and multiorgan dysfunction.[9,33,58,66] Although the virus disappears quickly from the bloodstream of survivors, it can persist in the eyes and testicles.[11,51] Indeed, Marburg virus was recovered from the semen of one patient some weeks after clinical recovery.

Studies of nonhuman primates show that filovirus infection triggers clotting disorders, but their clinical repercussions are variable. In survivors, they are limited to conjunctival hemorrhage, easy bruising, failure of venipuncture sites to clot, and bloody vomiting and diarrhea. Most fatalities are associated with disseminated intravascular coagulation and systemic bleeding.[14,16,23,26,27]

Mild leukopenia is a common development early in filovirus diseases, but leukocytosis may appear later in response to secondary bacterial infection (usually of the lungs). Thrombocytopenia (<100,000 platelets/mm^3) always is present, and coagulation studies are abnormal at the time of onset of bleeding. Fibrin split products indicative of disseminated intravascular coagulation have been detected in humans and experimentally infected primates. Serum transaminase activity is markedly elevated, whereas the bilirubin level is normal and clinical jaundice is a rare development. Serum protein levels are depressed, the hematocrit is increased, and proteinuria is a common finding.

PATHOLOGY

Limited information is available on human filovirus infections, whereas a large body of data has been obtained with experimentally infected rodents and nonhuman primates. Why some people recover from filovirus fevers and others die remains unclear.

No major difference exists in the pathogenesis of the different filovirus diseases. Studies of *Zaire ebolavirus* show that dendritic cells and macrophages are the initial host targets at the entry site.[12,23,27] These infected cells then disseminate through the bloodstream and lymphatics to all organs, where they release virions that infect monocytic cells in lymph nodes, spleen, liver, and other organs and tissues.[1] In the later stages of the disease, the virus also infects other cell types, such as hepatocytes, adrenal cortical cells, and endothelial cells, causing lysis and necrosis.[26] Lymphocytes do not seem to be infected, but they nonetheless undergo bystander apoptotic lysis.[24]

The pathogenetic mechanisms of filovirus infection involve both direct damage to infected cells[70,78] and indirect insults through interactions between the virus and the adaptive immune system.[53] By contrast with other viruses, filoviruses can infect and kill a wide variety of cells throughout the body. This apparent lack of target cell specificity may be due to the binding of the viral surface glycoprotein to widely distributed cell surface lectins.[64] Infection of macrophages and dendritic cells can suppress various cellular functions, including the production of proinflammatory mediators such as interferon-α, interleukin-1β, and tumor necrosis factor-α (TNF-α),[4,8,29] contributing to the suppression of innate antiviral responses and weakening adaptive immune responses. Studies of serial samples from infected nonhuman primates have shown that lymphocytes undergo apoptosis in vivo, probably through the TRAIL and FAS pathways.[23,24] These findings are compatible with the massive intravascular apoptosis of peripheral monocytic cells observed in patients who died (but not in those who survived) during the 1996 outbreaks in Gabon.[3] This bystander lymphocyte destruction has been suggested to play a major role both in immunosuppression and in the onset of septic shock observed in nonsurvivors. Various studies have shown that filovirus infection of endothelial cells causes their lysis and a loss of vascular integrity, leading to disseminated intravascular coagulation, vascular dysfunction, and finally shock.

DIAGNOSIS AND DIFFERENTIAL DIAGNOSIS

In the clinical setting, Marburg and Ebola virus fevers must be distinguished from other viral hemorrhagic diseases occurring in Africa as well as from the many bacterial, rickettsial, and protozoal diseases that can cause similar initial signs and symptoms. The absence of jaundice helps eliminate yellow fever and Rift Valley fever. For patients seen outside Africa, the travel history is the most important diagnostic sign available to physicians. Early laboratory diagnoses of Ebola virus and Marburg virus infection are based on antigen-capture ELISA, reverse transcriptase–polymerase chain reaction (RT-PCR), real-time PCR, and virus isolation.[38-40,44,74] These tests are the most accurate tools for diagnosis of acute Ebola virus fever. Later in the course of the disease, or after recovery, diagnosis relies on immunoglobulin M (IgM)-capture ELISA or IgG antibody detection. Interestingly, however, specific antibodies do not develop in a significant proportion of patients in whom the disease is fatal. Recently, a study

showed that these diagnostic tools also could be used for oral fluid specimens obtained from clinical patients.[19] Because large quantities of Ebola virus are present in dermal tissue, skin necropsy with immunohistochemical analysis has been proposed for postmortem diagnostic confirmation.[85] These specimens are easy to collect, and formalin fixation renders them safe for transport. Skin biopsy testing is not sufficiently sensitive for early diagnosis, however, and thus should be reserved for dead or dying patients.

Cases have been classified into three categories, depending on the patient's clinical manifestations and epidemiologic data:

- Suspect: a person with fever and a history of contact with a potential case (i.e., a person with unexplained bleeding, or with fever plus three or more of the following symptoms: headache, vomiting, loss of appetite, diarrhea, weakness or severe fatigue, abdominal pain, body aches or joint pains, difficulty swallowing, difficulty breathing, and hiccups; and any person having died of unknown causes).
- Probable: a case that meets the preceding criteria and is assessed and reported by a physician.
- Confirmed (laboratory-confirmed case): a case that meets the surveillance case definitions, and the patient is positive for Ebola virus antigens, Ebola virus IgG antibodies, or Ebola virus genomic sequences (RT-PCR).

TREATMENT AND PREVENTION

No specific treatment of filoviral hemorrhagic fevers is available. Ribavirin and interferon have no effect on filoviruses, and no other antiviral compounds have been found to be effective.[35] Treatment relies on symptomatic measures aimed at maintaining hydration and nutritional status and at preventing or curing bacterial and parasitic infections. Convalescent plasma has been used, but its efficacy has not been proved. In a study conducted during the 1995 Kikwit Ebola epidemic, seven of eight patients who received blood collected from convalescent donors recovered, warranting a thorough evaluation of passive immune therapy.[55] One person infected by *Sudan ebolavirus* after a laboratory accident survived after receiving plasma from *Zaire ebolavirus* survivors. Likewise, several patients who received early treatment with Marburg virus immune plasma appeared to have milder disease. Hyperimmune globulin raised in horses protected baboons in recent experimental studies.[52] Goat immune globulin has been tested in laboratory animals and also was given to laboratory researchers suspected of being infected by Ebola virus[41]; one researcher with highly probable infection made a full recovery. Recently, administration of recombinant nematode anticoagulant protein c2 (rNAPc2), a potent inhibitor of tissue factor–initiated blood coagulation, attenuated proinflammatory and coagulopathic responses and prolonged the survival of macaques that had received a lethal inoculum of Ebola virus.[22] This opens up interesting new possibilities for postexposure protection.

Attempts also are being made to develop a vaccine against Ebola virus. Several candidate vaccines were effective in guinea pigs but less so in macaques.[25] Experimental vaccines have so far been based on viral particles inactivated by heat, formalin, or gamma irradiation[48]; plasmids coding for the Ebola virus glycoprotein (GP) or nucleoprotein (NP)[84]; recombinant GP-expressing Venezuelan equine encephalitis virus[59] or variola virus; and Ebola virus particles encapsulated in liposomes.[60] In contrast, a vaccine candidate based on three injections of GP-encoding DNA given 4 weeks apart, followed by a booster with recombinant inactivated GP-expressing adenovirus, protected macaques from infectious challenge 1 week later.[71] However, the small inoculum used to challenge the macaques (6 plaque-forming units [pfu]), together with the lengthy immunization protocol, renders this candidate vaccine impractical.

A new advance was made recently with the development of two candidate vaccines that appear to be effective in both guinea pigs and macaques. The first consists of a single injection of 2×10^{12} particles of recombinant inactivated adenovirus 5 expressing both the GP and the NP (ADV-GP/NP). A variant consisting of two injections 9 weeks apart also has been tested. The two variants gave total protection and induced interferon-γ production by $CD8^+$ T cells, as well as a humoral response, after both low-dose challenge (13 pfu) and high-dose challenge (1500 pfu) 1 or 4 weeks later. No change in $CD4^+$ T lymphocyte status was observed. The single injection offers faster but shorter protection than does the dual injection.[69] The second candidate vaccine consists of a single dose of 10^7 pfu of live attenuated, recombinant, vesicular stomatitis virus expressing the Ebola GP (VSVΔG/*Zaire ebolavirus*). This immunization fully protected macaques from a high-dose challenge (1000 pfu) 28 days later. It induced a moderate increase in anti-GP IgG (mainly neutralizing antibodies) and a strong increase in interferon-γ^+ and TNF-α^+ CD8 T cells and interferon-γ^+ and TNF-α^+ CD4 T cells. Nearly 30 macaques so far have received this immunization, and none of them has developed any clinical signs after infectious challenge.[37]

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SUBSECTION 10

Coronaviridae and *Toroviridae*

CHAPTER 200

Coronaviruses and Toroviruses, Including Severe Acute Respiratory Syndrome

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The family *Coronaviridae* is composed of two genera, *Coronavirus* and *Torovirus*. The two genera are related in that they appear similar on electron microscopy and share similar strategies of replication (along with other members of the order Nidovirales). They differ, however, in the size of their RNA genome and structural proteins as well as in the morphologic features of their nucleocapsids. Until recently, coronaviruses were considered to cause upper respiratory tract illness and probably also some undetermined fraction of viral diarrhea. In the final months of 2002 and extending into 2003, a new coronavirus, the severe acute respiratory syndrome (SARS) coronavirus (SARS-CoV), was found to be the cause of an acute, severe, frequently fatal respiratory disease with prominent systemic symptoms (severe acute respiratory syndrome, or SARS). The outbreak spread worldwide from its origin in southern China and probably represented the transmission from animal (possibly the palm civet) to human. In retrospect, the emergence of SARS and the subsequent explosion of new information about coronaviruses are consistent with what was already known about coronaviruses: they are widespread pathogens in the animal kingdom, they evolve rapidly through mutation and recombination, and they jump species barriers.[104] Coronaviruses cause a wide variety of important diseases in animals, including infectious bronchitis and nephrosis in chickens; gastroenteritis and encephalitis in young piglets; enteritis in turkeys, dogs, and calves; hepatitis and encephalitis in mice; pneumonitis and sialodacryoadenitis in rats; and infectious peritonitis in cats. Recently, multiple strains of unknown pathogenicity have been recovered from multiple species of bats.[223] Toroviruses are, at least as presently known, exclusively enteric pathogens, both in animals and in humans.

Since the first report of human coronavirus (HCoV) isolation in 1965,[203] the HCoV group of RNA viruses has been confirmed as a frequent cause of the common cold in children and adults. They also have been implicated as contributors to lower respiratory infections in children and adults, and more recently, they have been shown to be important causes of respiratory disease in the elderly.[56,214] Their role in acute or chronic gastroenteritis is less clear.

The first HCoV was cultivated by Tyrrell and Bynoe[203] at the Common Cold Unit in Salisbury, England, with the use of human embryonic tracheal and nasal epithelial mucosal organ cultures. The authors were able to produce colds regularly in volunteers inoculated with organ culture fluid from the first and later passages of an agent, B814, obtained from a boy with a cold.[15]

Working independently, Hamre and Procknow[73] in 1966 described the isolation of five viruses, including the prototype strain HCoV-229E, in primary human embryonic kidney cell cultures. Four of these agents were obtained from medical students with upper respiratory illnesses and one from a healthy student. The growth of six additional HCoVs, including the second human prototype HCoV-OC43, was reported in 1967 by McIntosh and associates,[139,141] who used human embryonic tracheal organ cultures inoculated with secretions from adults with upper respiratory infections.

That the HCoVs were related to similar agents known to infect animals soon became evident. By electron microscopy, 229E and B814 were demonstrated to be morphologically identical to each other and to avian infectious bronchitis virus.[2] Subsequently, mouse hepatitis virus was demonstrated to be very similar morphologically and to be antigenically related to HCoV-OC43.[139,143] Shortly thereafter, these and similar agents were placed in the genus *Coronavirus* of the family *Coronaviridae*, named for the crownlike appearance of their surface projections on electron microscopy.[202]

Poor growth and a lack of cytopathic effect in cell culture were, until recently, major deterrents to research on HCoV. With the development of the polymerase chain reaction (PCR), and after the dramatic emergence of SARS in 2002 and 2003[25,111,175,201,238] and the discovery of the SARS-CoV,[50,101,168] the

field of coronavirology has developed widely and rapidly. Besides the SARS-CoV and its related animal strains,[70,106,123] two more human coronaviruses have now been described, NL-63[54,60,207] and HKU-1,[107,222] both of which clinically resemble traditional respiratory coronaviruses 229E and OC43 more than the SARS-CoV. The full spectrum of viruses in the human host likely is still to be delineated because several HCoVs, including the very first isolate, HCoV-B814, remain antigenically and genetically uncharacterized.[14,16,143,203]

Toroviruses were, like coronaviruses, first described in animals. They were detected in the 1970s and 1980s in the feces of cattle (Breda virus) and horses (Berne virus).[218,225] Shortly thereafter, Beards and colleagues[10] reported finding particles with a similar appearance in feces of adults and children with gastroenteritis. These particles aggregated in the presence of antiserum to the bovine and equine viruses. Neither the human nor the bovine viruses have been grown in tissue culture.

ETIOLOGIC AGENTS

CORONAVIRUSES

Coronaviruses are medium to large (80 to 220 nm) pleomorphic, spherical or elliptical, enveloped RNA viruses with widely spaced, petal-shaped, 20-nm-long surface projections giving the virus the appearance of a solar corona.[138] They are labile to heat, lipid solvents, and acid pH. The RNA genome is 27 to 32 kb (large for an RNA virus), single stranded, positive sense, and infectious.[104] The genomes of multiple coronaviruses have been sequenced completely, including those of HCoV-229E, HCoV-OC43, NL-63, HKU-1, and multiple strains of the SARS-CoV.[60,70,76,207,212,213,222] The genome and its surrounding capsid are arranged in helical symmetry and enclosed within a lipoprotein envelope.

Within the coronavirus particle, a nucleoprotein (N) surrounds the RNA genome, and together they appear as a coiled tubular helix within the bilayer lipid-containing envelope. The envelope contains two or three glycoproteins: a matrix protein M, which is embedded in the envelope; a surface component S, which is the structural protein of the petal-shaped spikes; and a hemagglutinin esterase HE, which is found in several of the group II viruses, including HCoV-OC43, and contains sequences closely related to influenza C hemagglutinin. Before the discovery of the SARS-CoV, the antigenic interrelationships of these four proteins permitted both the animal coronaviruses and HCoVs to be arranged into three groups, and recent genetic analyses confirm this classification scheme. The SARS-CoV and the related animal coronaviruses may form a fourth group, although they appear also to be related most closely to group II, and the final taxonomy has not yet been established.[65] The four respiratory HCoV serotypes, each along with several other mammalian coronaviruses, have been placed in group I (229E and NL-63) or group II (OC43 and HKU-1), and avian infectious bronchitis virus and several other avian coronaviruses belong to group III.

Coronaviruses contain a positive-sense, single-stranded RNA genome of 26 to 31 kb, the largest of any RNA virus group. In coronavirus replication, all processes take place in the cytoplasm. In the first step, the virus attaches to the cell membrane by using its HE or S protein in the petal-shaped spike. HCoV-229E uses aminopeptidase N (CD-13) as a cellular receptor,[231] and both the SARS-CoV and NL-63 use angiotensin-converting enzyme 2,[79,122] whereas the receptor for HCoV-OC43 still has not been identified definitively. Penetration occurs as a result of S protein–mediated fusion of the viral envelope with the plasma membrane. The genome then codes for the formation of an RNA-dependent RNA polymerase, which initiates the transcription of subgenomic mRNA through a negative-stranded intermediary. The mRNA molecules, as in other members of the virus order Nidovirales, form a nested set; the sequences of the first open-reading frame at the 5′ end (after a leader sequence) contain the coding region, and subsequent sequences through to the polyadenylated 3′ end are untranslated.[164] Virions then are assembled by budding into cytoplasmic vesicles in the rough endoplasmic reticulum and Golgi region.[72] Virus particles are released by cell lysis or fusion of post-Golgi, virion-containing vesicles with the plasma membrane.

TOROVIRUSES

Toroviruses are morphologically similar to coronaviruses in that they are pleomorphic, membrane-coated viruses, 100 to 120 nm in largest diameter, and bear club-shaped surface projections.[217] A photomicrograph of a torovirus particle is shown in Figure 200–1, juxtaposed to an intestinal coronavirus at the same magnification. The nucleic acid–containing core of the virus has a unique appearance on electron microscopy: it assumes a doughnut shape (i.e., a torus) if it is viewed from the right angle.[9,51,218] Berne virus, first isolated in the 1970s from horses with diarrhea, grows in equine cell tissue culture and has been characterized most thoroughly. The human toroviruses, like the bovine toroviruses, do not grow in tissue culture. Toroviruses differ from enteric coronaviruses in being somewhat smaller and more pleomorphic and in having somewhat less distinct surface projections.[51]

The complete bovine torovirus genome has been sequenced recently.[49] Toroviruses contain S glycoproteins on their surface, but no significant sequence homology exists between them and the S proteins of coronaviruses.[67,196] A second surface protein with HE activity and sequence homology to the HE proteins of both influenza C virus and mouse hepatitis virus has been found on Breda virus, the bovine torovirus.[45] Whether this molecule exists on human toroviruses is not known, although human toroviruses do hemagglutinate rabbit erythrocytes.[51] Toroviruses contain membrane and nucleoproteins similar to those of coronaviruses but with no sequence homology. Although the replicase contains sequence similarity to that of coronaviruses, because their mRNAs do not have 5′ leader sequences, their replication strategy must have important differences.[164,196]

EPIDEMIOLOGY

Early epidemiologic surveys of HCoV infections were carried out by serology with 229E or OC43 viruses used as antigens.[20,99,131] Recently, PCR-based surveys of 229E, OC43, NL-63, and HKU-1 have been performed in various collections of respiratory samples. These collections usually have represented frozen samples from patients of various ages with respiratory illness but sometimes include samples from asymptomatic subjects. As expected, the results of such surveys depend heavily on the selection of subjects (hospitalized or not; age; symptomatic or not) as well as the method of sample collection (nasopharyngeal aspirate, throat or nasopharyngeal swabs, bronchoalveolar lavage), season, and number of years represented. Because of selection in such surveys, the information obtained almost always is biased or limited in one way or another.

SARS-CoV epidemiology is quite separate and has been described both by PCR testing of respiratory and stool samples and by serology, which is reviewed in the section devoted to the outbreak below.

Because enteric HCoVs rarely have been propagated in culture, epidemiologic study of these agents has been hindered by lack of antigens for serology. Likewise, the epidemiology of

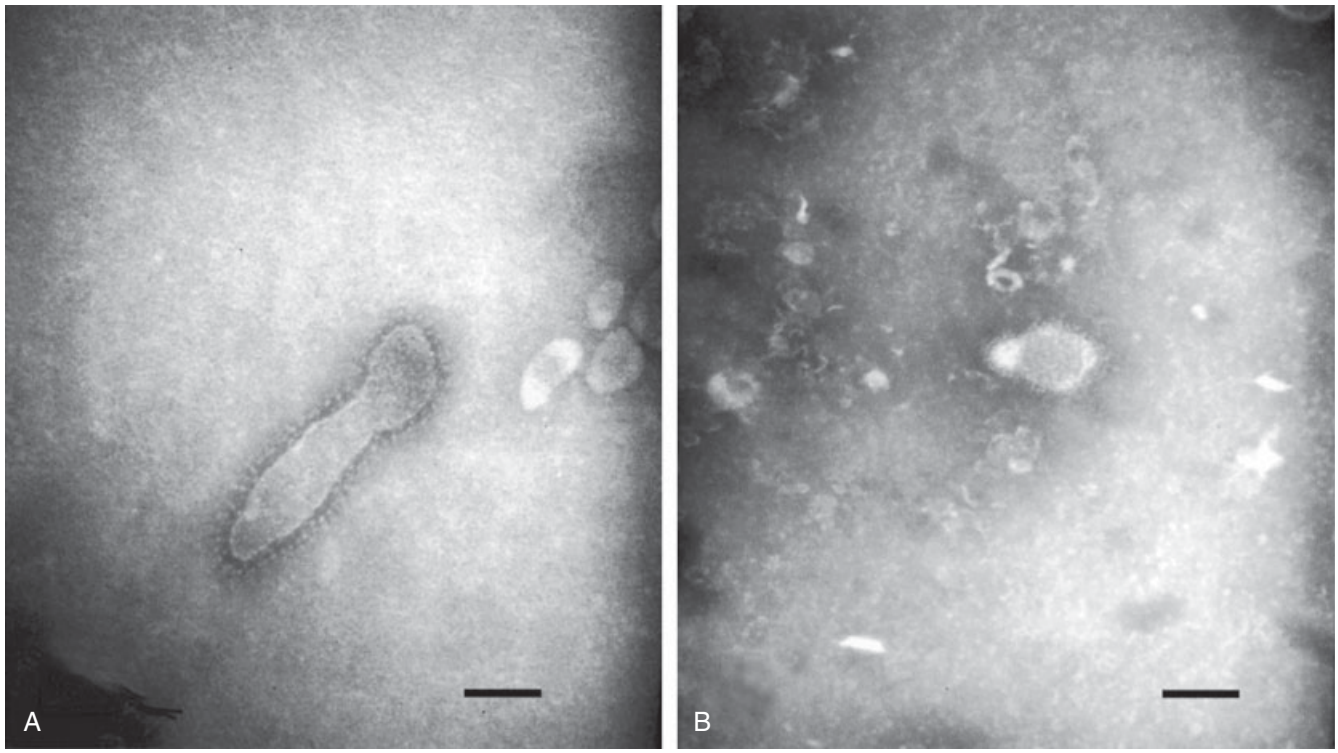

Figure 200–1 Negatively stained virus particles in stool samples representing a typical intestinal coronavirus (**A**) and torovirus (**B**). The bar represents 100 nm. The particles both show the typical petal-shaped projections, but those of the coronaviruses are more finely formed and distinct than those of the toroviruses. *(Photomicrograph kindly provided by Dr. Martin Petric, Department of Laboratory Medicine, Hospital for Sick Children, Toronto, Canada.)*

human toroviruses has not been well delineated. What information is available about the epidemiology of both enteric coronaviruses and toroviruses is outlined in the later section on clinical manifestations.

GEOGRAPHIC PREVALENCE

Respiratory coronavirus infections occur worldwide. Seroprevalence studies using 229E and OC43 antigens have been conducted in the United States, Europe, Brazil, and Iraq.[16,24,74,75,94,95,144] With enzyme-linked immunosorbent assay (ELISA), prevalence of antibody in adults from all areas where they have been examined approaches 90 to 100 percent. Coronavirus NL-63 has been found in PCR studies in Europe, the United States, Canada, Australia, Japan, Korea, Taiwan, and Hong Kong.* Coronavirus HKU-1 has been found in Hong Kong, the United States, Europe, and Australia.[55,195,205,224]

SEASONAL INCIDENCE AND ANNUAL RECYCLING PATTERN

Although HCoV infections may occur at any time of the year, most are seen in midwinter to early spring. The irregular year-to-year pattern that has been described for OC43 and 229E, namely, that individual serotypes predominated during a given year, followed by one or more years of low activity,[147,148] may apply also to NL-63 and HKU-1. An alternative pattern was seen in healthy children in Georgia, prospectively examined for 229E and OC43 infection from 1960 through 1968, where a considerable HCoV-229E outbreak occurred in 1961 to 1962, followed by 2 years with a low incidence and 4 subsequent years in which the incidence was moderate and similar from year to year.[94,95]

During outbreaks, individual coronavirus types may be responsible for a large fraction of respiratory infections. HCoV-229E was associated with a significant outbreak in medical students at the University of Chicago in 1966 and 1967, where 66 (35%) of 191 sampled students were infected.[71] In Tecumseh, Michigan, a large 229E outbreak that occurred between January and April 1967 affected 68 percent of 38 families and 34 percent of 159 individuals tested. Sharp outbreaks also can occur in hospitalized infants; at National Jewish Hospital in Denver, 16 of 20 hospitalized asthmatic infants were infected with HCoV-OC43 in December 1968,[142] and smaller outbreaks with OC43,[194] 229E,[62] and NL-63 have been seen in neonatal intensive care units.[54]

RATIO OF CLINICAL TO SUBCLINICAL ILLNESS

In healthy children and adults, HCoVs probably cause frequent asymptomatic infections. In the surveillance of healthy older children in Atlanta, Georgia, only 38 and 47 percent of 229E and OC43 seroconversions, respectively, were associated with respiratory illness.[94,95] In a group of infants and young children in metropolitan Washington, DC, tested for OC43 seroconversion, at least 50 percent of the infections were subclinical.[144] In contrast, in Denver, Colorado, of children hospitalized with atopic asthma, 19 were infected with either 229E or OC43 and 17 were symptomatic.[142]

It is not yet clear how frequent asymptomatic infections are with NL-63 and HKU-1. Only a single study using PCR for

*See references 5, 7, 13, 31, 40, 41, 52, 54, 60, 91, 207.

NL-63 has examined samples from asymptomatic control patients, and in this study, overall, 3 percent of symptomatic children and 1.7 percent of asymptomatic controls yielded NL-63.[13] The difference was not significant. No studies of HKU-1 in asymptomatic subjects have been published.

AGE SPECIFICITY OF INFECTION

Antibody to both 229E and OC43 appears in early childhood, and the prevalence increases rapidly with age. Asymptomatic and symptomatic infection occurs in individuals of all ages, including the elderly. In the Tecumseh families studied during a community-wide 229E epidemic in 1967, only 3 of 54 infections occurred in children younger than 4 years. The attack rate then rose to a peak of 14 percent in individuals 15 to 29 years of age and subsequently fell with increasing age.[24] The results were different in an outbreak of HCoV-OC43 infection. With this virus, the highest rate, 29 percent, occurred in children 4 years of age or younger, and rates decreased very little even into adult years, when the incidence was 22 percent.[148]

Prospective studies during the first year of life of viruses found by PCR in nasopharyngeal samples obtained during acute respiratory symptoms have found coronaviruses, including OC43, 229E, and NL-63, in 6 to 16 percent of episodes.[91,102,114] When samples from asymptomatic infants have been included, the percent positive was significant (4.4% versus 5.5% in symptomatics).[102]

Several studies have emphasized the importance of coronavirus infection in the elderly and the burden of infection, particularly in individuals in chronic care facilities and those with underlying cardiopulmonary disease.[160]

TRANSMISSION

Human volunteers can be infected readily through nosedrops, and a typical cold can develop 2 to 3 days later; therefore, natural infections are assumed to occur through the respiratory route. Nosocomial transmission of coronavirus respiratory infection has been reported. One study took place in a neonatal intensive care unit, where prospective surveillance by immunofluorescence testing of nasal aspirates performed for 15 months detected 10 infections with coronavirus OC43, all of them nosocomially acquired.[194] All infants had apnea, bradycardia, or abdominal distention, or a combination of these pathologic effects, at the time of infection. Another, conducted similarly, found that HCoV was the most frequent nosocomial viral infection in neonates and coincided with positive samples in members of the nursing staff.[62] The mode of transmission was not demonstrated in either study.

INFECTION AND IMMUNITY

PATHOGENESIS, INCUBATION PERIOD, AND SEROLOGIC RESPONSE

In healthy adults, HCoVs seem to replicate only in the upper respiratory tract and to produce little direct cytopathologic effect. In human embryonic tracheal organ culture, a decline in ciliary activity after serial passage was the only cytopathic effect observed.[141,203] Very similar events appear to take place in vivo. Electron microscopy of nasal epithelial biopsy specimens from a young girl with chronic rhinitis and bronchitis showed preservation of cellular structures and cilia despite replication of coronavirus particles.[1]

OC43 and 229E have been detected in nasopharyngeal cells,[145] and 229E virus titers from 10 to more than 1000 $TCID_{50}$ (median tissue culture infective dose) were found for a week or more in nasopharyngeal washings.[152] Bende and associates[11] studied the course of 229E colds in 24 volunteers and delineated the typical signs, symptoms, and virus shedding patterns; 8 volunteers were asymptomatic. The incubation period of HCoV colds is, on average, 2 days and usually lasts approximately 1 week.[15] In small infants, virus may be detectable in respiratory secretions for 3 weeks and longer.[91] Recovery is likely to be dependent on cell-mediated immunity. Infections in immunocompromised subjects may be severe and prolonged.[5,58,169]

Little is known about the pathogenesis or immunity of HCoV or torovirus infection of the gastrointestinal tract. In contrast, numerous studies have been devoted to the pathogenesis of SARS,[170] and this subject is discussed briefly later.

RE-INFECTION

Evidence from human volunteer experiments demonstrates that strain-specific antibody can be protective. Reed[178] infected volunteers with one of several 229E-like viruses. She found that immunity to homotypic challenge endured for at least 1 year but that immunity to heterotypic HCoV-229E strains in these same volunteers was much lower. IgA antibody may play an important protective role.

CLINICAL MANIFESTATIONS OF RESPIRATORY TRACT INFECTIONS

THE COMMON COLD AND OTHER UPPER RESPIRATORY TRACT ILLNESSES

A significant association of HCoVs 229E and OC43 with respiratory illnesses—most of them cold-like—has been demonstrated in prospective studies of adults and families with children. In the Chicago medical students described earlier, HCoV-229E attack rates were 31 percent during "illness periods" and only 9 percent in "wellness periods" ($p < .001$); the illnesses did not differ significantly from undifferentiated acute respiratory tract infections caused by respiratory syncytial virus, parainfluenza viruses, or rhinoviruses.[71] These findings have been confirmed in several other epidemiologic studies.[24,94,95]

Symptoms in the adults in the two investigations cited earlier were much like those in human volunteers inoculated with HCoV.[11,15,19,20,178] Infected volunteers contracted typical common colds, with perhaps more rhinorrhea than occurs in rhinovirus colds; sore throat, cough, malaise, and headache were noted in approximately 50 percent of volunteers. Twenty percent had fever.

The proportion of total respiratory disease or colds attributable to HCoV varies by season, from year to year, and also by the methodology used. Most serologic surveys have concluded that approximately 8 to 10 percent of all colds are associated with coronavirus infection.[134] A well-controlled study used PCR for 229E and OC43 to estimate the contribution of coronaviruses to illness at all ages leading to a visit to general practitioners in the Netherlands. Coronaviruses were found in 8 percent of subjects with acute respiratory tract infection, in comparison to 5.5 percent of nonrespiratory controls. Rhinoviruses were found most commonly in this group (24.7% versus 16.6% of controls).[210] One survey of first respiratory illnesses in the first year of life that used PCR with primers for 229E, OC43, and NL-63 found coronaviruses in 13 of 82 (16%) infants.[91] As more studies

are performed that span longer periods of time, that sample properly matched asymptomatic controls, and that include all known respiratory HCoV strains, the true incidence is likely to become clearer.

The possible role of coronaviruses in the etiology of otitis media with effusion has been the subject of several studies using PCR for 229E and OC43 in both nasal secretions and middle ear fluid. In one study, 92 children with acute otitis media were investigated. Coronavirus sequences were found in 16 children (17%), in the nasopharynx in 14 and in middle ear fluid in 7. This prevalence was less than that of both respiratory syncytial viruses (26%) and rhinoviruses (32%).[171] Another large study showed a much lower incidence of 2.4 percent.[161]

LOWER RESPIRATORY TRACT DISEASE

Asthma and Recurrent Wheezing

Substantial evidence indicates that HCoVs can precipitate asthma attacks,[85,90,142,146,165] although their role in this regard is clearly secondary to those of rhinoviruses and respiratory syncytial virus.[87] In a 2-year surveillance (1967 to 1969) of 32 mostly atopic children aged 1 to 5 years and hospitalized in Denver, Colorado, for severe, recurrent bouts of wheezing, 19 HCoV infections were diagnosed.[142] Six children were infected simultaneously with either parainfluenza virus or respiratory syncytial virus. Of the remaining 13 patients, all were symptomatic; three had mild wheezing, and seven had acute asthma attacks, two of which required intravenous therapy.

Pneumonia and Other Severe Lower Respiratory Tract Infections

The first evidence of a possible causative role of coronaviruses in lower respiratory tract disease was found in a study of hospitalized infants during 1967 to 1970.[140] Infections with both HCoV-229E and HCoV-OC43 were noted, and HCoV was the third most frequently occurring virus (behind respiratory syncytial virus and parainfluenza virus type 3), both in incidence and in specific association with pneumonia and bronchiolitis. Many of these infants required oxygen. HCoV-229E was grown in tissue culture from oropharyngeal swabs obtained from two of the infants with pneumonia.

With the advent of PCR for detection of coronaviruses and the addition of two new HCoV strains, NL-63 and HKU-1, that can be detected by PCR, the information about the presence of these viruses in children with serious respiratory disease has expanded greatly. Nonetheless, it is very difficult, from a perusal of published studies, to judge just how important coronaviruses are as serious pathogens in this setting. For example, despite the veritable explosion of publications on NL-63 with, at this writing, more than a dozen papers describing the presence of this virus in hospitalized children, only a single properly controlled study has been reported in which children of the same ages without respiratory symptoms were sampled during the same period and in the same hospital.[13] In this study, spanning two respiratory seasons, 3.0 percent of children with respiratory disease and 1.7 percent of controls carried NL-63. Similar ratios have been found when 229E and OC43 have been sought in children with and without respiratory symptoms (8.0% versus 5.5% in one study with all age groups, and 5.5% and 4.4% in a study during the first year of life).[102,210] In addition, coronaviruses frequently co-infect with other respiratory viruses, so the contribution of the coronavirus infection to the resultant illness is difficult to gauge. This difficulty is not different from that encountered with multiple other viruses, including human metapneumovirus, human bocaviruses, adenoviruses, parainfluenza viruses, and rhinoviruses.

With these many caveats in mind, Table 200–1 shows six studies of hospitalized patients in which coronaviruses, along with other recognized respiratory viruses, were sought. None of the six studies sought infections with all four presently recognized respiratory HCoV strains. Two studies, one of which included

TABLE 200–1 Results of Tests for Respiratory Viruses in Children Hospitalized for Respiratory Disease, Where Coronaviruses Have Been Assayed

Reference	63	88	13	40	41	140
Population sampled	Inpatients	Inpatients	Inpatients	Inpatients	Inpatients	Inpatients
Location	Pavia, Italy	Christchurch, New Zealand	Quebec City, Canada	Hong Kong	Seoul, Korea	Chicago
Type of respiratory disease	Any	Any	Any	Any	Any	Bronchiolitis or pneumonia
Number of patients	823	75	396	587	515	380
Age	61% < 5 years	Children	Children	Children	Children	Children
Number of respiratory seasons	2	1	2	1	5	4
Method for HCoV detection	PCR	PCR	PCR	PCR	PCR	Serology
Coronaviruses sought	229E, OC43, NL-63	229E, OC43	NL-63	229E, OC43, NL-63	229E, OC43, NL-63	229E, OC43
				Percent Positive		
All respiratory viruses	54.3	87	NR	36.3	60.6	55.0
Respiratory syncytial virus	14.6	48	50.2	7.0	23.7	27.9
Rhinovirus	13.1	15	NR	NR	5.8	NT
Influenza viruses	7.5	13	12.7	8.0	6.4	4.0
Parainfluenza viruses	0.9	9	NR	4.3	8.0	27.5
Human metapneumovirus	4.4	5	5.5	4.9	4.7	NT
Coronaviruses	**5.7**	**5**	**3.0**	**4.3**	**1.7**	**7.9**
Adenovirus	1.1	13	NR	5.5	6.8	6.8
Enteroviruses	NT	7	NR	NR	NT	NT
Human bocavirus	NT	NT	NT	NT	11.3	NT
Non-coronavirus co-infection rate	9.6	22.7	NR	2.2	11.5	NR
Coronavirus co-infection rate	29.8	75	60	20	NR	NR

NR, not reported; NT, not tested.

adults as well as children, used PCR for three of the four strains. From these studies, it seems likely that coronaviruses are found in the respiratory tracts of 5 to 8 percent of hospitalized pediatric patients with respiratory infections and that they probably play some role as pathogens (using inferences from their pathogenicity in adult volunteer inoculations and studies showing temporal coincidence of shedding with respiratory symptoms at all ages),[91,209] but because of high rates of co-infection and asymptomatic infection, their exact role is not clear.

In newborns, apnea, bradycardia, and increased demand for oxygen have been described during coronavirus infection.[62,193,194]

Whether differences exist in the clinical or epidemiologic features of infection between HCoVs 229E and OC43, on the one hand, and the more newly described NL-63 and HKU-1, on the other, remains unclear. HKU-1 has been studied mostly in adults, and those surveys that have included adequate numbers of children present an unclear picture of the clinical associations.[55,107,205] It does seem likely that NL-63 infections are common in infants and children hospitalized with respiratory disease.*

A suggestion has been made that NL-63 is seen particularly in croup.[41,208] Several children with either NL-63 or HKU-1 have had prominent gastrointestinal symptoms.[205,206] Information about the clinical spectrum of infections in children is thus far, however, scant. No volunteer inoculations with either virus have occurred.

Severe Acute Respiratory Syndrome

SARS is a new infectious disease that had its origin in Foshan, a city 24 kilometers from Guangzhou in Guangdong Province, China, in November 2002. After a few sporadic cases, probably independently contracted from contact with palm civets or other animals kept in markets for slaughter, the virus began spreading between humans.[36] A professor of nephrology who probably was infected in his teaching hospital in Guangzhou traveled to Hong Kong, became ill, and spread the virus during a stay in a hotel to individuals who then traveled to Vietnam, Singapore, Europe, Canada, and the United States. Meantime, the virus had spread within mainland China, where 2521 cases occurred in Beijing alone.[125] By the time the epidemic ended in the summer of 2003, 8096 probable cases had been reported in 29 countries and areas, with 774 deaths (9.6% mortality).[228] The total number of health care workers affected was 1706 (21.1% of all probable cases). Globally, children younger than 18 years accounted for only an estimated 5 percent of cases. Interestingly, the severity of the syndrome appears to have been greater in adults and adolescents than in young children, and no mortality was reported in children.[227]

ETIOLOGY

Although several putative viruses, and even *Chlamydia*, were associated with SARS when it first appeared, the cause, a novel coronavirus, the SARS-CoV, has been identified independently or collaboratively by numerous investigators.[50,101,168,175] SARS-CoV produces a cytopathic effect in Vero and FRhK-4 cells and can be identified by a reverse transcription–PCR in blood, plasma, respiratory specimens, and stool. Antibody to this coronavirus is detected in convalescent sera from patients by indirect fluorescent antibody testing and ELISA. IgG class neutralizing antibodies recognize amino acid sequence 441-700 of the spike protein (S protein) as the major epitope.[108]

This newly discovered coronavirus is the causative agent of SARS. Little doubt exists that it originated in animals, possibly bats, and then spread to exotic animals kept in cages in parts of southeastern China before they were slaughtered for human consumption.[36,70,92,106,123] The genome of the SARS-CoV has been sequenced by multiple investigators.[36,70,135,184,185] It is approximately 29,700 nucleotides in length, and the most widespread human strains had a 29–base pair deletion in the eighth open-reading frame, in comparison to strains isolated from palm civets. Its genetic organization is similar to that of other coronaviruses, and it appears to be an off-shoot of group II coronaviruses, although its final taxonomic position has not been determined.

As mentioned before, the SARS-CoV uses angiotensin-converting enzyme 2 as the key functional cellular receptor. The virus also binds to the C-type lectin CD209L (also known as L-SIGN) and DC-SIGN.[79,108,122] It has also been shown to require further protease cleavage by cathepsin L after attachment.[83,190]

EPIDEMIOLOGY

It is an enormous tribute to the collaborative efforts of many clinicians, public health departments, the World Health Organization, and numerous laboratories that the SARS epidemic was quenched after only approximately 8 months of activity. It is also a reflection of several important aspects of the infection in human hosts. The estimates for the incubation period of SARS converge at 2 to 10 days (range, 1 to 14 days). Most countries reported a median incubation period of 4 to 5 days and a mean of 4 to 6 days after exposure.[227] The disease begins with systemic symptoms such as fever, malaise, chills or rigor, headache, and myalgia, with respiratory symptoms, particularly cough and coryza. Dyspnea and, in some patients, diarrhea appear later. Most transmissions have been person to person through respiratory droplets, contact, or fomites. Because infected individuals become most infectious approximately 10 days after symptoms begin and only after severe respiratory symptoms become prominent, most presumed cases can be identified before they become highly infectious. This allowed isolation and institution of proper barrier precautions to inhibit spread within the community or hospital.[163,167,181] This protection was achieved despite the fact that the virus could, under the right circumstances, spread widely through aerosols, as demonstrated in one well-described cluster of cases that developed during a 3-hour airplane ride, as well as a community outbreak that probably resulted from infected air currents both inside and outside a densely populated high-rise housing complex in Hong Kong.[162,234]

Children with SARS are apparently less infectious than are their adult counterparts. Transmission from children has been documented but seems to be rare.[30] The extent of asymptomatic SARS-CoV infection in at-risk adult close contacts appears low.[29,34,43,78,84,105,109,118,216] Data on asymptomatic children, although fewer, were similar.[118,121,126]

PATHOPHYSIOLOGY

The SARS-CoV produces a disease in the human host quite different from that produced by respiratory or gastrointestinal coronaviruses. Although the primary site of disease is the respiratory tract, with radiologic evidence of patchy airspace disease, either ground-glass opacity or focal consolidation in the lungs,[6,12,111,115-117,175] early, essentially universal viremia with widespread involvement of multiple organs occurs. The tissue tropism of SARS-CoV includes not only the lungs but also the gastrointestinal tract, liver, spleen, pancreas, adrenals, heart, kidneys, skeletal muscles, sweat glands, parathyroid glands, pituitary, cerebrum, and lymph nodes.[47,57,159] SARS is thus a systemic disease with extrapulmonary dissemination, resulting in viral shedding in respiratory secretions, stool, urine, and possibly even sweat. There is prominent lymphopenia, with depression of both $CD4^+$ and $CD8^+$ T lymphocytes, leukopenia, thrombocytopenia, ele-

*See references 5, 7, 8, 40, 41, 52, 54, 63, 91, 199, 206, 207.

vated lactate dehydrogenase level, and mildly prolonged activated partial thromboplastin time. In comparison with adults, elevated serum values of alanine aminotransferase, aspartate aminotransferase, creatine kinase, and D-dimer are less commonly seen in children.[12,38,80,115-117,119,156,157] A prominent innate immune response to SARS-CoV infection includes acute-phase proteins, proinflammatory cytokines, chemokines, and C-type lectins such as mannose-binding lectin, which may play a protective role. In contrast, apparently no type 1 interferon response occurs.[108] Limited investigations have demonstrated the presence of very high circulating levels of interleukin-1β, interferon-γ–inducible protein 10, and monokine induced by interferon-γ, with normal or near-normal levels of interleukin-6, tumor necrosis factor–α, and RANTES (regulated on activation, normal T cell expressed and secreted).[154,155] These preliminary results suggest that SARS-CoV induces a selective activation of the caspase-1–dependent pathway in infected macrophages and predominantly induces a type 1 T-helper lymphocyte [T_H1]–mediated immune response, which promotes effective viral clearance but has relatively little effect on immune-mediated allergic reactions such as hyperreactive bronchoconstriction.[156] The immunologic profile and severity of lymphopenia during the course of illness correlated well with the clinical outcome in affected children.[120]

The tissue and organ damage produced by SARS-CoV likely is caused by both local viral replication and the immunopathologic consequences of the host response. Postmortem findings showed gross consolidation of the lungs and diffuse alveolar damage with pulmonary edema and hyaline membrane formation.[111,159,201] Other areas had cellular fibromyxoid organizing exudates in the airspaces. Interstitial spaces had only mild lymphocytic infiltrates. Vacuolated and multinucleated pneumocytes also were noted, along with changes of bronchiolitis obliterans.[158] Splenic and lymph node atrophy as well as depletion of all lymphocyte types, including dendritic cells, also was found.[69]

CLINICAL PRESENTATION

The largest series of proven, pediatric cases of SARS is that of Leung and coworkers in Hong Kong.[115,117] Forty-four children had infection confirmed by seroconversion to the SARS-CoV, and all also satisfied a modified clinical case definition: fever (rectal temperature ≥38.5° C or oral temperature ≥38° C); chest radiograph with pulmonary infiltrates or signs of acute respiratory distress syndrome (ARDS); likely or proven contact with another case within 10 days of onset; and at least one of a large number of general symptoms, such as chills, malaise, myalgia, cough, hypoxia, lymphopenia, or failure to respond to antibiotics for community-acquired pneumonia after 2 days of therapy.[115,117] The median age was 12 years, with more than 90 percent of patients being older than 5 years. The disease had many features similar to that in adults: onset with fever and, particularly in adolescents, systemic, influenza-like symptoms. Coryza was seen significantly more frequently in children younger than 13 years (61%) and much more frequently than in adults. The prodromal period was followed by cough in 64 percent and diarrhea in 20 percent, but the signs and symptoms of progressive pneumonia, occurring frequently in adults, were seen in a small minority and mostly in adolescents. Cough was not invariably present during the illness, even in severely affected children.[117] The combined relative frequency of presenting clinical features reported in the two largest series of serologically confirmed children with SARS is summarized in Table 200–2.

Chest radiographs and high-resolution computed tomography findings were similar to those in adults, showing peripheral consolidations and ground-glass opacities, with pleural effusions being rare (Figs. 200–2 to 200–4).[6] Radiographic features are nonspecific, but high-resolution computed tomography of the thorax in clinically suspected cases may be an early diagnostic aid when initial chest radiographs appear normal.[116] The most prominent laboratory findings were leukopenia and, particularly, lymphopenia, with reduction in all lymphocyte types. Children with more severe disease often had thrombocytopenia, laboratory evidence of disseminated intravascular coagulation, and elevated levels of lactate dehydrogenase, alanine aminotransferase, and creatine kinase.

TABLE 200–2 Frequency of Presenting Clinical Features of SARS in Children Younger than 18 Years*

Presenting Features	No. of Children (*N* = 64)	%
Fever	62	97
Malaise	36	56
Cough	36	56
Coryza	26	41
Chills or rigor	21	33
Sputum production	19	30
Headache	18	28
Myalgia	18	28
Anorexia	15	23
Nausea and vomiting	13	20
Dizziness	12	19
Diarrhea	11	17
Sore throat	7	11
Dyspnea	6	9
Abdominal pain	4	6
Lethargy	3	5
Chest pain	1	2
Cyanotic attack	1	2

**Pooled data based on references 38 and 117.*

Mortality in children has not been reported worldwide, although the disease can be severe in young infants or even life-threatening in adolescents.[35,59,191] Several independent observations have confirmed that SARS was less severe in children than in adults.* Nonetheless, follow-up imaging studies demonstrated persistent abnormalities in some children 6 months and even 1 year after the acute pneumonia.[44,120]

The performance characteristics of the modified clinical case definition used by Leung and colleagues[117] during the epidemic in Hong Kong, before the advent of serologic diagnosis, were respectable, with sensitivity of 97.8 percent, specificity of 92.7 percent, positive predictive value of 88 percent, and negative predictive value of 98.7 percent. The excellent negative predictive value was extremely useful in the outbreak situation to facilitate decision making on the need for isolation and cohorting of hospitalized children, where inadvertent mixing of infected and non-infected children could be disastrous.

In spite of the apparent universality of viremia, no evidence of perinatal transmission to infants born to infected pregnant women was found.[156,157,183,189,221,236] However, SARS-CoV infection has been associated with both maternal and fetal morbidity and mortality.[156,157,189,220,221]

DIFFERENTIAL DIAGNOSIS

The differential diagnosis of SARS in children includes the many viral, bacterial, mycoplasmal, and chlamydial agents that cause acute febrile respiratory illnesses in children (see Chapters 23, 25, 26, 27, 96, 149, 165, 168, 190, 191, 194, 195, 201, and 208). Particularly important to consider are agents that cause acute febrile respiratory illnesses in both adults and children. These agents include influenza viruses A (in particular, avian influenza

*See references 12, 38, 80, 103, 117, 119, 124, 129, 177, 230, 235.

Figure 200–2 Chest radiographs and high-resolution computed tomography scan of the thorax in two children with SARS. **A,** Admission chest radiograph of a 15-year-old girl showing left lower lobe consolidation 3 days from onset of fever. **B,** Progressive radiographic deterioration resulting in bilateral widespread consolidation at the time of intubation and mechanical ventilation of the same patient 12 days from onset of fever. **C,** Normal chest radiograph of a 16-year-old girl taken on admission 2 days from onset of fever. **D,** High-resolution computed tomography scan of the thorax of the same patient showing peripheral subpleural focal consolidation of the right lower lobe not evident on chest radiograph. *(From Leung, C. W., Kwan, Y. W., Ko, P. W., et al.: Severe acute respiratory syndrome among children. Pediatrics* 113:*e535-543, 2004.)*

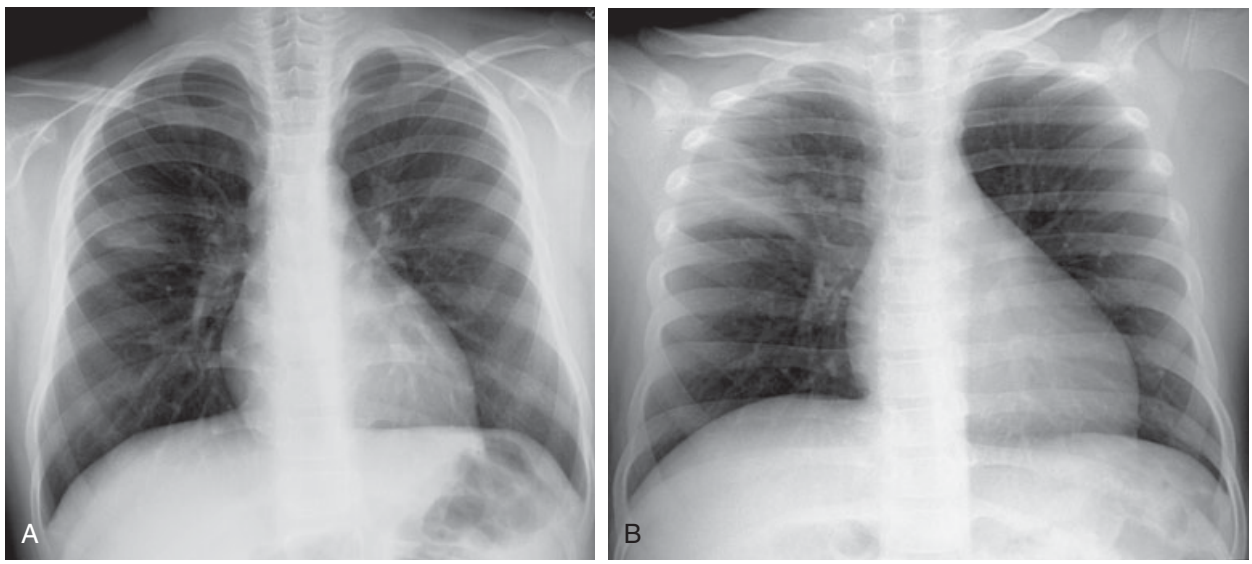

Figure 200–3 Serial chest radiographs in a 2-year-old boy who presented with fever and cough. **A,** Radiograph at presentation shows an ill-defined airspace consolidation in the periphery of the right upper lobe that abuts the horizontal fissure. **B,** This finding was followed by an increased consolidation in the right upper lung field on day 5. Complete resolution of the airspace consolidation occurred by day 14 (not shown). *(From Hon, K. L. E., Leung, C. W., Cheng, W. T. F., et al.: Clinical presentations and outcome of severe acute respiratory syndrome in children. Lancet* 361:*1701-1703, 2003.)*

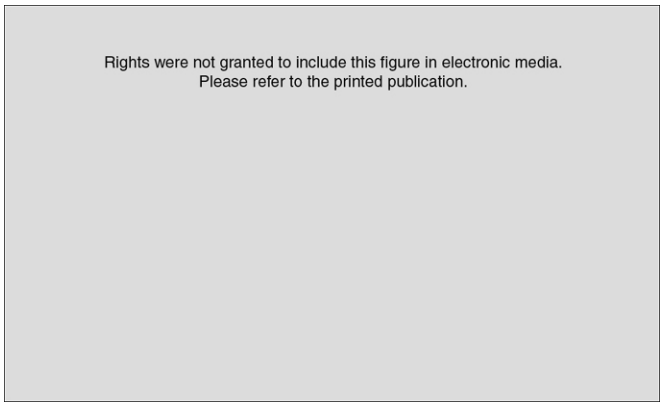

Figure 200–4 High-resolution computed tomography scan of a 13-year-old girl who presented with persistent fever for 1 week with chills, rigors, rhinorrhea, and myalgia. Mixed airspace consolidation and ground-glass opacity are seen in the left lower lobe. *(From Babyn, P. S., Chu, W. C., Tsou, I. Y., et al.: Severe acute respiratory syndrome [SARS]: Chest radiographic features in children. Pediatr. Radiol.* 34*:47-58, 2004.)*

H5N1) and B; parainfluenza viruses 1, 2, 3, and 4; respiratory syncytial virus; human metapneumovirus; several adenoviral types; *Chlamydia pneumoniae*, *Chlamydia psittaci*, *Mycoplasma pneumoniae*, and *Legionella pneumophila*; and the many causes of acute bacterial pneumonia.

SPECIFIC DIAGNOSIS

For surveillance purposes, both the World Health Organization and the Centers for Disease Control and Prevention (CDC) developed and revised case definitions as the SARS epidemic evolved.[26,226] The current CDC definition of a case has two levels, probable and confirmed, as well as two parts, clinical and epidemiologic.

A probable case is a person who meets the clinical criteria for severe respiratory illness and the epidemiologic criteria for likely exposure to SARS-CoV. Severe respiratory illness is defined as temperature above 38° C and one or more clinical findings of lower respiratory illness (e.g., cough, shortness of breath, difficulty breathing), plus one or more of the following findings: radiographic evidence of pneumonia, or ARDS, or autopsy findings consistent with pneumonia or ARDS without an identifiable cause. The epidemiologic criteria for likely exposure to SARS-CoV are close contact with a confirmed case of SARS-CoV disease or close contact with a person with mild-moderate or severe respiratory illness for whom a chain of transmission can be linked to a confirmed case of SARS-CoV disease in the 10 days before onset of symptoms.

A confirmed case of SARS is a person who has a clinically compatible illness (i.e., temperature above 38° C with constitutional symptoms or lower respiratory symptoms) that is laboratory confirmed.

In the absence of known cases anywhere in the world, the laboratory takes a particularly important place in establishing the specific diagnosis. The "gold standard" for laboratory diagnosis is a rise in antibody titer during illness. Rapid diagnosis can be accomplished by finding the virus, viral antigens, or viral nucleic acid in appropriate specimens obtained during illness. The most sensitive rapid diagnostic test appears to be conventional or, more recently, real-time quantitative, reverse transcription-polymerase chain reaction (RT-PCR) assays of either plasma or respiratory secretions (e.g., nasopharyngeal aspirate) obtained during the first two weeks of illness.[153,173] The improved RT-PCR assays are critical in establishing an early diagnosis of SARS, with sensitivity approaching 80 percent and specificity 100 percent in the first 3 days of illness when it is performed on nasopharyngeal aspirates, the preferred specimens.[174] A detection rate of 87.5 to 100 percent obtained in the plasma of eight pediatric patients within the first week of illness similarly suggests that plasma SARS-CoV RNA quantification is a very sensitive and potentially useful early diagnostic tool.[153] The overall diagnostic yield in the second week of illness is greater than 80 percent when stool specimens also are examined, with stool yielding better results than those of respiratory specimens.[28] A recently described real-time nested PCR assay performed on throat swabs is capable of detecting fewer than 10 copies of viral genome per reaction and achieves a much shorter turn-around time than conventional nested RT-PCR.[89]

Although antibody to the SARS-CoV, detected by indirect fluorescent antibody testing or ELISA, is seen specifically only after infection with this virus has occurred, a one-way cross-reactivity with OC43 and 229E (i.e., rises in reactivity are seen against these antigens during SARS) has occurred, with the OC43 cross-reactivity being the stronger of the two.[33] Absence of seroconversion to SARS-CoV beyond 28 days from disease onset generally excludes the diagnosis.[116]

Establishing the diagnosis of SARS by isolating SARS-CoV from specimens inoculated in appropriate cell cultures is limited by low sensitivity and slow growth in culture. The virus was isolated successfully from NPA cultures in only 16 percent of children in the largest pediatric series.[117] Cell culture is also technically demanding, and amplification of the viable virus is associated with potential biohazards necessitating biosafety level 3 containment. Currently, however, with the exception of animal inoculation, it is the only means to demonstrate the existence of viable SARS-CoV.

TREATMENT

When SARS first emerged, most cases were treated empirically with systemic ribavirin under the dire situation of an explosive epidemic of life-threatening infection in multiple countries and without a definitively identified etiologic agent. More recently, doubts have been expressed about the virologic efficacy of ribavirin in the treatment of SARS,[35] and some evidence indicates that the treated adult patients did more poorly than did the untreated ones.[37,215] The level of ribavirin required for inhibition of virus growth in vitro is such that adequate levels probably cannot be achieved without causing significant toxicity.[186]

High-dose corticosteroids (≥10 mg/kg intravenous methylprednisolone daily for up to 3 days plus a tapering regimen of oral prednisolone lasting several weeks) also were used widely during the epidemic and were, in several nonrandomized analyses, considered helpful.[77,238] Immunomodulation by corticosteroid therapy initially was considered beneficial in complementing empiric antiviral treatment with ribavirin. The rationale for use of corticosteroid in severe disease is the blunting of host damage caused by excessive proinflammatory cytokine responses, although use of such treatment in the early phase of illness has been shown to increase the plasma viral load in SARS.[110] In retrospect, ribavirin alone probably had no significant effect in arresting the progression of the disease, and corticosteroids are likely to be unnecessary for patients who do not develop moderate to severe hypoxemia. However, many clinicians who have treated patients with SARS successfully contend that corticosteroids may be life-saving in the later phase of illness in those threatened by impending acute respiratory failure. The place of corticosteroids in the rescue therapy of patients who clearly have experienced failure of supportive care remains to be determined. Interferon-α also has been considered to be possibly helpful in an uncontrolled study.[128] The role of other compounds with antiviral potentials against SARS-CoV remains to be elucidated.[116]

No evidence-based therapeutic approach for SARS currently exists. With more understanding of the pathogenesis as well as

the clinical course of the disease, treatment will evolve. Further studies are required to define the role of supportive care, antiviral therapy, and immunotherapy.

OUTCOME

The short-term outcome of SARS among children generally is good in comparison with that of adults.[117] Mild radiologic abnormalities detectable by high-resolution computed tomography and mild abnormalities in pulmonary function testing were observed 6 months after onset in 34 percent and 10.5 percent of a series of 47 children, respectively.[120] However, all children were asymptomatic and had normal clinical examination findings. In contrast, some adult patients have developed residual pulmonary fibrosis, persistent respiratory symptoms, and deranged lung function, despite recovery from the primary illness.[3,82] Follow-up study in a series of 27 children 15 months after the diagnosis of SARS was established suggested that aerobic capacity may be reduced in some despite normalization of lung function in all patients, likely a consequence of impairment in lung perfusion at peak exercise and post-SARS deconditioning.[233]

An important delayed complication in survivors of SARS, probably related to treatment with high-dose corticosteroids, is avascular necrosis (AVN) of the femoral head and, less commonly, the femoral condyle, humeral head, or talus. The incidence in Hong Kong, based on a territory-wide magnetic resonance imaging screening study of 1203 patients performed 6 to 9 months after onset of disease occurred, was 13.3 percent in adults and 9.3 percent in children between the ages of 10 and 18 years, many of whom were asymptomatic. Of the seven affected children in Hong Kong, five had AVN of the femoral head and four had knee involvement.[39] The incidences of AVN as reported in two series of symptomatic patients from Beijing, China, were 23 percent and 43 percent.[81,229] Cumulative steroid dosage appears to be the most important risk factor for predicting AVN. The risk of onset of post-SARS osteonecrosis was 0.6 percent for patients receiving less than 3 g and 13 percent for those receiving 3 g or more of prednisolone-equivalent dose.[68] Why none of the 44 children was affected by AVN in the largest pediatric series (C. W. Leung, unpublished data) whereas five of 21 (23.8%) children in another series were affected, although they all shared a similar treatment protocol, remains unexplained. The cumulative steroid dosage for the affected children ranged from 1.2 to 6.8 g prednisolone-equivalent administered for a total duration of 17 to 42 days.[27] Whether symptomatic AVN is more likely to progress than is asymptomatic AVN is not clear. However, it is reassuring, on the basis of past experience with steroid-related AVN, that not all lesions will progress necessarily to collapse of the articular surface without surgical intervention, and early AVN of the femoral head can resolve spontaneously. Preliminary follow-up data obtained at 12 to 18 months after detection of AVN suggest that the condition can remain static, progress, or regress.[39] The natural history of post-SARS AVN, especially in growing children, remains to be elucidated by longer term follow-up.

The long-term mental health impact of SARS on adult patients and infected health care workers has been the subject of recent research.[219] Data on the psychological outcomes of children recovering from SARS are limited.[117]

PREVENTION

The mainstay of prevention of SARS is implementation of effective infection-control practices in hospitals and quarantine procedures outside hospitals. In retrospect, many secondary cases of SARS in health care personnel were preventable. Patients with respiratory illnesses consistent with SARS should be isolated at triage and placed in a negative air pressure room for treatment. Because SARS is spread by contact, droplets, and aerosols, and because the nose, mouth, and eyes are sites for primary inoculation, health care workers should wear appropriate masks and goggles or face shields while tending patients, especially in performing procedures in which splashing and aerosolization are likely to occur. Because fomites appear to be important factors in the transmission, medical equipment should be restricted to patient rooms or adequately disinfected after contact with patients. Finally, adequate handwashing before and after contact with patients is critical.[42]

ENTERIC HUMAN CORONAVIRUS AND TOROVIRUS INFECTIONS

CORONAVIRUSES

From the earliest descriptions of HCoVs, because of the prominence of coronaviruses as a cause of diarrhea in young calves and pigs, attempts have been made to find coronaviruses in the stool and to associate them with enteric disease. The particles that have been seen, often called *coronavirus-like particles* (CVLPs), have appeared as frequently in stools from well subjects as in those with diarrhea in many studies, and at times they have been difficult to distinguish from cellular membrane fragments. Indeed, their authenticity as viruses has been called into doubt on occasion.[48,132,149,166]

This situation has been confused further by the more recent separation of toroviruses from enteric coronaviruses, although this separation probably will lead ultimately to clarification of this murky field. Many of the specimens that were considered to contain CVLPs in earlier papers very well may have contained toroviruses. At present, electron microscopists think that these virus genera can be distinguished in most instances on the basis of morphologic appearance alone,[51] and in many cases the presence of human toroviruses can be confirmed by serologic testing for stool antigens with bovine antisera containing bovine torovirus (Breda virus) antibody.[10,97,100]

The first reports of HCoV as a possible cause of human gastroenteritis appeared in 1975; CVLPs were found by electron microscopy in the stools of English adults in three sharp outbreaks of nonbacterial gastroenteritis.[21,23] In the same year, CVLPs were reported in the stools of healthy adults in India.[137] These rather contrasting publications from England and India heralded the beginning of a continuing controversy on the etiologic importance of these agents as enteric pathogens.[132,149]

The firmest link of CVLPs to human enteric disease is with gastroenteritis in the very young, especially neonatal necrotizing enterocolitis (NEC). A controlled epidemiologic investigation of NEC was conducted in two hospitals in France, one with and one without an NEC outbreak.[32] Within each hospital, newborns with "no pathologic occurrence" were used as controls. In the NEC-free hospital, no CVLPs were found in the stools of 21 controls, but two patients with mild diarrhea had CVLPs. In the NEC hospital, 23 of the 32 (72%) NEC patients had fecal CVLPs, whereas only 3 of 26 (11.5%) controls were positive ($p < .02$). Similarly, CVLPs were observed in infants who were part of an NEC outbreak in a special care nursery in Texas.[179] This outbreak yielded a virus that subsequently has been adapted to growth in tissue culture and to some extent characterized.[130]

In September 1979, an episode of acute, severe (bloody stools, bilious gastric aspirates, abdominal distention) gastroenteritis occurred in a neonatal intensive care unit in Arizona, and several clinical signs were associated significantly with CVLPs in patients' stools.[211] Two children died in this outbreak, and another infant death, with careful electron-microscopic description of coronavirus infection in the distal end of the small bowel,

occurred in Oklahoma.[180] In Italy, a case-control study of infants and young children with enteritis found a significant difference in the presence of fecal CVLP between the ill and control groups: 16.3 percent in ill children and 1.6 percent in controls ($p < .01$).[64]

Despite the foregoing evidence for a causal relationship between CVLPs and gastrointestinal disease, reservation about the role of CVLPs in children's enteric disease still remains, chiefly because CVLPs are found so often in the feces of healthy children, particularly, although not exclusively, in the developing world.[96,136,137,176,188,192] Many of these studies need to be repeated with use of PCR of stool for detection of the various coronaviruses.

Attempts have been made to cultivate CVLPs in cell and organ culture and to compare the resultant virus suspensions antigenically with other HCoVs or animal coronaviruses. As mentioned earlier, a possible enteric HCoV was cultured from a child with NEC, and this virus has been adapted further to growth in a mouse macrophage cell line and mosquito cells.[130] This virus is antigenically unrelated to HCoV-229E and HCoV-OC43. A hemagglutinating coronavirus antigenically and genetically related to a bovine coronavirus, BCV-LY138, has been isolated from a 6-year-old child with severe diarrhea.[237]

TOROVIRUSES

Studies of the clinical manifestations of human torovirus infection are still in their infancy, and certainty of the details is difficult to ascertain. Their pathogenicity still should be considered a matter of some doubt, although the few controlled studies that have been performed have been more consistent than have those of enteric coronaviruses; even though torovirus particles are found in the feces of both symptomatic and asymptomatic individuals, an excess in the symptomatic individuals clearly occurs.[86,98] These studies have included children from Canada and Brazil.

In the study from Brazil, 20 of 91 fecal samples from children in the community with diarrhea contained torovirus antigen detectable by ELISA, and toroviruses were associated significantly with both acute and chronic diarrhea ($p = .02$ in both instances).[98] In the study from Canada, symptomatic and asymptomatic hospitalized children were sampled for fecal viruses; toroviruses were found in 35.0 percent of the symptomatic children and 14.5 percent of the asymptomatic children. In comparison to those with stools containing either rotaviruses or astroviruses, torovirus-infected children were older (mean age of 4.0 versus 2.0 years) and their infections more often were acquired nosocomially (57.6% versus 31.3%). Vomiting occurred less commonly with torovirus infection, but occult blood was noted more frequently. A large proportion of symptomatic torovirus infections occurred in immunocompromised children.[86] One case of torovirus found in the feces of a child with acute abdomen has been described.[204]

A recent report described toroviruses in nonepidemic NEC of neonates.[127] During a 5-year period from 1996 through 2001, toroviruses were found by electron microscopy in 48 percent of infants with NEC, in contrast to 17 percent of controls during the same interval ($p < .001$). The mortality rate was 14 percent.

NEUROLOGIC DISEASES

Coronaviruses are the cause of some animal neurologic disorders, including a murine demyelinating disease with some features resembling multiple sclerosis. In humans, a serosurvey of HCoV infection in southern Finland discovered evidence of HCoV-OC43 infection in six patients with acute neurologic episodes, including one with polyradiculitis.[182] A recent case of acute demyelinating encephalitis accompanying OC43 respiratory infection was described in which OC43 RNA was detected by PCR in the spinal fluid.[232] Several observations have suggested a possible role of coronavirus infection in multiple sclerosis: (1) isolation of coronaviruses (SK and SD) from the central nervous system tissue of two patients with multiple sclerosis,[18] (2) demonstration that coronavirus SD can cause demyelination in a primate model,[151] (3) direct visualization of CVLPs in the brain of a patient with multiple sclerosis,[200] and (4) identification by in situ hybridization or PCR of coronavirus RNA in the brains of patients with multiple sclerosis.[4,150,198] However, coronaviruses SK and SD are antigenically and genetically very similar to mouse hepatitis virus and were isolated with the use of mouse tissues; the possibility that these isolates were of mouse origin has not been excluded. In addition, other studies have failed to demonstrate coronavirus RNA in the central nervous system tissue of patients with multiple sclerosis[197] or have found such RNA in the same proportion of patients with demyelinating diseases as in controls.[46] Further investigation will be needed to establish whether HCoVs are related causally to any neurologic disease in humans.

LABORATORY DIAGNOSIS

VIRUS ISOLATION

Respiratory Coronaviruses

Human embryo tracheal or nasal mucosal organ cultures were used first for primary isolation of multiple HCoV strains, including B814, OC43, and several less well characterized strains.[141,203] OC43 subsequently was adapted to growth in monkey kidney, BSC-1, and rhabdomyoma cells.[17,187] The 229E-type strains were shown to replicate and to produce a cytopathic effect in secondary human embryo kidney[73] or certain diploid cell lines: WI-38, MRC-5, and MA-177.[71,93] A recent report has described the growth and cytopathic effect of OC43-like strains in the HUH7 hepatocarcinoma cell line.[61] This line also has been used for primary isolation of HKU-1 strains.[205] NL-63 strains grow directly from clinical samples in tertiary monkey kidney cells, with subsequent growth in LLC or Vero cells.[60,207] The SARS-CoV was isolated in and grows well in Vero-E6 cells.[50,101]

Enteric Coronavirus-like Particles

Growth of these agents in cell or organ culture has been reported,[22,179,237] and one isolate has been adapted to growth in tissue culture.[130]

Toroviruses

Human toroviruses have not been grown in any culture system.

VIRUS DETECTION TECHNIQUES

Respiratory Coronaviruses

Respiratory coronaviruses can be sought successfully in clinical samples by direct antigen-detection techniques or by nucleic acid detection. Both immunofluorescence[88,145,194] and ELISA have been used successfully for establishing the diagnosis of HCoV infection.[85,133,146]

During the past decade, PCR has opened up the field of coronavirus diagnosis. Not only is the method the most sensitive for all the known strains of HCoV, but it also can be designed

to pick up new strains.[54] Attempts to design a set of "pan-coronavirus" primers have, however, always led to suboptimal sensitivity.[54,63] The exact design of the tests also influences greatly their sensitivity and specificity.

Enteric Coronaviruses

Enteric coronaviruses have been detected primarily by electron microscopy of negatively stained preparations from clarified stool specimens. The possible confusion of coronaviruses and toroviruses in such preparations has been discussed earlier. Immune electron microscopy has been helpful in identifying such viruses in purified preparations but not directly in stool samples.

An antigen-detection ELISA for enteric coronavirus has been described and applied to fecal specimens from healthy and diarrheal subjects in Thailand.[112,113] PCR has not been used widely to detect coronaviruses in diarrhea, although HKU-1 was found both in the respiratory tract and in the stool in two patients as part of a larger study of respiratory disease.[205]

Toroviruses

As with enteric coronaviruses, the primary detection method for toroviruses is electron microscopy of clarified fecal specimens. Torovirus identity can be specified further by either immune electron microscopy or antigen-detection ELISA with the use of antisera prepared against the Breda virus of calves.[9,97,98] With increasing experience, electron microscopists can differentiate toroviruses from enteric coronaviruses on the basis of morphologic features alone.[51,86] There are no reports of PCR detection of toroviruses in humans.

SERODIAGNOSIS

A rise in antibody titer after day 28 of onset of symptoms is the gold standard for diagnosis of SARS-CoV infection, most commonly with use of ELISA or indirect fluorescent antibody testing in Vero-E6 cells. The finding of antibody in convalescent serum is highly specific for SARS-CoV infection, although one-way cross-reactivities exist with both 229E and, especially, OC43.[53] For respiratory coronaviruses, complement fixation,[24,140,144,148,182] hemagglutination inhibition (for HCoV-OC43 only),[95] neutralization,[53] ELISA,[20,66,99,133] indirect hemagglutination,[94] and Western blot[172] have been used.

Serodiagnostic techniques specific for enteric coronaviruses have not been described. Serodiagnosis of torovirus infections has been performed by both immune electron microscopy and hemagglutination inhibition.[51]

PREVENTION AND TREATMENT

Barrier methods were extraordinarily successful in the limitation and final elimination of the SARS epidemic, and contact precautions likely would serve to reduce the spread of respiratory and gastrointestinal coronaviruses as well.

The high re-infection rate with the respiratory HCoVs as well as the number of related strains suggests that a vaccine may be ineffective in preventing HCoV-caused respiratory illness.

The value of antivirals in SARS is reviewed earlier. There are no known effective antivirals for the respiratory coronaviruses.

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SUBSECTION 11

Bunyaviridae

CHAPTER 201

HANTAVIRUSES

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HISTORICAL PERSPECTIVE

Hantaviruses are the etiologic agents of a diverse group of rodent-borne hemorrhagic fevers that are responsible for considerable morbidity and mortality worldwide. Although recognized by so-called modern medicine only relatively recently, clinical syndromes now known to be associated with these viruses have been described by traditional practitioners across the globe from antiquity.[16,85,102] In the 20th century, Soviet scientists reported sporadic outbreaks of febrile renal failure with hemorrhage in the eastern Soviet Union between 1913 and 1930,[11] and Japanese and Soviet scientists recognized annual outbreaks of a similar syndrome in Manchuria and Siberia between 1932 and 1935.[31,101,145,154]

In 1934, Swedish scientists described a novel disorder, characterized by fever, abdominal and back pain, and renal abnormalities.[110,176] Epidemic disease with these features occurred among German and Finnish troops stationed in Lapland during World War II.[60,150] Physicians called this syndrome *nephropathia epidemica*.

North American medical practitioners initially became acquainted with a similar syndrome in the early 1950s, when a previously unrecognized febrile illness characterized by shock, hemorrhage, and renal failure developed in thousands of soldiers serving with United Nation forces during the Korean War. Physicians called this syndrome, which had a mortality rate of 5 to 15 percent, *epidemic hemorrhagic fever*.[33,103]

In 1953, Gajdusek[40] noted similarities among epidemic hemorrhagic fever, the severe and frequently fatal Far Eastern diseases described by Soviet and Japanese scientists, and the Scandinavian disorder and proposed a common origin. Subsequent validation of this hypothesis (see "The Organism") ultimately prompted adoption of the collective term *hemorrhagic fever with renal syndrome* (HFRS) to describe this clinical entity characterized by varying degrees of renal dysfunction and hemorrhage with fever.

In June 1993, investigation of a cluster of unexplained respiratory deaths in previously healthy young adult residents of rural areas in the southwestern United States led to recognition of a "new" febrile disorder in North America. Application of sophisticated serologic and virologic tools to diagnostic specimens from patients with this disease quickly pointed to a hantavirus related to, but distinct from, those causing HFRS.[79,112] This disorder, now called *hantavirus pulmonary syndrome* (HPS) or *hantavirus cardiopulmonary syndrome* (HCPS), proved to be different clinically from classic HFRS, but as virologic and epidemiologic studies of the etiologic agent and its reservoirs were completed, similarities to other hantaviruses became apparent.

THE ORGANISM

CLASSIFICATION AND ANTIGENIC COMPOSITION

The genus *Hantavirus* of the family *Bunyaviridae* was defined in 1985.[135,138] The members of the genus *Hantavirus* are classified

among the bunyaviruses because of their shared morphologic, physicochemical, and molecular properties. Like other members of the family *Bunyaviridae*, hantaviruses are negative-stranded, lipid-enveloped RNA viruses with tripartite genomes; genomic segments are designated as large (L), medium (M), and small (S). Also like other bunyaviruses, they display Golgi-associated morphogenesis and usually acquire their envelopes by budding into intracytoplasmic vacuoles.[136] However, they are serologically distinct from other family members and possess unique terminal genomic sequences.[135,137] The genus *Hantavirus* contains the only members of *Bunyaviridae* that lack arthropod vectors. As a general rule, each hantavirus is highly adapted to a specific rodent species and depends on persistent asymptomatic infection in wild rodents for maintenance in nature.

PHYSICAL PROPERTIES

Morphologically, hantaviruses are spherical, 80 to 120 nm in diameter, and have surface glycoprotein projections embedded in a lipid bilayer envelope. Elongated particles (110 to 120 nm long) often are observed, and they display a characteristic grid-like pattern on their surfaces.[48,136]

SUSCEPTIBILITY

Hantaviruses are inactivated readily by lipid solvents and most disinfectants, including dilute hypochlorite solutions, detergents, ethyl alcohol (70%), most general-purpose household disinfectants, and beta-propiolactone (0.1% at 4° C for 3 days).[49,76,127] Limited studies with Hantaan virus have shown sensitivity to a pH of 5 or less and to temperatures of 56° C or higher.[49,88] Knowledge about the survival time in the environment in the absence of disinfection is somewhat limited. Studies have shown persistent infectivity in dried cell culture medium for as long as 2 days and in neutral solutions for several hours at 37° C or several days at lower temperatures.[13] Hantaan virus was detected after 96 days under "wet" conditions at 4° C, and it remained viable for 2 minutes in 30 percent ethanol.[51] Cell culture supernatants of Puumala and Tula hantaviruses remained infectious for 5 to 11 days at room temperature and for as long as 18 days at 4° C but were inactivated after 24 hours at 37° C.[139] That hantavirus transmission is possible through indirect contact with contaminated environmental surfaces was demonstrated through a study in which Puumala virus excreted by experimentally infected bank voles into bedding maintained infectivity to recipient voles at room temperature for 12 to 15 days.[66]

LABORATORY PROPAGATION AND TISSUE CULTURE GROWTH

Hantaviruses are fastidious but can be grown in culture through serial blind passage. They routinely establish persistent, noncytolytic infections and generally do not replicate to high titer.[49,136] The prototype member of the group, Hantaan virus, was isolated in 1978[92] and propagated successfully in cell culture in the A-549 cell line in 1981.[38] However, Vero E-6 cells subsequently were found to be a better cell culture system for this and other hantaviruses.[49] Hantaviruses have been isolated and propagated successfully through direct inoculation of homogenates of infected tissue from wild-caught rodent hosts onto cell lines or after amplification through serially infected, colonized rodent hosts.[34,38,47,49,92,113,134,170] Recovery of hantaviruses from human specimens has been difficult and infrequent. More than 25 hantaviruses currently are recognized as causing human disease, although many of them have not been fully characterized, and the reservoirs of a few have not been determined. The major viruses are listed in Table 201–1. Some hantaviruses have been isolated in cell culture, but many are known only from genetic material amplified from human or rodent tissue. This list will be expanded and modified as our knowledge of classification and new viruses increases. Laboratory infection of persons working with cell culture–adapted Hantaan virus has occurred occasionally, but laboratory transmission of hantaviruses from infected rodents to humans is a particularly common occurrence.[27,30,68,87,89,97,156,157] Consequently, attempts to propagate the virus should be performed only when using biosafety level 3 or 4 facilities and practices.[13,15]

TRANSMISSION

Hantaviruses are zoonotic; each hantavirus generally is associated with a distinct rodent species or subspecies in which it establishes a chronic, inapparent infection. Hantavirus infection of rodents produces brief viremia that results in dissemination of virus to the lungs, salivary glands, and kidneys. Once infected, the rodent sheds virus in urine, saliva, and (to a lesser extent) feces throughout its life, despite the development of neutralizing antibodies.[90] In general, infection does not diminish the longevity or the reproductive potential of the infected rodent, although juvenile mice infected with Sin Nombre virus may survive for a shorter time in nature than do uninfected juveniles.[29] Vertical transmission has not been demonstrated, and pups born to infected dams appear to be protected by maternal antibody.[28,67,107] Enzootic infection appears to be maintained by exposure to nesting materials contaminated with infectious secretions, by grooming behavior, and by intraspecies biting.[29,89,90]

Humans typically become infected with hantaviruses after contact with contaminated secreta or excreta from infected rodents by inhalation of small-particle aerosols, by direct contact of infectious materials with broken skin or mucous membranes, or, rarely, by percutaneous inoculation of infectious materials (e.g., rodent bite). The primacy of respiratory droplets or airborne particles as the mode of transmission to humans is supported by evidence from outbreaks of disease among laboratory workers and sporadic cases resulting from brief exposure to rodent-infected habitats.

The risk of human hantavirus infection is a function of the density of the local rodent reservoir population, the prevalence of infection among rodents, and the frequency of activities that result in contact between humans and rodents or rodent excreta. Individual hantavirus infections usually occur when humans disturb rodent habitats or rodents enter human housing.[175] The risk for contracting disease after rodent exposure remains unquantified; however, one serosurvey suggested that persons in North America whose occupation entails close physical contact with rodents have a low risk for acquiring hantavirus infection.[39] Epidemics generally are associated with changes in the behavior of human populations that result in large-scale exposure of persons to rodent-infested areas (e.g., military maneuvers, agricultural or forestry activities) or environmental changes that result in rapid increases in the density of rodent populations (e.g., increased abundance of food items). Person-to-person transmission of hantaviruses associated with HFRS has not been demonstrated. The experience to date with HPS has been similar in North and Central America.[163] However, interpersonal spread clearly occurred during a 1996 outbreak in southern Argentina.[36,165] Subsequent independent investigations support this observation by having identified clusters in Argentina and Chile that suggest person-to-person transmission associated with Andes virus.[82,100,161] Interpersonal transmission thus appears to be a feature peculiar to Andes virus.

TABLE 201–1 Some Currently Recognized Hantaviruses

Virus Strain	Principal Reservoir	Distribution	Disease Association
Hantaan	*Apodemus agrarius mantchuricus*	Far East Northern Asia	HFRS (severe)
Seoul	*Rattus norvegicus*	Worldwide	HFRS (mild/moderate)
Dobrava	*Apodemus flavicollis*	Balkans	HFRS (severe)
Puumala	*Myodes (Clethrionomys) glareolus*	Scandinavia Northern Europe Balkans	HFRS (mild) (nephropathia epidemica)
Tula	*Microtus arvalis/Microtus levis*	Western Russia, eastern Europe	HFRS
Saaremaa	*Apodemus agrarius agrarius*	Europe	HFRS (mild)
Amur	*Apodemus peninsulae*	Far Eastern Russia	HFRS
Sin Nombre	*Peromyscus maniculatus*	North America	HPS
New York	*Peromyscus leucopus*	United States	HPS
Monongahela	*Peromyscus maniculatus nubiterrae*	Eastern United States	HPS
Black Creek Canal	*Sigmodon hispidus spadicipygus*	Florida	HPS
Muleshoe	*Sigmodon hispidus texianus*	Texas, Louisiana	HPS
Bayou	*Oryzomys palustris*	Southeastern United States	HPS
Andes	*Oligoryzomys longicaudatus**	Southern Chile, Argentina	HPS
Oran	*Oligoryzomys longicaudatus**	Northern Argentina	HPS
Laguna Negra	*Calomys laucha*	Central South America	HPS
Choclo	*Oligoryzomys fulvescens*	Panama	HPS
Juquitiba	*Oligoryzomys nigripes*	Southeastern Brazil	HPS
Araraquara	*Necromys lasiurus*	Southeastern Brazil	HPS
Castelo dos Sonhos	Unknown	Central Brazil	HPS
Lechiguanas	*Oligoryzomys flavescens*	Central Argentina	HPS
Bermejo	*Oligoryzomys chacoensis*	Northwestern Argentina Central Brazil	HPS
Prospect Hill	*Microtus pennsylvanicus*	United States	None recognized
Thottapalayam	*Suncus murinus* (insectivore)	India	None recognized
Sangassou	*Hylomyscus simus*	West Africa	None recognized
Tanganya	*Crocidura theresae* (insectivore)	Guinea	None recognized

**The reservoirs of the Andes and Oran viruses are genetically distinct, but of uncertain taxonomic status. Nevertheless, both are currently referred to as* O. longicaudatus*. (D. Enria, personal communication). Additional hantaviruses have been associated with occasional human disease in Europe, Asia, and the Americas.*
HFRS, hemorrhagic fever with renal syndrome; HPS, hantavirus pulmonary syndrome.

EPIDEMIOLOGY

GEOGRAPHIC DISTRIBUTION

Hantaviruses have been found on every continent except Antarctica. With the exception of HFRS caused by Seoul virus, hantavirus diseases occur almost exclusively in rural areas. Hantaan virus, the cause of classic HFRS, is carried by the striped field mouse *Apodemus agrarius mantchuricus*. This rodent is distributed across eastern Russia, China, and the Korean peninsula.[168] The principal reservoir for Puumala virus, the etiologic agent of a milder HFRS variant found in Scandinavia, northern Europe, and Russia west of the Ural Mountains, is the bank vole, *Clethrionomys glareolus*.[91,141]

In Europe, at least four viruses have been linked etiologically with HFRS: Puumala, Tula, Saaremaa, and Dobrava; Tula virus is associated with voles (*Microtus* spp.); Saaremaa virus is hosted by a subspecies of the striped field mouse (*Apodemus agrarius agrarius)*; Dobrava virus is associated with *Apodemus flavicollis*, the yellow-necked field mouse.[2,4,5,47,124,125] *Rattus* spp. (primarily *Rattus norvegicus*) serve as reservoirs for the Seoul-like viruses identified worldwide; human infections have been seen most frequently in eastern Asia. Outbreaks of severe and occasionally fatal HFRS among animal handlers and laboratory scientists have been caused by inapparent infection of laboratory rats with Seoul-like viruses.[86]

Genome sequences of a novel hantavirus species were detected in an African wood mouse (*Hylomyscus simus*) in Guinea.[74] This agent, provisionally named "Sangassou" virus, was more similar to Eurasian hantaviruses than to those causing HPS in the Western Hemisphere. It was sufficiently distinct to represent a separate clade, but it has not been linked to clinical illness.

Numerous hantaviruses associated with native murid rodents are known to exist in the Americas. Several, all associated with rodents of the family Muridae, subfamily Sigmodontinae, are of medical importance in the United States.[121] The deer mouse, *Peromyscus maniculatus*, is the reservoir rodent for Sin Nombre virus, the agent associated most frequently with human disease in North America.[20,34] This rodent is distributed widely over the United States, Canada, and parts of Mexico. In addition, HPS cases in the eastern United States have been associated with viruses called *New York* and *Monongahela*, which are closely related to Sin Nombre virus and are found in *Peromyscus leucopus* (white-footed mouse) and *P. maniculatus nubiterrae*, respectively.[56,148] Clinical HPS has been recognized in areas outside the known ranges of *P. maniculatus* and *P. leucopus*, however, and a search for other virus-rodent pairings has yielded at least three additional U.S. hantaviruses. Black Creek Canal virus and Muleshoe virus are associated with two species of cotton rats, *Sigmodon hispidus spadicipygus* and *Sigmodon hispidus texianus*, respectively, and Bayou virus is hosted by the rice rat, *Oryzomys palustris*.[55,70,73,111,130,153] Several hantaviruses associated with sigmodontine rodents have not been implicated in human disease. El Moro Canyon virus is hosted by the harvest mouse (*Reithrodontomys megalotis*), and Limestone Canyon virus is hosted by the brush mouse (*Peromyscus boylii*). No human infections have been documented with these viruses, and the potential for causing disease, if any, is unknown.[54,132] Seoul virus introduced by *Rattus* has been associated with human infections in several large cities, but acute (HFRS-like) disease has been confirmed only in Recife, Brazil.[23,45,46,83] Several hantaviruses associated with voles (family

Muridae, subfamily Arvicolinae) in North America are not associated with human disease: Prospect Hill virus has been recovered from meadow voles (*Microtus pennsylvanicus*) in Maryland, and related but distinct hantaviruses are inferred to be present in other voles by detection of genetic sequences by reverse transcriptase-polymerase chain reaction (RT-PCR).[168,169]

In recent years, South America clearly has surpassed North America in terms of numbers of human HPS cases and numbers of hantaviruses and host species identified.[6,58,162,164] All the HPS-causing hantaviruses in South and Central America are associated with sigmodontine rodents. Some of the most important viruses are as follows: Andes virus, hosted by *Oligoryzomys longicaudatus* in southern Argentina and Chile; Lechiguanas virus, associated with *Oligoryzomys flavescens* in central Argentina; Laguna Negra virus, hosted by *Calomys laucha* in Northern Argentina, Paraguay, and Bolivia; Juquitiba virus, associated with *Oligoryzomys nigripes* in Brazil; and Choclo virus, associated with *Oligoryzomys fulvescens* in Panama. Several other hantaviruses responsible for significant disease in South America are listed in Table 201–1. The recognition of clinical HPS in Canada[149] and Central and South America clearly has established HPS as a panhemispheric rather than a geographically circumscribed disease. However, all HPS agents identified or suspected to date belong to a single genetic group of hantaviruses and are associated with rodents of the family Muridae, subfamily Sigmodontinae. These rodent species are restricted to the Americas, in accordance with the findings that HPS has been an exclusively Western Hemisphere disease.[110]

SEASONAL PATTERNS

The natural population cycles of rodents and the seasonal nature of certain human behavior result in a pattern of human hantavirus disease that varies both seasonally and annually.[11,15,16,41,85,102,154] Although the incidence of disease varies by season, cases of hantavirus-associated human disease are recognized year-round in all disease-endemic areas.[12,71,86,141]

In eastern Asia and Russia east of the Ural Mountains, HFRS occurs primarily during the late fall and early winter, with smaller peaks during the spring and summer. Most Scandinavian HFRS occurs between the late summer and early spring, whereas European HFRS in warmer regions (e.g., France, Belgium) tends to peak in the spring.[160] In the Balkans, the presence of multiple viral strains results in a more diffuse seasonal distribution of human disease. Persons whose occupations or avocations bring them to rural settings, such as agricultural workers, foresters, biologists, hunters, campers, and soldiers stationed in the field, are at greatest risk for contracting HFRS.

HFRS caused by Seoul virus tends to occur throughout the year, and descriptions of a seasonal occurrence of Seoul virus infection have been conflicted.[19,93] Cases of Seoul virus disease also have a more even age and sex distribution than do rural hantavirus infections, presumably because of the peridomestic nature of the agent's reservoir.

The temporal distribution of HPS cases suggests a mild spring-summer seasonality of human disease. However, environmental and geographic factors influence this pattern; HPS cases have been identified throughout the winter and early spring.[12,14,35,44,171]

PREVALENCE

Worldwide, human hantavirus infections may number in the hundreds of thousands of cases annually.[168] Because of the predominantly rural nature of the disease and its prevalence in developing regions of the Eurasian land mass (e.g., rural China), accurate case reporting (and statistical data) for HFRS is limited. Researchers have estimated that more than 100,000 cases of HFRS occur each year in China.[146,168] One report suggested an incidence of 1.6 to 29.6 per 100,000 population during 1980.[65] In the former Soviet Union, more than 4000 cases per year were recorded between 1978 and 1989, 96 percent of which occurred in "European" republics. Rates in western regions near the Ural Mountains were higher (20 to 40 per 100,000 population) than were those in eastern districts (2 to 5 per 100,000 population).[152] In Korea, approximately 500 persons with HFRS, approximately half of whom are soldiers, are hospitalized annually.[152] In central and northern Sweden, a mean annual incidence of 4.3 per 100,000 population has been reported, although northern locales have rates of more than 20 per 100,000 population.[141]

As of March 2007, 467 cases of HPS had been confirmed in the United States. Although a few cases were identified retrospectively, most have occurred since 1993. More than 2500 cases were documented in South America during approximately the same time-frame (including 864 cases in Brazil alone).

DEMOGRAPHIC FEATURES

Hantavirus infections are recognized infrequently in the pediatric age group.[3,42,71,72,141,152,172] The disease occurs principally in adults, with fewer than 10 percent of cases diagnosed in children. A slight male preponderance has been noted in reported cases.

The peak incidence of HFRS in Europe, the Balkans, and the Far East occurs in individuals who are 20 to 50 years of age.[80,86,119,154] Few cases have been reported in children younger than 10 years. Both HFRS and HPS cases in young children often are recognized in association with cases in other family members.[3,42,80,109,117] A male preponderance of cases has been observed for both the severe (Korean) and milder (European) forms of HFRS in children.[1,75,109,172] Although this pattern, also seen in adults with HFRS, suggests differential susceptibility or risk of exposure by gender, the number of recorded cases, particularly in the youngest age groups, is too small to draw firm conclusions.

The underrepresentation of children recognized to have HFRS and HPS may not be explained by age-related avoidance of activities resulting in exposure. The limited data available (see "Clinical Manifestations") suggest that hantavirus infections in children may induce milder disease than that seen in adults, although typical (even fatal) HPS has been described. Comprehensive population-based serosurveys are sparse and have been inadequate to define age-associated infection rates. The apparently immune-mediated pathologic process of this disease (see "Pathogenesis and Pathology") is possibly more evident in persons experiencing infection in adulthood.

CLINICAL MANIFESTATIONS

HEMORRHAGIC FEVER WITH RENAL SYNDROME

Two major clinical variants of HFRS have been recognized traditionally. Severe disease associated with high morbidity and mortality rates occurs primarily in areas of the world where Hantaan and Dobrava viruses are endemic: across the northern half of Asia, China, the Korean Peninsula, and the Balkan nations. Milder illness with little mortality occurs in areas where Puumala and related viruses have been recovered; this latter disease, known also as *nephropathia epidemica*, is found throughout northern Europe, Scandinavia, and western areas of the former Soviet Union. In the Balkans, the coexistence of Puumala virus–like strains with viruses causing more serious disease has resulted in a mixture of clinical findings in the former Yugoslavia and neigh-

boring countries. Benign manifestations of HFRS are well recognized in many regions where Hantaan virus is found, however, and clinically severe disease occasionally results from Puumala virus infection.[122] Hence, it is important to appreciate the protean nature of this disorder and to recognize the potential for disease of any severity whenever and wherever human infection with hantavirus occurs.

Severe HFRS, such as that associated with Hantaan or Dobrava virus, is a complex, multiphasic disorder that presents a substantial challenge in patient management. The clinical course of HFRS caused by Hantaan virus in adults spans a wide spectrum from mildly symptomatic disease to severe hemorrhagic fever and death. Subclinical infections probably occur infrequently.[126,172] In most cases, the clinical course is relatively benign, with severe disease developing in approximately 20 to 30 percent of patients. Modern-day case-fatality rates range from 2 to 7 percent. In Korea, approximately a third of recognized Hantaan virus infections follow a clinical course consisting of progression through five clinically and pathophysiologically defined stages: febrile, hypotensive, oliguric, diuretic, and convalescent.[93,142] Phases often blur, however, and in milder cases, one or more phases may not be discernible. After an incubation period of 2 to 3 weeks (range, 4 to 42 days), most patients report the abrupt onset of high fever, headache, chills, dizziness, myalgia, anorexia, and backache. Approximately one third of patients experience prodromal mild respiratory or gastrointestinal symptoms. Nausea, vomiting, abdominal pain, and intense thirst may be evident at initial evaluation but increase in severity in succeeding days. Photophobia, blurred vision, and eyeball pain are reported frequently. Physical examination reveals a restless, acutely ill patient with flushing of the face, neck, and upper thoracic region. Relative bradycardia is present. Conjunctival and pharyngeal injection, together with facial puffiness, is characteristic. In more than 90 percent of patients, petechiae develop on the soft palate, axillae, lateral aspect of the thorax, conjunctivae, or face, generally between the third and sixth days of illness. Tenderness occurs commonly over the costovertebral angles and diffusely throughout the abdomen. Hematologic studies in the first 3 to 4 days of illness reveal leukocytosis with a left shift in more than 90 percent of patients, as well as almost universal thrombocytopenia; the hematocrit at this stage generally is normal or slightly increased. Proteinuria develops by the third to fourth day, and microscopic hematuria, hyposthenuria, and mild pyuria are reported in most patients; fibrin clots in urine are a characteristic finding.

This febrile phase generally lasts approximately 1 week, followed by abrupt defervescence. Approximately 40 percent of patients then become hypotensive; in most cases, the drop in blood pressure is mild and brief, but in severely ill persons (30% to 50% of hypotensive patients), clinical shock develops. Tachycardia replaces bradycardia, the pulse pressure narrows, and cyanosis and mental confusion may be seen. The hypotensive phase may last from a few hours to 3 days, and 30 to 40 percent of deaths occur during this period.

As patients recover from hypotension, they enter a period of oliguria. This phase occurs in approximately 60 percent of patients and generally lasts for several days. Anuria develops in approximately 10 percent of patients. Blood pressure normalizes, and hypertension often develops. Clinical manifestations of uremia, including protracted vomiting and hiccups, may be seen. More extensive hemorrhagic manifestations such as ecchymoses, hemoptysis, hematemesis, melena, gross hematuria, and, rarely, bleeding in the central nervous system (CNS) become evident during the oliguric phase. Striking elevations in blood urea nitrogen and creatinine levels are common findings. Biochemical disturbances (electrolyte derangements, metabolic acidosis, uremia) may be severe, and in such cases, dialysis may be lifesaving. Approximately half of fatalities occur during the oliguric phase.

Between 10 and 14 days into the illness, renal function is restored spontaneously in most patients, and a period of diuresis follows. Polyuria may be substantial, and urine output frequently exceeds 3 to 6 L/day. With the onset of diuresis, clinical recovery is initiated; however, the rapid change in fluid status may precipitate further electrolyte disturbances, so close monitoring remains necessary.

Convalescence typically lasts from 3 to 6 weeks, but in many cases, a longer period passes before health is restored completely. Weight gain and strength are recovered slowly. Proteinuria resolves, but hyposthenuria persists for months. Most patients recover completely, although permanent sequelae may result from such complications as anterior pituitary or other CNS hemorrhage.

The disease has been reported infrequently in children.[42,75,172] However, the data available indicate that clinical manifestations are similar to and perhaps somewhat less severe than those observed in adults. In a series of 63 children identified retrospectively over a 15-year period in Korea, fever was universal, whereas abdominal pain, headache, and vomiting were present in 73 percent or more of patients (Table 201–2).[172] Proteinuria (100%), leukocytosis (71%), thrombocytopenia (80%), hypocholesterolemia (87%), and elevations in creatinine (94%), blood urea nitrogen (94%), and alanine transaminase (80%) were the laboratory abnormalities found most commonly. Petechiae and hypotension occurred infrequently (38% and 11%, respectively), and frank hemorrhage did so rarely. Eleven patients (18%) required dialysis. The mortality rate was 5 percent, and the remaining patients recovered without sequelae.

HFRS occurring in adults after they are infected with Seoul virus resembles that described for Hantaan virus infection, but the clinical manifestations generally are much milder.[93] Fever and constitutional symptoms are similar in the two types of infection, but hypotension occurs infrequently in persons infected with Seoul virus (10% of cases), and clinical shock is a rare event. The

TABLE 201–2 Prominent Clinical and Laboratory Features of Hemorrhagic Fever with Renal Syndrome in Children

Features	Korea[172] (%)	Sweden[1] (%)	Finland[109] (%)
Fever	100	100	100
Headache	76	100	59
Anorexia	33	100	NR
Nausea	62	86	81
Vomiting	73	91	72
Abdominal pain	91	93	59
Back/costovertebral angle pain	35	76	63
Dizziness	21	73	9
Thirst	NR	75	NR
Polyuria	NR	57	NR
Diarrhea	NR	57	9
Petechiae	38	NR	NR
Conjunctival hemorrhage	35	NR	3
Proteinuria	100	100	97
Hematuria	67	80	73
Pyuria	8	43	44
Glucosuria	NR	26	12
Casts	NR	33	NR
Leukocytosis	71	22	41
Thrombocytopenia	80	68	87
Elevated hemoglobin	39	NR	28
Elevated C-reactive protein	NR	28	89
Elevated erythrocyte sedimentation rate	NR	58	74
Elevated alanine transaminase	80	NR	53
Elevated creatinine	94	76	84

NR, not recorded.

frequency and severity of thrombocytopenia are less with Seoul virus infection, whereas elevations of transaminases occur commonly (>60% of cases). The mortality rate for HFRS caused by Seoul virus is 1 percent or less.

The Scandinavian or European form of HFRS (nephropathia epidemica) generally is a much more benign disease than that attributed to Hantaan virus; fatal outcomes are observed, but the case-fatality rate is less than 1 percent. In contrast to the findings with Hantaan virus, subclinical infection apparently occurs commonly with Puumala virus; one report suggested a case-to-infection ratio of 1:10.[115]

In adults, nephropathia epidemica typically is a biphasic disease of 1 to 3 weeks' duration.[24,119,159] The onset usually is abrupt, with no apparent prodrome. High fever is the initial symptom in 95 percent of patients. On examination, a facial flush is a usual finding. This febrile phase lasts from 3 to 6 days. The development of nausea, vomiting, abdominal pain, back pain, somnolence, and, occasionally, joint pain heralds the onset of the second, or renal, phase. The abdominal pain may be of such severity and character that an acute abdomen is suspected, and many patients have undergone surgery for suspected appendicitis before nephropathia epidemica was diagnosed. Visual disturbances are common manifestations, and hypotension, if it develops, usually is mild. Petechiae are seen relatively infrequently. The renal stage generally lasts 1 to 2 weeks and is characterized by the development of oliguria, proteinuria, hematuria, and hyposthenuria. Modest elevations in blood urea nitrogen and creatinine accompany the oliguria, which typically lasts for no more than a few days before diuresis begins. Mild leukocytosis and thrombocytopenia occur during the renal phase, whereas electrolyte disturbances sufficient to require dialysis are infrequent events. As with other forms of HFRS, convalescence may be prolonged, and hyposthenuria may persist for many months. Recovery typically is complete, although minor abnormalities in renal function and blood pressure have been described.[81,114]

As with Hantaan virus–associated HFRS, nephropathia epidemica in children is recognized infrequently. Clinically, the disease also appears to be similar to and, in most cases, milder than that seen in adults. Among 32 Swedish cases reported (18 identified retrospectively and 14 prospectively), fever (100%), headache (100%), anorexia (100%), abdominal pain (93%), vomiting (91%), nausea (86%), and back pain (76%) were the most prevalent symptoms (see Table 201–2).[1] Proteinuria (100%), microscopic hematuria (80%), elevated serum creatinine (76%), and thrombocytopenia (68%) were the laboratory abnormalities most frequently found. Leukocytosis was a relatively uncommon finding (22%) in this series. Six children (19%) had hemorrhagic manifestations, and one complained of blurred vision. In a separate 32-patient series from Finland, fever (100%), nausea (81%), vomiting (72%), and back pain (63%) again were prevalent, but headache and abdominal pain (59%) were reported less frequently (see Table 201–2).[109] Proteinuria (97%), hematuria (73%), and elevated serum creatinine (84%) were common findings. Thrombocytopenia (87%) was reported more prominently among Finnish than Swedish children. Approximately one fourth of Finnish children displayed hemorrhagic manifestations. The proportion of transient visual blurring was identical to that seen in adults (25%). Some disease manifestations (e.g., thrombocytopenia, renal function abnormalities) may be absent, however, and the disease should be considered in the differential diagnosis of "fever of unknown origin" in endemic areas.[158] Three children with Puumala virus nephropathy in the Czech Republic were admitted with interstitial nephritis following a flulike prodrome. All were mildly febrile, with anemia and elevated C-reactive protein, erythrocyte sedimentation rate, serum creatinine and proteinuria. Biopsy-confirmed renal abnormalities returned to normal within 4 weeks after steroid treatment.[32] No children in these series required dialysis.

HANTAVIRUS PULMONARY SYNDROME

Classic HPS in adults is a biphasic illness that challenges the diagnostic acumen and clinical management skills of physicians. The incubation period was 7 to 39 days (median, 18 days) for 20 people with defined exposure to Andes virus in a high-risk area,[161] an observation consistent with the incubation period of 9 to 33 days (median, 14 to 17 days) for 11 cases of patients with HPS and well-defined exposure in the United States[173] and the 3-week incubation period observed in two boys with HPS bitten by the same mouse.[144] The clinical features of the prodrome phase are not pathognomonic, and the diagnosis rarely is suspected before the abrupt clinical deterioration occurs that heralds onset of the cardiopulmonary phase. HPS characteristically begins with a prodrome that lasts on average 3 to 4 days but may extend as long as a week or more.[17,31,95,121,160] This phase is typified by fever and myalgia, particularly of the back or lower extremities. Although a cough may develop as the prodrome progresses, illnesses initially characterized predominantly by upper respiratory symptoms, such as cough and coryza, are unlikely to be HPS.[17,31,95,108] More than half of patients with HPS also have gastrointestinal symptoms (e.g., nausea, vomiting, diarrhea) that usually are mild.[17,31] Occasionally, HPS-associated gastrointestinal symptoms have been mistaken for an acute surgical abdomen or another intra-abdominal process. The presence of thrombocytopenia in the context of a compatible prodrome is highly suggestive of HPS.[17,31,77,108,143]

The onset of the cardiopulmonary phase is abrupt and often life-threatening. Patients usually are hospitalized within 12 to 24 hours of initial medical evaluation, and most deaths occur during the first 24 to 48 hours in the hospital.[17,18,31,95] Clear chest radiographs have progressed to diffuse bilateral pulmonary involvement during the course of several hours. Interstitial edema, present in only 5 percent of patients with adult respiratory distress syndrome, is present in most patients with HPS on initial radiography, and alveolar flooding that is usually indistinguishable from the peripheral pattern seen in the acute phase of adult respiratory distress syndrome develops in most patients.[69] This noncardiogenic pulmonary edema, resulting from a diffuse pulmonary capillary leak, can be differentiated from cardiac (hydrostatic) pulmonary edema by the presence of low pulmonary artery occlusion pressure and an increased protein content in edema fluid.[50] Secretions recovered when patients with HPS are intubated generally are acellular, resemble plasma or pulmonary edema fluid, and have been observed to clot in severe cases.[31,50] The presence of significant numbers of polymorphonuclear leukocytes in pulmonary secretions suggests an alternative cause.

The pulmonary decline usually is accompanied by the onset of shock caused by myocardial dysfunction and relative hypovolemia.[17,50,95] In severe cases, hemodynamic measurements show high systemic vascular resistance combined with low cardiac and stroke volume indices. Progression to death is associated with worsening cardiac dysfunction unresponsive to treatment, despite provision of adequate oxygenation.[50] At autopsy, the myocardium of patients who died of Araraquara virus–associated HPS contained endothelial cells and interstitial macrophage-type cells harboring hantavirus antigen and viral particles accompanied by a mononuclear cell inflammatory interstitial infiltrate that met the international consensus criteria for infectious inflammatory myocarditis and was absent from the autopsy myocardium of persons who died after having either necrotizing pancreatitis associated with acute lung injury or nonthoracic trauma.[129,131] This infectious inflammatory cardiomyopathy may be responsible for HPS-associated myocardial depression and shock. In one South American study, the absence of clinical and laboratory signs of circulatory shock on admission was associated with a favorable outcome.[133]

Thrombocytopenia may be present in the late prodrome and almost always is found during the cardiopulmonary phase. Addi-

tional laboratory abnormalities in hospitalized patients include hemoconcentration, prolonged prothrombin and partial thromboplastin times, elevated serum lactate dehydrogenase concentration, decreased serum protein concentration, mild leukocytosis with a marked left shift, and the frequent presence of myeloid precursors on the peripheral smear.[17,31,77,95,121] Proteinuria is a common occurrence. Serum creatinine often is elevated modestly in severe cases, although renal failure is not characteristic of infection with Sin Nombre virus, despite episodes of hypotension and other predisposing conditions that are present in many patients. Among patients who survive, recovery may be as dramatic as the decline; survivors may be extubated within 3 to 7 days after admission to the intensive care unit and may be discharged from the hospital within 2 weeks.[121]

Renal failure was more prominent in two reported cases of HPS caused by infection with Black Creek Canal virus and Bayou virus.[29,73] The hantavirus disease associated with Andes virus[98] in Argentina and Chile and that from other related virus infections in Brazil[111] and Paraguay[166] closely resemble Sin Nombre virus disease. Renal failure accompanied some cases in a focus in northern Argentina.[117]

Subclinical or mild disease rarely follows Sin Nombre virus infection.[143] However, although initial case-fatality rates of 70 to 80 percent were reported in 1993, a wider clinical spectrum became recognized once clinicians developed heightened suspicion and diagnostic assays became more readily available. As of March 2007, the overall case-fatality rate for HPS cases in the United States was 35 percent. This apparent decline in the incidence of mortality may reflect improvements in survival attributed to improved clinical management. However, it undoubtedly also reflects a decrease in the tendency to suspect this diagnosis only with the fatal and near-fatal disease that existed early in the clinical understanding of this syndrome.

HPS has been recognized infrequently in children younger than 17 years. Several case reports and reviews of pediatric patients with HPS (identified through the Centers for Disease Control and Prevention [CDC] surveillance and a university database) indicated that the geographic distribution, clinical course, and mortality rates in children and adolescents are similar to those described for adults.[3,10,72,84,116,128] Fever, headache, dyspnea or cough, gastrointestinal disturbances, and myalgia are common manifestations in the prodromal phase. Tachypnea and fever are frequent on hospital admission; in one case series, however, hypotension at presentation was an uncommon finding (33%).[128] In one patient, dizziness with an apparent vestibular component was described.[72] Thrombocytopenia, a left shift in the leukocyte differential count, elevated levels of hepatic transaminases and lactate dehydrogenase, and hypoalbuminemia typically are seen on admission. Although leukocytosis and hemoconcentration are not found early reliably, they may appear later in the course of illness. Investigators suggested that the mortality rate among prepubertal children may be somewhat lower than that seen in older individuals,[10] but as patient numbers have accumulated over time, overall case-fatality rates in pediatric and adolescent patients seem to be similar to those seen in adults (30% to 40%). In one series, hypotension and absence of fever at admission were found to be predictive of respiratory failure, whereas elevated prothrombin time (≥14 seconds) at admission was predictive of mortality.[128] In one small case series from Argentina, the case-fatality rate for HPS in 5- to 11-year-old children was somewhat higher (60%).[123] In the same report, the authors noted evidence of passive hantavirus antibody transfer from a pregnant mother to her fetus, without subsequent illness in the infant. Additionally, a woman who nursed a 7-month-old infant contracted HPS and subsequently died. Hantavirus antibodies were detected to high titer (>1:6,400) 8 and 15 months later in the baby (who remained healthy), a finding suggesting possible asymptomatic mother-to-child transmission of the infection through breast-feeding.[123]

COMPLICATIONS

In general, human infection with HFRS-associated hantaviruses results in an acute illness with prolonged incapacitation followed by complete recovery. Unless complicated by organ hemorrhage (e.g., CNS bleeding), residua have not been observed. However, epidemiologic associations between hypertensive renal disease and evidence of previous U.S. hantavirus infection have been reported.[45,46] In one study, 5 years after recovery, 50 percent of patients who had HFRS were hypertensive, with a higher glomerular filtration rate and urinary protein excretion compared with 21 percent of controls. These differences resolved by 10 years.[105] These data require further study to determine their significance.

Although prolonged prothrombin and partial thromboplastin times occur commonly in hospitalized patients with HPS, overt disseminated intravascular coagulation and overt hemorrhage occur infrequently.[17,174] Patients in whom disseminated intravascular coagulation is established rarely survive longer than 48 hours. One patient who did survive a cardiopulmonary phase accompanied by overt disseminated intravascular coagulation died 3 weeks later of gangrenous complications.

The South American viruses have been associated with more extrapulmonary manifestations than have the Sin Nombre infections in the United States. Evidence of bleeding is found more commonly, and other complications are reported with greater frequency.

PATHOGENESIS AND PATHOLOGY

Serum antibodies develop within 3 to 7 days of the onset of illness in patients infected with hantaviruses. Early clinical events are presumed to be accompanied by viremia; however, significant signs and symptoms develop in temporal association with the onset of a measurable antibody response. In HPS, a statistically significant association has been demonstrated between plasma viral RNA levels at hospital admission and the severity of disease.[167] Pathologic studies of fatal HFRS cases indicated that multiple organ systems are involved, but a triad of lesions consisting of hemorrhagic necrosis of the renal medulla, anterior pituitary, and cardiac right atrium is described as characteristic.[63] In HPS, multiple organ involvement with variable degrees of vascular congestion is noted in all fatal cases. However, the predominant findings at autopsy have involved the lungs, with pulmonary edema and serous effusions seen grossly and interstitial pneumonitis with a mononuclear cell infiltrate, intra-alveolar edema, and focal hyaline membrane formation described microscopically. Immunohistochemical studies demonstrated the widespread presence of hantavirus antigens in endothelial cells of the microvasculature, particularly in the lungs but also in the kidneys, heart, spleen, pancreas, lymph nodes, skeletal muscle, intestine, adrenal gland, adipose tissue, urinary bladder, and brain. However, few histologic changes have been noted in the kidney, brain, and heart of autopsied patients with HPS.[174]

Hantaviruses replicate primarily in human endothelial cells, but without apparent cytopathic effect.[174] However, endothelial cell mRNA responses to infection by pathogenic and nonpathogenic hantaviruses, observed by DNA array analyses, showed differential patterns of gene activation.[43] Pathogenic Hantaan and New York-1 viruses appeared to suppress early interferon responses that were activated by nonpathogenic Prospect Hill virus. Hantaan virus uniquely induced multiple chemokines (interleukin-8 [IL-8], IL-6, growth-regulated oncogene-beta [GRO-β]), cell adhesion molecules (intercellular cell adhesion

molecule [ICAM]), and complement cascade–associated factors that could play a role in HFRS immunopathogenesis. Although New York-1 virus failed to induce most of the cellular chemokines activated by Hantaan, it uniquely induced β_3 integrin–linked potassium channels, an action that could be important to HPS-induced vascular permeability. Whereas the mechanisms underlying the observed pathologic lesions are incompletely understood, both cellular and humoral immune-mediated mechanisms have been implicated.[7,25,33,63,155] Virus-specific cytotoxic T lymphocytes have been observed during acute phases of HFRS,[61] and elevated levels of inflammatory cytokines (interferon-γ [IFN-γ], tumor necrosis factor-α [TNF-α], TNF-β, IL-6, and IL-10) have been found in sera and renal biopsies of patients.[61,78,96,151] Animal studies suggested a role for T-helper cells and antibodies as well.[26] The abrupt onset of noncardiogenic pulmonary edema in HPS occurs after the development of an immune response directed against viral antigen present throughout the endothelial cells lining the pulmonary capillaries, and such edema leads to a pulmonary capillary leak syndrome.[120]

DIAGNOSIS AND DIFFERENTIAL DIAGNOSIS

A high index of suspicion is essential for recognition of hantavirus infection in persons living or traveling in disease-endemic areas. The protean nature of the disease in children is such that almost any ill-defined febrile disease (or fever of unknown origin) associated with abdominal or back pain in an appropriate geographic setting should stimulate consideration of the diagnosis, particularly if the clinical syndrome is accompanied by myalgia, if thrombocytopenia or proteinuria is present, or if a history of exposure to rodents or rural environments in disease-endemic areas is elicited.[143]

The differential diagnosis of HFRS includes rickettsial disease, leptospirosis, meningococcemia, other viral diseases, post-streptococcal syndromes, pyelonephritis, leukemia, and hemolytic-uremic syndrome. Differentiating from an acute intra-abdominal or pelvic process, such as appendicitis, may be extremely difficult on clinical grounds.

The differential diagnosis of HPS includes rickettsial diseases, leptospirosis, influenza, streptococcal pneumonia, legionellosis, *Yersinia pestis* infection (plague), meningococcemia, brucellosis, mycoplasmal and fungal pneumonias (including *Coccidioides immitis* and *Histoplasma pneumonias*), tularemia, psittacosis, pancreatitis accompanied by adult respiratory distress syndrome, and autoimmune disorders (including thrombotic thrombocytopenia purpura). A prominent cough or sore throat or a localized infiltrate on a chest radiograph that does not generalize within hours argues for a non-hantaviral cause.[17,53,108] A constellation of the absence of a cough in the presence of dizziness, nausea or vomiting, a low platelet count, a low serum bicarbonate level, and an elevated hematocrit discriminated HPS from similar patients with unexplained adult respiratory distress syndrome in two studies.[17,108] Thrombocytopenia or a falling platelet count provides a valuable clue late in the prodrome. After the onset of pulmonary edema, the presence of four of five findings (thrombocytopenia, myelocytosis, hemoconcentration, lack of significant toxic granulations in neutrophils, and more than 10% of lymphocytes with immunoblastic morphologic findings) has sensitivity for HPS of 96 percent and specificity of 99 percent.[77] Frequent use of pulse oximetry also is of assistance.

Laboratory diagnosis of HFRS or HPS is made by demonstrating specific anti-hantavirus immunoglobulin M (IgM) antibodies in acute-phase serum by enzyme immunoassay in the IgM capture format. A fourfold or greater rise in specific IgG antibodies by enzyme immunoassay or immunofluorescence[79,94] in sequential sera (ideally obtained ≥2 weeks apart) also is useful. The availability of purified recombinant hantavirus antigens has enhanced diagnostic specificity in both the enzyme immunoassay and Western blot assay systems.[37,64,177] Enzyme-linked immunosorbent assay and indirect fluorescent antibody tests are broadly cross-reactive, particularly among viruses from rodents of the same subfamily.[21,22] For example, a recombinant Sin Nombre virus antigen detects antibodies against Bayou, Black Creek Canal, Andes, and several other viruses from sigmodontine rodents, and it maintains reactivity with antibodies directed against arvicolid rodent–associated viruses (e.g., Puumala and Prospect Hill). Neutralizing antibody assays are more specific, but technical requirements preclude their routine use. Immunohistochemical techniques have been applied to tissue samples from patients infected with hantaviruses.[174] Nucleic acid primers from several hantavirus strains have been generated, and they have enabled nucleotide sequences from fresh or frozen tissues to be amplified by RT-PCR. RT-PCR usually is successful on whole blood or blood clots obtained within the first 7 to 10 days of illness. However, the expense and effort of performing the method are not justified unless the resulting genetic sequence information is needed for definition of the viral strain or for epidemiologic studies.[2,112] Attempts to isolate hantaviruses from human specimens are generally unrewarding.

TREATMENT

Cautious fluid management and hemodynamic and intensive care unit support are the most important aspects of the clinical management of any hantavirus disease.[50,95] Early hospitalization and avoidance of even minor trauma are essential to maintain the integrity of damaged vascular beds in these patients. Transport of patients should be minimized; the barotrauma associated with transport in underpressurized aircraft may be particularly hazardous.[9] Attention to fluid management and metabolic status is especially critical. In patients with HFRS, restriction of fluids may be necessary early in the course of disease as renal function diminishes, but large input may be required later during diuresis to cover massive losses. Electrolyte abnormalities and metabolic acidosis occur commonly. Peritoneal dialysis or hemodialysis may be lifesaving in severe cases.[9] In patients with HPS, the nature of the pulmonary disease predisposes to iatrogenic pulmonary edema; careful attention must be paid to maintaining appropriate central venous and pulmonary arterial pressure to avoid such complications. Tissue perfusion and adequate oxygenation are the goals of supportive therapy with this syndrome. Oxygen supplementation and mechanical ventilation are required nearly always. Inotropic agents may be necessary to maintain tissue perfusion.[50,95] Studies have suggested that cytotoxic T-lymphocyte activity and other aspects of inflammatory response contribute to the capillary leakage observed in patients with HFRS or HPS, and case reports have attributed clinical improvement to treatment with corticosteroids and veno-venous hemodiafiltration.[52,140] The safety and efficacy of steroid treatment have not been confirmed through controlled clinical trials.

Hantaan and Sin Nombre viruses exhibit similar in vitro sensitivity to ribavirin. One prospective, placebo-controlled trial suggested that intravenous ribavirin was effective in reducing the mortality and morbidity rates associated with HFRS in China.[62] In contrast, although 30 patients with HPS who received investigational, open-label intravenous ribavirin generally tolerated it well, treatment was accompanied by a low frequency of early drug-associated adverse events, most significantly anemia and resulting transfusion, and no clear evidence of benefit was obtained.[17,18] A subsequent randomized placebo-controlled trial was stopped short of a definitive end-point because of

small enrollment without trends supporting either efficacy or adverse events.[104] However, the study sample size was insufficient to exclude adverse events that occurred at a rate of less than 27 percent.[99] Similar doses of intravenous ribavirin resulted in hemolysis in 76 percent, a 2 g/dL hemoglobin level decrease in 49 percent, and discontinuation because of toxicity in 18 percent of 126 patients treated for sudden acute respiratory syndrome (SARS), findings consistent with observations during the open-label trial.[8,17,18,99] This contrast in clinical experience is not explained by differences in dosing schedules; patients in all three protocols received identical doses.[8,18,62] All hantaviruses have been sensitive to the antiviral effects of ribavirin in vitro. The lack of any dramatic effect by intravenous ribavirin in HPS is probably the result of the rapid progression of the disease; HPS-associated deaths usually occur within the first 48 hours after admission and therapeutic intervention.[16-18,31,50,62,95]

Ribavirin is not licensed for intravenous use in the United States. Teratogenic concerns mandate careful informed consent if the use of this drug is considered in children, pregnant women, or nursing mothers.

PREVENTION

PRIMARY PREVENTION

The most effective preventive measure available for hantavirus infection is the avoidance of rodents and their habitats. However, eradication of rodent reservoir hosts in disease-endemic areas is not feasible. Prevention efforts are directed more appropriately toward reducing the frequency of rodent-human interactions through environmental hygiene practices that minimize rodent density in home and work environments and avoidance of known rodent-infested areas and activities that increase the risk of human exposure to aerosolized infectious rodent excreta. Such avoidance may be particularly important during times of high rodent populations in specific localities. In fact, in the southwestern United States, remote sensing patterns from the year before can predict local areas of increased risk for acquiring the disease, thus allowing precisely targeted public health messages and interventions.[44]

Detailed guidelines on measures appropriate to eliminate rodents from homes in disease-endemic areas, to clean rodent-contaminated areas safely, and to minimize the risk for workers occupationally exposed to rodents and participants in outdoor recreational activities have been published.[106] Although rodent ectoparasites do not transmit hantaviruses, in the southwestern United States, several rodent species are also hosts to fleas that transmit *Y. pestis*. In such areas, insecticides should be used in conjunction with rodent extermination measures because eradication of rodents without concurrent control of the associated fleas may increase the risk of human plague.[106]

VACCINE PROSPECTS

Inactivated vaccines to Hantaan and Seoul viruses have been developed in South Korea and China. Some of these vaccines induce neutralizing antibody responses in humans and are being tested in field studies.[118,146,147] A recombinant vaccinia strain expressing Hantaan antigens has been tested in human volunteers in the United States and has been shown to induce a serum neutralizing antibody response.[138] Various other approaches to vaccine development use Puumala, Dobrava, or Andes viral elements (as well as Seoul or Hantaan), but none has progressed to clinical trials. An immediate need exists for a vaccine to Hantaan and perhaps other hantaviruses, but lack of a realistic animal model of human disease has hampered efforts to perform full preclinical evaluation of candidates. This is particularly important because the diseases are immunopathologic, and some monoclonal antibodies can enhance macrophage infection in vitro. The discovery that hamsters infected with Andes virus provide a reproducible model for HPS that mimics the human disease very closely[59] provides an avenue to explore mechanisms of disease and also vastly improves preclinical evaluation of hantavirus vaccines. Nonetheless, even more than with other viral vaccines, double-blinded, placebo-controlled trials are needed urgently to provide definitive evidence of protection and to exclude adverse effects.

HOSPITAL INFECTION CONTROL

The use of universal precautions in handling the blood and body fluids of all patients is prudent practice. In addition, a certified biologic safety cabinet should be used for all handling of human body fluids in situations in which splatter or aerosolization is possible.[13] Viral antigens can be detected in necropsy specimens, and RT-PCR readily detects viral genetic material in necropsy tissue and in blood and plasma obtained from hantavirus-infected persons early in the course of the disease.[57,174] However, secondary transmission of hantaviruses associated with HFRS has not been documented after contact with acutely ill persons or exposure to their clinical laboratory specimens. The experience with North American hantaviruses linked to HPS has been similar.[13,163] However, a single well-documented episode of person-to-person spread during an Andes virus outbreak in South America and subsequent cluster analyses, in addition to anecdotal experience with this agent, suggest that it may be an exception.[36,82,100,165] This latter incident notwithstanding, the many years of experience with HFRS and HPS indicate that once the diagnosis has been confirmed, isolation of hospitalized patients to prevent nosocomial transmission generally is not required. Sera and other specimens from hantavirus-infected persons can be handled safely by using biosafety level 2 facilities and practices. Higher biosafety levels are recommended for attempts to propagate hantaviruses.[13,16]

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CHAPTER 202

La Crosse Encephalitis and Other California Serogroup Viruses

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The term *California serogroup* does not describe the widespread geographic distribution of viruses in the serogroup but simply reflects the name of the prototype virus initially discovered in California in 1943.[58] In fact, La Crosse virus (LACV), the most prevalent and pathogenic member of the serogroup, produces disease in the Midwest and eastern United States but not in the West Coast region.[2,18,48,51,85,121,122] LACV is estimated to cause 8 to 30 percent of all cases of encephalitis in the United States annually and is the most common arboviral infection of children in North America,[107] occurring mostly East of the Mississippi River (Fig. 202–1). Although mortality from La Crosse encephalitis (LACE) is relatively low (0.3%)[107] compared with certain

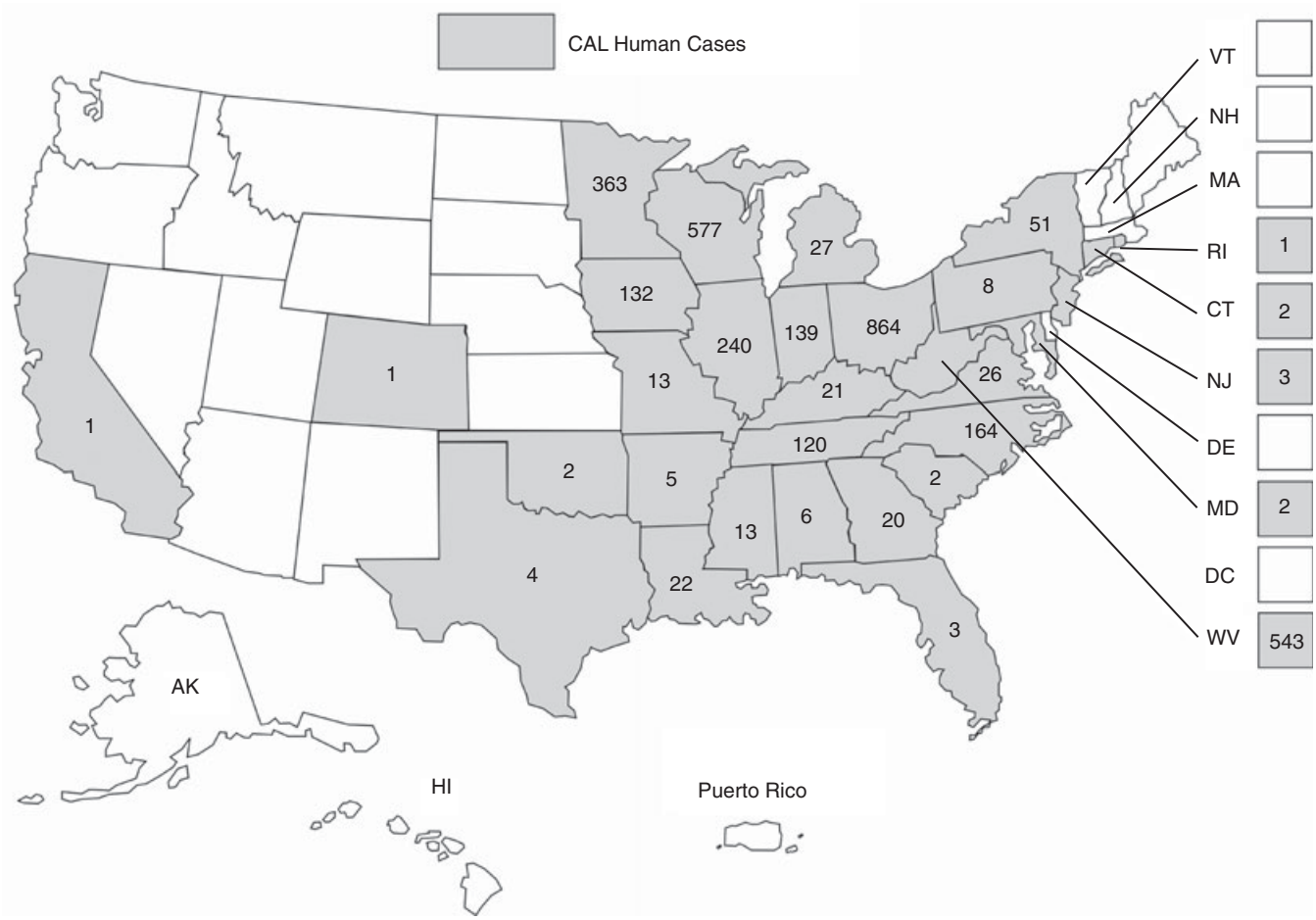

Figure 202–1 Reported cases of encephalitis attributable to California serogroup virus by state, 1964 to 1995.

other arboviral illnesses such as with West Nile virus (WNV) or Eastern equine encephalitis, the medical, psychosocial, and economic costs to affected individuals in endemic areas are significant. Acute morbidity includes seizures in roughly half of hospitalized cases, mental status changes in one third, cerebral edema in 16 percent, and cerebral herniation in 2 to 3 percent. One fourth of all hospitalized patients require mechanical ventilation.[87] Long-term morbidity following severe disease may include cognitive delays in up to one third and attention-deficit hyperactivity disorder (ADHD) in up to two thirds.[87] In a study of the economic and social impact of LACE in an endemic region of western North Carolina, the average direct and indirect medical costs per patient (from a group of 24 patients) were roughly $33,000 (≤$35,000), with the projected cost for five patients with neurologic sequelae ranging from approximately $50,000 to $3 million per patient.[124] Although the number of confirmed cases of LACE cases is in the range of 40 to 200 per year in the United States, underrecognition and underreporting are common problems. Because LACV is the most clinically significant member of the California serogroup, it is the main focus of this chapter, but other members of the serogroup are addressed briefly as well. Clinical information of greatest relevance to practitioners is contained in the sections on clinical manifestations, diagnosis, differential diagnosis, and treatment.

ETIOLOGIC AGENT

In 1960, a 4-year-old girl died of "rural encephalitis" in a hospital in La Crosse County, Wisconsin. Four years later, Dr. Wayne Thompson and colleagues isolated a novel virus from frozen homogenates of the child's brain by intracerebral inoculation of suckling mice.[118,120] Soon after its discovery, LACV was identified as a member of the California serogroup and is one of five (prototype California encephalitis [CE] virus, LACV, snowshoe hare virus, Jamestown Canyon [JC] virus, and trivittatus) such viruses causing human disease in North America. The California serogroup is 1 of 16 in the *Bunyavirus* genus. Inkoo and Tahyna viruses are members of the California serogroup distributed in Europe.

The LACV virion is described as pleomorphic and roughly 90 to 100 nm in diameter, having a host-derived viral envelope in which virus encoded glycoproteins G1 and G2 are located on spikes 5 to 10 nm long. The virus replicates in the cytoplasm and buds from the Golgi apparatus. Of the three segments of the RNA genome, the large (L) RNA segment encodes for the polymerase, the medium (M) RNA encodes for the G1 and G2 glycoproteins and a nonstructural protein (NSm), and the small RNA segment encodes for the nucleocapsid (N) protein and nonstructural protein NSs.[15]

The M segment that encodes the G1 and G2 glycoproteins is a major determinant of viral *neuroinvasiveness* in mice and susceptibility of *Ochlerotatus triseriatus* mosquitoes to oral and intrathoracic infection.[45,48,109] Neuroinvasiveness may be linked to the cell receptor and fusion functions associated with glycoproteins.[45,47] The G1 protein of LACV is involved in viral attachment to mammalian cell receptors, functioning as a type II fusion protein,[103] and is involved in hemagglutination and viral neutralization.[45,48,76,77,109] Group- and LACV-specific epitopes are present on G1. The G2 glycoprotein mediates viral attachment to insect cells,[84] but more recent work emphasizes that G1 also is required for infection of mosquito cells (in vitro and in vivo) to occur.[56] Although the M segment genome largely determines neuroinvasiveness (i.e., the ability to invade the central nervous system [CNS] from an extraneural site) through its glycoprotein products (especially G1), more recent evidence using viral reassortants of different virulence implicated the L segment as a major factor in determining neurovirulence (i.e., the ability to infect CNS tissue after direct cerebral injection).[35] Finally, a viral component encoded by the S segment seems to serve an emerging role in pathogenesis. This NSs was shown in insect and mammalian cells to inhibit the RNA interference (RNAi) pathway, thus inhibiting a mechanism (RNAi) of innate immune response to viral infection. NSs also plays a role in promoting apoptosis, the main mechanism by which LACV kills cells.[14,112] Investigators have shown that a truncated G1 protein preparation induces a protective immune response in the suckling mouse model through neutralizing antibody (see "Prevention").[100] Apparently, neutralizing antibody can prevent neuroinvasion by interruption of the transient viremia, which occurs just after virus inoculation.

Oligonucleotide maps of LACV isolates from various areas of the United States have grouped the viruses into three types. Upper Midwest viral strains are of two types, A and B; type C varieties are found in scattered areas of the eastern part of the United States.[39,79] Variations in strains indicate that changes in the viral genome occur by genetic drift. Genetic reassortment through exchange of RNA segments also has been demonstrated in nature, with one proposed mechanism involving dually infected mosquitoes, particularly those initially with transovarial infection before superinfection with a second closely related virus.[15] Study of the genome of viral isolates from three fatal cases indicated a high degree of conservation of nucleotide sequences among these isolates.[24,63]

ECOLOGY

LA CROSSE VIRUS

Borucki and coworkers provided an in-depth review of LACV replication in vertebrate and invertebrate hosts.[16] *Ochlerotatus triseriatus* (formerly known as *A. triseriatus*), the eastern tree hole mosquito, is the reservoir of LACV in nature and the vector for transmission of infection to humans.[51,126,127] Humans do not maintain prolonged viremia and therefore are dead-end hosts. The mosquito is distributed principally in eastern hardwood deciduous forests, where it breeds in tree holes; however, it also is adapted to breeding in small artificial containers that hold rainwater, such as discarded cans, bottles, and tires. The mosquito is diurnal and feeds actively during the day. Although these insects disperse over a wide area, fairly permanent foci of infected mosquitoes are observed in sharply delimited areas, and isolation of virus and human cases recur each summer in established foci.[8,51,78,116]

The most important mechanism for maintenance of LACV in nature is vertical (transovarial) transmission in *O. triseriatus*. The virus overwinters in infected eggs, which give rise to infected mosquito progeny the following year, thereby providing the mechanism of recurrent disease each summer in endemic areas.[91,115,127] Research indicated that transovarial transmission of LACV in *O. triseriatus* is controlled by a single gene locus.[50] The LACV also is maintained in nature through horizontal transmission by venereal propagation in *O. triseriatus* and through amplification of the virus in the vertebrate hosts on which vector mosquitoes feed.[91,126,127] Vector competence, related in part to the ability of the female mosquito to become infected in the first place, may be associated with malnutrition of the mosquito, which compromises her mesenteric barrier and may then allow infection to occur after taking an LACV-infected blood meal.[50,98] The principal amplifying hosts involved in horizontal transmission are chipmunks and squirrels.[132] Other wild vertebrates such as foxes and woodchucks also may contribute to amplification. Although domestic livestock and pets do not contribute to horizontal amplification of the virus, certain species do show seroconversion to California serogroup viruses, and these species may prove to be useful markers of viral presence in a given area.[44] For example, outbreaks of fatal encephalitis have occurred in puppies, and dogs have been used in an animal model of CNS infection.[13]

Concern that *Aedes albopictus*, the Asian tiger mosquito, has entered the transmission cycle for LACV [6,7,29,30,53,66] was heightened when LACV-infected *A. albopictus* mosquitoes were isolated in nature in areas proximate to human cases in 2001.[43] In addition, an elevated burden of *A. albopictus* near the residence of patients with LACE was associated significantly with LACV infection in cases versus controls.[41] This mosquito species, which is dispersed widely in the southeastern region of the United States, has significant potential to expand the geographic range and circumstances in which LACV (and possibly other California serogroup viruses) could be transmitted.

OTHER CALIFORNIA SEROGROUP VIRUSES

A brief discussion of the ecology of other California serogroup viruses follows. Although JC virus was isolated first in Colorado and is distributed widely in the West and Midwest, most cases of human illness have been reported in New York, New England, Ontario, and the upper Midwest. Seroconversion for JC virus also has been found frequently (18%) in native Alaskans.[125] Various *Aedes* mosquitoes (e.g., *A. communis* in the West, *Aedes stimulans* in the upper Midwest, *Aedes abserratus* in Connecticut) function as vectors.[32,51,52,54] As opposed to LACV, in which large mammals do not play a role in viral propagation in nature, JC virus horizontal amplification includes deer as the primary amplifying host, with up to 80 percent seroconversion found in adult deer populations in endemic areas.[96,97,133] The overwintering mechanism of JC virus has not been elucidated, although transovarial transmission in *Aedes provocans* has been demonstrated.[12]

Snowshoe hare virus is distributed throughout Canada, including the Yukon and Northwest Territories, in adjacent northern states, and in Russia and China. *Culiseta inorata* and various *Aedes* spp. transmit the virus to snowshoe hares (*Lepus americanus*), ground squirrels (*Citellus undulatus*), and other mammals. Transovarial transmission in vector mosquitoes has been demonstrated.[60]

Trivittatus virus is transmitted and maintained through vertical transmission by *Aedes trivittatus* in the Midwest and is vectored in the South by *Aedes infirmatus*. CE virus is distributed in the western part of the United States, where *Aedes melanimon* and *Aedes dorsalis* are the principal vectors.[50,57,104,119]

Inkoo and Tahyna viruses cause a febrile illness with CNS and respiratory tract infection, respectively, in Scandinavia, Central Europe, and western Russia. Viral recombinants

have been demonstrated.[35] Inkoo also has been found in native Alaskans.[125]

EPIDEMIOLOGY

LACE principally is a disease of children. Among reported cases in the United States from 1972 to 1981, 75 percent were in children younger than 10 years old, and only 3 percent occurred in persons 20 years or older.[74,130] Most cases are seen in boys, with a ratio of approximately 2:1 in most series.[93,118] Table 202–1 summarizes demographic data and clinical characteristics for a large series of hospitalized children.[87] The annual incidence of CNS LACV infection in individuals younger than 15 years in endemic regions is approximately 10 to 30 per 100,000.[75,129] LACV infections are endemic in the United States, with infections typically occurring from July through October, chiefly in rural areas of the east-central states.[74,121] As mentioned earlier, the medical and economic burden to affected individuals and society in endemic areas is significant.

The geographic distribution of LACV infection corresponds to natural divisions in which beech, oak, and maple woodlots are prevalent.[51] Although LACE has been considered a disease of the Midwestern and mid-Atlantic states (with highly endemic zones in Minnesota, Wisconsin, Illinois, Iowa, Ohio, and West Virginia), cases have been found in 30 states, mostly east of the Mississippi River (Fig. 202–2). One highly endemic focus has been recognized in western North Carolina for many years,[115] and sporadic cases have been found as far south as Louisiana and as far east as Connecticut (see Fig. 202–1). Although certain foci of infection appear to remain well localized,[92,115] investigations in eastern Tennessee that showed a low seroprevalence in the general population (only 0.5%) despite a recent marked increase in the number of pediatric cases in that region suggested that true increases in the prevalence of LACV infection (indicating a newly established endemic focus) are occurring in some areas as well.[71,72]

Active surveillance efforts can increase case findings markedly, as seen in West Virginia,[75] where after the death of a child from LACE in 1987, more than 150 cases were diagnosed during the next 8 years (versus only 15 cases ever previously reported in West Virginia).[89] The Centers for Disease Control and Prevention (CDC) records further showed that from 1987 to 1997, West Virginia became the most highly endemic state in the United States, often accounting for roughly half or more of the cases reported to the CDC annually.[22] Therefore, the public health importance of LACE nationally is not known fully because the virus is distributed discontinuously in the eastern part of the United States, where the disease could be endemic but underrecognized.[11,67,129] Improved recognition will require a high index of suspicion by clinicians because the virus typically is not recoverable on culture of cerebrospinal fluid (CSF) and therefore requires specific serologic testing (see "Diagnosis").

Further confounding efforts to understand the true prevalence of disease is the finding that inapparent infections are far more prevalent than is clinically evident disease (see "Clinical Manifestations").[74] Seroprevalence rises with age in endemic areas.[115,116] Serosurveys have shown that point-prevalence rates vary in endemic locations from 30 percent in rural areas to 15 percent in urban locations.[93] Although the risk appears to be highest in rural areas, many cases occur in suburban residential locations, and travel to forested recreational areas in endemic regions is reported by other patients.[33,67,119] A case-control study performed in a highly endemic county found several characteristics of the peridomestic environment to be associated with a risk of acquiring LACE: tree holes on the residential premise, proximity of the house to the forest edge, and the presence of artificial containers and large numbers of discarded tires.[129] A more recent case-control study in eastern Tennessee also showed an association of LACE with proximity (within 100 m) of tree holes to the residence, with increased time of patients spent outdoors, and with an increased burden of *A. albopictus* in the peridomestic environment.[41] These studies support other work pointing to the proximity and abundance of natural and artificial mosquito breeding sites as the principal risk factors from an environmental perspective.[92,95]

With regard to California serogroup viruses in the United States other than LACV, JC virus seroprevalence rates of 5 to 40 percent have been found in the upper Midwest. Most reported cases (21 of 29 in one series) have occurred in male patients.[113] Unlike LACE, cases are found in all age groups. As for snowshoe hare virus, human infections have been documented by serosurvey in Alaska and throughout Canada.[51] Antibody prevalence rates were more than 30 percent in some areas; seroprevalence in the male population was double the rate in the female population. Sporadic infection with CE viruses other than LACV probably occurs frequently in the western part of the United States.[104] However, symptomatic cases with California encephalitis prototype virus rarely occur.[38]

TABLE 202–1 Epidemiologic and Clinical Data on 127 Children with La Crosse Encephalitis*

Variable	Value
Sex: No. (%)	
Male	90 (71)
Female	37 (29)
Age in Years: No. (%)	
0.5-2	9 (7)
3-5	30 (24)
6-8	42 (33)
9-11	26 (20)
12-14	19 (15)
15	1 (1)
Month of presentation: No. (%)	
June	3 (2)
July	31 (24)
August	38 (30)
September	44 (35)
October	11 (9)
Symptoms on Presentation: No. with Findings/Total No. (%)†	
Headache	105/126 (83)
Fever	107/125 (86)
Vomiting	89/127 (70)
Disorientation	50/119 (42)
Seizures‡	58/127 (46)
Signs on Admission: No. with Finding/Notal No. (%)	
Nuchal rigidity	31/120 (26)
Glasgow Coma score ≤12	42/127 (33)
Focal neurologic signs	23/126 (18)

**For symptoms on presentation and signs on admission, data were missing for some patients. Because of rounding, not all percentages total 100.*

†Typically, the patients with headache or fever were admitted 3 or 4 days after presentation; those with vomiting were admitted 1 or 2 days after presentation; and those with disorientation or seizures were admitted on the day of presentation.

‡Seizures were generalized in 22 patients, partial in 24, and partial with secondary generalization in 22.

Data from McJunkin, J. E., de los Reyes, E. C., Irazuzta, J. E., et al.: La Crosse encephalitis in children. N. Engl. J. Med. 344:801-807, 2001.

PATHOGENESIS

LACV, which infects the salivary glands of the mosquito vector, is introduced into the host's skin and subcutaneous tissue during

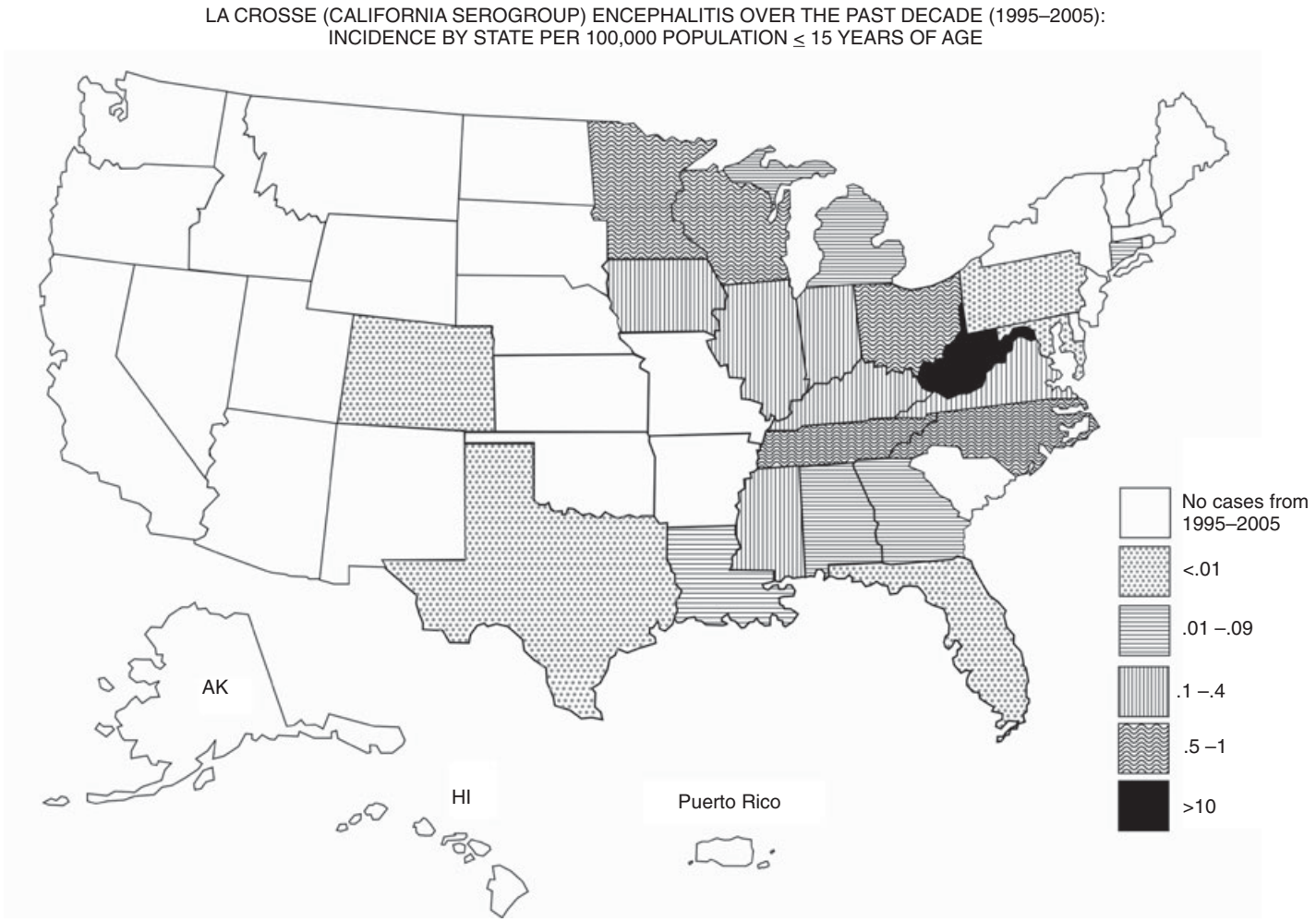

Figure 202–2 Incidence of La Crosse encephalitis over the past decade (1995-2005) per 100,000 population ≤15 years of age.[22,123]

feeding. Probing alone is infectious. Completion of a blood meal is not necessary for transmission to occur. Local viral replication in adjacent muscle tissue leads to systemic viremia that seeds the reticuloendothelial system, muscle, and chondrocytes; such seeding results in further amplification of the viremia and allows invasion of the CNS.[46,47,68,70] Entrance to the CNS probably is gained by infection of vascular endothelial cells initially, followed by infection of neurons and glial cells.[68,70] Case reports suggest that vascular involvement in human LACE may be important in the pathogenesis of focal deficits (from stroke) and more generalized cerebral edema (possibly from vasogenic edema).[83,90] Polymerase chain reaction (PCR) studies of the location of LACV within the CNS found evidence of viral RNA within the cortex but not in other tissues of the CNS.[24] The latter is consistent with the results of magnetic resonance imaging (MRI) in one study that showed findings predominantly consistent with lesions in cortical areas.[87]

Studies indicate that the extraneural phase of viral replication and neuroinvasiveness are mediated largely by the M segment of the genome primarily through G1 glycoprotein, which has cell receptor and fusion functions.[45,47,48,103,109] A more recent study in adult mice indicated that once the virus has gained access to the CNS, replication in CNS tissue (i.e., neurovirulence) is mediated largely by the L RNA segment, probably through viral polymerase.[40,48] Previous work had implicated the M segment in neurovirulence as well, but in that study neurovirulence was defined as time to death after intracutaneous inoculation.[109] The host immune response involves neutralizing antibody, which mediates viral aggregation, inhibition of viral attachment to cells, and inhibition of viral penetration and uncoating.[77] Other aspects of the immune response shown to play a role in protection against CNS LACV infection in animal models are $CD4^+$ T cells and MxA protein, the latter of which is induced by the interferon-α/β system.[61,80,105,108] Susceptibility and complications of LACE may have an immunogenic component, as indicated by an association of illness and seizures with certain human leukocyte antigens.[20] Neuronal cell death may be mediated largely by apoptosis (programmed cell death), as determined by studies in mice,[101] and the nonstructural protein NSs has been found to promote apoptosis, as well as interrupting the RNAi pathway.[14,112] It is interesting that bc1-2 expression can inhibit apoptosis in certain cell lines.

CLINICAL MANIFESTATIONS

The clinical spectrum of illness in LACV infections ranges from inapparent infection or a mild febrile illness to aseptic meningitis and fatal encephalitis.[4,5,25,26,28,55,62,117,131] The ratio of inapparent to clinical infection has ranged from 26:1 to 322:1.[74] Most clinically apparent infections are associated with signs and symptoms of meningoencephalitis. Severe cases tend to mimic herpes simplex encephalitis (HSE) at initial evaluation, whereas mild cases may manifest as aseptic meningitis. Although the case-fatality rate is less than 1 percent, a series of 127 hospitalized

patients included 3 with the near-lethal complication of cerebral herniation. In addition, nearly half of all patients required admission to intensive care, and one fourth required mechanical ventilation.[87]

Children typically have a 3- to 4-day prodrome of fever, headache, and vomiting; diarrhea usually is absent. The illness further evolves such that disorientation (42%) or seizures (46%), or both, may be seen frequently on the day of admission. Most seizures are partial or partial with secondary generalization (58%), and the remainder of seizures are generalized. Status epilepticus develops in approximately one fourth of children with seizures. Rarely, patients may have a sudden onset of seizures with little or no preceding fever or headache.[87]

On examination, signs of meningeal irritation and changes in mental status are found in approximately one quarter and one third of patients, respectively.[87] Focal neurologic signs, principally focal seizures, paresis, aphasia, and abnormal reflexes, are seen in 16 to 25 percent of patients with LACE. Focal and generalized seizures occur in 42 to 62 percent of cases, and status epilepticus develops in 10 to 15 percent. Focal neurologic signs, whether found on physical examination or on electroencephalographic (EEG) or imaging studies, should be taken as evidence of presumptive HSE, thus indicating the need to start empiric acyclovir until diagnostic studies for LACE and HSV are completed.[130] Table 202–1 summarizes clinical characteristics and demographic data, of a large series of hospitalized children with LACE.[87]

From the same study, which is the largest and most recent series studied (n = 127),[87] the hospital course was complicated by hyponatremia (21%) generally consistent with the syndrome of inappropriate antidiuretic hormone (SIADH) secretion, recurrent or de novo seizures (13%), signs of increased intracranial pressure (ICP) (13%), and, rarely, cerebral herniation (2.3%). Approximately 1 in 10 patients (including the 3 with herniation and 10 others with recurrent seizures, coma, or both) experienced neurologic deterioration after hospital admission, usually between 36 and 72 hours of admission. Risk factors associated with deterioration were the presence of disorientation or vomiting at admission and a trend toward seizures at admission. Such neurologic deterioration appeared to be related temporally to a decrease in serum sodium or an increase in body temperature in some cases, findings suggesting that these conditions may be risk factors for in-hospital deterioration as well. This tendency for 1 in 10 hospitalized children with LACE to deteriorate neurologically after admission represents a significant challenge for clinicians (see "Treatment").

Clinical findings in patients infected with other members of the California serogroup deserve brief mention. JC virus infections often are associated with prodromal respiratory symptoms in conjunction with aseptic meningitis or clinical encephalitis. Among 39 patients with proven or suspected CNS infection with JC virus, 10 had encephalitis, and 7 reported upper respiratory symptoms or pneumonia.[51,113]

The few reported patients with snowshoe hare virus infection have had manifestations ranging from an influenza-like illness to aseptic meningitis and fatal encephalitis. One fatality was reported in a 14-year-old girl who had symptoms and signs that suggested Reye syndrome.[36] In a 59-year-old man, radiologic and EEG findings showed a temporal lobe focus, which led to performance of a brain biopsy and a clinical and pathologic diagnosis of herpes encephalitis, although herpes virus was not isolated. Serologic examination later confirmed the diagnosis of snowshoe hare virus infection. Mild febrile illness attributed to infection with trivittatus virus has been described in some patients, and CNS infections have been identified in others.[93] Three cases of symptomatic infection caused by prototype CE virus have been reported. All three patients had signs and symptoms of encephalitis.[57]

Laboratory examinations for LACV infection were well outlined in one series (Table 202–2).[87] The total CSF leukocyte count typically was elevated only moderately (the median white blood cell [WBC] count in CSF was 75), and 25 percent of patients had red blood cells in CSF.[87] In numerous reports, elevated protein levels were observed in less than one third of cases.[4,26,28,62,87] Approximately 10 percent of children have negative or equivocal findings on initial CSF analysis, only to have numerous WBCs within 24 to 48 hours on repeat lumbar puncture. The findings that the peripheral WBC count is elevated in half the cases (>15,000 WBCs/mm^3) and that neutrophilia often is present frequently raise suspicion of bacterial meningitis or partially treated bacterial meningitis.

Brain imaging techniques usually are negative, but they have a higher yield in more advanced disease. In 127 children studied, 92 had computed tomography (CT) scans. Only 11 of these scans were positive; 8 showed generalized edema and 3 showed focal findings, all in supratentorial locations. In 3 children whose courses were complicated by cerebral herniation, CT scans became positive only after the herniation event (i.e., admission CT scans were negative [Fig. 202–3]). From this same series (n = 127), 10 children had MRI scans of the brain, 4 of which were abnormal and showed focal areas of gadolinium enhancement, predominantly in cortical areas (see Fig. 202–3).[86] Case reports suggest that MRI may reveal lesions not detected by CT in

TABLE 202–2 Laboratory Values at Admission in Patients with La Crosse Encephalitis

Variable	Mean Value ± SD	Range	Percentile					Remarks
			10th	25th	50th	75th	90th	
Cerebrospinal Fluid								
White blood cell count (per mm^3)	130 ± 151	2-867	10	26	75	184	316	<200/mm^3 in most cases
Differential count (% lymphocytes)	—	2-100	10	27	62	77	90	Predominance of lymphocytes
Red blood cell count (per mm^3)	71 ± 213	0-1,500	0	1	5	20	177	Elevated (≥20/mm^3) in 25%
Glucose (mg/dL)*	75 ± 20	37-149	56	62	71	83	105	Normal
Protein (mg/dL)	37 ± 15	10-85	20	27	34	45	56	Rarely elevated
Peripheral Blood								
White blood cell count (per mm^3)	15,700 ± 5900	6,800-49,700	8,900	11,500	14,800	19,000	22,600	Usually elevated (>15,000/mm^3)
Differential count (% polymorphonuclear leukocytes)	—	17-94	58	66	76	82	86	Predominance of polymorphonuclear leukocytes

**To convert values for glucose to millimoles per liter, multiply by 0.05551.*

Data from McJunkin, J. E., de los Reyes, E. C., Irazuzta, J. E., et al.: La Crosse encephalitis in children. N. Engl. J. Med. 344:801-807, 2001.

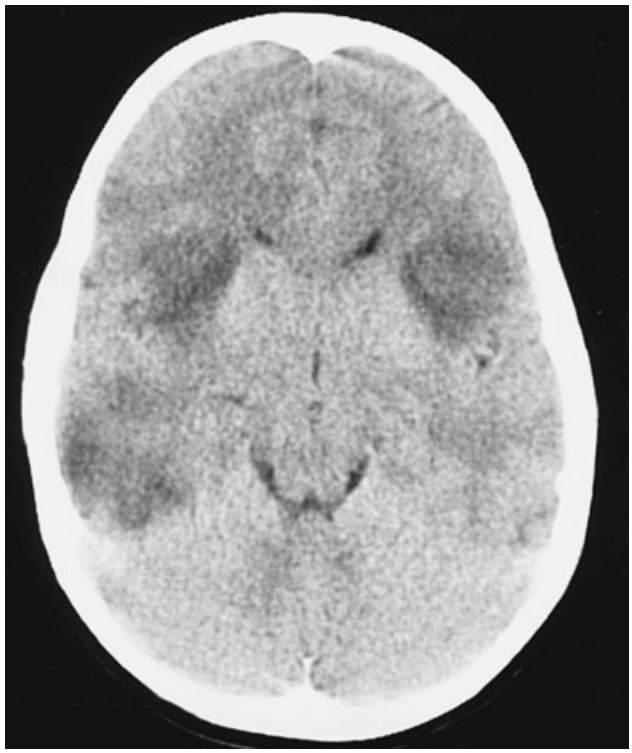

Figure 202–3 La Crosse encephalitis. A computed tomographic scan of an unusually severe case of La Crosse encephalitis shows small ventricles and multifocal hypodense lesions with loss of gray-white differentiation in the right frontotemporoparietal and left temporal regions. Computed tomographic scans are usually negative in La Crosse encephalitis (see text).

LACE. In a 20-month-old infant with right hemiparesis, CT on admission was negative, but MRI on day 1 showed evidence of acute infarction of the left basal ganglia.[83] In another 12-month-old child with clinical evidence of neurodegeneration, MRI showed areas of increased signal intensity in the periventricular white matter, even though the initial CT scan had been negative.[42] However, CT is still favored as the initial imaging study in children acutely ill with LACE because the need for anesthesia and intubation in performing MRI in children can confound examinations of mental status and supportive care.

EEG abnormalities have been found in 71 to 90 percent of patients tested. Focal EEG findings, usually slowing, may appear in as many as 44 percent of abnormal EEG tracings. Evidence indicates that periodic lateralizing epileptiform discharges (PLEDs), previously thought to be virtually pathognomonic of HSE, also are seen in LACE; 8 of 90 EEG tracings in one series were positive for PLEDs.[34,87] In 4 of these 8 cases, patients required continuous EEG surveillance to monitor treatment of nonconvulsive status epilepticus.[87] Patients with PLEDs represent a worrisome subgroup of all LACE patients because they have longer, more complicated, hospitalizations and probably worse long-term outcomes.[34a]

DIAGNOSIS

Clinicians must communicate with virology laboratory colleagues when choosing diagnostic tests for encephalitis so that the limitations of these tests are understood. To select appropriate laboratory tests, information about the clinical findings and travel history must be combined with knowledge of the local epidemiology and natural history of viral infections.

Although the preferred approach to the diagnosis of viral encephalitis is to identify the viral agent by isolation or detection of antigen or nucleic acid in CSF, limitations of these procedures for LACV require the diagnosis to be made by serologic methods at this time. For example, CSF culture is not a reliable means for establishing a diagnosis; in fact, no reports of successful isolation of LACV from CSF have been published. Isolation from brain tissue has occurred on three occasions.[73,92] Improvements of LACV RNA detection from previous methods[19,23,63,81] (including nucleic acid base amplification and reverse transcriptase PCR) have been used successfully in detection of viral RNA in vector mosquito samples and human brain tissue,[82] but the development of this method for making a clinical diagnosis using blood or CSF samples remains experimental. Therefore, serologic methods to detect LACV antibody continue to be the primary method of diagnosis. Immunoglobulin M (IgM)–specific antibody to LACV can be detected reliably in serum and CSF by two methods: (1) IgM capture enzyme immunoassay (EIA) or (2) indirect immunofluorescence (IF). With either method, detection of LACV IgM in CSF or a fourfold rise in LACV antibody titer on convalescent serum yields a *confirmatory diagnosis* of LACE, whereas an acute rise in LACV IgM in a single serum sample yields a *presumptive diagnosis*. IgM capture EIA is a sensitive serologic test that detects virus-specific IgM in 83 to 100 percent of cases.[9,10,13,17,35,37] An IgG capture EIA method has been developed to be used in tandem with IgM capture EIA.[69] The main advantage of IgM capture is that it is less operator-dependent than is the IF method, which requires careful observance of visual reading criteria by experienced personnel. For this reason, IgM capture EIA is the method used and is considered the gold standard by the CDC in their definition of LACE diagnosis. Conversely, possible limitations of the IgM capture method include longer turnaround time and the problem that standardized procedures and reagents are not available for commercial use, as opposed to the IF method.

In all diagnostic tests for LACE, false-positive results (compromised specificity) remain the key clinical issue. Unfortunately, neither currently available serologic method can guarantee 100 percent specificity, especially because in rare instances LACV IgM reportedly has remained elevated in serum for more than 1 year. A false-positive test could result in serious consequences if, for example, antiviral therapy for herpes simplex virus infection were discontinued erroneously or if antibiotic therapy for partially treated bacterial meningitis were discontinued. Therefore, clinicians should be in direct communication with laboratory colleagues concerning specificity data for their laboratory, particularly in cases in which treatable alternative diagnoses, such as HSE or partially treated meningitis, remain in the differential diagnosis on clinical grounds.

From a practical standpoint, the use of rapid diagnostic techniques for LACV (e.g., detection of IgM by indirect IF) may benefit patients by reducing unnecessary antibiotic or antiviral therapy, *provided the positive IgM test for LACV also is combined with reliably negative tests for treatable bacterial and viral agents (especially a negative PCR test for herpes simplex virus in patients with severe or focal encephalitis)*. No currently available Food and Drug Administration (FDA)–approved antiviral treatment for LACV exists.

DIFFERENTIAL DIAGNOSIS

The principal consideration in the differential diagnosis is HSE.[111,112] Clinical descriptions consistently disclose a high rate of focal neurologic findings or focal seizures, or both, in LACE (≈30%). The frontal and temporal lobe locations of abnormalities detected by EEG studies (including PLEDs in some cases) and

by brain imaging studies in some cases of LACE also may point to a presumptive diagnosis of HSE.[4,87] Hemorrhagic pleocytosis, which can be seen in both HSE and LACE, also may favor presumptive diagnosis and therapy of HSE, especially in the clinical setting described earlier. Because late institution of therapy in HSE is associated with a poor outcome, a reasonable approach is to make an early diagnosis of presumptive HSE in cases of encephalitis with focal findings or in severe encephalitis pending definitive diagnosis.[128]

In patients with less fulminant signs of CNS infection, enteroviral aseptic meningitis is a common consideration, especially because it often occurs in summer and early fall. The presence of rash, pharyngitis, myocarditis, or conjunctivitis is a clue to enterovirus and is not characteristic of LACV infection. In a study comparing enteroviral CNS infection with LACE, patients with LACE were significantly more likely to manifest aphasia, loss of consciousness, seizure, and admission to the pediatric intensive care unit than were patients with enteroviral CNS infection.[59] In areas where immunizations may be eschewed for religious reasons, poliomyelitis remains an important diagnostic consideration. Mumps encephalitis is another consideration, even in the absence of parotid swelling, and low CSF glucose levels may be a clue to this diagnosis.[94]

Patients with Rocky Mountain spotted fever (RMSF) may exhibit signs of CNS disturbance, and one should remember that rash may be absent in 20 percent of patients with RMSF. Catscratch or mycoplasma pneumonial encephalopathy may be valid considerations in the differential diagnosis. Certainly, other *Arboviridae* should be considered in the differential diagnosis, and many laboratories now test simultaneously for *Arboviridae* known to occur in the United States (St. Louis encephalitis virus, Eastern equine encephalitis, Western equine encephalitis virus, and WNV) in patients with severe seasonal (summer or fall) encephalitis. A rare and tragic consideration in a child presenting with encephalitis is rabies, which may occur in the absence of a known animal bite. Certainly, a wide array of viruses, bacteria, and even amebae and parasites can cause CNS infection, and they are reviewed in other chapters of this text.

TREATMENT

Because no FDA-approved specific antiviral therapy for LACE is available currently, careful supportive care is the mainstay of therapy. A reasonable general supportive strategy is to assess these patients as one would assess a child with closed head injury because, in both instances, (1) changes in the level of consciousness cannot be assumed to be caused by a normal need to sleep and therefore need to be monitored serially and (2) changes in the level of consciousness generally are the best and earliest indicator of evolving intracranial pathology, as opposed to brain stem signs (e.g., pupillary abnormalities, bradycardia with hypertension). Therefore, serial monitoring of the level of consciousness is the most important aspect of neurologic monitoring (using the Glasgow Coma Scale [GCS]), even if the child has minimal change in level of consciousness at the time of admission.

Three particularly important decision points in management depend on the presence or absence of (1) disorientation, (2) further deterioration in GCS scores, and (3) focal findings. Once the child becomes disoriented (GCS score typically <13), as shown by changes in mental status in an older child (or perhaps by lack of recognition of parents in an infant), the child is monitored best in an intensive care setting. Children presenting with seizures at admission also deserve consideration for admission to intensive care, as do those with unremitting vomiting and/or HA despite admission to the general pediatric floor.[87] Further deterioration in the GCS to a score of 8 or less generally indicates that the patient no longer is able to protect the airway adequately, and appropriate intervention is needed. Cases suggesting HSE because of focal findings or coma, or both, warrant presumptive treatment with acyclovir pending definitive diagnosis (see "Diagnosis").

Many of the same strategies that apply in treating children at risk for increased ICP are used in treating those with LACE. A reasonable approach is to perform funduscopy and consider CT of the brain before performing lumbar puncture and to note the opening pressure of the latter. General treatment strategies include the following: airway and (if necessary) ventilatory management designed to avoid hypercapnia; hemodynamic management to optimize mean arterial pressure (and presumably cerebral perfusion pressure); neurologic strategies to optimize seizure control and measures to avoid an excessive cerebral metabolic rate; and strategies designed to avoid hypo-osmolality, hyperthermia, and other factors that may exacerbate intracranial hypertension. Intubated patients should be sedated adequately and may need additional analgesia or sedation before noxious procedures such as endotracheal suctioning are performed.

In a large series of hospitalized patients with LACE, 1 in 10 patients with LACE deteriorated neurologically after admission. Risk factors identified to occur more often before in-hospital deterioration were the occurrence of GCS <13 disorientation (mainly seen as seizures and vomiting). In addition, the data suggested that hyponatremia and hyperthermia may be related temporally to clinical deterioration in patients with LACE.[87] Regarding therapeutic implications of the risk of developing hyponatremia, the recommendation is that isotonic fluids (normal saline or 5% dextrose in normal saline) be used at maintenance rates (in addition to deficit replacement) to minimize the tendency for the development of hyponatremia while maintaining intravascular volume.[87] Serum sodium should be monitored approximately every 8 hours during the acute phase of the illness. If evidence of SIADH is documented (and cerebral salt wasting is ruled out) by careful monitoring of fluid status and urine and serum sodium and osmolality, careful restriction of fluids may then be considered.

Because of data suggesting that hyperthermia may be associated with neurologic deterioration in some patients, having a low threshold for treatment of fever with antipyretics is recommended. In critically ill patients with evidence of increased ICP, cooling blankets may be considered in the presence of persistent hyperthermia, provided shivering and discomfort are controlled.

Patients suffering neurologic deterioration may need repeat EEG or brain imaging studies (or both) because nonconvulsive status epilepticus can occur and because the initial brain imaging results may be normal but later imaging may show evidence of cerebral edema. Some experience has been gained in the monitoring of ICP in LACE.[73] High ICP was documented in three of six patients in whom monitors were placed, and such monitoring was considered by clinicians to be a useful adjunct in those cases.[87] Establishing clinical and imaging criteria for which patients might benefit from insertion of an ICP monitoring device remains a dilemma, particularly because ictal/postictal events in LACE can confound the neurologic examination regarding possible signs of increased ICP.

Currently, no specific antiviral therapy is approved by the FDA for the treatment of LACE. Previous laboratory studies suggested ribavirin's potential as a therapeutic agent.[21,23,27,64,65,90,102,110,114] Intravenous ribavirin initially was administered on a compassionate-use basis in a human case of severe LACE in 1994,[88] and subsequently it was used similarly in six severe cases without serious adverse effects (McJunkin and associates, unpublished data). Despite this preliminary work, which was encouraging from a safety standpoint at modest doses, higher doses may be necessary for achievement of optimal CSF levels. Unfortunately, adult studies done at these higher doses and our anecdotal infor-

mation in two cases of pediatric LACE treated at this higher dosage range suggest a less favorable safety profile. In addition, the relatively low prevalence and mortality of LACE (as compared with WNV, for example) render studying ribavirin therapy to demonstrate efficacy a difficult task indeed.

OUTCOME

LACV infections are associated with a case-fatality rate of less than 1 percent. However, a series of hospitalized patients (n = 127) showed near-lethal disease from cerebral herniation in three cases and a sizable proportion of patients who required mechanical ventilation (25%) or intensive care (50%).[87]

Psychometric evaluation of recovered patients had failed to show significant differences in standard tests of cognitive ability when compared with controls or normative data in the general population.[86,106] However, a 2001 study of 28 recovered patients, most of whom had severe LACE (e.g., associated with seizures or coma, including 3 patients with cerebral herniation), showed a mean full-scale intelligence quotient (IQ) of 87.8 (95% confidence interval, 82.2 to 93.2); 35 percent of these patients had an IQ less than 80, and 46 percent demonstrated significant disparities between verbal and performance IQ scores. In addition, 60 percent of this group had positive tests for ADHD.[87] This study lacked a contemporaneous, matched control group, but certainly compared with normative data, these findings strongly suggest that neurocognitive and behavioral deficits do occur after LACE, particularly in severe cases of the disease. In addition, previous data indicating changes in performance on tests administered before and after illness suggested effects in children who were more seriously ill.[86] In another small series, abnormalities in visual-motor function and intellectual impairment were observed more often in patients who had focal abnormalities in the acute illness.[106]

Six to 15 percent of patients who have recovered from LACE have recurrent seizures.[26,31] The risk of having a recurrent convulsive disorder was approximately 25 percent in patients who experienced a seizure during the acute phase of illness.[31,49] The interval between the onset of recurrent seizures and recovery from acute infection ranged from a few days to years (mean, 4 years).[31] Persistent hemiparesis was a residual abnormality in 2 of 151 patients monitored for up to 6 years after recovery, but one patient had a brain biopsy performed on the opposite hemisphere.[26] Unilateral infarction of the basal ganglia and hemiparesis were described in the case of an infant.[83] Another infant with a presumed LACV infection initially had an apparent neurodegenerative disease secondary to acute disseminated encephalomyelitis.[42] See the description at the beginning of this chapter of the economic costs of LACE.

PATHOLOGY

Pathologic descriptions of two fatal cases were reported.[73] In gross appearance, the brain was swollen, and the meninges were congested. The principal brain lesions were neuronal degeneration, patchy inflammatory lesions, and vasculitis. The cerebrum and basal ganglia were the principal sites of involvement, but petechial hemorrhages and edema were noted in the spinal cord of one patient. In both cases, lesions in the cerebrum were confined to the frontal, parietal, and temporal lobes, and the cerebellum was not involved.

The focal inflammatory lesions and perivascular reactions are composed primarily of mononuclear cells. Neuronolysis and neuronophagia are observed in foci of inflammation and necrosis, along with reactive polymorphonuclear, mononuclear, and microglial responses. Small extravasations of erythrocytes may appear as well. Lymphocytic perivascular cuffs are seen, but inclusion bodies are not. In one case, a focal area of necrosis, hemorrhage, and formation of a hematoma was present in the temporal lobe, a finding corresponding to a mass lesion seen on CT.[18]

In one case, brain biopsy material examined by indirect IF demonstrated LACV antigen in neurons and perhaps in endothelial cells. The same biopsy material from this patient showed minimal necrosis and perivascular cuffing on light microscopy.[90] The finding of minimal necrosis despite abnormal CT findings and deep coma is interesting in view of the relatively benign outcome of LACE, even in patients who experience deep coma or exhibit CT abnormalities. This finding is in marked contradistinction to HSE, in which the presence of either deep coma or CT lesions is a poor prognostic factor consistent with the extensive necrosis seen pathologically.[128] Findings in a mouse model indicated that neuronal cell death in LACE may be mediated largely by apoptosis (as opposed to necrosis).[101]

PREVENTION

Large-scale spraying of insecticides is not an effective intervention for elimination of *O. triseriatus* because this tree-dwelling mosquito tends to be protected by the leaf canopy of its habitat. Public health prevention has focused on elimination of breeding sites of *O. triseriatus*. Such efforts in endemic areas have included removal of used tires and other containers that hold small pools of water and (less commonly) sealing tree holes with cement or gypsum wool insulation.[119] The sheer number of such potential breeding sites necessitates intervention on a community-wide basis.[91] Public health education on mosquito prevention is available through the "Fight the Bite Mosquito Prevention Program"[3] and through a CDC educational program called "Neato Mosquito" (the latter available at www.CDC.gov, as are fact sheets). Home visits to patients with new cases of LACE may identify risk factors around the home or in neighboring areas, with implications for uninfected siblings of the index case and possibly for children in the community. Counties identified with cases may be targeted during the subsequent spring for town meetings, television coverage, or tire removal campaigns. Such targeted interventions have been tracked in highly endemic areas of West Virginia in recent years. The proper use of insect repellents, playing in open fields, and avoidance of tree-shaded areas may confer a reduction in risk by minimizing exposure to the vector. Insect repellents acceptable for children may contain up to 30 percent DEET, but DEET-containing products should not be used on infants younger than 2 months of age. In 2005, the American Academy of Pediatrics and the CDC revised recommendations such that repellants containing picaridin are considered an acceptable alternative to DEET.[1] Less effective but perhaps acceptable alternatives include oil of lemon eucalyptus and 2 percent soybean oil. None of the alternatives to DEET have been proven to also repel ticks, a possible advantage of DEET-containing products, which do have efficacy against ticks. All products must be used as directed by the manufacturer.

The potential for development of a vaccine comes from work with a truncated G1 protein preparation that induces a protective immune response in suckling mice through neutralizing antibody.[100] Protection against neuroinvasion was achieved by interruption of the transient viremia, which occurs just after inoculation of the virus. More recently, a DNA-based vaccine that encodes for viral glycoproteins (G1 and G2) also induced a protective neutralizing antibody response in mice.[99,108] Should ecologic preventive measures fail to lower disease rates in endemic areas, development of a vaccine for use in persons living in these areas may prove worthwhile.

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CHAPTER 203

Other Bunyaviridae

CHAPTER 203a

Rift Valley Fever

C. J. Peters

Rift Valley fever (RVF) is primarily a disease of sheep and cattle. It is transmitted by mosquitoes and is caused by a virus with selective affinity for the parenchymal cells of the liver, which undergo characteristic eosinophilic degeneration. Infection with RVF virus causes a short but severe disease in sheep and cattle. Most pregnant ewes and cows abort, and more than 90 percent of newborn lambs die. Case-fatality rates in older sheep and cattle are lower but nonetheless significant. People usually acquire the infection from aerosols generated from the body fluids and tissues of animals dying of the disease and, less commonly, from bites of infected mosquitoes, especially during epidemics. See Swanepoel and Coetzer[10] for a comprehensive review.

HISTORY

RVF probably has occurred for many years in Africa and was recognized first at the beginning of the 20th century with the introduction of intensive livestock husbandry. In 1912, large numbers of newborn lambs died of an unknown disease in the Rift Valley of Kenya, and in the following year the clinical features were described. Daubney and associates[3] proved that the causal agent was a filterable virus, which they suspected was transmitted by mosquitoes because animals protected by screens did not contract the disease. In 1944, the virus was isolated from mosquitoes caught in the Semliki Forest in western Uganda, and it was proved later that *Erethmapodites chrysogaster* was able to transmit the infection under experimental conditions. Between 1950 and 1974, at least 15 major epizootics of RVF occurred in livestock in various areas of sub-Saharan Africa. During 1975, an extensive epizootic of RVF took place in South Africa, with many human cases and several deaths documented.[4]

An extensive epizootic of RVF also occurred in Lower Egypt in 1977. Hundreds of thousands of domestic animals, including cattle, buffaloes, goats, and sheep, were lost. Associated with this epizootic was the largest human epidemic of the disease ever known, in which 200,000 humans were infected and 600 died.[5] This outbreak emphasized the increasing threat of RVF to humans and domestic animals and showed that RVF virus was an important cause of hemorrhagic fever in Africa. In 1987, an epidemic of RVF occurred in Mauritania after flooding of the Senegal River basin following completion of the Diama Dam; at least 1200 human cases and 200 deaths occurred in one affected area alone. Epidemics and epizootics were reported in Madagascar in 1990,[7] in Egypt in 1993,[2] in eastern Africa in 1997 to 1998,[1] and in Yemen and Saudi Arabia in 2000.[8] The cases in the Arabian peninsula are the first outside Africa and demonstrate the potential of RVF to spread. Based on isolation of the virus, RVF probably occurs sporadically throughout most of sub-Saharan Africa. Zinga virus, previously described as a cause of sporadic human disease in central Africa, has been shown to be a strain of RVF virus.

ETIOLOGIC AGENT

RVF virus is destroyed by solvents such as ether and is inactivated readily by formalin; it is destroyed by heating at 56° C for 40 minutes. When stored at 4° C or –10° C, the virus loses its infectivity in about 3 months. The virus can be preserved indefinitely on dry ice at –70° C or in lyophilized form.

Fully formed virions are spherical and approximately 94 nm in diameter. They mature in the cytoplasm, although intranuclear inclusions occur in vivo and in cell culture. The virus multiplies readily in a variety of cell lines of animal and human origin. RVF virus has been assigned to the *Phlebovirus* genus of the family *Bunyaviridae*. The virus is highly pathogenic for mice, young rats, and hamsters, and death occurs in 95 to 100 percent of these animals 36 to 96 hours after inoculation.

VECTORS

In studies in South Africa, the virus has been transmitted experimentally by *Culex theileri*, *Culex zombaensis*, *Culex neavei*, *Aedes juppi*, and *Erethmapodites quinquevittatus*. Epizootics of RVF have occurred in years of unusually heavy rains that filled natural depressions in the land (pans or dambos), thereby favoring the proliferation of flood-water mosquitoes. Studies in Kenya have shown that transovarial transmission of the virus in pan-breeding *Aedes* of the subgenus *Neomelanoconion* is the probable mechanism of virus maintenance and periodic recrudescence. *Culex pipiens* was implicated as a vector in Egypt during the 1977 to 1978 epidemic. During epizootics, domestic livestock serve as viremic, amplifying hosts in the transmission cycle.

CLINICAL MANIFESTATIONS

Humans are very susceptible to RVF virus. During the epizootics in South Africa, most veterinarians and many farmers engaged in work with sick sheep and cattle became infected. In most cases, infection was linked to direct contact with the carcasses, tissues, and organs of animals that died of RVF. Transmission probably occurred by the aerosolized body fluids of the animals. Some patients gave no history of such contact; in these cases, the infection is presumed to have been transmitted by mosquitoes or possibly acquired by drinking infected milk. The virus can be transmitted readily to laboratory personnel by direct contact with infected animals or by the respiratory route from aerosol droplet infection.

The incubation period of RVF is 3 to 7 days; its onset is sudden, with chills, myalgia, joint pain, headache, and a biphasic fever that lasts approximately 1 week. Patients often feel nauseated and may vomit or complain of abdominal fullness and pain. The face is flushed, the conjunctivae are injected, and the tongue is furred. Bradycardia is present, and slight tenderness over the liver, which may be enlarged, can be present. Many patients become delirious, and some have hallucinations. In a small proportion (<1%) of patients, the infection is complicated by retinitis. Late in the course of the illness or early in convalescence, unclear vision may be noted, and the patient may have a central blind spot. This visual defect is associated with a cotton-wool exudate on the macula. Both eyes are involved occasionally, and the loss of vision is a severe handicap. These lesions gradually resolve, and the patient's vision returns to normal in most cases. Meningoencephalitis manifested as intense headache, confusion, and stupor may occur as a complication in fewer than 1 percent of patients during or after the second wave of fever. Lumbar puncture relieves the headache. Cerebrospinal fluid (CSF) shows slight pleocytosis, mostly of lymphocytes, a normal glucose level, and a slightly increased protein content. Antibody to RVF virus may be demonstrated in the fluid. Few patients with encephalitis die. Recovery usually is complete but may be prolonged. Occasionally, the patient is left with permanent sequelae.

Hemorrhagic fever, a complication with a case-fatality rate of 15 percent, develops in approximately 1 percent of patients with RVF. The mortality rate was disproportionately lower in children than in adults during the 1977 Egyptian epidemic. In cases of severe illness, a hemorrhagic diathesis may develop that includes epistaxis, hematemesis, melena, and, sometimes, cerebral hemorrhage. Profuse gastrointestinal hemorrhage may be fatal. Jaundice may be evident.

LABORATORY FINDINGS

The patient has initial leukocytosis that is followed by leukopenia. Profound thrombocytopenia and other defects in coagulation may be observed. Disturbance in liver and kidney function also may be documented. The diagnosis of RVF is suggested when human beings suffer from an acute, severe, but short febrile illness at the same time that an epizootic with high mortality is occurring in sheep.

The diagnosis usually can be confirmed by isolation of the virus in mice or cell culture from blood and, in fatal cases, from the liver. The development of antibodies can be demonstrated by immunofluorescence, immunoglobulin M (IgM) enzyme-linked immunosorbent assay, hemagglutination inhibition, complement fixation, and neutralization. In patients with encephalitis, IgM antibodies are detectable in CSF.

TREATMENT

Treatment is symptomatic. When a hemorrhagic diathesis develops, treatment should be directed toward controlling bleeding. Transfusions of fresh-frozen plasma and platelets may be beneficial. Disseminated intravascular coagulation has been documented in a monkey model, but its role in human disease is uncertain.

PREVENTION

RVF is mainly a disease of adults and usually is acquired occupationally. It is a serious hazard faced by veterinarians, ranchers, and laboratory personnel in the course of their work. Because RVF usually is acquired by direct contact with the tissues of infected sheep and cattle, the risk of acquiring infection can be reduced by wearing gloves, protective masks, and goggles when postmortem examinations are carried out on animals that have died of unknown causes. Because of the value of domestic animals in many economically depressed areas of Africa, sheep and cattle often are housed within family compounds. Sick or dying animals usually are killed to salvage their meat. In this peridomestic environment, children also can be exposed to virus aerosols and readily become infected. Infection from a mosquito bite is possible as well, and for this reason, the use of repellents and bed nets is recommended.

The primary strategy for preventing RVF in both humans and animals relies on vaccination of sheep and cattle, which are the amplifying hosts. Attenuated strains of the virus have been developed and used successfully on a mass scale to immunize livestock. These attenuated vaccines are associated with some abortions in pregnant ewes and cows. An inactivated vaccine is safe and is in widespread use in Africa.

A live attenuated vaccine designated MP-12 was developed by passage of RVF virus in the presence of the mutagen 5-fluorouracil.[6] The vaccine has proved safe for use in domestic

livestock, does not produce abortions, and offers considerable promise as a veterinary and human vaccine. Formalin-inactivated vaccines produced in cell culture[9] have been used on an investigational basis in more than 3000 persons with a seroconversion rate greater than 95 percent. A single case of Guillain-Barré syndrome was reported, but its relation to vaccination was uncertain. Human vaccination is recommended for high-risk occupational groups. Various genetically engineered vaccine candidates are under development.

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CHAPTER **203b**

Crimean-Congo Hemorrhagic Fever

Robert B. Tesh

Crimean-Congo hemorrhagic fever (CCHF) is a serious and potentially fatal viral illness, often having hemorrhagic manifestations. The clinical entity and its viral origin were described first in Russia by Chumakov in 1945, but the agent was not propagated or available for study until 1967.[8] Since then, CCHF virus has been reported from many countries in eastern Europe, central Asia, and Africa.[2,6,8] Exposure to ticks or contact with the blood of infected livestock or people are the usual sources of human infection.

ETIOLOGIC AGENT

CCHF virus, the etiologic agent, is a member of the genus *Nairovirus*, family *Bunyaviridae*. It is lethal to newborn mice after intracerebral inoculation. CCHF virus replicates in some vertebrate cell lines, but isolation in mice is more sensitive because the virus usually does not cause any discernible cytopathic effect under fluid overlay. Thus, indirect methods such as immunofluorescence or reverse transcriptase polymerase chain reaction (RT-PCR) must be used to detect CCHF viral antigen or nucleic acid in the cells.

A relatively high level of viremia develops in persons with CCHF and persists for 6 to 8 days after onset of the illness.[6] Blood from acutely ill CCHF patients is quite infectious, and numerous nosocomial infections have occurred in hospital personnel caring for patients with this disease.[1,2,5] CCHF virus is relatively stable in blood and serum and has been recovered from specimens stored as long as 2 or 3 weeks at 4° C.

EPIDEMIOLOGY

CCHF virus is known to occur over a wide geographic area, including eastern Europe, central Asia, and much of Africa (Fig. 203–1).[2] Most cases of the disease probably are unrecognized because of its sporadic occurrence and largely rural distribution.

The epidemiology of CCHF is complex and not fully understood because of the variety of tick and mammalian species that have been found to be naturally infected with the virus.[8] The basic transmission cycle of CCHF virus varies from region to region, depending on the developmental stage and species of ticks involved and their preferred mammalian hosts.[8] In addition, transovarial and trans-stadial transmissions of CCHF virus also have been demonstrated in some tick vectors. In general, human cases of CCHF usually are associated with periods of abundant prevalence of ticks and feeding activity.[2] For example, in eastern Europe, cases of CCHF typically occur between May and August, when the tick population is active. Humans usually acquire CCHF from a tick bite or from contact with blood or other tissues of infected livestock.[2,6,8] Most patients have a history of contact with large domestic animals (farmers, abattoir workers, and veterinarians). Health care workers are another high-risk group.

Numerous CCHF nosocomial outbreaks have occurred in hospital workers and laboratory technicians.[1,2,5] These persons usually have had direct contact with the blood of patients with CCHF. The mortality rate reported in secondary cases has been high, particularly in persons with needle-stick injury or those involved in management of patients with gastrointestinal bleeding.[2]

CLINICAL MANIFESTATIONS

The course of CCHF infection typically has four distinct phases: incubation, pre-hemorrhagic, hemorrhagic, and convalescent (Fig. 203–2).[2,7] The incubation period generally is 3 to 7 days. The pre-hemorrhagic phase begins suddenly with fever (39° C to 41° C), nausea, diarrhea, vomiting, myalgia, and general toxemia. Patients usually are flushed, with conjunctival and scleral injection. Hepatosplenomegaly occurs in as many as 30 percent of cases. This second phase lasts approximately 3 days (range, 1 to 7 days). The hemorrhagic phase usually begins between the third and fifth day of the disease and lasts only 2 to 3 days. The appearance is sudden and can vary from petechiae to large hematomas on the skin and mucous membranes. Profuse bleeding from the nose, gums, gastrointestinal and genitourinary tracts, and lungs can occur at this stage, resulting in tachycardia, shock, and, sometimes, death. Not infrequently, intra-abdominal bleeding occurs during this third phase and causes acute pain; some of these patients are subjected to surgery because of suspected appendicitis or intestinal perforation. If the patient survives the hemorrhagic phase, convalescence begins about the 10th day, with a slow convalescent period lasting 2 to 6 weeks.[2] Viremia is intense and prolonged in CCHF, especially in fatal cases, so blood from these patients should be treated with extreme care.

Leukopenia and thrombocytopenia are consistent features of CCHF and occur early in the disease.[2,3,7] Other abnormal laboratory findings usually include elevated levels of serum aspartate

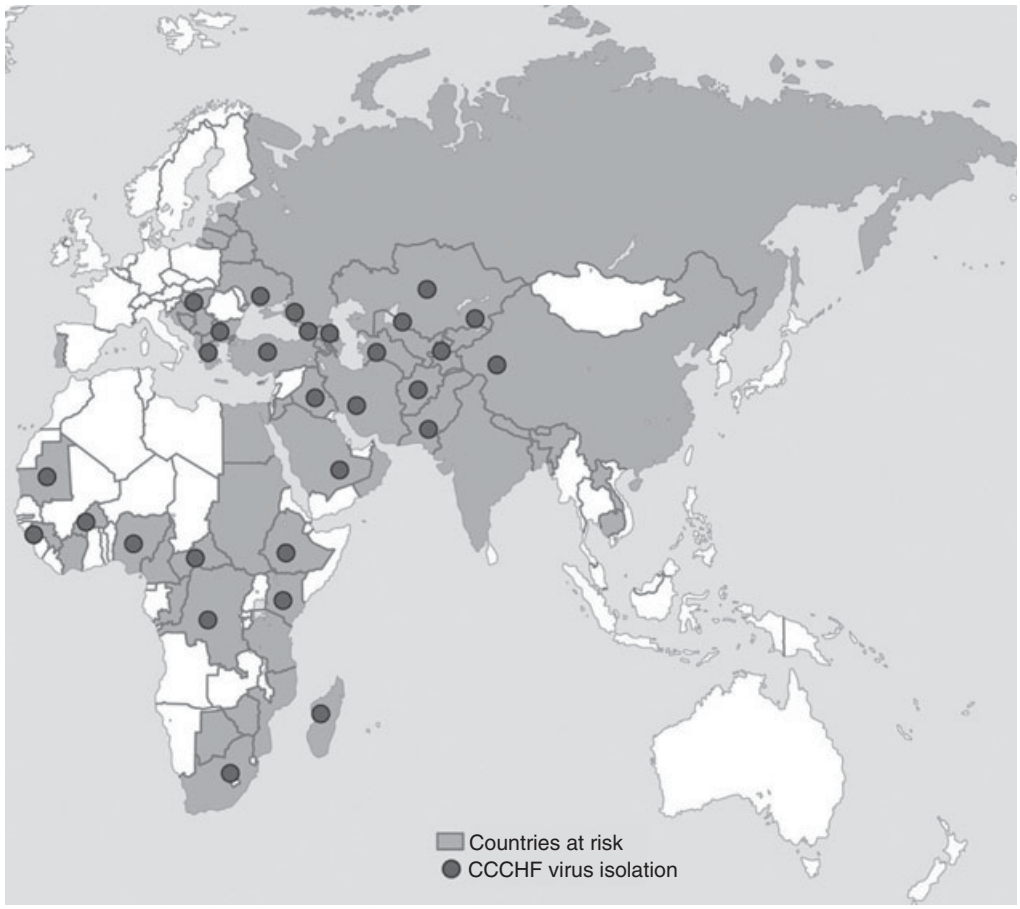

Figure 203–1 Known distribution of Crimean-Congo hemorrhagic fever (CCHF) virus in the world. (See companion Expert Consult web site for color version.) *(From Ergonul, O.: Crimean-Congo hemorrhagic fever. Lancet Infect. Dis. 6:203-214, 2006.)*

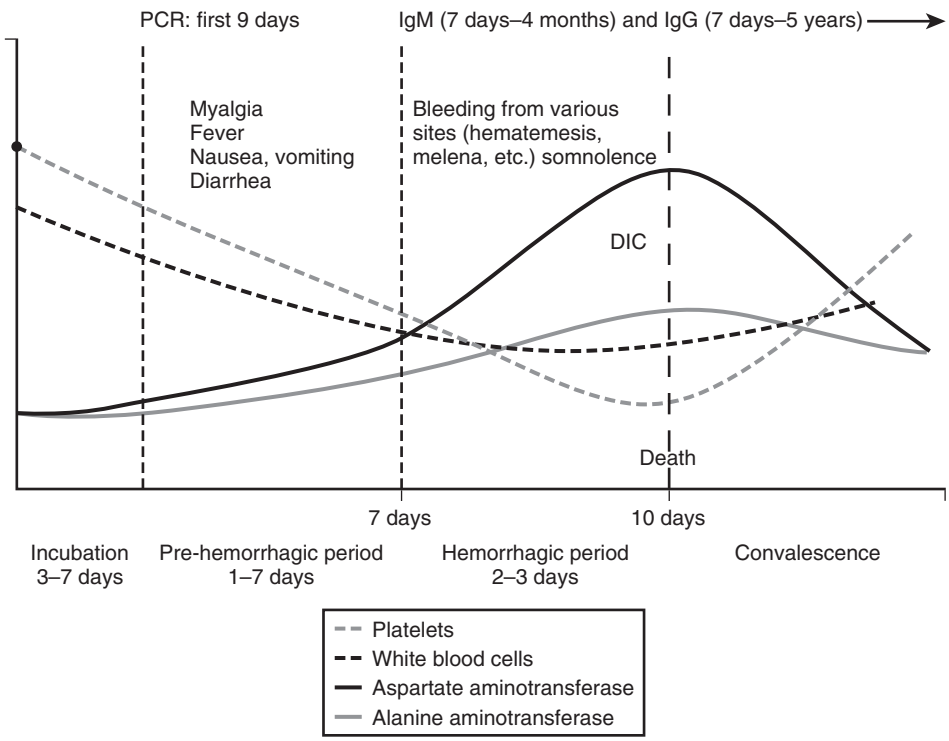

Figure 203–2 Clinical and laboratory course of Crimean-Congo hemorrhagic fever. DIC, disseminated intravascular coagulation; IgM, immunoglobulin M; PCR, polymerase chain reaction. *(From Ergonul, O.: Crimean-Congo hemorrhagic fever. Lancet Infect. Dis. 6:203-214, 2006.)*

and alanine aminotransferase, lactate dehydrogenase, bilirubin, creatinine, and urea. Coagulation tests such as prothrombin time and activated prothrombin time are prolonged. Fibrinogen may be decreased, and fibrin degradation products may be increased. These laboratory tests generally return to normal levels with the onset of convalescence.

PATHOGENESIS

The pathogenesis of CCHF is not well understood, but the available evidence suggests that endothelial damage and dysfunction play an important role in the hemostatic failure.[2,5,7] Fluorescent antibody studies on organs in fatal cases of CCHF show a concentration of viral antigen in the reticuloendothelial cells of the liver and spleen, a finding suggesting that these cells are major sites of virus replication.[7] Other pathologic changes observed at autopsy include generalized vascular lesions with endothelial damage that give rise to scattered focal hemorrhage and edema in most organs.[2] In severe cases, dysfunction of the coagulation system and disseminated intravascular coagulation appear to be prominent features of the disease.

DIAGNOSIS

A clinical diagnosis of CCHF is difficult to establish before the onset of hemorrhagic manifestations because the initial symptoms are nonspecific, and cases usually occur sporadically. As a result of the frequency and severity of the abdominal pain associated with the pre-hemorrhagic and hemorrhagic phases of CCHF, the disease sometimes is misdiagnosed as appendicitis or gastric ulcer. Such patients may be subjected to unnecessary surgery, which can be fatal to both the patient and the attending medical personnel.[1,2,6] Once hemorrhagic manifestations appear, the differential diagnosis should include erythema multiforme (Stevens-Johnson syndrome), leptospirosis, hemorrhagic fever with renal syndrome, Ebola virus infection, Lassa fever, Rift Valley fever, Q fever, and yellow fever, depending on the region of the world where the patient was exposed.

A definitive diagnosis of CCHF can be made by isolating the virus from the patient's serum or detecting viral nucleic acids by PCR, during the first week of illness. Immunoglobulin G (IgG) and IgM antibodies do not appear until 7 to 10 days after the onset of symptoms in nonfatal cases.[6,7] Establishing the diagnosis early is critical for proper management of the patient and prevention of potential nosocomial infections.

TREATMENT

Treatment of CCHF is largely supportive and should include immediate hospitalization and strict bed rest.[2,6] Because death usually results from acute blood loss and shock, patients may require transfusions of fresh blood, platelets, or plasma (or all three). Administration of immune plasma obtained from convalescent donors can be used early in the course of the illness or for persons, such as hospital personnel, who are exposed to the virus inadvertently. Reports have suggested that ribavirin may be beneficial for treatment of CCHF.[2-4] The mechanism of action is unknown. A World Health Organization report recommended ribavirin treatment for 10 days, with 30 mg/kg as an initial loading dose, then 15 mg/kg every 6 hours for 4 days, and then 7.5 mg/kg every 8 hours for 6 days.[2] Milder cases of CCHF probably do not require treatment with ribavirin.

PROGNOSIS

The mortality rates reported for CCHF have varied from approximately 15 to 70 percent,[2,6,8] although these figures are probably high, because milder cases of the disease may not be recognized or reported. If the patient survives the hemorrhagic phase of the disease, recovery is complete, and permanent immunity results.

PREVENTION

No vaccine of proven safety or efficacy for prevention of CCHF exists. Personal protection measures are recommended for people living or working in heavily tick-infested areas. People exposed occupationally to potentially infected animal blood should wear protective clothing and gloves to prevent skin contact with infected tissue or blood. Hospital personnel caring for patients with CCHF should follow barrier nursing and isolation protocols, which include use of gloves, gowns, face shields, and goggles.

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CHAPTER 203c

PHLEBOTOMUS FEVER (SANDFLY FEVER)

Robert B. Tesh

Phlebotomus fever is an acute, self-limited febrile illness of 2 to 4 days' duration that usually is acquired from the bite of infected phlebotomine sandflies. Phlebotomus fever also is known as *sandfly fever*, *pappataci fever*, and *3-day fever*. The disease is endemic in many areas of central Asia, northern Africa, and southern Europe. Historically, phlebotomus fever has been largely of military interest because the introduction of large numbers of susceptible troops into endemic areas often has resulted in epidemics of the disease.[8] More recently, the disease has been reported with increasing frequency in civilian populations and in tourists visiting endemic areas.[3,4,7]

In 1909, Doerr and associates first demonstrated that the causative agent of the illness was a virus transmitted by *Phlebotomus papatasi*. In 1954, Sabin successfully adapted the agent to

mice and described two distinct serologic types, designated the Naples and Sicilian strains.[8] Subsequently, 44 additional serotypes were isolated from various regions of the world; all these agents are included in the genus *Phlebovirus*, family *Bunyaviridae*. Although most of the phleboviruses probably are capable of producing human illness, the discussion here is limited to the three (Naples, Sicilian, and Toscana) that are associated most commonly with human illness. Rift Valley fever virus also is a member of this genus and frequently causes a phlebotomus fever–like illness in infected persons.[6] However, humans generally acquire Rift Valley fever from the bite of infected mosquitoes or from aerosol, and the sequelae of Rift Valley fever are more severe and occasionally fatal.

ETIOLOGIC AGENT

The phleboviruses are RNA-containing viruses, spherical, and 90 to 100 nm in diameter.[3,8] The Naples, Sicilian, and Toscana viruses produce a cytopathic effect, as well as plaques in Vero cells. Most laboratory animals are not susceptible to infection with these viruses, although the viruses usually can be adapted to newborn mice by serial passage intracerebrally. For this reason, tissue culture is recommended for primary isolation of these agents.

EPIDEMIOLOGY

The geographic distribution of the Naples and Sicilian virus types in the Old World closely parallels that of their presumed vector, *P. papatasi* (Fig. 203–3).[8] Toscana virus has been isolated in Portugal, Spain, France, Italy, and Cyprus and has been associated with two other peridomestic sandfly species, *Phlebotomus perniciosus* and *Phlebotomus perfiliewi*.[3,8]

These phlebotomine species are tiny, sand-colored, biting flies about 2 to 3 mm long. Because of their small size, they have little difficulty squeezing through ordinary screens and mosquito netting. They usually are nocturnal, and only the female bites. Sandflies move in short hops and usually do not travel more than a few hundred meters from their resting and breeding sites. During the day, peridomestic species such as *P. papatasi* and *P. perniciosus* rest in dark corners and crevices, often within houses. The larvae develop in loose soil and organic debris in stone walls, animal sheds, privies, open wells, and gardens. Because of their indoor habits, these peridomestic species are quite vulnerable to residual insecticides.

In central Asia and the Mediterranean region, sandflies are active during the late spring and summer. The incidence of phlebotomus fever follows the same seasonal pattern.

Many phlebotomus fever viruses appear to be maintained in the sandfly population by transovarial (vertical) transmission.[8] Thus, sandflies appear to serve as both vectors and reservoirs of these viruses. Unlike most other arboviruses, whose activity depends on the presence of susceptible and viremic vertebrate hosts, phlebotomus fever viruses are probably active continuously during each sandfly season, regardless of the immune status of the local human population. Serologic studies of persons living in endemic areas of the disease indicate that most of the residents are infected early in life.[8,9] In these communities, sporadic cases of phlebotomus fever occur in children because most of the adult population is already immune. However, because of the benign nature of the disease, its sporadic occurrence, and its similarity to many other viral diseases of childhood, most cases are unrecognized. When large numbers of susceptible persons (soldiers, tourists, refugees, and so on) enter an endemic area of phlebotomus fever, however, bite transmission occurs, and an epidemic of the disease quickly appears.[8]

CLINICAL MANIFESTATIONS

The incubation period for phlebotomus fever averages 3 to 5 days.[1,8] The illness begins suddenly with fever, severe frontal headache, retro-orbital pain, conjunctival injection, photophobia, malaise, anorexia, nausea, vomiting, myalgia, and lower back pain. The face may be flushed, but a true rash is absent. The disease is self-limited, and symptoms usually disappear within 1 to 3 days; however, a general feeling of weakness and depression often occurs for a week or more after the illness.

Meningitis and meningoencephalitis with Toscana virus infection have been described with increasing frequency in southern Europe.[2-4,7] In addition to the classic symptoms of phlebotomus fever, these patients exhibit nuchal rigidity, a positive Kernig sign, a clouded sensorium, and, occasionally, nystagmus and tremor. To date, no deaths have been recorded. Childhood cases of the meningoencephalitic form of Toscana virus infection have been reported,[2] but this form of the infection occurs more commonly in adults.

One attack of phlebotomus fever usually confers lifelong immunity against the infecting virus type but not against heterologous serotypes.[8] For this reason, second cases of the disease have been reported in the same individual in areas where two or more virus serotypes are active.

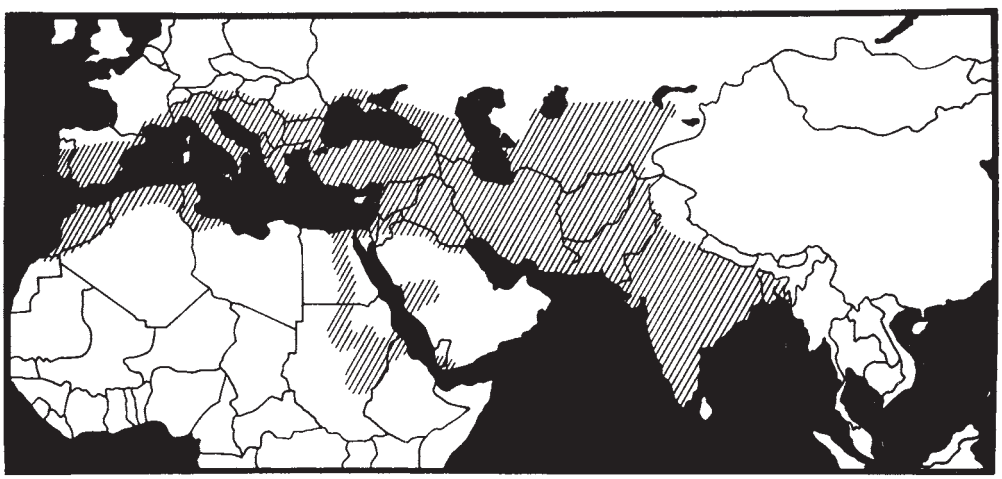

Figure 203–3 The known geographic distribution of *Phlebotomus papatasi*, the presumed vector of sandfly fever (Naples and Sicilian types), in central Asia, the Mediterranean region, and adjacent areas of Africa. *(Modified from Tesh, R. B., Saidi, S., Gajdamovic, S. J., et al.: Serological studies on the epidemiology of sandfly fever in the Old World. Bull. World Health Organ. 54:663-674, 1976.)*

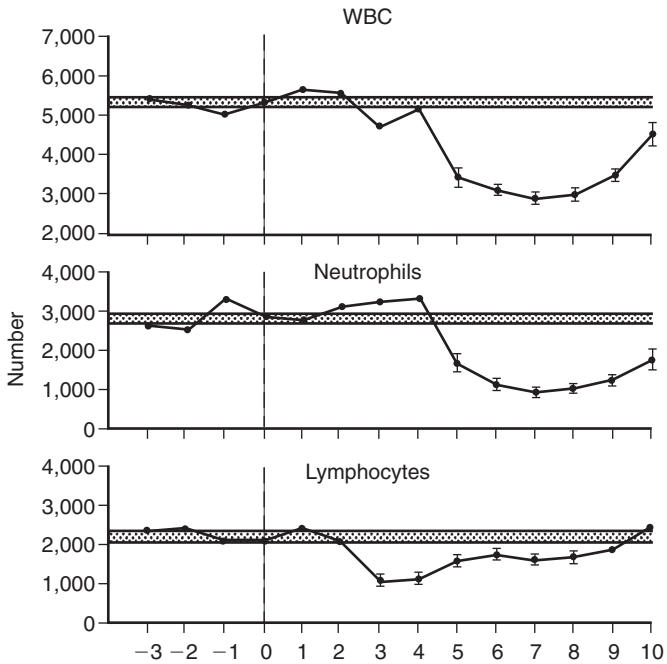

Figure 203–4 Mean total white blood cell (WBC) count as well as absolute neutrophil and lymphocyte counts in 11 adult volunteers inoculated (day 0) with the Sicilian strain of sandfly fever virus. The average incubation period in the subjects was approximately 70 hours. *(Modified from Bartelloni, P. J., and Tesh, R. B.: Clinical and serologic responses of volunteers infected with phlebotomus fever virus [Sicilian type]. Am. J. Trop. Med. Hyg. 25:456-462, 1976.)*

Marked leukopenia usually is observed in patients with phlebotomus fever.[1] It is characterized by initial lymphopenia, followed by protracted neutropenia (Fig. 203–4). In patients with central nervous system involvement, pleocytosis and an elevated protein content are observed in the cerebrospinal fluid (CSF).

PATHOLOGY

Fatalities caused by phlebotomus fever have not been reported, and little is known of the pathologic changes produced by these viruses.

DIAGNOSIS

The diagnosis of phlebotomus fever often can be made on the basis of clinical and epidemiologic evidence. A sudden outbreak during the summer months of a short febrile illness with severe headache and marked leukopenia in visitors or other newcomers to an endemic area where sandflies are abundant should suggest the disease. Depending on the region, the differential diagnosis may include dengue, West Nile fever, malaria, influenza, and numerous other respiratory and enteroviral infections.

Transient (24 to 36 hours), low-level viremia occurs during this illness; therefore, isolation of the virus from blood is unusual. In a few instances, Toscana virus has been isolated directly from the CSF of persons with central nervous system symptoms.[2] Molecular techniques (reverse transcriptase polymerase chain reaction [RT-PCR]) have been described for direct detection of Toscana virus in clinical samples[3]; however, one of the problems with using molecular methods to detect phleboviruses directly in clinical material is the wide genetic diversity among members of each serotype. In addition, limited sequence data are available for many of the phleboviruses. At present, serologic tests offer the simplest method for establishing a specific diagnosis of phlebotomus fever. Antibodies are present in serum 7 to 14 days after infection and can be demonstrated by immunofluorescence,[4] enzyme immunoassay,[5] or neutralization testing.[1] A fourfold rise in antibody titer from acute to convalescent sera or the presence of specific immunoglobulin M antibodies provides presumptive evidence of a recent phlebotomus fever virus infection. At present, these serologic tests are not generally available and usually are performed in only a few arbovirus laboratories.

TREATMENT

Treatment is symptomatic, and hospitalization usually is not necessary, except in more severe cases of Toscana virus infection. Occasionally, narcotics are required to relieve the severe headache associated with the disease.

PROGNOSIS

Phlebotomus fever is a self-limited, nonfatal illness. Recovery is complete.

PREVENTION

Control measures are directed primarily against the vector. Household spraying with residual insecticides is quite effective in reducing peridomestic sandfly vectors and controlling the disease. The use of personal insect repellents (e.g., diethyltoluamide) and fine-mesh bed nets is also effective in avoiding sandfly bites.

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CHAPTER 203d

OROPOUCHE FEVER

Francisco P. Pinheiro ● Amelia P. A. Travassos da Rosa ● Pedro Fernando da C. Vasconcelos

Oropouche (ORO) fever is an arbovirus infection that causes an acute febrile episode accompanied by headache, myalgia, arthral-

gia, and other systemic symptoms. The symptoms usually recur a few days after the end of the first febrile episode, at which time they generally are less severe. Aseptic meningitis may develop in some patients. Patients make a full recovery, without any apparent aftereffects, even in the most serious cases. No fatalities have been confirmed as being attributable to ORO fever. One of the most striking characteristics of ORO virus (OROV) is its ability to produce epidemics in urban population centers, most of which reportedly have occurred in the Brazilian Amazon region. Many of these outbreaks have had a major impact on the stricken cities.

The first case of the disease was described in 1955 in a resident of Vega de Oropouche, Trinidad, from whose blood the agent was isolated.[1] The disease was detected again in 1961, this time in the city of Belém, Pará State, northern Brazil, where it caused an epidemic that affected at least 11,000 people.[17] This epidemic was followed by many other epidemics, several of an explosive nature, in urban population centers throughout the Brazilian states of Pará, Amapá, Amazonas, Tocantins, Maranhão, Rondônia, and Acre.[7,12,17,21,22,24,27,28] Outside Brazil, epidemics of ORO fever were reported in Panama in 1989 (Quiroz, E., and associates, Panama, unpublished data, 1989) and in the Amazon region of Peru in 1992[5] and in 1994 (Ministry of Health, Peru, and U.S. Naval Medical Research Institute Detachment [NAMRID], Lima, unpublished data, 1994).

ETIOLOGIC AGENT

ORO fever is caused by OROV, which belongs to the genus *Orthobunyavirus* of the family *Bunyaviridae*.[23] The virus has enveloped spherical particles 90 to 100 nm in diameter; the capsid has helical symmetry; and the single-stranded, negative-sense RNA contains three segments, designated large (L), medium (M), and small (S) RNA.[11,23] Complete nucleotide sequences have been determined for all three RNA segments.[2,26,29] Phylogenetic analysis has revealed that all OROV strains form a monophylogenetic group consisting of three distinct lineages. Lineage I contains the prototype strain from Trinidad and most of the Brazilian strains, lineage II contains six Peruvian strains isolated between 1992 and 1998 and two strains from western Brazil isolated in 1991, and lineage III comprises four strains isolated from Panama during 1989.[26] Antigenically, OROV belongs to the Simbu group, which, in turn, is part of the Bunyamwera supergroup of arboviruses. The virus has a hemagglutinin that is active against geese erythrocytes, and it can be recovered from infected hamster serum treated with acetone (Travassos da Rosa, Belém, unpublished data, 1969). Intracerebral and intraperitoneal inoculation of OROV into baby mice and intracerebral, intraperitoneal, and subcutaneous inoculation of the virus into adult hamsters produce lethal infections. The virus replicates in numerous cell cultures, including Vero, BHK-21, and primary chicken embryo fibroblast, and it causes a cytopathic effect.[23] The agent is sensitive to the action of sodium deoxycholate.[10]

EPIDEMIOLOGY

GEOGRAPHIC DISTRIBUTION

Thus far, the only reported cases of ORO fever have occurred in Argentina, Brazil, Panama, Peru, and Trinidad (Fig. 203–5). However, most cases have been limited to the Brazilian Amazon region, with none reported in other areas of Brazil.

With a few exceptions, all episodes of ORO fever have been in the form of urban epidemics, including those in Belém and Manaus, the largest cities in the Brazilian Amazon region. The city of Belém, capital of Pará State, was struck by three major epidemics during a 20-year period. The city of Santarém and surrounding villages also were affected by a major epidemic in 1974 and 1975.[21] The first epidemics that occurred outside Pará State, those striking the cities of Manaus and Barcelos in Amazonas State[4] and the city of Mazagão in what was then the Amapá Territory, were reported early in the 1980s.[15] After a period of quiescence lasting until 1988, new outbreaks of the disease struck the cities of Porto Franco and Tocantinópolis in the states of Maranhão and Tocantins, respectively.[28] The next reported epidemics occurred in 1991, this time in more distant locations, namely, in the cities of Ariquemes and Ouro Preto D'Oeste in Rondônia State; the epidemic's impact on these cities was so great that it was reported in the national press. In 1994, another outbreak involving at least 6000 people was recorded in Serra Pelada, Pará State.[27] Other outbreaks were recorded in 1996 and affected at least five urban centers in the states of Pará, Amazonas, and Acre.[22] Thus, during 1961 to 1996, more than 30 epidemics of ORO fever were recorded in Brazil. Several small outbreaks also were recognized in Canãa dos Carajás in 2003 and in Tapará Village at Porto de Moz in 2004, both municipalities in Pará State (Vasconcelos, P. F. C., unpublished data, 2004). In April of 2006, a large epidemic of ORO fever occurred in northeastern Pará that affected several municipalities. Autochthonous cases of ORO fever also were diagnosed in the municipalities of Magalhães Barata, Maracanã, Marapanim, Igarapé-Açu, São Francisco do Pará, and Viseu. A retrospective serologic study conducted among inhabitants of urban and rural communities of the municipality of Magalhães Barata showed that 320 of 631 persons had immunoglobulin M (IgM) OROV antibodies detected by enzyme-linked immunosorbent assay (ELISA), thus suggesting that approximately half of the 8000 inhabitants had been infected. In this episode, a single isolate was obtained and sequenced, and the OROV genotype II was recognized (Vasconcelos, P. F. C., and Nunes, M. R. T., personal communication, 2006). The last recorded epidemic in those municipalities occurred in 1980.[7]

Serologic surveys[7,15,19,22] estimated that more than 360,000 people were infected during this period (Vasconcelos, P. F. C., and Nunes, M. R. T., unpublished data, 2006). However, this estimate actually is quite conservative because the incidence of this viral disease was not computed in many major outbreaks (Belém, 1968; Porto Franco and Tocantinópolis, 1988). Possibly more than a half million people in the Brazilian Amazon region may have been infected with the OROV since the beginning of the 1960s.

In addition to the aforementioned epidemic areas, countless small villages scattered throughout virtually the entire Amazon region have residents who show hemagglutination-inhibition antibodies against OROV. In general, the prevalence of these antibodies is less than 3 percent, with the exception of Ilha de Gurupá, where it is 10.7 percent.[22] An OROV strain was isolated from a novel primate host (*Callithrix* spp.) in Arinos, Minas Gerais State, southeastern Brazil,[14] thus extending the enzootic area further to southern Brazil.

Outside Brazil, outbreaks were reported in Panama and Peru. The outbreak in Panama occurred in 1989 in the village of Bejuco, which is located approximately 50 km west of the capital (Quiroz, E., and associates, Panama, unpublished data, 1989). The first epidemic in Peru was reported in 1992 in the city of Iquitos in the Peruvian Amazon region.[5] Subsequently, an outbreak occurred in Puerto Maldonado, Madre de Dios, also in the Peruvian Amazon region (Ministry of Health, Peru, and NAMRID, Lima, unpublished data, 1994). Studies performed in Peru suggested that transmission of OROV occurs continuously in the population of the city of Iquitos and surrounding villages.[30] Evidence of immunity to OROV was detected in nonhuman primates in Colombia, a finding thus suggesting the presence of OROV in that country as well.[10] OROV has been isolated from febrile persons in Jujuy, northern Argentina (Fabbri, C., Nunes,

Figure 203–5 Sites of outbreaks of Oropouche fever reported in the Americas from 1961 to 2006.

M. R. T., Vasconcelos, P. F. C., and Enria, D., unpublished data, 2006). The nucleotide sequencing of S RNA showed that the Argentinean strains constituted a separate phylogenetic group that was classified as a new genotype (genotype IV).

INCIDENCE

A significant characteristic of ORO fever is the exceptionally high attack rates seen during several outbreaks. Although incidence rates varied in different outbreaks, a rate of 30 percent was quite common. The proportion of those infected who suffered overt disease is not known with certainty, but in one epidemic, clinical disease developed in 63 percent of those infected.[7]

Gender-specific attack rates vary. Rates in female patients were slightly higher than those in male patients in villages in the Bragantina area, eastern Pará State, which was struck by the virus in 1979,[7] and the opposite was true in the outbreak in Belém that same year. However, in the reported epidemics in Santarém, the infection struck female patients twice as often as male patients.[6] ORO fever affects all age groups, although in certain outbreaks, the incidence was higher in children and young adults.

DIFFUSION OF EPIDEMICS

As indicated earlier, ORO fever epidemics have struck different locations at varying intervals. However, many outbreaks have been marked by bona fide epidemic sweeps, with countless numbers of villages within a particular geographic area affected by the virus. This diffusion phenomenon was observed in Bragança in 1967, in Santarém in 1974 and 1975, and even more dramatically in Belém and in the Bragantina area from 1978 to 1980 (where at least 10 towns were stricken), as well as in Rondônia in 1991. Spread of the virus most likely is attributable to the movement of viremic individuals throughout areas in which the virus vector is present.

SEASONAL FLUCTUATION

Most epidemics of ORO fever typically occur during the rainy season, which, in the case of the Pará State, corresponds to the period between January and June. However, many epidemics have extended into the dry season, although with less intensity. The seasonal nature of ORO fever most likely is linked to the higher density of populations of *Culicoides paraensis*, commonly known as the biting midge, the urban virus vector, in months with higher levels of rainfall, combined with a higher concentration of exposed individuals. Downward trends in epidemics of ORO fever generally are associated with the arrival of the dry season and the resulting lower density of biting midge populations and smaller numbers of exposed individuals.[11,24]

ENDEMIC TRANSMISSION

During interepidemic periods, isolated cases of ORO fever undoubtedly occur in Brazil but remain undiagnosed. Although no systematic studies have been conducted to investigate endemic transmission of OROV in Brazil, seroepidemiologic investigations have indicated that the prevalence of anti-OROV antibodies in humans is 0 to 2 percent in areas where endemic transmission has not been reported,[22] but it increases to 17 to 44 percent after outbreaks.[6,11,23] These data suggest that endemic transmission of OROV is quite low in Brazil.

TRANSMISSION MECHANISM

Laboratory studies and broad-based surveys conducted by the Evandro Chagas Institute of Belém during the course of epidemics point to the importance of the insect *C. paraensis* in the *Ceratopogonidae* family as the urban vector for OROV.[16,20] These tiny insects, commonly known as maruins (biting midges) in the Amazon region, are active during the day, particularly in the late afternoon hours. They crave human blood and bite people inside as well as outside their homes.[9,21,25] The disease is transmitted by inoculation of the virus into exposed individuals by bites of infected midges.

TRANSMISSION CYCLES

Studies conducted by the Evandro Chagas Institute[22] suggest that OROV is perpetuated in nature through two different cycles, namely, an urban cycle and a wild cycle. In the urban or epidemic cycle, the virus is transmitted from person to person by the bite of *C. paraensis*. One of the most conclusive pieces of evidence attesting to this assertion lies in demonstration of the ability of *C. paraensis*, after feeding on the blood of viremic patients, to transmit the virus to hamsters bitten by the midges 5 or more days later.[20] Moreover, these midges typically are found in high densities during periods of epidemics. They breed mostly in the decomposing trunks of felled banana trees, in rotting husks of cocoa beans,[8] and in piles of detritus formed in tree hollows.[13] They are scattered throughout tropical and subtropical areas of the Americas.[13]

Attempts to transmit the virus from one hamster to another through the bite of the *Culex quinquefasciatus* mosquito (a species commonly found in urban areas throughout the Amazon region) demonstrated that the virus was transmitted only in the presence of extremely high levels of viremia, which rarely occurs in infected humans.[16] Thus, this finding virtually rules out all likelihood that the epidemic vector is *C. quinquefasciatus*. Curiously, the virus isolation rate from *C. paraensis* during periods of epidemics is only 1:12,500,[11] a finding suggesting that we are dealing with a low-efficiency vector. Apparently, humans are the only vertebrates involved in the urban cycle of OROV because studies of domestic animals conducted during the course of numerous outbreaks ruled out the possibility that these animals play an amplifying role.

As far as the wild, silent cycle of the virus is concerned, evidence suggests that among vertebrates, the *Edentata* (sloth), nonhuman primates, and possibly certain species of wild birds serve as hosts. Although to date OROV has been isolated from a single pool of *Aedes serratus* in Brazil and once from *Coquillettidia venezuelensis* in Trinidad,[1,22] the possible involvement of biting midges in the virus' wild cycle nonetheless should be investigated.

The link between the two cycles most likely is humans themselves, who, after contracting the infection in enzootic forested areas and then returning to an urban setting during the viremic phase, become a source of infection for biting midges. The virus replicates in the tissues of biting midges, which, after the extrinsic incubation period, bite and infect exposed individuals. These individuals, in turn, serve as a source of infection for other midges, thereby forming a chain of transmission resulting in the unleashing of an epidemic.

INCUBATION PERIOD

Observations conducted during numerous epidemics suggest that the incubation period ranges from 4 to 8 days. A laboratory worker who accidentally was infected orally exhibited symptoms of the viral disease 3 days later, and another technician fell ill 4

days after being infected, probably through the respiratory route.[22]

TRANSMISSIBILITY PERIOD

The blood of infected patients is infectious to *C. paraensis* for the first 3 or 4 days after the onset of symptoms, when the level of viremia is high enough to infect the midges. Experimental studies have shown the length of the extrinsic incubation period to be 5 days or more.[20] The virus is not transmitted directly from one person to another.

RATIO OF SYMPTOMATIC CASES

A prospective study conducted in the city of Santa Izabel in Pará State during the course of the epidemic of 1979 showed the ratio of symptomatic to asymptomatic cases to be roughly 2:1.[7] The study was performed during the period from March through June of that year. It involved 274 individuals exposed to the virus who were monitored by clinical examination and laboratory testing on a weekly basis throughout the study. By the end of the study period, 78 (28.5%) of these individuals had serologic evidence of OROV infection, with clinical manifestations of the disease developing in 49 (63%) of the 78.

CLINICAL MANIFESTATIONS

In most cases of ORO fever, the infection takes the form of an acute febrile episode, which runs its course. However, certain patients may show typical signs and symptoms of aseptic meningitis, which also runs its course without complications.

CLASSIC FEBRILE FORM

This form of the disease is characterized by the sudden onset of symptoms after an incubation period ranging from 4 to 8 days. The first symptoms to appear are fever, headache, chills, dizziness, muscular pain, arthralgia, and photophobia. Retro-ocular pain and conjunctival congestion also may be present. In addition, some patients suffer from nausea, which may be accompanied by episodes of vomiting. Not uncommonly, patients have severe anorexia and insomnia. At times, cough and coryza are present as well, although these manifestations may be attributable to intercurrent infections. Certain patients complain of fleeting burning or stinging sensations in different parts of their body. The presence of an exanthem is a rare finding. Two laboratory workers who were infected accidentally reported a longer and heavier menstrual flow than usual.[21,22] The fever can be quite high, 39° C or 40° C, and in some cases it may be higher than 40° C. The headache usually is localized in the front or back part of the head, although it also may be diffuse. It generally is severe and, in some cases, may not respond readily to common analgesics. Generalized myalgia is present and sharpest in the neck, along the vertebral column, and in the area of the sacrum. The pain may be extremely severe. Patients generally describe feeling as though their body had been crushed or they had been beaten. Usually, generalized arthralgia also is present. Certain patients have dizzy spells so severe that in some cases they collapse. Any epigastric pain generally is mild. Patients have no sign of jaundice, hepatomegaly, or splenomegaly. Occasionally, swollen lymph nodes are detected in the submaxillary and occipital regions, and these findings could be totally unrelated to the viral infection.

The intensity of the clinical symptoms varies. In some cases, the symptoms are quite severe and even may cause prostration, whereas in others, they can be rather mild. Many patients are bedridden and, during epidemics, flood area hospitals, thus causing serious overcrowding.

The acute phase of the disease generally lasts 2 to 5 days but can be as long as a week. The myalgia, conversely, may persist for 3 to 5 days after the fever has disappeared. Some patients report having prolonged asthenia for as long as a month. Certain patients complain of a persistent headache lasting up to several weeks. No human deaths have been attributed to OROV infection.[11]

Nearly 60 percent of all patients have one or more recurrences in the first or second week after disappearance of the manifestations of the acute phase of the disease.[7,15,22] Relapses may take the form of reappearance of all the acute-phase symptoms of the disease, or they may be limited strictly to fever, asthenia, and dizziness. In some reported patients, relapses were accompanied by a urinary tract infection of bacterial origin. An abscess, most likely bacterial, developed in the oropharynx of one particular patient approximately 10 days after recovery from the original febrile condition. In some cases, patients may suffer a series of relapses during a period of 2 to 3 weeks.[22] All attempts to isolate OROV during relapses have failed.

Observations made during the course of the 1980 outbreak in Belém revealed that an exanthem developed in approximately 5 percent of all laboratory-confirmed cases.[15] The exanthem appeared between the third and sixth day after onset of the fever, disappeared 2 or 3 days later, and mainly involved the thorax, back, arms, and legs.[15,19] During the outbreak in Manaus, many patients exhibited a maculopapular exanthem beginning on the torso and spreading to the upper and lower extremities.[4] In another rare case, a 4-year-old child whose infection was confirmed by serodiagnosis experienced nystagmus, generalized tremors, and somnolence.[7] These symptoms lasted approximately 8 days, and the child apparently made a full recovery.

The effects of ORO fever on pregnancy are unknown. The only available data in this regard come from studies conducted in Manaus of nine pregnant patients, two of whom, both in the second month of pregnancy at the time, suffered miscarriages.[4]

ASEPTIC MENINGITIS

At first, patients exhibit manifestations typical of the initial acute phase of the infection. As the illness progresses, a few days later, the headache and dizziness become increasingly severe, and some patients begin to experience other neurologic symptoms, generally during the second week of the illness, that lead them to seek medical care. The main complaints cited by patients are fever, extremely severe headache in the back of the head, and dizziness. Approximately one third of all patients complain of nausea and vomiting. Some patients suffer from moderate lethargy. They also may have trouble holding themselves upright. Some patients complain of double vision or diplopia. They generally try to keep from moving their heads to avoid aggravating their pain. In most cases, physical examination of these patients reveals varying degrees of stiffness of the neck but no signs of paresis or paralysis. Some patients experience nystagmus. Despite the seriousness of these neurologic symptoms, patients make a full recovery. Electroencephalograms taken of four patients showed no abnormalities. The incidence of meningitis in patients who seek medical care is less than 5 percent.[18]

PATHOGENESIS

Little is known about the pathogenesis of ORO fever. Apparently, the agent produces a systemic infection in humans that

induces a viremic phase, but the organs in which the virus replicates have not been identified. Virtually all infected patients exhibit viremia during the first 2 days of their illness. By the third day, the rate of viremia drops to 72 percent, and it falls to 44 and 23 percent by the fourth and fifth days, respectively. Viremia titers generally are higher than 3.0 $\log_{10}$ median lethal dose per 0.02 mL in mice, with approximately 10 percent of patients exhibiting virus titers as high as 5.0 to 5.3 during the course of the first 2 days of their illness. By the third day, virus titers are 1 log lower than in the first 2 days, and titers plummet by the fourth day.[22]

Similarly, little is known about the pathogenesis of relapses, which occur commonly. That no sign of viremia could be detected in any of the countless patients examined while suffering relapses is noteworthy.

The finding that OROV is capable of causing aseptic meningitis, combined with isolation of the virus from the cerebrospinal fluid (CSF) in one patient with meningitis,[18] suggests that the virus has the ability to penetrate the blood-brain barrier. With no known confirmed fatalities attributable to OROV, no data are available on the possible organic lesions caused by this agent in humans.

Laboratory tests on young hamsters inoculated with OROV showed that the virus has essentially hepatoviscerotropic properties, with isolated necrosis of hepatocytes or focal necrosis and the involvement of Kupffer cells exhibiting reactive hyperplasia; animals invariably succumb to the infection. In newborn mice, the virus exhibits marked neurotropism, and animals show signs of focal encephalitis within 24 to 48 hours after being inoculated.[3]

LABORATORY FINDINGS

Leukopenia associated with neutropenia is found commonly, although in certain cases moderate leukocytosis may be present. The leukopenia can be severe, with reports of leukocyte counts as low as 2000/mm^3. No signs of cell abnormalities are present. Aspartate and alanine aminotransferase levels are normal or may show a moderate increase, but in no case do they exceed 135 U/mL of serum. Platelet counts usually are normal but occasionally may be slightly low. The erythrocyte sedimentation rate and levels of urea, creatinine, and glucose in blood are normal, as are urine tests.[22]

The CSF of patients with aseptic meningitis shows pleocytosis and an increased concentration of protein.[18] The cell count varies from 7 to 310 cells/mm^3 of CSF; both segmented and mononuclear cells are present, with a predominance of segmented cells. In one case, the cell count in CSF fell from 130 to 30 in a 1-week period, and another patient's cell count fell from 70 to 10 cells over a 3-week interval. In general, a moderate increase in protein levels occurs in the CSF, although one patient's protein level was more than 100 mg/mL. Glucose levels remain normal.

LABORATORY DIAGNOSIS

Specific confirmation of the infection is made by isolating the virus from patients' blood or by performing OROV-specific serologic assays.[23] To isolate the virus, blood samples need to be taken during the first 5 days of the illness, preferably in the first 2 days, when viremia is present in virtually all cases. The virus can be isolated by intracerebral or intraperitoneal inoculation of serum from infected patients into baby mice or young hamsters (in this case, subcutaneous inoculation can be used as well). Viral isolates also can be recovered in different cell cultures, such as Vero and BHK-21. The virus is identified by complement fixation or neutralization using OROV-specific ascitic fluid or antisera. Serodiagnosis is accomplished by the demonstration of an increase in antibody in paired serum samples taken during the acute and convalescent phases of the disease by hemagglutination inhibition, complement fixation, or neutralization. A positive IgM antibody capture ELISA (MAC-ELISA) on a single serum sample provides a presumptive diagnosis of recent infection, particularly in the presence of a clinical picture consistent with the disease; the test usually is positive after the fifth day of illness. A rapid and highly sensitive one-step TaqMan reverse transcriptase polymerase chain reaction (RT-PCR) assay has been described for detection of OROV and certain other orthobunyaviruses; it was found to be more sensitive than is the established nested PCR system.[31]

DIFFERENTIAL DIAGNOSIS

Because of the nonspecific nature of the symptoms, a clinical diagnosis of ORO fever is difficult to make, and often the disease is mistaken for other febrile illnesses such as dengue fever and malaria. In fact, malaria and dengue fever initially were suspected as the cause of numerous epidemics of ORO fever. Detailed clinical records combined with epidemiologic data can help establish a differential diagnosis, the certainty of which hinges on the absence of plasmodia in blood samples and the lack of laboratory evidence of dengue infection. Other viral and bacterial febrile diseases must be considered in the differential diagnosis. Accordingly, clinical and epidemiologic data and nonspecific tests will need to be taken into account, although establishing an accurate diagnosis requires specific tests.

Febrile forms of the disease accompanied by an exanthem need to be distinguished from other exanthematous febrile symptoms caused by dengue, measles, parvovirus B19 infection, infection with human herpesvirus type 6 or 5, enteroviral infections, and allergies to medication. Finally, differentiating cases of aseptic meningitis associated with OROV infection from cases of aseptic meningitis associated with other causative agents requires a specific etiologic diagnosis.

TREATMENT

Because ORO fever has no specific treatment, management is symptomatic. Rest is important and should be continued for several days after disappearance of the initial acute manifestations because relapses are thought to occur more often in patients who prematurely resume regular activities, particularly strenuous ones. Aspirin or another antipyretic should be taken to lower the fever, and the use of ordinary analgesics is recommended for headache, myalgia, and arthralgia. However, certain reported patients whose headaches failed to respond to this treatment were treated with morphine derivatives. Also recommended are fruit juices or glucose solutions. Severely dehydrated patients may be treated with intravenous fluids.

PREVENTION AND CONTROL

The most effective way to prevent, avert, or curb the impact of epidemics of ORO fever is to combat its vector, *C. paraensis*. To be effective, vector control effort needs to focus on the midge's adult and larval forms. Given that *C. paraensis* is habitually active during the day, application of insecticides to its habitats by thermonebulization or ultra-low-volume aerosolization may help to reduce populations of adult biting midges. Because this *Culicoides* species is most active during the late afternoon hours,[25] ultra-low-volume spraying may be more effective during this period. However, carefully planned studies are needed to assess how to

maximize the effectiveness of spraying by determining the type and concentration of insecticide to be used, the necessary volume of insecticide per treatment area, the size of the droplets, the frequency and timing of applications, and other factors. At the same time, making an effort to control the larvae by applying larvicides to corresponding habitats or, better yet, by conducting drives to eliminate or burn breeding sites such as rotting cocoa bean husks and the decomposing trunks of felled banana trees is essential.[8] Obviously, the success of these measures will depend largely on community involvement. Providing proper community education is important. Individuals can protect themselves by applying insect repellent directly to the skin. However, these types of products provide only temporary action, and they may be unaffordable to the poor.

No vaccine against ORO fever exists at this time. In light of the relatively benign nature of this viral disease, developing a general-purpose vaccine for at-risk populations living in areas where they are exposed to the disease is difficult to justify.

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CHAPTER 203e

TOSCANA VIRUS

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HISTORY

Toscana virus (TOSV) was isolated first in 1971 from the sandfly *Phlebotomus perniciosus* collected in Monte Argentario (Grosseto Province, central Italy).[52-54] Three strains were isolated from female sandflies. So far, most clinical and epidemiologic studies have been conducted in Italy, although studies from other Mediterranean countries have been published more recently. Results of these studies indicate that TOSV is a major viral pathogen involved in aseptic meningitis during summer months in these countries. A bibliographic search using "Toscana virus" as keywords in the PubMed database retrieved 65 research and review articles. Fewer than 50 percent of them reported imported or autochthonous human cases acquired in Italy, Spain, Portugal, France, or Cyprus; the diagnosis was established through viral isolation, polymerase chain reaction (PCR) and sequencing, serology, or a combination of these methods. Despite increasing evidence of its major role in human disease, TOSV remains poorly studied, and most physicians have little awareness concerning its potential to cause central nervous system (CNS) infections. Moreover, TOSV cases were reported from few countries where sandflies are prevalent; whether this factor is an accurate reflection of the reality or whether it relates to a lack of investigation is unknown. Thus, one of the major aims of this review is to disseminate knowledge of the role played by TOSV in human disease and to stimulate interest in conducting new studies to increase our understanding of the prevalence of TOSV in the countries surrounding the Mediterranean.

ETIOLOGIC AGENT: VIRUS PROPERTIES AND CLASSIFICATION

According the eighth report of the International Committee on Taxonomy of Viruses (ICTV), TOSV is a serotype of Sandfly fever Naples virus within the genus *Phlebovirus* in the *Bunyaviridae* family. TOSV is an arthropod-borne virus. The lack of biochemical and genetic data for most phleboviruses dictates that the species be defined by serologic relationships; these viruses are distinguishable by a fourfold difference in two-way neutralization tests. TOSV and Sandfly fever Naples virus cross-react serologically, except in the plaque reduction neutralization test (PRNT), in which a one-way specificity is observed (i.e., TOSV mouse immune ascitic fluid [MIAF] neutralizes Sandfly fever Naples virus and TOSV, whereas Sandfly fever Naples virus MIAF neutralizes only Sandfly fever Naples virus, not TOSV). By using other serologic tests (indirect immunofluorescence assay [IFA], hemagglutination inhibition, complement fixation, enzyme-linked immunosorbent assay [ELISA]), in most cases serologic cross-reactions are observed among TOSV, Sandfly fever Naples virus, and Sandfly fever Sicilian virus. Phleboviruses are arthropod-borne viruses that contain a negative-sense, single-stranded RNA genome that consists of three segments, designated large, medium, and small, that encode the RNA-dependent RNA polymerase,[22] the envelope glycoproteins, and the nucleoprotein, respectively. Phylogenetic analyses performed using the medium segment identified three major lineages separate from Rift Valley fever virus and Uukuniemi virus: lineage 1 includes Sandfly fever Sicilian virus and Sicilian-like viruses isolated from Italy, Cyprus, India, Iran, and Greece; lineage 2 includes Sandfly fever Naples, Naples-like viruses, and genotypes (Tehran and TOSV) isolated from Italy, Portugal, Iran, the former Yugoslavia, Egypt, Cyprus, and India; lineage 3 includes phleboviruses from the New World such as Punta Toro, Buenaventura, and related viruses.[27]

EPIDEMIOLOGY

Phlebotomus fever viruses have been isolated from sandflies in southern Europe, Africa, central Asia, and the Americas, and evidence exists for the presence of different viruses in the same sandfly population. The simultaneous occurrence of Sandfly fever Sicilian virus, Karimabad virus, and Isfahan virus (a *Vesiculovirus*) was documented in Iran,[45] and Verani and colleagues[52] isolated TOSV (associated with human disease) and Arbia (not yet shown to cause infection in man) viruses in Italy in the same sandfly population. Sandfly fever Naples (but not the TOSV serotype) and Sicilian viruses have the widest geographic distribution, parallel to their vector's *(Phlebotomus papatasi)* distribution. Isolation reports and serologic studies indicate that sandfly fever Naples and Sicilian viruses are present in the coastal Mediterranean regions of Europe and North Africa, the Nile valley, most of Southwest Asia, areas adjacent to the Black and Caspian Seas, and central Asia including Bangladesh.[46] Until recent years, the known distribution of TOSV was limited to Italy and Portugal.[18,31] In Italy, the virus was isolated both from the vectors (*P. perniciosus* and *Phlebotomus perfiliewi*) and from humans, whereas the presence of the virus in Portugal was suspected on the basis of a strain isolated from the cerebrospinal fluid (CSF) of a Swedish patient returning to his home country from Portugal. More recently, the geographic distribution of the virus has been extended to France, Spain, Slovenia, Greece, Cyprus, and Turkey, according to results from viral isolation and serologic surveys.[17,21,25,28,33] The geographic distribution of Toscana virus is shown in Figure 203–6.

Figure 203–6 Geographic distribution of Toscana virus in Europe. (See companion Expert Consult web site for color version.)

ITALY

Preliminary clues pointing to the role of TOSV in CNS infections in Italy were provided by case reports of imported cases diagnosed in the United States,[8] and Germany.[42] Italy was the first country where TOSV activity was demonstrated, and subsequently most studies concerning the epidemiology of TOSV were conducted there. A large study carried out between 1977 and 1988 showed that the virus was the cause of meningitis in two different regions of Italy, Tuscany and Marche, with a seasonal peak occurring in August, corresponding to the peak of sandfly activity.[31] Since then, the virus has been isolated in other regions of central and southern Italy. More recently, thanks to reverse transcriptase PCR (RT-PCR) and specific recombinant immunoenzymatic assays, research into TOSV as an etiologic agent of neurologic diseases has been performed in other regions of northern Italy (Emilia Romagna and Piedmont).[23]

Striking evidence that TOSV was the most prominent viral cause of summertime meningitis was reported in the late 1990s.[49] In one of the most comprehensive studies dedicated to CNS infections and viruses, the authors of the study reported that TOSV represented 81 percent of the viruses detected in CSF from patients presenting with meningitis and other CNS infections; TOSV sequences were detected in 85 of a total of 104 samples of CSF that provided positive results for viral sequences (enteroviruses [n = 12], mumps virus [n = 3], herpes simplex virus [HSV] type 1 [n = 3], measles virus [n = 1]). These results were obtained using a PCR system based on a nested protocol previously described by the same authors.[48] It would be interesting to perform a similar study using recently developed, more sensitive diagnostic tools for detecting DNA and RNA viruses that cause CNS infections, namely, real-time PCR assays.

A study of children living in rural or suburban areas of Siena (central Italy) showed that 40 percent of meningitis or encephalitis cases could be linked to infection with TOSV.[7] A 7-year study (1990 to 1996) performed in Siena showed that 52 percent of aseptic meningitis cases in adults were associated with TOSV (seroconversion, presence of immunoglobulin M [IgM], PCR detection).[5] All studies are in agreement regarding the monthly distribution of human cases of TOSV infections: the highest risk of acquiring TOSV is in August (++++), then July and September (++), and finally June and October (+). Populations living in rural areas and with high levels of outdoor activity are at the greatest risk of acquiring TOSV infection. An occupational risk study conducted on forestry workers in Siena, Florence, and Arezzo showed that 77.2% (278/360) of these workers had positive IgG for TOSV (ELISA test) by comparison with an urban population that exhibited a 22 percent prevalence for IgG (64/290). In con-

trast, only 6 percent of forestry workers of the Piedmont area showed TOSV IgG.[50]

The first evidence of TOSV infection in Umbria was published in 2003 in the form of a retrospective study of 93 aseptic meningitis and meningoencephalitis cases recorded from 1989 to 2001. A total of 36 percent and 6 percent of meningitis and meningoencephalitis cases, respectively, could be associated with TOSV through serology-based diagnosis. Of particular interest is the observed 16 percent of IgG-positive individuals in the healthy control population.[23]

TOSV infections in Emilia Romagna were documented for the first time in 2002. A total of nine cases were recorded over a 3-year period (1999 to 2201) with six meningitis cases, two meningoencephalitis cases, and one case of erythema with fever. Of these nine patients, six could be considered imported cases because these patients spent time in Tuscany before the onset of the disease; the three other cases are classified as autochthonous because no traveling out of the province of Emilia Romagna could be documented.[34] Most recently, TOSV was detected in the CSF of patients with meningitis and encephalitis in Naples, southern Italy.[14]

SPAIN

The first case of TOSV infection reported from Spain occurred in the late 1980s in a Swedish tourist after visiting Catalonia and was documented by PRNT.[19] Subsequently, Spanish researchers and physicians reported many cases of TOSV infection and conducted several comprehensive epidemiologic studies demonstrating that TOSV was one the three main causes of meningitis in Spain.[29] A total of 15 strains of TOSV were isolated between 1988 and 1996 from the CSF of patients with acute aseptic meningitis in Granada, Spain.[28] A total of 724 CSF samples from patients with suspected aseptic meningitis were tested through viral isolation in cell culture. TOSV was isolated from the CSF of 17 patients (7% of all viral isolates), 15 of whom were previously reported in the foregoing reference. The first case was diagnosed in June of 1988 and the last in August of 2002.[29] TOSV was ranked as the third most common cause of aseptic meningitis after enteroviruses ($n = 199$, 82% of virus isolation) and mumps virus ($n = 21$), a finding suggesting that its etiologic role in CNS infections in Spain is not as high as observed in central Italy. Investigation of 23 cases of meningitis in patients living in the Mediterranean coast of Spain and of 168 cases of meningitis in patients from all regions of Spain permitted the diagnosis of TOSV infection to be established in 4 (17%) and 5 cases (3%), respectively. These data suggest that the situation in Spain is similar to that observed in France, with lower prevalence of CNS infections compared with that observed in central Italy.

Sequence data obtained from Spanish isolates of TOSV and other phleboviruses confirmed that natural genome variability may hamper establishing the diagnosis of these agents by molecular methods, so this issue must be kept in mind when developing assays. Generic nested RT-PCR, followed by sequencing of the amplified products, was developed for the detection and specific identification of every member of the *Phlebovirus* genus, including TOSV (Table 203–1). A modification of this method, using newly designed primers, also allowed the researchers to make specific direct detection and identification of TOSV isolates originating from different geographic areas. The testing of clinical samples with these assays confirmed the role of TOSV as a causative agent of acute aseptic meningitis in the central region of Spain.[37] All clinical cases of sandfly fever among Swedish tourists that were serologically confirmed during 1986 to 1989 were investigated. Aside from the case of TOSV from Spain, 37 cases of Sandfly fever Sicilian virus infection and one case of Sandfly fever Naples virus infection were documented among tourists returning from Cyprus.[19] A collection of 88 serum and 53 CSF samples taken from 81 selected patients with acute aseptic meningitis of unknown origin, who were living in Madrid or on the southern Mediterranean coast of Spain, was studied retrospectively for the presence of TOSV-specific IgG and IgM antibodies. Specific IgM in serum or intrathecally produced anti-TOSV IgG was detected in 7 patients, 3 residents from the Mediterranean region and the remaining 4 from the Madrid region.[17] A large study conducted in different regions of Spain showed the presence of IgG antibodies to TOSV (26.2%), Sandfly fever Naples virus (2.2%) and Sandfly fever Sicilian virus (11.9%) in 1181 adults and 87 children.[28] A total of 457 serum samples taken from healthy individuals, 2 to 60 years old, from the south of the Madrid region was investigated for anti-TOSV IgG. The overall prevalence of anti-TOSV was 5 percent.[17]

In June of 2003, a collaborative, multidisciplinary network was created in Spain for the study of arthropod- and rodent-borne

TABLE 203–1 Primers Described in the Literature for Toscana Virus Reverse Transcriptase Polymerase Chain Reaction and Nested Polymerase Chain Reaction Detection

Primer Name	Primer Sequence	Gene	Assay	Reference
TV1	5′-CCAGAGGCCATGATGAAGAAGAT-3′	N	RT-PCR	
TV2	5′-CCACTCCTATGAGCAGCTTCT-3′	N	RT-PCR	18
TV3	5′-AACCTGATTTCAGTCTACCAGTT-3′	N	Nested	
TV4	5′-TTGTTCTCAGAGATGGATTTATG-3′	N	Nested	
TosN123	5′-GAGTTTGCTTACCAAGGGTTTG-3′	N	RT-PCR	
TosN829	5′-AATCCTAATTCCCCTAACCCCC-3′	N	RT-PCR	28
TosN234	5′-AACCTTGTCAGGGGNAACAAGCC-3′	N	Nested	
TosN794	5′-GCCAACCTTGGCGCGATACTTC-3′	N	Nested	
NPhlebo1+	5′-ATGGARGGITTTGTIWSICIICC-3′	L	RT-PCR	
Nphlebo1–	5′-AARTTRCTIGWIGCYTTIARIGTIGC-3′	L	RT-PCR	28
Nphlebo2+	5′-WTICCIAAICCIYMSAARATG-3′	L	Nested	
Nphlebo2–	5′-TCYTCYTTRTTYTTRARRTARCC-3′	L	Nested	
ATos2–	5′-RTGRAGCTGGAAKGGIGWIG-3′	L	Nested*	
T1	CTATCAACATGTCAGACGAG	N	RT-PCR	
T2	CGTGTCCTGTCAGAATCCCT	N	RT-PCR	47
T3	CATTGTTCAGTTGGTCAA	N	Nested	
T4	CGTGTCCTGTCAGAATCCCT	N	Nested	

**Primer used in combination with Nphlebo2+ for a nested reaction specific for Toscana virus.*
RT-PCR, reverse transcriptase polymerase chain reaction.

viral diseases (the EVITAR network). Within this context, a study on seroprevalence to TOSV infection in Granada (southern Spain) was conducted, for which an exhaustive population study was done. In this work, the overall seroprevalence rate was 24.9 percent. A statistically significant increase in the seroprevalence rate was observed with age (9.4% in individuals <15 years compared with 60.4% in individuals >65 years). In addition, several cases of TOSV were documented in numerous geographic areas of Spain (Badajoz, Málaga, Granada, Almería, Murcia, Alicante, Valencia, Madrid, and Barcelona), mostly in the southern, central, and Mediterranean areas of the country (EVITAR network, Tenorio, A., personal communication).

FRANCE

The first reported case of TOSV infection acquired in France was in a German traveler returning from southern France.[16] The story of TOSV in Italy led virologists in Marseille to develop the tools necessary for establishing the diagnosis based on serology, molecular biology, and traditional virology. A collection of 205 RNA samples comprising human clinical specimens sent to the virology laboratory from April to December of 2001 for suspected enterovirus infections was tested for the presence of TOSV RNA by a previously described nested RT-PCR protocol[48]; 5 of 205 samples were positive, and sequence analysis revealed a 100 percent identity with the homologous region of strain ISS.Phl.3. A total of 72 sera collected from April to September of 2003 in patients admitted for meningitis was tested for the presence of TOSV IgM and IgG using ELISA; 3 of 72 (4.2%) samples were IgM-positive, and 13 of 72 (18%) were IgG-positive (Charrel, R. N., and de Lamballerie, X., unpublished data). A total of 134 sera collected from patients who were admitted for febrile neurologic manifestations (encephalitis, meningoencephalitis, peripheral manifestations, but not meningitis) was tested for the presence of TOSV IgM and IgG; none of 134 samples was positive for IgM, whereas 26 of 134 (19.6%) samples were positive for IgG (Charrel, R. N., and de Lamballerie, X., unpublished data). A similar study was performed with 92 healthy blood donors from Marseille; 11 of 92 (12%) samples were positive for IgG, and 1 of 92 was positive for IgM. Values observed from the latter group were not significantly different from the values obtained across the two groups of patients.

During surveillance for West Nile virus in southern France, sera from suspected cases (meningitis) were tested for TOSV, and several contained specific IgM. Two cases of meningitis caused by TOSV were diagnosed by seroconversion and by viral isolation, the first instance of TOSV isolation reported in the literature from a clinical case.[33] Two other cases (one meningitis and one febrile illness) also have been published.[25] Other sporadic cases have been diagnosed through indirect IFA (presence of IgM, seroconversion) and PCR detection with sequencing, or a combination of both techniques. Sequence analysis of TOSV demonstrates that both the Italian and Spanish genotypes circulate in southern France in sandflies but also cause human infections.[10]

Together, data confirm that TOSV circulates in southeastern France and is responsible for cases of meningitis. Further investigation will be necessary to determine the importance of the role played by TOSV, in comparison with other viruses, in CNS infections in the summertime.

CYPRUS

Several studies were conducted in Swedish United Nations soldiers based in Cyprus in 1985. Blood samples were obtained from the 362-soldier battalion just before and immediately after their 6-month tour of duty. Of 298 serum pairs available, seroconversion for TOSV was observed in a single case without any clinical manifestations.[21] Seroprevalence studies showed that 20 percent (96/479) of the healthy population had TOSV IgG.[20] Every summer, numerous unexplained febrile cases are observed among the Greek Army Forces in Cyprus. For this reason, blood samples were collected from febrile patients in 2001 and 2002. Serology and molecular methods revealed that the causative agent was a phlebovirus. A virus strain was isolated from the blood sample of a patient taken on the first day of the disease. Sequence analysis of a partial L RNA segment revealed that the Sandfly fever virus strain from Cyprus differs from Sandfly fever Sicilian virus by 6.7 percent at the nucleotide level (Papa, A., unpublished data). For six patients from whom a convalescent serum sample was available, all had antibodies to phleboviruses (Sandfly fever Sicilian virus, Sandfly fever Naples virus, and TOSV), detected by indirect immunofluorescent assays. Cross-reactivity in serology was observed among the three serotypes of phleboviruses; a greater number of patients had antibodies to Sandfly fever Sicilian virus and higher antibody titers were present against Sandfly fever Sicilian virus than against Sandfly fever Naples virus and TOSV.

GREECE

Phleboviruses are present in Greece.[45] Results of a study showed that the prevalence of Sandfly fever Naples virus and Sandfly fever Sicilian virus neutralizing antibodies among Athens residents older than 30 years was 36 percent and 13 percent, respectively, whereas 4 percent of younger persons had Sandfly fever Naples virus antibodies, and all were negative to Sandfly fever Sicilian virus.[44] These differences were the result of the insecticide-spraying malaria eradication campaigns.[45] A phlebovirus, strain Corfu (Pa Ar 814), was isolated in 1981, on Corfu island from *Phlebotomus major*.[35] Using spot slides with Vero E-6 cells infected with this strain, researchers found a seroprevalence of 4 percent among healthy farmers, woodcutters, and shepherds.[2] Studies of populations living on the Ionian Islands and western mainland of Greece showed a seroprevalence of 60 percent and 35 percent, respectively, by electroimmunoassay (EIA) (Enzywell TOSV IgG/IgM Diesse) (unpublished data). To date, no studies have been performed of meningitis or encephalitis cases caused by TOSV in Greece.

PORTUGAL

One imported case of TOSV was reported in Portugal, in a man who presented with severe headache and fever without neck stiffness. A CSF sample showed 134 monocytes. Viral isolation was successful, and identification was performed by plaque neutralization.[18] In addition, one German patient returning from vacation in Portugal presented with meningitis; the diagnosis was established by ELISA and confirmed by immunoblot assay.[41]

GERMANY

In a seroepidemiologic survey of 859 health care workers and medical students, anti-TOSV IgG was detected in 1.0 percent of samples by IFA, and in 0.7 percent by EIA. In 2034 German patients hospitalized for various diseases, 1.6 percent tested positive for anti-TOS IgG by IFA and 0.8 percent by EIA. Anti-TOSV IgG was detected in 43 samples of commercial immunoglobulins at titers of 10 to 1000 by EIA. Although the seroprevalence of antibodies to TOSV is low in Germany, TOSV infection should be considered in patients returning from endemic areas who

complain of fever and headaches and who have symptoms of meningitis.[40]

CYCLE IN NATURE

THE VECTORS OF TOSCANA VIRUS

TOSV was isolated first from *P. perniciosus*. Other strains have been isolated from *P. perfiliewi* but never from *P. papatasi*. TOSV also has been isolated from the brains of the bat *Pipistrellus kuhli* trapped in areas where *P. perniciosus* and *P. perfiliewi* were present.[52-54] Isolation from bats has been reported for a large number of mosquito-borne arboviruses, including flaviviruses such as yellow fever virus, dengue virus, West Nile virus, and St. Louis encephalitis virus, with no clear information regarding implications for the ecologic cycle of these viruses. Transovarial transmission of TOSV in sandflies has been demonstrated in the laboratory and by viral isolation from male *Phlebotomus* species. Venereal transmission from infected males to uninfected females also has been demonstrated. *P. perniciosus* is distributed throughout the Mediterranean region as two races. The typical *P. perniciosus* race occurs in Italy as well as in Malta, Tunisia, and Morocco. It is replaced in southern Spain by the Iberian race (with the pni mtDNA sublineage).[32] TOSV was detected both in male and in female pools of phlebotomine sandflies captured during the summer seasons of 2003 and 2004 in Granada. The most probable transmission vector for TOSV in Spain is *P. perniciosus* because approximately 70 percent of captured individuals comprised this species. Detection in pools of males suggests vertical or sexual transmission of TOSV among phlebotomine sandflies. TOSV RNA was detected in *Sergentomyia minuta*, a sandfly known to feed on reptiles and assumed not to feed on humans; this property may prevent *S. minuta* from being a vector of human infection.[9] However, *Sergentomyia* flies have been reported to be infected with a variety of arboviruses.[9]

THE RESERVOIR OF TOSCANA VIRUS

The reservoir of TOSV is most likely the vector. Neither mammals nor birds have been recognized as a potential reservoir, although few studies have been carried out on mammals and almost none on birds. Whether humans can play a role in the virus cycle by infecting naïve sandflies is not known.

Although numerous phleboviruses have been isolated from the blood of sick persons and from wild animals, the importance of vertebrates in the maintenance of the transmission cycle of these viruses remains unclear. Transient and low-level viremia is present after phlebovirus infection in humans and in susceptible laboratory animals.[4,12,43] Moreover, a large quantity of virus must be ingested to infect sandflies.[11] It seems unlikely that a biting sandfly would often come into contact with an animal with viremia high enough to infect the insect orally. Verani and colleagues[52] examined different species of wild vertebrates (wild mouse, bank vole, stone marten, coypus, porcupine, bat, fox, and hedgehog) by serology and viral isolation; one virus strain was isolated from the brain of a bat *Pipistrellus khuli*, whereas no hemagglutination-inhibition antibodies were found in sera.[52]

CLINICAL MANIFESTATIONS

ASYMPTOMATIC OR MINIMALLY SYMPTOMATIC INFECTION

Seroprevalence studies suggest that some infections caused by TOSV are asymptomatic or minimally symptomatic. Additional studies will be necessary to evaluate the ratio of symptomatic to asymptomatic or minimally symptomatic infections.

FEBRILE ILLNESS

In some cases, TOSV infection causes a self-limiting febrile illness without CNS manifestations. These patients usually are neither hospitalized nor investigated further and may account for the probable underestimation of TOSV infection rates.

MENINGITIS

After an incubation period ranging from a few days to a maximum of 2 weeks, the onset of disease is brutal (70%), with headache (100%, 1 to 5 days), fever (76% to 97%), nausea and vomiting (67% to 88%), and myalgias (18%). On physical examination, neck rigidity (53% to 95%), Kernig signs (87%), poor levels of consciousness (12%), tremors (2.6%), paresis (1.7%), and nystagmus (5.2%) are apparent (Loredana Nicoletti, personal communication). In most cases reported so far, CSF samples contained more than 5 to 10 cells with normal levels of glucose and protein. Leukocytosis (29%) or leukopenia (6%) may be observed in the blood. The mean duration of the disease is 7 days, and the outcome usually is favorable.

OTHER CENTRAL NERVOUS SYSTEM MANIFESTATIONS

Although TOSV infection in most cases is a mild disease with a favorable outcome, a few severe cases have been reported in the literature. Two young brothers and a sister living in Umbria experienced TOSV infection in the form of severe meningoencephalitis with stiff neck, deep coma, maculopapular rash, diffuse lymphadenopathy, hepatosplenomegaly, renal involvement, skin rash with lamellar desquamation, tendency for bleeding, and diffuse intravascular coagulopathy. CNS manifestations occurred after 3 weeks of fever. Convalescence was marked by hydrocephalus that required a ventriculoatrial shunt. The diagnosis was established by serologic means and PCR sequencing. The occurrence of such severe manifestations in relatives suggests the existence of an immune defect, although this possibility was not tested.[3] Two cases of encephalitis without meningitis were diagnosed by serology and the detection of TOSV sequences in the CSF; for both patients, the outcome was favorable, without late manifestations.[15] One case of meningitis complicated by abducens nerve palsy was reported in a German tourist returning from Italy.[38] One patient developed chronic meningoencephalitis, with persistence of TOSV (detected by viral culture and RT-PCR) in CSF samples for more than 30 days (Navarro-Mari, J. M., personal communication).

OTHER DISEASE MANIFESTATIONS NOT INVOLVING THE CENTRAL NERVOUS SYSTEM

To date, no published data exist to suggest that TOSV could cause manifestations other than those previously mentioned. However, to our knowledge no study has been designed to investigate the potential of TOSV to cause other manifestations in humans.

DIAGNOSIS AND DIFFERENTIAL DIAGNOSIS

SEROLOGY

Immunofluorescence Assay

Seroconversion and the detection of IgG or IgM can be achieved in cells infected with TOSV. However, cross-reactivity exists

among members of the genus *Phlebovirus* and specifically between TOSV and other serotypes of Sandfly fever Naples virus.

Enzyme-Linked Immunosorbent Assay

ELISA tests have been developed with either crude antigens or purified virus obtained from infected cells. The advantage of ELISA resides in its capacity to test a large number of specimens rapidly; however, cross-reactions most likely also are observed. An ELISA test based on a recombinant nucleoprotein gene has been developed and is now available commercially from an Italian company. More recent seroprevalence studies were based on this test.[17,28]

Plaque Reduction Neutralization Test

PRNT is the test of choice when confirmation of the virus species is necessary. Therefore, careful interpretation of seroprevalence data is necessary because, in most cases, these data were obtained with ELISA or IFA tests that cannot discriminate among Sandfly fever Naples virus, Sandfly fever Sicilian virus, and TOSV.

Although seroconversion remains the serology gold standard for establishing the diagnosis of viral diseases, the presence of specific IgM in a single serum sample is considered to indicate a recent infection. This finding must be interpreted very carefully because, as demonstrated with West Nile virus and certain alphaviruses, TOSV infection may elicit specific IgM lasting for a very long time; Calisher and colleagues reported 418-day persistence in a 66-year-old immunocompetent patient.[8] This finding requires further investigation. TOSV serologic and molecular markers could be investigated concurrently with the screening of blood donors in the regions where the vector circulates and where human cases are reported. Long-term surveillance of TOSV-infected patients could indicate whether persistence of IgM is a common feature of TOSV infection or is rare. With this in mind, the presence of IgM in asymptomatic patients could result from an old infection instead of indicating an asymptomatic recent infection.[6] The major immune response elicited by TOSV infection is against the nucleoprotein. However, all sera that tested positive for IgG against TOSV also exhibited neutralizing properties.

VIRAL ISOLATION

Isolation from clinical samples can be achieved by using CSF but not serum. CSF samples yielding virus recovery by cell culture are collected in the first 2 to 4 days of the disease.

TOSV replicates in a variety of animals; intracerebral, intraperitoneal, and subcutaneous routes lead to death in the newborn mouse; intracerebral and intraperitoneal routes lead to death in the weanling mouse; in the guinea pig and rabbit, intracerebral inoculation results in paralysis and irregular death, whereas intraperitoneal inoculation is not fatal and results in antibody synthesis. *Macaca fascicularis* succumbs after intracerebral inoculation, but recovers with antibody production after intraperitoneal inoculation.[26]

TOSV replicates in Vero, BHK-21, CV-1, and SW13 cells with cytopathic effect but not in C6/36 cells.[26,54] However, cell culture appears to have a low sensitivity for detecting TOSV because only 14 percent of PCR-positive CSF specimens inoculated onto Vero cells led to viral isolation.

GENOME AMPLIFICATION BY REVERSE TRANSCIPTASE POLYMERASE CHAIN REACTION ASSAYS

In some cases, the relatively low level of virus in blood and CSF samples hampers attempts to isolate the virus. In such cases, molecular techniques based on PCR have been shown to be more sensitive than is IgM detection or viral isolation. Three different methods for establishing molecular diagnosis of TOSV have been developed. To date, all studies aimed at the molecular detection of TOSV sequences in the CSF of patients presenting with meningitis or other CNS manifestations have used classic PCR detection through single-round or nested protocols. Tests of an RT-PCR assay alone, without a further nested PCR step, showed that this method did not appear to be valid for the detection of TOSV, because no sample was positive after the first reaction. This result could stem from a very low amount of virus in CSF samples.[48] Two of the three molecular detection systems[39,48] use specific primers for amplification of regions of the S segment, and one is based on degenerate oligonucleotides designed in the L segment.[37] The most widely used system was designed by Valassina and coworkers.[48] It has been used successfully in Italy[49] and France (Charrel, R. N., et al., unpublished data) for establishing TOSV diagnoses. Their primers also have been used in combination with others able to detect enteroviruses in a fast duplex one-step RT-PCR for differential diagnosis of these major neurotropic viruses.[51] In 2003, another method for detecting TOSV was developed.[37] A nested RT-PCR for detecting TOSV of Italian or Spanish origin was produced using degenerate primers. The method is a modification of the generic method used for detecting phleboviruses and is able to detect all the members of the genus, including TOSV. The replacement of one of the generic primers used in the nested reaction by one able to detect only TOSV strains renders the diagnosis of this virus more specific. This method has been used in establishing the diagnosis of Spanish cases of TOSV infection. The description of two genotypes of TOSV according to geographic origin demonstrates a need for caution when designing molecular methods for diagnosis, to avoid obtaining false-negative results.[36] However, all these techniques are time-consuming and require sequence determination to confirm the diagnosis. One of the major drawbacks of molecular techniques, principally those requiring the manipulation of PCR tubes for gel loading and nested PCR reactions, is the risk of contamination. Real-time PCR systems, including a fluorescent dye–labeled probe, have reduced dramatically the risk of contamination because these systems rely on one-round RT-PCR assays without the need for gel loading or sequencing. The sensitivity of clinical tests for human viruses using real-time RT-PCR is close to that obtained by nested PCR protocols, and the results are obtained within 3 hours. Therefore, such technology must now be applied to TOSV diagnosis. However, to develop real-time PCR assays that detect all variants of TOSV circulating in Mediterranean countries and causing diseases in humans, a considerable amount of work on the sequence determination of strains reflecting viral heterogeneity observed in different countries will be necessary. The report of a Spanish genotype, genetically divergent from the strains circulating in Italy, which is not detected by PCR systems previously reported in Italy, underlines the requirement for a large program of strain isolation and full-length genome sequencing to achieve this goal.

GENETIC DIVERSITY OF TOSCANA VIRUS

STRAINS ISOLATED

TOSV has been isolated from sandflies of two different species, *P. perniciosus* and *P. perfiliewi*, as well as from a bat (*P. khuli*) and from humans. Whereas isolation from sandflies is quite common, at least in Italy, isolation from wild vertebrates is a rare event, and to date only one such strain has been isolated. The prototype TOSV strain, ISS Phl.3, was isolated from sandflies *P. perniciosus* in 1971. This strain has been sequenced completely and is used as reference for further genetic studies.[1,13,24] A total of 84 virus

strains was obtained from 16,374 male and female sandflies (*P. perniciosus* and *P. perfiliewi*) collected in two localities of the Tuscany region of Italy between 1980 and 1985. Thirty-seven (44%) strains were identified as TOSV and 47 (56%) were identified as a new member of the *Phlebotomus* fever serogroup, Arbia virus. The overall virus isolation rate from sandflies was 0.5 percent. Viral isolation rates for both viruses were similar in different years and in the two localities, a finding suggesting that the two virus types were active in the sandfly population simultaneously (maximum activity in July).[52] Seventeen strains of TOSV have been isolated in Spain from clinical cases.[29] Several strains have been isolated in southeastern France from clinical cases and remain to be characterized. Sequence analysis of the PCR products from Spanish TOSV isolates from phlebotomine sandflies and patients showed that homology within the nucleotide and amino acid sequences among them was 97 to 100 percent (EVITAR network). Overall, genetic characterization of the strains isolated since 1971 must be achieved through a collaborative network to address numerous unresolved questions rapidly.

GENETIC DIVERSITY

Numerous strains from Italy have been partially sequenced, and only minor differences in nucleoprotein (NP) sequences were found among strains isolated in the early 1980s from both species of sandflies, from the bat, and from humans, with no more than one amino acid substitution.[30] Similar results were described in a study on some variants in the NP gene of strains isolated from humans in the years 1995 to 1998; only one variant showed a single amino acid substitution out of an 80–amino acid region.[47] Changes in the amino acid sequence may render this protein less efficient in its interaction with the viral nucleic acid and possibly may be lethal for the virus and subject to negative selection.

A different situation has been described in Spain for partial sequences in the large segment encoding the polymerase activity. A phylogenetic analysis performed from L segment sequences obtained from 11 clinical isolates from Granada and compared with the homologous sequence of an Italian reference strain showed that Spanish sequences were closely related to one another and distantly related to the Italian strain.[37] This finding suggests the presence of at least two geographically distinct populations of TOSV. Both genetic populations of TOSV circulate in France, geographically located between Italy and Spain.

PHYLOGENY AND EVOLUTION

To date, sequence data are too scarce to perform significant phylogenetic analyses. Therefore, it will be necessary to set up an extensive program of complete genome sequencing of the strains collected in different regions and simultaneously encourage the development of viral isolation programs in all countries surrounding the Mediterranean, where vectors are circulating, to understand more clearly the genetic diversity, phylogenetic relationships, and mechanisms driving the evolution of TOSV.

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SUBSECTION 12

Retroviridae

CHAPTER 204

HUMAN RETROVIRUSES

CHAPTER 204a

ONCOVIRUSES (HUMAN T-CELL LYMPHOTROPIC VIRUSES) AND LENTIVIRUSES (HUMAN IMMUNODEFICIENCY VIRUS TYPE 2)

Susan L. Gillespie ● Gordon E. Schutze

The discovery of human T-cell lymphotropic virus type I (HTLV-I) in 1980 demonstrated for the first time that humans could be infected by retroviruses, which previously were known as animal pathogens that could cause malignant disease.[7,89,265] HTLV-I subsequently was found to cause adult T-cell leukemia/lymphoma (ATLL) and now is recognized to be associated with the following spectrum of disease manifestations: a chronic degenerative neurologic disease (HTLV-I–associated myelopathy [HAM]/tropical spastic paraparesis [TSP]); relapsing, severe generalized dermatitis in children (infective dermatitis); and numerous inflammatory or autoimmune conditions, or both, such as uveitis, arthropathy, polymyositis, bronchoalveolitis, and Sjögren syndrome.[43,193,208,306] In 1982, a second antigenically related retrovirus of the oncovirus subfamily, HTLV-II, was identified from a patient with a T-cell variant of hairy-cell leukemia.[148] The pathogenicity of this virus remains unclear, although the virus has been found in some individuals with $CD8^+$ lymphoproliferative diseases and neuromyopathies.[93] The more recent discovery of HTLV-III and HTLV-IV demonstrated that diversity among these viruses is greater than previously understood.[373]

This chapter retains selected contributions previously made by Lynne Mofensen.

CLASSIFICATION

Retroviruses form a family of single-stranded RNA viruses that are unusual because they contain a diploid RNA genome that replicates by flow of genetic information through a DNA intermediate, a process known as reverse transcription. The name *retrovirus* is derived from this characteristic. This unique capability results from the presence of a virally encoded, RNA-dependent DNA polymerase, reverse transcriptase, that catalyzes transcription of viral RNA into a double-stranded DNA copy. This viral DNA intermediary becomes integrated into host-cell DNA, where it then resides as a provirus; this process occurs by a specialized recombination mechanism requiring another viral protein, integrase. This capacity for genomic integration

correlates with the capability of retroviruses to cause lifelong infection, evade the usual mechanisms of immune clearance, and produce chronic diseases in the host that manifest only after a long asymptomatic period that may last years to decades. Retroviruses infect both animals and humans.

Retroviruses that infect humans have a more complicated genome that encodes for numerous regulatory genes involved in modulating viral replication. These retroviruses were divided historically into three subfamilies on the basis of nucleotide sequences and genetic organization. The subfamilies were the *Oncovirinae* (oncogenic or transforming viruses, which include HTLV), the *Lentivirinae* (slow viruses with cytopathic effects, which include human immunodeficiency virus types 1 [HIV-1] and 2 [HIV-2]), and the *Spumavirinae* (foamy viruses).

Although they share some similarities in genomic structure and life cycle, the different subfamilies of retroviruses have distinct in vitro and in vivo effects and different strategies for evading host immunity. Oncoviruses generally transform cells in culture, stimulate target-cell proliferation, and cause tumors in their hosts. Lentiviruses cause cell fusion and multinucleated giant-cell formation, are cytopathic in cell culture, and cause slow infections characterized by immunodeficiency in their hosts. The spumaviruses behave like other retroviruses because their viral RNA is transcribed into DNA and then integrated into the host genome. The reverse transcriptase step, however, occurs during budding and assembly, so the genome that is produced is actually DNA, as in the hepadnaviruses. These data suggest that spumaviruses may represent a link between hepadnaviruses and retroviruses.[229] Although no case of spumaviral disease in any natural or human host has been reported, concern exists about the potential threat of human disease because primate workers known to be infected with these viruses have donated blood in the United States.[115] Further data will need to be obtained to understand the potential threat these viruses may represent in the future.

MORPHOLOGY AND GENOMIC STRUCTURE

Retroviruses have a distinct morphology. They are enveloped RNA viruses that have diameters of 80 to 120 nm, a thin, electron-dense outer envelope, and an electron-dense core that is either spherical (HTLV) or cylindric (HIV). The envelope of all retroviruses is composed of a lipid bilayer derived from the host-cell plasma membrane during budding of the virus from the cell surface, with surface projections consisting of the viral envelope proteins (Fig. 204–1). The retroviral core protein encloses a ribonucleoprotein of genomic RNA complexed with viral reverse transcriptase and integrase.[49] The genome is a messenger-sense, linear, single-stranded RNA composed of two identical subunits held together by hydrogen bonds at their 5′ ends. The 5′ and 3′ ends of the RNA contain repeated sequences that give rise to elements in viral DNA called long terminal repeats (LTRs).

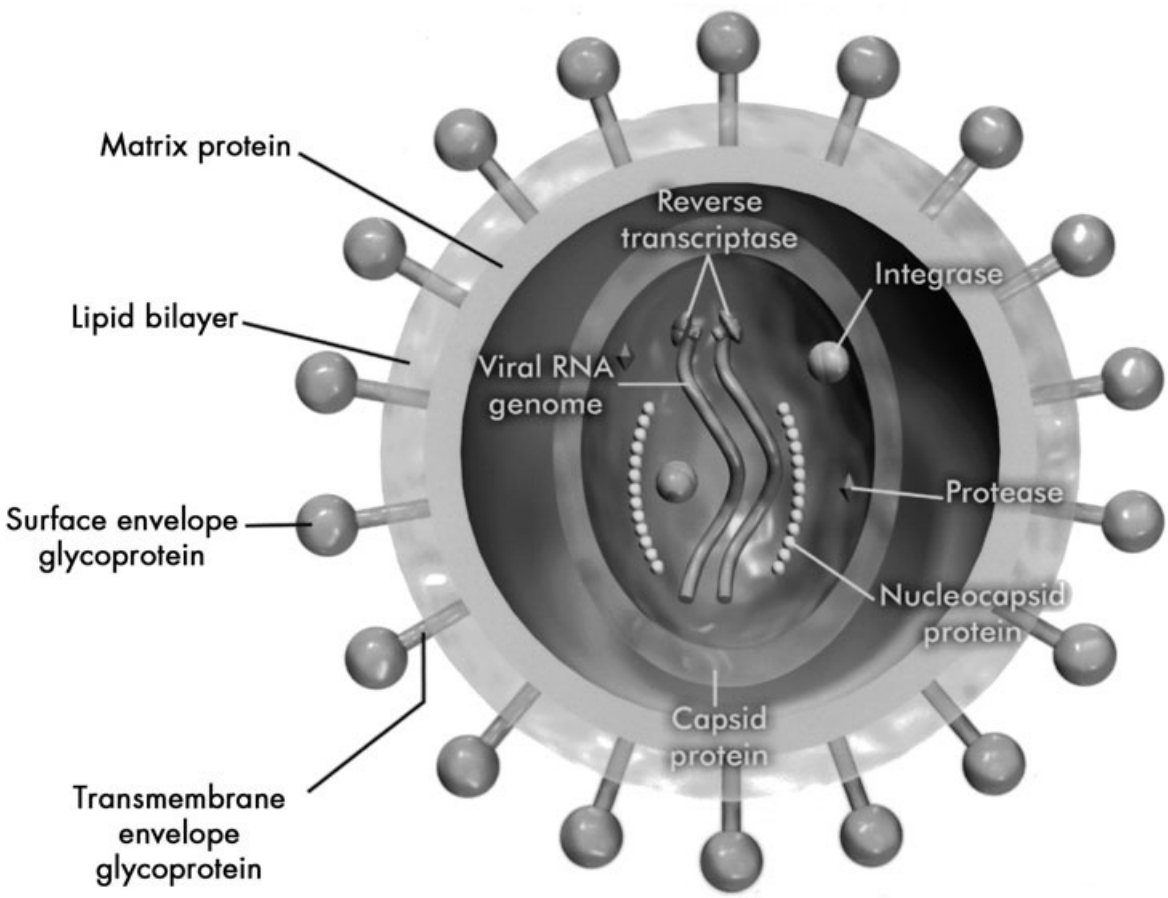

Figure 204–1 Retroviral structure. The mature retroviral virion is spherical; the central viral core is spherical in HTLV oncoviruses and cylindric in HIV lentiviruses. The core is surrounded by a lipid bilayer envelope derived from the host-cell membrane during bussing, the surface projections consisting of the viral surface and transmembrane envelope proteins. The viral matrix protein surrounds the virion core and is associated with the viral transmembrane envelope glycoproteins. The virion core is a structural shell composed of the viral capsid proteins. Within the shell are two copies of single-stranded viral RNA and multiple copies of the virally encoded reverse transcriptase, protease, and integrase enzymes. The viral nucleocapsid protein is bound to the RNA copies and may serve to condense the viral RNA into the capsid shell during virion assembly.

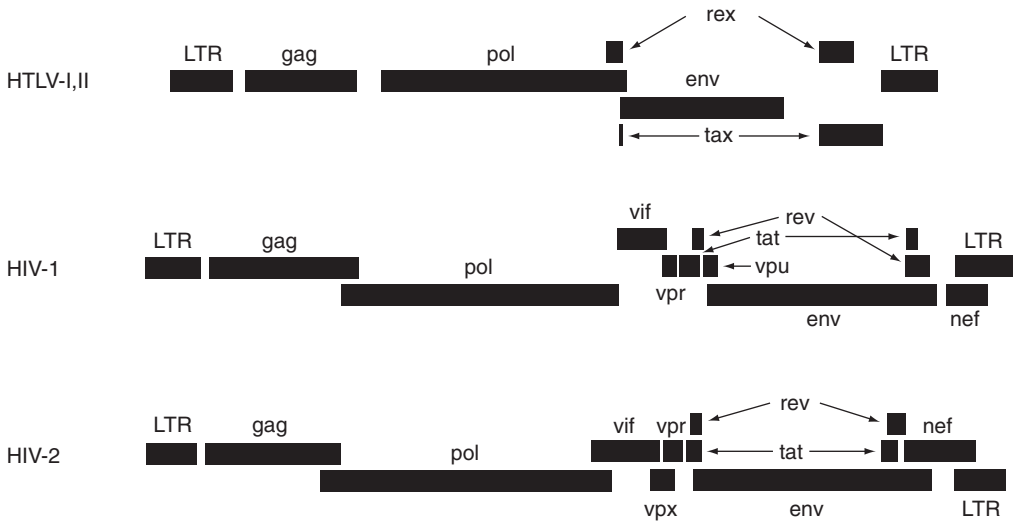

Figure 204–2 Retroviral genome. This schematic depiction of the human T-cell lymphotrophic virus (HTLV) and human immunodeficiency virus (HIV) genomes shows that HTLV has a more complex genome than most animal retroviruses do and that HIV, in turn, has additional regulatory genes. Details of the functions of these genes are summarized in the text. LTR, long terminal repeat. *(Reprinted by permission from Gallo, R. C., Wong-Staal, F., Montagnier, L., et al.: HIV/HTLV gene nomenclature. Nature 333:504, 1988. Copyright Macmillan Magazines, Ltd.)*

The LTRs are composed of nucleic acid sequences that provide signals critical for structural transformation of the viral genome, including initiation and progression of reverse transcription of viral RNA into DNA, integration of viral DNA into the host genome by viral integrase, and initiation of viral mRNA transcription from the integrated provirus and binding sites for viral and cellular proteins and that positively and negatively influence mRNA transcription.[273,328,340,374] Between the LTRs are the genes that encode the major structural proteins of the virus, the enzymes found in the viral particles, and additional proteins with specialized intracellular functions.

As noted earlier, all retroviruses contain a minimum of three genes, *gag* (group-specific antigen), *pol* (polymerase), and *env* (envelope). They are arranged in a 5′ to 3′ order, with LTRs at each end (Fig. 204–2).[105] The *gag* gene encodes the structural protein products that form the core particle of the virus, including nucleocapsid, capsid, and matrix proteins. The *pol* gene products include the enzymes required for genome replication (viral RNA-dependent DNA polymerase and ribonuclease H), proviral integration (integrase), and polyprotein processing (protease). The *env* gene encodes the major components of the viral coat, the surface and transmembrane glycoproteins. Retroviral genes generally are expressed first as large overlapping polyproteins that undergo processing into functional peptide products by viral or cellular proteases.

ONCOVIRAL REGULATORY AND ACCESSORY GENES

In addition to the standard retroviral genes, the oncoviruses HTLV-I and HTLV-II contain several regulatory and accessory genes important for viral replication and activation of host genes that are encoded in open-reading frames (ORFs) in a unique region at the 3′ end of the genome called the pX region.[6,134] The HTLV-I pX region contains four ORFs, whereas HTLV-II contains five ORFs. Two of these ORFs common to both viruses (ORF III and ORF IV) encode the regulatory proteins Rex and Tax.

The *rex* (regulator of expression of virion proteins) gene encodes a nucleolus-localizing phosphoprotein that affects mRNA splicing and export from the nucleus to the cytoplasm. For cellular genes, mRNA splicing and export are tightly coupled. After cellular genes are transcribed into mRNA, splicing occurs in the cell nucleus before mRNA can be exported into the cytoplasm for translation into proteins. Incompletely spliced mRNA molecules are retained in the nucleus.[206,328] However, for retroviral reproduction, the export of full-length, unspliced viral RNA into the cytoplasm is required to serve as genomic RNA for integration into progeny virions and to serve as mRNA for the production of viral structural proteins. Thus, retroviruses had to develop a mechanism to bypass the cellular regulatory process; for HTLV, the Rex protein serves this function. This protein is expressed predominantly at an early stage of viral gene expression and enhances the transport of single-spliced and unspliced viral mRNA coding for structural proteins, as well as transport of the viral genome from the nucleus to the cytoplasm. The Rex protein also exerts a negative effect on the transport of multiply spliced viral mRNA to the cytoplasm. These multiply spliced mRNA molecules code for regulatory proteins, including Rex and Tax.[340]

The *tax* (transactivator) gene encodes a nuclear protein that plays a critical role in the regulation of viral replication and also stimulates a large number of cellular genes involved in activation and proliferation of T cells, including lymphokines, lymphokine receptors, and nuclear proto-oncogenes.[381,382] The Tax protein does not bind directly to DNA but rather interacts with host-cell transcriptional factors to produce a multifaceted array of molecular effects.[22] The Tax protein activates mRNA transcription by binding to several different host-cell transcription-enhancing factors, including those of the nuclear factor NF-κB family. In addition to activating transcription of the HTLV genome, the Tax transcription factor complex acts to stimulate transcription of many cellular genes, including those encoding interleukin-2 (IL-2), IL-2 receptor-α, tumor necrosis factor, cyclooxygenase 2 (a prostaglandin synthetase), and some nuclear oncogenes.[215,381,382] Tax also represses transcription of tumor suppression genes and the expression of cellular DNA polymerase-β, a key enzyme for repair of damaged DNA. Additionally, Tax can bind directly to and inhibit some cell cycle regulation and tumor suppressor proteins, thereby interfering with cell cycle regulation and resulting in abnormal promotion of the cell cycle and enhancement of cellular proliferation.[382] Finally, Tax increases the genetic instability of the cell by impairing cellular DNA repair mechanisms, a process that leads to an accumulation of mutations and an increase in chromosomal anomalies. In vitro, Tax protein can immortalize human T cells and induce tumors in transgenic mice. However, in vivo, malignant disease develops in fewer than 5 percent of HTLV-1–infected individuals. Thus, although the *tax*

gene probably is involved in malignant transformation of infected T cells, it is not sufficient by itself to explain the final development of malignancy.[8,343,381,382]

Less is known about the function of the HTLV accessory proteins that are encoded by the remaining ORFs in the pX region (e.g., p12, p13, and p30 in HTLV-I and p10, p11, p22/20, and p28 in HTLV-II). The best characterized accessory protein is HTLV-I p12 from ORF I, which is a conserved, hydrophobic protein localized to the internal cell plasma membrane and perinuclear regions of the cell.[44] The p12 protein contains amino acid motifs commonly found in proteins involved in intracellular signaling pathways and probably interacts with cellular proteins, perhaps cellular kinases, to modulate intracellular signaling in infected cells. The protein also interacts with the β and γ chains of the IL-2 receptor. This protein may be required for activation of host cells during the early stages of infection, for cellular transformation, and for efficient viral infectivity, similar to the function of the *nef* gene of the lentivirus HIV-1.[4] The corresponding ORF I protein in HTLV-II, p10, does not bind the IL-2 receptor and is not associated with the constitutive activation of the IL-2 signaling pathway that is seen with HTLV-I–transformed cells.[44] These differences may be associated with differences in pathogenicity between the two oncoviruses.

LENTIVIRAL REGULATORY AND ACCESSORY GENES

More detailed data are available about the function of lentiviral genes.[73] The lentiviruses HIV-1 and HIV-2 have more complex genomes containing at least six regulatory genes in addition to *env*, *gag*, and *pol*. The lentiviral genes corresponding to the oncoviral *tax* and *rex* are called *tat* and *rev*; these genes are found in all known human and animal lentiviruses and appear to be essential for replication.[206]

Like the oncoviral *rex* gene, *rev* is expressed early in the viral replication cycle and undergoes splicing in the nucleus; multiply spliced *rev* mRNA transcripts then are exported to produce Rev protein in the cytoplasm. This protein contains a nuclear localization signal that facilitates entry of the protein back into the nucleus. Rev then binds to unspliced viral mRNA at a *cis*-acting Rev response element (RRE) located on the mRNA. A nuclear export signal sequence on the carboxy-terminal domain of Rev next targets the unspliced viral mRNA for export to the cytoplasm by binding to the cellular protein CRM1 (exportin 1) and exiting the nucleus through the host-cell export pathway.[53] The full-length unspliced viral mRNA then serves as a translational template for expression of the Gag and Pol proteins and also serves as genomic RNA for incorporation into new virions.

In contrast to the oncoviral Tax protein, which binds to host-cell proteins and has multiple effects in addition to promoting initiation of HTLV gene transcription, the lentiviral Tat protein binds to an RNA target, the Tat activation region (TAR), located immediately 3′ to the LTR transcription start site, and plays a primary role in the expression of HIV viral transcripts by enhancing mRNA elongation. At least two host-cell cofactors are involved in this process.[328] Cellular cyclin T binds to the activation domain of Tat, which increases both the affinity and specificity of the resulting complex for TAR. A host-cell encoded kinase then is recruited to the Tat-cyclin T-TAR complex and phosphorylates the carboxyl-terminal domain of the host-cell RNA polymerase II, which in turn enhances mRNA transcript elongation.

The remaining lentiviral accessory regulatory genes *nef*, *vpr*, and *vif* provide fine-tuning by enhancing or, less commonly, by diminishing viral replication. Another accessory gene, *vpu*, is found only in HIV-1, whereas HIV-2 and simian immunodeficiency virus (SIV) lack the *vpu* gene but contain a gene called *vpx*. The *nef* gene, like *tat* and *rev*, is expressed early in the replication cycle. Similar to the HTLV-I p12 protein, *nef* encodes a hydrophobic, membrane-associated protein that probably interacts with and modulates cellular signaling pathways and is required for optimal infectivity in vivo and for activation of infected host cells. Nef protein significantly enhances the cytoplasmic delivery of viral particles entering the cell through fusion at the plasma membrane, possibly by enhancing phosphorylation of the viral matrix protein and thereby allowing dissociation of the matrix and associated pre-integration nucleoprotein complex from the virion capsid proteins.[292,328] Nef also down-regulates cell surface expression of $CD4^+$ by accelerating $CD4^+$ receptor endocytosis through interaction with the cytoplasmic tail of $CD4^+$ and a protein complex (AP-2 adaptor complex) responsible for recruiting membrane-associated proteins to clathrin-coated pits for endocytosis.[73] This process reduces the potential interference of membrane-associated $CD4^+$ with the release of budding virions. Major histocompatibility complex (MHC) class I receptor expression also is down-regulated by Nef.

The Vpr protein is present in the virion itself, may act to regulate cellular events after penetration and uncoating of the virus to facilitate viral replication, and is involved in efficient localization of the nucleoprotein pre-integration complex into the nucleus.[49,114] Vpr appears to interact with a specific site on nucleoporins in the nuclear pore complex to facilitate nuclear entry.[328] Similar to the Vpr protein, the HIV-2 Vpx protein is found in the virion and appears to affect the efficiency of early replication events, but the precise mechanism is unknown.[49,179]

The *vif* (virion infectivity factor) accessory gene is expressed late, along with structural and enzymatic proteins. The Vif protein is predominantly cytoplasmic but also exists in a membrane-associated form.[206] Vif protein is phosphorylated by a host-cell protein kinase, which seems to be important for viral growth in primary cells.[104] Vif appears to stabilize the pre-integration provirus; viral particles that are produced in the absence of Vif are incapable of incorporating the provirus into host-cell chromosomes.[206] Vif also may function to prevent premature processing of Gag precursor proteins by viral protease in the cytoplasm, thereby ensuring that the Gag-derived peptides that form the viral nucleoprotein core are available at the plasma membrane for assembly with other viral components.

The HIV-1 *vpu* gene also is expressed late. The Vpu protein is associated with the endoplasmic reticulum in infected cells, where it binds the $CD4^+$ molecule and prevents it from translocating to the plasma membrane; instead, the $CD4^+$ molecule is targeted for proteolysis through the cytoplasmic ubiquitin-proteasome pathway.[73,328] This process prevents trapping and subsequent degradation of HIV envelope proteins by $CD4^+$ in the cytoplasm, thus increasing the ability of the viral envelope proteins to reach the cell membrane. Vpu, like Nef, also down-regulates MHC class I receptor expression. In addition, Vpu may be important in virion assembly and release of particles.[45,92,354]

VIRAL REPLICATION

The lentiviruses can infect and replicate in nondividing, terminally differentiated cells, and transmission can occur by cell-free or cell-associated virus. HIV-1 has high replication rates in vivo, with an average of 10^9 to 10^{10} viral particles produced each day. Free viral particles are estimated to have a half-life of less than 6 hours, and productively infected cells have a half-life of approximately 1 day.[132,243,363] The relentless rounds of reverse transcription rapidly generate extensive viral genetic variation within a single individual that results in a genetically related "swarm," or quasi-species, of viruses and provides the ability to escape the host immune response rapidly.

In contrast, the oncoviruses require that the host cell undergo division for productive infection to be established, and they replicate in vivo largely by mitosis rather than by reverse transcription.[195] In addition, transmission is predominantly, if not solely, accomplished by means of cell-cell contact.[60] The events that occur during primary HTLV infection are reverse transcription of the viral genome, integration of the provirus into DNA, transcription of mRNA, synthesis of viral proteins, and a burst of viral expression. However, after a period of active replication, viral persistence is facilitated by a prolonged phase of clonal proviral expansion produced by transactivation of host-cell genes by Tax, which results in cellular DNA replication and the production of new cells containing the HTLV proviral genome.[218] Unlike viral nucleic acid replication by the error-prone viral reverse transcriptase, cellular mitosis is much less prone to mutation and generates little viral genetic variation. Moreover, the provirus remains hidden from immune surveillance. Thus, oncoviruses have extraordinary genetic stability in comparison with lentiviruses. The genetic variability of the HIV-1 envelope protein that exists within the viral quasi-species in a single individual 5 years after infection is greater than the genetic variability found in all HTLV-I proteins identified worldwide to date.[360]

These differences are related to divergence among the retroviral subfamilies in genetic organization, transcription, and function of the major regulatory genes (*tax* and *rex* for the HTLVs and *tat* and *rev* for HIVs). The Tax and Tat proteins both are transactivating enhancer proteins. However, the HTLV Tax protein interacts with cellular proteins and activates cellular genes, thereby resulting in disruption of the cell cycle and induction of cell proliferation. In contrast, the HIV Tat protein has more focal effects; it recognizes a small RNA domain in the HIV LTR and serves primarily to transactivate HIV replication. The Rex and Rev proteins have similar functions; both interact with genomic mRNA structures (the Rex responsive element and Rev responsive element) to enhance transport of the unspliced and partly spliced viral mRNA molecules from the nucleus to the cytoplasm, and both also down-regulate the transport of multiply spliced mRNA transcripts that encode for early regulatory proteins, including the mRNA molecules coding for themselves. In the oncoviruses, Tax and Rex are derived from the same mRNA, and, therefore, down-regulation of Rex mRNA transport results in decreased Tax mRNA transport and protein production. This process leads to diminished transactivation of HTLV genome transcription and prevents massive viral production. In contrast, the HIV Tat protein comes in two functionally equivalent forms, a 72- and an 86-residue protein. The 72-residue Tat protein is derived from a joint Rev/Tat mRNA, but the 86-residue Tat protein comes from a small Rev-independent mRNA. Although Rev, like Rex, has a negative effect on its own expression, as well as that of Tat, this effect is mitigated by the expression of a Tat mRNA that is not dependent on expression of Rev mRNA, thereby resulting in continued production of the Tat transactivating protein and constant and massive transcription of the HIV genome.

RETROVIRAL LIFE CYCLE

All retroviruses have two phases in their life cycle: an infection phase (including viral attachment, entry, reverse transcription, and proviral integration) and an expression phase (including transcription, translation, assembly, and budding of the virion) (Fig. 204–3).[49] In general, more detail is known about replication in lentiviruses than in oncoviruses.

Infection Phase of Retroviral Replication

Retroviruses attach to cells through recognition and binding of viral outer surface envelope proteins to specific proteins on the surface of the host cell; such cell surface receptor specificity probably accounts for the species and cellular tropism of retroviruses.[145,239] The surface glycoprotein of the retroviral envelope has three structural and functional domains separated by conserved hinges and is noncovalently linked to the transmembrane protein by disulfide bonds. The envelope transmembrane protein anchors the entire envelope glycoprotein complex on the virion surface and is responsible for the fusogenic capacity of the viral envelope. The transmembrane protein of all retroviruses has several conserved motifs, including a "leucine zipper" coiled motif followed by the N-terminal hydrophobic "fusion peptide" responsible for fusion with the host-cell membrane. After binding of the viral surface protein to the host-cell receptor occurs, conformational changes permit correct exposure of the transmembrane fusion peptide to the host-cell membrane. The interaction of several fusion peptides may serve to destabilize the lipid bilayer of the target-cell membrane by forming a "fusion pore" between the two bilayers[82] to facilitate fusion of the viral and cellular membranes and release of the viral RNA-protein complex into the cytoplasm.[103,317,343]

The cell surface receptor for HTLV-I and HTLV-II has not been characterized completely. Oncoviruses transmit infection primarily by fusion of infected cells with uninfected cells and the subsequent formation of syncytia. In HTLV-I, fusion is thought to be mediated by the HTLV envelope proteins expressed on the surface of infected cells and by cell adhesion molecules, such as vascular cell adhesion molecule type 1 (VCAM-1), intracellular adhesion molecule type 1 (ICAM-1), ICAM-3, and the membrane permease CD98, on target-cell surfaces.[22] Although HTLV-I envelope–expressing infected cells can form syncytia with cells from most cell lines, in vivo, HTLV-1 is found only in $CD4^+$ lymphocytes.[60] In contrast, HTLV-II displays a preferential tropism for cells of the $CD8^+$ T-lymphocyte phenotype.[139,172]

The lentiviruses HIV-1 and HIV-2 infect cells that express $CD4^+$, the human leukocyte antigen (HLA) class II receptor, on their cell surface.[239] These cells include $CD4^+$ T lymphocytes, cells of the monocyte/macrophage lineage, and microglia in the brain. A high-affinity interaction between the surface glycoprotein and the $CD4^+$ receptor produces a conformational change that results in exposure of another site with high affinity for a secondary co-receptor required for the entry of HIV-1 and some HIV-2 isolates into the cell. The chemokine receptor CCR5 is the co-receptor used by monocyte/macrophage-tropic HIV-1, whereas the CXCR4 chemokine receptor is used by T-lymphocyte-tropic HIV-1; although most HIV-1 strains use only one receptor, some strains of HIV-1 are dually tropic and can use both receptors.[30,63,70,246] Additional co-receptors may support the entry of more restricted types of HIV isolates. For example, the chemokine receptor CCR3 is used by some macrophage-tropic HIV-1 strains and mediates entry of the virus into microglia; CCR2b, CR8, STLR/STRL-22, GPR15, GPR1, and the cytomegalovirus protein US28 also support entry by various HIV-1 and HIV-2 strains.[110,328] Whereas nearly all strains of HIV-2 can use $CD4^+$ together with the chemokine receptor CCR5 or CXCR4 (or both), most HIV-2 isolates also can interact with multiple other co-receptors.[216,296] Additionally, some HIV-2 isolates can use CXCR4 or other receptors, such as CCR3, as a primary receptor and infect cells lacking the $CD4^+$ molecule.[74]

Early post-entry events include uncoating of the virus, which appears to require interaction of viral structural proteins with host-cell components that may have been incorporated into the virion during viral assembly. In HIV-1, interaction of the viral capsid protein with the host-cell cyclophilin A that is associated with the virion core during viral assembly may destabilize the multimeric capsid complex.[180,328] Additionally, a virion-associated host-cell protein kinase phosphorylates the viral matrix protein and thereby allows dissociation of the capsid and nucleocapsid proteins from the rest of the virus at the cell membrane. The

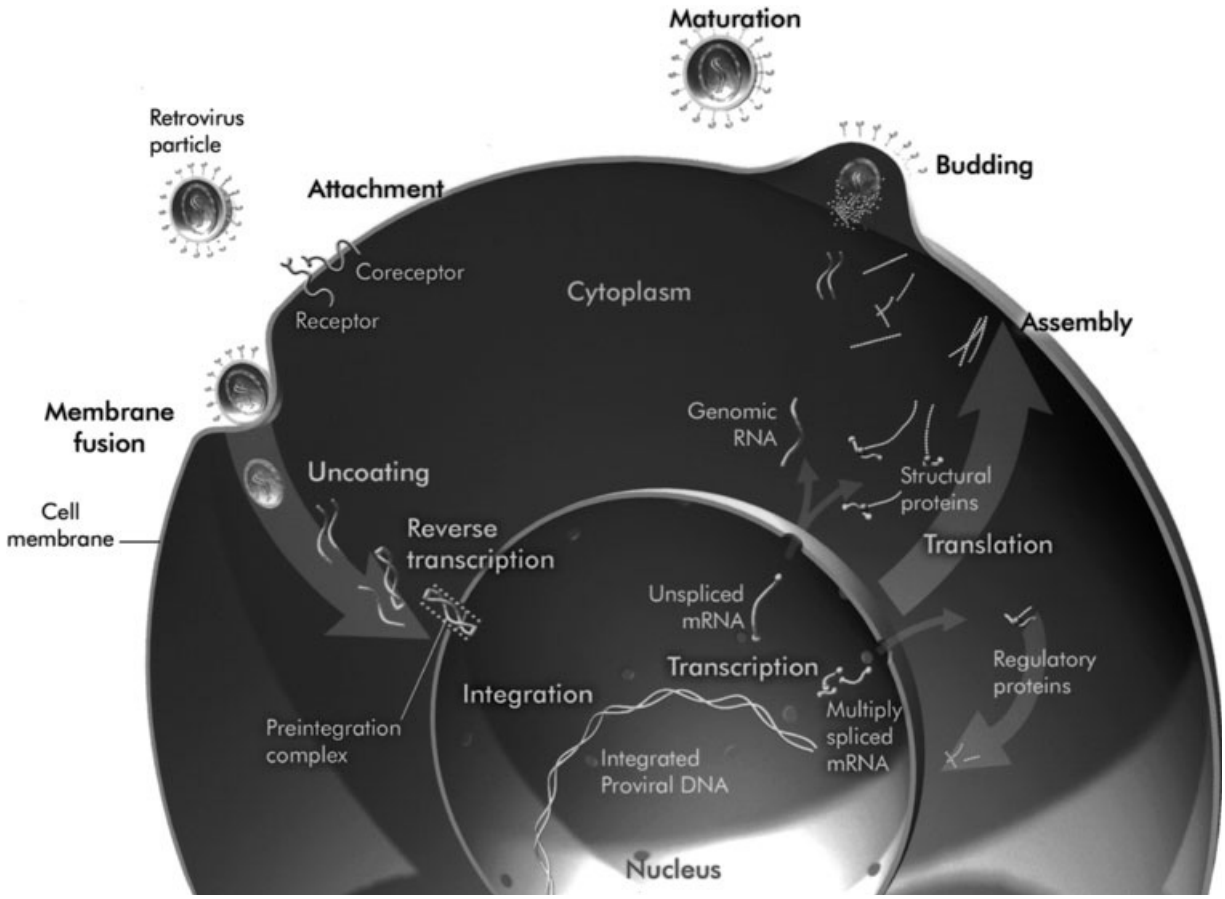

Figure 204–3 Life cycle of retroviruses. Details of viral replication are discussed in the text. *(Adapted from Furtado, M. R., Callaway, D. S., Phair, J. P., et al.: Persistence of HIV-1 transcription in peripheral blood mononuclear cells in patients receiving potent antiretroviral therapy. N. Engl. J. Med.* 340:*1620, 1999.)*

HIV-1 Vif protein, which co-localizes with the Gag-derived proteins, is thought to confer enhanced stability to the remaining cytoplasmic viral nucleoprotein complex.

Retroviral RNA-to-DNA transcriptional events are initiated in the cytoplasm through the action of the viral enzymes reverse transcriptase (RNA-directed DNA polymerase) and ribonuclease. Viral single-stranded RNA is transcribed into a viral RNA/DNA hybrid complex by the DNA polymerase; the ribonuclease then destroys the original RNA and permits the DNA polymerase to complete transcription of a second DNA strand to form a linear double-stranded DNA copy of the original viral RNA.[103] Retroviral integration is mediated by a large nucleoprotein complex that forms in the cytoplasm and is called the *pre-integration complex*. This complex includes the viral nucleic acids and a subset of viral proteins, including the matrix protein, reverse transcriptase, integrase, and, in the lentiviruses, Vpr.[328] The viral integrase enzyme component of this complex recognizes specific sequences within the viral LTR and cleaves two nucleotides from the 3′ ends as an initial processing step for integration; this process occurs while the pre-integration complex is still in the cytoplasm.[14] Integrase appears to be the principal viral determinant of integration specificity.[174] In the lentiviruses, which can infect nondividing cells, the pre-integration complex is imported actively into the nucleus during interphase. Nuclear transport is mediated by nuclear localization sequences on the matrix protein and on integrase and by interactions of Vpr with nucleoporins in the nuclear pore complex.

Once nuclear entry has been gained, the integrase joins the recessed 3′ ends to phosphates in the host DNA for insertion of the DNA duplex into the host genome.[14,50] A final step in integration is thought to involve cellular factors involved in DNA repair, in particular, a DNA-dependent protein kinase. This kinase is hypothesized to repair gaps left by the integrase around the sequence inserted into the host DNA; the result is a fully integrated provirus.[50,56]

Expression Phase of Retroviral Replication

Once viral DNA is integrated into the host cell, the proviral genome resembles other cellular genes and becomes highly dependent on the host cell for further replication, with the host-cell machinery used for replication, expression, and production of protein. Regulation of transcription is a complex process requiring interaction among the integrated proviral LTRs, host-cell DNA transcription factors, and the lentiviral Tat protein or oncoviral Tax protein.

The early phase of viral gene expression is characterized by the presence of spliced and unspliced viral mRNA in the nucleus, but only multiply spliced mRNA in the cytoplasm. In HTLVs, these mRNA molecules serve to direct production of the Tax and Rex regulatory proteins; in HIVs, they direct production of the Tat, Rev, and Nef proteins. The transition from early regulatory to late structural gene expression is characterized by the selective transport of unspliced and partially spliced mRNA into the cytoplasm; as discussed previously, in lentiviruses, this process depends on sufficient amounts of the Rev protein and, in oncoviruses, the Rex protein.[49,343] The unspliced or partially spiced mRNA transcripts that enter the cytoplasm encode for structural proteins

and are first expressed as large overlapping polyproteins. These polyproteins undergo processing into functional peptide viral structural components by the action of virally encoded and host-cell protease enzymes.

The retroviral Env protein is synthesized as a large precursor protein; it undergoes initial glycosylation in the endoplasmic reticulum, with subsequent disulfide bonding, folding, and oligomerization, and then is transported to the Golgi complex, where it is cleaved by a host-cell protease into surface and transmembrane proteins, which undergo further glycosylation.[60] The two mature glycoproteins are noncovalently associated through disulfide bonds. After cleavage occurs, the complexed proteins are transported and inserted into the plasma cell membrane by the cellular secretory pathway.[81]

The retroviral *gag* and *pol* genes are transcribed into a single mRNA transcript, which subsequently is translated into two separate polyprotein precursors, the Gag and Gag-Pol precursor proteins; the Gag-Pol polyprotein is translated by a ribosomal frame shift between the Gag and Pol mRNA reading frames. The Pol component of the Gag-Pol precursor protein is cleaved to form the viral enzymes reverse transcriptase, ribonuclease, and protease. The Gag precursor encodes the major structural proteins of the virion core, which do not undergo proteolytic processing into mature proteins until after assembly of the immature virion at the plasma membrane occurs.

Virion assembly and budding occur through targeting, accumulation, and association of different domains of the Gag and Gag-Pol precursor proteins at the inner face of the plasma membrane where the envelope proteins are being expressed.[81,83,275,353] The M domain, an amino acid sequence contained in the N-terminal area of the matrix portion of the Gag protein, has a membrane-targeting sequence that directs the Gag and Gag-Pol polyproteins to the cytoplasmic face of the plasma membrane. Myristylation of the N-terminal portion of some (lentiviruses) or all (oncoviruses) of the Gag precursor protein matrix domain is required for membrane targeting to occur and to allow Gag protein assembly at the membrane. In lentiviruses, the matrix domain interacts with the long cytoplasmic tail of the viral envelope transmembrane protein inserted into the membrane of the cell.[83] The I domain, an amino acid sequence contained in the nucleocapsid portion of the Gag polyprotein, participates in the Gag-Gag interactions to promote protein polymerization and also appears to be required for incorporation of the Gag-Pol precursor into virions.[81] The nucleocapsid domain of the Gag polyprotein interacts with viral genomic RNA through specific (sequence-specific nucleic acid binding) and nonspecific (interaction of basic residues with RNA) mechanisms and plays an important role in RNA binding, dimerization, and encapsidation and in facilitating Gag protein interactions and packaging into tight complexes.[48,81]

The interactions of these three domains in the precursor protein thus lead to assembly and emergence (but not release) of the viral "bud" from the cell surface.[260] On electron microscopy, large, electron-dense patches of Gag multimers are visualized under the plasma membrane that deform the membrane outward as they grow. More advanced intermediates appear as spheres connected to the cell by a thin stalk. During the process of budding, substantial amounts of cellular surface antigens, such as β_2-microglobulin and HLA-DR, are incorporated into the viral envelope. Additionally, during the process of virion assembly, host-cell proteins may be incorporated into the virion. For example, in HIV-1, a host-cell protein, cyclophilin A, interacts with the Gag polyprotein to form a complex in the virion core.[80,332] This host factor appears to be required for the formation of infectious virions, and it may play a role in early events after viral entry, as discussed earlier.[328]

In addition to the capsid, nucleocapsid, and matrix protein domains, other polypeptide segments that are required during the late stages of viral assembly are contained in the Gag polyprotein. They are referred to collectively as "late assembly" or "L" domains, and they are found in both lentiviruses and oncoviruses. In lentiviruses, the C-terminal portion of Gag (p6) includes a highly conserved amino acid motif that is needed to recruit the cellular machinery required for efficient release of budding virus from the cell membrane.[260,298,313,353] The Gag protein of oncoviruses also contains a late domain, although it is located in the N-terminal region of the protein.[313] Some investigators have suggested that these domains engage in interactions with host proteins that are located at the plasma membrane and that facilitate final release of the budding virus from the cell.

In HIV-1, the late domain is thought to interact with the cellular protein ubiquitin. Ubiquitin is a small protein present in cells that, together with cellular proteasomes, is involved in collecting or destroying cellular proteins that are damaged or no longer needed; it exists in cells as a free molecule or is covalently attached to lysines in a variety of proteins. When a protein is linked to multiple ubiquitin molecules (polyubiquitination), the protein becomes targeted for degradation by cellular proteasomes. However, when a protein is linked to a single ubiquitin molecule (mono-ubiquitination), instead of degradation, protein function is modulated; for example, mono-ubiquitination of plasma membrane receptor proteins promotes internalization and down-regulation of the receptor through endocytosis in a nonproteolytic process that has some similarities to virus budding. After protein degradation occurs, the ubiquitin molecule is recycled as a free molecule. HIV-1 particles have been shown to contain ubiquitin, predominantly as a free molecule, but 2 to 5 percent of the Gag-derived, late assembly domain protein p6 is mono-ubiquitinated.[313] One hypothesis is that the late domain of Gag contains an amino acid sequence that binds to residues on the ubiquitin ligase enzyme and thereby results in mono-ubiquitination of the Gag protein. The Gag-ubiquitin conjugate then may attract undefined cellular factors, perhaps those normally involved in endocytosis, that trigger release of the retrovirus from the cell membrane.[260] In the absence of the late domain, an infected cell becomes covered with virus that remains tethered to the plasma membrane by narrow stalks.

Maturation of the virion requires cleavage of the Gag precursor by the virally encoded protease and triggers structural changes that produce mature virions with the characteristic spherical oncoviral or cylindric lentiviral capsid. The Gag polyprotein gives rise to the following: the matrix protein, which lines the inner face of the viral membrane; the capsid protein, which forms a core shell surrounding the viral RNA genome and its associated proteins; and the nucleocapsid protein, which coats and condenses the RNA genome. This step occurs concomitantly with or immediately after the external budding process.

GENERAL METHODS OF DIAGNOSIS OF RETROVIRAL INFECTION

Detection of retroviral antibody has been the test most widely used to diagnose infection in older children and adults. However, because of transplacental passage of maternal antibody, antibody testing during infancy is not diagnostic of infection, and direct viral detection methods are necessary for establishing the diagnosis. In addition, during primary infection, a window period exists during which ongoing viral replication is detectable by virologic tests, but antibody tests will be negative or indeterminant because a detectable antibody response has not developed.

Antibody testing for retroviruses most frequently involves the use of an initial screening test followed by performance of a more specific confirmatory test. Initial screening generally involves the detection of viral antigens from disrupted whole virus or synthetic or recombinant viral antigens in an enzyme-linked

immunosorbent assay (ELISA). A positive ELISA is confirmed by the more specific immunoblot (or Western blot), which measures the presence of antibodies to a number of virus-associated proteins, both structural and nonstructural. Viral antigens (from disrupted whole virus or synthetic or recombinant antigens) undergo electrophoresis through polyacrylamide gel to separate the antigens by size. The separated antigens then are transferred to nitrocellulose paper, incubated with the patient's serum, and subsequently incubated with enzyme-linked antihuman antibody and chromogenic substrate, which results in visible bands where patient antibodies are bound by antigens. Analysis of band patterns permits more specific identification of the virus. Other techniques used to measure antibodies to retroviruses include indirect immunofluorescence assay (IFA), radioimmunoprecipitation assay (RIPA), and biologic assays for antibody to envelope glycoproteins (neutralization or syncytium inhibition assays).

Because antigenic differences exist between HIV-1 and HIV-2, HIV-1 screening tests are not reliable for the detection of HIV-2. A commercial HIV-1/HIV-2 combination assay is available that has high sensitivity for detection of both viruses; a supplemental immunoblot assay is used to confirm the viral subtype.[41,196] Serologic diagnosis of HTLV infection is similar to that of HIV and involves the use of a screening enzyme immunoassay (EIA) and confirmatory immunoblot or RIPA. However, these tests do not distinguish between antibodies directed at HTLV-I or HTLV-II.[43] In patients with positive serology, virologic detection tests, such as proviral amplification by polymerase chain reaction (PCR) or viral isolation, have been used.[57] Assays containing several synthetic peptides and recombinant proteins have been developed and appear to be capable of differentiating between HTLV-I and HTLV-II.[31,280]

Direct detection of virus by culture is intensive, expensive, and time-consuming; several weeks often are required for results. The ability to isolate HTLV and HIV depends on the patient's disease state, immune status, and viral load. The ability to culture retroviruses has been improved by co-cultivation of patient cells with human peripheral blood mononuclear cells that have been stimulated in vitro with mitogens (e.g., phytohemagglutinin) and growth factors (e.g., IL-2), as well as by removal of patient $CD8^+$ (suppressor) cells from the co-culture. In infants and children, the small volume of blood available for culture and the low virus load in some cases may render virus isolation challenging.

Other methods used for detecting virus include (1) antigen capture assays to detect free circulating viral antigens (e.g., p24 HIV-1 core antigen), (2) IFA and immunohistochemistry to detect viral antigens in tissue, (3) detection of viral nucleic acids by Southern blot analysis, (4) in situ hybridization or dot blots, (5) PCR to detect proviral DNA in leukocytes or tissue, and (6) assays to detect viral RNA in plasma or other fluids. DNA PCR can be used to quantitate virus and is highly sensitive because it is capable of amplifying tiny quantities of viral nucleic acids enzymatically to detectable levels with a system of specific nucleotide primers and probes. This technique can be modified to detect viral RNA by using reverse transcription to convert viral RNA to DNA and by performing PCR on the DNA product or by using other techniques such as branched-chain amplification or nucleic acid sequence-based amplification.[35,257,310]

ONCOVIRUSES: HUMAN T-CELL LYMPHOTROPHIC VIRUS TYPES

HUMAN T-CELL LYMPHOTROPHIC VIRUS TYPE I

HTLV-I was the first human retrovirus identified and was isolated initially from a patient with cutaneous T-cell lymphoma.[265] HTLV-I now is known to be the causative agent of adult ATLL, neurologic disorders (HAM/TSP), infective dermatitis in children, and numerous other disorders that are possibly autoimmune (Table 204–1).

EPIDEMIOLOGY

Evaluation of the global distribution of HTLV-I initially was complicated by the considerable antigenic similarity between HTLV-I and HTLV-II (≈60% homology in nucleotide sequences). The first ELISA and Western blot assays designed to detect HTLV were not able to discriminate between the presence of antibodies to HTLV-I and those to HTLV-II; DNA PCR was required to distinguish type-specific proviral sequences. Thus, early epidemiologic studies often were unable to distinguish between HTLV-I and HTLV-II infection. Second-generation assays use synthetic type-specific peptides that enable researchers to differentiate between HTLV-I and HTLV-II antibodies.[31,280] More recent studies have been able to discriminate the epidemiologic distribution of the two viruses more accurately.

On a global basis, an estimated 15 to 25 million individuals are infected with HTLV-I. Based on genetic analysis, HTLV-I is classified into three major types.[207,379] The Melanesian type, which was isolated from Melanesians in Papua New Guinea and

TABLE 204–1 Diseases Associated with Human T-Cell Lymphotrophic Virus Types I and II

Strength of Association	Human T-Cell Lymphotrophic Virus Type I	Human T-Cell Lymphotrophic Virus Type II
Strong	Adult T-cell leukemia/lymphoma	Neurologic disorders (spastic paraparesis/ myelopathy to more widespread central nervous system involvement)
	Myelopathy (HTLV-1–associated myelopathy tropical spastic paraparesis)	
	Infective dermatitis	
	Uveitis	
	Arthropathy	
	Interstitial pneumonitis	
	Immune deficiency with opportunistic infections	
Intermediate	Polymyositis	$CD8^+$ cell cancers
	Cutaneous lymphomata such as mycosis fungoides, Sézary syndrome	Immune deficiency with opportunistic infections
	Sjögren syndrome, sicca syndrome	
Weak	Renal failure	
	B-cell leukemias	
	Small cell lung cancer	

HTLV-I, human T-cell lymphotrophic virus type I.

the Solomon Islands in the South Pacific and from Australian aborigines, is the most divergent in that it exhibits only 93 percent sequence similarity in the envelope gene with other HTLV-I strains.[17,380] The other two HTLV-I types exhibit 97 percent sequence similarity. The second distinct genetic type is found in central Africa. The third, the Cosmopolitan type, includes isolates from various areas, and it represents the majority of HTLV-I isolates. The Cosmopolitan group has been divided further into four subtypes based on LTR sequencing: Transcontinental (A), Japanese (B), West African (C), and North African (D). Subtype A, the most widespread, has been isolated from Japan, the Caribbean basin, South America, India, Iran, far-eastern Russia, and South Africa (hence the name "Transcontinental"). Subtypes B and C are more restricted geographically, with subtype B found primarily in Japan and rarely in India and subtype C found in West Africa and the Caribbean basin. Subtype D was identified more recently in Morocco, Algeria, and North Africa.

Because the genetic variability and intra-subtype genetic drift of HTLV-I are limited, the geographic distribution of viral types has been hypothesized to represent the anthropologic movement of virus-carrying populations over the course of time.[379] Most HTLV-I isolates from the same ethnic group in different locations and from different groups inhabiting the same area have phylogenetic similarities. For example, in North and South America and the Caribbean, most HTLV-I isolates belong to subtype A and are thought to have originated from ancestral Mongoloid peoples who migrated from Asia to the American continents. In addition to subtype A, subtype C has been identified in the Caribbean and may reflect forced migration of black slaves from West Africa to the New World. Two HTLV-I subtypes have been found in Japan, with subtype A predominant in the northern and southern territories and subtype B more predominant in southwestern Japan; this dispersion has led to speculation that these differences reflect immigration of different populations to different areas of the Japanese archipelago.

HTLV-I is highly endemic in southwestern Japan, particularly on the islands of Kyushu, Shikoku, and Okinawa, where roughly 30 percent of the adult population is seropositive.[134,376] Geographic clustering of HTLV-I infection in southwestern Japan is observed, and marked variation may be observed within small geographic areas; such microclustering has been speculated to be attributable to the limited interchange, particularly intermarriage, between neighboring communities in Japan.[376]

Regions of moderate HTLV-I endemicity include areas of the Caribbean, such as Jamaica, Trinidad, Barbados, and the West Indies, where seroprevalence rates range between 2 to 5 percent in black adults.[18,189,223,361] Parts of Africa also appear to have large reservoirs of HTLV-I infection; possible endemic areas have been identified within Gabon, Chad, Nigeria, Cameroon, Guinea, Democratic Republic of Congo (formerly Zaire), and the Ivory Coast.[43,65,349] The seroprevalence rate in the African adult population is approximately 0.5 to 1 percent; however, the distribution of HTLV-I is not uniform and is characterized by clusters of high endemicity. Foci of HTLV-I infection also have been identified in Central and South America, including Panama, Brazil, Colombia, Venezuela, Surinam, Guyana, Ecuador, and Peru.[52,223] HTLV-I seroprevalence rates in Brazilian blood donors range from 0.08 percent in the south to 1.8 percent in the northeast, although seroprevalence in specific populations such as Brazilian Indians has been as high as 38 percent.[52,87,201] The HTLV-I seroprevalence rate in pregnant women in northeastern Brazil was 0.84 percent.[24]

HTLV-I seroprevalence in low-risk populations in the United States and Europe is less than 1 percent. Seroprevalence rates in blood donors in the United States in the year after initiation of blood screening were 0.016 to 0.021 percent. Female donors and blacks, Hispanics, and Asians were more likely to be seropositive than were male donors and whites.[40] During the same period, seropositivity in applicants for the U.S. Armed Forces was similarly relatively low but twice that observed in blood donors, 0.41 per 1000.[281] Similar to the demographics observed for seropositive blood donors, the prevalence in black applicants was more than 30 times that in white applicants, and a disproportionate rate of seropositivity in female applicants was observed. Clusters of HTLV-I infection also have been reported in blacks in the southern and southeastern United States and in immigrants from HTLV-I–endemic areas.[64,68,133,364] HTLV-I seroprevalence rates in blood donors in Europe have ranged between 0 and 0.02 percent.[333] In inner-city London, HTLV seroprevalence in pregnant women was 1.1 per 1000 women, with the highest prevalence noted in women from the Caribbean (17.0 per 1000) and western and central Africa (3.2 per 1000); in women born in nonendemic areas and who were not black Caribbean, seroprevalence was 0.06 to 0.12 per 1000.[3]

Factors that influence HTLV-I seropositivity are age, race, sex, and geography. In endemic areas, the prevalence in children is low but starts to increase during the teenage years; this age-related increase is more marked for girls and women than for boys and men. By 40 to 50 years of age, women are significantly more likely to be infected than are men. This sex- and age-related pattern may be the result of more efficient male-to-female sexual transmission of HTLV-I among sexually active adults.[29,324]

With the development of advanced immunoassays, differentiating the epidemiology of HTLV-II and HTLV-I infection has been possible. In a study of 1.7 million blood donors in the United States, the prevalence of HTLV-I and HTLV-II infection was 9.1 and 22.3 per 100,000 donors, respectively.[228] Thus, approximately 30 percent of volunteer blood donors found to be HTLV-seropositive are infected with HTLV-I, and 70 percent are infected with HTLV-II.[152] HTLV-I seroprevalence was associated with the following: older age; female sex; hepatitis C seropositivity; black, Hispanic, or Asian race/ethnicity; and birth outside the United States.[228] These data are consistent with HTLV-I infection in the United States in that it is concentrated primarily in persons born in or having sexual contact with persons from HTLV-I–endemic areas of Africa, the Caribbean, and Japan. In contrast, HTLV-II has been found primarily in injecting drug users and their sexual contacts.[152]

MODES OF TRANSMISSION

HTLV-I does not replicate efficiently in vivo and is transmitted through infected T cells in breast milk, semen, and blood. Modes of transmission of HTLV-I are similar to those of HIV-1: sexual contact, parenteral transmission through blood transfusion of a cellular blood product or through intravenous drug use, and perinatally, most often through breast milk. The efficiency of transmission of HTLV appears to be significantly lower than that of HIV because HTLV is highly cell-associated, whereas HIV can be transmitted in a cell-free and cell-associated manner.

Sexual Transmission

Sexual transmission is a significant source for acquisition of HTLV-I in adults. Male-to-female sexual transmission appears to be more efficient than is female-to-male transmission.[376] In one study from Japan, the risk of transmission of HTLV-I from an infected husband to his wife over a 10-year period was 61 percent, whereas the risk of transmission from an infected wife to her husband was less than 1 percent.[147] Sexual transmission of HTLV-I from infected men to their wives in Japan increased with older age of the male partner and elevated HTLV-I antibody titer, thus suggesting that a longer duration of infection and an elevated viral load may be associated with increased transmissibility.[314]

Data from studies in the United States are consistent with the findings in Japan. Risk factors for male-to-female sexual transmission of HTLV-I were examined in the Retrovirus Epidemiology Donation Study, which, since 1988, has enrolled from five participating blood centers volunteer blood donors who have been found to be seropositive for HTLV. The proportion of female sexual partners of infected men who were infected themselves (38%) was nearly twice that of male partners of infected female donors (20%).[152] In addition, ratios of transmitter-to-nontransmitter couples were similar for HTLV-I and HTLV-II, thus suggesting similar transmission efficacy of the two viruses. HTLV-transmitting men had been in their sexual relationships longer (mean, 225 versus 122 months) and had proviral loads higher than those of nontransmitters. Transmitting men also tended to have higher antibody titers against various viral proteins than did nontransmitters; in general, antibody titers correlated highly with viral load.

In Japan, rates of seropositivity also were found to be higher in persons with a history of sexually transmitted diseases, a finding suggesting that disruption of the genital mucosa or an increase in leukocytes in genital secretions and semen, or both, may increase the risk of transmission.[236] In studies from several different countries, prevalence of HTLV-I was found to be elevated in female sex workers.[61,99,236,371] The lack of consistent use of condoms, the duration of prostitution, older age, infection with *Chlamydia trachomatis* or syphilis, antibody to herpes simplex virus type 1, and concomitant HIV-1 infection were associated with elevated HTLV-I prevalence.[99,236] An increased risk for HTLV-I seropositivity also was observed in persons attending clinics that treat sexually transmitted diseases.[78,157,236,367] In such clinics in the United States, HTLV seropositivity ranged from 0.18 to 2.0 percent.[157] Risk factors for the acquisition of HTLV-I infection by women included multiple sexual partners, bruising during sex, syphilis, and concomitant HIV infection, whereas for men, hepatitis B antigenemia, bruising during sex, older age at onset of sexual activity, married status, and an agricultural occupation were associated with an increased risk.[78]

Homosexual men in endemic areas have an increased risk of acquiring HTLV-I, presumably through anal intercourse. In Trinidad, HTLV-I seropositivity was sixfold higher in homosexual men than in the general population, and in Baltimore, Maryland, male-to-male sexual activity was associated with an elevated risk of seropositivity in men seen in clinics for sexually transmitted diseases.[16,367] The higher rate of HIV than HTLV infection in homosexual men in Trinidad is an indication of the higher efficiency of HIV transmission.

Mother-to-Child Transmission

HTLV-I infection does not appear to interfere with the course of pregnancy and is not associated with congenital abnormalities.[24] However, mother-to-child transmission of HTLV-I is the major source of infection in children. Studies in Japan showed that more than 90 percent of HTLV-I–seropositive children have mothers who are seropositive themselves; overall, 15 to 25 percent of children born to infected women become infected.[86,123,147,341,369] Transmission to the infant appears to occur predominantly post partum, as reflected by infant seroconversion to seropositivity after the loss of passively transferred maternal antibodies. Depending on the study, 11 to 40 percent of breast-fed children of HTLV-1–infected women become infected versus 0 to 13 percent of bottle-fed children.[122,124,245,325,337,369,370,383] Numerous variables, including longer duration of breast-feeding, elevated maternal HTLV-I antibody titer, high maternal HTLV-I proviral load, presence of HTLV-I–bearing cells in breast milk, female gender of the child, older maternal age, long duration of membrane rupture (>4 hours), and lower maternal income, are associated with a risk of transmission of HTLV-I.[121,122,124,154,191,325,341,369,370]

Substantial evidence supports the conclusion that most incidents of transmission of HTLV-I occur through breast-feeding.[123] HTLV-I antigen has been detected in the breast milk of seropositive mothers, and HTLV-I proviral DNA has been identified by PCR in mononuclear cells from the breast milk of HTLV-I–carrier women.[158,233] HTLV-I infection of milk cells may not be restricted to T lymphocytes. Breast-derived luminal epithelial cells, which are present in human milk during early and long-term lactation, can be infected persistently with HTLV-I and undergo transformation in vitro. These cells can transmit HTLV-I infection to epithelial cells in breast milk or the intestines or to T cells derived from milk or blood.[312] In a marmoset animal model, transmission of HTLV-I was shown to occur by oral feeding of lymphocytes from the breast milk of HTLV-1–infected mothers.[159,378] Lymphocyte-facilitated infection of gastrointestinal epithelial cells has been hypothesized to be the mechanism for transmission.[385]

The risk of transmission through breast milk most likely is multifactorial and involves the duration of exposure, the amount and activation level of the virus, and the presence of protective or enhancing specific and nonspecific immunity. The median time to infection in a Jamaican cohort was estimated to be 11.9 months.[86] A duration of breast-feeding longer than 6 months appears to be associated with at least a threefold increased risk of transmission.[325,369,370] In one study in Japan, no child who was breast-fed for less than 6 months became infected, and in a study in Jamaica, children born to mothers with higher titers of HTLV-I antibody had a delayed time to seroconversion, a finding thus suggesting that passive transfer of maternal HTLV-I antibody may affect transmission timing and risk.[191,323,325] In an experimental rabbit model, passive immunization with HTLV-I hyperimmunoglobulin prevented milk-borne transmission of HTLV-I.[291] However, in some studies, higher levels of maternal antibody were associated with a higher risk of transmission, presumably because higher antibody levels may reflect a higher viral load.[369] Current recommendations are to provide formula feeding for infants born to HTLV-I–seropositive women.[43,122] However, in situations in which formula feeding is not possible, cessation of breast-feeding when the child reaches the age of 6 months, or earlier, may reduce the risk of transmission.[326]

Transmission may be possible, albeit infrequently, during the in utero or intrapartum period. Approximately 3 to 4 percent of infants born to carrier mothers are infected despite being bottle fed, a finding suggesting that intrauterine or intrapartum transmission may occur, but with much lower efficiency than with transmission through breast milk.[122,156,323] The finding that the duration of rupture of the membrane is associated with transmission of HTLV-I suggests that some percentage of transmission may occur intrapartum. HTLV-I has been shown to infect intestinal and cervical epithelial cell lines in vitro.[384,385] A study in 2002 used PCR to evaluate 41 children born to HTLV-1–infected mothers but who had not been breast-fed. No infected children were identified, but 81 percent of the babies were delivered by elective caesarian section.[26] This type of delivery has been demonstrated to be an effective measure against transmission of HIV, so it may affect the transmission of HTLV as well.

Parenteral Transmission

Parenteral transmission by transfusion and intravenous drug use is well documented and appears to be the most efficient mode of transmission, with a 15 to 60 percent chance of infection occurring in those receiving HTLV-1–infected cellular products.[250,279] Comparative rates of transmission of HTLV-I, HTLV-II, and HIV-1 were evaluated retrospectively in a large repository of U.S. blood donor serum from the Transfusion Safety Study.[67]

Consistent with a requirement for cell-cell contact for transmission of HTLV and in contrast to HIV-1, transmission of HTLV-I and HTLV-II was observed only with the transfusion of cellular blood components. Infectivity appeared to decrease with an increasing period of blood storage; no apparent transmission occurred after the transfusion of components stored more than 10 days, a finding suggesting that the known decrease in the ability of donor lymphocytes to be activated or to proliferate with storage renders the cells non-infectious. In this study, rates of transfusion-related transmission of HTLV-I and HTLV-II were similar; approximately 27 percent of recipients of blood components from seropositive donors became infected. In contrast, 89 percent of the recipients of HIV-1–positive blood became infected, regardless of the blood product component type, and no effect of storage on transmission risk was seen. The median time to seroconversion after the transfusion of HTLV-I–contaminated blood products is 51 days.[46]

In a study of HTLV-infected blood donors, both HTLV-I and HTLV-II infection was associated with low educational attainment, accidental needle-sticks or cuts, previous blood transfusion, seven or more sexual partners, and a sexual partner from an HTLV-I–endemic area.[297] However, injection drug use or having sex with injecting drug users was associated with HTLV-II only and not with HTLV-I infection.

Screening of blood donors in the United States since 1988, as well as in other countries such as Japan, reduced transfusion-related transmission markedly. However, in countries in which screening of blood for HTLV-I and HTLV-II is not performed, transfusions provide an important source of infection. In a study of hospitalized children in Gabon in Africa, multiple blood transfusions secondary to complications of sickle-cell disease were as predominant a mode of transmission of HTLV-I as was perinatal transmission.[62] Similarly, in Martinique, HTLV-I seroprevalence in patients with sickle-cell anemia was 10 percent versus 1 to 3 percent in normal blood donors, and HTLV-I–seropositive patients had received more transfusions than had seronegative persons.[290]

Descriptive studies have suggested that ecologic factors may influence the rate of seropositivity in a population; for example, residence in a lower-altitude, tropical environment was associated with higher rates of seropositivity in some reports.[189,204] A role for insect vectors, such as mosquitoes, was postulated. However, no evidence has shown that retroviruses can replicate in arthropods, and any hypothesized insect-borne transmission would need to occur mechanically by the mouth parts of biting insects that were contaminated with a significant amount of infected and infectious lymphocytes; such transmission, if it occurred, would be expected to be very unusual.[79] Additionally, the prevalence of antibodies to arboviruses was not significantly greater in HTLV-seropositive than in HTLV-seronegative persons.[221]

DISEASE ASSOCIATIONS

After being infected with HTLV-I, most infected individuals remain clinically asymptomatic for life. However, persistent viral infection and antigen production elicit a strong humoral and cellular immune response in infected individuals that results in high antibody titers to HTLV-I structural and regulatory proteins and increased circulating activated HTLV-I Tax protein–specific cytotoxic T lymphocytes.[54,138] In addition, even in asymptomatic patients, spontaneous in vitro proliferation of lymphocytes is observed.[164]

Early in the course of primary infection, the HTLV-I proviral load is high but becomes controlled rapidly. Within 90 days of primary infection, a narrow range of proviral load is observed in an individual that generally stays relatively constant over the course of time.[194,249,330] Antibody titer is highly correlated with proviral load after the initial set-point is reached. The HTLV-I proviral load in asymptomatic HTLV-I carriers can be as high as 5 percent of all peripheral blood mononuclear cells, and it is particularly high in individuals with HAM/TSP, in whom as many as 20 percent of peripheral blood mononuclear cells can be infected.[194,217] A high peripheral blood proviral load appears to predate the development of neurologic and ophthalmologic HTLV-I–associated disease entities.

In adults, the diseases associated with HTLV-I infection are malignancies and chronic degenerative neurologic syndromes. Pediatric manifestations of HTLV-I infection have been identified, and researchers now recognize that these diseases may occur more commonly in children and adolescents than previously recognized.[26] HTLV-I may be a prototype for other retroviruses yet to be discovered that predispose to the development of active diseases with long latency after exposure at birth or early in life.

ADULT T-CELL LEUKEMIA AND OTHER MALIGNANCIES

ATLL was the first clinical disease to be linked with HTLV-I infection. This aggressive form of leukemia/lymphoma first was described in Japan in 1977, before the discovery of HTLV-I. Although an infectious origin was postulated because of geographic clustering of ATLL in southern Japan, only after the discovery of HTLV-I in 1980 was a causal link established between HTLV-I seropositivity and ATLL.[265] ATLL is the most common form of leukemia in Japan, with approximately 700 new cases diagnosed yearly.[321,322]

ATLL is found primarily in HTLV-I–endemic areas or in migrants from such areas. The incidence of ATLL in the United States is low; cases are seen primarily in immigrants from HTLV-I–endemic areas or in blacks in the southeastern United States, in whom endemic HTLV-I infection has been documented.[274] In a study in central Brooklyn, which has a large Caribbean migrant population, the annual incidence of ATLL in African Americans was approximately 3.1 per 100,000 person-years.[176]

ATLL occurs more commonly in women in Africa and the Caribbean but more commonly in men in Japan (male- to-female ratio of 1.4:1).[126,261] The mean age at onset is between 40 and 60 years; however, the average age at onset of ATLL is somewhat lower in patients from the Caribbean and Africa (43 years) than in those from Japan (58 years).[376] The disease very rarely occurs in children. The incubation period for ATLL appears to be 20 to 30 years or longer after infection with HTLV-I, and researchers have postulated that ATLL results from HTLV-I infection acquired during the first few years of life.[227] The lifetime risk for development of ATLL is estimated to be 2 to 5 percent in individuals infected before reaching the age of 20 years.[43,193,220]

During the long latent period of HTLV-I infection, most infected individuals show polyclonal proliferation of cells harboring integrated HTLV-I provirus. Asymptomatic HTLV-I carriers may experience a pre-ATLL state associated with mild leukocytosis or the presence of abnormal lymphocytes with characteristic lobulated nuclei ("flower cells") that are found to have monoclonal or oligoclonal HTLV-I provirus integrated into the cell genome. More than 50 percent of such persons will experience resolution of the leukocytosis spontaneously. Why ATLL develops in only a small percentage of HTLV-1–infected individuals is not clear. Because malignant transformation probably is a multistep process, intermediate factors such as the host immune response or oncogenic environmental stimuli, or both, may be involved.[134] Diagnostic criteria for ATLL[307] are shown in Table 204–2.

Clinical manifestations of ATLL include lymphadenopathy in 50 to 80 percent of patients and hepatosplenomegaly in 25 to 67 percent (Table 204–3). Skin lesions, which occur in 40 to 60

percent of patients, include the following: large nodules; plaques; ulcers; a generalized papular rash appearing on the limbs, trunk, or face; or any combination of these lesions. An elevated white blood cell count ranging from 10,000 to 300,000/mm^3 occurs in approximately two thirds of patients, and abnormal T lymphocytes (flower cells) containing lobulated nuclei may be seen. Hypercalcemia occurs in 32 to 63 percent of patients. In patients with hypercalcemia, ATLL cells have been found to secrete excessive amounts of parathyroid hormone–related peptide, probably because of Tax-mediated transactivation of the gene for this protein.[72] Osteolytic bone lesions occur in 2.5 to 10 percent. The central nervous system (CNS), which is affected in 2.5 to 10 percent of patients, can act as a sanctuary and has been found to be an important site for relapse when chemotherapy is given to treat ATLL.[261] Opportunistic infections occur commonly because of the defective T-cell–mediated immunity observed in ATLL and are a primary cause of mortality. In patients with preexisting infestation with the roundworm *Strongyloides stercoralis*, a hyperinfection syndrome accompanied by gram-negative sepsis may occur and has a mortality rate of greater than 70 percent. Other common opportunistic infections include bacterial infections, *Pneumocystis* pneumonia, and serious fungal infections. Findings indicative of poor survival include age older than 40 years, increased tumor bulk, poor performance status, high lactate dehydrogenase level, hypercalcemia, and the presence of clones of cells containing multiple or defective copies of the HTLV-I provirus.[183,277]

TABLE 204–2 Requirements for Diagnosis of Adult T-Cell Leukemia/Lymphoma

1. HTLV-I seropositivity
2. Histologic or cytologic proven lymphoid malignancy with T-cell surface antigens present
3. Abnormal T lymphocytes ("flower cells") consistently present in peripheral blood (except for lymphoma subtype of ATLL)
4. Demonstration of clonality of proviral DNA as well as clonal integration of proviral DNA

ATLL, adult T-cell leukemia/lymphoma; HTLV-I, human T-cell lymphotrophic virus type I.

TABLE 204–3 Clinical Findings in Adult T-Cell Leukemia/Lymphoma

Median Age of Onset	40-60 yr
Gender	Caribbean, Africa: females > males Japan: males slightly > females (1.4:1)
Clinical/Laboratory Findings	
Generalized lymphadenopathy	50%-80%
Hepatosplenomegaly	25%-67%
Skin involvement	40%-60%
Elevated white blood cell count	60%-66%
Hypercalcemia	32%-63%
Pulmonary involvement	14%
Lytic bone lesions	2%-10%
Central nervous system involvement	2%-10%

Clinical and laboratory criteria have been used to differentiate ATLL into four subtypes: acute, chronic, lymphoma, and smoldering (Table 204–4).[42,261,307,376] The distribution of clinical ATLL subtypes varies geographically. In Japan, most cases are acute ATLL with leukemic manifestations; however, in the Caribbean, most cases are initially of the lymphoma subtype.[176,177]

Acute ATLL is the rapidly aggressive form of leukemia/lymphoma that was recognized first in Japan. It is the most common subtype and has the shortest survival time. Clinical findings include the following: characteristic cutaneous involvement in 40 percent of patients that ranges from a maculopapular rash to tumorous lesions; leukemia with circulating abnormal lymphocytes; generalized lymphadenopathy, hepatomegaly, splenomegaly, or any combination of these conditions; lytic bone lesions; hypercalcemia; and immunodeficiency leading to opportunistic infections. Either chronic or lymphoma-type ATLL is seen in approximately one fifth of patients with ATLL. Chronic ATLL has a more indolent clinical course than does acute ATLL, with a median survival of approximately 24 months. Chronic ATLL

TABLE 204–4 Clinical Subtypes of Adult T-Cell Leukemia/Lymphoma: Clinical and Laboratory Findings

	Adult T-Cell Leukemia/Lymphoma Clinical Subtype			
	Acute	**Chronic**	**Lymphoma**	**Smoldering**
Percentage with Subtype	57%	19%	19%	5%
Survival				
Median	6 mo	24 mo	10 mo	>24 mo
Four-year rate	5%	27%	6%	63%
Clinical Findings				
Lymphadenopathy	±	±	Yes	No
Hepatomegaly/splenomegaly	±	±	±	No
Skin involvement	±	±	±	±
Bone lesions	±	No	±	No
Bone marrow involvement	±	No	±	No
Laboratory Findings				
Absolute lymphocyte count ($\times 10^9$)	≥4	≥4	<4	<4
Abnormal circulating lymphocytes	≥5%	≥5%	≤1%	≥5%
Polylobulated lymphocytes ("flower cells")	Yes	Occasional	No	Occasional
Calcium	Normal or elevated	Normal	Normal or elevated	Normal
Lactate dehydrogenase	Normal or elevated	<2 times normal	Normal or elevated	<1.5 times normal

**If less than 5% of circulating abnormal lymphocytes are present in the smoldering adult T-cell leukemia/lymphoma subtype, at least one histologically proven lesion from the lungs or skin should be present.*

overlaps with cases of T-cell chronic lymphocytic leukemia and is associated with moderate leukocytosis in which 0.5 to 3 percent of circulating cells are malignant. Cutaneous manifestations may be observed, but nodal or extranodal involvement occurs rarely, and hypercalcemia is absent. Lymphoma-type ATLL has a median survival of approximately 10 months and overlaps with T-cell non-Hodgkin lymphoma, with prominent lymphadenopathy and the presence of monoclonally integrated HTLV-I in the malignant cells and little to no peripheral blood involvement. Smoldering ATLL occurs least commonly, in approximately 5 percent of patients, and is relatively indolent. It is characterized by abnormal cells in the absence of leukocytosis and resembles mycosis fungoides with slow progression, cutaneous involvement manifested as erythema or infiltrative plaques or tumors, and mild lymphadenopathy or splenomegaly, or both.

The pathogenesis of ATLL is not known. The typical phenotype of ATLL cells is $CD3^+$, $CD4^+$, $CD8^-$, and $CD25^+$ (IL-2 receptor-positive). Monoclonal HTLV-I provirus is found integrated into the DNA of ATLL malignant cells. HTLV-I is known to transform normal $CD4^+$ lymphocytes in vitro and results in immortalization, high levels of IL-2 expression, and increased expression of the IL-2 alpha-chain receptor on the cell surface. The presence of excessive receptors for IL-2, a known growth factor for T cells, may be linked to development of the proliferative leukemic process of ATLL.[376] Additionally, the HTLV-I Tax protein activates the promoters of many genes involved in cell growth and differentiation and interferes with DNA repair functions.[218] One hypothesis is that the continuous proliferation observed in HTLV-1–infected cells may render them more susceptible to spontaneous mutagenesis, as well as to the effect of external carcinogens.[1] Although tax does not directly transform cells and has no homology to known proto-oncogenes, the finding that persistent clonal expansion of HTLV-1–infected cells precedes the development of ATLL suggests that tumor cells may originate in clonally expanding nonmalignant cells through a multistep process that may include the acquisition of mutations in genes such as *p53* or *p16*.

The host immune response also may be involved in malignant transformation. An association between progression to ATLL and HTLV-I antibody titer has been reported. In a study of 5 cases of ATLL and 38 matched HTLV-1–infected controls without ATLL, researchers found a 1.6-fold increase in the risk of ATLL with every 2-fold increase in HTLV-I antibody titer.[125] However, despite having higher HTLV-I antibody levels, all patients with ATLL had low or undetectable levels of HTLV-I anti-Tax antibody for as long as 10 years preceding the diagnosis of ATLL when compared with HTLV-I carriers without ATLL. Malignant ATLL cells also are less likely to express detectable levels of Tax mRNA and are more likely to have partial deletion of the HTLV-I genome. The Tax protein is the major target of cytotoxic T cells in HTLV-I infection, and suppression or defective expression of the Tax protein on the surface of infected cells may permit escape from immune-mediated cell lysis.[125] Indeed, HTLV-1–infected patients with ATLL have poorly detectable Tax-specific cytotoxic T-cell activity,[150,248] which could permit more unrestrained proliferation of infected cells and lead to increased HTLV-I viral load, higher HTLV-I antibody levels to non-Tax viral proteins, and, eventually, malignant transformation.

Treatment regimens for ATLL remain unsatisfactory, with high rates of relapse. Combination chemotherapy with cytotoxic agents results in a complete response in 20 to 45 percent of individuals, but the duration of the response is short, generally lasting only a few months.[261,320] The combination of zidovudine and interferon-α has been shown to be active in the treatment of ATLL, even in patients in whom conventional multiagent chemotherapy has failed.[96,119] Because viral replication is not required for malignant transformation to ATLL and the level of viral replication during the leukemic phase of ATLL is barely detectable, clinical response is not likely to result directly from an antiretroviral effect of these drugs. Zidovudine produces cytostatic effects through termination of DNA replication and may exert direct antitumor effects; this agent has been shown to block HTLV-I–induced cell transformation in vitro. Interferon-α has multiple biologic effects, including an antiproliferative effect through inhibition of protein synthesis and cell growth, and it also may enhance immune recognition of tumor cells by inducing the expression of MHC molecules on the tumor cell surface. However, although survival is prolonged, this therapy is not curative, and relapse occurs. Novel treatments are being investigated. ATLL cells are known to overexpress IL-2 receptor on their surface. Therefore, immunotherapy with toxin-conjugated monoclonal antibodies to the alpha chain of the IL-2 receptor has been studied in clinical trials, with remissions lasting from 9 weeks to 3 years.[356-358] Topoisomerase inhibitors and retinoids also have been evaluated.[261] In vitro, ATLL cells are susceptible to lysis by Tax-specific cytotoxic T cells, a finding that led to the hypothesis that stimulation of cellular immunity to Tax may inhibit tumor growth.[150,248] In a rat model, the use of an HTLV-I Tax-directed DNA vaccine induced cytotoxic T-cell activity against Tax-expressing cells, and adoptive transfer of these cells effectively suppressed in vivo growth of HTLV-I–transformed tumor cells.[248]

Other malignant diseases have been associated with HTLV-I infection, although the supporting evidence for these associations is less clear than that for ATLL. Cutaneous T-cell lymphomata, such as mycosis fungoides and Sézary syndrome, have been described in adults and a child with HTLV-I infection.[18,107,355,388] In one small study of eight patients with Sézary syndrome, HTLV-I mRNA expression was found in four patients.[95] Multiple myeloma and B-cell chronic lymphocytic leukemia also have been described in patients infected with HTLV-I. These malignancies may arise as a result of chronic antigenic stimulation of B cells by HTLV-1–infected T cells, with such stimulation leading to uncontrolled B-cell expansion. In the Caribbean, HTLV-I has been associated with the development of T-cell non-Hodgkin lymphoma.[192] A single case of small cell cancer of the lung with monoclonally integrated HTLV-I in the tumor was reported. A pulmonary infiltrative syndrome resembling lymphoid pulmonary hyperplasia seen in HIV-1–infected children has been reported in patients with HTLV-I–associated myelopathy.[316]

HUMAN T-CELL LYMPHOTROPHIC VIRUS TYPE I–ASSOCIATED MYELOPATHY

In the 1880s, a "multiple neuritis" syndrome consisting of a predominantly ataxic motor neuropathy was reported in Jamaica. In subsequent years, similar syndromes of unknown origin were described in other geographic locales and given different names, including *Strachan disease*, *central neuritis*, *Jamaican neuropathy*, *tropical spastic paraplegia*, and *tropical spastic paraparesis*.[389] The identification of HTLV-I in 1980, the development of antibody tests to diagnose HTLV-I infection, and the recognition that HTLV-I and TSP were endemic in the same geographic locations led to the hypothesis that these entities could be associated. In 1985, Gessain and colleagues[94] performed a case-control study in Martinique in which patients with TSP of unknown cause were compared with a control group of nurses and blood donors without neurologic disease. Sixty percent of the patients with TSP versus only 4 percent of controls had HTLV-I serum antibodies detected.[84,94] In the same year, a report was published on the detection of HTLV-I antibodies in the cerebrospinal fluid (CSF) and serum of individuals afflicted with TSP.[284] In 1986, a series of Japanese patients with a similar clinical syndrome who

also had HTLV-I antibodies in their CSF were described, and these investigators proposed that the syndrome be called *HTLV-I–associated myelopathy*.[253] At a 1988 meeting of the World Health Organization's (WHO's) Scientific Group on HTLV-I Infections and Its Associated Disease, researchers proposed that HAM and HTLV-I–associated TSP were clinically and pathologically identical and recommended that the disorder be known by the acronym *HAM/TSP*.[375]

The disease usually occurs in HTLV-I–endemic areas, including the Caribbean, southern Japan, equatorial Africa, Central and South America, Melanesia, and southern Africa. Sporadic cases have been described in nonendemic areas, usually in immigrants from endemic areas or their sexual contacts or in recipients of blood transfusions (before the advent of HTLV blood screening). Incidence and prevalence estimates are unreliable because of the insidious nature of the disease and the lack of recognition of early symptoms by clinicians. In HTLV-I–seropositive persons in Japan, the incidence of HAM/TSP is 3.1 per 100,000 HTLV-1–infected persons per year. In contrast, the annual incidence of HAM/TSP in HTLV-1–infected persons in Jamaica and Trinidad was 22.1 per 100,000 persons.[187] This difference in incidence is hypothesized to reflect an association between the time that HTLV-I infection is acquired and the type of disease manifestation that occurs. In the Caribbean, most HTLV-I infection is acquired sexually during adult life, and HAM/TSP is the most common clinical manifestation of the disease; in contrast, in Japan, most infection is acquired by maternal-to-child transmission during infancy, and ATLL is the most common disease manifestation. Case reports of HAM/TSP occurring after receipt of blood transfusions and the finding in Japan and Martinique that 13 to 20 percent of patients with HAM/TSP had a history of receiving blood transfusions accelerated the decision of blood banks in the United States to screen for HTLV-I antibodies. In Japan, a 16 percent decrease in the number of HAM/TSP cases occurred in the 2 years after initiation of blood screening.[88,252]

Although the mean onset of disease is in the fourth decade of life, incubation of this disease appears to be shorter than that of ATLL. In patients with HTLV-I infection acquired by transfusion in whom HAM/TSP subsequently developed, the median time to development of symptoms after receiving a transfusion was 3.3 years, and in one report, HAM/TSP occurred 18 weeks after blood transfusion.[100,252] Onset of symptoms is uncommon in persons younger than 20 or older than 70 years. However, HAM/TSP was reported in a child as young as 6 years old after the development of transfusion-acquired HTLV-I infection.[252] The rate of HAM/TSP increases with age from 20 through 50 years and then declines.[184] The lifetime risk of HAM/TSP in an HTLV-1–infected person is estimated to be 1.9 percent overall, with a slightly higher incidence in women (1.8%) than in men (1.3%).[187]

The clinical features of HAM/TSP include chronic progressive spastic paraparesis and weakness of the limbs, particularly the legs, that results in an insidious onset of gait disturbance (Table 204–5). Mild sensory loss and painful paresthesias may develop and result in complaints of extremity numbness or dysesthesia and low back pain. Bowel and bladder sphincter impairment may occur and cause constipation, urinary frequency or incontinence, and impotence. Neurologic examination shows hyperactive deep tendon reflexes, clonus, extensor plantar reflexes, proximal muscle wasting, and a spastic paraparesis with a slow, scissoring gait; mild sensory changes may be observed. Cognitive function and the cranial nerves usually are spared. Systemic nonneurologic symptoms suggestive of an autoimmune process, such as pulmonary alveolitis, uveitis, arthropathy, Sjögren syndrome, and vasculitis, also may be noted.[134,135,316] Progression is variable; 10 years after onset, 30 percent of patients are bedridden, and 45 percent require crutches to walk.[97]

Nonspecific lesions of the brain are observed with magnetic resonance imaging (MRI) in as many as 75 percent of patients, but no clear correlation has been established between the lesions and symptoms. Atrophy of the spinal cord may occur, usually in the thoracic region. Multiple foci of increased T2 signal intensity are found in the periventricular white matter, similar to the findings observed in patients with multiple sclerosis. However, cognitive impairment may be noted in multiple sclerosis but is not found in HAM/TSP, and HTLV-I sequences have not been detected in the peripheral blood or CNS of patients with multiple sclerosis.[251]

High HTLV-I antibody levels are found in both peripheral blood and CSF. In CSF, mild to moderate pleocytosis, increased

TABLE 204–5 Clinical Findings in Human T-Cell Lymphotrophic Virus Type I–Associated Myelopathy/Tropical Spastic Paraparesis

Incidence	Primarily sporadic and occurring in adults, rare in childhood; female preponderance (2:1)
Onset	Usually insidious, rarely abrupt
Main neurologic findings	Chronic spastic paraparesis, slowly progressive
	Leg weakness, more marked proximally
	Bladder disturbance with urinary incontinence usually early, constipation later; impotence or decreased libido
	Paresthesias (tingling, pins/needles, burning) more prominent than objective physical signs
	Low lumbar pain with radiation to legs
	Impaired vibration sense, proprioception less often
	Hyperreflexia of lower limbs, often with clonus and Babinski signs
	Hyperreflexia of upper limbs; weakness may be absent
	Exaggerated jaw jerk in some patients
	Normal cognitive function
Less frequent neurologic findings	Cerebellar signs, optic atrophy, deafness, nystagmus, other cranial nerve deficits, hand tremor, absent or depressed ankle jerk
	Rarely, convulsions, cognitive impairment, dementia, impaired consciousness
Other possible neurologic findings	Muscular atrophy, fasciculation (rare), polymyositis, peripheral neuropathy, polyradiculopathy, cranial neuropathy, meningitis, encephalopathy
Possible systemic non-neurologic findings	Pulmonary alveolitis, uveitis, Sjögren syndrome, arthropathy, vasculitis, ichthyoses, cryoglobulinemia, monoclonal gammopathy, adult T-cell leukemia or lymphoma
Laboratory diagnosis	Detection of HTLV-I antibodies or antigens or viral isolation in blood and CSF
	Mild lymphocytic pleocytosis in CSF
	Lobulated lymphocytes in blood or CSF
	Mild to moderate increase in protein in CSF

CSF, cerebrospinal fluid; HTLV-I, human T-cell lymphotrophic virus type I.

protein, or oligoclonal immunoglobulin bands (or any combination of these findings) may be observed. Elevated CSF neopterin, an indicator of cellular immune activation, may be present. In approximately 50 percent of patients, atypical flower lymphocytes are observed and account for approximately 1 to 15 percent of peripheral blood lymphocytes; these cells also may be seen in CSF.[135] Unlike the monoclonal integration observed in ATLL, polyclonal integration of HTLV-I is noted in cells from patients with HAM/TSP.

Pathologically, HAM/TSP is characterized by perivascular demyelination and neuronal lesions. Macroscopic atrophy of the spinal cord occurs, with changes consistent with a chronic inflammatory process characterized by perivascular cuffing of mononuclear cells and lymphocytic infiltration of the brain and spinal cord. Early in the disease, these lymphocytes consist of both $CD8^+$ and $CD4^+$ T cells, along with B lymphocytes and macrophages in areas of parenchymal damage.[140] HTLV-I proviral DNA can be demonstrated in $CD4^+$ cells in the infiltrates by in situ PCR. Evidence suggests that these HTLV-1–infected cells migrate from the peripheral blood and cross the blood-brain barrier to enter the nervous system.[39] Later in the disease, the inflammatory cells are fewer in number and consist primarily of $CD8^+$ cytotoxic T cells. Marked myelin and axonal destruction and astrocytic gliosis are prominent. The lower thoracic spinal cord is particularly affected, and parenchymal damage of both white and gray matter of the cord may be present. In the brain, although perivascular mononuclear cell infiltration may be seen, parenchymal damage is an unusual finding. However, rare patients may have white matter lesions, cerebellar symptoms, amyotrophic lateral sclerosis–like symptoms, or neuropathy.[135]

The pathogenesis of HAM/TSP is not known. An increased HTLV-I viral load and an augmented humoral and cellular immune response to HTLV-I are reported in patients with HAM/TSP. When compared with asymptomatic HTLV-I–seropositive individuals or those with ATLL, patients with HAM/TSP have higher HTLV-I antibody titers, a higher proviral load, and elevated levels of spontaneous lymphocyte proliferation and proinflammatory cytokines, including IL-1, interferon-α, and tumor necrosis factor-α.[194,231,232] HTLV-I–specific $CD8^+$ cytotoxic T cells can be found in the CSF as well as the peripheral blood of patients with HAM/TSP, and a significant reduction in the naïve T-cell population occurs with a concomitant increase in the memory/effector $CD8^+$ cell population.[141,142,231] Examination of the T-cell receptor repertoire shows significant expansion of the $CD8^+$ T-cell population in patients with HAM/TSP as opposed to asymptomatic carriers. Many of these $CD8^+$ cells correspond to cytotoxic T lymphocytes directed against epitopes of the immunodominant Tax protein of HTLV-I.[342]

One postulated mechanism for nervous system damage is direct infection of CNS glial cells by HTLV-I; a direct cytotoxic immune response to the glial cell is generated and results in demyelination.[134] However, HTLV-I expression appears to be localized to infiltrating $CD4^+$ lymphocytes within the spinal cord lesions rather than nervous system parenchymal cells, and no clear evidence has established that HTLV-I infects CNS cells.[234] Alternatively, the heightened HTLV-I–specific immune response in patients with HAM/TSP and neuropathologic findings suggests that immune-mediated mechanisms may have a role in the pathogenesis of disease.[175] The activated HTLV-I–specific cytotoxic $CD8^+$ T cells observed in patients with HAM/TSP could secrete cytokines that may induce demyelination as well as increase the transmigration of additional HTLV-1–infected lymphocytes to the inflammatory lesion.[134,231,234]

Specific characteristics of the virus also may influence disease manifestations. In one study, a specific tax gene phylogenetic subgroup, *tax A*, was found to occur more commonly in patients with HAM/TSP than in healthy HTLV-I carriers, thus suggesting that functional or immunogenic differences in the transactivating Tax protein among HTLV-I viral types may play a role in causing disease.[85] Another hypothesized mechanism is more indirect and involves an HTLV-I–associated activation of autoreactive cells that could lead to an autoimmune process inducing myelin destruction. The finding that numerous autoimmune-like diseases may occur in HTLV-1–infected patients and may coexist with HAM/TSP is consistent with the latter hypothesis.[135] A genetic susceptibility to the development of HAM/TSP also may exist. HTLV-1–infected patients with the class I allele HLA-A*02 had a proviral load one third less than that of HTLV-I carriers who lack this allele, and they had half the odds for development of HAM/TSP.[144] Because the risk for HAM/TSP is related to proviral load, this relationship may be the result of a more efficient antiviral cellular immune response in individuals with a particular class I HLA allele.

Similar to ATLL, no curative treatment has been developed for HAM/TSP. However, prolonged survival may be seen. Mean survival after the onset of symptoms is 10 years, and the major causes of death are infection and cancer.[340] Symptomatic treatment includes measures to maintain muscle function and reduce spasticity. Treatment with systemic or intrathecal corticosteroids may induce a transient benefit in approximately 50 percent of patients, particularly those with early-stage disease, and zidovudine alone or combined with interferon-α therapy has shown some promise.[101,112,134]

HUMAN T-CELL LYMPHOTROPHIC VIRUS TYPE I–ASSOCIATED UVEITIS

Idiopathic, non-infectious uveitis has been reported to occur in HTLV-I–seropositive individuals, usually those who otherwise are asymptomatic.[208,209,235,306] In endemic areas in Japan, 38 percent of patients with idiopathic uveitis were infected with HTLV-I as compared with 19 percent of patients with nonuveitic ocular disease and 10 percent with uveitis of known origin.[210] In the younger age group (20 to 49 years) with idiopathic uveitis, HTLV-I seroprevalence was 49 percent. Similarly, in Brazil, an area of lower HTLV-I endemicity, HTLV-I seroprevalence also was elevated in patients with idiopathic uveitis; 1.8 percent of patients with idiopathic uveitis were HTLV-I–seropositive versus none of those with uveitis of known origin.[377] In a survey of 105 asymptomatic Brazilian HTLV-I carriers, uveitis was found in 2.8 percent of infected persons. In epidemiologic surveys in Japan, the prevalence rate of HTLV-I–associated uveitis in HTLV-1 carriers was lower, estimated to be approximately 0.1 percent.[377] The mean age at onset in a series of Japanese patients was 43 years in men and 48 years in women.[210] As noted for HAM/TSP, genetic factors also may be associated with a susceptibility to or the severity of HTLV-I–associated uveitis.[301]

The syndrome is characterized by the abrupt onset of blurred vision, foggy vision, floaters, or any combination of these findings. The predominant finding on ocular examination is intermediate uveitis with infiltrating cells and lacework-like membranous vitreous opacities. Approximately 14 percent of patients have panuveitis with uveoretinal lesions and mild retinal vasculitis, and 5 percent have iritis alone.[210] The uveitis is generally mild to moderate, and visual acuity is affected only slightly in most patients. Uveitis is unilateral in 57 percent and bilateral in 43 percent. The disease usually responds to topical or systemic steroids within a few weeks, although recurrence may be seen in 25 to 50 percent of cases within 2 months to 2 years after therapy is discontinued.[210,235] Many patients (≈20%) have hyperthyroidism (Graves disease) that precedes or follows the onset of uveitis. A study of 105 asymptomatic Brazilian HTLV-I–seropositive adults found abnormal results in at least one lacrimal film evaluation test in 40 percent versus 23 percent of uninfected controls,[377] a finding thus suggesting that abnormal early

ocular abnormalities may be present in asymptomatic HTLV-1 carriers.

HTLV-I antibody has been detected in the aqueous humor of patients with HTLV-I uveitis.[210] Additionally, proviral HTLV-I DNA has been demonstrated in T cells found in the intraocular fluid of 59 percent or more Japanese patients with HTLV-I uveitis. Production of cytokine by HTLV-1–infected T cells is hypothesized to be the cause of the intraocular inflammation.[210,289,340] Significant amounts of cytokines, including tumor necrosis factor-α, are produced by HTLV-1–infected T-cell clones that have been established from the ocular fluid of patients with HTLV-I–associated uveitis and by the retinal glial cells of rats experimentally infected with HTLV-I.[289,301]

PEDIATRIC MANIFESTATIONS

Infective Dermatitis

A severe, generalized, chronic relapsing dermatitis of childhood called *infective dermatitis* was described first in Jamaican children in 1966.[318] In 1967, researchers observed that cultures of the nares or skin lesions in children with infective dermatitis often were positive for *Staphylococcus aureus* or beta-hemolytic streptococci.[359] Patients with these infections responded well to antibiotic therapy but relapsed once therapy was withdrawn. The refractory nature of the disorder with frequent exacerbations, infections with bacteria that usually were nonvirulent, and resistance to treatment suggested an association with immune dysfunction. After HTLV-I was identified in 1980, epidemiologic studies demonstrated that HTLV-I was endemic in Jamaica; because HTLV-I infection was known to be associated with immune dysfunction and enhanced susceptibility to infections,[199] an association between infective dermatitis and HTLV-I infection was hypothesized.

In 1990, La Grenade and colleagues first described an association of infective dermatitis and HTLV-I infection in Jamaican children.[167] In a study of 14 children with typical infective dermatitis and 11 with atopic dermatitis, all children with infective dermatitis were found to be HTLV-I–seropositive versus none of those with atopic dermatitis. In a later, expanded report, all of 50 children with infective dermatitis were found to be HTLV-I–seropositive, as opposed to only 14 percent of 35 with atopic dermatitis.[164] Subsequently, cases of infective dermatitis were reported in HTLV-1–infected children in Trinidad, Colombia, Brazil, and Japan.[28,338]

The clinical features of infective dermatitis include the acute onset in early childhood of severe exudative eczema, typically without preceding infantile eczema. It is characterized by exudation and crusting involving the perinasal skin and nostrils, external ear and retroauricular areas, eyelid margins, scalp, neck, axillae, and groin. The eczema may be accompanied by a generalized, fine, papular rash on the trunk and back. A dermatopathic lymphadenitis with palpable lymph nodes also may be observed.[168] A chronic, watery nasal discharge in the absence of other causes of rhinitis is a common finding, and *S. aureus* or beta-hemolytic streptococci often are cultured from nose and skin lesions. Long-term antibiotic therapy is required to control the disease. Diagnostic criteria for HTLV-I–associated infective dermatitis are shown in Table 204–6.

In Jamaican children with infective dermatitis, HTLV-I antibody titers were significantly higher than those in asymptomatic HTLV-1–infected children. Children with infective dermatitis were more likely than those with atopic dermatitis to be anemic and to have lower serum albumin, higher white blood cell counts and erythrocyte sedimentation rates, lymphocytosis with atypical lymphocytes, and elevated serum immunoglobulins.[168] These findings are consistent with chronic inflammation and antigenic stimulation caused by the relapsing bacterial infections characteristic of infective dermatitis. However, elevated levels of T-cell activation also were seen in children with infective dermatitis, along with a higher $CD4^+$ and $CD8^+$ T-lymphocyte number, $CD4^+/CD8^+$ ratio, and percentage of HLA-DR antigen-positive T cells. These findings may be related primarily to HTLV-I infection rather than being secondary to infective dermatitis. In a cross-sectional study of asymptomatic HTLV-I–seropositive and HTLV-I–seronegative Jamaican children 11 to 31 months of age, HTLV-I infection was associated with an increase in $CD4^+$ cells expressing HLA-DR on their surface that was progressive and related to the duration of infection.[190] This finding may be an early marker for HTLV-I infection in children and indicative of an early perturbation in the immune system.

Complications of infective dermatitis occur in 30 to 35 percent of patients. The most common complications are crusted (Norwegian) scabies, corneal opacities, chronic bronchiectasis, and infection with parasitic worms such as *S. stercoralis*.[166] Other reported complications include lymphocytic interstitial pneumonitis and glomerulonephritis, which reflect the systemic complications of HTLV-I infection. Early death caused by severe bacterial infections with sepsis or the development of other later manifestations of HTLV-I infection may occur. Although ATLL and HAM/TSP in children are reported only rarely, a few cases of ATLL or HAM/TSP developing in patients with infective dermatitis after 12 to 25 years were reported.[25,108,169,338] The class II HLA haplotype DRB1*DQB1* was found in HTLV-1–infected children with infective dermatitis and also in infected Japanese

TABLE 204–6 Diagnosis of Human T-Cell Lymphotrophic Virus Type I–Associated Infective Dermatitis

Type of Criteria	Clinical/Laboratory Findings
Major criteria (diagnosis requires the presence of items 1, 2, and 5, with at least 2 items present from 1)	1. Crusting eczema involving the scalp, eyelid margins, perinasal skin, external ear and retroauricular areas, axillae, groin, and/or neck (must include 2 sites or more) 2. Chronic watery nasal discharge and/or crusting of the anterior nares without other signs of rhinitis 3. HTLV-I seropositivity 4. Chronic relapsing dermatitis with prompt response to appropriate antibiotic therapy but prompt recurrence as soon as therapy is stopped 5. Onset in early childhood
Minor or less specific criteria	1. Positive skin or anterior nares cultures for *Staphylococcus aureus* and/or beta-hemolytic streptococci 2. Fine, generalized papular rash 3. Generalized lymphadenopathy (with histologic dermatopathic lymphadenitis on biopsy, if performed) 4. Anemia 5. Elevated erythrocyte sedimentation rate 6. Hyperimmunoglobulinemia (IgD and IgE) 7. Elevated $CD4^+$ and $CD8^+$ T-cell count and elevated $CD4^+/CD8^+$ ratio

HTLV-I, human T-cell lymphotrophic virus type I; IgD and IgE, immunoglobulins D and E.

adults with HAM/TSP.[170] These findings suggest a possible genetically increased susceptibility to some HTLV-I disease manifestations, perhaps secondary to an exaggerated host immune response to HTLV-I infection in individuals with this haplotype.[170,188] Children with HTLV-I–associated infective dermatitis, therefore, may be at risk for development of more serious HTLV-I–associated disorders later in life.

Histologically, an inflammatory lymphocytic infiltrate is seen within the skin lesions. The HTLV-I genome was detected by PCR in lymphocytes cultured from biopsy specimens of skin lesions in patients with infectious dermatitis, although cultured fibroblasts were negative, thus suggesting that HTLV-1–infected lymphocytes had infiltrated the skin.[166] Cultured keratinocytes from children with infectious dermatitis were shown to exhibit overexpression of proinflammatory and anti-inflammatory cytokines that could be induced directly or indirectly by HTLV-I infection.[348] Secretion of cytokines by HTLV-1–infected cells may amplify or maintain a persistent inflammatory reaction in the skin and, when combined with the enhanced susceptibility to infection induced by HTLV-I–associated immunodysfunction, could result in the clinical manifestations of infective dermatitis. In a rabbit model, infection with HTLV-I by intravenous inoculation was associated with the development of generalized exfoliative papillary dermatitis characterized by T-cell infiltrates in the epidermis and epithelium of the hair follicle, similar to that seen in cutaneous T-cell lymphoma.[309] HTLV-I envelope sequences were detected by DNA PCR in rabbit skin biopsy samples, and HTLV-I was isolated from cultures of affected skin. Researchers postulated that infective dermatitis represents an HTLV-I–associated immunodeficiency syndrome resulting from exposure to HTLV-I in early life, primarily through mother-to-child transmission.

Treatment of infective dermatitis is symptomatic and targeted at controlling bacterial superinfection with the use of antibiotic therapy. However, long-term therapy is needed because of the relapsing nature of the illness. Occasionally, mild topical steroids are used for severe dermatitis.

Other Pediatric Manifestations

Renal manifestations rarely have been reported in association with infective dermatitis in children. Miller and colleagues reported a syndrome of infective dermatitis, glomerulonephritis, renal failure, severe hypertension with hypertensive encephalopathy, microangiopathic hemolytic anemia, and significant glomerulosclerosis with fibrosis on renal biopsy specimens in two Jamaican children infected with HTLV-I.[205] Pulse methylprednisolone was effective in reversing the renal failure in a child who was in the early stage of illness, but steroids were ineffective in a child treated later in the course of illness. In HTLV-1–infected adults, a syndrome of hemolytic-uremic syndrome or thrombotic thrombocytopenic purpura with severe hypertension, or both, has been reported.[66,339] Chronic renal failure has been reported rarely in adults with ATLL. In one patient with ATLL, HTLV-I antigen with bound immunoglobulin was demonstrated by immunofluorescence in the glomerulus, a finding suggesting that glomerular deposition of HTLV-I–associated immune complexes may be the cause of the renal damage.[230] Alternatively, concomitant infection with group A beta-hemolytic *Streptococcus* during the immunodeficiency associated with HTLV-I could result in streptococcal-associated glomerulonephritis.

OTHER DISORDERS

Numerous autoimmune disorders have been associated with HTLV-I. In a study of 113 HTLV-1–infected patients in southern Florida, rheumatologic or autoimmune diseases were not uncommon manifestations.[112] HTLV-I–associated large joint chronic arthropathy has been described. Clinically, the disorder is manifested as oligoarthritis of the shoulder, wrists, or knees, with a chronic course. Some patients may have accompanying myalgia or bronchitis.[238] Proliferative changes are observed in nonlymphocytic mesenchymal and synovial cells, along with synovial overgrowth. HTLV-I antibodies are detected in the synovial fluid of affected joints, and HTLV-I proviral DNA has been found in synovial cells and synovial fluid lymphocytes.[160,340] Chronic inflammatory arthropathy can develop in HTLV-I transgenic mice.[139] HTLV-I Tax protein may be associated with proliferation of synovial cells that leads to erosion of cartilage and bone.

HTLV-I–associated polymyositis has been described in patients with HAM/TSP, as well as in those without neurologic impairment.[213,256,376] Muscle biopsy is consistent with myositis with mononuclear interstitial infiltrates, necrosis, and regeneration. HTLV-I provirus has been identified by in situ hybridization in $CD4^+$ cells in the inflammatory cell infiltrate.[120] The mechanism by which HTLV-I produces disease is unknown. It is probably not a direct viral effect because HTLV-I does not appear to infect muscle cells.[256] As hypothesized for HAM/TSP, the pathologic process could be the result of an autoimmune response, or it could be caused by the production of cytokines in focal inflammatory infiltrates in muscle by activated HTLV-1–infected $CD4^+$ cells and subsequent bystander damage to the myofibers.

Asymptomatic, subclinical lymphocytic pneumonitis has been described in patients with HAM/TSP. Bronchoalveolar lavage has shown the presence of a T-lymphocyte alveolitis, with highly soluble IL-2 receptor levels also found in lavage fluid from these patients.[315,316] On lung biopsy, marked lymphocytic infiltration of the lung is seen. The presence of HTLV-I provirus in alveolar lymphocytes obtained by lavage was described in 7.5 to 30 percent of patients with HAM/TSP and in fewer than 5 percent of HTLV-1–infected patients without myelopathy.[135]

HTLV-I also has been associated with Sjögren syndrome, a chronic exocrinopathy causing keratoconjunctivitis sicca, xerostomia, and sialadenitis and characterized by a lymphocytic infiltration of the lacrimal and salivary glands; the origin has been hypothesized to be autoimmune. In a study in Japan, HTLV-I seroprevalence in patients with Sjögren syndrome was 23 percent, significantly higher than the 3 percent HTLV-I seroprevalence in blood donors.[331] HTLV-I antibody titers in HTLV-I–seropositive patients with Sjögren syndrome were significantly higher than those in asymptomatic HTLV-I carriers and similar to those seen in patients with HAM/TSP. However, in contrast to that in patients with HAM/TSP, the HTLV-I proviral load in peripheral blood was not always high. HTLV-I salivary immunoglobulin A (IgA) antibodies occurred commonly in HTLV-I–seropositive patients with Sjögren syndrome but very rarely in asymptomatic HTLV-I patients or those with HAM/TSP, a finding thus suggesting potential increased viral activity within the salivary glands.[331] In a study of HTLV-1–infected patients from Guadeloupe, French West Indies, a sicca-like syndrome was found in almost 80 percent of patients, approximately half of whom also had neurologic findings.[19] HTLV-I provirus has been identified by PCR in acini cells and inflammatory infiltrates in the labial salivary glands of patients with HAM/TSP, as well as in healthy HTLV-I carriers with the sicca syndrome.[329] In addition, one of the symptoms of Sjögren syndrome is impaired sweating, and HTLV-I pX sequences have been identified in samples of eccrine sweat gland epithelia from HTLV-1–infected individuals.[303] Transgenic animal models have indicated that HTLV-I is tropic for ductal epithelium of the salivary and lacrimal glands.[102]

HTLV-I infection, even in the absence of other disease manifestations, is associated with immune dysfunction. Perturbations in lymphokine and cytokine production have been observed with

HTLV-I in vitro, most likely caused by Tax-mediated transactivation of various host-cell pathways. Spontaneous lymphocyte proliferation is seen in asymptomatic seropositive patients, as well as in patients with HAM/TSP,[362] and changes in T-cell subsets reflecting an increase in activation markers, such as the IL-2 receptor, also may be observed. Opportunistic infections such as *Pneumocystis* pneumonia and Norwegian scabies can develop in HTLV-1–infected patients without other disease manifestations, and these patients are more likely to experience numerous other infections, including tuberculosis, leprosy, and strongyloidiasis.[224,225,282,350] HTLV-1–infected patients have lower eosinophil counts than those of uninfected patients, perhaps related to the increased susceptibility of HTLV-1–infected persons to parasitic diseases.[226]

DUAL INFECTION WITH HUMAN IMMUNODEFICIENCY VIRUS TYPE 1

Because some of the geographic areas endemic for HTLV-I also are endemic for HIV-1, dual infection with both viruses may occur.[15,16] In a study in the coastal port of Santos, Brazil, concurrent infection with HTLV-I and HTLV-II was seen in 6.0 and 7.4 percent of HIV-1–infected individuals, respectively.[75] Intravenous drug use and seropositivity to hepatitis C virus were correlated with co-infection with either HTLV-I or HTLV-II.

Based on in vitro studies indicating that the HTLV-I tax gene product can interact with the Tat response element of the HIV-1 LTR and enhance HIV-1 replication, researchers proposed that dual infection may increase the progression of HIV-1 disease or increase the frequency of HTLV-I–associated diseases.[308] However, whether enhanced replication of HIV-1 occurs in dually infected patients in vivo is unclear. In one study in Brazil, no significant difference in HIV-1 plasma RNA levels was observed in 23 patients with HIV-1/HTLV-I co-infection and in 92 patients with HIV-1 infection alone.[113]

The findings of several small clinical studies were consistent with the hypothesis of potential enhanced disease progression with co-infection. HTLV-I/HIV-1 co-infection in intravenous drug users was found to be associated with a threefold increase in mortality when compared with drug users who had only HIV-1 infection.[258] In a study in Trinidad, co-infected individuals had faster progression to acquired immunodeficiency syndrome (AIDS) than did those with HIV-1 infection alone, and in a study from Rio de Janeiro, patients dually infected with HIV-1 and HTLV-I had a more advanced WHO HIV disease stage.[13,293] Those patients with advanced disease and dual infection had higher $CD4^+$ lymphocyte counts than did those with a similar disease stage but who were infected with HIV-1 alone; similar findings also were reported in another study.[76,293] The $CD4^+$ number, therefore, may be an unreliable predictor of immunodeficiency in dually infected patients; HTLV-I may induce an elevated $CD4^+$ number through enhancement of lymphocyte proliferation, but the function of these cells may be abnormal.[36]

In addition, HIV-1 infection possibly increases the likelihood for development of some HTLV-I–associated diseases. Expression of HTLV-I and HTLV-II appears to be up-regulated in patients co-infected with HIV-1.[20] Several reports have been published of patients with dual infection in whom a typical HAM/TSP syndrome developed, and antibodies to HIV-1 and HTLV-I were present in the blood and CSF of one dually infected patient with neurologic disease.[2,21,202]

HUMAN T-CELL LYMPHOTROPHIC VIRUS TYPE II

HTLV-II was identified first in patients with hairy-cell leukemia.[148,285,286] HTLV-II is closely related to HTLV-I, with approximately 60 percent homology in nucleotide sequences.[165] HTLV-II infection is endemic in Native American populations and probably is an ancient infection that has been maintained in the population by sexual transmission and mother-to-child transmission through breast-feeding.[106,171,173] More recently, HTLV-II has been introduced into urban settings in the United States, Europe, and Asia, where transmission occurs primarily through intravenous drug use or transfusion of contaminated blood. The role of this virus in causation of disease has not been as definitively established as that for HTLV-I, but a possible association with lymphoproliferative and neurologic disorders has been suggested (see Table 204–1).

VIRAL PATHOGENESIS/MOLECULAR BIOLOGY

Like HTLV-I, HTLV-II can transform cells in vitro. However, HTLV-II displays a preferential tropism for and induces clonal expansion of cells of the $CD8^+$ T-lymphocyte phenotype.[47,136,182] In an evaluation of HTLV-I1–infected patients, HTLV-II was detected exclusively within $CD8^+$ lymphocytes in most individuals and less frequently in both $CD4^+$ and $CD8^+$ T cells in some individuals.[136,168] Whether the difference in cell tropism between HTLV-I and HTLV-II may lead to variations in pathogenicity between the two viruses is unknown.

Based on nucleotide sequencing of the viral envelope and phylogenetic analysis, two major molecular subtypes of HTLV-II have been identified and designated HTLV-IIA and HTLV-IIB; these subtypes have a 4 to 7 percent divergence in the gene encoding the envelope transmembrane protein.[107] Studies focusing on the LTR and pX regions of the provirus have shown additional differences. The Tax protein appears to differ in length between the two subtypes, with the HTLV-IIB Tax protein being 25 amino acids longer than the HTLV-IIA Tax protein.[259] Functionally, the HTLV-IIA Tax protein is a weaker inactivator of the *p53* tumor suppressor gene and a less potent transactivator of the viral LTR in vitro than is the Tax protein from HTLV-I or HTLV-IIB, and limited data suggest that the proviral load may be lower in HTLV-IIA–infected than HTLV-IIB–infected individuals.[71,184] Based on phylogenetic analysis of nucleotide sequences in the LTR region of the virus, two additional subtypes have been identified in restricted geographic locations, HTLV-IIC and HTLV-IID.[71,184] The Tax protein of HTLV-IIC is the same length as that of HTLV-IIB, and the length of Tax in HTLV-IID is intermediate between the A and B-C subtypes.

EPIDEMIOLOGY

HTLV-II is endemic in a large number of Native American populations in the United States (Navajo and Pueblo Indians in New Mexico and the Seminole Indians in Florida) and numerous indigenous Amerindian populations in Latin America, including Panama, Colombia, Argentina, Brazil, and Chile.[107,117,129,130,178,186,365] HTLV-II seroprevalence in these Amerindian tribes varies between 2 and 30 percent.[128,186,220,351] Although HTLV-I is endemic in Africa, HTLV-II seroprevalence appears to be relatively low; in one study, the prevalence of HTLV-II was 0.8 percent in the Ivory Coast, 0.05 percent in Guinea, and 0.02 percent in Senegal.[32] However, in a study in Eritrea, 2.1 percent of female sex workers were found to be HTLV-II–seropositive.[10]

In the United States, Europe, and Southeast Asia, HTLV-II infection is found primarily in intravenous drug users, with seroprevalence rates ranging from 3 to 18 percent.[33,34,157] In the United States, HTLV-II accounts for most of the HTLV infections in intravenous drug users.[43] In a study of HTLV-II and HIV seroprevalence in drug users from eight metropolitan areas in the

United States, the overall prevalence of HTLV-II alone was 15.1 percent, the prevalence of HIV-1 alone was 9.9 percent, and that of dual HIV-1/HTLV-II infection was 3.3 percent; HTLV-II prevalence was higher in the Southwest and Midwest than the Northeast, whereas HIV-1 prevalence was highest in the Northeast.[33] HTLV-II seroprevalence in drug users was highest in black and Hispanic persons, increased with age, and it was higher in women than in men in all age groups. The female preponderance of HTLV-II infection may indicate more efficient sexual transmission of HTLV-II from men to women than vice versa; similar findings have been noted with HTLV-I. In persons attending clinics for sexually transmitted diseases in the United States, nearly two thirds of HTLV infections are caused by HTLV-II.[157] In a study involving blood donors in the United States, HTLV-II prevalence was 22.3 per 100,000 donors and was highest in West Coast blood centers.[228] HTLV-II infection was associated with an age of 40 to 49 years, first-time blood donation, female sex, high school or lower education level, and hepatitis C seropositivity.

The geographic distribution of HTLV-II subtypes varies.[106] HTLV-IIA is the predominant infection in intravenous drug users in North America and Europe and is widespread worldwide. HTLV-IIB predominates in Paleoindian groups, such as the Native North American population, although a mixture of HTLV-IIA and HTLV-IIB also has been reported in some tribes.[131] In South America, HTLV-IIB infection is seen in native populations in Panama, Colombia, and Argentina, but an indigenous Brazilian Indian population in the Amazon appears to be infected with HTLV-IIC, also seen in intravenous drug users in urban areas of Brazil.[71] In Europe, a mixture of infections is noted; for example, HTLV-IIA is found primarily in Sweden, whereas HTLV-IIB is more prevalent in Spain and Italy. HTLV-IID has been detected in central African Pygmies.

MODES OF TRANSMISSION

HTLV-II, like HTLV-I, is transmitted by transfusion of contaminated cellular blood products, by injection with contaminated needles, sexually, and from mother to child. Whether differences in efficiency of transmission exist between the two viruses is unclear.

Sexual transmission is an important mode of HTLV-II acquisition, although data are more limited for HTLV-II than for HTLV-I. Studies in non–drug-using native populations have shown a strong association of HTLV-II infection between spouses.[185,351] Among Guaymi Indians in Panama, HTLV-II seroprevalence approaches 10 percent. Seropositivity in women is associated with an early age at first sexual intercourse, the number of lifetime sexual partners, and the number of long-term sexual relationships; in men, sexual intercourse with prostitutes is associated with seropositivity.[185] In HTLV-II–seropositive injecting drug users, higher rates of seropositivity are found in individuals with a history of sexually transmitted diseases.[33,299,352] Preferential male-to-female transmission has been reported, as with HTLV-I. In HTLV-II–seropositive blood donors, a history of sexual contact with an intravenous drug user or an HTLV-I1–infected sexual partner was associated with increased risk of HTLV-II seropositivity.[297] In addition, a high prevalence of HTLV-II seropositivity in patients who are not intravenous drug users but who have sexually transmitted diseases also supports the role of sexual contact in HTLV-II transmission.[297]

Injecting drug use is the primary mode of HTLV-II acquisition in the United States. In a study of HTLV-infected blood donors (the Retrovirus Epidemiology Donor Study), HTLV-II infection was associated significantly with injection drug use or sexual contact with an injecting drug user, whereas HTLV-I infection was not.[297] Having had an abortion also was an independent risk factor for the acquisition of HTLV-II infection by female blood donors, probably because abortion is a marker of increased unprotected sexual activity. Other risk factors, such as seven or more sexual partners, were common to both HTLV-I and HTLV-II. Sex with an injecting drug user was a particular risk factor for women; 65 percent of HTLV-I1–infected women reported that they had a sexual partner who used injection drugs, and 20 percent of women reported that they also injected drugs themselves. HTLV-II infection in drug users has been associated with nonwhite race, older age, markers of previous hepatitis B virus infection, the use of a specific needle-sharing practice called backloading, a history of herpes simplex virus type 2 infection, and a history of receiving money for sex.[33,299,352] In an Italian study of risk factors for development of HTLV in patients seen in a clinic for sexually transmitted diseases, injecting drug use was associated with HTLV-II infection, whereas HTLV-I infection was associated more strongly with sexual behavior.[97] Among injecting drug users, seroprevalence for HTLV-II was 8.2 percent versus 2.1 percent for HTLV-I. HTLV-I infection was associated with exposure to syphilis and non-European nationality.

HTLV-II also has been detected in the breast milk of carrier mothers, and mother-to-child transmission has been described in those who breast-feed.[118,167,169,343] The rate of transmission of HTLV-II through breast milk described in the few small studies available (≈14%) is slightly lower than that found for HTLV-I. Like HTLV-I, HTLV-II appears to be transmitted infrequently in the absence of breast-feeding.[88,106,151,347] Passive immunization with HTLV-II hyperimmunoglobulin in rabbits prevented blood-borne transmission of HTLV-I1–infected cells.[214] Only HTLV-II and not HTLV-I immunoglobulin was effective in preventing HTLV-II transmission, a finding thus suggesting that despite some cross-reactivity on conventional ELISAs, cross-neutralization between the viruses is minimal or nonexistent.[214]

CLINICAL DISEASE

When compared with HTLV-I, HTLV-II infection appears to be associated with a much lower prevalence of virus-associated neoplasia. Although HTLV-II was isolated initially from the cells of patients with hairy-cell leukemia,[30,148] subsequent studies did not confirm this association. In an HTLV-II–endemic population of Native Americans in New Mexico, no apparent increase in the incidence of hairy-cell leukemia, mycosis fungoides, and chronic lymphocytic leukemia was observed over that in other ethnic groups.[129]

When cancers do develop, they are of the $CD8^+$ cell phenotype. Unusual skin disorders resembling cutaneous lymphoma have been reported in a small number of patients co-infected with HIV-1 and HTLV-II. Severe erythroderma with subsequent exfoliation of the skin accompanied by eosinophilia and dermatopathic lymphadenopathy was reported in two intravenous drug users with dual infection.[153] In contrast to most cutaneous lymphomata in the United States, which are of the $CD4^+$ cell phenotype, the infiltrating T cells were of the $CD8^+$ phenotype. In a third HIV-1/HTLV-II co-infected patient with a cutaneous $CD8^+$ T-cell lymphoma, the skin infiltrate was found to contain $CD8^+$ cells with HTLV-II DNA.[264] HTLV-II infection of $CD8^+$ cells was described in one patient with large granular cell lymphocytosis and in another patient with large granular lymphocytic leukemia.[181,200] However, HTLV-II was found in only 2 percent of patients with large granular lymphocytic leukemia in a subsequent study of 51 patients.[116] HTLV-II infection also was described in one patient with mycosis fungoides.[387]

Evidence suggests that HTLV-II may be associated with neurologic disorders ranging from a spastic paraparesis-myelopathy

similar to HAM/TSP to more widespread involvement of the CNS.[222] Myeloneuropathies indistinguishable from HAM/TSP have been reported in patients infected with HTLV-II alone or co-infected with HIV-1.[21,127,220,222,228] In addition, a chronic neurodegenerative disorder with ataxia as a prominent feature has been reported in patients with HTLV-II infection.[111,305] In patients in whom HTLV-II was subtyped, type A was identified. Most cases occurred in women, similar to the observed female preponderance in patients with HAM/TSP. Patients with HIV-1 infection who have sensory peripheral polyneuropathy have been found to have a higher prevalence of HTLV-II co-infection, as diagnosed by serology and DNA PCR, than have HIV-1–infected patients without neuropathy, thus suggesting that HTLV-II may be involved in the pathogenesis of the neuropathy.[386]

Although immune deficiency and an increased risk of infections have been reported in HTLV-1–infected individuals, the data are less clear for HTLV-II. An initial report from San Francisco described an association of HTLV-II seropositivity and bacterial infections, particularly skin and soft tissue infections and bacterial pneumonia, but the results were confounded by intravenous drug use, which in itself could increase the risk for infections, in nearly all HTLV-II–seropositive patients.[211] In a case-control study of intravenous drug users from Baltimore with an overall HTLV-II seroprevalence of 7 percent, no significant association between HTLV-II seropositivity and the development of bacterial pneumonia, infective endocarditis, and skin abscess was found.[288] However, in a study of blood donors infected with HTLV-I or HTLV-II who were also HIV-1–seronegative, HTLV-II infection was associated with an increased incidence of bronchitis, bladder or kidney infection, and oral herpes simplex virus infection and with a borderline increase in the incidence of pneumonia.[225] Clearly, the natural history and clinical manifestations of HTLV-II need further delineation in the context of ongoing prospective natural history studies.

OTHER FORMS OF HUMAN T-CELL LYMPHOTROPHIC VIRUS

Additional forms of HTLV that are distinctly different from HTLV-I and HTLV-II have emerged.[37,373] The identification of HTLV-III and HTLV-IV among central African bushmeat hunters demonstrates greater HTLV diversity than was recognized previously. Preliminary data would suggest that HTLV-III comes from a simian origin, whereas HTLV-IV does not appear to have a known simian counterpart. Both these newer forms of HTLV are serologically indistinguishable from HTLV-I and HTLV-II by current assays and highlight the need for accurate testing. Although no clinical maladies have been attributed to HTLV-III or HTLV-IV to date, concern exists over the spread of infection by blood donations from infected persons or by those who participate in primate hunting. Further research will be required to understand more clearly what implications these newer forms of HTLV have on human disease.

LENTIVIRUSES: HUMAN IMMUNODEFICIENCY VIRUS TYPE 2

HIV-2 has a morphology and life cycle similar to those of HIV-1 but with significant antigenic, biologic, epidemiologic, and clinical differences (Table 204–7). Both viruses have similar modes of transmission and can result in immune depletion and AIDS. However, whereas HIV-1 has a global distribution, HIV-2 is confined primarily to West Africa and is found only sporadically in Europe, the United States, and other countries. The clinical latency period and rates of perinatal and sexual transmission also are lower than those for HIV-1. The basis of such

TABLE 204–7 Comparison of Human Immunodeficiency Virus Types 1 and 2

Characteristic	HIV-2	HIV-1
Viral Genome	*vpx* gene, no *vpu* gene *nef* gene longer in HIV-2, defective *nef* gene in 10% Difference in number and identity of transcription enhancers for LTR (less responsive to stimulation?)	*vpu* gene, no *vpx* gene Defective *nef* gene in <1% Greater responsiveness of LTR to transcription enhancers?
Geographic Distribution	West Africa Restricted distribution outside Africa, primarily in countries with ties to West Africa Europe: Portugal, France, Germany, Spain South America: Brazil India	Global
Modes of Transmission	Sexual, blood-borne, mother-to-child	Sexual, blood-borne, mother-to-child
Risk of Sexual Transmission	3-fold lower than HIV-1	
Seroconversion in commercial sex workers in Senegal[149,197]	Incidence of seroconversion in commercial sex workers in Senegal: 1% per year	Incidence of seroconversion in commercial sex workers in Senegal: 2%-3% per year
Risk of Mother-to-Child Transmission (without Antiretroviral Prophylaxis)	0%-4%	25%-35%
Viral Load (without Therapy)	28- to 30-fold lower than HIV-1	
Viral set-point:		
Seroconversion in Guinea-Bissau[9]	Median RNA, 2,500 copies/mL	Median RNA, 70,000 copies/mL
Commercial sex workers in Senegal[266,267]	Median RNA, 263 copies/mL	Median RNA, 7,182 copies/mL
Pregnant women in the Gambia[247]	Geometric mean RNA, 410 copies/mL	Geometric mean RNA, 15,100 copies/mL
Rate of $CD4^+$ T-Cell Decline	1% per year	10% per year
Time to Development of AIDS	<0.5% per year	3%-5% per year
Increase in Mortality Above that of HIV-Seronegative Individuals	2- to 4-fold increase	10-fold increase

HIV, human immunodeficiency virus; LTR, long terminal repeat.

differences in natural history between these two lentiviruses is unknown and may result from characteristics of the virus, the host, or both.

VIRAL GENOME/PATHOGENESIS

Seven genetic groups of HIV-2 (A to G) have been identified. However, current data suggest that only HIV-2 subtypes A and B are established in significant amounts in human populations.[23] No differences in replication potential between the two subtypes appear to exist.[55] HIV-1 and HIV-2 differ in the number and size of their accessory genes. HIV-2 lacks the genetic equivalent of the HIV-1 *vpu* gene. The HIV-1 vpu protein plays an important role in intracellular processing of $CD4^+$, specifically the down-regulation of $CD4^+$ receptor expression on HIV-1–infected cells. This characteristic could enhance the ability of cytoplasmic HIV-1 envelope proteins to reach the cell membrane and also enhance release of budding virions; no equivalent functional protein exists in HIV-2. HIV-2 contains the *vpx* gene, which is not found in HIV-1 but shares homology with the HIV-1 *vpr* gene and may affect early events in the replication cycle. Additionally, the *nef* ORF in HIV-1 is shorter than that in HIV-2, and the prevalence of defective *nef* genes in patients with HIV-2 infection (≈10%) is significantly higher than previously seen in HIV-1–infected individuals (<1%).[319] In HIV-1, deletions in the *nef* gene have been described in patients with long-term nonprogression.

The seven-transmembrane G-protein–coupled chemokine receptors CCR5 and CXCR4 are the major co-receptors used for HIV-1 and HIV-2 infection in vivo. Cell tropism of HIV is determined by the expression of $CD4^+$ and these co-receptors. Cell tropism is thought to play a role in both virus transmission and disease progression. Most transmitted HIV-1 viruses are R5 tropic, highlighted by the substantial protection from infection observed in individuals homozygous for a 32-bp deletion in CCR5. CXCR4 binding viruses emerge late in disease in as many as 50 percent of patients with AIDS. This change in co-receptor use correlates with progression of disease in infected individuals, although it is not a prerequisite as not all infected individuals demonstrate this co-receptor switch.

X4 strains target primarily new populations of T cells that express CXCR4 but not CCR5. Multiple other members of the chemokine receptor family and other receptor molecules can support infection of indicator cell lines in vitro. They include CCR3, CCR8, GPR1, GPR15, CXCR6, RDC1, and apj. In general, HIV-2 and SIV strains use a wider range of these alternative co-receptors than HIV-1.

Chemokine receptors together with $CD4^+$ molecules allow binding of HIV and ultimately entry into host cells. Binding of HIV to these host-cell surface molecules permits close interaction between or fusion of the virus and cell membranes to occur. Whereas both HIV-1 and HIV-2 infect $CD4^+$ T lymphocytes and use the CXCR4 or CCR5 co-receptors for cell entry, HIV-2 can use multiple additional co-receptors.[216] The cytopathicity of HIV-2 appears similar to that of HIV-1 and is determined by the type of co-receptor used for cell entry. In an in vitro study comparing HIV-1 and HIV-2 co-receptor use and cytopathicity in human lymphoid cells, HIV-2 specificity for the CCR5 co-receptor alone or in combination with other co-receptors was associated with restricted cytopathicity, whereas specificity for CXCR4 was linked to a more virulent phenotype, as observed for HIV-1.[296]

More recently, co-receptor use of HIV variants isolated from individuals who had undetectable viral loads, preserved $CD4^+$ cell counts, and a nonprogressive clinical course was compared with those from individuals with more progressive disease. In this comparison, HIV-2 variants were found to use CCR5, GPR15, and CXCR6 with high efficiency regardless of the clinical course. Consistent with prior studies, only HIV-2 variants isolated from individuals with viremia or overt progressive disease could use CXCR4. X4 variants were not observed in all individuals with progressive infection, a finding indicating that, although the capacity to use CXCR4 is likely a determinant of HIV-2 virulence, it is not the only one.

Studies have shown that the use of CCR1, CCR2b, and CCR3 co-receptors is rare.[27] Thus, the lesser virulence of HIV-2 compared with that of HIV-1 does not appear to be the result of a restriction in co-receptor use or lower intrinsic cytopathic potential. The linkage between syncytium-inducing phenotype and CXCR4 co-receptor usage was not observed in HIV-2 as it was in HIV-1. In addition, the broadening of the co-receptor usage did not necessarily increase the pathogenic potential of HIV 2 strains.[345]

In a study of sero-incident cases of HIV-1 and HIV-2 infection in Guinea-Bissau, the viral set-point after seroconversion was 28-fold lower in HIV-2 than in HIV-1 recent seroconverters.[9,242] Other studies have shown a broader-based neutralizing antibody response and higher antibody levels in HIV-2- than HIV-1–infected individuals.[197,327] These findings suggest that HIV-2 may have low rates of viral replication or a reduced ability to mutate and escape the host immune response, or both.

Productive lentiviral infection depends on continued activation of target cells. The lower rate of viral production in HIV-2–infected cells also could reflect a lesser ability of infected cells to respond to activation or could indicate that the cells themselves have lower activation states. The transcriptional enhancer/promoter region of the HIV-2 LTR differs from that of the HIV-1 LTR in the number and identity of enhancer elements, and transcriptional up-regulation of viral production may be disrupted more easily than for HIV-1.[196,266] This factor could result in lower responsiveness of the HIV-2 LTR to transcription factors in activated T cells. For example, the HIV-2 LTR has been shown to be less responsive to stimulation of viral gene expression by tumor necrosis factor-α than is the HIV-1 LTR.[109]

Lentiviral envelope glycoproteins can induce production of cytokines and other immunologic disturbances; differences in the effect of the HIV-1 and HIV-2 envelope glycoprotein also could contribute to differences in the pathogenicity of the viruses. Recombinant HIV-2 envelope glycoprotein is superior to the HIV-1 envelope in stimulating the production of interferon-γ and IL-16, both of which inhibit viral replication, and less effective in producing expression of IL-4, which stimulates viral replication.[237,302] The HIV-2 envelope glycoprotein inhibits T-cell proliferation more than does the glycoprotein of HIV-1 in vitro, and it also was found to inhibit expression of cell surface activation markers.[38] The presence of an HIV-2 protein that reduces immune system stimulation may decrease replication, result in lower levels of HIV-2 viremia, and also decrease $CD4^+$ cell apoptosis. The rate of total lymphocyte apoptosis has been found to be lower in HIV-2 than in HIV-1 infection.[203] In one study, 50 percent of peripheral blood mononuclear cells from HIV-2–infected commercial sex workers resisted in vitro challenge with CCR5-dependent HIV-1 but not CXCR4-dependent HIV-1.[163] Additionally, high levels of beta-chemokines RANTES (regulated on activation, normal T-cell expressed and secreted), macrophage inflammatory protein 1α (MIP-1α), and MIP-1 β, the natural ligands of the CCR5 receptor, were secreted when these resistant peripheral blood mononuclear cells from HIV-2–infected individuals underwent stimulation. These investigators hypothesized that beta-chemokine–mediated resistance could play a role in the potential protection of some HIV-2–infected individuals from secondary HIV-1 infection.

EPIDEMIOLOGY

HIV-2 is endemic in the former Portuguese and French colonies in West and South Central Africa, including Guinea-Bissau, Burkina Faso, the Gambia, Cape Verde, Senegal, and the Ivory Coast, as well as in Angola and Mozambique.[196] Transmission occurs principally through heterosexual contact. The overall prevalence of HIV-2 in these areas is approximately 1 to 2 percent, but seroprevalence rates of 8 percent in pregnant women in Guinea-Bissau have been observed.[334] In high-risk groups such as urban commercial sex workers, HIV-2 prevalence rates of 15 to 64 percent have been reported.[196]

HIV-2 group A infections have been identified predominantly in the western part of West Africa, mainly Guinea-Bissau, Senegal, the Gambia, and Mali.[22] HIV-2 group B infections have been found in central and eastern West African countries such as the Ivory Coast, Ghana, and Nigeria. HIV-2 has only limited distribution outside West Africa and has been described primarily in regions in Europe, South America, and India with historical socioeconomic ties to West Africa. In Europe, the greatest numbers of HIV-2 cases have been reported in Portugal, France, and Germany.[272] The cultural and economic ties of Portugal to its former colonies in West Africa probably facilitated the spread of HIV-2 to Europe and possibly Brazil, also a former Portuguese colony. As many as 4.5 percent of cases of AIDS in Portugal are caused by HIV-2, and in northern Portugal, HIV-2 accounts for 12 percent of HIV infections.[311] In France, 1 to 2 percent of all pediatric HIV infections are caused by HIV-2.[77] HIV-2–infected individuals have been identified in England, Wales, and Northern Ireland in increasing numbers. The prevalence of HIV-2 infection among clinic attendees with sexually transmitted diseases was 0.006 percent (6/104,006) in 2002. Most of these patients likely were infected through heterosexual intercourse in West Africa, including Nigeria (40%), Ghana (24%), Ivory Coast (17%), Sierra Leone (5%), and the Gambia (5%). Of 1324 HIV-infected adults, 917 (69%) were HIV-1 infected and 52 (6%) were HIV-2 or HIV-1/HIV-2 co-infected; the HIV type was not reported in the remaining 355 (27%). The ratio of HIV-2 and HIV-1/HIV-2 dual infections to HIV-1 infections varied greatly by country of infection, with the Gambia (11.7% to 15.2%) and Ivory Coast (7.2% to 9.8%) having a high proportion of HIV-2 infections and Nigeria (0.7% to 1.0%) a low proportion.[69] However, indigenous HIV-2 transmission also may occur, inasmuch as HIV-2 infections not directly linked to West Africa have been reported in Portugal, France, and Spain.

HIV-2 infection has been reported in India; as many as 7 to 8 percent of all HIV infections in certain regions are caused by HIV-2. In the United States, fewer than 100 cases of HIV-2 infection have been reported to the Centers for Disease Control and Prevention (CDC), and since the implementation of combination HIV-1/HIV-2 screening of the blood supply in June of 1992, no transfusion-acquired HIV-2 cases have been reported to the CDC.[40,41]

DUAL INFECTIONS WITH HUMAN IMMUNODEFICIENCY VIRUS TYPES 1 AND 2

Although some reports suggested that infection with HIV-2 may be protective against HIV-1 infection, that suggestion was not confirmed in other studies.[13,240,335,336,368] In two studies, HIV-2–positive subjects actually had a tendency toward a higher risk of acquiring HIV-1 infection than did seronegative individuals.[240,368] Dual infection with HIV-1 and HIV-2, confirmed by PCR and culture, has been observed in areas that are endemic for both viruses.[91,262] Susceptibility to dual HIV-1 and HIV-2 infection was studied in a macaque model of HIV infection.[255] The timing of secondary virus exposure was found to be a critical factor in the risk of acquiring infection. Productive mixed infections were established with simultaneous exposure or when viral challenge occurred within 4 weeks after primary infection developed. However, animals exposed at 8 weeks or more after primary inoculation were resistant to secondary infection. The mechanism of protection is not known.

Although HIV-1 infection predominated among a sampling of women living in Dakar, Senegal, HIV-2 and HIV-1/HIV-2 dual infections occurred more commonly among female sex workers compared with other women.[300] In studies conducted in England, Wales, Northern Ireland, Guinea-Bissau, and Dakar, when compared with HIV-1, the seroprevalence of HIV-2 was higher in older age groups. For example, in Guinea-Bissau, among individuals older than 50 years, HIV-2 prevalence was 12 percent in men and 16 percent in women.[69,300] The older age of those infected with HIV-2 probably reflects the longer asymptomatic period of HIV-2 infection, older age at the time of infection or at diagnosis, or possible differences in patterns of migration between HIV-1 and HIV-2 infected women.

MODE OF TRANSMISSION

The modes of acquisition of HIV-2 appear to be identical to those of HIV-1: heterosexual and homosexual intercourse, intravenous drug use, receipt of contaminated blood products, and vertical transmission. However, the infectivity of HIV-2 is lower than that of HIV-1, and transmission of HIV-2 by sexual intercourse appears to be less frequent than that observed with HIV-1.[149]

Like other sexually transmitted infections, the likelihood of transmission of HIV depends in part on the quantity of HIV in genital tract secretions. HIV-1 is detected more commonly in semen than is HIV-2. The viral loads in semen correlate with the plasma viral loads. In one report, HIV-2–infected subjects had 0.7 $\log_{10}$ lower HIV semen viral load than those with HIV-1, even after adjusting for plasma load.[98] Female genital shedding of HIV is important in both heterosexual and mother-to-child transmission of the virus. In a cross-sectional sampling of women living in Dakar, Senegal, HIV-1 shedding occurred more commonly than did HIV-2 shedding. HIV-2 shedding was associated with older age, severe vitamin A deficiency, advanced HIV disease, and $CD4^+$ cell counts lower than 200. Viral shedding was not detected in any women with dual HIV-1/HIV-2 infection.[300] Researchers estimated that the likelihood of sexual transmission of HIV-2 per sexual exposure is threefold less than with HIV-1.[147,194] The pandemic spread of HIV-1 compared with the more limited geographic spread of HIV-2 is likely the result of different rates of transmission of these two viruses. This difference in transmission of the two HIV viruses is reflected in the declining prevalence rates of HIV-2 in endemic areas over time.

In a study in Guinea-Bissau, the overall prevalence of HIV-2 infection in 1996 was 9.7 percent in police officers and 5.5 percent in pregnant women, as opposed to 0.9 percent in each group for HIV-1.[240] However, during the 7-year period from 1990 to 1996, the incidence of HIV-1 infection increased significantly in both groups, whereas the incidence of HIV-2 decreased, thus implying more efficient transmission of HIV-1. In a similar study of commercial sex workers in Senegal, the incidence of HIV-1 infection increased 12-fold despite a prevalence of HIV-2 at baseline that was approximately 2-fold higher than that for HIV-1.[149,197]

PERINATAL TRANSMISSION

Data from most studies of mother-infant pairs indicate that perinatal transmission of HIV-2 appears to be a rare occurrence, with mother-to-child transmission rates ranging from 0 to 4

percent.[5,93,247,269,334] In a large prospective study from the Ivory Coast, the risk of perinatal transmission from HIV-1–infected mothers was 21-fold greater than from HIV-2–infected mothers; transmission rates were 1.2 percent from HIV-2–infected mothers versus 24.7 percent from HIV-1–infected mothers.[5] In another large study in the Gambia, the estimated rate of mother-to-child transmission of HIV-2 was 4.0 percent versus 24.4 percent for HIV-1.[243] In this study, three of eight HIV-2–infected infants were infected after reaching 2 months of age, a finding suggesting that HIV-2, like HIV-1, can be transmitted postnatally through breast milk. The maternal plasma viral load is a significant risk factor for perinatal transmission of both HIV-1 and HIV-2 infection. In the study in the Gambia, for every 1 $\log_{10}$ increase in plasma RNA, the odds of transmission were 2.7 for HIV-1 and 2.8 for HIV-2.[247] Maternal RNA levels were significantly lower in HIV-2–infected than in HIV-1–infected mothers. After adjusting for viral load, the odds of transmitting HIV-1 were similar to those for HIV-2; this finding suggests that the level of viremia, as opposed to the type of virus, was the major determinant of the difference in mother-to-child transmission rates between HIV-1 and HIV-2. Transmission from women who are dually seropositive for HIV-1 and HIV-2 has been described; HIV-1 appeared to be transmitted more efficiently than does HIV-2, and HIV-1 transmission rates from dually infected women were similar to those from women infected with HIV-1 alone. Transmission of dual infection to the infant, though a rare occurrence, has been described. In the Ivory Coast cohort, 19.0 percent of women with dual HIV-1 and HIV-2 infection transmitted HIV to their infants; of 11 infected infants, 10 were infected with HIV-1 alone, and 1 was dually infected.[5]

DIAGNOSIS OF HUMAN IMMUNODEFICIENCY VIRUS TYPE 2

The diagnosis of HIV-2 can be accomplished by culture of HIV-2 from peripheral blood mononuclear cells (PBMCs), measurement of HIV-2 proviral DNA, detection of HIV-2 RNA in peripheral blood, or detection of antibodies to HIV-2. Although HIV-1 and HIV-2 share a similar genetic organization, significant divergence in genetic sequence exists, with differences of 58 percent for the *gag* gene, 59 percent for the *pol* gene, and 39 percent for the *env* gene. More similarity is seen between HIV-2 and primate simian immunodeficiency virus (SIV), in which the nucleic acid sequence homology between HIV-2 and SIV_{SM} and SIV_{MAC} strains is approximately 75 percent.[196] Because of this antigenic variation, screening EIAs for HIV-1 antibody may not detect antibody to HIV-2, and thus combination screening tests containing antigens from both viruses have been developed.[42] Confirmatory Western blot testing to detect HIV-2 requires the use of HIV-2–specific assays because not all samples from persons infected with HIV-2 will be positive on HIV-1 Western blot.

Rapid HIV detection tests have proven to be valuable tools for establishing the diagnosis of HIV. In several patient populations, when compared with ELISAs, rapid tests have been shown to have specificities and sensitivities close to 100 percent for the detection of either HIV-1 or HIV-2. The accurate detection of dually infected individuals has been less reliable; most individuals who were identified as dually infected by rapid test ultimately were diagnosed with HIV-2 only. When dual infections were identified by sequential rapid tests, performance of a supplementary test was necessary to allow the accurate discrimination between HIV-1 and HIV-2.[162,287]

The significance of this phenomenon is uncertain, but it may reflect the poor replication level of the HIV-2 virus and the resultant low end of antibody production. Host-related factors may include altered humoral or cellular responses to the HIV-2 virus. Another report showed HIV-2–specific cytotoxic T-lymphocyte activity in individuals in West Africa who may have been exposed to HIV-2 but who remained seronegative.[146]

VIRAL LOADS

Assays for the detection of HIV-2 RNA are not currently available commercially. However, in multiple studies examining HIV-2 viral load, an inverse relationship was found between $CD4^+$ lymphocyte counts and plasma HIV-1 and HIV-2 viral loads in singly infected individuals. Plasma HIV-1 levels were approximately 10 times higher than plasma HIV-2 levels throughout the comparable span of $CD4^+$ lymphocyte counts, and HIV-1 and HIV-2 regression lines had very similar slopes. Plasma HIV RNA levels were influenced independently by the type of HIV and $CD4^+$ lymphocyte counts, but not by sex or age. Among dually infected individuals, no correlation was found between $CD4^+$ counts and either plasma HIV-1 or HIV-2 levels.

The viral set-point is significantly lower in HIV-2 infection than it is for HIV-1 infection. Recent HIV-2 seroconverters have plasma HIV RNA levels 28 times lower than those of recent HIV-1 seroconverters. For HIV-1, the viral set-point has been demonstrated to be a very good predictor of outcome of disease. Differences between HIV-1 and HIV-2 in natural course, transmissibility, and epidemiologic features result, in large part, from lower viral levels in plasma. HIV-2 infection may resemble a slowly progressive or nonprogressive HIV-1 infection. The finding that HIV-2 is contained to a higher degree than is HIV-1 may reflect differences in the interaction between the immune system and the two HIV types or inherent differences in the biologic characteristics of the two viruses. Autologous neutralizing antibodies more frequently are found in HIV-2–infected individuals than in HIV-1–infected individuals and also may contribute to the slower disease progression in HIV-2.

In a study in Guinea-Bissau of individuals dually infected with HIV-1 and HIV-2, the interaction between the two viruses and the host defense mechanisms was less predictable. Although an inverse relationship existed between levels of $CD4^+$ lymphocytes and proviral HIV-2 DNA as compared with singly infected individuals, no correlation was found between levels of $CD4^+$ lymphocytes and plasma RNA or between HIV-1 and HIV-2 RNA load.[9]

A different study compared the $CD4^+$ cell counts and viral loads of individuals infected with HIV-1, HIV-2, and both HIV-1 and HIV-2. $CD4^+$ cell counts were significantly higher in those patients infected with HIV-2 compared with dually infected and HIV-1–infected individuals (582×10^6 cells/L, 165×10^6 cells/L, and 107×10^6 cells/L, respectively). HIV-1 viral loads were similar between HIV-1–infected and dually infected subjects, as were HIV-2 viral loads in HIV-2–infected and dually infected subjects. The HIV-1 viral loads were two times higher than HIV-2 viral loads.[161]

Studies indicate that levels of integrated proviral HIV-2 DNA in peripheral blood mononuclear cells are similar to those in comparable groups of HIV-1–infected individuals.[11,139,266] Despite similar proviral levels in HIV-1 and HIV-2, patients infected with HIV-2 have a significantly lower plasma viral load, a finding thus suggesting a lower rate of expression of the HIV-2 proviral DNA template.[58,266,267,304,334]

CLINICAL MANIFESTATIONS

HIV-2 is fully pathogenic in humans, and the clinical spectrum of disease caused by HIV-2 is similar to that of HIV-1. However, the rate of $CD4^+$ T-cell depletion and clinical progression to AIDS is slower, and more favorable survival is observed, although the mortality rate is increased in comparison with seronegative

persons.[143,155,196,198,358,366] As for HIV-1, the HIV-2 viral load significantly correlates with progression of disease as measured by $CD4^+$ T-cell decline or mortality, and differences in viral load throughout most of the natural history of HIV-1 and HIV-2 infection probably account for the differences in progression of disease and transmissibility reported between the two viruses.[9,12,266,267,311]

In a cohort study of HIV-2–infected and HIV-1–infected women in Senegal, the rate of decline in $CD4^+$ T-lymphocyte count was approximately 1 percent per year for women infected with HIV-2 versus 10 percent per year for HIV-1.[198] The median time to development of AIDS in HIV-1–infected women was 10 years; in contrast, HIV-2–related AIDS-free survival was so high that a median estimate of the time to a diagnosis of AIDS could not be determined.[197] Once the immune status deteriorates, however, the mortality rate does not differ greatly from that of HIV-1 infection. In community-based studies comparing the risk of death in infected versus uninfected adults, the relative risk of dying with HIV-2 infection was increased by 2- to 4-fold, in contrast to a 10-fold increase with HIV-1 infection.[219,268,278] Immunologic findings are similar to those in HIV-1 infection, with polyclonal hypergammaglobulinemia, increased T-cell activation, decreased lymphocyte proliferation, reduced antigen recall, and mildly elevated $CD8^+$ and reduced $CD4^+$ T-lymphocyte counts. However, in most cases, these changes are not as severe as those observed with HIV-1 infection. In a comparison of HIV-1–infected and HIV-2–infected persons with similar $CD4^+$ T-cell counts, the quantitative viral load was significantly lower in those infected with HIV-2.[304] In patients with a $CD4^+$ cell count greater than 500/mm^3, none of the HIV-2–infected individuals had a detectable plasma viral load, as compared with 52 percent of HIV-1–infected persons. In a study of sero-incident cases of single or dual infection with HIV-1 and HIV-2 in Guinea-Bissau, dually infected individuals had lower plasma RNA levels than did singly infected individuals.[9]

Similar to the clinical outcome of HIV-2 infection in adults, slower rates of progression of disease and better survival rates have been observed in HIV-2–infected versus HIV-1–infected or dually infected children.[5,59,241,254,271] In a prospective study in the Gambia, the 18-month mortality rate of children born to HIV-2–infected mothers did not differ significantly from that of children born to HIV-seronegative women (7% versus 6%, respectively).[254] In contrast, the relative risk of death in children born to HIV-1–infected versus HIV-2–infected or seronegative mothers was increased by 2.3- and 2.6-fold, respectively. The excess number of deaths in children of HIV-1–infected mothers was caused primarily by HIV-1 infection in the child; the mortality rate was 35 percent in HIV-1–infected children, as opposed to 9 percent in uninfected children born to HIV-1–infected mothers. In contrast, no deaths occurred in HIV-2–infected children.

In Guinea-Bissau, the overall child mortality rates were similar in children born to HIV-2–seropositive and HIV-seronegative women (16.3% versus 14.6%, respectively).[241] However, despite generally slower progression in HIV-2–infected children, severe immunodeficiency and AIDS occurring early in life rarely have been reported with HIV-2 infection.[90,212] In a cohort study in the Gambia, among HIV-infected patients with $CD4^+$ counts 500 cells/μL or greater, HIV-2–infected patients had a significantly lower mortality rate than that of HIV-1–infected or HIV-1/HIV-2–dually infected patients. Among HIV-infected patients with advanced disease ($CD4^+$ counts <200 cells/μL), all had a poor prognosis, and no difference in mortality rates occurred among HIV-1, HIV-2, or dually infected patients. Mortality was associated with increasing age and male sex and was inversely related to $CD4^+$ count. The mortality hazards ratio for men was 1.63 times higher than that for women even after adjusting for $CD4^+$ count, HIV type, and age.[295] Mortality in infants born to HIV-1–infected or dually infected mothers was 2.6 to 4.2 times higher than that in infants born to HIV-2–infected mothers (mortality rates of 133, 82, and 32 per 1000, respectively).[58]

This finding is similar to that observed in the Gambia, where the childhood mortality rate was 15 percent for children born to HIV-1–infected mothers, compared with 7 percent for those born to HIV-2–positive mothers and 6 percent for those born to HIV-seronegative mothers.[254] In a small cohort study investigating the survival of children born to HIV-1–positive and HIV-2–positive mothers, the mortality rates for HIV-1–infected and HIV-2–infected children were significantly higher than those of uninfected children.[294] The mortality hazards ratio of HIV-1–infected children was 3.1 times that of HIV-2–infected children but was not statistically significant. The overall mortality of HIV-uninfected children of HIV-1–seropositive and HIV-2–seropositive mothers was not significantly different from that in children of HIV-uninfected children.[295]

TREATMENT OF HUMAN IMMUNODEFICIENCY VIRUS TYPE 2 INFECTION

HIV-1 and HIV-2 reverse transcriptase (RT) enzyme regulates the formation of double-stranded viral DNA that is to be integrated into the host-cell genome. RT catalyzes the incorporation of deoxynucleoside triphosphates (dNTPs) forming a negative-sense DNA strand by using HIV-positive sense RNA as a template. The RT RNase activity catalyzes the degradation of the positive-sense RNA from the negative-sense DNA. The RT DNA polymerase then uses the negative-sense DNA as a template to form double-stranded proviral DNA. Nucleoside reverse transcriptase inhibitors (NRTIs) resemble endogenous dNTPs. These agents inhibit the formation of viral DNA by competitively inhibiting dNTPs for the RT enzyme and, then once incorporated into the HIV DNA strand, terminating the chain by preventing formation of 3′,5′-phosphodiester bonds. In vitro studies of antiretroviral activity against HIV-2 show NRTIs to have inhibitory effects against wild type and mutant HIV-2 clones.[263] The effective concentrations of lamivudine (3TC), didanosine (ddI), zalcitabine (ddC), and stavudine (d4T) needed to inhibit wild-type HIV-2 are very similar to what is effective against wild-type HIV-2. Researchers initially thought that HIV-2 was inhibited by zidovudine (ZDV, formerly AZT), but more recent studies suggested that wild-type HIV-2 harbors polymorphisms that confer resistance to zidovudine and significantly reduces its susceptibility to this agent.[276]

HIV-1 NRTI resistance results from base changes within the RT genome, provoking amino acid substitutions in the transcribed enzyme that, in turn, confer structural changes at the enzyme active site or at associated functional areas. In HIV-1, each NRTI induces a predictable set of genetic alterations in a step-wise fashion. Primary mutations generally arise first, with secondary mutations developing during continued therapy. HIV-2 RT resistance can be conferred by mutations at sites homologous to those from HIV-1 RT NRTI resistance. In a cell-free system, side-directed mutagenesis of HIV-2 RT amino acid residues homologous to residues in HIV-1 RT reduces the sensitivity of mutated HIV-2 to NRTIs. HIV-1 RT mutation T215Y confers resistance to AZT. Clinical studies of HIV-2 clones taken from patients receiving NRTI-containing antiretroviral therapy (ART) confirmed that phenotypic resistance is associated with genotypic changes. Amino acid changes associated with HIV-1 NRTI resistance at M184 and Q151M also seem to occur in HIV-2 and have been associated with NRTI treatment failure.[344] The significance of other mutations in the HIV-2 RT gene after exposure to NRTI is unknown; also not known is whether mutations in the NRTI gene accumulate in a step-wise fashion conferring increasing resistance to NRTI therapy. Non-nucleoside

reverse transcriptase inhibitors (NNRTIs) bind to a hydrophobic pocket near the polymerase site of HIV-1 RT. In vitro studies that measure HIV-2 susceptibility to inhibition by NNRTs supported the conclusion that these types of drugs have no effect against HIV-2.[372] Natural resistance of HIV-2 to this class of agents is thought to be conferred by a single amino acid, Leu-188. A change in amino acid at this site renders HIV-2 RT sensitive to efavirenz and delavirdine but not to nevirapine.[137] Newer NNRTIs inhibit HIV-2 at effective concentrations at least 50 times higher than those that inhibit HIV-1. These concentrations limit the clinical utility of these agents against HIV-2. NNRTIs generally show little to no inhibition of HIV-2 in vitro at nontoxic levels.[365]

Studies indicated that some HIV-1 protease inhibitors (PIs) also may inhibit HIV-2.[197] HIV-1, HIV-2, and SIV proteases belong to the aspartyl protease family. They are responsible for the post-translational processing and cleavage of the polyprotein products Pr GAG and PrGAG-POL into functional core proteins and essential enzymes including RT, integrase, and protease. Proteases are necessary to produce a mature retrovirus. PIs competitively bind to the protease substrate site, with resulting production of immature non-infectious particles. PIs are effective against HIV-2 protease, but they bind with a 10 to 100 times weaker affinity to HIV-2 protease, depending on the inhibitor. Studies of individual PIs showed nelfinavir and saquinavir to exert the same inhibitory activity against HIV-2 as against HIV-1, whereas ritonavir and indinavir were one to two orders of magnitude less inhibitory against HIV-2. ART against HIV-2 with dual PI or triple NRTI/PI-based therapy results in decreased HIV-2 viral load, increased $CD4^+$ T-cell count, and an improvement in AIDS related symptoms. Clinical studies examining PI susceptibility of HIV-2 clones from individuals both before and after receiving ART showed that indinavir, saquinavir, lopinavir, and tipranavir had full activity against wild-type HIV-2. Nelfinavir and amprenavir were less active against these clones. During ART with PIs, HIV-2 mutations were present, including in a patient who had been treated with lopinavir and who developed the proV47A, which translated into high-level resistance to indinavir, lopinavir, and amprenavir and hypersusceptibility to saquinavir. In isolates from the second patient, several different mutations resulted in decreased susceptibility to all PIs tested except saquinavir.[283] Naturally occurring polymorphisms or secondary mutations may decrease the activity of nelfinavir and amprenavir. On selection of primary resistance, mutations and preexisting secondary changes may play a role in the acquisition of a multi-PI resistance phenotype in HIV-2. Although PI resistance to HIV-1 is not completely understood, the HIV-2 protease active sites appear to differ, and this may confer natural HIV-2 resistance to ritonavir and indinavir. The D30N mutation as a natural polymorphism in HIV-2 reduces the efficacy of nelfinavir against HIV-2. A genotypic analysis of four HIV-2–infected individuals demonstrated the M46I mutation in all four individuals. V82F in one patient was associated with progressive decline in $CD4^+$ lymphocytes, despite treatment with indinavir. In a case report of two patients infected with both HIV-1 and HIV2, ART successfully suppressed HIV-1 but failed to suppress HIV-2. Treatment-experienced HIV-2–infected patients achieved HIV-2 viral suppression on a ritonavir-boosted indinavir or lopinavir highly active ART (HAART) regimen.[346]

In a cohort study on patients in Abidjan, Cote d'Ivoire, HIV-2–infected patients treated with dual ART or nelfinavir-containing regimens had incomplete HIV-2 viral suppression, whereas those treated with indinavir-containing regimens had complete and sustained viral suppression.[4] HIV-2 protease is 50 percent homologous to that of HIV-1. Researchers showed that mutations that confer resistance to PIs in HIV-1 were found in homologous positions in the HIV-2 protease. Natural polymorphism patterns were different in HIV-1 and HIV-2 proteases. In particular, highly polymorphic positions of HIV-2 (positions 14, 40, 43, 46, 65, and 70) from ART-naïve patients were highly conserved in HIV-1 strains. The highest levels of natural polymorphism in HIV-1 were observed at positions 35, 37, 41, 62, 63, 64, 77, and 93. Three regions of both HIV-1 and HIV-2 protease were highly conserved: positions 25 through 32, which includes the protease active site; the flap region; and positions 78 through 87. In protease sequences from ARV-naïve HIV-2 infected individuals, HIV-1 drug resistance-associated codons were identified in most cases (amino acids 36I, 46I, and 71V) or in all cases (amino acids 10V, 32I, 47V, and 73A). Fifty-nine percent of sequences from untreated HIV-2 patients harbored 46I. This finding corresponds in HIV-1 to a major drug resistance mutation to indinavir and to a minor drug resistance mutation to ritonavir, nelfinavir, and amprenavir, lopinavir/ritonavir, and atazanavir. These findings suggest a naturally reduced susceptibility of HIV-2 to these agents, even in an untreated individual.[51] In vitro studies using serial cultured cells passaged in the presence of serially diluted PIs documented the emergence of drug resistance over time. At baseline, HIV-2 was found to express natural protease polymorphisms at several positions that could be implicated in emergent drug resistance. Certain mutations were selected with different PIs. Whereas treatment-associated mutations all were associated with homologous mutations in the HIV-1 protease, mutations in the HIV-2 sequence were identified that had not been associated previously with drug resistance in either HIV-1 or HIV-2.[244] Genetic diversity of the HIV-2 genome renders it unlikely to be susceptible to the currently available HIV-1 fusion inhibitors.[270]

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CHAPTER 204b

Impact of Human Immunodeficiency Virus and Acquired Immunodeficiency Syndrome

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Human immunodeficiency virus (HIV) infection continues to have a significant clinical impact on our world. The World Health Organization (WHO) and Joint United Nations Programme on HIV/AIDS (acquired immunodeficiency syndrome) estimates that across the globe, 4.3 million people were newly infected with HIV-1 in 2006 (Table 204–8).[216] Of these newly infected individuals, 530,000 (10%) were children younger than 15 years of age. In 2004, half of the 40,000 new cases of HIV infection in the United States were in patients aged 13 to 25 years, a reflection of the change in the route of HIV infection for youth from perinatal to heterosexual transmission.[35] Racial and ethnic disparities among newly HIV-infected individuals continue to be observed worldwide, including in developed nations such as the United States. Between 2001 and 2004, black male and female patients accounted for more than 50 percent of newly diagnosed U.S. infections.[40] In 2005, the annual U.S. rate for HIV infection in Hispanics was three times greater than that for non-Hispanics. Despite the advances that have been made in care during the decades since the HIV infection first was described, HIV/AIDS continues its worldwide spread.[37] Eradicating racial disparities and preventing HIV infections in the changing face of the U.S. and global epidemic will be the medical and public health challenges for this century.[34,204]

DEFINITION AND STAGING

Pediatric AIDS was reported to the Centers for Disease Control and Prevention (CDC) first in 1982; AIDS was first reported in adults in 1981.[34] The first definitions of pediatric AIDS were published in 1985. In 1994, the classification system again was revised to include more advanced viral diagnostic technology and a system for staging pediatric HIV disease that involved both clinical and immunologic axes.[30] Table 204–9 outlines the clinical

TABLE 204–8 Estimated Number of Adults and Children Newly Infected with Human Immunodeficiency Virus during 2006

Country	Number of Infected Adults/Children 2006
African Continent	2.81 million (2.41-3.21 million)
East/South Asia	960,000 (610,000-2.6 million)
Eastern Europe/Central Asia	270,000 (170,000-820,000)
Latin America	140,000 (100,000-410,000)
North America	43,000 (34,000-65,000)
Caribbean	27,000 (20,000-41,000)
Western/Central Europe	22,000 (18,000-33,000)
Oceania	7,100 (3,400-54,000)
TOTAL	4.3 (3.6-6.6 million)

Adapted from UNAIDS: AIDS Epidemic Update: Special Report on HIV/AIDS. New York, UNAIDS, publication no. UNAIDS/06.29E, 2006, p. 66.

TABLE 204–9 Pediatric Human Immunodeficiency Virus Classification: Clinical Categories

Immunologic	N: No Signs/ symptoms	A: Mild Signs/ Symptoms	B: Moderate Signs/ Symptoms	C: Severe Categories Signs/Symptoms
1. No evidence of suppression	N1	A1	B1	C1
2. Evidence of moderate suppression	N2	A2	B2	C2
3. Severe suppression	N3	A3	B3	C3

Adapted from Centers for Disease Control and Prevention: 1994 Revised classification system for human immunodeficiency virus infection in children less than 13 years of age. M. M. W. R. Morb. Mortal. Wkly. Rep. 43:*2, 1994.*

TABLE 204–10 Immunologic Categories Based on Age-Specific $CD4^+$ T-Lymphocyte Counts and Percentage of Total Lymphocytes

	Age of Child					
	<12 mo		1-5 yr		6-12 yr	
Immunologic Category	μL	%	μL	%	μL	%
1. No evidence of suppression	≥1500	≥25	≥1000	≥25	≥500	≥25
2. Evidence of moderate suppression	750-1,499	15-24	500-999	15-24	200-499	15-24
3. Severe suppression	<750	<15	<500	<15	<200	<15

Adapted from Centers for Disease Control and Prevention: 1994 revised classification system for human immunodeficiency virus infection in children less than 13 years of age. M. M. W. R. Morb. Mortal. Wkly. Rep. 43:*4, 1994.*

axis of the staging system, which is composed of four clinical categories (N, A, B, C).[30] The clinical axis is intended for unidirectional use in any individual child; that is, severity of disease proceeds from N (asymptomatic), to A (mildly symptomatic), to B (moderately symptomatic), to C (severely symptomatic), and then to HIV-associated death. Clinical category C describes children with AIDS-defining characteristics, namely, wasting, encephalopathy, opportunistic infections, and malignancies, but it excludes lymphoid interstitial pneumonitis/pulmonary lymphoid hyperplasia (LIP/PLH). Clinical category B describes children with other specific HIV-associated illnesses (e.g., single episodes of bacteremia, leiomyosarcomas, lymphoproliferative lung disorders, anemia, thrombocytopenia) and excludes children with a clinical category C event. Clinical category A describes children with two or more specific HIV-associated illnesses, namely, lymphadenopathy, hepatomegaly, splenomegaly, upper respiratory tract infections/sinusitis/otitis media, parotitis, and dermatitis, and excludes children with a clinical category C or B event. Clinical category N describes children without clinical category C, B, or A events; these children may or may not be completely asymptomatic (single category A events, such as lymphadenopathy, may exist).

Table 204–10 provides an expanded description of the immunologic axis of the staging system, which includes categories 1 (no evidence of immune suppression), 2 (moderate immunosuppression), and 3 (severe immunosuppression).[30] The immune axis is limited to quantitative assessment (absolute count, percentage, or both) of the peripheral blood helper T-cell ($CD4^+$) population. Each category is linked to age-matched $CD4^+$ cell counts and percentages; age groups include 0 to 11 months, 12 to 71 months, and 72 months and older. Age-matched $CD4^+$ percentages and count standards for moderate to severe immunosuppression are identical to those provided in the 1995 revised guidelines for pediatric *Pneumocystis jiroveci* prophylaxis interventions.[31]

EPIDEMIOLOGY AND TRANSMISSION AND PREVENTION

TRANSMISSION BY BLOOD PRODUCTS

Pediatric HIV transmission has been documented by blood and blood products, by perinatal transmission from mothers with identified risk factors, by breast milk,[66] and by sexual transmission to children and adolescents,[86] a development that may become epidemic. Since HIV screening techniques, including nucleic acid amplification testing to pooled blood products, were adapted and recombinant factor products were used increasingly, transmission of HIV by blood products rarely has been reported in the United States.[61]

PERINATAL TRANSMISSION

In the United States, the total annual number of AIDS cases resulting from perinatal transmission peaked in 1992, with just fewer than 1000 newly affected children reported.[16] Predominance of perinatal transmission is found worldwide, especially in countries where adult heterosexual transmission is uniquely prevalent (e.g., in Africa).[176,204] Before the use of perinatal antiretroviral interventions, the transmission rate for perinatally acquired HIV infection was reported to be between 12 and 25 percent in the United States and Europe and is probably higher in Africa.[54,174,213] The perinatal transmission rates reported here define transmission only in the context of live infant outcomes. The incidence of spontaneous fetal loss in HIV-infected pregnant women has been suggested to be increased and may represent increased HIV perinatal morbidity.[130]

Maternal risk factors associated with enhanced perinatal HIV transmission and identified through national surveillance in reported AIDS cases include drug abuse, heterosexual infection by sexual partners with risk factors for acquiring HIV disease, and maternal transfusion before 1985. Prospective and retrospective evaluations of maternal predictors for perinatal HIV transmission have been the focus of multiple studies. Maternal transmission predictors identified to date include maternal viremia (measured as quantitative HIV RNA polymerase chain reaction [PCR] or viral load),[71,81] maternal immunosuppression, or an inadequate immune response (measured by the $CD4^+$ cell count, neutralizing antibody production)[71,141] and viral characteristics (chemokine receptor tropism, resistance patterns of maternal virus at delivery or infant virus at birth).[17] Pregnancy and placental variables (delivery mode, duration of rupture of membranes, chorioamnionitis) also may influence the risk of perinatal transmission of HIV.[161,202,211] Infant variables evaluated as predictors of transmission of HIV include specific human leukocyte

antigen (HLA) markers and the infant cellular immune response (cytokine production, activated T-cell function).[70,73,140] In addition, immunogenetic factors (chemokine co-receptor expression) have been suggested to confer protection against progression of disease.[184]

Perinatal transmission of HIV has been proposed to take place in the antepartum, peripartum, and postpartum periods.[198] The distribution of transmission across these timing periods has not been defined precisely, but in non–breast-feeding populations, more than 50 percent of transmission is estimated to occur at the end of pregnancy.[123] The timing of the first positive culture in perinatally infected infants—that is, early (the first 7 days of life) versus late (second week of life or later)—has been used epidemiologically to differentiate children potentially infected in utero from those with intrapartum infection.[22] Such distinctions and a need for more precise definition of the timing of distribution of perinatal transmission are of paramount importance in defining interventions for interruption of transmission. Such interruption is especially important because investigators have suggested that early HIV-1 infection can have an impact on the rate of neurodevelopmental dysfunction within the first 2 years of life.[196]

In an African study, evaluation of gender-specific risks for mother-to-child transmission of HIV showed preference for intrapartum transmission to female infants.[208] In this cohort, the overall transmission rate at birth was 9.5 percent. HIV-exposed male infants (n = 966) had a 6.3 percent transmission rate as compared with 998 HIV-exposed female infants, who had a 12.6 percent transmission rate. Attempts to assess progression of disease have led to descriptions of subpopulations of HIV-infected children: rapid, usual, and slow progressors.[111,120] Standardized definitions for rapid or usual progressors are lacking. A stringent definition of slow progressors has been proposed (8 years of age without evidence of clinical or immunologic decline).[96] In a multicenter, longitudinal U.S. study of 360 HIV-infected children, maternal advanced disease (clinical class C symptoms, lowered $CD4^+$ cell count, and increased viral load) was associated with faster progression of disease in infected infants.[2]

Advances in perinatal primary antiretroviral therapy (ART; antepartum, peripartum, and postpartum delivery of zidovudine in non–breast-feeding HIV-infected women without severe immunosuppression) in the United States and developing nations led to a decrease in perinatal transmission rates. In 1994, Pediatric AIDS Clinical Trials Group (PACTG) protocol 076 documented the remarkable effectiveness of zidovudine in reducing the maternal-infant transmission of HIV-1 by 66 percent.[54] The three-part regimen consisted of the following: (1) zidovudine initiated during 14 to 34 weeks' gestation (100 mg orally five times daily) and continued throughout pregnancy, (2) intrapartum zidovudine (intravenous loading dose of 2 mg/kg followed by a continuous infusion of 1 mg/kg until delivery), and (3) oral zidovudine for the newborn infant (2 mg/kg per dose every 6 hours orally for 6 weeks) or intravenous dosing for infants unable to tolerate oral administration (1.5 mg/kg per dose every 6 hours). Recommendations for prophylaxis continue to promote use of zidovudine for prevention of mother-to-child transmission; a more thorough review is available at http://AIDSinfo.nih.gov. Inclusion of highly active ART (HAART) and zidovudine to suppress HIV viral load and restore or preserve immune function in pregnant HIV-infected women resulted in a further reduction in transmission rates in the United States, now reported as less than 2 percent.[56] Guidelines to prevent perinatal transmission include promotion of early maternal and infant testing, prevention counseling for women of child-bearing age, and treatment intervention in women of child-bearing age with newly identified HIV infection.[166] The selection of ART to reduce perinatal HIV transmission must include consideration of optimal treatment for the infected pregnant women and balance potential for fetal harm. Maternal factors that should be considered include the mother's viral load, degree of immunosuppression, medications for use in treatment of co-morbid conditions (e.g., tuberculosis, hepatitis C virus) and the potential for inducing viral resistance. Transmission of resistance HIV to infants has been described in the literature, but it is an infrequent event.

Other considerations to prevent perinatal transmission of HIV include scheduled delivery to minimize prolonged rupture of membranes, including a recommendation from the American College of Obstetrics for scheduled cesarean section for women with viral loads exceeding HIV RNA levels of 1000 copies/mL.[53] In a meta-analysis of 15 North American and European cohorts of HIV-infected pregnant women (n = 100 women), vertical transmission rates differed by 5 percent for those with scheduled delivery by cesarean section (transmission rate, 2%) as compared with those with other delivery modes (transmission rate, 7.3%).[211] Evaluation of the morbidity and mortality related to such scheduled cesarean sections suggests that HIV-infected pregnant women may have a higher incidence of postpartum hemorrhage with resultant transfusion, sepsis, pneumonia, and death than do their non-infected pregnant peers undergoing the same scheduled procedure (odds ratio, 1.6).[137] HIV-infected pregnant women without detectable viral loads should be informed preoperatively of their risk for having morbidity related to cesarean section performed to prevent perinatal HIV transmission.

If the maternal HIV status is unknown at the time of delivery, options for perinatal transmission prophylaxis may not be available for use in the antepartum or postpartum period. Early and rapid HIV testing is being promoted as a public health measure to prevent perinatal HIV transmission. In the multicenter Mother-Infant Rapid Intervention at Delivery (MIRIAD) study, voluntary rapid HIV testing and 24-hour counseling were conducted at 16 U.S. hospitals, with mean turn-around time of 66 minutes for test results.[23] The sensitivity and specificity of the rapid test performed on whole blood were 100 and 99 percent, respectively, and the positive predictive value of the testing compared favorably with the standard of enzyme immunoassay (EIA).

SEXUAL TRANSMISSION: SECOND WAVE OF PEDIATRIC ACQUIRED IMMUNODEFICIENCY SYNDROME

In the adult, young adult, and adolescent populations, sexual acquisition of HIV infection is the predominant transmission pattern. The intersection of pediatric sexual abuse and transmission of HIV has been reported. Sexual abuse in young children and infants may be associated with the transmission of sexual disease (e.g., syphilis, gonorrhea, human papillomavirus). HIV infection should be considered in the differential diagnosis of possible sexually transmitted diseases in children assessed for sexual assault. In contrast to the infrequent reports of sexual transmission in infants and children younger than 13 years of age, adolescents, who account for 1 percent of AIDS cases in the United States, are infected frequently by sexual transmission.[133] In an inner city urban U.S. community, 41 percent of sexually active female youth described having vaginal intercourse and receptive anal intercourse in casual relationships.[107] Use of condoms was reported in 64 percent of youth, but receptive anal intercourse was used by some as a form of contraception, and condom use in this setting was reported in only 41 percent of these encounters. The American Academy of Pediatrics (AAP) stressed the importance of preadolescent and adolescent care and encouraged performing examinations in patients who are 11 to 14 years of age. Health care providers can take advantage of these pre-teen/teen visits as an opportunity to explore sexual exposures

of youth in their care and to educate sexually active youth on options for contraception.

Because the symptom-free period for clinical expression of HIV infection in adolescents may approach adult standards (i.e., >10 years), many young adults (20 to 29 years of age) with AIDS probably became infected with HIV during adolescence.[133] Adolescents with AIDS, like affected infants and children, are more likely to be poor and black or Hispanic. Reported risks for transmission in adolescents vary by age, sex, and race or ethnicity. Behavioral transmission (men who have sex with men [MSM] and heterosexual contact) and transmission of unknown risk predominate as reported risk behavior in U.S. youth. The category of heterosexual contact accounts for the largest proportional increase in the reported risk of transmission in both female and male adolescent cases of AIDS. The nature of youth (experimentation, search for self and sexual identity, and inability to access health care easily) places adolescents at risk for heterosexual transmission of HIV infection.

Recommendations for post-exposure prophylaxis (PEP) for nonoccupational exposure to blood, genital, or other body fluids were updated by the CDC in 2005.[194] With exposure to these fluids from an HIV-infected individual and with determination of significant exposure risk, a 28-day regimen of HAART is recommended if administration can begin within 72 hours of exposure. For these exposures, risk is defined as 1 per 10,000 exposures to the infected source. Other risk assignments are blood transfusions (9000 per 10,000 exposures), percutaneous needle-stick injuries (30 per 10,000 exposures), and sexual exposure risk ranging from 50 (anal receptive intercourse) to 0.5 (oral intercourse). Merchant and colleagues[151] published a stratified regimen for PEP in children and youth that includes modification of the dosing regimen, evaluates adverse events commonly associated with PEP, and recommends institution of antiretrovirals within 1 hour of exposure when possible.

OTHER MODES OF TRANSMISSION

Less frequently reported transmission routes of HIV infection are important for pediatric health care providers to acknowledge and target. The risk of transmission of HIV through breast-feeding is reported infrequently in the United States. In a study of HIV-infected, breast-feeding mothers and their exposed infants, Dunn and associates[66] documented a 14 percent risk of HIV transmission through breast-feeding above and beyond the established perinatal transmission risk. These studies prompted the adoption of guidelines for preventing transmission by this route by avoiding breast-feeding in HIV-seropositive postpartum mothers in the United States. Transmission of HIV to infants by breast-feeding represents approximately half of all perinatal transmission worldwide, and the risk of transmission continues with prolonged breast-feeding after the first year of life.[122]

Children may be prone to needle-stick or sharps injuries that occur in a non-hospital, non-medical setting (e.g., needle-stick injury associated with inappropriately discarded needles in public places such as public parks, bathrooms). Recommendations for psychological support, discussion of adherence to prescribed medications, and toxicity monitoring are recommended by the AAP Committee on Pediatric AIDS.[101]

To date, no data suggest that HIV infection is transmitted casually from HIV-infected children to siblings, playmates, or caregivers.[190] Documentation of transmission routes and lack of casual transmission have prompted defining appropriate measures for "mainstreaming" of HIV-infected children and families.[63] This measure includes school, daycare, and foster care and adoption placement for HIV-infected children and promotes social incorporation of these children.

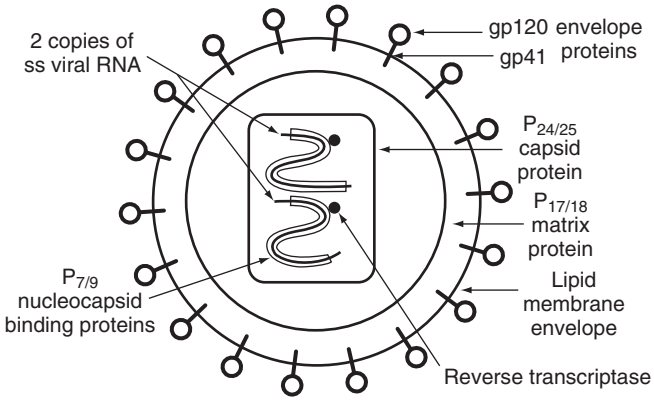

Figure 204–4 Diagram of the virion of human immunodeficiency virus type 1 (HIV-1). *(From Demmler, G. J., and Taber, L. H.: Virology of HIV-1. Semin. Pediatr. Infect. Dis. 1:17-20, 1990.)*

ETIOLOGY

Identification of the origin of AIDS followed closely behind the clinical description of this complex immunodeficiency. In 1983, investigators worldwide identified a viral cause for AIDS and described human lymphotropic virus III, lymphadenopathy-associated virus, and a virus associated with persistent generalized lymphadenopathy.[51,85] These viruses subsequently were identified as similar and now are called HIV-1, or simply HIV (Fig. 204–4).[58] Two species of HIV are recognized: HIV-1 and HIV-2. HIV-1 has been the more prevalent pathogenic species and, especially in the United States, almost uniformly has been associated with reported AIDS cases. Viral divergence is not uncommon, and HIV-1 can be divided into three groups: M, major; O, outliers; and N, non-M/non-O; the M group represents the major group with subtypes (clades) showing significant symmetry.[6] The major subtypes have been identified, and data from the WHO HIV Vaccine Initiative identified clade C in 50 percent of global infections in 2004.[102] Clade B is identified most frequently in industrialized countries, including the United States. In 2004, recombinant subtypes were responsible for 18 percent of worldwide infections; continued collection of such data will be important in the successful development of a vaccine that can be applied universally to stem transmission of HIV. A detailed description of these RNA viruses, their method of viral introduction into the affected host, and their impact on critical immune components is provided in Chapter 204a.

PATHOGENESIS

THE DEVELOPING IMMUNE SYSTEM

In perinatally infected infants, the disruption that HIV creates in the fetal/newborn immune system is envisioned best by considering the intricate normal differentiation pathways of the bone marrow stem cell.[222] Lymphocyte (T- and B-lymphocyte) differentiation pathways are presented in Figure 204–5 to illustrate points where genetic defects can occur and then cause congenital immunodeficiency. In many regards, the immunodeficiency of neonatal HIV infection parallels that of genetically inherited immune disorders.

HIV affects almost all immune cell types and alters their ability to produce important chemokines and interleukins that are integral to immunologic homeostasis. Independent of cell damage, host response also can vary according to individual

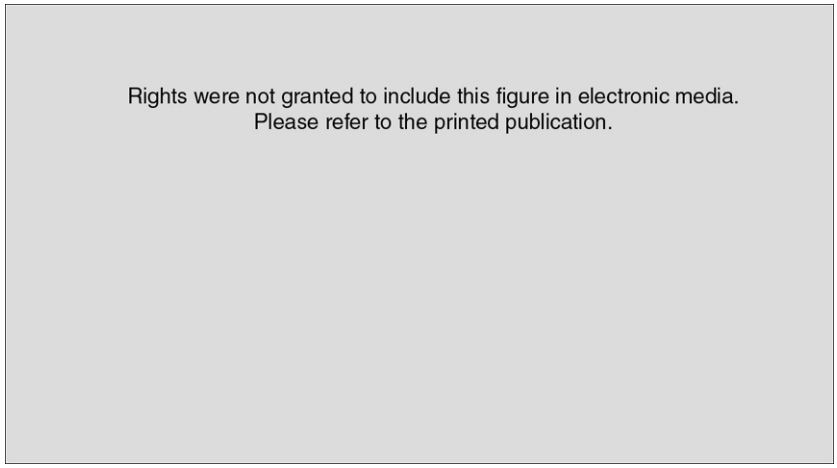

Figure 204–5 Model of the events of cellular maturation, cellular interaction, and cellular biosynthesis required for a normal immune response. The *arrows* indicate presumed defects in various immunodeficiency states. *Position 1*, Failure of B- and T-cell development (e.g., severe combined immunodeficiency disease). *Position 2*, Failure of development of the thymus (e.g., DiGeorge syndrome). *Position 3*, Failure of maturation of stem cells into pre-B cells (e.g., thymoma, hypogammaglobulinemia). *Position 4*, Failure of maturation of pre-B cells into B cells (e.g., X-linked hypogammaglobulinemia). *Position 5*, Failure of maturation of B cells into plasma cells (e.g., common variable hypogammaglobulinemia). *Position 6*, Hypercatabolism of immunoglobulin (e.g., myotonic dystrophy). *Position 7*, Reduced helper T cells (e.g., subset of common variable hypogammaglobulinemia). *Position 8*, Increase in suppressor T-cell activity (e.g., subset of common variable hypogammaglobulinemia). *Position 9*, Excessive loss of immunoglobulins and lymphocytes (e.g., intestinal lymphangiectasia). *(From Waldmann, T. A.: Immunodeficiency diseases: Primary and acquired.* In *Samter, M., Talmage, D. W., Frank, M. M., et al.: [eds.]: Immunological Diseases. 4th ed. Boston, Little, Brown, 1988, pp. 411-465.)*

genetic HLA representation and predict either probability of infection occurring following exposure or progression of disease.[76] $CD4^+$ cells bear a receptor (the $CD4^+$ molecule itself) for the gp120 component of the HIV virion coat, and through this mechanism viral entry into the host cell is allowed. This entry does not occur in isolation, and host expression of a chemokine receptor (CXCR4 for T-lymphotropic HIV strains; CCR5 for monocyte tropic HIV strains) interacts in promoting viral particle binding to the host-cell membrane and release of viral particles into the cytoplasm.[47] Data suggest that heterozygous allele expression of these co-receptors may affect expression and progression of HIV disease. Density expression of CCR5 molecules in infants has been correlated with progression of disease even in the presence of ART and with the slope of $CD4^+$ cell decline from birth.[42]

Once infected, the infant with perinatal transmission exhibits a quick rise in detectable HIV viral load that can exceed 750,000 copies/mL in the first weeks or months of life and persists unchecked without institution of antiviral therapy through the first years of life.[1] This persistent and lengthy viremia in early perinatal HIV infection differs from acute HIV-1 infection in adults, in which viremia initially may be equally elevated but abates with host immunologic response (albeit without complete elimination) over the course of a shorter time frame (weeks to months). These distinctions in host response reflect the more ineffective infant immunologic host response to HIV infection. Proposed mechanisms of immune dysfunction associated with continued viremia in infants include thymic damage with thymocyte depletion, disruption of thymic architecture, early $CD4^+$ cell depletion that affects $CD8^+$ cell response to HIV antigens, early lymphocyte activation as measured by $CD8^+DR^+CD38^+$ T cells that impede effective host T-cell response, and genetic predisposition to allow enhanced HIV entry into host cells.[180,227] Most clinicians opt for early treatment of HIV-infected infants younger than 1 year of age, independent of the clinical or immunologic ($CD4^+$ cell count) presentation. Studies have documented both viral control and improved response to HIV-specific antigens with such efforts.[226]

IMMUNE DYSFUNCTION

Abnormalities in adult HIV infection include leukopenia, lymphopenia, and decreased $CD4^+$ helper-inducer cells with an expanded $CD8^+$ cell population, which results in an inverted CD4/CD8 ratio, typically less than 1.0. Early in the course of infection, T-lymphocyte function has been noted to be diminished (<50%), with decreased in vitro responses to soluble antigens preceding $CD4^+$ cell depletion. Although in vitro responses to plant lectins, such as phytohemagglutinin, concanavalin A, and pokeweed, appear to be normal early in the course of HIV infection, these responses begin to decrease with clinical decline; characteristically, mitogen responses to pokeweed disappear first. Anergy is not unusual to commonly tested antigens (tetanus, *Candida*). Because of HIV-related paralysis of $CD4^+$ cells, production of interleukin-2 (IL-2) is reduced, thereby weakening the immune amplification system.[75] HIV-infected $CD4^+$ cells release soluble IL-2 receptors. These elevated serum IL-2 receptor levels produce a blockade of cell-bound IL-2 receptors by competition for IL-2. $CD8^+$ cell response to HIV-specific antigens is impaired.[165] Children with HIV infection seldom are lymphopenic in terms of values observed in adults. If lymphopenia is observed in children with HIV infection, it usually is seen in older children or in children who have progressed to end-stage HIV disease. In fact, $CD4^+$ cell counts in HIV-infected children commonly exceed 400 cells/mm^3. Moreover, Denny and associates[59] showed that in infants aged 12 to 24 months, a $CD4^+$ cell value of less than 1000 cells/mm^3 is significant for selective HIV-related $CD4^+$ depletion; in infants younger than 12 months, a value of less than 1500 cells/mm^3 similarly applies. Lymphoproliferative responses to recall antigens have been documented as relatively normal findings early in the course of infection; decline is correlated with the onset of clinical symptoms. As described earlier in discussions of pathogenesis, HIV-1–specific $CD8^+$ cytotoxic-suppressor cell function has been documented as being ineffective in infants with primary infection (early perinatal infection).

Following administration of HAART, T-cell function against specific HIV antigens continues to be imperfect. Despite lower-

ing of viral load, $CD4^+$ cell depletion may not be completely restored for many weeks. Differential restoration of subpopulations of $CD4^+$ cells (effector memory cells more commonly encountered than naïve cells) may occur. In 643 children with significant $CD4^+$ depletion ($CD4^+$ cells <250 cells/mm^3) and viremia, HAART resulted in a median rise of $CD4^+$ cell count of 100 cells/mm^3 over the course of 2 years, but anergy was reported in 80 percent, lack of proliferative response to the specific antigen of tetanus in 73 percent, and impaired serologic response to hepatitis A virus vaccination in 54 percent.[132] Hence, despite administration of ART, T-cell immune restoration may not be complete in all affected children.

B-cell dysfunction is identified similarly in HIV-infected adults and children and manifests as hypergammaglobulinemia. HIV-infected adults also demonstrate circulating immune complexes and production of autoantibodies secondary to polyclonal activation of B cells by HIV itself or concomitant viral infection with cytomegalovirus (CMV) or Epstein-Barr virus (EBV).[129] In particular, immunoglobulin G (IgG) levels may rise to 2 to 3 standard deviations (SDs) above the mean of normal values, and serum IgA, IgM, and IgE levels may be elevated as well. This extreme hypergammaglobulinemia in children may be caused by polyclonal stimulation of B cells by HIV or co-infection with CMV or EBV, or it may be caused by the absence of normal $CD4^+$ immunoregulatory cells. Specific antibody functions measured as childhood vaccine responses often are abnormal and are not always completely restored in the most severely immunocompromised individuals following administration of HAART therapy.[132]

Other host defense cells affected by HIV infection include natural killer (NK) cells. In adults with chronic HIV infection, reported NK abnormalities include differential representation of certain NK subpopulations, down-regulation of NK ligand expression that affects cytotoxicity of HIV-infected cells, and NK-dendritic cell interactions that switch from a T_H1 response (interferon-γ [IFN-γ]) to a T_H2 response.

Monocytes and macrophages play important roles in the pathogenesis of HIV infection by serving as reservoirs of HIV. Defective chemotaxis and bacterial killing have been observed in HIV-infected monocytes and macrophages, as has defective induction of IL-1, which possibly accounts for the decreased IL-2 response in $CD4^+$ cells.[195] The immune system also seems to contribute directly to HIV-induced encephalopathy (Fig. 204–6).[21,105] HIV-induced encephalopathy is particularly devastating in young infants, who fail to reach early motor milestones or, worse, regress from acquired early development. The incomplete state of myelination of central nervous system (CNS) tissue may account for susceptibility to the neurologic effects of HIV infection. In adults with progression of disease, depletion of $CD4^+$ cells has been linked to shifts in co-receptor use (from CXCR4 to CCR5), and these individuals with more severe disease appear to harbor predominantly CCR5-dependent (R5) viral strains.[88] HIV-containing monocytes are thought to transport HIV into the CNS and infect microglial cells including astrocytes.[50] Also implicated in HIV-associated neurologic complications are glial soluble factors that may have a direct neurotoxic effect.[127] Cytokines and reactive oxygen species released by HIV-infected lymphocytes and macrophages in the CNS may damage exposed nerve tissue.[221] The normal CNS immune defense system can attempt to enhance neuronal survival.

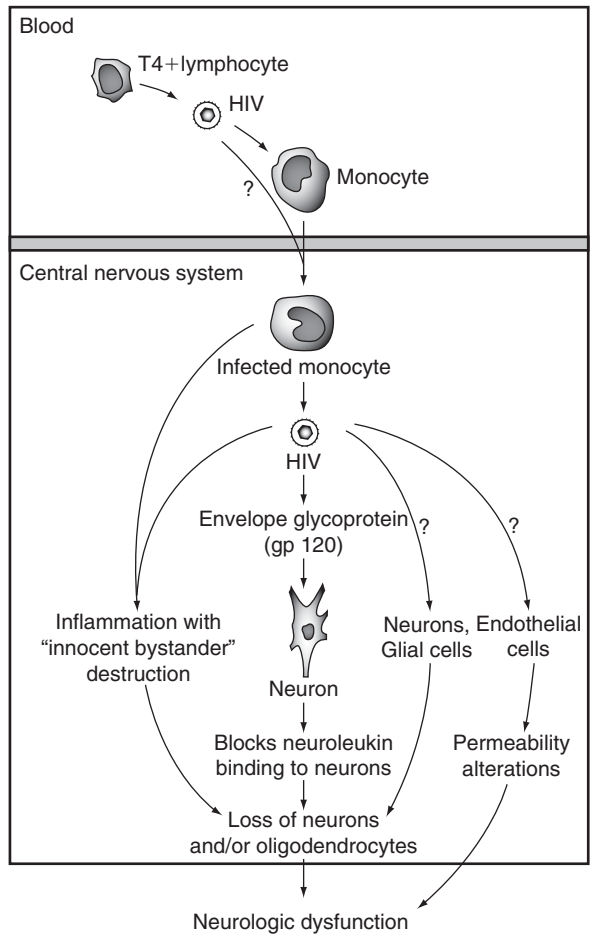

Figure 204–6 Role of the immune system in pediatric human immunodeficiency virus (HIV)–induced encephalopathy. HIV-infected $CD4^+$ lymphocytes and macrophages enter the central nervous system. HIV may escape from these transport cells and infect nerve cells directly or cause indirect damage to neurons by the release of cytokines such as tumor necrosis factor and interleukin-2. *(Reprinted by permission from Ho, D., Pomerantz, R. J., and Kaplan, J. C.: Pathogenesis of infection with human immunodeficiency virus. N. Engl. J. Med.* 317*:278-286, 1987.)*

ORIGIN OF IMMUNODEFICIENCY

The use of HAART has provided important opportunities to evaluate immune restoration in infected hosts. $CD4^+$ cell-depletion hypotheses of programmed cell death (apoptosis), depletion of tissue-associated $CD4^+CD45^+$ effector memory cells during acute infection, chronic ineffective immune activation (cellular and humoral), and switch from CXCR4-dependent virus (X4 virus) in acute infection to CCR5-dependent virus (R5 virus) in chronic infection all have been implicated as causes of HIV-induced immunodeficiency.

Ligands that induce apoptosis have been studied in HIV-infected individuals, and tumor necrosis factor–related apoptosis inducing ligand (TRAIL) has been documented to result in neuronal death.[152] TRAIL-associated apoptosis appears to require CXCR4 co-receptor expression but not CCR5 or CD4 receptor expression.[139]

Investigators hypothesize that HIV first depletes mucosal $CCR5^+CD4^+$ effector memory cells, thus leaving short-lived central memory or naïve $CD4^+$ cells and other immunoregulatory cells in a chronic state of activation that leads to an ineffective host response.[60,95] Infection of $CCR5^+CD4^+$ effector memory cells also is associated with higher expression of CTLA-4, a potent negative regulator for $CD4^+$ cells.[228] High expression of CTLA-4 may interrupt IL-2 production by $CD4^+$ cells, thereby impeding the ability of these cells to proliferate and hence depleting the population. The resultant chronic immune activation may lead to disorganization of the host immune response and further deplete $CD4^+$ cell regeneration. For patients with the most

profound CD4+ T-cell depletion, reduction of viral load with antiviral agents does not immediately result in correction of their immune defects.[88] The study of immune reconstitution with HAART may illuminate the immunopathogenesis of HIV infection. For example, after administration of HAART, CD4+ NK T cells that play an important part in regulatory immune response to viral infections are restored rapidly. Restoration of such CD4+ cell populations after administration of HAART and their important immune function may explain why, in the presence of persistently skewed CD4+ cell expression in peripheral blood, clinical disease appears to improve dramatically.[219]

In perinatal HIV infection, the role of the placenta in affording protection or permitting infection continues to be defined, and potential perinatal cofactors are beginning to be understood more clearly. Langston and associates[130] suggested that HIV may be fetotoxic, with an impact most notably reported in the thymus, where precocious involution, epithelial injury, and, occasionally, severe thymitis are described. Loss of lymphocytes at the corticomedullary junction of the thymus implies a defect induced by HIV in the immunologic selection process.

CLINICAL MANIFESTATIONS

In children and adults, HIV infection causes a spectrum of clinical abnormalities that affect multiple organ systems and include the symptom constellation of AIDS. Table 204–11 lists the pre-HAART frequency of AIDS-defining events per 100 child-years.[155]

Infection with HIV, independent of a diagnosis of AIDS, may be attended by nonspecific clinical findings, including mild failure to thrive, hepatosplenomegaly, acquired microcephaly, parotitis, generalized lymphadenopathy, nonspecific intermittent diarrhea, intermittent fever, and chronic skin disease. These clinical symptoms are shared by other pediatric disease processes and can, when manifested singly in an HIV-infected host, cause a delay in establishing the diagnosis. However, a careful and detailed history should provide helpful insight into potential HIV risk factors and should prompt inclusion of HIV infection in the differential diagnosis. With the use of HAART, HIV infection has become a chronic disease, and new clinical disease manifestations (e.g., lipodystrophy, hyperlipidemia), most closely related to chronic antiviral use, have been described. In general, the health of HIV-infected children has improved during the last decade. Data from the Pediatric Spectrum of HIV Disease Study shows lower hospitalization rates from 30.4 percent in 1990 to 12.9 percent in 2002.[13]

TABLE 204–11 Frequency of Opportunistic Infections in Human Immunodeficiency Virus–Infected Children before Widespread Use of Highly Active Antiretroviral Therapy

Pre-HAART Era (Event Rates >1/100 Child-Years)
Candidiasis
Disseminated *Mycobacterium avium* complex
Serious bacterial infections (pneumonia)
Herpes zoster
Pneumocystis jiroveci (formerly *P. carinii*) pneumonia
Serious bacterial infections (pneumonia)
Pre-HAART Era (Event Rates <1/100 Child-Years)
Cryptosporidiosis
Cytomegalovirus disease
Systemic fungal disease
Tuberculosis
Toxoplasmosis

HAART, highly active antiretroviral therapy.

Adapted from Mofenson, L.M., Oleske, J., Serchuck, L., et al.: Treating opportunistic infections among HIV-exposed and infected children: Recommendations from CDC, the National Institutes of Health, and the Infectious Diseases Society of America. M.M.W.R. Recomm. Rep. 53:1-92;2004.

OPPORTUNISTIC INFECTIONS

Opportunistic infections plague HIV-infected individuals and are the most prominent cause of morbidity and mortality in this cohort. Before the use of HAART intervention, opportunistic infections in children more often were primary infections as compared with the case in adults, who had reactivation of infection.[155] The introduction of effective HAART with associated host immune restoration significantly modified the expression of certain opportunistic infections in adults and children.[207] In children, the overall incidence of these infections declined from 14.4 cases per 100 patient-years before 1997 to 1.1 cases per 100 patient-years.[163] Additionally, in pre-HAART years, the age at clinical presentation was much younger, and age younger than 3 years was associated with very rapid CD4+ cell decline. Although the number of these opportunistic diseases has diminished, their prevalence appears to be unaffected. The administration of combination HIV therapy, including protease inhibitors (PIs) in adults and children, has improved the survival of perinatally infected children.[89]

Pneumocystis Pneumonia

P. jiroveci (formerly *P. carinii*) pneumonia (PCP) is the opportunistic infection most commonly reported in HIV-infected children. Declines in the incidence of PCP have been documented, with rates of 5.8 cases per 100 patient-years reported before 1997 and 0.3 cases per 100 patient-years reported after 1997.[163] PCP has a fulminant course in the pediatric population, with the highest mortality rates occurring in affected children younger than 1 year old. In a study of 172 children with perinatal HIV infection without HIV-specific intervention, 9 percent had PCP when they were younger than 1 year, with a median survival of 1 month.[183] Clinical expression of pediatric PCP often can be distinguished from other pulmonary diseases by the severity of the hypoxemia (higher alveolar-arterial oxygen gradients), elevated serum lactate dehydrogenase levels, the rapidity of progression of disease with tachypnea and fever, characteristic diffuse interstitial infiltrates on radiography, and the usual lack of digital clubbing.[98] The more insidious manifestation of PCP characteristic of HIV-infected adults, namely, prolonged fever (>7 weeks), cough, and dyspnea (averaging 3 weeks), is appreciated less commonly in infants and children. Radiographically, pediatric PCP is associated with diffuse interstitial markings progressing to the "white-out" picture of adult respiratory distress syndrome (Fig. 204–7).[99] However, PCP can be seen initially as a unilateral streaky pneumonic infiltrate, as lobar consolidation, or with accompanying pleural effusions.

Aggressive diagnostic measures may be indicated to establish a diagnosis of PCP. Bronchoscopic alveolar lavage (BAL) is the preferred diagnostic tool in adults and children and should be considered a first choice for presumed PCP in children who cannot provide sputum specimens for evaluation. In older children and adults, evaluation of sputum for the presence of *Pneumocystis* with appropriate stains or monoclonal antibodies may preempt the need for using invasive diagnostic measures. Lung biopsy carries the highest sensitivity for identifying the organism but has attendant morbidity related to thoracotomy or video-assisted thoracoscopy (VATS) and placement of a chest tube.

Acute PCP is treated with parenteral trimethoprim-sulfamethoxazole (TMP-SMX) or pentamidine or atovaquone for patients with TMP-SMX or pentamidine hypersensitivity. TMP-SMX treatment consists of 15 to 20 mg/kg/day of the

TMP component delivered intravenously three to four times daily. Adjunctive corticosteroid therapy given early in moderate to severe PCP provides significant benefit with only limited evidence of concomitant immune suppression and attendant infectious complications.[83,193] Concomitant CMV and PCP has been documented in HIV-infected children. In this clinical situation of concomitant viral infection, no existing data recommend delay of corticosteroid use. Treatment of PCP should not be altered. Dosing regimens for corticosteroids are published and include options for use of oral prednisone or oral methylprednisolone or intravenous methylprednisolone.[32]

Following the occurrence of PCP infection, secondary PCP prophylaxis (therapy initiated after acute disease treatment and resolution) is recommended. Proposed pediatric regimens include TMP-SMX at 150 mg/m^2/day and 750 mg/m^2/day, respectively, in two divided doses on 3 consecutive days/week (acceptable alternate regimens include a single dose on 3 consecutive days/week, two divided doses on 3 alternate days a week, two divided doses daily), dapsone for children 1 month or older at 2 mg/kg daily (single dosing not to exceed 100 mg/day) or 4 mg/kg weekly (single dosing not to exceed 200 mg), for children 5 years or older, pentamidine (aerosolized [300 mg by Respirgard II inhaler monthly]), and atovoquone (30 mg/kg daily for children 1 to 3 months and >24 months and 45 mg/kg daily for children 4 to 24 months).[31] In adults and adolescents, recommendations to discontinue secondary PCP prophylaxis are proposed once there is documentation of immune restoration (CD4$^+$ cell counts that have increased to >200 cells/mm^3 for ≥3 months as a result of HAART).[32] Data from the European PCP-Withdrawal Study Group suggest that with immunorestoration of HAART, the risk of PCP development is low, and withdrawal of PCP prophylaxis as recommended in the adult host appears to be a reasonable risk.[218] U.S. guidelines continue to recommend lifelong primary PCP prophylaxis in individuals who acquired their PCP before reaching13 years of age.[155]

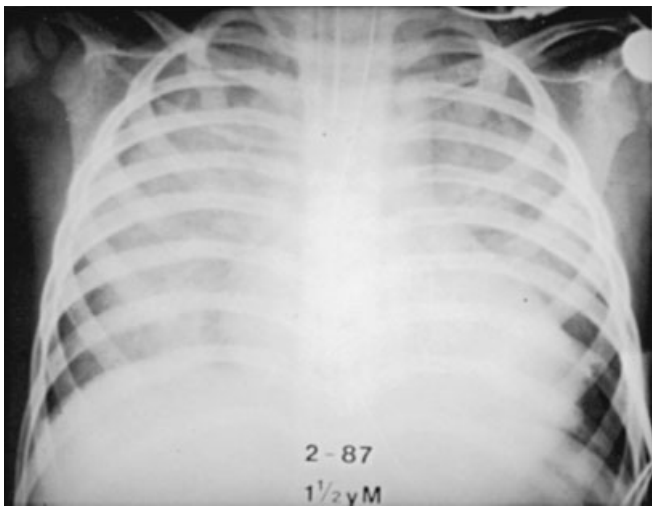

Figure 204–7 Classic diffuse interstitial infiltrates of acute and fatal *Pneumocystis carinii* pneumonia in a 15-month-old child with perinatal acquired immunodeficiency syndrome. *(From Hanson, I. C.: Respiratory infections in HIV-infected children. Immunol. Allergy Clin. North Am. 13:205-217, 1993.)*

Primary PCP prophylaxis (therapy initiated to prevent primary infection) for adults and children is accepted as standard care.[32] Pediatric regimens and dosing for secondary PCP prophylaxis do not differ from those outlined earlier. In 1993, the CDC published the first PCP prophylaxis guidelines for HIV-infected children; these guidelines linked the institution of therapy to the level of immunosuppression as measured by the CD4$^+$ cell count or percentage.[28] After implementation of these guidelines, Simonds and associates[191] reviewed the incidence of reported PCP cases to the CDC and noted no decline in case numbers. Of 300 children with PCP reported to the CDC between January of 1991 and June of 1993, 66 percent had never received prophylaxis. In addition, 18 percent of infants younger than 1 year old experienced PCP with CD4$^+$ counts that were higher than the guideline thresholds for immunosuppression. Based on these data, guidelines for PCP prophylaxis in children were modified in 1995, and Table 204–12 outlines the institution of therapy by age and CD4$^+$ cell monitoring.[31] The most significant changes in the revised guidelines included (1) an emphasis on early detection of HIV infection in infants for optimal implementation of the guidelines, (2) provision of PCP prophylaxis to all HIV-exposed infants in the first months of life, and (3) emphasis on quarterly immune monitoring. The low potential for associated morbidity from the administration of PCP prophylaxis allows for implementation of therapy to all infants, independent of HIV status, in the first months of life, when their risk has been documented to be highest and is least likely to correlate with immune monitoring. Data from Pediatric AIDS Clinical Trials Group protocol 1088 documented that primary PCP prophylaxis in HIV-infected children could be discontinued safely with immune restoration on stable ART.[162]

Mycobacterium avium–Intracellulare Complex

The *M. avium–intracellulare* complex (MAC), which caused disseminated infections in both adults and children with HIV infection in the early 1980s and 1990s, waned significantly in incidence with the increasing and successful use of HAART. In a 1990 review of opportunistic infections in pediatric patients with AIDS, 43 of 552 (7.8%) children from birth to 9 years of age

TABLE 204–12 Recommendations for *Pneumocystis* Pneumonia Prophylaxis and CD4$^+$ Monitoring for Infants Exposed to and Children Infected with Human Immunodeficiency Virus by Age and Human Immunodeficiency Virus Infection Status

Age/HIV Infection Status	PCP Prophylaxis	CD4$^+$ Monitoring
Birth to 4-6 wk, HIV exposed	No prophylaxis	1 mo
4-6 wk to 4 mo, HIV exposed	Prophylaxis	3 mo
4-12 mo HIV Infected or indeterminate	Prophylaxis	
6, 9, and 12 mo HIV infection reasonably excluded	No prophylaxis	None
1-5 yr, HIV infected	Prophylaxis *if* CD4$^+$ count is <500 cells/μL or CD4$^+$ percentage is <15%	Every 3-4 mo
6-12 yr, HIV infected	Prophylaxis *if* CD4$^+$ count is <200 cells/μL or CD4$^+$ percentage is <15%	Every 3-4 mo

HIV, human immunodeficiency virus; PCP, Pneumocystis *pneumonia prophylaxis.*

From Centers for Disease Control and Prevention: 1995 Revised guidelines for prophylaxis against Pneumocystis carinii *pneumonia for children infected with or perinatally exposed to human immunodeficiency virus. National Pediatric and Family HIV Resource Center and National Center for Infectious Diseases, Centers for Disease Control and Prevention: M. M. W. R. Recomm. Rep. 44:6, 1995.*

were found to have disseminated MAC infection.[99] The incidence of pediatric MAC infection in HIV-infected children dropped from 1.3 cases per 100 patient-years reported before 1997 to 0.2 cases per 100 patient-years after 1997.[163] Epidemiologically, MAC has been linked to significant immunosuppression ($CD4^+$ cell counts <50 cells/mm^3) in adults.[32,106,156] Researchers noted that the development of MAC infection in children may be associated with $CD4^+$ counts greater than 50 cells/mm^3, especially in children younger than 6 years old.[175] Symptoms of MAC infection include fever, malaise, weight loss, anorexia, and night sweats. Gastrointestinal manifestations are not uncommon and have included abdominal pain, diarrhea, malabsorption, and intestinal perforation. Rarely, MAC has been reported with extrabiliary obstructive jaundice (presumed to be secondary to lymphadenopathy) and endobronchial masses. The diagnosis of disseminated MAC infection relies on identification of these microorganisms from the blood, lymph tissue, bone marrow, liver, lungs, and gastrointestinal tract. Therapy for disseminated MAC infection in the pediatric population includes some combination of clarithromycin or azithromycin (maximal dosing of 500 mg and 250 mg, respectively) and/or rifabutin (maximal dosing of 300 mg). The epidemiologic link with severe immunosuppression and the advent of therapeutic prophylactic interventions (clarithromycin or azithromycin and/or rifabutin) prompted the implementation of MAC prophylaxis guidelines for adults, adolescents, and older children (6 years and older) with $CD4^+$ cell counts lower than 50 cells/mm^3.[29]

An unusual immune restoration inflammatory syndrome (IRIS) has been described as a paradoxical event after immune recovery has been achieved in adults and adolescents receiving HAART.[203] IRIS is associated with reactivation of opportunistic infections such as MAC. Activation of other latent complications, including carcinoma and neurologic symptoms, has been described.[215] Specific recommendations regarding prophylaxis in the context of IRIS are not yet defined. In IRIS, treatment of the reactivated opportunistic infection may include administration of anti-inflammatory agents including systemic steroids.

Mycobacterium tuberculosis

The increasing incidence of tuberculosis globally has affected the HIV-infected population because factors that increase the transmission of tuberculosis also affect the transmission of HIV. In a cohort of pregnant and nonpregnant HIV-infected women monitored in a multicenter longitudinal study, the prevalence of tuberculosis determined by medical history or positive skin tests was 14 percent.[155] In tuberculosis-positive individuals aged 25 to 44 years in the United States, the incidence of co-infection with HIV ranged from 9.9 to 24.2 percent in low- to higher-reporting states.[33] Factors associated with the acquisition of pediatric tuberculosis have included primarily family and caregiver risks.[9] In a study of 60 HIV-infected families, the incidence of tuberculosis was approximately 6 percent, with both HIV-infected and HIV-uninfected children affected. The vector for transmission was identified as an infected family member or caregiver. Tuberculosis should be considered in the differential diagnosis of pulmonary disease in HIV-exposed children. Treatment of active tuberculosis in an HIV-infected child includes at least a four-drug regimen, and the duration of treatment is 9 to 12 months, with the longest duration being in children with extrapulmonary disease. Careful monitoring of ART is necessary in HIV-infected children receiving concomitant antituberculosis therapy because drug-drug interactions can occur. For example, rifampin induces hepatic cytochrome P-450 and may have an impact on ART agents metabolized through that same pathway. Each HIV-infected child should be evaluated annually for exposure to tuberculosis.

Cytomegalovirus

In 1987 reports to the CDC, disseminated CMV infection occurred in 19 percent of pediatric patients with AIDS.[174] The adult spectrum of CMV disease, including retinitis, pneumonitis, esophagitis, gastritis, colitis, hepatitis, cholangitis, and encephalitis, is not reported uniformly in the pediatric AIDS literature. CMV clearly can cause primary pneumonitis in children or can be found in association with other pulmonary pathogens, especially *Pneumocystis*. Unusual gastrointestinal manifestations have included pyloric obstruction, enterocolitis, and oral and esophageal ulcers. CMV retinitis in children, in contrast to adults, is described infrequently and appears to be decreasing in incidence. Reported in 1.4 cases per 100 child-years before 1997, the incidence appears to be 0.1 cases per 100 child-years since 1997 and the wider use of HAART.[163]

Treatment of CMV disease associated with HIV infection focuses on progression of disease and not on a curative outcome. Therapeutic intervention includes the use of ganciclovir, valganciclovir, foscarnet, or cidofovir. These drugs have significant side effects, including bone marrow suppression and renal toxicity. Administration of these agents is divided into induction and maintenance dosing. The optimal interval for efficacy has not been determined and often is selected on an individual basis. Although many adults report experiencing subjective and objective improvement while receiving therapy, discontinuation of therapy is associated with high relapse rates, independent of the affected site. Careful monitoring of children receiving treatment for CMV and ART is imperative because the use of both classes of agents may depress bone marrow function. Guidelines to prevent CMV infection in HIV-infected persons have been published and include the use of oral ganciclovir.[80] Oral ganciclovir may provide primary prophylaxis in HIV- and CMV-infected children with severe immunosuppression ($CD4^+$ cell count <50 cells/mm^3). Prevention of complications of CMV in HIV-infected children with severe immunosuppression should include careful and regular (every 4 to 6 months) retinal monitoring for evidence of eye disease. Once secondary prophylaxis for disseminated CMV disease is initiated, its discontinuation should be considered on a case-by-case basis because current data to support discontinuation are limited. As reported with MAC, reactivation of CMV disease in the IRIS has been described.

Other Herpesvirus Infections

Infections with the herpesvirus family are not uncommon events in HIV-infected children. Chronic herpes simplex virus (HSV) infection was reported in 5 percent of children with AIDS before the use of HAART.[174] In general, HSV infection in pediatric AIDS has been limited to mild to severe localized infections without reports of dissemination. Varicella-zoster virus (VZV) and herpes-zoster virus (HZV) infections have contributed significant morbidity to HIV-infected persons. Disseminated and chronic HZV infections are reported in pediatric patients with AIDS. The judicious use of acyclovir for chronic infection is warranted to lessen the probability of emergence of resistant strains. Prevention of herpes infections in school-aged children is especially important because exposure to varicella may be considerable. Administering varicella-zoster immune globulin, if available, should be considered for susceptible HIV-infected children with exposure to VZV. Because the varicella vaccine is an attenuated live viral vaccine, its use in HIV-infected children is limited currently to children with asymptomatic or mildly symptomatic HIV disease and no evidence of immune suppression.[39]

Fungal Diseases

Chronic *Candida* infection plagues HIV-infected children. Affected mucous membranes or skin often does not respond well

to treatment with topical antifungal agents. Fluconazole has been documented in an open multicenter study to be as effective and safe as is ketoconazole for the treatment of oropharyngeal candidiasis.[104] In severe fungal infections, amphotericin B or other agents effective against the *Candida* spp. should be administered intravenously. Prophylaxis for candidal infections is not recommended because of concern for development of resistance to azoles such as fluconazole, which could limit the use of these agents in the care of disseminated or mucosal disease.[32]

Other disseminated fungal diseases, such as histoplasmosis and cryptococcosis, less commonly affect pediatric patients with AIDS. Histoplasmosis may be prevalent in HIV-infected children who reside in parts of the United States where *Histoplasma capsulatum* is endemic. Symptoms of histoplasmosis include fever, rash, cough, lymphadenopathy, splenomegaly, thrombocytopenia, low-grade disseminated intravascular coagulopathy, adult respiratory distress syndrome, meningoencephalitis, and neurologic abnormalities consistent with intracranial mass lesions. Symptoms of cryptococcosis include fever, headache, pulmonary involvement, and subacute meningitis or meningoencephalitis. Primary prophylaxis for adults and adolescents is suggested for those with compromised immune systems. Specifically, fluconazole is suggested as primary cryptococcosis prophylaxis for adults and adolescents with $CD4^+$ cell counts of less than 50 cells/mm^3 and itraconazole for histoplasmosis prophylaxis in adults and adolescents with $CD4^+$ cell counts of less than 100 cells/mm^3. Recommendations to discontinue prophylaxis as a result of HAART-related improved immune function have been developed for cryptococcosis and include sustained (≥6 months) $CD4^+$ cell counts higher than 100 to 200 cells/mm^3. In contrast, no such recommendations exist for discontinuation of histoplasmosis prophylaxis in HIV-infected adults or adolescents with evidence of HAART-related reconstitution of immunity.

Bacterial Diseases

Pediatric patients have an increased incidence of severe bacterial infections, including infections with *Streptococcus pneumoniae*, *Staphylococcus aureus*, and various gram-negative organisms. The risk of contracting community-acquired invasive bacterial infections has been estimated to be three times higher than the rate in non–HIV-infected children.[5] In a study of 372 HIV-infected children monitored for a median of 17 months, 14 percent experienced one or more laboratory-proven serious bacterial infections.[212] More recent findings suggested that the incidence of bacterial disease dropped from rates of 4.7 cases per 100 patient-years before 1997 to 0.2 cases per 100 patient-years after 1997.[163] Reported clinical infections included bacteremia, pneumonia, osteomyelitis, meningitis, and sinusitis. Therapy is directed against the specific isolated bacterial pathogen, and the dosing and duration of therapy depend on the affected site. Prophylaxis against bacterial infections initially should include vaccination with appropriate childhood vaccines, including *Haemophilus influenzae* type b, pneumococcal conjugate vaccines, and meningococcal conjugate vaccines.[41] Other medications available for bacterial prophylaxis include intravenous immunoglobulin (IVIG) and TMP-SMX. In a study that predated the widespread use of ART agents in children, the use of IVIG (400 mg/kg every 28 days) increased the time free from serious bacterial infections in those with $CD4^+$ cell counts exceeding 199 cells/mm^3 and decreased morbidity as measured by hospitalizations.[154] A subsequent analysis of concomitant IVIG and zidovudine use documented the efficacy of IVIG in children not also receiving TMP-SMX for PCP prophylaxis.[199] Given the expense and cumbersome route of administration, IVIG has been reserved as prophylaxis for HIV-infected children with evidence of hypogammaglobulinemia or those with more than two invasive bacterial infections in a 1-year period.

PULMONARY COMPLICATIONS

In addition to PCP and chronic sinopulmonary infection, noninfectious pulmonary complications of pediatric AIDS are associated with morbidity. In a series of more than 150 children with perinatal HIV infection early in the U.S. epidemic, the lymphoproliferative lung disorders lymphoid interstitial pneumonitis (LIP)/pulmonary lymphoid hyperplasia (PLH) were reported most frequently, and 17 percent of these children were affected.[183] Histopathologically, LIP and PLH appear to be distinct entities, although whether these disorders represent a continuum of reactive hyperplasia of lymphoid tissue is somewhat controversial. In LIP, small lymphoid infiltrates are dispersed throughout parenchymal lung tissue and often are accompanied by alveolar epithelial hyperplasia and interstitial widening. PLH describes larger, dense nodular aggregates of lymphoid tissue both in distal parenchymal tissue and in the walls of bronchi and bronchioles (Fig. 204–8A). Compression of blood and lymphatic vessels by these nodules may contribute to the accompanying clinical interstitial widening. The cause of LIP/PLH has not been defined. Associations with in situ EBV and the HIV genome have been noted, and an increase in local nonspecific and HIV-specific IgG and IgA production has been described.

Clinically, LIP/PLH is characterized by a nonproductive cough and the insidious onset of progressive hypoxia. The hypoxemia may be subtle and best appreciated during febrile, upper respiratory tract illnesses. Digital clubbing, generalized lymphadenopathy, chronic parotitis, or failure to thrive may accompany LIP/PLH. Radiographically, LIP/PLH often demonstrates characteristic interstitial infiltrates with a nodular pattern (see Fig. 204–8B). This radiographic picture frequently mimics that of miliary tuberculosis and warrants exclusion of this pulmonary infection. In an immunocompromised, anergic, HIV-positive child, simple delayed hypersensitivity skin testing for exclusion of tuberculosis may not suffice, and BAL or gastric aspiration for detection of acid-fast microorganisms may be necessary. Lung biopsy is the definitive diagnostic procedure for LIP/PLH. However, in HIV-infected children, a presumed diagnosis of LIP/PLH by the less invasive exclusion of infectious pathogens is preferred.

Therapy for LIP/PLH is not defined clearly because the clinical outcome is variable. HIV-infected children with LIP/PLH have been noted to have spontaneous remissions without therapeutic intervention, whereas other affected children progress to respiratory insufficiency and failure. Therapeutic interventions have included IVIG supplementation, ART, and corticosteroids (daily or alternate-day dosing ranging from 0.5 to 2.0 mg/kg/day), and observation. No significant associated infectious sequelae (bacteremia, fungemia) of corticosteroid use were reported in treated children. Supportive therapy consisting of oxygen supplementation, chest physical therapy, and attention given to adequate nutritional intake is helpful adjunctive treatment.

Other non-infectious pulmonary complications have been reported less frequently in the pediatric HIV-infected population and include bronchiectasis, vasculitis, and diffuse interstitial pneumonitis. An association of overrepresentation of asthma in HIV-infected children and youth treated with HAART has been reported that appears to correlate with the immune reconstitution of $CD4^+$ cell counts.[79]

CENTRAL NERVOUS SYSTEM COMPLICATIONS

Neurologic abnormalities have been documented in persons with HIV and can be attributed to opportunistic infections, adverse events of primary treatment, or primary infection with HIV, especially in light of HIV's described tropism for monocytes. Ten percent of adults with AIDS initially have neurologic symptoms,

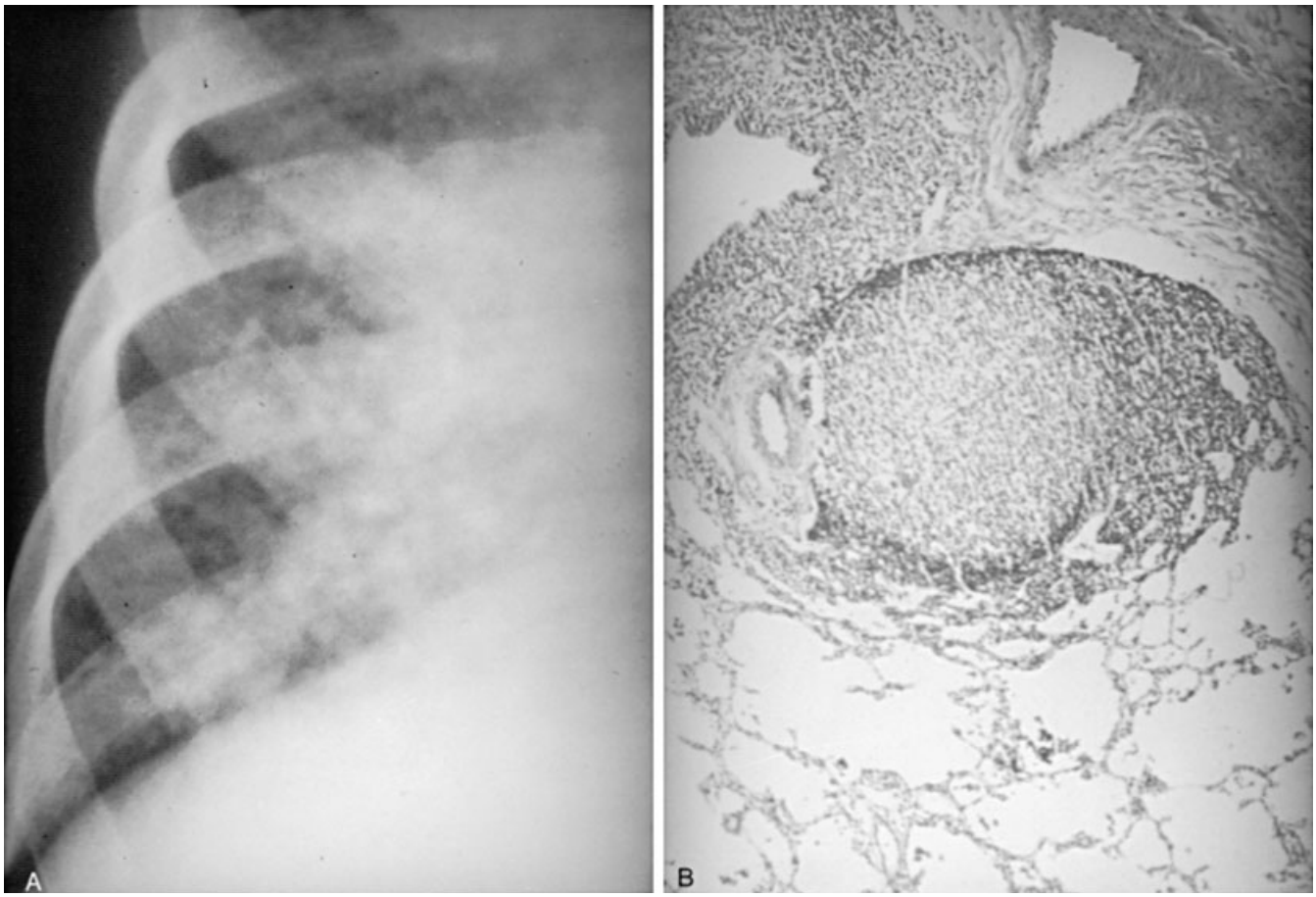

Figure 204–8 **A**, Chest radiograph documenting parenchymal nodularity in a 3-year-old child with perinatal acquired immunodeficiency syndrome (AIDS). **B,** Pulmonary lymphoid hyperplasia in the same infant with perinatal AIDS and histologic changes, including interstitial widening and prominent peribronchial lung nodes.

and 40 percent are affected during their clinical course.[146] Belman and associates[12] and Epstein and colleagues[69] described significant neurologic complications in children, including seizure disorders, attention-deficit disorders, developmental delay, and acquired microcephaly and encephalopathy. In 1987, expansion of the CDC surveillance criteria for the diagnosis of AIDS incorporated neurologic deficits by including AIDS dementia or encephalopathy.[27] In adults, dementia included "clinical findings of disabling cognitive and/or motor dysfunction interfering with occupation or activities of daily living." In 1994, the definition of HIV encephalopathy was adapted directly to children and consists of at least one of the following features present for at least 2 months: (1) failure to attain or loss of developmental milestones or loss of intellectual ability, verified by a standard developmental scale or neuropsychological tests; (2) impaired brain growth or acquired microcephaly demonstrated by head circumference measurements or brain atrophy identified by computed tomography (CT) or magnetic resonance imaging (MRI) (serial imaging is required for children younger than 2 years old); and (3) acquired symmetric motor deficit manifested by two or more of the following: paresis, pathologic reflexes, ataxia, or gait disturbance.[30] Before the use of potent antiviral treatment, the incidence of HIV encephalopathy in the pediatric AIDS population was 23 percent.[135]

The precise pathogenesis of the encephalopathic changes associated with HIV infection remains elusive, although cellular tropism of HIV has been implicated. Histopathologically, most children with involvement of the CNS show significant brain atrophy. Inflammatory lesions usually are sparse and alone cannot account for the significant amount of atrophy observed. Other purported factors potentially contributing to diminished brain size have included the following: (1) direct or indirect interference of HIV with brain growth (HIV toxic effect versus competition with brain growth factors such as neuroleukin), (2) severe malnutrition, (3) severe hypoxia from cardiac and pulmonary compromise, and (4) therapeutic regimens for infectious or noninfectious processes that mandate the prolonged use of medications that may inhibit brain growth. In a cohort of perinatally infected children followed with repeated developmental examinations over a 4-year period (3 to 7 years), cognitive development was depressed for those with an AIDS-defining event or severe immunosuppression as measured by low $CD4^+$ cells.[197] In this cohort of 422 infected children evaluated in The Women and Infants Transmission Study, early AIDS-defining illnesses increased the risk of developing chronic static encephalopathy during preschool and early school years. Infants with less severe disease had cognitive function equal to their uninfected controls. Attention-deficit hyperactivity disorder, learning disability, and graphomotor weakness have been described in higher proportion in HIV-infected children with transfusion-acquired infection.

Behavioral, neurodevelopmental, and, occasionally, imaging improvements have been documented with nucleoside analogue intervention. In a study of 146 perinatally HIV-infected children born between 1990 and 2003, the prevalence of encephalopathy (progressive or static) decreased from 40.7 percent before 1996 (limited combination antiviral therapy) to 18.2 percent after that

year.[185] In this cohort, neurocognitive scores remained stable over the course of a 6-month period in children receiving combined antiviral therapy, and only a weak association with lowered viral load could be found. Clearly, antiviral therapy not only must achieve effective serum and plasma concentrations but also must be able to cross the blood-brain barrier and affect HIV-infected CNS monocytes.

Children with neurologic or behavioral deficits and HIV infection should be evaluated by a pediatric neurologist, and developmental, audiologic, and ophthalmologic assessments are warranted. These children may need support systems including physical therapy, medications to assist with seizure control or contractures, specialized equipment (e.g., braces, splints, wheelchairs), and, as needed, medications or devices to assist with swallowing or food ingestion.

A clinical syndrome of aseptic meningitis, often recurring and presumed to be solely related to HIV infection, has been described in adults.[146] In pediatric AIDS, bacterial pathogens predominate as the most common cause of meningitis, and the aforementioned recurrent aseptic meningitis syndrome appears to be a rare occurrence.[8] Assessments of cerebrospinal fluid (CSF) in HIV-infected children without overt clinical CNS involvement usually are normal but occasionally reveal pleocytosis, elevated protein content, and elevated intrathecal antibody synthesis directed toward HIV itself or HIV antigen.[90] HIV rarely is isolated from CSF. Other pathogens reported in the pediatric HIV-infected population include *Candida* spp., CMV, *M. tuberculosis*, *Toxoplasma*, and *Cryptococcus*. In the reported adult cases of IRIS-associated progressive multifocal leukoencephalopathy, brain biopsy lesions reveal severe demyelination and perivascular inflammatory response of monocytes and T lymphocytes.[92]

GASTROINTESTINAL COMPLICATIONS

Gastrointestinal complications of HIV disease are encountered frequently in both children and adults. Among children with AIDS reported to the CDC in 1988 and 1989, wasting syndrome was described in 16 percent.[173] Wasting has been revised from the 1987 definition to its present definition (1994): (1) persistent weight loss greater than 10 percent of baseline, (2) downward crossing of at least two of the percentile lines on the weight-for-age chart in a child 1 year or older, or (3) less than the 5th percentile on a weight-for-height chart on two consecutive measurements at least 30 days apart, plus (a) chronic diarrhea (at least two loose stools per day for ≥30 days) or (b) documented fever (≥30 days, intermittent or constant).[30] Associated protein and micronutrient (zinc, selenium) deficiencies have been documented in HIV-infected adults and children.[45] Despite use of HAART, gastrointestinal conditions, nutritional deficiencies, and fluid and electrolyte disorders continue to be the most frequent described diagnoses for hospitalized HIV-infected children.[124]

More widespread use of maternal ART to prevent HIV transmission has resulted in a decline in the percentage of HIV-exposed infants with low birth weight.[182] A study of more than 11,000 HIV-exposed or infected infants observed between 1989 and 2004 showed a 14 percent decline in low birth weight, from 35 to 21 percent, and this decline occurred independent of race or ethnicity. In contrast, growth and metabolic abnormalities persist in HIV-infected children either as a direct consequence of HIV infection or as an adverse outcome of antiviral therapy. Wasting and weight loss in HIV-infected individuals appear to be independent predictors of mortality and are associated with lower $CD4^+$ cell counts.[142] These studies have prompted careful attention to the nutritional needs of HIV-infected children. In addition to provision of optimal caloric and nutritional supplementation, therapies that serve as appetite stimulants have been used increasingly and include cyproheptadine, megestrol acetate (Megace), and dronabinol (Marinol).[210] The efficacy of these agents is not permanent, especially when concomitant opportunistic infections are evident.

The use of more potent ART regimens, particularly those including PIs, has resulted in unwanted biochemical lipid abnormalities and changes in body composition for some HIV-infected adults and children. In a 2004 study of 94 infected children receiving long-term PI-containing antiviral regimens, 10 percent developed redistribution of fat, 52 percent developed dyslipidemia without associated somatic changes, and 38 percent had no associated findings.[209] In this cohort, changes in redistribution of fat and dyslipidemia were most likely to occur near ages consistent with the start of puberty (10 to 15 years). In a multicenter U.S. Perinatal AIDS Collaboration Transmission Study, 178 HIV-infected children were evaluated for hypercholesterolemia or hypertriglyceridemia, and 47 and 67 percent, respectively, met criteria for these diagnoses at least once during their follow-up.[25] In this cohort, hypercholesterolemia was associated with multiple PI-containing regimens and undetectable HIV viral load as measured by RNA PCR. Hypertriglyceridemia was predicted by elevated body mass index and use of the PI ritonavir (RTV). These clinical findings of lipodystrophy and dyslipidemia have been treated with some success with diet, exercise, or lipid-lowering medications, although the lipid-lowering medications may result in drug-drug interactions when they are combined with antivirals.

MALIGNANCY

B-cell non-Hodgkin lymphoma occurs in 3 to 4 percent of patients, and Kaposi sarcoma occurs in as many as 40 percent of HIV-infected MSM.[7] In a retrospective study of 4954 children with AIDS in the United States (1978 to 1996), approximately 2.5 percent had documented malignant diseases: non-Hodgkin lymphoma (n = 100), Kaposi sarcoma (n = 8), leiomyosarcoma (n = 4), and Hodgkin disease (n = 2); 10 others had unspecified cancers.[14] With increasing use of HAART in the mid-1990s, the incidence of primary CNS lymphoma in HIV-infected adults declined, but malignancy continues to be reported.[113] Associated in situ infection with HIV, EBV, or both, has been documented in pediatric malignancies, although the precise role in pathogenesis is not defined. Early chemotherapeutic intervention has enhanced the quality of life and longevity and often has been provided in conjunction with ART.

Kaposi sarcoma clearly occurs much less commonly in HIV-infected children. The pathogenesis of Kaposi sarcoma, both HIV and non-HIV related, has been elucidated, and human herpesvirus-8 has been documented in lesions.[103,157] Pathogenesis has been linked to oncogenesis and cytokine-induced growth. The clinical manifestations of Kaposi sarcoma associated with AIDS affect multiple organs. The skin, gastrointestinal tract, lungs, and heart have been affected by Kaposi sarcoma proliferations. Management of Kaposi sarcoma has improved with increasing use of HAART to restore immune function in HIV-infected individuals.[26]

Since 1987, more than 13 children with AIDS have been reported with smooth muscle malignancies, specifically, leiomyosarcomas of the gastrointestinal tract.[44,147] McClain and colleagues[147] documented (by PCR and in situ hybridization techniques) the association of EBV with leiomyosarcoma in children with AIDS. Since the advent of HAART, HIV-related malignant disease in the U.S. is reported less frequently. In a study of 2969 HIV-infected children followed in the Pediatric AIDS Clinical Trials Group 219/219C from 1993 to 2003, only 37 cases of malignant disease were reported, representing a prevalence of 0.6 percent malignancies and an incidence of 1.56 per 1000 person-years.[117] In this analysis, the standardized incidence

cancer rate for infected children was 10.08 as compared with uninfected exposed controls. The incidence of cancer was highest in children with severe immunocompromise and in those who had received HAART for less than 2 years. In contrast, developing nations continue to report high incidences of malignant disease.[192]

OTHER COMPLICATIONS

Cardiac Abnormalities

Cardiac manifestations of HIV infection in children have been described and include progressive left ventricular dysfunction, as measured by a diminished shortening fraction that may be associated with immune suppression.[134] Clinically, HIV-infected children have been described with congestive heart failure and cardiomegaly, cardiac tamponade, nonbacterial thrombotic endocarditis, conduction disturbances, and sudden death, presumably secondary to primary ventricular arrhythmia associated with severe cardiomyopathy. In a postmortem analysis of 30 HIV-infected children followed longitudinally in the prospective P2C2 HIV Multicenter Study, 50 percent had cardiomegaly with associated clinical findings of increased heart rate, increased left ventricular mass, and chronic heart disease.[115] A higher prevalence of postmortem pericardial effusions was also noted.

In a study of 81 HIV-infected children, unexpected cardiorespiratory arrest occurred in 9 percent, chronic congestive heart failure in 10 percent, and dysrhythmias in 35 percent of children.[138] Asymptomatic children with HIV infection additionally have been documented to exhibit cardiac abnormalities. No evidence suggests that HIV is directly cardiotoxic, although HIV has been documented within myocardial cells by in situ hybridization.[77] Other factors may have an impact on the development of cardiac abnormalities and include malnutrition and infectious agents such as CMV and EBV. In one study of HIV-infected children receiving nucleoside analogue ART (zidovudine or didanosine), the odds of developing cardiomyopathy were 8.4 times greater in children receiving zidovudine but not didanosine.[62]

The increasing use of HAART and, specifically, PIs in the pediatric HIV-infected population prompted careful management of associated hypercholesterolemia and hyperlipidemia.[104] In univariate analysis, hypercholesterolemia in children with HIV infection treated with a PI regimen was associated with elevated systolic blood pressure but not with body mass.[74] Careful follow-up of lipid profiles should be evaluated routinely in HIV-infected children receiving antiviral therapy.

Renal Dysfunction

Renal disease is yet another clinical manifestation of HIV infection. In a retrospective evaluation of 155 pediatric patients with AIDS, 12 were noted to have significant proteinuria.[205] The renal abnormalities described included nephritis (focal glomerulosclerosis and mesangial hyperplasia) and nephrosis. In fact, focal sclerosis and segmental sclerosis were described in more than half of reported children.[206] The immunopathologic characteristics of HIV-associated nephropathy include inflammatory infiltrations predominantly composed of activated T cells ($CD4^+$ cells usually exceeding $CD8^+$ cells).[172]

Persistent metabolic acidosis associated with renal tubular disease and high anion gap acidosis has been reported in pediatric HIV infection.[172] In a group of 202 HIV-infected children, 34 (17%) had evidence of persistent acidosis. Renal disease in HIV-infected children often appears in concert with profound immunodeficiency and end-stage HIV disease. Because most pediatric patients with AIDS and renal disease have perinatally acquired HIV infection, the importance of congenital or early concomitant infections (e.g., CMV) has been postulated to affect pathogenesis. The nephrosis of HIV disease can be particularly difficult to treat in an already malnourished, hypoproteinemic, HIV-infected child. Corticosteroid therapy may be attempted but often ameliorates symptoms with variable efficacy. Many of the reported children with renal disease have associated growth failure. Nutritional supplementation and dietary restriction may be supportive adjunctive therapy.

Bone Marrow Suppression

Hematologic abnormalities associated with HIV infection in children include leukopenia, anemia, and thrombocytopenia. Neutropenia often has been described in association with circulating antineutrophil antibodies and may respond to blockade therapy with IVIG. Granulocyte colony-stimulating factor has been used successfully in neutropenia, both drug-induced and HIV-associated.[94,160]

The anemia of HIV infection may be microcytic, hypochromic as seen in chronic infection, autoimmune with positive Coombs testing, or typical of nutritional deprivation (iron or vitamin B_{12} deficiency). The origin of anemia in AIDS is difficult to sort out and is confounded by multiple cofactors that affect red blood cell counts, that is, poor nutritional status and concomitant use of toxic therapeutic agents (zidovudine). In adults and children, recombinant erythropoietin has been beneficial in treating anemia associated with antiviral therapy.[18] In developing nations, infant anemia of HIV exposure or infection coupled with acute local parasitic infections (malaria) may significantly affect infant morbidity. The risk for developing severe and potentially fatal malarial anemia is increased for HIV-exposed or infected children with concomitant acute parasitemia, as compared with HIV-negative controls.[164]

Immune thrombocytopenia has been reported in 13 percent of children with symptomatic HIV infection with an onset as early as the first year of life.[67] This phenomenon appears to be immune-mediated, although its pathogenesis is not defined clearly. The presence of platelet-associated IgG in HIV-infected children has been reported to have a sensitivity of 93 percent; however, its specificity is only 13 percent, a finding thus suggesting that platelet-associated IgG is unlikely to be a cause of thrombocytopenia.[68] Therapy for thrombocytopenia in children and adults has included no intervention, platelet transfusions, systemic corticosteroids, IVIG, ART, and, most recently in adults, IFN-α. Immune thrombocytopenia has resolved spontaneously in some children with simple supportive measures.

DIAGNOSIS OF HUMAN IMMUNODEFICIENCY VIRUS INFECTION IN INFANTS AND CHILDREN

EARLY TRANSMISSION TO FETUSES

Information is scant on the role of the human placenta in transmitting HIV infection from mother to fetus. Studies suggested that fetal infection can take place as early as 9 to 11 weeks of gestational age.[20,145] Preliminary examination of birth placental tissue from HIV-infected mothers in another study indicated that HIV core antigens could be detected in approximately 50 percent of the cases studied (23 of 51); furthermore, HIV antigens were localized in the Hofbauer cells.[217] Finally, investigators documented that placental trophoblasts can be infected by CD4-independent isolates of HIV-1 in vitro.[3]

An increase in the incidence of intrauterine fetal demise was demonstrated in the literature in HIV-infected pregnant women.[65,130] In HIV-negative fetuses, placental lesions known to be associated with fetal demise were identified: abruption, infarc-

TABLE 204–13 Guidelines for the Diagnosis of Human Immunodeficiency Virus Infection in Infants Born to Human Immunodeficiency Virus–Infected Mothers

Definitive HIV Diagnosis for Infants 18 mo or Older
Two positive enzyme-linked immunosorbent assays and a positive confirmatory serologic test, e.g., Western blot or immunofluorescence assay
OR
Any two positive viral detection assays on separate specimens:
HIV culture
HIV polymerase chain reaction
p24 antigen test
OR
Documentation of a pediatric AIDS-defining illness
Presumptive Diagnosis for Infants Younger Than 18 mo
A single positive viral detection assay (excluding cord blood):
HIV culture
HIV polymerase chain reaction
p24 antigen assay
Definitive Diagnosis for Infants Younger Than 18 mo
Any two positive viral detection assays on separate specimens:
HIV culture
HIV polymerase chain reaction
p24 antigen test
OR
Documentation of a pediatric AIDS-defining illness

AIDS, acquired immunodeficiency syndrome; HIV, human immunodeficiency virus.
From Hanson, I.C., and Shearer, W.T.: Diagnosis of HIV infection. Semin. Pediatr. Infect. Dis. 5:266-271, 1994.

tion, and other infections (CMV). In HIV-positive fetuses, similar placental lesions could not be identified, and death was attributed to HIV infection of fetal tissue as detected by in situ hybridization. Two hypotheses for transmission are proposed: (1) direct cell-to-cell spread of HIV from infected maternal mononuclear cells through the placental cells and eventually to fetal tissue itself and (2) infected maternal cells that gain access to the fetal circulation.

METHODS USED TO DIAGNOSE INFECTION IN CHILDREN OLDER THAN 18 MONTHS

In children older than 18 months in whom the presence of maternal anti-HIV antibody no longer is a confounding variable, the conventional tests used to diagnose HIV infection in adults are applicable (Table 204–13). Thus, enzyme-linked immunoassay (ELISA), Western blot analysis, indirect fluorescent antibody assay, p24 HIV antigen analysis (p24Ag), HIV culture, and HIV PCR may be used. Of these tests, ELISA and Western blot analysis are the most practical, and two positive ELISAs in addition to a positive Western blot confirm a diagnosis of HIV infection. Combination kits that test for both HIV-1 and HIV-2 are now used.

METHODS USED TO MAKE AN EARLY DIAGNOSIS OF INFECTION IN INFANTS AND CHILDREN YOUNGER THAN 18 MONTHS

The clinical manifestations of HIV infection in children are varied and nonspecific and include chronic pneumonitis, failure to thrive, hepatosplenomegaly, thrombocytopenia, and chronic diarrhea. In children younger than 18 months, a positive serologic determination of HIV is not accepted as indicative of the presence of HIV infection because of passive maternal antibody. Documentation of HIV infection in children younger than 18 months requires identification of viral components from blood. The most commonly used virologic assay in the United States is HIV PCR (DNA or RNA) or virologic culture.[166] In resource-poor countries where virologic laboratories are not available, the CD4/CD8 ratio may be used to diagnose infants with HIV infection.[188]

Table 204–13 outlines the current guidelines for determining positive HIV infection status in infants younger than 18 months who are born to HIV-infected mothers. HIV DNA PCR is the preferred pediatric testing tool because it can be used to identify integrated proviral HIV DNA in the mononuclear cells of infants. Cord blood determinations for HIV are problematic because of contamination by maternal cells. Experience with the use of PCR or culture technology in neonates born to HIV-infected women has documented HIV infection as early as the first days or month of life.[153] The estimated sensitivity of HIV DNA PCR is 38 percent from birth to 48 hours of life, 93 percent by 14 days of age, and 96 percent by 28 days of age. HIV-1 RNA PCR measures extracellular viral RNA and has sensitivity of 25 to 40 percent in the first weeks of life and 90 to 100 percent by the time the child reaches 2 to 3 months of age.[226]

Current pediatric PCP prophylaxis guidelines suggest that infants with two negative virologic assays (HIV culture or HIV PCR), both performed at 1 month or older and at 4 months or older, most likely are not infected with HIV, and interruption of the therapeutic intervention is warranted.[31] Hence, early diagnosis in HIV-exposed infants has been improved both for identification of HIV-infected children (as early as the first month of life) and for uninfected children labeled as seroreverters (as early as the fourth month of life). Schedules for testing of HIV-exposed infants vary nationally. However, advances in diagnostic technology allow for early testing of HIV-exposed infants, including shortly after birth through 14 days of life, at 1 to 2 months of age, and then again at 3 to 6 months. Confirmation of negative virologic assays (HIV PCR) should be performed with typical serologic testing (ELISA or Western blot) when the child is 12 to 18 months of age.

The assays outlined earlier easily detect HIV-1 subtype B HIV commonly found in developed nations. To detect non-B HIV infection in individuals from or in developing nations, HIV RNA PCR may be preferred, because it has a lower incidence of false-negative assays as compared with HIV DNA PCR. If non-subtype B HIV is suspected, then use of HIV RNA testing that is sensitive for these subtypes (e.g., more sensitive RNA PCR assays or branched DNA assays that are commercially available in the United States) should be considered.

IMMUNOLOGIC AND CLINICAL MONITORING OF HUMAN IMMUNODEFICIENCY VIRUS–INFECTED CHILDREN

Laboratory monitoring of HIV-infected children is linked to those variables that predict morbidity and mortality, namely, $CD4^+$ T-cell percentage and HIV RNA PCR. Clinical evaluations should occur at a minimum of every 3 months, or sooner if the patient has clinical signs or AIDS-defining events, and measures of $CD4^+$ T-cell and HIV RNA PCR should be performed. Because of variability across commercially available RNA PCR assays that may be as great as twofold, the individual HIV-infected patient should, when possible, be tested with a single assay type.[226] Recommendations for changes in treatment based on changes in $CD4^+$ T-cell percentages or HIV RNA PCR should be undertaken in consultation with a specialist who routinely provides care to HIV-infected individuals. Evaluation of the individual HIV-

infected child for viral resistance can be accomplished with use of commercially available phenotyping or genotyping assays. Such studies should be performed in consultation with an HIV specialist. Depending on treatment regimens, most children also should have laboratory studies performed to assess their potential for developing treatment-induced toxicities including bone marrow suppression, metabolic acidosis, dyslipidemia disorders, and pancreatitis. It is recommended that this cohort annually receive dental, ophthalmologic, cardiologic, and neurodevelopment assessments. Routine recommendations for childhood vaccine delivery are outlined later.

TREATMENT

PRIMARY ANTI–HUMAN IMMUNODEFICIENCY VIRUS TREATMENT

Implementation of HAART consisting of at least three antiviral drugs has enhanced survival rates, improved immune status, and reduced opportunistic infections, as previously outlined.[19,87,148,171] Increased survival of HIV-infected children brings challenges in selecting successive new ART regimens for now chronically ill children, and specific issues to address include short- and long-term toxicities related to therapy and development of drug resistance.

In general, the principles of therapy are similar in all affected age groups. Treatment should be aggressive and use multiple drugs as early in the course of the infection as possible to suppress viral replication fully, reduce development of resistant viral strains, minimize drug-related toxicity, and preserve immune function. Considerations specific to the pediatric host include the following: (1) early clinical severity of HIV disease, viral load, and level of immune suppression; (2) availability of appropriate drug formulations and dosing; (3) unique pharmacokinetics (body composition, renal excretion, liver metabolism, enzyme maturation); (4) regimen potency, complexity, short- and long-term toxicity potential; (5) impact of changing therapy; (6) co-morbidities that affect drug choice; (7) drug interactions; and (8) the ability of the parent or child to adhere to a prescribed regimen.

Guidelines for initiation of therapy differ somewhat for adults and children. Current pediatric recommendations include initiation of therapy for symptomatic and immunosuppressed HIV-infected children, and for children aged less than 12 months regardless of clinical, immunologic, or virologic status (Table 204–14). Current ART intervention is designed to target different steps in the replication cycle of HIV (Fig. 204–9).[118] The Working Group on Antiretroviral Therapy and Medical Management of HIV-Infected Children currently recommends aggressive combination of at least three medications from at least two drug classes for initial treatment of HIV-infected infants, children, and adolescents.[226] With improvements in treatment formulations, dosing recommendations for antiviral agents can alter over time. Hence, for updated specific dosing and drug-drug interactions of antiviral agents, the reader is referred to The Working Group on Antiretroviral Therapy and Medical Management of HIV-Infected Children guidelines available at http://AIDSinfo.nih.gov.

INHIBITORS OF HUMAN IMMUNODEFICIENCY VIRUS CELL ENTRY

Entry of HIV-1 into target cells begins with interactions of the viral envelope protein gp120 with CD4 and a chemokine co-receptor, usually CCR5 or CXCR4. Both cellular receptors and structures in envelope proteins associated with membrane attachment and fusion are targets for therapeutic intervention.

Blockade of Human Immunodeficiency Virus by CD4-Receptor Inhibitors

CD4-based molecules can neutralize HIV by several mechanisms. Initial studies demonstrated the efficacy of soluble preparations of CD4 (sCD4) against HIV in vitro.[108,181,214] A chimeric molecule consisting of recombinant CD4 and immunoglobulin G (rCD4-IgG) was developed with an extended half-life and enhanced efficacy. Shearer and colleagues[186] documented safety, evidence of placental transfer, and appropriate elimination after intravenous administration of rCD4-IgG2 in pregnant women. In a phase I/II study, 18 children infected with HIV-1 received single or multiple intravenous doses of rCD4-IgG2 and demonstrated evidence of antiviral activity and tolerance.[187] Certain small molecules may competitively inhibit viral entry by binding gp120, thus blocking the gp120-CD4 interaction. BMS-806, a prototype of these small molecules, demonstrates its antiviral activity by blocking conformational changes in the viral envelope glycoproteins.[229]

Blockade of Human Immunodeficiency Virus by Chemokine (Co-receptor) Inhibitors

Agents that target blockade of CXCR4 and CCR5 currently are being evaluated in clinical trials. Concerns about the use of co-receptor inhibitors include potential for unintended immune modulation and for viral escape to occur from tropism selection with resultant disease progression.[159] Maraviroc (Selzentry), a

TABLE 204–14 Indications for Use of Antiretroviral Therapy by Age in Human Immunodeficiency Virus–Infected Children*

Age	HIV-Related Symptoms	CD4/Viral Studies	Recommendation
All	Significant	—	Treat
	AIDS-defining event	—	Treat
<12 mo	Asymptomatic *and*	CD4 <25%	Treat
	Any	—	Treat
1-<4 yr	Asymptomatic	*and* CD4 <20%	Treat
	Mild symptoms *and*	CD4 <20%	Treat
≥4-12 yr	Asymptomatic	*and* CD4 <15%	Treat
	Mild symptoms *and*	CD4 <15%	Treat
≥13 yr	Asymptomatic	*and* CD4 <200 cells/mm^3	Treat
	Mild symptoms *and*	CD4 <200 cells/mm^3	Treat

AIDS, acquired immunodeficiency syndrome; HIV, human immunodeficiency virus.

**Treatment considerations and deferral options for all age groups can be reviewed at http://AIDSinfo.nih.gov, Working Group on Antiretroviral Therapy and Medical Management of HIV-Infected Children. Guidelines for the Use of Antiretroviral Agents in Pediatric HIV Infection. October 26, 2006 1-126. Available at ttp://aidsinfo.nih.gov/ContentFiles/PediatricGuidelines.pdf. Accessed (October 2007 [pp.57].)*

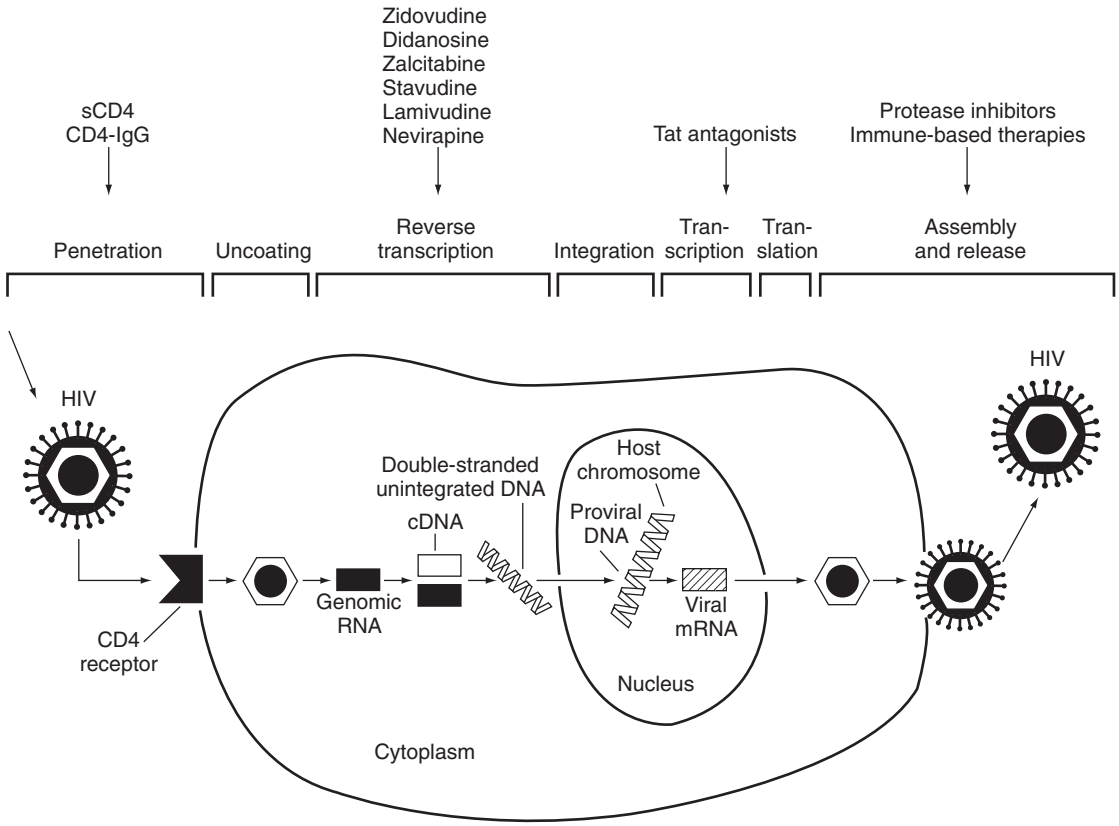

Figure 204–9 Life cycle of human immunodeficiency virus (HIV), with illustration of targets for pharmacologic intervention. *(From Kline, M. W., and Shearer, W. T.: HIV infection and AIDS in children.* In *Rich, R. R., Fleischer, T. A., Schwartz, W. T., et al.: [eds.]: Clinical Immunology. St. Louis, Mosby, 1996, pp. 739-750.)*

first-in-its-class, selective CCR5-co-receptor antagonist, received approval by the FDA for use in adults in August 2007. It is recommended as adjunct therapy to combination antiviral regimens and targets treatment of CCR5-tropic HIV-1 (R5 virus). The most common adverse effects are cough, fever, upper respiratory infections, rash, musculoskeletal symptoms, abdominal pain, and dizziness.

Blockade of Human Immunodeficiency Virus by Interference with gp120-CD4 Interactions

Polyanionic compounds such as dextran sulfate and heparin inhibit binding of HIV to the cell membrane, targeting the V3 loop of gp120.[158] Because of their systemic toxicity, most of these compounds have been evaluated in clinical trials as topical microbicides to prevent sexual transmission of HIV in humans. PRO 2000, a naphthalene sulfonate polymer exhibiting its effect by binding to CD4, is a potent inhibitor of M-tropic strains of HIV (R5 viruses) in human cervical explants.[78] PRO 2000 gel (0.5%) is effective and well tolerated in clinical trials following vaginal application. An additional substance, the green tea flavonoid, epigallocatechin gallate (EGCG), binds to the CD4 molecule at the gp120 binding site, thus inhibiting gp120-CD4 interaction in a dose-dependent manner.[225] Such substances promise adjunctive mechanisms to restrict HIV infection.

Blockade of Human Immunodeficiency Virus by Fusion Inhibitors

As previously described, fusion of the HIV envelope (glycoprotein gp41) with the target-cell membrane is an important step in host-cell infection. During fusion, FP of gp41 inserts into the target-cell membrane, and gp41 heptad repeats (HR1 and HR2) alter their conformation to form a 6-helix bundle. This process results in the formation of a fusion pore through which the HIV capsid passes into the target cell. Fusion inhibitors represent the first class of entry inhibitors for which there is a licensed drug. Enfuvirtide (T-20) is a synthetic peptide that binds to the HR1 domain of gp41, prevents six-helix formation, and thus inhibits fusion.[43,170] Enfuvirtide is approved for use in HIV-infected adults and children aged 6 years or older whose HIV infection has not been controlled by other anti-HIV drugs. Addition of enfuvirtide to an optimized background regimen of ART agents has demonstrated modest decreases of HIV viral load for long periods.[49] The most common adverse effects include mild to moderate erythema, induration, pain, lymph node swelling, itching, and tenderness at the injection site. Other side effects reported are diarrhea, nausea, fatigue, headache, dizziness, peripheral neuropathy, and hypersensitivity reactions.[48,57] However, in the absence of a fully suppressive ART regimen, mutations associated with resistance to enfuvirtide are demonstrated to be rapid.

NUCLEOSIDE ANALOGUE REVERSE TRANSCRIPTASE INHIBITORS

Nucleoside analogues have no direct effect on HIV reverse transcriptase. Following phosphorylation by the host-cell kinases, they inhibit viral reverse transcriptase by competing with the natural substrate at the same binding site on the enzyme. With their incorporation into the newly forming proviral DNA, further viral transcription is prematurely terminated. Six nucleoside

analogue reverse transcriptase inhibitors (NRTIs) are licensed and currently available in the United States: zidovudine (ZDV), didanosine (ddI), stavudine (d4T), lamivudine (3TC), abacavir (ABC), and emtricitabine (FTC).

Zidovudine

Early pediatric studies demonstrated clinical, immunologic, and virologic improvements after intravenous and oral administrations of ZDV.[149] However, sustained CD4+ cell restoration with ZDV monotherapy could not be achieved.[150]

ZDV is available in capsules, oral solution, tablets, and intravenous infusion forms. ZDV has good oral bioavailability (65%) and CNS penetration, with a mean serum half-life of 0.5 to 3 hours. It is rapidly metabolized in the liver by glucuronidation and is eliminated primarily by glomerular filtration and active tubular secretion. The most frequent adverse effects of ZDV are granulocytopenia and macrocytic anemia, often requiring limitations on doses. Some clinicians administer growth factors to counteract these adverse effects.[93] Other toxic effects include symptomatic myopathy, lactic acidosis, and hepatosteatosis.

In 1994, PACTG protocol 076 documented the remarkable effectiveness of ZDV in reducing the rate of maternal-infant transmission of HIV-1 by 66 percent (Fig. 204–10).[54] The short-term toxicity most frequently described among infants was mild and reversible anemia. Most clinicians continue to use ZDV as part of their regimen to prevent maternal transmission, but maternal dosing often includes additional antiviral medications to suppress maternal viral load optimally, and the administration of ZDV follows current adult standards of twice-daily dosing and not those used in the original PACTG 076 formulation.

ZDV and other nucleoside analogues are suspected of inducing mitochondrial dysfunction secondary to their affinity for mitochondrial gamma DNA polymerase. This effect has been a source of particular concern for the ZDV- and HIV-exposed fetus and infant. Adding to this concern was a report from a large French study that 12 of 2644 NRTI- (predominantly ZDV) and HIV-exposed infants had evidence of mitochondrial dysfunction.[11] An additional 14 children in this cohort had unexplained symptoms that could be attributed to mitochondrial dysfunction.

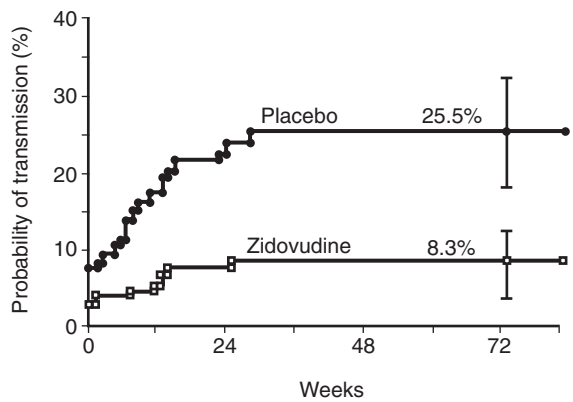

Figure 204–10 Analysis demonstrating a 66 percent reduction in perinatal transmission rates from 25 to 8 percent with antepartum, peripartum, and postpartum zidovudine therapy in human immunodeficiency virus–infected pregnant women and their infants. *(Reprinted by permission from Connor, E. M., Sperling, R. S., Gelber, R., et al.: Reduction of maternal-infant transmission of human immunodeficiency virus type 1 with zidovudine treatment: Pediatric AIDS Clinical Trials Group Protocol 076 Study Group. N. Engl. J. Med. 331:1173-1180, 1994.)*

In response to this and other smaller cohort publications, the Perinatal Safety Review Working Group reviewed deaths in 5 large HIV-exposed perinatal cohorts (total of >11,000 NRTI- and HIV-exposed infants and children) in the United States to determine whether similar cases of severe mitochondrial toxicity could be documented.[64] Prophylaxis with ZDV was not associated with findings consistent with mitochondrial dysfunction, including a careful review of the cohort's 85 deaths. In early treatment studies, low birth weight and premature delivery were ascribed to nucleoside analogue exposure in small study cohorts.[136] With further treatment including HAART and specifically ZDV, low birth weight appears to be reduced in incidence.[182] Concerns about NRTI therapy in HIV-exposed but uninfected infants led to other large-scale studies that documented safety of the maternal-infant ZDV regimen through age 18 to 38 months.[100,200]

Resistance to ZDV has been described, and the characteristic finding is a mutation at codon 215 of the reverse transcriptase.[110] In 1995, ACTG protocol 116B/117 performed a randomized comparison of didanosine with continued ZDV therapy in 187 patients with advanced HIV-1 disease who had received at least 4 months of ZDV treatment.

Didanosine

In early studies, ddI was well tolerated, and improvements in clinical and immunologic measures were documented in previously untreated HIV-infected children and those who had received prior ZDV treatment.[15,109,110] ddI is formulated as an enteric capsule with fewer gastrointestinal side effects and should be administered on an empty stomach. Common toxicities are diarrhea, abdominal pain, and nausea. The frequency of severe clinical toxicities, pancreatitis, and peripheral neuropathy is dose-related. Lactic acidosis, severe hepatomegaly with steatosis, and retinal and visual changes also have been reported in patients treated with ddI. This drug may interact with other ART agents and produce toxicity or unwanted clinical outcomes. For example, ddI should be used cautiously in combination with tenofovir, a nucleotide analogue, because of increasing plasma levels of ddI and decreasing CD4+ cell counts.[224]

Stavudine

Like ZDV and ddI, d4T is a potent inhibitor of in vitro HIV replication. In a phase I/II clinical trial of d4T in HIV-infected children, d4T was well tolerated without dose-related clinical or laboratory adverse events. The oral bioavailability ranges from 61 to 78 percent, and the CSF concentrations range from 16 to 97 percent 2 to 3 hours following oral administration.[119] Compared with ZDV, d4T is associated with better preservation of CD4+ lymphocyte counts.[121] d4T is available in capsule and oral solution forms. Reported severe toxicities include peripheral neuropathy, pancreatitis, lipodystrophy, and lactic acidosis. d4T and ddI should not be combined in the same regimen because of in vitro antagonism.

Lamivudine

3TC has good antiviral potency to both HIV-1 and hepatitis B virus (HBV), and the drug is well tolerated orally.[167] 3TC is available in both tablet and solution forms; its bioavailability is not affected by food. A combined product (Combivir) provides a preformed tablet formulation of ZDV (300 mg) and 3TC (150 mg). Toxicities are rare but include pancreatitis and lactic acidosis.

Abacavir

ABC is approved for use in combination with other ART agents in children and adults. It is well absorbed, is unaffected by food,

is well tolerated, and has good in vivo and in vitro potency in reducing HIV viral load when combined with other ART agents.[178] An important clinical side effect of this drug is ABC hypersensitivity reaction that occurs in approximately 5 percent of patients at a mean of 11 days after initiation of this agent.[57] Symptoms include fever, rash, malaise or fatigue, and gastrointestinal symptoms such as nausea and vomiting. HLA-B*5701 is documented to be a risk factor for development of hypersensitivity to ABC.[201] Suspected hypersensitivity to ABC requires permanent discontinuation of this agent because hypotension and death have occurred on repeat challenge. ABC is available in tablet and oral solution forms. A combined product, Trizivir, provides a preformed tablet formulation of ZDV (300 mg), 3TC (150 mg), and ABC (300 mg). Epzicom, another preformed tablet, contains 3TC (300 mg) and ABC (600 mg).

Emtricitabine

FTC was approved for use in combination with other anti-HIV medications. It is similar in many aspects to lamivudine and, like that drug, is active against both HIV-1 and HBV.[177] FTC has potent in vivo antiretroviral activity, 4 to 10 times more potency than 3TC in vitro, and is well tolerated.[223] Because of cross-resistance of FTC to the M184V resistance, mutations commonly observed in patients in whom a 3TC-containing regimen has failed, this agent has little utility in 3TC-experienced patients. It is available as a hard gelatin capsule and as an oral solution. Skin discoloration that manifests as hyperpigmentation on the palms and soles is a unique toxicity of FTC therapy and is usually mild.

NUCLEOTIDE REVERSE TRANSCRIPTASE INHIBITORS

Nucleotide reverse transcriptase inhibitors (NtRTIs) function like previously described NRTIs, but they do not require phosphorylation. Tenofovir disoproxil fumarate (tenofovir DF, TDF), is an orally bioavailable prodrug of tenofovir. It is the first NtRTI approved for use in the United States, but its use is limited to adolescents and adults. Tenofovir has good in vivo potency against both HIV-1 and HBV.[84] Tenofovir has long serum and intracellular half-lives that allow once-daily dosing. High-dose tenofovir treatment has been associated with skeletal abnormalities in young animals[220] and a significant decrease in absolute bone mineral density in HIV-infected children.[82] These changes in bone mineral density seemed to stabilize by 24 weeks of the treatment, and none of the patients experienced fractures during the study period. A combined product, Truvada, provides a preformed tablet formulation of FTC (200 mg) and TDF (300 mg) available for use once daily.

NON-NUCLEOSIDE REVERSE TRANSCRIPTASE INHIBITORS

Non-nucleoside reverse transcriptase inhibitors (NNRTIs) work by binding directly to the reverse-transcriptase enzyme, thus rendering it nonfunctional. Three available drugs in this class are approved for use in the United States: nevirapine (NVP), delavirdine, and efavirenz (EFV). Only NVP and EFV have been approved for use in pediatric HIV-infected populations and hence only these drugs are described. The major disadvantage of the current NNRTI agents is the rapid development of resistance with resultant cross-resistance across all class-related drugs.

Nevirapine

NVP has been shown to have antiretroviral efficacy in a number of different combination regimens in children. The most promising use of nevirapine has been documented in perinatal HIV transmission. The HIVNET 012 study in Uganda demonstrated that a single 200-mg dose of NVP taken by an HIV-infected breast-feeding mother at the onset of labor and a 2 mg/kg dose of NVP given to the infant within 72 hours of birth could reduce the mother-to-child transmission rate by 42 percent by 6 weeks of age when compared with short-course ZDV regimens.[97] The reduction in the relative risk of transmission persisted through 18 months of age.[112] NVP is available in both tablet and oral solution forms. Severe hepatotoxicity, hepatic necrosis, and hepatic failure have been reported in children as early as the first 12 weeks of life. NVP should be discontinued permanently in children or adults who develop severe rash or rash with constitutional symptoms and hepatotoxicity. Women with $CD4^+$ counts greater than 250 cells/mm^3 appear to have particularly increased susceptibility to nevirapine toxicity.

Efavirenz

EFV is approved as a single-daily dosing agent and for that reason has been attractive for use in adolescents and older children. It is available in capsule and tablet forms only. A combined product, Atripla, provides a preformed tablet formulation of FTC (200 mg), TDF (300 mg), and EFV (600 mg) available for use once daily in adults. In addition to toxicities of rash and elevated transaminase levels, CNS abnormalities including somnolence, insomnia, bad dreams, irritability and depression have been reported within the first 2 weeks of treatment. EFV has been reclassified as Food and Drug Administration pregnancy category D because in animal models EFV was associated with cleft palate and anencephaly.[24] Use in women of child-bearing age should include intermittent pregnancy testing and counseling for pregnancy prevention.

PROTEASE INHIBITORS

PIs are relatively small peptides that share structural features with the substrates for viral protease. These molecules bind to and inhibit viral protease, thereby preventing the virus from maturing into an infectious virion. These drugs are extremely potent in reducing plasma RNA viral loads. They exhibit a high barrier for development of drug resistance. As a class, they interact with the hepatic cytochrome P-450 enzyme system, and many induce their own metabolism. Thus, the potential for significant drug-drug interactions is considerable. Examples of such interactions include those with oral contraceptives, antituberculous drugs, antiepileptic drugs, some antihistamines, and methadone. Adverse events of use of PI include previously described metabolic complications (dyslipidemia, lipodystrophy, insulin resistance, gastrointestinal effects of nausea or diarrhea), higher pill burden than with NRTI- or NNRTI-based regimens, and poor palatability of liquid preparations. Currently, 10 PIs are approved for treatment of HIV infection: saquinavir, RTV, lopinavir (LPV)/RTV, indinavir, nelfinavir (NFV), atazanavir, amprenavir (APV), fosamprenavir, tipranavir, and darunavir. Only 4 of these PIs are approved for use in HIV-infected children: RTV, LPV/RTV, NFV, and APV.

Ritonavir

RTV is a potent inhibitor of the hepatic cytochrome P450 3A4 isoenzyme and therefore can improve the bioavailability and prolong the elimination half-life of most co-administered PIs.[55] This PI has been used as a pharmacokinetic booster in low doses combined with indinavir, LPV, saquinavir, APV, fosamprenavir, atazanavir, and tipranavir to enhance these PI serum levels. It is available in capsule and oral solution forms. When RTV is used

at lower doses as a pharmacokinetic booster of other PIs in adults, it is used as 100 mg twice daily or 200 mg once daily. Dose escalation at 2- to 3-day intervals is necessary to minimize the common side effect of nausea or vomiting. Other side effects include paresthesias, hyperlipidemia, and lipodystrophy.

Lopinavir/Ritonavir

LPV/RTV (LPV/r) is a co-formulation of LPV and RTV. RTV acts as a pharmacologic enhancer rather than as an ART agent by inhibiting the metabolism of LPV and increasing LPV plasma concentration. It is available in tablet (200 mg LPV/50 mg RTV) and oral solution (80 mg LPV/20 mg RTV per mL; contains 42.4% alcohol by volume) forms. Currently, it is not approved for use in neonates and infants. Clinical trials are under way for infants younger than 6 months. Major toxicities are diarrhea, nausea, vomiting, headache, asthenia, and lipid abnormalities. The 42.4 percent alcohol content in this oral formulation leads to almost uniform symptoms of nausea or vomiting and often can be overcome with concomitant delivery of foods with strong tastes. Dosing compliance may be affected by taste and caregiver discomfort in delivery.

Nelfinavir

NFV has been used widely in pediatric populations. It is available in tablet and oral powder forms. The most common side effects of NFV are diarrhea and lipodystrophy.

Amprenavir

APV is contraindicated in children younger than 4 years old, pregnant women, or patients with hepatic or renal failure because of the large amount of propylene glycol content of the oral solution. Patients should be switched from oral solution to liquid-filled capsules as soon as they are able to take the capsule formulation, to reduce exposure to the propylene glycol content of the oral solution. Because both the capsule and oral solution forms of APV contain vitamin E, patients receiving either form of APV formulation should not take vitamin E supplements. APV is a sulfonamide. The potential for cross-sensitivity between other sulfonamides and APV is unknown. APV should be used with caution in patients with a known sulfonamide allergy. Adverse events with this drug include gastrointestinal intolerance (nausea, vomiting, diarrhea), life-threatening rash (including Stevens-Johnson syndrome), and lipodystrophy.

INTEGRASE INHIBITORS

Joining of the viral genome and the host's chromosomal DNA is the hallmark of a retroviral infection. Integrase, the enzyme that catalyzes this step of HIV life cycle, is a promising therapeutic target. On binding to specific sequences in viral DNA, HIV-1 integrase catalyzes two consecutive steps during integration: 3′-processing, endonucleolytic cleavage of the 3′-ends of the viral cDNA and strand transfer, ligation of the viral 3′-OH cDNA ends to the 5v-DNA phosphate of an acceptor DNA (host chromosome).[169] Several compounds have been developed to block integration at different steps, and some are being assessed in phase II and phase III human clinical trials. The most advanced compounds belong to the class known as strand-transfer inhibitors (STIs), namely diketo aryl (DKA) and DKA-like inhibitors. Raltegravir (MK-0518) is a first-in-its-class oral integrase inhibitor (INI). It is the first INI approved for use in the United States (October 2007) in combination with other anti-HIV agents in HIV-infected adults. In a phase III clinical trial, 16 weeks or longer of treatment in addition to an optimized treatment regimen resulted in a significant decrease in HIV RNA PCR and improvement in CD4 counts. Even more striking, 61 percent of patients taking MK-0518 alone experienced reduced viral load versus 5 percent of patients taking placebo alone. $CD4^+$ counts increased two to three times more in patients taking MK-0518 than in those receiving placebo. MK-0518 is available in tablet form (400 mg) and may be a promising option for adolescents and children in the future. The treatment-related adverse effects most commonly reported are diarrhea, nausea, fatigue, headache, and itching. Another potent STI, GS-9137, is being investigated in phase II trials in treatment-naïve and treatment-experienced adult patients with HIV infection.[131]

SUPPORTIVE TREATMENT

Preventive measures applicable to immunodeficient individuals should be applied to the HIV-infected child. Examples include measles, influenza, and varicella prophylaxis after exposure. Additionally, routine childhood health care preventive measures, including adequate nutritional support, age and developmentally appropriate stimulation, dental and skin hygiene, and immunizations, should be offered to children with HIV infection or AIDS. Table 204–15 outlines suggested immunizations for HIV-infected children. The live viral measles-mumps-rubella vaccines are now recommended for all HIV-infected children, regardless of symptoms. Varicella vaccine is recommended for HIV-infected children without significant immune compromise. A second dose of varicella vaccine should be administered to children at age 4 to 6 years of age, following the first dose given at 12 to 15 months. Immunization of healthy household infants with the varicella vaccine also is recommended. Influenza vaccine should be administered yearly starting at age 6 months. Patients with HIV infection are at increased risk for acquiring meningococcal disease. Meningococcal conjugate vaccine (MCV4) is preferred in patients aged 11 to 55 years and now is approved for use in 2- to 10-year-old children.[38]

The newer quadrivalent human papillomavirus (HPV) vaccine, Gardasil, is a vaccine consisting of non-infectious HPV-like particles. It is now recommended in a three-dose schedule for girls aged 11 to 12 years, although it may be administered in girls as young as 9 years.[36]

IMMUNE-BASED THERAPIES

The cardinal immune manifestation of HIV infection is the development of progressive $CD4^+$ lymphopenia with resultant

TABLE 204–15 Suggested Immunizations for Children with Suspected or Confirmed Human Immunodeficiency Virus Infection*

TOPV	No
IPV	Yes
DTaP/TdaP	Yes
MMR	Yes
HAV	Yes
HBV	Yes
Hib	Yes
Pneumococcal	Yes
Influenza	Yes
Meningococcal	Yes
HPV	Yes

**Authors' recommendations compiled from guidelines suggested by the Centers for Disease Control and Prevention.*[38,39,41]

DTaP/TdaP, diphtheria-tetanus-acellular pertussis; HAV, hepatitis A; HBV, hepatitis B virus; Hib, HPV, human papilloma virus; Haemophilus influenzae *type b; IPV, inactivated poliovirus vaccine; MMR, measles-mumps-rubella; TOPV, trivalent oral poliovirus vaccine.*

clinical disease associated with severe immunodeficiency. Therefore, replacement of the $CD4^+$ T-lymphocyte pool is a logical approach for immune-based therapies. Immune modulators have been assessed as single therapeutic agents or in combination regimens in adult clinical trials. Using IL-2 infusions for 5-day cycles every 8 weeks, in addition to ART medications, Kovacs and associates[126] documented sustained elevations in $CD4^+$ T-cell counts without increase in plasma HIV RNA levels in adult patients with HIV infection and $CD4^+$ T-cell counts of 200 cells/mm^3 or higher. The same investigators also demonstrated that intermittent IL-2 therapy preferentially increased proliferation of $CD4^+$ naïve and memory T-cell populations in HIV-infected patients.[125] Katlama and associates[114] reported sustained increases in $CD4^+$ T-cell counts in HAART-treated patients with persistent $CD4^+$ T-cell counts less than 200 cells/mm^3 using subcutaneous IL-2 for 5 days every 6 weeks at doses of 4.5 million IU twice daily.

IFN-α has been documented to produce in vitro antiretroviral activity. IFN-α is presumed to inhibit HIV replication by diminishing the assembly and release of mature virus particles from infected cell surfaces.[168] A significant antiretroviral effect of IFN-α in resting $CD4^+$ T cells also has been documented.[46] INF-α initially was studied as a therapeutic agent for Kaposi sarcoma with good response rates, but significant side effects limit its use in HIV infection. The utility of immune-based therapies in pediatric or adolescent HIV infection may include their use in treatment of concomitant chronic HBV infection or ZDV-resistant, HIV-associated immune thrombocytopenia.[143]

VACCINES

Goals for the development of an HIV vaccine include prevention of persistent HIV infection, prevention of severe clinical disease (AIDS), and therapeutic immunization to control HIV infection. The first candidate preventive vaccines tested in human trials in late 1980s were based on the HIV-1 envelope (Env) glycoproteins gp120 or gp160, an approach aiming to induce neutralizing antibodies. The major limitations of this approach were lack of neutralizing antibody activity against a diversity of primary patient isolates of HIV-1 because of genetic variability and structural complexity of the HIV-1 envelope glycoproteins and absence of envelope-specific cytotoxic T-lymphocyte (CTL) responses.[91,116,144] Because of the growing recognition of the limitations of these traditional vaccine strategies, much effort currently is being focused on the development and assessment of two novel strategies for vaccination: live vector-based approaches and plasmid DNA immunogens.

The live vectors studied most extensively are poxviruses. A study performed in HIV-uninfected, low-risk human volunteers showed that recombinant canarypox vaccine carrying Env, Gag, Pol, and Nef epitopes elicited low titer HIV-1–specific antibody responses in as many as 70 percent of vaccine recipients. Moreover, HIV-1–specific CTL responses were observed to a broad range of epitopes up to 10 months following the last canarypox immunization.[72] A phase I human clinical trial with modified vaccinia virus Ankara (MVA) is ongoing. Studies suggest that replication-incompetent adenovirus vectors (Adenovirus serotype 5; Ad5) are very effective in eliciting HIV-1-specific CTL responses in small laboratory animals and monkeys.[189] The major limitation of this approach is that most humans have preexisting immunity to the Ad5 vector from natural exposure. To overcome this problem, strategies exploring vaccine vectors from rare human Ad serotypes are being developed.[10] A consecutive immunization strategy, known as "prime-boost," involving priming with DNA and boosting with a live recombinant vector, is being explored. A study that was performed in rhesus macaques showed that a DNA priming with a single recombinant MVA booster containing multiple immunodeficiency virus proteins elicited highly effective memory immune response on mucosal challenge with a chimera of simian and HIVs (SHIV-89.6P).[4] However, this approach did not prevent the macaques from acquiring SHIV infection. A study of an adenovirus vector-141V T-cell epitope vaccine in 3000 HIV at-risk subjects was halted in 2007 because the vaccine recipients developed as many HIV infections as did the placebo group, bringing into question the feasibility of this approach.[52]

Numerous HIV vaccine trials have been performed in HIV-exposed and HIV-infected children. The vaccines were safe, but the immunogenicity was poor compared with the results in adult studies.[128,179]

PROGNOSIS

Since the mid-1990s, mortality and morbidity rates for children and adolescents with HIV infection have declined dramatically in developed nations. Effective efforts directed toward early diagnosis have allowed for earlier administration of primary viral therapy to prevent HIV transmission and prompt treatment and prevention of opportunistic infections. In the United States and developed nations, opportunistic disease and death still affect the youngest infants, and public health strategies to slow these continued outcomes are ongoing. Pediatric research in developed nations should continue to address therapeutic interventions that allow for care of now chronically infected children and adolescents who are surviving and striving to contribute to their world. In addition, careful follow-up of HIV-exposed and neonatal antiviral-exposed individuals should be a priority effort in developed nations in which mother-to-child prevention regimens are established.

For developing nations, resources and research to stem the continued tide of HIV infection are of paramount importance. Efforts must be made to reduce global perinatal transmission rates further and to define HIV detection methods that allow earliest intervention in affected children. Effective vaccine development clearly would have a significant and worldwide impact on adults and children exposed to HIV.

Research and prevention initiatives must now be coupled with socioeconomic and political efforts to find the will to sustain a global impact on HIV transmission and treatment. Duplication of the achieved successes in preventing perinatal transmission in industrialized countries is needed desperately in sub-Saharan Africa, East Asia, and Central America, where competing medical needs limit available local funding for prevention of HIV transmission and for HIV treatment. When viewed from this perspective, placing the highest priority on global confrontation of this epidemic that threatens the future of our children is imperative.

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SUBSECTION 13

Prion-Related Diseases

CHAPTER 205

Transmissible Spongiform Encephalopathies (Creutzfeldt-Jakob Disease, Gerstmann-Sträussler-Scheinker Disease, Kuru, Fatal Familial Insomnia, New Variant Creutzfeldt-Jakob Disease, Sporadic Fatal Insomnia)

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The group of neurodegenerative diseases classified as transmissible spongiform encephalopathies (TSE) represents a subset within a group of diseases that also have been termed infectious amyloidoses or cerebral proteopathies.[185,231] These diseases are characterized by progressive cortical dysfunction linked to the accumulation of insoluble, proteinaceous aggregates in the central nervous system (CNS) (Table 205–1). The protein aggregates characteristic of these diseases are composed of polymeric fibrils of a host-encoded protein deposited in several different regions of the CNS and within other organ systems, particularly in lymphoid organs. Amyloid may be present in TSE, and its role in the pathogenesis of these diseases is uncertain. However, the symptoms associated with the spongiform encephalopathies are thought to arise from cellular and end-organ dysfunction associated with the abnormal deposition of an isoform of a normal cellular protein, PrP^c, encoded by the *PRNP* gene.[36,71,185] The physiologic function of PrP^c remains incompletely defined, and no null alleles of the *PRNP* gene have been identified, raising the possibility that some of the symptoms associated with these diseases are caused by a loss of normal PrP^c function.[168] The TSE share several clinical features with other cerebral amyloidoses, and the insoluble protein aggregates exhibit biophysical properties similar to plaques found in Alzheimer disease. In contrast to these diseases, the TSE can by definition be transmitted by parenteral inoculation or by other less efficient routes, such as ingestion of contaminated tissue from the nervous system.

TABLE 205–1 Naturally Occurring Transmissible Spongiform Encephalopathies

Host	Disease
Human	Kuru
	Creutzfeldt-Jakob disease
	Sporadic
	Familial
	Infectious
	Variant Creutzfeldt-Jakob disease
	Gerstmann-Sträussler-Scheinker syndrome
	Fatal familial insomnia, sporadic fatal insomnia
Sheep, goats	Scrapie
Mink	Transmissible mink encephalopathy
Deer, elk	Chronic wasting disease
Cattle	Bovine spongiform encephalopathy, bovine amyloidotic spongiform encephalopathy
Cats	Feline spongiform encephalopathy

TSE were diseases recognized only in domestic livestock in northern Europe until late in the 20th century. Scrapie, a disease of sheep, was described first in the mid-1700s.[36] Farmers realized early on that the agent responsible for scrapie is communicable and instituted control measures that included isolation and destruction of affected animals and herds.[36] Recent evidence has suggested that the scrapie agent can persist in contaminated soil for at least 16 years, a finding that explains the reported recurrences of this disease in flocks that had been culled decades earlier.[96] Although farmers were aware of the communicability of scrapie between herds of sheep and had documented its spread among sheep, its transmissibility was not formally demonstrated until 1936.[80] Shortly thereafter, scrapie developed in a flock of sheep that were inoculated with a vaccine for louping ill prepared from CNS tissue derived from sheep that had been exposed to scrapie.[36] Together, these reports provided definitive evidence of the transmissible nature of scrapie. The term *slow virus disease* was

initially coined by veterinarians to describe the natural history of this curious group of diseases in domestic animals that included a prolonged incubation period measured in years and a relentlessly progressive clinical deterioration once symptoms appeared.[206] This term was used to describe this group of diseases until the 1980s, when investigators began to consider that although the agent responsible for the spongiform changes in scrapie-infected mice is transmissible, it cannot be classified as a conventional virus. A decade earlier, the observational and laboratory studies of kuru, a progressive cerebellar ataxia and dementia that occurred predominantly in adolescent and young adults of the Fore tribe in Papua New Guinea, provided an important piece of the puzzle of the etiology of this group of diseases.[3,100] The natural history of kuru was described initially by Vincent Zigas and Carleton Gajdusek and was noted to be associated with the ritual cannibalism practiced by this tribe. In 1959, an astute veterinary neuropathologist, William Hadlow, observed a striking similarity of the spongiform changes in the brains of patients with kuru to the brains of sheep with scrapie and suggested that these two diseases could be caused by similar mechanisms, possibly a transmissible agent.[109] Considering the possibility that kuru is caused by a transmissible agent, Gajdusek and coworkers[92-94] eventually demonstrated that the clinical and histopathologic findings of kuru can be transmitted to chimpanzees and other nonhuman primates by inoculation of brain tissue from patients with kuru. Interestingly, Gajdusek and coworkers initially thought that the experiment was a failure because the animals did not become symptomatic until nearly 2 years (18 to 21 months) after intracranial inoculation was performed, and later reports from this same laboratory indicated that nearly 20 years elapsed between the inoculation and the development of disease in some chimpanzees.[14,36] Subsequent studies by other investigators have demonstrated that brain homogenates from patients with Creutzfeldt-Jakob disease (CJD), Gerstmann-Sträussler-Scheinker (GSS) disease, fatal familial insomnia (FFI), and most recently variant CJD (vCJD) can transmit spongiform changes to nonhuman primates.[25,183] More recently, a variant of FFI, sporadic fatal insomnia, has been identified and added to this group of transmissible neurodegenerative diseases.[165,177] The hypothesis that transmissible neurodegenerative diseases are caused by a protein was suggested first by Griffith[108] in 1967 and later more extensively developed by Prusiner[183] and coworkers. The claim that the etiologic agent of scrapie is an isoform of a normal host protein, PrP^c (alternatively referred to as prion protein), that can transmit disease in the absence of nucleic acid was met initially with great skepticism and even today continues to evoke debate.[63,64,160,183] During the ensuing decade, the hypothesis that a protein can promote the formation of a polymeric fibrillary, insoluble plaque without the requirement of nucleic acid encoding a protein of an infectious agent has been supported by studies from several laboratories. This hypothesis represented a paradigm shift in biology, and Stanley Prusiner was awarded a Nobel Prize in 1997 for his groundbreaking studies of prion diseases. Interestingly, approximately 20 years earlier, Gajdusek also was awarded a Nobel Prize for his description of the transmissible nature of kuru and similar diseases of the CNS.

The most recent chapter in the TSE saga is the appearance of mad cow disease (bovine spongiform encephalopathy [BSE]) in Great Britain in the mid-1980s, and by the late 1990s, nearly 200,000 diseased animals had been destroyed.[48] Mad cow disease has been discovered in cattle in several countries within Europe and most recently in the United States. Of greatest concern has been the transmission of BSE to more than 100 individuals in Great Britain and at least three cases in France.[48] BSE has been reported in the United States, and a significant increase in the incidence of TSE of elk and deer (chronic wasting disease) has been reported in wild game farms in the western United States as well as in wild deer herds in the midwestern United States.[166,232] Because of the concern that large numbers of human cases could eventually develop secondary to contamination of food products and pharmaceutical reagents with BSE or through contact with contaminated tissue from deer or elk, substantial interest has developed in the study of TSE.

EPIDEMIOLOGY

The natural history of human TSE usually is described in the context of the epidemiology of CJD. Three different modes of acquisition of disease have been described: (1) sporadic, (2) inherited mutations in the cellular gene encoding PrP^c (genetic), and (3) acquired or infectious following exogenous exposure to prion-contaminated material, such as after neurologic procedures (infectious) (see Table 205–1). Little is known about genetic or environmental factors that contribute to the development of sporadic forms of these diseases, although numerous epidemiologic studies have claimed associations ranging from ingestion of mutton to exposure to blood products after surgery.[4,40,78,164] Studies in cases of CJD have revealed numerous genetic mutations in the cellular gene encoding the normal cellular prion protein, PrP^c, associated with the development of disease, and they have been defined as genetic causes of CJD and other TSE (see following section). Polymorphism in the methionine at position 129 in the coding sequence of PrP^c is the genetic polymorphism most commonly recognized as being associated with sporadic CJD.[176] Homozygosity at methionine 129 of PrP^c is seen in approximately 64 to 81 percent of patients with sporadic CJD yet is present in only approximately 39 percent of the general population and is thought to predispose an individual to both iatrogenic and sporadic CJD.[2,176,240] Interestingly, valine homozygosity at this position was associated more frequently with cases of CJD with onset in those younger than 40 years.[2] Furthermore, a retrospective analysis of a small number of patients with kuru revealed that all were homozygous for methionine at position 129 and all had more rapidly progressive disease with a shorter incubation period than did other patients with kuru from this cohort.[150] Fore women surviving exposure to contaminated material during the ritual cannibalistic feasts were shown to be more likely to be heterozygous at methionine 129 (76%) than were similarly aged Fore women who had not been exposed to the cannibalistic feasts.[168] This result suggested that heterozygosity at methionine 129 provided a survival advantage. The codon at position 129 also appears to modulate the clinical course of inherited TSE such as FFI, and patients homozygous for methionine at position 129 have a more rapid clinical course than do patients heterozygous at this codon.[171] Polymorphisms in the PrP^c, including those that occur at position 129, have not been associated with the development of vCJD after exposure to BSE-contaminated material; in fact, all human cases in Great Britain have been homozygous for methionine at position 129.[130] Heterozygosity at methionine 129 is thought to confer resistance by inhibiting PrP^c protein:protein interactions.[176] Interestingly, sequence analysis of the *PRNP* gene from populations throughout the world suggested that the observed increased frequency of heterozygosity at position 129 could be explained by widespread cannibalism among primitive humans and selection of methionine 129 heterozygotes secondary to increased disease penetrance associated with exposure of susceptible methionine 129 homozygotes to prion-contaminated tissue.[168] This conclusion has been challenged by other investigators.[209]

Sporadic CJD is the most common TSE in humans and accounts for approximately 85 percent of all cases of CJD.[40] It occurs worldwide at a rate of approximately $1/10^6$ population.[40] CJD is a disease of late middle age, with a mean age at onset of 55 to 65 years. Cases of CJD range from 20 to 84 years, with the youngest cases being described in patients 16 to 18 years old.[39,88,170]

Little evidence exists for case-to-case transmission of CJD, with the obvious exception of cases resulting from iatrogenic transmission of CJD.[61] Vertical transmission of CJD has not been reported in studies of human CJD. Anecdotal reports of occurrence of CJD in the spouses of individuals with CJD raise the possibility of horizontal transmission.[42] Alternatively, disease in spouses could result from shared environmental exposures. Sporadic cases of non-CJD TSE previously thought to be caused solely by genetic mutations have been reported, thus extending the spectrum of TSE in humans.[151,165] With the exception of cases that can be linked to an inherited genetic mutation, no epidemics of CJD have been reported, an observation that led British epidemiologists to suspect that a link exists between what was initially thought to be an increased frequency of CJD in young adults and the outbreak of BSE in the British Isles.[6,13,79,103,226,238]

Genetic mutations in the cellular prion gene, *PRNP*, can result in a variety of inherited TSE and are likely to account for the reported outbreaks of TSE in isolated populations. Because of the relatively late onset of disease even in patients with well-characterized mutations in *PRNP*, the possibility that TSE are inherited diseases of the CNS was not considered until more recent studies of the host cell origin of prions and the discovery that a cellular gene encoded the pathogenic protein associated with TSE. Estimates from various studies suggest that between 5 and 15 percent of cases of CJD may arise from mutations in the cellular *PRNP* gene.[40] Genetic linkage studies have demonstrated that TSE are inherited as an autosomal dominant trait, an observation consistent with several studies in families with FFI and GSS.[21,126,169] In addition, defined mutations in the *PRNP* gene have been shown to account for the increased rate of TSE in either geographically or ethnically isolated populations, such as Libyan-born Jews, an ethnic group with an incidence of TSE nearly 40 times greater than that observed in other populations.[61,82,102,127,128,134,211] A large number (>20) of mutations in *PRNP* have been described and appeared to be scattered throughout the gene. Reported mutations include both point mutations in the coding sequence changes, resulting in amino acid substitutions, and octapeptide insertions.[55,61,101,128,180,188] Because mutations in *PRNP* by definition are not present in cases of sporadic CJD, mutations of this cellular gene are not thought to play a direct role in the development of CJD in these individuals. Thus, the mode of acquisition of the TSE agent in these individuals has not been defined. In sporadic cases of CJD, the proposal has been made that spontaneous generation of the TSE agent could be occurring extremely infrequently and result in CJD. However, when scrapie was eliminated in sheep in New Zealand and Australia by culling diseased herds, spontaneous development of scrapie has not been described in these herds, suggesting that spontaneous development of the agent of TSE leading to scrapie in sheep is almost nonexistent.[64] In most cases, disease secondary to a genetic mutation in *PRNP* develops in patients of middle age, suggesting that although these diseases exhibit an autosomal dominance inheritance, the genetic background of the host contributes significantly to the observed phenotypes.

Although farmers and neuropathologists were well aware that scrapie is readily transmissible, the possibility that human TSE also are transmissible was not demonstrated until Gajdusek and coworkers defined the natural history of kuru in the Fore tribe in Papua New Guinea.[3] Kuru was seen most commonly in adolescent and young adults of both sexes but only in older women. The disease was common, with an incidence of 1 percent in this tribe, and was a significant cause of death in this population.[3] Gajdusek carried out a detailed observational study of the Fore people, including their ritualistic cannibalism of the dead. This ritual included homogenization of the brains of dead relatives in bamboo cylinders with use of crude hand tools. The brain was reportedly ingested as well as smeared over the body by relatives and other members of the tribe. During most of their childhood, Fore children remained with their mothers and other women of the village, including the time spent preparing the brains of dead relatives for ritual cannibalism. It is during this time that the exposure to the agent responsible for kuru was thought to occur, either by ingestion of the contaminated brain material or through skin abrasions that were present secondary to the nearly universal scabies infestation. Gajdusek and coworkers hypothesized that kuru is caused by an infectious agent and injected homogenates prepared from the brain of a deceased kuru patient into nonhuman primates; following an incubation period of more than 2 years, a disease similar to kuru was observed in the experimental primates.[14] After the transmissibility of kuru was demonstrated in experimental animals, several other TSE were shown to be transmissible in nonhuman primates and other experimental animals.[14,44,74,217,219] Subsequent studies demonstrated that kuru could be transmitted to nonhuman primates by oral ingestion of contaminated tissue.[98] Although the origin of the TSE agent responsible for kuru in the Fore tribe is unknown, the most plausible explanation is that a spontaneous mutation in the cellular PrP^c gene resulted in a case of TSE in the Fore tribe that then was introduced and propagated in the population by cannibalism. After ritual cannibalism was halted by the government of these districts in Papua New Guinea, new cases of the disease disappeared, and the last case of kuru in an individual younger than 30 years was reported in 1985.[3] Cases of kuru have developed some 40 to 50 years after exposure to infectious material, illustrating the prolonged incubation period of these diseases.[76,142]

Before the 1980s, cases of TSE occurring in children and adolescents, with the exception of kuru, were either extremely rare or not recognized. In the late 1980s, cases of TSE resembling CJD were reported in young adults who as young children had received injections of human growth hormone prepared from cadaveric pituitary glands.[35,44,46] The thought was that the practice of pooling pituitary glands from a large number of cadavers increased the risk of contamination of the preparation with prions from subclinical cases of TSE. More than 100 cases of TSE occurring after patients were injected with contaminated growth hormone preparations have been reported.[61] Whether new cases of TSE will develop in adults given cadaveric growth hormone before the mid-1980s remains uncertain, but with a mean incubation period of 12 years in documented cases, it is unlikely that significant numbers of new cases will be reported.[37,46] Other sources of TSE include dura grafts, corneal transplants, contaminated stereotaxic neurosurgical equipment, and, more recently, blood transfusions.[7,37,41,83,107,110,123,174,180] An alarming case of TSE occurred in a chimpanzee after the use of a stereotaxic electrode that 2 years previously had been used in a procedure involving a patient with CJD. Because this electrode had been decontaminated numerous times with conventional sterilization methods before its use in this experimental animal, this case illustrated the potential for the occurrence of iatrogenic transmission of TSE to humans undergoing neurosurgical procedures.[99] Two confirmed and two probable cases of vCJD occurring after receipt of blood transfusions have been described in Great Britain.[115] Studies of experimental models of TSE, primarily scrapie in mice, have shown that the scrapie agent initially amplifies in titer in spleen and regional lymph nodes and enters the CNS by a blood-borne route or by infection of the peripheral nervous system.[1,136,138,140,148,159] In addition, studies in mice have demonstrated that brain tissue from animals injected with hamster scrapie can harbor the infectious agent for a prolonged time without evidence of disease.[118,191] Even during a period of inactive persistence and replication, the scrapie prion could be detected, suggesting that transmission of prion disease by contaminated blood products or surgical instruments may occur even if screening assays for the detection of prions are mandated.[189] To further cloud the issue, other investigators have postulated that less

virulent strains of prions can attenuate the virulence of other strains of prions, which then can be transmitted readily during asymptomatic periods.[163,228] After transmission to a secondary host has occurred, the phenotype of the more virulent strain could be expressed. These findings are particularly worrisome because of the possibility that food products from cattle with subclinical BSE could continue to be consumed. Similarly, the possibility exists that the low incidence of vCJD in Great Britain, in spite of what most authorities consider was a much larger exposure to BSE before eradication of BSE-containing herds, could be explained by persistence of infectious agents in genetically resistant individuals or possibly be secondary to persistence in non-CNS organ systems.[28,118] Previously, these studies raised the possibility that TSE could be acquired by transfusion of blood and blood products obtained from infected hosts.[149]

Considerable controversy existed about the importance of transmissibility of TSE by blood and blood products. Investigators argued that the risk is exceedingly small on the basis of experimental studies in rodents and epidemiologic studies involving patients receiving quantities of blood products, such as hemophiliacs.[1,32,34,85,223,225,239] However, BSE has been transmitted to sheep by blood transfusions, and buffy coat preparations have been shown to transmit TSE to experimental primates.[24,125] The U.S. Food and Drug Administration has recommended a very conservative policy for blood and blood products because of these theoretical concerns, and more recently, this policy has been reinforced by the reports of blood transfusion–acquired vCJD.[114,152,244] In addition to the exclusion of donors with symptoms consistent with TSE, individuals who have lived or visited Great Britain and other countries with BSE for a cumulative period of 3 months during the interval of 1980 to 1996 are also excluded as donors (*http://www.redcross.org/services/biomed/0,1082,0_557_,00.html)*. With the possible exception of kuru, little evidence was available previously to suggest that TSE such as CJD could be acquired by ingestion of contaminated tissue.[40] This claim was challenged in the early 1990s by the reports of several young adults with CJD in Great Britain.[79,238] Inspection of the rates of CJD in England revealed that the incidence of CJD was approximately 15-fold higher than earlier, based on surveillance studies from previous decades.[31] In the years preceding the startling increase in rate of CJD, the cattle industry in Great Britain was being devastated by the spreading epidemic of BSE, a disease ultimately shown to be a TSE.[112,208,236] A similar disease had been noted in domestic cats as well as in animals housed in zoos, where they had been fed British beef and beef byproducts.[25,75,208] The first case of human disease thought to be associated with BSE was termed vCJD and was described in 1995, about 10 years after the first cases of BSE were reported in cows, although more careful analysis suggested that the first cases of BSE probably occurred in the 1970s.[208,238] During the ensuing years, investigators have closed the circle of evidence, showing that vCJD represents the transmission of BSE to humans, presumably by ingestion of contaminated beef or byproducts from BSE-infected cattle.[72,120,207,210] These byproducts include many common household and medical products, such as gelatin in food products and in pharmaceutical capsules, bouillon cubes used for food preparation, and a wide variety of foodstuffs containing beef.

Although the origin of BSE is far from settled, most investigators concur that changes in the rendering of beef and sheep carcasses to remove fat for the preparation of meat and bone meal for use as a protein supplement in animal feeds led to the epidemic. Before the late 1970s, beef carcasses were heated and extracted with hydrocarbons such as chloroform to delipidate the homogenate.[33,81,218,236,238] Omitting the hydrocarbon-extraction step apparently decreased inactivation of bovine prions, thus allowing introduction of this agent into the food products of British cattle. The epidemic began slowly in cattle, with the first case documented in 1986; by the mid-1990s, more than 3500 new cases of BSE were being reported each year. As a result of the BSE epidemic, 200,000 diseased cattle died as a result of BSE and nearly 4.5 million cattle were slaughtered preemptively, at a cost of approximately 4 billion English pounds, to curtail the epidemic.[5,48,208,243] BSE has been documented in U.S. herds, resulting in a ban of exportation of beef to several economically important markets.[195] As noted before, transmission to humans was suggested first in 1995 after the findings of CJD in three young adults were reported; and by 1996, additional cases of CJD in young adults were thought to be secondary to transmission from exposure to beef from BSE-infected cows.[72] Of the initial 20 human cases, only one individual was older than 40 years, and the mean age of human cases was 28 years.[6,184,226,228,245] Additional evidence from in vitro studies of the protein agents responsible for BSE and vCJD and from animal inoculation indicated that vCJD represented a human infection with the agent responsible for BSE.[31,75,119,147] Because the BSE epidemic was recognized first in 1985, researchers argued that the incubation period is approximately 10 years. On the basis of studies in kuru and experimental animal models of TSE, this estimate was determined to be dependent on exposure, genetic susceptibility, and as yet other unrecognized risk factors.[60,153] For this and other reasons, mathematical projections of the extent of the human epidemic have been based on theoretical estimates of exposure rates, possible dose-dependent incubation periods, and as yet undefined host genes that may alter susceptibility to TSE. Even though most of these variables cannot be quantified, arguments have been put forth that the epidemic in humans has peaked and the 120 or so cases of vCJD that have resulted from contaminated beef will be the extent of human disease associated with BSE.[97,228] In contrast, other models of the current data suggest that as many as 100,000 people may be affected. Perhaps the most compelling argument against such a large number of human cases has been the slowly evolving epidemic of vCJD in humans, with the number of new cases declining, although several investigators have contested this hypothesis.[69,97,228,237]

PATHOLOGY AND PATHOGENESIS

Although the histopathologic features of TSE have been described well, the pathogenesis of these diseases remains unknown and the nature of the etiologic agent continues to evoke controversy. The initial claim that TSE resulted from a transmissible protein was met with considerable skepticism both because a paradigm of biology was challenged and because key elements of the supporting scientific data were incomplete. The vast majority of experimental studies have been performed with the agent responsible for scrapie in sheep, which had been adapted for growth in mice and other small animals. Early studies that argued that the scrapie agent did not contain a nucleic acid were based on studies with ultraviolet radiation inactivation of the agent. An important note is that these studies did not exclude the possibility that the scrapie agent contained nucleic acid but instead argued that if it contained nucleic acids, the coding sequence was so limited that it could not encode any replicative functions.[16,17,183] In fact, later calculations based on inactivation by gamma rays suggest that the scrapie agent could contain between 2 and 4 kilobase pairs of DNA.[197] Other experiments demonstrated that the infectivity of the scrapie agent could be eliminated or significantly reduced by treatment with agents that denatured proteins, such as guanidine hydrochloride, sodium hypochlorite (bleach), and proteases.[183] Together, these data led several groups of investigators to postulate that the agent is an infectious protein.[23,108,187] Prusiner and coworkers[187] isolated an infectious fraction from a scrapie-infected hamster brain homogenate and obtained partial amino acid sequence. This fundamental finding led to the identification of

the prion (PrP^c) and eventually the finding that a cellular gene, *PRNP*, present on chromosome 20, encoded the transmissible agent responsible for the development of scrapie in mice and hamsters.[12,65] Not all investigators have been convinced that the scrapie agent is an infectious protein, and recently published data have been cited as evidence that it is a small virus.[160,162] Once the protein encoded by the *PRNP* gene had been identified and definitively linked to scrapie and other TSE, many investigators thought that the pathogenesis of these diseases would be elucidated quickly. However, the pathogenesis of these diseases remains uncertain, perhaps because of the inability to assign a function to the normal protein product, PrP^c. The conformation of the normal cellular form of PrP^c is primarily alpha-helical and is susceptible to protease digestion. Various functions have been proposed for the normal nonpathogenic isoform of the cellular PrP^c, yet transgenic mice lacking the gene encoding PrP^c have a normal life span and exhibit only minor variations in normal sleep patterns.[53,54,77,186] Most interestingly, these mice are completely resistant to prion disease regardless of the source of exposure, a finding that is consistent with the hypothesis that PrP^c is necessary for the disease process associated with scrapie and other TSE.[1,53] In contrast to the normal cellular form of PrP^c that is composed of four alpha-helical domains, the pathogenic form of PrP^{sc} (scrapie prion protein; also termed PrP^{res}) exists in an extended beta sheet, is insoluble, and is partially resistant to protease digestion (PrP protease resistant or PrP^{res}). The partial resistance of PrP^{res} to proteinase K digestion is a characteristic that allows pathogenic prions to be detected in tissue specimens and has been widely used to identify products of the conversion reaction of PrP^c to PrP^{res}.[167,201] The conversion of PrP^c to PrP^{res} represents a central paradigm of the prion hypothesis and is thought to occur either as the result of a process similar to the nucleation of crystal formation by seeding the normal cellular pool of PrP^c with PrP^{res} or, alternatively, as the result of a template-directed misfolding of a normal cellular protein. Thus, PrP^{res} can be viewed as a transmissible, infectious agent that catalyzes the conversion of the normal cellular protein to a form associated with protein deposition in the CNS, cell death, and eventually clinical disease in the host.

The normal cellular protein, PrP^c, is a small membrane glycoprotein that is covalently linked to cellular membranes, including the plasma membrane, by a glycosylphosphoinositol (GPI) linkage.[212-214] This cell surface form can be released by treating cellular membranes with phospholipases.[214] In addition, a secreted form of the protein also is expressed. Genetic mutations in the cellular PrP^c gene appear to predispose the protein to assume other topologies, including a transmembrane form.[111,222] Membrane-associated forms are postulated to play an important role in the neurodegeneration associated with the accumulation of the misfolded forms of PrP^c, and secreted forms can accelerate disease in a genetically susceptible animal.[111] Chesebro and coworkers[66] demonstrated that transgenic mice encoding a GPI-anchorless form of PrP^c could generate an abnormal protease-resistant PrP^{res} (see later) that could be transmitted to other animals and induce formation of amyloid plaques but failed to cause clinical spongiform encephalopathy (scrapie equivalent in mice) in experimental animals. Interestingly, when the anchorless form was co-expressed with the wild-type form of PrP^c, accelerated disease was seen.[66] Thus, it appears that a cell surface–linked PrP^c is required for induction of disease yet that amyloid-like deposits can be formed from the secreted form of PrP^c and disease can be induced when both secreted and wild-type PrP^c are expressed.[66] Consistent with this observation has been the description of a transmissible PrP^c amyloid disease without spongiform encephalopathy after inoculation of tissue containing amyloid deposits from a patient with GSS without CNS spongiform degeneration.[182] Together, these studies argue that PrP^c misfolding, protease resistance, and amyloid formation are sufficient to confer transmissibility to the PrP^{res} but that these characteristics in themselves may not be directly responsible for transmissible disease. The challenge to existing paradigms of molecular biology and genetics, including the self-replication of a protein, were quickly noted by investigators in this field. Importantly, several investigators previously demonstrated that distinctive strains of the scrapie agent exist and that these strains induce definable and reproducible phenotypes of disease in experimental animals.[18,27,49,50,137] These well-accepted and reproducible studies strongly argued for the presence of a genetic program in the scrapie agent that was most consistent with a nucleic acid–containing agent. More recently, several studies have suggested that the phenotypic behavior of scrapie strains may be entirely dependent on the pathogenic conformation that the PrP^c assumes when it is exposed to pathogenic forms of PrP^c, which in turn is dependent on its post-translational modifications.[19,58,59,216,228,229,233] Thus, researchers argued that the phenotypes of scrapie strains are entirely dependent on the protein structure of PrP^c and independent of any nucleic acid–encoded genetic trait. A second characteristic of etiologic agents of TSE that was difficult to ascribe to an infectious protein is the species barrier for transmission of prions between species. This phenotypic characteristic was noted when the scrapie agent from sheep was first adapted to mice and subsequently to other species, including hamsters. Other examples of a species barrier include BSE, in which 1000 lethal doses (as defined in cattle) must be given to a mouse to induce disease.[234] Interestingly, the species barrier not only contributes to the absolute resistance of species to infection with prions derived from other species but also can lengthen the mean incubation period in a population. The species barrier often is invoked to explain the rarity of TSE, even in populations repeatedly exposed to TSE in other species (e.g., humans exposed to scrapie-infected sheep), and possibly the relatively low number of human cases of vCJD that develop after exposure to beef contaminated with BSE.[72] A more worrisome aspect of the phenomenon of species barrier is that subclinical infections could result from exposure to BSE and subsequent transmission, leading to secondary cases with clinical symptoms.[118,228] A unifying explanation for the restriction of the transmission of prion disease between species that has been proposed argues that a species restriction is related to the efficiency with which exogenous prions interact with endogenous host-derived PrP^c to form PrP^{res}.[1,58,59,71,124,144,192,216,227] Although considerable gaps in the understanding of the infectious agent responsible for TSE persist and at times have contributed to the controversy that surrounds the infectious protein hypothesis, more recent in vitro studies have shifted the view of the field toward the infectious protein hypothesis. These studies include the findings that protease-resistant prions (PrP^{res}) that appear identical biochemically to PrP^{res} derived from infected brain can be produced in cell-free systems in vitro.[1,58,143] However, until recently, these preparations could not transmit disease to uninfected animals unless they were supplemented with brain tissue derived from diseased animals, presumably because their titer was too low.[116] A more recent study using a system to amplify prion production through sequential folding, sonication, and feeding of new substrate produced titers of in vitro–derived prions that could transmit TSE to susceptible animals and accelerate the development of symptoms in infected animals.[57] The results from this in vitro system have provided compelling evidence for a mechanism of recruitment of cellular proteins by a misfolded protein and subsequent protein deposition as an etiology of diseases classified as TSE.

Although several mechanisms can be envisioned to explain how a misfolded host protein can lead to the recruitment and misfolding of additional copies of the protein, how the deposition of PrP^{res} can lead to disease remains unclear. The loss of normal protein function and the development of disease in humans have several well-studied examples, such as cystic fibrosis.[106,241] These diseases are associated with loss of function of an essential cellular

protein. In contrast, the accumulation of cellular proteins, such as amyloid secondary to overproduction of a normal protein, can lead to disease and usually is associated with deposition and loss of organ function. In the case of TSE, the proposed mechanism for the generation of pathogenic prions appears fundamentally different in that the conformational change from a predominance of alpha-helical structure of PrP^{c} to molecules containing extensive amounts of an extended beta sheet, PrP^{res}, results in the accumulation of insoluble, protease-resistant proteins that can recruit additional normal cellular proteins into this conformation. In vitro cell-free systems and studies from kindred with inherited TSE such as GSS and FFI have shown that exposure to the misfolded prion protein such as PrP^{res} can result in this conversion.[1,58,59,62,91,196] In genetic TSE such as GSS and FFI, the genetic mutation is inherited as an autosomal dominant trait, and interestingly, the PrP^{res} pathogenic form of the prion protein has been shown to be derived from the mutant allele individual heterozygous for this allele.[1,58,62] The energetics required for the conversion in conformation of PrP^{c} to PrP^{res} are thought to be unfavorable and could require a mutant template or perhaps a chaperone protein (protein X).[58,135,143,220] Because the conversion reaction is essentially irreversible, this change in protein conformation results in the accumulation of the insoluble and protease-resistant PrP^{res} protein. Arguments have been put forth that certain conformations of PrP^{c} favor the conversion to pathogenic forms of PrP^{res}, thus providing additional constraints on the expression of disease that could explain the non-PrP^{c} genetic contributions to disease susceptibility and also provide a final common pathway for genetic and infectious forms of the disease.[1,222] Interestingly, several studies have argued that different protease fragments that are generated after PrP^{res} treatment with protease K may be associated with different patterns of disease in the CNS and thus provide additional diversity in disease phenotype attributed to this single, small protein.[178,179,228] Once the pathogenic PrP^{res} accumulates, additional newly synthesized molecules are converted into PrP^{res}, and clinical symptoms develop, depending on the site of cell loss in the CNS. Accumulation of the PrP^{res} isoform probably involves a combination of new synthesis of PrP^{res} and the decreased clearance of the misfolded aggregate. The failure in the clearance of the misfolded pathogenic isoform may ultimately be the major pathway leading to accumulation of PrP^{res} and disease.[1,200] Finally, studies have linked copper binding to the product of the PrP^{c} and oxidation of the prion protein.[1,29,193,204,242] Alternatively, a copper-containing prion complex localized to the synaptic junction could protect normal synapses from oxidants or perhaps play a direct role in copper metabolism or transport.[132,204] Loss of normal prion function by misfolding could then lead to neuronal loss. The importance of these newly described characteristics of PrP^{c} remains incompletely defined at this time.

Although inoculation of a susceptible host with prion-contaminated cadaveric growth hormone or the use of contaminated instruments during neurosurgical procedures clearly can transmit CJD, the route of infection leading to vCJD is only assumed to be through oral ingestion of contaminated beef, based on studies in nonhuman primates.[25,228] As noted earlier, studies have demonstrated that other TSE, including kuru, can be transmitted by oral ingestion of infectious material, but only ingestion of large inoculums and often requiring extended incubation periods.[98] In studies in mice, researchers have shown that 10 g of BSE-infected cow brain can infect and kill most mice.[11] Even though extrapolating from these data a comparable dose required for human infection will be difficult, these findings demonstrate that the BSE agent can be transmitted by oral ingestion.

The pathway leading from ingestion of PrP^{res} tissue to deposition in the CNS has been shown to require local replication in lymphoid tissue, which is thought to be followed by blood-borne dissemination to the nervous system.[1,113,138,140] High titers of infectious prions can be found in the liver and the spleen as well as in lymphoid tissue such as the tonsil and other lymphoid tissue of the oropharynx and gut, suggesting that the titer of the infecting PrP^{res} is amplified in these tissues before spread to the CNS. In vCJD, PrP^{res} can be found in the tonsils at levels as high as 10 percent of the infected brain.[116,230] However, these findings were reported to be restricted to vCJD, reflecting a characteristic of the PrP^{res} of vCJD or possibly secondary to the route of inoculation. Studies in mice have demonstrated that performing splenectomy before inoculating with the scrapie agent can prolong the disease course, and subsequent studies have demonstrated the importance of replication in cells derived from bone marrow in neuroinvasion.[68,133,145] Other studies have demonstrated that expression of PrP^{res} in the peripheral nervous system is sufficient to transmit disease, suggesting that direct hematogenous dissemination to the brain is not necessary for transmission of TSE.[190] Prions are thought to replicate in these tissues before spreading to the CNS, and a study documented the presence of PrP^{res} in the appendix of an individual that was removed 8 months before the onset of TSE.[121] Investigators once thought the B lymphocytes represented a likely candidate for the cell transmitting the PrP^{res} to the CNS; however, studies have provided data inconsistent with this mechanism of transmission.[9,30,73,140,172,173,205] Studies have provided evidence for the role of follicular dendritic cells in lymphoid tissue as sites of prion replication and may direct the prion to the CNS.[133,145] Other investigators have suggested that host cell molecules as yet unidentified may provide the necessary interactions with the prion molecule, which permits their trafficking with migratory myeloid cells and, ultimately, entry into the CNS. Candidate molecules included components of the complement system based on the study of PrP^{res} in transgenic mice lacking specific components of the complement system, perhaps secondary to the expression of Fcγ and complement receptors CD21/CD35 by follicular dendritic cells.[20,141,155]

The pathologic findings of TSE include the hallmark of a triad of histologic findings: (1) neuronal loss, (2) proliferation of reactive astrocytes, and (3) status spongiosis.[15] *Status spongiosis* is a descriptive term of degeneration of neurons and collapse of the cortical cytoarchitecture leading to vacuolation of the neuropil.[15] Amyloid plaques also are observed in some patients, and in some cases PrP^{res} is present in these plaques.[15,52,139] Whether the distribution of spongiform lesions is related to the pathogenesis of these disorders or merely reflects the duration of disease before the onset of clinical symptoms is unknown; however, some TSE, such as FFI, exhibit a preponderance of histopathologic changes in specific locations, such as the thalamus.[15,52,158,177,221] Recent descriptions of the neuropathologic process of vCJD have suggested that distinctive histopathologic changes may be present in the cases thus far studied[228] and include a distinctive plaque containing a central amyloid core and fibrillary periphery with extensive spongiform changes in the immediate periphery of the plaque.[89,130] The surrounding spongiform changes are proximal to the plaque, and the remaining neuropil is relatively intact.[52,130] These findings are unique to vCJD cases and are not seen in sporadic CJD cases from outside Great Britain.[51,131] Histopathologic lesions in the cerebral and cerebellar cortices, basal ganglia, thalamus, and brain stem also have been described.[131] Involvement of the cerebellum appears to be more common in vCJD than in reported cases of sporadic CJD, but this finding may be related to the differences in the age at onset of disease in these two populations, as recent findings from young patients with sporadic CJD have suggested that these patients present with a syndrome more closely resembling vCJD than sporadic CJD in older patients.[22] Last, electron microscopic studies of plaques from vCJD brain tissue have suggested subtle but recognizable differences between plaques obtained from patients with vCJD and CJD.[89]

CLINICAL MANIFESTATIONS

Only the clinical manifestations of sporadic CJD and vCJD are discussed; other TSE, such as GSS and FFI, are diseases that are unlikely to be encountered in pediatrics (Table 205–2). The interested reader is referred to discussions of these diseases in adults.[38,86,95,154,158] Clinical signs and symptoms during the early stages of CJD are subtle and often complex and include both motor and sensory dysfunction. Abnormalities in gait and vision and complaints of headache, dizziness, and paresthesias often are noted.[38,47,175,198] Intellectual dysfunction, including loss of memory, speech abnormalities, anxiety, and depression, also are often presenting complaints of patients with early stages of CJD.[22,38,47] Neurologic abnormalities may include corticospinal tract dysfunction as manifested by hyperreflexia, spasticity, and extensor plantar reflexes. Visual disturbances include visual field abnormalities and, in some cases, cortical blindness. Seizures are not a frequent clinical symptom of patients with CJD. Other less frequent findings include evidence of autonomic system dysfunction and, rarely, lower motor neuron disease.[175,202] Although death secondary to complications associated with the vegetative state usually occurs within 1 year of onset of symptoms, a small percentage of patients may exhibit a prolonged disease course.[47]

The clinical symptoms of vCJD are distinct from those of sporadic CJD, and because this disease has been reported in an adolescent, it could potentially be encountered by pediatricians. The age range for reported cases of vCJD is 16 to 48 years, a distribution clearly outside that of sporadic CJD. Symptoms associated with this TSE have included psychiatric and sensory disturbances, such as dysesthesias and paresthesias with electromyographic evidence of denervation. Psychiatric symptoms included anxiety, depression, anorexia, social withdrawal, and other nonspecific complaints. As the disease progressed, more familiar findings of TSE, including pyramidal tract dysfunction, rigidity, cerebellar dysfunction, and myoclonus, developed. Visual disturbances also were noted late in the disease. The duration of illness has exceeded 1 year in most patients with vCJD and follows complications associated with the vegetative state. As noted before, the clinical presentation of sporadic CJD in young individuals more closely resembles that of vCJD than of sporadic CJD in older individuals.[22]

DIAGNOSIS

The diagnoses of CJD and vCJD require a high index of suspicion and, in the case of vCJD, a history compatible with exposure to contaminated beef or beef byproducts or, possibly, a previous neurosurgical procedure. Routine laboratory findings are nonspecific and not helpful. No consistent reports demonstrate systemic or CNS inflammation; thus, routine laboratory analysis of cerebrospinal fluid often is nondiagnostic. Assays for specific cerebrospinal fluid proteins, such as the 14-3-3 protein, tau, or neuron-specific enolase, may provide helpful laboratory evidence of CJD or vCJD. [104,105,129,146,203,246] Definitive premortem diagnosis requires biopsy tissue, and histopathologic diagnosis can be facilitated by use of PrP^{res}-specific antibodies in both immunocytochemistry and Western blot assays.[8,52,228,230] The use of monoclonal antibodies specific for PrP^{res} has permitted a more thorough understanding of the distribution of prions in CNS and non-CNS tissue in patients with TSE. Hill and coworkers have reported that tonsillar biopsy followed by detection of PrP^{res} by both Western blot and immunocytochemistry is useful for establishing the diagnosis of vCJD disease.[90,117] This approach has been particularly valuable for defining the incidence of vCJD infection in populations such as has been carried out in studies in Great Britain.[90,122]

Imaging of patients with suspected prion disease has been extremely helpful in establishing the diagnosis of these rare diseases. Although computed tomography may reveal a variety of structural abnormalities, none is sufficiently specific to be considered diagnostic. In contrast, several investigators have claimed that magnetic resonance imaging findings of increased T2 signals from the striatum and thalamus are specific for prion diseases,

TABLE 205–2 Human Transmissible Spongiform Encephalopathies

Disease	Acquisition	Clinical Features
Kuru	Ingestion of or percutaneous inoculation with contaminated central nervous system tissue during ritual cannibalism in Fore tribe in New Guinea Last case reported in 1985	Bimodal age distribution with disease in adolescence and in older adults Adolescents presented with ataxia, dementia with prominent cerebellar involvement Rapidly progressive disease
Creutzfeldt-Jakob disease (CJD)	Sporadic cases account for 85% of reported cases of CJD; approximately 15% are secondary to defined mutation in PrP^c, and an unknown number are secondary to infection Well-documented cases after neurosurgical procedures and injection of cadaveric growth hormone	Classic presentation of dementia, sensory abnormalities followed by loss of motor functions Rapid progression of symptoms with death usually occurring <1 year of onset Disease of late middle age except in cases acquired after iatrogenic transmission
Variant Creutzfeldt-Jakob disease (vCJD)	Acquired through exposure to beef or beef byproducts contaminated with bovine spongiform encephalopathy Route of acquisition unknown but presumed to be oral	Presentation similar to CJD but disease progresses more slowly, with death occurring >12 months after symptoms develop Mean age of cases approximately 28 years, with documented cases in adolescents
Gerstmann-Sträussler-Scheinker syndrome (GSS)	Autosomal dominant inheritance with documented point mutations in PrP^c gene	Disease of middle age, with mean age at onset of 45 years Motor abnormalities and progressive dementia
Familial fatal insomnia (FFI)	Autosomal dominant inheritance with point mutations in PrP^c gene (missense mutation codon 178)	Disease of middle age Presentation includes insomnia, dysautonomia, and motor dysfunction
Sporadic fatal insomnia	No genetic mutations identified, but homozygosity at methionine 129 characteristic	Presentation similar to FFI and neuropathologic changes similar

especially vCJD.[10,87] Other investigators have suggested that abnormal signals originating from the pulvinar in the thalamus are distinctive for vCJD and should be considered highly predictive.[10,70,157] Similarly, electroencephalographic tracings from patients with CJD and vCJD are characteristic but not diagnostic. The classic findings are synchronous bilateral biphasic or triphasic periodic sharp waves on a background of generalized slowing.[26,67,215] The lack of a characteristic electroencephalographic tracing and magnetic resonance imaging findings should cast doubt on the diagnosis of CJD or vCJD, especially several months after onset of clinical symptoms.

PREVENTION AND TREATMENT

Because effective treatments of TSE remain as yet undefined, prevention of disease remains the goal of current medical practice. Infection that occurs after exposure during neurosurgical procedures (including dural grafts and stereotaxic electrodes), exposure to contaminated CNS tissue in the form of growth hormone or gonadotropic hormone preparations, and corneal grafts are well-known modes of transmission of TSE that are preventable in most cases. Because transmissions by blood and blood products have been reported, the American Red Cross has placed restrictions on donors to exclude those who may have been exposed to agents associated with TSE (see previous sections). Cases of TSE that follow exposure to human feces, urine, or mucosal secretions have not been reported; therefore, current universal precautions employed in health care settings should suffice to prevent nosocomial transmission during routine care of the patient with TSE. The inactivation of tissue and fluid suspected of being contaminated with prions is problematic. Early studies indicated that subjecting prion-containing brain tissue to temperatures in excess of 360° C resulted in only approximately a 90 percent reduction in infectivity.[45] In addition, CJD has been transmitted from paraffin-embedded tissue sections.[43] Several different approaches for decontamination of prion-contaminated tissue and instruments have been proposed.[161,194,199] Suffice it to say that the concentration of prions in lymphoid and nervous tissue should be emphasized to anyone in contact with patients with TSE, and material that may be contaminated with prions should be handled in a rigorous manner to ensure safe disposal. To date, no cases of transmission after routine dental procedures have been reported.

There currently are no known treatments of TSE. In vitro models of PrP^{c} folding have suggested that a variety of agents may limit production of pathogenic forms of PrP^{res} in neuroblastoma cells in vitro. Comprehensive reviews of treatment modalities have been published, and the interested reader is referred to these publications.[56,224] Derivatives of Congo red, polyene antibiotics related to amphotericin B, branched polyamines, and phenothiazines all have been reported to exhibit some activity and to delay onset of disease in experimental animals.[224] To date, no evidence that any of these proposed therapies would be effective in human TSE has been reported. Interestingly, at least two recent reports have suggested that anti-prion antibodies may offer a therapeutic approach to TSE. These reports suggested that anti-prion antibodies directed at cell surface PrP^{c} prevented infection of susceptible cells and also eliminated infection in a chronically infected cell line.[84,181,235] These findings argued that impaired clearance of misfolded PrP^{c} contributed to disease in animals infected with PrP^{res} and that passive antibody therapy could potentially cure similar infections in vivo. Treatment of mice with soluble lymphotoxin-β blocked follicular dendritic cell maturation and inhibited the development of scrapie in inoculated, susceptible mice, suggesting that interventions with agents that can block neuroinvasion may be efficacious.[156,173]

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SECTION XVIII

CHLAMYDIA

CHAPTER 206

CHLAMYDIA INFECTIONS

Margaret R. Hammerschlag

Chlamydiae are obligate intracellular pathogens that have established a unique niche within the host cell. They cause a variety of diseases in animal species at virtually all phylogenic levels. Until fairly recently, the order has contained one genus with four recognized species: *Chlamydia trachomatis*, *Chlamydia psittaci*, *Chlamydia pneumoniae*, and *Chlamydia pecorum*.[29,35,39,49] *C. trachomatis* and *C. pneumoniae* are the most significant human pathogens, and *C. psittaci* is an important zoonosis. *C. pecorum* primarily infects cattle and other ruminants; there is no description of disease in humans. Recent taxonomic analysis involving the 16S and 23S rRNA genes have found that the order Chlamydiales contains at least four distinct groups at the family level and that within the family Chlamydiaceae are two distinct lineages.[35] This analysis has suggested splitting of the genus *Chlamydia* into two genera, *Chlamydia* and *Chlamydophila*. Two new species, *Chlamydia muridarum* (formerly the agent of mouse pneumonitis) and *Chlamydia suis* (an important pathogen of swine), would join *C. trachomatis*. *Chlamydophila* would contain *C. pecorum*, *C. pneumoniae*, and *C. psittaci* and three new species split off from *C. psittaci*: *Chlamydophila abortus* (causes abortion in cattle and sheep; rarely has caused abortion in humans), *Chlamydophila caviae* (formerly *C. psittaci* guinea pig conjunctivitis strain), and *Chlamydophila felis* (causes epidemic keratoconjunctivitis in cats). Controversy about this reclassification is continuing, but for the purposes of this chapter, I refer to these organisms as *Chlamydia*.

Recently, several chlamydia-like organisms that are endosymbionts of free-living amebae have been identified.[51,84] These organisms, which include *Parachlamydia acanthamoebae*, *Simkania negavensis*, and *Neochlamydia hartmannellae*, have been termed environmental chlamydiae. Analyses of nearly full length 16S rRNA gene sequences of these isolates showed that they clustered with other members of the order Chlamydiales but in a lineage separate from those of the genera *Chlamydia* and *Chlamydophila* (16S rRNA sequence similarities <88%). This bacteria-protozoa interaction might have been a driving force for the development of effective mechanisms by bacteria to survive phagocytosis by unicellular eukaryotes, which in turn may have been a first step in the evolution of intracellular bacterial pathogens of higher organisms.

Chlamydiae are characterized by a unique developmental cycle with morphologically distinct infectious and reproductive forms: the elementary body and the reticulate body. They have a gram-negative envelope without detectable peptidoglycan, although recent genomic analysis has revealed that both *C. trachomatis* and *C. pneumoniae* encode proteins forming a nearly complete pathway for the synthesis of peptidoglycan, including penicillin-binding proteins.[77,123] This finding is the basis for the so-called chlamydial peptidoglycan paradox, given that it has been known for decades that chlamydial development is sensitive to β-lactam antibiotics. Chlamydiae also share a group-specific lipopolysaccharide (LPS) antigen and use host adenosine triphosphate (ATP) for the synthesis of chlamydial protein.[108] Although chlamydiae are auxotrophic for three of four nucleoside triphosphates, they do encode functional glucose-catabolizing enzymes that can be used for the generation of ATP. As with peptidoglycan synthesis, for some reason these genes are turned off.[123] All chlamydiae also encode an abundant protein, the major outer membrane protein (MOMP or OmpA), that is surface exposed in *C. trachomatis* and *C. psittaci* but apparently not in *C. pneumoniae*.[123] MOMP is the major determinant of the serologic classification of *C. trachomatis* and *C. psittaci* isolates.

After infection develops, the infectious elementary bodies, which are 200 to 400 μm in diameter, attach to the host cell by a process of electrostatic binding and are taken into the cell by endocytosis that does not depend on the microtubule system. Within the host cell, the elementary body remains within a membrane-lined phagosome. The phagosome does not fuse with the host cell lysosome. The inclusion membrane is devoid of host cell markers, but lipid markers traffic to the inclusion, which suggests functional interaction with the Golgi apparatus. The elementary bodies then differentiate into reticulate bodies that undergo binary fission. After approximately 36 hours, reticulate bodies differentiate into elementary bodies. At approximately 48 hours, release may occur by cytolysis or by a process of exocytosis or extrusion of the whole inclusion, with the host cell left intact. Chlamydiae also may enter a persistent state after treatment with certain cytokines such as interferon-γ, treatment with antibiotics, or restriction of certain nutrients. In the persistent state, metabolic activity is reduced. The ability to cause prolonged, often subclinical infection is one of the major characteristics of chlamydiae.

INFECTION CAUSED BY *CHLAMYDIA TRACHOMATIS*

EPIDEMIOLOGY

C. trachomatis infection is the most prevalent sexually transmitted infection and infectious disease in the United States today.[22,23] The Centers for Disease Control and Prevention (CDC) estimates that the number of new *C. trachomatis* infections exceeds 4 million annually.[23] The prevalence of chlamydial infection is associated less with socioeconomic status, urban or rural residence, and race or ethnicity than is the case with gonorrhea and syphilis. The prevalence of *C. trachomatis* infection consistently is higher than 5 percent among sexually active adolescent and young adult women attending outpatient clinics, regardless of the region of the country, the location of the clinic (urban or rural), and the race or ethnicity of the population.[40] In sexually active adolescents, the prevalence commonly exceeds 10 percent and may exceed 20 percent.[45] Decreasing age at first intercourse and increasing age at marriage have contributed importantly to the

higher prevalence of infection with *C. trachomatis*. Infection with *C. trachomatis* tends to be asymptomatic and of long duration. If a pregnant woman has active infection during delivery, the infant may acquire the infection and, as a consequence, either conjunctivitis or pneumonia.[100] Rarely, children also may acquire chlamydial infection as a result of sexual abuse.

INFECTIONS IN OLDER CHILDREN

C. trachomatis has not been associated with any specific clinical syndrome in older infants and children. Most attention given to *C. trachomatis* infection in these children has concentrated on its relationship to sexual abuse. Isolation of *C. trachomatis* from a rectal or genital site in children without previous sexual activity may be a marker of sexual abuse; moreover, evidence for other modes of spread, such as through fomites, is lacking for this organism. Perinatal maternal-to-infant transmission resulting in vaginal or rectal infection has been documented, with prolonged infection lasting for periods up to 3 years. This duration can be an important confounding variable in evaluation of possible sexual abuse in a young child.

Vaginal infection with *C. trachomatis* was reported uncommonly in prepubertal children before 1980. The possibility of sexual contact frequently was not even discussed. In 1981, Rettig and Nelson[120] reported concurrent or subsequent chlamydial infection in 9 of 33 (27%) episodes of gonorrhea in a group of prepubertal children. However, *C. trachomatis* was not found in any of 31 children with urethritis or vaginitis that was not gonococcal. No information was given about possible sexual activity.

Most studies have identified rectogenital chlamydial infection in 2 to 3 percent of sexually abused children when these children underwent routine culture for the organism.[23] Most of those with chlamydial infection were asymptomatic. In two early studies that had control groups, similar percentages of control patients also were infected.[56,71] A larger study subsequently conducted by Ingram and colleagues[72] found a stronger association between vaginal chlamydial infection and a history of sexual abuse but not with pharyngeal infection, which was found in a similar number of controls. Rectal infection was detected in only 1 of 124 abused children. A recent CDC-sponsored multicenter study of sexually transmitted infections in children, birth to 13 years of age, being evaluated for suspected sexual abuse found infection with *C. trachomatis* in 15 of 536 (2.8%), all of whom were asymptomatic. Two (0.37%) of these children were positive in the rectum; the others were positive in the vagina. Sixty percent of the children who were positive for *C. trachomatis* gave a clear, credible detailed description of molestation.

In the setting of repeated abuse by a family member during the course of a long time, the development of infection would be difficult to demonstrate. The 2006 guidelines for the treatment of sexually transmitted diseases from the CDC do not recommend that samples for culture of *C. trachomatis* be obtained routinely from the pharynx and urethra in children who are suspected victims of sexual abuse.[23] The major reasons were the low yield from the urethra, the tendency for longer persistence of perinatally acquired pharyngeal infection, and the potential confusion with *C. pneumoniae*.[7]

Although asymptomatic, perinatally acquired nasopharyngeal infection with *C. trachomatis* may persist for at least 2 years; respiratory tract infection in older children and adults appears to be a distinctly uncommon occurrence, the reasons for which are not clear. *C. trachomatis* has been isolated from the pharynx of some adults and apparently is related to certain sexual practices. These infections have been asymptomatic. Two earlier studies based entirely on serology suggested that *C. trachomatis* might be a cause of pharyngitis and community-acquired pneumonia in adults.[81,82] Subsequent studies using culture methods did not confirm these findings.[44,67] The original studies probably detected cross-reacting antibody to *C. pneumoniae*.

C. trachomatis can cause pneumonia in older children or adults in two specific situations. One is in immunosuppressed persons. Several well-documented cases of *C. trachomatis* pneumonia in persons with leukemia, bone marrow transplant recipients, and those with acquired immunodeficiency syndrome (AIDS) have been reported.[73,83,98,102] In all these cases, *C. trachomatis* was isolated from biopsy specimens of lung tissue or bronchoalveolar lavage fluid. Several patients also had a serologic response that was diagnostic of acute *C. trachomatis* infection. Unfortunately, the clinical findings were nonspecific. These adults had none of the features that are distinctive of infantile chlamydial pneumonia.

Several cases of pulmonary infection after exposure to *C. trachomatis* serovars L1 and L2 in the laboratory also have been reported.[8] These infections probably were acquired by inhalation of aerosolized organisms. Clinically, these patients had high fever, night sweats, and cough and were found to have mediastinal lymphadenopathy, pneumonitis, or splenomegaly alone or in any combination. In two cases, the diagnosis of lymphoma was seriously considered. These findings are not unexpected, given the severity of lymphogranuloma venereum genital infection. Accidental exposure to the aerosolized trachoma biovar of *C. trachomatis* has not been associated with the development of significant illness.

DIAGNOSIS OF *CHLAMYDIA TRACHOMATIS* INFECTIONS

Culture remains the "gold standard" of diagnosis for detection of *C. trachomatis* from the vagina or rectum in prepubertal children. *Chlamydia* culture has been defined further by the CDC as isolation of the organism in tissue culture and confirmation by microscopic identification of the characteristic inclusions by fluorescent antibody staining.[22,23] Since the 1980s, numerous nonculture tests, including enzyme immunoassay (EIA), direct fluorescent antibody tests, and nucleic acid amplification tests (NAATs), have been approved for establishing the diagnosis of *C. trachomatis* genital infections in adults and adolescents. The only EIA and direct fluorescent antibody tests still available in the United States are Pathfinder *Chlamydia* direct fluorescent assay and EIA microplate (Bio-Rad Laboratories). Although these tests are approved by the Food and Drug Administration (FDA) for vaginal and urethral specimens from adolescents and adults, they are not approved for these sites in children. The DNA probe PACE 2 (Gen-Probe, San Diego, CA), which is still used in many laboratories, does not have approval for any site in children, including the conjunctiva.

A major advance in the diagnosis of *C. trachomatis* infection during the past decade has been the introduction of NAATs. These tests have high sensitivity, perhaps even detecting 10 to 20 percent more cases than is possible with culture, while retaining high specificity.[23] Currently, three commercially available NAATs are FDA approved: polymerase chain reaction (Amplicor; Roche Molecular Diagnostics, Nutley, NJ), transcription-mediated amplification (Gen-Probe), and strand displacement amplification (ProbeTec; Becton Dickson, Sparks, MD). Polymerase chain reaction and strand displacement amplification are DNA amplification tests; both use primers that target gene sequences on the cryptogenic *C. trachomatis* plasmid, which has approximately 10 copies per cell. Transcription-mediated amplification is an RNA amplification assay. A fourth DNA NAAT, ligase chain reaction (LCx; Abbott Diagnostics, Abbott Park, IL), was withdrawn from the market by the manufacturer in 2002. NAATs that are currently commercially available have FDA approval for cervical swabs from women, urethral swabs from men, and urine from men and women. The transcription-mediated amplification also has approval for vaginal swabs from

adults and adolescent women. Several studies have demonstrated that self-collected swabs also are suitable specimens.

Nonculture tests should never be used for rectal or vaginal sites in children or for any forensic purposes in adolescents or adults[22,23]; only culture should be used. Isolation of the organism in tissue culture should be confirmed by microscopic identification of the inclusions by staining with a fluorescein-conjugated, *C. trachomatis* species–specific monoclonal antibody. EIAs are not acceptable for confirmation of culture results, and their use has led to false-positive reports.[54,117] Isolates of *C. trachomatis* also should be preserved for further testing if necessary. The use of nonculture tests for detection of *C. trachomatis* in vaginal and rectal specimens has been associated with a large number of false-positive results.[53,117] Fecal material can give false-positive reactions with any EIA; none is approved for this site in adults. Common bowel organisms, including *Escherichia coli*, *Proteus* spp., vaginal organisms such as group B streptococcus, and *Gardnerella vaginalis*, and even some respiratory tract flora, such as group A streptococcus, also can yield positive reactions with EIAs.[117] Because all of the available EIAs use genus-specific antibodies, if they are performed on respiratory tract specimens, these tests will detect *C. pneumoniae* as well.[8] NAATs also are not approved for the detection of *C. trachomatis* in rectogenital specimens from prepubertal children and rectal specimens in adults. The major problem with rectal specimens is the presence of inhibitors of DNA polymerase, which can lead to false-negative results. Data on the use of NAATs for vaginal specimens or urine from children are very limited and insufficient to allow unqualified recommendation of their use.[32,45,78,93] Several studies used ligase chain reaction, which no longer is available.[32,45,78] The CDC-sponsored multicenter study of sexually transmitted diseases in children, birth to 13 years of age, being evaluated for suspected sexual abuse also compared NAATs (ProbeTec and TMA) and use of urine to culture for establishing the diagnosis of *C. trachomatis* infection.[10] The NAATs appeared to be more sensitive than culture for vaginal specimens with retention of specificity. Of the 15 children who had positive results for *C. trachomatis*, 7 were positive by culture, 10 were positive by NAAT of vaginal swab, and 13 were positive by NAAT of urine. All positive NAAT results were confirmed as *C. trachomatis* by *ompA* genotyping. Two of the seven culture-positive children were positive in the rectum but not in the vagina. Thus, although use of urine for NAAT testing correlated with positive vaginal culture or NAAT results, it obviously will miss rectal infection. Because the prevalence of *C. trachomatis* infection in children being evaluated for suspected sexual abuse has remained less than 3 percent, a potential problem exists with the positive predictive value of a single NAAT result. The 2006 CDC sexually transmitted diseases treatment guidelines recommend that NAATs be used as an alternative to culture *only* if confirmation is available.[23] Confirmation tests should consist of a second FDA-approved NAAT that targets a gene sequence different from the one used in the initial test.

TREATMENT

Chlamydial infections in older children may be treated with oral erythromycin (50 mg/kg/day four times a day orally to a maximum of 2 g/day for 7 to 14 days). Children older than 8 years may be treated with tetracycline (25 to 50 mg/kg/day four times a day orally for 7 days). Azithromycin, 1 g orally as a single dose, also may be used in children who weigh at least 45 kg, are 8 years of age or older, or both.[57]

INFECTION CAUSED BY *CHLAMYDIA PSITTACI*

Human infection with *C. psittaci* was described first probably by Juergensen in 1874 or Ritter in 1876. Ritter described seven cases of an unusual pneumonia that appeared to be caused by parrots and finches that were caged in the study of his brother's home in Switzerland. After these reports, several outbreaks of a similar disease in Europe established the association with exposure to birds. The term *psittacosis* was coined by Morange in 1892 from the Greek word for parrots, *psittakos*.

THE ORGANISM

C. psittaci is a diverse species that affects psittacine and nonpsittacine birds.[1] The known host range includes 259 avian species.[63] Birds that have been reported to have *C. psittaci* infection contain six major domestic species: chickens, turkeys, Pekin ducks, Muscovy ducks, geese, and pigeons. Infections in these species have been associated with significant outbreaks in humans.[20,21] In the past, chlamydial infection in psittacine birds was termed psittacosis, whereas disease in wild and domestic fowl was called ornithosis. However, as *C. psittaci* strains isolated from psittacine or nonpsittacine species have been shown to produce identical disease in birds of either grouping, this distinction is artificial. The more universally applicable term *chlamydiosis* can be usefully employed to describe chlamydial infections of all animal species.

EPIDEMIOLOGY

Chlamydial infections in birds are important because they represent a biologic hazard to human health as well as economic loss to the poultry industry.[20,21] *C. psittaci* has been recovered from symptomatic and from apparently healthy birds. Infection principally involves the gastrointestinal tract, and the chlamydiae are shed in feces or through infectious respiratory tract discharges.[135] Clinically inapparent, latent infections may be the predominant state. Overt clinical disease may be activated by stress factors, including overcrowding, poor nutrition, other bacterial and viral infections, and transportation.[21,128]

Apparently healthy birds shedding chlamydiae can infect other birds or humans through contact. Infectious chlamydiae in respiratory secretions or feces may remain viable for several months. Transmission of disease is mainly through aerosols of fecal or feather dust, but oral infection is an alternative route. Transmission through eggs has been shown in chickens, ducks, seagulls, and psittacine birds.[135] However, most infected eggs do not hatch. In the nest, parent birds may infect their young, which may carry the infectious agent for many years. Young birds are more susceptible to infection than are older birds, and some species seem to be more susceptible than others are. Often, disease carriers may be identified by the transmission of disease to other susceptible birds or by the sudden death of young nestlings with apparently healthy parents.[135]

From 1988 through 2003, 935 human cases of psittacosis were reported to the CDC.[128] According to the most recent report from the CDC, 85 percent of cases of psittacosis in the United States were associated with exposure to birds; 70 percent of these reported cases were the result of exposure to caged pet birds.[21] Individuals at highest risk of acquiring psittacosis included bird owners or fanciers and pet shop employees. Since 1984, several major outbreaks of psittacosis have occurred in the United States in turkey-processing plants, where approximately 300 persons were infected.[20,63] Workers exposed to turkey viscera were at the highest risk of acquiring infection.[20] In 1995, the CDC investigated an outbreak of avian chlamydiosis in a shipment of more than 700 pet birds from a Florida bird distributor to the Atlanta area.[105] Affected birds included parrots, parakeets, finches, lovebirds, cockatiels, conures, and canaries. Clinical psittacosis or

serologic evidence of *C. psittaci* infection was found in 30.7 percent of households with birds from the infected flock. An average of 21 days (range, 1 to 47 days) elapsed between purchase of the bird and the onset of symptoms. Most of the infected individuals had mild or asymptomatic illnesses. Among persons in exposed households, illness occurred more frequently if the recently purchased bird had become sick or had died. Kissing or nuzzling, handling, and feeding the bird all were significantly associated with the development of clinical psittacosis, but in contrast to earlier studies, cleaning the bird's cage was not. The risk for acquiring clinical psittacosis varied significantly by type of bird to which the individual was exposed. The attack rate was highest for individuals exposed to parrots.

Inhalation of infectious aerosols derived from the feces, fecal dust, or secretions of *C. psittaci*–infected animals is thought to be the primary route of infection. The source birds can be infected asymptomatically or can show signs of infection such as anorexia, ruffled feathers, depression, and watery green droppings. Psittacosis frequently is a systemic infection in birds, and the turkey strains can induce severe pericarditis.[135]

Psittacosis is an uncommon occurrence in children. In a series of 135 cases from Australia observed during a 15-year period, the youngest patient was 17 years of age.[141] Children may be less likely to be exposed to birds. Bird keeping is more commonly a hobby of adults, and the parents are the ones who usually clean the cage of the family's pet bird. An outbreak of psittacosis involving two adolescents was reported from a small village in Scotland. The source of the infection appeared to be the local pet shop, which had taken delivery of four lovebirds, two of which died shortly after arrival.[106] Another report described a family outbreak during which three members of a family of nine persons contracted severe pneumonia.[15] A newly purchased cockatiel appeared to be the primary source, but person-to-person transmission was likely between 19-year-old twin brothers who shared a bedroom, one of whom had no direct contact with the bird. A retrospective review of the records of the Public Health Laboratory at Leeds, England, for cases with a fourfold rise in *Chlamydia* complement-fixation (CF) titer identified 219 patients during a 24-year period from 1965 to 1989.[27] The ages ranged from 9 months to 87 years; only five (2.2%) of the patients were younger than 10 years, but on review, only 34 of the total cases were thought to be psittacosis. All involved antecedent avian exposure, but the ages were not specified.

CLINICAL MANIFESTATIONS

Infection with *C. psittaci* in humans may range from clinically inapparent to severe infection involving multiple organ systems as well as pneumonia.[141] Symptomatic *C. psittaci* infection in humans may present as two forms: a severe atypical pneumonia or pneumonitis and a systemic toxic or septic form without respiratory involvement. In both cases, fever, chills, muscle aches and pains, and severe headache are typical. Other manifestations may be diarrhea, nausea, and vomiting. The mean incubation period is 15 days after exposure (range, 5 to 21 days). The onset usually is abrupt, with complaints of fever, cough, and headache. The fever is high and frequently associated with rigors and sweats. The headache can be so severe that meningitis can be considered a possibility; 33 percent of patients in the Australian series underwent lumbar puncture.[141] The cough usually is nonproductive, and rales may be heard on auscultation. Chest radiographs generally are abnormal, with variable infiltrates. Pleural effusions also may be present. In contrast, most of the individuals in the Atlanta outbreak had very mild disease characterized by fever, headache, and cough.[105]

LABORATORY FINDINGS

The white blood cell count usually is not elevated, but mild leukocytosis may be present. Almost 50 percent of patients in the Australian series had abnormal liver function test results, including elevated levels of aspartate aminotransferase, alkaline phosphatase, and bilirubin.[141]

DIAGNOSIS

Because of the varying clinical findings, establishing the diagnosis of psittacosis can be difficult. A history of exposure to birds is important, although as many as 20 percent of patients with psittacosis may not have a history of such contact.[128] In the Australian series, 85 percent of patients had a history of recent bird contact, 71 percent of whom described a strong history of contact with birds.[141] Only five patients had been exposed to poultry. Pneumonia caused by *C. pneumoniae* also can have similar clinical findings.[114,115] Data from both Sweden and Denmark have suggested that many cases of *psittacosis* caused by *C. pneumoniae* with no history of exposure to birds probably have occurred, and the diagnosis has been established serologically with CF.[38,104] An outbreak of suspected psittacosis in a boys' boarding school in England was reported in 1984.[114] The outbreak involved 20 children, 13 to 18 years of age, and four adults. The illness was mild and characterized by pharyngitis and influenza-like symptoms, including headache, fever, and cough. The diagnosis was established on the basis of serology (CF titers). No avian source was identified. Because the cases occurred during a 3-month period, person-to-person spread was suggested. Subsequent analysis of the sera suggested that the outbreak was caused by *C. pneumoniae*.[115] Person-to-person spread rarely occurs in psittacosis; secondary cases tend to be severe, however.

Other infections that can produce the syndrome of pneumonia with high fever, unusually severe headache, and myalgia include *Mycoplasma pneumoniae* infection, tularemia, tuberculosis, fungal infection, legionnaires' disease, and various bacterial infections. The diagnosis of psittacosis in the human population is based primarily on clinical findings, epidemiology, and serology.

The diagnosis of human infection with *C. psittaci* has not changed substantially for many years. The mainstay of diagnosis remains serology with the CF test. According to the 2000 recommendations from the CDC,[21] a confirmed case of psittacosis requires a compatible clinical illness, usually with a good history of avian exposure. Laboratory confirmation can be made by one of the three following methods: (1) culture of *C. psittaci* from respiratory secretions, (2) fourfold or higher increase in CF or microimmunofluorescence (MIF) titer in sera collected at least 2 weeks apart, and (3) an MIF IgM titer of 16 or higher. A probable case should be epidemiologically linked to a confirmed case or have a single CF or MIF antibody titer of 32 or higher in at least one serum sample obtained after the onset of symptoms. As with use of MIF for the diagnosis of *C. pneumoniae* infection, cross-reactions with other *Chlamydia* spp. and bacteria can occur.[107] Cross-reaction with *Legionella longbeachae* also has been described in a patient with fulminant pneumonia caused by *C. psittaci*.[129]

Although *C. psittaci* will grow in the same culture systems used for the isolation of *C. trachomatis* and *C. pneumoniae*, very few laboratories culture for *C. psittaci*, mainly because of the potential biohazard.

CF is a genus-specific test; thus, infection caused by *C. pneumoniae* can give titers of 32 or higher. Early treatment with tetracycline also may suppress the antibody response. Even though many laboratories can isolate *C. psittaci*, it is not a service provided routinely by most clinical microbiology laboratories. The

CDC reported using a modification of the MIF test for the serodiagnosis of human psittacosis.[137] In the 78 patients examined, psittacosis was diagnosed on the basis of compatible clinical symptoms after exposure to sick birds. Conventional CF was positive in 36 (46%) of the patients. The MIF test detected diagnostic antibody responses in all the CF-positive patients and in another 12 patients whose sera were negative or anticomplementary according to CF. Seven other patients were thought to have *C. pneumoniae* infection because of their MIF antibody response.

Several reports also have examined the use of nonculture methods, including direct fluorescent antibody, EIA, and polymerase chain reaction (PCR), for the direct identification of *C. psittaci* in clinical specimens. Several in-house PCR assays for detection of *C. psittaci* have been reported in the literature.[97,111] Most reported studies have assessed only the ability of these assays to amplify laboratory isolates; they have not been evaluated extensively for detection of *C. psittaci* in clinical specimens from humans with suspected psittacosis. Only a small number of human cases of psittacosis documented by PCR have been reported in the literature.[97,111] In 1997, the CDC reported one of the most extensive evaluations of the use of PCR in the investigation of a psittacosis outbreak.[97] Most of the specimens tested were from birds. The target sequence of the assay was the 16S rRNA gene. The first amplification was genus specific, and the second was multiplexed and could differentiate among *C. psittaci*, *C. pneumoniae*, and *C. trachomatis* on the basis of the molecular weight of the amplicon. With the use of this assay, *C. psittaci* was detected in 13 (17.3%) of 75 sick or dead birds involved in three avian psittacosis outbreaks; 5 of the 13 PCR-positive birds were also culture positive. None of the throat swab specimens from four humans involved in this outbreak were positive by PCR or culture, but one individual had a *C. psittaci*–specific MIF IgG titer of 512. The CDC [21] provides a list of laboratories that test human specimens in its current recommendations for the control of *C. psittaci* infection in humans and pet birds (Table 206–1).

TREATMENT

The recommended treatment of psittacosis in adults and children older than 8 years is 500 mg of tetracycline orally every 6 hours for 7 to 10 days. Erythromycin (50 mg/kg/day, up to 2 g/day, for 7 to 10 days) also can be used. The experience in the Australian series[141] and anecdotal reports suggest that tetracycline may be more effective than erythromycin. The initial infection does not appear to be followed by long-term immunity. Re-infection and clinical disease can develop within 2 months of treatment; two well-documented cases of re-infection are reported in the literature. A pet shop employee had two episodes of psittacosis 11 months apart. Each episode met the CDC's confirmed case definition.[19]

TABLE 206–1 Laboratories that Test Human Specimens for *Chlamydia psittaci*

Laboratory	Tests Performed	Telephone Number
Respiratory Diseases Laboratory Section, Centers for Disease Control and Prevention, Atlanta	MIF CF PCR Culture	(404) 639-3563
Focus Technology, Cypress, CA	IFA PCR Culture	(800) 445-4032
Laboratory Corporation of America, Burlington, NC	Culture Polyclonal antibody	(800) 334-5161
Specialty Laboratories, Santa Monica, CA	MIF	(800) 421-4449

CF, complement fixation; IFA, immunofluorescent antibody; MIF, microimmunofluorescence; PCR, polymerase chain reaction.

From Compendium of measures to control Chlamydia psittaci *infection among humans (psittacosis) and pet birds (avian chlamydiosis). M. M. W. R. Recomm. Rep. 49(RR-8):3-17, 2000.*

INFECTION CAUSED BY *CHLAMYDIA PNEUMONIAE*

THE ORGANISM

The first isolates of *C. pneumoniae* were obtained serendipitously during trachoma studies in the 1960s.[48] After the recovery of a similar isolate from the respiratory tract of a college student with pneumonia in Seattle, Grayston and colleagues[50] applied the designation TWAR after their first two isolates, TW-183 and AR-39. On the basis of inclusion morphology and staining characteristics in cell culture, *C. pneumoniae* initially was considered a *C. psittaci* strain. Subsequent analysis, however, has demonstrated that this organism is distinct from both *C. psittaci* and *C. trachomatis*.[49] Sequencing has revealed that *C. pneumoniae* differs significantly from *C. trachomatis* in several areas. *C. pneumoniae* encodes 21 polymorphic membrane proteins versus 9 in *C. trachomatis*. Polymorphic membrane proteins may be surface exposed in *C. pneumoniae*.[77] Ultrastructural studies have demonstrated an elementary body morphology distinct from that of *C. trachomatis* and *C. psittaci* (Fig. 206–1).[24] However, some isolates of *C. pneumoniae*, including IOL-207, have been found to have round elementary bodies.[18,116] Thus, it may not be a consistent species characteristic. Restriction endonuclease pattern analysis, nucleic acid hybridization studies, and amplified fragment length polymorphism analysis suggest a high degree of genetic relatedness (>95%) among the *C. pneumoniae* isolates examined thus far and less than 10 percent homology with either *C. trachomatis* or *C. psittaci*.[17,26,35,49,96] At this point, we do not have a strain typing system for *C. pneumoniae*. A plasmid has been detected in a single isolate obtained from a horse but not from any human isolate of *C. pneumoniae*.

EPIDEMIOLOGY

C. pneumoniae appears to be a common human respiratory pathogen. The organism also has been isolated from nonhuman mammalian species, including a horse, koalas, bandicoots, and reptiles and amphibians, although the role that these infections may play in human disease is unknown.[13,75,85,119] The mode of transmission remains uncertain but probably involves infected respiratory tract secretions. Acquisition of infection by droplet aerosol was described during a laboratory accident.[68] *C. pneumoniae* can remain viable on Formica countertops for 30 hours and can survive small-particle aerosolization.[36,131] Spread of *C. pneumoniae* within families and enclosed populations such as military recruits has been described.[55,80,139]

Several serologic surveys have documented rising chlamydial antibody prevalence rates in school-age children that reach 30 to 45 percent in adolescents.[16] This increasing prevalence of chlamydial antibody during childhood probably is attributable to *C. pneumoniae*. The proportion of community-acquired pneumonia associated with *C. pneumoniae* infection has ranged from 0 to 19

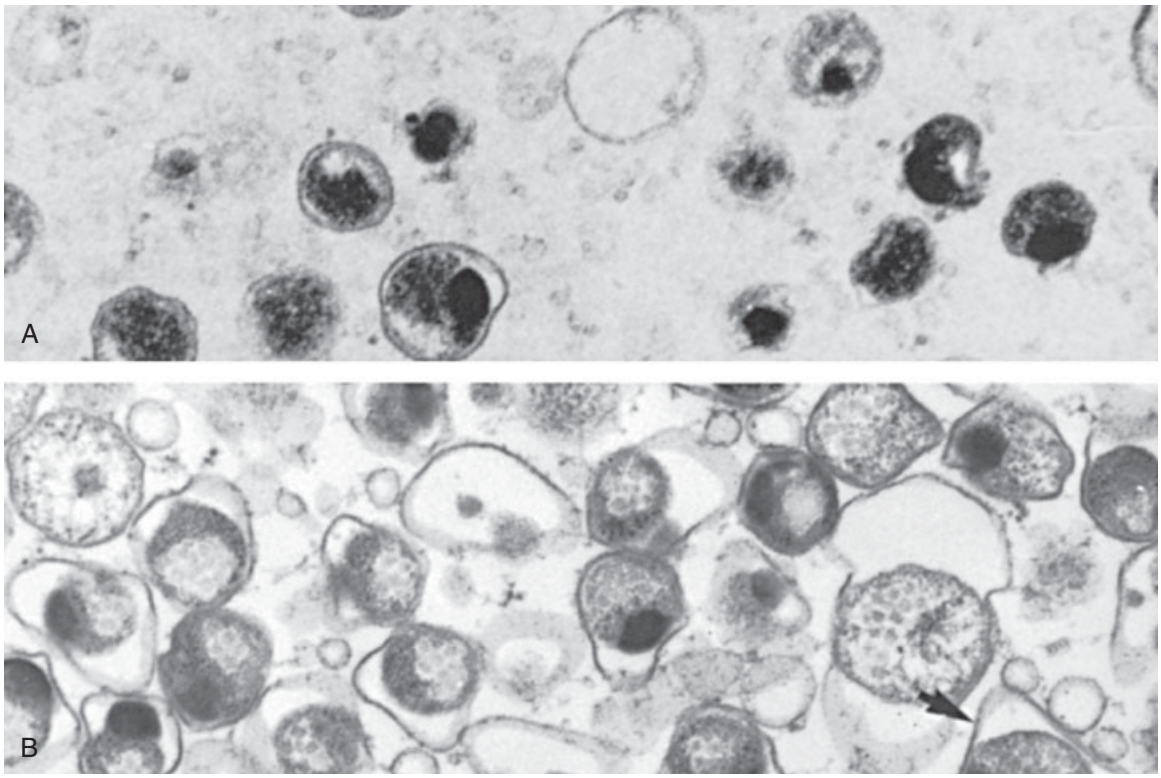

Figure 206–1 Electron micrograph of *Chlamydia trachomatis* (**A**) and *Chlamydia pneumoniae* (**B**) inclusions demonstrating the morphology of the elementary body. The elementary bodies of *C. pneumoniae* have a pear shape because of a loose periplasmic membrane *(arrow)*, unlike the typically round elementary bodies of *C. trachomatis* and *C. psittaci*.

TABLE 206–2 Selected Studies of *Chlamydia pneumoniae* Lower Respiratory Tract Infection in Children

				No. Tested/No. Positive Results (%)			
Study	**Year**	**Country**	**Age**	**No.**	**Culture**	**PCR**	**Serology**
Saikku et al.[124]	1988	Philippines	<5 yr	220	ND	ND	14 (6.4)
Forgie et al.[37]	1991	Gambia	1-9 yr	74	ND	ND	9 (12.1)
Jantos et al.[76]	1995	Germany	2 d-15 yr	290	1 (0.3)	2/290 (0.7)	2/101 (2)
Block et al.[12]	1995	United States	3-12 yr	260	34 (13)	ND	48 (18.5)
Harris et al.[61]	1998	United States	6 mo-16 yr	456	31 (7.3)	ND	37 (8.8)
Principi et al.[118]	2001	Italy	2-14 yr	613	ND	48 (7.8)	52 (11.4)
Baer et al.[6]	2003	Switzerland	1-18 yr	50	1 (2)	1 (2)	2 (4)
Likitnukul et al.[89]	2003	Thailand	1 mo-18 yr	333	ND	ND	149 (44.7)
Tsolia et al.[133]	2004	Greece	5-14 yr	75	ND	0	2 (3)
Michelow et al.[99]	2004	United States	6 wk-18 yr	154	ND	ND	14 (9)
Liu et al.[90]	2005	China	≤5 yr	85	ND	4 (2.2)	ND

ND, not done; PCR, polymerase chain reaction.

percent, varying with the geographic location, the age group examined, and the diagnostic methods used.*

Studies of the role of *C. pneumoniae* in lower respiratory tract infections in pediatric populations have found evidence of infection in 0.3 to more than 44 percent (Table 206–2). Most of these studies have relied entirely on serology for diagnosis. Early studies that relied on serology suggested that infection in children younger than 5 years was rare[48,140]; however, in a study of Filipino children younger than 5 years with lower respiratory tract infection, nearly 10 percent had either acute or chronic antibody to *C. pneumoniae*.[124] In Brooklyn, the proportion of lower respiratory tract infections associated with *C. pneumoniae*, as determined by culture, increased from 9 percent in children younger than 5 years to 19 percent in children and adolescents 5 to 16 years of age.[25]

Studies that have used culture have found a poor correlation with serology, especially in children.[12,33,61] Although 7 to 13 percent of children 6 months to 16 years of age enrolled in two multicenter pneumonia treatment studies were culture positive and 7 to 18 percent met the serologic criteria for acute infection with the MIF test, they were not the same patients.[12,61] Only 1 to 3 percent of the culture-positive children met the serologic criteria, and approximately 70 percent were seronegative.

In studies to date, acute infection with *C. pneumoniae* does not appear to vary by season. Studies have found much lower preva-

*See references 6, 12, 25, 37, 41, 47, 61, 65, 76, 84, 86, 89, 90, 99, 118, 124, 125, 132, 133.

lence of infection than reported in the 1990s, suggesting that cycling during the course of time may occur.[84,86]

Prolonged culture positivity lasting several weeks to several years after acute infection has been reported.[25,29,33,55] Asymptomatic nasopharyngeal carriage also occurs in 2 to 5 percent of adults and children.[11,33,46,69,84] The role that asymptomatic carriage plays in the epidemiology of *C. pneumoniae* is not known, but possibly these persons serve as a reservoir for spread of infection.

CLINICAL MANIFESTATIONS

The spectrum of disease associated with *C. pneumoniae* is expanding. Most infections probably are mild or asymptomatic. Longitudinal serologic data obtained during an epidemic among military recruits in Finland suggest that only approximately 10 percent of infections result in clinically apparent pneumonia.[80] Initial reports emphasized mild atypical pneumonia clinically resembling that associated with *M. pneumoniae*.[47,48,125] In several subsequent studies, however, pneumonia associated with *C. pneumoniae* was clinically indistinguishable from other pneumonias.[12,61] Co-infection with other pathogens, especially *M. pneumoniae* and *Streptococcus pneumoniae*, can be a frequent occurrence.[12,61] In one multicenter pneumonia treatment study, 20 percent of the children with positive *C. pneumoniae* cultures were co-infected with *M. pneumoniae;* they could not be distinguished from children who were infected with either organism alone.[12] *C. pneumoniae* has been associated with severe illness and even death, although the role of preexisting chronic conditions as contributing factors in many of these patients is difficult to assess. In some cases, however, *C. pneumoniae* clearly appears to be implicated as a serious pathogen, even in the absence of underlying disease. *C. pneumoniae* was isolated from the respiratory tract and the pleural fluid of a previously healthy adolescent boy with severe pneumonia complicated by respiratory tract failure and pleural effusions (Fig. 206–2).[5]

The role of host factors remains to be determined. Although *C. pneumoniae* has been detected in bronchoalveolar lavage fluid from 10 percent of a group of patients with AIDS and pneumonia, its clinical role in these patients is uncertain because most of these patients were co-infected with other well-recognized pathogens, such as *Pneumocystis carinii* and *Mycobacterium tuberculosis*.[4] Gaydos and colleagues[42] identified *C. pneumoniae* infection by PCR in 11 percent of a group of immunocompromised adults with human immunodeficiency virus (HIV) infection, malignant neoplasms, and other immune disorders, including systemic lupus erythematosus, sarcoidosis, and common variable immunodeficiency. *C. pneumoniae* appeared to be responsible for 14 to 19 percent of episodes of acute chest syndrome in children with sickle-cell disease.[30,101] *C. pneumoniae* infection in these patients appeared to be associated with more severe hypoxia than was infection with *M. pneumoniae*.

C. pneumoniae may act as an inflammatory trigger for asthma. Several cases of culture-documented *C. pneumoniae* infection in patients with significant bronchospasm have been reported.[53,55] Asthmatic bronchitis was diagnosed in one patient, and she was receiving systemic and topical steroids.[65] This patient did not improve until her chlamydial infection was treated. Hahn and associates[53] reported an association between serologic evidence of acute *C. pneumoniae* infection and wheezing in adults seen for lower respiratory tract illness. However, they were able to isolate the organism from only 1 of 365 patients. As part of a study in children, *C. pneumoniae* was isolated from 13 of 118 children (11%) 5 to 15 years of age who were evaluated initially for either new or acute exacerbations of asthma.[33] Treatment of the infec-

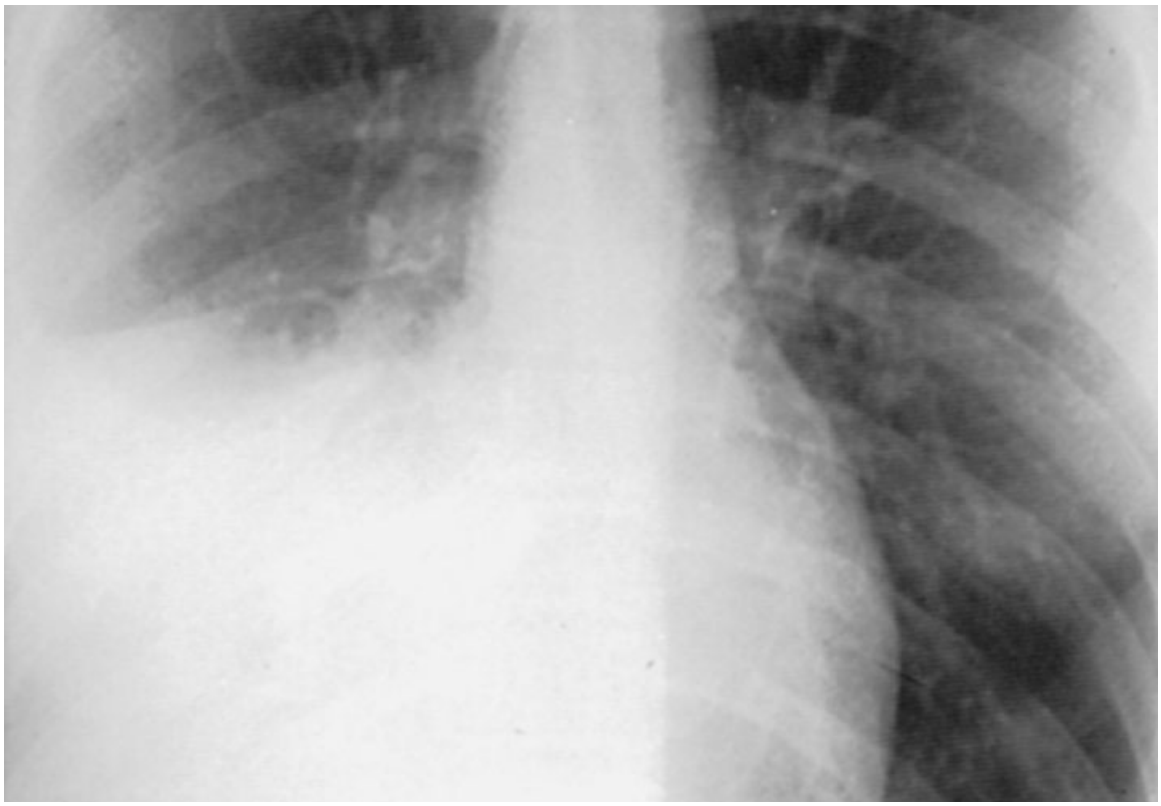

Figure 206–2 Chest radiograph of a 19-year-old man with *Chlamydia pneumoniae* pneumonia demonstrating pleural effusion in the right lung. *C. pneumoniae* was isolated from the pleural fluid.

tion appeared to result in both clinical improvement and improvement in pulmonary function test scores. Only five of the children with confirmed infection had detectable IgG antibody to *C. pneumoniae*. One child who did not comply with his antibiotic therapy was culture positive on five occasions during a 3-month period. Anti–*C. pneumoniae* antibody as determined by MIF was never detected. Specific anti–*C. pneumoniae* IgE was, however, detected in 85.7 percent of the culture-positive asthmatics compared with 9 percent of the children with *C. pneumoniae* pneumonia who were not wheezing.[34] This finding suggests that the bronchial reactivity seen with *C. pneumoniae* infection may be IgE mediated. The potential prolonged, persistent *C. pneumoniae* infection may produce chronic inflammation and trigger bronchospasm in susceptible persons. *C. pneumoniae* has been demonstrated to induce in vitro ciliostasis in ciliated bronchial epithelial cells.[127] Animal studies also suggest that steroids can reactivate *C. pneumoniae* lung infection in mice.[92] In addition, immune-mediated phenomena, including erythema nodosum and iritis, have been described as complicating *C. pneumoniae* infection.[130,138]

The role of *C. pneumoniae* in upper respiratory infections is not as well defined. *C. pneumoniae* has been isolated from the middle ear fluid of children and adults with otitis media.[11,110] Block and colleagues[11] recovered *C. pneumoniae* from the middle ear fluid of eight children, 3 months to 14 years of age, with acute otitis media. *C. pneumoniae* was the sole pathogen isolated in two patients. Co-pathogens in the remaining six patients included β-lactamase–positive *Haemophilus influenzae* and *Moraxella catarrhalis*, along with penicillin-resistant and penicillin-sensitive *S. pneumoniae*. Five of the children who were positive for *C. pneumoniae* responded favorably despite not being treated with antibiotics active against the organism, either a course of an oral β-lactam or single-dose intramuscular ceftriaxone. Symptoms suggestive of sinus involvement are not uncommon in patients with upper respiratory tract infection associated with *C. pneumoniae*, but only one case of isolation of the organism, from a 47-year-old man with sinusitis, has been reported.[67] Cultrara and colleagues[28] examined nasopharyngeal swabs, ethmoid and maxillary sinus biopsy samples, and adenoids from 20 children, 3 to 16 years of age, with chronic sinusitis. *C. pneumoniae* was not isolated from any sinus tissue but was isolated from the nasopharyngeal swab and adenoid tissue of a 6-year-old child. The results of this study suggest that *C. pneumoniae* does not play a significant role in chronic sinusitis in children. Data also are limited on the potential role of *C. pneumoniae* in pharyngitis. Although one study found serologic evidence of *C. pneumoniae* in 8.5 percent of adult patients with pharyngitis,[66] Hyman and associates[69] reported that 19 percent of asymptomatic adults who were culture or PCR negative (or negative by both modalities) fulfilled the serologic criteria for acute *C. pneumoniae* infection.

DIAGNOSIS

A specific laboratory diagnosis of *C. pneumoniae* infection can be made by isolation of the organism from nasopharyngeal or throat swabs, sputum, or pleural fluid, if present. The nasopharynx appears to be the optimal site for isolation of the organism.[12] The relative yield from throat swabs and sputum is not known. Isolation of *C. pneumoniae* requires culture in tissue; the organism cannot be propagated in cell-free media. Initial studies suggested that *C. pneumoniae* was more difficult to isolate in tissue culture than was *C. trachomatis*.[48] Originally, the same methods were used: HeLa or McCoy cells pretreated with dextran diethylaminoethyl. Multiple passages were needed, the inclusions were small and difficult to see, and, in general, the yield was poor. *C. pneumoniae* grows more readily in other cell lines derived from respiratory tract tissue, specifically, HEp-2 and HL cells.[121] Omission of pretreatment with dextran diethylaminoethyl results in much larger inclusions, and specimens need only one passage. Culture with an initial inoculation and one passage should take 4 to 7 days.

Nasopharyngeal cultures can be obtained with Dacron-tipped, wire-shafted swabs. Each lot of swabs should be treated in a mock infection system to ensure that no inhibitory effects occur on either the viability of cells or recovery of chlamydiae. Specimens for culture should be placed in appropriate transport media, usually a sucrose phosphate buffer with antibiotics and fetal calf serum, and stored immediately at 4° C for no longer than 24 hours. Viability decreases if specimens are held at room temperature. If the specimen cannot be processed within 24 hours, it should be frozen at –70° C until culture can be performed. After 72 hours of incubation, culture confirmation can be performed by staining with either a *C. pneumoniae* species–specific or a *Chlamydia* genus–specific (anti-LPS) fluorescein-conjugated monoclonal antibody.[103] Inclusions of *C. pneumoniae* do not contain glycogen and thus do not stain with iodine. Unfortunately, no commercially produced *C. pneumoniae*–specific culture-confirmation reagents are available. If a genus-specific antibody is used, *C. pneumoniae* should be confirmed by differential staining with a specific *C. trachomatis* antibody; if that result is negative, the isolate is either *C. pneumoniae* or *C. psittaci*. If the patient has not had avian exposure, psittacosis would be highly unlikely.

Because isolation of *C. pneumoniae* initially was considered to be difficult and limited, emphasis was placed on serologic diagnosis. However, performance of the MIF test also is limited to a small number of research laboratories. The MIF test was modified from the test used for *C. trachomatis* by use of elementary bodies from TW-183 or other *C. pneumoniae* strains as the antigen. With the MIF test, one can detect IgG, IgM, and IgA antibodies. Grayston and colleagues[48] proposed a set of criteria for the serologic diagnosis of *C. pneumoniae* infection with the MIF test that have been used by many laboratories and clinicians. For acute infection, the patient should have a fourfold rise in IgG titer, a single IgM titer of 16 or higher, or a single IgG titer of 512 or higher. Past or preexisting infection is defined as an IgG titer of 16 or higher but lower than 512. These researchers further proposed that the pattern of antibody response in primary infection differed from that in re-infection. In initial infection, the IgM response appears approximately 3 weeks after the onset of illness and the IgG response at 6 to 8 weeks. In re-infection, the IgM response may be absent and the IgG response occurs earlier, within 1 to 2 weeks.[48] A fourfold titer rise or a titer of 64 or higher with CF also is considered to be diagnostic. Initially, Grayston and colleagues[50] found that fewer than one third of hospitalized patients with suspected *C. pneumoniae* infection had detectable CF antibody. However, in a report of a small outbreak of *C. pneumoniae* infection in University of Washington students, all seven patients with pneumonia had CF titers of 64 or higher.[48] The CF test is genus specific.

Because of the relatively long period until the development of a serologic response in primary infection, the antibody response may be missed if convalescent sera are obtained too soon (i.e., earlier than 3 weeks after the onset of illness). The use of paired sera also affords only a retrospective diagnosis, which is of little help in terms of deciding how to treat a patient. The criteria for use of a single serum sample have not been correlated with the results of culture and are based primarily on data from adults. The antibody response in acute infection may take longer than 3 months to develop. Acute, culture-documented infection also can occur without seroconversion, especially in children.[12,25,33,43,61] Only 28 percent of the culture-positive children enrolled in a multicenter pneumonia treatment study had detectable anti–*C. pneumoniae* antibody by MIF, and only 1 percent met the serologic criteria for acute infection.[12] Most of them had no detectable antibody by the MIF test, even after 3 months of follow-up.[12] However, the results of immunoblotting revealed

that these children have antibody to numerous *C. pneumoniae* proteins but that less than 30 percent react with MOMP, which is the antigen presented in the MIF test.[87] Although MOMP has been demonstrated to be immunodominant in *C. trachomatis* infection, it does not appear to be immunodominant for *C. pneumoniae*.[9,70,87]

Background rates of seropositivity also can be high in some populations. Hyman and associates,[69] as part of a study of asymptomatic *C. pneumoniae* infection in subjectively healthy adults in Brooklyn, found 81 percent to have IgG or IgM titers of 16 or higher. Seventeen percent had evidence of "acute infection" (IgG titer > 512, IgM titer > 16, or both). However, none of these persons was culture or PCR positive. Similar results were reported by Kern and associates[79] in healthy firefighters and policemen in Rhode Island. The specificity of the MIF IgM assay can be affected by the presence of rheumatoid factor. A study from the Netherlands found an increased probability of false-positive results caused by rheumatoid factor with increasing age.[136] Sera should be routinely absorbed before MIF IgM testing is performed. Hyman and colleagues[69] absorbed all the IgM-positive sera, and the titers did not change. Some IgG antibody may result from a heterotypic response to other chlamydial species because cross-reactions with MOMP occur among the three species, as do cross-reactions caused by the genus LPS antigen. Moss and colleagues[107] reported that antibodies to *C. pneumoniae* and *C. psittaci* were found in as many as half of all chlamydial IgG–positive persons attending a clinic for sexually transmitted diseases. This point is reinforced by the observation that studies from the early 1980s suggesting *C. trachomatis* as a cause of community-acquired pneumonia and pharyngitis in adults and children were probably detecting antibody to *C. pneumoniae* rather than *C. trachomatis*.[81,82] Other organisms that have been reported as possibly causing cross-reactions on MIF are *Bartonella*[94] and *Bordetella pertussis*.[74] The latter could be significant because adults with pertussis frequently have a chronic cough or severe bronchitis, which is a clinical feature often ascribed to *C. pneumoniae*. Other studies have found significant homology between human and *C. pneumoniae* heat shock protein 60 (HSP-60) and *E. coli* GroEL.[109] Picornavirus proteins also have been reported to share antigenic determinants with HSP-60/65, including the HSP-60 of humans and *C. pneumoniae*, which conceivably could lead also to cross-reactions in the MIF assay.[60]

Moreover, the MIF test is not standardized, and reading of the slides has a large subjective component and requires an experienced microscopist. A study by Peeling and coworkers[112] attempted to address the problem of interlaboratory variation in performance of the MIF test by sending a panel of 22 acute and convalescent sera to 14 different laboratories. Nine of the laboratories used an in-house MIF. The antigens in most of the assays were either AR-39 or TW-183. Three laboratories used one of two commercially available kits, which included either AR-39 or Kajaani 6 as the antigen. The remaining two laboratories used the Washington Research Foundation kit, which uses TW-183. The overall agreement in assay results (to get within one twofold dilution of the gold standard, as read at the University of Washington) was 80 percent. The range was 50 to 100 percent, depending on the isotype. Agreement in serodiagnostic criteria was 69 percent for negative, 68 percent for "chronic," and 87 percent for a fourfold increase in IgG. This observation has been confirmed in subsequent evaluations of the performance of the MIF assay.[86]

Although EIA serology test kits offer the promise of standardized performance and objective end-points, none has been evaluated adequately in comparison to culture or PCR.[31] Most have been compared with only MIF. None has FDA clearance or approval for use in the United States. One commercial assay, the Medac recombinant enzyme linked immunosorbent assay, uses a recombinant LPS antigen; others are based on LPS-extracted elementary bodies or synthetic peptides.[31,88,113] These kits can measure IgG, IgM, and IgA antibodies, but cutoffs vary from kit to kit, and the criteria for a positive result (acute infection, past infection) can be complex.[32,103,134] A study from the United States compared recombinant LPS EIA with culture and found that the sensitivity ranged from 13 percent for IgM antibody in children to 78 percent for either IgA or IgG antibody in adults with respiratory infection.[88] Specificity in comparison to culture ranged from 21 to 91 percent. Persson and Haidl[113] reported cross-reaction with recombinant EIA and parvovirus, primarily for IgM, but it also was seen to a lesser extent with IgG and IgA. This cross-reaction was not seen with MIF.

The CDC has proposed some modifications of the serologic criteria for establishing the diagnosis of *C. pneumoniae* infection.[31] Although the MIF test was considered to be the only serologic test currently acceptable, the criteria were made significantly more stringent. Acute infection as determined by MIF was defined as a fourfold rise in IgG or an IgM titer of 16 or higher, and the use of a single elevated IgG titer was discouraged. An IgG titer of 16 or higher was considered to indicate past exposure, but neither elevated IgA titer nor any other serologic marker was thought to be a validated indicator of persistent or chronic infection. The CDC did not recommend the use of any EIA for detection of antibody to *C. pneumoniae*.

The number of *C. pneumoniae* organisms present in the respiratory tract of persons with pneumonia or other respiratory tract diseases is smaller than the number found in genital *C. trachomatis* infection. PCR appears to be the most promising technology in the development of a rapid, nonculture method for detection of *C. pneumoniae*. More than 25 in-house PCR assays for detection of *C. pneumoniae* in clinical specimens have been reported in the literature.[14,86] None of these assays is standardized or extensively validated in comparison to culture for detection of *C. pneumoniae* in respiratory specimens. None is commercially available or has FDA approval. Major variations in these methods include collection and processing of specimens, primer design, nucleic acid extraction, detection and identification of amplification products, and ways to prevent possible false-positive and inhibitory reactions. The primers used most frequently have been those based on the *omp1* gene, the 16S rRNA gene, the 16S and 16S-23S spacer rRNA genes, and a *C. pneumoniae*–specific cloned *Pst*I fragment.[14,86] Some assays have used single amplification; some have been nested. Methods for detecting the amplification product include agarose gel electrophoresis, Southern blot, EIA, and polyacrylamide gel electrophoresis.[14,86] Studies suggest significant interlaboratory variation in performance of PCR for *C. pneumoniae*.[3,91] Use of nested PCRs has been associated with a high risk of contamination due to amplicon carryover.[3] Real-time PCR may be the method of choice; several assays have been reported in the literature, but none has been validated compared with culture.[2,3]

The CDC did not recommend any specific assay because of a lack of comparative data[31] and suggested that more studies need to be conducted with proper controls and larger numbers of clinical specimens from patients. The CDC also suggested that any new PCR assay be compared with a sensitive culture system.

TREATMENT

Chlamydia spp. are susceptible to the tetracyclines, macrolides, and quinolones.[126] *C. pneumoniae*, like *C. psittaci*, is resistant to sulfonamides.[126] To date, few published data have described the response of *C. pneumoniae* to antimicrobial therapy. Most of the treatment studies of pneumonia caused by *C. pneumoniae* published thus far have relied entirely on diagnosis by serology; consequently, microbiologic efficacy could not be assessed. Anecdotal reports have suggested that prolonged courses, up to

3 weeks, of either tetracyclines or erythromycin may be needed to eradicate *C. pneumoniae* from the nasopharynx of adults with influenza-like illness and pharyngitis.[55] The results of two pediatric multicenter pneumonia treatment studies found that 10-day courses of erythromycin and clarithromycin and 5 days of azithromycin suspension were equally efficacious; they eradicated the organism in 79 to 86 percent of children.[12,61,122] Quinolones, including levofloxacin and moxifloxacin, also have been demonstrated to have 70 to 80 percent efficacy in eradicating *C. pneumoniae* from adults with community-acquired pneumonia.[58,59] Most patients improved clinically despite persistence of the organism. Persistence does not appear to be secondary to the development of antibiotic resistance.[58,59,122,126]

On the basis of these limited data, the following regimens for respiratory tract infection caused by *C. pneumoniae* can be suggested: in adults, doxycycline, 100 mg twice a day for 14 to 21 days; tetracycline, 250 mg four times a day for 14 to 21 days; azithromycin, 1.5 g during a period of 5 days; levofloxacin, 500 mg/day orally or intravenously for 7 to 14 days; and moxifloxacin, 400 mg/day orally for 10 days. For children, suggested regimens include erythromycin suspension, 50 mg/kg/day for 10 to 14 days; clarithromycin suspension, 15 mg/kg/day for 10 days; and azithromycin suspension, 10 mg/kg on day 1 followed by 5 mg/kg/day once daily on days 2 to 5. Some patients may require re-treatment.

INFECTIONS DUE TO OTHER CHLAMYDIALES SPECIES

The most studied member of this group of organisms is *Simkania negevensis*, which was first identified as a contaminant in tissue culture in Israel.[84] The *S. negevensis* 16S and 23S ribosomal DNA both have 80 to 87 percent sequence identity with members of the Chlamydiales. The organism has been associated with a variety of respiratory illnesses, including bronchiolitis in infants from Canada and Israel, community-acquired pneumonia and chronic obstructive pulmonary disease in adults from Israel, and respiratory tract infection in children and adults from Cornwall, England. *S. negevensis* has a life cycle similar to that of Chlamydiales, with a biphasic intracellular morphology with electron-dense nonreplicating elementary bodies and replicating reticulate bodies of comparable sizes. Distinct differences exist in that the growth cycle is longer, 12 to15 days compared with 48 to 72 hours for Chlamydiaceae. Unlike *Chlamydia*, *S. negevensis* is totally resistant to penicillin. *S. negevensis* has been found to be present in drinking water and reclaimed waste water in the Negev, Israel. The organism has also been shown to be able to replicate in amebae and to survive for long periods in amebal cysts, which would allow it to survive in water. On the basis of this, it has been proposed that *S. negevensis* may be transmitted to humans by drinking water.

Parachlamydia acanthamoebae, previously called Hall's coccus, is an endosymbiont of *Acanthamoeba*.[51,52] The organism was described initially as bacteria-like structures in trophozoites of *Acanthamoeba* isolated from patients with fever and pneumonia associated with use of humidifiers.[51] The bacteria-like structures subsequently were identified as *P. acanthamoebae*. The organism has been isolated from nasal mucosa of healthy human volunteers and a bronchoalveolar lavage specimen from a patient with a diagnosis of viral pneumonia. However, *P. acanthamoebae* appears to be a rare cause of pneumonia in humans. Greub and colleagues[52] were able to identify *P. acanthamoebae* DNA by PCR in only 1 of 1200 bronchoalveolar lavage specimens from patients with pneumonia of unknown etiology. This patient was a 31-year-old HIV-positive man who presented with cough, fever, and bilateral infiltrates on chest radiography. No other pathogens, including *C. pneumoniae*, *Legionella pneumophila*, *Pneumocystis carinii*, or mycobacteria, were detected. A subsequent study from the same group using serology suggested that *P. acanthamoebae* might be responsible for approximately 8 percent of pneumonias in a series of patients with head trauma and suspected aspiration pneumonia. The in vitro susceptibilities of *P. acanthamoebae* appear to be similar to those of *Chlamydia* except that they are constitutively resistant to quinolones.[95]

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Rickettsial Diseases

CHAPTER 207

Rickettsial and Ehrlichial Diseases

Morven S. Edwards ❂ Ralph D. Feigin

The Rickettsiaceae family was named to honor H. T. Ricketts. Dr. Ricketts discovered and described the cycle of the rickettsial agent causing Rocky Mountain spotted fever (RMSF) early in the 20th century. He died in 1910 from typhus fever while in Mexico investigating that disease.

Rickettsial diseases were grouped historically because they possessed a number of common characteristics: the agents are similar in size and shape visible by light microscopy as coccobacillary forms; they multiply intracellularly in susceptible hosts; the characteristic pathologic lesion is a widespread vasculitis of small blood vessels, except for Q fever, in which pneumonitis can be of equal importance; all are acute illnesses characterized by fever, headache, and rash, with the exception of Q fever, which has no rash, and ehrlichiosis, which often has no rash; the infecting agents are susceptible, early in the course of infection, to several broad-spectrum antibiotics; and all occur under natural conditions in either insects (lice and fleas) or arachnids (ticks and mites), and these vectors are, with the exception of Q fever, the primary means by which these diseases are transmitted to humans.

Within the past 2 decades, sequencing of genes has refined the classification and phylogeny of Rickettsiaceae.[29] On the basis of genetic and antigenic information, rickettsiae traditionally are divided into groups as shown in Table 207–1: the spotted fever group, the typhus group, and the scrub typhus group. The agent of scrub typhus, *Orientia tsutsugamushi*, is in the genus *Orientia* within the Rickettsiales group. The *Ehrlichia* group has four genera; two of these, *Ehrlichia chaffeensis* and *Anaplasma phagocytophilum*, are the agents of human monocytic and human granulocytic anaplasmosis, respectively. This chapter considers the rickettsial and ehrlichial diseases of importance in childhood that are listed in Table 207–1 as well as Q fever, although the agent of Q fever, *Coxiella burnetii*, now is classified in the gamma group of Proteobacteria.

In each of the rickettsial and ehrlichial infections except louse-borne typhus, humans are an incidental or accidental blind-end host and do not contribute to the survival of the vector. These diseases vary enormously in severity, from benign, self-limited illnesses without mortality to fulminating infections. A high index of suspicion leading to prompt establishment of diagnosis and institution of appropriate therapy is an important factor in enhancing the survival of children with rickettsial diseases.

THE SPOTTED FEVERS

The spotted fever group is composed primarily of rickettsial infections that are transmitted by ticks, but rickettsialpox, caused by *Rickettsia akari*, is a mite-borne infection and is included in this group. By comparison with other rickettsiae that grow exclusively in cytoplasm, the spotted fever group of rickettsiae also can multiply in the nuclei of susceptible animal cells.

RMSF is the most severe and important disease in the spotted fever group; it occurs throughout the temperate zone of North America. An illness apparently identical to RMSF occurs in South America, where it is called São Paulo disease. Other less severe forms of tick-borne spotted fevers occur in Europe, Asia, Africa, and Australia; they are distinguished from each other by geographic location as well as by differences in the spotted fever rickettsiae that cause them.[34]

ROCKY MOUNTAIN SPOTTED FEVER

HISTORICAL ASPECTS. RMSF, caused by *Rickettsia rickettsii*, was recognized first in Idaho and Montana at the turn of the 20th century. For decades, it was considered limited to the Rocky Mountain region. Beginning in the 1930s, RMSF was identified in the eastern United States and subsequently in all geographic areas of the country. For a decade after the introduction of broad-spectrum antibiotics around 1950, the incidence declined in both the east and the west. In recent years, infection rates have fluctuated (Fig. 207–1). From 1997 through 2002, 3649 cases were reported. More than half of the reported cases were from only five states: North Carolina, South Carolina, Tennessee, Oklahoma, and Arkansas.[6,7] The absolute number reported in 2004, 1514 cases, was the highest in United States history.[12]

The incidence of RMSF in the Rocky Mountain region had begun an inexplicable steady decline even before the antibiotic era; by 1988, fewer than 20 cases were reported in the Rocky Mountain and Pacific coastal areas. This trend has continued, and the major endemic regions currently are the south Atlantic, southeastern, and south central states (Fig. 207–2).

ETIOLOGY, MORPHOLOGY, GROWTH, AND METABOLISM. *R. rickettsii*, the etiologic agent of RMSF, is a small coccobacillus measuring 0.3 to 0.5 μm in diameter and 0.3 to 4 μm in length. Organisms usually occur singly but can appear in strands or as diploids with slightly pointed ends and a transparent band between the two bacilli. Electron microscopy reveals a two-layered cell wall and a cytoplasmic membrane. The chemical composition of rickettsiae is similar to that of gram-negative bacteria. Rickettsiae must penetrate into living cells to grow and multiply. They are grown most readily in the yolk sacs of embryonated eggs; but under special conditions, they also grow well in certain tissue culture cells. Once inside, rickettsial cells multiply by transverse binary fission.

Rickettsiae take on a characteristic red color when stained with Gimenez stain. *R. rickettsii* possess a soluble antigenic moiety that is shared with their antigenic variants in the spotted fever group and rickettsialpox. Living *R. rickettsii* organisms contain a toxin; when these organisms are injected intravenously, mice die within 6 to 12 hours, long before significant multiplication has occurred.

This chapter retains contributions by Edward S. Murray, now deceased.

TABLE 207–1 Characteristics of Some Important Rickettsiae and Ehrlichiae Causing Infections in Children

Disease	Agent	Distribution	Transmission	Mammalian Host
Spotted fever group				
Rocky Mountain spotted fever	*R. rickettsii*	Western Hemisphere	Tick bite	Wild rodents, dogs
Mediterranean spotted fever	*R. conorii**	Mediterranean, Caspian, and Black Sea coastal regions; Africa, Southeast Asia	Tick bite	Wild rodents, dogs
Rickettsialpox	*R. akari*	Worldwide	Mite bite	Mice
Typhus group				
Epidemic typhus	*R. prowazekii*	Worldwide	Infected louse feces rubbed into broken skin or as aerosol to membranes	Humans, flying squirrels
Murine typhus	*R. typhi*	Scattered pockets, worldwide	Flea bite	Rodents
Scrub typhus group	*Orientia tsutsugamushi*	Japan, Southeast Asia, west and southwest Pacific	Mite bite	Wild rodents
Ehrlichiosis group				
Human monocytic ehrlichiosis	*Ehrlichia chaffeensis*†	Worldwide	Tick bite	Deer, dogs, humans
Human granulocytic anaplasmosis	*Anaplasma phagocytophilum*	United States, Europe	Tick bite	Deer, humans, other mammals

**In addition*, Rickettsia australis *(Queensland tick typhus)*, Rickettsia sibirica *(Siberian tick typhus)*, Rickettsia slovaca, *and* Rickettsia japonica *(Japanese spotted fever)*, *among others, are antigenically and geographically distinct entities.*
†E. ewingii *also causes granulocytotropic ehrlichiosis in humans.*

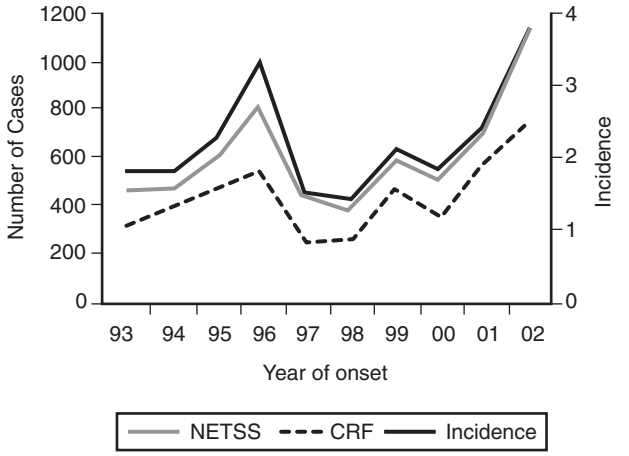

Figure 207–1 Number and incidence of Rocky Mountain spotted fever per million population in the United States, 1993-2002, as determined by the cases reported to the Centers for Disease Control and Prevention by the states through the National Electronic Telecommunications System for Surveillance (NETSS) and case report form (CRF). *(Data from Chapman, A. S., Murphy, S. M., Demma, L. J., et al.: Rocky Mountain spotted fever in the United States, 1997-2002. Vector Borne Zoonotic Dis 6:170-178, 2006.)*

EPIDEMIOLOGY AND TRANSMISSION. Because RMSF rickettsiae are primarily parasites of ticks, the epidemiology of human disease is associated intimately with the biology of the ticks that transmit it. Disease can be acquired in the laboratory, and workers must comply with protective measures. RMSF has been transmitted by blood transfusion[41] and by aerosol.[19,25]

The Rocky Mountain wood tick *(Dermacentor andersoni)* in the west and the American dog tick *(Dermacentor variabilis)* in the east and central United States are natural carriers and vectors of the disease. The brown dog tick *(Rhipicephalus sanguineus)* is implicated as a vector of *R. rickettsii* in eastern Arizona.[9] RMSF rickettsiae do not kill their arthropod hosts but are passed transovarially to subsequent generations of ticks. Congenitally acquired rickettsiae in tick eggs can persist through larval and nymphal stages to the adult stage during the course of a 2-year cycle; infected adult ticks can survive for as long as 4 years without feeding. Mammals act as the blood meal source for ticks during their various metamorphoses. A study in Maryland and Virginia in the late 1960s showed that 15 different mammals, including field mice, and 18 types of birds could be intermediate hosts. Many small wild animals as well as dogs possess antibodies to RMSF, indicating that they participate in the tick-mammal-tick cycle.[35]

Dogs are thought to be an important link in the RMSF cycle in nature. Rickettsial strains were isolated for the first time from the blood of sick or dying dogs in the 1970s; the rickettsial strains isolated were closely antigenically related to or identical to human disease–causing *R. rickettsii*. In addition, rising titers of RMSF antibodies were demonstrated in clinically ill dogs. Dogs appear to be susceptible to virulent *R. rickettsii*, but dogs can have RMSF antibodies without having any history of previous illness.

Moreover, all but a few of the rickettsiae found in ticks appear to be antigenically distinct from the rickettsiae isolated from both sick dogs and humans. The data presently available do not clarify the role of dogs in the RMSF cycle. Dogs may be accidental hosts of RMSF rickettsiae, much as humans are; they may mechanically transport ticks from infected tick islands to the proximity of humans, or they may play an important role in maintaining or increasing the reservoir of virulent *R. rickettsii* in nature.

A large serosurvey of children living in the southeast and south central regions of the United States found that as many as 12 percent had immunofluorescence antibody titers consistent with prior infection caused by *R. rickettsii* or antigenic spotted fever group rickettsiae, suggesting that subclinical infection occurs commonly.[23] Additional prominent epidemiologic features of RMSF are its seasonal character—most cases occur during the period of greatest tick activity, from April to September; its prevalence—it is the most common rickettsial disease in the United States and a growing infectious disease problem, with nearly two thirds of cases occurring in children younger than 15 years and the peak incidence occurring among children 5 to 9 years of age[35]; and its focal nature—relatively small areas in a state can account for a high percentage of that state's recorded cases; for example, 89 percent or more of all cases of RMSF occurring in New York State are reported from Long Island, Clermont County in Ohio reports approximately 10 percent of all Ohio cases of RMSF, and Cape Cod and the offshore islands generally account for almost all the cases reported in Massachusetts.

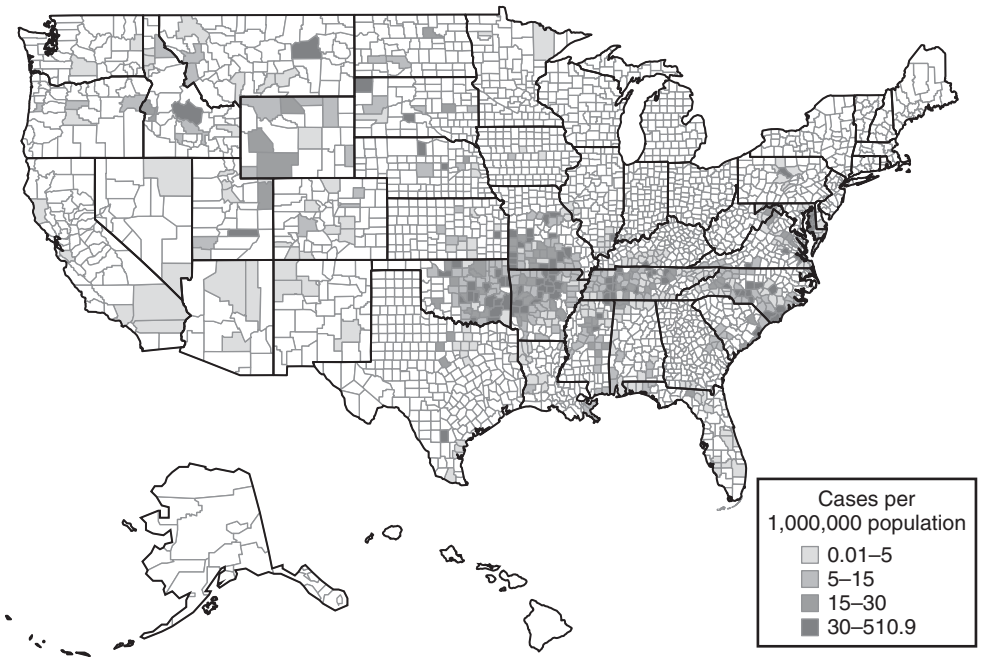

Figure 207–2 Annual incidence of Rocky Mountain spotted fever per million population by county in the United States, 1997-2002, based on reports from the National Electronic Telecommunications System for Surveillance and case report forms submitted to the Centers for Disease Control and Prevention. *(Data from Chapman, A. S., Murphy, S. M., Demma, L. J., et al.: Rocky Mountain spotted fever in the United States, 1997-2002. Vector Borne Zoonotic Dis 6:170-178, 2006.)*

PATHOGENESIS. The primary pathologic lesion of RMSF is found in the vascular system after the individual has been bitten by an infected tick. Rickettsiae multiply in endothelial cells lining small blood vessels and disseminate by the bloodstream. They can be demonstrated in both the cytoplasm and the nucleus of cells.[35] Focal areas of endothelial proliferation and perivascular mononuclear cell infiltration lead to thrombosis and leakage of red blood cells into the surrounding tissues. Potential mechanisms of cellular injury that are postulated include injury to cell membranes caused by the penetration of multiple rickettsiae, followed by a crescendo of rickettsial release; depletion of adenosine triphosphate by intracellular rickettsiae, which causes failure of the sodium pump and an influx of water; competition by *R. rickettsii* for crucial metabolic substrates; and damage to the cell by toxic products of rickettsial metabolism.[40,43]

Vascular lesions probably account for the more prominent clinical manifestations, such as rash, headache, and mental confusion, as well as terminal heart failure and shock. Vascular lesions can be found everywhere but are appreciated most readily in the skin, gonads, and adrenal glands. Parenchymatous inflammation accompanies vasculitis in the heart and central nervous system. Interstitial myocarditis, patchy in distribution, can be demonstrated regularly in fatal cases. The location of rickettsiae by immunofluorescence coincides with the patchy distribution of the myocarditis. Pathologic examination reveals interstitial edema and inflammation with relative preservation of myocardial fibers.[39] In neural parenchyma, both mononuclear infiltration and focal proliferative glial nodules usually are topographically related to inflamed blood vessels.[13] In the kidney, inflammation involves both vessels and the interstitium in most patients. Acute tubular necrosis occurs. In the lung, rickettsial involvement of the pulmonary microcirculation results in interstitial pneumonia, evidenced by alveolar septal congestion and interstitial edema, alveolar edema and hemorrhage with a fibrinous and mononuclear exudate, and interlobular septal edema. Organisms are demonstrable in the pulmonary microcirculation by immunofluorescence, indicating that the pathologic changes most likely are caused by direct infection and are not toxin mediated. Hepatic lesions include portal triaditis consisting of polymorphonuclear leukocytes and large mononuclear cells, portal vasculitis, sinusoidal leukocytosis, erythrophagocytosis by Kupffer cells, and increased hepatic weight; rickettsial organisms are demonstrable in portal blood vessels and sinusoidal lining cells.[18,27]

Negative nitrogen balance can be extreme. Early in infection, it can be related to excretion of nitrogen in urine; but after several days, it is related to insufficient intake of protein. The serum albumin concentration can be depressed as a result of protein loss, hepatic dysfunction related to the disease process itself, and leakage of protein through the damaged endothelium of the blood vessel walls.

Profound hyponatremia occurs, commonly related to a shift in water from the intracellular to the extracellular space, the loss of sodium through excretion in urine, and an exchange of sodium for potassium at the cellular level.[21] Intracellular sodium increases slightly. Destruction of some cells results in increased serum concentrations of potassium and massive losses of potassium in urine. Intracellular overhydration of the medulla oblongata has been proposed to contribute to a fatal outcome in some patients. Plasma concentrations of aldosterone and antidiuretic hormone are increased in some but not all patients.[20,30]

CLINICAL MANIFESTATIONS. A history of tick bite is elicited in only 50 to 60 percent of children with RMSF.[26] Fever, headache, rash, toxicity, mental confusion, and myalgia are the major clinical features of RMSF.[35] The onset of the disease in humans, which usually occurs approximately 7 days (range, 2 to 14 days) after being bitten by an infected tick, can be gradual or abrupt. The temperature rises rapidly to 40° C or 40.6° C (104° F or 105° F). Although the pattern of fever can remain persistently high, a considerable number of patients show dramatic temperature oscillations during a period of a few hours.

The rash of RMSF is an almost pathognomonic feature of the disease; it appears by the second or third day of illness in most patients but occasionally is delayed until the sixth day or later. As many as 15 percent of patients never develop a rash. The initial lesions are small erythematous macules that blanch on pressure; they rapidly become maculopapular and petechial and later, in untreated patients, can become confluently hemorrhagic. Rarely, they progress to massive skin necrosis.[16]

Usually, the rash first appears peripherally on the wrists and ankles and, within a few hours, spreads up the extremities to the trunk. An especially striking feature of the rash of RMSF is its regular occurrence on the palms and soles. Eschars, which are characteristic of other rickettsial diseases, rarely are reported with RMSF.[36] On occasion, RMSF can be "spotless" or "almost spotless."[31] The absence of a rash should not delay the institution of appropriate therapy if the historical and clinical features suggest a diagnosis of RMSF.[15,28]

The headache in adults and older children is characteristic. It is intense, persistent night and day, and intractable to all efforts at alleviation. However, young children may not complain of headaches. Toxicity is a salient feature of the disease. Signs of meningoencephalitis are common findings; the patient is restless, irritable, and apprehensive, and this stage can progress rapidly to mental confusion and delirium. Children can become comatose. Meningismus is not always accompanied by abnormalities in cerebrospinal fluid; cerebrospinal fluid is generally clear, with minor elevations in the lymphocyte count (<10/mm^3). A case of eosinophilic meningitis caused by RMSF has been reported.[8] Grand mal or focal seizures can occur. Cortical blindness and central deafness (transient or persistent) have been reported.[4] Other neurologic manifestations can include ataxia, spastic paralysis, and sixth nerve palsy. These signs and symptoms usually are short-lived, but they can persist beyond the acute illness.[14,35] RMSF exerts a mild but consistent effect on intellectual functioning that can result in learning disability and a corresponding difficulty with school performance in children who previously had RMSF.[44]

Cardiac involvement occurs frequently in RMSF. Close observation and evaluation with electrocardiography, echocardiography, and chest radiography are indicated. Congestive heart failure and arrhythmias are common events.[22]

Pulmonary involvement occurs in 10 to 40 percent of reported cases and is manifested as rales, abnormal chest radiograph, or abnormal arterial blood gas measurements. The chest radiograph can show focal infiltrates or pulmonary edema and cardiomegaly.[10,24]

Myalgia or muscle tenderness is a common feature. Characteristically, the patient complains bitterly when the calf or thigh muscles are squeezed.

Ocular manifestations occur most commonly in the retina and include venous engorgement, retinal edema, papilledema, cotton-wool spots, retinal hemorrhage, and retinal artery occlusion. One patient has had severe anterior segment uveitis and an iris nodule, presumed to be due to a widespread RMSF vasculitic process.[11]

Other signs include edema of the extremities or face, a stiff neck, and conjunctival suffusion. Enlargement of the spleen or liver occurs relatively infrequently, yet gastrointestinal symptoms and signs are common manifestations during the early course of RMSF. In one series of 131 patients, 56 percent reported nausea or vomiting, 34 percent had abdominal pain, and 20 percent had diarrhea at the initial visit for medical care. Jaundice also has occurred with RMSF.[38]

As noted with other rickettsial diseases but not adequately explained, patients with glucose-6-phosphate dehydrogenase deficiency appear to account for a disproportionate number of those who die of RMSF.[37]

DIAGNOSIS. Specific treatment must be initiated promptly, and it is imperative that physicians establish a provisional diagnosis based on presenting clinical features and epidemiologic considerations.

Presenting laboratory features are not distinctive but can assist in establishing the diagnosis. For the first 4 or 5 days of illness, the white blood cell count is normal or low. As the disease progresses, leukocyte counts can rise to between 11,000 and 30,000 cells/mm^3. Thrombocytopenia of varying severity occurs in most cases. The adherence of platelets to the surface of *Rickettsia*-infected endothelial cells contributes to the reduction in the number of circulating platelets.[32] Thrombocytopenia and hyponatremia are presenting laboratory findings in more than half of children with RMSF.[5]

Rickettsia rickettsii can be identified by immunohistochemical staining or polymerase chain reaction (PCR) of tissue specimens.[2,42] Assays for PCR-based detection of *R. rickettsia* in blood or tissue specimens are available at the Centers for Disease Control and Prevention (CDC) reference laboratories.[3]

The diagnosis is confirmed by demonstration of a fourfold or greater change in titer between acute and convalescent serum specimens obtained 2 to 3 weeks apart by one of a number of rickettsial group–specific serologic tests.[3] These include enzyme immunoassay (EIA), indirect immunofluorescence antibody (IFA), latex-agglutination, indirect hemagglutination, microagglutination, and complement-fixation tests. The most widely available of these confirmatory tests is the IFA assay. An interval of 7 to 10 days after the onset of illness is required before an antibody response is detectable. On occasion, it is necessary to arrive at a probable diagnosis from a single high-titer convalescent serum specimen. A probable diagnosis is constituted by a single serum titer of 1:64 or greater by IFA; 1:128 or greater by indirect hemagglutination, latex agglutination, or microagglutination; or 1:16 by complement fixation in convalescent serum. The Weil-Felix serologic test is insensitive and nonspecific and should not be used.

DIFFERENTIAL DIAGNOSIS. Measles and meningococcemia are the infections for which RMSF is most frequently mistaken. A petechial rash that involves the palms and soles and spreads centripetally is unlike true or modified measles. A centripetally spreading rash can be seen in the atypical measles syndrome. Thus, a careful history of previous measles immunization, particularly with killed vaccine, should be obtained. Meningococcemia can be a more difficult problem; early in both diseases, a normal or low leukocyte count, signs of meningeal irritation, and cerebrospinal fluid pleocytosis can be present. An inability to definitively differentiate RMSF from meningococcemia should not delay initiation of empiric antimicrobial therapy because both infections are potentially fulminating and fatal. Treatment should be initiated promptly with a tetracycline and with an expanded-spectrum cephalosporin until the diagnosis is confirmed.

The differential diagnosis also includes typhoid fever, leptospirosis, rubella, scarlet fever, disseminated gonococcal disease, infectious mononucleosis, secondary syphilis, rheumatic fever, enteroviral infection, immune thrombocytopenic purpura, thrombotic thrombocytopenic purpura, immune complex vasculitis, hypersensitivity reactions to drugs, murine typhus, rickettsialpox, recrudescent typhus, ehrlichiosis, and sylvatic *Rickettsia prowazekii* infection, which is enzootic in flying squirrels.[35]

General Considerations for the Treatment of Rickettsial Diseases

The rickettsial diseases, especially RMSF, louse-borne typhus, and scrub typhus, are potentially fatal infections for which specific therapy is available. Adequate antibiotic treatment is highly effective, so patients who are treated during the first week of illness almost invariably improve promptly. If the disease is allowed to proceed into the second week untreated, even optimal therapy becomes progressively less effective.

Because rickettsial diseases can be fulminant, prompt initiation of optimal specific therapy is important. Initiation of treatment before the fifth day of illness optimizes the likelihood of having a good outcome.

Doxycycline is the drug of choice for treatment. Although tetracyclines generally should not be used in children younger than 8 years, the rationale for their use in this setting is severalfold: (1) staining of teeth by tetracycline is dose related and unlikely to occur in association with one or two short courses of therapy; (2) doxycycline is less likely to stain developing teeth than are other tetracyclines, possibly because it binds less to calcium; (3) doxycycline is also the treatment of choice for ehrlichiosis.[1] Chloramphenicol may not be effective against ehrlichiosis. A fluoroquinolone is an alternative drug.[33]

For children weighing less than 45 kg, the dosage of doxycycline is 2.2 mg/kg orally or intravenously twice daily. Children weighing 45 kg or more should receive 100 mg orally or intravenously twice daily.[6]

The thrombocytopenia and blood coagulation deficiencies can herald disseminated intravascular coagulation. Prompt initiation of adequate antimicrobial therapy is the first essential step. Severe thrombocytopenia can require platelet transfusion.

Because of the widespread endothelial damage that occurs in severe rickettsial infection, a severely ill patient can be more ill than is apparent. Providing supportive care to the patient is important. Careful sequential evaluation of serum and urine electrolytes, renal function, and body weight is essential for planning and guiding fluid therapy. Hyponatremia is managed by providing maintenance fluids or by instituting modest fluid restriction.

TREATMENT OF ROCKY MOUNTAIN SPOTTED FEVER. Although doxycycline is effective against RMSF, pediatric caregivers must consider RMSF in the differential diagnosis to ensure an accurate diagnosis and institution of proper therapy. Treatment should continue for 3 days after the child is afebrile. The usual duration of therapy is 7 to 10 days.

PROGNOSIS. The fatality rate from RMSF in the preantibiotic era was approximately 25 percent. Recovery is optimized when appropriate antimicrobials are provided within the first 5 days of illness. Nonetheless, the overall mortality rate from RMSF in the United States remains 5 to 7 percent; death occurs primarily in patients in whom establishment of the diagnosis is delayed beyond the first week of illness. Death occurs, usually between the 9th and 12th day of illness, in association with vascular collapse, thrombocytopenia, and renal or heart failure, alone or in combination. Central nervous system involvement and disseminated intravascular clotting commonly occur.

Bronchopneumonia can develop in seriously ill patients, and overzealous administration of parenteral fluid can precipitate cardiac failure. Neurologic deficits such as speech or swallowing dysfunction, global encephalopathy, ataxia or gait disturbance, and cortical blindness are sequelae of infection in approximately 15 percent of children.[5] Digital necrosis occurs rarely. Solid immunity follows recovery from RMSF, even if therapy is initiated as early as a day or two after the onset of symptoms.

PREVENTION. Control of ticks in their habitat would be a costly undertaking and is not practical.[17] In contrast, personal measures for reducing tick contact within infested areas are inexpensive and highly effective. Wearing pants tucked into boots, limiting access to exposed skin around the neck and wrists, and frequently inspecting for ticks reaching these areas are important means of reducing contact. Early de-ticking is particularly valuable because infected ticks must be attached and feeding for 4 to 6 hours or more before they can transmit the disease. The application of repellents such as dimethyl phthalate to clothes and exposed parts of the body affords additional protection. Immunization has the potential to prevent RMSF, but no licensed *R. rickettsii* vaccine is available in the United States.

MEDITERRANEAN SPOTTED FEVER

HISTORICAL ASPECTS. Mediterranean spotted fever was described by Connor in 1910 and is a tick-borne infection caused by *Rickettsia conorii.* The name *Mediterranean spotted fever* was adopted in 1932. Other names given to this illness include boutonneuse fever, Kenya tick bite fever, African tick typhus, India tick typhus, Israeli spotted fever, and Marseilles fever. A resurgence of this disease has been reported, especially in Spain, Italy, and Israel.[51]

EPIDEMIOLOGY. Mediterranean spotted fever is an acute disease caused by *R. conorii*. In the Mediterranean region, the vector is the brown dog tick, *Rhipicephalus sanguineus.* The tick is the reservoir as well as the vector for *R. conorii*, so direct contact with dogs is not the sole risk factor for infection, but habitual contact with dogs is a feature in some children who acquire the infection.

Mediterranean spotted fever occurs in all age groups of both sexes. The epidemiologic pattern is determined by the biology of the tick; consequently, a consistent seasonal peak occurs from June to October. The prevalence of the disease is unknown. The incidence seems to be rising, however, perhaps because of an increase in recognition of the infection and an increase in the number of dogs in urban areas. The incidence in certain occupational subgroups can be reduced through better standards of hygiene.[51]

CLINICAL MANIFESTATIONS. The infecting bite is unnoticed in most cases. Conjunctival inoculation occurs occasionally. The incubation period ranges from 6 to 10 days. The primary lesion, tache noire (black spot), described and named by Pieri in the 1920s, develops at the site of the tick bite, is not painful, and rarely is pruritic.[57] It occurs most often on the head in children and on the legs in adults. The lesion develops central necrosis, an eschar develops, and regional lymph nodes enlarge. The initial lesion heals slowly and resolves after 10 to 20 days without scarring, although residual pigmentation can persist indefinitely.

Tache noire is pathognomonic but is evident in only 30 to 90 percent of patients. Multiple lesions occur occasionally. Formation of the lesion requires a substance secreted by the tick and another of rickettsial origin; it is not reproduced by a separate bite of an uninfected tick or by experimental inoculation of *R. conorii.* An inflammatory infiltrate, predominantly mononuclear, accumulates at the tache noire site, suggesting the importance of T cell–mediated immunity in local host defense.

The onset of disease usually is abrupt, with malaise and fever that reaches temperatures of 39° C to 40° C (102.2° F to 104° F) within the first 2 or 3 days. The fever continues for 6 to 12 days, but antibiotics can shorten the febrile period. Severe and unremitting headache is a typical feature of the disease in adults.[46] Headache is a feature of infection in less than one third of pediatric patients; its lower frequency contributes to the milder presentation in this age group.[48] Arthralgia and myalgia, especially of the leg muscles, is a prominent feature in adults; in children, it is rarely of sufficient severity to restrict mobility.

A maculopapular exanthem that develops on the third to the fifth febrile day is almost universally present. Lesions are present initially on the extremities, spreading within a day or so to the trunk, neck, face, buttocks, palms, and soles. The initial lesions are macular, pink, and irregularly defined but become maculopapular within hours. They generally measure 1 to 4 mm in diameter. The rash persists for 10 to 20 days after the remission of clinical symptoms. It can become purpuric or intensely pruritic. Atypical cutaneous manifestations such as petechial, purpuric, or papulovesicular rashes occur occasionally.[48,51]

The cutaneous manifestations are caused by involvement of the vascular structures of the dermis. Rickettsemia during the

incubation period probably seeds the endothelial cells of the capillaries, arterioles, and venules. The vasculitis produced is much like that seen in RMSF. It gradually disappears during convalescence, and as the maculopapules fade, a brown discoloration of the skin may be noticed.

Cardiovascular and respiratory changes are transient and nonspecific. Bradycardia is the most consistent finding, but other dysrhythmias can occur. In more seriously ill patients, pericarditis, heart failure, and myocarditis have developed.

Phlebitis of the lower limbs is the main vascular complication. Venous thrombosis is a recognized complication, and pregnant patients are particularly prone to venous thrombosis. Pneumonitis, pleuritis, pleuropericarditis, and adult respiratory distress syndrome have been described in association with Mediterranean spotted fever.

In addition to the headache that is characteristic of rickettsial illnesses, varying degrees of impaired consciousness can occur. Rarely, stupor, delirium, convulsions, and transient hypoacusis may be noted. Neurologic sequelae have been observed after rickettsial encephalitis.

Renal function is not altered in most cases, although nephritis with acute renal failure has occurred occasionally. The liver is palpable in a third of patients, and the spleen can be enlarged in 20 percent of children. Tests of hepatic function reveal an increase in serum transaminase levels in one fourth to one half of pediatric cases. Alkaline phosphatase levels are elevated in one third of patients. Needle biopsy of the liver reveals foci of hepatocellular necrosis and a predominantly mononuclear reaction to the necrosis at sites of infection by *R. conorii*. The lesion differs from a true granuloma in that it is not an aggregate of epithelioid macrophages.[58]

A variety of other systemic symptoms can occur. Photophobia and bilateral conjunctivitis have been reported. Severe unilateral conjunctivitis suggests that transmission of the disease occurs by the conjunctival route. Uveitis, choroiditis, retinal artery occlusion, and neuroretinitis are uncommon ocular disturbances.[45,50]

Hematologic abnormalities include isolated cases of autoimmune anemia and mixed cryoglobulinemia associated with Mediterranean spotted fever. Some studies report a high incidence of hypoproteinemia. Immune complex–mediated vasculitis associated with Mediterranean spotted fever has been described.[49]

On occasion, Mediterranean spotted fever can have a malignant, rapidly fatal course, even in previously healthy children. The illness is consistent with a widespread vasculitis characterized by irreversible shock, encephalopathy, disseminated intravascular coagulopathy, and renal failure.[60]

DIAGNOSIS. Rickettsial organisms can be detected by immunofluorescence if biopsy is performed early in the course of disease or by restriction fragment length polymorphism analysis of a PCR product from the tache noire.[55,59] *R. conorii* cannot be isolated from blood cultures by routine laboratory procedures. The clinical findings, geographic location, and epidemiologic considerations help establish the diagnosis.

The diagnosis can be established by one of multiple serologic tests that include complement-fixation, latex-agglutination, microagglutination, Western blot, and indirect IFA assays.[51,53,56] An IFA assay for *R. conorii* is commercially available. Identification of specific IgM by immunofluorescence can identify acute infection, although some patients with proven Mediterranean spotted fever treated early with antibiotics have normal IgM levels.

DIFFERENTIAL DIAGNOSIS. Before the rash appears, differentiation of Mediterranean spotted fever from other acute infections is difficult. Even after appearance of the rash, the disease can be confused with measles, meningococcemia, and secondary syphilis. Other rickettsial diseases should be considered, especially in the absence of a tache noire. Cross-reactions among rickettsiae occur with indirect immunofluorescence.

TREATMENT AND PREVENTION. Mediterranean spotted fever generally has a benign course, and fatalities rarely occur. Doxycycline is the drug of choice. Alternative regimens are clarithromycin or azithromycin administered orally at a dosage of 15 mg/kg/day in two doses for 7 days and 10 mg/kg once daily for 3 days.[47,48] Use of chloramphenicol or ciprofloxacin also is effective therapy. The optimal duration of specific therapy has not been definitively established, and regimens ranging from single doses to treatment for up to 15 days have been reported.[52]

The major effective methods of control involve avoidance of tick bites. Natural immunity occurs after infection, and antibodies persist for as long as 4 years after acute illness. Effective vaccines are not available.[54]

RICKETTSIALPOX

HISTORICAL ASPECTS. First described in New York City in 1946 by Sussman as Kew Gardens spotted fever, rickettsialpox is a benign spotted fever group rickettsial disease caused by *Rickettsia akari*.[65,67,73] Reporting of rickettsialpox declined in the latter decades of the 20th century; fewer than 50 cases were reported by the New York City Department of Health between 1980 and 1999. At least 34 cases were confirmed in 2001 and 2002, possibly as the result of increased awareness and detection of the disease after the bioterrorism anthrax attacks of 2001.[71]

THE ORGANISM. The etiologic agent, *R. akari*, is an obligate intracellular gram-negative coccobacillus that is morphologically identical to *R. rickettsii*. The principal vector is the house mouse mite, *Liponyssoides sanguineus*. Rodents, particularly the house mouse, *Mus musculus*, serve as a reservoir for *R. akari*.

EPIDEMIOLOGY AND TRANSMISSION. Most rickettsialpox in the United States has been reported from New York City, but cases also have occurred in many other cities in the United States and worldwide in Russia, Korea, and South Africa. Whereas house mice are the natural hosts of the mite transmitting rickettsialpox in the United States, rats have been shown to host the mite in Russia, and wild rodents are suspected of carrying the disease in South Africa. The reservoir for rickettsialpox may extend to additional, as yet unidentified, wild or domestic animals.

The disease has a natural cycle between the mite vector and the house mouse.[66] The mite passes the disease transovarially, so it is both a reservoir and vector. Humans acquire infection when depletion of mouse hosts caused by reduced availability of food, poison, disease, or trapping forces infected mites to seek an alternative host, namely, people. The disease affects persons of all ages. Males and females are equally susceptible.[62,63]

PATHOLOGY. Biopsy specimens from eschars are characterized by variable degrees of epidermal and dermal necrosis. Ulcerated lesions typically have neutrophils and thrombosed vessels in the ulcer base. The primary pattern of inflammation is a deep perivascular mononuclear cell infiltrate. Immunohistochemical staining reveals spotted fever group rickettsiae predominantly within the macrophages or mononuclear cells of the perivascular infiltrates associated with eschars or papules.[69]

CLINICAL MANIFESTATIONS. The incubation period of rickettsialpox is 9 to 14 days after the usually painless mite bite

occurs.[62,68] The primary lesion is a papule that develops at the site of the mite bite. This lesion progresses through a papulonodular stage to a tense 0.5- to 2-cm vesicle that ruptures to form an eschar with surrounding induration, which is the hallmark of the disease.[72] Two eschars can be seen. Regional lymph nodes related to the primary eschar are almost invariably enlarged.

Constitutional symptoms develop approximately a week after the mite bite. Fever and malaise are commonly present. The fever is irregular, with temperature fluctuating between 37.8° C and 39.5° C (100° F and 103° F), and rarely lasts longer than 6 or 7 days. It usually is accompanied by the frontal headache characteristic of rickettsial diseases. Rhinorrhea, cough, sore throat, nausea, vomiting, and abdominal pain are occasional findings.[64]

The rash is the most remarkable aspect of the disease. It usually develops within several days of the onset of fever as scattered nonpruritic macules, which rapidly become firm maculopapules; within a day or two, vesicles develop on the summits of the papules. The lesions usually appear on the face, trunk, and extremities, with sparing of the palms and soles.[61] The number of lesions ranges from 5 or 6 to more than 100.

DIAGNOSIS. The diagnosis can be made by immunohistochemical staining of skin biopsy specimens, by PCR of amplified gene products from eschars or culture of *R. akari* from eschars, and by serologic assays. These tests, used singly or in combination to establish the diagnosis, are available through the CDC. A fourfold increase in serum antibodies to *R. akari* between acute and convalescent sera or a single titer of more than a 1:64 dilution is considered diagnostic. Cross-reaction is seen between antibodies to *R. akari* and *R. rickettsii*, but titers are greater when reacted with *R. akari* than with *R. rickettsii* antigen.[71]

DIFFERENTIAL DIAGNOSIS. Anthrax is a differential consideration. Rickettsialpox lacks the striking brawny, nonpitting edema surrounding the eschar that is characteristic of anthrax. Anthrax is not associated with the characteristic papulovesicular rash of rickettsialpox. The haphazard distribution of the characteristic papulovesicles is similar in appearance to chickenpox rash in an adult. Infectious mononucleosis, gonococcemia, and infection with echovirus or coxsackieviruses also should be considered.[70] Patients often give a history of having worked in basements, around incinerators, or in similar areas that might be infested by house mice and their mites.

TREATMENT. Rickettsialpox is a self-limited nonfatal disease that resolves within 7 to 10 days in untreated patients. Treatment shortens the duration of symptoms, usually to 24 to 48 hours.[62] Doxycycline is the drug of choice. A treatment course of 3 to 5 days is sufficient.

OTHER SPOTTED FEVER GROUP RICKETTSIOSES

A number of antigenically distinct *Rickettsia* spp. cause disease in humans. Among these, *Rickettsia japonica* is the etiologic agent of Japanese spotted fever, *Rickettsia sibirica* of Siberian tick typhus, and *Rickettsia australis* of Queensland tick typhus. Dogs are the principal mammalian reservoir; ticks also act as reservoirs by virtue of transovarial transmission. Other newly recognized tick-borne diseases, such as *Rickettsia slovaca* infection, for which the wild boar is the main host, have been described in Europe.[74] *Rickettsia felis* has the unusual niche of a flea host.[75] The diseases caused by these and other rickettsial species have similar clinical, pathologic, and epidemiologic patterns. They produce a mild disease, similar to that of Mediterranean spotted fever.

Treatment is similar to that of RMSF.

TYPHUS GROUP

Two diseases in the typhus group, epidemic typhus and endemic typhus, are considered. Clinically and pathologically, these illnesses are similar; epidemiologically, they are different and hence are described under separate headings.

EPIDEMIC TYPHUS

HISTORICAL ASPECTS. Epidemic typhus, or louse-borne typhus, is transmitted by the human body louse. Epidemic typhus is a classic human plague and has had a major role in the history of nations for centuries. It has undoubtedly been more decisive than some military campaigns, as Zinsser[92] has convincingly described in his book *Rats, Lice and History.*

Epidemic typhus occurs only in the presence of the lice, which multiply to astronomical numbers during periods of war, famine, and poverty. Epidemics occurred in the 19th century in Europe, Asia, Africa, and the United States; the last recorded American epidemic occurred in Philadelphia in 1893. After World War I, more than 30 million people in eastern Europe had epidemic typhus, and an estimated 3 million died. During World War II, typhus affected millions of people in prison camps, the eastern European combat zone, and North Africa. In the 1970s, tens of thousands of cases occurred in uncontrolled epidemics in Burundi and Rwanda in Central Africa. In the 1980s, Ethiopia and Nigeria reported the greatest number of cases worldwide since World War II.[86] A small outbreak occurred in Russia in 1997.[87]

Since 1976, at least 30 cases of disease caused by *Rickettsia prowazekii* have been documented in the United States. They have occurred sporadically. The presumed source of infection is the flying squirrel *(Glaucomys volans)*.[80,85,88]

THE ORGANISM. The etiologic agent is *Rickettsia prowazekii.* Its morphologic features, growth, metabolism, toxin production, and staining characteristics are similar to those of rickettsiae of the spotted fever group. Antigenically, the organisms of louse- and flea-borne typhus form a separate group, although they show some minor antigenic cross-reactivity with the spotted fever group.

EPIDEMIOLOGY AND TRANSMISSION. Humans are the primary reservoir for *R. prowazekii*. Persons who recover from typhus are a reservoir of *R. prowazekii* in interepidemic periods. Relapses in chronically infected persons, known as Brill-Zinsser disease, can occur many years after the primary attack.[83] The presence of sporadic *R. prowazekii* infection, however, suggests that perpetuation of epidemic typhus is possible because it may persist in an animal reservoir.

Transmission of typhus is initiated when a louse becomes infected during a blood meal from a person with *R. prowazekii* infection. After 5 to 10 days of incubation, great numbers of rickettsiae are shed in louse feces. Transmission from an infected louse to a new host can occur by several mechanisms. Because a louse defecates as it feeds, infected feces can be rubbed into a louse bite wound. In addition, dried louse feces can gain access to the mucous membranes of the eye or respiratory tract. The epidemic spread of typhus reflects louse temperature preferences. Lice prefer blood meals from humans with a normal temperature and tend to leave febrile patients (as well as the dead). Crowding renders transfer to new hosts easy.

PATHOLOGY. The pathologic process is similar to that described for the spotted fever group of diseases.

CLINICAL MANIFESTATIONS. The onset of illness usually is abrupt, occurring 1 to 2 weeks after the individual has been

bitten by an infected louse. Because of partial immunity from the primary typhus attack, recrudescent infection almost always is a milder, shorter, and less debilitating illness. The major clinical signs and symptoms of primary disease are fever, headache, and a rash. Body temperature generally rises rapidly to 40° C (104° F) or higher. In untreated patients, it remains at this level with minor fluctuations until death or recovery occurs. The rash usually appears on the trunk by the fourth to seventh day; it spreads peripherally to the extremities and typically spares the face, palms, and soles. Initially, the rash consists of macules that fade on pressure; they soon become fixed as maculopapules and later become petechial or hemorrhagic. A severe, intractable headache is a characteristic manifestation. Typhus fever manifesting as encephalitis, meningitis, or meningoencephalitis can occur. Severe, untreated cases can progress to prostration, stupor, or delirium with terminal myocardial and renal failure. Complications occur uncommonly but can include gangrene, parotitis, otitis media, acute pericarditis, myocarditis, pericardial effusion, pleurisy, pleural effusion, and pneumonia.[79,90,91]

DIAGNOSIS. The diagnosis is established by immunohistochemical staining or PCR of amplified gene products in tissues, by isolation of the organism, or serologically by demonstration of a fourfold rise in antibody titer between acute and convalescent serum specimens obtained 2 to 3 weeks apart. These tests are available through the CDC. The IFA test is the preferred serologic assay, but EIA, dot immunoassay, and latex-agglutination testing are available.[78]

DIFFERENTIAL DIAGNOSIS. The rash of louse-borne typhus begins centrally on the trunk and spreads peripherally to the extremities, whereas the reverse is true for RMSF. Moreover, a rash on the palms and soles, a common event in RMSF, rarely is observed in typhus. Typhus usually occurs in epidemics under conditions of crowding and high louse populations.

TREATMENT. Doxycycline is the treatment of choice. Therapy is given until the child is afebrile for at least 3 days and clinical improvement is evident; the usual duration is 7 to 10 days. Fluoroquinolones or chloramphenicol are alternative agents. Doxycycline is the preferred treatment for relapse of epidemic typhus. Louse-infested people should be treated with gel or cream pediculicides containing pyrethrins, piperonyl butoxide, crotamiton, or lindane to halt the spread of disease.[78]

PROGNOSIS. Case-fatality rates in untreated cases correlate with age. A fatal outcome is uncommon in children; the fatality rate is 10 percent in young adults and as high as 60 to 70 percent in those older than 50 years.

PREVENTION. The insecticides lindane and malathion are effective in reducing louse infestation during typhus epidemics. Dusting insecticides onto the clothes of louse-infested populations is effective for delousing and curtailing louse-borne typhus epidemics. Washing clothing in hot water kills lice and their eggs.

ENDEMIC TYPHUS

HISTORICAL ASPECTS. Endemic or murine typhus is a zoonotic disease passed among rats by the rat flea and only occasionally and accidentally transmitted to humans by the bite of an infected rat flea. The disease occurs worldwide, primarily along coastal areas and around granaries where rats abound. During the first half of the 20th century, endemic typhus was prevalent along the Atlantic seaboard and Gulf Coast areas, where 2000 to 5000 cases were reported annually. Only 60 to 80 cases are currently reported annually, and most of these are from southern California, southern Texas, the southeastern Gulf Coast, and Hawaii.[76,82]

THE ORGANISM. The causative organism, *Rickettsia typhi* (formerly *Rickettsia mooseri*), is similar to *R. prowazekii* in metabolism, growth, toxin production, and staining characteristics, although it is slightly smaller and more uniform in size. The rat flea, *Xenopsylla cheopis*, is the usual vector that transmits *R. typhi* to humans.

EPIDEMIOLOGY AND TRANSMISSION. The rat flea acquires infection when feeding on an acutely ill rat. The rickettsiae multiply in the flea without causing ill effects, but infected flea feces teem with rickettsiae for the rest of the flea's life. Rat fleas prefer to feed on rats but will feed on people if rats are not available. If the flea bites a person, infected feces can be rubbed into the bite wound or transferred in a dried aerosol to the conjunctivae or respiratory tract.

Humans are not related to the maintenance of *R. typhi* in nature. However, serologic and molecular analysis suggests that the cat flea, *Ctenocephalides felis*, which has a propensity to feed on humans, also can serve as a vector.[81] In California, sporadic cases have been related to transmission of *R. typhi* by fleas from opossums to humans.[76] The overall seroprevalence of 0.6 percent in children and the finding of antibody-positive children residing in Kentucky, Oklahoma, and Missouri suggest that typhus may be widely prevalent in the United States.[84]

PATHOLOGY. The pathologic process is analogous to that described for the spotted fever group of organisms.

CLINICAL MANIFESTATIONS. The incubation period is 5 to 10 days after a flea bite occurs. Only half of affected children report an antecedent bite. The most common clinical features are fever, found in all patients, and rash and headache, each observed in three fourths or more of affected children. The classic triad of fever, headache, and rash occurs in only half of affected children.[89] The symptoms and signs are similar to those of louse-borne typhus; the principal differences are that endemic typhus is milder and of shorter duration. The temperature does not rise much above 39° C (102° F). The headache is less severe, and the maculopapular rash is less extensive and of shorter duration. Children who receive appropriate antibiotics undergo defervescence in 1 to 3 days; without appropriate treatment, defervescence occurs after 2 to 3 weeks.[82] Complications seldom occur, and the mortality rate is 1 percent or less.

DIAGNOSIS. A fourfold change in titer between acute and convalescent sera by indirect IFA, latex agglutination, complement fixation, or EIA is diagnostic. Differentiation among antibodies produced in response to epidemic typhus is difficult but may be accomplished by use of an IgM-specific EIA, if needed. Immunohistochemical staining or PCR of amplified gene product of tissues and culture, available through the CDC, also can be used for confirmation.

DIFFERENTIAL DIAGNOSIS. Because endemic typhus is a mild illness and the rash can be evanescent, the disease can be confused with any disease that causes a fever of unknown origin in a patient who does not generally appear to be acutely ill.

TREATMENT. The treatment of choice is doxycycline. Treatment should be continued for at least 3 days after defervescence and evidence of clinical improvement is evident, usually for 5 to 10 days.[77]

PREVENTION. Limiting of rat populations is the principal mode of prevention. Insecticides applied to rat runs can reduce the flea population. Rat populations can be reduced by poisoning, trapping, and eliminating rat harborages and by rat-proofing buildings.

SCRUB TYPHUS

HISTORICAL ASPECTS. Scrub typhus is a febrile illness caused by *Orientia tsutsugamushi*. The infection is transmitted to humans by the bite of trombiculid mites. Scrub typhus was a prominent cause of acute febrile illness among military personnel deployed in endemic regions during World War II and in the Korean and Vietnam conflicts.

THE ORGANISM. The causative organism, *Orientia* (formerly *Rickettsia*) *tsutsugamushi*, is distinguished by remarkable antigenic heterogeneity. Marked strain differences in scrub typhus rickettsiae also appear to contribute to striking differences in severity of disease in the same or different localities. The antigenic heterogeneity thus far has thwarted efforts to develop an effective vaccine.

EPIDEMIOLOGY AND TRANSMISSION. Scrub typhus is confined geographically to a roughly triangular area extending from northern Japan and eastern Russia in the north, to northern Australia in the south, to Pakistan and Afghanistan in the west, thus including Korea, China, Thailand, and other South Asian countries as the region of endemicity.[102] Scrub typhus cases reported from other geographic locales reflect international travel to the endemic area.

Trombiculid mites serve as vectors for infection and transmit the rickettsiae to their own progeny through infected ova. Only one of the four stages of trombiculid mite maturation, the six-legged larval form, feeds on field rodents that are the reservoir hosts. Other stages of the mite are spent in the soil, where they feed on organic matter. Infection occurs most commonly in the rainy months from June to November.

PATHOLOGY. The basic pathologic process of scrub typhus is a perivasculitis of the small blood vessels analogous to the other rickettsial diseases. In addition, an eschar or necrotic inflammatory lesion develops at the site of the mite bite, with subsequent development of regional lymphadenopathy similar to that caused by rickettsialpox. General lymphadenopathy occurs commonly in scrub typhus.

CLINICAL MANIFESTATIONS. In at least two thirds of cases, the initial mite bite lesion develops into a necrotic eschar. The preferential site for eschar distribution in adults is truncal[99]; in children, eschars are seen commonly in moist intertriginous areas such as the genitalia and perineum.[102] The incubation period is approximately 1 to 2 weeks, and characteristic features of the disease develop at approximately the same time that the eschar is noted. After regression of the eschar, a scar often remains and has been shown to persist for up to 25 years.[97]

In a contemporary prospective report of scrub typhus in 30 children presenting with febrile illness of at least 5 days' duration in Thailand, common physical signs included lymphadenopathy (93%), hepatomegaly (73%), eschar (68%), conjunctival hyperemia (33%), maculopapular rash (30%), interstitial pneumonia (30%), and splenomegaly (23%).[102] The rash of typhus fever commonly occurs between the fifth and eighth day of illness; in children, it is not easily recognized without careful observation. The lymphadenopathy is generalized and is especially prominent in the axilla, neck, and inguinal areas. Myocarditis and disseminated intravascular coagulation also have been reported.[100] Central nervous system involvement, observed in one of 30 children in the prospective report, ranges from aseptic meningitis to frank meningoencephalitis.[102] The severity of the clinical manifestations varies widely.

DIAGNOSIS. The mainstay of scrub typhus diagnosis is serologic testing. The "gold standard" is the IFA assay; however, there is little consensus in IFA methodology and positivity cutoff limits. Blacksell and colleagues[93] reviewed the evidence base for various methodologies and the criteria for positive IFA results and concluded that the diagnosis should be based on a fourfold or greater increase in the IFA titer in paired serum specimens and on a single sample only when there is an adequate local evidence base.

Other tests, such as enzyme-linked immunosorbent assay, dot immunoassay, indirect immunoperoxidase, and Weil-Felix assays, are also used.[96,101] The last can be the only serologic test available in less developed countries, but it is poorly sensitive; OX-K agglutinins develop in only a little more than 50 percent of scrub typhus patients. Moreover, OX-K agglutinins also are produced by relapsing fever.

Identification of the rickettsial strain in infected patients by use of strain-specific monoclonal antibodies in an inhibition enzyme-linked immunosorbent assay or PCR with strain-specific primers offers potential for establishing the diagnosis in the acute stage of the illness.[94,95] An eschar PCR assay, evaluated prospectively, demonstrated high sensitivity and specificity compared with IFA.[98]

DIFFERENTIAL DIAGNOSIS. Scrub typhus can be suspected when a patient gives a history of recent exposure in a geographic area where scrub typhus occurs. If, in addition, a local eschar, evanescent rash, and general and regional lymphadenopathy along with fever, headache, and conjunctival suffusion are present, the physician should be alerted to suspect scrub typhus; however, it cannot be differentiated with certainty from dengue, leptospirosis, malaria, or typhoid fever.

TREATMENT. Doxycycline is the treatment of choice. Children have responded well to treatment for 4 to 7 days.[102] Doxycycline is rickettsiostatic, and patients with scrub typhus who are treated in the first week of illness can require sporadic short courses of antibiotic therapy for prevention of relapse.[103] Chloramphenicol also is effective. Strains with reduced susceptibility to antibiotics have been observed in northern Thailand. Rifampin may offer an alternative for treatment of infection acquired in that locale.[104]

PROGNOSIS. The prognosis varies widely because of significant differences in the severity of disease caused by different strains in different populations and in various geographic areas. Mortality rates in the pre-antibiotic era ranged from 1 to 60 percent. With the use of antimicrobials, fatalities rarely occur. When treatment is begun early in the course of scrub typhus, relapses as well as definite second attacks of the disease can occur. The heterogeneity of scrub typhus strains probably accounts for re-infections.

PREVENTION. Vector control involves impregnating clothing and smearing exposed skin surfaces with dimethyl or dibutyl phthalate. Short-term vector control of camping grounds can be accomplished by cutting, burning, or bulldozing vegetation, along with heavy spraying with insecticides such as lindane. Chemoprophylaxis is feasible for persons with high-risk exposure for short periods. Doxycycline given once a week provides effective chemoprophylaxis against naturally transmitted scrub typhus if the prophylaxis is started before exposure to infection and con-

tinued for 6 weeks after exposure.[103] No satisfactory vaccine has been produced.

Q FEVER

HISTORICAL ASPECTS. Edward Derrick named the illness he described in 1937 Q (for query) fever.[109] The Q fever rickettsia was discovered in the late 1930s independently by Burnet and Freeman in Australia and by Cox in the United States; the organism was named *Coxiella burnetii.* Q fever is an acute rickettsial infection worldwide in distribution that is characterized in humans by fever, headache, and an associated pneumonitis in more than 50 percent of cases.[124]

THE ORGANISM. *C. burnetii* is distinctive among rickettsiae in being highly resistant to heat, desiccation, and chemicals. It is a pleomorphic coccobacillus that exhibits antigenic phase variation. Phase I variants are associated with mammalian infection; phase II variants, of reduced virulence, follow propagation and are associated with truncation of the lipopolysaccharide of *C. burnetii*. Acute Q fever is characterized by antibodies against phase II antigens; chronic Q fever is characterized by the presence of anti–phase I antibodies.

EPIDEMIOLOGY AND TRANSMISSION. Q fever is unique among the human rickettsial infections in that it is primarily a disease of animals transmitted to humans by contact with infected animals rather than by an arthropod bite. Q fever is primarily a zoonosis infecting cattle, sheep, goats, and rodents worldwide as well as marsupials in Australia and cats in Canada.[123,131]

In domestic livestock, the infection usually is inapparent and remains latent until some stress or physiologic alteration, such as parturition, leads to multiplication of the organism in birth tissues and excretion of rickettsiae in milk, urine, and feces.[115] At the time of parturition, the placental tissues and fluids of sheep, cattle, and goats contaminate the ground; dried dust particles containing the markedly resistant organisms can be blown about and remain potential sources of infection for many months. Epidemics of Q fever can occur in abattoirs, in textile plants where bales of wool are processed, in tanneries, and in shearing camps as well as in rural areas in children who are exposed at the annual spring lambing time.[112] Outbreaks have been reported in research laboratories.[111,117]

Infection, in most cases, occurs by inhalation. Ticks are a negligible factor in passing the disease to humans but appear important in transmission of the disease to small wild rodents and some domestic animals. Chronic Q fever in pregnancy can involve the placenta. The use of a suppressive regimen that controls the mother's placentitis may contribute to the delivery of a healthy baby.[106] At least two confirmed cases of *C. burnetii* infection in a human fetus have been reported; however, no teratogenic effects have been described. Human milk can serve as a source of infection in breast-fed babies.[125]

The prevalence of Q fever probably is underestimated. More than 40 percent of persons who have frequent contact with farm animals such as goats, cattle, and sheep are seropositive for Q fever antibodies. The disease has been diagnosed in an increasing number of children younger than 3 years and should be considered during an evaluation for fever of unknown origin.[125]

PATHOLOGY. Mortality from Q fever is rare, but the pathologic process has been well defined with the use of both autopsy and biopsy specimens. *C. burnetii* can be seen in lung macrophages at autopsy and in lung biopsy specimens.[122] Liver biopsy specimens demonstrate granulomatous changes with a dense fibrin ring surrounding a lipid vacuole. Rickettsial organisms are not found in these lesions, which are consistent with but not pathognomonic for Q fever.[114] Similar granulomata have been noted in bone marrow.[130] Valvular vegetations are seen when endocarditis complicates Q fever. Organisms have been detected intracellularly from affected valves.

CLINICAL MANIFESTATIONS. The incubation period is 9 to 20 days. A self-limited febrile illness and pneumonia are the most common manifestations of Q fever in children.[116] The most common presentation in adults is an influenza-like illness with varying degrees of pneumonia and hepatitis.[120] The disease usually begins abruptly, and presenting findings can include chills, high fever, malaise, myalgia, chest pain, and headache; rash is not present.[126] Despite a paucity of physical findings, chest radiographs reveal multiple round segmental opacities in more than 50 percent of patients. Pleural effusion, lobar consolidation, and linear atelectasis occur less commonly.[118] Hepatitis is a common clinical feature of Q fever, especially in younger patients.[124] Other findings can include pericarditis, rhabdomyelitis, petechial rash, and hemolytic anemia.[108,113,129] Other reported complications include myocarditis, pericarditis, meningoencephalitis,[110] glomerulonephritis,[128] and inappropriate secretion of antidiuretic hormone.[107] The disease usually is mild and lasts only 1 or 2 weeks. The overall mortality rate is approximately 1 percent.

Adults in whom chronic Q fever and endocarditis develop can have a mortality rate as high as 30 to 60 percent.[127] Chronic disease is rare in childhood. Children with Q fever endocarditis usually have underlying congenital heart disease.[116] Q fever osteomyelitis can involve one or several bones and should be suspected in children with a history of exposure to farm animals or when granulomatous lesions are present on bone biopsy specimens.[119]

DIAGNOSIS. Confirmation of the diagnosis requires one of the following: a fourfold change in antibody titer between acute and convalescent specimens taken 2 to 3 weeks apart to *C. burnetii* antigen by IFA, EIA, or complement-fixation assays; anti–phase II antibody is present in early primary disease, whereas anti–phase I antibody is present in higher titer than anti–phase II antibody in patients with chronic disease[121]; positive PCR result; positive immunostaining of *C. burnetii* in tissues; or isolation of *C. burnetii* from a clinical specimen.[105]

Attempts to isolate the organism should be undertaken only in reference laboratories.

DIFFERENTIAL DIAGNOSIS. Q fever should be considered in children with fever of unknown origin when the history suggests possible exposure to *C. burnetii*. The often severe and puzzling cases of myocarditis, pericarditis, or endocarditis can develop months after the original infection, so Q fever should be included in the differential diagnosis of these processes. In such patients, serologic tests using Q fever antigens can reveal extremely high Q fever antibodies.

TREATMENT. Doxycycline is the drug of choice; treatment reduces the duration of symptoms by several days. The acute disease usually is self-limited. Fluoroquinolones and chloramphenicol are alternatives. Patients with endocarditis should receive prolonged treatment with doxycycline and hydroxychloroquine. Children with Q fever endocarditis have required valve replacement to achieve cure of infection.

PROGNOSIS. The overall mortality rate from uncomplicated Q fever is approximately 1 percent. Most patients recover completely with or without treatment. In the rare instances of complications such as myocarditis, pericarditis, and especially

endocarditis, permanent disability and even fatal outcome can occur.[127]

PREVENTION. Whole-cell and acellular vaccines have been developed for use in animals and high-risk individuals. A whole-cell vaccine is licensed in Australia. Q fever vaccine for children is not licensed in the United States. Adherence to proper hygiene when handling parturient animals can reduce the risk of contracting Q fever in the farm setting. Preventive measures should be in place in animal research facilities.

EHRLICHIOSIS

HISTORICAL ASPECTS. Human ehrlichioses are named after Paul Ehrlich. In the United States, these illnesses are caused by at least three pathogens: *Ehrlichia chaffeensis* is the agent of human monocytic ehrlichiosis (HME),[133] *Anaplasma phagocytophilum* (formerly *Ehrlichia phagocytophila*) is the agent of human granulocytic anaplasmosis (HGA, formerly human granulocytic ehrlichiosis), and *Ehrlichia ewingii* is the agent of *E. ewingii* ehrlichiosis.[140] Each pathogen is tick borne and produces a febrile illness characterized by headache, anorexia, and myalgia. Before the initial description of human illness by Maeda and associates in 1987,[157] the ehrlichioses were considered primarily veterinary diseases. *Ehrlichia canis*, closely related to *E. chaffeensis*, was studied extensively since its recognition in the 1930s as causing an acute febrile illness in dogs. During the 1960s in Vietnam, the illness became known as tropical canine pancytopenia; a large number of trained military working dogs died with symptoms of anorexia, weight loss, and pancytopenia followed by fatal epistaxis.[171] *E. chaffeensis*, a name derived from Fort Chaffee, Arkansas, where the isolate originated, was identified in 1991 as the agent causing HME. In 1994, *A. phagocytophilum* was identified as the causative agent of HGA.[141]

THE ORGANISMS. *Ehrlichia* and *Anaplasma* (formerly *Ehrlichia*) are small gram-negative obligately intracellular bacteria. *E. chaffeensis* and *E. ewingii* multiply preferentially in granulocytes, and *A. phagocytophilum* multiplies in macrophages.

EPIDEMIOLOGY AND TRANSMISSION. Since the initial report of human illness was published, hundreds of cases of HME in adults and many in children have been reported.* Cases of ehrlichiosis have been diagnosed from every state except North Dakota and South Dakota.[161] Reported cases of HME are concentrated in the southeastern and south central areas of the country. Illness occurs in the months when ticks are prevalent, from March to October,[149] and approximately 80 percent of the children in whom the infection is diagnosed have a history of having a tick bite within the 3 weeks before the onset of symptoms. The seroprevalence of *E. chaffeensis* titers of 1:160 or higher was 3 percent among children residing in the southeast and south central regions of the United States, indicating that childhood ehrlichial infections often can be asymptomatic or unrecognized.[159]

The lone star tick, *Amblyomma americanum*, is the principal vector of HME and *E. ewingii* ehrlichiosis, although alternative tick vectors, such as *Dermacentor variabilis*, the American dog tick, and *Ixodes pacificus*, the Western black-legged tick, occasionally can transmit infection in some geographic regions. The reservoir for HME has not been fully clarified, but deer and livestock are the preferred hosts of *A. americanum*. Proximity to a wildlife reserve served as a risk factor for acquiring HME in one reported cluster of cases.[169]

The initial reports of HGA were from the upper Midwestern states of Wisconsin and Minnesota. Most infections have been reported from the northeastern and north central regions, but cases also are reported from the Pacific western states, especially California. Many hundreds of human cases have been identified. A tick bite frequently precedes the illness, and the black-legged or deer tick, *Ixodes scapularis*, is the principal vector in the northeastern and north central states. The Western black-legged tick, *I. pacificus*, is the primary vector in the Pacific western states. Because the predominant host of the deer tick, the white-tailed deer, has a wide geographic distribution, HGA can be more prevalent than is currently documented.[152,163,166]

Perinatal transmission of ehrlichiosis has been documented. The mother apparently was infected with the agent of HGE toward the end of her pregnancy and gave birth to a normal infant. Symptoms in both the mother and infant resolved after treatment with doxycycline.[155]

PATHOGENESIS AND PATHOLOGY. The pathogenesis of ehrlichiosis is incompletely elucidated. Although it is capable of establishing infection in numerous organs and tissues, the primary target cell for HGE is the granulocyte, and for HMA, it is the macrophage. Granulomata of the bone marrow occur frequently in HME, suggesting that involvement of the reticuloendothelial system may be important in pathogenesis.[144]

Organisms enter the cytoplasm of host cells and proliferate in phagosomes into elementary bodies. These individual organisms multiply by binary fission into immature inclusions called initial bodies. Mature groups of elementary bodies form morulae, which are released by rupture of the cell to re-initiate the infecting process.[157]

CLINICAL MANIFESTATIONS. The estimated incubation period for HME is 12 to 14 days. Like RMSF, HME is an acute febrile illness manifested as fever, headache, anorexia with or without vomiting, and myalgia.[150,164,170] The features of HME are shown in Table 207–2. Rash, which can be macular, maculopapular, or petechial, seldom develops in adult infections. In pediatric infections, rash appears to be a common symptom, with a distribution often including both the trunk and extremities.

Meningitis as a manifestation of HME has been reported in children. Symptoms can range from irritability and meningismus to obtundation with response only to painful stimuli.[139,142,151] Initial examination of cerebrospinal fluid reveals pleocytosis

TABLE 207–2 Clinical and Laboratory Features of Adult and Pediatric Monocytic Ehrlichiosis

Feature*	Percentage of Cases	
	Adult (*N* = 46)	Pediatric (*N* = 20)
Fever	96	100
Anorexia	76	78
Headache	80	100
Myalgia	74	67
Rash	20	65
Leukopenia†	61	72
Thrombocytopenia‡	52	78
Elevated aspartate aminotransferase§	76	83

**Some features not specified for all patients.*
†Fewer than 4000 white blood cells/mm³.
‡Fewer than 150,000 platelets/mm³.
§More than 55 U/L.

*See references 136-138, 143, 146, 151, 153, 154, 156, 158, 160.

ranging from approximately 50 to 1400 white blood cells, usually with a predominance of mononuclear cells; a range of 5 to 40 red blood cells; mildly elevated protein; and a normal to slightly low glucose value. Complete recovery is the rule.

One half to two thirds of adults and children have mild leukopenia and thrombocytopenia. One child had a documented decline in the white blood cell count from 13,000 to 1600 during an interval of several hours.[147] Usually, thrombocytopenia is not associated with clinical bleeding, but disseminated intravascular coagulopathy has been reported.[157] Elevations of aspartate aminotransferase, usually modest, peak at approximately 1 week into the illness, with values ranging from twice normal to several thousand. Uncommon manifestations of illness include protracted fever, renal dysfunction occasionally of sufficient severity to require dialysis, hyponatremia, hypoalbuminemia, and toxic shock syndrome.[148,168] Persistent infection during the course of a 2-month interval and infection complicating human immunodeficiency virus disease have been documented.[145,162]

The clinical features of HGA are similar to those of HME, with fever, malaise, myalgia, and headache occurring consistently. Rash occurs in less than 10 percent of cases. Morulae can be demonstrated in the cytoplasm of neutrophils but not mononuclear cells. Leukopenia, generally mild, is a feature of the illness in approximately half of the patients, and thrombocytopenia develops in most. As with HME, serum aspartate aminotransferase activity is elevated in most cases.

DIAGNOSIS. The diagnosis of HME or HGA is confirmed by one of the following: (1) isolation of *Ehrlichia* or *Anaplasma* from blood or cerebrospinal fluid, (2) a fourfold or greater change in antibody titer by indirect IFA in serum samples collected 2 to 3 weeks apart, (3) PCR amplification of ehrlichial DNA from a clinical sample,[134,135] or (4) detection of intraleukocytic morulae and a single IFA titer of 64 or greater.[132] A probable case is defined as a single IFA titer of 64 or greater or the presence of morulae within infected leukocytes.[161]

DIFFERENTIAL DIAGNOSIS. Human ehrlichiosis must be distinguished from other tick-borne diseases, especially RMSF. The illnesses are similar in that both have manifestations of diffuse vasculitis. Ehrlichiosis is less likely to be accompanied by rash and more often is associated with leukopenia or pancytopenia. The similarity of ehrlichiosis and RMSF is emphasized by two retrospective serosurveys in which approximately 10 percent of specimens from patients lacking the serologic criteria for a diagnosis of RMSF fulfilled diagnostic criteria for ehrlichiosis.[154,167] Other tick-borne illnesses, such as Lyme disease, babesiosis, Colorado tick fever, relapsing fever, and tularemia, should be included in the differential diagnosis.

Simultaneous HME and *Borrelia burgdorferi* infection has been described.[136] Whether it represents dual infection or is an instance of antigenic cross-reactivity is unknown. Kawasaki disease can have features mimicking ehrlichiosis; paired sera from a group of children with Kawasaki disease, however, failed to react with a panel of *Ehrlichia* antigens.[165]

TREATMENT. The drug of choice for treatment of human ehrlichiosis is doxycycline. The recommended dosage is 4 mg/kg/day in two divided doses (maximum, 200 mg/day). Treatment should continue for at least 3 days after defervescence for a course of 5 to 10 days.[132] Mild clinical illness can resolve without antimicrobial treatment, although fever can be protracted.[149,150,164] However, human ehrlichiosis can have a fatal outcome, so doxycycline treatment should be initiated when the diagnosis is suspected, without regard for the age of the child. Severe cases can require a longer course of treatment.

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MYCOPLASMA

CHAPTER 208

MYCOPLASMA AND UREAPLASMA INFECTIONS

James D. Cherry

Mycoplasmas and ureaplasmas, the smallest free-living microorganisms, are ubiquitous in nature. More than 100 species have been recovered from many animals, including human beings.[548,669,687,688] Of this group, 17 have been identified as human pathogens or as part of the "normal" human flora, with *Mycoplasma pneumoniae*, *Mycoplasma hominis*, and *Ureaplasma urealyticum* found to cause disease frequently in children. A protean array of illnesses in children is caused by infection with these organisms.

The generic name *Mycoplasma* is derived from Greek and Latin. *Myco* refers to the mycelial, or filamentous, characteristic, and *plasma* indicates the plasticity and pleomorphism of the organism.[646] *Urea* in *Ureaplasma* indicates the presence of urease in this genus.[228] (For neonatal infections, see Chapter 75).

HISTORY

In 1898 Nocard and Roux[509] recovered the first *Mycoplasma* organism from cattle with contagious pleuropneumonia. Shortly after the original discovery, many other mycoplasmas were recovered from several different animals.[265] These other mycoplasmas, which frequently were not associated with disease, originally were called *pleuropneumonia-like organisms;* this designation was abbreviated to PPLO. The term PPLO enjoyed general use until the early 1960s.

The first isolation of a mycoplasma from a human was reported in 1937 by Dienes and Edsall.[152] This organism, now recognized as *M. hominis*, was recovered from an abscessed Bartholin gland. In 1944 Eaton and colleagues[167] reported the recovery of an organism, originally called the *Eaton agent*, from persons ill with primary atypical pneumonia. For many years, the Eaton agent was considered to be a virus, even though it was inhibited by streptomycin and chlortetracycline.[165,166] In 1961 Marmion and Goodburn[431] noted that the Eaton agent was morphologically similar to PPLOs. In 1962 the organism now known as *M. pneumoniae* was cultivated on a cell-free agar medium and was shown in human volunteer studies to be the etiologic agent of primary atypical pneumonia.[87,89,265]

In 1954 Shepard[590] reported the recovery of PPLOs with a distinctive small-colony characteristic from men with and without nongonococcal urethritis. These T strains (T for tiny), as they were called, now are classified as *Ureaplasma.* More recently, another mycoplasma, *Mycoplasma genitalium*, has been associated with genital tract infections, and three mycoplasma species have been noted to co-infect patients with human immunodeficiency virus (HIV) infection.[470,651]

CLASSIFICATION

Mycoplasma and *Ureaplasma* are the two genera of the family Mycoplasmataceae, in the order Mycoplasmatales, which belongs to the class Mollicutes.[228,548,687,688] Both *Mycoplasma* and *Ureaplasma* require cholesterol for growth, have a genome with a molecular weight of approximately 4.5×10^8, and have nicotinamide adenine dinucleotide (NADH) oxidase localized in the cytoplasm. These species lack a cell wall, as do all organisms within the class Mollicutes.[297] Members of the genus *Mycoplasma* do not hydrolyze urea, whereas the two species within the genus *Ureaplasma* do.

The "normal" human mollicate flora includes 14 *Mycoplasma*, 2 *Ureaplasma*, and 1 *Acholeplasma* spp.[82,170,228,392,661,669] These organisms are listed by site of most common isolation and frequency of occurrence in Table 208–1. *Mycoplasma salivarium* and *Mycoplasma orale* are commonly part of the normal respiratory flora and have not been associated with illness in nonimmunocompromised persons. *M. hominis*, *U. urealyticum*, and *Ureaplasma parvum* also are recovered commonly from humans, and frequently they are related causally to illness.

Mycoplasma buccale, *Mycoplasma faucium*, *Mycoplasma amphoriforne*, *Mycoplasma primatum*, *Acholeplasma laidlawii*, and *Mycoplasma lipophilum* rarely are isolated and are not thought to cause disease in non-immunocompromised humans. *M. genitalium* and *Mycoplasma fermentans* have biologic and morphologic features that suggest they may be pathogenic in people, but their role in human genital disease is debated.[647,668,687] Persistent infections in blood, bones, joints, and kidneys with *M. fermentans*, *U. urealyticum*, *Mycoplasma penetrans*, *Mycoplasma pirum*, and *M. hominis* have occurred in patients with immunodeficiencies.[25,468,668] *M. pneumoniae* is a common cause of respiratory and other human illness.

MYCOPLASMA PNEUMONIAE

PROPERTIES

Morphology

Because mycoplasmas lack a cell wall, they all tend to be pleomorphic. Kammer and colleagues[325] studied the morphologic characteristics of *M. pneumoniae* organisms grown in broth medium by scanning electron microscopy and grouped their observations by days of incubation. From 0.3 to 2 days, the predominant morphologic feature consisted of 0.51 ± 0.011-μm symmetric round forms in tightly packed clusters. During the interval from day 2 to day 6, branched and straight filaments and bulbs were the predominant forms. The bulbous elements had a diameter of 0.25 ± 0.006 μm, and the filamentous forms were 0.19 ± 0.005 μm in diameter. The filaments were intertwined, and occasional round forms were observed. From day 6 to day 10, the organisms had a rounded shape but were asymmetric. Their diameter was 0.72 ± 0.027 μm, and they occurred in groups of three or four cells. Biberfeld and Biberfeld[38] noted that *M. pneumoniae* filamentous forms varied in length from 1 to 5 μm.

TABLE 208–1 Mollicutes Flora of Humans Listed by Site of Most Common Isolation and Prevalence

Organism	Prevalence
Respiratory Tract	
Mycoplasma salivarium	Very common
Mycoplasma orale	Very common
Mycoplasma buccale	Rare
Mycoplasma faucium	Rare
Mycoplasma lipophilum	Rare
Mycoplasma pneumoniae	Common
Mycoplasma amphoriferme	Rare
Acholeplasma laidlawii	Rare
Genitourinary Tract	
Mycoplasma hominis	Very common
Mycoplasma genitalium	Rare
Mycoplasma fermentans	Rare
Mycoplasma primatum	Rare
Mycoplasma spermatophilum	Rare
Mycoplasma penetrans	Rare
Ureaplasma urealyticum	Very common
Ureaplasma parvum	Very common
Blood	
Mycoplasma pirum	Rare

Data from references 392, 429, 617, 661, 668, 672, 687, 688.

The ultrastructure of *M. pneumoniae*, as well as that of all members of the family Mycoplasmataceae, is relatively simple and consists of a cell membrane and cytoplasm.[228,667] In 7-day *M. pneumoniae* cultures, Domermuth and associates[153] noted the following characteristics: elementary bodies 105 to 120 nm in diameter, mature cells with a maximal diameter of 690 to 750 nm and an average diameter of 440 to 590 nm, asymmetry of the limiting membrane, electron-dense lines outside the limiting membrane, and dense bodies as cytoplasmic inclusions.

Wilson and Collier[707] studied the ultrastructure of *M. pneumoniae* in hamster tracheal organ culture and noted filamentous organisms with trilaminar membranes; the cells were polymorphic, but each had a specialized terminal structure at the site of attachment to the organ culture. This terminal structure had a dense central core containing a denser central filament. Between the organism and the organ culture cell, fusion was not detected, but a loose network of fibrils was noted between the two surfaces. The bodies of the mycoplasmas contained densely staining fibrillar material and cytoplasmic granules, both of which contained nucleic acid.

Motility and Multiplication

Bredt[59] studied *M. pneumoniae* motility and multiplication on a glass surface in liquid medium by phase-contrast microscopy. The organisms were observed to multiply by binary fission; first, short filamentous structures were formed, which then separated into two cells. A growth cycle between two separations lasted approximately 3 hours. After division, the new cells moved by means of a gliding motion. The gliding speed has been noted to be approximately 0.2 to 0.5 μm/sec, but maximal speeds of 1.5 to 2.0 μm/sec have been observed.[541]

Composition

Mycoplasmas are composed of approximately 40 to 60 percent protein, 10 to 20 percent lipid, and a variable amount of carbohydrate. The *M. pneumoniae* genome is circular, double-stranded DNA with a contour length of 4.8×10^8 d.[474] The guanosine plus cytosine content of the DNA is 38.6 to 40.8 percent.[54,499,548,649]

Growth Characteristics and Physical Properties[84,265,548,646,667]

M. pneumoniae grows in *Mycoplasma* broth medium and on agar enriched with yeast extract and animal serum. *M. pneumoniae* ferments carbohydrates and requires sterol for growth. It grows under both anaerobic and aerobic conditions, but growth is more consistent when it is incubated in nitrogen and 5 percent carbon dioxide. When compared with other mycoplasmas isolated from humans, *M. pneumoniae* grows relatively slowly, with visible formation of colonies rarely occurring in less than 1 week and possibly taking 3 weeks or more. Repeated agar passage results in more rapid growth, and laboratory strains thus treated produce colonies in 3 days.

M. pneumoniae colonies on agar generally appear different from the classic *Mycoplasma* "fried egg" look noted with other types recovered from humans. The *M. pneumoniae* colony is spherical and dense, with a rough ("mulberry") surface.

M. pneumoniae has the following enzyme systems: $NADH_2$ oxidase, nicotinamide adenine dinucleotide phosphate ($NADPH_2$) oxidase, lactate dehydrogenase, probably succinic dehydrogenase, and diaphorases.[265] In liquid medium the following can be noted: acid color change in medium with added glucose and phenol red as a result of glucose metabolism, reduction of methylene blue in medium as a result of dehydrogenase activity, and reduction of 2-3-5 tetrazolium chloride to red formazan by dehydrogenase activity.[646]

On agar, *M. pneumoniae* hemolyzes erythrocytes in an agar overlay by liberation of peroxide. Erythrocytes and other cells adsorb to *M. pneumoniae* colonies, and organisms in suspension cause hemagglutination.

Mycoplasmas including *M. pneumoniae* are heat sensitive. They have a half-life of less than 2 minutes at 50° C and lose viability within 1 week at room temperature.[667] They can be stored for several years at –20° C, but –70° C is optimal for long-term storage.

Mycoplasmas are resistant to osmotic environmental changes but are sensitive to detergents. They are inhibited by gold salts and antibiotics not directed against cell wall synthesis.

Antigenic Composition

The following immunologic reactions have been noted in association with *M. pneumoniae*–host serum interactions: specific complement fixation; precipitation in gel; growth inhibition; indirect hemagglutination; metabolic inhibition; antigen-antibody union identified by immunofluorescence, enzyme-linked immunosorbent assay (ELISA), and radioimmunoassay; adherence inhibition assay; and nonspecific complement fixation (positive serologic test result for syphilis) and agglutination (cold and *Streptococcus* MG agglutinins).[73,291,306,392,538,646] *M. pneumoniae* organisms have both membrane and cytoplasmic antigens.[192,334] Two membrane antigens can be identified by immunodiffusion,[531] with the major membrane antigen being found in the lipid fraction of the organism.[334] The antigens are glycolipids and are of major importance in complement fixation, metabolic inhibition, and mycoplasmacidal reactions.[77] The cytoplasmic (soluble) antigen, which also contains lipid, can be identified by complement fixation when the antigen is prepared by phenol extraction.

Humans have an IgG immune response to six principal protein antigens.[91,140,141,226,358,364,547,685] These six polypeptides have the following molecular masses: 170, 130, 90, 45, 35, and 30 kd. The 170-kd antigen is the P1 protein. This protein is localized at the surface of the terminal organelle (terminal structure), is the major adhesin responsible for attachment, and is the cause of the gliding motility of the organism. Antibodies to P1

protein inhibit hemadsorption and adherence to respiratory epithelium.[287,289,290,305,323,359,630] Another important adhesin is the 30-kd protein (P30 adhesin). In addition to the P1 and P30 proteins, at least six accessory proteins are involved in the adherence of *M. pneumoniae* to host cells.[548] These accessory proteins are HMW1, HMW2, HMW3, and A, B, and C.

M. pneumoniae strains can be divided into two types on the basis of the sequence variation in the P1 gene.[157,158,163] Five subtypes of the type 1 strain and three subtypes of the type 2 strain have been noted by restriction fragment length polymorphism (RFLP). Kannan and Baseman have recently shown that *M. pneumoniae* liberates a 68-kDa protein that possesses ADP-ribosyltransferase (ART) activity.[327] This toxin is immunogenic and is synthesized in vivo, suggesting that it might be responsible for airway cellular damage.

Membrane determinants of *M. pneumoniae* cross-react with the erythrocyte glycoprotein containing the I antigen and the related sugar chain (F1)[271,309]; with pneumococcal serotypes 23 and 32[8]; with the glycolipids of spinach, parsnips, carrots, and selected strains of *Staphylococcus aureus* and group A streptococci; and with perhaps the filamentous hemagglutinin of *Bordetella pertussis*.[250,303,335,681]

Animal Susceptibility

M. pneumoniae grows and causes pneumonia in hamsters and cotton rats and causes inapparent infection of the bronchial epithelium of chicken embryos.[538]

EPIDEMIOLOGY

Epidemic Pattern

In large urban areas *M. pneumoniae* is endemic; infection and disease occur throughout the year. Foy and associates[208,214] noted cultural or serologic evidence of *M. pneumoniae* infection in a Seattle prepaid medical care group during all seasons throughout an 11-year period. Similar endemicity has been reported in other studies.[465,471,508] In addition to the background endemic pattern, *M. pneumoniae* enjoys a cyclic epidemic pattern that is specific for a particular urban community. Epidemics have occurred at 3- to 7-year intervals.[177,214,321,394,508] Epidemics, which develop slowly, usually start in the fall and persist in the community for 12 to 30 months.

As part of an influenza surveillance program, Layani-Milon and associates[373] studied acute respiratory infections in outpatients for six winters in the Rhône-Alpes region of France by using a polymerase chain reaction (PCR) plus a hybridization–based detection system. Each year from 1992 to 1997, at least one peak of *M. pneumoniae* infection was noted in the late fall. Overall, they studied 3897 children and adults with acute respiratory illnesses, and 7.3 percent were found to be infected with *M. pneumoniae*. The yearly rate of *M. pneumoniae* cases fell from 10.1 percent in 1992 to 1993 to 2.0 percent in 1995 to 1996; in the subsequent year (1996 to 1997), it rose to 4.2 percent.

Incidence of Infection and Disease

In the past, most pediatricians and other physicians considered *M. pneumoniae* illness to be uncommon in the general population. The initial epidemiologic studies were concerned mainly with the occurrence of pneumonia in closed populations such as the military and boarding schools.[88,465,568,626,628,679] During the past 43 years, however, many large studies in civilian populations, coupled with more sensitive serologic techniques, have indicated that both infection and disease with *M. pneumoniae* occur commonly.*

Hornsleth[283] examined 367 serum samples collected from children hospitalized in Copenhagen from September 1963 to May 1965 for complement-fixing antibodies. He noted that 42 percent of infants 6 to 11 months of age had demonstrable antibody; from 1 to 9 years of age, more than two thirds of the children had antibody. Suhs and Feldman[634] found a similar high prevalence of hemagglutination-inhibiting antibody in the sera of residents of Point Barrow, Alaska, but only 5 percent of the serum samples from a children's home in Syracuse had measurable antibody.

Brunner and associates[69] detected serum antibody by the sensitive radioimmunoprecipitation test in 28 percent of 7- to 12-month-old infants, 55 percent of 13- to 24-month-old children, 67 percent of 25- to 60-month-old children, and 97 percent of persons older than 17 years. The high antibody prevalence noted in this study, as well as the findings in Copenhagen and Alaska, suggests an infection incidence rate of approximately 20 to 30 percent per year in a susceptible population of young children. The prevalent antibody noted at an early age in these studies possibly is not caused specifically by *M. pneumoniae* infection but instead could be the result of exposure to the many cross-reacting antigens in nature.[8,234,335] However, studies by Fernald and colleagues,[194] in which infants and children in a daycare center were monitored systematically, indicated a yearly infection rate of approximately 12 percent.

Monto and colleagues,[471] in a large study involving 3243 persons, investigated the incidence of infection in six yearly cohort groups of children and adults. Infection was determined by significant rises in titer of complement-fixing antibody on three serum specimens collected during a 1-year period from each subject. The overall yearly infection rate was found to be 5.3 percent. The highest rate (8.8%) occurred in the 5- to 9-year-old group. Infants younger than 1 year had a rate of 2.8 percent.

Brunner and associates[69] noted that geometric mean antibody titers tended to increase with increasing age, which suggested that the older children were being re-infected. Fernald and colleagues[194] reported that 5 of 22 children infected with *M. pneumoniae* in their investigation experienced re-infections during the 5-year observation period. In the study of Monto and associates,[471] 24.4 percent of the 172 infections detected in subjects of all ages were re-infections. During a 12-year serologic surveillance period, Foy and coworkers[214] noted great variation in the incidence of *M. pneumoniae* infection; in the period from October 1965 to May 1966, only 0.2 percent of 398 children had fourfold rises in complement-fixation antibody titer, whereas during the May 1973 to May 1974 period, 35 percent of 246 subjects 10 to 20 years old had serologic evidence of infection.

The incidence of disease caused by *M. pneumoniae* depends on the endemic or epidemic prevalence of the organism in the community and is age related. The incidence of *M. pneumoniae* by age for two epidemics and the surrounding endemic periods in Seattle was studied by Foy and associates.[214] The highest epidemic attack rate was 14 per 1000 children 5 to 9 years of age, and the highest endemic attack rate (4 per 1000) occurred in the same age group. Ten- to 14-year-old children had the second highest attack rate during both epidemic and endemic periods. The attack rate in children younger than 5 years was about twice that observed in young adults.

The ratio of symptomatic to asymptomatic infection has varied in different studies. In family studies, both Balassanian and

*See references 20, 22, 42, 69, 80, 132, 151, 155, 161, 170, 174, 194, 209-212, 214, 217, 219, 239, 240, 242, 248, 283, 311, 318, 321, 394, 402, 408, 454, 471, 472, 503, 508, 519, 589, 634, 666.

Robbins[22] and Foy and associates[212] noted that only 15 percent of infections were asymptomatic, whereas Saliba and associates[568] noted that 55 percent of the residents of a boys' home had asymptomatic infection. Chanock and colleagues[88] observed that only 1 of 30 infections in Marine recruits was manifested as clinically apparent pneumonia.

Incubation Period

The reported incubation period has varied from a mean of approximately 1 week in volunteer studies and point-source epidemics to 3 weeks in community outbreaks.* In a volunteer study, Rifkind and colleagues[553] administered tissue culture–grown *M. pneumoniae* in a concentration of 320 to 1280 EID_{50} (mean egg-infective dose) into the nose and posterior pharyngeal area of 27 men with no demonstrable antibody. In this study, pneumonia occurred 9 to 12 days after inoculation, following 1 to 3 days of upper respiratory illness. In six volunteers, only upper respiratory illness was noted, and the incubation period varied from 4 to 9 days. In a similar study in which volunteers received 10^6 to 10^7 broth-grown organisms, the incubation period was 8 to 10 days.[607]

In an interesting common-source outbreak caused by an intense 8-hour exposure at a party, the peak incubation period was 13 days, and most of the cases occurred between days 11 and 14.[180] In another probable point-source outbreak, which may have resulted from a room aerosol, the incubation period varied from 4 to 9 days.[570] In studies of case-to-case intervals in families, Foy and associates[212] reported a median incubation time of 23 days, with most of the cases occurring between days 16 and 25. In similar studies, Copps and colleagues[132] noted an average interval of 21 days, and Biberfeld and Sterner[42] found a modal value of 20 days. A point-source outbreak in a family unit was described in which all seven family members became ill 10 to 16 days after the onset of symptoms in the index case.[336]

The longer incubation period in the family situation than in the volunteer studies and the point-source outbreaks may be the result of larger inocula in the last instances. In contrast to other respiratory illnesses such as measles and influenza, the spread of disease caused by *M. pneumoniae* in both closed populations such as military training units and boarding schools and open communities is usually slow. For example, the introduction of influenza or measles into a family most often results in infection of all susceptible persons from the primary case. In contrast, the spread of *M. pneumoniae* through a family of six people probably would require three or four passages. Foy and associates[212] noted secondary attack rates in families of 64 percent for children and 17 percent for adults. Biberfeld and Sterner[42] reported secondary infection rates of 41 and 84 percent, respectively, for adults and children in families. In contrast to family groups, the spread of *M. pneumoniae* in schools and other situations of brief exposure is low.[209,218] In one Seattle elementary school, the infection rate was 18 percent. Foy and Alexander[208] believe that neighborhood spread among playmates is more important than school exposure in community transmission of *M. pneumoniae*.

Transmission in families occurs during the acute phase of illness, and transmission by persons with asymptomatic infection has not been documented.[209]

Geography

The endemic and epidemic presence of *M. pneumoniae* has been demonstrated in the urban areas of developed countries with temperate climates throughout the world.† Serologic investigations in more remote areas including both arctic and tropical zones also indicate the presence of *M. pneumoniae* infection. Suhs and Feldman[634] found measurable antibody in the serum of 68 percent of 169 persons in Point Barrow, and Golubjatnikov and associates,[242] in a study of children in a remote Mexican highland community, detected seropositivity in 16 percent of 637 children. Serologic evidence of infection also has been noted in Cairo, Singapore, Hong Kong, the West Indies, and southern Africa.[85,321] Incidence studies have not been performed in rural areas, but patterns of infection probably would be characterized by epidemic periods of a year or so and then complete absence of *M. pneumoniae* circulation for several years.

Sex

Although the results of studies have varied, the difference in the incidence of *M. pneumoniae* disease by sex is small. During the 11 years of study in Seattle, Foy and associates[214] noted that the rate of *M. pneumoniae* pneumonia was higher in women than in men in the 30- to 39-year age group (1.8 versus 1.2 per 1000); in infants, boys were afflicted more often than girls, but otherwise the rates for children by sex were virtually identical. Jensen and associates[318] found that pneumonia, otitis media, and nasopharyngitis in various combinations occurred more commonly in boys than in girls. Monto and colleagues[471] noted that boys younger than 5 years of age had more infections, but that the reverse was true for children 5 to 14 years of age. In other studies involving all age groups, males have shown a slightly greater frequency of illness than females.[430,508] In the Seattle family studies, symptoms were more severe in boys than in girls.

PATHOGENESIS AND PATHOLOGY

Sequence of Events in Infection

M. pneumoniae infection is acquired via the respiratory route from the respiratory secretions of an ill person infected with this agent. Spread can be accomplished by small-particle aerosols or large droplets of secretions coming in contact with the epithelial surface of the nasopharynx and perhaps the surfaces of the lower respiratory tract (trachea, bronchi, and bronchioles) as well. In volunteer studies, Couch[135] observed that the 50 percent human infectious dose by small-particle aerosol was 1 colony-forming unit (nasal instillation required a dose that was 100 times greater).

Because epidemiologic data indicate the need for close and perhaps prolonged personal contact for transmission of infection, transmission by small-particle aerosol probably occurs rarely under natural conditions. After acquisition of the infectious agent, multiplication occurs extracellularly on mucous membrane surfaces. The incubation period, which varies from 4 days to more than 3 weeks, probably depends strongly on the size of the original inoculum. The extent of the respiratory infection increases during the incubation period, and shedding of organisms in respiratory secretions can be observed 2 to 8 days before clinical illness.[135] Initial symptoms of infection include headache, malaise, fever, sore throat, and cough; evidence of lower respiratory tract disease is present within the succeeding 3 days.[151,553] The method of extension of infection within the respiratory tract is unknown. The extent of disease possibly depends totally on the initial distribution of the infectious agent at the time of acquisition rather than on spread of infection from a primary upper respiratory site.

After the onset of clinical symptoms, the concentration of *M. pneumoniae* in respiratory secretions peaks, remains high for approximately 1 week, and then persists for 4 to 6 weeks or longer.[135,151] The associated symptoms and signs in disease caused

*See references 42, 132, 180, 209, 212, 216, 553, 570, 608, 669.
†See references 42, 151, 187, 209, 214, 311, 394, 417, 430, 490, 508, 622.

by *M. pneumoniae* (meningitis, arthritis, hemolytic anemia, rash, pericarditis) suggest the possibility of frequent dissemination of the organism from the respiratory tract. Some reviews on the subject tend to discount the possibility of generalized *M. pneumoniae* infection and attribute the associated systemic clinical findings to immunologic events related to respiratory infection.[135,151] However, little published evidence indicates that the organism has been sought carefully in the blood and other sites of dissemination. In many instances *M. pneumoniae* has been recovered from or identified by PCR in the blood, pericardial fluid, middle ear fluid, vesicular skin lesions, pleural fluid, kidneys, brain, and cerebrospinal fluid (CSF).* In addition, the observation of low CSF glucose in *M. pneumoniae* meningoencephalitis suggests direct involvement by the organism.[346]

Pathology

Pathologic findings in *M. pneumoniae* disease of children have not been reported, and only minimal data from adults are available. However, a reasonable understanding of the pathologic process of *M. pneumoniae* disease can be constructed from studies in the hamster, various tracheal organ cultures, and human biopsy and postmortem material.[156,322,419,456,523,656,714] The primary damage in *M. pneumoniae* infection is to the epithelial lining of the mucosal surfaces of the respiratory tract. This damage has been observed on the surface of bronchi, bronchioles, and alveoli, and clinical symptoms in children suggest that similar pathologic changes occur in the trachea and upper respiratory tract as well. Specifically conspicuous is destruction of the ciliated epithelium of the bronchi and bronchioles. Because of mucosal desquamation and ulceration, the lumina contain considerable debris; added to this debris is an inflammatory exudate consisting of fibrin, mononuclear cells, and neutrophils. The alveolar spaces contain similar exudate and edema fluid.

The walls of the bronchi and bronchioles are thickened by edema and contain an infiltrate of macrophages, lymphocytes, and plasma cells. The alveolar walls are also thickened and contain lymphocytes, mononuclear cells, and erythrocytes. Dilation of the septal capillaries occurs. Edema and cellular infiltration extend into the interstitial spaces. Gross examination of the lungs reveals areas of hemorrhage and congestion. The pleura may contain patches of fibrinous exudate, and pleural fluid may be present. The pneumonic areas may be discrete or widespread.

A biopsy specimen of a vesiculopustular skin lesion revealed an epidermis with mild acanthosis and marked edema that primarily was intracellular.[656] The papillary and upper reticular dermis contained neutrophils and round cells, and hemorrhagic foci were present within the upper corium and epidermis. The blister fluid contained plasma protein and neutrophils. Findings in other organs include mesenteric lymphadenitis, focal hepatic necrosis, follicular splenitis, acute myocarditis, and hemorrhagic encephalitis.

Immunologic Events

SPECIFIC ANTIBODY. A specific serum antibody response usually occurs after infection with *M. pneumoniae*, and it can be measured by many different serologic techniques: immunofluorescence, complement fixation, indirect hemagglutination, precipitation, growth inhibition, mycoplasmacidal antibody test, ELISA, radioimmunoassay, adherence inhibition assay, and radioimmunoprecipitation test.†

Complement-fixing antibodies occur early in *M. pneumoniae* disease, reach a peak titer in approximately 1 month, and then decline slowly over a variable period. Fluorescent-staining antibodies and antibody determined by ELISA have temporal patterns similar to those of complement-fixing antibodies. Growth-inhibiting antibodies appear later (2 to 3 weeks after the onset of illness), peak later, and persist longer than complement-fixing antibodies do. The initial serum immune response includes specific IgM, IgG, and IgA antibodies. After clinical illness and convalescence, specific antibody is located mainly in the IgG serum fraction. Occasionally, significant levels of IgM antibody persist for several months or years after infection.[37,70,173,319] Antibody titer responses in infected children are generally of a lesser magnitude than those in adults.[161] Asymptomatic infections in children may not be associated with a measurable serum antibody response. *M. pneumoniae*–specific IgE antibodies have been detected in the sera of patients with asthma, atopic dermatitis, or both conditions.[662]

After infection, specific antibody is also present in nasal secretions and sputum.[36,66] In volunteer studies, Brunner and colleagues[66] noted that 42 and 73 percent of subjects had respective IgA nasal and sputum responses. Biberfeld and Sterner[43] found specific antibody in 44 of 55 sputum specimens from patients with *M. pneumoniae* infection of the lower respiratory tract. They noted IgA antibody in all specimens tested, IgG antibody in 24 of 31 specimens, and IgM antibody in 13 of 27 specimens.

SPECIFIC CELL-MEDIATED IMMUNITY. Fernald and coworkers[190,191] have shown that lymphocytes from adults previously infected with *M. pneumoniae* undergo blast transformation when cultured in vitro in the presence of *M. pneumoniae* organisms. In age-related studies, researchers noted that only one of nine children younger than 4 years with documented previous infection had evidence of specific cell-mediated immunity as measured by lymphocyte stimulation.[194] In contrast, 7 of 12 children older than 4 years and 87 percent of an adult group demonstrated specific lymphocyte stimulation. This study suggests that specific cell-mediated immunity increases as a function of age and depends on repeated infection. Koh and colleagues[347] noted high levels of interleukin-4 (IL-4) and a high IL-4/interferon-γ ratio in the bronchoalveolar lavage fluid of children with *M. pneumoniae* pneumonia, thus suggesting a predominant T_H2-like cytokine response.

Martin and colleagues[433] found that leukocytes from volunteers with *M. pneumoniae* infection demonstrated chemotaxis in the presence of the organism, whereas leukocytes collected before infection did not. Patients infected with *M. pneumoniae* also respond with interferon-α in their blood and nasopharyngeal secretions early in infection and with interferon-γ during convalescence.[491,492]

NONSPECIFIC RESPONSES. Antibodies to several diverse antigens develop during human infection with *M. pneumoniae*. The best known of these antibodies are cold agglutinins, and they are useful in the diagnosis of *M. pneumoniae* pneumonia.[134,186,266,311,610] Cold agglutinins are directed against the I antigen of erythrocytes.[309,395,610] Most pneumonias in which serum cold agglutinins are noted are caused by *M. pneumoniae*. Cold agglutinins are detected in the serum of approximately 75 percent of patients with *M. pneumoniae* pneumonia; they are less common in *M. pneumoniae* infection without pneumonia.[84]

Patients with *M. pneumoniae* also frequently develop antibodies to the MG strain of nonhemolytic streptococci[393] and occasionally to *M. genitalium*,[397] *M. hominis*,[573] *Mycoplasma hyorhinis*, *Mycoplasma orale*, *Mycoplasma pulmonis*, *M. salivarium*, and *Mycoplasma mycoides* variety *mycoides*, the etiologic agent of contagious pleuropneumonia of cattle.[385] Cross-reacting antibodies to filamentous hemagglutinin and pertactin of *Bordetella pertussis* also

*See references 1, 26, 30, 46, 146, 205, 295, 328, 349, 372, 410, 411, 415, 486, 489, 496, 497, 548, 613, 614, 638, 663.

†See references 67-69, 73, 160, 193, 266, 291, 306, 392, 403, 538, 579, 653.

occur.[681] Other heterologous antibodies found in the serum of patients with *M. pneumoniae* infection include those to smooth muscle, the mitotic spindle apparatus, brain, lung, liver, and Wasserman (WR) cardiolipin antigen.[36,44,396] In addition to these findings, Biberfeld and Norberg[40] detected immune complexes by the platelet aggregation technique in the sera of 16 of 39 patients with acute respiratory illness caused by *M. pneumoniae*. Mizutani and Mizutani[462] demonstrated the presence of circulating immune complexes in most patients with pneumonia caused by *M. pneumoniae*. The same investigators also detected rheumatoid factor in the sera of patients with *M. pneumoniae disease*.[463]

Possible Mechanisms of Disease Production

Numerous studies have been performed in an attempt to understand the pathogenesis of respiratory disease caused by mycoplasmas.[69,102-104,117-119,125,127-130] Of particular interest in human *M. pneumoniae* infection is the apparent high prevalence of infection in infants, children, adolescents, and young adults but the frequently mild nature of disease in infants and young children in comparison with older patients. Some studies suggest that the more severe disease in older patients is associated with reinfection and is mediated somewhat by immunologic responses.

Organ culture and animal studies indicate that damage at the site of primary infection—the respiratory epithelium—is the result of close organism-cell attachment.[102,128,130,288,399,400,484,536,675] This attachment of organism to cell uses neuraminic acid receptors on the cell.[612] In an organ culture system with *M. mycoides* variety *capri*, ciliary damage was decreased when the cellular receptor sites were treated with receptor-destroying enzyme.[102] Lipman and associates[399,400] noted that one attenuated *M. pneumoniae* strain had lost its ability to cytadsorb. The close association of organism and cell allows the transport of specific damaging material to the cell. Although the precise nature of this substance is not known, the data available suggest that it might be hydrogen peroxide. It is liberated by *M. pneumoniae* in vitro; in organ culture studies, hydrogen peroxide has been shown to be the damaging factor in another *Mycoplasma* infection.[102,400,401] In cell culture, *M. pneumoniae* inhibits host-cell catalase activity.[10] This inhibition of catalase activity enhances the toxicity of the hydrogen peroxide generated by the microorganism. *M. pneumoniae* also enters host cells and persists intracellularly for at least 7 days.[24]

Damage to ciliated respiratory epithelial cells also could be caused by the recently identified ADP-ribosylating cytotoxin.[327] Another possible important component of *M. pneumoniae* pathogenicity is the induction of proinflammatory and other cytokines during infection.[710]

Fernald and associates[194] noted that specific cell-mediated immunity to *M. pneumoniae* as measured by lymphocyte transformation becomes more prevalent with increasing age, as does specific antibody. Their studies suggest that more than one exposure to antigen may be necessary to elicit both humoral and cellular responses. Fernald and Glezen,[195] in an inactivated *M. pneumoniae* vaccine trial in children, noted that lymphocyte sensitivity developed in many recipients, but not a humoral antibody response. In a trial of an inactivated vaccine in adults, Smith and associates[607] found that on challenge infection, an exaggerated illness occurred in vaccinees who failed to form humoral antibodies after immunization. These findings have led to the consideration that persistent specific cell-mediated responsiveness might contribute to the pulmonary process in *M. pneumoniae* infection. However, the incubation period of illness in adults and children is similar, which argues against the sensitization theory.[114]

Foy and associates[215] noted that complement-fixing antibodies remained elevated for 2 to 9 years after infection with pneumonia but fell quickly after the second year in persons with mild illness. Protection against re-infection was better in those who previously had pneumonia than in those with mild symptoms.

CLINICAL MANIFESTATIONS

Pneumonia

Pneumonia is the most important clinical manifestation of *M. pneumoniae* infection, and this agent is responsible for 10 to 20 percent of all cases of pneumonia.* The highest incidence of pneumonia caused by *M. pneumoniae* in Seattle occurred in children 5 to 14 years of age.[214] In a study in Chiba Prefecture, Japan, researchers noted that the peak age of lower respiratory tract illness caused by *M. pneumoniae* was 4 years.[488] In a prospective study of community-acquired pneumonia in Finland, 11 percent of the children with *M. pneumoniae* infection were younger than 5 years of age, 32 percent were between 5 and 9 years of age, and 57 percent were 10 to 14 years of age.[267] Although researchers frequently state that *M. pneumoniae* pneumonia rarely occurs in children younger than 5 years of age, in actuality, the incidence in this group was found to be about twice that noted in young adults in Seattle.[214] Pneumonia caused by *M. pneumoniae* occurs less commonly in children younger than 2 years of age and rarely in infants younger than 6 months of age. The apparent frequency of *M. pneumoniae* pneumonia is influenced by the relative occurrence of pneumonia caused by other pathogens. During the child's first 5 years of life, *M. pneumoniae* is only one of many agents (e.g., respiratory syncytial virus, adenoviruses, parainfluenza viruses, influenza viruses, *Streptococcus pneumoniae*, *Haemophilus influenzae*) that cause pneumonia. During later childhood and adolescence, pneumonia as a consequence of infection with these other agents is a rare event; therefore *M. pneumoniae* is the leading cause of pneumonia in these persons.

Because isolation rates of *M. pneumoniae* during both endemic and epidemic periods do not vary greatly by season, as do those of common respiratory viruses, the proportion of patients with *M. pneumoniae* pneumonia increases during the summer months.

SYMPTOMS AND SIGNS. Since 1961, a large number of studies have reported the frequency of signs and symptoms in *M. pneumoniae* infection.† Unfortunately, many studies have included only special populations such as the military and, with few exceptions, community investigations have failed to indicate differences by age. In only three investigations have data regarding children been itemized separately.[64,211,629] Table 208–2 presents the relative frequency of symptoms and signs as compiled from eight studies in which both children and adults were included. The hallmark of pneumonia caused by *M. pneumoniae* is fever and cough. The onset of illness usually cannot be demarcated clearly, but malaise, fever, and headache are early complaints. Cough has an onset 3 to 5 days after the beginning of illness and is initially nonproductive. Foy and colleagues[218] and Biberfeld and coworkers[41] noted that 77 and 100 percent, respectively, of the patients that they studied had maximal temperatures greater than 38.9° C (102° F). Copps and associates[132] found that 58 percent of the group that they evaluated had temperatures higher than 39.4° C (103° F) and that 4 percent had temperatures higher than 40.6° C (105° F).

The reporting of headache in association with *M. pneumoniae* pneumonia has varied considerably. Nakao and associates[490]

*See references 121, 151, 218, 219, 225, 267, 268, 284, 402, 448, 454, 503, 519, 666.

†See references 39, 41, 64, 96, 106, 121, 132, 177, 179, 187, 211, 225, 234, 261, 269, 298, 312, 424, 465, 479, 490, 565, 629.

TABLE 208–2 Frequency of Clinical Findings in Children and Adults with *Mycoplasma pneumoniae* Pneumonia

Finding	Frequency
Symptoms	
Fever	++++
Cough	++++
Malaise	+++
Headache	++
Sputum	++
Chills	++
Hoarseness	+
Earache	+
Coryza	+
Sore throat	+
Diarrhea	+
Nausea and/or vomiting	+
Chest pain	+
Signs	
Rales	+++
Pharyngitis	++
Lymphadenopathy	+
Conjunctivitis	±
Rash	±
Otitis media	±

++++, close to 100 percent; +++, 75 percent; ++, 50 percent; +, 25 percent; ±, 0 to 10 percent.

Compiled from eight studies in which both children and adults were included: references 39, 41, 132, 218, 225, 312, 424, 490.

noted this complaint in only 8 percent of subjects, whereas Biberfeld and associates[41] and Foy and colleagues[218] reported it in two thirds of those studied. Chills and the production of sputum are present in about 50 percent of ill patients. Again, great differences among investigations are noted, and they probably are related to the relative ages of the patients.

Coryza is unusual in *M. pneumoniae* pneumonia; therefore its occurrence should suggest another etiologic agent for illness in a specific patient. In a study involving children exclusively, Stevens and colleagues[629] noted that coryza occurred more commonly in young children; as might be expected, they found productive cough more frequently in their older patients. Hoarseness, earache, sore throat, gastrointestinal complaints, and chest pain occur in approximately 25 percent of patients.

On physical examination, about 75 percent of patients have auscultatory evidence of pneumonia, and approximately half have pharyngitis. Remarkable lymphadenopathy, particularly with cervical involvement, is noted in approximately 25 percent of patients. Twenty-one percent of the patients studied by Foy and associates[218] had otitis media. In other studies, this manifestation was noted in about 5 to 10 percent of cases. Conjunctivitis was reported in almost half the patients reported by Fransen and associates.[224] In contrast, except for Jansson and colleagues,[312] who noted conjunctivitis in 3 percent of their study group, this finding was not mentioned in the other reports. Similarly, rash was reported in 6, 11, and 17 percent, respectively, in the studies in Minnesota, Wisconsin, and Seattle,[132,219,424] but it was not mentioned in the other studies.

The most common finding on chest auscultation is dry rales, but musical rales with expiration are noted occasionally. Rales usually persist for 2 weeks, and hearing them a month or more after the onset of disease is not unusual. Occasionally, patients have no auscultatory evidence of pulmonary disease throughout their illness, in spite of the presence of abnormalities on chest radiographs. During illness, cough becomes increasingly prominent; initially it is nonproductive, but later, it may produce a frothy white sputum in older children and adolescents. The sputum also may appear purulent and contain blood. Cough persists for 3 to 4 weeks and longer after nonrespiratory symptoms such as fever and headache have subsided.

In a study of 44 children with lower respiratory illness caused by *M. pneumoniae*, Stevens and associates[629] noted the following frequencies of symptoms and signs: cough, 97 percent; malaise, 82 percent; vomiting, 40 percent; abdominal pain, 35 percent; headache, 32 percent; rash, 20 percent; fever higher than 38° C (>100.4° F), 78 percent; rales, 78 percent; pharyngitis, 32 percent; rhonchi, 30 percent; bronchial breathing, 27 percent; and otitis media, 27 percent. Foy and coworkers[211] found that chills and productive cough were more common in adults than children and that temperatures tended to be higher in children.

In a large study involving 108 children with *M. pneumoniae infection*, wheezing occurred with the acute illness in 40 percent.[565] When the children in this study were evaluated 3 years after their acute illness, they were found to have three indicators of lung function that had mean values significantly lower than those in control children.

Few reports specifically describe pneumonia caused by *M. pneumoniae* in young children.[21,95,122,212,254,488,603,629] However, a review of the case descriptions available indicates that illness, when it occurs, can be severe and relatively prolonged when compared with common viral and bacterial infections. Singer and DeVoe[603] reported a 3-year-old severely ill child who had a temperature of 39.4° C, a pulse of 150, and a respiratory rate of 40. Diffuse pulmonary involvement of the right upper lobe and lingular segments of the left lower lobe was observed by radiography, although rales and altered breath sounds could not be heard. The patient's condition worsened over a 6-day period. At this time, specific therapy with erythromycin was instituted, and slow recovery followed. The child had a normal white blood cell count, transiently elevated serum aspartate aminotransferase and alanine aminotransferase values, and microscopic hematuria. Grix and Giammona[254] observed two 5-year-old children with extensive pneumonia, pleural effusions, and febrile periods of 10 and 16 days. Stevens and associates[629] reported a 5-year-old boy with pulmonary consolidation and aseptic meningitis, and Clyde and Denny[122] described an asymptomatic 3-year-old boy with a "feathery infiltrate" in the right upper lung field. In a family study, Foy and colleagues[212] investigated four children younger than 6 years. In a 4-year-old child, the pneumonia persisted for more than 1 month, and in this child's brother, the illness lasted approximately 2 weeks. I have seen a 4½-year-old girl with scattered infiltrates throughout both lung fields and febrile illness of 14 days' duration.[95]

Severe and extensive pulmonary disease occurs occasionally in *M. pneumoniae* infection.* Massive lobar pneumonia is noted on occasion, and pleural effusions are fairly common findings.† A number of cases of necrotizing pneumonitis with massive pleural effusions have been described.[109,691] All these children had protracted periods of fever and respiratory distress. The adult respiratory distress syndrome has been observed, and illness has suggested pulmonary embolism with infarction.[202,542,601,678] Chronic interstitial pulmonary fibrosis and fulminant fatal diffuse interstitial fibrosis have been noted in two adults with *M. pneumoniae* pneumonia, and localized bronchiectasis developed in a 20-year-old man at the site of previous acute lung infection.[108,244,323,639] Children who had a delay in onset or inadequate duration of macrolide treatment were found by Marc and associates[426] to have reduced lung diffusion capacity after

*See references 97, 100, 108, 109, 112, 131, 199, 202, 209, 254, 257, 331, 391, 404, 421, 426, 452, 479, 489, 510, 514, 523, 542, 554, 596, 599, 601, 603, 616, 621, 691.

†See references 20, 100, 107, 109, 123, 149, 199, 202, 254, 407, 410, 480, 487, 489, 510, 603, 621, 691.

contracting *M. pneumoniae* pneumonia. A 5-year-old girl had severe necrotizing pneumonitis, and an adult had bronchiolitis obliterans organizing pneumonia.[404,514] *M. pneumoniae* pneumonia is generally more severe in patients with preexisting cardiorespiratory problems, immunodeficiencies, and sickle-cell disease.[31,112,223,231,314,452,528,596,616]

Four patients—one child, one adolescent, and two adults—have been found to have lung abscesses in association with *M. pneumoniae* infection.[108,391,599] The illness in the adolescents and adults was characterized by productive cough and chest pain for 2 to 4 weeks. In one patient, clinical recovery and clearance of the pulmonary lesion were dramatic with tetracycline therapy; two other patients received suboptimal therapy but eventually recovered. The child with a lung abscess was a 6-year-old girl who had a 7-day history of cough and a 4-day history of fever.[108] In addition to the abscess, she had pleural effusion, thrombocytopenia, and disseminated intravascular coagulation. One 18-year-old man with extensive consolidation of the right lower lung field had residual pleural scarring 8 months after the acute illness.[480]

A newborn who contracted congenital pneumonia, probably via vertical transmission of *M. pneumoniae*, has been described.[677]

Clyde[121] reported factors that correlated with *M. pneumoniae* pneumonia in a study of 1139 subjects with community-acquired pneumonia. Positive factors were sore throat, headache, fever of 38.9° C or higher, exanthem, family size of four or more, and ear infection. In the same study, pneumonia did not correlate with coryza, leukocytosis (15×10^9/L or more and 10×10^9/L or more), preexisting disease, recurrent pneumonia, hospitalization for treatment, or cigarette smoking.

Although recovery from *M. pneumoniae* pneumonia is usually complete, two studies suggest that persistent abnormalities in lung function can occur after illness.[469,565]

RADIOGRAPHY. Because the classic clinical entity, primary atypical pneumonia, has numerous causes but often is used as a synonym for *M. pneumoniae* pneumonia, much confusion has ensued about the spectrum of the radiographic appearance of the specific mycoplasmal infection. The radiographic pattern of primary atypical pneumonia is varied, but bilateral, diffuse, reticular infiltrates are common components.[409,580,606] Subsequent study has indicated that the diffuse interstitial pattern is an uncommon finding in *M. pneumoniae* infection and more often is the result of infection with other agents such as viruses, fungi, and *Chlamydia*.[63,233,498,540,674]

Brolin and Wernstedt[63] carefully evaluated the radiographic findings in 56 patients with significant *M. pneumoniae* pneumonia; 21 of the patients were younger than 20 years. They noted the following distribution of different patterns: typical lobar pneumonia, 8 patients; predominantly alveolar but not total consolidation, 13; interstitial (either reticular or nodularetic), 20; a combination of lobar pneumonia and other alveolar involvement without total consolidation, 2; a combination of lobar involvement and interstitial, 10; and a combination of alveolar involvement without total consolidation and interstitial, 3. Alveolar patterns were more common in females; interstitial involvement was found more frequently in males. Twenty-two percent of patients had enlargement of the hilar or paratracheal lymph nodes, and 14 percent had pleural effusion.

The persistence of radiographic changes is variable. Brolin and Wernstedt[63] noted that 13 percent of their patients who underwent follow-up studies had abnormal findings more than 4 weeks after the initial study. They observed that persistence tended to be longer in patients with alveolar disease than in those with interstitial patterns. The degree of clinical symptoms and pulmonary physical findings frequently correlates poorly with the apparent degree of involvement noted on radiographs. In many patients with significant symptoms, only minimal interstitial changes are observed. In other instances, patients with lobar pneumonia often have few clinical findings indicating pulmonary disease.

Lee and colleagues[376] reported the chest CT features in 11 children and 5 adults with *M. pneumoniae* pneumonia. The children had the following findings: lobar or segmental consolidation 100%, pleural effusion 82%, lymphadenopathy 82%, and volume decrease of the involved lobe 73%. None of the children had diffuse nodules of ground-glass attenuation, septical thickening, or bronchial wall thickening. In contrast with the children, the adults had diffuse nodules of ground-glass attenuation 80%, septical thickening 40%, lymphadenopathy 40%, bronchial wall thickening 40%, and volume decrease of the involved lobe 20%. None of the adults had lobar or segmental consolidation and pleural effusion.

Kim and associates[339] performed high-resolution computed tomography on 38 children who had been hospitalized with *M. pneumoniae* pneumonia 1 to 2.2 years previously. Abnormalities in two or more lobes, which corresponded to the initial chest radiographic infiltrates, were found in 37 percent of the children. Young age (<8 years) at the time of hospitalization and high *M. pneumoniae* antibody titer were found to be risk factors for the subsequent abnormalities.

NONSPECIFIC LABORATORY DATA. The total leukocyte count in patients with pneumonia caused by *M. pneumoniae* is most often normal, but variation is considerable.* In a group of more than 250 children younger than 15 years, Foy and associates[211] noted that 30 percent and 6 percent had total leukocyte counts greater than 10,000 and 15,000 cells/mm^3, respectively. In a group of 45 children, Stevens and colleagues[629] observed leukocytosis in 33 percent of patients and leukopenia in one patient. Sixty-seven percent of the children had neutrophilia, and one patient had neutropenia. An increased percentage of band form neutrophils in *M. pneumoniae* pneumonia is an unusual finding.

The erythrocyte sedimentation rate is elevated in all cases,[39,132,311] and this elevation is usually marked. Biberfeld and colleagues[39] noted that 16 of 37 patients had erythrocyte sedimentation rates of 50 mm/hr. Serologic tests for syphilis are found to be falsely positive on occasion, and serum cold agglutinins and antibodies to *Streptococcus* MG antigen are common findings.[37,84,88,151] Results of the direct Coombs test are frequently positive, and elevated levels of serum IgM are noted.[37,186] Urinalysis results are generally normal.

Respiratory Disease Other than Pneumonia

COMMON COLD AND UNSPECIFIED UPPER RESPIRATORY ILLNESS. By strict definition (significant nasal symptoms, no pharyngitis, and minimal fever), *M. pneumoniae* rarely causes the common cold. However, mild upper respiratory illness is noted frequently as the only manifestation of *M. pneumoniae* infection in children, adolescents, and young adults.† The frequency of unspecified upper respiratory tract illness as a manifestation of *M. pneumoniae* infection, when compared with other manifestations resulting from infection with this agent, varies considerably among studies. Feizi[185] studied patients from a country practice in England and noted that 50 percent of the patients had upper respiratory tract symptoms. Illness in these persons was often prolonged, however, and lasted as long as 7 to 10 weeks. In a review in Scotland, only 3 percent of 596 *M.*

*See references 39, 132, 177, 211, 218, 225, 311, 465, 629, 674.
†See references 85, 133, 151, 161, 183, 185, 187, 194, 212, 218, 282, 373, 408, 465, 490, 502, 568.

pneumoniae infections were classified as upper respiratory tract illness. In studies of common respiratory illnesses in children in which viruses and other agents were sought, *M. pneumoniae* was noted in 2 to 5 percent of patients with upper respiratory tract illness.[85,151,408]

PHARYNGITIS AND NASOPHARYNGITIS. As noted in Table 208–2, pharyngitis is observed in approximately half of all patients with *M. pneumoniae* pneumonia. However, pharyngitis as the major manifestation of *M. pneumoniae* infection occurs less commonly. Parrott[525] found that 12 percent of children admitted to the hospital with severe "bronchitis-pharyngitis" had *M. pneumoniae* infection. Jensen and associates[318] noted the frequent occurrence of pharyngitis and otitis media in children infected with *M. pneumoniae*. In a study of 715 children and adolescents with pharyngitis, Glezen and colleagues[238] reported that 36.8 percent had group A streptococcal infection and 3.1 percent were infected with *M. pneumoniae*. When the *M. pneumoniae* infections were grouped by age, the peak (11.4%) occurred in the 12- to 14-year-old group, and none were observed in children younger than 6 years. Five patients with *M. pneumoniae* infection had concomitant group A streptococcal infection, but the illnesses in these cases could not be distinguished clinically from those caused by either agent alone. Cervical lymphadenopathy occurred in approximately 50 percent of those infected, and the pharyngeal lesion was exudative in 43 percent. In a study of 127 children with acute pharyngitis, 25 (20%) were found to have serologic evidence of *M. pneumoniae* infection.[174] Seven (28%) of these children had pharyngeal exudates and 10 (40%) had cervical lymphadenopathy.

In a study involving 131 adult patients with pharyngitis, 10.6 percent were found to have serologic evidence of *M. pneumoniae* infection.[351]

OTITIS MEDIA AND BULLOUS HEMORRHAGIC MYRINGITIS. Although the incidence has varied in different studies, otitis media is noted in approximately 5 percent of children and adolescents with *M. pneumoniae* pneumonia. The role of *M. pneumoniae* as an etiologic agent in common acute otitis media in children is unclear. Halsted and associates[258] noted that 12 percent of children with otitis media had serologic evidence of *M. pneumoniae* infection, but they were unable to recover the agent from middle ear fluid. In a study in which children were selected because of *M. pneumoniae* infection in a family member, 47 of 49 children with otitis media had *M. pneumoniae* infection.[318] Räty and Kleemola[545] used PCR to detect *M. pneumoniae* in 16 of 380 (4%) middle ear fluid samples from 138 children with acute otitis media.

In a volunteer study, myringitis developed in 13 of 52 subjects.[553] Findings were usually bilateral and associated with throbbing pain. The appearance of the tympanic membrane varied from mild injection to severe inflammation with edema. Hemorrhagic areas on the drum were noted in five subjects, and serous-appearing blebs containing blood were observed in two. Bullous myringitis also has been noted only occasionally with natural *M. pneumoniae* infection.[64,122,212,218,424,613] In one study of 148 children and adults with *M. pneumoniae* pneumonia, 27 (18%) were found to have bullous myringitis.[424] Using PCR Kotikoski and associates[355] studied middle ear fluid from 30 children and blister fluid from 12 children with acute meningitis and found no evidence of *M. pneumoniae* DNA.

SINUSITIS. Although clinically recognized sinusitis has been reported rarely in patients with *M. pneumoniae* infection, Griffin and Klein[253] found radiographic evidence of sinusitis in approximately two thirds of a group of Navy recruits with *M. pneumoniae* pneumonia. In general, the patients with sinusitis had more prolonged illness than did recruits without sinusitis. Savolainen and colleagues[575] noted that 11 of 310 patients with acute maxillary sinusitis had fourfold or greater rises in complement-fixing antibody to *M. pneumoniae*. In chronic suppurative maxillary sinusitis, cultures for *M. pneumoniae* have been performed but no organisms were isolated.[35,619]

ACUTE BRONCHITIS. Acute bronchitis characterized by fever, cough, and rhonchi, with or without associated pharyngitis, is a frequent manifestation of *M. pneumoniae* infection.* Of 40 patients with *M. pneumoniae* infection, Feizi[185] reported that 6 had bronchitis, 3 had upper respiratory tract illness plus bronchitis, and 1 had sinusitis plus bronchitis. In contrast to these findings, Hornsleth[283] noted that only 1 of 25 patients with *M. pneumoniae* infection had acute bronchitis. In the differential diagnosis of acute bronchitis, *M. pneumoniae* infection accounts for 10 to 20 percent of cases.[86,90,151,178,531]

CROUP. *M. pneumoniae* infection has been associated only occasionally with croup. Parrott[525] found no instances of *M. pneumoniae* infection in a large number of children with croup, and Chanock and Parrott[90] did not list this agent as an etiologic consideration in croup. In contrast, extensive studies in both Seattle and Chapel Hill have revealed that approximately 2 percent of croup cases are associated with *M. pneumoniae* infection.[151,210,240,407] Because no descriptions of clinical illness are available, a reasonable assumption is that croup caused by *M. pneumoniae* infection is generally mild and without distinguishing characteristics.

BRONCHIOLITIS AND INFECTIOUS ASTHMA. Approximately 5 percent of cases of bronchiolitis are caused by infection with *M. pneumoniae*, but the percentage varies among studies.[85,86,90,151,161,210,240,407,525] In two large studies, no instances of *M. pneumoniae*–associated bronchiolitis were reported.[282,283] *M. pneumoniae* also is a relatively common cause of asthmatic bronchitis and recurrent wheezing in asthmatic children.[34,281,292,519,589] Horn and associates[281] noted that *M. pneumoniae* was isolated from 6.6 percent of children with wheezy bronchitis, and Berkovich and associates[34] found *M. pneumoniae* infection in 7 of 33 episodes of wheezing in asthmatic children. Biscardi and coworkers[45] studied children 2 to 15 years of age hospitalized for severe asthma. Of 119 children with previously diagnosed asthma, 24 (20%) had *M. pneumoniae* infections during the exacerbation that caused their hospital admission. In 51 children with first asthma episodes, *M. pneumoniae* infection was demonstrated in 26 (50%). Children with a first attack of asthma associated with *M. pneumonia* infection were significantly more likely to have asthma recurrences than children whose primary episodes were not related to *M. pneumniae* infection. Lehtinen and colleagues[381] studied 220 children with wheezing associated with viral infection and found that 5% were co-infected with *M. pneumoniae*. Wheezing also occurs during *M. pneumoniae* pneumonia.[21,565] Freymuth and colleagues[228] identified *M. pneumoniae* in nasal aspirate samples by PCR in 3 of 132 children (2%) with acute exacerbations of asthma.

OTHER. Exacerbations of chronic obstructive pulmonary disease have been associated with *M. pneumoniae* infection.[105,368,449,609,701] By culture, Cherry and associates[105] did not obtain any *M. pneumoniae* isolates from the bronchial specimens of adults with chronic bronchitis. However, more recently, Kraft and colleagues,[357] using PCR, detected *M. pneumoniae* in the bronchoalveolar lavage or bronchial biopsy specimens of 9 of 18 adults with chronic asthma. Smith and associates[609] were unable to show that patients with chronic obstructive pulmonary disease had increased susceptibility to infection in comparison with

*See references 85, 86, 90, 178, 185, 282, 283, 490, 502, 525, 568.

normal subjects. Illness suggestive of pertussis has been described in three children.[369,629] Teig and associates[655] studied nasal brush specimens and induced sputum from 38 children with stable chronic lung disease and from 42 healthy controls for the presence of *M. pneumoniae* DNA by PCR. Specimens from 4 (10.5%) of the children with chronic lung disease had *M. pneumoniae* DNA, whereas none of the specimens from control patients were positive. In two provocative studies Esposito and coworkers[175,176] found that *M. pneumoniae* infections may play a role in recurrent respiratory infections in children. In their studies they found that children with tonsillopharyngitis or other respiratory infections due to *M. pneumonia* who were treated with azithromycin (10 mg/kg/d for 3 days weekly, for 3 weeks) were significantly less likely to have recurrent repiratory tract infections than children with similar *M. pneumoniae* infections who did not receive azithromycin treatment.

Exanthem and Enanthem

Exanthem as a manifestation of *M. pneumoniae* infection is a common occurrence, but its incidence has varied considerably among different studies.* In large studies involving children in which *M. pneumoniae* infection was evaluated in a specific geographic area, the incidence of exanthem has varied from 3 to 33 percent.† Foy and associates[218] noted rash in 17 percent of 319 patients with *M. pneumoniae* pneumonia during a 5-year surveillance period. In a study involving only children, Stevens and colleagues[629] noted exanthem in 9 percent of their patients. Copps and coworkers,[132] in a community outbreak in La Crosse, Wisconsin, found that 11 percent of their patients with pneumonia also had rash.

The cutaneous manifestations in *M. pneumoniae* infection are protean. Most common is an erythematous maculopapular rash that is most prominent on the trunk and back; the lesions may be discrete (rubelliform) or confluent (morbilliform). Though not the most common cutaneous manifestations of *M. pneumoniae* infection, erythema multiforme and Stevens-Johnson syndrome are the most often reported and the most serious.‡

In Table 208–3 the clinical findings in 29 well-documented cases of *M. pneumoniae* infection with exanthem are presented; in Table 208–4 the specific mucocutaneous findings in 20 of the 29 patients are itemized. Of the total group, all but 8 were males, 24 of the 29 were younger than 20 years, and 12 of the 20 were younger than 11 years. The duration of exanthem was longer than 7 days in all but 2 patients; all patients were febrile, and in 17 cases, the rash occurred during fever.

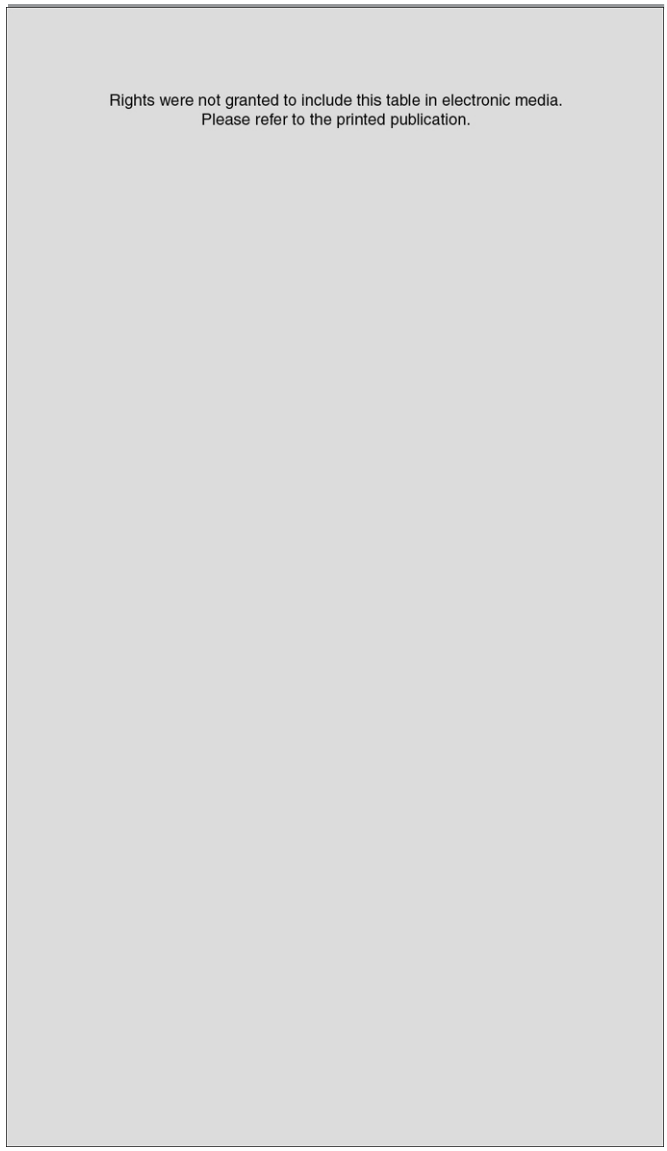
Fourteen patients had generalized ulcerative stomatitis, and seven had tonsillitis or pharyngitis. Severe conjunctivitis was observed in eight patients, and this manifestation was seen only in those with vesicular or bullous cutaneous lesions. All eight patients with severe conjunctivitis also had generalized ulcerative stomatitis. Surprisingly, vesicular or bullous exanthems with oral and eye lesions (Stevens-Johnson syndrome) rarely occur in females.[20,437,629]

As noted in Table 208–3, 25 of the 29 patients had pneumonia. The occurrence of rash as the major manifestation of *M. pneumoniae* infection is probably rare. In a study of 112 patients who had suspected infectious exanthems without pneumonia, Cherry and associates[99] could find none with *M. pneumoniae* infection. Foy and colleagues[212] noted a 2-year-old child with only rash, Stutman[633] reported a 15-year-old boy with Stevens-Johnson syndrome but not pneumonia from whom the organism was recovered from a vesicular lesion, and Ruhrmann and Holthusen[562] observed frequent cases of mild erythema multiforme without pneumonia.

In an analysis of 42 cases of erythema multiforme seen during a 20-year period, Villiger and associates[680] noted a presumptive or definite diagnosis of *M. pneumoniae* infection in 14 children. Nine of the children were boys (64%), and eight of the illnesses had mucous membrane involvement.

Many patients with *M. pneumoniae* infection and exanthem have a history of antibiotic administration before development of the rash; this observation suggests the possibility that the rash is drug induced rather than a result of the infectious process. As noted in Table 208–3, 17 patients had received antibiotics before the rash appeared, and the exanthem was present before the administration of antibiotic therapy in 10. Although these data incriminate the infection as a cause of exanthem, the large number

TABLE 208–3 Selected Clinical Findings in 29 Patients with *Mycoplasma pneumoniae* Infection and Exanthem



From Cherry, J. D.: Anemia and mucocutaneous lesions due to Mycoplasma pneumoniae *infections. Clin. Infect. Dis.* 17*(Suppl. 1):47-51, 1993.*

*See references 6, 77, 98, 100, 112, 131, 132, 177, 187, 206, 212, 216, 218, 243, 248, 257, 282, 311, 330, 369, 371, 374, 413, 415, 417, 424, 489, 490, 502, 546, 562, 571, 623, 629, 633, 656, 674.

†See references 132, 187, 212, 218, 282, 311, 369, 417, 424, 490, 502, 623, 629.

‡See references 20, 77, 100, 131, 185, 187, 216, 324, 330, 413, 415, 437, 502, 546, 551, 571, 577, 598, 629, 633, 674, 680.

TABLE 208–4 Mucocutaneous Findings in 20 Patients with *Mycoplasma pneumoniae* Infections and Exanthem

Case	Reference	Age (yr)	Sex	Distinguishing Characteristic of Exanthem	Generalized Ulcerative Stomatitis	Conjunctivitis
1	100	16	M	Fiery-red confluent maculopapular	0	0
2	100	17	F	Blotchy erythematous	0	0
3	95	4.5	F	Morbilliform	0	0
4	205	9	M	Erythematous maculopapular	+	0
5	205	8	M	Papulovesicular; "target" appearance	+	0
6	215	14	M	Symmetric macular and bullous	+	+
7	330	19	M	Erythematous maculopapular, vesicles; "iris" lesions	+	0
8	369	10	F	Macular and petechial	0	0
9	371	16	M	Varicella-like	+	+
10	674	8	M	Macular	+	0
11	674	6	M	Diagnosed as measles	0	0
12	413	16	M	Scattered vesicular; generalized	+	+
13	413	6	M	Vesicular; generalized	+	0
14	489	7	M	Urticarial	0	0
15	489	9	M	Maculopapular	0	0
16	571	10	M	Vesiculobullous and maculopapular	+	+
17	656	27	M	Vesiculopustular to papular; "pityriasis-like"	0	0
18	208	10	M	Vesiculobullous; generalized	+	0
19	374	5	M	Maculopapular	0	0
20	417	11	F	Papular; most marked on hands and feet	0	0

Modified from Cherry, J. D., Hurwitz, E. S., and Welliver, R. C.: Mycoplasma pneumoniae *infections and exanthems. J. Pediatr.* 87*:369-373, 1975.*

of cases in association with the administration of antibiotic raises the possibility that the antibiotic intensifies the dermosensitive potential of the infectious agent in a manner similar to that noted between Epstein-Barr virus and ampicillin in infectious mononucleosis. *M. pneumoniae* has been recovered from the blister fluid of two patients with erythema multiforme.[415]

Other unusual cutaneous manifestations include erythema nodosum, pityriasis rosea, varicella-like, urticaria, and Cockade purpura.[100,243,248,371,489,562,583,656]

Cardiac Manifestations

Cardiac involvement during *M. pneumoniae* infection generally is considered to be unusual.* However, studies of Pönkä[534] and Sands and associates[572] and survey data of Noah[508] and Assaad and Borecka[18] indicate that *M. pneumoniae* myocarditis and pericarditis are important causes of both morbidity and mortality. In a study of fatal viral and mycoplasmal infections, Assaad and Borecka[18] noted six cardiovascular deaths related to *M. pneumoniae* infection during a 9-year period. Noah[508] found that 1 percent of 700 patients with *M. pneumoniae* infection had cardiac manifestations as the main clinical feature. Pönkä[534] published findings of a 7-year study involving 560 patients with serologic evidence of *M. pneumoniae* infection. In this group, 69 patients with cardiac manifestations were detected; of these 69 patients, 25 had carditis for which no causal agent other than *M. pneumoniae* could be incriminated. Pönkä[534] also reviewed the world literature and found a total of 33 other cases of carditis.

Of 25 carefully studied patients, 17 had respiratory symptoms before the diagnosis of carditis and 10 had radiologically confirmed pneumonia. All but four patients had fever. Of the 25 patients, 2 were younger than 10 years and 2 were in the 10- to 19-year age group. Of the 33 cases in the literature, 7 were 20 years or younger. Of this survey group, 25 of the 33 had respiratory illness and in 19 it was recorded as pneumonia.

Of the 25 patients in Finland reviewed by Pönkä, 6 had pericarditis and the remainder had perimyocarditis. Antibiotic therapy in 11 patients did not appear to shorten the duration of illness or diminish the number of cardiac sequelae. At a 16-month follow-up, 11 patients had persistent cardiac damage. Another interesting aspect of this study was the finding of rises in *M. pneumoniae* complement-fixing antibody titer in five adults with myocardial infarcts.

Chergui and associates[94] described a 45-year-old man with bilateral interstitial infiltrates and second-degree heart block. He was treated with erythromycin, and the heart block, fever, and respiratory distress cleared on hospital day 5.

Hematologic Manifestations

Severe hemolytic anemia has been reported on several occasions in patients with *M. pneumoniae* infection.* Most cases of *M. pneumoniae* hemolytic anemia have been associated with marked pulmonary involvement. Stevens-Johnson syndrome is a common associated finding, and myocarditis has been noted on two occasions.[131,184,185,337,629] In general, severity of illness correlates with high titers of cold agglutinins.

The hemolysis may be severe and acute, often with a 50 percent reduction in hemoglobin concentration. In contrast to uncomplicated pulmonary disease, the leukocyte count in patients with hemolytic anemia frequently is elevated markedly with a predominance of neutrophils. Results of the direct Coombs test are usually positive. Clinical experience suggests that administration of steroids in conjunction with proper antibiotic therapy may be beneficial in this illness. Boccardi and associates[53] observed a 7-year-old boy with hemolytic anemia and transitory paroxysmal cold hemoglobinuria.

Feizi[186] has demonstrated that clinically inapparent compensated hemolysis frequently is associated with *M. pneumoniae* pulmonary infection. Fiala and associates[198] noted that bone marrow suppression also contributed to the anemia in a patient whom they studied. Ruhrmann and Holthusen[562] reported a hemorrhagic variant of erythema multiforme (Cockade purpura) similar to Henoch-Schönlein disease in *M. pneumoniae* infection; they

*See references 98, 131, 145, 184, 186, 198, 284, 337, 419, 557, 585, 602, 629, 643.

*See references 93, 94, 106, 181, 184, 230, 248, 333, 337, 369, 390, 486, 507, 638.

also noted severe thrombocytopenia not associated with hemolytic anemia. Gill and Marrie[236] reported a 27-year-old man who had hemophagocytosis with an *M. pneumoniae* infection. Two children with hemophagocytic lymphohistiocytosis in association with *M. pneumoniae* infections have been reported.[301] Also noted has been a 4-year-old girl with neutropenia, thrombocytopenia, and acute hepatitis.[92] Severe *M. pneumoniae* infection with pneumonia, thrombocytopenia, and disseminated intravascular coagulation has been described.[108,111]

Gastrointestinal Findings

NONSPECIFIC FINDINGS. Approximately 25 percent of patients with *M. pneumoniae* pneumonia have nausea, vomiting, diarrhea, or some combination thereof (see Table 208–2). Aside from these complaints, gastrointestinal problems in association with *M. pneumoniae* infection are rare events. Stevens and associates[629] found that 15 percent of a group of 44 children with infection had notable abdominal pain. Referred abdominal pain with pneumonia also has been observed.[185]

LIVER INVOLVEMENT. Liver involvement in *M. pneumoniae* infection is a surprisingly rare event. Levine and Lerner[389] reported that mild increases in transaminases occur and that acute and chronic active hepatitis has been noted with respiratory symptoms and proven *M. pneumoniae* infection, but they provided no further information. MacLean[417] reported a 13-year-old girl who initially had a sore throat and then 9 days later had clinical and laboratory evidence of hepatitis. Murray and associates[483] presented a case report of an adult with typical *M. pneumoniae* pneumonia in whom liver function and enzyme studies indicated hepatitis. Helms and colleagues[269] noted that 6 of 17 patients with *M. pneumoniae* pneumonia had elevated aspartate aminotransferase values. Enzyme changes have been observed in other case evaluations,[391,424,603] and hepatic necrosis has occurred.[419]

Simonian and Janner[602] reported a 12-year-old boy who had pleural effusion, hepatitis, and hemolytic anemia. Narita and associates[497] noted two children with lymphadenopathy and liver dysfunction. These two patients are of interest because both had mycoplasmemia and neither had pneumonia.

SPLENIC INFARCT. Two children (a 13-year-old girl and a 10-year-old boy) with pneumonia due to *M. pneumoniae* had splenic infarcts and transient antiphospholipid antibodies.[708]

PANCREATITIS. In 1974 Märdh and Ursing[429] reported six patients with respiratory illnesses, serologic evidence of *M. pneumoniae* infection, and pancreatitis. In four patients, pancreatic symptoms began 1 to 2 weeks after the onset of respiratory illness; in the other two patients, the pancreatitis was subclinical. Diabetes developed in two patients, one of whom died. At postmortem examination, pneumonitis and pancreatitis were confirmed. In a study of pancreatitis, Leinikki and Pantzar[383] noted that sera from 18 of 56 patients had rises in complement-fixing antibody titer to *M. pneumoniae*. Because none of the patients in this study had respiratory illness suggestive of *M. pneumoniae*, the investigators suggested that perhaps the antibody responses were not specific for *M. pneumoniae* infection but were caused by a cross-reacting infection or were the result of autoantigens from pancreatic damage. Leinikki and associates[384] have conducted further studies; their belief is that the antibody response is nonspecific, but their data neither confirm nor refute this assumption. In another study, Freeman and McMahon[227] also noted serologic evidence of *M. pneumoniae* infection in 33 percent of patients with pancreatitis. Oderda and Kraut[513] reported a 22-month-old girl with pancreatitis and a rise in complement-fixing antibody titer to *M. pneumoniae*. This child had no respiratory symptoms.

Arthritis

Mycoplasmas other than *M. pneumoniae* are a common cause of arthritis in animals other than human beings.[126,310] In many instances the animal diseases suggest human rheumatoid arthritis. Consequently, researchers have searched extensively for mycoplasmas in humans with rheumatoid arthritis, but to date, no associations have been established. However, *M. pneumoniae* infection clearly is associated occasionally with joint manifestations.* In a review of 1259 patients with *M. pneumoniae* infection, Pönkä[533] noted transient arthritis in 0.9 percent. Two patients were found to have Reiter syndrome. Hernandez and colleagues[273] reported seven instances of arthritis in 38 persons with *M. pneumoniae* respiratory disease. In one patient, the illness lasted 18 months and was associated with the development of rheumatoid factor.

Twenty-one instances of illness suggestive of rheumatic fever have been described.[32,115,320,367,473,486,493,584,699] In all 21 patients, large joints were involved; 18 patients had joint swelling or effusion, whereas 3 had only pain. Most patients had a history of preceding respiratory illness with sore throat, and 10 of the 21 had radiographic evidence of pneumonia. The sedimentation rate was elevated in all patients in whom the test was performed. One child had an erythema marginatum–like rash, as well as polyarthritis and fever.[473] Poggio and colleagues[530] reported a child with reactive arthritis 3 weeks after the onset of diarrhea and a respiratory tract infection. Leukocytoclastic vasculitis plus polyarthritis associated with *M. pneumoniae* infection has been described in a young adult.[527]

Muscular Disease

Berger and Wadowksy[33] reported a 15-year-old girl with right lower lobe pneumonia, hepatitis, and rhabdomyolysis associated with *M. pneumoniae* infection. Minami and associates[458] have reported a 4-year-old boy with pneumonia and a rhabdomyolysis associated with a marked titer rise to *M. pneumoniae* using a passive agglutination assay. Polymyositis has been noted in association with *M. pneumoniae* infection on six occasions.[526]

Neurologic Disease

Several large community and military studies of *M. pneumoniae* pneumonia and other respiratory illnesses are notable in that neurologic disease is not described.[88,132,177,218,311,465,479] However, other studies, particularly those performed more recently, indicate a surprising spectrum of neurologic illness associated with *M. pneumoniae* infection.† The failure to find neurologic disease in the initial large studies mentioned was probably due not to its absence but to the orientation of the investigators; neurologic disease was not studied under the respiratory investigation protocols. In three large studies of *M. pneumoniae* illness involving 1856 cases, 2.6 to 4.8 percent had neurologic illness.[502,508,535] Assaad and Borecka[18] noted five fatal *M. pneumoniae* infections in which central nervous system (CNS) findings were the major clinical manifestations.

In 1973 Lerer and Kalavsky[388] reported 5 cases of neurologic disease associated with *M. pneumoniae* infection and analyzed 45 cases from the literature. They noted the following frequencies

*See references 32, 144, 231, 234, 273, 320, 367, 369, 429, 484, 493, 533, 564, 699.

†See references 1, 3-5, 11, 13, 14, 16, 18, 19, 22, 26, 29, 30, 39, 42, 46, 65, 76, 110, 120, 124, 147, 156, 162, 179, 185, 196, 203, 225, 241, 275, 276, 284, 302, 328, 332, 338, 341, 346, 350, 352-354, 361, 370, 382, 386, 398, 414, 436, 457, 476, 481, 494, 502, 506, 508, 512, 514, 516, 522, 535, 561, 567, 578, 594, 604, 614, 623, 625, 629, 642, 644, 657, 663, 665, 675, 684, 695, 702.

of specific clinical involvement: generalized encephalitis, 30 percent; spinal nerve roots, 30 percent; meningitis, 20 percent; cranial nerves, 20 percent; focal encephalitis, 16 percent; cerebellum, 14 percent; psychosis, 8 percent; and spinal cord, 2 percent. Combined involvement was noted in 36 percent of cases; in 79 percent, a history of antecedent respiratory illness was elicited. Fifty-three percent of the patients were 20 years or younger, and 15 percent were younger than 10 years. Eighty percent of the children and adolescents were males. The onset of neurologic disease occurred 3 to 23 days after the onset of respiratory illness, with a mean value of 10 days. Five deaths were noted, and 22 percent of the survivors had residual neurologic deficits.

In a review of 61 patients with *M. pneumoniae*–associated neurologic disease during a 24-year period in Helsinki, Finland, Koskiniemi[354] noted that 45 of the patients were children and that all these children had encephalitis. Of the total group, 5 patients (8%) died and 14 (23%) had severe sequelae.

In a study of acute childhood encephalitis in Toronto, Canada, 9 of 50 children (18%) with adequate microbiologic study had evidence of *M. pneumoniae* infection.[350] Interestingly, four of the nine children also had evidence of concomitant viral infection. In a further study of encephalitis at the same center during a 5-year-period, researchers noted that 50 of 159 children (31%) with encephalitis had evidence of *M. pneumoniae* infection[46]; in this analysis, 30 of the 50 cases (60%) had microbiologic evidence of other concomitant infections. Respiratory prodromal symptoms preceded the encephalitis in approximately 67 percent of the patients in whom the diagnosis was based on culture, PCR, or both. Two children had acute demyelinating encephalomyelitis. Long-term neurologic sequelae occurred in 48 to 64 percent of cases. During the past decade 1988 patients were referred to the California Encephalitis Project.[110] *M. pneumoniae* was the most common agent implicated; there were 111 patients with evidence of *M. pneumoniae* infection and 84 (76%) of these patients were children.

In other studies, aseptic meningitis is reported more frequently; other less common findings include poliomyelitis-like syndrome, bilateral sensorineural deafness, Reye syndrome, cerebral infarction, optic disk swelling, brain stem syndrome, transverse myelitis, psychosis, cerebellar syndrome, radiculopathy, brachial plexus neuropathy, demyelinating polyneuropathy with syndrome of inappropriate secretion of antidiuretic hormone (SIADH), acute disseminated encephalomyelitis (ADEM), bilateral striatal necrosis, Tourette syndrome, intracranial hypertension, Bell palsy, and Guillain-Barré syndrome.* Arthur and Margolis[16] noted the appearance of *Mycoplasma*-like structures in granulomatous angiitis of the CNS at postmortem examination of a 35-year-old man.

Mixed Infections

In many studies of *M. pneumoniae* infection, cultural or serologic evidence of concomitant or sequential infection with other infectious agents has been noted.† In a large study of patients hospitalized with acute respiratory illness, Fransen and associates[224] found that 64 percent of patients with rises in complement-fixing antibody titer to *M. pneumoniae* also had antibody titer rises to viral, chlamydial, or bacterial agents. In this group, the most common concomitant infections were with parainfluenza viruses. The occurrence of mixed infection did not appear to have a pronounced effect on clinical manifestations; the only significant difference between patients with mixed infection and those with single *M. pneumoniae* infection was the more common finding of a high erythrocyte sedimentation rate in the former group. In several other large studies of disease caused by *M. pneumoniae*, concomitant infections were common, but no evidence of synergistic or antagonistic roles of one agent for another was noted.[187,238,466,622,623] Renner and associates[550] found a lower than expected frequency of seropositivity to *M. pneumoniae* in 91 serum pairs with seroconversion to influenza A virus.

The high rate of possible mixed infections in the large encephalitis study in Toronto is of particular interest.[46] In addition to common respiratory viruses and enteroviruses, they noted herpes group viruses, *Bartonella henselae*, and *Mycobacterium tuberculosis*. In studies of *Bordetella pertussis* infection, evidence of mixed infection with *M. pneumoniae* or perhaps serologic cross-reactivity has been found repeatedly.[303,681]

The observation by Grady and Gilfillan[245] that 81 percent of patients with legionnaires' disease also had serologic evidence of *M. pneumoniae* infection is interesting. In the same study, 29 percent of all patients seropositive for *M. pneumoniae* also were seropositive for the legionnaires' disease antigen. Comparable studies performed by the Centers for Disease Control and Prevention failed to find a similar rate of high co-positivity in sera obtained in other legionnaires' disease epidemics. Another study found no serologic relationship between *M. pneumoniae* and *Legionella pneumophila*.[549] Severe bacterial disease after *M. pneumoniae* infection has been reported occasionally. Stadel and colleagues[620] noted *H. influenzae* pneumonia and bacteremia after a mild *M. pneumoniae* illness; Biberfeld and colleagues[41] reported staphylococcal septicemia in two cases of *M. pneumoniae* pneumonia, and Rykner and associates[564] recovered pneumococci from the pleural exudate of a patient with *M. pneumoniae* pneumonia.

Kleemola and Kayhty[343] found fourfold or greater increases in *M. pneumoniae* complement-fixing antibody titer in 40.7 percent of 54 patients with bacterial meningitis. However, they thought that this antibody response was not caused by specific *M. pneumoniae* infection but by cross-reactive glycolipids resulting from the bacterial infection.[343,344]

Lind and associates[398] reported that 4 of 19 patients with neurologic disease and *M. pneumoniae* infection had serologic evidence of concomitant viral infection.

Other Disease Associations

Foy and colleagues[212] noted that both ear involvement and pneumonia as manifestations of *M. pneumoniae* infection occurred more commonly in children with previous tonsillectomy. Putman and associates[539] found that in all but 3 of 31 patients with sarcoidosis, the serum complement-fixing antibody titer to *M. pneumoniae* was 1:32 or higher, whereas in a similar-sized control group without sarcoidosis, only 2 persons had titers of 1:32 and none had higher titers. Other interesting observations include multiple birth defects in a newborn exposed to *M. pneumoniae* in utero,[58] a tubo-ovarian abscess in a young woman from whom *M. pneumoniae* was isolated in pure culture,[660] fever of unknown origin in a 32-year-old man,[362] glomerulonephritis in a few patients with pneumonia,[532] inappropriate secretion of antidiuretic hormone in a 6-year-old boy,[401] acute IgA nephropathy,[636] and optic disk swelling and iritis.[569]

Recurrent Disease

The results of several investigations suggest that recurrent *M. pneumoniae* infection is a frequent finding.[66,196,626] Repeat infections can be associated with severe disease such as pneumonia. In the Seattle studies, second attacks of pneumonia have been documented, and similar findings have been observed in England.[208,220,222,282]

*See references 1, 11, 13, 14, 19, 29, 124, 156, 162, 241, 264, 275, 302, 338, 341, 346, 353, 370, 386, 398, 436, 476, 481, 506, 511, 522, 524, 561, 567, 578, 594, 623, 629, 642, 657, 665, 675, 684, 695, 702.

†See references 6, 22, 46, 169, 187, 188, 225, 238, 245, 251, 303, 350, 387, 395, 425, 432, 465, 466, 494, 550, 620, 622, 623, 681, 698.

DIAGNOSIS

Differential Diagnosis

Because the clinical manifestations of *M. pneumoniae* infection are protean and because infections occur commonly in children and adolescents, this agent should be considered in the differential diagnosis of most infectious illnesses. Most important is its consideration in patients with pulmonary disease, in whom illnesses caused by viruses (particularly adenoviruses, parainfluenza viruses, influenza viruses); *Chlamydia psittaci; Chlamydia pneumoniae; Coxiella burnetii;* bacteria (particularly *S. pneumoniae*, *B. pertussis*, *H. influenzae*, *M. tuberculosis);* and fungi (particularly *Histoplasma capsulatum* and *Coccidioides immitis)* are the main differential possibilities. Because the clinical manifestations, including the radiographic appearance of the lungs, are frequently similar in the various differential possibilities, the following other factors are important: status of the host (normal or immunologically compromised), the environment (human, animal, or inanimate source), the age of the patient, the incubation period, and the season.

In otherwise healthy children, *M. pneumoniae* is a common cause of pneumonia in those older than 3 years and is the leading cause of pneumonia in older children and adolescents. The lack of coryza is sometimes useful in differentiating *M. pneumoniae* pneumonia from that caused by common viral agents, and elevation of the white blood cell count along with an increase in band form neutrophils is evidence against a mycoplasmal etiologic agent, except in patients with concomitant hemolytic anemia. The occurrence of exanthem and, in particular, Stevens-Johnson syndrome should lead the physician to suspect *M. pneumoniae;* similarly, the occurrence of hemolytic anemia, joint manifestations, or neurologic signs and symptoms with pneumonia should lead the physician to strongly suspect *M. pneumoniae* as the etiologic agent. Because the pulmonary manifestations of *M. pneumoniae* infection are not always clinically apparent, a physician investigating an unusual acute or subacute case (aseptic meningitis or other neurologic illness; exanthem; enanthem; hepatitis; pancreatitis; pericarditis, myocarditis, or both; and arthritis) would be wise to consider the possibility of *M. pneumoniae* as the etiologic agent and to obtain appropriate chest radiographs, as well as definitive cultures and serologic studies.

Specific Diagnosis

SERUM COLD AGGLUTININS. Despite the considerable confusion in the literature and by physicians in general regarding the diagnostic value of the serum cold agglutination test for *M. pneumoniae* infection, my opinion is that when it is used appropriately, the test is a simple and useful procedure. One cause for confusion was a report in 1966 in which only 1 of 28 children with positive cold agglutination titers actually had serologic evidence of *M. pneumoniae* infection by complement fixation.[635] However, this report can be criticized because the study population consisted of 444 children younger than 4 years and only 170 of this group had pneumonia. Because cold agglutinins are noted occasionally in the sera of patients of all ages with a variety of illnesses, for useful results, their study should have been restricted to patients likely to have *M. pneumoniae* lower respiratory tract disease.[100,200,234,394] In various studies of pneumonia, serum cold agglutinins at a titer of 1:32 or higher were found in 50 to 90 percent of patients with *M. pneumoniae* infection.[42,84,88,225,234,311,479,629,679] In general, the cold agglutinin response correlates directly with the severity of pulmonary involvement; patients with extensive lobar involvement nearly always have positive titers[3] (1:32), whereas those with only minimal findings on radiographic study frequently have equivocal or negative titers. Positive cold agglutination titers have been observed in 18 percent of adenoviral pneumonias in a study involving a military population.[234] In general, the higher the cold agglutinin titer, the more likely that a particular illness is caused by *M. pneumoniae*.

A rapid screening test for cold agglutinins is available and useful.[231,252] This test is performed by adding 4 drops of blood to a tube containing sodium citrate or another anticoagulant. The tube is placed in ice water (0° C to 4° C) in a freezer for approximately 30 seconds and then examined immediately for coarse agglutination by tilting the tube on its side. When the tube is warmed, the agglutination should resolve, and it can be reproduced again by repeating the ice water cooling procedure. A modified version of this rapid test was used in 126 children in an emergency department who had asthma exacerbations.[83] The test had a sensitivity of 78.3 percent and a specificity of 41.3 percent when compared with an IgM ELISA.

SPECIFIC ANTIBODY DETERMINATIONS. Several specific antibody tests (growth inhibition, immunofluorescence, indirect hemagglutination, precipitation, mycoplasmacidal antibody, complement fixation, ELISA, adherence inhibition assay, radioimmunoassay, and radioimmunoprecipitation) can be used to measure serum antibodies to *M. pneumoniae*. In the past only the complement-fixation test was available routinely. A fourfold rise in complement-fixation antibody titer indicates acute *M. pneumoniae* infection. Because complement-fixation antibody in *M. pneumoniae* infection is of relatively short duration, the observation of a fourfold fall in titer also can be useful on occasion in assigning etiologic significance in a particular illness. High single titers (≥1:256) usually indicate recent infection but rarely can be used to relate the cause of an illness specifically to *M. pneumoniae.* Because *M. pneumoniae* infection is associated with a relatively long incubation period, the development of antibodies is occurring at the time of acute disease. As a consequence, fourfold changes in titer can occur in a short interval (5 days), and collection of paired sera 5 to 7 days apart usually reveals a significant rise in complement-fixation antibody titer.

In recent years most diagnostic laboratories have replaced the complement-fixation test with commercial immunofluorescence or ELISA for demonstration of antibodies to *M. pneumoniae* antigens.* These tests, in addition to demonstrating rises in antibody values in paired sera, can identify specific IgM and IgA antibodies in single serum samples. In general, when used by experienced laboratory personnel, both immunofluorescence and ELISA have sensitivities and specificities similar to those of the complement-fixation test for determining significant increases in antibody values in paired serum specimens. In addition, demonstration of specific IgM or IgA antibody in a single serum sample suggests a recent infection. Today the most common method used for diagnosing *M. pneumoniae* infection in clinical practice in the United States is the single serum IgM assay. However, it should be pointed out that most available tests have low specificity so that overdiagnosis is common.[28,138,164,505,640] Furthermore, the specific IgM and IgA responses after infection may last for several months; therefore demonstration of these antibodies in a single serum sample may be misleading with regard to the diagnosis of specific illness. Hence in most instances, paired sera (5 to 14 days apart) should be examined to confirm a clinical diagnosis. Also useful is the determination of cold agglutinin titers at the time of IgM ELISA. If an ELISA IgM test is positive and a cold agglutinin test is negative, the physician should be skeptical of the former value.

CULTURE. With proper media, experienced personnel have little difficulty isolating *M. pneumoniae* from throat swabs of

*See references 7, 28, 78, 138, 164, 168, 182, 201, 304, 348, 378, 477, 505, 543, 600, 611, 640, 658, 659, 676, 694.

infected patients.[9,671] However, because *M. pneumoniae* is relatively slow growing, in most instances requiring more than 1 week of incubation, culture is of less use for diagnosis of routine cases than serologic study is. Cultures should be performed in all unusual situations; specifically, joint fluid, CSF, pericardial fluid, and biopsy material should be cultured. The modified SP-4 medium, which is more sensitive than conventional mycoplasma culture media, coupled with the agar plate immunofluorescence identification procedure, may assist the cultural diagnosis of *M. pneumoniae*.[671]

DETECTION BY POLYMERASE CHAIN REACTION. A large number of studies have indicated the usefulness of PCR for demonstration of specific *M. pneumoniae* DNA in sputum, throat, nasopharyngeal, blood, CSF, urine, and tissue specimens.* Numerous different primers have been used to identify gene sequences of the P1 cytoadhesin protein, the adenosine triphosphatase asperon, or 16S ribosomal RNA gene sequences. Using culture plus serologic criteria as the comparative standard, several studies have shown excellent sensitivity and specificity.

TREATMENT

Antimicrobial Therapy

M. pneumoniae is sensitive in vitro to erythromycin, tetracyclines, chloramphenicol, clarithromycin, azithromycin, several aminoglycosides, and quinolones.[27,151,299,300,313,427,504,605] It is resistant to all penicillins and for practical purposes to the cephalosporins. In spite of this demonstrated in vitro sensitivity of the organism, plus several studies that have shown clinical therapeutic effectiveness,† a common misconception of many physicians is that antibiotic therapy is of little value in the treatment of illness caused by *M. pneumoniae*. This idea had its origin before the present era, when many patients with viral pneumonia were given a diagnosis of primary atypical pneumonia and treated unsuccessfully with antibiotics.

In 1961 Kingston and associates[340] demonstrated the therapeutic effectiveness of demethylchlortetracycline for pneumonia caused by *M. pneumoniae*. Since then, several other antibiotics have been studied carefully and also have been found to be effective against *M. pneumoniae* pneumonia.[84,151,218,234,311,562,586,605,608] The drugs of choice for pneumonia caused by *M. pneumoniae* are either erythromycins or tetracyclines. Because of the adverse effects of tetracyclines on teeth, a macrolide is the drug of choice in children. In *M. pneumoniae* pneumonia, the dose of erythromycin for children is 40 to 50 mg/kg every 24 hours administered every 6 hours for a minimum of 10 days. For adolescents and adults, the dose of erythromycin or tetracycline is 2 g every 24 hours administered every 6 hours. In general, the effectiveness of antibiotic therapy correlates directly with the severity of pneumonia and the elapsed time of illness before the initiation of therapy.

Azithromycin and clarithromycin both are approved for the treatment of community-acquired pneumonia in children.[51,262] Their advantage is less frequent dosing, shorter duration of therapy, and less gastrointestinal disturbance in older patients. The azithromycin dosing schedule is 10 mg/kg/day (maximal dose, 1 g/day) on day 1, followed by 5 mg/kg daily (maximal dose, 500 mg/day) for 4 days. The clarithromycin dose is 15 mg/kg/day administered every 12 hours for 10 days (maximal dose, 1 g/day). Macrolide-resistant strains of *M. pneumoniae* from clinical isolates have been noted in Japan.[434] The importance of this finding is presently not known.

In all other clinical manifestations of *M. pneumoniae* infection except pneumonia (e.g., nonpulmonary respiratory infection, neurologic disease, Stevens-Johnson syndrome), antibiotic therapy has not been evaluated adequately. In general, otitis media, pharyngitis, croup, and bronchiolitis appear to be mild, self-limited illnesses that require no therapy. In more serious illness such as Stevens-Johnson syndrome and neurologic disease, individual case studies have indicated little evidence of therapeutic benefit with either erythromycin or tetracycline therapy. However, my opinion is that when diagnosed, most *M. pneumoniae* infections should be treated because there is little to lose and in vitro data suggest the possibility of efficacy. Jensen and colleagues[318] noted that prophylactic administration of oxytetracycline to family contacts prevented disease but not infection. Azithromycin prophylaxis has been used successfully in two hospital outbreaks of *M. pneumoniae* pneumonia.[294,342]

Corticosteroid Therapy

Steroids have been used in the management of severe pulmonary disease, Stevens-Johnson syndrome, encephalitis, and hemolytic anemia. Although definitive data are lacking, several case studies suggest associated clinical benefit; steroids seem to be particularly useful in treating severe hemolytic anemia. A 6-year-old girl with brain stem and striatal encephalitis complicating *M. pneumoniae* pneumonia experienced neurologic improvement within 48 hours of administration of intravenous immunoglobulin.[567]

Recently Lee and associates[377] reported on the use of prednisolone in the treatment of 15 children with *M. pneumoniae* pneumonia in whom the clinical course worsened while receiving appropriate macrolide therapy. Corticosteroid treatment in these children was related to clinical and radiologic improvement. Cimolai[113] noted dramatic improvement in a 7-year-old girl treated with methylprednisolone, and in 1964 Copps[131] noted dramatic improvement in an adolescent with persistent fever, severe pneumonia, and hemolytic anemia when treated with a corticosteroid and erythromycin.

General Management

Children and adolescents with *M. pneumoniae* pneumonia should be discouraged from engaging in excessive physical activity during the acute illness and for a 2-week period during convalescence because clearance, as observed by radiography, is slow and lags behind apparent clinical well-being. Older children and adolescents should be advised of their contagiousness to others; this risk period exists as long as cough persists, even with successful antibiotic therapy.

PREVENTION

Because of the marked and prolonged morbidity associated with *M. pneumoniae* infection, which has been particularly troublesome in the military, much effort was directed toward the development of vaccines. In 1965 Jensen and associates[317] reported encouraging initial trials with an inactivated vaccine. In this study, significant rises in *M. pneumoniae* growth-inhibiting antibody titer developed in 25 of 30 volunteers. Later challenge studies with the same vaccine indicated that 9 of 10 volunteers with serum antibody were protected, but illness more severe than that in the unvaccinated control group occurred in vaccinees who did not have an antibody response after initial immunization.[607] This altered reactivity on challenge suggested a sensitization process perhaps similar to that observed with other inactivated

*See references 1, 46, 75, 148, 159, 237, 280, 296, 316, 420, 447, 455, 461, 475, 485, 495, 529, 544, 552, 581, 587, 614, 641, 706, 709.
†See references 51, 84, 151, 212, 218, 234, 311, 340, 562, 582, 586, 605, 608.

antigen vaccines and indicated the need for caution in further trials.[137,196] Other trials of inactivated vaccines in both adults and children have had varying degrees of success.[451,467,700]

A trial with a live attenuated vaccine (a temperature-sensitive mutant) gave encouraging results.[249] However, because further study of natural *M. pneumoniae* disease indicated that re-infection occurs commonly and because sensitization may play a role in pathogenesis, proceeding slowly in conducting further vaccine trials in children seems prudent.[194]

The degree of contagion of *M. pneumoniae* is relatively low, so isolation methods should be effective in preventing spread of disease. The studies of Jensen and associates[318] and the hospital azithromycin trials[294,342] indicate that in certain circumstances (in particular, high-risk subjects such as patients with sickle-cell disease and high-risk populations), prophylactic administration of antibiotics may be justified.

UREAPLASMA

PROPERTIES

Ureaplasmas (formerly *T-strain mycoplasmas*) are distinguished from all other members of the order Mycoplasmatales by their production of urease and their ability to hydrolyze urea.[592,593,650] The genus *Ureaplasma* contains two species, *U. parvum* and *U. urealyticum*.[687] These two species were previously described as two biovars of *U. urealyticum*. Biovar 1 is now *U. parvum* and biovar 2 is *U. urealyticum*. The morphologic characteristics of *Ureaplasma* sp. in young liquid medium culture are similar to those of other mycoplasmas. Round-ovoid elements approximately 330 nm in diameter with a range of 100 to 850 nm are found; rod-shaped and filamentous structures also occur, and the latter have a length of 2 μm and a width of 50 to 300 nm.[47,593,704] In clinical material, short, bacillary forms with monopointed ends are common findings. Organisms are surrounded by a single trilaminar membrane approximately 10 nm thick with pilus-like structures radiating from the surface. Multiplication occurs by a simple budding process and perhaps by binary fission.

On unbuffered standard *Mycoplasma* agar with a pH of 6.0, *Ureaplasma* sp. colonies are small (20 to 30 μm) and circular, with irregular borders, and grow downward into the agar.[593] On buffered agar, *Ureaplasma* sp. colonies are bigger and often have the "fried egg" appearance of typical large colony-forming mycoplasmas.[421]

Isolation of *Ureaplasma* sp. from clinical material is assisted by the demonstration of urease activity.[197,591,652] In liquid medium containing urea and phenol red, growth of *Ureaplasma* sp. results in the production of ammonia, with a resultant increase in pH and a change in color. Subculture from broth to agar medium that contains urea and manganese sulfate yields dark brown ureaplasmal colonies.

EPIDEMIOLOGY

The main reservoirs of human strains of *Ureaplasma* sp. are the genital tracts of adult men and women.[15,217,441-443,652,669,675,687] Infants become colonized during passage through the birth canal of an infected woman.[221,345,379,637,687] With ruptured membranes, the infant can be infected in utero.[345] *Ureaplasma* sp. have been recovered from the following sites in newborn infants: throat, nose, genitourinary tract of girls, urine of boys, external auditory canal, umbilicus, and perineum. Not all infants of infected women become colonized, and neonatal colonization tends to not persist. In one study in which *Ureaplasma* sp. were recovered from 38 percent of girls and 6 percent of boys at birth, follow-up during a 2-year period revealed a decreasing prevalence of colonization; at 2 years, none of the children had positive cultures.[217]

During prepubertal childhood, *Ureaplasma* sp. are recovered only rarely from urine or genital specimens.[213,379] After puberty, colonization is a common occurrence and is primarily the result of sexual contact.[439,441,443] Colonization in adults is related directly to sexual activity. In population studies, *Ureaplasma* sp. rarely are isolated from persons with no sexual experience, but they occur in approximately 50 percent of men and 75 percent of women for whom sexual intercourse with three or more partners is reported.

CLINICAL MANIFESTATIONS (see Chapter 75 for Neonatal Infections)

Because *Ureaplasma* can be recovered with considerable frequency from the throat, eyes, and genitourinary tract of babies and from the genitourinary tract of postpubertal males and females who are well, establishing cause-and-effect relationships in disease frequently has been difficult. Studies suggest the following disease associations with *Ureaplasma* in human genitourinary and reproductive disease: good to strong association with nongonococcal urethritis, prostatitis, and urethral syndrome; moderate association with epididymitis, involuntary infertility, repeated spontaneous abortion and stillbirth, chorioamnionitis, and low birth weight; weak association with urinary calculi, pyelonephritis, Reiter disease, and pelvic inflammatory disease; and no association with Bartholin gland abscess, vaginitis, cervicitis, postabortal fever, and postpartum fever.* Most illnesses related to or possibly related to *Ureaplasma* infection are not pediatric problems; only those of direct or indirect importance in pediatric and adolescent medicine are considered here.

Nongonococcal Urethritis

Nongonococcal urethritis occurs more commonly than gonococcal urethritis in men in most developed countries.[263,308,438,686] Approximately 40 percent of cases of nongonococcal urethritis are caused by *Chlamydia trachomatis*, and 20 to 30 percent are the result of *Ureaplasma* infection.[55,56,136,277,442,576] Clinical differentiation of disease caused by *C. trachomatis* and *Ureaplasma* has not been studied, but nongonococcal and gonococcal urethritis have been evaluated comparatively.[260,308,380,683]

The incubation period in nongonococcal urethritis is relatively long, with most cases occurring 10 to 20 days after exposure, whereas with gonorrhea, the period is shorter, usually less than 1 week.[260] The onset of symptoms in nongonococcal urethritis is generally more gradual than that associated with gonorrhea. Virtually all men with gonorrhea have a urethral discharge, and most have both discharge and dysuria. In contrast, Jacobs and Kraus[308] found that only 38 percent of men with nongonococcal urethritis had both dysuria and discharge. In the same study, 15 percent of patients with nongonococcal urethritis had only dysuria, whereas only 2 percent of those with gonococcal urethritis had a similar complaint. On examination, Handsfield[260] found the discharge in nongonococcal urethritis to be purulent in 36 percent of his cases, nonpurulent in 9 percent, and of an intermediate character in the remaining 55 percent. In contrast, 73 percent of patients with gonorrhea had a purulent discharge, 27 percent had an intermediate discharge, and none had a nonpurulent discharge. Because of the more gradual onset and the usually less severe symptoms, patients with nongonococcal urethritis are less prompt in seeking medical care than those with gonorrhea are. Jacobs and Kraus[308] found that 76 percent of patients with discharge and gonococcal infection came to the clinic within 4 days of onset, whereas only 43 percent of similar

*See references 71, 81, 82, 172, 363, 366, 446, 624, 632, 652, 654, 664.

nongonococcal urethritis patients visited the clinic within 4 days of disease onset. Without treatment, nongonococcal urethritis symptoms subside gradually in some patients during a 1- to 3-month period.[517]

After penicillin, ampicillin, or spectinomycin treatment of men with urethral gonorrhea, urethritis recurs (postgonococcal urethritis) in many patients.[279] Studies of postgonococcal urethritis indicate an etiologic role for *C. trachomatis; U. urealyticum* is probably responsible for some cases.[518]

A 7½-year-old sexually inactive boy with recurrent urethritis associated with *U. urealyticum* infection has been described.[588]

Chorioamnionitis

In a study of 249 puerperal women and their babies, Shurin and colleagues[597] noted on histologic examination of the placentas that *Ureaplasma* sp. were recovered from 37.5 percent of babies whose placentas showed chorioamnionitis and from only 19 percent of those with normal placentas. In this study, no adverse effects could be attributed to either the placental lesions or colonization of the babies. Caspi and associates[79] reported a 32-year-old woman with amnionitis in whom *Ureaplasma* was recovered from her blood. After delivery, the same organism was recovered from the blood of one of the twin infants.

Other Infections

Ureaplasma was determined to be the cause of postoperative mediastinitis in an adult after undergoing coronary artery bypass surgery.[232,258]

DIAGNOSIS AND TREATMENT

Demonstration of infection by *Ureaplasma* sp. can be established easily by presently available culture techniques. However, assigning causation of disease is more difficult because of its ubiquitous presence in normal persons. In clinical practice the most important differential consideration is between gonococcal infection and nongonococcal urethritis. Although the symptoms of the two illnesses are frequently different, sufficient overlap exists to render arriving at a specific diagnosis without laboratory aid hazardous. Microscopic examination of a urethral specimen is essential. In most instances the observation of gram-negative cell-associated diplococci on Gram stain is sufficient for a diagnosis of *Neisseria gonorrhoeae* infection. When smears reveal polymorphonuclear neutrophils without organisms suggestive of gonococci, a specific bacterial culture should be performed. Because infection with multiple agents occurs commonly and postgonococcal urethritis is a frequent problem, one is advised to investigate initial illnesses completely in adolescents with cultures for bacteria, *Chlamydia*, and *Ureaplasma. Ureaplasma* also can be detected by PCR.[48,556,627,712]

Patients with nongonococcal urethritis should be treated with tetracycline (40 mg/kg/24 hr every 6 hours; persons weighing more than 50 kg should receive 500 mg every 6 hours) for 10 days.[260,278] *Chlamydia* and *Ureaplasma* are also sensitive to erythromycin, so this antibiotic is a useful alternative for patients in whom tetracycline is contraindicated. With adolescent patients, it is prudent to seek out and treat the sex partners whenever possible.

MYCOPLASMA HOMINIS

PROPERTIES

Three basic morphologic forms of *M. hominis* have been observed by phase-contrast microscopy: coccoidal cells 30 to 80 nm in diameter, diploforms and filamentous forms with a thickness of 30 to 40 nm, and forms with variable lengths reaching 40 μm or more.[52,60,62,153] Bredt[61] studied newly isolated strains and noted that coccoid forms and ring- or disk-shaped cells were predominant; with some strains, filamentous forms of variable length also were noted. Multiplication occurs by binary fission, by fragmentation of filaments and rings, and by budding.[52,555]

Anderson and Barile[12] studied the ultrastructure of *M. hominis* and noted considerable variability in internal components. In some cells, ribosome-like granules in the cytoplasm and a more central area of netlike strands were present, suggestive of a nucleus. Other cells had only irregular densities within the cytoplasm. In some instances, dense cytoplasmic bodies were observed; in other cells, vacuoles were seen.

On *Mycoplasma* agar, *M. hominis* colonies are approximately 200 to 300 μm in diameter and have the typical mycoplasmal "fried egg" appearance.[652] *M. hominis* grows on ordinary blood agar and produces pinpoint nonhemolytic colonies. *M. hominis* metabolizes arginine to ammonia, so arginine-supplemented liquid medium with a pH indicator (phenol red) can be used for primary isolation. *M. hominis* can be identified specifically and differentiated from other human mycoplasmas that metabolize arginine by growth inhibition by specific antibody.

M. hominis has two cytoadhesins that are membrane proteins; they allow attachment to cells of the urogenital tract.[272] Attachment is to sulfated glycolipids of the host cells.[515]

EPIDEMIOLOGY

Like those of *Ureaplasma* sp., the main reservoirs of *M. hominis* are the genital tracts of adult men and women.[221,439,441,443-445,652,687] Infants become colonized during passage through the birth canal, but such colonization tends to not persist. In a recent study of 208 women at delivery, *M. hominis* was recovered from cervicovaginal specimens in 11 percent and the gastric secretions of 1 percent of newborns.[246] In prepubertal children, *M. hominis* only rarely is recovered from urine or genital specimens. Postpubertal genital tract colonization results primarily from sexual contact.

M. hominis can be recovered from the oral cavity of 1 to 5 percent of normal adults.[617]

CLINICAL MANIFESTATIONS (for Neonatal Infections see Chapter 75)

Studies suggest the following disease associations with *M. hominis* in human genitourinary and reproductive diseases: good to strong association with pyelonephritis, pelvic inflammatory disease, postabortal fever, and postpartum fever; moderate association with prostatitis, vaginitis, and cervicitis; weak association with Bartholin gland abscess; and no association with nongonococcal urethritis, epididymitis, urinary calculi, Reiter disease, urethral syndrome, involuntary infertility, repeated spontaneous abortion and stillbirth, and chorioamnionitis.* With the exception of pelvic inflammatory disease and complications of pregnancy, which occur in adolescents, the other disease associations reported do not involve pediatric patients.

M. hominis has been recovered on two occasions from the CSF of a 2½-year-old girl with a ventriculoperitoneal shunt.[690] Because she had no complications from the infection and only minimal CSF inflammation, no treatment was initiated. Three months later, the organism could not be isolated from CSF and the child was doing well.

*See references 50, 71, 81, 171, 172, 235, 256, 365, 520, 521, 560, 595, 624, 652, 654, 672.

In volunteer studies in adults, researchers found that *M. hominis* could produce exudative pharyngitis.[478] Moffet and associates[464] isolated *M. hominis* from the throat of 1 of 174 infants and children with pharyngitis but made no similar isolation from a control group of children without pharyngitis. Neu and Ellner[500] recovered *M. hominis* from the throat of 1 child in a group of 56 with exudative pharyngitis. Other *M. hominis* infections include septicemia in a 10-month-old burned infant,[143] chronic multifocal osteomyelitis in an 8-year-old,[293] septicemia after heart surgery in a 5-year-old girl,[142] and exudative vaginitis in a 10-year-old girl.[689] In one study 10 patients with sickle cell disease were found to have acute chest syndrome in association with *M. hominis* recovery from sputum or bronchoscopy specimens.[501] A 4-year-old girl was found to have *M. hominis* endocarditis following biventricular repair of her congenital heart defect.[154] Surgical wound infections have been due to *M. hominis* infections.[360,558]

In adult patients the following clinical manifestations have been caused by *M. hominis:* mediastinitis,[435] endocarditis,[189,285] pneumonia in a previously healthy man,[566] wound infections as surgical sites,[460] brain abscess,[286,364,713] and bacteremia in a patient with multiple injuries.[255]

DIAGNOSIS AND TREATMENT

Illness caused by *M. hominis* infection rarely occurs in children. This organism should be considered an etiologic possibility in wound infections and a variety of illnesses in which routine cultures are negative. The possibility of *M. hominis* as an etiologic agent also should be considered in adolescent girls with pelvic inflammatory disease. *M. hominis* is readily isolated on routine mycoplasma media and infection can also be determined by PCR.

M. hominis is usually sensitive to tetracycline, and this antibiotic is the drug of choice unless it is otherwise contraindicated.[57,74,631,645,646,703] During the past decade, resistance of *M. hominis* to tetracyclines has increased.[139,440] The organism also is usually sensitive to clindamycin, rifampicin, quinolones, and chloramphenicol. In contrast to *U. urealyticum* and *M. pneumoniae*, *M. hominis* is markedly resistant to erythromycin.

MYCOPLASMA FERMENTANS, MYCOPLASMA GENITALIUM, MYCOPLASMA PENETRANS, MYCOPLASMA PIRUM, AND AIDS-ASSOCIATED MYCOPLASMAL INFECTIONS

MYCOPLASMA FERMENTANS

M. fermentans originally was isolated from the genital tract of men and women 50 years ago, but it has not been established as a cause of genitourinary disease.[563,648,669] This organism has been isolated from the blood of leukemia patients, from the joint fluid of patients with arthritis, and from the blood and urine of patients with acquired immunodeficiency syndrome (AIDS).[24,49,428,470,482,696,705] *M. fermentans* has been identified by PCR in the peripheral blood mononuclear cells and lymph nodes of HIV-infected patients.[264,329,358,574] The organism also has been recovered from the blood of homosexual men without HIV infection.[329,358,418] *M. fermentans* has been identified in synovial fluid samples from 15 patients with inflammatory arthritic diseases including rheumatoid arthritis.[615]

MYCOPLASMA GENITALIUM

M. genitalium first was identified and reported in 1981.[651,654,669,672] It was cultured from the urethral swabs of two men with nongonococcal urethritis. The organism also has been recovered from the respiratory tract of patients with pneumonia who were participating in an *M. pneumoniae* vaccine trial.[23] *M. genitalium* has biologic features that indicate its pathogenic potential and it has caused infection and disease in experimentally infected chimpanzees.[651,669,673] With the use of PCR, Jensen and associates[315] presented evidence suggesting a causative role in some cases of nongonococcal urethritis. *M. genitalium* is an emerging sexually transmitted infection[423] and a cause of mucopurulent cervicitis in women[422] and nongonococcal urethritis (NGU) in males.[450] The organism also has been recovered in mixed culture with *M. pneumoniae* from the synovial fluid of a patient with pneumonia and subsequent polyarthritis.[670]

MYCOPLASMA PENETRANS

M. penetrans is a relatively newly recognized species isolated from the urogenital tract of patients with AIDS.[405,406] In a seroprevalence study, Wang and associates[692] found that 35.4 percent of HIV-infected patients had antibody versus only 0.4 percent of HIV-seronegative subjects. They subsequently noted a high prevalence of antibody to *M. penetrans* in the sera of homosexual men but not in the sera of other HIV transmission groups.[693] In a more recent study, Grau and colleagues[247] found that 18.2 percent of HIV-infected patients had antibody to *M. penetrans*, whereas only 1.3 percent of HIV-seronegative persons had antibody. *M. penetrans* antibody seroprevalence increased with progression of HIV-associated disease, and it was associated predominantly with homosexual practices in the HIV-infected patients. No pediatric data relating to *M. penetrans* seroprevalence are available.

MYCOPLASMA PIRUM

M. pirum originally was recovered from eukaryotic cell cultures, and its origin was traced to a human tumor cell line.[7,150,375] It has been recovered more recently from primary lymphocyte cells in patients with AIDS.[49]

AIDS-ASSOCIATED MYCOPLASMAL INFECTIONS

The frequent identification of *M. fermentans*, *M. pirum*, and *M. penetrans* infections in HIV-infected patients has led to the consideration that they may function as cofactors in the progression of HIV infection.[49,356,470] Although these mycoplasmas have the capacity to invade cells and to be potent immunomodulators, their pathogenic role, if any, in association with HIV has not been determined yet.

MYCOPLASMA AND *UREAPLASMA* INFECTIONS IN IMMUNOCOMPROMISED PATIENTS

Patients with hypogammaglobulinemia are susceptible to severe persistent infection with *Ureaplasma* sp., *M. hominis*, *M. amphoriforne*, *M. pneumoniae*, and *M. orale*.[17,207,224,468,537,559] Clinical manifestations include osteomyelitis, arthritis, cellulitis, and chronic respiratory illness. Patients need to be treated for prolonged periods with high-dose intravenous immunoglobulin and antibiotics to which the specific agents are susceptible. Severe and persistent infections also have occurred in liver, kidney, and bone marrow transplant recipients, as well as other immunocompromised patients.[116,307,326,416,459,528,618,682]

Yechouron and associates[711] reported a 64-year-old man with Hodgkin lymphoma who died of septicemia caused by

Mycoplasma arginini, an animal pathogen.[711] Three children with cancer had pneumonia in which bronchoalveolar lavage fluid specimens yielded *U. urealyticum*.[72] Two of the patients died, and the survivor's improvement coincided with erythromycin treatment. A 9-year-old boy had an abdominal infection with *M. hominis* after liver transplantation,[412] and a 15-year-old girl had septic arthritis due to *M. hominis* 2 months after renal transplantation.[453]

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SECTION XXI

FUNGAL DISEASES

CHAPTER 209

CLASSIFICATION OF FUNGI

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Fungi are prominent causes of serious infections in immunocompromised and hospitalized individuals. Organisms once thought to be contaminants can play important roles in pathogenesis in humans. The laboratorian must learn the basic structures of fungi and principles of classification to recognize and to identify medically important fungi.

The medically important fungi are contained in a biologic kingdom called Fungi.[9] These organisms are divided into two groups on the basis of their basic growth pattern: yeasts and molds. Yeasts are unicellular fungi that reproduce by budding or by fission. Molds are multicellular fungi that grow by means of filamentous threads called hyphae. Developed reproductive propagules can be found on the hyphae and are used to propagate or to disseminate the organism.[2,3]

Fungi may reproduce sexually or asexually. The sexual form of growth is called the teleomorph, and the asexual form is called the anamorph. The teleomorph and the anamorph forms of the same fungus have different names. *Pseudallescheria boydii*, for example, is the teleomorph of two anamorphs, *Scedosporium apiospermum* and *Graphium eumorphum*. Although the sexual means of reproduction forms the basis of the taxonomy of fungi, it is the anamorph that is isolated predominantly from specimens of patients. For this reason, the anamorph name usually is used in reports from a clinical microbiology laboratory.

The kingdom Fungi is composed of four phyla.[6] The phylum name is defined by the type of sexual spore produced. Sexual spores are specialized structures that house the products of meiosis after sexual reproduction. The phylum names and the type of sexual spore formed by its members are as follows:

1. Zygomycota (zygospores)
2. Ascomycota (ascospores)
3. Basidiomycota (basidiospores)
4. Chytridiomycota (motile oospores)

The phylum Chytridiomycota does not contain any human pathogens. Another group, which is not taxonomically referred to as a phylum, contains fungi that do not produce sexual spores or for which no association has been made between the anamorph and teleomorph forms. This group is referred to as *anamorphic fungi*[8] or *fungi imperfecti*. The teleomorph phase has not been detected or described for many human pathogens; therefore, a genus name may be found under a well-described phylum as well as under the anamorphic fungi.

The taxonomic scheme depicted is based on various sources.[1,4,6,9,11] The original draft of the scheme was published in 2003.[6]

The basic taxonomic units are the species, and they are grouped into a hierarchical system that includes genera, families, orders, classes, and phyla. The categories may by subdivided (e.g., subphylum, subclass, suborder) to indicate degrees of relationship. Populations within a given species that have some characteristics in common may be set apart as tribes or varieties or some other subset designation. Delineation of the zoopathogen *Ajellomyces capsulatus*, the teleomorph of *Histoplasma capsulatum*, is as follows:

Kingdom: Fungi
- Phylum: Ascomycota
  - Class: Ascomycetes
    - Order: Onygenales
      - Family: Onygenaceae
        - Genus: *Ajellomyces*
          - Species: *Ajellomyces capsulatus* (anamorph: *Histoplasma capsulatum*)
            - Variety: The varietal state applies to the anamorph.[9]
              - *H. capsulatum* var. *capsulatum*
              - *H. capsulatum* var. *duboisii*
              - *H. capsulatum* var. *farciminosum*

This chapter includes taxa known to contain medically important fungi. Classes, orders, and families not known to contain pathogens are omitted. More detailed considerations can be obtained by consulting the references.[1-11]

Kingdom: Fungi
- Phylum: Zygomycota. The teleomorph (sexual form) consists of zygospores. The anamorph (asexual form) consists of sporangiospores within a sporangium.
  - Class: Zygomycetes
    - Order: Mucorales
      - Family: Mucoraceae
        - Genus: *Apophysomyces*
          - *Absidia*
          - *Mucor*
          - *Rhizomucor*
          - *Rhizopus*
      - Family: Cunninghamella
        - Genus: *Cunninghamella*
      - Family: Mortierellaceae
        - Genus: *Mortierella*
      - Family: Saksenaeaceae
        - Genus: *Saksenaea*
      - Family: Syncephalastraceae
        - Genus: *Syncephalastrum*
      - Family: Thamnidiaceae
        - Genus: *Cokeromyces*
    - Order: Entomophthorales—agents of entomophthoromycoses
      - Family: Basidiobolaceae
        - Genus: *Basidiobolus*
      - Family: Ancylistaceae
        - Genus: *Conidiobolus*

Phylum: Ascomycota. The teleomorph (sexual phase) consists of ascospores borne in an ascus. The anamorph (asexual phase) may be unicellular yeasts or multicellular molds. Molecular phylogeny studies have been used to define this group, even when a known teleomorph for a given anamorph has not been described.

- Class: Hemiascomycetes (Endomycetes)—yeasts (unicellular fungi)
  - Order: Saccharomycetales—ascomata absent. Asci are formed singly or in chains. Yeast cells divide by budding or fission.
    - Family: Saccharomycetaceae
      - Genus: *Candida*
        - *Saccharomyces*
- Class: Ascomycetes—molds. For more complete descriptions, see references 4 and 11.
  - Order: Onygenales—ascomata are cleistothecia
    - Family: Arthrodermataceae—agents of tinea (ringworm)
      - Genus: *Arthroderma*
        - *Microsporum* (anamorph)
        - *Trichophyton* (anamorph)
        - *Epidermophyton* (closely related, but no teleomorph has been described)
    - Family: Onygenaceae—agents of endemic pulmonary mycoses
      - Genus: *Ajellomyces* (teleomorph)
        - *Histoplasma* (anamorph)
        - *Blastomyces* (anamorph)
        - *Emmonsia* (anamorph)
        - *Coccidioides* (anamorph; no teleomorph has been described)
        - *Paracoccidioides* (anamorph; no teleomorph has been described)
  - Order: Eurotiales—ascomata are cleistothecia
    - Family: Trichomaceae—contains the greatest number of human pathogens
      - Genus: *Aspergillus* (anamorph)
        - *Penicillium* (anamorph)
        - *Paecilomyces* (anamorph; no teleomorph has been described)
  - Order: Ophiostomales—ascomata are perithecia with long necks
    - Family: Ophiostomaceae
      - Genus: *Ophiostoma* (teleomorph)
        - *Sporothrix* (anamorph)
  - Order: Hypocreales—ascomata are mostly perithecia
    - Family: Hypocreaceae—contains agents of mycoses
      - Genus: *Acremonium* (anamorph)
        - *Fusarium* (anamorph)
        - *Trichoderma* (anamorph)
        - *Verticillium* (anamorph)
  - Order: Microascales—ascomata are cleistothecia or perithecia
    - Family: Microascaceae
      - Genus: *Pseudallescheria*
        - *Scedosporium* (anamorph)
        - *Microascus*
        - *Scopulariopsis* (anamorph)
  - Order: Sordariales—ascomata are cleistothecia or perithecia; rarely pathogens
  - Order: Dothideales—ascomata are cleistothecia
    - Family: Didymosphaeriaceae
      - Genus: *Neostudina* (teleomorph)
    - Family: Herpotrichiellaceae—agents of phaeohyphomycoses and chromoblastomycoses
      - Genus: *Cladophialophora* (anamorph)
        - *Exophiala* (anamorph)
        - *Fonsecaea* (anamorph)
        - *Phialophora* (anamorph)
        - *Rhinocladiella* (anamorph)
    - Family: Mycosphaerellaceae
      - Genus: *Cladosporium* (anamorph)
    - Family: Piedraiaceae
      - Genus: *Piedraia* (teleomorph)
  - Order: Pleosporales
    - Family: Pleosporaceae
      - Genus: *Cochliobolus* (teleomorph)
        - *Bipolaris* (anamorph)
        - *Curvularia* (anamorph)
        - *Drechsleria* (anamorph)
- Class: Archiascomycetes[4]—found as trophozoites, cysts, and intracystic bodies; originally considered members of the kingdom Protozoa[7]
  - Order: Pneumocystidales
    - Family: Pneumocystidaceae
      - Genus: *Pneumocystis*[10]

Phylum: Basidiomycota. The teleomorph is a basidium bearing basidiospores. The anamorphs are yeasts or molds. Arthroconidia are reproductive propagules of some molds.

- Class: Hymenomycetes
  - Order: Tremellales
    - Family: Filobasidiaceae
      - Genus: *Filobasidiella* (teleomorph)
        - *Cryptococcus* (anamorph)
  - Order: Trichosporonales
    - Family: Trichosporonaceae
      - Genus: *Trichosporon* (anamorph)
- Class: Ustilaginomycetes
  - Order: Malasseziales
    - Family: Malasseziaceae
      - Genus: *Malassezia* (anamorph)
- Class: Urediniomycetes
  - Order: Sporidiales
    - Family: Sporidiobolaceae
      - Genus: *Rhodotorula* (anamorph)
        - *Sporobolomyces* (anamorph)

ANAMORPHIC OR FUNGI IMPERFECTI

These fungi have no known teleomorph phase and have been placed in a separate category for the convenience of discussion. At one time, these fungi were placed in the phylum Deuteromycota; however, this designation no longer is used in medical mycology. The anamorphic fungi are divided into subgroups but are not given names associated with orders or families.

SUBGROUP: BLASTOMYCETES. These organisms are unicellular fungi that reproduce by budding or fission. Although these yeasts have no described teleomorphic phase, they are related to the ascomycetes and basidiomycetes.

Genus: *Candida*
Cryptococcus
Rhodotorula
Trichosporon

SUBGROUP: HYPHOMYCETES. These molds produce asexual conidia. Moniliaceous forms have hyaline hyphae and conidia. Like the Blastomycetes, these forms have ascomycete or basidiomycete affinities. Moniliaceous hyphomycetes are the following:

Genus: *Acremonium*
Aspergillus
Blastomyces

Coccidioides
Epidermophyton
Fusarium
Microsporum
Penicillium
Trichophyton

Dematiaceous forms have dark conidia and hyphae and are agents of chromoblastomycosis and phaeohyphomycosis.

Genus: *Bipolaris*
Cladophialophora
Cladosporium
Curvularia
Phialophora
Rhinocladiella
Sporothrix

SUBGROUP: COELOMYCETES. These are molds that produce reproductive elements within pycnidia or on acervuli. They are agents of mycetoma and wound infections.

Genus: *Phoma* (pycnidium forming)
Colletotrichum (acervulus forming)

SUBGROUP: AGONOMYCETES (*MYCELIA STERILIA*). Molds with no reproductive propagules belong to this group.

Genus: *Rhizoctonia*

ORGANISMS OF UNCERTAIN POSITION

The taxonomic position of *Rhinosporidium seeberi* has always been uncertain.[5] Rhinosporidiosis presents as tumor-like masses in the nasal mucosa or on conjunctiva of humans.[9] Studies involving 18S rRNA genes from infected tissue have shown a phylogenetic relationship to a novel group of protists infecting fish and amphibians. The fungus is "the first known human pathogen from the DRIPs clade, a novel clade of aquatic protistan parasites (Ichthyosporea)."[5]

Prototheco sis is a rare cutaneous disease caused by *Prototheca* spp. The taxonomic position of these microorganisms is not established, but they are thought to be achloric algae. Although not considered to be fungi, they often are included in medical mycology texts.[9]

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CHAPTER 210

ASPERGILLUS AND ASPERGILLOSIS

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The incidence of *Aspergillus* infection is increasing and carries a dismal mortality.[5,85,116] Invasive aspergillosis is the most common cause of mortality due to invasive mycoses, generally attributable to the success with prophylactic antifungal regimens and easier diagnostic examinations for *Candida* infection. Invasive aspergillosis is gaining prominence probably as a result of the use of more intensive immunosuppressive therapies for graft-versus-host disease (GVHD) and rheumatologic diseases, increased use of mismatched or unrelated donor transplants, use of newer preparative regimens to avoid rejection or relapse, and increased early post-transplantation survival due to better control of bacterial and cytomegalovirus infections.[85,153] Unfortunately, at present, there are not optimal methods for either establishing early and accurate diagnosis or consistently providing effective treatment. Currently, the field is in a state of rapid advancement, both of newer molecular tools and of novel antifungal strategies.

THE ORGANISM

Although the genus *Aspergillus* contains approximately 185 species, most human disease is caused primarily by *Aspergillus fumigatus*, *Aspergillus flavus*, *Aspergillus niger*, *Aspergillus terreus*, and *Aspergillus nidulans*. *A. fumigatus* causes approximately 90 percent of cases of invasive aspergillosis.[24] A review of *Aspergillus* cultures found that amphotericin B–resistant *A. terreus* was seen in only 3 percent of isolates in cases of invasive aspergillosis but exclusively in cases of invasive aspergillosis (i.e., no cases of colonization).[106] *A. fumigatus* is responsible for most pulmonary disease, whereas most isolated sinus disease is caused by *A. niger* and *A. flavus*.[106]

Aspergillus is a ubiquitous organism, but its ecologic niche is the soil, where it functions as a saprophytic fungus growing on organic debris and recycling carbon and nitrogen.[163] Currently, no sexual stage has been identified for *A. fumigatus*, thereby eliminating the possibility for performing classic genetic studies, but genomic evidence suggests that it is capable of sexual reproduction.[103] Asexual reproduction is abundant and characterized by the production of green pigmented echinulate asexual conidia (spores). The conidia are produced in chains basipetally from the phialides borne on clavate vesicles. This reproductive structure is where the genus name *Aspergillus* is derived, from the aspergillum used to sprinkle holy water in the Catholic Mass. The morphologic features of the conidia and conidiophores are largely used to distinguish the individual *Aspergillus* species.

Aspergillus aerosolize conidia, which immunocompetent people asymptomatically breathe every day, and most people inhale an estimated several hundred *A. fumigatus* conidia a day.[44] Infection in immunocompromised patients usually is acquired through inhalation of airborne conidia. Two lines of host defense exist against *Aspergillus*, macrophages and neutrophils, and both mechanisms of host defense appear to be required for resistance to invasive disease. The first defense is formed by alveolar macrophages, and in vitro murine studies have suggested that resident pulmonary macrophages are responsible for eliminating inhaled *Aspergillus* conidia from the lung.[122,123] If conidia escape, they develop into invasive *Aspergillus* hyphae and become susceptible to neutrophil killing by the release of toxic reactive oxygen species.[125] The host can develop disease through neutropenia, high challenge doses of conidia overcoming macrophages, or corticosteroid suppression of macrophage conidiacidal activity.[34]

A. fumigatus, in particular, is highly successful at causing invasive disease in immunocompromised patients because of several characteristics, such as thermotolerance, small and abundant pigmented conidia, fast growth rates, production of toxic secondary metabolites, and numerous enzymes involved in breaking down complex polysaccharides. The ability to grow at 37° C is crucial for the development of human disease. Some *Aspergillus* spp. can grow at this high temperature, reportedly *A. fumigatus* can survive at temperatures up to 75° C, and this thermotolerance is likely to be a major factor in its ability to cause human morbidity.[7] In addition, at 37° C in a nutrient-rich environment, *A. fumigatus* can grow at an extremely fast rate.

Conidia are 2.5 to 3 μm in diameter, and this small size allows them to remain buoyant in the air for prolonged periods and to be inhaled deep into the lung alveoli. When inhaled by an immunocompetent person, conidia rarely have deleterious effects because they are cleared by phagocytic cells of the innate immune system. However, severe allergic disease can occur with repeated exposure to large doses of conidia. Pigments found on the conidial surface are thought to play important roles in mediating the effects of damaging reactive oxygen species produced by phagocytic cells.[108] Mutants of *A. fumigatus* that lack these conidial pigments are more susceptible to killing by alveolar macrophages than are their pigmented counterparts.[71] Whole genome sequencing of several *Aspergillus* spp. has revealed that these fungi contain large numbers of enzymes involved in degrading complex polysaccharides.[39] Although these enzymes allow *Aspergillus* spp. to be enormously successful as saprophytes, these enzymes also are thought to contribute to their ability to cause invasive mycoses.

Species of *Aspergillus* also are well known for their production of secondary metabolites. Perhaps the most well known *Aspergillus* metabolite is the carcinogen aflatoxin produced by some strains of *A. flavus*. Although aflatoxin is extremely carcinogenic, its role in the development of human mycoses has not been thoroughly examined to date. Various suspected putative *Aspergillus* virulence factors have been reviewed extensively,[74] and earlier gene disruption studies examined proteases, toxins, hemolysins, melanin pigmentation, and other gene products with little success. The true virulence of *A. fumigatus* is likely to be a multifactorial combination of these described attributes.

CLINICAL PRESENTATION

Aspergillus spp. are unique among pathogens; they are responsible for a gamut of infections extending across the clinical spectrum to include primary allergic reactions, saprophytic involvement, and invasive disease.[86] The type of *Aspergillus* infection generally depends on the immunologic background of the infected host; immunodeficient patients develop invasive disease, and immunoreactive patients develop an allergic disease. Most frequently encountered among the myriad clinical presentations are presentations involving the lungs, such as allergic bronchopulmonary aspergillosis and acute or chronic invasive pulmonary aspergillosis (IPA). Other common infections caused by *Aspergillus* spp. include invasive chronic sinusitis, cutaneous aspergillosis, aspergilloma, and cerebral aspergillosis.[24,86] The clinical manifestations of these infections are subtle and nonspecific and commonly occur late in the course of disease. As a result, a high index of suspicion needs to be maintained to implement treatment in the early stages of disease. Unfortunately, the patients most vulnerable to *Aspergillus* infections are the least likely to display significant symptoms, adding to the difficulty of establishing the diagnosis.[24]

PULMONARY ASPERGILLOSIS

Aspergillus spp. are ubiquitous in the environment, and one major portal of entry is the respiratory tract. Most people inhale the conidia daily, which usually is not a problem for the immunocompetent host as the functioning immune system will quickly clear the conidia. In some immunocompetent patients, this inhalation could result in nonpathogenic saprophytic colonization, whereas this conidial acquisition in the immunocompromised patient likely will result in establishment of invasive disease.[144] IPA is the most frequently documented form of aspergillosis and contributes considerably to morbidity and mortality among high-risk patients.[27] In a systematic review of the literature regarding the case-fatality rate of aspergillosis (1995-1999), 70 percent of infections were found to be invasive pulmonary disease.[77] As with other forms of *Aspergillus* infections, the burden of IPA is sustained by immunocompromised patients, including hematopoietic and solid organ transplant recipients, cancer patients, patients with various congenital immune deficiencies such as chronic granulomatous disease (CGD), and patients treated for autoimmune diseases.[117]

The clinical manifestation of IPA is heterogeneous; typically, it may include fever unresponsive to broad-spectrum antibiotics, dry cough, shortness of breath, pleuritic chest pain, hemoptysis, and pulmonary infiltrates on radiography.[2] Whereas neutropenic patients usually present with fever, approximately 20 percent of patients, specifically those receiving high-dose corticosteroid therapy,[54] will have no fever or cough the first several days of infection.[86] In general, disease often is bilateral diffuse pulmonary infection, and dyspnea is a more common presentation in these patients. Progression of infection is characterized by invasion of small vessels leading to hemoptysis as a primary symptom of IPA in some neutropenic patients. Two patterns of hemorrhage may be identified: (1) hemorrhagic infarction due to vascular invasion; and (2) during recovery from neutropenia, formation of mycotic aneurysms, which can rupture and result in fatal hemoptysis.[94]

Although patients undergoing treatment of cancer constitute the majority of individuals at risk for acquisition of *Aspergillus* infections, another largely pediatric population deserving a separate discussion is those with underlying CGD because IPA is the leading cause of mortality in these patients. In fact, patients with CGD have an estimated 33 percent lifetime risk of acquiring invasive aspergillosis, and IPA may be the first manifestation of CGD. Unlike the acute, rapidly progressive illness described for other immunocompromised patients, the clinical course in patients with CGD is characterized by an insidious onset of fatigue, fever, increased sedimentation rate, and pneumonia.[127] Presentation in a patient with CGD often does not have typical clinical symptoms (including a completely asymptomatic patient) and may consist of only an elevated erythrocyte sedimentation rate in the setting of no fever. In the early course of the disease, an acute neutrophilic response occurs in which the neutrophils surround hyphae. However, in patients with CGD, the hyphae

remain intact as a result of impaired neutrophil-mediated killing of hyphae. In this setting, pulmonary aspergillosis is a chronic progressive infection that may spread locally to involve pleura, vertebrae, and the chest wall. In contrast to patients with neutropenia, patients with CGD do not have hyphal angioinvasion as a feature of their disease. The halo sign (angioinvasion with surrounding tissue ischemia), cavitated lesions, and pulmonary infarcts are not typical findings in CGD. In a long-term follow-up study of 39 patients with CGD, the lungs were the site of infection in 23 percent of the 478 cases observed overall, and 67 percent of the patients had at least one episode of pneumonia.[76] Furthermore, among the 151 cases in which an infectious agent was identified, 12 (8%) of these isolates were *Aspergillus* spp.

INVASIVE *ASPERGILLUS* SINUSITIS

Fungal sinusitis can be manifested as allergic, saprophytic, or invasive disease. Invasive *Aspergillus* sinusitis probably is underdiagnosed because of its variable clinical presentation and the difficulty in establishing the diagnosis[24,54,86] due to a decreased inflammatory response in affected patients. Patients can present with nasal congestion, discharge, headache, facial pain or swelling, and abnormal findings of the nasal cavity, such as pallor of the nasal septum or turbinate mucosa. Epistaxis, orbital swelling, and high fever also can be present.[97] However, definitive diagnosis can be established best by endoscopic evaluation and selective biopsy. Common findings on endoscopy include pallor of the mucosa, discoloration or granulation of the mucosa due to ischemia that develops as a result of angioinvasion, and, as the disease progresses, a blackened necrotic focus (Figs. 210–1 and 210–2). Extension into bony structures can occur at the site of necrosis, leading to spread of the disease into adjacent structures, such as the orbit and the brain, which carries a high mortality rate.

Although imaging is not diagnostic, it can aid in establishing the diagnosis because it can be used as a road map for endoscopy by showing which sinuses are involved (Fig. 210–3). In a review of 25 patients with rhinosinusitis, 44 percent showed evidence of invasion beyond the sinus cavities on computed tomography (CT).[42] In the same study, however, 12 percent of patients had a normal CT scan, again highlighting that a high index of clinical suspicion must guide establishment of the diagnosis to ensure the best outcome.

CEREBRAL ASPERGILLOSIS

Aspergillus infections most commonly involve the lungs, but disease can disseminate through the bloodstream and involve distant organs. One of the most frequent sites of dissemination

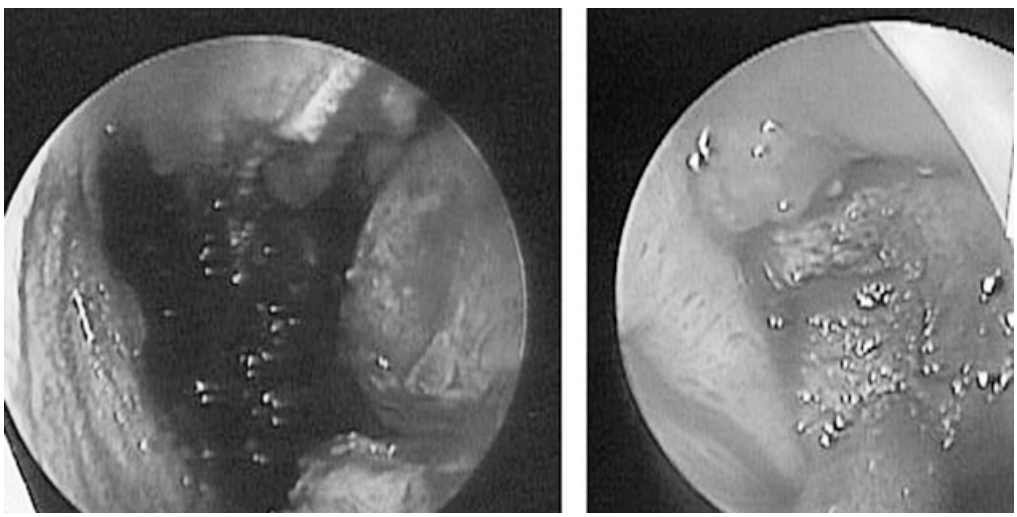

Figure 210–1 A 12-year-old with acute myelogenous leukemia and unrelated cord blood transplantation who developed *A. fumigatus* sinusitis. Endoscopy shows classic pale mucosa. (See companion Expert Consult web site for color version.)

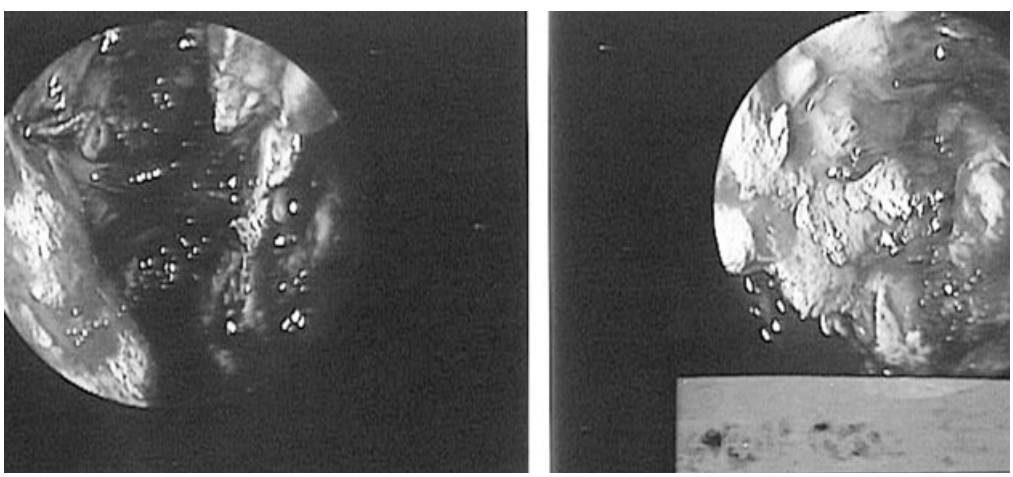

Figure 210–2 In the same patient as in Figure 210–1, endoscopy shows necrotic mucosa at the basilar skull area. (See companion Expert Consult web site for color version.)

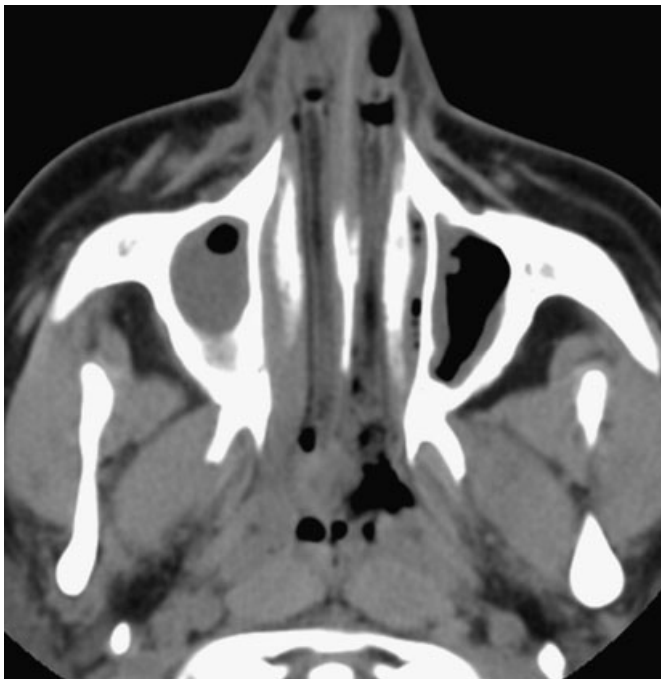

Figure 210–3 In the same patient as in Figure 210–1, CT shows complete opacification of the right maxillary sinus.

is the central nervous system (CNS).[47] Cerebral aspergillosis also may be a result of direct extension through the sinuses. Studies have shown that CNS aspergillosis may be found in an estimated 40 to 50 percent of patients with invasive aspergillosis and acute leukemia or allogeneic stem cell transplantation.[57,121] As with other *Aspergillus* infections, *A. fumigatus* is the species most frequently encountered in cerebral aspergillosis, but other implicated species are *A. flavus*, *A. niger*, and *A. nidulans*.[58] Clinical presentation often does not include more classic symptoms for an intracranial process, such as headache, nausea, or vomiting. Instead, patients present with alterations in mental status, convulsions, hemiplegia or hemiparesis, ophthalmoplegia, and loss of consciousness. Severely immunocompromised patients may not display these symptoms, and disease progresses more rapidly.

Aspergillus hyphae are angioinvasive and thrombose arteries to create hemorrhagic infarcts; as a result, CNS aspergillosis can present as solitary or multiple abscesses and, less commonly, as mycotic aneurysms and carotid artery invasion. Cerebral aspergillosis also can appear as meningitis or granuloma.[58] Cerebral aspergillosis presents as multiple areas of low density and no enhancement even with the administration of contrast material on CT, and lesions usually are located within the basal ganglia and gray-white matter junction. On magnetic resonance imaging (MRI), these same abnormalities appear as foci of intermediate T2 signal surrounded by a rim of higher signal.[101,143] Aspergillosis of the CNS carries a case-fatality rate of nearly 90 percent,[77] so prompt diagnosis and treatment are key to survival. Unfortunately, definitive diagnosis requires biopsy, and typically these patients are too coagulopathic to undergo such a procedure.

CUTANEOUS ASPERGILLOSIS

Neonates, burn victims, hematopoietic stem cell transplant (HSCT) recipients, solid organ transplant recipients, and other immunocompromised patients are the ones most frequently found to develop cutaneous aspergillosis. Although it is not the most commonly encountered *Aspergillus* infection, cutaneous infection has a reported incidence between 4 and 11 percent.[22,161] Cutaneous aspergillosis can be primary, a result of direct skin injury or traumatic inoculation, or secondary, a result of hematogenous spread or extension from infected underlying structures.[147] Primary cutaneous aspergillosis has been associated with intravenous access devices, adhesive dressings, and sites of burns or surgery. Premature infants are at particular risk because their skin is immature and most vulnerable. HSCT recipients generally develop disease as a result of hematogenous seeding from a primary source, usually the lungs. Lesions often begin as erythematous, indurated papules that progress to ulcerative, painful, and necrotic lesions.[134] Treatment involves débridement and excision of necrotic tissue, which provides diagnostic material, and systemic intravenous antifungals as well as topical preparations. The role of topical antifungal treatment alone is unclear, and it is probably inadequate if it is used alone, as often the cutaneous aspergillosis is a harbinger of underlying undiagnosed systemic disease.

CHRONIC ASPERGILLOSIS

Chronic aspergillosis is encountered less frequently than is acute disease, and the patient population affected typically is distinct as well. A degree of immune suppression does exist in these patients, and exposure to corticosteroids is a common finding. Underlying conditions include corticosteroid use for chronic lung disease, diabetes mellitus, and alcoholism.[24] Three separate categories are used to describe chronic aspergillosis.[30] The first is chronic cavitary pulmonary aspergillosis, in which multiple cavities form and expand and may be occupied by fungus balls. The second is chronic fibrosing pulmonary aspergillosis, which, as the name connotes, is characterized by progression of the cavities into extensive pulmonary fibrosis. The final category has been defined as chronic necrotizing pulmonary aspergillosis, also known as either subacute invasive or semi-invasive pulmonary aspergillosis.[30] The third category is an indolent pulmonary infection of patients with mild or moderate defect in immune function such as in the setting of prolonged corticosteroid use, unlike the other categories, and may be seen after resolution of neutropenia.[86] Chronic necrotizing pulmonary aspergillosis is a slowly progressive disease, and clinical signs observed include cough, fever, fatigue, and weight loss. Diagnosis requires biopsy, and treatment is with long-term antifungal therapy.[63]

ASPERGILLOMA

Aspergillomas consist of a mass of tangled hyphae, cellular debris, fibrin, and few inflammatory cells. They develop as a complication of existing cavitary lesions and are typically found in the upper lobes where there is poor drainage.[86,144] The cavitary lesions may be a result of tuberculosis infection, sarcoidosis, bullous emphysema, and other diseases associated with cavitary lung lesions. *A. fumigatus* typically causes pulmonary aspergilloma, and patients may remain asymptomatic, although many present with recurrent hemoptysis. Perhaps the earliest radiographic sign is pleural thickening of a preexisting lung cavity. Later, as the fungus ball develops, CT or plain radiography will show a solid and round mass within the cavity separated from the thickened wall by an air crescent sign.[46] Aspergillomas can be classified as simple or complex on the basis of radiologic criteria; simple indicates those lacking constitutional symptoms, paracystic lung opacities, cyst expansion, or progressive pleural thickening.[64] Treatment of active disease is aimed at control and prevention of complicating symptoms such as hemoptysis, and surgical resection is the definitive treatment. However, given the high morbidity and mortality rate in complex aspergilloma, surgi-

cal intervention is reserved for high-risk patients. Other treatment modalities, such as endobronchial and intracavitary instillation of antifungals, have been attempted with some success given that systemic therapy is ineffective at diffusing into the cavity containing the fungus ball.[64,86]

ALLERGIC BRONCHOPULMONARY ASPERGILLOSIS

Allergic bronchopulmonary aspergillosis occurs in asthmatic and cystic fibrosis patients and is a pulmonary disease caused by type I and type III hypersensitivity reactions to *A. fumigatus* allergens. Exposure to and inhalation of *Aspergillus* spores result in saprophytic colonization of the bronchial airways, which then triggers an IgE-mediated allergic inflammatory response. Over time, bronchial obstruction takes place, and patients can present with productive cough, wheezing, and chest pain; fever and malaise also are presenting symptoms.[135] Diagnosis is based on eight criteria: (1) history of bronchial obstruction (asthma), (2) peripheral blood eosinophilia, (3) elevated serum IgE levels, (4) specific IgE and IgG to *Aspergillus* antigen in serum, (5) immediate (type I) skin reactivity to *Aspergillus* antigen, (6) precipitating antibodies to *Aspergillus*, (7) transient pulmonary infiltrates, and (8) central bronchiectasis. Allergic bronchopulmonary aspergillosis is characterized radiologically by bronchial wall thickening, pulmonary infiltrates, and central bronchiectasis, a result of the type III hypersensitivity reaction.[86,144] Therapy consists of administration of corticosteroids to effectively control inflammatory responses and the antifungal itraconazole. Itraconazole has been established as the first-line agent for allergic bronchopulmonary aspergillosis in several studies.[16,19,136]

EPIDEMIOLOGY

It is critical to understand both the individual patient with invasive aspergillosis and the epidemiology of the pathogen to predict the likelihood of disease development as well as to prognosticate the outcome. Host factors are paramount, and the clinician needs to take into consideration the underlying and concomitant diseases as well as the degree and type of immunosuppression. Aspergillosis is the most common mold infection, and a review of more than 5500 patients who underwent HSCT during a 15-year period found that more than 7 percent had mold infections, including 342 patients with proven or probable *Aspergillus*, 31 patients with *Fusarium*, 29 with Zygomycetes, and 10 with *Scedosporium* infections.[85] Invasive aspergillosis is the leading cause of infectious death in HSCT recipients, and one study showed that 36 percent of all confirmed nosocomial pneumonia in these patients was caused by *Aspergillus* infection, with a crude mortality rate of 95 percent.[102] The incidence of *Aspergillus* infection in HSCT recipients has ranged from 3 to 7 percent,[90,164] but the true incidence is likely to be dependent on the follow-up duration of individual studies. A review of more than 5500 HSCT patients from 1990 to 1998 found that the yearly incidence of invasive aspergillosis increased in both allogeneic transplants (5% to 12%) and autologous transplants (1% to 5%), whereas the incidence of non–*A. fumigatus* spp. as the cause of invasive pulmonary disease dramatically increased after 1995 (18% to 34%).[85] In another study of 13 HSCT recipients with invasive aspergillosis, the risk for development of the disease was 12.8 times higher among recipients of allogeneic than of autologous HSCT.[99] A retrospective review of 409 patients showed 13.1 percent had invasive aspergillosis, which was 17.2 percent of all isolated pathogens in allogeneic transplant recipients and 3.8 percent in autologous transplant recipients.[68]

A well-characterized bimodal distribution of aspergillosis in HSCT recipients correlates with pre-engraftment neutropenia (median of 16 days after transplantation) and the peak of GVHD (median of 96 days after transplantation).[152] This correlation probably relates to the two major mechanisms of protection against invasive aspergillosis, alveolar macrophages and granulocytes. Most patients (86%) with autologous transplants were diagnosed with invasive aspergillosis while they were neutropenic, whereas patients with allogeneic transplants were at greatest risk after engraftment or during impairment of cell-mediated immunity caused by cytomegalovirus infection or GVHD.[152] In large reviews of patients undergoing allogeneic HSCT, invasive aspergillosis was diagnosed at a mean of 88 to 115 days after transplantation,[87,99,116,121] with mortality rates exceeding 80 percent. In some studies,[87,99] most cases were diagnosed after day +30; in another study,[56] no cases of invasive aspergillosis were diagnosed during the neutropenic period after transplantation. Pediatric patients undergoing transplantation generally are less vulnerable to all infections than are their adult counterparts,[37] and a review of 585 infections in 276 children who underwent HSCT found 38 (6%) mold infections in the first year after transplantation, including 28 *Aspergillus* infections.[6]

The risk of invasive aspergillosis is calculated to increase from 1 percent per day after the first 3 weeks of neutropenia to 4 to 5 percent per day after 5 weeks,[126] with a 70 percent incidence after neutropenia exceeds 34 days.[41] Prolonged or marked macrophage dysfunctions that result from underlying disease and its treatment also can predispose patients to invasive aspergillosis. Therefore, risk for development of infection is higher with advanced underlying disease, transplantation performed during relapse of malignant disease or chemotherapeutic rescue therapy, GVHD, or concurrent infection such as with cytomegalovirus.[126] In a survey of 24 medical centers of 148 invasive aspergillosis patients, 30 percent also had a bacterial infection, 20 percent also had a viral infection, and 19 percent also had another fungal infection.[106]

Corticosteroids also are well-known major risk factors for the development of invasive aspergillosis and can suppress the ability of monocytes-macrophages to kill conidia through inhibition of nonoxidative processes and impairment of lysosomal activity. Corticosteroids also inhibit polymorphonuclear neutrophils in their chemotaxis, oxidative bursts, and activity against hyphae.[35] In general, corticosteroids suppress macrophages, whereas cytotoxic chemotherapy decreases neutrophil number and function. Corticosteroids also greatly accelerate the growth of *A. fumigatus*, in one study increasing the doubling time to 48 minutes.[98]

In several HSCT recipient risk-factor studies, only moderate-severe GVHD and steroid prophylaxis for GVHD[6,87,116,166] or total-body irradiation[166] were significant variables in the multivariate analyses. In one study, several parameters were found to influence survival in the period from HSCT to diagnosis of fungal infection, all related to cumulative dose of prednisolone. In the multivariate analysis, a relative risk of 8.78 of death from invasive aspergillosis was noted in patients with acute active GVHD (grade 2 or more) or extensive chronic GVHD combined with a cumulative total prednisolone dose of more than 7 mg/kg in the 1 week before diagnosis.[116]

Only a few pediatric studies have been performed to examine risk factors for development of invasive aspergillosis. One study found that the highest incidence of invasive aspergillosis was seen in children who had undergone allogeneic bone marrow transplantation (4.5%) and those with acute myelogenous leukemia (4%). Specifically, the incidence of invasive aspergillosis in patients with acute myelogenous leukemia was significantly greater than the incidence in patients with acute lymphoblastic leukemia (RR, 5.6; 95% CI, 4.6-7.0).[167]

Little specific information is available on the fundamental epidemiology of pediatric invasive aspergillosis, and most epidemiologic investigations do not offer pediatric analyses.[20,38,68,166] One study of 327 patients with invasive aspergillosis from 1985 to 1999 did stratify patients into three age groups.[85] A total of 13

percent of patients were younger than 19 years, 34 percent were between 19 and 40 years, and 53 percent were older than 40 years. The number of transplants performed in that youngest age group was not reported, so a true incidence of pediatric disease cannot be calculated. The true incidence of invasive aspergillosis in children, therefore, remains unknown despite numerous epidemiologic studies of the disease.

Even large-scale studies of infections in pediatric HSCT recipients do not answer the fundamental questions. In a study of 148 pediatric HSCT recipients from 1986 to 1996 with 8 proven patients with invasive aspergillosis, 48 patients had suspected invasive fungal infection, but the results were not stratified between *Candida* and *Aspergillus* infection, and no specific invasive aspergillosis analyses were performed.[55] A larger report reviewed 510 pediatric HSCTs in 485 patients from 1990 to 1998.[6] There were 26 cases of invasive aspergillosis (4.79% of infections) in 584 culture-proven infections during the first year after transplantation. This pediatric report was the first to employ tools beyond descriptive statistics to analyze pediatric invasive aspergillosis. A multivariable analysis showed that invasive aspergillosis was more likely to be associated with severe GVHD (RR, 7.5%; 95% CI, 3.0-18.4). Additional analyses revealed that there were 10 cases of invasive aspergillosis in the first 30 days, 13 cases from 31 to 100 days after transplantation, and 3 cases from days 101 to 365 after transplantation.

Autopsy data from Japan for patients with invasive aspergillosis from years 1989, 1993, and 1997 analyzed the patients on the basis of age into single-decade blocks.[70] Of a total of 412 autopsies of patients with invasive aspergillosis, 14 invasive aspergillosis cases were in children aged 0 to 9 years and 24 were in those aged 10 to 19 years. By comparison, 92 cases were in patients aged 50 to 59 years, and 102 cases were in patients aged 60 to 69 years. The study did not report the total number of children in each range who underwent autopsy, so again, calculation of an incidence of pediatric invasive aspergillosis is impossible.

In an excellent review of invasive aspergillosis case-fatality rates pooled from 1941 patients from clinical trials, cohort or case-control studies, and case series of 10 or more patients with definite or probable invasive aspergillosis from 1995 to 1999, some stratification of case-fatality rate by decade of life was noted.[77] The mean age of all patients was 44.2 years (range, 3 to 91 years), and the youngest cohort (≤20 years) had a case-fatality rate of 68.2 percent (15/22). The next highest case-fatality rate was 59.3 percent in the age group 21 to 30 years. The investigators concluded that little variation exists in mortality by age, but the one pediatric case-fatality rate was considerably higher than that of the other age cohorts, suggesting the epidemiologic possibility that pediatric and adult invasive aspergillosis differ in outcome.

DIAGNOSIS

The diagnosis of invasive aspergillosis often is not straightforward. Because of the myriad clinical presentations, diagnosis of invasive aspergillosis is categorized as "proven," "probable," or "possible" disease on the basis of meeting certain clinical, microbiologic, and radiologic criteria designed by the European Organization for the Research and Treatment of Cancer and the Mycoses Study Group.[4] These criteria have served as a standard in clinical trials to group patients with similar disease characteristics, but these criteria are not perfect, and the designers have specifically cautioned against their implementation in routine clinical practice. Nonetheless, these distinctions have served the community well to establish a common framework for discussion about disease in the complicated high-risk patient. In general, proven and probable invasive aspergillosis can be considered as one entity because numerous clinical trials have shown their general equivalency in patient outcomes. However, possible disease is a vague entity and should be excluded from clinical trial design, and the clinical diagnosis should be strongly reconsidered.

CULTURES

A proven diagnosis of aspergillosis requires isolation from an otherwise sterile culture and histologic demonstration (Fig. 210–4).[4] However, this "gold standard" of tissue biopsy often is considered too invasive and complicated by bleeding or secondary infection in HSCT recipients. In a prospective study of 3857 clinical specimens from 230 patients, there were 86 positive mold cultures, including 95 percent as *Aspergillus* spp.[166] However, the important distinction for a positive *Aspergillus* culture must be made between disease and colonization. A 1-year retrospective study found that only 12 percent of patients with *Aspergillus*-positive cultures met criteria for invasive aspergillosis. Among high-risk patients, a positive culture was associated with invasive aspergillosis in 50 to 65 percent of cases; among intermediate risk, in 8 to 28 percent; and with low risk, rarely.[106]

In one study, the predictive value of respiratory tract cultures from patients with IPA was 40 to 100 percent.[145] Even in patients with established disease, the sputum specimens commonly are negative,[54,118] which is probably because IPA is predominantly infiltrative and does not have aerial growth in the bronchial tree.[54] Colonization with *Aspergillus* spp. has been a marker for reduced short-term survival, as 12 percent of colonized patients died within 3 months. IPA was diagnosed in 12 percent of patients with a positive culture, but this figure is likely to be an underestimate as culture specimens were not always obtained because diagnosis often was made by radiographic imaging.[106] In one study of heart transplant recipients, during a 10-year study period, *Aspergillus* spp. were recovered from 30 episodes from 27 heart transplant recipients (incidence: 10.5%). The overall positive predictive value was 60 to 70 percent, but it increased to 88 to 100 percent when it was recovered from a respiratory specimen other than sputum and decreased to 50 to 67 percent when it was

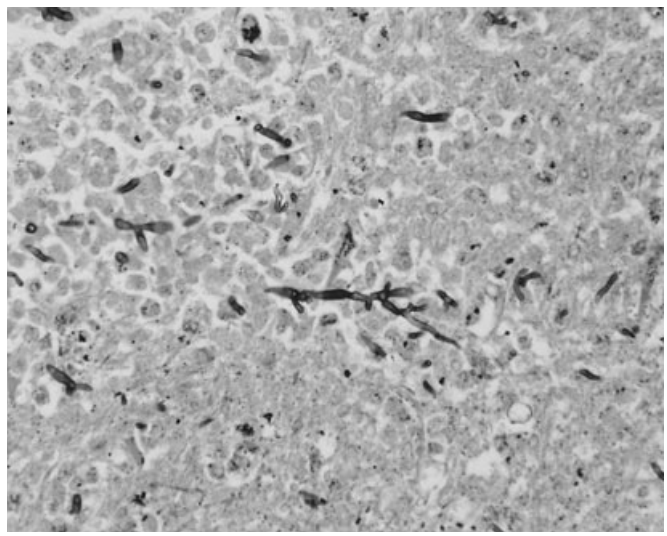

Figure 210–4 Histopathologic examination of invasive aspergillosis with Gomori methenamine silver stain of lung biopsy specimen shows septate hyphae. (See companion Expert Consult web site for color version.) *(Photograph courtesy of Dr. Thomas J. Cummings, Duke University Medical Center.)*

recovered from sputum. The sensitivities of fungal and conventional media for the recovery of *Aspergillus* spp. were 95 to 100 percent and 33 to 38 percent, respectively.[95]

Prior nasal colonization before receipt of a transplant has been associated as a predictor of subsequent IPA in some studies,[1] but the results have not been duplicated in others.[59] A prior episode of invasive aspergillosis does not eliminate the chance of having a successful HSCT, as one study reported recurrences in 9 patients with invasive aspergillosis before transplantation, and all patients showed no signs of delayed engraftment compared with transplant recipients without a history of invasive aspergillosis.[129]

Aspergillosis rarely is diagnosed by blood culture.[43] In a study of 1477 separate positive cultures, more than a dozen were positive, but most were associated with pseudofungemia or terminal events noted at autopsy.[106] In general, the *Aspergillus* hyphal mass that develops in the lumen during angioinvasion remains in place until the force of blood flow causes hyphal breakage, which then allows the mass to circulate. The likelihood of a blood culture capturing these irregularly and infrequently discharged units is small. This difficulty in detection of *A. fumigatus* in blood culture stands in contrast to other angioinvasive filamentous fungi (e.g., *Fusarium* spp., *Paecilomyces lilacinus*, *Scedosporium prolificans*, and *Acremonium* spp.) that have the ability to discharge a steady series of unicellular spores into the bloodstream, which are more likely to be captured in a blood sample. This ability to sporulate in tissue and blood has been termed adventitious sporulation.[124] As *A. terreus* also displays adventitious sporulation, histopathologic and potassium hydroxide examination of these spores also can allow rapid, presumptive identification of *A. terreus*. Therefore, a positive blood culture with *A. terreus* or another mold that demonstrates adventitious sporulation should not be ignored, and a positive blood culture with other *Aspergillus* spp. should be further evaluated.

RADIOLOGY

IPA characteristically is manifested on radiographs as multiple, ill-defined, 1- to 3-cm peripheral nodules that gradually coalesce into larger masses or areas of subsegmental and segmental consolidation. Lobar or diffuse pulmonary consolidation is a common finding.[18] Chest radiographs can be abnormal, but in one series they were normal in approximately 30 percent of patients in the week preceding death.[115]

IPA has two classic radiologic signs. The halo sign occurs in neutropenic patients with a hemorrhagic nodule caused by angioinvasion. An early CT finding of the halo sign is a rim of ground-glass opacity surrounding the nodule. In one study, the halo sign was seen in all patients with biopsy-proven IPA, but it is so nonspecific it was seen also in patients with zygomycosis, organizing pneumonia, or pulmonary hemorrhage.[165] These early lesions subsequently change into a cavitary lesion or lesion with an air crescent sign 2 to 3 weeks later when neutropenia recovers.[18,69] In one study, this sign was seen in 48 percent of patients 3 to 10 days after recovery of neutropenia.[121] Cavitation of the nodules or masses occurs in approximately 40 percent of patients and is characterized by an intracavitary mass composed of sloughed lung and a surrounding rim of air. A retrospective review of the CT findings for 47 autopsy-proven cases of IPA showed that nodular lesions and cavitation occurred significantly more often compared with controls.[48]

Just as the clinical presentation is nonspecific and heterogeneous, so are the findings in radiologic studies (Figs. 210–5 and 210–6). Although in most patients disease is seen as round infiltrates, peripheral wedge-shaped lesions, and the typical halo sign early in the course of IPA during the neutropenic period, hyphal angioinvasion is not a feature of disease in patients with CGD, and therefore the halo sign, cavitated lesions, and pulmonary infarcts are not typically found (Fig. 210–7). Instead, areas of tissue destruction secondary to the reactive acute and granulomatous inflammatory process are seen.[93]

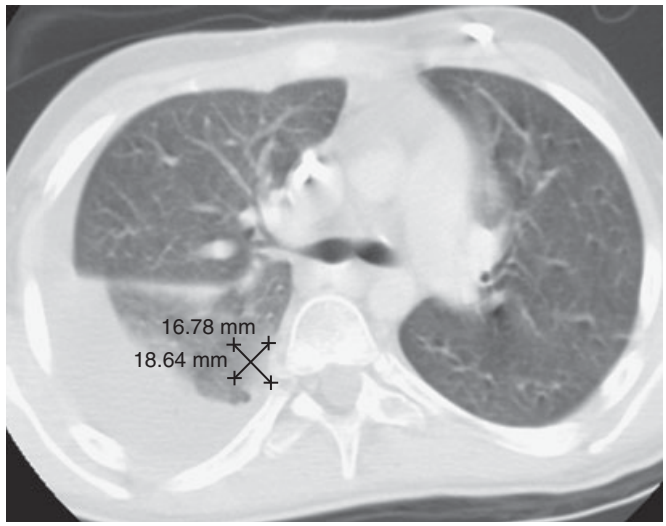

Figure 210–5 In a 15-year-old with Shwachman-Diamond syndrome and acute myelogenous leukemia, CT shows right lower lobe pulmonary nodules and pleural effusion.

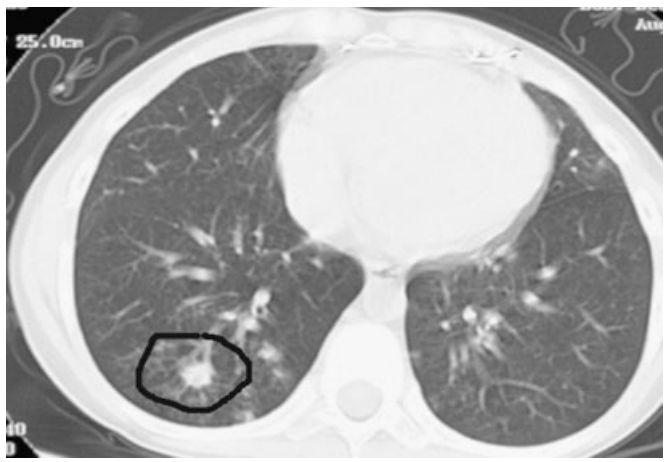

Figure 210–6 In an 11-year-old with cystic fibrosis after bilateral lung transplantation, CT shows bibasilar nodular opacities. Encircled is a 1-cm opacity associated with some peripheral speculation.

A long-term CT follow-up in 40 non–human immunodeficiency virus immunocompromised patients with IPA showed that formation of cavitation most strongly predicted the time until radiologic remission and beneficial outcome occurred. In that study, the natural history of early IPA lesions was evaluated, and 90 percent of patients were found to experience an increase in lesion size and number followed by a plateau in size and a decrease in number. Cavitation of the lesions developed in 55 percent of patients, and complete radiologic remission within a median 80 days was observed in 42.5 percent of patients. The number of days until remission without cavitation (50 days) was less than that for those with cavitation (95 days), so formation of no cavitation was strongly predictive of radiologic remission.[11] In one study, the appearance of the air crescent sign had no relationship to duration of neutropenia, and it showed a tendency to appear in large lesions, such as consolidation or mass, rather than in small lesions, such as nodules.[66]

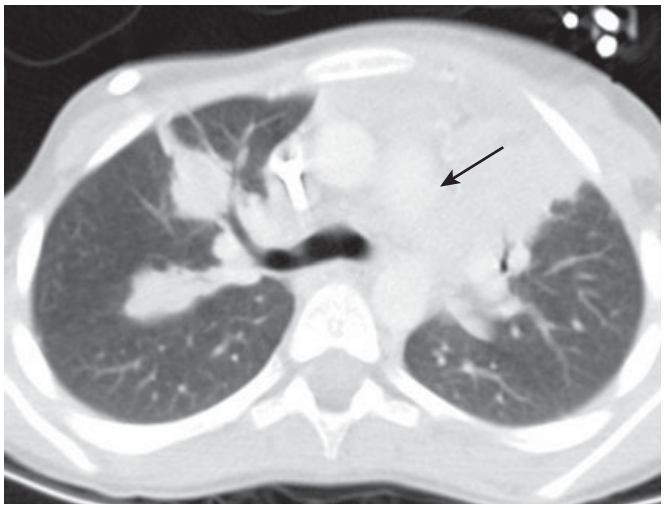

Figure 210–7 In a 9-year-old with chronic granulomatous disease, CT shows a soft tissue mass within the anterior mediastinum with heterogeneous enhancement. The mass caused convex deformity of the left anterior chest wall.

CT still remains a cornerstone to diagnosis. In a study of 37 cases of IPA, the mean time to diagnosis in bone marrow transplant recipients was reduced from 7 days to 1.9 days by chest CT.[14] One study comparing the usefulness of CT of the chest, latex agglutination test, and determination of plasma (1,3)-β-D-glucan levels for early diagnosis of IPA in 215 patients concluded that CT is the most beneficial. CT scan signs preceded a positive latex agglutination test result by 7.1 days and a positive (1,3)-β-D-glucan assay by 11.5 days.[61]

Radiologic differences for invasive aspergillosis may exist between adult and pediatric patients. In adult series of pulmonary aspergillosis, approximately 50 percent of cases show cavitation and 40 percent air crescent formation.[40] In one 10-year review of pediatric patients (mean age, 5 years), central cavitation of small nodules was found in only 25 percent of children, and no evidence of air crescent formation within any area of consolidation was found.[143] Another pediatric report had a 22 percent (6/27) rate of cavitation on chest radiography,[3] and yet another had a 43 percent (6/14) rate of cavitation on CT.[141] In these last two pediatric series, the mean ages were higher than those in the other report of lower rates of cavitation with no air crescent formation, suggesting that a spectrum of radiologic disease presentation exists that is related directly to age, with cavitation and air crescent formation more likely to develop in the older child and adult than in the younger child.

A review of 27 pediatric patients showed that radiographic changes developed after HSCT, but before diagnosis of pulmonary fungal infection, and included unilateral infiltrates (52%) slightly more often than bilateral infiltrates. At the onset, the infiltrates were interstitial (41%), alveolar (41%), and mixed (18%). Hilar or mediastinal lymphadenopathy and pleural effusion or thickening were rare findings. By the time the diagnosis of pulmonary fungal infection was established, the infiltrates were largely bilateral (66%) and alveolar or nodular (74%), including 22 percent of patients having cavitary lesions.[3]

Thoracic MRI findings are not as characteristic as are chest CT findings, and the typical MRI sign is the target sign, a nodular lesion with a lower signal in the center compared with a higher, contrast-enhancing signal intensity in the rim on T1-weighted images. This sign is highly suggestive of late-stage disease.[8] MRI is the modality of choice for diagnosis of cerebral aspergillosis, being preferred to CT for sensitivity (Fig. 210–8). Findings often show multiple lesions located in the basal ganglia that include an intermediate signal intensity, lack of contrast enhancement, and absence of mass effect.[91] CT of the head often reveals one or multiple hypodense, well-demarcated lesions. Hemorrhage and mass effect are unusual findings, but for patients with adequate peripheral white blood cell counts, a ring enhancement and surrounding edema are more frequent findings.[24] T2-weighted MRI shows very decreased signal intensity compared with that of bacterial sinusitis, which shows increased signal intensity.[24]

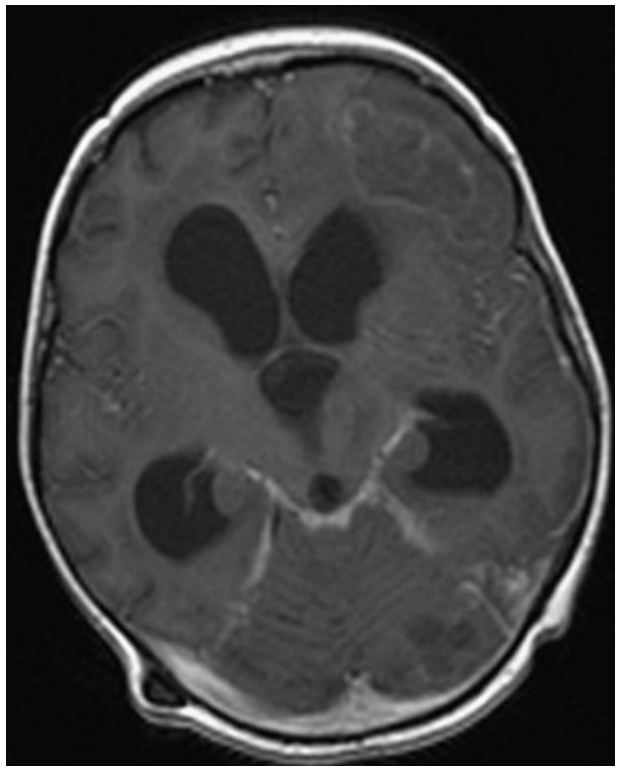

Figure 210–8 A 3-year-old with medulloblastoma and chemotherapy and radiation therapy who presented with new-onset acute mental status change. T1-weighted axial MRI shows multiple lesions in the subcortical area.

BRONCHOALVEOLAR LAVAGE

Bronchoalveolar lavage (BAL) often is useful in diagnosis of invasive aspergillosis, but a negative BAL result does not conclusively rule out disease. In one study, the BAL fluid was analyzed in 23 consecutive patients with histologically proven invasive aspergillosis, and only 7 patients (30%) had BAL specimens diagnostic for invasive aspergillosis. In the group in which BAL was diagnostic, 71 percent had multiple changes on thoracic CT compared with 25 percent of patients with negative BAL results. The diagnostic yield of BAL was not associated with clinical symptoms or duration of neutropenia. A thorough review of the diagnostic yield of BAL specimens in patients with histologically proven invasive aspergillosis yielded sensitivities of approximately 40 percent (range, 0-67%),[114] but in one study, BAL had a sensitivity of only 50 percent even in patients with focal invasive aspergillosis.[88] The sensitivity of respiratory tract culture specimens in general has ranged from 15 to 69 percent,[106] and in one study it increased to 50 to 70 percent in high-risk invasive aspergillosis groups. However, the sensitivity of BAL samples may be increased by use of a galactomannan enzyme immunoassay and by quantitative polymerase chain reaction (PCR), thus increasing

the yield of bronchoscopy and possibly precluding the need of further invasive procedures. In a large retrospective study at a major cancer center, these assays were applied to BAL fluid samples, and researchers demonstrated that each assay yielded a sensitivity higher than that commonly reported for BAL fluid culture. Concurrent use of both assays yielded the highest sensitivity (82%), detecting *Aspergillus* spp. in two thirds of the culture-negative samples from patients with IPA.[96]

SEROLOGIC TESTING

Serologic testing for antibodies to *Aspergillus* antigens is helpful to establish the diagnosis of aspergilloma or allergic bronchopulmonary aspergillosis in immunocompetent individuals, but serology unfortunately plays little role in the immunocompromised patient because growth of *Aspergillus* does not correlate with an increase in anti-*Aspergillus* antibody titers.[74] For example, serologic examination of 18 patients with invasive aspergillosis showed that anti-*Aspergillus* antibody detection was negative in all cases, probably because of profound immunosuppression.[121]

However, despite the historical lack of success with antibody testing, a movement is under way to use antibody testing in patients before immunosuppression in an attempt to discern who is most likely to develop invasive aspergillosis subsequently. A study to evaluate antibody responses by use of an enzyme-linked immunosorbent assay (ELISA) format in different types of aspergillosis patients showed that three recombinant antigens may have an important diagnostic potential.[120] The recombinant antigens with possible diagnostic utility are 18-kd ribonuclease, 360-kd catalase, and 88-kd dipeptidyl-peptidase V. In aspergilloma patients, all three of the antigens were equally useful for establishing the diagnosis, but only catalase was found to be useful in both immunocompetent and immunocompromised patients.

GALACTOMANNAN ANTIGEN TESTING

Galactomannan (GM) is a major cell wall component of *Aspergillus*, and the highest concentrations of GM are always released in the terminal phases of the disease.[74] An ELISA technique was introduced using a rat anti-GM monoclonal antibody, EBA-2, which recognizes the 1→5-β-D-galactofuranoside side chains of the GM molecule.[80] A sandwich ELISA technique was introduced in 1995,[137] and by use of the same antibody as both a capture and detector antibody in the sandwich ELISA (Platelia *Aspergillus*, Bio-Rad, France), one can lower the threshold for detection to 1 ng/mL. This technique is employed in the currently commercially available GM assay for diagnosis of invasive aspergillosis.

Positive assay results are suggestive of invasive disease, but false-positives are especially high during the neutropenic period after HSCT. A 3-year prospective trial showed that the sensitivity of serial monitoring was 90 percent, specificity was 98 percent, and negative predictive value was 98 percent. All 30 patients with proven invasive aspergillosis tested positive, with no false-negatives. GM detection preceded the development of infiltrates on chest radiography in 68 percent of patients. The false-positive rate was 14 percent, and therefore the improved sensitivity over that of latex agglutination is counterbalanced by the loss of specificity and greater number of false-positives.[81] Other studies have reported the incidence of false-positives at 5 to 8 percent and suggested they were due to cytotoxic agents, increased resorption of GM, or cross-reacting factors from the intestines.[149] In a meta-analysis conducted to characterize the clinical utility of GM assays, 27 studies from 1996 to February 28, 2005, were identified, and overall, the GM assay had a sensitivity of 71 percent and a specificity of 89 percent for proven cases of invasive aspergillosis as defined by the specific clinical criteria.[107] Of note, considerable heterogeneity was noted among the studies, and in subgroup analysis, the performance of the test differed by patient population as well as by which reference diagnostic standard was used. In patients with hematologic malignant disease or who had undergone hematopoietic transplantation, the assay was most useful, but the sensitivity and specificity in solid organ transplant recipients were 22 percent and 84 percent, respectively.

Serial testing performed at least twice a week has been recommended.[26] In one study, an increase in value during the first week of observation was predictive of treatment failure in allogeneic HSCT recipients.[10] In the largest prospective study of hematology and HSCT recipients with confirmed or probable invasive aspergillosis, GM was detected in 65 percent of patients an average of 8.4 days before CT scans or cultures were positive, and GM was detected in 40 percent of patients before the onset of clinical symptoms by a mean of 6.9 days. The sensitivity of GM detection in bone marrow transplant recipients was 89 percent, with a specificity of 98 percent. The test was performed easily, with results being available in 4 hours, and two successive samples with elevated GM values were required for positivity.[138] One study found the sandwich ELISA with a sensitivity of 60 percent and a specificity of 82 percent.[82] Another prospective study involved 186 consecutive patients at risk for invasive aspergillosis monitored twice weekly and yielded a sensitivity of 93 percent and specificity of 95 percent; in most cases, antigenemia was detected a median of 6 days before clinical symptoms were manifested.[80] Unfortunately, the GM assay has decreased sensitivity in the setting of a patient receiving anti-*Aspergillus* antifungals, although the specificity for detection does not change.[83] Therefore, assay results must be interpreted in the proper context.

Establishing the diagnosis of pediatric invasive aspergillosis with new antigen tests, such as the GM assay that is approved for use in adult patients and recently in pediatric patients, is difficult; studies have shown repeated differences in pediatric and adult values. In one prospective study from 1995 to 1998 of 450 adult allogeneic HSCT recipients (3883 samples) and 347 children with hematologic malignant neoplasms (2376 samples), the false-positive rate was 2.5 percent (10/406) in adult patients and 10.1 percent (34/338) in children. Although the sensitivity and specificity of the test with use of an optical density of greater than 1.5 in at least two sequential samples were 88.6 percent and 97.5 percent, respectively, the sensitivity in adult patients increased to 100 percent and the specificity dropped to 89.9 percent in children.[139] In another study of 797 episodes, including 48 pediatric patients, the false-positive rate in the group with fever of unknown origin was 0.9 percent in adults and 44.0 percent ($p < .0001$) in children. In addition, the specificity of the test was lower in children at 47.6 percent compared with 98.2 percent ($p < .0001$) in adult patients.[53] Numerous theories have sought to explain the increased false-positivity in children, ranging from *Bifidobacterium bifidum* spp. in the gut microflora that mimics the epitope recognized by the EBA-2 in the ELISA kit[89] to GM-positive infant formula used in pediatric patients,[160] but the complete answer remains elusive.

Questions remain about the GM false-positive results already mentioned as well as about false-negative results in some specific pediatric patients, such as those with CGD. One report describes a non-neutropenic 4-year-old child with CGD and invasive aspergillosis diagnosed by lung biopsy who had persistent false-negative serum GM test results.[150] Another study that evaluated patients with CGD ($n = 10$) and Job syndrome ($n = 6$) and invasive aspergillosis found that GM antigenemia was detected in 4 of 15 cases of CGD and Job syndrome versus 24 of 30 cases of all other immunocompromised conditions ($p = .0004$).[157] The GM assay will play an important role in noninvasive diagnosis of invasive

aspergillosis in the future, but complete elucidation of how to manage and to analyze this assay in children is lacking.

(1,3)-β-D-GLUCAN

(1,3)-β-D-Glucan is an integral cell wall component and, in contrast to GM, is not normally released from the fungal cell.[74] Factor G, a coagulation factor of the horseshoe crab, is a highly sensitive natural detector of (1,3)-β-D-glucan.[100] The "G test" detects (1,3)-β-D-glucan by a modified limulus endotoxin assay and detects *Aspergillus*, *Candida*, and even *Cryptococcus*, but it does not identify the genus of the fungi detected.[100] The G test is used widely in Japan, but these tests yield positive results only at advanced stages of infection in most patients.[62] A 1-year prospective study of patients with hematologic malignant disease and controls found the sensitivities of real-time PCR to be 79 percent, of the GM assay to be 58 percent, and of the G test to be 67 percent, with specificities of 92 percent, 97 percent, and 84 percent, respectively.[60] Currently, the (1,3)-β-D-glucan assay is available in the United States as the Fungitell assay (Associates of Cape Cod, Falmouth, MA).

In a study comparing (1,3)-β-D-glucan and GM, the sensitivity, specificity, and positive and negative predictive values for GM and β-glucan tests were identical. False-positive reactions occurred at a rate of 10.3 percent in both tests, but the patients showing false-positive results were different in each test. Both tests anticipated the clinical diagnosis and CT abnormalities, but (1,3)-β-D-glucan test results tended to become positive earlier than GM test results. A combination of the two tests improved the specificity (to 100%) and positive predictive value (to 100%) of each individual test without affecting the sensitivity and negative predictive values.[105] Another study compared GM, PCR, and (1,3)-β-D-glucan testing of patients with hematologic disorders, and the receiver operating characteristic (ROC) analysis showed that an area under the ROC curve was greatest for the GM assay, using two consecutive positive results. This result suggests that the GM assay was the most sensitive at predicting the diagnosis of invasive aspergillosis in high-risk patients with hematologic disorders.[65]

POLYMERASE CHAIN REACTION

Although GM assays created a noninvasive test with improved sensitivity and specificity, recent efforts have focused on defining an optimal primer sequence for a PCR detection method. At present, this diagnostic method is not commercially available, and reports can be difficult to interpret because of the lack of experimental standardization between centers. Because of the ubiquitous nature of the mold, the value of this test will probably be its high negative predictive value. Issues remaining unresolved in the use of PCR are the best source of material (e.g., whole blood, serum, plasma, BAL specimens), the amplification protocol (e.g., real-time, sample volume, extraction methods), and the primer selection (e.g., "panfungal," 18S rRNA, 28S rRNA, mitochondrial DNA).[67] Use of PCR in BAL specimens compared with blood samples seems less promising because of the higher number of false-positives. Real-time PCR assays seem to decrease the risk of getting false-positive results and have better reliability than conventional PCR.[21]

Reported sensitivities in numerous retrospective reports of PCR are 55 to 100 percent, with negative predictive values generally around 100 percent.[113] The high negative predictive value is consistent with PCR as a sensitive marker for any colonizing or infecting aspergilli, and a patient with a suspected invasive aspergillosis who has a negative PCR result most likely does not have the organism.[112] In a 2-year prospective study of 121 patients with hematologic malignant neoplasms analyzed twice weekly with screening of whole blood samples, the negative predictive value of two or three consecutive positive PCR results was 98 percent, with a sensitivity of 75 percent and a specificity of 96 percent. When PCR was detected only once, it was never associated with disease and resolved without antifungal treatment, thus indicating only a transient *Aspergillus* DNAemia.[72]

In another prospective PCR study in 84 allogeneic HSCT recipients, all patients with proven or probable invasive aspergillosis were PCR positive, and PCR was found to be the earliest indicator for patients with new-onset invasive aspergillosis, preceding the first clinical signs by a median of 2 days and preceding the diagnosis based on typical radiographic findings by a median of 9 days. For patients without a history of invasive aspergillosis who tested positive with PCR twice, sensitivity was 100 percent, and the negative predictive value was 100 percent.[50] In another study of patients with invasive aspergillosis, diagnosis by a positive PCR preceded radiologic signs by a median of 4 days. In addition, the disappearance of fungal DNA correlated with successful therapy, and the patients who died of invasive aspergillosis remained PCR positive.[36] In one study, PCR positivity preceded radiologic diagnosis in 11 of 18 patients, and an ELISA did not precede a positive PCR on any patient. PCR was more sensitive than was ELISA, although ELISA had no false-positives.[162]

PCR has been used to detect *Aspergillus* in BAL specimens, but that does not allow distinction between colonization and invasive disease. A prospective evaluation of 197 BAL samples in febrile, neutropenic patients with lung infiltrates showed that all immunocompromised patients with a negative PCR had no evidence of fungal disease, and only a few patients without immunocompromise showed positive results without evidence of disease. Further dividing into a high-risk group (patients with acute leukemia undergoing chemotherapy), the negative predictive value was 100 percent.[12] A two-step PCR was shown to detect down to as little as 10 fg of *Aspergillus* DNA (approximately 5 CFU/mL) and was tested in 100 consecutive patients with BAL and 278 consecutive patient blood samples. BAL PCR was positive in 17 immunocompromised patients, with a specificity of 93 percent and negative predictive value of 100 percent. Comparatively, in the blood samples, sensitivity was only 20 percent, but negative predictive value was 100 percent.[13]

TREATMENT

Overall success in treatment of invasive aspergillosis is dependent on numerous factors, not simply the choice of specific antifungal therapy. As with all infectious pathogens, the clinician should understand the epidemiology in the local setting, as different *Aspergillus* spp. will respond uniquely to different therapies. Any antifungal prophylaxis used before establishment of the diagnosis of invasive aspergillosis could have an effect on the ultimate use of agents designed for targeted therapy, including drug interactions and development of antifungal resistance. The cornerstone of antifungal therapy for invasive aspergillosis is prompt and aggressive institution of antifungal therapy, largely based on clinical suspicion of infection. Diagnostic work-up needs to be aggressive, but it should never delay initiation of therapy.

Since its initial approval for use in 1958, amphotericin B deoxycholate was the first-line therapy for invasive aspergillosis and the gold standard against which other therapies were measured. However, with overall survival rates of approximately 34 percent among patients treated with amphotericin B, in addition to limited tolerance as a result of acute and chronic toxicities, a better antifungal clearly was needed. Itraconazole became available in 1990 for the treatment of *Aspergillus* infection, but it was not without flaws, the most prominent of which were decreased fungicidal activity compared with amphotericin B, unpredictable

bioavailability in high-risk patients, and significant drug interactions.[132] The next step taken in the search of a better antifungal was the development of three different lipid formulations of amphotericin B, the advantages of which included an increase in the daily dose, high tissue concentrations with better delivery to reticuloendothelial organs, decrease in infusion-related side effects, and decrease in renal toxicity.[33] These three lipid formulations, amphotericin B lipid complex, amphotericin B colloidal dispersion, and liposomal amphotericin B, currently are indicated for patients with systemic mycoses, primarily invasive aspergillosis, who cannot tolerate or are refractory to conventional amphotericin B.[132] To date, no large randomized study has been performed to determine if any of the lipid formulations has improved efficacy over conventional amphotericin B, but data show that liposomal amphotericin B has less infusion-related toxicity.[45] A study to determine if lipid formulations are better than conventional amphotericin B, although informative, may not ever take place because of the introduction of voriconazole and a whole new class of antifungals, the echinocandins.

Voriconazole is a second-generation triazole and synthetic derivative of fluconazole that was approved for use in the United States in May 2002 for primary therapy for invasive aspergillosis. Among the instrumental trials leading to this approval is the multicenter noncomparative, open study of 116 patients treated with voriconazole as primary therapy (60 patients) or salvage therapy (56), which showed a response rate of 59 percent in the primary treatment of invasive aspergillosis and 38 percent rate for salvage treatment. The response rate for patients with IPA was 60 percent.[29] The other critical trial is that by Herbrecht and coworkers,[52] a large prospective comparative study that showed that voriconazole had a better response rate in the primary treatment of invasive aspergillosis compared with amphotericin B, 52.8 percent compared with 31.6 percent. Survival rates also increased, from 57.9 percent for amphotericin B to 70.8 percent for voriconazole. This improved response rate has translated well to the pediatric population, as shown by a review of 42 children treated for invasive aspergillosis with voriconazole.[155] An analysis of the compassionate open-label use of voriconazole in children for refractory invasive aspergillosis demonstrated a 43 percent complete or partial response. Voriconazole also was shown in these studies to be better tolerated and to have less toxicity in both adults and children. The main side effects of voriconazole are reversible visual disturbances in as many as 40 percent of patients, elevated transaminases, and occasional skin reactions most likely due to photosensitization.[119] It is available in both intravenous and oral formulations.

Another new triazole is the recently approved posaconazole, a derivative of itraconazole. No randomized studies have examined posaconazole for primary therapy for invasive aspergillosis, but numerous in vitro and in vivo data suggest that this triazole will be as effective as voriconazole as a potential first-line agent against invasive aspergillosis.

The echinocandins are an entirely new class of antifungals that interfere with cell wall biosynthesis. This is a distinct mechanism of action compared with the second-generation triazoles that work by inhibiting cytochrome P450 enzymes responsible for conversion of lanosterol to sterol and thus compromising the fungal cell membrane.[159] Three compounds, caspofungin, micafungin, and anidulafungin, currently are approved in the United States. Caspofungin, the first in the echinocandin class, has shown potent efficacy against *Aspergillus*.[78] The first clinical trial, conducted by Maertens and colleagues,[79] was an open-label, noncomparative study in 90 immunocompromised patients with definite or probable aspergillosis. Caspofungin produced a complete or partial response in 45 percent of patients, including 50 percent of 64 patients with probable invasive aspergillosis and 23 percent of 13 patients with disseminated aspergillosis. This result was validated by two subsequent clinical trials in which caspofungin resulted in complete resolution or stabilization of disease in 74 percent of patients (n = 31) in one trial and had an overall response rate of 56 percent (n = 32) in the other.[15,146]

Micafungin is an echinocandin lipopeptide compound that is fungistatic in vitro, like others in its class, with median minimum inhibitory concentration (MIC) values for *Aspergillus* spp. substantially lower than those of other non-echinocandins.[142] A noncomparative, open-label, multicenter study in adult and pediatric patients to examine the safety and efficacy of micafungin in the treatment of invasive aspergillosis that had failed to respond to prior therapy or for which other therapy could not be tolerated was conducted during 1998 to 2002. Of the 225 patients who met diagnostic criteria, a favorable response rate was reported in 35.6 percent (80/225), and of those treated with only micafungin, a favorable response was seen in 6 of 12 (50%) of the primary and 9 of 22 (40.9%) of the salvage therapy group.[28] Anidulafungin is another echinocandin that has been approved in the United States for treatment of candidemia and esophageal candidiasis, and an in vitro study looking at its efficacy against *Aspergillus* spp. isolates showed it to be the most active antifungal agent of those tested, with an MIC_{90} of less than 0.03 mg/L.[128] Currently, no prospective, comparative trials of any echinocandins as primary therapy for invasive aspergillosis have been performed; instead, most clinicians use the fungistatic echinocandins in a role for salvage therapy.

Beyond controlled clinical trials, very little investigation has been conducted into the treatment practice patterns or outcomes of pediatric patients with invasive aspergillosis. The largest study of treatment practices and outcomes in overall invasive aspergillosis reviewed 595 patients treated by 89 physicians. The patients analyzed were 0 to 86 years old, and the mean age was 42.3 years.[104] In a European review of diagnosis and therapeutic outcome, 123 patients were analyzed, with a mean age of 46 years (range, 9-83 years).[27] An older report of 2121 published cases[31] and a report on therapeutic outcome in 1223 cases of invasive aspergillosis[25] also did not specifically exclude children, but no specific data were supplied on the pediatric patients with invasive aspergillosis.

No dedicated, prospective, large-scale investigation into treatment of pediatric invasive aspergillosis has been conducted. One large data set analyzed adult and pediatric invasive aspergillosis outcomes separately. The adult data were an analysis from the open-label, multicenter clinical study of emergency use of amphotericin B lipid complex at 5 mg/kg/day from 1990 to 1995 in the treatment of patients with proven or probable invasive fungal infections who failed to respond to previous systemic antifungal therapy (including amphotericin B at a cumulative dosage of at least 500 mg), developed severe nephrotoxicity (serum creatinine concentration ≥2.5 mg/dL in adults or ≥1.5 mg/dL in children), had pretreatment renal insufficiency, or developed severe acute toxicity.[154] Patients were excluded if a systemic antifungal or investigational drug was administered concurrently with the amphotericin B lipid complex. This study evaluated 551 patients with 556 courses of amphotericin B lipid complex therapy, of which 291 patients fulfilled criteria for evaluation of efficacy. The mean age of all enrolled patients was 37.2 years (range, 21 days to 93 years). There were 130 cases of invasive aspergillosis, with a complete or partial response rate of 42 percent, stable response in 12 percent, and failure in 45 percent of patients. The complete or partial response rate of probable invasive aspergillosis (n = 74) was 38 percent; of disseminated invasive aspergillosis (n = 27), 30 percent; of sinusitis (n = 14), 64 percent; and of single-organ extrapulmonary invasive aspergillosis (n = 15), 67 percent. The analysis of 556 treatment episodes did not stratify pediatric responses, but a subsequent analysis was performed of 54 of the 111 pediatric patients (<18 years) from that study.[158] The mean patient age was 9.3 years (range, 21 days to 16 years). There were 25 cases of invasive aspergillosis with a

complete or partial response rate of 56 percent, stable response in 8 percent, and failure in 36 percent of patients. The complete or partial response rate of probable invasive aspergillosis ($n = 10$) was 50 percent; of disseminated invasive aspergillosis ($n = 7$), 29 percent; of sinusitis ($n = 5$), 100 percent; and of single-organ extrapulmonary invasive aspergillosis ($n = 3$), 67 percent.

In a retrospective French study of 46 pediatric patients treated with amphotericin B lipid complex for invasive fungal infections from 1994 to 1997,[51] the mean age of 23 patients with invasive aspergillosis was 9.7 years (3 months to 18 years). Eighteen of 23 (78%) patients showed cure (52%) or improvement (26%); therapy failed in 22 percent. Three patients who initially improved later relapsed, dropping the cure or improvement rate to 15 of 23 (65%).

An analysis of the compassionate open-label use of voriconazole in children for refractory invasive aspergillosis with clinical or radiologic progression of disease after 7 days or more of systemic antifungal therapy in children younger than 16 years included 42 patients with proven or probable invasive aspergillosis.[155] The mean age of all 58 children with invasive fungal infections, including invasive aspergillosis, and 16 patients with other systemic fungal disease was 8.2 years (range, 9 months to 15 years). Analysis of the 42 patients with invasive aspergillosis revealed a complete or partial response rate of 43 percent and a stable response in 7 percent of patients; 10 percent of patients were intolerant of therapy, and therapy failed in 40 percent. The complete or partial response rate of probable invasive aspergillosis ($n = 12$) was 33 percent; of CNS invasive aspergillosis ($n = 6$), 50 percent; of disseminated invasive aspergillosis ($n = 7$), 86 percent; of sinusitis ($n = 7$), 29 percent; and of single-organ (bone, liver, or skin) invasive aspergillosis ($n = 10$), 30 percent.

Although several reviews of combination therapy in systemic mycoses have been published,[75,109] only a few retrospective clinical reviews of combination invasive aspergillosis therapy exist.[31,111] There has been only a single published combination antifungal clinical trial for invasive aspergillosis,[148] and it was never completed. That study enrolled 18 patients with documented pulmonary aspergillosis; only 1 of 9 patients receiving amphotericin B monotherapy survived, and 2 of 9 treated with amphotericin B plus 5-flucytosine survived. The study was terminated because of poor outcomes in both arms, but outcomes might have been poor because a lower dose of amphotericin B (0.5 mg/kg/day) was used and a definite diagnosis was required. Therefore, in view of the low number of patients and the poor outcomes in both arms, no firm conclusion could be drawn about the possible superiority of that combination therapy.

Although no controlled clinical trial supports the use of combination therapy for invasive aspergillosis and its efficacy has not been conclusively established,[110] clinicians are desperately seeking new strategies for improved outcome. However, the experimental and clinical data do not reveal proven benefits with combinations, nor do they fully define the preferred combination for further exploration in a large clinical trial. The range of data from synergy to antagonism actually parallels the wide range of unproven treatment practices used by clinicians today searching for the best care for their patients.

Denning and Stevens[31] previously reviewed a total of 2121 cases reported in 497 articles concerning clinical aspects of invasive aspergillosis from 1966 to 1990, analyzing 446 treatment courses in 379 patients from 210 articles. That 1990 review[31] revealed 89 clinical cases of combination therapy reported in 54 articles: amphotericin B plus rifampin (26 cases) and amphotericin B plus 5-flucytosine (63 cases). The largest analysis of combination therapy for invasive aspergillosis reviewed 6281 total cases of invasive aspergillosis management and added an additional 12 years and 386 clinical cases of combination antifungal therapy reported in 236 articles[133] to that original 1990 study. After closer inspection and exclusion of 11 cases in 7 articles from the 1990 review and 215 cases in 154 articles from 1990 to 2001 that did not meet inclusion criteria, that large combination analysis analyzed a total of 249 clinical combination cases reported in 128 articles.

In that analysis, three combination regimens represented the majority of reported clinical experience; many (49%) involved amphotericin B plus 5-flucytosine, whereas amphotericin B plus itraconazole (17%) and amphotericin B plus rifampin (11%) were used less frequently. However, after inclusion of the lipid formulations of amphotericin B (i.e., amphotericin B lipid complex, amphotericin B colloidal dispersion, and liposomal amphotericin B), the frequency of those three combinations increased from 77 to 89 percent of the total number of combinations analyzed. Finally, inclusion of those nine patients who were treated with one combination regimen and then switched to another increased the contribution of these three regimens to 91 percent of combination strategies ever reported. Although these data helped lay the foundation of combination therapy in the past, the combinations used historically are not necessarily the combinations employed by today's clinician.

The 249 clinical cases represented a total of 23 different antifungal combinations, including 16 unique double antifungal and 7 triple antifungal regimens. A total of 64 percent of patients showed improvement, with the mortality rate from invasive aspergillosis at 34 percent. This response rate is higher than the general reporting of approximately 34 percent survival in patients treated with customary monotherapy.[77] There were also 27 reports of in vitro combination antifungal therapy against *Aspergillus* spp. published from 1974 to 2001, analyzing 34 different combinations. Analysis revealed in vitro synergy (38%), additivity (25%), indifference (27%), and antagonism (10%). An additional 18 reports of in vivo combination antifungal therapy in animals published from 1975 to 2001 analyzed 15 different combinations and their outcomes. The most frequently tested combination was amphotericin B plus 5-flucytosine, followed by amphotericin B plus itraconazole. The in vivo results were positive less frequently, with synergy (14%), additivity (20%), indifference (52%), and antagonism (14%). As a whole, the previous experimental data and clinical experience offer hope that newer strategies will improve outcomes.

Regardless of the continuous stream of data, the in vitro and in vivo interactions must continue to be questioned because of so many confounding patient variables, leaving clinical experience still the most accurate tool. Numerous factors that contribute to clinical efficacy include the complex interaction of the fungal virulence, the intrinsic or acquired fungal resistance, and the host immune condition and its interaction with the therapeutic agents. However, clinical relevance might best be related to patient factors (e.g., recovery of neutropenia, cessation of glucocorticoid therapy) and not intrinsically related to the susceptibility of the fungus itself.

One study to help address this issue of combination therapy of voriconazole plus caspofungin was a retrospective review of 47 patients with proven or probable invasive aspergillosis from 1997 to 2001 who experienced failure of primary therapy with amphotericin B formulations.[84] Patients initially received therapy with amphotericin B (1 mg/kg/day) or a lipid formulation (amphotericin B lipid complex or liposomal amphotericin B, both at 5 mg/kg/day). Salvage therapy was begun with voriconazole ($n = 31$) versus voriconazole plus caspofungin ($n = 16$) after 7 days or more of amphotericin B therapy. The change to the combination antifungal therapy was made in the institution's protocol in February 2001 in the middle of the retrospective window analyzed. In early 2001, the protocol was altered so caspofungin (70-mg load, then 50 mg daily) was used in combination with voriconazole therapy, thus creating the combination therapy arm of this retrospective analysis. Importantly, most patients received salvage therapy

because of clinical failure, not antifungal intolerance, yet no patients received their voriconazole or voriconazole plus caspofungin as primary therapy for invasive aspergillosis.

The overall survival rate 3 months after the day of diagnosis of invasive aspergillosis was higher among those who received combination therapy ($p = .048$). Similarly, 3-month survival after the start of salvage therapy was greatest among patients who received combination therapy (HR, 0.43; 95% CI, 0.17-1.1; $p = .050$). Finally, the probability of death also was lower in those patients receiving combination therapy ($p = .024$). In the bivariable analysis (controlling for antifungal therapy and receipt of HSCT), the combination showed an improved 3-month overall survival rate (HR, 0.27; 95% CI, 0.09-0.78; $p = .008$).

Others have suggested that the preclinical data with combination therapy seem promising yet inconsistent.[151] The point was made that usually historical control studies tend to underestimate results in the control group and show larger treatment effects than randomized studies do. Second, the advent of newer diagnostic tools for invasive aspergillosis (galactomannan assay, high-resolution computed tomography) cannot be adequately included in a retrospective analysis, and finally is the issue of consistency in shifting to salvage therapy. Marr and colleagues responded to other comments[17] and in analyzing the patients at 1 year found no difference in overall survival rates ($p = .26$). Importantly, at that 1-year mark, only seven patients in the voriconazole arm and one patient in the combination arm were alive. Subsequent analyses of these eight patients did reveal a decreased probability of dying of invasive aspergillosis ($p = .024$) and a larger probability of dying of other causes ($p = .042$), but again, only eight total patients were analyzed.

The current clinical use of combination therapy for invasive aspergillosis is rampant, even in the absence of a definitive clinical trial advocating its benefit. There are many editorials on combination therapy design questions, calls to arms to begin a combination antifungal clinical trial, standardization attempts for animal models, and opinions on the value of in vitro checkerboard testing. The data continue to mount with no clear indication. Perhaps we will never achieve the same improvement in mortality rates seen from amphotericin B to voriconazole clinically but will only be able to obtain a slight increase in benefit with combination strategies. Perhaps the decrease in fungal burden is all we can hope to achieve, and this will translate into clinical benefit. However, survival does remain the experimental standard, as survival incorporates host response as well as antifungal activity itself.

Antifungal resistance is not considered for invasive aspergillosis in the same fashion as one would depict resistance of bacteria to antibacterial agents or even of *Candida* spp. to antifungals. In those two examples, resistance often is inherent (e.g., *Candida krusei* and fluconazole) or develops after prolonged exposure pressure (e.g., antibacterials). Although it has not been proved in any large-scale format, the prevailing thought is that *Aspergillus* spp. do not develop resistance in this format but that certain species are intrinsically resistant to certain classes of antifungals.

For years, *A. terreus* was a recalcitrant *Aspergillus* spp. because of its now well-described in vitro resistance to amphotericin B,[140] which has been confirmed in animal models.[156] A review of in vitro analyses, multiple animals, and previously reported clinical cases showed nearly uniform failure with amphotericin B against *A. terreus*.[131] A multicenter retrospective analysis of more than 80 cases from 1997 to 2002 demonstrated that mortality was decreased in those patients who received voriconazole instead of amphotericin B therapy.[130] Other studies have shown that patients with *A. terreus* disease are more likely to be neutropenic,[49] are more likely to have disseminated disease, and have a decreased response to all classes of antifungals compared with patients infected with *A. fumigatus*.[73]

Antifungal resistance among *A. fumigatus* isolates is not a common finding. Itraconazole resistance was described first in 1997,[32] and one study estimated that 2.1 percent of more than 900 *A. fumigatus* isolates were resistant to itraconazole.[92] A large study examining 200 sequential isolates of *A. fumigatus* from 26 immunocompromised patients found similar antifungal susceptibility both before and after treatment with both amphotericin B and itraconazole, suggesting that emergence of resistance while receiving antifungal therapy is likely to be a rare event.[23]

Other interventional therapies used in the treatment of invasive aspergillosis include growth factors such as granulocyte colony-stimulating factor and granulocyte-macrophage colony-stimulating factor, granulocyte transfusions, interferon-γ, and surgery. Granulocyte colony-stimulating factor increases the number of circulating polymorphonuclear neutrophils and enhances phagocytic activity and oxidative burst metabolism. Interferon-γ promotes tumor necrosis factor–α production and enhances polymorphonuclear neutrophil and mononuclear cell–induced damage. It has been proved to help prevent invasive aspergillosis in patients with CGD and is used as prophylactic therapy in these patients.[132] Surgical intervention is indicated in patients with acute hemoptysis, for relapse prophylaxis before HSCT or the next cycle of chemotherapy, and for lesions impinging on great vessels or major airways.[9]

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CHAPTER 211

Blastomycosis

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Blastomyces dermatitidis is a dimorphic fungus that is responsible for a systemic disease characterized by granulomatous and suppurative lesions. Illness occurs when fungal spores are inhaled into the lungs and undergo transition into an invasive yeast phase. Once in the yeast phase, these organisms may not be cleared by bronchopulmonary phagocytes and may proliferate before immunity develops. The infection may be halted and resolved by the host at this point, or progression of the infection leading to localized pulmonary involvement or extrapulmonary disease may occur. Such dissemination results in the involvement of other parts of the body, most notably the skin and bones. Although the highest prevalence of disease with this organism is observed in North America, blastomycosis has been documented to occur worldwide.

Gilchrist and Stokes[27,28] were the first to describe blastomycosis in the late 1890s. During the next decade, the heightened awareness of the disease by the medical community produced reports from various regions of the United States, with most cases occurring in the Chicago area.[60] Because of this finding, blastomycosis became known as Chicago disease or Gilchrist disease. A widely accepted concept during these early studies was that two forms of the disease (cutaneous and systemic) existed and that each represented a different portal of entry by the organism. Many years later, Schwarz and Baum[66] established that blastomycosis is a primary pulmonary process and that the cutaneous manifestations are secondary to dissemination from the lung. More recently, blastomycosis became known as North American blastomycosis because of an erroneous perception that the disease is limited to North America; but because this disease now is recognized as having a worldwide distribution, it is known at present as blastomycosis.

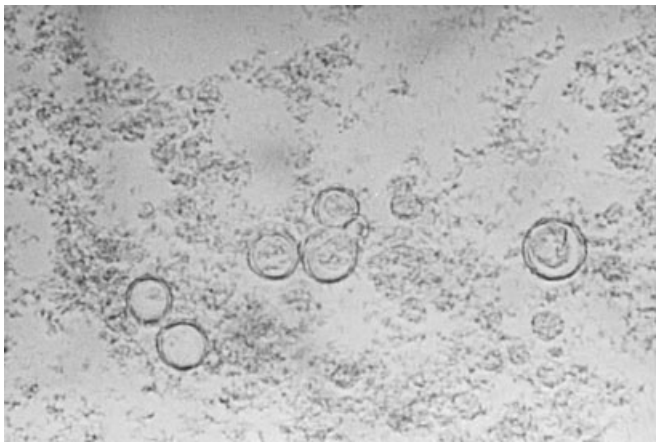

Figure 211–1 Wet preparation demonstrating the spherical shape of *Blastomyces dermatitidis*.

THE ORGANISM

B. dermatitidis is the causative agent of blastomycosis. The organism has two forms: sexual (the teleomorphic or perfect state) and asexual (the anamorphic or imperfect state). The sexual form of the fungus is named *Ajellomyces dermatitidis*, whereas the asexual form is actually *B. dermatitidis*. The asexual stage exhibits dimorphism, during which the fungus grows as a yeast form at body temperature and as a mycelial form at room temperature. At 37° C, the yeast form appears as a large round organism (6 to 15 μm in diameter) with a thick wall that can produce a single bud (Fig. 211–1). Characteristically, this bud is connected to the parent yeast by a wide base of attachment and usually will equal the size of the parent before detachment. At 25° C to 30° C on laboratory medium, *B. dermatitidis* grows as a white to gray-brown mold (mycelia) with a delicate, silky appearance. On microscopic examination, the mold is characterized by filamentous colonies composed of thin, uniform septate hyphae (1 to 2 μm in width) that produce conidiophores that branch at right angles to the main hyphal segment. The solitary conidia (spores), which are located at the end of the conidiophores, are small (2 to 10 μm in diameter) and may be oval or pyriform (pear shaped). These conidia are similar to the microconidia of *Histoplasma capsulatum*, but the conidia of *B. dermatitidis* do not form tuberculate macroconidia as do those of *Histoplasma*. The appearance of *B. dermatitidis* mycelia is not pathognomonic for the organism, and definitive identification depends on conversion of the organism to the yeast form by culture at 37° C.

ECOLOGY AND EPIDEMIOLOGY

Only a paucity of data concerning the ecology and epidemiology of this disease exists. Without data on the ecology of the organism, knowledge about the epidemiology of disease caused by this organism is derived from studies of sporadic cases. Although significant data on the epidemiology of the disease have been gathered with such studies, gaps in our knowledge will become clarified when the ecology of the agent is defined better. Because *B. dermatitidis* cannot be recovered easily from nature and an adequate skin test antigen is not available for conducting population surveys, the present knowledge of this disease is based on case reports of sporadic infections and outbreaks of human and canine disease. The use of new molecular techniques such as polymerase chain reaction may provide fresh insight into the epidemiology of this organism in the coming years.[46]

The natural reservoir of *B. dermatitidis* is not defined as precisely as are the reservoirs for other systemic mycoses (e.g., bird droppings, *H. capsulatum*), but this organism is assumed to have its reservoir in a habitat similar to that of the other dimorphic fungi. *B. dermatitidis* appears to be a soil saprophyte and presumably exists in the mycelial form in nature. The organism probably thrives in a location that is high in organic material, is abundant in moisture, has an acid pH, and even may be enriched with animal excreta.[39] The yeast and mycelial phases of the organism seem to disappear rapidly when they are placed in the soil, whereas conidia can survive for several weeks.[47] Desiccation of such an area with subsequent disturbance of the site results in an infectious aerosol of mycelial fragments and conidia.[59] Conidia also may be released from mycelia on the soil or from decaying material when it is wetted or disturbed.[35,37]

Speculation on the natural source of this organism began shortly after it was recognized. Several investigators have noted an association between water and *B. dermatitidis*. Denton and Di Salvo[19] were able to recover the organism in 10 of 356 samples along 1 mile of road by the Savannah River. Furcolow and associates[26] published an extensive review of cases of canine and human blastomycosis that occurred during a prolonged period (1885 to 1968); a high incidence of disease was demonstrated south of the Ohio River and east of the Mississippi River. Recently, Baumgardner and colleagues[4] reported a disproportionate number of human and canine cases near the waterways of the western Eagle River in Wisconsin. In addition, at least five human outbreaks of blastomycosis have occurred along river banks.[14,38,39,72] The exact role of the water, however, currently is not understood.

Blastomycosis has been reported to occur worldwide, but many of these reported cases have not withstood scrutiny. Unequivocal cases of blastomycosis from South America have not been described in the past 4 decades. Isolated cases have been reported from England, Switzerland, and Poland, and sporadic infections have been found in the Middle East and India. Most cases, however, are reported from the eastern portion of North America (east of the 100th meridian), with a smaller number scattered throughout Africa.[2]

All age groups are susceptible to blastomycosis. The age distribution varies with each series of cases published because of differences in the populations examined. Most patients, however, are between the ages of 20 and 70 years; only 3 to 13 percent of reported cases occur in patients younger than 20 years.[26,44,48,50] Two patients who were 3 weeks of age or younger have been described.[45,74] Exceptions to this age spectrum occur in outbreaks, in which cases in children can predominate. Although some studies in adults have demonstrated a preponderance of men infected with *B. dermatitidis*, previous studies in children and adolescents have not shown such a trend.[41,58,69,77] Case rates for blastomycosis have been described to be highest for African American and Aboriginal patients, but the general speculation is that the racial difference in case rates reflects the population in the community of the endemic region. The age, sex, and race distributions of children with blastomycosis, therefore, appear to be a matter of exposure and not susceptibility of the individual.

Domestic dogs and cats also are susceptible to *B. dermatitidis*.[9,29] Dogs, in particular, seem to be as susceptible to *B. dermatitidis* as humans are. Because of this finding, canine blastomycosis has become a surrogate marker for human blastomycosis. The incidence and prevalence of canine blastomycosis have been studied extensively to supplement our knowledge about the geographic distribution of the disease. Menges and associates[49] were among the first to conclude that humans and dogs acquire their infections from the same source and that no evidence has been found of passive transmission of illness from dog to human or vice versa.

PATHOGENESIS AND PATHOLOGY

Five methods of transmission of *B. dermatitidis* have been described: inhalation, accidental inoculation, dog bites, conjugal transmission, and intrauterine transmission. By far the most common portal of entry into the body is through the lungs. Not until the work of Schwarz and Baum[66] in the early 1950s did researchers recognize that *B. dermatitidis* is inhaled from the environment and results in a subclinical or mild respiratory illness. In most situations, disease at other body sites is a result of hematogenous spread from the lungs, even if it is not recognized clinically. Primary cutaneous blastomycosis has occurred secondary to accidental needle inoculation, often in a veterinarian or pathologist, or by dog bites.[30,40] Person-to-person spread of blastomycosis does not occur, except in certain situations. One well-recognized case of conjugal transmission and two of intrauterine transmission to neonates have been reported.[16,45,74]

Blastomycosis begins with inhalation of the conidia of *B. dermatitidis* into the lungs, followed by development of an inflammatory response with neutrophils and macrophages. Most conidia are killed easily by these phagocytes, but those that succeed in changing into the yeast form are more resistant to phagocytosis. The large size of the yeast form and resistance to the oxidative mechanisms of killing used by phagocytes render the yeast form more difficult to kill. During the next 4 to 8 weeks, unphagocytosed yeast forms proliferate, and patients may be asymptomatic or complain of an influenza-like illness with fever, arthralgia, myalgia, productive cough, and pleuritic chest pain.[64] The infection may be halted by the host at this stage, or progression of the infection may lead to localized pulmonary and extrapulmonary disease.

Subclinical cases of blastomycosis probably develop more commonly than do symptomatic ones.[73] The high number of asymptomatic infections supports the theory that healthy individuals are fairly resistant to infection. The exact mechanisms of both natural and acquired resistance to infection are not understood. Natural resistance is thought to be mediated by neutrophils, monocytes, and alveolar macrophages. Human neutrophils phagocytose the conidia of *B. dermatitidis* rapidly and effectively; within 2 hours, 90 percent of the conidia are located intracellularly,[22] whereas the yeast forms are moderately resistant to killing by neutrophils. Human alveolar macrophages exhibit phase transition–associated fungicidal and fungistatic activities by irreversibly blocking conidial phase transition to the yeast form or reversibly inhibiting phase transition by causing the accumulation of unusual intermediate forms.[70]

Only recently have we been able to characterize features of acquired resistance in blastomycosis. No relationship between the presence of specific antibody against *B. dermatitidis* and the development of resistance to disease has been found. Specific cellular immunity, however, appears to occur in all patients infected with this organism. BAD1 (*Blastomyces* adhesin 1) serves as an adhesin and a virulence factor by inducing suppression of tumor necrosis factor (TNF)–α.[25] Antigen-specific T cells are stimulated to produce lymphokines (e.g., interferon-γ) that activate macrophages, thereby resulting in enhanced fungicidal activity.[6] This enhanced fungicidal activity is due, at least in part, to up-regulation of TNF-α production by macrophages. In vivo data of experimental infection show that neutralization of TNF-α exacerbates pulmonary infection.[24] Kethineni and colleagues[34] note that young murine macrophages co-cultured with *B. dermatitidis* produced half the amount of TNF-α as did mature macrophages. Treatment with interferon-γ increased fungicidal activity of bronchoalveolar macrophages and polymorphonuclear leukocytes. In addition, a pilot experiment in blastomyces-infected young mice revealed increased survival in those treated with interferon-γ.

CLINICAL MANIFESTATIONS

PULMONARY DISEASE

Symptomatic illness develops in approximately 50 percent of infected children, with most of these illnesses appearing initially as an acute or chronic pulmonary process. Patients with acute pulmonary blastomycosis have symptoms similar to those of an acute bacterial process. Cough (which may be productive), fever, malaise, and chest pain are the most common complaints of patients.[58] In many instances, patients may respond initially to routine antimicrobial therapy, only to have their constitutional symptoms return at a later date. Unlike disease in adults, which may take years to progress to a chronic pulmonary process, diseases in children rarely progress longer than 6 months without a return of symptoms.[77]

A mass effect, fibronodular patterns, and consolidation are frequent findings on chest radiography in adults with blastomycosis.[10,18,57] Little information exists on the chest radiographic patterns commonly encountered in children, but extensive consolidation of the involved lobes appears to be the most common finding.[41,58,65] Consolidation has been described to involve all lobes of the lung and may be multilobar or bilateral. Multiple small cavitary lesions, pleural effusions, hilar adenopathy, and nodular infiltrates also have been described. Mass lesions, which occur commonly in adults, frequently are not demonstrated in children, probably because mass lesions are associated more commonly with chronic disease, whereas lobar consolidation is more consistent with acute disease. The consolidation demonstrated on chest radiography may mimic that of an acute bacterial process (Fig. 211–2), whereas the chronic chest abnormalities associated with blastomycosis (e.g., mass lesions, cavitary lesions) may be confused with tuberculosis or neoplasms. Mass lesions, consolidation, air bronchograms, nodular infiltrates, and satellite lesions

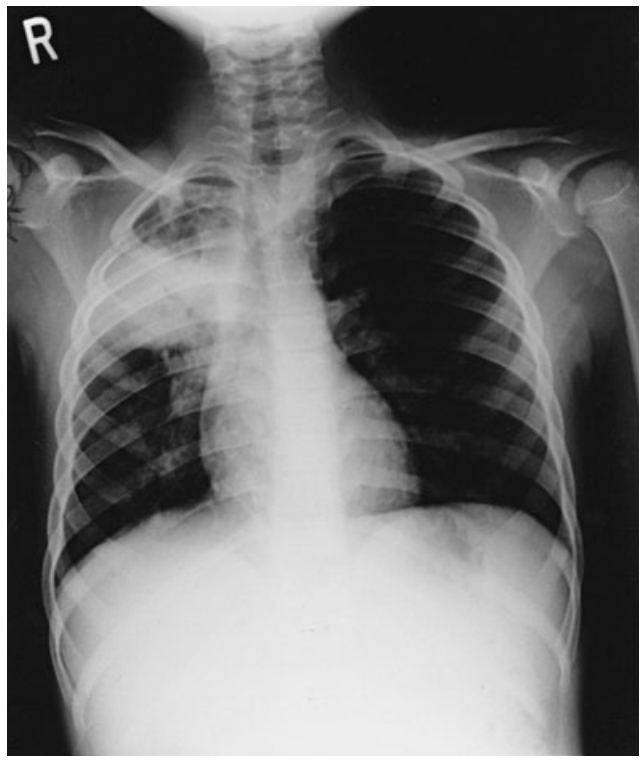

Figure 211–2 Chest radiograph of a right upper lobe infiltrate secondary to infection with *Blastomyces dermatitidis.*

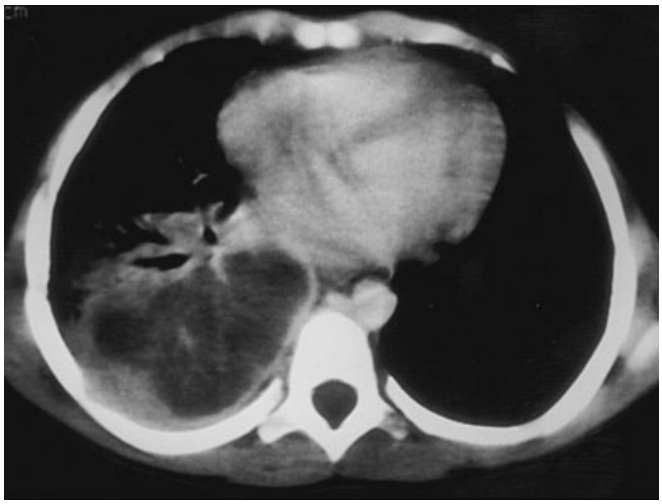

Figure 211–3 Computed tomographic scan of the chest revealing consolidation and abscess formation in the right lower lobe of the lung.

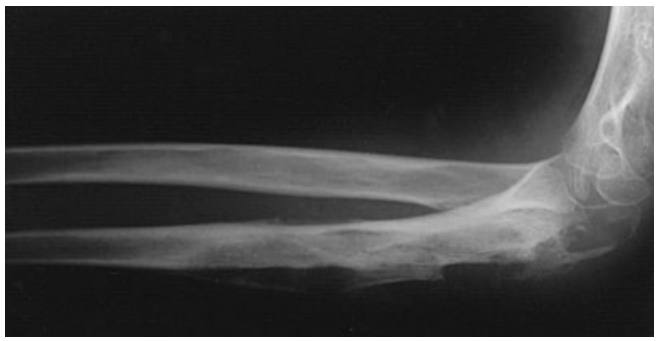

Figure 211–4 Bone destruction of the ulna by blastomycosis.

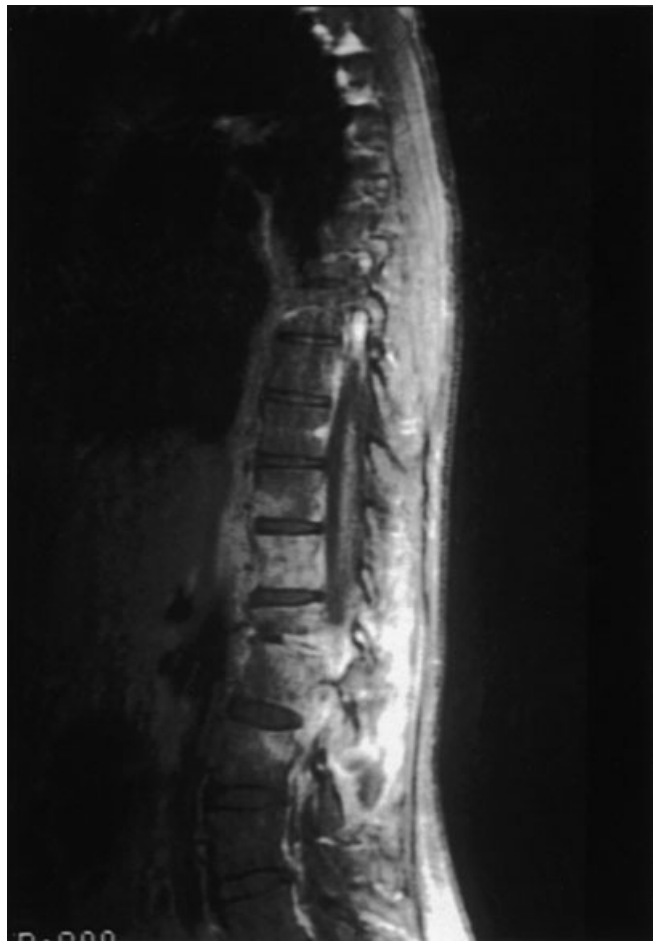

Figure 211–5 Magnetic resonance image of the spine demonstrating signal abnormalities at T11 through L3 with a severe compression deformity of the L1 vertebral body and multiple oval lytic lesions caused by blastomycosis.

commonly are found in adults with pulmonary blastomycosis who have undergone computed tomography of the chest.[75] Computed tomographic findings in children have been limited to consolidation, pulmonary abscess, and paratracheal adenopathy[65] (Fig. 211–3). Patients with severe radiographic disease may have slow pulmonary recovery and suffer from long-term sequelae, but most patients with pulmonary blastomycosis have normal pulmonary function testing at follow-up.[1]

DISSEMINATED DISEASE

Even though the numbers are small, disseminated disease appears to be present in 50 to 80 percent of children in whom blastomycosis is diagnosed.[65,69,77] The most common site of disseminated disease in children is the bones (Fig. 211–4). Although the long bones (e.g., the tibia, humerus), ribs, and vertebrae (Fig. 211–5) are involved most frequently, almost any bone is vulnerable.[61] Skin disease has been described in children, but the classic verrucous lesions seen in adults are uncommon findings in children (Fig. 211–6). Pustular or ulcerative lesions may be demonstrated, but they usually occur in children as a result of underlying bone involvement (Figs. 211–7 and 211–8). Skin lesions involving sun-exposed areas of the body (e.g., the nose, ears) also may be seen (Fig. 211–9). Other areas of involvement that have been described include the liver, spleen, heart, lymph nodes, psoas muscle, kidney, middle ear, and central nervous system (Fig. 211–10).[32,33,77]

DISEASE IN IMMUNOCOMPROMISED PATIENTS

Information concerning infection with *B. dermatitidis* in immunocompromised children is limited[79]; however, data exist on blastomycosis in immunocompromised adults and in adults with diabetes mellitus.[42,56,67,71,76] Unlike other fungi (e.g., *H. capsulatum*, *Cryptococcus neoformans*), this agent has caused infection in only a small number of patients with acquired immunodeficiency syndrome (AIDS), probably because many of these patients have less exposure to regions endemic for the organism. Pulmonary involvement is the most common manifestation of disease in immunocompromised patients, and adult respiratory distress syndrome is encountered more frequently than in immunologically normal hosts. Torres and colleagues[71] described a 13-year experience of endemic mycoses at a cancer hospital. Only two patients were found to have blastomycosis. Both patients were lymphopenic. Neither patient developed disseminated disease or died of fungal disease. Increased mortality rates (30-54%) have been described, with a large proportion of patients who die of other causes having evidence of persistent blastomycosis at the time of death. Many issues regarding therapy for this group of patients are not understood, but lifetime suppressive therapy appears to be indicated.

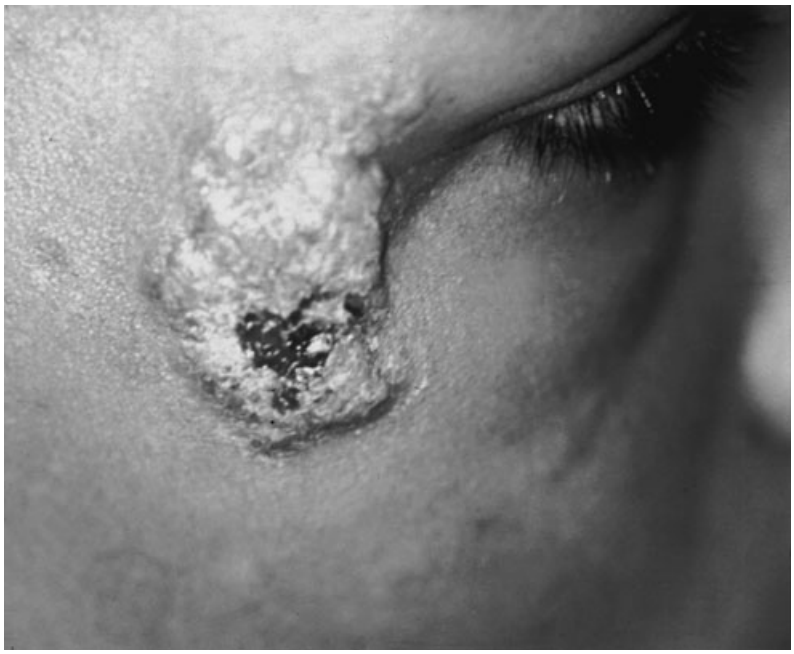

Figure 211–6 Verrucous lesion of blastomycosis on the face of a child.

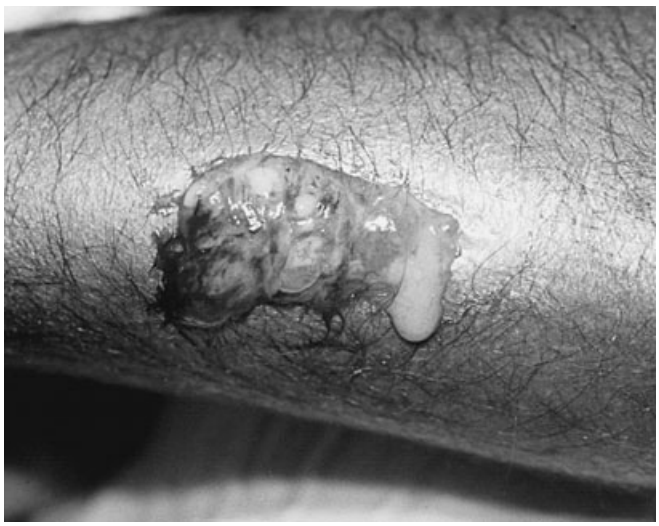

Figure 211–7 Purulent wound drainage overlying a region of osteomyelitis with blastomycosis.

DISEASE DURING PREGNANCY

Prenatal diagnosis of blastomycosis in pregnant women has been well documented.[42] Of the 19 women described in these reports, evidence of disseminated disease was noted in 10; 6 had pulmonary disease alone, 1 had isolated skin disease, 1 had adult respiratory distress syndrome requiring ventilatory support and early cesarean section, and 1 was not described. These 19 pregnancies culminated in the birth of 20 infants (one set of twins). None of the infants was found to be infected at birth. The placentas of the seven women were examined for evidence of *B. dermatitidis*. Only the mother with adult respiratory distress syndrome had organisms in the placenta. Organisms were located on the maternal as well as on the fetal side of the placenta. Two neonates developed disease during the first month of life.

NEONATAL DISEASE

Two neonates had an acute onset of respiratory distress within 3 weeks of birth.[45,74] Both infants died of their illness, and abnormalities on autopsy were limited to the lungs. One mother was noted to have lesions consistent with blastomycosis on her face and thigh,[74] whereas the second mother had a lesion on her right lower extremity.[45] Only one mother allowed an extensive physical examination to be performed, and her genital examination was unremarkable. The second mother denied having genital lesions; she refused therapy for blastomycosis and 2.5 years later died of a disseminated illness caused by *B. dermatitidis*. Autopsy findings revealed that her uterus and both ovaries had multiple abscesses, with the left ovary being almost entirely unrecognizable as a result of chronic infection.[78] The pathophysiologic process of neonatal disease is not understood entirely. Because the pathologic changes caused by *B. dermatitidis* were limited to the lungs in these neonates, this illness certainly could have been caused by aspiration of vaginal secretions that were colonized with this organism at birth. Autopsy findings in the second mother, however, certainly raise the question of transplacental passage of the organism.

DIAGNOSIS

Because no clinical syndrome is characteristic of blastomycosis, an unequivocal diagnosis requires isolation of the organism from a clinical specimen. A presumptive diagnosis can be made when the characteristic yeast is visualized in respiratory secretions, purulent material, or histopathologic sections. For patients with pulmonary disease, respiratory secretions can be obtained through sputum production, bronchoscopy with bronchoalveolar lavage, or open lung biopsy. Sputum samples usually are more helpful in older patients; such specimens are difficult to obtain in young children. Likewise, obtaining adequate specimens with the use of bronchoscopy and bronchoalveolar lavage in young children has been shown to be problematic. The need to perform open lung biopsy in patients with pulmonary blastomycosis after they receive negative results from flexible bronchoscopy and bron-

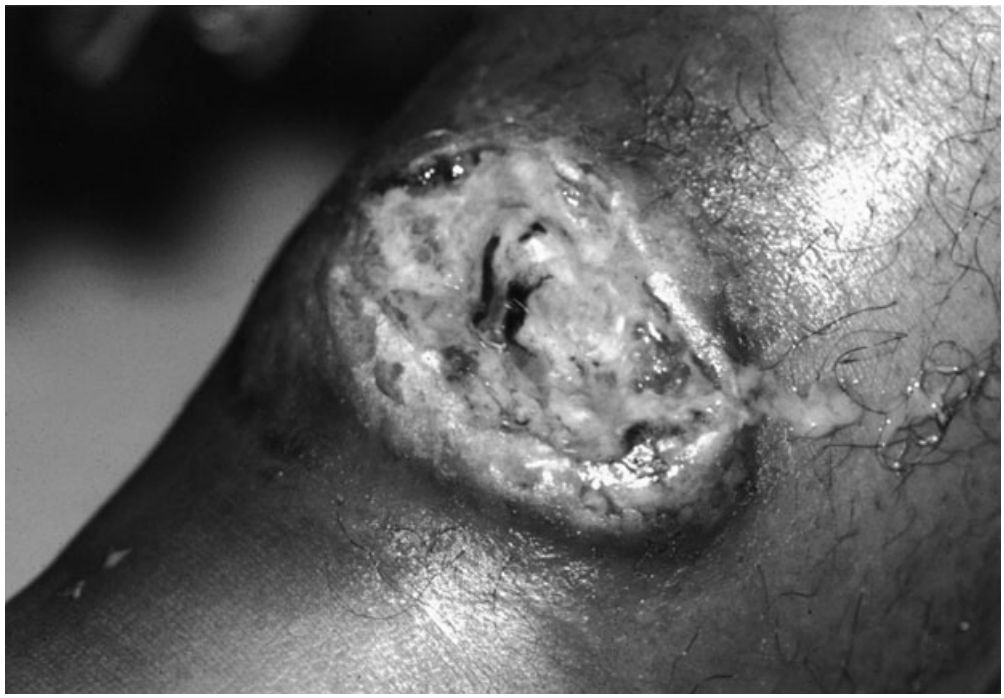

Figure 211–8 Ulcerative lesion overlying a region of osteomyelitis secondary to blastomycosis.

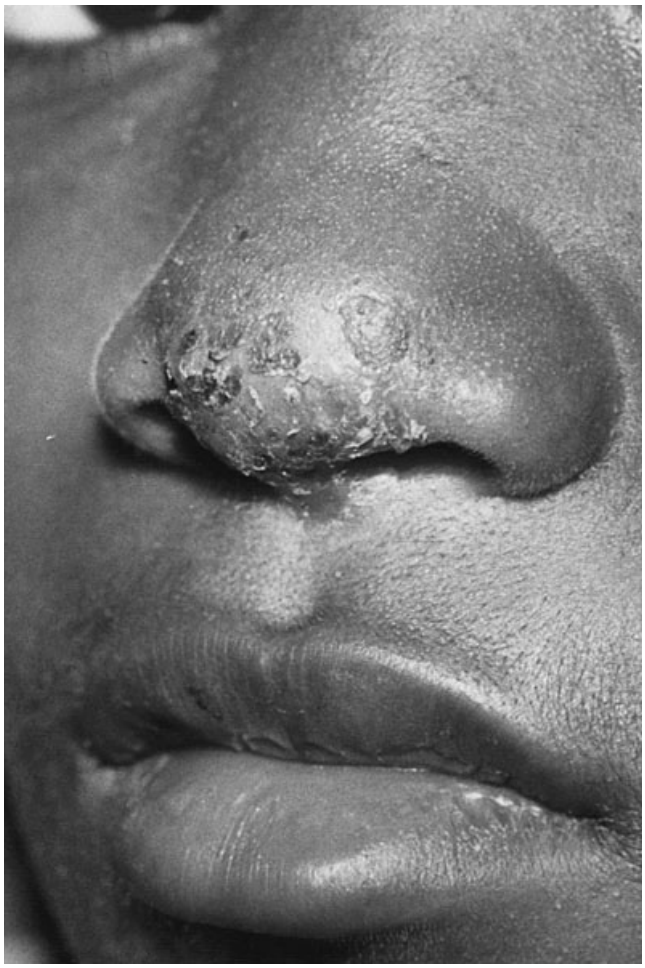

Figure 211–9 Crusted skin lesion on the nose caused by *Blastomyces dermatitidis*.

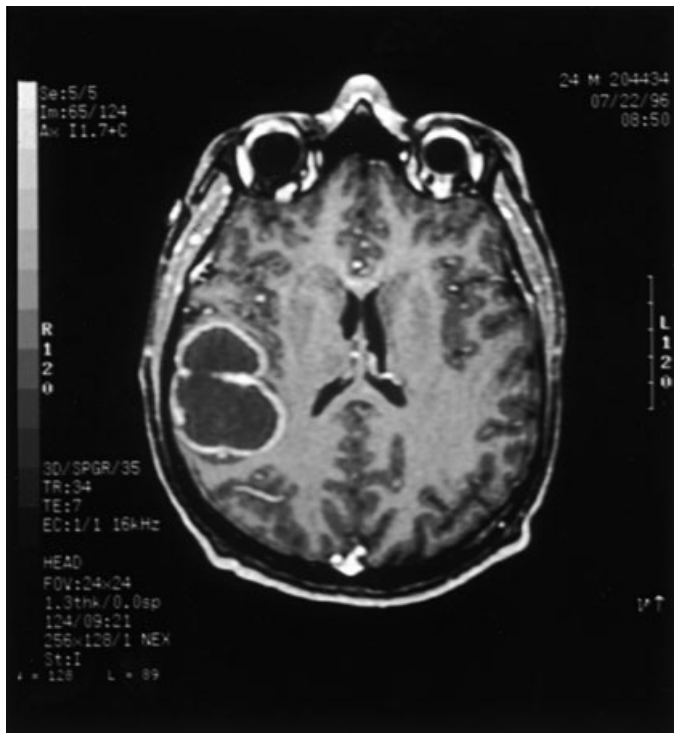

Figure 211–10 Computed tomographic scan of the brain demonstrating a multiloculated abscess in the right temporal lobe as a result of infection with *Blastomyces dermatitidis*.

choalveolar lavage demonstrates the technical limitations of this procedure in the pediatric population.[65] Bronchial brushings and bronchoscopically directed biopsy would improve recovery of the organism, but these procedures are technically difficult to perform in younger patients at this time. Because of the difficulty of establishing the diagnosis of pulmonary blastomycosis in young patients, it is recommended that children and adolescents who are thought to have blastomycosis undergo lung biopsy if sputum and bronchoscopic examination are nondiagnostic.[65]

Respiratory or purulent wound secretions can be examined by light microscopy of wet preparations with or without the use of potassium hydroxide to visualize the characteristic yeast forms. Other staining techniques, such as the calcofluor white stain, can be useful for analyzing specimens when the number of organisms in the specimen is limited. Gomori methenamine silver or periodic acid–Schiff stain may be useful for examining histopathologic specimens[44] (Fig. 211–11). The presence of pyogranulomata in a pathologic specimen should alert one to the diagnosis of blastomycosis, but organisms may be difficult to locate with the use of hematoxylin and eosin stains; in such instances, Gomori methenamine silver or periodic acid–Schiff stain may be helpful. Specimens collected for culture should be placed on a culture medium that will ensure recovery of all clinically significant fungi. The use of a more enriched agar, such as Sabouraud agar with brain-heart infusion, is essential for the recovery of such organisms as *B. dermatitidis*. Specimens potentially contaminated with bacteria or other fungal agents should be placed on an additional agar containing antimicrobial agents (e.g., chloramphenicol, cycloheximide) to inhibit the growth of these contaminants.

In some situations, however, isolation attempts are unsuccessful, and alternative laboratory tests may be used. Most currently available serologic tests are performed by complement fixation, immunodiffusion, or enzyme immunoassay with a yeast-phase antigen (A antigen). These assays have been most useful as epidemiologic tools, not for the clinical diagnosis of disease, because of poor sensitivity and specificity resulting from cross-reactivity with the antigens of other fungi (e.g., *H. capsulatum*). The most sensitive of these tests is the commercially available enzyme immunoassay.[7] However, a 120-kd protein that reacts with antibodies to *Blastomyces* has been identified.[36] This surface protein, designated WI-1, has been demonstrated to be a key antigenic target of humoral and cellular responses during infection. In a study comparing WI-1 and A antigen, WI-1 was found to be more reactive and specific for the binding of serum antibodies to *Blastomyces*.[31] Preliminary testing with WI-1 used as a target in a radioimmunoassay demonstrated that 93 percent of 27 patients with blastomycosis had positive serologic results within 60 days of diagnosis and that only 5 percent of 84 patients from whom the fungus was not identified had positive results.[68] Even though advances have been made, the role of serology remains limited in establishing a diagnosis. If serology is used, the most accurate method of testing is enzyme immunoassay. Antigen testing in body fluids also is a potentially helpful alternative; however, although the sensitivity is good, it is lacking in specificity. Of patients with known blastomycosis, 93 percent had positive urinary antigen assays. Unfortunately, cross-reactivity was noted in 96 percent of patients with histoplasmosis, 7 of 10 with penicilliosis marneffei, and all patients with paracoccidioidomycoses.[23]

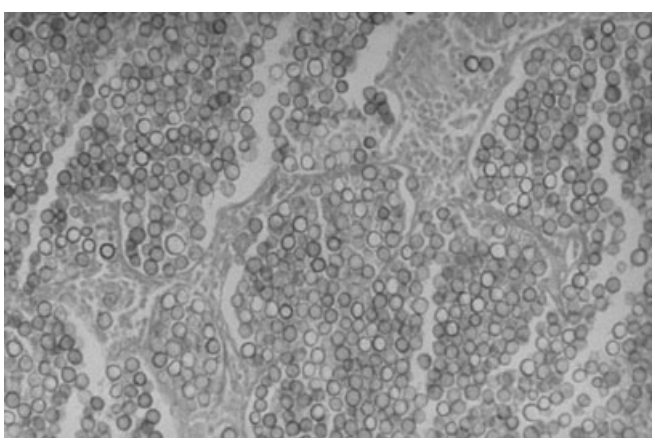

Figure 211–11 Overwhelming infection with *Blastomyces dermatitidis* demonstrated in lung tissue with a silver stain.

TREATMENT

Before antifungal medications were available, the mortality rate associated with blastomycosis usually exceeded 60 percent. After the introduction of effective medications for the treatment of blastomycosis, all patients received therapy when the diagnosis was established. This concept has been challenged because of the toxicity of amphotericin B and the recognition that self-limited cases of pulmonary blastomycosis exist.[63] Since the advent of safe and effective oral medications for the treatment of blastomycosis in adults, this controversy has lessened.[12] However, for children with blastomycosis, of whom as many as 80 percent have disseminated disease, the decision to withhold therapy for pneumonia secondary to blastomycosis can be dangerous. If a diagnosis of blastomycosis is established by culture after the patient has made a spontaneous recovery without therapy, the use of antifungal therapy might be questioned. If therapy is withheld, patients must be monitored carefully for months or even years for evidence of reactivation or dissemination of disease. Accidental introduction of blastomycosis into the skin through needle puncture or bite wounds usually can be treated locally by vigorous cleaning with tincture of iodine, an iodophor, or chlorhexidine.

The use of ketoconazole (400 to 800 mg/day for 6 months) for blastomycosis in adults has achieved cure rates of up to 89 percent.[8] However, little is known about the use of ketoconazole for blastomycosis in children. The recommended dose of ketoconazole in children, as established for the treatment of *Candida* infection, is 5 to 10 mg/kg/day given as a once-daily dose,[17] which would approximate the 400 to 800 mg suggested for blastomycosis in a 70-kg individual. When such a dose was used in five children with blastomycosis, two had relapses (one associated with noncompliance) and two had progression of their disease. The one patient who was cured also underwent a lobectomy, which would have eliminated the area with a large number of organisms.[65] Until more data become available, ketoconazole appears not to be effective for the treatment of blastomycosis in pediatric patients.

Fluconazole has been demonstrated to be effective against blastomycosis in 65 percent of adults at a dose of 200 to 400 mg daily.[55] A treatment failure rate of 30 percent was observed in the high-dose (400 mg/day) group, which included the youngest patient in the study. The investigators concluded that fluconazole is moderately effective against blastomycosis in adults and that its efficacy is similar to that of ketoconazole. No data on the treatment of blastomycosis with fluconazole are available for patients younger than 18 years. Hence, fluconazole does not have a role in the treatment of blastomycosis in pediatric patients.

The recommended therapy of choice for acute non–life-threatening blastomycosis in adults is itraconazole (400 mg/day) for 6 months.[12,21,62] Few data exist on the use of itraconazole in pediatric patients with blastomycosis, but doses of 5 to 10 mg/kg daily have been used safely and effectively in infants and children in other situations.[5,20,51,53] One study used itraconazole (5 to 7 mg/

kg/day; maximum, 200 mg/day) to treat four pediatric patients with blastomycosis.[65] Two patients were treated successfully with itraconazole after an initial treatment failure with a previous antifungal regimen. A third patient's initial trial with itraconazole for his pulmonary blastomycosis failed despite directly observed therapy. This patient had undetectable itraconazole levels in his serum, presumably caused by drug-drug interactions between primidone and itraconazole. The fourth patient completed a 6-month course of itraconazole for her pulmonary blastomycosis without relapse. Although data are limited because of the extremely small numbers of patients studied, itraconazole appears to be superior to ketoconazole or fluconazole for the treatment of blastomycosis in children. A dosage of 10 mg/kg (maximum of 400 mg/day) for itraconazole for 6 to 12 months is currently recommended.[12] In addition, serum levels of itraconazole should be determined after the patient has received it for at least 2 weeks to ensure adequate drug levels.[12]

Data are limited on the efficacy of newer antifungal agents in the treatment of blastomycosis. Both in vitro data and animal studies have shown efficacy of voriconazole and posaconazole.[31] Use of these newer agents in humans is limited to case reports.[3,54]

The agent with the greatest proven success for the treatment of blastomycosis in pediatric patients remains amphotericin B deoxycholate.[58,65,69,77] A total course of 25 to 30 mg/kg approaches a 100 percent cure rate. Therefore, for patients with life-threatening or central nervous system disease, neonates, and pregnant or immunocompromised patients, amphotericin B deoxycholate is recommended. Lipid formulstions of amphotericin B at a dose of 3 to 5 mg/kg/day may also be used.[12]

Specific treatment recommendations are outlined in Table 211–1. In cases of mild to moderate pulmonary disease or mild to moderate non–central nervous system disseminated disease, a combination of amphotericin B and itraconazole may be used. Treatment can be initiated with amphotericin B and changed to itraconazole to complete 6 months of therapy once the patient has been stabilized. In most instances, this change occurs after approximately 10 to 14 doses of amphotericin B deoxycholate at 1 mg/kg per dose. Caution should be used in patients with osteomyelitis because many experts suggest that these patients should receive a total of 1 year of itraconazole therapy.[12]

TABLE 211–1 Treatment Recommendations for Blastomycosis in Children

Type of Disease	Drug of Choice	Alternative
Pulmonary		
Life-threatening	Amphotericin B	May change to itraconazole when patient improves
Mild-moderate	Itraconazole	
Central nervous system	Amphotericin B	
Non–central nervous system		
Life-threatening	Amphotericin B	May change to itraconazole when patient improves
Mild-moderate	Itraconazole	
Immunocompromised host	Amphotericin B	Suppressive therapy should be continued with itraconazole
Neonate	Amphotericin B	

Because of the unpredictable nature of drug interactions, any patient receiving itraconazole with other medications that may interact with itraconazole should be monitored closely and have documentation of adequate serum levels. Close follow-up of these patients for identification of those with progression or relapse of their disease is mandatory. If patients do not have a clinical response within 2 to 4 weeks, if adequate serum levels cannot be obtained, or if clinical deterioration is documented, amphotericin B should be substituted for itraconazole for the treatment of blastomycosis.

Surgery, other than for establishment of the diagnosis, has a limited role in therapy for blastomycosis. Surgery is indicated for drainage of large abscesses or for removal of devitalized tissue in the occasional patient with osteomyelitis who is responding poorly to therapy. Surgery never should be considered curative and always should be performed in association with appropriate antifungal therapy. The duration of therapy should not be shortened simply because the patient has undergone surgical resection of the involved area.

Isolation of patients with pulmonary disease caused by blastomycosis is not required because the person-to-person spread of this disease has never been attributed to respiratory secretions. Furthermore, no special precautions are required for patients with open wounds caused by blastomycosis.

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CHAPTER 212

Candidiasis

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Candidiasis refers to infection with any of the yeast species of the genus *Candida* and encompasses a broad range of clinical manifestations. Most candidiasis is caused by *Candida albicans*, although other non-*albicans* species have been reported with increasing frequency in recent years. Hippocrates noted oral candidiasis (thrush) in infants, and for centuries afterward, candidiasis was regarded merely as a superficial and nonthreatening infection. A benign course is typical for *Candida* infections in otherwise healthy individuals, but for those whose immune systems are compromised, *Candida* spp. are opportunistic pathogens that may lead to disseminated disease and even death. Medical advances that have improved survival rates for many conditions that once were frequently fatal, such as prematurity and cancer, also have affected host defenses and microbial ecology, leading to an increased population at risk for acquisition of invasive candidiasis.

THE ORGANISM

Members of the genus *Candida* characteristically are round to oval vegetative cells possessing the ability to produce pseudohyphae (chains of elongated yeast forms) under certain conditions and the inability to develop ascospores (spores within a parent cell). The genus name *Candida* is derived from the Latin word *candidus* ("white") and reflects the creamy white appearance of *Candida albicans*, the species name of which is derived from the Latin for "to become white."

Candida spp. yeast forms are easily distinguished from *Cryptococcus* organisms by their lack of a capsule. Rapid laboratory identification of *C. albicans* may be made by provoking formation of germ tubes (septated cylindrical extensions) by the blastospore on incubation in human serum. *Candida* spp. may be distinguished from one another and from other yeasts by their use of carbohydrate substrates, which differs sufficiently among the *Candida* spp. to allow a biochemical profile for identification. These profiles have been established on the basis of the species' use of selected carbohydrates as carbon sources in the presence of oxygen (assimilation) and in the absence of oxygen (fermentation).

More than 150 species of *Candida* have been described, but only a small number of them are considered to be of medical importance. However, as infections caused by non-*albicans* species are recognized increasingly, more of these species may be reported in immunocompromised patients in the future. *C. albicans* remains the species most frequently causing disease in humans. However, azole-resistant *C. albicans* organisms are identified increasingly, and this finding, along with the observed increase in non-*albicans* infections, has implications for appropriate treatment of antifungal-resistant yeast infections. Non-*albicans* species that have been reported to cause disease in humans include *Candida glabrata*, *Candida guilliermondii*, *Candida kefyr* (formerly *Candida pseudotropicalis*), *Candida krusei*, *Candida lusitaniae*, *Candida parapsilosis*, and *Candida tropicalis*.

HOST SUSCEPTIBILITY

The relationship of *Candida* spp. to their human hosts is dependent on the number of organisms, the virulence of the species, and the host resistance. *Candida* spp. are frequent colonizers of human skin and mucous membranes but do not typically cause disease in immunocompetent individuals. Compared with other organisms that do cause disease in the immunocompetent host, *Candida* spp. have relatively low virulence factors. Therefore, for *Candida* spp. to establish infection, the number of organisms must be high, the host resistance must be impaired, or some combination of the two factors must exist.

Usually the number of organisms to which an individual has been exposed is not known. In one experiment with a known quantity of organisms, a healthy adult volunteer ingested a suspension containing more than 10^{12} organisms of *C. albicans*, and within 3 hours, he was febrile and *C. albicans* was cultured from both blood and urine samples.[49] Because the number of organisms will be unknown in most cases and because virulence is essentially constant, the predominant determinant for disease is the degree of host defense impairment.

Studies of responses of the immunocompetent host to *Candida* spp. suggest that eventually all components of the immune system are affected. The ability of *Candida* spp. to adhere to and invade host tissue is a major determinant of the virulence of these organisms. Although the microbial flora of the skin and mucosal surfaces serve as an important defense in the host's resistance to colonization and infection with *Candida* spp., the organisms are able to adhere to and invade host tissues. The host's antibody response to *Candida* spp. includes generation of secretory and humoral anti-*Candida* IgA[67] and specific anti-*Candida* IgE,[61] a transient IgM response with the infection, and circulating IgG found in most immunocompetent adults.[124] The IgG antibodies effectively opsonize *C. albicans*. *Candida* activates the alternative complement pathway.[108] Polymorphonuclear leukocytes,[19] monocytes, and eosinophils[52,53] ingest and kill *Candida*. A polysaccharide component of *C. albicans* can induce the formation of suppressor lymphocytes.[89]

Individuals with impaired host response to *Candida* spp. include neonates, pregnant women, and those who have congenital or acquired immunodeficiencies, immunosuppression due to medication (chemotherapy, corticosteroids) or radiation therapy, malignant neoplasms, endocrinopathies, debilitation caused by trauma or surgery, or alterations in mucosal barriers or normal flora due to catheterization or medications (particularly antimicrobials).

EPIDEMIOLOGY

Candida spp. are ubiquitous, are found in the environment in soil and water, and are frequent colonizers of human skin and mucosal membranes. *Candida* spp. also have been isolated from various species of domesticated animals. The incidence of invasive disease has been increasing coincident with advances in medical technology allowing improved survival of at-risk individuals, such as premature newborns, patients with cancer, and trauma victims.

In normal circumstances, transmission of *Candida* spp. requires contact of a colonized site with a mucous membrane or skin surface. Newborns may develop oral and cutaneous *Candida* infections after delivery through colonized vaginal mucosa.[48,69] Interestingly, however, a study suggests that vaginal mucosa may not be the main route of transmission of *Candida* spp. to the neonate.[15] Caramalac and colleagues[15] cultured vaginal mucosa of

100 women at the time of delivery and cultured the oral mucosa of their newborns. The incidence of *Candida* colonization was similar in the women who delivered vaginally (47.2%) and in those who delivered by cesarean section (46.4%), but oral mucosal colonization was more common in the neonates who were delivered vaginally (25%) compared with those who were delivered by cesarean section (3.6%). However, although infants who were delivered vaginally were more likely to be colonized with *Candida* spp., in only two cases (6%) were genotypic-phenotypic concordances between the maternal and neonatal isolates identified.

Although *C. albicans* has been shown to be cultured readily from air in the rooms of patients with cutaneous candidiasis, the significance of airborne transmission of *Candida* has not been determined.[66] Although nosocomial transmission has been reported frequently in neonatal and other closed hospital units, the source of transmission is not always clear. A recent Cochrane Database review found no evidence either to support or to refute use of measures such as cohorting or patient isolation rooms for neonates colonized with *Candida* spp.[68] The authors of that review stated that no standard policy of isolation measures exists and emphasized the importance of research into appropriate isolation measures. Studies using restriction enzyme fragment analyses have demonstrated that *Candida* colonization may be acquired from the environment or from staff members in closed hospital units.[12,102]

Several reports have indicated an increasing trend of non-*albicans Candida* infections in both adults and children.[47,58,109,132] *C. parapsilosis* is the most common non-*albicans* species isolated in pediatric patients, and it is isolated more commonly in children than in adults, for whom *C. glabrata* is the non-*albicans* species most frequently identified.[1,47,55,88,131,132] *C. parapsilosis* has been identified also as an emerging pathogen among adult oncology patients.[100] In some neonatal units, *C. parapsilosis* has surpassed *C. albicans* as the most common *Candida* species isolated. The increased rates of non-*albicans* species are possibly related to increased use of fluconazole.

The increased use of azole antifungals has been associated also with an increase in azole-resistant *Candida* spp. Increases in fluconazole-resistant species of *Candida*, especially *C. krusei* and *C. glabrata*, were recognized soon after fluconazole was introduced as a prophylactic agent in bone marrow transplant recipients.[126] Trifilio and colleagues[110] reported 10 breakthrough fungal infections in patients receiving voriconazole prophylaxis: 5 were caused by *C. glabrata* (which has variable susceptibility to azoles) and 1 was caused by *C. krusei* (which is inherently resistant to fluconazole). *Candida rugosa* is another species, not recognized until 1985, that has been reported increasingly and is resistant to azole antifungals.[87]

PATHOGENESIS AND PATHOLOGY

C. albicans, in addition to being the most common of the *Candida* spp., is the most pathogenic. *Candida* virulence factors include the ability of the organism to express adhesins, to transition from yeast to hyphal form, and to produce secretory aspartyl proteinases (SAPs) and phospholipases that facilitate invasion of tissue.[75,114] The initial step in colonization is adherence of the *Candida* blastospore (yeast form) to the mucosal or dermal epithelial cells. Once the yeast transforms to the hyphal stage, infection may become established. The exact mechanism by which *C. albicans* invades mucosal tissues is not well understood. Data support a proposed mechanism by which *C. albicans* invades mucosal tissues by proteolytic degradation of E-cadherin, the major protein in the epithelial adherens junctions.[114] Another proposed mechanism suggests that SAPs facilitate adhesion to and degradation of mucins (mucus O-linked glycoproteins) in the oral cavity and gastrointestinal tract.[18]

The SAP and phospholipase B (PLB) genes have been shown to be differentially expressed in humans.[75] Naglik and colleagues[75] analyzed gene expression in 137 cases of human oral or vaginal *Candida* colonization or disease. They found that *SAP2* and *SAP5* were the genes most commonly expressed during both colonization and disease states. Expression of other genes was correlated with disease and not carriage. *SAP1*, *SAP3*, and *SAP8* were expressed in both oral and vaginal disease but were preferentially expressed in vaginal infections. *PLB1* was correlated with oral but not vaginal disease.

Invasion of the mucous membrane leads to the formation of an adherent pseudomembrane, which is composed of epithelial cells, leukocytes, keratin, and food debris.[21] Mucosal lesions may progress to sharply demarcated ulcers with a base of granulation tissue covered by a fibrinous exudate and granulocytes intermixed with the organisms. Disseminated *Candida* infection is spread hematogenously from portals of entry, primarily from mucous membrane lesions. All organ systems may be involved in disseminated disease, but the kidneys, lungs, liver, brain, and spleen are affected most frequently. Microabscesses are formed in the body's pyogenic response to systemic disease. Granulomatous reactions occur infrequently.

Candida catheter-related infections also are associated with formation of biofilm.[20,77] *Candida* organisms within this sessile environment can be much more highly resistant to antifungal agents, rendering treatment difficult without removal of the catheter.

CLINICAL TYPES

Candida spp. may cause disease at any body site, although some areas are affected more commonly than are others, and the site and extent of disease indicate the relative immunocompetence of the host. For example, candidiasis involving the mouth and diaper area is a common occurrence in infants, most of whom are healthy children. On the other hand, chronic mucocutaneous candidiasis or disseminated disease suggests an underlying immune defect in the host.

The following sections describe manifestations of candidiasis classified by site of infection.

OROPHARYNGEAL CANDIDIASIS

Thrush (Acute Pseudomembranous Candidiasis)

Thrush is the most common type of candidiasis in infants and children and is caused almost exclusively by *C. albicans*. The lesions of thrush usually appear as white patches resembling milk curds affecting the dorsal and lateral aspects of the tongue, pharynx, gingivae, and buccal mucosa. These lesions coalesce in the formation of an adherent pseudomembrane composed of desquamated epithelial cells, leukocytes, keratin, necrotic tissue, and food deposits.[21] Removal of the pseudomembrane leaves an erythematous denuded lesion. Although pseudohyphal forms are abundant in thrush, the organisms rarely penetrate deeper than the stratum corneum, and in mild cases, the lesions are not painful. The subepithelial tissues may be involved, leading to edema and microabscess formation.

Acute Atrophic Candidiasis (Glossitis)

Glossitis is characterized by a smooth and erythematous tongue surface from erosion of the papillae of the tongue dorsum. Glossitis results from alteration of the bacterial flora of the oral mucosa due to use of broad-spectrum antibiotics. Typically, the white pseudomembranous lesions seen in thrush are absent or

minimal. Glossodynia (painful tongue) is a common complaint. The symptoms and lesions typically resolve with discontinuation of broad-spectrum antibiotics.

Angular Cheilosis (Perlèche)

Perlèche results from habitual licking at the corners of the mouth, which creates an environment in which *Candida* can establish infection. The infection is manifested as painful fissuring and erythema at the corners of the mouth. The lesions may evolve into shiny erythematous lesions associated with desquamation of the epithelium and surrounding hyperkeratosis.

Leukoplakia and chronic atrophic candidiasis are additional oropharyngeal manifestations of *Candida* infection that rarely are reported in the pediatric population.

ESOPHAGEAL CANDIDIASIS

Dysphagia is the characteristic finding in esophageal candidiasis, although in many cases affected individuals will be asymptomatic. Pain may occur with swallowing or may be a persistent finding. Patients may complain of retrosternal, paravertebral, intrascapular, or subscapular pain. Patients may also experience nausea and vomiting. Given that the distal third of the esophagus is the site most frequently affected, it is perhaps not surprising that many patients will not have thrush. Endoscopy reveals white patches similar in appearance to thrush on erythematous and friable esophageal mucosal tissue. An esophagram may reveal mucosal ulcerations in a cobblestone pattern. In advanced disease, larger lesions and strictures may develop, as may fistulas or esophageal perforation.

GASTROINTESTINAL CANDIDIASIS

The true incidence of gastrointestinal candidiasis is not known. Adequately documented cases have been associated with an underlying abnormality. Lesions localized to the stomach have been found predominantly in patients with peptic ulcer disease or malignant neoplasms or who have undergone gastric resection.

Although diarrhea and abdominal pain have been reported in patients with *Candida* spp. recovered from their stool, the relevance of the *Candida* to the symptoms is not always clear. In one review of the literature, the authors concluded that the available data show a strong correlation between antifungal treatment in patients with *Candida* spp. in their stools and resolution of diarrhea in those patients.[54]

Gastrointestinal *Candida* lesions are common manifestations in immunocompromised pediatric patients. In a review of 109 children with cancer and deep organ candidiasis studied ante mortem and at autopsy, *Candida* lesions were found in the esophagus in 49 patients, in the stomach in 40 patients, in the small intestine in 37 patients, and in the colon in 48 patients.[39] *Candida* spp. have been implicated also in typhlitis in immunocompromised patients. *Candida* infections of the gallbladder and biliary system also have been reported.

PERITONEAL CANDIDIASIS

Peritonitis caused by *Candida* spp. may occur as a complication of peritoneal dialysis, intestinal surgery, or bowel perforation. Abdominal distention, fever, and vomiting may occur, but in many cases, the typical signs of peritonitis may be absent.[41] Dissemination to other organs seldom occurs in cases of peritoneal candidiasis.[6,43,106] Clinical features of fungal peritonitis cannot be differentiated from bacterial peritonitis, and diagnosis must be made by microbiologic examination and culture of peritoneal fluid.[23] The outcome of fungal peritonitis in pediatric dialysis patients usually is more favorable than that in adults.[121]

A recent retrospective review of fungal peritonitis in pediatric patients receiving peritoneal dialysis described risk factors and evaluated treatment outcomes.[93] The authors reviewed 321 episodes of peritonitis in 159 patients, nine of whom were diagnosed with fungal peritonitis (2.9%). *Candida* spp. accounted for 78 percent of the fungal infections, and *C. albicans* specifically for 44 percent of the fungal infections. As with previous studies, preceding antibiotic use was found to be a risk factor for development of fungal peritonitis; 78 percent of the patients had received antibiotics in the previous month, 86 percent of them for bacterial peritonitis. Prior infection with gram-negative bacteria in particular is a risk factor for development of fungal peritonitis.

CANDIDIASIS OF THE URINARY TRACT

The presence of *Candida* in voided urine specimens is not always indicative of infection of the urinary tract. *Candida* spp. frequently are recovered in the urine of immunocompromised patients, in those with indwelling urinary catheters, and in those receiving antibiotics. Furthermore, data indicate that counts of 100,000 or more colonies of *Candida* spp. per milliliter of urine are no more indicative of urinary tract infection than are lower counts.[81] The symptoms of urinary tract candidiasis are similar to those that occur with analogous bacterial infections.[28] Lesions similar to those seen with thrush or esophageal candidiasis may be seen by cystoscopy on the bladder mucosa in patients with cystitis. *Candida* spp. also may cause renal microabscesses, papillary necrosis, and obstructive renal lesions. Many cases of urinary tract candidiasis are associated with underlying structural defects. In 26 cases with candidiasis and candiduria at the Mayo Clinic, 23 (88%) had urinary tract abnormalities.[2]

VAGINAL CANDIDIASIS

Vaginitis caused by *C. albicans* occurs commonly and is not indicative of a serious underlying disease. Vaginal candidiasis is characterized by pruritus and a white or watery discharge. The vaginal mucosa is erythematous with white lesions like those seen in thrush and other forms of mucosal candidiasis. Candidiasis also may affect the skin of the perineum, causing papular or ulcerative lesions.

RESPIRATORY TRACT CANDIDIASIS

Candida spp. are known to colonize respiratory tract mucosa, and their presence in cultures taken from respiratory specimens is not necessarily indicative of infection. Candidiasis may affect any site within the respiratory tract.

Respiratory involvement may be associated with oropharyngeal candidiasis. A syndrome of extensive oral candidiasis and hoarseness has been described in patients infected with human immunodeficiency virus (HIV) and in patients receiving immunosuppressive chemotherapy. Laryngoscopy has demonstrated discrete plaques of *Candida* on the vocal cords in such patients.[51,98]

Candidiasis limited to the bronchi occurs rarely and typically is associated with pulmonary parenchymal and systemic disease. Manifestations of pulmonary candidiasis include localized or diffuse pneumonia, nodular lesions, abscesses, and empyema.[34,81,127] Pulmonary candidiasis typically presents with nonspecific findings of fever and tachypnea. Definitive diagnosis requires an

invasive procedure such as lung biopsy to demonstrate the organism in tissue.

In addition to causing disease by infiltrating deeper tissues, *Candida* spp. within the respiratory tract may lead to an allergic response. Pulmonary allergy is not well defined, but both polysaccharide and protein extracts of *C. albicans* have been shown to provoke asthmatic attacks in atopic patients.[44]

MUSCULOSKELETAL CANDIDIASIS

Most cases of *Candida* arthritis have been reported in association with systemic candidiasis. The knee is the site most often reported to be affected. Joints may be infected by direct inoculation or by hematogenous spread and may be associated with a contiguous osteomyelitis. Nonsystemic candidal infection occurring after prosthetic arthroplasty has been reported.[17]

Candida osteomyelitis has been reported in several sites, including the spine, upper and lower extremities, ribs and costochondral junctions, mandible, and sternum.[22,81] Many of the reported cases of *Candida* osteomyelitis have occurred in infants younger than 14 weeks. In such cases, the lesion typically affects the lower extremity, which is shown to be osteolytic with cortical bone erosion.[123]

CARDIAC CANDIDIASIS

Candida spp. may infect any part of the heart. The clinical manifestations of *Candida* endocarditis are similar to those of subacute bacterial endocarditis. However, in contrast to bacterial endocarditis, blood cultures frequently are sterile in *Candida* endocarditis. Most cases of fungal endocarditis are caused by *Candida* spp. In one review of 319 cases of fungal endocarditis, *Candida* accounted for 67 percent of cases.[65] *Candida* spp. are not a common cause of endocarditis but are associated more frequently with endocarditis occurring in patients with systemic candidiasis and indwelling central venous catheters. In a review of 109 children with systemic candidiasis, 28 were found to have cardiac lesions.[39] The presence of an indwelling central venous catheter was associated with incidence of cardiac involvement in these children; only 3.7 percent of those without evidence of carditis had a central venous catheter compared with 18 percent of those with carditis. The aortic and mitral valves are the valves most frequently affected. Valvular involvement may be difficult to visualize by two-dimensional echocardiography. In addition to invasion of the endocardium, *Candida* spp. may cause embolization with occlusion of major arteries, necrosis, and microabscesses. *Candida* infection of the myocardium may be manifested as nonspecific electrocardiographic changes, such as supraventricular arrhythmias, QRS changes, and marked T-wave changes.[32]

CENTRAL NERVOUS SYSTEM CANDIDIASIS

The central nervous system (CNS) frequently is involved in disseminated candidiasis, although involvement of the CNS in the absence of disseminated disease rarely occurs. Autopsy studies have shown the incidence of CNS candidiasis to be less than 1 percent.[85,115] In one review of 29,659 autopsies, CNS candidiasis was noted in only seven cases (0.023%); however, all but two of those cases involved disseminated disease.[115] Involvement of the CNS has been described in approximately 25 to 50 percent of cases of systemic candidiasis.[39,56]

Candida spp. may cause meningitis, ependymitis, vasculitis and thrombosis, mycotic aneurysm, demyelination, transverse myelitis, and parenchymal lesions including macroabscesses and microabscesses, noncaseating granulomas, and nodules.[56]

Patients with CNS candidiasis may not demonstrate any signs indicative of neurologic involvement, or they may show nonspecific signs and symptoms of meningitis or encephalitis. *Candida* meningitis may be manifested as respiratory decompensation in premature neonates. Approximately half of all infants with *Candida* meningitis will not have positive blood cultures.[8] Many patients with *Candida* meningitis will not have evidence of cerebrospinal fluid pleocytosis or hypoglycorrhachia. Examination of cerebrospinal fluid and computed tomography or magnetic resonance imaging should be performed in cases of suspected *Candida* CNS disease and in cases of cardiac candidiasis, as CNS and cardiac candidiasis frequently co-occur in patients with disseminated disease.

In a report of pediatric oncology patients, duration of profound neutropenia with fever, antibiotic therapy, and administration of total parenteral nutrition were found to be significant factors associated with a diagnosis of *Candida* meningitis.[63] In that review, 11 of 12 cases were caused by *C. tropicalis*, and all cases were fatal.

OPHTHALMIC CANDIDIASIS

Careful routine ophthalmic examination should be performed in all patients with disseminated candidiasis. Retinitis is seen frequently in low-birth-weight neonates with disseminated candidiasis.[4] Chorioretinal involvement typically is manifested as white fluffy lesions that may extend to the vitreous. Patients may complain of eye pain, blurred vision, scotomata, and photophobia. Because retinal lesions may not be seen in severely neutropenic patients, careful ophthalmic examination in these patients after resolution of the neutropenia is vital.[36]

Candida endophthalmitis also has been described in as many as 20 percent of patients receiving total parenteral nutrition; hence, maintaining a high index of suspicion in this population and considering routine ophthalmologic evaluation are prudent.[35,70]

CUTANEOUS CANDIDIASIS

Some cutaneous manifestations of *Candida* infection are seen commonly in otherwise healthy children. *Candida* is a frequent cause of diaper dermatitis in healthy children. Infants with *Candida* diaper dermatitis will demonstrate obvious discomfort when urine comes in contact with the affected skin. Nail infection (paronychia) caused by *Candida* spp. is a frequent complication of thumb sucking, or it may develop after other trauma to the nail or surrounding tissue; it is characterized by intense erythema of the affected area.

Cutaneous *Candida* infections are seen frequently in immunocompromised patients, such as those with HIV infection or lymphoproliferative disease and those receiving immunosuppressive medications. Very-low-birth-weight infants may present with diffuse erythema and crusting of dependent and intertriginous areas, and many of these infants are found to have disseminated candidiasis.

CANDIDEMIA

Candidemia is associated with significant morbidity and with mortality rates of approximately 20 to 25 percent in children in general, 37 percent in pediatric patients in intensive care units, and approximately 45 to 55 percent in neonates and infants.[57,84,97,101,107,111,130] Neutropenia, endotracheal intubation, location in the intensive care unit, and presence of an arterial catheter have been identified as risk factors for death in pediatric patients with candidemia.[84,130]

Neonates with candidemia frequently have multiple positive blood cultures even after beginning antifungal therapy, and 10 percent may have candidemia for 14 days or more.[8]

DISSEMINATED CANDIDIASIS

Disseminated candidiasis may be designated acute (abrupt onset, with fever, fungemia, and evidence of organ involvement) or chronic (i.e., hepatosplenic candidiasis, usually seen in leukemic patients following recovery of neutrophil counts after chemotherapy).

In most cases of disseminated candidiasis, the infection is concentrated in two or three areas. The lungs, kidneys, liver, spleen, and brain are affected most frequently. *Candida* organisms are disseminated to these organs hematogenously from lesions of the gastrointestinal tract, oral mucosa, or skin. Although transient candidemia may occur without discernible foci of infection, most severely immunocompromised patients with candidemia will have disseminated disease.[128] Clinical manifestations of disseminated candidiasis depend on the sites and extent of involvement. Underlying conditions that predispose patients to development of disseminated candidiasis include cancer, HIV infection or acquired immunodeficiency syndrome (AIDS), history of solid organ or hematopoietic stem cell transplantation, premature birth, and presence of indwelling central venous catheters.

The retinal lesions seen in *Candida* endophthalmitis and a maculopapular rash are two clinical manifestations that are strongly indicative of disseminated candidiasis. The skin lesions have been described in patients with hematologic malignant neoplasms and typically are discrete, firm, erythematous papules measuring 0.5 to 1.0 cm in diameter. The rash frequently is characterized by a nodular center surrounded by a halo of erythema and may be generalized. Biopsy specimens of the rash will show both yeast and pseudohyphal forms. Biopsy may be vital for establishing a definitive diagnosis to exclude other infectious causes in the susceptible immunocompromised host.

Clinical manifestations of 109 pediatric oncology patients with disseminated candidiasis evaluated for 2 months before death were compared with findings at autopsy.[39] In approximately 90 percent of the cases, patients had developed fever or neutropenia, had received antibiotics or immunosuppressive agents, or were known to be in relapse of malignant disease in the 2 months before death. The major organs involved, in order of frequency, were the lungs, spleen, kidneys, liver, heart, and brain. More than one organ was involved in 88 percent of the patients, and in many cases, the organ involvement was not suspected before autopsy. For example, lesions were not detected by radiographs performed in the last 10 days of life for half of the patients with *Candida* involvement of the lungs, and laboratory evaluations of renal and hepatic function were normal in half of the patients with *Candida* involvement of the kidneys and liver detected at autopsy. Although 93 percent of the children were colonized with *Candida* spp., in only 17 percent of the patients was *Candida* cultured from antemortem blood specimens. Other studies have demonstrated an increased incidence of systemic candidiasis in pediatric patients colonized with *Candida* spp. at multiple sites.[60,62]

Zaoutis and colleagues[132] retrospectively reviewed cases of candidemia at a tertiary care children's hospital and identified risk factors for disseminated disease. In that series, only 24 percent of the children had an underlying oncologic diagnosis. Of 153 patients with candidemia, 17 percent had evidence of disseminated disease, and a third of those had involvement of more than one organ. The organs most commonly involved were the lungs (58%), liver (23%), kidney (16%), and brain (12%). In that series, candidemia persisting for more than 3 days with a central venous catheter in place, and immunosuppression were independent risk factors for development of disseminated disease in affected pediatric patients.

DIAGNOSIS

The diagnosis of candidiasis requires the association of clinical observations with laboratory tests identifying the organism. In some cases, a biopsy may be indicated to establish the diagnosis of candidiasis in diseased tissue.

Direct microscopic examination of specimens may help establish a diagnosis quickly. Specimens swabbed or scraped from surface lesions mounted in 20 percent potassium hydroxide or calcofluor will reveal the 3- to 7-mm-diameter ovoid budding yeast cells or pseudohyphae. *Candida* organisms stain well with Gram stain or with periodic acid–Schiff, Gomori methenamine silver nitrate, and toluidine blue stains. Organisms can be identified only tentatively as *Candida* spp. by direct microscopic examination, and in some preparations, differentiation of *Candida* spp. from other yeasts or even molds such as *Aspergillus* spp. may be difficult.

Both yeast and pseudohyphal forms may be seen in biopsy specimens. The early tissue reaction to infection is acute suppurative inflammation that may progress to granulomatous inflammation. Microabscesses frequently are seen in biopsy specimens.

Candida spp. may be cultured on Sabouraud dextrose media, which typically is prepared with an antibiotic to prevent overgrowth of bacteria contaminating the specimen. Cycloheximide, which is sometimes included in media to prevent saprophytic fungal overgrowth, also may inhibit some strains of *Candida* spp. and should not be used. Blood culture bottles should be vented for optimal growth. On solid media, *Candida* spp. appear as moist white or cream-colored colonies with well-demarcated borders. In contrast to most other *Candida* spp., *C. albicans* produces germ tubes when suspended in serum for a period of 1 to 4 hours, a characteristic that is used by laboratories for rapid presumptive diagnosis of *C. albicans* colonization or infection. *Candida* spp. are definitively identified by biochemical fermentation and assimilation tests.

Antibody testing is not useful in establishing the diagnosis of invasive candidiasis. *Candida* colonization may lead to positive antibody test results, even in the absence of invasive disease. Furthermore, immunocompromised patients may not mount sufficient antibody response even in the presence of invasive candidiasis. Antigen testing for SAPs has shown promise in vitro and in animal experiments.[24,72,74] Many new molecular diagnostic tests hold promise but are not readily available or standardized.

To date, an assay for β-1,3-glucan is the only non–culture-based diagnostic approved by the Food and Drug Administration (FDA) for diagnosis of invasive candidiasis.[77,82] β-1,3-glucan is a polysaccharide component of the *Candida* cell wall that is not found in humans, and therefore its presence in a sample of human tissue is indicative of fungal infection. Recent data suggest that the β-1,3-glucan test could be used also in establishing the diagnosis of *Candida* biofilms on implanted devices.[77] Although removal of a catheter is recommended in candidemia, doing so is not always a viable option, and use of a test to identify biofilm catheter-associated infection would allow clinicians to identify those patients for whom removal of the device is most critical.

Polymerase chain reaction testing for *Candida* spp. was reported first in 1990 and shows great promise for establishing rapidly and accurately a diagnosis of invasive candidiasis.[14,24] Currently, molecular diagnostic tests lack standardized and commercially available DNA extraction procedures, and many methods of polymerase chain reaction format and design need to be standardized.[24]

The definitive diagnosis of disseminated candidiasis is difficult to establish, but the diagnosis may be made if *Candida* is recov-

ered from otherwise sterile body fluids (blood, spinal fluid, bone marrow) in an immunocompromised patient with compatible clinical features in whom other causes of infection have been excluded.[40] Computed tomographic scans are useful in recognizing lesions of the liver, spleen, kidneys, or brain that are sufficiently characteristic for a presumptive diagnosis of systemic candidiasis.[5,30]

TREATMENT AND PREVENTION

In recent years, several new drugs have become available for the treatment of candidiasis. The appropriate drug or combination of drugs depends on the location and extent of infection. The following recommendations are based on the comprehensive and authoritative guidelines for the treatment of candidiasis developed by an expert panel of the Infectious Diseases Society of America.[83]

ANTIFUNGAL AGENTS

Until recently, treatment options for invasive candidiasis were limited. Amphotericin B deoxycholate was developed in the 1950s and had been the mainstay of therapy for decades until the recent development of several new agents and classes of antifungals. There are three main classes of antifungal drugs: the polyenes (amphotericin B deoxycholate and its lipid formulations), azoles, and echinocandins.

Polyenes

Use of amphotericin B deoxycholate, or "conventional" amphotericin B, has largely been supplanted by its less toxic lipid formulations. Amphotericin B is associated with infusion-related effects of high fevers and chills and with dose-limiting nephrotoxicity. In the 1980s, scientists used liposome technology to develop new formulations of amphotericin B that were better tolerated, allowing administration of larger doses.[78]

There are currently three lipid formulations of amphotericin B: amphotericin B lipid complex, amphotericin B cholesteryl sulfate complex (amphotericin B colloidal dispersion), and liposomal amphotericin B. Use of the cholesteryl sulfate complex form has not been widespread in the United States, and in one study, infusion-related events were more common with this formulation compared with conventional amphotericin B.[13] Wingard and colleagues[125] evaluated the safety of liposomal amphotericin B compared with amphotericin B lipid complex as empiric therapy in patients with fever and neutropenia and found that liposomal amphotericin B was better tolerated, with significantly less fever, chills, nephrotoxicity, and treatment-related discontinuation of antifungal therapy. Infusion-related events may occur with any of the formulations, and intolerance of one product does not necessarily indicate that a patient will have an adverse reaction to a different formulation.[33,45]

Higher doses of amphotericin B are possible with the better-tolerated lipid formulations. Although the usual recommended dosing of liposomal amphotericin B is 3 to 5 mg/kg daily, Walsh and colleagues[119] found that dosages as high as 15 mg/kg daily were effective and well tolerated.

Liposomal amphotericin B (AmBisome) is FDA approved for use in pediatric patients older than 1 month as empiric therapy in febrile neutropenia and for systemic candidiasis when risk of toxicity precludes the use of conventional amphotericin B. Amphotericin B lipid complex (Abelcet) is FDA approved for systemic candidiasis in pediatric patients who are intolerant of or refractory to conventional amphotericin B therapy (recommended dose, 5 mg/kg daily).

Amphotericin B products are active against most species of *Candida*, but resistance may be likely in isolates of *C. guilliermondii* and *C. lusitaniae*.

Azoles

Azole antifungals act by disrupting synthesis of ergosterol in the fungal cell membrane, by inhibiting the cytochrome P450 enzyme 14α-demethylase. The azoles include fluconazole, itraconazole, voriconazole, posaconazole, and ravuconazole, all of which are active against *Candida* spp.

Fluconazole, available in both oral and intravenous formulations, has been widely used and is well tolerated, and its increased use has been associated with an increased incidence of fluconazole-resistant organisms. Many fluconazole-resistant organisms are susceptible to the newer azoles. Fluconazole (Diflucan) has an FDA-approved indication for pediatric patients older than 6 months for treatment of systemic disease and candidiasis of the mouth and esophagus.

Itraconazole is poorly absorbed orally. Absorption is improved with the liquid formulation, but it is not well tolerated. An intravenous formulation of itraconazole also is available. Given the availability of more palatable options, itraconazole has a limited role in the treatment of *Candida* infections.

The second-generation triazoles have broader antifungal coverage than does fluconazole and may have activity against fluconazole-resistant organisms. Voriconazole (Vfend) is FDA approved for pediatric patients aged 12 years or older for treatment of esophageal candidiasis. The recommended dosing for this indication is 200 mg every 12 hours for patients weighing 40 kg or more or 100 mg every 12 hours for patients weighing less than 40 kg. Posaconazole (Noxafil) is available only as a liquid oral formulation, which must be taken with high-fat meals to optimize absorption. It is FDA approved for prophylaxis in pediatric patients aged 13 years and older who are severely immunocompromised.[16,112] The recommended dose for this indication is 200 mg (5 mL) three times a day with a full meal or liquid nutritional supplement.

Echinocandins

The echinocandins are the first new class of antifungal agents developed in decades. Echinocandins act on the fungal cell wall. Because the human host has no cellular equivalent of the fungal cell wall, the echinocandins are very well tolerated. All echinocandins are available as parenteral formulations administered once daily, and all have the same general spectrum of coverage. Anidulafungin differs from the other echinocandins in that it slowly degrades in plasma rather than being metabolized, and it may also have activity against *Candida* spp. (i.e., *C. parapsilosis*) that are resistant to other echinocandins.[113] Caspofungin has an FDA-approved pediatric indication. Available data from clinical trials and clinical experience suggest that the echinocandins are safe and effective in pediatric patients.[7,31,103,116] Pharmacokinetic studies of caspofungin in children indicate that the initial proposed pediatric dosing of 1 mg/kg daily is suboptimal and that 50 mg/m^2 daily dosing is comparable to adult dosing of 50 mg daily.[116] A study of the pharmacokinetics of micafungin in pediatric patients aged 2 to 17 years with febrile neutropenia showed linear pharmacokinetics and increased clearance as a function of decreased age.[103] Clearance was approximately 1.35 times greater in those aged 2 to 8 years compared with those aged 9 to 17 years. Dosing in that study was initiated at 0.5 mg/kg/day and increased up to 4.0 mg/kg/day. Micafungin does have a pediatric indication

in Japan, where the usual dose recommended for candidiasis is 1 mg/kg/day, which may be increased as indicated up to 6 mg/kg/day. A study of safety and pharmacokinetics of anidulafungin in pediatric patients with neutropenia demonstrated that doses of 0.75 mg/kg/day or 1.5 mg/kg/day are comparable to adult dosing of 50 mg daily or 100 mg daily, respectively.[7]

The echinocandins have been shown to be highly effective alternatives to previously available standard treatment options for invasive candidiasis. In studies of treatment of invasive candidiasis, caspofungin has been shown to be at least as effective as amphotericin B,[71] and micafungin compared favorably to liposomal amphotericin B,[50] and anidulafungin to fluconazole,[94] in noninferiority trials.

OROPHARYNGEAL CANDIDIASIS

Oropharyngeal candidiasis typically may be treated topically. Children who are able to hold the suspension in the mouth as long as possible before swallowing may receive nystatin 400,000 to 600,000 units (4 to 6 mL) four times a day. In infants 200,000 units (2 mL) nystatin should be applied to the affected areas four times daily. Clotrimazole also is effective and is given five or six times daily as a 10-mg troche that is held in the mouth until it dissolves completely. Use of gentian violet as a 0.5 or 1.0 percent solution swabbed onto the buccal mucosa twice daily has largely been supplanted by use of nystatin suspension. Gentian violet is moderately effective but causes irritation and ulceration of the mucosa with prolonged use, and the purple staining can be messy and unattractive.

For infants with thrush, it is important to address sites that may be colonized with *Candida* to effectively treat the infection. Nystatin cream may be applied four to six times daily to areas that have sustained contact with the infected infant's mouth (e.g., the mother's nipple area for breast-fed infants and the appropriate digits of the hands for those infants who habitually suck their thumbs or fingers). For bottle-fed infants, if the bottle nipple is to be reused, it should be boiled after each use. Pacifiers or other objects with sustained contact with the infant's mouth also should be boiled after each use. The avoidance of unnecessary antibacterials also is an important aspect in the prevention of thrush.

More severe or refractory cases of oropharyngeal candidiasis, particularly in immunocompromised children, may require treatment with an oral or intravenous medication. A 14-day course of fluconazole suspension given once daily, as a 6 mg/kg dose on day 1 and then at 3 mg/kg, has proved to be superior to nystatin for the treatment of oropharyngeal candidiasis in immunocompromised children.[29] Itraconazole and ketoconazole[38] also may be used but are less effective than is fluconazole.[83] Neutropenic patients, in particular, with severe cases of oral candidiasis may benefit from short courses of an intravenous antifungal agent.

ESOPHAGEAL CANDIDIASIS

Esophageal candidiasis is treated usually with a 2- to 3-week course of fluconazole. Treatment should continue for 1 to 2 weeks after resolution of symptoms. Dosing for *Candida* esophagitis is the same as for oropharyngeal candidiasis: a loading dose of 6 mg/kg, followed by 3 mg/kg daily for the remainder of the treatment course. Voriconazole also is FDA approved for children at least 12 years of age for esophageal candidiasis. Refractory cases may be treated with intravenous agents. Esophageal candidiasis may lead to complications of stricture, secondary infection, and perforation.

PERITONEAL CANDIDIASIS

For pediatric patients receiving peritoneal dialysis who develop *Candida* peritonitis, fluconazole has been recommended as first-line therapy because of its excellent bioavailability and peritoneal absorption and its broad activity against most *Candida* spp.[93,122] However, the most recent adult guidelines recommend amphotericin B (or fluconazole or voriconazole or caspofungin) combined with flucytosine as first-line therapy.[90,93] Flucytosine has a questionable role now that fluconazole has been shown to be safe and effective and to achieve good systemic levels when it is given both orally and intraperitoneally. The catheter should be removed, but no consensus exists about timing of removal. Early but not immediate catheter removal, to provide peritoneal lavage with fluconazole to help prevent peritoneal adhesions and to maintain peritoneal membrane viability, is a rational approach supported by available data.[93,121]

CANDIDIASIS OF THE URINARY TRACT

Candiduria can be cleared with a 2-week course of oral fluconazole, but recurrence is common.[105] Other treatment options for *Candida* cystitis in the absence of renal or systemic involvement include oral flucytosine and bladder irrigation with amphotericin B.[26,27] Serial urine cultures should be obtained to document clearance of candiduria. Intravenous therapy for 4 to 6 weeks is indicated for patients with renal candidiasis and those in whom disseminated infection is suspected.

VAGINAL CANDIDIASIS

Vaginal candidiasis may be effectively treated topically with azoles (e.g., clotrimazole or miconazole) or with nystatin suppositories. A single 150 mg dose of oral fluconazole is a simple and effective treatment option for adults.[73,86]

CENTRAL NERVOUS SYSTEM CANDIDIASIS

CNS candidiasis should be treated with intravenous antifungals, typically as part of combination therapy. The combination of amphotericin B (0.5 to 1 mg/kg/day) or one of its lipid formulations plus flucytosine (150 mg/kg/day in four equally divided doses) may be synergistic for *Candida* meningitis. Amphotericin B penetrates into the cerebrospinal fluid poorly, whereas flucytosine penetrates readily. Duration of treatment depends on clinical response and should be continued for a minimum of 4 weeks.

Candida meningitis is a frequent complication of candidemia in neonates and is associated with high morbidity and mortality rates. Accordingly, *Candida* meningitis in a neonate should be treated aggressively. Few data are published about the use of fluconazole in meningitis, and, although it has been reported to treat meningitis successfully,[42] an amphotericin B product, alone or in combination with flucytosine, is recommended for treatment of neonatal *Candida* meningitis.

CANDIDEMIA

Any of the main classes of antifungal agents may be used for treatment of candidemia or systemic disease. Fluconazole is an acceptable option for susceptible organisms, but clinicians must be aware of the increasing incidence of azole-resistant organisms. Clinicians also should be aware that some species of *Candida* may be inherently resistant to polyenes (e.g., *C. lusitaniae*) and echi-

nocandins (e.g., *C. parapsilosis*) as well. Combination therapy with an amphotericin B product plus fluconazole is also recommended as an alternative option for treatment of candidemia.[83,95] A study of this combination of antifungals in non-neutropenic subjects showed a trend toward improved successful treatment and more rapid clearance of *Candida* spp. from the bloodstream.[95]

Removal of vascular catheters is recommended in all cases of candidemia.[83] However, removal of a catheter is not always feasible given the cost and complications that may be associated with replacing it. Nucci and Anaissie[79] conducted a review of the literature to evaluate the impact of removing a catheter on outcomes. They identified 203 studies of candidemia, only 14 of which evaluated outcome of removal or retention of a catheter. Of those 14 studies, only four met the selection criteria of including multivariate analysis with odds ratios and 95 percent confidence intervals and included confounding variables. Those four studies, none of which was a prospective randomized trial, had conflicting results, and the authors concluded that available data do not support the consensus recommendation for universal catheter removal. However, the authors agreed with the consensus recommendations but proposed expanding them to include those situations in which removal of a catheter is not feasible.

In neonates with candidemia, delay (more than 1 day after beginning antifungal treatment) in removing or replacing catheters is associated with increased mortality rates and neurodevelopmental impairment.[8] Although no large randomized trials of catheter management in neonatal candidiasis have been performed, available data support prompt removal or replacement of vascular catheters in neonates with candidemia.[8,11]

Candidemia in neonates also is associated with significant morbidity. Benjamin and colleagues[9] have provided suggestions for end-organ evaluation in neonates with candidemia. These suggestions include routine evaluation of cerebrospinal fluid (including culture), head ultrasonography, ophthalmologic examination, and echocardiography in all neonates with candidemia.

DISSEMINATED CANDIDIASIS

Intravenous therapy is indicated initially for patients with disseminated candidiasis as well as for those with CNS, cardiac, or respiratory involvement. For patients who respond to initial intravenous therapy, are clinically stable, and are infected with an azole-susceptible strain, a switch to oral fluconazole therapy at 6 mg/kg/day is acceptable for continuation therapy. Duration of treatment depends on clinical response and should be continued for a minimum of 4 weeks. Broad-spectrum antibiotics and immunosuppressive drugs should be avoided if at all possible.

EMPIRIC THERAPY

For patients with febrile neutropenia who do not respond to broad-spectrum antibiotics within a week of therapy, the likelihood of systemic fungal infection is sufficient (approximately 30%) to warrant initiation of empiric antifungal treatment.[37,91] Amphotericin B has been the empiric antifungal therapy of choice for fever with neutropenia, but some concern exists that some *Candida* spp. isolated from severely immunocompromised patients may be resistant to usual concentrations of amphotericin B achieved in vivo.[92] In one study, all episodes of candidemia caused by isolates with minimal inhibitory concentrations greater than 0.8 μg/mL of amphotericin B and half of those with minimal inhibitory concentrations of 0.8 μg/mL or less were fatal.[92] However, another study using the National Committee for Clinical Laboratory Standards method failed to demonstrate that the results of in vitro susceptibility testing for amphotericin B and fluconazole predicted outcome of therapy in patients with candidemia.[96]

Although amphotericin B has been the standard empiric therapy in fever with neutropenia, newer antifungal agents have been shown to be safe and effective when they are used for this purpose.[117,118,120] A multicenter, randomized, double-blind study comparing conventional amphotericin B with liposomal amphotericin B found that the liposomal formulation was as effective as conventional amphotericin B in empiric therapy and was associated with fewer breakthrough fungal infections and less treatment-related toxicity.[120] In that study, only three breakthrough *Candida* infections occurred in the group treated with liposomal amphotericin B (two caused by *C. parapsilosis* and one by *C. krusei*), compared with 12 in the group treated with conventional amphotericin B ($p = .03$). Walsh and colleagues[118] compared voriconazole with liposomal amphotericin B in a multicenter randomized study and determined that voriconazole is an acceptable alternative to amphotericin B formulations for empiric therapy in fever with neutropenia. The frequency of breakthrough fungal infections was significantly less in the voriconazole-treated group ($p = .02$); two invasive *Candida* infections occurred in the voriconazole-treated group compared with six in the group treated with liposomal amphotericin B.[118] In another randomized, double-blind, multicenter trial, Walsh and colleagues[120] found caspofungin to be as effective as is liposomal amphotericin B for empiric therapy. In that study, breakthrough fungal infections and therapy-related adverse events were similar in incidence in the two groups (16 cases of invasive candidiasis in the caspofungin-treated group compared with 15 in the group treated with liposomal amphotericin B), but fever, chills, nausea, and nephrotoxicity were less common manifestations in the caspofungin group.[120]

A clinical predictive model for neonatal candidemia has been developed that may assist clinicians in deciding when to begin empiric antifungal therapy in at-risk neonates. Benjamin and associates[10] suggest considering initiation of antifungal therapy when blood cultures are obtained in neonates who are less than 25 weeks' estimated gestational age, have thrombocytopenia, or are 25 to 27 weeks' estimated gestational age and have received a third-generation cephalosporin or carbapenem in the previous 7 days. The authors caution that before widespread use of antifungals is initiated, these recommendations need to be evaluated prospectively, as was the predictive model for febrile neutropenia.

PROPHYLAXIS

Prophylactic administration of antifungal agents has been shown to reduce the incidence of *Candida* infections in those at risk for acquiring fungal infection. However, associations between use of azole antifungal prophylaxis and increases in incidence of azole-resistant *Candida* spp. have been reported.[80,99,104,126]

Fluconazole prophylaxis began to be used routinely in some neonatal intensive care units after the publication of a single-center, randomized trial supporting its efficacy in preventing candidiasis in extremely low-birth-weight neonates (those with birth weight of less than 1000 g).[46,59] One propensity analysis suggested that for every eight extremely low-birth-weight infants for whom candidiasis could be prevented, one life could be saved.[129] Routine fluconazole prophylaxis had not been recommended because of the lack of data about safety and resistance and because it had not been evaluated in large prospective studies.[3,25,59,64,76] However, a recent multicenter, prospective, randomized, double-blind, placebo-controlled trial demonstrated that fluconazole prophylaxis reduces incidence of *Candida* colonization and invasive disease in very-low-birth-weight infants (those with birth weight below 1500 g).[59]

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CHAPTER 213

Coccidioidomycosis

Ziad M. Shehab

Coccidioidomycosis is an infection caused by a dimorphic fungus of the genus *Coccidioides*. The primary pulmonary infection produced by this organism usually is self-limited, but disseminated and fatal disease may occur. In the United States, coccidioidomycosis is an endemic disease of the southwestern states and results in 150,000 infections per year.[68] In other parts of the United States, it also is seen in individuals who have traveled or lived in the endemic areas of the Southwest or Mexico.[26,33,50] The disease is endemic in other areas of the Western Hemisphere, most notably in northern Mexico and in certain countries in South and Central America.[111] With population growth in the endemic areas of the Southwest and increased population mobility, clinicians in nonendemic parts of the country likely will encounter this disease, particularly in its more severe or disseminated forms.

Coccidioidal granuloma, a form of disseminated coccidioidomycosis, was described first by Posadas in 1892 in Argentina.[48] The disease initially was thought to be a form of skin tumor. Rixford and Gilchrist associated it with what they thought was a protozoon resembling coccidia and named it *Coccidioides*. They divided it into two species, *Coccidioides immitis* and *Coccidioides pyogenes*. Ophuls and Moffitt, in 1900, were the first to attribute coccidioidal granuloma to the fungus *C. immitis*. Although earlier investigators had noted fungal growth on cultures from pathologic specimens, they dismissed these organisms as contaminants. Between 1900 and 1936, numerous reports of coccidioidal granuloma appeared in the medical literature, but the association between coccidioidal granuloma and acute pulmonary infection remained unrecognized. In 1936, Gifford and associates[72] and Dickson[53] were responsible for the concept that *C. immitis* could cause either a primary or a secondary type of illness. The primary form previously had been known in California as *San Joaquin fever* or *valley fever*.

In the period that followed, the epidemiology of the disease[134,138] and ecology of the organism[57,102] were studied carefully by many investigators, led by the efforts of Charles Smith. Many different forms of therapy were tried for the disseminated disease during this era, but none proved satisfactory.[60] In 1957, with the use of amphotericin B, effective antifungal therapy became available, and the prognosis associated with the disseminated form of the disease improved.

As with many pathogenic fungi, the life cycle of *C. immitis* demonstrates two distinct phases: a saprophytic, or vegetative, phase and a parasitic phase. In nature and on most laboratory media, the organism grows as a mycelium with branching, septate hyphae. After 5 to 7 days, the aerial mycelia show development of rectangular spores (arthrospores, arthroconidia) separated by empty nonviable cells (Fig. 213–1). At this stage, the hyphae

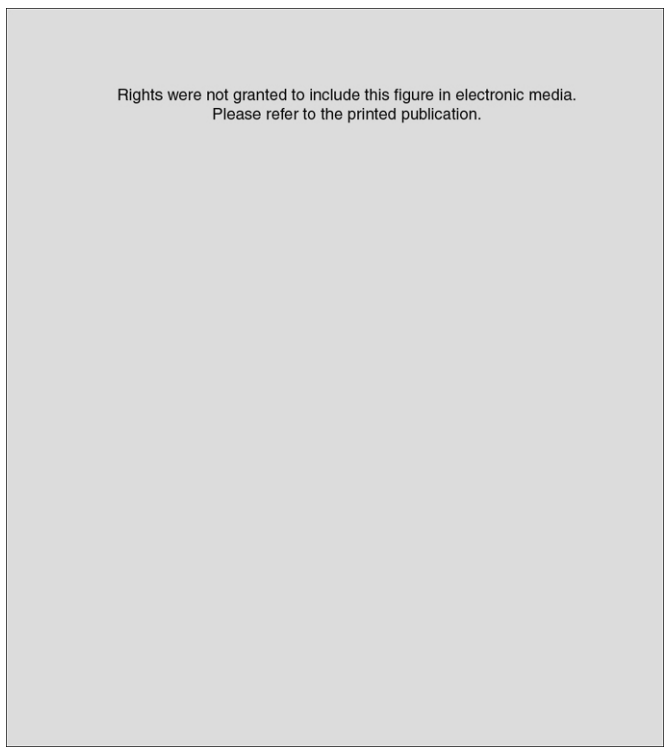

Figure 213–1 Mycelial form of *Coccidioides immitis*. Arthroconidia are separated by vacuolated cells. *(From Davis, B. D., Delbecco, R., Eisen, H. W., et al.: Microbiology: Including Immunology and Molecular Genetics, 3rd ed. Hagerstown, Harper & Row, 1980.)*

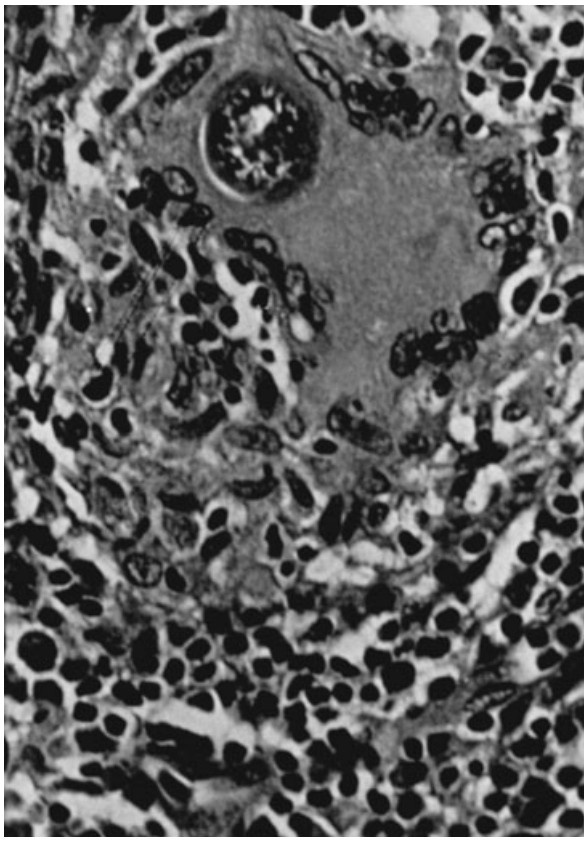

Figure 213–2 Coccidioidal granuloma showing a multinucleated giant cell containing a spherule filled with endospores. The adjacent field contains lymphocytes and plasma cells.

become fragile, and the arthroconidia, measuring 2 to 8 μm in diameter, easily become airborne. Because of their size, they can reach the alveolar spaces of the lung when they are inhaled.

On gaining access to the tissues of the mammalian host, the arthroconidia begin the parasitic phase of the life cycle. They enlarge and develop into spherules during the course of approximately 48 hours by undergoing internal segmentation containing endospores that are 2 to 5 μm in size. The spherules (Fig. 213–2) are round, double-walled structures measuring 20 to 100 μm in diameter. The endospores are released into the surrounding tissues by rupture of the spherule wall. They, in turn, may grow into mature spherules by maturation of the endospores in vivo to spherules, repeating the tissue phase of the life cycle of the organism. Alternatively, when the spherule ruptures, releasing the endospores into the environment, hyphal formation can occur and the cycle, thereby, is repeated in nature.[156]

C. immitis grows on laboratory media relatively easily, producing colonies that become visible in 3 to 4 days. They represent the mycelial phase of the fungus and appear as flat, smooth, and gray colonies, from which spicules may project in some, whereas others have the look of a velvety membrane. The colonies appear as tufts in the second week and then develop cobweb-like aerial hyphae. Pigmentation may be seen after 2 weeks and is observed best as a brownish undersurface. Variability in colony morphology can be significant. Of the two species of *Coccidioides* that are recognized, *C. immitis* is prevalent in California; *C. posadasii* accounts for isolates from Texas, Mexico, and South America. Both species can be found in Arizona.[62] No clinical differences have been identified between the two species. Identification is accomplished by inoculation of the mycelia into laboratory animals and demonstration of the conversion of the fungus to the spherule phase. However, reliable techniques showing in vitro conversion to the spherule stage, demonstration of specific exoantigens, or identification of the organism by genetic probes have been developed and obviate the need for animal inoculation.[129]

EPIDEMIOLOGY

Coccidioidomycosis is endemic in the Western Hemisphere between 40 degrees of latitude north and south. In the United States, these areas lie in the southwestern states, especially in Arizona, California, western Texas, and southern New Mexico. The areas in which coccidioidomycosis is prevalent generally correspond to the lower Sonoran life zone.[111] This life zone is characterized by an arid to semiarid climate, with hot summers and relatively short winters with limited rainfall and few freezes. Most of the fungal growth occurs during the rainy season in alkaline soil and at low altitude, conditions that favor the growth of the creosote bush that often coexists with *C. immitis*.[57] The fungus is found in the soil to a depth of 20 cm, especially in the walls of rodent burrows, and is isolated infrequently from surface soil during hot, dry weather[57] or in soils rich with other organisms.[102] Environmental conditions in endemic regions apparently inhibit growth of competitive organisms.[57] A variety of animals, including rodents, cattle, sheep, and dogs, have been shown to develop naturally acquired infection. Arthroconidia become airborne during wind storms or during disruption of soil by construction work and farming.[32,63] Prolonged droughts followed by heavy rains also have resulted in an increase in the number of cases, such as occurred in California in 1992 and 1993.[31] Archeologic excavations and digging by children in soil containing the organism have been reported to result in local outbreaks.[34,124,158] In addition, transmission has been reported to occur by contaminated fomites, such as dusty clothing and farm products.[1,127] Because of the ease by which arthroconidia become airborne, the organism is dangerous to laboratory personnel. Epidemics have resulted from inadvertent opening of a single culture plate.[90]

Primary coccidioidal infection occurs most frequently in summer and fall, following the rainy seasons. Arthroconidia are more likely to become dispersed because of the dry weather after promotion of greater hyphal growth by the wet environment.[57,66,94,111,134,135] Variations in seasonal infection rates also are explained partially by the occurrence of dust storms[111,135] and earthquake activity.[32] Significant increases in the number of cases are being seen in the Southwest, with high incidence periods in the winter being associated with environmental and climactic changes.[66,114]

Susceptibility to primary coccidioidal infection is unaffected by age, sex, or racial background.[60] Estimates of infection rates in endemic areas, which are based on the risk for development of infection in children, have been declining. For example, infection rates measured by skin test reactivity to skin test antigens have declined from approximately 10 percent in 1937-1939 to 2 percent in 1959 and to less than 1 percent thereafter in kindergarten and first-grade students who had lived all of their 5 to 7 years in Kern County, California.[97] Similarly, the annual risk has been estimated to be 2 and 4 percent in college students in Tucson, Arizona.[94] Incidence rates are higher in older male children, in rural areas, during wind storms, and for those with occupational exposure.[123,125,134] In contrast with susceptibility to primary infection, the frequency of dissemination varies considerably, being higher in infants,[37,38,87,132] Filipinos, Hispanics, and blacks.[63,72,83] Filipinos have 170 times and blacks 10 times the incidence of dissemination in comparison with non-Hispanic whites. However, some of this increased risk may reflect environmental exposure to high inocula of *C. immitis*.[6] Immunosuppressed hosts also are at increased risk for development of disseminated infection. In particular, patients with active coccidi-

oidomycosis and human immunodeficiency virus (HIV) infection are at greatly increased risk for development of severe pulmonary disease and disseminated infection.[3,160]

The tissue phase of the organism is the spherule, which is not infectious. Although mycelial growth can be found in cavities when they are carefully searched, no evidence exists of person-to-person spread of *C. immitis*,[78,156] except in special situations in which the fungus is allowed to revert to its airborne form, such as growth from wound drainage on a plaster cast.[56] Patients with coccidioidomycosis do not require isolation, even when draining wounds are present. In such circumstances, dressings should be changed frequently to prevent growth of the fungus and the formation of arthroconidia.

Once limited to the lower Sonoran life zone, the disease is seen throughout the country because of the ease and frequency of travel, increased populations in endemic areas, and reactivation of infection in immunocompromised hosts, including transplant recipients, patients with acquired immunodeficiency syndrome (AIDS), and recipients of tumor necrosis factor–α inhibitors. In these patients, delays in establishing the diagnosis can be fatal, and rapid recognition of the diagnosis and prompt institution of appropriate therapy mandate that a timely and accurate diagnosis be established.

PATHOGENESIS AND PATHOLOGY

Acquisition of *Coccidioides* spp. infection usually is through the respiratory tract. Researchers surmise that most human infections may result from exposure to only a single spore.[65] Rarely, direct cutaneous inoculation may occur by puncture of the skin with a contaminated object.[52,82,107] Growth of the organism stimulates an intense inflammatory response, and, in most patients, the infection remains localized to the lung and hilar nodes. In a minority of patients, clinically significant extrapulmonary dissemination occurs by way of lymphatics or the bloodstream.

The initial inflammatory response of acute pulmonary coccidioidomycosis predominantly is a polymorphonuclear leukocyte reaction, possibly related to a chemotactic effect of endospores or complement activation from other *C. immitis* antigens. Tissue necrosis, spherules, and a few mononuclear cells are present at this stage of the disease, but epithelial giant cells are infrequent findings. This bronchopneumonic process can occur in any lobe of the lung. The inflammatory response, however, is ineffective because neutrophils do not show any killing of coccidioidal forms at any stage of growth of the organism. Although this response may slow the progress of the infection temporarily, it ultimately cannot arrest the disease process.[65] Killing has been demonstrated with natural killer cells and mononuclear leukocytes. Numerous studies have demonstrated the importance of T cells in controlling the infection, as evidenced by delayed cutaneous hypersensitivity, peripheral lymphocyte transformation, and production of interferon-γ.[65] Dermal hypersensitivity correlates well with other measures of peripheral blood lymphocyte responsiveness, such as lymphocyte transformation and cytokine production.[4] As the disease progresses, cell-mediated immune defenses become defective, possibly as a result of antigen overload, suppressor cells, immune complexes, or fungal immunosuppressive substances, resulting in an ineffective response by type 2 helper cells.[42,141]

Disseminated coccidioidomycosis resembles progressive tuberculosis of childhood by its spread, which usually occurs within weeks or months after the initial infection develops. However, endogenous reactivation of treated primary disease may occur, particularly among individuals receiving immunosuppressive therapy[21] or among those with HIV infection.[3,66] Extrapulmonary spread may occur anywhere in the body, but lesions are found most frequently in bone, soft tissue, lymph nodes, and meninges. Bone lesions resemble chronic osteomyelitis (Fig. 213–3). Infection of the brain substance is a rare event, but meningitis occurs commonly and frequently localizes in the basilar area.[22,83]

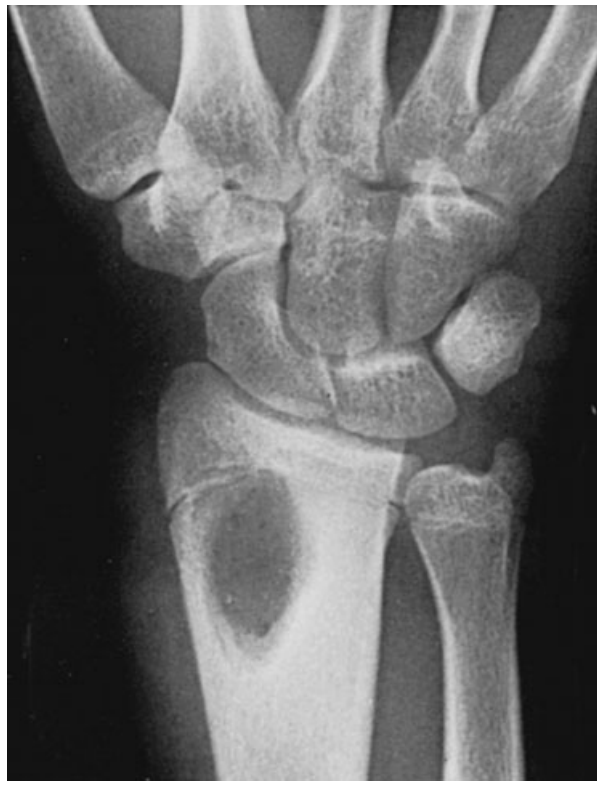

Figure 213–3 Osteomyelitis in a 14-year-old boy with disseminated coccidioidomycosis. An osteolytic lesion involves the distal radius.

The pathologic findings of fatal coccidioidomycosis have been reviewed extensively.[84] The tissue reaction in disseminated disease predominantly is granulomatous but can be accompanied by elements of acute inflammation. Typically, the granulomatous lesions contain abundant giant cells and histiocytes. Caseous necrosis is a common occurrence, and spherules usually can be identified lying freely and within macrophages (see Fig. 213–2). Fibrous tissue may surround areas of inflammation, but calcification is an infrequent occurrence. Autopsy studies in patients with coccidioidomycosis and AIDS show poor granulomatous responses and larger numbers of organisms in lung tissue than those seen in non-AIDS patients.[75]

Most cases of disseminated coccidioidal infection in the first months of life have been associated with heavy exposure to dust; these infants apparently acquire their infection by the respiratory route.[39,87] Nearly all the described infants in this age group have had severe disease,[37,38,87,144] but primary infection sometimes may go unrecognized.[38] Although women who develop coccidioidomycosis late in pregnancy may be at increased risk for development of disseminated disease,[151] with a few exceptions,[35,99] their infants are born free of infection.[39,40,133,149] In several patients with apparent perinatal transmission, the mothers had coccidioidal endometritis, and infected amniotic fluid was the most likely source of their infants' infections.[17,99,130,140]

Dissemination of coccidioidomycosis occurs more frequently in immunosuppressed patients who have resided in an endemic area,[66,103] but immunologic abnormalities have not been detected in other groups, such as Filipinos, who also are at high risk for development of disseminated disease. Once dissemination occurs, the patient's cell-mediated immunity frequently is

impaired,[29,42,43,139] especially if extensive infection is present.[14,137] Dissemination can occur in patients who have a selective lack of response to coccidioidal antigens, as evidenced by negative skin test results to coccidioidin or spherulin, and who do not have evidence of generalized anergy. In vitro measurements of lymphocyte transformation to phytohemagglutinin or to specific cell wall antigens of *C. immitis* also are depressed.[14,42] In contrast, patients with disseminated disease with positive skin test results usually have normal in vitro lymphocyte responses that are similar to those found in healthy persons who have recovered from primary infection.[43,109,162] Furthermore, patients who have recovered from severe disseminated disease may show return of specific and nonspecific cell-mediated immunity.[14,29,60,139] Infected children demonstrate immunologic findings similar to those described in adults, but they have not been studied as well.

CLINICAL MANIFESTATIONS

PRIMARY INFECTION

The clinical features of acute coccidioidomycosis in children are thought to be similar to the manifestations observed in adults (Fig. 213–4).[136] Studies in Air Force personnel indicate that infection is subclinical or indistinguishable from a mild upper respiratory tract infection in 60 percent of nonimmunocompromised hosts.[136,139] Twenty-five percent experience an influenza-like illness lasting 1 to 2 days, whereas the remainder have more severe lower respiratory illnesses including lobar pneumonia, pleural effusions, and, occasionally, pericarditis.[60,65,116] With intensive exposure such as occurs during military field exercises, the attack rate may be high and a large proportion of infections may be symptomatic.[44] The disease may mimic bacterial pneumonia and sepsis.[10,101] When serologic studies are used routinely in the evaluation of community-acquired pneumonia in endemic areas, almost 30 percent of such pneumonias in adults are the result of coccidioidal infection.[148] The most common form of symptomatic infection is a subacute, self-limited pulmonary illness. Some patients, however, will experience more complicated pulmonary infections or even extrapulmonary disease; these patients are those who are most likely to be seen by physicians outside the endemic areas and may require extensive work-ups, and delays in establishing the diagnosis may occur if coccidioidomycosis is not considered.[26,50] Severe disease is more likely to develop in individuals with diabetes or those with a recent history of cigarette smoking.[126]

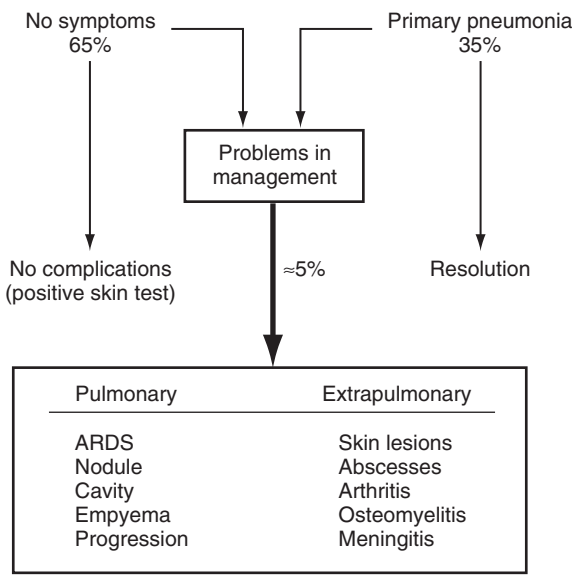

Figure 213–4 Clinical spectrum of coccidioidomycosis. ARDS, adult respiratory distress syndrome. *(From Galgiani, J. N.: Coccidioidomycosis. Reprinted by permission of the Western Journal of Medicine* 159*:153-171, 1993.)*

The usual incubation period is 10 to 16 days, but it may range from less than a week to almost a month.[60] In young adults, fatigue (77%), cough (64%), chest pain (53%), and dyspnea (17%) are the most common symptoms. Fever was present in 46 percent, with arthralgias, myalgias, and headaches being reported in 22 percent each.[143] In infants, stridor rarely may be present as a result of primary infection of subglottic tissue.[71,79] Chest pain sometimes is severe and usually is pleuritic.[143] It may be followed by vague chest pain that persists for several months.[55,60,65]

Transient rashes probably occur more frequently in children than in adults and are observed in slightly more than half of symptomatic children.[82,123] Two types of rashes are seen, based on time of presentation and immune status. Those that present early in the illness are erythematous and maculopapular.[60,82,154,158] They vary in severity, ranging from diffuse eruptions resembling measles or scarlet fever to more common and less extensive processes localized to the lower trunk and thighs.[60,82] In a few patients, urticarial lesions may be present.[154] Erythema nodosum and erythema multiforme–like lesions appear somewhat later in the course of illness, usually after the third day to as late as 3 weeks.[52,60,164] Erythema nodosum correlates with the development of cell-mediated immunity and is associated with a low incidence of dissemination.[13,55,60,134] This symptom complex may occur in other diseases, including tuberculosis, histoplasmosis, and group A beta-hemolytic streptococcal infections and inflammatory bowel processes. However, its presence in a child residing in some endemic areas, such as the San Joaquin Valley, nearly always signifies acute coccidioidal infection. For unknown reasons, erythema nodosum occurs less frequently in infected children inhabiting other endemic areas, such as Tucson.[123] The condition is self-limited, usually resolving within a few days to several weeks. Erythema nodosum occurs two to four times more frequently in adult women than in adult men, but this difference is not apparent in childhood infection.[134] The rash is an infrequent manifestation in blacks, Hispanics, and Filipinos. Erythema multiforme is seen more commonly in children.

Acute arthritis or arthralgia is an additional hypersensitivity manifestation, and occasionally one or both accompany primary coccidioidal infection. Because these findings usually are transient and do not signify dissemination, the presumption is that spherules are not present in the involved joints at this stage of the disease.[55]

The radiographic appearance of primary coccidioidomycosis is not specific.[15,36,77,123] Bronchopneumonic infiltrates are the most frequent finding and often are associated with hilar lymphadenopathy. Segmental or lobar consolidation and nodular or patchy pulmonary infiltrates also can occur. Small pleural effusions or pleuropericardial reactions also occur frequently (Fig. 213–5) and usually are sterile.[100] These radiographic findings resolve in 90 to 95 percent of symptomatic cases, albeit slowly in some, and usually do not necessitate specific therapy.

In a minority of patients, cavitation, nodule formation, bronchiectasis, or calcification may develop at the site of the pulmonic infiltrate.[19,27] The cavities usually are thin walled and asymptomatic and rarely require surgical therapy. Many resolve spontaneously[95] but result, nonetheless, in the need for prolonged care and convalescence. Rarely, the cavities lead to the development of an empyema or a bronchopleural fistula, which is more likely to occur in immunosuppressed patients or in diabetics.[88] Nodules and thin-walled cavities develop in 5 percent of patients with coccidioidal pneumonia.[141] These lesions are well circumscribed,

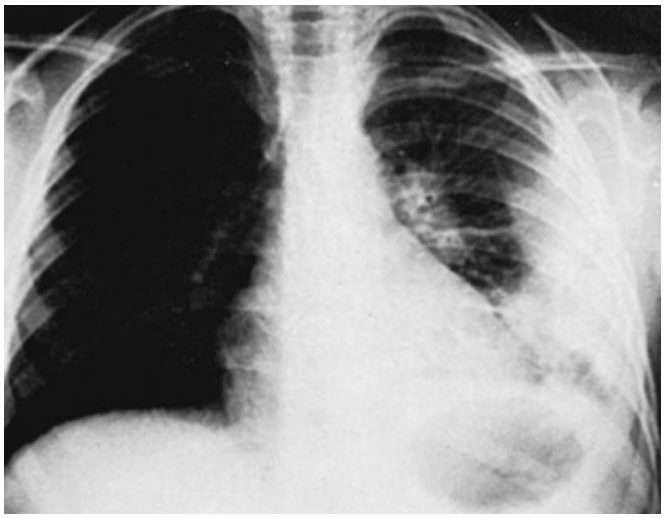

Figure 213–5 Chest radiograph of a patient with acute pulmonary coccidioidomycosis showing pulmonary infiltrates, extensive pleural fluid, and enlargement of the left hilum.

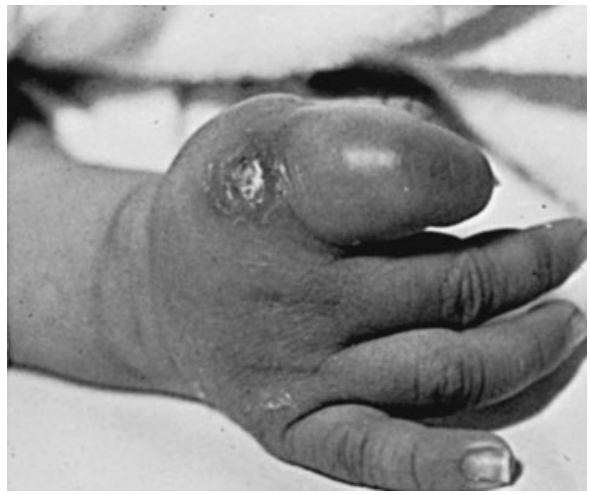

Figure 213–6 Swelling and chronic draining sinus over the proximal phalanx of the index finger. The infant has disseminated coccidioidomycosis with involvement of the underlying bone.

typically single, and less than 6 cm in diameter. In most patients, these lesions are asymptomatic. In adults, these nodules may be confused with carcinoma of the lung, thus requiring excision or diagnostic fine-needle aspiration.[54] A miliary pattern, indicating hematogenous or lymphatic spread, sometimes can be appreciated in immunocompromised hosts and, rarely, in immunocompetent hosts as well.[12] On occasion, endotracheal or endobronchial lesions can be demonstrated by endoscopy.[117]

In neonates, the constellation of radiographic findings of focal consolidation with diffuse nodular densities associated with nonspecific symptoms and minimal clinical evidence of respiratory tract infection has been described but is not specific for coccidioidomycosis at this age.[17,36] Chorioretinitis as a manifestation of systemic disease also has been reported.[73]

In contrast with primary pulmonary infection, primary cutaneous coccidioidomycosis is an uncommon occurrence.[52,107] Most cases have been reported in laboratory workers,[90] but this form of infection also is recognized in children.[107] The lesion of primary cutaneous disease resembles a chancre and is associated with regional lymphadenitis. In adults, usually only mild constitutional symptoms are present, and the process spontaneously resolves within 2 to 3 months. However, in children, progressive and prolonged infection may occur commonly; antifungal therapy may be necessary.[107,157]

The manifestations of primary coccidioidal disease thus are varied, and no constellation of symptoms and signs is specific enough to establish a diagnosis, rendering the use of specific laboratory tests a requirement.

DISSEMINATED COCCIDIOIDOMYCOSIS

Except in very young children, dissemination appears to occur less frequently in pediatric patients than in adults (0.5%), although this view has been questioned.[92,132] Spread of infection usually becomes apparent within a few weeks to a few months after the initial infection occurs and is heralded by persistent fever, toxicity, and insidious development of lesions outside the chest. An occasional patient develops disseminated disease after having an asymptomatic primary infection.[47,59,92] Disseminated disease rarely can be manifested with peritonitis, pericarditis, empyema, laryngeal lesions, or neck or gluteal abscesses.[23,46]

Skin Disease

The most common cutaneous manifestation of disseminated coccidioidomycosis is verrucous granuloma that characteristically is located at the nasolabial fold.[60,82] These lesions may heal or may continue to progress. The lesions mimic those caused by other fungi, tuberculosis, actinomycetes, and syphilis. The subcutaneous tissues also may be involved and result in large "cold" abscesses and the development of sinus tracks leading to chronic ulcers.[55]

Bone and Joint Disease

Involvement of bone results in chronic osteomyelitis,[20,49,89] which may drain into soft tissue (Fig. 213–6) and form fistulas to the overlying skin.[47] The bones most frequently infected are the vertebrae, tibia, metatarsals, skull, and metacarpals. The lesions are present in a single bone in 60 percent of the cases; two bones are involved in 20 percent, three in another 10 percent, and four or more bones in the remainder. On radiographic examination, the lesions typically are lytic (see Fig. 213–3). Vertebral osteomyelitis is characterized by involvement of all parts of the vertebra with relative sparing of the disk.[55] Spread of infection locally leading to meningitis is a serious concern with vertebral osteomyelitis.

Meningitis

Meningitis may be the sole site of extrapulmonary disease, particularly in whites, but it also occurs as part of widespread dissemination.[22,81,91,131] It may present acutely with the primary infection or appear up to 6 months later. The most common symptom is headache. Altered mental status, sluggishness, ataxia, and vomiting are other common symptoms. The child often lacks signs of meningeal irritation and sometimes presents with signs of focal neurologic deficits. The pathologic process is that of granulomatous and suppurative basilar meningitis, with frequent presence of parenchymal involvement with granulomata and abscesses of the spinal cord and brain.[58,106] Vasculitic complications with infarction and stroke-like findings may be abrupt in onset.[58,155] Examination of the cerebrospinal fluid (CSF) usually reveals moderate pleocytosis (few to more than 10,000) with mononuclear cell predominance, low CSF glucose concentration, and elevated protein level.[30,81,131] These findings are typical for

CSF sampled from the lumbosacral space. However, considerable variation occurs in the cell count, chemistry, and antibody content of fluid obtained from the ventricles, cisterna magna, or lumbosacral space[74,81,131]; the lumbosacral space exhibits the more severe changes, that is, lower glucose concentration, higher protein level, higher cell count, and higher CSF coccidioidal complement-fixation titer. Eosinophilic pleocytosis in the CSF is suggestive of coccidioidal meningitis and should prompt performance of an appropriate diagnostic work-up; it is of no prognostic value.[121] The CSF culture often is negative for the fungus, whereas CSF antibody often is detectable in 75 percent of specimens.[30] The diagnosis is confirmed by a positive CSF culture, serology, or both. The diagnosis is supported by a positive serum serologic result or culture of *Coccidioides* spp. from a nonpulmonary site. Before amphotericin B became available, coccidioidal meningitis was uniformly fatal, and the average length of survival in children was 5.5 months. Death with coccidioidal meningitis now is a relatively rare event. However, adults with neuroimaging abnormalities, especially hydrocephalus with or without cerebral infarction, have a high mortality rate.[11]

Genital Coccidioidomycosis

Like tuberculosis, coccidioidomycosis can involve the pelvic organs. Early studies had indicated an alarming risk of dissemination and death in women who acquired coccidioidomycosis, especially during the third trimester of pregnancy.[45] In a population-based study, Wack and colleagues[151] showed that coccidioidomycosis occurs infrequently during pregnancy but remains associated with serious complications when the disease develops in the third trimester or soon after delivery. Women with erythema nodosum are much less likely to experience disseminated disease than are pregnant women without erythema nodosum.[13] Wide variations are reported in the rates of dissemination and fatality among pregnant women, although all studies agree that the rate of dissemination in pregnant women is higher than that in the general population.[25,44] Coccidioiduria may be a silent manifestation of disseminated disease.[115]

Coccidioidomycosis in the Immunocompromised Host

Conditions that result in immune suppression, particularly T-lymphocyte dysfunction, such as those present in patients with lymphoma or bone marrow or solid organ transplantation, predispose the individual to more fulminant forms of coccidioidomycosis.[21,124] Infections may be the result of primary infection or of reactivation. Solid organ transplantation recipients are at the highest risk in the first year after undergoing transplantation, especially during their primary infection. Dissemination also can occur late as a result of reactivation of an old infection. Control of coccidioidal infection depends primarily on cell-mediated immunity. The risk is increased by a prior history of coccidioidomycosis or a positive serologic reaction just before transplantation.[21] Dissemination occurs in as many as 75 percent of such patients, and multiple sites of infection are common findings. An increased risk of acquiring symptomatic disease and disseminated coccidioidomycosis was described in patients receiving therapy with tumor necrosis factor–α antagonists.[16] Patients infected with HIV are at a greatly increased risk for development of severe forms of pulmonary coccidioidomycosis[3] and extrapulmonary diseases, including meningitis.[160] In HIV-infected patients, active coccidioidomycosis may represent recrudescence of old infection or may be the result of primary infection.[3] A CD4 count of less than 250/μL is associated significantly with the development of active disease. In HIV-infected patients who are relatively immunocompetent, the disease often is manifested as a community-acquired pneumonia similar to that of HIV-uninfected individuals. In HIV-infected patients, the clinical manifestations vary, ranging from minimal systemic symptoms without a pulmonary focus to severe cough and dyspnea associated with diffuse pulmonary disease and a radiographic pattern showing a discretely nodular appearance resembling *Pneumocystis* in those with CD4 counts of less than 250/μL. The severity of presentation is correlated inversely with CD4 counts. Even with appropriate antifungal therapy, the prognosis of HIV-infected patients with depressed CD4 counts who develop diffuse pulmonary coccidioidomycosis is poor, with a mortality rate of 70 percent.[61,67] HIV-infected patients also may present with evidence of extrapulmonary dissemination, typically meningitis or lymph node or cutaneous involvement. An important note is that many HIV-infected patients who have positive serologic results without evidence of active disease go on to develop active coccidioidomycosis during the course of time[9] and may be candidates for therapy.

DIAGNOSIS

In endemic areas where an awareness of the disease exists, the diagnosis of coccidioidomycosis usually is established readily by obtaining appropriate laboratory studies. However, even in these areas, coccidioidal pneumonia may be more prevalent than previously thought and may be underdiagnosed.[148] In nonendemic areas, the diagnosis is not considered unless a travel history is obtained. Cases have occurred even after brief exposure in an endemic area. Primary pulmonary coccidioidomycosis resembles other lower respiratory illnesses, including those caused by viruses, bacteria, mycoplasma, *Mycobacterium tuberculosis*, and other fungi (e.g., *Histoplasma*).

The hematologic findings in primary coccidioidal infection consist of elevation of the erythrocyte sedimentation rate, leukocytosis, and, frequently, eosinophilia.[60] Marked eosinophilia may be a clue that dissemination has occurred.[80] Specific diagnosis usually is based on the results of serologic reactions, cultures, and sputum examination.

CULTURE AND IDENTIFICATION OF THE FUNGUS

The organism is detected readily by direct examination and culture from purulent material. The yield from other sources, such as pleural fluid, blood, and gastric aspirates, is somewhat lower. Only approximately a third of CSF samples are culture positive, and direct examination of the CSF almost always yields negative results.[82,143] Detection of the fungus from the blood is an uncommon occurrence and is associated with severe forms of disseminated disease.[5]

In severe pulmonary or disseminated disease, microscopic examination of bronchopulmonary lavage specimens, exudates, or biopsy specimens is diagnostic if typical spherules containing endospores are seen. Hematoxylin and eosin–stained sections can be used to demonstrate spherules, but they are used mainly to show the inflammatory process. The periodic acid–Schiff stain is useful for demonstrating the spherule contents (Fig. 213–7), whereas methenamine silver stains highlight the wall of the spherule. Potassium hydroxide stains are useful but lack sensitivity and specificity; calcofluor white fluorescent stains are more sensitive but require experienced technologists.[129] Cytologic examination of sputa is more sensitive than are potassium hydroxide stains.[152] The spherules do not pick up the Gram stain.

In HIV-infected patients who present with pulmonary infiltrates, coccidioidal spherules may be identified in bronchopulmonary lavage specimens by the same methenamine silver stain used to identify *Pneumocystis*. Indeed, in areas endemic for coccidioidomycosis, the possibility of concomitant pulmonary infections with both *Coccidioides* and *Pneumocystis* or other pathogens should be kept in mind in this patient group.[104] Cytologic exami-

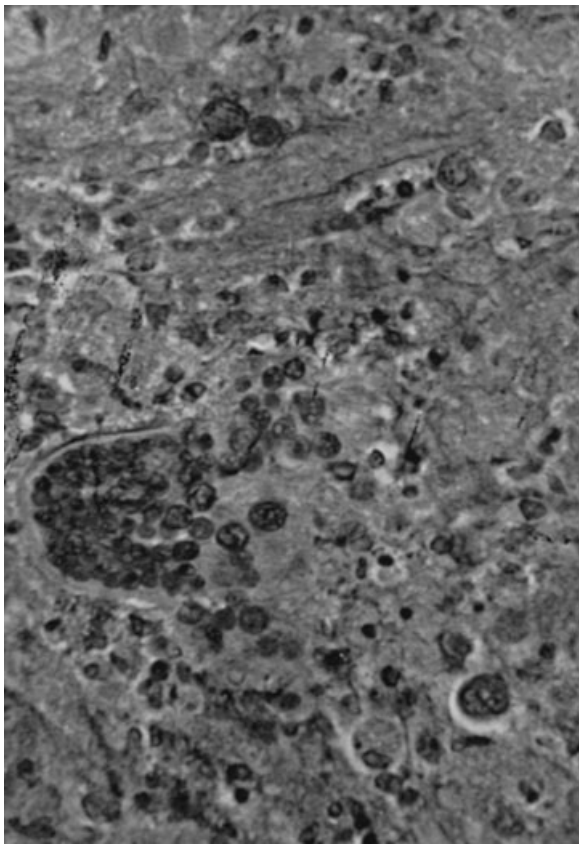

Figure 213–7 A spherule that recently has ruptured and is in the process of releasing endospores.

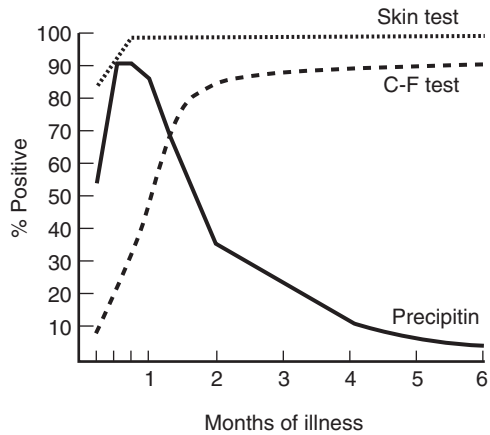

Figure 213–8 Immunologic reactions in symptomatic primary coccidioidomycosis, relating time of appearance and duration to the frequency of positive reactions. *(Based on Smith, C. E., Beard, R. R., Rosenberger, H. G., et al.: Effect of season and dust control on coccidioidomycosis. J. A. M. A.* 132*:833-838, 1946.)*

nation of bronchial wash or bronchoalveolar fluid is diagnostic in only approximately a third of persons with or without HIV infection and is less sensitive than culture.[54]

With the rare exception of persons with primary cutaneous infection, the finding of spherules in tissues outside the thoracic cavity is evidence that the patient has disseminated disease. For this reason, biopsy may be useful for establishing a diagnosis, particularly in patients with borderline or low complement-fixation antibody titers. Cultures of *Coccidioides* spp. from body fluids, sputum, or exudates can be performed on most laboratory media. If coccidioidomycosis is suspected, the use of mycologic media containing cycloheximide can be helpful. The handling of the cultures is hazardous and requires special biosafety precautions.[90] *Coccidioides* spp. are designated Select Agents by the Centers for Disease Control and Prevention, requiring handling in secure and contained environments. *Coccidioides* spp. grow on routine bacterial, fungal, and *Legionella* media. Once a nonpigmented mold is grown, confirmation is achieved best by use of genus-specific genetic probes that allow rapid confirmation, usually within hours.[110] The probe does not differentiate between *C. immitis* and *C. posadasii*, and it is not clinically relevant to differentiate the two species. Real-time polymerase chain reaction assays are being developed for rapid detection of *Coccidioides* spp. in clinical specimens but are not commercially available.[18]

SKIN TEST

Intradermal skin tests using lysates of either the mycelial phase (coccidioidin) or the spherule phase (spherulin) have been used to elicit delayed hypersensitivity. They are used as an epidemiologic tool and are no longer commercially available.

SEROLOGIC STUDIES

Figure 213–8 illustrates the serologic and skin test responses of symptomatic patients with primary coccidioidomycosis. The initial antibody response to coccidioidal infection predominantly is in the IgM fraction and is responsible for the positive precipitin test result that accompanies primary infection. These responses can be measured by tube precipitins, latex agglutination, enzyme immunoassay, or immunodiffusion methods.[113] Fifty percent of patients yield positive results in the first week, and 90 percent show precipitins within 2 to 3 weeks. Thereafter, antibody reversion occurs, and by 5 months, only 10 percent of patients with uncomplicated infection yield positive results.[138] Serum precipitins may persist in some patients with disseminated infection or may reappear with reactivation of infection. Precipitating IgM antibody detected by the tube precipitin assay or by immunodiffusion usually indicates acute infection.[138] In contrast with that seen with complement fixation, the magnitude of the precipitating antibody titer does not correlate with an increased risk of dissemination.[137] On occasion, IgM antibody has been detected in cord blood of newborns whose mothers had detectable antibody. These infants did not have any evidence of infection on follow-up.[113] Latex agglutination is sensitive, rapid, and easy to perform but frequently yields false-positive reactions,[113] and positive results should be confirmed by a second method. It also gives false-positive results in spinal fluids and in diluted sera and should not be used for these specimens.[112]

Serum IgG antibodies that fix complement usually appear later and last 6 to 8 months.[113] They are seen more commonly in more symptomatic infections and are detected in 50 to 90 percent by 3 months after the onset of symptoms. At least 90 percent of persons show a positive IgM or IgG response after having symptomatic primary infections. The main methods of detection of these antibodies are immunodiffusion, immunoassays, and complement fixation.[105,113,153,161] These assays correlate well, and immunodiffusion, particularly, is useful for detecting antibody in patients whose sera are anticomplementary.[113]

Immunodiffusion yields few false-positive results and can detect early asymptomatic infection in persons infected with HIV.[9] This method seems to be slightly more sensitive than is complement fixation in detecting early infections.[93]

More recently, enzyme immunoassay has become available. This assay compares well with more traditional assays (complement fixation, immunodiffusion, latex agglutination) and does not suffer from the subjectivity required to interpret the other

assays.[64,105] The IgG enzyme immunoassay has a sensitivity of 92 percent, whereas the IgM assay has a sensitivity of 77 percent. When both assays are combined, the sensitivity is excellent,[93,105] but the test suffers from false-positive results in patients with blastomycosis or suspected noncoccidioidal pulmonary illness.[93]

Antibodies measured by complement fixation are slower to develop and primarily are in the IgG fraction.[113] Usually, they are not detected in serum samples obtained during the first week of infection, and their rise may occur as late as 3 months after onset of symptoms.[86,138]

The magnitude of the complement-fixation antibody response correlates closely with the severity of infection and the likelihood of dissemination.[138] Although the antibody titer cannot be used as the sole indicator of dissemination, titers of 1:32 or greater in most laboratories are highly suggestive of extrapulmonary spread of infection[85,138]; 61 percent of these patients have a titer of at least 1:16, whereas 95 to 100 percent of those without dissemination have a titer lower than 1:16. The titer of complement-fixation antibody parallels disease activity and is useful in following the progress of patients with disseminated disease. A few patients, especially those with extensive pulmonary involvement and pleural effusion, develop high titers without other clinical evidence of dissemination.[100] Conversely, some patients with dissemination, particularly those with single lesions in skin, bone, or meninges, have lower titers.

Some patients with immunodeficiency disease, in particular HIV infection, may have extensive disease with low or undetectable antibody levels.[3,113] Bone marrow transplant recipients may yield no serologic or skin test response before or shortly after bone marrow transplantation, and aggressive attempts may be required to culture the fungus for diagnosis.[124] Some patients with HIV infection may have persistently positive serologic test results in the absence of any clinical disease. These patients are at high risk for development of active coccidioidomycosis and should be treated early.[9]

The complement-fixation antibody is detectable in the CSF of 70 percent of patients at the time of diagnosis of coccidioidal meningitis and eventually becomes detectable in almost all. The antibody measured is not a reflection of serum levels but rather is thought to indicate specific immunoglobulin biosynthesis by cells residing within or contiguous to the central nervous system. On occasion, patients with vertebral osteomyelitis or epidural abscesses yield low complement-fixation titers in the CSF without other evidence of meningeal involvement.

TREATMENT

PRIMARY INFECTION

In more than 90 percent of children, primary coccidioidomycosis is a self-limited illness, and antifungal therapy is not needed. In some children with severe primary disease, therapy with antifungals may be justified either to abbreviate the period of morbidity or to lessen the chance of dissemination in those with an elevated complement-fixation titer.

Which patients with primary uncomplicated pulmonary infections should receive therapy remains controversial. In general, evidence of severe infection or of concurrent risk factors such as immunosuppression should lead to the initiation of therapy. Severe infections usually are heralded in adults by weight loss of more than 10 percent, intense night sweats for a duration of longer than 3 weeks, infiltrates involving more than half of one lung or portions of both lungs, prominent or persistent hilar adenopathy, complement-fixation titer to *C. immitis* of greater than 1:16, failure to develop normal skin test hypersensitivity to coccidioidal antigens, inability to work, or symptoms that persist for longer than 2 months.[68] Diagnosis of primary coccidioidomycosis during the third trimester of pregnancy or immediately post partum should raise consideration of treatment. Amphotericin B is recommended for pregnant women because of the teratogenic potential of fluconazole and possibly of the other azoles.[2] Because of their high risk of dissemination, persons of African or Filipino ancestry should be considered for antifungal therapy, which typically consists of oral azoles for 3 to 6 months. When reticulonodular or miliary disease is seen, therapy usually is initiated with amphotericin B or high-dose fluconazole. Amphotericin B seems to yield a faster response and is replaced by an azole after a few weeks. The total course of therapy should last for at least 1 year and should be followed by oral azole therapy for secondary prophylaxis.[68]

Asymptomatic pulmonary cavities generally do not require therapy. Consideration should be given to excision of some cavities that persist for longer than 2 years, are progressively enlarging, or are adjacent to the pleural cavity. In patients with cavities that result in local discomfort, superinfection, or hemoptysis, therapy with oral azoles may alleviate the symptoms. Resection of localized cavities is an alternative to recurrent courses of antifungal therapy.[68] Pyopneumothorax results from rupture of a cavity into the pleural space. Its treatment is surgical with or without a course of antifungal therapy. Chronic fibrocavitary disease usually is treated with oral azoles for at least a year. In patients with inadequate responses, increasing the dose of azole, switching to a different azole, and administering amphotericin B are options. Surgical resection may be helpful in localized disease or when significant hemoptysis has occurred.[68]

DISSEMINATED DISEASE

Nonmeningeal Dissemination

Patients with disseminated disease who have clinically apparent lesions outside the thoracic cavity almost always should receive antifungal therapy, usually with an azole (fluconazole or itraconazole). In fulminant infections, amphotericin B is the agent of choice and usually yields faster responses.

Treatment is indicated for patients with extrapulmonary dissemination. The classic therapy consists of amphotericin B deoxycholate administered initially at a dose of 1 to 1.5 mg/kg/day and then tapered to 1 to 1.5 mg/kg/day three times a week. The usual total dose in adults is 1 to 2.5 g. In children, the maximal dose has not been defined because children better tolerate the toxicities of amphotericin B. Local instillation or irrigation of abscesses and cavities with amphotericin B or other antifungal agents may be beneficial but has not been studied systematically.[20,89] Amphotericin lipid-complexed preparations offer the possibility of administering higher doses with less toxicity and have largely replaced intravenous amphotericin B deoxycholate.

The total dose of amphotericin B depends on the patient's age and severity of disease. Most patients respond to between 15 and 45 mg/kg, with total dose and duration of therapy determined by the patient's response. The azoles have been tested extensively in this form of the disease. The main agents used for this purpose are itraconazole and fluconazole. In a randomized, double-blind study of progressive nonmeningeal coccidioidomycosis, a trend toward better efficacy with itraconazole, especially in the subgroup of patients with skeletal infections, was demonstrated.[70] This approach must be weighed in children against the need for more frequent drug administration and diet limitations related to the absorption of itraconazole. Ketoconazole has the advantage of being significantly less costly than are the other two azoles; it is associated, however, with more gastrointestinal discomfort and may impair testosterone secretion and adrenal steroid synthesis without causing an adrenal crisis.[24,119] Itraconazole and ketocon-

azole have important drug interactions, most notably with cyclosporine and diphenylhydantoin.[76] Anecdotal reports suggest that voriconazole and posaconazole can be effective therapies in patients refractory to fluconazole therapy.[7,41,120] A single case report demonstrates beneficial results with the concurrent use of interferon-γ.[96] In general, the azoles are used for a few months after resolution of symptoms or for at least a year of therapy. Caution should be exercised with the use of fluconazole in pregnancy, as cases of multiple congenital malformations somewhat reminiscent of the Antley-Bixler syndrome have been described.[2] The role of echinocandins is unclear at present.

Surgical therapy seldom is required in patients with primary disease, but therapeutic thoracentesis may be indicated rarely when pneumonitis is complicated by large pleural effusions. Surgery also is necessary when pericardial involvement is complicated by tamponade. Patients with persistent coccidioidal cavities (>1 year) may require lobectomy, especially if the lesions are symptomatic.[68] Coccidioidal lymphadenopathy occasionally requires excision. In general, bone and joint disease requires a combined surgical and medical approach. Coccidioidal arthritis typically responds poorly to systemic therapy, and synovectomy may aid in the control of symptoms.[20,118,159] Osteomyelitis optimally is dealt with by curettage and drainage of the involved bone, with the addition of amphotericin B therapy, itraconazole, or fluconazole.[20,28,49,70] Within endemic areas, screening of HIV-infected patients serologically by immunodiffusion or complement fixation may be useful to identify active cases early. Consideration should be given to offering antifungal prophylaxis to such persons. Treatment is recommended for all HIV-infected individuals with active coccidioidomycosis and CD4 counts of less than 250/μL. Once the infection is controlled, discontinuation of the therapy is reasonable if the CD4 count has risen to more than 250/μL, except in cases of meningitis, in which the therapy should be lifelong. Azoles have been used for prophylaxis in patients with solid organ transplants, both primary and secondary. The duration of treatment usually is 6 to 12 months or more, and the optimal duration is unknown. Because of their inhibition of the cytochrome P450 system, levels of cyclosporine and tacrolimus should be monitored.[21] Whether such prophylaxis is effective remains unclear, and the potential for drug resistance should be weighed. In a retrospective cohort study, clinical response, the lowest complement-fixation titer, the end-of-therapy complement-fixation titer, and a fourfold drop in complement-fixation titer were not predictive of relapse in nonmeningeal forms of disseminated coccidioidomycosis, whereas a peak complement-fixation titer of greater than 1:256 and a persistently negative coccidioidin skin test result were independently associated with increased risk.[108]

Meningeal Disease

Amphotericin therapy of coccidioidal meningitis has been associated with a marked improvement in survival rates. However, this treatment needs to include prolonged intrathecal therapy through lumbar, ventricular, or cisternal administration. This therapy is associated with significant side effects that include headache, nausea, vomiting, chills and fever, arachnoiditis, and, occasionally, paralysis, seizures, and coma.[81,142] The symptoms of arachnoiditis may be indistinguishable from those of microbiologic relapses.[81] Ketoconazole has been used with some success in meningitis, but it also is associated with a high relapse rate[51]; in high doses (1200 mg daily in adults, 15 to 23 mg/kg/day in children), therapy has been successful,[81,131] but such doses tend to be tolerated poorly in adults.[65]

Studies using 400 mg of fluconazole or itraconazole in the treatment of meningitis without concurrent use of amphotericin B show good results, with response rates of approximately 80 percent.[69,145-147] Many patients who do not show improvement with this regimen demonstrate clinical improvement when the dose is increased up to 1000 mg of fluconazole or 600 mg of itraconazole. Fluconazole currently is the preferred drug.[68] On the basis of limited data, the pediatric dose of fluconazole is approximately 12 mg/kg/day.[128] Patients generally respond clinically within 1 to 2 months, although abnormalities of the CSF may persist in some patients for a prolonged period. When therapy has been stopped, increases in CSF cell count or complement-fixation antibody titer may be the only indications of reactivation of meningeal disease and signify a need to intensify therapy.[55] Some investigators use intra-CSF administration of amphotericin in conjunction with azole therapy in the hope of achieving a faster response.[142]

Significant attention should be paid to CSF flow dynamics because almost all children with coccidioidal meningitis develop obstructive hydrocephalus, which requires ventriculoperitoneal shunting and rarely a second shunt to drain a "trapped" fourth ventricle.[81,131] Hence, the advantage of a drug such as fluconazole, which can be given systemically, obviates the problems of impaired CSF flow faced when intrathecal or intraventricular therapy is used.

Studies have indicated that the relapse rate after discontinuation of azole therapy is high. Of 14 persons treated for coccidioidal meningitis with azoles (ketoconazole, itraconazole, or fluconazole) for periods ranging from 8 to 101 months, 11 (78%) had a relapse of disseminated coccidioidomycosis within 0.5 to 30 months after therapy was stopped. There were no clinical or laboratory predictors of patients at risk for having a relapse. The data thus indicate that moderately prolonged azole therapy for coccidioidal meningitis suppresses but does not eradicate the infection, suggesting the need for prolonged if not lifelong therapy.[41,51,120] Voriconazole rarely has been used as a rescue agent after failure of fluconazole. The data on its use in meningitis remain scanty. Posaconazole has limited CSF penetration, and its role in central nervous system infections, if any, remains to be defined.

PROGNOSIS

Primary pulmonary coccidioidal infection usually is self-limited, with complete recovery occurring in 1 to 3 weeks. In a small proportion of cases, localized complications of the primary infection, such as pleural effusion or pericarditis, prolong the clinical course. Dissemination is a rare event in whites and is less likely to occur in patients with positive skin test results. Until the late 1950s, no effective therapy was available for the more severe forms of coccidioidomycosis, and the mortality rate of patients with disseminated disease was approximately 50 percent.[60] The mortality rate of patients with multiple sites of dissemination and those with coccidioidal meningitis approached 100 percent.[150] Presently, therapy with amphotericin B or azoles may cure some of these patients and in many others prolongs useful life. Certain forms of disseminated infection, such as joint involvement, particularly are resistant to systemic therapy and may persist for many years without other signs of dissemination. For patients with HIV infection, therapy for disseminated coccidioidomycosis usually is not curative, and lifelong suppressive therapy with intermittent doses of amphotericin B or oral azoles may be required to prevent relapses in those with CD4 counts of less than 250/μL.

PREVENTION

T_H1 responses have been associated with protection against human coccidioidomycosis. Persons recovering from infection with *Coccidioides* are immune to exogenous reinfection. The for-

malin-killed spherule vaccine, which had been immunogenic in mice and monkeys, proved not to be effective in humans. The 27K vaccine seems the most promising at present, but it is heterogeneous and its protective component has not been identified. Efforts are ongoing for the identification of a suitable protein or mixture of proteins for use in a vaccine.[42] Other efforts at prevention have been aimed at control of dust and eradication of the organisms from the soil.[135]

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CHAPTER 214

Paracoccidioidomycosis

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Paracoccidioidomycosis, formerly known as South American blastomycosis, usually is a progressive chronic disease that preferentially affects the lungs, skin, mucous membranes, adrenals, and reticuloendothelial system. Benign, self-limited infections also have been documented occasionally. Two types of clinical presentations are described: the acute-subacute (juvenile) and the chronic (adult) forms of the disease. Children generally present with the acute form, which affects predominantly the reticuloendothelial system organs. The mycosis is limited geographically to various Latin American countries.[18,24,29,80,103,105,109,142,187]

The disease was described originally by Lutz[103] in Brazil in 1908 and initially observed in children in 1911 by Montenegro.[107]

THE ORGANISM

Paracoccidioides brasiliensis, the etiologic agent of this disease, is a dimorphic fungus. The recognition of *P. brasiliensis* as an anamorph in the phylum Ascomycota, order Onygenales, family Onygenaceae has been accomplished by means of molecular tools.[20] The fungus has four or five DNA chromosomes showing molecular sizes ranging from 2 to 10 Mb.[70,134] *P. brasiliensis* genome size was estimated to be approximately 30 Mb.[43] A study of *P. brasiliensis* gene density suggests that this fungus contains between 7500 and 9000 genes, which is in agreement with the estimated gene number for ascomycete fungi genomes. Different groups have addressed the overall scenario of gene expression in *P. brasiliensis* yeast versus mycelium, or undergoing phase transition in vitro, making use of different molecular methods.[71,86,113,148] The existence of three different phylogenetic species (S1, PS2, and PS3) of *P. brasiliensis* has been demonstrated.[122]

It grows as a mold at room temperature (14° C to 24° C) and as yeast at 37° C. In the mycelial phase, growth is slow, requiring approximately 3 to 4 weeks; the colony is white to tan and compact, with short aerial mycelia. On microscopic examination, hyphae are fine (0.8 to 2.5 μm in diameter), septate, interlaced, and hyaline. Chlamydoconidia (20 to 50 μm in diameter), round to subspherical, terminal and intercalary, as well as arthroconidia may be observed.[103] Some isolates can produce aleurioconidia, uninucleate pear-shaped pedunculated or terminal structures, after long periods of incubation and under nutritional deprivation (media with reduced carbohydrate content).[29,173] The small size of these conidia (<5 μm) renders them compatible with alveolar deposition; furthermore, such conidia have been shown to be infectious when they are inhaled.[124]

The mycelial phase is not distinctive, and subcultures at 37° C are required for complete identification. At this temperature, *P. brasiliensis* produces soft, wrinkled, cream-colored colonies that develop in approximately 6 to 10 days. On microscopic examination, the most characteristic feature is the presence of multiple budding yeast cells; the parental cell produces various peripheral buds and acquires the appearance of a pilot's wheel. *P. brasiliensis* may possess alternative control mechanisms during cell growth to manage multiple budding and its multinucleate nature.[1] Single buds and short chains also are produced, but they are not diagnostic. Cells are variable in size (2 to 40 μm) and have a thick cell wall and internal vacuoles. This phase of growth is identical to the one observed in tissue and pathologic materials.[29,80,103,133,142,163] The organism does not readily take most bacterial or hematologic stains. *P. brasiliensis* is aerobic and, in its mycelial phase, grows well in the regular mycologic media to which antibiotics and cycloheximide have been added to reduce growth of bacteria and saprophytic fungi.[29,103] The isolation should be attempted by the use of modified Sabouraud agar, yeast extract agar plus antibiotics and cycloheximide, yeast extract–phosphate agar, brain-heart infusion agar plus blood and antibiotics, or tryptic soy agar plus fungus water extract and horse serum, incubated at room temperature.[102]

TRANSMISSION

Paracoccidioidomycosis is not a contagious disease.[29,80,103,142,172,216] A single case of placental involvement that did not result in fetal infection has been reported.[23] Epidemic outbreaks have not been reported, and only a few cases in family members have been documented,[103-106,142,216] which could be attributed to the long periods of mycosis latency between the moment of infection and the appearance of overt clinical manifestations.[109,171] Consequently, the memory of the activity contributing to the infection of the patient or of the accompanying individuals usually has been forgotten,[27] which prompted Borelli[27] to coin the name "reservarea" for those places in the endemic areas where the fungus has its habitat and humans acquire the infection. This concept differs from that of an endemic area, which implicates the place where the disease is diagnosed.

P. brasiliensis infection in animals as proved by culture or histologic examination has been shown consistently in armadillos, mammals that probably acquire the infection when they disturb the terrestrial habitat of the fungus,[33,140,173,196] and more recently in a dog.[176] Unfortunately, the microniche of *P. brasiliensis* has not been identified properly; soil has been incriminated, but the number of isolates from this source is small.[165] The inhibitory effect of pesticides on *P. brasiliensis* may suggest that they can interfere with attempts to isolate *P. brasiliensis* from soil.[151] Recently, the role played by deforestation in increasing both exposure and incidence rates has been documented in Brazil in Amerindians and in children.[48,75,176] Species-specific inner primers derived from rDNA regions (internal transcribed spacer [ITS], 5.8S gene) have been described, and the nested polymerase chain reaction (PCR) sensitivity was higher for the detection of *P. brasiliensis* in soil than in culture and animal inoculation. The fungus was detected in soil artificially seeded (positive soil control) and from environmental samples collected in an armadillo burrow.[204] This approach probably would improve the fungal microniche studies. The ecologic factors that prevail in the reserve area also have been characterized, with the following being significantly associated with the disease: altitude from 1000 to 1499 meters above sea level, rainfall from 2000 to 2999 mm, and presence of humid forests and of coffee and tobacco crops.[35] Environmental correlates (soil texture and precipitation) indicate that moisture availability plays an important role in paracoccidioidomycosis distribution.[197] The fertile regions of endemic areas have the highest incidence rates of paracoccidioidomycosis.[35,207]

Paracoccidioidomycosis was thought, and still is by some, to be acquired by trauma. However, clinical and experimental data indicate that infection is acquired most commonly by inhalation.[29,84,106,133,143]

EPIDEMIOLOGY

AGE

More than 70 percent of the patients are 30 years of age or older. Until recently, the number of childhood cases was shown to be only 2.1 percent in the first decade of life.[8,12,45,103,107,216] In 1976, Castro and del Negro[45] published one of the largest series of children with paracoccidioidomycosis diagnosed in a 30-year period. They found that among 1899 patients with this disease seen at the Hospital das Clinicas in São Paulo, Brazil, only 70 (3.6%) of the patients were 14 years of age or younger. However, in 1999 and in the same state, Blotta and colleagues[24] found that 5.6 percent of the paracoccidioidomycosis patients diagnosed between 1988 and 1996 were younger than 14 years, thus showing an increase over the l976 figure. In 1998, Rios-Gonçalves and associates[176] analyzed the records of 36 children who had been diagnosed in the state of Rio de Janeiro during a period of 25 years. In a similar period, Fonseca's group[75] described 13 children, 12.7 percent of all cases, who had been diagnosed in the Amazonian states of Pará and Tocantins, forest areas that have been colonized since the 1970s and where paracoccidioidomycosis formerly had been regarded as rare. In certain places, the infection rates measured by a positive skin test reaction also were high. In 1976, Pedrosa[156] conducted a skin testing survey among rural children (6 to 11 years of age) in a particular county of Rio de Janeiro where paracoccidioidomycosis had been diagnosed previously in a 3-year-old boy. He found that 34 percent of the children tested positive for paracoccidioidin, a figure indicative of early exposure to *P. brasiliensis*.

In 1996, Londero and colleagues[107] compiled the records of 106 children with the mycosis, including those formerly analyzed by Castro and del Negro.[45] This series, some compilations,[15,147,158] several individual reports, and a thesis[8] have revealed that the disease occurs more frequently in individuals 12 to 15 years of age than in younger children (Table 214–1). More than 250 reports of paracoccidioidomycosis in children aged 3 to 14 years, including those analyzed by Londero and colleagues,[107] have been found in a literature search.[174] The prevalence of infection with *P. brasiliensis* was evaluated in a cross-sectional study of 298 asymptomatic schoolchildren in the Brazilian Amazon region. The reactivity of children to two different *P. brasiliensis* antigen preparations, paracoccidioidin and a purified 43-kd glycoprotein (gp43), was 4.6 percent. However, in the group of individuals receiving paracoccidioidin who had a positive histoplasmin skin test result, the prevalence of exposure to *P. brasiliensis* was 44 percent, suggesting that the use of the paracoccidioidin prepara-

TABLE 214–1 Age and Gender Distribution in 354 Children with Paracoccidioidomycosis

	Age		
Gender*	3-9 yr	10-14 yr	Total by Gender (%)
Male	95	125	220
Female	63	71	134
Total by age	158 (41.8%)	196 (58.2%)	354 (100%)

**Male-to-female ratio = 1.6:1.*
Data from references 24, 34, 47, 49, 75, 131, 147, 156, 158, 177, 178.

tion may result in cross-reactions with other environmental fungi.[95]

The relative paucity of children with this mycosis in comparison with the figures reported in adults[18,24,45,103,105,106,117,191,211] may be explained, in part, by the effective control exerted by most hosts on the progression of the primary infection, by the long incubation period characteristic of the mycosis, and by the establishment of latent foci that may become apparent clinically many years after initial exposure.[11,29,46,79,84,106,109,171] Benard and associates[14] consider that the association with different age groups is related to the epidemiology of paracoccidioidomycosis because in the endemic areas, as previously shown, children become exposed to the fungus at an early age. In healthy children, appropriate defenses usually curtail development of the mycosis, but in infants of low socioeconomic conditions, the transition from infection to overt disease may be accelerated and aggravated by the mycosis itself, by malnutrition, or by other associated conditions.[14,15,24,45,75,176] In addition, unknown susceptibility factors, the intensity of exposure to the infectious source, the virulence of certain *P. brasiliensis* isolates, and other factors may well contribute to the development of the disease in children.[11,12,15,49,75,107,216]

GENDER

Adult men are afflicted with much greater frequency than are women (male-to-female ratio, 14:1).[18,24,29,75,103,106,142,216] In children, however, the disease affects boys and girls in approximately equal proportions.[18,24,45,42,107,147,176,216] In the review published by Londero and associates,[107] which included children up to 14 years of age, a slight predominance of male patients was noted in the older group (see Table 214–1). These findings suggest that the sex hormones, the hormone-dependent immunologic factors, or both play a role in determining the outcome of the host-parasite interaction.[4,5,11,29,168] Researchers have shown that in vitro estrogens inhibit *P. brasiliensis* mycelium to yeast transition, thereby hindering the progression of the infection.[4,29,168] Analysis of the mortality profile from paracoccidioidomycosis according to gender and age showed similar death totals for both genders in the groups younger than 20 years. The majority of deaths among male patients (97.14%) occurred in individuals older than 15 years, with only 2.37 percent among individuals younger than 15 years. Among female patients, most deaths from paracoccidioidomycosis (89.21%) also occurred in those older than 15 years, but 9.75 percent did occur in those younger than 15 years; that rate is nearly five times the percentage observed in males. These findings corroborate the literature, according to which the infection and disease occur equally between the genders during childhood, with a slight predominance of males with the disease, compared with an absolute predominance of males among adults with the chronic form of the mycosis.[52]

OCCUPATION AND RACE

Almost half of the reported cases in adults occur in individuals whose occupations require extensive exposure to the soil.* Adult residents of areas in which paracoccidioidomycosis is endemic generally develop disease that is less severe than that seen in immigrants or in people who migrate to highly endemic settings. These people often develop disseminated infection, much like the juvenile cases do.† The influence of genetic traits on the mycosis has not been elucidated,[61] but in patients with the unifocal chronic form of the disease, a mild clinical presentation in which lesions are restricted or localized, the HLA allele most commonly seen was DRB1*11, suggesting that the participation of HLA antigens may influence the outcome of the host-parasite interaction in paracoccidioidomycosis.[181]

GEOGRAPHIC DISTRIBUTION

Paracoccidioidomycosis is restricted to Latin America, from Mexico to Argentina; some countries within this area (Chile and some of the Caribbean Islands), however, are free of the disorder. In endemic countries, the disease's distribution is uneven, and most cases occur in individuals living in regions corresponding to the humid tropical and subtropical forests. The endemic area is centered in Brazil; 7000 of the about 10,000 patients described to date were natives of this country.[18,24,80,103,105,106,117,142,216] Although paracoccidioidomycosis has been reported in patients not living in endemic areas, prior residence in Latin America has been documented in every case.[96,109,171] In some of these patients, the interval between residence in the endemic area and clinical manifestations of the disease has been 10 to 40 years.[103,171] Within endemic areas, 10 to 50 percent of healthy adult individuals react to the intradermal administration of paracoccidioidin, suggesting previous contact or subclinical infection with *P. brasiliensis*.[25,29,33,48,95,107,156]

Molecular biology studies have indicated the existence of at least five different groups of *P. brasiliensis* strains and their close correspondence with the borders of the various endemic countries.[145] Recently, this fungus was described as having at least three distinct, previously unrecognized species: S1 (species 1 with 38 isolates), PS2 (phylogenetic species 2 with six isolates), and PS3 (phylogenetic species 3 with 21 isolates). Genealogies of four of the regions studied strongly supported the PS2 clade, composed of five Brazilian isolates and one Venezuelan isolate. The second clade, PS3, was composed solely of 21 Colombian isolates. The remaining 38 individuals formed S1. Two of the three lineages of *P. brasiliensis*, S1 and PS2, are sympatric across their range, suggesting barriers to gene flow other than geographic isolation.[122] Despite the apparently restricted ecologic niche, the habitat of the etiologic agent has not been determined precisely.[170,173]

PATHOGENESIS AND PATHOLOGY

The initial stages of the host-parasite interaction are unknown because of our inability to detect the precise moment when infection does occur.[79] In the past, traumatic implantation of the fungal propagules into skin and mucosa was thought to cause primary lesions, whereas other manifestations were regarded as secondary. However, through the study of many cases, including autopsies, what has become apparent is that pulmonary lesions are primary, with the infection taking place by inhalation of fungal propagules.[29,84] In experimental animals, the inhalatory route has been shown to give rise to disseminated disease.[124] In most cases, the initial pulmonary infection does not cause undue symptoms.[79,105] Once the conidia reach the terminal bronchi or the alveolar spaces, yeast transformation ensues.[124] In some cases, the fungus promptly disseminates by the lymphohematogenous route, producing distant subclinical and quiescent foci. Apparently, the local defenses are capable of controlling fungal spread, but some viable yeast cells may remain dormant in such foci in the pulmonary and mediastinal lymph nodes. Rarely, the initial pulmonary infection outweighs the host's immune defenses, causing an acute-subacute disease, the juvenile type, with predominant involvement of the reticuloendothelial system.[80] The disease in children falls into this category. Often, the quiescent foci remain so throughout life, as demonstrated by the high

*See references 24, 29, 80, 105, 106, 142, 172, 210.
†See references 11, 14, 46, 102, 105, 106, 107, 126, 142, 167, 216.

number of subclinical infections compared with the low disease incidence of this mycosis in the endemic regions.[77,216] Nonetheless, the most common clinical presentation of the disease is the chronic form, or adult-type disease, thought to result from fungal reactivation in these foci.[93] This reactivation frequently occurs when the patient has left the endemic area, as demonstrated by the cases reported outside Latin America, in countries where paracoccidioidomycosis is considered an imported disease.[46] Of note is that these cases may be manifested decades after leaving the endemic area, leading to difficult and delayed diagnosis.[91,123,198,212]

Thus, in the acute or subacute form, the initial lung infection may pass unnoticed but is followed by prompt dissemination to other organs and tissues.[11] In the chronic form, lung disease frequently occurs. Disease is restricted to the lungs in some of these patients, but in most, other organs also are afflicted. This spread may be caused by lymphohematogenous dissemination from reactivated pulmonary foci or directly from foci at virtually any organ. Actually, paracoccidioidomycosis, in the acute, subacute, or chronic forms, is more frequently a disseminated disease, even though clinical manifestations appear to be restricted to a sole organ.[80,126,142] However, determination of the degree of dissemination certainly is influenced by the availability of diagnostic procedures. After appropriate therapy, residual lesions, mostly fibrotic, become established.[79,126]

As already mentioned, the disease in children is acute or subacute and progressive (the juvenile form).[15,45,107] The time that elapses between infection and the onset of symptoms is not known precisely but has been estimated to be short, a few months.[79,80] In such patients, paracoccidioidomycosis is a severe, systemic disorder that involves preferentially the reticuloendothelial system to such an extent that it is the hallmark of the process.[8,15,107]

In adults, pulmonary lesions as seen on chest plain films may be defined as micronodular or miliary, nodular, infiltrative or interstitial, cavitary, fibrotic, and mixed types.[126,211] Emphysematous areas, pleural thickening, and enlarged hilar and mediastinal lymph nodes also can be observed. Right ventricular hypertrophy may be found in cases of long duration.[211] Lung involvement probably is secondary to a chronic lymphangitic process provoked by the fungus itself and to the host's response represented by formation of granulomata and fibrosis, which predominates at the perihilar region. This aspect correlates with the butterfly-like (perihilar) micronodular and interstitial infiltration observed on plain films.[210] Obstruction and reversal of lymphatic flow lead to the spread of the inflammatory process throughout the lungs. The pulmonary lesions as seen on computed tomographic imaging have been recently reassessed.[200]

In children and adolescents, the pulmonary component tends to pass unnoticed, and neither clinical examination nor radiologic studies reflect the real damage.[84] Only a minor proportion of the cases reported have had lung symptoms.[107] However, lack of notification may be more apparent than actual because the weight of the extrapulmonary lesions tends to minimize the less intense respiratory manifestations. Frequently, the mycosis is misdiagnosed, especially with tuberculosis and certain lymphomatous disorders.[107] Nonetheless, a careful search of pulmonary samples, including induced sputum, may reveal the characteristic multiple budding *P. brasiliensis* yeast cells.[107,169] Moreover, gallium scanning and computed tomography have allowed detection of incipient or discrete interstitial pulmonary lesions not revealed by plain radiographs.[34,81,218]

Lesions in the oropharyngeal and laryngeal mucosa occur frequently in the chronic form. They may be infiltrative, ulcerated, nodular, or vegetative and usually have a granulomatous aspect and may mimic squamous cell carcinoma.[21,126,130,186] The base of the ulcerated lesions usually is covered by small abscesses (the mulberry-like lesions) that probably represent fungal dissemination through the lymphatic system because they usually are accompanied by regional lymph node involvement.[44]

In children, such mucosal lesions are rather exceptional, but skin involvement occurs more commonly and tends to be multiple, in contrast with the adult chronic form.[118] In the adult chronic form, lesions are represented mostly by contiguous involvement of the periorificial mucosal lesions or draining lymph nodes. In children or young adults, and eventually in adults with the chronic severe disseminated disease, they represent hematogenous spread of the fungus. In this case, the lesions may appear as ulcerated or ulcerovegetative lesions, papules, or crust-covered ulcers and warts, usually at the same stage of development. Reports of septic shock caused by septicemia by *P. brasiliensis* show that the fungus can be blood borne.[126,209]

The reticuloendothelial system is the target organ in both children and young adults.[8] Almost every child with paracoccidioidomycosis exhibits involvement of the superficial or deep lymph node chains. Lymph nodes vary in size, number, consistency, and location; with time, they may liquefy, forming abscesses or fistulas. The spleen and liver frequently are involved in these same groups. Abdominal lymph node involvement also is a common occurrence in children.[8,107] Hypertrophied lymph nodes, usually generalized but particularly periaortic, around the hepatic hilum, and retroperitoneally, can be detected by ultrasonography, computed tomography, or magnetic resonance imaging.[119] Coalescent masses may become palpable and may result in disease caused by extrinsic compression of adjacent structures, such as jaundice by compression of the biliary duct,[25] pancreatitis,[25] or an intestinal obstruction.[120]

Splenic lesions are nodular or miliary. Gross hepatic lesions may not be apparent, but histopathologic examination regularly reveals fungal invasion of this organ.[8,15,107,133] In a series of fatal cases of paracoccidioidomycosis, 56.7 percent revealed the presence of yeast cells in the liver, associated with a granulomatous tissue response. Thirty percent of the cases had only widening of the portal tracts by fibrosis (31.6%), whereas 11.6 percent had an essentially normal liver.[203]

The intestinal mucosa may be affected, but similar to the lungs, its involvement is secondary to blockade of the regional lymphatic flow, with retrograde progression of *P. brasiliensis* to the mucosa, a process resulting in mycotic enteritis.[25,76,120] In this case, the submucosal inflammatory process is granulomatous; fungal cells are visualized; and the intestinal changes may vary from dilated loops, edema, congestion, and nodule formation to multiple mucosal ulcers.[76,120]

Studies also have shown bone marrow infiltration mainly, but not exclusively, in the acute-subacute form of the disease; 25.7 percent of the patients examined during active disease and 36.4 percent of those who died of paracoccidioidomycosis revealed this type of involvement.[164] The histologic pattern was variable, but appropriate staining always displayed fungal cells. Bone marrow invasion frequently is associated with marked eosinophilia.[164,192] In addition, bone and joint lesions are frequent occurrences in the acute-subacute form of the disease[3,15,66,107,209] and appear closely related to bone marrow infiltration.[164]

The adrenals often are involved in patients with the chronic form of the mycoses, many of whom suffer from adrenal hypofunction or insufficiency (Addison disease). The glands contain multiple granulomatous foci, and diffuse necrosis may be seen in the most severe cases. Hyperplasia of the adrenal glands also occurs commonly.[126]

On histologic examination, formation of granulomata is the rule, except in patients with severe disseminated disease, such as those with the acute-subacute form.[80] The granulomatous inflammation is associated with a mixed pyogenic component, especially in the case of ulcerated skin lesions or ruptured lymph nodes. Caseation and central necrosis may be present. In compact granulomata, abundant epithelial cells, Langerhans or foreign body

giant cells, plasmacytes, and lymphocytes are seen; often, phagocytosis of the yeast cells can be observed. $CD4^+$ lymphocytes dominate over $CD8^+$ lymphocytes and appear as peripheral mantles around aggregates of macrophages and histiocytes.[136] In the juvenile disseminated disease, the inflammatory reaction is diffuse, with abundance of both mononuclear and yeast cells but sparse formation of compact granulomata.[14,45,167] Loose granulomata appear unable to circumscribe fungal antigens, and at their periphery, *P. brasiliensis* antigens may permeate throughout the intercellular space.[185] Skin and mucous membrane lesions usually exhibit pseudoepitheliomatous hyperplasia and intraepithelial microabscesses.[133,153,154,185]

An interesting aspect drawn from the previously described histologic studies is the frequent description of areas of extremely active disease characterized by pyogenic reaction and loose granulomata, rich in budding fungal cells, intermingled with areas with compact granulomata, rare fungal cells, and variable degrees of fibrosis. This mixed aspect can be observed in lymph nodes, skin, or pulmonary lesions, suggesting that the disease evolves through localized new bouts of fungal multiplication and tissue invasion, whereas the adjacent older lesions are on the way to fibrotic resolution. Computed tomographic imaging of the lung confirmed this aspect by depicting areas with alveolar condensation along with fibrotic and emphysematous zones in one study and the simultaneous presence of features of more recent and chronic lesions.[81,200]

Tissue reactions are nonspecific; thus, diagnosis depends on finding *P. brasiliensis*. If the parasite is abundant, it may be identified by hematoxylin and eosin stains. Special fungal stains (e.g., Grocott silver methenamine) always should be employed, however, especially when granulomata are examined. The typical multiple budding yeast cells must be found to establish a diagnosis. The presence of fungal cells of different sizes (2 to 40 μm) suggests the presence of *P. brasiliensis*. In some cases, short chains and cells with single buds also are observed, and in these patients, differentiation of *P. brasiliensis* from *Cryptococcus neoformans*, *Blastomyces dermatitidis*, and even *Histoplasma capsulatum* must be made. When the disease is chronic, most of the fungal cells are found inside the macrophages, but free yeast cells predominate in disseminated cases. Internalized yeast cells exhibit altered morphology.[133,171]

CLINICAL MANIFESTATIONS

Paracoccidioidomycosis is a polymorphic disorder that at a particular time may involve more than one organ or system. Thus, making a topographic classification is unrealistic, and the classification of the mycosis currently accepted takes into consideration not only the organs involved but also the host's immune condition and the disease's natural history.[79] The infection is categorized as a *subclinical form*, and the overt process is subdivided into *acute-subacute* or *chronic disease*. The acute-subacute pattern predominates in children and young adults who are at greater risk than are patients exhibiting the chronic disease. According to the severity of the process, juvenile patients are assigned to two subgroups, severe and moderate. The adult type, which is the chronic progressive form of paracoccidioidomycosis, may be localized to pulmonary lesions, the unifocal disease, or disseminated from its primary foci, the multifocal process. Disseminated disease is characterized by involvement of the skin, mucosa, reticuloendothelial system, adrenal glands, and, less frequently, the gastrointestinal or the genitourinary tract, bones, and central nervous system.[8,23,84,118,126,133,167] The chronic form can be mild, moderate, or severe.[79]

Patients with acute or subacute disease develop signs and symptoms of a wasting process. Fever, malaise, listlessness, weight loss, and emaciation are recorded frequently. The severity of these symptoms is proportional to the degree of the organic involvement. Londero and associates[107] gathered the information published up to 1994 and found 269 cases in children younger than 14 years; however, sufficient clinical data for analysis of the prominent organic involvement were provided for only 77 children. The clinical characteristics exhibited by these children and by other patients described more recently, including the series of patients reported at two centers, are shown in Table 214–2. These findings support the notion that juvenile paracoccidioidomycosis is a disease of the reticuloendothelial system resulting in damage of the corresponding organs caused by severe macrophage dysfunction, as suggested previously.[80] Superficial lymph node enlargement was the predominant sign (81.6%) in these cases. Cervical and submandibular lymph node chains were involved most commonly, followed by those of the supraclavicular and axillary regions; however, any chain can be affected (Fig. 214–1). Lymph nodes may vary in size from slightly enlarged to

TABLE 214–2 Clinical Findings on Admission Diagnosis in Children with Paracoccidioidomycosis

Clinical Findings	No. of Children (%)
Lymph node enlargement	
Superficial	81.6
Thoracic	25
Abdominal	36.6
Abdominal masses	19.9
Hepatomegaly or splenomegaly	60.7
Ascites	19.1
Jaundice	10.5
Diarrhea, vomiting, abdominal pain, or distention	19.1
Joint or bone lesions	37.8
Skin lesions	29.1
Respiratory symptoms	9.1
Pulmonary consolidations or infiltrates	10.2
Pleural effusion	5.1
Oral and upper respiratory tract mucosa lesions	9.2

Total number of children investigated for each clinical finding varied from 98 to 199. Data from references 3, 34, 49, 66, 75, 107, 131, 146, 157, 166.

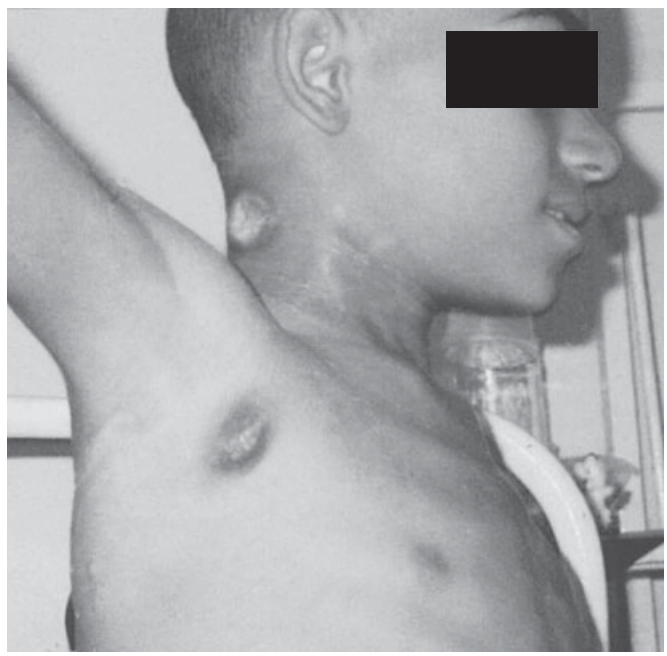

Figure 214–1 An 11-year-old boy with a 5-month history of fever and cervical and axillary lymph node enlargement, with fistulization. *(See companion Expert Consult web site for color version.)*

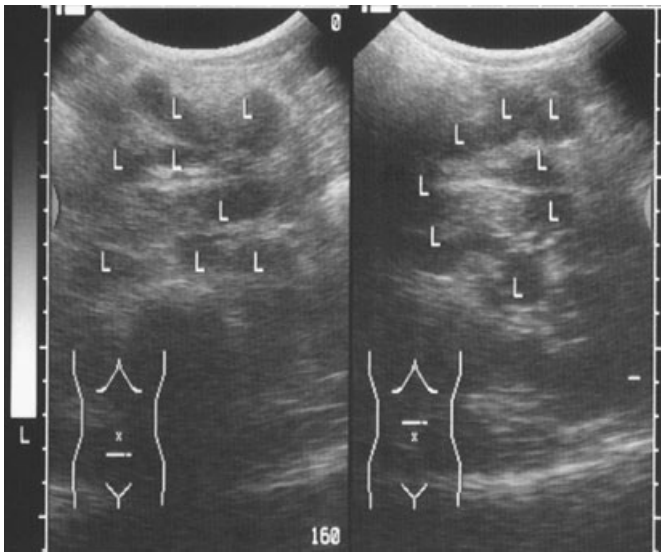

Figure 214–2 Extensive mesenteric lymph node enlargement in a 10-year-old boy (L) as shown by ultrasonography imaging.

large, painful, coalescent masses; they may be mobile and of elastic consistency or fixed to the adjacent tissues; hypertrophied nodes may progress to fistulization and discharge purulent material rich in *P. brasiliensis* yeast cells.

The next most important finding was hepatomegaly or splenomegaly (60.7%), which usually was asymptomatic. Jaundice is an infrequent occurrence and more closely relates to extrinsic compression of the biliary tree.[25] Liver enzymes, especially alkaline phosphatase, were abnormal in some cases but not markedly increased.[25,146] Portal hypertension also was a rare occurrence. Findings and complaints relating to the abdomen and digestive tract, such as abdominal masses, lymph node enlargement, diarrhea, vomiting, abdominal distention or pain, and ascites, also have also been recorded in a sizable proportion of patients (Fig. 214–2; see Table 214–2). Signs and symptoms of an acute abdomen, caused by masses formed by hypertrophied lymph nodes or perforation, also have been reported; this problem may lead to intestinal occlusion, blockage of lymphatic drainage, and, later, ascites.[8,15,107,147,158]

In a series comprising predominantly patients with the juvenile form of the disease, abdominal radiographic studies (double-contrast barium study) revealed ileal and jejunal alterations in 42 to 51 percent.[76,120] The main findings were distortion and coarsening of the mucosal pattern and loop dilation. However, part of these alterations was probably nonspecific because jejunal biopsies performed in a small number of patients revealed neither granulomatous response nor *P. brasiliensis* yeast cells.[120] On the other hand, histopathologic examination of autopsy specimens (probably the more severe cases) showed a specific granulomatous enteritis in 80 percent.[76] In addition, in a magnetic resonance imaging study of patients, mostly with the juvenile form, 48 percent had abdominal lymph node enlargement, even if no abdominal signs and symptoms had been recorded.[119] This lymph node involvement causes mesenteric lymphatic stasis and enteric mucosal edema that may progress to fungal enteritis accompanied by abnormal intestinal function, such as reduced absorption of fat.[121,147,158] Computed tomography and magnetic resonance imaging have proved helpful in determining the extent and nature of abdominal involvement.[119] These consequences of the primary fungal process adversely affect the health status of the patient.[12,193] Abdominal complications continue to be reported occasionally in the most recent series.[147,158]

Bone damage and articular problems also were important components (37.8%) of disseminated disease, especially in younger children. This proportion is higher than in earlier observations[121] and may be explained by the more frequent active search for bone lesions, irrespective of the presence or absence of symptoms in the more recent reports.[147,158] In these patients, the long bones frequently were affected, with the lytic lesions located at the diaphyseal or metaphyseal-epiphyseal regions,[3] probably because of their higher vascularization, emphasizing the hematogenous dissemination that typically occurs in this form of the disease (Fig. 214–3). Ribs, skull, phalanges, and vertebral lytic lesions also have been documented (see Fig. 214–3). Different from the painful, motion-restriction joint lesions, the bone lesions usually were silent.[3,15,66,132] A pathologic fracture occasionally may occur.[132]

Skin lesions were noted in 29.1 percent of the juvenile cases, with a tendency toward higher frequency with increasing age of the patient. Distribution of cutaneous lesions was variable, but face and trunk were involved more frequently.[118] Lung abnormalities were recorded in a smaller proportion of cases. However, even in the absence of clinical and radiologic involvement, colonization of the lung by *P. brasiliensis* can be demonstrated by direct examination and by culture.[169] When chest radiographs were abnormal, enlarged hilar lymph nodes and miliary infiltrates predominated.[84,107,169] In contrast with the adult form of the disease, in which adrenal involvement is a serious concern, it is an uncommon manifestation in children. A recent survey of adrenal function before and after treatment in 23 children has shown normal adrenal function.[159]

Anemia, increased erythrocyte sedimentation rate, severe hypoalbuminemia, and hypergammaglobulinemia with high IgG serum concentrations are found regularly.[8,15,80,146] Nonetheless, anti–*P. brasiliensis* antibodies may prove undetectable in some patients.[58,59] Eosinophilia and elevated IgE antibody titers have been detected in most patients.[146,192,219]

The association between human immunodeficiency virus (HIV) infection and paracoccidioidomycosis was reviewed.[11] The youngest patient described was a 15-year-old boy who had enlargement of superficial and mediastinal lymph nodes, skin nodules, and pulmonary infiltrates.[47] The mycosis also was diagnosed occasionally in other patients whose immune function was depressed by certain conditions or medications.[115] Of interest, however, is that the incidence of this mycosis has not increased in HIV-infected patients, as would have been expected.[11] No report of paracoccidioidomycosis and HIV co-infection in children has been published so far.

In the chronic, progressive, adult form of paracoccidioidomycosis, signs and symptoms differ substantially from those found in children. Descriptions of the disease in adults are beyond the scope of this text but are referenced.[80,126,142,172,186]

DIAGNOSIS

The diagnosis of paracoccidioidomycosis frequently is established by the direct examination of the clinical sample and confirmed by culture or serologic tests. Specific diagnosis depends solely on laboratory confirmation. The recommended way of diagnosing this mycosis, in the mycology laboratory, is the direct microscopic observation of the characteristic *P. brasiliensis* in a drop of physiologic saline or potassium hydroxide test (Fig. 214–4).[102] Usually, the sources of clinical specimens are sputum, secretion, and scraps or debris from ulcerated skin and mucosal lesions, draining material from suppurating lymph nodes or abscesses, bronchioalveolar fluid, biopsy tissue, and the like. Bronchioalveolar, articular, cerebrospinal, or other fluids must be centrifuged before examination. Draining material from suppurating lymph nodes usually is rich in fungal cells with multiple buds. The large

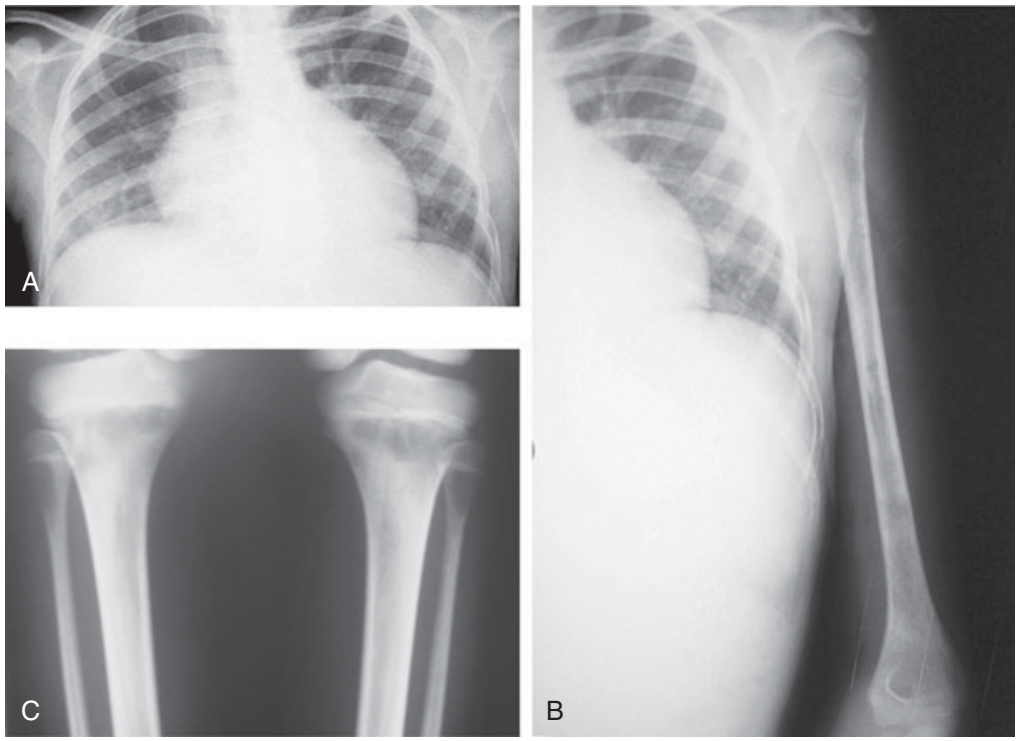

Figure 214–3 Severe disseminated subacute paracoccidioidomycosis in a 9-year-old boy. The chest radiograph (**A**) shows mediastinal lymph node enlargement and mild bilateral infiltrates in the middle and lower fields. Osteolytic lesions of long bones: humerus (**B**) and tibia and fibula bilaterally (**C**).

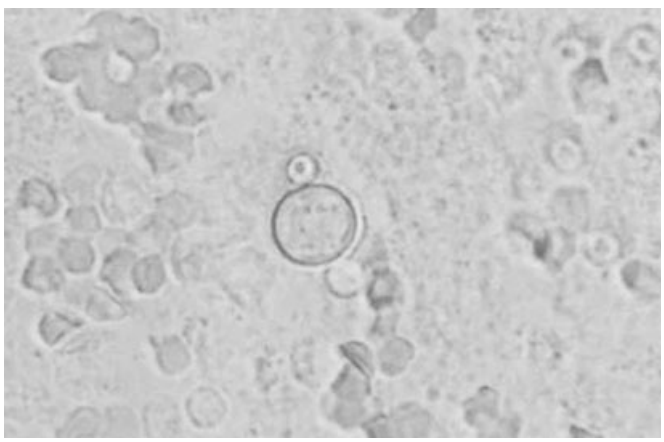

Figure 214–4 Direct examination of a potassium hydroxide mount of a patient's sputum specimen showing a budding *Paracoccidioides brasiliensis* yeast form–like cell.

cell, with multiple exosporulation, resembling a pilot's wheel, is pathognomonic of the disease. Sputum is one of the most important specimens used for diagnosis, and various procedures are used to improve the detection of the organism in this specimen. A cell block preparation method for sputum proved more sensitive (93%) than simple direct examination,[204] but few laboratories have employed it routinely. When the first examination of the sputum is negative, it can be homogenized with an equal volume of *N*-acetyl-L-cysteine and then centrifuged again. Four percent sodium hydroxide can be used, but only in direct examination because this treatment reduces the fungus isolation. The sensitivity of the direct examination (wet mount, smears, and histopathologic examination) varies from 80 to 100 percent or less and depends on the laboratory.[29,102] Biopsy samples should be taken only when routine direct examinations have failed, and the specimen should be placed in two separate flasks: one with sterile saline and the other with formalin for mycologic and histopathologic examinations, respectively. As the morphologic appearance of the parasitic forms of *P. brasiliensis* is pathognomonic, the diagnosis of the mycosis can be established by histopathologic examination of a biopsy specimen and confirmed by either culture or serology. If the parasite is abundant, it may be identified by hematoxylin and eosin stain and confirmed by a special fungal stain such as periodic acid–Schiff or Gomori methods.[102,133] Pereira and colleagues[161] established the diagnosis of 63 children with paracoccidioidomycosis by the identification of *P. brasiliensis* in lymph node biopsy (84%), bone biopsy (9%), or skin biopsy (7%) specimens.[158]

When the disease is chronic, most of the fungal cells are found inside the macrophages, but free yeast cells predominate in disseminated cases. Pauciparasitic forms of the *P. brasiliensis* granuloma that show a sarcoidic pattern with rare yeast forms may appear. In these cases, the diagnosis may rely on epidemiologic data plus serologic and molecular diagnostic tools such as PCR on paraffin-embedded tissue.[128,129] Visualization of the multiple budding yeast cells, in which the buds are connected to the parent cells by a narrow bridge with a well-defined refringent double wall, is mandatory because the *P. brasiliensis* yeast cells may range from a few microns to 30 to 40 μm. Sometimes, in the lesions, the parasite is present as diminutive yeast cells, measuring about 3 to 5 μm.[103] *P. brasiliensis* blastoconidia may be confused with *B. dermatitidis*, capsule-deficient *C. neoformans*, endospores and small *Coccidioides immitis* empty spherules, large *H. capsulatum* yeast cells,[29,103] or even *Pneumocystis carinii*.[194] For this reason, the multiple budding structures always should be found for specific diagnosis. In these cases, the molecular tests such as PCR and immunocytochemistry with specific polyclonal or monoclonal anti–*P. brasiliensis* antibody could be used.[40,184]

Disseminated and pulmonary paracoccidioidomycosis both can be confused with tuberculosis, histoplasmosis, leukemia, malignant neoplasms, or Hodgkin disease. When the skin or mucous membranes are affected, paracoccidioidomycosis must be differentiated from histoplasmosis, leishmaniasis, leprosy, syphilis, lupus erythematosus, and a variety of malignant neoplasms. In children, tuberculosis, acute abdominal syndrome, intestinal obstruction, osteomyelitis, and rheumatic fever also are important considerations in the differential diagnosis of this disorder.

Recently, several *P. brasiliensis* DNA sequences of potential diagnostic use have been reported, but few have been applied to clinical samples, needing further study before they are introduced in diagnosis. Until now, *P. brasiliensis* DNA sequences of potential diagnostic use are a species-specific 110-bp DNA fragment derived from the rat β-actin gene,[85] the 5.8S rRNA gene and its flanking internal transcribed spacer regions,[93,104,134] and the gp43 gene.[20,87] In addition, a species-specific 14-base DNA probe originating from the 28S ribosomal gene was used for in situ hybridization to detect *P. brasiliensis* in oral biopsy specimens from patients with paracoccidioidomycosis. However, this probe detected only 2 to 3 percent of the fungal cells present in the tissues; consequently, this technique exhibited low sensitivity for routine diagnostic purposes.[56,184] A PCR assay based on sequence of the gene coding for the gp43 antigen presented the highest sensitivity and specificity and gave a single band of 600 bp in sputum from a patient with chronic paracoccidioidomycosis. In addition, a PCR assay based on the 5′ nuclease assay using a fluorescent probe could detect at least 10 copies of this DNA sequence, providing sufficient accuracy to be useful for diagnosis of paracoccidioidomycosis.[188] Morais and colleagues[135] showed that some sequences of gp43 could undergo substitutions and need further testing to verify their universality and specificity for *P. brasiliensis*. Recently, the species-specific gp43 gene of *P. brasiliensis* was detected by loop-mediated isothermal amplification in DNA extracted from a paraffin-embedded tissue sample of paracoccidioidomycosis, suggesting that this method may achieve clinical application.[69] The primers reported by San Blas and colleagues[183] in 2005 produced positive identification bands, mainly in sputum samples; however, experiments with serum and blood samples were unsuccessful. Interestingly, the molecular test produced results that preceded, by 1 week or more, the information of classic tests as well as detected *P. brasiliensis* in a cerebrospinal fluid sample. Further experience will be necessary to include molecular tests in routine diagnosis.

Culture samples should be obtained to support the diagnosis and to establish the viability of the fungus. However, they are not always positive because the presence of other, more rapidly growing microorganisms in the samples renders isolation difficult. Culture methods, although essential for establishing the diagnosis in some cases, have limitations for rapid diagnosis, namely, insensitivity, need for invasive procedures, and delayed growth. At room temperature, *P. brasiliensis* is a slow-growing fungus that can be overgrown readily by bacteria, yeasts (especially *Candida*), and contaminant molds. Isolation should be attempted by the concomitant use of modified Sabouraud agar and yeast extract agar plus antibiotics and cycloheximide. In noncontaminated samples, the use of brain-heart infusion agar plus blood and antibiotics (without cycloheximide), a hemoglobin-containing agar incubated at 37° C, or both also is advisable. Cultures should be observed for 4 to 6 weeks, depending on temperature of incubation, with a definitive classification being accomplished only in the yeast phase.[29,103,142,172] The isolation rate of the fungus in culture varies from 80 to 100 percent but may be less in other nonspecialized centers.[29,102] As the mycelial form is not characteristic, subcultures in brain-heart infusion plus blood, Fava Netto's agar, or peptone–yeast extract–glucose agar, for up to 14 days at 35° C to 37° C, allow yeast conversion to occur and definitive diagnosis to be established.[102] Several sequences of *P. brasiliensis* DNA are of use in the identification of the fungus, as is the exoantigen test with specific antiserum, mainly for atypical isolates.[99] The 110-bp DNA fragment, a 14-base DNA probe, sense PbITS1s, and antisense PbITS3a have been used as a diagnostic marker for the identification and classification of different *P. brasiliensis* isolates.[85] Two primers, sense PbITS1s and antisense PbITS3a, were capable of identifying 29 strains of *P. brasiliensis* by means of a specific 418-bp fragment.[93] In addition, primers OL5 and OL3, specifically designed for 5.8S and 28S ribosomal DNA regions, were able to discriminate between *P. brasiliensis* and other human pathogenic fungi by PCR. OL5 was used in combination with primer ITS1 and OL3 with primer UNI-R in nested PCR and generated a 203-bp fragment present only in *P. brasiliensis*.[134] Universal fungal primers ITS1 and ITS4, directed to the conserved regions of ribosomal DNA, were found to be highly specific and could distinguish yeast-like organisms.[104] The species-specific gp43 gene of *P. brasiliensis* was detected by loop-mediated isothermal amplification in DNA from *P. brasiliensis* strains in 3 hours, suggesting that this method may be applied to rapid identification of this fungus.[69] Recently, the identity of the *P. brasiliensis* typical and atypical strains could be confirmed by its dimorphism, by the expression of gp43, and by PCR using specific primers for gp43 as well as ribosomal internal transcribed spacer regions.[26,51] However, an important note is that one sequence (483 bp) of the *Lacazia loboi* gp43-like gene had 85 percent identity at the nucleotide level and 75 percent identity with the deduced amino acid sequences of the *P. brasiliensis* gp43 protein.[215]

Patients with paracoccidioidomycosis not only have a polyclonal B cell activation but also produce large amounts of anti–*P. brasiliensis* antibodies, which are long-lasting and generally correlate with the severity of the disease.[7,13,22,108,219] Assessment of the serologic response has long been used in paracoccidioidomycosis. It has been used as a diagnostic tool mainly because, in a significant proportion of the patients, it is difficult to obtain clinical specimens for visualization or cultivation of the fungus when only deep-sited lesions are present. On some occasions, a serologic result is the first indication of the mycotic nature of the patient's illness. In recent years, a great interest has been generated in the development of techniques to improve paracoccidioidomycosis diagnosis. These developments have come in the fields of either antibody or antigen detection. Thus, serologic tests are of value for the diagnosis and may be useful in monitoring the evolution of the disease and its response to treatment. Antibodies are encountered in patients with clinical symptoms, and generally the titers of antibodies can be related to the severity of the disease. Patients with paracoccidioidomycosis have a polyclonal activation of the humoral system with high serum concentrations of specific IgA, IgG, and IgE isotypes.[7,19,22,108,129] However, antibodies usually are impaired or absent in some patients with severe juvenile forms and in immunocompromised individuals.[58,214] On occasion, titer fluctuations may result in values below the level of detection by current methods, which may result in false-negative results. Experience over the years has resulted in various highly sensitive and widely employed serologic tests; the frequency of positive test results in patients with paracoccidioidomycosis varies from 70 to 95 percent and may even reach 100 percent in particular cases, depending on the type of test used and the stage of the disease. Of note is that discrepancies between serologic tests performed in different laboratories represent a problem because of the variation in reagents used, laboratory procedures adopted, and varying criteria used for evaluation of the results obtained. Some of the most critical reasons for discrepancies are variations in the type of antigenic preparations, different strains, and, indeed, the mycelial or yeast cell phase chosen. Culture filtrates, cytoplasmic extracts, and antigens derived from the cell wall have been used as reagents in serologic tests for

paracoccidioidomycosis, although culture filtrates are used in most laboratories.[17,59,94,128]

Various fractions that have been cloned, sequenced, and applied to the diagnosis include HSP60,[53] gp43,[208] 29-kd,[161] and 27-kd fractions[50] as well as the combinations of the previously described 27-kd recombinant antigen and the 87-kd heat shock protein.[63] The main diagnostic antigen of paracoccidioidomycosis is the 43-kd glycoprotein[162] because 100 percent of these patients' sera display antibodies against it, depending on the test.[40,82] This fraction has been extensively and successfully employed as an antigen in establishing the serologic diagnosis of paracoccidioidomycosis.

Many serologic tests have been developed and extensively studied. Agar gel immunodiffusion, complement fixation, and counterimmunoelectrophoresis tests have been employed extensively.[129] Complement fixation tends to be used less often because it is a difficult and time-consuming technique. Also available are indirect immunofluorescence, indirect hemagglutination, enzyme-linked immunosorbent assay (ELISA), and dot-blot immunobinding and immunoblotting.[129,201] Some of the newer tests have employed purified antigens, such as glycoprotein 43, which are well characterized and more specific.[208] Immunodiffusion and counterimmunoelectrophoresis, which employ crude antigens derived from the yeast phase, can detect 90 percent or more of active cases.[59] Most authorities recommend the use of two different tests because none of these tests guarantees more than 90 percent sensitivity when it is employed alone. Immunodiffusion, for instance, is very simple and specific. Three precipitin bands, two of which are specific for *P. brasiliensis*, have been identified.[19,34]

A conjoint effort of several diagnostic centers assessing the value of this test with use of a reference crude antigen (Ag7) demonstrated it to be 84.3 percent sensitive and 98.9 percent specific[213]; but 15 percent represented false-negative results and constituted a failure of this diagnostic test, or they may be related to the production of low-avidity IgG2 antibodies directed against carbohydrate epitopes.[144] Patients with very localized, benign disease may present negative results in these tests, but with more sensitive techniques (e.g., immunoblotting or gp43-ELISA), a diagnosis usually is reached. In our laboratory, three tests (immunodiffusion, counterimmunoelectrophoresis, immunoblotting) are used in a routine serologic diagnosis. The sensitivities of these tests were 85.3 percent, 92.9 percent, and 100.0 percent, respectively, evaluated against 120 paracoccidioidomycosis sera. Limitations of these tests include cross-reactivity to other mycotic disorders, especially histoplasmosis and *Lacazia loboi* antigens, mainly in more sensitive tests,[162] and difficulties in standardization of the various tests and reagents.[39] The gp43 cross-reactivities were predominantly attributed to periodate-sensitive carbohydrate epitopes containing galactosyl.[162]

The value of serologic tests in the follow-up of patients undergoing treatment also has been addressed by several centers. The value of these tests in monitoring patients also is problematic, partly because of the diversity and complexity of the humoral response in patients with paracoccidioidomycosis. Serial serologic evaluations, usually done 3 months apart, together with the clinical, mycologic, and radiologic surveys can guide the treatment schedule, and two negative results in two of the serologic tests routinely employed is an additional criterion that helps in the difficult decision of therapy discontinuation.[173] The immunodiffusion, complement fixation, counterimmunoelectrophoresis, and ELISA tests were shown to be able to document the decline in antibody levels that parallels the clinical improvement; serum antibodies are cleared 1 to 2 years after cessation of therapy, depending on the clinical form.[30,59,129] Conversely, in these circumstances, rising antibody levels mean a relapse. Nonetheless, a considerable number of patients maintain, sometimes all their lives, the so-called healing titers (i.e., the persistence of low serologic titers in the tests). This is seen more frequently in patients with the acute form or the chronic multifocal form of the disease.[59] The debate on the clinical relevance of this phenomenon—whether it represents the persistence of residual active lesions, thus predicting possible future relapses—is inconclusive. During treatment of paracoccidioidomycosis, an important consideration is to be able to establish cure or remission criteria. A significant fall in reactivity of anti-gp43 antibodies has been shown to correlate with clinical improvement. During relapses, the levels of specific antibodies rose, and some patients considered cured exhibited a residual reactivity. Some patients have a negative immunodiffusion and counterimmunoelectrophoresis test result but a residual reactivity with gp43 and gp70 by immunoblotting.[29,59,67,129,142,172]

On the other hand, gp43 and gp70 can be detected in the biologic fluids. Furthermore, several studies have shown that the detection of antigens in blood and urine instead of antibodies is a potent means of establishing a diagnosis, monitoring treatment, and maintaining cure control.[88,111,112,129,213] They allow a more precise diagnosis to be established for patients with disseminated childhood and adult multifocal disease, in which antibodies are undetectable because of their coupling to excess antigen or their incapacity to raise antibodies. Antigenemia follow-up studies permit a more precise determination of improvement as antigen load decreases with treatment response. The detection of circulating antigen may represent a more practical approach to the early and rapid diagnosis of the disease. It also will be very helpful in establishing the diagnosis of immunocompromised patients, especially those with acquired immunodeficiency syndrome (AIDS), in whom searching for an antibody response is not a completely reliable diagnostic tool.

The skin test with paracoccidioidin is not considered a diagnostic test because 30 to 50 percent of the patients prove nonreactive when they are tested initially.[19,22,80,85] Conversely, a positive skin test result indicates previous contact with the fungus but not necessarily active disease. When histoplasmin skin test material is used, cross-reactions have been verified.[19,32,80,85] When the result of the skin test with paracoccidioidin is negative and the patient is treated, the skin test result may become positive, in which case the patient's prognosis is considered good.[29,142]

TREATMENT

The treatment of paracoccidioidomycosis usually is divided into two phases: attack and maintenance. Two different drugs can be used for each phase, or the same drug can be used for the two phases but at different dosages. Among the reasons for this two-phase prolonged treatment are the chronic progressive nature of the disease and the knowledge that all currently available drugs, albeit with apparently different efficacies, are only fungistatic. Thus, an adequate humoral and cellular immune response is required to control the mycosis. Cellular immunity, however, may be impaired in a large number of patients because of malnutrition or the disease process itself.[8,15,137] Provision of supportive therapy, therefore, is imperative. Rest, adequate nutrition, correction of anemia, and treatment of other concomitant infections are essential.[127] Paracoccidioidomycosis is the only fungal disease that can be treated successfully with sulfa drugs. Until 1958, when amphotericin B was introduced, no other treatment was available. In children, sulfadiazine can be provided orally at daily doses of 60 to 100 mg/kg divided into four to six equal parts; in adults, a maximum daily dose of 6 g can be used. Water intake and urine alkalinization (usually by bicarbonated water intake) should be encouraged during therapy to prevent crystalluria and tubular deposits of sulfadiazine.[127,191] Duration of the attack treat-

ment is dictated by the patient's response after clinical and serologic improvement has been attained; in general, in the chronic form, 2 to 6 months are required. A slow-acting sulfa drug (sulfamethoxypyridazine or sulfadimethoxine) can be provided as maintenance treatment, at a maximum daily dosage of 1 g.

In children, the precise total duration of therapy varies according to the severity of the disease, and it should also be dictated by the clinical and serologic responses of the patient to treatment. The importance of continuous treatment must be emphasized because relapses occur if the drug is not taken regularly. Moreover, if the drug is interrupted prematurely, the patient's isolate may become resistant to sulfonamides.[127,166] Sulfonamides can be used by patients with mild or moderately severe disease. They also can be used for patients who have been treated initially with amphotericin B.[127] Brazilian physicians most often employ trimethoprim-sulfamethoxazole (80 mg of trimethoprim and 400 mg of sulfamethoxazole per tablet) given at a dose of two tablets and administered orally at 12-hour intervals with satisfactory results. This drug also is used in association with amphotericin B. Tolerability is good; myelotoxicity (leukopenia), the main side effect, can be monitored and controlled by folinic acid administration without modification of the therapeutic regimen. In general, children should be given half the dose given to adults.[8,127] A Brazilian consensus on paracoccidioidomycosis recommends 8 to 10 mg/kg of trimethoprim daily.[190] This combination has the advantage of permitting alternative parenteral administration whenever necessary. Serum concentrations of sulfonamides should be determined when this drug is used and should not be less than 50 μm/mL. Duration of the acute treatment with this drug varies in each case, but it usually lasts for 6 months. Maintenance treatment in these cases can be achieved by using half the dose of the attack treatment or by using a slow-acting sulfa. Development of resistance to trimethoprim-sulfamethoxazole has been documented in an adult patient with the subacute form.[90]

Amphotericin B is effective but should be reserved for severely disseminated cases.[15,127] It also can be used by patients who relapse during the course of or after treatment with a sulfonamide or any orally administered drug because gastrointestinal involvement may impair drug absorption in these cases. The effectiveness of therapy with azoles has curtailed the need for more aggressive regimens. Amphotericin B should be provided as described in other chapters of this book. More than one course of therapy may be required in some patients.

Some investigators have suggested the use of a combined amphotericin B–sulfonamide treatment. In adults, a total cumulative dose of amphotericin B of 1 to 2 g followed by sulfa drugs, given as indicated previously, usually is sufficient.[116] With this drug combination, clinical improvement can be achieved in approximately 75 percent of patients; approximately 10 percent do not respond as well, and the remainder die during treatment. Relapses may be expected in approximately 10 to 15 percent of optimally treated patients.[127] Data regarding effectiveness of the new amphotericin B lipid formulations in paracoccidioidomycosis are insufficient.[62]

The use of oral ketoconazole has improved greatly the prognosis of patients with the progressive forms of this disease.[60,64,110,116] A comparative study of ketoconazole with amphotericin B plus sulfonamides showed equivalent to better responses in the ketoconazole group.[116] Ketoconazole is not as toxic as amphotericin B and does not require parenteral administration. Adults have been treated with 200 to 400 mg once daily for periods of 6 to 12 months with no problems and have shown a lower relapse rate (10%).[127,172] However, other studies have indicated that patients may need 18 months or more of treatment to achieve good results.[59,64,110,116] The use of ketoconazole for treatment of childhood paracoccidioidomycosis has been reported recently, with good results.[147] However, the role of ketoconazole in treating this mycosis remains uncertain because relatively few children have been treated to date. The results available suggest that the drug is effective. Children may be treated with doses varying from 5 to 8 mg/kg/day, in accordance with the manufacturer's indications. Children treated with ketoconazole should be monitored for changes in liver enzymes.[116,126,142] In adults, liver toxicity and gonadal changes also should be evaluated, especially during prolonged therapy.[127]

Ketoconazole no longer is considered the therapy of choice because of its side effects and numerous drug interactions. When the new triazole derivatives for oral administration (itraconazole, fluconazole) became available, they were tested in patients with paracoccidioidomycosis.

Today, more experience has been gained with itraconazole.[127,141,147,158,172] This triazole is more potent and less toxic than ketoconazole and is equally effective as or even more effective than the parent compound. A good experience in treating children with disseminated histoplasmosis has been reported.[205] It is administered in 100-mg capsules that should be given with or shortly after a meal. One or two capsules, depending on the severity of the fungal process, taken daily for 6 months have been shown to be effective in reducing all active lesions. Most (98%) of the patients, including children and young adults, respond.[127,141] Post-therapy observations indicate variable proportions (ranging from 2.1% to 21%) of relapses, depending on the severity of the disease, duration of therapy, and time of follow-up.[114,206] Side effects have been few and include transient elevation of hepatic enzymes.[127,141,142,206] At present, several well-conducted (but noncomparative) trials have been reported favoring this new triazole, which has become the first choice for the treatment of paracoccidioidomycosis in many reference centers. As for ketoconazole, the experience in treating childhood paracoccidioidomycosis with itraconazole still is limited compared with that with amphotericin B and trimethoprim-sulfamethoxazole, but the few reports are encouraging.[147,158] A randomized trial with sulfadiazine, ketoconazole, and itraconazole for the treatment of adult patients with disease of moderate severity failed to show higher efficacy of a particular regimen over the others.[191] However, long-term follow-up was not evaluated in this study. Itraconazole maintenance therapy is recommended at a dose of 5 to 10 mg/kg daily, with a maximum dosage of 200 mg twice daily. A liquid formulation of itraconazole in cyclodextrin was suggested initially as a promising alternative for treatment of children, but it has not been marketed in countries where the disease is endemic, and thus far no clinical experience could be gathered to support its recommendation.

Fluconazole is not as effective in this disorder; higher doses, up to 600 mg/day, and longer treatment periods are required. Recrudescence and relapse of disease occur more frequently than when itraconazole is used.[15,127,142] Fluconazole may be useful in severely ill patients who must be treated intravenously.[127] No experience in children is available. The literature has only one report of the successful use of terbinafine in an adult patient.[150]

Many questions remain to be answered about optimal treatment of paracoccidioidomycosis. The role of adjunct therapy with immunomodulators, such as cytokines, for cases refractory to conventional therapy remains to be investigated. α-Glucan obtained from *Saccharomyces cerevisiae* has been investigated.[125] The clinical and laboratory criteria that allow the transition from the attack to the maintenance treatment phase have not been standardized, nor have the parameters that could be used to end treatment with the assurance that the patient is cured. Progressive decrease in the titers of the serologic tests currently available is one of the laboratory parameters used most frequently; restoration of some cellular immunity responses, such as reactivity to the paracoccidioidin skin test, also is used. Newer methods (e.g.,

antigenemia detection and molecular biology approaches) are being standardized.[68,87,88,93,111,112,138] Tests for detection of antigenemia in serum have been developed in some centers and showed good efficacy in the follow-up of patients, but it has not yet become widely available.[88,111,112] Meanwhile, most specialists agree that treatment decisions need to be tailored according to the patient.

PROGNOSIS

Paracoccidioidomycosis is considered to be progressive in most cases and fatal if it is left untreated. However, residual lesions have been observed in a few patients with no known history of active mycotic infection.[79,84,126] Prognosis depends on the status of the patient at the time of diagnosis. Children and young adults in whom fungemia has taken place and who have multiple-organ involvement do not respond well to therapy. In less disseminated cases, the response to treatment depends on the severity of disease at the time of diagnosis. The fatality rate in young patients at one time was reported to be 31 percent,[45] but it now is much lower as a result of earlier diagnoses and better treatments. Nonetheless, it remains a life-threatening disease, with a rate of 9.5 to 13 percent fatalities in the most recent series. This rate was attributed to delayed diagnosis or searching for medical assistance, irregular treatment, or treatment not monitored. Abdominal complications, specially ascites, and malnutrition were associated with the lethal prognosis.[8,147,158] In the adult group, patients have a greater chance of survival because of the usually less severely disseminated nature of the chronic form. Once specific treatment is instituted, lesions regress promptly; skin lesions may heal completely in 2 to 4 weeks.[118,127] Complete remission is possible in most patients with the acute-subacute and chronic forms of the disease. Prognosis has improved as a result of establishing the diagnosis earlier, new antifungal drugs that facilitated compliance, and better knowledge of the disease; the last of the three has provided better clinical and laboratory follow-up and the notion that the disease needs prolonged surveillance, as shown by the decrease in positive mycological test results during and after therapy.[127,142,206] Some investigators consider the term *cure* inappropriate because of the inability to confirm complete eradication of the organism; the term *apparent cure* should be used instead.[127]

Paracoccidioidomycosis persists as a disease with a low mortality rate but high morbidity. Complications vary, and like the prognosis, their occurrence depends on the extent of fungal invasion. In the juvenile form, early complications that may lead to surgical intervention are intestinal obstruction and jaundice, both of which result from enlarged mesenteric lymph nodes. Disabsorptive syndromes may be associated, aggravating the nutritional status of the patients.[12,193] Patients also may present with ascites or chylothorax. Lymph nodes may suppurate, and fistulas can develop. A late complication that has been described recently is abdominal malakoplakia.[178] In the adult form, acute complications of mucosal involvement are dysphagia and dysphonia, edema of the glottis, respiratory insufficiency, and Addison disease, among others. Sequelae are not seen as commonly in children as in adults. In general, fibrosis is the cause of serious problems in patients who respond to therapy. Despite the newer, very effective therapies, these sequelae preclude, in many cases, the complete restoration of the patients' previous health status.[126,127,141,142,172,206] In the acute-subacute group, scarring and fibrosis of the affected nodes and residual pulmonary fibrosis have been noted.[107] Malabsorption syndrome probably is the most serious sequela in the juvenile form because of the enteric loss of proteins and inflammatory cells that results in immunodeficiency and opportunistic infections.[12,193] In adults, the sequelae described most frequently are microstomia, laryngeal stenosis leading to permanent tracheostomy, adrenal insufficiency, pulmonary fibrosis, dysphonia, and emphysema.[133,211,217]

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CHAPTER 215

Cryptococcosis

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Cryptococcosis is a life-threatening systemic fungal infection caused by the basidiomycetous yeast *Cryptococcus neoformans*. Historically, the disease has been referred to as *torulosis*, *European blastomycosis*, and *Busse-Buschke disease*. The organism and the disease that it causes were discovered independently more than a century ago. In 1894, Busse, a German pathologist, found the yeast in the tibial lesion of a 31-year-old woman who later died of systemic infection under the medical care of Buschke, her physician. Meanwhile, in 1895, Sanfelice, an Italian, had cultured for the first time the encapsulated yeast *Saccharomyces* (later *Cryptococcus*) *neoformans* from peach juice, suggesting that this organism is not an obligate human pathogen. Sanfelice pointed out the similarity between the isolates from Busse's case and the yeast from the peach juice. The first case in which *C. neoformans* was recognized as the cause of meningitis was reported in 1914 by Verse; in 1916, Stoddard and Cutler described the central nervous system (CNS) pathology of the organism.

Cryptococcosis involves predominantly the CNS, lungs, skin, and bones, but other organs also have been affected. Although most cases occur in immunocompromised patients, the systemic disease also may occur in seemingly immunocompetent individuals.

ORGANISM

Of the 19 species of *Cryptococcus*, *C. neoformans* causes almost all cryptococcal infections. Only rarely do other species of *Cryptococcus*, such as *Cryptococcus laurentii*[33] or *Cryptococcus albidus*,[14] cause disease. *C. neoformans* originally was thought to include two varieties: var. *neoformans* and var. *gattii*. *C. neoformans* var. *gattii* has been recognized more recently, however, as a separate species, *Cryptococcus gattii*.[35]

C. neoformans is an encapsulated, basidiomycetous yeast, measuring 4 to 10 μm in diameter. Based on capsular agglutination reactions, the five serotypes of *C. neoformans* are A, B, C, D, and AD hybrid.[38] Molecular studies and genome sequences have detected significant genetic variability between serotypes A and D, and more recently, serotype A has been distinguished as a new variety, var. *grubii*.[24] Currently, the organism is classified into two varieties: *C. neoformans* var. *neoformans* (serotype D) and var. *grubii* (serotype A), and *C. gattii* (serotypes B and C).

C. neoformans has two forms of life cycle: asexual and sexual. In the asexual stage, it replicates by budding, and haploid unicellular yeasts are recovered from the environment and human infections. In the sexual cycle, the organism has two mating types,

"alpha" and "a," which, when placed in contact, undergo conjugation. The end product is a basidia form that undergoes meiosis and produces basidiospores, thought to be infectious propagules that can convert to a yeast form when located in the lungs.[38]

A feature of all *C. neoformans* strains is the mucopolysaccharide capsule surrounding the cell wall. This capsule may vary in size from twice the diameter of the yeast to a barely detectable thickness. The presence of this capsule may be recognized with the use of a mucicarmine stain or with negative staining using India ink. The Fontana-Masson stain detects melanin precursors and is useful in identifying *C. neoformans* in fixed tissues.[48]

C. neoformans forms smooth, mucoid colonies on solid media. Initially cream-colored, the colonies become dry and tan, pinkish, or yellow with aging. The fungus produces urease, is nonfermentative, and uses several carbohydrates. Species of the genus are separated with carbohydrate assimilation tests and potassium nitrate. The organism grows at 37° C, is lactose-negative, and does not produce pseudomycelia on cornmeal agar. It does not grow well at room temperature (25° C). *C. neoformans* serotypes can be identified by growth on agar containing canavanine, glycine, and thymol-blue indicator, which serves to differentiate serotypes B and C from A and D. Changes in color occur with serotypes B and C, but not with serotypes A and D.[42] Antibodies that distinguish differences in capsular structures are commercially available now. Serotypes also can be identified on the basis of DNA sequence polymorphisms detected by polymerase chain reaction (PCR) fingerprinting, amplified fragment length polymorphism, restriction fragment length polymorphism, and multilocus sequence typing analysis.[38]

EPIDEMIOLOGY

Cryptococcosis occurs worldwide as a sporadic infection but not in epidemics. It is found in soil, especially in sites enriched with avian guano. Serotypes A, D, and AD of *C. neoformans* are found worldwide and usually associated with pigeon excreta, whereas serotypes B and C more often are located in tropical and subtropical countries and have not been isolated from bird guano. Serotype B has been found in the environment of the river red gum tree (*Eucalyptus camaldulensis*).[20] Naturally acquired infections occur in domestic animals, such as cats, dogs, cattle, pigs, rabbits, sheep, and horses, but neither animal-to-human nor human-to-human infections have been reported.[10] The clinical presentation of cryptococcosis in animals differs from disease in humans.[10] Subclinical infection and nasal colonization are known to occur in cats and dogs.[17]

Limited serologic data indicate that most children and adults have had subclinical or mild cryptococcal infection, possibly manifested as a brief respiratory illness with cough.[35] *Cryptococcus* can be found transiently as part of the flora, and isolation from the flora does not indicate disease. Clinical cryptococcal infections occur sporadically and usually are self-limited in immunocompetent individuals.

The initial cryptococcal infection is acquired by inhalation of the yeast into the lungs from an environmental source, such as soil contaminated with avian guano[10] (*C. neoformans* var. *neoformans* and var. *grubii*) or eucalyptus trees and decaying wood (*C. gattii*).[9] In avian nesting areas, the yeast has a minimal capsule and can be aerosolized more easily and inhaled to the level of the alveoli. Viable *Cryptococcus* has been isolated from dried soil particles measuring less than 2 μm in diameter.[51,58] These small particles become airborne easily and are capable of alveolar deposition. The organism can colonize the respiratory tract without producing any symptoms and can be cleared or enter a dormant stage. From the lungs, hematogenous dissemination may occur. Systemic infection may occur in otherwise normal individuals, but most cases of cryptococcosis occur in immunosuppressed individuals. Some underlying immunosuppressive conditions include leukemia, lymphoma, Hodgkin disease, systemic lupus erythematosus, corticosteroid therapy, sarcoidosis, organ transplantation, diabetes mellitus, congenital immunodeficiency disorders, idiopathic $CD4^+$ T-lymphocytopenia, and human immunodeficiency virus (HIV) infection.[10,18,29,43] The acquired immunodeficiency syndrome (AIDS) epidemic has become a major factor in the increased prevalence of cryptococcosis since 1980.[16] Cryptococcosis occurs in an estimated 8 percent of patients with AIDS in the United States.[43] For reasons that are not understood, almost all cryptococcal infections in AIDS patients are caused by *C. neoformans* var. *neoformans*, even in areas where *C. neoformans* var. *gattii* is environmentally prevalent.[34,60]

An active, population-based surveillance from 1992 to 2000 in two U.S. cities showed the incidence of cryptococcosis to decrease from 5 cases per 100,000 population in 1993 to 1.3 cases per 100,000 population in 2000.[14] Of 1491 cases, 89 percent occurred in patients known to be HIV-infected. Only 10 of the patients with cryptococcosis were younger than 16 years old.[29,43] Cryptococcal infections occur much less frequently in children than in adults. It primarily affects HIV-infected children aged 6 to 12 years old, in whom the reported prevalence is 0.5 to 1 percent,[1,27,69] and most frequently in children whose $CD4^+$ cell counts indicate profound immunosuppression.[1,27,28] Surveillance studies in children show a 0.85 to 1 percent frequency of cryptococcal infection in HIV-infected children.[27,37] The reasons for the disparity in the frequency of cryptococcal disease between the adult and the pediatric population are unknown. Some researchers have postulated that the difference might be explained by the lack of exposure to *C. neoformans*.[13] The lower frequency of cryptococcal disease among children compared with adults seems unlikely to reflect differences in exposure because serologic surveillance performed in one U.S. city showed a 70 percent seroprevalence among children 15 years or younger.[2]

PATHOPHYSIOLOGY

Considerable evidence suggests that *C. neoformans* becomes airborne from soil or dried pigeon excreta. These organisms, or fomites, usually measure less than 2 μm in diameter and are of a size that easily can reach the lung parenchyma through the airways of the lower respiratory tract.[23] After being inhaled, the yeast may colonize the host respiratory tract without producing any disease. Infection typically is asymptomatic, and it can either be cleared or enter a dormant, latent form.[53] Impaired cell-mediated immunity of the host, virulence of the strain, and size of the inoculum are the major determinants in the infection and progression to disease. When host immunity is compromised, the dormant form can reactivate and disseminate hematogenously to cause systemic infection.[38,53] Early lesions are characterized by collections of encapsulated yeasts surrounded by a gelatinous-like material.

Four basic patterns of reaction are seen in the lung: (1) one or more peripheral granulomas, (2) granulomatous pneumonia with intra-alveolar organisms and varying degrees of inflammatory response, (3) diffuse invasion of organisms within alveolar capillaries and interstitial tissues with little or no inflammation, and (4) massive intra-alveolar and intravascular organisms.[26] The lesions of the brain and meninges are cystlike, especially in the cerebral cortex. They represent masses of yeast forms, and little inflammatory reaction, in which macrophages predominate, and *C. neoformans* may be found in the cytoplasm of these cells. Granulomatous lesions are uncommon findings in the brain. The meninges may be thickened with the gelatinous polysaccharide of the yeast capsule.[53] Bone lesions are found in approximately 5 percent of cases of disseminated cryptococcosis. Acute and

chronic inflammation is seen and often is associated with giant cells and granulomata.[3]

The variety of *C. neoformans* may be a factor in the pathology of the infection. Some studies show that infection with *C. gattii* (serotypes B and C) more often is associated with immunocompetent individuals with cerebral mass lesions (with or without hydrocephalus) and pulmonary lesions than is infection with *C. neoformans* var. *neoformans*. The histopathologic features are indistinguishable, however.[44]

The most important virulence factors of *C. neoformans* are polysaccharide capsule, production of melanin, and growth at 37° C. The extracellular polysaccharide capsule is composed of glucuronoxylomannan and galactosylomannan, and has been shown to interfere with various immunologic functions.[8,12] *C. neoformans* sheds capsular polysaccharide into tissue during infection, interfering with local neutrophil migration, cytokine production, and phagocytosis; systemically, polysaccharide shedding elicits decreased antibody responses.[53] *C. neoformans* also synthesizes melanin, which is produced in human tissues during infection and contributes to virulence by protecting the organism from oxidative antifungal mechanisms of host immune effector cells and may down-regulate T-cell response.[30]

Studies in rats show that *C. neoformans* penetrates the lung parenchyma shortly (2 hours) after infection occurs, and that control of infection and decreased fungal burden are temporarily associated with a granulomatous tissue reaction.[53] The cryptococcal capsule inhibits phagocytosis,[32] which is the main defense against the organism. Monoclonal antibodies to the capsular polysaccharide are protective against experimental infection in mice, confirming the importance of the capsule in the pathogenesis of *C. neoformans* infection.[47] The high susceptibility of athymic (nude) mice and T cell–depleted mice to *C. neoformans* is consistent with the importance of cell-mediated immunity in the defense against *C. neoformans* infection. In contrast, when normal mice are challenged with *C. neoformans*, the infection is limited by $CD4^+$ and $CD8^+$ T cells.[45]

More recent evidence indicates that *C. neoformans* is killed primarily by $CD4^+$ T cells via the effector molecule granulysin.[72] In the presence of antibody and complement, *C. neoformans* is ingested by phagocytes. With production of polysaccharide within the phagocyte, however, the yeast can survive as a facultative intracellular pathogen.[21] *C. neoformans* can transform in vivo to a more pathogenic mucoid phenotype, which may be responsible for some cases of reactivation of disease in immunocompromised hosts.[25] Despite persistent infection of pulmonary macrophages in experimental mice, a vigorous immune response to secondary challenge shows that cell-mediated immunity is not impaired systemically during persistent infection.[39]

In humans, containment of the infection in the lung is associated with the formation of a granulomatous subpleural nodule.[5] Macrophages are considered the main effector cells against *C. neoformans*. They ingest and kill the organism and produce proinflammatory cytokines, such as interleukin-12. T cells contribute as well with a T-helper (T_H1)–polarized response that activates macrophages.[53] Humoral immunity also may play a role because specific antibodies can provide opsonins for promoting efficient phagocytosis, enhancing natural killer cell function, and clearing capsular polysaccharide.[71]

At a molecular level, the enzyme inositol phosphosphingophospholipase C is crucial to the yeast's resistance to pulmonary macrophages.[65] Production of melanin by *C. neoformans* also is protective against host immune defense mechanisms.[11] Glycosphingolipid glucosylceramide is essential for the growth of *C. neoformans* in alveolar spaces and in the bloodstream.[61]

A key pathogenic feature of *C. neoformans* is its ability to grow at 37° C. The disaccharide trehalose is a stress protectant, and the trehalose synthesis pathway is essential to permissive growth at body temperature.[54] Because this pathway also is important to the pathogenicity of *C. neoformans* in homeotherms, it is thought to have a role beyond protection from temperature stress.

The genome of *C. neoformans* was sequenced more recently.[41] This information, coupled with new insight into the life cycle of the pathogen,[38] should lead to advances in understanding of the pathogenesis of cryptococcosis.

CLINICAL MANIFESTATIONS

Two primary sites of infection by *C. neoformans* are the lungs and the CNS. In HIV-negative patients infected with *C. neoformans*, 36 percent of infections affect the lung only, and 51 percent of infections have CNS involvement.[52] In HIV-infected patients, CNS and extrapulmonary infection are more frequent.[53] In a pediatric systemic fungal infection surveillance conducted in Houston, Texas, nine cases of cryptococcosis were identified over the course of 9 years. Three children presented with meningitis: two with cutaneous disease, two with overwhelming fungemia, one with gastroenteritis, and one with pulmonary involvement (Table 215–1) (R. J. Hamill et al., 2002, data not published).

Bone and joint involvement occur in 5 percent or more of cases of extrapulmonary cryptococcosis. Acute and chronic inflammation occurs, often associated with giant cells and granulomata; sarcoidosis frequently is an underlying condition.[6,53] Other sites of dissemination include skin, eye, urinary tract, adrenal, liver, lymph nodes, sinuses, gastrointestinal tract, breasts, and the female reproductive system.

PULMONARY INVOLVEMENT

Although the lung is the most common portal of entry for infection, pulmonary cryptococcosis is uncommon in children.[69] One third of pulmonary infections are asymptomatic. Approximately half of patients have cough or chest pain, however, and smaller percentages have sputum production (32%), weight loss (26%), fever (26%), and hemoptysis (18%).[50] In immunocompromised hosts, the onset may be more severe, and the course more rapid.[53] Radiographic lesions in non-immunosuppressed hosts include well-defined, noncalcified, single or multiple nodules, masslike infiltrates, hilar lymphadenopathy, pleural effusions, and lung cavitations.[53,73] In immunosuppressed hosts, radiographic findings include alveolar and interstitial infiltrates as well.[53] A comprehensive review of pediatric pulmonary cryptococcosis revealed that most affected children were immunocompromised, and almost half of them were asymptomatic. Chest radiographs showed a lower lobe nodular pattern in most cases.[69]

CENTRAL NERVOUS SYSTEM INVOLVEMENT

Meningitis occurs in more than half of cryptococcal infections affecting HIV-infected children.[27,37] Headache and fever are the most common symptoms. Nausea and vomiting occur in half of cases. Stiff neck is seen in 75 percent of patients who are not immunocompromised and in 33 percent of patients with AIDS. Other, less frequent, manifestations include alteration of consciousness, impaired mental function, cranial nerve lesions, visual deficits, papilledema, seizures, diplopia, focal neurologic deficits, photophobia, and abnormal cerebellar signs.[1,28,53] The duration of symptoms before a diagnosis is established ranges from less than 1 week to 18 months and tends to be shorter in patients with AIDS.

TABLE 215–1 Clinical Manifestations of Cryptococcal Infections in Children

Age	Comorbid Condition	Clinical Presentation	Treatment	Outcome	Reference
14 yr	AIDS ($CD4^+$ = 3/μL)	Meningitis	AMB, then FLU	Survived, residual blindness	*
6 yr	Acute lymphocytic leukemia	Cutaneous	LAMB + 5-FC, then FLU	Resolved	*
3 yr	Unknown	Cutaneous	Unknown	Survived	*
18 yr	Neurocysticercosis, ventriculoperitoneal shunt	Meningitis, peritoneal pseudocyst	FLU, shunt removal	Survived	*
4 yr	Unknown	Disseminated	Unknown	Unknown	*
9 yr	AIDS	Disseminated	FLU	Died	*
13 yr	AIDS, hemophilia	Meningitis	FLU	Survived	*
15 yr	Tuberculosis	Pulmonary	None	Survived	*
16 yr	None	Pulmonary	AMB, then FLU	Survived	47
11-16 yr	AIDS ($CD4^+$ = 15/μL)	Pulmonary (1), disseminated (3)	AMB + 5-FC, then FLU	All survived	22
5 mo-16 yr	HIV ($CD4^+$ = 0-386/μL, median 54/μL)	Meningitis, disseminated, pulmonary	None, AMB, AMB + 5-FC, then FLU	Died (23%)	1
5 mo-12 yr	HIV ($CD4^+$ count not done)	Disseminated	AMB (8/15 patients received treatment)	Died (46%)	23
13 yr	Systemic lupus erythematosus	Meningitis	AMB + FLU	Survived	31
3-17 yr	Acute lymphoblastic leukemia	Meningitis (5), cutaneous (2), disseminated (2)	AMB + 5-FC, then FLU	All survived	28

**R. J. Hamill et al.: 2002, data not published.*
AIDS, acquired immunodeficiency syndrome; AMB, amphotericin B; 5-FC, flucytosine; FLU, fluconazole; HIV, human immunodeficiency virus; LAMB, liposomal amphotericin B.

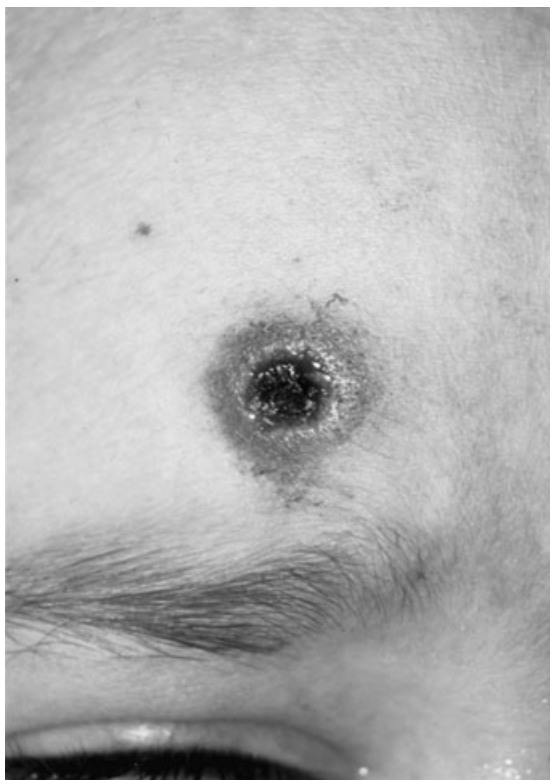

Figure 215–1 Purpuric and papular skin lesions in a 3-year-old boy who presented with acute lymphoblastic leukemia and disseminated cryptococcosis. (See companion Expert Consult web site for color version.)

CUTANEOUS LESIONS

Various skin lesions caused by *C. neoformans*, including ulcers,[70] nodules,[36] vesicles, abscesses, papules, cellulitis, acneiform plaques, and purpuric and sinus tracts, have been described (Fig. 215–1; see Fig. 64–24). Skin lesions usually represent metastases to the skin of a disseminated infection and are difficult to diagnose clinically, given the multiple presentations. Histologic examination and culture are essential to identify specifically the cryptococcal skin lesion.

EXTRAPULMONARY CRYPTOCOCCOSIS IN IMMUNOCOMPROMISED INFANTS AND CHILDREN

The extent of knowledge about cryptococcosis in infants and children is reflected by reports and reviews.[36,37] From 1966 to 1997, only 22 immunosuppressed children without AIDS and 30 children with AIDS and extrapulmonary cryptococcosis were reported. Aspects of these cases are summarized in Table 215–1.

DIAGNOSIS

The diagnosis is established by showing *C. neoformans* during the disease state. *C. neoformans* may be shown by direct examination of clinical specimens from cerebrospinal fluid (CSF), urine, sputum, bronchoalveolar lavage fluid, or aspirates of skin lesions using India ink preparations, Gram staining of smears of cytocentrifugated specimens, or mucicarmine and Masson-Fontana silver stains, which distinctively show the cell wall and the capsule of the organism; by histologic examination; by isolation in culture; and by the detection of cryptococcal antigen. During cryptococcal meningitis, the India ink CSF preparation reveals the yeast in 80 percent of cases in patients with AIDS and 50 percent of cases in patients not infected with AIDS[53]; the culture yields the organism in 87 to 100 percent, and the cryptococcal antigen test is positive in 83 to 100 percent of cases. The leukocyte count of the CSF may be normal or increased to low levels, rarely exceeding 100 cells/mm^3, and the glucose is less than 50 mg/dL in 65 to 75 percent of patients.[62]

C. neoformans can be isolated in most routine mycologic or bacteriologic media, especially chocolate agar, 3 to 7 days after being inoculated into the media.[53] Sabouraud agar and Niger seed agar medium can be used, and cultures are maintained at 30° C to 32° C (not 37° C or 25° C). The sediment from centrifuged CSF or urine specimens is spread on several plates or slants.

Alternatively, 2 to 5 mL of CSF can be inoculated into Sabouraud broth and incubated on a shaker. Cycloheximide should not be used because it may inhibit the growth of *C. neoformans*. Blood can be cultured using the automated and the lysis-centrifugation procedure (Isolator tube; Dupont, Wilmington, DE). Although blood cultures may become positive within 3 days, they should be held for 3 to 4 weeks before being considered sterile.

Detection of cryptococcal antigen by latex agglutination and enzyme immunoassay in CSF, serum, and urine offers a highly sensitive and specific diagnostic tool. Latex agglutination uses hyperimmune rabbit immunoglobulin anti–*C. neoformans* antibodies bound to latex particles. Titers of 1:4 and greater suggest cryptococcal infection. Commercial kits detect 10 ng of cryptococcal antigen and have a sensitivity rate of approximately 95 percent in experienced hands. Enzyme immunoassays use monoclonal or polyclonal antibody to detect antigen. Close agreement between latex agglutination and enzyme immunoassays has been shown.[22] There are very few false-positive results, such as the cross-reactive polysaccharide of *Trichosporon asahii* (*beigelii*) or IgM antibody complexes (i.e., rheumatoid factor). False-negative results also are unusual and may be caused by low titers, early infection, presence of immune complexes, prozone effect of high titers, or "unencapsulated" strains with low production of polysaccharide.[53]

Despite the good diagnostic utility of the cryptococcal antigen tests for screening febrile, high-risk patients, such as patients with HIV infection, they are not recommended as treatment response indicators. High titers (≥1:1024) generally reflect, however, high burden of yeasts, a poor host immune response, and a greater likelihood of therapeutic failure.[53]

TREATMENT

Three factors must be considered in guiding the treatment of cryptococcosis: (1) the degree of immunosuppression, (2) extent of infection (pulmonary or extrapulmonary with and without neural involvement), and (3) choice of drugs. Untreated, cryptococcal infection in immunosuppressed patients is uniformly fatal. Controlled studies of treatment for cryptococcal disease in pediatric populations are scarce, and most of the recommendations are obtained from the adult literature.[46,63] Several antifungal agents with therapeutic efficacy are available and include amphotericin B or one of its lipid-associated formulations, flucytosine, fluconazole, and itraconazole. Other agents include voriconazole and posaconazole, although no in vitro or clinical studies have evaluated their use in children (Table 215–2).

Standardized methods for in vitro susceptibility testing are available. The clinical utility of in vitro susceptibility testing is not clearly defined, however. Detection of resistance in vitro seems to correlate with clinical resistance[3]; however, most reported cases are relapse isolates, rather than isolates associated with initial infection.[7,68]

TREATMENT OF CRYPTOCOCCOSIS IN IMMUNOCOMPETENT HOSTS

Pulmonary and Non–Central Nervous System Disease

Some patients with normal immunocompetence and cryptococcosis of limited extent and nonprogressive disease may recover without receiving treatment. Pulmonary cryptococcal infection may resolve without treatment in immunocompetent hosts, and observation has been suggested as a reasonable course of management.[50] Given the potential for dissemination, the seriousness of CNS involvement, and the availability of effective and well-tolerated antifungal agents, however, this approach should be considered only when the patient can be followed closely and reliably.

In most infants and children, excluding extrapulmonary dissemination is difficult, and treatment is warranted. Performing a lumbar puncture is essential in all cases of pulmonary disease. Immunocompetent patients with asymptomatic and mild-to-moderate disease should be treated with fluconazole, 3 to 6 mg/kg/day for 3 to 6 months for asymptomatic disease or 6 to 12 months for mild-to-moderate disease. Itraconazole is an alternative drug for patients unable to take fluconazole (see Table 215–2). If oral azoles cannot be taken, or progression of the disease occurs, amphotericin B, 0.4 to 0.7 mg/kg/day, is recommended. Severe pulmonary disease is treated similar to CNS disease.

Central Nervous System Disease

For CNS disease, the combination of amphotericin B, 0.7 to 1 mg/kg/day, and flucytosine, 100 mg/kg/day, is used as induction therapy for 2 weeks, followed by fluconazole, 12 mg/kg/day, for a minimum of 10 weeks. Alternatively, the amphotericin B and flucytosine combination may be continued for 6 to 10 weeks. To avoid development of neutropenia, the dosage of flucytosine should be adjusted to achieve a serum level of 30 to 80 μg/mL, measured 2 hours after administration of the dose. The CSF should be examined after the first 2 weeks of therapy, at which time 60 to 90 percent of patients have sterile CSF. A positive culture at this point is an indication for extending the treatment course beyond the minimum recommendation. Fluconazole, 3 to 6 mg/kg/day, should be administered for a minimum of 10 weeks.[55,64]

Intraventricular and intrathecal amphotericin B may be needed for refractory cases. Lipid formulations of amphotericin B can be used in place of amphotericin B in patients with renal dysfunction, with similar therapeutic efficacy. Regardless of the initial regimen, some experts recommend a subsequent course of fluconazole, 3 to 6 mg/kg/day for 6 to 12 months. Treatment outcome is poor if CNS disease is not recognized and treated early.[4,19]

TABLE 215–2 Therapeutic Agents Available for Treatment of Cryptococcosis*

Drug	Dose	Route	Excretion	Major Side Effects
Amphotericin B	0.5-1.0 mg/kg/day	IV	Renal—40% over 7 days; biliary—minimal; dialysis—poorly dialyzable	Infusion-related reactions, hypokalemia, hypomagnesemia, renal impairment, renal tubular acidosis, anemia, thrombophlebitis
Flucytosine	100 mg/kg/day	PO	Renal—>90%	Nausea, vomiting, anemia, leukopenia, thrombocytopenia, hemorrhagic colitis, hepatotoxicity
Fluconazole	12 mg/kg/day	IV, PO	Renal—80%	Hepatotoxicity, nausea, headache, skin rash

**Other drugs with in vitro efficacy against C. neoformans include itraconazole, voriconazole, and posaconazole.*

TREATMENT OF CRYPTOCOCCOSIS IN IMMUNOCOMPROMISED HOSTS WITH ACQUIRED IMMUNODEFICIENCY SYNDROME

Cryptococcal Pneumonia

All HIV-infected patients with pulmonary cryptococcal infection must be treated because they are at high risk for developing disseminated infection. For children with mild-to-moderate disease, treatment with fluconazole alone is appropriate and should be followed by lifelong suppressive therapy. An alternative to fluconazole is itraconazole.[46] AIDS-infected patients responding with immune reconstitution after receiving effective highly active antiretroviral therapy (HAART) may not require lifelong maintenance therapy. For patients with severe pneumonia, amphotericin B should be used until the patient is asymptomatic, at which time fluconazole can be substituted for maintenance therapy. Patients with cancer and pulmonary cryptococcosis treated with fluconazole monotherapy generally had good outcomes.[31]

Cryptococcal Meningitis

For meningeal cryptococcosis, induction therapy consists of a combination of amphotericin B (0.7-1.0 mg/kg body weight/day) and flucytosine (25 mg/kg/dose four times daily) for a minimum of 2 weeks.[63] Induction is followed by consolidation treatment with fluconazole (12 mg/kg/daily) for a minimum of 10 weeks, or until CSF cultures are sterile.[59] Management principles of elevated intracranial pressure in children with cryptococcal meningitis are the same as for adults and include measurement of the CSF pressure and frequent therapeutic lumbar punctures or ventricular shunting for hydrocephalus and prolonged clinical or microbiologic failures.[63]

Because of the relapse rate of 50 percent, maintenance therapy is important after an acute episode of meningitis in HIV-infected individuals. Fluconazole given daily is the preferred drug for maintenance, but intravenous amphotericin B one to three times a week is a reasonable alternative, although this form of maintenance therapy may be complicated by bacterial infections related to vascular access.[57] Fluconazole has a more rapid clearance and shorter half-life in children than in adults; higher doses are recommended. Oral itraconazole or amphotericin B given one to three times weekly are alternative maintenance regimens. HIV-infected patients who are treated initially with fluconazole are at risk of relapse despite continued fluconazole.[7] Neurosurgical procedures may be required in severe cases to control cerebral edema. A ventricular shunt may be required to treat hydrocephalus.

The safety of discontinuation of secondary prophylaxis in children treated with HAART has not been studied extensively. Data from adult studies show that after successful treatment of cryptococcal meningitis, secondary prophylaxis can be discontinued in individuals who have a satisfactory response to HAART ($CD4^+$ cell count >100 to 200 cells/μL for at least 6 months).[49]

Immune reconstitution inflammatory syndrome has been described in adult and pediatric patients co-infected with HIV and *C. neoformans* who are treated with HAART.[59,66] Patients with *C. neoformans*–related immune reconstitution inflammatory syndrome present with higher CSF opening pressures, glucose levels, and cell counts.

PROGNOSIS

Based on the reports of extrapulmonary cryptococcosis in immunosuppressed infants and children, the prognosis for severe systemic cryptococcosis is reasonably good. Of the 13 children with AIDS and cryptococcosis,[37] 2 untreated patients died, and 10 of the 11 treated patients had a clinical response to amphotericin B with or without flucytosine, although 7 of the 10 had died of HIV infection at the time of the report. Of the 15 non–AIDS-infected but immunosuppressed patients reviewed by Leggiadro and associates,[36] responses also were favorable but difficult to evaluate clearly because of the presence of other diseases and complications.

Factors that suggest poor prognosis in cryptococcal meningitis are burden of yeast at presentation, patient's level of sensorium at presentation, high cryptococcal antigen titer (1:1024) and low cell count in CSF (<20 cells/mm^3), and inability to manage underlying disease.[15,56] Among patients receiving organ transplants, patients receiving liver transplants have a particularly unfavorable prognosis.[67] Patients receiving a calcineurin inhibitor have a significantly better prognosis than patients receiving other immunosuppressive therapies.[67]

PREVENTION

Immunocompromised individuals should avoid exposure to dust, especially in areas contaminated with bird droppings, particularly pigeon droppings. Long-term prophylactic therapy with fluconazole is effective in preventing recurrent cryptococcal infection. Prophylaxis with fluconazole should be considered for patients who continue to be severely immunocompromised at the end of therapy for cryptococcal infection. Prophylaxis generally is not needed by patients with cancer who experience remission and immune recovery, but should be considered until such time. Prophylaxis is indicated for patients with AIDS who have had cryptococcosis, as discussed earlier.

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CHAPTER 216

Histoplasmosis

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Histoplasmosis is the most common pulmonary and systemic mycosis in humans and affects millions of people.[200,460] Although most infections occur in areas in which the etiologic agent, *Histoplasma capsulatum*, is endemic, increasingly it is being reported from areas not previously known to be endemic. A substantial proportion of infections occur in the United States, where approximately 500,000 individuals are infected annually.[429] Of these, an estimated 55,000 to 200,000 infected individuals become symptomatic, and 1500 to 4000 require hospitalization.[275] The public health significance of *H. capsulatum* as an opportunistic infection has escalated in proportion to the increasing numbers of individuals who are immunosuppressed.[268]

H. capsulatum was described[367] first in 1906 by Darling, a pathologist in the Panama Canal Zone,[73] while examining autopsy specimens of a man from Martinique who had died of a chronic, wasting illness. Darling observed an organism within histiocytes and thought it to be an encapsulated plasmodium; the illness caused by this new pathogen was named *reticuloendothelial cytomycosis*.[271] In 1932, Dodd and Tompkins[96] showed intracellular *H. capsulatum* in the peripheral blood smear of a febrile child, and DeMonbreum[90] subsequently grew the organism from this same child and correctly classified it as a fungus. In 1944, Christie and Peterson[59] developed histoplasmin and, after subcutaneous inoculation, showed a positive cutaneous reaction in a child with histoplasmosis.

In 1945, Parsons and Zarafonetis[306] characterized the disease as rare and usually fatal after studying 71 patients with disseminated histoplasmosis. With the use of histoplasmin skin testing, however, Christie and Peterson[59] soon showed histoplasmosis to be a common and usually a mild disease. They correctly recognized that histoplasmosis was the cause of pulmonary calcifications in large numbers of patients who were presumed to have tuberculosis, but in whom tuberculin skin tests were negative. Further characterization of the natural habitat and transmission of the fungus occurred in the late 1940s and early 1950s with demonstration of the organism in soil and air samples.[3,111,339,430]

ORGANISM

H. capsulatum var. *capsulatum*, the anamorphic (asexual) form of the organism, is a thermally dimorphic, saprophytic fungus of the class Ascomycetes, family Gymnoascaceae, subdivision Ascomycotina. The mold form displays heterothallic sexual reproduction with plus (+) and minus (–) mating types. Ninety percent of infections are caused by the minus type. The ascomycetous teleomorph produced by mating opposite types was isolated in 1972 and termed *Emmonsiella capsulata*[218]; the current taxonomy of this perfect (sexual) form is *Ajellomyces capsulatus*.[261,340] Because the fungus is isolated in the laboratory as the anamorph, the more common name *H. capsulatum* is used in this text.

At temperatures of 25° C to 30° C, the mold form grows as a fluffy colony with an aerial mycelium that varies from white to buff brown. Mycelia, small oval microconidia (3 to 5 μm) that are attached laterally to hyphae, and tuberculate and nontuberculate macroconidia (8 to 16 μm) are seen microscopically (Fig. 216–1).[423] The organism grows slowly, with 1 to 2 weeks required for laboratory strains and 8 to 12 weeks for growth from clinical specimens. When cultured at 37° C on enriched media containing cysteine, the mold transforms to the yeast form in 7 to 10 days.[275] The small, heaped, yellow-white, pasty colonies appear microscopically as ovoid, budding yeasts (1 to 3 μm × 3 to 5 μm) with rare pseudohyphae.[275,297]

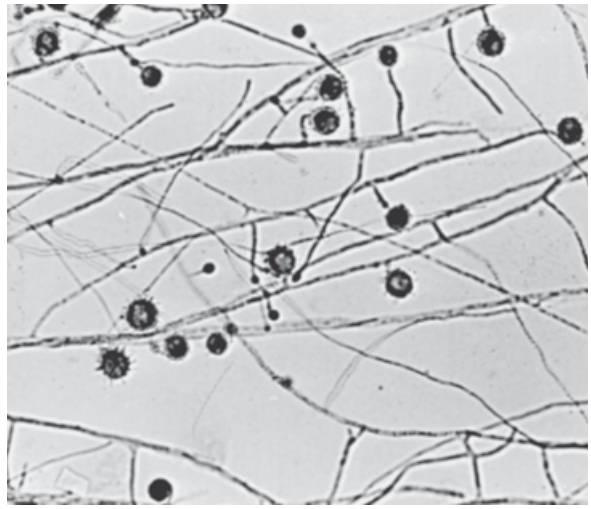

Figure 216–1 Culture of *Histoplasma capsulatum* from sputum illustrating tuberculate macroaleuriospores and microaleuriospores (lactophenol cotton blue, ×52).

In its natural habitat, the soil, *H. capsulatum* exists as mycelia and spores. It survives best in moist (95 to 100% humidity), nitrogen-rich soil at temperatures of 37° C or greater and can be found 1 mile below the soil surface.[417,429] The droppings in roosting sites of chickens, blackbirds, and starlings contain nutrients that promote fungal growth and, at least in the case of chickens, may contain substances that discourage the growth of competitive organisms in the soil.[376] Many species of colonial New World bats become infected and excrete the fungus in their guano; bat guano also contains substances that encourage growth of the fungus.[110,244,392,393] Although birds excrete the fungus in droppings, they remain uninfected, probably because of their high body temperature. *H. capsulatum* can remain viable in droppings or other contaminated sites for many years.[475]

Early techniques used to differentiate strains of *H. capsulatum* included serotyping and chemotyping.[97] Examinations of restriction fragment length polymorphisms of DNA have delineated three broad classes that exhibit geographic and possibly clinical differences.[103,207,420] Class 2 strains are found most frequently in North America, and class 3 strains are found in Central and South America. Class 1 strains are isolated almost exclusively from immunocompromised patients. Individual strains within each class have been examined further with additional nucleic acid–based and genome-wide approaches to reveal considerable genetic diversity.[50,183] Such strain differences are useful for molecular epidemiologic investigations.[183,195,279,337,394,493] Studies have suggested that patients with acquired immunodeficiency syndrome (AIDS) may be infected with less virulent, temperature-sensitive variants of *H. capsulatum*.[381] Additional investigations using molecular methodology have suggested that genetic differences in the pathogen may underlie clinical differences.[168,195]

Histoplasma duboisii, the cause of African histoplasmosis, was reclassified as a variant of *H. capsulatum* when investigators

showed that mating of isolates from each type produced cleistothecia and ascospores identical to those of *E. capsulata* (now termed *A. capsulatus*).[219,339] *H. capsulatum* var. *duboisii* is indistinguishable from *H. capsulatum* var. *capsulatum* in its mycelial stage, but its tissue phase is much larger and consists of thick-walled oval yeast forms that are 10 to 15 μm in diameter. Infections caused by *H. capsulatum* var. *duboisii* have been described across central and western Africa, and cases occur along with histoplasmosis caused by *H. capsulatum*.[155,297] A more recent phylogeographic analysis[196] of both varieties that cause human disease and another, *H. capsulatum* var. *farciniminosum* (an Old World horse pathogen), using DNA sequencing of four independent protein-coding genes questioned the validity of the classification.

EPIDEMIOLOGY

Histoplasmosis is acquired almost exclusively when environmental sites that are contaminated with *H. capsulatum* are disturbed, and spores become aerosolized and are inhaled. Early understanding of its epidemiology was influenced heavily by analyses of point-source outbreaks of acute infection among small groups of people exposed to relatively large inocula. Because most infections are either asymptomatic or mild and self-limited, however, such investigations skewed the true epidemiology. A more comprehensive understanding was achieved when the results of skin test epidemiology; cultures of environmental sources, birds, and other animals; and careful analysis of the history of exposure also were considered.[49]

Histoplasmosis has been reported worldwide in tropical, subtropical, and, most frequently, temperate areas.[118] Regions with a high incidence of infection are termed *endemic*, and most infections occur in these areas. Most infections occur sporadically and cannot be associated with exposure to a specific site or activity. They are presumed to result when environmental conditions are conducive to growth of the fungus, and dry windy weather or other events or activities facilitate the airborne transmission of spores. Although early reports emphasized that the infection usually was associated with occupational exposure in primarily rural environments, infection is recognized commonly in urban areas,[184,249,356] where it may affect numerous people.[468] Infections occur commonly in children and frequently remain unrecognized because, as investigations of outbreaks have shown, children often remain asymptomatic.[37,157,177] In a skin test survey of a highly endemic area in Tennessee, skin test reactivity was found in 80 percent of children 10 years old.[494]

The incidence of human infection varies widely in endemic regions. Within short distances of areas in which residents show only a moderate incidence of skin test reactivity, reactivity may be hyperendemic in areas in which the incidence of infection is considerably higher. Still more localized and very heavily contaminated sites, termed *microfoci*, may be found in which localized, sometimes severe, outbreaks occur. Because the droppings of birds and bats[92] accelerate the growth of the fungus in the environment, sites that have been implicated in localized outbreaks have included blackbird and pigeon roosting areas, chicken houses, bat-infested caves,[21,251] attics, chimneys, old buildings, and decaying woodpiles and trees. Activities that disturb these types of sites have been implicated in localized outbreaks.[177,321,429] Infections in children have been associated with exploring caves; playing in barns or hollow trees; cleaning abandoned buildings; cutting firewood or decayed tree stumps; renovating the walls, attics, or basements of older homes; digging in contaminated sites; being downwind of the excavation/demolition of buildings; cleaning seldom-used fireplaces; and being in indoor environments contaminated by fungal aerosols gaining access to air intakes.[55,249]

Edwards and colleagues[105,106] described the geographic distribution of *H. capsulatum* in the United States by performing histoplasmin skin testing on 275,558 Navy recruits between 1958 and 1965. The highest incidence of reactivity was found in residents of the Ohio-Mississippi-Missouri, the St. Lawrence, and the Rio Grande River Valleys.[275] In four states (Arkansas, Kentucky, Missouri, and Tennessee), the percentage of positive adult reactors ranged from 57 to 68 percent. In the adjacent states (Illinois, Indiana, Ohio, and Oklahoma), skin test reactivity ranged from 50 to 73 percent among adults in farm areas. Of the other seven states in the endemic area, lifetime prevalence rates exceeding 50 percent were shown in one or more counties.[2] Epidemiologic studies of this scale have not been repeated, and, as has been shown with other endemic mycoses,[224] population shifts and changes in land use may alter these patterns.

Numerous histoplasmosis outbreaks have been documented and offer some means of assessing the epidemiology and clinical spectrum of infections acquired in point-source and sustained outbreaks. The largest sustained outbreak was reported to occur in Indianapolis, Indiana, where it involved an estimated 100,000 individuals within a 400-square-mile area and lasted nearly 1 year.[468] Symptomatic cases were identified in 435 individuals (including 49 <15 years old), and disseminated disease developed in 46. Individuals 15 to 34 years old were more likely to become infected than were other age groups. An equal sex distribution of cases occurred before puberty; males predominated in a 3:1 ratio in older individuals. Localized outbreaks related to high-risk sites and activities[44,351,414] are well documented. Isolated cases may not be associated readily with such activities. Infections acquired during travel[19,116,190,191,305,452] to endemic regions[18,114,131,428] are being reported more frequently. Infections acquired in areas not previously recognized to be endemic, such as occurred in a large group of college students whose only known common exposure was vacationing at a hotel in Mexico,[278] require consideration of the diagnosis of histoplasmosis in patients presenting with compatible clinical findings.[16,41,47,350] Rarely, infections may occur in non-endemic areas and result from reactivation of quiescent infection in patients who become immunosuppressed.[180,405]

Although various wild and domestic animals may become infected, transmission of histoplasmosis to humans does not occur.[272] Infection of an infant after exposure to a pillow that contained contaminated feathers has been reported.[48] Although a case of sexual transmission that resulted from exposure to the mucosal lesions of a patient with disseminated histoplasmosis has been reported,[374] human-to-human transmission is extremely rare. Transmission has been confirmed, with the use of molecular typing methodology, in the recipients of two cadaveric organs transplanted from a donor who had resided in an endemic area.[235] Infections caused by direct inoculation of contaminated material in the environment or from laboratory accidents are rare.

PATHOPHYSIOLOGY

H. capsulatum has unique biologic interactions with human macrophages, dendritic cells, and neutrophils,[288] the outcome of which contributes to its success as a human pathogen. Key among these interactions are its ability to infect and gain access to macrophages[484]; to alter the intracellular environment, allowing it to survive, replicate, and disseminate; to remain viable during clinically unapparent infection; and to become reactivated when host immune factors permit.[45,485] The onset of infection results in a brisk innate immune response that is followed by an acquired immune response after about 2 weeks. During these events, the fungus induces an orderly modulation of the host inflammatory and cytokine responses. Production of cytokine begins within 24 hours of primary infection and continues for approximately 3 weeks, bridging the innate and acquired immune reactions. Suc-

cessful clearance of the infection[20,192,296,332,496] depends primarily on the functional integrity of cellular immunity. The clinical manifestations and outcome of the infection are determined by this complex interplay of events.

The incubation period of histoplasmosis varies depending on the size of the inoculum, the integrity of the host immune response, the presence of immunity from previous infection, and strain-to-strain differences in fungal virulence.[143,213] Although the range of incubation periods is reported to be 1 to 3 weeks in non-immune hosts, it is based largely on data gathered from point-source outbreaks in which the time of exposure is readily determined. In these settings, the size of the infecting inoculum probably is larger, however, than that resulting in sporadic infections.[49] Because most infections occur sporadically and either are asymptomatic or result in nonspecific flulike illnesses that are not diagnosed, the upper range may be longer. In patients who retain specific cellular (protective) immunity from previous infection, re-exposure results in less severe symptoms and shorter incubation periods, usually approximately 4 to 7 days.[49] Infants and individuals with a primary or acquired deficiency in cellular immune function are more likely to experience symptomatic illness after exposure.[143]

In experimental animals, acute pneumonitis develops after inhalation of *H. capsulatum* microconidia or mycelial fragments.[24] Because of their smaller size, microconidia gain access to the terminal alveoli and bronchioles more readily than macroconidia do. Within 2 to 3 days after being inhaled, spores germinate and convert to the yeast form of the fungus. This step apparently is a critical determinant of virulence because chemical blockage of the conversion from the mycelial to the yeast phase impairs virulence[285] in a murine model.[270] Progress in understanding the molecular cell biology and genetics of the mold-to-yeast transition has been reviewed more recently.[178] Molecular techniques also have identified two genes (*URA5*, *CBP1*) that have a role in pathogenesis.[483,485]

Additional determinants of fungal virulence have been described and include a role for α-1,3-glucan.[333] In the first week, a neutrophil response occurs at the site of the infection[24]; it is followed in approximately 2 weeks by the accumulation of helper T lymphocytes and macrophages.[323] The yeast forms attach to integrins of the CD11/CD18 group of cell surface receptors and enter neutrophils[363] and macrophages.[40,100] This process may be an adaptive microbe entry mechanism that enables the organism to evade the host oxidative burst response associated with host-directed opsonophagocytosis.[485] Human neutrophils possess fungistatic activity that resides within azurophilic granules.[38,290] Neutrophils have been shown to play a role in the primary immune response in a murine model[498]; however, depletion of neutrophils in a systemic model of secondary infection did not alter the course.

Traditional opsonophagocytosis facilitated by binding to complement or to immunoglobulin after the development of humoral immunity also occurs.[485] This process exposes the yeast to the full array of antimicrobial defense mechanisms.[484] Microbe-directed binding using a specific ligand (adhesin) on the yeast's surface has been identified as *H. capsulatum* HSP 60.[15,42,245] This heat-shock protein also has been shown to induce immunity to lethal and sublethal infection in a mouse model, a complex effect related to its ability to stimulate $CD4^+$ T lymphocytes.[82]

Yeast seems to have a competitive advantage within the membrane-bound vacuoles of macrophages,[286,287] and the asylum seems to protect it from pulmonary collectin-mediated killing.[260] In mouse models, additional strategies used by the organism to survive and replicate in this ordinarily hostile environment include increasing the alkalinity, tolerating nutrient starvation, and resisting the toxic effects of host reactive oxygen and nitrogen intermediates, degradative enzymes, and antimicrobial peptides.[485] Mouse macrophages differ from human macrophages because the latter mount a respiratory burst without the need for opsonization, as is required for murine macrophages. Lysis of infected cells results in the release of additional yeasts and infection of additional macrophages. During this period of fungal sequestration and replication, lymphohematogenous dissemination distributes yeast from the lungs to the reticuloendothelial system and to other organs.[286] Without the development of specific cellular immunity, unrestricted growth of the fungus is lethal.[287,323]

Although infection usually results in a brisk antibody response, humoral immunity does not contribute substantially to primary or secondary immunity.[255,294,352] A mouse model has shown, however, that antibodies to a cell surface protein of the fungus altered the intracellular fate of the fungus and mediated protection.[17,291] Human dendritic cells that reside in the lung link the innate and adaptive immune responses. More efficient antigen-presenting cells than alveolar macrophages, dendritic cells use the fibronectin receptor, phagocytose and rapidly degrade the organism, present *H. capsulatum* antigen for lymphocyte proliferation, and facilitate the induction of cell-mediated immunity.[137,287] Dendritic cells also have been shown to present exogenous antigen through uptake of apoptotic macrophage-associated fungal antigens (cross-presentation).[237] Some of the strategies used by the yeast that facilitate its survival and replication within macrophages, such as prevention of phagolysosomal fusion, do not occur in dendritic cells. The resultant rapid destruction of yeast within human dendritic cells constitutes an important role for these cells in the innate immune response[287] and seems to result from exposure to lysosomal hydrolases.[136]

After they participate in the innate immune response, several endogenous cytokines induced by the development of acquired cellular immunity[287] activate macrophages, which act as the primary cellular effectors that control the infection.[286] Cytokines that play key roles in the resolution of primary or secondary infection (protective immunity) in animal models include tumor necrosis factor-α (TNF-α),[8,10,11,78,80,488,498] interferon-γ (IFN-γ),[8,9,64,222,419] interleukin-4 (IL-4),[78] IL-12,[9,46,499,500] IL-1,[86] and granulocyte-macrophage colony-stimulating factor.[7,9,10,80,85,377] The effect of IL-12 results from its induction of IFN-γ; it is active in primary infection, but not in re-infection.[9] Deficiency of IL-10 has been shown to confer a salutary effect on histoplasmosis in a mouse model.[83] Apoptosis, a crucial element of protective immunity in the mouse model, when inhibited, is accompanied by elevated production of IL-4 and IL-10 and exacerbation of the severity of infection.[6]

Cytokines that activate human macrophages and induce them to become fungistatic are granulocyte-macrophage colony-stimulating factor, macrophage-stimulating factor, and IL-3[78,289]; the mechanisms by which the colony-stimulating factors stimulate fungistatic activity are not fully elucidated, but have been shown to not require acidification to kill and degrade yeast.[291] TNF-α[43,78,84,226,482] is a central and critical mediator of resistance in mice and humans, although its effect on cellular targets differs.[161] Human macrophages also express antifungal activity that is not cytokine-dependent. The latter mechanism is stimulated by attachment of macrophages to type 1 collagen matrices and is mediated at least partly by overcoming the ability of yeasts to inhibit phagolysosomal fusion.[292]

In addition to macrophages, specific T-cell subpopulations also are capable of killing *H. capsulatum*. $CD4^+$ cells have been shown to be essential for fungal clearance in mouse models.[79,140,236] The importance of these cells in human infection is supported by observations showing that the risk of severe histoplasmosis developing in adults with human immunodeficiency virus (HIV) infection increases when the $CD4^+$ count decreases to less than 200/μL.[447] $CD8^+$ cells also mediate immunity in mice,[236] but their contribution is not as significant as that of $CD4^+$ cells. $CD8^+$ cell clearance of *H. capsulatum* involves perforin-dependent and

perforin-independent mechanisms.[497] Perforin also plays an independent and essential role in primary immunity in a mouse model.[497] Cytokines such as IFN-γ and TNF-α also work in concert with cytolytic mechanisms to provide protective immunity against *H. capsulatum*.[497] The role of natural killer cells has been studied in murine models.[308] Natural killer cells participate to a limited degree in the immune response in immunocompetent animals and play a more substantial role in animals depleted of T cells.[287]

In immunocompetent hosts, cellular immunity is suppressed[312] early in the course of histoplasmosis, especially in disseminated histoplasmosis of infancy.[61,296] Cellular immune function returns to normal after 4 to 6 weeks of treatment. In the mouse model, *H. capsulatum* infection is associated with the generation of suppressor T-cell activity; this activity depresses the production of T cell–dependent, delayed-type hypersensitivity, mitogen-induced lymphocyte transformation, and cytotoxic activity.[20] T-cell immunity develops approximately 10 to 21 days after exposure, and splenic suppressor T-cell numbers decrease, while helper T cells increase.

In a study of the cellular immune response of patients infected with HIV-1 and with recent onset of histoplasmosis, all had nonreactive skin tests. Lymphoproliferative responses and IFN-γ production were depressed in patients with $CD4^+$ counts of 200 to 500 cells/mm^3; lymphoproliferative responses approached normal in patients with $CD4^+$ counts greater than 500 cells/mm^3.[412] In addition to having defective cellular immunity resulting from decreased numbers of $CD4^+$ cells, patients with HIV infection have macrophages that show a diminished ability to control *H. capsulatum*. These macrophages have a profound defect in their ability to recognize and bind to yeast and show permissiveness for intracellular growth of the fungus.[56] The HIV envelope glycoprotein gp120 seems to play a role in inhibiting phagocytosis of *H. capsulatum* by macrophages, but it is not responsible for the capacity for accelerated growth of yeast within macrophages.[57]

PATHOLOGY

With the exception of patients who have severe progressive disseminated infection, particularly patients with preexisting primary or acquired cellular immune dysfunction, histopathologic findings in histoplasmosis are characterized by granulomatous inflammation, usually in association with caseating and noncaseating granulomata. The mature granuloma serves the host by isolating the inflammation, protecting adjacent healthy tissues, inhibiting growth of the organism, and preventing systemic dissemination. The effectiveness of this pathologic response is underscored by the wide prevalence of asymptomatic and self-limited infections in geographic regions where skin test reactivity exceeds 90 percent. At the same time, a benefit that accrues to the pathogen in this microenvironment is long-term latency, sometimes ending with reactivation in the setting of late, acquired immune dysfunction.[171] An animal model that permits investigation of this interface between the pathogen and its host has been described.[171]

Typical granulomata appear after the development of an effective acquired immune response; the cellular elements in these lesions consist primarily of mononuclear phagocytes and lymphocytes, largely T cells. Langerhans giant cells often are present, and typical yeast forms occasionally, but not consistently, are visible within macrophages. Inflammation ultimately progresses to fibrosis and often is accompanied by calcification.[143] The rate of calcification is age-dependent, and it may occur within months in children and over several years in adults.[143,385] Exuberant granulomatous inflammation or fibrosis or both can result in obstruction or dysfunction of adjacent mediastinal or, less commonly, abdominal[335] structures. Although viable yeasts remain in quiescent granulomata, they are few in number and rarely seen. In areas endemic for histoplasmosis, old granulomata in the lung, bone marrow, or other sites may be seen as incidental findings, the significance of which depends on clinical assessment. Yeasts almost never are observed in histologic sections of extensive fibrosis in late histoplasmosis.

In patients with acute progressive manifestations of histoplasmosis, especially patients with preexisting cellular immune dysfunction,[118,274] or in infants, the inflammatory response is impaired, and granuloma formation is poor.[145] In these instances, extensive parasitization of macrophages by yeasts occurs, and organisms may be seen readily in various reticuloendothelial structures, especially the bone marrow, and sometimes within leukocytes in the peripheral blood.[296] Many other organ systems are involved. The histopathologic findings in central nervous system (CNS) infection consist of granulomatous basilar meningeal and vascular inflammation, perineural inflammatory changes in cranial nerves, and the presence of many organisms at the periphery of mass lesions.[437] In disseminated infections, the adrenals frequently are affected,[154,217,390] and a wide spectrum of pathologic abnormalities of the gastrointestinal tract is seen.[22,221]

CLINICAL MANIFESTATIONS

Approximately 95 percent of infections caused by *H. capsulatum* occur in normal individuals, and either are asymptomatic or cause brief, self-limited illness with no sequelae. The only residual findings are the incidental radiographic demonstration of typical granulomata in the lung parenchyma or calcifications in the hilar/mediastinal lymph nodes or the spleen. In immunocompetent hosts, the size of the inoculum probably is the principal determinant of whether infection is accompanied by clinical symptoms. After exposure to small inocula, only 1 percent of individuals develop symptoms, but after heavy exposure, 50 to 100 percent become ill.[429] Additionally, the symptoms that develop are more severe in individuals who are exposed to large inocula. Severity may be reduced by preexisting immunity derived from previous infection. Protection is incomplete, however, and severe disease can occur after re-exposure to a heavy inoculum.

A crucial determinant of susceptibility and severity also is host-dependent. Individuals with primary or acquired disorders of cellular immunity and otherwise normal infants younger than 1 year have a disproportionately high risk for developing symptoms after exposure. These groups also are at far greater risk for progression of early fungal dissemination. The higher risk for dissemination during infancy is thought to result from age-related, relative immaturity of immune function, rather than a primary immunodeficiency disorder.

As with the other endemic mycoses, histoplasmosis begins as an acute inflammatory pneumonitis, undergoes self-limited or progressive dissemination, and requires an effective host immune response for its control. Clinical manifestations (Table 216–1) vary because they result from any or all of the following: the nonspecific systemic effects caused by the infection, symptoms resulting from either the primary inflammatory focus or perturbation of function of anatomic structures adjacent to primary sites of inflammation, hypersensitivity phenomena resulting from the acquired immune response, symptoms caused by hematogenously infected sites, life-threatening multisystem symptoms resulting from progressive parasitization of the reticuloendothelial and other organ systems, symptoms caused by chronically progressive forms of pneumonitis or dissemination, or exacerbation of quiescent disease. Recognition, confirmation of the diagnosis, and selection of prudent therapeutic options require understanding of these diverse clinical manifestations. Aside from patients with preexisting conditions or receiving therapy that

TABLE 216–1 Clinical Manifestations of Histoplasmosis

Asymptomatic infection
Pulmonary disease
Acute (mild, moderate, severe forms)
Mediastinal adenitis
Mediastinal granuloma*
Obstruction/dysfunction of contiguous mediastinal structures by granulomatous inflammation of lymph nodes
Pericarditis
Chronic disease with cavitation†
Broncholithiasis†
Lithoptysis†
Mediastinal fibrosis‡
Progressive disseminated infection
Acute
Infancy (PDH of infancy) with or without meningitis
Immunocompromised (non-HIV)
HIV
Subacute†
Chronic†
Presumed ocular histoplasmosis†
Primary cutaneous infection†
Isolated meningitis†

**Complications similar to those of mediastinal adenitis, but presentation either unassociated with acute infection or following asymptomatic or unrecognized infection.*
†*Rare in children.*
‡*Infrequent manifestation during childhood.*
HIV, human immunodeficiency virus; PDH, progressive disseminated histoplasmosis.

impairs immune function, all patients with serious infections, disseminated disease, persistent antigenuria after the completion of therapy, relapse, or recurrent infection should undergo comprehensive assessment of immune function.

PULMONARY HISTOPLASMOSIS

The most common symptoms of acute primary histoplasmosis encompass a spectrum[441] of respiratory and systemic complaints of varying severity. Eighty percent of infections that are accompanied by symptoms are mild, undifferentiated, flulike illnesses with cough, myalgia, headache, and variable low-grade fever. Symptoms usually are self-limited and resolve in 3 to 5 days.[429] In cases of more significant fungal exposure, fever is present, and symptoms can include headache, myalgia, chills, persistent cough, and nonpleuritic chest pain.[143] Illness may persist for 2 weeks and be associated with nausea, asthenia, weight loss, or fatigue. This manifestation also is self-limited, although fatigue and weight loss improve slowly after the fever resolves.

In primary infections that last longer than 2 weeks, fever persists and is accompanied by greater weight loss, chills, night sweats, fatigue, and chest pain. The chest pain that occurs in histoplasmosis typically is nonpleuritic, may be substernal or lateralized, usually is brief in duration, and recurs frequently. This pattern of chest pain may last 1 week to a few months.[143] Wheezing may be an early symptom.[43,427] Hepatosplenomegaly occasionally is present, although its occurrence should raise suspicion of early dissemination. In these prolonged illnesses, resolution without treatment may occur, but with the safe and effective oral antifungal agents available, most experts recommend treatment with antifungal therapy.

Finally, in acute primary infections that occur after intense exposure to numerous spores, the resulting diffuse pneumonitis may be associated with dyspnea or adult respiratory distress syndrome early in the infection. In these instances, the ordinarily self-limited early dissemination has a high risk of becoming progressive, and treatment is required. After development of the respiratory complaints that accompany primary infection, rheumatologic syndromes may be seen within several months of infection. They usually consist of erythema multiforme,[269] erythema nodosum,[143,242,304,368] acute migratory polyarthritis, or any combination of these conditions.[63,345] Although histoplasmosis-induced erythema nodosum is seen during childhood and adolescence, arthritis is uncommon. The rheumatologic symptoms are immune-mediated and usually self-limited or respond to anti-inflammatory therapy.

Various symptoms may result from complications arising from intrathoracic or, less commonly, intra-abdominal lymphadenitis caused by *H. capsulatum*. One of the most problematic, often termed *mediastinal lymphadenitis*, is acute primary infection accompanied by fever, weight loss, and a mediastinal mass visible on chest radiograph. This scenario and the presence of mediastinal lymphadenopathy in the absence of any recognized clinical symptoms often require definitive diagnosis to exclude neoplasm, especially lymphoma.[127,456,487] Pediatric series that have examined the definitive diagnosis of such manifestations have found rates of histoplasmosis to range from 1 to 57 percent,[33,127,426,487] a discrepancy best explained by considering whether the study population lived in an endemic region. In a highly endemic region, the cause of a mass within the middle mediastinum was histoplasmosis if the *Histoplasma* complement-fixation (CF) titer was greater than 1:16.[127]

Complications of acute primary histoplasmosis may be seen when granulomatous lymphadenitis results in inflammation, compression, or obstruction of contiguous structures within the thorax; this manifestation occasionally is termed *mediastinal granuloma*. Structures most commonly affected are, in decreasing order of frequency, the bronchi, trachea, pericardium, pulmonary vasculature and great vessels, lymphatics,[407] esophagus, and nerves.[102,307,338,486] Symptoms include bronchial compression or obstruction, or both,[383] which sometimes is associated with distal pneumonitis; pericarditis; pulmonary infarction; esophageal diverticula, fistula formation, or dysmotility, alone or in combination; tracheoesophageal fistula formation; and phrenic or recurrent laryngeal nerve palsy. Presumably because of their more pliable airways, children are more susceptible than adults to tracheobronchial compression as a result of encroachment by enlarged lymph nodes.[129] The risk of involvement of contiguous structures by such mediastinal granulomata may be related to the thickness of the capsule, rather than the overall size of the node.[143]

In highly endemic regions, histoplasmosis is the cause of 25 percent of acute pericarditis cases; approximately one fourth of patients have symptoms of tamponade,[430] although it rarely is seen in children. In almost all instances, pericardial effusion results from inflammation caused by infected lymph nodes adjacent to the pericardium, and not by frank fungal pericarditis.[492] The pericardial fluid is exudative, bloody, and almost always sterile.[468] Most patients with *Histoplasma* pericarditis quickly improve with administration of nonsteroidal anti-inflammatory drugs, and neither acute drainage nor pericardiectomy is needed. Rarely, contiguous adenitis or broncholiths erode through the pericardium and cause fungal contamination of the pericardial space.[143,492] Pleural effusions occur very rarely in children with histoplasmosis, and, similar to pericarditis, seem to result from pleural inflammatory reactions to adjacent granulomata; cultures of pleural fluid are negative.[329] Broncholithiasis may result from calcifications that erode through bronchi,[28] but they rarely occur in children. Lower cervical[262,430] or supraclavicular adenitis is an infrequent manifestation of histoplasmosis and usually is seen in association with mediastinal involvement. Lymphadenitis occasionally affects the intra-abdominal lymph nodes and has been reported to cause biliary tract obstruction[309,335] in children.

Subacute or chronic manifestations of intrathoracic histoplasmosis may occur in children, although they most commonly affect older adults. Of these entities, mediastinal fibrosis may

affect adolescents. In these rare instances, granulomatous inflammation progresses to dense fibrosis and may cause stenosis, obstruction, or malfunction of contiguous critical mediastinal structures. Although symptoms are similar to those caused by active granulomatous inflammation that impairs the function of these structures, the fibrosis is progressive and irreversible and responds to neither antifungal therapy nor anti-inflammatory agents.[248] Common complications include the superior vena cava syndrome and stenosis or obstruction of the trachea, bronchi, pulmonary artery, or esophagus.[161]

Late constrictive pericarditis was reported[143] to develop in 14 percent of adults with *Histoplasma* pericarditis, but data have been descriptive. Long-term data in children are lacking; however, a 30-year experience in a highly endemic area has failed to identify histoplasmosis-induced constrictive pericarditis during childhood (M. Kleiman, 2007, personal experience). Additional late complications of intrathoracic histoplasmosis seen primarily in adults include pulmonary histoplasmomas (lung nodules), which are granulomata in the peripheral lung fields that become encased in dense fibrous tissue, often with concentric layers of calcification. They enlarge over several years and may cause symptoms of a mass lesion[146] or manifest as an isolated pulmonary mass. Endobronchial lesions characteristically manifest with stricture, obstruction, or hemoptysis, and mimic bronchogenic neoplasms.[194,346]

Cavitary (also termed *chronic*) pulmonary histoplasmosis rarely has been reported in a child,[27,95] and typically is seen in adults with underlying chronic obstructive pulmonary disease. Symptoms include episodes of low-grade fever, productive cough, weakness, weight loss, and fatigue.[148,200] Early pulmonary findings consist of apical interstitial pneumonitis that eventually progresses to fibrosis, cavitation, and gradual spread to uninvolved areas of the lung.[460] If chronic histoplasmosis goes untreated, the disease is fatal.[200] Disseminated infection eventually develops in approximately 80 percent of patients with this disorder.[123,348]

PRIMARY CUTANEOUS HISTOPLASMOSIS

Numerous reports[298,418] describe apparently immunocompetent patients who present with mucosal,[132,273,344,374] corneal,[401] or cutaneous ulcerated lesions that show characteristic pathologic changes and morphologic and cultural documentation of *H. capsulatum* and resolve without treatment. These lesions have been considered to be primary, localized infections.[399] Aside from several cases that reasonably may be attributed to documented instances of direct inoculation,[216,374] many of these reports fail to provide sufficient evidence[113,225] to exclude confidently low-grade, chronic dissemination with histoplasmosis.[460] Comprehensive clinical and laboratory evaluation and long-term follow-up[69,491] are needed to attribute with confidence mucocutaneous lesions to localized infections with *H. capsulatum*.

PROGRESSIVE DISSEMINATED HISTOPLASMOSIS

Progressive disseminated histoplasmosis (PDH) occurs most frequently in immunodeficient patients and patients at the extremes of age.[145,358,460] The clinical entity is defined as an illness that is accompanied by active replication of *H. capsulatum* in multiple organ systems. Symptoms are prolonged and usually last longer than 3 weeks. Laboratory evidence of dissemination may include anemia, thrombocytopenia, or leukopenia; persistent antigenuria or antigenemia or both; demonstration of granulomas in extrapulmonary tissue; histopathologic demonstration of compatible yeast; or growth of the fungus in culture.[452] This definition excludes the transient fungal dissemination that uniformly occurs in early primary infection and is aborted by the host's acquired cellular immune response. It does encompass, however, the rare instance in which intense exposure to a large fungal inoculum results in severe primary infection that overwhelms the host's ability to mount an effective cellular immune response, allowing the usually self-limited primary dissemination to progress.

Distinct clinical presentations of PDH in adults permit subclassification into acute, subacute, and chronic forms. In contrast, PDH in children occurs almost exclusively as an acute, progressive, life-threatening infection.[186] Most of these infections develop in immunocompetent infants younger than 1 year, or in children with acquired or primary cellular immunodeficiency. Rarely, PDH occurs in an immunocompetent child.[74] Transplacental infection has been reported to occur in infants of women with PDH complicating HIV infection.[474]

PDH may result from exogenous exposure of a susceptible or immune host or from reactivation of endogenous quiescent foci of infection. Precise differentiation of these mechanisms of pathogenesis is complicated by the risk for re-exposure in endemic areas and the relative unavailability of a method that easily distinguishes strain differences in isolates. Although reactivation of infection may occur in an immunosuppressed host,[180,405] epidemiologic data in immunosuppressed individuals who reside in areas highly endemic for histoplasmosis favor exogenous exposure as the most common mechanism. Supporting evidence consists of observations showing that rates of PDH in immunocompromised patients increase only during periods in which infection rates increase in the general population and do not increase in interepidemic periods.[314,362,413,471] Using the results of histoplasmin skin testing obtained at the time of diagnosis of malignancy in a large cohort of children, Hughes[176] found that skin test reactivity was absent in all children in whom PDH subsequently developed, and that in none of those known to be skin test positive at the time of diagnosis of neoplastic diseases did PDH develop later.

Progressive Disseminated Histoplasmosis of Infancy

PDH of infancy usually affects children younger than 1 year and has been reported to occur in a 6-week-old infant.[229,296] Rare instances of transplacental[474] infection have been reported. It usually is a subacute illness in which symptoms often are reported to have been present for 1 to 12 weeks before the patient undergoes an initial evaluation; as the disease progresses, patients eventually become profoundly ill and usually die unless treated. Early symptoms include variable fever, failure to thrive,[382] and hepatosplenomegaly in almost all instances. Pallor, cough, tachypnea, oropharyngeal ulcerations, lymphadenopathy, gastrointestinal bleeding, or hemorrhagic skin lesions may develop. Abnormalities that are seen frequently in laboratory studies, and inexorably worsen, include anemia, thrombocytopenia, leukopenia,[145,229] disseminated intravascular coagulopathy, and marked depression of immunoregulatory cells[61,296]; these abnormalities reflect the overwhelming fungal parasitization of the reticuloendothelial system and often suggest the diagnosis of a lymphoreticular malignancy.[1] The chest radiograph may show signs of focal pneumonitis, mediastinal adenopathy, or miliary disease, but more than 50 percent of chest radiographs are normal at the time the diagnosis is made. Organs commonly affected are the spleen, liver, bone marrow, lymph nodes, gastrointestinal tract, and adrenal glands.[52,154,232] Abnormalities in the cerebrospinal fluid (CSF) were found in 62 percent of a large series of PDH cases in Costa Rican infants.[296]

PDH of infancy was uniformly fatal before effective antifungal agents became available[229]; survival rates are now excellent. In a series of Costa Rican children that included some infants with malnutrition, 90 percent of patients treated with amphotericin B survived; of the four who died, two had received amphotericin B for either 33 or 35 days and thereafter were treated with keto-

conazole orally. Both families declined further treatment and were lost to follow-up (C. Odio, 2007, personal communication); each returned, within 5 weeks of discharge, with massive gastrointestinal hemorrhage and recurrent dissemination. The other two were gravely ill and died after having received only either two or three doses. Survival was 100 percent in an Indiana series of PDH of infancy (M. Kleiman, 2008, unpublished data).

Progressive Disseminated Histoplasmosis in Immunocompromised Hosts

Immunocompromised individuals, including individuals in whom the primary medical condition or therapy[246] has depressed cellular immunity, are at risk for dissemination if they acquire histoplasmosis.[176] Common predisposing conditions include AIDS, primary immunodeficiency disorders,[174] immunosuppressive therapy for reticuloendothelial malignancies,[176] organ transplantation,[263] and chronic renal failure. More recently, an increased number of severe PDH infections has been recognized in patients treated with TNF-α antagonists for inflammatory disorders.[482] This effect seems to be associated most closely with the use of infliximab,[108,226] a chimeric mouse monoclonal IgG1 antibody directed against soluble and cellular TNF-α that blocks it from binding with its endogenous cell surface TNF-α receptor.[78,126]

In children who are receiving chemotherapy for malignancies, disseminated histoplasmosis can occur during either remission or relapse.[1,176] The most common initial symptom is persistent fever, often without localizing symptoms and unassociated with toxicity. As the illness evolves, fever continues, and liver and spleen enlargement may occur. Laboratory abnormalities include progressively worsening anemia, leukopenia, and thrombocytopenia; disseminated intravascular coagulopathy may ensue eventually. The chest radiograph may be normal.[176] *Histoplasma* antibody assays usually are negative, and the diagnosis can be confirmed most readily using *Histoplasma* antigen assay of urine or serum.

Another common symptom complex consists of respiratory complaints and tachypnea. In these instances, persistent fever is followed in several days by dyspnea. Chest radiographs, although occasionally normal at the onset of symptoms, show diffuse interstitial infiltrates[1] that progressively worsen. Hypoxemia usually accompanies the symptoms and is progressive. This clinical complex is not specific for histoplasmosis, however, and may be indistinguishable from that caused by *Pneumocystis jiroveci*, cytomegalovirus, various viral respiratory pathogens, other opportunistic fungi, and bacterial septicemia.[152]

The diagnosis of histoplasmosis may be established noninvasively with the urine antigen assay. If this assay is negative, lung biopsy and culture are the most sensitive and specific diagnostic methods and often provide diagnostic information about other potential causes. Examination of bronchoalveolar lavage (BAL)[159] fluid for *Histoplasma* antigen is sensitive and specific in adults, but has not been studied adequately in children.

A subacute form of PDH is seen primarily in adults,[460] although in one report of 19 cases, it was found in 7 infants and 1 child.[145] The principal clinical features that distinguish the subacute from the acute form of PDH are more prolonged symptoms at initial evaluation, a higher frequency of focal lesions, less pronounced fever, and fewer hematologic abnormalities in subacute infection. Early complaints are nonspecific and may be present for 1 to 6 months; malaise and weight loss occur frequently. Hepatosplenomegaly is seen almost uniformly, and intestinal ulceration,[192] sometimes with perforation, occurs commonly.

Gastrointestinal histoplasmosis displays protean manifestations that may occur as a result of mediastinal histoplasmosis[189] or in the setting of PDH.[452] The terminal ileum often is involved, and findings may mimic Crohn's disease.[51,383a] Colonic lesions may result in bleeding or obstruction,[172] or mimic neoplasms.[227] Isolated colonic lesions without evidence of dissemination are rare.[179] Oropharyngeal ulcerations occur and are large and deep and can mimic neoplasms.[113,145,273] Seventy-five percent of patients have adrenal involvement, sometimes unilateral[390]; adrenal insufficiency occurs less frequently, but is reported in 15 percent of adults.[145,149,378] CNS infections are seen occasionally and may be manifested as chronic meningitis, focal mass lesions, or focal areas of cerebritis. Endovascular infections occur and may involve abnormal or normal native valves. The aortic or mitral valves or prosthetic valves[5,29,58,109,133] most commonly are affected. Valvular disease generally is typical of fungal endocarditis, with large vegetations and a high frequency of embolic phenomena.[89]

Numerous isolated lesions[29,72,257,401,481] have been described; some occur in the setting of PDH, and others are found to be isolated manifestations of infection.[189] Vaginal and penile ulcers, soft tissue nodules, recurrent panniculitis, tenosynovitis, carpal tunnel syndrome, osteomyelitis and arthritis, enteropathies, immune hemolytic anemia, and epididymitis all have occurred.[188,202,210,302,324,345,374,386,427] Although the progression of subacute PDH is slower than that seen in acute PDH, it is nonetheless a progressive infection that is fatal in 2 to 24 months if untreated.[145]

Chronic disseminated histoplasmosis is seen almost exclusively in adults in association with cavitary pulmonary lesions.[145] Oropharyngeal ulcers are accompanied by chronic, mild, intermittent constitutional symptoms. A chronic relapsing disseminated form of the disease has been described in two younger patients, 9 and 20 years old, with chronic mucocutaneous candidiasis[118,274,310] and in a child with IFN-γ receptor-1 deficiency.[496]

Progressive Disseminated Histoplasmosis in Human Immunodeficiency Virus–Infected Patients

Histoplasmosis is an AIDS-defining opportunistic infection,[54] and commonly occurs in HIV-infected patients residing in endemic areas. Disseminated infection develops in more than 90 percent of HIV-infected adults with histoplasmosis.[447] In 1988, less than 0.5 percent of adult patients with AIDS had disseminated disease.[147] In endemic areas, it has been reported in approximately 5 percent of patients,[163,258,266] and in 21 to 53 percent during epidemic periods.[32,447,469,470]

In a multivariate analysis of risk factors for the development of histoplasmosis in HIV-infected adults residing in endemic areas, receipt of antiretroviral therapy and triazole drugs was associated independently with decreased risk[164,267]; one study found the risk to be increased with positive baseline serology, $CD4^+$ cell counts less than 150/μL, and exposure to chicken coops.[266] Risk factors for death in two large cohorts of adults also have been determined.[70,89,439] Mortality rates were greater in HIV-infected individuals with PDH treated with antifungal therapy compared with HIV-negative patients with PDH; in HIV-positive patients receiving highly active antiretroviral (HAART) therapy along with antifungal therapy, clinical outcomes were similar to those of patients with PDH unassociated with HIV infection.[402] Comparable studies have not been done in children. In Arkansas, a highly endemic area, histoplasmosis was the AIDS-defining illness in 8 percent of 40 children who acquired HIV in the perinatal period.[366] Histoplasmosis occasionally is seen in non-endemic regions, perhaps because of reactivation of quiescent foci of infection.[19,47,116,353,355]

In HIV-infected adults with disseminated histoplasmosis, symptoms often are nonspecific; prolonged unexplained fever and weight loss are seen almost uniformly, and respiratory complaints occur in approximately half of patients.[158,186,355,447] Features are not distinctive, and in areas in which *Penicillium marneffei* also is endemic, clinical, laboratory, and radiographic findings overlap.[277] Gastrointestinal symptoms[189] are reported commonly and usually are nonspecific; abdominal pain, weight loss, and diarrhea occur in 50 to 70 percent of patients.[22,162,165,221,387]

Multiple intestinal pathogens may be present along with *H. capsulatum*.[319] Enlargement of the liver and spleen is found in approximately 25 percent of patients. Ten percent of patients are initially gravely ill with signs of a septic shock–like syndrome.[447] Mucocutaneous lesions occur in approximately 10 percent of adult patients and include nonspecific maculopapular rash, papules, nodules, pustules, ulcerative lesions, acneiform lesions, mucosal ulcers, and vegetative plaques[153,160]; histopathologic examination of skin biopsy specimens often is diagnostic. The frequency of skin lesions in a cohort of Brazilian patients was 66 percent, a difference attributed to geographic strain differences.[198]

CNS involvement, including meningitis, encephalitis, and focal brain lesions, occurs in approximately 18 percent of patients with PDH complicating HIV infection.[14,15,32,185,254,355,437] Symptoms include headache, encephalopathy, or complaints arising from focal neurologic abnormalities.[437] Published reports of histoplasmosis complicating HIV infection in children are few; in four HIV-infected children in Indiana, fever was present in all, and cutaneous lesions were absent; hepatosplenomegaly was found in one child, and an abdominal mass caused by an infected abdominal lymph node was seen in one child. In adults, initial chest radiographs are normal in approximately 40 percent, show diffuse interstitial or reticulonodular infiltrates in approximately 50 percent, and show localized lesions in approximately 5 percent. Diffuse abnormalities frequently appear during treatment of individuals whose chest radiographs initially were normal.[355]

With the widespread use of HAART for treating advanced HIV infections, an immune reconstitution inflammatory syndrome has been described.[71,280,372] The immunopathogenesis of immune reconstitution inflammatory syndrome is presumed to result from interaction between HAART-induced improvement of host immune function and residual microbial antigens.[240,342] *H. capsulatum* is among other granuloma-inducing pathogens reported to induce immune reconstitution inflammatory syndrome.[36,372]

Central Nervous System Infection

CNS manifestations occur in 5 to 10 percent of adults with PDH,[437,466] but they also may occur as chronic meningitis without other signs of apparent dissemination.[212,348,358,378,437,466] Acute or chronic meningitis occurs in 60 percent of CNS infections; single or multiple focal lesions affecting the brain or spinal cord, stroke syndromes, and encephalitis constitute the balance of manifestations. Symptoms of meningitis often are present 1 to 6 months before the diagnosis is established, but they have been reported to be present for 7 years.[134] Meningitis has been reported to occur years after apparent total resolution of PDH.[36,401] This situation may result from failure of antifungal agents to penetrate the CNS compartment sufficiently to eradicate the infection fully from this sequestered site.[23,402] Symptoms include headache, decreased level of consciousness, confusion, and cranial nerve deficits in 28 to 56 percent of cases.[437]

Diagnosis is most problematic in patients with meningitis and no features of localized or disseminated infection. It is suspected ante mortem in only 40 percent of cases; symptoms and findings mimic those caused by other granulomatous infections, sarcoidosis,[437] cerebral vasculitis,[384] neoplasms,[193] or conditions leading to normal-pressure hydrocephalus. CSF findings are nonspecific and include mild pleocytosis, elevated protein, and depressed glucose. The diagnosis is confirmed by isolating *H. capsulatum* from CSF, detecting *Histoplasma* antigen or antibody in CSF, or identifying the organism in non-CNS sites. Although the specificity of culture and antigen detection are high, sensitivity is variable. CSF antigen sensitivity is 38 percent in non–HIV-infected patients and 67 percent in HIV-infected patients. CSF antibody sensitivity ranges from 80 to 89 percent, but cross-reactions with *Cryptococcus* occur in 28 percent of cases.[466]

CNS infection in children is unusual and, when seen, usually is reported in association with dissemination[365,370]; in one series,[296] meningitis was found in 62 percent of cases of PDH of infancy. A chronic, progressive infection of the CNS has been reported in a child.[341] Other reports describe isolated meningitis with focal neurologic abnormalities occurring after an acute respiratory illness,[26] cerebellar ataxia that was diagnosed only presumptively as histoplasmosis,[371] and cerebellar and medullary lesions.[421] A child has been described with a symptom complex of cervical lymphadenopathy, CSF pleocytosis, arthritis, and interstitial nephritis.[427]

Presumed Ocular Histoplasmosis Syndrome

The entity termed *presumed ocular histoplasmosis syndrome* consists of a triad of findings: (1) discrete atrophic choroidal scars in the macula or midperiphery (histo spots), (2) peripapillary atrophy, and (3) choroidal neovascularization that can lead to loss of central vision.[60,361] In contrast to the acute endophthalmitis[142] or choroiditis[303] that occurs as a manifestation of disseminated infection, the abnormalities seen with presumed ocular histoplasmosis syndrome occur in the absence of inflammatory changes in the vitreous or anterior chambers.

Commonly diagnosed in endemic areas, the cause-and-effect relationship of the findings in presumed ocular histoplasmosis syndrome and histoplasmosis has been based largely on a weak epidemiologic association with histoplasmin skin test reactivity. The clinical syndrome also occurs in individuals residing in non-endemic regions, however.[301,389,425] Little pathologic evidence exists to support histoplasmosis as its cause.[209,300,326] Only a single report detected *H. capsulatum* DNA in cells of an enucleated eye from an affected patient; however, this report used a nonstandardized polymerase chain reaction–based methodology (see section on molecular diagnosis). Presumed ocular histoplasmosis does not seem to occur in children younger than 10 years, although among patients included in a review of the operative management of this entity were 11 children 12 to 18 years old and one child 7 years old.[408]

ILLNESS CAUSED BY INFECTION WITH *HISTOPLASMA CAPSULATUM* VAR. *DUBOISII*

In patients infected with *H. capsulatum* var. *duboisii*, focal lesions are seen more commonly in bones (usually the femur, ribs, or skull) and skin (cutaneous or subcutaneous). The lungs, gastrointestinal tract, liver, spleen, and lymph nodes rarely are involved. A progressive disseminated form of infection with *H. capsulatum* var. *duboisii* occurs and is associated with pyogranulomatous inflammation involving multiple organs.[65,250,476]

RADIOGRAPHIC FINDINGS

The radiographic findings seen most commonly in children with histoplasmosis are not pathognomonic[211] and may mimic the findings seen in tuberculosis or other granulomatous processes and, in some cases, neoplastic conditions, especially lymphoma.[127] After small or moderate degrees of fungal exposure, the plain chest radiograph is normal in approximately 75 percent of skin test converters.[143,473] Computed tomography (CT) is more sensitive and likely to reveal parenchymal infiltrates that are invisible on plain radiographs. The most common pulmonary parenchymal changes are "soft" single or multiple, poorly defined areas of airspace consolidation[156] often found in the basilar portions of the lungs (Fig. 216–2). They either may fully resolve or may consolidate into granulomata that persist, with or without calcification,

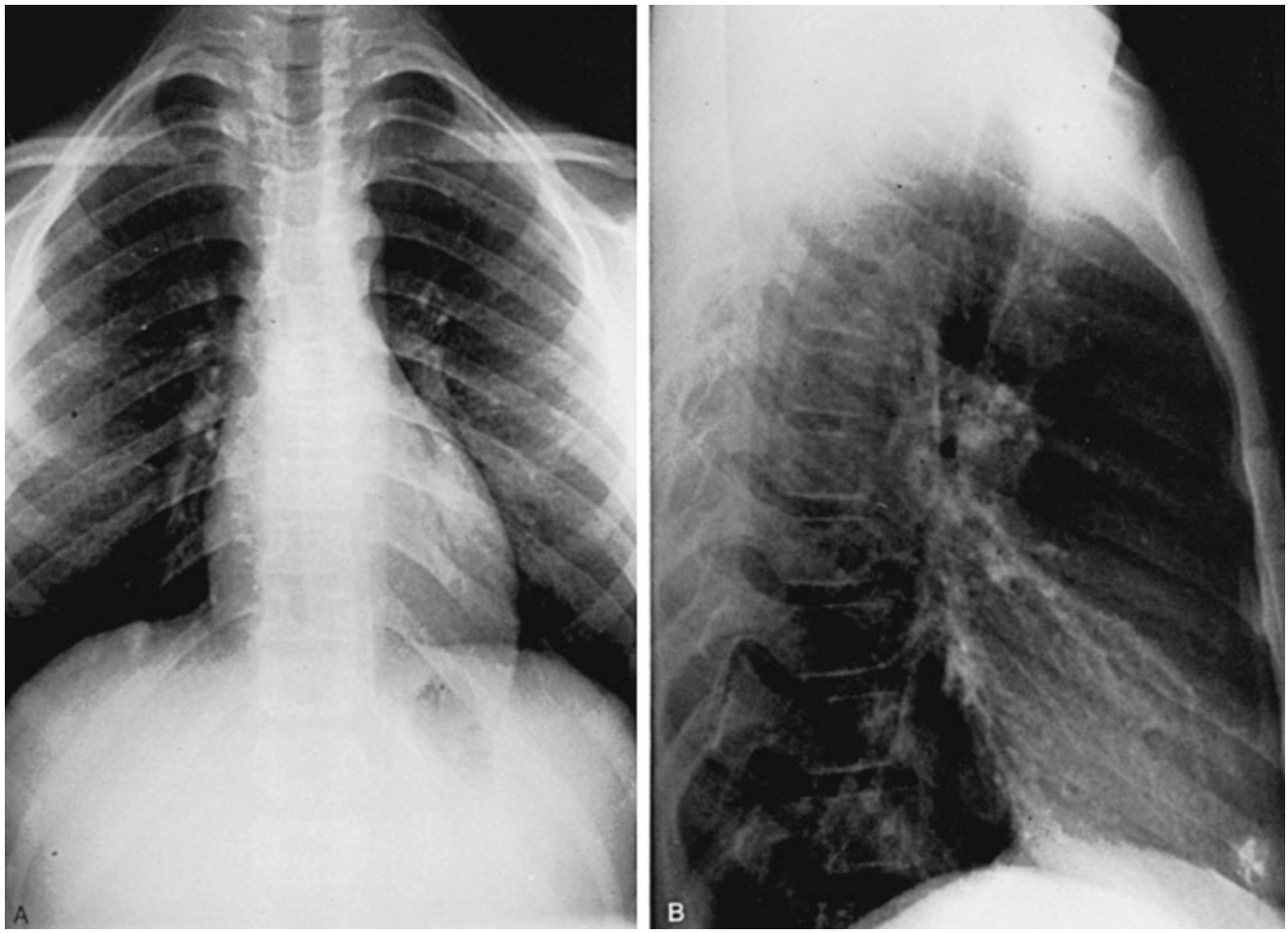

Figure 216–2 **A** and **B,** Chest radiograph of an asymptomatic boy shows in the right lower lobe a calcification overlying the diaphragm between ribs 10 and 11 in the posteroanterior view **(A)** and overlying the apex of the heart in the lateral view **(B)**. These films also showed punctate calcifications overlying the spleen and right axilla (not shown here).

as common findings in residents of or travelers to endemic areas.[299]

The appearance of enlarged hilar/mediastinal nodes, either in association with pulmonary infiltrates or as isolated findings, also is a common radiographic finding of acute pulmonary histoplasmosis. Isolated hilar or mediastinal adenopathy occurs in 20 percent of cases, 80 percent of which are asymptomatic.[67] With CT, low signal intensity within nodes sometimes is shown, although frank suppuration is unusual. Infected nodes may enlarge sufficiently to compress or obstruct adjacent structures. Nodes usually have reached their maximal size at the time of diagnosis and become smaller or return to normal in several months; calcification may, but does not consistently, occur. In infants and young children, clusters of small infiltrates occasionally coalesce into a larger bronchopneumonic lesion.[143] Although small pleural effusions are present in 10 percent of adults with acute histoplasmosis,[468] they occur infrequently in children.[1,327,407] Isolated calcifications may be seen in the spleen or liver months to years after infection. These calcifications result from the self-limited fungal dissemination that occurs during primary infection and often are appreciated as incidental findings in individuals who have lived in endemic regions.

Three distinct chest radiographic patterns have been described in individuals who have had intense exposure to the fungus.[143] Individuals from non-endemic areas initially may have scattered infiltrates that later evolve into "buckshot" calcifications. The second and most common pattern is the presence of smaller, nodular lesions (Fig. 216–3). This form is seen in individuals from endemic and non-endemic areas. Finally, a diffuse miliary or interstitial pattern (Fig. 216–4) may occur, often in a patient with protective immunity.

In PDH, the chest radiograph in adults may show abnormalities in only 25 to 50 percent of patients.[66] Diffuse abnormalities commonly appear during treatment of individuals for whom chest radiographs initially were normal.[355] Diffuse interstitial or reticulonodular infiltrates are present in approximately 50 percent, and localized lesions are present in the remainder. In immunocompromised children, diffuse interstitial infiltrates are the most common radiographic findings; they worsen rapidly and in concert with progressive hypoxemia, especially in patients with HIV infection or patients receiving immunosuppressive therapy.[66] The presence of interstitial infiltrates at admission has been reported in 60 percent of children with PDH of infancy[296] in a Costa Rican series, but chest radiographs were uniformly normal in a series of Indiana patients (M. Kleiman, 2007, unpublished data). Abdominal ultrasound or CT, or both, may show evidence of adrenal enlargement in chronic disseminated disease in adults.[247,480]

Fibrosing mediastinitis caused by histoplasmosis is seen radiographically as pronounced thickening of the mediastinum that often compresses or obstructs the superior vena cava, major

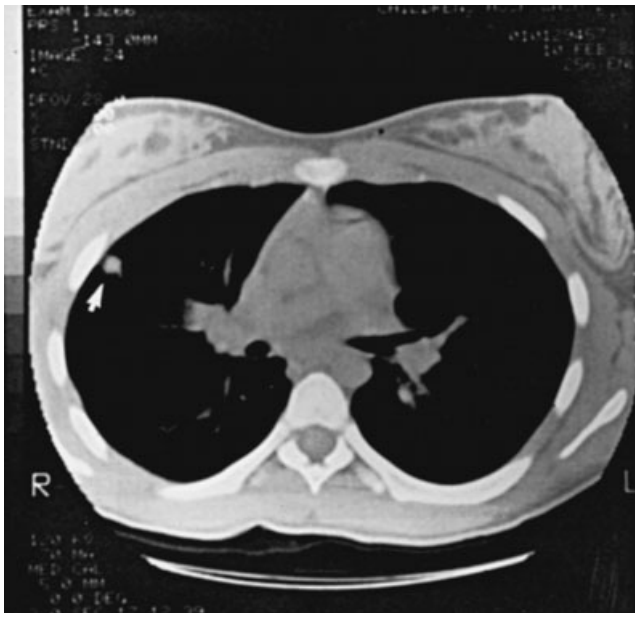

Figure 216–3 CT scan of the chest of a teenage girl from Tennessee complaining of right arm pain when swimming. A nodular density is seen in the periphery of the right lung field *(arrow)*, and the right hilum is enlarged slightly. Serologic tests and needle biopsy of the lesion were nondiagnostic, but excisional biopsy showed necrotizing granulomata with numerous histoplasmal yeast forms.

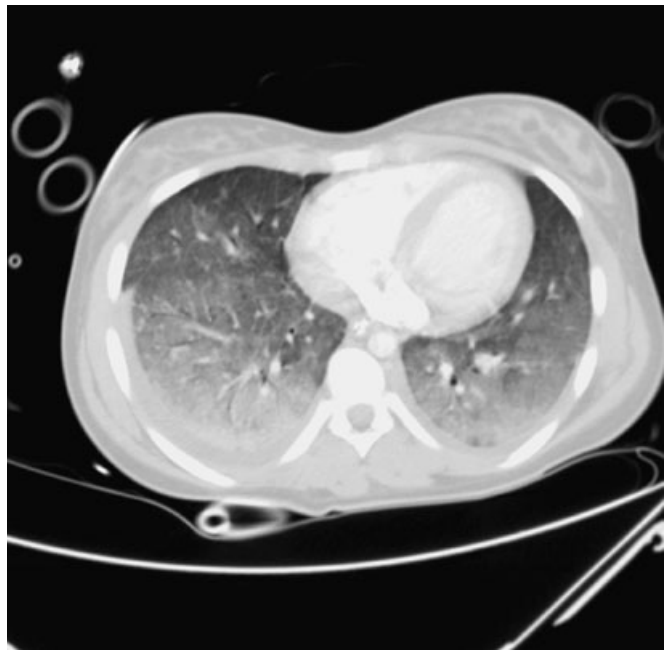

Figure 216–4 CT scan of the chest of a 16-year-old with a history of recent demolition and renovation in the home. The patient developed fever, cough, and progressive respiratory failure requiring maximal ventilatory support using an oscillator. CT scan shows diffuse interstitial infiltrates and a pleural effusion. A urine *Histoplasma* antigen assay was strongly positive.

TABLE 216–2 Interpretive Guidelines for Laboratory Tests in Children with Acute Histoplasmosis

		Interpretation*	
Test	**Result**	***Normal Host***	***Immunocompromised Host***
Complement fixation antibody assay†	≤1:8	–	(–)
	1:16	+	(–)
	≥1:32	++	++
Immunodiffusion antibody assay†	M bands and H bands negative	–	(–)
	M bands present only	+	+
	H bands present only	++	++
	M bands and H bands present	++	++
Urine antigen‡	Negative	(–)‖	(–)‖
	Positive (low) 0.6-4 ng/mL	+	+
	Positive >4 ng/mL	++§	++§¶
Histologic findings			
Node/mass	Negative	(–)	(–)
Infected organ	Granulomata, no yeast form	++	++
	Granulomata, yeast forms	+++	+++
Bone marrow/blood	No yeast forms	(–)	(–)
	Yeast forms seen	+++¶	+++¶
Culture any site	Negative	(–)	(–)
	Positive	+++	+++
Bone marrow/blood culture	Positive	+++¶	+++¶

**(–), does not exclude infection; –, recent infection unlikely (≤5% probability); +, recent infection possible, ++, strongly suggestive of acute or recent infection; +++, confirms current infection.*
†Seroconversion confirms acute/recent infection.
‡Positive urine antigen tests should be confirmed, especially if additional laboratory examinations do not strongly support active infection.
§Compatible with early infection or disseminated infection.
¶Strongly suggestive of progressive disseminated infection or acute primary infection.
‖Progressive disseminated infection unlikely.
Modified from Long, S., Pickering, L. K., and Prober, C. G. (eds.): Principles and Practice of Pediatric Infectious Diseases. New York, Churchill Livingstone, 2003, p. 1236.

bronchi, esophagus, or other critical structures. It can be found in a localized pattern that frequently contains calcification. It must be carefully differentiated from idiopathic fibrosis or fibrosis not induced by infection, which may cause similar symptoms.[373] CT and magnetic resonance imaging help differentiate active inflammation from fibrosis and aid in monitoring progression.[343,347]

DIAGNOSIS

Several laboratory tests play key roles in establishing the diagnosis of histoplasmosis. Although each has value, their optimal use depends on an understanding of their differing sensitivities and specificities in various clinical settings.[436] Table 216–2 provides general guidelines for their interpretation.

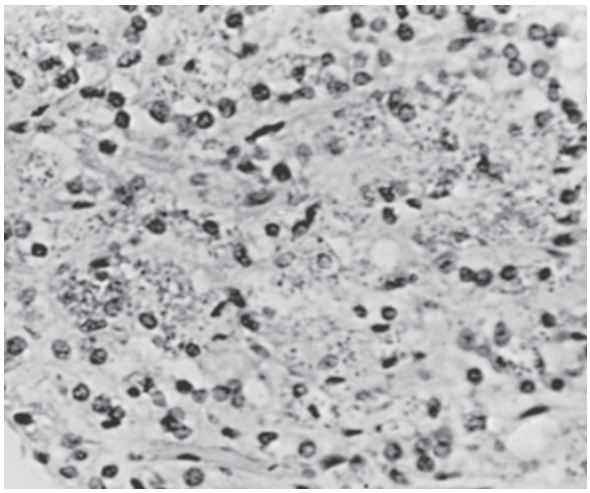

Figure 216–5 Histoplasmosis of the adrenal gland illustrating the histiocytic response with numerous cells of *Histoplasma capsulatum* within the cytoplasm (hematoxylin and eosin, ×52).

TABLE 216–3 Sensitivity of Diagnostic Studies in Different Histoplasmosis Syndromes

Test	Acute Pulmonary	Subacute Pulmonary	Progressive Disseminated
Antigen (%)	75-81	19-34	91-92
Antibody (%)	40-80	78-89	63-81
Histopathology (%)	47	9-38	12-43
Culture (%)	34	9-15	75-85

Modified from Wheat, L. J., Expert Opin. Biol. Ther. 6:1207-1221, 2006.

ORGANISMS SHOWN ON HISTOLOGY

In clinical and epidemiologic settings compatible with histoplasmosis, observation of 2- to 4-μm typical yeast forms in histopathologic specimens is strong supportive evidence of histoplasmosis (Fig. 216–5). Rarely, typical yeast may be observed but result from past infection.[436] Care must be taken to differentiate *H. capsulatum* from the intracellular pathogens *Leishmania donovani* and *Toxoplasma gondii*, small variants of *Blastomyces dermatitidis*, endospores and young spherules of *Coccidioides immitis*, *P. marneffei*, and the yeast forms of *Cryptococcus neoformans*. Giemsa and hematoxylin and eosin stains show intracellular yeasts in sputum, blood smears, bone aspirates, and biopsy specimens. The Gomori methenamine silver stain is the most sensitive reagent and has the advantage that calcification artifacts dissolve during the staining procedure[297,423]; its disadvantage is that it does not provide adequate cellular detail.

Although observation of typical yeast forms in specimens provides important diagnostic information, it requires either a body fluid with a large fungal load or tissue that must be obtained by biopsy. Test sensitivity (Table 216–3) is better in disseminated forms of histoplasmosis, in which yeasts are shown easily in bone marrow, in affected tissue, and often in blood smears.[185] It is less sensitive in self-limited primary infections,[477] including acute pulmonary infection following heavy exposure, in which cytology and histopathology sensitivities are 25 percent.[436] Histopathologic demonstration of typical yeast forms was 18 to 62 percent in three large Indianapolis outbreaks.[436] The technique usually is unrewarding in patients with calcified and fibrotic lesions.[253,258,477]

CULTURE

Normally, sterile specimens and minced or homogenized tissue can be inoculated onto suitable media, such as brain-heart infusion agar, inhibitory mold agar, Sabouraud's glucose (dextrose) agar, and enriched broth such as brain-heart infusion broth. The optimal method for recovery of *H. capsulatum* from blood is the lysis-centrifugation technique, which lyses white blood cells, inhibits complement, and prevents coagulation; after processing is completed, the resulting concentrate is transferred to culture media. Specimens from nonsterile sites should be cultured on media that inhibit bacteria and saprophytic fungi; non-cycloheximide–containing media also should be used to permit the growth of other opportunistic pathogens.[423] Cultures are incubated aerobically at 25° C to 30° C and held for at least 12 weeks before being considered negative. Most isolates grow in 3 to 4 weeks.[456] Use of the lysis-centrifugation system for blood culture has shortened the time for identification of a positive culture from approximately 16 days to 9 days.[31,311] The fungus is recovered in its mycelial form, and confirmation of identification requires that it be converted to the yeast form, be shown to produce the H or M exoantigen, or react positively with a specific nucleic acid probe (AccuProbe; Gen-Probe, Inc., San Diego, CA).[384] Laboratory-based rapid identification and differentiation of fungal pathogens using polymerase chain reaction methodology have been developed, but they are not yet available in clinical laboratories.[325,328]

Recovery of *H. capsulatum* from a clinical specimen obtained from a symptomatic patient confirms the diagnosis of active histoplasmosis. The only exception is the infrequent event in which an incidental granuloma is found to be culture-positive, but histoplasmosis is not a likely cause of the clinical symptoms. The sensitivity of culture depends heavily on the severity of the infection and the fungal burden (see Table 216–3).[478] In primary acute, self-limited histoplasmosis, 40 percent of patients have positive cultures when obtained from sputum (23%), BAL fluid (39%), lung, and extrapulmonary sites (37%).[436] In contrast, cultures are positive in 75 to 85 percent of adults with PDH. In the latter patients, sites from which *H. capsulatum* commonly is recovered include the lower respiratory tract, blood, bone marrow, CSF, liver, spleen, skin lesions, and synovium.[12,296] The highest yield in PDH is from bone marrow, which is positive in 75 percent of instances.[358,430] Urine is positive in 40 to 70 percent, and sputum is positive in 60 percent.[378] In adults with PDH, rates of positive lysis-centrifugation cultures of peripheral blood are 90 to 100 percent in acute dissemination and 50 percent in subacute dissemination; positive cultures are very rarely seen in chronic dissemination.[247]

In forms of pulmonary histoplasmosis other than low-inoculum infection, sputum may be a reliable site for recovery of the organism in culture, but not the best choice of diagnostic methods. High-inoculum infection after exposure to heavily contaminated sites usually results in moderate-to-severe infection in which patients seek medical attention within 2 weeks of inhaling spores. In these instances, the fungal burden is high, and respiratory secretions or lung tissue may show the organism, allowing the diagnosis to be made promptly; culture also may be positive, but the 2- to 4-week delay in achieving growth renders culture less desirable than other diagnostic methods, especially antigen detection. In adults with chronic cavitary pulmonary infection, sputum culture is positive in 50 to 85 percent of instances.[144,434] The sensitivity of sputum culture may be increased with multiple sampling.[454]

Bronchoscopy with BAL may be helpful in patients with pulmonary disease. In a study of bronchoscopy in 71 adults, BAL was positive in only 4 percent of individuals with a single pulmonary nodule, but it increased to 55 percent in the remainder of the patient group. The highest yield of 88 percent (7 of 8)

was in patients with infiltrates or cavitary disease.[327] Bronchoscopy did not seem to be helpful in evaluating patients with adenopathy, chronic pleural effusion, or bronchopleural fistulas. In children, culture plus staining of BAL fluid has been rewarding in diagnosing high fungal burden infection in patients with HIV, but it is less sensitive than lung biopsy in other immunocompromised patients, especially patients receiving chemotherapy for reticuloendothelial malignancy. In patients with mediastinal granuloma and fibrosis, cultures were positive in only 3.8 to 10 percent.[91,248]

Cultures usually are negative in rheumatologic manifestations of histoplasmosis, such as arthritis, erythema nodosum, erythema multiforme, and pericarditis, because these reactive manifestations generally occur months after low-burden infection in patients whose acquired cellular immune response has controlled fungal replication. Isolated CNS infection is uncommon in children, but meningitis was reported to occur in 25 of 40 cases of PDH of infancy; yeasts were observed in the CSF, and *H. capsulatum* was recovered in culture from 4 patients.[296] Recognizing CNS infection in adults is difficult, unless concurrent dissemination, which occurs in approximately 20 to 40 percent of cases, is present.[434,437,466] Invasive procedures sometimes are needed to confirm the diagnosis. Culture is positive in 27 to 65 percent of CSF specimens and in 50 percent of cultures obtained from other sites in adults.[434] The number of organisms in CSF is small, so the yield is improved substantially by culturing large volumes (10 to 20 mL in adults) on at least two occasions.[466] In isolated CNS infections, culture is usually unrewarding.

ANTIBODY AND ANTIGEN DETECTION

The serologic methods that are used most commonly for the diagnosis of histoplasmosis are immunodiffusion (ID) and CF. These tests are useful because, despite documentation of skin test reactivity in 50 to 70 percent of young healthy adults residing in an endemic area, seropositivity in this population has been found to be 0.5 percent by ID and 2 percent by CF using the mycelial antigen, and 4 percent with the yeast antigen.[453] These tests serve to make the probability of histoplasmosis high in a patient with a compatible illness and elevated titers.

Both methods have equal sensitivity (75 to 85%); however, ID is slightly more specific (>95% versus 85 to 90%).[430,431] In immunocompetent patients, either or both tests are positive in 95 percent of patients with acute primary pulmonary infection. CF titers often become positive 2 to 4 weeks earlier, usually by 4 to 6 weeks after exposure.[430] In patients with CF antibody, 25 percent have a negative ID result; only 1 percent of patients reactive by ID are CF-negative.[75] When the ID test is reactive, however, it remains so for a longer time. In addition to the 4- to 6-week lag in developing elevated titers, an important limitation of both serologic assays is their reduced sensitivity in immunosuppressed patients. Only 50 percent of immunosuppressed children and adults with disseminated histoplasmosis are seropositive.[76,176] Of immunocompetent infants and young children with severe PDH during infancy and early childhood, 93 percent had elevated antibody titers.[296]

Cross-reactivity with other fungal antigens affects CF and ID assays.[456] CF cross-reactivity occurs most commonly with *B. dermatiditis* (40%) and C. *immitis* (16%).[456] Cross-reactions also rarely occur with candidiasis, tuberculosis, aspergillosis, and cryptococcosis.[253,456] Cross-reactions with blastomycosis are common in the histoplasmosis CF assay, in which titers of 1:8 to 1:16 frequently are observed[205]; comparison of results of a single serum sample performed in the same assay often reveals CF antibody concentrations to be higher for the homologous infection. In these instances, ID tests sometimes are corroborative because the blastomycosis ID method is more specific and less likely to cross-react in histoplasmosis.[239] ID has been noted to show false-positivity in 5 percent of other fungal infections, however.[456] Cross-reactivity also is seen occasionally in patients with chronic cavitary tuberculosis, although simultaneous infection with tuberculosis and fungal pathogens can occur.[125,456] Variability in the specificity of commercial reagents has been reported to cause false-negative results.[230]

Complement Fixation

CF uses sensitized sheep red blood cells, killed whole *H. capsulatum* yeast cells, and histoplasmin, a soluble mycelium-form filtrate antigen.[275] In a common-source outbreak of acute primary pulmonary histoplasmosis, the titer becomes positive in 6 percent of individuals at 3 weeks, 73 percent at 4 weeks, and 77 percent at 6 weeks.[75,456] With resolution of infection, titers decrease to 1:8 to 1:16 within 4 to 6 months and become undetectable (<1:8) by 9 months in most instances. Reported sensitivities for a single titer vary from 70 to 95 percent and depend on the threshold for considering a result positive. Single titers of 1:32 performed by an experienced laboratory are strong supportive evidence of acute or recent infection, especially when the accompanying clinical symptoms are compatible. One report[398] showed that 12 of 28 patients with non-*Histoplasma* febrile pneumonia had false-positive CF titers of 1:32 or greater, so other laboratory and clinical data need to be considered.

The presence of a fourfold increase between acute and convalescent sera is the best serologic evidence of recent infection. The individual yeast (CF-Y) and mycelial (CF-M) phases can be measured. CF-Y is more sensitive than CF-M for recent or active infection. In a series of 11 children with acute pulmonary histoplasmosis, CF-Y was 1:32 or greater in 9, but CF-M was 1:32 or greater in only 3 children.[487] In endemic regions, background low-titer CF serologic reactions may be present in 5 to 15 percent of adults,[283] but they have been reported to occur in 30 percent.[135,143,247,457]

The serologic response seems to correlate with the severity of disease in immunocompetent individuals with acute infection.[429] In an outbreak of histoplasmosis, seropositivity was 90 to 100 percent in severe acute histoplasmosis, 86 percent in moderate histoplasmosis, 75 percent in mild histoplasmosis, and 18 percent in asymptomatic histoplasmosis.[223] Twenty-five percent of patients with acute histoplasmosis have CF titers that could be considered borderline positive, between 1:8 and 1:16.[75] Although they may result from previous infection, these titers should not be disregarded if clinical symptoms suggest acute infection. In adults with chronic pulmonary histoplasmosis, the CF titer is greater than 1:32 in almost all patients.[199] In children who present with middle mediastinal masses caused by histoplasmosis, 67 percent have CF titers of 1:32 or greater.[127]

The serologic diagnosis of patients with isolated meningitis caused by *H. capsulatum* often is problematic[466] because no single test exhibits high sensitivity. CF and ID can be positive in CSF, but half of patients with other chronic fungal meningeal infections may show false-positive results.[322,455] CF-M antibody seems to be the most sensitive and specific test for the diagnosis of meningitis caused by histoplasmosis. In one study, no false-positive CSF CF-M titers occurred in patients with cryptococcal meningitis, but CF-Y was false-positive in 5 of 18 patients.[455] Individuals with fibrosing mediastinitis or mediastinal granuloma generally have negative or very low levels of CF antibody.[429]

Immunodiffusion

The micro-ID method[204] detects precipitins (reported as bands) against the H and M glycoprotein antigens of *H. capsulatum*.[275,457] The M band is present in 25 percent of infections by the fourth week of infection and in 50 to 86 percent by the sixth week.[25,75,315]

It can persist for 18 to 36 months after recovery, but eventually becomes nonreactive. In endemic areas, in which serologic surveys show that 24 percent of healthy adult blood donors have CF-Y titers of 1:8 or 1:16, ID serology is positive in less than 1 percent.[135] Because it is more specific, ID can be of value to confirm the diagnosis of histoplasmosis in patients with *Histoplasma* CF titers in the borderline 1:8 to 1:16 range.[424]

The H band is present infrequently in patients with histoplasmosis; when seen, it is transient, and its presence suggests active infection.[143] In patients with active pulmonary histoplasmosis, one half to three fourths have an M band alone. The H band is present in only 10 to 20 percent of acute infections,[12,148,205,457] and only 10 percent of individuals have M and H bands present.[12] The latter finding is highly suggestive of active histoplasmosis.[204] The H band is detected less consistently in children with histoplasmosis.[427] In adults with disseminated disease, both bands are present in 25 percent of individuals.[12] The M band has been detected in 52 percent and 57 percent of patients with chronic pulmonary and disseminated disease.[148] The ID test is not approved for use in CSF specimens and has not been evaluated as a test for the diagnosis of meningitis.[436]

Antibody Detection by Radioimmunoassay and Enzyme Immunoassay

Antibody detection methods that use radioimmunoassay and enzyme immunoassay have been developed. They can detect antibody slightly earlier than is possible with ID and CF, can detect antibody to histoplasmin and yeast antigens, and can measure IgG-specific and IgM-specific *Histoplasma* antibodies.[75,220,315,457,461,501] The disadvantages of cross-reactivity,[456] complexity and cost of the methodology, poor reproducibility, and negligible clinically relevant superiority over standard serologic methods have restricted their commercial availability. Neither radioimmunoassay nor enzyme immunoassay is recommended for diagnosis.

Antigen Detection

The development in 1986[462] and further refinements of the *Histoplasma* antigen assay fill an important gap in laboratory diagnosis. When performed on serum, urine, or other selected body fluids, antigen detection provides rapid, accurate, noninvasive, diagnostic information for the most serious manifestations of disease. It is especially useful for the evaluation of infection in an immunocompromised host, in whom serologic methods often are negative.[462] Although evaluated almost exclusively in adults, antigen detection occupies the same diagnostic niche for managing infection during childhood.

Antigen detection is most sensitive in infections accompanied by high fungal burdens.[478] These include progressive disseminated infections in intensely exposed or immunocompromised hosts and primary pulmonary infection, in whom antigen detection reflects the early hematogenous dissemination that occurs before it is aborted by the cellular immune response. In addition to its usefulness in diagnosis, antigen assay provides a parameter with which to assess the adequacy of response to therapy and, after treatment, a monitor to predict relapse in patients who are at high risk for recurrence.[444,448-450]

The sensitivity of urine antigen detection (see Table 216–3) in patients with acute primary pulmonary infection is 75 to 81 percent, with the highest rates seen in patients tested within a few weeks of exposure, in patients with intense exposure, and in patients with extensive pulmonary involvement.[434,477] Sensitivity is less in subacute pulmonary and chronic cavitary infection in adults. The sensitivity of antigen detection is very high in progressive disseminating forms of histoplasmosis. In adults with disseminated histoplasmosis, antigen is detected in 91 to 92 percent of immunosuppressed and non-immunosuppressed patients and in 95 percent of patients with AIDS.[434] In 22 children with PDH, urinary antigen testing was positive in 100 percent.[119] From 1991 through 2006 at Riley Hospital for Children in Indianapolis, urine antigen testing was positive in all instances of disseminated histoplasmosis (M. Kleiman, unpublished data).

Measurement of antigen initially was developed using radioimmunoassay technology and was replaced with an enzyme-linked immunoassay.[104] Two generations of refinement of the enzyme-linked immunoassay have greatly increased its sensitivity and specificity. The results of these semiquantitative assays were reported in "antigen units" that were determined by comparison with a negative control. Assay-to-assay variability with radioimmunoassay and enzyme-linked immunoassay required that sequential specimens were stored and retested in the same assay to determine whether a significant change had occurred in response to therapy. A fully quantitative, third-generation assay, introduced in 2006, has eliminated this need, but has altered recommendations for follow-up of patients using antigen assay.

Although *Histoplasma* antigen may be detected in serum, the sensitivity is less than that of urine. Of immunosuppressed adults with disseminated histoplasmosis, antigen was found in 50 percent of serum samples and 92 percent of urine specimens. Similarly, in patients with disseminated histoplasmosis complicating AIDS, antigenuria was seen in 95 percent versus 85 percent in serum.[462,477] Antigen often is found in BAL fluid of patients after intense exposure and in immunocompromised patients with hematogenous dissemination and lung involvement.[447,451] In adults with isolated meningitis, antigen detection in CSF may be the sole basis for confirming the diagnosis.[462,463,466] The range of antigen positivity in all cases of meningitis is 40 to 66 percent; the highest rates are found in severe infections with heavy fungal burdens, especially infections that occur in immunosuppressed patients.[466] In patients with clinical findings suggestive of meningitis but in whom antigen is not present in CSF, antigen found in extrameningeal sites sometimes can confirm the diagnosis.

Antigen levels in blood or urine decrease during treatment and should clear completely in infections that are treated adequately.[459] Conversely, the persistence of moderate antigenuria has been associated with a risk of recrudescence. This risk has been shown best in patients with AIDS and histoplasmosis, in whom significant antigenuria often persists after cessation of therapy.[448,449,465] Monitoring urine antigen in such patients has shown that increasing urine antigen concentrations foretell recurrence.[448] The sensitivity of the antigen assay is high, however, and the pattern of antigen clearance has not been established firmly in other clinical settings. Infants with PDH who have been treated adequately, have had a good clinical response, and in whom urine antigen levels have substantially decreased during therapy often have persistence of low levels of urine antigen after completion of amphotericin B therapy. In these infants, all of whom were immunocompetent, no relapse has been observed after treatment with a total dose of 30 mg/kg of amphotericin B (M. Kleiman, 2007, unpublished data). Residual excretion of urine antigen continues to decrease and eventually ceases; monitoring is recommended to confirm resolution.[119]

With the introduction in 2006 of the third-generation quantitative *Histoplasma* antigen method,[436] interassay variability was reduced by extrapolating antigen concentrations from a calibration curve that was created using the results of assays of known quantities of antigen. Because this calibration curve is linear at values less than a concentration of 40 ng/mL, measurements in excess of this are reported as greater than 39 ng/mL, and changes cannot be determined until they fall below this value. Although the quantitative method has increased precision and eliminates the need to evaluate current specimens in tandem with earlier specimens,[436] as required in earlier enzyme-linked immunoassays, it also has necessitated changes in recommendations

for using the antigen assay to monitor the effectiveness of therapy.[448]

Although urine samples could be used conveniently for this purpose with the older, semiquantitative methods, urine antigen concentrations measured with the quantitative assay often exceed the upper end of the calibration curve, especially in disseminated infections. If urine concentrations are found to exceed 39 ng/mL, monitoring patients for adequacy of response is more informative if serum antigen concentrations are initially followed because maximum serum concentrations are lower than the concentrations of concomitant urine samples and more likely to fall within the range in which differences in concentration could be measured accurately. When antigenemia is resolved, urine concentrations can be followed with the quantitative assay. Testing should be performed at 3- to 4-month intervals during treatment, at the end of therapy, if symptoms recur, and periodically for another year to monitor for relapse.

Antigen concentration decreases during effective therapy. If the baseline value is less than 20 ng/mL, antigen concentration should decline at least 3 ng/mL during the first month and during subsequent 3-month intervals. If the baseline antigen is greater than 20 ng/mL, the concentration should decrease by at least 15 percent during similar time periods. Failure of the antigen concentration to decline or documentation of progressive increase may indicate treatment failure. It is unknown whether treatment should be continued until antigenuria clears completely; some patients who have stopped therapy despite having low levels of antigenuria (<4 ng/mL) seem to have done well.

Cross-reactions in the *Histoplasma* antigen assay occur in paracoccidioidomycosis, African histoplasmosis caused by *H. capsulatum* var. *duboisii*, blastomycosis, and infections caused by *P. marneffei*.[472] Because these infections have distinguishing epidemiologic and clinical features, and supportive diagnostic tests may aid further in differentiating them from histoplasmosis, cross-reactions usually create little difficulty in interpreting positive test results. Treatment is similar for these endemic mycoses, so appropriate therapy may be given while confirmatory data are sought. Blood, urine, BAL fluid, and CSF specimens yield reliable results in the antigen assay. False-positive results may occur with pleural fluid and other high protein–containing body fluids, such as peritoneal, synovial, and pericardial fluids; these fluids and tissue specimens are unsuitable specimens. The test is commercially available (MiraVista Diagnostics, Indianapolis, IN).

SKIN TESTING

A skin test for histoplasmosis has been a valuable epidemiologic and investigational tool, but has little diagnostic usefulness[143]; it no longer is commercially available. Skin testing is performed with standardized histoplasmin antigen prepared from mycelial-phase culture filtrate. After intradermal administration, an area of induration of 5 mm or larger at 48 hours is considered positive and indicative of cellular immunity.[275] Skin test reactivity usually develops within 2 to 4 weeks after infection.[123] In chronic pulmonary disease, including cavitary disease, the skin test is positive in three fourths of patients.[123] In contrast, individuals with disseminated disease seldom have positive results.[13] Eighty percent of children who are older than 12 years and reside in endemic areas may be reactive.[429] Cutaneous reactivity may diminish, wax, or wane with re-exposure,[143,429,494] but it usually persists indefinitely in most individuals. The problem of serologic boosting by skin testing also has limited its diagnostic use.[12,13,39,173,203,206,275] Cross-reactions occur in patients with blastomycosis and coccidioidomycosis.[105-107]

MOLECULAR METHODS

Epidemiologic studies have used restriction fragment length polymorphisms of DNA. Nucleic acid–based and genome-wide approaches have been used to explore the genetic diversity of the pathogen.[207,420] Molecular methods also have been used to examine strains recovered from patients who have failed therapy[464] and to identify relatedness in isolates recovered after organ transplantation.[235]

Sensitive and specific oligonucleotide probes for yeastlike fungi have been developed and hold promise for application to clinical specimens.[238] Molecular methods that reliably detect *H. capsulatum* in clinical samples have yet to show superiority, however, to standard diagnostic tests.[58] Polymerase chain reaction methodology has been evaluated for identifying *H. capsulatum* in tissue and body fluids, but false-negative results were encountered in a third of specimens.[30] Polymerase chain reaction methodology used with clinical specimens (urine, serum, BAL, CSF) containing urine antigen showed specificities of 80 to 100 percent, but sensitivities of only 0 to 22 percent.[436]

TREATMENT

Most patients with symptomatic histoplasmosis recover without receiving antifungal therapy.[143] Treatment almost always is required for patients with severe symptoms, prolonged illness, or evidence suggestive of progressive dissemination. Evidence-based, consensus practice guidelines for treatment of histoplasmosis have been published[467] and revised in 2007[452] to include recommendations for treatment of children.

No controlled therapeutic trials have been conducted in children. Chronic pulmonary infection and subacute and chronic dissemination syndromes rarely occur in children. Table 216–4 summarizes recommendations for treating the clinical manifestations of histoplasmosis seen most frequently in children. These recommendations have been derived from anecdotal published reports, clinical experience, and extrapolation from experience in adults. Surgical management of histoplasmosis rarely is indicated.

MEDICAL MANAGEMENT FOR MANIFESTATIONS REQUIRING ANTIFUNGAL THERAPY

Because most patients with light or moderate exposure to fungal spores recover without treatment, the first decision is to determine whether to observe the patient carefully or to begin administering an antifungal agent. The criteria that are key to making this decision are clinical assessment of the character, severity, and duration of symptoms, and assessment of the adequacy of the patient's cellular immunity. When treatment with antifungals has been elected, a regimen is selected that is appropriate for the initial findings. Manifestations of histoplasmosis in children that most often require antifungal treatment are severe or protracted symptoms resulting from acute primary pulmonary infection, mediastinal adenitis that is causing compression of adjacent structures, disseminated infection of infancy,[119,296] and infection in an immunocompromised host.[1,388] Treatment recommendations are summarized in Table 216–4.

The antifungal agents that play primary roles in medical management include amphotericin B deoxycholate,[357] liposomal amphotericin B,[187] amphotericin B lipid complex,[316] and itraconazole.[93,458] Amphotericin B is fungicidal for *H. capsulatum*, whereas itraconazole is fungistatic. Combinations of the two agents are not synergistic. Based largely on the results of its superiority in animal studies[68] and its faster clearance of fungemia in patients

TABLE 216–4 Summary of Treatment Recommendations for Children with Histoplasmosis

Manifestation	Treatment	
	Severe Illness	***Moderate or Mild Illness***
Acute pulmonary	AmB,* 1-2 wk, then Itr for 12 wk†; use of concomitant steroids is controversial	Symptoms <4 wk, none; persistent symptoms for >4 wk, Itr for 6-12 wk
Disseminated (non-HIV) ± meningitis	AmB,‡or AmB for 2 wk, then Itr for 6 mo‡	Itr† for 6-8 mo or same as for severe§
Disseminated (with HIV)	AmB,‡¶ or AmB followed by Itr§	Same as for severe; Itr secondary prophylaxis if immune dysfunction persists (see text for criteria)
Meningitis, isolated	AmB¶ for 3 mo, then Itr for 12 mo	Some as for severe because of poor outcome
Fibrosing mediastinitis	Itr for 3 mo¶	Same as for severe
Pericarditis	Pericardial drainage for severe tamponade + NSAID for 2-12 wk**	NSAID for 2-12 wk
Rheumatologic	NSAID for 2-12 wk	Same as for severe
Compression of contiguous structures by mediastinal adenitis/granuloma	Prednisone 2 mg/kg/day, concurrent Itr (see text)	

**Amphotericin B deoxycholate is preferred, 1 mg/kg/day.*
†In severe manifestations, itraconazole may be given at 150% of the recommended dose in three divided doses for the first 3 days of treatment only. Itraconazole levels are recommended for severe infections, and in patients in whom clinical response is suboptimal. Serum levels of 2 μg/mL are recommended when steady state is reached (approximately 2 weeks). Itraconazole dose is 5 to 10 mg/kg/day divided twice a day.
‡If amphotericin B is used for the entire course of treatment, 30 to 40 mg/kg should be given over 4 to 6 weeks.
§Therapy should continue until Histoplasma *urine antigen concentrations are stable and <4 ng/mL; prolonged low-level excretions may persist (see text).*
¶Liposomal amphotericin B (3 to 5 mg/kg/day) may be superior to amphotericin B deoxycholate in patients with HIV (see text).
¶Probably ineffective if fibrotic; when granulomatous mediastinitis could be present, it may be considered.
***Prompt administration of NSAID may obviate need for drainage in non–life-threatening manifestation of pericarditis.*
AmB, amphotericin B; HIV, human immunodeficiency virus; Itr, itraconazole; NSAID, nonsteroidal anti-inflammatory drug (indomethacin 1 to 3 mg/kg/day).
Modified from Wheat, L. J., Freifeld, A., Kleiman, M. B., et al.: Practice guidelines for management of patients with histoplasmosis. Clin. Infect. Dis. 45:807-825, 2007.

with AIDS,[187] amphotericin B is more effective for severe disease than itraconazole.[458] Amphotericin B is used most commonly as "induction" therapy; after substantial improvement has occurred, it is stopped, and step-down therapy with itraconazole is used to complete treatment. Monotherapy with itraconazole is effective for treating patients who have mild or moderately severe symptoms. In addition to regimens that are used to treat acute infections, suppressive (secondary prophylactic) regimens have been developed for treating patients with AIDS because the rate of relapse is high if antifungal treatment is stopped, and immune function has not been improved by HAART.[447] Primary prophylactic regimens also have been developed for use by patients with AIDS who live in highly endemic areas.[267]

Chapter 252 contains a detailed discussion of the antifungal agents used to treat histoplasmosis. Amphotericin B deoxycholate usually is well tolerated and very effective in children. Its lipid formulations, although less nephrotoxic, are substantially more expensive.[395] Liposomal amphotericin B is the best evaluated of the lipid preparations. A comparative trial of amphotericin B deoxycholate and liposomal amphotericin B in adults with disseminated histoplasmosis and AIDS showed faster improvement and lower mortality rates with the liposomal preparation.[187] Liposomal and standard amphotericin B formulations clear fungemia faster than itraconazole does.[440]

If amphotericin B is used as monotherapy for severe or disseminated infection in a normal or immunocompromised host, a dosage of 1 mg/kg/day administered for 4 to 6 weeks is recommended. Failures of 30 and 35 mg/kg total dose have been reported in PDH of infancy,[296] and because relapse has been observed in immunosuppressed patients,[176] close follow-up, preferably with monitoring of urine antigen concentrations, is required after completion. When amphotericin B is used as induction therapy in severely ill children, it should be continued for 2 to 4 weeks and until substantial improvement in clinical and laboratory findings is achieved. Short courses of therapy for PDH of infancy have been successful.[120,229,241] One report described cure in five infants[120] (only two with microbiologic confirmation and another in which PDH was highly probable) with an initial dose of 0.25 mg/kg, 0.5 mg/kg given on the second day, and 1 mg/kg/day administered for 7 to 11 days thereafter. Insufficient data are available to recommend short courses of amphotericin B for treatment as monotherapy, and because of the availability of itraconazole, such regimens seldom are needed.

Itraconazole generally is well tolerated by children and, in adults, is more effective[142] and less likely to induce resistance than the other azoles.[112,128,166,169,283,318,379,416] Although clinical trials using itraconazole have not been conducted in children, clinical experience has confirmed its effectiveness as the oral azole of choice.[452] The erratic bioavailability of the capsule form can be improved when it is taken with liquids with low pH and caloric content (concomitant food and a cola drink are recommended).[181] Serum levels should be monitored,[138] particularly if symptoms persist. The liquid solution of itraconazole is better absorbed, but may have adverse gastrointestinal effects that affect compliance. The liquid solution is better absorbed when taken on an empty stomach. The 90 percent minimal inhibitory concentration (MIC_{90}) of itraconazole for *H. capsulatum* is less than 0.01 μg/mL; the optimal therapeutic level has not been determined, but serum concentrations greater than 2 μg/mL should be effective, easily achieved, and well tolerated.[168] With its long half-life, serum concentrations vary little after steady state is achieved (approximately 2 weeks). When used as monotherapy, or as step-down treatment after amphotericin B induction to treat mild to moderately severe infections in adults, a loading dose consisting of 150 percent of the total daily dose is recommended for the initial 3 days of therapy. Itraconazole is quite expensive. As with the other azoles, important drug-drug interactions need to be considered in patients receiving agents known to affect excretion of the azole or whose excretion is affected by the antifungal.

Fluconazole was less effective than itraconazole in treating adults with chronic pulmonary histoplasmosis[265] or disseminated histoplasmosis.[464] It also has been associated with relapse in disseminated infection,[465] is less effective than itraconazole for secondary prophylaxis in adults with disseminated infection,[267] and clears fungemia more slowly in adults with disseminated

infection than itraconazole.[444] Fluconazole is recommended for use in patients who either do not tolerate itraconazole or fail to absorb it. When fluconazole is used, careful follow-up with urine antigen monitoring is needed to detect relapse. Because itraconazole does not enter the CSF easily, fluconazole may be considered in patients with CNS infection. The relationship between CSF concentrations and outcome has not been determined, however.[467] Antagonism is a concern when fluconazole is used in conjunction with amphotericin B to treat meningitis.[231] Fluconazole is reliably absorbed and metabolized, and drug monitoring seldom is needed.[465]

Ketoconazole is less well tolerated than either itraconazole or fluconazole.[35,88,170,233,406] It is reasonably effective in adults with chronic pulmonary infection[94,233,282,375] and in patients with disseminated infection, although relapse is common.[330] In histoplasmosis complicating AIDS, the response to ketoconazole was only 9 percent versus 74 to 88 percent with amphotericin B and 85 percent with itraconazole.[284,411,433] Ketoconazole is inexpensive and may be an alternative consideration for treatment if high cost precludes the use of itraconazole.

The newer azoles, voriconazole and posaconazole, have activity against *H. capsulatum* in vitro. Posaconazole has shown greater activity[446] and seems to be more active in experimental models.[68] Resistance that seems to have been induced by fluconazole when used in patients with AIDS also was accompanied by increase in MIC values to voriconazole,[446] suggesting that resistance may emerge during treatment with voriconazole.[445] Voriconazole[4,121,175,281,404] and posaconazole[336] have been effective in isolated reports describing differing manifestations of infection. These agents and fluconazole remain second-line alternatives to itraconazole.[62,320,452] If these agents are elected for use, therapeutic drug monitoring is recommended.[452] Echinocandins have been examined in vitro with conflicting results; human data are lacking.

Primary Pulmonary Infection

Primary pulmonary histoplasmosis that occurs after intense exposure to spores may result in life-threatening illness with high fever, diffuse pulmonary infiltrates, hypoxemia, and adult respiratory distress syndrome.[99,143,145,432,467] Infection of this severity should be treated promptly, and induction with amphotericin B is recommended until substantial clinical improvement occurs. Only anecdotal evidence indicates that concomitant use of corticosteroids may be beneficial in treating patients with severe inhalation and adult respiratory distress syndrome.[150,197,397,490] After improvement results from amphotericin B induction, itraconazole should be continued for at least 3 months. In patients who may not appear as ill, but in whom laboratory or clinical evidence is suggestive of progressive primary dissemination, empiric therapy should be initiated.

Patients without respiratory distress but in whom severe systemic complaints, such as high fever, fatigue, and weight loss, persist for 2 to 4 weeks or longer also are candidates for antifungal therapy. In these instances, firm criteria to guide the timing of this decision are not established, and treatment must be individualized. Still, as observed when amphotericin B was the only effective treatment, and its toxicity and inconvenience resulted in deferring therapy for longer than currently practiced, a significant proportion of these symptoms could be expected to undergo spontaneous resolution. The convenience of oral antifungal agents renders the decision less problematic because these protracted but mild to moderately severe symptoms may be treated with itraconazole. Itraconazole should be used for at least 6 weeks in these instances (see Table 216–4). No clinical studies have been conducted to show whether treatment shortens the duration of symptoms or prevents later complications.[452]

Mediastinal Adenitis

One of the most common initial presentations is mediastinal adenitis that is associated with acute pulmonary infection. In this setting, affected nodes enlarge, coalesce and compress, or obstruct adjacent structures. Bronchi and the trachea are most often affected; less commonly, the superior vena cava and esophagus are affected. The phrenic or recurrent laryngeal nerves rarely are affected. Symptoms usually associated with airway compression are cough, mild tachypnea, exertion with activity, wheezing, and nonpleuritic chest pain. The superior vena cava syndrome may accompany obstruction of the superior vena cava. Fever is an uncommon manifestation, unless secondary bacterial pneumonitis caused by obstruction is present. The illness generally is self-limited, but may persist for several weeks. Although only anecdotal evidence supports the benefit of treatment, itraconazole may be helpful in patients with active inflammation. An elevated erythrocyte sedimentation rate and CF titer could be considered markers compatible with active inflammation. As also shown in a study involving dogs with mediastinal granuloma,[364] adjunctive treatment with steroids sometimes is beneficial[150,397] and, if effective, results in prompt improvement. If steroids are used, they should be given with an antifungal agent because of the risk of dissemination resulting from steroid-induced suppression of cellular immunity. Itraconazole should be continued for 6 to 12 weeks. Although fibrosing mediastinitis is a late complication of histoplasmosis, no evidence has shown that mediastinal adenitis is its precursor, and that antifungal therapy would prevent its occurrence.

The clinical entity termed *mediastinal granuloma* affects adults and is seen less frequently in children. Mediastinal granuloma describes a large (3 to 10 cm in adults) mass of mostly caseous lymph nodes that coalesce into a single lesion usually located in the right paratracheal region (Fig. 216–6). Lesions often are asymptomatic and discovered as incidental findings in imaging studies. Symptoms may result from compression of bronchi,

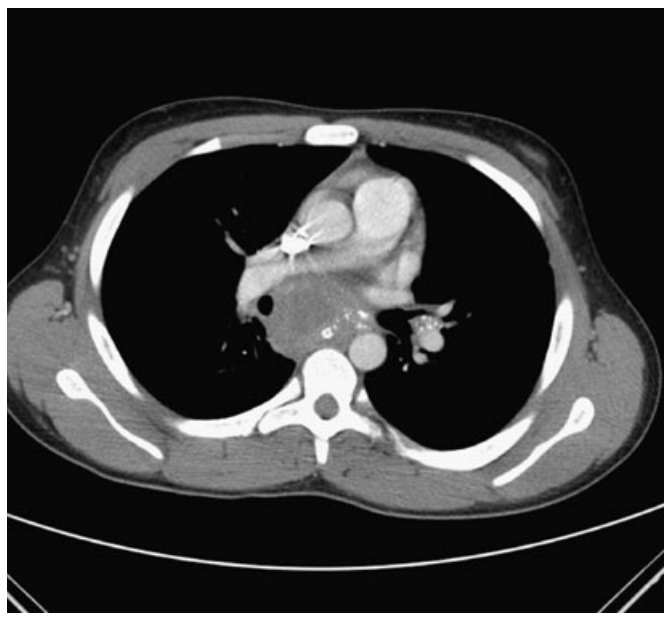

Figure 216–6 A 16-year-old boy was present when a barn was cleaned using a power blower. One week later, he developed a low-grade fever followed by intermittent, nonpleuritic, right parasternal chest pain. CT scan of the chest shows a subcarinal mass with low signal intensity centrally. There is scattered calcification. Histoplasmosis was confirmed by the laboratory, and was compatible with re-exposure to the fungus.

esophagus, or vena cava. Drainage of caseous material may occur spontaneously and result in fistulous tracts to the esophagus,[383] bronchi, or skin. Treatment with itraconazole is appropriate, although efficacy is unproven. Operative intervention sometimes is indicated in the event that compression of the superior vena cava or esophagus occurs. No evidence indicates that this entity is a precursor to mediastinal fibrosis (see section on surgical treatment).

Disseminated Infection

Progressive disseminated infections in children occur in several settings, and all require antifungal therapy.[229,241] Among immunocompetent children, they occur primarily in infants younger than 1 year old and infrequently in children at any age after intense exposure.[296] Among immunocompromised children, dissemination is seen most often in patients who are receiving immunosuppressive agents that impair the cellular immune system, in patients with AIDS, and less commonly in patients with primary disorders of cellular or combined immunity.[174,496] Table 216–4 summarizes treatment recommendations.

Immunocompetent Patients and Immunosuppressed Patients without Human Immunodeficiency Virus Infection

If not treated with antifungal agents,[145,229,241,445] patients with acute progressive disseminated histoplasmosis have a mortality rate approaching 100 percent.[93,122,187,458] Treatment with amphotericin B results in survival of more than 90 percent of infants with PDH.[119,176,229,296] In contrast to adults, in whom monotherapy with itraconazole for patients with nonsevere dissemination has been successful,[93,458] only one report[403] has described the outcome of itraconazole monotherapy in children with disseminated histoplasmosis. Seven children 2 to 14 years old were treated. One child died shortly after treatment was stopped at 1 month. "Marked improvement" was noted in patients treated for 3 months (four patients) and 6 months (one patient), and complete resolution was reported in one patient after 12 months of therapy. Monotherapy with itraconazole is not recommended for disseminated histoplasmosis.[452] Based largely on its demonstrated effectiveness in clinical trials in adults, however, and its ease of administration and tolerability in children, itraconazole is recommended for step-down therapy after induction with amphotericin B. Serum levels of itraconazole should be monitored (see section on antifungal therapy) along with serum/urine antigen concentrations during therapy and in follow-up to ensure that the fungal burden is decreasing. If amphotericin B is elected as monotherapy, a total dose of 30 to 40 mg/kg over 4 to 6 weeks is recommended.

Disseminated Infection in Patients with Acquired Immunodeficiency Syndrome

The severity of the initial signs of illness heavily influences the outcome and recommended treatment regimens for patients with disseminated histoplasmosis complicating HIV infection (see Table 216–4). Regimens are based on trials conducted in adults. Amphotericin B was effective in 74 to 88 percent of all cases,[433,447] whereas patients treated for infections that were sufficiently severe to require hospitalization had a 50 percent mortality rate.[433] In patients with moderate-to-severe disseminated histoplasmosis, liposomal amphotericin B provided a survival benefit compared with amphotericin B deoxycholate.[187] Adults with only mild or moderate symptoms were treated successfully with itraconazole monotherapy in 85 percent of instances.[458]

Recommendations for children are similar to the recommendations for adults; amphotericin B deoxycholate usually is well tolerated in children and is recommended as monotherapy at a dose of 1 mg/kg/day given for 4 to 6 weeks. Alternatively, it may be used for 2 weeks as induction therapy and followed thereafter by itraconazole, 5 to 10 mg/kg/day for 12 months. Liposomal amphotericin B, a more costly preparation, may be substituted for the deoxycholate preparation with either regimen in the event of renal complications. Serum followed by urine antigen monitoring[293,448] (see section on antigen detection) is recommended during therapy and for 1 year after its completion to monitor response and to assess for relapse. After the completion of therapy, the significance of low levels of antigenuria in the absence of antigenemia and clinical symptoms is unclear and not an indication for prolonged therapy. Prolonged follow-up is recommended in these instances.

Because of a high rate of relapse after cessation of therapy in patients with AIDS in whom there is persistently impaired immune function, long-term maintenance therapy (secondary prophylaxis) is recommended.[186,438,447,469] In adults with mild to moderately severe disseminated infection, amphotericin B (50 mg weekly or biweekly) is effective in 81 to 97 percent of cases,[264,447] and itraconazole is effective in 90 percent.[168,459] Itraconazole is the azole of choice for mildly or moderately ill patients, in whom it may be used as induction therapy and secondary prophylaxis. Fluconazole is not recommended for secondary prophylaxis (see section on medical management), but may be considered if patients received amphotericin B as induction therapy, if itraconazole is poorly tolerated, or if itraconazole's cost precludes its use.

A prospective observational study[139] of adults with AIDS and disseminated histoplasmosis found it safe to discontinue secondary prophylaxis after 12 months of primary antifungal therapy and demonstration of sustained immunologic improvement resulting from HAART therapy. Specific criteria for withholding secondary prophylaxis include negative blood cultures, *Histoplasma* urine antigen concentration of less than 4 ng/mL, and $CD4^+$ T-cell counts greater than 150 cells/mm^3 in patients continuing to receive HAART.[139] Primary prophylaxis with itraconazole is effective for patients with AIDS who live in hyperendemic areas[267] (areas in which the incidence of histoplasmosis is >5 cases per 100 patients per year).

Prophylaxis of Immunosuppressed Patients

Clusters of infection occasionally are reported in high-risk populations, sometimes in geographic regions with low background rates.[121] Based on the results of a large clinical trial, however, the need for prophylaxis for patients residing in a hyperendemic area and who are undergoing immunosuppression for management of neoplasms, inflammatory syndromes, or transplantation of allogeneic bone marrow or solid organs seems to be low.[413] Included were patients with CF titers to *H. capsulatum* of 1:8 or 1:16, positive M bands by ID, or radiographic evidence suggestive of past infection (calcified splenic or lung nodules). In contrast, patients receiving TNF-α antagonists seem to be at greater risk of developing disseminated histoplasmosis,[422,482] perhaps attributable to reactivation of latent infection. Active histoplasmosis during the 2 years preceding the need for immunosuppression may be a basis for consideration of prophylaxis. Although firm evidence is lacking, criteria for such evidence may include chest radiographic abnormalities compatible with histoplasmosis, *Histoplasma* antigenuria, or anti-*Histoplasma* antibody titers 1:32 or greater. Duration is unclear, but 12 to 24 weeks has been recommended.[452]

Central Nervous System Infection

With the exception of CNS infections that accompany PDH, histoplasmosis of the CNS in children[175] rarely is seen.[296] Adults with CNS infections[23,360,466] can present with meningitis, focal infection of the brain[212] or spinal cord, thrombotic events caused by focal vasculitis or emboli, or diffuse encephalitis. As with children, some infections occur in association with disseminated infection, others occur as an isolated illness, and others occur in the context of relapse in a sequestered site that is penetrated inadequately by antifungal agents.[466] Mortality is almost uniformly invariable if untreated. Treatment is effective in only 20 to 40 percent of patients with meningitis, and 50 percent of responders relapse after cessation of treatment.[437]

Although amphotericin B deoxycholate and liposomal amphotericin B enter the CSF poorly,[101,151,228] liposomal amphotericin B concentrations are higher in brain tissue in animal models.[228] Comparative studies of these agents for the treatment of CNS infections caused by histoplasmosis have not been performed. In a more recent report,[360] five adults were treated initially with amphotericin B deoxycholate at doses of 40 mg/kg for 8 weeks followed by fluconazole, 200-400 mg/day for 1 year, resulting in cure of four patients and minor residual sequelae in one. These reports, the ability of liposomal amphotericin B to achieve higher brain tissue levels than occur with amphotericin B deoxycholate, and the superiority of itraconazole over fluconazole in an animal model all contribute to the basis for the recommendation[221] that adults with CNS infections receive liposomal amphotericin B (5 mg/kg/day) for 4 to 6 weeks followed by itraconazole for 1 year.

Meningitis was present in 25 of 40 children in a series[296] of progressive disseminated histoplasmosis. All children received 40 mg/kg of amphotericin B deoxycholate followed by ketoconazole for 3 months. The overall mortality rate in the series was 10 percent; however, two deaths were in children who failed to complete therapy, and two died within 4 days of presentation, suggesting that this regimen cured meningitis in most cases. In the rare setting in which meningitis is found in a child as an isolated infection, recommendations reasonably might include the use of liposomal amphotericin B (in place of amphotericin B deoxycholate) followed by itraconazole for 12 months.

The role of combination therapy for CNS infection has not been examined in humans. Antagonism has been shown in an animal model[167] that used a regimen of fluconazole and amphotericin B. In another animal model of CNS infection, itraconazole in association with amphotericin B provided no benefit compared with amphotericin B alone.[231]

Despite isolated case reports that have reported success with ketoconazole, itraconazole, fluconazole, the combination of itraconazole and fluconazole, and voriconazole,[404,452] the efficacy of azole monotherapy for the treatment of CNS infections has not been adequately studied. Posaconazole[320,336] has been used successfully as salvage therapy in one patient after therapeutic failure with several prior regimens. The evidence is insufficient to recommend azole monotherapy for CNS infection.

MEDICAL MANAGEMENT OF MANIFESTATIONS THAT DO NOT REQUIRE ANTIFUNGAL THERAPY

Several common and uncommon manifestations of histoplasmosis do not require antifungal treatment. Apart from mild primary pulmonary infection, one of the most common is pericarditis. Because it rarely is associated with frank fungal infection of the pericardium, it usually resolves spontaneously or with nonsteroidal anti-inflammatory drugs. Indomethacin usually is effective[492] in rapidly reducing inflammation and can reverse signs of impending tamponade. Steroids have been reported to be effective, but, if used, should be accompanied by antifungal therapy. Rheumatologic symptoms of arthritis, with or without erythema multiforme or erythema nodosum, also respond to nonsteroidal anti-inflammatory drugs.[368] Fibrosing mediastinitis is a late complication of histoplasmosis, but it occurs occasionally in children. When granulomatous lesions evolve to become densely fibrotic, antifungal and anti-inflammatory therapies are ineffective. The clinical and laboratory criteria that are used to assess whether active inflammation is present are inexact, however, and a trial of antifungal therapy often is warranted if clinical features or laboratory evidence of active inflammation, or both, is present. Treatment with antifungals almost always is unsuccessful, but improvement has been reported.[409] Presumed ocular histoplasmosis is seen almost exclusively in adults and does not improve with antifungal therapy; its management has been reviewed extensively.[60]

SURGICAL TREATMENT

Operative intervention rarely is needed to manage patients with histoplasmosis. In a review of 94 patients, 10 to 40 years old, the most common reason for evaluation was obstruction of thoracic structures by mediastinal masses.[129] Seventy-five patients underwent surgery or endoscopy to relieve obstruction of the pulmonary artery, superior vena cava, bronchus, or esophagus. Recurrent pneumonia, tracheoesophageal fistula, hemoptysis, and broncholithiasis were other indications for surgical procedures.[129,214,495] Because attempts to excise caseous nodes completely sometimes can damage contiguous structures, the preferable approach is to incise and evacuate debris from such lesions and leave the adherent portion of the capsule intact.[117,129,313,479]

Surgery has little place in the management of fibrosing mediastinitis resulting from histoplasmosis. The dense fibrous consolidation of mediastinal structures coupled with the hypervascularity of mediastinal tissues renders the risk for development of serious hemorrhage and other life-threatening surgical complications high.[259] Excisional biopsy carries a prohibitive risk.[77] Bronchoscopy coupled with coagulation of hyperemic vessels within bronchi in individuals presenting with hemoptysis has been useful.[256] When calcification has occurred in fibrosing mediastinitis, surgical repair is not feasible,[253] and the fibrosis continues after surgery. Some success has been achieved with relief of vascular obstruction by percutaneous placement of intravascular stents,[98,400] but complications occur.[334] An intravascular stent has been used successfully to relieve obstruction in a child with superior vena cava syndrome (M. Kleiman, unpublished observation, 2008). Prophylactic excision of large mediastinal nodes has not been shown to prevent fibrosis, and patients with large mediastinal lymph nodes who are otherwise asymptomatic rarely progress to develop mediastinal fibrosis.[429]

PROGNOSIS

Histoplasmosis is either unrecognized or self-limited in most individuals who sustain this most common of the endemic mycoses. Histoplasmosis results in serious illness in infants, immunocompromised patients, and older individuals, particularly individuals with emphysematous pulmonary disease.[34,275] The cure rate for children who receive therapy for serious acute manifestations is high. Little prospective information is available about the long-term outcome in acute pulmonary histoplasmosis. A study that examined pulmonary function in six children and their parents after intense exposure found restrictive (three patients) and obstructive (two patients) patterns and a reduction in carbon monoxide diffusing capacity in five of six tested. At 2 years, a diffusing capacity abnormality remained in three children, and hypoxemia persisted in the most severely affected

patient.[215] No data that can be used to predict the occurrence of long-term complications, such as mediastinal fibrosis, are available. When it does occur, mediastinal fibrosis is progressive, and therapeutic options are few; because by definition mediastinal fibrosis invades the airways and pulmonary vasculature, its prognosis is influenced heavily by whether it affects one or both lungs.[77] The prognosis is poor in the latter instance.

PREVENTION

Complete prevention of histoplasmosis is currently impossible, but reasonable precautions can decrease greatly the risk to individuals who are highly likely to experience serious complications if they become infected. Individuals with impaired cellular immunity who reside in, or plan travel to, endemic regions should be counseled about the potentially serious consequences of infection. Counseling should include education about the sites likely to be contaminated with *H. capsulatum* and the activities that potentially may expose them to spores; primary prophylactic regimens using itraconazole may be considered for HIV-infected patients with exposure to soil mixed with bird or bat droppings (see section on epidemiology).[164] If such activities are unavoidable, the use of National Institute for Occupational Safety and Health (NIOSH)–certified high-efficiency mask filtration devices[410] should be encouraged; however, devices appropriate for use by small children may be unavailable. Sterilization of sites by formaldehyde spray[53,410] have been attempted, but the toxicity of this agent renders its use undesirable and, in some settings, ineffective. When potentially contaminated sites are disturbed, aerosols containing spores can be reduced substantially by thoroughly dampening the material with water before it is manipulated. Although educating and counseling high-risk patients afford some protection against acquiring infection from microfoci, some of which can result in intense exposure, they do not protect against "sporadic" infections. For the general population, education in endemic areas also is beneficial, particularly for protecting individuals whose occupational and recreational activities may expose them to *H. capsulatum*.

A vaccine for prevention of histoplasmosis has not been developed, although active investigation in animal models[295,354] is under way to identify potential vaccine targets[81,87,485,489] and delineate the regulatory elements needed for immunity against *H. capsulatum*.[82,359] An intriguing area of inquiry is identifying vaccine targets integral to dimorphism, a requisite for the pathogenicity of all of the endemic fungi.[285] Other areas of inquiry have explored the feasibility of developing vaccines against invasive fungal pathogens in the setting of severe immunodeficiency, including individuals with $CD4^+$ T-cell deficiency.[489] A recombinant protein vaccine has been made from cloned sequences of DNA coding for the cell wall glycoprotein HSP 60, which has been found to be protective in mice against a lethal intravenous inoculum of *H. capsulatum*.[141] This vaccine protected mice in a model of pulmonary infection.

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CHAPTER 217

Sporotrichosis

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Sporotrichosis is an infection caused by the fungus *Sporothrix schenckii* that is manifested most commonly as an ulcerating nodule at a site of local trauma, with spread occurring along regional lymphatic channels. Infection of other tissues or widespread dissemination is rare. It is an uncommon problem in children, but young adults seem to be infected more frequently, presumably owing to more frequent exposure to soil, plants, and decaying vegetable matter that harbor the organism. The disease was described first by a medical student, Schenck, in 1898, and most of what is known about the clinical syndrome has been learned from studies of large outbreaks, particularly one occurring in gold miners near Johannesburg, South Africa, in the early 1940s.[77,87]

ORGANISM

S. schenckii is a dimorphic fungus found principally in decaying vegetable matter or plant debris, although it does not seem to be a plant pathogen. A molecular phylogenetic study of 60 isolates from South America and Europe showed three major clades that tended to group according to geographic origin.[57] The organism grows well on most culture media and is resistant to cycloheximide. Preferred culture media are Sabouraud glucose agar and blood agar, incubated at 25° C to 27° C. Most isolates grow readily from clinical material within 3 to 5 days, although occasional instances of slow growth have been reported, and cultures should be held at least 4 weeks before being considered negative. Incubation on blood agar at higher temperatures (37° C) allows growth of the yeast phase, which is necessary for specific identification of the organism in culture.[72]

EPIDEMIOLOGY

Most cases of sporotrichosis are reported from Central and South America, especially from Mexico and Brazil, and cases in the United States appear to cluster in the Midwest, particularly along the Mississippi and Missouri River areas.[32,66] Disease in humans usually results from inoculation of minor wounds by debris containing *S. schenckii*, and gardeners and nursery workers, forestry workers, miners, and other individuals exposed to contaminated plant materials are at higher risk for acquiring the disease.[12,16] Disseminated disease has been the initial presentation of some human immunodeficiency virus–infected individuals.[2] Laboratory personnel working with the organism have become infected after incurring needle-stick injuries.[86] The large South African epidemic in the gold mines of Transvaal probably resulted from the miners' brushing against rotting timbers in the mines, and a cluster of cases that occurred after a brick-throwing incident in Florida was traced to *S. schenckii* in the packing straw of the bricks.[75,87] Contaminated hay was the source of one outbreak linked to a Halloween haunted house.[22] Pulmonary disease may result from inhalation of spores.[66] Uncommonly, transmission of disease from animals, particularly domestic cats with cutaneous disease, or from family members occurs.[24,28,29,70] One adult

acquired the disease after being bitten by a squirrel.[76] In South America, armadillo hunters are at risk for developing sporotrichosis, but it is acquired from the decaying plant debris in armadillo nests, rather than from the animals themselves.[56] Cats have been implicated in a large outbreak in Brazil.[78] Molecular typing of isolates can be useful in investigations of outbreaks.[15,59,60]

PATHOGENESIS AND PATHOLOGY

As indicated, the most common mode of acquisition of sporotrichosis is by inoculation of the organism into skin structures, although disease also may develop from inhalation of spores of the organism. The incubation period varies considerably, commonly ranging from 7 to 30 days after cutaneous inoculation, but possibly as long as 6 months.[72] The disease usually remains localized; of the 2825 cases of sporotrichosis in the Transvaal mine epidemic, none had systemic spread.[87]

S. schenckii, similar to other yeasts, seems to bind specifically to the glycosphingolipid lactosylceramide, which is present on the cell surface of animal cells.[41] This mechanism may be one by which the organism establishes a foothold in the host. Cell-mediated immune responses probably are important for containment of infection. One study documented intact responses in patients with cutaneous forms of the disease, whereas patients with systemic sporotrichosis had impaired cell-mediated immunity.[65] This study is supported by the observation that systemic disease tends to occur in individuals with underlying diseases that alter cell-mediated immunity.

Histopathologic examination of primary cutaneous lesions usually reveals changes in the epithelium, with hyperkeratosis, parakeratosis, and pseudoepitheliomatous hyperplasia. Intraepidermal microabscesses may be seen as well. In more established lesions, the pathologic process involves the dermis and below, with inflammatory infiltrate extending perivascularly.[53] The classic lesion on microscopic examination is a granuloma with an asteroid body at the center, although this picture is not pathognomonic for sporotrichosis. The asteroid body is an antigen-antibody complex deposited on the surface of the organism.[52] Showing fungi in tissue sections often is difficult, even with special staining techniques, because of the paucity of organisms in tissue. They may be seen on Gram stain as gram-positive, but irregularly staining bodies, sometimes as cigar-shaped, 3- to 5-μm yeast forms. Periodic acid–Schiff and silver stains probably are better suited for detection of fungi in tissue sections.[53,72] Immunohistochemical staining techniques may prove superior to standard methods.[58,63]

CLINICAL MANIFESTATIONS

Sporotrichosis can occur in cutaneous and extracutaneous forms, with the cutaneous varieties accounting for approximately 80 percent of all cases.[72] These categories can be broken down further into the organ system involved (Table 217–1).

TABLE 217–1 Clinical Forms of Sporotrichosis

Cutaneous	Extracutaneous
Lymphocutaneous	Osteoarticular
Fixed cutaneous	Pulmonary
	Muscular
	Ocular
	Genitourinary
	Central nervous system

Data from references 1, 35, 36, 49, 66, 73.

CUTANEOUS SPOROTRICHOSIS

Cutaneous disease with *S. schenckii* can be either lymphocutaneous or fixed cutaneous, the latter of which occurs less frequently. In lymphocutaneous cases, the initial lesion appears as a firm, slightly tender subcutaneous nodule. It progresses along local lymphatic channels, with multiple nodules appearing. The lesions typically enlarge, and may ulcerate and suppurate (Fig. 217–1). Untreated, they may heal slowly over months or persist; recurrences are frequent. Differential diagnosis includes cutaneous nocardiosis, atypical mycobacterial disease, leishmaniasis, rosacea, syphilis, pyoderma gangrenosum, and disseminated *Acanthamoeba* disease, and cutaneous manifestations of other fungal diseases.[20,47,69,85,94]

The fixed cutaneous form of the disease is just as the name implies, with no evidence of lymphatic spread. The primary lesions are identical to the lesions seen in the lymphocutaneous form. Although some researchers have suggested that sporotrichosis in children is more likely to appear in the fixed cutaneous form, compared with adults, this theory has not been borne out in prior studies. Table 217–2 shows results from six reports of series of cases of sporotrichosis in children. The 60 percent rate for the lymphocutaneous form may not differ much from the 90 percent figure quoted for adults when one takes into account that many pediatric studies are reports of small numbers of cases and may have resulted from selection bias. A large series of patients from Peru documented a disproportionate number of children with facial lesions (86 of 143 children versus 23 of 95 adults) but did not classify fixed versus lymphocutaneous forms by age.[64] Fixed cutaneous disease in children has been confused with

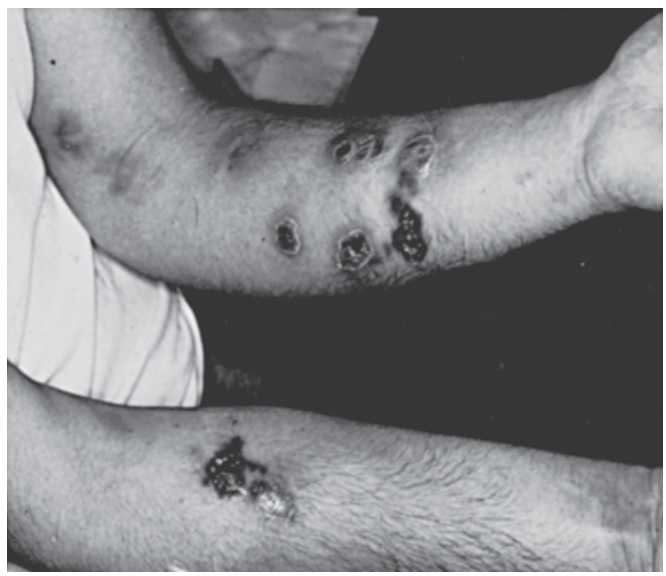

Figure 217–1 Cutaneous lymphatic sporotrichosis of both arms. Note the characteristic involvement of lymphatics that drain the sites of the primary lesions. *(Courtesy of G. Medoff and G. S. Kobayashi.)*

TABLE 217–2 Summary of 42 Cases of Pediatric Sporotrichosis

Duration of symptoms before diagnosis	3-10 wk
Lymphatic involvement	28/47*
Culture-positive	39/44*
Relapse after therapy	1/21*

**Numerator is number of patients positive; denominator is number of patients tested. Data from references 10, 13, 18, 31, 54, and 61.*

impetigo.[10] A study from Peru also documented a predilection of children to develop lymphocutaneous lesions on the face and neck.[55] Risk factors for disease in children in this study included playing in fields, ownership of a cat, and residence in a house with dirt floors.

EXTRACUTANEOUS SPOROTRICHOSIS

Sporotrichosis occurring in extracutaneous sites either may be localized (related to an unusual area of trauma) or may represent disseminated disease. In the absence of trauma, the presence of extracutaneous sporotrichosis should raise suspicion of disseminated disease and consideration of immunodeficiency states. Overall, infection of bones and joints is the most common form of extracutaneous disease.[92] Sporotrichal arthritis usually is an indolent and slowly progressive disease that may occur with or without cutaneous or lymphatic disease, suggesting a hematogenous route of infection for most cases. Diagnosis generally requires synovial biopsy with culture for showing the organism.[79,92] Two studies showed diagnostic delays averaging 17 and 25 months from the onset of symptoms.[5,17] Sporotrichal osteomyelitis usually occurs with concomitant arthritis, but isolated bone involvement has been recorded. Lytic lesions and periosteal changes are noted most frequently.[34,92]

Pulmonary sporotrichosis seldom occurs with cases of disseminated disease and probably develops after inhalation of spores, as with primary pulmonary histoplasmosis.[66] The presence of cavitary lesions in the upper lobes often leads to a diagnosis of tuberculosis, and fungal culture of sputum, bronchoscopic specimens, lung tissue, or gastric aspirate usually is needed for establishing the diagnosis.[25,66,90] Pleural involvement is uncommon (3 of 47 cases in one review), and complications such as massive hemoptysis rarely occur.[26,37,66]

Sporotrichosis has been found to involve virtually every organ system in the body as part of disseminated disease.[92] Most commonly, underlying immunodeficiency states, such as diabetes, prolonged steroid therapy, alcoholism, tumor necrosis factor-α antagonist therapy, and acquired immunodeficiency syndrome, are present.[1,27,33,35,38,49,84,92] Fungemia in the absence of disseminated disease has been documented in one otherwise healthy adult with a lysis-centrifugation blood culture system, and laryngeal and cutaneous sporotrichosis have been reported in a healthy child.[46,48] Dissemination in patients with neoplasia seldom occurs.

DIAGNOSIS

A high index of suspicion is necessary to establish the diagnosis of sporotrichosis. In the largest outbreak in the United States, which occurred in 1988 among horticulturists and forestry workers, only 15 percent of cases were diagnosed at the time of initial presentation to a physician.[14] Culture is the gold standard for diagnosing sporotrichosis. Although organisms occasionally are seen on pathologic specimens, the yield is low enough to render biopsy unnecessary. If the diagnosis is suspected, scrapings of cutaneous lesions for culture should be sufficient to establish the diagnosis.[72]

A skin test antigen has been available for many years, but, as with the histoplasmin skin test for histoplasmosis, it is useful mainly as an epidemiologic tool. Polymerase chain reaction may prove useful in diagnosis in the future.[42] Serodiagnosis has been explored in a few studies. Immunoprecipitation or commercially available slide latex agglutination can be useful when material for culture is difficult to obtain, or cultures are negative, such as with sporotrichal meningitis.[21,80] Similarly, an enzyme-linked immunosorbent assay has been used for diagnosis. Antibody titers in cerebrospinal fluid tend to decrease with successful therapy, and such tests might prove useful for monitoring response to therapy.[6,80] Western blotting has been used to detect sporotrichal antibody.[81] Using a crude antigen preparation, Scott and Muchmore[81] determined that detection of antibody to three antigens, 32 kd, 40 kd, and 70 kd, seems to be sensitive and specific for diagnosis of active sporotrichosis. Patients with extracutaneous disease seemed to form antibody to a greater number of *S. schenckii* organisms than patients with cutaneous disease. Further studies of the immune response in sporotrichosis should enable development of better serodiagnostic tests than those that now are routinely available.

TREATMENT AND PROGNOSIS

Sporotrichosis may resolve spontaneously, but treatment is indicated in most circumstances.[4,67] The Mycoses Study Group of the Infectious Diseases Society of America has published practice guidelines for management of patients with sporotrichosis.[45]

Heat applied to the site of cutaneous disease has been reported anecdotally to cause resolution of lesions in patients in whom medical therapy was contraindicated.[30,73,88] One prospective study showed good results using benzene pocket warmers for heat therapy of facial lesions in children.[39] Surgical removal of infected skin and soft tissue has been used, but skin grafting may be required.[9] This form of treatment usually is unnecessary with the availability of medical management except possibly for some cases of pulmonary infection (particularly if it is confined to one lobe) and other extracutaneous disease.[17,34,66] Hyperthermia remains an option for treating sporotrichosis in pregnancy, for which other agents may be contraindicated.[42]

A saturated solution of potassium iodide (SSKI), a proteolytic agent for which the mechanism of action in sporotrichosis is unclear,[51] can be used for uncomplicated sporotrichosis. The in vitro growth of *S. schenckii* is not inhibited appreciably by iodide, but free iodine has a marked inhibitory effect on growth.[91] The small amount of free iodine in a saturated solution of potassium iodide may be sufficient to cause resolution of disease. The pediatric dosage of a saturated solution of potassium iodide is empiric; it usually is given three times daily in juice or milk, starting at a low dose (e.g., 1 to 2 drops per year of age) and increasing the dose over the course of several days to a maximum of 30 to 40 drops per dose.[13,54,61] For younger children, lower dosages may be acceptable.[54,68] Treatment is continued until a few weeks after all lesions have resolved. Adverse reactions, such as salivary gland swelling, excessive lacrimation or salivation, nausea, vomiting, and abdominal pain, may resolve with temporary cessation of therapy followed by re-institution at a lower dosage. SSKI currently is only an alternative treatment for lymphocutaneous and cutaneous forms of the disease.[42]

In vitro susceptibility testing is not used widely, and results may not always agree with clinical response.[74,89] Itraconazole, an oral azole derivative, is the drug of choice for treatment of cutaneous, lymphocutaneous, and osteoarticular forms of sporotrichosis.[8,40-42,83] Restrepo and colleagues[71] showed clinical cures in all 17 patients with cutaneous forms of sporotrichosis treated with itraconazole (100 mg/day), with no major side effects. Borelli[7] reported a treatment failure, however, in a patient treated with 100 mg/day, who subsequently responded when the dose was increased to 200 mg/day. Systemic disease also has been treated with itraconazole with favorable clinical responses, although all information is from cases in adults.[3,50,62,83,93]

Intravenous amphotericin B remains the drug of choice for treating patients with disseminated or severe sporotrichosis.[42,92] Duration of therapy in children has not been studied but probably should require 30 mg/kg as a total dose. Occasionally, intra-articular amphotericin B is used for treating sporotrichal arthritis

if response to other therapy is poor.[23] Amphotericin B therapy in patients with pulmonary sporotrichosis is less effective than with other forms of sporotrichosis, which has prompted many clinicians to use a combined medical-surgical approach.[45,66] Itraconazole is an alternative therapy and may be useful for long-term suppression.[45] Amphotericin B seems to be effective in treating sporotrichal meningitis when it is given early in the course of the disease, but adjunctive therapy with flucytosine or rifampin might be helpful.[79] Itraconazole and fluconazole also may have roles in treating meningitis.[40,45]

S. schenckii does not seem to be particularly susceptible to ketoconazole in vitro, and clinical experience with ketoconazole treatment of sporotrichosis has been mixed.[11,19,82] Possibly, relatively large doses of ketoconazole are needed to produce a good clinical response. Terbinafine, an allylamine, cured five adults with cutaneous sporotrichosis, but further experience with this agent is needed.[40]

Prognosis for cutaneous forms of disease is excellent, but the extracutaneous forms are associated with significant morbidity and mortality, in part related to the underlying conditions predisposing these patients to disseminated disease. Many cases of osteoarticular disease result in permanent disability.[17]

PREVENTION

The key to prevention of sporotrichosis is the elimination of exposure to the organism, particularly with regard to skin surfaces and mucous membranes. Usually, elimination of exposure can be accomplished by the use of protective clothing during high-risk activities, such as working with sphagnum moss or other decaying, moist plant material.[12,14] Nursery workers should be educated about the hazards and early signs of sporotrichosis, and physicians and veterinarians should be aware of the uncommon circumstances of spread of the infection from family members and domestic cats. The epidemic in the Transvaal gold mines was stopped when timbers in the mine shafts were sprayed with a fungicide.[87] Although reporting of individual cases of sporotrichosis is not required in the United States, reporting of clusters of cases can aid epidemiologic investigations and stop the spread of the disease.

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CHAPTER 218

ZYGOMYCOSIS

Theoklis E. Zaoutis ✪ William J. Steinbach

Zygomycosis (formerly *mucormycosis* or *phycomycosis*) is a relatively new term that refers to a group of uncommon but frequently fatal mycoses caused by the fungi of the class Zygomycetes. Organisms of the class Zygomycetes have been reported to cause disease in humans in publications since the 1800s. Platauf[86] is credited with providing the first description of zygomycosis in humans in his paper entitled "Mycosis Mucorina." In this German language manuscript, Platauf[86] described a case of disseminated mycosis in a patient with cancer. The first English language description of the disease appeared in 1940 when Wade[120] described a case of cutaneous mucormycosis infection of the face.

The class Zygomycetes is divided into two orders, the Mucorales and the Entomophthorales.[94] The Mucorales organisms are responsible for most of the disease seen in humans, predominately affecting immunocompromised hosts and often characterized by a rapidly evolving clinical course with tissue destruction and invasion of blood vessels. The mycoses caused by Entomophthorales historically have been limited to immunocompetent patients residing in tropical and subtropical areas of the world. Infections caused by Entomophthorales usually are chronic, subcutaneous, and limited without blood vessel invasion.[94] This chapter focuses on the more relevant infections caused by Mucorales, with a brief description of infections caused by Entomophthorales.

ORGANISMS

The organisms of the class Zygomycetes are ubiquitous in nature and have a wide geographic distribution. The class consists of

Figure 218–1 Classification of Zygomycetes. *(From Ribes, J. A., Vanover-Sams, C. L., and Baker, D. J.: Zygomycetes in human disease. Clin. Microbiol. Rev.* 13*:236, 2000.)*

two orders, Mucorales and Entomophthorales, each containing several species reported to cause disease in humans (Fig. 218–1).[94] The most common causative organisms in humans are the *Mucor*; *Rhizopus*, *Absidia*, and *Rhizomucor* genera of the Mucoraceae family. These organisms grow readily on common laboratory media, but are inhibited by cycloheximide. On solid media, members of Mucorales show rapid growth of woolly colonies, often with spores seen as tiny dark dots to the naked eye. Hyphae characteristically are large (10 to 30 μm in diameter), nonseptate, and, often, twisted or ribbon-like. The lack of septation and the tendency of hyphae to branch at right angles usually serve to distinguish them from *Aspergillus* spp., which are septate, are smaller, and branch at acute angles. Genera usually are differentiated from one another by examination of mycelia. Differentiating the members of Zygomycetes into species often is difficult.

Growth of members of Entomophthorales also occurs readily on solid media, but appears as flat, gray, or pale yellow waxy colonies with velvety white mycelia on the surface.[94,96] The hyphae often are septate, and differentiation between genera and among species is based on morphologic characteristics of cultured organisms.

EPIDEMIOLOGY

All members of Zygomycetes are ubiquitous in nature and are found in all parts of the world, regardless of climate or other factors. Most reported human disease caused by members of Mucorales is concentrated in North America, perhaps because of a concentration of immunocompromised hosts.[7] Infections caused by the Entomophthorales genera *Basidiobolus* and *Conidiobolus* occur predominantly in Africa, India, and the Far East, although a few cases have been reported in North and South America.[94]

These fungi are found commonly in soil, in decaying organic material such as vegetation, bread, seeds, and fruits, and in manure. Many of them are animal pathogens. Members of Mucorales also are isolated occasionally from the hospital environment.[51,54,94,125,128] Studies have suggested that a relationship exists between activities involved in hospital construction and development of zygomycosis in immunocompromised hosts.[54,99,128] *Rhizopus* spp. frequently are found in moldy bread and fruits.[94] Members of Entomophthorales commonly are found in feces of reptiles and other animals and in decaying vegetable matter, and some are insect pathogens.[94]

The ubiquitous nature of these organisms, coupled with the paucity of human disease associated with them, is strong evidence for their saprophytic characteristics. Infections caused by the Zygomycetes are rare, occurring at an annual rate of 1.7 infections per 1 million population in the United States.[91] Since the 1990s, the frequency and incidence of zygomycosis have increased, however, particularly among hematopoietic stem cell transplant (HSCT) recipients and patients with hematologic malignancies (Fig. 218–2).[32,52,65] Despite these increases since the 1990s, Zygomycetes were seen at autopsy in 1.9 percent of patients dying of malignancy, contrasting with the high incidence of aspergillosis at autopsy (19.6%) at the same center.[53]

The incidence of zygomycosis complicating HSCT was 2.5 percent in one study; it may be less common than other molds because evaluation of indoor environments shows Zygomycetes rarely are isolated.[57] A review of 1500 patients who underwent HSCT over the course of 26 years at one center found only 0.9 percent of patients had zygomycosis.[74] In the largest systematic review of reported cases of zygomycosis, *Rhizopus* spp. and

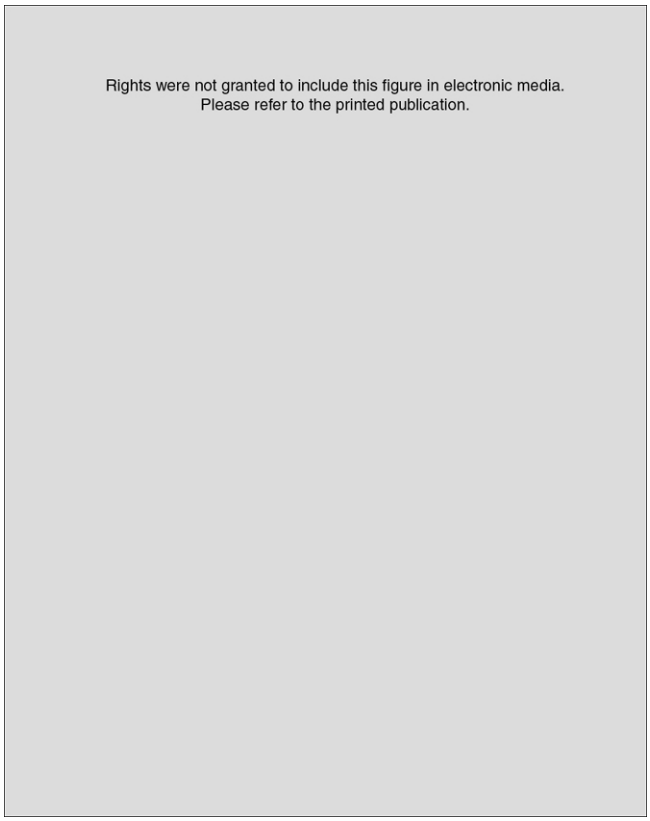

Figure 218–2 **A,** Changing frequency of non-*Aspergillus* molds in blood and marrow transplantation recipients at Fred Hutchinson Cancer Research Center (Seattle, WA), 1985-1999. **B,** Changing spectrum of *Aspergillus* and Zygomycetes at M. D. Anderson Cancer Center (Houston, TX). *(**A** from Marr, K. A., Carter, R. A., Crippa, F., et al.: Epidemiology and outcome of mould infections in hematopoietic stem cell transplant recipients. Clin. Infect. Dis.* 34*:909, 2002; **B** from Kontoyiannis, D. P., Lionakis, M. S., Lewis, R. E., et al.: Zygomycosis in a tertiary-care cancer center in the era of Aspergillus-active antifungal therapy: A case-control observational study of 27 recent cases. J. Infect. Dis.* 191*:1350, 2005.)*

Mucor spp. were the causative organisms most commonly reported, being identified in 47 percent and 18 percent of cases.[98] The review included 157 published cases of pediatric zygomycosis, and a similar distribution of species causing infection was seen in children and adults.[133]

Infection with zygomycetes commonly manifests as an opportunistic infection in patients with underlying risk factors. Predisposing risk factors include immunosuppression, metabolic disorders, breakdown of skin or soft tissue integrity such as occurs in burn patients or surgical patients, and prematurity (Table 218–1).[51,53,98,133] The underlying conditions most commonly reported for adults and children are diabetes mellitus and neutropenia.[53,98,133] More recent evidence has suggested that the increasing use of voriconazole as prophylaxis and as empiric, preemptive, and targeted therapy for invasive aspergillosis is associated with an increased risk for development of zygomycosis.[40,52,66,105] One hypothesis is that voriconazole, because of its lack of activity against Zygomycetes, has exerted selection pressure favoring the emergence of zygomycosis.[128]

MUCORMYCOSIS

PATHOGENESIS AND PATHOLOGY

Mucormycosis usually is acquired by humans after inhalation of spores from environmental sources, and the organisms may colonize the sinuses and nasopharynx of some patients.[94] Occasionally, mucormycosis results from cutaneous inoculation of spores secondary to trauma, as has occurred with contaminated elastic adhesive tape[24,34,94] and ingestion of fermented milk or dried bread products.[55,78] After airborne spores are inhaled, primary infection usually develops in the upper and lower respiratory tract and later by contiguous or hematogenous dissemination. Disseminated disease most commonly arises from the lungs and spreads hematogenously to the central nervous system, kidneys, gastrointestinal tract, and heart. Dissemination also can occur from cutaneous or subcutaneous sites of infection.[24,34,109] Person-to-person transmission has not been documented.

TABLE 218–1 Underlying Diseases Commonly Associated with Increased Risk for Mucormycosis

Pediatric-Specific Risk Factors	Risk Factors Seen in Adults and Children
Prematurity	Neutropenia or neutrophil dysfunction
Methylmalonicaciduria	Diabetes mellitus
Malnourishment	Malignancy
	Organ transplantation
	Corticosteroid therapy
	HIV infection
	Diabetes mellitus
	Deferoxamine therapy
	Intravenous drug use
	Trauma or surgery
	Burns

The immune status of the host is fundamental to production of disease. In normal hosts, Zygomycetes are killed by mononuclear cells and neutrophils via the generation of oxidative metabolites and cationic peptide defensins.[25,121,123] The neutrophil seems to be the primary component of the immune response against these organisms and may serve to prevent germination of inhaled spores.[25] Neutropenia and qualitative deficiencies of neutrophil function are associated with an increased risk of development of zygomycosis.[17,109] In addition, corticosteroid treatment impairs the ability of macrophages to prevent germination of the spores.[122] Zygomycosis reported in patients with acquired immunodeficiency syndrome (AIDS) tends to occur in intravenous drug users and may reflect direct inoculation as the risk factor, rather than the immunodeficiency itself.[75,107] Disease may follow a less fulminant course in patients with AIDS compared with other immunocompromised populations.[8]

Diabetes mellitus, in particular diabetic ketoacidosis, is the most common underlying disease in patients with mucormycosis.[50,51,98] Serum from patients with diabetic ketoacidosis does not inhibit the growth of *Rhizopus* spp., but serum from those same patients after treatment of the ketoacidosis does show inhibitory properties similar to those of normal human serum.[3,17,33,94] Hyperglycemia and acidosis, commonly seen in diabetic patients, are known to impair the ability of phagocytes for chemotaxis and to kill the organisms by oxidative and non-oxidative mechanisms.[17]

Iron levels in serum also may play a role in the pathogenesis and immune response to the organisms. Patients treated with the iron chelator deferoxamine have a markedly increased risk of developing invasive mucormycosis, as shown by the tendency for deferoxamine therapy to result in progression of natural and experimental mucormycosis.[11,12,22,23,36,93,101,117] This phenomenon seems to occur commonly, particularly in patients undergoing hemodialysis.[10,11,131] Studies in experimental animals suggest that deferoxamine may serve as a siderophore for *Rhizopus* spp., with resultant increased fungal growth and increased mortality rates in a guinea pig model.[9,23,117] In diabetic patients, the presence of systemic acidosis can lead to elevated levels of serum iron, likely

TABLE 218–2 Clinical Forms of Mucormycosis

Rhinocerebral
Cutaneous
Pulmonary
Gastrointestinal
Disseminated
Miscellaneous

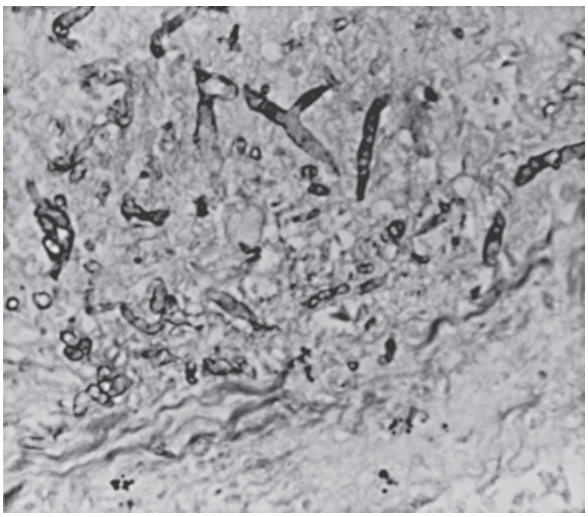

Figure 218–3 Zygomycosis caused by *Rhizopus* spp. Note the varied morphologic features of the coenocytic hyphae and large size (hematoxylin and eosin, original magnification ×64).

as a result of the release of iron from binding proteins.[3] In vitro data conflict, however, on the exact effects of iron or deferoxamine on promoting the growth of members of Mucorales.[80] Much remains to be understood about the pathogenesis of mucormycosis, and a unifying concept would have to incorporate the effects of steroids, acidosis, and iron on host neutrophils and macrophages in failing to suppress fungal replication.

The hallmark of histologic examination of mucormycosis is vascular invasion with resultant thrombosis and tissue necrosis and accompanying acute and chronic inflammation.[14,39,109] Most of the clinical findings of progressive disease can be related to these effects on blood vessels. Septic emboli may affect all parts of the body. Perineural invasion also occurs commonly.[31] In the rhinocerebral form, the disease may progress along nerve roots during intracranial spread. Although sparse, hyphal forms may be seen in tissue on routine hematoxylin and eosin staining more commonly than with silver or other special fungal stains (Fig. 218–3).[96]

CLINICAL MANIFESTATIONS

The clinical forms of zygomycosis are best considered by type of organ system involvement (Table 218–2). The primary site of infection at the time of initial diagnosis varies as a function of the host population (Table 218–3). Rhinocerebral, cutaneous, and pulmonary diseases are the most common clinical manifestations of zygomycosis.[98] Other forms of invasive zygomycosis include gastrointestinal, disseminated, and other rare presentations.[51,98]

Acute Rhinocerebral Mucormycosis

The acute rhinocerebral form of mucormycosis, including sinus and sino-orbital infections, is the most common presentation of zygomycosis and occurs most often in the setting of diabetes mellitus.[53,98] Rhinocerebral zygomycosis spreads from the nares, palate, or sinuses to the paranasal sinuses, and then to the retro-orbital region and brain by hematogenous spread. Symptoms include facial pain and headache; physical examination reveals brownish, blood-tinged nasal discharge; black eschar on the palate; proptosis; or cranial nerve involvement.[29] Involved tissues become red, then violaceous, and finally black as vessels are thrombosed, and tissues undergo necrosis. Extension into the periorbital region can be manifested as proptosis or orbital edema, and ocular or optic nerve involvement can be suggested by pain, blurring, or loss of vision.[108] Initial symptoms of invasive rhinocerebral disease include bloody nasal discharge,[94] seizures,[79] and cerebrovascular accident.[68] The mortality rate for rhinocerebral disease is greater than 50 percent.[98]

TABLE 218–3 Patterns of Zygomycosis by Host Population

Predisposing Condition	Predominant Sites of Infection*
Diabetes mellitus	Rhinocerebral, pulmonary, sino-orbital cutaneous
Malignancy (neutropenia)	Pulmonary, sinus, cutaneous, sino-orbital
Hematopoietic stem cell transplantation	Pulmonary, disseminated, rhinocerebral
Solid organ transplantation	Sinus, cutaneous, pulmonary, rhinocerebral, disseminated
Intravenous drug use/abuse	Cerebral, endocarditis, cutaneous, disseminated
Malnutrition	Gastrointestinal, disseminated
Deferoxamine therapy	Disseminated, pulmonary, rhinocerebral, cerebral, cutaneous, gastrointestinal
No underlying condition	Cutaneous, pulmonary, sino-orbital, rhinocerebral, gastrointestinal

**In order of frequency.*
From Kontoyiannis, D. P., and Lewis, R. E.: Invasive zygomycosis: Update on pathogenesis, clinical manifestations, and management. Infect. Dis. Clin. North Am. 20:581, 2006.

Cutaneous Mucormycosis

Cutaneous zygomycosis represents approximately 20 percent of reported cases in adults and a slightly higher proportion of pediatric cases.[98,133] Primary cutaneous infection usually occurs at the site of trauma, burns, or adhesive dressings, or invasive procedures in immunocompromised hosts.[20,34,49,114,129,130] Among neonates, the skin is the most common point of entry, with involvement of adhesive tape, monitor leads, or central venous access sites predominating. Premature infants represent most neonatal cases, and the overall mortality rate reported in the literature is 75 percent.[60,81,97] Secondary cutaneous disease results from hematogenous dissemination.[49] Primary disease may be very invasive locally and by direct extension affect adjacent bone and tissue. With vessel invasion, frankly disseminated disease may arise.

Primary cutaneous disease has lesions, which appear erythematous and indurated and progress to form black eschars (Fig. 218–4). In contrast, cutaneous disease arising from disseminated disease usually manifests in patients as nodular subcutaneous lesions, which may ulcerate.[94] Cutaneous zygomycosis can be evidence of disseminated disease, but primary cutaneous zygomycosis associated with occlusive bandages, burns, or wounds rarely extends beyond local involvement.[42] Most cutaneous cases are caused by *Rhizopus* and manifest with lesions that typically are black necrotic ulcers surrounded by erythematous and indurated painful cellulitis.[43] There can be rapid progression and skin

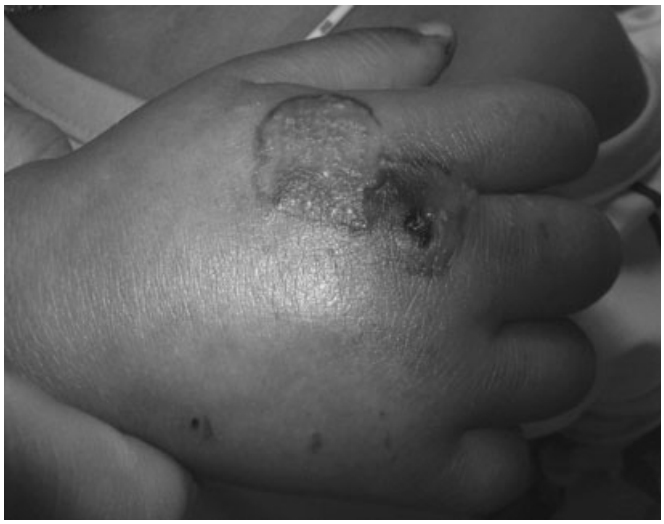

Figure 218–4 Cutaneous zygomycosis in a child with neutropenia. (See companion Expert Consult web site for color version.)

necrosis caused by hyphae invading the vasculature and causing tissue infarction.[58] Cutaneous zygomycosis should be considered when skin necrosis continues despite administration of appropriate antibiotics.[83]

Pulmonary Mucormycosis

Pulmonary zygomycosis may develop as a result of inhalation or by hematogenous or lymphatic spread. The clinical manifestations of pulmonary zygomycosis are similar to the manifestations of invasive pulmonary aspergillosis and often are indistinguishable.[16] Mucormycotic pneumonia typically develops in severely immunocompromised patients, such as patients with malignancy and neutropenia,[65,74,109] but it also has occurred in patients with juvenile diabetes mellitus and in normal hosts.[18,45,98] Patients with pulmonary zygomycosis often present with fever, cough, chest pain, and rapidly progressive dyspnea.[111] Angioinvasion results in necrosis of lung tissue, which may lead to cavitation and hemoptysis. Massive pulmonary hemorrhage may result from vascular erosion.[37,127] Chest radiographs and computed tomography (CT) scans may reveal a lobar pneumonia pattern, isolated masses, nodular disease, cavitation, or wedge-shaped areas of infarction.[48,64,68,111] Because of the lack of inflammatory cells that are capable of infiltrating the lung in neutropenic patients, however, radiography may not be dramatically abnormal.

Distinguishing pulmonary zygomycosis from invasive pulmonary aspergillosis is a clinical challenge. In patients with hematologic malignancies, clues for distinguishing pulmonary zygomycosis are the presence of concomitant pansinusitis, a history of antifungal prophylaxis with *Aspergillus*-active agents such as voriconazole and echinocandins, and possibly the repeated absence of detectable *Aspergillus* galactomannan antigen in serum. In addition, the presence of multiple nodules (>10) and, to a lesser degree, pleural effusions on CT scans favors the diagnosis of pulmonary zygomycosis over invasive pulmonary aspergillosis in patients with cancer.[16] Some atypical presentations of pulmonary zygomycosis include chronic infection with constitutional symptoms in relatively immunocompetent hosts.[71] Rarely, fungus balls have been noted within a preexisting lung cavity.[94] Hypersensitivity pneumonitis caused by *Rhizopus* spp. has been described in farm workers and Scandinavian sawmill workers (so-called wood trimmer's disease).[27] The disease usually is fulminant, but may be milder in hosts with less severe immunocompromise.

Gastrointestinal Mucormycosis

Gastrointestinal involvement probably results from ingestion of fungal spores, either from the environment or from colonized upper airways. Occasionally, hematogenous or direct extension routes result in intestinal tract involvement. Malnutrition, prematurity, uremia, and underlying gastrointestinal disease, such as typhoid fever or amebiasis, are predisposing factors.[59,70,72,78] Gastrointestinal zygomycosis is the most common clinical manifestation among neonates, representing two thirds of neonatal cases.[133] Most gastrointestinal disease in neonates is seen in premature neonates, some occurring in association with progressive necrotizing skin lesions and others manifesting with necrotizing enterocolitis.[115,132] All sites along the intestinal tract can be involved, with the stomach and colon being the most common sites.[78] Nonspecific abdominal pain with hematemesis, hematochezia, or melena may occur. Dissemination to other sites may occur by hematogenous routes. Perforation after bowel wall necrosis is common. Diagnosis usually is made at autopsy.

Disseminated Mucormycosis

Disseminated mucormycosis occurs in the most severely immunocompromised patients and usually is diagnosed post mortem.[42,118] Clinical findings are nonspecific, and a high index of suspicion and readily accessible tissue for biopsy are needed to establish the diagnosis. The pulmonary system is affected most commonly, followed by the central nervous system, but virtually any organ can be involved. A subacute form of disseminated disease with a protracted but fatal course has been reported.[82] The combination of severe immunocompromise and subtle clinical findings renders this form of mucormycosis the most difficult to treat.

Miscellaneous Forms of Mucormycosis

A variety of case reports of mucormycosis involving isolated areas of the body are found in the literature and include reports of endocarditis,[71,100,134] brain abscess,[98] peritonitis,[15,76,87] osteomyelitis,[28] mediastinitis,[67] tracheitis,[103] corneal infection,[112] otitis externa,[112] superior vena cava syndrome,[13] and renal disease.[62] Common to these disease manifestations is some type of local accidental or surgical trauma that results in a ready access site for invasion of fungal organisms. Outcome varies and depends on the underlying condition of the host and the extent of disease.

DIAGNOSIS

The presence of Zygomycetes in clinical specimens need not always represent clinically significant isolates. The spores from asexual reproduction are easily airborne and may be laboratory contaminants of culture medium or a result of oral ingestion in food or nasal inhalation of air before patient sampling. A positive culture linked to hyphal identification in cytologic specimens or tissue sections is considered diagnostic.[4,94] One study showed that the isolation of Zygomycetes from a patient with malignancy with no risk factors or clinically evident infection probably represents colonization; however, the pretest probability of a culture positive for Zygomycetes in a patient with leukemia was high.[53]

In most patients, the clinical presentation is assumed to be invasive aspergillosis, and in one series of 185 cases of disseminated zygomycosis, the correct diagnosis was made ante mortem in only 17 cases.[42] In the largest single-center review of zygomycosis, chest radiographs were abnormal in all patients with pulmonary zygomycosis, showing patterns such as focal consolidation (30%), cavitation (24%), widespread infiltrates (24%), or nodules (18%).[53] Cavitation is a common finding on chest radiograph, but the "air crescent sign" is seen less frequently than in patients with

invasive aspergillosis. In acidotic patients, pulmonary findings mostly manifest as lobar consolidation that may cavitate to form a lung abscess.[19] In one series, most manifestations in patients with pulmonary involvement were nodular or cavitary lesions.[74]

Noninvasive diagnostic procedures for zygomycosis are nonspecific, and a tissue diagnosis must be sought, even in situations in which other infectious agents have been isolated. Cultures of blood and bodily fluid rarely detect the disease. Immunofluorescent antibody staining of formaldehyde-fixed tissue is available through the mycology section of the Centers for Disease Control and Prevention, which might be helpful in confirming a suspected diagnosis.[29,42] Many specimens, including blood, sputum, gastric fluid, or nasal swabs, are either difficult to culture or of no diagnostic value, and invasive zygomycosis is correctly diagnosed with tissue biopsy.[29]

Antemortem respiratory tract specimens from autopsy-confirmed cases found zero of eight positive results in patients, including blood, urine, and cerebrospinal fluid cultures.[85] Cultures of lower respiratory specimens, including bronchoalveolar lavage and sputum specimens, were negative for zygomycosis in 75 percent of cases of histopathologically proven pulmonary zygomycosis, possibly owing to the high incidence of concomitant pulmonary infections.[53] Pulmonary zygomycosis has been diagnosed by fine-needle aspiration,[5] and an analysis of 87 cases of zygomycosis revealed that 40 percent of pulmonary disease was diagnosed by transbronchial biopsy, and 20 percent required open lung biopsy or surgical resection for diagnosis.[56]

Serologic diagnostic tests for detecting zygomycosis are not clinically useful. Antibody tests lack specificity, whereas several antigen detection systems have been used in tissue specimens. Very few molecular techniques are in use for establishing the diagnosis of zygomycosis, and techniques that are available are still experimental. Polymerase chain reaction amplification of the 18S rRNA sequences may be useful in epidemiologic studies of outbreaks, but little is currently being offered for primary diagnosis.[94] Primers from ribosomal DNA for Zygomycetes most implicated in clinical infection have been designed for use in a polymerase chain reaction assay for rapid and accurate identification.[95,119]

TREATMENT

Of overall great importance is the combination of early and aggressive surgical excision of the necrotic lesions, restoration of immune function, and intense antifungal therapy. Because tissue infarction is a prominent feature, removal of devitalized tissue is crucial because antifungals alone, especially in the setting of continued immunosuppression, would not elicit cure.[57,85] All necrotic-appearing tissue should be removed, and often patients require repeated surgical procedures for removal of devitalized tissue. Surgical débridement alone may be sufficient to cure localized cutaneous disease.[89]

Currently, the recommended antifungal therapy for zygomycosis is amphotericin B, given at maximal dosages of 1 to 1.5 mg/kg/day, based on the individual's tolerance of side effects.[51] Amphotericin B lipid complex also has been used successfully.[84] Whether lipid formulations of amphotericin B confer any advantage over conventional amphotericin B for mucormycosis is unclear,[53,84] but avoiding the nephrotoxicity associated with conventional amphotericin B may prove beneficial in delivering a longer duration of therapy.

Most azoles, including fluconazole and voriconazole, have no meaningful activity against Zygomycetes fungi. This point is crucial because voriconazole is an excellent antifungal against invasive aspergillosis, but has no clinical activity against zygomycosis. Posaconazole, a more recently approved orally available triazole, does possess potent activity against Zygomycetes.[113] In several more recent salvage therapy reports and studies, the overall success rate was 60 to 70 percent in patients with refractory disease.[90,116,104] The drug was well tolerated with only minimal gastrointestinal side effects. These encouraging results suggest that posaconazole may be a significant advance in the treatment of zygomycosis, but dedicated prospective clinical trial data are lacking. Echinocandins lack significant activity against Zygomycetes in vitro, and several cases of breakthrough zygomycosis have occurred in patients receiving echinocandin-based therapy.[52] This is a second crucial treatment point because the echinocandins also possess antifungal activity against invasive aspergillosis, but are not clinically useful against zygomycosis.

Hyperbaric oxygen therapy has been used as an adjunct to surgical and antifungal therapy for some patients in an attempt to limit the extent of gangrene and tissue necrosis.[21,30,46,88] The increased oxygen pressure achieved with hyperbaric therapy seems to improve the ability of neutrophils to kill organisms and inhibits fungal growth in vitro.[6] Although this regimen is apparently promising, the number of patients evaluated with this form of treatment is much too small to permit making any firm conclusions on its benefit. The role of other adjunctive therapies for zygomycosis has not been well studied. Granulocyte transfusions or cytokines that enhance phagocytic activity, such as interferon-γ and granulocyte-macrophage colony-stimulating factor, have been used occasionally to treat patients with zygomycosis.[1,35,106] Case reports have indicated favorable outcomes in patients treated with these adjunctive therapies,[1,106] but further studies are warranted before these therapies are recommended routinely.

PROGNOSIS AND PREVENTION

The disease site and host factors are key determinants of prognosis for zygomycosis. The reported mortality rate is highest for disseminated disease (100%) followed by gastrointestinal (85%), cerebral (79%), pulmonary (76%), and rhinocerebral (46%) disease.[98] Localized cutaneous disease is associated with a low mortality rate of 10 percent. Significant risk factors for mortality include disseminated disease and renal failure. Patients who undergo surgery as primary therapy and patients who receive antifungal therapy are significantly more likely to survive.[98] Among children, age younger than 1 year is associated with higher mortality.[133]

ENTOMOPHTHOROMYCOSIS

PATHOGENESIS AND PATHOLOGY

Entomophthoromycosis presumably develops through inhalation of spores to cause sinus disease or cutaneous inoculation to cause cutaneous or subcutaneous infection. The exact mechanisms are unclear, however, and no specific animal model has been developed. The organisms are of extremely low virulence, as manifested by the rare occurrence of these infections despite the ubiquitous nature of the fungi. Although discrete clinical syndromes have been assigned to the two genera causing disease, this distinction is artificial, and one should recognize that either group could cause either form of entomophthoromycosis or even a syndrome more suggestive of mucormycosis.[94,102,124,126]

Histopathologic examination of affected tissues shows some similarities to mucormycosis. Areas of acute and chronic inflammation are found in association with broad hyphal elements that may or may not display septations. The hyphae are more visible with hematoxylin and eosin staining than with more specific fungal stains.[94] The tendency for vascular invasion typical of mucormycosis does not occur with entomophthoromycosis, however, and necrosis is uncommon. Instead, a Splendore-

Hoeppli phenomenon, likely consisting of hyphae surrounded by eosinophilic material in a stellate pattern, occurs.[94] This feature is not pathognomonic of entomophthoromycosis, however, and has been seen in cases of mucormycosis.

CLINICAL MANIFESTATIONS

Chronic Rhinofacial Zygomycosis

Chronic rhinofacial zygomycosis, also called *entomophthoromycosis conidiobolae* because most infections are caused by *Conidiobolus coronatus*, is an indolent subcutaneous infection involving the face.[38,73,77] Typically, bilateral intranasal swelling eventually progresses to invasion of the sinuses and soft tissue swelling of the face, unaccompanied by fever or pain. One infant developed orbital involvement from *C. coronatus* dacryocystitis.[61] Symptoms commonly persist for weeks or months, but deep tissue progression is unlikely to occur.

Chronic Subcutaneous Zygomycosis

Chronic subcutaneous zygomycosis, also known as *entomophthoromycosis basidiobolae*, is similar to chronic rhinofacial disease with the exception that the lesions usually are on the trunk or extremities. The etiologic agent is *Basidiobolus ranarum*. Painless subcutaneous nodules may progress to invade deeper soft tissues, and massive soft tissue swelling may develop. Facial infection similar to that of chronic rhinofacial zygomycosis also may occur.[26] *Conidiobolus incongruus* has caused orbitofacial disease in a child residing in the Middle East.[2] With rare exceptions, most cases of subcutaneous zygomycosis are slowly progressive.[44,94]

Gastrointestinal Basidiobolomycosis

Gastrointestinal basidiobolomycosis is an extremely rare condition resulting from *B. ranarum* infection of the gastrointestinal tract. A cluster of cases was identified in Arizona in the late 1990s, suggesting a possible emerging infection in the region. In a case-control study including seven adult patients in Arizona, infected patients tended to be more likely to use ranitidine and to have resided in Arizona for a longer time compared with controls.[63] Clinical features included abdominal pain, leukocytosis, and eosinophilia. Combined surgical resection and itraconazole seemed to be effective treatment.

DIAGNOSIS

Entomophthoromycosis often can be diagnosed presumptively on the basis of clinical presentation and geographic origin of the patient, but usually biopsy is required.[94] More recent work on serodiagnosis by immunodiffusion has had promising results.[41,47] Onchocerciasis, filariasis, and Burkitt lymphoma sometimes are in the differential diagnosis.[44,94]

TREATMENT

Evaluating therapy for this group of diseases is difficult because of occasional reports of spontaneous resolution. Many agents have been tried with variable success. Taylor and colleagues[110] treated a young man with rhinofacial disease with amphotericin B, corticosteroids, miconazole, and multiple surgical procedures before noting resolution months after any therapy had been given. Susceptibility testing of the isolate on the patient showed minimal inhibitory effects of amphotericin B and no benefit of adding flucytosine or rifampin; miconazole, ketoconazole, and potassium iodide did not have impressive in vitro activity. Other investigators recommend potassium iodide therapy, with trimethoprim-sulfamethoxazole as an alternative.[92]

Other anecdotal reports of efficacy of trimethoprim-sulfamethoxazole, ketoconazole, or itraconazole raise hopes for the future development of effective therapy, but such hope must be tempered by the fact that only small numbers of patients have been treated.[38,63,110] Currently, no antifungal is the preferred treatment for this condition, although some type of medical therapy seems indicated for all cases. Data regarding the activity of posaconazole against this group of fungi are unavailable. The role of surgery is less for this group of disorders than for mucormycosis, but patients with well-circumscribed areas, such as nodular lesions, may benefit from surgical excision. Despite surgery, recurrence of disease after surgery commonly occurs in such patients.[110]

PROGNOSIS AND PREVENTION

Mortality caused by entomophthoromycoses is unlikely to occur, but morbidity and disfigurement are common. The efficacy of medical and surgical therapy is unclear, but all patients likely should receive attempts at treatment because the natural history of the disease is one of slow progression. Specific means for preventing disease are unavailable because so little is known about factors predisposing individuals to these chronic zygomycotic syndromes.

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CHAPTER 219

Miscellaneous Mycoses

Judith L. Rowen

ENTITIES

Fungi other than the classic mycoses generally cause disease in two distinct ways: (1) Organisms present in the environment are inoculated accidentally into the host, and (2) immunocompromised hosts cannot defend against the ubiquitous fungi and acquire systemic infection with organisms that rarely are seen as pathogens in a normal host. When the infection is caused by inoculation, several distinct entities occur that may be caused by many different fungi. Table 219–1 lists etiologies reported for these clinical syndromes.

TABLE 219–1 Selected Miscellaneous Fungi Implicated in Common Clinical Syndromes

Clinical Syndrome	Genus	References
Keratitis	*Acremonium*	76, 93
	Alternaria	37, 181
	Aureobasidium	86
	Bipolaris	86
	Blastoschizomyces	125
	Curvularia	181
	Exophiala	6
	Fusarium	181
	Geotrichum	183
	Lasiodiplodia	231
	Metarrhizium	55
	Paecilomyces	181
	Penicillium	181
	Phoma	203
	Scedosporium/ Pseudoallescheria	37, 100, 180
	Scopulariopsis	37
Mycetoma/soft tissue infection	*Acremonium*	75, 93, 249
	Alternaria	34
	Curvularia	207
	Exophiala jeanselmei	249
	Fusarium	249
	Madurella grisea	30, 38
	Madurella mycetomatis	38, 249
	Pseudallescheria boydii/ Scedosporium apiospermum	38, 249
Peritonitis	*Acremonium*	75, 245
	Alternaria	206
	Bipolaris	2, 114, 179
	Exophiala	114
	Fusarium	79, 114
	Paecilomyces	50, 140, 202, 245
	Penicillium	245
	Pichia	161
	Rhodotorula	24, 65, 245
	Saccharomyces	155
	Scedosporium	214
	Trichosporon	45, 58, 150, 245
Sinusitis	*Acremonium*	76
	Alternaria	62, 159
	Bipolaris	62, 247
	Curvularia	62, 106, 165
	Exserohilum	2, 62
	Fusarium	62
	Pseudallescheria	245
	Scopulariopsis	67, 119
	Ulocladium	184

NOTE: The fungi listed are those within the purview of this section on "miscellaneous mycoses." Many of these syndromes are commonly caused by Candida, Aspergillus, and the Zygomycetes as well.

MYCOTIC KERATITIS

In a review of 51 cases of microbial keratitis in children, 18 percent were attributed to fungal pathogens.[52] The proportion of cases of keratitis caused by fungal pathogens is higher in adults, and the outcome generally is worse than in children.[231] *Fusarium* and *Aspergillus* are the most commonly isolated fungi, followed by *Curvularia* and *Alternaria*.[181,231] Corneal infection with fungi often occurs after trauma to the eye (e.g., by a nylon-line trimmer, frequently with contamination of the injury by organic matter).[48,181,231] More recently, contact lens use has been implicated in numerous cases of mycotic keratitis.[109] The diagnosis often is delayed because the child has been presumptively treated with antibacterial, antiviral, or corticosteroid drops before the acquisition of corneal scrapings.[181] The most common manifestations include a grayish surface and anterior chamber reaction; serrated margins, raised slough, satellite lesions, and any color other than yellow suggests a fungal rather than bacterial etiology.[231,232]

Gram stain or potassium hydroxide wet-mount examination of eye drainage or scrapings frequently results in a proper diagnosis.[231,238] Appropriate therapy with topical or oral antifungal agents, or both, is successful in most cases; surgical therapy is required for patients with large areas of infiltrate or who have failed medical treatment.[81,86] Natamycin, a topical polyene, is used most frequently, but successful treatment with topical and systemic voriconazole is reported increasingly.[37,231] New products, such as collagen shields soaked in antifungal medications, may improve drug delivery over drops, which must be applied at frequent intervals.[231]

SOFT TISSUE INFECTION, INCLUDING MYCETOMA

Smoldering infection may occur when environmental fungi are inoculated directly into normal tissue. The classic manifestation of this process is mycetoma, a chronic granulomatous disorder affecting all the soft tissues in the area of inoculation and occasionally involving bone and distant organs.[249] The incubation period ranges from months to years after the original trauma occurs.[132] The condition is characterized by abscesses, nodules, and sinuses that may drain characteristic granules. Figure 219–1 depicts a characteristic granule.

Approximately two thirds of mycetomata actually are caused by bacteria, such as *Nocardia* spp., whereas the remaining third (also referred to as *eumycetoma*) are caused by fungi.[38] In the United States, the pathogen found most frequently is *Scedosporium apiospermum* (sexual phase, *Pseudallescheria boydii*),[38] but in the subtropics, where the condition is more prevalent, the organism isolated most frequently is *Madurella mycetomatis*.[249] The disease occurs more commonly in men than women and is more likely to affect adults than children.[249] In one case series, however, 4.5 percent of cases occurred in children.[29] The age and gender differences in the incidence of disease have been ascribed to hormonal influences, although another potential contributing factor is increased occupational exposure in male farm workers.[29] The lower extremity, especially the foot, is the body part most commonly involved, followed by the thorax, but cases have been reported that involve all body areas.[38,249]

The differential diagnosis includes cutaneous tuberculosis, tumor, osteomyelitis, and sporotrichosis.[249] Examination of exudates from draining sinuses may reveal the diagnosis, but surgical biopsy might be required.[38,249] Therapy should be directed at the pathogen isolated; itraconazole has shown some promise in selected cases.[30,249]

FUNGAL PERITONITIS

Patients undergoing peritoneal dialysis are compromised hosts because of direct violation of the mucocutaneous barrier by the dialysis catheter and the immunosuppressive effect of the underlying renal disease.[19] Fungal peritonitis develops after contamination of the dialysis system occurs. *Candida* spp. are the most common causative agents,[246] but several other yeasts and filamentous fungi have been reported as the etiology (see Table 219–1). Fungi are responsible for 2.9 percent of episodes of peritonitis that occur in children undergoing peritoneal dialysis.[246] Treatment includes removal of the dialysis catheter and initiation of antifungal therapy, but the ideal timing for removal of the catheter is unclear.[27,246] Amphotericin has very poor penetration into the dialysate when given intravenously[27] and is irritating when given intraperitoneally.[74] Penetration of fluconazole into

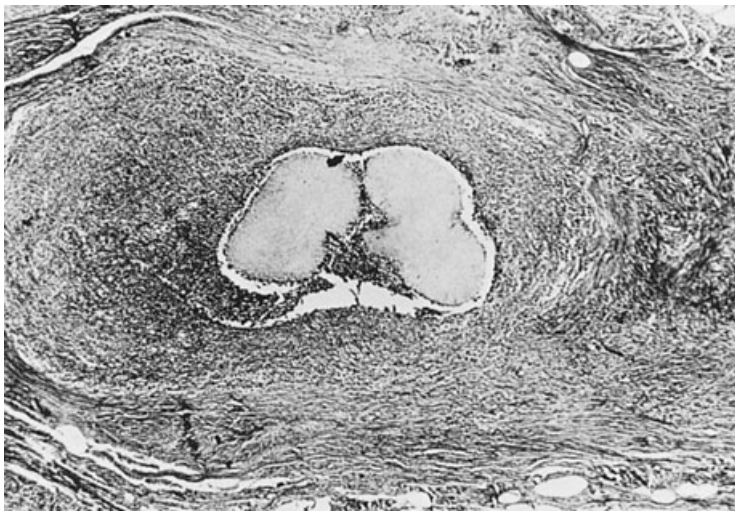

Figure 219–1 Large granule of *P. boydii* in the sinus tract of a mycetoma. Extensive fibrosis is seen in the periphery of the tract, and the tract is filled with a mixed granulomatous and pyogenic infiltrate (hematoxylin and eosin, ×100). *(Courtesy of Centers for Disease Control and Prevention.)*

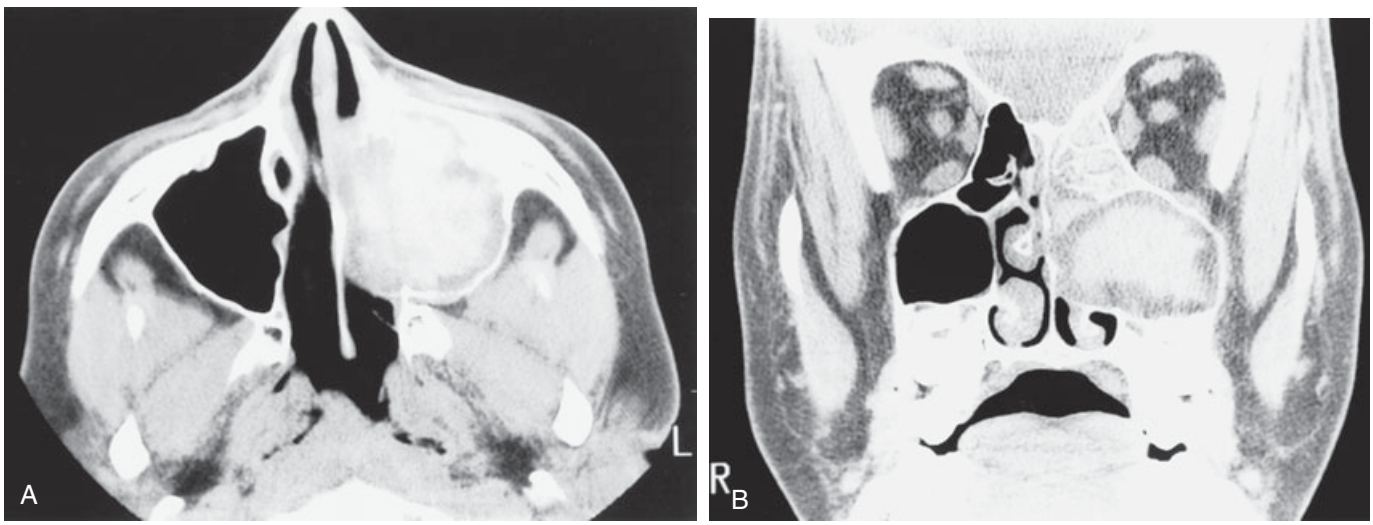

Figure 219–2 Allergic *Curvularia* sinusitis in a 12-year-old girl. **A,** CT scan shows a large space-occupying mass involving the left maxillary sinus with expansion of its medial and lateral posterior walls. The mass has high density centrally with a peripheral zone of decreased density. **B,** Coronal image shows the mass with centrally increased density and low density in the periphery. Additionally, the ethmoid air cells are involved. *(Photos courtesy of Eric Hendrick, M.D.)*

dialysate is rapid and efficient.[27] Fluconazole and itraconazole have been used successfully as therapy.[27,43,83,156,245] The outcome of fungal peritonitis may be better in children than adults, with a larger percentage successfully continuing peritoneal dialysis.[246]

FUNGAL SINUSITIS

Fungal sinusitis apparently is a spectrum of diseases ranging from a purely allergic condition through invasive forms that may progress to brain abscess as erosion through the sinus walls continues.[59] Noninvasive sinusitis takes two forms: allergic fungal sinusitis and sinus mycetoma. In allergic fungal sinusitis, the sinus (most commonly a maxillary sinus) fills with a gelatinous material often described as having the consistency of peanut butter or cottage cheese.[59] This material consists of mucin, eosinophils, Charcot-Leyden crystals, and sparse fungal hyphae.[62] Patients have signs and symptoms of chronic sinusitis, and proptosis and distortion of facial features may develop, more commonly in children.[62,143,165] Most case series include children, with the youngest patients being early elementary school age.[62,120,211] The disease is more common in humid, coastal areas.[59] The fungi implicated most commonly include *Curvularia* and *Bipolaris* spp.[50,62,211]; *Aspergillus*, *Exserohilum*, and *Alternaria* spp. also have been reported.[50,59]

The diagnosis of allergic fungal sinusitis should be suspected in patients with chronic sinusitis, especially patients with a history of atopy. Nasal polyps are found frequently, and many patients may recall production of nasal casts.[62,211] Computed tomography (CT) and magnetic resonance imaging (MRI) may highly suggest the diagnosis; on non–contrast-enhanced CT, the sinuses contain areas of high attenuation, and by MRI, areas of T2-weighted signal void are seen.[133] Figure 219–2 shows a typical CT scan from a patient with allergic fungal sinusitis. Many patients show loss of bony margins; this loss is not from invasive destruction, but from bone compression caused by a mass effect.[62] The diagnosis is confirmed by histopathology; to meet the criteria for allergic fungal sinusitis, the patient must be immunocompetent; have radiographic evidence of sinusitis; and have histologic

specimens with eosinophil-rich allergic mucin, positive fungal stain or culture, and no evidence of invasion.[62]

Treatment predominantly is surgical, usually endoscopic removal of the allergic mucin and improvement in aeration and drainage.[59,62,120,137] The pathophysiology is assumed to be similar to that of allergic bronchopulmonary aspergillosis, in that the disease is the result of the host's response to inhaled fungi that lodge in the sinuses, rather than the result of true infection.[50] Elevated IgE is noted frequently.[62,211] Systemic and nasally inhaled corticosteroids are administered after surgical drainage has been performed.[59,137,212] Immunotherapy with fungal allergens also has been used by some clinicians.[137] Recurrences are common, especially if use of corticosteroids is stopped prematurely.[212] Itraconazole has been reported to be helpful in treating allergic bronchopulmonary aspergillosis[224]; it has been used in some cases of allergic fungal sinusitis, but its utility is yet unproven.[13]

The other form of noninvasive fungal sinusitis is sinus mycetoma, which essentially is a fungus ball in the sinuses, usually the maxillary sinuses.[60,117] The patient initially has nasal obstruction, facial pain, and a fetid odor (cacosmia).[59,60] Similar to allergic fungal sinusitis, distortion of the bony margins may occur,[60] although one large series noted this finding in only 4 percent of patients.[117] Although *Aspergillus fumigatus* is the fungus most frequently implicated, *Pseudallescheria* and *Alternaria* also are reported.[60,117] Claylike, cheesy material, similar in appearance to the material present in allergic fungal sinusitis, but without the findings of allergic mucin on histopathology, is seen at surgery.[59] Although hyphae are sparse in allergic fungal sinusitis, they are abundant in mycetoma, but frequently fail to grow on culture.[59] Treatment is surgical, with wide opening of the sinuses and removal of the mycetoma; recurrences are rare.[117]

Acute, fulminant invasive fungal sinusitis is associated most frequently with Zygomycetes or *Aspergillus* in immunocompromised individuals.[59] Reports of this entity increasingly implicate other fungi, however, such as *Pseudallescheria*, *Fusarium*, *Alternaria*, and *Scopulariopsis*.[59,67,119,159] Affected patients usually are immunocompromised as a result of having cancer, transplantation, or poorly controlled diabetes mellitus.[61] Four percent of children treated at one center for hematologic or lymphoid malignancies developed invasive fungal sinusitis; all were neutropenic for a prolonged period (mean 21.3 days).[184] Initial signs and symptoms may be limited to fever or facial pain, with some patients developing headache and epistaxis; careful physical examination or nasal endoscopy may reveal nasal mucosal ulcers or eschars.[59,184] The hallmark of this entity is histopathologic evidence of invasion into the mucosa or other tissues.[59,61] The disease may progress rapidly and lead to orbital apex syndrome and further intracranial involvement.[59] Therapy includes wide surgical excision and administration of amphotericin B; lipid formulations of amphotericin B and combination therapy may be useful in treating this syndrome.[59,184]

ETIOLOGIES

The taxonomy of fungi has undergone significant revision with the addition of molecular techniques to the classic morphologic methods. When presented with a patient with a fungal infection, the clinician rarely can identify the taxonomic group of the invading organism until final culture results are available. Instead, the appearance of the organism in tissue specimens is what is appreciated first. In the following discussion of infection caused by specific pathogens, the organisms are grouped by their appearance in tissue. For detailed review of fungal taxonomy, the reader is referred to several excellent resources.[228,254]

YEASTS

In contrast to the filamentous fungi, which tend to invade locally at the site of initial inoculation, with later dissemination if at all, yeasts tend to cause disease after hematogenous dissemination without significant local disease. End-organ deposition may lead to focal disease, however, after hematogenous spread. In addition to *Candida* and the classic dimorphic fungi of endemic infections, several yeastlike pathogens have emerged as significant pathogens.

Malassezia Species

Also known by the obsolete term *Pityrosporum*, *Malassezia* spp. are most familiar as the cause of a minor skin infection, tinea versicolor. *Malassezia* spp. also have a putative role in neonatal cephalic pustulosis, seborrheic dermatitis, and atopic dermatitis.[17] *Malassezia furfur* and *Malassezia pachydermatis* also cause bloodstream infection, usually in association with central venous catheters. *M. furfur* is obligatorily lipophilic, which probably explains the concordance of systemic disease with the use of intravenous lipid emulsions. Systemic infection with *Malassezia* was described first in 1981 by Redline and Dahms[199]; the patient was an extremely low-birth-weight infant, which is typical for this disease. Catheter-related *Malassezia* fungemia also has been described in older children and adults with immunocompromising conditions.[20] In a review of 55 cases in neonates, the most common underlying condition was prematurity, followed by short-gut syndrome; all infants were receiving lipid supplementation.[136] In contrast, *M. pachydermatis* does not require lipids for growth. This organism is most familiar as a causative agent of otitis externa in dogs, and at least one nursery outbreak has been associated with colonized pets of health care workers.[44]

Adults are colonized nearly universally with *Malassezia* organisms.[136] Colonization in neonates varies from 30 to 100 percent of those sampled and occurs early in life.[5,123,194,216] Factors correlated with colonization include length of hospitalization, lower gestational age, time in an isolette, and use of occlusive dressings.[136] The organisms resist superficial cleaning and may persist on plastic surfaces for prolonged periods of at least 3 months, which may facilitate outbreaks within an intensive care nursery.[239]

Clinically, infected infants have fever (53%), respiratory distress (53%), lethargy, poor feeding, bradycardia, and hepatosplenomegaly.[136] Thrombocytopenia occurs in 48 percent. Frequently, blood drawn directly from the central catheter is culture-positive, with negative peripheral blood cultures. The laboratory should be alerted that *Malassezia* is suspected because lipid supplementation may enhance recovery of the organism.[173] The 2.5- to 6-μm yeasts may be seen on a smear of blood drawn through the catheter.[91,135] Treatment should include removal of the implicated central catheter. Antifungal therapy usually is provided, although testing suggests only moderate susceptibility to amphotericin B.[135] Most infants recover; however, infants dying of infection frequently have heavy involvement of the lungs and heart at autopsy.[135,136]

Trichosporon Species

Most infections caused by *Trichosporon* are attributed to *Trichosporon beigelii* in the literature; however, revision of the genus has suggested that most invasive disease is caused by *Trichosporon asahii*, with other species implicated predominantly in superficial disease, such as white piedra.[228] White piedra affects the shafts of terminal hairs and results in nodules or coalescent concretions; one epidemiologic survey found it in 40 percent of men and 14 percent of women tested.[112] Invasive disease usually affects cancer patients with neutropenia or neonates.[10,78,95,99,102] Table 219–2

TABLE 219–2 Summary of Reported *Trichosporon* Infections

Type of Infection	Underlying Condition	References
Bloodstream	Burns	94
Cholangitis	Natural killer cell deficiency	111
Disseminated	Hemophagocytic syndrome	56, 101
Endocarditis	Repaired congenital heart disease	15
Invasive fungal dermatitis	Prematurity	207
Liver abscess	Leukemia	153
Lung abscess	Chronic granulomatous disease	191
Lymphadenitis	Job syndrome	41
Meningitis	Acute lymphocytic leukemia	227
Necrotizing fasciitis	Trauma	54
Oral mucositis	Acute myelogenous leukemia	103
Paravertebral abscess	Acute lymphoblastic leukemia	21
Peritonitis	Continuous ambulatory peritoneal dialysis for renal failure	150
Pneumonia	Trauma, chronic granulomatous disease	154, 162
Sepsis with shock	Diabetes mellitus	64
Septic arthritis	Acute myelogenous leukemia	149
Shunt infection	Arachnoid cyst	18
Urinary tract infection	Renal transplant	128

summarizes other reports of *Trichosporon* infection causing unusual manifestations of disease or affecting patients with other underlying conditions.

Trichosporon is isolated from blood cultures as a yeast that is germ tube–negative and urease-positive. In tissue sections, hyaline hyphae with septations and rectangular arthroconidia (2 to 4 μm × 3 to 7 μm) are seen, with pseudohyphae and blastoconidia noted occasionally.[228] The organism may be found in soil, water, vegetation, and animal excreta.[102,228] *Trichosporon* shares a heat-stable antigen with *Cryptococcus neoformans*, which may cause the latex agglutination test for cryptococcal capsular polysaccharide to be positive in the setting of disseminated *Trichosporon* infection.[148] The results of the cryptococcal antigen test have been followed in an experimental model and in patients to document response to therapy; however, the initial antigen testing may be negative despite disseminated disease.[94,227,243]

Most affected neonates have been less than 1000 g birth weight and born between 23 and 25 weeks' gestational age.[78,209,229,256] Disease was identified most frequently in the second week of life, with the bloodstream and skin being the sites most commonly involved. Respiratory cultures usually are positive, suggesting the lung as a possible portal of entry.[209] Most of these infants died of their infection.

The incidence of trichosporonosis in patients with hematologic malignancies has been reported to be 0.4 percent.[90] Immunocompromised hosts frequently have fever during periods of neutropenia. Skin involvement occurs with disseminated disease and is described as papular, purpuric, or necrotic lesions.[70,108] The organism is recovered from blood cultures, skin lesions, urine, sputum, and liver and kidney biopsy specimens, indicating the organism's widespread involvement.[70,102] Isolated fungemia (including catheter sepsis) and infection limited to the lungs also have been reported.[9,118,157] Mortality rates are high; one cancer center reported 53 percent crude mortality.[70,102,118] Although most immunocompromised hosts with *Trichosporon* infection have been patients with cancer, infection also has been reported in patients with congenital immunodeficiencies, such as chronic granulomatous disease[162]; see Table 219–2 for other examples.

Although amphotericin B often is used, some experts recommend therapy with one of the newer azoles. The organism has been described as tolerant to amphotericin B,[244] but animal models have shown good efficacy with fluconazole.[10,243] Voriconazole has good activity in vitro, and reports of successful clinical use are increasing.[16,134,141,146] Successful therapy with lipid-based amphotericin B and with posaconazole has been reported.[54,229] Echinocandins are not sufficiently active against *Trichosporon*, and breakthrough infections have occurred in patients receiving these drugs for antifungal prophylaxis.[92,141]

Penicillium marneffei

Occasional reports describe opportunistic infection with *Penicillium* molds,[130] but the major pathogen in this genus is *Penicillium marneffei*, which is the only species that is a dimorphic fungus. At 25° C, *P. marneffei* grows as a filamentous mold and produces a diffusible red pigment, whereas at 37° C, it grows as a yeast form that reproduces by fission rather than budding.[49] The hyphae are septate and hyaline, and the yeast forms are 3 to 5 μm in size, similar to the size of *Histoplasma*.[228] Initial reports confused the organism with *Histoplasma*; one histopathologic feature that may be helpful is that the yeasts of *P. marneffei* frequently have visible cross walls that form during fission.[49,57] Adding to the potential confusion, infection with *P. marneffei* may lead to a false-positive *Histoplasma* antigen test.[49]

The fungus was isolated first from bamboo rats captured in Vietnam, and the disease is endemic to Southeast Asia, including areas of Myanmar, Cambodia, southern China, Indonesia, Laos, Malaysia, Thailand, and Vietnam.[171] Disease caused by *P. marneffei* was listed as an acquired immunodeficiency syndrome (AIDS)–defining condition in 1992, and it is the third most common opportunistic infection in the Chiang Mai region of Thailand, with 16 percent of all patients infected with human immunodeficiency virus (HIV).[49] The exact mode of transmission is unclear; bamboo rats and humans are thought to be infected from a common source, rather than transmission being from rats to humans.[63] An inhalational route of infection is supported by an infection in a physician from Africa who was HIV-positive and whose only possible exposure was during a visit to the Pasteur Institute, where other laboratory workers were handling the fungus.[171] The incubation period may be only 2 weeks or very prolonged; the first recognized human case was in a U.S. missionary with Hodgkin disease who had traveled in Southeast Asia 2 years before the discovery of his infection.[171]

In most cases, the disease is disseminated, with prominent involvement of the skin by umbilicated papules reminiscent of molluscum contagiosum (Fig. 219–3).[49] As with the other endemic mycoses caused by dimorphic fungi, the disease is thought to disseminate from a pulmonary source.[240] In a review of 21 children with HIV and penicilliosis, the initial signs and symptoms included generalized lymphadenopathy (90%), hepatomegaly

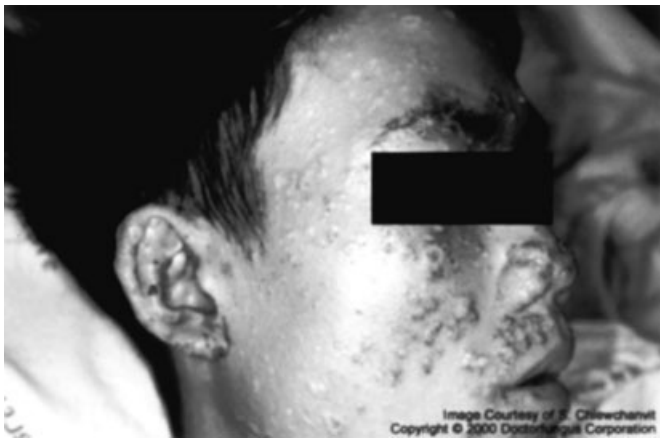

Figure 219–3 Skin lesions from *P. marneffei*. *(Photograph courtesy of S. Chiewchanvit, provided by www.doctorfungus.org.)*

(90%), fever (81%), skin lesions (67%), splenomegaly (67%), failure to thrive (52%), severe anemia (43%), and thrombocytopenia (21%).[222] The infection was manifested at a later age than most opportunistic infections in this population (32 months of age versus 7 months of age). In adults, fever, weight loss, and anemia also are prominent, and a painful cough is noted frequently.[63] The organism usually is isolated from blood cultures; skin biopsy cultures also frequently yield the organism.[63,222]

More focal involvement of lymph nodes may occur. In children with HIV, mesenteric adenitis with prolonged fever and abdominal pain may mimic acute appendicitis.[237] Chronic lymphadenopathy resembling scrofula has been reported in a previously healthy child.[257] This child and another without underlying disease had transient lymphocyte abnormalities that resolved after treatment of the infection.[121,257]

The diagnosis depends primarily on isolation of the organism from cultures or characteristic findings on histopathologic examination with a consistent clinical history. Bone marrow aspirates, touch smears of skin, or lymph node biopsy specimens can be Wright stained for rapid, presumptive diagnosis.[240] Occasionally, the organism is seen on peripheral blood smears.[240] Serologic and DNA amplification tests have been devised, but remain experimental.[63,171,240] The antibody used in galactomannan assays may cross-react with *P. marneffei.* Amphotericin B followed by itraconazole is the usual mode of therapy. As with many fungal infections in patients with HIV, relapse occurs frequently when therapy is halted.[225] In a placebo-controlled trial of itraconazole maintenance therapy, relapse did not occur in the treatment arm, whereas 20 of 35 placebo recipients relapsed at a mean of 24 weeks after therapy.[226]

Pichia Species

Pichia anomala (formerly known as *Hansenula anomala*) and *Pichia angusta* (formerly known as *Hansenula polymorpha*) are rare causes of human disease. *P. anomala* is the teleomorph, or sexual stage, of *Candida pelliculosa.* It characteristically has one to four hat-shaped ascospores when asci are produced.[228] It may colonize the human gastrointestinal tract and is found in pigeon excreta, plants, and fruit.[14] Another species, *Pichia ohmeri* (synonym, *Kodamaea ohmeri*) has been reported to cause disease, with several reports in pediatric patients.[190]

Human infection has been reported primarily in immunocompromised hosts and premature neonates.[8,14,147,161,166,213,220,233,255] Nursery outbreaks with high levels of colonization have been described.[14,166,220] Neonates with fungemia are lethargic and have mottling, apnea, bradycardia, and an increased need for ventilatory support.[166] Abscesses may occur at the site of insertion of peripheral intravenous catheters.[166] Jaundice and fever also have been described.[14] Almost every patient reported had recently received broad-spectrum antibiotics and had a central catheter in place, frequently for parenteral nutrition.[14] The organism often is isolated from blood cultures and the catheter tip.[8,255] Other infections have included ventriculitis, lymphadenitis, and necrotizing enteritis.[147,161,166]

Despite the fragile nature of the patients affected, disease caused by *Pichia* rarely is fatal. Most reported cases have been treated with amphotericin B plus 5-flucytosine and removal of central venous catheters. Although many isolates are susceptible to fluconazole, elevated minimal inhibitory concentrations (MICs) have been found in patients' isolates associated with poor clinical response to this drug.[8,255]

Rhodotorula Species

Rhodotorula grows as glistening pink-to-red colonies. Usually, no pseudohyphae are formed; the yeast cells are ovoid or elongate (2 to 5.5 μm × 2.5 to 14 μm).[228] The organism is urease-positive and does not ferment carbohydrates.[228] It can be found colonizing normal human skin, mucous membranes, shower curtains, bathtubs, and toothbrushes, and can be isolated from cheese, milk, air, soil, and water.[116,218,228] Most human infections have been associated with catheters in patients with cancer or HIV.[116,218,242] Endocarditis in a child with presumed underlying rheumatic heart disease also has been described, as have nursery outbreaks.[169,189] The pathogen seems to be of low virulence, with few deaths attributable to infection. Resistance to fluconazole is seen frequently, and disease has been reported in patients receiving fluconazole prophylaxis; in vitro testing suggests that most strains are susceptible to voriconazole, itraconazole, and amphotericin B, with ravuconazole having the highest activity of the newer agents.[71,116,127] Clinical cases have been treated successfully with amphotericin B or 5-flucytosine monotherapy and by removal of catheters, without antifungal therapy.[116,169]

Saccharomyces cerevisiae

Saccharomyces cerevisiae is a yeast best known as baker's yeast or brewer's yeast. Strains (often called *Saccharomyces boulardii*) also have been used as a biotherapeutic agent for severe diarrhea, especially *Clostridium difficile*–associated diarrhea.[97] The yeast is distributed ubiquitously in the environment and may be found as a commensal in the human gastrointestinal tract.[254] Clinical isolates have enhanced virulence compared with environmental strains when evaluated in animal models.[193] *Saccharomyces* is responsible for 3 percent of cases of fungal vaginitis, especially in women with a history of recurrent or chronic vaginitis.[193,223] Invasive infection develops in patients with multiple medical problems, usually with a central venous catheter in place.[9,77,156,234] The source of infection is presumed to be via translocation from the gastrointestinal tract or from indwelling venous lines.[234] Mortality rates with these infections are high, with reports ranging from 35 to almost 50 percent.[68,77,163]

Organisms have been isolated from nearly every body site.[68,77,163] Infections associated with the use of *S. boulardii* (actually not a separate species) seem to be less clinically devastating, although septic shock developed in one patient, who later recovered.[68,97,192] Environmental sampling has indicated that the organisms can be isolated from the air, environmental surfaces, and the hands of caretakers opening packets for administration to the patient, so direct contamination of central venous catheters may be the source, although gut translocation also has been postulated.[97,192] In a review of 60 cases, probiotic use was noted in 43 percent.[163] The authors recommend caution when considering probiotics in immunosuppressed or critically ill patients. Therapy

includes removal of implicated catheters and administration of antifungal agents. MICs are low for amphotericin B and 5-flucytosine, and itraconazole and voriconazole seem to have better activity than fluconazole.[71,234]

Blastoschizomyces capitatus

Blastoschizomyces capitatus was formerly known as *Trichosporon capitatum* and *Geotrichum capitatum*. It may be found in soil and beach sand and as part of the normal human flora of the gastrointestinal tract.[228] Infection has been seen predominantly in immunocompromised hosts, especially patients with hematologic malignancies; one European consortium reported an incidence of 0.5 percent in their patients.[90] In a review of 99 patients with *Blastoschizomyces* infection, blood cultures were positive in 77 percent.[90] In another case series, 8 patients had lesions comparable to those seen with hepatosplenic candidiasis, and the fungus grew from liver specimens.[139] Focal lesions of the brain and kidney also have been described.[90,139] Chronic meningitis that developed in a patient after bone marrow transplantation has been described.[89] Organisms have been isolated from the blood, heart, lungs, liver, spleen, cerebrospinal fluid (CSF), skin, stomach, urine, kidneys, and intracerebral lesions.[15,89,139] The lung lesions may resemble lesions of an aspergilloma, with cavitation and a typical crescent sign.[139] In tissue, pleomorphic yeasts that are 3 to 8 μm in diameter and septate hyphae are seen. Successful therapy generally requires recovery from neutropenia. Amphotericin B with and without 5-flucytosine and fluconazole have been used. A patient with chronic meningitis responded clinically to a prolonged course of fluconazole, but fungi were noted in the meninges at autopsy, so the infection was not eradicated.[89] Voriconazole has better activity in vitro than the other azoles do; clinical correlation has not been reported.[71,90] The mortality rate is high, reported at 56 percent in a large case review.[90]

MOLDS

In contrast to the pathogens found in tissues as yeasts, molds rarely are part of the normal human flora and instead have arisen from the environment. A portal of entry, be it a minor wound, a central catheter, or the lungs, often is recognizable. With increasing numbers of immunocompromised hosts, the variety of fungi assuming greater clinical importance has expanded similarly. In tissue specimens, making a definitive diagnosis of the particular mold involved rarely is possible. One useful differentiation is between the "black molds," or dematiaceous fungi, which lead to clinical infections collectively known as *phaeohyphomycoses*, and the hyaline molds, which cause hyalohyphomycoses. Dematiaceous fungi contain melanin in their cell walls, which can be seen in tissue sections, especially with Masson-Fontana staining. Table 219–3 lists the genera included in this group. In addition to phaeohyphomycosis, other clinical syndromes associated with the dematiaceous fungi, such as mycetoma (discussed previously) and chromoblastomycosis, are sufficiently unique to be discussed separately.

TABLE 219–3 Dematiaceous Fungi

Alternaria*	***Madurella***
Aureobasidium	*Microascus*
Bipolaris	*Mycocentrospora*
Botryomyces	*Nigrospora*
Byssoascus	*Ochroconus*
Cephalotrichum	*Oidiodendron*
Chaetomium	*Periconia*
Chloridium	*Phaeoacremonium*
Cladophialophora	*Phialemonium*
Cladosporium	***Phialophora***
Coniothyrium	*Phoma*
Curvularia	*Pithomyces*
Dactylaria	*Pleurophragmium*
Doratomyces	*Ramichloridium*
Drechslera	*Rhinocladiella*
Echinobotryum	*Rosellinia*
Epicoccum	*Scedosporium prolificans*
Exophiala	*Scolecobasidium*
Exserohilum	*Scytalidium*
Fonsecaea	*Sporidesmium*
Gamsia	*Stachybotrys*
Gliomastix	*Stemphylium*
Hannebertia	*Stephanosporium*
Helminthosporium	*Taeniolella*
Hormonema	*Torula*
Humicola	*Ulocladium*
Hypoxylon	*Wangiella*
Khuskia	*Wardomyces*
Lecythophora	
Leptodontium	
Leptosphaeria	

**Genera in bold type are most frequently implicated in human disease.*

TABLE 219–4 Agents of Hyalohyphomycosis

Acremonium*
Aphanoascus (anamorph = *Chrysosporium*)
Arthrographis
Beauveria
Chrysosporium
Coprinus (actually a mushroom)
Cylindrocarpon
Fusarium
Myriodontium
Paecilomyces
Penicillium
Schizophyllum commune (actually a bracket fungus)
Trichoderma
Tritirachium
Volutella

**The most frequently implicated genera are listed in bold type.*

Agents of hyalohyphomycosis are listed in Table 219–4. In tissue section, the hyaline molds may be presumed to be *Aspergillus* or *Candida*. Although the distinction is difficult to make, a few characteristics may allow differentiation of these molds from the more common *Aspergillus*. The hyphae are septate, frequently with marked variation in diameter.[126] Whereas *Aspergillus* nearly uniformly branches at 45 degrees, the hyaline molds may exhibit 45-degree and 90-degree branching.[126] Careful investigation frequently reveals evidence of adventitious sporulation through the presence of phialoconidia and phialides; *Aspergillus* generally undergoes in vivo sporulation only in aerated spaces, such as lung cavities.[126] Confusion with *Zygomycetes* occurs less commonly, but the hyphae of hyaline molds are narrower and more frequently septate than those of Zygomycetes and are less likely to twist in a ribbon-like pattern.[126]

Phaeohyphomycosis

The range of clinical diseases attributed to the phaeoid, or dematiaceous, fungi is broad. The term *phaeohyphomycosis* literally means "condition of fungi with dark hyphae," and does not reflect

the disease produced, but rather the pathogen producing the disease. The presence of melanin in the cell wall may act as a virulence factor, as has been shown for *Wangiella dermatitidis* in animal models.[142] Focal diseases attributed to this group of fungi include bone and joint infection after local trauma, sinusitis, peritonitis associated with dialysis, and keratitis. Implicated genera include *Alternaria*, *Curvularia*, *Bipolaris*, *Exserohilum*, and *Exophiala*.[2,34,75,142] Allergic bronchopulmonary pneumonitis also has been described.[2]

Phaeohyphomycosis has been termed an *emerging disease*,[200] and increasing reports of health care–associated acquisition have appeared. Bloodstream infections with *Exophiala* were associated with contaminated hospital water, and disseminated disease with *Phialemonium* was seen in a group of patients undergoing hemodialysis who had been dialyzed on the same unit.[177,195] Another outbreak involved *Exophiala* infections of the central nervous system (CNS) traced to contaminated injectable steroids.[69]

Phaeomycotic brain abscess often is caused by *Cladophialophora bantiana* (formerly named *Xylohypha bantiana*) or *Ochroconus gallopavum* (formerly named *Dactylaria gallopavum*).[142,221] In the Middle East, *Ramichloridium mackenziei* is a prominent pathogen.[69] *Bipolaris* also has been reported as a cause of brain abscess and granulomatous meningoencephalitis.[2,160] Many of these patients are immunocompetent, without underlying disease. Brain abscess caused by *C. bantiana* is manifested as chronic headache, fever, and hemiparesis.[142] Boys are affected predominantly, and the frontal lobes are involved most frequently. Lumbar puncture reveals elevated opening pressure, high protein and depressed glucose levels, and negative cultures. CSF eosinophilia may be seen.[201] Abscess cultures must be obtained.[142] CNS disease also may manifest as meningitis, encephalitis, myelitis, or arachnoiditis.[201]

Disseminated disease usually occurs in the setting of altered host immunity. Most patients have hematologic malignancies with resultant neutropenia. In a review of 72 cases of disseminated phaeohyphomycosis, the mortality rate was 79 percent.[200] Fever was present in 76 percent, with skin manifestations in 33 percent, CNS symptoms in 31 percent, gastrointestinal symptoms in 31 percent, and apparent sepsis in 11 percent.[200] Blood cultures were positive in more than half of the patients.[200] Fungemia with *Exophiala jeanselmei* has been described in association with central venous catheters.[176] Eosinophilia may be a hint that disseminated phaeomycosis is the cause of fever in an immunocompromised host, although it was noted in only 11 percent of such patients.[200]

Infection in organ transplant recipients is manifested differently from infection in patients with hematologic malignancies. The length of time post-transplant until the development of symptomatic disease is prolonged, with a median time of 22 months.[221] Skin, soft tissue, or joint infection develops in most organ transplant recipients, with *Exophiala* being the pathogen implicated most frequently.[221] The second most common manifestation is brain abscess, which occurs much earlier in the course, at a mean of 3 months post-transplant.[221]

Premature neonates are another population of immunocompromised hosts susceptible to acquiring unusual pathogens. Infection caused by the agents of phaeohyphomycosis in premature infants predominantly have been in the form of invasive fungal dermatitis with necrotic ulcers. Two cases caused by *Curvularia* and one caused by *Bipolaris* have been described.[36,75,206] Endocarditis caused by *Acremonium* also has been reported in a premature neonate.[87]

Skin lesions attributed to phaeohyphomycosis may take many forms, including papules, plaques, pustules, nodules, and nonhealing ulcers, most commonly on the extremities.[221] In a review of 89 cases of cutaneous alternariosis, the lesions were noted to be shallow-based, nonhealing ulcers that evolved from nodules, subcutaneous noninflammatory cysts, verrucous lesions, and confluent scaly patches.[129] In the setting of disseminated disease, skin manifestations may take the form of a rash rather than discrete lesions.[199]

Therapy for phaeohyphomycosis depends on the site of infection. If surgical resection is feasible, it should be pursued. Complete resection, rather than aspiration, of brain abscesses has been associated with improved outcome.[201] Generally, amphotericin B does not have good activity, whereas itraconazole has more consistent activity.[200] In vitro results of 15 species of dematiaceous fungi showed low MICs for itraconazole and terbinafine.[145] Itraconazole led to clinical improvement, remission, or stabilization in 11 of 17 patients.[215] Cases of successful treatment with posaconazole and terbinafine also have been reported.[3,170]

STACHYBOTRYS

A cluster of 10 cases of pulmonary hemorrhage and hemosiderosis in infants in Cleveland, Ohio, initially was attributed to environmental exposure to *Stachybotrys chartarum* (also referred to as *Stachybotrys atra*).[66,73] This mold produces trichothecene mycotoxins, so researchers postulated that the disease in infants was caused by exposure to mycotoxins from fungi growing in water-damaged households. Further evaluation by the Centers for Disease Control and Prevention found that the association was not substantiated.[40] If similar outbreaks recur, more careful analysis of the role of fungi and mycotoxins is warranted.

Chromoblastomycosis

Chromoblastomycosis is a chronic infection of skin and subcutaneous tissue; the agent isolated most commonly is *Fonsecaea pedrosoi*; other implicated fungi are *Cladophialophora carrionii* and *Phialophora verrucosa.* The disease is limited to the tropical and subtropical regions of the world, usually in warm, humid areas.[28] Infection begins with inoculation of the organism, typically through minor trauma, such as a splinter or thorn.[72] Most affected patients are men, frequently farmers.[28] The median age of affected patients is 35 years, but pediatric patients have been reported.[28] The mean duration of illness before initial medical evaluation is sought is 3 years, attesting to the slow-growing nature of this process.[28]

The lesions are markedly hyperkeratotic and may be nodular, verrucous, or psoriaform (Fig. 219–4).[28] Common symptoms include pruritus and pain.[28] Scratching the lesions may be the mode of spread for the frequent development of satellite lesions.[144] Bacterial superinfection is a common complication; the fungi

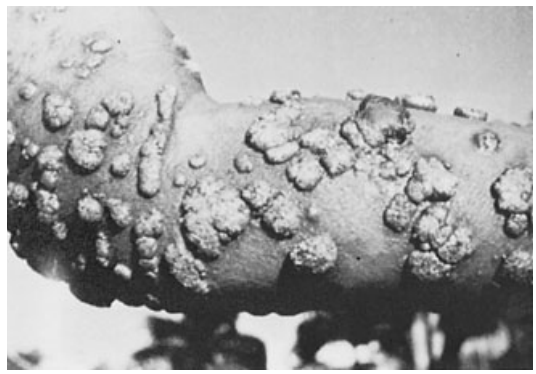

Figure 219–4 Chromoblastomycosis. The warty-like growth of epidermis and dermis is a result of traumatic implantation of the etiologic agent and subsequent autoinoculation from scratching. The agent was *Fonsecaea pedrosoi.*

usually do not disseminate to deeper tissues, although brain involvement has been reported.[28,144] A late complication is fibrosis leading to lymphatic obstruction and a clinical picture similar to that of elephantiasis.[144]

Most cases can be diagnosed by direct potassium hydroxide smears, which reveal the pathognomonic sclerotic bodies.[28] Sclerotic bodies also have been referred to as muriform cells and Medlar bodies. They are round to polyhedral, chestnut brown, thick-walled cells that are 5 to 12 μm in diameter with horizontal or vertical septa.[144] If biopsy is performed, the sclerotic bodies are noted, and the dermis reveals granulomata.[28,144] The fungi readily grow in culture, but colonies may not be evident for a few weeks. A new polymerase chain reaction assay holds promise for more rapid diagnosis.[53] The differential diagnosis includes verrucous cutaneous tuberculosis, sporotrichosis, mycetoma, leishmaniasis, coccidioidomycosis, psoriasis, hyperkeratotic tinea, blastomycosis, leprosy, and tertiary syphilis.[28,144] Differentiating features include the distinct margins and frequent satellite lesions of chromoblastomycosis.[144] Treatment has included local excision when feasible, with some practitioners using either cryosurgery or the application of heat.[28,144] Surgery plus antifungal therapy with itraconazole or terbinafine is considered the standard of care.[72]

SCEDOSPORIUM/PSEUDALLESCHERIA

Classification of these organisms as agents of phaeohyphomycosis versus hyalohyphomycosis has been controversial.[142] *Scedosporium prolificans* is listed more commonly as dematiaceous, and *Scedosporium apiospermum* infection usually is listed as a hyalohyphomycosis. *S. apiospermum* is the asexual anamorph of *Pseudallescheria boydii*; other obsolete names for this fungus include *Petriellidium boydii*, *Monosporium apiospermum*, and *Allescheria boydii. S. prolificans* previously was referred to as *Scedosporium inflatum*, but was found to be conspecific with a fungus named *Lomentospora prolificans* isolated from the soil of potted plants in a Belgian greenhouse; the previously assigned specific nomenclature was adopted.[25] The two species differ morphologically, with *S. prolificans* having annellides with a distinctive, swollen base.[253] In addition, *P. boydii* grows on Sabouraud agar containing cycloheximide, whereas *S. prolificans* does not.[253]

Both species of *Scedosporium* cause disseminated disease in immunocompromised hosts, but differences in the form of localized disease most associated with the species do occur. In the United States, *P. boydii* is the causative agent most commonly isolated from mycetoma.[38] *P. boydii* is distributed widely in soil, sewage, and contaminated water.[228] Its presence in polluted water most likely is responsible for the unique occurrence of brain abscess caused by this fungus in patients who have survived near-drowning. In a review of 38 cases of CNS infection, most of the patients had immunocompromising conditions, but 28 percent had sustained near-drowning.[174] The time of onset from the near-drowning episode to symptoms ranges from 7 to 135 days (mean 37 days).[182] Abscesses may be single or multiple.[174] Although the fungus may be isolated from CSF, direct inspection of brain tissue usually is required to establish the diagnosis.[115] Near-drowning victims with CNS involvement often have evidence of pulmonary disease, suggesting the lung as a portal of entry.[113] Even in immunocompetent patients, mortality is high (70%), perhaps related to a delay in establishing the diagnosis.[113,182]

P. boydii may be isolated from respiratory secretions in the absence of disease.[235] In patients with cystic fibrosis, 8.6 percent had *S. apiospermum* isolated from sputum cultures, the most frequent filamentous fungus after *A. fumigatus.*[46] Two of the patients had signs and symptoms compatible with allergic bronchopulmonary disease occurring after chronic colonization with *S. apiospermum.*[46] Invasive pulmonary disease also develops in immunocompetent and immunocompromised hosts.[110,164,235] Clinically, it may resemble aspergillosis. In addition to pulmonary infection, other focal forms of the disease have included endocarditis related to an indwelling catheter, osteomyelitis, surgical wound infection, sinusitis, and olecranon bursitis.[80,235,250]

S. prolificans initially was recognized as a cause of focally invasive disease, especially musculoskeletal infections occurring after penetrating trauma or surgery.[253] More recently, the organism has been recognized as an emerging pathogen of immunocompromised hosts.[188,193] Some clusters of cases have occurred in association with hospital renovation, suggesting a possible environmental source, but this source has not been conclusively shown.[25] Because many of the cases have been reported from Spain, Australia, and California, some researchers have suggested that climactic factors may influence the likelihood of infection. Cases in California have been suspected to be coccidioidomycosis.[175]

The usual finding of disseminated *S. prolificans* infection is fever unresponsive to broad-spectrum antibiotics, followed by pulmonary involvement and later neurologic manifestations, skin lesions, and, finally, widespread involvement with frequent renal failure.[193,197] In a review of 16 cases of disseminated infection, chest radiographs were abnormal in 12 patients, with bilateral focal infiltrates in 6, diffuse infiltrates in 5, and a solitary pulmonary nodule in 1.[25] The skin lesions usually are multiple and may be erythematous or nodular or have necrotic centers.[25,175] One patient had intense myalgias preceding the eruption of skin lesions.[175] Meningoencephalitis may occur; one case was attributed to direct inoculation during lumbar puncture for intrathecal chemotherapy.[131] At autopsy, the fungus is found most commonly in the kidney, lung, brain, and spleen.[25] The mortality rate is very high—87.5 percent in one series.[25] Blood cultures frequently are positive, with the organism growing in 9 to 15 days in routine blood culture detection systems, but much more rapidly in fungal isolator tubes.[25]

Both clinically important species of *Scedosporium* are resistant to amphotericin B.[25,71] Therapy usually includes wide surgical débridement whenever possible. Some joint infections apparently have responded to débridement plus administration of intra-articular amphotericin B; the high local concentration may improve its effectiveness.[253] As with most mold infections, recovery of neutrophil counts in immunocompromised hosts is crucial for improvement. *P. boydii* is susceptible to some of the newer triazoles, such as posaconazole, ravuconazole, and voriconazole.[193] Some strains of *S. prolificans* also were susceptible, and successful treatment with voriconazole combined with terbinafine has been described.[193,251] Successful treatment of *P. boydii* with voriconazole and with posaconazole has been reported.[151,164,174] Itraconazole has some activity and has proved effective in localized disease, but decreased concentrations in brain tissue may limit its effectiveness.[81,174] Adjuvant treatment with interferon-γ, granulocyte colony-stimulating factor, and granulocyte-monocyte colony-stimulating factor also has been reported.[1,251]

Hyalohyphomycosis

FUSARIUM SPECIES

Fusarium initially was recognized as a plant pathogen that caused crown rot on cereal grains.[172] The organisms are distributed widely in soil and vegetation and in all climates, and they may be airborne.[172] Pathogenic species also have been isolated from hospital water systems, suggesting the possibility of nosocomial acquisition.[12] The species most commonly associated with invasive human disease are *Fusarium solani*, *Fusarium oxysporum*, and *Fusarium verticilloides* (also known as *Fusarium moniliforme*).[98]

The first human disease states attributed to *Fusarium* were secondary not to invasion, but to elaboration of mycotoxins. Alimentary toxic aleukia was responsible for epidemics in the Soviet

Union during World War II.[172] This disease results from the consumption of grains colonized with *Fusarium sporotrichioides* after overwintering. The fungus elaborates a toxin designated T-2, which is a potent protein synthesis inhibitor. The disease begins with a burning sensation from the mouth to the stomach, followed by vomiting and diarrhea, abdominal pain, headache, and fatigue. Progressive leukopenia develops, and hemorrhage and necrosis eventually may lead to death. A similar disease known as scabby grain intoxication, or akakabi-byo, has occurred in Japan.[172] Mycotoxins have never been shown conclusively during invasive fusariosis, but they have been postulated to contribute to virulence through further suppression of the host's immune system.[172] Other possible virulence factors include production of enzymes and adherence and invasion of prosthetic materials.[172]

In immunocompetent hosts, *Fusarium* may cause localized disease. Onychomycosis from *Fusarium* may result in a characteristic milky discoloration of the nail.[172] Burn wounds also are colonized frequently with *Fusarium*.[188] In central Africa, *Fusarium* is a frequent cause of mycotic external otitis.[172] Bone and joint infections have been reported after traumatic inoculation has occurred,[32,172] and hematogenous osteomyelitis has been described in an adolescent with leukemia.[35] Peritonitis associated with continuous ambulatory peritoneal dialysis catheters also may occur.[79,114,172]

Fusarium is the most common fungal etiology of keratitis in the United States.[172] Corneal injury accompanied by contamination with fungal material may progress to keratitis, possibly aided by the elaboration of proteases by *F. solani*.[172,181] The organism can survive on contact lenses, affording another possible mode of entry.[172] In 2005 to 2006, an epidemic of *Fusarium* keratitis in wearers of contact lenses was associated with use of a specific storage solution.[23] The isolates were genetically diverse, suggesting multiple sources of contamination.[178] In vitro studies showed that conditions simulating noncompliant use of contact lenses (e.g., overnight wear) enhanced the risk for having corneal damage and fungal overgrowth.[39,124,205,258]

The incidence of infection caused by *Fusarium* has been increasing, most probably in concert with the increased use of immunosuppressive regimens. Most cases are described in patients with hematologic malignancies, but other underlying conditions include aplastic anemia, AIDS, solid tumors, and burns.[138] Eight percent of all non-*Candida* fungal infections occurring after bone marrow transplantation are caused by *Fusarium*.[158] Most patients are neutropenic at the time infection is acquired. In a review of 81 cases, the infection was disseminated in 88 percent.[138] Common sites of infection were the skin (79%), lungs (42%), and upper respiratory tract, including the sinuses (22%).[138] Localized disease, specifically onychomycosis, precedes disseminated infection in many cases, underscoring the need for careful inspection and treatment of local infection before the institution of immunosuppressive therapy.[138,172] Infection in patients after receiving solid organ transplants usually is localized and occurs at a median of 9 months post-transplant.[210]

In immunocompromised hosts, skin involvement is characteristic (Fig. 219–5). The lesions are found predominantly on the extremities and begin as tender, erythematous papules that may become vesicular and later develop a necrotic center reminiscent of ecthyma gangrenosum.[85,167,172,210] The lesions usually occur in the setting of disseminated disease with hematogenous spread, but they also may follow direct inoculation. Nasal lesions may resemble the lesions of zygomycosis or aspergillosis, with painless, black necrotic eschars.[85]

Infection also has been reported in neonates. One 28-week-gestation infant has been described with a fungal mass in the renal pelvis akin to renal fungus balls seen after candidiasis; the infant recovered after resection of the mass.[168] Endocarditis, most likely related to a central venous catheter, has been described in a term neonate.[104]

In a large case series, histopathology or blood cultures, or both, were positive in 80 percent of cases; blood cultures were positive in 47 percent.[138] This prevalence is in contrast to aspergillosis, in which positive blood cultures are distinctly unusual. The high rate of positive blood cultures in fusariosis most likely is related to the production of adventitious unicellular propagules and the vasoinvasive nature of the fungus.[188,193] In tissue sections, the fungi often have a perivascular distribution, with frank vascular invasion leading to thrombosis and necrosis.[85,172] The fungus grows rapidly in culture, and mature colonies develop by 4 to 5

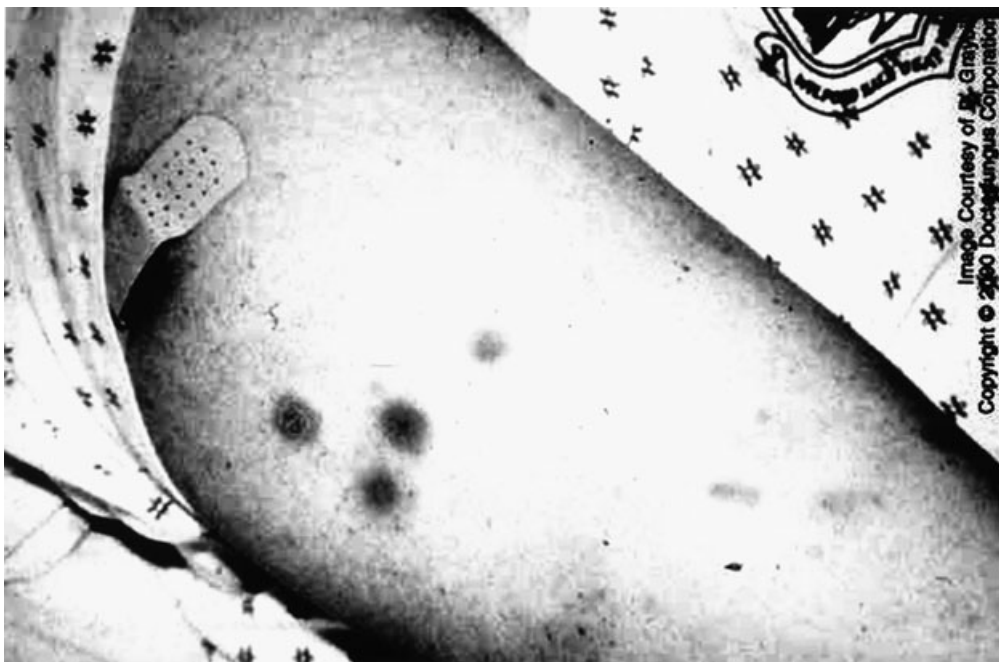

Figure 219–5 Skin lesions of disseminated fusariosis in an immunocompromised host. The lesions often resemble ecthyma gangrenosum. *(Photograph courtesy of D. Graybill, provided by www.doctorfungus.org.)*

days.[85] Sickle-shaped or banana-shaped macroconidia are noted, and the reverse is deeply pigmented.[85]

Mortality rates from *Fusarium* infection are high: 76 percent in one case series, 57 percent in another, and 26 percent in a third report.[98,138,198] The outcome seems to be better in patients with solid organ transplants than in patients with bone marrow transplants, with resolution achieved in five of six patients in one series.[210] Therapy generally has been disappointing in neutropenic patients, and recovery of neutrophil counts seems to be mandatory for clinical resolution.[85] Catheter-related infections respond well with removal of the catheter and administration of antifungal therapy; however, most reported cases also had normal neutrophil counts.[241] In burn patients, very high-dose topical nystatin therapy has been reported to be effective.[22] Whenever feasible, wide surgical resection is necessary.

Antifungal therapy for disseminated infection has been disappointing. The organisms are resistant to amphotericin in animal models.[11] The activity of amphotericin B may be enhanced, however, by the addition of agents providing synergy; a more recent report describes encouraging in vitro results with the combination of amphotericin B plus azithromycin.[35] Amphotericin B lipid complex has been used to achieve levels above the MIC.[33,187] Because neutrophils are clearly critical to recovery, granulocyte transfusions and colony-stimulating factors have been used.[26,33,88,187,193,198] In vitro testing shows the activity of voriconazole to be similar to that of amphotericin B and itraconazole against *Fusarium*.[71]

PAECILOMYCES SPECIES

Paecilomyces lilacinus and *Paecilomyces variotii* have been reported as human pathogens. The organisms are widely distributed in soil and decomposing vegetation. They were classified previously with *Penicillium* and bear some resemblance microscopically, but never have the blue-green coloration frequently seen in *Penicillium* colonies. *P. lilacinus* has a faint violet, mauve, or reddish gray tint, whereas *P. variotii* has powdery colonies that initially are buff, but eventually turn yellowish brown.[228] Localized infection, such as peritonitis complicating continuous ambulatory peritoneal dialysis and keratitis, has been described.[51,140,181,202,245] Resistance to commonly used disinfectants has contributed to outbreaks from contaminated solutions and topical agents; an outbreak occurring after implantation of intraocular lenses was traced to contaminated bicarbonate solution, and skin infection in immunocompromised patients was linked to contaminated moisturizing lotion.[107,248] Cases of sinusitis, endophthalmitis, endocarditis after placement of cardiac grafts, orbital cellulitis, pulmonary infection, and cutaneous and subcutaneous infection have been reported.[188,230]

In a review of 119 cases of *P. lilacinus* infection, 51 percent affected the eye, and 35 percent were cutaneous/subcutaneous.[186] Infection has been noted in patients with underlying hematologic malignancies, chronic granulomatous disease, and diabetes mellitus, and after receiving bone marrow transplants.[107,208,219,230,252] Even in immunocompromised hosts, the infection usually remains localized, but dissemination has been described.[107,188] Skin lesions may resemble the lesions seen in fusariosis, and they range from erythematous macules to nodules, pustules, vesicular lesions, and necrotic crusts.[107] Blood cultures may be positive, especially in infection related to indwelling vascular access devices.[188,220] Skin biopsy samples frequently yield positive cultures.[107] In contrast to most fungi, biopsies may reveal evidence of sporulation, allowing presumptive diagnosis before culture results are known.[186] Clinical outcome was poor in 30 of 47 cases reviewed.[230] Removal of vascular devices and surgical débridement are recommended.[188,234]

Speciation is important when devising optimal therapy because *P. lilacinus* is frankly resistant to amphotericin B and fluconazole. Clinical success has been reported with voriconazole, and posaconazole has the lowest MIC values in vitro.[186] *P. variotii* generally is susceptible to amphotericin B and the azoles, although breakthrough infection during voriconazole prophylaxis has been reported.[4,42]

ACREMONIUM SPECIES

Acremonium previously was named *Cephalosporium*, and this name for the genus is the one most familiar to clinicians; cephalosporin was isolated first from *Acremonium chrysogenium*.[93] The organisms are widely distributed in soil, plant debris, and rotting mushrooms.[93] The most common form of infection caused by *Acremonium* is focally invasive disease after trauma, with mycetoma and keratitis predominating.[76,93] Other reported manifestations include hypersensitivity pneumonitis, fungus balls, sinusitis, bone and joint infection, arteriovenous fistula infection, peritonitis, empyema, meningitis, and endocarditis.[76] Many patients have had recent surgery, injury, intravenous drug injection, or immunosuppressive therapy as predisposing factors.[76] In immunocompromised hosts, fungemia, including catheter-related fungemia, and papular rashes have been reported.[93,204] Recovery usually depends on restoration of normal neutrophil function.

Acremonium is resistant to most antifungals when tested in vitro; amphotericin B has the best activity.[93] Many different therapeutic regimens have been attempted, with variable success.[93] Catheter-related fungemia has responded to removal of the catheter followed by liposomal amphotericin B.[204]

"MYCOSES" CAUSED BY ORGANISMS OTHER THAN FUNGI

Three diseases—protothecosis, rhinosporidiosis, and pythiosis—generally were considered mycoses until molecular genetic techniques revealed the true nature of the pathogens. Protothecosis is caused by an achlorophyllic alga, and rhinosporidiosis and pythiosis more recently have been attributed to protistan parasites.

Rhinosporidiosis

Rhinosporidiosis consists of warty, vegetative growths, usually found in the nose or eyes (Fig. 219–6). The growths do not cause pain, and grow very slowly. Epidemiologically, the disease is associated with exposure to stagnant pools or fresh water. Of cases, 88 percent are reported from India; however, cases also are reported from South America and the United States.[7,84] The lesions may resemble nasal polyps, but mucous cysts are absent, and histopathology reveals the characteristic "sporangia." The spherules of *Rhinosporidium seeberi* are visible with most commonly used tissue stains. The spherules also stain with mucicarmine, which stains *C. neoformans* as well. The only known therapy to date is surgical removal, which may lead to copious bleeding and secondary infection. The organism never has been grown in culture, but more recent evaluation of 18S ribosomal RNA has revealed that *R. seeberi* is most closely related to the DRIP clade (named for *Dermocystidium* rosette agent, *Ichthyophonus*, and *Psorospermium*) clade of aquatic protistan parasites; close phylogenetic relatives include known fish parasites.[82] Further evaluation of these related parasites may result in better understanding of the natural history of infection and the ideal approach to therapy.

Protothecosis

The algae responsible for protothecosis grow readily on Sabouraud agar, unless cycloheximide is incorporated.[228] The species most commonly implicated is *Prototheca wickerhamii*, but *Prototheca zopfii* and *Prototheca stagnora* also have been reported.[105] Forms of infection include cutaneous or subcutaneous infection,

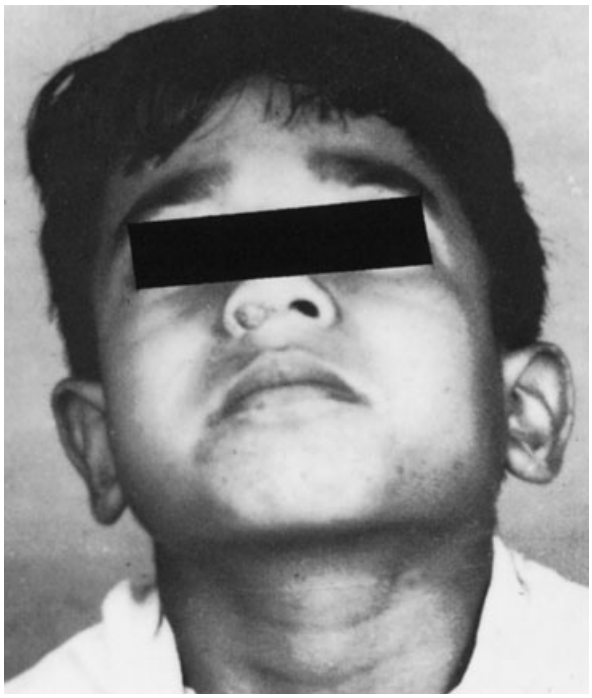

Figure 219–6 Rhinosporidiosis. Polyps are developing in the nose. *(Courtesy of S. Banerjee.)*

olecranon bursitis, catheter-related infection, and systemic disease.[105] Half the reported patients have an underlying immunocompromising condition.[105] Infection frequently occurs after local trauma. Histologically, granulomata often are noted.[106] Disseminated disease usually involves the abdominal cavity, with the development of multiple nodular lesions.[105] The organisms usually are susceptible to amphotericin B, so treatment generally is surgical excision (if feasible), followed by administration of amphotericin B, although olecranon bursitis frequently is treated with bursectomy alone.[105,229]

Pythiosis

Pythiosis is best known as a veterinary condition characterized by chronic subcutaneous granulomas; the equine form of the disease is referred to as *swamp cancer* and *Florida horse leeches*.[96,229] The aquatic organism seems to grow hyphae in culture, but the cell walls do not contain ergosterol.[96] Motile zoospores also are produced, with an affinity for attachment to plant stems and human hair.[96] Most human disease has been reported in tropical and subtropical areas, including the United States. Many cases have been reported from Thailand, usually affecting patients with thalassemia and related hemoglobinopathies.[196] The disease usually manifests with cutaneous, subcutaneous, vascular, or ophthalmic involvement; pneumonia with subsequent development of pleural and pericardial infection has been reported in a child with leukemia.[96] Patients often report contact with swampy water or with horses.[196,236] In tissue specimens, the organisms may resemble Zygomycetes, and misidentification as such has been reported.[31,152,217] Silver stains show the hyphae, but the organisms are not well visualized with periodic acid–Schiff stains.[236] Successful therapy usually includes surgical débridement; given the absence of ergosterol, treatment with standard antifungal agents would not be expected to be useful, but survival has been reported after use of various agents, including terbinafine, itraconazole, amphotericin B (deoxycholate and liposomal forms), and potassium iodide, alone and in combination.[31,96,196,217]

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SECTION XXII

Parasitic Diseases

CHAPTER 220

Classification and Nomenclature of Human Parasites

Lynne S. Garcia

Although common names frequently are used to describe parasitic organisms, these names may represent different parasites in different parts of the world. To eliminate these problems, a binomial system of nomenclature in which the scientific name consists of the genus and species is used. These names generally are of Greek or Latin origin. In certain publications, the scientific name often is followed by the name of the individual who originally named the parasite. The date of naming also may be provided. If the name of the individual is in parentheses, it means that the person used a generic name no longer considered to be correct.

On the basis of life histories and morphologic characteristics, systems of classification have been developed to indicate the relationship among the various parasite species. Closely related species are placed in the same genus, related genera in the same family, related families in the same order, related orders in the same class, and related classes in the same phylum, one of the major categories in the animal kingdom. As one moves up the classification schema, each category becomes broader; however, each category still has characteristics in common.

Parasites of humans are classified in five major divisions: (1) the Protozoa (amebae, flagellates, ciliates, sporozoans, coccidia, and microsporidia); (2) the Platyhelminthes or flatworms (cestodes, trematodes); (3) the Acanthocephala or thorny-headed worms; (4) the Nematoda or roundworms; and (5) the Arthropoda (insects, spiders, mites, ticks). Although these categories seem to be well defined, often considerable confusion occurs in attempting to classify parasitic organisms. One of the primary reasons is the lack of known specimens. Some organisms recovered from humans are very rare; difficulty arises in determining morphologic and physiologic variation among such groups. Type specimens must be deposited for study before a legitimate species name can be given. Even when certain parasites are numerous, they may represent strains or races of the same species with slightly different characteristics.

Generally, reproductive mechanisms are a valid concept in determining definitions of species, but so many exceptions exist within parasite groups that taking into consideration properties such as sexual reproduction, parthenogenesis, and asexual reproduction is difficult. Another difficulty in recognizing species is the ability and tendency of the organisms to alter their morphologic forms according to age, host, or nutrition, which often results in several names being given to the same organism. An additional problem involves alternation of parasitic and free-living phases in the life cycle. These organisms may be very different and difficult to recognize as belonging to the same species. Despite these difficulties, newer, more sophisticated molecular methods of grouping organisms often have confirmed taxonomic conclusions reached hundreds of years earlier by experienced taxonomists.

As investigations continue in parasitic genetics, immunology, and biochemistry, the species designation is expected to be defined more clearly. Originally, these species designations were determined primarily by morphologic differences, resulting in a phenotypic approach. With the use of highly sophisticated molecular techniques, the approach is expected to continue to be more genotypic. Benefits of these studies also include the development of highly specific and sensitive diagnostic tests and the ability to diagnose parasitic infections based on molecular parameters, rather than merely phenotypic characteristics.

Although gaps in knowledge concerning classification of all human parasites remain, the binomial system has allowed the classification of 1.5 million species of organisms in the animal kingdom such that all published information can be retrieved, regardless of the language spoken. The difficulty for the clinician arises when one considers the rapid increase in information concerning microbiology that has transpired in recent years and the changing considerations such as the role of immunosuppression in the host-parasite interaction and the modified definitions of "normal flora" and "nonpathogenic" in this patient population.

The classification of parasites is presented in tabular form. Although certain designations of species may be controversial, this classification scheme is designed to provide some order and meaning to a widely divergent group of organisms. No attempt has been made to include every possible organism, and only organisms considered clinically relevant in the context of human parasitology are included. The main groups that are presented include protozoa, nematodes (roundworms), cestodes (tapeworms), and trematodes (flukes). Some relevant information on arthropods is presented in Tables 220–1 and 220–2. It is hoped that this information will provide some insight into the parasite groupings, leading to a better understanding of parasitic infections and the appropriate diagnostic and clinical approach.

PROTOZOA

AMEBAE—INTESTINAL

These organisms are characterized by having pseudopods (motility) and trophozoite and cyst stages in the life cycle and include some exceptions in which a cyst form has not been identified. Amebae usually are acquired by humans through fecal-oral transmission or mouth-to-mouth contact (*Entamoeba gingivalis*).

TABLE 220–1 Human Vector-Borne Infections

Infection (Disease)	Causative Agent	Vector (Common Name)
Protozoal		
Malaria	*Plasmodium* species	Mosquitoes
Leishmaniasis	*Leishmania* species	Sandflies
Chagas disease	*Trypanosoma cruzi*	Triatomid bugs
East African trypanosomiasis	*Trypanosoma brucei rhodesiense*	Tsetse flies
West African trypanosomiasis	*Trypanosoma brucei gambiense*	Tsetse flies
Babesiosis	*Babesia* species	Ticks
Helminthic		
Filariasis	*Wuchereria bancrofti*	Mosquitoes
Filariasis	*Brugia malayi*	Mosquitoes
Filariasis	*Dirofilaria* spp.	Mosquitoes
Filariasis	*Mansonella persans*	Biting midges
Filariasis	*Mansonella streptocerca*	Biting midges
Filariasis	*Mansonella ozzardi*	Biting midges
Onchocerciasis	*Onchocerca volvulus*	Black flies
Loiasis	*Loa loa*	Deer flies
Dog tapeworm infection	*Dipylidium caninum*	Dog lice and fleas, human fleas
Rat tapeworm infection	*Hymenolepis diminuta*	Rat fleas, beetles, grain beetles
Dwarf tapeworm	*Hymenolepis nana*	Grain beetles (rare)

TABLE 220–2 Medically Important Arthropods

Local or Systemic Problems	Vector (Common Name)	Local or Systemic Problems	Vector (Common Name)
Skin reaction to bites	Sucking lice	Painful sting, potential anaphylaxis	Honeybees
	Bedbugs		Bumblebees
	Kissing bugs		Wasps, hornets, yellow jackets
	Biting midges		Fire ants
	Sandflies		Scorpions
	Black flies	Dermatitis, ulcerations	Fleas
	Mosquitoes	Nodular ulceration with subsequent secondary infection	Chigoe flea
	Deer flies		
	Tsetse flies		
	Soft ticks	Blistering of skin after contact with adult beetles	Blister beetles
	Hard ticks		
Painful bite	Horseflies	Bite, usually painless, delayed systemic reaction	Black widow spiders
	Fire ants		
	Centipedes		
Intense itching	Human itch mites	Initial blister followed by extensive necrosis and slow healing	Brown recluse spiders
	Chiggers		South American brown spider

Current Name
*Entamoeba histolytica**
*Entamoeba dispar**
Entamoeba hartmanni†
Entamoeba coli
Entamoeba polecki
Entamoeba gingivalis
Endolimax nana
Iodamoeba bütschlii
*Blastocystis hominis**

**E. histolytica* is being used to designate the true pathogen, whereas *E. dispar* now is being used to designate a nonpathogen. Unless trophozoites containing ingested red blood cells (*E. histolytica*) are seen, the two organisms cannot be differentiated on the basis of morphology seen in permanent stained smears of fecal specimens and are reported as *E. histolytica/E. dispar*. Immunoassays are available commercially for differentiating *E. histolytica* from *E. dispar*. Because the differences in pathogenicity are genetic and not just phenotypic, the decision to treat must be made by the physician. Finding of organisms in the *E. histolytica/E. dispar* group in patient specimens must continue to be reported to state and county Departments of Public Health (follow your particular state reporting regulations).

†*E. hartmanni* is nonpathogenic and is totally different from *E. histolytica*. "Small race *E. histolytica*" is incorrect and should not be used at any time to designate *E. hartmanni*.

FLAGELLATES—INTESTINAL

These organisms move by means of flagella and are acquired by fecal-oral transmission. With the exception of *Dientamoeba fragilis* (internal flagella) and species in the genera *Trichomonas* and *Pentatrichomonas*, they have the trophozoite and cyst stages in the life cycle. *D. fragilis*, *Trichomonas*, and *Pentatrichomonas* spp. do not have a cyst stage.

*The taxonomic position of *Blastocystis* has always been confusing, but it has been placed in the kingdom Chromista (plantlike organisms, mainly algae), and this group lies close to the sporozoans and ciliates.

Current Name

*Giardia lamblia**
Chilomastix mesnili
Dientamoeba fragilis
Pentatrichomonas hominis
Trichomonas tenax
Enteromonas hominis
Retortamonas intestinalis

Ciliates—Intestinal

These organisms, which move by means of cilia, are acquired by humans through fecal-oral transmission. They have the trophozoite and cyst forms in the life cycle.

Current Name

Balantidium coli

COCCIDIA, MICROSPORIDIA—INTESTINAL

These organisms are acquired by humans by ingestion of various meats or through fecal-oral transmission via contaminated food or water.

Current Name

COCCIDIA

Cryptosporidium hominis
Cryptosporidium parvum
Cryptosporidium spp.
Cyclospora cayetanensis
Isospora belli
Sarcocystis hominis
Sarcocystis suihominis
Sarcocystis bovihominis

MICROSPORIDIA†

Enterocytozoon bieneusi
Encephalitozoon (Septata) intestinalis‡

AMEBAE, FLAGELLATES—OTHER BODY SITES

The amebae are pathogenic, free-living organisms that may be associated with warm, fresh-water areas. They have been found in the central nervous system, the eye, and other sites. *Trichomonas vaginalis* usually is acquired by sexual transmission. This particular flagellate is found in the genitourinary system.

Current Name

AMEBAE

Naegleria fowleri
Acanthamoeba spp.
Hartmannella spp.
Balamuthia mandrillaris
Sappinia diploidea

FLAGELLATES

Trichomonas vaginalis

COCCIDIA, MICROSPORIDIA, UNDECIDED CLASSIFICATION—OTHER BODY SITES

These organisms are particularly important in immunocompromised patients. They also may infect many individuals who have no apparent symptoms. On the basis of several RNA studies, *Pneumocystis jiroveci* is linked more closely to the fungi and has been reclassified with those organisms. Consequently, it is removed from the list.

Current Name

COCCIDIA

Toxoplasma gondii

MICROSPORIDIA

Nosema ocularum
Brachiola connori
Brachiola algerae
Brachiola vesicularum
Vittaforma corneae
Pleistophora ronneafiei
Trachipleistophora hominis
Trachipleistophora anthropophthera
Encephalitozoon hellem
Encephalitozoon cuniculi
*Encephalitozoon (Septata) intestinalis**
Enterocytozoon bieneusi†
Microsporidium africanum‡
Microsporidium ceylonensis‡

SPOROZOA, FLAGELLATES—BLOOD AND TISSUES

All of these organisms are arthropod-borne. Diagnosis may be more difficult to make than is diagnosis of the intestinal protozoa, particularly if automated blood differential systems are used. The *Leishmania* spp. have undergone extensive revisions in classification. From a clinical perspective, however, recovery and identification of the organisms still are related to body site. Recovery of the organisms is limited to the site of the lesion in infections other than those caused by the *Leishmania donovani* complex (visceral leishmaniasis).

Current Name

SPOROZOA (MALARIA, BABESIOSIS)

Malaria
Plasmodium vivax
Plasmodium ovale

*Although some individuals have changed the species designation for the genus *Giardia* to *G. intestinalis* or *G. duodenalis*, no consensus exists. For this listing, we retain the name *Giardia lamblia*.
†The microsporidia are now thought to be more closely related to fungi than to protozoa; however, parasitologists have been reluctant to part with this group, whereas mycologists have been equally reluctant to accept it. Consequently, the microsporidia are retained within this listing for parasites.
‡Formerly called *Septata intestinalis*.

*Formerly called *Septata intestinalis*.
†*E. bieneusi* has been recovered from sites other than the intestinal tract.
‡This designation is now written as a true genus but remains a "catch-all" for organisms that have not been (or may never be) identified to the true genus or species levels.

Plasmodium malariae
Plasmodium falciparum

Babesiosis
Babesia microti
Babesia divergens
Babesia gibsoni
Babesia spp.

FLAGELLATES (LEISHMANIASIS, TRYPANOSOMIASIS)

Leishmaniasis
Leishmania tropica complex (cutaneous leishmaniasis)
Leishmania mexicana complex (cutaneous leishmaniasis)
Leishmania braziliensis complex (mucocutaneous leishmaniasis)
Leishmania donovani complex (visceral leishmaniasis)

Trypanosomiasis
Trypanosoma brucei gambiense (West African trypanosomiasis)
Trypanosoma brucei rhodesiense (East African trypanosomiasis)
Trypanosoma cruzi (American trypanosomiasis)
Trypanosoma rangeli

NEMATODES

INTESTINAL

These organisms normally are acquired by ingestion of eggs or penetration of the skin by larval forms from the soil.

Current Name

Ascaris lumbricoides
Enterobius vermicularis (pinworm)
Ancylostoma duodenale (Old World hookworm)
Necator americanus (New World hookworm)
Strongyloides stercoralis
Strongyloides fuelleborni
Trichostrongylus spp.
Trichuris trichiura (whipworm)
Capillaria philippinensis
Oesophagostomum spp. (*O. bifurcum* most common in humans—West Africa)
Ternidens diminutus (80% in Zimbabwe)

TISSUE

For the most part, these organisms rarely are seen within the United States; however, the first three are more important.

Current Name

Trichinella spiralis
Trichinella spp.
Toxocara canis or *Toxocara cati* (visceral or ocular larva migrans)
Ancylostoma braziliense or *Ancylostoma caninum* (cutaneous larva migrans)
Baylisascaris procyonis (severe systemic visceral larva migrans, neural larva migrans)
Dracunculus medinensis
Angiostrongylus cantonensis
Angiostrongylus costaricensis
Gnathostoma spinigerum
Gnathostoma spp.
Anisakiasis (larvae from salt-water fish)
Anisakis spp.
Phocanema spp.
Contracaecum spp.
Pseudoterranova spp.
Hysterothylacium spp.
Porrocaecum spp.
Capillaria hepatica
Thelazia spp.

FILARIAL WORMS—BLOOD, OTHER BODY FLUIDS, SKIN

These organisms also are arthropod-borne. The adult worms tend to live in the tissues of lymphatics. Diagnosis is made on the basis of the recovery and identification of the larval worms (microfilariae) in the blood, other body fluids, or skin. Elephantiasis may be associated with some of the organisms listed.

Current Name

Wuchereria bancrofti
Brugia malayi
Brugia timori
Loa loa
Onchocerca volvulus
Mansonella ozzardi
Mansonella streptocerca
Mansonella perstans
Dirofilaria immitis ("coin" lesion in the lung) (dog heartworm)
Dirofilaria spp. (may be found in subcutaneous nodules)

CESTODES

INTESTINAL

The adult form of these organisms is acquired by humans through ingestion of the larval forms contained in poorly cooked or raw meats or fresh-water fish. In the case of *Dipylidium caninum*, infection is acquired by the accidental ingestion of dog fleas. *Hymenolepis nana* and *Hymenolepis diminuta* are transmitted by ingestion of certain arthropods (fleas, beetles). Also, *H. nana* can be transmitted through ingestion of eggs (life cycle can bypass the intermediate beetle host). Humans can serve as the intermediate and definitive hosts in *H. nana* and *Taenia solium* infections.

Current Name

Diphyllobothrium latum (broad, fish tapeworm)
Dipylidium caninum (dog tapeworm)
Hymenolepis (Rodentolepis) nana (dwarf tapeworm)
Hymenolepis diminuta (rat tapeworm)
Taenia solium (pork tapeworm)
Taenia saginata (beef tapeworm)
Taenia asiatica (Taiwanese variant of *T. saginata*)

LARVAL FORMS—TISSUE

The ingestion of certain tapeworm eggs or accidental contact with certain larval forms can lead to the diseases shown in parentheses.

Current Name

Taenia solium (cysticercosis)
Echinococcus granulosus (hydatid disease)
Echinococcus multilocularis (alveolar hydatid disease)
Echinococcus oligarthrus (polycystic hydatid disease)
Multiceps multiceps (coenurosis)

Diphyllobothrium spp. (sparganosis)
Spirometra mansonoides (sparganosis)

TREMATODES

INTESTINAL

These organisms are uncommon within the United States except for four species of *Alaria*, which are endemic within North America.

Current Name

Fasciolopsis buski (giant intestinal fluke)
Echinostoma ilocanum
Eurytrema pancreaticum
Heterophyes heterophyes
Metagonimus yokogawai
Alaria spp.

LIVER AND LUNG

These organisms are not seen commonly within the United States; however, some Southeast Asian refugees do harbor some of these parasites.

Current Name

Clonorchis (Opisthorchis) sinensis (Chinese liver fluke)
Opisthorchis viverrini
Fasciola hepatica (sheep liver fluke)
Paragonimus westermani (lung fluke)
Paragonimus spp.
Metorchis conjunctus (North American liver fluke)

BLOOD

The schistosomes are acquired by penetration of the skin by the cercarial forms that are released from fresh-water snails. Although they are not endemic within the United States, occasionally patients are seen who may have these infections.

Current Name

Schistosoma mansoni
Schistosoma haematobium
Schistosoma japonicum
Schistosoma intercalatum
Schistosoma mekongi

ARTHROPODS

See Tables 220–1 and 220–2.

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SUBSECTION 1

Protozoa

A. Amebae

CHAPTER 221

AMEBIASIS

Shinjiro Hamano ✪ William A. Petri, Jr.

Diarrheal diseases continue to be major causes of morbidity and mortality in children in developing countries. In Bangladesh, 1 in 30 children dies of diarrhea or dysentery by age 5 years.[75] Amebiasis is an infection caused by the protozoan parasite *Entamoeba histolytica*. Infection occurs via ingestion of the parasite's cyst from fecally contaminated food, water, or hands. Approximately 50 million illnesses and 100,000 deaths occur annually from amebiasis, rendering it the third leading cause of death by parasitic disease in humans.[75] Long-term consequences of amebiasis in children include malnutrition and reduced cognitive

abilities.[69,93] Although amebiasis is present worldwide, it occurs most commonly in underdeveloped areas, especially Central and South America, Africa, and Asia. In the United States and other developed countries, cases of amebiasis are most likely to occur in immigrants from and travelers to endemic regions, but it can affect populations of the developed world, as shown by the epidemic that occurred in Tbilissi, Republic of Georgia, caused by contaminated municipal water.[13] Currently, there is no vaccine to prevent the childhood morbidity and mortality resulting from infection with *E. histolytica*.

ETIOLOGY

E. histolytica is named for the pathologic evidence of "lysis" of tissues. The first demonstration of the organism in human tissues was made by Lambl in 1859 in the postmortem examination of the colon of a child who died as a result of having excessive diarrhea.[16,71] No connection of the organism with the disease was made until 1875, when Losch, in St. Petersburg, Russia, found the organism at autopsy in the colon of a woodcutter. Losch induced diarrhea and ulcerations in a dog given feces from the patient.[59] He did not think, however, that a connection existed between the organism and the disease. The first patient described in the United States was a physician treated by Osler for an amebic liver abscess in 1890.[71] Councilman and Lafleur described the organism and the disease in 1891.[20,42] Further investigation of the disease was delayed until a better understanding of the life cycle of *E. histolytica* could be obtained.[26] In recent years, the application of modern molecular biology techniques to the study of *E. histolytica* and *Entamoeba dispar* has resulted in an explosion of information about the mechanisms of virulence, pathogenicity, and immune responses to these organisms.[85,87]

E. histolytica is the pathogenic species, having the capacity to invade tissue and cause symptomatic disease, whereas *E. dispar* (and *E. histolytica*) is associated with the asymptomatic carrier state.[6,85] More recently, a study revealed that all genotypes of *E. histolytica* are not equally capable of causing disease.[6] Morphologically distinct members of the genus *Entamoeba*, such as *Entamoeba coli* and *Entamoeba hartmanni*, also are nonpathogenic. *Dientamoeba fragilis* and *Entamoeba polecki* have been associated with diarrhea, and *Entamoeba gingivalis* has been associated with periodontal disease.

Members of the genus *Entamoeba*, which are protozoan organisms belonging to the subphylum Sarcodina and close to *Dictyostelium discoideum* on one of the lowest branches of the eukaryotic tree, have trophozoite and cyst forms.[37] The cysts of *E. histolytica* and *E. dispar* are almost spherical, being surrounded by a cell wall composed of chitin. The cysts may have one to four nuclei, although quadrinucleate cysts are most typical. This feature allows differentiation from *Escherichia coli*, which usually has 6 to 8 nuclei in the cysts and may have 32 nuclei.[72] Cysts of *E. histolytica* are 5 to 20 μm in diameter (average 12 μm) and have a greenish tint in the unstained condition.[60] Young cysts contain chromatoid bodies, which are composed of ribosome particles in crystalline arrays.[12] The cysts of *E. hartmanni* appear identical to those of *E. histolytica* except for being a smaller size (4 to 10 μm). *E. histolytica* cysts can survive for days in the dried state at 30° C or for months at 0° C to 4° C. They can be killed by temperatures greater than 50° C retained for 5 minutes.[42] They are completely resistant to the concentrations of chlorine used in water supplies, but may be killed with hyperchlorination or with iodine solutions.[60,72] They are filtered from water supplies that pass through a sand filtration phase. They resist acids well.

When these quadrinucleate cysts are ingested, they resist the acid pH of the stomach and ultimately excyst in the alkaline environment of the bowel. The process of excystation results in the release of four trophozoites that divide by binary fission to produce eight trophozoites. The usual trophozoites have a diameter of 25 μm (range 10 to 60 μm).[26,85] They have a single nucleus that is 3 to 5 μm in diameter and contains fine peripheral chromatin with a slightly eccentric karyosome. They have a granular endoplasm that typically contains vacuoles in which bacteria and debris can be seen. Some glycogen is present and can be stained with periodic acid–Schiff stain.

Although amebae were thought to lack organelles, such as mitochondria, endoplasmic reticulum, and Golgi apparatus, evidence to the contrary is coming to light. The existence of nuclear-encoded mitochondrial genes and a remnant mitochondrial organelle was reported more recently.[61,94] The presence of ingested erythrocytes is a characteristic feature of *E. histolytica*, but not *E. dispar*.[85] Movement is accomplished by extension of clear pseudopodia. Replication is by binary fission. These protozoa live in the colon of humans and other mammals. Trophozoites die quickly outside the body and are quite sensitive to acid—they generally are not considered to be infective.[31] When cooled (as when feces are expelled and gradually cooled from body temperature) or stimulated by as-yet-undefined luminal conditions, the trophozoites form cysts that can remain viable for weeks to months on excretion.[85]

Trophozoites of *E. coli* are 15 to 50 μm in diameter; have much more sluggish motility than the trophozoites of *E. histolytica*; and have blunt pseudopodia, rather than the sharp, finger-like pseudopodia of *E. histolytica*. Trophozoites of *E. hartmanni* are 4 to 14 μm in diameter and have much less glycogen than the trophozoites of *E. histolytica*.[26]

EPIDEMIOLOGY

Amebiasis is distributed throughout the world. The number of people infected with either *E. histolytica* or *E. dispar* per year is estimated to be 500 million. Although most individuals remain asymptomatic, perpetuating the natural cycle of the organism through fecal excretion of infective cysts, approximately 50 million people experience the severe morbidity associated with invasive disease, with an estimated 100,000 dying annually.[75,92] In the United States, 50 percent of amebiasis is observed in Hispanic/Asian/Pacific Islanders. Travelers from developing countries, men, and residents of institutions for the mentally retarded are considered to be at higher risk for amebiasis (Table 221–1).

During the 1990s, enough evidence had accumulated to support the formal separation of two morphologically identical species of ameba: the nonpathogenic *E. dispar* from the potentially pathogenic *E. histolytica*.[1,14,29,30,36,92] Morbidity and mortality data in absolute numbers that existed before this time pertaining to cases of invasive disease were not greatly affected by this reclassification because all invasive disease was known to be caused by *E. histolytica*.[92] Because most prevalence and incidence data previously collected pertained to asymptomatic individuals, however, and it was clear that most asymptomatic individuals with cysts detected in their stool were infected with nonpathogenic *E. dispar*, the true prevalence and incidence of *E. histolytica* became a matter of speculation.[92]

TABLE 221–1 Risk Factors for Amebiasis in the United States

Hispanic/Asian/Pacific Islanders—50% of U.S. cases reported to CDC
Travelers—0.3% incidence in one study
Institutions for mentally retarded
Men who have sex with men
Men—90% amebic liver abscesses in men, but rare in children

CDC, Centers for Disease Control and Prevention.

Estimates of *E. histolytica* infections have been based primarily on examinations of stool for cysts and parasites, but these tests are insensitive and cannot differentiate *E. histolytica* from morphologically identical species that are nonpathogenic, such as *E. dispar* and *Entamoeba moshkovskii*.[5,22] Specific and sensitive means to detect *E. histolytica* in stool are now available and include antigen detection and polymerase chain reaction (PCR).[34,41,56]

A prospective study of preschool children in a slum of Dhaka, Bangladesh, showed *E. histolytica*–associated diarrhea in 9 percent and *E. histolytica*–associated dysentery in 3 percent of the children annually.[39] Not all individuals are equally susceptible to amebiasis, with certain HLA-DR and HLA-DQ alleles associated with resistance to infection and disease.[23] The annual incidence of amebic liver abscess was reported to be 21 cases per 100,000 inhabitants in Hue City, Vietnam.[14] Carefully conducted serologic studies in Mexico, where amebiasis is endemic, showed antibody to *E. histolytica* in 8.4 percent of the population.[17] In the urban slum of Fortaleza, Brazil, 25 percent of all individuals tested carried antibody to *E. histolytica*; the prevalence of antiamebic antibodies in children 6 to 14 years old was 40 percent.[15]

PATHOGENESIS AND PATHOLOGY

The cysts are transported through the digestive tract to the intestine, where they release their mobile, disease-producing form, the trophozoite. *E. histolytica* trophozoites can live in the large intestine and form new cysts without causing disease. They also can invade the lining of the colon, killing host cells and causing diarrhea, amebic colitis, acute dysentery, or chronic diarrhea. The trophozoites also can be carried through the blood to other organs, most commonly the liver and occasionally the brain, where they form potentially life-threatening abscesses (Fig. 221–1). Important virulence factors include the trophozoite cell surface galactose and *N*-acetyl-D-galactosamine (Gal/GalNAc)–specific lectin that mediates adherence to colonic mucins and host cells,[74,86] cysteine proteinases that likely promote invasion by degrading extracellular matrix and serum components, and amoebapore pore-forming proteins involved in killing of bacteria and host cells.[57,88]

The interface of the Gal/GalNAc lectin with the host mucins lining the intestine is the defining moment of the infection.[19] If the parasite lectin attaches to the host mucin glycoproteins that line the intestinal lumen, a noninvasive gut infection ensues. The life cycle continues as the trophozoites reproduce by clonal expansion in the mucin layer. Subsequently, the Gal/GalNAc lectin, along with mucin glycoproteins or other gut bacteria, initiates the developmental pathway leading to encystment.[25,92]

Colitis is caused when the trophozoite penetrates the intestinal mucous layer, which otherwise acts as a barrier to invasion by inhibiting amebic adherence to the underlying epithelium and by slowing trophozoite motility.[19] Invasion is mediated by the killing of epithelial cells, neutrophils, and lymphocytes by trophozoites, which occurs only after the parasite lectin engages host GalNAc on O-linked cell surface oligosaccharides.[75] The interaction of the lectin with glycoconjugates is stereospecific and multivalent.[100] The identity of the high-affinity intestinal epithelial cell receptor is unknown. Secretion of amoebapore, a 5-kd pore-forming protein, by the ameba may contribute to killing.[55] Activation of human caspase 3, a distal effector molecule in the apoptotic pathway, occurs rapidly after amebic contact, and caspases are required for cell killing in vitro and for the formation of amebic liver abscesses in vivo.[45,99]

Interaction of the parasite with the intestinal epithelium causes an inflammatory response marked by the activation of nuclear factor κB and the secretion of cytokines.[24,89] The development of this epithelial response may depend on trophozoite virulence factors, such as cysteine proteinase, and leads to intestinal abnormalities through neutrophil-mediated damage. Neutrophils also can be protective, and activation of neutrophils or macrophages by tumor necrosis factor-α or interferon-γ kills amebae in vitro and limits the size of amebic liver abscesses.[7,21] In contrast to the intense inflammatory response, typical of early invasive amebiasis, inflammation surrounding well-established colonic ulcers and liver abscesses is minimal, given the degree of tissue damage.[16]

The initial lesions of clinical amebiasis often are small interglandular ulcers with a diameter of approximately 1 mm. They extend only to the muscularis mucosa.[16,64] The margins may be hyperemic, and slight edema of the surrounding mucosa is present. *E. histolytica* organisms seen in these ulcers stain well with periodic acid–Schiff stain.[76] Bleeding and friability are not prominent at this stage, although proctoscopic examination may find mucus coming from these ulcers, with an abundant number of amebae present.

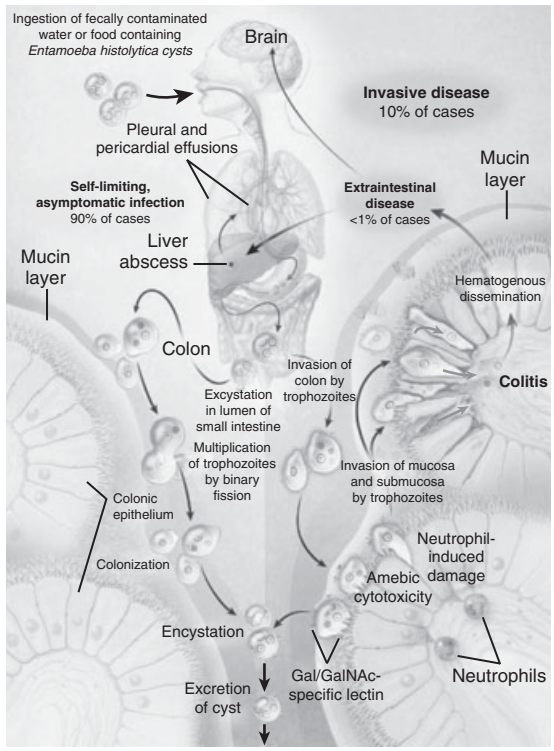

Figure 221–1 Life cycle of *Entamoeba histolytica*. Infection normally is initiated by the ingestion of fecally contaminated water or food containing *E. histolytica* cysts. The infective cyst form of the parasite survives passage through the stomach and small intestine. Excystation occurs in the bowel lumen, where motile and potentially invasive trophozoites are formed. In most infections, the trophozoites aggregate in the intestinal mucin layer and form new cysts, resulting in a self-limited and asymptomatic infection. In some cases, adherence to and lysis of the colonic epithelium, mediated by the galactose and *N*-acetyl-D-galactosamine (Gal/GalNAc)–specific lectin, initiates invasion of the colon by trophozoites. Neutrophils responding to the invasion contribute to cellular damage at the site of invasion. When the intestinal epithelium is invaded, extraintestinal spread to the peritoneum, liver, and other sites may follow. Factors controlling invasion, as opposed to encystation, most likely include parasite "quorum sensing" signaled by the Gal/GalNAc-specific lectin, interactions of amebae with the bacterial flora of the intestine, and innate and acquired immune responses of the host. (See companion Expert Consult web site for color version.) *(From Haque, R., Huston, C. D., Hughes, E., et al.: Amebiasis. N. Engl. J. Med.* 348*:1565-1573.)*

The next stage of intestinal disease is the production of deeper ulcers. These "buttonhole" ulcers may be 1 cm in diameter and may extend into the submucosa.[16,76] The ulcer often extends laterally under normal-appearing mucosa, forming a characteristic flask shape. Occasional perforation through the serosa leads to peritonitis or pneumoperitoneum.[91] Extensive necrosis may be present, but usually only very little inflammation occurs. The edema is more intense, but the mucosa between ulcers is normal, in contrast to the marked inflammatory response seen in bacterial enteritis. When ulceration is more extensive, the edema surrounding the ulcers becomes confluent, and the mucosa appears gelatinous. In young children, this condition can progress to a fulminant necrotizing colitis associated with transmural necrosis. The pathologic events associated with this phenomenon are not understood. Rarely, an inflammatory response is present, resulting in granulation of the tissue with a fibrous outer wall.[72] It is given the name *ameboma*. Occasionally, an ameboma fills a significant portion of the lumen, which causes stricture or obstruction. Other complications of intestinal amebiasis result from direct extension of the ulcers. This extension may result in cutaneous involvement of the perianal area or lesions of the penis, vulva, vagina, or cervix.[2,72] Cutaneous and ophthalmologic amebiasis also is caused by fecal contamination of the face.[67]

Amebas disseminate to the liver in 50 percent of patients with fulminant amebiasis.[2,3] Dissemination to other organs directly from the intestine probably does not occur, but dissemination from the liver to lung, heart, brain, spleen, scapula, larynx, stomach, and aorta has been described.[16] Amebic abscess of the liver occurs more often in men than in women by a ratio of 16:1, but occurs equally often in prepubertal children of both sexes.[3,16] Abscesses occur more commonly in adults, but occur in children as young as 4 months of age.[70] These abscesses vary from microscopic lesions to massive necrosis of 90 percent of the liver. Fever, right upper quadrant pain, and the presence of serum antibodies to amebae point to hepatic amebic abscess.[84] Examination of the fluid from such an abscess frequently reveals a reddish, "anchovy paste" fluid that rarely may appear white or green. The fluid is acidic, with a pH ranging from 5.2 to 6.7.[82] Amebas are found in the walls of the abscess and only rarely in the fluid of the abscess. Many patients with amebic liver abscess also have anaerobic bacteria in the abscess fluid.[83] The walls are composed of a thin connective tissue capsule. The right lobe of the liver is involved with amebic liver abscess about six times as often as the left lobe. Abscesses in the right lobe can perforate and cause disease below the diaphragm or in the thoracic cavity. Abscesses in the left lobe can lead to pericardial effusions, which are less common than pleural effusions.[32,47]

Pleural effusions can remain loculated or lead to cutaneous fistulas or to bronchopleural fistulas. Drainage from these fistulas is acidic, in contrast to the neutral secretions in the normal lung. Seeding of the cardiac valves and of the brain has been described.[16] Cerebral abscesses have the same microscopic findings as do liver abscesses, with a thin capsule of connective tissue surrounding a fluid with little or no associated inflammatory response.

IMMUNITY

Protection from amebiasis, including acquired immunity to infection and invasion by *E. histolytica*, is associated with a mucosal IgA antibody response against the carbohydrate recognition domain of the parasite Gal/GalNAc lectin.[37,35,43,53] Cell-mediated immunity in protection from invasive amebiasis, but not infection per se, also has been shown. There is substantial evidence from in vitro, animal model, and most recently human studies of an important role for interferon-γ in protection from amebic colitis, acting in part by activating of macrophages to kill the parasite.[40,44] Invasive amebiasis rarely occurs in individuals with human immunodeficiency virus/acquired immunodeficiency syndrome, even in areas where amebiasis is common, suggesting an important role also exists for natural immunity or innate immune responses, or both, in protection from infection.[7,34]

CLINICAL MANIFESTATIONS

INTESTINAL AMEBIASIS

Asymptomatic Intraluminal Amebiasis

The most common type of amebic infestation is an asymptomatic cyst-passing carrier state. All *E. dispar* infections and 90 percent of *E. histolytica* infections are asymptomatic, manifesting with only *Entamoeba* cysts in the feces.[29,78] Some investigators have suggested that stools of these individuals generally are more liquid than stools of individuals without trophozoites.[26]

Entamoeba histolytica–Associated Diarrhea

Diarrhea is the most common manifestation of amebic disease, present in 9 percent of children in the Mirpur cohort each year, compared with only 3 percent of children having amebic colitis each year.[39] *E. histolytica*–associated diarrhea is defined as three or more unformed stools in a 24-hour period accompanied by a new episode of *E. histolytica* infection. This definition was validated previously in the cohort by (1) showing that diarrhea was approximately five times more common in the setting of a new infection (age-adjusted odds ratio for the association of new *E. histolytica* infection with diarrhea of 4.7; 95% confidence interval 2.9 to 7.6), and (2) showing by a complete bacteriologic, virologic, and parasitic work-up that only 32 percent of *E. histolytica*–associated diarrhea cases were co-infected with another pathogen compared with identification of an enteropathogen in 59 percent of all cases of diarrhea.[37]

Acute Amebic Colitis

Amebic dysentery was defined as a diarrheal stool sample containing occult or gross blood that was positive for *E. histolytica* antigen. Seventy percent of patients have a gradual onset of symptoms over 3 or 4 weeks after infestation, with increasingly severe diarrhea as the primary complaint, accompanied by general abdominal tenderness. Occasionally, the onset may be acute or may be delayed for several months after infestation. This onset differs from bacterial causes of dysentery, in which patients usually have only symptoms of 1 to 2 days' duration. The diarrhea is usually associated with pain in children. Pain may be of such severity that an acute abdomen is suspected.[2,10,48,76] The stools contain blood and mucus in virtually all cases.[2,76,77] Fever is present in only a few patients with amebic colitis. Abdominal distention and dehydration occur in less than 10 percent of patients. In young children, intussusception, perforation, peritonitis, or necrotizing colitis may develop rapidly.[10,48,91]

Ameboma

Unusual manifestations of amebic colitis include toxic megacolon (0.5% of cases, usually requires surgical intervention), ameboma (granulation tissue in colonic lumen mimicking colonic cancer in appearance), and a chronic nondysenteric form of infection that can manifest as years of waxing and waning diarrhea, abdominal pain, and weight loss (easily misdiagnosed as inflammatory bowel disease).

EXTRAINTESTINAL AMEBIASIS

Amebic Liver Abscess

The typical patient with an amebic liver abscess in the United States is an immigrant, usually a Hispanic/Asian/Pacific Islander; male; 20 to 40 years old; who presents with fever, right upper quadrant pain, leukocytosis, abnormal serum transaminases and alkaline phosphatase, and a defect on hepatic imaging study. Roughly 90 percent of patients with liver abscess are men. The abscess usually is single and is in the right lobe of the liver 80 percent of the time.[49] Most frequently, patients present with liver abscess without concurrent colitis. Amebae are seen infrequently in the stool at the time of diagnosis of liver abscess.[3] Liver abscess can manifest acutely with fever and right upper abdominal tenderness and pain, or subacutely, with prominent weight loss and less frequent fever and abdominal pain. The peripheral white blood cell count is elevated, as is the alkaline phosphatase level, in many patients.

Early evaluation of the hepatobiliary system with ultrasound or computed tomography (CT) is essential to show the abscess in the liver. The differential diagnosis of the lesion in the liver includes pyogenic abscess, hepatoma, and echinococcal cyst. Aspiration of the abscess occasionally is required to diagnose amebiasis (although amebae are visualized in the pus in only a few cases; if the abscess is pyogenic, the responsible bacteria are seen or cultured). Antibodies to *E. histolytica* are present in the serum of 92 to 97 percent of patients on acute presentation with amebic liver abscess and are very useful diagnostically. Unusual extraintestinal manifestations of amebiasis include direct extension of the liver abscess to pleura or pericardium and brain abscess. In a patient who presents with right upper quadrant pain, ultrasound, CT, or magnetic resonance imaging (MRI) should be performed to examine the liver and gallbladder.

If a space-filling defect in the liver is observed, the differential diagnosis includes (1) amebiasis (most common in men with a history of travel or residence in a developing country); (2) pyogenic or bacterial abscess (suspect in women, patients with cholecystitis, elderly individuals, individuals with diabetes, and patients presenting with jaundice); (3) echinococcal abscess (an incidental finding because echinococcal abscess should not cause pain or fever); and (4) cancer. Most patients with amebic liver abscess have detectable circulating antigen in serum and serum anti-amebic antibodies.[34]

In children, abdominal pain is reported infrequently with amebic liver abscess.[33,68] More commonly, high fever, abdominal distention, irritability, and tachypnea are noted. Some children are admitted to the hospital with a fever of unknown origin. Hepatomegaly occurs frequently, but elicitation of hepatic tenderness is not well documented. In one report, four of five children younger than 5 years died with amebic liver abscesses because the diagnosis was not suspected.[54] Death usually results from rupture of the liver abscess into the peritoneum, thorax, or pericardium, but may follow extensive hepatic damage and liver failure.[3,81]

Metastatic Amebiasis

Extra-abdominal amebiasis presumably follows direct extension from liver abscesses, rather than direct dissemination from the intestine.[3,16] Thoracic amebiasis is the most common type of extra-abdominal amebiasis and occurs in approximately 10 percent of patients with amebic liver abscess.[16,47] Symptoms depend on the type of involvement. Empyema, bronchohepatic fistulas, or extension of a pleuropulmonary abscess into the pericardium may occur.

Pericardial amebiasis is the next most common form of extraintestinal involvement and may result from rupture of a liver abscess in the left lobe of the liver into the pericardium or through extension of the right-sided pleural amebiasis.[16,27,28,32] It is estimated to occur in 3 percent of patients with hepatic abscesses.[28] It manifests as acute pericarditis with tamponade and, occasionally, as pneumopericardium.[27] Amebic liver abscess in the left lobe also may rupture directly into the left chest.[63]

Cerebral amebic abscesses were found in 8 percent of patients with amebic infections discovered at autopsy in one study.[58] In other studies, lower rates of 0.66 to 4.7 percent of patients with amebic liver abscess having brain abscesses were reported.[46] Patients with cerebral amebiasis frequently are so ill from the intestinal, liver, and possibly lung involvement that neurologic signs are not always assessed easily. In 18 patients with proven cerebral amebiasis, initial neurologic examination was normal in 13, and only 1 patient later developed seizures.

Other foci of infection are rare findings, but amebic rectovesical fistula formation and involvement of pharynx, heart, aorta, and scapula have been reported. Cutaneous extension after the adherence of perforated, inflamed bowel to the skin is an extremely painful and rare complication.[16,72] This situation also may arise after invasion of the skin by trophozoites emerging out from the rectum occurs.

DIAGNOSIS

A heightened suspicion of amebiasis should be present if the patient has been in a developing country as a resident or traveler. The diagnosis of amebiasis should be considered in any child who is passing diarrhea, bloody stools, or stools with mucus; any child with a hepatic abscess; and any febrile child with right upper quadrant pain, abdominal distention, or tachypnea.[54,68] In a patient with diarrhea, if blood is present in the stool (grossly bloody or occult blood positive), infectious (Shiga toxin–producing *E. coli*, *Salmonella*, *Shigella*, *Campylobacter*, and *E. histolytica*) and noninfectious (inflammatory bowel disease, diverticulosis, arteriovenous malformations, cancer) causes should be considered.

IMMUNOLOGIC OR MOLECULAR EXAMINATION OF STOOL OR SERA

E. histolytica infection was defined as a positive test for antigen in stool. Antigen was detected using the TechLab (Blacksburg, VA) *E. histolytica* II stool antigen detection test, which specifically detects *E. histolytica* and does not cross-react with *E. dispar* or *E. moshkovskii*. The antigen test is 95 percent sensitive and specific compared with the "gold standard" of *E. histolytica* culture and zymodeme determination. It also is 80 percent sensitive compared with real-time PCR for *E. histolytica* DNA in stool. Although real-time PCR is excellent in sensitivity and specificity, real-time PCR still is not practical for the measurement of infection in the thousands of stool samples analyzed in this prospective study, so we usually use the less sensitive but highly specific antigen detection test, recognizing that in doing so we may underestimate the incidence of amebiasis.[41,56] In addition, immunohistochemical staining of amebae is useful in a difficult-to-diagnose case. Serologic tests for anti-amebic antibodies also are a very useful tool in diagnosis, with sensitivity of 70 to 80 percent early in disease and approaching 100 percent sensitivity on convalescence. The combined use of serology and stool antigen detection test offers the best diagnostic approach.

MICROSCOPIC EXAMINATION OF STOOL

Before the development of new antigen detection and PCR tests, amebiasis was diagnosed by examining a stool sample through a microscope to determine whether *E. histolytica* cysts were present. This method often requires more than one specimen, however, because the number of cysts in the stool varies greatly. In addition, stool microscopy has limited sensitivity and specificity. The body's own immune system produces macrophage cells that can look like the amebae. Three different amebae—*E. histolytica*, which causes amebiasis, and *E. dispar* and *E. moshkovskii*, which do not cause disease—look identical under a microscope.[22]

NONINVASIVE DIAGNOSIS OF EXTRAINTESTINAL AMEBIASIS

Amebiasis outside the intestine has been even more difficult to diagnose. Clinical manifestations of extraintestinal disease vary widely, and less than 10 percent of individuals with amebic liver abscesses have identifiable *E. histolytica* in their stools. The TechLab *E. histolytica* II test, which differentiates the true pathogen *E. histolytica* from *E. dispar*, was reported to detect Gal/GalNAc lectin in the sera of 22 of 23 (96%) patients with amebic liver abscess tested before treatment with the anti-amebic drug metronidazole and 0 of 70 (0%) controls. After 1 week of treatment with metronidazole, more than 80 percent of patients became serum lectin antigen–negative. Detection of *E. histolytica* Gal/GalNAc lectin in the sera using the TechLab *E. histolytica* II kit is sensitive to diagnose hepatic and intestinal amebiasis before the institution of metronidazole treatment.[38]

Noninvasive diagnostic procedures such as ultrasound, CT, and MRI can detect extracolonic amebiasis in the liver, paracecal masses, brain, and other sites, but they cannot distinguish between abscesses caused by amebae and those caused by bacteria, hampering proper treatment of the condition. Most patients with amebic liver abscess have a single abscess in the right lobe of the liver, although multiple lesions also can occur.[4] Chest radiographs show elevation of the right diaphragm in 56 percent of patients with hepatic abscess.[3] The diagnosis of cerebral amebiasis requires careful neurologic evaluation and radiographic evaluation with either CT or MRI.[16,46,58] Because of the risk for perforation, barium studies are relatively contraindicated in patients with amebic colitis.

BIOPSY STUDIES

The colonic and rectal mucosa in amebic colitis usually reveals ulcerations with a diameter of 1 to 10 mm. Amebic trophozoites often are at the periphery of these necrotic areas, which can be sampled through a biopsy specimen taken during sigmoidoscopy or colonoscopy.[42,49] Because of the potential for perforation, colonoscopy should be undertaken with caution.

In patients with amebic liver abscesses, amebic trophozoites are found near the capsule of the abscess. Until more recently, the most accurate diagnostic test involved the examination of a sample collected from the abscess tissue by needle aspiration, a procedure that is painful, potentially dangerous, and relatively insensitive, identifying amebic trophozoites only 20 percent of the time.

DIFFERENTIAL DIAGNOSIS

Invasive amebic colitis may resemble ulcerative colitis, Crohn disease of the colon (inflammatory bowel disease), bacillary dysentery, or tuberculous colitis.[11,18,42,89] Stool examinations, colonoscopic examination with biopsies, and serologic examination should be able to differentiate amebic colitis from these diseases. Histologic examination of involved colonic mucosa should differentiate amebic colitis, with its lack of inflammation and rare granulation tissue, from the inflammatory responses seen in ulcerative colitis, bacillary dysentery, and Crohn disease of the colon. Tuberculous colitis and Crohn disease are more likely to show granuloma formation than amebiasis. Ileocecal or small bowel involvement as seen on barium studies would suggest Crohn disease or tuberculosis of the gastrointestinal tract, rather than amebiasis. Tuberculous colitis usually is associated with pulmonary tuberculosis and with a strong reaction to tuberculin skin testing. In some cases, differentiating between invasive amebic colitis and inflammatory bowel disease may be impossible. If a patient with this differential diagnosis is placed on corticosteroids and deteriorates, the corticosteroids should be stopped, and repeat investigation for amebiasis should be performed.[18,68,72]

Amebic liver abscess must be differentiated from pyogenic abscesses and neoplastic lesions. Detection of *E. histolytica* Gal/GalNAc lectin in the sera using the TechLab *E. histolytica* II kit is quite helpful to diagnose hepatic and intestinal amebiasis before the institution of metronidazole treatment.[38] Total leukocyte counts and cultures of blood may help to differentiate pyogenic and amebic abscesses. Many children with pyogenic liver abscesses have negative blood cultures, however. Often, amebic and pyogenic liver abscesses show similar features on CT and MRI. Occasionally, nuclear imaging with gallium is helpful because, in contrast to a pyogenic abscess, very few neutrophils are contained within an amebic liver abscess.[85,87] Gallium scanning of an amebic liver abscess may reveal a cold spot, possibly with a bright rim. Several investigators recommend a trial using an appropriate drug for amebic abscess for 3 or 4 days while serologic and culture results are awaited.[68,98] Patients with amebic liver abscess should respond to treatment in this length of time by becoming afebrile. No change in size of the liver or size of the abscess should be noted at this time because resolution of the abscess usually takes 2 months to several years.[4,79,80,90,97]

COMPLICATIONS

Complications of amebiasis may be prevented by early establishment of diagnosis and initiation of treatment with appropriate agents.[46,68] When complications occur, the prognosis generally is poor.

Invasive intestinal amebiasis has been associated most commonly with perforation and peritonitis,[8,10,48,68,91,96] which apparently are an end result of "necrotizing" or "toxic" amebic colitis. In children, perforation may be heralded by the appearance of an acute abdomen or pneumoperitoneum, with rapid progression to death, presumably from sepsis.[8,68,96] Surgical resection and therapy for endotoxic shock improve the prognosis.[96] This complication is not rare and accounts for more than 30 percent of deaths from amebiasis in children.[11,50] Massive intestinal hemorrhage causes approximately 3 percent of deaths from amebiasis. Intussusception occasionally occurs and can be reduced with gentle barium enema. Multiple colonic strictures also can occur and cause obstructive symptoms. Fistulas to other organs or to the skin may develop.

Liver abscesses and their resultant complications account for approximately 40 percent of deaths from amebiasis.[50] Liver abscess also was found in 13 percent of patients with amebiasis who had postmortem examinations. Liver abscess with rupture into the abdomen was present in 8 percent of patients who died with amebiasis, and rupture of a liver abscess into the right pleural space was found in 12 percent.[50] Many patients with amebic liver abscess also have anaerobic bacteria in the abscess fluid.[83] In cases free of bacterial contamination, the fluid has few

inflammatory cells and an acidic pH. Amebic pericarditis or pneumopericardium occurs rarely and is found in only 1 percent of patients whose deaths were caused by amebiasis.[27,28,32,50] The fluid is similar to that found in the pleural space. A cerebral abscess was found in 4 percent of patients with amebiasis who died.[50] It has been reported in fewer than 10 children, only one of whom survived.[9,16,46,58] Other complications include infections of the retroperitoneal space, stomach, spleen, esophagus, and duodenum.[58]

TREATMENT

INTESTINAL AMEBIASIS

Asymptomatic Intraluminal Amebiasis

Therapy for asymptomatic and noninvasive infection differs from therapy for invasive infection. Asymptomatic infections may be treated with intraluminal agents, such as paromomycin or diloxanide furoate. Each agent has a high rate of success for eradication of cyst passage.[65,66] Paromomycin is a nonabsorbable aminoglycoside that is active against the cyst and trophozoite stages. High cure rates have been reported with a 7-day oral dose of paromomycin at 25 to 35 mg/kg/day in three divided doses (Table 221–2). Diloxanide furoate (Furamide) is a poorly absorbed agent that is quite active against only intraluminal amebiasis, but treats symptomatic and asymptomatic disease.[62,98] Cure rates have been greater than 90 percent with a 10-day oral course of diloxanide furoate at 20 mg/kg/day in three divided doses (maximum dose of 1500 mg/day).[65,66,73]

Acute Amebic Colitis

Nitroimidazoles, particularly metronidazole, are the mainstay of therapy for invasive amebiasis.[37] The oral dosage of metronidazole is 35 to 50 mg/kg/day (to a maximum of 2250 mg/day) in three divided doses for 7 to 10 days for severe intestinal or extraintestinal amebiasis. Metronidazole is concentrated in the ameba, probably via reduction of its nitro group by ferredoxin or flavodoxin-like electron transport proteins, which maintain a gradient for the entry of the unchanged drug. Metabolic intermediates of metronidazole damage DNA and possibly other macromolecules, and they deprive the organism of reducing equivalents by acting as an electron sink. Nitroimidazoles with longer half-lives (tinidazole, secnidazole, and ornidazole) are better tolerated and allow shorter periods of treatment.[38] The oral dosage of tinidazole is 60 mg/kg/day (to a maximum of 2000 mg/day) for 5 days for severe intestinal or extraintestinal amebiasis (see Table 221–2) (see http://www.medletter.com/).

Approximately 90 percent of patients who present with mild-to-moderate amebic dysentery have a response to nitroimidazole therapy. In the rare case of fulminant amebic colitis, adding broad-spectrum antibiotics to treat intestinal bacteria that may spill into the peritoneum is prudent; surgical intervention occasionally is required for acute abdomen, gastrointestinal bleeding, or toxic megacolon.[37] Agents such as metronidazole that are active against invasive and extraintestinal amebiasis are well absorbed and do not stay in the lumen long enough to have an effect on intestinal amebiasis. Parasites persist in the intestine in 40 to 60 percent of patients who receive nitroimidazole. Nitroimidazole treatment should be followed with paromomycin or the second-line agent diloxanide furoate to cure luminal infection.[38] Metronidazole and paromomycin should not be given at the same time because the diarrhea that is a common side effect of paromomycin may render assessing the patient's response to therapy difficult.[50-52]

EXTRAINTESTINAL AMEBIASIS

Amebic Liver Abscess and Metastatic Amebiasis

Extraintestinal and severe intestinal amebiasis must be treated with the tissue-active agents. Metronidazole (35 to 50 mg/kg/day in three divided doses for 7 to 10 days) is the preferred drug because it is effective and relatively free of serious side effects (see Table 221–2).[2,3,62,85,87,98] It is effective for extraintestinal amebiasis in any location, although amebic brain abscesses usually are not treated successfully with any medications. Most patients with amebic liver abscess respond to metronidazole within 72 hours. For amebic colitis, follow-up therapy with a luminal agent is very important because of the high rates of asymptomatic intestinal colonization in patients with amebic liver abscess.

Therapeutic aspiration of an amebic liver abscess occasionally is required as an adjunct to antiparasitic therapy. Drainage of the abscess should be considered in patients who have no clinical response to drug therapy within 5 to 7 days or patients with a high risk of experiencing rupture of the abscess, as defined by a cavity with a diameter of more than 5 cm or by the presence of lesions in the left lobe.[95] Because many patients with amebic liver abscess also have anaerobic bacteria in the abscess fluid,[83] addition of antibiotics, drainage, or both to the treatment regimen in the absence of a prompt response to nitroimidazole therapy is reasonable. Imaging-guided percutaneous treatment (needle aspiration or catheter drainage) has replaced surgical intervention as the procedure of choice for reducing the size of an abscess.[95]

PROGNOSIS

Invasive disease develops in 50 million people each year, and 50,000 to 100,000 deaths per year are caused by the invasive disease.[78,84,85] The case-fatality ratio is between 1 in 500 and 1 in 1000 diagnosed cases. Among patients with illness severe enough to require hospitalization, the case-fatality ratio is higher. One small study in children reported a 9 percent mortality rate and a 27 percent morbidity rate.[68]

Bowel necrosis or perforation is the cause of death from purely intestinal amebiasis, and early surgical intervention can reduce the mortality rate of these complications from 100 to 28 percent.[96] Amebic liver abscess has a case-fatality rate of 10 to 15 percent in combined figures of adults and children.[54,70,81] The mortality rate when pleural involvement is noted is 14 percent.[47,54] Amebic pericarditis has a case-fatality rate of 40 percent.[32] Cerebral amebiasis has a case-fatality rate of 96 percent.

TABLE 221–2 Pediatric Dosage of Drugs for Amebiasis

Symptomatic	Paromomycin	25-35 mg/kg/day in 3 doses × 7 days
Mild-to-moderate intestinal disease	Metronidazole	35-50 mg/kg/day in 3 doses × 7-10 days
	Trinidazole	50 mg/kg (maximum 2000 mg) qd × 3 days
Severe intestinal and extraintestinal disease	Metronidazole	35-50 mg/kg/day in 3 doses × 7-10 days
	Trinidazole	60 mg/kg/day (maximum 2000 mg) × 5 days

FUTURE

In a perfect world, amebiasis would be prevented by eradicating fecal contamination of food and water. Providing safe food and water for all children in developing countries would require massive societal changes and monetary investments. An effective vaccine would be much less costly, and for several reasons, a vaccine is a desirable and feasible goal. The high incidence of amebiasis in more recent community-based studies suggests that an effective vaccine would improve child health in developing countries.

The fact that humans naturally acquire partial immunity against intestinal infection indicates that barriers to stimulating an effective acquired immune response should not be insurmountable. Aiding vaccine design is the demonstration that several recombinant antigens, including the Gal/GalNAc-specific lectin, provide protection in animal models of amebiasis, and that human immunity is linked to intestinal IgA against the lectin.[35,37,43] The high degree of sequence conservation of the Gal/GalNAc-specific lectin suggests that a vaccine could be broadly protective. Finally, the absence of epidemiologically significant animal reservoirs suggests that herd immunity could interrupt fecal-oral transmission in humans. The challenges will be to design vaccines capable of eliciting durable mucosal immunity, to understand the correlates of acquired immunity, and, most important, to enlist the continued support of industrialized nations to combat diarrheal diseases of children in developing countries.

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CHAPTER 222

BLASTOCYSTIS HOMINIS INFECTION

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Blastocystis hominis is one of the most common gastrointestinal protozoa of humans, with a worldwide prevalence that may be greater than 50 percent.[26,27] Other *Blastocystis* spp. have been described from numerous other vertebrates and insects.[27] Although new information about the molecular and cell biology of this organism has been acquired in recent years, considerable controversy regarding its true taxonomy and life cycle remains.[4,26,27,29,30] The pathogenicity of *B. hominis* and its ability to cause gastrointestinal illness in humans are equally controversial.[10,11,13,16,24]

ETIOLOGY AND PATHOGENESIS

Since its discovery in the early part of the 20th century by Alexeieff[1] and then Brumpt,[6] *B. hominis* has been assigned to many different phyla in animal and plant kingdoms. Early on, it was identified by various workers as vegetable material, a yeast, a fungus, or a protozoan. Although the organism was identified as a protozoan parasite in 1967, its subphylum status bounced between the Sporozoa and the Sarcodina.[4] Nucleic acid sequencing data now suggest that *B. hominis* does not belong to either category,[13,14] but instead probably constitutes its own group (class Blastocystea) more closely related to the Straemenopiles, a heterogeneous group of unicellular and multicellular protists that includes slime nets, water molds, and brown algae.[27] Additional molecular taxonomic data suggest that the intraspecific variation among stocks of *B. hominis* is sufficiently different to warrant multiple separate species assignments for the organism.[5,27] This observation ultimately may have a bearing on the current controversies surrounding the pathogenicity of the organism and different modes of transmission (i.e., zoonotic and human-to-human).[27]

Ultrastructural data gathered from light and electron microscopy on organisms obtained from in vitro culture and from fresh fecal material indicate the existence of several different parasite forms, including cyst forms; ameboid forms; and the so-called granular, avacuolar, and vacuolar forms.[4] The *B. hominis* vacuolar cell is the most distinctive and appears as a thin peripheral band of cytoplasm surrounding a large membrane-enclosed central vacuole.[4] The central vacuole may have a storage function. Although *B. hominis* is thought to have predominantly anaerobic metabolism, structures that look like mitochondria have been identified on transmission electron microscopy. *Blastocystis* mitochondria may function only in lipid biosynthesis and not oxidative phosphorylation.[4,29] Alternatively, what appears to be a *Blastocystis* mitochondrion on electron microscopy actually may be a hydrogenosome.[4]

Several highly speculative life cycles of *B. hominis* have been proposed.[4,14] The infective stage probably is a dormant cyst form that undergoes excystation in response to host gastric acid and intestinal enzymes. Excystation may result in the release of avacuolar forms that can undergo facultative transformation to either an ameboid or a multivacuolar form. The multivacuolar form either may encyst to an infective stage or may coalesce to the vacuolar form.[4] These stages are thought to predominate in the large intestine, although organisms also have been recovered from duodenal aspirates. Which, if any, of these life cycle stages invades tissue or causes disease is unknown. Acquisition of knowledge in this area has been hampered by lack of a suitable animal model, although infection of gnotobiotic guinea pigs with this organism was reported to result in mild intestinal hyperemia and superficial invasion of *B. hominis* into the mucosa of the cecum. Nonspecific inflammation (infiltration of lymphocytes and plasmocytes) and edema of the colonic mucosa have been seen during sigmoidoscopy with biopsy in some patients.[4] So far, no serologic antibody response to the organism has been shown[7] except in a subset of patients with irritable bowel syndrome who reportedly show elevated IgG2 antibody.[12] The possibility remains that *B. hominis* is entirely commensal in humans.

EPIDEMIOLOGY AND CLINICAL MANIFESTATIONS

B. hominis has a worldwide distribution in tropical and temperate regions, with infection rates of 54 percent occurring among some populations.[2-4,8,9,20,21,24,28] High rates of infection also have been reported in individuals with a recent history of travel,[23] with exposure to pets or farm animals,[8] and living in institutionalized settings.[4] In many of these individuals, however, *B. hominis* probably is a commensal parasite. Although *B. hominis* is found commonly in preschool-age and school-age children,[20-22] children overall do not seem to be at increased risk for acquisition of infection.[4]

Most studies investigating the association between *B. hominis* infection and disease are based primarily on clinical laboratory isolates of the organism in patients exhibiting gastrointestinal symptoms. Common clinical complaints include abdominal discomfort, bloating, cramping, diarrhea, and vomiting.[2] As noted earlier, IgG2 seroconversion to *B. hominis* has been linked to irritable bowel syndrome.[12] Weight loss associated with a protein-losing enteropathy also has been described. Infective arthritis also has been reported.[16] Because *B. hominis* commonly is found in symptomatic and asymptomatic individuals, some investigators have proposed that only very heavy infections result in disease.[17,25] Many of these studies were not controlled, however.

Shlim and colleagues[25] conducted a large prospective controlled study among a population of expatriates and tourists in Katmandu, Nepal, who were at high risk for developing traveler's diarrhea. They concluded that *B. hominis* in high concentrations was not associated with diarrhea, and the presence of higher concentrations of the organism in stool was not associated with more severe symptoms.[25] In a subsequent editorial, several design features were cited that may "weaken the authors' conclusions."[15] At least four other prospective trials have been conducted, with one supporting *B. hominis* as a cause of diarrhea, one arguing against it, and two others that were inconclusive.[23] The confusion may be resolved partly by the identification of separate human "demes" of *Blastocystis*,[5,14] which might lead to improved molecular diagnostic techniques for distinguishing pathogenic from nonpathogenic species. This situation is analogous to the morphologically identical species of *Entamoeba* (*Entamoeba histolytica* and *Entamoeba dispar*), only the former of which causes colitis.

DIAGNOSIS

Light microscopy of wet preparations of fresh or concentrated stool usually identify *B. hominis*. Staining of preparations with iodine or trichrome also is beneficial. Many laboratories attempt to identify the characteristic vacuolar forms, which may be underrepresented in clinical material.[4] Under these circumstances, the services of an experienced technologist are required to identify the less distinctive fecal cyst form.[27] Organisms also can be recovered from biopsy material obtained during sigmoidoscopy and colonoscopy.

TREATMENT

Given the controversy surrounding the pathogenicity of *B. hominis*, a prudent approach is to refrain from treating asymptomatic immunocompetent individuals.[18] In individuals who have gastrointestinal illness and in whom other pathogens have been excluded, administering a course of antiprotozoal chemotherapy may be reasonable. Some investigators have reported symptomatic improvement in patients receiving either metronidazole or tinidazole.[4,9] Using an in vitro assay that employed metabolic labeling, researchers found that the drugs emetine, satranidazole, furazolidone, and quinacrine were superior in activity to either metronidazole or tinidazole.[4] The authors caution, however, that the in vitro assay does not take into account the pharmacokinetic properties of the drugs. Furazolidone is available in suspension and may be suitable for pediatric use.[11] In two prospective, randomized, double-blind, placebo-controlled studies, children and adults who received 500 mg of nitazoxanide were shown to respond significantly better to treatment parasitologically and

clinically compared with placebo controls.[23] Trimethoprim-sulfamethoxazole also has been reported as an alternative regimen.[19]

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CHAPTER 223

ENTAMOEBA COLI INFECTION

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Until more recently, *Entamoeba coli* was considered to be entirely nonpathogenic and was of interest to the clinician only because of its morphologic similarities to *Entamoeba histolytica* that might result in misdiagnosis. In 1991, several case reports from northern Europe appeared, however, that implicated *Entamoeba coli* as a possible cause of infectious diarrhea.[2,5,7] Two cases of diarrhea associated with *Entamoeba coli* have been described in children.[2]

ETIOLOGY AND PATHOGENESIS

Entamoeba coli, similar to other members of the genus *Entamoeba*, has trophozoite and cyst forms. The trophozoite is similar in size to *E. histolytica* (15 to 50 μm), but has a more sluggish motility with short pseudopodia.[4] The cytoplasm is described as granular, coarse, or frothy, and contains numerous bacteria, yeasts, and other food materials.[4,6] Occasionally, red blood cells are seen in the cytoplasm, but their occurrence is not nearly as common as in pathogenic strains of *E. histolytica*. During passage through the colon, the trophozoite rounds up and synthesizes a chitin-containing cyst wall. *Entamoeba coli* cysts measure 10 to 35 μm and usually contain 8 to 16 nuclei, although occasionally 32 nuclei are seen.

Transmission of *Entamoeba coli* infection occurs through the fecal-oral route in the same manner as *E. histolytica* infection. On cyst ingestion, the total number of excysting trophozoites usually is fewer than eight.[4] The *Entamoeba coli* trophozoites colonize the lumen of the large intestine. Very little is known about the events by which *Entamoeba coli* trophozoites occasionally cause human gastrointestinal illness. Even with a pathogenic *Entamoeba coli* strain, the invasive potential of this organism is presumed not to be nearly as great as that of *E. histolytica* because diarrheal disease in patients infected with *Entamoeba coli* is not associated with dysentery or accompanied by a leukocytosis or elevated serum IgA.[7]

EPIDEMIOLOGY AND CLINICAL MANIFESTATIONS

Entamoeba coli is worldwide in distribution, although it occurs more commonly in warmer climates and in some populations of homosexual men.[4] In 1991, Wahlgren[7] described eight patients from Sweden with mild or persistent diarrhea who harbored *Entamoeba coli*. Before receiving specific anti-amebic chemotherapy, all eight patients had their stools examined repeatedly by (1)

light microscopy, to exclude other protozoa and helminths; (2) electron microscopy, to exclude the presence of some pathogenic viruses; and (3) aerobic and anaerobic culture, to exclude pathogenic bacteria. These patients typically complained of a long history of loose but not watery stools (without blood or mucus), flatulence, and colicky pain. One patient was a parasitology laboratory technician who had symptoms for more than 15 years. Every patient responded to specific anti-amebic chemotherapy.[7] Two children with similar symptoms who also responded to anti-amebic chemotherapy subsequently were described in Ireland.[2]

DIAGNOSIS AND TREATMENT

Entamoeba coli sometimes is difficult to distinguish from *E. histolytica*, particularly because the nuclear structures of their trophozoite stages are similar.[3] Some differences exist, however, including the karyosome, which is eccentric in *Entamoeba coli*, but central in *E. histolytica*, and the cytoplasm, which is coarse and seldom contains red blood cells in *Entamoeba coli*, in contrast to *E. histolytica*.[3] The differences between the cyst stages of *Entamoeba coli* and *E. histolytica* (and *Entamoeba dispar*) are more apparent. The *Entamoeba coli* cyst typically has two to four times more nuclei than cysts in *E. histolytica*. The *Entamoeba coli* cyst has been reported to become more refractive during fixation so that it often is visualized better in a wet preparation.[4]

Generally, *Entamoeba coli* still is regarded by most investigators as a commensal organism. In patients with persistent diarrhea whose diagnostic fecal evaluation reveals only the presence of *Entamoeba coli*, administering a course of specific anti-amebic therapy is reasonable.[1] All of the Swedish patients were reported to respond to a 10-day course of diloxanide furoate in a dose of 500 mg three times daily.[7] Children also may respond to an equivalent pediatric dose of 20 mg/kg/day in three divided doses for 10 days. As of 1994, diloxanide furoate was available in the United States from the Centers for Disease Control and Prevention Drug Service. Alternatively, two children from Ireland (where diloxanide furoate was unavailable) were treated successfully for *Entamoeba coli*–associated diarrhea with metronidazole.[2]

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B. Flagellates (Intestinal)

CHAPTER 224

GIARDIASIS

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Giardiasis is caused by an infection with the flagellated, binucleated protozoan parasite *Giardia lamblia* (also known as *Giardia duodenalis* or *Giardia intestinalis*). Worldwide, *G. lamblia* is one of the most prevalent and important infectious causes of diarrheal illness in humans. Van Leeuwenhoek first described the parasite when examining his own stool under a microscope in 1681. Lambl described the trophozoite in 1859, and Grassi described the cyst in 1879.[64] This flagellated parasite, which belongs to the family Hexamitidae, is one of the most ancient eukaryotes and lacks many organelles, including Golgi apparatus and mitochondria.[30] Mitochondrial genes have been discovered in the organism more recently, however, and the organism has been shown to possess specialized membrane structures that perform the respiratory chain activities of the inner mitochondrial membrane. This finding lends support to the theory that *Giardia* organisms have become highly evolved and have lost many of their ancestral characteristics.[1,4,59,60] For centuries, *Giardia* were thought to be nonpathogenic organisms; however, during the past 30 years, data have shown that *G. lamblia* can be a pathogen that causes sporadic and epidemic disease.[40]

ETIOLOGIC AGENTS

G. lamblia has two morphologic forms: the infectious cyst, which is resistant to chemicals and environmental stresses, and the trophozoite, which is responsible for the disease. The trophozoite is pear-shaped, with a dorsal convexity and a spiral organelle on its ventral surface. The spiral organelle or sucking disk is the means by which the organism attaches to mucosal surfaces. The trophozoite usually measures 10 to 21 μm in length and 5 to 15 μm in width. It has four pairs of flagella and two symmetric nuclei. Trophozoites live in the small intestine and divide by binary fission. The cyst form usually is seen in stool samples and is oval-shaped, measuring 8 to 12 μm in length and 5 to 10 μm in width. It contains two to four nuclei and remnants of organelles. Cysts can remain viable for long periods (≥3 months) in cold water and are resistant to killing by iodine and chlorine; however, they can be destroyed by heating to 50° C and by desiccation.[2]

Six species of *Giardia* have been identified based on the morphologic characteristics of the trophozoite: *G. duodenalis*, *G. agilis*, *G. muris*, *G. ardeae*, *G. psittaci*, and *G. microti*. *G. lamblia*, also called *G. duodenalis*, is the primary pathogenic species in humans and other mammals. All the other species infect primarily animal species and cause no significant disease in humans. The species that infects humans is morphologically indistinguishable from that of other mammals.[2,40] Isoenzyme and genetic analyses indicate that isolates from infected individuals exhibit high levels of genetic diversity and comprise a large number of genotypes that can be placed into four groups.[13] The different isolates within groups show some specificity for infection in various mammals

versus humans, but no consistent genetic differences have been identified between isolates that cause symptomatic versus isolates that cause asymptomatic disease. The genotypes are grouped into two major genetic assemblages: assemblage A and assemblage B. More recent studies have shown that in symptomatic infection a strong correlation exists between isolate assemblage type and the severity of infection; however, the infectivity and pathogenicity of *Giardia* genotypes seem to vary depending on the age of the host.[2,44,69,77]

EPIDEMIOLOGY

Giardia infections are ubiquitous, and outbreaks occur in developed and underdeveloped countries throughout the world. Cross-transmission and infection occur between domestic and wild animals and humans and between humans.[20] Transmission involves the fecal-oral, water-borne, and food-borne transmission of cysts; the level of sanitation and a high intradomiciliary/peridomiciliary concentration of domestic animals are related directly to the prevalence of infection.[6,24,46,51,61,84,85]

G. lamblia is one of the most common parasites in the United States, affecting individuals of all ages, and is found in 4.2 to 7 percent of specimens submitted to the laboratory for examination.[50] The populations that are affected most frequently include children 0 to 5 years old, adults 31 to 40 years old, backpackers, campers, hunters, and travelers to areas where the disease is endemic.[45] Data from the National Giardiasis Surveillance System of the Centers for Disease Control and Prevention estimate that 2.5 million cases occur each year in the United States.[103] Cases per 100,000 population range from 1.4 to 30, with seven states reporting more than 15 cases per 100,000 population. In 2005, Vermont had the highest incidence, with 30 cases per 100,000 population. Cases are more prevalent in males, and rates are highest in children 1 to 4 years old (children in daycare centers and their close contacts), followed closely by children 5 to 9 years old and adults 31 to 40 years old. Most cases are reported in the early summer through early fall.[103]

Giardia is a frequent cause of diarrhea in daycare centers around the world, with infection rates of 1 to 55 percent.[3,8,53,70,73,75] Most children are symptomatic, and chronic passage of cysts in some preschool-age children in daycare centers may persist for 5 to 6 months after the initial diagnosis is established. Transmission from these infants and children to adult family members is common; 25 percent of the children have *Giardia*-positive stools. The prevalence rates are highest in centers with many non–toilet-trained children and staff members who change diapers and prepare food without adequate handwashing. Prevalence rates decline after children are toilet trained.[8]

Sexual transmission may occur with heterosexual and homosexual contact; however, male homosexual behavior is an established risk factor for acquiring *G. lamblia* infection, with cyst passage rates reaching 20 percent.[43,52,57] Other individuals at risk include campers, backpackers, hunters, and other travelers to national parks, wilderness areas, and disease-endemic areas because of vertical transmission from animals and ingestion of untreated drinking water.[99] Patients who were infected during travel had longer exposure times in countries where the prevalence was high.[48] In addition, many outbreaks have been reported in municipal water supplies that have not been treated with flocculation or filtration.

From 1985 to 1994, *Giardia* was responsible for 44 percent of the outbreaks of water-borne diarrheal illness in which an etiology could be determined. Sixty-four percent of these outbreaks were associated with unfiltered water. According to a sample survey of 66 reservoir sites in Canada and the United States, 81 percent contained *Giardia* cysts at a concentration of 3 cysts per liter before treatment and 1.7 cysts per liter after treatment.[54,55] Treatment of water with iodine preparations kills most cysts, but they are resistant to chlorination. Swimming pools may be another source of infection when used by diapered infants and developmentally delayed individuals.[76] A less common mode of transmission is through ingestion of contaminated food, in which only 10 cysts may be required to establish infection.[66,79]

PATHOGENESIS

Humans are infected by the oral ingestion of 10 to 25 cysts.[79] Excystation occurs in the stomach and small intestine, and the trophozoites are found in large numbers in the upper part of the small intestine, where they closely apply their sucking disks to the mucosa (Fig. 224–1). They may penetrate into the secretory tubules of the mucosa and sometimes are found in the gallbladder

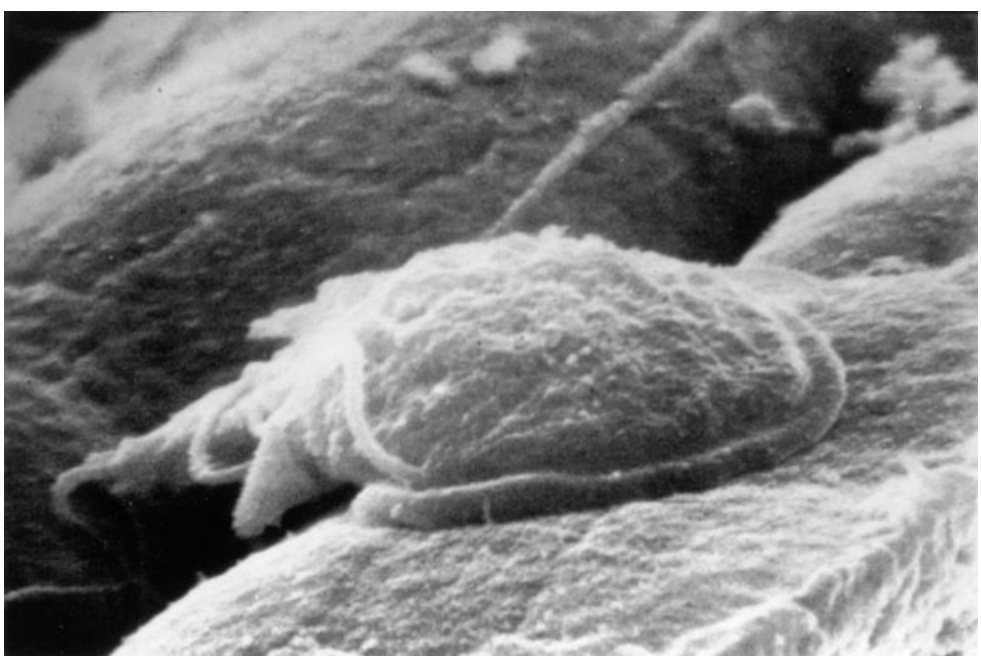

Figure 224–1 Trophozoite of *Giardia lamblia* adhering to the intestinal surface.

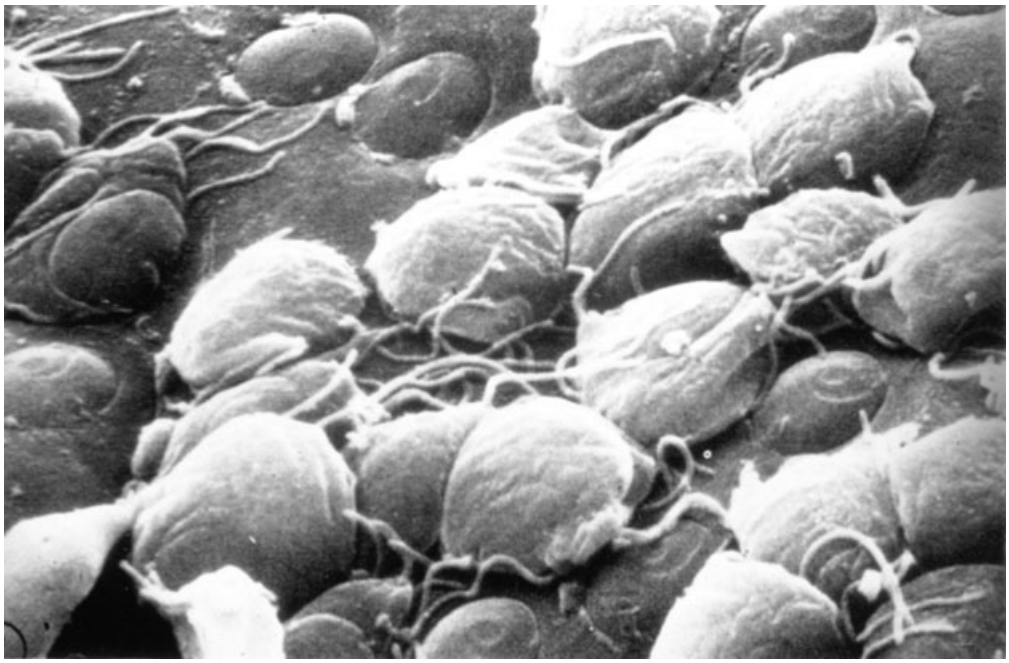

Figure 224–2 Scanning electron micrograph of trophozoites of *Giardia lamblia* from an intestinal biopsy specimen. Note the indentations left on the mucosal surface by the parasite's sucking disk.

and in the biliary drainage. Scanning electron micrographs of the intestinal mucosa (Fig. 224–2) show the mechanical damage caused by the presence of the organisms on the mucosal surface.

The histologic changes seen in the tissues do not always correlate with the presence or absence of symptoms. Biopsy specimens of the small intestine in children and adults show normal histology despite the presence of diarrhea and other symptoms.[82] Flattening of the brush border, damage to mucosal epithelial cells, and slight flattening of the villi with increased mitotic index, increased goblet cells, alteration of bile content or duodenal flora, and infiltration with inflammatory cells also may be present.[19,71,80,102] Studies have shown that *Giardia* disrupts tight junctional zona occludens, increases permeability, and induces apoptosis in small intestinal epithelial cells. Disruption of the intestinal epithelial cell brush border may explain the lactose intolerance that commonly develops.[11,15] In vivo and in vitro studies have established that *Giardia* causes malabsorption of glucose, sodium, and water, and reduces disaccharidase activity owing to loss of epithelial absorptive surface area.[10] Disruption of digestion by bile and proteases may produce malabsorption, diarrhea, and resulting malnutrition.

The causes of symptoms are not well understood. Hypotheses include (1) the parasite as a physical barrier to absorption, (2) disruption of the brush border with loss of enzymatic activity, (3) elaboration of toxins by the parasite, (4) changes in fat absorption in the small intestine, and (5) activation of cytokines after damage to enterocytes.

Host immunity, including the humoral and the cellular components, is important in the clearance and protection against reinfection with *Giardia*. Persistence of infection in nude mice suggests a role for T-cell activity.[81,89] Humans produce serum IgG, IgM, IgA, and IgE in response to infection.[31,34,74,87,97] Studies have shown that IgM and IgG antibody with complement is lethal to *Giardia* trophozoites. In the intestinal lumen, the secretory IgA response seems to play a role in the modulation of infection, as does the migration of infiltrating lymphocytes in the intestinal mucosa. The absence of secretory IgA is associated with the inability to clear *Giardia* infection and is associated with the development of chronic giardiasis in humans.[14] In a study of human volunteers, 84 percent of volunteers self-cured in 18.4 days, whereas the remainder became chronically infected.[79] Human milk has been shown to kill the trophozoites of *G. lamblia* by generating toxic lipolytic products.[30,31,78]

Infection may be severe and difficult to eradicate in immunodeficient individuals with antibody deficiencies (e.g., common variable immunodeficiency, X-linked agammaglobulinemia) or in patients with reduced gastric acidity. Giardiasis long has been associated with hypogammaglobulinemia, nodular lymphoid hyperplasia of the small intestine, and chronic diarrhea.[41] Patients with hypogammaglobulinemia, absence of plasma cells in the intestinal lamina propria, cystic fibrosis, protein-calorie malnutrition, and human immunodeficiency virus (or acquired immunodeficiency syndrome), and patients who are receiving immunosuppressive therapy are more likely to develop chronic giardiasis than individuals with X-linked hypogammaglobulinemia, selective IgA deficiency, and Wiskott-Aldrich or Nezelof syndrome.[47,72,86]

CLINICAL MANIFESTATIONS

Infection with *Giardia* organisms can result in asymptomatic infection (5 to 15%), an acute self-limited diarrheal illness (25 to 50%), or chronic diarrhea and malabsorption depending on host susceptibility and pathogen genotype virulence.[38,45] The incubation period for giardiasis is 7 to 14 days. Symptoms vary from mild abdominal discomfort and diarrhea to severe cramping; bloating; and severe, explosive, watery, greasy, foul-smelling diarrhea (Table 224–1).[43] Patients and caregivers describe a constellation of signs and symptoms, including abdominal bloating, flatulence, and frequent foul-smelling diarrhea. Young infants may exhibit anorexia, weight loss, or a malabsorption syndrome that resembles sprue. Symptoms usually last more than 7 to 10 days and in most cases clear spontaneously. Patterns of stools vary from normal to mushy, foul-smelling diarrhea (Table 224–2). Gross blood, pus, and mucus usually are not present in the stool, and signs of inflammatory diarrhea, such as tenesmus and bloody diarrhea, do not occur. A few patients develop persistent, chronic infection that is associated with severe malaise, headache, weight

TABLE 224–1 Symptoms in Patients with Giardiasis

Symptom	Frequency (%)
Diarrhea	89
Malaise	84
Nausea	58-68
Foul-smelling stool	57-72
Flatulence	56-74
Abdominal cramps	55-80
Bloating	55-69
Weight loss	48-64
Anorexia	40-64
Abdominal distention	31
Belching	30
Fever	17-28

TABLE 224–2 Stool Characteristics in Patients with Giardiasis

Stool Characteristic	Frequency (%)
Mushy	52
Formed	33
Watery	12
Mucus	3
Bloody	0

loss, and diffuse abdominal and epigastric discomfort that is exacerbated by eating.

Episodes of diarrhea typically alternate with periods of normal bowel movements and may persist for months. Lactose intolerance may develop in 20 to 40 percent of cases and persist for weeks after the infection has been eradicated.[86,100] Children with symptomatic giardiasis may develop steatorrhea and malabsorption of vitamin A, vitamin B_{12}, protein, D-xylose, and iron.[33,88] Anecdotal reports have attributed various other symptoms, including rash, urticaria, arthralgia, reactive arthritis, constipation, biliary tract disease, and gastric infection, to giardiasis, but no firm evidence supports *G. lamblia* as the cause of these symptoms.

Individuals passing cysts may be asymptomatic and may serve as a reservoir of infection for others. This fact is particularly important for outbreaks among food handlers and in daycare centers. Severe prolonged illness that requires hospitalization is found in 2 per 100,000 cases, which is similar to that for shigellosis.[56] Young children and pregnant women usually are admitted to the hospital because of volume depletion and failure to thrive.[88]

Parasitic infection, including giardiasis, should be considered in the evaluation of a child who fails to thrive and in immunocompromised infants and children who have diarrhea or gastrointestinal complaints. It also should be considered in the differential diagnosis of any child who is in a daycare center or who has traveled outside of the United States and has gastrointestinal symptoms.[39,70]

DIAGNOSIS

Obtaining a thorough travel and potential exposure history is important in establishing the diagnosis of giardiasis. Recent travel to the wilderness or a national park and travel to developing areas or other endemic areas of the world where fecal-oral hygiene is a problem are important parts of the history in any patient with persistent diarrhea. Demonstration of trophozoites or cysts in the stool on ova and parasite examination is the traditional standard for establishing the diagnosis.[65] Examination of preserved fecal specimens reveals cysts or trophozoites in most infections. Organisms are excreted in a highly variable pattern; multiple samples taken on different days are required for detection.[18] A single stool sample misses 20 to 40 percent of the infections, whereas three stool samples miss less than 10 percent of infections.[42]

Commercially available tests to detect antigen by enzyme-linked immunosorbent assay (ELISA) have been shown to be rapid and highly sensitive and specific, but are qualitative and are unable to distinguish between genotypes or detect low levels of infection.[49,98] ELISA may be particularly useful in screening mass outbreaks and assessing cure. Monoclonal antibodies usually are employed to detect antigen either by ELISA or by immunofluorescence assay. Some of these assays have a sensitivity of 91 to 95 percent and specificities of greater than 98 percent.[22,62,98] Antigen may be detected best when the stool is preserved in formalin.[22] These tests do not replace microscopic examination of the stool, which is considered to be the standard, because of the possibility of infection with multiple organisms in travelers.

Molecular techniques such as polymerase chain reaction (PCR) are alternative methods that may be used for the specific detection of *Giardia* organisms in the stool. PCR in combination with restriction fragment length polymorphism or nested PCR may be used to genotype the organisms. The sensitivity of detection by PCR is greater than that of microscopy and is much better for detection of low numbers of parasites in stool samples.[7] The use of real-time PCR is a more recent advancement in PCR-based technology for the detection of *G. lamblia* in stool. This method, using dual-labeled fluorescent probes targeting the β-giardin gene, is sensitive and rapid and may be used to detect *G. lamblia* in stool and to differentiate the major genotypes of the organism. It can be adapted to high-throughput detection to screen large numbers of samples, especially in outbreak situations.[12,36]

Even with the available diagnostic tests, infections may be missed in some patients, and in cases with a high index of suspicion, the organism may be recovered by duodenal biopsy or duodenal aspiration. Invasive techniques may be an important diagnostic adjunct in immunosuppressed individuals and in patients with sprue, for whom histology of the bowel also may be important in planning therapy.

TREATMENT

All patients with either cysts or trophozoites in the stool should be treated. Multiple classes of drugs are available for the treatment of giardiasis, each with different clinical properties and efficacies (Table 224–3).[5,27,101] The nitroimidazole class of agents includes metronidazole, tinidazole, ornidazole, and secnidazole and is the mainstay of treatment for giardiasis. These drugs are administered orally in their inactive probiotic form.[27]

Of the nitroimidazoles, metronidazole has been the one most extensively studied and widely used worldwide. The mechanism of killing of *Giardia* organisms by the nitroimidazoles involves the use of the anaerobic metabolic pathways of the organism. The drug diffuses into the trophozoite, where it is activated and inhibits trophozoite respiration and causes cellular damage by the production of toxic radicals and loss of DNA helical structure resulting in trophozoite death.[32,94]

Of the nitroimidazoles, tinidazole and metronidazole have shown the greatest in vitro activity.[17,35] Metronidazole is considered the drug of choice for treatment, with tinidazole being an alternative.[25,27] Although albendazole has been shown to be curative in single doses or a 5-day course, it is not approved for use in giardiasis in the United States.[37,105] Quinacrine, which is inexpensive and has excellent efficacy, also is no longer available in the United States.[27] It may cause hemolysis in children with

TABLE 224–3 Drugs for the Treatment of Giardiasis

Drug	Dosage	Efficacy (%)	Side Effects
Metronidazole	15 mg/kg/day PO divided tid × 5-10 days (maximum 750 mg/day)	80-100	Metallic taste, headache, nausea, vomiting, rash, peripheral neuropathy, neutropenia, disulfiram-like effects
Tinidazole	50 mg/kg, single dose (maximum 2 g)	80-96	Metallic taste, nausea, vomiting, headache, rash, peripheral neuropathy, neutropenia, disulfiram-like effects
Ornidazole*	40-50 mg/kg, single dose (maximum 2 g)	96-100	Metallic taste, nausea, vomiting, headache, rash, peripheral neuropathy, neutropenia, disulfiram-like effects
Secnidazole	30 mg/kg, single dose (maximum 2 g)	90	Nausea, anorexia, abdominal pain
Albendazole†	15 mg/kg/day × 5-7 days (maximum 400 mg)	94-100	Anorexia, constipation, neutropenia, elevated liver function tests
Furazolidone	6-8 mg/kg/day PO divided qid × 1-10 days	81-96	Allergic reactions, headache, nausea, vomiting, diarrhea, brown discoloration of urine, hemolysis, disulfiram-like effects
Paromomycin	30 mg/kg/day divided tid × 5-10 days (maximum 2 g/day)	55-88	Nausea, vomiting, ototoxicity, nephrotoxicity
Quinacrine*	6 mg/kg/day divided tid × 7 days	92-95	Nausea, vomiting, dizziness, headache, yellow/orange skin and mucous membrane discoloration, hemolysis, toxic psychosis
Nitazoxanide	1-3 years old—200 mg/day divided bid × 3 days 4-11 years old—400 mg/day divided bid × 3 days ≥12 years old—1000 mg/day divided bid × 3 days	85-90	Nausea, abdominal pain, diarrhea, anorexia, flatulence, headache, yellow eyes, discolored urine, increased creatinine and serum alanine aminotransferase

**No longer produced in the United States; can be obtained from Panorama Pharmacy, Panorama City, CA.*
†Not approved by the Food and Drug Administration for this indication in the United States.

glucose-6-phosphate dehydrogenase deficiency. Furazolidone is an effective alternative and is available in a liquid form. Furazolidone is administered four times a day for 7 to 10 days and is associated with side effects including rash, nausea, and vomiting. Tinidazole has been shown to eliminate the parasite after single-dose therapy.[25] Secnidazole, another member of the 5-nitromidazoles, has been shown to be well tolerated at a dose of 30 mg/kg/day (maximum 2 g) and is an option for therapy.[32] Paromomycin, an aminoglycoside that is not well absorbed, is the only drug that can be used in pregnancy. It is not as effective as other medications in eradicating the parasite, but may induce clinical improvement.

A 3-day course of a new drug licensed by the U.S. Food and Drug Administration, nitazoxanide (Alinia), has been shown to treat successfully 85 percent of children with diarrhea caused by *Giardia*.[23,83] Although outbreaks of *Giardia* in daycare centers are common, treating all children in the daycare center is not efficacious because recurrence rates are high, and a chance of a drug reaction always exists. Treatment is reserved for symptomatic children.

Treatment failures and the development of drug resistance have been reported with all of the common agents used to treat *Giardia*, including albendazole, furazolidone, metronidazole, and quinacrine.[5] The prevalence of clinical metronidazole-resistant cases is reported to be 20 percent[9,21,90,93,96] with recurrence rates reaching 90 percent.[104] Metronidazole-resistant *Giardia* strains also have manifested cross-resistance to tinidazole.[90,93,96] Furazolidone-resistant *Giardia* spp. can be induced in vitro and in vivo, and this may induce the organism to become resistant more easily to quinacrine.[63,91,95] *Giardia* resistant to albendazole has been reported to develop rapidly in vitro. Albendazole resistance also develops more easily in *Giardia* strains that are furazolidone-resistant, giving rise to multidrug-resistant phenotypes.[57,92]

Patients with clinically resistant strains have been treated with longer repeat courses or higher doses of the initial agent[28,68]; however, the most effective means of eradicating these infections seems to be the use of an anti-*Giardia* agent from a different class to avoid potential cross-resistance.[16,29] For *Giardia* strains that are phenotypically considered to be multidrug-resistant, administration of anti-*Giardia* drugs in combination may be used for the successful treatment of the infection.

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CHAPTER 225

DIENTAMOEBA FRAGILIS INFECTIONS

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Dientamoeba fragilis is a protozoan that may inhabit the human gastrointestinal tract. In 1918, *D. fragilis* was recognized as a distinct species by Jepps and Dobell, who considered it to be a rare intestinal commensal.[36] In unpreserved feces, the morphologic characteristics of *D. fragilis* do not persist, which most likely accounts for its perceived rarity in some surveys. Careful studies using preserved and stained fecal specimens have found an association between *D. fragilis* infection and acute and chronic gastrointestinal symptoms, which has led to its recognition as a pathogenic protozoan.

ORGANISM

D. fragilis, in contrast to most other intestinal protozoa, has no known cyst form. The trophozoite was classified initially in the genus *Entamoeba*. The similarity of *D. fragilis* to flagellates, however, specifically to *Histomonas meleagridis*, the cause of "blackhead" enterohepatitis in fowl, was noted on careful examination under the light microscope.[18] *H. meleagridis* is a flagellate when it is found in the cecum of fowl; however, its flagella are lost when it invades tissues.[18] *D. fragilis* in the binucleate form resembles the tissue form of *Histomonas*.[18] *D. fragilis* has not been found to invade tissues, however. Antigenic and ultrastructural relatedness to *Histomonas* has been based on fluorescent antibody,[12] electron microscopic,[19] and molecular[19] studies.[15,27,65] *D. fragilis* was reclassified in 1974 by Honigberg as a nonflagellate trichomonad of the order Trichomonadida, family Monocercomonadidae, subfamily Dientamoebinae, and genus *Dientamoeba*.[8,21] Genetic studies confirmed recent evolution with trichomonads,[15,27,65] with two genotypes detected in DNA encoding ribosomal RNA.[38] Genotype 1 seems to predominate in epidemiologic studies.[13,38,54,69,79]

D. fragilis infects the mucosal crypts of the large intestine in close proximity to mucosal epithelium from the cecum to the rectum. The organism ranges in size from 3 to 18 μm in diameter, but it usually is 7 to 12 μm. The pseudopodia of *D. fragilis* have a delicate, leaflike appearance and serrated margins.[35] This protozoan is seen to move actively in fresh feces, but quickly becomes rounded after standing. *D. fragilis* is easy to isolate and grow in vitro in media containing solid rice-starch.[18] Cultures should be maintained at 37° C to 38° C (98.6° F to 100.4° F) because the organism rounds up and stops moving and feeding at lower temperatures. *D. fragilis* thrives in temperatures up to 41° C (105.8° F). This organism feeds on bacteria and starch grains and ingests human red blood cells.

D. fragilis reproduces by binary fission.[18] Although the organisms are found most commonly in the binucleate form, approximately 20 percent are in the uninucleate form, and a few are multinucleate.[35,36,41,78,83] Each nucleus contains a large, fragmented (four to eight granules) karyosome surrounded by a clear zone with no peripheral chromatin and a fine nuclear membrane.[36,82] Occasionally, aberrant forms may be found that usually are uninucleate and may be 20 μm. They do not reproduce. Humans seem to be the natural host of *D. fragilis*. *D. fragilis* infection has been reported in two simian species.[18] Multiple attempts to infect other animals have been unsuccessful so far. The atypical morphologic and biologic characteristics of the organism and the absence of an animal model have impeded classification and defining the epidemiology and pathogenicity of *D. fragilis*.[37]

EPIDEMIOLOGY AND TRANSMISSION

D. fragilis was thought to be uncommon until improved techniques for preserving the organism were used.[25,30,63] The tropho-

zoites of *D. fragilis* have been noted to be quite sensitive to an aerobic environment.[12] They die and disintegrate within 1 hour in an isotonic salt solution at room temperature; when smeared on slides, they round up and become granular within 15 minutes during microscopic examination at room temperature and low humidity.[12]

D. fragilis has been reported worldwide with a prevalence in selected populations of 1.4 to 38 percent.* Higher prevalence rates of 19 to 69 percent have been reported in crowded living situations, such as institutions and communal groups[5,45,47,48,75] and in individuals traveling outside the United States.[42,59,68,71] A serologic survey in Canada using indirect immunofluorescence techniques detected antibodies to *D. fragilis* in 87 to 100 percent of healthy children 1 to 19 years old,[10] suggesting that infection occurs in most individuals during childhood. Although the antibodies detected in this survey did not absorb when incubated with *Klebsiella pneumoniae* or *Bacteroides vulgatus*, known to contaminate the antigen source of *D. fragilis*, additional studies are needed to confirm the specificity of the assay and to define the seroprevalence in other populations.

The mode of transmission of *D. fragilis* is unknown; however, two mechanisms have been postulated. One hypothesis is that *D. fragilis* is transmitted in the eggs of *Enterobius vermicularis* (pinworm) in a manner similar to the transmission of *H. meleagridis* in the eggs of the avian nematode *Heterakis gallinae*.[6,18,76,81] In support of this theory, many investigators have noted a high frequency of concomitant infection with *D. fragilis* and *E. vermicularis*.[6,18,31,76] Ockert[51] provided the most convincing support for this theory when, by ingesting eggs of *E. vermicularis*, which he had washed with water and exposed to pepsin and hydrochloric acid, he became infected with *E. vermicularis* and *D. fragilis*. Other investigators, noting a high rate of concomitant infection of *D. fragilis* with organisms causing intestinal infections[44,45,48,81] and negative tests for *E. vermicularis*,[14,69] suggest fecal-oral transmission.

CLINICAL MANIFESTATIONS

Gastrointestinal and, less frequently, systemic symptoms have been reported to occur in association with *D. fragilis* in the fecal specimens of children and adults.† Symptomatic infections occur in 15 to 85 percent of infected individuals.‡ Acute watery diarrhea and a chronic recurrent abdominal pain syndrome have been associated with *D. fragilis* in children and adults.§

Individuals with acute diarrhea also have reported abdominal pain, anorexia, nausea, and vomiting, and, less frequently, fever, weight loss, headache, malaise, fatigue, irritability, and weakness.¶ The stools have been described as greenish brown, mushy or sticky, with a foul odor, and sometimes bloody and with mucus.[39,40] Abdominal tenderness has been found commonly on physical examination[14,39,67] and may mimic appendicitis.[64,76]

When symptomatic, *D. fragilis* infection is associated most frequently with chronic abdominal pain,[14,46,66,67] which may persist for months to years.[32,83] The pain is described commonly as dull, achy, crampy, or colicky, and usually is located in the lower abdominal quadrants.[63,67,83] Complaints of flatulence, fatigue, and alternating diarrhea and constipation are common in individuals with *D. fragilis* infections.[36,67] Laboratory and radiologic studies usually are normal. Eosinophilia, not usually seen with protozoal infections, has been reported in infected children and adults, most commonly in association with chronic symptoms.[14,66,67,72] Eosinophilia has not been observed by others, however.[39] The high prevalence of *D. fragilis* in fecal surveys generally without symptoms and the nonprogressive nature of symptomatic infections should steer clinicians to look for alternative diagnoses in seriously ill children.

DIAGNOSIS

Infection with *D. fragilis* should be considered when abdominal pain or diarrhea or both persist beyond 1 week, particularly if the child lives in an institution, has lived or traveled to a location where sanitary practices are poor, or is infected with pinworms. Investigation for *D. fragilis* infection should include the collection of at least three stool specimens that are immediately placed in a stool preservative such as polyvinyl alcohol stool preservative to retain the morphologic characteristics of the delicate trophozoite.[63] Diagnosis also can be made by permanent stained smear of a fresh or purged fecal specimen.[6,30,78] Detection of the organism seems not to be compromised by use of "environmentally friendly" mercury-free stool preservative and stain (EcoFix and EcoStain, Meridian Diagnostics, Inc., Cincinnati, Ohio).[26]

Three fecal specimens properly collected and stained leads to the identification of this intestinal protozoan in 70 to 93 percent of infected individuals.[34,62] Stool specimens should be collected on alternate days because excretion of *D. fragilis* seems to manifest a cyclic pattern.[16] Stool samples should be collected before radiologic studies with barium are done because barium interferes with detection of the protozoa.[24] Other medications interfering with parasite identification include antibiotics, mineral oil, antimalarials, antiprotozoan agents, nonabsorbable diarrheal preparations, and bismuth.[24] These substances may interfere with the detection of parasites for 3 weeks.[24]

After arrival in the laboratory, stool specimens are processed with the use of a formalin-ether sedimentation concentration technique and stained with either iron hematoxylin or trichrome and examined by qualified and experienced individuals for the proper identification of *D. fragilis*.[22,26] Garcia and associates[24,25] reported 92.2 percent of *D. fragilis* trophozoites were determined solely on the basis of the trichrome-stained smear, and subsequent studies indicate a comparable sensitivity with the use of Ecostain.[26] Diagnostic characteristics of *D. fragilis* on a permanently stained smear include a high percentage of binucleate trophozoites and nuclei without peripheral chromatin, but with four to eight chromatin granules in a central mass.[21] Detection of *D. fragilis* by indirect immunofluorescence[11] or by culture[61,85] seems promising in limited investigations. Assays using polymerase chain reaction that are sensitive and specific for detection of *D. fragilis* have been described.[54,70] One serologic test has been developed; however, the high seroprevalence of *D. fragilis* precludes its use as a diagnostic tool.[10]

TREATMENT

Several different agents have been used in the treatment of *D. fragilis* infection. Presently, one of four drugs is recommended for the treatment of *D. fragilis* infection: iodoquinol, tetracycline, paromomycin, or metronidazole.[2,11,43,67,80] However, owing to a paucity of clinical studies, tetracycline and paromomycin are considered investigational by the U.S. Food and Drug Administration.[1,2] Among newer agents, secnidazole[28] and ornidazole[90] seem effective in limited studies, and tinidazole is active in vitro.[23]

Iodoquinol, 650 mg three times a day for 20 days, is recommended for adults and 40 mg/kg/day divided three times a day

*See references 3-5, 9, 12, 13, 20, 42, 44-46, 49, 53, 55, 58, 60, 73-75, 78, 79, 81, 84, 88.
†See references 7, 11, 13, 14, 17, 29, 31-36, 39, 40, 47, 48, 52, 53, 56, 57, 59, 67-69, 74, 77-79, 83, 86, 89.
‡See references 39, 43, 44, 47, 53, 59, 66-68, 86, 88.
§See references 13, 14, 17, 30-36, 45, 46, 48, 50, 52, 53, 55, 56, 61-64, 66, 69, 73, 74, 83.
¶See references 14, 16, 17, 32, 39, 40, 56, 64, 66, 67, 83, 89.

for children. The tablets should be taken with meals. Side effects include abdominal discomfort, diarrhea, anal irritation and pruritus, headache, and dysesthesias of the hands and feet. Paromomycin in a dosage of 500 mg three times a day for adults and 25 to 35 mg/kg/day in three divided doses in children for 7 days may be more effective than iodoquinol.[87] Adverse reactions to paromomycin include nausea, abdominal cramps, and diarrhea. It is absorbed poorly after oral administration and is no longer available in syrup form in the United States.

Recrudescence or relapse has been reported after one or more 7-day courses of paromomycin in a few treated children.[80] Alternative therapy is tetracycline hydrochloride or metronidazole. Tetracycline is recommended at a dosage of 500 mg four times a day for adults and 40 mg/kg/day in four divided doses for children for 10 days. Tetracycline may cause gastrointestinal and central nervous system symptoms. It should not be given to children younger than 9 years old because it may cause discoloration of the teeth. Metronidazole treatment is recommended at a dosage of 500 to 750 mg three times daily for 10 days for adults and 20 to 40 mg/kg/day divided into three doses for children. Treatment with metronidazole may cause gastrointestinal and central nervous system symptoms. Because of the adverse reactions associated with these drugs, the clinician should evaluate carefully the need for therapy in each case and discuss treatment options with the child's family.

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CHAPTER 226

TRICHOMONAS INFECTIONS

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Trichomonas spp. are found in animals and humans. The most widely studied member of this species is *Trichomonas vaginalis* because it is the trichomonad most relevant to human disease. Donne first described *T. vaginalis* in 1836 as motile microorganisms in the purulent frothy leukorrhea of women with vaginal discharge and genital irritation.[17] Epidemiologic information concerning *Trichomonas* infection is most detailed in the adult population; nonetheless, studies that document the epidemiology of *T. vaginalis* in children and adolescents are available.*

BACTERIOLOGY

Trichomonads are acellular flagellated protozoans. The Trichomonadidae family is characterized as mononucleate with an axial organelle that has an undulating membrane.[73] The *Trichomonas* genus includes protozoans that have organelles with three to four anterior flagella and an undulating membrane composed of the posterior flagellum.[73] Five *Trichomonas* spp. infect humans, and three *Trichomonas* spp. infect animals. In humans, the five species of *Trichomonas* include *T. tenax*, *T. ardin delteili*, *T. faecalis*, *T. hominis*, and *T. vaginalis*.[73]

T. vaginalis is the most clinically relevant human trichomonad; the other four species are nonpathogenic. *T. vaginalis* consists of four anterior flagella and a posterior flagellum incorporated into the undulating membrane. *T. vaginalis* can survive but cannot multiply at room temperature. In contrast, *T. hominis* has five anterior flagella and a trailing posterior flagellum, and can survive and multiply at room temperature. Only *T. hominis* can survive in media without serum and in feces for 24 hours.[73]

PATHOGENESIS

Virulence factors associated with *T. vaginalis* infection have been defined and include adherence, contact-independent factors, hemolysis, and host macromolecule acquisition. Epithelial cell

*See references 9, 11, 18, 22-24, 27, 28, 36, 38, 39, 50, 58, 69.

adherence depends on an intact cytoskeleton and *Trichomonas* protein ligands and proteases, which are necessary to activate adherence molecules.[12,19] Cell contact-independent factors include pH variability and cell-detaching factor, which in vitro inhibit reorganization of cells infected with *T. vaginalis*. *T. vaginalis* produces lactic and acetic acid as by-products of glucose metabolism.[12] These acids lower the pH, which is cytotoxic to epithelial cells. Another metabolic by-product of *T. vaginalis* is cell-detaching factor, which has a cytopathic effect on epithelial cells and increases subepithelial vascularity, producing the clinical sign of "strawberry cervix."[12] The activity of cell-detaching factor is optimal at pH 5.0 or greater. Hemolysis is seen only in the presence of live trichomonads. Cysteine proteases seem to be important for hemolysis because introduction of their inhibitors in vitro eliminates hemolysis by *T. vaginalis*.[10,12,30]

The addition of metronidazole reduces levels of hemolysis by 50 percent.[12] The hemolytic activity of *T. vaginalis* is temperature-dependent, with maximal hemolysis at 37° C. Hemolysis is inhibited via separation of trichomonads from erythrocytes by a 3-μm filter, suggesting a contact-dependent mechanism.[12] As a parasite, *T. vaginalis* also depends on host macromolecules for nutrition, including plasma proteins and lactoferrin.

The host responds to *T. vaginalis* infection at the cellular level with polymorphonuclear cells and lymphocyte activity. *T. vaginalis* secretes proteases that are chemotactic to polymorphonuclear leukocytes, with resultant phagocytosis and killing of the trichomonad by oxidative mechanisms.[12] *T. vaginalis* secretions are mitogenic to lymphocytes; they enhance phagocytosis by polymorphonuclear cells and may suppress the host immune response if numerous suppressor lymphocytes are activated.

Clinically, *T. vaginalis* infection has gender differences. Women are largely symptomatic, whereas only a few men have symptoms with *T. vaginalis* infection, many of which undergo spontaneous cure. Estrogen levels in females directly correlate with infection at peak estradiol levels. In an early study of premenarcheal vaginitis in children 3 months to 9 years old, *T. vaginalis* infection accounted for only 2.8 to 4.4 percent of cases of vaginitis in unestrogenized vaginas compared with 50 percent of infections in fully estrogenized vaginas of patients nearing puberty.[20] In asymptomatic males infected with *T. vaginalis*, the prostate gland serves as a reservoir. Men may remain asymptomatic because of the concentration of zinc salts in prostatic fluid, which is cytocidal for trichomonads.[34] In vitro, testosterone decreases the growth of *T. vaginalis* as well.[19] In symptomatic males, *T. vaginalis* may be isolated in specimens from urine, urethral discharge, and semen.[29]

In the host, trichomonads may serve as a vector for bacteria and viruses, as shown by the high co-infection rate with *T. vaginalis* and human papillomavirus. Although bacteria contaminate trichomonads externally, trichomonads are thought to ingest virus-infected cells and destroy them, with the active virus left intact.[22] *T. vaginalis* has been implicated in pelvic inflammatory disease via ascension from the vagina to the fallopian tubes. In vitro studies have shown *Escherichia coli* strains, a part of the normal vaginal flora, intimately attached to trichomonads by glycoprotein strands.[30] Trichomonads contaminated with bacteria serve as a vector for the bacteria to produce pelvic infection on reaching the uterus, fallopian tubes, or peritoneum.[30] Likewise, a more recent study involving Dutch women attending a sexually transmitted infection treatment clinic found that 71 percent of the women infected with *T. vaginalis* were co-infected with *Mycoplasma hominis*. Because of the small sample size, however, whether infection with *T. vaginalis* predisposes females to infection with *M. hominis*, or whether this co-infection reflects the sexual behavior of the individual, remains unclear.[6]

In contrast, vaginal colonization with *Lactobacillus* spp. was thought to inhibit trichomonal invasion by lowering vaginal pH. In a study of 336 African women 15 to 49 years old, 199 of whom were pregnant, 31 percent were culture-positive for *T. vaginalis*, whereas only 40 percent of the patients tested positive for *Lactobacillus*. Although the rate of *Lactobacillus* colonization in these African women was low, the high rate of *T. vaginalis* infection was not related to the absence of *Lactobacillus*. Trichomonads may not alter the vaginal flora substantially.[45] In women infected with *T. vaginalis* in the lower genital tract, however, the aggressive inflammatory response with resultant punctate hemorrhages allows transmission and infection with human immunodeficiency virus (HIV)-1.[63]

A spectrum of severity of disease exists in patients infected with *T. vaginalis*. Some patients are asymptomatic, whereas others experience severe symptomatic inflammation and discomfort. The virulence of *T. vaginalis* isolates varies; whether this variance is caused by the host response or inherent properties of the parasite is unknown. The Golgi apparatus in *T. vaginalis* is a prominent structure and seems to be a key station in the production of adhesins.[7] Evidence indicates, however, that the dramatic heterogeneity that exists on the surface of the parasite leads to antigenic diversity among different isolates of *T. vaginalis*. In one study, prominent immunogens absent on the surface of *T. vaginalis* isolates led to an enhanced ability of the parasite to cause cytoadherence-dependent killing of HeLa cells in monolayer culture. In addition, only the adherent parasites possessed adhesins, which directly affected cytoadherence and cytotoxicity of the parasites.[11] The cysteine proteases of *T. vaginalis* may be responsible for the cytoadherence, nutrient acquisition, and cytotoxicity of *T. vaginalis*. These proteinases are shed during the life cycle and growth of *T. vaginalis*. One hundred percent of sera from women infected with *T. vaginalis*, but none from normal uninfected women, possessed IgG to numerous trichomonad cysteine proteinases. This serum antiproteinase antibody disappeared after the women received effective therapy for the infection.[3]

IMMUNOLOGY

The interaction between *T. vaginalis* and host immunoglobulins is unclear. In women infected with *T. vaginalis*, specific local antibodies, IgG and IgA, are seen in vaginal secretions. IgA may serve to increase opsonization of the parasite by IgG and result in enhanced phagocytosis. IgG specific for *Trichomonas* cysteine proteases and surface proteins is seen, but it does not help rid the host of infection.[3] *T. vaginalis* synthesizes high-molecular-weight proteins with variable surface expression.[2] Of samples obtained from women infected with *T. vaginalis*, 70 percent of vaginal washes and 80 percent of vaginal mucus samples had IgG to a specific *T. vaginalis* surface protein immunogen with a molecular mass of 230,000 d (P230). In contrast, no antibody to P230 was detected in uninfected women or in detergent extract depleted of P230, suggesting a highly specific antibody.[2] Clinically, this finding may account for the lack of resistance to repeated *Trichomonas* infections and variable host antibody titers in infected individuals.

EPIDEMIOLOGY

As noted previously, five species of *Trichomonas* have been identified in humans; the most clinically important one is *T. vaginalis*. *T. vaginalis* is not part of the normal flora, but is found in the human vagina, urethra, and prostate. Inoculation experiments with *T. vaginalis* in the mouth and intestines failed to establish infection in these sites.[73] *T. tenax* is found in the mouth, whereas *T. hominis*, *T. ardin delteili*, and *T. faecalis* are found in the bowel. *T. tenax* is detected in approximately 5 percent of patients with *T. vaginalis*.[73] *T. tenax*, *T. ardin delteili*, and *T. faecalis* are part of the normal flora and are nonpathogenic.[73] *T. hominis* can infect

humans and is associated with gastrointestinal dysentery.[73] The incidence of *T. hominis* in humans is 0.4 to 3.5 percent. *T. hominis* is found only rarely in the stools of patients who concomitantly have *T. vaginalis*.[73]

T. vaginalis infection may be the most commonly encountered sexually transmitted disease. A vast amount of international prevalence data on *T. vaginalis* sexually transmitted infections stems from research done in clinics and populations in Africa.[4,13] The prevalence of *T. vaginalis* urogenital infection varies from 5 to 65 percent in different studies conducted predominantly in adults. Many of the studies of urogenital trichomoniasis are biased because of lack of random sampling, variance in the sensitivity and specificity of the diagnostic tests used, and sample selection, often from sexually transmitted disease clinics.[43] One of the reasons for the high prevalence of *T. vaginalis* infection is its rate of asymptomatic carriage; reliance on clinical symptoms alone would cause a practitioner to miss 80 percent of infections.[48]

Trichomonal infection is found in all age groups, from neonates to adults. It has been detected in newborns and infants, presumably from contamination on passage through an infected birth canal.[58] Most of the cases reported have involved premature infant girls. Vertical transmission of the organism occurs as the infant passes through the infected maternal birth canal.

T. vaginalis is seen most commonly in postmenarcheal sexually active adolescent girls and women; however, *T. vaginalis* vaginitis is noted occasionally in children.[22] *T. vaginalis* in a prepubertal girl or non–sexually active adolescent girl must raise suspicion of sexual abuse and prompt an evaluation for other sexually transmitted diseases.[18,50,73] In a study of 409 children suspected of having been sexually abused, *T. vaginalis* was diagnosed in 4 children, 10 to 12 years old, by wet-mount examination of vaginal secretions. This study may have underrepresented cases of *T. vaginalis* in patients suspected of having been sexually abused because a saline wet-mount preparation was available in only 18 of the 409 children.[72] One study of 54 premenarcheal girls (median age 5.8 years) with vulvovaginitis failed to identify *T. vaginalis*.[48,72] Lang and others[23,39] found *T. vaginalis* in 3.6 percent of 9- to 12-year-old girls. In adolescents, the prevalence of *T. vaginalis* is 5.5 to 34 percent.[18]

Trichomonal infections can be found in males and females, although the incidence is greater in females. The incidence of *T. vaginalis* infection is 10 to 25 percent in sexually active adolescent girls and women worldwide. Approximately 180 million women worldwide may be infected with *T. vaginalis*; prevalence data depend on the population studied. The prevalence of *T. vaginalis* is 5 to 74 percent in women, and 5 to 29 percent in men.[1,2] In men attending a sexually transmitted disease clinic, the prevalence of *T. vaginalis* was 22 percent in sexual contacts of women with trichomoniasis and 6 percent in homosexual men attending the same clinic.[32] *T. vaginalis* was found in 58 percent of 85 young black men 16 to 22 years old who were sexually active.[57] Sixty-nine percent previously had a sexually transmitted infection.

The rate of infection with *T. vaginalis* is increased in black women and in women with other sexually transmitted diseases. It also is increased in women who have gonococcal cervicitis with a vaginal pH shift greater than 4.5 and in women who are pregnant, and it may be increased around the time of menarche and after menopause.[16,47,73] Trichomoniasis apparently does not have a seasonal pattern of infection.[43] Urogenital trichomoniasis is seen more commonly in inner-city patients and patients in the 20- to 30-year-old age group.[43]

Infection with *T. vaginalis* increases in direct relation to number of sexual partners. An increased number of sexual partners also increases the risk of co-infection with other sexually transmitted organisms, including, but not limited to, *Chlamydia trachomatis*, *Neisseria gonorrhea*, *M. hominis*, and HIV.[3] Oral contraceptives diminish the rate of infection with *T. vaginalis* compared with an intrauterine device or tubal ligation.[43] The use of nonoxinol 9 spermicidal cream was not related significantly to a decrease in *Trichomonas* infection.[5]

In another study of 226 women attending a sexually transmitted disease clinic, trichomonal infection was noted in 44 percent of the patients.[47] No association was found among patient age, frequency of coitus, date of most recent coitus, day of menstrual cycle, antibiotic use, contraceptive methods, or symptoms of discharge or pruritus.[47] Other risk factors for women may involve a change in the normal vaginal flora, such as overgrowth of *Gardnerella vaginalis*, *Bacteroides*, or *Peptostreptococcus*. These bacteria may serve as sources of nutrients for trichomonads and allow them to thrive. In men, sexual contact with a woman infected with *T. vaginalis*, nongonococcal urethritis, or nongonococcal nonchlamydial urethritis was associated with an increased risk for acquiring infection with *T. vaginalis*.[35] In trichomoniasis, lactobacilli are absent from the vagina. This absence promotes alkalinity of the vaginal pH, enhancing the overgrowth of anaerobes and trichomonads.[65] Another study performed in Africa failed to show a significant role for *Lactobacillus* in patients with *T. vaginalis* infection.[15,45]

T. vaginalis usually is transmitted sexually in adolescents and adults. Large numbers of trichomonads are found in the prostatic secretions of husbands of women with recurrent *T. vaginalis* infection. In a clinic for patients with sexually transmitted diseases, 60 percent of husbands of women who had chronic, repeated *T. vaginitis* infection were culture-positive for *T. vaginalis*, in contrast to 8 percent in a control group of men attending the same clinic.[70] In a study from the United Kingdom of child and adolescent sexual assault victims, *T. vaginalis* was found in 1 of the 16 girls who were 12 years or younger.[28] These data support the concept of sexual transmission of *T. vaginalis*.

Nonsexual transmission of *T. vaginalis* has been reported. In rural India, a point prevalence survey of random samples from preadolescent and adolescent girls complaining of leukorrhea revealed that 76 percent were infected with *T. vaginalis*. Of the girls who were infected, 38 percent were younger than 12 years old. In this study, poor genital hygiene and underwear use were correlated with a higher incidence of infection. In addition, a significantly higher risk occurred in girls who washed or bathed in tanks or rivers versus girls who used pipe or well water. In the tropics, nonsexual transmission may account for infection with *T. vaginalis* in preadolescent and adolescent girls.[11] *T. vaginalis* can survive on toilet seats for 1 hour, on wet clothes for 3 hours, in fresh water for 30 minutes, in warm mineral water for 2 or 3 days, and in urine for hours. According to the literature, transmission of *Trichomonas* may occur via fomites (washcloths, towels), particularly when people are living together in crowded, confined spaces.[50]

CLINICAL MANIFESTATIONS

Trichomonas has been isolated from the tracheal aspirates in preterm neonates with respiratory failure and from nasal secretions in a full-term newborn with suppurative nasal discharge and respiratory distress.[55,67,68] The vagina of an infant may serve as a reservoir of infection that goes unnoticed until the infant is evaluated 5 to 6 weeks after birth for fever. Motile trichomonads and pyuria are seen on examination of the urine. Symptoms resolve after treatment with metronidazole.[58] Premenarcheal girls have diffuse bubbly leukorrhea and pruritus.[39] Vaginitis with a purulent foul-smelling discharge is the most common manifestation of infection with *T. vaginalis* in girls; in some patients, trichomonads may be found in the urine initially, with the development of frank signs of vaginitis 7 to 28 days later.[27]

Fifty percent of girls and women and 90 percent of boys and men infected with *T. vaginalis* are asymptomatic.[66] When one controls for co-infection with other organisms, *T. vaginalis* infection is associated significantly with purulent discharge, vulvar itching, colpitis macularis (strawberry cervix), and vaginal and vulvar erythema. The sensitivity of the other signs and symptoms of *Trichomonas* vaginitis, including vaginal burning, dysuria, urinary frequency, dyspareunia, frothy discharge, and cervical friability, is low.[72] Although frothy leukorrhea was associated most frequently with *Trichomonas* infection, 29 percent of the patients with frothy discharge in one study did not have *Trichomonas* infection.[72] Strawberry cervix was pathognomonic of *T. vaginalis* infection, but it was noted in only 2 to 3 percent of patients. One cannot depend on this finding to establish a clinical diagnosis of *T. vaginalis* vaginitis in most patients.[16] The diagnosis of *Trichomonas* infection should be considered in any girl or woman with a vaginal discharge.

Trichomonads may ascend the fallopian tubes and, if contaminated with bacteria, can produce the syndrome of pelvic inflammatory disease.[9,30] In HIV-infected girls and women, the risk of developing pelvic inflammatory disease increases significantly with *T. vaginalis* vaginitis.[49] Vaginal *Trichomonas* has been associated with adverse pregnancy outcomes, primarily premature rupture of membranes, preterm labor, and low-birth-weight infants.[31]

In boys and men, *T. vaginalis* may be manifested as symptomatic urethritis with dysuria secondary to urethral inflammation and discharge. On examination, the discharge often is not visualized. When a discharge is present, it is clear to cloudy, but not grossly purulent. On microscopy, numerous inflammatory cells are seen.[32,37] *T. vaginalis* also may be a cause of chronic nonbacterial prostatitis and may be manifested as chronic prostatitis resistant to standard therapy.[26,37] In addition, *T. vaginalis* has been reported to be an etiologic agent of epididymitis in men, with purulent urethral discharge, scrotal swelling, and enlargement of the epididymis.[16] Although rare, *T. vaginalis* has been reported to infect the median raphe of the penis.[64]

DIAGNOSIS

CLINICAL EXAMINATION

In girls and women, the presence of a frothy purulent discharge, vulvar/vaginal erythema, and a strawberry cervix should suggest *Trichomonas* infection. Boys and men tend to be asymptomatic or, if symptomatic, have a purulent urethral discharge and urethral inflammation. If the physician relies on clinical examination alone, 80 percent of infections may be missed.[17,65]

WET-MOUNT EXAMINATION

The diagnosis of trichomoniasis in women usually is made by wet-mount examination of vaginal secretions. Wet-mount preparations are obtained by swabbing the lateral and anterior vaginal fornices to obtain discharge material with vaginal epithelial cells; the secretions are placed in a tube with normal saline and mounted on a slide. These preparations are easy to prepare, and the method is cost-effective, but in women its sensitivity is only approximately 60 to 70 percent.[10] This method also may be used for urethral specimens in men, but it is insensitive. In addition, the validity of the result depends on the technical skill of the examiner and the rapidity with which the specimen is examined; cooling greatly affects the motility of trichomonads, which may hinder identification.[36] Wet-mount examinations are tests now regulated by Clinical Laboratory Improvement Amendments, which may be a barrier in performing them in office-based laboratories.

PAPANICOLAOU SMEAR

Conventional Papanicolaou smears can detect trichomonads and have the advantage of being fixed and read at a later time. This method is considered unreliable, however, for the diagnosis of trichomoniasis. *T. vaginalis* does not always appear in its typical pear-shaped morphology after fixation on the slide. Rather, it may appear to be more rounded, similar to a polymorphonuclear leukocyte. Cytopathologic diagnostic criteria for *Trichomonas* infection include the presence of perinuclear halos and a dirty granular background. These features are nonspecific, however, and a similar background and halos can be found with certain other conditions.

The sensitivity of Papanicolaou smears for diagnosing *Trichomonas* in culture-proven infection is 56 percent.[34] In a study of 1199 women, *T. vaginalis* infection would have been diagnosed falsely in 37 percent based on Papanicolaou smear results. In a second study, *T. vaginalis* infection would not have been diagnosed in 44 percent of patients with culture-proven infection because of negative Papanicolaou smear findings.[54] More recent data show the liquid-based Papanicolaou smears as being highly specific for the diagnosis of *T. vaginalis* and may warrant treatment without confirmation. Compared with culture, the liquid-based test has a low sensitivity of 61 percent. It has a specificity equal to culture (99%).[40]

STAINING TECHNIQUES

Acridine orange is a compound that differentially stains DNA (yellow-green) and RNA (bright red). *T. vaginalis* stains brick-red with an oval, yellow-green nucleus. The flagella do not stain. Unfixed smears are kept at room temperature for 24 hours; fixed slides may be kept 5 days. Acridine orange stains may permit a rapid, accurate diagnosis of *T. vaginalis* infection. The diagnosis can be confirmed by other diagnostic methods in 93 percent of cases.[46] Acridine orange staining seems to be at least as sensitive as wet-mount examination.[8,59]

CULTURE

Diagnosis of *T. vaginalis* by culture continues to be considered the gold standard. Although generally used in clinical research, this diagnostic method should be considered strongly when trichomoniasis is suggested, but cannot be confirmed by other easily available methods. Secretions are collected from the vagina or urethra with a cotton-tipped swab and placed directly on culture media. The reformulated media currently prepared commercially are Diamond, Kupferberg, and Lash media.[61] These media contain antibiotics to inhibit bacterial overgrowth and may contain yeast extract, horse or sheep serum, or both.[60] Cultures are incubated for 7 days, and a drop of sediment from the culture tube is placed on a slide, and a wet-mount examination is performed.

Culture of *T. vaginalis* on these media detected less than 10 trichomonads; cultures were not affected by douching in the previous 24 hours. In contrast, the sensitivity of wet-mount preparations decreased from 57 to 22 percent after douching.[17] Limitations of culture include the failure of culture media to support the growth of trichomonads in some cases by culture day 7. Growth in culture media also is inoculum-dependent; less sensitive media require higher inocula of trichomonads.[60] In men, the combination of prostatic massage before collection of the speci-

men and culture of urethral samples and urinary sediment has a sensitivity of 94 to 98 percent.[35,57]

The plastic envelope or pouch method is a simplified and now standard commercially available method of culture for *T. vaginalis*. The most common test is the InPouch (Biomed Diagnostics, San Jose, CA). Vaginal secretions are placed in a liquid medium contained in a pouch with the growth medium. The medium allows the organism to grow so that it can be visualized on direct microscopy for 5 days. Compared with wet-mount examination, the pouch method can increase *T. vaginalis* detection rates by twofold. The sensitivity and specificity of culture via the pouch method are 94 percent and 96 percent; sensitivity and specificity of vaginal wet-mount examination are 58 percent and 100 percent.[41,51]

DIRECT IMMUNOFLUORESCENCE AND ENZYME IMMUNOASSAYS

Direct immunofluorescence and enzyme immunoassays are more sensitive than the wet-mount preparation and give fairly rapid results. They are not readily available, however. The sensitivity of direct fluorescent antibody (DFA) staining approaches 86 percent compared with culture and is not related to the number of trichomonads seen on wet-mount examination. The sensitivity of DFA staining is superior to that of wet-mount examination or acridine orange staining in women who have *T. vaginalis* infection and is comparable to the sensitivity of these techniques in women infected with multiple organisms.[7,32,59] Interpretation of DFA staining may be accomplished in less than 1 hour.[32] In a study of high-risk men who underwent prostatic massage before collection of the specimen, DFA staining on urethral samples had a sensitivity of 63 percent.[57]

A monoclonal-based enzyme-linked immunosorbent assay was developed for use with a monoclonal antibody specific for a 65-kd surface polypeptide of *T. vaginalis*. Polyclonal rabbit anti–*T. vaginalis* antibody labeled with horseradish peroxidase was used as the probe. In a limited study of 36 women, this enzyme-linked immunosorbent assay had a sensitivity of 89 percent and a specificity of 97 percent.[42]

NUCLEIC ACID HYBRIDIZATION AND AMPLIFICATION TESTS

Rapid diagnostic tests such as DNA hybridization techniques are now available for clinical use with vaginal secretions. Two point-of-care tests have been approved by the U.S. Food and Drug Administration: OSOM Trichomonas Rapid Test (Genzyme Diagnostics, Cambridge, MA), an immunochromatographic capillary flow dipstick technology, and the Affirm VP III (Becton Dickinson, San Jose, CA), a nucleic acid probe test that evaluates for *T. vaginalis*, *G. vaginalis*, and *Candida albicans*. The results with these tests can be obtained in 10 minutes and 45 minutes. Although the sensitivity of both tests on vaginal secretions is greater than 83 percent, and the specificity is greater than 97 percent, false-positive tests might occur in low-prevalence populations[6] available for clinical use. Several primer sets have been described, and sensitivities using vaginal swabs have ranged from 8.5 to 100 percent.[10,25] In contrast, the sensitivity of using a urine specimen to detect trichomoniasis is 64 percent.[41]

No Food and Drug Administration–approved nucleic acid hybridization and amplification tests such as a polymerase chain reaction test for *T. vaginalis* are available in the United States. Research has shown that sensitivities using polymerase chain reaction vaginal swabs have ranged from 8.5 to 100 percent, in contrast to the 64 percent sensitivity of a urine specimen used to detect trichomonas.[41]

TREATMENT

T. vaginalis infection in girls and women should be treated to relieve symptoms, prevent further transmission of disease, and prevent chronic inflammation of the Bartholin and Skene glands.[69] In boys and men, chronic infection may lead to prostatitis or urethral stricture.[13] Adolescent boys and men who are asymptomatic may re-infect their partners. Male partners of women infected with *T. vaginalis* should be treated.[70]

Metronidazole or tinidazole are the treatments of choice for *T. vaginalis* infection and are available for treatment of trichomoniasis in the United States. Worldwide, other drugs used for trichomoniasis include nifuratel, nimorazole, secnidazole, and carnidazole. Despite the availability of these other drugs, metronidazole remains the standard therapy for trichomoniasis. Metronidazole enters the trichomonad via passive diffusion, and its nitro group is reduced to a cytotoxic intermediate that reacts with DNA and causes cell death. Metronidazole is 93 to 95 percent bioavailable, and after oral administration, peak serum levels are attained in 1 to 3 hours, and a steady state is attained in 2 to 3 days. Metronidazole is metabolized by the liver, with only 20 percent being protein-bound; the drug is distributed well in the body.[44]

Side effects reported with the use of metronidazole include nausea, vomiting, anorexia, a metallic taste, headache, dizziness, diarrhea, and darkening of the urine. Urticaria, reversible peripheral neuropathy, seizures, and ataxia have been reported with intravenous use. The side effects tend to be dose-related and self-limited.[44] In a study of 1199 women with *T. vaginalis* infection treated with metronidazole, only 4 to 5 percent experienced symptoms of nausea, coated tongue, dryness of the mouth, anorexia, or diarrhea. All symptoms disappeared within a few days of completion of treatment, and in only one case was treatment discontinued because of side effects. In addition, relative and absolute leukopenia was not observed in these subjects. Metronidazole enhances or reactivates the growth of *C. albicans* in the vagina.[54] Metronidazole may potentiate the actions of anticonvulsants and warfarin. Because a significant disulfiram-like effect is produced when the drug is combined with moderate intake of alcohol,[44] alcohol should be avoided during and for 48 hours after completion of a course of therapy with metronidazole.

In the United States, two treatment regimens are recommended for treatment of *T. vaginalis*. The first is metronidazole, 2 g orally in a single dose in adolescents and adults. This regimen is 90 to 95 percent effective.[10] Metronidazole vaginal suppositories are not recommended for the treatment of trichomoniasis. The second regimen is tinidazole, 2 g orally in a single dose. This regimen is 86 to 100 percent effective. An alternative regimen is metronidazole, 500 mg twice daily for 7 days. Some strains of *T. vaginalis* have reduced susceptibility to metronidazole, with low-level metronidazole-resistant strains reported in 2 to 5 percent of infected cases. Although rare, higher level resistance also has been reported.[42,61,62] Treatment options for metronidazole-resistant cases are limited. If the infection fails to respond to metronidazole, it should be re-treated with tinidazole or metronidazole, 500 mg twice daily for 7 days.[10] If treatment failure continues, the patient can be re-treated with 2 g of metronidazole or tinidazole once daily for 5 days.[10]

Metronidazole is a pregnancy category B drug. To date, studies have been unable to show consistently an association between metronidazole use during pregnancy and teratogenic effects in infants.[9] In addition, although some data suggest increased risk of having a premature or low-birth-weight infant after undergoing metronidazole treatment, limitations of the studies prevent definitive conclusions regarding risks of treatment.[10,31] Treatment during pregnancy may relieve symptoms and prevent pregnancy and perinatal complications. The Centers for Disease Control and Prevention[10] recommends counseling

TABLE 226–1 Dosing of Metronidazole in Children

Age (yr)	Weight (kg)	Metronidazole Administered (mg)	
		*Orally**	*Locally*†
0-1	10	150	10
1-6	20.5	250	50
7-12	40	500	150
>12	>40	500-1000	250-500

**Oral dose = one third of the total dose administered every 8 hours for 5 to 10 days.*
†*Local dose = total dose administered intravaginally once daily or half the total dose administered twice daily for 5 to 10 days.*
Adapted from Kurnatowska, A., and Komorowska, A.: Urogenital trichomoniasis in children. In *Honigberg, B. M. (ed.): Trichomonads Parasitic in Humans. New York, Springer-Verlag, 1989, p. 268.*

patients on the potential risks and benefits of treatment. In symptomatic women, some specialists offer treatment during the first trimester, and some do so after the first trimester. In asymptomatic women, some clinicians defer treatment to after 37 weeks of gestation.

Tinidazole is a pregnancy category C drug, and its safety in pregnancy has not been evaluated. It is not offered during pregnancy.[10] A breast-fed infant consumes approximately 1 percent of a single 2 g oral dose of metronidazole; infants of mothers who are breast-feeding and who are treated with a single dose of metronidazole for trichomoniasis should be removed from the breast for at least 24 hours after treatment.[10,44] In the case of treatment with tinidazole, breast-feeding can be resumed 3 days after the last dose.

In children with infections of the genital organs, local therapy with metronidazole is preferred because it has fewer systemic side effects. Cotton-tipped swabs saturated with metronidazole are introduced into the hymen and applied locally. In multifocal infections, genital infections that fail to respond to local therapy, and infections in newborn or infant boys, oral therapy with metronidazole is given (Table 226–1).[38] Before administering oral or intravenous metronidazole to children, the clinician should obtain baseline hematologic, renal, and liver function tests to monitor changes as therapy continues. Resolution of the signs and symptoms of infection indicates a response to treatment. Eradication of *T. vaginalis* should be confirmed by wet-mount examination or culture (if available) 3 to 5 days after completion of treatment in children.[38]

PROGNOSIS

If untreated, *T. vaginalis* can lead to chronic inflammation of the Bartholin and Skene glands in women and to prostatitis and urethritis with urethral stricture formation in men.[62] Complete resolution of symptoms plus eradication of *T. vaginalis* usually is noted when treatment is provided promptly.

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C. Ciliates (Intestinal)

CHAPTER 227

BALANTIDIUM COLI INFECTION

Peter J. Hotez

Balantidium coli is the largest protozoan parasite and the only ciliate to infect humans.[6,9,15] The organism has a worldwide distribution, but it usually is found in the less developed nations of the tropics. Because pigs are the principal animal reservoir, most human infections have been reported in tropical regions where swine have close contact with humans, such as the islands of the South Pacific (including Papua New Guinea) and Central and South America.[10] Balantidiasis occurs in areas where other swine-associated parasitic zoonoses (e.g., taeniasis, trichinellosis, ascariasis) also are prevalent, although infection with *B. coli* occurs rarely. In Iran where pig raising no longer is permitted, wild boar serve as an animal reservoir.[11] Less than 1000 cases of human balantidiasis are reported in the literature.[9] The organism first was described in 1857 by Malmsten, who observed the ciliates from two patients in Sweden.[3]

ETIOLOGY AND PATHOGENESIS

The organism has trophozoite and cyst stages. The trophozoite is a large, pear-shaped organism covered with cilia. Estimates of size range from 50 to 100 μm in length and 40 to 70 μm in width.[6] This enormous protozoan organism typically can be seen by light microscopy using only low-power magnification.[6] Subcellular organelles, such as a cytostome and many large vacuoles containing bacteria and debris, are visualized under higher magnifica-

tion. Similar to many other ciliates, *B. coli* trophozoites have a macronucleus and a micronucleus. The trophozoites colonize the large intestine, where ultimately they round up and secrete a cyst wall as they pass down the lumen. The cysts measure 50 to 70 μm and contain a macronucleus and a micronucleus. The cyst stages can survive in the outside environment and are infectious to a wide range of animals, including humans (discussed later).

Frequently, *B. coli* does not invade human tissues and does not cause clinical disease. Under conditions that are not understood well, however, *B. coli* also has the potential for highly aggressive invasion and destruction of tissue. Whether the invasive potential of *B. coli* results from parasite virulence, compromised host defenses, or some combination of these two factors is unclear. The observation that invasive disease occurs more commonly in debilitated patients and in patients with polyparasitism suggests that host defenses have an important role in limiting tissue destruction by *B. coli*.[1,9]

When parasite invasion occurs, it begins in the colonic mucosa, where ulcerations and secondary microabscesses can result. Extensive tissue damage in the cecum and appendix results in clinical presentations of typhlitis and appendicitis.[4,5,9] Histopathologic examination of these tissues reveals flask-shaped ulcerations and necrosis with an extensive inflammatory infiltrate composed predominantly of polymorphonuclear leukocytes.[4,9] *B. coli* probably creates mucosal ulcerations through the release of histolytic enzymes similar to those described from *Entamoeba histolytica*.[12] Ulcerations can lead to hemorrhage or even colonic perforation. A second type of histopathology has been reported wherein patients harboring *B. coli* develop inflammatory polyposis of the rectum and sigmoid colon.[8]

When tissue invasion is extensive, the organism can metastasize to extraintestinal sites and cause hepatic and pulmonary involvement. Polymorphonuclear inflammatory cell infiltration results in abscesses at these sites.[3,5,8] Most patients with metastatic balantidiasis have recognizable defects in host defenses.[8]

EPIDEMIOLOGY

As noted earlier, human balantidiasis has a worldwide distribution, but epidemic foci have been reported in the swine-producing areas of Papua New Guinea, Micronesia, the Seychelles Islands, and Central and South America.[3,10,13] Incidence rates can be high among swine farmers and slaughterhouse workers. The potential for development of human *B. coli* infections is thought to be high in areas of poor hygiene where extensive contact occurs between humans and pigs. A notorious outbreak of human balantidiasis occurred after a devastating typhoon on the Pacific island of Truk caused widespread contamination of ground and surface water supplies with pig feces.[13] Many other animals, including nonhuman primates, guinea pigs, horses, cattle, and rats, also potentially can serve as reservoir hosts. *B. coli* also colonizes many great apes, including baboons, orangutans, chimpanzees, and gorillas, and clinical balantidiasis has been reported in these primates when they are maintained in captivity.[9] Human epidemics also have been described in institutional settings, especially where crowding mixes with low levels of personal hygiene.[13] In these instances, human-to-human spread has been postulated. Although *B. coli* is not known as an opportunistic pathogen in patients infected with human immunodeficiency virus, at least one case of *B. coli* in this setting has been described.[3]

CLINICAL MANIFESTATIONS

ASYMPTOMATIC INFECTION

Most infections are asymptomatic or cause occasional loose stools. This situation probably accounts for 85 percent of patients harboring *B. coli*.[9] Asymptomatic infection may occur more commonly in children than in adults.[14]

DIARRHEA

The next most frequent presentation of *B. coli* infections is in patients who have intermittent diarrhea, abdominal pain, and weight loss.[14] Sometimes, discrete ulcerations can be observed during sigmoidoscopy.[9] Chronic diarrhea has been described in a patient with acquired immunodeficiency syndrome.[2] A subset of patients with balantidiasis develop invasive disease subsequently.

INVASIVE COLONIC BALANTIDIASIS

The hallmark of *Balantidium* colitis is dysentery with bloody and mucous stools, colonic tenderness, leukocytosis, and fever. Sigmoidoscopy and colonoscopy of these patients reveal ulcerations and formation of mucosal granulomata.[1,9,13] Involvement of the large intestine can be diffuse, although in some cases, right-sided colonic lesions predominate. Right-sided colonic lesions can progress to typhlitis or appendicitis.[4,5,9] Transmural involvement of the colon frequently results in intestinal obstruction, hemorrhage, and balantidial peritonitis. Colonic perforation is an ominous complication that is associated with extremely high mortality.[9]

METASTATIC BALANTIDIASIS

Highly invasive balantidiasis leading to metastatic disease of the mesenteric lymph nodes, liver, and lung is a rare complication that can occur in malnourished, debilitated, and immunocompromised patients.

DIAGNOSIS

Stools from patients harboring *B. coli* have been described as having a pigpen odor.[14] The examination of wet preparations of fresh or concentrated stools usually reveals cyst and trophozoite forms of *B. coli*. Cilia motility and rapid rotary motion of the trophozoites occasionally can be appreciated under low-power magnification. Because the organism takes up heavy concentrations of dye, stained preparations typically do not reveal internal structures or even cilia.[6] These large organisms can be confused with helminth ova, especially on stained preparations.[6] As an adjunct to direct fecal examinations, sigmoidoscopy can show ulcerations from which abundant trophozoites may be obtained for diagnosis.[14]

TREATMENT

For the treatment of intestinal balantidiasis, numerous chemotherapeutic regimens have been tried, usually with some improvement. In many cases, the parasite is not eradicated, however.[9] For children older than 8 years, tetracycline (40 mg/kg/day in four doses for 10 days [maximum 2 g/day]) is the treatment of choice. Tetracycline is considered to be investigational for this condition by the U.S. Food and Drug Administration. Also considered investigational for balantidiasis are the drugs iodoquinol (30-40 mg/kg/day in three doses for 20 days) and metronidazole (35 to 50 mg/kg/day in three doses for 5 days).[7] Alternative chemotherapeutic agents that have been tried with variable success include paromomycin and chloroquine.[9] Surgical intervention

often is required for gastrointestinal invasive complications of *B. coli*, such as typhlitis, appendicitis, and peritonitis.

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D. Coccidia, Inicnosporidia (Intestinal)

CHAPTER 228

CRYPTOSPORIDIOSIS

Theresa J. Ochoa ✪ A. Clinton White, Jr.

Cryptosporidium is an important cause of acute diarrhea in normal hosts worldwide, of persistent diarrhea in children in developing countries, and of chronic diarrhea in immunocompromised hosts including patients with acquired immunodeficiency syndrome (AIDS).[42,56,64,91] These protozoan parasites were identified first in the stomach of mice in 1907,[229] and for decades were considered to be veterinary pathogens. The first human cases of cryptosporidiosis were reported in 1976.[151,170] In the early 1980s, large numbers of cases were noted with the emerging epidemic of AIDS.[11,52] Thereafter, *Cryptosporidium* was identified in animal handlers and children[52,244] and was associated with large waterborne outbreaks of diarrhea.[55,140] Studies have shown that *Cryptosporidium* is a common cause of diarrheal disease in immunocompetent and immunocompromised hosts.

MICROBIOLOGY

Cryptosporidium spp. are ubiquitous, small (2 to 6 μm) obligate intracellular protozoan parasites that infect the epithelium of the gastrointestinal tract of vertebrates. The genus *Cryptosporidium* has been classified within the subclass Coccidia (coccidia) along with *Eimeria*, *Isospora*, *Cyclospora*, *Sarcocystis*, and *Toxoplasma*. Species names were given according to the animal that they infected.[250] Initial cross-transmission studies revealed that little host specificity exists.[66,230] Subsequent genetic studies have identified at least 13 accepted species of *Cryptosporidium* and more than 30 *Cryptosporidium* genotypes.[249] Most human infections are caused by separate species, *Cryptosporidium hominis* (formerly *Cryptosporidium parvum* genotype 1) and *Cryptosporidium parvum* (formerly *C. parvum* genotype 2). *C. hominis* is found only in humans (but can infect gnotobiotic pigs), and *C. parvum* is found in a wide range of animals (particularly cattle and sheep) and in humans.[164,180,248] The relative frequency of *C. hominis* and *C. parvum* in humans differs in geographic regions, probably as the result of differences in transmission routes. In European countries, *C. parvum* is found more commonly in human cases than *C. hominis*, although a more recent study in the United Kingdom has shown a comparable rate for both pathogens.[134] In the rest of the world, *C. hominis* is the predominant species in humans.[31,32,248] Humans also can be infected rarely with *Cryptosporidium meleagridis*, *Cryptosporidium canis*, *Cryptosporidium felis*, *Cryptosporidium muris*, *Cryptosporidium suis*, and *Cryptosporidium andersoni*.[62,76,77,147,179,228,247]

LIFE CYCLE

Cryptosporidium spp. can complete their entire life cycles, including asexual (merogony) and sexual (sporogony) reproductive cycles, within a single host (homoxenous). The life cycle is characterized by six major developmental stages: (1) excystation, or release of infective sporozoites; (2) merogony, or asexual replication in the host; (3) gametogony, or the formation of microgametocytes and macrogametocytes; (4) fertilization, or the union of microgametocytes and macrogametocytes; (5) oocyst formation; and (6) sporogony, or the formation of infectious sporozoites within the oocyst wall (Fig. 228–1).[51,100]

The life cycle begins with ingestion of the infectious oocyst. The oocysts are activated in the stomach and upper intestines, which allows the organisms to excyst, releasing four infective sporozoites.[69,174] The motile sporozoites bind to receptors on the surface of the intestinal epithelium, inducing actin polymerization and protrusion of the intestinal epithelial cell membrane.[41,61] The membrane surrounds the sporozoite and fuses to form the parasitophorous vacuole, which remains in the microvillus layer on the surface of the epithelium. Inside the parasitophorous vacuole, the parasites undergo asexual reproduction (merogony). They enlarge into trophozoite forms and divide to form type I meronts, which mature and rupture to release the motile merozoites. The merozoites bind to receptors on the epithelial cells and are engulfed by the cells. They then either repeat the process of merogony or undergo sexual differentiation (sporogony).

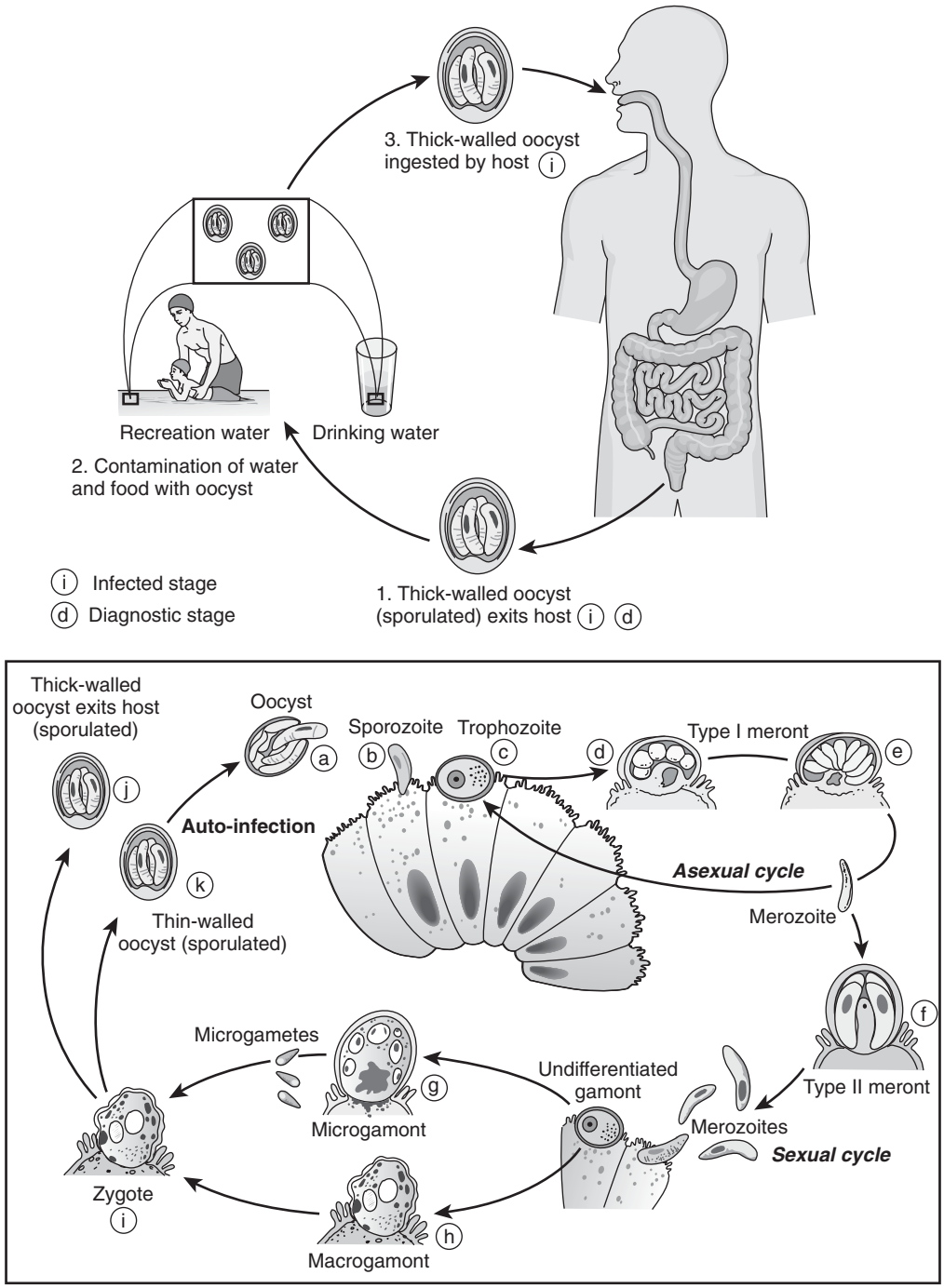

Figure 228–1 Life cycle of oocyst. After ingestion, the oocysts (a) release the sporozoite (b), which attach to and are engulfed by the epithelial cell. Inside the parasitophorous vacuole, the organisms enlarge to form trophozoites (c) and divide to form meronts (d, e, f). After reinvasion, they may differentiate into the sexual forms (macro- and microgamont, g, h), which fuse to form the zygote (i). The zygote releases the thin-walled oocysts (k), which cause autoinfection, or thick-walled oocysts (j), which are the infectious form.

In the case of sexual differentiation, the merozoites differentiate into the microgametocytes and macrogametocytes. The microgametocyte releases the microgametes, which penetrate the cells infected with macrogametocytes. The macrogametocyte and microgametes fuse to form the zygote form, which then undergoes meiosis to form the oocyst, containing four sporozoites. Two morphologic forms of the oocyst have been described: thin-walled oocysts, which excyst within the same host in a process of self-infection, and thick-walled oocysts, which are shed into the environment.[100]

EPIDEMIOLOGY

Cryptosporidium is distributed worldwide. Infection generally is more common in warm, humid months. Prevalence rates generally are higher in developing countries than in industrialized countries. Older surveys not involving patients with human immunodeficiency virus (HIV) documented oocysts in 1 to 3 percent of specimens from industrialized countries of Europe and North America and in 5 to 10 percent of specimens from developing countries.[91] Most studies on the prevalence of infection

have relied on detection of oocysts in fecal samples. With the development of improved diagnostic techniques, more cases are being identified. Using direct immunofluorescence, *Cryptosporidium* was found in 19 percent of children with diarrhea in Brazil.[180] With the use of polymerase chain reaction, *Cryptosporidium* was found in 18 percent of hospitalized patients and schoolchildren in South Africa,[210] in 25 percent of children with diarrhea in Uganda,[228] and in 36 percent of children prospectively followed in an urban slum in Brazil.[31] The prevalence may be higher than suggested by earlier stool studies.

Cryptosporidium has been reported more frequently in children than in adults.[103] The prevalence of asymptomatic infection with *Cryptosporidium* in children younger than 5 years old is estimated to be generally less than 1 percent, but varies with geographic location. In a longitudinal study in Italy, asymptomatic infection was seen in 6 percent of immunocompetent children and 22 percent of immunodeficient children.[182] In a Peruvian cohort study, 36 of 57 children (63%) followed for 2 years had asymptomatic *Cryptosporidium* infection.[38] Infection is particularly common in the first 2 years of life. Young children in daycare centers are at high risk for acquiring infection. Outbreaks of cryptosporidiosis in daycare centers have been reported from the United States and many other countries.[12,48,67,82,153,237]

The seroprevalence of antibodies to *Cryptosporidium* is 25 to 35 percent in adults in industrialized countries of North America and Europe, and is 65 percent in developing countries of South America.[233,252] Most serosurveys have documented a seroprevalence of approximately 30 percent in U.S. adults. Studies from the United States–Mexico border showed seroprevalence rates of 80 percent, however, in urban border communities.[128] Seroprevalence increases progressively with age. In a study in Oklahoma, 31 percent of children were seropositive, with rates increasing from 13 percent in children younger than 5 years to 58 percent in children 14 to 21 years old.[125] In a study in rural communities of China, no detectable antibody was found in infants 2 to 6 months old, but the proportion that were seropositive steadily increased after they reached 1 year of age.[252] In a Brazilian shantytown, less than 90 percent of children seroconverted during the first year of life, showing the high prevalence of this infection.[252] In a Peruvian birth cohort study of diarrheal disease, the incidence rate of cryptosporidiosis determined by a serologic assay was higher than the rate determined by stool microscopy (0.77 versus 0.41 infection/child-year of surveillance).[187]

Cryptosporidium is a common cause of diarrhea in patients infected with HIV. Cryptosporidiosis occurs in an estimated 10 to 15 percent of patients with AIDS in developed countries and in 30 to 50 percent of patients with AIDS in developing countries.[91] Among patients with HIV, the infection rate is proportional to the $CD4^+$ cell count. In a longitudinal study of patients with HIV, the infection rate varied from 23 percent of patients with $CD4^+$ cell counts greater than 1000/μL to 46 percent of patients with $CD4^+$ cell counts less than 100/μL.[186] In recent years, the widespread use of highly active antiretroviral therapy has resulted in the restoration of immune function and a reduction in the incidence of cryptosporidiosis and other opportunistic infections.[133]

Cryptosporidium is a common cause of persistent diarrhea in immunocompetent and immunocompromised children, especially in developing countries.[168,171,220,227,228] A study in West African children investigated episode-specific determinants for the progression of an acute episode of diarrhea to chronic diarrhea; current infection with *C. parvum* was the most significant risk factor.[220] Among 243 children with persistent diarrhea in Uganda, 76 (31%) had *Cryptosporidium*.[227] Ninety-one of the 243 children had HIV, of whom 67 (74%) had *Cryptosporidium*. Children with $CD4^+$ cell counts less than 25 percent were more likely to have *Cryptosporidium*.[227]

Cryptosporidiosis also has been noted in other immunodeficient hosts, including patients with primary immunodeficiencies, organ transplantation, cancer, and diabetics.[104] A particularly high prevalence of cryptosporidiosis occurs in patients with X-linked hyper-IgM syndrome. In two case series of hyper-IgM syndrome, the prevalence of cryptosporidiosis was 24 percent.[95,135]

TRANSMISSION

Humans can acquire *Cryptosporidium* infections through several transmission routes, such as direct contact with infected individuals (person-to-person transmission) or animals (zoonotic transmission) and ingestion of contaminated water (water-borne transmission) and food (food-borne transmission). The relative importance of these transmission routes varies from country to country.[248,249]

The infectious dose of *Cryptosporidium* is low, but considerable variability exists among isolates, ranging from approximately 1000 oocysts (UCP strain) to less than 10 oocysts (Texas isolate).[60,173] Because the infectious dose is low, *Cryptosporidium* is transmitted easily from person to person. This route of transmission was recognized initially in outbreaks associated with contact with daycare centers[8,48,96,244] and with nosocomial transmission.[122,166,211] Secondary transmission within households also is common.[141] During the acute diarrheal illness, large quantities of oocytes are excreted in stool and are highly infectious. Asymptomatic shedding of oocytes may continue for 5 weeks after an acute episode of diarrhea.[222] Contact with an ill individual is a major risk factor for cryptosporidiosis.[189,193] Sexual transmission has been postulated to occur in association with anogenital sex.[97]

Numerous outbreaks have been attributed to animal contact.[158,184] *C. parvum* (bovine genotype) is thought to infect primarily domestic animals, with zoonotic transmission to people. In England, most endemic cases of cryptosporidiosis are caused by *C. parvum*.[147] In addition to cattle, sheep, pigs, and pets have been implicated in zoonotic infection.[36,147,159,246,247]

Cryptosporidium oocysts are resistant to environmental conditions; oocysts can remain infectious for at least 6 months if kept moist,[65] but viability decreases rapidly with desiccation.[194] Oocysts are killed by heat, pasteurization, hydrogen peroxide, ozone, and ultraviolet radiation.[63] Oocysts are highly resistant to chlorination, however.[123] Surveys have shown that most surface sources of drinking water are contaminated with oocysts before treatment,[131,200] and even a low-grade contamination has been documented in samples of treated water.[130,200]

Water-borne outbreaks of cryptosporidiosis have been linked to contaminated drinking water, such as water from artesian wells, surface water, and filtered public drinking water,[56,64] and to contaminated recreational water, such as swimming pools, water parks, lakes, rivers, beaches, and fountains.[22,64,132] The largest documented water-borne outbreak of diarrhea occurred in Milwaukee in 1993, affecting an estimated 400,000 individuals.[140] Many of the water-borne outbreaks, including the outbreak in Milwaukee, have been caused by *C. hominis*.

Food-borne infections occur less commonly. Outbreaks have been associated with contaminated apple cider, unpasteurized milk, chicken salad, and raw produce.[64,79,156,190] Oocysts are found commonly on vegetables in developing countries.[177] *Cryptosporidium* is associated with international travel[120] and causes approximately 2 percent of traveler's diarrhea.[110]

PATHOLOGY AND PATHOGENESIS

In immunocompetent hosts, the organisms are localized primarily to the distal small intestines and proximal colon. In immunodeficient hosts, the parasites have been identified throughout the gut, in the biliary tract, and in the respiratory tract.[46,85,118] Children with persistent cryptosporidiosis may have villous atrophy and a mild increase in lamina propria lymphocytes.[183] Heavier infection is associated with villous atrophy; crypt hyperplasia; marked infiltration with lymphocytes, plasma cells, and neutrophils[46,80,83,85,138]; and with extraintestinal involvement.

Cryptosporidium causes watery diarrhea and malabsorption. These symptoms are thought to be related to sodium malabsorption, electrogenic chloride secretion, and increased intestinal permeability. The profuse, watery diarrhea suggests a toxin-mediated illness. Some investigators have reported enterotoxic activity in fecal samples from patients with cryptosporidiosis.[89] No secretory activity has been detected in formal studies, however.[89,119]

Cryptosporidiosis also is characterized by defects in intestinal permeability. Increased permeability may result in decreased absorption of fluids and electrolytes and solute fluxes into the gut. *Cryptosporidium* infection directly induces defects in intestinal epithelial cell barrier function in vitro.[2,86,199] Studies in children with cryptosporidiosis have shown a correlation between the severity of disease and altered intestinal permeability.[251]

Infection of intestinal epithelial cells leads to activation of nuclear factor κB[43,146] and increased expression of proinflammatory cytokines and markers of inflammation, including tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), IL-8, CXCL10, and lactoferrin.[5,116,121,126,196,214,245] The chemokines and cytokines recruit inflammatory and immune cells into the intestines. Infection also leads to increased expression of cyclooxygenase-2, production of prostaglandins by the epithelial cells, and production of neuropeptides by the inflammatory cells.[127,198] Prostaglandins are thought to mediate decreased sodium and to stimulate chloride secretion.[18,47,116,127] Prostaglandin inhibitors have not proven to be effective symptomatic therapy in human cryptosporidiosis, however. The neuropeptide substance P correlates with severity of diarrhea in patients with cryptosporidiosis and AIDS.[198] Octreotide, a substance P antagonist, partially suppresses diarrhea in patients with chronic cryptosporidiosis.[18,88]

Cryptosporidiosis leads to villous atrophy and crypt hyperplasia.[17,28,47,83,124,163,183] Increased epithelial cell apoptosis has been shown in cells from tissue cultures and in biopsy specimens from infected intestines.[138] Loss of villous surface area is associated with malabsorption.[46,83,124,215] Patients with AIDS and severe cryptosporidiosis have malabsorption of bile acids, vitamin B_{12}, and fatty acids,[83,191,213] which is partly responsible for the severe wasting seen in these patients.

IMMUNOLOGY

Innate and acquired immunity are important in the defense and clearance of *Cryptosporidium*. $CD4^+$ T cells play a key role in the control of cryptosporidiosis. Among patients with HIV infection, cryptosporidiosis is self-limited in patients with $CD4^+$ cell counts greater than 180/μL, chronic in patients with $CD4^+$ cell counts less than 100/μL, and fulminant in some patients with $CD4^+$ cell counts less than 50/μL.[27,68,93,144] Resolution of cryptosporidiosis in patients with AIDS in response to highly active antiretroviral therapy is associated with an influx of $CD4^+$ cells into the intestines.[212] The role of other cell populations has been less clear.

Hyper-IgM syndrome, owing to defects in CD40 ligand, is associated with increased frequency and severity of *Cryptosporidium* infection.[95,135] Hyper-IgM syndrome is associated with profound defects in the ability of antigen-presenting cells to produce IL-12 and TNF-α and to stimulate production of interferon-γ (IFN-γ).[107]

IFN-γ is an important mediator of the immune clearance of *Cryptosporidium*. Depletion of IFN-γ causes exacerbation of infection[39,231,234]; inactivation IL-12, the major factor stimulating production of IFN-γ, causes chronic infection.[33,235] Treatment of intestinal epithelial cell lines with IFN-γ can activate the cells directly to clear partially *C. parvum* infection.[185] Lymphocytes from individuals who have recovered from cryptosporidiosis produced IFN-γ after antigen stimulation in vitro.[81] Approximately half of volunteers challenged with *C. parvum* express IFN-γ in the intestinal mucosa.[239] IFN-γ production by cells from patients with HIV during active cryptosporidiosis and from Haitian children with active cryptosporidiosis was very low, however, despite the fact that they had self-limited disease.[81,121] Similarly, IFN-γ expression in normal volunteers was limited to the subset with evidence of prior exposure.[239] In seronegative, normal volunteers experimentally infected and in patients with AIDS recovering from cryptosporidiosis, control of infection was associated with expression of IL-15.[176,197] This effect likely is mediated by activation of natural killer cells.[54]

In murine models, inactivation of IFN-γ expression resulted in only a mild chronic infection in BALB/c mice and was associated with expression of IL-12, IL-4, and TNF-α.[126,149,219] In the absence of IFN-γ, IL-12 treatment worsened cryptosporidiosis.[218] IL-4 synergizes with IFN-γ in eliminating infection of epithelial cells; however, IL-4 treatment did not modulate infection in IFN-γ knockout mice.[4,129,218] By contrast, TNF-α limited infection in IFN-γ knockout mice, activated human epithelial cells to limit infection, and has been associated with control of infection in cattle.[126,185,245]

The role of antibody in the immune response to cryptosporidiosis is controversial.[192] Early studies noted cases of chronic cryptosporidiosis in patients with low antibody levels, but, in most cases, studies were not performed to exclude coexisting T-cell dysfunction. In animal models, inactivation of B cells did not affect clearance of cryptosporidiosis.[40,223] By contrast, treatment with high concentrations of anti-*Cryptosporidium* antibody did facilitate clearance.[20,111,181,192,206] High levels of serum and fecal antibodies to *C. parvum* have been found in patients with AIDS with chronic cryptosporidiosis and in volunteers challenged with *C. parvum*.[24,49] The presence and timing of antibody correlated with shedding of oocysts, however, rather than with clearance or resistance to infection. Similarly, cytokines such as transforming growth factor-β that stimulate IgA production often develop only after resolution of illness.[195] In a longitudinal study of diarrheal disease in Peruvian children, the magnitude of the antibody response (IgG) was related to the number of previous infections with *Cryptosporidium*.[188]

CLINICAL MANIFESTATIONS

The clinical spectrum of disease caused by *Cryptosporidium* is broad and depends on the immunologic status of the host (Table 228–1). Symptoms of cryptosporidiosis develop after a prepatent period, during which the parasites invade the intestinal epithelium and proliferate. The prepatent period is approximately 1 week (range 1 to 30 days).[113,140,173,175]

Regardless of the immunologic status of the patient, diarrhea is the most common manifestation of cryptosporidiosis. In developing countries, cryptosporidiosis accounts for 5 to 10 percent of cases of childhood diarrhea.[3,108,121,162,168,169,208,211,228] Children present with an acute diarrheal syndrome characterized by watery diarrhea and crampy abdominal pain with frequent, foul-smelling, bulky stools.[209] Other, less common clinical findings include low-grade fever, flatulence, nausea, and vomiting. The stools rarely contain blood and fecal leukocytes, with occasional

TABLE 228–1 Clinical Manifestation of Cryptosporidiosis

Host	Clinical Manifestations	Comments
Normal host	Acute watery diarrhea	Relapses are common Persistent diarrhea is common
Children in developing countries	Acute watery diarrhea Persistent diarrhea	Diarrhea more severe in children with malnutrition Persistent diarrhea affects nutritional status, growth, and intellectual function
Immunocompromised host	Acute watery diarrhea Relapsing diarrhea Persistent/chronic diarrhea Cholera-like illness Extraintestinal involvement	Transient, self-limited, similar to normal host Very common Usually found in patients with low CD4 or malnutrition Voluminous watery diarrhea, only with very low CD4 Respiratory tract, biliary tract, and pancreas

TABLE 228–2 Diagnosis of *Cryptosporidium* Infection

	Method	Comments
Microscopic examination of stools	Modified acid-fast stain of stools	Inexpensive and widely available diagnostic test
	Fluorescent stains (auramine O, auramine-rhodamine)	Faster than other acid-fast stains and may improve sensitivity
	Immunofluorescent assays (IFA)	More sensitive than acid-fast staining but also more expensive
Antigen-detection assays	Enzyme immunoassay (EIA), and immunochromatographic tests: direct and indirect immunofluorescence assay (DFA and IIF)	Good sensitivity (66-100%) and excellent specificity (93-100%), but occasional lots associated with false positive tests
Molecular methods	Polymerase chain reaction (PCR)	Increased sensitivity compared to microscopic or antigen-detection studies

mucus. In immunocompetent patients, the onset of diarrhea is abrupt, and the illness usually is self-limited. Recurrent symptoms develop in 40 percent of cases after initial resolution. Relapses may occur after a diarrhea-free period of several days to weeks.[140,141,168] Forty-five percent of cases develop diarrhea lasting more than 14 days.[169,220]

Cryptosporidium is one of the most common causes of persistent diarrhea in developing countries, causing about one third of cases.[168,209,220,228] Children with persistent diarrhea, especially if caused by *Cryptosporidium*, are at high risk for developing additional gastrointestinal infections after the initial illness, weight loss, and premature death.[3,9,136,161] Persistent diarrhea in children seriously affects nutritional status, growth, and intellectual function.[25,90] A long-term follow-up study of children with onset of cryptosporidiosis before age 1 year suggests an association with poorer physical fitness and poorer cognitive development that persists for years.[90] In a second study, long-term effects of early childhood malnutrition were noted, but no significant association with *Cryptosporidium* compared with other pathogens was found.[25]

Cryptosporidiosis is more severe in children with malnutrition. Malnourished children tend to have a protracted course and shed oocysts longer. They often require hospitalization, and their diseases may have a fatal outcome.* Prospective cohort studies of children followed from birth have shown significant differences in nutritional status before acquisition of *Cryptosporidium* infection.[3,37,160] In addition, the onset of cryptosporidiosis is associated with growth faltering. Older children eventually recovered and experienced catch-up growth, but children infected before 1 year of age often never recovered.[3,37,160] Asymptomatic infection can lead to malnutrition even without diarrhea.[38] *Cryptosporidium* infection causes acute malnutrition, and the long-term consequences of this interaction are likely to be worse in children infected in infancy or with prior malnutrition.

*See references 9, 104, 108, 109, 139, 168, 207, 209, 211, 228.

The clinical manifestations of cryptosporidiosis in patients with HIV vary. Four distinct gastrointestinal syndromes have been described in HIV-infected patients: (1) transient, self-limited diarrhea, similar to diarrhea in normal hosts, usually in patients with $CD4^+$ cell counts greater than 150/μL; (2) voluminous watery diarrhea or cholera-like illness that requires rehydration, seen in a few cases, usually in patients with $CD4^+$ cell counts less than 50/μL; (3) relapsing diarrhea; and (4) a chronic diarrheal illness resulting in wasting, usually in patients with $CD4^+$ cell counts less than 150/μL.[27,93,138,144] Other conditions associated with *Cryptosporidium* infection include cancer, hypogammaglobulinemia, severe combined immunodeficiency,[104] bone marrow and renal transplantation, and concurrent viral infections such as measles and cytomegalovirus. The severity and duration of illness generally depend on the degree of immunodeficiency.

In immunocompromised patients, extraintestinal involvement may occur in the respiratory tract, biliary tract, and pancreas.[45,93,137,154,236] Respiratory tract involvement often is asymptomatic, but may cause cough, shortness of breath, wheezing, croup, and hoarseness. *Cryptosporidium* has been reported as a cause of laryngobronchitis in children.[92] Biliary tract involvement in cryptosporidiosis has been limited to patients with profound immunodeficiency.[93,236] Patients may present with acalculous cholecystitis or cholangitis and, less frequently, with sclerosing cholangitis or hepatitis.[93,224,236] Pancreatitis is an uncommon finding, but it has been reported in adults and children infected with HIV.[94,137,157] Concomitant infection with cytomegalovirus can occur and frequently is associated with irregularities of the intrahepatic duct.[23,101,115]

DIAGNOSIS

Many laboratories do not test for *Cryptosporidium* unless the tests are specifically requested (Table 228–2). *Cryptosporidium* infection usually is diagnosed by microscopic examination of stools. Fresh, preserved, and frozen stools can be used for testing.[15,19]

Numerous concentration methods have been attempted to improve the yield, but all are laborious and are used mainly in research laboratories.[19,44]

Traditional approaches to stool examinations usually miss the organism because the oocysts are small, 4 to 6 μm in diameter, and similar in size and shape to yeast forms normally found in stool. Several special staining methods have been explored, including auramine-rhodamine, auramine-carbolfuchsin, Giemsa stain, safranin–methylene blue stain, aniline-carbolmethyl violet, and acridine orange.[50] The stains that are used routinely to detect other intestinal parasites (trichrome and iron hematoxylin) do not detect *Cryptosporidium*.

Traditionally, the method of choice for clinical laboratories to detect *Cryptosporidium* oocysts in stools has been the modified acid-fast stain.[16,19,142] Oocysts stain pink or red, whereas yeast cells and fecal debris are green or blue (Fig. 228–2). The stain used most frequently is a modification of the Ziehl-Neelsen stain.[15,19] Many other modifications that have been described include hot and cold techniques, incorporation of dimethyl sulfoxide, and use of detergent. The sensitivity of stool examination with acid-fast staining is poor, requiring an oocyst concentration of greater than 500,000/mL in formed stools,[238] with fewer cases detected than with fluorescent methods.[7,112] Fluorescent stains (e.g., auramine O, auramine-rhodamine) can be read more quickly than other acid-fast stains and may have improved sensitivity.[226] These assays can yield false-positive results, however; all of the acid-fast stains detect other parasites that may cause similar illnesses (e.g., *Isospora* and *Cyclospora*).[15,19]

Immunofluorescent assays employing oocysts specific for monoclonal antibodies can be used to test for cryptosporidiosis. Immunofluorescent assays have been reported to be 10 times more sensitive than acid-fast staining.[53,71,106] Direct immunofluorescent staining using monoclonal antibodies now is the gold standard for stool examination.

Antigen-detection assays are being used increasingly for diagnosis of stools. Commercial kits for *Cryptosporidium* are available in enzyme-linked immunosorbent assay (ELISA) and immunochromatographic formats. The ELISA kits for *Cryptosporidium* have good sensitivity (66 to 100%) and excellent specificity (93 to 100%).[19,53,71-73,217a] The sensitivity has been poor in some studies,[105,112,167] however, and pseudo-outbreaks from false-positive results have been reported.[14,58] Commercial ELISA kits also may test for *Giardia* and *Entamoeba* antigens.[72] Immunochromatographic tests are rapid tests for *Cryptosporidium* and *Giardia* antigen.[73,112] The sensitivity is less than with other assays, but the specificity is excellent, and results are available in minutes.[73,112] Antigen assays as a group have the advantage of not requiring skills in microscopic identification of organisms.

Polymerase chain reactions for *C. parvum* DNA also have been employed to detect organisms. They also have increased sensitivity compared with microscopic or antigen-detection studies of stool.[21,148,217a] In one study of children with primary immunodeficiencies, polymerase chain reaction was able to detect significantly more cases of biliary cryptosporidiosis than were detected by stool studies.[148]

Biopsy of the intestine typically is unnecessary for the diagnosis of intestinal infection, although the unique apical location within the intestinal epithelium is distinctive. The parasite can be visualized by light microscopy after staining with hematoxylin and eosin or by electron microscopy.[50]

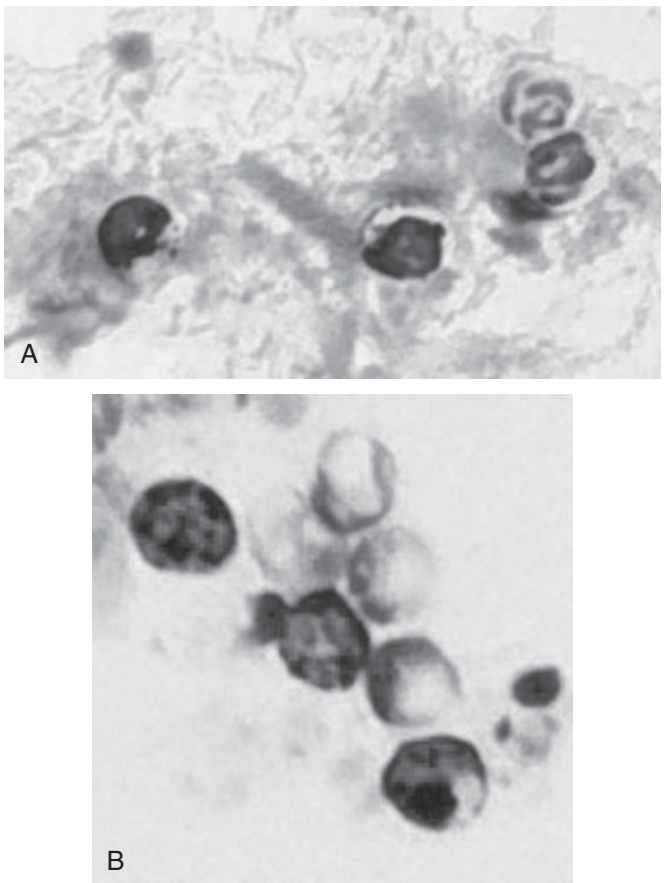

Figure 228–2 **A** and **B,** Staining of oocysts. (See companion Expert Consult web site for color version.)

MANAGEMENT

Replacement of fluids and electrolytes is a crucial first step in management, as with all cases of diarrhea. Oral rehydration is preferred, but severely ill patients may require parenteral fluids. Glutamine supplementation may improve fluid absorption.[34] Dietary management is crucial in cryptosporidiosis, especially if it is associated with persistent diarrhea. Supportive care should include initially a lactose-free diet because lactase activity is decreased on the apical border of epithelial cells.[183]

Cryptosporidiosis is associated with increased intestinal transit, which could interfere with absorption of fluids, electrolytes, and drugs.[29,215] Antimotility and antisecretory agents (i.e., tincture of opium, loperamide, octreotide, acetorphan) have roles in therapy, but their use generally is limited to refractory cases in adults with AIDS.[74,216] These agents may control the diarrhea, but they do not eradicate the parasite.

In immunocompromised hosts, management of intestinal cryptosporidiosis is problematic. For patients with AIDS with chronic cryptosporidiosis, effective antiretroviral therapy can result in dramatic improvement in diarrhea.[35,70,87,143,155,176] The HIV protease inhibitors have anticryptosporidial activity in vitro and reduced infection by 90 percent in an animal model,[102,152] and most of the studies reporting responses have used protease inhibitors. By contrast, cryptosporidiosis is associated with malabsorption of nucleosides.[30]

The role of antiparasitic therapy in cryptosporidiosis is controversial. Because cryptosporidiosis usually is self-limited in immunocompetent hosts and can be variable in compromised hosts, controlled trials are crucial. No agent has proven reliably curative in patients with advanced AIDS. A meta-analysis of seven randomized controlled clinical trials found no clear evidence for efficacy of antiparasitic agents in the management of cryptosporidiosis in compromised hosts.[1]

Nitazoxanide is a nitrothiazolyl-salicylamide derivative with a broad spectrum of antiprotozoal and antihelmintic activity.[241]

Nitazoxanide is active against *Cryptosporidium* in vitro and in animal models.[75,225] In the initial study in patients with AIDS and cryptosporidiosis, a 7-day course of nitazoxanide was associated with improvement in 7 of 12 patients, but diarrhea resolved completely in only 4.[59]

A placebo-controlled study of nitazoxanide in patients with cryptosporidiosis showed significant clinical improvement in apparently immunocompetent children and adults with persistent diarrhea.[202] By day 7, diarrhea had resolved in 39 of 49 (80%) patients treated with nitazoxanide compared with 20 of 49 (41%) treated with placebo. Nitazoxanide also reduced the duration of shedding of oocysts. In parallel randomized studies in severely malnourished HIV-infected and HIV-negative children in Zambia with chronic cryptosporidiosis, a 3-day course of therapy not only led to clinical and parasitologic improvement, but also improved survival in HIV-negative children.[10] All children were treated with nitazoxanide suspension (100 mg twice a day for 3 days) or matching placebo. Among non–HIV-infected patients, resolution by day 7 was noted in 14 of 25 (56%) of patients treated with nitazoxanide compared with 5 of 22 (23%) for the placebo group. Similar findings were reported in immunocompetent patients 12 years old and older. A good clinical response was seen in 27 of 28 (96%) patients receiving nitazoxanide compared with 11 of 27 (41%) receiving placebo.[204] Among the HIV-infected children, most with severe malnutrition, no significant response occurred.[10]

Higher doses and longer duration were effective in adults with HIV.[203] Among patients with $CD4^+$ cell counts greater than 50/μL, 10 of 14 (71%) responded to 1 g/day, and 9 of 10 (90%) responded to 2 g/day compared with 3 of 15 (20%) treated with placebo. The response was no better than placebo for patients with $CD4^+$ cell counts of 50/μL or less.[150] In expanded access programs, most patients improved on therapy.[201]

Nitazoxanide suspension was approved in the United States in 2002 for treatment of cryptosporidiosis and giardiasis in children. For most indications, it is dosed every 12 hours for 3 days. The recommended dose is 100 mg (5 mL) for children 12 to 47 months old, 200 mg for children 4 to 11 years old, and 500 mg for older children and adults (approximately 15 mg/kg/day).[240] Nitazoxanide generally is well tolerated; adverse events occur at a frequency similar to that for placebo and include abdominal pain, diarrhea, vomiting, and headache.[240]

Paromomycin is an orally administered nonabsorbable aminoglycoside. Initial in vitro studies noted poor activity against *C. parvum*. When patients with AIDS and cryptosporidiosis were treated with available antiparasitic drugs, however, some improved when treated with paromomycin.[78] Three randomized, controlled trials examined the effects of paromomycin in patients with AIDS and cryptosporidiosis.[98,117,242] Paromomycin was associated with a significant reduction in shedding of oocysts and decreased stool frequency, but not cure.[242] In another study, no significant difference was found between groups.[98]

Macrolide antibiotics including spiramycin, azithromycin, roxithromycin, and clarithromycin have some activity against *Cryptosporidium*.[26] In one study, children treated with spiramycin had shorter duration of symptoms and shedding of oocysts,[205] but a second trial showed no effect.[243] Case series noted improvement in cryptosporidiosis in patients with HIV and cancer treated with azithromycin,[57,99,114,165] but subsequent trials in patients with AIDS did not show changes in frequency of stools or shedding of oocysts.[26] A study in Egyptian schoolchildren suggested more rapid resolution of symptoms was achieved with azithromycin.[6] Roxithromycin treatment was associated with improvement in AIDS-associated cryptosporidiosis in two uncontrolled studies.[221,232]

Case reports noted improvement in chronic cryptosporidiosis in patients treated with oral anti-*Cryptosporidium* immunoglobulin preparations.[84] Two well-controlled trials with oral bovine anti-*Cryptosporidium* immunoglobulin preparations in cryptosporidiosis showed no significant decrease in symptoms or shedding of oocysts, however.[172]

PREVENTION

In hospitals and daycare centers, handwashing is the most important measure to prevent the spread of any enteric pathogen. In hospitals, enteric precautions (washing hands, wearing gloves, and wearing gowns if soiling is likely) are important measures to prevent nosocomial spread.

Swimming pools are an important source of infection. Contamination of treated recreational water, such as a fecal accident in a swimming pool, should prompt aggressive measures, including closing the pool temporarily. Recreational waters, such as lakes, may pose a danger for compromised hosts, who should avoid contact with untreated water.[145]

Control of water-borne transmission of cryptosporidiosis is a major public health concern. This issue is complicated by the ability of *Cryptosporidium* to escape the filtration system used in most public water facilities and its resistance to chlorination. The Centers for Disease Control and Prevention recommends that during outbreak situations, compromised patients boil tap water for 1 minute, filter tap water using a filtration system to remove particles 1 μ or smaller, or use bottled water prepared by distillation or reverse osmotic filtration. In situations not associated with an outbreak, no special measures are recommended. Routinely using the outbreak measures outlined earlier for severe immunosuppressed patients may be prudent, however.[13] The Centers for Disease Control and Prevention web site contains an excellent patient fact sheet about *Cryptosporidium* and its prevention at www.cdc.gov/ncidod/dpd/parasites/cryptosporidiosis/. Currently, no vaccine is available to protect against *Cryptosporidium*.

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CHAPTER 229

Cyclosporiasis, Isosporiasis, and Microsporidiosis

Chaouki K. Khoury ✪ Jane T. Atkins

CYCLOSPORIASIS

Cyclospora cayetanensis is a coccidian parasite that infects the gastrointestinal tract of immunocompetent and immunocompromised patients.[73,137,207] This organism was described in feces from humans without enteritis in 1979.[7] The first documented cases of diarrheal disease attributed to *Cyclospora* were reported in 1986 in four immunocompetent patients who had traveled from the United States to either Haiti or Mexico.[164] At that time, it was referred to as an unsporulated, coccidian body or a fungal spore. Before the mid-1980s, reports of diarrheal illness caused by *Cyclospora* were rare. With the advent of the epidemic of acquired immunodeficiency syndrome (AIDS), this organism gained increasing recognition as an enteric pathogen.[137,159,207]

MICROBIOLOGY AND LIFE CYCLE

Cyclospora is a round-to-ovoid, variable acid-fast organism that measures 8 to 10 μm in diameter (Fig. 229–1).[130] It also has been referred to as a *Cyanobacterium*-like body, a blue-green alga, a coccidian-like body, *Cryptosporidium*-like, or big *Cryptosporidium*.[8,12,79,130,133,135,143,158,166] In 1993, Ortega and associates[130,133] assigned this organism to the family Eimeriidae and the genus *Cyclospora* and proposed the name *C. cayetanensis* for the species that infects humans. This classification was based on its sporulation characteristics, and the name was derived from the institution where the original research was performed (Universidad Peruana Cayetano Heredia in Lima, Peru).[206]

Cyclospora, similar to *Isospora*, sporulates exogenously and has two sporocysts per oocyst. It differs from *Isospora* in that it has two sporozoites per sporocyst, whereas *Isospora* has four per sporocyst. *Cyclospora* spp. are ubiquitous and infect various animals, including vipers, moles, rodents, and myriapods. Humans are the only known host of *C. cayetanensis*. Organisms resembling *C. cayetanensis* have been found in the stool of chimpanzees living in Uganda.[206]

Sexual and asexual stages of *Cyclospora* are found in jejunal biopsy specimens from infected individuals, showing that this coccidian parasite can complete its entire life cycle in a single host.[131] Infected patients pass unsporulated oocysts into the environment. A period outside the host is required for maturation into an infectious, sporulated oocyst. A warm, moist environment seems to favor sporulation in nature. In vitro, sporulation occurs 1 to 2 weeks after incubation in distilled water or 2.5 percent potassium dichromate at 25° C to 35° C.[163,169] Sporulation has been delayed by storage of oocysts at 4° C for 6 months or by storage at 37° C for 14 days, and can be completely aborted if oocysts are subjected to –20° C for longer than 24 hours or 60° C for longer than 1 hour.[162] In vivo, excystation of mature sporulated oocysts most likely occurs in the small bowel. In vitro, a combination of bile salts, sodium taurocholate, and mechanical pressure is required for excystation.[169]

EPIDEMIOLOGY AND TRANSMISSION

Cyclospora is distributed worldwide. Initial reports of cyclosporiasis involved residents of developing countries or travelers return-

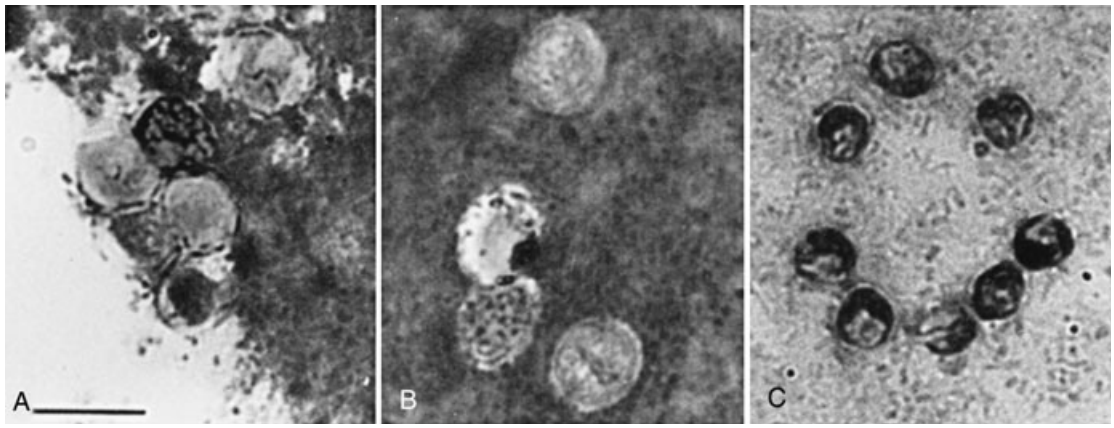

Figure 229–1 **A-C,** Modified acid-fast stain of *Cyclospora* sp. (**A**), *Cryptosporidium muris* (**B**), and *Cryptosporidium parvum* (**C**). Bar = 10 μm. *(From Ortega, Y. R., Sterling, C. R., Gilman, R. H., et al.:* Cyclospora *species: A new protozoan pathogen of humans. N. Engl. J. Med.* 328:*1308-1312, 1993.)*

ing from such countries.[78,130,133,143,158] It has been identified in individuals residing in or traveling to numerous geographic areas, including North, Central, and South America; the Caribbean islands; England; eastern Europe; Turkey; Egypt; India; eastern Asia; Nigeria; South Africa; and Australia.[3,4,15,48,152,163,169,209] In one study of diarrheic stool from subjects returning from tropical areas, *C. cayetanensis* was identified in 5 percent of samples, rendering it the second most common pathogen after *Giardia*.[186] Seasonal variation has been described. In Nepal, most cases occur during the rainy season, between May and October.[78] In Peru, the peak is during the fall, between April and June.[133] The prevalence in endemic regions varies with the age and immunologic status of the host. Prevalence rates in children from Peru and Nepal are reported to range from 2 to 18 percent.[77,133] In one series from Haiti, *Cyclospora* was reported in 11 percent of adults infected with human immunodeficiency virus (HIV), but was not detected in non–HIV-infected adults or infants younger than 6 months.[137]

Direct person-to-person transmission is unlikely to occur because a period outside the host is required for maturation into a sporulated oocyst.[169] The infectious dose of *Cyclospora* is unknown, but is hypothesized to be low, as with other coccidian parasites. Contamination of the environment with oocysts plays a major role in the transmission of *Cyclospora*. Food-borne and water-borne transmission of *Cyclospora* have been described.[169] Water-borne outbreaks have been reported in Chicago and in Nepal.[80,135,206] In one area of Egypt, the average prevalence of *Cyclospora* spores in stool samples was 5.6 percent in patients with diarrhea, in contrast to 2.3 percent in asymptomatic subjects, with higher prevalence in places with higher water contamination.[48] High levels of aquatic contamination also were documented in Hanoi (Vietnam), reaching 63.6 percent in certain areas, again emphasizing the important role of contaminated water in the spread of the pathogen.[118]

Food-borne transmission of this organism via the consumption of uncooked meat and poultry was suspected in 1979.[7] In recent years, an increasing number of cases of food-borne outbreaks have been reported.[73-75] Widespread recognition of this organism as a food-borne pathogen occurred in 1996 and 1997, when outbreaks of cyclosporiasis occurred in the United States and Canada and were associated with the consumption of raspberries imported from Guatemala.[73-75] Researchers postulated that the raspberries were contaminated with insecticide or fungicides that were mixed with contaminated water, or that the irrigation water was contaminated with *Cyclospora* oocysts.[134,163] During 1997, clusters of cases were identified in which fresh basil and mesclun lettuce were implicated.[144] Eleven food-borne outbreaks occurred in North America between 1990 and 2000, affecting 3600 people.[107] *Cyclospora* oocysts contaminating vegetables are not removed easily by routine washing.[132]

CLINICAL MANIFESTATIONS

Cyclospora causes disease in immunocompetent and immunocompromised patients.[133,137,143,158,206] *Cyclospora* spores also have been detected along with other pathogens, such as with *Giardia* in stool,[95] or rarely with tuberculosis in sputum.[82]

The incubation period typically is 2 to 11 days. In the Chicago outbreak, cases occurred 12 hours to 7 days after the suspected contamination of the water supply occurred.[135] In an immunocompetent host, diarrheal symptoms may last 7 weeks and may be remitting.[206] A cyclic, relapsing pattern of diarrhea alternating with constipation may occur.[163] Resolution of symptoms usually correlates with disappearance of the organism from the stool.[78,138] Asymptomatic excretion of cysts has been reported, however.[12,143] In one prevalence study in a Venezuelan community, *C. cayetanensis* spores were identified in 6.1 percent of subjects, with an asymptomatic carrier state in 84.6 percent of these.[26]

In immunocompromised hosts, the duration of diarrhea is highly variable, ranging from a few days to several months; in most cases, it is protracted. The illness is characterized by an abrupt onset of watery diarrhea. Flulike symptoms, including malaise, myalgia, and anorexia, also occur.[27,206] Low-grade fever is reported in approximately 25 percent of patients.[135,158,206] Vomiting may occur, but is less common than diarrhea. Other associated symptoms include abdominal cramping, heartburn, and indigestion.[163] Weight loss from malabsorption occurs in immunocompetent and immunocompromised patients.[78,164,207] Cyclosporiasis also was described in an infant, in whom it presented as irritability and refusal to feed.[83]

The median duration of disease in various U.S. outbreaks has been 10 to 20 days (range 1 to 60 days).[73] In HIV-infected patients, *Cyclospora* causes symptoms that are indistinguishable

from symptoms of *Cryptosporidium* and *Isospora* infection.[206] Biliary disease was reported in two patients with AIDS infected with *Cyclospora*. These patients had clinical and radiographic confirmation of biliary disease and did not have evidence of infection with other pathogens. Both patients had acalculous cholecystitis that was responsive to therapy with trimethoprim-sulfamethoxazole.[159] Finally, *Cyclospora* may play a role as a pulmonary pathogen in immunocompromised patients.[82]

DIAGNOSIS

Cyclospora, in contrast to *Cryptosporidium*, can be visualized by light microscopy after formol-ether concentration of the stool. In fresh stool, the oocysts are unsporulated. They appear as refractile spherical bodies measuring 8 to 10 μm and have a central greenish morula that contains six to nine refractile globules.[130] Safranin staining enhances the outline of the membrane, but does not stain internal structures. The sensitivity of wet mounts is 75 percent.[137] Oocysts exhibit bright blue autofluorescence when exposed to ultraviolet light. Staining is variable with modified Ziehl-Neelsen stain (see Fig. 229–1).[133] Fluorescence with auramine-rhodamine staining enhances the visualization of internal structures, but this stain usually is weak and irregular. Lacto-phenol cotton blue staining also allows visualization of the oocysts.[139] *Cyclospora* is not visualized by Gram, Giemsa, Grocott-Gomori methenamine–silver nitrate, Lugol iodine, periodic acid–Schiff, or hematoxylin and eosin staining.[139,206]

A direct comparison of formalin-ether sedimentation, direct smear examination, and sucrose centrifugal flotation yielded the highest recovery of *Cyclospora* oocysts in stool with the last method.[93] More recently, real-time polymerase chain reaction (PCR)[183,186] and flow cytometry[44] were advocated as an operator-independent, relatively rapid quantitative tool for the screening of stool samples for *Cyclospora* oocysts that seems to be more sensitive than microscopy. Real-time PCR may detect 1 oocyst/5 μL volume.[183]

TREATMENT

Disease seems to be self-limited in immunocompetent hosts. When treatment is indicated because of the severity or persistence of symptoms, trimethoprim-sulfamethoxazole is the drug of choice. In a small, uncontrolled study, therapy with trimethoprim-sulfamethoxazole resulted in resolution of symptoms and reduction of the duration of shedding oocysts from 9 to 13 days.[106] In a placebo-controlled trial involving travelers to Nepal, trimethoprim-sulfamethoxazole given for 7 days eradicated *Cyclospora* from the stool in 96 percent of patients.[78] In contrast, 88 percent of the placebo group still had detectable *Cyclospora* in the stool at the end of 7 days. Eradication of the organism correlated with resolution of symptoms. In one study, patients with *Cyclospora* did not improve when treated with empiric therapy (norfloxacin, tinidazole, quinacrine, nalidixic acid, and diloxanide furoate)[158] that was aimed at other enteric pathogens. Prophylaxis with trimethoprim-sulfamethoxazole 3 days a week seems to prevent recurrent episodes in HIV-infected patients.[137]

ISOSPORIASIS

Isospora belli is an enteric coccidian parasite that is related closely to *Toxoplasma*, *Cryptosporidium*, *Sarcocystis*, and *Cyclospora* (Fig. 229–2). Numerous species of *Isospora* infect reptiles, birds, and mammals. *I. belli* is the only species that infects humans.[49] *Isospora*, similar to the other enteric coccidian parasites, causes a self-limited diarrheal illness in immunocompetent hosts and a prolonged diarrheal illness in immunocompromised hosts. Cases of human isosporiasis were first reported in 1915.[200,205] Similar to the other enteric coccidian diseases, isosporiasis was reported infrequently before the AIDS epidemic.

MICROBIOLOGY AND LIFE CYCLE

I. belli is a monoxeonous coccidian parasite that is related closely to *Cyclospora*.[84] The mature oocyst of *I. belli* is oval-shaped, measures 10 to 20 × 20 to 33 μm, and has a translucent thin wall that contains two round sporoblasts, each with four crescent-shaped sporozoites (Fig. 229–3).[28] Infection occurs after the ingestion of a mature sporulated oocyst. Excystation occurs in the proximal part of the small intestine and results in the release of sporozoites that invade enterocytes of the distal duodenum and proximal jejunum and develop into trophozoites. The trophozoites reproduce asexually to form merozoites. The merozoites undergo asexual replication (schizogony or merogony) or sexual replication (gametogony) that results in the production of an immature unsporulated oocyst. This latter form is excreted in stool and requires 12 to 48 hours to mature into the infectious form, a sporulated oocyst.[17]

EPIDEMIOLOGY AND TRANSMISSION

The true prevalence of *I. belli* is unknown. It is more common in the tropical and subtropical regions[50a,104] of Africa, Southeast Asia, and Central and South America. In North America, it has been implicated as a cause of diarrhea in institutionalized patients[86] and as a cause of traveler's diarrhea.[66,157] Before the AIDS pandemic, *Isospora* was an uncommon cause of diarrhea, even in endemic regions. Between 1976 and 1980, *Isospora* oocysts were detected in 0.17 percent of stool specimens from 1139 Southeast Asian refugees.[17] *Isospora* is found in 3 to 18 percent of patients with AIDS from developing countries and in less than 0.2 percent of patients with AIDS from the United States and Europe.[81,136,165] In Haiti, *Isospora* accounts for 15 percent of chronic diarrhea in patients with AIDS.[37] It is thought to be the most frequent human coccidiosis.[39]

The consensus is that humans are the only host of *I. belli*, and that no animal reservoirs exist. Organisms resembling *I. belli* have been identified in the stool of dogs.[65] Transmission is by the fecal-oral route. Sexual transmission of *Isospora* also has been suggested.[55] Infection occurs after the ingestion of oocysts in fecally contaminated food or water or from environmental sources.[55,138,165] The incubation period is thought to be 3 to 14 days.[17,72] The infectious dose has not been established. In one investigation, symptoms developed in a single volunteer after 3000 organisms were ingested, but they failed to develop with a second challenge.[111] If untreated, the organism is shed in the stool for 11 to 120 days.[72] The oocysts of *I. belli* are highly resistant to commonly used disinfectants and may remain viable for months in a cool, moist environment.

PATHOPHYSIOLOGY

The pathologic findings in patients with isosporiasis are nonspecific. Histopathologic changes include shortening of the villi; hypertrophy of the crypts; and infiltration of the lamina propria with plasma cells, lymphocytes, polymorphonuclear leukocytes, and eosinophils.[17,20,28,136,176] All stages of the life cycle have been identified within the villous epithelium (Fig. 229–4) and always are enclosed within a parasitiferous vessel.[20,136,176] Extracellular

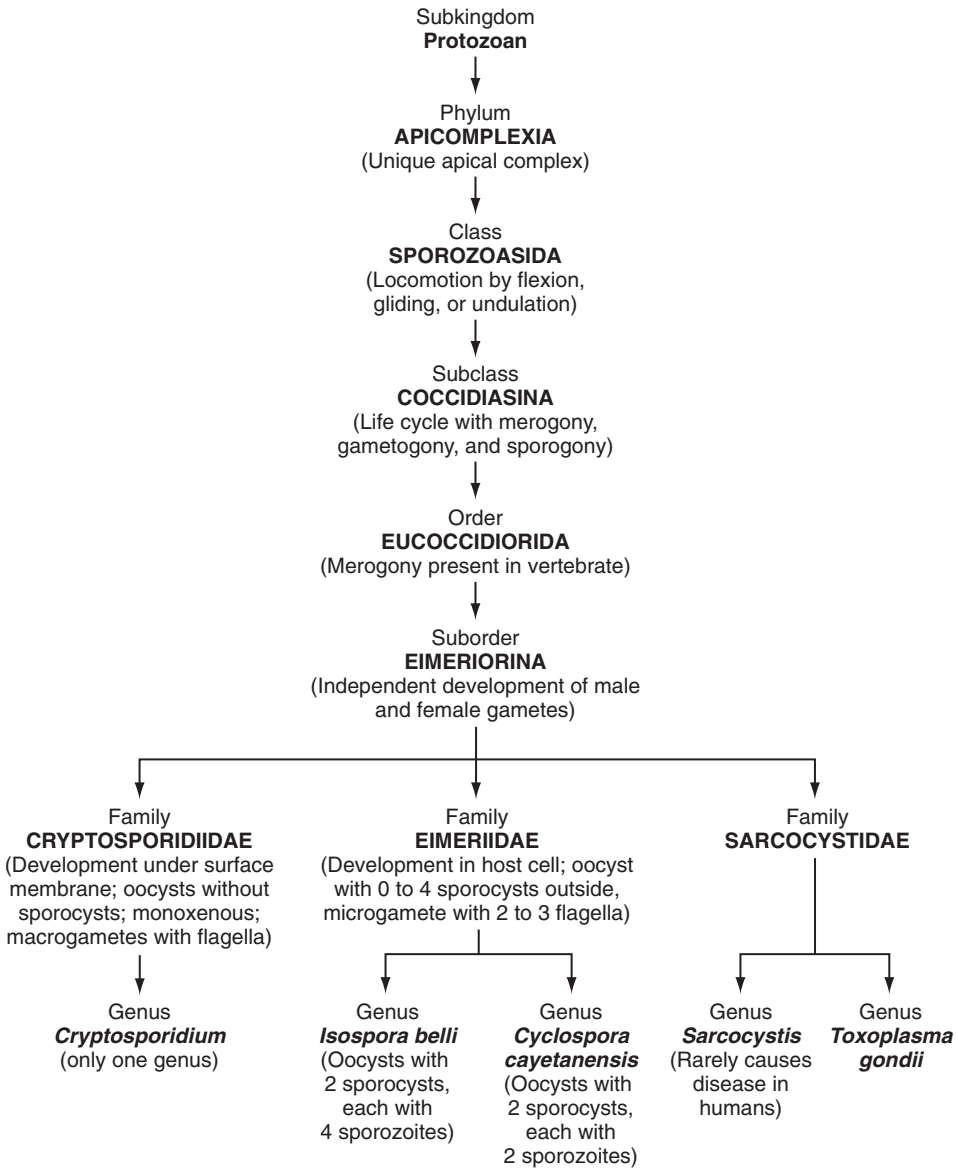

Figure 229–2 Taxonomic classification of *Cryptosporidium*, *Isospora*, *Cyclospora*, and *Sarcocystis*.

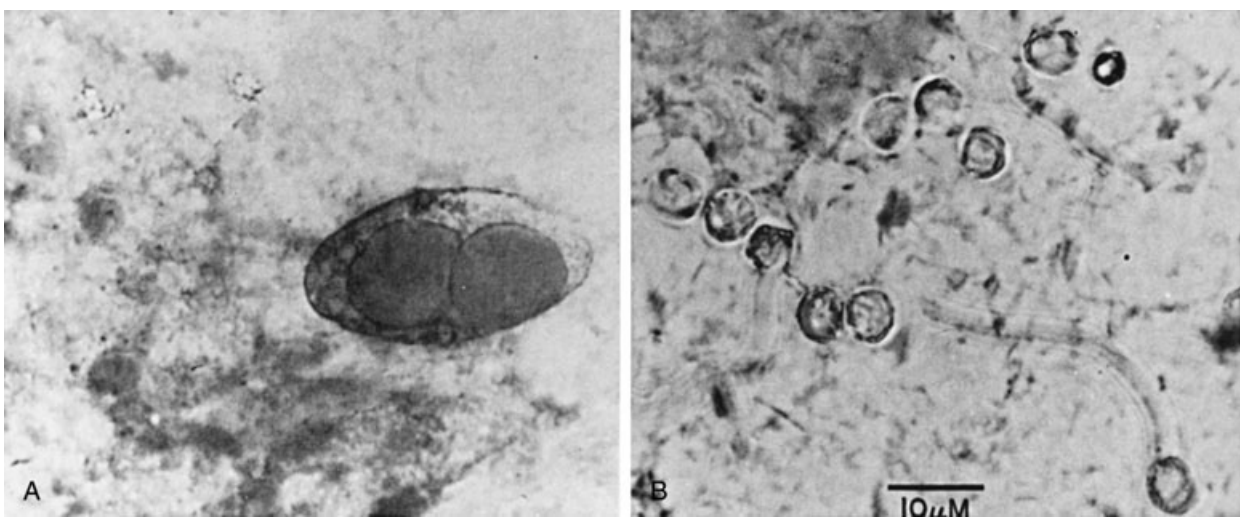

Figure 229–3 Unconcentrated fresh stool sample from two patients with AIDS stained with modified Kinyoun stain. **A,** *Isospora belli*. **B,** *Cryptosporidium*. Note the difference in size and shape of the two coccidian parasites; *Isospora* averages 25 × 15 μm and contains two sporoblasts, whereas *Cryptosporidium* averages about 5 μm in diameter. *(From DeHovitz, J. A., Pape, J. W., Boncy, M., et al.: Clinical manifestations and therapy of* Isospora belli *infection in patients with the acquired immunodeficiency syndrome. N. Engl. J. Med.* 315:87-90, *1986.)*

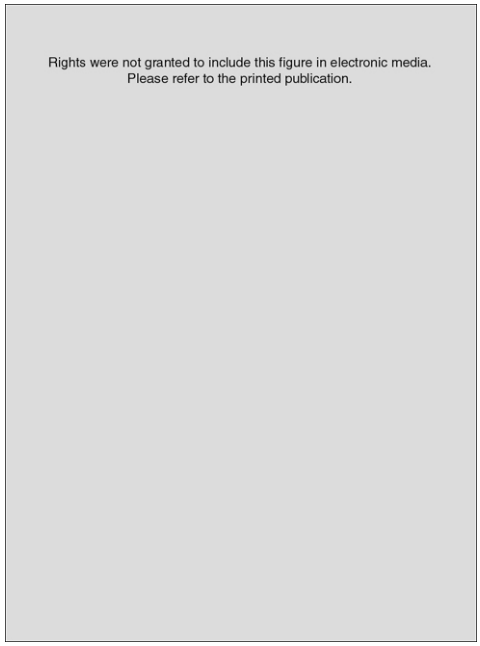

Figure 229–4 *Isospora* infecting the jejunum of a patient with severe diarrhea. Trophozoites (T) divide within enterocytes by schizogony to form merozoites (M). *(From Garcia, L. S., Owen, R. L., and Current, W. L.: Isosporiasis.* In *Balows, A., Hausler, W. J., Jr., Ohashi, M., and Turano, A. [eds.]: The Laboratory Diagnosis of Infectious Diseases: Principles and Practice. Vol. 1. New York, Springer-Verlag, 1988, pp. 899-903.)*

merozoites rarely are found in the intestinal lumen or the lamina propria.[28]

Isosporiasis is characterized by massive fluid loss suggestive of toxin-mediated hypersecretion, but no enterotoxin has been identified.[136] The pathophysiology of isosporiasis has not been defined; however, cell-mediated immunity seems to be important in the pathogenesis of villous changes. Activated T cells are mitogenic to enterocytes, and this mitogenic activity results in crypt hyperplasia, which leads to villous atrophy. Local T-cell activation results in the release of lymphokines, increase in intraepithelial lymphocytes, and enhanced expression of HLA-DR antigens on enterocytes of the villi and crypts. Intestinal mast cells also may play a role in the pathogenesis by releasing a collagen IV protease.[21]

CLINICAL MANIFESTATIONS

The clinical findings are indistinguishable from the findings of other enteric coccidian parasites. The spectrum of disease ranges from asymptomatic infection to severe, protracted, life-threatening diarrhea. Immunocompetent patients usually have a self-limited illness that resolves spontaneously over several weeks.[136,165,203] Headache, malaise, vomiting, fever, and dehydration may be noted. Recurrent symptoms and chronic illness may occur.[104] The disease is more severe in infants and children than in adults,[103,175] and intractable diarrhea has been reported in infants.[86]

In patients with HIV/AIDS, *I. belli* infections occur more frequently when the $CD4^+$ count is less than 200 cells/mm^3, and asymptomatic infection is uncommon in this patient population (2% of all infections).[25] These patients tend to have a more severe illness that may be life-threatening. Other conditions that predispose to a more severe illness include alpha-chain disease, acute lymphoblastic leukemia, Hodgkin disease, human T-cell lymphotropic virus type 1–related T-cell leukemia, and non-Hodgkin lymphoma.[147,150] In these patients, the onset of illness is insidious and associated with nonspecific symptoms, such as low-grade fever, headache, malaise, myalgia, and anorexia.[37,136] Nausea, vomiting, and diffuse crampy abdominal pain also are present. The stool is watery and may contain mucus, but does not contain blood or leukocytes. Chronic intermittent diarrhea with a mean duration of 7.9 months (range 2 to 26 months) is the major clinical manifestation.[138] Dehydration occurs in 70 percent of patients and requires intravenous fluids in 10 percent. The average daily fluid loss is 2 L, with some patients losing 20 L.[136] Malabsorption, steatorrhea, severe weight loss (>10% of body weight), and lactose intolerance have been reported.[20,37,176,201] Charcot-Leyden crystals are a common finding in stool. Peripheral eosinophilia occurs in more than 50 percent of patients.[85,138] Nonspecific radiographic findings include prominent mucosal folds, thickening of the intestinal wall, and disordered motility.[165]

Biliary disease and extraintestinal manifestations of *I. belli* are rare, but have been reported in patients with AIDS.[21,117,148] The organism has been identified in tracheobronchial, mediastinal, and mesenteric lymph nodes; the spleen; and the liver.[117,148] Acalculous cholecystitis has been reported.[11] A case of coccidial endometriosis in an immunocompetent patient also was attributed to *I. belli* based on the microscopic identification of intracytoplasmic oocysts that contain sporoblasts.[39]

DIAGNOSIS

The diagnosis is made by identifying oocysts in stool or by visualizing the parasite in biopsy specimens of the small intestine.[17,138,165] Similar to *Cryptosporidium*, *Isospora* can be detected in stool by using modified acid-fast or auramine-rhodamine stain. Organisms are sparse, so concentration techniques such as zinc sulfate, hypertonic sodium chloride, formalin-ether sedimentation, and Sheather sucrose solution are used to enhance recovery.[136,165] The oocysts also can be identified in stool by using lacto-phenol cotton blue staining.[139] The thin translucent wall of the oocyst may be difficult to identify in stool preserved in polyvinyl alcohol.[108]

Isospora oocysts are distinguished easily from *Cryptosporidium* oocysts. They are oval, contain one or two sporoblasts, and are 10 times larger than *Cryptosporidium* oocysts. In comparison, *Cryptosporidium* oocysts are round, contain four sporozoites, and measure 2 to 5 μm in diameter (see Fig. 229–3). The oocysts of *I. belli* exhibit autofluorescence with an ultraviolet epifluorescent illuminator (450 to 490 nm excitation filter).[108] The sensitivity of autofluorescence is significantly higher than that of light microscopy using iodine staining (95.7% versus 48.4%).[14] The sensitivity and specificity of the other methods for detection of *Isospora* in stool are unknown.[136] It is possible to detect the parasite by biopsy and not visualize oocysts in stool.[20] The parasite usually is found in the proximal part of the small bowel (Fig. 229–5). In patients with AIDS, it can be found in the small and large intestines.

TREATMENT

The treatment of choice is trimethoprim-sulfamethoxazole given four times a day for 10 days.[37,138,165,201] Patients who have been symptomatic for months usually have resolution in less than 1 week with this treatment. Pyrimethamine-sulfadoxine (Fansidar) is an alternative agent. For a sulfonamide-allergic patient, ciprofloxacin[185] and pyrimethamine[199] alone are alterna-

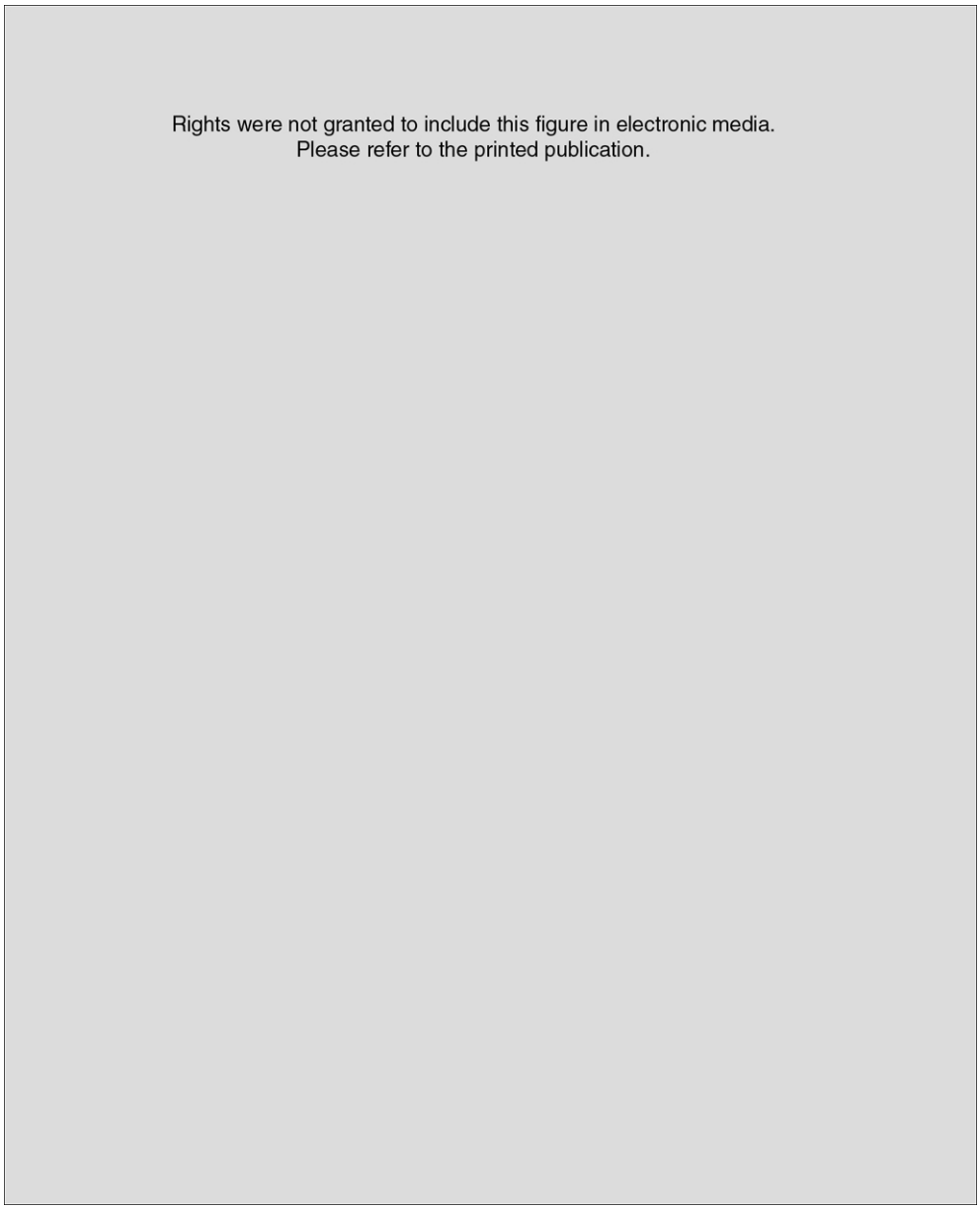

Figure 229–5 Jejunal biopsy from a patient with AIDS and severe diarrhea. Various developmental stages of the intestinal microsporidian *Enterocytozoon* infect almost every enterocyte. Stages include proliferative plasmodia *(1)*, early sporogonial plasmodia *(2)*, late sporogonial plasmodia *(3)*, and mature spores *(4)*. A spore *(arrow)* can be seen within a necrotic enterocyte, which appears ready to slough into the lumen. *(From Cali, A., and Owen, R. L.: Intracellular development of* Enterocytozoon*: A unique microsporidian found in the intestine of AIDS patients. J. Protozool. 37:145, 1990.)*

tive agents. Ciprofloxacin[185] is not as effective as trimethoprim-sulfamethoxazole and is not approved by the U.S. Food and Drug Administration for children younger than 18 years. Data on the use of macrolides and metronidazole are limited to uncontrolled studies.[55,104]

Fifty percent of patients with AIDS and isosporiasis have recurrences.[37,138] Prophylaxis with trimethoprim-sulfamethoxazole, (160 mg/800 mg) three times a week, or sulfadoxine-pyrimethamine, (500 mg/25 mg) once a week, is necessary to prevent relapse.[138] Ciprofloxacin can be used as an alternative prophylaxis agent for sulfonamide-allergic adolescent patients older than 17 years.[185] Pyrimethamine is an alternative prophylaxis agent, but data on its use are limited, especially in pediatric patients.[199]

MICROSPORIDIOSIS

Microsporidiosis is caused by an extensive group of obligate intracellular, phylogenetically ancient spore-forming parasites that infect vertebrate and invertebrate animals.[10,193] In humans, microsporidia were detected first in cerebrospinal fluid in 1959 in Japan in a child with seizures.[110] This infection has been reported infrequently in normal hosts. The number of cases described has increased since 1985, after recognition of the diarrhea and wasting syndrome in patients with AIDS. The pathogenic role in other immunocompromised individuals remains to be studied, as do the mode of transmission and sources of infection. The broad range of clinical manifestations includes kerato-

conjunctivitis, sinopulmonary infection, myositis, cholecystitis, hepatitis, and disseminated infection with tubulointerstitial nephritis.[193]

MICROBIOLOGY AND LIFE CYCLE

Microsporidians were reclassified more recently. Based on genomic and phylogenetic studies, they now are part of the domain Eukaryota, supergroup Opisthokonta, first rank subgroup Fungi, second rank subgroup Microsporidia.[2] They are characterized by the presence in the spore stage of an extrusion apparatus with a polar tubule that allows transfer of protoplasmic material into the host cell to occur.[155,193] The number of genera and species known to cause human disease increases as diagnostic abilities improve.[172] In addition, in vitro studies of a nonhuman microsporidian, *Tubulinosema ratisbonensis*, show that it is able to infect human cell lines, indicating that it could cause opportunistic zoonoses when the host immune defenses are impaired.[56] To date, nine genera have been identified that infect humans: *Enterocytozoon*, *Encephalitozoon*, *Nosema*, *Trachipleistophora*, *Vittaforma*, *Pleistophora*, *Anncaliia*, *Brachiola*, and *Microsporidium*.[38,109]

The genus *Microsporidium* groups together all unclassified microsporidia. Subclassification of these organisms traditionally has been based on morphologic characteristics of the stages of the parasite. More recent advances in differentiation are based on phenotypic (protein antigenic patterns) and genotypic characteristics as determined by PCR techniques, small-subunit ribosomal RNA patterns, restriction fragment length polymorphism, and subspecies-specific monoclonal antibodies.[36,70,120,153,173,187] These studies have allowed the genus *Septata* to be reclassified into the previously known *Encephalitozoon*.[36] *Encephalitozoon hellem* is differentiated from *Encephalitozoon cuniculi* by antigenic and molecular analysis.[123,151] A final taxonomic classification may develop after more precise molecular analysis is conducted.[193]

These spore-forming unicellular organisms are true eukaryotes. Historically, they were thought to lack mitochondria, and their small ribosomal RNA and ribosomes caused them to be thought of as an early phylogenetic group that diverged from prokaryotes before the endosymbiosis of mitochondria by the other eukaryotes. Nonetheless, their nuclear genes encode mitochondrial heat shock protein Hsp70, and anti-Hsp70 antibodies show localization within a small organelle with a double membrane, reminiscent of mitochondria, although it has lost its aerobic function.[202]

The asexual life cycle in the host cell is divided into two phases: the merogonic or proliferative vegetative phase and the sporogonic phase, which results in the production of mature spores.[155] Intracellular stages proliferate by binary or multiple fission and appear as multinucleated elements that can be in direct contact with the cell cytoplasm (e.g., *Enterocytozoon bieneusi*, *Nosema* spp.) or engulfed in a parasitiferous vacuole (*Encephalitozoon* spp.). In the case of *Encephalitozoon intestinalis*, septa are formed by a fibrillar structure.[193] The spore consists of a surrounding protective three-layered wall and internal infective nucleated material that is injected into the host cell by extrusion of a coiled polar tubule attached to an anchoring disk. The average size of the vegetative stages ranges from 2 to 6 × 1 to 3 μm, and the average size of the spores ranges from 0.7 × 1.64 μm (*E. bieneusi*) to 3 × 5 μm (*Nosema ocularum*). Cell culture systems are available for a few species, including *Nosema corneum*, *E. hellem*, and *E. intestinalis*.[36,70,122,156,180,196] These isolated organisms are useful for the development of specific antisera that may allow the microsporidial species to be differentiated.

EPIDEMIOLOGY AND TRANSMISSION

Precise data on the incidence, relevant reservoirs, source of infection, and mode of transmission are undefined. Symptomatic cases have been documented in non–AIDS-infected patients, and half of them had evidence of altered immune status.[68,191] Few cases of self-limited diarrhea have been reported in apparently normal hosts.[53,191] In HIV-infected patients, approximately 25 to 50 percent of cases of chronic diarrhea of undetermined etiology, or roughly 15 percent of the total cases of diarrhea, may be caused by these organisms.[16,97,123,181,182] The percentages have varied, depending on the population studied and the diagnostic techniques used. Young homosexual or bisexual adults[9,51,145,173] and severely immunosuppressed children with congenital HIV can be affected.[197] The prevalence of microsporidia (as detected by light microscopy) in the stool of HIV-infected patients is almost 4 percent in Lima, Peru.[171] *E. bieneusi* has been the most prevalent microsporidium and is found primarily in individuals with $CD4^+$ cell counts less than 100/mm^3.[3,41,51] Second in frequency are *E. intestinalis*, *E. hellem*, and *E. cuniculi*.[193] Asymptomatic infection and persistent carriage in immunosuppressed patients have been reported.[145] The other microsporidia infecting humans have been reported much less frequently.[40]

Microsporidia are ubiquitous in nature, and some of the species infecting humans are present in animals: *E. bieneusi* (pigs, nonhuman primates), *E. hellem* (parakeets), *E. cuniculi* (rabbits, dogs, horses), *E. intestinalis* (gorillas), and *Pleistophora* spp. (fish).[40,46,67,102] The major human microsporidian pathogens all have been identified in animals that commonly are in contact with human populations, such as pigeons in urban parks[69] or aquatic birds,[161] suggesting that microsporidiosis may be a zoonotic infection or may be transmitted via water, or both. The zoonotic potential of these organisms is supported by genotypic studies that show identical genotypes across species (e.g., human and monkey).[46]

Disease caused by contamination of water and food has been reported.[30] Ways of eliminating the infectivity of the microsporidian spores are being studied. Food that undergoes high-pressure processing of at least 345 MPa eliminates infectivity of the spores.[88] Similarly, treatment with commercial bleach, 70 percent ethanol, and the commercial disinfectants HiTor and Roccal also can eliminate spore infectivity. The duration of treatment necessary to do so increases as the solution used is diluted further.[87] Disinfection protocols for water also are being evaluated. They include filtration, coagulation, chlorination, gamma-radiation, and ozonation.[42]

An aerosol route of bronchopulmonary infection with species that are not found in the gut, such as *Encephalitozoon*, is supported by histopathologic findings in the bronchial tract.[196] Direct contact with conjunctival mucosa is postulated to be the source of ocular infections.[129] Serologic surveys using *E. cuniculi* antigens have found antibodies in multiple population groups, but they probably represent cross-reactivity with either various microsporidia species or other organisms.[155] *Nosema* is the only member of this group not reported in AIDS-infected patients; rather, it has been described as a disseminated infection in children with thymic aplasia and as local keratitis in normal hosts with or without previous trauma.[156,193] *Pleistophora* infection may be manifested as myositis infiltrating muscle fibers and may cause atrophy and degeneration.[99]

Co-infection with other opportunistic agents, including cytomegalovirus, *Mycobacterium avium–intracellulare*, *Cryptosporidium*,[113] and other parasites, has been reported in 5 to 60 percent of cases.[9,59,97,123,128,145] Unsuspected *Cryptosporidium* infection may be found in 20 percent of patients with microsporidia.[63]

PATHOPHYSIOLOGY

Despite the growing number of reports of microsporidiosis, the mechanism of disease in humans has remained largely unknown.[193] Latent asymptomatic infection or acute disease has not been described fully in humans. With the exception of local ocular disease, the parasite proliferates when immunologic defenses are defective, especially cell-mediated immunity.[45,122] The reason behind the different clinical pictures seen with the different microsporidioses also is unclear.[58] The immunopathogenic response of the human hosts to microsporidia has begun to be elucidated only more recently.

The portal of entry of the microsporidia into human cells always has been thought to be through cellular impalement by the deployed apical polar tube.[100] Some debate has ensued, however, about the role that parasite phagocytosis may play. Studies of *E. cuniculi* infection of human cell lines revealed that spores gain intracellular access through a parasitophorous vacuole more frequently than by polar tube impalement. Some of these spores would extrude their polar tube within the vacuole to gain access to the cytoplasm.[50,60] Whether or not this vacuole is a phagocytic vacuole is still debatable. Study of the membrane protein composition of the parasitophorous vacuole of *E. cuniculi* shows absence of host membrane proteins, especially markers of the endophagocytic pathway, 1 minute post-infection, the significance of which is unknown. If not phagocytic in nature, the origin of the parasitophorous vacuole remains to be elucidated.[50]

Other studies of human cell lines infected with *E. intestinalis* revealed that the parasite can gain access into mature cell lines only through impalement, where there was a modest role for phagocytosis into undifferentiated cell lines. The attachment of the parasite to the cell surface can be inhibited by chondroitin sulfate A (a sulfated glycan), and certain cells show a higher attachment propensity than others, which correlates with higher infectivity of these cells.[100] The use of exogenous nonsulfated glycans does not decrease attachment,[71] indicating a role for cell-surface sulfated glycosaminoglycans in mediating parasite attachment, which correlates with infectivity.[71,100] It is possible that the importance of cell membrane impalement with the polar tube as a means of entry into the host cells varies among the different microsporidian species.

As for the variability of the clinical picture with the different microsporidioses, cellular uptake studies showed a difference in the extent of uptake of microsporidian spores by different human cell lines, but all cell lines were able to internalize microsporidian spores. This finding suggests that a combination of cellular specificity and route of transmission might be at play in deciding what ensuing clinical picture might emerge.[58]

After entry into the cell, several pathogenic processes occur, the actual sequences of which remain to be elucidated. In vitro studies of infected macrophages revealed a specific pattern of inflammatory chemokine up-regulation 6 hours post-infection. These chemokines lead to a 2.9-fold increase in migration of naive cells about 48 hours post-infection. The different chemokines are involved in the recruitment of different cell types (monocytes, neutrophils, lymphocytes, eosinophils), consistent with the histopathologic observation of cell aggregates from different cell lines in patients with microsporidiosis. Neutralization of these chemokines resulted in blunting of the cellular chemotactic response. The fact that a delay occurs before the chemotactic response is noted could represent a parasitic immune-evasion characteristic, to provide enough time for invasion of and replication in human cells.[52]

Other studies showed that the different *Encephalitozoon* spp. inhibit caspase-3 cleavage and the activation of the p53 pathway, failing to activate the cellular apoptotic pathway, which allows it to replicate intracellularly.[38] The nitric oxide response similarly is inhibited.[57] Investigators also have shown that *E. cuniculi* expresses a microsporidial aquaporin (EcAQP) onto the surface of the spore and the infected cells. EcAQP is similar to the human aquaporin channel (AQP), but it lacks the cysteine residues that line AQP, which renders it resistant to mercury inhibition. This EcAQP is purported to mediate a rapid water influx into the spores, a step considered to be necessary for the infectious process.[64]

Finally, whether it is important for cell entry or extruded after entry into the cell, the polar tube plays an important role in the infectivity of the microsporidians. The polar tube of *E. hellem* consists of three proteins (PTP1, PTP2, and PTP3). PTP1 is the major protein. It is modified post-translationally by *O*-mannosylation, which seems to allow it to interact with some intracellular mannose-binding protein, which is essential for its infectivity because pretreatment of the cell culture with mannose decreased the infectivity of *E. hellem*. Presumably, this factor is due to competitive inhibition of the yet-to-be-identified cellular mannose-binding protein.[208]

These in vitro studies elucidate the first steps in understanding the microsporidioses. Whether the infected macrophages act as a carrier for dissemination of the microsporidians and what the subsequent steps are in the infection process remain to be elucidated.[52]

The different microsporidians exhibit diverse histopathologic characteristics. *E. intestinalis* can reach the submucosa; its presence in the kidneys and lower airways presumably results from systemic dissemination.[45,122] Mucosal injury, characterized by partial villus atrophy and crypt hyperplasia, correlates with xylose malabsorption and decreased activity of mucosal disaccharidases.[96]

Other types of encephalitozoonosis, including infection with *E. cuniculi* and *E. hellem*, have greater potential for dissemination.[153] *Encephalitozoon* infects macrophages and can disseminate to the liver, brain, kidneys, or sinuses and cause randomly distributed granulomatous lesions.[40] *E. cuniculi* has been reported in children with seizures,[13,110] hepatitis, peritonitis, and disseminated infection. It has been found in conjunctival and sinopulmonary infections associated with colonization of the intestinal tract.[61] The portal of entry for the organism in these cases is uncertain. *E. hellem* may involve the urinary tract, bronchial epithelium, and conjunctiva.[153] In sections of corneal scrapings, the cytoplasm of superficial epithelial cells contains vacuoles with the granular organisms, but these vacuoles cause minimal nuclear distortion.[22] *E. bieneusi* almost always is limited to the intestinal and biliary tract; it has a preference for enterocytes in the small intestine. The organism produces a limited inflammatory reaction and abnormalities in villi.[51] It rarely invades the lamina propria, but, exceptionally, respiratory epithelia may be infected.[195]

When infection occurs, the immune response that the host mounts determines the outcome. IgM antibodies against the polar tube seem to be protective. Their highest level is in young healthy adults, with levels decreasing with age. HIV-infected individuals with $CD4^+$ cell counts less than $250/mm^3$ do not produce these antibodies.[127]

CLINICAL MANIFESTATIONS

INTESTINAL AND BILIARY TRACT MICROSPORIDIOSIS

Typically, microsporidiosis manifests as chronic diarrhea and wasting syndrome in severely immunodeficient HIV-infected patients ($CD4^+$ cell count $<100/mm^3$).[9,52,123,173] AIDS-infected

patients with $CD4^+$ cell counts greater than 200/mm^3 tend to have self-limited diarrhea. In some cases, microsporidiosis is the AIDS-defining opportunistic infection.[112,128] Transplant recipients also are at risk for acquiring these pathogens.[30] The usual symptom is afebrile, loose to watery, nonbloody, nonmucoid diarrhea consisting of 3 to 20 bowel movements per day, which is worsened by food intake and associated with progressive weight loss, malabsorption, and anorexia.[9,129] Absorption of fat, D-xylose, and zinc is abnormal.[9,123] Persistent or intermittent symptoms may lead to severe cachexia by a combination of decreased intake and malabsorption.[173] Half of patients have abdominal pain; some complain of nausea and vomiting.[128] Affected children may have failure to thrive, chronic diarrhea, and intermittent abdominal pain.[197] Sporadic cases of *E. bieneusi* and *E. intestinalis* causing self-limited diarrhea with diffuse abdominal pain and nausea in immunocompetent individuals have been reported.[53,189,191]

Patients with cholangitis and acalculous cholecystitis have right upper quadrant abdominal pain. Imaging studies may reveal dilation of the intrahepatic and common bile ducts or irregularities of the bile duct and gallbladder wall. AIDS cholangiopathy is similar to cholangiopathy associated with cytomegalovirus and cryptosporidial infection.[142]

OCULAR INFECTION

Immunocompetent patients with histologically confirmed ocular infection with *Microsporidium ceylonensis*, *Nosema* spp. (*Nosema corneum* and *Nosema ocularum*), *Vittaforma corneae*, and *Trachipleistophora hominis* have been reported.[32,146,156,193] In India, the 2-year incidence of microsporidial keratoconjunctivitis in immunocompetent patients is approximately 0.4 percent, with a presentation duration of symptoms of approximately 1 week (range 1 day to 2 years). The infection in these patients is mostly unilateral and may be associated with previous trauma or bathing in contaminated water. The infection may lead to a progressive decrease in visual acuity secondary to severe corneal stromal disruption or corneal ulcer. The prognosis for full recovery usually is favorable.[91] The most common finding on slit-lamp examination in immunocompetent individuals is a stromal keratitis,[92] although atypical punctate epithelial keratitis with a mild nonpurulent conjunctivitis also has been reported.[91] Involvement of the posterior segment has been reported in a patient with longstanding psoriasis. She had sclerouveitis with retinal detachment caused by *Nosema*.[119]

Encephalitozoon spp. (in particular, *E. hellem*, but also others) are a cause of keratoconjunctivitis and scleritis in HIV-infected patients and other immunodeficient patients; symptoms in these patients include conjunctival inflammation, photophobia, blurred vision, a foreign body sensation, decreased visual acuity, and punctate epithelial keratopathy.[94,129,154] The lesion usually is bilateral and not associated with ocular trauma. Occasionally, ocular involvement is accompanied by evidence of disseminated infection.[68,94] Intrastromal keratitis caused by *Trachipleistophora anthropopthera* also has been reported in an HIV-infected patient.[140]

SYSTEMIC MICROSPORIDIOSIS

Disseminated systemic infection may develop in HIV-positive patients. *E. cuniculi* and *E. hellem* have been associated with tubulointerstitial nephritis, ureteritis, cystitis, conjunctivitis, and colonization or infection of the respiratory tract; these findings may occur in the absence of gastrointestinal symptoms.[36,68,187,191,196] Flank pain, hematuria, and dysuria are symptoms of urinary tract involvement, and progressive nonproductive cough, wheezing, and pleuritic pain are seen with lower respiratory involvement. Disseminated *E. cuniculi* infection involving the intestinal tract has been reported.[61] *E. intestinalis* infection usually is limited to the intestinal tract, although systemic manifestations consisting of urinary tract involvement (interstitial nephritis) and sinopulmonary dissemination may occur after invasion of the intestinal lamina propria.[45,68,122] Disseminated disease also may occur in other immunosuppressed, non–HIV-infected patients, such as kidney transplant recipients.[62] Serologic evidence (enzyme immunoassay and counterimmunoelectrophoresis) also shows that *E. intestinalis* causes central nervous system infection characterized by severe headache and seizures in HIV-infected patients.[182]

Disseminated *Nosema conorii* infection has been described in an immunodeficient athymic child with chronic diarrhea, fever, and weight loss. The parasite was found in the myocardium, diaphragm, kidney tubules, liver, and lungs.[155]

Severely immunosuppressed hosts may be predisposed to the development of myositis (caused by *Pleistophora ronneafiei*, *T. hominis*, or *Brachiola algerae*) with nonspecific symptoms of generalized muscle weakness or myalgias and elevated creatinine phosphokinase. The myocardium also may be involved, and the infection may be fatal in this instance. Muscle biopsy provides a definitive diagnosis.[23,24,31,33,101]

Sinusitis with a mucopurulent nasal discharge or lower respiratory tract involvement with bronchiolitis, pneumonia, and respiratory failure has been described with *E. bieneusi* and *Encephalitozoon* spp.[19,174,195] The source of the organism is unknown and may represent primary respiratory acquisition or secondary dissemination from other mucosal surfaces.[174]

DIAGNOSIS

A definitive diagnosis is made by direct morphologic demonstration of organisms in stool, body fluids (duodenal aspirates, bile, bronchoalveolar lavage fluid, nasal secretions, urine, conjunctival smears, cerebrospinal fluid), or tissue sections[46,68,122,123,193,194]; microsporidia sometimes can be cultured.[122] Light microscopy is reliable, although the small size and the staining properties of microsporidia render recognition difficult to establish. Electron microscopy usually is necessary to define the ultrastructural features of the different genera. Immunologic, molecular, antigenic, and biochemical analysis of isolated organisms can be performed if ultrastructure does not allow differentiation among similar species.[36,70,153,196]

Weber chromotrope–based stain,[192] with modifications[151] that allow better resolution from background, has shown good sensitivity and specificity for the analysis of unconcentrated stool samples and other body fluids, including urine.[9,35,41,45,68,196,198] Under oil immersion, the pinkish red spore wall of microsporidia must be differentiated morphologically from some yeast elements and bacteria.[173] Uvitex 2B[35,179,181] and calcofluor white[105] are useful, but fungi can give false-positive results.[41] Giemsa stain seems less satisfactory for stool analysis.[173] It is unclear whether intermittent shedding occurs, so multiple stool samples are needed for detection.[10,123] Acid-fast staining has been attempted with some success in the cytologic examination of centrifuged fluids.[142] In corneal scrapings, potassium hydroxide with calcofluor white and acid-fast stains yield the highest accuracy (96.7% and 93.3%).[89]

Recognition of microsporidia by light microscopy[96,123,195] in paraffin-embedded tissue sections has been reported with routine techniques, including hematoxylin and eosin,[129,153] tissue Gram,[129,153,173] periodic acid–Schiff, silver,[51] and Giemsa stains.[149,173] The accuracy of each technique is related to the intensity of infection[181] and the level of training of the individual performing the analysis. The spores often are on the surface of the villi; the multinucleated sporogonial stage appears as a col-

lection of granules that is confused easily with cytoplasmic organelles.[128,129] Some workers prefer touch preparations of small intestine stained with Giemsa.[10,123,160] Histologic examination of ultrathin plastic sections stained with toluidine blue or methylene blue azure II fuchsin stain may increase the sensitivity.[129,173] Differentiation of the spores by immunofluorescence assay may allow investigators to identify the species.[6,63,154,192,196] Monoclonal antibodies produced against isolated microsporidia may permit speciation in cytologic and histologic analysis.[5,187] Cross-reactivity of *Encephalitozoon* antisera has been used in establishing the diagnosis of *E. bieneusi* infection in stool and intestinal biopsy tissue.[5,210] In ocular microsporidiosis, conjunctival and corneal scrapings or biopsy samples prepared with Giemsa and other routine histologic stains can be used in establishing a diagnosis.[22,129] Less invasive conjunctival swabs may be positive, with the same stains as used for stool samples.[122,196]

Electron microscopy of stool, body fluids, and tissue sections[68,122,129,142,145,173,196] is considered the gold standard for confirmation of infection; it allows evaluation of the multiple stages of the parasite in tissues. The sensitivity may be lower than that of other techniques for the detection of spores in stool and urine specimens.[34] Successful isolation of *Encephalitozoon* and *N. corneum* is possible with the use of several cell lines.[36,70,122,156,180,196]

Serologic tests were available first for *E. cuniculi* infection.[13] The sensitivity and specificity are unknown, and cross-reactivity is likely. Presumed *E. intestinalis* infection has been diagnosed in AIDS-infected patients by enzyme-linked immunosorbent assay and counterimmunoelectrophoresis techniques.[182] Another method that has been applied to the rapid identification of whole organisms and spores is the matrix-assisted desorption ionization time of flight mass spectroscopy, which identifies unique spectral markers of each of the different species.[124] PCR testing also is helpful in establishing the diagnosis of microsporidiosis[59,76,121] and in identifying the different species in clinical samples, whether on intestinal biopsy specimens in disseminated disease,[36,187,196] on corneal scrapings or biopsy sample in microsporidial keratitis,[29,90] or on stool samples in patients with diarrhea.[1,98,204] Differential hybridization, in conjunction with PCR, can allow specific species identification with high sensitivity and specificity (95% and 100%).[126,178,188] When DNA extraction uses the FTA filter method (whatman Bioscience, Cambridge, United Kingdom) the PCR sensitivity reaches 100 percent.[170] In addition, real-time PCR can be used to monitor response to treatment and to monitor dissemination to blood or serum.[114,115] Direct comparison of PCR with light microscopy revealed that lower spore levels, such as seen in asymptomatic cases, can be detected by PCR, but are missed with light microscopy.[125] The sensitivity and specificity of light microscopy is approximately 86.7 percent and 100 percent.[170]

TREATMENT

In ocular infection, keratoplasty often is required to clear the infection. Keratoplasty usually is followed by systemic (albendazole, itraconazole) and usually topical (fumagillin, propamidine isothionate) therapy.[54,140,168,184] In systemic disease, control of HIV infection is the most effective means of controlling infections caused by microsporidia. In patients who fail to respond to antiretroviral therapy, other measures may be necessary.

At present, albendazole is the only treatment that may be useful for enteric and systemic infections caused by *E. intestinalis* and other *Encephalitozoon* spp.[9,47,51,121,122,167,198] It binds tubulin-inhibiting microtubule assembly.[177] Intestinal *E. bieneusi* infection responds inconsistently to albendazole.[16,189] Treatment with albendazole may lead to improvement in diarrhea without eradication of spores in the stool specimens obtained; diarrhea eventually may recur.[122,123,198] Stool volume and frequency are reduced with a low-fat, low-residue diet.[9] Fumagillin, an antibiotic made by *Aspergillus fumigatus*, is an effective topical therapy for microsporidial corneal disease, and an oral preparation (not available in the United States) is the drug of choice for intestinal *E. bieneusi* infection.[43] Fumagillin is an inhibitor of the microsporidian methionine aminopeptidase type 2 (MetAP2), which is essential for microsporidia growth.[177] Given this lack of effective therapies, a search is on for novel treatments. They include the following:

1. Glycosaminoglycan inhibitors would inhibit spore binding to the cell surface.[100]
2. Anti–polar tube antibodies, especially against the post-translational *O*-mannosyl moiety of PTP1, have been shown to be protective.[208] One potentially could devise vaccines that lead to the production of these antibodies.[141]
3. Microsporidian aquaporin inhibitors would impair spore germination.[64]
4. Growth inhibitors, such as inhibitors of MetAP2, would be less toxic than fumagillin[18,177,190] or inhibitors of aspartyl protease, another enzyme essential for microsporidia growth. This latter enzyme was identified as a potential therapeutic target when it was noted that HIV-positive patients treated with protease inhibitors showed disease clearance beyond what would be expected from immune reconstitution alone.[116]
5. Competitive inhibitors of chitin synthesis would be helpful, chitin being an essential element in the wall of these organisms. In vitro studies of polyoxin D and nikkomycin Z seem promising, with the former being 10-fold more effective.[167]
6. Flavonoids and isoflavones have been shown in in vitro studies to be effective against microsporidia, either alone or in conjunction with other drugs to augment their efficacy.[113]

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E. Sporozoa, Blood Flagellates, and Free-Living Amebae

CHAPTER 230

BABESIOSIS

Peter J. Krause

The first reference to babesiosis may have been in the Bible, where a widespread murrain or plague in cattle and other domestic animals is described in Exodus, Chapter 9, Verse 3: "Behold, the hand of the Lord is upon thy cattle which is in the field, upon the horses, upon the asses, upon the camels, upon the oxen, and upon the sheep: there shall be a very grievous murrain." The word "murrain" still is used to describe red-water fever, a form of babesiosis found in cattle in parts of Ireland.[23] Babesiosis long has been recognized as an important disease in livestock, having a significant economic impact in many parts of the world; the health burden of babesiosis in humans has been recognized only more recently.[23,33,56]

Babesiosis is a disease caused by an intraerythrocytic protozoon that is transmitted by ticks and has many clinical features similar to those of malaria. The parasite first was described in animals in 1888 by Babes.[4] In 1893, it became the first microorganism shown to be transmitted by arthropods when Smith and Kilbourne[115] identified a tick as the vector for a species of babesiosis (*Babesia bigemina*) in Texas cattle. The first human case was described in 1957.[112] During the past 30 years, the epidemiology of the disease has changed from a few isolated cases to the establishment of endemic areas in southern New England, New York, and the north-central Midwest, and reports have come from a wide geographic range in North America, Europe, Asia, Africa, and South America. Evidence indicates that the disease is more common in children and adults than is currently reported.[58,61]

EPIDEMIOLOGY

Worldwide, more than 70 species in the genus *Babesia* infect a wide variety of wild and domestic animals. *B. bigemina*, *Babesia bovis*, *Babesia divergens*, and *Babesia major* are found in cattle; *Babesia equi* is found in horses; *Babesia canis* is found in dogs; *Babesia felis* is found in cats; and *Babesia microti* is found in rodents.[68] Each species previously was thought to be host-specific, but the host range of some species now is recognized to be quite broad.[43,67,68,119,125] Some confusion has occurred in taxonomy because the identification of different *Babesia* spp. has been based largely on morphology and the vertebrate host.[41] Most *Babesia* spp. are small (1 to 5 μm in length) and pear-shaped, round, or oval.[67] Seven *Babesia* spp. have been found to cause disease in humans: *B. microti*, *Babesia duncani*, *B. divergens*, EU1, MO1, KO1, and TW1.[21a,44,44a,45,64,90,110,129a]

Human babesiosis is a zoonotic disease transmitted by a tick vector from an infected animal reservoir (Fig. 230–1). Humans are an uncommon and terminal host for *Babesia* spp., which depend on other species for survival. The primary reservoir for *B. microti* in eastern North America is the white-footed mouse (*Peromyscus leucopus*), but the parasite also has been found in shrews, chipmunks, voles, and rats.[42,119,126] Two thirds of *P. leucopus* have been found to be parasitemic in endemic areas.[120] *Babesia* spp. are transmitted by hard-bodied (ixodid) ticks. The primary vector in the northeastern United States is *Ixodes scapularis* (also known as *Ixodes dammini*), which is the same

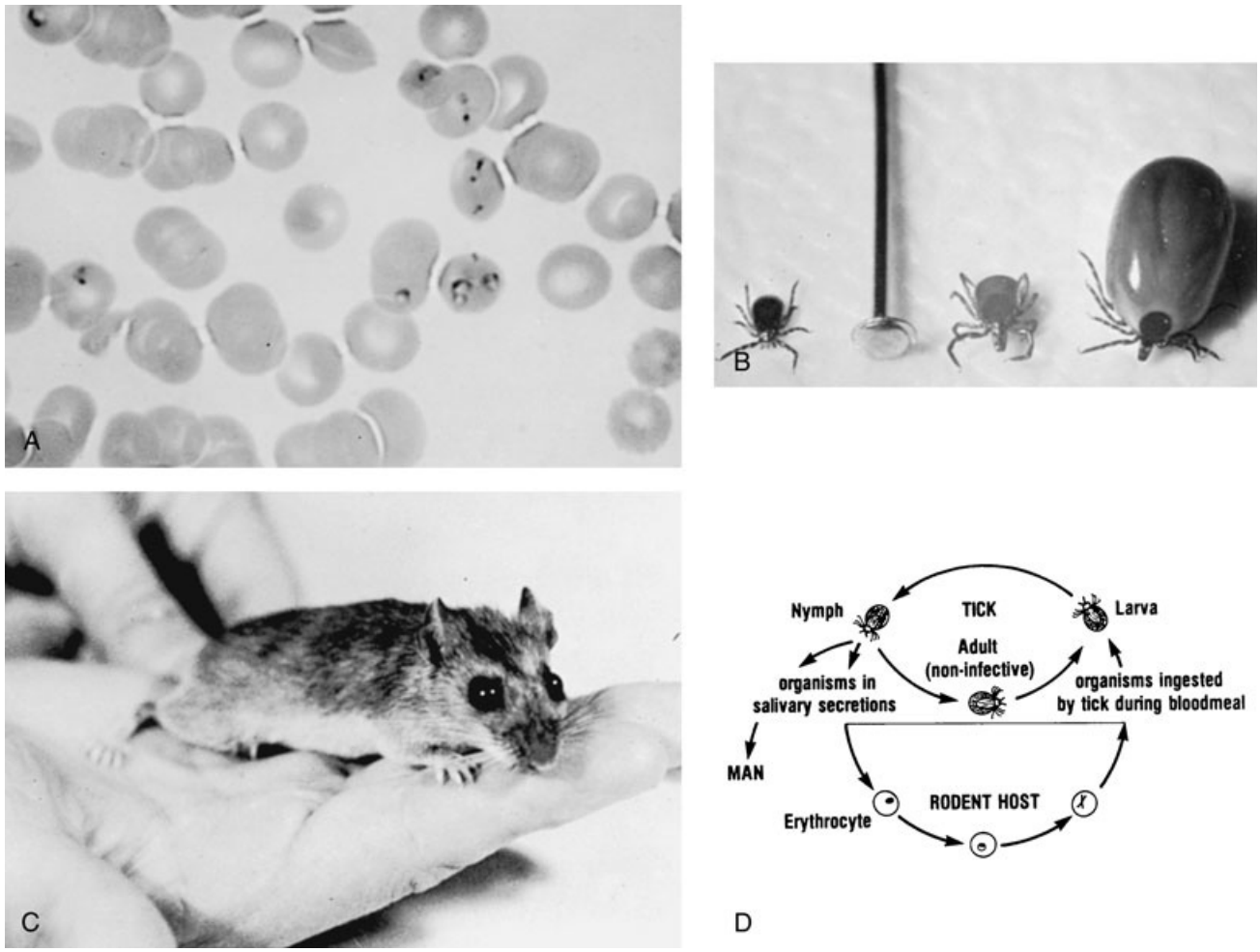

Figure 230–1 Life cycle of *Babesia microti*. **A,** Ring forms of *B. microti* in human blood film (original magnification ×1000). **B,** *Ixodes dammini* ticks and a common pin. The ticks are an adult male, an adult female, and an engorged female. **C,** White-footed mouse (*Peromyscus leucopus*). **D,** Life cycle of *B. microti*. *(**B** courtesy of Mike Frigione, Pfizer, Inc., New York, NY; **D** modified from Ruebush, T. K., II: Babesiosis.* In *Strickland, G. T. [ed.]: Hunter's Tropical Medicine. Philadelphia, W. B. Saunders, 1984.)*

tick that transmits *Borrelia burgdorferi*, the etiologic agent of Lyme disease, and the agent of human granulocytic anaplasmosis.[51,53,116-121,124,130] *I. scapularis* ticks may be infected simultaneously with *B. burgdorferi*, *B. microti*, and the agent of human granulocytic anaplasmosis.[73,86,130] Clinical and laboratory evidence exists for simultaneous human infection with two or more of these pathogens.[7,61,64,70,79,127]

Each of the three active stages in the life cycle (larva, nymph, and adult) of *I. scapularis* takes a blood meal from a vertebrate host to mature to the next stage. Ingested babesial organisms infect intestinal tissue of the tick and subsequently travel to the salivary glands, from which they may be introduced into a new vertebrate host.[87] *Babesia* spp. are transmitted to the subsequent tick stage (transstadial passage). In some species of *Babesia*, such as *B. bovis*, the organisms may invade the ovaries and pass transovarially to the larvae. The tick transmission cycle begins in late summer, when newly hatched larvae ingest the parasite with a blood meal from an infected rodent and maintain the parasite to the nymphal stage. Nymphs transmit the *Babesia* spp. to rodents in late spring and summer of the following year.[117,120] Larvae, nymphs, and adults can feed on humans, but the nymph is the primary vector.[87] All active tick stages also feed on the white-tailed deer (*Odocoileus virginianus*), which is an important host for the tick, but is not a reservoir for *B. microti*.[88,120] An increase in the deer population during the past few decades is thought to be a major factor in the spread of *I. scapularis* and the resulting increase in human cases.[40,117,120] Domestic animals such as the dog may carry the adult *I. scapularis*, but do not seem to be important hosts for the tick and are not infected with *B. microti*.[105,118]

Since the 1980s, human babesiosis has been described with increasing frequency at mainland sites, and more recent studies suggest that the endemic range continues to expand. In certain sites, in certain years of high transmission, babesiosis may constitute a significant public health burden.[58] *B. microti* has been identified in rodent populations in several regions of the United States,[2,3,34,100,119] and human cases have been reported in Connecticut, Massachusetts, Rhode Island, New York, Minnesota, Wisconsin, and New Jersey.[15,24,27,62,77,100,122]

Human babesiosis caused by organisms that morphologically are distinct from *B. microti* has been reported in Georgia and Mexico, although the precise species in these cases have not been identified.[43,109,129a] Moderately severe illness caused by *B. duncani*

occurs in Washington state and California.[84,90] *B. duncani* morphologically is indistinguishable from *B. microti*, but is antigenically and genotypically distinct.[84,90] A case of human babesiosis caused by MO1, a species that is similar to *B. divergens*, has been described in Missouri.[44] In Europe, *B. divergens* and EU1 infections are thought to be transmitted by the cattle tick *I. ricinus*, and human cases have been reported in the former Yugoslavia, France, Ireland, Great Britain, and Russia.[31,32,44a,45,129a,140] Human cases also have been reported in Asia, Africa, and South America.[11,95,110,131] An absence of clinical cases of babesiosis in the tropics may be due to cross-immunity from other endemic protozoal diseases.[52]

Most human cases of babesiosis occur in the summer and in areas where the vector tick, rodents, and deer are in close proximity to humans.[117,119,122] Rarely, babesiosis is acquired through blood transfusions.[29,32a,37,49,66a,71,114,134] Whole blood, frozen erythrocytes, and platelets have been implicated. The incubation period in these cases seems to be 6 to 9 weeks. Transplacental/perinatal transmission of babesiosis also has been described.[26,29]

PATHOGENESIS AND PATHOLOGY

Understanding of the pathogenesis and pathology of babesiosis in humans is incomplete and is based largely on information gathered from studies of babesiosis in other animals. The early cellular events of babesial entry into erythrocytes were investigated by Jack and Ward,[48] who presented data suggesting that *Babesia rodhani* penetrates the erythrocyte by activation of the alternative complement pathway. These authors found that the C3b receptor plays a key role in modification of the parasite or red blood cell, allowing entry of the parasite into the cell. Rudzinska and colleagues[53,97,98] studied the life cycle of *B. microti* in erythrocytes using electron microscopy. After adhesion and entry into the erythrocyte occur, the organism multiplies by asexual budding into two to four daughter cells, or merozoites. In contrast to *Plasmodium* spp. merozoites, which are released from the erythrocytes all at once (synchrony), *Babesia* spp. merozoites are released at varying intervals. New erythrocytes are infected, and the cycle is repeated.

It is unknown whether the initial merozoite release leads to destruction of the host erythrocyte, but alteration of the erythrocyte membrane and eventual lysis occur.[97,123] Erythrocyte lysis is associated with many of the clinical manifestations and complications of the disease, including fever, hemolytic anemia, jaundice, hemoglobinemia, hemoglobinuria, and renal insufficiency. The absence of synchrony decreases the possibility of massive hemolysis and may explain why patients heavily parasitized with *Babesia* spp. may be less ill than patients with *Plasmodium* spp.[123]

Babesia spp. are intraerythrocytic protozoa, and extracellular forms are seen only in heavily parasitized cases.[1,123] *Babesia* organisms do not invade fixed tissue, and evidence in animals suggests that disease results from the excessive production of proinflammatory cytokines (e.g., tumor necrosis factor), as is thought to occur with malaria.[20,65a] A more recent report of fatal human babesiosis with cerebral involvement that was not associated with erythrocyte adherence and vascular occlusion is consistent with this hypothesis.[19] Release of a molecule similar to endotoxin has been hypothesized to trigger the release of proinflammatory cytokines in patients with babesiosis. These cytokines subsequently stimulate release of downstream mediators, such as nitric oxide, which kill parasites, but also may cause direct cellular damage if produced in excess.[18,20,65a]

In addition to proinflammatory cytokine release, obstruction of blood vessels by parasitized erythrocytes with accompanying ischemia and necrosis possibly may result in cerebral abnormalities, hepatomegaly and hepatic dysfunction, and splenomegaly.[23,94,102] Clinical manifestations such as hypotension, vascular congestion, and anoxia also may result from the activation of fibronectin, kallikreins, and complement.[25,50,137,138]

Innate and adaptive immune mechanisms limit the severity of babesial infections, but immunity is incomplete because parasitemia may exist for months to years in animals and up to 27 months in humans after recovery from the initial illness.[60] Reinfection may occur, although it is uncommon. Age is an important factor in host defense against babesial disease in animals and humans. Most clinically apparent cases are reported in adults, but serologic surveys indicate that children are equally susceptible to infection and presumably are exposed to ticks to the same extent.[61,100,105] Almost all of the pediatric cases reported have been in neonates.[29,74,134]

More recent data from a murine model of babesiosis have suggested that resistance to *B. microti* infection conferred by the adaptive immune system is genetically determined and associated with age.[129] Alternatively, the increased severity of babesiosis observed in elderly patients and in neonates may result from impaired splenic function. The spleen plays a critical role in protection against *Babesia* spp. by (1) removing parasites from infected erythrocytes through a process known as "pitting," (2) ingesting parasites by resident reticuloendothelial cells and mononuclear phagocytes, and (3) producing antibabesial antibody.[13,22,25,96,134,137] It long has been known that splenectomized animals have more severe babesiosis than animals with intact spleens. Animals that have recovered from babesiosis and have had negative blood smears have developed parasitemia again after undergoing splenectomy.[69,106] Most fatal cases of babesiosis in humans have occurred in splenectomized individuals, although asplenia does not always result in death or even severe illness.[12,96] Other host defense mechanisms that may help limit babesial infection include macrophages and macrophage products, such as tumor necrosis factor,[20,25,137] B lymphocytes,[76] T lymphocytes,[13,76,99,135] polymorphonuclear leukocytes,[111] antibody,[13,50,65b] and complement,[7,137,151]

CLINICAL MANIFESTATIONS

Clinical manifestations of babesiosis range from subclinical illness to fulminating disease resulting in death. Overt signs and symptoms begin after an incubation period of 1 to 6 weeks from the beginning of tick feeding.[105] The unengorged *I. scapularis* nymph is about 2 mm in length, and the patient often has no recollection of having a tick bite. In most cases, the patient has a gradual onset of malaise, anorexia, and fatigue followed by intermittent fever as high as 40° C (104° F) and one or more of the following: chills, sweats, myalgia, arthralgia, nausea, and vomiting.[1,31,57,64,74,100,104,122] Less commonly noted are emotional lability and depression, hyperesthesia, headache, sore throat, abdominal pain, conjunctival injection, photophobia, weight loss, and nonproductive cough.[55,102,122,123] In contrast to other tick-borne illnesses, such as Lyme disease, Rocky Mountain spotted fever, or tularemia, rash seldom is noted.[24] Ecchymoses and petechiae have been described.[54,123] Erythema chronicum migrans has been noted in patients with babesiosis, but these patients likely had babesiosis and Lyme disease co-infection.[6]

The findings on physical examination generally are minimal, often consisting only of fever.[1,44,64,104,122] Mild splenomegaly, hepatomegaly, or both are noted occasionally.[102,134] Slight pharyngeal erythema, jaundice, and retinopathy with splinter hemorrhages and retinal infarcts also have been reported.[55,74,82] Several abnormal laboratory findings in patients with babesiosis reflect the invasion and subsequent lysis of erythrocytes by the parasite.[55,74,100,102] Mild to moderately severe hemolytic anemia occurs, with an elevated reticulocyte count. Elevated liver enzyme levels may be detected in serum.[100] The leukocyte count is normal to slightly decreased, with a "left shift." Thrombocytopenia may occur.[102] The

erythrocyte sedimentation rate is elevated. Proteinuria and an elevated blood urea nitrogen and creatinine also may be noted.[55,72,102] The illness usually lasts a few weeks to several months, with prolonged recovery taking up to 18 months.[1,6,60,102,104,122] Parasitemia may continue even after the patient feels well. Persistent parasitemia and relapse of illness, as noted with malaria, has been described 27 months after the initial episode.[60]

Some patients, especially patients who are immunocompromised or patients with *B. divergens* or *B. duncani* infection, have a more severe form of the disease consisting of fulminant illness lasting approximately 1 week and ending in death or a prolonged convalescence.[32,39,54,70,82,123] Signs and symptoms include high fever, hemolytic anemia, hemoglobinemia and hemoglobinuria, jaundice, ecchymoses, petechiae, congestive heart failure, pulmonary edema, renal failure, adult respiratory distress syndrome, and coma.[35,36,39,54,122,123,132] Patients with babesiosis who are co-infected with Lyme disease also have more severe acute illness than patients with babesiosis alone.[38,64,70,127] Co-infected patients usually experience moderate to severe acute illness often followed by persistent fatigue. In a recent case-control study, patients with severe immunosuppression who became infected with *B. microti* developed a prolonged, relapsing course of illness despite multiple courses of antibabesial therapy. Recurrent illness sometimes lasted more than a year and a fifth of these patients died.[65b]

Inapparent infection occurs in approximately a quarter of adults and half of children.[58] Serosurveys provide evidence of asymptomatic infection because of the disparity between seroprevalence rates and the number of indigenous reported cases of babesiosis. In a survey on Nantucket Island in Massachusetts, 2 percent of 577 random blood samples and 7.5 percent of 133 blood samples from patients with a history of tick bite or fever had *B. microti* indirect immunofluorescent antibody (IFA) titers of 1:64 or greater.[104] A survey of adults living on Shelter Island, New York, showed that 6 of 136 (4.4%) and 7 of 102 (6.9%) had *B. microti* IFA titers of 1:64 or greater.[28] In a survey of Massachusetts blood donors, 29 of 779 (37%) from Cape Cod had *B. microti* IFA titers of 1:16 compared with 7 of 148 (4.7%) from metropolitan Boston.[89] In a serosurvey in Connecticut, 72 of 735 (9.5%) residents who were seropositive for *B. burgdorferi* had positive *B. microti* IFA titers of 1:64 compared with 8 of 299 (2.7%) seronegative for *B. burgdorferi*.[62] Serosurveys in Mexico, Nigeria, and Taiwan also showed high *B. microti* seroprevalence rates compared with the number of indigenous reported cases of babesiosis.[37a,46,66,83]

DIAGNOSIS

Specific diagnosis of babesiosis is made by microscopic identification of the organism by Giemsa or Wright stains of thick or thin blood smears and by detection of babesial antibodies by one of several serologic tests. *Babesia* spp. are round, oval, or pear-shaped and have a blue cytoplasm with a red chromatin. The ring form is most common and is similar to the rings of *Plasmodium falciparum*.[41] *Babesia* spp. can be distinguished from *Plasmodium* spp. by (1) the absence of pigment, which is present in older trophozoites of *Plasmodium* spp.; (2) the absence of schizonts and gametocytes; (3) the absence of synchronous stages within the erythrocytes; and (4) the presence of the infrequently noted tetrad or Maltese cross forms, in which four compact masses, each containing nuclear material, are joined by strands of cytoplasm.[41]

Multiple thick and thin blood smears should be examined because only a few erythrocytes are infected in the early stage of the illness, when most people seek medical attention.[41] Rapid automated differential blood analyzers may fail to distinguish erythrocytic inclusions.[10] In thick smears, the *Babesia* organism appears as a tiny red-to-purple nucleus with a thin tail of light blue cytoplasm. Maximum erythrocyte infection is approximately 10 percent in normal hosts, but up to 85 percent in asplenic individuals.[122] Usually less than 1 percent of erythrocytes are parasitized early in the course of the illness, and the laboratory investigation of possible babesiosis should include more than an examination of blood smears.

In cases in which the presence of *Babesia* spp. is suspected but not shown by blood smears, babesial DNA can be amplified and detected using the polymerase chain reaction.[65,85] Blood from the patient also can be injected by the intravenous or intraperitoneal route into small laboratory animals such as hamsters or gerbils. If present in the patient, *B. microti* usually appears in the blood of the inoculated animal within 2 to 4 weeks.[8] This diagnostic technique is less sensitive and more time-consuming and costly than the polymerase chain reaction.[65]

Numerous serologic tests have been developed to detect babesial antibodies. Of the commonly used serologic tests, the IFA assay is the most reliable.[16,59,63,102] The IFA test is simpler, less expensive, and more rapid than the complement-fixation test. IgG and IgM IFAs can be detected.[16,59,63] During the acute phase of the illness, titers usually exceed 1:1024, but decline to 1:64 or less within 8 to 12 months. A babesial IFA titer of 1:1024 or greater usually signifies active or recent infection.[16,103] Although cross-reactions occur to different *Babesia* spp. and *Plasmodium* spp. with the IFA test, these titers almost always are low (≤1:16).[16,17] The problem of cross-reactivity with *Plasmodium* spp. is minimized in areas that have no indigenous malaria. A reliable immunoblot assay for detection of *B. microti* antibody has been developed.[108] Enzyme-linked immunosorbent assays for detection of *B. divergens* and *B. major* have been found to be superior to complement fixation and IFA procedures.[9,93,125,128]

PREVENTION AND TREATMENT

The combination of either atovaquone plus azithromycin or clindamycin plus quinine for 7 to 10 days is the initial therapy that should be considered for patients with babesiosis. Clindamycin and quinine should be given to patients with severe babesiosis. In such patients, clindamycin should be administered intravenously rather than orally, and exchange transfusion should be considered. The dosage recommendations are as follows:[14,29,35,91,136]

- Atovaquone—children, 20 mg/kg every 12 hours (maximum of 750 mg per dose); adults, 750 mg orally every 12 hours
- Azithromycin—children, 10 mg/kg once per day on day 1 (maximum of 500 mg per dose) and 5 mg/kg once per day (maximum of 250 mg per dose) and orally thereafter; adults, 500 to 1000 mg on day 1 and 250 mg orally once per day thereafter; immunocompromised adults, 600 to 1000 mg/day
- Clindamycin—children, 7 to 10 mg/kg given intravenously or orally every 6 to 8 hours (maximum of 600 mg per dose); adults, 300 to 600 mg every 6 hours intravenously or 600 mg every 8 hours orally
- Quinine—children, 8 mg/kg given orally every 8 hours (maximum of 650 mg per dose); adults, 650 mg every 6 to 8 hours orally

The clindamycin and quinine combination was first used in a case of babesiosis in an 8-week-old infant girl who contracted the disease from a blood transfusion.[134] Initially, she was thought to have malaria. Clindamycin and quinine were given after failure with chloroquine. Her favorable outcome suggested the prospective use of this combination in adults. Numerous children and adults subsequently have been treated with clindamycin and quinine, with prompt clearing of parasitemia and resolution of clinical signs and symptoms.[14,15,74,122]

The successful use of atovaquone and azithromycin for the treatment of malaria and for babesiosis in hamsters prompted a

clinical trial to determine whether the combination would be effective in human babesiosis.[47,133] In the first prospective trial of antibabesial therapy in humans, atovaquone and azithromycin were compared with clindamycin and quinine in adults.[57] Adverse effects were reported in 15 percent of subjects who received atovaquone and azithromycin compared with 72 percent of subjects who received clindamycin and quinine. In approximately one third of subjects taking clindamycin and quinine, the apparent drug reactions were severe enough that the drugs were discontinued or the dosages decreased compared with only 2 percent of subjects taking atovaquone and azithromycin. Both drug combinations were equally effective in clearing symptoms and parasitemia. Although the combination of atovaquone and azithromycin has had limited use in children, the mild course of disease in children and the favorable safety profile of this combination justify its use in children who have mild to moderate disease.[29,91]

Other antimicrobial agents that have been used to treat babesiosis generally are ineffective. Although chloroquine may give some symptomatic relief of fever and myalgia by its antiinflammatory action, it often fails to clear parasitemia in guinea pigs and humans and is not recommended.[14,78] Other antimalarial drugs, such as quinacrine, primaquine, pyrimethamine, pyrimethamine and sulfadoxine, sulfadiazine, and tetracycline, have no effect on parasitemia in animals. Pentamidine isothionate has been found to decrease fever and parasitemia, but the organisms are not eradicated, and the drug has proved to be ineffective in animals and humans.[30] Diminazene aceturate was effective in clearing parasitemia and clinical symptoms in one patient, but he developed Guillain-Barré syndrome during recovery, possibly as a result of receiving the drug.[107] Pentamidine and trimethoprim-sulfamethoxazole were used successfully to treat a case of *B. divergens* infection in France.[92]

Partial or complete red blood cell exchange transfusion can decrease the degree of parasitemia rapidly and remove toxic byproducts of babesial infections.[12,49,123] Exchange transfusion is indicated for patients with severe babesiosis, as indicated by high-grade parasitemia (≥10%); significant hemolysis; or renal, hepatic, or pulmonary compromise. No data are available to determine whether partial exchange transfusion is preferable to whole-blood exchange transfusion.[136]

Babesiosis can be prevented by avoiding areas in May through September where ticks, deer, and mice are known to thrive. It is especially important for asplenic individuals in endemic areas to avoid tall grass and brush where ticks may abound. Use of clothing that covers the lower part of the body and that is sprayed or impregnated with diethyltoluamide, dimethyl phthalate, or permethrin (Permanone) is recommended for individuals who travel into the foliage of endemic areas.[23,113] A search for ticks on people and pets should be carried out and the ticks removed as soon as possible.[23] Tick removal is accomplished best with tweezers by grasping the mouth parts without squeezing the body of the tick.[24,81] Attempts to reduce the tick, mouse, or deer populations in endemic areas have either been unsuccessful or have encountered opposition.[74,113,117,120a,132a] It is recommended that prospective blood donors who reside in endemic areas and who present with a history of fever within the preceding 1 to 2 months be excluded from giving blood to prevent transfusion-related cases.[101] The American Red Cross defers blood donors who have had a history of babesiosis.[75] Effective *B. bovis* and *B. bigemina* vaccines have been developed for use in cattle, but no *B. microti* vaccine has been developed.[80,139]

Babesiosis is a tick-borne zoonosis that is endemic in parts of North America (*B. microti*) and Europe (*B. divergens*). It commonly manifests as a viral-like illness, and the incidence in the United States and throughout the rest of the world likely is greater than currently recognized. The clinical symptoms of babesiosis usually are nonspecific, and detecting the organism in blood smears may be difficult. Although fatalities have been reported in patients experiencing babesial infection (primarily in immunocompromised hosts), complete recovery with antibabesial chemotherapy is the rule.

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CHAPTER 231

Malaria

Elizabeth D. Barnett

Malaria is a disease of global importance, affecting approximately 270 million people and causing 1 to 2.5 million deaths yearly. Most malaria deaths worldwide occur in children.[128] Malaria is transmitted regularly in parts of Africa, Asia, the Middle East, Central and South America, Hispaniola, and Oceania. It has been imported by travel to nearly every part of the world. Because of the ubiquity of malaria, the ability to recognize its signs and symptoms and knowledge about methods of prevention and treatment are important for health care professionals wherever they practice.[32,39]

Malaria usually is transmitted by bites of infected female anopheline mosquitoes. Disease typically results from infection with one or more of the four species of *Plasmodium* (*Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium ovale*, or *Plasmodium malariae*) that infect humans. A fifth species, *Plasmodium knowlesi*, has also been identified to cause human malaria.[18a] These protozoa have complex life cycles involving arthropod and vertebrate hosts. Untreated, *P. falciparum* malaria can progress to coma, renal failure, pulmonary edema, and death. Asymptomatic carriage may last decades in the case of *P. malariae*. Relapses are common with *P. vivax* and *P. ovale*. Resistance of the parasites to antimalarial agents and incomplete success in developing and maintaining programs to eradicate the mosquito vectors have contributed to making malaria a persistent worldwide challenge.

HISTORY

Malaria has been known since antiquity and probably affected prehistoric humans. Fossilized mosquitoes have been found in geologic strata 30 million years old. Descriptions of the signs and symptoms of malaria have been found in early Hindu and Chinese writing, and Hippocrates described seasonal and geographic aspects of the disease.[120] Numerous references to malaria occur in literature, ranging from Shakespeare's *The Tempest* to Laura Ingalls Wilders' *Little House on the Prairie*. The name is derived from the Italian *mal aria* ("bad air") based on recognition of the connection between malaria and swamps. The first methods of malaria control, draining of swamps, were based on this association.

The first malaria treatment known to Europeans, bark of the cinchona tree, was identified in the early 17th century almost 200 years before the active ingredient (quinine) was isolated. Not until the late 1800s was the vector-borne nature of malaria understood and described; for their roles in this discovery, Laveran, a French military surgeon working in Algeria, and Ross, a British military physician working in India, were awarded Nobel prizes. Further work by many investigators rounded out the current understanding of the life cycle of the malaria parasites and the complex interrelationship with vector mosquitoes.

During the 20th century, parallel efforts directed toward vector control and discovery and development of drugs to treat malaria were undertaken. The development of larvicides and insecticides permitted control of the mosquito vector. The spectacular success of house spraying with dichlorodiphenyltrichloroethane (DDT) was instrumental in eradicating malaria from most of North America and Europe and in significantly decreasing its prevalence in the Mediterranean area, the Middle East, the Far East, and parts of southern Africa.[100] The development of antimalarial drugs, stimulated largely by the need to protect soldiers during World Wars I and II during shortages of the standard antimalarial agent quinine, allowed for successful treatment of malaria cases.

The World Health Organization (WHO) launched a global campaign for eradication of malaria in 1957. Early successes, attributable to the efficacy of DDT and development of new antimalarial agents, subsequently were hindered by resistance of mosquito vectors to DDT and resistance of the parasites to antimalarial drugs. In 1969, the WHO philosophy on malaria was altered to emphasize the development of health services and research. The goals of malaria control since then have been refined and broadened to include implementation of selective and sustainable preventive measures, early diagnosis and prompt treatment, early detection or prevention of epidemics, and strengthening of local infrastructures to allow better understanding of the determinants of local transmission and malaria control.[100,120]

During the past several decades, transmission of malaria has increased in many areas despite adoption of these measures. Factors contributing to increases in the number of cases of malaria in many areas and re-emergence in areas thought to be free of disease include lapses in local control measures; resistance to antimalarial drugs; changes in climate; and population movement as a result of urbanization, mass displacement of populations, and international travel.[8,58,65,69]

ORGANISM

The life cycle of malaria parasites is complex and requires a suitable population of anopheline mosquitoes and infected humans for completion (Fig. 231–1). To begin the cycle, the female anopheline mosquito injects sporozoites along with saliva in preparation for taking a blood meal from a vertebrate host. Sporozoites, the infective stage of *Plasmodium*, remain in the circulation for less than 1 hour and then migrate to the liver, where they invade hepatocytes and multiply asexually. Proliferation within hepatocytes takes approximately 1 week for *P. falciparum* and *P. vivax* and approximately 2 weeks for *P. malariae*. At the end of this period, mature tissue schizonts rupture and release thousands of merozoites, which then invade red blood cells (RBCs). *P. vivax* and *P. ovale* have a second type of exoerythrocytic form, the hypnozoite, which can remain dormant for weeks to years. Dormant hypnozoites may develop weeks, months, or years later into merozoites, which then can enter RBCs and cause relapse of malaria. The factors that influence which exoerythrocytic form develops are not understood.

Merozoites released from tissue schizonts invade RBCs, where the erythrocytic phase of the life cycle occurs. Two pathways exist in the erythrocytic, or blood, phase: asexual and sexual. In the asexual phase, development of the parasite begins with the youngest stage, the trophozoite, or ring form. The parasite undergoes nuclear division to form schizonts and then merozoites in the asexual multiplication process, called *erythrocytic schizogony/merogony*. Lysis of RBCs releases the merozoites, which invade other RBCs, perpetuating the asexual erythrocytic cycle. The cycle continues until interrupted by treatment or by the host's immune response.

In the sexual phase, subpopulations of merozoites in the erythrocytic phase differentiate into gametocytes, or sexual forms, which then are available for ingestion by mosquitoes to complete the life cycle within the mosquito. Female macrogametocytes and male microgametocytes appear in the circulation within 3 to 15 days of the onset of symptoms. Gametocytes of *P. vivax* may appear in 4 days, whereas gametocytes of *P. falciparum* may require 10 days for development.

In the stomach (midgut) of the mosquito, male gametocyte nuclei divide into four to eight nuclei and form motile gametocytes that fertilize the female gametocytes. The zygotes become motile ookinetes that migrate through the wall of the midgut, attach to its outer surface, and form oocysts. The oocysts rupture 9 to 14 days later and release sporozoites that invade the mosquito salivary glands, where they are ready for inoculation into the next vertebrate host.

EPIDEMIOLOGY

Transmission of malaria occurs in large parts of Africa, the Indian subcontinent, Southeast Asia, the Middle East, Oceania, and Central and South America (Fig. 231–2). Although indigenous transmission of malaria has been eradicated almost completely from the United States, Canada, northern Europe, most of the Caribbean, parts of South America, Israel, Lebanon, Reunion, Singapore, Hong Kong, Japan, Korea, Taiwan, Brunei, and Australia, many cases of imported malaria occur in these countries each year.[120]

P. falciparum is the major malaria species in sub-Saharan Africa and the island of Hispaniola, with *P. malariae* assuming a more minor role. *P. vivax* occurs alongside *P. falciparum* in the Indian subcontinent, Central and South America, Mexico, Southeast Asia, and Oceania. *P. ovale* occurs mainly in Africa. *P. vivax* occurs rarely in sub-Saharan Africa because most Africans lack the Duffy blood group antigen necessary for parasite invasion.

TRANSMISSION

EPIDEMIOLOGIC TERMINOLOGY

Patterns of transmission of malaria include stable endemic malaria (natural transmission occurring over many years, with a

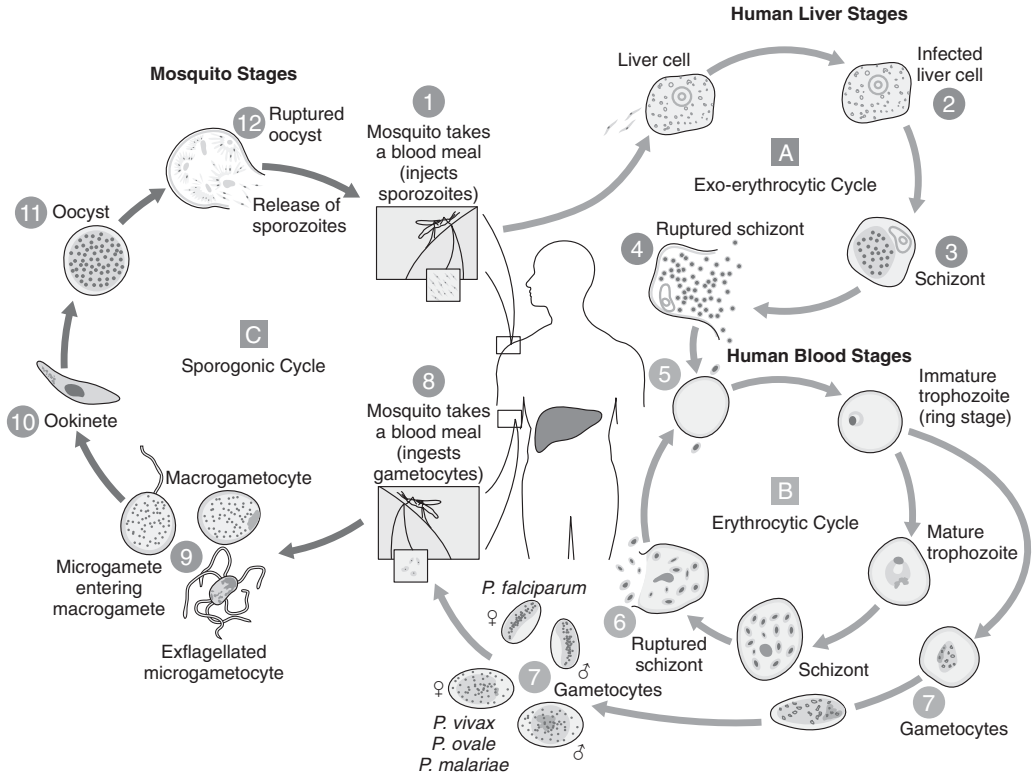
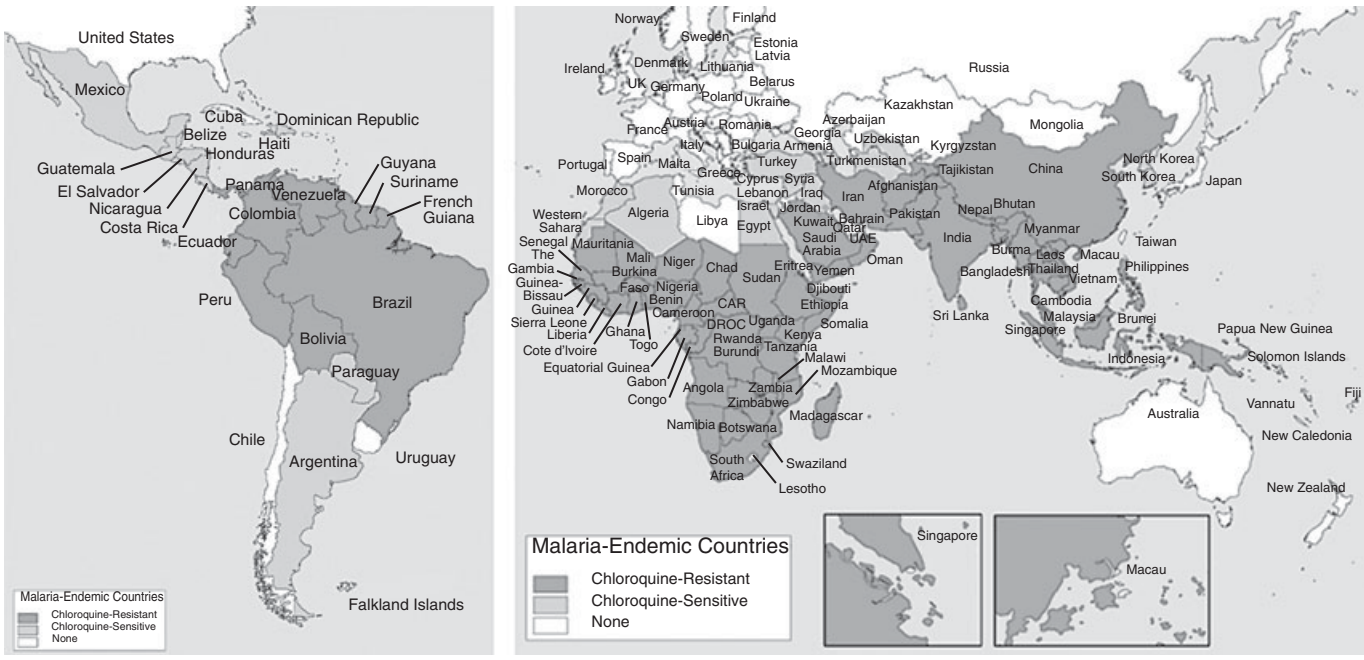


Figure 231–1 Life cycle of the human malaria parasites. The malaria parasite life cycle involves two hosts. During a blood meal, a malaria-infected female *Anopheles* mosquito inoculates sporozoites into the human host ❶. Sporozoites infect liver cells ❷ and mature into schizonts ❸, which rupture and release merozoites ❹. (Of note, in *P. vivax* and *P. ovale* a dormant stage [hypnozoites] can persist in the liver and cause relapses by invading the bloodstream weeks, or even years later.) After this initial replication in the liver (exo-erythrocytic schizogony **A**), the parasites undergo asexual multiplication in the erythrocytes (erythrocytic schizogony **B**). Merozoites infect red blood cells ❺. The ring stage trophozoites mature into schizonts, which rupture releasing merozoites ❻. Some parasites differentiate into sexual erythrocytic stages (gametocytes) ❼. Blood stage parasites are responsible for the clinical manifestations of the disease. The gametocytes, male (microgametocytes) and female (macrogametocytes), are ingested by an *Anopheles* mosquito during a blood meal ❽. The parasites' multiplication in the mosquito is known as the sporogonic cycle **C**. While in the mosquito's stomach, the microgametes penetrate the macrogametes generating zygotes ❾. The zygotes in turn become motile and elongated (ookinetes) ❿, which invade the midgut wall of the mosquito where they develop into oocysts ⓫. The oocysts grow, rupture, and release sporozoites ⓬, which make their way to the mosquito's salivary glands. Inoculation of the sporozoites ❶ into a new human host perpetuates the malaria life cycle. (See companion Expert Consult web site for color version.) (*From http://www.cdc.gov/malaria/biology/life_cycle.htm.*)



A B

Figure 231–2 **A** and **B,** Malaria endemic countries in the Western (**A**) and Eastern (**B**) Hemispheres, 2007. (See companion Expert Consult web site for color version.) *(From Centers for Disease Control and Prevention. Available at http://wwwn.cdc.gov/travel/yellowBookCh4-Malaria.aspx. Accessed March 8, 2007.)*

predictable incidence of illness and prevalence of infection) and unstable malaria (transmission rates vary from year to year, and immunity is low, with a greater likelihood of epidemics occurring). The degree of endemic malaria is based on the parasite rate in children 2 to 9 years old. Types of endemic malaria include hypoendemic (parasite rate of 0 to 10%), mesoendemic (parasite rate of 11 to 50%), hyperendemic (parasite rate consistently >50%, with a high proportion of adults having enlarged spleens), and holoendemic (parasite rate consistently >75%, with a low proportion of adults having enlarged spleens).[120]

Autochthonous malaria is acquired locally and may be indigenous or introduced. Introduced malaria may occur when migrant populations with asymptomatic infection provide blood meals for feeding anopheline mosquitoes under conditions that allow for the life cycle to be completed in the mosquito and enable the mosquito to infect others. Imported malaria cases may occur in non-endemic areas, but result from infection in an endemic area. Induced malaria is acquired by exposure to infected blood, such as from blood transfusion, needle-stick injury, laboratory accident, or, historically, medical treatments. Cryptic malaria cases are cases for which no explanation can be found, and no epidemiologic link to other cases can be identified.

MOSQUITO-BORNE TRANSMISSION

The most typical means of transmission of malaria is through the bite of an infected anopheline mosquito. Although more than 350 species of anopheline mosquito exist, only approximately 45 have been shown to be effective vectors of malaria. A population of infected humans is necessary to sustain transmission because of the short life span of mosquitoes (5 to 20 days) and the long incubation period required in the mosquito (8 to ≥10 days).

BLOOD-BORNE TRANSMISSION

Transmission of malaria via blood transfusion is well documented.[79] Transmission also may occur through organ donation or needle-stick injury.[35,132] Relapses of malaria cannot occur with blood-borne transmission, even if the infecting species is *P. vivax* or *P. ovale*, because the infection is produced by the transmission of infected RBCs, rather than forms that invade the liver.

CONGENITAL MALARIA

Infants can acquire malaria from their mothers during pregnancy. Transplacental transmission of parasites has been proposed as the most likely route of transmission, although breakdown of placental barriers allowing transmission of maternal blood cells to the infant during labor or delivery also has been suggested as a mechanism of transmission.[42,75] *P. vivax* most often is associated with this phenomenon, but it may occur with all species. Congenital malaria is less likely to occur in infants of semi-immune mothers because of passage of maternal antibody at the time of birth. As with transfusion-associated malaria, relapses do not occur.

CRYPTIC MALARIA

The category of cryptic malaria includes cases for which no source of infection can be identified. Typical cases include confirmed malaria in U.S. residents who have never traveled to or resided in malarial areas, who have not received blood transfusions, and who are not linked epidemiologically with other cases.[56,95,132] Airport malaria, one kind of cryptic malaria, occurs in proximity to international airports and is thought to occur when mosquitoes arriving with airplanes from endemic areas infect individuals working in or living near airports.[51]

HOST-PARASITE INTERACTION

The intensity of transmission of malaria depends on factors that affect the density of vectors and the extent of vector-human contact. Transmission of malaria may be continuous or seasonal, or it may depend on local site-specific factors, such as the presence of irrigation projects or intermittent flooding. Mosquito vectors differ in their efficiency to transmit malaria; the principal vector in sub-Saharan Africa, *Anopheles gambiense*, is known for being a highly effective vector. Variations in climate may affect the viability of mosquitoes.

The incidence and severity of malaria are affected by the intensity of exposure, the presence of immunity, and genetic factors. Distinction must be made between malaria infection (presence of parasitemia) and malaria illness. A major puzzle in malaria is why individuals with similar degrees of parasitemia may exhibit radically different clinical manifestations.

Individuals who reside in endemic areas and are exposed continually to infected mosquitoes acquire immunity to malaria illness. Adults in these areas continue to become infected, but they have lower levels of parasitemia. Infants born to mothers with acquired immunity may be protected transiently by placental passage of maternal antibody. The highest incidence of infection with malaria occurs in infants and young children who are no longer protected by maternal antibody, but who are too young for significant acquired immunity to have developed. Children also are more susceptible to certain manifestations of severe illness, such as cerebral malaria. Lack of acquired immunity in infants and young children accounts partly for their increased risk of acquiring disease and having severe manifestations. Acquired immunity diminishes during pregnancy, and pregnant women are at high risk for development of severe complications of malaria. Individuals in the population who remain asymptomatic but harbor gametocytes in their blood are reservoirs of infection when bitten by mosquitoes.

Clinical manifestations of malaria may be severe in non-immune patients. Malaria is the most common life-threatening infection acquired by travelers to malaria-endemic regions. Individuals with acquired immunity who then leave endemic areas for long periods may lose their immunity and be at risk for severe disease if re-exposed.

Genetic factors determine the risk of acquiring malaria and having severe infection. Individuals who have a Duffy-negative blood type lack specific receptors for invasion of the merozoites of *P. vivax* and are resistant to infection with *P. vivax*.[76] This resistance is the basis for the low incidence of vivax malaria in Africa. Specific human leukocyte antigens present in individuals from West Africa may protect against the development of severe complications of malaria, including cerebral malaria and severe anemia. The best-known example of the relationship between malaria and genetics is the association between sickle hemoglobinopathies and protection against severe falciparum malaria. This balanced polymorphism is thought to have helped ensure survival of the gene for hemoglobin S in the population because of the selective advantage provided on a population basis to those who are heterozygous for sickle-cell disease. Individuals with sickle hemoglobinopathies still may be infected and manifest signs and symptoms of malaria, although the risk of acquiring severe malaria or dying of malaria may be 60-fold to 70-fold less in children with hemoglobin AS than in children with hemoglobin AA.[53]

PATHOPHYSIOLOGY

The pathogenesis of malaria is multifactorial because of the effects of blood-stage parasites, and it involves multiple organ systems. Pathophysiologic changes are caused by the destruction of RBCs, production of cytokines, stimulation of intravascular synthesis of nitric oxide, and sequestration of infected erythrocytes.

Lysis of RBCs leads to anemia, which may be severe, and its attendant hemodynamic consequences. Anemia may develop as a result of hemolysis, impaired erythropoiesis, or bone marrow depression secondary to folic acid deficiency.[91] Intravascular hemolysis may be so severe that it results in pronounced hemoglobinuria ("blackwater fever"), which may be a precipitating event in the development of renal failure. This complication has been noted in association with treatment using quinine. Hematopoiesis is suppressed during acute infection, and such suppression may not be reversed as readily in iron-deficient individuals, contributing to chronic anemia.

Cytokines such as tumor necrosis factor (TNF) and interleukin-1 have important roles in the pathogenesis of malaria.[10,54] Severe disease has been associated with higher concentrations of TNF, and TNF polymorphisms may play a role in the development of specific complications.[71,72] Parasite factors responsible for release of cytokines have not been identified. One possible role of TNF in malaria is to stimulate nitric oxide, a short-lived neurotransmitter. Nitric oxide may have a role in cerebral malaria, and its transient nature may provide a partial explanation for the complete recovery noted in some patients with severe cerebral malaria. TNF and nitric oxide have harmful and beneficial roles in the pathogenesis of malaria; both have been shown to be correlated with clearance of parasites and eventual recovery and the severity of illness.

Sequestration of infected RBCs has long been thought to contribute to the clinical manifestations of malaria, particularly that caused by *P. falciparum*. Late-stage parasites induce host cells to develop knobs on the surface of erythrocytes that facilitate adherence of these cells to vascular endothelium. The effects of these sequestered RBCs on the perfusion, nutrition, and oxygenation of surrounding tissues may be responsible for the complications of *P. falciparum* infection, including cerebral malaria, renal failure, and watery diarrhea.[81,94] Consumption of glucose by metabolically active late-stage parasites contributes to hypoglycemia and lactic acidosis. Despite these changes, the histopathologic appearance of tissue is remarkably benign, consistent with the reversible nature of the changes. This phenomenon also lends support to the role of cytokines and secondary messengers in the pathogenesis of complications of cerebral malaria.[53,120]

CLINICAL FEATURES

The clinical manifestations of malaria depend on the species of malaria parasite causing the infection, the immune status of the individual, the mode of transmission of infection, whether the individual was taking prophylaxis, and host immune factors (Table 231–1). Acute malaria generally is understood to refer to the signs and symptoms associated with disease caused by infection with malaria parasites.

Recurrent infections are of three types: relapse, recrudescence, and re-infection. Relapses occur as a result of delayed maturation of the dormant liver stages (hypnozoites) of *P. vivax* or *P. ovale*. Recrudescence occurs when parasitemia caused by the same parasite responsible for the initial infection recurs after clearance or a significant reduction in the initial parasitemia. It occurs most commonly with *P. falciparum* because of drug resistance. Re-infection with different parasites and infection with more than one type of *Plasmodium* occur especially in areas with a high intensity of transmission. Persistent infection is noted with *P. malariae*. Hypnozoites have not been identified with *P. malariae*, so the organism is thought to persist as a low-level parasitemia that can exist for years without causing symptoms.

ACUTE MALARIA

A classic description of malaria includes features of the malaria paroxysm resulting from the lysis of parasitized RBCs and release of merozoites into the circulation at the completion of asexual reproduction. The paroxysm is characterized by fever and chills accompanied by constitutional symptoms of headache, body ache, fatigue, dizziness, and malaise. Gastrointestinal symptoms include nausea and vomiting, abdominal pain, and diarrhea. Cough and dyspnea may accompany an attack. Although periodicity of the paroxysms in primary attacks is thought to be pathognomonic for malaria species, this periodicity may take several days to become established, may not occur at all in asynchronous infections, or may be modified by previous immunity or treatment.

In children, fever and headache may be the sole symptoms, or gastrointestinal symptoms may predominate. Physical signs of malaria include anemia, jaundice, and hepatosplenomegaly. Rash and lymphadenopathy typically are not associated with malaria, although malaria may precipitate recrudescence of latent herpes infections.

TABLE 231–1 Characteristics of the Four *Plasmodium* Species Responsible for Human Malaria

	P. falciparum	*P. vivax*	*P. ovale*	*P. malariae*
Incubation period in days (range)	12 (8-25)	14 (8-27)	17 (15-≥18)	28 (15-≥40)
Periodicity of febrile attacks (hr)	None	48	48	72
Earliest appearance of gametocytes (days)	10	3	?	?
Relapse	No	Yes	Yes	No
Duration of untreated infection (yr)	1-2	1.5-4	1.5-4	3-50
RBC preference	Younger cells (but can invade cells of all ages)	Reticulocytes	Reticulocytes	Older cells
Characteristic morphology	Ring forms Multiply infected cells Banana-shaped gametocytes	Schüffner dots Enlarged RBCs	Schüffner dots Enlarged RBCs	Normal-sized cells Band or rectangular forms of trophozoites

RBCs, red blood cells.

LABORATORY FINDINGS

Anemia is the most common abnormality in malaria. Thrombocytopenia is common and may be the first manifestation in patients with uncomplicated malaria.[53] Leukopenia also may occur, but high white blood cell counts are less usual and should provoke investigation for other conditions. Liver function test abnormalities may be present and can be mistaken for evidence of hepatitis, especially in patients with jaundice and tender hepatosplenomegaly. Hypoglycemia occurs frequently with falciparum malaria and may develop before or as a consequence of treatment with quinine. When present in children before treatment, hypoglycemia is associated with a poor prognosis.[119] Hyponatremia may occur as part of the syndrome of inappropriate secretion of antidiuretic hormone in some patients. Serum creatinine and blood urea nitrogen may be elevated transiently or may increase significantly with acute renal failure.

Malaria stimulates a polyclonal increase in immunoglobulins associated with rapid production of malaria-specific antibodies and reduced complement levels. False-positive tests for syphilis, rheumatoid factor, heterophil agglutinins, and cold agglutinins may occur.[120]

MALARIA IN SPECIAL POPULATIONS

MALARIA IN CHILDREN

The signs and symptoms of malaria in children range from asymptomatic infection to life-threatening illness. Severe anemia is most likely to develop in children younger than 2 years old, whereas cerebral malaria is most likely to occur in older children (mean age 3.5 years). The greatest burden of disease is borne by infants and young children, although the impact of disease on adolescents has not been studied extensively. Malaria in pregnant adolescents may be of particular concern.[55]

In endemic areas, lack of diagnostic facilities, limited resources, and encroaching drug resistance complicate the ability to make a rapid, accurate diagnosis and provide expedient treatment to children with malaria. Malaria often is difficult to distinguish from other common illnesses such as pneumonia. Clinical algorithms that can distinguish children with malaria from children with pneumonia or other illnesses have been developed and studied, but do not eliminate completely the overlap in conditions with similar clinical manifestations.[97,98,102] Risk factors for a fatal outcome in children with malaria were studied in Kenya; the presence of coma or respiratory distress, or both, at initial evaluation identified children at high risk of dying.[68]

Imported malaria occurs in children in many non-endemic countries. The diagnosis often is delayed because of lack of consideration of malaria as a cause of illness and unfamiliarity with the disease. In children with acquired immunity, the signs and symptoms of disease may be subtle and nonspecific, but fever or a history of fever is universal.[23,62] Other symptoms include anorexia, vomiting, diarrhea, headache, lethargy, and abdominal pain. Laboratory findings include anemia, thrombocytopenia, and leukopenia. The diagnosis of malaria should be considered in every child with fever or a history of recent fever who has visited an area where malaria occurs.

CONGENITAL MALARIA

All four types of human malaria can be transmitted congenitally, but the disease most often is associated with *P. vivax*. That congenital malaria is not seen more frequently is due partly to the effective barrier function of the placenta. Congenital malaria develops in infants of approximately 0.1 percent of immune mothers and 10 percent of non-immune mothers in endemic areas, although placental infection occurs in one third of pregnant women.[47] In endemic areas, distinguishing malaria acquired congenitally from malaria acquired by transmission from mosquitoes is difficult. In non–malaria endemic countries, congenital malaria is very rare; five cases have been reported in the United States since 2000.[11]

The onset of symptoms is insidious and usually occurs at 2 to 8 weeks of age. The typical malaria paroxysm is absent, with the infant instead having poor feeding, fever, vomiting, diarrhea, irritability, and hepatosplenomegaly on physical examination.[42] The most common laboratory finding is anemia, but thrombocytopenia and hyperbilirubinemia also are common findings. Therapy for the infected species of malaria is curative, and the infant does not need treatment of the exoerythrocytic stages of the parasite (although the mother does).

MALARIA IN PREGNANCY

The effects of malaria on the mother depend on the degree of immunity that she has attained and her parity.[75] Relapses and recrudescence of malaria are common during pregnancy, probably because of the immunosuppression associated with the pregnant state. Malaria can exacerbate the anemia occurring during pregnancy, and hypoglycemia and renal insufficiency may complicate falciparum malaria during pregnancy.[31]

Placental infection during pregnancy may be associated with low birth weight, particularly in primigravidas. Vivax malaria has been linked to maternal anemia and low birth weight in multigravidas and primigravidas.[83] Severe falciparum malaria is harmful to the fetus because of fetal tachycardia and distress secondary to maternal fever; disruption of maternal-fetal blood flow and exchange of metabolic substrates by malaria parasites trapped in the placenta; and the potential for reduction in fetal glucose supply, which may be exacerbated by treatment with quinine, a drug that is able to stimulate the release of insulin.[60] Heavy infection of the placenta interferes with transfer of tetanus antibodies from the mother to the fetus, but the effect on other antibodies is unknown.[9] Prompt treatment of pregnant women with severe malaria is critical to survival of the mother and the fetus.

SEVERE AND COMPLICATED MALARIA

Non-immune individuals are most susceptible to the severe complications of falciparum malaria, which include cerebral malaria, pulmonary failure or acute respiratory distress syndrome, renal failure, and severe anemia.[63] Hypoglycemia and metabolic acidosis may occur. Falciparum malaria in non-immune patients should be considered a medical emergency, and treatment of *P. falciparum* should be initiated in all ill patients with malaria until the species can be confirmed.

CEREBRAL MALARIA

Cerebral malaria is the most common complication of falciparum malaria in children and occurs most often in children 3 to 6 years old.[44,92] Alteration of consciousness in a patient with falciparum malaria and no other explanation for the condition constitutes the general definition. Clinical manifestations are broad. Patients may be comatose without response to stimuli and may assume an opisthotonic posture. Generalized convulsions may occur, but focal findings are uncommon. Intracranial pressure often is increased in children with cerebral malaria, but a relationship between the presence of increased intracranial pressure and morbidity and mortality has not been established. Factors associated

with neurologic sequelae include prolonged coma, severe anemia, and multiple seizures. Mortality rates range from 15 to 30 percent of affected children; most survivors recover completely, but approximately 10 percent may have neurologic sequelae. The most common neurologic sequelae in children noted in a study in the Gambia were hemiplegia, cortical blindness, aphasia, and ataxia.[10]

Many factors, including hypoglycemia, anemia, microvascular obstruction, acidosis, and elaboration of inflammatory mediators, contribute to the syndrome of cerebral malaria. Histopathologic features are minor, with occasional hemorrhages and perivascular infiltrates.

SEVERE ANEMIA

Children younger than 1 year old, especially in sub-Saharan Africa, are those most likely to experience severe malarial anemia. This complication is thought to occur most often in areas with year-round transmission. Clinical consequences of anemia are determined by rate of development of anemia and by severity of anemia, with high risk of complications as hemoglobin decreases to less than 5 g/dL.[128]

HYPOGLYCEMIA

Hypoglycemia (blood glucose <40 mg/dL) may be present on initial evaluation in 20 percent of children with severe malaria and is associated with a poor prognosis. The etiology of pretreatment hypoglycemia is thought to be a combination of parasite consumption of glucose and inadequate gluconeogenesis in the liver. Hypoglycemia also can occur as a result of treatment, most typically with quinine. Rapid intravenous infusion of quinine may cause hypoglycemia by stimulating insulin secretion; pregnant women seem to be especially susceptible to this complication. In addition, hypoglycemia may occur several days into a course of oral quinine, presumably caused by resolution of the reduced tissue sensitivity to insulin that is a feature of acute malaria.

ACID-BASE CHANGES

Metabolic acidosis is a marker of severity and clinically may be manifested as hyperpnea. Acidosis often is associated with hypoglycemia. Fluid resuscitation and treatment with antimalarial drugs often result in rapid resolution of acidosis, although persistence of acidosis may occur in patients who eventually die of malaria.

RENAL COMPLICATIONS

Acute renal failure is a potentially life-threatening consequence of acute malaria that occurs more commonly in adults than in children. It typically is oliguric in nature and often is reversible if the patient can be supported by dialysis through the oliguric phase. Acute renal failure is a rare development in residents of endemic areas and long has been thought to occur more frequently in patients treated with quinine or quinidine (blackwater fever). The histologic changes resemble those of acute tubular necrosis.

Nephrotic syndrome and chronic renal failure occur more frequently in areas where malaria is endemic and usually are associated with *P. malariae*. Symptoms occur in individuals younger than 15 years old in approximately half the cases, with gradual progression to renal failure over 3 to 5 years. Most patients have asymptomatic proteinuria and the gradual development of hypertension and deterioration in renal function. Adults more commonly have hematuria and azotemia; adults and children may have hematuria. The disease does not respond to antimalarial agents. Treatments with steroids, cyclophosphamide, and azathioprine have had variable results, with remission occurring only in patients with mild changes on renal biopsy.[120]

PULMONARY EDEMA

Pulmonary edema typically develops late in the course of severe malaria when other complications are already present, and it occurs more commonly in adults than in children. The pathogenesis is consistent with capillary leak syndrome. Supplemental oxygen or mechanical ventilation with positive end-expiratory pressure may be necessary to manage respiratory complications.

HYPERREACTIVE MALARIAL SYNDROME (TROPICAL SPLENOMEGALY SYNDROME, HYPERREACTIVE MALARIAL SPLENOMEGALY)

Hyperreactive malarial syndrome is characterized by massive splenomegaly, high concentrations of total serum IgM and malarial antibodies of multiple immunoglobulin classes, and clinical and immunologic response to antimalarial agents.[135] Hyperreactive malarial syndrome is correlated with malaria endemicity, with an incidence ranging from 0.5 to 80 percent of the adult population. The pathogenesis is unknown, but seems to involve chronic exposure to malaria, resulting in chronic stimulation of the immune system, and genetic factors. Findings on physical examination include a huge spleen and an enlarged liver. Laboratory findings include anemia and an increased reticulocyte count; some patients may have thrombocytopenia or neutropenia. Patients may have an increased risk of acquiring bacterial infections, and some researchers have suggested that hyperreactive malarial syndrome is a premalignant condition.[5] Lifelong treatment with antimalarial agents is the treatment of choice for patients who reside in endemic areas.[120] Treatment of patients who have left endemic areas has not been standardized; some experts recommend a single treatment course of antimalarials with close monitoring of the size of the spleen.

DIAGNOSIS

The most important first step in establishing the diagnosis of malaria is to consider the diagnosis in all individuals with febrile illness, especially febrile individuals with a history of travel to endemic areas. Having a high index of suspicion for the diagnosis of malaria cannot be overemphasized; failure to diagnose and treat malaria promptly contributed to fatal outcomes in U.S. civilians who died of malaria between 1963 and 2001.[82] Manifestations of the disease are most classic in non-immune individuals or in individuals in areas where malaria transmission is seasonal. The signs and symptoms of disease may be nonspecific in semi-immune individuals, individuals who have received malaria prophylaxis, or individuals who have been partially treated.

In non-endemic areas, a history of travel to an endemic area should suggest the diagnosis in all individuals with a febrile illness, regardless of the accompanying signs and symptoms. Common diagnoses mistakenly assigned to patients ultimately determined to have malaria include gastroenteritis and viral syndrome. The course of disease may be modified by exposure to antimalarial drugs, such as agents that may have been used for prophylaxis, and the incubation period may be prolonged after the administration of antimalarial chemoprophylaxis. Because malaria may be transmitted by blood transfusion or an organ

transplant, may be congenitally acquired, and, rarely, occurs cryptically, the diagnosis also should be considered in patients with compatible signs and symptoms of malaria, anemia, or thrombocytopenia, and no other explanation for their illness.

Microscopy is the technique used most commonly for establishing the diagnosis of malaria. Alternatives to microscopic diagnostic techniques for malaria include tests using reagents based on parasite antigens or enzymes, fluorescent microscopy, DNA probes, polymerase chain reaction (PCR), antibody detection, and flow cytometry. Only a few of these methods meet the requirements of low cost, high reliability and reproducibility, and rapid turnaround time. Some of them are suitable for use in field conditions, however, and have been used in endemic areas under field conditions and for self-diagnosis.

MICROSCOPY

Microscopy is the gold standard for establishing the diagnosis of malaria. Identification of typical parasite forms by an experienced microscopist is the mainstay of diagnosis worldwide (Figs. 231–3 to 231–6). There are many advantages of using light microscopy: it can be performed at low cost, it can be done rapidly, it allows identification of the infecting species and estimation of parasite load, and it can be performed with a small amount of blood. The major disadvantage of microscopy is the need for an experienced microscopist. In settings where malaria is not endemic, the need for specialists to review microscope slides may lead to a delay in establishing the diagnosis.

Thin smears are the most useful in diagnosing malaria. They are easy to prepare, with only a single drop of blood required. The drop of blood is placed at one end of the slide, and the edge of a second slide is placed at the edge of the blood smear and drawn across the slide.[113] RBC morphology is preserved, so invasion of large RBCs by the parasites can be identified, and speciation of the organism is possible (see Table 231–1). Oil-immersion magnification (×1000) should be used for viewing the slide because many young asexual intraerythrocytic parasites are only 2 to 3 μm in diameter and may be missed when using high-dry (×440) magnification. Examining a thin smear is easiest, so the microscopist begins by looking at the thin edge of the blood film, farthest from where the drop of blood was placed. Giemsa stain is preferred to Wright stain when available because it preserves details such as Schüffner dots in *P. vivax* and *P. ovale* infections. When individual RBCs can be seen at low magnification (×100), switching to oil immersion allows examination for parasites within the cells. The major disadvantage of thin films is low sensitivity. With low parasite loads (<100 to 300/μL), the amount of blood on the smear may be too small to detect the parasites.

Thick smears have greater sensitivity than thin smears do as a result of the larger quantity of blood used. Because the RBCs are lysed during preparation of the slide, the types of cells containing parasites cannot be identified. To make a thick smear, a drop of blood is placed on the slide and spread in a circle. The slide is stained without using methanol fixation, a procedure that lyses RBCs.

Estimating parasite density often is useful for assessing the likelihood of development of complications associated with high parasite density and for evaluating response to therapy. The density of parasites can be determined on thick or thin smears.[53] When thin smears are used, the proportion of RBCs infected is counted while the smear is viewed under an oil-immersion lens.

FLUORESCENT MICROSCOPY

The quantitative buffy coat test relies on identification of parasitized RBCs stained with acridine orange in the RBC layer of centrifuged blood.[116] Experienced personnel can perform the test rapidly, but the reagents are costly when compared with those needed for microscopy, and the species of parasite cannot be identified. If a fluorescent microscope is available, identification of parasitized RBCs can be accomplished by staining a thick smear with acridine orange and examining it under fluorescent light.

DETECTION OF PARASITE ANTIGEN

Antigen capture dipstick assays based on detection of histidine-rich protein 2 (HRP-2) (ParaSight F; Becton Dickinson), HRP-2 and aldolase (ICT Malaria Pf/Pv; ICT Diagnostics), or parasite lactate dehydrogenase (LDH) (OptiMAL-IT; Flow, Inc) have been developed.[22,64,78] These assays use a finger-prick sample of blood and give a result in 10 to 15 minutes. Assays detecting HRP-2 are specific for *P. falciparum*, whereas assays based on parasite LDH or HRP-2 and aldolase can identify all four species of *Plasmodium*.[16,78] The Binax Now malaria test, based on detection of a pan-malaria antigen and a circulating antigen specific to *P. falciparum*, was licensed recently in the United States.[24a] The ParaSight F dipstick test, when studied in field trials in Kenya, showed a sensitivity of 96.5 to 100 percent in patients whose parasitemia was greater than 60 parasites/μL, with lower sensitivity noted at lower levels of parasitemia. Antigen persisted to day 6 in 11.9 percent of children whose blood smears were clear at that time.[6] When studied in travelers, the sensitivity of the ParaSight F test ranged from 40 percent in subjects with less than 50 parasites/μL to 93 percent or more in subjects with more than 100 parasites/μL, and positive dipstick tests occurred in 68 percent of blood smear–negative patients on day 7 and in 20 percent on day 28.[43]

Compared with PCR, the HRP-2 assays performed well, with sensitivity of 88 to 95 percent and specificity of 95 to 97 percent; compared with microscopy, sensitivity ranged from 80 to 95 percent, and specificity ranged from 85 to 100 percent.[87] The test also performed well when used in non-immune travelers; a meta-analysis of 21 studies identified sensitivity of 88 to 98 percent and specificity of 95 to 100 percent for detection of *P. falciparum*.[70] The HRP-2/aldolase-based assay that can detect all four species of malaria has a lower sensitivity for diagnosis of *vivax* malaria, although this assay has a higher sensitivity for detection of malaria than the HRP-2 assay.[17,45,78] A limitation of HRP-2-based assays is that they remain positive after therapy because HRP persists in the blood after acute infection.

LDH-based assays are able to distinguish between *P. falciparum* and other malaria species, although the test is unreliable in detecting *P. vivax* in the presence of *P. falciparum*.[96] The sensitivity of OptiMAL-IT was 85 to 95 percent, with specificity of nearly 100 percent for *P. falciparum* and lower specificity (75 to 85%) for *P. vivax*. Sensitivity decreased with decreasing parasite density. An advantage of LDH-based assays is the ability to distinguish between viable and nonviable parasites, rendering the test useful for monitoring response to therapy.[18,45,78,89] Dipstick tests may have a role in cost-effective diagnosis of malaria in situations in which laboratory services are inadequate, in mobile clinics, in locations where levels of malaria transmission are low and drug resistance is high, when the cost of treatment exceeds the cost of the dipstick test, and when blood films are negative and determining the diagnosis is critical. In developed countries, dipstick assays would benefit laboratories with less experienced microscopists by helping them establish a rapid diagnosis, confirm a diagnosis made by microscopy, or determine the species an infection. Current limitations of the tests are decreased sensitivity compared with expert microscopy, especially at low levels of parasite density; cost; and lack of approval by the Food and Drug Administration for use in the United States of some of the tests.

Dipstick malaria tests have been proposed for use by travelers in the self-diagnosis of malaria and have been tested for this

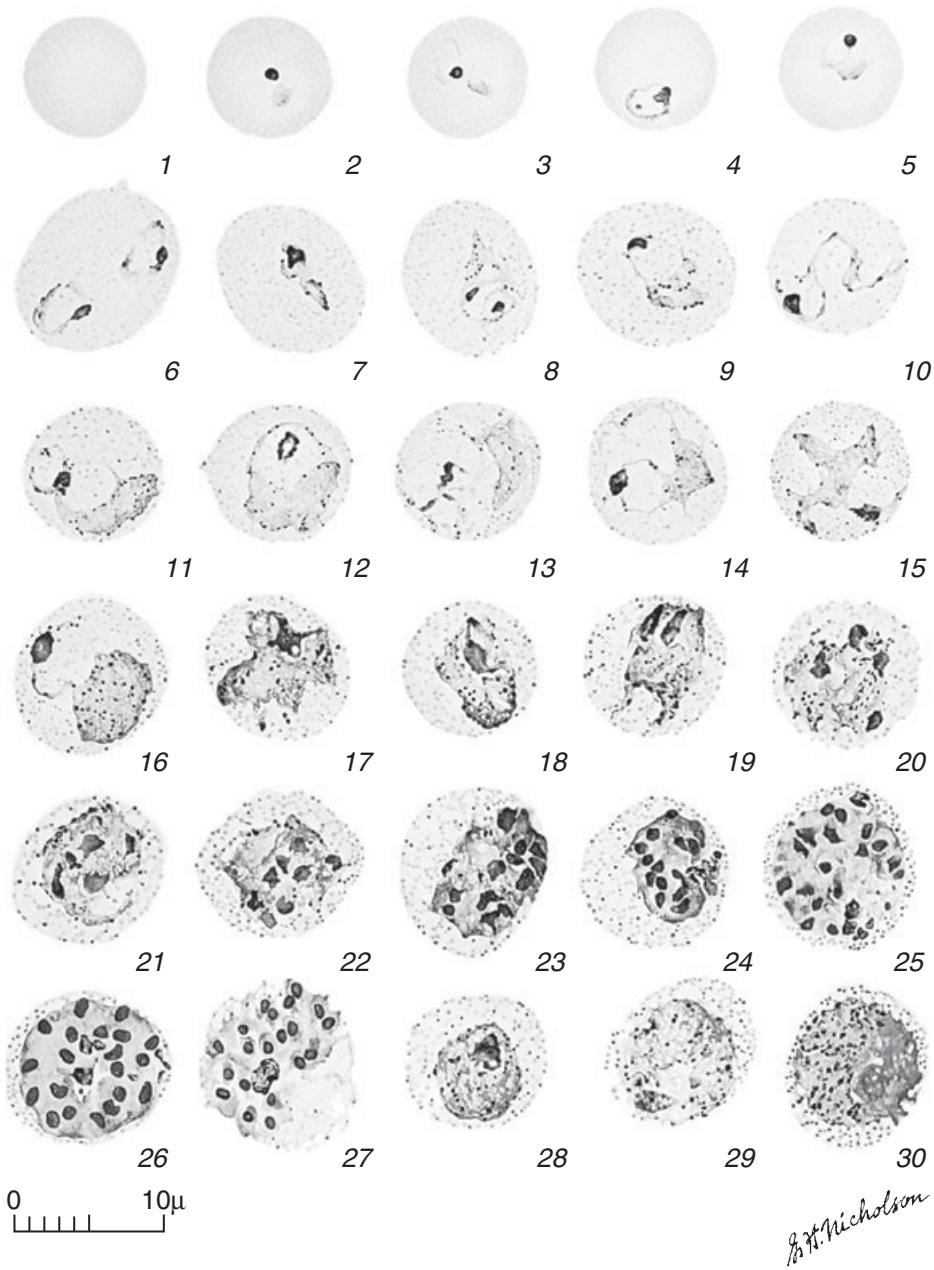


Figure 231–3 *Plasmodium vivax. 1*, Normal erythrocyte; *2-5*, young trophozoites; *6-16*, growing trophozoites; *17*, *18*, mature trophozoites; *19-21*, early schizonts; *22*, *23*, developing schizonts; *24-27*, nearly mature and mature schizonts; *28*, *29*, nearly mature and mature macrogametocytes; *30*, mature microgametocyte. (See companion Expert Consult web site for color version.) *(From Coatney, G. R., Collins, W. E., Warren, M., et al.: The Primate Malarias. Bethesda, MD, U.S. Department of Health and Human Services, National Institutes of Health, National Institute of Allergy and Infectious Diseases, 1971.)*

purpose. The use of these tests for self-diagnosis is controversial, with some experts supporting this use and others contending that technical improvements in the tests are necessary before they can be used accurately to guide decisions about self-treatment of malaria.[46,106]

DNA PROBE

Specific DNA probes for identification of *P. falciparum* have been developed and tested in field conditions in Thailand.[4] The technique compared favorably with microscopy and could be used to test large numbers of samples at a time, but it had the disadvantage of needing a radiolabeled probe. Non-isotope probes have been used in other field trials in Madagascar, where an enzyme-linked probe was compared with microscopy for the diagnosis of *falciparum* malaria. The results were comparable to examination of thick smears by microscopy for *P. falciparum* infection, but the assay could not identify infection with *P. vivax*, *P. ovale*, or *P. malariae* alone.[73]

POLYMERASE CHAIN REACTION

PCR-based techniques used for establishing the diagnosis of malaria are highly sensitive and now are able to detect mixed infections. Compared with thick and thin smears, sensitivity and specificity are nearly 100 percent. PCR techniques can be useful for determining the species of an infection when microscopy is equivocal. Early detection of resistance to antimalarial drugs is another benefit of PCR-based techniques, which have been used

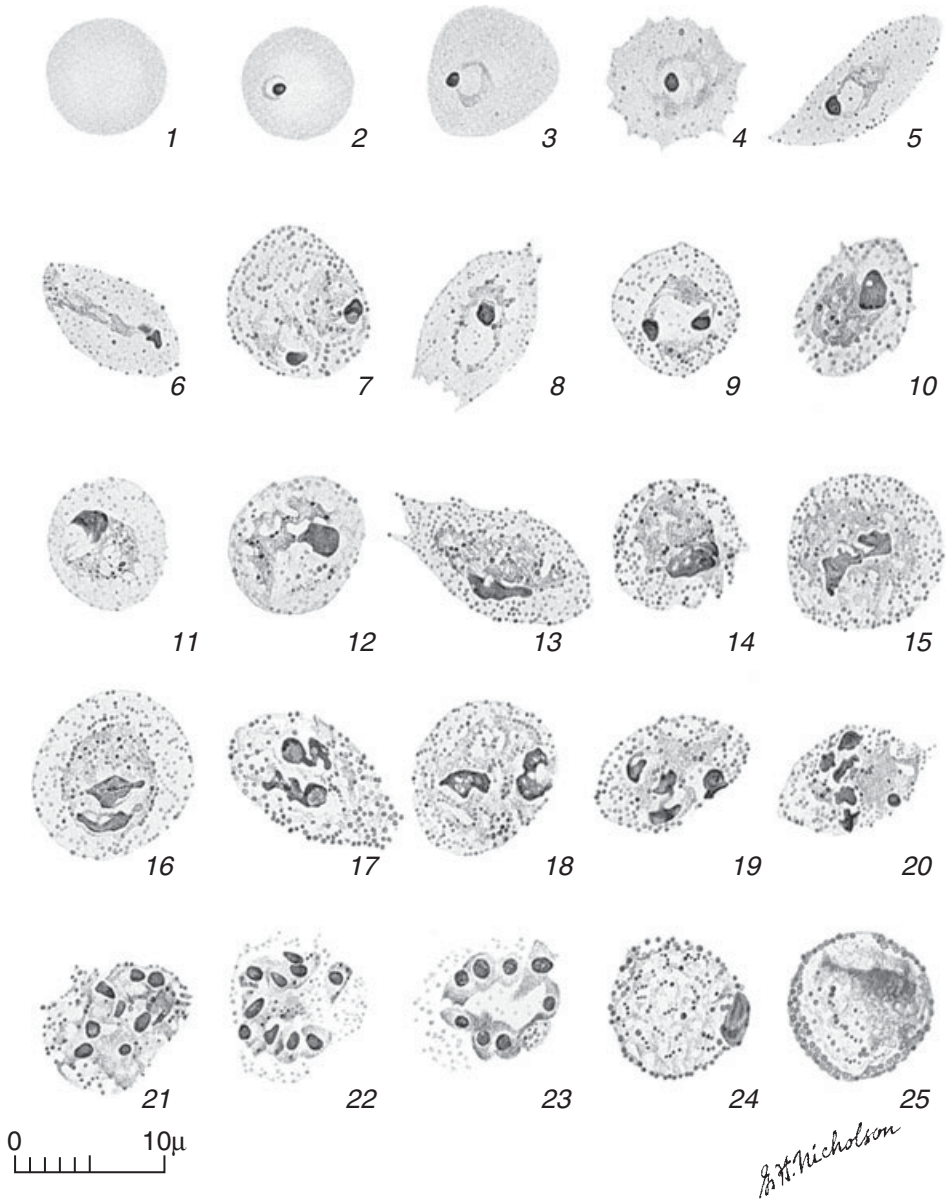


Figure 231–4 *Plasmodium ovale*. *1*, Normal erythrocyte; *2-5*, young trophozoites; *6-12*, growing trophozoites; *13-15*, mature trophozoites; *16-22*, developing schizonts; *23*, mature schizont; *24*, adult macrogametocyte; *25*, adult microgametocyte. (See companion Expert Consult web site for color version.) *(From Coatney, G. R., Collins, W. E., Warren, M., et al.: The Primate Malarias. Bethesda, MD, U.S. Department of Health and Human Services, National Institutes of Health, National Institute of Allergy and Infectious Diseases, 1971.)*

to detect a mutation of *P. falciparum* associated with resistance to chloroquine.[24,48]

Major disadvantages of PCR include the need for expensive equipment and reagents, significant technical expertise, and the length of time required to perform the testing. At this time, PCR may be better suited for use in large-scale epidemiologic surveys, rather than for establishing the diagnosis of acute malaria in the clinical setting.

FLOW CYTOMETRY

Automated blood cell analyzers based on flow cytometry have been shown to display cells containing malaria pigment as a population of cells distinct from other blood components. To date, this discovery has been serendipitous in patients not suspected to have malaria, and the diagnosis must be confirmed by conventional methods.[34,49] A future application of this technology could be to modify automated blood cell analyzers to flag specimens that need to be examined further by thin or thick smears (or both).[49]

ANTIBODY DETECTION

Malaria antibodies develop rapidly after infection and remain present for years; they are of limited value in diagnosing malaria in individual patients. Occasionally, antibody tests might prove useful in the diagnosis of non-immune individuals with cryptic febrile illnesses or on a population basis to assess the degree of community-wide immunity. Measurement of antibody may have a role in the diagnosis of hyperreactive malarial syndrome.[135]

TREATMENT

Treatment of malaria depends on identification of the species of *Plasmodium* causing infection, knowledge of the presence of resis-

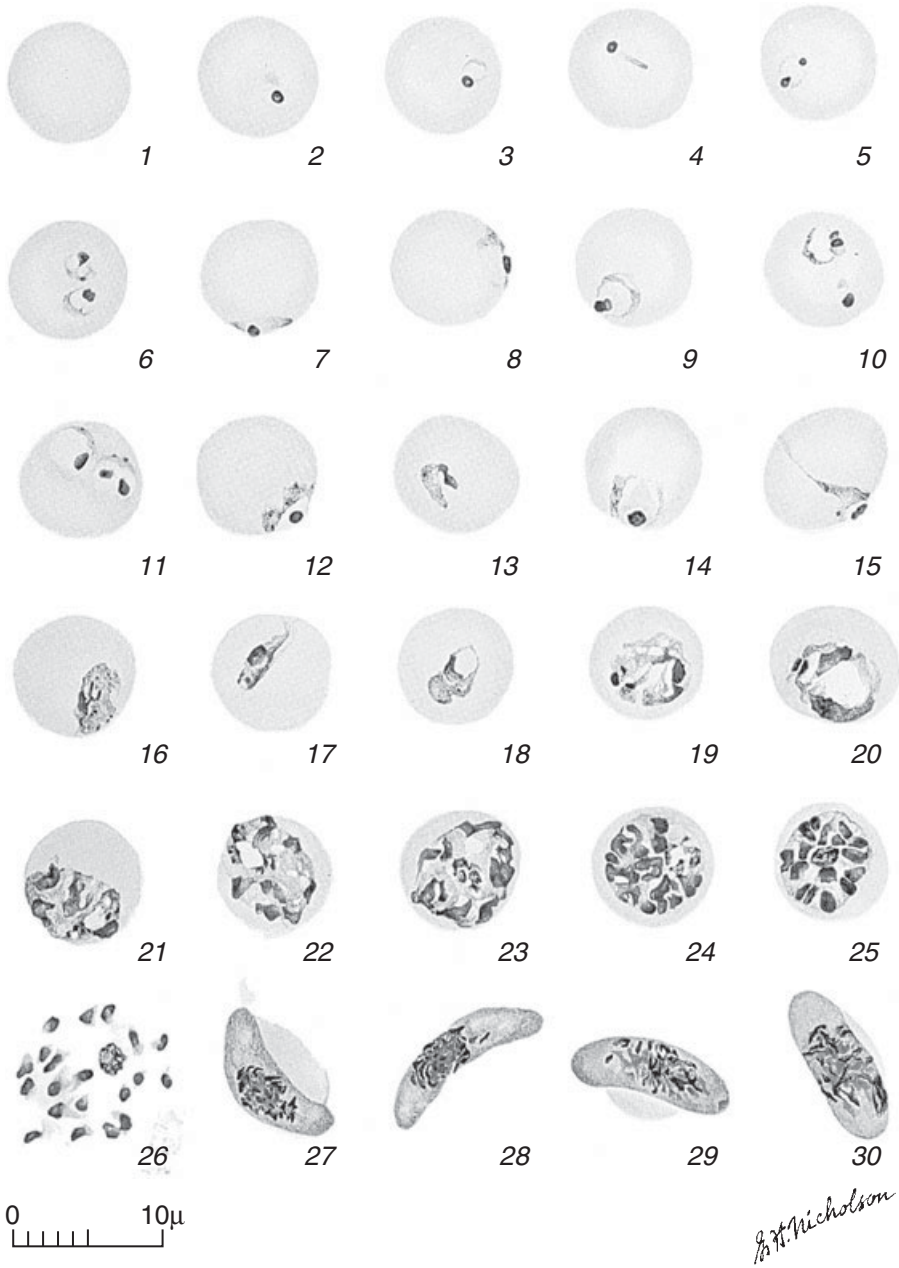


Figure 231–5 *Plasmodium falciparum*. *1*, Normal erythrocyte; *2-11*, young trophozoites; *12-15*, growing trophozoites; *16-18*, mature trophozoites; *19-22*, developing schizonts; *23-26*, nearly mature and mature schizonts; *27*, *28*, mature macrogametocytes; *29*, *30*, mature microgametocytes. (See companion Expert Consult web site for color version.) *(From Coatney, G. R., Collins, W. E., Warren, M., et al.: The Primate Malarias. Bethesda, MD, U.S. Department of Health and Human Services, National Institutes of Health, National Institute of Allergy and Infectious Diseases, 1971.)*

tance to chloroquine in the area in which malaria was contracted, and therapeutic goals (treatment of acute illness, eradication of the exoerythrocytic phase of malaria, or prevention of infection). Drugs and classes of drugs currently in use for treating malaria include 4-aminoquinolines and related compounds (chloroquine, mefloquine [Lariam], amodiaquine); primaquine; sulfadoxine-pyrimethamine (Fansidar); halofantrine; atovaquone-proguanil (Malarone); artemisinin (qinghaosu) and other artemisinins (artemether, artesunate); cinchona alkaloids (quinine, quinidine); and the antimicrobial agents doxycycline, tetracycline, and clindamycin. Other combination drugs are available outside the United States, and multiple investigational compounds are in various stages of development. Drugs that may be used for the treatment of malaria are listed in Table 231–2. Drugs used for the prevention of malaria are discussed in a subsequent section.

The choice of agent to treat symptomatic malaria depends on the presence of resistance to chloroquine and other antimalarial drugs, the availability of drugs in the local area, and the age of the patient. Local and national guidelines for the treatment of malaria differ, and practitioners treating patients with malaria should consult local resources and experts. Information about countries and regions with chloroquine-resistant malaria is available in the United States from the Centers for Disease Control and Prevention (CDC) (http://www.cdc.gov/malaria/diagnosis_treatment/tx_clinicians.htm) and outside the United States from local and national health organizations and WHO (http://www.who.int/malaria/diagnosisandtreatment.html). In malaria endemic areas, strategies such as intermittent treatment of infants and pregnant women are being employed in combination with use of insecticides and treated bed nets to prevent and treat

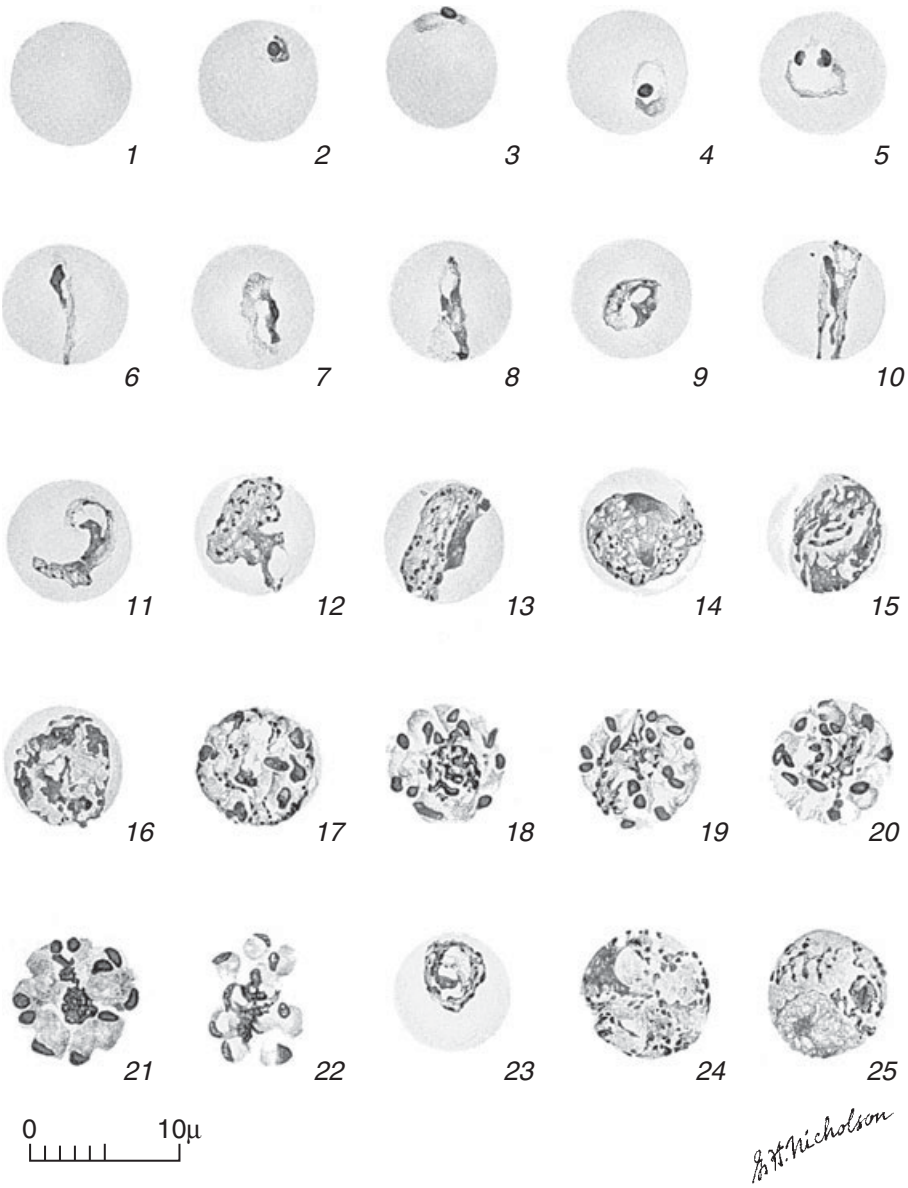


Figure 231–6 *Plasmodium malariae*. *1*, Normal erythrocyte; *2-5*, young trophozoites; *6-11*, growing trophozoites; *12*, *13*, nearly mature and mature trophozoites; *14-20*, developing schizonts; *21*, *22*, mature schizonts; *23*, developing gametocyte; *24*, mature macrogametocyte; *25*, mature microgametocyte. (See companion Expert Consult web site for color version.) *(From Coatney, G. R., Collins, W. E., Warren, M., et al.: The Primate Malarias. Bethesda, MD, U.S. Department of Health and Human Services, National Institutes of Health, National Institute of Allergy and Infectious Diseases, 1971.)*

malaria.[105] A full discussion of these programs is beyond the scope of this chapter.

ANTIMALARIAL AGENTS AVAILABLE FOR USE IN THE UNITED STATES

Chloroquine

Chloroquine is the drug of choice for chloroquine-susceptible malaria caused by *P. falciparum* and *P. vivax* and for all infections with *P. ovale* and *P. malariae*. Chloroquine is a 4-aminoquinoline with rapid schizonticidal activity. It is not effective against exoerythrocytic forms of the parasite, and patients treated with chloroquine after acquiring *P. vivax* or *P. ovale* infection need to have additional treatment to eliminate these forms. Chloroquine is most active against late ring stages and mature trophozoites. Response to treatment is rapid, with disappearance of parasitemia within 48 to 72 hours after initiation of treatment when the parasite is susceptible.

Chloroquine is available as an oral preparation and, in many areas, as intramuscular and intravascular formulations. Oral absorption is excellent, and the drug is safe and effective when given by nasogastric or orogastric tube in comatose children.[130] Intramuscular and intravenous administration may be associated with an increased risk for cardiac arrhythmias.

Adverse reactions to chloroquine rarely occur at the doses used for suppression or treatment of malaria. Side effects include gastrointestinal symptoms such as nausea, vomiting, and diarrhea; dizziness; headache; and fatigue. Pruritus may be a troubling adverse event, especially in black patients. Chloroquine has no known teratogenic effects, and it may be used in pregnant and lactating women. The drug is not contraindicated in glucose-6-phosphate dehydrogenase (G6PD)–deficient individuals. Serious adverse events, including hypotension, cardiac arrest, and circulatory failure, may occur after intravenous or intramuscular use of the drug, especially in children. Psychotic symptoms may occur rarely with chloroquine.[28] Chloroquine always should be given

TABLE 231–2 Guidelines for Treatment of Malaria in the United States*

Clinical Diagnosis/ *Plasmodium* species	Region Infection Acquired	Recommended Drug and Adult Dose[1,8]	Recommended Drug and Pediatric Dose[1,8] *Pediatric dose should NEVER exceed adult dose*
Uncomplicated malaria/*P. falciparum* or Species not identified If "species not identified" is subsequently diagnosed as *P. vivax* or *P. ovale:* see *P. vivax* and *P. ovale* on p. 2912. retreatment with primaquine	**Chloroquine-sensitive** (Central America west of Panama Canal; Haiti; the Dominican Republic; and most of the Middle East)	**Chloroquine phosphate (Aralen and generics)** 600 mg base (=1000 mg salt) PO immediately, followed by 300 mg base (=500 mg salt) PO at 6, 24, and 48 hr Total dose: 1500 mg base (=2500 mg salt) ***2nd line alternative for treatment:*** **Hydroxychloroquine (Plaquenil and generics)** 620 mg base (=800 mg salt) PO immediately, followed by 310 mg base (=400 mg salt) PO at 6, 24, and 48 hr Total dose: 1550 mg base (=2000 mg salt)	**Chloroquine phosphate (Aralen and generics)** 10 mg base/kg PO immediately, followed by 5 mg base/kg PO at 6, 24, and 48 hr Total dose: 25 mg base/kg ***2nd line alternative for treatment:*** **Hydroxychloroquine (Plaquenil and generics)** 10 mg base/kg PO immediately, followed by 5 mg base/kg PO at 6, 24, and 48 hr Total dose: 25 mg base/kg
	Chloroquine-resistant or unknown resistance[1] (All malarious regions except those specified as chloroquine-sensitive listed above. Middle Eastern countries with chloroquine-resistant *P. falciparum* include Iran, Oman, Saudi Arabia, and Yemen. Of note, infections acquired in the newly independent states of the former Soviet Union and Korea to date have been uniformly caused by *P. vivax* and should therefore be treated as chloroquine-sensitive infections.)	**A. Quinine sulfate[2] plus one of the following:** **Doxycycline, tetracycline, or clindamycin** **Quinine sulfate:** 542 mg base (=650 mg salt)[3] PO tid × 3 to 7 days **Doxycline:** 100 mg PO bid × 7 days **Tetracycline:** 250 mg PO qid × 7 days **Clindamycin:** 20 mg base/kg/day PO divided tid × 7days	**A. Quinine sulfate[2,3] plus one of the following:** **Doxycycline,[4] tetracycline,[4] or clindamycin** **Quinine sulfate:** 8.3 mg base/kg (=10 mg salt/kg)[3] PO tid × 3 to 7 days **Doxycycline:** 2.2 mg/kg PO every 12 hr × 7 days **Tetracycline:** 25 mg/kg/day PO divided qid × 7 days **Clindamycin:** 20 mg base/kg/day PO divided tid × 7 days
		B. Atovaquone-proguanil (Malarone)[5] **Adult tab** = 250 mg atovaquone/100 mg proguanil 4 adult tabs PO qd × 3 days	**B. Atovaquone-proguanil (Malarone)[5]** **Adult tab = 250 mg atovaquone/100 mg proguanil** **Peds tab = 62.5 mg atovaquone/100 mg proguanil** 5-8 kg: 2 peds tabs PO qd × 3 d 9-10 kg: 3 peds tabs PO qd × 3 d 11-20 kg: 1 adult tabs PO qd × 3 d 21-30 kg: 2 adult tabs PO qd × 3 d 31-40 kg: 3 adult tabs PO qd × 3 d >40 kg: 4 adult tabs PO qd × 3 d
		C. Mefloquine (Lariam and generics)[6] 684 mg base (=750 mg salt) PO as initial dose, followed by 456 mg base (=500 mg salt) PO given 6-12 hr after initial dose Total dose = 1250 mg salt	**C. Mefloquine (Lariam and generics)[6]** 13.7 mg base/kg (=15 mg salt/kg) PO as intial dose, followed by 9.1 mg base/kg (=10 mg salt/kg) PO given 6-12 hr after initial dose Total dose = 25 mg salt/kg
Uncomplicated malaria/*P. malariae*	**All regions**	**Chloroquine phosphate:** Treatment as for mefloquine ***2nd line alternative for treatment:*** **Hydroxychloroquine:** Treatment as for mefloquine	**Chloroquine phosphate:** Treatment as for mefloquine ***2nd line alternative for treatment:*** **Hydroxychloroquine:** Treatment as for mefloquine

**Based on drugs currently available for use in the United States. If outside the United States, contact national authorities about current recommendations.*

[1]*Note: There are three options (A, B, or C) available for treatment of uncomplicated malaria caused by chloroquine-resistant P. falciparum. Options A and B are equally recommended. Because of a higher rate of severe neuropsychiatric reactions seen at treatment doses, we do not recommend option C (mefloquine) unless options A and B cannot be used. For option A, because there is more data on the efficacy of quinine in combination with doxycycline or tetracycline, these treatment combinations are generally preferred to quinine in combination with clindamycin.*

[2]*For infections acquired in Southeast Asia, quinine treatment should continue for 7 days. For infections acquired in Africa and South America, quinine treatment should continue for 3 days.*

[3]*U.S.-manufactured quinine sulfate capsule is in a 324 mg dosage; therefore, 2 capsules should be sufficient for adult dosing. Pediatric dosing may be difficult due to unavailability of non–capsule forms of quinine. If unable to provide pediatric doses of quinine, consider malarone (recommendation B) or mefloquine (recommendation C).*

[4]*Doxycycline and tetracycline are not indicated for use in children younger than 8 years of age. For children younger than 8 years of age with chloroquine-resistant P. falciparum, quinine (given alone for 7 days or given in combination with clindamycin) and atovaquone-proguanil are recommended treatment options; mefloquine can be considered if no other options are available. For children younger than 8 years of age with chloroquine-resistant P. vivax, quinine (given alone for 7 days) or mefloquine is the recommended treatment option. If none of these treatment options is available or is not being tolerated and if the treatment benefits outweigh the risks, doxycycline or tetracycline may be given to children younger than 8 years of age.*

[5]*Give atovaquone-proguanil with food. If patient vomits within 30 minutes of taking a dose, then the dose should be repeated.*

[6]*Treatement with mefloquine is not recommended in persons who have acquired infections from the Southeast Asian region of Myanmar, Thailand, and Cambodia due to resistant strains.*

Continued

TABLE 231–2 Guidelines for Treatment of Malaria in the United States—cont'd

Clinical Diagnosis/ *Plasmodium* species	Region Infection Acquired	Recommended Drug and Adult Dose[1,8]	Recommended Drug and Pediatric Dose[1,8] *Pediatric dose should NEVER exceed adult dose*
Uncomplicated malaria/*P. vivax* or *P. ovale*	**All regions**[8] *Note:* for suspected chloroquine-resistant *P. vivax*, see row below	**Chloroquine phosphate plus primaquine phosphate**[7] **Chloroquine phosphate:** Treatment as for mefloquine **Primaquine phosphate:** 30 mg base PO qd × 14 days ***2nd line alternative for treatment:*** **Hydroxychloroquine plus primaquine phosphate**[7] **Hydroxychloroquine:** Treatment as for mefloquine **Primaquine phosphate:** 30 mg base PO qd × 14 days	**Chloroquine phosphate plus primaquine phosphate**[7] **Chloroquine phosphate:** Treatment as for mefloquine **Primaquine phosphate:** 0.5 mg base/kg PO qd × 14 days ***2nd line alternative for treatment:*** **Hydroxychloroquine plus primaquine phosphate**[7] **Hydroxychloroquine:** Treatment as for primaquine phosphate **Primaquine phosphate:** 30 mg base PO qd × 14 days
Uncomplicated malaria/*P. vivax*	**Chloroquine-resistant**[8] (Papua New Guinea and Indonesia)	**A. Quinine sulfate**[2] **plus either doxycycline or tetracycline plus primaquine phosphate**[7] **Quinine sulfate:** Treatment as for primaquine phosphate **Doxycycline or Tetracycline:** Treatment as above **Primaquine phosphate:** Treatment as for primaquine phosphate	**A. Quinine sulfate**[2,3] **plus either doxycycline**[4] **or tetracycline**[4] **plus primaquine phosphate**[7] **Quinine sulfate:** Treatment as for primaquine phosphate **Doxycline or tetracycline:** Treatment as for primaquine phosphate **Primaquine phosphate:** Treatment as for primaquine phosphate
		B. Mefloquine plus primaquine phosphate[7] Mefloquine: Treatment as for primaquine phosphate Primaquine phosphate: Treatment as above	**B. Mefloquine plus primaquine phosphate**[7] Mefloquine: Treatment as for primaquine phosphate Primaquine phosphate: Treatment as for primaquine phosphate
Uncomplicated malaria: Alternative for pregnant women[9-12]	**Chloroquine-sensitive**[12] (see uncomplicated malaria sections above for chloroquine-sensitive *Plasmodium* species by region)	**Chloroquine phosphate:** Treatment as for primaquine phosphate ***2nd line alternative for treatment:*** **Hydroxychloroquine:** Treatment as for primaquine phosphate	Not applicable
	Chloroquine-resistant *P. falciparum*[9-11] (see uncomplicated malaria sections above for regions with known chloroquine resistant *P. falciparum*)	**Quinine sulfate**[2] **plus clindamycin** **Quinine sulfate:** Treatment as for primaquine phosphate **Clindamycin:** Treatment as for primaquine phosphate	Not applicable
	Chloroquine-resistant *P. vivax*[9-12] (see uncomplicated malaria sections above for regions with chloroquine-resistant *P. vivax*)	**Quinine sulfate** **Quinine sulfate:** 650 mg salt PO tid × 7 days	Not applicable
Severe malaria[13,14,15,16]	**All regions**	**Quinidine gluconate**[14] **plus one of the following:** **Doxycline, tetracycline, or clinidamycin** **Quinidine gluconate:** 6.25 mg base/kg (=10 mg salt/kg) loading dose IV over 1-2 hr, then 0.0125 mg base/kg/min (=0.02 mg salt/kg/min) continuous infusion for at least 24 hr. An alternative regimen is 15 mg base/kg (=24 mg salt/kg) loading dose IV infused over 4 hr, followed by 7.5 mg base/kg (=12 mg salt/kg) infused over 4 hr every 8 hr, starting 8 hr after the loading dose (see package insert). Once parasite density <1% and patient can take oral medication, complete treatment with oral quinine, dose as above. Quinidine/quinine course = 7 days in Southest Asia; = 3 days in Africa or South America.	**Quinidine gluconate**[14] **plus one of the following:** **Doxycline,**[4] **tetracycline,**[4] **or clinidamycin** **Quinidine gluconate:** Same mg/kg dosing and recommendations as for adults. **Doxycycline:** Treatment as for primaquine phosphate. If patient not able to take oral medication, may give IV. For children <45 kg, give 2.2 mg/kg IV every 12 hr and then switch to oral doxycycline (dose as above) as soon as patient can take oral medication. For children ≥45 kg, use same dosing as for adults. For IV use, avoid rapid administration. Treatment course = 7 days. **Tetracycline:** Treatment as for primaquine phosphate **Clindamycin:** Treatment as for primaquine phosphate. If patient not able to take oral medication, give 10 mg base/kg loading dose IV followed by 5 mg base/kg IV every 8 hr. Switch to oral clindamycin (oral dose as for primaquine phosphate) as soon as patient can take oral medication. For IV use, avoid rapid administration. Treatment course = 7 days.

	Doxycycline: Treatment as for quinidine gluconate. If patient not able to take oral medication, give 100 mg IV every 12 hr and then switch to oral doxycycline (as above) as soon as patient can take oral medication. For IV use, avoid rapid administration. Treatment course = 7 days. **Tetracycline:** Treatment as for quinidine gluconate **Clindamycin:** Treatment as for quinidine gluconate. If patient not able to take oral medication, give 10 mg base/kg loading dose IV followed by 5 mg base/kg IV every 8 hr. Switch to oral clindamycin (oral dose as above) as soon as patient can take oral medication. For IV use, avoid rapid administration. Treatment course = 7 days. ***Investigational new drug (contact CDC for information):*** **Artesunate followed by one of the following: Atovaquone-proguanil (Malarone),[5] Doxycycline (clindamycin in pregnant women), or mefloquine**	***Investigational new drug (contact CDC for information):*** **Artesunate followed by one of the follow: Atovaquone-proguanil (Malarone),[5] clindamycin, or mefloquine**

[7] *Primaquine is used to eradicate any hypnozoite forms that may remain dormant in the liver, and thus prevent relapses, in P. vivax and P. ovale infections. Because primaquine can cause hemolytic anemia in persons with G6PD deficiency, patients must be screened for G6PD deficiency prior to starting treatment with primaquine. For persons with borderline G6PD deficiency, or as an alternate to the above regimen, primaquine may be given 45 mg orally one time per week for 8 weeks; consultation with an expert in infectious disease and/or tropical medicine is advised if this alternative regimen is considered in G6PD-deficient persons. Primaquine must not be used during pregnancy.*

[8] *Note: There are two options (A or B) available for treatment of uncomplicated malaria caused by chloroquine-resistant P. vivax. High treatment failure rates due to chloroquine-resistant P. vivax have been well documented in Papua New Guinea and Indonesia. Rare case reports of chloroquine-resistant P. vivax have also been documented in Burma (Myanmar), India, and Central and South America. Persons acquiring P. vivax infections outside of Papua New Guinea or Indonesia should be started on chloroquine. If the patient does not respond, the treatment should be changed to a chloroquine-resistant P. vivax regimen and the CDC should be notified (Malaria Hotline number: 770-488-7788 or 770-488-7100). For treatment of chloroquine-resistant P. vivax infections, options A and B are equally recommended.*

[9] *For pregnant women diagnosed with uncomplicated malaria caused by chloroquine-resistant P. falciparum or chloroquine-resistant P. vivax infection, treatment with doxycycline or tetracycline is generally not indicated. However, doxycycline or tetracycline may be used in combination with quinine (as recommended for nonpregnant adults) if other treatment options are not available or are not being tolerated, and the benefit is judged to outweigh the risks.*

[10] *Because there are no adequate, well-controlled studies of atovaquone and/or proguanil hydrochloride in pregnant women, atovaquone-proguanil is generally not recommended for use in pregnant women. For pregnant women diagnosed with uncomplicated malaria caused by chloroquine-resistant P. falciparum infection, atovaquone-proguanil may be used if other treatment options are not available or are not being tolerated, and if the potential benefit is judged to outweigh the potential risks. There are no data on the efficacy of atovaquone-proguanil in the treatment of chloroquine-resistant P. vivax infections.*

[11] *Because of a possible association with mefloquine treatment during pregnancy and an increase in stillbirths, mefloquine is generally not recommended for treatment in pregnant women. However, mefloquine may be used if it is the only treatment option available and if the potential benefit is judged to outweigh the potential risks.*

[12] *For P. vivax and P. ovale infections, primaquine phosphate for radical treatment of hypnozoites should not be given during pregnancy. Pregnant patients with P. vivax and P. ovale infections should be maintained on chloroquine prophylaxis for the duration of their pregnancy. The chemoprophylactic dose of chloroquine phosphate is 300 mg base (=500 mg salt) orally once per week. After delivery, pregnant patients who do not have G6PD deficiency should be treated with primaquine.*

[13] *Persons with a positive blood smear OR history of recent possible exposure and no other recognized pathology who have one or more of the following clinical criteria (impaired consciousness/coma, severe normocytic anemia, renal failure, pulmonary edema, acute respiratory distress syndrome, circulatory shock, disseminated intravascular coagulation, spontaneous bleeding, acidosis, hemoglobinuria, jaundice, repeated generalized convulsions, and/or parasitemia of >5%) are considered to have manifestations of more severe disease. Severe malaria is practically always due to P. falciparum.*

[14] *Patients diagnosed with severe malaria should be treated aggressively with parenteral antimalarial therapy. Treatment with IV quinidine should be initiated as soon as possible after the diagnosis has been made. Patients with severe malaria should be given an intravenous loading dose of quinidine unless they have received more than 40 mg/kg of quinine in the preceding 48 hours or if they have received mefloquine within the preceding 12 hours. Consultation with a cardiologist and a physician with experience in treating malaria is advised when treating malaria patients with quinidine. During administration of quinidine, blood pressure monitoring (for hypotension) and cardiac monitoring (for widening of the QRS complex and/or lengthening of the QTc interval) should be monitored continuously and blood glucose (for hypoglycemia) should be monitored periodically. Cardiac complications, if severe, may warrant temporary discontinuation of the drug or slowing of the intravenous infusion.*

[15] *Consider exchange transfusion if the parasite density (i.e., parasitemia) is >10% OR if the patient has altered mental status, nonvolume overload pulmonary edema, or renal complications. The parasite density can be estimated by examining a monolayer of red blood cells (RBCs) on the thin smear under oil immersion magnification. The slide should be examined where the RBCs are more or less touching (approximately 400 RBCs per field). The parasite density can then be estimated from the percentage of infected RBCs and should be monitored every 12 hours. Exchange transfusion should be continued until the parasite density is <1% (usually requires 8-10 units). IV quinidine administration should not be delayed for an exchange transfusion and can be given concurrently throughout the exchange transfusion.*

[16] *Pregnant women diagnosed with severe malaria should be treated aggressively with parenteral antimalarial therapy.*

From http://www.cdc.gov/malaria/pdf/treatmenttable.pdf

slowly when administered intravenously and in small doses when given by intramuscular injection; some experts discourage the use of parenteral chloroquine. Oral doses are absorbed rapidly. The risk of aspiration occurring while giving a dose by tube to a comatose patient must be weighed against the risk of delaying therapy if parenteral treatment is unavailable or of complications developing when giving chloroquine by the intravenous or intramuscular route. The lethal dose of chloroquine is 1 g in children and 4 g in adults. Cumulative doses exceeding 100 g are associated with an increased risk for development of retinopathy. Dose reduction may be required for patients with renal and hepatic disease.

Atovaquone-Proguanil

A fixed-dose combination (available in adult and pediatric formulations) of atovaquone and proguanil hydrochloride is available for treatment of malaria caused by *P. falciparum* for individuals who were not taking this agent for prophylaxis. The combination is significantly more effective than either drug alone, and is effective for the treatment of malaria strains resistant to other antimalarial drugs. It has fewer reported adverse events than quinine-based regimens. The combination also is likely to be active against the blood stages of *P. vivax*, *P. ovale*, and *P. malariae*, but the regimen does not have activity against the hypnozoites of *P. vivax*. Atovaquone-proguanil is significantly more effective than mefloquine, chloroquine, amodiaquine, or pyrimethamine-sulfadoxine in locations where parasites are resistant to these drugs.[59]

Mefloquine

The development of mefloquine was a major advance in the treatment of chloroquine-resistant malaria, although resistance has developed rapidly in some parts of the world where it is used widely. Mefloquine is a 4-quinolone-carbinolamine whose exact mechanism is unknown. Its main target is early trophozoites. Similar to chloroquine, it is not active against exoerythrocytic phases. Mefloquine has been associated with neurologic and psychiatric side effects when used for treatment or prophylaxis.[120] Vomiting occurs commonly when mefloquine is given to children younger than 5 years old and may decrease its efficacy.[114] Mefloquine should not be administered concurrently with quinine, quinidine, or halofantrine because of concern about arrhythmias. The drug has been used in the second and third trimesters of pregnancy without adverse outcome.

Quinine and Quinidine

Quinine has been used for the treatment of malaria for centuries.[120] It is active against the mature asexual erythrocytic forms of all four species of human malaria and against the gametes of all species except *P. falciparum*, where it has activity against only immature gametes. It is not effective against exoerythrocytic forms. Quinine is the drug of choice for severe or complicated malaria. It may be given orally, but intravenous and intramuscular forms are available. Quinine crosses the placenta easily and may be used in the treatment of pregnant women, although close monitoring for hypoglycemia is warranted.[60]

Adverse reactions to quinine include tinnitus, headache, nausea, visual and hearing disturbances, and tremors, a constellation of symptoms called *cinchonism*. Symptoms may appear during the first 1 to 3 days of therapy and stop when treatment is terminated. Hypoglycemia may occur, particularly when the drug is used to treat pregnant women. Severe adverse events are rare, but may occur idiosyncratically. Blackwater fever (hemoglobinuria) seems to occur after treatment with quinine, but the causal mechanism is unknown.

Recrudescence rates may be high when quinine is used alone because of the presence of quinine-resistant strains of *P. falciparum*. A second drug usually is added to treat the remaining forms. Drugs that may be used with quinine include doxycycline, tetracycline, and clindamycin.

Quinidine is a related drug that may be given intravenously; in some developed countries where intravenous quinine is unavailable, it is the drug of choice for severe malaria. The mechanisms of action and adverse events are similar, although cardiac arrhythmias occur more commonly with quinidine, and cardiac monitoring is advised.[129]

Tetracycline and Doxycycline

Tetracycline, doxycycline, and the related drug minocycline have very slow activity against malarial schizonts.[120] Doxycycline and minocycline may be given once daily. They are active against chloroquine-resistant and pyrimethamine-sulfadoxine–resistant *P. falciparum*. Resistance to tetracycline has not been shown. Because of their slow activity, these agents should be given in combination with a rapidly acting drug such as quinine. These drugs should not be used in children younger than 8 years old or by pregnant or nursing women.

Primaquine

Primaquine, an 8-aminoquinoline derivative that is effective against exoerythrocytic hypnozoites, is used primarily to prevent relapses of *P. vivax* and *P. ovale*, although it may not be completely effective against some strains of *P. vivax*. It is not an effective blood schizonticide, but is an active gametocidal and sporontocidal drug for all four species of *Plasmodium* that cause human malaria. Primaquine can cause intravascular hemolysis in individuals with G6PD deficiency. Other adverse events include gastrointestinal symptoms such as nausea, epigastric pain, anorexia, and abdominal cramps. Other rare side effects include methemoglobinemia, hemoglobinuria, and bone marrow suppression. Safety in pregnancy has not been established, and the drug should not be used by pregnant women. Patients should be tested for G6PD deficiency before being treated with primaquine.

ANTIMALARIAL AGENTS NOT CURRENTLY AVAILABLE OR RECOMMENDED FOR TREATMENT OF MALARIA IN THE UNITED STATES

Artemesinin Derivatives

Artemesinin (qinghaosu) is a sesquiterpene lactone that, along with its derivatives (artemether, artesunate, arteether, and others), has antimalarial activity thought to be due to the ability to cause free radical damage in parasite membrane systems.[38] These compounds are unique in their ability to cure malaria more rapidly than other agents, with no apparent adverse events. Except for parenteral artemesinin, available on an emergency basis only from the CDC, they are unavailable in the United States, but are used widely in other parts of the world. When used alone, recrudescence rates are high, and the drug class seems to have limited potential for prophylaxis. Oral preparations, suppositories, and oil-based preparations for intramuscular injection are available, although standardization of preparations is lacking.[99] The major utility of these drugs at this time is for severe malaria in areas where quinine resistance is appearing or, in combination with other antimalarials, in areas of multidrug-resistant *P. falciparum*.[1,37] In clinical trials in the Gambia, artemether was as effective as quinine and chloroquine for treatment of cerebral malaria in children.[125,131] When used in sequence with mefloquine, the combination was effective and tolerated well by patients with acute, uncomplicated *falciparum* malaria in Thailand, an area with multidrug resistance.[61] Later studies showed that the use of this combination may have helped halt the progressions of mefloquine resistance in the region.[86]

Artemether-Lumefantrine

The fixed dose combination of artemether (20 mg) and lumefantrine (120 mg) will be available in the United States, and is considered a first-line agent for treatment of uncomplicated *falciparum* malaria in countries where it is available. It is well tolerated and effective against multidrug-resistant *falciparum* malaria.[128] A six-dose regimen compared favorably with other agents in African children and adults with uncomplicated *falciparum* malaria.[80,93]

Pyrimethamine-Sulfadoxine

The combination pyrimethamine-sulfadoxine, a sulfonamide plus a dihydrofolate reductase inhibitor, exhibits synergy in its action against malaria parasites; the combination is active even against strains resistant to the individual drug components. Although pyrimethamine-sulfadoxine has been used for presumptive treatment of malaria in travelers who are taking other drugs for prophylaxis in areas where chloroquine resistance occurs, widespread resistance to the drug combination and its slow action should limit its use in non-immune individuals with *falciparum* malaria. The CDC no longer recommends pyrimethamine-sulfadoxine for treatment of malaria. Although safety has not been established in pregnancy, the drug has been used effectively for intermittent presumptive treatment of pregnant women in endemic areas to decrease the risk for severe anemia.[111]

Amodiaquine

Amodiaquine, similar to chloroquine, is a 4-aminoquinolone. It has similar efficacy, but may have increased activity compared with chloroquine for the treatment of strains of *P. falciparum* resistant to chloroquine.[88] It is available in an oral preparation and is more palatable. Significant adverse events, including agranulocytosis, hepatotoxicity, and death, when used as a prophylactic agent have limited its use as a first-line agent. It is not licensed or marketed in the United States.

Halofantrine

Halofantrine is a 9-phenanthrene-methanol that has been used to treat malaria caused by chloroquine-resistant *P. falciparum*. Its major side effect is dose-related prolongation of the QT interval, and it has been associated with cardiac arrhythmias and sudden death, especially in individuals taking mefloquine prophylaxis.[84,118] Its major use was in uncomplicated malaria in areas of multidrug resistance, but cardiac toxicity at the doses required to be effective may limit its widespread use.[121] It is contraindicated in pregnant and lactating women because of embryotoxic effects. Resistance to halofantrine has limited its use in some areas. Travelers who are taking mefloquine for prophylaxis should be cautioned against accepting treatment with halofantrine if they are diagnosed with malaria during their travels. Halofantrine is unavailable in the United States.

INVESTIGATIONAL DRUGS

Targets for new antimalarial drugs include malaria proteases, which play a crucial role in malaria pathogenesis.[101] Adjuvant therapy that would modify or prevent the effects of inflammatory mediators also may have a role in the treatment of complications of malaria.

SUPPORTIVE AND ADJUNCTIVE THERAPY

Malaria, especially that caused by *P. falciparum*, is a potentially fatal illness even in patients who may have developed partial immunity. Patients, especially children, with falciparum malaria should have immediate treatment and generally should be admitted to the hospital, especially in non-endemic countries such as the United States. Patients with severe malaria should be managed in intensive care units, and experts in treatment of malaria and its complications should be consulted. Blood smears should be examined at least every 12 hours (every 4 to 6 hours for severe malaria) until parasitemia is less than 1 percent. Most patients can be treated with oral medication, although patients should be switched promptly to parenteral medication if oral medication is not tolerated. WHO guidelines for treatment of malaria, including management of severe malaria, are available (http://www.who.int/malaria/diagnosisandtreatment.html).

The goals of supportive therapy are to maintain oxygenation and treat acidosis and hypoglycemia. Treatment with glucose infusion, oxygen, and blood transfusion may be necessary. Mechanical ventilation with positive-pressure ventilation may be needed to manage acidosis and acute respiratory distress syndrome. In some cases, hemodialysis may be needed. A high index of suspicion should be maintained for other concomitant or nosocomial infections, such as meningitis or septicemia.

Adjunctive therapies that have been shown to be beneficial include antipyretics and anticonvulsants. Exchange transfusion remains a controversial option.[53,129] Some therapies, such as high-dose corticosteroids for cerebral malaria or heparin, have been shown to be harmful.[129,134] The search for effective adjunctive therapy for severe and complicated malaria continues.

PREVENTION

Prevention of malaria can be accomplished by reducing the mosquito population, using personal protection methods to prevent mosquito bites, and initiating chemoprophylaxis. Reduction of mosquito vectors is most successful when locally controlled programs are developed and maintained.[52] Strategies that take into account local circumstances, such as applying insecticide to cattle in communities where families share their homes with domestic animals, are most likely to be successful.[103] Most programs in endemic areas require a combination of preventive strategies and effective treatment modalities. Many non-endemic countries have developed recommendations for prevention of malaria in travelers.

PERSONAL PROTECTIVE MEASURES

A significant reduction in the incidence of malaria in children in communities where bed nets, especially when impregnated with insecticide, are used consistently has been reported from many countries.[21,29,115] In northern Ghana, community-wide use of bed nets was associated with a reduction in all-cause child mortality in young children.[7] The use of bed nets, especially bed nets impregnated with insecticide, is recommended for travelers to malarial areas. Other personal protective measures include using mosquito repellents and insecticide-impregnated clothing or gear; wearing clothing that covers areas likely to be bitten; and remaining, if possible, in air-conditioned or well-screened areas during times when mosquitoes are biting. DEET-containing repellents are recommended most commonly for prevention of mosquito bites and are highly effective.[25] Other agents, such as picaridin and oil of lemon eucalyptus, are available. Picaridin is available only in a 7 percent formulation, which may not have the same duration of protection as higher concentrations of DEET. Other agents have not been studied extensively against *Anopheles* mosquitoes.[74]

CHEMOPROPHYLAXIS

Country-specific information regarding recommendations for the use of antimalarial drugs may differ, and readers are urged to seek recommendations from national or local authorities. In the United

TABLE 231–3 Drug Regimens Used for Prevention of Malaria

Drug	Adult Dosage	Pediatric Dosage	Comments
Chloroquine-Sensitive Areas			
Chloroquine phosphate (drug of choice)	500 mg salt (300 mg base) orally once/wk beginning the week before exposure and continuing for 4 wk after exposure	5 mg/kg base (8.3 mg/kg salt) once/wk, up to adult dose of 300 mg base beginning the week before exposure and continuing for 4 wk after exposure	May be used in pregnant women
Chloroquine-Resistant Areas			
Mefloquine	250 mg salt (228 mg base) salt once/wk orally beginning the week before exposure and continuing for 4 wk after exposure	<15 kg: 5 mg/kg salt 15-19 kg: 1/4 tablet 20-30 kg: 1/2 tablet 31-45 kg: 3/4 tablet >45 kg: 1 tablet All doses: orally, once/wk beginning the week before exposure and continuing for 4 wk after exposure	See text for contraindications
or			
Doxycycline	100 mg daily beginning 1-2 days before exposure and continuing for 4 wk after exposure	2 mg/kg/day up to 100 mg/day	Not to be used in children <8 yr old or pregnant women
or			
Atovaquone-proguanil	250 mg/100 mg (1 tablet) daily beginning 1-2 days before exposure and continuing for 7 days after exposure	11-20 kg: 62.5 mg/25 mg (1 pediatric tablet) 21-30 kg: 125 mg/50 mg (2 pediatric tablets) 31-40 kg: 187.5 mg/75 mg (3 pediatric tablets) >40 kg: 1 adult tablet daily	
alternatives			
Primaquine	30 mg base daily up to 30 mg daily and glucose-6-phosphate	0.6 mg/kg base daily in pregnancy, lactation, dehydrogenase deficiency	Contraindicated; use in consultation with malaria experts
or			
Chloroquine phosphate *plus*	Same as chloroquine-sensitive	Same as chloroquine-sensitive	
Proguanil	200 mg daily	<2 yr: 50 mg daily 2-6 yr: 100 mg 7-10 yr: 150 mg >10 yr: 200 mg	Breakthroughs may occur frequently in areas of intense transmission with this combination

States, these recommendations are available widely in publications from the CDC[12] (http://wwwn.cdc.gov/travel/yellowBookCh4-Malaria.aspx) or national health information sources. Many excellent sources of health information for travelers include discussions of malaria prevention.[13-15,26,67,104,107,110,117] The Infectious Diseases Society of America has published detailed pre-travel guidelines that include recommendations for malaria prevention.[39] Table 231–3 lists drugs available for malaria prevention. Prevention of malaria for travelers is best discussed in the context of providing complete pre-travel services, advice, and information.

ANTIMALARIAL AGENTS AVAILABLE IN THE UNITED STATES FOR PREVENTION OF MALARIA

Chloroquine

A major determinant of appropriate chemoprophylaxis is whether a child is to travel to an area of chloroquine resistance. In areas of the world that have no resistance to chloroquine, the drug of choice for prevention of malaria is chloroquine. This drug is tolerated well and may be given to children of all ages, including infants. Dosing of chloroquine in children is based on the child's weight and can be found in Table 231–3. In the United States, chloroquine is available only in tablets that have a very bitter taste, although some compounding pharmacies may be willing to make liquid preparations. Pharmacies can prepare appropriate doses in capsules, and the contents of the capsule can be mixed with a small amount of liquid or semisolid food for administration. Although liquid preparations are widely available overseas and are stable for long periods,[77] many U.S. pharmacies are reluctant to prepare them. If patients are considering purchasing liquid preparations overseas, the family should be given information with the child's medication dose calculated as the base and the salt because concentrations of liquid preparations may vary. Families also should be cautioned about the greater incidence of ineffective or counterfeit medications available in some areas.

Adverse events caused by chloroquine are uncommon when the medication is used at doses needed for malaria prophylaxis. Minor side effects include gastrointestinal upset, headache, diz-

ziness, blurred vision, and pruritus. Adverse events rarely require discontinuation of medication. Chloroquine may be taken during pregnancy and is not contraindicated in children with G6PD deficiency.

The choice of antimalarial agents in locations where drug-resistant parasites have been identified is more challenging. Chloroquine-containing regimens are not recommended for travel to areas with known chloroquine-resistant *P. falciparum*, and deaths from malaria have occurred when these regimens have been used.[66] The choice of regimen depends on the type of drug resistance, length of stay, age of the traveler, and individual medical history. Drugs currently available in the United States include mefloquine, doxycycline, atovaquone-proguanil, and primaquine. Tafenoquine may be available in the future.[41,50,109]

Atovaquone-Proguanil

Atovaquone-proguanil is becoming one of the most popular choices for malaria chemoprophylaxis because of the favorable profile of side effects and the need to take the medication for only 1 to 2 days before and 7 days after leaving the area of malaria risk. The drug is effective against most *P. falciparum*, including resistant strains. It is available in adult and pediatric dosing forms. It must be taken daily, preferably at the same time each day. Adverse events most commonly reported with atovaquone-proguanil are abdominal pain, nausea, vomiting, and headache. It is not recommended at this time for prophylaxis of infants weighing less than 5 kg, for mothers nursing such infants, or for pregnant women.

Mefloquine

Mefloquine generally is well tolerated in children and can be given to infants. Dosing is based on weight, and no liquid preparation is available. Doses for older children can be supplied by breaking tablets; when small fractions of tablets are needed, pharmacists can provide individual doses in capsules. The medication has a pasty consistency, but does not taste bitter. Infants tend to swallow doses better when given liquids or semisolid foods after the medication. Adverse events with mefloquine are self-limited in most cases and include gastrointestinal disturbance, insomnia, and dizziness.[57] Withdrawal rates in adults, presumably caused by the incidence of these adverse events, may impair the efficacy of mefloquine.[19] Rarely, serious adverse events, such as psychosis or seizures, have occurred after prophylactic doses of mefloquine. Consequently, mefloquine should not be used in individuals who have seizures or neuropsychiatric disorders or in individuals who have had previous adverse events with mefloquine. Mefloquine also is contraindicated in patients with cardiac conduction abnormalities, although it may be used in individuals concurrently taking beta blockers for indications other than arrhythmias.

Mefloquine must be taken weekly, beginning 1 to 2 weeks before travel and continuing weekly during travel and for 4 weeks after leaving the malarial area. Families may be counseled to choose a day of the week to take the medicine and to take their first dose or doses on that day of the week 1 to 2 weeks before departure. Mefloquine may be used by pregnant women, preferably after the first trimester.[85,124]

Doxycycline

Doxycycline is an effective antimalarial drug in parts of the world where chloroquine resistance is present. It also has been shown to be effective in areas where mefloquine-resistant *P. falciparum* exists, such as portions of Cambodia, Burma (Myanmar), and Thailand. Limitations in the use of this drug include contraindications in children younger than 8 years, the need for daily dosing, and photosensitivity. In women, daily use of doxycycline may be associated with an increased risk of *Candida* vulvovaginitis. Gastrointestinal side effects, including nausea and vomiting, can be limited by taking the medication with meals and by avoiding bedtime dosing. Doxycycline must not be used by pregnant or lactating women. Prophylaxis with doxycycline needs to begin 1 to 2 days before travel and should be continued daily for 4 weeks after leaving the malarial area.

Primaquine

Primaquine, used most often to eradicate the exoerythrocytic stages of *P. vivax* and *P. ovale*, has been licensed as a prophylactic agent for individuals who are unable to tolerate any other drug. Individuals must be tested and shown not to be deficient in G6PD before they take this drug. Primaquine can be used to prevent *vivax* and *falciparum* malaria in non–G6PD-deficient individuals, and its efficacy compared favorably with that of mefloquine, doxycycline, and chloroquine plus proguanil in children in the holoendemic area of western Kenya.[27,108,127] It cannot be used in pregnant women.

ANTIMALARIAL AGENTS NOT AVAILABLE OR RECOMMENDED IN THE UNITED STATES FOR PREVENTION OF MALARIA

Tafenoquine

Tafenoquine is an investigational long-acting primaquine analogue that kills parasites in the liver and in blood. It is thought to be more effective and less toxic than primaquine, but it cannot be used by individuals with G6PD deficiency or by pregnant women. No dosing regimen has been established, and it is not licensed for use.[33,109,126]

Proguanil

Proguanil has been used in combination with chloroquine to prevent chloroquine-resistant *P. falciparum*, but is less effective than mefloquine, and is not recommended or available in the United States.

Pyrimethamine-Sulfadoxine

Pyrimethamine-sulfadoxine has been used for prevention of malaria in areas of chloroquine-resistant *P. falciparum*. An unacceptably high incidence of Stevens-Johnson reactions (fatalities in 1 in 11,000 to 25,000 users) resulted in withdrawal of the indication for using this drug for malaria prophylaxis.

VACCINE

Vaccination against malaria has been a subject of active research for several decades, but a licensed vaccine still is not on the immediate horizon. Development of a vaccine is challenging because of the complexity of the parasite life cycle, heterogeneity of the host immune response, lack of animal models, and absence of surrogate markers of protection. Testing of vaccines is difficult because vaccine efficacy depends on conditions of transmission and the degree of immunity present in the population being studied.[122] Targets for development of a vaccine include the sporozoite (prevention of infection), the merozoite (prevention of disease manifestations by preventing invasion of RBCs), or the gametocyte (prevention of transmission by interfering with development of the parasite within the mosquito). Hundreds of candidate vaccine antigens have been identified by the *P. falciparum* genome project; choosing the most promising ones is challenging.[30]

The first malaria vaccines were directed against circumsporozoite antigen, an antigen present over most of the surface of the sporozoite. Development of these vaccines was based on the concept that sporozoites should be susceptible to antibody-mediated destruction in the extracellular space. Results of early studies with these vaccines were disappointing, with inadequate protection provided against sporozoite challenge, inconsistent relationships between antibody response and clinical efficacy, and numerous local side effects.[36,40] More recently, the candidate vaccines RTS,S/AS02A and RTS,S/ASOIE have shown promise in trials in Mozambique[2] and Kenya. Merozoite surface proteins are another group of antigens that have been studied in connection with the development of a blood-stage, or asexual, malaria vaccine.[90] The SPf66 vaccine, derived from three merozoite proteins linked to a 4-amino acid from a *P. falciparum* circumsporozoite protein, has been tested in clinical trials in Colombia, Tanzania, and the Gambia.[3,20,123] Although initial studies in countries in South America with low malaria endemicity showed promise, investigations in African countries with high endemicity have been disappointing, with protective efficacy ranging from 31 percent (95% confidence interval 0 to 52%) in Tanzania to 8 percent (95% confidence interval –18 to 29%) for first or only clinical episodes and 3 percent (95% confidence interval –24 to 24%) in overall incidence of clinical episodes in the Gambia.

Vaccines that block transmission from humans to mosquitoes would reduce the reservoir of infected mosquitoes and reduce transmission on a population level. The poor immunogenicity and diversity of gametocyte antigens have proved challenging to this approach to the development of a vaccine, and no vaccines of this type have been used yet in clinical trials. Future vaccines are likely to use a combination approach that incorporates target antigens from all three stages of the parasite. To have a significant impact on decreasing worldwide burden of malaria, vaccines ultimately may need to be combined with other prevention strategies, including protection against mosquito bites with insecticides and treated bed nets, and intermittent preventive treatment with antimalarial drugs.[30]

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CHAPTER 232

Leishmaniasis

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Leishmaniasis consists of a group of diseases that may affect the skin, mucous membranes, and viscera with a wide range of clinical presentations caused by obligate intracellular hemoflagellates of the genus *Leishmania*. Infection is transmitted by several genera and species of bloodsucking sandflies. Three major clinical syndromes usually are recognized: cutaneous (CL), mucocutaneous (MCL), and visceral (VL) leishmaniasis. Macrophages of the skin; mucous membranes; and spleen, liver, and bone marrow (reticuloendothelial system) are parasitized.

Studies on the components of sandfly saliva suggest that substances may influence partially the tropism of the parasite and the resulting disease. The amount of a vasodilatory peptide, maxadilan, in sandfly saliva seems to correlate with the type of human infection.[40] Sandflies in Brazil that transmit *Leishmania chagasi* VL have large amounts of maxadilan in their saliva, which causes marked vasodilation and may encourage visceralization of the parasites. The same complex of sandflies in Costa Rica, which have small amounts of the vasodilatory peptide in their saliva, transmit organisms that produce a non-ulcerating and nonvisceralizing *L. chagasi* infection that is limited to the dermis.

The clinical manifestations of leishmaniasis seem to depend on a complex set of factors, including tropism and virulence of the parasite strain, and the susceptibility of the host, which may be determined genetically.[27] Cell-mediated immune mechanisms seem to be the major factors in modulating these diseases (Table 232–1). Disease syndromes range from self-healing cutaneous lesions to debilitating mucocutaneous infections, subclinical viscerotropic dissemination, and fatal visceral involvement.

In cutaneous disease, *Leishmania tropica* (with some notable exceptions), *Leishmania major*, *Leishmania aethiopica*, and *Leishmania mexicana* tend to be restricted to reticuloendothelial cells of the skin, and infections generally are self-limited, with spontaneous healing. Similarly, *Leishmania braziliensis* can invade the reticuloendothelial cells of the skin and the mucous membranes of the nose, mouth, and pharynx, resulting in serious disfigurement or even death. Some strains of *L. tropica* may cause viscerotropic disease. In cases of VL (kala-azar), *Leishmania donovani*, *L. chagasi*, and *Leishmania infantum* invade cells of the reticuloendothelium of viscera, usually causing splenomegaly and occasionally hepatomegaly and pancytopenia; untreated, the disease is progressive and generally fatal.

Individuals with acquired immunodeficiency syndrome (AIDS) may have atypical manifestations. Dermatotropic strains of *L. infantum* are known to cause visceral infection in immunocompromised patients with AIDS.[39] VL is quite prevalent in patients with AIDS in certain geographic areas (e.g., southern Spain), with a high proportion of cases being subclinical. Similar to other opportunistic infections, subclinical VL can be found at any stage of human immunodeficiency virus (HIV)-1 infection, but symptomatic cases occur mainly when severe immunosuppression is present.[5] HIV-*Leishmania* co-infection is being seen with increasing frequency in the Mediterranean basin, especially in Spain, France, and Italy.

ORGANISM

In vertebrate hosts, *Leishmania* spp. are obligate intracellular parasites that exist only in the amastigote stage. The species that infect humans usually are morphologically indistinguishable from

TABLE 232–1 Clinical Syndromes Caused by *Leishmania* Species and Their Geographic Distribution

Clinical Syndromes	*Leishmania* Species	Location
Visceral Leishmaniasis		
Kala-azar: generalized involvement of the reticuloendothelial system (spleen, bone marrow, liver)	*L. (L.) donovani*	Indian subcontinent, northern and eastern China, Pakistan, Nepal
	L. (L.) infantum	Middle East, Mediterranean littoral, Baikans, central and southwestern Asia, northern and northwestern China, northern and sub-Saharan Africa
	L. (L.) donovani (archibaldi)	Sudan, Kenya, Ethiopia
	L. (L.) spp.	Kenya, Ethiopia, Somalia
	L. (L.) chagasi	Latin America
	L. (L.) amazonensis	Brazil (Bahia State)
	L. (L.) tropica	Israel, India, and viscerotropic disease in Saudi Arabia (U.S. troops)
Post–kala-azar dermal leishmaniasis	*L. (L.) donovani*	Indian subcontinent, East Africa
Old World Cutaneous Leishmanias		
Single or limited number of skin lesions	*L. (L.) major*	Middle East, northwestern China, northwestern India, Pakistan, Africa
	L. (L.) tropica	Mediterranean littoral, Middle East, western Asiatic area, Indian subcontinent
	L. (L.) aethiopica	Ethiopian highlands, Kenya, Yemen
	L. (L.) infantum	Mediterranean basin
	L. (L.) donovani (archibaldi)	Sudan and East Africa
	L. (L.) spp.	Kenya, Ethiopia, Somalia
Diffuse cutaneous leishmaniasis	*L. (L.) aethiopica*	Ethiopian highlands, Kenya, Yemen
New World Cutaneous Leishmaniasis		
Single or limited number of skin lesions	*L. (L.) mexicana (chiclero ulcer)*	Central America, Mexico, Texas
	L. (L.) amazonensis	Amazon basin and neighboring areas, Bahia and other states in Brazil
	L. (V.) braziliensis	Multiple areas of Central and South America
	L. (V.) guyanensis (forest yaws)	Guyana, Suriname, northern Amazon basin
	L. (V.) peruviana (uta)	Peru (western Andes) and Argentinean highlands
	L. (V.) panamensis	Panama, Costa Rica, Colombia
	L. (V.) pifanoi	Venezuela
	L. (V.) garnhami	Venezuela
	L. (V.) venezuelensis	Venezuela
	L. (V.) colombiensis	Colombia and Panama
	L. (V.) chagasi	Central and South America
Diffuse cutaneous leishmaniasis	*L. (L.) amazonensis*	Amazon basin and neighboring areas, Bahia and other states in Brazil
	L. (V.) pifanoi	Venezuela
	L. (L.) mexicana	Mexico and Central America
	L. (L.) spp.	Dominican Republic
Mucosal leishmaniasis	*L. (V.) braziliensis* (espundia)	Multiple areas in Latin America

(L.), subgenus Leishmania*; (V.), subgenus* Viannia.

Data from Laison, R., and Shaw, J. J.: Evolution, classification and geographic distribution. In *Peters, W., Killick-Kendrick, R. (eds.): The Leishmaniases in Biology and Medicine. London, Academic Press, 1987, pp. 1-120; modified from Pearson, R. D., and Sousa, A. Q.: Clinical spectrum of leishmaniasis. Clin. Infect. Dis. 22:1-13, 1996; from Pearson, R. D., Jeronimo, S. M. B., and de Queiroz, A.: Leishmaniasis. In Guerrant, R. L., Walker, D. H., and Weller, P. F. (eds.): Tropical Infectious Diseases: Principles and Practice. Philadelphia, Churchill Livingstone, 1999.*

one another at the light microscopic and ultrastructural levels. The organisms are round-to-oval bodies approximately 2 to 4 μm in diameter with a single nucleus, a specialized mitochondrial structure that has extranuclear DNA termed a *kinetoplast*, and no free flagellum. Amastigotes are engulfed by macrophages and reside within the parasitophorous vacuole of the macrophage host. They multiply by binary fission and eventually destroy the host cell. Subsequently, they are phagocytized, and the process occurs repeatedly.

When the vector, a female sandfly, feeds on an infected person, it may ingest an infected cell from blood or tissue. Amastigotes are liberated in the fly's midgut, and within a few hours, transformation to the promastigote stage occurs.[21] Promastigotes are elongated flagellates 15 to 25 μm long by 1.5 to 3.5 μm wide, each having an anterior, free flagellum that measures approximately 15 to 28 μm in length and may vary morphologically from short and stumpy to an elongated form. Binary fission begins, and large numbers of promastigotes are produced and gradually move forward to the pharynx, buccal cavity, and mouth parts. Depending on temperature and the species of sandfly, at 8 to 20 days, the mouth parts of the fly may be blocked partially or completely by huge numbers of promastigotes. These organisms may be dislodged into the bite wound when the female sandfly (*Phlebotomus*, *Lutzomyia*) next takes a blood meal. Promastigotes have surface molecules that bind to several macrophage receptors. The phagocytized promastigote forms transform into amastigotes within the parasitophorous vacuole, and multiplication occurs.

Amastigotes can be seen in Giemsa-stained or Wright-stained tissues or smears by light microscopy with the nucleus and kinetoplast staining bright red and the cytoplasm staining pale blue. In Novy-McNeal-Nicolle (NNN) culture medium at 24° C, the organisms grow readily and assume the promastigote or insect form.

In the past, specific and subspecific taxonomy designations were determined by the clinical syndrome caused by an isolate in a particular geographic area. More recently, various molecular

techniques have been used to characterize the strains and species of clinical isolates, including endonuclease restriction studies of kinetoplast DNA, buoyant density of kinetoplast DNA and mitochondrial DNA on cesium chloride, leishmanial isozyme patterns, monoclonal antibody specificity, and exoantigen secretory factor 4 serotyping.[3]

EPIDEMIOLOGY

Leishmaniasis is mainly a zoonosis, although primarily human-vector-human transmission occurs in certain areas of the world. The World Health Organization estimates that 1.5 million cases of CL and 500,000 cases of VL occur every year in 88 countries.[16] Estimates indicate that approximately 350 million people are at risk for acquiring leishmaniasis, with 12 million currently infected.[4] With more recent outbreaks in many areas of the world, including Brazil, India, Italy, Spain, Sudan, and Kenya, leishmaniasis has become more widely recognized as an important emerging infectious disease in many developed and underdeveloped countries.[10,22] Although the parasite usually is transmitted via the bite of the sandfly vector, it also may be transmitted as a result of a laboratory accident, direct person-to-person transmission, and blood transfusion.[30] In addition, evidence indicates that it may be transmitted either in utero or during the peripartum period.

The bloodsucking sandfly, the vector for leishmaniasis, was identified first by Bonanni in 1691.[15] The name of the genus *Phlebotomus* was coined in 1840 by Rondani and Berte. In 1907, the first description of an American bloodsucking sandfly was made by Coquillet. In 1909, Vianna in Brazil identified the parasites responsible for CL, later identified as *L. braziliensis* in 1911.[14] Concurrently, Lutz and Neiva identified the first three species of sandflies in Brazil. *Lutzomyia* sandflies were later named after Lutz. To date, 229 species have been identified. It was not until 1921, however, that the Sergent brothers implicated the bloodsucking sandflies in the transmission of CL in Algeria. The evidence was substantiated by the work of Aragao in 1922 and Pessoa and Pestana in Brazil in 1940. In 1950, researchers discovered that the different forms of leishmaniasis infesting the American continent were caused by distinct species of the parasite. The research on leishmaniasis and sandflies has shed light on the transmission of other zoonotic illnesses, including Trypanosomatids (*Crithidia*, *Endotrypanum*, *Trypanosoma*), and several viruses, including Rhabdoviridae, Bunyaviridae, and Reoviridae, all isolated from bloodsucking sandflies. In the Amazon region, 69 serotypes of different arboviruses isolated from sandflies have been identified.[15]

OLD WORLD CUTANEOUS LEISHMANIASIS

DEFINITION AND EPIDEMIOLOGY

Old World CL is caused by *L. major* (rural), *L. tropica* (urban), and *L. aethiopica*. It is found throughout the Middle East; along the Mediterranean basin and islands; and in East and West Africa, India, and southwestern Asia.[43] In humans, infection by *L. tropica* usually produces skin ulcers in which intracellular (amastigote) parasites can be found within macrophages in and around the lesions. These organisms are rarely found in spleen, liver, or bone marrow; however, several more recent reports have described *L. tropica* isolates from patients with VL. In many areas, dogs or rodents are found to be naturally infected and are thought to be the natural reservoirs of infection. Various *Phlebotomus* spp. transmit the infection, although person-to-person transmission is possible and is the basis of the long-time practice in middle and central Asia of immunizing inoculation—that is, "vaccination" to prevent possible disfigurement by a natural infection (Fig. 232–1).

Figure 232–1 *Leishmania tropica.* Immunization by an induced lesion. *(From a nonprofit cooperative endeavor by numerous colleagues under the editorship of Dr. Herman Zaiman, New York.)*

Old World leishmaniasis, or Oriental sore (Delhi boil, Aleppo button) can be classified into "wet" and "dry" types. The wet or rural form is caused by *L. major*; and is found chiefly in various rodents on the edge of deserts. The dry or urban type is caused by *L. tropica*, and is transmitted by bloodsucking species that frequently feed on humans and dogs. The dry or urban form of Oriental sore has a long incubation period, long duration of active infection, and numerous parasites in the dermis. The moist or rural type has a short incubation period, with rapid healing and few parasites in the skin. *L. aethiopica* is restricted to the mountain valleys of the Rift Valley of Ethiopia and Kenya, where rock and tree hyraxes are infected regularly. Humans become infected when they intrude in these areas. This form of CL is usually self-limited,[53] although in a few individuals (1 per 100,000), nonhealing diffuse cutaneous leishmaniasis (DCL) disease has been reported.

PATHOLOGY

At the bite site, promastigotes are engulfed by histiocytes, in which they multiply. The histiocytes are destroyed, and amastigotes are released into tissues, where the process is repeated. Lymphocytic and plasma cell infiltration along with histiocytic hyperplasia occurs, and in some lesions, epithelioid and giant cells may be seen. Hypertrophy of the stratum corneum and hyperplasia of dermal papillae occur early, usually followed by necrosis of the area caused by capillary obstruction and endothelial proliferation eventually. The epithelium overlying the center of the lesion becomes necrotic and is sloughed, with the formation of a characteristic ulcer. Secondary neutrophil infiltration also occurs. At this point, a depressed ulcer with a raised purpuric indurated border and a base of friable granulation tissue is present. Amastigotes are located within the cells, although during the period of necrosis, organisms may be seen outside cells, but division does not occur.

With *L. tropica*, development of the lesion may take weeks to months, with large numbers of parasites present in nests of mac-

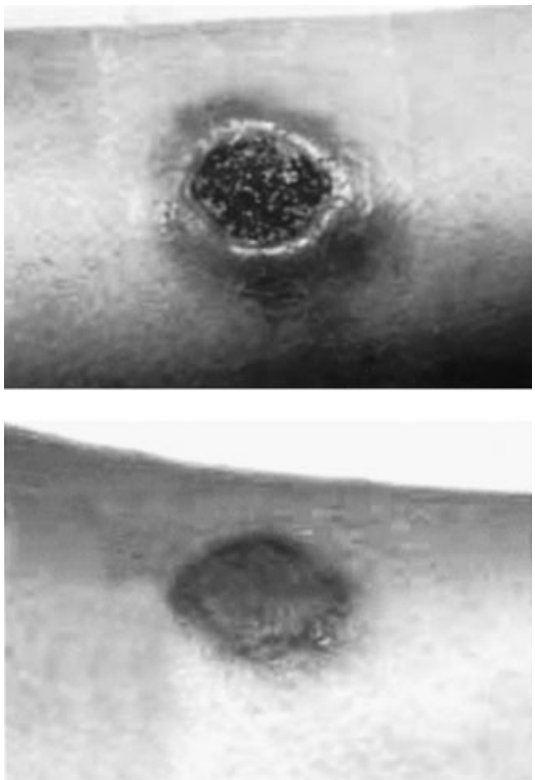

Figure 232–2 *Upper*, Typical cutaneous leishmaniasis ulcer. *Lower*, Healed cutaneous leishmaniasis ulcer.

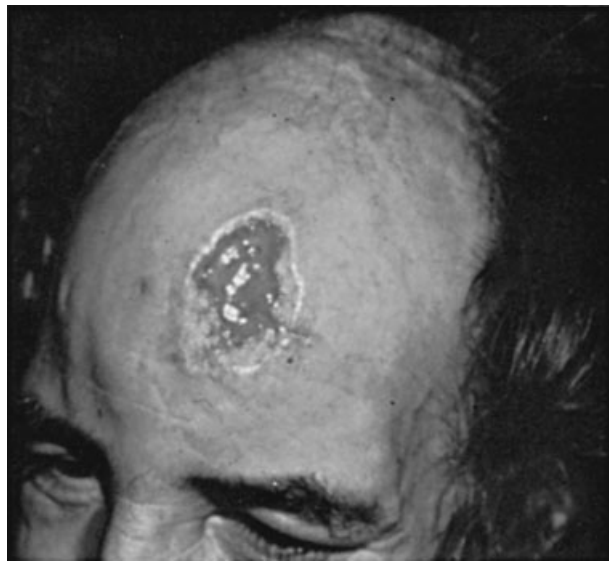

Figure 232–3 Healing cutaneous leishmaniasis in a patient from Venezuela.

rophages. In *L. major* infection, the onset is rapid, with an outpouring of lymphocytes and plasma cells; parasites sometimes are difficult to find. In some cases, satellite lesions form close to the primary lesion so that local spread is seen. The pathologic reaction may be florid, with marked pseudoepitheliomatous hyperplasia that can be mistaken for carcinoma. Secondary bacterial infection may complicate the lesion and delay healing. After the ulcer heals, however, usually by fibrosis, the patient has long-lasting immunity (Fig. 232–2).

Several manifestations of CL have been described and seem to be associated with the ability of the patient to respond to the infection by cell-mediated immune mechanisms. Whether these mechanisms are directly responsible for protection in humans still is uncertain. In a few patients, the inability to mount a suitable cell-mediated immune reaction is associated with specific anergy to leishmanin, however, and an indolent nonhealing lesion.[12] This condition is known as DCL. Characteristically, lesions in DCL are filled with large, parasite-containing histiocytes, and lymphocytes are absent. More recent studies of DCL from the Dominican Republic suggest that immunosuppression plays an important role in this form of the disease.

At the other extreme are a few patients whose cell-mediated immune response to infection with leishmanial organisms is exaggerated, and the lesions heal by scarring. At the edge of the scar, new lesions appear, however, so the disease seems to extend from the margins. Eventually, tissue damage may be extensive. On histologic examination, many lymphocytes, plasma cells, epithelioid cells, and large multinucleated giant cells are seen. Organisms are difficult to locate, but sometimes can be cultured from these lesions. This form of CL is called *leishmaniasis recidivans*. Patients exhibit marked delayed hypersensitivity to leishmanin. The work of Turk and Bryceson[50] has provided the concept that CL may be a spectrum of diseases analogous to leprosy. They consider DCL to be at one end of the spectrum and representative of anergy, and leishmaniasis recidivans to be at the other end and representative of marked delayed hypersensitivity, with an ordinary Oriental sore as the center in which balance exists.

CLINICAL MANIFESTATIONS

The disease usually begins with a pruritic, red, vesicular papule that appears weeks to months after the bite of a sandfly. The papule gradually enlarges, often measuring 1 to 2 cm in diameter. When the surface of the papule dries, it encrusts and drops off to reveal a shallow ulcer. The ulcer may or may not enlarge progressively, and characteristically has raised, sharp, indurated, deep purpuric margins. Healing usually occurs in 3 to 18 months, with an obvious hypopigmented or hyperpigmented depressed scar frequently remaining (Fig. 232–3). Single or multiple papules often heal directly without extensive ulceration, however. If the lesions do not become infected secondarily, usually no complications occur.

DIAGNOSIS

Definitive diagnosis depends on showing the amastigotes in tissue specimens or the promastigotes in culture. Microscopic examination of Giemsa-stained or Wright-stained smears of tissue obtained from non-necrotic areas of the ulcer or from the base should be performed. Aspiration and culture of tissue fluid taken from the ulcer margin can be positive; however, biopsy material taken from the edge of the ulcer is preferred and should be examined histologically, as should small fragments macerated in saline and inoculated into NNN medium or Schneider's insect medium supplemented with antibiotics. The specimen of choice would be a collection of several punch biopsy specimens taken from the most active lesion areas. Clinically, the lesions often are

characteristic, so the diagnosis should be suspected in a patient who has visited an endemic area.

Although the leishmanin test usually is positive in patients with ulcerated lesions, the material is not readily available in the United States. A positive leishmanin skin test may help distinguish various skin lesions, however, such as syphilis, tropical phagedenic ulcer, yaws, tuberculosis, and various fungal diseases. The indirect fluorescent antibody test or direct agglutination test may be positive in this infection, although often at low titer and of little value.

TREATMENT

Uncomplicated *L. tropica* lesions generally respond well to chemotherapy or to conservative management. Because Old World CL usually remains a local lesion, if the ulcer is not disfiguring and appears to be healing, allowing the lesion to heal spontaneously is appropriate.[53] In most cases, systemic therapy with stibogluconate sodium is effective.[23] Several reports have indicated limited success in treating skin lesions with heat. Generally, increasing intralesional temperature to 40° C to 42° C for 12 hours a day was necessary to obtain a satisfactory result. Recombinant interferon-γ has been reported to accelerate healing. Various parenteral, topical, and oral agents have been used, but the data are anecdotal or conflicting, or both. Allopurinol and various imidazoles (e.g., ketoconazole, itraconazole) have been used and seem to have limited antileishmanial activity. An ointment containing paromomycin (aminosidine) and methylbenzethonium chloride (Leshcutan) has been reported to show some promise in treating cutaneous lesions, especially in Israel.[46]

A 31-year-old man who since age 3 had had DCL, a disease with profound physical and psychosocial repercussions and no effective treatment at present, was treated with miltefosine.[55] The patient was treated for 120 days, 100 mg/day for 1 week, and then 150 mg/day subsequently. Lesions were free of parasites at 43 days, and no signs of infiltration were present at day 76. No adverse side effects were observed. The dramatic clinical effect of miltefosine in this patient seems to justify fully further evaluation of this experimental therapy in DCL.

PREVENTION

Residual spraying for sandflies, eradication of reservoir hosts, and vaccination procedures have reduced and limited Old World CL in many areas of the Middle East and central Asia. Because of the indolent nature of the healing with vaccination and the possibility of visceralization, this practice has been discontinued in Israel. The customary method for controlling leishmaniasis and sandfly bites in Israel involves the spraying of large quantities of residual insecticides on walls of houses and neighboring surfaces. The high summer temperatures, strong radiation, and dust limit the efficacy of the method, however.[36]

AMERICAN CUTANEOUS LEISHMANIASIS

The epidemiology and etiology of American CL are quite complex. In South and Central America, many varieties of leishmaniasis exist, including MCL, or espundia. In contrast to Old World CL, American CL is tied closely to the forests of South and Central America, and each variety has its own distinct epidemiologic, pathologic, and clinical picture.[20] Whether designating each of the clinical types of American CL by a separate species of *Leishmania* is justified is unclear. With regard to the American cutaneous forms, two main groups of organisms are distinguished: the *L. mexicana* and *L. braziliensis* complexes (Figs. 232–4 and 232–5). The former often are characterized by rapid growth in culture medium and in hamsters, and the latter are organisms that grow slowly in culture and hamsters.

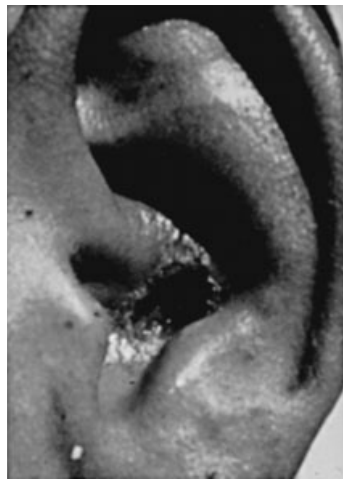

Figure 232–4 Chicle ulcer *Leishmania (mexicana) mexicana*. A typical chronic lesion of the external ear is evident. Such lesions never metastasize.

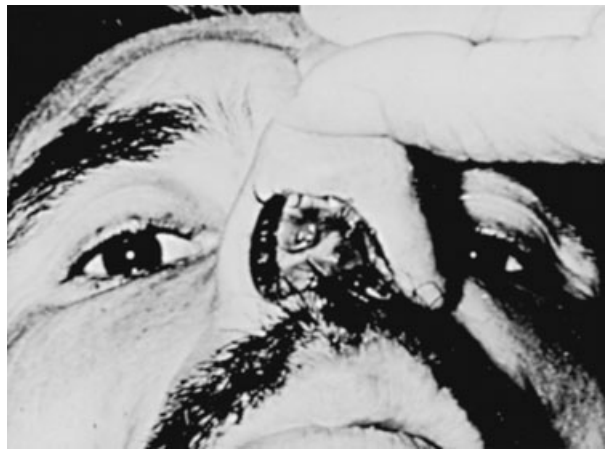

Figure 232–5 Mucocutaneous leishmaniasis: *Leishmania (braziliensis) braziliensis*. The entire nasal septum has been eroded. Few organisms could be found by smear or biopsy; however, promastigote forms were cultured from the lesion.

LEISHMANIA MEXICANA COMPLEX

L. (mexicana) mexicana is transmitted by species of sandflies of the genus *Lutzomyia* (Fig. 232–6). Many rodent reservoir hosts exist. This species is found in Mexico, Guatemala, and Belize. It causes mild infection, often a single cutaneous lesion that is self-limited, persistent chronic ear lesions, or chiclero ulcer.[2] One case of disseminated disease has been reported. This species probably is responsible for the occasional cases of CL found in the southern portion of the United States. The range of endemic infection has been expanded within the United States to include areas within Arizona that are farther north than the southern Texas site previously identified.[44] Apparently, wood rats serve as the primary wild reservoir hosts in these two states.

L. (mexicana) amazonensis is found along the Amazon basin and in Trinidad. It rarely infects humans, but is transmitted by various species of *Lutzomyia* in rodents. In humans, it causes a mild and

Figure 232–6 Sandfly.

self-limited skin lesion. Occasionally, disseminated disease reportedly develops.

LEISHMANIA BRAZILIENSIS COMPLEX

L. (braziliensis) braziliensis or *L. (viannia) braziliensis* is transmitted by various species of *Lutzomyia* in Brazil and the forest areas east of the Andes. It was described originally by Vianna in Brazil as the cause of Bauru ulcer.[14] This organism is the "prototype" of American CL or MCL, or espundia. It may cause destructive ulcerative lesions of the naso-oropharynx as a result of early or late metastases from a more superficial site.

Leishmania (braziliensis) guyanensis is transmitted by species of *Lutzomyia* in Guyana, Suriname, Brazil, and Venezuela. It causes single or multiple spreading cutaneous ulcers over many parts of the body, and is thought to metastasize along the lymphatics, but does not visceralize. The organism sometimes spreads to the naso-oropharynx and causes mucosal disease. It sometimes is referred to as *pian bois*, or *forest yaws*.

L. (braziliensis) panamensis is transmitted by species of *Lutzomyia* in Panama and possibly farther north and south. It may cause single to several superficial ulcers and may metastasize along the lymphatics to the naso-oropharynx.

Leishmania (braziliensis) peruviana is seen in Peru on the western slopes of the Andes to an altitude of 3000 m. It causes a single or a few self-healing ulcers. No oronasopharyngeal spread occurs. Often, it is referred to as *uta*. Dogs are regarded as the reservoir hosts.

In MCL as represented by espundia, researchers estimate that nasal involvement may occur in 80 percent of infections, 30 percent of which eventually mutilate the mucous membranes of the mouth, nose, palate, larynx, and trachea. These cases often are fatal because of the intervening sepsis. Lesions of the mucous membranes often occur years to decades after a cutaneous ulcer has healed. When mucous membrane involvement occurs, the infection may be difficult to cure using chemotherapy.

DIAGNOSIS

The discussion of Old World CL includes the methods for diagnosis. In MCL, the fluorescent antibody test using amastigote antigen is most useful in that it is positive in 75 to 85 percent of cases, with declining titers after therapeutic cure.[52] A direct agglutination test using promastigotes also is used frequently, as is enzyme-linked immunosorbent assay (ELISA).[52] More recently, a DNA-DNA hybridization or dot-blot test, which is highly sensitive and species-specific in tissue or biopsy specimens, has been used.[32] Isozyme analysis of isolated organisms currently is being used to help identify the species causing the infection.

TREATMENT

As in Old World CL, treatment of most lesions is effective using pentavalent antimonials. Because prompt and adequate therapy for primary cutaneous lesions may reduce the risk of subsequent development of metastatic disease in potentially mucocutaneous infections, pentavalent antimony should be used. If lesions should prove unresponsive to antimony therapy, amphotericin B should be tried. Although the experience is limited, liposomal amphotericin treatment has been useful in selected studies.[41] Relapses with this form of leishmaniasis are common and must be retreated. Pentavalent antimonials are moderately effective in treating mild mucosal disease, but often are unsatisfactory with severe mucosal involvement. Diffuse CL, a disseminated form of the disease, should be treated with stibogluconate sodium[23]; when relapses occur, amphotericin B should be used next because this disease usually is refractory to further antimony therapy.[41] Pentamidine also has been used with limited success when lesions have proved resistant to antimony compounds. Pentavalent antimony-resistant CL, such as leishmaniasis recidivans, has been treated with limited success with ketoconazole, 400 to 600 mg daily for 4 weeks. Miltefosine, an oral antileishmanial agent effective against VL, has shown promise for the treatment of American CL (*Leishmania panamensis/Leishmania amazonensis*).[48]

PREVENTION

American CL is extremely difficult to prevent because it is a forest disease. It can be avoided only by sleeping in tents under fine-mesh netting, wearing long-sleeved clothing, and using insect repellents. The presence of *Leishmania* parasites in the unaffected skin and peripheral blood monocytes of a high proportion of patients even after treatment and the acquisition of infection by sandflies support the plausibility of anthroponotic transmission of American CL.[51] As travel to Latin America has become more common, CL increasingly is seen among returning travelers; the number of observed cases has doubled in the Netherlands and tripled in the United Kingdom in the past decade.[45] A high proportion of cases are acquired in rural or jungle areas of the Amazon basin. In children acquiring cutaneous *L. (viannia) braziliensis* infections, the major risk factor for acquisition of disease was the presence of an adult in the household with CL within the past year.[1] Humans may serve as reservoir and source of infection for young children. Development of a vaccine against American CL is under way.[25,38,42]

VISCERAL LEISHMANIASIS

VL is caused by various organisms in the *L. donovani* spp. complex, although more recently, strains of *L. tropica* from the Middle East and *Leishmania amazonensis* from Latin America have been found to cause this syndrome. VL, or kala-azar, is found in a broad belt that extends from the Strait of Gibraltar across the Mediterranean through Asia to the east coast of China, at latitudes between 30 and 48 degrees north. It is transmitted by various species of sandfly, although congenital and blood-borne infections also can

occur.[54] VL has been reported from 47 countries, but the Sudan and India account for more than half of the cases. In the Western Hemisphere, it is found in Brazil, northern Argentina, Paraguay, Venezuela, Colombia, Guatemala, and Mexico. Kala-azar seems to exist in at least three epidemiologic forms:

1. The Mediterranean type of VL, with a canine reservoir, infects young children (1 to 4 years old). Dogs, foxes, or feral animals are the reservoirs (*L. infantum*). This type extends from the Mediterranean littoral through central Asia into China; it also is present in parts of South America (*L. chagasi*), where foxes and dogs are reservoir hosts. In Brazil, boys are infected most often.
2. An Indian type (*L. donovani*) of VL with a human reservoir predominates in Indian children 5 to 15 years old; humans are the only known reservoir. Although sought, evidence of natural infection in dogs has not been found. No evidence of rodent reservoirs exists.
3. The African type of VL has rodents for the reservoir hosts. The Nile rat in the Sudan and probably the gerbil in Kenya are the reservoirs. In Kenya, researchers have noted that kala-azar often is related to old or eroded termite mounds where boys often congregate.

PATHOLOGY

The main pathologic lesions are the result of reticuloendothelial cell hyperplasia, especially in the spleen and liver (Fig. 232–7). Later, the bone marrow and lymph nodes are filled with infected macrophages, and a concomitant leukopenia and anemia develop (i.e., pancytopenia). Also, the kidneys may be filled with infected macrophages, and invasion of the submucosa and mucosa of the duodenum and jejunum results in hypertrophic congested and edematous villi. Small ulcerations and hemorrhages may occur. The spleen gradually enlarges, sometimes assuming enormous proportions, and eventually extends into the pelvis. Splenic infarcts frequently develop. The capsule is thickened, and, more deeply, the sinuses are dilated. Erythrophagocytosis by histiocytes is seen commonly, and the anemia so typical of kala-azar may be partly the result of such sequestration of red blood cells. Kupffer cells of the liver, filled with amastigotes, are swollen and hyperplastic, and centrilobular necrosis or fatty infiltration of the hepatic parenchyma often is observed. In late-stage or chronic disease, increased hepatic fibrosis may give a nodular cirrhotic appearance. Lymphadenopathy, especially of the mesenteric glands, is an early finding, and numerous parasite-filled macrophages are present. The bone marrow often is filled with parasitized histiocytes that replace the normal marrow elements; such replacement results in myelophthisic anemia.

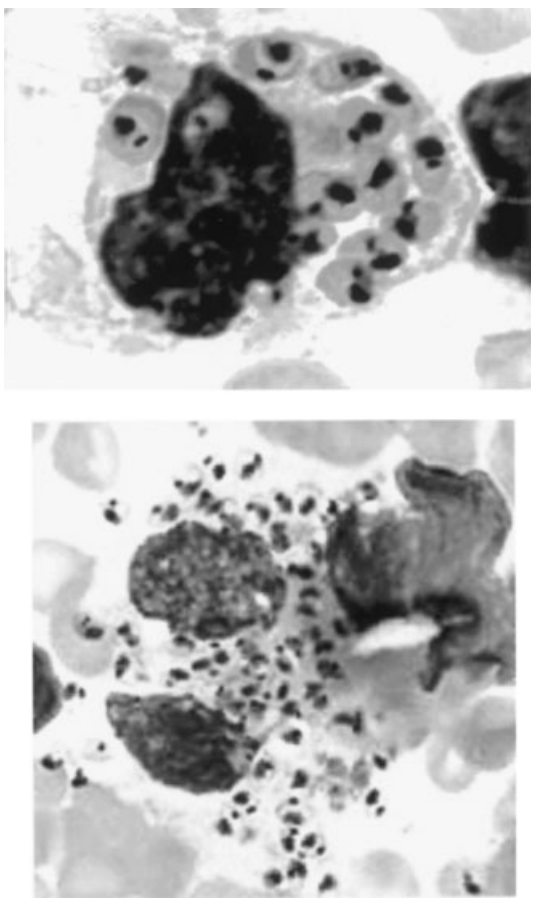

Figure 232–7 *Upper*, *Leishmania tropica* in a macrophage. *Lower*, *Leishmania donovani* in a liver touch preparation.

The immunologic response to kala-azar infection is imperfectly understood. At the bite site, a small, pea-sized dermal lesion may form (i.e., a leishmanioma); the parasites, initially localized in dermal macrophages, disseminate within the macrophages to the spleen, liver, bone marrow, and lymph nodes.

The infection outcome depends on the host's ability to raise a suitable cell-mediated immune response and the virulence of the invading organism. Experimentally, resistance in mice seems to be determined by a single autosomal gene. Researchers have shown in experimental infections that the disease is controlled by the T_H1 subset of $CD4^+$ and $CD8^+$ T cells; these cells are related to the production of cytokines such as interferon-γ, interleukin-2, and tumor necrosis factor-α.[31] Infection with *L. donovani* amastigotes induces a T_H1 cytokine milieu in dendritic cells and T cells.[19] Cytokines activate macrophages to kill intracellular amastigotes by oxidative and non-oxidative mechanisms.

Studies in mice indicate that nitric oxide is an important factor in killing amastigotes. If the infection is not eliminated or controlled by the host's cellular immune response, it becomes clinically evident. Lymphocytogenesis and histiocytogenesis occur in affected organs, with resultant hepatosplenomegaly and lymphadenopathy. Polyclonal B-cell activation ensues and causes hyperglobulinemia. This outpouring of humoral antibodies, chiefly IgG and largely nonspecific, is not protective and may represent more than half the total serum proteins of the patient. The specific antibodies produced during active disease have diagnostic significance. Fluorescent antibody, ELISA, indirect hemagglutination, and complement-fixation tests are reasonably reliable diagnostic procedures.

Resistance to kala-azar is essentially absent when the infection has become evident clinically. After chemotherapeutic cure, acquired immunity emerges; delayed hypersensitivity, as shown by the Montenegro (leishmanin) skin test (see later), also becomes apparent. The hypergammaglobulinemia abates concomitant with chemical cure and the appearance of delayed hypersensitivity. Usually, immunity to VL is complete and long-lasting after chemotherapeutic cure. Relapse, as seen in post–kala-azar dermal leishmaniasis, is characterized by delayed hypersensitivity, dermal localization of parasites, and moderate hypergammaglobulinemia. Although macrophage activation results in enhanced phagocytosis of parasites, macrophages remain unable to eliminate parasites. The occurrence of dermal delayed hypersensitivity at the time that acquired immunity occurs suggests that cell-mediated immunity plays an important role in protection. Further work on this aspect of VL is needed. The genetics of resistance in humans remains unclear.

CLINICAL MANIFESTATIONS

The incubation period varies from 6 weeks to 6 months, but has been reported to be as short as 10 to 14 days and as long as 10 years. A primary skin nodule rarely is seen, although in African leishmaniasis, it is a more regular feature. Infantile VL may begin either suddenly with high fever and vomiting or insidiously with irregular daily fever, anorexia, weight loss, lassitude, and pallor. When fever is present, double daily spikes are a characteristic sign, with temperatures reaching 40° C to 40.6° C. The spleen gradually enlarges so that by the end of the first month, it can be palpated. If the symptoms continue unabated, the spleen may extend to the umbilicus or into the pelvis. Diarrhea or frank dysentery is not an unusual development, and blood sometimes is observed. A general bleeding diathesis often becomes evident shortly before death. After several months, if the disease is untreated, patients usually die. Acute fulminant disease is seen more often in infants and young children.

In other cases, the clinical course is more protracted and generally ends fatally after 1 or 2 years. In older age groups, the disease tends to assume a more chronic course, with marked emaciation, brittle hair, massive splenomegaly, lymphadenopathy, and a dusky slate-gray complexion. Hyperglobulinemia, leukopenia, and anemia typically are found. As a result of general debility, death results from concurrent infections, such as pneumonia, amebic or bacillary dysentery, malaria, or cancrum oris, in more than 90 percent of cases. Infantile VL has been associated with alterations in lipoprotein metabolism.[6] A handful of cases of presumed congenital VL have been reported.[18,54] These infants were born of infected mothers, and in some, evidence of parasitism of the placenta was found.[18] However, whether these cases represent congenital infection or peripartum infection is unclear because sophisticated serologic techniques were unavailable.

Cutaneous manifestations of kala-azar are encountered frequently. In India, the dark gray appearance of the skin is known as kala-azar (black sickness). In some cases of inadequately treated VL, a skin condition termed *post–kala-azar dermal leishmaniasis* may ensue. In Indian VL, this complication is encountered in 15 to 20 percent of cases and appears several years after therapy. Individuals with VL or kala-azar and individuals with post–kala-azar dermal leishmaniasis are considered to be reservoirs of transmission of *L. donovani* in India.[47] In African disease, post–kala-azar dermal leishmaniasis occurs much less commonly, often during therapy in approximately 2 to 3 percent of cases, and it heals spontaneously in a few months. The lesions are characterized by the appearance of hypopigmented, erythematous, or nodular lesions on the skin of the face, chest, neck, and buttocks. At times, the nodular lesions of the face may resemble lepromatous leprosy.[50] The lesions are thought to represent a modified form of *L. donovani* infection, in which the parasites no longer invade the viscera and are localized to the skin. These lesions seem to be related to the host's immune response. This change to dermal tropism is said to coincide with recovery from VL and to disappear with relapse. Infection with *L. chagasi* causes VL in young children. More recently, atypical CL has been reported in older children with this infection.[7]

Pancytopenia is not unusual. Characteristically, anemia is always evident, with hemoglobin levels less than 8 g/dL. Survival of red blood cells is shortened as a result of several possible factors, including Coombs-positive hemolytic anemia and hypersplenism. Leukopenia of 2000 to 3000 cells/mm^3 typically is found with neutropenia, relative lymphocytosis, an almost total absence of eosinophils, and thrombocytopenia. Serum albumin usually is less than 3 g/dL, and globulin levels (mostly IgG) often are greater than 5 g/dL (5 to 10 g/dL).

Kala-azar has been reported as an important opportunistic infection in patients infected with human immunodeficiency virus type 1 (HIV-1) and not known to have contracted *Leishmania* infection previously. These patients seem to have a more severe and fulminant form of kala-azar. In this regard, inapparent *Leishmania* infection may become evident after immunosuppression, such as chemotherapy for malignant disease. The diagnosis may be particularly difficult to make inasmuch as the findings often are atypical and consist of low-grade fever, fatigue, cough, and gastrointestinal complaints. In patients with AIDS, VL is a recurrent disease that is highly prevalent, and the clinical course is modified by HIV. VL is very prevalent among HIV-1-infected patients in southern Spain, with a high proportion of cases being subclinical.[33] Symptomatic cases occur mainly when severe immunosuppression is present. An association also exists among VL, male gender, and intravenous drug use. Similarly, atypical visceral disease caused by *L. tropica* was seen in individuals who participated in Operation Desert Storm in the Persian Gulf.[28]

DIAGNOSIS

VL is diagnosed by finding the organism in stained smears of spleen aspirate,[11] peripheral blood, or bone marrow (see Fig. 232–7). In Indian kala-azar, the parasites may be found regularly in peripheral blood monocytes (i.e., buffy coat), but in the African and Mediterranean forms, they may be difficult to find by this technique. Blood and marrow cultures grown on NNN medium or in Schneider insect medium with 15 to 20 percent fetal calf serum are most useful (Fig. 232–8). Some investigators regard splenic rather than bone marrow aspiration as the most sensitive procedure, although it can be especially hazardous in individuals with a bleeding diathesis. Contraindications include a soft or diffluent, acutely enlarging spleen. Patients with low platelet counts or a prolonged prothrombin time (or both) should not undergo the needle biopsy procedure. In children younger than 5 years old, splenic aspiration should be performed only by a physician fully experienced in the procedure. Spleen and bone marrow aspirates should be placed in culture medium and smeared on slides, and saline-diluted aspirates should be inoculated into the peritoneal cavity of hamsters.

In a study in pediatric patients in the Mediterranean region, antibody detection techniques, antigen detection in urine (KAtex kit), and *Leishmania*-nested polymerase chain reaction (LnPCR) analysis of the blood were useful for diagnosis of the first clinical episode.[13] After treatment, clinical improvement was associated with negative NNN cultures and microscopy of bone marrow aspirate, KAtex test, and LnPCR blood analysis results. New

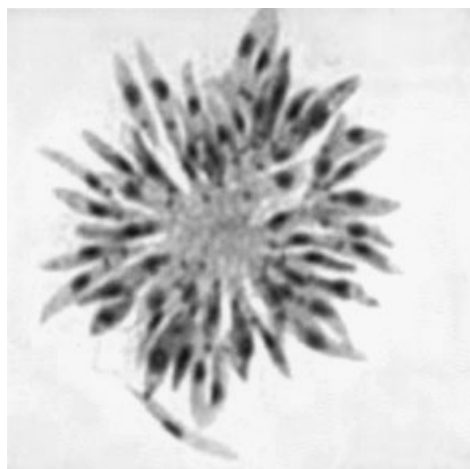

Figure 232–8 *Leishmania* sp. promastigotes from culture system.

noninvasive techniques tested showed high diagnostic sensitivity. LnPCR analysis of the bone marrow was the most sensitive, however, and was able to detect the persistence of parasites and predict potential relapses.[13]

Nonspecific tests reflecting the markedly elevated serum globulins, such as the formol gel test, are helpful in acute disease and are performed readily in the field. Antileishmanial antibodies usually are present and can be used to aid in the diagnosis. The fluorescent antibody test is highly specific, as are the indirect hemagglutination and gel diffusion tests. The complement-fixation test is positive in only 65 to 70 percent of cases, however. Sera from patients with VL are known to give false-positive results when antibodies to *Trypanosoma cruzi* are present; consequently, in the Western Hemisphere, absorbing out these antibodies may be necessary. Fluorescent antibody titers usually decline after complete cure, so a negative titer often is regarded as a sign of successful therapy. DNA-DNA hybridization tests are being evaluated and promise exquisite specificity and sensitivity for the diagnosis of leishmaniasis.[32] Serologic tests may be positive as a result of past or subclinical inapparent infection. An ELISA that detects antibodies to a cloned recombinant antigen, K39, of *L. chagasi* has been shown to be specific in active VL and could detect antibodies in patients with AIDS. A study has shown that this test could be applied to field situations.

In HIV-1-infected patients with active VL, the sensitivity of a peripheral blood smear is approximately 50 percent, owing to a high parasitemia in these patients. In patients with HIV-1 and subclinical VL, the sensitivity of a routine blood smear is less than 10 percent.[5] In these patients, serology and *Leishmania* skin tests have low sensitivities and are not very helpful. Cultures have proven to be effective in patients with VL who are co-infected with HIV-1. The usual diagnostic tests also may be negative in patients with *L. tropica* infection or in otherwise profoundly immunocompromised patients. More recently, two genomic fragments encoding portions of a single 210-kd *L. tropica* protein have proved useful for the diagnosis of viscerotropic *L. tropica* infection in Desert Storm patients.[17]

The leishmanin or Montenegro skin test, similar to the lepromin and tuberculin skin tests, is a measure of delayed hypersensitivity to leishmanial antigen. It consists of 10^6 phenol-killed, culture-grown promastigotes in 1 mL of 0.5 percent phenol in saline. The test is performed similar to the tuberculin test—0.1 mL is injected intradermally. A positive result is a palpable area of induration at least 5 mm in diameter in 48 to 72 hours. Because the leishmanin test can be positive in CL or VL, the results must be evaluated carefully. In VL, the test remains negative throughout the period of active disease. When chemotherapeutic control starts to take effect, and immunocompetent lymphocytes are able to respond, the test begins to turn positive.[9] Recovery from kala-azar is characterized by the development of cell-mediated immunity. The change from a negative to a positive leishmanin test in VL is regarded as an important prognostic sign that protective immunity is developing or has developed. Because numerous reports noting positive leishmanin tests in individuals who have had no history of VL have appeared more recently, researchers have postulated that many individuals in an endemic area may become immune by previous inapparent infection.

PROGNOSIS

Untreated VL is fatal in 75 to 85 percent of infantile cases and 90 percent of adult cases. Properly treated at an early stage, VL can be cured in 85 to 95 percent of cases. The prognosis usually is poor for patients in whom pancytopenia or bleeding diatheses develop or in whom a delayed hypersensitivity skin reaction fails to develop.

TREATMENT

VL generally responds to treatment with pentavalent antimonials, such as stibogluconate sodium (Pentostam, Triostam), which is the usual drug of choice in the United States and is available through the Drug Service of the Centers for Disease Control and Prevention.[23] Meglumine antimoniate (Glucantime) is available in French-speaking countries and Latin America.[8] The pediatric and adult dose is 20 mg/kg daily administered intramuscularly or intravenously for 28 days. Treatment can be repeated. In areas where leishmanial parasites may have acquired relative resistance to pentavalent antimonials, such as India, Nepal, and East Africa, extending therapy for more than 4 weeks may be necessary.[26] Side effects occur commonly and include nausea, vomiting, headache, anorexia, and abdominal pain. Elevated levels of serum amylase and lipase, indicative of pancreatitis, are encountered occasionally and can be severe. Electrocardiographic changes, including decreased T-wave amplitude and T-wave inversion, prolongation of the QTc interval, and nonspecific ST-T wave changes, are seen. They resolve shortly after therapy is concluded. (Treatment should be discontinued if the QTc interval is >0.5 second.) Deaths, presumably caused by arrhythmias, have been reported in patients who were receiving more than 20 mg/kg/day.

Primary antimony resistance and relapses after receiving pentavalent antimony therapy occur with all types of VL, but most often is encountered with the Indian form. The mechanism of antimonial resistance is unclear.[26] Among HIV-infected patients, almost 25 percent fail to respond to antimony therapy, and almost 40 percent of responders subsequently relapse.[35,37]

Liposomal amphotericin B (AmBisome) has been approved by the U.S. Food and Drug Administration for the treatment of VL.[34] This compound is taken up by cells of the reticuloendothelial system, where amastigotes reside, and is less nephrotoxic, allowing for higher daily doses with shorter courses of therapy. The approved, recommended regimen for immunocompetent VL patients is 3 mg/kg/day on days 1 through 5 and days 14 and 21. In immunosuppressed and HIV-infected patients, treatment of VL with AmBisome has resulted in an almost 100 percent response rate. Most of these patients relapse, however.[35,37] The recommended treatment is 4 mg/kg/day on days 1 through 5, 10, 17, 24, 31, and 38. Without maintenance therapy, almost all patients relapse. Because resistance to pentavalent antimonials has developed in Bihar, India, amphotericin B is used and is highly efficacious. Several more recent studies have used liposomal amphotericin B successfully for the treatment of VL presumably caused by *L. infantum* in immunocompetent children.[29]

Parenteral pentamidine isethionate (Pentam 300) is administered intramuscularly. The dosage is 4 mg/kg given three times weekly for up to 15 doses, depending on side effects, such as hypotension, vomiting, and blood dyscrasias. Pentamidine occasionally may exacerbate diabetes mellitus or precipitate latent diabetes. Shock and liver and renal damage have been reported.

In several more recent clinical trials, miltefosine, an oral alkyl phospholipid agent initially developed as an oral antineoplastic drug, has been used as an effective agent for the treatment of antimony-resistant Indian VL.[24,49] The treatment dose was 2.5 mg/kg/day for 4 weeks.

Patients may require hospitalization for therapy; supportive and corrective measures should be instituted in the event that other infections are present. Occasionally, a patient may be encountered who may require splenectomy to relieve the profound hypersplenism and the resulting anemia. Response to therapy often can be assessed by return of the patient's temperature to normal, a brisk reticulocytosis, a gradual reduction in spleen size, and the reappearance of eosinophils on the peripheral blood smear.

Allopurinol has been used with pentavalent antimonials to treat cases of VL that did not respond to pentavalent antimonials

alone. Several reports suggest that recombinant interferon-γ is helpful, along with pentavalent antimonial drugs, in successfully treating this disease. Because assessing whether a cure has been achieved is difficult, patients must be monitored at 6-month intervals for 2 years. Fluorescent antibody titers should be absent by the end of 1 year, and complement-fixation titers should be absent by 6 to 8 months. If post–kala-azar dermal leishmaniasis occurs, treatment should be re-instituted.

PREVENTION

Control of VL has many aspects. Sandflies (*Phlebotomus* and *Lutzomyia*) can be eliminated readily by residual spraying. Because sandflies ordinarily do not fly very high, sleeping quarters should be above ground level. Permethrin-impregnated bed nets can be highly effective in preventing sandfly bites. Animal reservoirs, such as infected dogs and rodents, should be destroyed. Early therapy prevents family and neighborhood transmission. A vaccine against different forms of leishmaniasis should be feasible, considering the wealth of information on genetics and biology of the parasite, clinical and experimental immunology of leishmaniasis, and the availability of vaccines that can protect experimental animals against challenge with different *Leishmania* spp. At present, however, there is no vaccine against any form of leishmaniasis for human use. One major obstacle is the lack of a confirmed market for human leishmaniasis vaccines. Ninety percent of visceral leishmaniasis occurs in five countries (Bangladesh, Brazil, India, Nepal, and Sudan). Nonetheless, local studies for vaccine development are ongoing in India and Brazil.

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CHAPTER 233

TRYPANOSOMIASIS

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AMERICAN TRYPANOSOMIASIS

American trypanosomiasis, or Chagas disease, is caused by the protozoan hemoflagellate *Trypanosoma cruzi*, which was discovered first by Chagas in 1909 in the blood of a seriously ill, wasted Brazilian child with fever, lymphadenopathy, and anemia.[56] The disease, transmitted by reduviid bugs, is limited to the Western Hemisphere and is a major health problem in Latin American countries. Chagas disease presently is identified in 18 countries within the Western Hemisphere. In endemic areas, it occurs most commonly in the rural poor who live in an environment with many feral reservoir hosts. Several autochthonous and laboratory-acquired cases have been reported from the United States in recent years.[36] Chagas disease in some endemic areas is the most important cause of heart disease and may result in serious chronic digestive tract pathology.

Collaborative efforts, in particular the Southern Cone and Andean initiatives, have resulted in substantial strides in vector control. These efforts, combined with screening of potential blood donors, has diminished the estimated number of individuals at risk of acquiring Chagas disease from 100 million to 40 million.[64,60] An estimated 16 to 18 million people are actually infected, with approximately 200,000 new cases and 20,000 deaths occurring each year. In certain areas of endemic infection, approximately 10 percent of all adult deaths are caused by Chagas disease. The southern United States, particularly Texas, is identified as having many cases. The geographic distribution of *T. cruzi* infection overlaps that of *Trypanosoma rangeli* infection; the trypomastigotes may be misidentified.

ORGANISM

Two evolutionary lineages have been identified in *T. cruzi—T. cruzi* I and II. Before 2005, epidemiologic and immunologic data suggested that chronic infections occurring in Brazil and Argentina were caused primarily by *T. cruzi* II strains. Using polymerase chain reaction (PCR) techniques, this hypothesis has been confirmed, and future studies can establish which strains are responsible for Chagas disease in other geographic locations.[30]

T. cruzi is a pleomorphic, spindle-shaped organism with a quite variable size, depending on the strain. The length of the blood forms (trypomastigote) in humans may vary widely, from 11 to 30 μm, including a free anterior flagellum. Most trypomastigotes assume a C or S shape, with the nucleus located just anterior to the middle of the cell. Trypomastigotes occur in the blood in two forms, a long slender form and a short stubby one. The nucleus is situated in the center of the body, with a large oval kinetoplast located at the posterior extremity. The kinetoplast consists of a small blepharoplast and a large oval parabasal body; the kinetoplast often appears to protrude from the cell surface (Fig. 233–1). The kinetoplast contains thousands of minicircle and maxicircle DNA that serve in the synthesis of mitochondrial proteins. A flagellum arises from the blepharoplast and extends along the outer edge of an undulating membrane until it reaches the anterior end of the body, where it projects as a free flagellum. When the trypomastigotes are stained with Giemsa stain, the cytoplasm stains blue, and the nucleus, kinetoplast, and flagellum stain red or violet.

Trypomastigotes are ingested by the reduviid bug as it obtains a blood meal. Within a few hours after taking an infective blood meal, short, spindle-shaped forms lacking a free flagellum can be found in the insect's foregut. These forms develop into small epimastigotes that continue to divide and give rise to large (35 to 40 μm) epimastigotes; by the third or fourth day after the blood meal, these organisms can be found attached to the rectal epithelium. By about the fifth day, the epimastigotes have begun to round up and gradually develop into short, stout trypomastigotes, which then proceed to elongate, and by the seventh and eighth days, they become the long, slender (17 to 22 μm), infective, metacyclic trypomastigotes. After 8 to 10 days, metacyclic trypomastigotes develop from the epimastigotes and are passed in the feces to infect humans when rubbed into the insect's puncture wound or rubbed onto exposed mucous membranes. These forms do not divide again, but are passed out with the feces some time after the blood meal. There may be 3000 to 4000 organisms per microliter of excreta. Transmission may occur by means of infected insect excreta, and metacyclic forms readily negotiate mucosa, conjunctiva, and abraded or otherwise broken skin (puncture site). Extensive experimental evidence has shown that infection is by contamination rather than insect inoculation.

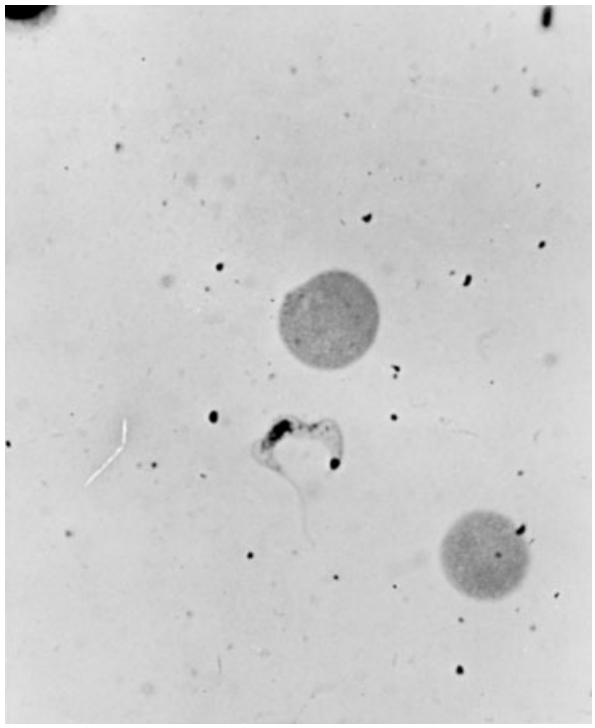

Figure 233–1 *Trypanosoma cruzi* trypomastigote forms in a blood smear. Note the large posterior kinetoplast (original magnification ×288).

On invasion of a cell, the trypomastigote loses its flagellum and undulating membrane and divides by binary fission to form an amastigote. The amastigote continues to divide and eventually fills and destroys the infected cell. Amastigote and trypomastigote forms are released from the cell. The amastigote is indistinguishable from those found in leishmanial infections. It is 2 to 6 μm in diameter and contains a large nucleus and rod-shaped kinetoplast that stains red or violet with Giemsa stain. The cytoplasm stains blue. Only the trypomastigotes are found free in the peripheral blood.

When the infective metacyclic trypomastigotes traverse the skin, they invade and multiply locally in many cell types after having transformed into intracellular amastigote forms. Host-cell entry by the parasite is a complex process involving many host and parasite factors. Trypomastigotes released from infected host cells may infect adjacent uninfected cells or enter the bloodstream and lymphatics to infect distant tissues. No tissue is spared from infection, but *T. cruzi* strains may vary in tissue tropism. The cells of the reticuloendothelial system and nervous and muscular (striated and cardiac) systems seem to be particularly vulnerable. In the ensuing weeks, during which time repeated cycles of multiplication, cell destruction, and cell re-invasion have occurred, flagellates enter the bloodstream as trypomastigotes, and the infection becomes disseminated.

EPIDEMIOLOGY

Chagas disease is a zoonosis occurring throughout American continents and involves reduviid bugs living in close association with human reservoirs (dogs, cats, armadillos, opossums, raccoons, and rodents). The most ubiquitous sylvatic reservoir host is the opossum, *Didelphis*, which is found throughout much of the range of *T. cruzi* in the Americas. Multiple nesting or resting sites of the opossum encompass many types of triatomine habitat. High *T. cruzi* prevalence rates are due partly to the fact that opossums eat triatomid bugs and may transmit infection via anal gland secretions. Sylvatic cycles of *T. cruzi* transmission extend from southern Argentina and Chile to northern California.

The vectors are distributed in rural wooded areas closely associated with feral animal species; humans intervene only occasionally. This infection usually is a zoonosis. Many species have become adapted to houses, however, where they may live in cracks; on roofs, walls, and floors; and beneath seldom-moved objects. Other triatomine species have developed regular visiting habits to human dwellings, but have not taken up residence as yet. Although Chagas disease always has been associated with rural areas and low socioeconomic groups, some vectors have adapted to areas undergoing urbanization. Various suitable hosts that often live close to humans, such as the opossum, may provide the transition between wild and domestic infection. Investigators frequently have observed that domestic animals such as pigs, cattle, goats, and sheep are kept in close contact with the domicile, and that triatomid bugs closely associated with these animals can be carried into the home.

Although 12 species of reduviids occur within the United States, they have not adapted themselves to household habitation. Humans should avoid sleeping in thatch, mud, or adobe houses; bed nets should be used by individuals sleeping in these types of houses. Travelers planning to stay in hotels, resorts, or other well-constructed housing facilities are not at high risk for contracting Chagas disease. Insecticides can be used to kill the vectors and reduce the risk of transmission.

Aside from natural transmission via the vector, other modes of transmission include congenital, blood transfusion, maternal milk, organ transplantation, and laboratory accident. The overall incidence of congenital Chagas disease has been reported as ranging from 2 to 6.7 percent.[5,10,52]

Blood transfusion is an important mode of transmission. In many large urban areas of South America, 3 to 15 percent of blood donors were reported to have a positive serology. The problem is compounded when one considers that in stored refrigerated blood, blood forms can live for weeks without losing their infectivity. In areas where seroprevalence is high, rather than discarding all positive blood units, laboratorians add gentian violet to the units, which are stored at 4° C for 24 hours before use, to kill the organism. In recent years, an influx of immigrants has occurred from these areas into North America and Europe. Some of these individuals are seropositive for Chagas disease.[44] With this immigration, an increase in the diagnosis of clinical Chagas disease has occurred in the United States, as has the recognition of blood transfusion–associated Chagas disease in nonendemic areas.[32]

Ingestion of infected mice by cats or cannibalism among rodents has been shown to transmit the infection successfully. Several reports have indicated the acquisition of infection by humans from infected meat. How important these routes of infection might be in maintaining the disease in nature or in humans is unclear, however. In endemic areas, large numbers of feral and domestic reservoirs of *T. cruzi* exist. Dogs and cats, especially in Brazil and Chile, have been shown to have a high index of infection and are thought to represent important domestic reservoirs. In some areas, armadillos have been implicated. In the United States, naturally occurring Chagas disease has been reported from Louisiana, Oklahoma, and Texas. In addition, the wood rat, raccoon, armadillo, and opossum are important feral hosts.

PATHOGENESIS AND PATHOLOGY

When metacyclic trypomastigotes penetrate the skin or mucous membrane, they may be phagocytized by tissue macrophages or

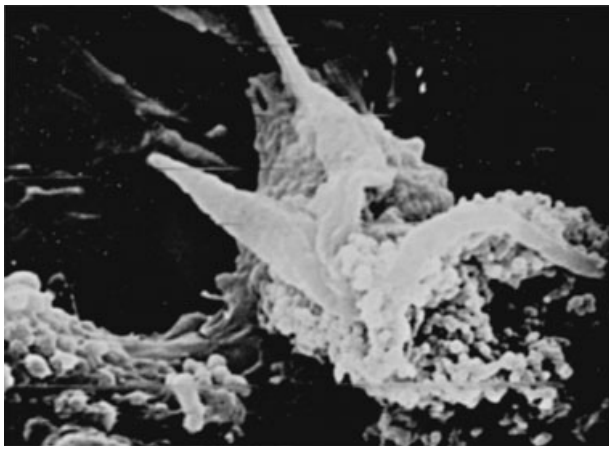

Figure 233–2 *Trypanosoma cruzi* phagocytized by a mouse peritoneal macrophage (scanning electron micrograph ×6000).

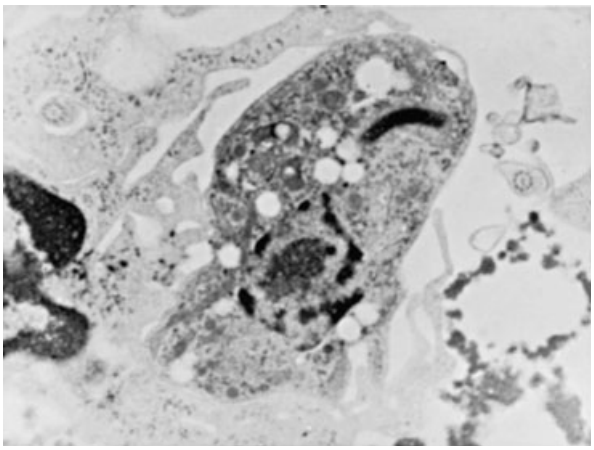

Figure 233–3 *Trypanosoma cruzi* phagocytized by a mouse peritoneal macrophage (transmission electron micrograph ×7000).

actively penetrate other cells (Figs. 233–2 and 233–3).[33] Within the cell, trypomastigotes rapidly transform into rounded amastigotes, and division begins (Fig. 233–4). In vitro studies have shown that parasites within macrophage vacuoles may be killed by the cytocidal mechanisms of the host cell, which include nitric oxide. The parasite may escape the parasitophorous vacuole by elaborating a hemolysin and reach the cytoplasm, where it reproduces. Within these cells, large numbers of amastigotes are formed, and within a few days, the greatly distended cell ruptures and frees trypomastigotes and amastigotes into tissues. They actively invade previously uninfected cells, and the process is repeated.

A nodular swelling, or chagoma, develops at the site of entry (Fig. 233–5). This area soon is infiltrated with macrophages surrounded by lymphocytes, eosinophils, and neutrophils. The process spreads to the regional lymph nodes, where focal lymphadenitis may be seen. Shortly thereafter, blood forms appear and disseminate throughout the body. Acute pathology is caused by the invasion and subsequent destruction of cells by the replicating intracellular parasites. There also is a marked host inflammatory reaction characterized by local accumulation of neutrophils, lymphocytes, and plasma cells. Although any tissue can be involved, cardiac and skeletal muscle and the cells of the reticuloendothelial and nervous systems are infected more frequently.[45]

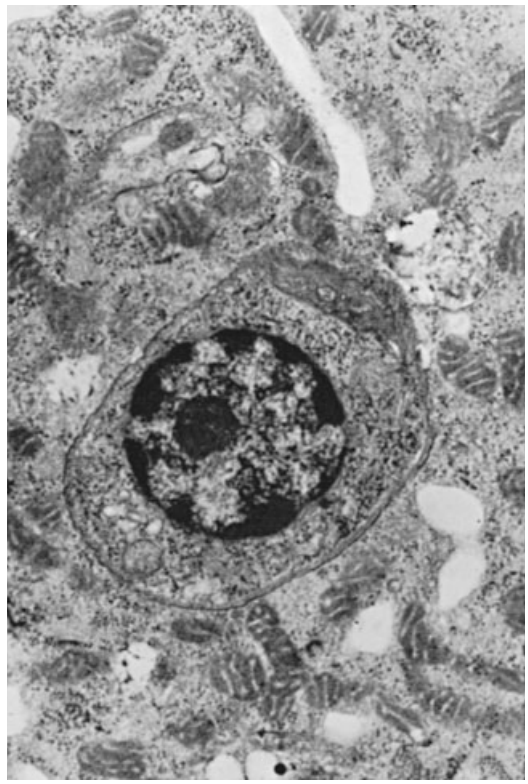

Figure 233–4 *Trypanosoma cruzi* amastigote forms in the cytoplasm of a macrophage (transmission electron micrograph ×7000).

Figure 233–5 Chagas disease. A chagoma on the mucocutaneous junction of the lip and moderate unilateral periorbital edema are present. *(From a nonprofit cooperative endeavor by numerous colleagues under the editorship of Dr. Herman Zaiman, New York.)*

The myocardium reveals focal myonecrosis, contraction band necrosis, interstitial fibrosis, and lymphocytic infiltration. Interspersed among the degenerating fibers is a marked mixed inflammatory cell exudate, which with time becomes predominantly mononuclear. The course of the acute infection can be quite

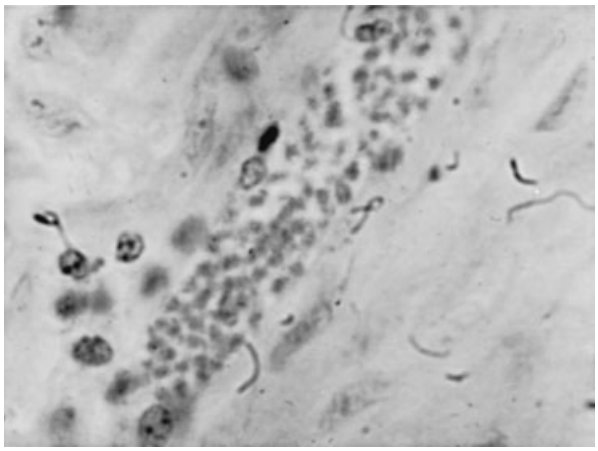

Figure 233–6 *Trypanosoma cruzi* amastigote forms in cardiac muscle (original magnification ×288).

variable, with severe tissue destruction, or the infection can be silent, with little or no obvious pathology. Nonetheless, the parasites have entered various cells of the body successfully and have formed pseudocysts, each containing hundreds to thousands of amastigotes (Fig. 233–6). Individuals who recover from the acute episode, including those who have remained asymptomatic, probably harbor these intracellular parasites for the rest of their lives.

The immunology of Chagas disease in experimental animals and humans is very complex. It is clear, however, that cell-mediated immunity and antibody play important roles in the immune response.[49] Cytokines such as interferon-γ and nitric oxide contribute to the intracellular killing of this parasite and to myocardial and gastrointestinal tract dysfunction.

Chagasic heart disease represents the interplay of inflammation and ischemia. During acute infection, foci of myonecrosis, myocytolysis, and vasculitis are observed, along with an inflammatory exudate consisting primarily of leukocytes. Pseudocysts containing amastigotes can be found interspersed among the degenerating fibers. *T. cruzi* gains access to cardiac myocytes by first invading endothelial cells, the interstitial areas of the vascular wall, and the myocardium. Parasites can be seen in and around the endothelium of infected mice. The trypomastigotes pass two basal laminae and two layers of extracellular matrix. Parasite enzymes such as proteases, gelatinases, and collagenases degrade native type I collagen, heat-denatured type I collagen, and native type IV collagen. Proteolytic activities against laminin and fibronectin also have been detected. These enzymes may play an important role in the degradation of extracellular matrix and subsequent parasite invasion.

Degradation of the collagen matrix, evident in acute murine Chagas disease, has been proposed to result in chronic pathology such as apical thinning of the ventricle. When damage such as that caused by ischemia and necrosis occurs, extracellular matrix degradation ensues and leads to slippage of the ventricular layers, with mural thinning and formation of an aneurysm. Damage to this area of the heart and remodeling of the wall frequently are encountered in chagasic heart disease. Remodeling refers to the structural changes associated with inflammation, necrosis, hypertrophy, and ventricular dilation. In the course of chronic chagasic heart disease, myonecrosis, myocytolysis, and contraction band necrosis are evident. The necrosis results from transient hypoperfusion followed by reperfusion, such as occurs after local spasm of the coronary microvasculature. Focal and diffuse areas of myocellular hypertrophy may be observed with or without inflammatory infiltrates. In other areas, focal fibrosis replacing previously damaged myocardial tissue is evident.

An important feature of chagasic heart disease is a dense accumulation of extracellular collagen that encloses fibers or groups of fibers. All areas of the heart, including the conduction pathways, may be involved. Microvascular involvement, manifested by basement membrane thickening, has been shown. These irreversible changes lead to functional disturbances. The remodeling process results in replacement of cardiac myocytes and cells of the vasculature by fibrous tissue, which leads to thinning of the myocardium and hypertrophy of the remaining cardiac myocytes. Chronic chagasic cardiomyopathy is characterized by focal lymphocytic infiltrates (predominately $CD8^+$), myocyte necrosis, and marked reactive and reparative fibrosis. The role of apoptosis in the pathogenesis of chronic chagasic cardiomyopathy is unclear.

In the chronic stage of Chagas disease, the heart usually is enlarged. The chambers may be dilated and associated with an apical left or right ventricular aneurysm, which rarely ruptures. Mural thrombi, especially of the right atrium and apex of the left ventricle, commonly are seen. They may cause widespread embolization, especially to the brain, lungs, spleen, and kidneys. The myocardium often reveals diffuse fibrosis with small numbers of mononuclear cells scattered throughout, and in many patients, finding parasites in the myocardium may be quite difficult.

The apparent absence of parasites in the myocardium during routine histologic examination in chronic Chagas cardiomyopathy, even in the presence of marked morphologic and functional changes, has engendered much speculation regarding possible pathogenic etiologies. Parasite persistence, autoimmunity, disturbances in the autonomic nervous system, and vascular compromise have emerged as possible important factors. Experimental studies indicate that endothelin-1 and thromboxane A_2, potent vasoconstrictors, and nitric oxide may contribute to the pathogenesis of Chagas cardiomyopathy.[46] In addition, genetic epidemiologic studies suggest that susceptibility to infection and progression to chronic disease may be partly genetically determined. Researchers have estimated that destruction of more than 95 percent of the ganglion cells of the myenteric plexus in the esophagus and colon is required for evidence of megaesophagus and megacolon. In experimental studies, destruction of ganglion cells mediated by inflammatory mediators is the result of parasitic destruction during the acute phase of the disease.

CLINICAL MANIFESTATIONS

Clinically, Chagas disease can be viewed as having three phases: acute, indeterminate, and chronic. Seroepidemiologic studies indicate that overt or clinically recognizable disease fails to develop in most individuals infected with *T. cruzi*, and researchers estimate that 99 percent of individuals infected have inapparent or subclinical infection. Most individuals in whom clinical disease develops as a result of natural infection are infants unable to ward off the bite of triatomes, or "kissing bugs," and other young children up to 10 years old. After an incubation period of approximately 7 to 14 days, acute clinical symptoms may begin with anorexia, lassitude, headache, and intermittent or remitting fever with temperatures of 38° C to 40.5° C. In young infants, swelling and edema of the Bichet fat pad may make feeding painful.

Early Disease

Locally, or near the site of initial infection, a characteristic unilateral, painless palpebral edema, often with conjunctivitis, may be recognized. This so-called Romaña's sign, when present in an endemic area (25 to 30% of patients), is highly suggestive of early acute Chagas disease. The edema may be generalized and mistaken for nephrotic syndrome, however, or swelling of the preauricular region may be so severe that it suggests mumps. The

edema does not pit on pressure, and the skin is dry. Generalized lymphadenopathy, hepatosplenomegaly, vomiting, diarrhea, and signs of meningeal irritation commonly are encountered. Skin lesions can vary from a generalized maculopapular or morbilliform eruption to urticaria. Neurologic symptoms, found more often in younger infants than in older patients, include focal to generalized seizures.

Acute-Stage Disease

Acute-stage symptoms occur in only approximately 1 percent of patients, usually are seen in younger children, and are less obvious in older individuals because of the nonspecific nature of the symptoms and the lack of availability of health care. Symptoms generally last 4 to 8 weeks and then may subside, even without therapy. Acute systemic signs occur around week 2 to 3 of infection and are characterized by high fevers, which may be intermittent, remitting, or continuous; hepatosplenomegaly; myalgia; erythematous rash; acute myocarditis; lymphadenopathy; keratitis; and subcutaneous edema of the face, legs, and feet. There may be signs of central nervous system (CNS) involvement, including meningoencephalitis, which has a very poor prognosis. Myocarditis is manifested by electrocardiographic changes, tachycardia, chest pain, and weakness. Amastigotes proliferate within the cardiac muscle cells and destroy the cells, leading to conduction defects and a loss of heart contractility. Death may occur secondary to myocardial insufficiency or cardiac arrest. In infants and very young children with acute Chagas disease, swelling of the brain can develop, causing death.[42] Also, in some areas of endemic infection, a high frequency of early electrocardiographic abnormalities in children who are seropositive for *T. cruzi* suggests a rapid evolution from infection to disease. Under such conditions, a public health chemotherapy program targeted to this population would be recommended.

During acute infection, echocardiography may reveal abnormal segmental left ventricular wall motion, most commonly in the anterior or apical regions (or both). The overall size of the left ventricle and ejection fraction are typically normal. Fatal acute myocarditis may occur in a few patients. Examination of blood during the acute infection often reveals trypomastigote forms, leukocytosis with a relative lymphocytosis, and an elevated erythrocyte sedimentation rate. Trypomastigotes may be present in cerebrospinal fluid (CSF), even in the absence of neurologic abnormalities. Although most patients recover, apparently without sequelae, 2 to 10 percent may die of severe myocarditis, meningoencephalitis, or an underlying bacterial or viral infection. As the acute stage subsides in 8 to 12 weeks, fewer and fewer parasites can be found in the peripheral blood as antibody levels increase. The disease enters the indeterminate period, and evidence of infection may be detected by special techniques described later. During acute infection, most of the antibody is IgM. Later, in the indeterminate and chronic stages, antibodies predominantly are IgG. From a global perspective, *T. cruzi* infection is recognized as the most common cause of myocarditis.

Indeterminate Phase

The indeterminate phase of Chagas disease represents the period between acute infection and the onset of chronic signs and symptoms. The duration of this period can span 10 to 40 years, and in most cases, overt clinical disease never develops despite the fact that in asymptomatic individuals, organisms often can be identified by culture or molecular methods and by xenodiagnosis. During the indeterminate phase, electrocardiographic or radiologic abnormalities may not be detectable. Echocardiography often reveals significant changes, however. Many of these individuals (10 to 30%) eventually experience the serious and potentially fatal chronic phase of the disease. A report from a hospital in Brazil identified Chagas heart disease in 11 percent of patients who died of a cardiovascular cause.[26]

The patient may have a subpatent parasitemia and develop antibodies to various *T. cruzi* antigens. Chronic Chagas disease is diagnosed more commonly than the acute disease. The chronic stage may be initially asymptomatic (indeterminate stage), and although trypomastigotes seldom are seen in peripheral blood, transmission by blood transfusion is a serious problem in areas where the disease is endemic. There are regional variations in disease severity, which may account for the geographic differences in morbidity. Approximately 8 to 10 weeks after infection, the indeterminate stage begins, during which the patients do not have any symptoms.

Chronic Stage

Chronic Chagas disease shows close interaction between the parasite and the host, causing many different clinical syndromes. Approximately 30 percent of patients may develop chronic Chagas disease, including cardiomyopathy, megacolon, and megaesophagus. Symptoms of the chronic stage are related to the damage sustained during the acute stage of the disease, the state of the host's immune system, and the inflammatory response. Other contributing factors include the host genetic background, environmental and social factors, the genetic composition of the parasite, and mixed infections and re-infections. The inflammatory response undergoes periods of exacerbation, and is probably responsible for neuronal damage, microcirculatory changes, heart matrix deformations, and possible cardiac failure. Selenium deficiency also has been linked with some cardiomyopathies and apparently is a biologic marker for Chagas disease and related to progressive pathology.

Chronic chagasic heart disease occurs insidiously or abruptly with an arrhythmia, dilated congestive cardiomyopathy, thromboembolic phenomena, and sudden death. An apical aneurysm, with or without thrombus formation, is a hallmark of this disease and is commonly found, although not exclusively, in the left ventricle. Although significant major coronary artery lesions are usually absent, several instances of myocardial infarction in individuals with Chagas disease have been reported. Destruction of conduction tissue results in conduction abnormalities.[41]

In endemic areas, electrocardiographic abnormalities may suggest chagasic cardiomyopathy. A right bundle branch block alone and a right bundle branch block together with an anterior fascicular block are highly suggestive of chagasic heart disease. These abnormalities have been reported in teenagers and young adults. Premature ventricular contractions and right bundle branch block occur in 50 percent of cases, atrioventricular block of varying degrees develops in about 30 percent of cases, complete atrioventricular block is seen much less commonly, and left bundle branch block is not characteristic. Chest radiographs may reveal cardiomegaly. Heart sounds may be distant, and murmurs of functional mitral and tricuspid insufficiency sometimes are heard. More recently, echocardiography and cardiac magnetic resonance imaging have been used to diagnose and suggest prognosis of chronic chagasic cardiomyopathy.[61] When congestive heart failure ensues, it is usually intractable and difficult to treat. Models have been formulated to identify risk scores predictive of survival probabilities from Chagas heart disease. Such models may facilitate development of clinical trials for therapeutic intervention.[48]

Megaesophagus and megacolon are the result of destruction of the ganglion cells of the autonomic plexus. Although these complications are not usually fatal except in congenital disease, the marked dilation of the colon and esophagus can be debilitating.[21] Any hollow viscus, including the gallbladder and ureters, can be involved, but esophageal and colonic disease are encountered most frequently. Dysphagia, gastroesophageal reflux, and

paroxysmal night coughs, presumably caused by aspiration while sleeping, are associated with megaesophagus; chronic constipation, long-term fecal retention, impaction, and volvulus are observed with megacolon. In some regions, patients with digestive disease have symptomatic chagasic heart disease as well. Wide geographic variation in the prevalence of cardiac and gastrointestinal disease has been noted. In Panama, Venezuela, and Colombia, megaesophagus and megacolon are rarely encountered, whereas in parts of Brazil, both forms of Chagas disease may be seen in the same individual.

Congenital Disease

Congenital Chagas disease is caused by vertical transmission of the infection.[8,29] Pregnant women in endemic areas may experience abortion or placentitis and may give birth to infants with congenital infection associated with prematurity. In its acute stage, congenital Chagas disease often resembles the acquired disease. The onset may be at birth or a few months later, with the infant manifesting hepatosplenomegaly, anemia, jaundice, edema, thrombocytopenia, petechiae, tremors, and seizure disorders. The clinical and laboratory findings may resemble those of congenital toxoplasmosis, cytomegalovirus and herpes simplex virus, or erythroblastosis fetalis. Although meningoencephalitis has been observed, it is more likely to be silent. The prognosis of meningoencephalitis in congenital disease is better than in the acquired form of disease. Examination of CSF often reveals trypomastigotes. Necrotic and hemorrhagic lesions of the oral mucosa and skin are thought to represent hematogenously disseminated chagomas. Megacolon and megaesophagus can be present and cause constipation and aspiration pneumonitis.[9,19]

Immunocompromised Patients

Chagas disease has been reported in patients with human immunodeficiency virus (HIV), and neurologic sequelae caused by *T. cruzi* have been major findings in these patients.[17,39] Individuals who were previously infected with *T. cruzi* and later became positive for HIV are at risk of reactivation of Chagas disease.[50] These patients develop a severe multifocal or diffuse meningoencephalitis with numerous tissue parasites.[51] Meningoencephalitis is rare in patients without acquired immunodeficiency syndrome and is seen most frequently in immunocompetent children younger than 4 years. Because of the immunodeficiency associated with HIV infections, concomitant infection with *T. cruzi* may be difficult to recognize, particularly in individuals who have moved to areas where the disease is not endemic. *T. cruzi* infection also may have a protracted asymptomatic course in immunosuppressed HIV patients. Although a positive blood smear has been considered the key indicator of Chagas disease reactivation in immunocompromised patients with chronic disease, this finding may occur late, rather than early, in the reactivation process. Apparently, there is no association between given *T. cruzi* genotypes and specific clinical forms of Chagas disease–HIV associations.

Recrudescence of *T. cruzi* infections in immunosuppressed patients, particularly transplantation patients, is concerning. Transplant recipients also can become infected through receipt of infected organs.[14] Treatment guidelines for HIV-infected adults and adolescents with Chagas disease have been formulated.[6] In patients with end-stage Chagas cardiomyopathy, heart transplantation is an option that has had variable success. Reactivation of the disease with the development of cutaneous lesions has been seen. Bone marrow transplant recipients also are at risk of Chagas disease owing to reactivation or transfusion. Prophylactic treatment of these patients has led to favorable outcomes.

There seems to be a link between *T. cruzi* seropositivity and human T-cell lymphotrophic virus 2 (HTLV-2) infection in studies of the Indians in western Paraguay. In this particular group, an individual infected with HTLV-2 is 2.28 times more likely to be *T. cruzi*–positive than an HTLV-2-negative individual. The public health significance of these findings is emphasized by the fact that approximately 18 million people are infected with *T. cruzi* in the Americas, and the frequency of HTLV-2 continues to increase.[27]

DIAGNOSIS

Routine Methods

The diagnosis of Chagas disease should be considered if there is a history of consistent exposure to reduviid bug bites, residence in or travel to areas where the disease is endemic, laboratory accident, or recent blood transfusion in an area where the disease is endemic, or if cardiac or digestive tract lesions are compatible with Chagas disease. A definitive diagnosis depends, however, on demonstration of the trypomastigotes in the blood, amastigote stages in tissues, or positive serologic reactions. Methods used for examination of blood are similar to the methods used for the diagnosis of African trypanosomiasis. Trypomastigote stages may be detected easily in the blood in young children; however, in chronic disease, this stage is rare or absent except during febrile exacerbations. Trypomastigotes may be detected in blood by using thin and thick blood films or by buffy coat concentration techniques. The most sensitive methods to detect trypomastigotes are concentration techniques, such as the Strout method of buffy coat preparations.[31] With the Strout method, blood is collected without anticoagulant and allowed to clot. The serum is centrifuged at low speed to remove the remaining blood cells and then at a higher speed ($600 \times g$) to concentrate the parasites in the sediment. The stain of choice is Giemsa for trypomastigote and amastigote stages; other blood stains also are acceptable.

Examination of peripheral blood is valuable only during the initial acute disease (6 to 12 weeks) or during chronic exacerbation. Although inoculation of animals with the patient's blood often aids in the diagnosis, this is not a practical approach in most diagnostic laboratories. In chronic stages, the investigator frequently must take 30 to 50 mL of the patient's blood and inoculate numerous cultures to obtain a positive diagnosis. Aspirates, blood, and tissues also can be cultured, which is valuable in detecting low-grade parasitemias. The medium of choice is Novy, McNeal, and Nicolle medium. Cultures should be incubated at 25° C and observed for epimastigote stages for 30 days before they are considered negative.

Aspirates from chagomas and enlarged lymph nodes can be examined for amastigotes and trypomastigotes. Histologic examination of biopsy specimens also may be performed. Liver biopsy, bone marrow aspiration, or splenic puncture also can be tried to confirm the diagnosis. Sometimes, xenodiagnosis may be the only means by which organisms can be found. In this technique, laboratory-reared reduviid bugs are permitted to feed on the patient or the patient's fresh blood; subsequently, the bugs are allowed to feed on uninfected guinea pigs. The guinea pigs are examined for trypomastigotes after approximately 45 days. Although 100 percent specific, this test is less than 50 to 80 percent sensitive. If organisms are present in the blood meal, the parasites multiply and are detected in the bug's intestinal contents, which should be examined monthly for flagellated forms over 3 months.

Serologic Tests

Serologic tests can be useful during various stages of the disease. The precipitin test may be positive during the acute episode, whereas complement fixation (Machado-Guerreiro test) is useful for the diagnosis of chronic disease. The indirect fluorescent antibody and indirect hemagglutination tests are valuable tools

for the diagnosis of Chagas disease. Patients with leishmaniasis, malaria, collagen vascular disease, and syphilis may give false-positive reactions, however. In congenital disease, an indirect fluorescent antibody test using anti-IgM or an enzyme-linked immunosorbent assay for IgA antibodies may provide evidence of congenital disease.[23,40] Tests also have been developed to detect antigen in serum and urine.

Polymerase Chain Reaction

Tests using recombinant methods that have the potential for high specificity and high sensitivity have been developed. During chronic infection, the number of circulating parasites is very low, but PCR-based assays can detect low numbers of organisms because the parasite has highly repetitive nuclear and kinetoplast DNA sequences that can be amplified by PCR.[43] During the chronic stage, serologic methods may be the only means to arrive at a diagnosis. The differential diagnosis of chronic chagasic cardiomyopathy includes rheumatic and arteriosclerotic heart disease and cardiomyopathies of other etiologies.

Immunoassays

Immunoassays have been used to detect antigens in urine and sera in patients with congenital infections and patients with chronic Chagas disease.[7] Determination of antigenuria can be valuable for early diagnosis of Chagas disease and for diagnosis of chronic cases in patients with conflicting serologic test results. A highly sensitive and specific chemiluminescent enzyme-linked immunosorbent assay has been developed for blood bank screening and to monitor patients who are undergoing chemotherapy.[15]

TREATMENT

Although numerous drugs have been tried, including agents used to treat African trypanosomiasis and leishmaniasis, few have proven to be effective for therapy of Chagas disease.[37,58] In acute and congenital Chagas disease and infections caused by laboratory accidents, treatment should be administered as soon as possible, even though in some cases symptoms are self-limited. At present, therapy for Chagas disease is recommended for patients with acute disease, children with congenital infection, and patients in the early phase of chronic disease (in particular, children <15 years old).[3,55] In addition, therapy should be prescribed for patients co-infected with HIV who experience Chagas reactivation. Whether therapy can modify the progression of chronic Chagas disease has not been conclusively shown. Data published more recently suggest a beneficial effect of therapy in diminishing the risk of progression to severe chronic cardiomyopathy.[62] A randomized trial in progress is expected to define more clearly the role of therapy on cardiac outcomes in chronic Chagas disease.[22]

Nifurtimox (Lampit), a nitrofurfurylidine derivative, is tolerated better in younger than older patients and should not be used during pregnancy. It reduces the duration and severity of illness and decreases mortality resulting from acute and congenital Chagas disease. Reversible gastrointestinal, cutaneous, and neurologic adverse effects are common. Treatment success varies from one country to another, possibly indicating differences in the susceptibility of strains of *T. cruzi*. There is no indication that treatment of patients with chronic Chagas disease is beneficial. Nifurtimox must be taken orally for prolonged periods, and there can be severe side effects, including abdominal pain, nausea, vomiting, anorexia, and neurologic symptoms. Nifurtimox is available in the United States from the Parasitic Drug Service of the Centers for Disease Control and Prevention. It is supplied in 30-mg and 120-mg tablets. The usual dose for adults is 8 to 10 mg/kg body weight per day. For adolescents (11 to 16 years old), it is 12.5 to 15 mg/kg/day, and for children (1 to 10 years old), the dose is 15 to 20 mg/kg/day. It is given in four divided doses daily for 90 to 120 days. This drug has many untoward reactions, including abdominal pain, nausea, vomiting, anorexia, restlessness, disorientation, insomnia, twitching, paresthesia, polyneuritis, and seizures. Skin reactions also may be observed.

Benznidazole (Rochagan) is similar to nifurtimox and seems to have similar efficacy. Some researchers consider it to be tolerated well, with fewer adverse reactions. It is used extensively in Brazil at 5 mg/kg/day for 30 to 120 days in two divided doses (adult dose). For children up to 12 years old, the dose is 10 mg/kg/day in two doses for 30 to 90 days. Bone marrow depression and peripheral neuritis have limited its use, however. Additional chemotherapeutic agents are urgently needed and are under investigation.[59]

Interferon-γ has been used in conjunction with drug therapy because experimental data suggest that it is a useful adjunct in treatment by activating macrophage killing. Allopurinol and antifungal drugs have been suggested as alternatives in some patients. Patients with chronic chagasic cardiomyopathy may be helped for a varying period by implantation of a cardiac pacemaker, but the congestive heart failure is often refractory to cardiac glycosides and vasoactive drugs. More recently, inhibitors of angiotensin-converting enzymes have been advocated to reduce cardiac remodeling in these individuals. Heart transplantation also has been performed for the management of chronic cardiomyopathy. The use of high-fiber diets, occasional laxatives, and enemas may assist in the management of megacolon. When these measures fail, surgical resection may be required. Megaesophagus is usually amenable to therapy consisting of a combination of diet and dilation of the esophagogastric region. In more severe disease, various surgical procedures have been used with variable success to relieve the symptoms of achalasia.

PREVENTION

Regular insecticide spraying programs with benzene hexachloride could reduce transmission of the infection, at least around domiciles. Proper screening and improved housing for individuals in endemic areas also help reduce transmission. In endemic areas, thorough screening of blood donors would help prevent transmission by transfusion. The development of a chemoprophylactic agent or vaccine holds the best hope for prevention and subsequent eradication of Chagas disease.

Transfusion-acquired disease is a major public health problem in endemic areas. Treatment of blood with gentian violet inactivates the parasite. Transfusion-acquired infection has been reported in North America. Because no effective rapid means of detecting the parasite exists, researchers have suggested that individuals from endemic areas be rejected as blood donors.

AFRICAN TRYPANOSOMIASIS

African sleeping sickness is caused by two morphologically identical subspecies of hemoflagellate protozoans: *Trypanosoma brucei gambiense*, the cause of West African or Gambian sleeping sickness, and *Trypanosoma brucei rhodesiense*, the cause of East African or Rhodesian disease. Although these parasites produce similar disease, the Gambian form is usually chronic and evolves slowly, often over many years, and ends fatally if untreated, whereas the Rhodesian form is characterized by being acute, usually killing the host in a matter of weeks or months. These diseases exist

wherever the various species of *Glossina*, the tsetse fly, are found. The completion of the *Trypanosoma brucei* genome should facilitate greatly the discovery of new drug targets and genetic markers.[12]

ORGANISM

T. b. gambiense and *T. b. rhodesiense* are pleomorphic flagellates 15 to 30 μm in length by 1.5 to 3.5 μm in breadth. In Giemsa-stained blood smears, they may appear long and slender with an undulating membrane and free anterior flagellum or appear short and broad without a free anterior flagellum. No intracellular forms exist. At various stages of disease, trypomastigote forms may be found in the peripheral blood, lymphatics, lymph nodes, CSF, and neural tissue. Although the two species are morphologically indistinguishable and share major biochemical features, isozyme and molecular analysis has shown substantial variation among species, particularly regarding clinical disease. In addition, the two species maintain separate biologic characteristics, especially with regard to virulence in cross-inoculation experiments. Humans are the only important reservoir host for *T. b. gambiense*, whereas *T. b. rhodesiense* naturally infects wild game animals.

The haploid genome size of *T. brucei* spp. is approximately 40 Mb, with 14 percent variation within the same subspecies and 29 percent variation among different subspecies. Homologous chromosomes, when probed by Southern blot, can differ in size by 20 percent. In addition to the large chromosome pairs, *T. brucei* has approximately 100 linear minichromosomes ranging in size from 50 to 150 kb. These minichromosomes contain transcriptionally silent copies of variant surface glycoprotein (VSG) genes. *T. brucei* has approximately 1000 genes capable of coding for *VSG* genes; these genes are switched at a rate of 10^{-2} to 10^{-6} switches per generation, which serves as the main mechanism of immune evasion for *T. brucei*.[24] Only one VSG expression site is active at any given time. Studies comparing homologous nuclear genes between *T. cruzi* and *T. brucei* have shown large evolutionary divergence in codon usage. Comparison of the nuclear small-subunit and large-subunit rRNA gene sequences yields genetic distances comparable to that between plants and animals.

Within the insect vector, the tsetse fly, that has ingested a blood meal, settles in the posterior portion of the midgut and multiplies by binary fission for approximately 7 to 10 days. The slender trypomastigotes migrate anteriorly to the foregut, where they remain for the next 2 to 3 weeks. They next move further forward and finally enter the salivary glands, in which they transform into epimastigote forms—forms in which the kinetoplast has migrated just anterior to the nucleus—and continue to replicate. After multiplication cycles, they transform into infective metacyclic trypomastigote forms, which are the small, broad or "stumpy" forms that lack a free anterior flagellum. When next feeding, the infective tsetse fly may inoculate into the bite wound thousands of these infective trypomastigotes. The entire life cycle of the tsetse fly spans 15 to 35 days. Within the human host, trypomastigotes multiply by binary fission in blood, lymph, and extracellular spaces. The CNS eventually is invaded, at which time multiplication continues unabated. Transmission by blood transfusion, hypodermic needle, other insects, and congenital transmission has been reported.

EPIDEMIOLOGY

African sleeping sickness (*T. b. gambiense* and *T. b. rhodesiense*) and veterinary trypanosomiasis caused by *T. brucei* subgroup parasites are responsible for much human suffering and economic loss. Factors contributing to disease resurgence include socioeconomic unrest resulting in population shifts of animal reservoirs, insufficient financial allocations, emergence of drug-resistant parasitic strains, climatologic alterations, and altered susceptibility of populations at risk.[35] Because the larval stages of the tsetse fly are vulnerable to desiccation, *Trypanosoma* spp. are restricted to an area spanning 10 million km^2 south of the Sahara, encompassing 200 separate foci from 36 countries, where the annual rainfall exceeds 500 mm (20 inches). The most recent estimates are that 25,000 new cases occur each year, and a human population of 60 million is at risk (only 4 to 5 million of whom are under surveillance), along with cattle and other species of agricultural importance. In the past, 10,500 cases have been reported annually in Zaire. These figures are probably low in light of more recent military activity in the Sudan, Democratic Republic of Congo (formerly Zaire), Angola, and Rwanda. Movement of populations in war areas has increased the risk of epidemics in these and neighboring regions.

The Gambian form occurs mainly in the western portion of tropical Africa, with focal incursions eastward north of Lake Victoria into the Sudan. *Glossina palpalis* is the main tsetse fly vector, although other related species, such as *Glossina tachinoides* and *Glossina fuscipes*, are implicated as well. The Rhodesian form is found in the southeastern portion of Africa. Of great concern is the recent identification of a focus of *T.b. rhodesiense* in Uganda, which has substantially diminished the geographic separation of the two species of African trypanosomiasis. Because control of human African trypanosomiasis requires different strategies for the two parasites, emergence of geographic overlap would add greatly to costs.[28,47]

Although *T. b. gambiense* can infect various mammals, humans seem to be more susceptible and maintain a high enough parasitemia to sustain the fly-human-fly cycle. The prolonged chronicity of Gambian disease with infectious individuals continually exposed to tsetse flies undoubtedly helps sustain the disease. Asymptomatic carriers of the infection also may be an important factor in maintaining the disease in a community. Among the important factors that may limit Gambian infection to humans is the transient and low parasitemia that results from an infective tsetse fly bite in mammals, usually ungulates. Researchers have observed that Gambian disease usually is sustained only when a close and repeated relationship exists between humans and tsetse flies. The practical result of all this is that West African sleeping sickness is maintained by members of the *G. palpalis* group of tsetse flies that use or prefer human blood almost exclusively.

In contrast to Gambian disease, *T. b. rhodesiense* infection generally is maintained in wild mammals. The fly-human-fly cycle tends to be unimportant inasmuch as the severe nature of the disease usually quickly removes acutely ill humans as an infective source. The intervention of wild game animals, in which the disease tends to be less acute and whose blood seems to be more attractive, seems to have relegated humans to an occasional or facultative host for *T. b. rhodesiense*. The *Glossina morsitans* group of tsetse flies that inhabit the dry East African savannah readily feed on wild ungulates, especially the bushbuck *Tragelaphus scriptus* and the hartebeest *Alcelaphus buselaphus*. Occasionally, Rhodesian disease may reach epidemic proportions, and during these epidemics, direct human-fly-human cycles may occur.

All age groups are susceptible to infection. The factors that influence human prevalence rest more on occupational exposure to suitable tsetse flies and the flies' breeding and feeding habits. Young men are found to be infected most frequently. During epidemics, all groups are infected, however, and mechanical transmission probably occurs. Tourists on safari are possibly at risk. In that regard, reports of African trypanosomiasis in Europe, the United States, and Australia have been increasing, although fewer than 50 cases/year of human African trypanosomiasis are diagnosed outside of Africa.

PATHOGENESIS AND PATHOLOGY

The metacyclic infective trypomastigote forms are inoculated by the tsetse fly into the skin, where they multiply at the inoculation site. A characteristic, hard, sometimes painful chancre develops. By approximately the 10th day, long slender forms are found in the bloodstream and lymphatics, and for the next several days, their numbers increase logarithmically. Soon thereafter, the organisms nearly disappear from the bloodstream as a result of immune lysis, only to reappear again.

The interval between waves of parasitemia may vary from 1 to 8 days, with clinical symptoms accompanying each bout of parasitemia. These parasitemic waves can be accounted for by the highly developed antigenic variation strategy of the parasite. The trypomastigote is covered with VSG, and with each peak of parasitemia, a predominant variable antigen type is displayed by the organism. The specific antibody response to this coat protein (VSG) leads to the destruction of parasites that display the predominant variable antigen type or homotype. Numerous heterotypes are found within each population of parasites, one of which, not recognized by the host's immune system, becomes the next homotype. The parasite in each successive wave of parasitemia bears a different variable antigen type. A single trypomastigote may contain 1000 genes, each encoding for a specific VSG. Each successive parasitemic wave represents a new antigenic variant that has emerged to elude the host's antibody response to the previous antigen.

As a result of successive waves of immune lysis and parasitemia, a marked early humoral antibody response, predominantly involving IgM, is seen regularly. *In an immunocompetent host, the absence of elevated IgM levels in serum rules out trypanosomiasis.* These macroglobulins contain not only antitrypanosomal antibodies, which are directed against the surface antigens, but also a variety of other antibodies such as heterophile and rheumatoid factor. Researchers have shown experimentally that because of polyclonal B-cell activation, many antibodies are produced to a wide variety of antigens, including brain-specific autoantibodies directed against myelin basic protein, gangliosides, and cerebrosides. Circulating immune complexes have been reported regularly and may be responsible for the glomerulonephritis, hypocomplementemia, and hemolytic anemia that often accompany acute and chronic disease. Cell-mediated immunity also is important in African trypanosomiasis, and increased production of nitric oxide may be important in depression of T-cell responsiveness and generalized immunodepression.

The main pathologic lesions involve the posterior cervical, submaxillary, supraclavicular, and mesenteric lymph nodes and the CNS. The lymphatic tissue usually reveals generalized hyperplasia with diffuse proliferation of lymphocytes. Later, the nodes may become small and fibrotic; however, they initially are markedly hemorrhagic and contain large numbers of trypomastigotes. The CNS remains normal until invaded by organisms, but then a progressive chronic leptomeningitis develops. The brain becomes edematous, and prominent perivascular cuffing by glial cells, lymphocytes, and plasma cells is present. When the latter become vacuolated with pyknotic nuclei, they often are referred to as the morula cells of Mott and Marshalko. Organisms often can be found in brain tissue in proximity to vessels and may be detected in CSF. Glomerulonephritis, myocarditis, pericardial effusion, pulmonary edema, and hypoplastic bone marrow with associated anemia may be seen.

The pathogenesis of the neuropsychiatric manifestations is not understood. More recent experimental studies suggest that changes in levels of brain neurotransmitters; deposition of immune complexes; and alteration in production of prostaglandin, cytokine, and nitric oxide may account partly for these behavioral changes.[2,53]

CLINICAL MANIFESTATIONS

The clinical manifestations of *T. b. gambiense* and *T. b. rhodesiense* disease are similar except that Rhodesian infection is a more fulminant, acute disease that may run its course in several weeks to 6 to 9 months, whereas Gambian infection may last for years. Typically, the incubation period in Rhodesian infection is brief (3 to 21 days), whereas the onset of symptoms with Gambian infection may be delayed for several weeks or years. Approximately 1 week after infection and at the site of the tsetse fly bite, a hard and painful chancre sometimes appears and lasts several weeks. During the early stages, when recurrent bouts of fever may be the only symptom, the blood and lymphatics primarily are involved, and infection often is mistaken for malaria, especially if a chancre is not obvious, or a history of exposure to tsetse flies is not obtained. The period of intermittent fever may last months to years with Gambian infection. During this time, persistent headache and tachycardia often are encountered, and a circinate erythematous rash or erythema multiforme sometimes is noted. The fever abates gradually.

In many patients, characteristic posterior cervical lymphadenopathy becomes evident (Winterbottom sign). The nodes are nontender and attain a diameter of approximately 1 cm. They tend to become small and fibrotic in approximately 6 months.

Signs of CNS invasion often develop in untreated patients with Gambian disease. The initial signs and symptoms of neurologic involvement can be difficult to assess. They may consist of alterations in behavior or personality (or both) that eventually may be manifested as a severe psychosis. Severe headache, loss of nocturnal sleep, and a feeling of impending doom typically are described. Next, progressive mental deterioration may occur, and with unrelenting deterioration, patients become incapable of caring for themselves. Tremors, especially of the tongue, hands, or feet, and generalized or focal convulsive episodes may occur. Almost any neurologic or psychiatric manifestation can be seen, and with progressive mental deterioration, patients finally lapse into a coma and die.

During the final period of the disease, patients often die of concurrent infections such as bacterial pneumonia, amebiasis, and malaria. Wasting and malnutrition are a large component of the progressive deterioration in these patients. Sleeping sickness can be a difficult disease to diagnose early in young children. It usually is found only after a child is evaluated for obtundation, seizures, or psychomotor retardation.[13,42]

T. b. rhodesiense produces a more rapid, fulminating disease than *T. b. gambiense*. Fever, severe headaches, irritability, extreme fatigue, swollen lymph nodes, and aching muscles and joints are common symptoms. Progressive confusion, personality changes, slurred speech, seizures, and difficulty walking and talking occur as the organisms invade the CNS. The early stages of the pathologic process parallel those of *T. b. gambiense* infections; however, the disease progresses more rapidly, such that death may occur before there is extensive CNS involvement, even though CNS invasion occurs early. The incubation period is short, often within 1 to 4 weeks, with trypomastigotes being more numerous and appearing earlier in the blood. Lymph node involvement is less pronounced, and Winterbottom sign may be absent. Febrile paroxysms are more frequent, and the patients are more anemic and more likely to develop myocarditis or jaundice. Some patients may develop persistent tachycardia, and death may result from arrhythmia and congestive heart failure owing to pancarditis.

Second-Stage Human Disease

The clinical symptoms and signs of patients with second-stage human African trypanosomiasis are described for a large cohort ($N = 2541$) of patients treated in a prospective multicenter, multinational study.[11] Special emphasis was given to the influence of

disease stage (duration, number of white blood cells in CSF) and patient age on the clinical picture. Although the frequencies of symptoms and signs varied greatly among centers, the clinical picture of the disease was similar for all countries. Headache (78.7%), sleeping disorder (74.4%), and lymphadenopathy (56.1%) were the most frequent symptoms and signs, and they were similar for all stages of the disease. Lymphadenopathy tended to be highest in the advanced second stage (59%). Neurologic and psychiatric symptoms increased significantly with the number of white blood cells in the CSF indicating the stage of progression of the disease. Pruritus was observed in all stages and increased with the number of white blood cells in CSF from 30 to 55 percent. In children younger than 7 years, lymphadenopathy was reported less frequently (11.8 to 37.3%) than in older children or adults (56.4 to 61.2%). Fever was reported most frequently in children 2 to 14 years old (26.1 to 28.7%), and malnutrition was observed significantly more frequently in children of all ages (43 to 56%) than in adults (23.5%).

DIAGNOSIS

Diagnostic options for human African trypanosomiasis are limited in field settings.[16] Even in field settings, however, positive results from screening assays, such as the card agglutination test, must be confirmed by demonstration of the parasite. Absence of confirmatory testing may lead to unnecessary administration of toxic drugs.[34]

Routine Methods

As indicated earlier, a definitive diagnosis can be made only by finding trypanosomes in blood and bone marrow smears, in lymph node aspirates in early or acute disease, and in CSF in late or chronic disease.[63] For examination of thick and thin smears and the buffy coat, 10 to 20 mL of citrated whole blood or the sediment from 5 mL of centrifuged CSF is essential. Numerous microscopic techniques, including capillary tube centrifugation, quantitative buffy coat examination, miniature anion-exchange centrifugation, density gradient centrifugation, and acridine orange staining, have been used to enhance parasite detection. Culture or animal inoculation, or both, is sometimes the only successful means of making a diagnosis. In advanced CNS disease, lymphocytosis and elevated IgM are detected in the CSF.

Serologic Tests

A serologic diagnosis of African trypanosomiasis usually depends on detection of high levels of nonspecific serum IgM. Detection of specific antibody by indirect fluorescent antibody is helpful, especially when the serum is tested with trypanosomes of the homologous human species. Direct agglutination and a microscale version of enzyme-linked immunosorbent assay are highly reliable methods for making a specific diagnosis of trypanosomiasis.[4]

Polymerase Chain Reaction

Antigen-detection and PCR tests have been developed to detect circulating parasites.[18] The development of a peptide nucleic acid fluorescence in situ hybridization probe seems to offer an excellent diagnostic tool. By combining this test with a cytospin step, the limit of detection from blood can be improved to 5 parasites/mL, a level of sensitivity that equals an optimal PCR detection limit on blood specimens. A newer PCR technique targets the gene encoding the small ribosomal subunit to identify and differentiate all clinically important African trypanosome species and some subspecies. This method seems to be more economical, simple, and sensitive than some other screening methods and yields more detailed information.[18]

Immunoglobulin Levels

In advanced, untreated sleeping sickness, the IgM level in CSF often is elevated, but it has no relationship to the presence of trypanosomes in CSF. After successful treatment, the IgM level declines gradually and disappears after approximately 1 year. If 1 year after therapy a constant high level is present, or an abrupt increase in IgM occurs, however, relapse should be suspected. Nonetheless, the IgM level should not be used as the sole method to arrive at a diagnosis or prognosis.

The differential diagnosis of African trypanosomiasis includes many diseases, but chronic relapsing fever associated with enlarged cervical lymph nodes in an individual who has been to Africa is suggestive. As indicated earlier, considering diseases such as malaria, syphilis, lymphoma, visceral leishmaniasis, and leprosy is not unusual. Later, encephalitis of other etiologies must be excluded. An important diagnostic feature is the frequent finding of a strikingly elevated erythrocyte sedimentation rate, which is a reflection of the markedly increased serum macroglobulins.

Molecular Dipstick

The development and the first-phase evaluation of a simple and rapid test (human African trypanosomiasis–PCR–oligochromatography) for detection of amplified *T. brucei* DNA has been reported more recently.[20] Through hybridization with a gold-conjugated probe (oligochromatography), PCR products are visualized on a dipstick. Visualization is simple and takes only 5 minutes. Controls for the PCR and for DNA migration are incorporated into the assay. The lower detection limit of the test is 5 fg of pure *T. brucei* DNA. One parasite in 180 μL of blood is still detectable. Sensitivity and specificity for *T. brucei* were calculated at 100 percent when tested on blood samples from 26 confirmed sleeping sickness patients, 18 negative controls (nonendemic region), and 50 negative control blood samples from an endemic region. Human African trypanosomiasis–PCR–oligochromatography is a promising new tool for diagnosis of sleeping sickness in laboratory settings.

Animal Inoculation and Culture

T. b. gambiense isolation in small laboratory animals is usually unsuccessful; in contrast, *T. b. rhodesiense* readily infects animals. Cultivation is not practical for most diagnostic laboratories, but is more successful than animal inoculation.[31]

PROGNOSIS

If therapy is initiated before significant CSF involvement, the outcome is usually favorable. Untreated infection often ends fatally.

TREATMENT

All drugs currently used in the treatment of African trypanosomiasis are toxic and require prolonged administration. Treatment should be started as soon as possible and is based on the patient's symptoms and laboratory findings. The choice of antiparasitic drug depends on whether the CNS is infected.[38] Pentamidine isethionate or suramin can be used when the CNS is not infected. The main chemotherapeutic agents for human trypanosomiasis are pentamidine and suramin for early-stage disease.

Pentamidine Isethionate

Pentamidine is a water-soluble aromatic diamidine that has been in use for nearly 60 years. It is administered as either the isethionate form (Pentam 300) or methanesulfonate (Lomidine). The dose is 4 mg/kg/day for 10 days for adults and children. Pentamidine is effective against early-stage *T. b. gambiense* infection, but less effective against *T. b. rhodesiense* infection and ineffective against late-stage disease. African trypanosomes have a nucleoside (adenine/adenosine: P_2) transporter that takes up pentamidine, which results in concentrations of the agent many times that in plasma. Although laboratory-induced resistant strains seem to have reduced transport properties, naturally derived clinical isolates seem to have retained transport, indicating that most resistance may be caused by alteration of a metabolic target, rather than drug uptake. Pentamidine isethionate does not cross the blood-brain barrier and is administered intramuscularly; side effects include an immediate hypotensive reaction, nausea, vomiting, and Herxheimer-type (inflammatory) reactions.

Suramin

Suramin is a sulfonated naphthylamine that has been used successfully against early-stage sleeping sickness caused chiefly by *T. b. rhodesiense*. It was first used in 1922 and was developed from the closely related azo dyes trypan red and trypan blue. Suramin is administered intravenously. The adult dose is 100 to 200 mg (test dose), then 1 g on days 1, 3, 7, 14, and 21. The pediatric dose is 20 mg/kg on days 1, 3, 7, 14, and 21. Suramin has an extremely long half-life in humans (44 to 54 days), which is the result of avid binding to serum proteins. Suramin binds to many plasma proteins, including low-density lipoprotein, which trypanosomes avidly bind and endocytose as a result of specific membrane receptors. Low-density lipoprotein is a prime source of sterols for bloodstream trypanosomes. Suramin is taken up as a protein complex and has been shown to inhibit all the glycolytic enzymes in *T. b. brucei* in low concentrations, which in most cases are severalfold lower than for the corresponding mammalian enzyme. Suramin also has been found to affect thymidine kinase and dihydrofolate reductase.

Suramin (Bayer 205), a diamidine, also is effective in treating the hemolymphatic stage and CNS disease of *T. b. gambiense*. When using suramin, a test dose should be given to the patient to ensure the drug can be tolerated. It should not be given to patients with renal disease, and if protein, red blood cells, or casts are detected in the urine, treatment should be stopped. Suramin is given intravenously; its side effects include nausea, vomiting, loss of consciousness, seizures, pruritus, edema, and hepatitis. Although rare, fatalities have been reported during suramin therapy. Suramin can be obtained under an investigational new drug protocol from the CDC Drug Service, Centers for Disease Control and Prevention, Atlanta, Georgia, 30333; 404-639-3670 (evenings, weekends, or holidays: 404-639-2888).

Melarsoprol

Melarsoprol (mel B; Arsobal) is an arsenical created by the efforts of Freidheim in the late 1940s. His initial compound, melarsen oxide, *p*-(4,6-diamino-*s*-triazinyl-2-yl) aminophenylarsenoxide, was complexed with dimercaptopropanol (British antilewisite) to form a less toxic complex, melarsoprol. Until 1990, it was the only agent available for curing late-stage (CNS) disease of East African and West African origin. Usually, each series is followed by a week interval before the next series. Melarsoprol is insoluble in water and must be dissolved in propylene glycol. It must be given intravenously; otherwise, it induces a severe localized inflammatory reaction. This drug is administered intravenously. The adult dosing schedule is 2 to 3.6 mg/kg/day for 3 days. This is followed in 1 week with 3.6 mg/kg/day for 3 days. This schedule is repeated after 10 to 21 days. The pediatric dose is 18 to 25 mg/kg total over 1 month; the initial dose is 0.36 mg/kg at intervals of 1 to 5 days for a total of 9 to 10 doses.

Side effects include nausea, vomiting, encephalopathy, and exfoliative dermatitis. Melarsoprol has been used for CNS involvement for years; however, relapse occurs in 6 percent of patients. Arsenic-related encephalopathy occurs in 10 percent of patients, and prednisolone treatment may help reduce the incidence. The mortality resulting from this side effect depends on whether supportive treatment is given, but it is often greater than 50 percent. Resistance to melarsoprol by *T. b. gambiense* and *T. b. rhodesiense* has been noted.

Reactive arsenical-induced encephalopathy is an important toxicity of melarsoprol and is followed by pulmonary edema and death within 48 hours in more than half the cases. The incidence of reactive arsenical-induced encephalopathy has been estimated at 2 to 10 percent. In one clinical study, the co-administration of steroids significantly reduced the incidence of reactive arsenical-induced encephalopathy–related deaths during therapy with melarsoprol. Although the mechanism of action of melarsoprol has been studied extensively, it still remains unclear.[1]

Eflornithine

Eflornithine (DL-a-difluoromethylornithine; DFMO) is an enzyme-activated inhibitor of ornithine decarboxylase, the lead enzyme of polyamine biosynthesis. DFMO was developed as an antitumor agent. After initial testing in model infections, DFMO was studied extensively in human trials in Africa, with some trials in Europe and the United States. The standard treatment regimens resulting from trials indicate that DFMO is more than 95 percent active when given intravenously at 400 mg/kg/day in four doses every 6 hours for 14 days. In these studies, DFMO cured children and adults, patients with melarsoprol-refractory strains, and patients with late-stage disease.[25] The short plasma half-life of DFMO necessitates constant dosing when given as an intravenous drip. The most frequent toxic reaction was reversible bone marrow suppression, which was reversed by reducing the dosage.

The major drawbacks of DFMO are its cost and the duration of treatment and availability. DFMO is not trypanocidal and depends on a functional immune system to rid the host of nondividing forms. It is curative for laboratory infections of *T. b. brucei* and *T. b. gambiense*, but not all strains of *T. b. rhodesiense*. The reason for this selectivity is not evident, although it is not caused by uptake of DFMO because the drug enters by passive diffusion, not transport. DFMO treatment leads to intracellular concentrations of approximately 5 mmol/L, an increase of approximately 50-fold over untreated parasites. The basis for DFMO selectivity lies in the rapid turnover of the mammalian enzyme as opposed to trypanosome ornithine decarboxylase. Because mammalian ornithine decarboxylase is synthesized constantly at a high rate, DFMO must be continuously available, and this availability is made more difficult by rapid excretion.

DFMO has been used for more than 10 years for melarsoprol-resistant *T. b. gambiense* infection with or without CNS involvement; treatment is started intravenously and is followed by oral therapy. In one study, 47 patients with a relapse after a first treatment were treated with a 7-day course of intravenous eflornithine (100 mg/kg every 6 hours) and monitored for 2 years; the failure rate was 6.5 percent. This approach seems to provide adequate treatment in cases of Gambian trypanosomiasis relapsing after treatment with another drug. Disadvantages of eflornithine therapy include the prolonged course of treatment and frequent side effects of diarrhea and anemia. With adequate treatment, non-CNS disease has a cure rate greater than 80 percent.

Newer Therapeutic Agents

As previously noted, available treatment regimens are limited in number, are associated with serious adverse effects, and are potentially compromised by the emergence of drug-resistant parasites. These considerations underscore the urgent need to develop inexpensive, safe, and efficacious new drugs.[37] It is hoped that efforts led by the Tropical Disease Research/World Health Organization Committee on Genomics and Discovery Research and the Drugs for Neglected Diseases Initiative will encourage further pharmaceutical efforts.[12]

PROPHYLAXIS AND PREVENTION

Chemoprophylaxis with suramin, 0.3 to 0.7 g intravenously every 2 to 3 months, is reported to be highly effective. With few exceptions, the vector has proved to be difficult to control. Preventing tsetse fly bites and chemoprophylaxis may be the best means of eliminating the disease from an area. Development of a vaccine has been hindered by antigenic variation.

At present, there are no endemic areas where *T. b. gambiense* and *T. b. rhodesiense* coexist. Although foci of both exist in Uganda, they apparently are geographically separate. Based on evidence of the spread of cattle carrying *T. b. rhodesiense* further north, a merger of the two foci could create tremendous problems because the diagnosis and treatment of the two diseases differ significantly.

Vector control measures have met with limited success. Methods have included clearing streams of underbrush, eliminating breeding grounds, using insecticides, using fly traps, and releasing sterile male flies. Because tsetse flies have a slow reproductive rate and have not developed insecticide resistance, *Glossina* spp. are tempting control targets. The most effective control measures include an integrated approach to reduce the human reservoir of infection and to use insecticides and fly traps.

In regions where the disease is endemic, natives seem to be more resistant to infection than new arrivals to the area, although there is no evidence of acquired immunity. West African sleeping sickness affects primarily rural populations, and tourists are rarely infected. Chemoprophylaxis is not recommended because of drug toxicity, and vaccines are unavailable. Despite antigenic variation, it may be possible to develop a vaccine because immunity to reinfection occurs with *T. b. gambiense*.

Tourists are usually not at great risk unless they are spending long periods in rural areas of western and central Africa.[54] Individuals visiting areas where the disease is endemic should avoid tsetse fly bites by wearing protective clothing (long-sleeved shirts and long pants); khaki or olive-colored clothing is optimal because the tsetse fly is attracted to bright colors and very dark colors. Because heavy clothing is not always practical owing to heat and humidity, other measures, including the use of insect repellents, bed netting, and screens, are recommended. It also has been recommended not to ride in the back of jeeps, pickup trucks, or other open vehicles; the tsetse fly is attracted to the dust created by moving vehicles and wild animals. Bushes should be avoided because the tsetse fly is less active during the hot period of the day and rests in bushes, but bites if disturbed.

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CHAPTER 234

NAEGLERIA, ACANTHAMOEBA, AND *BALAMUTHIA*

Patrick J. Gavin ✪ Tina Q. Tan

Interest in free-living amebae has increased since demonstration of their pathogenicity and, more recently, their role as reservoirs and vectors for potentially pathogenic bacteria, such as *Legionella pneumophila* and *Mycobacterium avium*. Small free-living amebae of the genera *Naegleria*, *Acanthamoeba*, and *Balamuthia* cause severe central nervous system (CNS) infections in humans and animals that are characterized by few distinguishing symptoms and an almost uniformly poor prognosis. Although these severe infections are uncommon, for optimal treatment, it is important to recognize and diagnose them early.

Although small free-living amebae share structural and pathogenic similarities, the infections they cause have distinctive epidemiology, clinical presentation and course, neuroimaging, immunology, and pathology. Primary amebic meningoencephalitis caused by *Naegleria fowleri* is an acute fulminant illness that affects healthy children and young adults, and usually results in death within 1 week of presentation. Almost invariably, the patient has a recent history of swimming in bodies of warm fresh water. In contrast, granulomatous amebic encephalitis caused by *Acanthamoeba* spp. or *Balamuthia mandrillaris* (formerly leptomyxid amebae) is an insidious, subacute, and protracted illness that affects primarily, but not exclusively, immunocompromised or debilitated patients and leads to death in weeks to months. Typically, the patient has no history of recent exposure to fresh water.

Despite these differences, the prognosis of CNS infection caused by *N. fowleri*, *Acanthamoeba* spp., and *B. mandrillaris* is similarly dismal, with few reports of survival. In the handful of cases that survived, recognition, early diagnosis, and timely administration of appropriate antimicrobial treatment were crucial to achieving a successful outcome. *Acanthamoeba* spp. also are associated with disseminated cutaneous, pulmonary, or sinus infection in immunocompromised hosts, and with chronic painful and potentially sight-threatening keratitis in association with use of contact lenses or corneal trauma in otherwise healthy patients.

The pathogenic potential of small free-living amebae was recognized first during polio vaccine trials in the 1950s, when they were found to cause cytotoxicity in contaminated cell cultures.[22,53] Subsequently, the fulminant fatal encephalitis that followed intracerebral injection or intranasal instillation of tissue culture fluid in experimental mice and monkeys showed pathogenicity and a potential route for human infection.[19,22,23] The first cases of human infection caused by small free-living amebae were recog-

nized in four children from southern Australia by Fowler and Carter[36] in 1965, and soon afterward by Butt[12] in Florida.

More recently, Visvesvara and colleagues[126,129] isolated and named *B. mandrillaris*, a new agent of amebic meningoencephalitis, from the brain of a mandrill baboon. The first reported human case of *B. mandrillaris* encephalitis was in a patient with acquired immunodeficiency syndrome (AIDS).[3] Reports of infection in immunocompetent hosts have followed.[6,25,38,59,94] Subsequently, infections caused by free-living amebae have been reported worldwide.[4,60,69,72,79,96] Finally, the first case report of nonfatal CNS disease, in an otherwise healthy adult, caused by *Sappinia diploidea*, a free-living ameba normally found in soil contaminated with elk and buffalo dung, adds to the list of pathogenic small free-living amebae, and suggests that others may be identified in the future.[41]

EPIDEMIOLOGY

Free-living amebae are found worldwide and are cosmopolitan in their distribution, living primarily in soil, but also in water, inside vertebrates, on plants, and in the air.[93] They are spread readily by wind and water currents. In humans, infection with free-living amebae causes primary amebic meningoencephalitis, granulomatous amebic meningoencephalitis, disseminated granulomatous disease (cutaneous, pulmonary, or sinus), and keratitis.[69,72,106]

Although they are rare, cases of infection caused by *N. fowleri* have been reported throughout the world.[35] As of 2004, more than 200 cases of primary amebic encephalitis were documented worldwide, with approximately half occurring in the United States.[20,106] In the United States between 1989 and 2000, 24 fatal cases of primary amebic meningoencephalitis were reported to the Centers for Disease Control and Prevention (CDC).[82] Most of them occurred in the summer and in young children. Increased water temperatures, whether from thermal pollution or sunlight, provide the ideal environment for proliferation of *N. fowleri*.[122,130] Cases typically are associated with a history of swimming, diving, or playing in bodies of warm fresh water, brackish habitats with a soil and water interface, artificial lakes, hot springs, and thermally polluted rivers and streams.[20,26,35,70,82,122] Researchers have postulated that thermal pollution, particularly from overflow of power plants, or inadequate chlorination of water, kills off normal thermosensitive and chlorine-sensitive fauna, shifting the balance in favor of thermotolerant, less chlorine-sensitive, pathogenic *N. fowleri*.[106]

In North America, cases of primary amebic meningoencephalitis tend to be clustered in the warmer southern states and Mexico.[106] In one study, almost 50 percent of fresh-water lakes in Florida contained *N. fowleri* when the temperature was 30° C or higher.[130] *N. fowleri* may be found in colder areas, however. In temperate regions, distribution of *N. fowleri* is limited by seasons, being greatest during summer and early fall.[122] *N. fowleri, Acanthamoeba* spp., and *B. mandrillaris* were present year-round in lakes in the Tulsa region of Oklahoma.[55] Although it is most prevalent in areas where water temperatures are higher, *N. fowleri* has the ability to overwinter, in cyst form, in lake bottom sediment.[130]

Small outbreaks of primary amebic meningoencephalitis have been described. Over a 3-year period, from 1962 to 1965, 16 swimmers died after being exposed to warm contaminated and inadequately chlorinated water in an indoor pool in Czechoslovakia.[16,106] Similarly, 8 cases of fulminant meningoencephalitis associated with an artificial lake in Richmond, Virginia, were recognized retrospectively as being caused by *N. fowleri*.[14] Finally, 5 cases of primary amebic meningoencephalitis caused by *N. fowleri* occurred in 1990 in patients swimming in a drainage canal in Mexicali, Mexico.[65]

Disease also has been documented in patients with no history of exposure to fresh water. Atypical exposures have included bathing in domestic bath water, playing in a warm muddy puddle, and being baptized by full body immersion.[4,8,74] *N. fowleri* also has been shown in domestic tap water.[29,74] Although cases of infection have not followed ingestion of drinking water contaminated by amebae, fatal infection was associated with washing in tap water heated to high temperatures in pipes by the summer sun in South Australia, and with exposure to contaminated domestic water in Arizona.[2,29,74]

Infections occur far more frequently in previously healthy children and young adults than in other age groups, presumably because youngsters are most likely to remain in water for prolonged periods and to disturb sediment containing amebae by diving and swimming underwater.[20,82] In one series of primary amebic meningoencephalitis cases, boys outnumbered girls 12 to 1, presumably because boys are more likely than girls to engage in aquatic horseplay.[128] The incubation period in natural human infection is 2 to 15 days after exposure.[8,20,82] Death usually ensues within 1 week of presentation (generally within 72 hours).

Acanthamoeba spp. are among the most prevalent protozoa found in the environment, being found in soils from tropical to arctic regions. They are important components of the food chain and promote plant growth by predation on soil bacteria. *Acanthamoeba* spp. are prevalent in natural and artificial habitats that include fresh, brackish, potable, or stagnant water; in sewage; in improperly treated swimming pools; and in taps, sinks, hot tubs, air-conditioning units, aquaria, flowerpots, showers, ventilators, and ventilation in homes and hospitals.[17,33,72,104,106] Although warm waters might enhance their numbers, pathogenic amebae may be isolated year-round from water samples in the United States.[130] Their distribution and viability are governed primarily by availability of a bacterial food source; soil texture; water temperature, pH, and salinity; and ultraviolet light and desiccation.[93,106] Growth of *Acanthamoeba* spp. is inhibited by temperatures of 35° C to 39° C. Although *Acanthamoeba* spp. seemingly are ubiquitous in water, *Acanthamoeba* infections generally are not associated with a history of recent swimming or water activities.[106] Acquisition occurs by inhalation or direct contact with contaminated soil or water.

In contrast to *N. fowleri*, *Acanthamoeba* spp. are opportunistic pathogens. Granulomatous amebic encephalitis and disseminated disease caused by *Acanthamoeba* spp. generally are seen in immunocompromised hosts or in patients with underlying disease or debilitation, such as that due to malignancy, cancer chemotherapy, steroid treatment, organ transplantation, human immunodeficiency virus, diabetes, or malnutrition.[69,72] *Acanthamoeba* spp. cause keratitis in otherwise healthy individuals by directly attacking the corneal surface, usually in association with poor contact lens care or corneal trauma. Although cases of CNS or disseminated *Acanthamoeba* infection are extremely rare, in 2004, estimates of cases of amebic keratitis worldwide exceeded 3000.[106]

Comparatively less is known about *B. mandrillaris*. It is closely related to *Acanthamoeba* and causes a similar spectrum of infections that includes granulomatous amebic encephalitis and cutaneous and sinus disease.[106] Although initial cases of *B. mandrillaris* infection were confined to immunocompromised and debilitated hosts, its ability to cause disease in apparently healthy children is well documented.[6,25,39,59,94] By 2004, more than 100 cases of human infection had been reported.[106] *Balamuthia* is assumed to inhabit soil and water; it has been isolated more recently in soil from a flowerpot associated with a case of amebic encephalitis in a child from northern California.[100]

As with *Acanthamoeba* spp., granulomatous encephalitis caused by *B. mandrillaris* seems to occur after inhalation of airborne cysts or soil contamination of breaks in the skin. The patient generally has no history of recent exposure to fresh water. To date, 50 percent of cases of *Balamuthia* infection reported in the United

States have occurred in patients of Hispanic ethnicity.[6,102] Although this statistic most likely is a result of environmental exposure, a genetic predisposition may exist. Among California cases of balamuthiasis, most cluster in the southern part of the state. Researchers have postulated that the area's dry warm soil is conducive for the amebae, and that large-scale agriculture in these areas increases the likelihood of workers being exposed to soil or wind-blown particles. Cases of *B. mandrillaris* infection linked directly to incidental exposure to soil or to blowing soil while traveling in an open car or by motorcycle are reported.[25,59,100,104] Although *Balamuthia* encephalitis is a zoonosis, animals do not represent a source of infection for humans.

Despite their seeming ubiquity in the environment and ample opportunity for contact with humans compared with other parasitic protozoa, such as *Entamoebae*, malaria and trypanosome infections caused by free-living amebae are extremely rare. Given the enormous number of exposures to water containing *Naegleria*, why so few individuals become infected remains unknown. Only seven cases of primary amebic meningoencephalitis were reported in Florida during a 14-year period, despite estimates of billions of potential exposures in contaminated fresh-water lakes during that time period.[130] Epidemiologic investigations of cases typically find that many other individuals swam in the same water at the same time, but did not become ill. Factors other than the presence of amebae, such as host susceptibility, anatomy, inoculum size, and activity at the time of exposure, have been implicated.[130] Nonetheless, in recent years, an increase in the number of diagnoses of primary amebic meningoencephalitis cases has been noted.[67,135] The increase probably reflects a combination of greater recognition of the pathogenic potential of free-living amebae, improved diagnostic techniques, and a growth in the numbers of immunocompromised hosts.

Acanthamoeba organisms can infect the cornea directly, after trauma, or, most commonly, in association with the combination of extended-wear soft contact lens use and subsequent exposure to contaminated water.[108,117] Major risk factors for development of amebic keratitis include poor compliance with care of daily wear soft contact lenses, particularly use of homemade saline solutions using nonsterile tap water that may be contaminated with amebae; use of chlorine release lens disinfection systems; and inadequate cleansing of lens cases, which encourages growth of a bacterial biofilm that acts as a nidus and a food source for amebae.[90,116] Wearing contact lenses while swimming or in hot tubs, which potentially may be contaminated with amebae, is similarly risky.[95]

Since the first cases of amebic keratitis were described in the early 1970s, the incidence has increased dramatically. Between 1973 and 1984, slightly more than 200 cases of amebic keratitis were reported to the CDC. By 2002, in the United States, greater than 3000 cases had been reported, and 1 in 250,000 people were estimated to be affected.[57,64,84,106] The increased incidence most likely is due to increased use of contact lenses, particularly of the soft, extended-wear variety. Although many of these lenses were marketed as products requiring "low care," many patients interpreted this designation as meaning "no care."[90]

ORGANISMS

In contrast to the related true parasite protozoa *Entamoeba* spp., *Naegleria*, *Acanthamoeba*, and *Balamuthia* spp. are amphizoic protozoa, capable of existing as free-living amebae and as parasitic pathogens.[106] *Naegleria* spp. are ameboflagellates found primarily in moist soil and warm fresh water.[70] Although more than 27 separate *Naegleria* spp. have been identified, to date only *N. fowleri* is associated with human infections.[26,106] *Naegleria* spp. can exist in three forms: an active feeding trophozoite, a rarely seen dormant cyst (Fig. 234–1), and a flagellate form (Fig. 234–2).[70]

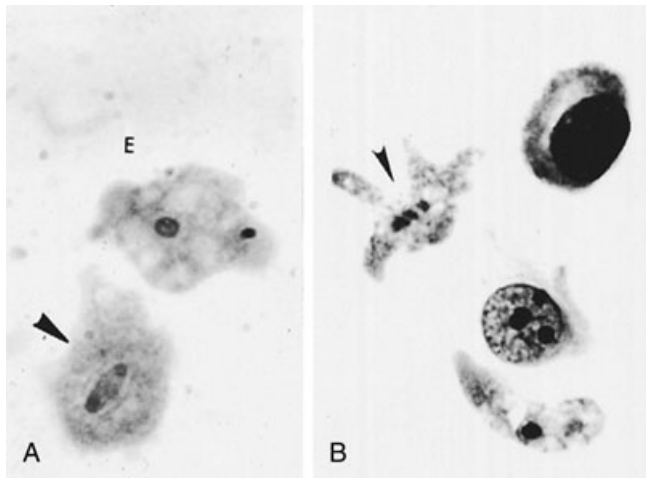

Figure 234–1 **A** and **B,** Trophozoites of *Naegleria*.

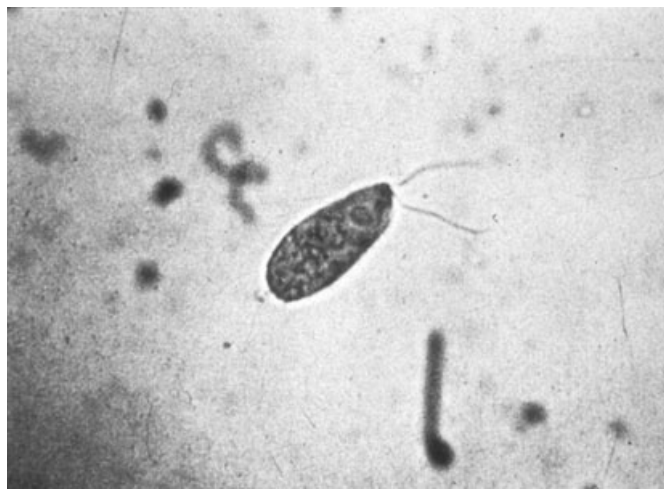

Figure 234–2 Flagellate form of *Naegleria fowleri*.

Of these forms, only the trophozoite is found in tissue or cerebrospinal fluid (CSF). The trophozoite of *N. fowleri* is smaller than that of *Acanthamoeba* spp., measuring 8 to 15 μm in diameter, with a conspicuous nucleus containing a large, dense central karyosome without peripheral chromatin lining the membrane.[78] *Naegleria* trophozoites move slowly by extension of broad, rounded anterior pseudopodia (lobopodia). This feature may be noted on a warm saline preparation or in CSF specimens. *Naegleria* cysts are spherical, measure 7 to 12 μm in diameter, and have a single-layered outer membrane.

Approximately 20 *Acanthamoeba* spp. are identified by morphologic criteria.[106] Because morphologic distinctions do not always correlate with results of molecular typing, however, classification is complex and is currently under review.[72] Molecular classification is ongoing. The life cycle of *Acanthamoeba* spp. consists of two stages: an actively feeding and dividing trophozoite and a dormant cyst stage (Fig. 234–3).[86] The trophozoite is 15 to 40 μm. The nucleus is vesicular; has a large, dense central "targetoid" nucleolus; and is surrounded by vacuolated cytoplasm. Locomotion typically is sluggish, by extrusion of multiple fine spiny pseudopodia (acanthopodia).[78] *Acanthamoeba* trophozoites feed on bacteria, yeasts, and algae. *Acanthamoeba* cysts are smaller, 15 to 20 μm, with a distinct, wrinkled, thick double wall. Encystation occurs in response to adverse environmental condi-

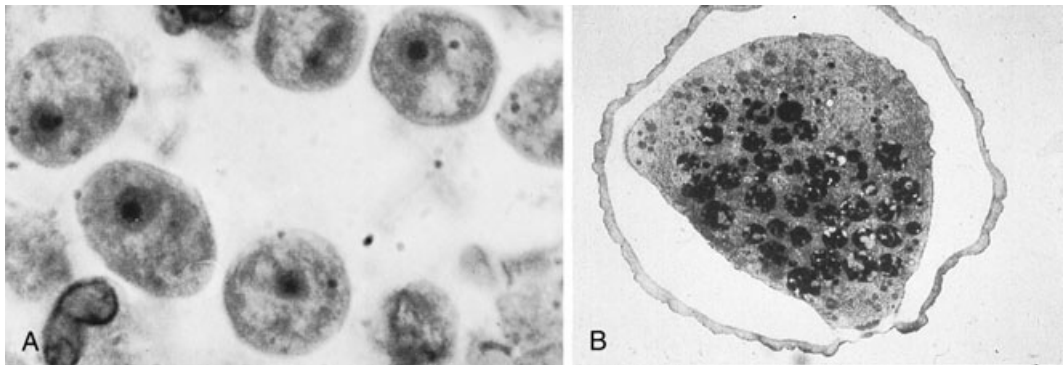

Figure 234–3 **A,** Trophozoite of *Acanthamoeba*. **B,** Cyst of *Acanthamoeba* (scanning electron micrograph). *(A from Jager, B. V., and Stamm, W. P.: Brain abscesses caused by free-living amoeba probably of the genus Hartmannella living in a patient with Hodgkin disease. Lancet 2:1343-1345, 1972. Copyright © by the Lancet Ltd.)*

tions, such as desiccation, scarcity of food, and changes in pH or temperature. *Acanthamoeba* cysts are particularly hardy; they are intrinsically resistant to chlorination and sterilization of potable water, to biocides used for disinfecting bronchoscopes and contact lenses, and to antibiotics; they may remain viable for decades after freezing.[27,62,80] *Acanthamoeba* is the only pathogenic free-living species isolated from marine water. *Naegleria* spp. do not tolerate seawater.

Few *Acanthamoeba* spp. have been associated with human infections. Among the potentially pathogenic species, *Acanthamoeba culbertsoni*, *Acanthamoeba castellanii*, *Acanthamoeba astronyxis*, and *Acanthamoeba polyphaga* are prominent.[79] On the basis of 18S rRNA gene sequences *Acanthamoeba* spp. are divided into 15 genotypes (T1 through T15). At present, no clear consensus has been reached regarding which genotypes are pathogenic. Genotypes T1, T4, T7, T10, and T12 are found among cases of granulomatous encephalitis, whereas isolates associated with keratitis fall into genotypes T3, T4, and T11.[10,34,72,119] Genotype T4 makes up 94 percent and 80 percent of keratitis and nonkeratitis *Acanthamoeba* isolates,[10] presumably reflecting the preponderance of this genotype in the environment. Rare genotypes, such as T1, T10, and T12, are found only in CNS disease and are not found in the environment.

B. mandrillaris is the only pathogenic *Balamuthia* spp. identified to date.[106] It exists as either a vegetative trophozoite or a dormant cyst (Fig. 234–4). Trophozoites are similar in appearance to *Acanthamoeba* spp., but larger (50 to 60 μm in diameter), and the spherical cyst stage (10 to 30 μm in diameter) has a triple-layered wall on electron microscopy. *Balamuthia* trophozoites typically have a single vesicular nucleus, often with multiple large, dense nucleoli. Movement occurs by extension of broad pseudopodia. *Balamuthia* has been isolated more recently from environmental soil samples.[31] Because of its slow growth, it may have been obscured in environmental cultures by overgrowth of more rapidly growing bacteria, fungi, or other amebae. In contrast to *Naegleria* and *Acanthamoeba* spp., *Balamuthia* feeds on smaller amebae and protozoa and does not prey on bacteria.

S. diploidea is a species of free-living amebae that is found worldwide. The life cycle of this organism involves animal feces. The trophozoites are 40 to 70 μm in diameter and can be distinguished by the presence of a large cytoplasmic vacuole, mitochondria with characteristic tubular crystal patterns, a juxtanuclear Golgi-like network, and a double nucleus.[41]

CLINICAL MANIFESTATIONS

NAEGLERIA FOWLERI

Primary amebic meningoencephalitis caused by *N. fowleri* is an acute, rapidly progressive illness that almost uniformly is fatal. Patients present with abrupt onset of severe headache, fever, nausea and vomiting, malaise, rhinitis, meningitis, and changes in mental status 2 to 15 days after exposure, most frequently after swimming or bathing in warm contaminated fresh water.[35,69,82,110] A history of disturbances of taste and smell has been noted in many cases. Progression from fever to signs of meningitis and encephalitis is unrelenting and rapid. Seizures are common. Coma is present at or develops soon after hospital admission. In

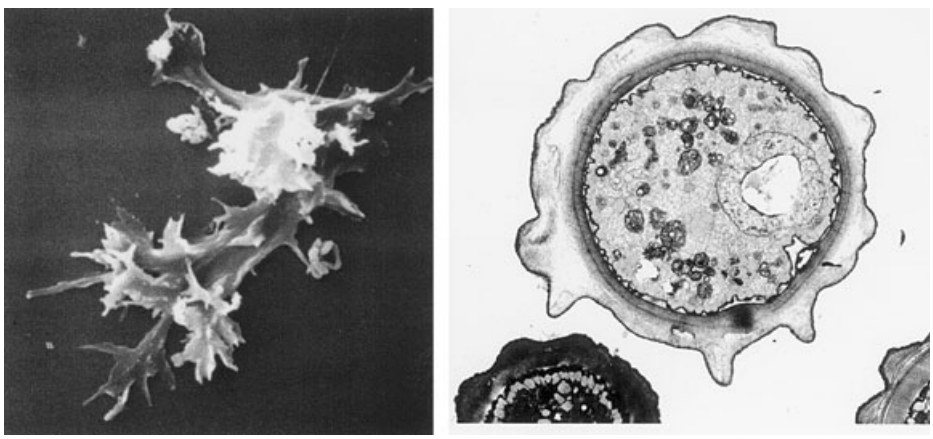

Figure 234–4 Trophozoite and cyst of *Balamuthia mandrillaris*. *(Courtesy of Dr. G. S. Visvesvara, Centers for Disease Control and Prevention.)*

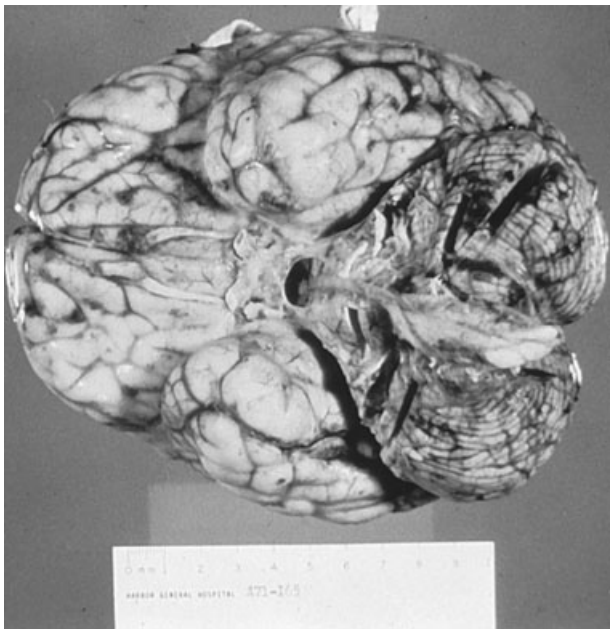

Figure 234–5 Brain from a fatal case of *Naegleria* meningoencephalitis. Note the areas of necrosis on the basilar surface of the brain.

the absence of early diagnosis and appropriate treatment, the illness characteristically progresses rapidly to death within 1 week of onset of symptoms (median 72 hours).[8]

Other than the clue of a recent history of exposure to warm water, there is little to distinguish primary amebic meningoencephalitis from fulminant bacterial meningitis. A high peripheral polymorphonuclear leukocytosis may be present. CSF findings are similarly nonspecific, with increased pressure, elevated protein, a normal to low glucose, and modest polymorphonuclear pleocytosis, but no bacteria, mycobacteria, or fungi are identified. Patients with a suggestive history and CSF indices suggestive of purulent meningitis, but without bacteria on Gram stain should have a wet mount examined for the presence of motile trophozoites (Fig. 234–5).

ACANTHAMOEBA SPECIES

Acanthamoeba spp. and *B. mandrillaris* cause granulomatous amebic encephalitis. The course typically is prolonged, with progression from focal neurologic signs to diffuse meningoencephalitis over weeks to months after exposure.[72,106] Patients most susceptible to contracting this disease include very young, old, debilitated, immunosuppressed, and chronically ill patients. The clinical picture resembles that of a bacterial brain abscess or brain tumor, with insidious headache, intermittent low-grade fever, nausea, vomiting, and symptoms of increased intracranial pressure. Altered mental status is particularly prominent. As the infection progresses, depending on the area of the brain affected, focal neurologic signs, hemiparesis, drowsiness, aphasia, behavioral and personality changes, and seizures develop. The incubation period after exposure to onset of CNS disease is unknown, but is of the order of several weeks to months.

Acanthamoeba skin lesions often have been present for months before the onset of CNS disease. Routine CSF findings frequently are abnormal but nonspecific, with elevated protein, low glucose, and, typically, a mononuclear pleocytosis. *Acanthamoeba* spp. also may cause cutaneous, pulmonary, bone, and nasopharyngeal or sinus disease.[42,68,76] Cutaneous *Acanthamoeba* infection is characterized by pustules and hard erythematous nodules, which develop into nonhealing indurated draining ulcers or subcutaneous abscesses.[37,72] Disseminated disease without CNS involvement has a slightly better prognosis. In patients with AIDS, mortality rates of 70 percent and 100 percent are reported for *Acanthamoeba* skin disease alone and in association with CNS disease.[58]

Acanthamoeba Keratitis

In contrast to its role as an opportunistic pathogen in CNS and skin disease, most cases of *Acanthamoeba* keratitis occur in otherwise healthy individuals who wear contact lenses or who have a history of corneal trauma. Contact lenses of any type, including daily wear and disposable soft and extended-wear lenses, have been implicated in infection. All soft contact lenses contain 50 to 75 percent water. These lenses can absorb pathogens from contaminated cleaning solutions, carrying cases, and hands. When the lens comes into contact with the contaminated fluid, the amebae quickly adhere to the lens surface. If corneal trauma is present, the organisms invade the corneal tissue and produce infection.[56] The nidus for infection most likely is trauma to the cornea, but also may be related to preexisting herpesvirus or bacterial conjunctivitis (Fig. 234–6).

Amebic keratitis characteristically is painful, progressive, and sight-threatening, and fails to respond to conventional antibacterial or antiviral treatment.[5,72] Typically, only one eye is involved. Early symptoms include severe eye pain, lacrimation, foreign body sensation, and photophobia. *Acanthamoeba* keratitis frequently is misdiagnosed, initially, as herpetic, bacterial, or fungal infection. The course usually is more indolent, however. Periods of temporary remission delay establishing the diagnosis further. Recurrent corneal epithelial breakdown with dendritic infiltrates progresses to nonsuppurative keratitis, which waxes and wanes over several months and ends in a characteristic ring-shaped stromal abscess with secondary uveitis leading to loss of the cornea.[5,124] Infection that follows severe trauma deteriorates more rapidly than that which follows contact lens wear. In addition, complications of infection include iritis, cataracts, hypopyon, glaucoma, scleritis, and penetrating keratitis.[5]

BALAMUTHIA MANDRILLARIS

B. mandrillaris causes granulomatous encephalitis and cutaneous, sinus, and nasopharyngeal disease.[106] The incubation period of CNS disease is unknown, but is thought to be weeks to months (not <10 days).[79] In animal models, extensive CNS necrosis develops 1 to 3 weeks after inoculation.[77] Onset of symptoms of granulomatous encephalitis is insidious, with low-grade fever, severe frontal headache, nausea and vomiting, meningismus, personality change, and prominent focal neurologic signs that have included cranial nerve palsies, hemiparesis, dysarthria/aphasia, focal seizures, and ataxia.[6,47] A history of recent otitis was noted by many authors.[6,47] A slow relentless deterioration to coma and death ensues over several weeks to months (as long as 2 years afterward). Death usually results from cardiorespiratory failure secondary to severe cerebral edema. In many reports, manifestation of cutaneous lesions (facial, nasal, and limb) has preceded development of CNS disease.[25,52,88,91,131] Although normal CSF parameters have been described in *B. mandrillaris* CNS infection, moderately increased protein (may be >1 g/dL), normal or mildly decreased glucose, and a pleocytosis (about 500 cells/mm^3), which commonly but not invariably has a mononuclear predominance, are the typical findings.[6,39,47,77,106]

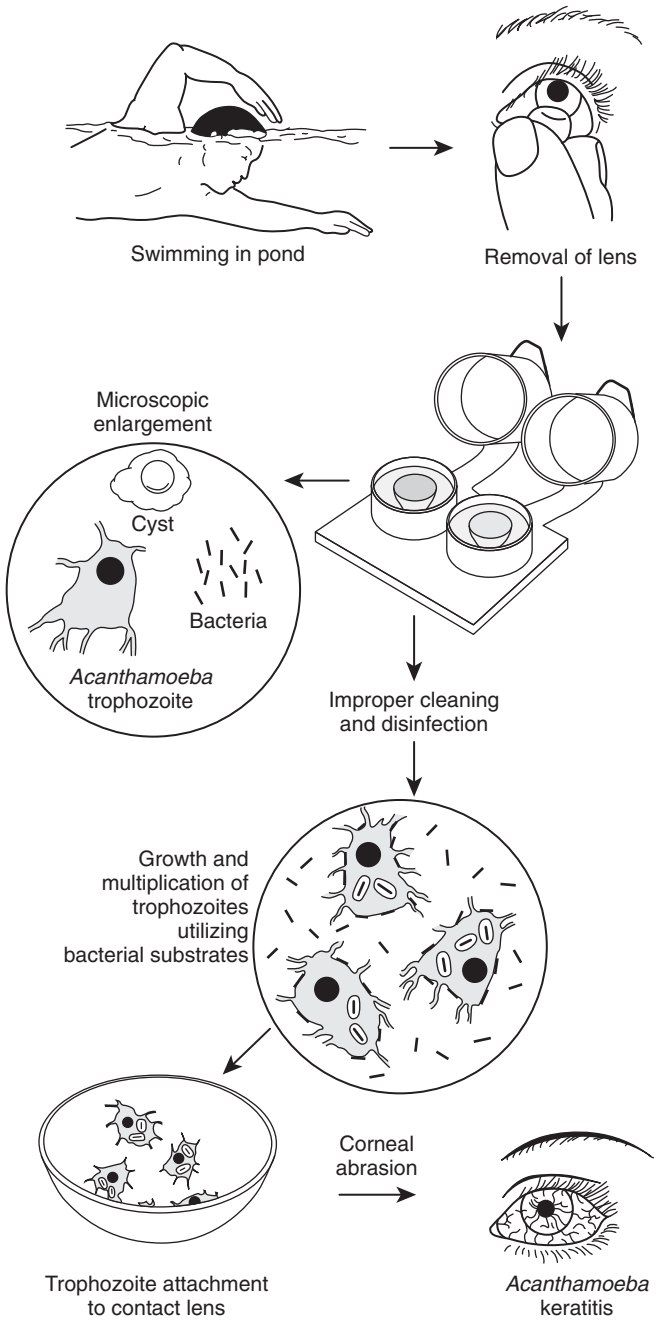

Figure 234–6 Proposed mechanism for *Acanthamoeba* keratitis. Bacterial contaminants in the water on the lens support the growth of amoebae, which leads to an increased number of invading organisms. The organisms adhere to the contact lens and enter tissue through the damaged cornea, with the subsequent development of keratitis.

NEUROIMAGING

Neuroimaging findings in infections caused by free-living amebae are nonspecific, and alone do not provide clues to an earlier diagnosis. In addition, in the early stages of infection, computed tomography (CT) and magnetic resonance imaging (MRI) may be normal. In primary amebic meningoencephalitis, cranial CT and MRI show obliteration of basal cisterns and signs of diffuse brain edema, focal and basilar meningeal enhancement, and infarction.[63,97,111] In granulomatous encephalitis, neuroimaging reveals variable but nondiagnostic abnormalities that include, alone or in combination, discrete corticomedullary lesions; large, solitary, masslike lesions (which may be confused with brain abscess or tumor); large arterial occlusions; and spinal cord infarctions.[63,97,111] Similarly, in *Balamuthia* infection, neuroimaging is helpful, but nondiagnostic; CT scans show a focal enhancing mass or ring-enhancing cystic lesions or both, vascular occlusion, and diffuse edema that frequently progresses to communicating hydrocephalus.[6,25,47]

PATHOGENESIS

Naegleria trophozoites or cysts enter the nasal cavity by aspiration of water or inhalation of soil. After active phagocytosis by sustentacular neuroepithelial cells of the olfactory nerve occurs, trophozoites migrate along the nerve, pass through the cribriform plate, and gain entry to the CNS.[54,79] From the olfactory bulb, which is bathed in CSF and lies in the highly vascularized subarachnoid space, *N. fowleri* disseminate throughout the CSF and CNS. Only *Naegleria* trophozoites are found in the CNS lesions. In contrast, most *Acanthamoeba* spp. infections seem to follow hematogenous dissemination from a primary lower respiratory or cutaneous focus.[79] Researchers have postulated that *Acanthamoeba* spp. are inhaled or inoculated through skin in the cyst phase. Less commonly, *Acanthamoeba* is capable of causing CNS infection directly via the olfactory neuroepithelium.

Virulence factors described for free-living amebae include the ability to adhere to brain microvascular epithelium, production of extracellular protease and elastases, and phagocytosis by foodcups or amebostomes.[1,33,48,61,70,72] Among *Naegleria* spp., pathogenicity is closely related to thermotolerance.[26] *N. fowleri*, the only species pathogenic for humans, is unusually thermophilic and can tolerate temperatures of 45° C.[106] In addition, *N. fowleri* has less demanding growth requirements than nonpathogenic *Naegleria* spp. Trophozoites of pathogenic *Acanthamoeba* spp. also are thermophilic, being able to tolerate temperatures of 37° C, relative to their nonpathogenic counterparts.[98,106] Thermotolerance seems to be less important for *Acanthamoeba* strains that cause keratitis, presumably because corneal temperatures are lower (32° C to 35° C).[106]

PATHOLOGY

Pathologic changes of infection with small free-living amebae are most marked in the CNS. The pathology of primary and granulomatous amebic encephalitis differs fundamentally.[79] Macroscopically, *N. fowleri* produces diffuse meningoencephalitis, most severely affecting the cortical gray matter. It is characterized by marked cerebral edema and purulent leptomeninges, particularly adjacent to the olfactory bulbs, at the base of the frontal and temporal lobes and at the hypothalamus.[78] Frequently, the olfactory bulbs are necrotic and hemorrhagic. Microscopically, an acute mononuclear and polymorphonuclear inflammatory infiltrate is seen throughout the cerebrum, brainstem and cerebellum, and upper spinal cord. Trophozoites tend to concentrate in the perivascular spaces and adventitia of arteries, and they may contain ingested erythrocytes and brain tissue. Characteristically, only trophozoites are seen in the CSF or brain tissue.[79] Presumably, *N. fowleri* cysts are not found in clinical specimens because progression of *Naegleria* infection is so rapid and fatal that the patient dies before transformation from trophozoite into the cyst can occur.[69]

CNS infections caused by *Acanthamoeba* spp. and *B. mandrillaris* are characterized macroscopically by diffuse cerebral edema and hemorrhage, with areas of softening and abscess formation. The meninges are spared except for areas overlying involved cerebrum. Additional areas of hemorrhagic necrosis are concen-

trated in the posterior fossa, brainstem and cerebellum, midbrain, cerebral cortex, and basal ganglia.[6,25,28,69] Narrowing of the sulci and flattening of the gyri are noted in areas of active infection. Microscopic changes consist of multinucleated giant cells, with trophozoites and cysts seen within the lesions.[78] The olfactory bulbs and spinal cord are relatively spared.

Microscopically, necrotizing hemorrhagic encephalitis and subacute or chronic necrotizing granulomatous infiltrates and multinucleate giant cells are scattered throughout the CNS, particularly in the cerebral hemispheres, basal ganglia, midbrain, and brainstem. Typically, trophozoites and cysts of *Acanthamoeba* spp. or *B. mandrillaris* are present within necrotic cerebral tissue, particularly in perivascular spaces. Invasion of blood vessels causes severe necrotizing arteritis and hemorrhagic necrosis. The angiotropic distribution of *Acanthamoeba* or *Balamuthia* infection supports a hematogenous mechanism of spread. In severely immunocompromised patients, the inflammatory reaction may be negligible and lacks a granulomatous component.[9,77] In the absence of a vigorous granulomatous response, hemorrhagic necrosis that resembles primary amebic meningoencephalitis may occur.[132]

Skin lesions of acanthamebiasis and balamuthiasis are characterized by nodules, ulcers, and abscesses, and the presence of amebic trophozoites, cysts, and granulomas in tissue.[76,79,131] Amebic keratitis is characterized by moderate chronic inflammation of corneal stroma, a mixed polymorphonuclear and lymphocytic infiltrate, and presence of amebic trophozoites and cysts within the corneal ulcerations.[128]

DIAGNOSIS

Previously, most cases of amebic meningoencephalitis were diagnosed with certainty only at autopsy or by brain biopsy.[9] Routine laboratory tests did not distinguish amebic meningoencephalitis from other causes of meningoencephalitis. The first and most important diagnostic step is to recognize the possibility that small free-living amebae may cause infection in the appropriate clinical setting. Among the few reported therapeutic successes, all are notable for early diagnosis and timely institution of appropriate antimicrobial therapy.[25,87]

Primary amebic meningoencephalitis should be included in the differential diagnosis of any child or young adult with meningoencephalitis. A history of recent exposure to fresh water should be sought, and a wet mount should be performed on centrifuged CSF. *Naegleria* is isolated most readily from CSF, but may be isolated from brain tissue, particularly olfactory lobes. In contrast, *Acanthamoeba* trophozoites characteristically are absent from CSF, and *B. mandrillaris* has yet to be seen in CSF. *Acanthamoeba* spp. are more likely to be isolated from brain tissue or skin biopsy, or from corneal scrapings in cases of keratitis.[98] Diagnostic techniques include direct microscopy of wet-mount and stained CSF specimens, histologic stains of tissue, fluorescent and immunofluorescent antibody stains of CSF and tissue, direct immunohistochemical stains of tissue, serology, molecular diagnostics, and culture.[127]

DIRECT MICROSCOPIC IDENTIFICATION

If amebic infection of the CNS is suspected, a wet-mount preparation of fresh CSF should be examined for the presence of motile amebae.[69,78,79,127] This method remains the most rapid means of diagnosis. Ideally, the CSF specimen should be kept at room temperature and examined soon after it has been collected. *Naegleria* is particularly fragile. Although the specimen may be kept at 4° C for short periods, it never should be frozen.[127] If present, amebae tend to attach to the test tube wall or surface of the container, so gentle shaking and low centrifugation at 150 *g* for 5 minutes are recommended to dislodge and concentrate the amebae.[78,127] The supernatant should be placed gently on a slide. The wet mount is examined best by light microscope under low power (10× and 40× objectives), phase-contrast microscopy, or darkfield illumination, and may be warmed to promote amebic motility. *Naegleria* trophozoites move in a characteristic "slug-like" fashion by extrusion of a few broad pseudopodia (lobopodia) anteriorly.[106] Leftover specimens can be frozen at –20° C for later antigen or antibody testing. Although *N. fowleri* may be shown by histologic stains of fixed CSF smears, Gram stain is not useful because it does not stain amebae, and the heat fixation causes lysis.

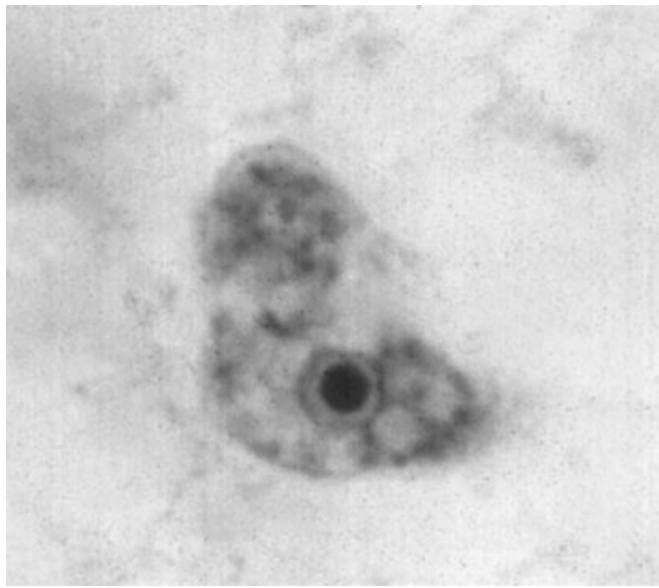

Figure 234–7 Trichrome stain of a trophozoite of *Naegleria fowleri*.

Naegleria, *Acanthamoeba* spp., and *Balamuthia* may be shown in fixed sections of brain tissue biopsy or autopsy specimens by hematoxylin and eosin, Masson trichrome, periodic acid–Schiff, Wright-Giemsa stain, and Gomori–methenamine silver stains (Fig. 234–7).[69,72,78,79] Reliably differentiating *Acanthamoeba* and *Balamuthia* by histology alone is difficult. In addition, *Acanthamoeba* trophozoites have been variously mistaken for macrophages or "atypical mononuclear" or epithelial cells on routine histology.[78,132]

Immunofluorescent stains using monoclonal and polyclonal anti-*Acanthamoeba* and anti–*B. mandrillaris* antibodies (available at the CDC), immunoperoxidase staining, and electron microscopy of tissue sections have been used to confirm the diagnosis (Fig. 234–8).[25,59,72,127,129] Amebic keratitis may be diagnosed by direct detection of trophozoites or cysts in smears of deep corneal scraping or biopsy using Wright-Giemsa, trichrome, periodic acid–Schiff, calcofluor white, or indirect fluorescent antibody stains.[69,78,133]

CULTURE

Culture has a limited role in diagnosis, but is useful for confirmation, speciation, and antimicrobial susceptibility testing. *N. fowleri* and *Acanthamoeba* spp. can be cultivated using bacteria as a food source (xenic cultures) or without bacteria, in an enriched cell-free nutrient (axenic) medium that contains antimicrobials to inhibit growth of contaminating bacteria.[21,98,109,127] *N. fowleri* and *Acanthamoeba* spp. are readily cultured from CSF, brain, and lung

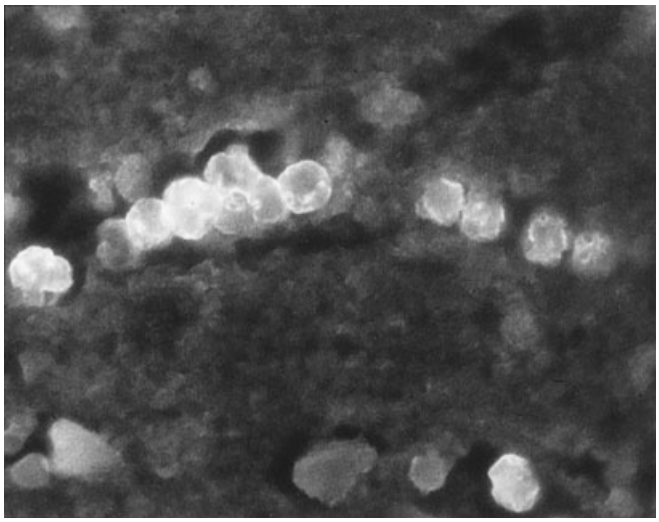

Figure 234–8 Indirect fluorescent antibody examination of *Naegleria fowleri*. *(Courtesy of Dr. G. S. Visvesvara, Centers for Disease Control and Prevention.)*

tissue on various non-nutrient agars or with low concentrations of nutrients, in the presence of a lawn of living or killed bacteria (e.g., most commonly nonmucoid strains of *Escherichia coli* or *Enterobacter* spp.).[78,98,127] Amebae feed on the lawn of bacteria and grow to cover the plate after 1 to 2 days' incubation at 37° C. Culture plates are incubated at 37° C and examined under low power daily for 10 days.[78,127]

Amebae can be visualized directly through the back of the inverted agar plate with a conventional microscope under low power.[98] The characteristic slow, sluglike movement of *Naegleria* trophozoites that is readily seen in a wet mount of CSF is difficult to distinguish on an agar plate.[98] The ability of *Naegleria* to transform into the flagellated stage after suspension in distilled water is useful diagnostically.[98,127] Trophozoites of *Acanthamoeba* spp. move slowly by extrusion of multiple, finger-like pseudopodia (acanthopodia).

Culture and isolation of *Balamuthia* is more challenging. Generally, *B. mandrillaris* is more fastidious, does not use bacteria as a food source, grows slowly, and requires a heavily enriched basic medium. *N. fowleri*, *Acanthamoeba* spp., and *B. mandrillaris* all grow vigorously, however, and produce detectable cytopathic effects in mammalian cell cultures (monkey kidney, HeLa, and lung fibroblast tissue), which can be examined for cytopathic effect.[78,129] Typically, *Balamuthia* grows slowly and may require several weeks to proliferate.[129] Use of a positive control culture is advised to ensure correct methodology and to aid recognition.[98]

Although no outbreaks of infection caused by free-living amebae in laboratory workers have been reported, care must be taken when handling specimens to avoid getting culture materials on skin and in cuts or abrasions. Specimens should be handled in a biologic safety cabinet by technologists wearing masks and gloves.[78,127]

SEROLOGY

Although specific *N. fowleri* antibody has been shown in survivors of primary encephalitis, serologic tests are of no use diagnostically.[110] Onset of primary amebic meningoencephalitis is rapid, and almost all patients die before significant antibody production can occur. In contrast, because the clinical courses of *Acanthamoeba* spp. and *B. mandrillaris* infections usually are chronic, there is time for robust antibody responses to develop. Detection of serum or CSF antibody against *Acanthamoeba* spp. or *Balamuthia* by indirect immunofluorescence staining has helped establish the diagnosis in many cases.[25,59,106] By this method, amebae fixed on a slide bind antibody present in the patient's serum or CSF, which then is detected by fluorescein-conjugated, antihuman antibody.

Serologic testing has been most useful as an epidemiologic tool. Although the incidence of infection with free-living amebae seems low, results of seroepidemiology studies suggest that human exposure to amebae occurs frequently. Sera from healthy adults and children contain antibody against *N. fowleri*, *Acanthamoeba* spp., and *B. mandrillaris.*[24,50,73,101,104] Detection of antibodies to *N. fowleri* and *Acanthamoeba* spp. in asymptomatic adult and pediatric populations from North Carolina, Virginia, and Pennsylvania in the United States and from Czechoslovakia and New Zealand presumably reflects unavoidable environmental contact with these ubiquitous organisms.[15,73] All specimens from a group of 93 asymptomatic patients from New Zealand contained anti-*Naegleria* and anti-*Acanthamoeba* IgG and IgM antibodies.[24] Antibodies were neutralizing against *Acanthamoeba* spp., but not against *N. fowleri*. In virtually all cases of infection caused by *Acanthamoeba* spp., disease occurs in the setting of immunocompromise or in an immunologically privileged site, such as the cornea. Whether neutralizing antibodies play a role in the very low incidence of infections in healthy individuals, despite seemingly frequent environmental exposure, is unknown.

Immunofluorescent antibody staining of serum for antibodies against *Acanthamoeba* spp. and *B. mandrillaris* has proved to be a rapid noninvasive diagnostic test in many clinical cases.[29,59,134] Potentially, this test may be useful clinically for establishing an earlier definitive diagnosis and initiation of therapy. Because the presence of *Balamuthia* antibodies may represent asymptomatic environmental exposure, confirmation of results of immunofluorescent antibody testing by histology, indirect immunofluorescent staining of tissue, or polymerase chain reaction (PCR) of CSF is advised. More recently, immunofluorescent antibody staining of serum from patients with encephalitis, predominantly from California, revealed seven previously unrecognized or misdiagnosed cases of *Balamuthia* and one case of *Acanthamoeba* encephalitis.[104] Most serum specimens showed some evidence of antibody (<1:64), which was attributed to cross-reactivity or subclinical exposure to contaminated soil. A cutoff value of greater than 1:64 for *Balamuthia* serology was considered positive pending results of confirmatory tests (e.g., histology, immunohistochemistry, or PCR).[104] Low levels of antibody may reflect preceding subacute disease or contact with environmental amebae.[104]

MOLECULAR DIAGNOSTICS

Although microscopic assessment of a wet-mount preparation or histology may be useful in experienced hands, definitive identification in most centers awaits results of specimens sent out for immunofluorescence tests. Advances in molecular diagnostics offer the real possibility, however, of sensitive, specific, and rapid diagnosis being made by any laboratory with the capability to perform PCR. PCR of DNA extracted from frozen brain tissue has been used to confirm *N. fowleri* infection diagnosed by histopathology and immunofluorescent staining.[20] Similarly, nested and real-time PCR provide rapid, sensitive, and specific screening of environmental and recreational water sources for the presence of *N. fowleri.*[74,92] PCR, using mitochondrial 16S rRNA gene DNA as the target, has been used to detect *B. mandrillaris* in clinical samples of CSF and formalin-fixed brain and lung tissue of patients with encephalitis.[118,134] These assays have the ability to detect a single ameba per reaction mixture.[134]

Finally, the development of a multiplex real-time PCR assay for simultaneous detection of *N. fowleri*, *Acanthamoeba* spp., and *B. mandrillaris* in clinical specimens of CSF and brain tissue offers the possibility of highly sensitive and specific rapid confirmatory diagnosis of CNS infection by free-living ameba and the chance of initiating earlier, more effective treatment.[89] The assay is sensitive down to detection of a single ameba in a specimen and is species-specific for *N. fowleri* and *B. mandrillaris*, and genus-specific for *Acanthamoeba*.[89]

TREATMENT

Mortality among patients with primary and granulomatous amebic encephalitis is greater than 95 percent.[8,107] The high fatality rate undoubtedly is due partly to late diagnosis or misdiagnosis and late initiation of potentially effective treatment. Inclusion of amebic infection in the differential diagnosis, in the appropriate clinical setting, and timely administration of effective antiamebic treatment are emphasized in successful cases.[11,87] In the future, availability of real-time PCR may facilitate accurately establishing an early diagnosis. Although no randomized trials of optimal treatment for infection caused by free-living amebae have been performed, potentially effective treatment is available if the condition is recognized early.

If infection is suspected, treatment should not be withheld pending the results of confirmatory testing. Treatment regimens are based on results of in vitro susceptibility, in vivo animal experiments, and empiric treatment of individual cases. If amebic infection is suspected, accurate determination of the species is important to ensure optimal treatment. No one drug is effective against all free-living amebae (e.g., amphotericin is the drug of choice for *N. fowleri* infection, but is neither static nor cidal for *Acanthamoeba* spp., and varies in its efficacy against *Balamuthia*).[30,105] Previously, authors have suggested initiating treatment with a combination of agents such as amphotericin B and flucytosine, which have activity against *Naegleria* and *Acanthamoeba* or *Balamuthia*, until amebae can be identified definitively.[132] Among newer antibiotics, miltefosine and voriconazole are potentially attractive as treatments for infection caused by free-living amebae. Both drugs penetrate into brain tissue and have a low toxicity profile. Although differences in susceptibility exist among strains, voriconazole has activity in vitro against *Acanthamoeba* spp. and *N. fowleri*, whereas miltefosine has in vitro activity against *Acanthamoeba* spp. and *Balamuthia*.[103]

NAEGLERIA MENINGOENCEPHALITIS

N. fowleri is exquisitely susceptible in vitro to amphotericin B, which remains the treatment of choice for primary amebic meningoencephalitis.[107] All successfully treated cases have included amphotericin B in the treatment regimen. *Naegleria* also is susceptible in vitro to azithromycin and miconazole, with synergism in vitro and in the animal model with the combination of amphotericin and miconazole or tetracycline.[30,43,110,114,125] Treatment regimens in cases with successful outcomes have included intravenous amphotericin, high-dose intravenous and intrathecal amphotericin and miconazole, oral rifampin, intravenous sulfisoxazole, and intravenous and intrathecal amphotericin in combination with oral rifampin.[4,11,110] Rifampin has yielded conflicting results in in vitro testing.[44] Liposomal amphotericin B seems less effective in vitro and in the animal model than conventional amphotericin (10-fold higher minimum inhibitory concentration), and is not recommended.[44] In contrast to *Acanthamoeba* spp. and *B. mandrillaris*, *N. fowleri* remains a trophozoite and does not encyst in tissues, so after it is destroyed by antimicrobial therapy, infection with *N. fowleri* should not recur.

Optimal treatment for CNS disease caused by *Acanthamoeba* spp. has not been established, and the prognosis remains grim, with few reports of survivors.[107] Most cases are diagnosed at autopsy or a few days before death, leaving little time to evaluate treatment. Treatment successes have been attributed to initiation of treatment before spread of infection into the CNS. Combination treatment is favored because no single drug is active against trophozoites and cysts, and many drugs are amebostatic rather than amebocidal.[72] Among the few reports of successful treatment, oral trimethoprim-sulfamethoxazole, ketoconazole, and rifampin were associated with a favorable outcome in two apparently immunocompetent children with CNS disease.[112] Fluconazole, rifampin, metronidazole, and sulfadiazine were used successfully in a 64-year-old immunocompetent host with early disease.[87]

Similarly, Slater and colleagues[113] reported successful treatment of disseminated cutaneous acanthamebiasis in a renal transplant recipient using 4 weeks of intravenous pentamidine, topical chlorhexidine, and 2 percent ketoconazole followed by maintenance oral itraconazole. A combination of pentamidine, 5-fluorocytosine, itraconazole, and topical chlorhexidine gluconate/ketoconazole cream was used to treat disseminated acanthamebiasis successfully in a lung transplant recipient.[85] Pentamidine is considered the most suitable treatment before CNS invasion has occurred because of its low penetration into the CNS. Because of its low penetration across the blood-brain barrier and nephrotoxicity, 5-fluorocytosine was preferred to pentamidine for CNS infection and in renal transplant recipients.[30,72]

Optimal treatment for *B. mandrillaris* infection also remains to be determined. Reports of successful treatment for granulomatous encephalitis caused by *Balamuthia* based on empiric regimens are few. If it is recognized early in its course, however, a more favorable outcome apparently is possible. In two successful cases, treatment regimens included flucytosine, pentamidine, fluconazole, sulfadiazine, a macrolide, and a phenothiazine.[25] Pentamidine treatment frequently was discontinued or interrupted because of side effects. Both patients recovered and showed no evidence of relapse 5 and 7 years later.[25] Treatment failures have been reported in patients treated with similar regimens, however, possibly because of late initiation of treatment.[39] Pentamidine and azithromycin were more effective in vitro than fluconazole, flucytosine, or sulfasalazine.[107] Some researchers have postulated, however, that some of these drugs may be synergistic in vivo.[107] Because of concern about reactivation from dormant cysts in the brain, both patients remained on long-term fluconazole and on sulfadiazine or clarithromycin prophylaxis.

Use of corticosteroids to treat cerebral edema and inflammation seems to exacerbate *Acanthamoeba* infection. A precipitous decline in the clinical condition of one patient has been reported after administration of corticosteroid therapy.[39] At present, the risk of corticosteroid treatment probably outweighs any potential benefit and is best avoided.[39,72,94]

AMEBIC KERATITIS

Untreated, amebic keratitis invariably progresses to visual loss and enucleation. Although amebic keratitis is more amenable to antimicrobial treatment than systemic infection, treating it is still difficult. The presence of *Acanthamoeba* cysts in deeper corneal layers renders exposure to adequate drug levels problematic. In addition, *Acanthamoeba* cysts are resistant to most antibacterial agents at concentrations that are achievable but nontoxic to the cornea. As with systemic infection, establishing

the diagnosis early is required for treatment to be most effective.

In the early stages of infection, prolonged frequent application of drug is recommended. Combination treatment is preferred during prolonged treatment because of concerns for development of resistance. Nonetheless, cures have been reported with a variety of topical agents; the cationic antiseptic, chlorhexidine gluconate, and 0.02 percent polyhexamethylene biguanide, a swimming pool disinfectant, alone or in combination with propamidine are among the drugs of choice for amebic keratitis.[32,66,107,108] Both drugs are effective against trophozoites and dormant cysts and are well tolerated in the eye.[66] Neomycin and oral azoles (e.g., miconazole or itraconazole) also have proved useful. Newer agents, such as myristamidopropyl dimethylamine and miltefosine, are effective *Acanthamoeba* cysticidal agents, possess excellent antifungal and antibacterial activity, and show promise in treating recalcitrant keratitis.[51,99,107]

Many cases of keratitis, particularly when chronic or where medical treatment alone fails, require keratoplasty and corneal grafting. In these cases, prolonged medical treatment aims to cure or control disease to allow for successful transplantation. Timing of surgery is controversial. Antiamebic drugs usually are continued for months after surgery to prevent late excystation of residual dormant cysts.[5] Use of topical or systemic corticosteroid to treat severe pain or inflammation is controversial. Severe complications have been described in clinical cases, and dexamethasone induces encystment and increases cytopathogenicity of emerging trophozoites in animal models.[81,119]

ROLE OF *ACANTHAMOEBA* SPECIES AS RESERVOIRS OF INTRACELLULAR PATHOGENS

Although most bacteria are prey for free-living amebae, some bacteria have evolved to survive uptake by amebae. Many *Acanthamoeba* spp. act as environmental reservoirs of recognized intracellular pathogens for humans, including *Coxiella burnetii*, *Legionella pneumophila*, *Mycobacterium* spp., *Pseudomonas aeruginosa*, and *Cryptococcus neoformans*, and for potential emerging pathogens, such as *Rickettsia*-like organisms and *Parachlamydia* spp.[45,46,49,75,121] Some ameba-resistant microorganisms coexist with amebae as endobacterial symbionts, whereas others exploit the amebae as hosts for survival, for multiplication, or as vectors of disease.[7] Free-living amebae act as a "Trojan horse" in the transmission of bacterial passengers, such as for *L. pneumophila* or *M. avium*, or as an "evolutionary crib" for the selection of virulence traits and for adaptation of microorganisms to life within human macrophages, such as for *C. neoformans*.[7,46]

For *L. pneumophila*, prior multiplication within free-living amebae, principally *Acanthamoeba* spp., seems to be a prerequisite for acquisition of infection in humans.[120] In addition, many bacteria, such as *M. avium*, survive protected from chlorination, biocides, antibiotics, and desiccation within ameba cysts.[71,118] Similarly, growth of *M. avium* in amebae results in enhanced survival in macrophages. Researchers also have postulated that free-living amebae may have adapted some microorganisms for successful intracellular survival within macrophages.[40,115] Free-living amebae also apparently promote virulence traits and antimicrobial resistance in *L. pneumophila*, *M. avium*, and *C. neoformans*.[18,19,83] Researchers have postulated that capsule formation evolved in *C. neoformans* in response to selective pressure to resist environmental amebae. Production and maintenance of a capsule allows *Cryptococcus* to survive in human macrophages.[115] Some microorganisms resistant to amebae seem to behave as endosymbionts and to influence development and maintenance of virulence traits that enhance pathogenicity of free-living amebae.[38,46] Clinical and environmental *Naegleria* spp. do not support bacterial endosymbionts.

PREVENTION

Infections caused by free-living amebae are reported to be characterized by an absence of distinguishing symptoms, delayed diagnosis or misdiagnosis, and an almost uniformly poor prognosis without early appropriate treatment. Prevention of infection is of paramount importance. Strategies to prevent *Naegleria* infection exist; *N. fowleri* is susceptible to 1 μg/mL or less of chlorine, and adequate chlorination of water in swimming pools or hot tubs is a simple and sensible preventive measure. If water temperatures are higher, use of increased concentrations of chlorine (>2 to 3 mg/L) is recommended.[79] In certain parts of the world, environmental levels of *N. fowleri* are monitored to ensure it is safe to swim, and that the probability of acquiring an infection is low.[13] A prudent measure is to educate the public on the potential dangers associated with exposure to warm bodies of fresh or thermally polluted water, especially when temperatures are high and water levels are low, and to avoid immersion, diving, jumping, or horseplay that might force water up the nasal passages or disturb sediment.[135]

Prevention of infection caused by *Acanthamoeba* spp. or *B. mandrillaris* is problematic and involves regular inspections of hot-water tanks, plumbing, and eye-wash stations. *Acanthamoeba* keratitis associated with use of contact lenses is preventable, however. Contact lens wearers should be aware of the importance of proper care of the lenses. Lenses should be cleansed in sterile benzalkonium-preserved saline or disinfected by heat. Use of homemade cleansing solutions and wearing lenses while swimming are contraindicated.

Although the incidence of infections caused by free-living amebae is small at present, the number of these infections is likely to grow with increasing numbers of transplant patients and patients with AIDS. Similarly, some researchers have predicted that climate change associated with global warming may increase further numbers of free-living amebae in the environment.[20]

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CHAPTER 235

TOXOPLASMOSIS

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Toxoplasma gondii is an obligate intracellular protozoan parasite (phylum Apicomplexa, class Sporozoasida, order Eucoccidiida).[181] Infection may be clinically inapparent or result in disease, called *toxoplasmosis*. This parasite was observed first in 1908 by Nicolle and Manceaux[136,137] in mononuclear cells in the spleen and liver of a North African rodent, the gundi (*Ctenodactylus gundi*). The organism soon was identified as a cause of disease in other animals,[177] and in 1923, Janku[85] first recognized a case in a human. He described a parasite found in the retina of an infant; it was recognized later by Levaditi[102] as *Toxoplasma*.

In 1937, Wolf and Cowen[189] reported a case of congenital granulomatous encephalitis that they considered to be caused by an "encephalitozoon." Sabin,[155] who previously had encountered *T. gondii* in guinea pigs, made the correct diagnosis. The discovery of *Toxoplasma* as a cause of disease acquired later in life has been credited to Pinkerton and Weinman,[141] who in 1940 described a generalized fatal illness caused by this organism in a young man. In retrospect, a case of acquired toxoplasmosis had been reported in 1908 by Darling.[41] In 1948, Sabin and Feldman[156] described a serologic test, the dye test, which allowed numerous investigators to study the epidemiologic and clinical aspects of toxoplasmosis and define the spectrum of disease in humans. In 1969, some 60 years after the parasite was discovered, Frenkel and colleagues[54,60,61] established that *Toxoplasma* was a coccidian protozoan, and that its definitive host was the cat.

ORGANISM AND TRANSMISSION

T. gondii exists in three forms, or stages—the proliferative stage, or tachyzoite; a tissue cyst that contains bradyzoites; and an oocyst, within which sporozoites develop. The oocyst is formed during the intestinal epithelial stage of infection, exclusively in members of the cat family, the definitive host. The tachyzoite and tissue cyst are found in the extraintestinal tissues of cats and in other mammalian and avian hosts. Each stage of the organism has antigens in common with the other stages and unique antigens. Many of these antigens have been cloned, sequenced, and localized to microanatomic structures.[123]

The tachyzoite form (Fig. 235–1A to C) is crescent-shaped or oval, is approximately 3 to 7 μm, and is seen during the acute stage of infection. It stains well with Wright or Giemsa stain. Ultrastructural features include the apical complex of microtubules and rings, secretory organelles called *rhoptries*, and a chloroplast-like structure with its own unique DNA.[27,181] Tachyzoites can invade all mammalian cells except perhaps non-nucleated red blood cells. They cannot withstand freezing and thawing, desiccation, or brief exposure to gastric or duodenal digestive juices. After penetration occurs, the tachyzoite multiplies by endodyogeny, ultimately causing disruption of the cell and cell death.

The bradyzoite (see Fig. 235–1D to F) is able to persist in encysted form in all tissues and cause a chronic (latent) infection for the entire life span of the infected host. Cysts are demonstrable in tissues the first week of infection and range in size from approximately 10 to 100 μm. They have an argyrophilic wall, but stand out most clearly from surrounding tissue when stained with periodic acid–Schiff stain. Usually, no inflammatory reaction occurs around cysts. Because this form may persist for many years in the tissues of clinically normal children and adults, its demonstration in histologic sections does not signify recent infection. Peptic or tryptic digestive fluids immediately disrupt the cyst wall, but the liberated bradyzoites (which resemble the tachyzoite form under light microscopy) can survive in these fluids for several hours, which allows time for invasion of local cells. The cyst is destroyed by heating to 66° C, by freezing (<–20° C) and thawing, and by desiccation. It can survive for some months at refrigeration temperatures (4° C) if it is in tissue. Infection in humans may be acquired by eating inadequately cooked meat that contains cysts (Fig. 235–2). In carnivorous animals, infection may be acquired by eating raw meat or prey species that contain encysted organisms or by ingesting sporulated oocysts as discussed subsequently.

The oocyst form (see Fig. 235–1G to I) is found only in feces of members of the cat family, the definitive host for *Toxoplasma*, and is the result of gametogony and schizogony, which occur in the intestinal epithelium.[83] The oocyst is ovoid and approximately 10 to 12 μm. Infected cats may shed 10 million oocysts each day, which may be excreted for 3 weeks after primary (acute) infection develops, but rarely thereafter. Excreted oocysts become infectious only after they undergo sporulation (eight sporozoites form in each oocyst); sporulation occurs 1 to 21 days (most commonly 2 to 8 days) after excretion occurs, depending on temperature and the availability of oxygen. The oocyst is far more resilient than the other life cycle forms and can survive for months in water and for 1 year or more in moist soil. Ingestion of sporulated oocysts transmits the infection. This fact suggests that the oocyst plays a major role in transmission by the fecal-oral route in animal reservoirs and by inadvertent ingestion in humans.

The genome of *T. gondii* consists of 8×10^7 base pairs distributed among 12 chromosomes. Much of the genome has been sequenced, a genetic linkage map has been constructed, and three clonal genotypes (I, II, and III) have been identified.[95,163,181] Some of the factors that regulate stage conversion from tachyzoite to bradyzoite have been defined.[123,181] The shikimate pathway, an enzyme system that is absent in animals, is important for the production of many essential aromatic compounds in plants, bacteria, and fungi. This biochemical pathway has been identified in *Toxoplasma* and in other apicomplexan parasites, such as *Plasmodium falciparum* and *Cryptosporidium parvum*.[148] Biochemical inhibition of this pathway suppresses the growth of these parasites.[145] Understanding of the regulation of life cycle stages and the unique pathways of intermediary metabolism in *T. gondii* may provide new approaches to therapy.

EPIDEMIOLOGY

ACQUIRED INFECTION

The cat is central in the parasite's life cycle, and humans and other mammals are intermediate hosts. If infected tissue (e.g., a mouse) is consumed by a susceptible cat, the sexual cycle is induced in the cat intestine; oocysts are excreted and are infectious for mammals and birds, in which the life cycle (tachyzoites and cysts) is perpetuated. Cats shed oocysts for only brief durations (days to weeks), but in extremely large numbers (1×10^7/day). A cat is more likely to become infected if it is an outdoor cat or a predator or is fed fresh, uncooked table scraps. Humans

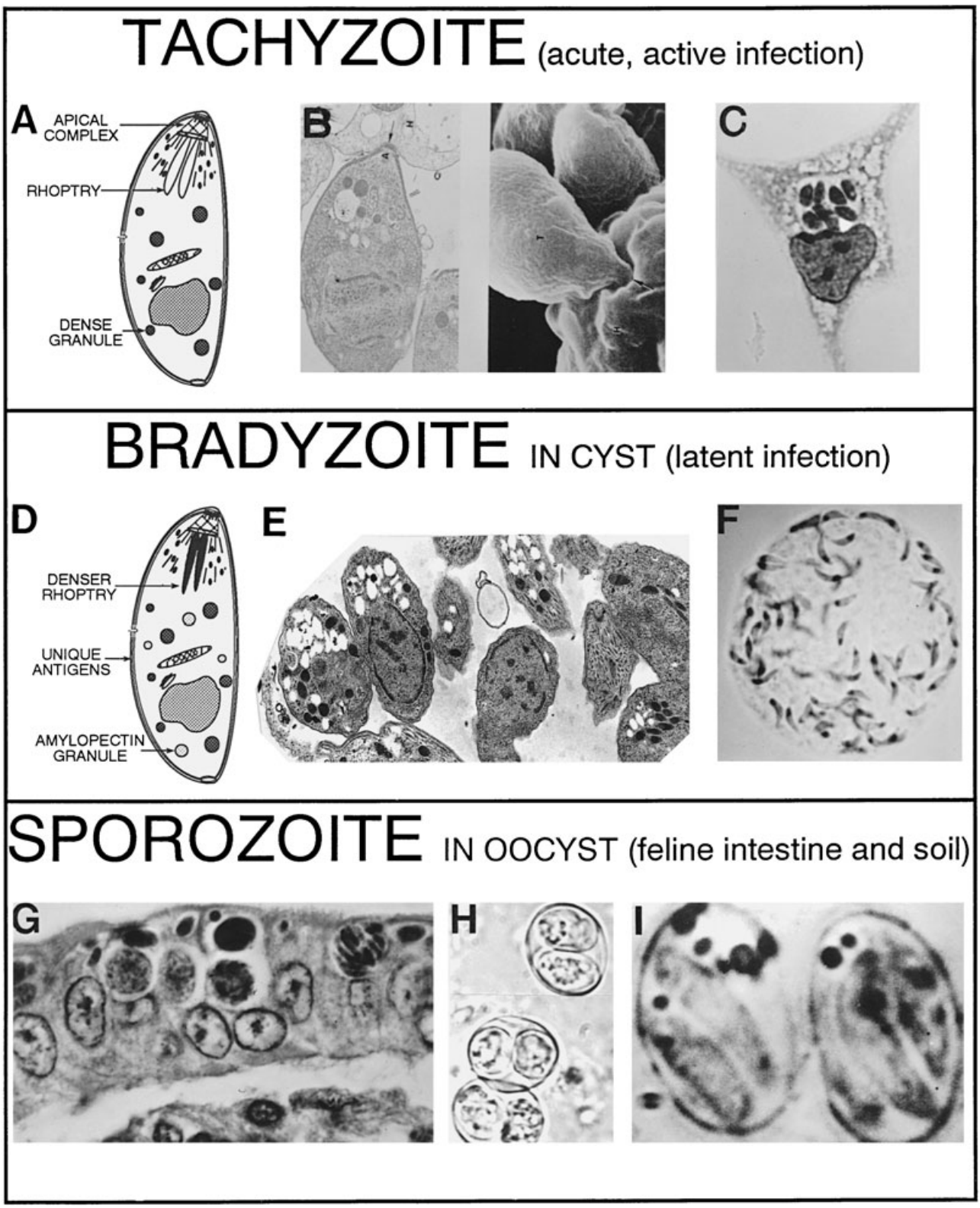

Figure 235–1 Stages of *Toxoplasma gondii*. **A,** Schematic diagram of a tachyzoite. **B,** Transmission and scanning electron micrographs of a tachyzoite invading a host cell. **C,** Light micrograph of tachyzoites replicating within a parasitiferous vacuole in the host cell cytoplasm. **D,** Schematic diagram of a bradyzoite. **E,** Transmission electron micrograph of a cyst containing bradyzoites (*arrow* indicates amylopectin granules). **F,** Light micrograph of a cyst containing bradyzoites. **G,** Development of oocysts in cat intestine. **H,** Oocysts in the lumen of cat intestine. **I,** Sporulating oocysts that contain sporozoites. *(From Boyer, K. M., and McLeod, R. L.: Toxoplasma gondii [toxoplasmosis].* In *Long, S. S., Pickering, L. K., and Prober, C. G. [eds.]: Principles and Practice of Pediatric Infectious Diseases. New York, Churchill Livingstone, 1997, p. 1423.)*

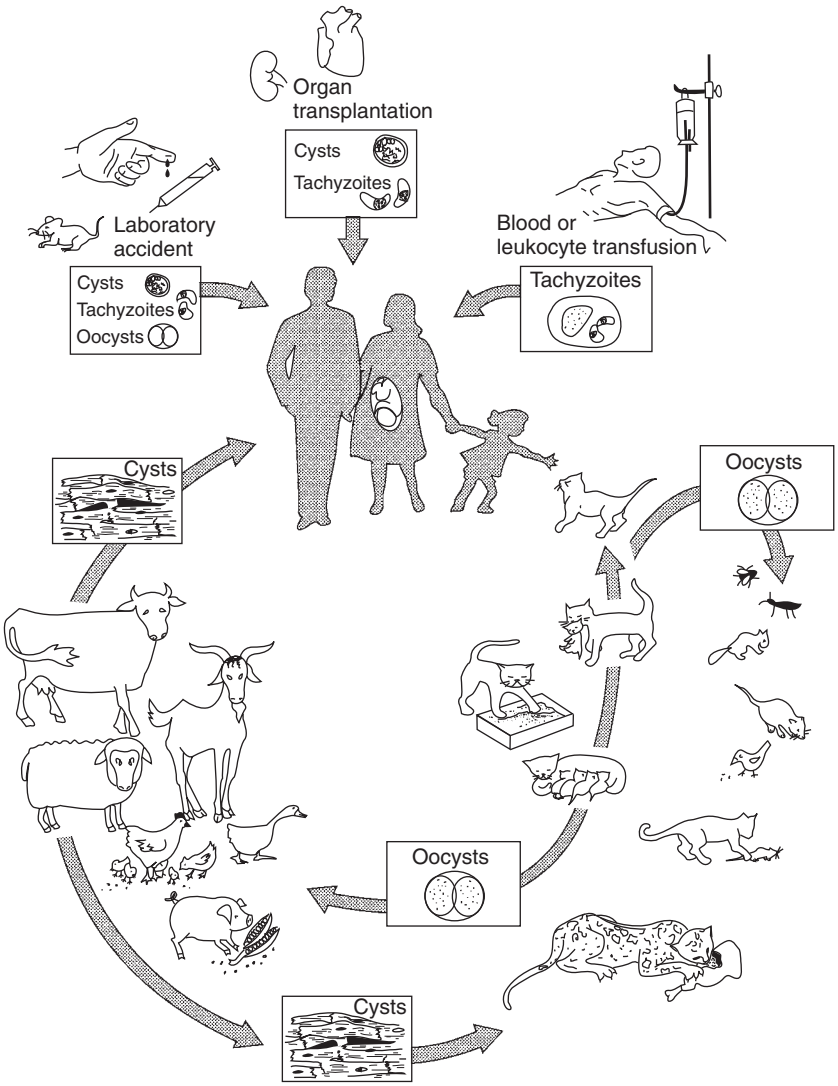

Figure 235–2 Life cycle of *Toxoplasma gondii*. Cats are definitive hosts, with humans and other mammals being intermediate hosts. *(From Remington, J. S., and McLeod, R.: Toxoplasmosis. In Braude, A. I. [ed.]: International Textbook of Medicine. Vol. II. Medical Microbiology and Infectious Disease. Philadelphia, W. B. Saunders, 1981, p. 1818.)*

come in contact with cat excrement either directly (e.g., emptying the litter pan) or by more insidious means (e.g., cleaning a horse stall, weeding the garden, playing in a sandbox). Meat for human consumption may serve as a source of infection if it is eaten raw or undercooked. Accidental ingestion may occur under circumstances that are unsuspected. Examples include an Amish farm wife preparing sausage, a couple consuming steak tartare in an expensive restaurant, and a rancher butchering a deer.[16]

Infection with *Toxoplasma* occurs commonly in humans. In the United States, the prevalence of seropositivity has been determined from studies of military recruits, surveys in major cities, and most comprehensively from the National Health and Nutrition Examination and Surveys (NHANES). The NHANES surveys are periodic cluster sample surveys representing the entire population of the United States. In the early 1960s, the overall prevalence in military recruits was 14 percent, with the lowest rates in the Mountain (3%) and Pacific (8%) states, and the highest rates in the Northeastern (20%) and East South Central (19%) states.[164] A more recent study from the late 1980s showed a similar geographic distribution, but prevalence rates were approximately a third lower throughout the country.[164] This decline has continued as documented in the NHANES surveys.

The NHANES III survey (1988-1994) showed an overall seroprevalence in individuals of child-bearing age (12 to 49 years old) of 14.1 percent compared with a rate of 9 percent for the same age cohort in the NHANES 1999-2004 survey.[87] The consistent gradual increase in seropositivity with increasing age in the NHANES surveys suggests dietary exposure as a major mode of acquisition in the United States.[88] Previous estimates were that approximately 8 percent of commercial beef, 20 percent of commercial pork, and 25 percent of commercial lamb contain encysted *Toxoplasma* bradyzoites.[144] More recent data suggest much lower levels of viable tissue cysts in commercial meat.[53] The NHANES III survey failed to show an association, however, with specific dietary habits such as meat consumption. *Toxoplasma* infection was associated with foreign birth, low educational level, living in crowded conditions, and having a soil-related occupation.[88]

During these decades of declining prevalence of *Toxoplasma* infection, the rate of cat ownership has increased in the United States.[87,88] The decline may be explained partly by improved meat safety, with decreasing prevalence of viable *Toxoplasma* in commercial meat.[53] A common practice is to freeze commercial meat before releasing it for sale, which, combined with the use of home freezers, also may be having a beneficial effect.[163] Additional modes of transmission, such as water-borne,[10,14,52] have been described. Their exact contribution to the overall prevalence of toxoplasmosis in the United States is unclear, however. During the decades of the NHANES studies, an increasing proportion

of U.S. water supplies have added filtration to decrease *Giardia* and *Cryptosporidium* transmission. This filtration also likely would eliminate *Toxoplasma* oocysts.[88]

Urban studies in U.S. women of child-bearing age have yielded variable prevalence rates: Denver, 3 percent; Palo Alto, 10 percent; Chicago, 12 percent; Boston, 14 percent; and Birmingham, 30 percent.[144] Internationally, rates also vary: Thailand, 3 percent; Japan, 6 percent; Australia, 23 percent; United Kingdom, 35 percent; Poland, 36 percent; Belgium, 53 percent; Tahiti, 77 percent; and France, 87 percent.[144] The prevalence of infection is determined partly by climate; colder regions and regions that are hot and dry or at high altitude have lower rates of human infection than warmer and moister regions. Prevalences are declining in Europe, as in the United States, owing to educational efforts and improved meat safety.

Common-source outbreaks of acute acquired *Toxoplasma* infection have been authenticated. Unique and clear-cut sources that have been documented to be highly likely[14] include exposure to aerosolized cat excrement in a riding stable[173] and consumption of unpasteurized goat milk.[157] In other circumstances, extensive investigations have failed to yield convincing answers.[110,161] Because of the possibility of common-source exposure, however, families of patients with acute acquired infection should be evaluated for subclinical infection.[107]

Accidental self-inoculation with a needle contaminated with *Toxoplasma* has resulted in many acquired infections in laboratory workers. Infections also have been ascribed to blood transfusions and organ transplantation.[59]

CONGENITAL INFECTION

Congenital infection is thought to occur, with rare exception, only after primary maternal infection with *Toxoplasma*.[46,144] The only two well-documented exceptions to this rule occurred in immunodeficient pregnant women.[127,144] In a large series that carefully studied more than 800 women who had given birth to congenitally infected children, not a single congenital infection occurred in subsequent pregnancies.[144] A general consensus is that only acute infection beginning *during* pregnancy can lead to congenital infection. Despite the existence of a few well-documented cases of biopsy-proven lymphadenopathic toxoplasmosis occurring 2 months *before* conception that resulted in congenitally infected infants,[177] this pattern of occurrence also seems to be extremely rare.[65]

The prevalence of congenital toxoplasmosis in a population is determined by the risk of a woman's experiencing primary infection while she is pregnant. This prevalence depends on three factors: (1) the age-specific incidence of primary infection during the child-bearing years, (2) the age distribution of pregnant women in the population, and (3) the fetal transmission rate in primary infection. A theoretical analysis by Frenkel[60] based on these three factors, assuming a child-bearing age group of 20 to 29 years and an overall fetal transmission rate of 40 percent, showed that maximal risk occurs when the age-specific incidence rate in a population is 3 to 5 percent per year, which corresponds to a seropositivity rate of 50 to 80 percent in women of child-bearing age. In such a population, the predicted prevalence of congenital toxoplasmosis would be 4.4 to 4.6 per 1000 pregnancies. At higher age-specific incidences, rates of congenital toxoplasmosis would be lower because nearly all pregnant women already would be infected chronically. At lower incidences, rates also would be lower, but because of less frequent infection.

Studies in the early 1970s revealed a prevalence of congenital toxoplasmosis in the United States of approximately 2 per 1000 births.[1,11,96,144] The only prospective data currently available in the United States are derived from the screening of approximately 600,000 newborns in Massachusetts and New Hampshire with an IgM enzyme-linked immunosorbent assay (ELISA) as applied to filter paper blood samples.[76] The prevalence of infection identifiable at birth in this more recent study was approximately 1 in 12,000. The limitations of the testing method (small serum volumes, a test that yields positive results in only 75% of cases) undoubtedly render this estimate a conservative one, but it supports the notion that the prevalence has declined in recent years.

PATHOLOGY

After intracellular multiplication at the site of entry occurs, tachyzoites are disseminated in blood and may invade all organs and tissues. The severity of infection probably is a function of strain virulence, host susceptibility, tissue tropism, and the immune privilege of the eyes and central nervous system (CNS).[73,109,144] Proliferation of tachyzoites results in death of the invaded cells and, eventually, small necrotic foci surrounded by intense cellular reaction. With recovery, cysts without an inflammatory response around them may persist in the brain, bone marrow, lymph nodes, liver, spleen, and lungs, and in skeletal, heart, and smooth muscle.[59-61]

In active infection of the CNS, *Toxoplasma*-filled cells are scattered throughout the gray matter, where they produce diffuse meningoencephalitis with miliary microglial nodules and foci of perivascular inflammation.[30] Large lesions may mimic cerebral tumors. Areas of basal ganglial and periventricular inflammation may calcify in the fetus.[50,139] Obstruction of the aqueduct of Sylvius or the foramen of Monro may result in hydrocephalus in congenital infection.

The enlarged lymph nodes in acute acquired toxoplasmosis show characteristic pathologic changes that may warrant making a presumptive diagnosis.[51,177] Such changes include reactive follicular hyperplasia, epithelioid histiocytes encroaching on and blurring the margins of germinal centers, and distention of subcapsular and trabecular sinuses by monocytoid cells. Tachyzoites and cysts rarely are seen.[51]

In the eye, active chorioretinitis begins in the retina with severe inflammation and necrosis and exudation into the vitreous. Single or multiple foci occur, and secondary involvement of the choroid is always present. Tachyzoites and cysts have been found in these lesions.[17,144,146,147]

In immunocompromised hosts, widespread necrotizing lesions may be seen in the heart, muscle, brain, and other organs. These lesions, particularly those involving the CNS, have been found frequently with reactivation of toxoplasmosis in patients with acquired immunodeficiency syndrome (AIDS).[105]

IMMUNOLOGY

Cell-mediated immune responses are the major immunologic mechanisms that prevent reactivation of *T. gondii* in a chronically infected normal host.[28,39] Numerous effector mechanisms contribute to protection in murine models and human infection: $CD8^+$ cytotoxic T lymphocytes, $CD4^+$ T lymphocytes, monocyte oxidative mechanisms, production of interferon-γ by natural killer cells, and activation of macrophages by interferon-γ and tumor necrosis factor-α.[67,77,89,91,162,169] Killing of the parasite within activated macrophages is associated with the intracellular production of nitric oxide. Interleukin-2 and interleukin-12 enhance host resistance to *T. gondii*; interleukin-10 impairs the ability of macrophages to kill the parasite.[66]

In murine models, evidence exists for genetic determination of host resistance to infection.[18,19,109,119] Presence of the DQ3 allele seems to be associated with toxoplasmic encephalitis in patients with AIDS and hydrocephalus in infants with congenital

toxoplasmosis.[19] Successful immunization of mice[20] and sheep[21] with *T. gondii* components and live tachyzoites of an "incomplete" strain[22] raises the possibility of developing a human vaccine.[86,120,181]

CLINICAL SYNDROMES

The disease in children may be considered in three categories: postnatally acquired, congenital, and ocular (which may be congenital or acquired). Clinically apparent infection in older children may have been acquired recently or caused by reactivation of latent congenital or postnatally acquired infection. Congenital and acquired infections usually are subclinical, but congenital infection ultimately leads to serious sequelae in most cases.

ACUTE ACQUIRED TOXOPLASMOSIS

Acquired *Toxoplasma* infection is asymptomatic in most cases and frequently goes unrecognized because only 10 to 15 percent of infected individuals have clinical symptoms and signs. In certain outbreaks related to infection by oocysts, however, more than half of infected patients have been symptomatic.[173] The most common findings are lymphadenopathy and fatigue without fever.[112] The nodes are discrete and may or may not be tender. They do not suppurate. The groups of nodes most commonly involved are the cervical, suboccipital, supraclavicular, axillary, and inguinal. The adenopathy may be localized or involve multiple areas, including the retroperitoneal and mesenteric nodes. Uncommonly, the lymphadenopathy is accompanied by fever, malaise, fatigue, sore throat, and myalgia, a picture that closely simulates that of infectious mononucleosis, but without serologic evidence of acute Epstein-Barr virus infection. The differential diagnosis of the lymphadenopathy often includes lymphoma. Chorioretinitis may develop during acute acquired infection, but it does not occur commonly.[110,132] The liver may be involved, and liver function tests may reflect hepatocellular damage. In individuals with normal immunologic function and no severe underlying disease, the infection usually is self-limited and rarely requires treatment.

In contrast, more severe and frequently fulminant infections are seen in patients receiving immunosuppressive therapy, in patients who have disease of the bone marrow or reticuloendothelial system, in patients with agammaglobulinemia, in recipients of bone marrow or stem cell transplants, and in patients with AIDS.[43,84,103,105,111,154,176] Encephalitis and rarely pneumonitis and myocarditis are the most important localized forms that may be encountered in immunocompromised patients. In toxoplasmic encephalitis, the predominant neurologic symptoms are headache, disorientation, and drowsiness. These symptoms may simulate aseptic meningitis or a mass lesion. In view of the various clinical manifestations of CNS involvement, it is important to consider toxoplasmosis whenever evidence of acute CNS disease is present.

CONGENITAL TOXOPLASMOSIS

Congenital infection usually is the result of an asymptomatic acute infection in the mother.[16,57] In a small proportion of cases, spontaneous abortion, prematurity, or stillbirth may result. Congenital toxoplasmosis has a wide spectrum of clinical manifestations, but usually is subclinical in newborns. When clinically apparent, it may mimic other diseases of the newborn. Fever, hydrocephalus or microcephaly, hepatosplenomegaly, jaundice, convulsions, chorioretinitis (usually bilateral), cerebral calcifications, and abnormal cerebrospinal fluid (CSF) (markedly increased protein and mononuclear pleocytosis) are considered the classic features of congenital toxoplasmosis.[50,125,144] These manifestations occurred commonly in an early series of patients reported by Eichenwald[56] (Table 235–1). The case-fatality rate was 12 percent. In survivors in this series, sequelae included mental retardation in 86 percent; convulsions, spasticity, and palsies in almost 75 percent; and severely impaired vision in 60 percent.[56] Other occasional findings included rash (maculopapular, petechial, or both), myocarditis, pneumonitis and respiratory distress, hearing defects, an erythroblastosis-like picture, thrombocytopenia, lymphocytosis, monocytosis, and nephrotic syndrome. These signs now are known to be most typical of the severe form of the infection in the absence of treatment.

The often subclinical nature of congenital toxoplasmosis in the newborn was shown in a French prospective study of 154 mothers who had acquired *Toxoplasma* infection during pregnancy and did not receive treatment.[46] Nine pregnancies (6%) ended in stillbirth, and 85 (55%) resulted in the birth of infected live-born infants. Of the live-born infants who were infected, 64 (75%) had subclinical infection, 14 (16%) had mild disease, and only 7 (8%) had clinically obvious severe disease at birth.[46,144]

TABLE 235–1 Signs and Symptoms Occurring before Diagnosis or during the Course of Untreated Acute Congenital Toxoplasmosis*

Signs and Symptoms	Frequency of Occurrence† in Patients with	
	Neurologic Disease‡	*Generalized Disease*§
Infants	*N* = 108	*N* = 44
Chorioretinitis	102 (94)	29 (66)
Abnormal spinal fluid	59 (55)	37 (84)
Anemia	55 (51)	34 (77)
Jaundice	31 (29)	35 (80)
Splenomegaly	23 (21)	40 (90)
Convulsions	54 (50)	8 (18)
Fever	27 (25)	34 (77)
Intracranial calcification	54 (50)	2 (4)
Hepatomegaly	18 (17)	34 (77)
Lymphadenopathy	18 (17)	30 (68)
Vomiting	17 (16)	21 (48)
Hydrocephalus	30 (28)	0 (0)
Diarrhea	7 (6)	11 (25)
Pneumonitis	0 (0)	18 (41)
Microcephalus	14 (13)	0 (0)
Eosinophilia	6 (4)	8 (18)
Rash	1 (1)	11 (25)
Abnormal bleeding	3 (3)	8 (18)
Hypothermia	2 (2)	9 (20)
Cataracts	5 (5)	0 (0)
Glaucoma	2 (2)	0 (0)
Optic atrophy	2 (2)	0 (0)
Microphthalmos	2 (2)	0 (0)
Children ≥4 years old	*N* = 70	*N* = 31
Mental retardation	62 (89)	25 (81)
Convulsions	58 (83)	24 (77)
Spasticity and palsies	53 (76)	18 (58)
Severely impaired vision	48 (69)	13 (42)
Hydrocephalus or microcephalus	31 (44)	2 (6)
Deafness	12 (17)	3 (10)
Normal	6 (9)	5 (16)

**In 152 infants and 101 of these same patients after ≥4 years of follow-up.*
†Data indicate numbers of patients, with percentages in parentheses.
‡Patients with central nervous system diseases in the first year of life.
§Patients with non-neurologic diseases during the first 2 months of life.
Modified from Eichenwald, H. G.: A study of congenital toxoplasmosis, with particular emphasis on clinical manifestations, sequelae, and therapy. In *Siim, J. C. (ed.): Human Toxoplasmosis. Copenhagen, Munksgaard, 1960, p. 44.*

The risk of transmission to the fetus varies significantly with the trimester of gestation in which the mother becomes infected. For untreated women, the rate is approximately 25 percent in the first trimester, 54 percent in the second trimester, and 65 percent in the third trimester; these figures are minimal estimates derived from placental isolation studies.[46] In contrast, the severity of clinical disease in congenitally infected infants is related inversely to the gestational age at the time of primary maternal infection. In two studies from France,[34,46] severe disease or fetal/neonatal death occurred in approximately 40 to 79 percent of infants born to mothers with first-trimester infection, in 15 to 18 percent with second-trimester infection, and in 0 to 3 percent with third-trimester infection.

Another French prospective study of 210 congenitally infected infants born to mothers who were identified to have primary infection acquired during pregnancy revealed significant morbidity in 94 newborns.[34] Overall, 2 (0.9%) cases were fatal, 21 (10%) were severe, and 71 (33.8%) were mild; 116 cases were asymptomatic. Approximately 40 to 45 percent of the mothers in this study had been treated with spiramycin during pregnancy. These observations confirm that most congenital infections are subclinical at birth. Obvious manifestations occur infrequently. In the same study by Couvreur and associates,[34] 116 infants initially were thought not to be infected on the basis of a routine newborn physical examination. On more intensive examination, however, 39 (34%) of them were found to have one or more abnormalities. Twenty-two (19%) had abnormal CSF on lumbar puncture, 17 (15%) had chorioretinitis on indirect ophthalmoscopic examination, and 10 (9%) had intracranial calcifications on head radiographs or computed tomography (CT) scans. Guerina and colleagues[76] made remarkably similar observations in the congenitally infected newborns that they identified in New England by heel-stick blood sampling.

Some infected children without overt disease as neonates may escape serious sequelae of the infection; however, a significant number (24 to 85%) develop chorioretinitis, strabismus, blindness, hydrocephalus or microcephaly, cerebral calcifications, developmental delay, epilepsy, or deafness months or years later. Three studies provide data that define the incidence of these late sequelae.[96,97,99]

In a study from Paris,[99] 26,402 apparently healthy infants were tested routinely for serologic evidence of *Toxoplasma* infection at 10 months of age. Of these infants, 51 had positive serologic results for *Toxoplasma*, indicative of congenital infection. None had been treated for *Toxoplasma* infection in infancy. Of the 51, 5 were found to have chorioretinal scars by ophthalmologic examination, and chorioretinal lesions had developed in another 4 children by the time they reached 4 years of age, the longest period of follow-up. Some eventually lost functional vision in one eye. Three had intracranial calcifications.

Similarly, in a study from Holland in which a cohort of 1821 pregnant women were screened serologically, 12 congenitally infected infants were detected, and 11 of them were monitored for 20 years.[96,97] Of the 11, 5 were treated as neonates for 1 month only, and 6 were not. Of the 5 treated infants, 4 had eye disease as neonates, and 1 had parasites in CSF, which prompted therapy. Nine of these 11 (82%) had chorioretinal scars by the time that they reached 20 years of age, and 4 of them, including 2 who were initially normal, had severe visual impairment or blindness in one eye. The onset of disease leading to blindness occurred as late as 18 years of age. No neurologic or cognitive sequelae were observed.

The results of this prospective study are similar to those previously reported by Wilson and associates[187] from Alabama in a retrospective analysis of patients from the United States. Over a mean follow-up period of 8.3 years, sequelae developed in 11 of 13 congenitally infected children (85%) who had no signs of disease on detailed examination in the newborn period. Sequelae included chorioretinal lesions in 11 children (85%), severe neurologic disability in 1 child (8%), and mental retardation in 2 children (15%). Sequelae first were noted at ages ranging from 1 month to 9 years. These 13 children were detected either as a result of routine screening of cord serum for IgM antibodies to *Toxoplasma*,[9] performed because acute *Toxoplasma* infection was diagnosed in the mother,[2,188] or as a result of nonspecific findings in the neonatal period (2 were small for gestational age, and 1 had transient borderline thrombocytopenia).

That treatment may decrease the frequency or severity of sequelae is suggested by the Alabama and Paris studies, in which chorioretinitis developed only in untreated infants between 10 months and 4 years old. Taken together, these data indicate that most congenitally infected children who receive no or brief treatment, including children with inapparent infection as neonates, experience untoward sequelae during childhood. Current treatment regimens—prolonged for at least 1 year and often initiated before birth—seem to be associated with substantially less frequent and severe sequelae (see section on treatment).

Congenital toxoplasmosis may mimic or coexist with infection by other organisms. It must be differentiated from other perinatal infections caused by cytomegalovirus, herpes simplex virus, rubella virus, *Treponema pallidum* (syphilis), human immunodeficiency virus type 1 (HIV-1), lymphocytic choriomeningitis (LCM) and certain bacteria (e.g., *Listeria*). Herpesvirus and cytomegalovirus infection, syphilis, and rubella may cause chorioretinitis; cytomegalovirus and HIV-1 may cause encephalopathies associated with cerebral calcifications. Degenerative encephalopathies and storage diseases in older children also may resemble congenital toxoplasmosis.

Many infants or preschool children with coexisting HIV infection and toxoplasmosis have been reported.[126,127] In at least six of these patients, HIV and *Toxoplasma* infection seem to have been acquired in utero. Of these six patients, all but one had clinical evidence of CNS disease, and in most of these children, the CNS disease was associated with other findings common in congenital infection. These findings included hepatosplenomegaly, fever, and chorioretinitis, and were evident at birth (one infant) or developed by the time the infant reached 4 months of age. Two of the other infants remained asymptomatic; one was treated for *Toxoplasma* infection, and the other was not treated. One additional infant who acquired HIV infection at 18 months of age from a blood transfusion died at 5 years of age of toxoplasmic encephalitis. The findings in this patient resembled those in adults with AIDS and toxoplasmic encephalitis.[105] The advent of highly active antiretroviral therapy (HAART) along with the widespread use of trimethoprim-sulfamethoxazole for *Pneumocystis jiroveci* pneumonia prophylaxis has decreased greatly the frequency of clinical toxoplasmic encephalitis in children and adults with HIV.

OCULAR TOXOPLASMOSIS

In active congenital toxoplasmosis, the retinal lesions usually are bilateral.[125] In older children, chorioretinitis may involve only one eye and may be the sole manifestation of congenital toxoplasmosis. Toxoplasmic chorioretinitis, even in older children and adults, usually is considered to be the result of congenital infection.[132] A report of 38 children diagnosed with symptomatic chorioretinitis between 2002 and 2004 in the United Kingdom confirmed that 58 percent of cases were the result of congenital infection.[69] In some studies, *Toxoplasma* infection has accounted for 5 percent of severe visual impairments in children.[90] Active lesions on the fundus appear as white or yellowish foci with elevated, edematous margins surrounded by a zone of hyperemia (Fig. 235–3). Cells and fibrinous exudate in the vitreous may obscure the fundus. Older lesions appear as glial scars, and in

areas in which the retina has been destroyed, the choroid and sclera are visible. Around the depigmented areas, deposition of pigment from the destroyed retina is present. The position of the lesion may be macular, juxtapapillary, or peripheral.

Patients may experience loss of central vision (caused by a perimacular lesion), hazy vision (caused by accumulated exudate), or "floaters" (caused by reactivation of peripheral foci). Neonates or infants with toxoplasmic eye disease may have microphthalmos, small corneas, posterior cortical cataract, anisometropia, strabismus, and nystagmus.[125] Strabismus and nystagmus in a child of any age should raise the possibility of congenital toxoplasmosis. The appearance of lesions in the fundus is not specific for toxoplasmosis. Similar lesions may occur with other, less common granulomatous diseases in the eye, such as toxocariasis, LCM virus, cat-scratch disease, and tuberculosis. Chorioretinitis may be recurrent, most commonly with reactivation at the margins of preexisting lesions.

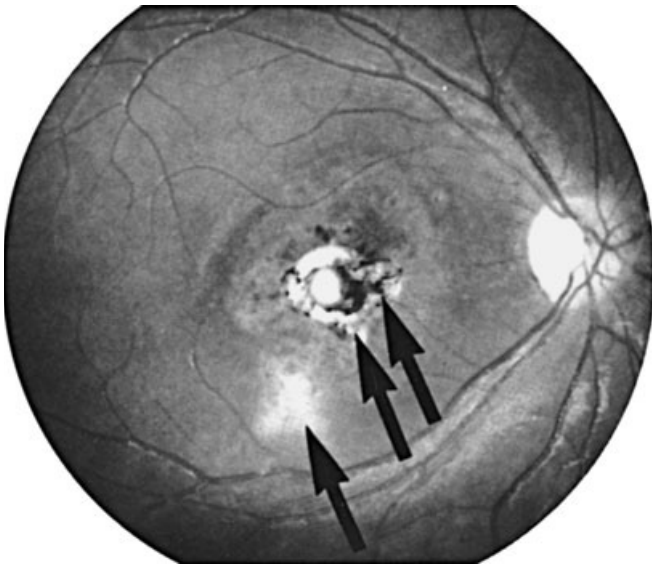

Figure 235–3 Example of active and quiescent chorioretinitis caused by congenital toxoplasmosis in a 12-year-old patient. The active lesion *(single arrow)* is a satellite of an old chorioretinal scar *(two arrows). (From Mets, M., Holfels, E., Boyer, K. M., et al.: Eye manifestations of congenital toxoplasmosis. Am. J. Ophthalmol.* 122*:309-324, 1996. Published with permission from the* American Journal of Ophthalmology. *Copyright © by The Ophthalmic Publishing Company.)*

LABORATORY DIAGNOSIS

Acute infection can be diagnosed by isolation of *T. gondii* from blood or body fluids; demonstration of tachyzoites in histologic sections of tissue or cytologic preparations of body fluids; characteristic lymph node histology; demonstration of *Toxoplasma* cysts in the placenta, fetus, or neonate; detection of the *Toxoplasma* genome by polymerase chain reaction (PCR) in body fluids; and characteristic serologic test results.[15,129,188] Each of these methods is discussed, but serologic tests are emphasized because they are the most common method of establishing the diagnosis (Table 235–2).[188]

SEROLOGIC METHODS

Measurements of IgG Antibody

The most useful tests for detection of IgG antibodies to *Toxoplasma* include the Sabin-Feldman dye test,[156] indirect immunofluorescent antibody (IFA) test, agglutination tests, and ELISA. Titers in ELISA are expressed in different terms for different commercial kits, precluding a discussion of IgG ELISA titers per se in relation to the diagnosis of acute infection.[49,180,188] In these

TABLE 235–2 Guidelines for Interpretation of Serologic Tests for Toxoplasmosis

IgG	IgM	IgG Avidity	Interpretation
Positive	Negative	—	Remote infection, immune. IgG avidity testing is best used when both IgG and IgM are positive, and timing of infection is crucial, as in pregnancy (see below) For evaluation of infection in the newborn, false-negative IgM occurs in approximately 25% of cases. If infection is suspected in this setting, further testing (dye test, IgM EIA, IgA EIA, IgE EIA/ISAGA, PCRs, ideally with paired maternal serologies—see text) is necessary in a reference laboratory (*Toxoplasma* Serology Laboratory, Palo Alto Medical Foundation, 860 Bryant Street, Palo Alto, CA 94301, 415-326-8120)
Positive	Positive or equivocal	High	Infection within the past 18 mo, but likely >12 wk ago. If pregnant and beyond first trimester, consider sending specimen to reference laboratory for dye test, repeat IgG avidity and IgM EIA, IgA EIA, IgE EIA/ISAGA, and AC/HS testing (see above)
Positive	Positive or equivocal	Low	Infection within the past 12 wk. Consider sending specimen to a reference laboratory (see above) to time infection more accurately (dye test, repeat IgG avidity and IgM EIA, IgA EIA, IgE EIA/ISAGA, and AC/HS) in the setting of pregnancy
Equivocal	Negative	—	Indeterminate. Test a new specimen or consider a different assay (IFA or ELISA)
Equivocal	Equivocal	—	Indeterminate. Test a new specimen or consider a different assay (IFA or ELISA)
Equivocal	Positive	—	Acute infection or false-positive IgM. Test a second specimen; if IgG becomes positive or remains equivocal, consider sending specimen to a reference laboratory to time infection more accurately (dye test, IgG avidity, IgM EIA, IgA EIA, IgE EIA/ISAGA, AC/HS—see text) in the setting of pregnancy
Negative	Negative	—	No evidence of *Toxoplasma* infection. Not immune
Negative	Equivocal	—	Either false-positive IgM result or possible recent infection. Obtain a new specimen and retest. If infection is recent, IgM and IgG should become positive, with low IgG avidity. If repeat testing is still IgG negative and IgM equivocal, patient is likely uninfected. Consider IgM ISAGA
Negative	Positive	—	Acute infection or false-positive IgM. Repeat testing on new specimen. If results the same, likely a false-positive IgM. Consider IgM ISAGA

AC/HS, differential agglutination test; EIA, enzyme immunoassay; ELISA, enzyme-linked immunosorbent assay; IFA, immunofluorescence assay; ISAGA, immunosorbent agglutination assay; PCR, polymerase chain reaction.

tests, IgG antibodies appear within the first week of primary infection and reach peak titers (usually ≥1:500) within 1 to 2 months; detectable titers usually persist for life. Although the dye test is the most reliable method, it is available in only a few reference laboratories. IFA test and ELISA are the most widely available and, when properly performed, yield results similar to those obtained with the dye test; however, many laboratories use commercially available kits that are not consistently reliable. Some sera that contain antinuclear antibodies yield false-positive IFA results. The direct agglutination tests that currently are available use formalin-fixed tachyzoites or antigen-coated latex particles, are simple to perform, and are accurate.[164,188]

Initially after primary infection occurs, the avidity of IgG antibody for *T. gondii* antigen is low. Urea dissociates low-avidity antibodies, and a test has been developed that determines the percentage of antibodies that resist elution by 6 mol/L urea. This test is useful in the first 12 weeks of gestation in that the presence of high-avidity antibodies excludes the acquisition of infection in the previous 3 months.[23,188]

Other tests vary in their reliability. Indirect hemagglutination is widely available, but the results frequently are negative in newborns with congenital infection.[188] This test should *not* be used for screening pregnant women because detectable increases in titer are delayed compared with the increases detected by ELISA and by the dye, IFA, and agglutination tests. Meaningful interpretation of changes in titer on sequential sera requires that assays on each sample be performed in the same run by a reliable laboratory.[188]

Measurements of IgM Antibody

IgM antibodies are detected most commonly by IgM IFA, IgM immunosorbent agglutination assay (ISAGA), or IgM ELISA. IgM antibodies appear in the first week of primary infection and peak within 1 month. Depending on the sensitivity of the method used, IgM antibodies may be demonstrable for 2 to 3 months or 1 year or longer. IgM ELISA and IgM ISAGA are much more sensitive than IgM IFA. Absence of IgM ELISA or IgM ISAGA antibodies in an immunologically normal older child (>1 year old) or adult essentially rules out a recently acquired infection.[187] A negative IgM IFA result is not as sensitive in ruling out recently acquired infection. Of sera that were negative with IgM IFA and obtained from adults who had acquired toxoplasmosis recently, 93 percent were strongly positive in IgM ELISA.[134] IgM IFA detects specific IgM antibody in only 25 percent of infants with proven congenital infection, whereas IgM ELISA detects antibody in approximately 75 percent of such cases.[134] The presence of rheumatoid factor or antinuclear antibodies may cause false-positive results in IgM IFA.[2]

The "double-sandwich" IgM ELISA avoids the false-positive results caused by the presence of rheumatoid factor, which the infant can produce in utero, and the false-negative results caused by competition from the high levels of maternal IgG antibody that occur in IgM IFA.[133,188] In addition, the false-positive results in IgM IFA caused by antinuclear antibodies are not found in IgM ELISA.[2,133]

ISAGAs are used widely in Europe. Similar to IgM ELISA, they capture IgM on a solid surface, detect specific IgM, and involve the addition of whole formalin-fixed organisms or *Toxoplasma* antigen–coated latex particles.[48,142] These assays are available commercially and give results comparable to those of IgM ELISA, are simpler to perform, and do not require expensive equipment.

Measurements of IgA and IgE Antibodies

Demonstration of IgA and IgE antibodies in the fetus or newborn by ELISA and ISAGA seems to be at least comparable in sensitivity for the diagnosis of congenital *Toxoplasma* infection compared with demonstration of IgM antibody.[42,167,190] The IgA test also seems to be more sensitive than the IgM test for detection of acquired infection. Specificity remains an issue, however, so neither IgA/IgE ELISA nor IgA/IgE ISAGA has superseded the IgM test for diagnosis of acquired infection. IgA and IgE antibodies persist longer than of IgM and may be useful in cases of subacute illness or when IgM titers are low.

Differential Agglutination

Acetone-fixed and formalin-fixed *T. gondii* tachyzoites may yield differing agglutination titers depending on the acuity of the infection. The test based on this phenomenon is called the AC/HS differential agglutination test.[38] Generally, a disproportionately high agglutination titer with acetone-fixed organisms suggests acute infection; a disproportionately high titer with formalin-fixed organisms suggests chronic infection. Interpretative norms for this test have been established.[38] This test, when combined with the dye test, IgM ELISA, IgA ELISA, IgE ELISA/ISAGA, and avidity studies, yields a "toxoplasmic serologic profile" that permits the most accurate evaluation of infection acuity in pregnant women,[131,188,191] and often can resolve any inconclusive or discrepant results obtained in a hospital or commercial laboratory.

NONSEROLOGIC METHODS

Nonserologic methods are used less commonly for establishing the diagnosis of *Toxoplasma* infection because they are not widely available and require tissue specimens.[188]

Isolation of the Organism

Isolation of *Toxoplasma* from blood or body fluids (e.g., CSF) establishes that the infection is acute. In the case of a neonate, isolation from the placenta or the infant's tissues is sufficient to diagnose congenital *Toxoplasma* infection. In 90 percent of placentas from which *T. gondii* is isolated, congenital infection occurs.[144] Isolation of *Toxoplasma* from the tissues of older children or adults may reflect, however, only the presence of latent infection (cyst form). The organism may be isolated by inoculation of body fluids, leukocytes, or tissue specimens into the peritoneal cavities of mice or into tissue cultures. Specimens should be processed and inoculated immediately; however, tissue and blood may be stored at 4° C overnight. Freezing and thawing or formalin treatment kills the organism. Establishing definitive diagnosis by isolating *Toxoplasma* from tissues usually takes 4 to 6 weeks by mouse inoculation; tissue culture is less sensitive for recovering *Toxoplasma*, but the results are available sooner.

Histology

Demonstration of tachyzoites, but not cysts, in tissue sections or smears of body fluids (e.g., CSF) establishes a diagnosis of acute infection. The organism may be difficult to see with routine stains. The peroxidase-antiperoxidase technique is exquisitely sensitive and has been used with a high degree of sensitivity and specificity to show the organism in the CNS of patients with AIDS.[30] In older children and adults, the histopathologic changes in toxoplasmic lymphadenitis are sufficiently distinctive to enable pathologists to make a presumptive diagnosis of acute acquired toxoplasmosis (see section on pathology).[51,177] Histologic demonstration of cysts establishes that a patient has *Toxoplasma* infection, but they are diagnostic of toxoplasmosis only in the placenta, fetus, or newborn.

Antigen-Specific Lymphocyte Transformation

Lymphocyte transformation in response to *Toxoplasma* antigens is a specific and sensitive indicator of previous *Toxoplasma* infection in adults, and has been used successfully to diagnose congenital *Toxoplasma* infection in infants 2 months or older.[116,168,185] Lymphocyte transformation often is absent in the newborn period, however, particularly in more severely affected infants, because of specific immune tolerance.[116,122]

Polymerase Chain Reaction

Amplification of the B1 genome of *T. gondii* DNA by PCR permits detection of the parasite in body fluids or tissues such as CSF, amniotic fluid, and lymph nodes.[29,75,78,153,177] Experience with the test in France, where PCR of amniotic fluid was compared with the results of percutaneous umbilical blood sampling in 339 pregnant women, has shown that agreement of the two methods is almost 100 percent for the diagnosis of intrauterine infection.[78] In view of the considerably lower risk accompanying amniocentesis than percutaneous umbilical blood sampling, and the fact that amniocentesis potentially can be performed earlier in gestation, this diagnostic procedure seems to be a major advance in prenatal diagnosis. A prospective study in France has shown, however, that only 48 (64%) of 75 amniotic fluid specimens from congenitally infected infants were positive by PCR on a single sample obtained soon after maternal seroconversion. The test had a 100 percent positive predictive value, but a single negative assay did not rule out fetal infection.[153]

DIAGNOSIS IN SPECIFIC CLINICAL SITUATIONS

Acute Acquired Toxoplasmosis

If IgM and IgG antibody are not detectable, the diagnosis of acute *Toxoplasma* infection in an immunocompetent child virtually is excluded.[188] The diagnosis of recently acquired infection is confirmed if seroconversion from a negative to a positive titer is noted, or if a serial fourfold increase in titer to high levels is observed when sera drawn at 3-week intervals are run in parallel. A single high titer in any test is not diagnostic. A dye test or IFA titer of 1:500 or greater in the presence of a high IgM antibody titer probably is diagnostic of recent acute infection. Because IgM can remain positive for 18 months after acute infection, a serum IgG avidity test can be performed to time the infection more accurately. Low IgG avidity suggests infection has occurred within the past 12 weeks, whereas high avidity suggests more remote infection. The absence of IgM antibodies in IgM ELISA or IgM ISAGA essentially excludes the diagnosis of acute infection. In contrast, the absence of IgM antibodies in IgM IFA does not mean that the infection is not acute; in one series, 25 percent of results in adults with acute infection were negative by IgM IFA.[134]

Toxoplasma Infection in Immunodeficient Children

Serologic tests should be done to identify individuals at risk of acquiring toxoplasmosis, such as recipients of organ, stem cell, and bone marrow transplants. The available serologic tests may be inadequate to detect acute active infection in some immunodeficient patients because their antibody response may be abnormal, which is especially the case in stem cell and bone marrow transplant recipients.[44,111] Experience in the Palo Alto, California, reference laboratory has revealed that acute infection may be present in patients with AIDS and in bone marrow transplant recipients without any demonstrable IgM antibody, and in some immunocompromised patients who have little or no IgG antibody.[106] In patients with AIDS and active *Toxoplasma* infection, antibody titers in the modified direct agglutination test[49] may be elevated in the presence of low or undetectable titers in the dye or IFA test.[113] These and other immunodeficient patients can have progressive, lethal toxoplasmosis. In almost all cases, encephalitis, brain abscesses, or both are the predominant findings; hepatic involvement, pneumonitis, and myocarditis may be present. A high index of suspicion is necessary in these patients, and immunoperoxidase staining of appropriate biopsy specimens often is required to establish the diagnosis. Real-time PCR may be a valuable aid in these situations, although tissue immunoperoxidase staining remains the most reliable test.[29]

Toxoplasma Infection in Pregnant Women

Toxoplasma infection acquired during pregnancy is associated with clinical signs (e.g., lymphadenopathy) in only 10 to 15 percent of patients. The fetus is at risk, however, for contracting the infection regardless of whether the mother is symptomatic. To detect acute infection in a pregnant woman in the absence of a routine screening program in which serologic tests are performed periodically throughout pregnancy, a suitable test for IgM antibody (IgM ELISA or IgM ISAGA) should be performed if other serologic tests are positive at any titer. If a suitable IgM antibody test is unavailable, and the original serum contains IgG antibodies, the IgG antibody test should be repeated in 3 weeks, in parallel with the original serum, to determine whether the titer is stable or increasing.[188] More recent experience with IgG avidity testing on a single maternal specimen suggests this additional tool may be helpful in attempting to define maternal infection as acute (within the previous 12 weeks) or remote.[23]

If the IgM ELISA or IgM ISAGA result is negative, and the IgG antibody titer is stable and less than 1:500, no further evaluation is necessary. Because IgG titers usually stabilize at high levels (e.g., the dye test or IFA titer ≥1:500) at 6 to 8 weeks or longer after acquisition of the infection, if the dye test or IFA titer is 1:500 or less and stable (regardless of IgM antibody titer), infection was acquired at least 4 weeks, and probably more than 8 weeks, before the serum was obtained. In the United States, an asymptomatic woman commonly is evaluated for the first time more than 8 weeks after conception, however. If her dye test or IFA titer is 1:500 or greater, her IgM ELISA or IgM ISAGA result is negative, and no significant increase in titer in any test can be shown, her infection almost certainly was acquired before conception. In women with elevated IgM titers or increasing IgG test titers, infection possibly was acquired during pregnancy. A complete toxoplasmic serologic profile[131] in a reference serologic laboratory is recommended to settle the question.

Fetal Diagnosis

As noted earlier, severe disease almost always is associated with primary maternal infection in the first or second trimester of pregnancy, but only 25 percent and 54 percent of such maternal infections result in fetal infection. These rates in exposed fetuses may be reduced by half by maternal treatment with spiramycin. Identification of cases in which the fetus already is infected permits a parental decision to terminate the pregnancy or treat the fetal infection more aggressively with pyrimethamine-sulfadiazine.

Studies by workers in Paris have established an approach that allows establishment of definitive diagnosis and treatment of fetal infection in utero.[36,47,78] These researchers initially sought to establish the diagnosis of fetal infection at 20 to 29 weeks of gestation by isolation of *Toxoplasma* from amniotic fluid or from fetal blood obtained by percutaneous umbilical blood sampling and use of the sensitive mouse inoculation method. Prenatal diagnosis was attempted in 746 pregnancies in which seroconver-

sion occurred near the time of conception or before the 26th week of gestation. In 39 of these pregnancies, fetal infection was diagnosed in utero. *Toxoplasma* was isolated from fetal blood alone in 12 cases, from amniotic fluid alone in 7 cases, and from both in 15 cases, for a total of 34. *Toxoplasma*-specific IgM antibodies were detected in fetal blood in only 9 cases with the highly sensitive ISAGA, and none was positive before 24 weeks of gestation. By follow-up examination until 3 months postpartum or by examination of aborted fetal tissue, a total of 42 cases were proved to have been infected. These researchers were able to detect 39 (93%) of 42 cases of fetal infection occurring before the 26th week of gestation; no false-positive diagnoses occurred.

These investigators subsequently extended their series and reported that a definitive diagnosis of infection was established in utero in 80 (90%) of 89 cases in which the fetus was infected.[79] These results may not be reproduced easily by other investigators for various reasons: the ability to detect maternal infection soon after it occurs depends on monthly monitoring for new maternal infection, sampling of fetal blood and amniotic fluid on more than one occasion, the use of optimal laboratory methods and conditions, and experience in these procedures.

With the use of PCR to amplify the B1 gene of *Toxoplasma* in amniotic fluid obtained after 18 weeks of gestation, these same investigators were able to achieve a sensitivity and specificity for the diagnosis of fetal infection that approached 100 percent.[78] In the more recent multicenter collaborative French study, in which only single specimens were studied, the specificity and positive predictive value remained 100 percent, but the sensitivity was only 64 percent.[153] The sensitivity and specificity for this test as specimens currently are processed and handled in the United States remains to be determined.

Most of the mothers in these French studies had received treatment with spiramycin soon after primary maternal infection was diagnosed, with the intention of continuing therapy until delivery. In contrast to its beneficial effect on maternal-to-fetal transmission, spiramycin does not seem to decrease the severity of fetal infection after it has been established. The impetus for proving fetal infection in early to middle gestation is to allow for intervention either to terminate the pregnancy or to treat the fetus more aggressively. In an extended series of 89 infected fetuses reported by Hohlfeld and colleagues,[79] 34 pregnancies were terminated at the request of the parents. In each of these cases, which were selected either for detectable CNS disease on ultrasound examination or for onset of maternal infection before 12 weeks of gestation, brain necrosis was found on examination of tissue.

These data indicate the value of specifically identifying fetuses that truly are infected and are at high risk for acquiring severe disease because selective termination of these pregnancies allowed successful completion of most pregnancies in which fetal infection did not occur. In addition to the 34 terminated pregnancies with proven fetal infection, 52 pregnancies with 55 offspring were carried to term. In these 52 pregnancies, 43 had fetal infection proven prenatally, and 9 additional offspring were found to be infected by postnatal examination. In the 43 pregnancies in which prenatal diagnosis of fetal infection was established, more aggressive therapy with pyrimethamine and a sulfonamide compound alternating with spiramycin was given in the latter part of pregnancy. This treatment seemed to decrease the number of parasites, as indicated by an approximate 50 percent reduction in the fraction of placentas from which *Toxoplasma* was isolated at delivery compared with cases in which no treatment or spiramycin alone was given.

Regarding clinical outcome, the authors interpreted their data to indicate that such treatment decreased the incidence of severe disease.[79] This conclusion was based, however, on a retrospective comparison with a group of historical controls in which data regarding fetal deaths and termination of pregnancy were unavailable. Because pregnancies with proven or probable severe fetal infection were terminated selectively at the parents' request, one cannot conclude with certainty that the outcome of the infants born to mothers with first-trimester infection was improved by the administration of more aggressive therapy; the outcome of infants born to mothers with second-trimester infection seems to have been improved.[184] A meta-analysis of selected published cohorts on prenatal treatment of congenital toxoplasmosis showed only a weak association between early treatment and a reduced risk of acquiring congenital toxoplasmosis.[174]

It would be desirable to have data from studies in which the effects of such therapy are compared directly in a randomized, concurrent study[174,175]; such studies are unlikely to be performed, however. Nonetheless, these published studies have established the utility of an aggressive approach to prenatal diagnosis in allowing physicians to make an accurate, early diagnosis of fetal infection. If the fetus is infected and affected, as determined by ultrasound, a decision for selective termination of pregnancy can be made rationally. Aggressive therapy with pyrimethamine-sulfadiazine may be offered in cases in which termination is not considered desirable, and it may improve the outcome.

Diagnosis of Congenital Toxoplasmosis after Birth

Performing a thorough clinical and laboratory evaluation is necessary to evaluate fully the existence and extent of congenital toxoplasmosis in a newborn (Table 235–3). Demonstration of

TABLE 235–3 Evaluation of a Neonate When Serology of the Mother or Illness of the Neonate Indicates That a Diagnosis of Congenital Toxoplasmosis Is Suspected or Probable

In addition to a careful general examination, the infant is examined by the following:
Clinical Evaluation and Nonspecific Tests
Pediatric ophthalmologist
Pediatric neurologist
Brain CT (data suggest excellent agreement between CT and ultrasound,[99] which can be obtained more quickly and without the need for sedation)
Blood tests
Complete blood cell count with differential and platelet counts
Serum total IgM, IgG, IgA, and albumin
Serum alanine aminotransferase, total and direct bilirubin
CSF cell count, glucose, protein, and total IgG
***Toxoplasma gondii*–Specific Tests**
Newborn serum analyzed for antibody detected by Sabin-Feldman dye test, IgM ISAGA, IgA EIA, IgE EIA/ISAGA (0.5 mL serum to *Toxoplasma* Serology Laboratory, Palo Alto Medical Foundation, 860 Bryant Street, Palo Alto, CA 94301, 415-326-8120)
Newborn blood for inoculation into mice (1-2 mL clotted whole blood in red-topped tube to *Toxoplasma* Serology Laboratory)
Lumbar puncture: CSF dye test and IgM EIA (0.5 mL CSF to *Toxoplasma* Serology Laboratory); consider PCR (1 mL frozen CSF to *Toxoplasma* Serology Laboratory)
Sterile placental tissue (100 g in saline, from fetal side near insertion of cord, no formalin, to *Toxoplasma* Serology Laboratory for subinoculation)
Maternal serum analyzed for antibody detected by dye test, IgM EIA, IgA EIA, IgE EIA/ISAGA, and AC/HS

AC/HS, differential agglutination test; CSF, cerebrospinal fluid; CT, computed tomography; EIA, enzyme immunoassay; ISAGA, immunosorbent agglutination assay; PCR, polymerase chain reaction.

Modified from McLeod, R., Wisner, J., and Boyer, K.: Toxoplasmosis. In *Krugman, S., Katz, S. L., and Gershon, A. A. (eds.): Infectious Diseases of Children. St. Louis, Mosby-Year Book, 1992, p. 539.*

IgM, IgA, or IgE antibody in an infant's blood or CSF at any time is diagnostic of congenital infection if contamination by maternal blood can be reasonably excluded.[188] Specimens obtained after the infant reaches 10 days of age are more reliable in this regard. If the much less sensitive IgM IFA is used, the presence of antinuclear antibody and rheumatoid factor also must be excluded. As mentioned earlier, the detection rate of congenitally infected infants is 25 percent for IgM IFA and 75 percent for IgM ELISA and IgM ISAGA. Data are insufficient for predicting how often IgA and IgE antibodies are detected. IgM antibodies may be demonstrable in the first few days of life or may appear at variable times after birth.

If *Toxoplasma* is not isolated, and IgM, IgA, or IgE antibodies are not detected, follow-up serologic testing is the only means of establishing the diagnosis. Maternally transmitted IgG antibodies may persist for 6 to 12 months or longer, depending on the original titer. The higher the original titer, the longer maternal antibody is detectable in the infant. The presence of IgG antibody at 8 to 12 months of age does not prove that the infant is infected. Synthesis of IgG *Toxoplasma* antibody usually can be shown by the third month of life if the infant is not treated; it may be delayed until the sixth or ninth month if the infant is treated. At the time that the infant begins to synthesize IgG antibody, infection may be documented by computing the specific "antibody load"—the ratio of specific serum antibody titer to the level of serum IgG in the infant.[144] In the absence of infection, the antibody load decreases in the second or third month as the infant begins to produce IgG that does not contain specific *Toxoplasma* antibodies. In the presence of *Toxoplasma* infection, the infant produces specific antibodies, and the antibody load remains the same or increases. Most infected infants who are treated during the first year of life have a substantial increase in antibody after termination of therapy ("serologic rebound").[178] This phenomenon permits the diagnosis to be confirmed in some uncertain cases.

Ocular Toxoplasmosis

Toxoplasma has been estimated to cause 35 percent of cases of chorioretinitis in the United States and central and western Europe.[81,158,172] Acquired toxoplasmosis usually is not accompanied by chorioretinitis. Most cases are thought to result from congenital infection that does not become clinically apparent until after reactivation. This event occurs most commonly in adolescence. Although the presence of chorioretinitis should prompt a search for *Toxoplasma* infection, proof that *Toxoplasma* caused the eye disease often is lacking. The titer of antibody in serum does not correlate with the presence of active lesions in the fundus. Low titers of IgG antibody are the usual finding in patients with reactivation *Toxoplasma* chorioretinitis. IgM antibodies generally are absent.

Toxoplasma probably is excluded as a cause of chorioretinitis if the results of serologic tests are negative in undiluted serum. If the retinal lesions are characteristic, and serologic test results are positive, the diagnosis is probable. If the retinal lesions are atypical, and the serologic test results are positive, the diagnosis of *Toxoplasma* chorioretinitis is less certain because of the increasing prevalence of *Toxoplasma* antibodies with age in the normal population. Finding *Toxoplasma* antibodies in the child's mother supports the possibility of congenital infection, as does detection of intracranial calcification on CT scan of the patient. Demonstration of local antibody production in aqueous humor obtained by paracentesis of the anterior chamber can be used to establish the diagnosis of *Toxoplasma* chorioretinitis in equivocal cases.[144,188] The risk of this procedure in a situation of threatened vision, when weighed against the low risk of a short course of treatment, is such that it seldom is performed.

TREATMENT

The need for therapy and the duration of therapy are determined by the nature and severity of the clinical illness and by the immune status of the infected patient. Antibody titers are not useful indicators of therapeutic response, and an increasing antibody titer soon after discontinuation of therapy ("serologic rebound") is not an indication of therapeutic failure. Specific therapy acts primarily against the tachyzoite form; the drugs currently available do not eradicate the encysted form containing bradyzoites. Close, longitudinal follow-up and supportive interventions are crucial contributors to therapeutic success.

THERAPEUTIC AGENTS

The therapeutic agents used, their dosages, and indications for their use in the management of toxoplasmosis are included in Table 235–4 and discussed next.

Spiramycin

Spiramycin is a macrolide that has been used extensively in Europe to reduce transmission of infection from an acutely infected mother to the fetus in utero.[31] It is concentrated in the placenta and is reported to reduce transmission by 50 to 60 percent. It reduces the ability to isolate the organism from the placentas of definitively infected newborns from 95 to 80 percent.[33] Spiramycin is less effective than pyrimethamine-sulfadiazine in the treatment of congenital infection and toxoplasmic encephalitis.[33,45,159,160] Toxoplasmic encephalitis has developed in patients receiving spiramycin and has been treated effectively with pyrimethamine-sulfadiazine. Toxicities include allergic manifestations, gastrointestinal intolerance, and paresthesias.

Spiramycin apparently does not treat manifestations of *T. gondii* infection in the fetus in utero.[33,45,79,159] It formerly was used in alternate-month regimens with pyrimethamine and sulfadiazine for treatment of congenital toxoplasmosis in France.[144] Spiramycin is not approved by the U.S. Food and Drug Administration, but may be obtained with compassionate clearance by calling 301-827-2127. The manufacturer (Aventis-Pasteur) provides the drug after the diagnosis has been documented and Food and Drug Administration forms have been completed.

Pyrimethamine

Pyrimethamine has been shown to be effective against *T. gondii* in vitro,[121,124] in animal models,[63] and in human infections.[35,37,63,108,124,191] When used in conjunction with sulfadiazine, synergy can be shown. The pharmacokinetics of pyrimethamine have been studied in infants and adults.[124] Pyrimethamine is metabolized in the liver. Its pharmacokinetics are not altered by renal insufficiency, but are affected by concomitantly administered drugs (e.g., phenobarbital). Pyrimethamine toxicities include reversible marrow suppression (most commonly) and allergy. Aplastic anemia, hepatotoxicity, and various allergic manifestations (including Stevens-Johnson syndrome) also have been listed as toxicities of this medication. Pyrimethamine always should be administered in conjunction with leukovorin (i.e., folinic acid) because human cells can use folinic acid for synthesis of nucleic acids, but *T. gondii* cannot.[124]

Leucovorin

Leucovorin (folinic acid) always is administered during treatment with pyrimethamine. Folinic acid has a protective effect for human cells that folic acid does not. Increased doses of leucovorin are used in the event of marrow suppression. Because of the long

TABLE 235–4 Treatment of Toxoplasmosis

Disease	Medication	Dosage	Length of Therapy
Acute acquired—generally not treated unless severe/ persistent symptoms or vital organ damage or host is immunosuppressed	Pyrimethamine *plus*	2 mg/kg/day for 2 days, then 1 mg/kg/ day	4-6 wk or 2 wk after symptoms resolve for normal host; 4-6 wk beyond resolution for immunosuppressed hosts. In AIDS, treat until $CD4^+$ count >200
	Sulfadiazine *plus*	75-100 mg/kg/day divided twice daily (maximum 4 g/day). Consider the lower dose in children >20 kg (see text)	
	Folinic acid	5-20 mg 3 times weekly. Use higher doses if marrow suppression	
Ocular, older child	Pyrimethamine *plus*	2 mg/kg/day for 2 days, then 1 mg/kg/ day (maximum 50 mg/day)	4-6 wk or 2 wk after symptoms resolve
	Sulfadiazine *plus*	75-100 mg/kg/day divided twice daily (maximum 4 g/day). Consider the lower dose in children >20 kg (see text)	Prednisone should be continued until resolution of sight-threatening active chorioretinitis
	Folinic acid *plus*	5-20 mg 3 times weekly	
	Prednisone	1 mg/kg/day divided twice daily	
Congenital	Pyrimethamine *plus*	2 mg/kg/day for 2 days, then 1 mg/kg/ day for 6 mo, then 3 times weekly (M-W-F) for 6 mo	1 yr
	Sulfadiazine *plus*	100 mg/kg/day divided twice daily	
	Folinic acid *plus*	5-10 mg 3 times weekly	
	Prednisone	1 mg/kg/day divided twice daily	Until resolution of elevated CSF protein or sight-threatening active chorioretinitis
Pregnant women—acute infection first 21 wk of gestation	Spiramycin	3 g/day divided twice daily without food	Until fetal infection documented or excluded at 21 wk of gestation. If fetus infected, change to pyrimethamine plus sulfadiazine plus folinic acid until delivery
Pregnant women—fetal infection confirmed (amniotic fluid PCR positive)	Pyrimethamine *plus*	100 mg/day divided twice daily for 2 days, then 50 mg/day	Until delivery
	Sulfadiazine *plus*	3 g/day divided twice daily	
	Folinic acid	5-20 mg/day	

AIDS, acquired immunodeficiency syndrome; CSF, cerebrospinal fluid; PCR, polymerase chain reaction.

half-life of pyrimethamine, continuation of leucovorin therapy for 1 week after discontinuing pyrimethamine is recommended.

Sulfadiazine, Sulfamerazine, and Sulfamethazine

The three sulfonamides sulfadiazine, sulfamerazine, and sulfamethazine (known as *triple sulfa* when used in combination) are the most active of the sulfonamides against *T. gondii*, and are synergistic with pyrimethamine in their activity against *T. gondii*. Of the three, only sulfadiazine is available in the United States. All other sulfonamides are less active in vitro.[121] The sulfonamides are excreted by the kidney, and the dosage must be adjusted for patients with renal insufficiency. Nephrolithiasis can occur in older children and adults, in whom urinary acidification is more effective, and fluid requirements are lower on a weight basis than in infancy. The risk of development of stones in these groups can be reduced by aggressive fluid supplementation (1 to 2 L above maintenance) and urine alkalinization.[26] In older children who weigh more than 20 kg, a dose of 75 mg/kg/day (rather than the conventional 100 mg/kg/day) should be considered because this dose more closely approximates the calculated adult dose based on body surface area. Other sulfonamide toxicities include allergy (including Stevens-Johnson and DRESS syndromes), marrow suppression, and hepatotoxicity.[120a] Sulfonamide pharmacokinetics have been studied in infants.[144]

Clindamycin

Although the effect of clindamycin is delayed, it does have an effect in vitro against *T. gondii* with prolonged time in culture.[140] It also has been shown to be effective in murine models. Clindamycin has been found to be comparable in efficacy to sulfadiazine for the treatment of toxoplasmic encephalitis in adult patients with AIDS when used in a combined high-dose regimen with pyrimethamine.[37,108] High-dose pyrimethamine also is effective alone. It was not compared directly with the other two regimens in the latter study.

Other Antimicrobial Agents

Numerous other antimicrobial agents have been shown to be effective in vitro or in animal models against either tachyzoites or encysted bradyzoites,[3-6,80,82] but their role, if any, in the treatment of human disease remains to be defined. Atovaquone (5-hydroxynaphthoquinone) was effective against bradyzoites within cysts in vitro.[82] Forty percent of patients with AIDS had a relapse of toxoplasmic encephalitis, however, while being treated with this antimicrobial agent. Other antimicrobial agents with an effect on *T. gondii* in vitro or in vivo include cycloguanil[80]; artemisinin[80]; pyrimethamine-sulfadoxine (Fansidar)[121]; rifabutin[7]; trovafloxacin (no longer available owing to liver toxicity)[92]; and the newer macrolides clarithromycin, azithromycin, and roxithromycin.[3,5,6]

Because the activity of sulfamethoxazole is less than that of sulfadiazine, trimethoprim-sulfamethoxazole has been considered less effective as treatment for toxoplasmosis. Many investigators have used this combination successfully, however, to treat toxoplasmic encephalitis in adults with AIDS. Doses of trimethoprim-sulfamethoxazole, as used to prevent *P. jiroveci* pneumonia in the context of HIV infection, also seem to prevent episodes of reactivated toxoplasmosis.[24] A randomized controlled trial has shown that trimethoprim-sulfamethoxazole is as

effective as pyrimethamine-sulfadiazine in the treatment of isolated ocular toxoplasmosis in young adults.[165] Pyrimethamine combined with sulfadoxine, despite having lower in vitro activity than that of pyrimethamine-sulfadiazine, also has been used in Europe to treat reactivated and congenital infection.[12]

THERAPY IN SPECIFIC CLINICAL SETTINGS

Acquired Toxoplasmosis

Most immunologically normal patients with the lymphadenopathic form of toxoplasmosis do not require specific treatment. Indications for treatment in these cases are the presence of severe and persistent symptoms or damage to vital organs. Because of the high incidence of severe morbidity and mortality in immunocompromised patients, toxoplasmosis should be treated in this population. Most immunocompromised patients in whom the diagnosis is established ante mortem improve when specific therapy is administered. The major problem lies in establishing the diagnosis early enough to institute treatment.

The optimal duration of specific therapy for toxoplasmosis is unknown. Patients who seem to be immunologically normal, but who have severe and persistent symptoms or damage to vital organs should receive specific therapy for 2 to 6 weeks, provided that symptoms resolve. In immunocompromised patients, therapy should continue at least 4 to 6 weeks beyond complete resolution of all signs and symptoms of active disease. Careful follow-up of these patients is imperative because relapses may occur and require prompt re-institution of therapy. In patients with AIDS in whom toxoplasmosis develops, suppressive therapy with pyrimethamine-sulfadiazine, pyrimethamine-clindamycin, or trimethoprim sulfamethoxazole should be continued for life, or until immune reconstitution has been sustained for at least 6 months.

Pregnant Women

Treatment of an acutely infected woman during pregnancy may prevent transmission of the infection to her fetus. The rationale for such treatment is derived from the observation that the lag period between the onset of maternal infection and acquisition of infection in the fetus may be significant. Data from France,[45,46] where women were treated with spiramycin, and from Austria[8,9] and Germany,[98] where women were treated with pyrimethamine and sulfonamides, indicate that the incidence of congenital infection in the offspring of mothers treated during gestation is at least 50 percent less than that in the offspring of untreated mothers. None of these studies was controlled rigidly. A meta-analysis including 20 cohorts with 1721 infected mothers and 506 infected children suggested a reduction in the incidence of transmission (odds ratio 0.48, 95% confidence interval 0.28 to 0.80) was achieved if therapy was started within 3 weeks of maternal seroconversion.[174] These results combined with the numerous women studied by the group from France (154 untreated and 388 treated patients) strongly suggest that intrauterine treatment does reduce the incidence of transmission of maternal infection to the fetus.[51,167] The meta-analysis failed to show that intrauterine treatment ameliorated the manifestations of congenital infection in children who were born infected, but the large single-site studies from France suggest a benefit.

Spiramycin treatment of pregnant women with recently acquired primary infection should be instituted empirically in the hope of preventing spread of infection to the fetus. When fetal infection has occurred, however, maternal treatment with spiramycin does not seem to alter the evolution and severity of disease in the fetus, which is why evaluation of a potentially exposed fetus by PCR amplification of amniotic fluid permits informed decisions to be made about termination of pregnancy or treatment of the fetus in utero with pyrimethamine-sulfadiazine.

Congenital Infection

POSTNATAL TREATMENT

Data regarding the efficacy of postnatal treatment of infants with congenital *Toxoplasma* infection are becoming available. Uncontrolled studies in humans and controlled studies in experimental animals[144] have been interpreted as indicating beneficial effects of postnatal treatment on the development of sequelae in symptomatic and asymptomatic infants with congenital *Toxoplasma* infection. The controlled National Collaborative Treatment Trial is now in progress in Chicago. This study seeks to define optimal therapeutic regimens. Physicians treating patients with congenital *Toxoplasma* infection who are younger than 2.5 months may wish to contact this multidisciplinary group regarding potential enrollment of their patients in that study (773-834-4152).

Outcomes to date from the National Collaborative Treatment Trial are substantially better for most, but not all, infants treated from the neonatal period for 12 months with pyrimethamine-sulfadiazine and leucovorin than for historical controls receiving no or short-course therapy.[117] Signs of active infection resolve within weeks of initiation of treatment. In many children, the appearance of brain CT scans has improved remarkably. Cerebral calcifications have diminished in size or resolved in most such treated children.[139] In conjunction with this improvement in brain CT scans, cognitive function has been in the normal range for 69 percent of treated children.[151,170] Overall, 18 of 66 (27%) children in the National Collaborative Treatment Trial have IQ results less than 70 compared with 86 of 101 (85%) in the reported literature (see Table 235–1).[48,117]

No significant diminution in cognitive function occurs over time, and most treated children function well in regular school classrooms. Although the number of children compared is limited, for a small subset of these children, measures of cognitive function seem to be less than those for their siblings. No sensorineural hearing loss has been ascribable to congenital toxoplasmosis in treated children.[117] Despite the much improved neurologic outlook for most of these children, a subset of children with significant irreversible neurologic damage already present in the perinatal period have manifested profound developmental delay, motor impairment, and seizures. For the most part, these were children with hydrocephalus, high CSF protein, minimal improvement in brain CT scans after shunting, and often substantial delays in shunt placement or needed revision for shunt failure or other intercurrent medical problems.[170] This experience emphasizes the importance of recognizing hydrocephalus and managing it aggressively.

Although treatment during the first year of life arrests all signs of active disease, results in normal cognitive and motor outcomes for most children, and may result in resolution of seizures without recurrence for some treated children, the drugs currently available do not eradicate all cysts containing bradyzoites. In most children, serologic titers of *T. gondii*–specific antibodies rebound in the 3 to 4 months after treatment is discontinued.[117,178]

To date, new retinal lesions have occurred in 17 of 58 children in the National Collaborative Treatment Trial during 3 to 10 years' follow-up after the 1-year course of treatment.[117] These active lesions have responded to brief courses of treatment with pyrimethamine, sulfadiazine, and leucovorin without subsequent loss of visual acuity. Although follow-up durations are shorter, this result contrasts with the almost uniform eventual development of retinal lesions in studies of untreated or briefly treated children.[31,56,96,97,187] We recommend that infected children undergo retinal examination each month for 3 months after

discontinuing treatment around their first birthday, then every 3 months until they are old enough to describe visual symptoms accurately, and then every 6 months. In addition, an ophthalmologic evaluation should be performed promptly for any acute visual signs or symptoms that may be related to recrudescence of congenital ocular toxoplasmosis.[125]

SEQUENTIAL FETAL AND POSTNATAL TREATMENT

Hohlfeld and colleagues[36,79] described outcomes in patients treated in utero with continuing treatment during the first year of life. As noted earlier, however, pregnancies in which fetuses had obvious manifestations on ultrasound examination and most pregnancies with definite first-trimester infection were terminated.

When the French method of initiating aggressive treatment of fetuses in utero was applied, retinal disease was reported in only 3 of 50 such infants monitored to 2 years of age. This finding contrasts with the presence of retinal or neurologic involvement in 50 percent of asymptomatic newborns detected by serologic screening in Massachusetts[76] and in 75 percent of children whose pediatricians referred them to our National Collaborative Treatment Trial for treatment in the perinatal period.[125] A prospective, carefully controlled study (as part of the National Collaborative Study) is under way to compare directly outcomes in infants identified and treated in utero, as detected by systematic neonatal screening and by pediatricians in the neonatal period and then referred. The outcome of pregnancies with infection acquired in the first trimester after in utero treatment also has been reported to be favorable in another study,[13] in which only pregnancies in which the fetus had hydrocephalus were terminated.

Toxoplasmosis and coexistent HIV infection in children is increasingly rare since the advent of HAART. Most children reported with toxoplasmosis and HIV have had congenital toxoplasmosis and have been symptomatic. For such children, therapy with pyrimethamine and sulfadiazine plus folinic acid is recommended in the doses described in Table 235–4. Adult and adolescent patients receiving secondary prophylaxis (i.e., long-term maintenance therapy) for acquired toxoplasmic encephalitis are at low risk for recurrence when they have successfully completed initial therapy, remain asymptomatic with regard to signs and symptoms of toxoplasmic encephalitis, and have a sustained increase in $CD4^+$ T lymphocyte counts of more than 200 cells/μL after receiving HAART (e.g., >6 months).

Although the numbers of patients who have been evaluated remain limited, and occasional recurrences have been reported, on the basis of these observations and inference from more extensive cumulative data indicating the safety of discontinuing secondary prophylaxis for other opportunistic infections during advanced HIV disease, discontinuing long-term maintenance therapy among such patients is a reasonable consideration. The highest risk for relapse seems to occur within the first 6 months of discontinuing secondary prophylaxis. Certain specialists would obtain a magnetic resonance image of the brain as part of their evaluation to determine whether discontinuing therapy is appropriate. Zidovudine antagonizes the toxoplasmacidal effect of pyrimethamine and its in vitro synergy with sulfonamide. Whether this effect occurs in vivo is unknown.[101]

Prompt initiation of specific treatment in active ocular toxoplasmosis is mandatory to preserve vision. Inflammatory reactions in the vitreous frequently are a major pathogenetic phenomenon in patients with active disease, and in such cases, administration of corticosteroids in addition to specific anti-*Toxoplasma* therapy is recommended strongly.[160] Their use also is recommended for cases of retinochoroiditis involving the macula, maculopapillary bundle, or optic nerve. The initial daily dosage of prednisone is 1 mg/kg orally to a maximum of 75 mg in 24 hours. The equivalent dosage of another corticosteroid may be given. The dosage of corticosteroid may be reduced gradually when the lesion appears to be well demarcated and pigmentation has begun. Some physicians have used systemic or intraocular clindamycin to treat patients in whom the use of corticosteroids and pyrimethamine plus sulfadiazine has failed[171]; its efficacy has not been proved in humans.

PREVENTION

Congenital infection may be avoided by preventing primary *Toxoplasma* infection during pregnancy.[58,114,191] The responsibility of all physicians caring for pregnant women at risk is to inform them of specific hygienic measures (Table 235–5) for avoiding *Toxoplasma* infection. Similar measures are useful for prevention of acquired infection in other settings as well. The effectiveness of a 10-minute education program, offered as part of prenatal care, to reduce the risk of acquiring *Toxoplasma* infection by modifying the behavior of pregnant women with regard to cats, food, and personal hygiene has been shown.[25] Pamphlets that describe methods to prevent toxoplasmosis in pregnant women are available from the March of Dimes (312-435-4007), from Abbott Diagnostics (800-323-9100), and on the Internet (http://www.iit.edu/~toxo/pamphlet).

When primary maternal infection has occurred, several problems are inherent in the secondary prevention of congenital toxoplasmosis by therapeutic abortion or by treatment of the pregnant woman. Because 80 to 90 percent of women with primary *Toxoplasma* infection are asymptomatic, most primary infections are overlooked, unless sequential serologic testing is performed routinely in pregnant women. The cost-effectiveness of routine screening in the prevention of congenital toxoplasmosis is clear in some European countries where screening is mandated by law.[45] In countries with a lower incidence, cost-efficiency has not been proved.[148] In the absence of such data, physicians may choose to screen patients on an individual basis.[185] If screening is undertaken, a reliable serologic test for IgG antibodies (see section on laboratory diagnosis) should be performed before conception occurs or as soon as possible thereafter, and then repeated every 2 to 3 months until the time of delivery. Serologic test results that suggest the acquisition of primary infection during

TABLE 235–5 Prevention of *Toxoplasma* Infection

Prevention of Acquired Infection (Primary Prevention)
Cook meat to medium (66° C [150° F]), smoke it, or cure it in brine
Wash fruits and vegetables before consumption
Avoid touching mucous membranes of the mouth and eyes while handling uncooked meat or unwashed fruits or vegetables
Wash hands and kitchen surfaces thoroughly after contact with raw meat or unwashed fruits or vegetables
Prevent access of flies, cockroaches, and other coprophagous insects to fruits and vegetables
Avoid contact with materials that potentially are contaminated with cat feces, such as cat litter boxes, or wear gloves when handling such materials and when gardening
Disinfect cat litter boxes for 5 min with nearly boiling water
Prevention of Congenital Infection (Secondary Prevention)
Identify women at risk by serologic testing
Treatment during pregnancy results in an approximately 50% reduction in the incidence of infection in infants
Therapeutic abortion prevents birth of an infected infant—consider only for women who acquire infection in the first or second trimester

Adapted from Remington, J. S., and Wilson, C. B.: Toxoplasmosis. In *Kass, E. H., and Platt, R. (eds.): Current Therapy in Infectious Disease: 1983-1984. Philadelphia, B. C. Decker, 1983, pp. 149-153.*

pregnancy should be confirmed by a reference laboratory. Decisions regarding treatment or therapeutic abortion should be based on a consideration of whether the fetus is infected or affected, as determined by amniocentesis and ultrasonography.

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CHAPTER 236

PNEUMOCYSTIS PNEUMONIA

Walter T. Hughes

Pneumocystis pneumonia (PCP) is an opportunistic infection characterized by a life-threatening bilateral diffuse alveolar disease occurring almost exclusively in the immunocompromised host. Even in fatal cases, the organism and the disease are limited to the lungs. Since 1980, the pneumonia has gained prominence as a major opportunistic infection in infants, children, and adults with acquired immunodeficiency syndrome (AIDS), while continuing as a complication of cancer, congenital immunodeficiency disorders, and organ transplantation. The causative organism is a fungus-like microbe best known as *Pneumocystis carinii*, although some investigators prefer the controversial term *Pneumocystis jiroveci* Frenkel to designate the organism found in human lungs. Effective chemoprophylaxis and treatment are available.

ORGANISM

In 1909, Chagas[22] identified what he interpreted as spherical and sickle-shaped forms of the parasite *Trypanosoma cruzi* in the lungs of experimentally infected guinea pigs. The same organism was found later by Carini[18] in animals with trypanosome infections. In 1912, Delanoe and Delanoe[34] identified this parasite in the lungs of rats and guinea pigs not infected with trypanosomes and proposed an independent genus of *P. carinii*.[74] These and other investigators, including Chagas, subsequently showed that this agent was not related to the trypanosome.[52] *P. carinii* was first found in human lungs as a cause of pneumonitis in 1942,[183] and immunologic studies in 1972[100] showed a difference in *P. carinii* from humans and rats. During the past 2 decades, the application of molecular technology has resulted in extensive data to delineate the genetic diversity of the organism. Researchers have established that *P. carinii* found in each mammalian host is specific for that host.

Although previously considered a protozoan, *P. carinii* generally is accepted as belonging to the Fungi kingdom. In support of the fungal nature of the organism are the observations that ribosomal RNA sequences are homologous to fungi[43]; the dihydrofolate reductase, in contrast to that of protozoa, is not a bifunctional polypeptide with thymidylate synthetase[42]; the cyst wall contains chitin and beta-1,3-glucan[121]; poorly developed mitochondria contain lamellar cristae; and the organism is susceptible to echinocandins.[165] The antifungal drugs amphotericin B, ketoconazole, nystatin, 5-flucytosine, fluconazole, and miconazole have no effect against PCP, whereas the drugs with demonstrated anti–*P. carinii* activity also are antiprotozoan drugs: pentamidine (*Leishmania donovani* and *Trypanosoma gambiense*), trimethoprim-sulfamethoxazole (*Isospora belli*, *Toxoplasma gondii*), and pyrimethamine-sulfadiazine and atovaquone (*T. gondii* and *Plasmodium falciparum*).

Three developmental forms of this organism have been identified by light microscopy: cysts, sporozoites, and trophozoites. Cysts occur in lung tissue or respiratory secretions as spherical or crescent-shaped structures approximately 5 μm in diameter (Figs. 236–1 and 236–2). They may contain eight oval bodies or sporozoites 1 to 2 μm in diameter. A third extracystic pleomorphic structure, called a *trophozoite*, which varies in size from 2 to 5 μm in diameter, is identified in association with cysts. The ultrastructural morphologic features identified by electron microscopy and scanning electron microscopy are well described in the literature.[17,148,151] The major protein component of trophozoites and cysts[57,58] is referred to as the major surface glycoprotein or glycoprotein A, with molecular weight ranging from 95 to 140 kd. Genes encoding the major surface glycoprotein are repeated, highly polymorphic, and distributed among all of the

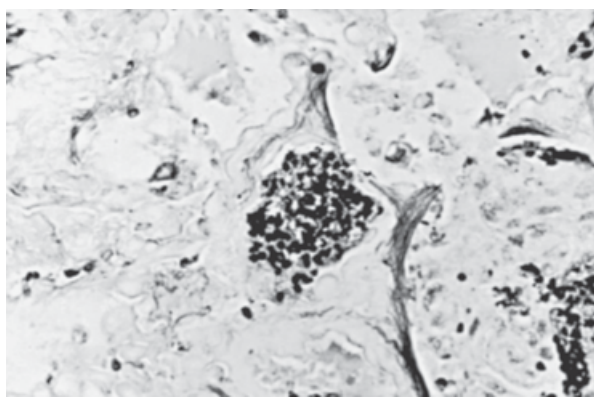

Figure 236–1 Typical cyst forms shown by Gomori silver methenamine stain of lung tissue obtained by open lung biopsy from a 20-month-old child with severe combined immunodeficiency disease (×100).

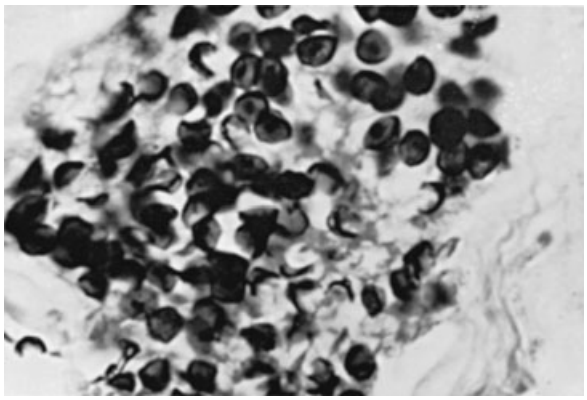

Figure 236–2 Typical cyst forms shown by Gomori silver methenamine stain of lung tissue obtained by open lung biopsy from a 20-month-old child with severe combined immunodeficiency disease (×1000).

14 to 15 chromosomes.[106,189] The major surface glycoprotein is antigenically specific for *P. carinii* of various animal hosts.[55,58,106] This mannose-containing carbohydrate is important in attachment of organisms to mammalian host cells.[180] To date, none of the culture systems reported[7,30,107,123,148] has been established and standardized to provide prolonged propagation and growth of the organism.

Efforts have been made to adopt new nomenclature for *P. carinii*, primarily to distinguish between strains derived from humans and strains from lower mammals. In 1976, Frenkel[49] suggested *P. jiroveci* n. sp. for isolates from humans. During the ensuing 3 decades, this term was not used. In 1988, Hughes and Gigliotti[81] proposed a nomenclature merely designating the host from which the organism was obtained (e.g., *P. carinii*, *humanus*; *P. carinii*, *rattus*). In 1994, Stringer and the Pneumocystis Workshop[177] used DNA sequence data to employ a "special forms" designation (e.g., *P. carinii* f. sp. *hominis*). A subsequent system put forth in 2002 by Stringer and colleagues[177] is currently used by some investigators and designates the human form as *P. jiroveci* Frenkel. Because of issues discussed elsewhere[56,73] and the expected future proposals for change, I continue to use *P. carinii* here to designate the organism in human disease.

TRANSMISSION AND EPIDEMIOLOGY

TRANSMISSION

P. carinii has been recognized in many wild and laboratory animal species over a wide geographic distribution. An association between animal reservoirs and human infection has not been established. Animal-to-animal transmission by the airborne route has been shown in laboratory rats.[69,76] DNA sequences identical to those of *P. carinii* have been detected in ambient air.[190] Genotypic studies of human *P. carinii* isolates in Britain suggest the clustering of specific genotypes in residential areas and are consistent with person-to-person transmission of the organism by the airborne route.[129] Evidence for transmission of *P. carinii* DNA from an infected infant to close hospital contacts has been reported.[185] Before and during World War II, epidemics of interstitial plasma cell pneumonitis secondary to *P. carinii* were recognized in debilitated and premature infants throughout European institutions and nursing homes.[12,164] In 1942, Van der Meer and Brug[183] identified *P. carinii* in the lungs of humans with interstitial plasma cell pneumonitis. Subsequent studies confirmed this organism as the cause of the pneumonitis. Interruption of outbreaks by the introduction of strict isolation of affected patients within these institutions suggests the probable importance of person-to-person spread of disease within that setting.[168] Most investigators believe the organism is acquired very soon after birth in rats and humans, and these neonates serve as reservoirs of infection.[29,134,180]

In contrast to the early European patterns, American cases largely have been sporadic and have occurred almost exclusively in children with impaired host defenses.[32,144] A few outbreaks of PCP within families[197] or among closely associated groups of hospitalized cancer patients have been reported, further suggesting the importance of contagion in the spread of this disease.[13,162,173]

Autopsy studies have shown the occasional presence of *Pneumocystis* organisms in the lungs of patients without evidence of underlying host defense disorders or pulmonary disease,[111] and have shown that unapparent (asymptomatic) infection occurs frequently in patients with cancer and in other immunocompromised patients.[146,201] The epidemiologic importance of asymptomatic carriers in the transmission of *Pneumocystis* disease is unknown. Organisms are detected frequently in sputum, pharyngeal secretions, and tracheal aspirates of symptomatic patients.[66] Cysts have been shown to remain intact for several months in dried lung specimens maintained at room temperature.[88] These observations, in addition to the almost universal localization of disease within the lungs of affected patients, suggest that infection probably results from inhalation of the organism. In view of this possibility, respiratory isolation of symptomatic patients should be maintained to prevent exposure of other highly susceptible patients. No data support the isolation of such patients from otherwise healthy individuals.

Although intrauterine transmission of *P. carinii* has been documented in one stillborn infant and has been implicated in three siblings with no demonstrable immunologic abnormalities who were infected in the neonatal period,[8] the paucity of such cases does not support the epidemiologic importance of this mode of transmission. Of the infants of eight women with AIDS and PCP during pregnancy, one infant had evidence of *P. carinii* infection.[70,133] Studies in severe combined immunodeficiency mice have failed to show transplacental passage of this organism.[92]

EPIDEMIOLOGY IN THE NORMAL HOST

Asymptomatic *P. carinii* infection is highly prevalent in humans. Serologic studies have shown that more than 90 percent of normal adults have antibody to *P. carinii*, and that approximately 75 percent have acquired *P. carinii* antibody before reaching 4 years of age.[104,126,145,149] Molecular techniques show that during periods of immunosuppression, PCP can occur either by reactivation or by the acquisition of new organisms.[72]

Some evidence suggests that *P. carinii* pneumonitis may occur in immunocompetent infants.[175] In a prospective study of 67 infants 2 to 12 weeks old with pneumonitis in Birmingham, Alabama, 10 were found to have serologic evidence of this infection. In 1 of the 10 infants, the diagnosis was proved by lung biopsy. In serial observations conducted every 2 months on a cohort of 107 normal healthy infants in Chile, *P. carinii* DNA was detected in nasopharyngeal aspirates obtained during episodes of mild respiratory infection in 24 (32%) of 74 infants from whom specimens were available. Three (12.5%) of the 24 infants versus 0 of 50 infants who tested negative for *P. carinii* had apnea episodes. Of the infants, 85 percent seroconverted by 20 months of age.[184] Further delineation of the clinical features of *P. carinii* infection in normal hosts is needed.

Studies in Santiago, Chile, and Oxford, United Kingdom, found *P. carinii* in 15 to 35 percent of infants with sudden infant

death syndrome (SIDS) versus 2.9 percent of infants in the same age groups who died of other causes.[186] In another report of 112 infants younger than 1 year who died in Santiago from 1998 to 2000, *P. carinii* DNA was detected in 51.7 percent of 89 infants (75 had SIDS) from the community and 20 percent of 25 infants who died in the hospital ($p = .006$). Viral infections were found more commonly in hospitalized patients than in patients in the community.[187] Because of the scarcity of organisms, the terminal event in SIDS cannot be explained by PCP; the association remains unexplained.

EPIDEMIOLOGY IN HOSTS NOT COMPROMISED BY ACQUIRED IMMUNODEFICIENCY SYNDROME

The European outbreaks of PCP in debilitated infants, the studies of *P. carinii* in malnourished children in Africa, and the well-known association of malnutrition and impaired resistance to various infectious disorders suggest the importance of malnutrition as an additional host determinant for the development of *P. carinii* infection.[89,96,167] Before the AIDS epidemic, PCP occurred almost exclusively in patients with primary immunologic disorders or in patients receiving immunosuppressive therapy for oncologic disease or organ transplantation.[54,146,188,195] Studies before the advent of chemoprophylaxis for PCP reflect the natural pattern of PCP in the compromised host.

Of 194 cases of PCP reported to the Centers for Disease Control and Prevention (CDC) between 1967 and 1970, 29 occurred in infants younger than 1 year; 83 percent of these infants had primary immunodeficiency disorders. In contrast, acute lymphocytic leukemia was the most common underlying disease in children older than 1 year.[195] Of 1251 children with malignancies at the St. Jude Research Hospital (1962 to 1971), PCP occurred in 51 (4.1%), whereas in 379 patients with other types of neoplasias and in 1669 children without malignant disease, *P. carinii* infection was not encountered.[88] *P. carinii* infection occurs more commonly in patients with cancer involving generalized lymphoproliferative malignancies than in patients with solid tumors.[15,172] The extent of malignant disease and the intensity of the chemotherapy or radiotherapy provided were associated with an increased risk for the development of *Pneumocystis* infection.[78,146,172]

Chemoprophylaxis for PCP has been in widespread use for 3 decades in the management of severely immunosuppressed hosts. Changes have occurred in the treatment of the primary underlying diseases, raising the question as to whether PCP is still the threat to the compromised host that was revealed in earlier studies. Reports from several cancer centers show that the incidence or prevalence, or both, of PCP has increased during the past decade.[73,142,203] Investigators at University Hospitals in Leiden[5] and Basel[136] show the emergence of PCP in patients with organ transplants and others with vasculitis/autoimmune diseases. During 1990 to 2003 at the University of Texas M.D. Anderson Cancer Center, Houston, 80 episodes of PCP occurred in 79 patients; 29 percent of the patients had undergone hematopoietic stem cell transplantation.[181]

Among 519 patients undergoing allogeneic hematopoietic stem cell transplantation in Paris, 13 cases of PCP occurred (10 were not on prophylaxis). The median $CD4^+$ T cell count was 131/μL at diagnosis.[33] In a review of 4581 solid organ transplant recipients who were not receiving prophylaxis, 4.9 percent had PCP, with considerable variation seen among organ groups (33% of 106 lung, 4.2% of 895 heart, 11% of 278 liver, and 3.8% of 3301 kidney transplants).[60] During the decade 1991 to 2001, 118 episodes (108 patients) of PCP occurred at one hospital in Goteborg, Sweden. The most common underlying entities were human immunodeficiency virus (HIV) infection in 29 cases and organ transplantation in 26 cases. Of the episodes, 75 percent were in non-AIDS patients. The number of PCP cases did not increase during the study period.[128]

More recent evidence suggests that patients with connective tissue diseases, such as lupus and rheumatoid arthritis, who are receiving immunosuppressive therapy and have $CD4^+$ T lymphocyte counts less than 250/μL are at risk for development of PCP.[115] In 88 consecutive patients with cystic fibrosis, *P. carinii* mitochondrial RNA was detected in 21.5 percent of cases. None of the patients developed PCP or clinical evidence of infection.[155]

EPIDEMIOLOGY IN ACQUIRED IMMUNODEFICIENCY SYNDROME PATIENTS

On June 5, 1981, the CDC reported five cases of *P. carinii* pneumonia in previously healthy men. These were the first cases of AIDS in the United States, discovered because the opportunistic *P. carinii* infection served as a badge of immunodeficiency and led to a search for a host defect. Since that time, HIV/AIDS and the associated opportunistic infections have caused the deaths of more than 22 million people worldwide and 500,000 people in the United States.[21]

Individuals infected with HIV type 1 (HIV-1) have a remarkably high risk for the development of *P. carinii* pneumonitis. The pneumonitis was diagnosed in 1080 (39%) of the 2786 pediatric patients with AIDS reported to the CDC through 1990.[171] Pneumonitis develops in approximately 75 percent of untreated adults with AIDS. Among children with AIDS, *P. carinii* pneumonitis may occur at any age, but most frequently it is found in infants 3 to 6 months old.[103,169,171] The introduction of *P. carinii* prophylaxis alone without antivirals in the treatment of AIDS provided a per-person survival benefit of 3.1 months.[193] The use of highly active antiretroviral therapy (HAART) plus chemoprophylaxis for PCP has reduced drastically the incidence of PCP infections in children with AIDS. Gona and associates[59] found the incidence rate of PCP per 100 person-years to be 1.3 in 3331 HIV-infected children from 1981 to 1988, before the introduction of HAART, and less than 0.5 in 2767 HIV-infected infants from 2001 to 2004 with use of HAART.

PATHOGENESIS

P. carinii is an organism of low virulence found almost exclusively in the alveoli of the lung with the potential for provoking life-threatening diffuse alveolar disease. This paradox is based on the fact that the extent of the disease is due primarily to the magnitude of the host's defensive response.

In recent years, the application of molecular technology has provided a vast amount of information to aid in the understanding of the pathogenesis of this infection and disease. Thomas and Limper[180] provided a comprehensive composite of these data.

When *P. carinii* reaches the lungs by the airborne route, the organism may elicit no host response, and the subject remains asymptomatic; alternatively, if the host is severely immunocompromised, a life-threatening pneumonitis may evolve. Cyst and trophozoite forms may bind avidly to the epithelial cells of the alveolus, activating specific signaling pathways in the organisms.[180] Alveolar macrophages ingest and degrade the organisms. The macrophages elaborate proinflammatory cytokines, which, in addition to aiding in removal of organisms, evoke pulmonary injury. Tumor necrosis factor–α promotes neutrophil, lymphocyte, and monocyte infiltration, which may cause alveolar damage and diminished gas exchange, and result in respiratory failure.[26,64] The $CD4^+$ T lymphocyte is of major importance in the recruitment and activation of other immune effector cells, including monocytes and macrophages.[9,61] The $CD4^+$ cell mediates inter-

leukin-1 and macrophage-derived tumor necrosis factor–α, which are necessary for pulmonary responses to the organism. The lymphocytes also proliferate in response to *P. carinii* antigens to produce lymphotactin and interferon-γ.[9] With pneumonitis, an increase in $CD8^+$ lymphocytes occurs in the lungs.[10]

Surfactant phospholipids and apoproteins are affected by *P. carinii*.[11,139,202] Reduction in lung surfactant has been shown in animal studies.[196,202]

CLINICAL STUDIES TO ELUCIDATE PATHOGENESIS

The ability of corticosteroids to induce *P. carinii* infection in laboratory animals, the occurrence of PCP in patients with AIDS and pure T-cell deficiency disease, and the development of PCP in malnourished hosts with significantly impaired cellular immune responses[89,96,110,167] provide indirect evidence of the potential importance of cellular immunity in protecting the host against this opportunist. Because corticosteroids, cytotoxic agents, and malnutrition variably depress humoral and cellular immune mechanisms and nonspecific immune responses (inflammation), no absolute statement can be made regarding the importance of each defense mechanism, other than that strong evidence has implicated the importance of T-lymphocyte competence in the pathogenesis of PCP. Experimental studies in rats show provocation of the pneumonitis after the administration of cyclosporine, a compound that specifically affects T cell–mediated immune responses related to impairment of interleukin-2 production and receptor site inhibition and with no direct effect on other components of the immune system.[91] Especially convincing is the remarkable susceptibility of patients with AIDS to acquisition of PCP. In this syndrome, host compromise is limited primarily to impaired T-cell function, and PCP develops in at least 50 percent of affected untreated adults.

In infants, children, and adults with AIDS, the quantity of peripheral blood $CD4^+$ helper T lymphocytes serves as a useful risk predictor of development of *P. carinii* pneumonitis. As the $CD4^+$ lymphocyte count decreases, the risk for acquiring *P. carinii* pneumonitis increases. In adults, a $CD4^+$ cell count less than 200/μL is highly predictive of impending *P. carinii* pneumonitis. Because infants and children have relatively higher total lymphocyte counts, absolute $CD4^+$ cell counts are higher than in adults. The threshold for the risk of acquiring *P. carinii* infection is age-related.[103,170]

No characteristic pattern of serum immunoglobulin abnormality has been shown. Low levels of IgG were the most consistent finding in two reported series of children with immunodeficiency disease.[15,195] In contrast, normal or elevated levels of IgG were documented in 70 percent of leukemic children with PCP. Administration of serum immunoglobulin to infected children with these disorders usually provided no therapeutic benefit.[195] The role of IgA antibody in host defense against *P. carinii* probably is not of major importance because secretory IgA levels are normal in most affected patients, and patients with immunodeficiency states characterized by IgA deficits are not unduly susceptible.[15,195]

The development of specific antibodies to *P. carinii* in infected patients has been inconsistent, in part because of the lack of a standardized method for testing. During "epidemic" disease in malnourished infants, IgM values frequently increase markedly, with variable changes occurring in IgG and IgA values. IgG antibody concentrations increase in serum 4 to 6 weeks after infection and are thought to provide permanent immunity in these infants.[102] In selected instances, specific antibody responses (IgG, IgA, and IgM) have been shown in normal individuals who have been associated closely with infected patients.[15,125]

Using immunofluorescent staining techniques, Brzosko and colleagues[14] showed IgG and IgM antibody with smaller amounts of IgA antibody and β_{1c}-globulin deposits on the surface of *Pneumocystis* organisms within the alveoli of infants with "epidemic" PCP. Late in their disease, less immunoglobulin was present within alveoli, whereas increased numbers of plasma cells and alveolar macrophages containing fluorescent material were identified. Possibly, specific antibody fixes complement (β_{1c}-globulin) on the surface of *Pneumocystis* organisms, allowing subsequent phagocytosis by alveolar macrophages.[14]

PATHOLOGY

P. carinii infections are unique in that the pathologic findings, with rare exceptions, are limited to the lungs, even in fatal cases. In the infantile "epidemic" form of disease, essentially all alveoli contain large numbers of organisms. Extensive interstitial plasma cell infiltrates distend the alveolar walls 5 to 20 times over their normal thickness, and almost no intra-alveolar fibrinous exudate is noted.[40,66]

In the childhood and adult forms of PCP, the histogenesis has been described in three stages.[66,88,151] An initial stage is characterized by the presence of cysts and trophozoites attached by fibronectin to the alveolar walls.[150] No septal inflammatory or cellular response is evident, and no clinical disease is associated with this stage. A second stage, which may or may not be associated with clinical signs and symptoms, is characterized by desquamation of alveolar cells and an increase in the number of cysts within alveolar macrophages. Tumor necrosis factor may be a major mediator involved in the killing of *P. carinii* by activated alveolar macrophages and may be induced by oxidative stress in the alveoli.[147] The final stage is typified by extensive reactive and desquamative alveolitis manifested by marked cytoplasmic vacuolization of macrophages, mononuclear and plasma cell infiltrates within alveolar septa, and clusters of organisms located predominantly within macrophages in the lumen of alveoli. The histopathology of this final stage definitely is associated with clinical manifestations of pneumonitis (see Figs. 236–1 and 236–2).[151]

Rarely, *P. carinii* organisms have been detected in the lymph nodes, spleen, liver, retina, bone marrow, gastrointestinal tract, pancreas, heart, adrenals, and peripheral blood.[62,152,179] A fatal case of disseminated *P. carinii* infection in a 13-month-old infant with thymic alymphoplasia has been reported.[152]

CLINICAL MANIFESTATIONS

The natural course of *P. carinii* infection in children varies greatly and depends primarily on the status of host defenses in individual patients. The onset may be insidious, with a clinical course of 3 or more weeks, or be fulminant and rapidly progressive over a few days.

The clinical course of infantile epidemic pneumocystosis is typified in premature, debilitated, or marasmic infants 2 to 6 months of age. These patients often have chronic diarrhea and weight loss before developing respiratory symptoms. Characteristically, the onset is insidious, with progression of cough, tachypnea, and respiratory distress occurring over a 1- to 4-week interval. Fever is either absent or of low grade in most cases.[39]

Symptoms in immunosuppressed children or adults without AIDS may be more abrupt in onset and more rapidly progressive than in patients in infantile epidemic cases. Even with these patients, the course also varies greatly.[15,54,66,88,153,195] The mortality rate is approximately 100 percent in untreated cases because of the overall severity of the disease in immunocompromised patients.[66] In cases in children and adults, in contrast to infantile cases, fever generally is present and of high grade. It often precedes the onset of nonproductive cough, tachypnea, and severe dyspnea. Fever, tachypnea, and the radiographic appearance of

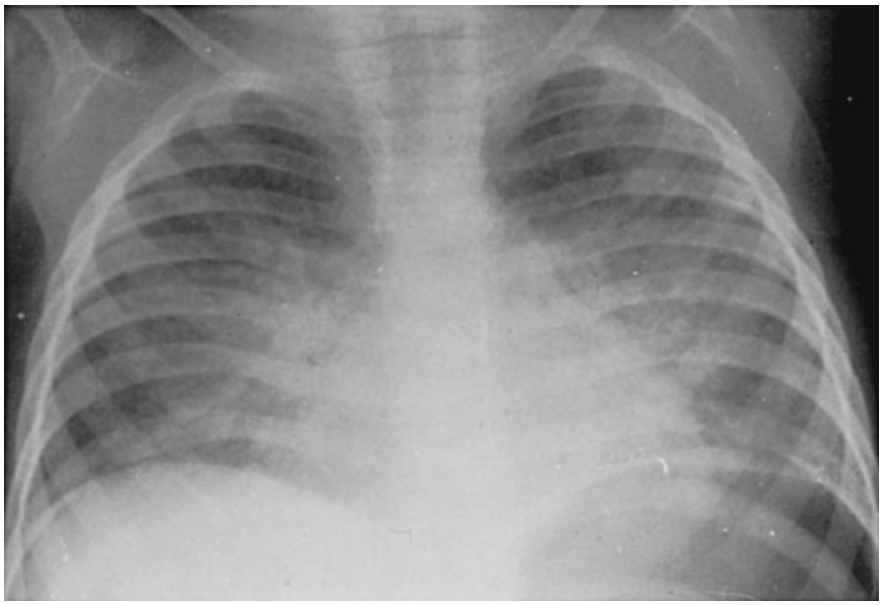

Figure 236–3 Typical diffuse interstitial infiltrates seen in a 20-month-old child with severe combined immunodeficiency disease at the time of evaluation for clinical *Pneumocystis carinii* pneumonitis.

pulmonary infiltrates, in that sequence, 1 to 21 days before diagnosis was established, occurred in a group of children with malignancies.[88] In half of these patients, signs and symptoms occurred within 5 days before initiation of treatment. In a select group of 10 untreated patients, the extent of fever, respiratory distress, and radiographic abnormalities varied from mild to severe. Pulmonary infiltrates were apparent 1 to 13 days before death, and the total course of infection ranged from 4 to 21 days.

The time of onset of clinical disease in non-AIDS, high-risk patients is unpredictable, but disease often occurs after discontinuance or a reduction in the dose of corticosteroid therapy. Rifkind and coworkers[156] noted the clinical onset of infection in transplant recipients when the prednisone dosage was reduced to less than 1 mg/kg body weight per 24 hours. In another series, PCP developed in 9 of 46 patients while steroid therapy was being reduced.[15] The relationship may be to the duration of corticosteroid therapy, however, rather than the withdrawal or reduction in dosage. Patients with severe combined immunodeficiency disease who receive a bone marrow transplant subsequently may contract fulminant PCP when apparent immunologic reconstitution has occurred.[15] These observations imply that the development of clinical disease depends partly on normal inflammatory responses, which may be impaired as a result of the patient's underlying disease, the therapeutic regimen, or both.

Infants and children with AIDS usually are acutely ill with fever (79%), cough (86%), dyspnea (88%), tachypnea (88%), and an alveolar-arterial oxygen gradient greater than 30 mm Hg (95%) at the time of onset of *P. carinii* pneumonitis. The median length of survival after the diagnosis of *P. carinii* pneumonitis was established was only 2 months in a study by Connor and associates.[27]

In children and adults with and without AIDS, physical examination at the time of initial evaluation may reveal tachypnea; nasal flaring; and intercostal, subcostal, or supracostal retractions. An ashen color or cyanosis may be present or may develop rapidly. Auscultation of the chest frequently is characterized by a conspicuous absence of adventitious sounds despite the presence of rapid (80 to 100/min), shallow respirations. Scattered rales, rhonchi, or wheezes most often are detected later in the clinical course as resolution occurs. Aside from variable elevation in temperature, few other physical abnormalities are noted except those referable to pulmonary disease or secondary to the patient's underlying disease or treatment.[15,66,88,195]

Various radiographic abnormalities have been observed in documented cases of isolated PCP.[36,47] These variations result partly from observations at different stages in the course of disease. Bilateral diffuse parenchymal infiltrates (Fig. 236–3) occur most commonly, but no pattern is sufficiently specific either to exclude or to confirm a consideration of *P. carinii* disease. Although initially a reticulogranular interstitial process, *Pneumocystis* pneumonitis progresses to a predominantly alveolar process with coalescence and air bronchogram formation. Late in the course of the disease, lung fields may opacify completely.[36,47] Hilar adenopathy and pleural effusion are not characteristic, unless they are a result of an underlying disorder. During treatment, radiographs show gradual clearing after a variable latent period, during which they may appear worse. Residual interstitial fibrosis occurs in a small percentage of patients. Unusual radiographic findings, including an asymmetric distribution, consolidated lobar infiltrates, pneumothorax and pneumomediastinum, localized parenchymal nodular densities, and pleural effusion, have been documented. One investigator noted the occurrence of at least one "atypical" radiographic finding in 56 percent of 30 cases of PCP.[36] In 23 episodes of PCP in stem cell transplant recipients, 23 percent had nodular infiltrates on the chest radiograph.[181]

DIAGNOSIS

Characteristic clinical features are not sufficiently specific to differentiate PCP from other opportunistic pulmonary infections in highly susceptible pediatric patients. Mixed infections with viral, bacterial, fungal, or parasitic agents have been documented along with *P. carinii*.[15] Implicit in these observations is the importance and urgency of establishing a definitive diagnosis before the institution of specific therapy. An etiologic diagnosis can be ascertained only by the demonstration of *P. carinii* organisms in lung tissue or respiratory secretions.

Numerous techniques have been used to obtain suitable material for diagnostic purposes. Although specimens obtained by noninvasive methods from sputum[48,116] or pharyngeal,[45] tracheal,[109] or gastric[24,178] secretions occasionally reveal *P. carinii* in infected patients, these sources are not sufficiently reliable to exclude the diagnosis if organisms are not identified.[66] Bronchopulmonary lavage,[37,66,137] endobronchial brush biopsy,[46,154] and

transbronchial lung biopsy[63,97] have been used successfully to establish a diagnosis of PCP in adult patients.

Invasive techniques, including open lung biopsy, closed needle biopsy, and percutaneous needle aspiration, are the most reliable methods for confirming a diagnosis.[66,127,158,194] Open lung biopsy provides the most dependable specimen from which identification of the organism and the extent of the infection can be ascertained.[1,204] The chief disadvantage is the need for general anesthesia. A closed needle biopsy procedure is less reliable in providing adequate tissue and is associated with significantly greater morbidity than open thoracotomy.[98] Percutaneous needle aspiration has proved to be a consistent and safe procedure in selected centers.[66,99]

Bronchoalveolar lavage has been safe and successful, especially in patients with AIDS, in whom organisms are in great abundance.[143,65] Bye and colleagues[16] used bronchoalveolar lavage in infants 2 months old. Regardless of the procedure selected, it is crucial to avoid undue delay in establishing a diagnosis before the patient's condition deteriorates sufficiently to preclude any definitive diagnostic procedure.

For diagnostic purposes, the methenamine silver nitrate method of Gomori and the less widely used, but more rapid, toluidine blue O[23] and calcofluor white[180] stains are most useful for showing cyst forms in tissue sections, aspirates, or imprints. For more detailed morphologic study of intracystic sporozoites and trophozoites, polychrome stains, including Giemsa, Wright, Gram, and methylene blue stains, are more suitable.[99] In tissue sections, the Gomori stain in combination with hematoxylin and eosin staining allows study of the organism and host tissue.[79] A direct immunofluorescent monoclonal antibody technique has become commercially available (Merifluor; Meridian Bioscience, Inc., Cincinnati, OH). This technique has no definite advantage over the more conventional methods, although one report describes slightly higher detection rates of *P. carinii* in sputum samples by the immunofluorescence method.[104,105]

Serologic methods, including complement fixation, immunofluorescence,[135] enzyme-linked immunosorbent assay, and latex agglutination, have been developed, but are not useful for diagnostic purposes. A lack of specificity or sensitivity inherent in the procedures themselves, in addition to the likelihood of impaired immune responses in affected patients, precludes interpretation of results in most cases.

A method for the detection of *P. carinii* antigenemia by counterimmunoelectrophoresis was described in 1978.[149] Although this study indicated promise for use of counterimmunoelectrophoresis as a diagnostic test, subsequent studies have yielded conflicting results.

Several investigators[4,38,118,138,163,190,192] have amplified *P. carinii* DNA by polymerase chain reaction (PCR) as a molecular diagnostic method. In a study of bronchoalveolar lavage or lung biopsy specimens (or both) from 47 HIV-infected patients, all of the 18 with documented PCP and 4 of 29 with other pulmonary conditions were positive by PCR analysis (sensitivity 100%, specificity 86%).[182] In a study using nested and quantitative real-time PCR for the amplification of *Pneumocystis* dihydropteroate synthase gene, in 71 confirmed cases of PCP and 70 cases without PCP, the sensitivities and specificities were 94 percent and 81 percent for nested PCR and 94 percent and 96 percent for real-time PCR, favoring real-time PCR ($p = .015$) with few false-positive results.[3] At this time, PCR remains a research tool and is not applicable to general clinical practice.

Hematologic studies are of no diagnostic value, primarily because of baseline abnormalities reflecting the underlying disease of affected patients. Eosinophilia has been reported in isolated cases, but usually is not present. Depressed serum total protein and albumin values frequently are noted because of the poor nutritional status of representative patients.[89] Lactate dehydrogenase activity is increased, but it is not a specific reaction for PCP and is of little diagnostic benefit.

PROGNOSIS, TREATMENT, AND PREVENTION

PROGNOSIS

Before the availability of specific therapeutic agents, the overall prognosis of patients with PCP was poor. Despite supportive care, almost 100 percent of infected patients with underlying neoplastic or immunodeficiency disorders died, whereas approximately 50 percent of infants in the European epidemics died as a result of this pulmonary infection.[72,112,130] To control the European epidemics, Ivady and Paldy[93] first suggested the use of pentamidine isethionate, a diamidine with previously shown antifungal and antiprotozoal activity. Use of this therapeutic agent in infants during the next several years resulted in a dramatic reduction in the mortality rate from 50 to 3.5 percent.[94]

Pentamidine became available to investigators in the United States through the CDC in 1967. During the next 3 years, of 163 children and adults with documented PCP who were treated with pentamidine, 43 percent recovered.[194,199] Of 404 patients to whom the drug was administered for suspected or documented *P. carinii* infection, 189 (47%) experienced significant toxic manifestations. Toxicity ranged from localized reaction at injection sites (18%) to systemic effects, including impaired renal function (24%), liver toxicity (10%), hypoglycemia (6%), hematologic abnormalities (4%), hypotension (10%), and hypocalcemia (1%).[199] Although pentamidine was effective, the high incidence of toxicity emphasized the need for an alternative therapeutic agent.

An immunosuppressed rat model has been useful in the discovery and development of therapeutic and prophylactic drugs for PCP.[50,87] Initial studies of trimethoprim and sulfamethoxazole (co-trimoxazole) in infected rats showed therapeutic efficacy equal to that of pentamidine and successful prevention of *Pneumocystis* infection.[87] The efficacy of co-trimoxazole was shown in the treatment of PCP complicating childhood leukemia.[79,80] A controlled, randomized study involving 50 leukemic children with PCP was reported. Overall recovery rates of 75 percent for pentamidine and 77 percent for co-trimoxazole suggest that these drugs are equally effective in the treatment of PCP in this patient population. No significant toxicity secondary to co-trimoxazole therapy was observed in this investigation.[79] Subsequent studies have confirmed the efficacy of this drug combination in the treatment of pneumonitis.[67,68,108]

TREATMENT

Co-trimoxazole presently is the drug of choice for the treatment and prevention of PCP. A minimum of 2 weeks of therapy is recommended for non-AIDS patients; 3 weeks may be optimal for patients with AIDS. The CDC, the National Institutes of Health, and the Infectious Diseases Society of America have provided consensus guidelines for the treatment of PCP in patients with AIDS (Table 236–1).[20] This scheme also can be applied to non-AIDS patients with PCP. Patients with AIDS have a remarkably high rate of adverse reactions to co-trimoxazole[16] and pentamidine, and should be treated with the alternative drugs recommended in Table 236–1.

Atovaquone, a hydroxynaphthoquinone approved by the U.S. Food and Drug Administration, is effective and has few side effects (rash, nausea, and diarrhea). It can be administered only by the oral route.[83,86] The sulfone dapsone[71] has been evaluated in children[53,122] and may be used alone, but is synergistic with trimethoprim[114] against *P. carinii*. Dapsone

TABLE 236–1 Recommendations for the Treatment of *Pneumocystis carinii* Pneumonia in HIV-Exposed and HIV-Infected Infants and Children

Preferred Therapies and Duration

Trimethoprim-sulfamethoxazole (TMP/SMX) 15-20 mg/kg body weight TMP plus 75-100 mg/kg body weight SMX administered IV or PO 3-4 times daily. After acute pneumonitis has resolved in mild to moderate disease, IV TMP/SMX may be changed to PO

Treatment Duration

21 days for AIDS and about 14 days for non-AIDS, followed by long-term suppressive prophylaxis doses

Alternative Therapies (if TMP/SMX Intolerant or Clinical Treatment Fails after 5-7 Days of TMP/SMX)

Pentamidine 4 mg/kg body weight IV once daily is first-choice alternative regimen. Pentamidine may be changed to atovaquone after 7-10 days IV therapy; *or*

Atovaquone 30-40 mg/kg body weight (maximum 1500-mg dose) PO in two divided doses daily with food; infants 3-24 mo may require a higher dose of 45 mg/kg/day

Other Options and Issues

Dapsone 2 mg/kg once daily (maximum 100 mg/day) plus trimethoprim 15 mg/kg PO in three divided doses daily has been used in adults, but data on children are limited

Primaquine base 0.3 mg/kg PO once daily (maximum 30 mg/day) plus clindamycin 10 mg/kg IV or PO (maximum 600 mg administered IV and 300-450 mg administered PO) q6h has been used in adults, but data in children are unavailable

Indications for Corticosteroids

Pao_2 <70 mm Hg at room air or alveolar-arterial oxygen gradient >35 mm Hg

Prednisone 1 mg/kg PO twice daily for 5 days, then 1 mg/kg PO daily for 5 days, then 0.5 mg/kg PO daily for days 11-21

Secondary Prophylaxis

Recommended for lifetime after initial therapy course, or until immunodeficiency has resolved

From Centers for Disease Control and Prevention: Guidelines for the prevention and treatment of opportunistic infections among HIV-exposed and infected children: Recommendations from CDC, the National Institutes of Health and the Infectious Diseases Society of America. M. M. W. R. (in press). Available at http://aidsinfo.nih.gov/contentfiles/Pediatric_01.pdf (accessed 22 Dec. 2008).

may cause rashes, neutropenia, thrombocytopenia, anemia, and methemoglobinemia.

Several other promising drugs are in various stages of clinical evaluation, although none has been investigated systematically in children. Agents with evidence of efficacy include pyrimethamine plus sulfadoxine,[101,160] trimetrexate,[2] and clindamycin plus primaquine.[161] Animal studies show that the combination of erythromycin-sulfisoxazole has strong anti–*P. carinii* activity through a synergistic mechanism.[84]

Some studies in adults with AIDS and *P. carinii* pneumonitis suggest that the administration of corticosteroids early in the course of moderately severe pneumonitis reduces the occurrence of respiratory failure and improves oxygenation.[28] Other studies show no benefit from corticosteroids.[35] More limited studies of this supportive therapy have been reported to increase survival in children.[6,174] If used, a reasonable approach (see Table 236–1) seems to be to withdraw corticosteroids as soon as pulmonary function has become stabilized.

PREVENTION

P. carinii pneumonitis can be prevented in more than 95 percent of patients at high risk for acquiring the disease.[85,90] Patients in high-risk groups, such as patients with cancer, acquired and congenital immunodeficiency disorders, and organ transplants, should be placed on a chemoprophylaxis regimen throughout the risk period. Underlying non-AIDS conditions at high risk for associated acquisition of PCP are lymphoproliferative malignancies, solid tumors with intensive immunosuppressive therapy, corticosteroid therapy, bone marrow transplantation, certain solid organ transplants, prior episode of PCP, severe malnutrition, brain tumor on intensive immunosuppressive therapy, severe combined immunodeficiency syndrome, certain other congenital immunodeficiency disorders, and $CD4^+$ T-lymphocyte counts less than 200/μL.[75,158] Revised guidelines from the CDC, National Institutes of Health, and Infectious Diseases Society of America for *P. carinii* prophylaxis in infants and children with AIDS[20] are given in Table 236–2, and generally can be applied to non-AIDS patients, although the significance of the $CD4^+$ lymphocyte has not been well established in this group.

Because of the high risk for acquiring PCP during the first year of life, often before HIV infection is recognized,[169,170] PCP prophylaxis should be initiated at 4 to 6 weeks of age in all infants

TABLE 236–2 Recommendations for *Pneumocystis carinii* Pneumonia Prophylaxis for HIV-Exposed Infants and HIV-Infected Children

	Preventive Regimens	
Indications	***First Choice***	***Alternatives***
HIV-infected or HIV-indeterminate* infants 1-12 mo	Trimethoprim-sulfamethoxazole 150/750 mg/m^2/day in 2 divided doses PO 3 times/wk on consecutive days	Dapsone (≥1 mo), 2 mg/kg (maximum 100 mg) PO qd, or 4 mg/kg (maximum 200 mg) PO per wk
HIV-infected children 1-5 yr with $CD4^+$ count <500/μL or $CD4^+$ percentage <15%	Acceptable alternative schedules	Aerosolized pentamidine (≥5 yr), 300 mg every month via Respirgard II nebulizer
HIV-infected children 6-12 yr with $CD4^+$ count <200/μL or $CD4^+$ percentage <15%	Single dose PO 3 times/wk on consecutive days	
Previous PCP	2 divided doses PO qd	Atovaquone 30 mg/kg PO qd (1-3 mo and >24 mo), 45 mg/kg PO qd (4-24 mo)
	2 divided doses PO 3 times/wk on alternate days	

**No prophylaxis for infants <1 month because of the rarity of* Pneumocystis carinii *pneumonia (PCP) at this age. Human immunodeficiency virus (HIV) infection can be reasonably excluded in infants who have had two or more negative HIV diagnostic tests (i.e., HIV culture or polymerase chain reaction, both of which are performed at ≥1 month old and one of which is performed at ≥4 months old, or two or more negative HIV IgG antibody tests performed at >6 months old in children who have no clinical evidence of HIV disease. Prophylaxis is not recommended for infants who meet criteria for HIV-uninfected and presumptively uninfected with HIV status.*

From Centers for Disease Control and Prevention: Guidelines for the prevention and treatment of opportunistic infections among HIV-infected persons—2002. Recommendations of the U. S. Public Health Service and Infectious Diseases Society of America. M. M. W. R. (in press). Available at http://aidsinfo.nih.gov/contentfiles/Pediatric_01.pdf (accessed 22 Dec. 2008).

born of HIV-infected women, regardless of $CD4^+$ lymphocyte cell counts. The use of chemoprophylaxis in this age group has been highly effective in HIV-infected infants.[157] When infants are shown not to be infected with HIV, prophylaxis may be discontinued. HIV-infected infants and infants with undetermined status should be continued on prophylaxis during the first year of life. In addition, at 1 year of age and subsequently, the use of chemoprophylaxis is based on the $CD4^+$ lymphocyte count and other AIDS-defining features (see Table 236–2).

Co-trimoxazole given 3 days per week is the preferred drug for chemoprophylaxis. For patients unable to take co-trimoxazole, dapsone (2 mg/kg/day or 4 mg/kg/wk) is suggested.[53,131] Studies in adults show that daily doses of dapsone[124] or one dose of dapsone per week[82] is effective prophylaxis.[122] An alternative to dapsone is aerosolized pentamidine (300 mg via Respirgard II inhaler monthly).[132] The dosage for aerosolized pentamidine is the same as the dosage for adults.[113,131] Atovaquone is effective and safe in children[31] and adults.[44] A controlled study compared prophylaxis with atovaquone and azithromycin with co-trimoxazole in 366 high-risk, HIV-infected children.[77] The regimens were similar in effectiveness in prevention of bacterial infections, and PCP occurred with equal frequency. Table 236–2 summarizes the approach to selection of patients at risk.

Several studies in adults[41,51,166,200] show that PCP prophylaxis can be discontinued safely in patients responding to HAART with a sustained increase in $CD4^+$ T lymphocytes from less than 200 cells/μL to greater than 200 cells/μL. Similar consideration may be given to children in accordance with age-related $CD4^+$ T-lymphocyte counts (see Table 236–2).

Although currently available anti–*P. carinii* drugs may prevent activation of latent infection, they do not eradicate the organism. Patients are protected from pneumonitis only while receiving chemoprophylaxis and become susceptible again when use of the drugs is discontinued. No vaccine is available, although mice immunized with a recombinant mouse *P. carinii* antigen exhibited evidence of reduced infection.[198]

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SUBSECTION 2

Nematodes

CHAPTER 237

Parasitic Nematode Infections

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INTESTINAL NEMATODES

The three major intestinal nematodes of children, *Ascaris lumbricoides*, *Trichuris trichiura*, and the hookworms, together have a substantial impact on the health and well-being of children living in less developed nations of the world.[1] This "unholy trinity" of soil-transmitted helminths (STHs), or "geohelminths," deprives hundreds of millions of young children of their full intellectual and growth potential. Current estimates suggest that 2 billion people are infected with STHs, 300 million of whom have severe morbidity. An estimated 400 million schoolchildren are infected with STHs. Building on studies from the early part of the 20th century,[9,10] extensive new data from many different geographic regions confirm that chronic intestinal nematode infections acquired during childhood suppress cognitive and intellectual development,[3,7,10] and impair physical growth and fitness.[4,11-13] Because many of these detrimental effects are reversible with the use of anthelmintic drugs to eliminate nematodes from the intes-

tinal tract,[12,13] several investigators and international relief agencies have advocated the administration of benzimidazole anthelmintics (sometimes known as "deworming") as a cornerstone of public health programs directed at school-age children.[1,2,8]

A 2001 World Health Assembly resolution urges its member states to deworm at least 75 percent and up to 100 percent of school-aged children at risk for acquiring STH infections (www.who.int/wormcontrol). Because in highly endemic areas reinfection with *A. lumbricoides*, *T. trichiura*, and hookworms sometimes occurs within 6 months of receiving anthelmintic treatment,[14] however, this strategy is not always a practical means of control, unless the drugs are used frequently. Concern about the potential for emerging anthelmintic drug resistance also exists. Some researchers have pointed to the use of genetically engineered antihelminth vaccines as one possible solution to the problem of worm re-infection and disease during childhood.[5] Still another public health concern about the chronic effects of STH infections is new evidence suggesting that they may promote the susceptibility to, or worsen the severity of, malaria and human immunodeficiency virus/acquired immunodeficiency syndrome.[6] Interest has developed in examining the impact of large-scale deworming on these killer diseases.

In addition to the chronic effects seen when children harbor numerous intestinal nematodes are the increasingly recognized unique neonatal and infantile syndromes that new information indicates may result from vertical transmission of the infective stages of some intestinal nematodes, probably in colostrum and breast milk.[1,4] The best documented example of vertical transmission in infants results in the "swollen belly syndrome" caused by *Strongyloides fuelleborni*, although other perinatal nematode infections probably also occur (discussed later). Finally, children experience significant morbidity during zoonotic transmission of the infective stages of intestinal nematodes of companion animals such as dogs and cats. The resultant aberrant migration of these foreign nematode larvae (visceral larva migrans) has become a major pediatric public health problem in large urban areas of the United States and Europe.

ASCARIS LUMBRICOIDES

Ascariasis is one of the most prevalent infections in the world,[21] with an estimated 800 million cases in the developing world.[1] Accurate determinations based on 1,477,742 fecal examinations conducted during the 1990s indicated that more than 500 million cases of ascariasis occur in China alone[34]; however, revised estimates now suggest that those numbers have declined significantly, especially in economically developed regions of Eastern China.[1] Currently, the greatest number of cases of ascariasis occur in Southeast Asia and the Indian subcontinent and sub-Saharan Africa.[1] Surveys conducted in the rural southern communities of the United States during the 1970s indicated that the prevalence of ascariasis was 20 to 67 percent.[16] Those rates most likely have declined significantly over the past few decades, however.

Ascaris infection, when relatively light, is usually inapparent until the patient passes a worm through the rectum. In heavy infections, constitutional symptoms may occur during the early phase, and intestinal malabsorption and obstruction may occur in the later phase. The infection is acquired by ingestion of the infective eggs, which hatch in the upper part of the small intestine and free the larvae. The larvae penetrate the intestinal wall, reach venules or lymphatics, and pass through the portal circulation to the liver, the right side of the heart, and the lungs.

In the lungs, the larvae break out of the capillaries and begin ascending through the respiratory radicles until they reach the glottis; passing over the epiglottis, they enter the esophagus and are carried down to the small intestine, where they mature and become adult worms. The adult female *Ascaris* produces huge numbers of eggs—possibly 200,000 a day. To ensure adequate quantities of egg-requiring cholesterol, the parasite sequesters oxygen through a specially modified hemoglobin.[22] The entire cycle, beginning with the infective eggs and resulting in ovipositing females, lasts approximately 2 months. Infection is maintained in the community by the deposition of human stool in soil, which permits embryonated eggs to develop into the infective stage. This process takes approximately 2 weeks. The high prevalence of infection results not only from deficient sanitary facilities for disposal of human excreta, but also from the deliberate use of human feces as fertilizer.

EPIDEMIOLOGY

In many regions of the developing world, *Ascaris* eggs are almost ubiquitous in the environment. All ages are affected by the parasite; young children, who are exposed more often to the contaminated soil, are affected most frequently. These children usually harbor greater numbers of adult worms in their intestine than do adults living under similar conditions.[11-13,20] High worm burdens in children also occur with *Trichuris* infections (see later). Because the phenomenon of heavy *Ascaris* and *Trichuris* worm burdens in childhood is found throughout most of the developing world, pediatric predisposition to "worminess" is widely thought to have a possible genetic or immunologic basis.[4]

Ascaris eggs are extremely hardy and resistant to extremes of temperature and desiccation, which may explain the common finding of high rates of ascariasis in impoverished urban environments (e.g., Guatemala City, Mexico City) and in rural environments. The eggs also are resistant to chemical disinfectants and are not destroyed readily by sewage treatment. In some areas, pigs may serve as a reservoir for zoonotic *Ascaris* infection.[15]

PATHOPHYSIOLOGY

During the migratory phase of the infection, the larvae evoke an inflammatory response associated with eosinophilic infiltration. *Ascaris* antigens—so-called ABA-1 allergen—released during the molting of larvae evoke an immune response, and IgE antibodies directed against ABA-1 may be associated with resistance.[30] *Ascaris* larvae also release immunomodulatory glycosphingolipids that inhibit T_H1 responses.[1,20] A controversial role of IgE anti-*Ascaris* antibodies is in protecting children against atopic states, including asthma.[29] During the intestinal stage of infection, symptoms derive primarily from the physical presence of the worms in the gut, from aberrant migration into other lumina, or from perforation into the peritoneum. As a protective mechanism for its own survival, *Ascaris* secretes peptides that block the action of pancreatic digestive enzymes (trypsin, chymotrypsin, elastase),[23,26] which may play a role in parasite-associated nutrient malabsorption, including the malabsorption of vitamin A.[19] Ascariasis also results in lactose intolerance.[19] Whether these phenomena provide the basis of observed *Ascaris*-associated physical growth retardation during childhood[3,11-13,20,31] is unknown.

CLINICAL MANIFESTATIONS

The degree of disease induced by the migratory phase of *Ascaris* is related directly to the number of larvae migrating simultaneously. In light infections, this phase is typically unrecognized. Heavy infection, such as that induced in himself by Koino,[28] who swallowed 2000 infected eggs, may cause severe pneumonitis. In

some regions of the world, pediatric *Ascaris* pneumonitis is seasonal.[1]

In the lumen of the intestine, *Ascaris* adult worms may become matted together and form a bolus large enough to cause intestinal obstruction, sometimes leading to bowel infarction and intestinal perforation. This is a particular problem in very young children because of the small luminal diameter of their intestines.[27] The incidence of this complication has been estimated at 2 per 1000 infected children per year.[16] Children with *Ascaris* obstruction exhibit a toxic appearance, often with signs and symptoms of peritonitis.[1] When recognized early, the obstruction can be treated with medical management, but in many cases surgical intervention is mandatory.

Hepatobiliary and pancreatic ascariasis (HPA) results from blockage of the bile duct and pancreatic duct. Patients with HPA are subject to cholecystitis, acute cholangitis, "biliary colic," acute pancreatitis, or hepatic abscess.[27] In contrast to intestinal obstruction, HPA tends to be more common in adults, especially women.[27] Certain irritants, such as halogenated hydrocarbons (e.g., carbon tetrachloride and tetrachloroethylene, used in the past to treat certain hookworm infections), elevation of body temperature, and general anesthesia, have been known to precipitate aberrant migration. *Ascaris* screening by fecal examination should be considered before performing elective surgery on a child who might have immigrated recently from an impoverished area of the tropics.

Whatever the mechanism of interference by *Ascaris* with growth or nutrition, treatment with anthelmintics leads to substantial catch-up growth in previously parasitized children.[3,11-13,29,31] Ascariasis in pregnant women results in intrauterine growth retardation.[33] Most individuals with light infections rarely are symptomatic (they become aware of the parasites by passage of adult worms in stool or through regurgitating and vomiting the adult worms), although researchers have conjectured that even these individuals may exhibit subtle deficits in cognitive and intellectual development.[3]

Several cases of neonatal ascariasis have been described in the literature.[17,18] The mode of acquisition of these infections is unknown, but canine and feline ascarid infections commonly are acquired by a transplacental route, suggesting the possibility that this route also may occur in humans.

DIFFERENTIAL AND SPECIFIC DIAGNOSES

The differential diagnosis of pneumonia caused by *Ascaris* suggests a parasitic etiology because of the peripheral eosinophilia. Any nematode with a migratory phase through the lungs can mimic this infection, however. *Ascaris* must be considered as a cause of intestinal obstruction in any geographic locale where its prevalence is high.

The diagnosis of intestinal ascariasis is established by identifying the characteristic ascarid eggs through microscopic examination of stool. Efforts have been made more recently to detect *Ascaris* metabolites in the urine by gas-liquid chromatography.[24,25] HPA is suspected in heavily infected children who have signs of biliary obstruction. Ultrasonography and endoscopic retrograde cholangiopancreatography are useful adjunctive diagnostic procedures for these conditions.[27]

TREATMENT

For an ordinary *Ascaris* infection, either albendazole administered at a fixed dose of 400 mg once or mebendazole administered at a fixed dose of 100 mg twice daily for 3 days is effective. A single fixed dose of 500 mg of mebendazole also may be effective, but it may be unsuitable for the treatment of other STH co-infections. As an alternative, pyrantel pamoate administered in a single dose of 11 mg/kg, not to exceed 1 g, is effective. The benzimidazoles and pyrantel pamoate are not approved for children younger than 2 years old, and all the printed statements caution against the use of these drugs in the younger age group. An examination of the use of benzimidazoles in children 12 to 24 months old concluded, however, that these agents are probably safe and should be used if the circumstances warrant it.[31] In such cases, the dose of albendazole in these young children should be reduced to 200 mg.[1] The risk of treatment in this younger age group currently is undergoing more rigorous investigation because of mounting evidence that the growth and cognitive delay caused by STHs may be corrected with albendazole or mebendazole.[11-13,31,32] These drugs have a potential for embryotoxicity, however, so judicious use in young children is warranted. The World Health Organization is evaluating the use of benzimidazoles during pregnancy, particularly for use during the second and third trimesters.

In cases of intestinal obstruction, piperazine citrate may be effective because this drug paralyzes the myoneural junction of *Ascaris* and may result in relaxation of the matted bolus of worms. It is antagonistic to pyrantel pamoate, and these two drugs should not be administered together.

Management of intestinal obstruction or HPA often is surgical. For some patients, biliary decompression may be performed with endoscopic retrograde cholangiopancreatography.[27]

PROGNOSIS

The prognosis is excellent in most cases of ascariasis. In patients with obstruction or perforation, the prognosis depends entirely on the speed of recognition and therapy.

PREVENTION

Ascaris infection could be eliminated entirely through proper disposal of human excreta. As an isolated means of health improvement in the world, this approach seldom is successful. Elimination of *Ascaris* from a community or a substantial reduction in the incidence of this infection usually occurs after general poverty reduction efforts and improvements in the standard of living have been achieved. Periodic administration of community-wide therapy with anthelmintics has been effective in reducing worm burden as a short-term strategy. In the absence of aggressive sanitation and other control measures, however, re-infection can occur within 6 months after treatment in areas of high transmission,[14] although in areas of periodic therapy the intensity of infection is decreased, even if the prevalence is little affected.

TRICHURIS TRICHIURA

Trichuriasis has a prevalence approximating that of the other major intestinal nematode infections, or approximately 600 million cases worldwide,[1] but the infection usually is asymptomatic because in most cases it is light. Children with so-called asymptomatic light infection may have deficits in cognition, however.[3,7,44]

The infection is acquired by the ingestion of embryonated eggs acquired from the soil on hands or through contaminated food. The eggs hatch in the upper part of the small intestine, and the liberated larvae penetrate the villi. In contrast to the larvae of *Ascaris*, *Trichuris* larvae do not undergo extraintestinal migration, but remain in situ for approximately 1 week, at which time they begin a progressive descent into the cecum and the colon.

They mature there, and the attenuated anterior end of the adult worm embeds itself in the colonic mucosa. Creation of syncytial tunnels (derived from the columnar epithelium of the colon) is facilitated by the release of a parasite-derived, pore-forming protein.[38] The parasites derive their nourishment from these colonic mucosal tunnels. The entire cycle from the ingestion of embryonated ova to the development of sexually mature adults takes approximately 2 months. Persistence of the infection in a community depends on continual contamination of the soil with human feces.

EPIDEMIOLOGY

These parasites are found most commonly throughout the developing countries of the tropics and subtropics. The distribution of this worm often parallels that of *Ascaris*. In addition, as in the case of *Ascaris*, children with trichuriasis usually harbor greater numbers of worms than adults living under similar conditions.[35,36] Consequently, children have greater morbidity from trichuriasis than adults do. The mechanistic basis of added worminess in children is unknown, although researchers have observed that for all of the soil-transmitted helminthiases, including trichuriasis, infections are aggregated, with a few children having particularly heavy infections. These heavily infected children seem to have a genetic or immunologic predisposition to *Trichuris* infection.[35,36] Children also are the major source of *Trichuris* eggs in the environment, which when deposited in the soil become infective in approximately 1 month and remain viable for several months. They are killed by exposure to temperatures greater than 40° C within 1 hour. Freezing temperatures less than –8° C also destroy these eggs. Similar to ascarid eggs, they are resistant to chemical disinfectants.

PATHOPHYSIOLOGY

At the site of attachment, the adult worm elicits characteristic changes in the colonic mucosa as noted earlier. Inflammatory cells also are found at these sites, but they do not seem to account entirely for the clinical resemblance of trichuriasis to some forms of inflammatory bowel disease.[35,42] Similar to many other intestinal worms, *Trichuris* is expelled through the action of the host's immune system. This expulsion results from a combined effect of antibody and lymphoid cells.[45] Regulation of *Trichuris* populations in the gut has been ascribed to a carefully orchestrated balance of host-derived cytokines.[39] Although anemia has been attributed to trichuriasis,[40] the amount of blood loss caused by this parasite is much less than hookworm-associated blood loss and is insufficient to account for anemia.[41] One possibility is that *Trichuris*-induced anemia results from chronic inflammation, similar to the anemia of inflammatory bowel disease.[35,36]

CLINICAL MANIFESTATIONS

Two major disease syndromes are caused by heavy *Trichuris* infection during childhood.[35,36] *Trichuris* dysentery syndrome (TDS) is associated with severe diarrhea with blood and mucus. Children with TDS are anemic and frequently manifest growth retardation and failure to thrive.[37] Infants and toddlers with TDS are at risk for protracted tenesmus, which leads to rectal prolapse. *Trichuris* colitis is a more chronic manifestation of moderate to heavy infection that is characterized by a form of inflammatory bowel disease similar to what occurs in Crohn disease or ulcerative colitis. Children with this form of colitis also can have chronic malnutrition and short stature. Moderately and heavily infected (and possibly even lightly infected) children are at risk for having deficits in cognition and intellectual development.[44]

DIFFERENTIAL AND SPECIFIC DIAGNOSES

The diagnosis is established by identification of the characteristic barrel-shaped eggs through microscopic examination of stool. Clinically, heavily infected children with TDS may resemble children with amebic or bacillary dysentery. Children with *Trichuris* colitis may have signs and symptoms that resemble other forms of inflammatory bowel disease. The erythrocyte sedimentation rate is not elevated in children with *Trichuris* colitis, however.

TREATMENT

In the past, when noxious or toxic drugs had to be used, only patients with heavy infection were treated. Currently, any patient with this infection can be treated by the administration of either albendazole, administered as a single fixed dose of 400 mg (except for very heavy infections, in which consecutive doses for 3 days may be required), or mebendazole, 100 mg twice daily for 3 days.[43] A single dose of 500 mg of mebendazole also is used for mass drug administration in some developing countries. The dose does not need to be adjusted to the weight of the patient because the drug is not well absorbed from the gastrointestinal tract. As noted earlier, the safety of the benzimidazoles in very young children has not been established, but this may not preclude its use in the practice of pediatrics.[26] As an alternative, the drug oxantel is effective for the treatment of trichuriasis. In some countries, oxantel is formulated with pyrantel pamoate.[35]

PREVENTION

As in the case of other nematodes, sanitary disposal of excreta—but more important, improvement in the standard of living—tends to reduce the incidence of infection. Frequent mass treatments with mebendazole reduce the worm burden in a community.

HOOKWORMS

Hookworm infection is one of the most important infections of adults and children in the developing world, with an estimated prevalence of approximately 600 million cases.[1,5] Hookworms exert their pathogenic effect by causing intestinal blood loss, which leads to iron-deficiency anemia.[51] By considering the average daily blood loss induced by each worm, a moderate hookworm infection comprising 40 adult hookworms produces sufficient blood loss to rob a child of his or her daily iron requirement. Because loss of blood represents loss of erythrocytes *and* plasma, heavy infections also contribute to protein malnutrition.[51]

Two major species of hookworms infect the human intestine: *Ancylostoma duodenale* and *Necator americanus*, although *N. americanus* is found more commonly worldwide, especially in sub-Saharan Africa, Southeast Asia, and the Americas.[51] Two other members of the genus *Ancylostoma*, *Ancylostoma ceylanicum* and the dog hookworm *Ancylostoma caninum*,[52] are much less frequent causes of intestinal pathology in humans.[51]

The infection is acquired either by exposure of skin to moist soil infested with the larvae of these worms (*A. duodenale* and *N. americanus*) or by ingestion of the infective larvae (*A. duodenale* only). The most propitious circumstances for infection are shady areas and sandy or loamy soil. Infection is particularly likely to

occur early in the morning, when the ground is moist with dew, or after a rainfall. After the larvae enter the host, they initiate a developmental program that continues until they enter the intestine.[4,51] This process is coupled to the release of parasite-derived proteases and other virulence factors.[46,57] Larvae that enter through the skin are carried by the venous circulation to the right side of the heart and there follow the route described for *Ascaris*, whereas larvae that are ingested may develop entirely within the gastrointestinal tract.

On reaching the small intestine, the larvae mature to adult worms, which become attached with their mouth parts to the intestinal mucosa. The worms sustain themselves by releasing hydrolytic enzymes that degrade the intestinal mucosa and then feed on cellular and connective tissue debris.[51] During this process, capillaries and arterioles are eroded and lacerated, with subsequent extravasation of blood. The adult worms also ingest hemoglobin in blood through the action of parasite gut brush border proteases.[56] Anticoagulants that block the activity of host factor Xa and VIIa/tissue factor facilitate blood flow and are responsible for continued bleeding from the original site after the worm has moved to a new one.[47]

The entire cycle from penetration of the skin or ingestion of larvae to the development of mature worms usually takes approximately 6 to 8 weeks. At that time, hookworm eggs appear in the feces. *A. duodenale* larvae also may undergo a period of developmental arrest within the human host that lasts weeks or months. Intestinal ancylostomiasis can occur up to 1 year (and possibly longer) after initial exposure to infective larvae has occurred.[55] Investigators have conjectured that the reservoir of arrested *A. duodenale* larvae enters the mammary glands and breast milk.[50] This sequence of events may account for cases of infantile ancylostomiasis noted in Africa, India, and China.[50] Humans are the major reservoir of these organisms, and this infection is maintained by continual contamination of soil by human feces.

EPIDEMIOLOGY

Although in ancient times these parasites had a worldwide distribution, they currently are most prevalent in areas of rural poverty in the tropics and subtropics. *A. duodenale* predominates in many parts of the Indian subcontinent and focal areas of China north of the Yangtze River.[4] *N. americanus* is the predominant hookworm in the world, especially in the Western Hemisphere, most of Africa, China south of the Yangtze River, Southeast Asia and Indonesia, and certain islands of the Pacific. *A. duodenale* also is a major parasite in Egypt and other parts of the Mediterranean region, Africa, and a few focal areas in South America, including Paraguay and northern Argentina. A small Central American focus also exists in southern Honduras and El Salvador, and one exists among Aboriginal populations in Australia. This differential distribution is not always absolute, and small numbers of either parasite can be present where the other predominates. Mixed infections with both species are common. *A. ceylanicum* occurs in focally endemic areas of southern Asia,[51] whereas the dog hookworm *A. caninum* has been described as a cause of human eosinophilic enteritis in Australia and possibly elsewhere.[52]

Larvae survive in soil for 6 weeks. They are destroyed by drying, freezing temperatures, and heat greater than 45° C. Hookworm infection occurs in areas with high agricultural intensity and is not found frequently in urban areas, where *Ascaris* and *Trichuris* might predominate. Because shade and moisture are essential for survival at the infective larval stage, a not surprising finding is high rates of hookworm infection in families that harvest tea in India and Bangladesh, mulberry leaves (for the silkworm industry) in eastern China, sweet potatoes and corn in western China, coffee and bananas in Central and South America, and rubber in Africa.

Similar to other STHs, hookworm infections usually are aggregated such that most individuals harbor light worm burdens, whereas a substantial minority harbor moderate or heavy infections.[51,55] Even after receiving specific anthelmintic chemotherapy, moderately and heavily infected individuals seem to be predisposed to the reacquisition of heavy infection.[55] A predisposition to hookworm infection may have a genetic or immunologic basis.

In contrast to the other STHs, high rates of hookworm infection occur in adults and children. In some regions, the age-associated prevalence and intensity increase linearly, with the heaviest infections occurring in elderly populations.[48]

PATHOPHYSIOLOGY

During the migratory phase of the infection, the larvae evoke an inflammatory response associated with eosinophilic infiltration. Immune responses to the infection have been difficult to study in humans, but in an animal model, a dog infected with *A. caninum* can be rendered immune to challenge infection by receiving repeated dosing with the infective larvae. This observation has provided a means of producing a live attenuated larval vaccine in the laboratory, but one that is unsuitable for humans. Reproduction of this larval vaccine effect by using specifically genetically engineered polypeptides is in progress.[5,46,49]

The major source of injury to the host is loss of blood, with *A. duodenale* producing greater blood loss than *N. americanus*. Rarely, massive bleeding has been reported.[53] Iron-deficiency anemia results when iron loss exceeds the host's iron reserves. Because children and women of reproductive age generally have lower iron reserves than do other populations, they are at the highest risk for developing hookworm disease and anemia.[51] Long-standing moderate and heavy infections result in the characteristic features of severe iron deficiency. Loss of protein contributes further to malnutrition. Together the iron and protein malnutrition result in impaired growth and physical fitness.[9,11-13,51] In some cases, hypoproteinemia can be corrected by a high-protein diet without deworming the patient,[53] although the World Food Program and other international agencies now frequently incorporate deworming into their nutritional supplementation programs. In addition to the physical deficits noted earlier, hookworms may affect cognitive development because iron is important for the development of dopaminergic neurons and for the biosynthesis of some neurotransmitters.[51]

CLINICAL MANIFESTATIONS

Penetration by larvae causes "ground itch" or "dew itch," a pruritus that occurs after walking in the morning dew. These cutaneous manifestations can occur on almost any part of the body. This phase of early infection is followed within days to weeks by a pneumonitis, which typically is less pronounced than *Ascaris* pneumonitis.

The acute intestinal phase in heavy infections is characterized by abdominal pain, diarrhea, nausea, and anorexia.[50] Eosinophilia occurs approximately at the onset of the intestinal phase. Subsequently, well-nourished individuals with light infections have mild gastrointestinal symptoms, but no evidence of anemia or malnutrition. At the other extreme, heavily infected children can have hemoglobin values of 2 g/dL and edema or anasarca caused by hypoproteinemia. Infants with severe ancylostomiasis can exhibit failure to thrive, profound pallor, and melena.[50]

Deficits in physical and intellectual growth as a result of chronic hookworm infection in childhood have been reported

since the early part of the 20th century.[3,4,9,10,51] Some recovery has been described after "deworming," although some deficits occurring in infancy may be irreversible.

DIFFERENTIAL AND SPECIFIC DIAGNOSES

Hookworm pneumonitis resulting from the early migratory stages typically is nonspecific in its presentation and difficult to diagnose. Hookworm anemia is caused by blood loss; it must be distinguished from all other causes of intestinal loss of blood. In the developing countries, where severe hookworm anemia is common, the probability of the occurrence of rarer causes of intestinal blood loss, such as Meckel diverticulum and polyps, is low. The opposite holds true for regions where hookworm infections are light or infrequent. In sub-Saharan Africa, the distribution of hookworm overlaps with other significant causes of so-called agricultural anemias, including malaria, schistosomiasis, malnutrition, and hemoglobin polymorphisms.[6]

The diagnosis is established by identifying the characteristic eggs by microscopic examination of stool. The eggs of *A. duodenale* and *N. americanus* cannot be distinguished from each other by morphologic criteria, but the worms can be differentiated by direct examination of the infective larvae or adults. One must assume that it is one or the other species on the basis of the geographic origin of the patient. Although this decision is not of paramount importance, it does have some therapeutic implications, including the possibility that arrested larvae of *A. duodenale* would become reactivated and either repopulate the intestine at some point after treatment or enter breast milk during or after parturition.

TREATMENT

For *N. americanus* infection, the drug of choice is either albendazole, administered at a fixed dose of 400 mg, or mebendazole, given as 100 mg twice daily for 3 days. Pyrantel pamoate given as a dose of 11 mg/kg, not to exceed 1 g, for 3 days is a suitable alternative drug. In areas endemic for ancylostomiasis, the health care provider should be aware of the potential of arrested tissue larvae to repopulate the intestine or enter breast milk and infect infants during the perinatal period. Iron supplementation and transfusion are occasional important adjunctive therapies for severe hookworm disease.

PREVENTION

As in all cases of all STH infections, the sanitary control of the disposal of excreta represents an important public health control measure. Accomplishing effective sanitation in resource-poor settings has not been feasible in most of the developing world. The popular recommendation to wear shoes is naive because *N. americanus* larvae also enter through the upper extremities, torso, and legs, and because *A. duodenale* also is orally infective. Studies to develop and test a first-generation recombinant hookworm vaccine are in progress.[5,46,49]

ENTEROBIUS VERMICULARIS

Pinworm infection (enterobiasis), or oxyuriasis (the older term), is one of the most frequent of all human helminth infections and one that is common in North America and Europe.[63] Some investigators think that the overall prevalence of enterobiasis is declining in the United States, however. Most infected individuals are children, and the infection is found in all socioeconomic classes. It is acquired by the ingestion of infective eggs picked up on the perianal skin, in the air, or on bedclothes and underwear. Swallowed eggs, transmitted to the mouth by fingers or through inhalation, hatch in the duodenum, and the liberated larvae undergo additional maturational steps in the small intestine before reaching the cecum. There, the sexually mature worms copulate and proceed to the rectum and eventually to the perianal skin, where the gravid females lay eggs. The eggs become infective within 2 to 4 hours after deposition.

The entire cycle from ingestion of the egg to the egg-laying phase of the gravid female is 4 to 6 weeks. Rarely, a retrograde infection occurs in which eggs hatch on the anal mucosa, and the larvae migrate up the bowel and mature to adult worms. Although enterobiasis is a human infection, anthropoid apes can be infected experimentally.

EPIDEMIOLOGY

The infection is worldwide in distribution, and children are infected most frequently. Communal living, especially assembling in school gymnasia and living in crowded households, promotes transmission of the infection. Adults tend to be infected through their contact with children; parents and teachers are the most vulnerable. Orphanages and daycare centers frequently are affected.[63]

PATHOPHYSIOLOGY

No intestinal reactions occur during the migratory phase, and because no tissue migration takes place, no eosinophilia occurs to the degree seen with some other nematodes. Occasionally, hypersensitive individuals may have a slight increase in eosinophils. The deposited ova induce pruritus on the perianal skin. No evidence indicates that some individuals are more susceptible than others to this infection.

Enterobius occasionally has been found in vermiform appendixes removed at surgery. A causal relationship to appendicitis has not been established, but some evidence points to the possibility that this worm induces granuloma formation and may cause obstruction of this vestigial structure.[58]

When the adult gravid female migrates along the perineal skin into the vagina, it may cause vulvitis as a reaction to the eggs deposited in that region. Some investigators speculate that migrating pinworms may introduce bacteria into the lower urinary tract and result in urinary tract infection.[62] Other aberrant infections, such as those causing hepatic granuloma, occur more infrequently.[61]

CLINICAL MANIFESTATIONS

Pruritus is the most common symptom; its intensity varies from mild itching to acute, intractable pain. Secondary cellulitis also may occur in severely pruritic cases.[60] Vaginal discharge and vulval itching are symptoms in the rare cases in which a worm has migrated into the vagina. Insomnia, restlessness, irritability, loss of appetite, loss of weight, and grinding of teeth all have been reported anecdotally in individuals with pinworm infection, but no evidence has shown that any of these symptoms is related causally to *Enterobius* infection. Enuresis also has been blamed on the pinworm, but one epidemiologic study failed to determine the causality.[64] An unusual form of eosinophilic ileocolitis resulting from massive infestation with many *E. vermicularis* larvae has been described in a homosexual man.[59]

SPECIFIC DIAGNOSIS

E. vermicularis eggs are identified readily by low-power microscopic examination of transparent adhesive tape previously applied to the perianal skin and then affixed to a microscope slide.

TREATMENT

Single-dose therapy with mebendazole (100 mg), albendazole (400 mg), or pyrantel pamoate (11 mg/kg, not to exceed 1 g) is effective. With the current availability of these three highly effective drugs, the intensive laundering of underwear and bed clothing, recommended in the older literature, no longer is necessary. Treating all members of the household is advisable, however, because they all must be presumed to be infected. Re-treatment in 2 or 3 weeks to destroy any adult worms that have hatched from the eggs swallowed at the time of initial therapy may be necessary. None of these drugs destroys the eggs.

One of the most important aspects of management of this infection is reassurance that its ubiquity virtually precludes effective eradication. Re-infection can be anticipated in any family infected with pinworms because of the high prevalence of this worm in the community. It also is important to reassure families that the presence of pinworms does not suggest poor hygienic standards in the family.

STRONGYLOIDES STERCORALIS AND *STRONGYLOIDES FUELLEBORNI*

Strongyloides stercoralis and *S. fuelleborni* are among the most virulent helminthic pathogens of humans, although they are much less prevalent than *Ascaris* or hookworm. *S. stercoralis* has the unusual ability to cause autoinfection, which can lead to hyperinfection and disseminated infection in immunocompromised hosts.[68,70,72-77,79,83,84] *S. fuelleborni* causes an aggressive infantile protein-losing enteropathy that leads to ascites and high mortality rates.[65,66]

S. stercoralis infection is acquired by the exposure of skin to infective larvae in the soil, much as in the case of hookworm infection. Similar circumstances that promote the survival of hookworm larvae in soil (i.e., moisture, sandy or loamy soil, and shade) promote the survival of *Strongyloides*. Larvae penetrate skin, facilitated by a potent histolytic protease that they secrete.[67,81] From the moment of penetration of the skin to the arrival of the worms in the intestine, the cycle commonly is thought to be similar to that of hookworms, although experimental evidence suggests that *S. stercoralis* also may explore routes of migration that bypass the lungs.[86] Within the intestine, the small adult worms do not attach to the mucosa as hookworms do, but instead lie embedded in its folds. The cycle from penetration of skin to development of mature worms in the intestine is approximately 28 days.

No parasitic adult male worms exist to fertilize the eggs. Instead, the mature eggs develop by parthenogenesis. In addition, in contrast to the eggs of other parasitic nematodes, these eggs usually are not found in feces but instead embryonate within the intestine and develop into larvae, which are deposited in soil with human stool. These so-called rhabditiform larvae must molt before they become infective.

This cycle has two variations. One permits the development of nonparasitic male and female adults in soil, which can maintain infestation of the soil for a certain period; this free-living phase sometimes is called the *heterogonic life cycle*. The second variation has much greater clinical relevance. Under certain conditions that are not well defined, the rhabditiform larvae molt to new infective larvae while still in the intestine. These new infective larvae can penetrate the intestine and set up a new cycle, commonly called *autoinfection* or the *autoinfective cycle*.[75] In this fashion, this nematode, in contrast to most other intestinal nematodes of humans, actually can increase in number without re-infection from the outside world. This phenomenon also is responsible for persistence of this infection for decades in an untreated host.[77] Some investigators think that low levels of autoinfection occur in most patients with strongyloidiasis.

When host defenses are impaired, especially through use of high-dose steroids (discussed later), *S. stercoralis* can undergo multiple rounds of autoinfection, leading to the production of thousands to hundreds of thousands of adult parasites in the intestine. This phenomenon is known as *hyperinfection*.[68,72,76,83] One possible consequence of hyperinfection is disseminated infection, in which larval and adult worms are identified at extraintestinal sites.

S. fuelleborni larvae are passed to infants by ingestion in breast milk.[65,69] Transmammary infection by nematode larvae is an extremely common route of transmission in nonhuman nematode infections[8]; although not well studied, it probably also occurs commonly in humans.

EPIDEMIOLOGY

S. stercoralis infection has worldwide distribution, but it is most prevalent in tropical and subtropical regions. In North America, strongyloidiasis is focally endemic in some parts of Appalachia[70] and is common in Southeast Asian immigrants.[78] In one study, 76.6 percent of Kampuchean immigrants and 55.6 percent of Laotian immigrants were seropositive for *S. stercoralis* infection.[78] Strongyloidiasis also is endemic in Jamaica and presumably elsewhere in the Caribbean.[84] Because of the possibility of autoinfection and, by extension, infection through contamination of skin by infested feces, strongyloidiasis is highly prevalent in mental hospitals, prisons, and homes for retarded children. Dogs and anthropoid apes may serve as animal reservoir hosts for *S. stercoralis*. *S. fuelleborni* infection is endemic in Papua New Guinea and parts of sub-Saharan Africa.[65,66]

PATHOPHYSIOLOGY

During the migratory phase of the infection, the larvae of *S. stercoralis* evoke an inflammatory response associated with eosinophilic infiltration. The adult phase in the intestine, even in moderate infection, may be associated with an inflammatory reaction sufficient to be symptomatic. Some evidence suggests that *Strongyloides* induces a malabsorption syndrome, which has been treated effectively by deworming.[70,82] It also has been noted, however, that the diarrhea associated with strongyloidiasis in young children occurs more frequently in those with underlying malnutrition.[72] Young children with *S. stercoralis*–induced malabsorption experience growth stunting and failure to thrive.[70]

The deficits in host defense that promote hyperinfection and disseminated strongyloidiasis are not well understood. Although cell-mediated immune deficits, such as those occurring in immunosuppression, organ transplantation, severe malnutrition, and cytotoxic chemotherapy for neoplasms and collagen vascular disease, are associated with this phenomenon, certain established deficits in cell-mediated immunity, such as those in human immunodeficiency virus infection, do not trigger hyperinfection.[75] Researchers have suggested that patients receiving large

doses of corticosteroids are particularly susceptible to hyperinfection because the corticosteroids themselves function as direct signals or ligands for the parasite to undergo autoinfection.[75] Patients in Japan and Jamaica with human T-cell lymphotropic virus type I seem to be at high risk for acquiring opportunistic strongyloidiasis,[73,79] possibly because of a specific deficit in their effector IgE immune response.[84] The pathogenesis of the marked protein-losing enteropathy that leads to ascites in the swollen belly syndrome of *S. fuelleborni* infection has not been established.

CLINICAL MANIFESTATIONS

During the migratory phase of larval strongyloidiasis, patients may be susceptible to the development of pneumonitis associated with eosinophilia. Larval migration through the skin can result in larva currens. Although most patients harboring *S. stercoralis* in their intestine are asymptomatic, patients with moderate or heavy infection classically have intense diarrhea productive of watery, mucous stool. Periods of alternating diarrhea and constipation may occur. Anorexia and cachexia, which lead to failure to thrive and other deficits in physical growth, are common features of pediatric strongyloidiasis.[70]

In disseminated strongyloidiasis caused by the hyperinfective cycle, larvae may invade all tissues, including the central nervous system (CNS). Because larvae penetrate the intestine, they may carry with them enteric flora and cause sepsis or meningoencephalitis.[68] Although diarrhea is the most commonly recognized consequence of *Strongyloides* infection, the hyperinfective cycle has the greatest portent for immunosuppressed patients. Infants with *S. fuelleborni* infection may manifest the swollen belly syndrome with marked abdominal ascites and pleural effusions that can be fatal.

DIFFERENTIAL AND SPECIFIC DIAGNOSES

Strongyloides pneumonitis can resemble the clinical manifestations associated with the lung migration of other nematode parasites, such as *Ascaris* and hookworm. The differential diagnosis of diarrhea must include causes of chronic diarrheal disease. In some patients, eosinophilia is a presenting sign of strongyloidiasis, either in patients with diarrhea or even in asymptomatic immigrants from developing countries.

The diagnosis is established by identification of the characteristic larvae during microscopic examination of stool, which is not easy because rhabditiform larvae usually are not produced in abundance. Specific stool concentration techniques are available to increase the sensitivity of fecal examination, although they are not as effective as amplifying the heterogonic life cycle by the Baermann technique or by looking for characteristic larval tracks on nutrient agar plates.[87] The stool of all immunosuppressed individuals, including those given corticosteroids for any reason, who have ever been in a region where *Strongyloides* is found must be examined to rule out this infection. If routine stool examination results are negative, the stool should be processed as outlined earlier. In addition, examination of duodenal contents can be attempted by the string test (Enterotest). This examination only divulges the contents of the duodenum, however, and can miss the larvae in the lower part of the small intestine. An enzyme-linked immunosorbent assay (ELISA) for detection of *Strongyloides*-specific antibodies is available on a research basis.[78,84]

Children with the swollen belly syndrome from *S. fuelleborni* infection shed eggs rather than larvae in their feces. Large numbers of eggs are common findings in clinical cases.

TREATMENT

Previously, the drug of choice for *S. stercoralis* and *S. fuelleborni* infection was thiabendazole,[71] administered at a dose of 50 mg/kg/24 hours divided into two equal doses on each of 2 successive days. The drug has high toxicity, however, which frequently includes nausea, vomiting, and vertigo, and sometimes requires interruption of therapy. Rarely, it induces leukopenia, rash, and Stevens-Johnson syndrome. Because the drug is detoxified in the liver, its dose may have to be reduced for patients with liver failure. Another benzimidazole used with increasing frequency is albendazole, 400 mg twice daily for 7 days. In the United States, albendazole is still considered investigational for this purpose.

Ivermectin is considered the treatment of choice for strongyloidiasis. It is administered at a dose of 200 μg/kg/day for 2 days. Treatment with ivermectin gives a cure rate of 80 percent.[79,80] Because the mortality rate of patients with disseminated strongyloidiasis remains high despite specific anthelmintic therapy, a common practice is to treat with a prolonged course or with a repeat course. In addition, several case reports have now described the use of veterinary parenteral formulations in patients too ill to take oral medications,[80] or use of ivermectin combined with albendazole.[82] Secondary bacterial complications, such as sepsis and meningitis, are common with disseminated strongyloidiasis, so judicious use of broad-spectrum antimicrobial agents frequently is indicated for this condition. As an additional supportive measure, patients who have hyperinfective strongyloidiasis and are receiving high-dose corticosteroid therapy probably benefit from steroid taper. Patients with transplants and cyclosporine immunosuppression may benefit from some of the direct helminthotoxic properties of this compound.[85]

PROGNOSIS

The prognosis is excellent in patients who do not have disseminated infection and are treated promptly. Unrecognized disseminated infection can be lethal.

PREVENTION

Proper disposal of human excreta substantially reduces the prevalence of strongyloidiasis in any community. In closed institutions, where control of direct spread is not likely to be achieved, identification plus treatment of infected individuals is the only feasible control.[74]

ABERRANT INFECTIONS WITH INTESTINAL NEMATODES

TOXOCARA CANIS

As indicated in the previous sections, the life cycles of the intestinal nematodes are adjusted precisely through evolutionary selection. In many instances, only one host in which the cycle can be completed is parasitized by the nematode. Infection of an unnatural host, in most cases, leads to complete failure of development and causes no disease. In a few instances, an infection may be established, but the cycle is not completed. Under such circumstances, the process of aberrant migration of larvae may be more pathogenic than steps in the natural cycle.

One of the most dramatic examples of an aberrant infection is visceral larva migrans, caused by infection with *Toxocara canis*. This roundworm causes intestinal infection in the dog, in which

its cycle resembles that of *A. lumbricoides* in humans. Humans become infected by *Toxocara* through the ingestion of an embryonated egg, much as in human infection with *Ascaris*. Larvae hatch in the small intestine, penetrate the villi, and begin a migration that takes them through every organ and tissue of the body. Because they cannot mature, the larvae tend to migrate for months until they are overcome by the inflammatory reaction of the host and die. Although larvae of other toxocarids such as *Toxocara cati* and *Toxascaris leonina* have been suggested as possible causes of visceral larva migrans, they are probably much less important as zoonotic pathogens in humans.

Because population-based serologic testing is limited to only a handful of research studies,[88,97,104,105] the prevalence of toxocariasis in North America and elsewhere may be underestimated. *T. canis* infection may have replaced *E. vermicularis* as the most common helminth parasite in the United States.

EPIDEMIOLOGY

The prevalence of toxocariasis is difficult to assess because of the failure of establishing the diagnosis in many cases. The disease has been reported from many parts of the world, including temperate climates, and one can assume that it is found wherever humans and dogs coexist. Young children often come into contact with *T. canis* eggs while playing in sandboxes and on playgrounds that were contaminated by a family pet.[99,100] The level of contamination of public areas also is difficult to ascertain. In several studies, ova were present in 5 to 25 percent of soil samples obtained, and surveys of dogs in urban communities have shown the occurrence of frequent infections, particularly in puppies, which are infected almost universally with *T. canis* (canine infection occurs transplacentally).[94,95]

Subsequent dissemination of *T. canis* eggs in the environment probably is aided by migrating earthworms and other soil invertebrates.[92] The seroprevalence of toxocariasis in the United States is high, and the parasite should be considered an emerging pathogen in some poor urban areas. In some groups of socioeconomically disadvantaged black children, the seroprevalence is 30 percent,[98,103] with even higher rates occurring among U.S. inner-city Hispanic children.[105] Toxocariasis is endemic to Puerto Rico. Major risk factors for acquiring toxocariasis include having a litter of puppies in the home and the habit of geophagia.[101] The latter risk factor probably accounts for the observed association between toxocariasis and elevated lead levels.[101] In Ireland, the prevalence of ophthalmologist-diagnosed ocular toxocariasis is 9.7 per 100,000.[98]

The presence of a positive skin test for toxocariasis is associated with poliomyelitis statistically.[108] No direct causal relationship exists; possibly, the circumstances leading to ingestion of *Toxocara* ova also are conducive to ingestion of poliomyelitis virus. Likewise, seizures are correlated with seropositivity for *Toxocara* antibodies, but a causal relationship has not been established.[88] Some investigators have postulated that toxocariasis may be an important cause of so-called idiopathic seizures in young children.[96,99]

PATHOPHYSIOLOGY

The entire infection is restricted to the migratory phase and represents an "exaggeration" of the symptoms found during the early phases of *A. lumbricoides* infection. Symptoms are protean and depend on which organ or tissue is infected. For unknown reasons, visceral migration through the liver, lungs, and brain occurs more commonly in toddlers and children younger than 5 years old, whereas older children tend to have ocular involvement almost exclusively.[99,100] Epidemiologic evidence suggests that this infection produces two distinct syndromes, visceral and ocular, because involvement of one tends to occur in the absence of the other.[97] Visceral migration elicits eosinophilic granuloma formation in the target organs and leads to hepatitis, pneumonitis, or cerebritis. Larval migration in the retina results in ocular larva migrans, which includes granuloma formation in the retina.[89,93,106] The lesion can resemble retinoblastoma so that it often is confused with it. Endophthalmitis[93] or papillitis[92] also may develop. Invasion of other organs and tissues induces granuloma formation there.

CLINICAL MANIFESTATIONS

Most patients infected with *T. canis* are thought to be asymptomatic. Some of these individuals have isolated findings, including eosinophilia, or wheezing and asthma. The term *covert toxocariasis* is used by some investigators to describe these patients, who often are identified by their circulating anti–*T. canis* antibody titers. An association between asthma and covert toxocariasis has been well described in Europe, but as yet this association is unproven in North America.[105]

Visceral larva migrans, the extreme form of toxocariasis, typically occurs in a toddler with the symptoms and signs of a multisystem disease. It is associated with fever, hepatosplenomegaly, lung infiltrates accompanied by wheezing, a high degree of eosinophilia (approaching 80%), and elevated immunoglobulin levels, particularly of the IgM class.[96,99-101] Seizures and neuropsychiatric disturbances also are common. In one case report, the child's major neurologic manifestation was a static encephalopathy.[96]

In contrast, ocular larva migrans is characterized by a unilateral vision deficit and, sometimes, strabismus. Ophthalmologic examination frequently reveals one or more posterior poles or peripheral pole granulomas.[93,100,106] More global eye inflammation also can occur (discussed earlier). Children with ocular involvement usually have few, if any, systemic manifestations. Often, no laboratory abnormalities are detected.

DIFFERENTIAL AND SPECIFIC DIAGNOSES

Visceral larva migrans must be distinguished from the migratory phase of the other nematode infections. Because of hepatosplenomegaly and hypereosinophilia, eosinophilic leukemia occasionally has been suspected, but it can be ruled out readily by examining bone marrow.

T. canis larvae can be identified in tissues in liver biopsy specimens, but the diagnostic yield is low. One must resort to indirect means and be aware that a multisystem disease with elevated IgM and hypereosinophilia fits the diagnostic criteria. An ELISA test available at the Centers for Disease Control and Prevention is highly specific and diagnostic.[100,103,104]

Ocular larva migrans usually is diagnosed by an experienced ophthalmologist who recognizes the characteristic granulomas and larval tracks on retinal examination. Presumably because of minimal antigen presentation by a few migrating larvae in the eye, often no measurable immune response occurs in this condition. For this reason, ELISA frequently is unreliable for establishing the diagnosis of ocular larva migrans.[98,100,101,103,104]

TREATMENT

Traditionally, treatment of visceral larva migrans was primarily symptomatic, especially because much of the morbidity is associated with immunopathologic responses against dying parasites. In the 1960s, thiabendazole and diethylcarbamazine were determined to be effective against migrating larvae.[102] Since then, new

agents of the benzimidazole class have been claimed to be equally effective, but associated with fewer drug toxicities.[99] In a comparative study with thiabendazole, the drug albendazole (10 mg/kg/day in two divided doses for 5 days) was shown to be well tolerated and less toxic.[107] Another benzimidazole, mebendazole, also may be effective when given in doses high enough to achieve significant extraintestinal levels.[90] Albendazole (400 mg twice daily for 5 days) is the treatment of choice. Although anecdotal experience with albendazole and mebendazole overseas suggests that these drugs are safe in children,[91] the large doses required for the treatment of larva migrans may be associated with hepatic and other toxicities (including embryotoxicities), and they have not been approved for this purpose.

Treatment of ocular larva migrans often requires surgical management, particularly in cases associated with tractional retinal detachment.[93,106] Specific anthelmintic adjunctive chemotherapy seems to be beneficial in some cases.[94,99,106]

PROGNOSIS

Except for patients in whom blindness develops as a consequence of retinal damage and a rare fatal case resulting from the intensity of the acute clinical reaction, most patients recover. The recovery phase may be slow, however, and may take 2 years.

PREVENTION

Theoretically, the disease can be prevented by elimination of dog feces from the human environment, but in practice, it is no less difficult to achieve than control of human excrement disposal.

OTHER ABERRANT INFECTIONS WITH INTESTINAL NEMATODES

Baylisascaris procyonis, a parasite of raccoons, also can cause visceral larva migrans. *Baylisascaris* infection occurs when humans accidentally ingest parasite eggs that are shed in barn lofts and attics accessible to raccoons.[101,113,114,116,118] In at least one reported human case, the infection was fatal in an infant.[116] Similar to *Toxocara* infection, *Baylisascaris* larvae within aberrant hosts cannot complete their cycle and continue their aimless migration through the tissues of these hosts. Baylisascariasis is probably more severe owing to the propensity of the larval stages of the parasite to invade the CNS, however, along with the continued growth of the larvae during migration resulting in greater mechanical damage.[113,114] The lesions caused by the larvae are eosinophilic granulomas, which tend to be concentrated in the CNS and result in eosinophilic meningitis. Neither the frequency nor the range of severity of this infection in humans is known. Most human cases have been diagnosed at autopsy. Because baylisascariasis is so uncommon, no studies are available to evaluate different anthelmintic chemotherapy regimens systematically. Some clinicians have recommended prolonged treatments with high doses of albendazole.[113,114]

Other, less severe aberrant infections of importance to humans are those caused by the dog hookworm, primarily *Ancylostoma braziliense*, but also *A. caninum* and *Uncinaria stenocephala*.[50,113,115,126] Infection with the larvae of *A. braziliense* and *U. stenocephala* cannot be completed, and larvae remain viable and migrate in the skin (usually between the epidermis and dermis); hence the terms *cutaneous larva migrans* and *creeping eruption* are used. In North America, cutaneous larva migrans is common along the Gulf Coast and along the Atlantic seaboard.[51] It also is common in the Caribbean. Failure of these zoonotic hookworms to complete entry through the human skin may reflect differences in the hydrolytic enzymes released.[115] Infection is acquired in the same fashion as that of the human hookworms. Children who expose their whole bodies to contaminated soil may be infected at any site. Adults are most likely to have infection in the lower extremities, but plumbers in the tropics, who often must crawl beneath houses, acquire infection on the elbows and knees.

The interval from exposure to appearance of the first symptoms is approximately 2 weeks; papules 2 mm in diameter then begin to appear on the skin. Behind them usually are serpiginous, erythematous, intracutaneous tunnels. The entire area itches intensely. Left untreated, cutaneous larva migrans tends to last 2 months. Albendazole (400 mg daily for 3 days) or ivermectin (200 μg/kg daily for 1 to 2 days) is effective treatment when administered orally. Topical therapy with a 15 percent aqueous suspension of thiabendazole was successful in one reported series. Forty-seven of 50 patients achieved permanent cure in 2 weeks, and 2 more patients were cured after a third week of treatment.[129] Placebo-treated patients were used as controls in this study. In contrast with skin penetration of zoonotic hookworms, oral ingestion of the dog hookworm *A. caninum* results in an eosinophilic enteritis syndrome (discussed in the section on hookworm infection).

Rarer aberrant infections include those caused by various species of *Trichostrongylus*, *Oesophagostomum*, *Angiostrongylus*, *Capillaria*, and *Anisakis*. *Trichostrongylus* is a common parasite of many mammals, and it has been found in the small intestine of humans, mainly in Asia, Africa, and Australia. Ingestion of larvae leads to the development of adult worms in the small intestine. Whether this development results in any disease remains a moot point because infections tend to be mild and usually are associated with other helminth infections.[114] *Oesophagostomum bifurcum* is a common nematode of subhuman primates in Africa and has been reported to be a common intestinal nematode that causes nodular disease of the intestines in humans living in West Africa.[120,123] Human oesophagostomiasis has been treated successfully with pyrantel pamoate.[120]

Land snails and slugs serve as intermediate hosts for *Angiostrongylus* spp. *Angiostrongylus cantonensis* is a cause of eosinophilic meningitis throughout East Asia and Hawaii[111,119,124,125]; *Angiostrongylus costaricensis* is a cause of mesenteric arteritis and abdominal pain in Central and South America and in Latin American immigrants to the United States.[117] The rat serves as the natural host of these parasites, which live either in the lung (*A. cantonensis*) or in mesenteric arteries (*A. costaricensis*). The rat eats the infected mollusks and ingests the larvae, which migrate to their final destination. An incomplete infection develops in individuals who ingest either the mollusks or food contaminated by the mollusks. *A. cantonensis* infection usually is limited to the CNS and is manifested as eosinophilic meningitis.[111,119,124,125]

Signs and symptoms include meningismus, severe headache, paresthesias, and, less commonly, cranial nerve palsies. No specific treatment is available (although the anthelmintics thiabendazole and ivermectin are effective in some experimental animal models[111]), but the disease is self-limited and lasts no longer than 2 weeks. Symptomatic relief has been reported with the use of prednisone. In contrast, *A. costaricensis* infection typically is manifested as abdominal or right iliac fossa pain, fever, and eosinophilia. In children with this condition, appendicitis or Meckel diverticulum may be diagnosed.[117] High doses of mebendazole have been tried as therapy for this condition, as well as albendazole.

Capillaria philippinensis is a common parasite of water fowl in the Philippines.[110] The mode of transmission of this parasite to humans is unknown, but human cases have been reported in which 40,000 adult worms were found embedded in the crypts of the small intestine. *C. philippinensis*, similar to *S. stercoralis*, can undergo autoinfection and hyperinfection in humans.[110] No associated inflammatory reaction occurs, but flattening of villi, loss

of epithelial surface area, and severe malabsorption have been reported.[110,112,128] In one series of 1000 cases of *C. philippinensis* infection, a mortality rate of 10 percent was reported.[112] Thiabendazole may be effective in shortening the course of the infection,[128] although albendazole has become the treatment of choice more recently.[110] Another member of the genus, *Capillaria hepatica*, a rare zoonosis of humans, has been known to disseminate to the lungs, liver, and other viscera.[122]

Anisakis spp. are nematode parasites of marine mammals, with fish being intermediate hosts. When the infective larvae of the parasite are ingested as a result of eating raw or poorly cooked fish, they may become embedded in the gastric mucosa and cause eosinophilic granuloma.[51,109,121,127,130,132] In adults, it may resemble carcinoma of the stomach clinically and radiographically. Human anisakiasis occurs frequently in Japan, where raw marine fish are eaten commonly, and in Holland, where lightly pickled herring is considered a delicacy.

FILARIAL PARASITES

Except for rare instances of zoonotic *Brugia* infection, the filarial worms parasitizing humans affect people within the geographic area almost entirely limited to the developing world, especially sub-Saharan Africa and India. Although accurate data are lacking, approximately 100 million people are infected with lymphatic filariasis (LF), and 18 million are infected with onchocerciasis. The various human parasites in this category have certain characteristics in common. They all are spread by vectors, and the adults invade and occupy the lymphatics, skin, connective tissue, or blood. They produce live embryos called *microfilariae* that enter the bloodstream or skin, where they can survive for months or years without further development. The range of disease caused by these worms is wide; some produce no symptoms, whereas others can be responsible for severe clinical disorders.

The life cycles of the filarial worms are similar in that infections are acquired through an insect bite, during which transmission is effected by introduction of infective larvae onto the skin of the host from the mouth parts of the insect. The larvae enter the wound in the skin and make their way to the respective tissue, where they mature into adult worms. The adults mate and produce live microfilariae, which, in LF, migrate to the blood through the walls of the lymphatics or through the thoracic duct. To complete the life cycle, the microfilariae are ingested by blood-sucking insects, in which they undergo metamorphosis through two larval stages until they reach the third, infective stage. The interval from the infective bite to the appearance of microfilariae in the blood of the host can be 6 to 12 months.

Mass drug administration with ivermectin alone (for onchocerciasis), or ivermectin together with albendazole (for LF), or diethylcarbamazine with albendazole (also for LF) can reduce the microfilarial load of human populations living in endemic regions.[131] For onchocerciasis, reductions in microfilarial loads lead to reductions in chronic morbidity, with marked improvement in the serious skin manifestations of the infection and the prevention of blindness. For LF, widespread implementation of mass drug administration one day may reduce transmission to the point of elimination. Such programs are currently under way in 10 countries of sub-Saharan Africa. In Egypt, five rounds of mass drug distribution already may have effectively eliminated transmission.[132] Mass drug administrations provide the core activities of several international public and private partnerships, including the Global Alliance to Eliminate Lymphatic Filariasis, the African Programme for Onchocerciasis Control, and the Onchocerciasis Elimination Program for the Americas.[133,134]

LYMPHATIC FILARIASIS: *WUCHERERIA BANCROFTI* AND *BRUGIA* SPECIES

EPIDEMIOLOGY

Wuchereria bancrofti is prevalent primarily between the two Tropics, but also is encountered north of the Tropic of Cancer in Africa. An estimated 100 million infections exist in 80 countries worldwide, with approximately half of the infected individuals living in sub-Saharan Africa.[132] In each of its geographic locales, it has a specific anopheline, culicine, or aedine mosquito vector. In the Caribbean area, South America, Asia, East and West Africa, and Papua New Guinea, the microfilariae of this worm exhibit nocturnal periodicity; in the South Pacific, their periodicity is diurnal. *W. bancrofti* has no animal hosts.

Brugia malayi occurs in India, Malaysia, and other parts of Southeast Asia. Some strains of *B. malayi* are associated with animal reservoirs, as are certain other members of the genus *Brugia*, such as *Brugia timori*. In the United States, zoonotic *Brugia* infections caused by *Brugia beaveri* and *Brugia lepori* may infect humans, but cannot develop patent infections.[134,137,138]

PATHOPHYSIOLOGY

The pathology of LF is caused principally by the adult-stage parasite, the host response, and bacterial superinfection of tissues with compromised lymphatic function. Adult worms induce lymphatic dilation that results in lymphatic dysfunction, lymphedema, and a greater susceptibility to bacterial infection. The consequent inflammation, plus that caused by host responses to dying parasites, damages the delicate lymphatic vessels further and compromises lymphatic function further. When such processes occur in the lymphatic vessels of the scrotum, hydrocele develops. Microfilaremia often is "asymptomatic," but frequently is associated with immune complex nephritis; more rarely, the microfilariae can be the target of immunologic hyperresponsiveness and result in a severe "tropical pulmonary eosinophilia" syndrome.[135]

CLINICAL MANIFESTATIONS

Asymptomatic microfilaremia often develops in children living in endemic areas. Using highly sensitive diagnostic tests, including antigen detection and ultrasound, one third of children living in endemic areas can be seen to be infected before age 5 years. Damage to the lymphatics of these young children frequently is subclinical, however.[139] With repeated exposure, these children begin to have episodes of acute adenolymphangitis, sometimes associated with fever and lymphangitis. These "filarial fevers" may reflect either an inflammatory response to dying parasites (with "retrograde" lymphangitis) or bacterial superinfection.[133] Progression to lymphedema of the upper and lower extremities, the most common sequela of chronic LF, is an uncommon occurrence in children until after the age of puberty. Benign lymphedema has been described in a few patients in the United States with zoonotic *Brugia* infection.[134,138] Chronic, recurrent eosinophilic pneumonitis associated with wheezing, cough, chest pain, pulmonary infiltrations, and hypereosinophilia (tropical pulmonary eosinophilia) is an IgE-mediated hypersensitivity reaction to microfilariae trapped in the lungs and, if left untreated, leads to debilitating interstitial pneumonitis.[135]

DIFFERENTIAL AND SPECIFIC DIAGNOSES

The differential diagnosis of lymphatic obstruction in children should rule out other more likely conditions before focusing on

filariasis. In older children and adults, familial lymphedema (Milroy disease) can mimic filariasis.

In endemic regions, LF typically is diagnosed on the basis of appropriate clinical findings in a region with known endemicity for the disease. In young children living in endemic areas, however, early infection is largely subclinical.[139] A definitive diagnosis of LF still relies on detecting adult parasites in the lymphatics through ultrasonography or by recovering microfilariae from the blood. In view of the circadian periodicity of the appearance of numerous microfilariae in blood, a specimen should be collected at the appropriate time, in many regions of the world between 10 P.M. and 2 A.M., for microscopic examination by a staining or concentration technique. Increasingly, an immunochromatographic card test that measures specific filarial antigen is being used, especially in amicrofilaremic individuals.[133]

TREATMENT

New agents or drug regimens are needed that would be more effective against the adult filarial worm and at the same time elicit minimal immunopathologic damage. Currently, the major drugs used are especially effective against microfilariae. Diethylcarbamazine is the treatment of choice and typically is given in a 12-day course (6 mg/kg/day) for a total dose of 72 mg/kg. In the United States, it is common practice to administer first small doses of 1 to 2 mg/kg (maximum of 50 to 100 mg) before beginning this regimen. More recently, a single dose of 6 mg/kg has been recognized to produce a similar therapeutic result.[133] Diethylcarbamazine is available under an Investigational New Drug Protocol from the CDC Drug Service, Centers for Disease Control and Prevention, Atlanta, Georgia, 30333; 404-639-3670 (evenings, weekends, or holidays: 404-639-2888).

Side effects of specific antifilarial therapy include allergic and febrile reactions that are caused by the inflammatory response to dying microfilariae rather than by the drug itself, and that occur primarily in patients with high levels of circulating microfilariae. These systemic manifestations can be treated symptomatically with antihistamines or corticosteroids. Some of the allergic manifestations also may be avoided by administering diethylcarbamazine in a graded, stepwise manner. This drug also is the treatment of choice for tropical pulmonary eosinophilia, a syndrome caused by circulating microfilariae. Ivermectin also may have a role in the medical treatment of LF because it is very effective in clearing circulating microfilariae.[136] Albendazole, in high doses, is effective in killing adult worms and, in lower doses, inhibits microfilarial production, but it is not registered for use in LF in the United States.[132]

The difficulty in management of filariasis by drugs is that late symptoms, such as elephantiasis, do not abate. The main usefulness of chemotherapy is in cases recognized early, before the anatomic abnormalities develop. Hydrocele can be treated surgically. Of critical importance to the management of lymphedema and elephantiasis are attention to hygiene, wearing shoes to prevent injury, and reduction of lymphostasis with exercise and elevation of the lower extremity.

The more recent discovery that *W. bancrofti* harbors bacterial endosymbionts has led to efforts to use tetracycline and related antimicrobial agents as part of the therapeutic regimen for LF.[138] The therapeutic use of such regimens is under active investigation. As of this writing, the Centers for Disease Control and Prevention (see earlier) and the Clinical Center of the National Institutes of Health (Bethesda, MD) have extensive experience and expertise in the management and treatment of human filarial infections, including *W. bancrofti*, *Loa loa*, and *Onchocerca volvulus* infections.

PREVENTION

Prevention in the past depended principally on vector control, which was unsatisfactory, primarily because of the difficulty in developing effective insecticides that also would be nontoxic to the rest of the environment. Successful experience in China has shown that large-scale chemoprophylaxis approaches to prevention are possible in endemic countries. Currently, through the Global Alliance to Eliminate Lymphatic Filariasis, the international community is directing a large-scale LF control campaign on this principle. Typically, this control is being accomplished through single-dose combination of albendazole (400 mg) with either ivermectin (200 μg/kg) or diethylcarbamazine (6 mg/kg). On this basis, long-term targets for the elimination of LF have been proposed.[131,132] The finding of extensive subclinical infection among children living in endemic regions highlights the importance of targeting this group for mass drug administration as well as adults.[139]

LOA LOA

L. loa infection is limited to a small area of western and central Africa and is spread by *Chrysops* flies. Periodicity in *L. loa* microfilariae is diurnal. The parasite elicits numerous allergic inflammatory responses that are most evident in expatriates.[140,141] Infection leads to high eosinophilia and recurrent angioedema, which when localized develop into painful, pruritic, subcutaneous swellings on the extremities and the face known as Calabar swellings. Rarely, some cases of lymphatic obstruction of the lower extremities and hydroceles have been reported.

The most dramatic manifestation of this infection is the occasional appearance of a migrating *L. loa* adult under the conjunctiva of the eye. It does not damage the eye and can be removed surgically. Treatment with diethylcarbamazine, as indicated earlier, effectively destroys the adults, but reactions may be more intense than in the treatment of *Wuchereria* and *Brugia* infections. Diethylcarbamazine should be administered in a gradual, stepwise manner as described earlier, particularly in patients with high levels of circulating microfilariae. Co-administration of corticosteroids often is required during treatment. Encephalopathy has been described in patients receiving diethylcarbamazine, especially when heavily infected.[140] Ivermectin elicits fewer inflammatory symptoms during treatment and may be less toxic in general, but still encephalopathy can occur in patients with heavy *Loa* infections.[142]

ONCHOCERCA VOLVULUS

Onchocerca volvulus infection is acquired through the bite of a *Simulium* fly, which tends to breed along rivers and streams (hence the name of the disease—river blindness).

EPIDEMIOLOGY

Disease is limited to sub-Saharan Africa, where approximately 99 percent of the world's 18 million cases occur (approximately half of the cases occur in Nigeria and Congo), and focal pockets in Central America (primarily Guatemala and Chiapas State, Mexico), and the northern parts of South America (near the border between Venezuela and Brazil and in Ecuador). In view of human dependence on water and the establishment of settlements along rivers, the frequency of infection tends to be high in areas where *Simulium* prevails. The development of hydroelectric power based on the construction of large dams can increase

the breeding sites of *Simulium* and increase the incidence of *Onchocerca* infection.

PATHOPHYSIOLOGY

Larvae deposited by a *Simulium* bite remain in the subcutaneous tissue and develop into adult worms there. Adult worms tend to become coiled, and worms of both sexes become enveloped by fibrous tissue and form nodules within which they reproduce. The larvae produced by fertilized females invade the skin, where they remain until they are picked up by a *Simulium* bite, or they die about 30 months later. In addition to the skin, microfilariae penetrate the eye and affect every layer from the conjunctiva to the optic nerve. In African onchocerciasis, chorioretinitis and optic atrophy occur commonly; in the Central American disease, iritis is the primary lesion.

The probability of the development of eye disease is related to the location of the adult worms. When the nodules are situated around the head, eye lesions are common; when they are in the lower parts of the body, eye lesions occur less frequently. In Africa, the nodules tend to be distributed primarily in the lower parts of the body, but because of the high prevalence of the infection, onchocercal blindness is common. In Central America, the lesions tend to be on the upper part of the body.

CLINICAL MANIFESTATIONS

The appearance of the skin nodules and the presence of live microfilariae within the eye (readily seen with a slit-lamp ophthalmoscopic examination) are the manifestations of early and intermediate disease. Later, the eye involvement includes keratitis, iridocyclitis, chorioretinitis, and, eventually, blindness. Microfilariae in the skin cause an inflammatory reaction that includes acute papular onchodermatitis and chronic changes such as edema, lichenification, atrophy, and depigmentation.

DIFFERENTIAL AND SPECIFIC DIAGNOSES

Because the skin invasion is associated with itching, the pruritus of onchocercal infection must be differentiated from contact dermatitis, prickly heat, insect bites, and scabies. Onchocerciasis is identified by examination of a skin snip. Examination of sectioned and stained tissue or a stained impression smear reveals microfilariae.

TREATMENT

Surgical removal of all visible nodules may radically extirpate the source of new microfilariae that invade the eye. The routine use of nodulectomy is still controversial, however. Chemotherapy with ivermectin (single oral dose of 150 μg/kg administered every 6 to 12 months) until asymptomatic is the treatment of choice. Ivermectin reduces the number of microfilariae in the skin within days, and subsequently the number of microfilariae in the eye, and prevents the onset of blindness.[143] Ivermectin also can be used in mass treatment to diminish transmission by the vector and control the incidence of this infection.[131,143,144]

PREVENTION

Vector control, periodic treatment of infected individuals, and possibly surgical removal of subcutaneous nodules all are means of prevention, and when carried out effectively, can be remarkably successful.[143]

MANSONELLA PERSTANS AND *MANSONELLA OZZARDI*

Neither *Mansonella perstans* nor *Mansonella ozzardi* is known to cause significant human pathology, but the microfilariae present in blood must be distinguished morphologically from the microfilariae of the other more pathogenic filariae. *M. ozzardi* is found throughout the Caribbean (especially Haiti) and Central America. It has been suggested as a cause of chronic arthritis in these regions. *M. perstans* also is found in Africa, where it has been identified as a cause of painless nodules in the conjunctiva and secondary eyelid swelling. For that reason, it sometimes is called the Kampala or Ugandan eye worm.[145] Albendazole, mebendazole, or ivermectin is the treatment of choice for *M. perstans* infection.

DIROFILARIA IMMITIS

Dirofilaria immitis is a filarial worm commonly found in dogs, in which it occupies the right ventricle of the heart. The microfilariae produced circulate in blood and are transmitted to new animals through the bite of culicine mosquitoes. Fewer than 100 cases of human infection have been reported, none of them in children. Most human hosts infected with *Dirofilaria* were asymptomatic, but individuals who had symptoms complained of chest pain, wheezing, and cough. All infected individuals had coin lesions detected on pulmonary radiographs.[146,147] Human infection is transmitted through a mosquito bite. As with visceral larva migrans and the zoonotic *Brugia* infections, *D. immitis* cannot complete its life cycle in humans. No microfilariae of this worm have ever been shown in human peripheral blood.

All patients evaluated for pulmonary dirofilariasis have had mild peripheral eosinophilia, usually not exceeding 10 percent. Because the radiographic picture is not diagnostic, and in view of the potential seriousness of a coin lesion,[146] the lesion must be examined histologically. If a worm is found, the diagnosis of dirofilariasis can be made; if it is not found, the diagnosis is still tenable. In the presence of eosinophilia and pneumonitis, however, a whole range of other diagnostic possibilities must be considered, including eosinophilic pneumonia, polyarteritis nodosa, Wegener granulomatosis, and histiocytosis X. No treatment is necessary for this infection in humans.

DRACUNCULUS MEDINENSIS

Infection by *Dracunculus medinensis* (guinea worm) also is limited largely to sub-Saharan Africa, with the greatest number of cases occurring in Sudan, Ghana, and Nigeria. Currently, fewer than 50,000 cases are thought to remain in these regions, and the disease is slated for elimination. The adult female worm lies in subcutaneous tissue and can extend over a length of 50 to 120 cm. No information about the fate of the male exists. The adult lives for 18 months. At the end of the first year of infection, the adult female migrates to subcutaneous tissue, where it produces an indurated papule that tends to vesiculate and ulcerate. When the surface of the ulcer comes in contact with water, the worm discharges motile larvae. These larvae are ingested by a crustacean, *Cyclops*, in which they undergo additional maturation and development.

Humans become infected by swallowing *Cyclops* in drinking water. The larvae penetrate through the gut into subcutaneous tissue by a route not fully understood. Multiple infections occur

commonly. In Nigeria, this disease previously was reported to be responsible for 25 percent of absenteeism in schoolchildren.[149] Secondary bacterial infections leading to cellulitis are common, as are secondary arthritis and contractures that can lead to permanent disability.[149]

Stagnant water is necessary for maintenance of the infection, which tends to be infrequent when running water and properly constructed wells are available. The diagnosis is established readily by observing the adult worm emerging from a cutaneous ulcer. The outline of the worm can be seen easily under the skin.

The classic treatment of this infection involves incision of the skin and tying the end of the worm to a small piece of wood. By turning the wood daily, the worm can be extracted over several weeks. This therapy is unsatisfactory and often results in failure of complete extraction. If the worm tears, an intense inflammatory reaction with skin sloughing develops. Treatment with chemotherapeutic agents such as metronidazole is controversial. Some investigators report that treatment with these agents helps decrease inflammation and facilitate removal of the worm.

Because filtering of drinking water can prevent this infection effectively, authorities are optimistic that this parasite can be eradicated through appropriate control measures.[148-150] Through an initiative led by the Carter Center (Atlanta), success has been achieved in eradicating guinea worm infection in many parts of Africa and Asia. The highest rates of infection still exist in the Sudan because of the difficulty encountered in implementing public health control measures in this war-torn region.

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Aberrant Infections with Intestinal Nematodes

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SUBSECTION 3

Cestodes

CHAPTER 238

CESTODES

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OVERVIEW OF CESTODES AND THEIR IMPORTANCE FOR CHILDREN

Cestodes are multicellular helminth parasites.[228] Children may harbor the adult worms (tapeworms) or larval stages (e.g., cysticercosis or hydatid disease). The adult tapeworms are segmented worms that contain a scolex (attachment organ), a neck region where the segments are formed, and segments termed *proglottids*. The scolex contains either sucking grooves (*Diphyllobothrium*, *Spirometra*) or round suckers (other species). In some species

(e.g., *Taenia solium*), the scolex is armed with rows of hooks, which also aids in attachment. The proglottids develop in the neck region and move distally as they are displaced by newer proglottids. Eventually, chains of proglottids form and compose the body of the adult worm. The external surface of the proglottid forms an absorptive surface, which functions as the parasite gut. As the proglottids are displaced from the scolex, they gradually mature, with both male and female sexual organs developing in each proglottid. The terminal proglottids contain the uterus, which are full of ova. The ova of *Spirometra* and *Diphyllobothrium* are shed into water, where they infect a series of aquatic intermediate hosts. The ova of other species infect the immediate host after being ingested. Both larval and adult forms of *Hymenolepis nana* live in the human intestines, and the entire life cycle can take place in a single host. Humans are dead-end hosts for numerous zoonotic tapeworms, including *T. solium* (cysticercosis), *Echinococcus* spp. (hydatid disease), and *Spirometra* (sparganosis). The term *cysticercosis* (literally bladder-tail) refers to infection of tissues with an intermediate form of the parasite containing a cystic parasite with one (or a small number) invaginated scolex. Human cysticercosis usually is caused by *T. solium*. However, human cysticercosis is also rarely caused by *Taenia crassiceps* or *Taenia multiceps*. *Hydatid disease* refers to infection of tissues with the intermediate form of the parasites of the genus *Echinococcus*, which form cystic lesions filled with innumerable protoscolices, each of which can turn into a tapeworm or another hydatid lesion, depending on the tissues. Infections with cestodes have been common human infections since antiquity and have been found in mummies.[18,91] Currently, approximately 200 million people worldwide are infected by cestodes.

TAENIA SAGINATA (BEEF TAPEWORM) INFECTION

The different life cycle forms and designation of species of the beef tapeworm were identified in the 18th and 19th centuries.[227] Although other species names have been used in the past, *Taenia saginata* is accepted now as an inclusive term for all of the species of tapeworms acquired from beef. In recent years, however, *Taenia asiatica* has been identified as a distinct species similar to *T. saginata* but acquired from pork. Differentiation of these species morphologically, however, is difficult, and identification of species is aided by DNA analysis.[31,91]

THE ORGANISM

The scolex of *T. saginata* measures 1 to 2 mm in diameter and has four muscular suckers but bears no hooks. The length of the parasite usually varies from 4 to 10 m but may reach 25 m. The size of the proglottids depends on their stage of development and their state of muscular relaxation. Gravid proglottids typically are 16 to 20 mm in length and 5 to 7 mm in width. The linear, central uterine stem has 12 to 30 main lateral branches on each side. The eggs measure 30 to 40 μm in diameter. The six-hooked embryo is surrounded by a brown, radially striated embryophore. When the bovine host ingests the ova, embryos hatch and burrow through the intestinal mucosa, where they gain access to the circulation. After lodging in capillaries, the embryo develops into a larval cysticercus. This cystic, ovoid structure measures 7 to 10 mm by 4 to 6 mm and contains an invaginated scolex.

Cattle are the principal intermediate hosts. Humans are the only definitive host. Ingestion of *T. saginata* eggs is of no particular danger to children because humans are not susceptible to development of the cysticercus stage.

TRANSMISSION

The life cycle of *T. saginata* requires transmission of the infection to the intermediate host and subsequently to humans, who in turn transmit the organism to other intermediate hosts. Contamination of pastures or feed lots with human feces or untreated sewage leads to bovine cysticercosis. When humans ingest raw or poorly cooked beef containing viable cysticerci, the scolex evaginates from the cysticercus and attaches to the intestinal wall. Proglottids develop from the neck region and gradually enlarge to form the adult tapeworm. Gravid proglottids, which are at the distal end of the worm, appear only 84 to 120 days after infection.[204] Infection may persist for years. In most geographic areas, parasitism is with a single worm, but in highly endemic areas, multiple infections may be found.[204]

Ova may be found within feces. However, eggs are shed more frequently when gravid proglottids actively migrate from the anus. As these muscular proglottids crawl about, eggs are expressed from the anterior margin. The ova of *T. solium* and *T. saginata* are not distinguishable on morphologic grounds.

EPIDEMIOLOGY

Worldwide, 45 to 60 million people are thought to be infected with *T. saginata*. Infection is most important in tropical and subtropical cattle-raising areas of Africa, the Middle East, Europe, Asia, and South America.[31,117,227] The prevalence of human taeniasis caused by *T. saginata* is poorly understood. In general, the prevalence of human infections is less than 1 percent of stool examinations in all but highly endemic areas, but because of the poor sensitivity of the assays, this figure may understate the prevalence. In recent studies, prevalence rates of more than 20 percent have been noted in East Africa, Bali, and Tibet.[31] Although the incidence of taeniasis in the United States is low, transmission does occur. However, less than 0.1 percent of fecal specimens examined in state health laboratories were positive for *Taenia* eggs.[102] With socioeconomic changes, improvements in the cattle industry, and decreasing instances of ingestion of undercooked beef, the prevalence of intestinal taeniasis likely will continue to drop in much of the world.

PATHOLOGY AND PATHOGENESIS

Little is known of the pathology or pathogenesis of intestinal taeniasis. Speculation about local mucosal trauma, irritation, production of toxic substances, or induction of clinically significant hypersensitivity has scant documentation. Rare, ectopic localization of worms or proglottids may stimulate local inflammatory reactions. Because adult worms "rob" the infected host of nutrients, malnutrition could result from the child's incomplete uptake of ingested food, but little evidence exists to support this possibility.

CLINICAL MANIFESTATIONS

A variety of symptoms have been attributed to *T. saginata*, but the parasite infection likely is often merely coincidental to the concurrently observed symptoms in the child.[31] The most common complaint is the discomfort caused by the migration of gravid proglottids from the anus. The patient's awareness of the infection frequently causes a preoccupation with gastrointestinal function. Some studies of *T. saginata* infection revealed abdominal pain, nausea, weakness, and loss of weight as the most common symptoms recorded.[31,227] Alterations in appetite and bowel habits were reported inconsistently. Intestinal obstruction and

symptoms related to ectopic localization of proglottids are extremely rare. Although taeniasis usually is not associated with significant eosinophilia, some patients occasionally have significant eosinophilia between the sixth and ninth weeks after the initial infection occurs. One study reported that by the time the patients first began passing proglottids, the eosinophil counts had returned to normal or nearly normal.[80] Another series of observations showed a syndrome of marked eosinophilia and severe colicky abdominal pain that occurred approximately 1 month before the patients passed *Taenia* proglottids.[8] A single experimental infection produced symptoms of nausea, headache, and disturbed sleep at approximately the time that gravid proglottids appeared in the stool, 84 days after infection. A 10.5 percent increase in the number of eosinophils and a 13 percent increase in the number of lymphocytes were noted.[204]

DIAGNOSIS

The scolex of *T. saginata* lacks hooks and is differentiated easily from the scolex of *T. solium*, which bears a circle of hooks. However, the scolex usually is not recovered, even after successful treatment. Studies suggest that use of polyethylene glycol salts as a purgative may improve recovery of proglottids.[101] Eggs may be seen on routine fecal wet mounts, but the likelihood of their being detected is enhanced by concentration methods. Even then, ova are shed only intermittently, and the yield of a single stool examination is low. Cellulose tape swabs applied to the anal and perianal skin, as used for the diagnosis of *Enterobius* infection, have been found to be even more efficient than is fecal concentration.[208]

The eggs of *T. saginata* and *T. solium* cannot be differentiated morphologically. Thus, examination of gravid proglottids is the usual method for determining species. The patient is instructed to collect proglottids in a vial of saline and to deliver the specimen to the laboratory as soon as possible. Fixatives such as alcohol and formalin tend to render the proglottids rigid and opaque. The proglottid should be compressed between two microscope slides. If the uterus contains enough ova, counting the main branches of the uterine stem is relatively easy. The uterus is more readily identified by injecting a small amount of India ink into the midportion. Counts of 13 or fewer branches on one side of the stem are considered diagnostic for *T. solium*. If the count is 14 or more, the species is designated *T. saginata*. Because of morphologic variations, this method has been criticized, particularly when the decision is based on gravid proglottids with counts in the range of 12 to 15. Analysis of many specimens of *Taenia*, as identified by their scolex, showed that mature proglottids with fully developed sex organs could be differentiated by several characteristics. The most prominent features are a vaginal sphincter seen only in *T. saginata* and a third ovarian lobe that is present only in *T. solium*. To define these and other features, the proglottids require painstaking staining and clearing as well as examination by a skilled parasitologist.

Antigen detection tests are becoming increasingly useful for the identification of *T. saginata*. Tests for *T. solium* currently are more specific than are tests for *T. saginata*.[3] Polymerase chain reaction testing has been useful in diagnosis of *T. saginata* in field situations.[31] On occasion, the diagnosis of tapeworm is established when larger portions of a worm appear as a ribbon-like defect in the barium column during contrast studies of the gastrointestinal tract. In plain films of the abdomen, a tapeworm may be seen as a linear density in a gas-filled loop of bowel.

TREATMENT

Praziquantel, a pyrazinoisoquinoline derivative, is the primary medication used to treat *T. saginata*. It is given as a single oral dose of 5 to 10 mg/kg. An alternative to praziquantel for treatment of intestinal taeniasis is niclosamide (marketed as Yomesan in parts of Europe but not consistently available in the United States); this agent is given as a single oral dose of 50 mg/kg (up to 2 g for large children or for adults). Nitazoxanide, a newer thiazolide agent approved for use in children with other parasitic infections, has been effective in treating taeniasis that has been incompletely responsive to both praziquantel and niclosamide.[117]

Because infection with *T. saginata* is not contagious, no routine follow-up is needed after treatment. If, however, proglottids are noted through the anus or in the stool more than a week after treatment is completed, subsequent therapy may be considered, perhaps with an alternative agent.

PROGNOSIS

Symptoms, when present, may be annoying but do not usually alter health significantly. Treatment is highly effective, and the prognosis is excellent.

PREVENTION

T. saginata infection can be prevented by avoiding the ingestion of raw or undercooked beef. In addition, visual meat inspection is effective in detecting all but light infections. Cooking beef to a temperature of 56° C kills cysticerci. Education of workers in the cattle-raising industry, coupled with detection and prompt treatment of human infection, may reduce the incidence of transmission. Vaccines to prevent bovine *T. saginata* cysticercosis are feasible, but development has been slowed by limited commercial interest.[122]

TAENIA ASIATICA (ASIAN PORK TAPEWORM)

T. asiatica was identified in East and Southeast Asia, where humans become infected by eating small cysts contained in raw porcine liver. The proglottids are morphologically similar to those of *T. saginata*, such that it was not recognized as a separate species until 1993.[31,98]

THE ORGANISM

Although adult tapeworm proglottids of *T. asiatica* are morphologically similar to those of *T. saginata*, the scolex contains a rostellum (like *T. solium*) but no hooks (like *T. saginata*). Proglottids usually contain 16 to 21 uterine branches, similar to *T. saginata*. The larval cysticercus stage is also similar to that of *T. saginata*, but the cysticerci generally are smaller. The cysticerci also are located primarily in the liver, not generally in muscle. *T. asiatica* cysticerci are found mainly in pigs but have been identified in cattle, goats, monkeys, and wild boar.[98]

TRANSMISSION

Pigs (but also cattle, wild boar, and goats) become infected by ingesting *T. asiatica* eggs, which hatch, releasing the larvae, which in turn invade and develop into the cysticercal stage primarily in the liver. Animals, including humans, become infected when they ingest cysticerci (usually in raw porcine liver) and then develop the intestinal adult forms of infection. An experimental human infection demonstrated that a period of 76 days transpired

between ingestion of the cysticercus and the appearance of gravid proglottids.[53]

EPIDEMIOLOGY

Asian taeniasis has been identified in Taiwan, Korea, China, Philippines, Vietnam, Thailand, Indonesia, and Malaysia.[31,53] It may be that other parts of the Asia-Pacific region are also endemic for *T. asiatica* but that previous surveys incorrectly identified the offending organism as *T. saginata*. The prevalence of infection varies among regions, probably related to religious and cultural choices about food; 0 to 21 percent of people in various parts of Indonesia are infected.[221]

PATHOLOGY AND PATHOGENESIS

Observations in humans and studies in mice suggest that the pathogenesis of infection by *T. asiatica* is similar to that of *T. saginata*.[99] No human cysticercal form has been identified.

CLINICAL MANIFESTATIONS, DIAGNOSIS, TREATMENT, AND PROGNOSIS

The clinical manifestations, diagnosis, treatment, and prognosis of *T. asiatica* have not been described completely, but they appear to be similar or identical to those of *T. saginata*.

PREVENTION

Human *T. asiatica* infection can be prevented by avoiding ingestion of undercooked liver and other viscera (especially porcine).

TAENIA SOLIUM

Cysticercosis was first described in ancient Greece, where cysticerci (literally bladder-tails) were noted in infected pork.[75] The life cycle was clarified by the 19th century. The main clinical manifestation results from infection of the central nervous system (CNS) and is termed neurocysticercosis. Large case series describing most of the clinical manifestations were published in the early 20th century.[47] Neurocysticercosis, however, was diagnosed only rarely until the late 1970s. Subsequent advances in neuroimaging led to a dramatic increase in recognition.[165] Neurocysticercosis is now recognized as among the more common causes of disease worldwide.[14,69,75,176,222]

THE ORGANISM

T. solium, referred to as the pork tapeworm, takes two distinct forms in the human host: taeniasis (tapeworm infection of the gut lumen) and cysticercosis (infection of tissues with the larval cysticercus form).[69,75] Neurocysticercosis refers to cysticercosis involving the CNS (including the subarachnoid space, spinal cord, and eyes). Humans develop the adult tapeworm after ingesting undercooked pork containing the cysticercus. The scolex evaginates in the intestines, attaches, and develops chains of proglottids. The tapeworms can be several meters long. Eggs and gravid proglottids are shed intermittently into stool. The off-white proglottids appear as flattened segments 1 mm thick and up to 2 cm in length and 1 cm in width. Pigs are infected after ingesting eggs or proglottids from human fecal material. Once ingested, the larval oncospheres emerge from the eggs, penetrate the wall of the gut, enter the bloodstream, and migrate to the tissues, where they develop into cysticerci during a period of a few weeks.

TRANSMISSION

Field studies of both taeniasis and cysticercosis are associated with tight clustering.[66,67,119,175] Taeniasis is acquired by ingestion of undercooked pork and is closely tied to pig-raising areas in endemic countries. Most carriers are from families that raise pigs and butcher them informally in the household. Cysticercosis is acquired from the human tapeworm carriers, who not only shed eggs into the environment but also harbor eggs on their hands and fingernails. These eggs can then autoinfect the tapeworm carrier or infect other people. Thus, most transmission occurs among close contacts of tapeworm carriers. Human cysticercosis is not always acquired directly from pork, as illustrated by outbreaks noted among vegetarians in India and orthodox Jews in the United States.[178,188,216] In both cases, transmission has been associated with domestic servants who are tapeworm carriers, a factor also seen in urban areas lacking infected pigs.[93]

EPIDEMIOLOGY

The global prevalence of human cysticercosis is not clearly defined. Between 50 and 100 million people are thought to be infected with cysticercosis, but far fewer with intestinal taeniasis. Nearly all areas where pigs are raised that have access to human fecal material are endemic for cysticercosis.[176] In endemic areas, pigs often are reared on small farms, where they are allowed to forage for food rather than being fed. Improved sanitation, meat inspection, and animal husbandry led to the eradication of cysticercosis from western Europe, which previously was highly endemic. However, similar approaches have not proved sustainable in poorer countries. Currently, porcine cysticercosis remains highly endemic in Latin America, sub-Saharan Africa, and South and Southeast Asia as well as parts of Korea, China, Indonesia, and Papua New Guinea.[14,98,128,188]

In the United States, porcine cysticercosis is a very rare occurrence. By contrast, human neurocysticercosis is widespread among immigrants from endemic countries.[46,146,185,197,198,213] Limited transmission occurs in the United States, mainly linked to tapeworm carriers infected in endemic areas.[38,199,220]

PATHOLOGY AND PATHOGENESIS

Cysticerci can develop in a wide range of tissues. In most tissues, the cysticerci cause few symptoms, and their survival appears to be limited. For example, many of those infected have cigar-shaped calcifications noted on radiographs of skeletal muscle, despite the absence of musculoskeletal symptoms. By contrast, infection of the CNS often lasts for years and can cause severe symptoms.

Cysticerci mature to their full size (typically 10 to 20 mm in diameter) within a few weeks after infection occurs; but as illustrated in studies of British subjects returning from India and Hispanic immigrants to the United States, symptoms do not develop for several years.[46,47] This delay may reflect the parasites' complex array of molecules used to modulate the host inflammatory response.[75,223] When the parasites lose the ability to evade immune attack, the cysticerci are attacked by a granulomatous response with a mixed cell population, including lymphocytes and mononuclear cells, with variable numbers of eosinophils and neutrophils.[87,123,163,164,182] This granulomatous host response to the parasite, when it is present in the brain parenchyma, is thought

to be the cause of seizures, the characteristic clinical feature of parenchymal neurocysticercosis.[201] During the course of several months, the inflamed parasite is invaded by inflammatory cells, the cyst cavity collapses, and the parasite material is replaced by the host granulomatous response.[87] The lesion eventually either resolves or is replaced by a small calcified granuloma. The calcified lesions contain a mixture of fibrosis and residual parasitic debris. An inflammatory response to persistent parasitic antigen may be the cause of recurrent seizures in patients with cerebral calcifications. Rarely, massive infection is accompanied by a diffuse cerebral edema.[162,207]

Extraparenchymal neurocysticercosis is associated with a poorer prognosis, largely because of production of hydrocephalus.[10,64,65,75] Parasites can cause obstructive hydrocephalus by lodging in the outflow tracks of the cerebral ventricles. Cysticerci in the basilar cisterns often are associated with chronic arachnoiditis. This basilar inflammation may lead to chronic meningitis (with headache and nuchal rigidity but usually without fever), vasculitis and strokes, or communicating hydrocephalus. Less frequently, cysticerci in fissures enlarge to 5 cm or larger (termed giant cysticerci). The parasites, along with accompanying cerebral edema, may cause a mass effect. In patients with multiple cysticerci, several forms of the disease can be present at the same time.

CLINICAL MANIFESTATIONS

Taeniasis

Taeniasis typically causes few symptoms. Cases have been associated with vague abdominal complaints and occasionally pruritus. The most specific symptom is the passage of proglottids in the stool. The proglottids appear flattened (typically 0.3 to 0.5 cm wide by 1 to 2 cm long and 1 mm thick). Each segment has 13 or fewer uterine branches. Unlike *T. saginata*, the proglottids usually are single and seldom are noted to be motile.

Cysticercosis

The clinical manifestations of cysticercosis are extremely variable, depending on the location of the parasites and the host inflammatory response. Experts now group neurocysticercosis into a range of syndromes that differ in pathogenesis, clinical manifestations, prognosis, and management.[64,65,75,144,183]

SINGLE ENHANCING LESIONS. The most common presentation of children with neurocysticercosis is with seizures and a single enhancing lesion on neuroimaging studies.[113,136,168,169,172,190,192,207,219] Onset typically occurs in those 5 to 40 years old, but it has been noted in infants. Most cases are manifested with seizures, which can be isolated or recurrent. The seizures typically are focal with secondary generalization, although they may be focal or generalized.[190,219] Some children will complain only of severe headaches,[156] which can resemble either tension headaches or migraines. Imaging studies of children reveal either a focal area of enhancement or a ring of enhancing lesions, often with surrounding edema. Lesions typically are found within the cortex.

MULTIPLE CYSTIC (VIABLE) LESIONS. Similar to children with single enhancing lesions, patients with multiple parenchymal cysticerci also present with seizures. The seizures more often are generalized or focal with secondary generalization but may also be focal.[21,22,45,74,192] Neuroimaging studies demonstrate edema or contrast enhancement or both for one or more parasites,[186,222] and symptoms are thought to result from the host inflammatory response. Noninflamed cysts cause few symptoms, even when they are numerous,[62] but they pose a risk for development of recurrent symptoms when they degenerate.

Other symptoms include headaches. Infected children may develop learning disabilities,[120,169] but in many cases they may be the result of either poorly controlled seizures or hydrocephalus.[203] An association of cysticercosis with depression and occasionally psychotic episodes also may exist.[59]

CYSTICERCAL ENCEPHALITIS. Some children present with diffuse cerebral edema from large numbers of inflamed cysticerci, termed cysticercal encephalitis. The clinical presentation includes symptoms or signs of raised intracranial pressure, seizures, and altered mental status.[162,207] Cysticercal encephalitis is thought to result from a brisk inflammatory response to a massive infection and is seen more frequently in children and women than in adult men. This pathogenesis is the direct result of the host inflammatory response, and the key to management is to address inflammatory and cerebral edema. Antiparasitic drugs are contraindicated because they can worsen the cerebral edema.

PARENCHYMAL CALCIFICATIONS. Resolution of neurocysticercosis can be associated with the formation of calcified granulomata within the brain parenchyma.[142] The calcifications appear as well-defined calcified nodules measuring 2 to 10 mm. The calcified lesions contain fibrotic reactions, parasite debris, and calcium deposits, with variable degrees of inflammation. Patients frequently present with seizures.[189] The most common imaging finding from neurocysticercosis in population-based studies in endemic villages is with focal calcifications.[44,57,139,160] Few patients will have focal abnormalities on electroencephalographic studies.[24,45] Among patients with seizures, calcified lesions are a risk factor for having recurrent seizures.[39,78,159] Patients with seizures and calcifications should be treated with antiepileptic therapy indefinitely. Some patients with neurocysticercosis and seizures have calcified lesions and associated enhancement and edema on magnetic resonance imaging (MRI) studies.[7,86,143,187] Rather than revealing viable parasites, the enhancement may result from breakdown of the calcified granulomata, with release of antigen resulting in restimulation of the host inflammation.[86]

VENTRICULAR NEUROCYSTICERCOSIS. Approximately 10 to 20 percent of adult patients with cysticercosis have cysticerci in the ventricles,[75,64,144,173,222] but the numbers often are lower than those in children.[172] Cysticerci are found in any of the ventricles, but symptoms are particularly associated with obstruction of the outflow of the third or fourth ventricle at the cerebral aqueduct or foramina of Luschka and Magendie. In contrast to parenchymal disease that largely results from the host inflammatory response, symptoms of ventricular disease result from mechanical obstruction typically caused by viable parasites.[104] Within the thin-walled viable cysticerci, the cyst fluid often is isodense with cerebrospinal fluid (CSF), rendering them difficult to detect. Computed tomography (CT) scanning may show only obstructive hydrocephalus or distortion of the shapes of the involved ventricle. Even on MRI, the findings may be subtle and can be missed by inexperienced observers unless the patients have concomitant parenchymal cysticerci.[89,233,234]

Symptoms from ventricular neurocysticercosis usually result from raised intracranial pressure and include headache, nausea or vomiting, altered mental status, papilledema with visual changes, or dizziness. The onset is extremely variable from chronic intermittent headache to sudden loss of consciousness.

SUBARACHNOID CYSTICERCOSIS. Subarachnoid cysticercosis can also be quite variable. Small cysticerci in the gyri have a clinical presentation and prognosis similar to that of parenchymal cysts, although they are associated more often with

CSF pleocytosis, may be slightly larger, and respond less well to antiparasitic drugs.[173] Cysticerci in the basilar cistern can cause arachnoiditis, leading to CSF outflow obstruction, communicating hydrocephalus, vasculitis, and strokes.[10,63,200] In the era before antiparasitic drugs were available, this form carried a high case-fatality rate.[200] By contrast, more recent case series have been characterized by low mortality rates.[40,144]

Most patients with cysticerci in the basilar cisterns are infected with large numbers of parasites, and they frequently have coexisting ventricular or parenchymal as well as subarachnoid disease. Symptomatic cysticerci in the basilar cisterns typically are accompanied by chronic arachnoiditis. This arachnoiditis may present as meningitis with headache, stiff neck, and CSF pleocytosis, but it usually is not accompanied by fever. Patients often develop communicating hydrocephalus (headaches, nausea, vomiting, and dizziness). Basilar cysticercosis frequently involves the basilar vasculature either by direct invasion or by inducing vasculitis. Thus, patients may present with cerebrovascular accidents, which may involve either small vessels with lacunar infarctions from vasculitis or large-vessel strokes (associated with invasion of the vessel wall by the parasite).[11,41,64]

GIANT CYSTICERCI. Cysticerci, termed giant cysticerci, can develop in the fissures (especially the sylvian fissure) and may enlarge to more than 5 cm in diameter.[151] In some cases, the cysticerci can lose the scolex and may grow as clusters of cyst walls (termed racemose cysticercosis). Giant cysticerci not only are associated with symptoms of parenchymal inflammation but also can cause mass effects such as midline shift. Frequently, giant cysticerci are accompanied by cysticerci in the parenchyma or basilar cisterns. Giant cysticerci are readily visualized by CT or MRI, but the accompanying basilar cysticerci may not be seen as easily.

OTHER FORMS OF CYSTICERCOSIS. Neurocysticercosis rarely involves the spine.[10] Most patients present with radicular pain and less frequently myelitis. In most cases, cysticerci are in the spinal fluid, but they can also be intramedullary cysticerci.[118] The intramedullary form typically presents with myelitis. Orbital involvement includes subconjunctival cysticerci and involvement of the extraocular muscles.[155,181] Intraocular disease may be subretinal, intravitreal, or within the anterior chamber. Skeletal muscle involvement typically is asymptomatic, but it may result in pseudohypertrophy or weakness with massive infection. Subcutaneous lesions typically present as one or more painless mobile cystic lesions. Subcutaneous disease is more commonly noted in Asia and Africa than in the Western Hemisphere.

DIAGNOSIS

Taeniasis

Taeniasis has been diagnosed traditionally by examination of the stool for proglottids, ova, or both. The ova and proglottids are shed only intermittently, and collection of stool specimens from children can prove difficult. Furthermore, the sensitivity of stool microscopy for ova is only 26 percent,[5] and the ova of *T. solium* and *T. saginata* are indistinguishable, which limits the specificity of the assay. Identification of species requires collection of the proglottids, which can be facilitated by treating the patient with polyethylene glycol.[101] The proglottids of the two species can be distinguished by counting the number of uterine branches, which are lower for *T. solium*.

More sensitive and specific techniques are available only as research tools. Antigen-detection assays have a specificity of 99.2 percent and a sensitivity of 70 to 92 percent,[4,5,166] but they cannot distinguish *Taenia* spp. Enzyme-linked immunosorbent assay (ELISA) to identify serum antibodies to *T. solium* tapeworm-stage antigens may be more sensitive than are stool-based tests and seem to be specific for the *T. solium* tapeworm stage.[88,121] However, the antibody may persist after treatment. Polymerase chain reaction assays also can be used to improve sensitivity and specificity.[133]

Neurocysticercosis

Establishing the diagnosis of neurocysticercosis is more difficult than with many other parasitic infections. The major clinical presentations are nonspecific, and the location of the parasites within the CNS usually precludes their being observed directly. Serologic tests using crude antigens (including a number of commercially available ELISAs) are plagued by poor sensitivity and specificity. However, computerized imaging methods (e.g., CT and MRI) have led to a dramatic increase in the ability to recognize cases and are now the mainstay of diagnosis (Figs. 238–1 to 238–3), although even these techniques cannot always distinguish neurocysticercosis from other neurologic processes.

An expert group proposed diagnostic criteria based on neuroimaging studies, serologic tests, clinical history, and exposure.[42] The presence of either a single absolute criterion or two major criteria along with two minor or epidemiologic criteria is considered diagnostic. One major criterion plus two other criteria or three minor criteria along with exposure define a probable diagnosis.

Direct visualization of the parasite on ophthalmoscopic examination or histology is considered diagnostic of neurocysticercosis.[42] However, biopsy or autopsy material demonstrating *T. solium* parasites rarely is available, and parasites seldom are visualized in the eye. The neuroimaging pattern of a cystic lesion with

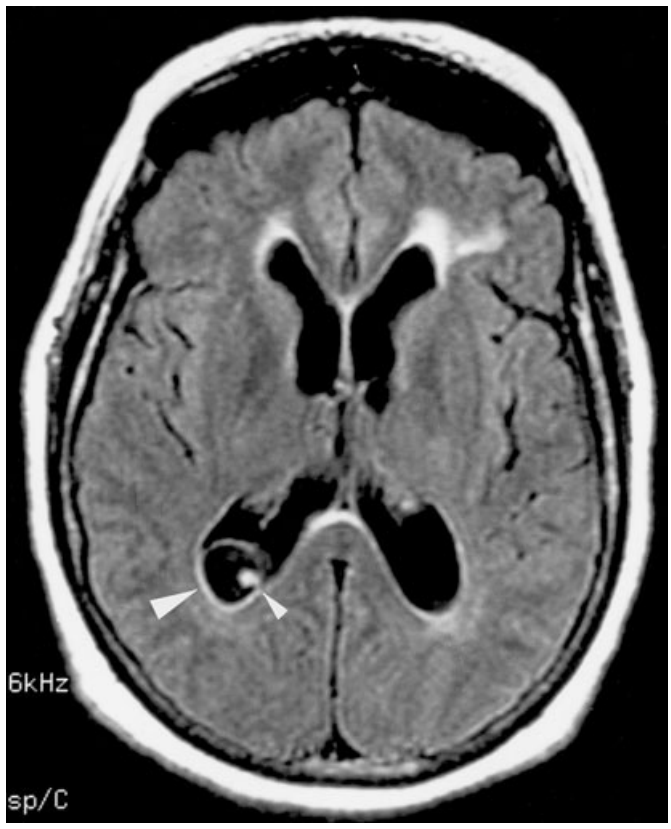

Figure 238–1 Ventricular neurocysticercosis. *(Courtesy of C. Mark Mehringer, MD, Harbor-UCLA Medical Center.)*

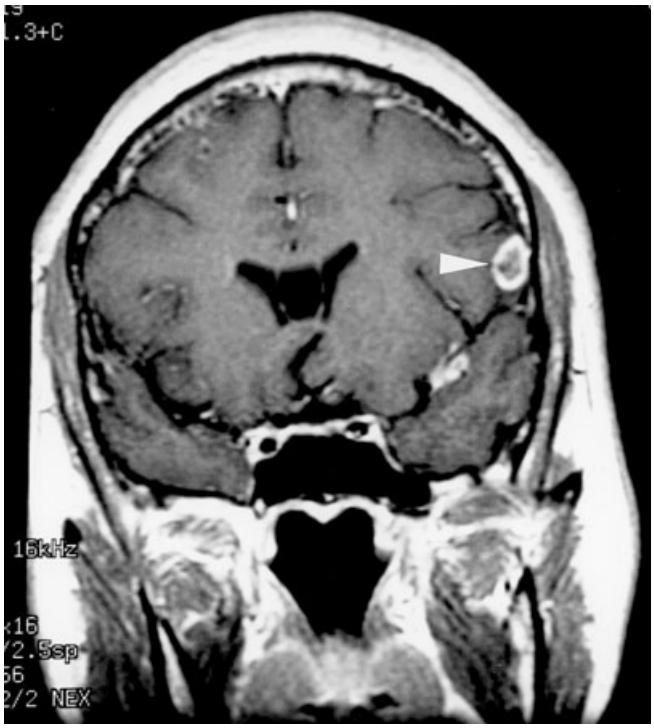

Figure 238–2 Neurocysticercosis with subarachnoid involvement. *(Courtesy of C. Mark Mehringer, MD, Harbor-UCLA Medical Center.)*

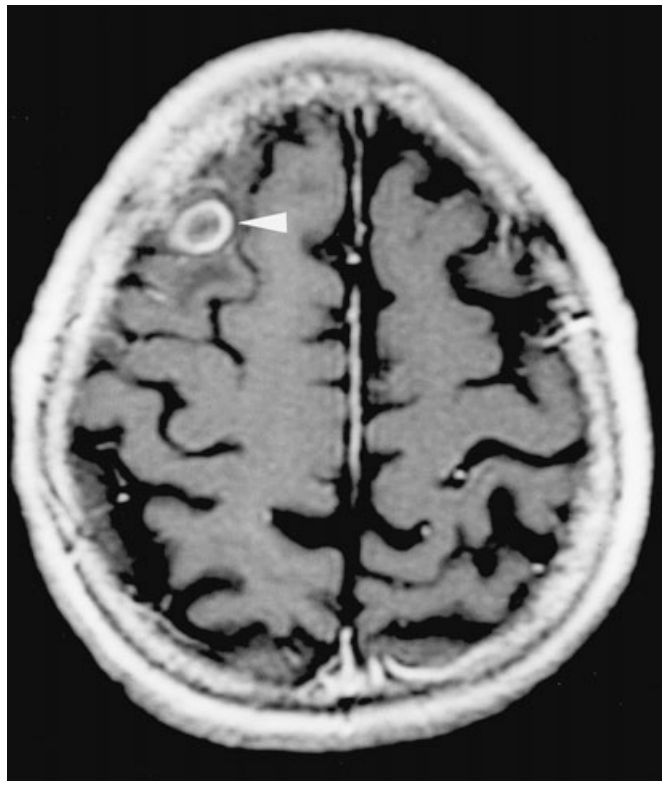

Figure 238–3 Neurocysticercosis with parenchymal involvement. *(Courtesy of C. Mark Mehringer, MD, Harbor-UCLA Medical Center.)*

a mural nodule measuring 1 to 3 mm (consistent with a scolex) is thought to be pathognomonic for cysticercosis.[232,234] However, rare cases have been reported in which similar findings have been noted in other diseases, and even in neurocysticercosis, the scolex cannot be visualized in most cases.

The main major diagnostic criterion for neurocysticercosis is one or more lesions on neuroimaging studies highly suggestive of neurocysticercosis.[42] Viable cysticerci appear on CT or MRI scans as rounded fluid collections typically 1 to 2 cm in diameter. The cyst fluid is isodense with CSF (hypodense compared with brain tissue on CT or T1 imaging). The scolex sometimes is visible as a small nodule found attached to the cyst wall. The wall of the cysticercus initially is isodense with the parenchyma and not easily visualized. On T2-weighted and fluid attenuated inversion recovery images, the fluid is seen as hyperintense. Inflamed cysticerci are characterized by perilesional edema and contrast enhancement.[201] Subsequently, the cyst fluid increases in density and becomes infiltrated by host inflammatory cells. The cyst cavity eventually collapses, forming a solid area of focal enhancement. At that point, the granulomatous inflammation either resolves or leads to the formation of a calcified nodule typically measuring 2 to 6 mm in diameter.

Although enhancing lesions are typical of neurocysticercosis, similar lesions can be noted with tuberculomas, brain abscesses, and tumors. Rajshekhar and Chandy[158] noted that cysticercal lesions typically have a diameter smaller than 20 mm and rarely cause midline shift. A single, round, enhancing lesion less than 20 mm in diameter without midline shift on imaging studies in patients lacking signs or symptoms of systemic disease, focal neurologic deficits, or increased intracranial pressure was highly suggestive of neurocysticercosis in a prospective study of 401 patients in southern India.[160] However, the results are less specific in areas with a lower prevalence of cysticercosis. Spontaneous resolution or resolution achieved with antiparasitic drugs is another major criterion.[157]

Serodiagnosis has proved problematic for cysticercosis. ELISAs employing unfractionated antigens, such as cyst fluid, have poor sensitivity and specificity.[152,161] The preferred serodiagnostic test is the enzyme-linked immunotransfer blot employing semi-purified parasite glycoproteins.[214] A positive reaction to any one of seven glycoprotein bands is considered positive. ELISAs using recombinant versions of these antigens are being developed.[64] Studies have confirmed a specificity of nearly 100 percent in suspected cases, but the sensitivity is limited in subjects with either a single lesion or with only calcified lesions.[81,152,179,226] For this assay, tests of serum are more sensitive than are tests of CSF.[226] Assays to detect parasite antigens may prove to be important diagnostic studies.[37,54,70,72,73,148] Although several different antigen detection tests are in development, none is widely available commercially.

Minor criteria include (1) lesions on neuroimaging studies consistent with neurocysticercosis but less suggestive (e.g., isolated basilar meningitis, hydrocephalus without demonstration of cystic lesions, or filling defects in the spinal subarachnoid space without discrete cysticerci), (2) symptoms suggestive of seizures or hydrocephalus, and (3) cysticercosis outside the nervous system (e.g., cigar-shaped muscle calcifications or subcutaneous nodules).[42] Epidemiologic criteria include residence or prolonged visits to endemic areas and contact with a tapeworm carrier.

Numerous other tests may prove useful in excluding other illnesses but are not diagnostic of neurocysticercosis. These tests include blood counts, lumbar puncture for testing of CSF, and stool studies. Lumbar CSF frequently has elevated protein levels and may show hypoglycorrhachia. Cell counts are variable and may demonstrate a predominance of lymphocytes, neutrophils, or eosinophils.

TREATMENT

For patients with seizures caused by neurocysticercosis, initial management should focus on control of the seizures, which usually can be accomplished with a single antiepileptic drug. Most published studies describe use of phenytoin or carbamazepine. Newer agents (e.g., valproate, lamotrigine, levetiracetam, topiramate, oxcarbazepine, or clobazam) may be more effective, but they are not affordable in endemic areas.[103] Breakthrough seizures usually occur with subtherapeutic antiepileptic drug levels, often from poor adherence to medication therapies.[74] Seizure therapy can be tapered off for many patients after they have a seizure-free period if radiographic resolution of the lesion is seen. However, a substantial risk exists for recurrence of seizures if residual inflamed or calcified lesions are present.[22,74,159,219] Antiepileptic drugs should be continued indefinitely if calcifications develop.

Patients with obstructive hydrocephalus usually require surgery.[65,144,183] For patients with mild or intermittent symptoms (e.g., a cysticercus in the lateral ventricles not associated with midline shift), elective endoscopic removal of the cysticercus is the treatment of choice. Cases with altered mental status or impending herniation should undergo emergency CSF diversion by a ventriculostomy or placement of a ventriculoperitoneal shunt. A high rate of shunt failure exists if shunting is not followed by administration of antiparasitic therapy or corticosteroids.[75,104,200] Patients with communicating hydrocephalus often require placement of a ventriculoperitoneal shunt.[104] In some patients, cysticerci and associated edema may lead to mass effects. If the symptoms are life-threatening or if the cysticercus is easily approachable (e.g., in the sylvian fissure), surgical decompression of the cysticercus is the preferred approach.

A consensus is emerging among experts about the proper role of antiparasitic drugs and corticosteroids in the management of neurocysticercosis.[144,183] Experts agree that neurocysticercosis represents a spectrum of diseases that differ in optimal management.

SINGLE ENHANCING LESIONS. The most common presentation of children with neurocysticercosis is seizures and a single enhancing lesion. Seizures generally can be controlled with antiepileptic medications.[103,159,219] Controlled trials of antiparasitic drugs in patients with single enhancing lesions have demonstrated a favorable prognosis with symptomatic therapy. However, the length of time until radiologic resolution is achieved and the duration of time for risk of experiencing seizures appear to be somewhat shorter with antiparasitic drug treatment.[43,191,209] By contrast, these treatments do not appear to affect the formation of calcified lesions or development of chronic epilepsy. The role of steroids is better defined; several studies showed clear benefit in reducing seizure activity and faster resolution of the lesion on CT when steroids were used in conjunction with antiepileptic medications.[77,79,112,130,150] Overall, the best evidence points to a small benefit achieved from use of antiparasitic drugs (e.g., albendazole, 15 mg/kg/day for 8 days) and a short course of corticosteroids (e.g., prednisone, 1 mg/kg/day for 8 to 10 days).

MULTIPLE PARENCHYMAL LESIONS. Patients presenting with seizure activity with multiple cysticerci usually will have one or more lesions in the process of degenerating (e.g., edema or contrast enhancement on imaging studies). However, other cysticerci may be in the viable stage, posing a prolonged risk for having seizures. A placebo-controlled trial from Peru showed a modest reduction in the number of generalized seizures in patients treated with albendazole and corticosteroid,[74] but no decrease occurred in the development of chronic calcifications. A meta-analysis of trials also supported this approach.[43] One recent trial suggested a worse outcome with this treatment,[36] an effect not seen in any other study. Overall, the current expert consensus is that patients with multiple parenchymal cysticerci usually should be treated with a course of an antiparasitic agent (albendazole, 15 mg/kg/day in two daily doses for 8 days) and a simultaneous course of corticosteroids.[144] Even so, symptomatic therapy (e.g., antiepileptic drugs) remains a key to management.

CYSTICERCAL ENCEPHALITIS (NUMEROUS CYSTICERCI WITH CEREBRAL EDEMA). In this context of diffuse cerebral edema (cysticercal encephalitis), antiparasitic drugs are contraindicated because of their potential to exacerbate host inflammatory responses.[65,162,207] Most cases will resolve spontaneously with anti-inflammatory medications.

CALCIFIED PARENCHYMAL CYSTICERCI. Parenchymal brain calcifications can be seen in more than 10 percent of residents in some neurocysticercosis endemic areas.[142,160] These parenchymal calcifications are a significant risk factor for having continued seizure activity.[76,159,219] If a patient with a history of seizures has a calcified lesion, that patient should be treated with antiepileptic therapy indefinitely. Recurrent seizures may be due to concomitant mesiotemporal sclerosis.[218] Antiparasitic medications and anti-inflammatory drugs are not indicated. Surgical removal of the calcified focus has been used in some cases of intractable seizures.[225] By contrast, most cases can be managed with a single antiepileptic medication.

VENTRICULAR NEUROCYSTICERCOSIS. Patients with obstructive hydrocephalus usually require surgery. For mild or intermittent symptoms (e.g., a cysticercus in the lateral ventricles not associated with midline shift), elective endoscopic removal of the cysticercus is the treatment of choice.[12,65,94,144,154,202] Cases with altered mental status or impending herniation should undergo emergency CSF diversion by a ventriculostomy or placement of a ventriculoperitoneal shunt. A high rate of shunt failure exists if shunting is not followed by administration of antiparasitic therapy. Cysticerci in the lateral and third ventricles usually can be removed through a rigid endoscope. A flexible endoscope is required to approach the fourth ventricle or basilar cisterns.[95,212,235] Endoscopic foraminotomy and third ventriculostomy often can be used to treat residual hydrocephalus. Antiparasitic medications should not be given before the procedure is performed as they can cause the cysticerci to be friable or to adhere to the ventricular wall. The cysticerci frequently rupture during removal, but this rupture has not been associated with any adverse effects. Endoscopic third ventriculostomy has been used as an alternative to shunting procedures.[12,94,114,153]

A ventriculoperitoneal shunt may be used to treat hydrocephalus. The rate of shunt failure is high in patients treated only with ventriculoperitoneal shunting. However, shunt failure appears to occur less frequently when patients receive adjunct therapy with corticosteroids and antiparasitic drugs.[12] Although some patients have been treated with only chemotherapy and steroids, a substantial risk exists for development of acute hydro-

cephalus from cysticerci or accompanying inflammation causing obstruction of the foramina.[13]

SUBARACHNOID NEUROCYSTICERCOSIS. Basilar subarachnoid cysticercosis is perhaps the most severe form of infection and carries risks for communicating hydrocephalus, vasculitis, and strokes.[64,75,144] Data on how best to treat this form are limited, but most experts agree that patients should be treated with antiparasitic drugs, corticosteroids, and, in most cases, CSF diversion procedures.[151] Most experts recommend treatment with albendazole (typically 15 mg/kg/day in divided doses for at least 28 days), but cure rarely is achieved with a single course of albendazole. Repeated courses, prolonged therapy, higher doses, switching to praziquantel, and combination of the two drugs have been tried in some cases.[131] Improved responses with endoscopic debulking of a number of parasites are anecdotal,[85,144] and this approach has not yet been studied systematically.

Inflammation seems to drive much of the pathogenesis, and the residual symptoms may reflect a chronic inflammatory response to cysticercal antigens rather than viable cysticerci. Prednisone (doses up to 60 mg per day) or dexamethasone (doses up to 24 mg per day) should be used along with the antiparasitic drugs.[63] After 2 to 4 weeks, the dose often can be tapered. In cases that require more prolonged steroid therapy, methotrexate has been used as a steroid-sparing agent.[137]

GIANT SUBARACHNOID NEUROCYSTICERCOSIS. Some cysticerci, particularly in the sylvian fissure or basilar cisterns, may enlarge to more than 5 cm in diameter, causing mass effect either directly or by surrounding edema. If mass effect cannot be reversed quickly by corticosteroids and even mannitol, surgical decompression may be required. In the absence of symptomatic mass effect, however, giant cysticerci can be treated similarly to other subarachnoid cysticerci.[144,151]

OTHER FORMS OF NEUROCYSTICERCOSIS. Cysticercosis of the eye, spine, or subcutaneous tissue generally is treated by surgical removal.[75] There are, however, anecdotes of medical therapy.

PROGNOSIS

The prognosis of neurocysticercosis is extremely variable, depending on the sort of infection. Patients with single enhancing lesions generally do well and often are seizure free and discontinue therapy within 1 to 2 years. Patients with calcified lesions may require chronic antiepileptic therapy. Patients with numerous subarachnoid cysticerci often require repeated courses of therapy and even repeated surgeries. However, with optimal management of hydrocephalus, cases are rarely fatal.

PREVENTION

Cysticercosis was eliminated from western Europe by improved sanitation, animal husbandry, and meat inspection.[68,83] For example, porcine cysticercosis can be prevented by not allowing pigs access to human feces.[217] However, confined pigs must be fed, which is not affordable for peasant farmers. Human infection with adult tapeworms also can be prevented by adequate cooking or freezing of infected pork, but meat inspection has not been successful in areas where pigs are slaughtered informally.[205] Mass chemotherapy of endemic populations for carriage of the human tapeworm or porcine cysticercosis has resulted in significant short-term decreases in prevalence,[4,35,71,83,174] but prevalence rates rapidly return unless mass treatment is continued. Development of an effective vaccine against cysticercosis may provide the best potential tool for eradication of the disease. There are promising studies that this will be possible for human and porcine cysticercosis.[58,84]

COENUROSIS (*TAENIA MULTICEPS,* OTHERS) AND CYSTICERCOSIS CAUSED BY *TAENIA CRASSICEPS*

Several species of *Taenia* have a larval stage called a *coenurus* that consists of a cystic membranous structure measuring up to 6 cm in diameter from which multiple scolices bud internally or externally.[87,97] On the basis of this morphologic appearance, the parasite was given the species name *multiceps*. Currently, the preferred genus name is *Taenia* because of the morphology of the adult worms and the inability to justify a genus designation based solely on the form of larvae. The following species generally are accepted for purposes of discussion. The adult tapeworm of *Taenia multiceps* is found in dogs, and the larval stages occur in herbivores. The larvae often develop in the CNS in sheep. In sheep-raising areas of Europe and Asia, the infection is still endemic in animals. *Taenia serialis* adult worms are found in dogs and other canids, but common intermediate hosts are rabbits, hares, and rodents. The coenurus develops in subcutaneous and intramuscular tissue. *T. serialis* has been reported from the United States, Canada, France, and Africa. *Taenia brauni* is the name given to the tapeworms of dogs, jackals, foxes, and genets found in tropical Africa. The larvae develop in gerbils and other rodents. Humans are a rare accidental host for the larval stages of these worms. Fewer than 100 human infections have been recorded. Most infections are from Africa, with many occurring in children. Giving a species designation based solely on the morphologic features of organisms recovered from human tissue is nearly impossible. The infection is presumed to be acquired by the ingestion of eggs excreted by the definitive hosts, usually dogs. Because of the subcutaneous and subconjunctival locations of cysts in infections in tropical Africa, some researchers have suggested that direct contact of the eggs on skin or the conjunctiva is a mode of transmission. A review of the six human infections in North America has been published.[87,97]

The clinical manifestations of coenurosis are related to the location of the parasite. Involvement of the CNS produces a spectrum of illness that resembles that of cysticercosis (discussed earlier), including meningeal reactions. Larvae have been seen in the subconjunctival and subretinal tissue, the extraocular muscles, the anterior chamber, and the vitreous. Subcutaneous and intramuscular lesions occur most commonly in the abdomen and chest wall. A definitive diagnosis is made by demonstrating the characteristic morphologic features of the larva recovered at surgery. The multiple scolices that bud from the delicate cyst membrane have double rows of hooklets of a typical shape, size, and number. In instances in which no scolices are found, differentiation of a coenurus from a cysticercus of *T. solium* is impossible. The diagnosis of intramuscular coenurosis has been made by examination of fine-needle aspirates.[87,97] Radiographic studies such as CT are useful in cerebral coenurosis but do not differentiate the parasite from other cystic lesions. Treatment is surgical. Mortality rates in cerebral disease are high. Organisms in other locations, with the exception of subretinal lesions, are removed easily. A combination of praziquantel and a corticosteroid administered to a patient with a subretinal coenurus caused death of the parasite, which resulted in a severe inflammatory reaction, retinal detachment, and permanent loss of vision.[96] Severe reactions were reported in the two additional cases described in this review. Praziquantel should be used with great caution, if at all, in cases

of human coenurosis. Although the exact mode of infection in humans has not been identified, avoidance of close contact with dogs and dog excreta, which are the most likely sources of infection, is prudent.

Taenia crassiceps cysticerci usually are found in field mice, with the tapeworm found in foxes, other canids, and felines. A limited number of cases have been noted to cause ocular disease or mass lesions in compromised hosts (e.g., patients with acquired immunodeficiency syndrome).[25,28,61,90,129] Diagnosis and management require surgery.

DIPHYLLOBOTHRIUM SPECIES (FISH TAPEWORM)

Diphyllobothriasis can occur in children who ingest raw or undercooked fresh-water fish. The prevalence of infection increases with age in endemic areas. Nonspecific gastrointestinal complaints may occur in infected individuals, and megaloblastic anemia has resulted.[31,227]

Several species of *Diphyllobothrium* have been reported in humans. *Diphyllobothrium latum* was described first in 1592 from specimens found in Switzerland; it is now estimated to infect approximately 9,000,000 individuals worldwide, with most cases occurring in Europe and some in South America and Asia.[49] *Diphyllobothrium pacificum* is a similar fish tapeworm found infecting humans in Japan and South America, and *Diphyllobothrium nihonkaiense* has been found in Pacific salmon (and, hence, in humans) in Japan and Europe.[31] The *Diphyllobothrium* spp. typically have fish-eating birds, marine mammals, bears, dogs, and foxes as definitive hosts, but children living in endemic areas may be infected when they eat raw or undercooked fish.

THE ORGANISM

The scolex of *D. latum* possesses neither discrete suckers nor hooks but has two deep grooves. The worm may be as long as 15 m. The gravid proglottids have a characteristic rosette-shaped central uterus. Eggs measure 55 to 61 μm by 37 to 56 μm and have light brown, operculated shells. The eggs are shed in an unembryonated state from a uterine pore into the fecal stream. The embryos develop within the eggs in fresh water. The ciliated embryos hatch and are ingested by copepods to develop further. After the fish have ingested the infected copepod, the larval stages develop into the plerocercoid stage in the muscles of fish. As the smaller fish are eaten by larger species, the larvae parasitize the muscles of the new host. Eventually, the larvae are found in fish that are sources of food for humans, such as species of pike, perch, turbot, lake trout, and whitefish. The plerocercoid larvae or spargana are white, ribbon-like worms approximately 5 cm in length.

TRANSMISSION

Children can be infected by ingesting the plerocercoid forms in raw or undercooked fish muscle that are infectious when ingested by people. Cooking and freezing kill the larvae. The cycle of infection is perpetuated by the discharge of untreated human sewage into fresh-water lakes and streams. Some fish-eating mammals also may serve as definitive hosts for *D. latum* and maintain the cycle of infection in the absence of humans. Because *D. latum* requires intermediate hosts, direct transmission cannot occur, and no techniques of isolation or special precautions are required for infected patients.

EPIDEMIOLOGY

The prevalence of infection increases with age in endemic areas. However, infections have been reported in children younger than 1 year.[31] The disease is worldwide in distribution, but higher endemicity is associated with eating fresh-water fish raw, lightly salted, or pickled without cooking. Infection occurs frequently in the Baltic countries and Russia; South America; Scandinavia; Switzerland and the adjacent lake regions of Italy, France, and Germany; the Danube River delta; the lake areas of the northern United States; Canada; and the river deltas of Alaska. Five decades ago in northern Canada, 83 percent of Eskimos older than 2 years were infected with *D. latum*. Pediatric infection by *D. latum* has been identified recently in school-age children in India, Taiwan, Korea, Argentina, and Hawaii. Salmonid fish (salmon, trout, whitefish) transmit *D. nihonkaiense* to humans in Japan. Infection of humans with a tapeworm parasite of seals, *D. pacificum*, has been reported from Peru.

PATHOLOGY AND PATHOGENESIS

Little is known about the direct effects of parasitization, with the exception of megaloblastic anemia.[1] The following factors may be significant: (1) the strains of *D. latum* found in Finland, where anemia occurs, absorb seven times more vitamin B_{12} than do strains from North America, where anemia has not been reported; (2) interference with absorption of vitamin B_{12} occurs in nonanemic carriers as well as in those with anemia, and deficient stores of vitamin B_{12} plus malabsorption of vitamin B_{12} may contribute to the anemic state; (3) dietary intake of vitamin B_{12} may be low in anemic patients, and oral vitamin B_{12} has been shown to cause reticulocytosis in the presence of the worm; (4) worms found in anemic patients take up more of an oral dose of vitamin B_{12} than do worms in asymptomatic carriers, and worms are attached more proximally in the small intestine in anemic patients; and (5) secretion of intrinsic factor is reduced in anemic patients.

CLINICAL MANIFESTATIONS

Most infections are asymptomatic, but patients may notice proglottids or chains of segments in the stool. In Finland, comparison of nonanemic infected patients with uninfected controls revealed an increase in the symptoms of fatigue, weakness, craving for salt, lack of well-being, dizziness, and numbness of the extremities in the infected group.[170] Significant gastrointestinal symptoms occur infrequently. However, an experimental infection with seven larvae produced nausea, severe periumbilical pain, and marked weight loss. Episodes of intestinal obstruction associated with the vomiting of masses of tapeworms have been reported. Eosinophilia is an uncommon finding. Patients with *D. pacificum* complained of abdominal pain, vomiting, diarrhea, and flatulence.[125] Megaloblastic anemia caused by *D. latum* infection is extremely rare outside Finland and neighboring endemic areas. Usually, it is seen in patients older than 50 years (and may occur in as many as 2 percent of adult tapeworm carriers), but it has been reported in children as young as 9 years.

DIAGNOSIS

Identification of the characteristic eggs (oval, operculated, approximately 60 μm across) or proglottids provides the

diagnosis. The parasite produces nearly 1 million eggs per day, and ova usually are detected easily by fecal examination.

TREATMENT AND PREVENTION

Treatment with praziquantel (5 to 10 mg/kg as a single oral dose) is highly effective. An alternative medical treatment is niclosamide (50 mg/kg as a single oral dose). Tapeworm-related anemia is reversible by treatment of the infection, but the affected child should receive supplementation initially with vitamin B_{12}.

A significant reduction in the transmission of *D. latum* has been accomplished in many areas through the introduction of sewage treatment and targeted drug treatment of human infections. Raw and undercooked fish should not be consumed in areas where a possibility of larval infection exists. Larvae do not survive in fish that is either cooked for 5 minutes or more at temperatures of at least 55° C or frozen at temperatures of at least –10° C for 8 to 72 hours.[49]

DIPYLIDIUM CANINUM (DOG TAPEWORM)

Dipylidium caninum, the common tapeworm of dogs and cats, also infects infants and young children.[31,227] Transmission occurs through the accidental ingestion of infected fleas (*Ctenocephalides* spp.) or body lice (*Trichodectes* spp.). Although it usually is asymptomatic, infection may be associated with decreased appetite, abdominal discomfort, or, perhaps, diarrhea.

THE ORGANISM

D. caninum adult worms have a scolex with four cup-shaped suckers and a rostellum that bears one to seven rows of small hooks. The worm ranges from 10 to 50 cm in length. The gravid proglottids resemble cucumber seeds in size and shape. The proglottids have two pairs of sex organs, and a genital pore opens on each lateral margin. The eggs measure 35 to 65 µm in diameter and appear in packets of 5 to 30 enclosed in a membrane. The gravid proglottids are excreted in feces or migrate actively from the anus. The eggs are liberated as the proglottid disintegrates. Several species of flea serve as intermediate hosts. The larval flea ingests the egg of *D. caninum*. Development of the tapeworm's larval stages takes place as the flea metamorphoses into an adult. When the flea is ingested by the definitive host, the adult worm develops in the small intestine.

TRANSMISSION AND EPIDEMIOLOGY

Humans acquire the infection by the accidental ingestion of infected fleas. Dogs may transfer infective larvae to humans by licking children after crushing fleas orally. Because of the motility of the proglottids, eggs may be disseminated widely in the environment of animal hosts. Fleas are so ubiquitous that transmission to the intermediate host is accomplished readily.

Infection of dogs and cats is a common occurrence worldwide. *D. caninum* also has been found in a variety of wild canines and felids. Reports of dipylidiasis in humans in the United States, however, are uncommon.[23,138]

CLINICAL MANIFESTATIONS, DIAGNOSIS, PROGNOSIS, AND TREATMENT

Although the incubation period in humans is unknown, infection has been seen in infants aged 5 weeks. Frequently, the only evidence of infection is the finding of proglottids in stool or in an infant's diapers. Varying degrees of abdominal pain, diarrhea, irritability, and even pruritus have been recorded in symptomatic children.

The characteristic proglottids must be differentiated from other parasites. A frequent error is to assume, from the parents' description, that the small, motile proglottids that migrate from the anus are pinworms. Proglottids also may be mistaken for fly larvae. Examination findings of stool specimens may be spuriously negative because proglottids tend to migrate from the fecal mass and disintegrate on the walls of the specimen container. Treatment with praziquantel (5 to 10 mg/kg as a single oral dose) is effective. An alternative treatment is niclosamide (50 mg/kg as a single oral dose).

Infected dogs and cats also should be treated. Flea control requires the use of appropriate insecticides on pets. In addition, particular attention must be given to carpets and areas where pets sleep because these are the sites of development of the larval fleas.

HYMENOLEPIS NANA (DWARF TAPEWORM)

Infection with *Hymenolepis nana*, the dwarf tapeworm, occurs worldwide and probably is maintained by direct fecal-oral transmission from person to person.[31,227] Insects may serve as intermediate hosts. Infection may be asymptomatic, but gastrointestinal, neurologic, and allergic symptoms have been reported.

THE ORGANISM

Adult worms of *H. nana* measure 1 to 5 cm in length and are less than 1 mm in maximal width. The tiny scolex has four cup-shaped suckers and a rostellum with a circle of 20 to 30 small hooks. Gravid proglottids disintegrate within the intestine, and eggs are found in the feces. The oval eggs measure 30 to 50 µm in diameter and contain a six-hooked embryo within two envelopes. The space between the two envelopes contains filaments that extend from two small polar protrusions on the inner envelope. Larval stages develop in insect intermediate hosts after they have ingested the eggs. However, if the definitive host ingests eggs, larval stages develop in the intestinal villi in 4 to 5 days and then break into the lumen and develop into adult worms. Ova appear in feces 2 to 4 weeks after infection occurs. A morphologically identical organism found in rodents may be a separate species. The rodent strains are alternatively called *Hymenolepis fraterna* or *H. nana* var. *fraterna*.[31,127] The rodent strain occasionally infects humans.

TRANSMISSION

Human infection is acquired most commonly by the ingestion of eggs from the feces of infected persons. Humans serve as both intermediate and definitive hosts in this direct cycle. Poor hygienic habits, overcrowding, lack of running water, and any other factor that fosters fecal-oral transmission enhance transmission through this route. The rodent strains of *H. nana* are infectious for humans, and food contaminated by rodent feces is a possible source of infection. Eggs may be produced for periods longer than 1 year, but the possibility of recurrent autoinfection obscures determination of the duration of the initial infection. Internal autoinfection may occur when ova from gravid proglottids are exposed to appropriate conditions within the intestinal lumen. Hatching occurs, and the embryos penetrate the mucosa, undergo larval development, and eventually emerge as adult worms.

EPIDEMIOLOGY

H. nana is a common parasite of rats and mice worldwide, and 50 to 75 million people are thought to be infected.[31,227] The distribution of infection in humans is worldwide, with an increased prevalence in some urban areas. Stool surveys from endemic areas commonly show prevalence rates of 5 to 25 percent, which may approach 50 percent in children aged 4 to 10 years.[34]

PATHOLOGY AND PATHOGENESIS

Little is known of the pathogenesis and pathology of *H. nana* infection in humans. The larval stage of *H. nana* is a cystlike structure called a *cysticercoid* that is approximately 250 μm in diameter. Local reactions consisting of mucosal inflammation or atrophy may occur at the site of attachment of the adult worms in the small intestine. Rare cases of disseminated infection in children with depressed cellular immunity have been reported.[124]

CLINICAL MANIFESTATIONS

A wide variety of symptoms have been ascribed to *H. nana* infection, but few well-controlled clinical studies have been presented. Heavy burdens of worms have been associated with abdominal pain, diarrhea or loose stools, malnutrition, retardation of growth, and lethargy, whereas children with low burdens did not have significant symptoms.[26,27,193]

DIAGNOSIS

Detection of the characteristic eggs in fecal material by examination of direct saline wet mounts or by concentration techniques usually is not difficult. However, a single examination is not always adequate to rule out infection.

TREATMENT AND PROGNOSIS

Praziquantel (25 mg/kg as a single oral dose) is effective in treating *H. nana* infection. Nitazoxanide (100 mg by mouth twice daily for 3 days for children 1 to 3 years of age, 200 mg by mouth twice daily for 3 days for children 4 to 11 years of age, and 500 mg by mouth twice daily for 3 days for older children) is a reasonable alternative therapy; efficacy is approximately 75 to 82 percent.[26,147] Family members should be examined and treated if they are infected. Infection with *H. nana* rarely is severe and responds well to appropriate treatment.

PREVENTION

Transmission of *H. nana* infection largely could be eliminated if proper methods of personal hygiene and disposal of human waste could be invoked throughout the world. Infected food handlers always should be treated and monitored to ensure that treatment has been successful. Stored food should be protected from rodents and insects.

HYMENOLEPIS DIMINUTA (RAT TAPEWORM)

Hymenolepis diminuta is a rat tapeworm that only rarely presents as a human infection. Adult worms are 20 to 90 cm long; eggs are round.[31,227] Children become infected after ingesting larva-infected insects. Infection often is asymptomatic, but abdominal discomfort and pruritus have been reported. Treatment is with praziquantel (25 mg/kg in a single oral dose) or niclosamide.

SPARGANOSIS (INTERMEDIATE-STAGE *SPIROMETRA* SPECIES INFECTION)

Sparganosis is a zoonotic infection seen most frequently in eastern Asia.[87,227] It occurs when humans are infected with a metacestode larval stage of *Spirometra* spp.

THE ORGANISM

Evidence indicates that the amorphous, thin, ribbon-like spargana recovered from human infections belong to the genus *Spirometra*.[87,227] The adult tapeworms are found in dogs, cats, raccoons, and a variety of wild carnivores. Eggs passed in the feces of these definitive hosts hatch in fresh water. The embryo that emerges from the egg is ingested by a copepod, where it undergoes larval development. When a second intermediate host ingests the infected copepod, the larva develops into a sparganum (plerocercoid). The range of second intermediate hosts includes amphibians, reptiles, birds, and mammals. When the second intermediate host is eaten by the definitive host, the sparganum attaches to the intestinal mucosa and develops into the adult tapeworm. The designation of species found in humans is based largely on epidemiologic relationships to species described in local animals. The species found in Africa, Asia, Europe, and South America usually is called *Spirometra mansoni* or *Spirometra erinacei*, and the species infecting humans in the United States is called *Spirometra mansonoides*. A rare form of sparganosis that buds, branches, and multiplies asexually to massive numbers in humans is called *sparganum proliferum*.[141]

TRANSMISSION

In parts of Asia, application of poultices of amphibian or reptile flesh to wounds or sores is a common practice, and children can be infected when the spargana migrate from the poultice into human tissues. Human sparganosis in the United States often is associated with drinking well water or untreated surface water that could contain the minute copepods containing the procercoid form. Transmission by ingestion of the raw or inadequately cooked flesh of second intermediate hosts has been demonstrated experimentally in humans. Animal sources that have been incriminated in human infection are snakes, frogs, chickens, and pigs.

EPIDEMIOLOGY

Human infection is an uncommon occurrence. In the United States, almost all reports have come from the southern or southeastern states and from Puerto Rico or were reported in persons who had been in these areas. The only report of infection in a child in the United States came from southern California.[30] Reports of human infection have come mainly from China, Korea, Southeast Asia, Japan, India, Indonesia, the Philippines, Australia, Africa, Italy, South America, and the former Soviet Union.[87,227,229]

PATHOLOGY, PATHOGENESIS, AND CLINICAL MANIFESTATIONS

Ocular sparganosis, usually acquired by contact, may involve conjunctival, retro-orbital, or palpebral tissues and cause

conjunctivitis, periorbital and palpebral edema, exophthalmos, chemosis, and corneal ulceration.[87,227,229] Subcutaneous sparganosis is the most common form of the infection, with the trunk frequently being involved. The lesion usually is nodular. Tenderness and inflammation may be absent, intermittent, or constant. Some lesions are migratory. Eosinophils may or may not be present. Some infections have had an associated peripheral eosinophilia, but it is not a constant finding. Spargana have been identified in a wide range of other tissues. Sparganosis of the CNS may be associated with a variety of neurologic manifestations.[87,111,196,227,229] Brain imaging of patients with cerebral sparganosis shows edema and degeneration of cerebral white matter commonly with a "tunnel sign" of hypodensity and ring enhancement.[196] Sequential MRI imaging has revealed migration of larva between various regions of the brain in some children. The disease caused by sparganum proliferum consists of progressive replacement of host tissues by the multiplying organisms. Eleven cases reported from the world literature were reviewed in 1990.[141]

DIAGNOSIS

The diagnosis may be suspected in persons with typical subcutaneous lesions and a suggestive epidemiologic background. Usually, however, the diagnosis is made during surgical removal of a painful or cosmetically disturbing lump without anticipation of the parasitic etiology. Spargana vary considerably in width and length. They usually are several centimeters in length, whitish, and opaque, and they may have the grooved indentations on the anterior end that are precursors of the bothria or suckers of the scolex of the adult worm. Small portions of spargana are difficult to distinguish grossly or microscopically from cysticerci and other less common larval cestodes. Neuroimaging may yield characteristic findings in cerebral sparganosis.[111,196] Obtaining an ELISA for serum and CSF antibody appears to be useful in endemic areas, but this test is not generally available.[227]

TREATMENT AND PREVENTION

Surgical removal is the only known form of therapy. Praziquantel has been used in some cases, but no convincing evidence of clinical activity exists. Filtration of water prevents the ingestion of copepods from wells or ponds. Proper cooking of meat eliminates second intermediate hosts as sources of infection. Educational effort should be made to warn of the dangers of applying raw flesh poultices in areas where it is a cultural practice.

ECHINOCOCCUS GRANULOSUS AND RELATED SPECIES (CYSTIC HYDATID DISEASE)

Humans may be infected with the larval stages of parasites of the genus *Echinococcus*.[32,33,51,52,100,177] Humans acquire the infection by the ingestion of ova from the feces of carnivorous definitive hosts. The disease in humans and intermediate hosts is called hydatid disease and is characterized further according to the morphologic features of the larval stages: cystic echinococcosis caused by *Echinococcus granulosus* and related organisms, alveolar echinococcosis caused by *Echinococcus multilocularis*, and polycystic echinococcosis caused by *Echinococcus vogeli* or *Echinococcus oligarthrus*. Most cases in children present with cystic hydatid disease, which is the focus of this section of the chapter.[32,33,51,52,100,177]

THE ORGANISMS

Cystic hydatid disease is caused by *E. granulosus* and related species. On the basis of morphologic features, all cases initially were considered to belong to a single species. However, genetic studies have demonstrated 10 separate genotypes that infect different intermediate hosts.[51,100,177] Significantly different strains are found in sheep, Tasmanian sheep, cattle, pigs, horses, camels, and cervids (deer, reindeer, moose, elk). Most human infections are caused by the sheep strain. However, Tasmanian sheep, cattle, camel, and cervid strains also can cause human infection. The life cycles of the species of *Echinococcus* are similar, but the geographic distributions, types of hosts, and morphologic features of the parasites differ significantly. Adult tapeworms of the sheep strain are found in dogs. The cervid strain involves primarily wolves, but dogs also may be infected. The adult tapeworm is only 3 to 8 mm long and has two to five segments. Dogs often are hosts to thousands of adult worms. When sheep or humans ingest eggs from the feces of infected dogs, the embryos hatch in the intestine and burrow through the intestinal wall to gain access to the portal circulation. The embryos that survive are able to develop in many tissues, but they do so most commonly in the liver or lungs, where they become the cystic larval structures called *hydatid cysts*. A hydatid cyst is composed of an outer laminated, acellular membrane that is lined by a thin, cellular germinal membrane. Spherical structures called *brood capsules* grow from the germinal membrane. Protoscolices develop within the brood capsules. Each protoscolex has suckers and hooks and the potential to become the scolex of an adult worm if it is eaten by a dog. Compression and reaction from growth of the cyst produce a "pericyst" of compact, collagen-rich host tissue around its exterior. In older cysts, so-called daughter cysts may develop within the primary cyst cavity.

TRANSMISSION

Humans acquire cystic hydatid disease by ingestion of ova shed by the definite host, typically dogs.[33,51,135,140,171,177] Close contact with dogs can result in infection because tapeworm eggs can be found on the dog's perianal hair, muzzle, and paws. Contaminated food, drink, or fomites (e.g., flies and other insects) may disseminate the eggs from dogs' feces. However, most cases occur in pastoral families and are associated with close contact with infected dogs.

EPIDEMIOLOGY

The sheep strain of *E. granulosus* is found worldwide in most areas where sheep are raised.[33,51,100,135,140,177] Studies of the global burden of disease and economic impact of cystic hydatid disease suggest that the impact is significantly underestimated compared with other illnesses.[19] The disease is endemic in the sheep-raising areas of South America, the Middle East, the Mediterranean basin, China, and the former Soviet Union.[33,51,100,135,140,171,177] One of the highest morbidity rates is seen in rural Africans in Kenya and Uganda, where people live in close association with their dogs. The custom of feeding sheep viscera to sheepdogs maintains foci of infection. In the United States, Basques in central California, Mormon ranchers in Utah, and Native Americans in Arizona and New Mexico have been infected. The cervid strain of *E. granulosus* is found in northern parts of the Western Hemisphere. In Alaska and Canada, human infection usually is limited to Eskimos and Native Americans who have working dogs that are fed moose and reindeer viscera.[206] The strain found in this region often is manifested as giant pulmonary cysts.

PATHOLOGY AND PATHOGENESIS

In cystic hydatid disease, the pathologic process is related to compression or displacement of the host's tissue.[51,149,177] Cyst growth is variable but probably averages approximately 1 cm in diameter per year. Rates of up to 4 to 5 cm a year have been reported, but growth may be more rapid in the lung. The cyst may exceed 35 cm in diameter in the abdominal cavity. When rupture or leakage of a cyst occurs, an allergic reaction caused by the antigenic cyst contents may develop. Cysts may calcify after many years, which usually signifies death of the parasite. Hydatid cysts may form foci for secondary bacterial infection.

CLINICAL MANIFESTATIONS

Most children with *E. granulosus* infection have a single unilocular cyst, but multiple cysts are seen in 15 to 30 percent of patients, usually in a single organ system.[51,149,177] The most common site of the cysts is in the liver. In clinical series, approximately one in five children with a pulmonary cyst also has a concurrent liver cyst, but the proportion outside the liver is lower in population-based screening.[116] Approximately 10 percent of cysts are found in sites other than the liver or lung, including spleen, kidney, peritoneum, genitourinary tract, bone, muscles, heart, eye, and brain. The cervid strain of *E. granulosus* found in Canada and Alaska characteristically produces pulmonary cysts.[51,149,177] CNS hydatid disease occurs much more commonly in children than in adults.[29,108] Bone cysts, which may be seen in preschool children, occur most frequently in vertebral and long bones.[149,177] Unlike cysts in other sites, bone cysts characteristically are multilocular and contain little fluid. Eye, bone, and brain cysts typically are small when they are discovered, whereas cysts in other sites may exceed 35 cm in diameter before being detected. Cysts grow approximately 1 to 3 cm in diameter each year, but growth may be more rapid with pulmonary cysts.[116] Complications usually occur only if cysts become large and include secondary bacterial infection of hydatid cysts and leakage or rupture with resultant hypotension, urticaria, and eosinophilia. Rupture may occur spontaneously or secondary to trauma or surgery, but it generally is considered to be an uncommon complication of hydatid disease in children.

The wide spectrum of symptoms in cystic hydatid disease depends on the number, size, and location of the cysts.[51,149,177] Slowly growing cysts in liver or lung often are asymptomatic for years. Symptomatic intrahepatic cysts cause constant or intermittent right upper quadrant or epigastric pain, enlargement of the liver, or nausea and vomiting. Unruptured cysts rarely cause fever. Rupture of a cyst may be precipitated by a traumatic event and may cause a range of symptoms, including fever, abdominal pain, hypotension, and allergic manifestations, including eosinophilia, urticaria, and anaphylaxis. Rupture into the biliary tract may cause cholangitis with fever and right upper quadrant pain. Some children with hydatid disease also have retarded growth patterns.[134]

Intact pulmonary cysts typically do not cause symptoms.[51,149,177,184,211] Rupture or leakage, however, may cause cough, chest pain, dyspnea, and hemoptysis. Fever may be present, but it is often a marker for secondary bacterial infection. As many as a third of pulmonary cysts rupture into the pleural space or into a bronchus. In the latter case, the patient may describe coughing up portions of the membranes, which may resemble grape skins, or noting a characteristic salty taste. Bone cysts are seen in patients with bone pain or pathologic fracture. Vertebral hydatid disease causes signs and symptoms of spinal cord and radicular compression; severe pain on palpation of the affected portion of the spine is characteristic. Fifty to 75 percent of intracranial cysts are seen in children.[29,82,108,215] Increased intracranial pressure with headache, vomiting, and focal neurologic complaints are common findings. Seizures also may occur.

DIAGNOSIS

The diagnosis of cystic echinococcosis usually is suspected on the basis of clinical or radiologic findings plus a history of residence in an endemic area. Physical examination rarely is definitive. Only half of patients with hepatic cysts have abnormal liver enzyme results. Eosinophilia typically is low grade or absent. The initial diagnosis of cystic hydatid disease often is based on imaging findings. An unruptured pulmonary hydatid cyst has a sharply demarcated, round or oval smooth border. It has a homogeneous cannonball appearance and sometimes is surrounded by a layer of atelectatic lung.[51,149,177] After the cyst has ruptured into a bronchus, a crescent-shaped air layer may be seen that is virtually diagnostic. In addition to the arc of air between the parasite and the host cyst wall, air in the cyst lumen also may be present. The membrane of a collapsed cyst floating on the surface of the fluid in a ruptured pulmonary cyst has a characteristic "water lily" appearance.

Cysts in the liver usually are visualized easily and measured with ultrasonography, CT, or MRI. Ultrasound techniques are useful for defining most cysts within the abdomen and can differentiate fluid-filled cysts from solid tumors. Because ultrasonography is portable and relatively inexpensive, it has been the main technique used in epidemiologic studies and clinical cases in developing countries. A World Health Organization staging system has been developed that has been used to define management (Fig. 238–4).[6,167,224] Stage CL cysts are unilocular collections without a discrete wall and with uniform anechoic contents. This appearance is not specific for *Echinococcus*. Stage CE1 cysts have a dense wall and may have internal echoes. This so-called hydatid sand is caused by protoscolices within the cyst. CE2 cysts have internal septa or daughter cysts, forming honeycomb patterns, that are highly characteristic of hydatid cysts. CE3 cysts demonstrate detachment of the cyst wall or early collapse. CE4 cysts demonstrate inhomogeneous material instead of a cyst cavity. CE5 cysts demonstrate a thick rim of calcification. Radiographically apparent cyst wall calcification occurs only in liver or spleen cysts and generally takes more than 5 to 10 years to develop. Bone cysts typically produce radiolucencies without periosteal reaction. MRI offers little advantage over CT for the imaging of cysts except in the CNS.

Fine-needle aspiration of cysts for establishment of the diagnosis can be dangerous if it is not performed carefully because of the risk of spillage of hydatid fluid, which can induce anaphylactic shock and possibly secondary cyst development. However, the percutaneous route has been used for both diagnosis and treatment, with few untoward events (see later). The fluid obtained should be examined for evidence of protoscolices, hooks, or antigen.

Serologic tests can be useful in confirmation of the diagnosis.[51,149,177] Antibody tests using crude antigen of either *E. granulosus* or *E. multilocularis* tested by ELISA, indirect hemagglutination, and fluorescent antibody assays are positive in 50 to 90 percent of cases. False-negative results are more common in patients with pulmonary, brain, and splenic cysts. Because the tests available may cross-react with cysticercosis or other parasitic infections, positive results should be confirmed by a more specific test, either an immunoblot assay or a gel diffusion assay looking for the specific arc 5.

TREATMENT AND PROGNOSIS

The treatment of cystic hydatid disease is in evolution. Surgery was the traditional treatment of choice for nearly all cases of

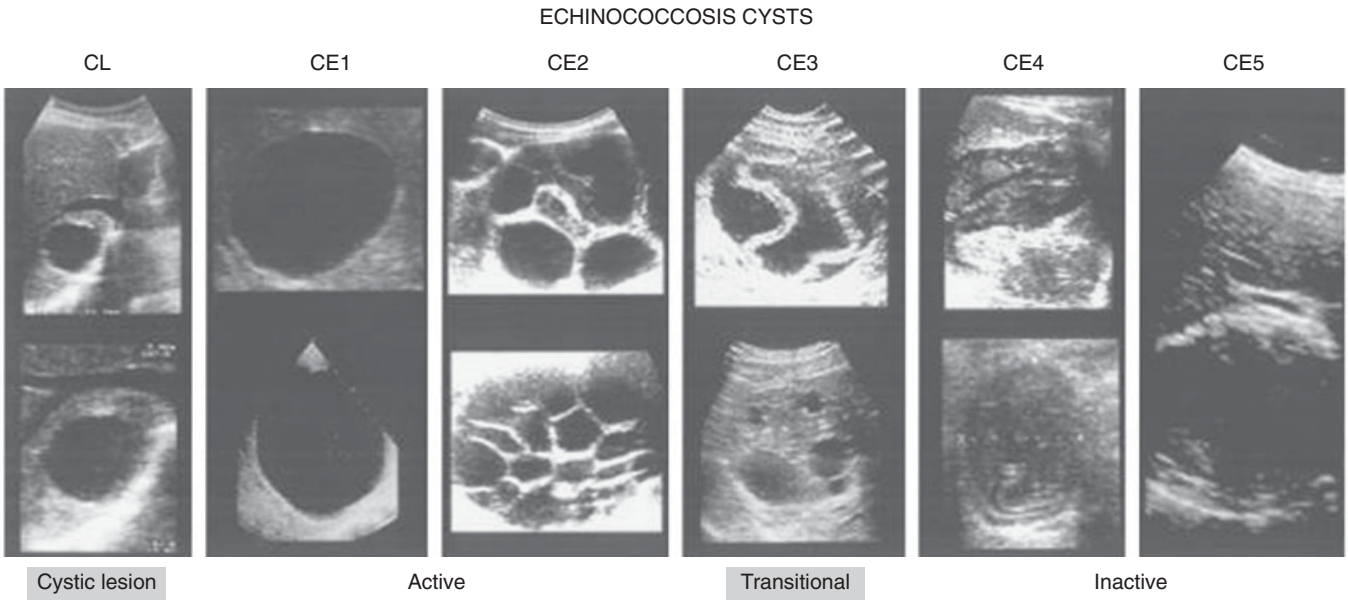

Figure 238–4 Staging of echinococcosis. *(From World Health Organization Informal Working Group on Echinococcosis: International classification of ultrasound images of cystic echinococcosis. Reproduced with the permission of the World Health Organization.)*

cystic hydatid disease, but that approach has given way gradually to a range of approaches including surgery, antiparasitic chemotherapy, percutaneous drainage termed PAIR (*p*ercutaneous *a*spiration, *i*ntroduction of scolicide, and *r*easpiration), and "watch and wait."[51,177] Currently, expert opinion suggests that treatment should vary on the basis of the location of the cyst or cysts, the nature and size of the cysts, the condition of the patient, and the available expertise with surgery required by only a minority of cases. For example, in a prospective study, only 10 percent of children with hepatic cysts detected in a population-based ultrasound survey required surgery during the subsequent 5 years.[115]

The benzimidazoles albendazole and mebendazole have been used for chemotherapy.[51,55,60,92,107,115,177,210] Approximately a third of hepatic cysts will resolve in response to benzimidazoles, and 30 to 50 percent improve. Many of the residual lesions considered treatment failures were shown subsequently to be nonviable. The response to chemotherapy is better with smaller lesions. It is now thought to be the treatment of choice for small (<5 cm) CL, CE1, and perhaps CE3 lesions.[51,177] Chemotherapy also has proved to be successful for treatment of small pulmonary cysts.[48] However, a high failure rate exists with chemotherapy alone for larger lesions in either the liver or the lung. Albendazole is thought to be more effective than is mebendazole. Albendazole typically is dosed at 10 to 15 mg/kg/day in two daily doses continued for 3 to 6 months. Side effects may include neutropenia, elevated liver enzymes, and alopecia. Prospective studies of surgically removed specimens show that protoscolices can remain viable for as long as 3 months, which is the minimum duration of chemotherapy.[15,55] Treatment failures have been successfully managed by further chemotherapy, PAIR, or surgery. In some cases, PAIR may demonstrate that protoscolices no longer are viable, even with persistent lesions. Because chemotherapy can be associated with relapses or treatment failure, children should receive follow-up imaging studies for years after receiving chemotherapy. Chemotherapy also is an important adjunct to surgical therapy.

The PAIR technique offers a less invasive option than the traditional surgical approaches do.[17,56,110,145,177,195,230,231] Results have been similar to those with surgery, but with fewer perioperative complications. PAIR is thought to be the treatment of choice for uncomplicated CL and CE1 lesions larger than 5 cm and CE3 lesions. CE2 lesions can be treated with a modification of PAIR that employs a larger bore catheter and drainage.[17,180] Although the data comparing different treatments are limited, PAIR was associated with similar efficacy and less morbidity than was surgery in one randomized trial. PAIR should be performed after initiation of albendazole chemotherapy for at least 4 hours but preferably for several days. In a controlled trial, PAIR plus chemotherapy was superior to either chemotherapy or PAIR alone.[109,145] Aspiration generally should go through solid tissue (e.g., liver) rather than directly into the cyst (to limit spillage). The cyst fluid should be examined for protoscolices or hooks (to confirm the diagnosis). Before scolicidal agents are injected, the cyst fluid should be checked for bile staining (by dipstick or, preferably, by injection of contrast medium) because these agents can cause sclerosing cholangitis if they are injected into the biliary tract. Alcohol and hypertonic saline have been used as scolicidal agents in this procedure. Contraindications to PAIR include communication with the biliary tract, inaccessibility, superficial cysts, and cysts that are free in the abdomen, heart, brain, or spine. If the cyst is found after puncture to communicate with the biliary tract, contrast media can be used to further define the communication. Contrast material also is mildly scolicidal.

Surgery has been the traditional approach to therapy for hydatid disease and remains the treatment of choice for complicated infections and some CE3 lesions.[51,149,177] The goal of surgery is to remove the entire cyst without spilling its contents. Rupture of the cyst contents at the time of surgery carries a risk of causing disseminated echinococcosis and a small risk of anaphylaxis occurring at the time of operation. Surgical approaches have included pericystectomy (removal of the entire cyst and a rim of surrounding tissues), resection of the involved organ, simple drainage, omentoplasty, and capitonnage. In general, the more radical procedures have been associated with fewer relapses, but at the cost of more perioperative complications. However, laparoscopic approaches have been reported recently to be associated with a high success rate and less morbidity.[20,50,126,231] Chemotherapy with albendazole with or without praziquantel is performed routinely.[9,55,177] With administration of chemotherapy, pressure within the cyst may be reduced, and removal of cyst membranes and contents will be facilitated. In addition, treat-

ment decreases the likelihood of development of secondary cysts. With albendazole alone, cysts remain viable for as long as 3 months.[15] The preoperative administration of praziquantel combined with a benzimidazole has been suggested because of the scolicidal properties of praziquantel.[9] The practice of injecting the cyst with scolicidal drugs before removing it is associated with complications and is not thought to be necessary in the setting of preoperative and postoperative chemotherapy.[177]

Pulmonary cysts have been treated successfully surgically, medically, and with PAIR.[2,48,105,132,194] No controlled trials of these different strategies have been performed. However, retrospective series suggest that smaller cysts often respond to antiparasitic chemotherapy.[48] Larger cysts may be treated with PAIR or surgery. The cervid strains of *E. granulosus* from Alaska and Canada do not produce anaphylaxis on rupture, and pulmonary cysts spontaneously resolve after evacuating into bronchi. If the cyst wall remains in place after the patient has undergone chemotherapy or PAIR, residual parasite material can serve as a nidus for bacterial superinfections.[2]

The prognosis of hydatid disease varies widely from self-limited asymptomatic infection to fatal infection associated with rupture of the cyst. All of the modes of therapy are associated with relapse during the first few years of treatment. Thus, careful follow-up is essential. With appropriate therapy, however, few cases are fatal.

PREVENTION

Control programs for cystic hydatid disease have met with variable success.[32,33,177] *E. granulosus* was eliminated from Iceland by banning home slaughter of sheep. Banning of home slaughter, education, arecoline purging of dogs, and treatment of infected dogs with praziquantel have led to eradication in New Zealand and Tasmania. However, these eradication programs required significant resources and have been less successful elsewhere. A recombinant vaccine has proved to be highly effective in field studies but awaits implementation.[33,122] Other tools in developmental stages include coproantigen tests to detect infected dogs and improved therapy for ovine infection.

OTHER *ECHINOCOCCUS* SPECIES

Echinococcus multilocularis is the cause of alveolar hydatid disease.[51,100,106,149,177] Humans are an incidental host in a life cycle that typically involves foxes as definitive hosts (but also wolves and occasionally domestic dogs) and rodents as intermediate hosts. The larval parasite does not form a large cystic structure but grows by progressive external budding. The laminated membrane and host pericyst are thin. The larval mass slowly enlarges and replaces liver tissue much as a malignant neoplasm does. *E. multilocularis* is endemic in arctic and alpine areas, including western China, central Europe, and scattered foci in North America. In recent years, disease is emerging in much of central Europe. The larvae may invade contiguous structures and rarely metastasize. Human infection almost invariably involves the liver, but it may spread to adjacent tissues or metastasize to the brain. Because the larval mass grows slowly, with a typical latent period of 5 to 15 years before clinical presentation, clinical disease is a rare finding in children. Diagnosis involves imaging studies with serologic confirmation. Management requires surgical resection along with prolonged chemotherapy. Comprehensive reviews of epidemiology, clinical manifestations, diagnosis, and management are available.[16,51,100,106,149,177]

Echinococcus vogeli is a parasite of bush dogs and feral dogs in South America.[177] The intermediate hosts are rodents (pacas, agoutis, spiny rats). When humans are infected, the germinal membrane grows externally to form additional cysts, and septa develop within the original cyst. This manifestation is the "polycystic" variety of hydatid disease. A rare polycystic hydatid disease in humans has been attributed to *E. oligarthrus*, a fourth species that is found as adult tapeworms in wild felids such as pumas and jaguars. The intermediate hosts are the same as those for *E. vogeli*. Most infections occur in Central and South America. Polycystic hydatid disease is limited to South and Central America, where fewer than 100 human infections have been reported. Initial management should include administration of albendazole.

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SUBSECTION 4

Trematodes

CHAPTER 239

TREMATODES

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Humans are infected with a number of trematode parasites, which are also referred to as flukes. In fact, an estimated 40 to 50 million people are infected with food-borne trematode infections, and approximately 10 percent of the world's population is at risk of acquiring infection.

Flukes are flatworms (members of the phylum Platyhelminthes and of the subphylum Trematoda). Although schistosomes are trematodes, they differ from other flukes in morphology, biology (e.g., separate sexes), and clinical manifestations, so they are discussed separately. Trematodes other than schistosomes share common characteristics. The adult worm usually is flat and leaf shaped, with an oral and ventral sucker and a bifurcated, blind-ended gastrointestinal tract. Unlike schistosomes, other trematodes are hermaphroditic, producing operculated eggs that typically are passed in host feces. Eggs possess an operculum at one end, the opening through which a ciliated larva will hatch. Fresh-water snails are intermediate hosts for all trematodes and are infected by either direct penetration by the ciliated larva or by ingestion of the unhatched egg. Complex larval development and multiplication occur within the snail host, with large numbers of larvae called cercariae ultimately produced. In most trematode life cycles, the cercariae emerge from the snails and swim about until they attach to the appropriate second intermediate host, which may be fish, crustaceans, mollusks, or aquatic vegetation, depending on the species of fluke. After attachment occurs, the larvae develop into metacercariae within protective cyst walls. Humans are infected by ingestion of the metacercariae. In Southeast Asia and the western Pacific region, food-borne trematodiasis has become an emerging public health problem, probably because of the exponential growth of aquaculture.[22] Many of the medically important flukes have a wide range of definitive hosts. Diagnosis of fluke infections typically is accomplished by examination of fecal specimens for characteristic ova. Immunodiagnostic tests are available to aid in establishing the diagnosis of some flukes and are becoming the preferred method of diagnosis for certain species. Common nonschistosomal intestinal, liver, and lung flukes are reviewed in this chapter.

INTESTINAL FLUKES

FASCIOLOPSIASIS

LIFE CYCLE

Fasciolopsiasis is a food-borne, intestinal zoonotic parasitosis that is caused by *Fasciolopsis buski*, the giant intestinal fluke. The fluke attaches to the proximal end of the small intestine and, in its adult form, measures up to 7.5 cm in length. The life span of the adult worm is approximately 6 months. Eggs, usually produced about 3 months after infection occurs, are oval and measure 130 to 150 µm in length by 80 to 85 µm in breadth. A single parasite may excrete 25,000 eggs daily. The eggs are released in the feces, and after several weeks in fresh water, ciliated larvae hatch from the eggs and penetrate snails, where they undergo further larval development. Cercariae emerge from the snail approximately 1 to 2 months later and encyst on a wide variety of aquatic vegetation and debris as well as on the water surface. The infective larvae cause infection when ingested. Water chestnuts, caltrop, and water bamboo are common vegetative sources of infection. Ingestion of larvae also can occur through drinking of untreated water or handling or processing of water-derived plants (e.g., peeling water chestnuts that have encysted larvae attached to the outer surface of the nut with the teeth).

EPIDEMIOLOGY

Fasciolopsiasis occurs most often in Asia and the Indian subcontinent. Pigs are the reservoir host, and fasciolopsiasis is particularly prevalent in areas where pigs are raised and aquatic plants are commonly consumed. Human infection has been reported most frequently in China, Bangladesh, Southeast Asia, and parts of India. Fasciolopsiasis is most prevalent in school-age children, in whom high worm burdens are not uncommon findings.

PATHOLOGY

The parasites usually are found in the duodenum and jejunum, but heavy infections also have been observed to migrate to the stomach, ileum, and colon. Inflammation, ulceration, and small abscesses sometimes develop in the intestinal mucosa where the parasite is attached. Increased mucus secretion and mild bleeding can result. Massive infections can lead to intestinal obstruction. Light infections typically are asymptomatic. Systemic manifestations, including malabsorption and protein-losing enteropathy, are common occurrences in heavy infections and can lead to generalized edema and ascites. Toxic and allergic metabolites of the parasite also are thought to contribute to the edema.

CLINICAL MANIFESTATIONS

The severity of symptoms usually is correlated with the number of parasites. Epigastric pains resembling "hunger pains" or peptic ulcer disease have been reported as early as 30 days after exposure. Diarrhea and abdominal pain may be intermittent and may occur separately or simultaneously. In heavy infections, nausea and vomiting may develop. Facial edema, anasarca, and ascites are encountered in advanced, severe infections. Eosinophilia with counts greater than 30 percent is not an uncommon occurrence. Leukocytosis and mild anemia may be noted.

In endemic areas, it is not unusual for a child to have multiple intestinal parasites and a borderline nutritional status. Fasciolopsiasis adds an additional burden to the host defense mechanisms and may be responsible, in concert with the other stresses, for significant morbidity.

DIAGNOSIS

The eggs of *Fasciolopsis* are demonstrated easily by routine fecal examination, but they are virtually indistinguishable from those of the liver fluke *Fasciola hepatica* and the echinostomes. Epidemiologic information, such as travel history and exposure to sources of infection, may be helpful in determining the diagnosis. Because the life span of the adult worm is short, anyone who has been out of an endemic area for longer than 9 months is not likely to have persisting infection with *Fasciolopsis*. Therefore, eggs present in stool long after a potential exposure would be attributable to the longer-lived *Fasciola*.

TREATMENT AND PROGNOSIS

Praziquantel, 75 mg/kg divided into three doses given in 1 day, is effective therapy and has few side effects. Although this drug is approved by the U.S. Food and Drug Administration, it is considered investigational for *Fasciolopsis* infection.

Infections treated early and most light infections, even when they are untreated, have an excellent prognosis. Heavy infections in children, especially when they are complicated by intestinal obstruction, edema, or concomitant secondary infections, portend a much poorer prognosis.

PREVENTION

The most practical method to prevent fasciolopsiasis is to avoid eating raw, water-derived food. Cooking aquatic vegetation or immersing the plants or nuts briefly in boiling water usually is sufficient to prevent infection. Also, discouraging the use of human feces as fertilizer and promoting the institution of modern pig farming both can aid in the control of fasciolopsiasis. Successful efforts in health education have reduced the transmission of this parasite in some endemic areas, such as Taiwan[25]; however, in areas where fasciolopsiasis was presumed controlled, such as Uttar Pradesh, re-emerging infection has been reported.[3]

HETEROPHYIASIS

More than 10 different species of the family Heterophyidae have been reported to cause human infection. Except for two species, *Heterophyes heterophyes* and *Metagonimus yokogawai*, these infections are relatively uncommon occurrences and are incidental to the prevalence in other mammals and birds.

THE ORGANISMS

H. heterophyes and *M. yokogawai* adult worms attach to the mucosa of the small intestine. The parasites are minute, measuring 1 to 2.5 mm long and less than 1 mm wide. They often burrow deeply into the mucosa. The eggs measure 26 to 30 µm in length and 15 to 17 µm in width. The eggs of the two species are essentially identical. Eggs excreted in the stool are fully embryonated. The snail intermediate host becomes infected by ingesting the trematode egg. After multiplication in the snail, cercariae emerge and encyst under the scales or in the skin or flesh of a variety of freshwater fish. After being ingested by the definitive host, the metacercariae are freed from their cysts and develop into adults in as little as 5 days.

TRANSMISSION AND EPIDEMIOLOGY

Humans acquire the infection by eating fresh-water fish that is raw, inadequately cooked, pickled, or salted. Heterophyiasis occurs worldwide. Areas of endemic infection are Southeast Asia, the Middle East, the Nile Delta, China, Japan, Taiwan, the Philippines, and parts of the former Soviet Union. Many reservoir hosts, such as dogs, cats, and fish-eating birds, may play an important role in maintenance of infection in some endemic areas.

PATHOLOGY

The small heterophyid flukes cause variable degrees of inflammatory reactions, including superficial inflammation and erosions at the sites of mucosal attachment and granulomatous lesions that contain eosinophils due to eggs deposited beneath the mucosa. Eggs may invade the circulatory system through the intestinal capillaries or lymphatics and embolize to distant sites such as the heart, brain, and spinal cord, causing significant disease that can be fatal. The complications of embolic heterophyiasis appear to be particularly frequent in the Philippines, where heart disease often is attributed to heterophyiasis. The embolized eggs induce a granulomatous response, with the eventual production of fibrosis in the infected areas.

CLINICAL MANIFESTATIONS

Light infections without the deposition of ectopic eggs usually are asymptomatic, although eosinophilia may be noted. In heavier infections, epigastric pain, diarrhea, malabsorption, and weight loss may occur. Seizures may result from eggs carried to the brain. Congestive heart failure or arrhythmias may occur after cardiac involvement.

DIAGNOSIS

Routine fecal examination demonstrates eggs, but because the eggs closely resemble the eggs of the liver flukes, establishing a specific diagnosis of these flukes often is difficult. Eggs recovered from biliary drainage or from persons who have been out of endemic areas for more than 2 years can be assumed to be those of the liver flukes *Opisthorchis* or *Clonorchis*. The heterophyid worms have a relatively short life span compared with that of the liver flukes.

TREATMENT AND PROGNOSIS

Praziquantel, 75 mg/kg/day in three divided doses for 1 day, is the recommended treatment. With the exception of the complication of egg emboli to distant organs, the prognosis is excellent.

LIVER FLUKES

FASCIOLIASIS

Fascioliasis is a parasitic disease caused by the flukes *Fasciola hepatica* and *Fasciola gigantica*. With 91 million people at risk of acquiring infection,[1] human fascioliasis is an important public health problem with increasing incidence of human cases in 51 countries.[12,30] Fascioliasis is primarily a zoonosis of ungulates (e.g., cattle and sheep), and humans are an incidental host.

THE ORGANISM

The adult *Fasciola hepatica* measures up to 30 mm in length and 13 mm in width. The surface has scalelike spines, and the anterior portion of the worm is cone shaped. The worms reside in the bile ducts, and eggs appear in the bile and eventually are excreted in feces. Eggs measure approximately 130 μm in length and up to 90 μm in width. The eggs are indistinguishable from those of *Fasciolopsis buski*. After being incubated in water for several days, the ciliated larva hatches from the egg and swims about in search of the snail intermediate host. The larva penetrates the snail and undergoes a complex cycle of asexual multiplication. Cercariae, which are the final stage of this asexual cycle, leave the snail and encyst on fresh-water vegetation, including watercress and alfalfa. These encysted metacercariae initiate infection when the mammalian host ingests the raw aquatic vegetation or drinks water contaminated with metacercariae. The metacercariae excyst in the intestine, penetrate the intestinal wall, and migrate in the peritoneal cavity to the liver. The developing worms penetrate the liver capsule and slowly burrow through the parenchyma to the bile ducts, where they mature into the adult worms. The adult worms feed on liver cells and duct epithelium. The life span of the adult fluke in humans ranges from 9 to 13.5 years.

TRANSMISSION AND EPIDEMIOLOGY

Ingestion of infected raw aquatic plants, such as watercress, is the most frequent cause of infection in humans. In a study from Peru, alfalfa juice seemed to be the major food source.[29] Water from ponds or marshes with infected vegetation may contain metacercariae. Infections occur in humans who have no history of eating watercress but have ingested water from sources that potentially are infected. The distribution of fascioliasis is worldwide, with the highest numbers of infected humans in Andean countries (Peru, Bolivia, Ecuador, Chile), the Caribbean, western Europe, northern Africa, and the Caspian Sea.[31] The reservoir host range is broad and includes herbivorous mammals, the most common or which are sheep, cattle, and goats. Intermediate hosts are fresh-water snail species. *F. gigantica* infections are predominantly infections in cattle. Human infection with *F. gigantica* has been reported in Africa, the former Soviet Union, Vietnam, Hawaii, and Iraq.

PATHOLOGY AND PATHOGENESIS

Fascioliasis includes an acute phase as the juvenile forms migrate through the liver and a chronic phase associated with the adult worms and eggs in the biliary tract. The pathogenesis of acute fascioliasis is related to damage occurring to liver tissues as the juvenile worms migrate through the liver tissue to the bile ducts. Linear necrotic lesions containing eosinophils form as worms progress through the liver parenchyma. Flukes that die before reaching the bile ducts may produce necrotic cavities that eventually evolve into fibrous scar tissue. Juvenile flukes that fail to migrate into the liver may wander about and cause ectopic fascioliasis. They may appear in the intestine, pancreas, subcutaneous tissue, brain, eye, and other locations. During the chronic stage, adult worms in the bile ducts cause inflammation and adenomatous changes in biliary epithelium, leading to duct thickening and dilatation. Ductal and periductal fibrosis occurs. The gallbladder and extrahepatic ducts may be invaded and undergo similar

inflammatory and fibrotic reactions. Adult worms may migrate back into liver parenchyma through eroded biliary epithelium and cause formation of abscesses.

CLINICAL MANIFESTATIONS

The first symptoms of fascioliasis occur approximately 4 to 6 weeks after infection develops, but this period varies widely, depending on host response and the number of parasites. The acute stage of infection occurs during the migration of worms in the liver. Children often have severe symptoms of right upper quadrant or generalized abdominal pain, tender hepatomegaly, fever, and anemia. Patients typically have marked eosinophilia. Sweating, dizziness, wheezing, and urticaria may occur. This stage may last 1 to 3 months. The chronic form of the disease is defined less well and includes a variety of symptoms related to the biliary system. These symptoms frequently are identical to those of gallbladder disease, cholangitis, and pancreatitis caused by nonparasitic conditions. Elevation in alkaline phosphatase levels is a common manifestation. Patients often endure years of having biliary tract symptoms before the diagnosis of fascioliasis is considered. Heavy infections eventually may cause sufficient liver damage to produce cirrhosis with all of the classic complications of this condition. Chronic infections with *F. hepatica* also may be asymptomatic but often are associated with eosinophilia. If ectopic localization of immature *F. hepatica* flukes occurs, it usually is in the subcutaneous tissues of the thorax, back, and extremities but has also been observed in lungs, heart, brain, and intestinal wall. A detailed review of the clinical aspects of human fascioliasis has been published.[2] The clinical manifestations of fascioliasis in a series of 16 Egyptian children included fever, abdominal pain, and a marked peripheral eosinophilia.[11]

Because the acute manifestations of human fascioliasis may precede the appearance of eggs in the stool by 7 to 12 weeks, immunodiagnostic tests are required to identify early infections. Obtaining a history of eating raw watercress or drinking surface water from an area that may be contaminated by domestic animals is helpful. The syndrome of fever, hepatomegaly, and eosinophilia is consistent with the diagnosis. Radiologic imaging of the liver may demonstrate findings of tracklike small abscesses, subcapsular lesions, and slow evolution of the lesions on follow-up examinations.[15] Computed tomography detects parenchymal lesions, and ultrasonography effectively evaluates the biliary tract and gallbladder. Magnetic resonance imaging findings also have been described.[6] A variety of immunologic tests have been developed. Presently, the tests used most frequently are enzyme immunoassays with excretory-secretory antigens combined with confirmation of positive results by immunoblot. *Fasciola*-specific antibodies may be detected within 2 to 4 weeks after infection or several weeks before eggs appear in stool. The reported sensitivities of the FAST-ELISA (the Falcon assay screening test–enzyme-linked immunosorbent assay) format of enzyme immunoassay and the confirmatory immunoblot are 95 percent and 100 percent, respectively.[17] Serologic tests are also useful in the diagnosis of ectopic fascioliasis.

During chronic infection, routine stool examination with use of a formalin–ethyl acetate concentration should reveal ova of *Fasciola* in established infections. As discussed previously, *Fasciola* eggs are indistinguishable from those of *Fasciolopsis buski*. False-positive stool examinations may occur if the patient recently ingested infected livers containing eggs. To avoid false-positive results in this situation, keep the patient on a liver-free diet several days before the stool specimen is collected. Chronic fascioliasis may be detected during radiographic studies of the biliary tract or gallbladder. Adult worms may be seen on ultrasonography or appear as curvilinear lucent areas in the contrast medium at cholangiography. Endoscopic retrograde cholangiopancreatography may directly visualize the adult worms, which are leaf shaped and bile stained. Eggs also may be identified in bile.

TREATMENT AND PROGNOSIS

Unlike other trematode infections, fascioliasis is relatively resistant to praziquantel. The drug of choice is triclabendazole at a dose of 10 mg/kg with a meal once or twice. The formulation manufactured for human use as Egaten is currently not available in the United States. Triclabendazole (Fasinex) has been used for many years by veterinarians for treatment of livestock in endemic areas. Anecdotally, it has been used safely in humans. However, resistance is emerging in isolates from domestic animals. Triclabendazole has been used successfully in a variety of open clinical trials in adults and children for both acute and chronic fascioliasis.[10,21] It is effective against both migrating worms and established infections. Treatment with bithionol also is effective. Bithionol no longer is manufactured, but a small supply is available in the United States for investigational use only from the Centers for Disease Control and Prevention (CDC). Bithionol usually is administered in a dose of 30 mg/kg given either daily for 5 days or on alternate days for a total of five doses. More intense courses of 30 to 50 mg/kg of bithionol on alternate days for 10 to 15 doses have been recommended as well. After successful treatment, the eosinophilia resolves slowly and immunologic test results become negative. Nitazoxanide was effective in two trials in chronic fascioliasis.[14,35]

In one study, children with severe acute infections were treated with 5 to 10 mg of prednisone before receiving specific fasciolicidal drugs.[13]

Heavy infections in children may be fatal during the acute stage of the disease. However, most heavy infections become chronic or, possibly, asymptomatic. The variable reaction of the human host and the number of parasites determine the outcome. In heavy infections, hepatic damage may be significant, with fibrotic scarring or abscess formation. Chronic or recurrent biliary tract problems are common.

PREVENTION

Watercress grown for human consumption should be protected from human and animal fecal contamination. Animal fascioliasis can be targeted for chemotherapeutic control, and control of the snail intermediate hosts with molluscicides can be attempted. Effort should be made to educate the population at risk about the danger of eating raw watercress and other metacercariae-carrying aquatic plants harvested from unprotected waters and the hazard of drinking untreated or filtered surface water.

CLONORCHIASIS AND OPISTHORCHIASIS

Three similar trematodes of the genus *Opisthorchis* infect the bile ducts of humans. Although the name *Opisthorchis sinensis* is proper parasitologically, this organism more commonly is called *Clonorchis sinensis*, and the name of the infection, clonorchiasis, is well entrenched in the clinical literature. This organism also is called the Chinese or Oriental liver fluke. *Opisthorchis viverrini* and *Opisthorchis felineus* have similar life cycles and produce similar lesions and illnesses in humans.

THE ORGANISMS

The adult flukes measure 4 to 25 mm in length by 2 to 5 mm in breadth and have an anterior oral sucker. The life cycle involves

mammals (primarily humans, dogs, and cats) as definitive hosts, snails, and fresh-water fish or shrimp. In the definitive hosts, they are found mainly in the intrahepatic biliary ducts and occasionally in the extrahepatic and pancreatic ducts. The small operculate eggs, similar to those of the heterophyid flukes, appear in bile and are excreted in feces. The snail intermediate host ingests the embryonated egg, and free-swimming cercariae emerge from the snail approximately 6 to 8 weeks later. The cercariae encyst under the scales or in the musculature of a variety of fresh-water fish. When the raw, inadequately cooked or pickled fish is eaten, the larvae excyst and migrate to the intrahepatic bile ducts, usually through the ampulla of Vater and the common duct. Juvenile flukes attach themselves to the bile duct epithelium with their suckers and travel along the biliary tree against bile flow. Adult worms begin producing eggs approximately 4 weeks after ingestion of metacercariae. The worms probably survive for as long as 30 years.

TRANSMISSION AND EPIDEMIOLOGY

Infection is initiated by the ingestion of raw, inadequately cooked, cured, or pickled fresh-water fish that have encysted larvae in their tissues. *C. sinensis* is endemic in China, Japan, Korea, Taiwan, and Vietnam. The high prevalence in Hong Kong is attributed to the importation of fish from mainland China. Natural reservoir hosts are cats, dogs, pigs, and rats. *O. viverrini* is localized to the northern areas of Thailand, Laos, and Cambodia. In northern Thailand, where a dish prepared from chopped raw fish, called *koi pla*, is popular, researchers have estimated that more than 7 million residents are infected. Cats, civet cats, dogs, and other fish-eating mammals serve as reservoirs. In China and other Asian countries, edible fish often are raised in ponds that are fertilized with human feces, thus providing perfect conditions for the entire life cycle of the parasite.

O. felineus is endemic in central Siberia and in eastern and southeastern Europe. Cats, dogs, and foxes serve as major reservoirs. Human infection is limited to groups that habitually consume raw, dried, or freshly salted fish or fish lightly pickled in garlic juice. Sporadic infections with *O. felineus* have been reported from several Asian countries.

The distribution of infection by all species in human infections depends on the eating habits of the population. In most areas, patterns of infection are similar. Typically, the lowest prevalence and intensity of infection is found in the youngest age groups; intensity of egg excretion often increases with age and reaches a plateau by the late teens. Prevalence is usually highest in older persons.[41]

PATHOLOGY AND PATHOGENESIS

The epithelium of infected bile ducts reacts with desquamation, adenomatous hyperplasia, and metaplasia of goblet cells, accompanied by an increase in mucus production. The pathogenesis of the hepatobiliary damage may be due to mechanical irritation from the fluke suckers or metabolites from the worms. Other proposed mechanisms include immunopathologic processes, especially those involving host immune responses.[16] Ductal dilatation and bile stasis probably increase susceptibility to bacterial cholangitis. Inflammation of the bile duct wall usually indicates secondary infection. Numerous factors determine the severity of these changes and include the duration and intensity of infection, host susceptibility, and number of reinfections. Chronic infection may lead to extensive periportal and periductal fibrosis.[38] The presence of the parasites in addition to other factors appears to render the host susceptible to cholangiocarcinoma. Experiments in hamsters have shown that repeated infection with *O. viverrini* induces overproduction of nitric oxide, which may play a significant role in the development of cholangiocarcinoma through oxidative and nitrosative DNA damage to the bile ducts.[32] An extensive analysis of the available data concerning cancer and liver flukes has concluded that infection with *O. viverrini* is carcinogenic to humans and that infection with *C. sinensis* probably is carcinogenic.[39] Data were not sufficient to determine the status of *O. felineus* infections. *O. viverrini* infections frequently involve the gallbladder and may lead to complications such as gallbladder sludge, cholecystitis, and formation of gallstones.[28] The parasites often are found in the pancreatic ducts, where reactions of the epithelium are similar to those in the bile duct. Pancreatitis occurs infrequently in these liver fluke infections and is usually mild.

CLINICAL MANIFESTATIONS

The occurrence of symptoms probably correlates with the intensity of infection but is variable. In endemic areas, infections may begin early in life and often are asymptomatic. Patients with heavy infections may complain of a dull pain or discomfort in the right upper quadrant, weakness, or malaise, and they may have significant hepatic enlargement. An uncontrolled study of patients with *O. viverrini* infections in Thailand showed general improvement in well-being after treatment.[33] Symptoms of abdominal distress and epigastric pain declined significantly. A community study showed significant improvement in the infected population after treatment based on symptoms, laboratory test results, and ultrasound abnormalities.[34]

Acute symptoms starting 2 to 3 weeks after exposure have been reported with *C. sinensis* and *O. felineus* infections and include fever, malaise, anorexia, diarrhea, tender hepatomegaly, and eosinophilia. The eosinophilia of acute infection gradually decreases and in chronic infection disappears.

Complications are more likely to occur in heavy infections. Relapsing cholangitis, cholecystitis, bilirubin gallstones, pancreatitis, and cholangiocarcinoma all have been associated with infection. Clonorchiasis often is cited as a cause of pancreatitis in individuals from endemic areas.[23,36] Cholangiocarcinoma, which may result from chronic or repeated infections, accounts for only approximately 7 to 8 percent of liver cancer in the United States but is presumed to be responsible for more than 60 percent of liver tumors in northeastern Thailand.[39]

DIAGNOSIS

Symptoms of acute infection may develop 3 to 4 weeks before eggs appear in the stool. The diagnosis may be suggested by the epidemiologic information. Serologic tests are sometimes used to establish the diagnosis in some endemic areas[1,18,40] but are not always readily available or practical to perform. Ultrasonography has been shown to be a useful diagnostic tool. Sonographic findings consist of the pathologic changes of the bile duct or gallbladder that result from infection with *Clonorchis* or *Opisthorchis* species and include intrahepatic bile duct dilatation, increased periductal echogenicity, and gallbladder sludge.[8] In chronic infections, eggs should be evident in routine fecal examinations. Filtration of fluid obtained at duodenal intubation is claimed to be diagnostically more sensitive than is examination of two fecal specimens. A monoclonal antibody–based ELISA has been developed for the demonstration of *O. viverrini* antigen in fecal specimens.[19] This test appears to be specific and highly sensitive.

Cholangiography often shows multiple cystic dilatations of the ducts, elliptic or filamentous filling defects within the peripheral intrahepatic ducts, and intrahepatic duct haziness.[7] A combination of large cystic dilatations and small cystic ectasias or

mulberry-like dilatations is considered diagnostic. The flukes may be evident as linear radiolucencies on cholangiography. The adult worms or eggs also may be visible on endoscopic retrograde cholangiopancreatography.

The eggs of *O. viverrini* and *C. sinensis* are virtually identical. *O. felineus* is reported to have a narrower egg, as determined by the ratio of length to width. Differentiation between the eggs of these species of liver flukes and the eggs of small heterophyid flukes is difficult. Fortunately, treatment with praziquantel is identical for all these parasites.

TREATMENT AND PROGNOSIS

These liver fluke infections respond well to treatment with praziquantel, 75 mg/kg divided into three doses given in a single day. Side effects of headache and dizziness are common occurrences. Although untreated light and moderate infections appear asymptomatic and may have little if any clinical significance, the availability of a safe, easily administered drug renders treatment an appropriate approach, especially for those who have left an endemic area. Treatment also may decrease the risk for development of cholangiocarcinoma in those infected. Albendazole also has been found to be effective in treating clonorchiasis. A dose of 10 mg/kg is given daily for 7 days. Effective treatment has reversed the biliary tract abnormalities in *O. viverrini* infections and would be expected to have a similar salutary effect in clonorchiasis and *O. felineus* infections.

The prognosis of heavy infections depends on the complications. Superimposed bacterial infections are the most common cause of morbidity and mortality.

PREVENTION

Education of the population at risk concerning the consumption of raw fish in its various forms (dried, pickled, salted, or smoked) is about the only hope for reducing prevalence in humans. A large reservoir exists in domestic and wild animals; therefore, attempts to eliminate infection in humans by mass treatment are unlikely to succeed. Prohibiting the fertilization of fishponds with raw human sewage surely would reduce the incidence of transmission. In Thailand, control programs for opisthorchiasis have included examinations of stools with treatment of positive cases, health education to discourage the consumption of raw fish, and promotion of hygienic defecation.[20]

METORCHIS INFECTION

Metorchis conjunctus, the North American liver fluke, is a member of the Opisthorchiidae family and has a life cycle similar to that of the other opisthorchids described earlier. A wide variety of fish-eating mammals may serve as definitive hosts. This infection is a significant cause of morbidity and mortality in sled dogs in Canada. It is an occasional incidental finding on fecal examination in native communities in northern Canada. An outbreak of 19 cases of *Metorchis* infection was related to fish prepared raw as sashimi.[26] The symptoms began 1 to 15 days after the infected fish were ingested. The severity and duration of the symptoms correlated with the amount of fish eaten. Symptoms were fatigue, upper abdominal tenderness, fever, epigastric abdominal pain, headache, weight loss, anorexia, nausea, diarrhea, vomiting, muscle pain, backache, cough, and rash (in descending order of frequency). The degree of eosinophilia and elevation in liver enzyme levels were proportional to the amount of fish ingested. Eggs were noted in stool 10 days after infection developed. All symptoms resolved after treatment with the standard course of praziquantel. Serologic testing indicates that a similar liver fluke, *Metorchis bilis*, is a frequent parasite of residents of the Novosibirsk areas of Russia.

LUNG FLUKE

PARAGONIMIASIS

Many species of *Paragonimus* have been identified, at least 10 of which have been recovered from humans and in some instances have been found in distinct endemic foci. Of these, *Paragonimus westermani* is the species of lung fluke that causes most human infections.[4]

THE ORGANISM

The adult forms of *Paragonimus* measure about 15 mm long by about 6 mm wide. They are nearly as thick as they are wide. The worms typically are located in the lungs. Eggs measuring 80 to 118 μm by 48 to 60 μm are discharged into the bronchi and are either expectorated or swallowed and excreted in feces. The larva hatches from the egg after at least 2 weeks of development in water. The free-swimming larva penetrates the snail intermediate host and undergoes development and multiplication for several weeks. Cercariae emerge and encyst in the tissues of fresh-water crabs and crayfish. When humans or reservoir hosts ingest the flesh of these second intermediate hosts, the larvae penetrate the wall of the intestines and migrate through or around the diaphragm to reach the lungs. In some instances, the worms may lodge in ectopic sites within the abdomen, in subcutaneous tissue, or in the central nervous system. The worms usually are found singly or in pairs within a capsule or cyst of reactive host tissue. Approximately 2 to 3 months are required from the time of ingestion until the worms are fully mature. In most infections, the worms die within 10 years; however, production of eggs for 20 years after the individual has left the endemic area has been reported.

TRANSMISSION AND EPIDEMIOLOGY

The infection is acquired by eating fresh-water crabs, crayfish, or shrimp that are raw, inadequately cooked, salted, pickled, or soaked in wine. Cooked foods may be contaminated with viable larvae from the hands, utensils, or cutting boards used in the preparation of crabs or crayfish.

Paragonimiasis is a zoonotic disease that is estimated to affect 20 million people worldwide.[27] Significant reservoir hosts for *P. westermani* include cats, civet cats, wild felids, foxes, wolves, dogs, pigs, and mongooses; main endemic foci are in China, Taiwan, Korea, Japan, eastern India, Sri Lanka, Southeast Asia, Indonesia, and some areas of the former Soviet Union. Instances of paragonimiasis being acquired in North America are extremely rare.

More than nine different species of *Paragonimus* infect humans. In many areas, the distribution of these species often overlaps that of *P. westermani*. Endemic areas with other species can be found in Central Africa (Cameroon and Nigeria), East Asia (China, Japan), and the Pacific coast of Latin America (Mexico, Peru, Ecuador, and Central America). The potential exists for transmission of paragonimiasis in the United States.[9]

PATHOLOGY AND PATHOGENESIS

After penetration of the pleura occurs, the worms reside near larger bronchioles or bronchi, where an exudate of neutrophils

and eosinophils forms around them. In time, this inflammatory response around the parasite organizes into a fibrotic wall that may measure up to several millimeters thick. The cysts usually are 1 to 2 cm in diameter and often are filled with a brownish material that likely contains hematin. The cyst may communicate with a bronchiole or bronchus, which provides a route for discharge of the eggs into sputum. Complications of paragonimiasis include bronchiectasis, fibrosis, interstitial pneumonia, and secondary bacterial infections.

The central nervous system is a common location for extrapulmonary involvement. All areas of the brain and meninges are susceptible to invasion. The adult worms and the eggs cause areas of central necrosis and granuloma formation with dense collagenous walls surrounded by lymphocytes, plasma cells, eosinophils, and Charcot-Leyden crystals. The lesions vary in size and may be several centimeters in diameter and may appear cystic. Eventually, the wall may calcify. Spinal cord lesions are similar. Other sites at which worms may cause cysts or abscesses are the intestinal wall, mesentery, peritoneal cavity, liver, diaphragm, myocardium, and subcutaneous tissue.

CLINICAL MANIFESTATIONS

Migration of the worms from the intestinal tract to the lungs usually causes no symptoms, but diarrhea, abdominal pain, and urticaria may occur in the first 3 weeks after exposure. These symptoms may be followed by fever, chest pain, dyspnea, cough, malaise, and night sweats.

The pulmonary clinical manifestations are mainly a chronic cough that is productive of mucus, and rust-colored or blood-streaked sputum is present. Hemoptysis usually is intermittent and occasionally may be severe. Eosinophilia is a common finding in the early stages of infection but may return to normal during a period of months or years. Heavy infections may be associated with complications such as pneumothorax, pleural effusion, empyema, and pneumonia. The presence of hemoptysis and cavitary lung lesions can be confused with tuberculosis, but tuberculosis typically has more systemic symptoms (e.g., fevers, night sweats, and weight loss).

Cerebral paragonimiasis occurs most commonly in endemic areas of Asia and may result in serious morbidity and often death.[24] Cerebral involvement is more common in children, with more than half the infections occurring before the child reaches 10 years of age. This form of paragonimiasis may present initially as a mass lesion, a seizure disorder, meningitis, or a cerebrovascular accident. Seizures often begin as the focal motor type but may generalize. Visual disturbances, headache, and elevated cerebrospinal fluid pressure are common events. Pleocytosis with eosinophilia in the cerebrospinal fluid may be noted. The variety of neurologic manifestations depends on the number, location, and size of the lesions. Spinal cord lesions often are extradural and mimic mass lesions caused by tumors or infection.

Cutaneous paragonimiasis is identified by the appearance of subcutaneous nodules, which may be fixed or migratory. In China, species of *Paragonimus* found in the northern region often cause subcutaneous, migratory lesions associated with fever and eosinophilia. Worms recovered from these lesions are immature.

DIAGNOSIS

Normal results on chest radiographs are found in 10 to 20 percent of patients. Radiographic abnormalities may develop as the worms enter the lungs. Initially, the chest film shows poorly defined basilar pneumonic infiltrates. These areas may evolve into cysts or nodules within a few weeks. Other radiographic findings include cavitations, fibrosis, and pleural thickening, which occasionally can be associated with a pleural effusion. Although the nodules and cysts of the chronic stage of infection may develop in any area of the lung, including the apices, they tend to localize in the periphery of the middle and lower lung fields. A common radiographic diagnostic feature is a "ring shadow." This finding represents the circular or oval thin-walled cyst with a crescent-shaped opacity along one side.

Cerebral paragonimiasis may be seen as an avascular mass on computed tomography. In long-standing cerebral infections, the cystlike structures may calcify and be seen as a cluster of "soap bubbles." The individual oval or spherical bubbles may measure 2 to 40 mm in diameter, and a cluster of these structures may extend 10 cm. Magnetic resonance imaging is not as effective in demonstrating calcification, but it is a useful supplement in defining the lesions of cerebral infection. Magnetic resonance imaging of infected children can show hemorrhage and irregular, conglomerated lesions.[42]

Pulmonary paragonimiasis is diagnosed definitively by finding the characteristic eggs in sputum or stool from a patient with symptoms suggesting the infection. Eggs are not present until 2 to 3 months after infection develops. Concentration techniques may help identify eggs in patients with light infections. Several immunodiagnostic tests, complement fixation, ELISA, DNA probes, and immunoblotting have been developed, which may assist with confirming the presence of infections and with monitoring responses to treatment. Immunodiagnosis is especially useful in infections in which eggs are not demonstrated easily, such as in cerebral paragonimiasis. The immunoblot assay performed with a crude antigen extract of *P. westermani* has a sensitivity of 96 percent and specificity of 99 percent and is the serodiagnostic test of choice.[37] The complement-fixation test and enzyme immunoassay tests also have been used. Antibody levels detected by enzyme immunoassay and immunoblot decline slowly after successful treatment, so antigen-detection systems may be a more useful approach to monitor treatment efficacy.[43] Intradermal testing cannot distinguish between past and current infections, but it has been useful in performing epidemiologic studies.

TREATMENT AND PROGNOSIS

Praziquantel, 75 mg/kg divided into three doses, is given daily for 2 days. Bithionol is no longer manufactured, but a small supply is available in the United States through the CDC in Atlanta. Bithionol is given in single doses of 30 to 50 mg/kg on alternate days for a total of 10 to 15 doses. Side effects occur much more commonly with bithionol than with praziquantel. Data from case reports and clinical trials have revealed the potential usefulness of triclabendazole as an alternative treatment for humans infected with *Paragonimus* species.[21] One study demonstrated 84 to 90 percent efficacy with single-day regimens of triclabendazole for the treatment of pulmonary paragonimiasis, indicating possible value for use of this regimen in community-based treatment programs.[5]

The prognosis is good in most pulmonary infections, even if they go untreated, although symptoms may persist for many years. Treatment effectively resolves the pulmonary lesions and symptoms. The prognosis for central nervous system involvement depends on the location and extent of the lesions but is usually grave.

PREVENTION

The key in prevention is education of the population in endemic areas concerning the source of infection. Crabs and crayfish,

prepared in a manner that transmits paragonimiasis, are delicacies in many parts of the world. Changing attitudes about food habits is not an easy task, and the likelihood of eliminating the occurrence of human infections by mass treatment and improved sanitation is doubtful because of animal reservoirs.

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CHAPTER 240

SCHISTOSOMIASIS

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Approximately 200,000,000 individuals are infected with parasites of the genus *Schistosoma*.[28,66,113,148] Many are unaware of their infection, although approximately 20 million people have severe disease with potentially fatal portal hypertension, urinary tract involvement, renal failure, and bladder cancer. Initial infection usually occurs during childhood. The prevalence typically peaks in the teen years, and although most of the morbidity occurs in adults, symptomatic disease develops in millions of children.

There is historical evidence of schistosomiasis occurring as early as the second millennium B.C.E.[130,132] For example, *Schistosoma* eggs have been found in mummies from the 20th Dynasty in ancient Egypt and in ancient China. In 1851, Theodor

Bilharz identified the parasitic etiology of endemic hematuria. At the same time, the association between *Schistosoma* and Katayama fever was recognized in Japan. The complete life cycle was identified during the first decades of the 20th century. Before the development of effective curative medication in the 1970s, schistosomiasis was considered to be the "most dreadful of the remaining plagues of Egypt."[66,67] Efforts to control the disease continue, and eradication measures are still in the early stages of being implemented.

EPIDEMIOLOGY

Schistosomiasis is caused by helminth parasites of the class Trematoda, which includes the flukes. Schistosomes differ from other human flukes in that they live within the vascular system and have separate male and female sexes. Humans are the definitive host for five species within the *Schistosoma* genus.[130,132] *Schistosoma haematobium* is the cause of urinary schistosomiasis. *Schistosoma japonicum* and *Schistosoma mansoni* are common causes of disease resulting from infection of the intestinal vasculature. *Schistosoma mekongi* and *Schistosoma intercalatum* also cause infection along the intestinal tract but are restricted geographically. Other mammalian *Schistosoma* spp. occasionally infect humans.

Each species of *Schistosoma* uses fresh-water snails as obligate intermediate hosts,[130,132] and the geographic distribution of *Schistosoma* is limited by the habitat of these snails (Fig. 240–1). Updated World Health Organization figures on the prevalence and distribution of schistosomiasis are available at *http://www.who.int/wormcontrol/en/*. *Oncomelania* snails transmitting *S. japonicum* live in the moist soil along slow-flowing streams and irrigation canals, limited primarily to parts of China, the Philippines, and Indonesia. The *Bulinus* snails associated with *S. haematobium* and the *Biomphalaria* snails associated with *S. mansoni* live in shaded, slow-flowing, shallow water. *S. haematobium* is seen primarily in tropical Africa, along the Nile River, and in the Middle East. *S. mansoni* is found across tropical Africa, along the Atlantic coast of South America, and on some Caribbean islands. *Tricula*, the snail host for *S. mekongi*, is limited to the Mekong River in Laos and Cambodia. *S. intercalatum* and its snail host *Bulinus* are found only in Cameroon and the Democratic Republic of Congo. In the United States, schistosomiasis occasionally is diagnosed in immigrants and travelers from endemic regions.

Most human infections occur when children come into contact with fresh-water streams, rivers, or lakes inhabited by infected snails (Fig. 240–2).[132] Thus, for most of the world, transmission of disease occurs during routine activities such as obtaining water for household use, bathing, fishing, and irrigating fields. Humans are infected by the fork-tailed schistosomal cercariae. Cercariae penetrate the skin with the aid of secretory serine proteases.[88,118] During penetration, the parasites lose their tails and modify their tegument to form the schistosomula stage. Schistosomulae enter the bloodstream and then migrate to the lungs. They move from the pulmonary arterial to the venous circulation without exiting the vessels. The parasites then travel through the systemic circulation until they reach splanchnic vessels and gain access to the portal system.

During the course of a few weeks, the schistosomulae develop into mature male and female adults that mate continuously.[131,132] Adult worms are 1 to 3 cm in length and usually live for 3 to 7 years. *S. haematobium* localizes to the venules of the urinary bladder, *S. japonicum* goes to the superior mesenteric vessels, and *S. mansoni* goes to the inferior mesenteric vessels. The gravid females migrate upstream to lay eggs near the bladder or intestines. Each adult female can produce hundreds (in the case of African species) to thousands (for Asian species) of eggs per day.[57] Most of the eggs become embedded in the walls of the peripheral vessel, where they were laid. However, some of them flow with the blood and can stimulate reactions and cause disease at other sites. A localized granulomatous response allows the eggs to pass through the wall of the bladder (for *S. haematobium*) or the intestine (for other species) to be released in urine or stool. Released eggs mature in a week's time and then hatch to release miracidia. The miracidia penetrate the snail to continue their life cycle and eventually develop into hundreds of cercariae.

Infection is seen in children as young as 6 months; exposure to cercariae often is greatest in boys between the ages of 5 and 10 years.[66,71,130] In some lakeside African villages, however, nearly half the children are infected during the first 3 years of life.[101] The peak intensity of infection, as measured by excretion of eggs, occurs in children as young as 8 to 12 years in heavily infected areas and takes place during the teenage years in more lightly infected areas. The intensity and the incidence of excretion of eggs decline after the adolescent years, and hormonal changes during puberty are associated with increased resistance to infection.[76]

PATHOGENESIS AND IMMUNITY

Within hours of penetration of the skin, local erythema and inflammation may result from the activity of parasite proteases.[87,117,136] In individuals previously sensitized, a delayed immune reaction may cause papular or vesicular lesions.[74,130] Infiltration of the epidermis and dermis with a mixture of mononuclear cells and eosinophils causes papules. This reaction (termed *swimmer's itch*) is more pronounced for avian schistosomes, which are unable to penetrate into the human circulation and die in situ. During the initial migration of schistosomes through the lungs, patients may develop fever, pneumonitis, pulmonary infiltrates, and eosinophilia. However, these reactions resolve spontaneously.

Acute schistosomiasis typically begins 4 to 8 weeks after exposure, at the time that the female parasites begin to produce eggs.[39] This condition is recognized more commonly with *S. japonicum* than with *S. mansoni* and rarely is described with *S. haematobium*.[74,130] Patients characteristically have marked eosinophilia along with IgE and IgG antibodies to the parasites, consistent with an active T_H2 response.[74,130] However, studies suggest that proinflammatory cytokines play an important role.[36,97] The antibodies react with egg antigens, which cross-react with antigens from the cercaria or schistosomula stages.[8,62,63] During early production of eggs, the quantity of antigen exceeds that of antibody, and soluble immune complexes are formed. Immune activation and immune complex deposition lead to the clinical manifestations, including fever, myalgia, urticarial rash, and bloody diarrhea. Pulmonary nodules and infiltrates may be noted during acute infection.[30,119] In addition, acute infection can be complicated by ectopic egg production. Patients with acute *S. mansoni* or *S. haematobium* infection occasionally can have neurologic involvement.[23,45,108] Interestingly, acute disease occurs rarely in endemic populations, perhaps because of modulation of the immune response from exposure to parasite antigens in utero.

CHRONIC SCHISTOSOMIASIS

Despite the fact that the adult worms live within the bloodstream, they cause little host response. The parasite surface incorporates blood group glycoproteins and major histocompatibility antigens and down-regulates expression of its own surface proteins, which may help the adults evade immune system attack.[55,106,123]

Chronic infection is characterized by localized granulomata surrounding the parasite eggs.[74,113,130] Excretory products of the eggs (termed *soluble egg antigens*) are the major antigens. The egg antigens are cytotoxic to host cells, such that infection of

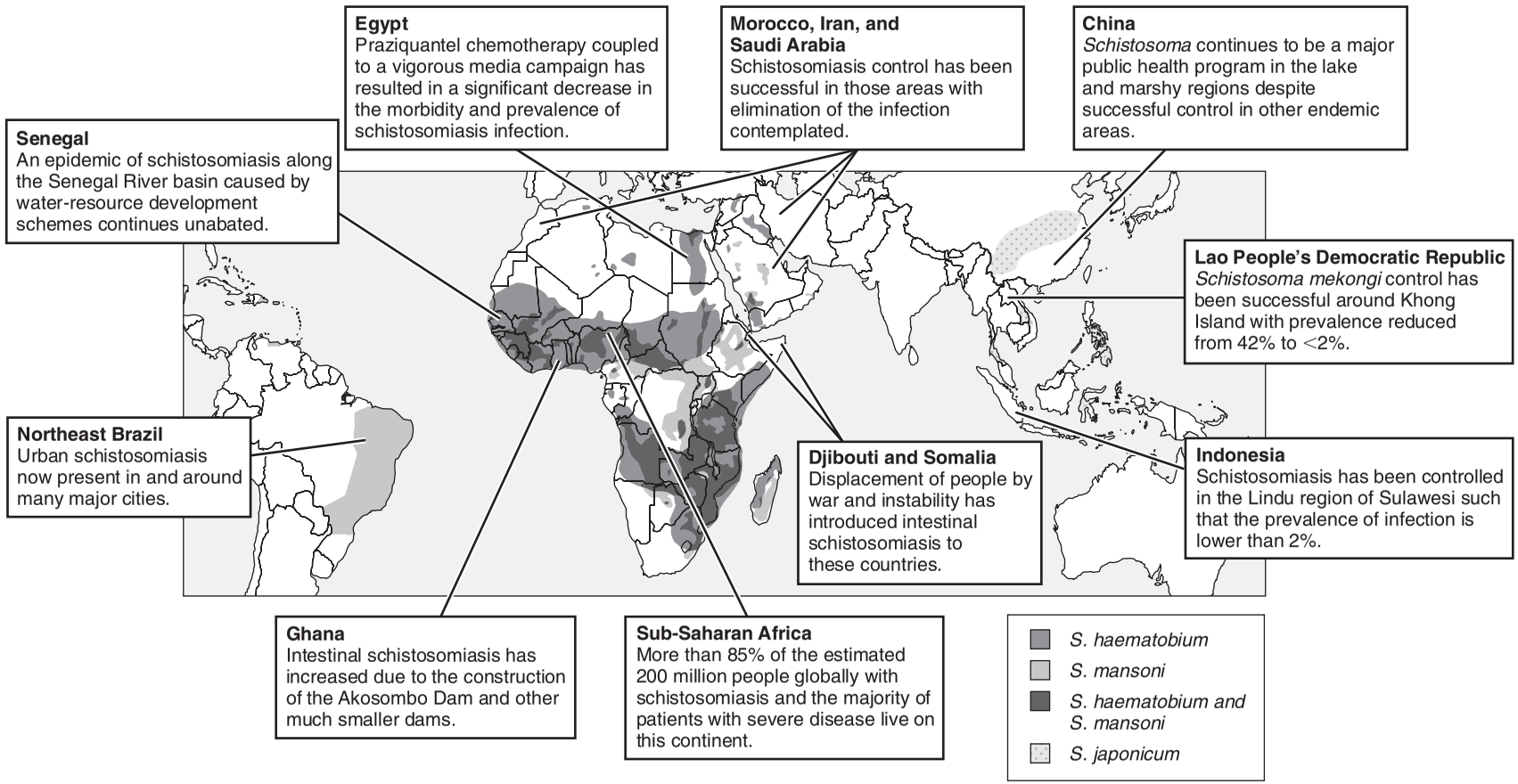

Figure 240–1 Geographic distribution of human schistosome species. *(From http://www.who.int/ctd/schisto/epidemio.htm.)*

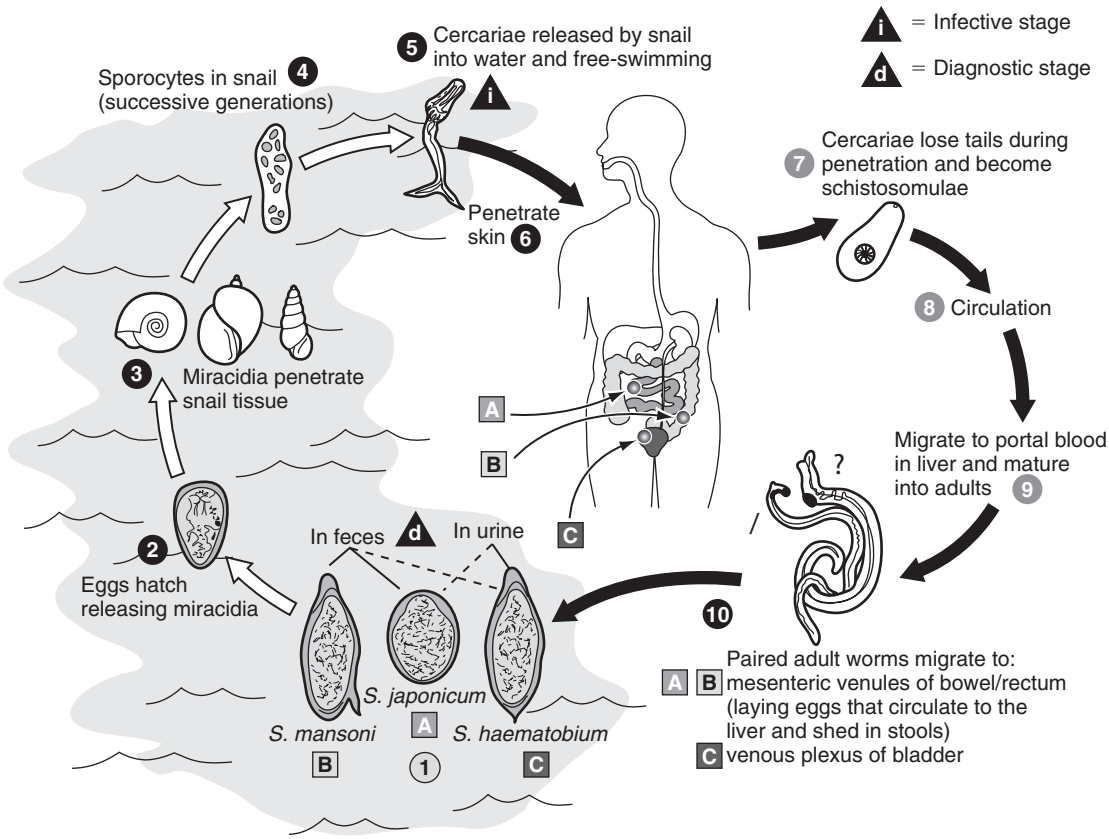

Figure 240–2 Life cycle of the human schistosome species. *(From Centers for Disease Control and Prevention: Schistosomiasis. Available at: http://www.dpd.cdc.gov/dpdx/HTML/Schistosomiasis.htm.)*

mice lacking T cells results in increased localized necrosis surrounding the eggs; the granulomata help protect the host from the ravages of the eggs. The granulomata also facilitate transit of the eggs into stool and urine as illustrated by the fact that immunodeficient hosts tend to have lower rates of shedding of eggs despite having high concentrations of eggs in tissues.[38,70,99]

The granulomata also contribute to the pathologic process. They are composed of a mixture of eosinophils, lymphocytes, macrophages, and granulocytes.[16] Late-stage granulomata also contain fibroblasts, collagen, and giant cells. Initially, antigen-presenting cells stimulate $CD4^+$ T cells to produce interleukin (IL)-2, tumor necrosis factor–α (TNF-α), and interferon-γ.[98] Early expression of TNF-α plays a key role in the initial formation of granulomata.[4] A subsequent shift occurs toward production of T_H2 cytokines (e.g., IL-4, IL-5, IL-10, and IL-13) along with antibody.[107] This event is accompanied by eosinophil recruitment. IL-4 and IL-13 also play a primary role in the development of hepatic granulomata, and IL-13 expression is linked closely to the development of fibrosis in murine models.[26,27] Subsequent immunomodulation, largely mediated by IL-10 and transforming growth factor–β (TGF-β), results in downsizing of the granuloma and suppression of the inflammation.[7,42,47,64,90,97,122,145] When granulomata are limited to the intestines, the cytokine response is dominated by anti-inflammatory cytokines (e.g., IL-10 and TGF-β), and patients have few symptoms.[32] By contrast, patients with granulomata in the liver are more likely to be symptomatic. They express more inflammatory cytokines, including IL-13 and TNF-α. TGF-β also is associated with fibrosis in primate models,[42] whereas T_H1 cytokines (e.g., interferon-γ and IL-12) suppress fibrosis.[17,149,154]

IL-13 in particular stimulates the formation of fibrosis in the portal tracts.[26] Portal fibrosis, in turn, leads to the development of portal hypertension (i.e., hepatosplenomegaly, ascites, and esophageal varices).[74] Death from hepatic schistosomiasis typically results from gastrointestinal bleeding. Overt bleeding is an uncommon occurrence in children, but it can occur in adolescents and young adults. In patients with isolated schistosomiasis, hepatocellular function is surprisingly normal.[16] Patients are thought to tolerate even gastrointestinal bleeding better than cirrhotic patients do. By contrast, progressive hepatocellular damage and rapid progression to liver failure develop in patients co-infected with hepatitis viruses, particularly hepatitis C.[5,29,69] The immunomodulation that develops in response to schistosomiasis is thought to suppress immune control of the hepatitis virus. Whether schistosomiasis-associated immune changes alter susceptibility to or expression of human immunodeficiency virus is unknown.[142]

Interestingly, patients with schistosomiasis are less susceptible to allergies.[141] Evidence indicates that asthmatics with schistosomiasis produce lower levels of IL-5 and greater levels of IL-10 than do asthmatics without schistosomiasis, and it is this suppression of T_H2 responses that might modulate the expression of asthma and atopic disease in patients with schistosomiasis.[6] Alternatively, it is possible that a common genetic predisposition alters the risk of subsequent development of both atopic disease and schistosomiasis.[10] Similarly, parasite-induced changes in the T_H1/T_H2 balance are hypothesized to explain how young children with underlying schistosomiasis are less likely to develop malaria.[89]

Several means exist by which schistosomiasis might be related to anemia, and the exact pathologic mechanisms are not fully understood.[49] At least sometimes, however, the anemia is due to inflammation rather than to iron deficiency.[79] Generalized undernutrition is associated with schistosomiasis; proinflammatory

cytokines (IL-1β) and generalized inflammation (as manifested by elevated levels of C-reactive protein) are contributing factors.[34] Even without acute inflammation, schistosomal infection and periportal fibrosis are associated with reduced vitamin A status and high oxidative stress (increased serum hydroperoxides).[14]

Approximately 40,000,000 women of child-bearing age are infected with schistosomes. Although mechanisms are not clear, some evidence indicates that schistosomiasis during pregnancy is associated with decreased birth weight and a higher risk of preterm delivery.[50]

URINARY SCHISTOSOMIASIS

Genital and urinary schistosomiasis also is the result of granuloma formation. Severity of disease correlates with increased production of TNF-α and decreased production of IL-10.[75] Direct inflammatory responses to eggs along the ureters or from obstruction to ureteral drainage caused by space-occupying granulomata in the bladder wall block ureteral emptying.[72,124] Abnormal ureteral flow, dilatation, and hydronephrosis develop.[124] In some communities where *S. haematobium* is common, ureteral deformities demonstrable on intravenous pyelography are noted in half of affected children, and hydronephrosis develops in more than 10 percent.[73] Eventually, scarring and calcifications form and may be associated with chronic hydronephrosis, even with resolved or light chronic infection.[75,124] The hydronephrosis and urinary stasis predispose patients to chronic bacteriuria and urinary tract infections.

In *S. mansoni* infection, immune complexes may mediate glomerulonephritis, as evidenced by the finding of schistosome-derived immune complex deposits in glomeruli.[11,125] Nephrotic syndrome has been reported in association with chronic schistosomiasis.[91,92] Amyloid deposits have been found in the kidneys of children with chronic schistosomiasis, and this condition probably is caused by immune responses that are altered by the chronic parasitic infection.[12,143]

Squamous cell carcinoma of the bladder is a common occurrence in areas of heavy infection with *S. haematobium*, but otherwise, it is a rare form of bladder cancer.[75,124,130] The prolonged irritation of bladder epithelium by schistosome eggs and the resulting immune response are thought to trigger hyperplasia and subsequent malignant disease.[80] This condition could be aggravated by urinary stasis in the bladder with secondary increases in pH that favor malignant transformation of epithelial cells. Carcinogenic tryptophan metabolites also have been postulated to link the nutritional activities of parasites near the bladder with subsequent carcinogenesis.

CLINICAL MANIFESTATIONS

The main clinical manifestations of schistosomiasis vary with the stage of the parasite's life cycle and also differ among species. Many patients remain asymptomatic. Patients with asymptomatic schistosomiasis may be identified during community screening programs in an endemic area, through immigrant or returned traveler evaluations in a nonendemic area, or when diagnostic tests such as urine or stool microscopy are performed to evaluate other, seemingly unrelated symptoms. The minority of children with heavy infections are most likely to develop early symptoms and also are at greatest risk for subsequent major health complications.

CERCARIAL PENETRATION

Within a few minutes of coming into contact with cercariae, some children develop pruritus. This response to initial contact with the parasite occurs more commonly in nonimmune visitors to endemic areas than it does in indigenous residents of endemic areas, but it seems to be more pronounced after repeated exposure to cercariae than on the first exposure. It may occur after contact with any of the human *Schistosoma* spp. but more commonly results after contact with the cercariae of avian schistosomes. An erythematous and, sometimes, papular rash may develop.[74,130] The rash usually subsides during a period of 2 to 10 days without scarring, regardless of whether specific treatment is given.

ACUTE SCHISTOSOMIASIS (KATAYAMA FEVER)

Acute schistosomiasis, referred to as Katayama fever, typically begins 4 to 8 weeks after exposure, when the adult worms begin to produce eggs.[6,39,65] Clinical manifestations of acute schistosomiasis usually include high fever, chills, myalgia, headache, and a general ill appearance.[36,39] An urticarial rash, which may include giant urticaria, and diffuse lymphadenopathy may be seen.[112,156] Cough, rales, and pulmonary infiltrates may be noted, even in the absence of fever.[30,119] Gastrointestinal symptoms of anorexia, abdominal pain, and loose stools sometimes are observed. Bloody diarrhea may be seen acutely with heavy infections by *S. japonicum* and *S. mansoni*. Tender hepatomegaly and mild splenic enlargement develop in approximately 30 percent of children with Katayama fever. Some reports suggest that myelopathies are a common accompaniment of acute schistosomiasis.[45,108] Genital symptoms (primarily hematospermia) also are frequent initial symptoms.[31,110] Marked eosinophilia often is seen with acute schistosomiasis.

URINARY SCHISTOSOMIASIS

Hematuria is the classic finding of *S. haematobium* infection.[20,37,72,130,147] In some highly endemic areas, for boys nearing puberty not to display this evidence of "male menstruation" is considered abnormal. Typically, hematuria results from the release of blood from irritated, inflamed areas around granulomata in the bladder wall as the bladder contracts during micturition.[124] Thus, the blood is most obvious at the end of the urine stream ("terminal hematuria"). The hematuria usually is not associated with pain or discomfort, but dysuria can be present.[1,20] With chronic infection in children who either were not treated initially or were re-infected, obstructive uropathy can develop. In community surveys in endemic areas, as many as 40 percent of children were found to have significant renal or ureteral abnormalities (or both).[1,61,144] Hematuria and dysuria occur more commonly in children than in adults, presumably because they have heavier parasite loads. However, hydronephrosis, which develops more slowly, is seen more often in adults.[72,73,96,124]

Bacterial urinary tract infection may coexist with urinary schistosomiasis, probably secondary to obstruction of urinary outflow.[124] Chronic infection can lead to renal failure. Obstructive uropathy, with or without incident or recurrent urinary tract infections, can lead to loss of renal function. Children with schistosomal infection also can have glomerulonephritis and nephrotic syndrome. Bladder cancer, when it occurs, typically develops in the setting of untreated, heavy chronic urinary schistosomiasis.

GENITAL SCHISTOSOMIASIS

Studies have emphasized the genital tract as an important site of schistosomiasis in both men and women.[22,81,108,109] In women, egg granulomata may be found in the cervix, uterus, or fallopian

tubes.[43,81,82,109] Cervical irritation and ulceration, vaginal bleeding, or ectopic pregnancy may develop. Genital schistosomiasis can be confused with cancer.[134] Adolescent boys may have involvement of the prostate and seminal vesicles.[81,110] Eggs were noted in semen from 43 percent of men in an area endemic for *S. haematobium*.[81] The main clinical finding is hematospermia,[81,94] which may occur with acute infection or during chronic disease. In Africa in particular, genital schistosomiasis may be a cofactor in transmission of human immunodeficiency virus infection.

INTESTINAL DISEASE

Most children with intestinal schistosomiasis do not have intestinal symptoms.[128,130] Thus, even finding eggs in stool does not mean that symptoms are related to the schistosomiasis.[59] Nonetheless, both the small and large intestines may be involved with schistosomal disease. Irritation of the bowel wall from inflammatory reactions induced by eggs may lead to diarrhea that sometimes contains blood or mucus.[24,25,128-130] Crampy abdominal pain and generalized malaise may occur. Endoscopy can reveal granular inflammation with hyperemic areas, ulceration, and hemorrhage. Polyps, which may develop around granulomata, may be identified by contrast radiography or endoscopy. Protein-losing enteropathy and blood loss can result in malnutrition and iron deficiency, especially in cases with significant small bowel disease. The role of schistosomiasis in malnutrition was illustrated by studies of mass chemotherapy, which resulted in improved nutrition and a lower prevalence of anemia.[9,102,135]

HEPATOSPLENIC DISEASE (HEPATOMEGALY, SPLENOMEGALY, AND PORTAL HYPERTENSION)

The life-threatening complications caused by *S. japonicum* and *S. mansoni* are the result of eggs that remain in the venous vasculature and migrate back to the liver.[16] Egg burden seems to be the greatest predictor of hepatic involvement with schistosomiasis, but some link with HLA type has been suggested.[93,94,121,146]

Children with compensated disease initially have few symptoms. These symptoms may include anorexia, malaise, and abdominal fullness.[16,25,77,103,110,147] Children have hepatomegaly even without significant portal hypertension. The hepatomegaly generally is firm and either minimally tender or nontender. Splenic enlargement usually is noted as well. Liver function is affected only late in the course of hepatic schistosomiasis, so jaundice and liver enzyme elevations are unusual findings.

As fibrosis develops around eggs in the liver, portal hypertension ensues. Liver function usually remains intact, but esophageal varices can cause death during the late adolescent and early adult years.[16,25,103] Ascites gradually develops during a period of years, and the spleen may become very large. Bleeding esophageal varices are a common cause of demise in individuals who had heavy schistosomal infections in childhood. Such patients may come to medical attention for melena or hematemesis. Initial episodes are well tolerated. However, with progressive liver decompensation, variceal bleeding can be fatal.

Children with severe hepatosplenic schistosomiasis do not grow as well as do other children. Debate has ensued about whether this deficiency is due to a direct effect of the parasitic infection or poor nutritional intake caused by poor general health. Some recent data suggest that a decrease in insulin-like growth factor–1 activity is linked to hepatic schistosomiasis.[105] Extensive formation of hepatic granulomata may hinder the liver's structure and function in such a way that growth-promoting factors are not produced normally.

PNEUMONITIS AND COR PULMONALE

Sometimes, children initially have low-grade fever and cough as schistosome larvae migrate through their lungs. This condition can occur with an initial heavy infection (when eggs will not be detectable) or with a heavy re-infection (when eggs will be detectable in urine or stool from the preceding underlying infection). This larval pneumonitis can be manifested on lung examination as basilar rales and wheezing.[30,119] Radiographs may show basilar mottling in the lung fields. Eosinophilia is a common occurrence with this type of pneumonitis. Resolution, even without treatment, usually occurs in 2 to 4 weeks. Similar symptoms also may be seen as a reactive pneumonitis when patients with heavy parasite burdens are treated.

In children with advanced hepatosplenic schistosomiasis and portal hypertension, eggs may bypass the liver and flow from the abdominal and pelvic veins into the small lung vessels. Localized granulomata form around eggs lodged in the lungs. During the course of time, children may be subject to fatigue, cough, and right-sided heart failure. Medical therapy stops the progress of disease and may decrease the ongoing inflammatory responses, but right-sided heart failure, once it is established, is not fully reversible.

CENTRAL NERVOUS SYSTEM INVOLVEMENT

Neurologic manifestations of schistosomiasis often are dramatic, even if not common.[45,100,108,120] Worms do not always follow the typical routes described for urinary and intestinal schistosomiasis. On occasion, worms migrate to cerebral blood vessels. Production of eggs in that location can cause seizures and headaches in children.[108,120] Sometimes, optic field defects and dysarthria are noted; these findings seem to result from localized space-occupying inflammatory reactions that develop around worms or eggs. Spinal fluid pressure may be elevated, and both protein concentrations and lymphocyte counts in spinal fluid may be increased. This cerebral schistosomiasis occurs more commonly with *S. japonicum* infection and is thought to develop in as many as 2 percent of infected children.

S. mansoni infection and, to a lesser extent, *S. haematobium* infection can produce eggs that embolize to the spinal cord. The eggs or associated granulomata can cause transverse myelitis. Paraplegia along with urinary and fecal incontinence often is the initial problem in children with spinal schistosomiasis.[45,120]

CHRONIC OR RECURRENT SALMONELLOSIS

In endemic areas, some children have salmonellosis and schistosomiasis concurrently. Children present with chronic low-grade fever, fatigue, malaise, and poor growth.[54,77,111,116,152] Blood cultures frequently demonstrate *Salmonella* bacteremia, but stool may not contain these bacteria. Some children have chronic *Salmonella* bacteriuria. Sepsis and mortality are unusual findings despite these persistent bacterial infections. Relapse of the *Salmonella* infection occurs commonly unless the coexisting schistosomal infection is treated. The bacteria are thought to hide within the parasitic worms, where antibiotic penetration is poor, and thereby evade host defenses.

DIAGNOSIS

A clinical diagnosis of schistosomiasis should be entertained in the presence of typical clinical features. These features might be as obvious as painless hematuria in a child in a region endemic for *S. haematobium*, or portal hypertension may be noted late in

the second decade of life in an otherwise healthy adolescent from an area known to be endemic for *S. mansoni* or *S. japonicum*.[110,118] The other clinical findings described also should prompt the clinician to consider a diagnosis of schistosomiasis when the child has resided in or traveled through a *Schistosoma*-endemic area.[133] In traveling children, the index of suspicion must be particularly high because patients may be asymptomatic or have atypical symptoms, they may not have clear history of exposure to contaminated water, and parasitologic examination findings often are negative.[15,110]

Outside endemic areas, re-infection is not expected. Thus, a reasonable approach is to test all children, including asymptomatic children, who have returned or emigrated from an endemic area where they had potentially significant exposure to fresh water. Such testing would involve all travelers who swam in, waded in, or even touched suspicious water, and it could involve immigrants who have spent long periods in endemic areas. Certainly, any child outside an endemic area who has even a remote history of having had possible contact with cercariae and clinical findings suggestive of any form of schistosomiasis should be tested. Testing is appropriate because even late therapy can favorably alter the course and outcome of schistosomiasis. In some highly endemic areas, diagnostic testing is of limited feasibility or questionable reliability (or both), and curative medications are readily available. In such settings, establishing a proven diagnosis might be less necessary. For example, in a highly endemic setting, a urine paper strip test result positive for hematuria could provide sufficient suspicion of infection to warrant administration of specific antischistosomal therapy. In populations with known high rates of infection, mass treatment (without expensive diagnostic testing) is reasonable.

In established heavy infections, eggs usually are readily apparent on microscopic examination of urine or stool. For lighter infections, concentrating techniques are useful. Urine can be centrifuged and filtered to increase the yield of eggs. The result of urine examination is most likely to be positive when urine is voided at midday and when it is collected at the end of the urine stream. In the event of high suspicion and negative urine findings, bladder wall biopsy can be performed. The procedure usually is not necessary, however, because urine microscopy, especially with filtered or concentrated samples, generally is positive. Examination of multiple samples increases the yield.

The eggs of *S. haematobium* and *S. mansoni* are approximately 90 mm in diameter, whereas the eggs of *S. japonicum* and *S. mekongi* are somewhat smaller. As demonstrated in Figure 240–3, identification of species is aided by observation of the spine—small for *S. japonicum* and *S. mekongi*, at one end for *S. haematobium*, lateral for *S. mansoni*, and broad at each end for *S. intercalatum*. Eggs usually are detectable in voided urine. Plain radiographs might demonstrate some calcification in the bladder wall around chronic granulomata. Cystoscopy, if it is performed, reveals bladder wall hyperemia and, subsequently, nodular lesions and fibrosis that give rise to "sandy patches" on the bladder wall. Granulomata may protrude from the bladder wall into the lumen. Both edema and granulomata may be seen obstructing ureteral orifices.

In light infections, examination of stool or urine samples may not reveal ova because of intermittent or low-volume shedding. Thus, negative stool or urine test results do not adequately rule out intestinal schistosomiasis.[15,110] When stools repeatedly are negative for ova, rectal biopsy may be performed. Samples may be obtained by random punch biopsy, but the yield is increased when samples are taken from inflamed sites under direct visualization. Eggs are detected in unstained smears made by pressing the tissue sample between a coverslip and a glass slide, but eggs also can be seen with standard histologic stains.

Nonviable eggs may be excreted for months or years after therapy has been successful. With good medical treatment, eggs usually are not viable when they are passed more than a week after the initiation of therapy. With ineffective treatment or re-infection, however, viable eggs may continue to be passed. On microscopic examination, living ova contain transparent miracidia within the egg, which often are motile. For further documentation of their viability, the eggs may be hatched by placing them in fresh water exposed to light for 20 minutes; observation with a hand lens then can reveal swimming miracidia.

Serologic tests may be needed in acute infection, which may occur before the eggs are excreted or with eggs in ectopic locations.[60,137] For travelers with limited exposure and the likelihood of having either an acute or a limited chronic infection, serology is the main diagnostic tool. Currently, the most common screening serologic test is the FAST-ELISA (the Falcon assay screening test–enzyme-linked immunosorbent assay) using *S. mansoni* adult worm microsomal antigen.[138] It also cross-reacts with *S. haematobium* but is less sensitive for *S. japonicum* or *S. mekongi* infection. Species can be confirmed by use of an immunoblot assay (enzyme-linked immunotransfer blot).[137] An immunoblot assay also is available for *S. japonicum*. Antigen-detection assays may be helpful, but they are not available in the United States.[2]

Ultrasonography is the main imaging procedure used in schistosomiasis.[61] It can document involvement of the portal and urinary tracts and, in selected cases, be diagnostic of schistosomiasis. Ultrasonography can identify bladder polyps, ureteral dilatation, hydronephrosis, and calcifications within the urinary tract. When resources are available, an ultrasound evaluation of the urinary tract should be performed in any child found to be

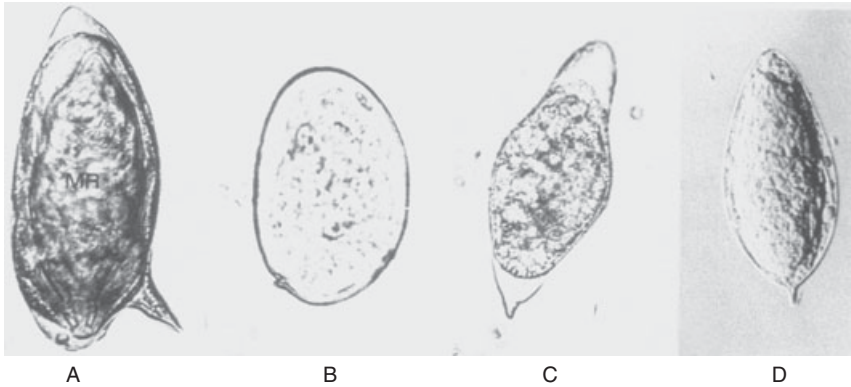

Figure 240–3 Human schistosome eggs. **A,** *S. mansoni.* **B,** *S. japonicum.* **C,** *S. intercalatum.* **D,** *S. haematobium.* *(From Mahmoud, A. A. F. [ed.]: Schistosomiasis. London, Imperial College Press, 2001. Reprinted with permission.)*

infected with *S. haematobium*. For children with persistent urinary symptoms (dysuria, suprapubic pain, hematuria) despite having received good medical treatment, cystoscopy should be considered, in part to screen for bladder cancer. Other radiographs and tests of renal function and excretion generally are not indicated unless the ultrasound evaluation reveals significant urologic disease.

Portal ultrasonography is the imaging study of choice for hepatic involvement. The presence of periportal fibrosis with thickening of the portal tracts and vein walls (so-called clay-pipe stem or Symmer fibrosis) correlates with parasite burden and hepatic disease. In experienced hands, schistosomiasis can be distinguished reproducibly from cirrhosis and other chronic liver diseases.

TREATMENT

Optimal treatment of schistosomiasis varies with the stage of the disease. Cercarial dermatitis resolves spontaneously and requires no antiparasitic treatment. Oral antihistaminic agents, however, may be given for severe itching. Medical therapy for schistosomiasis caused by the human schistosomes is effective and well tolerated without significant complications. No good medical reason exists to allow schistosomiasis to go untreated. The cost of therapy, however, is a limiting factor in resource-poor areas of the world. Several different medications can be considered for curative treatment.

Praziquantel, a mixture of stereoisomers of pyrazinoisoquinoline ring structures, is a broad-spectrum oral anthelmintic agent that is effective against each of the five human schistosome species. The mechanism of action was thought to involve unmasking of parasite antigens with subsequent killing by the host immune response.[19] However, patients with acquired immunodeficiency syndrome respond well to praziquantel, raising questions about this hypothesis. Praziquantel is given in divided doses on a single day as full curative treatment. For infection with *S. haematobium*, *S. mansoni*, and *S. intercalatum*, the dose is 40 mg/kg in two divided doses given on the same day. For children infected by *S. japonicum* or *S. mekongi*, the dose is 60 mg/kg in three divided doses given on the same day.[3,40] Heavily infected children sometimes experience some nausea, vomiting, and abdominal cramping with treatment. Rare side effects, including headache, pruritus, bloody stools, and fever, are transient and resolve within 1 to 2 days after the initiation of treatment. Because praziquantel primarily kills mature adult worms, a second course is recommended for the treatment of acute infection. Praziquantel is effective in more than 90 percent of treated children. Some studies suggest that resistance may be emerging.[84,85] However, those studies were performed in areas with frequent re-infection and with high parasite burden. Thus, the poor response may be due to the fact that juvenile forms are not as susceptible to praziquantel. Studies from nonendemic areas, however, continue to demonstrate excellent efficacy.[147] In addition to curing infection, treatment with praziquantel is associated with improved growth and reduction in anemia.[33] In endemic areas with high rates of infection, school-based and community-based (when many children in the area do not regularly attend school) treatment strategies (without confirmatory diagnostic testing) are effective; cost issues, however, raise concerns of program sustainability.[51]

An alternative treatment effective only against *S. mansoni* is oxamniquine, a tetrahydroquinoline compound.[127] The mechanism by which this agent acts is unknown. The effective dose has varied with the geographic origin of the schistosomal infection, but some resistance has been reported. Current recommendations are that children treated with oxamniquine be given 40 to 60 mg/kg divided into two doses administered on 1 day or four separate doses given during the course of 2 days. Mild side effects include nausea, headache, and fever. Seizures are a rare side effect, so administration of this medication should be avoided in children with seizure disorders. Cure rates of more than 90 percent are reported with oxamniquine.

Metrifonate is an organophosphate compound that causes paralysis of the parasite. It is 70 to 80 percent effective against *S. haematobium* when it is used at a dose of 10 mg/kg orally once every 2 weeks for 6 weeks. It does decrease the child's own plasma and erythrocyte cholinesterase activity, but actual cholinergic symptoms seldom occur. This medication is less expensive than the other options, but it generally is not used when praziquantel is available.

Medical therapy usually is effective for children with schistosomiasis, even with advanced disease.[86,115,126,153] Clear improvement in the patient's disease burden occurs when treatment is given. Even established uropathy and portal hypertension often are reversible.[88,115,126] Surgical procedures usually are reserved for children with complications related to long-term infection, such as persistent portal hypertension. Propranolol prophylaxis and sclerotherapy or banding can reduce rebleeding from esophageal varices.[41] Some cases require surgical decompression of portal hypertension. Splenorenal shunts are associated with high rates of hepatic encephalopathy. Comparative studies suggest that in experienced hands, esophagogastric devascularization with splenectomy is the procedure of choice.[46,53]

Systemic steroids may be useful for severely ill children with Katayama fever or severe larval pneumonitis. They also may be helpful in cases with granulomata in the central nervous system.[45,48] However, corticosteroids have not been proved effective treatments in other forms of the disease.

PREVENTION

Travelers and expatriates in endemic areas can prevent disease by limiting exposure to infectious fresh water. In endemic areas, they should avoid swimming in fresh-water streams and lakes. In addition, the cercaria can be eliminated by chlorination or by allowing water to settle for 24 hours before bathing or washing.

The use of effective medical therapy coupled with improved urine and stool hygiene has the potential to eradicate schistosomiasis from endemic areas.[148] Elimination of the snails that serve as intermediate hosts also would be effective, but attempts at implementation of programs administering molluscicides have been unsuccessful to date. Historically, national control programs in China focusing on snail control, sanitation, and improved water supplies have led to sustained reductions in infection rates, even apart from the influence of chemotherapy.[140,150] For specific populations, health education can lead to significant favorable changes in knowledge about the illness, water-exposure activities, and re-infection rates in school-age children.[58] Recently, control efforts have focused on mass chemotherapy, with particular emphasis on school-based therapy.[9,59,102,104,148] Treatment programs in which the entire school-age population is treated, typically with praziquantel, have resulted in overall improvement in levels of nutrition and in a reduction in the prevalence of anemia. In areas where re-infection commonly occurs, however, the effects of mass treatment programs are of limited duration, and treatment must be repeated every 6 months to 1 year. Sustainability of national control programs and of favorable outcomes remains challenging in Africa.[52,68,44]

Several studies suggest that artemisinin derivatives can prevent development of infection and decrease the worm burden in those exposed to *S. mansoni* and *S. japonicum*.[83,113,139] However, they are less effective for chemotherapy than is praziquantel,[18,35] probably because parasitologic activity is limited to the juvenile forms.

Furthermore, the public health role of chemoprophylaxis in the prevention of infection has not been established.

Immunization of experimental animals with irradiated cercariae can prevent experimental schistosomiasis. Thus, development of a vaccine to prevent human schistosomiasis is theoretically possible. Several potentially protective antigens have been identified on schistosomes, and they are being used as targets for vaccine development. However, the correlates of protective immunity in humans are not well defined. Currently, vaccines using combinations of antigens are being studied for *S. mansoni*.[151] Early-stage clinical trials have been performed for *S. haematobium* glutathione *S*-transferase–containing vaccines.[21] Vaccines related to several *S. japonicum* antigens also are being studied in animals, but efficacy is limited.[95,114,155] Whether development of a vaccine to prevent human infection is a real possibility remains far from certain, and no vaccine is likely to be ready for clinical use in the near future.[13,56]

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SUBSECTION 5

Arthropods

CHAPTER 241

ARTHROPODS

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Arthropods comprise nearly three fourths of the world's animal species. Although some arthropods are capable of producing damage to agriculture and homes and others serve as reservoirs, hosts, and vectors of human and animal pathogens, the majority have no significant impact in causing human disease.[59] However, among the more significant diseases transmitted to humans by arthropods are Chagas disease, malaria, dengue, and bubonic plague. In the United States, ticks transmit more vector-borne diseases caused by bacteria, rickettsia, viruses, and protozoa than do any other species.

TICKS

Ticks, mites, spiders, and scorpions all belong to a class of arthropods known as Arachnida. Two of three families of ticks belonging to this class contain species capable of transmitting pathogens to humans. Ixodidae, the hard tick family, and Argasidae, the soft tick family, serve as both vectors and reservoirs for many rickettsiae (Table 241–1). By use of a fluorescent technique, these rickettsiae can be identified in the tick hemolymph.

Hard ticks are so named because of their scutum, a dorsal sclerotized shield. Owing to the limited scutum coverage of the female, she is more capable of becoming engorged with blood than is the male, a factor significant in the reproductive process. During feedings, these ticks remain attached to the host for hours or days at a time.[15,52] Three genera of Ixodidae are known to transmit disease to humans in the United States: *Amblyomma*, *Dermacentor*, and *Ixodes*.[51]

Soft ticks have no scutum and tend to live for long periods of time, often surviving for years without eating. Both the nymph form and the adult tick have a tendency to eat often but for brief periods often lasting less than 30 minutes.[15,52] Of the family Argasidae, only ticks of the genus *Ornithodoros* are known to transmit pathogens to humans in the United States.[51] Specifically, they are the vectors of tick-borne relapsing fever.

Local reaction from tick bites appears to be mediated by complement. The reaction from these bites may persist and subsequently develop into a so-called tick bite granuloma. Systemic reactions such as fever, chills, nausea, vomiting, abdominal pain, and headache can be associated with tick bites.

As noted, tick-borne diseases can result from infection with pathogens that include bacteria, rickettsia, viruses, and protozoa.[53] The major tick-borne diseases occurring in the United States include tick paralysis (discussed here) as well as tularemia (Chapter 144), tick-borne relapsing fever (Chapter 152), Lyme disease (Chapter 153), Colorado tick fever (Chapter 184), Rocky Mountain spotted fever (Chapter 207), ehrlichiosis (Chapter 207), and babesiosis (Chapter 230).

Tick-borne rickettsial diseases are clinically similar yet epidemiologically and etiologically distinct illnesses. In the United States, they include Rocky Mountain spotted fever, human monocytotropic (or monocytic) ehrlichiosis, human granulocytotropic (or granulocytic) anaplasmosis (formerly known as human granulocytotropic ehrlichiosis), *Ehrlichia ewingii* infection, and other emerging tick-borne rickettsial diseases.[10] Mediterranean spotted fever, a tick-borne disease caused by *Rickettsia conorii* (Chapter 207), is found almost exclusively in the Mediterranean area.

TABLE 241–1 Human Infectious Diseases for Which Ticks Are a Vector

Disease	Agent
Relapsing fever	*Borrelia duttonii*
Q fever	*Coxiella burnetii*
Tularemia	*Francisella tularensis*
Queensland tick typhus	*Rickettsia australis*
Fièvre boutonneuse	*Rickettsia conorii*
Rocky Mountain spotted fever	*Rickettsia rickettsii*
Asian tick typhus	*Rickettsia sibirica*
Colorado tick fever	Arbovirus
Encephalitis	Arbovirus
Lyme disease	*Borrelia burgdorferi*
Human monocytic ehrlichiosis	*Ehrlichia chaffeensis*
Human granulocytic ehrlichiosis	*Ehrlichia* species
Babesiosis	*Babesia microti*

TICK PARALYSIS

Tick paralysis is a neurologic syndrome characterized chiefly by an ascending flaccid paralysis in association with the attachment of certain species of ticks. A well-known disease of animals, it was reported to occur in humans in North America first in 1912.[56] Most cases have occurred in the Pacific Northwest and Rocky Mountain states, in spring and summer.[53] It has been reported to occur more frequently in children, especially in girls between 2 and 5 years of age, than in adults. Numerous genera of ticks are known to be associated with tick paralysis. In North America, *Dermacentor andersoni* (wood tick) and *Dermacentor variabilis* (dog tick) are the primary species responsible for tick paralysis.[36] In Australia, ticks implicated most commonly are *Ixodes holocyclus* and *Ixodes cornuatus*. Both adult female and male as well as immature ticks have been implicated in this nervous system disorder.

Pathogenesis

Tick paralysis is thought to be caused by a neurotoxin (holocyclotoxin) produced in the tick's salivary glands.[34] It usually is released by gravid female ticks at the site of attachment, usually the scalp. The neurotoxin is a protein with temperature-dependent activity.[13] The exact mechanism and location of the

toxin's action are not known but may include decreased entry of calcium into motor nerve terminals or interference with presynaptic excitation-secretion coupling, which leads to a reduction of acetylcholine release at the motor end-plate.

Investigations in children with tick paralysis have implicated primarily peripheral nerve dysfunction, as nerve conduction velocities were noted to be diminished somewhat.[28,54] Compound muscle action potentials that are abnormally low or low-normal in amplitude when the patient has maximal neurologic deficits generally are reversed rapidly once the tick is removed.[36] Swift and Ignacio[54] postulated that the major effect of the toxin is to prevent depolarization in the terminal portions of the motor neurons.

Clinical Manifestations

Onset of tick paralysis usually occurs 5 to 7 days after the female tick attaches to the skin. Until mating occurs with the male, engorgement of the female tick is relatively limited. Mating leads to rapid engorgement, fertilization of the eggs, and production of the neurotoxin. In turn, production of neurotoxin coincides with initial paralytic symptoms, and continual feeding accelerates production of toxin, accounting for rapid clinical deterioration. Once the female becomes engorged, she disengages from the skin to deposit her eggs.[22]

Symmetric weakness of the lower extremities progresses to an ascending flaccid paralysis during the course of several hours or days. Sensory function usually is spared, and the sensorium is clear.[53] Alternatively, the disease can present as acute ataxia without muscle weakness.[37] If the paralysis involves the cranial nerves, the patient may have changes in voice and have difficulty in swallowing and handling secretions. Nystagmus, strabismus, and convulsions may occur as well.[28] Temperature elevation generally is not present. After tick removal, symptoms tend to resolve within several hours or days. Left untreated, tick paralysis can be fatal, with reported mortality rates of 10 to 12 percent.[48]

Routine laboratory tests are not helpful in establishing the diagnosis. The white blood cell count, urine analysis, cerebrospinal fluid, and erythrocyte sedimentation rate usually are normal.

Diagnosis

The diagnosis of tick paralysis can be established when the patient shows the typical clinical picture and improves when a tick is removed. A fine-toothed comb can aid in the detection of ticks attached to the scalp. The differential diagnosis of tick paralysis includes Guillain-Barré syndrome, botulism, poliomyelitis, myelitis, spinal cord neoplasm, syringomyelia, and porphyria. Botulism is characterized by slow, descending paralysis involving cranial nerves first, usually with extraocular palsy and large, poorly reactive pupils.[22]

The neurologic impairment caused by *I. holocyclus* ticks in Australia is more severe than that caused by *Dermacentor* spp. in North America. With exposure to *I. holocyclus* neurotoxin, the weakness and bulbar symptoms often intensify during the first 24 to 48 hours after tick removal. Clinical recovery is much slower than with *Dermacentor*-related paralysis. *I. holocyclus* antitoxin must be administered before removal of the ticks, and a longer observation period is required.[22]

Treatment

The earlier the tick is removed in the course of the syndrome, the more promptly the syndrome will clear. Forceps, not fingers, should be used to remove the tick. The tick's head should be gently but firmly grasped, and slow, reverse traction should be applied to remove all body parts. Recovery generally is complete within 1 to 5 days. In one reported case, weakness did not resolve for several months.[16] Intensive supportive care is required if the patient has cranial nerve dysfunction. Ineffective ventilation and ensuing respiratory failure require assisted ventilation.

MYIASIS

Myiasis is the invasion of a host's tissues by the larval stage (maggot) of nonbiting flies. Because of the immaturity of maggots, differentiating the various species is difficult. Myiasis can be classified according to the anatomic site of infestation (i.e., aural myiasis, ophthalmomyiasis, or cutaneous myiasis) or on the basis of the clinical syndrome (i.e., furuncular cutaneous myiasis, migratory cutaneous myiasis, or wound myiasis).[50] In the past, parasitic diseases were limited to their endemic areas. With the relative ease of worldwide travel, however, they are appearing with increasing frequency in the United States and other developed countries.[27]

ETIOLOGY

The true flies of the order *Diptera* undergo metamorphosis in four stages: egg, larva, pupa, and adult. Some larvae of the suborder *Cyclorrhapha* have adapted to a parasitic relationship with humans to different degrees. Some are classified as obligate, facultative, or accidental parasites in humans. In each case, the larval stage of the fly is able to invade the tissues of the host and progress in the stages of metamorphosis.

EPIDEMIOLOGY

The occurrence of human myiasis has been linked to humid and warm climates that favor the breeding of flies. Epizootics in livestock, marginal housing, poor disposal of refuse, and undernutrition also are important factors in the development of human myiasis.[40] In the United States, myiasis has been reported both from flies native to North America and from the larvae of flies acquired during foreign travel. More than 50 species of flies have been reported to cause human myiasis.

PATHOGENESIS

The pathogenesis of human myiasis differs with the degree of parasitic adaptation of each fly. *Dermatobia hominis* (the human botfly) uses a bloodsucking insect as a vector to deposit its eggs on a warm-blooded host. The larvae emerge from the eggs and then penetrate the host's skin, frequently using the puncture site of the carrier insect. The larvae develop within the dermal layer of skin, which leads to a boil-like swelling. During this period, the human host develops clinical symptoms. *D. hominis* and *Cochliomyia hominivorax*, the primary screwworm, are examples causing obligate myiasis.[40] *C. hominivorax* can be responsible for aural or nasal myiasis.

The genus *Sarcophaga* (flesh flies) is capable of causing facultative myiasis. The adult fly is attracted to wounds or ulcers containing purulent and necrotic material. The adult fly deposits eggs in the open wound, where the larvae hatch.

Maggots seldom are found in the human intestinal or urinary tract.[50] Accidental myiasis can occur when humans ingest eggs or larvae and the larvae remain in the intestinal tract. Genitourinary myiasis is thought to occur by the deposition of eggs around the external urethral orifice. The larvae then may migrate into and up the urethra. Such situations should be called *pseudomyiasis*[62] because the maggots are not living parasitically.

CLINICAL MANIFESTATIONS

The lesions of cutaneous myiasis generally are located over the exposed area of the body. Early in the course of cutaneous myiasis, pruritus is the predominant symptom. As the larvae grow after the first week of infestation, a serous exudate may drain from the penetrating site. At this point, pain and pruritus are prominent symptoms and the lesion appears as a small furuncle (furuncular myiasis). Tissue destruction by the larvae may continue, and secondary bacterial infection can occur. *Staphylococcus aureus* and group A streptococci, as well as gram-negative organisms, have been isolated from infected cutaneous myiasis wounds.

Abdominal pain, diarrhea, and anal bleeding are the symptoms of intestinal myiasis, which is self-limited and may last 2 to 6 weeks. Larvae within the genitourinary tract may lead to proteinuria, dysuria, hematuria, and pyuria. Nasal myiasis can extend into bone, sinus cavities, and even the meninges.[3] Aural myiasis has been described in a child without underlying disease.[14] Ophthalmomyiasis is characterized by an acute catarrhal conjunctivitis.[17] Penetration into the brain has been associated with intracerebral hematomas.[35]

DIAGNOSIS

A careful history of travel, occupation, and exposure is necessary to establish the diagnosis when the physician is confronted with unusual skin lesions that are pruritic and have not resolved with usual local care.[26,32] Myiasis is confirmed if larvae are demonstrated within the wound. A parasitologist or entomologist may be able to identify the species of larvae responsible.

TREATMENT

The removal of the larvae is necessary in any of the forms of myiasis. Endoscopic removal of nasal infestation is recommended. Surgical intervention may be required to expose the larvae in the wound. Forceps are used to pick out the larvae; the application of 5 percent chloroform in olive oil may facilitate removal. Occlusive coverings of the wound opening are helpful in extruding *Dermatobia* larvae because this maneuver diminishes the oxygen supply to the larvae. A thick layer of petroleum jelly (Vaseline) effectively interrupts air flow to larvae. Local or systemic antibiotics may be required if secondary bacterial infection is present.

The prevention of human myiasis requires good wound care, adequate personal hygiene, screening to protect against flies, and the prevention of myiasis in domestic animals.

MITES

As mentioned earlier, mites belong to the same class of arthropods (Arachnida) as do ticks, spiders, and scorpions. They, too, can be vectors for infectious agents. The house mite, *Liponyssoides sanguineus*, serves as the vector for rickettsialpox (Chapter 207) agent. Chiggers, which are larvae of mites (family Trombiculidae), transmit to humans the agent responsible for scrub typhus, *Rickettsia tsutsugamushi* (Chapter 207).

Some mites transmit no specific disease but can cause an annoying pruritic rash. In 2004, the microscopic itch mite (*Pyemotes herfsi*) was identified as a likely cause of an intensely pruritic skin reaction in individuals living in several Midwestern states as well as Texas and Oklahoma. Skin lesions were papular and erythematous and occurred primarily on the face, neck, and limbs.[11]

Scabies, an extremely pruritic skin infestation, has been known for more than 2500 years. The etiologic agent for this condition, *Sarcoptes scabiei*, is an obligate human parasite that burrows into the epidermis, no deeper than the stratum granulosum.[12] Scabies appears to have an increased incidence in 15-year cycles[49] and is transmitted person to person through direct and usually prolonged contact.

PATHOGENESIS

Mites of all developmental stages tunnel into the stratum corneum and deposit feces (scybala) behind them. Characteristically, the gravid female lays eggs in the tunnels. Clothing and bed items are thought to be less important in the transmission of the *Sarcoptes* mite. Because scabies is transmitted by skin-to-skin contact, sexual transmission occurs commonly, as does nonsexual spread in family settings.[12] Epidemic outbreaks of scabies have been reported in hospitals and other institutions where people were living closely together and especially where poor sanitation predominated.[4]

Hypersensitivity of both immediate and delayed types has been implicated in the development of lesions other than burrows.[12] Papulovesicular scabies is characterized by perivascular lymphohistiocytic infiltrates with eosinophils. The papillary dermis is edematous. The histologic appearance of nodular scabies is one of a dense, superficial, and deep perivascular lymphohistiocytic infiltrate with many plasma cells and eosinophils. Various vascular changes also may be apparent. Norwegian scabies (crusted scabies) is distinguished by numerous mites that are found in histologic sections of the stratum corneum, and hyperkeratosis is noted.[23] This form of scabies occurs most commonly in debilitated individuals, such as institutionalized retarded children or immunosuppressed children, including children with human immunodeficiency virus (HIV) infection.

CLINICAL MANIFESTATIONS

Scabies is characterized by moderate to severe pruritus that starts several weeks to months after infestation, at which time the host has become hypersensitive to the mite or its products. Itching, most evident at night, is the primary symptom of infection. A papular or vesicular eruption with pustules and linear burrows occurs and classically involves the webs between the fingers, flexures of the arms, axillae, and genital regions. In infants and children, scabetic lesions more typically occur on the palms, soles, head, and neck in the form of vesicles, pustules, or nodules. In this age group, scabies often is not suspected because of the atypical skin lesions that result from vigorous scratching and secondary infections.[31]

Acute glomerulonephritis may be associated with pyoderma in scabies. A careful history may reveal that other family members or child caretakers have pruritus and skin lesions consistent with scabies. Examination of the skin of family members for signs of scabies frequently is helpful. Norwegian scabies may be manifested with a nonspecific hyperkeratosis that may be generalized or localized to the hands or feet rather than with the typical pruritic papules.[18]

DIAGNOSIS

Definitive diagnosis can be made by microscopic identification of the mites, eggs, or mite feces.[32] To obtain this material, skin scrapings should be taken from papules or the end of a burrow or from underneath the surface of fingernails. In some cases, a variety of biopsy techniques can be useful.[7]

The diagnosis should be considered when the physician is faced with an unusual papular or bullous rash. The differential diagnosis of scabies in children includes impetigo, atopic eczema, seborrheic or contact dermatitis, psoriasis, histiocytosis, and chickenpox.[30,31]

TREATMENT

The management of scabies involves the application of a topical scabicide to all areas of skin (except the face) and subsequent removal in 8 to 24 hours, depending on the product applied. Effective scabicides are gamma benzene hexachloride (lindane), permethrin 5 percent, and 10 percent crotamiton.

The agent of choice for infants and young children is permethrin topical cream, which can be applied to the entire head, neck, and body of the infant.[31] All family members should be treated simultaneously.[44] Antibiotics may be necessary if secondary bacterial infection is present.

Ivermectin has proved to be highly efficacious as an oral therapy for scabies in a single dose for otherwise healthy or HIV-infected adults.[41] It works against several different parasites by interrupting GABA-induced neurotransmission.[12] Approval has not been granted for use of ivermectin in children, especially for those weighing less than 15 kg.

Articles of clothing and bed sheets should be machine washed in hot water at 60° C. Insecticidal powder or aerosol should be reserved for materials that cannot be washed.[12] Some evidence exists indicating that gamma benzene hexachloride may not be safe for use in infants and young children because of transcutaneous absorption and subsequent adverse central nervous system effects.[31,38] These adverse effects possibly may be prevented by a careful explanation of the application and removal of lindane after 8 to 12 hours.[16,22] Pruritus often persists for some time after successful treatment with a scabicide and may be relieved by an oral antihistamine or mild to moderate topical steroids.[8,44] In hospitalized patients, contact isolation is recommended to lessen the potential for nosocomial transmission.

Management of the intensely pruritic rash caused by the itch mite (*Pyemotes herfsi*) may include DEET-containing insect repellents to prevent the bites, Calamine lotion, an oral antihistamine, and an over-the-counter topical hydrocortisone preparation.[11]

PEDICULOSIS

Arthropods of the order Anoplura (sucking lice) are important as vectors of rickettsial or spirochetal illnesses. The body louse, *Pediculus humanus humanus*, is the vector for epidemic typhus (*Rickettsia prowazekii*), trench fever (*Bartonella quintana*), and louse-borne relapsing fever (*Borrelia recurrentis*). The body louse, head louse *(Pediculus humanus capitis*), and crab louse (*Phthirus pubis*) all are capable of achieving human infestation. Pediculosis has been a problem for humans for more than 10,000 years.[12]

PATHOGENESIS

Head lice are the most common type of louse. Tiny, they measure no more than 1 to 4 mm in length. They have six legs, each with powerful claws allowing firm attachment. Mouth parts consist of stylets (retracted when not in use) modified for piercing and sucking. When they are not feeding, lice are translucent with grayish white bodies; but when they are engorged with blood, they become red. Each female is capable of laying as many as 300 eggs (nits) in a brief lifetime of 1 to 3 months. These eggs are less than 1 mm in diameter and hatch in 6 to 10 days, giving rise to nymphs. During a period of 10 days, the nymphs become adults. School children of all socioeconomic groups are affected, with head-to-head contact being the most common means of transmission.

CLINICAL MANIFESTATIONS

Children or adults with head lice usually present with white or opalescent nits attached to individual strands of hair. The lice themselves firmly adhere to the base of the hair shaft, millimeters from the scalp. Once a louse pierces the skin, a poisonous salivary secretion is exuded, resulting in pruritic dermatitis. Itching is the primary symptom in acute cases.

Body lice occur when socioeconomic conditions are poor. Infestation occurs when people fail or are unable to wash their clothes regularly, such as those living in refugee camps. Intense itching is a major problem, with noticeable excoriations and occasional secondary infections.

Pubic lice are transmitted primarily through sexual contact, although not exclusively so. In children, pubic lice generally occur from nonsexually transmitted contact with an infected parent. Again, itching is a major presenting symptom. Pubic lice may become attached to other hairy areas of the body, such as the axillae of adolescents, the eyelashes of children, and the scalp hair of any age group.[12]

As mentioned, body lice can function as vectors for the transmission of several diseases. One of these is trench fever caused by *B. quintana*. Although some patients may have no symptoms, others may present with fever, myalgias, headache, meningoencephalitis, transient maculopapular rashes, or chronic adenopathies. Another disease is epidemic typhus caused by *R. prowazekii.* Symptoms of this infection may include fever, headache, rash, or confusion. Relapsing fever caused by *B. recurrentis* is the third group of louse vector–transmitted diseases.[12]

DIAGNOSIS

Head, body, and pubic lice are visibly recognizable. Nits are attached firmly to hair shafts and are distinguished readily from dandruff flakes, lint, and other debris easily removed from the hair. Viable nits, located close to the scalp, are oval and grayish to yellow-white. Empty nonviable nits, attached at some distance from the scalp, are almost completely clear. Although difficult to see with the naked eye, nits visibly fluoresce yellow-green under Wood's lamp. Polymerase chain reaction allows the identification of host DNA from lice through their blood meal, providing valuable information for rape, homicide, and child abuse cases.[39]

The body louse only lately has been demonstrated by polymerase chain reaction detection of *B. quintana* DNA in lice from infected patients as the true vector of trench fever.[6] Detection of rickettsial DNA in lice indicates current human typhus.

TREATMENT

Head Lice

Numerous forms of treatment for the eradication of head lice have been used. Gamma benzene hexachloride (lindane) shampoo was used almost exclusively until the early 1970s. Because of concern about potential neurotoxic effects for the patient, it no longer is recommended as a first-line medication for children. Subsequently, a 1 percent permethrin cream rinse used as a single treatment became the preferred form of therapy. This biodegradable and generally very safe product is recommended to be applied to wet hair and left in place for about 10 minutes before

rinsing. To remove the firmly adherent nits, a fine-toothed stainless steel comb (LiceMeister) has been recommended.

During the course of time, resistance to several forms of therapy has developed. Additional therapeutic intervention has included pyrethrin plus piperonyl butoxide, ivermectin, malathion, trimethoprim-sulfamethoxazole, and smothering agents (including olive oil, margarine, and petroleum jelly). Some success has been reported with use of a 5 percent permethrin preparation and wearing a shower cap overnight.[46]

Malathion, an organophosphate, when it is applied to hair and left in place for 8 to 12 hours, provides residual protection. A combination of 0.5 percent malathion and 78 percent isopropanol has been approved for use by the Food and Drug Administration. Because it is hydrolyzed and detoxified by plasma carboxylesterases much more rapidly in mammals than in insects, it is considered safe.[1] However, it is flammable and is not recommended for use in children younger than 6 months.[12]

Ivermectin has been used when other medications have failed. A dose of 200 μg/kg given on days 1 and 8 appears to be effective. Concern exists when the drug is used in patients weighing less than 15 kg, or those who are pregnant or breastfeeding.[8]

The antibiotic combination trimethoprim-sulfamethoxazole works by destroying essential gut bacteria in the louse. These bacteria, responsible for the production of vitamin B, are essential for life.

A nonchemical approach to treatment has been the application of a topical lotion (Cetaphil Skin Cleanser) to the hair with subsequent use of a blow drier to "shrink-wrap" and ultimately suffocate the lice.[43] Although this approach may result in significant success, the original study was not rigorous enough to prove its effectiveness.

The use of a high-volume, hot air blow drier alone has been reported to have killed 94 to 98 percent of lice eggs and 76 to 80 percent of hatched lice.[25] When examined 1 week later, virtually all of the patients were completely cured. Although it is promising, this approach necessitates further testing by independent investigators. If it is proved to be efficacious, it could eliminate the use of chemicals, to which some lice have become resistant.

Body Lice

A general recommendation has been thorough washing of the body with soap and water followed by the application of a pyrethrin or pyrethroid or malathion for 8 to 24 hours. Again, caution should be taken in the use of these preparations in children. Decontamination of clothing and bed linens is essential by either washing them in hot soapy water at a temperature of 130° F for 10 minutes or placing them in a hot clothes drier for 20 minutes.

Pubic Lice

Treatment should be similar to that used for managing head lice. Clothes and bed linen should be decontaminated. In the case of children, parents also should be treated; and in the case of adolescents, sexual partners require treatment.

BEDBUGS

There are numerous recent reports of the re-emergence of bedbugs (*Cimex lectularius*) as a cause of pruritic, erythematous papules.[55] Although they are known to harbor pathogens (e.g., plague and hepatitis B), bedbugs are considered to be incapable of transmitting these diseases to humans[33,57] and, therefore, are not thought to pose a medical threat. Secondary skin infections, however, can develop as a result of persistent scratching.

PATHOGENESIS AND CLINICAL MANIFESTATIONS

Bedbugs tend to be nocturnally active and are attracted to humans by warm body temperature and the exhalation of carbon dioxide. Survival is dependent on blood extracted from their respective hosts, which is achieved by piercing the skin with their beak-like proboscis containing a set of two hollow tubes. Through one of these tubes, they inject saliva, containing both anticoagulants and anesthetics; through the other one, blood is withdrawn.[55] Although they can live for up to 18 months without feeding, bedbugs typically seek blood every 5 to 10 days.

Total feeding time can range from as short as 5 minutes to as long as 30 minutes, after which the insect abandons the skin surface and seeks seclusion among bed sheets, blankets, mattresses, floorboard cracks, or other crevices.[55] The host generally does not experience itching or skin irritation until several minutes or hours after the insect has fed. In some cases, no skin reaction occurs at all.

DIAGNOSIS

Suspicion for the possible presence of bedbugs occurs when the host awakens with itching and visible bites not present before going to sleep. Clues to the presence of bedbugs include discrete reddish brown bloodstains on sheets and mattresses as well as flecks of excrement at the portals of hiding places.[55] Confirmation requires finding and identifying these bugs.

TREATMENT

Treatment of bites is relatively conservative, directed toward symptomatic relief of itching, irritation, and secondary infection. Antipruritic medication, either topical or oral preparations, may be considered. First-generation antihistamines can help alleviate the itching but may be sedating. Topical corticosteroids can help reduce the erythema and itching. Oral antibiotics alone are sufficient to treat secondary bacterial skin infections. To rid the living environment of bedbugs, a professional exterminator should be consulted.

SPIDERS

Of the thousands of species in the United States, only a few pose a threat to humans. Species of the genus *Loxosceles* are the spiders predominantly responsible for necrotic arachnidism in the United States. The two most common species are the black widow spider (*Loxosceles mactans*) and the brown recluse spider (*Loxosceles reclusa*). Both prefer dark, undisturbed habitats, such as outdoor lavatories, woodpiles, underside of stones, or dark corners of garages and attics. The black widow has a characteristic red hourglass shape on its ventral surface. The brown recluse has a violin-shaped marking over its dorsal surface. In the Pacific Northwest area of the United States, the hobo spider (*Tegenaria agrestis*) produces an envenomation similar to that of the brown recluse spider.[9]

PATHOGENESIS

Venom from the black widow contains a neurotoxin (α-lactotoxin), not a tissue toxin. The main effect is at the presynaptic membrane of the neuromuscular junction. Venom from the brown recluse spider contains sphingomyelinase D, a phospholipase that induces dermal necrosis and also causes system effects through its interaction with red blood cells,

platelets, and endothelium.[47] The necrosis caused by the venom is dependent on neutrophils, but the neutrophils are not activated by the venom itself. Rather, the venom is a potent stimulus for the inflammatory response of endothelial cells, which in turn activates the polymorphonuclear neutrophils to cause tissue destruction.[42]

CLINICAL MANIFESTATIONS

The bite of the black widow spider generally is painless, but a target lesion may develop. Within 30 to 120 minutes, some patients complain of regional lymph node tenderness.[61] The primary symptom that follows the bite is muscle cramping, generally involving the abdomen, chest, or back, depending on the location of the bite. Autonomic symptoms including profuse sweating, nausea, vomiting, and tachycardia may occur.[47] Hypertension can be a significant problem. The degree of systemic symptoms depends on the amount of venom injected and the number of bites. Severe cases may progress to internal hemorrhage, paralysis, and, rarely, even death.

The brown recluse spider bite may produce a mild or sharp stinging sensation. After several hours, the pain intensifies and itching occurs.[2] Eventually, a blister with surrounding erythema forms at the site. In the course of time, the lesion becomes larger, and central necrosis develops. Systemic signs developing in the first 24 to 48 hours may include headache, fever, nausea, vomiting, and joint pain. Disseminated intravascular coagulation, multiorgan failure, and death have been reported in children.[24,60]

Like that of the brown recluse spider, the bite of the hobo spider may result in a central necrotic area. The most common systemic symptom is a severe headache. Protracted systemic effects, including aplastic anemia, intractable vomiting, and profuse secretory diarrhea, are rare occurrences but may be associated with death.[58]

TREATMENT

For the patient who has been bitten by a black widow spider, analgesia is the mainstay of care.[47] An antivenin is available but only for the most severe cases of envenomation that are unresponsive to other measures.[5]

For bites of brown recluse spiders, numerous treatments have been used and include hyperbaric oxygen therapy, early excision, antibiotics, dapsone, and corticosteroids. Careful supportive care, including monitoring of electrolytes and renal function, is required if symptoms are severe.[47] Systemic corticosteroid therapy may be of some benefit if the patient has systemic symptoms. Dapsone, an inhibitor of neutrophil function, appears to decrease the development of wound complications and subsequent need for surgical excision.[45]

SCORPIONS

Scorpions, like ticks, mites, and spiders, belong to the Arachnida class of arthropods.[19] The striped bark scorpion (*Centruroides vittatus*) is the most common type of scorpion found in the United States.[20] The most dangerous and perhaps the most lethal scorpion in this country is *Centruroides exilicauda* (former species name, *sculpturatus*), occasionally referred to as the bark scorpion.[21,47] It is indigenous to the Desert Southwest, particularly Arizona. Depending on the species, scorpions tend to reside in cracks and crevices, in cupboards and closets, or under loose tree bark. They inject their venom when disturbed or threatened.

PATHOGENESIS AND CLINICAL MANIFESTATIONS

C. vittatus produces a neurotoxic venom stored in poison glands in the tip of the tail. The venom of these scorpions is deadly to insect prey and produces a painful sting for humans. For those allergic to the sting, it may produce an anaphylactic reaction.[20] The venom from the *Vaejovis carolinianus* (plain eastern stripeless scorpion) is considered to be mild, similar to that of a honeybee sting. Anecdotal reports indicate that on occasion, a severe reaction may occur.[19]

The *C. exilicauda* scorpion produces a neurotoxic venom that can lead to cardiac failure, respiratory paralysis, agitation, paresthesias, hypersalivation, hypertension, and gastrointestinal symptoms. Although tachycardia and hypertension are frequent reactions, opposite effects are a possibility when the venom activates the parasympathetic nervous system.[47] The absence of local tissue reaction is helpful in distinguishing *C. exilicauda* stings from those of other scorpions found in Arizona.[21] Children and elderly adults are most at risk as a result of envenomation from *C. exilicauda*.[20]

TREATMENT

Most scorpion stings necessitate only nonprescription analgesic medication. For the management of *C. exilicauda* envenomation, atropine can be used to control hypersalivation, although it could be contraindicated when the sting has been produced by scorpions foreign to the United States. In some of those cases, the use of atropine can exacerbate an adrenergic toxicity reaction created by the sting of the scorpion. Treatment of *C. exilicauda* envenomation with an antivenin (available in Arizona only; not approved by the Food and Drug Administration) has been reported to provide relief from the neurotoxic effects. It has the unfortunate potential of causing both immediate and delayed hypersensitivity reactions.[21,47]

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SECTION XXIII

Health Information for International Travel

CHAPTER 242

International Travel Issues for Children

Michelle Weinberg ✪ Susan A. Maloney

During the past half-century, the number of persons traveling internationally has increased tremendously. Between 1950 and 2006, the number of international arrivals worldwide grew from 25 million to 806 million, an annual growth rate of 7 percent. In 2006, travel to Africa increased by 10 percent, more than travel to any other region of the world, whereas travel to Asia and the Pacific was the world's second fastest growing region, with growth of 8 percent. In 2005, more than 38 million trips overseas were taken by U.S. residents. Approximately 55 percent of these trips were to destinations other than Europe or Canada; these destinations included almost 5 million trips to Asia, 5.6 million trips to Mexico, 2.4 million trips to South and Central America, 700,000 trips to Oceania, 500,000 trips to the Middle East, and more than 200,000 trips to the African continent.[148,174]

International travelers may be exposed to a variety of health risks, depending on the travel destination, trip itinerary, duration of stay, planned travel activities (i.e., business versus adventure), individual risk factors, and preventive measures taken. For persons from industrialized countries visiting developing countries, travel may expose them to a variety of infectious pathogens and environmental hazards, some of which they may rarely encounter at home. An estimated 15 to 70 percent of adult travelers report health problems during international travel; 1 to 8 percent of travelers seek medical care during travel, 0.1 to 1 percent are hospitalized abroad, 0.01 to 0.1 percent require emergency evacuation, and 1 in 100,000 dies.[55,142,144]

Infectious diseases are a major cause of morbidity among international travelers. Numerous authors have studied and estimated the incidence of infectious diseases in international travelers.[39,40,45,125,144] The most common illnesses are diarrhea, respiratory tract infections, malaria, and skin infections.[82-84,110,123] In a review of 917 travelers receiving treatment at two Australian Infectious Disease Units for illnesses acquired overseas, 19 percent had malaria, 15 percent had gastroenteritis or diarrhea, and 7 percent had upper respiratory tract infections.[110] Malaria is a potentially life-threatening problem for travelers; an estimated 30,000 North American and European travelers contract malaria annually.[87] Diarrhea has been estimated to occur in as many as 40 percent of persons traveling annually from industrialized to developing countries.[39,40,43,49,81,125,139,144] In a review of patients at GeoSentinel surveillance sites, a network of globally dispersed sentinel clinics providing care for international travelers, 7.8 percent of all infections in returned travelers were respiratory tract infections. Among the 1719 persons with respiratory tract infection, 1100 patients had upper respiratory tract infection, most of which were unspecified upper respiratory tract infection (47.2%) and pharyngitis (13%), and 680 had lower respiratory tract infection, primarily bronchitis (20.3%). Influenza was diagnosed in 96 patients (5.6%). Influenza was associated with travel to the Northern Hemisphere during December through February, travel involving visits to family or relatives, and trip duration of more than 30 days. Lower respiratory tract infections were associated with male sex and increasing age, whereas the risk of pharyngitis, sinusitis, or otitis media was significantly greater in younger persons.[83]

Infectious disease risks vary with destination. In a review of 17,353 travelers who returned ill from 30 GeoSentinel Surveillance Network sites, significant regional differences were detected. Systemic febrile illness without localizing features occurred disproportionately among travelers returned from sub-Saharan Africa or Southeast Asia, acute diarrhea among those returning from south central Asia, and dermatologic problems among those returning from the Caribbean or Central or South America.[55]

Other infectious diseases reported in travelers include parasitic infections, such as amebiasis, cutaneous larva migrans, giardiasis and schistosomiasis, hepatitis A and B, typhoid, sexually transmitted diseases, animal bites with a risk of rabies, cholera, legionellosis, human immunodeficiency virus (HIV) infection, and meningococcal disease.* Approximately 13 percent of the cases of hepatitis A reported in the United States, 42 percent of cases in Switzerland, and 40 percent of cases from Quebec, Canada, are associated with international travel.[24,105,122] Among Swiss travelers, the incidence of hepatitis A in travelers to countries of high or intermediate risk of transmission was 3.0 to 11.0 per 100,000 person-months abroad for all travelers and 6.0 to 28.0 per 100,000 for those presumed to be nonimmune; the highest risk was for children 0 to 14 years of age who were visiting family and friends.[105] In a review of 2445 cases of typhoid fever reported in the United States between 1985 and 1994, 72 percent were associated with international travel within 30 days of onset of illness.[99] Drug-resistant typhoid infection also is associated with international travel. Among 293 persons with symptomatic typhoid fever reported in the United States between 1996 and 1997, 81 percent were associated with international travel; patients with multidrug-resistant *Salmonella typhi* and nalidixic acid–resistant *S. typhi* were more likely to have traveled outside the United States, particularly to the Indian subcontinent (Bangladesh, India, and Pakistan).[1]

Fewer data have been collected on the incidence of noninfectious health problems among adult travelers, but those available suggest that injuries (especially from motor vehicle and sporting accidents and drowning) and complications from preexisting

*See references 10, 14, 48, 56, 65, 78, 80, 89, 98, 99, 103, 118, 130, 135, 140, 143, 144, 151.

medical conditions are substantial causes of serious morbidity and mortality.[113] Noninfectious health problems also are more common causes of mortality among adult travelers than infectious diseases are. In separate studies of deaths in adult international travelers from the United States, Australia, and Scotland, the most common cause of death was cardiovascular disease; among U.S. travelers, cardiovascular disease in men older than 60 years accounted for 50 percent of all deaths.[58,60,114,121,138]

Despite the well-documented health risks associated with international travel, surveys have shown that many travelers do not seek pre-travel health advice or obtain appropriate travel-related preventive medications or vaccinations. Surveys of U.S., European, and Australian travelers have shown that only 31 to 52.1 percent of the travelers sought travel advice before the trip. In an airport survey of 203 U.S. passengers traveling to high-risk, malaria-endemic areas, only 46 percent were carrying antimalarial medications with them,[59,156,162] and few were vaccinated for their travel: only 11 percent for tetanus, 14 percent for hepatitis A, 13 percent for hepatitis B, and 5 percent for yellow fever.[59]

Travelers who are visiting family and friends are at high risk for acquiring a variety of illnesses and are less likely to seek pre-travel medical advice.[6-8,84] In a review of travelers seeking medical care for travel-related illnesses in the GeoSentinel Surveillance Network, only 16 percent of those who were immigrants and were visiting family and friends sought pre-travel medical advice.[84] Systemic febrile illnesses (including malaria), nondiarrheal intestinal parasitic infections, respiratory syndromes, tuberculosis, and sexually transmitted diseases were more commonly diagnosed among immigrants visiting family and friends, compared with other travelers, whereas acute diarrhea was less frequent. Compared with other travelers, the immigrants who were visiting family and friends were more likely to travel to sub-Saharan Africa or Latin America and to take a trip of longer than 30 days in duration.[84] In a review of travelers presenting at one GeoSentinel Surveillance Network clinic site, the proportion of travelers with malaria who had been visiting family and friends was eight times greater than among tourists (16.9% versus 2.1%).[82]

PEDIATRIC TRAVELERS AND HEALTH RISKS ABROAD

Less specific information is available on the number of children traveling internationally or living abroad. By extrapolation from overseas travel data for U.S. residents, a conservative estimate is that at least 2.6 million trips overseas are taken by children traveling overseas annually (i.e., 7% of the 38 million U.S. residents traveling internationally in 2005 reported traveling with children). Information also is more limited on the causes of serious morbidity and mortality among pediatric travelers, and efforts to collect these data should be undertaken to direct prevention measures so that morbidity and mortality rates in pediatric travelers can be decreased.[12,64,120] In a review of 1743 patients in Belgium with fever after international travel, 117 (6%) of the fever cases occurred in children younger than 15 years, and 15 children required hospitalization. Among all patients (adults and children), a definitive diagnosis was established in 76 percent of the cases of fever. Tropical diseases accounted for 39 percent of all causes of fever, and the most common pathogen was malaria.[11] The data available include a 1-year prospective hospital-based study in the United Kingdom of children admitted with fever who had traveled recently to the tropics.[77] In this study, 31 children with a median age of 4 years (range, 5 months to 15 years) met the study entry criteria. Fourteen of these children had nonspecific, self-limited illnesses of presumed viral origin, and 17 children had conditions requiring hospital management and antimicrobial therapy. Conditions requiring hospital management included four cases of malaria (three of *Plasmodium falciparum* malaria and one of *Plasmodium vivax* malaria), three cases of bacillary dysentery, two cases each of dengue and typhoid fever, and one case each of acute hepatitis A infection, pneumonia (unspecified), *Pneumocystis jiroveci* pneumonia (in a child with newly diagnosed HIV infection), bacterial lymphadenitis, streptococcal throat infection, and acute myeloid leukemia; no deaths occurred. In another retrospective study of traveler's diarrhea in Swiss children who had visited the tropics or subtropics, Pitzinger and colleagues[120] found incidence rates of 40, 8.5, 21.7, and 36 percent in children aged 0 to 2, 3 to 6, 7 to 14, and 15 years and older, respectively. Other authors have reported substantial health risks for pediatric travelers from noninfectious causes such as injuries. Most deaths are associated with swimming or automobile accidents.[64,85] One study of British children drowning abroad while traveling showed that an average of eight British children drown each year abroad, primarily in swimming pools without adequate lifeguards.[38]

Although data from adult travelers can be extrapolated to use in the management of pediatric travelers, infants and children have special vulnerabilities and needs when preparing for travel abroad. These needs include (1) up-to-date and appropriate vaccinations (at times through accelerated or altered routine childhood vaccine schedules and also including special travel-related vaccines); (2) appropriate malaria and other chemoprophylaxis regimens tailored for use in pediatric travelers; (3) prevention counseling, particularly in the areas of insect barriers, food and water safety, and injury prevention; and (4) anticipatory guidance for the management of potential illness and successful location of medical resources overseas. This chapter provides recommendations for conducting a pediatric pre-travel assessment to ensure that the child has received the appropriate vaccination and chemoprophylaxis regimens, pre-travel prevention counseling, and means of accessing additional sources of travel information about health risks and the availability of medical resources at specific travel destinations.

GENERAL APPROACH TO PRE-TRAVEL ASSESSMENT FOR CHILDREN

Before embarking on international travel, children should have a pre-travel health assessment performed by a health care provider. The number of physicians who specialize in travel medicine for children is relatively limited, and most pre-travel assessments are performed by general pediatricians or pediatricians who specialize in infectious diseases. The visit should be conducted with the goal of preventing travel-related illnesses. Depending on the destination and the vaccinations recommended or required, the assessment should be conducted optimally up to 6 months before travel. Because families may not be aware of the need for multiple vaccinations, health care providers should be proactive and routinely ask patients and their families if they are anticipating any international travel in the next 6 months, especially before holiday periods.

During the pre-travel assessment, the provider should do the following:

1. Review the child's current and past medical history, including the status of routine childhood vaccinations.
2. Obtain specific details about the travel itinerary and planned activities.

Then, after review of endemic diseases and recent outbreaks in travel destinations:

3. Administer indicated routine and travel-related vaccinations.
4. Prescribe antimalarial and other prophylactic medication (based on risk assessment from the medical history, travel itinerary, and planned activities).

5. Counsel about prevention of travel-related illnesses.
6. Provide guidance about seeking medical assistance for travel-related illness.

The age, immunization status, and medical history of the child are important information to have in conducting the pre-travel assessment. The provider should consider three categories of vaccinations: routine childhood vaccinations, required travel-related vaccinations, and recommended travel-related vaccinations. Some routine vaccinations should be administered at an earlier age or after an accelerated schedule in preparation for international travel. In addition, the provider should review and administer travel-related vaccinations that are required for entry by various countries to prevent the importation of disease and development of outbreaks as well as vaccinations recommended for the prevention of illness in the individual traveler. Currently, only two vaccines, yellow fever and meningococcal, are required for entry by selected countries; however, these requirements may change in the future. In addition, some countries require documentation of HIV testing before allowing entry of long-term travelers (see the section on international travel information resources). Guidelines for standard and accelerated schedules for routine childhood immunizations and for required and recommended travel-related vaccinations by specific travel destinations are summarized in this chapter in the section on vaccination for international travel. A review of medical conditions and contraindications is critical before vaccinations are administered or medications such as antimalarial chemoprophylaxis are prescribed.

The pre-travel assessment should include a detailed review of the trip itinerary. The health care provider should ask about sources of water and food (homes versus hotel restaurants), type of accommodations (e.g., camping, hotel chains, or residing with local families), amount and type of contact with the local population, and planned activities during travel (adventure travel with exposure to animals, water, high altitude, or any combination of these factors). A template of a pre-travel assessment questionnaire for children that can be completed by the family at the visit is shown in Figure 242–1. Recommendations to prevent vector-borne diseases, including malaria, should be determined by a comprehensive review of the travel itinerary and planned activities. Guidance for determining appropriate regimens for antimalarial chemoprophylaxis in pediatric travelers is provided in this chapter in the section on malaria prevention. In addition, the risk of acquiring an infectious disease differs and is probably greater for an adolescent who is participating in a rural home-stay program for 3 months than for a 5-year-old child traveling with parents to a beach resort. Furthermore, expatriates who live abroad and foreign-born persons, especially immigrants, refugees, and their children visiting friends and relatives in their home country, have health risks different from those of short-term travelers who are visiting tourist destinations. These differential risk profiles should be taken into account during the pre-travel assessment because such knowledge is essential for devising a specific pre-travel management plan.

On the basis of risk assessment, preventive health counseling should include information about prevention of vector-borne illnesses, food and water safety, and prevention of other infectious and noninfectious illnesses, such as automobile accidents. General and specific prevention counseling recommendations are addressed later in the chapter. In addition, families should clarify with their health insurance company whether they are covered for health care provided abroad. If not, insurance can be purchased from several companies; such insurance may include coverage for health care providers in a specific country and airlift medical evacuation. If assistance for illness acquired abroad is needed, the U.S. embassy or consulate can provide names and addresses of English-speaking health care providers at the travel destination. This information can be obtained before departure by accessing embassy Internet sites or by calling the embassy. In addition, names of physicians abroad also can be obtained from some worldwide directories (see the section on international travel information resources). Parents should be told to carry all health-related documents with them for easy retrieval, including written prescriptions for medications, health insurance information, and medical contacts abroad. Counseling on ensuring the use of sterile needles and the safety of the local blood supply also should be included in pre-travel prevention counseling topics; blood screening and infection control practices may not be as stringent in some countries as in the United States.

Special consideration must be given to children who have chronic diseases. Children with medical conditions should take a summary of their medical history and treatment record. If the child has cardiac disease, the family should consider taking a copy of the child's recent electrocardiogram and echocardiography report. Children with diabetes who are traveling through more than one time zone should consult their pediatrician or endocrinologist for guidance on the need for altering insulin dosing. Prescription medications should be carried in original bottles, and a sufficient supply for the length of the planned trip should be taken. Required medications should be carried on the plane rather than packed in luggage because bags may be lost or exposed to unfavorable environmental conditions. Families should be counseled about the potential hazards of obtaining medications in pharmacies in developing countries, where many medications can be purchased over-the-counter without a prescription and where the names, content, and concentration of medications may be different from those of U.S. products. Parents carrying medical equipment (such as nebulizers) should remember to take adaptors for foreign electric current and bring a letter from a physician that documents the need for the equipment.[41] Similar documentation is encouraged for anaphylaxis kits that include syringes. Additional information on travel considerations for immunocompromised children is provided later in the chapter.

VACCINATION FOR INTERNATIONAL TRAVEL

Vaccination before international travel is among the most critical and complex components of the pre-travel assessment for children. The pre-travel assessment should include a review of both recommended routine childhood vaccinations and required and recommended travel-related vaccines. Vaccination recommendations change rapidly. Whereas this section provides guidance as of publication date, clinicians should obtain the most current recommendations available on the Centers for Disease Control and Prevention (CDC) Web site (see the section on international travel information resources).

ROUTINE CHILDHOOD VACCINATIONS FOR PEDIATRIC TRAVELERS

For children who will be traveling internationally, having their routine immunizations brought up to date is essential because many vaccine-preventable diseases are more prevalent in developing countries than in the United States. In particular, children who have nonmedical exemptions to routine vaccinations may be at high risk of acquiring vaccine-preventable diseases, such as measles and rubella, that no longer are endemic in the United States but still occur frequently in other countries. The pre-travel visit also may give the health care provider a unique opportunity to update the routine vaccination status of a patient who has fallen behind schedule. In certain cases, routine vaccinations may need to be accelerated to maximize protection, particularly against polio, diphtheria-tetanus-pertussis, and measles (e.g.,

Pretravel Health Consultation

Date of visit: _____

Name: _____

Age: _____ Date of birth: _____

Past medical problems, including any chronic diseases, and recent changes in medical history:

_____

_____

_____

Current medications: _____

Allergies (include any drugs, foods, environmental): _____

Vaccination history		
Routine and travel vaccinations	**Total number of doses**	**Date of last dose**
Bacille Calmette-Guérin (BCG)		
Diptheria, Pertussis, Tetanus (circle most recent vaccine type: DTaP/DPT/DT/dT/Tdap)		
Japanese encephalitis (JE)		
Haemophilus influenzae type b (Hib)		
Hepatitis A		
Hepatitis B		
Hepatitis A-B combination vaccine		
Human papilloma virus (HPV)		
Immune globulin (IG)		
Influenza (oral or injectable)		
Measles, mumps and rubella (MMR)		
Meningococcal, conjugate (MCV4)		
Meningococcal, polysaccharide (MPSV4)		
Pneumococcal, conjugate (PCV)		
Pneumococcal, polysaccharide (PPV23)		
Polio (OPV or IPV)		
Rabies		
Rotavirus		
Typhoid, oral (Ty21a)		
Typhoid, injectable (ViCPS)		
Varicella		
Yellow fever		

Have you ever had an adverse reaction (side effect) to any vaccine? ___ No ___ Yes
If yes, specify vaccine, when it occurred and type of reaction: _____

_____

Figure 242–1 Pre-travel assessment questionnaire for children.

measles vaccination may be recommended for children younger than 12 months).[119] The management plan for routine vaccinations will be determined by the travel destination and itinerary. For example, diphtheria and pertussis are prevalent in eastern Europe and many developing countries, and measles is still endemic in the developing world; therefore, if travel to developing countries is planned, ensuring immunity is imperative, and accelerated schedules should be considered. In addition, parents should check their own immune status because traveling with children can increase their risk of exposure to measles and other vaccine-preventable diseases. Trip activities also may increase the risk of acquiring infectious diseases such as measles; recently, numerous cases of serologically confirmed measles in internationally adopted children and their new parents and siblings who

had traveled to China to accompany them home have been reported. Data suggest that the exposure to measles occurred in China, probably at the orphanage, where an outbreak of measles was occurring.[95,96,104] In 2005, importation of measles by an unvaccinated U.S. traveler led to the largest outbreak of measles in the United States since 1996 and involved 34 confirmed cases. The index case was a 17-year-old student who had a nonmedical vaccination exemption and was working as a missionary in an orphanage in Romania, where a large measles outbreak subsequently was reported.[116] Travel in large groups on conveyances such as cruise ships can facilitate the transmission of infectious and vaccine-preventable diseases.[91] The CDC has provided consultation on managing outbreaks of varicella and meningococcal disease in pediatric travelers on international cruise ships (CDC, unpublished data).

Worldwide polio eradication efforts have decreased the number of countries where travelers are at risk for acquiring polio. In 2005, 1979 cases of wild poliovirus were reported from 16 countries; however, wild poliovirus is considered endemic in only four countries: Afghanistan, India, Nigeria, and Pakistan. These four countries never completely interrupted transmission of wild poliovirus and, through exportation, account for approximately 92 percent of all reported cases of poliomyelitis globally. Nigeria accounts for 65 percent, and India accounts for 25 percent. As of late 2006, eight countries, Angola, Bangladesh, Democratic Republic of Congo, Ethiopia, Namibia, Nepal, Niger, and Somalia, have ongoing transmission after importation.[166,167] In July 2000, an outbreak of vaccine-derived poliovirus type 1 was reported in the Dominican Republic and Haiti.[154] To ensure protection, pediatric travelers visiting countries where polio is epidemic or still endemic should be fully immunized, and clinicians should obtain up-to-date information about areas of transmission from the World Health Organization (WHO) or the CDC (see the section on international travel information resources).

Respiratory tract infections caused by influenza are a major cause of illness among travelers.[83] Influenza can occur throughout the year in tropical countries. Peak influenza rates in temperate regions of the Southern Hemisphere occur from April through September. In North America, influenza vaccine may not be available during the summer months. Although all children between the ages of 6 and 59 months and children older than 6 months with certain risk factors should have routine influenza vaccination annually, some pediatric travelers older than 59 months also should receive influenza vaccine (see Table 242–1). Travelers to the Southern Hemisphere should be vaccinated by the spring, if possible. Influenza vaccinations should be considered for travelers who are at high risk for complications from influenza for travel to (1) tropics any time of year, (2) Southern Hemisphere from April through September, (3) any destination with large groups of tourists at any time of year, or (4) any destination where influenza outbreaks are occurring.[23]

Avian influenza A (H5N1) has become a panzoonotic infection affecting regions of Asia, Africa, and the Middle East. As of December 2006, the WHO had reported 261 human cases and 157 deaths caused by avian influenza from 10 countries. Most of these cases were associated with direct contact with infected poultry.[27,168] The risk to travelers is low. However, clinicians should obtain up-to-date information about affected countries and counsel travelers going to those areas. Persons visiting areas with reports of outbreaks of H5N1 among poultry or of human H5N1 cases can reduce their risk of infection by (1) avoiding direct contact with poultry, including touching well-appearing, sick, or dead chickens; (2) avoiding poultry farms and bird markets; (3) avoiding handling surfaces contaminated with poultry feces or secretions; (4) practicing frequent and careful handwashing; and (5) consuming all foods from poultry, including eggs and poultry blood, only if they have been thoroughly cooked. Because influenza viruses are destroyed by heat, the cooking temperature for poultry meat should be 74° C (165° F).[27]

Hepatitis A, hepatitis B, *Haemophilus influenzae* type b, rotavirus, *Streptococcus pneumoniae*, and varicella are endemic in many developing countries, and vaccination should be ensured, particularly for young pediatric travelers to these regions. For detailed information about accelerating routine childhood vaccinations and indications for use in pediatric travelers, see Table 242–1.

COMMON TRAVEL-RELATED VACCINES FOR CHILDREN

An important consideration in determining travel vaccination needs is "required" versus "recommended" vaccinations. The most recent travel requirements and recommendations for vaccinations can be obtained from the CDC Travelers' Health Internet site (see the section on international travel information resources). Few vaccinations are required. The United States does not require arriving travelers to have any vaccinations for entry or return to the United States. However, immigrants, refugees, asylum seekers, and internationally adopted children migrating to the United States for permanent residence have different requirements. Some other countries may require proof of vaccination against yellow fever for entry, especially if the traveler is arriving from a country where yellow fever is present. Yellow fever vaccine is available only from certified yellow fever vaccination centers; providers can contact their state public health department to locate certified centers in their areas. Saudi Arabia requires meningococcal vaccine for travelers visiting Mecca for the Hajj or for the Umrah.[18,171] Some countries previously have required cholera vaccination, but currently no countries require vaccination as a condition of entry.[171] However, non-U.S. customs officials who "unofficially" request proof of vaccination have been reported. Cholera vaccine is not available in the United States.

Tables 242–1 and 242–2 provide general guidelines and indications for the use of routine and selected travel-related vaccines based on U.S. recommendations.[17,18,25,66,67,119] WHO recommendations may differ.[171] Depending on the complexity of the travel itinerary and the patient's medical history, the vaccination plan may need to be developed in consultation with a travel medicine or infectious disease specialist. Travel-related vaccine recommendations should be tailored carefully to the trip itinerary and based on the travel destination, season when the travel will occur, duration of the trip, and activities to be undertaken. For example, hepatitis A is endemic in most of the world, and travelers are at risk in any area where sanitation is poor. In 2006, hepatitis A vaccination became part of routine childhood recommendations in the United States for all children at 1 year of age (12 through 23 months), supplementing recommendations in 1999 for vaccination of children 2 to 18 years of age residing in states and communities with high incidence of disease and other high-risk groups. Vaccination is recommended for pediatric travelers aged 1 year or older who will be visiting countries with intermediate to high endemicity, such as Mexico, Central and South America, Asia (except Japan), Africa, and eastern Europe.[17,24,25] Intramuscular immune globulin is recommended for immunoprophylaxis against hepatitis A in children younger than 1 year. In addition, for children 1 year of age or older who will be departing sooner than 4 weeks after the pre-travel visit (and therefore may not have sufficient time for immunity to develop after hepatitis A vaccination), both immune globulin and vaccine can be given concurrently at different sites to ensure more immediate protection. Shortages of immune globulin have occurred recently, so obtaining it for hepatitis A prophylaxis may be difficult. In practice, some clinicians choose to administer hepatitis A vaccine rather than immune globulin to children who have 14 days but less than 1 month before their departure. Limited data are available about the timing of the appearance of neutralizing antibody. Among a

TABLE 242–1 Routine Childhood Vaccinations and Accelerated Schedules and Modifications for Pediatric Travelers

Vaccine	Minimum Age	Routine Schedule*	Accelerated Schedule and Modifications†	Indications for Use and Other Information‡
DTaP, DTP, DT	6 weeks	5 doses at ages 2, 4, 6, 15-18 months and 4-6 years	5 doses MI from dose 1 to dose 2: 4 weeks MI from dose 2 to dose 3: 4 weeks MI from dose 3 to dose 4: 6 months Minimum age for dose 4: 12 months MI from dose 4 to dose 5: 6 months Minimum recommended age for dose 5: 4-6 years Dose 5 is not necessary if dose 4 was administered after the 4th birthday	Use for children 6 weeks to 7 years of age. Use DT for children <7 years of age for primary series (instead of DTaP or DTP) when pertussis vaccination is contraindicated.
Tdap	11 years	One dose		Only 1 dose of Tdap is recommended. All subsequent doses of tetanus-diphtheria booster should be administered as Td. If vaccination to prevent tetanus or diphtheria disease is required for children 7-9 years old, Td should be administered. The preferred interval between Tdap and a previous dose of Td is 5 years.
Td	7 years	1 booster dose every 10 years Children ≥11 years should receive 1 dose of Tdap, followed by booster doses of Td every 10 years.	MI for booster for travel: 5 years	Td should be used rather than Tdap if Tdap is not available and for: Anybody who has already received Tdap Children 7-9 years of age See information for Tdap regarding timing of Td versus Tdap.
Haemophilus influenzae type b (Hib)	6 weeks	4 doses at ages 2, 4, 6, and 12-15 months *or* 3 doses at ages 2, 4, and 12-15 months (PRP-OMP vaccines only) Unvaccinated child >15 months: 1 dose	Accelerated schedule is available but varies by vaccine brand; see package insert for vaccine used. MI between doses: 4-8 weeks	Minimum interval between doses depends on age. Children receiving the first dose of vaccine at age ≥7 months require fewer doses to complete the series. See M. M. W. R. Morb. Mortal. Wkly. Rep. *54*:Q1-Q4, 2005, for indications. Vaccinate all children <5 years of age and children >5 years of age with special indications (e.g., asplenia, immunodeficiency).
Hepatitis A	1 year	2 doses at ages 12 and 18 months	MI from dose 1 to dose 2: 6 months	Recommended for all children at 1 year of age (12-23 months). Recommended for (1) international travelers to Mexico, Central and South America, Asia (except Japan), Africa, and eastern Europe; (2) all children residing in states, counties, and communities with existing hepatitis A vaccination programs based on high disease incidence and other high-risk groups.§ Immunogenicity: limited data are available for the timing of the appearance of neutralizing antibody. Among a sample of vaccinated persons, 54-62% were positive for neutralizing antibody 14 days after the first dose, and 94-100% were positive at 1 month.‡

TABLE 242–1 Routine Childhood Vaccinations and Accelerated Schedules and Modifications for Pediatric Travelers—cont'd

Vaccine	Minimum Age	Routine Schedule*	Accelerated Schedule and Modifications†	Indications for Use and Other Information‡
Hepatitis B¶	Birth	3 doses at ages 0-2, 1-4, 6-18 months For children 11-12 years: 3 doses at 0-, 1-, and 4-month intervals For unvaccinated adolescents 11-15 years: 2-dose vaccine series (only Recombivax HB, adult dose) at 0, 4-6 months or 3 doses of other vaccine brands as for children 11-12 years	3 doses (except 2 doses of Recombivax HB, adult dose for adolescents) MI from dose 1 to dose 2: 4 weeks MI from dose 2 to dose 3: 8 weeks MI from dose 1 to dose 3: 16 weeks Minimum age for dose 3: 6 months	Use for travelers to endemic areas, including Africa, Asia, Latin America, who plan to: Travel abroad for ≥6 months Have intimate or sexual contact with local population Work in health care setting or may have contact with blood, blood products, or body fluids Count all doses; do not restart series even if time lapse between doses is greater than recommended interval. **Accelerated schedules that may be associated with decreased immunogenicity:** 4 doses at 0, 1, 3 weeks and 6-12 months *or* 4 doses at 0, 7, 21 days and booster at 1 year
Human papillomavirus vaccine (HPV)	9 years Approved only for female patients 9-26 years	3 doses at 11-12 years at 0-, 2-, and 6-month intervals	Recommended interval dose 1 to dose 2: 2 months MI dose 1 to dose 2: 4 weeks Recommended interval dose 1 to dose 3: 6 months Recommended interval dose 2 to dose 3: 4 months MI dose 2 to dose 3: 3 months	
Influenza, trivalent inactivated vaccine (TIV) or live attenuated vaccine (LAIV)	TIV: 6 months; however, check product as minimum age varies by vaccine manufacturer LAIV: 2 years, approved for use only for persons aged 2-49 years	Recommended annually for healthy children aged 6-59 months and children aged >59 months with certain risk factors. See M. M. W. R. Recomm. Rep. *55*(RR-10):1-48, 2006. Children ≤8 years of age who are receiving influenza vaccine for first time: 2 doses Children >8 years of age and persons who previously received influenza vaccine: 1 dose	TIV: MI dose 1 to dose 2: 4 weeks LAIV: MI dose 1 to dose 2: 6 weeks	Influenza can occur throughout the year in tropical countries. Peak influenza in temperate regions of the Southern Hemisphere occurs from April through September. In North America, influenza vaccine may not be available during the summer months. Travelers to the Southern Hemisphere should be vaccinated by the spring, if possible. Consider for travelers who are at high risk for complications from influenza for travel to Tropics any time of year Southern Hemisphere from April through September Any destination with large groups of tourists at any time of year Any destination where influenza outbreaks are occurring
Measles, mumps, rubella (MMR)	6 months	2 doses at ages 12-15 months and 4-6 years	Children 6-11 months: 1 dose, then revaccinate at 12-15 months of age and 4-6 years of age Children >12 months vaccinated with 1 prior dose: 1 additional dose at least 4 weeks after dose 1 Unvaccinated children >12 months: 2 doses separated by 4 weeks MI between doses: 4 weeks	Do not administer MMR for 3 months after immune globulin for hepatitis A prophylaxis; see M. M. W. R. Morb. Mortal. Wkly. Rep. *54*:Q1-Q4, 2005, for deferral times for blood products and immune globulin administered for other indications. Defer immune globulin administration for 2 weeks after MMR vaccination. Monovalent vaccines are available for measles, mumps, and rubella. Combination MMRV (measles-mumps-rubella-varicella vaccine) can be used for children 12 months-12 years.

TABLE 242–1 Routine Childhood Vaccinations and Accelerated Schedules and Modifications for Pediatric Travelers—cont'd

Vaccine	Minimum Age	Routine Schedule*	Accelerated Schedule and Modifications†	Indications for Use and Other Information‡
Meningococcal (quadrivalent A, C, Y, W135) Conjugate vaccine (MCV4) Polysaccharide vaccine (MPSV4)	MCV4: 11 years MPSV4: 3 months for serogroup A protection, standard recommended minimum age: 2 years (see indications)	MCV4: routine vaccination, 1 dose for children at age 11-12 years MCV4: 1 dose for high-risk populations and selected travelers ≥2 years MCV4 for children >2 years	MCV4: no booster dose recommended MPSV4: every 3-5 years for high-risk persons¶	Meningococcal vaccination is required for travelers to annual Hajj in Mecca, Saudi Arabia. Vaccination is recommended for travel to sub-Saharan Africa during dry season (December through June) or for travel to any countries where epidemic is occurring. Recommended for all children at age 11-12 years, unvaccinated adolescents at high-school entry (15 years of age), all college freshmen living in dormitories, and certain high-risk children ≥2 years of age with terminal complement deficiencies or anatomic or functional asplenia and certain other high-risk groups. See M. M. W. R. Recomm. Rep. *54*(RR-7):1-21, 2005. For children ≥3 months of age traveling to risk areas (sub-Saharan Africa): Serogroup A meningococcal polysaccharide vaccine, given as MPSV4, can be immunogenic in children as young as 3 months. Responses to other serogroup components are poor or unknown in children ≤2 years of age. For children younger than 18 months, it is administered as 2 doses separated by a 3-month interval. See *Red Book 2006*.
Pneumococcal conjugate 7-valent (PCV)	6 weeks	4 doses at ages 2, 4, 6, and 12-15 months	MI between doses: 4-8 weeks, depending on age Number of doses varies with age at first dose administered and current age.	Minimum intervals between doses depend on age. Children receiving the first dose of vaccine at age ≥7 months require fewer doses to complete the series. See M. M. W. R. Morb. Mortal. Wkly. Rep. *54*:Q1-Q4, 2005. Pneumococcal polysaccharide vaccine (PPV23) is routinely recommended for high-risk persons aged ≥2 years. See M. M. W. R. Morb. Mortal. Wkly. Rep. *54*:Q1-Q4, 2005.
Polio-inactivated poliovirus vaccine (IPV)	6 weeks	4 doses at ages 2, 4, 6-18 months and 4-6 years	Previously unvaccinated children: 3 doses MI between doses: 4 weeks Preferred interval between dose 2 and dose 3: 2 months Minimum age for dose 2: 10 weeks Minimum age for dose 3: 14 weeks Minimum age for dose 4: 18 weeks Previously unvaccinated children <4 years at completion of dose 3: 4 doses Previously unvaccinated children ≥4 years at completion of dose 3: 3 doses Previously vaccinated adult (≥18 years): 1 booster recommended for travel to risk area MI between doses: 4 weeks	Primary series is recommended for all travelers; booster dose for adults is recommended for international travelers to Africa or Asia; not routinely recommended for travel to Latin America except to Haiti or Dominican Republic, where an outbreak occurred in 2001. Single booster of IPV for previously vaccinated adults confers lifetime immunity. Oral poliovirus vaccine (OPV) is no longer recommended or available in the United States. For use in other countries, the World Health Organization recommends birth as the minimum age of administration. The minimum interval between doses is 4 weeks. OPV can be used for children younger than 6 weeks traveling to areas with polio transmission.

TABLE 242–1 Routine Childhood Vaccinations and Accelerated Schedules and Modifications for Pediatric Travelers—cont'd

Vaccine	Minimum Age	Routine Schedule*	Accelerated Schedule and Modifications†	Indications for Use and Other Information‡
Rotavirus (RV)	6 weeks	3 doses at ages 2, 4, 6 months	First dose must be administered at age 6-12 weeks Maximum age of any dose: 32 weeks MI between doses: 4 weeks Minimum age for dose 2: 10 weeks Minimum age for dose 3: 14 weeks	Vaccine series should not be started at age 13 weeks. RV should not be administered to children aged 33 weeks regardless of the number of doses received at age 6-32 weeks. If possible, defer rotavirus vaccination for 6 weeks after receipt of antibody-containing product such as immune globulin. However, if the 6-week deferral would cause the first dose of RV to be scheduled for age ≥13 weeks, a shorter deferral should be used to ensure that the first dose of RV is administered no later than 13 weeks.
Varicella	12 months	2 doses at 12-15 months of age and 4-6 years	MI between dose 1 and dose 2 for children <13 years: 12 weeks MI between dose 1 and dose 2 for persons ≥13 years: 4 weeks Minimum age for dose 2: 15 months	Do not administer varicella vaccine for 3 months after immune globulin for hepatitis A prophylaxis; see M. M. W. R. Morb. Mortal. Wkly. Rep. *54*:Q1-Q4, 2005, for deferral times for blood products and immune globulin administered for other indications. Defer immune globulin administration for 2 weeks after varicella vaccination. Combination MMRV (measles-mumps-rubella-varicella vaccine) can be used for children aged 12 months-12 years.

**Number of doses and recommended ages. Minimum age is the U.S. recommendation unless otherwise noted. Recommendations in the U.S. schedule may be different from those of the World Health Organization and other countries.*

†Number of doses and minimum interval (MI) between doses. Note: interval represents time to dose.

‡Contraindications: anaphylactic reaction to prior dose or to any vaccine component or moderate to severe acute illness is a contraindication for all vaccines.

§See M. M. W. R. Recomm. Rep. 55(RR-7):1-23, 2006.

¶Combination hepatitis A–hepatitis B (TWINRIX) is available for use in persons 18 years of age and older. The vaccine, which is composed of inactivated viral components, is administered intramuscularly. The schedule involves 3 doses at 0, 1, and 6 months. It can be administered at an accelerated schedule of 4 doses at 0, 7, and 21 days and 1 year.

¶A second dose of meningococcal vaccine is recommended for persons previously vaccinated with MPSV who remain at high risk for meningococcal disease. MCV4 is preferred for revaccination of persons aged 2 to 55 years, but a second dose of MPSV is acceptable. Certain experts recommend a second dose of MPSV 3 years after the first dose for persons at increased risk for meningococcal disease.

sample of vaccinated persons, 54 to 62 percent were positive for neutralizing antibody 14 days after the first dose, and 94 to 100 percent were positive at 1 month.[24] If the first dose of hepatitis A vaccine is administered less than 30 days before departure, patients should be counseled that they may not be fully protected against hepatitis A virus and that they should take extra precautions with food and water to reduce exposure risk (see Table 242–4).[24]

Meningococcal disease occurs sporadically worldwide. Epidemic disease has been reported in India, Saudi Arabia, and sub-Saharan Africa; indeed, recurrent epidemics of meningococcal disease occur in sub-Saharan Africa, mainly from December to June (the dry season). Serogroup A is the most common cause of epidemics outside the United States, but serogroup C and other serogroups also have been associated with epidemics. Serogroup W-135 meningococcal infection has been reported recently in travelers returning from Saudi Arabia after visiting the Hajj.[151] Two meningococcal vaccines, a polysaccharide vaccine (MPSV4) and a conjugate vaccine (MCV4), are available in the United States. Both vaccines are quadrivalent and effective against serogroups A, C, Y, and W-135. MCV4 is expected to provide better, longer lasting protection. In the United States, routine MCV4 vaccination is recommended for all children at the 11- to 12-year-old visit, unvaccinated adolescents at high-school entry (15 years of age), and college freshmen living in dormitories. Meningococcal vaccination is recommended for pediatric travelers 2 years of age or older who are visiting sub-Saharan Africa during the dry season or any country where an epidemic caused by a vaccine serogroup is occurring. MCV4 is the preferred vaccine for children 2 years of age and older. MCV4 is also recommended for children 2 to 10 years old who are at risk. Although it is less immunogenic than when it is administered to older children and adults, MPSV4 also can be used in children as young as 3 months to 2 years of age to provide protection against serogroup A, such as travelers to areas with an epidemic or during seasonal transmission. These children should have two doses, 3 months apart.[22,25,120]

Yellow fever occurs year-round in the predominantly rural areas of sub-Saharan Africa and South America; however, outbreaks have been increasing in incidence, particularly in Africa.[173] The resurgence of yellow fever in Brazil has raised concern about increased risk in other areas of Latin America and the possibility of urban yellow fever transmission.[157,158] Although a rare disease, yellow fever continues to be reported in travelers, particularly unvaccinated travelers, and it usually is fatal. Preventive measures taken against yellow fever should include the use of personal protection against mosquitoes and vaccination. As discussed previously, some countries require yellow fever vaccination for travelers arriving from endemic regions; current requirements and recommendations for vaccination based on travel destination can be obtained from the CDC Travelers' Health Internet site. Yellow fever vaccine is largely considered to be a safe and effective vaccine. However, the vaccine has been found to be associated with an increased risk for development of encephalitis and other severe reactions in young infants.[119] The vaccine should not be used in children younger than 6 months. It should be used with caution in children 6 to 9 months of age after discussion with a travel medicine expert to weigh the risks and benefits.[18] Medical waivers can be given to children who are too young for vaccination and to those who have other contraindications to

TABLE 242–2 Common Travel-Related Vaccines and Immune Globulin for Children

Vaccine*	Vaccine Type	Route	Minimum Age	Primary Series†	Booster or Revaccination‡	Accelerated Schedule	Protection After	General Indications for Use§
BCG	Live attenuated bacteria	ID, SC	Birth	1 dose	None	None	2 months after dose (WHO)	Consider for children <1 year who will be long-term travelers residing in high-risk areas to protect against meningeal and miliary tuberculosis.
Immune globulin (IG)	Antibody from pooled human plasma	IM	Birth	1 dose	3-6 months	None	Immediate	Use to prevent hepatitis A infection in children <2 years of age who are traveling to risk areas or for persons departing within 4 weeks. Defer MMR and varicella vaccination for 3 months after IG administered for hepatitis A prevention; defer the administration of IG for hepatitis A prevention for 2 weeks after MMR or varicella vaccination. Defer rotavirus vaccine (RV) administration for 6 weeks if possible. However, if 6-week deferral would cause the first dose of RV to be scheduled for age ≥13 weeks, a shorter deferral interval should be used to ensure that the first dose of RV is administered no later than age 13 weeks. Defer immune globulin administration for 2 weeks after MMR or varicella vaccination. See M. M. W. R. Morb. Mortal. Wkly. Rep. *54*:Q1-Q4, 2005.
Japanese encephalitis	Inactivated virus	SC	1 year	3 doses at 0, 7, and 14 or 30 days	2-3 years Booster: 1 dose	None	10-14 days after dose 3	Use for travelers to selected areas of Asia, Oceania (Australia and Papua New Guinea), Russia, especially long-term travelers to rural, agricultural endemic areas or epidemic regions during seasonal transmission (usually May to September). Allergic reactions (urticaria, angioedema, respiratory distress, anaphylaxis) occur in approximately 0.6% of persons. Anaphylaxis can occur up to 10 days after vaccination; therefore, all doses of vaccine should be administered at least 10 days before departure. Persons vaccinated should remain in areas with access to health care. Observe for 30 minutes after vaccine administration and counsel about possible delayed hypersensitivity reactions. Persons with history of urticaria are at greater risk for development of allergic reactions. Mild adverse reactions (headache, fever, local reactions) occur in approximately 20% of vaccinees.

Rabies Human diploid cell vaccine (HDCV) Rabies vaccine adsorbed (RVA) Purified chick-embryo cell culture vaccine (PCEC)	Inactivated virus; cell culture derived	IM, ID (HDCV only)	US: None WHO: 1 year	3 doses at 0, 7, and 21 or 28 days	6 months-3 years, depending on risk or serologic tests Continuous risk: check antibody titer every 6 months Frequent exposures: check antibody titer after 2 years WHO: 1 year after primary series, then every 2-3 years Booster: 1 dose	None	14 days after dose 3	Use for travelers to rabies-endemic countries. Vaccination is recommended for long-term travel in rural areas or in areas with limited access to health care. Travelers with extensive unprotected outdoor, evening, and nighttime exposure in rural areas, such as backpacking, should consider vaccination even for trips of short duration. Widespread worldwide rabies distribution. See Health Information for Travelers for list of countries that have not reported cases of rabies recently. Complete intradermal rabies vaccine series at least 7 days before starting chloroquine or mefloquine. Postexposure vaccination is required even for persons who receive vaccination before exposure. After exposure in vaccinated person: 2 doses at 0, 3 days (no rabies immune globulin required) After exposure in unvaccinated person: rabies immune globulin (20 IU/kg) plus vaccination with 5 doses at 0, 3, 7, 14, and 28 days
Typhoid (ViCPS)	Capsular polysaccharide	SC	2 years	1 dose	2 years	None	14 days after dose	Use recommended for travelers to Africa, Asia, and Latin America for long-term stays, travel outside usual tourist destinations, or travelers who desire maximal protection. Patients should be counseled about protective efficacy of vaccine (approximately 50-74% when studied in endemic areas) and importance of other preventive measures.
Typhoid oral (Ty21a)	Live attenuated bacteria	Oral	6 years	4 doses at 0, 2, 4, 6 days	5 years	None	7-10 days after last dose	See indications for typhoid ViCPS. Patients should be counseled about protective efficacy of vaccine (approximately 60-85% when studied in endemic areas) and importance of other preventive measures. Must be swallowed (cannot be chewed) 1 hour before meal, and vaccine must be refrigerated until administration. Do not administer within 24 hours of mefloquine, atovaquone-proguanil, doxycycline, or any other antibiotic. Contraindicated for immunocompromised patients
Yellow fever	Live attenuated virus	SC	4 months	1 dose	10 years	None	10 days after dose	Use for travelers to selected areas of Africa and South America. Yellow fever has not been reported in Asia; however, some countries require proof of vaccination if the traveler is arriving from a yellow fever–endemic country. Yellow fever vaccination for children 4-9 months should be discussed with an expert to weigh risk of adverse events against risk of yellow fever. Reports of rare serious adverse events of multiorgan system failure after vaccination. Contraindicated for patients who are immunocompromised or have allergy to eggs.

**Cholera vaccine is no longer available in the United States. It is administered as a 2-dose series at 1- to 4-week intervals. It had limited efficacy and was associated with frequent adverse effects. Tick-borne encephalitis vaccine is not available in the United States. It is administered as a 4-dose series at 0, 28-day, 42-day, and 1-year intervals, with booster doses required every 3 to 5 years. The vaccine is available in Europe.*

†Number of doses and recommended ages and intervals. Note: interval represents time from dose.

‡Number of doses and recommended ages and intervals.

§Contraindications: anaphylactic reaction to prior dose or to any vaccine component or moderate to severe acute illness is a contraindication for all vaccines.

BCG, bacille Calmette-Guérin; MMR, measles, mumps, rubella; WHO, World Health Organization.

vaccination, such as immunodeficiency. More recently, life-threatening severe illness with major organ system failure has been reported in association with yellow fever vaccination. The syndrome usually consists of fever, jaundice, and multiple organ system failure, and more than half of the people who have developed these adverse effects have died. The risk is thought to be approximately 1 per 200,000 to 300,000 doses in persons younger than 60 years and 1 per 40,000 to 50,000 doses in persons 60 years of age and older. The risk is greater in persons with a history of thymectomy, thymoma, myasthenia gravis, or DiGeorge syndrome.[30] Further studies are being conducted.[33,92,157] In the interim, the CDC has recommended that given the risk of serious illness and death from yellow fever,[94] evidence of increasing transmission of the disease,[129] and the known effectiveness of the vaccine, clinicians should continue to use yellow fever vaccine to protect travelers.[18] Health care providers should carefully review travel itineraries to ensure that only people traveling to areas endemic for yellow fever or areas where yellow fever activity is reported receive yellow fever vaccine.[18,50,52,171]

Japanese encephalitis (JE) is a viral infection transmitted by *Culex* mosquitoes that bite from dusk to dawn. JE occurs year-round in tropical regions and primarily from May through October in temperate zones. The risk is greatest for travelers to rural Asia, where the mosquito breeds in rice fields and other agricultural areas. JE is associated with high rates of case fatalities and severe neurologic sequelae, especially in young children and the elderly. Vaccination should be considered for pediatric travelers 1 year of age or older who will visit and reside in areas where JE is endemic or epidemic, especially during the transmission season, or for pediatric travelers whose activities include trips to rural farming areas. Short-term travelers (<30 days) who visit only major urban areas are at lower risk for acquiring JE and generally do not need to be vaccinated.[18]

Rabies occurs worldwide except in Antarctica. In certain areas of the world, including (but not limited to) parts of Brazil, Bolivia, Colombia, Ecuador, El Salvador, Guatemala, India, Mexico, Nepal, Peru, the Philippines, Sri Lanka, Thailand, and Vietnam, canine rabies remains highly endemic. Rabies also occurs in other wild animals, including bats. Rabies vaccine should be considered for children visiting rabies-endemic countries for longer than 1 month, those undertaking extensive outdoor activities such as backpacking or camping in endemic countries, or children traveling to areas where access to health care is limited. To reduce the risk of acquiring rabies, children and their families should be counseled to stay away from stray dogs and other animals, especially if traveling to Latin America, Asia, or Africa.[18]

Typhoid vaccine is recommended for pediatric travelers visiting developing countries, especially for prolonged periods, or traveling outside the usual tourist destinations. Two vaccines are available in the United States: (1) an attenuated, live oral vaccine that can be administered to children aged 6 years and older and (2) an injectable Vi capsular polysaccharide vaccine that can be administered to children, starting at 2 years of age. Parents should be cautioned, however, that vaccination is not 100 percent effective, and precautions regarding safe food and water should be followed.[18]

Finally, bacille Calmette-Guérin (BCG) vaccine is a live vaccine prepared from attenuated strains of *Mycobacterium bovis;* BCG is used primarily in young infants to prevent disseminated and other forms of life-threatening disease caused by tuberculosis, such as tuberculous meningitis. BCG is recommended by the WHO for administration at birth; in the United States, BCG is recommended in only limited circumstances, such as unavoidable risk of exposure to *Mycobacterium tuberculosis.* Vaccination of a young pediatric traveler (non–HIV infected and with a negative tuberculin skin test response) might be considered, therefore, if long-term stay (such as children of missionaries or expatriates) is planned in a country with a high prevalence of tuberculosis and prolonged contact with active tuberculosis cases is thought to be a potential problem.[3,119,146] A rate of tuberculin skin test seroconversion of 1.8 percent was reported among travelers to areas endemic for tuberculosis.[34] More recently, 10 percent of 357 travelers to the Hajj for pilgrimage had evidence of seroconversion when tested with use of QuantiFERON, a whole-blood assay for tuberculosis antigens.[161] Multidrug-resistant tuberculosis has been increasing in incidence worldwide. Cases of extensively drug-resistant tuberculosis have been reported from several countries, including Estonia, Latvia, Lesotho, Peru, the Philippines, South Africa, Swaziland, and the United States.[169,170] From 2000 to 2004, a survey of an international network of tuberculosis laboratories determined that of 17,690 isolates, 20 percent were multidrug resistant and 2 percent were extensively drug resistant.[47] BCG vaccine can be obtained from the Canadian subdivisions of Sanofi Pasteur *(http://www.sanofipasteur.ca)*. More generally, children traveling to countries with a high prevalence of tuberculosis should be skin tested before and after travel to document possible exposure to tuberculosis. According to reports, U.S. children who had traveled to countries with a high prevalence of tuberculosis within the previous 12 months were 3.9 times more likely to have positive tuberculin skin test results than were children who lived in the same U.S. areas but had not traveled.[86] Additional information about preventing other infectious diseases is provided in this chapter in the section on preventing other infectious diseases in pediatric travelers.

One limitation to performing adequate travel-related vaccination is that patients may not seek medical assessment in sufficient time to complete vaccination and acquire protective immunity. Providers should ask patients routinely whether they are planning any travel in the future, particularly in the months before holidays and summer vacations. Multiple doses are required for some travel-related vaccines. In addition, other time-related vaccination issues also should be considered; for example, a patient who receives JE vaccine should complete the series of three doses at least 10 days before travel so that the patient can be observed for delayed allergic reactions.

PREVENTION OF MALARIA

EPIDEMIOLOGY OF MALARIA

Malaria is a parasitic infection caused by one of four species of *Plasmodium: Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium ovale*, or *Plasmodium malariae*. It is transmitted by the bite of an infective female *Anopheles* mosquito. Malaria is endemic in more than 100 countries on five continents; transmission occurs primarily in the tropic and subtropical regions of sub-Saharan Africa, Asia, Latin America, the Caribbean, the Middle East, and Oceania. Globally, malaria is responsible for 300 to 500 million clinical infections and 1.5 to 2.7 million deaths annually, with most caused by *P. falciparum*, principally in young children and pregnant women.[172]

An estimated 10,000 to 30,000 travelers from industrialized countries are thought to contract malaria each year, but these numbers probably are underestimated because they do not include travelers in whom malaria was diagnosed and treated abroad.[70,71,87] Of 5794 cases of malaria in U.S. civilians reported to the CDC from 1992 through 2000, 976 (17%) of the cases occurred in children younger than 18 years. Among children with malaria, 343 (35%) were 1 month to 5 years old, 215 (22%) were 6 to 9 years old, 226 (23%) were 10 to 14 years old, and 192 (20%) were 15 to 17 years old (CDC, unpublished data).

In 2003, 1268 imported malaria cases and seven deaths due to malaria among persons in the United States were reported to the CDC. Of 1201 imported cases in which the region of acquisition was known, the majority (70.0%) were acquired in Africa, 14.7

percent were acquired in Asia, 12.3 percent were acquired in the Americas, and 3.1 percent were acquired in Oceania. *P. falciparum* species was identified in 53.4 percent and *P. vivax* was identified in 22.9 percent of infected persons, with the species identified in blood films. Approximately 82.7 percent of imported cases of malaria among U.S. civilians occurred among persons who were either not taking prophylaxis or were taking nonrecommended prophylaxis for the region to which they were traveling. Reviews of deaths attributed to malaria in the United States indicate that failure to take or to adhere to recommended antimalarial chemoprophylaxis, to promptly seek medical case for post-travel illness, and to promptly diagnose and treat suspected malaria contributed to fatal outcomes.[46,90,108]

The majority (53.9%) of the malaria cases occurred among persons visiting family or relatives, whereas 12.5 percent occurred among tourists and 9.2 percent occurred among missionaries or their dependents. Retrospective reviews of malaria in children also have found that a substantial proportion of cases occurred in recent immigrants and the children of former immigrants who had traveled to visit their family's country of origin.[12,46,63,128] These data highlight the importance of pre-travel assessment and counseling, particularly for foreign-born persons, who may assume that they are protected by natural immunity or do not perceive the risk of acquisition of malaria by their children who accompany them on returning to visit their home country.

PREVENTING MALARIA IN PEDIATRIC TRAVELERS

The substantial proportion of U.S. civilian malaria cases reported in children younger than 18 years underscores the importance of pre-travel counseling and strategies for malaria prevention. Young children and nonimmune persons of any age are at greater risk for development of severe complications from malaria. Prevention of malaria in pediatric travelers depends first on obtaining current and accurate information about the risk of acquiring malaria in proposed travel destinations and determining whether planned activities, such as rural versus urban travel, and the season of travel place the traveler at increased risk of exposure. Information on the geographic- and country-specific risks of acquiring malaria is available from multiple sources. Selected Web sites with information about the country-specific risk of acquiring malaria are listed in this chapter in the section on international travel information resources.

After a thorough assessment of the risk of acquiring malaria has been made, prevention strategies for pediatric travelers are twofold: personal protection measures against contact with mosquitoes and antimalarial chemoprophylaxis. The first mainstay of prevention of malaria is appropriate and effective use of personal protection measures. All travelers to malaria-endemic areas should be counseled on recommended measures to avoid bites from *Anopheles* mosquitoes, which typically are evening and nighttime feeders. Such measures include wearing clothing that reduces the amount of exposed skin (such as long-sleeved shirts, long pants tucked into socks, hats, stroller nets) and, whenever possible, remaining in well-screened or enclosed air-conditioned areas. Travelers staying overnight in facilities without air conditioning or screens should use insecticide-treated mosquito nets over the beds. Stroller nets can be used for children in strollers. Bed nets that have been treated with insecticide such as permethrin are more effective in preventing malaria than are untreated bed nets and are safe for children.[109] Travelers can purchase permethrin to spray on the bed nets or purchase pretreated (or impregnated) bed nets. Permethrin also can be sprayed on clothing, but it should not be applied directly to skin. During the evening, insecticide also can be sprayed inside rooms. Another important focus of counseling for personal protection measures is appropriate use of insect repellent on exposed skin.

The CDC recommends the use of repellent products with active ingredients registered with the U.S. Environmental Protection Agency (EPA). In scientific studies, two registered products, DEET (*N*,*N*-dimethyl-*m*-toluamide) and picaridin (KBR 3023), have been demonstrated to have a higher degree of efficacy than that of products containing other repellents. In recent studies, repellent products containing oil of lemon eucalyptus were tested against mosquitoes in the United States and were found to provide protection similar to that of low concentrations of DEET. Other products have been evaluated for repellent activity. However, they have not been studied as well as has DEET and may not be safe for use in children. Most botanical products provide relatively limited or no protection.

Some controversy has occurred regarding the recommended concentration of DEET for pediatric use. In 1998, the EPA conducted an extensive review of DEET safety. The agency concluded that no evidence indicates that DEET is toxic to infants or children. Additional evaluations have not demonstrated a link between seizures and topical use.[18,21] DEET formulations of up to 50 percent can be used for children older than 2 months. The concentration of DEET affects the duration of protection. Higher concentrations provide longer protection; however, the duration of protection reaches a plateau at approximately 30 to 50 percent. Therefore, most clinicians recommend products having up to 30 percent for children. In a laboratory study, a product with 23.8 percent DEET provided an average of 5 hours of protection (range, 3 to 6 hours), and a product with 6.65 percent DEET provided an average of 2 hours of protection (range, 1.5 to 2.8 hours). Duration of protection may be affected by the environmental temperature, sweating, and wind conditions.[21,53] DEET should not be used on children younger than 2 months.

The EPA recommends the following precautions in use of insect repellents:

- Apply repellents only to exposed skin or clothing.
- Never use repellents over cuts, wounds, or irritated skin.
- Do not apply to eyes or mouth, and apply sparingly around ears. When using sprays, do not spray directly on face—spray on hands first and then apply to face.
- Do not allow children to handle the product. When using on children, apply to your own hands first and then put it on the child. You may not want to apply to children's hands.
- Use just enough repellent to cover exposed skin or clothing. Heavy application and saturation generally are unnecessary for effectiveness. If biting insects do not respond to a thin film of repellent, then apply a bit more.
- After returning indoors, wash treated skin with soap and water or bathe. This procedure is particularly important when repellents are used repeatedly in a day or on consecutive days. Also, wash treated clothing before wearing it again. (This precaution may vary with different repellents; check the product label.)
- If you or your child gets a rash or has other reactions from an insect repellent, stop using the repellent, wash the repellent off with mild soap and water, and call a local poison control center for further guidance. If you go to a physician because of the repellent, take the repellent with you to show the physician.[21,53]

Products that contain repellents and sunscreen generally are not recommended because of the need to reapply sunscreen more frequently than repellent.[5] Mosquito coils should be used with extreme caution in the presence of children to avoid burns and inadvertent ingestion.[29]

Clinicians are encouraged to stress to parents and children alike the importance of using personal protection measures. Despite the demonstrated efficacy of these measures, studies have found that only 17 percent of adult travelers with malaria reported

using insect protection methods and only 11 percent took the recommended chemoprophylaxis.[70]

The second mainstay of malaria prevention is chemoprophylaxis. Selection of the appropriate drug for antimalarial chemoprophylaxis must be based on numerous factors: the most recent information available about malaria in the proposed travel destinations; the trip itinerary; the age, weight, and medical history of the traveler; the personal preference for the frequency of dosing and the duration of chemoprophylaxis on return from the trip; and the cost of medication. These decisions can be challenging for primary care providers and clinicians with limited experience in infectious disease and travel medicine, so when in doubt, clinicians should seek the advice of a travel medicine or infectious disease expert. In addition, the CDC provides resources with guidance on appropriate use of and recommended regimens for antimalarial chemoprophylaxis (listed in the section on international travel information resources).

Figure 242–2 outlines an algorithm for determining appropriate antimalarial chemoprophylaxis regimens for pediatric travelers. Maps of malaria-endemic areas and zones of drug resistance, which can be used as visual aids, are presented in Figures 242–3 to 242–5. Because data on the distribution of drug-resistant malaria are evolving constantly, in addition to using the information in this chapter, clinicians always should obtain the most recent information about the risk of malaria and zones of drug resistance before prescribing malaria chemoprophylaxis.[19,137] For example, in November 2006, an outbreak of malaria caused by *P. falciparum* was reported in Kingston, Jamaica, an area not considered endemic for malaria. As of December 2006, 107 cases had been confirmed, and the CDC recommended malaria chemoprophylaxis for all travelers staying overnight in Kingston. Although the recommendation is expected to be temporary, it highlights the importance of obtaining current information about malaria for any overseas destination.[28]

The first decision point in selecting appropriate antimalarial chemoprophylaxis is whether travel is occurring in a region of chloroquine-sensitive or chloroquine-resistant malaria. For travel to areas with chloroquine-sensitive malaria, chloroquine is the drug of choice for antimalarial chemoprophylaxis. *P. ovale*, *P. malariae*, and most *P. vivax* are widely sensitive to chloroquine; however, chloroquine-resistant *P. vivax* is an emerging problem and has been reported from Guyana, Papua New Guinea, India, Burma (Myanmar), and areas of Indonesia.[19,72] In addition to chloroquine-resistant *P. vivax*, chloroquine-resistant *P. falciparum* has been reported from these areas, and consequently chloroquine would not be recommended as chemoprophylaxis for travelers to these regions.

If the traveler is visiting a region with chloroquine-resistant malaria, the next decision point is whether travel will include regions with chloroquine-resistant malaria only or both chloroquine- and mefloquine-resistant malaria. Chloroquine-resistant *P. falciparum* is widespread and exists in all malaria-endemic areas except Mexico, the Caribbean, Central America west of the former Panama Canal Zone, Argentina, and parts of the Middle

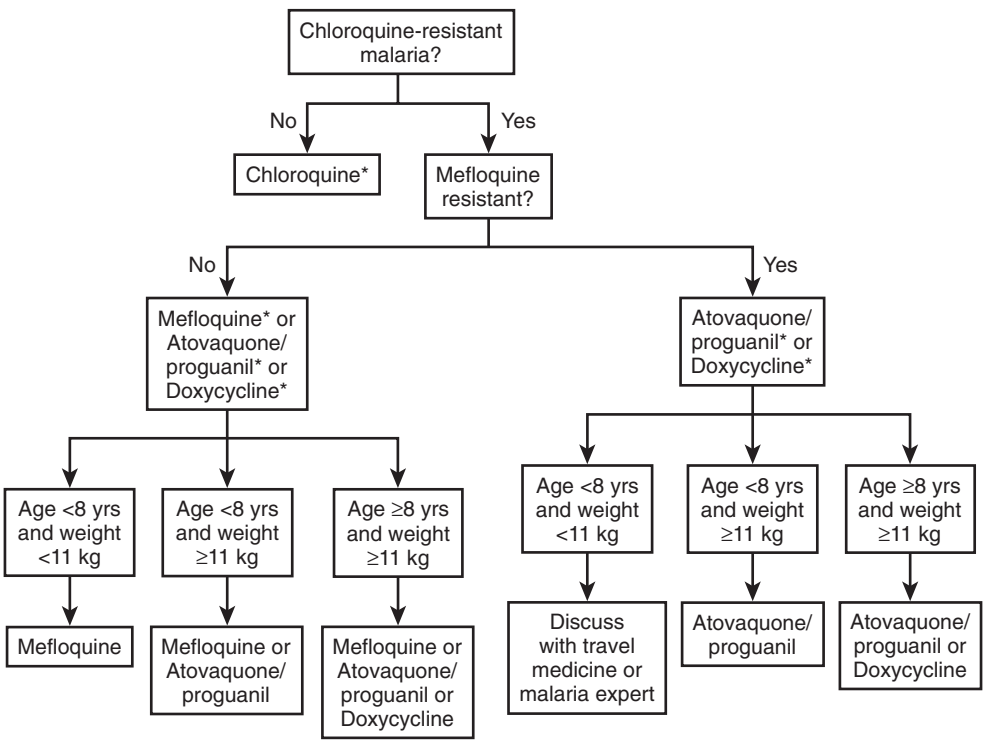

Figure 242–2 Algorithm for determining appropriate antimalarial chemoprophylaxis regimens for pediatric travelers.

Figure 242–3 Malaria-endemic countries in Africa, Asia, and Europe, areas of chloroquine-sensitive and chloroquine-resistant malaria. Maps of areas in which malaria is endemic and zones of drug resistance. These maps are for use as a visual aid only; more detailed information about country-specific malaria risk is available through on-line resources (see the section on international travel information resources). (See companion Expert Consult web site for color version.) *(From Centers for Disease Control and Prevention: Health Information for International Travel 2007-2008. Atlanta, U.S. Department of Health and Human Services, Public Health Service, 2007.)*

Figure 242–4 Malaria-endemic countries in North, Central, and South America and the Caribbean, areas of chloroquine-sensitive and chloroquine-resistant malaria. Maps of areas in which malaria is endemic and zones of drug resistance. These maps are for use as a visual aid only; more detailed information about country-specific malaria risk is available through on-line resources (see the section on international travel information resources). (See companion Expert Consult web site for color version.) *(From Centers for Disease Control and Prevention: Health Information for International Travel 2007-2008. Atlanta, U.S. Department of Health and Human Services, Public Health Service, 2007.)*

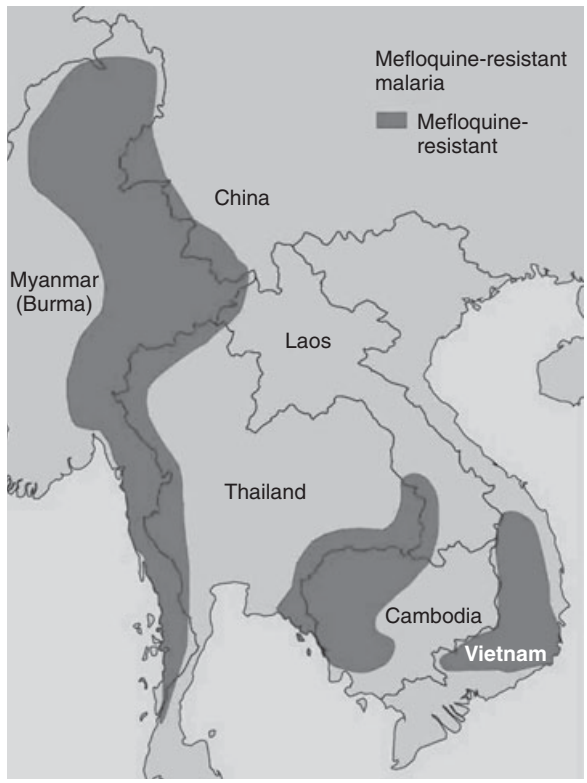

Figure 242–5 Area of mefloquine-resistant malaria. Maps of areas in which malaria is endemic and zones of drug resistance. These maps are for use as a visual aid only; more detailed information about country-specific malaria risk is available through on-line resources (see the section on international travel information resources). (See companion Expert Consult web site for color version.) *(From Centers for Disease Control and Prevention: Health Information for International Travel 2007-2008. Atlanta, U.S. Department of Health and Human Services, Public Health Service, 2007.)*

East and China.[72] In some regions, *P. falciparum* may be resistant to both chloroquine and mefloquine; these areas currently are limited to the borders of Thailand with Myanmar (Burma) and Cambodia, the western provinces of Cambodia that border Thailand, and the eastern states of Myanmar (Burma), including the states of Shah, Kayin, and Kayan.[18,72] For travel to areas with chloroquine-resistant malaria, three current antimalarial chemoprophylaxis options are mefloquine (Lariam), atovaquone-proguanil (Malarone), and doxycycline. The CDC no longer recommends the use of chloroquine-proguanil for chemoprophylaxis in chloroquine-resistant areas. For travel to areas with chloroquine- and mefloquine-resistant malaria, either atovaquone-proguanil or doxycycline can be used. Primaquine can be used as an option for primary prophylaxis in special circumstances. Clinicians should contact the CDC Malaria Hotline (770-488-7788) for additional information. Primaquine also can be used for terminal prophylaxis to prevent relapses of *P. vivax* or *P. ovale*. In general, terminal prophylaxis is indicated only for persons who have had prolonged exposure to malaria-endemic areas (missionaries, expatriates).[18] Tafenoquine, a long-acting primaquine analogue, is being investigated and may be approved for malaria chemoprophylaxis in the future.[32,72,79,136,137]

In evaluating options for antimalarial chemoprophylaxis, physicians should review each medication for contraindications and weight and age restrictions (Table 242–3). Chloroquine is relatively well tolerated in children. In the United States, chloroquine is available in tablet form; in Europe and other countries, it is also available as a syrup. Mefloquine can be used safely in children weighing less than 15 kg and may be useful for longer trips because it is administered once weekly.[88] However, it must be continued for 4 weeks after leaving the malarious area, and no liquid preparation is available. Doses for children are one fourth, one half, and three fourths of a tablet, depending on weight. No data are available on the use of atovaquone-proguanil in children weighing less than 11 kg; however, studies are in progress. For children who weigh more than 11 kg and are at risk of acquiring chloroquine-resistant *P. falciparum* infection, atovaquone-proguanil can be advantageous for short trips because it is started 1 to 2 days before the trip commences and can be stopped 7 days after the trip. However, the patient or parent must remember to take or to administer the medication daily. It is available in pediatric tablet form. Doxycycline is contraindicated in children younger than 8 years because of concern about the propensity of tetracyclines to stain growing teeth or to potentially affect developing bones. For older children, doxycycline must be administered daily and continued for 4 weeks after departing the malaria-endemic area.[18,72,79]

The importance of determining appropriate antimalarial chemoprophylaxis regimens for travelers and counseling to improve compliance cannot be overemphasized. In retrospective reviews of pediatric malaria cases, between 75 and 100 percent of infected children had received no or inadequate chemoprophylaxis.[46,128,159] Finally, effective pre-travel malaria prevention counseling includes anticipatory guidance for parents about recognition of and response to symptoms of malaria infection in young pediatric travelers. Parents should be counseled that although compliance with personal protection measures and chemoprophylaxis will decrease the risk, it cannot guarantee prevention of malaria infection. They should be instructed about the symptoms of malaria infection, such as fever, headache, vomiting, diarrhea, and myalgia, and be counseled to seek immediate medical attention if symptoms occur. Delay in recognition and treatment of malaria is associated directly with an increase in rates of morbidity and mortality; therefore, prompt and appropriate initiation of effective therapy is paramount.[18,57,70,71,145,165] Parents also should be advised that some types of malaria can become symptomatic several weeks to months after exposure, and therefore prompt medical attention should be sought even if illness develops in a child months after international travel to malaria-endemic areas.

PREVENTION OF OTHER INFECTIOUS DISEASES IN PEDIATRIC TRAVELERS

Because the epidemiology of many diseases is evolving, prevention hinges on the clinician's being knowledgeable about information on current outbreaks and risk in planned travel destinations, evaluating risk based on planned activities and the season of travel, and providing appropriate counseling and vaccination, if available. Information about specific infectious disease risks can be obtained from numerous sources listed in the section on international travel information resources. More detailed information about specific vaccinations is included in the section on vaccination for international travel.

A variety of pathogens increasingly are being recognized as emerging infectious diseases in travelers. In addition to malaria, other vector-borne infectious diseases are among the important diseases for consideration in travelers. Dengue is one of the most significant vector-borne viral infections worldwide and is endemic in Asia, the South Pacific, Africa, Latin America, and the Caribbean. Worldwide, an estimated 50 to 100 million cases of dengue fever occur annually; of these cases, 200,000 to 500,000 are dengue hemorrhagic fever. Dengue fever has been reported among 2 to 14 percent of ill travelers.[11,55,110,163] Epidemics of dengue hemorrhagic fever, the more severe clinical form of dengue fever, occur every 3 to 5 years in Southeast Asia and are an emerging problem in Latin America.[115] Epidemics caused by all four serotypes (1 to 4) have become progressively more frequent and larger in the past 25 years. As of 2004, dengue fever is endemic in most tropical countries of the South Pacific, Asia, the Caribbean, the Americas, and Africa. In addition, most tropical urban centers in these regions have multiple dengue virus serotypes co-circulating (hyperendemicity), which increases the risk of dengue transmission and the risk for dengue hemorrhagic fever.[18] Outbreaks of dengue fever also have occurred in Hawaii and along the United States–Mexican border.[26,124,150] Dengue is transmitted primarily by day-biting *Aedes aegypti* mosquitoes, which breed in flower vases, barrels, and discarded tires that collect water. Transmission occurs in rural and urban areas, but the risk is greatest in urban areas. Prevention should focus on protection against mosquito bites. Travelers to areas of risk should be counseled to apply repellent during the day, even while visiting cities. No vaccine is available, and previous infection with one of the four serotypes does not protect against infection with another serotype. The risk of contracting dengue hemorrhagic fever increases after subsequent infection with a different serotype.[147]

Chikungunya virus is an alphavirus that exists in tropical Africa and is transmitted by the bite of infected mosquitoes. Because chikungunya fever epidemics are sustained by human-mosquito-human transmission, the epidemic cycle is similar to those of dengue and urban yellow fever. Infection is associated with fever, headache, severe joint pain, and, in approximately half of the cases, a generalized maculopapular rash similar to that of dengue virus infection. Large outbreaks of chikungunya have been reported recently on several islands in the Indian Ocean and in India. During 2005 to 2006, 12 cases were reported in travelers who arrived in the United States from areas known to be epidemic or endemic for chikungunya virus.[16] No vaccines or specific preventive medications are available; therefore, prevention measures should focus on personal protection against mosquito bites and awareness of local epidemics.

African trypanosomiasis (sleeping sickness), a parasitic infection transmitted by the bite of a tsetse fly, occasionally has been reported in travelers, including children. Infection can result in

TABLE 242–3 Antimalarial Chemoprophylaxis Regimens for Pediatric Travelers

Medication	Regimen	Dose	Contraindications and Precautions	Side Effects	General Indications and Information for Use
Chloroquine (Aralen)	Weekly starting 1-2 weeks before trip Continue weekly during trip and for 4 weeks after trip	5 mg base/kg (8.3 mg salt/kg) up to 300 mg base (500 mg salt) Tablets: 300 mg base (500 mg salt)	Prior retinal or visual field changes Psoriasis (may be exacerbated by chloroquine) Do not administer with intradermal human diploid cell rabies vaccine (intradermal rabies vaccine series must be completed at least 7 days before starting chloroquine)	Gastrointestinal symptoms, seizures, rash headache, dizziness, pruritus (especially in dark-skinned persons), blurred vision, decreased hearing, tinnitus, retinal damage at high cumulative doses* High toxicity in overdoses, keep out of reach of children	Use only in areas of chloroquine-sensitive malaria Limited usefulness because of widespread chloroquine resistance Bitter taste
Mefloquine (Lariam)	Weekly starting 1 week before trip Continue weekly during trip and for 4 weeks after trip	≤15 kg: 4.6 mg/kg base (5 mg/kg salt) 15-19 kg: 1/4 tablet 20-30 kg: 1/2 tablet 31-45 kg: 3/4 tablet ≥46 kg: 1 tablet Tablets: 228 mg base (250 mg salt)	Psychiatric conditions, cardiac conduction disorders, seizure disorders, persons with known hypersensitivity to mefloquine Should not take with quinine-like drugs Do not administer with intradermal human diploid cell rabies vaccine (intradermal rabies vaccine series must be completed at least 7 days before starting mefloquine) Do not administer oral typhoid vaccine within 24 hours of mefloquine	Gastrointestinal symptoms, dizziness, insomnia Occasional serious adverse effects: seizures, nightmares, depression, anxiety, psychosis, especially in persons with these preexisting medical conditions	Use in areas with chloroquine-resistant malaria Advantageous for long-term travelers because of weekly dosing and less costly than atovaquone-proguanil Bitter taste
Atovaquone-proguanil (Malarone)	Daily starting 1-2 days before trip Continue daily during trip and for 7 days after trip	11-20 kg: 1 pediatric tablet (62.5 kg/25 mg) 21-30 kg: 2 pediatric tablets (125 mg/50 mg) 31-40 kg: 3 pediatric tablets (187.5 mg/75 mg) ≥40 kg: 1 adult tablet (250 mg atovaquone and 100 mg proguanil hydrochloride) Pediatric tablets: 62.5 mg atovaquone and 25 mg proguanil hydrochloride Adult tablets: 250 mg atovaquone and 100 mg proguanil hydrochloride	Contraindicated in severe renal failure Not recommended or use with caution for children <11 kg, pregnant or lactating women Do not take with tetracycline, metoclopramide, rifampin, or rifabutin (all reduce concentrations of atovaquone) Do not administer oral typhoid vaccine within 24 hours of atovaquone-proguanil	Gastrointestinal symptoms, headache, loss of appetite, dizziness, pruritus	Use in areas with chloroquine-resistant or mefloquine-resistant malaria Advantageous for short-term travelers because prophylaxis can be stopped 1 week after leaving malaria area Available in pediatric tablets Take with food or milk
Doxycycline	Daily starting 1-2 days before trip Continue daily during trip and for 4 weeks after trip	2 mg/kg up to 100 mg daily Tablets: 50 mg, 100 mg	Do not use for children <8 years, pregnant or lactating women Do not give simultaneously with antacids or Pepto-Bismol Do not administer oral typhoid vaccine within 24 hours of doxycycline	Gastrointestinal symptoms, photosensitivity, increased blood urea nitrogen level, hypersensitivity reactions, blood dyscrasias, vaginal candidiasis May decrease the effectiveness of oral contraceptives	Use in areas with chloroquine-resistant and mefloquine-resistant malaria (borders of Thailand with Myanmar (Burma) and Cambodia, the western provinces of Cambodia that border Thailand, and the eastern states of Myanmar (Burma), including the states of Shah, Kayin, and Kayan Take with food; taking with food or milk can decrease gastric irritation (absorption of doxycycline is not significantly decreased by simultaneous administration of milk or food)

**Despite the use of chloroquine as an antimalarial chemoprophylaxis agent for decades and the use of high-dose chloroquine for certain chronic diseases, the literature is inconclusive about the potential risk of retinopathy associated with long-term use of chloroquine for antimalarial prophylaxis. Retinopathy has rarely been reported in patients receiving weekly prophylaxis. Retinopathy appears to be related to dosage and accumulated dosage.*

severe neurologic sequelae and is 100 percent fatal if untreated. In 2001, significant increases in cases were reported among U.S. and European travelers to game parks in Tanzania and Kenya. Between 1967 and 2000, an imported case occurred on average every 1 to 2 years; however, in 2001, seven cases were reported in U.S. travelers.[51,100-102,154]

Schistosomiasis, another parasitic infection caused by flukes that live part of their life cycle in fresh-water snail hosts, affects more than 200 million people worldwide. Schistosomiasis has been reported in travelers to endemic areas of Africa, Asia, South America, and the Caribbean who participated in high-risk activities such as swimming and wading in fresh water.[18,31,37,49] Because most acute infections are asymptomatic, preventive counseling is critical. Children and their families should be counseled against swimming or wading in fresh water in risk areas.

Tick-borne encephalitis is transmitted primarily by the bite of *Ixodes* ticks. It also can be transmitted by the ingestion of unpasteurized dairy products from infected livestock. Transmission occurs during the summer months in western and central Europe, Scandinavia, and parts of the former Soviet Union. Persons who will be traveling for longer than 3 weeks in endemic rural areas or travelers who will be engaging in high-risk activities, such as camping, should be considered for vaccination. The vaccine is not available in the United States but can be obtained in Europe.[18]

Adventure travel can increase the risk of acquiring a variety of infectious diseases. Examples of recent outbreaks or cases of unusual pathogens affecting adventure travelers include fungal organisms (such as histoplasmosis and coccidioidomycosis), leptospirosis, and leishmaniasis. Histoplasmosis is a fungal infection acquired by the inhalation of spores, usually through exposure to bat, bird, or chicken droppings in barnyards and caves. The organism is endemic in the United States, Latin America, eastern Asia, parts of Europe, Africa, and Australia. Coccidioidomycosis, a fungal infection associated with the inhalation of organisms in soil from high-risk areas, is endemic in the southwestern part of the United States and Latin America. Both infections can cause a spectrum of illness from asymptomatic infection to acute pulmonary infection to severe, disseminated disease, especially in immunocompromised persons. Several outbreaks of histoplasmosis have been reported in groups of U.S. visitors who entered a cave with bats in Costa Rica[15] (CDC, unpublished data), Ecuador,[155] Peru,[14] and Nicaragua.[160] More than 200 college students became infected with histoplasmosis during a spring break trip to Acapulco, Mexico.[112,153] Two outbreaks of coccidioidomycosis have been reported in youth missionary groups involved in construction work in Mexico.[13,35] Most of these fungal outbreaks have two common features: high-risk group activities and high attack rates, even in young, nonimmunocompromised individuals. Because no vaccine is available, prevention involves counseling travelers to avoid exposure or to use special masks for high-risk individuals who cannot avoid exposure.[20]

Leptospirosis is a zoonotic infection that is transmitted by exposure to water or soil contaminated with organisms excreted by domestic and wild animals. Outbreaks have been reported in white-water rafters in Costa Rica[113] and in athletes from 26 countries who participated in the Eco-Challenge multisport expedition race in Borneo, Malaysia, in 2000.[111,152] Because no vaccine against leptospirosis exists, persons engaging in high-risk activities should be counseled to avoid exposure to water that may be contaminated or to wear protective clothing. The CDC recommends that persons engaging in high-risk activities consider the use of doxycycline (200 mg orally, once a week), begun 1 to 2 days before exposure and continuing through the period of exposure for prophylaxis; doxycycline should not be used routinely for children younger than 8 years.[18]

Leishmaniasis, a parasitic infection transmitted by the bite of a sandfly, can lead to cutaneous or visceral infection. It has been reported in students who traveled to the rain forest in Costa Rica and other travelers.[61,62,97,164] The appropriate use of insect repellent and other personal protection measures against sandfly bites is the only prevention tool available.

Finally, studies of sexual practices among travelers have shown that 5 to 50 percent of short-term travelers engage in casual sex while abroad; 40 to 60 percent of long-term travelers have reported engaging in casual sex while abroad.[93,126] A study of British travelers reported that 18.6 percent had had new sexual partners, two thirds of those who were sexually active did not use condoms on every occasion, and 5.7 percent contracted sexually transmitted diseases.[125] Adolescents should be counseled about the risks and prevention of sexually transmitted diseases, hepatitis B, and HIV infection associated with sexual contact, sharing needles, or receiving acupuncture or tattoos.

PREVENTION OF TRAVELER'S DIARRHEA IN CHILDREN

EPIDEMIOLOGY OF TRAVELER'S DIARRHEA

One of the most difficult tasks faced by international travelers of any age is ensuring the safety of food and water. Traveler's diarrhea, caused by the ingestion of contaminated food and water, typically is defined as the occurrence of four or more unformed stools in a 24-hour period or three or more unformed stools in an 8-hour period with at least one of the following signs or symptoms: temperature higher than 38° C, abdominal cramping, nausea, vomiting, fecal urgency, tenesmus, or blood or mucus in stools.[44,49,117] Traveler's diarrhea affects between approximately 20 and 50 percent of adult travelers and is the health problem most frequently reported by travelers to developing countries.[43,44] It can occur any time during travel but typically occurs within the first week or two of the trip. The illness usually is self-limited, and for adults the average duration of illness has been reported to be 3 to 5 days.[49] Less information about traveler's diarrhea in children is known. A retrospective study conducted by Pitzinger and associates[120] of Swiss children who had visited the tropics or subtropics reported finding similar incidence rates of traveler's diarrhea in children: 40, 8.5, 21.7, and 36 percent in children aged 0 to 2, 3 to 6, 7 to 14, and 15 years and older, respectively. In this study, the authors also found that small children (0 to 2 years old) most frequently were affected with traveler's diarrhea and that the clinical course tended to be more severe and prolonged than in older pediatric age groups. Overall, children were found to have longer lasting illness than that noted in adults, with an average duration of 11 days for all children combined and 29 days for small children.

Enteric pathogens typically are isolated from approximately 50 to 75 percent of stool specimens from adult travelers with diarrhea; in the remainder, usually no pathogen is isolated. *Escherichia coli*, especially enterotoxigenic *E. coli*, is the most common overall cause of traveler's diarrhea (although the incidence can vary by destination), followed by *Campylobacter*, *Salmonella*, and *Shigella*. Other etiologic agents include pathogenic bacteria, such as *Aeromonas* and *Plesiomonas*; protozoa (e.g., *Giardia lamblia*, *Entamoeba histolytica*, *Cryptosporidium* spp., and *Cyclospora cayetanensis*); viruses, such as rotavirus and Norwalk-like viruses; and, rarely, helminths.[117] Numerous risk factors for traveler's diarrhea, including the consumption of certain high-risk foods (raw foods such as meats, seafood, and vegetables; unpasteurized dairy products; ice and tap water) and travel to certain destinations, also have been identified.[139] Other authors previously categorized destinations by high, intermediate, and low levels of risk for acquiring traveler's diarrhea. Destinations generally considered to have the highest associated risk for contraction of traveler's diarrhea include Latin America, Africa, Asia, and the Middle East; destina-

tions with intermediate risk include southern Europe and the Caribbean; and low-risk travel destinations include North America, northern Europe, Australia, and New Zealand.[18] The location of food preparation also is a recognized risk factor for acquiring traveler's diarrhea, with a higher risk shown for travelers eating from street vendors and in local restaurants and a lower risk in luxury hotels and private homes. Although fewer data on traveler's diarrhea in children are available, children (especially toddlers) are probably more vulnerable to food- and water-borne pathogens for many reasons, including their propensity to touch multiple surfaces and to mouth objects. Of note, one study found that only 40 percent of parents said that they had practiced dietary prevention measures consistently, and only 5 percent reported using oral rehydration solutions (the mainstay of treatment of diarrhea in children).[120]

PREVENTIVE COUNSELING FOR TRAVELER'S DIARRHEA

Counseling parents of pediatric travelers about appropriate preventive behavior to avoid traveler's diarrhea and anticipatory guidance to ensure successful management of diarrhea are important aspects of the pre-travel assessment.

Food and Beverage Precautions

The most important aspect of preventive counseling for parents of pediatric travelers is appropriate food and beverage precautions (Table 242–4). In addition to preventing the diarrheal diseases already listed, compliance with food and water precautions also will decrease the risk of contracting other food-borne diseases such as hepatitis A. Parents should be counseled that in areas where chlorinated tap water is not available or where hygiene and sanitation are poor, tap water (including ice cubes) should be considered contaminated and hence should be avoided. In addition, travelers also should be advised to avoid brushing their teeth with tap water. Breast-feeding, boiled or bottled water, and bottled carbonated beverages are recommended as typically safe beverages for children; powdered drink mixes made with boiled water (e.g., formula, tea, Kool-Aid) also generally can be considered safe. Parents should dry wet cans and bottles before opening and wipe drinking surfaces clean before serving.[69]

In areas where access to bottled water is poor, water may be boiled for 1 minute (or for 3 minutes at altitudes above 2000 m [6562 feet]). These procedures will kill bacterial, parasitic, and viral pathogens. Chemical disinfection with iodine is an alternative method for water treatment when the water cannot be boiled; however, this method cannot be trusted to kill *Cryptosporidium* unless the water stands for 15 minutes after boiling before drinking. Two well-tested methods are tincture of iodine and tetraglycine hydroperiodide tablets (e.g., Globaline, Potable Aqua, or Coghlan's); these tablets are available at pharmacies, sporting goods stores, and camping outfitters. Chlorine also can be used for chemical disinfection, but its germicidal activity varies with pH, temperature, and organic content of the water; therefore, it can provide less consistent levels of disinfection in many types of water. Portable filters are available and provide various degrees of protection against microbes. Reverse-osmosis filters afford protection against viruses, bacteria, and protozoa, but they are large and expensive, and the small pores can be plugged by cloudy or muddy water. Microstrainer filters with pore sizes in the 0.1- to 0.3-μm range can remove bacteria and protozoa from drinking water, but they do not remove viruses. To kill viruses, parents using microstrainer filters should be advised to chemically disinfect water with iodine after filtration. Data are inadequate at present to evaluate the efficacy of specific brands or models of filters, and therefore the CDC cannot recommend specific brands or models of filters most likely to remove bacteria, viruses, and parasites.[18]

Parents of pediatric travelers also should be counseled on the importance of advance planning for food and beverage items, especially for infants and young children. Breast-feeding infants are considered relatively safe from contracting traveler's diarrhea because they are not exposed to local food and water; therefore, traveling mothers should be encouraged to continue breast-feeding for as long as practical. For infants receiving formula, formula concentrate and powdered forms are the most convenient for travel, but a clean water supply must be available or water must be boiled or chemically disinfected before preparation. For feeding toddlers and older children, the travel adage of "boil it, cook it, peel it, or forget it" applies. In general, unless beverages and food come from a can or can be completely cooked or peeled, they should be considered unsafe (including raw fruits and vegetables). Travelers should avoid unpasteurized dairy products, including cheese and ice cream. In addition, travelers should be advised to not consume raw seafood, shellfish, and meats. Freshly prepared steaming hot food, breads and cereals, hot pasta (including rice and noodles), and well-cooked meat or fish generally can be considered safe for consumption. Bringing crackers and peanut butter is a good suggestion for parents of hungry children when the availability of safe foods is uncertain. The incidence of traveler's diarrhea in children also can be reduced potentially by advising parents to encourage or to supervise frequent handwashing, including the use of alcohol-based hand sanitizer, and to try to prevent children from placing objects in their mouths.

Some species of fish and shellfish can contain poisonous biotoxins, such as ciguatoxin and scombrotoxin, even when they are well cooked. Ciguatera poisoning is a potential risk in all subtropical and tropical regions of the West Indies and the Pacific and Indian oceans where the implicated fish are consumed; such fish include barracuda, red snapper, grouper, amberjack, and sea bass. Symptoms of ciguatera poisoning include gastroenteritis and neurologic manifestations such as dysesthesias and weakness.

TABLE 242–4 Food and Beverage Precautions for International Travel

Generally Safe Food and Beverages	Generally Unsafe Food and Beverages
Boiled or chemically disinfected water	Tap water and ice
Powdered drink mixes made with boiled water (e.g., formula, teas, Kool-Aid)	Unpasteurized milk and dairy products (including cheese and ice cream)
Canned or bottled water or beverages	Cooled, standing, or reheated foods
Fruits and vegetables that can be peeled	Raw fruit and vegetables, including salads
Breads and cereals	Undercooked or raw meats
Hot, fully cooked pasta, vegetables, meat, or fish	Undercooked or raw fish and shellfish; fish with suspected biotoxins

Modified from Jenista, J. A.: The International Child Traveler. In *Jensen, H. B., and Baltimore, R. S. (eds.): Pediatric Infectious Diseases: Principles and Practice. Norwalk, CT, Appleton and Lange, 1995, p. 1518.*

Scombroid poisoning occurs in tropical as well as in temperate regions and is caused by high levels of histidine in the flesh of fish such as bluefin, yellowfin tuna, mackerel, bonito, mahi-mahi, herring, amberjack, and bluefish; if improperly refrigerated, histidine is converted to histamine, which can cause flushing, headache, nausea, vomiting, diarrhea, and urticaria.[18]

MANAGING TRAVELER'S DIARRHEA IN CHILDREN

Adults traveling with children should be counseled about the signs and symptoms of dehydration and the proper use of WHO oral rehydration solution (ORS). Immediate medical attention is required for an infant or young child with diarrhea who has signs of moderate to severe dehydration (Table 242–5), bloody diarrhea, temperature higher than 38.5°C (>101.5°F), or persistent vomiting. ORS should be provided to the infant by bottle or spoon while medical attention is being obtained.

Assessment and Treatment of Dehydration

The greatest risk to the infant with diarrhea and vomiting is dehydration. Fever or increased ambient temperature increases fluid losses and speeds dehydration. Parents should be advised

TABLE 242–5 Anticipatory Guidance for Management of Traveler's Diarrhea in Children

Signs	Minimal or No Dehydration (<3% loss of body weight)	Mild or Moderate Dehydration (3%-9% loss of body weight)	Severe Dehydration (>9% loss of body weight)
Mental status	Well; alert	Normal, fatigued or restless, irritable	Apathetic, lethargic, unconscious
Thirst	Drinks normally; might refuse liquids	Thirsty; eager to drink	Drinks poorly; unable to drink
Breathing	Normal	Normal; fast	Deep
Eyes	Normal	Slightly sunken	Deeply sunken
Tears	Present	Decreased	Absent
Mouth and tongue	Moist	Dry	Parched
Skin fold	Instant recoil	Recoil in <2 seconds	Recoil in >2 seconds
Capillary refill	Normal	Prolonged	Prolonged; minimal
Extremities	Warm	Cool	Cool; mottled; cyanotic
Urine output	Normal to decreased	Decreased	Minimal

1. Include oral rehydration solution (ORS) packets in travel kit. Products can be obtained from Jianas Brothers Packaging Company, 2533 Southwest Boulevard, Kansas City, Missouri 64108, USA (1-816-421-2880) for WHO ORS packets (glucose-based formula) or Cera Products, 9017 Mendenhall Court, Columbia, Maryland 21045, USA (1-410-309-1000 or 1-888-Ceralyte; *www.ceraproductsinc.com*) for CeraLyte, a rice cereal–based product.
2. Recognize the signs and symptoms of mild, moderate, and severe dehydration.
3. Begin ORS with first episode of watery stools or vomiting.
4. Mix 1 ORS packet with 1 liter of bottled or boiled water in a clean container or as instructed on the packet label; discard after 12 hours at room temperature.
5. Treatment should include two phases: rehydration and maintenance. In the rehydration phase, the fluid deficit is replaced quickly (i.e., during 3-4 hours). This is followed by a maintenance phase with the provision of calories and fluids.

   Rehydration

   During the rehydration phase, administer ORS as follows:

   In a nondehydrated child: 1 mL of fluid for each gram of output. When losses are not easily measured, 10 mL of additional fluid can be administered per kilogram of body weight for each watery stool or 2 mL/kg body weight for each episode of emesis. Alternatively, children can be hydrated with the same amounts as specified for children with mild too moderate dehydration as below.

   In mildly to moderately dehydrated child

   <10 kg body weight: 60-120 mL ORS for each diarrheal stool or vomiting episode

   ≥10 kg body weight: 120-240 mL ORS for each diarrheal stool or vomiting episode

   For children with vomiting, it may be necessary to start rehydration with small volumes of ORS (5 mL) every 5 minutes, with a gradual increase in the amount consumed. Administration with a spoon or syringe under close supervision can help guarantee a gradual progression in the amount taken.

   Feeding

   Continue normal, unrestricted feeding throughout illness if possible. Rapid realimentation should follow rapid rehydration with a goal of quickly returning the patient to an age-appropriate unrestricted diet. Gut rest is not indicated. The diet should be increased as soon as tolerated to compensate for lost calorie intake.

   In infants: Formula or breast-feeding. For formula-fed infants, diluted formula is not recommended, and special formula usually is not necessary.

   In older children: Encourage the intake of the usual diet. Foods high in simple sugars (carbonated soft drinks, juice, gelatin desserts) should be avoided because the osmotic load might worsen diarrhea. The practice of withholding food for ≥24 hours is inappropriate. Highly specific diets (e.g., the BRAT [bananas, rice, applesauce, and toast] diet) are unnecessarily restrictive and provide suboptimal nutrition for the patient's nourishment and recovering gut.

   In a severely dehydrated child: Intravenous or intraosseous therapy is required for severe dehydration. Severe dehydration is a medical emergency. Oral or nasogastric rehydration should also be provided.
6. Antimotility agents (i.e., Lomotil [active ingredient, diphenoxylate] and Imodium [active ingredient, loperamide] are not recommended.
7. Empiric treatment with antimicrobials is not generally recommended.
8. Seek urgent medical attention for diarrhea with high fever, bloody stools, moderate to severe dehydration, lethargy, severe vomiting, or diarrhea lasting more than 3 days.

Modified from Duggan, C., Santosham, M., and Glass, R. I.: The management of acute diarrhea in children: Oral rehydration, maintenance, and nutritional therapy. M. M. W. R. Recomm. Rep. 44(RR-16):1-20, 1992; Jenista, J. A.: The international child traveler. In *Jensen, H. B., and Baltimore, R. (eds.): Pediatric Infectious Diseases: Principles and Practice. Norwalk, CT, Appleton & Lange, 1995, p. 1520; King, C. K., Glass, R., Bresee, J. S., and Duggan, C.: Managing acute gastroenteritis among children: Oral rehydration, maintenance, and nutritional therapy. M. M. W. R. Recomm. Rep. 52(RR-16):1-16, 2003; Sandhu, B. K.: Practical guidelines for the management of gastroenteritis in children. J. Pediatr. Gastroenterol. Nutr. 33(Suppl. 2):S36-S39, 2001; World Health Organization: The Treatment of Diarrhea: A Manual for Physicians and Other Senior Health Workers. Geneva, World Health Organization, 1995.*

that dehydration is best prevented and treated by use of ORS, in addition to the infant's usual food. Rice-based and other cereal-based ORS, in which complex carbohydrates are substituted for glucose, also are available and may be more acceptable to young children. Adults traveling with children should be counseled that sports drinks, which are designed to replace water and electrolytes lost through sweat, do not contain the same proportions of electrolytes as those of the solution recommended by WHO for rehydration during diarrheal illness.

ORS packets are available at stores or pharmacies in almost all developing countries. (See information later about ORS availability in the United States.) ORS is prepared by adding one packet to boiled or treated water. Travelers should be advised to check packet instructions carefully to ensure that the salts are added to the correct volume of water. ORS should be consumed or discarded within 12 hours if it is held at room temperature or 24 hours if it is kept refrigerated. A dehydrated child will drink ORS avidly; travelers should be advised to give it to the child as long as the dehydration persists. An infant or child who vomits the ORS usually will keep it down if it is offered by spoon, oral syringe, or straw in frequent small sips.[19,76,132]

Children weighing less than 10 kg who have mild to moderate dehydration should be administered 60 to 120 mL of ORS for each diarrheal stool or vomiting episode. Children who weigh 10 kg or more should receive 120 to 240 mL of ORS for each diarrheal stool or vomiting episode. Severe dehydration is a medical emergency that usually requires administration of fluids by intravenous or intraosseous routes.[19,42,76,132]

Dietary Modification

Breast-fed infants should continue nursing on demand. Formula-fed infants should continue their usual formula during rehydration. They should receive a volume that is sufficient to satisfy energy and nutrient requirements. Lactose-free or lactose-reduced formulas usually are unnecessary. Dilution of formula may slow resolution of diarrhea and is not recommended. Older infants and children receiving semisolid or solid foods should continue to receive their usual diet during the illness. Recommended foods include starches, cereals, yogurt, fruits, and vegetables. Foods that are high in simple sugars, such as soft drinks, undiluted apple juice, gelatins, and presweetened cereals, can exacerbate diarrhea by osmotic effects and should be avoided. In addition, foods high in fat may not be tolerated because of their tendency to delay gastric emptying. The practice of withholding food for 24 hours or longer is inappropriate. Early feeding can decrease changes in intestinal permeability caused by infection, reduce illness duration, and improve nutritional outcome. Highly specific diets (e.g., the BRAT [bananas, rice, applesauce, and toast] diet) frequently have been recommended; however, similar to juice-centered and clear fluid diets, such severely restrictive diets used for prolonged periods can result in malnutrition and should be avoided.[76]

ORS packets are available in the United States from Jianas Brothers Packaging Company, 2533 Southwest Boulevard, Kansas City, Missouri 64108, USA (1-816-421-2880). In addition, Cera Products, 9017 Mendenhall Court, Columbia, Maryland 21045, USA (1-410-309-1000 or 1-888-Ceralyte; *www.ceraproductsinc.com*), markets a rice cereal (rather than a glucose-based product), CeraLyte, in different flavors. ORS packets also may be available at stores that sell outdoor recreation and camping supplies.

Other Measures

Parents should be particularly careful to wash hands well after changing diapers of infants with diarrhea to avoid spreading infection to themselves and other family members.

Oral syringes available in most pharmacies for oral medications can be useful for the administration of ORS and can be included as part of the traveler's health kit for young children. Straws also can be useful for older children.

The use of antimotility agents (e.g., Imodium, Lomotil) in children younger than 2 years is not recommended. Because overdoses of these types of drugs can be fatal, they should be used with extreme caution in children. Side effects of these drugs in adults include opiate-induced ileus, drowsiness, and nausea. Lomotil has been associated with fatal overdoses and other severe complications, including coma and respiratory depression. Antinausea medications, such as promethazine and prochlorperazine, are not recommended routinely. They are contraindicated for use in children younger than 2 years. Fatal respiratory depression in children has been reported with use of promethazine. Children with an acute illness, including gastroenteritis and dehydration, are more susceptible than are adults to neuromuscular reactions, especially dystonias, associated with prochlorperazine. The extrapyramidal side effects associated with these medications can be confused with symptoms of other undiagnosed primary diseases associated with vomiting, such as Reye syndrome. These medications should not be prescribed routinely for empiric treatment of children with possible traveler's diarrhea. Adults traveling with children should be fully counseled about the indications, dosage, frequency, and possible side effects if these medications are prescribed.[19,76]

Antibiotics

Few data are available for empiric administration of antibiotics for traveler's diarrhea in children. Furthermore, the antimicrobial options for empiric treatment in children are limited. Trimethoprim-sulfamethoxazole was used previously for empiric treatment of traveler's diarrhea in children; however, its effectiveness has been reduced by widespread drug resistance, and it is no longer routinely recommended. Fluoroquinolones are used frequently for the empiric treatment of traveler's diarrhea in adults. The use of fluoroquinolones generally is not recommended in children and adolescents younger than 18 years because of cartilage damage in animals tested. The only indication for fluoroquinolone use in children that has been approved by the Food and Drug Administration is for complicated urinary tract infections. The American Academy of Pediatrics suggests some special circumstances for fluoroquinolone use, including the treatment of gastrointestinal infection caused by multidrug-resistant *Shigella* spp., *Salmonella* spp., *Vibrio cholerae*, or *Campylobacter jejuni*. However, the routine use for empiric treatment of traveler's diarrhea is not recommended. Tetracyclines can cause teeth staining if they are used in children younger than 8 years.[19,119]

In some studies, azithromycin has been found to be as effective as fluoroquinolones in treating traveler's diarrhea in adults.[2] In practice, some clinicians prescribe azithromycin either as a single dose or at 10 mg/kg for 3 to 5 days for empiric treatment. Flavored oral suspension of azithromycin is available. The suspension does not require refrigeration; however, it should be used within 10 days of mixing. The unreconstituted form of azithromycin has a longer expiration period. In certain circumstances, the unreconstituted form can be provided with clear instructions for preparation and may be useful for children traveling for more than 10 days.

SPECIAL CONSIDERATIONS FOR IMMUNOCOMPROMISED PEDIATRIC TRAVELERS

Travel preparations for a child with an immunocompromising condition, including congenital immunodeficiencies and acquired

immunodeficiencies (such as HIV infection), need to be tailored to the specific underlying condition. Limited data are available on health risks in immunocompromised pediatric travelers, but data from adult travelers can be used to guide the management of children. Schuhwerk and colleagues[133] have published a comprehensive review of considerations for travelers with HIV infection. A key component of the pre-travel assessment is a careful review of routine and travel-related vaccinations. In addition, the child and family should receive individualized preventive counseling about how to reduce disease risk while traveling. The health care provider should ensure that parents of immunocompromised children are knowledgeable about the type of immune defect or condition, the types of infections to which their children will be more susceptible, and how these risks may be affected by planned travel and activities. As previously discussed, parents should be advised to carry the following items with them for easy retrieval: (1) a summary of their child's medical history and treatment record, (2) copies of all important health-related documents (i.e., written prescriptions for medications; health insurance information; and medical contacts abroad, including specialists in planned travel destinations), and (3) all required prescription medications (in original bottles and sufficient supply for the length of the trip). Physicians should stress the importance of bringing an adequate supply of prescription medications, especially for travelers with HIV infection, because many medications are unavailable outside the United States. At present, no countries require proof of a negative HIV test result for entry for tourism purposes; however, some countries require HIV testing for students and workers and for persons wishing to stay in the country longer than 3 months.[36]

VACCINATION OF IMMUNOCOMPROMISED PEDIATRIC TRAVELERS

The American Academy of Pediatrics, Committee on Infectious Diseases, reports that experience with vaccine administration in immunocompromised children is limited. In most situations, theoretical considerations are used to guide decisions about vaccine administration because data for specific vaccines in children with specific disorders are lacking.[119] Vaccination recommendations may change, so clinicians should obtain the most recent recommendations before vaccinating immunocompromised patients (see the section on international travel information resources and Advisory Committee on Immunization Practices general recommendations on immunization[17] for detailed recommendations). In general, children with immunocompromising conditions, including primary and secondary immune deficiencies, should receive all inactivated vaccines that are recommended for nonimmunocompromised pediatric travelers. The risk for development of complications from the administration of inactivated vaccines and immune globulin preparations has not been shown to be increased in immunocompromised persons; however, immune responses may vary and may be suboptimal (thereby substantially reducing vaccine immunogenicity). Children with a deficiency in antibody-synthesizing capacity usually are incapable of mounting an antibody response to vaccines and should receive regular doses of immune globulin to provide passive protection against many infectious diseases. Children with milder B lymphocyte and antibody deficiencies have an intermediate degree of vaccine responsiveness and may require monitoring of postimmunization antibody titers to confirm vaccine immunogenicity.[119] Consideration should be given to timing the dosing of intravenous immune globulin to coincide with the immediate pre-travel period.[36,119]

In general, immunocompromised children should not receive live vaccines, either viral or bacterial, because of the risk for development of disease from vaccine strains. The American Academy of Pediatrics, Committee on Infectious Diseases, notes, however, that some immunocompromised children may benefit from special-use as well as routinely administered immunizations.[119] In patients with primary immunodeficiencies, live vaccines are contraindicated for most individuals with B lymphocyte defects except immunoglobulin A (IgA) deficiency and for all patients with T lymphocyte–mediated disorders. Fatal poliomyelitis and measles vaccine virus infections have occurred in children with disorders in T-cell function after the administration of live viral vaccines. Children with phagocytic function disorders, including chronic granulomatous disease and leukocyte adhesion defect, can receive all immunizations except live bacterial vaccines (BCG and Ty21a *Salmonella typhi*). Live vaccines generally are contraindicated in patients with secondary immunodeficiencies (including children with HIV infection or acquired immunodeficiency syndrome [AIDS]), cancer, and organ transplants and in children receiving immunosuppressive or radiation therapy. Exceptions to this proviso are children with HIV infection who are not severely immunocompromised; measles-mumps-rubella vaccines are recommended in these children, and varicella vaccine should be considered if age-specific $CD4^+$ T-lymphocyte values are 15 percent or more. In the United States, BCG is contraindicated for HIV-infected children, although the WHO recommends giving BCG to asymptomatic HIV-infected children in regions with high tuberculosis prevalence. All HIV-infected children should, however, have tuberculin skin testing performed before and after traveling. Yellow fever vaccination is contraindicated in HIV-infected persons by the Advisory Committee on Immunization Practices, but it is recommended by the WHO for those with asymptomatic HIV infection who reside in endemic areas.[91,119,171]

PREVENTION OF MALARIA IN IMMUNOCOMPROMISED CHILDREN

No data have been published to indicate that immunocompromised travelers are at increased risk for acquiring malaria or having more severe disease if they are infected, except in persons who are HIV infected and pregnant.[18] Before prescribing malaria chemoprophylaxis, clinicians should check for possible drug interactions for immunocompromised children receiving other medications. Counseling should emphasize the importance of exercising personal protection measures against malaria and using recommended chemoprophylaxis. Immunocompromised patients may be at risk for acquiring a variety of infections that are manifested as febrile illnesses; parents should be advised to seek expert medical advice early in the course of a febrile illness to ensure that the child receives the appropriate diagnosis and effective treatment.[36]

PREVENTION OF TRAVELER'S DIARRHEA AND OTHER INFECTIOUS DISEASES IN IMMUNOCOMPROMISED CHILDREN

During travel to developing countries, immunocompromised children may be at higher risk for acquiring food- and water-borne diseases than when they are in the United States. At present, data are too limited to determine whether the risk and incidence of traveler's diarrhea are increased in this population; however, good evidence indicates that the disease is more severe or prolonged in immunocompromised travelers.[36] Intensive counseling on the effective use of food and beverage precautions for this population is essential to minimize and to prevent morbidity and mortality. Water-borne infections also can result from swallowing water during recreational water activities. To reduce the risk of acquiring diseases such as cryptosporidiosis and giardiasis, parents should be advised that children should avoid swimming in water that might be contaminated and that their children

should be encouraged to not swallow water during swimming. In addition, many tropical and developing countries have high rates of tuberculosis; some immunocompromised persons, such as HIV-infected travelers, may be at greater risk for acquiring tuberculosis, especially during long-term travel.[127] BCG vaccination is contraindicated in HIV-infected persons. Chemoprophylaxis may be appropriate for some individuals visiting or living in high-risk areas for a prolonged period. When immunocompromised children travel abroad, they may encounter new organisms not endemic in their own countries; these organisms will pose variable risks based on their degree of immunocompromise. Health care providers should identify travel-specific risks and instruct parents in ways to reduce the risk of contracting infection. Other pathogens that can lead to more severe infection in immunocompromised travelers include visceral leishmaniasis and fungal infections (including *Penicillium marneffei*, coccidioidomycosis, and histoplasmosis).[18] Leishmaniasis is endemic in the Mediterranean coastal regions of southern Europe and northern Africa. *P. marneffei* poses a risk for travelers to the Far East, and histoplasmosis affects those traveling to Latin America. Few data exist on the efficacy of primary prophylaxis for opportunistic infections such as leishmaniasis and penicilliosis; therefore, avoidance of areas of high prevalence may be prudent if the child has severe immunodeficiency or advanced HIV disease.[73]

TABLE 242–6 International Travelers' Health Information, Recommendations, and Outbreak Notices

Resource	Contact Information	Information and Services Provided
Centers for Disease Control and Prevention (CDC) Travelers' Health Information	www.cdc.gov/travel/	U.S. travelers' health recommendations by destination region and country, including malaria chemoprophylaxis and vaccinations; links to other sites, travel health warnings, advisories, and outbreak notices
CDC Health Information for International Travel (Yellow Book)	wwwn.cdc.gov/travel/contentYellowBook.aspx, or order from Elsevier Publishers at www.us.elsevierhealth.com	General travelers' health information, region- and destination-specific recommendations, including malaria chemoprophylaxis and vaccinations
CDC Malaria Branch	www.cdc.gov/malaria/travel/index.htm 770-488-7788 (8:00 AM to 4:30 PM EST, Monday through Friday) 404-639-2888 (4:30 PM to 8:00 AM EST, weekends and holidays, ask operator for staff on call for Malaria Epidemiology Branch)	Information about malaria prophylaxis and treatment, intended for use by health care professionals
CDC Morbidity and Mortality Weekly Report, Recommendations and Reports, Surveillance Summaries	www.cdc.gov/mmwr	Reports on U.S. and international outbreak investigations, disease surveillance summaries, reports and recommendations
CDC National Immunization Program	www.cdc.gov/vaccines/	Immunization information
U.S. Immunization Schedules	www.cdc.gov/nip/menus/vaccines.htm#Schedules	Immunization schedules for children, adolescents, and adults
Immunization Action Coalition	www.immunize.org	Immunization information and educational material for practitioners and parents
National Network for Immunization Information (NNii)	www.immunizationinfo.org	Information for public and health professionals about immunization Site includes immunization news, a vaccine information database, and a guide to evaluating vaccination information on the Web
U.S. State Department	www.travel.state.gov	General information about travel, including safety, visa requirements, links to individual embassies and consulates
World Health Organization (WHO) Health Topics	www.who.int/topics/en/	Information and maps of health topics and diseases
WHO Yellow Book, International Travel and Health	www.who.int/ith/en/	General travelers' health recommendations; country-specific malarial risks and recommendations
WHO, Outbreak News	www.who.int/csr/don/en/	Notifications of recent infectious disease outbreaks
WHO, Weekly Epidemiological Record	www.who.int/wer/en/	Global disease surveillance and WHO program updates
Health Canada, Travel Medicine Program	www.phac-aspc.gc.ca/tmp-pmv/index.htm	Canadian recommendations for travelers' health; outbreak information
EuroSurveillance	www.eurosurveillance.org	Surveillance and outbreak information for European nations; weekly and monthly surveillance reports and outbreak notices
ProMED (Program for Monitoring Emerging Diseases)	www.promedmail.org	E-mail postings; verified and unverified reports on emerging diseases and outbreaks
University of Texas at Austin	www.lib.utexas.edu/maps	World maps on-line
American Society of Tropical Medicine and Hygiene (ASTMH)	www.astmh.org 847-480-9592	Directory of travel medicine clinics and practitioners certified by ASTMH
International Society of Travel Medicine (ISTM)	www.istm.org 800-433-5256	Directory of travel medicine clinics and practitioners with ISTM affiliation

GENERAL TRAVEL HEALTH COUNSELING FOR CHILDREN

During the pre-travel assessment, the clinician should provide the parents with general advice and preventive counseling to avoid health risks and injuries in children. Simple personal protection measures can prevent children from incurring many types of travel-associated injuries and conditions. The most common skin condition reported by travelers is sunburn. To prevent sunburn and the subsequent risk of skin malignant neoplasms later in life, children should avoid sun exposure during peak hours and use sunscreen appropriate for the child's age. When sunscreen and insect repellent are used concomitantly, the sunscreen should be applied first. Injuries, drownings, and motor vehicle accidents pose a great risk for morbidity and mortality among international travelers.[60,125] The risk of motor vehicle–related death generally is many times higher in developing countries than in the United States. Parents traveling with infants or young children should be advised to bring their own child safety restraint seats for use during travel. The use of car seats and seat belts for children, preferably sitting in the rear seat, should be emphasized. Unfortunately, in some developing countries, cars with seat belts may not be readily available. Night driving can be more hazardous, especially outside urban areas in developing countries, and should be avoided.[18] Use of a helmet is imperative for bicycle travel. Fire injuries can be prevented by advising parents to inquire whether hotels have smoke detectors and sprinkler systems (they also may consider bringing their own smoke detector). Other major causes of injury and trauma include drowning and boating accidents. Parents and children should be counseled to be aware of weather conditions and forecasts, and parents should be advised that an adult should accompany children at all times when swimming. Personal floatation devices should be used when boating or water skiing, regardless of the distance to be traveled or swimming ability.[131]

Swimming in contaminated water can result in skin, eye, ear, and some intestinal infections; for prevention of infectious diseases, generally only pools that contain chlorinated water can be considered safe. Parents also should be advised that swimming in fresh-water lakes and streams in developing countries carries a risk of contracting schistosomiasis, leptospirosis, and primary amebic meningoencephalitis.[18] In salt-water bodies, biting and stinging fish, corals, and jellyfish also can be a risk. Parents of pediatric travelers, especially to areas endemic for rabies, should be reminded to warn children that they should not attempt to pet, handle, or feed domestic or wild animals (including monkeys). Travelers also should avoid snakes because most bites are the direct result of handling, harassing, or trying to kill snakes. In general, children should wear covered footwear rather than sandals, including when wading on reefs and swimming.[18,131] Children should avoid walking barefoot, particularly in rural areas, to prevent cutaneous larva migrans, hookworm, and *Strongyloides* infections.

THE PEDIATRIC TRAVELER'S HEALTH KIT

Children traveling to developing countries may have unique exposures, particularly if they are traveling to remote locations or will have close contact with other children. Consideration should be given to inclusion of the following medications as part of the traveler's health kit: rectal preparations (suppositories) of selected medications, such as acetaminophen; topical treatments for lice and scabies for pediatric travelers to developing countries who may have extensive contact with local children; and a topical antibiotic, such as mupirocin, for bacterial skin infections.[19] Adults accompanying these children and teenagers should consider obtaining basic first aid and safety training before departure.

INTERNATIONAL TRAVEL INFORMATION RESOURCES

One of the most important functions that a clinician can fulfill in preparing a pediatric patient and parents for international travel is to provide up-to-date and accurate travel health information and recommendations for preventing illness. Many varied sources of information are available, including software packages and databases designed specifically for use in travel medicine and pre-travel care.[54,75] Increasingly, the Internet and computer-based travel resources are being used by practitioners and consumers alike because they provide current information to appropriately and effectively counsel and treat international travelers. Two reviews have provided comprehensive summaries of travel medicine resources.[54,75] Table 242–6 is a summary of some selected travel health resources that can be useful for providing health care professionals and parents with essential information on health risks in specific travel destinations (including endemic or epidemic diseases) and current travel health recommendations (including immunizations and chemoprophylaxis). Travelers also should be aware of the need for health insurance and emergency evacuation coverage during travel and medical assistance in the event of a travel-related illness; a variety of companies offer these services, and travelers should investigate options before making any purchases. This summary is not meant to be comprehensive; because resources are constantly changing, clinicians are advised to review and to compare sites for availability and updated information and recommendations.

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CHAPTER 243

Infectious Disease Considerations in International Adoptees and Refugees

Margaret K. Hostetter

More than 20,000 internationally adopted children enter the United States each year to begin life with their new families. The demographics of this process have changed considerably since the mid-1980s, when approximately 70 percent of internationally adopted children spent their infancy in two-parent foster homes in Korea. Since 1991, China, Russia, and eastern Europe are the countries of origin for more than 70 percent of internationally adopted children; most of these children spend weeks to years in orphanages and other state-run institutions, variably called nurseries, baby homes, or hospitals. Although all internationally adopted children present special health considerations, the institutionalized child is particularly at risk for acquisition of numerous infectious diseases as well as for developmental and emotional handicaps ascribable to institutional care.

PRE-ADOPTION ENVIRONMENTS IN THE COUNTRIES OF ORIGIN

Children coming from the state-subsidized foster homes of Korea receive optimal nutrition, nurturing, and preventive health care. The Korean foster home system chooses only two-parent families who have successfully reared their own children. Foster parents must meet defined dates for medical follow-up, continuity of care, and immunizations. Both boys and girls may be adopted, but the Korean procedure has numerous stipulations about age, physical condition, and household income of the prospective adoptive parents. Korean infants typically are adopted when they are between 6 and 13 months of age.

Because Chinese culture stipulates that the son must care for his aging parents, most infants surrendered for adoption under China's "one child" policy are girls. These girls typically are adopted in the United States when they are between 8 and 15 months of age. The standard of care in Chinese orphanages is certainly acceptable in terms of nutrition, but hygiene and immunization practices can be variable. At times, the child's stated age may be younger than the actual age because growth parameters and developmental attainments then appear more favorable.

Children coming from Russian or eastern European orphanages have highly variable lengths of stay in pre-adoption care. A substantial number of Russian adoptees have remained in institutions for more than 2 years. Children can be removed from their biologic families at any time, and it is not uncommon to find sibling groups who have been separated because of parental abuse or alcoholism. Immunizations may be erratic and, although recorded, may not have been administered. Anecdotal reports of caseworkers filling out immunization sheets as the child is leaving with the parents are not unusual. The varying backgrounds of Russian or eastern European adoptees, the variable ages at the time of adoption, and the unpredictable nature of the pre-adoption environment carry implications not only for medical but also for developmental health.

TABLE 243–1 Screening Tests for Internationally Adopted Children

Hepatitis B virus surface antigen (HBsAg) and antibodies to hepatitis B surface and core antigens
Hepatitis C virus antibody
Human immunodeficiency virus (HIV) antibodies by ELISA and DNA PCR
Varicella-zoster virus antibody
Fecal examination for parasite ova and *Giardia* antigen
VDRL (or RPR) and FTA-ABS
PPD (Mantoux) skin test with control for adequacy of the delayed hypersensitivity response
Complete blood count with erythrocyte indices
Serum lead level
Thyroid-stimulating hormone level
Urinalysis
Hearing screening
Vision screening
Developmental evaluation

ELISA, enzyme-linked immunosorbent assay; FTA-ABS, fluorescent treponemal antibody absorption assay; PCR, polymerase chain reaction; PPD, purified protein derivative; RPR, rapid plasma reagin; VDRL, Venereal Disease Research Laboratory.

TABLE 243–2 Prevalence of Hepatitis B and C by Country of Origin

Country	Hepatitis B[16]	Hepatitis C[1,14,25]
Central and South America	2-7%	1-1.9%
China	8-10%	2-2.9%
Eastern Europe	2-7%	2-2.9%
Russia (former USSR)	2-10%	2-2.9%
South Korea	8-10%	1.7%

TABLE 243–3 Interpretation of Hepatitis B Profile

Status	HBsAg	Anti-HBc	Anti-HBs	AST and ALT
Carrier	+	–	–	Normal
Acute infection	+	IgM	–	Elevated
Chronic infection	+	IgG	–	Normal to elevated
Past infection	–	IgG	IgG	Normal
Passive transfer	–	IgG	IgG	Normal
Vaccination	–	–	IgG	Normal

ALT, alanine aminotransferase; AST, aspartate aminotransferase; IgG, immunoglobulin G.

Other countries also serving as sources of international adoptees include Central and South America, India, Vietnam, Cambodia, and Ethiopia. Often, little or no information about the child's birth circumstances, maternal health, or pre-adoption environment is available. In Guatemala, approximately 50 percent of adoptees spend time in foster homes for which subsidies are paid by the potential adopting parents. The remainder reside in orphanages or mixed-care environments before adoption.[17] Orphanages run by religious groups in Central America and Colombia frequently provide prenatal care for single mothers and excellent postnatal care for their infants. Most children from India come from orphanages where limited resources may impair the ability of the orphanage workers to meet all the needs of the children in their care. Children from Vietnam and Cambodia often are adopted at an early age, which mitigates the effects of less than optimal nutrition and truncates potential time of exposure to cases of tuberculosis among caregivers.

To my knowledge, screening for tuberculosis is not required in any of the countries in which children are adopted from orphanage settings, nor have standards of sanitation in orphanages been mandated or accepted worldwide. As a result, the highly varying environments from which these children come carry substantial implications for their health. This chapter addresses many of the issues with which the infectious disease specialist must be familiar.

SCREENING TESTS

Screening tests currently recommended by the American Academy of Pediatrics should be applied to internationally adopted children without regard to age, sex, or country of origin (Table 243–1). Many pre-adoption referrals will report results of medical testing done in the country of origin, but any test done in the country of origin should be repeated when the child arrives in the United States. In my experience, results of testing done in the country of origin are wrong at least 10 percent of the time, especially with regard to hepatitis B.

As shown in Table 243–2, the reported prevalence of hepatitis B and hepatitis C may vary widely, depending on the country of origin. The remainder of this section discusses specific infectious diseases of particular importance to children, parents, and physicians.

VIRAL INFECTIONS

Hepatitis A, B, and C

Because hepatitis A is endemic in the developing world, routine testing for hepatitis A immunity is not recommended. If the adoptee is involved in a situation in which vaccination for hepatitis A is a possibility (e.g., daycare outbreak, travel to a country in which the virus is endemic), antibody testing should be done to determine the need for vaccination.

The prevalence of hepatitis B among internationally adopted children varies with the carriage rate for this virus among women of child-bearing age (see Table 243–2). As a result, parents planning to adopt from most countries in Asia (Cambodia, China, India, or Vietnam) should be informed that the chance of the child's having hepatitis B infection may be as high as 10 percent in unvaccinated children; parents planning to adopt from Russia or eastern Europe should know that the prevalence of hepatitis B ranges from 2 to 20 percent, the latter in some regions of Romania.[13]

Accurate testing for hepatitis B infection, past infection, or vaccine-induced immunity involves the hepatitis B profile. Table 243–3 delineates common patterns of serologic responses to the three components of the hepatitis B profile: hepatitis B surface antigen (HBsAg) and antibodies to hepatitis B surface and core antigens (anti-surface and anti-core antibodies). The use of all three tests is essential to determine which category best fits the child's serologic pattern. A substantial proportion of hepatitis B infections in internationally adopted children is misdiagnosed as "carrier state" when HBsAg is present but testing for immunoglobulin G (IgG) anti-core antibodies is omitted.[12] The child who is positive for HBsAg and IgG anti-core antibodies for more than 6 months meets the definition for chronic hepatitis B; approximately 90 percent of children exposed to hepatitis B during the first 12 months of life will develop chronic infection, and approximately 10 percent of these children will go on to develop cirrhosis or hepatocellular carcinoma. Therefore, the finding of a positive HBsAg response never should be dismissed as indicative of the "carrier state" in the absence of testing for IgG anti-core antibodies. Treatment with interferon α-2b may be beneficial in cases of chronic infection. Interferon α-2b and ribavirin in combination or lamivudine alone is approvid for children older than 2 years of age.

Additional testing in children positive for HBsAg should include the hepatitis Be antigen, aspartate and alanine aminotransferases, and hepatitis D antibodies. The coexistence of hepatitis Be antigen increases the risk of infectivity. Hepatitis D (delta virus) occurs only in conjunction with hepatitis B and typically is acquired in countries such as southern Italy, parts of eastern Europe, South America, Africa, and the Middle East. Co-infection with hepatitis D enhances the development of chronic liver disease and the rate of cirrhosis in patients infected with hepatitis B. Practitioners should be aware that administration of the hepatitis B vaccine can lead to a brief period of antigenemia if testing for HBsAg is performed within 2 or 3 days of vaccination.

Hepatitis B immunization is given appropriately in Korea, and this national policy has virtually eliminated hepatitis B infection in Korean adoptees.[12] However, although the Korean government has practiced universal immunization for hepatitis B since 1989, premature infants or the rare abandoned child may not have been vaccinated within the first week of life. Hepatitis B immunizations are given erratically if at all in every other country from which internationally adopted children come. Health care providers should review the timing of hepatitis B immunizations: optimally, the first is given within the first week of life; the second, 1 month later; and the third, 6 months after the first. In many countries, the administration of the initial hepatitis B immunization is delayed for several weeks or months. This delay of course means that the child is not protected from the acquisition of hepatitis B either by vertical transmission from the mother or by horizontal transmission in the orphanage during this time.

Most children who have received at least two hepatitis B vaccines should have anti-surface antibodies (anti-HBs). Vaccination does not elicit anti-core antibodies (anti-HBc). Children who do not have anti-surface antibodies after receiving two or more recorded vaccines probably have not received the immunizations indicated on their certificates, and the entire hepatitis B series should be repeated.

Hepatitis B vaccination also is essential for household contacts, including family members and home daycare contacts. Although most infants in the United States will have received hepatitis B vaccine and thereby are protected from horizontal transmission in a daycare center, daycare mothers or other daycare workers themselves often are not immunized. The primary caregiver is the one to whom hepatitis B is transmitted most frequently; therefore, the physician should provide counseling to the adoptive parents as to the necessity of immunizing household contacts and home daycare providers who will be caring for any child who is HBsAg positive.

On the basis of prevalence rates determined by the World Health Organization (see Table 243–2), the risk of hepatitis C being present in internationally adopted children is thought to be significantly lower, perhaps around 1 percent,[21] although no study of the prevalence of this infection in internationally adopted children has been published. Between 2 and 8 percent of infants born to infected mothers will acquire the virus; data on transmissibility from breast milk are somewhat more controversial. Numerous studies have documented the presence of hepatitis C RNA in breast milk, but studies looking for infection after breastfeeding typically have found little if any evidence that breastfeeding is a major source of infection.[24,26]

The hepatitis C enzyme immunoassay is the screening test of choice. If the result is positive, the physician may choose either the recombinant immunoblot assay or a direct measure of viral RNA by polymerase chain reaction for confirmation. The polymerase chain reaction test is preferred by many adoption medicine specialists because it is specific for infection and helpful for identifying infection in infants in whom passive transfer of maternal antibody may confound the clinical picture. Documentation of aspartate and alanine aminotransferase levels for those children who are positive by antibody test also is important.

The prognosis of children with hepatitis C infection is unclear. Although the disease is thought to be benign in many children, there are anecdotal reports of internationally adopted children with more aggressive presentations. Children with bridging necrosis or active cirrhosis on liver biopsy or with persistently elevated serum transaminase concentrations exceeding twice the upper limits of normal should be referred to a gastroenterologist for further management, which typically includes tests for alpha-fetoprotein concentration and abdominal ultrasonography. Eight studies of interferon α-2b therapy have been undertaken in children, and one pediatric study has used combination therapy with interferon α-2b and ribavirin.[9]

Human Immunodeficiency Virus Infection

Detection of unsuspected human immunodeficiency virus (HIV) infection in internationally adopted children after arrival in the United States is quite rare. However, because the results of the HIV enzyme-linked immunosorbent assay (ELISA) may be confounded by the presence of passively transferred maternal antibodies in children younger than 18 months, some experts recommend testing for HIV DNA by polymerase chain reaction on arrival. A secondary advantage to this test is that horizontal transmission ensuing within the previous 1 to 2 months theoretically will be detected, even though the length of time has not been sufficient for the child to develop an antibody response. Other experts recommend a repeated ELISA for HIV 6 months after the child arrives in the United States. When the diagnosis is confirmed, management by a pediatric HIV infection specialist is essential.

BACTERIAL INFECTIONS

Helicobacter, Salmonella, Shigella, Campylobacter, and *Yersinia*

In a single retrospective study, colonization with *Helicobacter pylori* as assessed by antibodies to this organism was found in 31 percent of 226 adopted children from 18 countries.[19] Seropositive children were more likely to be older at the time of adoption and to have co-infection with intestinal parasites, but no significant differences in height, weight, anemia, or diarrhea among seropositive versus seronegative adoptees were found.[19] Interestingly, all seropositive children reported having spent some time in an orphanage; none of the 25 children who resided completely in foster care was seropositive. Because no prospective, controlled studies in adoptees have assessed the long-term effects of colonization or the utility of pediatric treatment in preventing resumption of colonization or progression to ulcers or cancer, neither routine testing for *H. pylori* nor presumptive therapy is indicated.

Although routine testing for ova and parasites is recommended for all international adoptees (see Table 243–1), the utility of stool culture for identifying bacterial pathogens in asymptomatic children is controversial. Prospective series put the rate of bacterial gastroenteritis between 2.5 and 7 percent.[12,18,23,27] Because of transmissibility, some practitioners test for salmonellae; although this organism may be present in asymptomatic children, treatment prolongs excretion and therefore is not recommended for otherwise normal children older than 3 months. Children with diarrhea should be evaluated for a number of pathogens, including *Salmonella*, *Shigella*, *Campylobacter*, and *Yersinia*; rotavirus and caliciviruses, such as *Norovirus;* and intestinal parasites (see later).

Syphilis

Maternal syphilis is a major problem in Russia, eastern Europe, and Guatemala. Treatment in these countries often is erratic or inadequate; foreshortened courses, use of oral agents, and poor documentation of treatment are common findings in pregnant women. Referral paperwork on many adoptees states that "preventive treatment for syphilis was given," but these regimens can vary dramatically from a few days of oral antibiotic therapy to the more appropriate 21-day course of intramuscular penicillin.

As a result, testing for syphilis with both a non-treponemal (Venereal Disease Research Laboratory [VDRL] or rapid plasma reagin) test and a treponemal test (fluorescent treponemal antibody absorption [FTA-ABS]) is essential for internationally adopted children, even in the face of purportedly negative test reports from abroad. The combination of the VDRL and FTA-ABS tests is extremely useful, especially in infants young enough to have experienced passive transfer of maternal antibodies. Mothers infected with syphilis before the child's birth will have a positive FTA-ABS assay response, and the antibody will be passed to the young infant, even if the VDRL test result in the child has been confounded by partial therapy in the nursery or orphanage. When the FTA-ABS or the VDRL test result is positive, consideration should be given to obtaining spinal fluid, long bone films, and hepatic transaminase values before the initiation of parenteral penicillin therapy. Because syphilis is an entirely curable disease and the complications of untreated or partially treated congenital syphilis involve not only intellectual impairment but disfiguring physical complications such as Hutchinson incisors, saber shins, and gummata, accurate determination of the adoptee's syphilis status is essential.

For the child found to have congenital infection, treatment with 10 days of intravenous penicillin G (100,000 to 150,000 units/kg/day) should be followed by repeated VDRL levels at 3- to 6-month intervals for up to 24 months to ensure a fourfold reduction in titer. Children with a positive VDRL test result in the spinal fluid will require a repeated lumbar puncture 6 months after receiving treatment.

Although congenital syphilis is by far the most common presentation, my experience also has included cases of primary and secondary syphilis acquired in situations of abuse. Differentiation among these causes of positive serologic results has important implications for treatment.

Tuberculosis

The American Academy of Pediatrics has mandated that the purified protein derivative (PPD; Mantoux test) be used to test skin reactivity in children coming from areas in which tuberculosis is endemic. Despite this recommendation, many pediatricians still use the tine test or do not test at all if the adoptee has received bacillus Calmette-Guérin (BCG) vaccine. Both of these practices are incorrect. All internationally adopted children should be tested with PPD (and a *Candida* skin test or other control for delayed-type hypersensitivity) as soon after adoption as is feasible. As many as 30 percent of adoptees may be anergic within the first 1 to 2 months after arrival; when both skin test results are negative shortly after adoption, the PPD test and the control for delayed-type hypersensitivity should be repeated within 4 months.[11,12] Children who have received the vaccine by report and whose left (or more rarely right) deltoid shows the typical scar may be tested with the PPD approximately 12 months after application of the BCG vaccine without inducing a false-positive reaction. Prospective studies in Native American children given BCG at birth have demonstrated that cross-reactivity between BCG and PPD is maximal 6 months after vaccination and wanes thereafter.[15] Concerns about a sloughing reaction if PPD is placed after administration of BCG vaccine are exaggerated and should not stand in the way of placing a Mantoux skin test.

The Mantoux test should be read by trained medical personnel, not by parents. If the PPD response shows more than 10 mm of induration in the transverse diameter, chest radiography should be performed. If the chest radiograph is normal, the child should receive 10 mg/kg of isoniazid once a day for 9 months. The 6-month course of isoniazid for latent tubercolosis is no longer recommended. Parents should be instructed that isoniazid as prepared by many commercial pharmacies may be poorly tolerated from the standpoint of taste and gastrointestinal complaints. Under those circumstances, physicians should instruct the pharmacist to prepare a low-sucrose formulation in an appealing flavor. A little extra work by the physician to ensure palatability of isoniazid goes a long way toward maintaining compliance.

A child with a positive PPD response and an abnormal chest radiograph should be managed by an infectious disease specialist. Acid-fast bacillus culture and stains should be performed on sputum or gastric aspirates, depending on the age of the child. Resistance to multiple antituberculous agents can be a problem in the countries of Southeast Asia and in some areas of Russia and eastern Europe; as a result, unusual regimens may need to be implemented for individual patients on the basis of the drug susceptibility of their isolates.

In children older than 5 years with a positive PPD reaction and a normal chest radiograph, consideration should be given to extrapulmonary disease of the spine or genitourinary tract. Standard evaluation in these cases should include a sedimentation rate, imaging of the lumbosacral spine with appropriate shielding, and three first-morning voided urine specimens as well as urinalysis to look for hematuria and sterile pyuria. Pulmonary or extrapulmonary disease requires at least three antituberculous agents for 2 months while awaiting sensitivity data.

Although transmission of tuberculosis to school or family contacts is rare in children younger than 6 years who cannot generate sufficient tussive force to spread respiratory droplets, household contacts of the child with a positive PPD reaction also should undergo PPD testing. Cavitary disease, although an unusual occurrence among children, is virtually always infectious for others, and failure to place a Mantoux skin test or to interpret it correctly can lead to widespread dissemination among multiple contacts.[8]

PARASITIC INFECTIONS

The presence of intestinal parasites is a common concomitant of orphanage confinement but is exceedingly rare in Korean adoptees raised in foster homes. A single stool sample for ova and parasites is informative approximately 85 percent of the time[7]; however, detection is improved when multiple specimens are examined.[10,22] Ova of *Ascaris lumbricoides* will not be found in the stool sample if the harbored worm is either a male or an unfertilized female, but the subsequent passage of the worm may cause great consternation for the parent. Apprising the parents of this possibility saves recriminations later.

Administration of albendazole before the child's arrival may limit the number of parasites found in internationally adopted children,[20] but certain parasites, such as *Giardia lamblia*, are not killed by this regimen. Because of the potential to transmit *Giardia* in the daycare setting, most children should be treated, even when they are asymptomatic. The drug of choice is metronidazole, although the liquid formulation of furazolidone typically is more palatable for children. Tinidazole and nitazoxanide also are appropriate. For symptomatic children in whom giardiasis is suspected, a stool antigen test, in addition to examination for ova and parasites, enhances the likelihood of establishing the diagnosis.[30]

Although not yet reported in internationally adopted children, *Cyclospora*, *Cryptosporidium* spp., *Isospora belli*, and *Microsporidia* spp. have caused diarrhea in immunocompetent children[30]; thus, these agents should be sought in diarrheal illness that does not yield other pathogens. Treatment of *Blastocystis hominis*, *Chilomastix mesnili*, *Trichomonas hominis*, *Trichomonas tenax*, *Entamoeba coli*, and *Iodamoeba buetschlii* is not necessary because these organisms are not pathogenic, although some experts recommend treating immunocompromised children with *B. hominis*. Repeated stool samples for ova and parasites should be performed at the close of a treatment course if the child remains persistently symptomatic or if more than one organism was isolated on the original screen.

LICE AND SCABIES

Scabies is a consequence of the orphanage environment and as such is found infrequently in Korean adoptees and more commonly (7% to 10%) in those from other countries.[21] Pruritic papules on the wrists, ankles, palms, or soles are the major diagnostic clues. Treatment entails the topical application of 5% permethrin cream; a second application one week later may be necessary. Head lice *(Pediculus capitis)* are found more frequently than are body lice *(Pediculus corporis)* or pubic lice *(Phthirus pubis)*. Topical 1 percent permethrin, 1 percent lindane, or 0.5 percent malathion is effective for most cases of head lice, but treatment failures due to emerging resistance may require two doses of oral ivermectin, spaced at a 10-day interval.

OTHER TESTS FOR INFECTIOUS DISEASES IN INTERNATIONAL ADOPTEES

The microcephalic child represents a perplexing dilemma. Studies have shown that head circumference may be inversely correlated with length of time in the orphanage setting,[13] and a downward trend of head circumference from the percentile recorded at birth is common in adoptees reared in orphanages. Microcephaly is a common feature of fetal alcohol syndrome, but testing for treatable causes, such as toxoplasmosis and syphilis, should be undertaken before the diagnosis of fetal alcohol syndrome is made. Although entities such as rubella, cytomegalovirus, and herpes simplex virus are prognostically highly important as a cause of microcephaly, routine testing of normocephalic adoptees for rubella, cytomegalovirus, herpes simplex virus, or toxoplasmosis is not recommended. Routine screening tests for malaria are not warranted in asymptomatic international adoptees.

Internationally adopted children from Mediterranean regions, Middle Eastern countries, and China should be tested for glucose-6-phosphate dehydrogenase deficiency if administration of nalidixic acid, nitrofurantoin, or sulfonamide is contemplated.

VALIDITY OF MEDICAL RECORDS AND MEDICAL TESTING IN THE COUNTRY OF ORIGIN

Parents may be inappropriately encouraged to accept "normal" results when screening is performed in the country of origin. Parents should be cautioned that many countries have no standard accreditation of laboratory personnel and that results may be haphazard, inaccurate, or falsified. For these reasons, medical testing should be repeated as soon as possible after the child arrives in the United States.

A major area of debate at present is the validity of immunization records from countries other than South Korea. A careful analysis of both the number of immunizations and the interval between them more often than not detects aberrancies. Immunizations that antedate a child's recorded birth date, that are given on the same day of the month (e.g., 8/7/00, 9/7/00, 2/7/01), or that are administered in orphanages without refrigeration are suspect.

DPT AND POLIO SERIES

In prospective studies, an evaluation of titers to diphtheria, tetanus, and polio in 98 Chinese orphans whose records indicated complete DPT and OPV series found that as many as 30 percent lacked detectable antibodies, as compared with less than 1 percent in age-matched Dutch children.[28] In a retrospective chart review, only 28 percent of 103 Guatemalan adoptees' vaccination records met U.S. standards.[17] Another retrospective study reviewed immunization records of 504 children from 16 countries; only 35 percent had documented vaccination for DPT (diphtheria, pertussis, tetanus), hepatitis B, or MMR (measles, mumps, rubella), and of these, only 62 percent were up to date with one or more series according to the U.S. schedule.[29]

On the basis of these reports, the Advisory Committee on Immunization Practices has recommended re-administration of the entire DPT series (and IPV) if any departure from the U.S. vaccination schedule is found in terms of the number of vaccinations, the interval between them, or the child's age at immunization. Table 243–4 presents some approaches to immunizations in international adoptees. In my 20 years of experience with reimmunization of children with questionable vaccination certificates, I have encountered not a single incident of toxicity with this approach. At issue here is not so much the infant who may have received one immunization abroad and will certainly receive a protective complement here in the United States; rather, the focus of concern should be the older child, age 2 years and older, whose immunization certificate may be inaccurate. If not reimmunized, such a child is at risk for acquiring tetanus in the United States or other diseases such as polio or diphtheria that may be acquired during international travel.

IMMUNIZATION FOR MEASLES, MUMPS, AND RUBELLA

Countries other than Korea either omit the MMR vaccine entirely or give only the measles component, typically when the child is 6 to 12 months of age. Two outbreaks of measles in Chinese adoptees and caregivers traveling to the United States impugn the reliability of vaccination certificates from orphanages. The first outbreak encompassed 14 cases: 10 among adoptees and 4 secondarily transmitted to siblings and caregivers aged 28 months to 47 years.[2] The second outbreak among 12 Chinese adoptees resulted in 10 cases across three states (Washington,

TABLE 243–4 Approaches to Immunizations in Internationally Adopted Children

Accept all appropriate pre-adoption records of immunization.
Measure serum antibodies against diphtheria, tetanus, poliovirus (serotypes 1, 2, and 3), hepatitis B, varicella, measles, mumps, and rubella. If antibody levels are appropriate for age, accept all corresponding pre-adoption records of immunization.
Measure serum antibodies against vaccine-preventable diseases (above); if normal for age, consider the child to have received one immunization in the series.
Accept no pre-adoption records of immunization; immunize the adoptee with all vaccinations appropriate for age.

Modified from Murray, T. S., Groth, M. E., Weitzman, C., et al.: Epidemiology and management of infectious diseases in international adoptees. Clin. Microbiol. Rev. 18*:510-520, 2005.*

Maryland, and New York); nine of these cases occurred in adoptees aged 12 to 18 months and the tenth in an exposed, unvaccinated adult aged 19 years.[3-6] In both outbreaks, the infected adoptees were old enough to have been vaccinated under Chinese policy. For children coming from countries other than Korea, MMR vaccine should be given when the child is 12 to 16 months old, 4 to 6 years of age, and again in middle school.

POLYSACCHARIDE VACCINES

Polysaccharide conjugate vaccines for *Haemophilus influenzae* type b and *Streptococcus pneumoniae* serotypes are not administered routinely in any countries other than the United States and western Europe. These vaccines should be given to internationally adopted children as part of the immunization series in the United States.

TRAVEL MEDICAL KIT

Many parents are encouraged by adoption agencies or by information on the Internet to take antibiotics to the country of origin. Although this action may seem prudent, no antibiotic known to the author is a panacea for all infectious diseases. A child with a fever whose parents are carrying a third-generation cephalosporin may have needed treatment delayed if the real cause of the illness is methicillin-resistant *Staphylococcus aureus* or tuberculosis. For these reasons, in our clinic, we instruct parents that fever should prompt medical attention, not random dosing with an antibiotic. Teaching parents how to take a rectal temperature accurately is more important than supplying them with an antibiotic that may be outdated, inefficacious, or contaminated with nonsterile water on dilution.

INTERNET RESOURCES

Informative Web sites for prospective adoptive parents can be found at *eeadopt.org* and *www.adopting.com*. A plethora of Web sites also provides first-hand information from parents, but subjective reports should not be confused with objective information. Physicians may find the Web site *www.adoptmed.org* of value if questions arise with regard to individual patients.

INFECTIOUS DISEASES IN REFUGEE CHILDREN

The widely varying conditions from which internationally adopted children come are no more predictable for refugee children. Some will have experienced only recent disruption, whereas other children may have been displaced from parents for months to years. An accompanying parent oftentimes is helpful in providing information about immunizations and illnesses in the past medical history, but refugees from war or persecution may arrive without a knowledgeable adult.

Except in the most chaotic circumstances, refugee children who have spent some time in a camp supervised by responsible international health organizations have many advantages over internationally adopted children, for whom there are no globally accepted screening protocols (Table 243–5).

TABLE 243–5 Comparison of Refugees and Adoptees

Refugees	Adoptees*
Organized screening in camps	No organized screening
Most diseases already identified	Many medical illnesses unidentified
Medical testing accurate in camps	Medical testing inaccurate
Preventive health care under way	Preventive health care delayed

**From countries other than South Korea.*

SCREENING TESTS

No universally accepted recommendations exist for medical evaluation of refugee children. In general, in the absence of documentation of immunizations or screening undertaken in refugee camps, the most prudent course is to screen for transmissible infectious diseases that are found more commonly in the child's country of origin or that may have been transmitted during the period of displacement. Such entities include but are not limited to hepatitis B, hepatitis C, HIV infection, tuberculosis, and diarrheal pathogens such as *Salmonella*, *Shigella*, *Campylobacter*, and *Yersinia*. Illnesses such as melioidosis, typhoid fever, malaria, filariasis, and flukes may be found more commonly in refugees than in adoptees because of regional prevalence. In refugees or immigrants from regions such as Asia, the Middle East, sub-Saharan Africa, eastern Europe, Latin America and the Caribbean, the frequency of intestinal parasitosis may be high; therefore, some experts recommend presumptive treatment with albendazole as a more cost-effective strategy than screening. The pediatric dosage is 400 mg given once. Practitioners should be aware that such treatment is not effective against *G. lamblia*.

A careful history and physical examination may suggest diseases caused by geographically circumscribed entities such as leprosy, schistosomiasis, and other rarely encountered parasitic diseases. Many travel medicine clinics find it particularly useful to have a parasitologist on staff or at least to have ready access to informed consultation. Last, consideration is indicated for both refugees and adoptees of sickle hemoglobinopathies in Africa, India, and Central and South America; hemoglobin E in Southeast Asia; glucose-6-phosphate dehydrogenase deficiency in Africa, the Mediterranean, the Middle East, and China; and delta hepatitis (hepatitis D) in hepatitis B–infected individuals from southern Italy, eastern Europe, South America, Africa, and the Middle East.

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SECTION XXIV

HEALTH CARE–ASSOCIATED INFECTIONS

CHAPTER 244

HEALTH CARE–ASSOCIATED INFECTIONS

W. Charles Huskins ❂ Donald A. Goldmann

Health care–associated (nosocomial) infections traditionally have been defined as infections that develop in hospitalized patients and are neither present nor incubating at the time of the patient's admission to the hospital.[208] A more inclusive view is that health care–associated infections are infections that occur as a consequence of health care, regardless of whether they arise during hospitalization. This definition includes a surgical-site infection that occurs after an outpatient surgical procedure or a case of varicella acquired during an emergency department visit. Infections in hospital personnel caused by microorganisms acquired in the hospital also are health care–associated infections. This expanded view of health care–associated infections fits well with the increased delivery of medical care in ambulatory settings, the more comprehensive assessment of outcomes of medical care, and the occupational risk medical personnel face of acquiring infections.

In some cases, determining whether an infection is a consequence of health care may be difficult. Infections with long or variable incubation periods, such as hepatitis C or late-onset prosthetic valve endocarditis, may not be manifested until long after patients have undergone medical procedures, raising doubt about their causation. Infections in immunocompromised patients occurring during hospitalization, such as cytomegalovirus enteritis, hepatitis, or pneumonia, and even aspergillosis, often are perceived as being attributable to the patients' underlying risk of acquiring infection, to previous infection, or to colonization, although the use of immunosuppressive medications may contribute directly to the occurrence of these infections.

Despite these ambiguities, defining health care–associated infections as infections that occur among persons who are exposed in the health care environment makes sense because doing so identifies the population at risk. This definition affords us the opportunity to understand the mechanisms leading to these infections better and to design and to evaluate interventions to prevent their occurrence. This chapter discusses the general epidemiology of health care–associated infections (including those that are introduced into the hospital from the community); infections related to invasive devices, procedures, and treatments; and infections in special populations. Programmatic approaches to the prevention and control of nosocomial infections are discussed in Chapter 245.

HISTORICAL ASPECTS

The history of health care–associated infections and their control is linked tightly to developments in institutional medical care. Two publications describe this history in considerable detail.[335,540] Unfortunately, historical data of health care–associated infections in children are relatively limited. Nonetheless, ample evidence indicates that efforts to study and to prevent infection among hospitalized children have contributed significantly to the development of infection control and prevention efforts in general.

Hospitals in Europe during the Middle Ages and the Renaissance were notorious for their overcrowding and unsanitary conditions, and one can imagine that children suffered greatly from the epidemics of contagious diseases that spread through hospitals during this period. The Sanitarian movement in England in the 19th century included a focus on the egregious conditions in many hospitals. Florence Nightingale and William Farr noted the high mortality rates in hospitals and campaigned tirelessly to improve hospital design and hygiene. By the 18th and 19th centuries, sketchy information about the impact of infections in hospitalized pediatric patients began to emerge. These data, summarized in a report presented in a landmark seminar on nosocomial infections in pediatric patients at the Sixth Northern Pediatric Congress in Stockholm in 1934,[198] provide dramatic evidence that health care–associated infections were the cause of considerable morbidity and mortality in hospitalized children.

Semmelweis' classic studies of puerperal fever in the Vienna Lying-In Hospital in the mid-1800s provided insights into the etiology of perinatal infections in newborn infants, not just their afflicted mothers.[541] Semmelweis noted that rates of mortality in infants born to women in the First Division of the hospital (the division where medical students who had come from the autopsy table cared for women in labor) were several-fold higher than those in infants born to women in the Second Division of the hospital (the division where midwives cared for women in labor). In support of his theories about the infectious etiology of puerperal fever, he noted that rates of infant mortality closely paralleled rates of maternal mortality from puerperal fever and that autopsy findings were remarkably similar in infants and mothers. The opening of wards and entire hospitals designated for the treatment of patients with infectious diseases in the early 20th century stimulated interest in the study of "cross-infection" with measles, chickenpox, scarlet fever, whooping cough, diphtheria, and invasive meningococcal disease.[540] The potential for cross-infection with these classic contagious diseases on pediatric wards, especially wards caring for infants, was well recognized in the leading hospitals of the day and stimulated a number of interventions to minimize this problem. Quarantine areas for new admissions, confinement of each child in an individual cubicle, cohorting of patients admitted during community epidemics, use of masks by persons caring for patients, exclusion of visitors, and strict control of the health of nurses and physicians caring for the patients were employed to minimize the spread of contagious diseases.[59,69,261,349,362] Some hospitals even used closed cubicles with outside exhaust of air for patients with measles or varicella.[261] Analysis of the effectiveness of these interventions contributed to a better understanding of how contagious diseases are spread in hospitals.

Undoubtedly because of this vigilance, the first systematic surveys of infections in pediatric patients in hospitals in Europe

and the United States published in the 1930s and 1940s demonstrated that spread of the classic contagious diseases in hospitals occurred but was relatively uncommon.[261,349,396,471,620] However, respiratory infections of various types were encountered frequently; gastrointestinal and skin infections also were relatively common occurrences.[261,349,396,620]

Although infections caused by beta-hemolytic streptococci had been a scourge of obstetric and surgical wards for centuries, advances in diagnostic microbiology and serotyping of streptococci in the mid-1900s led to greater appreciation of the etiologic role of this organism in hospital-acquired scarlet fever, postpartum infection, postoperative infection, and secondary infection in patients with burns, measles, and influenza.[530,540] The decline of this organism as a major hospital-acquired pathogen coincided with the introduction of antibiotic therapy in the 1940s and 1950s, although a causal link between these events has not been established.[530]

Outbreaks of *Staphylococcus aureus* infection in hospitalized newborn infants had been documented in late 1800s and early 1900s,[79,314] but the pandemic of *S. aureus* infections that plagued hospitals in the 1950s and 1960s drew special attention to the impact of infections caused by this organism. Outbreaks of staphylococcal disease were particularly devastating in newborn nurseries, where epidemics caused by specific phage types caused substantial morbidity and mortality.[530,533]

The seriousness of the problem of hospital-acquired staphylococcal infection spawned more comprehensive efforts to document the impact and consequences of hospital-acquired infections and served as the impetus for development of organized infection-control programs, particularly in Great Britain (with its tradition of infection control sisters) and North America. A 1-year study of nosocomial infections in pediatric patients at The Hospital for Sick Children in Toronto was conducted in 1959.[511-513] The cumulative incidence of these infections was 6.5 percent; respiratory and gastrointestinal infections occurred most commonly. *S. aureus* caused infection in only 2.6 percent of patients overall, but it accounted for most of the surgical-site infections. Even this early study recognized the important consequences of nosocomial infections, reporting that 16 deaths and 2070 extra hospital days were the result of these infections.

In 1970, a surveillance and control program for nosocomial infections was established at Children's Hospital in Boston, and data were reported to the nascent National Nosocomial Infections Study at the Centers for Disease Control and Prevention (CDC).[201] The cumulative incidence of infection was 4.6 percent. *S. aureus* was the pathogen encountered most frequently, but more than 60 percent of the pathogens were gram-negative bacilli, including *Pseudomonas aeruginosa*, *Escherichia coli*, *Klebsiella* spp., *Enterobacter* spp., *Proteus* spp., and *Serratia* spp. Study of the epidemiology of specific pathogens in this period was hampered because few clinical laboratories performed extensive speciation at the time. Moreover, the detection of hospital-acquired viral infections was hindered by the limited availability of suitable diagnostic techniques. The Children's Hospital study emphasized the association of nosocomial infections with exposure to invasive devices and procedures (e.g., surgical-site infections in patients undergoing surgery, urinary tract infections in patients with indwelling urinary catheters, bloodstream infections and septic phlebitis associated with intravascular catheters, and ventriculitis associated with cerebrospinal fluid shunts). Additional epidemiologic studies of infections in newborn infants and children cared for in intensive care units (ICUs) during the 1970s and early 1980s strengthened the association between the use of invasive devices and procedures and hospital-acquired infections.[229,267,272,372]

Advances in viral diagnostics in the 1970s led to greater appreciation of the importance of viruses as a significant cause of nosocomial infections, particularly in pediatric patients.[610,632] Spread of respiratory and gastrointestinal viruses, especially respiratory syncytial virus (RSV) and rotavirus, was documented to be a severe problem on pediatric wards.[148,253,404,519,634]

The past 30 years have witnessed several major new developments. Gram-positive bacteria, including coagulase-negative staphylococci, *S. aureus*, enterococci, and streptococci, have re-emerged as significant pathogens in hospitalized patients.[181,236,294,424,577] Antimicrobial-resistant bacteria, such as methicillin-resistant *S. aureus* (MRSA), vancomycin-resistant enterococci, and gram-negative bacilli resistant to third-generation cephalosporins, aminoglycosides, carbapenems, and quinolones, have become especially problematic.[211,424,607] Although MRSA has received the most attention in the media and peer-reviewed literature, gram-negative pathogens, such as *Burkholderia*, *Stenotrophomonas*, and *Acinetobacter*, are potentially even more problematic because they are resistant to most (or even all) available antibiotics, and the pipeline of new active agents is virtually nonexistent. Overuse of antibiotics is partly responsible for the continued increase in the incidence of *Clostridium difficile* infections; the clonal spread of new, more virulent stains of *C. difficile* (NAP-1) is particularly alarming. As the number of severely ill and immunosuppressed children receiving care in hospitals has increased, the incidence of fungal infection, especially infection caused by *Candida* and *Aspergillus*, has increased dramatically.[1,181,191] Health care–associated acquisition of common respiratory viruses, such as RSV, parainfluenza virus, and adenovirus, can lead to severe, sometimes lethal pneumonia in these patients. Finally, the potential for health care–associated infections caused by blood-borne pathogens, such as the human immunodeficiency virus (HIV), hepatitis B, and hepatitis C, to occur among patients as well as health care providers is well recognized.[58,94,444,462,638]

In summary, the history of health care–associated infections in children is tied closely to the progress of medicine itself. New therapies and invasive procedures have had the unwanted side effect of increasing the risk of acquiring infections. Longer survival from conditions formerly causing early death and increasing numbers of immunocompromised children have resulted in a growing population of children with impaired host defenses who are at increased risk for development of infections. The selective pressure of widespread use of new, broad-spectrum antimicrobial agents has resulted in the development of previously unknown forms of antimicrobial resistance and the emergence of fungi as serious health care–associated pathogens.

GENERAL EPIDEMIOLOGY OF HEALTH CARE–ASSOCIATED INFECTIONS

Perhaps more than any other area of infectious disease epidemiology, the methodology used to study the epidemiology of health care–associated infections has itself been subjected to intense investigation and validation. This section describes the epidemiology of health care–associated infections in pediatric patients in general terms, highlighting important methodologic issues. The epidemiology of specific health care–associated infections and pathogens is discussed in later sections.

RATES OF HEALTH CARE–ASSOCIATED INFECTION

During the 1960s to 1980s, numerous hospital surveys examined rates of nosocomial infections in pediatric patients.[116,181,201,294,398,511-513,632] These surveys were useful in documenting the nature and frequency of these infections and in illustrating trends in infections caused by various pathogens (see the section on historical aspects). However, methodologic differences rendered evaluation and comparison of infection rates among these studies difficult. The types of infections studied and the definitions used to identify infections varied considerably.

TABLE 244–1 Pooled Means of Device-Associated Infection Rates by Type of Intensive Care Unit in Hospitals Participating in the National Nosocomial Infection Surveillance System

Type of Intensive Care Unit	Pooled Mean		
	Central Line–Associated Bloodstream Infections/1000 Central Line Days	Ventilator-Associated Pneumonia/1000 Ventilator Days	Catheter-Associated Urinary Tract Infections/1000 Urinary Catheter Days
Adult ICUs			
Coronary	3.5	4.4	4.5
Cardiothoracic	2.7	7.2	3.0
Medical	5.0	4.9	5.1
Medical-surgical			
Major teaching	4.0	5.4	3.9
All others	3.2	5.1	3.3
Neurosurgical	4.6	11.2	6.7
Respiratory	4.8	4.9	6.4
Surgical	4.6	9.3	4.4
Trauma	7.4	15.2	6.0
Burn units	7.0	12.0	6.7
Pediatric ICUs	6.6	4.9	4.0

Data are from January 2002 to June 2004.
Modified from National Nosocomial Infection Surveillance System: National Nosocomial Infection Surveillance (NNIS) System Report, data summary from January 1992 through June 2004, issued October 2004. Am. J. Infect. Control 32:470-485, 2004.

The sensitivity of the case-finding techniques employed and the vigor with which viral infections were sought and confirmed also varied greatly. In most cases, there was insufficient adjustment for length of hospitalization, exposure to invasive devices and procedures, case mix, and severity of illness, all of which increase the risk of acquiring health care–associated infection.

Many but not all of these methodologic concerns have been alleviated by examining rates of health care–associated infection in pediatric patients provided by the National Nosocomial Infections Surveillance (NNIS) system, now called the National Healthcare Safety Network (NHSN),[606] coordinated by the Division of Healthcare Quality Promotion at the CDC. Similar methods have been used in other countries for surveillance of health care–associated infections in pediatric patients.[490] As of 2000, more than 320 U.S. acute-care hospitals, including 70 pediatric ICUs and more than 120 high-risk nurseries (the term used by the NNIS system for newborn ICUs), were reporting data to the NNIS on the incidence of health care–associated infections.[576] Participating hospitals follow NNIS/NHSN surveillance methods, which include use of published definitions of health care–associated infections,[208] standardized coding of data, structured data collection sheets, and a microcomputer surveillance software program designed to report data to the NNIS system.[167,274] Case-finding methods are not specified in the NNIS/NHSN methodology, although most hospitals identify health care–associated infections by reviewing microbiology result reports and patient charts.[167] Post-discharge surveillance for infections such as surgical-site infection is highly variable among participating hospitals.

The accuracy of data reported to the NNIS has been evaluated in a small subset of ICUs, which did not include any pediatric ICUs or high-risk nurseries.[166] The accuracy of reports of infection varied by the site of infection. The sensitivity of surveillance for bloodstream infection, pneumonia, surgical-site infection, urinary tract infection, and other sites of infection was 85, 68, 67, 59, and 30 percent, respectively; the specificity was 98, 98, 98, 99, and 99 percent, respectively. Consequently, although current surveillance procedures in these hospitals fail to identify some infections, those infections that are reported are very likely to be true infections.

The invasive devices that are a routine part of modern hospital care are especially important risk factors for health care–associated infection, and additional adjustment of infection rates is necessary to accurately reflect these risks. Central venous catheters increase the risk of acquiring bloodstream infection, mechanical ventilation increases the risk of developing pneumonia, and indwelling urinary catheters increase the risk of developing a urinary tract infection. To adjust for exposure (as well as the duration of exposure) to these devices, infection rates can be expressed as the number of infections among individuals exposed to the device per 1000 device-exposure days. Table 244–1 contains data on the device-associated incidence density of bloodstream infections, pneumonia, and urinary tract infections for adult and pediatric ICUs in hospitals participating in the NNIS system.[424] Table 244–2 contains data on device-associated incidence density of bloodstream infections and pneumonia stratified by birth weight for high-risk nurseries in hospitals participating in the NNIS system.[424] More recent data for adult and pediatric ICUs and high-risk nurseries have been reported from the NHSN, but they include only a subset of the facilities that previously reported data to the NNIS system.[159] For this reason, NNIS system data are discussed in this chapter. The NNIS system data illustrate that the pooled mean rate of bloodstream

TABLE 244–2 Pooled Means of Device-Associated Infection Rates by Birth Weight Category in High-Risk Nurseries in Hospitals Participating in the National Nosocomial Infection Surveillance System

Birth Weight Category	Pooled Means	
	Umbilical and Central Line–Associated Bloodstream Infections/1000 Umbilical or Central Line Days	Ventilator-Associated Pneumonia/1000 Ventilator Days
≤1000 g	9.1	3.5
1001-1500 g	5.4	2.4
1501-2500 g	4.1	1.9
>2500 g	3.5	1.4

Data are from January 2002 to June 2004.
The number of participating high-risk nurseries in each of the birth weight classes ranged from 94 to 104 for umbilical and central line–associated bloodstream infections and 86 to 102 for ventilator-associated pneumonia.
Modified from National Nosocomial Infection Surveillance System: National Nosocomial Infection Surveillance (NNIS) System Report, data summary from January 1992 through June 2004, issued October 2004. Am. J. Infect. Control 32:470-485, 2004.

infection is higher in pediatric ICUs and high-risk nurseries, particularly for lower birth weight categories, than in adult ICUs (except for adult trauma and burn ICUs). Conversely, the pooled mean rate of pneumonia is substantially lower in pediatric ICUs and high-risk nurseries than in adult ICUs. The pooled mean rate of urinary tract infections in pediatric ICUs is comparable to that in adult ICUs.

On the basis of seminal studies by Freeman and others, birth weight has long been recognized as an important risk factor for health care–associated infection in newborn infants.[186,267] For this reason, rates of device-associated bloodstream infections and pneumonia from high-risk nurseries participating in the NNIS system are stratified into four birth weight strata (see Table 244–2): 1000 g or less, 1001 to 1500 g, 1501 to 2500 g, and 2500 g and more.[424] In the future, the NHSN will stratify data for the lowest birth weight category into two categories: 750 g or less and 751 to 1000 g.[159] As seen in Table 244–2, there is a progressive and marked increase in rates of bloodstream infections in sequentially lower birth weight categories such that the rate of infection in infants weighing 1000 g or less is nearly threefold higher than the rate in infants weighing more than 2500 g. Higher rates of pneumonia also occur in lower birth weight categories, but this trend is much less marked than for bloodstream infections.

Despite adjustment made for length of hospitalization, exposure to invasive devices, and birth weight (for neonates), health care–associated infection rates still vary considerably among ICUs. Boxplots of the distribution of device-associated infection rates reported by pediatric ICU and high-risk nurseries in hospitals participating in the NNIS system are displayed in Figures 244–1 and 244–2, respectively.[424] These figures illustrate that device-associated infection rates vary as much as 10-fold among units.

A portion of this variability likely is due to differences in the nature and severity of patients' underlying illnesses. Simple systems for classifying severity of illness, such as the system proposed by McCabe and Jackson[389] and the American Society of Anesthesiologists Physical Status Classification (ASA score), have been in existence for decades.[309] In the 1980s and 1990s, investigators developed and refined severity-of-illness scoring systems for adult, pediatric, and newborn ICUs. The use of these measures to adjust rates of nosocomial infections in pediatric and newborn ICUs is discussed in the following paragraphs.

The Physiologic Stability Index (PSI) was developed and validated as a measure of severity of illness for pediatric ICU patients.[650] The Pediatric Risk of Mortality (PRISM) score subsequently provided a streamlined scoring system by reducing the number of variables in the PSI through regression analysis and weighting of physiologic variables to better reflect their contribution to the risk of mortality.[466] Although the utility of this score and its subsequent modifications in predicting mortality in pediatric ICU patients has been studied extensively,[464-466] only three studies have examined the use of the PRISM score as a predictor of the development of health care–associated infection.[467,565,567] The most recent study developed a multivariable model, including the PRISM III-24 score, for assessing the risk of acquiring a health care–associated infection.[565] The model consisted of three factors assessed on the day of admission: the use of invasive devices (i.e., central venous catheter, mechanical ventilation, indwelling urinary catheter), the administration of parenteral nutrition, and an interaction of the PRISM III-24 score and postoperative care. As a whole, the model predicted the risk of acquiring a health care–associated infection well. However, the datasets used to develop and to validate the model were limited in size and derived from only one pediatric ICU. In addition, the most common health care–associated infection was tracheitis, which accounted for more than a third of all health care–associated infections analyzed; bloodstream infections and pneumonia accounted for only 25 percent and 7 percent, respectively, of the infections analyzed. The implication of these observations on the generalizability of model is not clear.

Another score, the Paediatric Index of Mortality (PIM), and a second-generation index (PIM2) are used widely[545,570] but in limited investigation have not been found to be good predictors of health care–associated infections.[24]

Several severity-of-illness measures have been developed for newborn ICU patients.[233,279,498-500,603,655] The most extensively tested and applied score, the Score for Neonatal Acute Physiology (SNAP), was shown by Gray and colleagues[232] to be a strong predictor of health care–associated coagulase-negative staphylococcal bacteremia among infants of very low birth weight

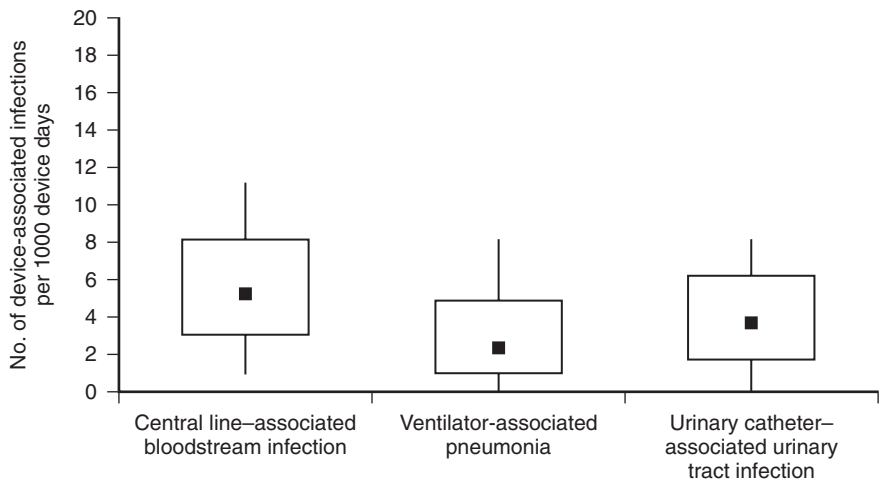

Figure 244–1 Distribution of device-associated infection rates in pediatric intensive care units in hospitals participating in the National Nosocomial Infection Surveillance system. Boxplots of the distribution of device-associated infection rates in pediatric intensive care units indicate the following: the solid square (■) represents the 50th percentile (median). The lower and upper bounds of the open vertical rectangle (□) represent the 25th and 75th percentiles, respectively. The lower and upper bounds of the lines extending above and below the open vertical rectangle represent the 10th and 90th percentiles, respectively. The number of participating pediatric intensive care units was 54 for central line–associated bloodstream infections and 52 each for ventilator-associated pneumonia and urinary catheter–associated urinary tract infections. *(Modified from National Nosocomial Infection Surveillance System: National Nosocomial Infection Surveillance [NNIS] System Report, data summary from January 1992 through June 2004, issued October 2004. Am. J. Infect. Control 32:470-485, 2004.)*

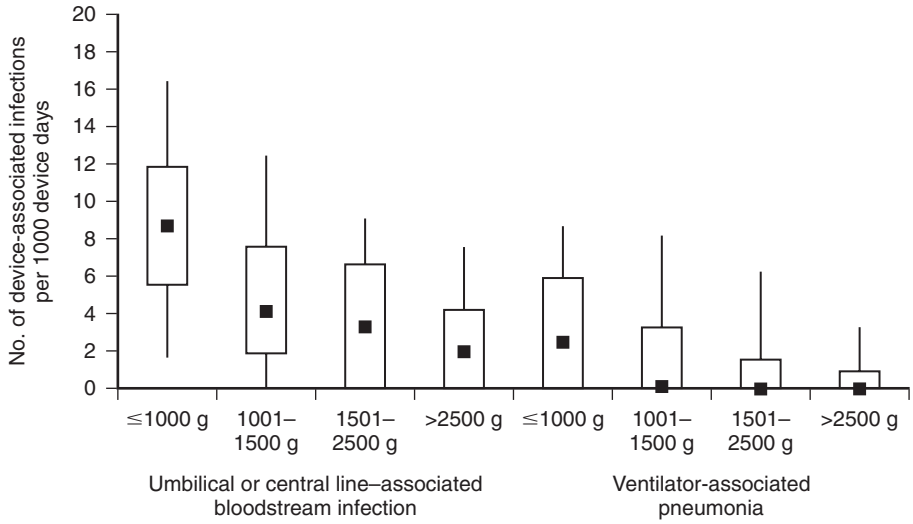

Figure 244–2 Distribution of device-associated infection rates in high-risk nurseries in hospitals participating in the National Nosocomial Infection Surveillance system. Boxplots of the distribution of device-associated infection rates in pediatric intensive care units indicate the following: the solid square (■) represents the 50th percentile (median). The lower and upper bounds of the open vertical rectangle (□) represent the 25th and 75th percentiles, respectively. The lower and upper bounds of the lines extending above and below the open vertical rectangle represent the 10th and 90th percentiles, respectively. The number of participating neonatal intensive care units in each of the birth weight classes ranged from 94 to 104 for umbilical and central line–associated bloodstream infections and 86 to 102 for ventilator-associated pneumonia. *(Modified from National Nosocomial Infection Surveillance System: National Nosocomial Infection Surveillance [NNIS] System Report, data summary from January 1992 through June 2004, issued October 2004. Am. J. Infect. Control 32:470-485, 2004.)*

(<1500 g) in an early study. However, rates of infection in this study were not adjusted for exposure to central or umbilical lines, and information about other significant risk factors, such as the administration of lipid emulsions, was not available. Two subsequent studies have examined SNAP as a predictor for development of bacteremia in more detail. In a follow-up case-control study conducted in the same units used by Gray, Avila-Figueroa and colleagues[30] found that SNAP on day 7 of hospitalization was not associated with coagulase-negative bacteremia, after adjustment for birth weight, length of hospital stay, and nearest date of discharge. In addition, Brodie and colleagues[70] conducted a cohort study in six newborn ICUs and found that after adjustment for birth weight and the presence of small-for-gestational age, SNAP was not a significant predictor of bloodstream infection. Therefore, in these two studies, SNAP did not add to adjustment by other intrinsic (i.e., host) risk factors. Consistent with a prior work,[185] both studies identified administration of parenteral nutrition with lipid emulsion as a highly significant risk factor for infection.[30,70] Interestingly, the study by Brodie found substantial variability in infection rates among newborn ICUs, even after applying rigorous methods of risk adjustment, suggesting that other patient care practices also may play a role in the observed variability in infection rates among newborn ICUs.[70] Although not explored in Brodie's study, higher nurse-to-patient staffing ratios have been linked to higher rates of health care–associated infections in various inpatient settings, including ICUs.[25,193,241,243,259,429,504]

Another approach to risk adjustment—use of a composite risk index calculated by several individual risk factors—has been applied to analysis of surgical-site infections by the NNIS system. Developed by analysis of 4 years of surgical-site infection data reported to the NNIS, the NNIS Basic Surgical-Site Infection (SSI) Risk Index is an example of a composite risk index that has been incorporated into routine surveillance in many hospitals.[125,213] This risk index is calculated by counting one point for each of the following three risk factors: a preoperative ASA score of 3, 4, or 5; an operation classified as either contaminated or dirty infected; and an operation with a duration of more than *T* hours, where *T* depends on the operative procedure performed.[125] The duration of a particular procedure is thought to reflect the complexity of the procedure. As seen in Table 244–3, this risk index provides a much better assessment of risk than does traditional wound classification alone. This index has not been applied to pediatric patients undergoing surgery. Moreover, although the NNIS Basic SSI Risk Index works well across a broad range of different surgical procedures, it does not work as well for some specific procedures, such as cardiac surgery, neurosurgery, and cesarean section.[165,214,275,495,510] Procedure-specific composite risk indices are being developed with use of NNIS data and multivariable modeling techniques.[212,273] The standardized infection ratio (a ratio that compares the observed rate of infection to the expected rate of infection generated with use of the regression

TABLE 244–3 Surgical-Site Infection Rates* by Traditional Wound Classification and the National Nosocomial Infection Surveillance (NNIS) System Basic Surgical-Site Infection Risk Index†

	Basic Surgical-Site Infection Risk Index†				
Wound Classification	**0**	**1**	**2**	**3**	**Cumulative**
Clean	1.0	2.3	5.4	—	2.1
Clean contaminated	2.1	4.0	9.5	—	3.3
Contaminated	—	3.4	6.8	13.2	6.4
Dirty infected	—	3.1	8.1	12.8	7.1
Cumulative	1.5	2.9	6.8	13.0	

**Surgical site infections per 100 operative procedures.*

†Calculated by counting one point for each of the following three risk factors: a preoperative American Society of Anesthesiologists Physical Status Classification (ASA) score of 3, 4, or 5; an operation classified as either contaminated or dirty infected; and an operation with a duration of more than T hours (T is the 75th percentile for the duration of surgery rounded to the nearest hour for procedures included in the NNIS database; see reference 125).

Modified from Culver, D. H., Horan, T. C., Gaynes, R. P, et al.: Surgical wound infection rates by wound class, operative procedure, and patient risk index. Am. J. Med. 91:152S-157S, 1991.

equation developed by multivariable modeling) has been proposed as a means of using procedure-specific composite risk indices for benchmarking SSI rates.[212,273] A similar approach could be applied to other infections.[565] Other risk-adjustment methods may be useful in benchmarking SSI rates. For example, the National Surgical Quality Improvement Program uses a robust risk-adjustment method to calculate expected versus observed rates of complications in the 30 days after surgery; this method, which was validated in adult patients in Veterans Administration hospitals, has not been studied systematically in pediatrics.

In summary, the methodology for meaningful comparison of rates of health care–associated infection among ICUs or hospitals has advanced remarkably in the past 2 decades. Hospitals have used risk-adjusted infection rates, such as those provided by the NNIS system, to benchmark their institution-specific rates and to target areas for improvement. They likely have achieved some success in this effort because rates of device-associated bloodstream infections, pneumonia, and urinary tract infections declined in adult and pediatric ICUs participating in the NNIS system during the 1990s.[95,576] In high-risk nurseries during the same period, rates of catheter-associated bloodstream infections declined among infants in all birth weight categories, and rates of ventilator pneumonia declined among all but the smallest infants.[576] More recently, requirements for public reporting of health care–associated infection rates and national patient safety and quality improvement initiatives are reshaping the focus of surveillance of health care–associated infections from refinements in risk adjustment and benchmarking to a perspective of "zero tolerance" and complete elimination of health care–associated infections.[52,292,397,654] Application of evidence-based care bundles appears to be especially effective in reducing rates of central venous catheter–associated bloodstream infection and ventilator-associated pneumonia.[127,475,492]

SITES OF HEALTH CARE–ASSOCIATED INFECTIONS

Figures 244–3 and 244–4 illustrate the site distribution of health care–associated infections among patients in pediatric ICUs and

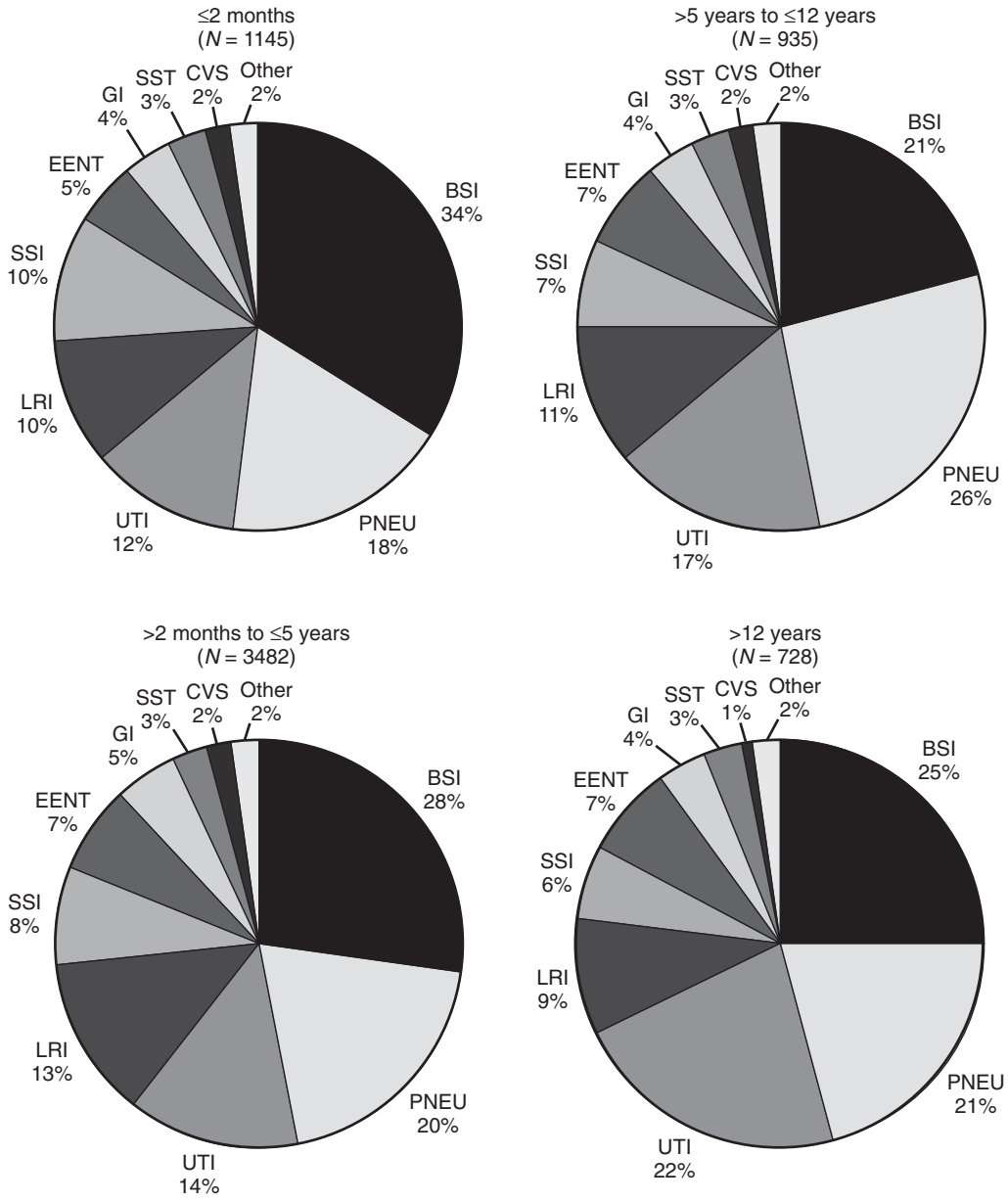

Figure 244–3 Site distribution of health care–associated infections in pediatric intensive care units in hospitals participating in the National Nosocomial Infection Surveillance system. BSI, bloodstream infection; CVS, cardiovascular infection; EENT, eye, ear, nose, or throat infection; GI, gastrointestinal infection; LRI, lower respiratory infection other than pneumonia; PNEU, pneumonia; SSI, surgical-site infection; SST, skin or soft tissue infection; UTI, urinary tract infection. *(Modified from Richards, M. J., Edwards, J. R., Culver, D. H., and Gaynes, R. P.: Nosocomial infections in pediatric intensive care units in the United States. National Nosocomial Infections Surveillance System. Pediatrics* 103*:e39, 1999.)*

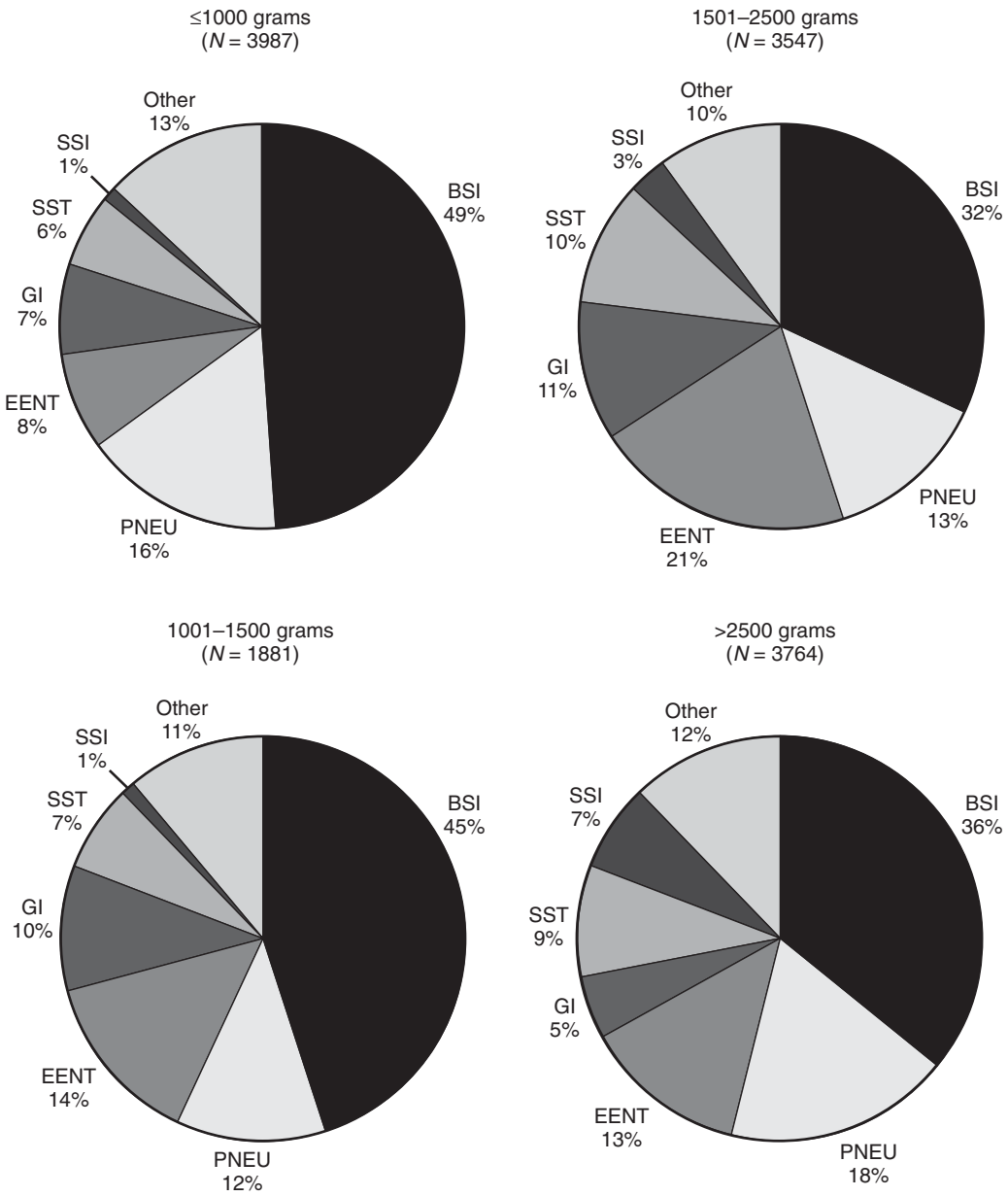

Figure 244–4 Site distribution of health care–associated infections in high-risk nurseries in hospitals participating in the National Nosocomial Infection Surveillance system. BSI, bloodstream infection; EENT, eye, ear, nose, or throat infection; GI, gastrointestinal infection; PNEU, pneumonia; SSI, surgical-site infection; SST, skin or soft tissue infection. *(Modified from Gaynes, R. P, Edwards, J. R., Jarvis, W. R., et al: Nosocomial infections among neonates in high-risk nurseries in the United States. National Nosocomial Infections Surveillance System. Pediatrics 98:357-361, 1996.)*

high-risk nurseries in hospitals participating in the NNIS system.[215,497] Bloodstream infection, pneumonia, urinary tract infection, other types of lower respiratory tract infection (e.g., tracheitis), and surgical-site infection represent the majority of health care–associated infections among patients in pediatric ICUs (see Fig. 244–3). The distribution of infections does not vary substantially by age group, although bacteremia is found more frequently and urinary tract infection less frequently among the youngest children (≤2 months). The majority of health care–associated infections among patients in high-risk nurseries are the following: bloodstream infection; pneumonia; eye, ear, nose, and throat infections; gastrointestinal infection; and skin and soft tissue infection (see Fig. 244–4). As noted previously, bloodstream infection occurs most frequently in lower birth weight categories of infants (≤1000 g and 1001 to 1500 g). A point prevalence survey conducted in pediatric and newborn ICUs in 29 U.S. hospitals participating in the Pediatric Prevention Network (PPN), a research collaboration between the National Association of Children's Hospitals and Related Institutions and the CDC, confirmed these general conclusions.[236,577]

The site distribution of health care–associated infections among children and newborns differs substantially from that among adults. Bloodstream infection represents a considerably greater proportion of health care–associated infections in children and newborns; conversely, urinary tract infection and, to a lesser extent, pneumonia and surgical-site infection represent lower proportions of infections in children and newborns.

Some of these differences are due to differences in exposure to invasive devices, but others are not. For example, urinary catheters are used much less frequently in pediatric and newborn ICUs than in adult ICUs,[424] decreasing the overall frequency of urinary tract infection, although the per-day risk of acquiring infection is probably similar. In contrast, the greater frequency of bloodstream infections in children and newborns is not attributable to catheter exposure because central lines (including central venous and umbilical catheters) are used less frequently

in pediatric and newborn ICUs than in most adult ICUs.[424] Indeed, as discussed previously (see the section on rates of infection), rates of bloodstream infections are higher among children and newborns than among adults even after adjustment for catheter exposure.[424] Mechanical ventilation is used roughly as frequently for children in pediatric ICUs and for very-low-birth-weight infants (≤1000 g) in newborn ICUs as in adult ICUs, indicating that other factors must play a role in the lower proportion and rates of pneumonia in children and newborns. Other factors playing a role in these differences are discussed in greater detail in other portions of this chapter (see the sections on health care–associated infections related to invasive devices, procedures, and treatments and health care–associated infections in special populations).

Health care–associated respiratory and gastrointestinal infections caused by seasonal pathogens circulating in the community, especially viruses such as RSV, influenza virus, and rotavirus, are important because of their impact on children and newborn infants. These infections are discussed in detail in other portions of this chapter (see the section on health care–associated infections due to spread of infections common in the community).

PATHOGENS CAUSING HEALTH CARE–ASSOCIATED INFECTIONS

Tables 244–4 and 244–5 list the distribution of pathogens for major sites of health care–associated infections among patients in pediatric ICUs and high-risk nurseries participating in the NNIS system.[215,497] *S. aureus*, coagulase-negative staphylococci, *Enterococcus* spp., a variety of gram-negative bacilli, and *Candida* spp. are responsible for most infections. Pathogens responsible for specific infections are discussed in more detail in other portions of this chapter (see the sections on health care–associated infections due to spread of infections common in the community; health care–associated infections related to invasive devices, procedures, and treatments; and health care–associated infections in special populations).

Several general trends in the microbial etiology of health care–associated infections that are relevant for children should be emphasized. The number of infections caused by coagulase-negative staphylococci has increased dramatically in the past 2 decades, almost entirely as a result of an increase in the frequency of bloodstream infections caused by these microorganisms.[215,497] This trend is particularly impressive in newborns, which is explained at least in part by the increased survival number of very-low-birth-weight infants who have long hospital stays and are highly dependent on intravascular catheters and parenteral nutrition.[185,186,188,558] Several studies using molecular epidemiologic techniques indicate that specific strains of coagulase-negative staphylococci may become endemic in newborn ICUs and may be transmitted by the hands of caregivers.[75,283,365,447,571,616] The frequency of *Candida* spp., especially *Candida albicans*, has increased for all major sites of infection.[191] Infections caused by these fungi have had a major impact among critically ill and immunocompromised children and premature infants.[191,293,486,527] The frequencies of infections caused by *S. aureus*, *Enterococcus* spp., *P. aeruginosa*, and *Enterobacter* spp. also have increased.

More alarming is the dramatic increase in antimicrobial resistance among common health care–associated pathogens. Project ICARE (Intensive Care Antimicrobial Resistance Epidemiology) studied the prevalence of antimicrobial resistance in adult and pediatric ICUs in more than 40 U.S. hospitals during 1996 to 1997.[192] This surveillance project subsequently became a part of the NNIS system and NHSN.[424] In its 2004 report, NNIS

TABLE 244–4 Commonly Reported Pathogens by Site of Nosocomial Infection in Pediatric Intensive Care Units Participating in the National Nosocomial Infection Surveillance System

Pathogen	% Bloodstream Infections (*N* = 1887)	% Pneumonia (*N* = 1459)	% Urinary Tract Infections (*N* = 1045)	% Lower Respiratory Tract Infections (*N* = 935)	% Surgical-Site Infections (*N* = 544)
Coagulase-negative staphylococci	37.8	0.9	4.3	1.5	14.0
Enterococcus	11.2	1.0	10.0	1.2	8.1
Staphylococcus aureus	9.3	16.9*	1.5	18.8	20.2†
Enterobacter spp.	6.2	9.3†	10.3	12.2	8.1
Candida albicans	5.5*¶	1.6	14.3§¶	3.6	5.0
Pseudomonas aeruginosa	4.9	21.8	13.1¶	15.1	14.5‡
Klebsiella pneumoniae	4.1	5.3	7.3	3.5	3.7
Other *Candida* spp.	3.4*	0.4	6.2	1.1	2.0
Escherichia coli	2.9	3.6	19.0‡	3.2	5.1
Acinetobacter spp.	2.0	3.1	0.4	3.1	0.7
Serratia marcescens	2.0	3.6	1.2	3.6	2.8
Streptococcus pneumoniae	0.6	3.4	0	2.6	0.6
Citrobacter	0.5	0.5	4.3	1.1	1.8
Candida glabrata	0.4*	0	0.6	0	0
Other fungi	0.2*	0.7	1.6	0.1	0.2
Group B streptococcus	0.1	0.2	0.1	0	0.4
Haemophilus influenzae	0.1	10.2	0	5.8	0.9
Aspergillus	0.1	0.5	0	0.1	0.7
Viruses	0.1	2.5†	0.2	10.1	0

Data are from 1992 to 1997.

**Reports of fungi from bloodstream infections and S. aureus from pneumonia occurred more frequently in children older than 5 years than in younger children* ($p < .001$).

†Reports of Enterobacter spp. and viruses from pneumonia and S. aureus from wound infections occurred more frequently in children 2 months and younger than in older children ($p < .001$).

‡Reports of E. coli from urinary tract infections and P. aeruginosa from surgical-site infections occurred more frequently in children older than 2 months ($p < .02$) *than in neonates.*

§Reports of C. albicans from urinary tract infections occurred more frequently in children older than 12 years than in younger children ($p < .002$).

¶Pathogens associated with use of an invasive device.

Modified from Richards, M. J., Edwards, J. R., Culver, D. H., and Gaynes, R. P.: Nosocomial infections in pediatric intensive care units in the United States. National Nosocomial Infections Surveillance System. Pediatrics 103:*e39*, *1999*.

TABLE 244–5 Commonly Reported Pathogens by Site of Nosocomial Infection in High-Risk Nurseries Participating in the National Nosocomial Infection Surveillance System

Pathogen	% Bloodstream Infections (*N* = 7521)	% Eye, Ear, Nose, and Throat Infections (*N* = 2685)	% Gastrointestinal Infections (*N* = 1058)	% Pneumonia (*N* = 2665)	% Surgical-Site Infections (*N* = 619)
Coagulase-negative staphylococci	51.0	29.3	9.6	16.5	19.2
Staphylococcus aureus	7.5	15.4	0	16.7	22.3
Group B streptococci	7.9*	0	0	5.7*	
Enterococci	6.2	3.4	0	4.6	8.9
Candida spp.	6.9	0	0	0	0
Escherichia coli	4.3	6.1	13.9	5.8	12.0
Other *Streptococcus* spp.	2.7	7.4	0	3.3	0
Enterobacter spp.	2.9	4.5	5.5	8.2	7.6
Klebsiella pneumoniae	2.5	2.8	9.8	5.8	6.3
Pseudomonas aeruginosa	0	6.6	0	11.7	0
Haemophilus influenzae	0	2.7	0	1.4	0
Viruses	0	5.1	30.0†	0	0
Gram-positive anaerobes	0	0	9.4	0	0
Other enteric bacilli	0	0	0.8	0	0
Others	8.1	16.7	21.0	21.7	23.7

Data are from 1986 to 1993.
**Group B streptococcal bloodstream infections and pneumonia occurred more frequently in infants weighing more than 2500 g.*
†Rotavirus constitutes 96.4% of viruses isolated from gastrointestinal infections.
Modified from Gaynes, R. P, Edwards, J. R., Jarvis, W. R., et al: Nosocomial infections among neonates in high-risk nurseries in the United States. National Nosocomial Infections Surveillance System. Pediatrics 98:357-361, 1996.

reported that the overall prevalence of methicillin resistance among *S. aureus* causing health care–associated infections was 60 percent (an increase of 11% in 2003 compared with 1998 to 2003), and the prevalence of vancomycin resistance among enterococci was 29 percent (an increase of 12% in 2003 compared with 1998 to 2003).[424] Resistance to methicillin is now virtually ubiquitous among coagulase-negative staphylococci. Resistance to third-generation cephalosporins is a common finding among gram-negative rods such as *E. coli*, *Klebsiella pneumoniae*, *Enterobacter* spp., and *P. aeruginosa*, and resistance to imipenem and quinolones is a rapidly growing problem among *P. aeruginosa* and other gram-negative pathogens.[424]

Hospitals caring for children have not escaped these trends, although the frequency of specific problems of resistance differs somewhat between pediatric and adult ICUs.

Infections caused by MRSA in pediatric patients have been recognized in children's hospitals since the 1980s.[295] However, data from the Project ICARE in the late 1990s indicated that the prevalence of MRSA in pediatric and neonatal ICUs is far less than in adult ICUs.[426] Similarly, a study of the prevalence of colonization with antimicrobial-resistant bacteria among patients in eight U.S. pediatric and newborn ICUs participating in the PPN found that colonization with this microorganism was uncommon.[560] Reasons for this difference are not known, but they may relate to the fact that a greater proportion of adult patients are transferred to the ICU from long-term care facilities, where colonization occurs more frequently, and the greater success of various control measures in containing or eradicating MRSA from an individual unit when the overall prevalence rate in the unit is relatively low. In recent years, the threat posed by MRSA has become accentuated by genotypically distinct strains of MRSA that have become widely disseminated in communities across the United States[415] and are an increasing cause of health care–associated infections.[319,320,544] Studies have documented that these strains also are a common cause of health care–associated MRSA infection in children at major pediatric referral centers.[284,653] Outbreaks of infection also have been described in otherwise healthy neonates and their mothers.[67,91,182,289,523]

The first reported infection with *S. aureus* with reduced susceptibility to vancomycin occurred in a child with a wound infection after undergoing cardiac surgery in Japan.[271] The experience thus far in the United States has involved infections in only a handful of adults.[103,563]

As previously noted, methicillin resistance among coagulase-negative staphylococci encountered in the hospital setting is now nearly universal in all inpatient care settings. Infections caused by *S. epidermidis* with reduced susceptibility to vancomycin also have been reported in adults.[209,562]

Viridans streptococci have emerged as a major cause of bloodstream infections among neutropenic patients, and the prevalence of resistance to penicillins and cephalosporins is increasing among these bacteria.[158,550]

Resistance among enterococci has become a particularly alarming problem, especially given the increasing frequency of health care–associated infections caused by these organisms. Enterococci are intrinsically resistant to many antimicrobial agents, including all cephalosporins, clindamycin, and trimethoprim-sulfamethoxazole.[98] In addition, enterococci, especially *Enterococcus faecium*, always have been relatively resistant to penicillin, but high-level resistance due to mutations in penicillin-binding proteins is now a relatively common finding.[98] High-level resistance to aminoglycosides emerged in the 1970s and became widespread in the 1980s; enterococci resistant to both streptomycin and gentamicin are resistant to the synergistic activity of all known antimicrobial combinations.[98] Penicillin resistance due to β-lactamase production in conjunction with high-level gentamicin resistance also has been reported.[494,633] As alarming as these trends were, the rapid increase in the rate of colonization and infection with vancomycin-resistant enterococci (VRE) observed across the United States during the 1990s was truly astounding.[86,425] Treatment of VRE-colonized adults with anti-anaerobic antibiotics promotes high-density colonization with VRE, which may facilitate dissemination of these bacteria.[151] Although colonization and infection with VRE have been demonstrated in pediatric patients,[56,57,234,356,382,514,566,608] data from Project ICARE and the PPN colonization study indicate that the problem is much less widespread among hospitalized children than among adults.[426,560]

In contrast, antimicrobial-resistant, gram-negative bacilli are both a common and serious threat to hospitalized children. The

introduction and widespread use of third-generation cephalosporins in the 1980s was followed quickly by the emergence and dissemination of gram-negative bacilli with mutations resulting in constitutive expression of chromosomally encoded AmpC β-lactamases.[355] In the late 1980s and throughout the 1990s, plasmid-borne, extended-spectrum β-lactamases capable of inactivating third-generation cephalosporins were identified in gram-negative rods from around the world and included *Klebsiella* spp., *Enterobacter* spp., *E. coli*, *Citrobacter* spp., *Morganella morganii*, and *P. aeruginosa*.[64,355] These pathogens often carry genes for resistance to aminoglycosides and other front-line antibiotics on their plasmids.[64,355] More recently, some gram-negative rods have been demonstrated to produce both an AmpC β-lactamase and an extended-spectrum β-lactamase, with both enzymes encoded on a plasmid.[456] Gram-negative rods producing AmpC β-lactamases or extended-spectrum β-lactamases have caused a number of outbreaks involving pediatric patients,[27,189,427,546] and data from Project ICARE and the PPN colonization study indicate that these bacteria, especially *Enterobacter* spp., are a significant and widespread problem in pediatric and newborn ICUs.[426,560] CTX-M extended-spectrum β-lactamases are now widespread in *E. coli* and *Klebsiella* both in the community and in hospitals in many countries and generally are associated with resistance to multiple antibiotics and reduced susceptibility to quinolones.[459] OXA-40 β-lactamase–producing *Acinetobacter* have caused outbreaks in Illinois and Indiana,[419] and carbapenemase-producing, gram-negative bacteria (both plasmid and chromosome mediated) are reducing the utility of this important class of antibiotics.[477] Aminoglycoside resistance due to production of aminoglycoside-inactivating enzymes by gram-negative rods has been a persistent but less pervasive problem. Resistance to imipenem and fluoroquinolones is a newer and growing concern, particularly among *P. aeruginosa*,[424] although the impact of these problems in pediatric patients has been limited thus far. Other gram-negative bacilli, including *Stenotrophomonas maltophilia*, *Acinetobacter* spp., *Citrobacter* spp., *Alcaligenes* spp., and *Burkholderia cepacia*, infrequently cause health care–associated infection but are notable because they usually exhibit multiple drug resistance.

Fluconazole resistance among *Candida* spp. is well documented; however, resistance is largely confined to non-albicans species of *Candida*, especially *Candida glabrata* and *Candida krusei*.[455] Although these species have become a more common cause of infection in patients undergoing treatment for cancer, they have not become widespread in other populations.[191]

ANTIMICROBIAL USE

The emergence, selection, amplification, and dissemination of antimicrobial-resistant microorganisms are closely related to the use of antimicrobial agents, although this connection may be easier to demonstrate at a population level than in an individual patient. Data regarding the use of antimicrobial agents in hospitals providing care to pediatric patients are very limited, although two multicenter studies provide general descriptive information.

Project ICARE tracked the use of antimicrobial agents in adult and pediatric ICUs. The initial Project ICARE report provided data from only a small number of pediatric ICUs,[192] but the 2001 NNIS system report included data from 16 pediatric ICUs.[425] Antimicrobial use in this report is quantified as the number of defined daily doses per 1000 patient days. The defined daily dose is a metric designed to quantify medication use in adult patients and does not make any adjustment for weight (e.g., the defined daily dose for cefotaxime is 3 g/day, whereas the usual dose prescribed for a child is 150 mg/kg/day). Consequently, the data in the NNIS system report probably underestimate the use of antimicrobial agents in children versus adults. Despite this caveat, these data provide a qualitative assessment of the frequency of use of specific agents in pediatric ICUs. In the participating units, third-generation cephalosporins are by far the most commonly used agents, followed by parenteral vancomycin, first-generation cephalosporins, ampicillin- or amoxicillin-containing agents, second-generation cephalosporins, antistaphylococcal penicillins, trimethoprim-sulfamethoxazole, antipseudomonal penicillins, fluoroquinolones, carbapenems, penicillins, oral vancomycin, and aztreonam.

The PPN point prevalence survey cited earlier found somewhat different results.[235] In this study, the antimicrobial agent most commonly used in pediatric ICUs was a first-generation cephalosporin, followed by third-generation cephalosporins and vancomycin. Most of the patients receiving cefazolin had undergone surgery recently. The agent most commonly used in neonatal ICUs was gentamicin, followed by ampicillin and vancomycin.

The reason for the discrepant results between the NNIS system report and the PPN study are not clear and may relate to differences in the ICUs and their patient populations or methodologic differences in measuring antimicrobial use. Although these two studies provide data on the relative frequency of use of specific antimicrobial agents, the determinants or the appropriateness of antimicrobial use in hospitals providing care to children in the United States has not been described in any detail. Moreover, a standardized approach to the measurement of antimicrobial use in children has not been defined and validated.

INTERACTIONS BETWEEN HOSTS AND PATHOGENS

In general, newborns begin life devoid of microbial flora, but they quickly become colonized with a broad array of microorganisms. Most of these microorganisms will not produce disease unless their human host's natural defenses against infection are compromised. In contrast, exposure to microbial pathogens may lead to infection unless the host has effective innate or specific immunity to these microorganisms. In hospitalized children, weakened host defenses coupled with the aggressive medical care required to sustain critically ill children tend to be more important factors than is the pathogenic potential of the specific microorganisms that happen to be circulating in the institution at any given moment.

Neonates are at particular risk for acquiring infection because of the relative immaturity of their immune systems, especially if they are born prematurely. A full review of the deficits in newborn immune function is available in other texts,[348] but a few of the most important problems deserve emphasis. Infants born before approximately 28 weeks of gestation do not have the benefit of transplacentally acquired maternal antibody, and even more mature newborns lack specific antibodies to many of the pathogens they can expect to encounter early in life. The alternative pathway of complement activation is designed to protect the host in the absence of specific antibody, but it may not function adequately against pathogens in the newborn infant. Neonates have limited neutrophil reserves, which may be exhausted quickly in the face of aggressive pathogens. In addition, migration of neutrophils is decreased, phagocytosis is less effective, and production of antibiotic proteins and peptides, such as bactericidal/permeability-increasing protein, is decreased.[347] Not surprisingly, opsonophagocytosis is compromised by the combination of inadequate specific antibody, suboptimal complement activation, and qualitative defects in recruitment, migration, and function of neutrophils. Immature cellular immunity also compromises the response of neonates to viral and other intracellular pathogens. Moreover, the neonate's lack of an established normal bacterial flora provides no natural "colonization resistance" against pathogens entering the upper respiratory or alimentary tracts, and the

fragile skin of premature infants is less resistant to trauma and the resulting microbial invasion.

Even after their immune system has matured and they are capable of mounting their own vigorous immune response to infection, infants and young children remain susceptible to communicable diseases that may be spread in hospitals, such as varicella, measles, and parvovirus, if they lack specific immunity. Some pathogens, such as RSV, provoke such a limited immune response that infants become susceptible again a short time after infection develops. Others, such as influenza virus, change their antigenic presentation so rapidly that immunity acquired in one year is of limited value in the next.

Underlying diseases, especially those that compromise the immune system, predispose the host to infections caused by a wide array of microorganisms that ordinarily do not cause disease in normal hosts. For instance, neutropenic children are particularly susceptible to filamentous fungal infections, such as invasive pulmonary aspergillosis, that rarely affect other children.[1,617] Immunocompromised children also may suffer more severe consequences from infections that would be relatively trivial in normal hosts. For example, RSV and adenovirus may cause prolonged lower respiratory tract infection or even fatal pneumonia in transplant recipients; rotavirus and *Cryptosporidium* may cause chronic diarrhea in children with acquired immune deficiency syndrome (AIDS). Pathogens that remain well localized in normal hosts may disseminate widely in compromised children (e.g., disseminated candidiasis in neutropenic children).

Medical treatment also has an important impact on the risk for development of an infection. Common features of modern medical care include the use of intravascular catheters and infusions, indwelling urinary catheters, and mechanical ventilation. Many pediatric patients require much more sophisticated care, including invasive hemodynamic monitoring, extracorporeal membrane oxygenation, intensive chemotherapy, bone marrow and solid organ transplantation, hemodialysis, plasmapheresis, and monitoring of intracranial pressure. Aggressive surgical procedures, such as reconstructive surgery and sophisticated cardiovascular surgery, not only are performed more frequently but also are performed on children at a very young age. Overuse of antibiotics in critically ill children predisposes them to colonization and infection with antimicrobial-resistant bacteria or fungi.

Although considerable knowledge exists about the host factors that influence the risk of acquiring a health care–associated infection, substantially less is known about the properties of specific microorganisms that render them more or less pathogenic in hospitalized patients. For the most part, reasons why some microorganisms do not disseminate widely in a hospital whereas others do, and why some microorganisms colonize many patients but produce few infections whereas others cause devastating epidemics of infection, are unclear. Why, for example, did the *S. aureus* phage type 80/81 cause a worldwide pandemic of staphylococcal disease, especially in nurseries, whereas other phage types have a much more limited range, appear to disseminate less readily on hospital wards, and produce serious infections less frequently?[530] Why have genotypically distinct strains of MRSA (USA 300 and USA 400) disseminated rapidly across the United States, causing both community and health care–associated infections?[319,320,415,544]

Contemporary laboratory techniques gradually are unraveling the pathogenic properties of specific microorganisms. For example, the genomes of nosocomial and community-acquired MRSA have been sequenced and analyzed, providing better insights into the origins and pathogenesis of virulent strains.[34,310,328] Interestingly, both phage type 80/81 and community-acquired MRSA strains have genes mediating production of a putative virulence factor, Panton-Valentine leukocidin. The genetic make-up of community MRSA strains may favor colonization of the skin. Studies of coagulase-negative staphylococci have determined that this bacterium produces a capsular polysaccharide that facilitates its adherence to prosthetic materials and "slime" that protects it from clearance by host defense mechanisms.[282,323,395] *Citrobacter diversus* (now classified as *Citrobacter koseri*) produces a surface protein that contributes to its propensity to produce destructive meningitis and cerebral abscesses[321,322]; *E. coli* with the K1 capsular serotype are more likely to invade the meninges[291]; group A streptococci that produce an exuberant hyaluronic acid capsule are more likely to evade opsonophagocytosis[636]; and *E. coli* that produce P pili, are hemolytic, and have specific capsular serotypes and colicin types are more likely to produce urinary tract infections.[300] Nonetheless, we are a long way from understanding the factors that govern the ecology and pathogenic potential of most microorganisms and using this knowledge to prevent infections caused by these microorganisms.

MODES OF TRANSMISSION OF HEALTH CARE–ASSOCIATED INFECTIONS

Modes of transmission are the general mechanisms involved in the transfer of microorganisms from the reservoirs where they live and replicate to susceptible hosts. Table 244–6 lists the important modes of transmission. Examples of specific health care–associated infections are listed, as are the relevant reservoirs, sources, and modes of transmission of microorganisms causing these infections. Because reservoirs often cannot be eliminated, strategies must be designed to interrupt modes of transmission. These strategies are discussed in detail in Chapter 245.

The three basic types of airborne transmission are dissemination of droplet nuclei, "shedding" of skin squames (or "rafts") by colonized or infected individuals, and aerosolization of fungal spores. Droplet nuclei are small particles (<5 μm) generated by the desiccation of larger droplets expelled by coughing, sneezing, or speaking letters such as *t* or *p* forcefully. Because they are extremely light, droplet nuclei can travel over very long distances on air currents. If ventilation is poor and the microorganisms in the droplet nuclei are hardy, these infectious particles may remain suspended in the air of enclosed spaces for relatively long periods in concentrations sufficient to cause infection even if the index patient is no longer present. Because they are so small, droplet nuclei can remain suspended in inhaled air, evading the mechanical host defenses of the upper respiratory tract, and reach the lungs.[417] Classic diseases spread by respiratory droplet nuclei included measles, tuberculosis, smallpox, and, in certain circumstances, influenza and varicella.[128,343,418,491,626] Legionnaires' disease may be spread by small aerosolized droplets generated by devices such as cooling towers, showerheads, and even bedpan cleaners.[595]

Certain individuals are heavy "shedders" of skin squames contaminated by staphylococci or, more rarely, group A streptococci and other skin microorganisms (e.g., *Rhodococcus*).[530] Shedders may have obvious dermatitis or a clinical infection, but they often are asymptomatic. Shedders have been implicated in outbreaks of infection, especially in operating rooms, but most personnel who are colonized with potential pathogens do not dispense large numbers of bacteria and do not pose a threat to patients.[530]

Spores of filamentous fungi, such as *Aspergillus* and Zygomycetes, are ubiquitous in the environment, especially where there is decaying organic matter and moisture. Their small size (<3 μm) and aerodynamic shape permit dispersion over long distances and facilitate penetration of hospital air-handling systems and the respiratory tract of susceptible individuals.

Aerosolization of other organisms, such as *Coxiella burnetii*, can occur in hospitals under special circumstances. For example, an outbreak of Q fever among personnel occurred when sheep were transported through the corridor of a university hospital for a research study.[399]

TABLE 244–6 Modes of Transmission with Examples of Specific Health Care–Associated Infections and the Reservoirs and Sources Involved in the Transmission of These Infections

Mode of Transmission	Health Care–Associated	Reservoir	Source
Airborne	Measles, varicella,* pulmonary tuberculosis	Infected persons	Airborne droplet nuclei
Contact			
Direct	Neonatal staphylococcal skin infection	Infected or colonized caregiver	Drainage from infected wound on the hand of a caregiver
Indirect	RSV infection	Infected persons	Hands of caregivers, fomites
	Infection with antimicrobial-resistant bacteria	Infected or colonized persons	Hands of caregivers, fomites
Droplet	Pertussis, invasive meningococcal disease, group A streptococcal infection	Infected or colonized persons	Large respiratory droplets
Endogenous (autoinfection)†	Coagulase-negative staphylococcal bacteremia associated with a central venous line	Skin at the site of the catheter insertion	Intravascular catheter
	Escherichia coli urinary tract infection associated with an indwelling urinary catheter	Periurethral skin and mucus membranes	Indwelling urinary catheter
Common vehicle	Gram-negative bacteremia associated with intravenous infusion	Liquid substances in the environment	Intrinsically or extrinsically contaminated intravenous fluids
	Post-transfusion infection with blood-borne pathogen (HIV, hepatitis B virus, hepatitis C virus, CMV)	Infected persons	Blood products from infected donors
	Salmonellosis	Infected or colonized persons	Contaminated food
Vector	Enteric infection	Infected persons or infectious material	Flies, pharaoh ants

**Varicella-zoster virus may be transmitted by airborne, direct contact, and droplet contact transmission.*
†See text.
CMV, cytomegalovirus; HIV; human immunodeficiency virus; RSV, respiratory syncytial virus.

Contact transmission is the principal mode of transmission for most nosocomial infections. Direct contact transmission involves physical contact between a person harboring the microorganism, such as a caregiver with a staphylococcal infection on a hand, and the host. Indirect contact transmission involves transfer of microorganisms through an intermediary person or object. The hands of caregivers are the most common source for indirect contact transmission, but fomites also are important for certain pathogens (e.g., RSV, *C. difficile*). Droplet contact transmission involves transfer of microorganisms by large respiratory droplets, such as those generated by coughing or sneezing, which typically travel short distances in the air before settling. Important pathogens spread by this route include *Bordetella pertussis*, *Neisseria meningitidis*, and group A streptococci.

Endogenous infection (or autoinfection) is caused by a patient's own flora. These generally harmless commensals cause disease when the patient's host defenses are compromised by severe underlying disease, immunosuppressive therapy, or invasive devices and procedures. Microorganisms that produce endogenous infections are not always part of a patient's normal flora that he or she brought into the hospital from the community. Commonly, these microorganisms are transferred from other patients by the hands of caregivers and become part of a patient's colonizing endogenous flora. Consequently, these infections can be considered a special case of contact transmission.

Common vehicle (common source) transmission involves the widespread dissemination of a microorganism to many persons through a contaminated item or substance. Many outbreaks of infection in hospitals have been caused by nonenteric, gram-negative bacilli, such as *P. aeruginosa*, which thrives in medications, solutions, or wet equipment and is relatively resistant to antimicrobial preservatives, antiseptics, and disinfectants.

Vector transmission of microorganisms either on (extrinsic) or within (intrinsic) insects is a rare occurrence in hospitals. Extrinsic vector transmission of enteric pathogens may occur when these microorganisms are transported on the legs of flies, roaches, or ants.[42,99,183] Intrinsic vector transmission, such as transmission of malaria or dengue, involves more than physical transfer of the microorganism because a portion of the life cycle of the microorganism is completed in the vector. Whereas intrinsic vector transmission in the hospital is possible theoretically, to the authors' knowledge, no cases have been reported.

Some infections may be spread by more than one mode of transmission. For example, varicella-zoster virus may be spread by airborne and direct contact transmission.

CONSEQUENCES AND COSTS OF HEALTH CARE–ASSOCIATED INFECTIONS

Valid, well-controlled studies of the consequences and costs of health care–associated infections in pediatric patients are limited, partly because of the difficulty in determining whether the consequences or costs are directly attributable to the patient's infection as opposed to the patient's underlying disease. To attribute a consequence to health care–associated infection, investigators must match infected and noninfected patients carefully, using criteria such as age, sex, presence of underlying conditions, severity of illness, operative procedures, and length of stay, or adjust for these potential confounding factors using multivariable statistical analyses. In addition, studies must quantify hospital costs, as opposed to hospital charges, which may be difficult to do precisely.[520]

Pediatric studies that have made a rigorous effort to measure attributable risk indicate that health care–associated infections have a substantial adverse impact on hospitalized children. For example, appropriately designed cohort studies of coagulase-negative staphylococcal bacteremia in newborn ICUs (including adjustment for birth weight and severity of illness) have found increased length of stay (approximately 14 days), increased use of antibiotics, and increased hospital charges (about $25,000) but no increased mortality from this infection.[185,232] More recent data from the Vermont Oxford Network, a network of neonatal ICUs in the United States, indicate that attributable excess costs and

increased length of stay for neonates are somewhat lower than these estimates and vary by birth weight categories.[449] The attributable cost of bloodstream infections in a pediatric ICU was estimated at approximately $39,000,[163] a cost similar to that reported previously in adult ICUs.[460]

HEALTH CARE–ASSOCIATED INFECTIONS DUE TO SPREAD OF INFECTIONS COMMON IN THE COMMUNITY

Health care–associated infections that occur as a result of the in-hospital transmission of infections common in the community are a major concern for all facilities providing health care to children. Several principles about the general epidemiology of these infections, modified from those initially published by Hall[247] in relation to the epidemiology of health care–associated respiratory virus infections are summarized here.

First, their appearance and spread on the wards parallel closely the activity of disease in the community. Second, significant exposure to these pathogens generally results in infection in any host that lacks specific immunity; consequently, a susceptible child is at risk regardless of the nature or severity of the underlying disease or specific medical treatment. Third, these infections often are more severe in hospitalized patients who have underlying diseases (e.g., pulmonary or cardiac disease) or who are immunocompromised. Fourth, children hospitalized as a result of the community-acquired infections are the most important reservoir for microorganisms causing these infections, but mildly symptomatic or asymptomatically colonized adult caregivers may be important reservoirs for some agents (e.g., RSV, pertussis). Fifth, prevention depends primarily on the timely implementation of and compliance with isolation precautions specifically designed to interrupt transmission of the microorganisms involved. In the case of some infections, other interventions also are indicated, such as antimicrobial therapy to reduce the risk of transmission from children with pertussis and passive immunization with varicella-zoster immune globulin to protect high-risk individuals exposed to varicella. Finally, unless post-discharge surveillance is performed, reports of the frequency of these infections are likely to be gross underestimations, especially given recent trends toward shorter lengths of hospital stay, as many of these infections may be in the incubation period at the time of discharge and become manifested only after the child returns home.

RESPIRATORY INFECTIONS

Respiratory Viruses

RSV infection is the most common health care–associated respiratory virus infection, especially among children in the first 2 years of life. Community outbreaks occur every year in the late fall or winter, although the precise timing, intensity, and duration of these outbreaks may vary.[244] Population-based studies indicate that RSV infection may account for a substantial number of hospital admissions in young children during epidemic periods, especially children with underlying medical conditions.[63,548]

Children admitted to the hospital with community-acquired RSV infection represent a substantial reservoir for transmission of RSV to other hospitalized children and health care providers. In a study conducted during a community outbreak in the 1970s, 45 percent of children who were hospitalized for 1 week or longer on an infant ward and 40 percent of the caregivers on this ward acquired RSV infection.[253] A study actively surveyed the frequency of health care–associated RSV infections in all children admitted to eight leading Canadian children's hospitals during the mid 1990s.[340] The percentage of health care–associated RSV cases among all RSV cases in the hospital varied from 2.8 to 13.0 percent among the participating hospitals. As demonstrated in several studies, risk factors for health care–associated infection are prematurity, heart and lung disease, and immunocompromising conditions.[246,251,340,367,461] Outbreaks in newborn ICUs have been reported, some in association with other viruses.[251,400,575,609,642]

In the absence of effective control programs, attack rates tend to be high because immunity after infection is short-lived and because inoculation of virus into the nose or eyes reliably leads to infection.[244,245,248] Moreover, RSV survives for relatively long periods in the environment, and infected children excrete high titers of virus in their copious secretions.[244,245,250] Not surprisingly, duration of hospitalization (and thus the duration of potential exposure) correlates strongly with the risk for development of infection because a greater opportunity exists for direct or indirect contact transmission to occur.[253] One study used polymerase chain reaction to detect RSV nucleic acid in air samples of patient rooms, including samples taken as far as 7 meters from the bedside.[8] However, the implication of this finding on infectivity is unclear.

Scrupulous attention given to hand hygiene and the use of barriers (gloves and gowns) when touching patients or their immediate inanimate environment can reduce markedly the transmission of RSV.[342] Goggles or masks or both may be effective in reducing the attack rate of RSV in personnel,[6,200] which in turn can reduce transmission of virus to patients. However, whether masks and eye protection are effective because they prevent direct deposition of respiratory droplets on the eyes or nose or merely because they reduce the likelihood that staff will rub their eyes or noses with contaminated hands remains unclear. Covering only the nose and mouth with a mask probably is not very effective.[249] The CDC's Healthcare Infection Control Practices Advisory Committee recommends Standard and Contact Precautions for care of hospitalized patients with RSV and does not require use of a mask.[559,594] Some investigators have demonstrated that cohorting of infected patients, combined with the use of barriers, can reduce spread of RSV.[149,327,370] However, it is not clear whether cohorting by clinical symptoms alone is effective or whether all admitted patients must be screened for RSV infection to identify children with minimal symptoms who may be excreting the virus. Moreover, the added value of strict cohorting, which may be expensive or difficult to implement, as opposed to the rigorous use of a barrier technique, has not been demonstrated. Various multidisciplinary programs to promote awareness of the potential for transmission of RSV and to optimize use of prevention measures have been described.[305,342,366] Two studies estimated that the cost savings associated with their programs were substantial.[305,366]

One group of investigators used a screening approach to reduce the frequency of RSV infection among children undergoing cardiac surgery.[11] They screened all children aged 16 days to 4 years admitted for cardiac surgery between September and March with RSV enzyme-linked immunoassay. Surgery was delayed in patients with symptomatic diseases as well as in asymptomatic patients with positive RSV test results (the majority of whom developed symptoms in the subsequent days after having the positive test result). Although this procedure was not a controlled trial, the incidence of postoperative infections with RSV and the attendant complications were reduced substantially during the screening period.

Treatment of RSV infection with ribavirin reduces shedding of RSV,[147] which may decrease the potential for transmission. However, the use of this agent has been limited because of a lack of clear evidence of its clinical benefit.[16] Palivizumab, a humanized murine monoclonal antibody, has demonstrated efficacy in preventing or reducing the severity of RSV infection in high-risk patients,[17] but it is unlikely to have a profound effect on nosoco-

mial transmission. Palivizumab has been used to help control an outbreak of RSV in a neonatal unit.[119] The development of vaccines against RSV may lead to more effective prevention strategies in the future.[244]

Health care–associated infections caused by parainfluenza virus and human metapneumovirus are similar to RSV infection in their epidemiology and prevention.[244,412,594]

Population-based studies indicate that rates of hospitalization in young children are substantially lower for influenza than for RSV infection.[63,287,430,548] Yet the potential for transmission of influenza virus to occur remains significant. Health care–associated influenza is a common cause of intercurrent fever in hospitalized children during epidemic periods,[253] and outbreaks in neonatal ICUs have been described.[126,420]

Although the modes of transmission of influenza have not been defined precisely, direct, indirect, and droplet contact are likely to be most important in health care settings. Nonetheless, data from animal models and the rapid spread of influenza in confined human populations suggest that airborne transmission may occur in some situations.[225,418] In addition to the precautions described for RSV, masks should be worn during close contact because of the potential for droplet transmission to occur. The need for isolation rooms with negative air pressure relative to hallways has not been established.[594] Placement of a patient in a private room without special air handling and cohorting of patients with proven influenza are recommended control strategies.[594]

Annual influenza vaccination of high-risk individuals and hospital staff limits the potential for large outbreaks of influenza to occur if the vaccination is offered at the appropriate time and is well accepted by patients and staff. Antiviral medications are an adjunct to vaccination for treatment and chemoprophylaxis after an exposure to influenza virus.[177] Oseltamivir and zanamivir are the only antiviral medications currently recommended for use in the United States.[177] These agents may reduce the risk for occurrence of transmission, but it has not been demonstrated.

Health care–associated adenovirus infections occur sporadically throughout the year, and outbreaks of respiratory infection and pharyngoconjunctival fever among hospitalized children are well described.[176,219,422,457,470,568,591,635] Outbreaks in ICUs have been associated with severe disease and substantial mortality rates.[568,635] Children undergoing liver transplantation also are at high risk for development of severe disease in the immediate post-transplantation period.[403] In addition to direct and indirect contact, adenovirus may be spread by droplet contact, necessitating the use of masks with eye protection during close contact.

Health care–associated rhinovirus infections generally are mild, although serious lower tract disease has been documented in children with underlying disorders.[105] The mode of transmission of rhinovirus infection remains controversial. Some studies suggest direct and indirect contact transmission as the primary modes of transmission; others suggest that droplet contact may be more important.[225] Hand hygiene is sufficient as a control measure in most situations.

Pertussis

Outbreaks of pertussis in hospitals and chronic care institutions are well documented.[178,329,353,549,583,599,611] In many situations, health care providers were responsible for spreading pertussis to hospitalized children.[104,329,353,611] Indeed, two studies have demonstrated that infection among health care providers is a relatively frequent occurrence, even in the absence of a defined outbreak.[146,647]

Prevention of health care–associated pertussis depends on the appropriate isolation of children in whom the infection is suspected, use of masks to prevent droplet contact transmission, antimicrobial treatment of confirmed cases to minimize the potential for transmission, and prophylactic treatment of exposed individuals. In addition, hospital staff who present with symptoms suggesting pertussis (upper respiratory tract infection with severe, prolonged cough) must be evaluated and treated promptly. The availability of clarithromycin and azithromycin has enhanced treatment of exposed individuals because most adults tolerate these agents much better than erythromycin.

The beneficial impact but relatively high cost of aggressive steps to control the intrahospital spread of pertussis was illustrated during a large community-wide epidemic of pertussis in Cincinnati in 1993.[106]

The availability of acellular pertussis vaccine enhances the potential to enhance immunity among health care providers to prevent spread of pertussis in health care settings. Acellular pertussis vaccine was used as an adjunctive control measure in a hospital outbreak of pertussis.[548] The vaccine was reasonably well tolerated, but no data about its efficacy were reported.

Diphtheria

The potential for health care–associated transmission of diphtheria has been emphasized by a large outbreak in Russia and the Ukraine in the 1990s. Diphtheria acquired during travel to these regions has been reported in U.S. citizens, and imported cases have been documented in other European countries.[84] An imported case of diphtheria in a child from Haiti resulted in exposure of a large number of hospital contacts in Florida. Secondary cases were reported among household contacts but not among hospital contacts.[171] Health care providers should wear masks while providing care of patients with pharyngeal diphtheria. Investigation and prophylaxis of contacts should be pursued aggressively.[171]

GASTROINTESTINAL INFECTIONS

Gastrointestinal Viruses

Rotavirus is a common cause of endemic and epidemic health care–associated gastrointestinal virus infections.[223,488,505] Risk of acquiring infection is closely associated with the duration of hospitalization.[114,144] Infection usually is self-limited, although prolonged duration of diarrhea may occur in immunocompromised patients. One study associated an outbreak of necrotizing enterocolitis in a newborn nursery with concurrent rotavirus infection.[508]

Patients infected with rotavirus in the hospital or community may shed the virus in their stools for many days after symptomatic infection occurs.[637] Asymptomatically infected patients also may shed virus.[114,640] Together, these patients represent a substantial reservoir of virus that may be transmitted easily to other patients. In addition, rotavirus can be transferred by hands and can survive for extended periods on environmental surfaces.[23,531] The primary mode of transmission is indirect contact through the contaminated hands of caregivers, and the scrupulous use of barriers (gowns and gloves), hand hygiene, and appropriate disinfection of environmental surfaces is critically important.[559] An experimental study suggests that rotavirus infection also can be spread by the respiratory route,[473] although this finding has not been demonstrated clinically.

The development and licensure of the rotavirus vaccine likely will reduce the number of hospitalizations due to rotavirus infection, which in turn will reduce the risk of spread of this virus in health care settings.

Hospital outbreaks of norovirus infection, most often due to a new genogroup II.4 variant that emerged earlier this decade in the United States and Europe, have become a major problem, although wards with elderly populations have been affected more significantly than have pediatric wards.[298,302] A variety of other gastrointestinal viruses, including enteric adenoviruses,[325] Norwalk-like viruses,[579] calicivirus,[580] astrovirus,[143,330] and torovi-

rus,[290] also have been documented to cause endemic and epidemic gastrointestinal infection.

Clostridium difficile

Although toxin-producing *C. difficile* is a well-recognized cause of antimicrobial-associated diarrhea in adults, investigation of the role of this organism as a cause of health care–associated diarrhea in hospitalized children and infants has been limited.

Studies conducted in the 1980s illustrated that toxin-producing *C. difficile* often can be found in the stools of neonates and young infants.[111] Because *C. difficile* seldom is found in the stools of healthy women and because clusters of colonized infants often can be detected in nurseries,[111] a presumption is that health care–associated transmission, as opposed to vertical transmission, plays a role in the acquisition of this microorganism by neonates. Nonetheless, *C. difficile* rarely produces significant disease in newborns or young infants,[111] although one study has reported that infants whose stools are positive for *C. difficile* toxin A have more stools per day than do infants whose stools are negative.[168]

On the other hand, numerous reports indicate that older children and even infants can develop symptomatic *C. difficile*–associated diarrhea and that some children may develop severe disease, including pseudomembranous colitis.[73,82,174,260,392,438,441,476,656] Disease usually occurs in association with antimicrobial therapy, and several reports have described the occurrence of the disease in children with cancer.[73,82,476,656] A prospective study of health care–associated diarrhea in hospitalized children and adolescents found that as a group, viral causes were most common; however, *C. difficile*–associated diarrhea was the most frequent single cause and was found more frequently in older children.[338]

An epidemic strain of *C. difficile* (NAP-1) emerged and disseminated in Canada and the United States during mid-2000 and involved patients in the community and health care facilities.[360,390] This strain has caused severe disease in populations thought to be at low risk, including peripartum women and children.[92]

Two studies have explored the relationship of humoral immunity and *C. difficile*–associated diarrhea in elderly hospitalized adults.[331,332] In one study, hospitalized adults who became asymptomatically colonized with *C. difficile* but did not develop disease had significantly higher serum levels of immunoglobulin G (IgG) antibody against toxin A than did those who developed disease.[332] A second study of patients who developed an initial case of *C. difficile*–associated diarrhea found that patients with higher serum concentrations of IgM and IgG antibodies against toxin A were less likely to develop recurrent disease than were patients with lower concentrations of antibody.[331] The implications of these findings on the epidemiology of this disease in children have not been investigated.

C. difficile can be found on the hands of personnel and in the patient's immediate environment,[221] where *C. difficile* spores can survive for prolonged periods and are relatively resistant to disinfectants. Therefore, direct or indirect contact is responsible for the spread of this microorganism from patient to patient. Barriers (gowns and gloves) and hand hygiene may reduce the risk of transmission. Vigorous environmental cleaning is important because *C. difficile* spores can heavily contaminate the environment and survive for long periods. Conventional cleaning agents may not be sporicidal, and some authorities recommend using dilute bleach to disinfect rooms of patients with *C. difficile* infection, particularly while they have diarrhea.[221]

If possible, antibiotic therapy should be discontinued. Diarrhea resolves spontaneously in 15 to 25 percent of patients without further intervention, although predicting which patients will respond to conservative management is difficult. Oral metronidazole and vancomycin are equivalent in terms of response and relapse rates,[600] although metronidazole is considerably less expensive. Vancomycin should be used for treatment of severely ill patients.[220]

Other Bacteria

Bacterial pathogens are rare causes of endemic health care–associated diarrhea in U.S. hospitals.[154] Consequently, in the absence of an outbreak, the yield of routine stool cultures in the evaluation of health care–associated diarrhea is extremely low.[65,120,652] One study developed and evaluated simple screening criteria for rejecting stool bacterial cultures for patients who were hospitalized for more than 3 days.[652] The reduction in volume of tests processed by the laboratory led to cost savings of $25,082 in the 6-month trial period.

Outbreaks of health care–associated salmonellosis have been documented throughout the world, although they are reported more frequently from developing countries.[239,255,313,373,416,569,589,648] Neonates are particularly susceptible to infection by *Salmonella* spp., and invasive disease, such as bacteremia, meningitis, and osteomyelitis, is a common occurrence.[239,255,313,373,569,589,648] Transmission occurs through a variety of means, including contaminated food, direct contact between patients, and indirect contact transmission by contaminated hands or instruments.

Outbreaks of health care–associated shigellosis occur much less frequently. Only one hospital outbreak has been reported in the United States,[45] although shigellosis can be a major problem in institutions caring for disabled children.[36,155] A study in a hospital in Kenya cultured *Shigella* spp. from the stools of 2.5 percent of patients with health care–associated diarrhea.[446] *Shigella* is transmitted easily by direct or indirect contact, and only a small inoculum is required to establish infection.[154]

Health care–associated cholera has been documented in developing countries.[402,518] One of these reports is notable because it provides evidence that acquisition of *Vibrio cholerae* by children who were discharged to home before becoming symptomatic was instrumental in initiating and sustaining an outbreak of cholera in the surrounding community.[402] The mechanism involved in transmission of cholera in these studies is not clear, but direct or indirect contact transmission, as opposed to transmission by contaminated water, was suspected.

Other bacterial pathogens, including *Campylobacter* spp.,[77,269,306,613] *Yersinia* spp.,[80,487] and various types of *E. coli*, have caused outbreaks of diarrhea.[217,542]

Protozoa

Health care–associated infections caused by protozoa are rare findings. An outbreak of cryptosporidiosis was reported from a pediatric hospital in Mexico.[428] The index case was a patient with AIDS who had chronic diarrhea caused by infection with *Cryptosporidium*. Although giardiasis is a common cause of diarrhea in institutionalized children,[601] infections in hospitalized children or neonates have not been reported. Given the large number of severely immunocompromised children in U.S. hospitals and the relative ease of transmitting protozoa by direct or indirect transmission in families and daycare centers, it is somewhat surprising that health care–associated gastroenteritis caused by *Giardia*, *Cryptosporidium*, *Microsporidium*, and other intestinal protozoa has not been reported more frequently.

VARICELLA-ZOSTER VIRUS

Outbreaks of health care–associated varicella in hospitals have been documented in numerous reports.[197,240,343,534] These reports demonstrate conclusively that varicella can be transmitted by the airborne route, although spread by direct and droplet contact may well be more efficient. In these outbreaks, secondary infections occurred in patients who had no face-to-face contact with the index patients and who were separated from the index patient by considerable physical distances (in some cases more than 30

meters).[240,343] Air flow studies indicated that air in the rooms of index patients flowed into the hallway and into other patient rooms.[240,343] In one report, contaminated air flowed through an open window in the index patient's room, traveled along the exterior of the building, and entered other patient rooms by through-the-wall ventilation units.[343] The majority of secondary infections in each of these outbreaks occurred in patients who had been discharged and were detected only by telephone contact or home visit.[240,343] Several susceptible hospital staff members also were infected.

These clinical observations of airborne spread of varicella-zoster virus (VZV) are supported by a study that used polymerase chain reaction to detect airborne virus in hospital rooms of patients with active infection.[532] VZV was detected in air samples collected 1.2 to 5.5 meters from patients' beds for 1 to 6 days after the onset of rash. VZV DNA also could be detected in some air samples obtained in the hallway just outside the patient's negative-pressure isolation rooms.

Transmission of varicella can be minimized if infected patients have single rooms with separate exhaust systems and negative air pressure relative to the hallway.[19] Because infected persons are infectious for 24 to 48 hours before distinctive symptoms and signs appear, prompt recognition and isolation of patients and visitors who may be in the contagious phase of varicella also are critical. Barriers to prevent direct and indirect contact transmission (gloves and gowns) can reduce the spread of this infection dramatically. In addition, caregivers who are not immune to varicella should not care for such patients. Varicella-zoster immune globulin (VZIG) can prevent infection or mitigate the consequences of infection in high-risk, exposed, susceptible individuals if it is administered within 96 hours, but preferably within 48 hours, of exposure.[12] Intravenous acyclovir should be administered to these individuals to limit the replication of virus if infection develops.[12] Hospitalized exposed children should remain in isolation from day 8 to day 21 after being exposed. If VZIG is administered, isolation should be continued until day 28 after the exposure occurs.[12] Management of exposed health care providers is discussed in Chapter 245.

The use of VZV vaccine should diminish the risk of transmission of health care–associated varicella by decreasing the number of children hospitalized with varicella, reducing the size of the pool of susceptible children in hospital wards, and providing protective immunity to health care providers who do not have a history of prior infection. However, data demonstrating these effects have not been published. The use of this vaccine in health care pro-viders is discussed in Chapter 245.

CYTOMEGALOVIRUS

Perhaps no issue is the subject of as much concern and misinformation among health care providers as is the risk of acquiring health care–associated cytomegalovirus (CMV) infection. Concern among caregivers undoubtedly has been heightened by reports of CMV infection occurring among staff at daycare centers. Daycare centers provide optimal conditions for CMV transmission because many children are excreting CMV in their saliva or urine, and abundant opportunities exist for sustained contact with contaminated secretions. However, even in the daycare setting, transmission of CMV to susceptible care providers occurs slowly.[5] This delay reflects the relative inefficiency of direct or indirect contact transmission of CMV, a virus that is inactivated easily by soaps, detergents, and disinfectants and is not stable on environmental surfaces for long periods.[5]

In hospitals, the risk to staff appears to be very low. A meta-analysis of numerous studies that have examined the risk of CMV being acquired by pediatric nurses indicates that CMV infection in this population occurs at a rate comparable to that in control populations (persons of comparable age and sex who are not nurses).[5] A subanalysis of these data suggests that nurses who work in nurseries may have a slightly higher rate of infection than do control populations.[5] However, studies using restriction enzyme analysis of CMV isolates have shown that these nurses did not acquire CMV from the infants in their care,[5] and they presumably contracted their infections from children in their own households, through sexual contact, or from other community sources.

In conclusion, few if any data suggest that health care–associated transmission of CMV is a significant risk factor for health care providers. Given the frequency of asymptomatic CMV excretion in children (e.g., approximately 1% of newborns excrete the virus), health care providers should assume that any child may be excreting virus and should practice handwashing and use of Standard Precautions (see Chapter 245) as a part of routine patient care. Additional interventions are not indicated, and pregnant staff need not be given special assignments.

Two restriction enzyme studies have documented probable patient-to-patient spread of CMV in a newborn ICU and a chronic care unit.[142,578] In both situations, the infected children had been in proximity and were provided care by common caregivers for extended periods.

HERPES SIMPLEX VIRUS

Health care–associated transmission of herpes simplex virus (HSV) type 1 to newborn infants has been confirmed through the restriction enzyme analysis.[256,352,528,614] The mode of transmission is not clear in all of these cases. Direct contact with a hospital worker with herpes labialis was implicated in one case.[614] In the other cases, indirect contact transmission from one infected infant to another is most likely, although direct contact transmission from an asymptomatic parent or caregiver cannot be ruled out.[256,352,528] Health care–associated HSV infections among health care providers, patients, and family members also have been described in ICUs.[4,453] Herpetic whitlow has been observed among nurses, presumably as a result of direct transmission during suctioning of oral and respiratory secretions from infected patients.[4] The risk associated with this procedure is substantial, given recent data that indicate that HSV is found commonly in mucosal and orofacial cultures obtained from intubated patients, including those without obvious lesions.[258] When present, lesions often are atypical in appearance and frequently are found in the distribution of tape used to secure endotracheal tubes.[258] Substantial risk of transmission from immunocompromised patients, in whom reactivation of latent HSV infection is a common occurrence, also exists.[619,644] The use of Standard Precautions (see Chapter 245), particularly the use of gloves during contact with oral and respiratory secretions and hand hygiene, will prevent transmission of HSV.

Surveys of health care–associated infections in pediatric wards indicate that HSV infections are uncommon occurrences.[181,610,632] When documented, these infections usually are attributed to reactivation of preexisting endogenous infection rather than to primary infection.[610] However, as with patients in ICUs, subclinical infection in other hospitalized children may be a more common occurrence than is recognized presently.

MEASLES, MUMPS, AND RUBELLA VIRUSES

Efforts to control measles worldwide have reduced markedly or eliminated endemic measles transmission in many developed

countries.[90] However, as past history indicates, transmission of measles in health care facilities remains a threat, and hospitals have the potential to serve as niduses for community-wide infection.[89a,394,481,503,582b]

Measles is transmitted by airborne droplet nuclei, as is demonstrated by a report of an outbreak in a pediatrician's office.[491] Four susceptible children visiting the office on the same day as the index child subsequently developed measles, even though none of these children had face-to-face contact with the index child or was even in the same room at the same time. Three children visited the office 60 to 75 minutes after the index case had left.

Prevention of health care–associated measles hinges on the prompt recognition of infected or potentially infected patients and placement of these patients in isolation rooms with negative air pressure relative to the hallway.[559] Prophylactic vaccination of exposed susceptible individuals within 3 days of the exposure reliably prevents measles, whereas administration of immune globulin within 3 days attenuates disease but does not guarantee that the exposed individual will not develop contagious infection.[14] Outbreaks of measles among health care providers can be avoided by having health care facilities require new employees involved in patient care to provide evidence of immunity or appropriate vaccination or to receive vaccination with either measles vaccine or MMR (measles, mumps, rubella) as a condition of employment (see Chapter 245).

Outbreaks of health care–associated mumps and rubella have been reported less commonly than has measles.[270,463,590,637] The primary concern with regard to spread of both of these infections relates to potential complications in health care providers. Mumps orchitis in adult men can cause sterility; rubella in pregnant staff can result in fetal infection and the congenital rubella syndrome. Information about the transmission of both of these viruses is limited, but it probably occurs primarily by droplet contact. Masks should be worn during close contact with infected patients.[559] Patients with congenital rubella syndrome may excrete large amounts of virus in their urine and respiratory secretions, and excretion of virus in the urine may persist for months or even years. Consequently, gowns and gloves should be worn for contact with these patients for their first year of life unless nasopharyngeal and urine cultures are negative by the time they are 3 months old.[559] No effective post-exposure prophylaxis for exposed individuals is known. As with prevention of measles, health care facilities should require new employees involved in patient care to provide evidence of immunity or appropriate vaccination or to receive vaccination with MMR (measles, mumps, rubella) as a condition of employment (see Chapter 245).

PARVOVIRUS B19

Parvovirus B19 causes the relatively benign syndrome of erythema infectiosum (occasionally accompanied by arthritis, particularly in older children and adults), acute aplastic crisis in children with hemoglobinopathies, and chronic infection and anemia in immunocompromised children. Some reports have suggested that an association exists between vasculitis and other immunologically mediated diseases.[175,645]

An experimental study of acute parvovirus B19 infection in normal adults detected virus in respiratory secretions in three of four patients during a period of viremia and systemic symptoms that developed 6 to 13 days after the patients were inoculated.[22] Detectable virus in respiratory secretions disappeared as viremia and systemic symptoms diminished, and virus was not demonstrable in respiratory secretions when rash, arthralgias, and arthritis occurred several days later.[22]

Recognition of the wide spectrum of disease caused by parvovirus, coupled with well-documented health care–associated outbreaks of infection, has fueled efforts to understand the transmission and control of this virus in hospitals.[46,169,326,363,458,489] These studies indicate that parvovirus B19 infection can be transmitted by acutely infected patients, probably as a result of the high levels of virus in their blood and in their respiratory secretions. The risk of transmission occurring from chronically infected, immunocompromised patients is less clear; however, one report indicates that these patients do transmit infection.[363] The mode of transmission is not clear but probably involves direct or indirect contact with respiratory secretions or droplet contact. Therefore, gloves and gowns should be worn during contact with infected patients or fomites contaminated with respiratory secretions, and a mask should be worn during close contact.[559] The description of a play therapist who acquired parvovirus despite her lack of close contact with an infected patient has led to concern about possible airborne transmission, but the need for use of isolation rooms with negative air pressure has not been established and currently is not recommended.[559]

Limited data in one of the outbreaks suggest that standard immune globulin preparations may be useful in mitigating the consequences of infection in high-risk, exposed, susceptible contacts.[458] However, data at present are sufficient to recommend the routine use of immune globulin for postexposure prophylaxis.

HEPATITIS A VIRUS

Although health care–associated outbreaks of hepatitis A seldom occur, this pathogen can cause major epidemics before infection is detected and contained. The largest outbreak of hepatitis A occurred as a result of a blood transfusion from a single donor who was viremic but had not yet developed symptomatic disease; 11 newborns received contaminated transfusions and 55 secondary cases occurred in two hospitals.[433] Other transfusion outbreaks have been described,[32,318] as have outbreaks traced to asymptomatic excretion by an infected child[152] and vertical transmission from mother to infant.[621]

Controlling an outbreak of health care–associated hepatitis A is difficult and usually requires the assistance of the local health department, especially if nonhospitalized contacts or more than one institution is involved. To limit the potential for indirect contact transmission, hospitalized infected children should be cohorted. Hand hygiene and use of barrier precautions should be emphasized to prevent direct contact transmission to caregivers and indirect contact transmission to other children.[559]

Immune globulin should be administered to all exposed, susceptible individuals.[60] Symptomatic health care providers should be furloughed until 1 week after the onset of symptomatic infection or until all susceptible persons have received immune globulin.[60] Tracing of contacts is necessary to prevent additional secondary cases. Hepatitis A vaccine can be administered concurrently with immune globulin to patients who have not been immunized if vaccination is indicated for these individuals.[60] Hepatitis A vaccination is not recommended routinely for health care providers.[60]

ENTEROVIRUSES

Numerous outbreaks of health care–associated enterovirus have been reported, most occurring in newborn nurseries.[37,101,266,299,317,483,596] In most cases, adult caregivers infected in the community transmitted the virus to one or more hospitalized children through direct contact, with subsequent indirect contact transmission from one child to another occurring. In one nursery outbreak, the virus was introduced by a perinatally infected

child.[483] Newborns with severe underlying illness were more likely to be infected, presumably because the prolonged and intensive care these infants required provided more opportunities for transmission.[317,483] Enterovirus infections in newborn infants can be severe—even fatal—but mild and subclinical illnesses also may occur.[410,483] The presence of maternally derived antibody may play a role in limiting the severity of disease in some infants.[410,483]

Surveys of infections in pediatric wards indicate that health care–associated enterovirus infections are uncommon occurrences,[181,610,632] yet the potential for transmission of these viruses is substantial. Large community outbreaks of echovirus and coxsackievirus infections occur predictably every summer and fall. Because virus is excreted in stool for long periods, a large number of hospitalized children and caregivers certainly are excreting virus during these periods. Detection of infection may be compromised because viral cultures may not be readily available, some enteroviruses are difficult to cultivate in tissue culture, and infection may not become manifested until after the patient is discharged.

Like hepatitis A, enteroviruses are picornaviruses, and the use of hand hygiene and barrier precautions are of paramount importance to prevent transmission, and cohorting of infected infants is prudent.[559] A report has demonstrated the value of early diagnosis by use of polymerase chain reaction and aggressive control measures.[29] The upper respiratory tract may be involved in acute enteroviral infection, but most authorities do not recommend the use of masks during the care of infected patients.[559] These same principles should be applied to confirmed or suspected enteroviral infections in children, especially young diapered children, although the need for cohorting is debatable and may not be feasible during summer and fall epidemics. The efficacy of immune globulin as treatment prophylaxis for individuals exposed during the course of an outbreak is unclear, but it may be helpful if the preparation used has significant titers of antibody to the outbreak strain, especially in newborn infants who are at risk for development of severe disease.[2] An antiviral agent, pleconaril, may be useful in treating serious infection.[507]

RABIES

Rabies has been transmitted by corneal and solid organ transplants,[21,581] but spread through contact with infected patients has not been reported.

TUBERCULOSIS

The number of new cases of tuberculosis in the United States has reached an all-time low.[89b] The proportion of cases of multidrug-resistant *Mycobacterium tuberculosis* has remained stable in recent years; rare cases of extensively drug-resistant *M. tuberculosis* have been reported. However, the threat of health care–associated transmission of tuberculosis remains real, heightened by the increasing proportion of cases of tuberculosis in the United States that occur in foreign-born persons and continuing problems with multidrug-resistant and extensively drug-resistant *M. tuberculosis* in many regions of the world.[89b]

This risk was demonstrated dramatically by outbreaks of multidrug-resistant *M. tuberculosis* among adult patients and health care providers in several hospitals in New York and Florida in the 1990s.[43,117,157,194,297,450] One of these outbreaks involved transmission of multidrug-resistant *M. tuberculosis* in the hospital nursery and resulted in infection among infants, mothers, and health care providers.[432] This outbreak and others illustrated that tuberculosis in hospitals caring for pediatric patients occurs almost exclusively as a result of transmission of *M. tuberculosis* from infected adults—parents, visitors, and health care providers—to other children and adults.[33,216,432,585,627] Probable transmission from infected children in health care settings has been described.[388,482] However, the risk of transmission from a child is very small because children infrequently have cavitary disease and consequently have fewer tubercle bacilli in their endobronchial secretions and because young children do not tend to generate aerosols of airborne droplet nuclei.[582a] Nonetheless, transmission from children can occur as evidenced by extensive transmission from a 9-year-old child with bilateral cavitary disease.[128]

The risk of health care–associated tuberculosis occurring in adult hospitals has been minimized by prompt recognition and treatment of pulmonary tuberculosis, adequate isolation of infectious patients in rooms with negative air pressure relative to the hallway, and proper use of personal protective equipment.[296] If rooms with appropriate ventilation are unavailable, alternative engineering solutions, such as well-placed and well-maintained ultraviolet lights, may be useful.[296] Personnel entering the rooms of infected patients should wear respiratory protection devices. Particulate respirators with a National Institute for Occupational Safety and Health certification of N95 or better satisfy the CDC specifications for these devices.[296] These devices must be fit tested on the individuals using them according to the standards of the Occupational Safety and Health Administration.[296] Prompt identification of tuberculosis in parents, visitors, and health care providers in hospitals caring for pediatric patients is an integral part of reducing the risk of transmission of tuberculosis. Screening of health care providers for latent or active tuberculosis is discussed in Chapter 245.

INVASIVE BACTERIAL INFECTIONS

Health care–associated transmission of *Neisseria meningitidis* is a rare occurrence and has been demonstrated in hospitalized patients only when the index patient has meningococcal pneumonia,[112,506] an uncommon clinical presentation of disease in children. Other situations in which transmission has occurred have involved special circumstances. For instance, a mother became infected after nursing her infant who was hospitalized with meningococcemia,[381] and several microbiology laboratory providers developed fatal disease after working with cultures of this microorganism.[87] Hospital personnel caring for patients, on the other hand, appear to be at minimal risk unless they have extensive face-to-face contact and fail to wear a mask.

Health care–associated transmission of *Haemophilus influenzae* or *Streptococcus pneumoniae* also has been regarded as a rare phenomenon but may be more common than previously recognized. A few reports from the 1980s documented transmission of *H. influenzae* type b among pediatric patients,[35,41] and cases among elderly adults and even hospital staff were reported in the late 1980s and early 1990s.[280,393,448,573] Widespread immunization of infants with *H. influenzae* type b conjugate vaccine has reduced considerably the risk of transmission of this microorganism in pediatric patients. Numerous outbreaks of health care–associated infection caused by nonencapsulated *H. influenzae* in elderly patients and hospital staff also have been reported.[20,280]

Numerous outbreaks of health care–associated infection caused by penicillin-resistant *S. pneumoniae* among hospitalized adults and residents of nursing homes have been documented in North America and Europe.[141,383,405,435,630] There are fewer reports of health care–associated multidrug-resistant pneumococcal infections in pediatric patients,[107,136] although this problem has been well documented among hospitalized children in South Africa for many years.[195]

N. meningitidis, *H. influenzae*, and *S. pneumoniae* are spread by droplet contact transmission. Masks should be worn by health care providers while caring for patients with suspected invasive meningococcal disease until the patient has completed 24 hours of parenteral antimicrobial therapy.[559] In the past, the use of masks during care of patients with infections caused by *H. influenzae* type b was variable in many hospitals. However, the CDC Healthcare Infection Control Practices Advisory Committee guideline recommends use of masks to prevent droplet contact transmission from patients infected with *H. influenzae* until the patient has completed 24 hours of parenteral antimicrobial therapy.[559] The guideline does not recommend the use of a mask in caring for patients with *S. pneumoniae* or even penicillin-resistant *S. pneumoniae* unless there is evidence of transmission within a patient care unit or facility.[559] However, given the reports of health care–associated transmission of *S. pneumoniae*, the authors recommend use of masks in caring for patients with infections caused by penicillin-resistant *S. pneumoniae*. Antimicrobial prophylaxis should be administered to persons with close, unprotected contact with patients infected with *N. meningitidis*, but it is not recommended for exposure to patients infected with *H. influenzae* or *S. pneumoniae* unless an outbreak clearly is in progress.

Invasive bacterial infections in neonates, including infections caused by *S. aureus*, group B streptococci, *Citrobacter* spp., and *Enterobacter sakazakii*, are discussed in another section of this chapter (see the section on health care–associated infections in special populations, newborn infants).

ECTOPARASITES

Scabies and pediculosis are common infections in children, and incidental diagnosis of these infections among hospitalized children is not an uncommon occurrence. A large number of outbreaks of scabies have been reported from a variety of health care institutions.[344] The presence of crusted scabies, which is associated with defects in cellular immunity, including HIV infection, increases the risk of transmission because of the large number of mites in these lesions.[344] Although health care–associated transmission of pediculosis can occur, the direct or indirect contact necessary to spread this infection (i.e., head-to-head contact or sharing of combs) is less likely to happen in medical settings than at home.

INTESTINAL HELMINTHS

Person-to-person transmission of four intestinal helminths, *Enterobius vermicularis* (pinworm), *Strongyloides stercoralis*, *Hymenolepis nana*, and *Taenia solium*, is possible because these microorganisms do not require an intermediate host and because the eggs or larvae excreted in the stool are infectious.[345] However, evidence for health care–associated transmission of these microorganisms is limited.[345]

UNUSUAL INFECTIONS INCLUDING INFECTIONS CAUSED BY AGENTS OF BIOLOGIC WARFARE

A variety of unusual, potentially fatal infections may be in transmitted in health care settings. Because international air travel has increased the mobility of the world's population, persons may travel long distances during the incubation period of infections acquired in remote settings.[622] In addition, some of the potential agents of bioterrorism are highly transmissible (e.g., smallpox virus, *Yersinia pestis*, hemorrhagic fever viruses) and could result in secondary health care–associated cases after a primary bioterrorism attack.[26,61,145,268,285,414] A high index of suspicion for these infections and prompt institution of appropriate isolation precautions as indicated are necessary to reduce the risk of health care–associated transmission.

Smallpox is the most feared of the potential agents of biologic warfare because it can be transmitted through droplet nuclei over large distances, the case-fatality ratio is approximately 30 percent, no effective treatment exists, and much of the world's population is either nonimmune or was immunized in the distant past.[268] Even a very limited number of cases of smallpox would be a global public health emergency, and detailed plans for containment would be needed. Hospitalized patients must be cared for with airborne and contact isolation precautions, and health care providers caring for these patients must be vaccinated.[268,592]

Plague is endemic in portions of the western United States and in other countries. No person-to-person transmission of plague has been identified in the United States for more than a half-century,[285] but an outbreak in India illustrated the potential for plague to be imported into other countries.[85] Droplet transmission of *Y. pestis* from persons with pulmonary involvement is well documented. Health care providers should wear masks while caring for patients with signs or symptoms of pulmonary involvement.[285] Tetracycline, doxycycline, sulfonamides, chloramphenicol, and perhaps fluoroquinolones can be used for prophylaxis of contacts.[285]

Ebola, Marburg, Lassa, and Crimean-Congo viruses and the New World arenaviruses have been spread in hospitals because of close contact with infectious body fluids or reuse of contaminated needles.* Health care–associated transmission of hantavirus in the United States has not been reported but has occurred with Andes virus, another cause of hantavirus pulmonary syndrome.[443] Ebola, Marburg, Lassa, Rift Valley fever, yellow fever, Omsk hemorrhagic fever, and Kyasanur Forest disease viruses and New World arenaviruses have been proposed as potential biologic weapons.[61] Of these, only Ebola, Marburg, and Lassa viruses and the New World arenaviruses are spread from person to person (see earlier).[61] The bulk of evidence suggests that airborne transmission of these viruses does not occur in clinical settings, although epidemiologic data are insufficient to exclude this possibility.[83] Health care providers, as well as family members and visitors, providing care to these patients should use airborne isolation precautions as well as additional viral hemorrhagic fever–specific precautions, which emphasize enhanced barrier precautions to avoid contact with the copious amounts of infected material typically encountered in the care of these patients.[61]

HEALTH CARE–ASSOCIATED INFECTIONS RELATED TO INVASIVE DEVICES AND PROCEDURES

This section reviews health care–associated infections related to intravascular catheters and infusion respiratory therapy, instrumentation of the urinary tract, and surgical procedures. Infants, children, and adolescents are at risk for acquiring other health care–associated infections in association with medical treatments, including infections associated with receipt of blood products or immunosuppressive treatments. Readers are referred to chapters in this text related to common bloodborne pathogens (i.e., HIV, hepatitis B and C viruses, and *Trypanosoma cruzi*). In addition, health care–associated infections may occur in relation to invasive procedures other than surgery, such as various endoscopic procedures and interventional radiographic procedures.

*See references 39, 61, 308, 311, 413, 454, 496, 572, 593, 622.

INFECTIONS RELATED TO INTRAVASCULAR CATHETERS AND INFUSIONS

Local infections related to the use of intravascular catheters include infection at the site where the catheter exits the skin (exit-site infection), infection along the subcutaneous track of a tunneled catheter (tunnel infection), and infection in the subcutaneous pocket containing an implanted catheter (pocket infection).[401,436,484] Phlebitis is a common local complication related to the use of intravascular catheters, but it usually is caused by chemical or mechanical irritation and is seen less frequently in children than in adults.[206] Suppurative thrombophlebitis is a rare finding in children.[312]

Systemic infections related to the use of intravascular catheters include bloodstream infections occurring as a result of microbial colonization of the catheter (catheter-related bloodstream infection) and contamination of fluids or medications infused through the catheter (infusate-related bloodstream infection).[401,436] Endocarditis, septic thrombophlebitis, and infection at other body sites as a result of hematogenous seeding (e.g., meningitis, pyelonephritis, hepatic or splenic abscesses, osteomyelitis, septic arthritis, and endophthalmitis) are serious complications of bloodstream infections.

The risk for development of endemic local and systemic infections associated with intravascular catheters varies by the type of catheter in use.[374] Percutaneously inserted peripheral intravenous catheters, especially catheters made with modern pliant, nonthrombogenic materials, are associated with a very low rate of local infection in children, and bloodstream infections are rare occurrences.[206,207,374,554] Rates of local and bloodstream infection associated with peripheral intravenous catheters also are low in newborn infants,[205] but infusion of parenteral nutrition with lipid emulsion through these catheters significantly increases the risk of acquiring bloodstream infection with coagulase-negative staphylococci and *Candida*.[30,70,187,527] Peripheral arterial catheters generally also have a low rate of endemic local and systemic infectious complications; two reports studying a total of more than 400 arterial catheters did not identify any catheter-related bloodstream infections.[153,199]

Most endemic catheter-related bloodstream infections in pediatric patients are associated with central venous catheters.[374] As shown in Tables 244–1 and 244–2, rates of bloodstream infection associated with central venous catheters in pediatric ICUs and central venous and umbilical catheters in newborn ICUs are higher than in adult ICUs participating in the NNIS system.[424] Rates of infection among low-birth-weight infants (<1000 g) in newborn ICUs are higher than those of any other ICU population.[424] These data do not distinguish rates of infection associated with specific catheter types, nor do they distinguish whether a specific catheter is the source of the infection in patients with multiple catheters in place at the same time.

Comparisons of bloodstream infection rates associated with different types of central venous catheters in pediatric patients are confounded by numerous factors.[636] Patient populations in whom these catheters are used have diverse underlying diseases (e.g., cancer, cystic fibrosis, prematurity, short bowel syndrome, AIDS) with widely variant intrinsic risk of bloodstream infection. Even among pediatric oncology patients, risk varies considerably with the type of cancer and the intensity of chemotherapy,[286] and younger children have a higher risk of acquiring infection even when the underlying disease and the type of catheter used are similar.[286,649] Use of the catheter for multiple purposes and infusion of parenteral nutrition with lipid emulsion also increase the risk of acquiring infection.[30,70,315]

With these caveats, rates of bloodstream infection appear lower in totally implanted than in tunneled catheters.[374,639] This conclusion is supported by several studies directly comparing rates of infection between these two types of catheters[334,407,543,649] as well as by a comprehensive review of the literature.[374] These studies demonstrated lower rates of infection with implanted catheters, although statistically significant differences were not seen in every report.

Among adults, rates of bloodstream infection associated with percutaneously inserted central venous catheters are at least several-fold higher than rates associated with either tunneled or implanted catheters,[374] and this observations appears to hold for infants and children as well.[281,584] Rates of infection associated with hemodialysis catheters or intracardiac catheters in pediatric patients who have undergone cardiac surgery have not been reported. Peripherally inserted central venous catheters are now used commonly in newborn ICUs, pediatric ICUs, and pediatric wards. A randomized trial comparing peripherally inserted central venous catheters with peripheral intravenous catheters in very-low-birth-weight infants found no difference in rates of bloodstream infection associated with these two types of catheters. A study of umbilical catheters in neonates found that bloodstream infection occurred in 5 percent of neonates with umbilical artery catheters and 3 percent of neonates with umbilical venous catheters (rates of infections per 1000 catheter days were not reported).[337]

In contrast to endemic infections, which are closely associated with the type of catheter used, epidemics of bloodstream infection generally are related to other factors.[44] In the past, improperly disinfected pressure transducers were the most frequent cause of epidemics of bloodstream infection.[44] Detailed investigation of practices often is necessary to identify and to eliminate causes of bloodstream infection outbreaks, as illustrated by an outbreak of candidemia in a newborn ICU related to retrograde administration of medications into intravenous tubing.[551] Several recent reports have associated the introduction of new mechanical valves in Luer lock catheter caps and hubs with increased infection rates.[386,515]

The principal risk factors for catheter-related bloodstream infection in adults have been identified.[122,123,436,479] Heavy colonization of the skin at the catheter exit site is an important risk factor for colonization of the external surface of catheters of all types and is correlated with increased risk of bloodstream infection.[122,123,436] Colonization of the catheter hub also is an important risk factor for development of bloodstream infections, especially disease occurring late in the course of central venous catheterization.[140,351] Other risk factors related to central venous catheters include longer duration of insertion (>3 days), insertion into the internal jugular vein as opposed to the subclavian vein, use of catheters with multiple lumens as opposed to single lumens, and presence of a mural or atrial thrombus.[122,123,436] Both the level and composition of nurse staffing have been associated with bloodstream infections in adult surgical ICUs—a low nurse-to-patient ratio and a low regular (as opposed to pool nurse) nurse-to-patient ratio were associated with a higher risk of acquisition of a bloodstream infection.[193,504]

Information about general risk factors in children and newborn infants is far more limited than in adults. Two studies of peripheral intravenous catheters in pediatric patients found that the likelihood of colonization occurring on the external surface of the catheter was slightly greater with increasing duration of insertion, but bloodstream infections were extremely uncommon occurrences, even in patients with colonized catheters.[206,207] A study of catheter colonization in patients in newborn ICUs found that duration of insertion longer than 3 days was a significant risk factor for colonization of peripheral intravenous catheters and umbilical catheters.[124] A similar trend was seen with central venous catheters in this patient population, although the number of catheters studied was limited and no conclusions could be drawn with regard to the risk of acquiring a bloodstream infection.[124] Current information is not sufficient to state whether the site of insertion of central venous catheters has an impact on

the risk of acquiring infection, although one study in pediatric ICU patients found similar rates of infection between catheters inserted in the femoral vein and catheters inserted into veins at other sites.[582a] A more recent study of risk factors for acquiring a bloodstream infection in a pediatric ICU identified a higher number of arterial catheter days and packed red blood cell transfusions as well as the presence of an underlying genetic syndrome as independent predictors.[162] Severity of illness, underlying illnesses, and medications were not independently associated with increased risk. As in adult patients, a higher risk for development of infection exists with the use of larger catheters (i.e., multiple-lumen versus single-lumen catheters).[108]

More detailed information is available about risks factors for specific pathogens. A series of studies of coagulase-negative staphylococcal bloodstream infection in patients in newborn ICUs found that length of stay, birth weight, and administration of lipid emulsions were significant risk factors.[30,185,186] Given the potential use of fluconazole for prophylaxis against candidemia, several studies have explored risk factors for development of candidemia in newborn ICUs. Two studies examined risk factors other than birth weight.[172,553] One study identified intravascular catheter use, previous bacterial bloodstream infection, and a history of gastrointestinal disease as risk factors.[172] Another study identified gestational age of less than 26 weeks, vaginal delivery, and abdominal surgery as risk factors.[553] A third study, which did not match or restrict the analysis by birth weight, identified birth weight of 400 to 750 g, receipt of cephalosporin antibiotics, male sex, and lack of enteral feeding as risk factors.[47]

Microorganisms associated with catheter-related bloodstream infections in pediatric and newborn ICUs participating in the NNIS system are displayed in Tables 244–4 and 244–5.[215,497] In both populations, the most common cause of bloodstream infections is coagulase-negative staphylococci. Other gram-positive bacteria, including *S. aureus*, *Enterococcus* spp., and various streptococci, are also relatively common. Ten to 20 percent of infections are caused by gram-negative bacteria, including *E. coli*, *P. aeruginosa*, *K. pneumoniae*, *Enterobacter* spp., *Acinetobacter* spp., *Serratia marcescens*, *Citrobacter* spp., and other aerobic gram-negative bacilli. *C. albicans*, other *Candida* spp., and other fungi together cause 5 to 10 percent of infections. A study from the NNIS system from 1995 to 2004 found that the incidence of *Candida* bloodstream infections in low-birth-weight infants declined during this period, and infection with species commonly resistant to azoles was extremely rare.[190] Atypical mycobacteria occasionally cause bloodstream infection.

A large number of studies in adults, including studies using molecular typing of isolates, have demonstrated that most infections arise from microorganisms that colonize the skin at the insertion site and migrate along the external surface of the catheter through its subcutaneous track and into the blood vessel (extraluminal contamination).[122,123,436,522] Colonization of the interior surface of the catheter occurs through contamination of the catheter hub (intraluminal contamination).[122,123,436,522] The relative contributions of these two mechanisms may depend on the duration of catheterization.[480] Detailed microbiologic studies and scanning electron microscopy have demonstrated that colonization of the external surface predominated in the first 10 days after insertion, whereas colonization of the internal surface increased with the length of catheterization and was significantly more common in catheters in place for more than 30 days.[480] Microorganisms on the hands of health care providers can contaminate the catheter, the insertion site, or the hub during insertion and maintenance of the infusion system.

There are few similarly detailed studies in pediatric patients. However, several studies in neonates suggest that intraluminal contamination is the most common source of organisms causing bloodstream infection. The catheter hub appears to be a common source of bloodstream infections acquired through the intraluminal route.[202,529]

A variety of microbial and host factors play roles in the pathogenesis of bloodstream infections. For example, capsular polysaccharide adhesin, which is a component of the capsule of coagulase-negative staphylococci, facilitates adherence of these bacteria to prosthetic material, formation of biofilm (slime), and intercellular adhesion.[282,395] *Candida* spp. also may produce slime, particularly in the presence of glucose-containing fluids.[66] Interactions among organisms in mixed biofilms also may contribute to colonization of catheter surfaces.[3]

Catheter materials are important factors in catheter colonization and associated infection. Catheters made of polyvinyl chloride or polyethylene are less resistant to colonization in vitro than are catheters made of Teflon, silicon elastomer, or polyurethane. Trauma to blood vessels is more likely to occur with use of stiff plastics (e.g., polyvinyl chloride) than with newer plastic materials (Teflon, silicon elastomer, or polyurethane), predisposing to development of phlebitis and infection. Other factors, such as surface irregularities and the thrombogenicity of catheter material, also may play roles. Catheters that are coated or impregnated with anti-infective agents (e.g., benzalkonium chloride, chlorhexidine–silver sulfadiazine, minocycline-rifampin, silver) to resist bacterial colonization have been developed (see additional discussion later).[122]

Contaminated intravenous infusions,[380,552] medications,[48,237] narcotics (as a result of criminal tampering),[378,442] blood products,[96] and hemodynamic monitoring systems[44] have resulted in serious outbreaks of infection. Purposeful contamination of infusates as a form of child abuse also has been reported.[354] The frequency of hematogenous seeding of the catheter from a distant site of infection is unknown but is likely to be uncommon.

Table 244–7 lists clinical criteria for establishing the diagnosis of infections related to intravascular catheters and infusions.[436] The utility of these definitions has been affirmed for pediatric patients.[484] Definitions used by the NNIS system are similar but do not specifically define separate local infections and include two subcategories of bloodstream infection (laboratory-confirmed bloodstream infection and clinical sepsis).[436] These definitions were revised in early 2008 to require two positive blood cultures within 48 hours for infections caused by common skin microorganisms.[88]

Potential bloodstream infections should be evaluated with at least two blood cultures, including at least one blood culture drawn by venipuncture, before antimicrobial therapy is instituted.[401] If there are indications for catheter removal (see additional discussion later), the catheter should be removed by an aseptic technique and cultured to determine whether it is colonized. Non-tunneled central venous catheters may be changed over a wire or removed and cultured. The semiquantitative roll-plate culture technique is the traditional and most widely available method for culturing the catheter.[379] More than 15 colony-forming units (CFU) is regarded as evidence of colonization of the external surface of the catheter but does not reliably predict risk for development of bloodstream infection; more than 100 CFU has greater positive predictive value for development of bloodstream infection.[379] However, the roll-plate technique does not detect colonization of the catheter hub or the intraluminal surface of the catheter. Quantitative cultures of catheter segments (i.e., catheter tip, subcutaneous segment, or hub), which involve flushing the segment with broth culture media or sonicating the segment in broth followed by serial dilutions of the broth and plating on blood agar, are more sensitive than is the roll-place technique because they detect colonization of both the external and internal surfaces of the catheter.[479,521,551,561] One study suggests that sonication is the most important procedure for increasing the recovery of bacteria from removed catheters.[550]

TABLE 244–7 Clinical Criteria for Infections Related to Intravascular Catheters and Infusions

Infection	Diagnostic Criteria
Catheter colonization	Significant growth of a microorganism (>15 CFU) from the catheter tip, subcutaneous segment of the catheter, or catheter hub
Exit-site infection	Erythema or induration within 2 cm of the catheter exit site, in the absence of concomitant bloodstream infection and without concomitant purulence
Tunnel infection	Tenderness, erythema, or site induration >2 cm from the catheter site along the subcutaneous track of a tunneled (e.g., Hickman or Broviac) catheter, in the absence of concomitant bloodstream infection
Pocket infection	Purulent fluid in the subcutaneous pocket of a totally implanted intravascular catheter that might or might not be associated with spontaneous rupture and drainage or necrosis of the overlying skin, in the absence of concomitant bloodstream infection
Bloodstream infection	
Infusate related	Concordant growth of the same organism from the infusate and blood cultures (preferably percutaneously drawn) with no other identifiable source of infection
Catheter related	Bacteremia or fungemia in a patient with an intravascular catheter with at least one positive blood culture obtained from a peripheral vein, clinical manifestations of infections (i.e., fever, chills, or hypotension), and no apparent source for the bloodstream infection except the catheter One of the following should be present: a positive semiquantitative (>15 CFU/catheter segment) or quantitative (>10^3 CFU/catheter segment catheter) culture whereby the same organism (species and antibiogram) is isolated from the catheter segment and peripheral blood; simultaneous quantitative blood cultures with a ratio ≥5:1 central venous catheter versus peripheral; differential period of central venous catheter culture versus peripheral blood culture positivity of >2 hours

CFU, colony-forming units.
Modified from O'Grady, N. P., Alexander, M., Dellinger, E. P., et al.: Guidelines for the prevention of intravascular catheter–related infections. Centers for Disease Control and Prevention. M. M. W. R. Recomm. Rep. 51(RR-10):1-29, 2002.

Unfortunately, quantitative catheter cultures are time-consuming and expensive, and they are not universally available.

Because these techniques require removal of the catheter, some investigators have attempted to diagnose catheter-related bloodstream infection by comparing quantitative blood cultures drawn through the catheter and by venipuncture (paired cultures) or by comparing multiple cultures drawn through the catheter only (unpaired cultures). A meta-analysis found that the paired quantitative blood culture technique was the most sensitive technique.[521] The differential time to positivity technique, which compares information (time to positivity) provided by existing automated systems between blood cultures drawn through catheter with those drawn peripherally, is nearly as sensitive as the paired quantitative blood culture technique and is less labor-intensive.[521] However, consistent volumes of blood must be inoculated into each of the paired culture bottles.

Other investigators have examined rapid techniques. The acridine orange leukocyte cytospin test is nearly as sensitive as the paired quantitative blood culture technique and the differential time to positivity technique.[521] In a study of infants, the Gram stain and acridine orange leukocyte cytospin test of blood drawn through a central venous catheter had 87 percent sensitivity and 94 percent specificity for the diagnosis of catheter-related bloodstream infections compared with quantitative blood cultures drawn from the catheter and by venipuncture.[517] An advantage of this test is that it can be completed in less than 1 hour. Buffy coat Gram stain of a blood sample drawn from the catheter may reveal yeast forms in some patients with candidemia. If the catheter is removed, Gram staining of adherent material also may facilitate rapid diagnosis.

In practice, several difficulties arise in establishing the diagnosis of catheter-associated bloodstream infections. Physicians' designation of microorganisms isolated from blood cultures as pathogens or contaminants may be confounded by the patient's age and underlying condition and the presence of an intravascular catheter, particularly a central venous catheter. For example, in one study, physicians were more likely to interpret the coagulase-negative staphylococci in blood cultures drawn from very-low-birth-weight infants as clinically significant.[188] Obtaining cultures by venipuncture is difficult in young children, and clinicians may be reluctant to obtain blood for more than one culture in low-birth-weight infants. Central venous catheters often are not removed for culture because of potential complications associated with reinsertion of a new catheter and the fact that many catheter-related bloodstream infections can be treated with the catheter in place. Finally, unless infusate-related bloodstream infection is suspected, obtaining cultures of intravenous fluids or medications often is overlooked.

The terminology used to describe the clinical manifestations of bloodstream infection in pediatric patients has been standardized recently.[231] Consensus definitions of the systemic inflammatory response syndrome (a condition that arises from a variety of processes including bloodstream infection), sepsis, severe sepsis and septic shock, and multiple organ dysfunction in neonates and children have been reported[231] but have not been validated.

Empiric antimicrobial therapy for suspected bloodstream infection instituted while awaiting culture results should be based on the severity of the patient's clinical disease, the nature and severity of the patient's underlying condition, and local knowledge of the relative frequency and susceptibility patterns of pathogens causing this infection. The guideline cited previously contains detailed recommendations for the management of these infections.[401] In most situations, empiric therapy should include an agent effective against gram-positive bacteria, such as nafcillin, oxacillin, or vancomycin, and an agent effective against most gram-negative bacteria including *Pseudomonas*, such as ceftazidime, cefeprime, or an aminoglycoside. MRSA has become such a common problem that many clinicians now prefer to initiate therapy with vancomycin until culture and sensitivity results are available. Gentamicin generally is equivalent to ceftazidime in its activity against gram-negative rods, is synergistic with vancomycin against susceptible *Enterococcus* spp., and is much less costly. Unless renal toxicity or ototoxicity is a major concern, gentamicin may be appropriate empiric coverage against gram-negative rods if the patient has no evidence of meningitis. Empiric use of two agents with activity against gram-negative rods (e.g., ceftazidime plus gentamicin) is appropriate in a severely ill patient or when infection with a resistant gram-negative rod is suspected because even a short delay in instituting appropriate therapy is associated with an increased risk of mortality. In settings where extended-spectrum β-lactamase–producing gram-negative rods are prevalent, choosing a carbapenem for empiric therapy may

be prudent. Empiric antifungal therapy also may be necessary, for instance, in situations when suspicion of fungemia is high (i.e., a severely ill patient who is colonized with *Candida* spp. or a neutropenic patient who is already receiving broad-spectrum antibacterial therapy). Once culture information is available, the treatment regimen can be tailored accordingly or discontinued if no infection is identified.

The aforementioned management guideline also reviews the efficacy of the "antibiotic lock" technique for treatment of tunneled catheter–related bacteremia.[401] The concept behind this approach is that organisms embedded in biofilms on the luminal surfaces of long-term, tunneled catheters may be killed more effectively by antibiotic concentrations 100 to 1000 times higher than standard concentrations of therapeutic parenteral antibiotics.[401] An antibiotic solution (usually vancomycin or gentamicin) in a concentration of 1 to 5 mg/mL with an appropriate concentration of heparin (usually 50 to 100 units) is infused into the catheter in sufficient volume (2 to 5 mL) to fill the lumen and is left in place while the catheter is not being used.[401] The volume of instilled antibiotic is removed before the next dose of antibiotic or intravenous medication or solution is infused. This treatment approach may be used with or without concomitant systemic therapy in carefully selected patients with tunneled catheter–related bacteremia caused by intraluminal colonization of the catheter.[401] Preliminary studies suggest that alcohol locks may provide an additional approach to treatment of recalcitrant catheter infections, although data are insufficient to support this approach.[479]

No evidence suggests a benefit for the use of thrombolytic agents for adjunctive treatment of catheter-related bloodstream infection. Two noncontrolled studies reported good responses to treatment of catheter-related bloodstream infections using a combination of antimicrobial therapy and low-dose urokinase.[303,537] However, two subsequent randomized, controlled trials found no benefit from the combination of urokinase and antimicrobial therapy.[28,333] Recombinant tissue plasminogen activator has proved effective in restoring flow to occluded central venous catheters,[288,469] but this agent has not been studied as adjunctive therapy for catheter-related bloodstream infection.

Most uncomplicated bloodstream infections associated with central venous catheters can be treated effectively without removal of the catheter.[401,639] However, treatment of catheter-associated fungemia without removal of the catheter has a relatively low success rate and may be associated with higher mortality rates as well as with other complications.[135,401] Strong consideration also should be given to removal of catheters associated with difficult-to-treat bacteria, such as antimicrobial-resistant enterococci or gram-negative rods. In the authors' experience, in situ treatment is not as likely to be successful with implanted catheters, but few published data specifically address this issue. Percutaneously inserted central venous catheters may be changed over a wire and the catheter tip sent for culture. If the culture of the tip is negative, the replacement catheter can be left in place. If the culture of the tip is positive, the replacement catheter should be removed and a new catheter inserted at a new site. Catheters should be removed immediately if evidence of embolic phenomena, septic thrombophlebitis, or endocarditis is present or the patient is hemodynamically unstable.

Few data address the optimal duration of therapy for bloodstream infections if the catheter remains in place, but experience suggests that 10 to 14 days usually is adequate. If the catheter is removed, shorter courses of therapy (5 to 7 days) are appropriate for uncomplicated infections caused by less virulent pathogens such as coagulase-negative staphylococci.

Persistently positive blood cultures despite antimicrobial therapy or recrudescence of infection shortly after therapy is completed should prompt removal of the catheter. The need to remove catheters from patients with persistent fever but no positive culture results and no evidence of infection at another site is controversial and should be approached on a case-by-case basis.

Some success has been reported in treating uncomplicated exit-site infections with the catheter in place.[401,639] A trial of a combination of local therapy and systemic antimicrobial treatment is warranted before a decision to remove the catheter is made. In contrast, treatment outcomes of tunnel or pocket infections generally are poor if the catheter is left in place.[401,639]

A guideline for preventing the development of infections related to intravascular catheters and infusions has been published.[436] In addition, several reviews have focused on the role of novel technologies in the prevention of these infections.[122,123,479] Key aspects of the prevention of these infections are emphasized in the following.

Intravascular catheters and infusions should be used only when necessary and only for as long as necessary. Caregivers should monitor catheterized patients closely for signs of local and systemic infection. Interventions directed at minimizing contamination of the catheter during insertion include use of an effective skin antiseptic (chlorhexidine-based agents are more effective than povidone-iodine or alcohol-based agents but are not approved by the Food and Drug Administration for use in neonates).[100,122,205,378] Hand hygiene should be performed before the procedure, and full sterile barrier precautions (e.g., sterile gowns, gloves, and large drapes) should be used while central venous catheters are being inserted.[478] Rigorous application of an "insertion bundle," which includes these interventions with a central line "cart" (to ensure that all necessary supplies and equipment are easily accessible) and a checklist to monitor compliance, has been associated with significant reductions in the incidence of bloodstream infections in several studies, including a statewide observational study in Michigan.[49,475,618] The impact of this type of bundled intervention has not been studied as extensively in pediatric patients, but it has been used in a quality improvement collaborative sponsored by the Child Health Corporation of America and the National Association of Children's Hospitals and Related Institutions.

Several studies, including a study involving neonates, have examined the efficacy of a chlorhexidine-impregnated sponge placed at the catheter insertion site.[204,257,346,376] This device reduced cutaneous colonization at the insertion site and colonization of the catheter tip; however, only one study demonstrated that use of the device resulted in lower bloodstream infection rates.[376] In the neonatal study, 15 percent of the neonates weighing less than 1000 g developed localized contact dermatitis from the device.[204] Routine application of topical antimicrobials to the catheter exit site is not recommended.[123,436] Transparent semipermeable dressings facilitate inspection of the catheter site. However, some studies suggest that they may increase microbial proliferation at the site of insertion and predispose the patient to development of bloodstream infection, whereas others have shown no difference in colonization of the site or infection rates in comparison to gauze and tape dressings.[436] Despite the controversy, transparent dressings are in widespread use, often in combination with a small piece of gauze placed at the site of the insertion to absorb moisture.

Several publications have reviewed the data on the effect of central venous catheters impregnated with antimicrobials and silver-impregnated subcutaneous catheter cuffs on the incidence of catheter-related bloodstream infection.[123,436,479] These studies have not involved any pediatric patients. Catheters impregnated with chlorhexidine–silver sulfadiazine (coating the external surface only) or minocycline-rifampin (coating both the internal and external surfaces) significantly reduce rates of bloodstream infection compared with unmedicated catheters.[122] The minocycline-rifampin catheter was associated with lower rates of bloodstream infection than was the chlorhexidine–silver sulfadiazine catheter in a head-to-head trial.[134] However, a new chlorhexi-

dine–silver sulfadiazine catheter designed with a higher level of antiseptic in the catheter material and with coating of the internal and external surfaces has been demonstrated to reduce catheter colonization.[516] Silver-impregnated catheters and catheters using active iontophoresis have been tested, but the results have been mixed.[479] The long-term impact of these catheters on antimicrobial resistance has not been evaluated.[123,479] These types of catheters have not been evaluated systematically in pediatric patients. In general, these catheters should be considered only if the conventional methods for prevention of infections as described before have been implemented in a highly reliable fashion and have failed (e.g., in high-risk patients prone to recurrent infections).

Catheter caps containing antiseptic agents also have been tested. A randomized controlled trial of a central venous catheter cap with a chamber containing 3 percent iodinated alcohol demonstrated a fourfold reduction in the rate of catheter-related bloodstream infection,[539] although these results were not confirmed in a subsequent study performed by a different group.[364] A small randomized controlled trial of a povidone-iodine–saturated sponge to encase the hub demonstrated significant reduction in the incidence of bloodstream infections.[254] These devices have not gained widespread acceptance, and the amount of iodine that may enter the bloodstream if these devices are used routinely remains unclear.

Needle-less connectors for intravenous tubing are important to reduce the risk of needle-stick injury among health care providers. However, increased rates of bloodstream infection have been observed when these devices have been used incorrectly.[115,130,391]

Reports associating increased rates of bloodstream infection with introduction of new mechanical valves in Luer lock catheter caps are cause for concern because they may indicate a design defect that facilitates contamination of the interior surfaces of the device.[386,515] However, because these reports do not document adherence with optimal cap care, incorrect practice may be a contributing factor.

Studies of the use of "prophylactic" vancomycin or other antibiotics added to intravenous fluids and flush solutions to prevent development of catheter-related infections have shown some promise.[436] A randomized, placebo-controlled trial of a vancomycin-heparin lock solution found that this intervention reduced the incidence of catheter-related bloodstream infections in high-risk neonates with long-term central catheters.[203] No vancomycin-resistant organisms were identified. Vancomycin was not detected in the blood of infants who did not receive systemic vancomycin therapy. A substantial proportion of infants in each arm of the study had asymptomatic hypoglycemia at the end of a catheter-lock period, but these episodes resolved promptly when glucose-containing intravenous fluids were restarted. The intervention appears to be safe, although with asymptomatic hypoglycemia in the infants receiving the lock. This type of intervention warrants further study.

Manipulation of the catheter and infusion sets should be minimized, and these sets should be maintained as closed systems whenever possible. When manipulation is necessary, hand hygiene and use of aseptic technique should be practiced and hubs should be disinfected with an alcohol swab. Infusion sets used for standard intravenous fluid administration need not be changed more frequently than every 72 hours.[436] If parenteral nutrition or blood products are administered, infusion sets should be changed at least every 24 hours.[436]

The apparatus for hemodynamic monitoring should be maintained as a closed system, and manipulations of this system should be kept to a minimum.[436] When manipulation is necessary, hand hygiene and use of aseptic technique should be practiced. Disposable domes and transducers used for hemodynamic monitoring should be changed every 96 hours.[436] Reused transducers should be reprocessed appropriately between uses.[436]

Stopcocks are common components of intravenous infusion and hemodynamic monitoring systems and are used as portals for injection of medications, infusion of fluids (e.g., parenteral nutrition), and collection of blood samples. These devices can become contaminated during use, although their role in bloodstream infections has not been studied well. Manipulation of these devices should be kept to a minimum, and the access port should be disinfected before it is entered.

Two randomized controlled trials have found fluconazole to be effective in preventing candidemia among low-birth-weight infants in neonatal ICUs,[307,385] although the rates of candidemia in both trials were substantially higher than those reported in a large network of newborn ICUs.[585] A survey of prescribing practices conducted between the publication of these two trials indicated that use of prophylactic fluconazole was not widespread.[76]

INFECTIONS RELATED TO RESPIRATORY THERAPY

Most cases of health care–associated pneumonia are caused by bacteria and are associated with mechanical ventilation. Pneumonia also can be caused by respiratory viruses, but these infections are caused by intrahospital transmission of common community infections rather than by exposure to medical devices or ICUs (see the section on health care–associated infections due to spread of infections common in the community, respiratory infections, respiratory viruses). *Legionella* and filamentous fungi also cause pneumonia, but these infections occur almost exclusively in immunocompromised patients and are related to inhalation of contaminated aerosols or fungal spores, not respiratory therapy.

Tables 244–1 and 244–2 display rates of pneumonia associated with mechanical ventilation in pediatric ICUs and newborn ICUs in U.S. hospitals participating in the NNIS system.[424] Rates of pneumonia are considerably lower in pediatric and newborn ICUs than in adult ICUs.[424]

Aside from these rates, very limited data exist on the epidemiology, pathogenesis, treatment, and prevention of ventilator-associated pneumonia in infants and children. The information discussed here draws largely from studies in adult patients. This same general information can be found in several reviews.[180,474,646]

Health care–associated pneumonia related to the use of equipment generating contaminated mists has not been reported recently in pediatric patients, although numerous outbreaks caused by "water bacteria" (e.g., *Flavobacterium meningosepticum*, *P. aeruginosa*, *Achromobacter* spp.) in pediatric patients in the 1950s and 1960s were linked to the use of centrifugal, Venturi, and ultrasonic mechanical nebulizers.[411] Such devices can aerosolize droplets that are small enough to reach the distal airways, so contamination of their fluid reservoirs is extremely hazardous. Most large-volume, mechanical nebulizers have been replaced by heated humidifiers. These devices are considerably less dangerous because they do not generate small-particle aerosols and are less prone to heavy contamination because heating of the reservoir retards the growth of most potential pathogens. Mist tents used today for patients with bronchiolitis and croup do have mechanical nebulizers, but they generate larger particles that are deposited in the environment, mouth, and pharynx and do not generally reach the lower respiratory tract. Nonetheless, an effective cleaning and disinfection program is required to minimize the likelihood for development of infection.

On the other hand, small-volume, hand-held nebulizers used commonly for inhalation therapy in pediatric patients are designed intentionally to generate particles small enough to reach the distal airways, and the potential for pneumonia related to the use of these devices is underappreciated by most caregivers. An outbreak of *Legionella* pneumonia caused by contaminated

medication administered with use of small-volume nebulizers has been reported.[387] In addition, the small-volume medication nebulizers used in conjunction with mechanical ventilators can become contaminated by bacteria colonizing the ventilator circuit and thus generate bacteria-laden aerosols.[121]

Mechanical ventilation is a major risk factor not only for health care–associated pneumonia but also for sinusitis and otitis media because the nasotracheal tube interferes with normal drainage of the ostia of the sinuses and the eustachian tube.[50,238] Detection of these infections requires a high index of suspicion because they often are "silent," with little in the way of symptoms other than fever.

Risk factors for ventilator-associated pneumonia in adults have been summarized in a review.[594] This review groups risk factors into several categories: host factors, such as chronic pulmonary disease and immunosuppression, which increase general susceptibility to pneumonia; factors that enhance bacterial colonization of the oropharynx and stomach with pathogenic bacteria, such as severe underlying disease, administration of antimicrobials, and, possibly, agents that raise gastric pH (e.g., antacids, H_2 blockers); factors that increase the likelihood of reflux of gastric contents and aspiration into the lower airway, such as depressed mental status, supine positioning, nasogastric tubes, and enteral feeding; conditions that require prolonged ventilation and hence increase potential exposure to contaminated respiratory equipment and contact with contaminated hands of caregivers; and factors that hinder adequate pulmonary toilet, such as thoracic or abdominal surgery and immobilization.[594] At least some of these risk factors are likely to be important in children as well. A study in a pediatric ICU found that the presence of a genetic syndrome, repeated intubation, and transport from the pediatric ICU independently predicted development of ventilator-associated pneumonia.[164]

Microorganisms associated with ventilator-associated pneumonia in pediatric and newborn ICUs participating in the NNIS system are displayed in Tables 244–4 and 244–5.[215,497] Not reflected in the tables is the fact that the majority of cases of pneumonia are polymicrobial in nature and often include both gram-positive and gram-negative bacteria.[594] Gram-negative bacilli, including *P. aeruginosa*, *Enterobacter* spp., and *Klebsiella* spp., are major pathogens, as is *S. aureus*. *S. pneumoniae* and *H. influenzae* have been recognized increasingly as causes of early-onset ventilator-associated pneumonia in adults.[591] *Moraxella catarrhalis* is an occasional pathogen. Anaerobic bacteria seldom are implicated as a cause of pneumonia,[594] but they may play a role in some polymicrobial infections, especially when pneumonia is caused by aspiration. Although *C. albicans* and other *Candida* spp. are isolated in some cases, they rarely if ever are the primary cause of pneumonia. The distribution of microorganisms causing sinusitis and otitis media in nasotracheally intubated patients is similar to that of pneumonia, although anaerobic bacteria may play a greater role.[71]

The pathogenesis of ventilator-associated pneumonia is complex but can be reduced to two general mechanisms: (1) aspiration of microorganisms colonizing the stomach and oropharynx and (2) inhalation of contaminated aerosols.[594] Aspiration is responsible for most cases of endemic pneumonia, whereas inhalation of contaminated aerosols tends to occur in the context of outbreaks of infection from common sources.

A variety of factors facilitate colonization of the oropharynx and upper respiratory tract with pathogenic bacteria. Adherence of gram-negative organisms to mucosal cells is enhanced in severely ill or debilitated patients by exposure of epithelial bacterial receptors and by changes in the amount and character of respiratory secretions.[594] Bacterial factors, such as the presence of pili in *P. aeruginosa*, also play a role.[594] Antimicrobial therapy reduces the concentration of normal flora, thereby reducing "colonization resistance" and allowing antimicrobial-resistant microorganisms to gain a foothold. Bacteria may reach the pharynx by the hands of caregivers or from contaminated equipment or aerosols, or they may be regurgitated into the pharynx from the stomach. Normal gastric pH prevents heavy contamination of stomach contents, but bacteria proliferate to high levels when stomach acid is neutralized by antacids or H_2 blockers.[594] Although the roles of these agents in fostering gastric colonization are supported by most studies, the degree to which they are associated with an increased risk of acquiring pneumonia is less clear.[591] Factors that increase reflux of gastric contents into the upper airway, such as bolus enteral feedings, nasogastric tubes, and supine position, probably increase the risk substantially, but they have not been studied extensively.

Regardless of how microorganisms reach the upper respiratory tract, contaminated secretions can be aspirated into the lower respiratory tract of mechanically ventilated patients during changes in position or deflation of the endotracheal tube cuff. Uncuffed endotracheal tubes are used in most pediatric patients, so the potential for aspiration is constant.

Pneumonia associated with inhalation of contaminated aerosols is much less common than is colonization of the oropharynx and aspiration.[591] As noted previously, such infections are likely to be caused by *P. aeruginosa*, *Legionella*, and a variety of other nonenteric gram-negative bacilli that are capable of surviving and proliferating in medications and solutions used in respiratory therapy.[594]

Once microorganisms have entered the lower airway, the status of normal defense mechanisms is extremely important in determining whether infection results. Diseases that compromise the mucociliary clearance system (i.e., cystic fibrosis, chronic lung disease) and the ability of the immune system to contain and to inactivate these microorganisms (i.e., chemotherapy, HIV infection) increase the risk of infection dramatically.

Methods used to diagnose community-acquired pneumonia, such as auscultation of the chest, examination of sputum, and chest radiography, are helpful but much less precise tools for establishing the diagnosis of ventilator-associated pneumonia. Auscultation often is hindered by sounds of the ventilation system itself and is virtually impossible in patients on high-frequency ventilation. Furthermore, a variety of underlying pulmonary diseases (e.g., bronchopulmonary dysplasia, adult respiratory distress syndrome, cystic fibrosis) and conditions (e.g., fluid overload) may produce sounds indistinguishable from those present in pneumonia. Cultures of tracheal aspirates may be misleading because the endotracheal tube often is colonized with potential pathogens, especially in patients ventilated more than a few days.[224] A Gram stain of the tracheal aspirate can semiquantitatively assess both the number of neutrophils and the number and type of microbial flora, but localized irritation or superficial infection of the trachea related to the endotracheal tube may produce purulent secretions that are laden with bacteria on Gram stain. Finally, the presence of new radiographic findings consistent with pneumonia may be extraordinarily difficult to assess in patients with underlying lung disease or patients recovering from thoracic or complicated cardiac surgery. Viral pneumonia requires demonstration of the pathogen by direct detection, culture, or serology.

A variety of techniques to improve the diagnosis of ventilator-associated pneumonia in adults have been evaluated. Bronchoscopic techniques (e.g., quantitative culture of protected brush specimens, bronchoalveolar lavage, and protected bronchoalveolar lavage) have been studied in detail. A meta-analysis and a recent randomized controlled trial failed to show a benefit in terms of clinical outcomes from the use of a bronchoscopic technique.[555,602] The utility of blind catheterization of the distal airway (i.e., mini–bronchoalveolar lavage) to obtain specimens for Gram stain and quantitative culture of the endotracheal aspirate also

has been investigated, albeit less rigorously.[72] Neither technique has been studied rigorously in ventilated children or infants.

Evaluation of fever in a mechanically ventilated patient should include examination for otitis media. If a cause of the fever is not established, consideration should be given to radiographic studies of the sinuses (e.g., sinus films or a computed tomographic scan), especially if the child has a nasotracheal tube. If otitis media or sinusitis is diagnosed, tympanocentesis or a tap of the sinuses for culture should be considered to guide antimicrobial therapy.

Administration of empiric antimicrobial therapy for ventilator-associated pneumonia while awaiting culture results should be based on the severity of the patient's clinical disease, the nature and severity of the patient's underlying condition, and local knowledge of the relative frequency and susceptibility patterns of pathogens causing this infection. Therapy with a broad-spectrum agent with good activity against gram-negative bacilli, including *P. aeruginosa*, possibly with inclusion of an agent active against MRSA as guided by local patterns of susceptibility, generally is appropriate. On the basis of the patient's clinical status and culture results, the treatment regimen can be tailored accordingly or discontinued (i.e., de-escalated). Prolonged treatment of patients in whom the diagnosis is questionable should be avoided because it often leads to endotracheal colonization with antimicrobial-resistant bacteria. Duration of treatment has not been studied in children. Uncomplicated cases may be treated with 7 to 10 days of therapy. If a necrotizing gram-negative pneumonia is diagnosed (rare in pediatric patients), therapy should be administered for at least 14 days.

Nasotracheally intubated patients with otitis media or sinusitis should have the nasotracheal tube changed to an orotracheal tube. Short-term treatment with a decongestant and lavage of the sinuses (often performed at the time of the sinus tap) may be beneficial in the treatment of sinusitis. Treatment of these infections also has not been studied extensively, but the authors generally use approximately 10 days of therapy for otitis media; extensive sinusitis warrants administration of therapy for at least 14 days.

A comprehensive guideline for the prevention of health care–associated pneumonia has been developed by the CDC and the Healthcare Infection Control Practices Advisory Committee.[594] Key features of this guideline are emphasized here.

Noninvasive approaches to enhance ventilation should be used as much as possible. Mechanical ventilation should be used only when necessary and for only as long as necessary. Care should be taken to avoid accidental extubation. Caregivers should monitor ventilated patients carefully for signs of pneumonia, otitis media, and sinusitis. New approaches to weaning of patients from mechanical ventilation can reduce the length of the period of mechanical ventilation significantly.[605] However, a randomized controlled trial in pediatric ICUs did not demonstrate reduced time to extubation with the use of a volume support weaning protocol, in part because most of the children were weaned from mechanical ventilator support in 2 days or less.[485]

The importance of prevention of cross-colonization with "hospital flora," especially antimicrobial-resistant bacteria, should be stressed. Hand hygiene and use of gloves during contact with respiratory secretions or objects or surfaces contaminated with respiratory secretions are important, especially in busy ICUs caring for many sick patients. Data are insufficient to recommend sterile rather than clean, nonsterile gloves.

A cuffed endotracheal tube with subglottic or in-line suctioning is recommended in adults. Cuffed endotracheal tubes are not used routinely in all children, especially younger children, and in-line suctioning has not been studied in a pediatric population. Suctioning should be performed gently with a sterile, single-use catheter, and sterile fluids should be used to loosen secretions and to clear the suction catheter. Several procedures to prevent reflux of gastric contents during enteral feeding, such as elevating the head of the bed, avoiding rapid infusion of large fluid volumes, and avoiding external pressure on the stomach, are simple to implement and probably helpful. Use of continuous rather than intermittent bolus feedings and duodenal rather than gastric placement of the feeding tube have not been studied in sufficient detail. Avoiding the use of agents that elevate gastric pH is prudent unless the patient is viewed as being at risk for stress-induced gastric bleeding. A retrospective study of ventilated children found that the strategy used to prevent gastrointestinal bleeding was not associated with the risk for development of ventilator-associated pneumonia.[361] Regular oral care should be performed. A recent randomized trial found that perioperative decontamination of the nasopharynx and oropharynx with 0.12 percent chlorhexidine gluconate reduced the incidence of lower respiratory tract infections and deep surgical-site infections in adult cardiothoracic surgery patients.[538] This strategy has not been tested in infants and children.

The experience of a children's hospital implementing a ventilator-associated pneumonia prevention bundle, derived from the interventions discussed before, is discussed in a recent review.[127]

Endotracheal tubes and ventilator circuits become contaminated with the patient's own oropharyngeal flora very quickly, and repeatedly changing this equipment does not reduce the risk for development of infection. Ventilator circuits and humidifiers should be changed no more frequently than every 48 hours. The maximum "permissible" period of use has not been established.[594] Care should be taken to prevent condensate that collects in the ventilator tubing from draining into the endotracheal tube because this fluid can be contaminated with a very large number of microorganisms. Tubing should be maintained in a dependent position relative to the endotracheal tube, and condensate should be discarded routinely. The impact of various innovations, such as traps to collect condensate, bacterial filters, hygroscopic condenser-humidifiers, or heat exchange humidifiers, on the risk for development of pneumonia has not been determined.

Large-volume, mechanical nebulizers should not be used unless they are scrupulously cleaned and reprocessed on a daily basis. Only sterile water should be used in these devices. Small, hand-held medication nebulizers should be rinsed with sterile water and allowed to air dry between uses. These devices should be reprocessed before use in another patient. Only sterile, aseptically dispensed medications should be used in these devices. Other respiratory therapy equipment should be reprocessed between uses in different patients.

Development and application of effective procedures for reprocessing of all reused respiratory therapy and ventilator equipment have played a major role in reducing the risk for development of serious gram-negative pneumonia. Items that have direct or indirect contact with the mucus membranes or respiratory secretions should be cleaned thoroughly and either sterilized or disinfected in a manner consistent with high-level disinfection.

INFECTIONS RELATED TO INSTRUMENTATION OF THE URINARY TRACT

Urinary tract infections (UTIs) are the most common health care–associated infection in hospitalized adults, accounting for approximately 40 percent of all infections in this group.[242] In contrast, UTIs represent a much smaller proportion (10% or less) of health care–associated infections in hospitalized children.[181,294,339,632] This difference can be explained in part by differences in urinary tract catheterization in these two populations. Data from the NNIS system indicate that indwelling urinary catheters are used approximately half as frequently in pediatric

ICUs as they are in adult ICUs.[424] Indwelling urinary catheters are used infrequently in newborn ICU patients. On the other hand, the infectious risk associated with the use of indwelling urinary catheters is similar in adults and children. As shown in Table 244–1, rates of UTIs associated with indwelling urinary catheters are only slightly lower in pediatric ICUs than in adult ICUs in U.S. hospitals participating in the NNIS system.[424]

The descriptive epidemiology of UTIs in pediatric patients has been examined in several studies.[138,339,357,358,440] In a prospective cohort study, catheterized patients were identified and prospectively observed for symptoms and signs of UTIs (in which case a urinalysis and culture were performed) and by weekly urinalyses and urine cultures regardless of symptoms.[357] Health care–associated UTIs in noncatheterized patients also were studied. UTIs were detected in 11 percent of all catheterized patients, including 11 percent of those in whom only indwelling catheterization was used, 9 percent with both indwelling and intermittent catheterization, and 12 percent with intermittent catheterization alone. The incidence density of infection was not reported. The median duration of catheterization preceding infection was 7 days (range, 2 to 77 days). Three quarters of the infections identified were symptomatic. No definite cases of secondary bacteremia were detected.

Additional information about the spectrum of health care–associated UTIs in this study was provided by combining data from catheterized and noncatheterized patients.[357] Catheterization and female sex were identified as risk factors for development of UTIs; 77 percent of all infections occurred in catheterized patients, and 75 percent of all infections occurred in female patients. The majority of infections in noncatheterized patients occurred in the newborn ICU and the preschool-age ward. A wide range of underlying diagnoses were reported, but neurologic, renal, oncologic, orthopedic, or trauma-related diagnoses accounted for 50 percent of cases.

No data have been published on rates of UTIs associated with different types of indwelling catheters (e.g., urethral, suprapubic, ureteral, nephrostomy) in pediatric patients. In the authors' experience, infection (or at least bacteriuria or candiduria) frequently occurs when catheters remain in place for long periods (i.e., more than 1 to 2 weeks), regardless of the type of catheter used. Data on the incidence of infection developing after cystoscopy, renal transplantation, or other types of urinary tract instrumentation in pediatric patients are not available. In the authors' experience, infections seldom develop after cystoscopy alone or urologic surgery in which preexisting infection or bacteriuria is not present and long-term use of indwelling catheters is not necessary. Bacteriuria and UTIs appear to occur more frequently among patients undergoing renal transplantation, although it is difficult to determine whether this is due to the use of urinary catheters in this population or to inherent risks of the transplantation procedure itself.

Outbreaks in adult patients in the 1970s and 1980s were attributed to contamination of the urinary collecting system by pathogens on the hands of hospital personnel, contaminated drainage pans and measuring containers, and use of inadequate or contaminated antiseptics.[74] Several outbreaks demonstrated that use of urinary catheters contributed to the emergence of antimicrobial resistance among gram-negative bacilli.[74] Antibiotics excreted in the urine provide selective pressure for the emergence of resistant populations of bacteria colonizing urine collection systems, and the large numbers of different types of bacteria present in these systems facilitate transfer of resistance plasmids between species.[74]

Microorganisms causing catheter-associated UTIs in pediatric ICUs participating in the NNIS system are displayed in Table 244–4.[497] Roughly a fifth of infections are caused by *E. coli* alone, and another quarter are caused by various other gram-negative bacteria. *Enterococcus* spp. and various *Candida* spp. account for 10 and 14 percent of infections, respectively. These data are similar to those reported from individual studies.[138,357,358] The rate of UTIs attributed to coagulase-negative infections in newborn ICU patients has been reported to be in excess of 30 percent.[138,358]

Microbial colonization of the urine is the first step in the pathogenesis of UTIs. A detailed culturing study of catheterized patients suggests that roughly two thirds of infections are caused by microorganisms that migrate up the extraluminal surface of the catheter, and the other third of infections arise from microorganisms that gain access to the bladder through the internal lumen of the catheter.[598] In addition, numerous studies in catheterized adults have correlated microorganisms colonizing the urethral meatus with those most frequently causing infection.[74] These microorganisms can be carried into the bladder during insertion of a catheter, or more commonly, once the catheter is in place, they can migrate along the external surface of the catheter into the bladder. The shorter length of the urethra in women has been suggested as an explanation for their increased risk of causing infection in women. However, meatal colonization with pathogenic microorganisms, which also is more common in women, also may be important.[74] Distention of the urethra by the catheter, obstruction of periurethral glands, adherence of pathogenic microbes to the uroepithelium and the external surface of the catheter, and formation of a biofilm composed of host proteins and microbial exopolysaccharide on the external surface of the catheter all play a role in initiation of infection, although the relative contributions of each of these factors are poorly understood.[74]

Microorganisms also may be introduced into the bladder from exogenous reservoirs. Although modern catheters and urine collecting systems are "closed" systems, microorganisms may be introduced into the interior of the catheter collecting system at three points: the junction between the urinary catheter and the collecting system, the port used to aspirate urine specimens and to irrigate the catheter, and the drainage spigot attached to the collection container. Some urinary catheters are fused to the collection system during manufacture, eliminating the possibility of junction disconnection, but such catheters are not in widespread use. Improper technique in the use of the aspiration and irrigation port or the spigot also may result in contamination of the interior surfaces of the system. Once microorganisms gain access to the interior of the catheter or collecting system, they can multiply quickly, reaching concentrations in the collection container exceeding 10^5 CFU/mL of urine in a matter of a few days. Encrustations on the interior surface of the system may serve as sites for microbial attachment and proliferation. Movement of microorganisms into the bladder is facilitated by obstruction of the urine flow and reflux of urine into the bladder (i.e., raising the collection system above the level of the bladder), but many gram-negative bacteria are motile and can "swim" upstream even if the system is maintained properly.

Microorganisms that reach the bladder multiply in the small, but persistent, reservoir of urine that is not completely drained by the catheter.[74] Infection (i.e., symptoms and signs of tissue invasion) is not an inevitable result of colonization of the bladder, and many chronically catheterized patients go for long periods with gross urine colonization but no clinical evidence of infection. Because many young children have vesicoureteral reflux, ascending infection involving the kidneys is a significant concern, but the epidemiologic studies discussed before have not demonstrated pyelonephritis and secondary bacteremia to be common problems.

The diagnosis of a health care–associated UTI depends heavily on the result of a quantitative urine culture. Urine specimens from noncatheterized patients should be obtained aseptically by one of three techniques: midstream, clean-catch collection; "straight" catheterization of the bladder; or suprapubic aspira-

tion. Cultures of urine specimens obtained from external bag collectors are of dubious value because they frequently are contaminated. Specimens from patients with indwelling urinary catheters should be obtained from the aspiration port because urine collected from the collection container may reflect colonization of this reservoir rather than infection.

Although diagnostic criteria for catheter-associated UTIs vary, several definitions established for surveillance purposes generally are well accepted.[208] Symptomatic UTI is defined as the presence of symptoms or signs of infection (e.g., fever, urgency, frequency, dysuria, or suprapubic tenderness) and a quantitative urine culture with more than 10^5 CFU/mL of urine of no more than two different species. Asymptomatic bacteriuria or candiduria (also termed asymptomatic urinary tract infection in some studies, including the cohort study discussed before[357]) is defined as the absence of symptoms or signs of infection and a quantitative urine culture with more than 10^5 CFU/mL of urine of no more than two different species.[208] The diagnosis of asymptomatic bacteriuria is complicated by the fact that approximately 5 percent of healthy school-aged and adolescent girls in the community have asymptomatic bacteriuria that may be detected only after they have been hospitalized. A urinalysis showing evidence of pyuria (e.g., dipstick positive for leukocyte esterase or ≥10 white blood cells/mL^3 of urine) or nitrate provides corroborating evidence of infection. Pyuria is a reasonably good predictor of the presence of significant concentrations of gram-negative rods in the urine of catheterized patients, but it is a poor predictor for the presence of significant concentrations of enterococci or *Candida*.[597] A Gram stain of an unspun urine specimen is useful in guiding initial treatment decisions. However, patients with indwelling catheters and low-level bacteriuria (<10^5 CFU/mL) tend to progress to frank bacteriuria with 10^5 CFU/mL or more within a few days if antibiotics are not administered.

The diagnosis of symptomatic UTI in patients with urinary catheters often is complicated by the fact that these patients do not have symptoms such as urgency, frequency, or dysuria, and the abdominal pain and tenderness may be difficult to assess in newborns and severely ill children in ICUs. Consequently, fever is the sign of infection most frequently identified in these patients, emphasizing the importance of a quantitative urine culture in the evaluation of a new fever in a hospitalized child.

Therapy for health care–associated UTIs is similar to that for community-acquired infections, although parenteral therapy usually is used initially until secondary bacteremia is excluded. Empiric antimicrobial therapy for urinary tract infection while awaiting culture results should be based on the severity of the patient's clinical disease, the nature and severity of the patient's underlying condition, and local knowledge of the relative frequency and susceptibility patterns of pathogens causing this infection. Therapy with a broad-spectrum agent with good activity against gram-negative bacilli, possibly including an agent or agents active against *P. aeruginosa* and enterococci, is generally appropriate. If the Gram stain reveals only gram-negative bacilli, enterococcal infection is unlikely. If renal toxicity from the use of an aminoglycoside is a serious concern, use of a third-generation cephalosporin, carbapenem, aztreonam, or a quinolone in older children is appropriate. Antifungal therapy generally can be withheld, unless yeast elements are identified on a urinalysis or Gram stain of the urine. On the basis of the patient's clinical status and culture results, the treatment regimen can be tailored accordingly or discontinued (i.e., de-escalated).

Whenever possible, indwelling urinary catheters should be removed. Cases of asymptomatic bacteriuria (or asymptomatic candiduria) often resolve with removal of the catheter alone.

Few data are available on the appropriate duration of therapy for health care–associated UTIs. Although simple community-acquired cystitis in older children and adolescents often can be treated successfully with 1 to 3 days of oral therapy, it is prudent to treat uncomplicated hospital-acquired UTIs for at least 7 days. If the infection is uncomplicated and the child has responded to therapy, oral therapy can be used to complete the course. Even pyelonephritis may be treated with oral agents if the pathogen is susceptible, but therapy should be extended to at least 14 days. Secondary bacteremia usually is treated parenterally. Treatment of asymptomatic candiduria with a short course (i.e., 5 days) of therapy is sufficient, but infection is likely to recur unless the catheter is removed. Likewise, infections involving nephrostomy or suprapubic catheters often recur if these catheters must remain in place. In cases in which nephrostomy tubes must be used for extended periods, suppressive antimicrobial therapy may be used after treatment is completed, although this approach involves a risk for development of secondary infection with resistant bacteria or yeast.

The prospective cohort study discussed previously examined the consequences of health care–associated UTIs during the 6 months after infection occurs.[357] Four patients (7.8%) suffered relapses and one patient (2.0%) suffered a re-infection. No deaths occurred, and no additional complications were identified. The need for evaluation of vesicoureteral reflux or anatomic abnormalities of the kidney or urinary collecting system after a health care–associated UTI in infants or young children has not been evaluated. The authors do not perform these studies routinely if the infection appears related to a urinary catheter.

A guideline for the prevention of health care–associated UTIs was published in the mid-1980s.[643] Key features of the prevention of these infections are emphasized here.

Reducing unnecessary use of urinary catheters is the most effective preventive measure. Davies and colleagues[138] reduced the rate of urinary tract infection in a pediatric ICU by 90 percent during the course of an 8-month period simply by instituting a policy whereby nurses automatically removed urinary catheters from patients after 48 to 72 hours unless a physician indicated that continued catheterization was necessary. Urinary catheters should be inserted by trained personnel using careful aseptic technique after the meatus has been cleansed and an effective antiseptic (e.g., povidone-iodine) applied to the meatus and surrounding skin.[643] Contamination of the interior surfaces of the catheter and collection system can be minimized by maintaining a closed system (i.e., avoiding disruption of the connection between the catheter and the collection system), disinfecting the aspiration and irrigation port before accessing this port, and minimizing contamination of the spigot on the collection container.[643] The collecting system should be examined regularly to ensure that urine flow is not impeded, and care always should be taken to maintain the collection system in a dependent position relative to the bladder to prevent reflux of urine.[643] Treatment of preexisting UTIs is important, especially among patients with underlying renal, urologic, or neurologic disease, in whom these infections are frequent occurrences.[643] Systemic antibiotics are effective in preventing UTIs for short periods (5 days or less)[74]; however, routine use of antibiotics for this purpose is likely to facilitate emergence of resistant pathogens. A wide variety of other interventions have been studied, most with limited or variable benefit.[74,375] Manufacturers continue to attempt to design a catheter that will resist adherence of microorganisms or minimize urethral trauma. A silver-hydrogel catheter that inhibits adherence of microorganisms to the catheter surface and antibiotic-impregnated catheters have been studied in adults with mixed results. No studies of novel urinary catheters have been performed in pediatric patients.

INFECTIONS RELATED TO SURGICAL PROCEDURES

Patients undergoing surgical procedures are at risk for a variety of health care–associated infections. A 1-year study of more than 600 pediatric patients undergoing surgical procedures found that

surgical-site infection was the most common infection after surgery, occurring in 3.5 percent of patients.[53] Bloodstream infection occurred in 2.3 percent of patients, with half of these infections occurring in patients whose surgical procedure was insertion of a tunneled central venous catheter. Other reported infections included pneumonia (1.6%), UTI (0.8%), gastrointestinal infection caused by rotavirus (0.3%), and necrotizing enterocolitis (0.2%). A report of health care–associated infections in pediatric patients undergoing cardiovascular surgery found that surgical-site infection and bacteremia accounted for 28 and 27 percent of all infections, respectively.[468]

A handful of reports have described the epidemiology of surgical-site infections in general pediatric surgery patients, although these reports are now dated.[54,137,139,150,369,547]

As expected, wound class was a powerful predictor in these studies of the likelihood for development of surgical-site infection. Considerable variability exists in infection rates within wound class categories among the pediatric studies, suggesting that other factors must have a strong impact on the risk for development of infection. A composite risk index developed through the analysis of data from the NNIS system, which includes measures of severity of illness (the ASA score) and duration of surgery in addition to wound class, is a much better predictor of risk of surgical-site infection than is wound class alone (see Table 244–4).[125] Children undergoing surgery were not included in the original development of this risk index.[125] Other risk factors for development of surgical-site infection are longer duration of surgery,[53,137,139,150] younger age,[150,369,547] prolonged preoperative hospital stay,[137,139,150] emergency surgery,[54,150] longer length of incision,[137,150] and presence of underlying diseases.[54] The experience of the surgeon (i.e., resident versus attending surgeon) performing the surgery was not found to be a risk factor for development of infection, although unnecessary trauma and cautery as well as residual hematoma probably do increase risk.

Two types of surgical procedures—cardiovascular procedures and neurosurgical procedures—are discussed because of the particularly serious nature of surgical-site infections associated with these procedures.

A prospective study of more than 300 children undergoing cardiovascular surgery found an infection rate of 7.1 percent.[468] Surprisingly, infection rates in this study were lower among patients undergoing open heart surgery with cardiopulmonary bypass (6.7%) than among patients undergoing closed heart surgery without bypass (8.1%), but this difference was not statistically significant. Infection rates were higher among patients in whom the sternum was left open after surgery (27.6% in patients with open sternums versus 5.0% in patients with closed sternums, $p < .001$) and among patients with higher PRISM scores (10.7% in patients with PRISM scores ≥10 versus 2.3% in patients with PRISM scores <10, $p < .01$). It is not clear from the data presented in this study whether an open sternum after surgery was an independent risk factor for development of infection or whether the increased risk in patients with open sternums was attributable entirely or in part to increased severity of illness (i.e., higher PRISM score). Subsequent studies documented prolonged duration of surgery and younger age as risk factors.[9,304,423] However, a study that examined the performance characteristics of a risk index specific for children undergoing cardiovascular surgery failed to identify a set of factors that adequately predicted the risk for development of surgical-site infection.[304]

Rates of infection after cerebrospinal fluid shunting procedures vary widely, but many studies are dated, and reported rates of infection related to ventriculoatrial and ventriculoperitoneal shunts (which now are used infrequently) involved small numbers of patients or did not routinely incorporate perioperative antimicrobial prophylaxis into the regimen of care.[651] A large pediatric series from the 1980s with more than 500 patients undergoing ventriculoperitoneal shunting procedures, most of whom received perioperative antimicrobial prophylaxis, reported an infection rate of 11 percent.[437] Rates of infection were higher among patients with myelomeningocele and patients who had undergone previous shunting procedures. Half of the infections occurred in the 2 weeks after surgery and were not commonly associated with simultaneous incisional infection.

Microorganisms causing surgical-site infections in pediatric ICUs participating in the NNIS system are displayed in Table 244–5.[497] Gram-positive cocci account for nearly half of the isolates. *S. aureus* is the most common isolate, but coagulase-negative staphylococci and *Enterococcus* spp. also are common findings. Gram-negative bacilli of various types represent approximately 40 percent of isolates. *Bacteroides fragilis* and other anaerobic bacteria are isolated infrequently, but how frequently anaerobic cultures were performed is unclear. *C. albicans* and other *Candida* spp. are isolated from a minority (4%) of surgical-site infections. This distribution of microorganisms is similar in the several studies of surgical-site infection in general pediatric surgery patients cited previously.[53,137,547] Studies of pediatric patients who undergo cardiovascular surgery have described a similar distribution of microorganisms.[160,468] Gram-positive bacteria more frequently are the cause of cerebrospinal fluid shunt infections. In the large study cited previously, gram-positive bacteria accounted for three fourths of the infections; coagulase-negative staphylococci were the cause of 40 percent of the infections.[437]

The pathogenesis of surgical-site infection relates to the degree of microbial contamination of the wound, the condition of the host (both resistance to infection and the ability to heal the wound), and the conduct of the procedure itself. Microbial contamination of the surgical site occurs almost exclusively during the time the incision is open, which likely is the reason that longer duration of surgery is associated with increased risk of infection, although longer procedures also may be reflective of other factors that influence risk (i.e., complexity of the surgery, skill of the surgeon, care in dissection). If the incision is closed primarily, subsequent contamination of the site from external sources (i.e., use of contaminated antiseptics or dressings) seldom occurs. Most of the microorganisms contaminating open wounds are endogenous in origin (i.e., microorganisms colonizing the skin and respiratory, gastrointestinal, and genitourinary tracts). Indeed, carriage of *S. aureus* in the anterior nares is associated with postoperative infection caused by this organism.[324,384] Infrequent outbreaks of surgical-site infection caused by *S. aureus*, group A streptococci, and group C streptococci have been traced to personnel in the operating room (but not necessarily participating in the surgery itself) who are heavy shedders of these microorganisms, usually as a result of rectal, vaginal, or nasal carriage.[230,502,530] The inanimate environment of the operating room (e.g., walls, floors, other surfaces, and surgical instruments) is an uncommon source of microorganisms causing surgical-site infections.[31] Postoperative hematogenous seeding of the surgical site can occur but seldom does. Airborne fungal spores can cause postoperative infections in immunocompromised patients and rarely in patients undergoing cardiovascular or neurosurgical procedures.

Host factors, such as underlying diseases (e.g., malignant neoplasm, uremia), the competence of the immune system, and nutritional status, are likely to be important, but the nature and degree to which these factors affect the risk for development of an infection are not well described. Hyperglycemia and frank diabetes have been demonstrated to be risk factors for development of infection in adults.

Even with careful preparation of the skin in clean surgery, small numbers of microorganisms are present at the surgical site of virtually every procedure, and careful surgical technique is necessary to avoid conditions that favor the growth of these microorganisms in the postoperative period. Damaged and devascularized tissue created by rough tissue handling and overuse

of electrocautery is less resistant to infection.[10] Hematomas and seromas, which provide optimal growth conditions for bacteria, may develop if dead spaces are not obliterated or if hemostasis is inadequate.[10] Low-pressure suction drains are indicated to facilitate drainage of blood and secretions and to facilitate adherence of tissues and surfaces to promote wound healing.[10] To the extent that they accomplish this goal, surgical drains are useful. However, unnecessary use of drains should be avoided because they provide a portal for entry of microorganisms and may inhibit healing.

Consensus criteria for surgical-site infections developed through the collaborative efforts of the CDC, surgeons, and infection control professionals have been published.[276] Surgical-site infections are subcategorized into superficial incisional infections (involving the skin or subcutaneous tissues), deep incisional infections (involving the muscle or fascia), and organ or space infections (involving visceral organs or deep body spaces or cavities). For the most part, these criteria rely on observations made by direct inspection of the surgical site. Cultures of wound drainage are useful to determine the microbial etiology of the infection, but these criteria rely on the results of wound cultures only when wound drainage is not clearly purulent (i.e., serosanguineous drainage must be culture positive for it to be considered evidence of an infection). Stitch abscesses are not considered surgical-site infections.

Principles of the treatment of surgical-site infections are described in other references.[10] Significant fluid collections, especially collections of purulent material, should be drained. Contemporary interventional radiology drainage techniques have revolutionized the approach to such collections. In some cases, fluid collections may be inaccessible or an attempt at drainage may seriously compromise the patient. In these cases, antimicrobial therapy alone may be successful in resolving the infection, although therapy needs to be chosen carefully to ensure adequate penetration into the area and to minimize the potential for emergence of antimicrobial resistance. Clinical response must be monitored closely. Devitalized tissue should be débrided, and any tension, pressure, or obstruction in the area should be relieved to allow adequate blood flow and drainage. Foreign bodies should be removed. For instance, cerebrospinal fluid shunt infections are not likely to resolve unless the shunt is removed.[651] In some special cases, antimicrobial therapy can successfully suppress infection with foreign bodies in place while postoperative healing takes place (i.e., plates and screws to stabilize bones during healing after orthopedic procedures). However, removal of these devices generally is required eventually to achieve complete cure. Systemic antimicrobial therapy should be prescribed for serious infections, but many superficial infections resolve with local care and application of topical antimicrobial agents.

A guideline for the prevention of surgical-site infections is available.[384] More recently, the Surgical Care Improvement Project has codified the most important prevention practices.[68] Key prevention measures that have the common goal of minimizing microbial contamination of the surgical site are summarized in the following paragraphs.

Preoperative interventions have a major impact on the nature and degree of microbial contamination of the surgical site; it is prudent to minimize the duration of preoperative hospitalization because hospitalized patients are more likely to become colonized with hospital flora (e.g., methicillin-resistant staphylococci). Treatment of preexisting infections is important because microorganisms causing these infections may contaminate the surgical site, but unnecessary therapy should be avoided as it increases the likelihood that colonization with antimicrobial-resistant microorganisms will occur.

Preoperative bathing with use of an agent with antimicrobial activity is a logical prevention measure. This practice does reduce colony counts of bacteria on the skin but has not been shown to reduce the rate of surgical-site infection definitively.[384] A study comparing chlorhexidine and iodophor shampoos before neurosurgical procedures in children demonstrated that chlorhexidine shampoos reduce the number of microorganisms on the scalp before surgery and the frequency of wound contamination; however, the number of patients studied was not sufficient to demonstrate any effect on infection rates.[341] A meta-analysis concluded that preoperative bathing with chlorhexidine is not beneficial in reducing the risk for development of an infection.[625]

A randomized trial examined the effect of topical mupirocin applied to the nares preoperatively to prevent surgical-site infections after general, gynecologic, neurologic, or cardiothoracic surgical procedures.[452] Children were not included in this trial. The overall rate of surgical-site infections was not different among patients receiving topical mupirocin versus those receiving placebo. However, the risk for development of infection with *S. aureus* at any site (surgical site, bloodstream, catheter, lower respiratory tract) among patients with nasal carriage of *S. aureus* was significantly lower among those who received mupirocin than among those who received placebo. Screening for colonization with *S. aureus* or MRSA in patients undergoing elective surgery remains controversial, but some authorities advocate MRSA screening for patients undergoing placement of prosthetic materials and devices in regions where the community prevalence of MRSA is high. If a carrier is detected, an attempt can be made to eradicate colonization before surgery, or the prophylactic antibiotic can be changed to an agent with activity against MRSA.

Although removal of hair appears to provide a "cleaner" operative site, shaving the skin, especially the day before surgery, has the paradoxical effect of increasing skin colonization by liberating resident skin flora from deeper skin structures and causing microscopic skin trauma that facilitates the growth of bacteria. If hair removal is necessary, hair should be clipped instead of shaved. If shaving is necessary, it should be done immediately before surgery.

Appropriately administered perioperative antimicrobial prophylaxis eradicates or at least retards the growth of bacteria that gain access to the surgical site. Numerous studies have examined the effectiveness of perioperative antimicrobial prophylaxis for various surgical procedures performed on adults, but few studies have been performed in pediatric patients. Consequently, recommendations for perioperative antimicrobial prophylaxis for pediatric patients undergoing surgery largely follow regimens recommended for use in adults.[13] Prophylaxis should be administered within 1 hour (2 hours for vancomycin) before the surgical incision is made.[110] If prophylaxis is administered outside this window period, effectiveness is greatly diminished; administration more than 6 hours after the incision is made has essentially no effect.[110] If the surgical procedure is prolonged, another dose should be administered after 2 half-lives of the administered agent have elapsed (i.e., 4 to 6 hours for cefazolin). Additional doses in the postoperative period are unnecessary. Ensuring appropriate use and timing of perioperative antimicrobial prophylaxis is an important quality of care issue; likewise, ensuring that prophylaxis is not used unnecessarily is important for containment of costs.

A variety of intraoperative practices and procedures are intended to prevent microbial contamination of the surgical site.[384] However, the effectiveness of many of these measures is either minimal or has not been studied rigorously. Cleaning of the skin and application of an antiseptic (e.g., alcohols, iodophors, chlorhexidine) reduce the number of viable bacteria at the surgical site. The surgical hand scrub performed by members of the operative team using an effective antiseptic (e.g., alcohols, iodophors, chlorhexidine) reduces the number of bacteria on the hands and the potential for contamination of the surgical site through visible or microscopic breaks in surgical gloves. Barriers worn by the surgical team (i.e., masks, caps, gowns) are logical

parts of operating room practice and provide protection for the operative team against exposure to blood and body fluids, but little evidence exists that they have a substantial impact on the risk for development of surgical-site infections. Traffic and activity in the operating room increase the bacterial count in the air, and limiting the number of persons and movement to a minimum is reasonable, but the effect on the risk for development of infection is likely to be minimal. Bacterial counts in the air also can be minimized by regularly servicing the operating room ventilation system and by maintaining adequate ventilation parameters (15 air changes with 3 changes of outside air per hour and use of a ventilation system with filters with at least 95% efficiency).[18] Laminar air flow and ultraviolet lights can decrease bacterial counts in the air to very low levels, but their effectiveness in reducing the incidence of infections remains controversial.

Proper surgical technique also is important in the prevention of surgical-site infections, as discussed previously. Many studies have demonstrated that confidential feedback of surgeon-specific rates of infection to individual surgeons reduces rates of infection.[601] This approach has been endorsed by the CDC, surgeons, and infection control professionals as a means by which individual surgeons may examine their own rates of infection and adjust their operative techniques accordingly.[604]

Other interventions that have been examined in adult patients include glycemic control, maintenance of normothermia, and supplemental oxygen. These interventions have not been investigated in infants and children undergoing surgery.

HEALTH CARE–ASSOCIATED INFECTIONS IN SPECIAL POPULATIONS

NEWBORN INFANTS

Healthy newborns are thrust into the world quickly, becoming colonized with microorganisms derived from their mothers and the immediate environment within several days. Predominant colonizers are coagulase-negative staphylococci on the skin and umbilicus and in the nose and alpha-hemolytic streptococci in the mouth. Colonization of the gastrointestinal tract is more complex. Lactobacilli predominate in breast-fed babies, whereas more "adult" flora composed of *Bacteroides* spp., other anaerobes, and *E. coli* colonize formula-fed babies.[229] These commensals help resist colonization with pathogenic microorganisms. Because pathogens can spread quickly in crowded nurseries, rooming-in and early discharge reduce the potential for transmission to normal newborns. However, early discharge also renders detection of significant problems when they occur more difficult. For instance, the incubation period for *S. aureus* infection generally is longer than a newborn's stay in the hospital, and significant outbreaks can escape detection unless an aggressive reporting system is established.[265,368]

Premature and full-term infants requiring care in newborn ICUs face a far different fate. Colonization of these infants is delayed substantially, perhaps because of limited contact with their mothers, delayed enteral feeding, and administration of parenteral antimicrobial agents.[222,228] When colonization does occur, it is with markedly different microorganisms. Coagulase-negative staphylococci colonize the skin, umbilicus, and nose of these infants as well.[228] However, molecular typing studies have demonstrated that particular strains of coagulase-negative staphylococci can persist in newborn ICUs for extended periods, being transmitted on the hands of caregivers and causing bloodstream infection in some infants.[283,365,447] *S. aureus* may colonize a variety of sites, particularly the umbilicus and nose.[228] Although lactobacilli and anaerobes still colonize the gut, aerobic gram-negative bacilli, including *E. coli*, *Klebsiella* spp., *Enterobacter* spp., *P. aeruginosa*, *S. marcescens*, and *Citrobacter* spp., represent a much larger proportion of the intestinal flora than in normal newborns. Antimicrobial-resistant strains of these bacteria are a particular problem in many newborn ICUs. As these bacteria can reach concentrations in the stool of 10^6 to 10^8 CFU/g, one is not surprised that the hands of caregivers are easily contaminated, providing an important route for transmission. *Enterococcus* and *Candida* can become established on the skin and umbilicus and in the gut,[228] again by contamination of the hands of caregivers and selection pressure imposed by treatment with antimicrobial agents. Unfortunately, the medical devices that help sustain these infants, such as intravascular catheters and mechanical ventilation, provide portals of entry for these microorganisms to invade sterile sites and cause infection.

The preceding sections have described a variety of microorganisms generally regarded as community pathogens that often cause outbreaks of health care–associated infection in nurseries and newborn ICUs, including RSV and other respiratory viruses, rotavirus, *Salmonella* and other enteric bacterial pathogens,[648] herpes simplex virus, cytomegalovirus, hepatitis A virus, and enteroviruses, and infections related to the use of invasive devices in neonates (see the sections on health care–associated infections due to spread of infections common in the community and health care–associated infections related to invasive devices, procedures, and treatments). Other important pathogens in normal newborn nurseries and newborn ICUs are highlighted here.

S. aureus remains a significant pathogen in newborn infants, although not nearly to the extent that it was in the 1950s and 1960s. Staphylococcal skin and soft tissue infections, including superficial skin infections, mastitis, and omphalitis, are the most common infections; severe staphylococcal pneumonia is now a rare occurrence.[530] Direct contact with colonized caregivers is the predominant mode of transmission.[530] Indirect contact transmission from infected or colonized infants and droplet contact transmission from infants with coexisting viral respiratory tract infection (so-called cloud babies) are uncommon occurrences.[530] Colonization of the skin, nose, umbilicus, or rectum precedes development of infection, but the correlation between rates of colonization and development of infection is poor. Typically, the number of colonized infants far exceeds the number of infected infants; conversely, outbreaks of staphylococcal infection can occur in nurseries with low colonization rates.[530] For this reason and because of cost considerations, surveillance cultures to detect colonized infants are not recommended except under outbreak conditions.

MRSA has been a persistent if somewhat sporadic problem in newborn ICUs for many years. However, infants in newborn ICUs as well as otherwise healthy newborns are being affected by broader trends in the incidence of MRSA infection (see the section on pathogens causing health care–associated infections). Outbreaks of MRSA in newborn ICUs have become more commonplace, even involving multiple separate units in the same city.[218] In addition, genotypically distinct strains of MRSA commonly associated with staphylococcal infection in the community have been identified increasingly as a cause of serious infection in otherwise healthy newborns and their mothers.[67,91,182,523] Risk factors for these infections include cesarean delivery (likely due to the longer postnatal hospital stay), male sex, improper infection control practices associated with circumcision, and contact with an infected health care worker.[51,91,431] Management of these outbreaks includes emphasis on standard infection control practices such as hand hygiene, use of isolation precautions, and proper disinfection or sterilization of patient care equipment. Although screening for colonization during the course of an outbreak clearly is useful to identify asymptomatically colonized individuals, the role of routine screening of infants—and potentially health care providers—is less clear.[91,218]

The use of hexachlorophene (3%) for bathing is effective in reducing colonization,[226,263] but this agent was found to cause cystic degenerative changes in the white matter of premature infants,[472,556,557] although no evidence was found that this agent posed a hazard to full-term infants. A warning against use of hexachlorophene was issued by the Food and Drug Administration in 1972. The use of this agent for infant bathing remains an option during outbreaks of *S. aureus* infection, although it should be diluted by 1:4 or 1:5 in water and should not be used for bathing very low-birth-weight infants.[226] Chlorhexidine is a reasonable alternative because it has good antistaphylococcal activity, and studies in neonates have demonstrated negligible absorption after bathing or cord care and no recognized toxicity. However, chlorhexidine is not approved by the Food and Drug Administration for use in neonates, even as an antiseptic for the insertion of vascular catheters.[7,118,301] Use of iodophors may cause adsorption of iodine, and alcohols may cause chemical burns, so these agents should not be used for bathing. A variety of agents, including triple dye (an aqueous mixture of brilliant green, proflavine hemisulfate, and crystal violet), alcohol, bacitracin, chlorhexidine, and mupirocin, have been used for care of the cord, although extensive efficacy and safety data are not available for any of these compounds.[226,263]

Fifty to 75 percent of women with group B streptococcal vaginal colonization will transmit this microorganism to their newborn infants, although only 1 to 2 percent of colonized infants will become infected. The CDC defines these and other infections transmitted through the birth canal as health care–associated infections.[208,215] The logic behind this designation has not been stated explicitly, but because these infections occur after an event (i.e., delivery) usually associated with medical care, it is reasonable to consider them health care–associated infections. However, the impact of conventional infection control interventions on early-onset (i.e., within the first 7 days of life) invasive group B streptococcal infection is likely to be limited. Intrapartum prophylaxis of women is a far more effective intervention to reduce infection rates in newborns.[97,536] An important note is that group B streptococci can be associated with health care–associated infection, not just perinatal infection. Outbreaks of group B streptococcal infection due to indirect contact transmission in nurseries have been well documented.[156,227,434]

Preterm infants are particularly susceptible to infection caused by a variety of yeasts and, to a lesser extent, filamentous fungi. *Candida* spp. are the most common pathogens. Infants probably become colonized in their gastrointestinal tracts because of carriage of *Candida*, especially *Candida parapsilosis*, on the hands of health care providers.[526] Cutaneous colonization also may lead to invasive infection.[509] Risk factors for colonization with *Candida* are treatment with H_2 blockers or third-generation cephalosporins and delayed enteral feeding.[526] Risk factors for development of bloodstream infections and the use of prophylactic fluconazole have been discussed previously (see the section on health care–associated infections related to invasive devices and procedures, infections related to intravascular catheters and infusions). Risk factors for development of *Malassezia furfur* infection are similar to those for *Candida*, but the association with lipid emulsion is particularly strong because this organism is obligately lipophilic.[129,359,501] Outbreaks of *Malassezia pachydermatis* infection have been reported, in one case in association with colonization of a health care provider's dogs.[102,628] Infections caused by filamentous fungi are rare and usually are associated with contaminated devices or practices that facilitate cutaneous invasion.[408,509]

Citrobacter diversus (now classified as *C. koseri*) has been responsible for numerous outbreaks of health care–associated infection in newborn infants.[350,445,641] Although most infants colonized with this microorganism do not develop clinical disease, infection almost always results in meningitis due to the particular neurotropism of this bacteria (see the section on general epidemiology of health care–associated infections, interactions between hosts and pathogens) and usually is accompanied by formation of one or more brain abscesses. Outbreaks of infection caused by this bacterium may occur sporadically for an extended time.[350,445,641] Transmission of this bacterium on the hands of caregivers has been implicated in several of these outbreaks.[445,641] On the other hand, molecular techniques have demonstrated that *C. koseri* also can be acquired perinatally by vertical transmission.[264]

Enterobacter sakazakii is a rare cause of health care–associated infection in neonates.[586] However, as is the case with infections caused by *C. koseri*, infections caused by this bacterium are usually severe, often involving meningitis and brain abscess.[55,93,109,336,564,612] Outbreaks of infection caused by *E. sakazakii* have been linked conclusively to consumption of intrinsically contaminated powdered milk formulas.[55,93,421,564,612] A variety of products have been implicated in these outbreaks.[93,336] Because powdered formulas are not marketed as sterile products, feeding practices in neonatal ICUs need to balance the relative nutritional benefits of particular products against the small but real risk of infection with *E. sakazakii* and other potential pathogens.

New obstetric practices, such as water birth, may pose infectious threats to infants, as demonstrated by the report of a case of *Legionella* pneumonia in an infant after a prolonged delivery in a pool with contaminated water.[184]

Hand hygiene is the most effective intervention to prevent spread of pathogenic microorganisms.[62] However, the hands of some staff may remain colonized with these microorganisms for prolonged periods despite scrupulous handwashing.[229] In addition, use of artificial nails or nail wraps has been associated with carriage of *P. aeruginosa* on the hands of health care providers in a neonatal ICU.[179] Use of these devices should be banned in these units.[524] Alcohol-based hand rubs are the most effective agents in reducing hand colonization with pathogenic bacteria and are the preferred method for hand hygiene when hands are not visibly soiled.[62]

Preceding sections contain specific recommendations for the prevention of health care–associated infections caused by community pathogens and infections related to the use of invasive devices (see the sections on health care–associated infections due to spread of infections common in the community and health care–associated infections related to invasive devices, procedures, and treatments). Other general references for the prevention of infections in normal nurseries and newborn ICUs, including recommendations for the design of facilities, appropriate staffing, and general infection prevention procedures, have been published.[15,263]

Several interventions designed specifically to prevent acquisition of health care–associated infections in high-risk infants deserve additional comment.

The Vermont Oxford Network Neonatal Intensive Care Quality Improvement (NIC/Q) Collaborative is a large multicenter network of neonatal units organized for the purpose of implementing evidence-based interventions to improve the quality of care of newborn infants *(www.nicq.org)*.[278] A subgroup of six units used quality improvement methods to implement 17 practices designed to reduce the incidence of health care–associated infections. The range of the number of these practices implemented per unit was 10 to 16. Compared with 65 units that did not participate in this initiative, overall rates of health care–associated infection and rates of coagulase-negative staphylococcal bacteremia declined more rapidly in the six units implementing these interventions.[277]

Many investigators have purposefully colonized infants in newborn ICUs with nonpathogenic microorganisms, including viridans streptococci and *Lactobacillus*, to "interfere" with colonization by potential pathogens.[226,406,493] Some of the studies using viridans streptococci demonstrated favorable results, but this approach has not been pursued in part owing to concern about

the potential for adverse events.[226] Studies using *Lactobacillus* have demonstrated little or no beneficial effect.[406,493]

Extremely-low-birth-weight infants have fragile skin, which is predisposed to drying and cracking and may be an inadequate barrier to prevent invasion by bacteria colonizing the skin. For this reason, products containing emollients that include fatty acids have been developed to reduce transepidermal fluid loss and to improve function of the skin barrier in very-low-birth-weight infants. A randomized controlled trial of the effect of an emollient ointment (Aquaphor) in infants with a birth weight of 501 to 1000 g and a gestational age of 30 weeks or less was conducted in 53 newborn ICUs participating in the Vermont Oxford Network.[161] The study found that treated infants had better skin condition on days 1 to 14 of life and fewer skin injuries on days 15 to 28 of life. However, the treatment group had a higher rate of late-onset bacterial sepsis, nearly entirely due to higher rates of bacterial sepsis infection in infants weighing 501 to 750 g. There was no effect on mortality rates. In contrast, two randomized controlled trials conducted in Bangladesh found that sunflower seed oil reduced the rates of late-onset infection and mortality in preterm infants with a gestational age of 33 weeks or less.[131,133] Interestingly, the second of these trials included Aquaphor as a comparator treatment and found a similar decrease in rates of mortality associated with application of this agent. An additional study conducted during the second trial provided indirect evidence that the effect of the treatment was mediated by decreased passage of skin pathogens into the bloodstream.[132]

Because premature infants lack sufficient levels of opsonizing antibodies, several well-designed trials have examined the efficacy of intravenous immune globulin in preventing health care–associated infection in premature infants.[38,170,316,371,628] Only one of these trials demonstrated any benefit in reducing overall health care–associated infection rates.[38] A systematic review of studies addressing the efficacy of intravenous immune globulin in preterm (<37 weeks) or low-birth-weight (<2500 g) infants found an overall 3 percent reduction in the incidence of sepsis and a 4 percent reduction in the incidence of serious infections associated with administration of immune globulin. However, no evidence of an impact on other outcomes, such as necrotizing enterocolitis, intraventricular hemorrhage, length of stay, or mortality, was noted.[439] An evaluation of commercially available intravenous immune globulin preparations found a large degree of variability in opsonic activity against common neonatal pathogens among lots produced by various manufacturers.[629] Individual lots also demonstrated variable levels of opsonic activity against different pathogens. This variability appeared to be a function of the donor pool rather than of the manufacturing method. Whether intravenous preparations with known pathogen-specific antibody content may be effective in reducing the rates of health care–associated infections with specific agents remains to be seen. Immunotherapy directed at antigens on the surface of staphylococci appeared promising in early clinical trials but failed to reduce the risk of development of *S. aureus* infections in premature neonates in a phase III trial.

Because preterm infants have limited neutrophil pools as well as limited neutrophil function, several randomized, controlled studies have examined the prophylactic and therapeutic effect of granulocyte-macrophage colony-stimulating factor (GM-CSF) and granulocyte colony-stimulating factor (G-CSF) on health care–associated infections in preterm infants. In all of these studies, infants receiving these agents had higher peripheral blood neutrophil counts than did infants receiving placebo. However, the benefits of treatment with these agents have been limited. A study of prophylactic treatment with GM-CSF for 5 days beginning shortly after birth found a trend toward decreased infection rates in treated infants, although the study was powered to address whether treatment reduced the incidence of neutropenia, not whether it reduced infection.[81] A second study of prophylactic treatment with GM-CSF during the first 28 days of life failed to demonstrate any benefit in terms of decreased health care–associated infection rates.[81] A study of the therapeutic effect of 3 days of treatment with G-CSF administered to infants with the clinical diagnosis of early-onset sepsis did not demonstrate any reduction in mortality rates but did find that infection rates were lower in treated infants in the 2 weeks after treatment was administered.[409]

Leukocytes present in stored blood products may have adverse effects on immune function, especially in preterm infants. However, a study examining the effect of universal pre-storage red blood cell leukoreduction programs found no effect on bloodstream infection and mortality rates, but it was associated with improvements in other clinical outcomes.[173]

Use of postnatal corticosteroids to prevent or to treat chronic lung disease in preterm infants has been associated with higher rates of health care–associated infection[588] as well as with other short- and long-term adverse outcomes.[113]

CHILDREN WITH BURNS

Information about the epidemiology of health care–associated infections in pediatric burn patients has been described in three reports.[210,535,624] The experience of a single pediatric burn facility that performed prospective surveillance using modified CDC definitions has been reported.[624] The definitions used in this study were modified to describe more accurately burn infections and secondary bloodstream infections in the burn population. On the basis of this and other studies, new definitions for burn infection have been proposed.[451] The overall frequency of infection in this unit was 14 percent or 16 infections/1000 patient days.[624] The frequency of burn infections was 10 percent or 5.6 burn infections/1000 patient days. Gram-positive cocci, including *S. aureus* and MRSA, were the most common causes of burn infections in patients with relatively small burns (<30% of body surface area). Gram-positive cocci and gram-negative bacteria (especially *P. aeruginosa*) were common causes in patients with extensive burns (>30% of body surface area). Rates of device-associated infections were 4.9 bloodstream infections/1000 central venous catheter days, 11.4 cases of ventilator pneumonia/1000 ventilator days, and 13.2 UTIs/1000 urinary catheter days. These rates are somewhat lower than the pooled means for adult burn units (see Table 244–1) and are roughly comparable to rates of infection in pediatric ICUs (see Table 244–1 and Fig. 244–1), although the incidence of UTIs is somewhat higher. When infection rates in this study were stratified by the extent of the burn, the incidence of bloodstream infection secondary to burn infection and catheter-associated UTIs increased with increasing size of the burn, although rates based on the number of patient days or device days reflected the risk of infection more accurately over time.

Interventions to reduce the incidence of health care–associated infections in burn patients include use of barrier techniques to reduce cross-colonization of patients, prevention of cross-colonization during hydrotherapy treatments, use of topical antibiotics to retard growth of microorganisms in the burn wound, appropriate use of systemic antibiotics, and early excision and closure of the burn wound. The success of the Shriners Burns Hospital in Boston in controlling infections in severely burned children by these methods and "bacteria-controlled nursing units" (a laminar air flow unit providing controlled temperature and humidity combined with barrier precautions) has been described.[623] A randomized, controlled trial of selective decontamination of the digestive tract using polymyxin E, tobramycin, and amphotericin B in severely burned children found no evidence of benefit.[40]

CHILDREN WITH CYSTIC FIBROSIS

Saiman and colleagues[525] reviewed the epidemiology of important pathogens in persons with cystic fibrosis in a document summarizing the conclusions of an international consensus conference convened to develop evidence-based guidelines for standardized microbiologic and infection control practices for these persons. They concluded that evidence of transmission of *Burkholderia cepacia* complex, *P. aeruginosa*, and MRSA among persons with cystic fibrosis in health care settings was substantial. They concluded that evidence was insufficient to determine whether *Stenotrophomonas maltophilia*, *Achromobacter xylosoxidans*, nontuberculous mycobacteria, or filamentous fungi could be transmitted from person to person.

The aforementioned guideline emphasizes standard infection control practices in the care of patients with cystic fibrosis, including appropriate hand hygiene and use of barriers by health care providers, proper use of isolation precautions, and effective procedures for cleaning and disinfection or sterilization of reused equipment contaminated with respiratory secretions.[525]

In inpatient settings, the guideline recommends contact isolation and a private room for patients who are colonized or infected with MRSA, *B. cepacia* complex, or multidrug-resistant *P. aeruginosa*. Patients who are not colonized with any of these bacteria may share a room with a patient who does not have cystic fibrosis and who is at low risk for acquiring infection with these bacteria. All patients, whether colonized with these bacteria or not, should avoid contact with other patients with cystic fibrosis.

The guideline makes no recommendation for the use of masks by patients with cystic fibrosis in either inpatient or outpatient settings because of lack of sufficient evidence for or against the efficacy of this practice.

In ambulatory settings, the guideline recommends that patients with cystic fibrosis who are colonized or infected with *B. cepacia* complex be segregated from other patients with cystic fibrosis and other patients colonized or infected with *B. cepacia* (to avoid replacement of one strain with another), that their visits be scheduled at the end of the clinic session or on another day, and that they be placed in an examination room immediately instead of sitting with other patients in the waiting room. Patients colonized or infected with multidrug-resistant *P. aeruginosa* also should be placed in an examination room immediately. For other patients, the guideline recommends a variety of clinic logistics to minimize the time that patients spend in waiting areas and emphasizes education of patients about appropriate waiting area behaviors to minimize contact among patients (e.g., discouraging handshakes and physical contact among patients, maintenance of at least 3 feet between patients, avoiding contact with common-use items). All patients should receive annual influenza vaccination and age-appropriate pneumococcal vaccination.

CHILDREN IN LONG-TERM CARE FACILITIES

Preceding sections have described a variety of microorganisms that have caused disease in long-term care facilities for children, including various viral infections and enteric pathogens. Specific interventions to prevent these infections have been discussed previously (see the section on health care–associated infections due to spread of infections common in the community).

A prospective, longitudinal study of infections in a pediatric long-term care facility illustrates the spectrum of endemic health care–associated infections in this population.[615] The cumulative incidence of infection was 40 percent among the more than 400 patients cared for in this facility during a 2-year period. Upper respiratory tract infections and UTIs accounted for 37 and 31 percent of infections, respectively. Nearly 80 percent of the UTIs occurred in a small group of patients with neural tube defects or neuromuscular disorders, but the study did not differentiate between symptomatic infections and asymptomatic bacteriuria. The rate of infection among children exposed to indwelling or intermittent catheterization also was not reported. Upper and lower respiratory tract infections were common findings among young children with tracheostomies. Skin infections accounted for 16 percent of infections, but the specific per-centage of decubitus ulcers was not reported. Gastrointestinal infections were remarkably uncommon (4% of all infections).

Similar findings regarding the overall distribution of infection have been described in a more recent report.[262] In addition, this report described the burden of device-related infections in this population. The incidence of catheter-associated UTIs was more than twice as frequent as in pediatric ICUs reporting data to the NNIS system, whereas the incidences of ventilator-associated pneumonia and central venous catheter–associated bloodstream infections were equivalent or slightly lower than those reported in pediatric ICUs.

Guidelines for the infrastructure and essential activities of infection control programs and key prevention practices in long-term care facilities have been published, although these documents do not deal with issues specific to the care of children.[196,574] Key prevention measures are effective hand hygiene, appropriate use of barriers and isolation precautions, proper care of patients who have indwelling devices (e.g., tracheostomies) or require invasive procedures (e.g., bladder catheterization), prevention of decubitus ulcers, age-appropriate immunization (including yearly influenza vaccine, conjugate or polysaccharide pneumococcal vaccine, hepatitis B vaccine, and hepatitis A vaccine, depending on local epidemiologic conditions), and early detection and control of outbreaks of infection.

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CHAPTER 245

Programs to Prevent and Control Health Care–Associated Infections

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The history of formal hospital programs to prevent and control hospital-acquired infections generally is traced to the organized efforts of hospitals in Great Britain and North America to control the pandemic of *Staphylococcus aureus* infections in the 1950s and early 1960s. However, programs to study and control the hospital spread of contagious diseases were well established in leading pediatric hospitals in several European countries and in North America even in the mid-1930s.[8,13,33,47,48,51]

Since the 1950s, the scope and complexity of programs to prevent and control hospital infections have expanded dramatically. Today, hospital inpatient populations include more severely ill children and children with compromised immune systems. Advances in medical care and technology have improved the survival rates of these vulnerable patients; however, this progress has gone hand in hand with an increased risk of health care–associated infection caused by a wide spectrum of microbial pathogens. These factors, in combination with the selective pressure of widespread use of broad-spectrum antimicrobial agents and suboptimal adherence to basic infection control practices, have resulted in the emergence and dissemination of antimicrobial-resistant bacteria, as well as in the rise of fungi as serious pathogens. The shift in the site of patient care from the inpatient to the outpatient setting has expanded the focus of infection control programs from the hospital to the entire health care system. The increased risk among medical professionals of acquiring an occupationally related infection has required infection control programs to work with employee health services to develop workplace policies and procedures to prevent these infections. Finally, many infection control professionals have assumed additional responsibility to address more general issues of health care quality and patient safety.

This chapter highlights key elements of a comprehensive program to prevent and control health care–associated infections in pediatric patients and health care workers. A detailed description of the development, implementation, and maintenance of an effective program is beyond the scope of this chapter, and the reader is referred to several excellent and authoritative references on this topic.[1,50,77] Specific health care–associated infections are discussed in Chapter 244.

EXTERNAL GROUPS AND ORGANIZATIONS INFLUENCING HEALTH CARE–ASSOCIATED INFECTION PREVENTION AND CONTROL PROGRAMS

Many external groups and organizations have significant influence on programs for the prevention and control of health care infection. The most influential groups and organizations are discussed in this section.

The Division of Healthcare Quality Promotion (DHQP), formerly the Hospital Infections Program, is a part of the Center for Disease Control and Prevention's (CDC's) National Center for Infectious Diseases (*http://www.cdc.gov/ncidod/dhqp/about.html*). The DHQP plays a major role in providing information and guidance to health care infection control programs, as well as in conducting its own investigations. The DHQP has the single largest experience in the investigation of outbreaks of health care–associated infections and can provide assistance to individual health care institutions. In general, health care institutions should consult their state public health departments before asking for assistance from the CDC.

Coordinated by the DHQP, the National Healthcare Safety Network (NHSN),[75] previously known as the National Nosocomial Infection Surveillance (NNIS) System, collects and reports data on the incidence of health care–associated infections, including device-associated infections in pediatric and newborn intensive care units (ICUs).[18,54] In the future, the NHSN will also report data on key process measures that are tightly linked with prevention of health care–associated infections.[75]

The Healthcare Infection Control Practices Advisory Committee (HICPAC) was established in 1991 to provide advice and guidance to the DHQP and the CDC regarding the practice of infection control and strategies for surveillance, prevention, and control of health care–associated infections in United States health care systems (*http://www.cdc.gov/ncidod/dhqp/hicpac_charter.html*). The HICPAC has published numerous guidelines regarding prevention of these infections.[9,10,49,58,71,72,74]

Numerous professional and trade associations also develop recommendations and guidelines related to infection control. The most prominent among these in the United States are the Society of Healthcare Epidemiology of America (SHEA) and the Association for Professionals in Infection Control and Epidemiology (APIC). The SHEA and the Infectious Diseases Society of America (IDSA) have formulated concise guidelines on major health care–associated infections (*http://www.idsociety.org/Content.aspx?id=9088*), and these guidelines have been endorsed by the APIC.

During the past several decades, the Joint Commission (formerly the Joint Commission on Accreditation of Healthcare Organizations), a private, not-for-profit organization, has had a major influence on the structure and activity of health care infection prevention and control programs (*http://www.jcaho.org*). Joint Commission standards regarding these programs have evolved to place greater emphasis on organizational performance regarding processes that have a significant impact on patient care. The Joint Commission has promulgated a set of annual National Patient Safety Goals that have included goals to prevent health care–associated infections (*http://www.jointcommission.org/PatientSafety/NationalPatientSafetyGoals*). These goals are revised or supplemented periodically. Currently, these goals involve optimizing hand hygiene practice and managing all identified cases of unanticipated death or major permanent loss of function related to a health care–associated infection as sentinel events.

The United States Department of Labor's Occupational Safety and Health Administration (OSHA) issues regulations to minimize the hazards of occupational exposure to bloodborne pathogens and tuberculosis (*http://www.osha.gov/SLTC/etools/*

hospital/hazards/bbp/bbp.html; *http://www.osha.gov/SLTC/etools/hospital/hazards/tb/tb.html*).

The Institute of Medicine issued a report in 2000, entitled *To Err Is Human*,[41] that helped launch a variety of national patient safety and quality improvement initiatives. These initiatives, combined with requirements for public reporting of health care–associated infection rates, are reshaping the focus of infection control and prevention programs from surveillance of infection rates and an emphasis on traditional infection prevention strategies to a perspective of "zero tolerance" and tactics that aim to eliminate these infections completely.[6,37,52,81] The Institute for Healthcare Improvement (IHI) conducted the 100,000 Lives campaign, from June 2005 to December 2006, that included application of evidence-based care "bundles" of interventions to reduce the incidence of central venous catheter–associated bloodstream infections, surgical-site infections, and ventilator-associated pneumonia. An additional "plank" on reducing infections caused by methicillin-resistant *S. aureus* (MRSA) was added when IHI launched the "Protecting 5 Million Lives from Harm Campaign" (*http://www.ihi.org/IHI/Programs/Campaign*). Programs using such care bundles appear to be effective.[16,63,64] Some of the most striking results were obtained in a collaborative of 108 ICUs in Michigan (the Keystone Project). The median rate of central venous catheter infections per 1000 central venous catheter days fell from 2.7 to zero 3 months after implementation.[63] Initiatives to reduce the incidence of bloodstream infections in newborn ICUs have been conducted by the Vermont Oxford Network (*http://www.vtoxford.org/*) and in pediatric ICUs by the National Association of Children's Hospitals and Related Institutions (*http://www.childrenshospitals.net*) and the Children's Hospital Corporation of America (*http://www.chca.com*). For adult patients, the Surgical Care Improvement Project (SCIP) has codified the most important prevention practices for surgical-site infections, and initiatives to reduce MRSA infections also have been launched by the Department of Veterans Affairs, the Voluntary Hospital Association, and the APIC. The Leapfrog Group is a voluntary group of large employers whose aim is to mobilize purchasing power improve health care safety, quality, and customer value (*http://www.leapfroggroup.org/*). These U.S. initiatives have their counterparts in other countries. For example, the National Health Service in the United Kingdom is engaged in an ambitious program to reduce bloodstream infections caused by MRSA by 50 percent. The effectiveness of these initiatives needs further study. Finally, prodded by consumer advocacy groups, legislators have entered the picture by considering and passing legislation related to public reporting of hospital-associated infection rates and MRSA control programs.[3]

ORGANIZATION AND ACTIVITIES OF HEALTH CARE INFECTION CONTROL PROGRAMS

The Study on the Efficacy of Nosocomial Infection Control (SENIC) project, conducted in U.S. hospitals in the mid-1970s, found that hospitals with a trained, effective infection control physician, 1 infection control nurse for every 250 acute care hospital beds, and a system for reporting surgical-site infection rates to surgeons could reduce their health care–associated infection rates by 32 percent compared with hospitals with no infection control program.[30] However, because relatively few hospitals had implemented these maximally effective programs, only 6 percent of the theoretically preventable infections nationwide were, in fact, prevented. In 1983, a repeat survey of a sample of the participating hospitals found the percentage of hospitals with 1 infection control nurse for every 250 acute care hospital beds had increased from 22 to 57 percent[31]; however, the percentage with a physician trained in infection control remained low (15%), and the percentage of hospitals performing surveillance of surgical-site infections and reporting these rates to surgeons actually decreased. The percentage of preventable infections that were avoided had risen to 9 percent. Apart from the landmark SENIC study, there has been little objective evaluation of the efficacy of various components of infection control programs.

Nonetheless, a large amount of practical experience has guided the development of health care infection control programs. In 1998, the report of a consensus panel of national experts on the requirements for infrastructure and essential activities of these programs was published with the endorsement of the SHEA, the APIC, and other key organizations (including the DHQP and the Joint Commission).[70] The report stated that the principal goals of these programs are to protect the patient, the health care worker, visitors, and others in the health care environment and to accomplish these goals in a cost-effective manner whenever possible.[70] The specific recommendations of the panel are listed in Table 245–1, accompanied by an indication of the strength of the recommendation. The recommendations are described in more detail in the published report.[70] A guideline for the prevention of health care infections in long-term care residents also has been published.[24,73] These publications do not deal specifically with pediatric issues, although the recommendations generally are applicable to facilities providing care to children.

SURVEILLANCE STRATEGIES

Surveillance of health care–associated infections is necessary to understand the specific infection-related problems of individual hospitals. Surveillance data can focus prevention and control efforts on the highest-risk patients and provide a means of evaluating the effectiveness of these interventions. By establishing endemic rates of health care–associated infections, surveillance also facilitates detection of outbreaks. For these reasons, surveillance has been regarded traditionally as an essential component of a hospital infection prevention and control program, a view validated by the SENIC study.[30] Recommended practices for surveillance have been defined and published.[46]

Prevalence surveys can be used for quickly gaining a perspective of the nature and scope of health care–associated infection problems. These surveys also can be used to validate the sensitivity and specificity of ongoing surveillance. For hospitals that focus surveillance efforts on high-risk patients, periodic prevalence surveys can provide reassurance that previously low-risk populations or low-priority problems have not become more problematic. In general, however, surveillance systems that measure the incidence of health care–associated infections provide a more detailed assessment risk of infection and are much more likely to detect outbreaks.

Hospital-wide surveillance, frequently performed in programs in the 1970s and 1980s, has largely been abandoned because of the substantial time commitment required for data collection, although some hospitals have developed computerized systems for case finding that have improved the efficiency of this approach considerably.[14,19] Most hospitals currently favor focused (or targeted) surveillance, which concentrates on specific high-risk groups (e.g., patients in ICUs, surgical patients), specific sites of infection (e.g., bloodstream infections, surgical-site infections), or specific pathogens (e.g., respiratory viruses, *Clostridium difficile*, multidrug-resistant microorganisms). This approach is designed to maximize the efficiency of surveillance and to focus on particularly problematic infections or pathogens.

A surveillance plan for an individual health care facility may incorporate various types of surveillance. A written plan for surveillance activity should be reviewed annually and modified as necessary. For each surveillance component, the plan should include a description of the following: (1) the rationale for surveillance; (2) the target population; (3) infection definition(s); (4) case-finding method(s); (5) source of denominators used for rate

TABLE 245–1 Consensus Panel Recommendations Regarding the Requirements for Infrastructure and Essential Activities of Infection Control and Epidemiology in Hospitals

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Please refer to the printed publication.

From Scheckler, W. E., Brimhall, D., Buck, A. S., et al.: Requirements for infrastructure and essential activities of infection control and epidemiology in hospitals: A consensus panel report. Infect. Control Hosp. Epidemiol. 19*:114-124, 1998.*

calculations; (6) collection of additional data regarding risk factors; (7) calculation of infection rates (including stratification by risk factors, if planned); (8) frequency of reporting to specific target groups (e.g., clinicians, infection control committee, leadership); and (9) estimated time commitment of staff for data collection, analysis, and reporting.

When choosing a particular surveillance strategy, it is important to consider its sensitivity and specificity. For example, self-reporting by clinicians is notoriously insensitive, and surveillance that relies on microbiology culture results alone is likely to be both insensitive and nonspecific. Considerable research regarding case-finding methods has been performed.[25,59] In general, active, concurrent case finding (i.e., active searching for cases by trained persons) should be used. The gold standard for case finding includes bedside examination, interviews with ward staff, review of patient records, and verification of all related microbiologic information by a trained surveyor.[23] However, few programs have the staff for this labor-intensive effort. Many programs rely on positive microbiology culture reports as a starting point for the investigation of infections for which cultures are obtained routinely, such as bloodstream infections, with subsequent review of patient records to confirm or refute the presence of a health care–associated infection. Strategies for detecting infections that may not be associated with collection of a microbiology culture,

such as a surgical-site infection, require other approaches to case finding. Strategies for surveillance that use electronic information to enhance surveillance also have been developed.[14,19,20,62] Sands and colleagues performed detailed studies of the usefulness of this approach in performing surveillance of surgical-site infections.[69]

Calculations of infection rates should include appropriate adjustments whenever possible (see Chapter 244). The simplest technique is merely to adjust for exposure to a particular device or procedure (e.g., number of bloodstream infections per 100 patients with central venous catheters). Adjustment for the duration of exposure to the hospital (e.g., number of infections per 1000 patient-days) or to a device (e.g., number of bloodstream infections per 1000 central venous catheter days) is more useful if the necessary denominator data can be obtained. Surgical-site infections should be stratified by wound class and, in adults, preferably by the surgical-site infection risk index or other method of risk stratification.[15] Some investigators have used severity-of-illness measures to adjust for host risk factors, but this type of adjustment has yet to be incorporated widely into routine surveillance data analysis (see Chapter 244).

Feedback of infection rates to clinicians should be done regularly using an easy-to-understand format. Graphic displays of data, such as statistical process control charts, are most effective.[4,5] Surveillance data also should be used to target areas for further investigation, additional staff education, and specific interventions.

Data mining strategies may be helpful in identifying undetected problems (e.g., an organism with a particular susceptibility profile in multiple different units) or unrecognized associations (e.g., patterns of antimicrobial prescription and the prevalence of specific antimicrobial-resistant organisms).[55]

Surveillance efforts also should examine adherence to processes that are tightly linked to outcomes. For example, the SCIP has examined adherence to three measures related to the administration of perioperative antimicrobial prophylaxis for prevention of surgical-site infections: (1) prophylactic antimicrobial received within 1 hour before surgical incision, (2) prophylactic antimicrobial administration consistent with published guidelines, and (3) prophylactic antimicrobial discontinued within 24 hours of surgery end time (≤48 hours for patients undergoing cardiac surgery).[12] Measurement of adherence to recommendations for hand hygiene and to isolation precautions during patient care is critical for reducing the transmission of epidemiologically important organisms. These process measures should be used to guide and monitor process improvement efforts. Surveillance and reporting of communicable infections to state departments of health are duties retained by some hospital infection prevention and control programs, although others delegate this responsibility primarily to the microbiology laboratory.

OUTBREAK INVESTIGATION

Investigators have estimated that a community hospital can expect to experience at least one outbreak of a health care–associated infection per year[32]; teaching hospitals can expect to experience several outbreaks every year.[78] Because outbreaks of health care–associated infections often are associated with significant rates of patient morbidity and mortality, clusters of infection should be investigated promptly. A single, highly unusual infection, such as a postoperative group A streptococcal infection, is sufficient cause for an investigation. Clusters of health care–associated infections caused by an uncommon microorganism, a common microorganism with an unusual antimicrobial susceptibility pattern, or a series of infections at the same anatomic site (i.e., an outbreak of diarrhea) also are obvious indications for an investigation. However, some outbreaks are more difficult to recognize because they occur intermittently, involve multiple microorganisms, or involve infection at various anatomic sites.

TABLE 245–2 Approach to the Investigation of Clusters of Nosocomial Infections

1. Confirm the diagnosis.
2. Make a case definition.
3. Search for additional cases.
4. Plot the epidemic curve.
5. Compare pre-epidemic rates to current rates using statistical tests to prove that an epidemic exists.
6. Perform a literature review.
7. Open lines of communication with leaders of relevant departments, the microbiology laboratory, and the hospital administration.
8. Keep detailed records of events and conversations.
9. Review charts of all cases and compile a line listing of relevant information.
10. Formulate a hypothesis about a likely reservoir and mode of transmission.
11. Institute temporary control measures.
12. Perform a case control study to develop epidemiologic evidence to support or refute the hypotheses.
13. Update control measures.
14. Document the reservoir and mode of transmission microbiologically. Confirm the relatedness of isolates, using molecular genotyping techniques, if necessary.
15. Document the efficacy of control measures.
16. Write a report and distribute it to appropriate individuals.
17. Change policies and procedures, if necessary.

A guide for investigating a cluster of health care–associated infections is provided in Table 245–2. Detailed discussions of the methodology for investigating outbreaks are found in other sources.[38] Published outbreak investigations are an invaluable resource because they often provide insight into potential causes of the outbreak. For example, an outbreak caused by *Stenotrophomonas (Xanthomonas) maltophilia*, *Burkholderia (Pseudomonas) cepacia*, or *Pseudomonas* spp. should suggest the possibility of a common source outbreak resulting from a contaminated solution or medication or an inadequately disinfected piece of equipment. Conversely, jumping to conclusions regarding the cause of a particular cluster of infections based on the results of a prior investigation is hazardous. Careful investigation is necessary to establish or refute epidemiologic links between cases and potential causes. Molecular genotyping techniques are important adjuncts to traditional epidemiologic methods (e.g., cohort or case-control studies) and can serve as powerful tools to confirm or refute a particular source or mode of transmission of a particular microorganism.

POLICIES AND PROCEDURES

Policies and procedures are necessary to optimize and standardize hospital routines and patient care practices. Certain generic policies and procedures, such as handwashing, isolation precautions, prevention of transmission of infectious diseases from and to visitors and health care workers, reprocessing of reusable patient care items, and disposal of medical waste, apply to all departments. Other policies and procedures should be tailored to the potential infection risks relevant to specific departmental activities. Numerous guidelines have been published that can help hospitals develop policies and procedures.[9,10,49,58,71,72,74]

As evidenced by numerous studies documenting poor adherence to policies for handwashing and isolation precautions,[10] adherence to policies and procedures often is less than optimal. Unfortunately, relatively few studies have attempted to elucidate

the reasons for non-adherence or to investigate methods for modifying the behavior of staff.[44,56,80] To have any chance of improving performance in such critical aspects of prevention of infections, hospital and departmental leaders must make adherence an organizational priority. Staff members responsible for implementation should be included in the process of developing policies and procedures to ensure that the resulting documents are workable, as well as to develop their sense of ownership and responsibility for successful implementation and sustained adherence. Staff education is important but not sufficient in and of itself. Prompt feedback of data concerning key outcome and process measures also is critical in motivating staff, and barriers to improvement must be identified and removed. For example, clinicians cannot be expected to comply with an isolation precaution policy if gloves are not available or are located inconveniently. Additional investigation into engineering solutions, cognitive approaches, behavioral modification, and training strategies also is needed. These efforts should be guided by principles of behavioral science and quality improvement.[36,79]

HAND HYGIENE

Proper hand hygiene, which encompasses both handwashing using soap and water and hand antisepsis using a waterless (alcohol-based) agent, reduces carriage of potential pathogens on the hands of health care workers.[10] Hand hygiene is widely regarded as the quintessential infection prevention measure.

Indications and procedures for hand hygiene are reviewed in detail in a HICPAC guideline.[10] In general, hand hygiene should be practiced under the following circumstances: before and after contact with patients; after contact with body fluids and substances, mucous membranes, nonintact skin, and objects that are likely to be contaminated; before performing invasive procedures; and after removing gloves (see "Isolation Precautions"). Handwashing must be performed when hands are visibly soiled, to remove organic material and associated microorganisms. When hands are not visibly soiled, hand antisepsis is preferred because is it likely to reduce more effectively than handwashing the number of pathogenic bacteria that transiently colonize the hands.[10,61] Hand hygiene also is necessary after removing gloves—an indication that many health care professionals fail to appreciate fully—because gloves may have macroscopic and microscopic holes that lead to contamination of hands[42] and because hands may be contaminated in the process of removing soiled gloves.

Before alcohol-based agents became widely available, adherence to hand hygiene (accomplished solely by handwashing) was notoriously poor among health care workers.[10] However, the hope is that the wider use of alcohol-based agents will boost hand hygiene adherence because these agents are faster and more convenient to use (i.e., they can be placed near the patient's bedside and require less than 30 seconds to use), are less drying and irritating to the skin than is handwashing,[11] and are more effective in reducing the numbers of transient flora.[60] Programs that combine ready access to these products at the point of care (i.e., close to the patient's bedside) with active efforts to promote their use have demonstrated improved hand hygiene adherence.[7,61] For these reasons, a HICPAC guideline on hand hygiene in health care facilities strongly recommends that these agents should be used preferentially for hand hygiene (when hands are not visibly soiled) and that facilities should develop active programs to promote the use of these agents.[10] However, alcohol has minimal activity against the spores of *C. difficile*, and some experts recommend handwashing after caring for patients with *C. difficile*–associated diarrhea.[27]

Persons wearing artificial fingernails are more likely to harbor pathogenic gram-negative bacilli and yeast on their fingertips, and these organisms are cleared less effectively on artificial nails by use of an alcohol-based agent than on natural nails.[34,53] Use of artificial nails or nail wraps was associated with carriage of *Pseudomonas aeruginosa* on the hands of health care workers and infection among high-risk infants in a neonatal ICU.[22,29] For these reasons, the HICPAC guideline on hand hygiene recommends that health care workers not wear artificial fingernails or extenders when providing patient care and that natural nails should be kept less than 1/4 inch long.[10]

ISOLATION PRECAUTIONS

Proper utilization of isolation precautions is important for all health care facilities but is especially critical in facilities caring for pediatric patients. Children hospitalized as the result of infections acquired in the community represent a substantial proportion of pediatric admissions, and transmission of these infections to other susceptible patients is a significant problem (see Chapter 244). Effective use of appropriate isolation precautions markedly reduces transmission of these agents.[2,45] A HICPAC guideline recommends the use of two tiers of precautions, Standard Precautions and Transmission-Based Precautions, which are described in greater detail in the following subsections.[71,72]

Standard Precautions

Standard Precautions synthesize the goals of protecting health care workers from bloodborne pathogens and protecting health care workers and patients from transmission of microorganisms from moist body substances.[72] Standard Precautions apply to the following: any planned or potential contact with blood; all body fluids, secretions, and excretions except sweat, regardless of whether they contain visible blood; nonintact skin; mucous membranes; and supplies, equipment, or surfaces contaminated with these substances. Standard Precautions should be used in the care of all patients at all times regardless of the diagnosis or presumed infection status.[72] New components of Standard Precautions include the following: use of respiratory hygiene/cough etiquette by patients and family members and visitors to prevent spread of respiratory infections; safe injection practices by health care providers to prevent spread of bloodborne pathogens; and infection control practices for special lumbar puncture procedures to prevent infections associated with these procedures.

Transmission-Based Precautions

Other precautions are needed to prevent transmission of contagious diseases (e.g., varicella, measles, tuberculosis, and pertussis) and other epidemiologically important microorganisms (e.g., multidrug resistant microorganisms, *C. difficile*) from infected or colonized patients. Transmission-Based Precautions are designed to provide the necessary measures, in addition to those already specified by Standard Precautions, to interrupt known modes of transmission of these microorganisms (see Chapter 244).[72] The three types of Transmission-Based Precautions are Airborne, Droplet, and Contact Precautions.

Airborne Precautions are designed to prevent transmission of microorganisms spread by droplet nuclei (e.g., measles, varicella, tuberculosis) that can be carried on air currents over substantial distances.[72] Special air handling and ventilation are required.[72] Droplet Precautions are designed to prevent transmission of microorganisms spread by large respiratory droplets that travel only short distances before settling.[72] Special air handling and ventilation are not required. Contact Precautions are designed to prevent transmission of microorganisms spread by direct and indirect contact.[72] Some infections are spread by more than one mode of transmission, so precautions systems may need to be combined for these infections (i.e., varicella requires Airborne

Precautions and Contact Precautions). Patients infected or colonized with more than one microorganism also may require a combination of precautions systems (i.e., a patient with active pulmonary tuberculosis and *C. difficile* enterocolitis requires both Airborne Precautions and Contact Precautions).

The HICPAC guideline lists clinical syndromes and conditions warranting empiric use of Transmission-Based Precautions, in addition to Standard Precautions, to prevent transmission of epidemiologically important pathogens until infection with these microorganisms is excluded.[72] To ensure that appropriate empiric precautions are implemented promptly, hospitals must have systems in place to evaluate patients for these infections as a part of routine preadmission and admission care.

VISITORS

Visitors with communicable diseases can expose hospitalized patients and health care workers inadvertently unless procedures to identify and exclude these infections are in place. Varicella, measles, and tuberculosis are the most problematic infections because these diseases are spread by airborne transmission, thus enabling infected visitors to expose a large number of individuals in a short period of time. In addition, visitors with pertussis, viral respiratory and gastrointestinal infections, parvovirus B19 infection, rubella, and mumps can pose a significant hazard to patients and health care workers with whom they have close contact.

Procedures to identify potentially infected visitors should include children who are visiting because they are the most likely persons to be infected with these agents. Parents or guardians of all such children should be asked a set of screening questions regarding the presence of fever, rash, and respiratory and gastrointestinal symptoms in the visiting child, as well as any recent exposure to other children with chickenpox, measles, or whooping cough. Children without any significant symptoms or exposures should be allowed to visit with no restrictions. Children who are otherwise well but who have a history of a recent minor illness may be allowed to visit but potentially with restrictions, such as avoiding close contact (<3 feet) with any patient, not visiting the activity room, and not sharing food, drinks, or toys. Children with upper respiratory infections should not be allowed to visit children with congenital heart disease, bronchopulmonary dysplasia, cellular immunodeficiency, or other conditions that predispose these vulnerable children to development of severe infection with respiratory viruses. Children with significant exposures to chickenpox, measles, or pertussis who may be in the incubation period of the disease should not be allowed to visit the hospital. This screening process should be repeated each day the child visits the hospital. This procedure does not guarantee that a child may not be in the incubation phase of a contagious disease at the time of the visit—varicella is an obvious example—but can be useful in limiting potential exposures.

OCCUPATIONAL HEALTH

Health care workers require protection from the significant infectious risks inherent in patient care; conversely, patients and other health care workers need to be protected from exposure to health care workers with communicable diseases. Integrating management and prevention strategies to accomplish these two goals requires close collaboration between hospital infection prevention and control programs and employee health departments. A HICPAC guideline for infection control in health care workers contains comprehensive recommendations for evaluation of illnesses, post-exposure evaluation and management, and prevention of occupationally acquired infections in health care workers.[9]

Evaluation of Ill Health Care Workers

Hospital staff and volunteers with symptoms such as persistent fever, conjunctivitis, skin lesions or rashes, diarrhea, and persistent cough should be evaluated for the presence of a contagious disease. Possible cases of varicella, herpes zoster on an exposed area of the body, herpetic whitlow, adenoviral conjunctivitis, measles, mumps, rubella, pertussis, staphylococcal skin infection, enteric infection in a food service worker, and active pulmonary tuberculosis should be investigated promptly and confirmed with laboratory tests, if necessary. Although many health care workers choose to have these problems evaluated by their primary care provider, employee health departments have an interest in completing these assessments, especially if a question exists about whether the condition was acquired in the workplace, requires a furlough from work, or may have exposed patients or other health care workers. Infection control staff can provide assistance in these evaluations as needed and should be kept abreast of the results.

Post-exposure Evaluation and Management of Health Care Workers

A structured approach to the assessment and management of exposures of health care workers to patients with infectious diseases is critical to provide post-exposure prophylaxis promptly, if indicated, and to allay anxiety while avoiding unnecessary interventions and loss of workdays. The first step is to develop criteria for assessing the nature of the exposure because many reported encounters are not significant. Some exposures (e.g., varicella, hepatitis B) may require an assessment of the susceptibility of the health care worker to infection, and procedures should describe the indications for laboratory tests as well as the interpretation of results. Post-exposure prophylaxis regimens are discussed in Chapter 244, in a HICPAC guideline,[9] and through the DHQP Web site (*http://www.cdc.gov/ncidod/dhqp/worker.html*). Counseling regarding the risks and consequences of the exposure is an important component of this service.

Prevention of Occupationally Acquired Infections in Health Care Workers

Because vaccination against infectious diseases is a highly cost-effective prevention strategy, hospitals should offer vaccinations free of charge. Vaccination against measles is so effective that ensuring immunity to measles (either as the result of natural immunity or vaccination) among hospital staff is widely regarded as an appropriate quality-of-care indicator for occupational health programs.[43] Because the combined measles-mumps-rubella (MMR) vaccine is readily available,[76] immunity against mumps and rubella can be ensured in an analogous fashion. Annual influenza vaccination of health care workers is recommended by the Immunization Practices Advisory Committee and is considered an essential quality measure by the Leapfrog Group; annual updates provide the latest recommendations.[21] However, acceptance of this vaccine by health care workers often is suboptimal. Consequently, employee health departments need to consider aggressive and innovative strategies to encourage employees to have an annual influenza vaccination. Given the significant occupational risk of hepatitis B infection in hospitals, hepatitis B vaccination should be offered routinely to health care workers. The OSHA bloodborne pathogens standard requires hospitals to offer hepatitis B vaccination free of charge; workers who do not wish to be vaccinated must sign a specific "informed

refusal." Other vaccines, such as the varicella vaccine and acellular pertussis vaccine, can help eliminate or at least drastically reduce the rate of occupational acquisition of these diseases as well.

In addition to hepatitis B vaccination, the OSHA bloodborne pathogens standard mandates other specific prevention measures, including the following: (1) the development of an exposure control plan that identifies employees with occupational risk of exposure to bloodborne pathogens; (2) annual training for these individuals regarding the risk of acquiring bloodborne infection and prevention measures; (3) provision of personal protective clothing and equipment; (4) work practice controls, including equipment and procedures for the safe handling and disposal of sharps; and (5) procedures for identification, transportation, storage, and disposal of contaminated items and waste.[57] The CDC also has published a detailed guideline for prevention and control measures to prevent transmission of *Mycobacterium tuberculosis* to health care workers.[39]

REPROCESSING OF REUSABLE PATIENT CARE ITEMS

Large numbers of outbreaks of health care–associated infections have been related to the use of contaminated equipment. Consequently, hospital infection prevention and control programs must work closely with all hospital departments that reprocess reusable patient care items to ensure proper selection, implementation, and quality monitoring of reprocessing methods. An APIC guideline discusses the characteristics and efficacy of various classes of disinfectants and provides recommendations for methods used to reprocess specific patient care items.[65] Newer methods for sterilization and disinfection have been reviewed.[67,68]

REGULATED MEDICAL WASTE

Regulated medical waste refers to waste that has at least the potential to transmit infectious agents to humans. However, infections resulting from exposure to regulated medical waste (other that those related to percutaneous exposures within hospitals) have not been documented.[66] Moreover, waste from hospitals represents 1 percent or less of the total municipal waste generated annually and has been demonstrated to have a lower microbial burden than does common household waste.[66]

Nonetheless, numerous national, state, and local regulations pertain to the identification, packaging, transport, storage, and disposal of regulated medical waste.[28] A full discussion of this topic is beyond the scope of this chapter, but hospital infection prevention and control programs can provide valuable input into the design of rational approaches to complying with these regulations. Because the management of regulated medical waste is considerably more expensive than that of traditional waste, ensuring proper sorting of regulated medical waste from nonregulated hospital waste can result in considerable cost savings.

EDUCATION AND TRAINING OF HEALTH CARE WORKERS

Providing education and training in both general principles and specific aspects of hospital infection prevention and control is one of the primary responsibilities of program staff. Staff members need to be familiar with principles of adult learning, assessing the educational needs of the audience, defining learning objectives, determining optimal instructional formats, using effective teaching and communication skills, and weighing the merits of various educational tools.[35]

ANTIBIOTIC UTILIZATION AND ANTIMICROBIAL RESISTANT MICROORGANISMS

The rapid emergence of pathogens resistant to multiple antimicrobial agents (e.g., vancomycin-resistant enterococci [VRE] and gram-negative, bacteria-producing, extended-spectrum beta-lactamases) and the widespread dissemination of these resistant microorganisms constitute an unprecedented crisis for hospitals worldwide. The mechanisms involved in the emergence and spread of antimicrobial resistance are complex but no doubt are facilitated by intense selection pressure caused by overuse and misuse of antimicrobial agents in hospitals, particularly newer, broad-spectrum agents. Dissemination of resistant strains is facilitated by suboptimal compliance with hand hygiene and isolation precautions.

A HICPAC guideline provides a detailed discussion of interventions to reduce the spread of antimicrobial resistant organisms, including administrative support, education, surveillance for resistant organisms, judicious antimicrobial use, isolation precautions, adherence monitoring, environmental disinfection, and decolonization.[71] The guideline provides general recommendations applicable to all facilities and recommendations for intensified interventions when the incidence or prevalence of multidrug-resistant organisms is not decreasing despite the use of routine control measures or when outbreaks or new isolations of epidemiologically important multidrug-resistant organisms are detected. A consensus guideline for preventing transmission of MRSA in newborn ICUs by a group of infection control professionals in Chicago also has been published.[26] One of the most controversial aspects of efforts to control MRSA and VRE is whether to perform active surveillance to identify asymptomatically colonized patients. Although screening for colonization during the course of an outbreak clearly is important, the role of routine screening of hospitalized children and infants is not clear.

The IDSA and SHEA have collaborated to publish a guideline for institutional antimicrobial stewardship programs.[17] The evidence base for the interventions recommended in the guideline, particularly those related to improving patient outcomes or controlling antimicrobial resistance, remains relatively weak. Few interventions have been specifically tested in health care facilities providing care to children, but many recommendations are likely to be applicable.

PRODUCT EVALUATION

Large numbers of new medical products are introduced to the health care market every year. Although some of these products have the potential to reduce the risk of acquiring infections, data to substantiate the safety and efficacy claims of manufacturers often are limited. Many devices marketed to hospitals caring for children have never actually been tested in children. Because these products often are substantially more expensive than are existing products, a compelling rationale must exist for their use. Hospital infection prevention and control staff can provide valuable assistance to hospital committees evaluating new products and, in some cases, can design and conduct appropriate clinical trials.[40]

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CHAPTER 246

Antibiotic Resistance

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Soil bacteria developed mechanisms of resistance to antibiotics millions of years ago as a means of protecting themselves against other antibiotic-producing microorganisms or their own antimicrobial products. The current crisis of antibiotic resistance, however, was prompted by the intense selective pressure posed by the worldwide use of antibiotics during the past half-century. The problem of antimicrobial resistance in humans has been exacerbated by the widespread administration of antibiotics to farm animals.[247,370,420] Resistant zoonotic bacteria selected by this practice contaminate the food supply and thereby spread to human consumers.

To understand antibiotic resistance fully, one must make a distinction between the in vitro phenomenon of resistance and the less frequently observed clinical consequences of this in vitro phenomenon. Much of the discussion of bacterial resistance to antibiotics derives from the apparent changes in susceptibility of pathogenic bacteria to the concentrations of antibiotic achieved in blood following standard antibiotic dosing.[163] These concentrations have been established as "breakpoints" based on an integration of data derived from both clinical and animal studies, along with the measurement of plasma and serum drug concentrations associated with successful outcomes. Unfortunately, such breakpoints are not absolute. Rather, they depend on an intricate interrelationship between the pharmacokinetics and pharmacodynamics of the drug in question.[58] As such, if one component of this complex interrelationship is changed, the apparent breakpoint distinguishing susceptibility from resistance possibly and most likely also will change. Consequently, in presenting information concerning the mechanisms of antibiotic resistance and their clinical contexts, one must remember that the discussion derives, in part, from certain artificial pretexts. Any changes in a drug-dosing paradigm (e.g., dose, infusion duration, formulation) may affect the clinical relevance of the in vitro observations dramatically.

Bacteria develop resistance to antimicrobial agents through three principal cellular mechanisms (Table 246–1). In some microorganisms, two or more mechanisms of resistance to a given agent exist simultaneously. Antibiotic-resistance genes may be encoded either on the bacterial chromosome or on extrachromosomal DNA. The spread of antibiotic resistance among bacteria has been facilitated by the inclusion of many resistance genes on transmissible extrachromosomal genetic elements. To date, three interrelated genetic elements have been implicated in the expression and spread of antibiotic resistance, namely, *plasmids*, *transposons*, and *integrons*.

Plasmids are composed of circular double-stranded extrachromosomal DNA. Although plasmids characteristically use bacterial DNA polymerase to replicate, their replication occurs autonomously.[390] Many different plasmids exist in nature; they vary in size, in the complexity of their genetic machinery, in their host range and ability to conjugate, and in their stability within the bacterium. Minimal plasmids (replicons) contain genetic sequences encoding the site at which their replication begins and sequences that determine the copy number produced per cell. However, many more genetic elements can be inserted into the replicon. Indeed, plasmids range in size from 1 kb to more than 400 kb; additional genetic machinery is required for conjugation, and, therefore, conjugative plasmids are at least 30 kb in size.[352] Groups of plasmids containing homologous replicative sequences generally are unable to be maintained in the same bacterial cell. This property has been exploited to categorize plasmids into different "incompatibility groups."

Recognition of the importance of plasmids to human disease increased substantially when researchers discovered that these elements could incorporate one or more antibiotic-resistance genes. These so-called resistance plasmids are found in a wide variety of gram-positive and gram-negative bacteria and encode determinants conferring resistance to many antibiotics. Resistance-conferring sequences producing resistance to several drugs may be included on the same plasmid, and multiple, different plasmids expressing different determinants can be included in the same microorganism.[31,390] Several additional classes of plasmid-borne determinants besides antibiotic-resistance genes can confer selective advantage to the bacterium. These determinants include genes rendering resistance to environmental heavy metals (e.g., mercury, antimony, arsenic, and silver) and a variety of toxic organic compounds (e.g., camphor and toluene), genes leading to the synthesis of virulence factors such as adhesion ligands promoting bacterial attachment to epithelial surfaces and those encoding exotoxins, and genes encoding compounds enabling acquisition of chemicals necessary for bacterial survival (e.g., siderophores).[390] The coexistence of these genes on plasmids carrying antibiotic-resistance determinants serves to maintain the plasmid, even in the absence of antibiotic exposure.

Plasmids are transmitted from microorganism to microorganism both vertically and horizontally.[390] Vertical transmission occurs during bacterial division as the plasmids segregate to each daughter cell. Horizontal transmission is affected through conjugation, the transmission of the plasmid from one bacterium to another. At a minimum, all conjugal plasmids encode a genetic sequence that serves as the origin of transmission, and most encode additional mobilization genes.[389] However, plasmids that are unable to conjugate on their own frequently are able to do so when a conjugal plasmid co-resides in the same cell.[390] Especially in gram-negative bacteria, the donor-recipient contact required for conjugation is effected through sex pili. Conjugation frequently is restricted to intragenus transfer, but conjugation across bacterial genera may occur, particularly in gram-negative organisms, and conjugation occasionally occurs across Gram-stain barriers.[242,389]

TABLE 246–1 Cellular Mechanisms of Antibiotic Resistance and Their Transfer

Mechanisms
Genes that encode enzymes that modify or degrade antibiotics
Genes that modify the molecular targets for antibiotics
Genes that code for changes in cell wall channels or active pumping mechanisms
Transfer
Plasmids: extrachromosomal genetic elements made of circular double-stranded DNA <10 to >400 kb pairs; autonomous, self-reproducing; may be conjugative or nonconjugative
Transposon: a mobile genetic element in the bacterial chromosome or plasmid that confers a recognizable phenotype characteristic; incapable of self-replication
Integrons: DNA sequences on bacterial chromosome or plasmid often linked to a resistance gene that facilitates recombination among nonhomologous DNA sequences

A second group of bacterial nucleic acid elements that carry antibiotic-resistance genes is termed *transposons*. Transposons move segments of nucleic acid from one location to another, such as from different sites on the bacterial chromosome or from chromosome to plasmid.[346,353] Unlike chromosomal and plasmid DNA, transposons do not replicate independently and therefore must be integrated into a molecule capable of replication to propagate. Antibiotic-resistance genes may be encoded on conjugative transposons, which, unlike nonconjugative transposons, are able to transfer from one bacterium to another.[353] Most antibiotic-resistant conjugative transposons characterized to date have been identified in gram-positive organisms, although transposons in *Bacteroides* have been well characterized, and transposons in some facultative gram-negative bacteria have been discovered as well.[341] Conjugative transposons are very promiscuous in two senses. First, they move easily from one species of bacterium to another.[341,353] Second, the integration of transposons into other nucleic acid elements is relatively sequence-nonspecific. Although a preference exists for integrating into host DNA sequences with long stretches rich in adenine and thymidine, integration of conjugative transposons targets no detectable consensus sequences.[341]

Intracellular shuttling of a transposon from DNA to DNA begins with the production of a staggered cut on either side of the transposon sequence to produce single-stranded overhangs. These overhang sequences (termed *coupling sequences*) join covalently to produce a circularized double-stranded intermediate that contains the non–base-paired coupling sequences derived from opposite ends of the transposon.[341,353] Integration of the circle into the target double-stranded DNA also results in flanking non–base-paired regions, which become complementary only after a round of replication occurs.[353] Conjugation of the transposon from cell to cell also begins with the circularized intermediate, which, after nicking at a presumed origin site, is transferred as a single strand from donor bacterium to recipient bacterium, whereupon it re-circularizes. Complementary strands then are added to both single strands in the donor and the recipient, and integration into target DNA in both cells ensues.[341]

Excision and integration of transposons are catalyzed by a transposon-encoded integrase.[341,353] The efficiency of excision varies widely and accounts for the differences in rates of transfer of transposons. Conjugation of many transposons appears to be regulated.[341] Unlike some plasmids, conjugative transposons do not exclude entry of other transposons.[341] Indeed, some transposons promote the conjugation of other transposons as well as co-resident plasmids; the result is the dissemination of resistance determinants that are not encoded on the transposon itself.[353]

Integrons, a third type of bacterial DNA important in antibiotic resistance, are composed of segments of DNA that are able to capture and excise gene cassettes coding antibiotic-resistance genes. Integrons are unable to transfer from bacterium to bacterium themselves. To disseminate, the integron first must insert into a plasmid or transposon.[101,309,317] So-called superintegrons, carrying more than 50 gene cassettes, have been identified on the chromosomes of some nonpathogenic environmental bacteria.[241] Superintegrons possess many of the organizational features of antibiotic-resistance integrons and may have been their progenitors. Integrons contain an integrase that catalyzes the incorporation and excision of the cassette into the integron, as well as a promoter that mediates the expression of the resistance determinant, both situated 5′ to the cassette. The cassettes themselves are small segments usually encoding a single antibiotic-resistance determinant. Cassettes have been identified that confer resistance to a broad range of antibiotics, including the β-lactam agents, the aminoglycosides, fluoroquinolones, trimethoprim (TMP), and choramphenicol.[101,309,317] At the 3′ end of each cassette is a variable region commonly termed the *attC site* or, alternatively, the *59-bp element*, although it varies in length from cassette to cassette from 57 bp to more than 100 bp.[241] This 3′ element contains the sequences recognized by the integrase for incorporation of the gene cassette into the integron.[241,317] Several classes of integrons have been defined, based on the sequence of the integrase and their preference for certain transposons. Most antibiotic-resistance integrons are derived from class 1.[241] A single integron may contain multiple antibiotic-resistance cassettes in tandem, all of which are controlled by the single integron promoter. Transcription of tandem cassettes may terminate prematurely, however, such that the cassettes closest to the integron promoter are transcribed at the highest level.[309,317]

The consequences of mobile resistance genes on plasmids and transposons cannot be overstated. They have given bacteria a profound replicative advantage in the age of antibiotics by enabling the instant acquisition of multiple, ready-made antibiotic-resistance genes without requiring each organism to independently undergo the same or similar resistance-conferring mutations again and again.[241] Hence, bacteria may become resistant almost immediately after exposure to antibiotics and may efficiently transmit this resistance to bacteria co-residing in the same host and to bacteria colonizing or infecting multiple hosts in the same community.

RESISTANCE TO SPECIFIC ANTIBIOTICS

β-LACTAM ANTIBIOTICS

The β-lactam antibiotics are a diverse, highly effective family of drugs with broad activity against a wide variety of hospital- and community-acquired pathogens. The β-lactams are composed of four subgroups, namely, the penicillins, the cephalosporins, the monobactams, and the carbapenems. Resistance to the β-lactams is mediated by one of two principal mechanisms: (1) production of enzymes capable of hydrolyzing the β-lactam ring and (2) alteration of the target bacterial molecules, the penicillin-binding proteins (PBPs).

β-Lactamase Production

CLINICAL RELEVANCE

The explosive development in antimicrobial therapeutics since the mid-20th century has been driven, to a great extent, by the impact of β-lactamase activity on the response to antibiotic therapy in the clinical setting.[320] Shortly after penicillin became incorporated into the clinical armamentarium for the treatment of systemic infections, clinical failures were reported in patients treated for infections caused by *Staphylococcus aureus*. On further analysis, these failures were attributed to the presence of an enzyme, "penicillinase," elaborated and excreted by the bacteria, that inactivated the antibiotic before it could manifest its clinical effect. This finding led to the development of numerous new antistaphylococcal penicillins (i.e., the isoxazolyl penicillins) that were not substrates for this enzyme.

At the same time, a newer class of β-lactam agents, the cephalosporins, was developed. One of the criteria employed in moving candidate drugs from this class to the clinic was their resistance to inactivation by the penicillinase elaborated by staphylococci. Further development of all four of the subgroups in the β-lactam family (i.e., penicillins, cephalosporins, monobactams, and carbapenems) was influenced significantly by the recognition that the penicillinase elaborated by staphylococci was representative of a larger class of enzymes that were also present in gram-negative organisms. In this regard, history repeated itself. As gram-negative organisms such as *Escherichia coli* emerged as important clinical pathogens, their emerging resistance to aminopenicillins on the basis of β-lactamase elaboration spawned the development

of a group of enzyme inhibitors including clavulanic acid, sulbactam, and tazobactam.[221,324] Moreover, even as it became necessary to expand the spectrum of both the penicillins and cephalosporins to include some of the more itinerant gram-negative organisms such as *Serratia* spp., *Enterobacter* spp., and even *Pseudomonas*, β-lactamase stability emerged as a necessary attribute for clinical success.[221,324] This expansion was accomplished either through the addition of one of the β-lactamase inhibitors to members of the carboxy and acylureido class of penicillins or through structural engineering such as that seen with the development of the second- and third-generation cephalosporins and carbapenems.

In pediatric practice, the importance of β-lactamase became apparent soon after the widespread use of antibiotics in hospitalized patients began. However, three sentinel clinical events were probably most responsible for establishing β-lactamase as a prominent pediatric clinical problem. In 1974, the first case of bacterial meningitis caused by ampicillin-resistant *Haemophilus influenzae* was reported.[270,388] This report resulted in marked changes in clinical practice. First, all infants and children with suspected bacterial meningitis were treated empirically with both ampicillin and chloramphenicol (a drug not susceptible to degradation by bacterial β-lactamase) instead of with ampicillin alone.[320] In addition, several newer antibiotics, particularly the second- and third-generation cephalosporins, were evaluated for their efficacy in this clinical setting. From these studies, ceftriaxone or cefotaxime emerged as the drug of choice for the empiric treatment of suspected or proven bacterial meningitis in children. This selection was based both on both greater potency against *H. influenzae* and β-lactamase stability.

The second circumstance resulting in the affirmation of β-lactamase as an important clinical problem in pediatrics was the recognition of *Moraxella catarrhalis*, a β-lactamase–producing organism, as the third leading cause of acute otitis media in infants and children, and the emergence of amoxicillin resistance among the nontypeable strains of *H. influenzae*. β-Lactamase production in *H. influenzae* was thought to be particularly associated with therapeutic failure, thus spawning the development of oral second- and third-generation cephalosporins and newer macrolides as therapeutic alternatives in childhood otitis media.

By the early 1980s, β-lactamase–producing bacteria garnered additional attention from pediatricians because of the role of these bacteria as nosocomial pathogens. These bacteria became prominent as children with severe but survivable conditions (e.g., hematologic malignancies and solid organ transplantation) required immunosuppressive therapy and prolonged hospitalization, both of which predisposed these children to acquisition of hospital-acquired infection. With each succeeding decade, the number and variety of β-lactamase–producing hospital-acquired bacteria increased. By the early 21st century, virtually all nosocomial *S. aureus*, coagulase-negative staphylococci, and enteric and nonenteric gram-negative pathogens elaborated β-lactamase. These organisms commonly encode multiple distinct β-lactamases and express them simultaneously. Consequently, many bacterial infections currently acquired in the hospital are resistant to all β-lactam agents except for the higher-generation β-lactam–β-lactamase inhibitor combinations, the advanced-generation cephalosporins, or the carbapenems. In the most extreme cases, particularly in *Pseudomonas* and *Acinetobacter* infections, the organisms are resistant to all available β-lactam drugs.[56,209,311]

MECHANISM OF RESISTANCE

The β-lactamases constitute a broad array of enzymes that hydrolyze the β-lactam ring of selected antibiotics (Fig. 246–1). Most β-lactamases are structurally similar to the PBPs, and the consensus is that at least some of the β-lactamases were derived evolutionarily from them.[252] β-lactamases can be identified in both gram-positive and gram-negative bacteria. In gram-positive bacteria, the enzyme is secreted into the cell-free environment, whereas in gram-negative organisms, most enzyme is confined and concentrated in the periplasmic space between the cell wall and the outer membrane.

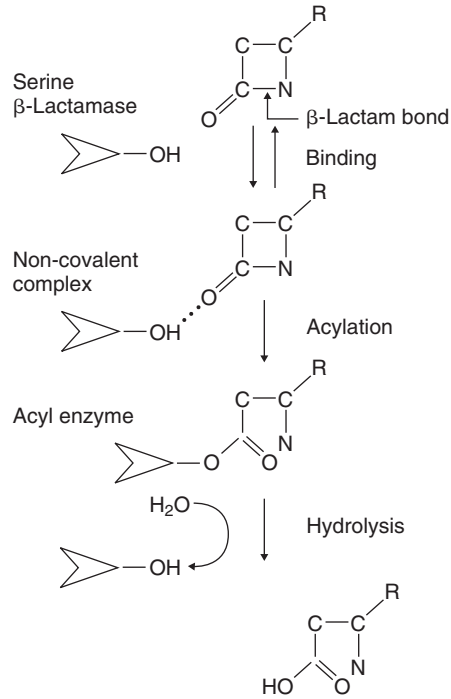

Figure 246–1 Mode of action of β-lactamases (classes A, C and D).

Hundreds of β-lactamases have been characterized,[170] and, doubtless, many more exist in nature. The β-lactamases have been categorized variously according to substrate preference, isoelectric focus, whether they are encoded on the bacterial chromosome or an episome, their ability to be inhibited by clavulanate, and their molecular structure. The molecular classification of Ambler[4] proposed in the 1980s is still widely employed. This classification divides the β-lactamases into four categories, A through D, although all but B, the metalloenzymes, have proved to have similar three-dimensional conformations.

The updated classification system of Bush, Jacoby, and Medeiros, proposed in 1995,[36] divides these enzymes into four groups (with subcategories) and uses virtually all the properties listed earlier to discriminate one class of enzyme from the others. The properties of the three most important groups proposed by Bush, Jacoby, and Medeiros are summarized in Table 246–2 and are described more fully here.

Group 1 AmpC β-Lactamases

The group 1 enzymes (also referred to as *AmpC* β-*lactamases*) hydrolyze virtually all β-lactam antibiotics except the carbapenems and are resistant to inhibition by clavulanate. These enzymes typically are chromosomally encoded on a sequence labeled *amp*C in a wide range of gram-negative bacteria; however, the AmpC β-lactamases are expressed in quantities sufficient to produce clinically important resistance only in selected species, including *Enterobacter cloacae*, *Serratia marcescens*, *Citrobacter freundii*, *Pseudomonas aeruginosa*, and *Morganella morganii*. In the absence of drug exposure, expression of the group 1 AmpC β-lactamases in these species is repressed by an upstream sequence labeled *amp*R (Fig. 246–2).[252,301] However, exposure of the bacteria to a β-lactam leads to the production and intraceullular incorporation of a bacterial cell wall metabolite that itself is pro-

TABLE 246–2 Properties of the Major Groups of β-Lactamases

BJM* group	1	2	3
Ambler class	C	A, D	B
Substrate preference	All but carbapenems	Variable†	Variable (carbapenems)
Chromosomal/plasmid	C	P	C/P
Distribution	Gram-neg	Gram-neg/gram-pos	Gram-neg
Clavulanate inhibition	No	Yes	No
Inducible‡	Yes	No	No

**Data from Bush, K., Jacoby, G. A., and Medeiros, A. A.: A functional classification scheme for beta-lactamases and its correlation with molecular structure. Antimicrob. Agents Chemother. 39:1211-1233, 1995.*
†May be divided into subgroups including penicillinases, cephalosporinases, cloxacillinases, and carbapenemases. Enzymes frequently can hydrolyze more than one group of β-lactam.
‡Indicates that normally repressed β-lactamase production can be induced by β-lactam exposure.

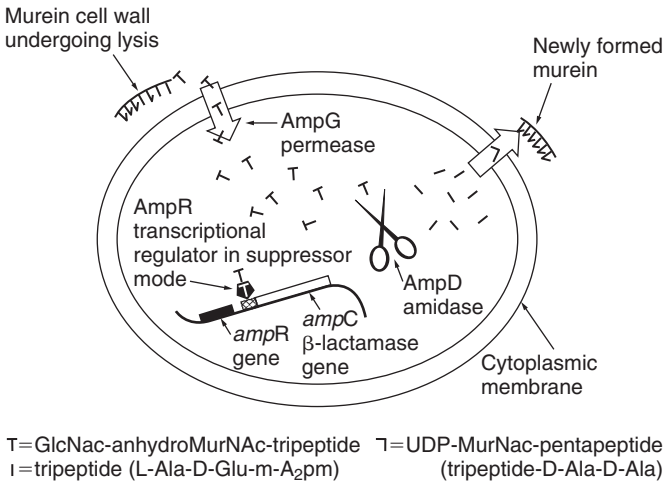

Figure 246–2 A model for peptidoglycan recycling and β-lacamase induction in enterobacteria. The lytic transglycosylases in the periplasm cleave the bond between *N*-acetylglucosamine (GlcNac) and *N*-acetylmuramic acid (MurNac) and form the peptidoglycan degradation product, *N*-acetylglucosaminyl-1,6-anhydro-*N*-acetylmuramyl-L-alanyl-D-glutamyl-meso-diaminopimelic acid (T). The AmpG permease transports T into the cytoplasm. The AmpD amidase recognizes specifically substrate containing anhydromuramic acid and cleaves the bond between it and L-alanine, releasing the stem tripeptide, L-Ala-D Glu-m-A_2pm (I). Unprocessed T activates the AmpR transcriptional regulator, thereby inducing β-lactamase production. The tripeptide, I, conversely, recycles to form new peptidoglycan. By regulating the relative amounts of T versus I, AmpD may control peptidoglycan composition. The regulatory function of the AmpC β-lactamase, if any, is unknown.

cessed by the enzyme AmpD.[165,219] In sufficient quantity, the AmpD substrate interacts with *amp*R, thus reversing the repression, and relevant quantities of the AmpC β-lactamase are thereby synthesized.

β-lactam antibiotics differ in their ability to induce AmpC β-lactamases by this mechanism. Ampicillin, cefoxitin, and imipenem are potent inducers, whereas most of the third-generation cephalosporins are weak. However, spontaneous mutations leading to a dysfunctional AmpD enzyme that occur at high frequency in nature result in permanent de-repression of the *amp*C gene, which, in turn, results in constitutive production of large quantities of the AmpC β-lactamase (see Fig. 246–2). De-repressed organisms are rendered highly resistant after this single mutational step. The third-generation cephalosporins are particularly prone to selecting such AmpD mutants, and the clinical emergence of resistant de-repressed isolates during the course of treatment with a third-generation cephalosporin has been documented.[49]

Group 2 β-Lactamases

The Bush, Jacoby, and Medeiros group 2 β-lactamases include a wide variety of enzymes that are encoded primarily on plasmids.[36,144,199,290,301] These plasmids commonly contain resistance determinants to other antibiotic classes as well. The plasmids are stable in nature and frequently are maintained within the bacteria even in the absence of antibiotic pressure. Moreover, they can be transmitted by conjugation from organism to organism and across species, sometimes leading to a "plasmid outbreak" in a confined environment (e.g., a nursing home or intensive care unit [ICU]) that is caused by different species containing the same plasmid. The group 2 enzymes have substrate preferences that have become increasingly broad with the introduction of each new class of β-lactam antibiotic. Characteristically, the group 2 enzymes are inhibited by clavulanate and are found in both gram-positive and gram-negative bacteria (see Table 246–2).[36,199,252]

The prototypical group 2 β-lactamases are included in the TEM and SHV families of enzymes, although large numbers of group 2 enzymes not belonging to either of these families also have been characterized. TEM-1, first identified in Europe in 1963, hydrolyzed ampicillin. During the ensuing decades, the TEM sequence mutated in a step-wise fashion (with each new iteration labeled sequentially: TEM-2, TEM-3, and so on), resulting in the ability of each new enzyme to degrade an ever-increasing number of β-lactam antibiotics.[88,169,252] Many of these mutations widen a critical cavity, formed by the three-dimensional configuration of the molecule, containing a serine-active site at its bottom that is key to hydrolysis of the β-lactam.[118,170] Although these mutations render the enzymes kinetically less efficient than their parents, they allow the entry of the newer β-lactams with their bulky side chains into the cavity, thereby enabling the serine-active site at the bottom to reach the β-lactam ring.[88,252]

Of increasing importance among the group 2 enzymes are the extended-spectrum β-lactamases (ESBLs). ESBL-producing organisms are distributed worldwide, but their incidence varies geographically.[299] Currently, bacteria elaborating ESBLs infect primarily critically ill, hospitalized patients,[291] although these enzymes are being reported in increasing numbers in the community.[53,256,307] Although they are molecularly diverse, ESBLs confer a common resistance phenotype, namely, reduced susceptibility to the third-generation cephalosporins, especially ceftazidime, and to aztreonam. Susceptibility to the carbapenems is preserved.[290] Common to other group 2 β-lactamases, hydrolytic activity typically is suppressed by the β-lactamase inhibitors. Most ESBLs are molecular descendents of TEM-1 and SHV-1 and are found in *E. coli*, *Klebsiella pneumoniae*, and to a lesser extent in *Proteus mirabilis*. Other families of ESBLs have been described. Among the more important ESBLs are the CTX-M enzymes, which are particularly active against cefotaxime, and some of the OXA-β-lactamases, the principal substrate of which is oxacillin but the spectrum of which has broadened to include

the advanced-generation cephalosporins.[290,299,307] Concomitant with their molecular evolution, ESBLs have been identified in an ever-widening range of bacteria, including *Pseudomonas*, *Salmonella*, and *Shigella* and *Enterobacter* spp.[290]

Although most ESBL-producing bacteria are overtly resistant, the third-generation cephalosporins have minimum inhibitory concentrations (MICs) against some ESBL-producing organisms that fall into the high end of the susceptible range. Consequently, the incidence of ESBL-producing bacteria is underestimated when conventional MIC breakpoints for resistance are used. Growing numbers of reports, however, indicate that infections caused by organisms expressing these "susceptible" MICs are clinically unresponsive to the third-generation cephalosporins.[315] Many hospital microbiology laboratories therefore screen all *E. coli*, *Klebsiella*, and *P. mirabilis* clinical isolates for reduced susceptibility to both ceftazidime and cefotaxime and alert the clinician accordingly. Although the optimal therapy for infection caused by ESBL-producing, gram-negative bacteria is not established, many authorities recommend a carbapenem.[170,290,315]

An additional family of group 2 β-lactamases, composed of carbapenemases, was identified first in the late 1990s.[26,154,367,426] These enzymes are found principally in hospital-acquired *K. pneumoniae*, and hence they are designated *K. pneumoniae* carbapenemases (KPCs).[26,154,368,426] By the early 2000s, they had become prominent in selected regions of the eastern United States. KPCs have been identified in a variety of other Enterobacteriaceae as well.[28] KPC enzymes are carried by conjugative plasmids expressing multiple other resistance genes. The carbapenem MICs for some KPC-containing organisms are at the high end of the susceptible range, whereas others are overtly resistant.

Group 3 Metalloenzymes

The Bush, Jacoby, and Medeiros group 3 β-lactamases are composed of the metalloenzymes (see Table 246–2).[35,411] These β-lactamases are genetically heterogeneous and structurally dissimilar to those of groups 1 and 2. The metalloenzymes are not suppressed by β-lactamase inhibitors. The active site requires the participation of zinc; hence hydrolysis of the β-lactam ring is inhibited by chelators such as ethylene diaminetetraacetic acid (EDTA). The active site itself is composed of a wide plastic groove that can accommodate many β-lactam substrates. Some metalloenzymes are chromosomal (e.g., in *Stenotrophomonas*), but many have been identified on integrons that are inserted on transferable elements.[411] These latter metalloenzymes, which characteristically encode aminoglycoside-resistance determinants as well, are identified primarily in *Pseudomonas* and *Acinetobacter* spp. and selected Enterobacteriaceae. During the latter 1990s and early 2000s, organisms harboring transferable metallo-β-lactamases, particularly those from the IMP and VIM family of metalloenzymes, were identified in many countries in eastern Asia, Europe, and South America. The substrate preference of the metallo-β-lactamases varies, but most organisms expressing these enzymes have reduced susceptibility or overt resistance to all the β-lactam antibiotics, including the carbapenems. Moreover, organisms encoding a metalloenzyme frequently express other chromosomal and plasmid-encoded β-lactamases as well, and this phenomenon, coupled with the coincident expression of resistance to other classes of antibiotic, often severely limits therapeutic options.

Although the properties summarized in Table 246–2 apply to most of the enzymes in each group, many exceptions have been noted. For example, enzymes that are genetically homologous to the group 2 family, typically plasmid-borne enzymes, have been identified on the bacterial chromosome in some isolates of *S. marcescens* and *E. cloacae*.[252] Conversely, group 1 AmpC enzymes, typically contained on the bacterial chromosome, have been identified on plasmids in genera such as *Klebsiella* and *Salmonella* that customarily do not express chromosomal *ampC* β-lactamase.[299-301] Some rare group 2–type enzymes are inducible.[252] TEM-type enzymes that are resistant to clavulanate have been identified (the so-called inhibitor-resistant TEMs [IRTs]).[42,192]

The degree of resistance exhibited by an organism encoding a β-lactamase frequently depends not only on the enzyme's in vitro substrate specificity and kinetics but also on the amount of enzyme produced. Hyperproduction of β-lactamase usually is the result of some alteration of a controlling sequence or an increase in number of copies of the β-lactamase gene within the bacterium.[117,287,431] High-level resistance results, and the susceptibility of the organism diminishes with increasing numbers of bacteria. Consequently, the organism may appear clinically unresponsive to a β-lactam antibiotic if hyperproduction occurs in the context of a high-inoculum disease. In some circumstances, hyperproduction operates in concert with other mechanisms to derive a resistant phenotype. Thus, some isolates of *E. cloacae* and *P. aeruginosa* have been rendered carbapenem-resistant through a combination of hyperproduction of an AmpC β-lactamase, which normally is ineffective in hydrolyzing the carbapenems, and the simultaneous loss of the outer-membrane poron through which imipenem traverses to reach the PBP.[45,182,266,311]

Alteration of Penicillin-Binding Proteins

CLINICAL RELEVANCE

Two species of clinical importance to pediatricians express resistance to β-lactam drugs through the alteration of PBPs, namely, penicillin-resistant *Streptococcus pneumoniae* and methicillin-resistant *S. aureus* (MRSA). Indeed, not until penicillin-resistant *S. pneumoniae* was recognized did pediatricians begin to realize that alterations in the PBPs in the bacterial cell wall could pose a major clinical problem.[186,244] The identification of patients with pneumococcal infections caused by organisms resistant to penicillin created tremendous consternation within the pediatric infectious disease community. However, to date, the precise clinical importance of these organisms remains unclear.

Part of the problem in assessing the clinical importance of altered penicillin binding among pneumococci is that, rather than simply conferring penicillin resistance, the genes coding for this altered enzyme activity often co-segregate with genes coding for resistance to other antibiotics, such as TMP-sulfamethoxazole (TMP-SMX) and the macrolides.[46,70,77,78,167,321,392] Thus, unlike resistance associated with the elaboration of β-lactamase, in which simply selecting a different class of antibiotic often will provide a reasonable solution, resistance associated with alteration in PBPs is multifarious and, if clinically important, may require significant alterations in clinical practice to provide an effective antimicrobial regimen. The more limited menu of antibiotic choices for the treatment of infections caused by these organisms led to some interesting therapeutic conventions. For example, because the basis for this type of resistance is akin to altered enzyme affinity, investigators recognized in the mid-1990s that overcoming the apparent decrease in efficacy could be possible by simply increasing the dose of β-lactam employed.[186,245,246] Of the available agents, only amoxicillin had the requisite therapeutic index to support the increased dosing strategy. On this basis (almost exclusively), the high-dose amoxicillin regimen (80 to 100 mg/kg/day) that is currently recommended was adopted. Additionally, the perceived need for a drug effective against antibiotic-resistant *S. pneumoniae* led to the widespread use of the parenteral third-generation cephalosporin, ceftriaxone, in the setting of common upper and lower respiratory tract infections because of its in vitro activity against most of these organisms.

As the clinical impact of the emergence of the antibiotic-resistant *S. pneumoniae* has been examined more carefully in recent years, several trends have emerged.[186,430] In immunocompetent patients with pneumonia and most other non–central

nervous system pneumococcal infections, no difference appears to exist in outcomes between patients infected with organisms that are susceptible to penicillin and those infected with organisms showing decreased susceptibility to penicillin.[286,298] Moreover, apparently no association exists between the intrinsic virulence of the organism and its antibiotic susceptibility. In contrast, in patients with pneumococcal meningitis caused by organisms with decreased penicillin susceptibility, there appears to be a delay in sterilization of cerebrospinal fluid in the presence of standard treatment.[225,429] For this reason, the current recommendation for empiric treatment of bacterial meningitis in infants and children consists of a combination of ceftriaxone or cefotaxime and vancomycin until the organism is identified and its antibiotic susceptibility is known.

S. pneumoniae remains the most prevalent bacterial cause of acute otitis media in infants and children. The clinical importance of the antibiotic susceptibility of these organisms in this clinical setting remains difficult to ascertain. The infection has a high rate of spontaneous resolution, and virtually all treatment is provided on an empiric basis. Consequently, any improved cure rate related to altered prescribing practices aimed at greater activity against organisms with decreased antibiotic susceptibility would be difficult to substantiate. Finally, the importance and virulence of these organisms in children who are either immunocompromised or immunosuppressed remain to be determined unequivocally.

The problem of penicillin-resistant *S. pneumoniae* has been at least partially addressed by the introduction of the conjugated heptavalent pneumococcal vaccine. The serotypes included in the vaccine account for most penicillin-resistant pneumococci in the United States.[393] Surveys conducted by the Centers for Disease Control and Prevention (CDC) indicated that the incidence of invasive *S. pneumoniae* infection caused by penicillin-resistant strains decreased by more than 50 percent in the years immediately following introduction of the vaccine.[206] The decrease was most pronounced in children younger than 2 years of age. This benefit has been mitigated in part by increases in disease caused by resistant serotypes not included in the vaccine, most notably serotype 19A.[17,206] Acute otitis media is caused by many pneumococcal serotypes not included in the heptavalent vaccine, and, consequently, the effect of immunization on reducing resistance in this and other noninvasive pneumococcal infection probably is marginal.

MRSA was isolated first from hospitalized adult patients in 1961 and became a prominent pathogen in critically ill patients during the ensuing decades. By the early 2000s, more than half of *S. aureus* infections in patients in ICUs in the United States were caused by MRSA.[267] Compared with those in adults, MRSA infections among hospitalized children occur relatively infrequently. In the mid-1990s, however, MRSA infections were identified among otherwise healthy children with no prior contact with hospitals or hospitalized patients.[145,161] These strains of community-associated MRSA (CA-MRSA) were molecularly distinct from MRSA infections previously identified in the hospital environment. By the mid-2000s, CA-MRSA had established a worldwide presence in both children and adults.[3] The infection causes primarily skin and soft tissue infection but occasionally bone and joint infection, severe necrotizing pneumonia, and other invasive, deep tissue disease. CA-MRSA infections are especially common in populations inhabiting close quarters and engaging in skin-abrading activities, including athletes participating in competitive sports, military personnel, and prison populations.[414]

Most MRSA isolates, both hospital and community-associated, are derived from a relatively small number of prominent, widely disseminated clones.[279] Several systems have been proposed to categorize the principal MRSA lineages. The system established by the CDC is based on *Sma*I macro-restriction patterns of bacterial DNA visualized after separation by pulse-field gel electrophoresis (PFGE). The PFGE types so defined have been labeled USA100 through USA800, in addition to two additional groups, named USA1000 and USA1100.[74,248] Currently, more than 90 percent of MRSA strains derived from patients in the United States fall within one of these PFGE types.[74,248] Most CA-MRSAs are included in the USA300 group, with a minority derived from the USA400 and USA1100 groups.[197,355]

MECHANISM OF RESISTANCE

β-Lactam antibiotics must bind to PBPs to confer their activity, and alteration of the structure of PBPs results in diminished affinity for drug and, consequently, antibiotic resistance. The PBPs are a heterogeneous group of compounds normally responsible for maintaining the peptidoglycan matrix supporting the bacterial cell wall. PBPs vary from species to species in number, size, copy number per cell, and affinity for β-lactam agents.[115,134,343] Typically, four to eight PBPs are present in any given bacterial isolate; they range in molecular weight from 35 to 120 kd.[115] PBPs possess two principal enzymatic activities. In general, the larger PBPs are responsible for peptidoglycan transpeptidase activity, whereas the smaller PBPs usually carry D,D-carboxypeptidase activity.[115] These activities constitute the final reactions in the formation of the peptidoglycan matrix and are responsible for the cross-linkage that significantly adds to the tensile strength of the bacterial cell surface.

Examination of PBP-mediated β-lactam resistance in *S. pneumoniae* and *S. aureus* serves to exemplify some of the features of this mechanism of resistance. Detailed investigations indicate that penicillin resistance in pneumococcus is associated with alternations in PBP 1A, 1B, 2A, 2X, and 2B; cephalosporin resistance has been correlated with structural abnormalities in PBP 1A, 2A, and 2X.[14,52,125,133,135,264] Confirmation that the resistant phenotype is related directly to structural abnormalities in various pneumococcal PBPs has been achieved through transformation experiments.[14,52,125,264] In these studies, penicillin-susceptible strains are converted to penicillin-resistant strains by addition of amplified DNA encoding the putative resistant PBPs. Patterns of PBPs from resistant pneumococci vary considerably from geographic location to location, a finding indicating that PBP-related pneumococcal resistance probably arose independently in various parts of the world.[133]

PBPs from penicillin-resistant *S. pneumoniae* contain blocks of amino acid sequences that are remarkably divergent from those seen in susceptible pneumococci. These sequences are homologous to those encoding for PBPs from other streptococcal species (e.g., *Streptococcus oralis* and *Streptococcus mitis*), a finding suggesting that the resistant pneumococci contain mosaic PBPs that arose after transformation and recombination of homologous genes from closely related bacteria.[84,85,132,208] These sequences then developed point mutations conferring resistance. A similar pattern of "resistance blocks" of amino acids has been identified in the PBPs of penicillin-resistant *Neisseria gonorrhoeae*, which appear to have originated from other *Neisseria* spp.[134] The degree of resistance conferred by a single mutated PBP is relatively small.[115,125] Acquisition of multiple abnormal PBPs results in incremental resistance and, ultimately, an organism that can survive routine β-lactam therapy.

Whether associated with the hospital or the community, all isolates of MRSA contain the *mecA* gene, a 2130-bp chromosomal sequence encoding the PBP 2A, which, unlike the other staphylococcal PBPs, has low affinity for β-lactam antibiotics.[62,63] The *mecA* gene is carried on a mobile element designated the *staphylococcal cassette chromosome mec (SCCmec) sequence*.[437] All MRSA strains contain an SCC*mec* complex, but their size and organization vary, thus allowing them to be grouped into five distinct SCC*mec* types, numbered I to V.[278,437] Hospital-acquired (HA)-MRSA strains contain SCC*mec* types I to III, whereas CA-MRSA

strains harbor type IV and, to a lesser extent, type V. In most HA-MRSA isolates, the SCC*mec* sequence contains resistance determinants additional to *mec*A, including those conferring resistance to clindamycin, the aminoglycosides, and the fluoroquinolones.[67,437] Hence, these organisms possess a selective advantage in ICUs, where exposure to multiple classes of antibiotics commonly occurs. By contrast, the CA-MRSA SCC*mec* type IV and V strains encode a smaller SCC*mec* sequence lacking the multiple antibiotic-resistance genes found in their hospital-associated counterparts.[67,437] These strains include certain virulence factors, however, most notably the Panton-Valentine leukocidin, possibly important for soft tissue breakdown and destruction. The smaller SCC*mec* sequences allow CA-MRSA to replicate more rapidly than does HA-MRSA,[277] because they can be maintained by the bacterium at a lower metabolic cost. The relatively small size of the CA-MRSA SCC*mec* sequences further enables efficient transfer to co-colonizing susceptible *S. aureus*.[437]

SCC*mec* elements likely originated in coagulase-negative staphylococci. Type IV SSC*mec* sequences, for example, have been identified in isolates of *Staphylococcus epidermidis* from the 1970s.[424] Additionally, sequences have been found in *S. epidermidis* and *Staphylococcus hominis* strains that contain the regulatory genes and insertion sequences of the SCC*mec* elements found in MRSA but that lack the *mecA* gene itself.[3]

The complexity of the genetics underlying PBP-associated β-lactam resistance is apparent in MRSA. In the presence of methicillin concentrations inhibitory to susceptible staphylococci, MRSA PBP 2A assumes virtually all responsibility for peptidoglycan synthesis, with the resultant production of a matrix of unusual muropeptide compositions. Some MRSA strains encode regulatory sequences for *mecA* termed *mecRI*, the product of which is a transmembrane sensor needed for induction of *mecA* expression in the presence of β-lactam, and an inhibitory controlling sequence (*mecI*).[134] However, the degree of methicillin resistance is not well correlated with the amount of expressed PBP 2A,[62,63] a finding indicating that other cellular factors are important for the production of the resistant phenotype. Transposon inactivation experiments have identified multiple additional sites on the staphylococcal chromosome critical for methicillin resistance. These sequences, termed *fem* (*f*actors *e*ssential for *m*ethicillin resistance) genes, express abundant substrate for PBP 2A that, in turn, is available to compete successfully with methicillin and other β-lactam antibiotics for the PBP 2A active site.[134] Dysfunction of the *fem* genes and absence of these peptidoglycan precursors result in reversion to the methicillin-susceptible phenotype, even in the presence of *mecA* encoded PBP 2A. Similar mechanisms appear to operate in methicillin-resistant, coagulase-negative staphylococci.

MACROLIDES, LINCOSAMIDES, AND SPREPTOGRAMINS

Clinical Relevance

The macrolides are compounds that contain 14-member (e.g., erythromycin and clarithromycin), 15-member (e.g., azithromycin), or 16-member (e.g., spiramycin) lactone rings. Their activity is directed primarily against gram-positive organisms and some gram-negative respiratory tract pathogens. In many bacteria, resistance to macrolides occurs concomitantly with that of the structurally unrelated lincosamides (including clindamycin) and streptogramin B families of antibiotics, two classes with activity also primarily against gram-positive bacteria. Because these three families of antibiotics interact competitively for ribosomal binding, they probably are associated with the same ribosomal site.

Resistance to the macrolides was noted in staphylococcal species soon after the introduction of erythromycin. More recently, however, macrolide resistance in streptococcal pathogens, particularly *S. pneumoniae* and *Streptococcus pyogenes*, emerged and assumed major clinical importance. Macrolide resistance among pneumococci is a result of both international dissemination of resistant clones and horizontal transmission of resistance determinants.[113,334] The late 20th century witnessed dramatic increases in the incidence of macrolide resistance in *S. pneumoniae* in many areas of the world.[146] In Southeast Asia, surveys indicated that nearly 40 percent of pneumococcal isolates were resistant to all macrolide antibiotics, and samplings of organisms from Europe and the Western Hemisphere showed similar but less extreme trends.[146,195] Perhaps most troubling, resistance to macrolides among pneumococci occurred particularly frequently in isolates co-expressing resistance to penicillin and other oral antibiotics.[78,162]

Similar to penicillin-resistant pneumococcus, the prevalence of macrolide-resistant *S. pneumoniae* has been reduced during the first decade of the 21st century by the introduction and widespread administration of the conjugated heptavalent pneumococcal vaccine, which includes serotypes that most commonly express both β-lactam and macrolide resistance.[393] Studies performed in Atlanta, Georgia, before release of the vaccine, for example, documented that 31 percent of pneumococcal isolates causing invasive disease were resistant to erythromycin.[112] Follow-up studies performed after the introduction of the vaccine recorded a drop in incidence of macrolide-resistant invasive pneumococcal disease by more than two thirds.[374] As in penicillin-resistant pneumococcus, this benefit has been partially offset by an increase in the incidence of invasive diseases by macrolide-resistant organisms derived from serotypes not included in the vaccine.[95]

Similar to *S. pneumoniae*, macrolide resistance in *S. pyogenes* has been unevenly distributed geographically.[191] By the start of the 21st century, most surveys in the Western Hemisphere, including the United States, indicated a prevalence of resistance less than 10 percent,[126,285,325,386,418] although the citywide clonal outbreak of macrolide-resistant group A streptococcus experienced in Pittsburgh, Pennsylvania, in 2001 underscored the potential for this phenotype to spread rapidly within a community.[234] Macrolide resistance in *S. pyogenes* is, on average, more prevalent in Europe, but the prevalence there also varies substantially from area to area, ranging (by 2007) from 3 percent in Norway,[220] to 13 percent in Belgium,[226] to more than 20 percent in Spain and France.[21,297] Molecular analysis of macrolide-resistant group A streptococcal isolates from geographically diverse regions suggested that the resistance genes were acquired multiple times by many lineages, some of which then disseminated worldwide.[330]

The increase in macrolide resistance has occurred concomitant with increased worldwide consumption of these compounds for respiratory tract infections beginning in the 1980s,[15,20,50,162,184,354] a finding suggesting a biologic association between these two phenomena. A nationwide survey of macrolide susceptibility among pneumococcal isolates in the United States, for example, documented an increase in erythromycin resistance from 10.6 percent in 1995 to 20.4 percent in 1999, a time when the number of prescriptions for macrolides increased by 13 percent across the board and by 320 percent in pediatric patients.[162] Seppälä and colleagues[354] reported an increase in resistance among *S. pyogenes* isolates in Finland from 5 percent in 1988 to 13 percent in 1990 after national macrolide consumption doubled during the 1980s. In response, a national campaign to reduce macrolide prescribing practices in Finland was launched in the early 1990s, after which macrolide resistance fell by half.[354] A similar campaign in Taiwan yielded similar results.[158]

Although the epidemiologic trends in macrolide resistance are alarming, the clinical consequences of the increases in macrolide resistance among streptococci are largely unknown. Current con-

vention employs an MIC of 4 μg/mL or greater to define resistance. The newer macrolide compounds, however, achieve tissue and intracellular concentrations that are many-fold higher than this designation.[93,276] Italian investigators, for example, noted a very high incidence of erythromycin resistance among group A streptococcal throat isolates, but neither clinical nor microbiologic failures were clearly associated with macrolide treatment.[404] The macrolides do not distribute in high concentrations to the blood compartment, however, and several reports document poor outcomes when this class of drugs is used for bacteremic pneumonia caused by macrolide-resistant pneumococcus.[194,222]

A newer group of related antibiotics, the ketolides, contain chemical modifications to the lactone ring. To date, they have proven in vitro activity against organisms that express high-level macrolide resistance.[94,260] Although the MICs of ketolides to macrolide-resistant organisms are higher than in macrolide-susceptible bacteria, they remain well within the range of concentrations achieved with standard dosing. A report suggesting a link between telithromycin, a prototypical ketolide, and severe hepatotoxicity in adults,[51] however, has slowed the development of this class of drugs for children.

Mechanism of Resistance

Two principal resistance phenotypes to macrolides and related antibiotics have been detected. The first, denoted the *MLS_B phenotype*, is characterized by resistance to all macrolides, regardless of the size of the ring, as well as to the lincosamides and the type B streptogramins. Most organisms expressing the MLS_B phenotype remain susceptible to the combination streptogramins (e.g., quinupristin-dalfopristin).[136,348] The second phenotype, denoted the *M phenotype*, is associated with resistance to the 14- and 15-member macrolides (including erythromycin, azithromycin, and clarithromycin), but not those with 16-member rings. The degree of resistance to the affected macrolides is lower, on average, compared with that seen with MLS_B resistance. Additionally, in organisms with the M phenotype, susceptibility to clindamycin is preserved.[239,380]

Most MLS_B resistance results from methylation of the 23S ribosomal RNA (rRNA) in the 50S subunit of the bacterial ribosome within the peptidyltransferase circle in domain V.[66,172,387,419] The methylases are encoded on transposons, with sequences found both on plasmids and in the bacterial chromosome.[120] Methylation of the 23S rRNA is encoded by a family of related genes labeled *erm* (*e*rythromycin *r*esistance *m*ethylases).[66,172,387,419] Historically, these methylases have been described and named in a haphazard fashion, although some investigators have proposed renaming many of these enzymes using a classification scheme based on genetic homology.[328]

Methylation is both inducible and constitutively expressed. Unlike many other inducible antibiotic-resistance factors, the mechanism of MLS_B resistance induction is at the level of translation, rather than transcription.[417] In the uninduced state, the leader sequence of the mRNA encoding ErmC, one of the methylases found in *S. aureus*, forms two stem loop structures that stall the ribosome's movement along the message, thereby preventing synthesis of the methylase enzyme. The attachment of erythromycin to the ribosome leads to a conformational alternation of this leader sequence that uncovers the appropriate ribosomal binding sites and allows the stalled ribosomes to proceed to translation.[417] Inducible macrolide resistance is detected in the clinical microbiology laboratory by the *double-disk D-test*. In this assay, an erythromycin disk and a clindamycin disk are placed side by side on a lawn of the test organism. Induction of clindamycin resistance in the presence of erythromycin is manifested by increased growth of bacteria on the erythromycin side of the clindamycin disk, producing a D-shaped zone of inhibition. Through the 1990s, greater numbers of *erm*-containing bacteria produced the rRNA methylase constitutively, a consequence of mutation of the leader sequence.[79,385]

In the mid-1990s, macrolide resistance mediated through a genetically transferable efflux pump was identified.[380] Most organisms expressing the M phenotype are resistant through this mechanism. Most macrolide-resistant pneumococci in North America express the macrolide efflux pump, and much of the expansion of macrolide resistance that occurred through the 1990s was the result of the appearance of organisms exhibiting this mechanism.[162,178,218,386] A family of related genes, termed *mef*, encodes the macrolide efflux pumps. The *mef* genes are included on transposons encoding associated functions, in particular an adenosine triphosphate (ATP)–binding protein.[112,113,120,239] As expected, ribosomes isolated from organisms resistant through *mef* genes bind to macrolide antibiotics as readily as do ribosomes from susceptible organisms. Radiolabeled erythromycin is excluded from the intracellular space in *mef*-positive bacteria, a phenomenon that is reversed by compounds that poison ATP-dependent pumps.[380]

Mechanisms of macrolide resistance besides rRNA methylation and efflux pumps have been identified but to date are uncommon. Some organisms modify the antibiotic by hydrolysis, acetylation, phosphorylation, or esterification.[328] Some evidence indicates that some erythromycin resistance is caused by altered ribosomal proteins and results in the production of ribosomes with diminished affinity for drug.[47]

TRIMETHOPRIM AND SULFAMETHOXAZOLE

Clinical Relevance

The combination antibiotic TMP-SMX inhibits bacterial folate synthesis at two successive steps. This mechanism accounted for its broad activity against a wide variety of gram-positive and gram-negative bacteria when it was first introduced in 1968. Despite the dual activity of this combination, resistance to TMP-SMX has risen steadily during the decades of its use and is now commonplace. Acquisition of resistance is potentiated by recent exposure to TMP-SMX.[96,373,428] When resistance occurs, the MIC expressed usually is very high,[159,160] and the affected organisms frequently are co-resistant to multiple other classes of antibiotics. Indeed, TMP-SMX resistance is sufficiently widespread that it precludes the use of the drug as a first-line antibiotic in clinical circumstances in which it was formerly the agent of choice, namely, respiratory tract infections and *Shigella*-associated dysentery. Its utility in urinary tract infection (UTI) also may be limited when the community prevalence of TMP-SMX resistance is sufficiently high. Ironically, its continued activity against CA-MRSA has rejuvenated the use of TMP-SMX among many practitioners as the incidence of infections caused by CA-MRSA organisms has mushroomed.

RESPIRATORY TRACT INFECTIONS (OTITIS MEDIA AND PNEUMONIA)

The frequency of TMP-SMX resistance in organisms causing respiratory tract infections has increased significantly. Among *S. pneumoniae* organisms, resistance to TMP-SMX was identified during the 1990s in many geographic locations, with resistance detected in 12 percent to more than 50 percent of isolates.[76,146,157,167,231,289,377] A worldwide survey conducted in the late 1990s noted the highest incidence of TMP-SMX pneumococcal resistance in the Asian Pacific region and in Latin America.[146] In surveys of U.S. isolates, TMP-SMX resistance is the most common resistance phenotype found in *S. pneumoniae*,[421] although the incidence of resistance varies widely from region to region.[76] TMP-SMX resistance is strongly associated with resistance to other classes of antibiotics commonly used in respiratory tract

infections, particularly penicillin and the macrolides. Among other organisms implicated in respiratory tract infections, TMP-SMX resistance also has been documented with increasing frequency in *H. influenza*, to which resistance ranges between 7 percent in Finland[231] and more than 50 percent in Taiwan.[157] The susceptibility of *M. catarrhalis* to TMP-SMX varies little from region to region. Although most *M. catarrhalis* organisms remain susceptible to TMP-SMX, the MICs cluster near achievable respiratory tract concentrations.[167]

The clinical consequences of TMP-SMX resistance in respiratory tract pathogens are not well defined. Direct evidence of the association between isolation of a TMP-SMX–resistant organism from the middle ear and failure of therapy by TMP-SMX in acute otitis media, for example, is lacking, but such an association is assumed to exist. The issue of the clinical relevance of TMP-SMX resistance in respiratory tract infections is particularly pressing in developing countries, where World Health Organization (WHO) guidelines recommend TMP-SMX as a first-line agent for pneumonia. A comparison of amoxicillin and TMP-SMX for treating pneumonia in Pakistan demonstrated superiority of amoxicillin in producing clinical cure, but treatment failure was not associated with resistance in the infecting organism.[378] These data and others[193,316,332] support the continued use of TMP-SMX therapy of pneumonia in the developing world, as least at present.

SHIGELLOSIS

Resistance of *Shigella* spp. to TMP-SMX rose dramatically during the 1980s in many parts of the world, including Bangladesh,[19] Thailand,[147] Somalia,[40] Israel,[10] and Canada.[138] In these surveys, the incidence of disease from TMP-SMX–resistant *Shigella* increased two- to threefold, to more than half the isolates; by 1998, more than 90 percent of *Shigella* organisms in Thailand,[147] as well as *Shigella* cultured from patients with acquired immunodeficiency syndrome (AIDS) in Kenya,[205] were resistant to TMP-SMX. Many of these *Shigella* organisms express extraordinarily high levels of resistance, with MICs to TMP exceeding 1000 μg/mL. In the United States, TMP-SMX resistance among *Shigella* isolates initially was confined to closed populations.[127] A more recent survey in Oregon, however, indicated that by the late 1990s, the incidence of TMP-SMX resistance among *Shigella* isolates in that state approached 60 percent. Resistant isolates were found disproportionately frequently in migrant workers traveling from Mexico and other areas of Latin America,[322] a finding emphasizing the importance of geographic spread of resistance in modern society. Subsequent surveys performed by the CDC in the early 2000s revealed that TMP-SMX–resistant *Shigella* had spread nationwide, with the prevalence highest in the East and West.[362] The incidence of TMP-SMX resistance in *Shigella* mirrors the increased TMP-SMX resistance among other enteric species as well,[127,401] notably *Salmonella*.[1,127]

TMP-SMX resistance in *Shigella* is virtually always borne on conjugative plasmids, and frequently these plasmids contain resistance determinants to several other antibiotic classes.[40,127,205,217,362,401] Co-resistance to four or more unrelated antibiotics, including β-lactams, chloramphenicol, tetracycline, and the aminoglycosides, is a common finding. Molecular analyses in a given geographic environment usually indicate fluid epidemiology, with multiple dominant chromosomal genotypes containing a variety of resistance plasmids.[27,217]

URINARY TRACT INFECTIONS

Through the 1990s and 2000s, TMP-SMX resistance in urinary tract isolates of *E. coli*, the most prominent pathogen implicated in UTIs, rose to 15 to 25 percent in the developed world and to as high as 60 percent in nonindustrialized countries.[129,130,190,229,438,439] Studies in the 1980s indicated that TMP-SMX–resistant *E. coli* can be transmitted to household contacts.[333] Indeed, one analysis suggested that TMP-SMX–resistant urinary tract isolates from geographically disparate areas of the United States may have emanated from a single clone.[229] Although urinary concentrations of both TMP and SMX are several-fold higher than concomitant concentrations in serum, resistance to TMP-SMX nonetheless is associated with treatment failure in UTIs. Bacterial and clinical response rates to TMP-SMX are approximately 80 to 95 percent in cystitis and pyelonephritis caused by susceptible organisms, but the response falls to approximately 50 percent when the infecting agent is TMP-SMX–resistant.[243,383] Considering all urinary tract pathogens together, however, the clinical and microbiologic response to TMP-SMX is nearly equivalent to comparator agents, and experts continue to recommend this drug as the first-line therapy for uncomplicated UTIs, based on its low cost.[98,153,413] Analyses have indicated that the total expense of treatment of UTIs with TMP-SMX begins to exceed that incurred by therapy with more expensive antibiotics, such as the fluoroquinolones, when the community TMP-SMX–resistance rates of urinary pathogens exceed approximately 20 percent, as a result of the additional expense of clinical treatment failure.[210,383] Alternatives to TMP-SMX also should be considered when the patient possesses risk factors for acquisition of infection with a TMP-SMX–resistant strain, namely, recent hospitalization, diabetes, or therapy with TMP-SMX within the previous 3 months.[153]

COMMUNITY-ASSOCIATED METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS*

Despite the explosive emergence of methicillin resistance in community-associated *S. aureus* infections in the latter half of the 1990s, these organisms have remained nearly uniformly susceptible to TMP-SMX.[29,185,259] Most infections caused by CA-MRSA are skin and soft tissue infections that can be treated without hospitalization, and TMP-SMX offers an inexpensive, oral therapeutic option in this setting. The clinical effectiveness of TMP-SMX in CA-MRSA infection relative to other oral agents is uncertain; indeed, whether any antibiotic is superior to incision and drainage is not clear.[254] Experiential reports, however, support the use of TMP-SMX as a principal or adjuvant therapy for CA-MRSA cellulitis.[382]

Mechanisms of Resistance

In susceptible bacteria, TMP-SMX inhibits sequential steps in the de novo synthesis of folate (Fig. 246–3). SMX inhibits the enzyme dihydropteroate synthetase (DHPS), which catalyzes the conversion of *para*-aminobenzoic acid to dihydrofolate. TMP inhibits the next enzyme in the pathway, namely, dihydrofolate reductase (DHFR), which catalyzes the conversion of dihydrofolate to tetrahydrofolate. The resulting depletion of folate within the bacterium interrupts the synthesis of critical cellular substrates, including purine nucleotides.[331] Eukaryotic DHFR is resistant to the activity of the sulfonamides, and eukaryotic cells do not express DHPS activity at all, thus accounting for the selective activity of these drugs against bacteria.[159,160]

The principal mechanism of resistance to TMP-SMX is alteration of the target enzymes. Altered genes for DHFR with reduced susceptibility to TMP have been identified on the chromosomes of intrinsically resistant organisms from a variety of species,[306] as well as on transferable elements. Many of the transferable resistance genes are encoded on transposons that can shuttle between plasmids and chromosomal DNA. Approximately 20 different transferable *dhfr* resistance genes have been identified.[160] Resistance usually is conferred by a single-point mutation

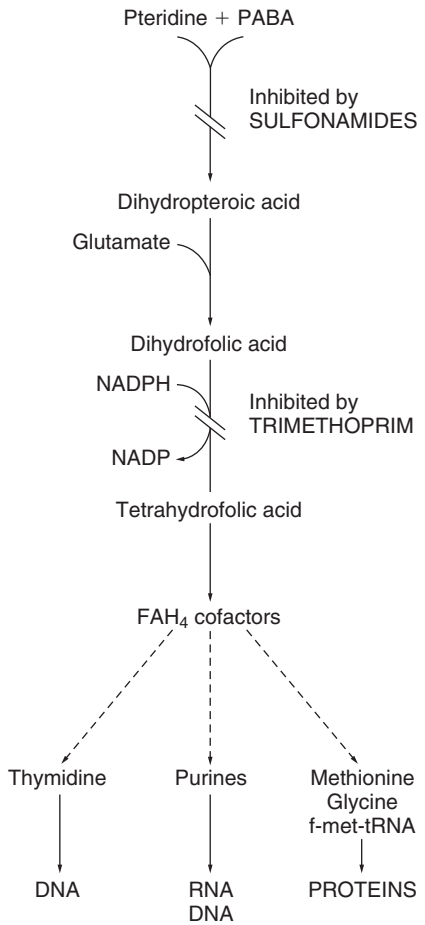

Figure 246–3 Trimethoprim-sulfamethoxazole inhibition of de novo folate synthesis. NADP, nicotinamide adenine dinucleotide phosphate; NADPH, reduced form of nicotinamide adenine dinucleotide phosphate; PABA, para-aminobenzoid acid.

that results in a disruption of the three-dimensional conformation of DHFR, thereby interrupting a hydrogen bond normally available for binding the antibiotic but leaving the affinity for its natural substrate intact.[61,160,306] These structural changes may occur in concert with mutations of controlling sequences and lead to overproduction of the altered DHFR.[306] Similarly, sulfonamide resistance usually is the result of mutated *dhps* genes. In TMP-SMX–resistant *Neisseria meningitides*, the abnormal chromosomal *dhps* sequence is different compared with that found in susceptible isolates over a large portion of the gene, a finding implying that resistance resulted from a recombination event after horizontal transfer from an unrelated *Neisseria* spp.[313] Similar recombination events may account for sulfonamide resistance in pneumococcus. In other species, the abnormal *dhps* gene is the result of a single amino acid mutation, and the resistance gene is encoded on a transmissible transposon,[284] frequently linked to a *dhfr* resistance gene.[160]

Some organisms, particularly *P. aeruginosa*, are intrinsically resistant to TMP-SMX on the basis of the expression of multidrug efflux pumps.[200] The same pumps are responsible for resistance to a wide variety of other antibiotics, including fluoroquinolones, chloramphenicol, and β-lactams. Homologous pumps occasionally have been identified in other gram-negative species as well. Finally, in some isolates, the mechanism of resistance to TMP-SMX is not attributable to any of the previously listed mechanisms and remains undefined.[160,284]

AMINOGLYCOSIDES

Clinical Relevance

The aminoglycosides are parenteral antibiotics that are employed primarily for serious infections in hospitalized patients. Resistance to aminoglycosides is encoded on transmissible elements that have disseminated throughout the world, both among gram-negative and gram-positive pathogens. Among gram-negative species, resistance to the aminoglycosides varies widely according to geographic location.[347,349,402] Surveys recording longitudinal trends in susceptibility patterns of hospital-acquired isolates between the 1990s and 2000s, however, documented that, relative to other agents, the increase in resistance to the aminoglycosides has been slow, and resistance has decreased in some areas.[18,105,188] Surveys of hospital isolates conducted throughout Europe and North America in the late 1990s and early 2000s indicated resistance rates of 1 to 15 percent among enteric bacilli and 10 to 50 percent among non–lactose fermenters.[100,181,272,347,349,403] In almost all cases, the incidence of resistance was lowest to amikacin.[105,273,347,349,402] Aminoglycoside resistance occurs with much higher frequency among gram-negative organisms with coexisting resistance to other antibiotics.[13,181,261] Not surprisingly, fecal colonization with aminoglycoside-resistant bacilli is an uncommon finding among healthy persons. Although resistance to some of the older aminoglycosides, such as streptomycin and kanamycin, has disseminated into the community, carriage of organisms resistant to gentamicin is a rare finding in ambulatory populations.[214,281,312]

Few data exist recording the endemic incidence of aminoglycoside resistance in gram-negative organisms specifically among hospitalized children. In a survey of pediatric patients in an ICU in Cleveland in the mid-1990s, approximately 10 percent of patients were colonized with a tobramycin-resistant, gram-negative bacillus before discharge.[394] A survey in the neonatal ICU in the same institution, where gentamicin frequently was employed, recorded an approximate 5 percent endemic incidence of colonization with gentamicin-resistant bacilli.[391] However, outbreaks caused by gram-negative rods resistant to aminoglycoside have been reported from numerous neonatal ICUs since the early 1980s.[54,175,249]

Although the aminoglycosides have no utility in the treatment of infections with gram-positive organisms when used alone, they improve bacterial killing when they are employed in combination with cell wall–active agents. For this reason, these drugs frequently are added to β-lactam agents or vancomycin in the treatment of difficult or persistent infections caused by *S. aureus*, coagulase-negative staphylococci, or enterococci. However, resistance to aminoglycosides has been documented in all these gram-positive microorganisms. Surveys in Europe indicated 20 to 30 percent resistance to aminoglycosides among hospital isolates of *S. aureus*.[347] The occurrence of aminoglycoside resistance is many-fold higher among MRSA isolates than in bacteria that are methicillin-susceptible.[65,100] As with gram-negative bacilli, aminoglycoside resistance among *S. aureus* isolates varies greatly from region to region,[347] a finding correlating largely with the incidence of MRSA. Aminoglycoside resistance in coagulase-negative staphylococci also is a common finding. As with *S. aureus*, resistance is much more frequent among organisms that are methicillin-resistant (seen in more than half of the isolates tested from centers around the world), as opposed to the unusual hospital-acquired methicillin-susceptible, coagulase-negative staphylococci, for which resistance to aminoglycosides is recorded in fewer than 10 percent.[73,198,347]

Since the 1970s, proliferation of enterococci that express MICs to the aminoglycosides exceeding 2000 μg/mL has occurred, thus rendering the organisms resistant to all synergistic effects when the aminoglycosides are used with a cell wall–active

TABLE 246–3 Aminoglycoside-Modifying Enzymes

Drug	Aminoglycoside* Acetyltransferase Acetylation	Aminoglycoside* Nucleotidyltransferase Adenylation	Aminoglycoside* Phosphotransferase Phosphorylation
Amikacin	X	X	
Gentamicin	X	X	X
Kanamycin	X	X	X
Netilmicin	X		
Neomycin	X	X	X
Streptomycin		X	X
Tobramycin	X	X	X

**Numbers of susceptible sites on each molecule vary.*
Adapted from Welton, A., & Neu, H. C. (eds.): The Aminoglycosides. New York, Marcel Dekker, 1982.

agent.[91,293] Such high-level aminoglycoside resistance has been recorded in approximately 30 percent of enterococcal samples from hospitals throughout the world.[223,361,436] Organisms expressing high-level aminoglycoside resistance frequently test highly resistant to ampicillin as well.[293] High-level aminoglycoside resistance also is a common finding in vancomycin-resistant enterococci, especially those identified in the United States.[223]

Mechanism of Resistance

The aminoglycosides are hydrophilic sugars composed of one to four rings, with amino groups substituting some of the hydroxyl groups at sites that vary from drug to drug. The amino groups render the drugs polycationic and allow them to bind with high affinity to selected sequences of nucleic acid.[319] The antibacterial activity of these drugs results from their binding to specific sites on the 16S ribosomal RNA that, in turn, interferes with the recognition of transfer RNA during translation and the translocation of tRNA to the peptidyl-tRNA site. Three-dimensional studies of the aminoglycoside-rRNA interaction suggest that this interference is the result of conformational changes in the shape of the rRNA.[204] The affinity of the aminoglycosides to ribosomal RNA is 10-fold higher in prokaryotes than in eukaryotes.[318]

The principal mechanism of resistance to the aminoglycosides is through the expression of aminoglycoside-modifying enzymes (Table 246–3). Many of these enzymes are encoded on transposons that reside in plasmids along with other resistance determinates. Three groups of modifying enzymes have been identified, namely, acetyltransferases (AAC), nucleotidyltransferases (ANT), and phosphotransferases (APH), each of which adds its respective moieties to selected side groups of the aminoglycoside sugar rings.[204,356] Researchers have hypothesized that these genes originated from bacterial housekeeping enzymes and then expanded to include aminoglycosides among their substrates through mutational evolution.[356] Transcription of these resistance genes is constitutive. Most members of each group of modifying enzymes share structural homologies, with minor amino acid variations resulting in specific substrate profiles.[356] The enzymes are subdesignated by the site they modify and by the resistance profile they confer (Fig. 246–4). Thus, for example, AAC(6′)-II denotes an AAC that substitutes an acetyl group for the amino group at the 6′-site of the aminoglycoside, with a resulting resistance pattern II (including, in this case, resistance to gentamicin, tobramycin, and sisomicin, but not amikacin). At least one enzyme has been discovered that possesses both AAC and APH activity [designated AAC[6′]-Ie-APH[2″]-Ia].[48,198,356,436] The modifications conferred by these enzymes result in altered electrostatic qualities of the antibiotic and consequent reduced ability to interact with rRNA.

The pharmaceutical industry has altered the structure of the aminoglycoside compounds to render them poorer substrates for the modifying enzymes. This exercise has been most successful in the development of amikacin, a kanamycin derivative with a relatively long amino hydroxybutyrate added at the 1 position.[255] This substitution does not interfere with rRNA binding but protects the compound from alteration by many of the modifying enzymes. Consequently, organisms resistant to gentamicin, for example, may remain susceptible to amikacin. Not all positions are protected, however, and amikacin-resistant phenotypes are conferred by certain modifying enzymes, most notably AAC(6′)-I.[255]

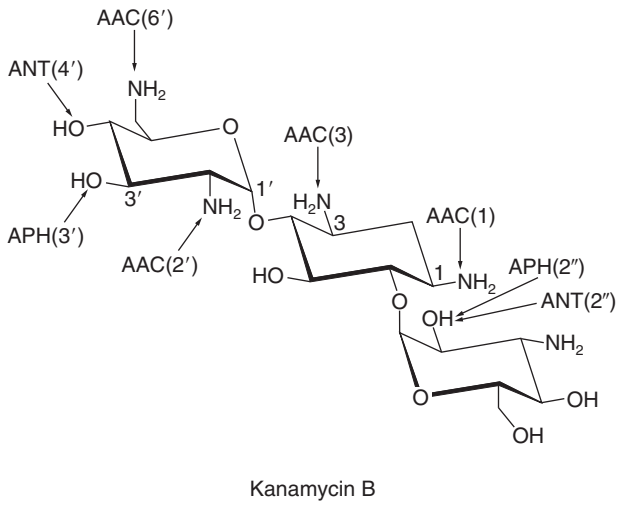

Figure 246–4 Sites of modification on kanamycin B by various aminoglycoside-modifying enzymes. The *arrows* point to the sites of modification by the specific enzymes, namely, acetyltransferases (AAC), phosphotransferases (APH), and nucleotidyltransferases (ANT).

Moderate-level resistance to the aminoglycosides has been achieved by some bacteria through decreased uptake of drug.[255] This mechanism has been noted most frequently in *Pseudomonas*. To date, modifications of the ribosomal binding sequences have been identified only rarely as a mechanism of resistance to the aminoglycosides.[255] The sequences involved in aminoglycoside binding likely are sufficiently critical to the survival of the organism that mutations within this site usually are fatal.[204]

GLYCOPEPTIDES AND OTHER ANTIBIOTICS DIRECTED AGAINST GRAM-POSITIVE BACTERIA

Clinical Relevance

Vancomycin is one of two glycopeptides available for clinical use. The second, teicoplanin, is licensed in Europe but not the United

States. Since the late 1980s, the prevalence of vancomycin resistance in organisms belonging to the genus *Enterococcus* (*vancomycin-resistant enterococci*, or VRE) has risen alarmingly. VRE frequently are highly resistant to many other antibiotics as well, and reported mortality from infection caused by these resistant organisms exceeds 30 percent.[71,262,359]

The appearance of VRE occurred coincident with an increased use of vancomycin in the United States and the use of the glycopeptide avoparcin as a growth promoter in animal feed in Europe, a practice banned by the European Union in 1997.[265] The incidence of VRE colonization and disease is much more prevalent in the United States than in other areas of the world,[72,223] but these organisms are present globally.[156,422] Approximately 10 to 20 percent of U.S. enterococcal isolates investigated in surveys in the late 1990s and early 2000s were resistant to vancomycin.[72,223] A disproportionate number of VRE in the United States are found in patients hospitalized in ICUs in large urban medical centers, especially among persons with prolonged hospitalizations and severe disease.[140,223,224,359] Additionally, a high incidence of VRE is seen among oncology patients,[90,238] transplant recipients,[89,251] and those with chronic renal failure who are receiving dialysis.[11,99,407] Some surveys have documented VRE among adults in chronic care facilities, who exported the organism into the community after acquisition in an acute care setting.[395] By contrast, the prevalence of VRE is relatively low in U.S. hospitals serving rural areas.[310,376]

Surveys of European centers also indicate a concentration of VRE in ICUs but in substantially smaller numbers compared with the United States.[350] A survey of resistance patterns among thousands of European hospital isolates collected during the early 2000s indicated that only 6 percent of enterococci expressed resistance to vancomycin.[337]

The genetic machinery conferring vancomycin resistance in *Enterococci* is complex, and resistance does not develop de novo by spontaneous mutation under antibiotic pressure.[265] The initial event in the appearance of VRE in a given unit is the importation of the resistant organism into the environment. The association between antecedent treatment with vancomycin and acquisition of VRE has been documented by many,[104,359] but not all, investigators.[38,81] Some researchers have established a stronger connection between the appearance of VRE and the use of extended-spectrum cephalosporins[24,283] and antibiotics with potent anti-anaerobic activity.[224] A study conducted in a Veteran's Affairs hospital reported that the stool density of VRE in patients who were treated with anti-anaerobic antibiotics increased exponentially, whereas the density decreased in those whose antibiotics had limited anaerobic activity.[80] The principal role of antecedent antibiotic exposure in VRE may be to open up ecologic niches in the gastrointestinal tract that allow a stable presence of VRE once the patient is exposed to the resistant organism.

Horizontal transmission of VRE is prominent in any closed environment and may outweigh antecedent antibiotic exposure as the principal determinant for acquisition.[24,140] Patient-to-patient spread in an ICU, presumably through the hands of caregivers, has been documented repeatedly, as has contamination of the immediate environment of the VRE-positive patient.[283,364] Indeed, perhaps the most significant risk factor for acquisition of VRE is the number of neighboring patients colonized with the organism at the same time.[12,24,60]

In many U.S. ICUs, multiple clones of VRE are present at any given moment, some imported into the unit and others spread after admission has occurred,[283] thus rendering control and containment very difficult to achieve. Extreme barrier isolation precautions, including surveillance for VRE on admission to a given unit, single-room isolation with dedicated medical instruments, and strict use of gowns and gloves, frequently are necessary to contain VRE once it has established an endemic presence, and even then these measures may not be entirely effective.[174,207,364]

With some exceptions,[124,250,396] to date VRE have been less a threat to pediatric patients than to adults, despite the wide use of vancomycin among hospitalized pediatric patients and their geographic proximity in many hospitals to sick adults colonized with the organism. Several outbreaks of VRE in the newborn nursery have been reported,[213,227] but they have resulted primarily from dissemination of a single clone within the unit and have been successfully eradicated with implementation of relatively simple barrier isolation and cohorting measures.

In the latter half of the 1990s, a second phenomenon involving reduced susceptibility to vancomycin was identified, namely, vancomycin-intermediate *S. aureus* (VISA).[103,367] These organisms express MICs to vancomycin in the intermediate range of approximately 8 μg/mL. Virtually all such isolates are coresistant to methicillin, but several have retained susceptibility to numerous other antimicrobial agents. To date, VISA isolates have been identified primarily in patients who have been treated with prolonged courses of vancomycin for MRSA infection. Disproportionate numbers of these patients have been undergoing dialysis.[367] Eradication of VISA has been difficult but achievable with high doses of vancomycin in combination with other antibiotics.[103,367] At present, infection with VISA is a rare occurrence, both in adults and in children.

In 2002, the first two cases of true vancomycin-resistant *S. aureus* (VRSA) in the United States were reported, one from Michigan and the other from Pennsylvania (MIC to vancomycin >128 μg/mL and =32 μg/mL, respectively).[398,399] Both patients were suffering from chronic foot ulcers from which the organisms were isolated. In both instances, the bacteria were susceptible to alternative agents, and the patients remained clinically stable. The potential for VRSA to emerge as a major public health hazard is substantial, but subsequent cases have been rare.[400]

Mechanisms of Resistance

By 2006, six genotypes for vancomycin resistance in *Enterococci* had been defined, labeled VanA through VanE and VanG, each associated with phenotypic nuances.[57,265] These genotypes differ in the species of enterococci affected, the relative resistance conferred to vancomycin and teicoplanin, and whether they are inducible or constitutively expressed (Table 246–4). The VanC phenotype is distinct from the others in that it is an intrinsically expressed, species-specific product of *Enterococcus gallinarum*, *Enterococcus casseliflavus*, and *Enterococcus flavescens*.[268] The other vancomycin phenotypes are acquired, although VanD resistance and VanE resistance are not readily transferable.

In susceptible bacteria, the glycopeptides are large molecules that interfere with cell wall peptidoglycan synthesis at a point proximal to that targeted by the β-lactam antibiotics. Specifically, the synthesis of peptidoglycan in vancomycin-susceptible bacteria includes the incorporation of a D-alanyl-D-alanine dipeptide onto the carboxyl end of the growing glycopeptide chain to form a pentapeptide precursor. The pentapeptide is translocated from the cytoplasm to the outer surface of the bacteria.[57] The D-Ala-D-Ala carboxy-terminal end of the pentapeptide is critical for the continued synthesis of the peptidoglycan chain and, ultimately, for peptidoglycan cross-linking and the formation of a stable cell wall. Vancomycin, which never enters the cell, binds to the D-Ala-D-Ala moiety of the pentapeptide through several hydrogen bonds along the outer surface of the cell wall,[16] thereby blocking synthesis of the remaining macromolecule.

The molecular basis for vancomycin resistance results from the complex, coordinated interactions of multiple genes usually encoded on a single genetic element. These interactions have been defined best for the VanA phenotype, although

TABLE 246–4 Characteristics of the Types of Resistance to Glycopeptide Antibiotics Found in Enterococci

Characteristics	Type					
	VanA	VanB	VanC	VanD	VanE	VanG
Genetic characteristics	Acquired	Acquired	Intrinsic	Acquired	Acquired	Acquired
Terminus of peptidoglycan Precursor	D-Ala-D-Lac	D-Ala-D-Lac	D-Ala-D-Ser	D-Ala-D-Lac	D-Ala-D-Ser	D-Ala-D-Ser
Minimal inhibitory concentration (μg/mL)						
Vancomycin	64->1000	4->1000	2-32	16-64	16	16
Teicoplanin	16-512	0.5->32*	0.5-1	2-4	0.5	0.5
Ligase gene	*vanA*	*vanB*	*vanC-1* and *vanC-2/*	*VanD* *vanC-3*†	*VanE*	*VanG*
Enterococci found to have these genes for resistance in nature	*E. faecalis*, *E. durans*, *E. mundtii*, *E. avium*, *E. gallinarum*, *E. casseliflavus*, *Bacillus circulans*	*E. faecalis*, *E. faecium*	*E. gallinarum* and *E. casseliflavus*	*E. faecium* *E. flavescens*‡	*E. faecalis*	*E. faecalis*

Most vanB-*containing isolates are susceptible to teicoplanin on testing, but the development of resistance in vivo and in vitro has been documented.*
†*The* vanC-3 *gene is 98 percent identical to* vanC-2.
‡E. flavescens *is probably the same species as* E. casseliflavus.

genetic homologues are found in organisms expressing the other vancomycin-resistant phenotypes. The genetic machinery for VanA resides on the 10,851-bp transposon Tn*1546*.[7,409] The molecules central to conferring the resistant phenotype are encoded by the contiguous genes labeled *vanH*, *vanA*, and *vanX*, all of which are essential for expression of vancomycin resistance. The *vanA* gene encodes a ligase that, unlike the native enterococcal ligase, produces an abnormal D-alanyl-D-lactate dimer over the normal D-alanyl-D-alanine.[32] This process results in the formation of the peptidoglycan precursor uridine diphosphate–MurNAc–L-Ala-D-Glu-L-Lys-D-Ala-D-Lac, the C-terminus of which has profoundly reduced affinity for vancomycin.[33] The homologous ligase in VanC-, VanE-, and VanG–expressing organisms synthesizes D-Ala-D-Ser (see Table 246–4) with similar consequences.[57,265] VanH catalyzes the reaction taking pyruvate to D-Lac and increases the availability of this substrate to VanA for production of the D-Ala-D-Lac dimer.[33] *vanX* and a second gene labeled *vanY* encode a cleaving enzyme for the D-Ala-D-Ala dipeptide, thus reducing the natural cellular substrate for the D-Ala-D-Ala adding enzyme and resulting in greater relative incorporation of D-Ala-D-Lac into the peptidoglycan precursor.[57,323]

The *vanS* and *vanR* genes included on the same vancomycin-resistance transposon regulate expression of resistance. The products of these genes are homologous to other compounds of so-called two-component regulatory systems.[397] Ultimately, the result is transcription of the resistance genes in the presence of vancomycin.[57] The functions of the last two gene products, VanY and VanZ, are not essential for expression of the resistant phenotype.[8,9]

Between 2002 and 2004, five adult patients were described who had fully vancomycin-resistant MRSA infections from *S. aureus* harboring the TN*1546* transposon found in VRE.[8,9,398-400] This finding indicated that true VRSA could result from the conjugal transfer of the vancomycin-resistance transposon from VRE to MRSA, a phenomenon that can be replicated in vitro.[274] By contrast, VISA isolates do not contain the resistance genes found in VRE; rather, these isolates acquire unusual extracellular material around the cell wall as detected by electron microscopy.[34,367] This layer represents accumulated peptidoglycan with reduced levels of cross-linking, thereby exposing an excess of D-Ala-D-Ala moieties that bind to vancomycin before it can reach the cell wall.[410] Other vancomycin-resistance phenomena in *S. aureus* have been described and include those in which subpopulations express MICs two- to eightfold higher than those in the parent population (termed *heteroresistance*),[103,183] as well as those in which *S. aureus* can be inhibited but not readily killed by vancomycin (termed *tolerance*).[240] Some investigations indicated that isolates of *S. aureus* expressing vancomycin heteroresistance develop into VISA with continued exposure to the drug.[410]

RESISTANCE TO OTHER ANTIBIOTICS USED FOR GRAM-POSITIVE BACTERIAL INFECTIONS

Two antibiotics introduced in the early 2000s, namely, linezolid and daptomycin, were developed to address the increasingly important problem of antibiotic-resistant, gram-positive pathogens, especially VRE and MRSA. In both instances, the antibiotic represented a novel class of molecules, in the hope that resistance would be slow to develop.

Linezolid, the first of the oxazolidinone antibiotics, has activity against all important human gram-positive pathogens, including MRSA, VISA, and VRE. The molecular target of the oxazolidinones is domain V of the 23S rRNA of the 50S bacterial ribosomal subunit. Binding to this domain results in the inhibition of the initial steps of translation.[381] Linezolid's binding site is unique to the oxazolidinones, and cross-resistance to other antiribosomal antibiotics does not occur. Because the oxazolidinones are entirely synthetic, likely no significant natural resistance to this class of drugs existed before the introduction of linezolid.[253] To date, resistance to linezolid in vivo has been a rare occurrence. Multinational surveys conducted throughout the first decade of the 2000s have identified remarkably few linezolid-resistant organisms, despite the widespread use of the agent.[68,179,273] The few linezolid-resistant clinical isolates that have been encountered have been derived primarily from enterococci and *S. aureus*, usually after prolonged exposure to drug.[25,423] All linezolid-resistant organisms possess a mutation in the gene encoding domain V of the 23S rRNA.[253] Most bacteria have multiple copy numbers of this gene. The degree of resistance is gene-dose–dependent, such that the MIC of linezolid usually remains in the susceptible range after a single mutation occurs but increases incrementally as each allele is affected.[253]

Daptomycin, a fermentation product of *Streptomyces roseoporus*, is a unique antimicrobial with activity against a full range of gram-positive bacteria. The compound is a cyclic lipopeptide that inserts into the lipid bilayer on the bacterial cell surface. In the

presence of calcium, this event disrupts the transmembrane electrical potential with a secondary interruption of ATP and macromolecular synthesis.[136,372] As with linezolid, laboratory surveys of large numbers of gram-positive clinical isolates from many areas of the world indicate that daptomycin resistance is rare.[335,336] Soon after daptomycin was introduced in the early 2000s, however, several cases were reported documenting the emergence of *S. aureus* with reduced susceptibility to daptomycin during prolonged therapy.[141,230,236] In these cases, the infection emanated from a bony or endovascular focus into which penetration of the drug may have been limited. The mechanism of daptomycin resistance remains undefined. Some investigators mapped the resistance phenotype to selected genetic foci in *S. aureus*, at least one of which encodes a protein important in maintaining the bacterial cell membrane.[107] One report correlated vancomycin-intermediate resistance in *S. aureus* with reduced susceptibility to daptomycin,[59] and the investigators hypothesized that the accumulated molecules surrounding the cell wall in VISA protected the bacteria from the effects of daptomycin much as it does with vancomycin.

QUINOLONES

Clinical Relevance

The quinolone family of antibiotics possesses activity against a broad variety of microorganisms. As of the early 21st century, the quinolones had not been labeled for pediatric use. However, the safety profile in children of the more recently developed quinolones (i.e., the fluoroquinolones) is similar to that recorded in adults,[114,173,344] and this class of antibiotics has been effectively employed in selected children with serious infections caused by bacteria resistant to alternative agents. The following subsections discuss clinical circumstances in which quinolone resistance is relevant to pediatric practice.

CYSTIC FIBROSIS

The lungs of patients with cystic fibrosis (CF) are chronically infected early in life, primarily with *S. aureus* and later with *P. aeruginosa*. Exacerbations of pulmonary symptoms are treated effectively with antibiotics directed against these microorganisms. Ciprofloxacin represents the first oral agent with significant anti-*Pseudomonas* activity and manifests potency generally superior to that of the other fluoroquinolones.[304] As a result, experts in many centers treat patients with CF with ciprofloxacin, even when the recipients are younger than 18 years of age.[114] In most circumstances, pediatric patients with CF treated with ciprofloxacin have experienced improvement in their respiratory status.[345]

Pseudomonas surveys conducted with organisms derived from patients with CF as well as those without CF indicated susceptibility to ciprofloxacin in approximately 50 to 90 percent of strains; CF isolates tended to express resistance more frequently than did other isolates.[39,102,123,143,232,340,358] Several investigators documented that decreased susceptibility of *Pseudomonas* lung isolates to ciprofloxacin occurs frequently during the course of ciprofloxacin treatment in patients with CF.[82,371] Indeed, high proportions of *Pseudomonas* strains infecting the lungs of patients with CF are "hypermutators," particularly prone to spontaneous mutation resulting from defects in genetic repair mechanisms,[280] that allow the ready emergence of antibiotic resistance. However, as a general rule, patients who improve clinically during therapy for an acute pulmonary exacerbation do not eradicate the infecting organisms, and the importance of emergence of resistant bacteria during therapy versus any of the commonly employed antibiotics is uncertain, as long as the infecting organisms were susceptible initially.[365] Moreover, frequently the *Pseudomonas* reverts back to a quinolone-susceptible phenotype several weeks after treatment with ciprofloxacin has been completed.[75,82] As a result, ciprofloxacin currently appears to be a useful option for patients with CF who have worsening pulmonary disease, if at least one of the organisms in the initial sputum culture is susceptible.

The usefulness of ciprofloxacin in the patient with CF who is infected with *S. aureus* also probably depends on the susceptibility of the pre-treatment isolates. A worldwide survey conducted in the late 1990s indicated that most methicillin-susceptible strains of *S. aureus* also were susceptible to ciprofloxacin and other fluoroquinolones. However, the fluoroquinolone susceptibility among methicillin-resistant strains was less than 20 percent, and susceptibility was extraordinarily low to ciprofloxacin in particular.[102,128,151]

Enteric Gram-Negative Bacilli: Uropathogens, Nosocomial Bacteria, and Agents of Bacterial Enteritis

Quinolone resistance in enteric gram-negative rods, particularly among uropathogens, has been an uncommon occurrence in North America. Studies of enteric urinary tract isolates in the United States and Canada documented susceptibility rates to ciprofloxacin of 95 percent or greater during the 1990s and 2000s among nonelderly patients,[438] a finding supporting the effectiveness of quinolones for community-acquired cystitis and uncomplicated pyelonephritis.[86,98,129,130,210,243,383] In contrast, in some regions of Europe, the incidence of quinolone resistance among uropathogens has increased as administration of this class of antibiotics has become more widespread.[110,122,155] A 1998 survey of urinary tract isolates from Latin America indicated an even more dramatic occurrence of quinolone resistance: 22 percent of *E. coli*, 30 percent of *P. mirabilis*, and more than 35 percent of *Enterobacter* were ciprofloxacin-resistant.[110] Quinolone resistance in gram-negative urinary pathogens frequently is associated with resistance to other classes of antibiotics as well.[189] Consequently, several experts continue to recommend TMP-SMX over the fluoroquinolones as the first-line agent for uncomplicated UTIs, because clinical and microbiologic efficacy is nearly equivalent for the two classes of drugs, and routine use of quinolone will needlessly promote quinolone resistance.[98,153,413]

The quinolones traditionally have been mainstays of treatment for nosocomial infections in adult patients with severe illness. During the late 1990s and early 2000s, however, quinolone resistance in nosocomial gram-negative pathogens increased in many areas of the world among both Enterobacteriaceae and non–lactose fermenters.[105,181,188,261,273] In several instances, quinolone resistance in gram-negative bacilli increased more rapidly than any other resistance phenotype.[105,181] Many of these hospital-acquired pathogens co-express ESBLs and other resistance determinants.[6,30,292]

Antibiotic therapy, including treatment with quinolones, for bacterial enteritis usually is not indicated.[425] That said, with the exception of *Campylobacter*, most agents that cause bacterial enteritis have remained susceptible to the quinolones by in vitro testing,[121] although epidemic strains of both *Shigella* and *Salmonella* expressing quinolone resistance have been identified in Asia.[109,295,384] *Salmonella typhimurium* DT104, which spread rapidly throughout Europe and the United States in the late 1990s, is resistant to nalidixic acid, but most isolates (except for some identified in the United Kingdom) are susceptible to the newer fluoroquinolones.[108,121,142,257] Similarly, surveys of other non-*typhi Salmonella* conducted in the early 2000s indicated that few were ciprofloxacin-resistant, although as many as one fifth of the strains had reduced susceptibility to the drug.[131,233,375]

In contrast to resistance among other enteritis-associated bacteria, quinolone resistance among *Campylobacter* isolates from many parts of the world has become prominent and high level.[147,171,302,427] *Campylobacter* can acquire quinolone resistance

shortly after exposure to the antibiotic. Indeed, clinical relapse with quinolone-resistant organisms after therapy for *Campylobacter* enteritis is relatively common.[302,427] The introduction of quinolones into poultry feed for animals bred for food has exacerbated the spread of *Campylobacter* resistance in humans.[271,366] In chickens obtained from several different supermarkets in Minnesota in the mid-1990s, nearly 90 percent of samples were culture-positive for *Campylobacter*, and approximately 20 percent of these samples were resistant to ciprofloxacin.[366] These circumstances prompted a Food and Drug Administration ruling withdrawing the use of quinolone in poultry in 2005. It is likely, however, that quinolone-resistant *Campylobacter* has found a stable ecologic niche in both animals and humans and will continue to be encountered despite this intervention.[271] Unlike most other bacteria, *Campylobacter* becomes highly resistant to the quinolones after a single mutation,[119,412] so most quinolone-resistant clinical isolates demonstrate MICs of 32 μg/mL or more, much higher than the conventional cutoff for defining quinolone resistance.

COMMUNITY-ACQUIRED RESPIRATORY TRACT INFECTIONS

The applicability of quinolones to respiratory tract infections has been shadowed by the marginal activity of the first modern quinolones, namely, ciprofloxacin, ofloxacin, and norfloxacin, against *S. pneumoniae*. MICs of these antibiotics against most clinical isolates of *S. pneumoniae* average 1 to 2 μg/mL, concentrations that are close to those achievable in bronchial secretions.[304] Several reports of clinical treatment failures were reported in which ciprofloxacin was administered in pneumococcal disease in the respiratory tract.[201,211,296] Moreover, even when *S. pneumoniae* was the intended target of therapy, the wide use of the early quinolone agents in treating community-acquired infections during the 1990s resulted in the inadvertent exposure of respiratory tract colonizers, including *S. pneumoniae*, to these agents; the result was an even greater, albeit gradual, increase in the MICs of community-acquired *S. pneumoniae* isolates to ciprofloxacin.[43,97,177,339] By the mid-2000s, surveys recorded a prevalence of ciprofloxacin resistance (MIC ≥ 4 μg/mL) in *S. pneumoniae* of approximately 2.5 percent in the United States,[78,326] 4 percent in Canada,[2] and 15 percent in Italy.[69]

Most of the more recently introduced fluoroquinolones are substantially more potent against *S. pneumoniae* than is ciprofloxacin[203] and express MICs that are easily achieved in the respiratory tract. Usually, susceptibility is maintained, even in isolates resistant to the older quinolone agents.[180,304,327] However, MICs against the newer quinolones roughly correlate with those expressed against ciprofloxacin.[43,177,180,327] Consequently, clinically relevant resistance also has begun to emerge against these newer agents, particularly levofloxacin.[2,69,308,326] To date, susceptibility of other respiratory tract pathogens, including *H. influenzae*, *M. catarrhalis*,[22,166,304] and the bacterial agents causing atypical pneumonias, remains very good to all the fluoroquinolones, although exceptions, particularly in *Haemophilus* spp.,[269,435] have been reported.

Mechanisms of Resistance

The two principal molecular targets of the quinolone antibiotics are the topoisomerases DNA gyrase and topoisomerase IV. These enzymes mediate the three-dimensional topologic configuration of the bacterial duplex DNA ring during cell division and transcription and allow the repair and replication of nucleic acid and the separation of daughter chromosomes in the cramped intracellular environment to occur.[139,148] Several reactions have been identified that are mediated by these two bacterial DNA topoisomerases, including negative supercoiling and catenation.[149,379] Both DNA gyrase and topoisomerase IV are composed of two nonidentical subunits. In DNA gyrase, these subunits are labeled GyrA and GyrB, which are constructed into an A_2B_2 tetramer.[149,379] Quinolones bind to the GyrA subunit of the DNA gyrase-DNA-ATP complex and prevent further progress of the enzyme along the nucleic acid.[149] Bacterial cell death is enhanced by the release of toxic DNA ends from the quinolone-gyrase DNA complex.[139] Topoisomerase IV is composed of two equivalent subunits, labeled ParC and ParE, which share sequence homologies to GyrA and B, respectively.[150]

Quinolone resistance is conferred through several molecular mechanisms. Most organisms resistant to the fluoroquinolones demonstrate one or more mutations in DNA gyrase, topoisomerase IV, or both. Resistance to quinolones among gram-negative bacilli usually is mediated through mutations in DNA gyrase, whereas resistance in gram-positive organisms is the result of alterations of topoisomerase IV.[139,288,443] However, this pattern varies from organism to organism (e.g., the primary target in *S. pneumoniae* appears to be DNA gyrase) and from quinolone to quinolone.[139,150] In gram-negative bacilli, most first-step resistance-conferring mutations occur in *gyrA*, the gene encoding the GyrA subunit. These mutations are clustered in a segment termed the *quinolone-resistance determining region* (QRDR).[303,342,433,434] Hybrid DNA gyrase molecules composed of the A subunit from a resistant bacterial strain and the B subunit from a susceptible isolate confer quinolone resistance.[37] Within the QRDR, most mutations have involved the substitution of a serine for a bulkier, nonpolar amino acid at a single, specific site. This mutation, first identified in *E. coli*,[405,433] has been documented since in a variety of organisms.[64,164,202,258,314] Other amino acid substitutions near this site also have been described, with multiple-site mutations resulting in increased quinolone resistance compared with a single mutation alone.[405] Mutations of the GyrB subunit, which result in a quinolone-resistant phenotype, also have been identified. In general, mutations affecting the GyrB subunit result in lower levels of resistance than those noted with GyrA.[434] As with GyrA mutations, those affecting GyrB preferentially localize to specific amino acids.[434]

In most quinolone-resistant, gram-positive organisms, mutations of topoisomerase IV occur at locations that are the equivalent to those noted in DNA gyrase. First-step mutations most frequently are noted in *parC*, which, like *gyrA*, contains a hot-spot segment around which most resistance-conferring mutations occur.[149,150,303] *GyrA* mutations in these organisms frequently are clinically silent unless they occur concomitantly with a mutation in *parC*.[149]

Three additional mechanisms of quinolone resistance, which may appear together and in conjunction with a topoisomerase mutation in the same cell, have been identified. Two of these additional mechanisms result in diminished intracellular accumulation of drug. The first mechanism involves decreased entry of antibiotic into the cell secondary to alterations in porin composition, a mutation that generally results in only moderate drug resistance and that affects individual quinolones differentially, depending on their hydrophilicity.[342] Particular attention has centered on alterations in outer member protein F (OmpF).[152,440] This mutation, identified principally in *E. coli*, frequently is associated with resistance to other classes of antibiotics as well, including chloramphenicol, tetracycline, and some of the β-lactams. The diminished expression of OmpF in these resistant isolates does not appear to result from mutations in the corresponding structural gene or its regulatory sequences. Rather, the decreased expression of this outer-membrane protein is mediated through the enhanced expression of the gene *micF*, which transcribes an antisense RNA complementary to the 5′ end of the *ompF* message,[5] thereby destabilizing its binding to the ribosome. Occasional mutants involving additional strains of Enterobacte-

riaceae have demonstrated other alterations in outer-membrane porin composition.[37]

In the second mechanism, many quinolone-resistant species mediate their resistance through increased drug efflux.[215,216,235] Both bacterial and eukaryotic cells normally encode several efflux pumps that provide protection from a range of potential exogenous toxins.[294] The overexpression of an efflux pump resulting from the mutation of a controlling sequence accounts for reduced susceptibility to quinolones in many clinical isolates.[168,235] Individual efflux pumps select substrates based on their physical chemical properties (e.g., hydrophobicity and net charge), rather than their gross structural similarities; therefore, the various quinolone compounds, which differ from one another regarding these properties, may be differentially susceptible to organisms expressing these pumps.[294]

The gene encoding the quinolone efflux pump in *S. aureus*, labeled *norA*,[432] has been cloned and sequenced. Transformation of susceptible bacteria with the putative gene results in a resistant phenotype, for which resistance can be abolished by the addition of inhibitors of energy-dependent cellular pumps.[432] This pump, however, appears to be able to transport only hydrophilic quinolones; intracellular accumulation of the hydrophobic drug sparfloxacin, for example, is unaffected.

A range of additional genes encoding efflux pumps capable of producing quinolone resistance has been identified in other species, such as *prmA* in *S. pneumoniae*,[303] *acrAB* in *E. coli*,[168,228] and the *mexAmexB-oprM* complex in *P. aeruginosa*.[237] This third complex gene encodes components that transport the drug through both the cell wall and across the periplasmic space, as well as an outer-membrane protein.[294] Most of these pumps are able to extrude a wide variety of exogenous substances, such as detergents, dyes, and other antibiotics, from the bacterial cytoplasm.[168] Therefore, reduced susceptibility to quinolones may be selected by exposures unrelated to the quinolones themselves.

The first decade of the 2000s has witnessed the rapid emergence of transmissible plasmid-mediated quinolone resistance, the third mechanism. All previously defined mechanisms of quinolone resistance were chromosomal. Organisms exhibiting the transmissible mechanism have been identified in Asia, Europe, and North America.[329] The products encoded by these plasmids have been labeled *Qnr proteins*, a group of molecules that bind to topoisomerase and protect it from the inhibitory effects of the quinolones.[275,329] Although all the Qnr molecules described to date belong to the so-called pentapeptide-repeat family of proteins, a finding suggesting similar three-dimensional conformations, distinct families of Qnr proteins with largely nonhomologous amino acid sequences have been characterized.[275,329] These families have been named QnrA, QnrB, and QnrS, with subtypes identified within each. The variety of plasmid and integron structures carrying Qnr proteins and the multiplicity of sequences of the proteins themselves support the notion that Qnr-mediated resistance arose through many independent molecular events in geographically distinct regions of the world. The plasmids encoding the Qnr proteins have been found in multiple species belonging to the Enterobacteriaceae, but to date they have not been identified in nonenteric bacteria such as *Pseudomonas* and *Acinetobacter*.[329] Qnr plasmids frequently carry other antibiotic-resistance genes, most notable those encoding ESBLs and the aminoglycoside-modifying gene *aac(6′)-Ib-cr*, a gene that is able to acetylate selected quinolones as well.[329] The plasmids also may carry resistance determinants against rifampin, tetracycline, sulfonamides, and chloramphenicol.[275]

Quinolone resistance occurs incrementally as the organism acquires a series of topoisomerase mutations, altered outer-membrane proteins, overexpressed efflux pumps, and Qnr proteins.[92] Thus, in gram-negative species, mutations of *gyrA* usually occur in first-step mutants producing relatively small increases in the MIC and frequently result in bacteria that still are susceptible to the antibiotic. Additional mutations to *gyrA*, or added mutations to *parC* or the overexpression of an efflux pump, which may in themselves confer undetectable or only minor resistance to the quinolones, result in substantial quinolone resistance when they operate in concert.[150,342] Therefore, choosing a fluoroquinolone that can achieve a sustained concentration at the site of infection several-fold higher than the MIC may suppress the emergence of resistance, because this concentration is sufficient to suppress growth of both the parent organism and first-step mutants.[87,342] The propensity of a bacterial strain to emerge resistant to the quinolones can be quantified empirically by measuring the *mutant prevention concentration* (MPC), that is, the lowest concentration of quinolone required to suppress the emergence of resistant subpopulations among a high inoculum (10^{10}) of bacteria.[87,139] The acquisition of each resistance determinant results in an increase in MPC disproportionate to the increase in MIC, a finding indicating that ever-increasing concentrations of drug are required to halt the escalation of resistance as new resistance mechanisms are gained.[139,168]

CHLORAMPHENICOL

Clinical Relevance

In industrialized nations, chloramphenicol has been supplanted by other agents with similar or improved antibacterial potency and less toxicity. However, the antibiotic remains a staple for pediatric bacteremia and meningitis in the developing world because of its broad antibacterial activity, low cost, favorable achievable serum concentrations after oral or intramuscular administration, and excellent penetration across the blood-brain barrier. The incidence of resistance to chloramphenicol among the principal organisms causing bacteremia and meningitis in children, namely, *H. influenzae*, *S. pneumoniae*, *N. meningitidis*, and *Salmonella*, varies greatly by geographic location. Between the late 1980s and the early 2000s, surveys of *H. influenzae*, both type b and non–type b, were completed in several areas of the world. In vitro resistance to chloramphenicol ranged between 0 percent of isolates (from the Central African Republic and various locations in South America) and more than 50 percent (in India and Kenya).[41,116,176,196,282,332,338,351,363,416] Studies in 2001 and 2003 of *H. influenzae* isolates in Canada and the United States, where infection by these organisms is almost always treated with an alternative agent, indicated chloramphenicol resistance in fewer than 0.5 percent of tested bacteria.[146,441]

International surveys completed in the mid- to late-1990s that measured in vitro susceptibility of *S. pneumoniae* to chloramphenicol indicated resistance exceeding 10 to 20 percent in many areas of the world.[167,212,282,332] Chloramphenicol resistance was recorded in approximately 10 percent of European isolates of *S. pneumoniae* and in 5 percent of isolates identified in the United States and Canada.[146] Resistance to chloramphenicol is seen more commonly and is of a higher grade in penicillin-resistant pneumococci than in isolates that are penicillin-susceptible.[146] Even in penicillin-intermediate or resistant strains that retain in vitro susceptibility to chloramphenicol, the clinical response to chloramphenicol in meningitis is poor. Some investigators have suggested that such bacteria express high minimal bactericidal concentrations to chloramphenicol; as a result, concentrations of drug achieved in the cerebrospinal fluid with routine dosing are lower than those required for good outcomes in central nervous system infections.[106]

Chloramphenicol retains excellent activity against most strains of *N. meningitidis*. However, investigators described meningococcal strains from Vietnam, Paris, and Australia that possessed

high-level resistance to chloramphenicol.[111,360] Chloramphenicol resistance in *Salmonella* spp., a fourth group of organisms that causes disseminated disease and meningitis in pediatric patients, varies geographically. Resistance, with a prevalence frequently exceeding 50 percent, usually is detected in developing countries,[187] where chloramphenicol has been a readily available oral agent for typhoidal and nontyphoidal *Salmonella* infections for decades. In most *Salmonella* isolates, chloramphenicol resistance occurs coincident with resistance to multiple other antibiotics, particularly ampicillin, sulfonamides, tetracycline, and some of the older aminoglycosides. Chloramphenicol resistance has become increasingly common in industrialized nations as well.[55,369,415] In the United States, the strain *Salmonella typhimurium* DT104, which similarly co-expresses resistance to several classes of antibiotics including chloramphenicol, has become a widespread health hazard.[1,406]

Mechanisms of Resistance

The antibacterial activity of chloramphenicol is conferred by its interference with the peptidyl transferase region of the 50S prokaryotic ribosome. The principal mechanism of resistance is through the expression of the modifying enzyme chloramphenicol acetyltransferase (CAT), which catalyzes the acetylation of the C3-hydroxy group of the drug and thereby prevents its binding to the bacterial ribosome.[357] CATs actually represent a family of enzymes. Many CATs share chemical properties and structural homologies, especially around the active site.[357] CATs frequently reside on plasmids or transposons that carry other antibiotic-resistance determinants.[408] In *Salmonella* in particular, the chloramphenicol-resistance gene frequently is encoded on a class 1 integron incorporated in the so-called *Salmonella* Genomic Island 1 (SGI1), which includes multiple antibiotic-resistance cassettes.[83,263] SGI1 is transmissible and has been identified in many different *Salmonella* serovars.

Alternative mechanisms of resistance to chloramphenicol also have been identified in certain gram-negative species. In most of these organisms, resistance is linked to decreased intracellular accumulation of drug. In chloramphenicol-resistant *Pseudomonas*, this phenomenon has been associated with the overproduction of an approximately 50-kd outer-membrane protein,[216] which in the past led to the speculation that entry of drug into the bacterium was diminished by an abnormal porin. More recently, however, cell membrane proteins of this size have been identified as components of complex efflux apparatus that include both the pump itself and proteins that channel the extruded substance across the periplasmic space and through the outer membrane.[294] More direct evidence of such multidrug-resistance efflux pumps have been identified in chloramphenicol-resistant *E. coli*,[137] *Pseudomonas*,[216] *Stenotrophomonas*,[442] and *Salmonella*.[23,44,305] Usually, these pumps result in co-resistance to multiple other antimicrobial agents, including tetracycline, sulfonamide, aminoglycosides, quinolones, and selected detergents.

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CHAPTER 247

The Pharmacokinetic-Pharmacodynamic Interface: Determinants of Anti-infective Drug Action and Efficacy in Pediatrics

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The success of anti-infective treatment of any infection depends wholly on proper drug selection and use. Without question, consideration of in vitro data that define the offending pathogen and provide information with regard to drug susceptibility is both valuable and vital. However, consideration of in vitro data in the absence of information that determines and governs drug exposure often can result in a therapeutic act of omission sufficient to bring about treatment failure. Thus, the conjoint consideration of drug action (i.e., pharmacodynamics) and drug disposition (i.e., pharmacokinetics) is of critical

importance in the selection and evaluation of any anti-infective drug regimen.

The *pharmacokinetic-pharmacodynamic interface* reflects an association between two determinants of drug effect, delivery of drug to the site of action and the intrinsic activity of a drug to alter cellular function once it reaches that site. In much of the contemporary literature in clinical pharmacology and therapeutics, *kinetics* generally is associated with a description of the rate processes associated with drug absorption, distribution, metabolism, and excretion (ADME). Although accurate in its definition concerning the movement of drugs into and out of the body, kinetics as a determinant of drug effect must be viewed in a much broader context, specifically, the sojourn and fate of a drug molecule in both the extracellular and intracellular milieu. Although understanding this facet of drug behavior admittedly is difficult because of a relative inability in the clinical context to track the intracellular fate of a drug in humans, achieving a high degree of control over anti-infective therapy by the application of well-characterized and understood kinetic principles is quite possible.

The focus of this chapter is the pharmacologic considerations necessary for the prudent use of anti-infective agents in pediatric patients, specifically, the pharmacokinetic and pharmacodynamic principles that, if embraced, can be the key to optimizing drug treatment and maximizing therapeutic efficacy and safety. The pediatric patient presents a particular challenge to the clinician. Although pediatric practitioners generally recognize that "children are not just miniature adults," one may not appreciate that the dramatic changes associated with normal human growth and development can, and many times do, exert a profound influence on drug disposition, drug action, and, ultimately, therapeutic outcome.[109] Failure to compensate for developmental differences in pharmacokinetics in the context of therapeutic drug use in pediatrics can increase the risk of occurrence of adverse drug effects associated with either overdose (i.e., drug toxicity) or underdose (i.e., lack of efficacy).[75,109]

The following sections of this chapter present the basic principles required to understand the pharmacokinetic-pharmacodynamic interface in pediatrics, namely, general principles in pharmacokinetics, the impact of development on drug disposition, the pharmacokinetic determinants of drug action, and methods to facilitate clinical integration of the pharmacokinetic and pharmacodynamic properties necessary to optimize anti-infective therapy.

PHARMACOKINETIC DETERMINANTS OF EXPOSURE

Implicit in the production of any pharmacologic effect is the association of a drug molecule with one or more receptors and the subsequent propagation of intracellular events that ultimately translate into drug action. In general terms, successful drug-receptor interactions are characterized by three principles: (1) *avidity*, the ability of a drug to combine with a receptor; (2) *affinity*, the physical combination of a drug with a receptor, and (3) *intrinsic activity*, the ability of the drug-receptor combination to generate one or more "impulses" or "signals" capable of activating biologic effector systems. The primary determinants of both avidity and affinity reside with the maintenance of structural specificity of both the drug and receptor to ensure that an association with one or more physicochemically distinct active sites can occur. Simply stated, a drug molecule must fit into a receptor as a key fits into a lock before intrinsic activity becomes a possibility. The receptor or receptors thus will function in the role of cellular targets for drug molecules when an effective combination is required for drug effect and, ultimately, therapeutic efficacy. The pharmacodynamic implications of these targets are discussed in the second half of this chapter and in other chapters in this textbook in the context of specific anti-infective agents.

The onset and offset of drug action are determined not only by receptor-modulated intrinsic activity but also by receptor occupancy. In almost all instances, these events are both concentration- and time-dependent. Hence, the disposition of a drug in the body (e.g., ADME) becomes the "driver" for its pharmacodynamics. This action is particularly true for anti-infective drugs when the therapeutic target, the infecting organism, must be exposed to a sufficient concentration of pharmacologically active (i.e., free) drug for a period sufficient for drug binding to critical cellular elements (e.g., penicillin-binding proteins [PBPs], intracellular enzymes) to occur and for subsequent disruption of normal cellular function (e.g., inhibition of protein biosynthesis, inhibition of cell wall synthesis) to result in cellular demise. Thus, the clinical determinants of drug efficacy (and safety), such as proper selection of both dose and dosing interval relative to the intrinsic sensitivity of the infecting pathogen or pathogens, and factors that determine delivery of the drug to the site or sites of infection embody the importance of pharmacokinetics in the selection and clinical use of anti-infective drugs.

BASIC TERMS

A complete discussion of pharmacokinetics is beyond the scope of this chapter because it would require a description of theory and a presentation of relatively sophisticated mathematic concepts. This information can be found in many excellent textbooks that provide both theoretical[74] and conceptual[109,181,225] presentations of pharmacokinetics and through Internet-based programmed instruction courses.[25] However, well within the scope of this chapter are a definition and glossary of pharmacokinetic terms sufficient to equip the reader with a conceptual, working knowledge of this pharmacotherapeutic tool. These definitions and concepts are presented as follows:

Absolute bioavailability (F) is the extent or fraction of drug absorbed after extravascular administration. It is determined by comparing the area under the plasma concentration versus time curve (AUC) after administration of an oral dose of a drug with the AUC after administration of an intravenous dose (e.g., $F = AUC_{PO} \times dose_{IV}/AUC_{IV} \times dose_{PO}$).

Absorption of drugs describes the process of drug uptake from a site of extravascular administration (e.g., oral, intramuscular, subcutaneous, intraperitoneal, intraosseous, intratracheal, intravaginal, intraurethral, sublingual, buccal, rectal, and dermal) into the systemic circulation. Drug absorption is conceptualized most accurately by considering both rate (e.g., absorption half-life, time to peak concentration) and extent (e.g., bioavailability), either of which can be influenced by biopharmaceutical (e.g., drug formulation), physicochemical (e.g., pH, solubility, hydrophilicity and lipophilicity, protein binding, complexation characteristics with food or drugs), and physiologic factors (e.g., barrier integrity, motility, volume and pH of body fluids at the absorptive site, protein-binding capacity, degradation/biotransformation potential).

Area under the curve (AUC) is, conceptually, a measure of both the extent of drug absorbed and its persistence in the body. It is the integral of drug blood levels over time from zero to either a predetermined post-dose time point (i.e., $AUC_{0\rightarrow tx}$) or extrapolated to infinity (i.e., $AUC_{0\rightarrow\infty}$) by using the apparent terminal elimination rate constant (similarly calculated from the observed plasma concentration versus time plot) (Fig. 247–1). AUC is therefore a pharmacokinetic parameter that is both time- and concentration-dependent.

Bioequivalence of a drug product is achieved if its extent and rate of absorption are not significantly different (i.e., within 80% to 125%) from those of a reference standard drug product when

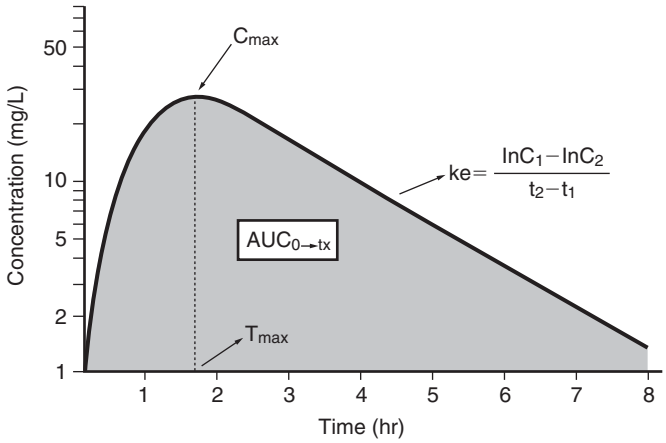

Figure 247–1 Representative plasma concentration versus time curve. Illustrated are commonly referenced pharmacokinetic parameters, including AUC, area under the curve; C_{max}, maximal plasma concentration; ke, elimination rate constant; and T_{max}, time to achieve maximal plasma concentration.

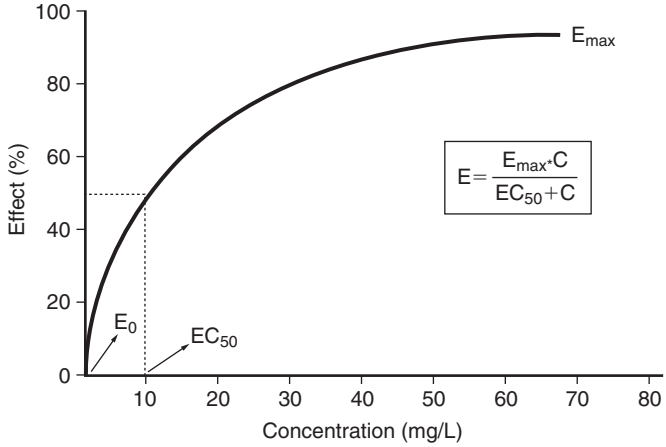

Figure 247–2 Representative nonlinear concentration (C) versus effect profile. Emax, maximal effect; EC50, drug concentration for which the observed effect is 50 percent of the maximal effect.

administered at the same molar dose. Bioequivalence is a pharmacokinetically determined parameter and does not entail a relative comparison of drug action or efficacy. Therefore, if a drug product (e.g., a generic drug) produces a rate and extent of absorption sufficiently similar to those of the reference formulation, the effect and toxicity profiles of the two drugs are assumed to be virtually identical.

Biopharmaceutics deals with the physical and chemical properties of the drug substance, the dosage form, and the body, as well as the biologic actions of a drug or drug product after administration. Biopharmaceutical considerations (e.g., rate and extent of the disintegration or dissolution of a dosage form, liberation of the active drug from a dosage form, solubility or binding of the drug at the site of absorption) can be rate-limiting for drug absorption or bioavailability, or for both, and thus may limit the efficacy of drug therapy.

Clearance (Cl) of a drug is represented conceptually by the volume of blood from which a certain amount of unmetabolized drug is removed (i.e., cleared) per unit of time by any and all pathways capable of drug removal (e.g., renal, hepatic, biliary, pulmonary, breast milk, sweat). In pharmacokinetics, clearance generally is represented as total-body (or plasma) clearance, renal clearance (Cl_{ren}), or nonrenal clearance (Cl_{nr}). Clearance is determined easily from knowledge of the drug dose and AUC and can be calculated as follows: Cl = Dose (mg/kg)/AUC (mg/L × hr), where AUC can represent either the $AUC_{0\rightarrow tx}$ for single-dose administration or the AUC from time zero to the end of the dosing interval at steady state (i.e., $AUCss_{0\rightarrow\tau}$). Calculation of renal clearance requires a complete, quantitative collection of urine (usually over the course of 24 hours) to determine the amount of drug excreted (Ae) unchanged. Nonrenal clearance generally is determined as the difference between total body clearance and renal clearance. For drug administration *by any extravascular route*, calculation of clearance yields an apparent value (e.g., Cl/F) in that it must be corrected for the extent of the drug dose absorbed (i.e., the bioavailability) from the site of administration.

A *compartment* in pharmacokinetics represents a *hypothetical space* into and out of which drug partitions as a function of time. *Compartment models* are used in pharmacokinetics to characterize the relationship between drug dose and concentration as a function of time. The most simple of all pharmacokinetic models is a *one-compartment open model* in which the only applicable rate processes represent drug ingress and egress from a single theoretical or central space. A *two-compartment model* has been used to characterize the disposition of many anti-infective agents in both pediatric and adult subjects. This particular model is composed of a central compartment and a peripheral compartment, both of which represent the various fluids and tissues where a drug may reside. Although a compartment often may not correspond to a true physiologic space that can be characterized by a specific volume of biologic fluid, compartment models can be used to conceptualize both drug distribution between physiologic spaces and elimination from the body. In the two-compartment model, for example, the central compartment often is used to represent a drug resident within the intravascular space and the highly exchangeable extracellular fluid of tissues and organs that are well perfused, whereas the peripheral compartment represents drug distribution to intracellular fluid and tissues and organs that are less well perfused and, in some instances, the association of the drug (e.g., binding) to specific tissues or tissue components. In all instances, compartmental models oversimplify the true processes of ADME. However, they have been demonstrated repeatedly to be useful as a reliable means to model, and therefore predict, the relationship between drug dose and concentration in both plasma and tissue.

Disposition refers collectively to the processes of ADME, all of which occur simultaneously after administration of a drug, as opposed to being discrete pharmacologic events.

Dose-response curve is a graphic representation of the pharmacologic effect as a function of either the drug dose or the concentration of the drug. A log dose-response curve is often sigmoidal, whereas a cartesian concentration-effect curve is hyperbolic (Fig. 247–2). Theoretically, a drug concentration of zero indicates no drug effect (i.e., E_0). As a drug travels to and interacts with a receptor, the effect (E) increases in a concentration-dependent manner (i.e., $E = [(E_{max} \times C)/(E_50 + C)]$) to a point at which a maximal effect (E_{max}) is attained and above which higher drug concentrations fail to enhance the effect. A pharmacologically important term that can be derived easily from a concentration-effect curve is the EC_{50}, or the drug concentration for which the observed effect is 50 percent of the E_{max}.

Elimination half-life of a drug is the time necessary to reduce the drug concentration (in blood serum or plasma) by 50 percent after absorption is complete and distribution between body compartments has attained equilibrium. Loss of drug from the body, as represented by the elimination half-life, reflects elimination of the administered parent drug molecule (i.e., not its metabolites)

by metabolism, urinary excretion, or other pathways capable of resulting in elimination of drug. Accordingly, many individuals use elimination half-life as a surrogate indicator of drug clearance. Such a determination should be made cautiously because this particular pharmacokinetic parameter is dependent on both clearance and the apparent volume of distribution (VD), as illustrated by the following equation: $t_{1/2}$ elimination = [(0.693 × VD)/Cl], where $t_{1/2}$ is half-life. Practically speaking, the elimination half-life is an important pharmacokinetic parameter because it can be used to determine the period of time required for a drug dosing regimen to produce steady-state plasma concentrations (e.g., 5 × $t_{1/2}$ elimination) and the dosing interval required to produce a desired excursion (i.e., peak and trough) in plasma drug concentrations.

First-pass effect describes a phenomenon whereby drugs may be metabolized or chemically degraded (or both) after extravascular administration before they reach the systemic circulation. Specific examples include biotransformation of selected drugs (e.g., cytochrome CYP3A4 substrates) in the enterocyte and nonenzymatic hydrolysis of an active drug (e.g., aspirin) in the lumen of the gastrointestinal (GI) tract (e.g., stomach, intestine, rectum). Drugs subject to a first-pass effect generally have a reduced rate or extent of relative bioavailability, or both, when compared with that achieved by parenteral administration.

Protein binding is the phenomenon that occurs when a drug (or metabolite) combines with plasma or extracellular or tissue proteins to form a drug-protein complex. In general, drug-protein binding usually is nonspecific and depends on the drug's affinity for the protein molecule (i.e., binding site), the number of protein-binding sites, and the drug and protein concentrations. With few exceptions, drugs that are bound to proteins are pharmacologically inactive and cannot be metabolized or excreted readily. The pharmacokinetic consequences of drug-protein binding can influence the drug dose versus the plasma concentration versus the effect relationship. For example, drugs with extensive tissue binding have apparent volumes of distribution that are far in excess of the total-body water space and, in general, have relatively long elimination half-lives. Conditions in which intravascular proteins escape to extravascular sites (e.g., nephrotic syndrome, severe burns, ascites) can increase the apparent volume (and elimination half-life) of drugs that are extensively (i.e., >70%) bound to albumin.

Relative bioavailability reflects the extent of drug absorbed from one dosage form given by an extravascular route of administration in comparison to a dose of a "standard" drug formulation administered by the same route. Generally, it reflects the relative extent of systemic availability (F) and is calculated by comparing the AUC of the test regimen relative to the standard formulation (e.g., $F = AUC_{test} \times dose_{standard}/AUC_{standard} \times dose_{test}$).

Steady state reflects the level of drug accumulation in the blood and tissue after multiple doses when input (i.e., the amount of drug placed into the systemic circulation) and output (i.e., drug clearance) are at equilibrium. When drugs are given at fixed doses and dosing intervals, the steady-state concentrations fluctuate between a maximum (C_{max}) and minimum (C_{min}) within a given dose interval that is identical between doses, provided that the size of the dose, method of administration, dosing interval, or drug pharmacokinetics (or any combination of these parameters) does not change. For drugs that follow first-order pharmacokinetics, steady-state plasma concentrations for a given dosing regimen are attained over a period of time that corresponds to four to five times the elimination half-life. In general, the pharmacokinetics of a drug at steady state provides the most accurate correlate for examining drug effect.

Volume of distribution (apparent volume of distribution) (VD) represents a hypothetic volume of body fluid that would be required to dissolve the total amount of a drug at the same concentration as that found in the blood and is illustrated by the following equation: $VD = Dose/Cp^0$, where Cp^0 represents the highest attainable plasma concentration after the administration of a single dose. As a proportionality constant, the VD is a determinant of plasma drug concentrations attained after administration of a given drug dose. For drugs that are not distributed extensively or that bind with great affinity to proteins and tissues, the apparent VD may correspond dimensionally to physiologic or anatomic body spaces (e.g., VD <0.1 L/kg approximates the intravascular space, 0.1 to 0.3 L/kg approximates the extracellular space, 0.6 to 0.7 L/kg approximates the total-body water space), which, when altered by disease or development, or by both, will influence the apparent VD and thus the achievable concentration for a given drug.

A working knowledge of these pharmacokinetic definitions allows one to have an understanding of the relationships among drug dose, concentration, and effect. The use of knowledge related to the physicochemical and pharmacologic properties of a drug, the impact of isolated or concurrent variables on drug disposition, and predictors of pharmacodynamics or drug response enables the clinician to optimize the selection of agents and dosing regimens and thus individualize drug therapy. To accomplish this goal, it is imperative that the relationship between drug pharmacokinetics and pharmacodynamics not be compartmentalized but rather, as illustrated in Figure 247–3, be conceptualized as multifactorial, in which the determinants of drug concentration and effect are dynamic and change as a function of a disease state and drug therapy.

IMPACT OF ONTOGENY ON PHARMACOKINETICS

Development represents a continuum of biologic events that enable adaptation, somatic growth, neurobehavioral maturation, and, eventually, reproduction. The impact of development on the pharmacokinetics of a given drug is determined, to a great degree, by age-related changes in body composition and the acquisition of function in organs and organ systems that are important in determining drug metabolism and excretion. Although classifying pediatric patients on the basis of postnatal age (e.g., neonates, ≤1 month of age; infants, 1 to 24 months of age; children, 2 to 12 years of age; and adolescents, 12 to 18 years of age) often is convenient for providing drug therapy, recognizing that the changes in physiology are not linearly related to age and may not correspond to these age-defined breakpoints is important. In fact, the most dramatic changes in drug disposition occur during the first 18 months of life, when the acquisition of organ function is most dynamic. Additionally, the pharmacokinetics of a given drug may be altered in pediatric patients as a result of intrinsic (e.g., gender, genotype, ethnicity, inherited diseases) or extrinsic (e.g., acquired disease states, xenobiotic exposure, diet) factors that may occur during the first 2 decades of life.

Selection of an appropriate drug dose for a neonate, infant, child, or adolescent requires an understanding of the basic pharmacokinetic properties of a given compound and how the process of development affects each facet of drug disposition. Accordingly, conceptualizing pediatric pharmacokinetics by examining the impact of development on the physiologic variables that govern ADME is most useful.

Drug Absorption

The rate and extent of GI absorption depend primarily on pH-dependent passive diffusion and motility of the stomach and small intestine, both of which control transit time. In full-term neonates, gastric pH ranges from 6 to 8 at birth and drops to 2 to 3 within the first few hours. After the first 24 hours of extrauterine life, gastric pH increases to approximately 6 to 7 as a result of immaturity of the parietal cells. A relative state of achlorhydria

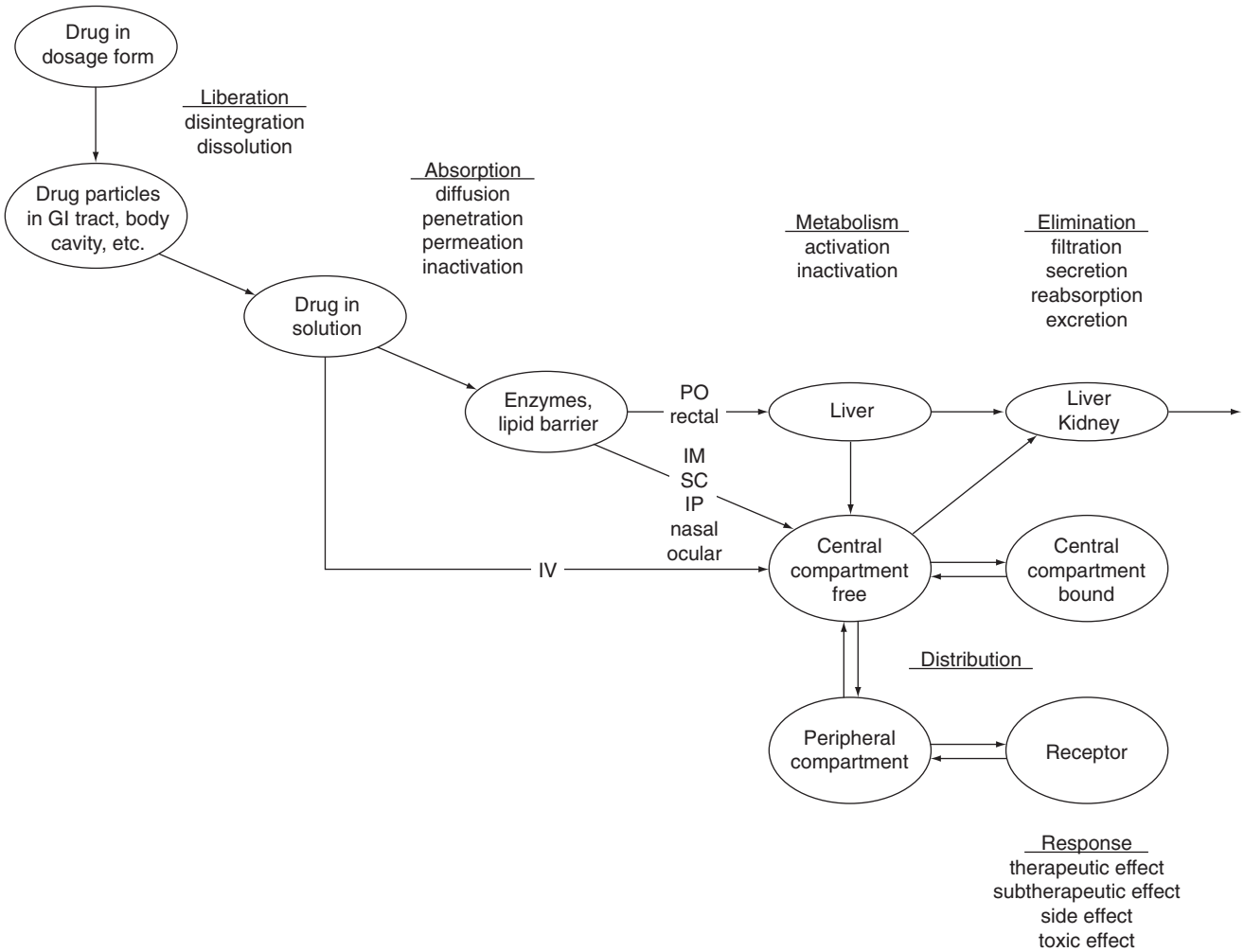

Figure 247–3 Graphic representation of the pharmacokinetic-pharmacodynamic interface. GI, gastrointestinal; IM, intramuscular; IP, intraperitoneal; PO, oral; SC, subcutaneous.

remains until adult values and diurnal patterns of gastric pH are reached at 20 to 30 months of age. Although basal gastric acid output can be quite similar in neonates, young infants, and adults, stimulated acid output can be three- to fourfold higher than that seen in adults.[52,53]

In neonates, GI transit time is prolonged as a result of reduced motility and peristalsis. Gastric emptying is both irregular and erratic and only partially dependent on feeding. Gastric emptying rates approximate adult values by the time the infant reaches 6 to 8 months of age. During infancy, intestinal transit time generally is reduced relative to adult values because of increased intestinal motility.[109] In contrast to developmental changes in motility, histologic examination of the luminal absorptive surface suggests that the adult pattern of architecture (and hence absorptive surface area relative to body size) is present at birth.[78,222] In neonates and young infants, additional factors, including diminished splanchnic blood flow, immature biliary function, variable microbial colonization, and an apparent reduction in the activity of intestinal drug-metabolizing enzymes, may play a role in intestinal drug absorption.[40,175]

These developmental changes in GI function and structure in the newborn period and early infancy produce alterations in drug absorption that are quite predictable. In general, the oral bioavailability of acid-labile compounds (e.g., β-lactam antibiotics) is increased, whereas that of weak organic acids (e.g., phenobarbital, phenytoin) is decreased. For orally administered drugs with limited water solubility (e.g., phenytoin, carbamazepine), the rate of absorption (i.e., T_{max}) can be altered dramatically as a result of changes in GI motility.[175] The pharmacokinetics of the antiviral agent pleconaril similarly provides an example of developmental differences in drug absorption. After administration of a 5-mg/kg dose, the AUC was much lower in neonates than either children or adults, thus demonstrating that the extent of pleconaril bioavailability in neonates was reduced. The reason for this difference may be attributed to the lipid-based pleconaril formulation and developmental differences in the ability to absorb lipids as a consequence of biliary immaturity and reduced lipase secretion in neonates.[112]

In newborns and young infants, both rectal and percutaneous absorption is highly efficient for properly formulated drug products. The bioavailability of many drugs administered by the rectal route is increased as a result of not only efficient translocation across the rectal mucosa but also a reduced first-pass effect caused by the immaturity of a number of drug-metabolizing enzymes in the liver. Both the rate and extent of percutaneous drug absorption are increased because of the thinner and better hydrated stratum corneum in young infants. As a consequence, systemic toxicity can occur with the percutaneous application of some drugs (e.g., hexachlorophene) to seemingly small areas of skin during the first 8 to 12 months of life. In contrast to older infants

TABLE 247–1 Summary of Drug Absorption in Neonates, Infants, and Children*

	Neonates	Infants	Children
Physiologic Alteration			
Gastric emptying time	Irregular	Increased	Slightly increased
Gastric pH	>5	4 to 2	Normal (2-3)
Intestinal motility	Reduced	Increased	Slightly increased
Intestinal surface area	Reduced	Near adult	Adult pattern
Microbial colonization	Reduced	Near adult	Adult pattern
Biliary function	Immature	Near adult	Adult pattern
Muscular blood flow	Reduced	Increased	Adult pattern
Skin permeability	Increased	Increased	Near adult pattern
Possible Pharmacokinetic Consequences			
Oral absorption	Erratic: reduced	Increased rate	Near adult pattern
Intramuscular absorption	Variable	Increased	Adult pattern
Percutaneous absorption	Increased	Increased	Near adult pattern
Rectal absorption	Very efficient	Efficient	Near adult pattern
Presystemic clearance	Less than adult	Greater than adult	Greater than adult (increased rate)

**The direction of alteration is given relative to the expected normal adult pattern.*
Adapted from Ritschel, W. A., and Kearns, G. L.: Pediatric pharmacokinetics. In *Ritschel, W. A., and Kearns, G. L. (eds.): Handbook of Basic Pharmacokinetics. 6th ed. Washington, D.C., American Pharmaceutical Association, 2004, pp. 227-240.*

and children, the rate of bioavailability for drugs administered by the intramuscular route may be altered (i.e., delayed T_{max}) more than the extent of absorption in a neonate. This developmental pharmacokinetic alteration is the consequence of relatively low muscular blood flow in the first few days of life, the relative inefficiency of muscular contractions (useful in dispersing an intramuscular drug dose), and an increased percentage of water per unit of muscle mass.[175]

Developmental differences in drug absorption among neonates, infants, and older children are summarized in Table 247–1. The data contained therein reflect developmental differences that may be expected to occur in healthy pediatric patients. Certain conditions or disease states that could modify the function or structure, or both, of the absorptive surface area, GI motility, or systemic blood flow can further affect either the rate or the extent of absorption for extravascularly administered drugs in pediatric patients.

Distribution

Development is associated with marked changes in body composition as reflected by examination of total-body water, extracellular water, and stores of body fat (Fig. 247–4). The most dynamic changes occur in the first year of life, with the exception of total-body fat, which has a distinctly different pattern in male and female children. Furthermore, the adipose tissue of neonates may contain as much as 57 percent water and 35 percent lipids, whereas values in adults approach 26.3 percent and 71.7 percent, respectively.[175]

In addition to age-related alterations in body composition, several physiologic changes that occur during the neonatal period are capable of altering the plasma protein binding of drugs (Table 247–2). In neonates, the free fraction of drugs that are bound extensively to circulating plasma proteins is increased markedly, largely because of lower concentrations of drug-binding proteins, reduced binding affinity of these proteins, the presence of a relatively acidic plasma pH, and endogenous competing ligands (e.g., bilirubin, free fatty acids). This consideration is exemplified by ceftriaxone, a weak acid that is approximately 95 percent bound to albumin in adults but only 70 percent bound in neonates and thus is capable of producing significant displacement of bilirubin.[88,142] The reduced plasma protein binding in combination with absolute and relative differences in the size of various body compartments (e.g., total-body water, extracellular fluid,

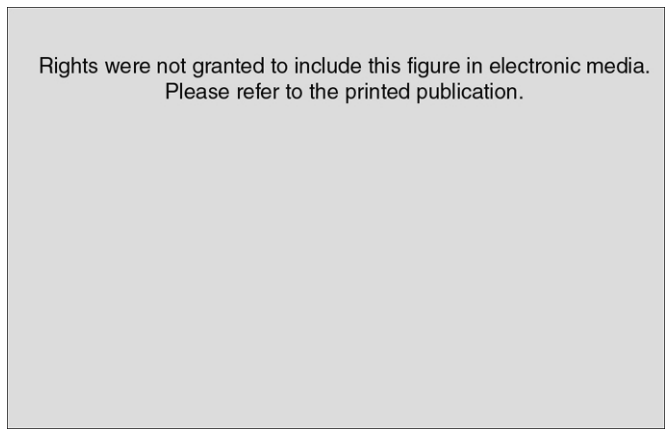

Figure 247–4 Body composition reflected as a percentage (y-axis) of total-body mass for total-body water (TBW), extracellular water (ECW), and body fat as a function of age (x-axis). *(From Ritschel, W. A., and Kearns, G. L.: Pediatric pharmacokinetics.* In *Ritschel, W. A., and Kearns, G. L. [eds.]: Handbook of Basic Pharmacokinetics. 6th ed. Washington, D.C., American Pharmaceutical Association, 2004, pp. 227-240.)*

composition of body tissues) frequently influences (i.e., increases) the apparent VD for many drugs and also their localization (i.e., both uptake and residence) in tissue, which, in turn, can alter their plasma elimination half-life.

Renal Excretion

The renal excretion of many drugs is directly proportional to age-dependent patterns in the acquisition of renal function, primarily glomerular filtration and active tubular secretion. Accordingly, developmental differences in renal function may serve as a major determinant of drug clearance in neonates and young infants for compounds that are not metabolized extensively.[175]

In preterm infants, renal function is reduced dramatically because of the continued development of functioning nephron units (i.e., nephrogenesis). In contrast, the acquisition of renal function in a term neonate represents, to a great degree, the recruitment of fully developed nephron units. In both term neonates and preterm infants who have birth weights greater than

TABLE 247–2 Plasma Protein Binding and Drug Distribution*

	Neonates	Infants	Children
Physiologic Alteration			
Plasma albumin	Reduced	Near normal	Near adult pattern
Fetal albumin	Present	Absent	Absent
Total protein	Reduced	Decreased	Near adult pattern
Serum bilirubin	Increased	Normal	Normal adult pattern
Serum free fatty acids	Increased	Normal	Normal adult pattern
Blood pH	7.1-7.3	7.4 (normal)	7.4 (normal)
Possible Pharmacokinetic Consequences			
Free fraction	Increased	Increased	Slightly increased
Apparent volume of distribution			
Hydrophilic drugs	Increased	Increased	Slightly increased
Hydrophobic drugs	Reduced	Reduced	Slightly decreased
Tissue-to-plasma ratio	Increased	Increased	Slightly increased

**The direction of alteration is given relative to the expected normal adult pattern.*
Adapted from Ritschel, W. A., and Kearns, G. L.: Pediatric pharmacokinetics. In *Ritschel, W. A., and Kearns, G. L. (eds.): Handbook of Basic Pharmacokinetics. 6th ed. Washington, D. C., American Pharmaceutical Association, 2004, pp. 227-240.*

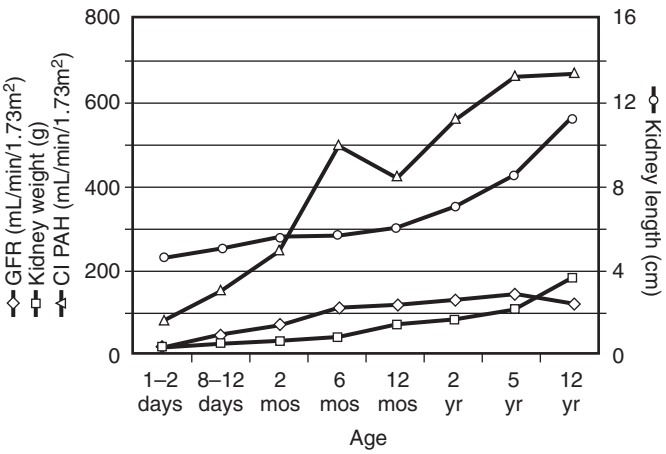

Figure 247–5 Ontogeny of renal function. Cl, clearance; GFR, glomerular filtration rate; PAH, *para*-aminohippuric acid. *(Adapted from Papadopoulou, Z. L., Tina, L. U., Sandler, P., et al.: Size and function of the kidneys.* In *Johnson, T. R., Moore, W. M., and Jeffries, J. E. [eds.]: Children Are Different: Developmental Physiology. 2nd ed. Columbus, OH, Ross Laboratories, 1978, pp. 97-104.)*

TABLE 247–3 Impact of Ontogeny on the Elimination Half-Life of Drugs with Predominant Renal Routes of Excretion*

Drug	Cl (mL/hr/kg)	VD (mL/kg)	Elimination $t_{1/2}$ (hr)	
			Neonate	Adult
Gentamicin	35-72	350-500	4.4-11.4	2.5
Tobramycin	41-74	590-840	8.2-11.3	2.0
Amikacin	50	570	8.4	2.5
Cefotaxime	50-100	310-790	3.4-6.4	1.2
Ceftriaxone	44-60	530-610	7.7-8.4	6.5
Ceftazidime	31-42	292-363	5.0-8.7	1.8
Vancomycin	36-78	480-680	8.0-17	6.0

**Data represent average or a range of average values reported from individual studies and summarized by van den Anker and Kearns[213] (for preterm infants) and Ritschel and Kearns[175] for adults.*
Cl, total plasma clearance; $t_{1/2}$, half-life; VD, apparent volume of distribution.

1500 g, glomerular filtration rates increase dramatically during the first 2 weeks of postnatal life (Fig. 247–5). This particular dynamic change in function is a direct consequence of postnatal adaptations in the distribution of renal blood flow (i.e., medullary distribution to the corticomedullary border) and results in a dramatic recruitment of functioning nephron units.[109] In addition, a glomerular-tubular imbalance exists in which the maturation of the glomerular function is more advanced than is the proximal tubular secretion. This imbalance may persist for up to 6 to 10 months of age, when both tubular function and glomerular function approach values approximately equal to those observed in healthy, young adults.[175]

The impact of development on each of the components of renal function can be characterized by a definable pattern during the first year of life (see Fig. 247–5).[161] Accordingly, the renal handling and, hence, excretion and elimination characteristics of virtually any drug in neonates, infants, and children can be predicted largely by considering the ontogeny of renal function and the specific pharmacologic characteristics of a given drug with regard to its renal excretion (e.g., routes of renal excretion, the percentage of a given dose excreted unchanged in urine) in adults. For antimicrobial agents, the impact of the ontogeny of the renal function on pharmacokinetics is reflected largely by alterations in the plasma drug clearance. As summarized by van den Anker and Kearns,[213] the elimination half-life of antimicrobial agents from different classes that share predominantly renal pathways of excretion is increased substantially in neonates and young infants (Table 247–3). This increase is not the case for antimicrobial and other agents that are not excreted primarily by the kidneys (e.g., ceftriaxone; see Table 247–3).

Metabolism

Simply stated, drug metabolism (or biotransformation) involves the modification of a drug molecule by one or more enzymes such that the hydrophilicity of the product is increased relative to the parent drug, thus enhancing elimination and excretion. In most instances, drug metabolism creates products that are either less pharmacologically active than is the parent drug (e.g., desacetylcefotaxime) or devoid of pharmacologic activity altogether (e.g., cefotaxime lactone metabolites).[108] In other instances, metabolism can result in drug bioactivation and produce metabolites with pharmacologic activity from an inactive parent drug (i.e., prodrug) or generate metabolites that can result in cellular toxicity in the host (e.g., sulfamethoxazole's nitroso and hydrox-

ylamine reactive metabolites, acetaminophen's NAPQI reactive metabolite).

Virtually every tissue has some ability to carry out drug biotransformation reactions. Although the liver is quantitatively the most important organ capable of drug metabolism, the small intestine, lungs, skin, and kidneys also have substantial drug biotransformation activities. In infants, children, and adolescents, developmental variations in drug metabolism have been associated with age, sex, maturation, and genetic constitution. For compounds that are extensively metabolized, developmental differences in drug metabolism can serve as the primary determinant for age-appropriate dose selection.[127] Much of the existing information regarding the impact of ontogeny on drug metabolism has been derived as a byproduct of pharmacokinetic investigations designed, in part, to determine whether age-dependent differences in drug disposition were evident.[109] The following paragraphs highlight important general issues regarding drug metabolism and its relationship with development.

PHASE I PATHWAYS

Phase I biotransformation reactions include oxidation, reduction, and hydrolysis reactions that, in general, introduce or unmask a functional group (e.g., hydroxyl, amine, sulfhydryl) that renders a drug more polar. Quantitatively, the P-450 cytochromes are the most important of the phase I enzymes and represent a superfamily of heme-containing proteins that catalyze the metabolism of many lipophilic endogenous substances (e.g., steroids, fatty acids and fat-soluble vitamins, prostaglandins, leukotrienes, and thromboxanes) and exogenous compounds. In humans, the P-450 cytochromes can be divided functionally into two distinct classes: steroidogenic enzymes expressed in specialized tissues, such as the adrenal glands, gonads, and placenta; and enzymes involved in the metabolism of drugs, pesticides, and environmental contaminants. P-450 cytochromes that have been identified as important in human drug metabolism are found predominantly in the CYP1, CYP2, and CYP3 gene families.[127]

As reviewed by Leeder and Kearns,[127] considerable interindividual variability exists in the hepatic expression of P-450 enzymes, and, for a given individual, the pathway and rate of a compound's metabolic clearance constitute that individual's unique phenotype with respect to the forms and amounts of P-450 isoforms expressed. Of the cytochromes P-450 involved in drug biotransformation, CYP2C19, CYP2C9, and CYP2D6 are examples of isoforms that are polymorphically expressed in humans. Polymorphic expression of CYP1A2 and CYP3A4 has been reported in the literature but not definitively established with regard to functional consequences. Within a P-450 subfamily, several isoforms can exist (e.g., CYP3A4, CYP3A5, and CYP3A7), each of which can demonstrate substrate specificity and a distinct developmental pattern with regard to enzyme activity. In addition, certain P-450 cytochromes (e.g., CYP3A4) can work cooperatively with transporters (e.g., MDR-1 or P-glycoprotein) located in distinct cells or tissues to alter the availability of specific drugs to the systemic circulation (e.g., first-pass metabolism of CYP3A4 substrates in the enterocyte). Moreover, for certain polymorphically expressed P-450 cytochromes (e.g., CYP2C19, CYP2D6), enzyme activity has been shown to vary as a consequence of racial or ethnic origin[171] and, within a given phenotypic distribution (e.g., extensive metabolizers for CYP2D6), as a function of single nucleotide polymorphisms that exist in either the gene or its regulatory regions.[169] Finally, throughout development, the activities of the P-450 cytochromes can vary widely (e.g., 5- to 25-fold) among individuals of the same phenotype,[127] an important factor with respect to the ability for induction or inhibition and, thus, the prediction of drug-drug or drug-xenobiotic interactions in vivo.

PHASE II PATHWAYS

In general, phase II reactions involve the coupling of a drug or drug metabolite with an endogenous substance to enhance its hydrophilicity further and to facilitate drug excretion in either urine or bile. These reactions require the participation of specific transferase enzymes (e.g., epoxide hydrolase, glucuronosyltransferases, glutathione *S*-transferases, sulfotransferases, *N*-acetyltransferases, methyltransferases, transacylases) and high-energy, activated endogenous substances. Although most conjugation reactions result in drug detoxification, examples of bioactivation by phase II enzymes do exist.[174] Similar to what has been found for certain of the P-450 cytochromes, different isoforms of phase II enzymes also occur in humans. This finding is exemplified by the review of de Wildt and colleagues,[41] who described substrate specificity for 10 different glucuronosyltransferase isoforms in humans and known polymorphisms in 5 different isoforms of this enzyme. The impact of age on the disposition of several glucuronosyltransferase substrates (e.g., morphine, acetaminophen, zidovudine, chloramphenicol) suggests that isoform-specific, age-related differences in activity occur to a degree sufficient to produce profound effects on drug clearance that translate directly into age-specific differences in a drug dose.[41]

Normal growth and development can have a profound effect on the activity of drug-metabolizing enzymes. Traditionally, the impact of ontogeny for all enzymes was viewed as being extremely limited in newborn infants, rapidly increasing in the first year of life to levels in toddlers and older children that may exceed adult capacity, and declining to adult levels by the conclusion of puberty. Experimental and clinical data previously reviewed demonstrated that this theory is not accurate.[109,127] As illustrated by the summary data contained in Table 247–4, the impact of ontogeny on the activity of both phase I and phase II drug-metabolizing enzymes is very much a substrate- and isoform-specific event; development is one dynamic factor in a multitude of conditions (e.g., nutritional status, gender, diurnal variation, menstrual cycle, disease states/organ dysfunction, pregnancy, concomitant drug therapy) capable of altering the activity of drug-metabolizing enzymes and, thus, the clearance of drugs (and their metabolites) from plasma. Although discussion of the impact of ontogeny on the disposition (pharmacokinetics) of specific drugs is beyond the scope of this chapter, the clinician can use much of the information concerning specific enzymes provided in Table 247–4 as a tool to facilitate inquiry or clinical decision-making, or both. It can be done simply by using readily available, updated information describing which enzymes are responsible for drug metabolism and then searching the published or unpublished (e.g., information available from pharmaceutical companies for drugs under development) literature for information describing the pharmacokinetics of the drug being considered.[57]

Clinicians must recognize that age-dependent differences in the activity of enzymes that catalyze drug biotransformation are not limited solely to P-450 cytochromes or the host of transferase enzymes responsible for phase II drug metabolism, and, in many cases, these differences represent a critical determinant for successful anti-infective drug therapy. This factor is exemplified by considering the example of two antimicrobial agents that have unique places in pediatric therapy: cefotaxime and linezolid. In the case of cefotaxime, non–P-450 enzymes (probably an esterase) capable of generating the active desacetylcefotaxime metabolite appear to be present and fully active by the third trimester of gestation.[113] Nonetheless, the elimination half-life of cefotaxime in neonates is approximately threefold greater (i.e., ≈3 to 4 hours) than that observed in older infants and children (i.e., ≈1 to 1.5 hours), a difference that permits extension of the dosing interval for the use of cefotaxime in neonates. The reasons for this developmental difference reside not with the enzymes responsible for

TABLE 247–4 Developmental Patterns for the Ontogeny of Important Drug-Metabolizing Enzymes in Humans

Enzyme(s)	Known Developmental Pattern
Phase I Enzymes	
CYP2D6	Low to absent in fetal liver but present at 1 wk of age; poor activity (i.e., 20% of adult) by 1 mo; adult competence by 1 to 3 yr of age
CYP2C9	Apparently absent in fetal liver; low activity in first 2 to 4 wk of life, with adult activity reached by ≈6 mo; activity may exceed adult levels during childhood and declines to adult levels after conclusion of puberty
CYP1A2	Not present in appreciable levels in human fetal liver; adult levels reached by ≈4 mo and exceeded in children at 1 to 2 yr of age; adult activity reached after puberty
CYP3A7	Fetal form of CYP3A that is functionally active (and inducible) during gestation; virtually disappears by 1 to 4 wk of postnatal life when CYP3A4 activity predominates, but remains present in ≈5% of individuals
CYP3A4	Extremely low activity at birth, reaching ≈30% to 40% of adult activity by 1 mo, and full adult activity by 6 mo; may exceed adult activity between 1 and 4 yr of age and decrease to adult levels after puberty
Phase II Enzymes	
NAT2	Some fetal activity by 16 wk of gestation; poor activity between birth and 2 mo of age; adult phenotype distribution reached by 4 to 6 mo, with adult activity reached by 1 to 3 yr
TPMT	Fetal levels ≈30% of adult values; in newborns, activity ≈50% higher than in adults, with phenotype distribution approximating that of adults; exception is Korean children, in whom adult activity is seen by 7 to 9 yr of age
UGT	Ontogeny isoform-specific; in general, adult activity reached by 6 to 24 mo of age
ST	Ontogeny isoform-specific and appears faster than that for UGT; activity for some isoforms may exceed adult levels during infancy and early childhood

CYP, cytochrome P-450; NAT2, N-acetyltransferase-2; ST, sulfotransferase; TPMT, thiopurine methyltransferase; UGT, glucuronosyltransferase.
Adapted from Leeder, J. S., and Kearns, G. L.: Pharmacogenetics in pediatrics: Implications for practice. Pediatr. Clin. North Am. 44:55-57, 1997.

generation of an active metabolite; instead, age-associated reductions in the activity of enzymes appear to be responsible for the generation of inactive metabolites of cefotaxime (e.g., cefotaxime lactone) and pathways involved in the renal clearance of desacetylcefotaxime.[110] Linezolid, an oxazolidinone antimicrobial approved in the United States for use in pediatric patients, undergoes extensive biotransformation in humans, not through a cytochrome P-450 enzyme, but rather by nonselective chemical oxidation.[115] As demonstrated by Kearns and associates,[111] the mean plasma clearance of linezolid in children was approximately threefold higher than that observed previously for adults, with the greatest increase noted in infants younger than 1 year. Given the mechanism of action for linezolid and properties that reflect time-dependent killing,[115] the clinical implications of the age-dependent pattern of increased plasma clearance for this drug in young infants and children suggest that for infections with selected pathogens that have relatively high (90%) minimal inhibitory concentration (MIC_{90}) values for the drug, shorter dosing intervals (e.g., every 8 hours) may be necessary to ensure sufficient exposure of the organism in blood and tissue through most of a dosing interval.[111]

PHARMACOKINETIC DETERMINANTS OF EFFECT

Collectively, the most important determinants of efficacy for anti-infective agents are the pharmacokinetic profile of the drug, the physicochemical and biochemical characteristics of the local environment (i.e., site of infection), and the susceptibility of the infecting organisms under local growth conditions. As previously reviewed by Barza,[14] the mechanisms and pharmacokinetics of drug transport to and accumulation at the site or sites of infection, as well as the subcellular localization of drugs, are critical to success and are poorly understood. In many circumstances, plasma drug concentrations may not be reflective of those in tissue. Pathophysiologic processes related to the host, the infection, and the physicochemical properties of the drug work in concert to regulate distribution into and retention of active drug at the site of infection. Because these considerations should be embraced by the clinician when a particular anti-infective drug is selected for treatment and its therapy is monitored for clinical evidence of success, some general examples are presented as follows.

In addition to age (as discussed earlier), many disease processes (e.g., trauma, malignancy, renal or hepatic disease) have an impact on both the quantity of circulating plasma proteins (e.g., albumin, α_1-acid glycoprotein) and their affinity to bind anti-infective agents. Given that only free drug is available to enter tissue, the degree of binding to protein components in the blood will affect tissue concentrations. Thus, despite the presence of total (i.e., free and bound) plasma drug concentrations well in excess of the MIC, these concentrations may not be predictive of concentrations of highly bound (i.e., >70%) drug at the site of infection. Properties of the capillary bed feeding the site of infection also dictate drug penetration. Highly vascularized tissue with a large ratio of capillary surface area to volume can be expected to accumulate higher drug concentrations than can tissue that is poorly vascularized, principally because of the rate and extent to which drug can be delivered to the site. As with protein binding, variability in capillary density may be a function of disease (e.g., severe atherosclerosis, diabetes), infection (e.g., abscess, cardiac vegetation), and age, and it should be expected to have an impact on drug delivery. Similarly, tissues with tight junctions and few fenestrations (e.g., eye, central nervous system [CNS]) afford lower tissue concentrations, given that drug entry is restricted to transport across the lipid bilayer of the endothelial cell (i.e., transcellular versus paracellular transport). Here, adequate drug penetration is restricted to agents with a favorable lipid-water partition coefficient and ionization constant. As such, many infectious processes warrant direct instillation of antibiotic at the site of infection (e.g., intracisternal, intrathecal, intra-articular). Additionally, cellular transporters (e.g., P-glycoprotein at the blood-brain barrier, organic anion and cation transporters at the choroid plexus) capable of pumping drug from the cell can limit distribution and retention of drug at the tissue level. Finally, the pH at the site of infection (e.g., in tissue fluids, exudates, transudates) relative to the pH in plasma also can govern distribution of a drug. For example, in cases of bacterial meningitis, the pH that occurs in the CNS is lower relative to plasma. Weak acids (e.g., penicillin) are more highly ionized in plasma than in cerebrospinal fluid (CSF) and pass more readily from the CSF into plasma than in the reverse direction. Similarly, when the pH of

the local environment drops in the presence of infection, as occurs in lung abscesses, aminoglycoside efficacy is reduced because of chemical inactivation of the drug by ionization and the formation of stable adducts with high DNA concentrations found in purulent secretions. Finally, the physicochemical association of an intact (i.e., unmetabolized) drug with a particular physiologic fluid as part of its normal excretion profile can serve to localize drug at the site of infection (e.g., accumulation of drugs excreted by the biliary route in patients with cholangitis and in the urine in patients with nephritis).

PHARMACODYNAMIC DETERMINANTS OF EFFECT

The desired effect of any drug is realized when sufficient concentrations are achieved and maintained at the active site for an adequate period. In many models of disease for which the treatment targets of modern chemotherapy remain fixed, the concentration-effect (i.e., pharmacodynamic) relationships can be described with relatively modest effort. In contrast, evaluating the concentration-effect relationship for antimicrobials can be anything but straightforward, given the dynamic nature of infection. The intended target of the anti-infective agent is in constant flux as the number of organisms changes, the quantity and affinity of target receptors evolve, and the contribution of the host response adapts accordingly during the course of infection. Moreover, antimicrobials demonstrate variable effects at different concentrations and under different physicochemical environments. In fact, evaluating whether an organism survives in the presence of antibiotic is only one criterion by which to predict or define drug effect. Rather, a variety of effects related to antimicrobial concentration—from the extremes of complete eradication to complete survival—can be observed in the invading organism. The previous sections of this chapter review the principles of pharmacokinetics, specifically, factors that link dose to concentration. The sections that follow explore the pharmacodynamics of anti-infective therapy and detail the multitude of factors that link concentration with effect.

EFFECTS DESCRIBED BY PHARMACOKINETIC PARAMETERS AND CONVENTIONAL SUSCEPTIBILITY END-POINTS

Among the many decision-making tools that clinicians have at their disposal to guide in the selection of antimicrobial therapy are qualitative (e.g., breakpoints) and quantitative (e.g., MIC, minimal bactericidal concentration [MBC]) estimates of susceptibility. Although numerous studies and years of clinical evidence support the correlation between qualitative end-points and clinical outcome, their predictive power is diminished in the presence of immunocompromise, severe underlying disease, mixed infections, and infection with organisms demonstrating heterogeneic resistance patterns.[30,36,56,136,144] Quantitative end-points provide a better assessment of dose-effect relationships, with antibiotic concentration serving as a surrogate for the dose. However, these data alone do not reveal the complete picture relative to the anticipated bacterial response because laboratory-based quantitative tests contain a notable number of artificial aspects. Factors appreciated to influence the activity of antimicrobial agents in vivo (e.g., protein binding, fluctuating drug concentrations, serial drug exposure, inoculum size, immune defense status, physicochemical environment, compound stability) essentially are neglected when determining MIC values. As such, agents with comparable MIC values in vitro may, in fact, demonstrate markedly different effect profiles in a patient.[1,29,63,103,120]

In an attempt to move beyond reliance on quantitative tests as the sole marker for predicting antimicrobial activity in vivo, the integration of susceptibility information with population- and patient-specific pharmacokinetic data continues to be explored. Surrogate end-points, defined by a combination of pharmacokinetic parameters and quantitative susceptibility data, have been suggested to predict more reliably the efficacy of different antimicrobials. By far, pharmacodynamic determinants of antimicrobial effect most frequently link attainable drug concentrations or estimates of total-body exposure, or both, with in vitro estimates of pathogen sensitivity. For antimicrobials for which the mechanism of action is determined to be time-dependent (concentration-independent), the percentage of time that plasma or tissue concentrations remain above the MIC has been closely linked to therapeutic response (Fig. 247–6). By comparison, the ratio of C_{max} to MIC has demonstrated a similar relationship for drugs with concentration-dependent killing (see Fig. 247–6). Furthermore, the ratio of AUC to MIC, which reflects the concentration profile over the course of time, has been used with both classes of agents (see Fig. 247–6).

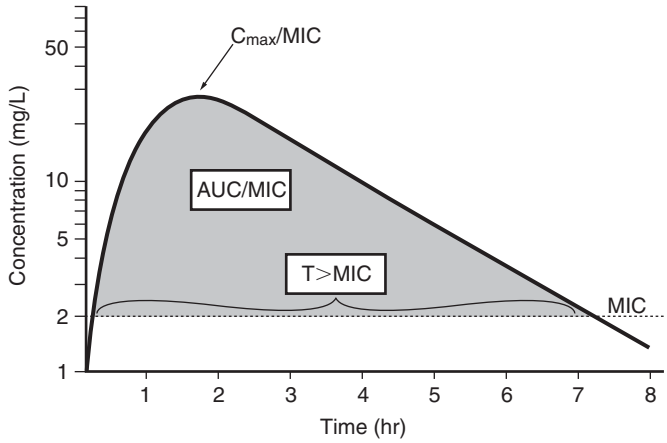

Figure 247–6 Representative plasma concentration versus time curve illustrating commonly referenced pharmacodynamic parameters. AUC, area under the curve; C_{max}, maximal plasma concentration; MIC, minimal inhibitory concentration; T, time.

Evidence to support the application of specific pharmacodynamic surrogates is reviewed in the following paragraphs. However, a large degree of interdependence exists among these pharmacokinetic parameters. With few exceptions, when the dose of a drug is increased, C_{max} and the total AUC increase, and with no change in clearance, so, too, does the percentage of time that plasma drug concentrations remain above the MIC. Accordingly, making distinctions among the various pharmacokinetic parameters in an attempt to determine the optimal pharmacodynamic end-point can be misleading. Given that many studies evaluate a limited number of doses or dosing intervals, restrict the number of pharmacokinetic parameters that are calculated, or neglect to perform multivariate analysis on the data, clear distinctions between parameters cannot always be made, and an appropriate degree of caution should be exercised when evaluating such data.[217]

Time Above the Minimal Inhibitory Concentration

Among the first class of antimicrobial agents to be discussed in the context of their concentration-effect relationships were the β-lactams, specifically penicillin. Although limited supporting evidence existed at the time, investigators were concerned with maintaining adequate blood levels of the drug in the host for a fixed period of time. This concern was reflected in their attempts to inhibit clearance pathways and formulate sustained-release preparations designed specifically for the purpose of extending

the time spent above some minimal concentration (T > MIC).[17,177] As it turns out, in vitro studies support the relative concentration independence of penicillin and demonstrate that a maximal rate of killing can be observed that is quickly saturated at reasonably low multiples of the MIC (Fig. 247–7A). As concentrations increase above this maximal level, no faster rates of kill can be demonstrated.[33] Rather, penicillin displays time-dependent killing in which the duration of time spent above some minimal concentration (typically ±1 to 2 dilutions of the MIC or MBC) appears to be the most important determinant of activity.[49] As newer antimicrobial agents with putative time-dependent activity became available for evaluation, those with longer plasma and tissue terminal elimination half-lives demonstrated enhanced bactericidal activity when compared with similar drugs. Those that simply resulted in higher maximal peak concentrations, with no extension of the time above the MIC, produced minimal if any differences in effect.[10,79,184,223] This time-dependent activity has been observed in vitro with virtually all β-lactams (e.g., cephalosporins, carbapenems, monobactams) against both gram-positive and gram-negative organisms.[58,79,197,229] Similarly, other drug classes, namely, the macrolides, azalides, glycylcyclines, glycopeptides, ketolides, and oxazolidinones, appear to possess this same characteristic.[8,85,139,214,215]

When evaluating whether the time-dependent activity observed in vitro can be reproduced in vivo by evaluating intrinsic antibiotic activity in the host (through restriction of observations to animals in which the element of both humoral and cell-mediated immunity has been removed), the strongest relationship between antimicrobial concentration and effect for the aforementioned agents remains the time spent above the MIC.[178] Elegant studies evaluating extensive numbers of dosing regimens and drug-microbe combinations demonstrated that β-lactam and macrolide efficacy correlates best with the percentage of time spent above the MIC, irrespective of whether gram-positive or gram-negative pathogens are involved.[217] Given the same total daily dose, therapy administered at more frequent intervals is required for a successful outcome.[12,128] Even when the drug-microbe interaction is such that only bacteriostatic activity results, a regimen consisting of more frequent doses is able to maintain an environment of no net growth, whereas positive net growth is observed with larger doses administered less frequently.[72] From such data, the efficacy of time-dependent agents appears to be maximized by maintaining antibiotic concentrations above the MIC for as much of the dosing interval as possible.

When evaluating whether these agents obey the same relationship in the presence of a competent immune system, animal models similarly demonstrate a significant correlation between antimicrobial efficacy and the percentage of time spent above the MIC.[2,64] In contrast to immunocompromised animals, animals with a functional immune system do not require that virtually 100 percent of the dosing interval be spent above the MIC to achieve adequate antimicrobial activity. Although the bactericidal activity of penicillin ceases when concentrations fall below the MIC, the remaining organisms do not resume multiplication for many hours, well in excess of the time observed for the same organisms in vitro.[51,104] This difference may reflect the time required for the organism to recover from the initial antibiotic insult, coupled with the complementary activity of subinhibitory antibiotic concentrations and the immune system (these pharmacodynamic principles are explored later in further detail).[69] Irrespective of the mechanism by which the protracted antimicrobial effect occurs, the pharmacodynamic goals for time-dependent agents are not as stringent in vivo as they are in vitro. However, the persistent suppression of bacterial growth is not indefinite, and a maximal amount of time can be spent below the MIC before a reduction in efficacy becomes apparent. Thus, a maximal dosing interval for these agents appears to exist in immunocompetent hosts, beyond which a decrease in efficacy can be expected.[50,189]

Clinical studies further confirm that for the agents discussed earlier, time above the MIC is a suitable pharmacodynamic endpoint in patients. In clinical studies of upper respiratory tract infection, the highest bacterial eradication rates for the β-lactams are observed when the time above the MIC exceeds 40 to 50 percent of the dosing interval.[37] For cefuroxime, cure rates drop from greater than 90 percent to approximately 75 percent when

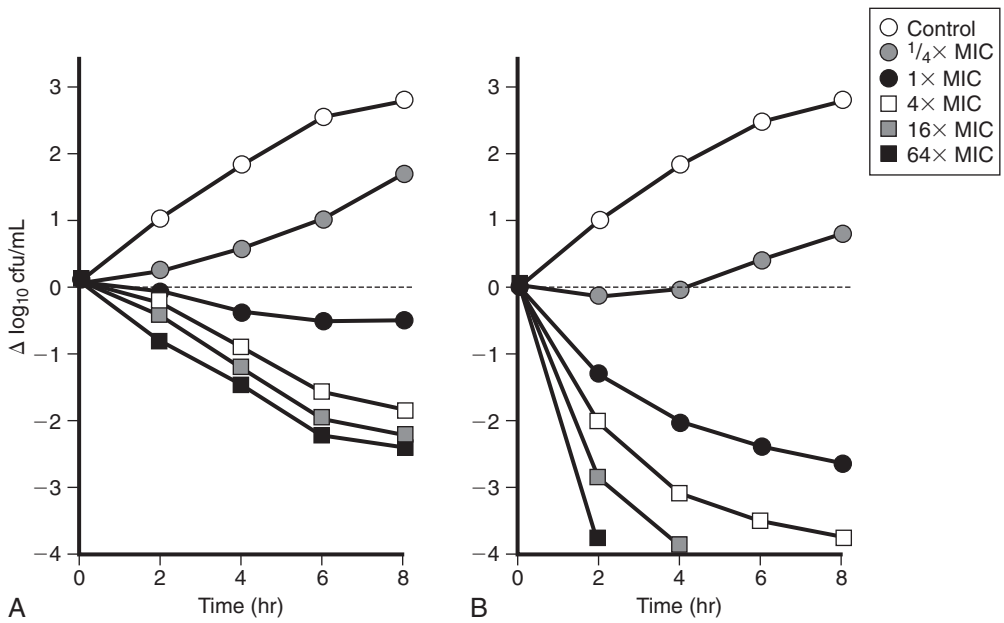

Figure 247–7 Representative log-kill curves. These curves illustrate the response to concentration-independent **(A)** (time-dependent) and concentration-dependent **(B)** antimicrobial agents. MIC, minimal inhibitory concentration. *(Adapted from Craig, W. A., and Ebert, S. C.: Killing and regrowth of bacteria in vitro: A review. Scand. J. Infect. Dis. 74[Suppl.]:63-70, 1991.)*

the time above the MIC falls to less than 40 percent.[67,76] Patients with a susceptible organism show 92 percent efficacy with a continuous-infusion regimen of cefamandole versus 63 percent efficacy with intermittent administration of the agent. Finally, in neutropenic patients, an overall efficacy rate of 65 percent was observed after continuous infusion of cefamandole as opposed to 21 percent efficacy in patients receiving intermittent administration.[24] For macrolide-susceptible pathogens, cure rates approaching 100 percent were observed when concentrations remained above the MIC for greater than 80 percent of the dosing interval.[37] As the MIC increases such that the percentage of time spent above the MIC falls, so too do cure rates. Macrolide concentrations rarely exceed the MIC for *Haemophilus influenzae*, and cure rates are comparable to those seen in placebo-treated individuals.[37] A few studies appear to contradict the aforementioned data, findings indicating that little difference in clinical response is observed when the same total daily dose of selected agents is administered as a continuous infusion or with increased frequency versus less frequent intermittent administration; however, a prolonged post-antibiotic effect (PAE) or protracted half-life, such that both of the regimens evaluated spend nearly 100 percent of the dosing interval above the MIC, may explain these discrepancies.[28,123,218]

Concentration Above the Minimal Inhibitory Concentration

In contrast to the aforementioned agents, a smaller group of agents demonstrate concentration dependence, with the magnitude of antimicrobial effect increasing in direct proportion to increasing drug concentrations (see Fig. 247–7B).[16,141] The aminoglycoside and fluoroquinolone antibiotics are the prototypic drugs of this pharmacodynamic class; however, concentration-dependent activity also has been observed with the lipopeptides and metronidazole.[5,39,168,196,212] Unlike the time-dependent agents, for which shortening the dosing interval results in increased efficacy, extending the dosing interval for concentration-dependent drugs affords an ability to increase the dose and results in a greater reduction in bacterial inoculum and, for some agents, a diminution in adaptive resistance.[38] Though highly variable, optimal ratios of peak plasma concentration to MIC that result in complete killing have been defined for many drug-microbe combinations. Similarly, ratios of C_{max} to MIC (C_{max}/MIC) can be defined, below which the organism is afforded the opportunity to regrow with increased selection of resistant subpopulations.[22,44,45]

Concentration-dependent killing for many of these agents has been demonstrated in both animal models and human clinical trials. In neutropenic mice with pseudomonal soft tissue infection, dosing schemes that deliver larger aminoglycoside doses less frequently result in greater killing than do schemes with the same total daily dose administered more frequently, even though all regimens achieved serum concentrations in excess of the MIC.[72] In patients receiving quinolone therapy for the treatment of respiratory tract, urinary tract, and skin and soft tissue infections, several pharmacodynamic parameters proved to correlate with clinical response; however, the best correlation was observed with the C_{max}/MIC ratio.[166] In clinical trials in which patients received aminoglycoside therapy for gram-negative infections, the strongest association between pharmacokinetic indices and clinical response was observed with the C_{max}/MIC ratio. In fact, the relationship between the C_{max}/MIC ratio and clinical response appeared to be linear over the range of clinically relevant plasma concentrations. No significant difference occurred in the time to achieve C_{max} between responders and nonresponders; therefore, the improved response rates in patients achieving higher peak plasma concentrations were not simply an artifact of drug accumulation as a result of longer duration of therapy.[148,149]

In addition to the absolute peak plasma concentration, the time that optimal peak levels are achieved appears to play a critical role in the response to concentration-dependent antimicrobial agents. A significant correlation with clinical improvement and reduction in mortality is observed if therapeutic peak plasma concentrations are reached early during the course of treatment.[150,153] The probable reason for this effect is that the response of the bacterial population to the same antibiotic concentration can change with subsequent doses. As alluded to earlier, subtherapeutic peak plasma concentrations serve to select out fewer susceptible variants within the population.[22,23,71] Based on these data, a key goal of therapy (i.e., in addition to proper drug selection) for concentration-dependent agents appears to be the attainment of high peak plasma concentrations early in therapy to afford rapid initial killing and minimize adaptive resistance.

Although prolonging the dosing interval affords the luxury of increasing the dose to optimize killing, the law of diminishing returns applies even for concentration-dependent agents, and it is possible to increase the dosing interval to excess and decrease efficacy. Despite the clear concentration dependence with escalating doses of fluoroquinolones, a simulated every-12-hour regimen was less effective than was a regimen using the same total daily dose administered every 8 hours.[15] Similarly, animal models of gram-negative lung and soft tissue infection suggest that delayed clearance of the infecting organism is observed in response to aminoglycoside and fluoroquinolone therapy when administered in protracted dosing regimens (e.g., dosing interval much greater than the half-life) as opposed to shorter dosing intervals.[128,152,178] Again, scattered studies appear to contradict these findings; however, they use agents with a longer half-life, and therefore the dosing interval may, in fact, not exceed the time spent above the MIC plus the residual PAE.[42] Thus, despite their concentration dependence, an element of time appears to be involved in the response to these agents. Unless peak concentrations are achieved in excess of those necessary to obtain maximal kill rates and the dosing interval does not markedly exceed time above the MIC plus the PAE, the efficacy of such agents is not only dose-dependent but also dose interval–dependent.

Total-Body Exposure Above the Minimal Inhibitory Concentration

The AUC, a reflection of total-body exposure, is a pharmacokinetic parameter that integrates both time and drug concentration. Estimates of AUC reflect the magnitude of the dose received and the drug half-life relative to the dosing interval. Accordingly, researchers have proposed that the ratio of AUC to MIC (AUC/MIC ratio) may serve as a better surrogate of pharmacodynamic activity for both time- and concentration-dependent agents. In several studies, the AUC/MIC ratio serves as the best correlate of the reduction in number of organisms for aminoglycosides, quinolones, streptogramins, rifamycins, isoniazid, evernimicin, and azole antifungals.* Because of the interdependence between AUC and the pharmacokinetic parameters previously noted, studies also can be identified that demonstrate a relationship between clinical or microbiologic outcome and the AUC/MIC ratio for agents with time-dependent activity (e.g., the β- lactams and glycopeptides). Often, however, these studies fail to evaluate multiple pharmacokinetic parameters.[147,188]

In an elegant study evaluating certain aminoglycoside dosing regimens against *Pseudomonas aeruginosa* and *Escherichia coli* infection, the log-normalized AUC provided the best correlation with efficacy. However, as was observed with concentration-dependent agents, when the dosing interval exceeded the time above the MIC plus the residual PAE, the best correlation with

*See references 4, 6, 7, 43, 54, 105, 106, 122, 132, 178.

efficacy was the percentage of time spent above the MIC, thus reaffirming that the dosing interval can be too long with the aminoglycosides.[217] A meta-analysis of 19 studies evaluating 8 quinolones and 6 different organisms in experimental endocarditis similarly demonstrated a correlation between the AUC/MIC and reduction in $\log_{10}$ colony-forming units (CFUs) per vegetation. However, given the relationship between the pharmacokinetic parameters, C_{max}/MIC and time greater than MIC also proved to correlate with a decrease in the size of the inoculum.[3] In human investigations, AUC/MIC appears to serve as the best predictor of clinical response, microbiologic response, and bacterial eradication for the fluoroquinolones. Nonetheless, C_{max}/MIC and time greater than MIC can be linked similarly to bacterial eradication for this class of agents.[61,62,163]

Clearly, the optimal AUC/MIC ratio will be different for different organisms despite sharing a similar quantitative susceptibility end-point. Similarly, optimal AUC/MIC ratios are highly variable and depend on the antimicrobial agent being evaluated. For example, effective eradication of organisms implicated in nosocomial pneumonia is observed with an AUC/MIC ratio of 540 for cefmenoxime, 34 for tobramycin, and 23 for ciprofloxacin.[187] Consequently, attempts have been made to standardize this pharmacodynamic end-point, and investigators have proposed a value of 125 as the cutoff below which a reduction in efficacy and increase in resistance may be expected.[96,186] However, optimal AUC/MIC ratios may differ, depending not only on a given organism and agent but also on the desired outcome and the disease state. A ratio greater than 125 resulted in bacterial eradication roughly 7 days after the initiation of β-lactam and quinolone therapy; however, an AUC/MIC ratio of 250 resulted in eradication of the pathogen within 1 to 2 days after initiating quinolone therapy.[186] For patients with acute exacerbations of chronic bronchitis who are receiving fluoroquinolones, an AUC/MIC ratio less than 276 was associated with longer time to clinical success, whereas a ratio greater than 576 was associated with a reduction in coughs per day and a ratio greater than 212 with decreasing days to a reduction in the volume of sputum.[145] In an animal model of bacterial endocarditis, significantly lower numbers of organism were found in the vegetation after 3 to 6 days of therapy when the AUC/MIC ratio exceeded 100.[3] Moreover, a value of 100 appeared to be the cutoff below which an increased risk of resistance was observed in a retrospective review of 107 acutely ill patients with lower respiratory tract infection.[207] Furthermore, in vitro models evaluating fluoroquinolone activity against *Streptococcus pneumoniae* demonstrated effective eradication with an AUC/MIC ratio between 30 and 65, much lower than the value of 125 reported as optimal for other pathogens.[122,132]

Although this hybrid parameter bridges concentration and time (i.e., extent of systemic drug exposure from a given dose) and has been demonstrated to correlate with efficacy for numerous drug classes, optimal criteria appear to vary with the organism, agent, disease state, and desired outcome. In addition, established relationships no longer may hold for some agents when a protracted dosing interval is used in therapy. As such, a general classification and standard outcome are not straightforward, and agents need to be evaluated with respect to known mechanisms of activity and the specific clinical and microbiologic situations in which they are used.

Optimal Surrogates for Drugs in Combination

Combination antimicrobial therapy is initiated in numerous circumstances: to ensure broad-spectrum coverage early in therapy, to treat polymicrobial infections, and to combat organisms that require multiple agents for effective eradication. Often, combination therapy entails the use of agents from different classes or agents that have different mechanisms of action (or both). This particular situation raises the question of how one applies the aforementioned pharmacodynamic principles to selecting or evaluating the efficacy of drug therapy when confronted with the combined use of agents that do not share similar pharmacodynamic targets. Although optimization of combination therapy may be possible by choosing the interval for one agent,[73] efficacy appears to be explained best when a combination of both pharmacodynamic properties is considered.[72,151]

Reasonable models of a concentration-effect relationship have been proposed for numerous agents alone and in combination; however, optimal ratios remain to be defined for many drug-microbe combinations. Moreover, it is probably more complex than simply defining the optimal ratio for specific combinations. Even though one can define the best correlate, killing rates, in fact, may vary with the site of infection because penetration of a drug into or clearance from tissue varies from site to site. For example, logAUC proves to be the best predictor for clearance of organisms from the lung regardless of the dosing interval used. In contrast, given faster drug clearance from the thigh, the time above the MIC was a more important predictor of eradication when a longer dosing interval was used, whereas the logAUC was the best predictor of clearance of organisms with the use of shorter dosing intervals.[129] Similarly, in animal models of bacterial meningitis, greater kill rates were observed as time greater than MBC increased. However, when concentrations remained above the MBC for the entire dosing interval, which may not be unexpected in situations in which the antibiotic half-life is longer in CSF than in plasma, kill rates were directly proportional to both peak/MBC and AUC/MBC ratios.[140] These data suggest that we may need to expand the characterization of pharmacodynamic surrogates to look at the pharmacokinetic-pharmacodynamic interface at the site of infection rather than simply defining dosing strategies based on in vitro susceptibility data and the disposition characteristics of drugs in plasma. Without question, many data need to be collected and verified in the human host. The maximal dose and dosing regimen ideally are selected so that the resultant pharmacokinetic profile affords optimal pharmacodynamic activity without causing unnecessary adverse events, therapeutic failure, or both, as may be observed with inappropriate dosing. Development of a successful therapeutic strategy requires the following: incorporation of knowledge regarding the infecting pathogen; the physicochemical, pharmacologic, and pharmacokinetic properties of the anti-infective agent; and specific factors of the host or disease that are capable of altering either drug disposition or action. Such an approach enables the clinician to make clearer distinctions among agents with similar susceptibility profiles, yet different mechanistic or pharmacokinetic profiles and to select an optimal drug regimen that will be most likely to result in successful therapy.

EFFECT OF SUPRAINHIBITORY ANTIMICROBIAL CONCENTRATIONS (EAGLE EFFECT, PARADOXICAL ZONE PHENOMENON, CONCENTRATION QUENCHING)

In simplest terms, one typically thinks of anti-infective activity's increasing with increasing antimicrobial concentration. More sophisticated models describe increasing activity with increasing drug concentration to a maximal effect until activity reaches a plateau and remains relatively constant despite further increases in drug concentration (see Fig. 247–4). However, for many antibiotics, an increase in drug concentration can, in fact, result in a reduction in antimicrobial activity (Fig. 247–8).[92] This paradoxical effect was observed as early as 1945 and described 3 years later by Eagle and Musselman, who reported the existence of antibiotic concentrations above the maximally effective concentration at which the killing rate of bacteria is paradoxically reduced.[48,118]

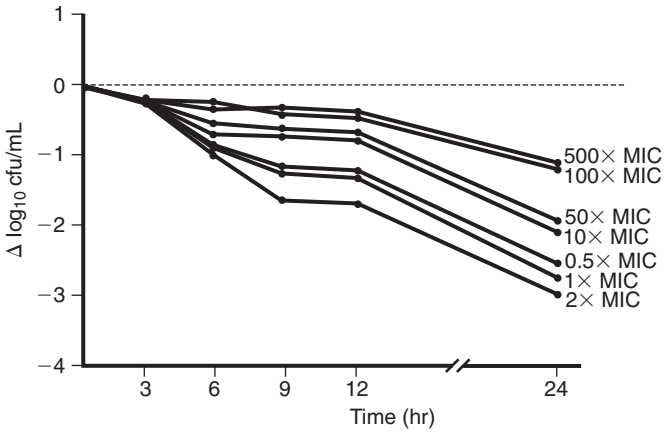

Figure 247–8 Representative log-kill curve demonstrating the paradoxical effects that can be observed with suprainhibitory antimicrobial concentrations. MIC, minimal inhibitory concentration. *(Adapted from Holm, S. E., Odenholdt-Tornqvist, I., and Cars, O.: Paradoxical effects of antibiotics. Scand. J. Infect. Dis. Suppl. 74:113-117, 1991.)*

This phenomenon has been described with a host of organisms and, although originally observed with penicillin, has been confirmed with other β-lactams, glycopeptides, aminoglycosides, and fluoroquinolones.[138,155,164,202,226] Given the diversity of agents that demonstrate a paradoxical reduction in activity with increasing drug concentration, this phenomenon probably cannot be explained by a single mechanism, and although they are not definitively elucidated, numerous mechanisms have been proposed to explain these observations.

For certain β-lactam antimicrobials, induction of β-lactamase may be responsible for the paradoxical effects observed in selected organisms. In vitro experiments performed with *Proteus vulgaris* suggested that the greatest paradoxical response seen with β-lactams was observed with agents that demonstrated the highest β-lactamase–inducing capacity and the poorest stability to degradation by this enzyme. When a mutant strain of this organism that was unable to induce β-lactamase production was evaluated, no paradoxical effect was noted.[98] In animal models of peritoneal infection, antibiotics with strong dose-dependent induction of β-lactamase similarly demonstrated a paradoxical effect against β-lactamase–positive strains of *P. vulgaris*. However, no paradoxical effect was observed against β-lactamase–negative strains. Agents that were only weak inducers of β-lactamase demonstrated no evidence of a paradoxical effect, regardless of whether the *P. vulgaris* strains produced β-lactamase.[99]

Because this paradoxical effect can be demonstrated with the β-lactams against organisms that do not produce β-lactamase, other mechanisms must also be responsible. One such mechanism may involve dysregulation of the bacterial autolytic system, which often confers tolerance to an organism but also may uncover an Eagle-type effect.[87] A paradoxical reduction in antimicrobial activity at high doses may be related to differences in regulation or expression of these autolytic enzymes. In *Enterococcus* strains that fully express only one of two recognized autolytic enzymes, diminished or deficient production of the second enzyme resulted in a paradoxical response to penicillin. Strains in which both enzymes were produced remained susceptible to the antibiotic irrespective of concentration, thus suggesting that a proportional dose-response relationship may require the synergistic activity of both enzymes.[59,60] Further investigation into the paradoxical effect observed in tolerant organisms suggests that organisms in the exponential growth phase demonstrate this response more consistently than do those in the stationary phase.[165,167] Animal models of *Staphylococcus aureus* endocarditis provide in vivo confirmation of the paradoxical response observed with tolerant organisms in vitro. Administration of continuous-infusion cloxacillin to animals infected with a nontolerant strain produced a dose-proportional reduction in bacterial density ($\log_{10}$CFUs/g) within the vegetation, whereas animals infected with a tolerant strain demonstrated a reduction and subsequent paradoxical increase in bacterial density with increasing dose. No statistical difference was observed in bacterial density within other organs or in overall survival, which raises questions regarding the in vivo significance of these observations.[219]

For antibiotics that demonstrate polyfunctional activity at supratherapeutic concentrations, a paradoxical response may occur as a result of secondary alterations in cellular functions that are requisite for antimicrobial activity. Despite a principal mechanism of action for the fluoroquinolones at the site of DNA gyrase, these agents appear to be bactericidal only in cells able to synthesize protein. As quinolone concentrations increase above those that are bactericidal, RNA synthesis and therefore protein synthesis are progressively inhibited, thus explaining the reduced rate of bactericidal activity observed at high quinolone concentrations.[176] Incompletely characterized genetic modifications also may confer a paradoxical response to antibiotics. β-Lactam resistance in methicillin-resistant *S. aureus* (MRSA) is mediated by de-repressing the production of a low-affinity PBP. However, because the modified PBP serves as an inefficient transpeptidase, it is regarded as an internal selective pressure in the bacterium, and a countermutation has arisen. Although it is not completely understood, the phenotypic expression of this countermutation is manifested as an Eagle-type resistance to methicillin.[119,183] Finally, an observed paradoxical response may not be a result of the drug-microbe combination alone but a result of the chemical environment as well. A paradoxical effect with penicillin, in which both cell count and the rate of killing were affected, was demonstrated in *Streptococcus agalactiae* when the pH was reduced under in vitro conditions.[100]

Most studies investigating the Eagle effect have been conducted in vitro, and the clinical implications of the paradoxical phenomenon have yet to be fully elucidated. However, anecdotal evidence appears to suggest that the clinical efficacy of antimicrobials can be compromised by high-dose antibiotic therapy. Case reports of β-lactam–susceptible streptococcal endocarditis and staphylococcal pneumonia describe patients who failed to respond to high-dose β-lactam therapy (plasma concentrations >100 times the MIC in one case), with subsequent improvement in each case when the dose was lowered.[70,80,205] For many agents, it is clearly advantageous to establish high peak drug concentrations early, the benefits of which include increasing log kill, preventing the selection of resistant mutants, overcoming refractoriness, and enhancing penetration of tissue. However, the relative safety of many drug classes (e.g., the β-lactams) often results in the use of doses that may be higher than necessary. When clear evidence is lacking for enhanced bactericidal activity at higher concentrations and the potential for diminished efficacy exists, escalation of antibiotic doses should be considered judiciously.

EFFECTS OF SUBINHIBITORY ANTIMICROBIAL CONCENTRATION

Individuals may be exposed to subtherapeutic antimicrobial concentrations for many reasons, including underdosing, poor compliance, and, perhaps most commonly, the inability of the pharmacokinetic profile of the agent in question to afford adequate penetration to the site of infection. Exposure of bacteria to antibiotic concentrations below the MIC can have variable effects, depending on the organism and the antimicrobial agent. In fact,

the effects observed with subinhibitory levels are not simply a milder extension of those observed at inhibitory concentrations but, instead, are qualitatively different from the effects observed at concentrations that equal or exceed the MIC.[134] Subinhibitory concentrations can have the following effects: enhance or impair opsonization and phagocytosis; affect the virulence of the bacteria by modifying the bacterial cell surface; alter adherence properties, antigen expression, or the excretion of enzymes and toxins (or any combination of these effects); and inhibit bacterial growth.[157,158,210] The result can be an increase or decrease in responsiveness to therapy, both of which are discussed later in this section. Before reviewing the current data, however, one should note that this discussion does not refer to subinhibitory concentrations occurring after initial suprainhibitory drug levels. These particular effects are distinguished as PAE and are addressed separately.

Even though antibiotic concentrations may fail to reach or exceed the MIC, these agents still can affect the growth of an invading organism adversely.[134,135,137] In fact, the term *minimal antibacterial concentration* (MAC), usually a fraction of the MIC, was coined to describe the lowest concentration of antibiotic required to produce either structural changes in the bacterium, a 1 $\log_{10}$ decrease in the number of organisms present, or a 10 percent delay in turbidimetric growth in vitro.[68,201] Rather than resulting in treatment failure, subinhibitory antimicrobial concentrations in concert with defenses in an immunocompetent host may be sufficient to clear the infection.[210] Many effects have been observed to occur as a result of exposure to subinhibitory antimicrobial concentrations, and they are mediated by a variable number of mechanisms.

Increased susceptibility to phagocytosis may be seen with subinhibitory antibiotic concentrations and can occur in many ways. Subinhibitory concentrations may induce morphologic changes in the bacterium such that its size and shape are altered markedly, and, as a result, the immune system is signaled to clear the bacterium.[11,201,220] However, subinhibitory concentrations of drugs that share a common intracellular target cannot be expected to have the same effects on bacterial morphology. Even though both penicillins and cephalosporins act through PBPs, subinhibitory concentrations of penicillins induce filaments in *Proteus mirabilis*, whereas cephalosporins induce the formation of globules.[135,137,205] A likely explanation lies in their different affinity for the various PBPs, whereby they can be expected to bind selectively at low concentrations and rather nonspecifically at therapeutic or supratherapeutic concentrations. The preferential binding of ceftibuten to PBP-3 of *E. coli* at subinhibitory concentrations results in filamentation because this PBP allows crosswall but not sidewall synthesis.[26] Similarly, subinhibitory concentrations of the same drug applied to different organisms can produce different morphologic changes. In *S. aureus*, subinhibitory concentrations of loracarbef induce no morphologic changes, whereas similar concentrations lead to elongated forms and short-chain forms in *E. coli* and elongated and filamentous forms in *H. influenzae*.[211]

Although size may be one factor stimulating enhanced clearance of the organism, increased uptake by the immune system can be demonstrated even for cells with little gross morphologic alteration. Subinhibitory antibiotic concentrations can impair the synthesis of antiphagocytic surface factors or liberate their release, thereby enhancing complement binding, opsonization, phagocytosis, and, ultimately, bacterial killing.[89,94,124] Production of the antiphagocytic A protein of *S. aureus* can be repressed by subinhibitory concentrations of selected protein synthesis inhibitors and fluoroquinolones.[143,195] Subinhibitory concentrations of β-lactam agents, but not vancomycin or protein synthesis inhibitors, increase the susceptibility of *S. agalactiae* to uptake by polymorphonuclear leukocytes (PMNs) because of the loss of antiphagocytic capsular material.[93] Carbapenems and cephalosporins at subinhibitory concentrations increase the serum sensitivity of K1-positive *E. coli* by reducing expression of the K1 capsular polysaccharide.[203]

Even drugs with no inherent bacteriostatic or bactericidal activity against a specific organism can induce sufficient changes in the cell surface to affect serum sensitivity. Pre-incubation of *P. aeruginosa* with subinhibitory concentrations of certain macrolides decreases cell surface hydrophobicity and enhances the sensitivity of the organism to human serum bactericidal activity.[206] Subinhibitory β-lactam concentrations are capable of sufficiently altering the peptidoglycan structure in MRSA to enhance susceptibility of the organism to the lysozyme present in sputum, even though the organism, because of altered PBP production, is no longer susceptible to the bactericidal activity of these agents.[97] Although most effects stimulating bacterial clearance by the host occur as a direct result of cellular changes in the organism, immune cells also can be altered by subinhibitory antibiotic concentrations. Erythromycin does not affect the generation of serum-derived leuko-attractants, nor does it increase PMN cell numbers, yet it appears capable of stimulating the migration of PMNs. Researchers suggested that this effect probably results from the ability of erythromycin to sustain cellular migration by reducing the level of leuko-attractant–induced auto-oxidation mediated by the myeloperoxidase–hydrogen peroxide–halide system.[55]

In addition to enhancing bacterial clearance, subinhibitory antibiotic concentrations also can impair bacterial virulence mechanisms. For many organisms, pili, fimbriae, and fimbriae-associated adhesins are responsible for the initial events surrounding adherence and colonization at the site of entry or infection.[185] Subinhibitory quinolone and clindamycin concentrations are capable of decreasing the adherence of nosocomial pathogens (e.g., *Staphylococcus epidermidis)* to the synthetic materials of implantable catheters.[31,193] Subinhibitory concentrations of penicillins, cephalosporins, azalides, and quinolones all have demonstrated the ability to decrease the formation of fimbriae or alter structures that mediate adherence. Specific examples include decreased adherence to oral and urinary tract epithelial cells for organisms such as *Porphyromonas gingivalis*, *E. coli*, *Salmonella typhimurium*, and *P. aeruginosa*.[26,133,195,204,220] Moreover, these effects do not appear to be growth phase–specific for select drug-microbe combinations.[27]

Subtherapeutic antibiotic concentrations also can have an impact on virulence by down-regulating bacterial toxin production. Subinhibitory concentrations of clindamycin can inhibit toxin production in *S. epidermidis* markedly without appreciably altering bacterial growth.[193] *Pseudomonas* exoenzymes that facilitate disease pathogenesis (e.g., exotoxin A, exoenzyme S, phospholipase C, total protease, elastase) are suppressed after exposure to subinhibitory concentrations of ciprofloxacin, tobramycin, and ceftazidime.[81] Finally, given the ability of subinhibitory fluoroquinolone concentrations to disrupt DNA structure sufficiently to interfere with effective mRNA production, continuous exposure of *S. aureus* to fluoroquinolones at subinhibitory concentrations decreases the production of nuclease and hemolytic alpha-toxin production.[195]

Although discerning which of these effects, alone or in combination, are responsible for the subinhibitory effects is difficult, one may see in vivo that the impact of the sum total of these effects can be observed at the level of the host. In a non-neutropenic rat model of *Klebsiella pneumoniae* pneumonia, a continuous infusion of ceftazidime resulting in steady-state concentrations of 0.06 mg/L protected all the animals from death, despite an MIC for the pathogen of 0.2 mg/L. The same could not be said, however, for immunocompromised animals, which required steady-state concentrations of 0.38 mg/L for adequate protection.[179,180] In a similar model of intraperitoneal *K. pneumoniae* infection, subinhibitory clindamycin concentrations resulted in an increase in bacterial clearance and a decrease in the

$\log_{10}$CFUs in blood at 72 hours.[9] In humans, low-dose ampicillin (such that concentrations in urine were no greater than one fourth to one half times the MIC) combined with large water intake was demonstrated to clear documented *E. coli* urinary tract infection in 16 of 20 patients within 2 days of initiating therapy, whereas none of the 18 control subjects receiving the fluid intervention alone demonstrated resolution of the infection.[173] Despite the resistance of *Pseudomonas* in patients with cystic fibrosis, the fluoroquinolones remain capable of eliciting a significant reduction in sputum colony counts and improvement in clinical signs and symptoms.[191,194]

Subinhibitory antimicrobial concentrations can be as potentially disadvantageous to the host as they can be beneficial. These concentrations can impair the ability of the host to respond to the bacterial inoculum, can augment bacterial defenses, and can serve to inactivate the antibiotic. For organisms that are susceptible to β-lactamase induction, strong inducers can up-regulate β-lactamase synthesis at concentrations below the MIC. Although β-lactamase induction typically is a reversible phenomenon, many antibiotics demonstrate the ability to select out genetically de-repressed mutants at subinhibitory concentrations.[192,198] Irrespective of whether this induction is phenotypic or genotypic, many antibiotics to which the organism originally may have been susceptible no longer retain their efficacy in light of β-lactamase production. This type of adaptive resistance, observed in response to sublethal antibiotic concentrations, also can be nonenzymatic. A single, one-time exposure of *P. aeruginosa* to subinhibitory concentrations of chlorhexidine results in unstable resistance, and repeated exposure leads to stable expression of altered cell surface macromolecules or efflux systems responsible for resistance.[208] In a clinical strain of this same organism exposed to subinhibitory concentrations of fluoroquinolones and carbapenems for 5 days, a 16- to 32-fold increase in MIC was observed in concert with an alteration in overall protein expression.[32]

Sublethal antibiotic concentrations also can stimulate the production of toxins that may be detrimental to the host. Exposure of enterohemorrhagic *E. coli* to subinhibitory concentrations of fluoroquinolones, cephalosporins, tetracyclines, and inhibitors of folic acid synthesis induces enhanced expression of a *Shiga*-like toxin (SLT-1), which is proposed to mediate events surrounding the development of hemolytic-uremic syndrome. Subinhibitory fluoroquinolone concentrations also can stimulate the production of verotoxins in this same organism.[221,227] Lincomycin and tetracycline at subinhibitory concentrations stimulate the production of heat-labile enterotoxin in enterotoxigenic *E. coli*, as well as *Vibrio cholerae* enterotoxin, even though these agents are principally protein synthesis inhibitors. Prior data suggest that the copy number of plasmids does not increase and proposed that the rate of enterotoxin liberation is enhanced as a result of antibiotic inhibition of the synthesis of proteins responsible for degrading the toxin.[131,228] The alpha-toxin of *S. aureus* is demonstrated to be hemolytic, dermonecrotic, and antichemotactic and appears to be up-regulated in the presence of subinhibitory nafcillin concentrations, irrespective of whether the isolate is susceptible to the agent. Greater hemolytic activity and increased lethality of the broth filtrate in a murine intraperitoneal model are observed, and this activity is ablated when incubated with anti–alpha-toxin antibody.[116] Other β-lactams (with the exception of aztreonam), ofloxacin, and trimethoprim are similarly capable of inducing alpha-toxin expression at subinhibitory concentrations.[159]

The up-regulation of protein expression by subinhibitory antibiotic concentrations not only is responsible for contributing to antibiotic and cellular destruction but also can enhance the binding of pathogens to host proteins and implantable devices. Subinhibitory concentrations of ciprofloxacin increase the transcription of fibronectin-binding proteins in *S. aureus* isolates and thereby result in greater adhesion to immobilized fibronectin and fibronectin-coated polymers, a particular concern with subcutaneously implanted devices that have polymer surfaces.[19,20] Subinhibitory concentrations of antibiotic also can alter the immunogenicity of the pathogen such that the host can no longer mount the same degree of immune response. *S. aureus* isolates co-incubated with subinhibitory concentrations of oxacillin demonstrated a diminished capacity to stimulate proinflammatory cytokine production (e.g., tumor necrosis factor-α, interleukin-1β [IL-1β], and IL-6) in human monocytes. Given that activation of the complement system and cytokine release are stimulated by peptidoglycan molecules of a certain size or tertiary structure, subinhibitory β-lactam concentrations probably distort the peptidoglycan structure sufficiently that the immune system no longer effectively responds to these immunostimulatory components.[190]

Unless unusual circumstances prevail, the goal of therapy probably will not be to target plasma concentrations below the MIC. However, given the reality of encountering this situation in the clinical setting, practitioners certainly need to be aware of the potential implications for therapeutic response associated with subinhibitory concentrations of antimicrobial agents.

EFFECTS THAT PERSIST AFTER ANTIMICROBIAL EXPOSURE (POST-ANTIBIOTIC EFFECT, POST-ANTIBIOTIC LEUKOCYTE ENHANCEMENT, AND POST–β-LACTAMASE INHIBITOR EFFECT)

Early investigations into antibiotic activity described a delay in the recovery and regrowth of penicillin-exposed staphylococci and streptococci when the drug was removed by enzymatic inactivation or the organism was removed to drug-free media.[18,47,162] In addition, in vivo evaluations of penicillin activity suggested that the effects of penicillin in an immunocompetent animal lasted well beyond the time when blood levels remained above quantifiable concentrations. Despite a half-life of minutes, many hours could elapse after the administration of a dose of penicillin before bacterial growth resumes.[51,104,189] The term *post-antibiotic effect* was coined to describe the suppression of bacterial growth that persists after brief exposure of the organism to an antimicrobial agent.[35] Unlike the subinhibitory effects discussed earlier in the chapter, the PAE occurs as a result of residual antimicrobial activity after initial exposure at inhibitory concentrations, as opposed to the subinhibitory concentrations themselves. The PAEs described to date typically are reversible, and although the mechanisms have not been elucidated fully, probably are caused by either nonlethal damage or persistence of antibiotic at the site of action. In the same vein as the PAEs, the term *post–β-lactamase inhibitor effect* (PLIE) was coined to describe the residual effects of β-lactamase inhibitors after exposure to inhibitory concentrations.[209] Similarly, one encounters the term *post-antibiotic leukocyte enhancement* (PALE), which is used to account for a PAE in vivo that lasts longer than that observed in vitro and is defined as exposure to subinhibitory concentrations of an antibiotic that render the organism more susceptible to the phagocytic and bactericidal action of neutrophils.

The PAE depends on many factors, the most discernible being the drug-microbe combination.[102] Since the initial descriptions with penicillin were reported, similar observations have been noted for a broad range of organisms, and essentially all antibiotic classes demonstrate a PAE against select organisms.[35] Virtually all agents evaluated demonstrate a PAE against susceptible gram-positive cocci. Inhibitors of protein and nucleic acid synthesis produce longer-lasting PAEs in vitro than do cell wall–acting agents, with an average of approximately 1 to 2 hours for β-lactams, 1 to 3 hours for fluoroquinolones, and 3 to 5 hours for protein synthesis inhibitors. For resistant gram-positive cocci, descriptions of a PAE are mixed. Against gram-negative bacilli,

no appreciable PAE is observed for trimethoprim or the β-lactams in vitro, with the exception of the carbapenems, which demonstrate a PAE of approximately 1 to 2 hours. The fluoroquinolones and protein synthesis inhibitors have PAEs of roughly 1 to 3 hours and 3 to 8 hours, respectively. Within this group of organisms, *P. aeruginosa* serves as an exception, and the fluoroquinolones and protein synthesis inhibitors display slightly shorter PAEs, 1 to 2 hours and 2 to 3 hours on average for the respective drug classes. Similar observations have been described for the gram-negative anaerobes, with little or no PAE seen with the β-lactams but a measurable PAE observed for protein synthesis inhibitors.[35]

Given that most drug classes demonstrate a PAE, multiple mechanisms must exist by which this effect arises. For the β-lactams, researchers proposed that the PAE corresponds to the time required for the organism to synthesize new PBPs. The initial high β-lactam concentrations are thought to bind irreversibly to and thus inactivate the PBPs. As such, cell multiplication subsequently is prolonged until a critical number of PBPs are resynthesized and cell division can resume.[210] Within the large class of β-lactam agents, however, further distinctions can be made based on the specific PBP bound and the subsequent morphologic alteration that is induced. Against *E. coli*, the longest-lasting PAEs are observed for agents that induce the formation of spheroplasts and the shortest for agents that induce the formation of filaments. On drug removal, filaments (which may contain a biomass corresponding to more than 20 bacteria) readily separate into individual bacteria, whereas spheroplasts require a longer period to resynthesize a normal cell wall and resume replication.[86] Analogous to the β-lactams, the time required for the resynthesis or recovery of ribosomal proteins is probably responsible for the PAEs observed after the administration of an aminoglycoside. Evaluation of the intracellular events taking place in *E. coli* during aminoglycoside-induced PAEs suggests that both DNA and RNA synthesis resume immediately after drug removal; however, synthesis of structural and functional protein does not resume for nearly 5 hours.[13,102] For the fluoroquinolones, the proposed mechanism behind the PAE is not as consistent with the antimicrobial activity of these agents. A progressive increase in ^{3}H-thymidine incorporation in *S. aureus* during the PAE suggests that DNA synthesis continues to occur, and thus, the PAE may represent the time needed to repair the damage to DNA gyrase, the time required to re-establish the function of DNA gyrase after dissociation of the antibiotic from the enzyme, or the time required to synthesize new DNA gyrase.[77]

In addition to the specific drug-microbe combination, the magnitude and duration of drug exposure also have an impact on the PAE. Cell wall–acting agents (e.g., β-lactams and glycopeptides) demonstrate a concentration-dependent PAE. Increases in the concentration and duration of exposure, (i.e., increasing AUC) prolong the PAE in both gram-positive and gram-negative organisms to a point of maximal effect, typically 2 to 6 hours, although the time may be shorter for some members of this class. In fact, the PAE appears to be related to the log-normalized AUC in a sigmoidal manner.[34,86,121] Similarly, an increasing concentration of protein synthesis inhibitors is associated with progressive prolongation in PAE to a point of maximal effect. However, it can be difficult to establish with the aminoglycosides and fluoroquinolones because complete killing at supratherapeutic concentrations can obscure accurate determination of the PAE.[35,102] Bacteriostatic agents generally demonstrate concentration-independent PAEs such that increasing the duration of antibiotic exposure, but not the concentration, results in an increase in PAE to some maximal effect.[35]

As expected, the magnitude of the PAE also depends on whether antimicrobial agents are administered alone or in combination. Indifferent, additive, and synergistic PAEs all have been described with the use of combination therapy.[65,83,102] However, for some drug-microbe combinations, the magnitude of the PAE may differ with the phenotypic expression of resistance. For *Enterococcus faecalis* isolates that demonstrate only low-level resistance to streptomycin or gentamicin, the effect of a penicillin-aminoglycoside combination on the PAE is markedly synergistic. In contrast, for isolates with high-level resistance to the aminoglycosides, no synergistic PAE is observed.[224] Additionally, physicochemical factors can affect in vitro determination of PAEs, and, although the in vivo relevance is questionable, some are worthy of consideration. In *S. aureus*, the PAEs of penicillin and gentamicin are markedly protracted at the slightly acidic pH 6 as compared with a physiologic pH of 7.4. Given that a relatively acidic pH can occur at the site of active infection, a disease-PAE interaction is possible. A similar pH-dependent effect on PAE is not observed with the fluoroquinolones or macrolides against *S. aureus*, nor is it noted for *E. coli* and *P. aeruginosa* with the β-lactams, fluoroquinolones, and macrolides.[66,82] An increase in PAE in vitro also is observed as temperature drops, a finding that raises the question whether the PAE is truly altered or the generation time is lengthened. Regardless, the temperatures evaluated were much lower than physiologic temperatures and probably have no clinical impact in the presence of infection. Finally, anaerobic conditions are observed to increase the PAE for ciprofloxacin–*E. coli* and gentamicin–*S. aureus* combinations, but not with other combinations.[66]

The attention paid to PAE of late highlights its significance, which lies in the flexibility that it affords to extend the dosing interval before reexposure to drug, essentially re-dosing, is necessary. Researchers suggested that agents with a small PAE require that a dosing interval be selected that will maintain concentrations above the MIC throughout most of the interval. Agents with a more protracted PAE can be given less often and thus at higher doses, thereby supporting the argument for once-daily dosing with drugs such as the aminoglycosides. However, many nuances can be identified between the in vitro conditions under which PAEs are determined and the in vivo conditions of infection such that the ultimate clinical (i.e., therapeutic) implications of these data have yet to be determined. In vitro, attempts are made to remove the drug completely (i.e., abruptly terminate drug exposure to the microbe) after initial exposure when determining the PAE. In contrast, drug concentrations fall in a more controlled, typically first-order fashion in vivo. The impact of this distinction on PAEs in patients remains unknown. Variable growth rates or metabolic states of the organism when growing in vivo similarly may have an impact on the PAE. In vitro, a prolonged PAE is observed when *Enterococcus faecium* is exposed to the combination of gentamicin and penicillin; yet, when the same drug concentrations and the same inoculum size are achieved in the vegetation of a murine aortic valve endocarditis model, no prolongation of PAE can be observed.[91] Similarly, a rat model of *P. aeruginosa* endocarditis failed to demonstrate a PAE in vivo for the combination of imipenem and gentamicin despite a PAE of nearly 5 hours determined in vitro; however, an observable PAE in vitro and in vivo was noted in the same model when ciprofloxacin was evaluated.[90,101]

IMPACT OF INOCULUM SIZE ON THE CONCENTRATION-EFFECT RELATIONSHIP

The antimicrobial effect observed at any given drug concentration may vary with the number of organisms at the site of infection. Accordingly, the size of the inoculum can be a primary determinant of drug efficacy. Early investigations clearly demonstrated that the duration of the infection before treatment is a significant predictor of clinical response.[46] When therapy is delayed, presumably leading to a larger inoculum, the efficacy of

that therapy often is affected adversely. In non-neutropenic rats, larger doses of antibiotic were needed to treat *K. pneumoniae*, whether administered by continuous or intermittent infusion, when the infection was allowed to progress 34 hours before therapy was initiated versus 5 hours.[179] Many potential mechanisms have been proposed to account for this event. Large inocula perhaps generate a higher local density of enzymes that effectively reduce active (i.e., functional) antibiotic concentrations at the site of infection. Large bacterial populations also are statistically more likely to contain resistant organisms that arise by spontaneous mutation and predominate within the population as a result of the selective pressure posed by the antibiotic. One such example has been observed in a murine model of group A streptococcal myositis, in which the activity of penicillin is compromised most severely in the presence of a large inoculum (10^8 to 10^9 CFUs/mL), with relatively little impact on the activity of clindamycin. Given that the isolate did not produce β-lactamase and any residual PAE was irrelevant because both regimens maintained drug concentrations above the MIC for the duration of the dosing interval, this effect of the inoculum was attributed in part to the selection of a cell wall–deficient mutant.[199] Clindamycin, however, is not devoid of susceptibility to the inoculum effect for all organisms; an increase in the size of the inoculum or an increase in the time until therapy resulted in a lower reduction in $\log_{10}$CFUs in a murine *Bacillus fragilis* abscess model.[107] Another proposed mechanism for the effect of the inoculum lies in the finding that dense populations of organisms may grow at a slower rate or be metabolically inactive when compared with their less dense counterparts. An effect arising by this mechanism probably will not affect antimicrobial agents that are bactericidal against both rapidly growing and stationary-phase organisms (e.g., fluoroquinolones). However, for agents that exert their bactericidal effects primarily on rapidly dividing organisms (e.g., β-lactams), activity may be compromised in the presence of an established infection in which the bacterial population is large and as many as 90 percent of the organisms may be slowly dividing and, thus, metabolically inactive. For clinical isolates of group G streptococci, time-kill studies demonstrated rapid complete bacterial killing when the organism was primarily in log-phase growth, regardless of whether 10^4 or 10^7 organisms were involved. In contrast, when the organism was principally in the stationary phase, rapid and complete killing was observed only for the smaller inoculum (10^4 organisms), with no appreciable killing at 10^8 organisms. Furthermore, in patients with a protracted clinical course of infection or recalcitrant infection, therapy failed despite high drug doses and the presence of a susceptible isolate.[125]

Attempting to link multiple pharmacodynamic effects in evaluating the outcome of anti-infective therapy poses an arduous task from both theoretical and practical perspectives, and, hence, a complete discussion is not undertaken in this chapter. Nonetheless, the combination of such effects has received some attention in the literature and merits mention. Specifically, large inocula appear to be capable of modulating the PAE and subinhibitory effects observed with numerous agents. Exposure of *E. coli* to subinhibitory sparfloxacin concentrations produced a pronounced inoculum-dependent subinhibitory effect. At a dose that was 0.3 times the MIC, growth of the organism was delayed by 0.3, 0.9, and 3.3 hours with inocula of 10^8, 10^6, and 10^4 CFUs, respectively, with similar observations noted for other drug-microbe combinations.[34,156]

CONCENTRATION-DEPENDENT COMBINATION EFFECTS (SYNERGY AND ANTAGONISM)

As discussed earlier and elsewhere in this text, antimicrobial combinations are used for many purposes: to prevent or delay the emergence of resistance, to enable dosage reduction and thereby minimize dose-related toxicities, and to treat polymicrobial infections. Combination therapy can be designed with the goal of synergistic or additive activity in mind, or, alternatively, these effects can occur without conscious consideration or forethought. Moreover, the undesirable outcomes of indifference or antagonism similarly can result through the combination of agents. Although a complete review of antagonism and synergy is beyond the scope of this section, we would be remiss not to mention concentration-dependent combination effects. We provide this brief discussion specifically to point out that a simple classification of drug combinations as synergistic, antagonist, and so forth is not always feasible. As discussed elsewhere, disagreement in the classification certainly can arise when different methods are used for evaluation.[21,154]

However, even when restricted to evaluations using the same experimental methods, certain combinations can be classified differently, depending on antibiotic concentration. Synergy is observed with cefoperazone and low imipenem concentrations against MRSA; however, higher concentrations of imipenem antagonize the activity of cefoperazone. Although antagonism with imipenem often is explained away as being β-lactamase–mediated, the concentration-dependent antagonism observed remains unexplained by this mechanism because antagonism was shown for strains that lacked β-lactamase production.[170] The combination of penicillin and clindamycin at subinhibitory concentrations demonstrates synergy against group A beta-hemolytic streptococci. At concentrations ranging from two to four times the MIC, the combination demonstrates antagonism, and at clinically relevant concentrations (≈100 times the MIC), indifference is observed, with the combination exhibiting no advantage over either agent alone.[200] Against isolates of vancomycin-intermediate *S. aureus*, antagonism is observed when methicillin and vancomycin are combined at subinhibitory concentrations. However, when the methicillin concentration exceeds the MIC, synergy is observed with the same combination. Although the mechanism behind this differential activity remains unclear, researchers proposed that the antagonism observed at lower methicillin concentrations results from an increase in the density of non–cross-linked D-alanyl-D-alanine side chains, the target site for vancomycin and a substrate for the PBPs. This increase would decrease the efficacy of methicillin as a PBP inhibitor by substrate competition.[95] For certain strains of methicillin-sensitive *S. aureus*, a combination of rifampin and methicillin below the MIC is synergistic, but above the MBC it is antagonistic. Similarly, a rifampin-vancomycin combination is synergistic at concentrations close to the MBC but indifferent as concentrations rise.[230] Furthermore, for some drug-microbe combinations, it is not the classification per se that is affected by the concentration, but rather the magnitude of the effect observed. Against *E. faecium*, fixed piperacillin and variable teicoplanin concentrations are synergistic. However, the degree of synergy depends on the teicoplanin concentration; comparable synergistic activity occurs at teicoplanin concentrations of 2, 4, or 8 mg/L; reduced synergistic activity occurs at teicoplanin concentrations of 16 and 32 mg/L; and the greatest degree of synergy is observed at a concentration of 64 mg/L.[172]

Thus, the available data support the finding that in many cases, the clinical assignment or "expectation" of synergy, additivity, or antagonism by specific combinations of anti-infective agents cannot be assumed simply by virtue of the drug class or drug-specific mechanisms of action, or both. With drug combinations for which the activity against an infecting pathogen is potentially concentration-dependent, prediction of therapeutic outcome must entail some assessment, be it actual or theoretical (e.g., the use of pharmacokinetic modeling), of the time-dependent drug concentration profile at the site or sites of infection.

PHARMACOGENETIC DETERMINANTS OF EFFECT

Although this chapter is designed to detail the pharmacokinetic and pharmacodynamic determinants of anti-infective drug response, we would be remiss not to introduce the emerging role of pharmacogenetics as a predictor of drug response and a driver of anti-infective drug therapy. Although the application of molecular biology to infectious diseases traditionally has been limited to diagnostics (e.g., polymerase chain reaction–based assays for the detection of viral pathogens and slow-growing microorganisms) and disease monitoring (e.g., viral loads), molecular tools increasingly are finding application with identifying the likelihood of drug response a priori and determining optimal treatment strategies for infectious pathogens. Genotyping strategies are used to identify mutations that confer drug resistance and guide the selection of antiretroviral therapy in management of human immunodeficiency virus infection.[130,216] Similarly, viral genotyping appears to be among the most robust predictors of response to combination therapy in the treatment of hepatitis C virus,[126,182] and it has prompted similar investigations for hepatitis B virus.[84,114] Finally, commercially available molecular-based assays have been developed for the determination of methicillin resistance in *S. aureus*, multidrug resistance in *M. tuberculosis*, and vancomycin resistance in *Enterococcus* in clinical isolates. These kits appear to perform well when compared with conventional phenotype-based methods.[117,146,160] However, the degree of integration for these kits into routine clinical practice remains to be determined.

CONCLUSIONS

Since the mid-1950s, the development of pharmacokinetics has produced a powerful and valuable tool, with applications for both science and clinical medicine. For the clinical scientist, pharmacokinetics can be used to characterize a given drug by providing a profile of its absorption, distribution, metabolism, and excretion in individuals with and without disease, during normal alterations of human physiology (e.g., pregnancy), as well as under conditions of normal altered organ function or body composition (e.g., growth and development, senescence). For the clinical practitioner, the tool of pharmacokinetics can provide a means to individualize drug therapy by characterizing the relationship between drug dose and resultant drug concentrations in plasma or other relevant biologic fluids (e.g., urine, CSF, synovial fluid, pleural fluid, peritoneal fluid). When linked with information regarding the pharmacodynamic behavior of the antibiotic, the susceptibility of the organism, and the status of the host, the application of pharmacokinetics affords the practitioner the ability to exercise some degree of adaptive control over drug therapy by selecting a drug and dosing regimen that have the greatest likelihood of producing both efficacy and safety.

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CHAPTER 248

Antibacterial Therapeutic Agents

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This review of the use of antimicrobial agents is divided into two sections: (1) the clinical pharmacology of currently available antibacterial drugs and (2) the various aspects of administration of antimicrobial agents to infants and children. The second section includes dosage schedules and routes, prophylactic use of antimicrobial agents, considerations in writing orders and prescriptions, and other aspects of administration. Drugs of value in the treatment of disease caused by viruses, fungi, mycobacteria, and parasites are discussed in other chapters dealing with these pathogens. For the most part, only antimicrobial agents approved for use in infants and children by the U.S. Food and Drug Administration (FDA) are discussed, although promising new agents that are likely to be approved are mentioned where relevant.

CLINICAL PHARMACOLOGY

The antibacterial agents of value in treating infectious diseases in infants and children may be classified into five groups:

1. The β-lactams, including penicillins, cephalosporins, carbacephems, monobactams, and carbapenems
2. The glycopeptides (i.e., vancomycin)
3. The aminoglycosides
4. The macrolides, including erythromycin, clarithromycin, and azithromycin
5. Miscellaneous antibacterial agents, including chloramphenicol, clindamycin, colistin, fluoroquinolones, linezolid, rifamycins, the sulfonamides, and the tetracyclines

The following properties that govern the use of each group of drugs in infants and children are considered: mechanism of action, mechanisms of resistance, in vitro efficacy, pharmacokinetics, therapeutic uses, available preparations, and side effects and toxicity.

β-LACTAMS

BIOCHEMICAL STRUCTURE

The β-lactams are a large group of compounds that have in common a four-membered β-lactam ring. The subclasses of β-lactams differ from one another with regard to their side chains and the presence of other ring structures: the penicillins contain

a five-membered thiazolidine α-ring fused to the β-lactam ring,[155] the cephalosporins have a six-membered dihydrothiazine instead of the thiazolidine ring, and the carbacephems have a methylene group replacing the sulfur atom in the dihydrothiazine ring of the cephalosporin nucleus. The β-lactam ring is essential for antibacterial activity, whereas the side chains influence the pharmacologic properties of the β-lactam and the spectrum of antibacterial activity.

MECHANISM OF ACTION

Our understanding of the mechanism of action of the β-lactams has evolved over the past 3 decades. Previously, researchers thought that binding of β-lactam to a bacterial cell membrane–associated enzyme (transpeptidase) blocked the terminal step in synthesis of the peptidoglycan layer of the bacterial cell wall. Cell death would ensue because the weakened cell wall could not withstand the osmotic and mechanical pressure resulting from a growing bacterium.[268] More recent evidence suggested that it is a more complex process involving activation of endogenous autolytic systems[196,270] and inhibition of bacterial enzymes called the *penicillin-binding proteins* (PBPs), which include transpeptidase, carboxypeptidase, and endopeptidase. These enzymes are located beneath the cell wall and are responsible for cell wall synthesis by achieving cross-linkage between peptide chains of peptidoglycans. PBPs are vital for cell division, cell shape, and structural integrity. Because the specific PBPs within each bacterial species and the affinity of each β-lactam antibiotic for a particular PBP differ, some β-lactams have better activity than do others against particular bacteria. The various β-lactam antibiotics can have different morphologic effects on the same bacterial species; this property is thought to be related to specific functions of the PBP to which the β-lactam binds.[257] Some bacteria have a deficiency in the system of autolytic enzymes that results in inhibition, but not killing of the bacteria by a β-lactam that otherwise would be bactericidal. This phenomenon is called *tolerance* and is demonstrated in vitro by a minimal inhibitory concentration (MIC) in the susceptible range and a ratio of minimal bactericidal concentration (MBC) to MIC of 32 or greater.[118]

β-Lactam antibiotics are bactericidal against most susceptible bacteria. The nature of the bactericidal activity has been described as time-dependent, as opposed to the concentration-dependent bactericidal activity of aminoglycosides.[169] Bactericidal activity is thought to be optimal when the concentration of β-lactam antibiotic at the site of infection is 4 to 10 times greater than that of the MIC (or MBC) of the infecting organism. The rapidity and extent of killing are not increased when concentrations exceed that ratio. A more important determinant of bactericidal activity for β-lactams is the length of time during the dosing interval that the concentration of antibiotic exceeds the MIC (time > MIC) for the infecting organism. Optimal bacterial killing correlates with time greater than MIC that exceeds 40 to 50 percent of the dosing interval.[71]

MECHANISMS OF RESISTANCE

Bacteria can acquire resistance to an antibiotic by at least four mechanisms: (1) alteration in the antimicrobial target, (2) decreased uptake of the antibiotic by expression of efflux pumps or by reduction of cell membrane permeability that is determined by porin channels, (3) production of an enzyme that inactivates the antibiotic, and (4) production of an alternative metabolic pathway that bypasses the action of the drug.[262] With respect to β-lactam antibiotics, resistance involves alterations in PBPs leading to decreased affinity for the β-lactam, decreased permeability of the bacterial cell wall resulting in diminished amounts of β-lactam reaching the PBPs, or production of β-lactamases that hydrolyze the β-lactam ring. Hydrolysis of β-lactams is the mechanism that is most significant clinically. Gram-positive bacteria excrete their β-lactamases outside the cell wall, whereas the β-lactamases of gram-negative bacteria remain in the periplasmic space. The spectrum of β-lactamase activity involves the following: narrow-spectrum penicillinases that preferentially hydrolyze penicillins; broad-spectrum β-lactamases that hydrolyze penicillins and cephalosporins equally well; cephalosporinases that preferentially hydrolyze cephalosporins and are resistant to inhibition by clavulanic acid; and ESBLs that hydrolyze first-, second-, and third-generation cephalosporins but are susceptible to inhibition by clavulanic acid; and carbapenemases that inactivate all β-lactams, including imipenem-cilastatin.[51]

PENICILLINS

Although first discovered by Fleming in the late 1920s, penicillin G was not available for general use in the United States for another 20 years. Since that time, numerous semisynthetic penicillins have been developed. The penicillins can be classified into four groups based on their antimicrobial activity, with some overlap (Table 248–1). The spectrum of activity among the compounds within each group usually is similar, whereas differences are attributable to their pharmacologic properties (Table 248–2).

Penicillin G and Penicillin V

Despite the more than 50 years that penicillin G has been in use, some bacteria continue to be exquisitely susceptible. Resistant strains of *Streptococcus pyogenes* (group A Streptococcus) and *Streptococcus agalactiae* (group B Streptococcus) have not emerged. Penicillin G remains the drug of choice for the treatment of disease caused by a wide variety of microorganisms (Table 248–3).

TABLE 248–1 Classification Scheme for Penicillins

Generic Name	Trade Name	Route
Natural Penicillins		
Penicillin G	Many	PO,* IM, IV
Penicillin V	Many	PO
Aminopenicillins		
Ampicillin	Many	PO, IM, IV
Amoxicillin	Many	PO
Amoxicillin/clavulanate	Augmentin	PO
Ampicillin/sulbactam	Unasyn	IM, IV
Bacampicillin	Spectrobid	PO
Penicillinase-Resistant Penicillins		
Cloxacillin	Cloxapen	PO
Dicloxacillin	Dycill, Dynapen, Pathocil	PO
Methicillin	Staphcillin	IM, IV
Nafcillin	Nafcil, Nallpen, Unipen,	IM, IV, PO
Oxacillin	Bactocill, Prostaphlin	IM, IV, PO
Extended-Spectrum Penicillins		
Ticarcillin	Ticar	IM, IV
Ticarcillin/clavulate‡	Timentin	IV
Mezlocillin	Mezlin	IM, IV
Piperacillin†	Pipracil	IM, IV
Piperacillin/tazobactam†	Zosyn	IV

**No longer available.*
†Safety and efficacy have not been established for children younger than 12 years.
‡Safety and efficacy have not been established for children younger than 1 month.
IM, Intramuscularly; IV, intravenously; PO, orally.
Modified from USP DI: Information for the Health Care Professional. Vol. 1, Thomson MICROMEDEX, 2006.

TABLE 248–2 Pharmacokinetics of Penicillins

Antibiotic	Oral Absorption (%)	Protein Binding (%)	Metabolized (%)	Urinary Recovery* (%)	Approximate Half-Life† (hr)
Natural Penicillins					
Penicillin G	—	60	20	20/60-90	0.5-0.7
Penicillin V	60-73	80	55	20-40	0.5-1
Aminopenicillins					
Ampicillin	35-50	20	10	40-45/75-90	1-1.5
Amoxicillin	75-90	20	10	60-75	1
Penicillinase-Resistant Penicillins					
Cloxacillin	50	95	20	30-60	0.5-1
Dicloxacillin	37-50	95-98	10	50-70	0.5-1
Methicillin		40	10	60-80	0.3-1
Nafcillin	Erratic	90	60-70	11-30	0.5-1.5
Oxacillin	30-35	90-94	45	55-60	0.4-0.7
Extended-Specturm Penicillins					
Ticarcillin		45-60	15	60-80	1.0-1.2
Mezlocillin		16-42	20-30	55-60	0.8-1.1
Piperacillin		16	20-30	60-80	0.6-1.2

**Urinary recovery after oral/parenteral administration.*
†With normal renal function.
Modified from USP DI: Information for the Health Care Professional. Vol. 1, Thomson MICROMEDEX, 2006.

TABLE 248–3 Microorganisms for Which Penicillin G or V Is the Drug of Choice

Actinomyces israelii
Bacillus anthracis
Clostridium species
Corynebacterium diphtheriae
Erysipelothrix rhusiopathiae
Leptospira species
*Neisseria gonorrhoeae**
*Neisseria meningitidis**
Pasteurella multocida
Spirillum minus
Staphylococcus aureus†*
Streptobacillus moniliformis
Streptococcus groups A, B, C, D, G; viridans group†; anaerobic strains
Streptococcus pneumoniae†
Treponema pallidum

**Strains that do not produce β-lactamase.*
†Only those without altered penicillin-binding proteins.

The mechanism by which most bacteria have acquired resistance to penicillin G is that of β-lactamase production; resistance caused by altered PBPs occurs less commonly. Most strains of *Staphylococcus aureus* and *Staphylococcus epidermidis* produce penicillinase. *Neisseria gonorrhoeae* gradually developed resistance to penicillin G beginning in the late 1960s by various mechanisms including plasmid-mediated production of a TEM-1–like β-lactamase, as well as chromosomal mutations in at least four genes (*penA*, *penB*, *ponA*, and *mtrR*) that encode increased expression of an efflux pump and alterations in PBPs and porins.[215] Mechanisms of β-lactam resistance in *Bacteroides* spp. and other gram-negative anaerobes include production of class A β-lactamases (penicillinase or cephalosporinase) and class B metallo-β-lactamases that hydrolyze carbapenems, as well as alteration of PBPs.[14] Historically, penicillin has been the antibiotic of choice for treatment of *Neisseria meningitidis* infections, but strains with reduced susceptibility to penicillin have been reported in Europe, South America, Asia, Australia, and the United States, where approximately 15 percent of strains have alterations in PBP2 encoded by the *penA* gene that likely results from mosaic genes derived through transformation with DNA from commensal *Neisseria* spp. in the nasopharynx of colonized individuals. Although penicillin resistance resulting from production of β-lactamase has been reported, such strains are extremely rare.[143]

Of particular concern is the global dissemination of strains of *Streptococcus pneumoniae* resistant to penicillin G as a result of altered PBPs. The first documented clinical case of infection caused by penicillin-resistant pneumococcus was reported in 1967 from Australia. Surveillance data in the United States estimated the average rate of penicillin nonsusceptibility to be 33 percent (high-level resistance, 16%) with the highest incidence of 44 percent reported in the south-central region.[138] However, since the introduction of the pneumococcal conjugate vaccine in 2000, disease in young children caused by vaccine serotypes of penicillin-nonsusceptible *S. pneumoniae* has decreased by approximately 90 percent. Conversely, invasive disease caused by non-vaccine serotypes increased.[162] Currently, approximately 6 percent of pneumococci in the United States exhibit a multidrug-resistance phenotype defined as resistance to penicillin, trimethoprim-sulfamethoxazole (TMP-SMX), erythromycin, clindamycin, and tetracycline.[138] Although penicillin-resistant pneumococcal meningitis has developed in patients who were receiving vancomycin for pneumococcal sepsis,[61] no treatment failures have been documented when the appropriate dosage of vancomycin was administered.

In 2008, the Clinical and Laboratory Standards Institute published new *S. pneumoniae* breakpoints for penicillin because of a reevaluation that showed clinical response to penicillin was being preserved in clinical studies of pneumococcal infection, despite reduced susceptibility response in vitro. Under the former criteria, susceptible, intermediate, and resistant MIC breakpoints for penicillin were <0.06, 0.12-1, and >2 μg/mL, respectively, for all pneumococcal isolates, regardless of clinical syndrome or route of penicillin administration. Those breakpoints remain unchanged for patients without meningitis who can be treated with oral penicillin. However, for patients without meningitis who are treated with intravenous penicillin, the new breakpoints are <2, 4, and >8 μg/mL, respectively. Furthermore, isolates from patients with meningitis are now categorized as either susceptible or resistant, with intravenous penicillin breakpoints of <0.06 or >0.12 μg/mL, respectively. Use of narrow-spectrum agents, such as penicillin, is encouraged to prevent the spread of antimicrobial-resistant *S. pneumoniae* and also the spread of methicillin-resistant *S. aureus* and *Clostridium difficile*, which can result from use of broader-spectrum antimicrobials.

Several oral and parenteral forms of penicillin G are available. Selection of a preparation is based on the pattern of antimicrobial activity, including the peak and duration of activity in serum and tissues, and factors that reflect absorption, distribution, and excretion of the drug. These characteristics of penicillins are as follows:

1. Aqueous (water-soluble) penicillin G produces high peak concentrations of antibacterial activity in serum within 30 minutes after intramuscular administration but is excreted rapidly; thus, the concentration in serum is low within 2 to 4 hours. If aqueous penicillin G is given by the intravenous route, the peak is higher and occurs earlier, and the duration of antibacterial activity in serum is shorter (≈2 hours). Aqueous penicillin G given intravenously is used for severe disease such as meningitis, complicated pneumonia, and endocarditis caused by susceptible pathogens. In such cases, the drug should be administered at frequent intervals, usually every 4 hours, until the infection has been controlled.

2. Procaine penicillin G given intramuscularly produces lower concentrations of serum antibacterial activity (≈10% to 30% of the peak concentration achieved by the same dosage of the aqueous form), but activity persists in serum for as long as 12 hours. Intramuscular administration of procaine penicillin G should be reserved for patients with mild to moderate disease who cannot tolerate oral preparations. A 10-day course of this agent may be used as an alternative to intravenously administered aqueous penicillin G for infants with documented or suspected congenital syphilis.[278]

3. Benzathine penicillin G given intramuscularly is a repository preparation that provides low concentrations of serum activity (≈1% to 2% of the peak concentration achieved by the same dosage of the aqueous form). After administration of this drug, concentrations of penicillin are measurable in serum for 3 weeks or more and in urine for several months. Pain at the site of injection is the major deterrent to widespread use of this unique antibiotic. A combination of the benzathine and procaine salts (900,000 and 300,000 U, respectively) is a less painful treatment and is comparable in efficacy to benzathine alone (1,200,000 U) for the treatment of streptococcal pharyngitis.[22] Benzathine penicillin G is appropriate for only highly sensitive organisms present in tissues that are well vascularized so that the drug can diffuse readily to the site of infection. Thus, benzathine penicillin G is suitable for treatment of children with group A streptococcal pharyngitis or impetigo and for prophylaxis of streptococcal infection in children who have had rheumatic carditis. Current recommendations by the Centers for Disease Control and Prevention (CDC)[278] for management of syphilis include the use of benzathine penicillin G for primary, secondary, and early latent syphilis (<1 year's duration). Benzathine penicillin G also is recommended for infants with suspected congenital syphilis who do not meet the criteria for therapy with a 10-day course of aqueous or procaine penicillin G and whose follow-up can be ensured for repeat serologic testing.

4. Oral preparations of buffered penicillin G (no longer available in the United States) and phenoxymethylpenicillin (penicillin V) are absorbed well from the gastrointestinal tract. The peak concentration of serum activity of penicillin V is approximately 40 percent and that of buffered penicillin G is approximately 20 percent of the concentration achieved by the same dosage of aqueous penicillin G administered intramuscularly. Therefore, oral penicillins may be satisfactory for treating mild to moderately severe infections caused by susceptible organisms. Penicillin V and penicillin G have equivalent activity in vitro against gram-positive cocci, but penicillin V is less active than is penicillin G against *N. meningitidis*, *N. gonorrhoeae*, and susceptible strains of *Haemophilus influenzae*.[155] The benefit of treating streptococcal tonsillopharyngitis with a 10-day regimen of penicillin V for the prevention of acute rheumatic fever is based on indirect evidence derived from rates of pharyngeal eradication of *S. pyogenes* equivalent to those of injectable penicillin, the treatment with proven efficacy.[80,252]

All penicillins are excreted by both glomerular filtration and tubular secretion. The concomitant use of probenecid, a drug that blocks tubular secretion of organic acids, with a penicillin can produce higher peaks and more sustained concentrations of antimicrobial activity. Dosages and dosing intervals may need adjustment when penicillins are administered to persons with altered renal function.

Penicillinase-Resistant Penicillins

The semisynthetic penicillinase-resistant penicillins were developed in response to the emergence of penicillinase-producing staphylococci. The acyl side chain, by means of steric hindrance, prevents hydrolysis of the β-lactam ring by penicillinases. Most strains of *S. aureus* produce penicillinase, regardless of whether the infection is health care–associated or community-acquired. Thus, penicillinase-resistant penicillins are the β-lactams of choice for the initial management of patients with suspected staphylococcal disease. However, alternative effective antimicrobials should be substituted or added if endemic rates of antibiotic resistance exceed 10 to 15 percent or if the severity of the disease warrants empiric treatment of methicillin-resistant *S. aureus* (MRSA).[110] With the exception of methicillin, these agents are active against streptococci and can be used for empiric treatment of infections commonly caused by both staphylococci and streptococci. Because these agents are less active than is penicillin G against streptococci, penicillin G should be used instead of these agents if streptococci alone are isolated from culture. Penicillinase-resistant penicillins have no activity against gram-negative bacteria or enterococci.[181]

Methicillin was the first penicillinase-resistant penicillin to be introduced and was available in parenteral form only. Oxacillin and nafcillin are available in both parenteral and oral preparations. Cloxacillin and dicloxacillin are available in oral forms only and are absorbed more efficiently from the gastrointestinal tract than are the other oral drugs. Differences among these five penicillins include routes of elimination, degree of binding to proteins and degradation by β-lactamases, and in vitro susceptibility.[207] However, all are effective in the treatment of susceptible strains of staphylococcal isolates, and clinical studies have shown them to be equivalent when used at appropriate dosage schedules. Suspension formulations are poorly tolerated because of their unpleasant taste. Disease caused by methicillin-resistant staphylococci was reported shortly after introduction of the drug in the 1960s. Resistance is caused by alterations in PBPs, rather than production of β-lactamase. The *mecA* gene encodes a new PBP2a that has low affinity for β-lactams, thereby resulting in resistance to all β-lactam antibiotics currently available, including penicillinase-resistant penicillins, cephalosporins, and carbapenems. The *mecA* gene is carried on one of four mobile genetic elements known as the staphylococcal chromosomal cassette (SCC)*mec* I to IV that differ in size and the presence of additional resistance genes. The mechanisms of resistance to other antibiotics are unrelated to PBPs and may be plasmid-mediated or chromosomal.[179] SSC*mec* IV is found predominantly in community-acquired strains of MRSA (CA-MRSA) that are not associated with multidrug resistance, whereas SSC*mec* I to III are found typically in health care–associated strains that are resistant to multiple antibiotics including aminoglycosides, clindamycin, fluoroquinolones, fusidic acid, macrolides, rifampin, sulfonamides (TMP-SMX), and tetracyclines.[232] However, these distinguishing characteristics are tending to merge as *S. aureus* becomes increasingly resistant. Coagulase-negative staphylococci, including *S. epidermidis*, are residents of the normal microbial flora of the skin and are occasional contaminants of body fluid cultures. These organisms can be pathogens in certain settings, such as in

neonates, or in infections of prosthetic devices, such as heart valves or cerebrospinal fluid (CSF) shunts. Most strains of coagulase-negative staphylococci produce a penicillinase that inactivates penicillin G, penicillin V, and ampicillin, and many strains have *mecA*-mediated altered PBP2a leading to methicillin resistance. In addition, these methicillin-resistant, coagulase-negative staphylococci frequently are resistant to cephalosporins, erythromycin, and clindamycin. Vancomycin is the drug of choice for pathogenic coagulase-negative staphylococci and severe disease known or suspected to be caused by MRSA. Some experts advocate the addition of nafcillin or oxacillin for empiric therapy for suspected MRSA in patients who are severely ill, although toxicity may be increased with combination therapy.[96,110]

Aminopenicillins

The aminopenicillins are semisynthetic β-lactam antibiotics formed by the addition of an amino group to benzylpenicillin. Amoxicillin differs from ampicillin by the presence of a hydroxyl group on the phenyl side chain. The aminopenicillins were the first penicillins that had activity against some gram-negative organisms, including *H. influenzae*, *Escherichia coli*, *Proteus mirabilis*, *Salmonella* spp., and *Shigella* spp., while retaining activity against penicillin-susceptible, gram-positive bacteria.[206]

When compared with penicillin G, aminopenicillins are significantly more active against *Listeria monocytogenes*, slightly more active against enterococci, equally active against *Actinomyces*, *N. meningitidis*, and clostridial and corynebacteria species, and slightly less active against group A streptococci, group B streptococci, and pneumococci. Aminopenicillins are the drugs of choice for the treatment of infections caused by *L. monocytogenes* and enterococci. Other organisms susceptible in vitro to ampicillin and amoxicillin include non–penicillinase-producing strains of *H. influenzae*, *Moraxella catarrhalis*, *N. gonorrhoeae*, *S. aureus*, *E. coli*, *Salmonella*, and *Shigella*. A survey of health care–associated and community-acquired *H. influenzae* and *M. catarrhalis* strains isolated from the blood and respiratory tracts of children in North America documented ampicillin resistance rates of 34 percent and 99 percent, respectively.[142] The in vitro spectrum of activity for amoxicillin and ampicillin is identical, except amoxicillin is two times less active against *Shigella* and two to four times more active against enterococci and *Salmonella* than is ampicillin.[206]

As seen with penicillin G, the primary means of acquired resistance to aminopenicillins is the production of β-lactamase. However, organisms that are resistant to penicillin G or to methicillin because of altered PBPs—*S. pneumoniae* and *S. aureus*, respectively—also are resistant to aminopenicillins. β-Lactamase–negative, ampicillin-resistant strains of *H. influenzae* have been identified in the United States much less frequently than in Japan (0.2% versus 2.6%, respectively).[134]

The broad-spectrum bactericidal activity of ampicillin and amoxicillin provides the basis for the use of these drugs as empiric therapy for lower respiratory infections and acute otitis media, for which amoxicillin remains the drug of choice.[9] Aminopenicillins are indicated for treatment of susceptible strains of *Shigella*- or *Salmonella*-associated enteric infections as well as susceptible acute and chronic infections of the urinary tract. By bacteriologic and clinical measures, amoxicillin is significantly less effective than is ampicillin for the treatment of shigellosis.[204]

Both drugs are available for oral administration; ampicillin alone is available in a parenteral form. Amoxicillin provides higher and more prolonged serum concentrations than those achieved with equivalent dosages of ampicillin; thus, amoxicillin can be given in lower dosage, two or three times a day rather than four times, as required for ampicillin. An additional advantage of amoxicillin is that absorption is not altered when the antibiotic is administered with food, whereas absorption of ampicillin is decreased significantly when it is given with food. Ampicillin is associated more frequently with diarrhea than is amoxicillin.

Extended-Spectrum Penicillins

Extended-spectrum penicillins are semisynthetic derivatives of ampicillin that have better activity against gram-negative organisms because of a higher affinity for PBPs and greater penetration through the gram-negative outer membrane. Carbenicillin and ticarcillin, the carboxypenicillins, have a carboxyl group replacing the amino group side chain of ampicillin, whereas the acylureidopenicillins have a ureido (urea) side chain (mezlocillin) or ureido and piperazine side chains (piperacillin). Their pharmacologic properties are similar and include susceptibility to hydrolysis by staphylococcal penicillinases and, to a lesser extent, the β-lactamases of gram-negative bacteria. They differ somewhat in their toxicities and spectrum of activity.

The activity of carbenicillin is equivalent to or slightly less than that of ampicillin against non–β-lactamase–producing *N. gonorrhoeae*, *N. meningitidis*, *H. influenzae*, *E. coli*, *P. mirabilis*, *Salmonella* spp., and *Shigella* spp. It is less active than ampicillin against group A streptococci, pneumococci, and enterococci. Its activity is variable against many *Bacteroides fragilis*, *Enterobacter*, and *Serratia* organisms, and it is not active against *Klebsiella*. An oral preparation, carbenicillin indanyl (Geocillin) is active against susceptible strains of *Pseudomonas aeruginosa*, but its production was discontinued in 2008.

The activity of ticarcillin is similar to that of carbenicillin, but it is two to four times more active against some strains of *P. aeruginosa* and less active against gram-positive cocci. In addition, ticarcillin can be used for infections caused by susceptible strains of *Acinetobacter*. Because of its increased activity, ticarcillin may be used in smaller doses than needed with carbenicillin for the treatment of disease caused by gram-negative organisms.

Piperacillin and mezlocillin are parenteral penicillins with a spectrum of activity similar to that of ticarcillin but greater activity in vitro against some gram-negative bacilli and anaerobic bacteria. In vitro studies against *P. aeruginosa* demonstrated that piperacillin has activity that is four times that of mezlocillin and ticarcillin and about eight times that of carbenicillin, the clinical significance of which is unknown. Piperacillin and mezlocillin are more active in vitro than are carbenicillin or ticarcillin against susceptible strains of *E. coli*, *Klebsiella*, *Enterobacter*, *Serratia*, and *B. fragilis*. Furthermore, piperacillin, mezlocillin, and ticarcillin are effective in the treatment of infections caused by susceptible strains of *Citrobacter*, indole-positive *Proteus*, and *Providencia*. Carbenicillin and ticarcillin are disodium salts and contain 4.7 mEq (108 mg) and 5.2 mEq (120 mg) of sodium per gram, respectively. The acylureidopenicillins are monosodium salts that have a lower sodium content (1.9 to 2.2 mEq) than those of the carboxypenicillins. The amount of sodium administered may be of concern when treating certain patients with renal or cardiac disease. Hypokalemia with metabolic alkalosis occasionally occurs with the administration of extended-spectrum penicillins, especially the carboxypenicillins. The penicillin acts as a nonreabsorbable anion in the distal renal tubules, where it affects normal hydrogen exchange and secondarily results in loss of potassium. These extended-spectrum penicillins can bind to platelet adeno-sine diphosphate receptors and thereby result in abnormal platelet aggregation and prolonged bleeding times. This dose-related phenomenon occurs more frequently with the administration of ticarcillin than with the acylureidopenicillins. The effects on platelet function may be a consideration when choosing empiric therapy for a thrombocytopenic patient with suspected gram-negative infection.[280]

When carboxypenicillins or ureidopenicillins have been used as single agents for empiric treatment of patients with severe gram-negative infections, fever and neutropenia, or polymicrobial intra-abdominal infections, bacterial resistance has developed

in 10 to 15 percent of cases during treatment. Combinations of extended-spectrum penicillins with an aminoglycoside demonstrate synergistic in vitro inhibition of certain gram-negative bacteria, including *P. aeruginosa*. Based on in vitro data and clinical experience, extended-spectrum penicillins should be combined with an aminoglycoside in some patients (particularly if immunocompromised) with severe infections, to prevent the development of bacterial resistance.[280] All the extended-spectrum penicillins are available in parenteral formulations only. Because of limited clinical experience in infants and children, piperacillin has not been approved for use in patients younger than 12 years old.

β-Lactam–β-Lactamase Inhibitor Combinations

β-Lactamase inhibitors are compounds that have weak antibacterial activity but can bind irreversibly to the catalytic site of many β-lactamases and render them inactive. The inhibitors currently in use are clavulanic acid, sulbactam, and tazobactam, the latter of which are halogenated penicillanic acid derivatives. All three inhibitors are identical in their mode of activity but differ to some degree in their potency and spectrum of enzyme inhibition. These agents primarily inhibit the plasmid encoded β-lactamases and less frequently the chromosomally encoded β-lactamases. β-Lactamases are classified on the basis of their (1) responses to β-lactamase inhibitors, (2) spectrum of antibiotic substrates (Bush classification, groups 1 to 4), or (3) primary molecular structure (Amber classification); serine β-lactamases are class A, C or D; metalol-β-lactamases are class B. Bush group 2 corresponds to Amber classes A and D, which are encoded frequently by genes carried on plasmids and easily spread among different species, whereas Bush groups 1 and 3 (Amber classes C and B, respectively) are frequently encoded by chromosomal genes and therefore are confined to certain species.[74,218] Chromosomally mediated, inducible Bush group 1 β-lactamases that are produced by certain species of *Citrobacter*, *Enterobacter*, *Morganella*, *Pseudomonas*, and *Serratia* are not inhibited by the β-lactamase inhibitors, and, consequently, they remain resistant to the accompanying β-lactam. However, *Bacteroides*, *Klebsiella*, *Legionella*, and *Moraxella* spp. produce chromosomally mediated β-lactamases that are effectively inactivated. The β-lactamase inhibitors are most effective against *S. aureus*, *H. influenzae*, *M. catarrhalis*, *Bacteroides* spp., *E. coli*, and other Enterobacteriaceae. Clavulanic acid and tazobactam are more potent inhibitors of β-lactamases. Furthermore, clavulanate induces Bush group 1 enzymes. Disks containing β-lactamase inhibitors can be used in the clinical microbiology laboratory to detect pathogens that produce Amber class A extended-spectrum β-lactamases (ESBLs) of the TEM and SHV types that may be overlooked otherwise.[52] β-Lactamase inhibitors have been formulated in a fixed ratio with a β-lactam antibiotic. The spectrum of activity of each combination is determined primarily by the spectrum of activity of the β-lactam. However, many determinants of the inhibitor influence its activity, including its affinity for the β-lactamase and its ability to traverse the gram-negative cell wall to bind to periplasmic β-lactamases.[173] From a clinical perspective, all three β-lactamase inhibitors are considered therapeutically equivalent. The main indication for the use of these combination antimicrobial agents is for the treatment of health care–associated infections or infections caused by susceptible β-lactamase–producing pathogens.

AMOXICILLIN-CLAVULANIC ACID

Amoxicillin combined with potassium clavulanate was introduced in 1984 for oral administration. The pharmacokinetic properties of the two drugs are similar; both are absorbed rapidly and are not affected when taken with meals. Gastrointestinal side effects, including nausea, vomiting, and diarrhea, occur more commonly with amoxicillin-clavulanate than with amoxicillin alone.[154]

The combination drug is equivalent to amoxicillin alone in activity against amoxicillin-susceptible organisms. The addition of clavulanic acid extends the activity of amoxicillin to include β-lactamase–producing strains of *S. aureus* (but not methicillin-resistant strains), *H. influenzae*, *M. catarrhalis*, *N. gonorrhoeae*, *E. coli*, *Proteus*, *Klebsiella*, *Providencia*, *Haemophilus ducreyi*, and some anaerobic bacteria, including *B. fragilis*. Some β-lactamase–producing, gram-negative bacilli are resistant to amoxicillin-clavulanic acid because of either hyperproduction of β-lactamase or production of a β-lactamase that is not susceptible to clavulanate. Amoxicillin-clavulanate is not active against penicillin-resistant *S. pneumoniae* or vancomycin-resistant enterococci.

Amoxicillin is considered the preferred therapy for low-risk children with uncomplicated, mild to moderately severe disease of the respiratory tract, including initial empiric therapy for otitis media. In children who are at risk for penicillin-nonsusceptible pneumococcal acute otitis media, a 90-mg/kg daily regimen in two divided doses is recommended. Alternative therapy, including amoxicillin-clavulanic acid, should be considered if a β-lactamase–producing organism is known or suspected to be the cause of the disease based on risk factors, treatment experience, or microbiologic results. In 2001, high-dose amoxicillin-clavulanic acid suspension (Augmentin ES-600; 600 mg of amoxicillin/5 mL) was approved for use in children in the United States. The recommended dosage of this formulation is 90 mg/kg/day in two divided doses for the treatment of high-risk patients with acute otitis media.[9] The high ratio (14:1) of amoxicillin to clavulanate optimizes the penicillin dose without intensifying the risks of undesirable adverse effects associated with the β-lactamase inhibitor. The combination drug is useful in areas where the proportion of β-lactamase–producing strains of *H. influenzae* is large (>30%) and where *M. catarrhalis* organisms (most of which are β-lactamase producers) are identified more frequently as pathogens in otitis media, sinusitis, and other respiratory tract infections. Amoxicillin-clavulanate also has been used successfully for the oral treatment of urinary tract, skin, and soft tissue infections, as well as for the treatment of human and animal bite wounds.[52]

AMPICILLIN-SULBACTAM

Ampicillin combined with sulbactam as a parenteral β-lactam–β-lactamase inhibitor combination was approved in 1987. The spectrum of activity of ampicillin-sulbactam is similar to that of amoxicillin-clavulanate. It is most useful as monotherapy for potential polymicrobial intra-abdominal or gynecologic infections and is effective for treatment of soft tissue, urinary tract, and respiratory tract infections. Ampicillin-sulbactam has been shown to be safe and efficacious for the treatment of skin and skin structure infections caused by susceptible organisms in children aged 3 months to 12 years.[16] The combination of ampicillin-sulbactam and an aminoglycoside has efficacy equivalent to that of a combination of ampicillin, clindamycin, and an aminoglycoside for empiric treatment of intra-abdominal infections in children.[66] Ampicillin-sulbactam is potentially useful for multidrug-resistant health care–associated infections caused by *Acinetobacter baumannii-calcoaceticus* complex.[199] Carbapenems and third-generation cephalosporins are comparatively more active against members of the Enterobacteriaceae. Because ampicillin-sulbactam has no activity against certain strains of the Enterobacteriaceae that produce Bush group 1 β-lactamases and *P. aeruginosa*, it should not be used as empiric single-agent therapy for febrile neutropenic patients or severely ill patients with probable bacteremia.

TICARCILLIN-POTASSIUM CLAVULANATE

Ticarcillin combined with potassium clavulanate was approved for use in 1985. It extends the spectrum of activity of ticarcillin

to include β-lactamase–producing strains of staphylococci (but not methicillin-resistant strains), *H. influenzae*, *M. catarrhalis*, *E. coli*, *Klebsiella*, *Proteus*, *Providencia*, *N. gonorrhoeae*, and *B. fragilis*.[63] Ticarcillin-clavulanate also often is active against multidrug-resistant *Stenotrophomonas maltophilia*. Enterococci are moderately resistant to this agent. Because clavulanic acid does not inhibit Bush group 1–inducible chromosomal β-lactamases, *Citrobacter*, *Enterobacter* and *Serratia* spp., as well as de-repressed mutant strains of *P. aeruginosa* that are resistant to ticarcillin because of inducible cephalosporinases, also are resistant to ticarcillin-potassium clavulanate. Ticarcillin-potassium clavulanate is approved for use in children older than 3 months of age for lower respiratory, skin and skin structure, urinary tract, bone and joint, and intra-abdominal infections caused by susceptible pathogens.[34] Treatment of febrile neutropenic adult patients with ticarcillin-clavulanate combined with an aminoglycoside has been shown to be effective.

PIPERACILLIN-TAZOBACTAM

Piperacillin combined with tazobactam was approved for use in adults in 1993. It extends the spectrum of activity of piperacillin to include β-lactamase–producing strains of oxacillin-susceptible *S. aureus*, many members of the Enterobacteriaceae, and virtually all gram-positive and gram-negative anaerobes; its spectrum of activity is superior to that of ceftazidime and other β-lactam–β-lactamase inhibitor combinations. It has greater activity than does ticarcillin-clavulanate against gram-positive and gram-negative bacteria and equivalent activity against gram-positive bacteria compared with ampicillin-sulbactam. Tazobactam, however, does not increase the activity of piperacillin against bacteria with resistance that is mediated by altered cell wall permeability to piperacillin, including certain strains of *P. aeruginosa*. Penicillin-resistant pneumococci, methicillin-resistant staphylococci, *Corynebacterium jeikeium*, most *Enterococcus faecium* strains, and most gram-negative bacteria that produce Bush group 1 β-lactamases (*Citrobacter*, *Enterobacter*, *Morganella*, certain strains of *Pseudomonas*, and *Serratia* spp.) are resistant to piperacillin-tazobactam.[52] Although studies have shown piperacillin-tazobactam to be safe and effective for the treatment of lower respiratory tract, intra-abdominal, pelvic, skin and skin structure, bone, and diabetes-related foot infections in adults, as well as for empiric treatment of febrile episodes in neutropenic adults,[40] the experience in children is limited. The safety and efficacy of piperacillin-tazobactam have not been established for children younger than 12 years of age or neonates. Nonetheless, it is a potentially useful agent that has been used for the treatment of serious or complicated polymicrobial infections in children, especially in febrile neutropenic children, as initial empiric treatment. This antimicrobial agent should be used in combination with an aminoglycoside for serious *P. aeruginosa* infections to prevent the development of resistance. Clinicians also should be aware that administration of piperacillin-tazobactam can cause false-positive *Aspergillus* galactomannan test results for up to 5 days after cessation of treatment.[13]

Adverse Effects and Sensitization

The penicillins are unique among antimicrobial agents in having little dose-related toxicity (Table 248–4). Seizures may occur under circumstances that result in high concentrations of penicillin in nervous tissue: rapid intravenous infusion of single large doses, substantial dosages for prolonged periods of time in patients with impaired renal function, or high concentrations given by the intrathecal route. Confusion, dizziness, seizures, and psychosis caused by toxic concentrations of procaine have been associated with the administration of procaine penicillin G.[255] Nephritis has been associated with the administration of some penicillins, most frequently after the use of methicillin. Bleeding because of drug-induced platelet aggregation has been noted after the administration of carbenicillin and penicillin G.

TABLE 248–4 Adverse Reactions to Penicillins

Type of Reaction	Frequency (%)	Most Frequent*
Electrolyte disturbance		
Sodium overload	Variable	Ticar
Hypokalemia	Variable	Ticar
Hyperkalemia, acute	Rare	PCN G
Gastrointtestinal		
Diarrhea	2-5	Amp
Enterocolitis	<1	Amp
Hematologic		
Hemolytic anemia	Rare	PCN G
Neutropenia	1-4	PCN G, Naf, Ox, Pip
Platelet dysfunction	3	Ticar
Hepatic		
Elevated AST	1-4	Ox, Naf
Neurologic		
Seizures	Rare	PCN G
Bizarre sensations	Rare	Procaine PCN
Renal		
Interstitial nephritis	1-2	Meth
Hemorrhagic cystitis	Rare	Meth
Allergic		
IgE medlated	0.004-0.4	PCN G
Cytotoxic antibody	Rare	PCN G
Immune complexes	Rare	PCN G
Delayed hypersensitivity	4-8	Amp

**All reactions can occur with any penicillin.*
Amp, ampicillin; AST, aspartate transaminase; Meth, methicillin; Naf, nafcillin; Ox, oxacillin; PCN G, penicillin G; Pip, piperacillin; Ticar, ticarcillin.
Modified from Chambers, H. F., and Neu, H.C.: Penicillins. In *Mandell, G.L., Bennett, J.E., and Dollin, R. (eds.): Mandell, Douglas and Bennett's Principles and Practice of Infectious Diseases. 4th ed. New York, Churchill Livingstone 1995.*

Although toxicity may not be a significant concern with the penicillins, sensitization is an important factor.[172] Penicillins are haptens, that is, they are low-molecular-weight compounds too small to elicit an immune response alone, but when bound to a carrier molecule (e.g., host tissues or proteins), they are highly immunogenic in humans. The native penicillin molecule can bind to a protein, as can its major (benzylpenicilloyl) and minor (benzylpenicillin, benzylpenicilloate, benzylpenilloate) determinants. Four types of immune-mediated reactions can occur after the administration of a penicillin (or any drug or antigen): immediate hypersensitivity (immunoglobulin E [IgE]–mediated) reactions, cytotoxic antibody reactions, immune complex reactions (Arthus reaction), and delayed (cell-mediated) hypersensitivity. Allergic reactions also can be classified as (1) immediate (onset ≤1 hour), accelerated (1 to 72 hours), or delayed (>72 hours). Researchers have estimated that an immediate serious reaction occurs in 2 of every 10,000 courses, and fatal reactions occur in 1 of 100,000 treatment courses.[24,266]

1. *Type 1 immediate hypersensitivity reactions* usually occur within 30 minutes after administration and are life-threatening events. The interaction of preformed mast cell–bound IgE antibody to the antigenic determinants of penicillin results in the release of mast cell mediators.[37] Clinical signs include hypotension or shock, urticaria, laryngeal edema, and bronchospasm. Acute anaphylaxis is a rare event after the administration of penicillin, but significant numbers of fatalities occur each year because of the extensive use of these drugs. Children are thought to have fewer systemic reactions than do adults, presumably because of fewer previous exposures to penicillin antigens. Oral preparations are less likely to result in an immediate reaction than are paren-

teral forms, perhaps because antigens are altered in the gastrointestinal tract, absorption is slower, dosages are smaller, or a combination of these factors.

2. *Type 2 cytotoxic antibody reactions* can occur after passive absorption of the benzylpenicilloyl hapten by the membrane of circulating blood cells or by renal interstitial cells, especially when high dosages of penicillins are used for prolonged periods of time. IgM antibody, IgG antibody, or both antibodies to the benzylpenicilloyl antigen bind to the altered cell surface; complement can be activated, and damage to the cell ensues. Cytotoxic antibody reactions usually are manifested more than 72 hours after initiation of antibiotic therapy and include hemolytic anemia, leukopenia, thrombocytopenia, and drug-induced interstitial nephritis.

3. *Type 3 immune complex (Arthus) reactions* occur after the formation of immune complexes between soluble penicillin antigens and IgG and IgM antibodies. The complexes lodge in the skin, joints, kidneys, or other tissue sites. Complement activation occurs, and the clinical manifestations of serum sickness ensue: cutaneous symptoms (urticaria, maculopapular rash, erythema multiforme), polyarthralgia, and fever. The onset typically is 7 to 21 days after initiation of penicillin therapy but can occur after use of the antibiotic has been discontinued. Although serum sickness has been associated with the administration of penicillins, it occurs more frequently after administration of cefaclor.

4. *Type 4 delayed hypersensitivity reactions* involve cellular rather than humoral immunity. On exposure to certain antibiotics, T lymphocytes become sensitized in a major histocompatibility restricted fashion, and they mediate mild to severe reactions that include contact dermatitis, some maculopapular rashes, fixed drug eruption at sites of previous tinea infections, and toxic epidermal necrolysis. Diagnostic tools to detect an antibiotic that induces a type 4 reaction include the delayed reading (>6 hours) intradermal skin test, patch test, and in vitro lymphocyte transformation test. However, these tests have not been adequately standardized for routine use.[266]

Idiopathic reactions are those for which an immune-mediated mechanism has not been proved, but more recent data suggest that these reactions may represent a type 4 hypersensitivity reaction.[266] Included in this category are the morbilliform exanthems, erythema multiforme, photosensitivity reactions, exfoliative dermatitis, and pruritus. Approximately 4 percent of courses of penicillin and up to 9 percent of courses of ampicillin or amoxicillin therapy are associated with a maculopapular rash. In some of these patients, the rash is a manifestation of a primary viral infection such as infectious mononucleosis, for which the penicillin was prescribed inappropriately.

Identifying patients who will have a significant reaction if penicillin is administered remains difficult. Serologic assays (radioallergosorbent tests) for the detection of IgE antibodies to major and minor penicillin determinants are available but are time-consuming, expensive, and less sensitive than is skin testing. Because the immediate reaction is mediated largely by IgE reagin or skin-sensitizing antibody, patients who are likely to respond subsequently with a life-threatening reaction can be identified by the use of intradermal tests with appropriate antigens. Selecting the most appropriate antigens to be used for skin testing, however, has been problematic because many different antigens may play roles in the allergic reaction. At least 10 metabolic breakdown products of the penicillin nucleus have been identified. Other potential antigens include macromolecular impurities present in solutions of the drug, high-molecular-weight penicillin polymers found in poorly buffered penicillin solutions standing for prolonged periods of time, side chains of the various penicillins, and the bacterial enzymes (amidases) used to prepare semisynthetic penicillins. Thus, investigators have had difficulty choosing sensitive and specific antigens to use for skin testing.

The most informative studies of skin test antigens have been those of Levine,[167] who identified two antigens, benzylpenicilloyl poly-L-lysine (Pre-Pen, Taylor Pharmacal, Decatur, IL) and a "minor determinant mixture," a preparation of a dilute solution of aqueous crystalline penicillin G that includes metabolic breakdown products. Only the Pre-Pen reagent is available commercially in the United States. The CDC currently recommends the use of benzylpenicilloyl poly-L-lysine and, if available, a freshly prepared mixture of dilute minor determinant precursors including benzylpenicillin G, benzylpenicilloate, and penicilloyl propylamine, as well as a positive control (histamine) and a negative control (diluent, phenol saline) after a protocol prepared by Beall.[24,278] If the full battery of minor antigens is not available, penicillin G should be used. An epicutaneous (prick) test is performed first. If the patient's history of penicillin allergy is that of a mild reaction, the skin test reagent can be used at full strength; however, if the previous history is suggestive of anaphylaxis, a 1:100 dilution of the reagent should be used for the first prick test, followed by a full-strength test if no reaction with the diluted reagent occurs. If the prick test result is negative, an intradermal test is performed using duplicate 0.02-mL volumes of antigen solutions, and the skin is observed for 20 minutes. A positive result is indicated by a wheal-and-flare reaction in 15 minutes that is more than 2 mm larger than the initial wheal; this result suggests a significant chance that a reaction will occur on subsequent administration of a penicillin. A negative result suggests that a significant allergic reaction will not take place. Although much effort has gone into clinical tests of these antigens, the predictive value of positive and negative results in children remains uncertain. Because of the risk of severe life-threatening reactions when a skin test is performed, having a physician present and resuscitation equipment and medications readily available is prudent.

At present, the physician must rely on the patient's history of an adverse reaction after administration of a penicillin to identify who is likely to be allergic. If the reaction appears to be related to the administration of a penicillin, the drug should be avoided for minor infections. More recent experience suggests that fewer than 10 percent of adults with a history of penicillin allergy have positive skin tests. Of those individuals with negative skin tests, IgE-mediated reactions occur in 4 percent or less if these patients receive penicillin, a rate that is similar to individuals without a history of penicillin allergy.[266] Because no proven alternative therapies to penicillin are available for treating patients with neurosyphilis, congenital syphilis, or syphilis in pregnancy, the CDC recommends skin testing and desensitization of patients considered to have reacted positively to the skin test antigens.[278] If a life-threatening infection should occur and penicillin is clearly the drug of choice, the physician may choose to administer the drug under carefully controlled conditions after desensitization. All penicillins are cross-reactive with regard to sensitization; allergy to any one implies sensitization to all, although cross-sensitivity is considerably less than 100 percent.

CEPHALOSPORINS

The cephalosporins have a broad range of activity that includes gram-positive cocci, gram-negative enteric bacilli, and anaerobic bacteria. Most cephalosporins are relatively resistant to hydrolysis by β-lactamases produced by *S. aureus*, but many are susceptible to gram-negative β-lactamases. Unlike other antimicrobial agents, this group has a high therapeutic-toxic index. For simplicity, the cephalosporins have been categorized as first-, second-, third-, and fourth-generation agents (Table 248–5), based on the pattern of in vitro activity.

Pharmacokinetics

The cephalosporins are available as parenteral and oral products (Table 248–6). Most of the parenteral drugs can be administered by the intravenous or intramuscular routes. Most of the oral

TABLE 248–5 Classification Scheme for Cephalosporins

Generic Name	Trade Name	Route
First Generation		
Cephalexin‡	Keflex, Keftab	PO
Cefadroxil	Duricef	PO
Cephradine	Velosef	PO
Cephalothin†		IM, IV
Cefazolin‡	Ancef, Kefzol	IM, IV
Cephapirin§	Cefadyl	IM, IV
Second Generation		
Cefaclor‡	Ceclor	PO
Cefuroxime axetil§	Ceftin	PO
Cefprozil*	Cefzil	PO
Ceftibuten*	Cedax	PO
Cefamandole†	Mandol	IM, IV
Cefonicid‖	Monocid	IM, IV
Cefuroxime§	Zinacef, Kefurox	IM, IV
Cephamycins		
Cefoxitin	Mefoxin	IM, IV
Cefotetan‖	Cefotan	IM, IV
Third Generation		
Cefixime‡	Suprax	PO
Cefpodoxime proxetil¶	Vantin	PO
Cefdinir*	Omnicef	PO
Cefditoren pivoxil**	Spectracef	PO
Ceftizoxime*	Cefizox	IM, IV
Cefotaxime	Claforan	IM, IV
Ceftriaxone	Rocephin	IM, IV
Ceftazidime	Fortax, Tazicef, Tazidime, Ceptaz	IM, IV
Cefoperazone‖	Cefobid	IM, IV
Fourth Generation		
Cefepime††	Maxipime	IM, IV

**Safety and efficacy have not been determined for infants younger than 6 months.*
†No longer available in the United States.
‡Safety and efficacy have not been determined for infants younger than 1 month.
§Safety and efficacy have not been established for infants younger than 3 months.
‖Not approved for use in children.
¶Safety and efficacy have not been established for infants younger than 5 months.
***Safety and efficacy have not been established for children younger than 12 years.*
††Safety and efficacy have not been established for infants younger than 2 months.
IM, intramuscularly; IV, intravenously; PO, orally.
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products are absorbed well from the gastrointestinal tract. Esterification of the base compounds of cefuroxime and cefpodoxime is required to enhance gastrointestinal absorption. The presence of food does not alter absorption and, for some antibiotics, even can enhance absorption. Cephalosporins penetrate most tissues and body fluids well, except CSF. The first- and second-generation cephalosporins do not achieve therapeutic CSF concentrations. CSF penetration of third-generation drugs varies but is generally adequate for most major meningeal pathogens, except penicillin-nonsusceptible pneumococci, for which vancomycin is added in the initial empiric regimen.[158] Cefepime, a fourth-generation cephalosporin, reaches adequate CSF concentrations for the treatment of most common invasive pathogens. Because glomerular filtration and tubular secretion are the major modes of excretion, the urinary concentrations achieved are sufficient for the treatment of urinary tract infections. Ceftriaxone has dual excretion by the kidneys and the biliary tract and achieves high concentrations in urine and bile. The presence of moderate to severe renal insufficiency may require adjustment of the dosage or dosing interval for all cephalosporins, except those agents with biliary excretion. Hepatic insufficiency can affect the metabolism of cephalosporins that undergo biliary excretion; however, adjustments in ceftriaxone dosage or dosing interval are required only in the presence of both hepatic and renal insufficiency.

Therapeutic advantages of cephalosporins include concentration-independent bactericidal activity, broad-spectrum antibacterial activity, lack of significant dose-related toxicity, and relative stability against staphylococcal β-lactamases. Certain cautions must be kept in mind when prescribing cephalosporins: none is effective against enterococci, methicillin-resistant staphylococci, *L. monocytogenes*, chlamydial species, and *Clostridium difficile*, and resistance may develop rapidly in closed communities, such as neonatal or pediatric intensive care units, because of inducible chromosomal β-lactamases produced by gram-negative bacteria. Recommended daily dosage schedules are shown in Table 248–10.

First-Generation Cephalosporins

First-generation cephalosporins are effective against gram-positive cocci, including β-lactamase–producing *S. aureus*, and they have variable activity against gram-negative enteric bacilli. Five first-generation cephalosporins currently are available for infants and children: the parenteral drugs cephapirin and cefazolin; the oral products cephalexin and cefadroxil; and cephradine. Cephalothin is not available in the United States. Because cephapirin is painful in intramuscular injections, the intravenous route is preferred. Cefazolin produces higher concentrations in blood than do the other parenteral first-generation drugs. Cefadroxil can be administered in twice-daily doses because of its longer serum half-life. None of these agents will attain appreciable concentrations in the central nervous system (CNS).

These drugs are of value as alternatives to penicillin for disease caused by *S. aureus*, *S. pyogenes*, and susceptible *S. pneumoniae*, and they are active against some strains of community-acquired gram-negative enteric bacilli such as *E. coli*, *P. mirabilis*, and *Klebsiella pneumoniae*. The three oral preparations have comparable activity in vitro and in vivo. First-generation cephalosporins are valuable in children who have a history of non-anaphylactic allergy to penicillin. These drugs have been used to treat staphylococcal and streptococcal skin and skin structure infections, bone and joint infections, pharyngitis, and uncomplicated community-acquired urinary tract infections caused by susceptible bacteria. A meta-analysis revealed that many first-generation and other-generation cephalosporins (cephalexin, cefadroxil, cefuroxime, cefprozil, cefdinir, cefixime, cefpodoxime, and ceftibuten) have equivalent or superior bacteriologic and clinical cure rates compared with penicillin for treatment of group A streptococcal pharyngotonsillitis.[56] Experts disagree on the significance of the results of this meta-analysis. Cefadroxil is effective for the treatment of streptococcal pharyngitis in a once-a-day dosage schedule. Cephalexin has been used for sequential parenteral-oral treatment of staphylococcal osteomyelitis and arthritis occurring after surgical intervention and an initial period of parenteral antibiotic therapy. Cefazolin and cefuroxime are the antibiotics of choice for perioperative prophylaxis in selected surgical procedures.[44] Because first-generation cephalosporins have minimal, if any, activity against *H. influenzae* and *M. catarrhalis* and inadequate activity against penicillin-resistant *S. pneumoniae*, they should not be used for empiric treatment of respiratory tract infections. First-generation cephalosporins should not be used for empiric therapy of suspected severe gram-negative health care–associated infections; a third- or fourth-generation cephalosporin in combination with an aminoglycoside, or other classes of antibiotics, should be selected for this purpose. The safety and efficacy of cefaclor, cefazolin or cephalexin, and cephapirin have not been established for infants younger than 1 month and 3 months, respectively.

Second-Generation Cephalosporins

The second-generation cephalosporins that are approved for use in children consist of two parenteral drugs (cefamandole and

TABLE 248–6 Pharmacokinetics of Cephalosporins

Antibiotic	Bioavailability* (%)	Protein Binding (%)	Metabolized (%)	Urinary Recovery (%/hr)	Approximate Half-Life† (hr)
First Generation					
Cephalexin	95	10-15	0	90/8	0.9-1.5
Cefadroxil	95	15-20	0	93/24	1.2-1.5
Cephradine	95	8-17	0	60-90/6	0.8-1.3
Cephalothin		70	20-30	60-70/6	0.5-1.0‡
Cefazolin		85	0	70-86/24	1.4-2.0‡
Cephapirin		44-50	40	70/6	0.5-0.8
Second Generation					
Cefaclor	95	25	0	60-85/8	0.6-0.9
Cefuroxime axetil	37/52	33-50	0§	50/12	1.2-1.9‡
Cefprozil	95	36-45	0	60-70/8	1.8-2.1
Ceftibuten	75-90/<75‖	65-77	10¶	95/24	1.4-2.6
Cefonicid		>90	0	99/24	3.5-4.5
Cefuroxime		33-50	0	96/24	1.2-1.9‡
Cefoxitin		70-80	<5	85/6	0.7-1.1‡
Third Generation					
Cefixime	40-50	65	0	16/24	3-4
Cefpodoxime proxetil	50/>50	21-40	0§	29-33/12	2.1-2.8
Cefdinir	16-25**	60-70	0	12-18/1.7	1.5-1.7
Cefditoren pivoxil	13-19	88	0§		1.6
Ceftizoxime		30	0	70-100/24	1.4-1.7
Cefotaxime		30-50	30-50	15-25/6	1.0
Ceftriaxone		83-96		33-67††/24	4.3-8.7‡‡
Ceftazidime		<10	0	80-90/24	1.4-2.0
Fourth Generation					
Cefepime	100	20	15	80-85/12	2.0

**Fasting/nonfasting.*
†Normal renal function.
‡Elimination half-life prolonged in neonates.
§Prodrug rapidly metabolized to active drug; otherwise, no significant metabolism.
‖Because the bioavailability of suspensions is decreased by food, ceftibuten should be administered at least 2 hours before or 1 hour after a meal.
*¶*Cis-*isomer converted to* trans-*isomer.*
***Suspension formulation has greater bioavailability than the capsule formulation.*
††Forty to 75 percent eliminated unchanged in bile.
‡‡The half-life is prolonged after intramuscular administration.
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cefuroxime) and three oral preparations (cefaclor, cefuroxime axetil, and cefprozil). Also classified with the second-generation cephalosporins are the cephamycins, of which only one, cefoxitin, is approved for use in children. When compared with the first-generation cephalosporins, the second-generation agents have similar or somewhat less activity against gram-positive cocci but better activity against *H. influenzae*, *M. catarrhalis*, *N. meningitidis*, *N. gonorrhoeae*, and some members of the Enterobacteriaceae. The cephamycins are more active than are the first- or other second-generation cephalosporins against gram-negative enteric bacteria and *B. fragilis*, but they have poor activity against gram-positive cocci.

CEFACLOR

Cefaclor is more active than is cephalexin against *H. influenzae*, *M. catarrhalis*, *E. coli*, and *P. mirabilis* and has activity against staphylococci similar to that of cephalexin. Cefaclor is an unstable compound and is destroyed within 2 hours in human plasma. It is susceptible in vitro to hydrolysis by the β-lactamases produced by approximately 14 percent of *H. influenzae* and 90 percent of *M. catarrhalis* isolates; presumed rates of resistance of *H. influenzae* are considerably higher when pharmacokinetic and pharmacodynamic parameters are taken into account.[134] Although cefaclor is effective therapy for susceptible bacteria causing otitis media, sinusitis, and mild to moderate cases of pneumonia, it is not the preferred empiric agent for these indications because it is not effective against penicillin-nonsusceptible strains of *S. pneumoniae*. The rare but potential risk of serum sickness, as well as the poor stability of this drug to some β-lactamases, has limited its use. The safety and efficacy of cefaclor have not been established for infants younger than 1 month, although the drug has been used successfully to treat neonatal otitis media.

CEFAMANDOLE

Cefamandole is active against gram-positive cocci, including β-lactamase–producing *S. aureus*, and some gram-negative enteric bacteria, and it was the first cephalosporin to be effective for infections caused by *H. influenzae*, including β-lactamase–producing strains. However, this agent is no longer available in the United States. Before the development of cefuroxime, cefamandole was used for respiratory tract, skin, and soft tissue infections in infants and children.[15] Once cefuroxime was approved for use, cefamandole seldom was used because, although its spectrum of activity was comparable with that of cefuroxime, some of its pharmacologic features made it less favorable. Cefamandole does not penetrate the CSF well, and because it contains a methylthiotetrazole side chain, altered hemostasis may occur. Clinical and microbiologic failure in cases of meningitis caused by *H. influenzae* (despite in vitro susceptibility and evidence of CSF penetration) has limited the use of cefamandole to disease in which the development of sepsis is not a concern. The safety and efficacy of this agent have not been established for infants younger than 1 month.

CEFOXITIN

Cefoxitin has excellent activity against anaerobic organisms and is the most active cephalosporin against *B. fragilis*.[27] It has selective activity against gram-negative enteric bacilli, but *Enterobacter* or *Pseudomonas* spp. are inherently resistant. Cefoxitin is resistant to hydrolysis by the β-lactamases produced by gram-positive bacteria and some of the gram-negative β-lactamases. Because it is a potent inducer of Bush group 1 chromosomal β-lactamases, its indiscriminate use should be avoided. Cefoxitin has been shown to be effective for infections involving facultative gram-negative bacilli and anaerobes, such as intra-abdominal, pelvic, and gynecologic infections. When combined with doxycycline, cefoxitin is effective for the treatment of pelvic inflammatory disease.[278] The use of β-lactam–β-lactamase inhibitor combinations or metronidazole, rather than cefoxitin, should be considered for empiric treatment of patients with life-threatening anaerobic infections because as many as 25 percent of *B. fragilis* strains can be resistant to cefoxitin.[4] Higher doses of cefoxitin have been associated with an increased incidence of eosinophilia and elevated aspartate aminotransferase (AST) concentration. A diminished zone of inhibition in cefoxitin disk diffusion assays are useful in the clinical microbiology laboratory to detect inducible or constitutive (stably derepressed) AmpC Bush group 1 β-lactamase production among strains of *Citrobacter*, *Enterobacter*, *E. coli*, *Klebsiella*, *Morganella*, *Providencia*, *Pseudomonas*, and *Serratia*. Identification of this phenotype in vitro is important because these strains have high-level resistance to many classes of β-lactam antibiotics that may not be detected by standard susceptibility assays. Fourth-generation cephalosporins and carbapenems are potentially effective antimicrobial agents for these multidrug-resistant pathogens.[140]

CEFPROZIL

Cefprozil has a structure similar to that of the first-generation cephalosporin cefadroxil. It is more active than are the oral first-generation agents against *S. pyogenes*, *S. pneumoniae*, *Neisseria* spp., *H. influenzae*, *M. catarrhalis*, *E. coli*, *P. mirabilis*, *Klebsiella*, and, to a lesser extent, staphylococci. This drug is hydrolyzed by the β-lactamases produced by approximately 11 percent of *H. influenzae* and 91 percent of *M. catarrhalis* isolates; rates of resistance of *H. influenzae* are likely considerably higher when pharmacokinetic and pharmacodynamic parameters are taken into account.[134] The rate of resistance among *S. pneumoniae* isolates in the United States is approximately 20 percent.[138] Because of its relatively long serum half-life, cefprozil can be administered twice daily. Cefprozil is comparable to penicillin, cefaclor, and erythromycin in the treatment of pharyngitis,[184] and it is equivalent or superior to cefaclor and erythromycin in the treatment of mild to moderate skin and skin structure infections.[211] Cefprozil is approved for the treatment of acute otitis media, mild lower respiratory tract infections, acute sinusitis, and skin and skin structure infections. However, it is not the preferred antibiotic for these indications because it is unlikely to be effective in children with penicillin-nonsusceptible *S. pneumoniae*, *H. influenzae*, or *M. catarrhalis*, on the basis of unfavorable pharmacodynamic parameters.[271] Cefprozil is safe and effective as part of a parenteral-oral antibiotic regimen for the treatment of suppurative skeletal infections in children.[272] The safety and efficacy of cefprozil have not been established for infants younger than 6 months.

CEFUROXIME

When compared with first-generation cephalosporins, this parenteral second-generation cephalosporin is slightly less active against staphylococci but is more active against group A streptococci and pneumococci. Cefuroxime has excellent activity against many members of the Enterobacteriaceae.[208] Its stability to β-lactamases is greater than that of first-generation agents, and it is the only first- or second-generation antibiotic that achieves substantial CSF concentrations. However, cefuroxime is not recommended for treatment of proven or suspected bacterial meningitis because of suboptimal CSF pharmacologic characteristics, particularly in the context of infections caused by *H. influenzae* or nonsusceptible *S. pneumoniae*, for which third-generation cephalosporins (cefotaxime and ceftriaxone) are indicated.[251] Cefuroxime has been approved for the treatment of skin and skin structure infections, lower respiratory tract infections, bone and joint infections, uncomplicated gonorrhea, and uncomplicated urinary tract infections caused by susceptible bacteria. Cefuroxime is most useful for the treatment of infections sufficiently severe to warrant parenteral therapy, in which susceptible *S. aureus*, *S. pneumoniae*, *S. pyogenes*, encapsulated or nonencapsulated *H. influenzae*, and *M. catarrhalis* are probable pathogens, such as suppurative arthritis, orbital cellulitis, or severe pneumonia. Cefuroxime offers the advantage of single-drug therapy for these diseases. The safety and efficacy of cefuroxime have not been established for infants younger than 3 months.

Cefuroxime Axetil

Cefuroxime axetil is an oral form of cefuroxime with a similar spectrum of activity. It is an ester prodrug of cefuroxime that is metabolized to the active drug by intestinal esterases. Oral absorption is increased by the presence of food. When crushed, the tablet has a bitter taste that renders it unpalatable. The suspension has an unpleasant flavor that makes it difficult for some children to tolerate and therefore may limit adherence. The drug may be considered a suitable alternative to amoxicillin for the treatment of otitis media[9] and sinusitis when coverage must include β-lactamase–producing bacteria. Cefuroxime axetil has limited activity against penicillin-nonsusceptible pneumococci and *M. catarrhalis*, of which approximately 22 percent and 50 percent, respectively, of isolates are resistant.[134,138] This agent has been approved for the treatment of uncomplicated urinary tract infection, skin and soft tissue infection, acute bacterial maxillary sinusitis, and lower respiratory tract infection caused by susceptible bacteria. Cefuroxime axetil is an effective alternative to doxycycline and amoxicillin for the treatment of early Lyme disease.[279] The safety and efficacy of cefuroxime axetil have not been established for infants younger than 3 months.

Third-Generation Cephalosporins

Third-generation cephalosporins approved for use in children include the parenteral agents cefotaxime, ceftizoxime, ceftriaxone, and ceftazidime and the oral agents cefixime, cefpodoxime proxetil, ceftibuten, cefdinir, and cefditoren pivoxil. Third- and fourth-generation cephalosporins are the most potent cephalosporins against gram-negative enteric bacteria.[142] Most of them have excellent activity against *H. influenzae*, *M. catarrhalis*, *N. gonorrhoeae*, *N. meningitidis*, group A streptococci, and penicillin-susceptible pneumococci but relatively poor activity against staphylococci. Ceftazidime is the only agent with activity against *P. aeruginosa*. A serious global health concern has emerged because members of the Enterobacteriaceae have developed increased resistance to third-generation cephalosporins on the basis of plasmid-mediated production of ESBLs and AmpC β-lactamases.[221] The parenteral cephalosporins provide high concentrations of drug in serum and adequate concentrations in CSF. Third-generation cephalosporins and aminoglycosides are synergistic in vitro against certain susceptible and resistant strains of *P. aeruginosa* as well as *Serratia marcescens* and other Enterobacteriaceae, including *Enterobacter cloacae*, *E. coli*, *K. pneumoniae*, and *P. mirabilis*.

CEFOTAXIME

Cefotaxime has excellent activity against group A streptococci, susceptible pneumococci, *H. influenzae*, *N. meningitidis*, and *N. gonorrhoeae*. Because cefotaxime can be hydrolyzed by AmpC Bush group 1–inducible chromosomal β-lactamases and by ESBLs, it is not active against strains of Enterobacteriaceae that produce these β-lactamases. Cefotaxime is metabolized in the liver to desacetyl cefotaxime, a less active metabolite that may act synergistically with cefotaxime. Although it is metabolized in the liver, cefotaxime is excreted by the kidneys. High serum, tissue, and CSF concentrations of cefotaxime can be achieved at the recommended dosages. The rapid development of resistance by colonizing gram-negative enteric bacilli when cefotaxime was used extensively for initial treatment of neonatal sepsis raised concern that extensive use of newer cephalosporins in the nursery or intensive care units could lead to more rapid emergence of drug-resistant bacteria than had been identified with the traditional regimens of a penicillin and an aminoglycoside. Because of its broad spectrum of activity against many of the common pathogens causing pediatric infections, cefotaxime is used widely for inpatient treatment of lower respiratory tract infections, urinary tract infections, sepsis, intra-abdominal infections, bone and joint infections, and meningitis or ventriculitis caused by susceptible organisms. Studies in neonates have raised concern that when cefotaxime is used routinely as part of an empiric regimen for neonatal sepsis, colonizing gram-negative enteric bacilli can rapidly develop resistance, and certain neonates have adverse outcomes.[62,79] Therefore, it is prudent to reserve cefotaxime for appropriate clinical and microbiologic indications. In 2003, the Clinical and Laboratory Standards Institute (formerly the National Committee for Clinical Laboratory Standards) published new breakpoints for defining the susceptibility of *S. pneumoniae* isolates to cefotaxime and ceftriaxone. The former breakpoints were based on attainable concentrations of these antibiotics in CSF and the level at which it was presumed that meningitis treatment failed because of elevated MICs. According to the former criteria, susceptible, intermediate, and resistant MIC breakpoints for cefotaxime and ceftriaxone were 0.5 μg/mL or less, 1 μg/mL, and 2 or more μg/mL, respectively, for pneumococci. Based on the newer criteria, the original breakpoints should be used for isolates from CSF in patients with suspected meningitis, but isolates causing nonmeningeal syndromes have breakpoints of 1 μg/mL or less, 2 μg/mL, and 4 or more μg/mL, respectively.[58]

CEFTIZOXIME

Ceftizoxime is a parenteral agent with a spectrum of activity similar to that of cefotaxime. Its safety and efficacy have not been established for infants younger than 6 months, and it rarely is used in pediatrics.

CEFTRIAXONE

The antibacterial spectrum of ceftriaxone is similar to that of cefotaxime, with activity against beta-hemolytic streptococci (including groups A, B, C, F, and G), susceptible pneumococci, *H. influenzae*, *M. catarrhalis*, *Neisseria* spp., and many members of the Enterobacteriaceae. Surveillance data from pediatric patients in the United States revealed that no significant resistance has been reported against ceftriaxone among oxacillin-susceptible strains of *S. aureus*.[142] Nonetheless, this drug should not be used as empiric monotherapy for severe infections presumed to be caused by staphylococci because it has minimal activity against MRSA, and its efficacy for treatment of susceptible strains has not been established in children. Ceftriaxone is not active against most strains of *Pseudomonas*. It differs from cefotaxime in its pharmacokinetic properties. Ceftriaxone undergoes extensive protein binding and has a long serum half-life. Because of its broad spectrum of activity against many of the pathogens that commonly cause sepsis, meningitis, and respiratory tract infections in older infants and children, along with its unique pharmacokinetic features, ceftriaxone has been used excessively for outpatient management of febrile infants and young children being evaluated for possible systemic bacterial infection. Single-dose therapy was approved for the treatment of acute otitis media in 1997; a 3-day regimen is recommended as alternative therapy for patients with refractory cases.[9,166]

Ceftriaxone is effective for various sexually transmitted diseases, including the following: chancroid, proctitis, epididymitis (in combination with doxycycline), and different forms of gonococcal disease (neonatal ophthalmia); uncomplicated urethral, endocervical, rectal, or pharyngeal gonorrhea (in combination with doxycycline for treatment of possible coexisting chlamydial infection); and disseminated gonococcal infection, meningitis, or endocarditis.[278] Short-course ceftriaxone therapy for typhoid fever in children is comparable in effectiveness to chloramphenicol therapy.[194] However, duration of ceftriaxone therapy greater than 5 days has been associated with fewer confirmed bacteriologic relapses.[261] Ceftriaxone (or cefotaxime) is recommended for treatment of acute Lyme disease with advanced heart block (14 to 21 days) or meningitis (14 to 28 days).[279] Although third-generation cephalosporins have been used for empiric treatment of health care–associated respiratory, wound, intra-abdominal, and urinary tract infections caused by gram-negative bacteria, administering an aminoglycoside instead of or in addition to a third-generation cephalosporin often is prudent because of the risk of encountering plasmid-mediated, ESBL resistance, or inducing the expression of chromosomally mediated AmpC Bush group 1 β-lactamases.

For infections requiring prolonged therapy (e.g., suppurative arthritis, osteomyelitis, brain abscess) and caused by susceptible organisms, ceftriaxone therapy is cost-effective for use outside the hospital. Once the acute signs of disease have diminished and the child remains in the hospital for only parenteral therapy, discharge and once-daily administration of ceftriaxone in the home or clinic can be considered.

Because of its extensive protein binding, ceftriaxone can displace bilirubin from albumin-binding sites, with the potential risk of inducing kernicterus, although to date this adverse clinical effect has not been reported. Cefotaxime is administered to neonates more often than is ceftriaxone because considerably more information is available on its safety and pharmacokinetics. Ceftriaxone is excreted and concentrated in bile. Gallbladder "sludge," diagnosed by abdominal sonography (and not identifiable by other radiographic techniques), has been demonstrated in some patients who received ceftriaxone. The material appears to be a calcium-ceftriaxone complex and resolves on cessation of the drug. Most patients with ceftriaxone-associated sludge are asymptomatic, but occasionally patients have symptoms of gallbladder disease, and incidents of acute cholecystitis have been reported in a few children.

CEFTAZIDIME

When compared with cefotaxime and ceftriaxone, ceftazidime has poor antibacterial activity against *S. aureus*, is less active against penicillin-susceptible *S. pneumoniae*, and is slightly less active against group A streptococci, but it is more active against *P. aeruginosa*. On a weight basis, ceftazidime is the most effective of all β-lactam antimicrobial agents in vitro against *P. aeruginosa*. It frequently is active in vitro against *P. aeruginosa* strains that are resistant to antipseudomonal penicillins. Resistance to ceftazidime can develop in gram-negative bacteria by the production of ESBLs or because of decreased bacterial cell permeability, as seen

with *P. aeruginosa*, *Acinetobacter*, and some *Serratia* strains. Ceftazidime is indicated for use in the following circumstances: infections suspected to be caused by *Pseudomonas*, including acute exacerbations of chronic pulmonary infections in patients with cystic fibrosis, chronic suppurative otitis media, or malignant otitis externa; thermal injuries, puncture wound infections of the foot, and complicated health care–associated infections; and febrile illnesses in neutropenic patients with cancer or other immunocompromised individuals.

CEFIXIME

Cefixime has a vinyl group instead of a chlorine atom at position 3 of the cephem nucleus and an aminothiazole oxime group, rather than a phenyl glycine side chain. These biochemical changes result in potent gram-negative activity. Cefixime was introduced in 1989 as an oral third-generation cephalosporin with a broad spectrum of activity, including activity against group A streptococci, *H. influenzae*, *M. catarrhalis*, susceptible *S. pneumoniae*, and many members of the Enterobacteriaceae, including *Shigella*, *Salmonella*, *E. coli*, *Klebsiella*, and *P. mirabilis*.[21] Cefixime, like ceftibuten, is distinguished from other extended-spectrum oral cephalosporins by its lack of activity against *S. aureus.* It also is inactive against *Citrobacter freundii*, *Enterobacter*, *P. aeruginosa*, and *Serratia*. Although cefixime has in vitro activity against penicillin-susceptible pneumococci, lower bacteriologic cure rates occurred with cefixime than with amoxicillin when cefixime was used to treat children with pneumococcal acute otitis media.[127] Cefixime is resistant to degradation by certain β-lactamases. Administration of cefixime is facilitated by once-daily dosing,[89] pleasant taste, and stability of the suspension at room temperature. Cefixime is effective therapy for uncomplicated urinary tract infections caused by *E. coli* or *P. mirabilis*, for shigellosis, and for acute otitis media or sinusitis caused by *H. influenzae* or *M. catarrhalis.*[32] Some specialists use cefixime to treat uncomplicated gonococcal infections in children because the drug can be administered orally; however, no reports have been published concerning the safety or effectiveness of cefixime used for this purpose.[278] Clinical studies indicate that the rate of laboratory-confirmed eradication of *Shigella sonnei* in an epidemic setting is higher with a 5-day than with a 2-day cefixime regimen,[182] cefixime alone is equally effective as sequential therapy with intravenous and oral antibiotics in selected febrile infants with urinary tract infections,[30,123] and a 14-day cefixime regimen for treatment of multidrug-resistant *S. typhi* septicemia in children has efficacy comparable to that of a 5-day course of ceftriaxone therapy.[106] Cefixime is not recommended for the treatment of infections frequently caused by staphylococci, such as skin or soft tissue infections, and it is not the most effective agent for the treatment of pneumococcal infections. The safety and efficacy of cefixime have not been established for infants younger than 1 month. Wyeth discontinued manufacturing cefixime in 2002, but the drug was reintroduced in 2004 as a generic formulation (Lapine Laboratory).

CEFPODOXIME PROXETIL

The antibacterial activity of cefpodoxime proxetil is similar to that of cefixime, with the exception of improved activity against staphylococci. Cefpodoxime is active against the following: group A streptococci; susceptible pneumococci; β-lactamase–producing strains of *N. gonorrhoeae*, *H. influenzae*, and *M. catarrhalis*; oxacillin-susceptible *S. aureus*; and many members of the Enterobacteriaceae. It is not active against *Enterobacter*, *Pseudomonas*, *Serratia*, or *Morganella*. Cefpodoxime proxetil, the ester prodrug of cefpodoxime, is cleaved by intestinal esterases to the active drug. Oral absorption is increased by the presence of food. Because of its longer serum half-life, cefpodoxime proxetil can be administered in twice-daily dosing intervals. Cefpodoxime is hydrolyzed by some ESBLs. The unfavorable palatability of cefpodoxime proxetil may adversely affect adherence.[258]

Treatment of group A streptococcal pharyngitis with a 5-day cefpodoxime proxetil regimen is equivalent to therapy with penicillin V and has been approved for this indication by the FDA.[72] A 5-day regimen is approved for the treatment of acute otitis media based on clinical outcomes that are comparable or superior to therapy with amoxicillin-clavulanate, cefaclor, or cefixime.[64] Cefpodoxime is not likely to be effective for the treatment of acute otitis media caused by penicillin-nonsusceptible pneumococci because of its unfavorable pharmacodynamic parameters.[276] It also has been approved for outpatient treatment of community-acquired respiratory tract infections, uncomplicated urinary tract infections, and mild skin and skin structure infections. The safety and efficacy of cefpodoxime proxetil have not been established for infants younger than 5 months.

CEFTIBUTEN

Ceftibuten was approved by the FDA for pediatric use in 1995. The primary active component is formulated as a *cis*-isomer that is converted in serum to the less active *trans*-isomer. The chemical structure and spectrum of antimicrobial activity are similar to those of cefixime. Ceftibuten, like cefixime, is distinguished from other extended-spectrum oral cephalosporins by its lack of activity against *S. aureus.* Its antimicrobial activity against groups A, C, F, and G beta-hemolytic streptococci is good, whereas its activity against groups B and D, as well as viridans streptococci, is poor. Ceftibuten exhibits MICs for penicillin-susceptible pneumococci in the susceptible range, whereas penicillin-nonsusceptible strains are ceftibuten-resistant. When compared with other oral cephalosporins, ceftibuten is the most stable to hydrolysis by β-lactamases produced by Neisseriaceae, *Moraxella*, *Haemophilus*, and Enterobacteriaceae, including most strains of *E. coli* and *K. pneumoniae* that produce Amber class A plasmid-mediated, ESBLs of the TEM, SHV, and OXA types. Ceftibuten is variably active against strains of *Citrobacter*, *Serratia*, and *Morganella*, but it is inactive against *Acinetobacter*, *Pseudomonas*, *Enterobacter*, *Bordetella*, and anaerobic species. It is also a weak inducer of AmpC Bush group 1 β-lactamases produced by *S. marcescens*, *E. cloacae*, and *Enterobacter aerogenes*.[114]

Ceftibuten has excellent bioavailability and a favorable pharmacokinetic profile that facilitates a once-daily dosing schedule.[20] Ten-day treatment regimens have been approved for streptococcal tonsillopharyngitis and acute otitis media caused by *H. influenzae*, *M. catarrhalis*, or *S. pyogenes* because of superior clinical and microbiologic efficacy data for ceftibuten in comparison with penicillin V in children with streptococcal tonsillopharyngitis[224] and comparable clinical efficacy data for ceftibuten versus amoxicillin, amoxicillin-clavulanate, cefaclor, and cefprozil for the treatment of children with clinically defined acute otitis media with or without effusion. However, cumulative data from clinical trials indicate that microbiologic cure rates of pneumococcal otitis media are lower for ceftibuten than for comparable antimicrobial agents.[114] Because of the increasing prevalence of nonsusceptible *S. pneumoniae* isolates, ceftibuten has almost no place in the treatment of acute otitis media, especially in young or otherwise at-risk infants and children.

Ceftibuten also should be considered as alternative therapy for sinusitis caused by susceptible organisms. Although not approved for this indication, ceftibuten achieved clinical success equivalent to and bacterial eradication superior to those reported with TMP-SMX in the treatment of complicated or recurrent urinary tract infections in children.[19] Adverse reactions to ceftibuten, which are limited mainly to the gastrointestinal tract,

occur in approximately 10 percent of children, a rate similar to comparable oral β-lactam antibiotics. The safety and efficacy of ceftibuten have not been established for infants younger than 6 months.

CEFDINIR

Cefdinir was approved in 1999 for the treatment of acute otitis media in children. It has a broad spectrum of in vitro antimicrobial activity, favorable pharmacokinetics, a convenient once- or twice-daily dosage schedule, superior palatability, a low rate of adverse events, and proven clinical efficacy against common childhood pathogens.[125,156] When compared with cephalexin, cefaclor, cefuroxime, cefixime, and cefpodoxime, cefdinir has equivalent or superior MICs for groups A, B, C, F, and G streptococci, viridans streptococci, and oxacillin-susceptible staphylococci. Its in vitro activity against penicillin-susceptible and penicillin-nonsusceptible strains of *S. pneumoniae* is equivalent to that of cefuroxime and cefpodoxime but superior to that of other oral cephalosporins. Cefixime and cefpodoxime have superior activity against *H. influenzae* (including β-lactamase–producing strains), whereas cefdinir and cefuroxime have comparable activity against this pathogen. The activity of cefdinir against *M. catarrhalis* is similar to that of cefixime, cefpodoxime, and cefuroxime but superior to that of earlier-generation cephalosporins. Cefdinir is stable to hydrolysis by many common Enterobacteriaceae-produced β-lactamases. However, activity against these pathogens is variable. Cefdinir is inactive against strains of enterococci, methicillin-resistant staphylococci, *Legionella*, *Listeria*, *Acinetobacter*, *Citrobacter*, *Enterobacter*, *P. aeruginosa*, *Serratia*, *Stenotrophomonas*, and most anaerobes.[115]

Based on comparative clinical and microbiologic efficacy trials, administration of cefdinir should be considered for children with the following: (1) acute bacterial otitis media caused by β-lactamase–positive or β-lactamase–negative strains of *H. influenzae* and *M. catarrhalis* or penicillin-susceptible strains of *S. pneumoniae* as either a 5- or 10-day regimen; cefdinir has efficacy equivalent to that of cefuroxime and cefpodoxime but superior palatability and therefore should be considered as an alternative to other second-line regimens; however, use of amoxicillin-clavulanate or of three single daily doses of parenteral ceftriaxone is recommended if penicillin-nonsusceptible pneumococci are suspected or proven[9,10,156]; (2) group A streptococcal tonsillopharyngitis in 5- or 10-day dosage schedules; (3) acute maxillary sinusitis caused by susceptible organisms; and (4) uncomplicated skin and skin structure infections caused by oxacillin-susceptible *S. aureus* or group A streptococci.[141] Although cefdinir is not approved for treatment of urinary tract infections, microbiologic data support its potential utility for community-acquired uncomplicated urinary infections.[39] The safety and efficacy of cefdinir have not been established for infants younger than 6 months.

CEFDITOREN PIVOXIL

Cefditoren pivoxil was approved by the FDA in 2002 for oral treatment of acute exacerbations of chronic bronchitis, pharyngitis, tonsillitis, and uncomplicated skin and soft tissue infections in adults and adolescents aged 12 years or older. Cefditoren is similar to cefdinir and cefpodoxime in its antibacterial activity. It is stable to hydrolysis by many common Enterobacteriaceae-produced β-lactamases. Cefditoren has the greatest in vitro antimicrobial activity of all oral cephalosporins against *S. pneumoniae*. The inactive metabolite pivalate is eliminated by the kidneys in combination with carnitine as pivaloylcarnitine. Although cefditoren transiently decreases serum concentrations of carnitine, the clinical significance of this is not clear, but no adverse effects have been reported.[183]

Fourth-Generation Cephalosporins

CEFEPIME

Cefepime, the prototypic agent of this class of antibiotics, was approved by the FDA in 1996. It is distinguished from other cephalosporins by the following characteristics: rapid penetration of outer-membrane porins into the periplasmic space of gram-negative bacteria, facilitated by its net neutral charge; enhanced stability against hydrolysis by inducible or constitutively expressed chromosomally mediated AmpC Bush group 1 β-lactamases and Amber class A plasmid-mediated SHV- and TEM-type ESBLs; and increased binding affinity to multiple PBPs. These factors contribute to cefepime's expanded spectrum of activity and improved efficacy against gram-negative pathogens when compared with third-generation cephalosporins.

The in vitro activity of cefepime encompasses a broad range of gram-positive and gram-negative organisms, including the following: oxacillin-susceptible staphylococci; approximately 75 percent of penicillin-resistant strains of *S. pneumoniae*; viridans streptococci; most strains of Enterobacteriaceae, including, most notably, ESBL-producing strains of *E. coli* and *K. pneumoniae*; and AmpC-mediated resistant *Enterobacter* spp., and *Citrobacter* spp., as well as approximately 90 percent of *P. aeruginosa* isolates. Furthermore, bacteria in which resistance to third-generation cephalosporins develops by means of single-step mutations usually remain susceptible to cefepime. Clinical and laboratory data also indicate that selection of cefepime resistance, specifically among *Enterobacter* spp., and *P. aeruginosa*, is rare. As with other cephalosporins, cefepime is inactive against enterococci, methicillin-resistant staphylococci, *S. maltophilia*, and many anaerobic organisms.[142,152]

Cefepime is indicated for the parenteral treatment of lower respiratory tract, urinary tract, skin and skin structure, and intra-abdominal (in combination with anaerobic antibacterial agents) infections, as well as for empiric monotherapy in pediatric patients with cancer who have fever and neutropenia, for whom cefepime has been shown to be as effective as is meropenem, piperacillin-tazobactam, or ceftazidime with or without an aminoglycoside. In comparative clinical and microbiologic efficacy trials, cefepime as a single antibacterial agent was equivalent to third-generation cephalosporins for the most common childhood pathogens. In addition, cefepime is as safe and well tolerated as are other cephalosporin antibiotics.[42,201,250]

Cefepime should be reserved for complicated community-acquired, health care–associated, or polymicrobial infections, penicillin-resistant and third-generation cephalosporin-resistant pathogens, and patients with cystic fibrosis and *P. aeruginosa* lung infections. Cefepime concentrations in CSF reach approximately 3 to 6 μg/mL (9% of the peak plasma concentration) in children with bacterial meningitis. Therefore, achievable CSF concentrations exceed the MICs of common CNS pathogens by at least 10-fold, except for penicillin- and cephalosporin-nonsusceptible *S. pneumoniae*.[36] Currently, data are insufficient to recommend cefepime as single-agent therapy for meningitis. The safety and efficacy of cefepime have not been established for neonates, but data extrapolated from pharmacokinetic studies among preterm and term infants support a dose of 30 mg/kg every 12 hours for infants younger than 14 days of age, regardless of gestational age. This dosage should provide antibiotic exposure equivalent to or greater than the approved dose of 50 mg/kg every 8 to 12 hours in older infants and children.[55]

Adverse Effects

The cephalosporins, like the penicillins, are safe for children and have almost no dose-related toxicity. The most common reactions are local, including pain at the injection site or thrombophlebitis with parenteral administration and mild gastrointestinal

complaints with oral dosing. Hypersensitivity reactions occur in approximately 1 to 3 percent of treatment courses and include morbilliform rash, urticaria, and pruritus. Drug fever has been associated with the administration of cephalosporins. Nonspecific antibiotic-associated diarrhea and, less commonly, *C. difficile* toxin–mediated colitis can occur after use of cephalosporin.

Other adverse effects are rare, and some are unique to one or a few cephalosporins. Physicians should be alert for uncommon reactions, including reversible neutropenia, which can occur after prolonged use of high-dosage cephalosporins, Coombs-positive hemolytic anemia, and bleeding. Altered hemostasis because of hypoprothrombinemia can result when using any cephalosporin that contains a methylthiotetrazole side chain (cefamandole, cefotetan, and moxalactam). These agents act as competitive inhibitors of vitamin K–dependent carboxylase, which converts clotting factors II, VII, IX, and X to their active forms. Gallbladder sludging, biliary pseudolithiasis, and symptomatic obstructive biliary disease rarely have been associated with the administration of ceftriaxone.[284]

The cephalosporins may produce allergic reactions similar to those caused by the penicillins. Cross-sensitization exists among the cephalosporins, and allergy to one cephalosporin implies allergy to all. Various degrees of immunologic cross-reaction of penicillins and cephalosporins have been demonstrated in vitro and in animal models.[220] Previously quoted studies suggested that the frequency of allergic reactions to cephalosporins ranges from 5.4 to 16.5 percent in adult patients with a history of penicillin allergy and from 1 to 2.5 percent in those without such a history. The incidence of hypersensitivity to unrelated drugs is increased in some patients who are allergic to penicillin, a finding thus suggesting that excipients in antibiotic preparations may be responsible for these reactions.[247] Most patients who are thought to be allergic to penicillin receive cephalosporins without adverse reaction. Data indicate that the risk of an allergic reaction to a cephalosporin challenge among penicillin-allergic subjects with positive skin test responses is 4 percent. Therefore, the following options are available for individuals with positive skin test responses for penicillin hypersensitivity: administration of an alternative non–β-lactam antibiotic, administration of a cephalosporin by graded challenge, or desensitization to the cephalosporin. Individuals with a history of penicillin allergy who have negative penicillin skin test responses may receive cephalosporins because they are at no higher risk than the general population of experiencing allergic reactions.[171] In the absence of penicillin skin testing, a cephalosporin may be used with caution as an alternative to penicillin in children who have an ambiguous history of rash; however, cephalosporins should be avoided in patients with a known immediate or accelerated reaction to a penicillin. Currently, skin testing to evaluate for cephalosporin hypersensitivity is not possible because the potential cephalosporin haptens are unknown, and no standardized antigen exists.

Some products may cause unexpected reactions, such as bile sludge attributed to ceftriaxone and serum sickness–like disease described with cefaclor. A generalized pruritic rash, similar to erythema multiforme, developed in some children treated with cefaclor along with fever, purpura, and arthritis with pain and swelling in the knees and ankles. The signs appeared 5 to 19 days after the start of therapy and generally disappeared within 4 to 5 days after discontinuing use of the drug. The children had no previous history of allergy to a penicillin or cephalosporin.[200] Levine compared adverse reactions in children who received cefaclor (1017 patients, 2513 courses) or amoxicillin (1009 patients, 2358 courses). Serum sickness (defined as arthritis or arthralgia in addition to a rash or urticaria) or erythema multiforme occurred in 11 children (1.1%) who received cefaclor but in none of those who received amoxicillin.[168] Studies suggest that serum sickness–like reactions to cefaclor are associated with lymphocyte sensitization.[150]

CARBACEPHEMS

Carbacephems have a carbon atom at position 1 of the dihydrothiazine ring (cephem nucleus), rather than a sulfur atom. The only carbacephem currently available is loracarbef. Loracarbef is structurally similar to cefaclor but has greater chemical stability in solution. The antibacterial spectrum of loracarbef is similar to that of second-generation cephalosporins. When compared with cefaclor, loracarbef has similar activity against penicillin-susceptible pneumococci, group A streptococci, and oxacillin-susceptible *S. aureus* and greater activity against *H. influenzae* and *M. catarrhalis*, including β-lactamase–producing strains.[84] Loracarbef is not active against *P. aeruginosa*, *Enterobacter*, *Citrobacter*, indole-positive *Proteus*, or *B. fragilis*.

Loracarbef is more stable than cefaclor to hydrolysis by β-lactamases but can be hydrolyzed by ESBLs. Loracarbef is available for oral administration only. It is absorbed rapidly and well; however, absorption is decreased when the agent is taken with food. Loracarbef has a longer serum half-life than do some of the penicillins and can be given twice daily. It is excreted by the kidneys. Loracarbef is relatively well tolerated. Gastrointestinal complaints, including diarrhea, nausea, and vomiting, are the adverse effects most frequently reported, but they occur in less than 5 percent of treatment courses. The safety and efficacy of loracarbef have not been established for infants younger than 6 months.

Loracarbef can be used as an alternative to penicillin or erythromycin for the treatment of group A streptococcal pharyngitis.[83] The effectiveness of loracarbef is similar to that of amoxicillin and amoxicillin-clavulanate for the treatment of acute otitis media caused by susceptible bacteria.[94] Loracarbef is not effective against penicillin-nonsusceptible pneumococci; therefore, it is not considered appropriate for empiric therapy of acute otitis media.[9] For the treatment of mild to moderate skin and skin structure infections, the efficacy of loracarbef is comparable to that of cefaclor. Loracarbef has been approved for use in the treatment of acute bacterial sinusitis and uncomplicated urinary tract infections caused by susceptible bacteria.

MONOBACTAMS

Aztreonam is the prototype monobactam, a name that refers to the unique monocyclic nucleus. This drug has aerobic, gram-negative antibacterial activity similar to that of ceftazidime, but it has no significant gram-positive activity. Unlike most β-lactam antibiotics, aztreonam has the advantage of not inducing β-lactamase activity, and its molecular structure confers a high degree of stability to hydrolysis by β-lactamases. The mechanisms of antibiotic resistance of some gram-negative bacteria include expression of efflux pumps, reduced expression of outer-membrane porin proteins, and production of AmpC-type β-lactamases or ESBLs. Approximately 15 percent of Enterobacteriaceae in North America are resistant to aztreonam.[242] Although it is a β-lactam, aztreonam is weakly immunogenic and can be used in patients with minor forms of β-lactam allergy. Its use in pediatrics is indicated as a secondary agent for the intravenous treatment of lower respiratory tract, skin and soft tissue, urinary tract, and intra-abdominal infections caused by susceptible pathogens. Higher doses of aztreonam may be warranted for pediatric patients with cystic fibrosis. Aztreonam and aminoglycosides are synergistic in vitro against most strains of *P. aeruginosa*, many strains of Enterobacteriaceae, and other aerobic gram-negative bacilli. Aztreonam lysinate for inhalation is a novel monobactam formulation being investigated for *P. aeruginosa* respiratory infections in patients with cystic fibrosis. The safety and efficacy of aztreonam have not been established for infants younger than 9 months or for children with impaired renal function. However,

aztreonam has been used with favorable outcomes in premature and term neonates, as well as in young infants.

CARBAPENEMS

Carbapenems differ from penicillin by virtue of the substitution of a sulfur atom by a carbon atom at position 1 of the β-lactam ring and possession of an unsaturated bond between carbon atoms at positions 2 and 3 in the structure. The carbapenem class of antimicrobial agents exhibits the broadest spectrum of activity of all β-lactam antibiotics; these agents are active against most clinically significant gram-positive and gram-negative pathogens, including anaerobic organisms. Because they are acid-labile in the stomach, they must be administered parenterally.[70] Three carbapenems, imipenem-cilastatin, meropenem, and ertapenem, are approved for use in children.

Imipenem-Cilastatin

Imipenem-cilastatin was the first carbapenem evaluated for the treatment of severe bacterial infections in children. Extensive hydrolysis of imipenem by dehydropeptidase I in the proximal renal tubule results in the production of a potentially nephrotoxic inactive metabolite. Consequently, imipenem must be administered with cilastatin, an inhibitor of dehydropeptidase I. Despite favorable in vitro efficacy against a broad spectrum of bacterial pathogens, the clinical suitability of this antibiotic is limited because of the drug's epileptogenic potential, especially in children with bacterial meningitis. Imipenem causes seizures presumably by acting as a competitive inhibitor of γ-aminobutyric acid, an inhibitory neurotransmitter.[70] Of concern is that imipenem acts as a strong inducer of production of ESBL and may select for β-lactam resistance.[74] Imipenem-cilastatin is indicated for children with severe non-CNS infections caused by susceptible organisms. The dose must be reduced for children with impaired renal function. With the advent of meropenem, imipenem-cilastatin became used less frequently in pediatrics.

Meropenem

Meropenem was approved for use in children in 1996. It is structurally related to imipenem. However, it is more stable against degradation by renal dehydropeptidase I and therefore does not require co-administration with cilastatin. On the basis of its superior safety and efficacy, meropenem generally is considered the preferred carbapenem for treatment of childhood infections.

MECHANISMS OF ACTION

As with other β-lactam antibiotics, meropenem is bactericidal against susceptible bacteria because it inhibits bacterial cell wall synthesis. The *trans* configuration of the hydroxyethyl side chain and hydrogen atoms protect the parent β-lactam structure from inactivation by the most common β-lactamases, including almost all Bush groups 1 and 2 (Amber classes A, C, and D) β-lactamase–producing organisms, including those that produce ESBLs (*Citrobacter*, *Enterobacter*, *E. coli*, *Klebsiella* spp., and *P. mirabilis*) or AmpC β-lactamases (*Citrobacter*, *Enterobacter*, *Pseudomonas*, and *Serratia*). In addition, the pyrrolidine side chain enhances the compound's antipseudomonal activity.[74]

MECHANISMS OF RESISTANCE

Resistance may be intrinsic or acquired and is mediated by numerous mechanisms: (1) some strains of *P. aeruginosa* are deficient in the cell wall porin proteins (OprD) that usually facilitate intracellular penetration; (2) efflux pumps in some gram-negative bacteria are capable of excreting carbapenems; (3) *S. maltophilia* and some other gram-negative pathogens possess uncommon metallo-β-lactamases, the so-called carbapenemases (Bush groups 3a and 3b; Amber class B), which form a diverse group of β-lactamases comprising the IMP- and VIM-families, and SPM-1 and GIM-1 enzymes; these carbapenemases have the ESBL and AmpC antibiotic substrate profile; (4) some gram-negative bacteria possess clavulanic acid-inhibited carbapenemases consisting of the *K. pneumoniae* carbapenemases (KPCs) (Bush group 2f; Amber class A) or OXA enzymes (Bush group 2d, Amber class D); and (5) MRSA strains and *E. faecium* have altered PBPs, which account for their inherent resistance. Furthermore, meropenem acts as a weak inducer of ESBLs.[41,74] At present, the risk that a single-step spontaneous mutation causes resistance among *Pseudomonas* spp. appears to be low.

IN VITRO ACTIVITY

Meropenem has equivalent or slightly less in vitro potency than imipenem does against gram-positive pathogens, but it is significantly more active against gram-negative organisms. The spectrum of activity of meropenem encompasses streptococci (excluding many strains of penicillin- and cefotaxime-nonsusceptible *S. pneumoniae)*, oxacillin-susceptible staphylococci, ampicillin-susceptible enterococci, *L. monocytogenes*, *H. influenzae*, *N. meningitidis*, Enterobacteriaceae, most strains of *P. aeruginosa*, and anaerobes (including β-lactamase–positive strains of *B. fragilis*). Pathogens resistant to meropenem are *Stenotrophomonas*, MRSA, *E. faecium*, approximately 10 percent of *Pseudomonas* strains, and most strains of penicillin- and cefotaxime-nonsusceptible *S. pneumoniae*.[41,49,275] In vitro tests indicate that meropenem acts synergistically with aminoglycoside antibiotics against some isolates of *P. aeruginosa*.[131]

PHARMACOKINETICS

Meropenem exhibits nearly linear pharmacokinetics; increases in dosage result in approximately proportional increases in the peak plasma concentration and area under the plasma concentration-time curve (AUC).[33] Only 2 percent of the drug is bound to plasma proteins, and the drug is distributed widely into tissues and fluids.[70] The elimination half-life declines with increasing age (premature neonates, 2.9 hours; term neonates, 2 hours; children, 1.1 hours; adults, 1 hour) and is longer than that for imipenem. Because meropenem is cleared primarily by glomerular filtration, the dosage should be reduced in children with renal dysfunction.[41] Furthermore, meropenem is cleared efficiently by hemodialysis; therefore, it should be administered after the patient undergoes this procedure. Pharmacodynamic studies indicate that a favorable bacteriologic outcome is best predicted by the duration of the dosing interval for which the meropenem plasma concentration exceeds the MIC_{90} of the target pathogen.[70] Accordingly, a dose of 20 mg/kg every 8 hours achieves optimal plasma concentrations (50 to 60 μg/mL) for systemic infections that do not involve the CNS,[35] whereas a dose of 40 mg/kg every 8 hours is recommended for patients with cystic fibrosis because of accelerated drug excretion.[124] Penetration of meropenem through inflamed meninges is approximately 8 percent of the mean plasma concentration. Consequently, a dose of 40 mg/kg every 8 hours is required to achieve mean peak CSF concentrations of 0.9 to 6.5 μg/mL and to ensure effective treatment of bacterial meningitis.[70,213] An every-12-hour administration schedule probably is appropriate for neonates because of their immature renal function.[33]

INDICATIONS FOR USE

The FDA has approved meropenem as monotherapy for susceptible organisms causing intra-abdominal infections and bacterial

meningitis in children older than 2 months. Meropenem should be reserved for the treatment of cephalosporin-resistant nosocomial pathogens, severe polymicrobial infections with favorable susceptibility profiles, and infections that fail to respond to other antimicrobial agents. Meropenem monotherapy has clinical and microbiologic efficacy equivalent to that of third-generation cephalosporin-based regimens for septicemia and infections of the CNS, abdominal cavity, lower respiratory tract, urinary tract, and skin.[43,213] In addition, meropenem monotherapy has been shown to be equivalent to ceftazidime and amikacin and to piperacillin-tazobactam for empiric treatment of febrile children with neutropenia.[67,219] Clinical efficacy against cephalosporin-resistant pathogens also has been demonstrated in patients with cystic fibrosis.[124] In view of increasing resistance of pneumococci to β-lactam antibiotics, it is prudent to administer vancomycin with meropenem for empiric treatment of suspected bacterial meningitis until CSF microbiologic results are available. Combination therapy with aminoglycosides should be considered for suspected or proven *P. aeruginosa* infections in view of emerging resistant strains and potential antibiotic synergy. The effectiveness of meropenem for neonatal infections has been assessed in small, noncomparative studies that demonstrated a favorable clinical response in neonates in whom previous conventional therapy had failed.[131]

ADVERSE EFFECTS

Data from well-designed clinical trials indicate that meropenem has clinical and laboratory adverse event profiles that are equivalent to those of comparable antibiotic regimens. In contrast to treatment with imipenem-cilastatin, no increased risk of seizures exists in children with or without meningitis who are treated with meropenem,[157] presumably because meropenem has less affinity than does imipenem for the γ-aminobutyric acid receptor. Meropenem can be given intravenously as small-volume bolus injections (over a course of 3 to 5 minutes) without inducing nausea or vomiting. The most common adverse reactions include diarrhea (4%), rash (2%), and vomiting (1%).[41]

β-LACTAM ANTIBIOTICS NOT APPROVED FOR USE IN CHILDREN

Several β-lactam antibiotics available in the United States are not approved for use in children. These agents include the following: cefonicid, a second-generation cephalosporin; cefotetan, a cephamycin; and cefoperazone, a third-generation cephalosporin. Several extended-spectrum cephalosporins and carbapenems that have activity against MRSA are undergoing clinical investigation.

Ertapenem

Ertapenem is a newer, long-acting, parenteral carbapenem that initially was approved for use in adults in 2001. An indication for children was approved in 2005. However, clinical experience with this agent in children is limited. Ertapenem is distinctive because it is administered in a single daily dose in adolescents and adults and is stable against hydrolysis by many β-lactamases except metallo-β-lactamases produced by certain bacterial nonfermenters, particularly *Pseudomonas* and *Acinetobacter*.[183] Apart from these organisms, its antimicrobial spectrum is similar to that of meropenem. Ertapenem has restricted activity against methicillin-resistant staphylococci, penicillin-nonsusceptible pneumococci, enterococci, *Pseudomonas* spp., and *Acinetobacter* spp.[151] The agent may be administered by intravenous or intramuscular routes. It exhibits nonlinear pharmacokinetics because of its concentration-dependent protein binding. The drug is excreted renally, and dosages need to be adjusted in patients with renal insufficiency.

Ertapenem is indicated for the treatment of patients 3 months of age and older with complicated intra-abdominal infections, complicated skin and soft tissue infections, community-acquired pneumonia, complicated urinary tract infections, and acute pelvic infections. Ertapenem is not recommended for the treatment of meningitis because drug concentrations in the CSF are subtherapeutic. The recommended dose in patients 3 months to 12 years of age is 15 mg/kg given parenterally twice daily, up to a maximum of 1 g/day. In older children and adults, the recommended dose is 1 g given once daily. The recommended duration is up to 14 days for intravenous administration. The most common adverse reactions, which include diarrhea, vomiting, and pain at the site of infusion, have comparable rates to those of other parenteral broad-spectrum β-lactam antimicrobials. Seizures have been reported in patients receiving ertapenem and may occur more commonly in individuals with underlying seizure disorders, brain lesions, or renal dysfunction.

VANCOMYCIN

Vancomycin, first isolated from *Amycolatopsis orientalis* (formerly called *Streptomyces*, then *Nocardia*) in soil samples from Borneo, is a high-molecular-weight, complex, soluble glycopeptide.[222] Because of a lack of adequate therapy for penicillinase-producing staphylococci, vancomycin was approved expeditiously by the FDA in 1956 before exhaustive pharmacologic and toxicologic studies had been performed. Original preparations contained fermentation byproducts that caused significant toxicity. Once the penicillinase-resistant penicillins were developed, vancomycin no longer was indispensable until the emergence of methicillin-resistant staphylococci in the late 1970s.

MECHANISMS OF ACTION

Vancomycin is slowly bactericidal against most susceptible gram-positive bacteria, except enterococci, for which it is bacteriostatic. It forms complexes with the D-alanyl-D-alanine portion of peptide precursor units and thus prevents polymerization of the phospho-disaccharide-pentapeptide-lipid complex and cross-linking of peptidoglycan during the second stage of cell wall synthesis. Because the site of action of vancomycin is distinct from that of β-lactam antibiotics, no cross-resistance among the drugs and no competitive inhibition exist. Like penicillin, vancomycin exerts its antimicrobial effect on bacteria in the active growth phase. Additionally, vancomycin alters cytoplasmic membrane permeability and impairs RNA synthesis. Animal infection models suggest that the drug exhibits time-dependent bactericidal activity against susceptible organisms, as well as a moderately long in vitro post-antibiotic effect. The most important pharmacokinetic-pharmacodynamic parameter that correlates with antimicrobial efficacy is the ratio of the 24-hour AUC to the MIC (24-hour AUC/MIC).[71] A lag phase before the onset of rapid killing has been demonstrated in serum-killing studies.[2]

MECHANISMS OF RESISTANCE

Resistance can be categorized into three types: tolerance, acquired resistance, and inherent resistance. Some susceptible gram-positive bacteria, especially enterococci, are tolerant to the bactericidal activity of vancomycin; that is, vancomycin inhibits but does not kill the bacteria (MBC/MIC ratio > 32). Staphylococci tolerant to vancomycin can have autolysin deficiencies. Although most resistance to vancomycin by gram-positive bacteria is

acquired, three genera of gram-positive organisms are inherently resistant to vancomycin: *Erysipelothrix*, *Leuconostoc*, and *Pediococcus*. Five genes code for acquired vancomycin resistance in enterococci: *VanA*, *VanB*, *VanC*, *VanD*, and *VanE*.[198] The *VanA* gene that encodes the VanA phenotype (vancomycin MIC > 256 and teicoplanin resistance) is on a plasmid that is easily transferable by conjugation to other enterococci. The VanB phenotype also is transferable and encodes vancomycin but not teicoplanin resistance. The presence of an inducer such as vancomycin or teicoplanin, another glycopeptide that has not been approved for use in the United States, activates the transcription of genes that encode ligases necessary for resistance to vancomycin. Some enzymes make cell wall precursors ending in D-alanyl-D-lactate located at the end of the pentapeptide side chain, to which vancomycin binds with very low affinity. Other enzymes inhibit or alter the synthesis of endogenous cell wall precursors ending in D-alanyl-D-alanine, to which vancomycin is unable to bind. The finding that vancomycin resistance genes can be transferred experimentally from vancomycin-resistant enterococci to staphylococci highlights the potential risk of spread of resistance by means of naturally occurring plasmid- or transposon-mediated conjugative systems.[198] Although vancomycin resistance can affect susceptibility to other investigational glycopeptides, no cross-resistance exists between vancomycin and other unrelated antibiotics. Resistance to vancomycin rarely develops during appropriate therapy, perhaps because of its multiple mechanisms of action, but prolonged or indiscriminate use of the drug can contribute to selective pressure and result in the development of vancomycin-resistant enterococci colonizing the gut. Of great concern is the emergence of invasive strains of multidrug-resistant enterococci (≤14% of health care–associated enterococcal strains)[178] and staphylococci, for which only a few therapeutic options exist, including linezolid and other antimicrobials not approved for use in children such as quinupristin-dalfopristin, daptomycin, and tigecycline (a novel glycylcycline antibiotic).The first strains of *S. aureus* with intermediate resistance to vancomycin/glycopeptide (VISA/GISA), defined as having MICs of 8 or 16 μg/mL, were detected in the United States in 1997. Soon thereafter, three cases of vancomycin-resistant *S. aureus* (VRSA; MIC = 32 μg/mL) were isolated. The mechanism for this high-level vancomycin resistance involves the horizontal transfer of a transposon containing *vanA* and associated genes from vancomycin-resistant enterococci.[232] Glycopeptide-resistant isolates were found to have thicker extracellular matrices, a characteristic that may cause vancomycin to become trapped in the outer layers of the cell wall and may limit access to the cytoplasmic membrane where the functional targets are located. Pneumococci tolerant to vancomycin were described first in 1999.[212] These strains escape lysis and killing by vancomycin because of an alteration in the regulation of autolysin. Their prevalence is 4 to 10 percent of nasopharyngeal and invasive isolates, and they are associated with increased mortality when etiologic in meningitis.[239] The CDC recommends that the use of vancomycin be restricted appropriately to reduce the risk of inducing vancomycin resistance.[57]

IN VITRO ACTIVITY

The in vitro spectrum of activity for vancomycin is limited to gram-positive aerobic and anaerobic bacteria, with little, if any, activity against aerobic or anaerobic gram-negative bacilli, except *Chryseobacterium meningosepticum*. Group A streptococci, pneumococci, *Corynebacterium* spp., and *C. difficile* are highly susceptible to vancomycin, whereas *L. monocytogenes*, microaerophilic and anaerobic streptococci, enterococci, coagulase-positive and coagulase-negative staphylococci, *Bacillus anthracis* and other spp., *Lactobacillus*, *Actinomyces*, and other *Clostridium* spp. have higher MIC values that are still in the susceptible range. Pathogens resistant to vancomycin include *Erysipelothrix* and *Leuconostoc* and *Pediococcus* spp. that can cause serious infections in immunocompromised patients. The bactericidal activity of vancomycin combined with gentamicin has been shown to be synergistic in vitro against strains of enterococci (provided there is no high-level gentamicin resistance; MIC > 2000 μg/mL), nonenterococcal group D streptococci, viridans streptococci, and most methicillin-susceptible *S. aureus* and MRSA. Addition of rifampin, gentamicin, or both, results in improved cure rates for *S. epidermidis* prosthetic valve endocarditis.[178] Although vancomycin has activity against many gram-positive bacteria, it is not the most active agent for these organisms, but it is the drug of choice for multidrug-resistant bacteria such as methicillin-resistant staphylococci and highly penicillin-resistant and cephalosporin-resistant pneumococci.

PHARMACOKINETICS

Intravenous administration of vancomycin is preferred because intramuscular injection causes pain and tissue necrosis. Intravenous preparations must be diluted further in normal saline or dextrose solutions before beginning slow infusion. Vancomycin is approximately 55 percent bound to serum proteins and diffuses well into most body tissues, with adequate concentrations achieved in pericardial, pleural, ascitic, bone, and synovial fluids, but not in aqueous humor or bile. Vancomycin does not diffuse well into CSF in the absence of inflamed meninges, but CSF concentrations of 7 to 21 percent of concomitant serum levels can be achieved during therapy for meningitis when higher doses (15 mg/kg every 6 hours) are administered. The bone-to-serum ratio of vancomycin is 10 percent, but this figure increases up to 30 percent in infected bone. Vancomycin remains active at pH 6.5 to 8, and concentrations achieved in abscess fluid approximate serum levels.[178] Intrathecal or intraventricular administration has been used infrequently for CNS infections that are difficult to eradicate.[177] Vancomycin is not metabolized significantly and is excreted by glomerular filtration. The mean serum elimination half-life in adults with normal renal function is 6 hours. For children, it ranges from 5 to 10 hours in newborns and 4 hours in older infants to 2 to 3 hours in children. In anephric patients, the elimination half-life extends to 7 or more days. Hemodialysis removes small amounts of vancomycin, whereas peritoneal dialysis may reduce serum concentrations by approximately 40 percent. Nomograms and patient-individualized bayesian dosing regimens have been used for vancomycin dosing in renal failure in adults.[163,192]

INDICATIONS FOR USE

Because of the potential for development of resistance, vancomycin should be reserved for patients with moderate to severe infections caused by vancomycin-susceptible bacteria that are resistant to other antibiotics. Vancomycin can be used for the treatment of infections caused by β-lactam–susceptible and vancomycin-susceptible bacteria in patients with hypersensitivity reactions to β-lactam antimicrobial agents. Empiric therapy for patients with ventricular shunt-related and catheter-associated infections frequently includes vancomycin to provide activity against coagulase-negative staphylococci. Vancomycin can be used in combination with gentamicin, rifampin, or both, for bactericidal synergistic activity to treat documented prosthetic device–related *S. epidermidis* infections, methicillin-resistant staphylococcal endocarditis, or endocarditis caused by high-level penicillin-resistant, aminoglycoside-susceptible strains of enterococci. *S. epidermidis* infections of long-term intravenous catheters usually

can be cured without removal of the device.[178] In addition, vancomycin is the drug of choice in patients with immediate-type hypersensitivity to penicillins and other β-lactam antibiotics for prophylaxis (gastrointestinal or genitourinary procedures) or treatment of endocarditis caused by streptococci, enterococci, and staphylococci.[17,73] Empiric treatment of bacterial meningitis suspected or proved to be caused by *S. pneumoniae* in children 1 month old or older should consist of vancomycin in addition to a third-generation cephalosporin until susceptibility data are available; if cephalosporin resistance is documented, rifampin should be added to the regimen. However, vancomycin need not be used if compelling evidence implicates pathogens other than *S. pneumoniae*, such as the observation of gram-negative diplococci on a CSF smear. Vancomycin also is indicated for the treatment of infections with penicillin-resistant streptococci, *C. jeikeium*, and *Bacillus* spp. penicillin-resistant enterococci, as well as for bacteremia, pneumonia, cellulitis, and osteomyelitis caused by methicillin-resistant staphylococci. Some experts advocate the addition of nafcillin, oxacillin, gentamicin, or rifampin for optimal coverage of oxacillin-susceptible *S. aureus* in severely ill patients who are receiving empiric treatment with vancomycin because certain strains of staphylococci are tolerant to vancomycin.[110] Proof of enhanced efficacy with combination therapy in this setting is lacking. Although nonabsorbable oral vancomycin is effective therapy for *C. difficile* colitis, oral metronidazole represents first-line therapy; vancomycin should be given only for metronidazole treatment failures, to limit the development of vancomycin-resistant enterococci. Vancomycin is among the agents of choice for the treatment of meningitis caused by *C. meningosepticum*.

ADVERSE EFFECTS

The purification of vancomycin allegedly decreased the frequency of adverse reactions noted several decades ago. The most common adverse effect is "red man" or "red neck" syndrome, or glycopeptide-induced anaphylactoid reaction, manifested as flushing of the face and upper part of the trunk, pruritus during vancomycin infusion, angioedema, and, rarely, hypotension. Vancomycin directly causes release of histamine from mast cells by non–immune-mediated mechanisms. Because it is a dose- and rate-dependent reaction, administering doses less than 500 mg, prolonging the infusion period to at least 1 hour, or doing both, decreases the risk of occurrence. Pretreatment with H_1-receptor antagonists (diphenhydramine, hydroxyzine) prevents the development of this reaction, and symptoms usually resolve promptly after discontinuation of the infusion.

Controversy surrounds the issue of vancomycin-induced ototoxicity and nephrotoxicity.[48] Such toxicity has not been demonstrated in experimental animal models. Although numerous cases of toxicity in humans have been reported, the literature is difficult to interpret because of confounding variables, including recent or concurrent use of aminoglycosides or other ototoxic or nephrotoxic agents, lack of identification of antecedent otologic or renal disease, and inconsistencies in sampling methods when measuring serum vancomycin concentrations. Tinnitus and high-tone hearing loss have been associated with the administration of vancomycin, particularly when the peak serum levels exceed 50 μg/mL. If vancomycin is ototoxic, whether toxic peak or toxic trough serum concentrations are responsible is not clear. Several studies suggest an increased risk of nephrotoxicity when aminoglycosides are used concurrently with vancomycin, especially when they are administered for longer than 21 days.[107] Other adverse effects seen occasionally with the use of vancomycin include reversible neutropenia, thrombocytopenia, drug fever, and macular rash.

The usefulness of serum vancomycin concentration determinations is controversial because a definitive relationship between vancomycin concentration and either adverse effects or clinical outcome has not been proved.[55,191] Until the significance of vancomycin serum concentration determinations has been clarified, monitoring has been suggested for the following clinical situations only: (1) patients with altered renal function, including premature infants; (2) patients receiving larger than normal dosages, especially those with meningitis in whom adequate CSF values are necessary; (3) anephric patients undergoing hemodialysis (to avoid subtherapeutic vancomycin concentrations during prolonged dosing intervals); and (4) patients receiving concomitant therapy with nephrotoxic agents.[191] Because serum vancomycin values commonly are subtherapeutic in the first days of treatment for severe sepsis, presumably because of larger volumes of distribution of vancomycin in these ill children, we recommend an initial dosage of 60 mg/kg/day in four divided doses.

Daptomycin has been approved for treatment of adults with complicated skin and soft tissue infections and with endocarditis and sepsis. It is a parenteral lipopeptide that disrupts cell membrane potential, and, unlike vancomycin, it is rapidly bactericidal against MRSA strains. Pediatric experience is limited. Other semisynthetic glycopeptides in development such as televancin or the glycopeptide approved for adults, dalbavancin, may have some advantage over vancomycin and daptomycin in that resistance of MRSA is less likely because of their dual mechanisms of action.

AMINOGLYCOSIDES

Aminoglycosides are natural and semisynthetic compounds that consist of at least two amino sugars bound by a glycosidic linkage to a hexose nucleus, the aminocyclitol ring. A more appropriate name would be aminoglycosidic aminocyclitols. Streptomycin, isolated from *Streptomyces griseus*, was the first aminoglycoside and was available for use in 1944. Many aminoglycosides have been isolated or developed since that time. The suffix denotes the origin of the aminoglycoside: those ending with the suffix *-mycin* were derived from *Streptomyces* spp., whereas those ending with *-micin* were derived from *Micromonospora* spp. Currently, eight aminoglycosides are approved for use in the United States (Table 248–7), and several more are available in other countries. Despite the development of less toxic antibiotics with broad-spectrum activity, aminoglycosides continue to fulfill an essential role in the treatment of severe infections caused by aerobic gram-negative bacilli and enterococci.

MECHANISMS OF ACTION

Against susceptible bacteria, aminoglycosides demonstrate rapid, concentration-dependent bactericidal activity.[87,274] They

TABLE 248–7 Classfication Scheme for Aminoglycosides

Aminocyclitol Ring	Family	Member
Streptidine	Streptomycin	Streptomycin
2-Deoxystreptamine	Kanamycin	Kanamycin Amikacin Tobramycin
2-Deoxystreptamine	Gentamicin	Gentamicin Netilmicin
2-Deoxystreptamine	Neomycin	Neomycin Paromomycin

exert their effect by binding irreversibly to the 30S subunit of the bacterial ribosome, which results in inhibition of protein synthesis and induction of translational errors. Bacterial uptake of aminoglycosides can be facilitated by concomitant therapy with cell wall–active antibiotics such as β-lactams or vancomycin. Penetration of the outer membrane of gram-negative bacteria is mediated by a self-promoted uptake process involving aminoglycoside-induced disruption of magnesium bridges between adjacent lipopolysaccharide molecules. Passage through porin channels is unlikely because of the large size of aminoglycosides. Subsequent transport into cytoplasm and attachment to the 30S ribosomal subunit require two energy- and oxygen-dependent steps, energy-dependent phases I and II (EDP-I and EDP-II), that use an electrochemical proton gradient. EDP-I is inhibited by hyperosmolarity, low pH, and anaerobic conditions. Unlike other protein synthesis inhibitors, which usually are bacteriostatic, aminoglycosides are bactericidal. Binding of aminoglycosides to the 30S subunit does not prevent formation of the initiation complex of peptide synthesis. However, it disrupts elongation of the peptide chain by impairing the proofreading process, an impairment that results in translational inaccuracy. Aberrant protein products may be inserted in the cell membrane and cause altered permeability. The primed amino sugar and 2-deoxystreptamine groups are essential for the ribosome-specific activity of aminoglycosides.[189]

MECHANISMS OF RESISTANCE

The prevalence of acquired aminoglycoside resistance is relatively low, and its development during therapy is an unusual event. Bacteria can acquire resistance to aminoglycosides because of alterations in the bacterial target, reduced bacterial cell permeability or uptake, or modification of the antibiotic by bacterial enzymes, the last being most significant clinically.[76] Mutations in the aminoglycoside-binding site of the 30S ribosome have been associated with high-level resistance to streptomycin but not to other aminoglycosides, possibly because, unlike streptomycin, they bind to multiple sites on the ribosome. Facultative aerobic bacteria causing infection in sites with reduced oxygen tension, anaerobic bacteria, and such fermentative bacteria as streptococci are inherently resistant to aminoglycosides because they are unable to generate an electrochemical proton gradient sufficient for aminoglycoside transport into the cytoplasm. Other bacteria can acquire resistance because of reduced permeability or lack of transport, as demonstrated in staphylococci, in which resistance to aminoglycosides quickly develops when monotherapy is administered. *P. aeruginosa*, other nonfermenting gram-negative bacilli, and, less frequently, members of the Enterobacteriaceae can acquire a moderate level of resistance to all aminoglycosides as a result of various mechanisms that interfere with uptake, cytoplasmic transport, or regulation of the anaerobic respiratory pathway. Plasmid-mediated production of aminoglycoside-modifying enzymes is the most common mechanism of acquired resistance. Many enzymes have different substrate specificities, several of which can be elaborated simultaneously in the same bacterium. These acetyltransferases, nucleotidyltransferases, and phosphotransferases interact with amino or hydroxyl groups on the aminoglycoside and modify the aminoglycoside so that it binds poorly to the 30S ribosome, thereby usually resulting in high-level resistance.[189] Gentamicin and tobramycin each is susceptible to at least five modifying enzymes, whereas amikacin possesses an aminohydroxybutyryl group that prevents enzymatic modification at multiple sites. Consequently, more than 80 percent of gentamicin-resistant strains of Enterobacteriaceae and 25 to 85 percent of gentamicin-resistant *P. aeruginosa* strains are susceptible to amikacin.[160]

IN VITRO ACTIVITY

The in vitro antibacterial spectrum of aminoglycosides includes a wide range of aerobic gram-negative bacilli, many oxacillin-susceptible staphylococci, many enterococci, and some mycobacteria.[190] Some gram-negative bacilli, including *Burkholderia cepacia* and *S. maltophilia*, are consistently resistant to all aminoglycosides. The spectra of activity of gentamicin, tobramycin, and netilmicin are similar, and strains resistant to one usually are resistant to the others. The major advantage of tobramycin currently is its activity against some strains of *Acinetobacter* and indole-positive Proteae (*Morganella* spp., *Proteus* spp., *Providencia* spp.) that are resistant to gentamicin.[231] Because many aminoglycoside-modifying enzymes are active against gentamicin, tobramycin, and netilmicin but inactive against amikacin, amikacin frequently is prescribed for empiric treatment of nosocomial gram-negative bacillary infections. Amikacin also has activity against the *Mycobacterium avium* complex (MAC), some rapidly growing mycobacteria, and *Nocardia asteroides*. Netilmicin is less active than are other aminoglycosides against *P. aeruginosa*, but it can have activity against some gentamicin-resistant, aerobic, gram-negative bacilli. Although gentamicin, tobramycin, amikacin, and netilmicin have similar spectra of activity, susceptibility testing is recommended because of geographic and interhospital variation in resistance patterns. Lack of activity against *P. aeruginosa*, *Klebsiella*, and *Serratia* has limited the use of kanamycin. Streptomycin is inactive against many gram-negative enteric bacilli but does have activity against *Francisella tularensis*, *Yersinia pestis*, and *Mycobacterium tuberculosis*.

PHARMACOKINETICS

Aminoglycosides have in common many pharmacokinetic characteristics. They are highly polar, water-soluble compounds that are positively charged cations at neutral pH. Their antibacterial activity is pH-dependent, with increased activity at higher pH. They are relatively resistant to degradation at various temperatures and pH values. After parenteral administration, aminoglycosides are distributed rapidly in extracellular body water, with slow accumulation in tissues. The volume of distribution is decreased (with respect to total-body weight) in obese patients and is increased in patients with illnesses associated with edema, such as severe infections, burns, congestive heart failure, and ascites. With the exception of proximal renal tubular cells and possibly inner ear hair cells, penetration into other body compartments is impaired because of lipid insolubility, polycationic charge, and size of the aminoglycoside. Proximal renal tubular cells absorb aminoglycosides through carrier-mediated pinocytosis, and as a result, renal cortical concentrations exceed those in plasma. Aminoglycosides do not penetrate the blood-brain barrier in the absence of meningeal inflammation, but with inflammation, approximately 20 to 25 percent of the serum concentration penetrates the CSF. These CSF concentrations are low in relation to the MIC of the meningeal pathogen for which it is given. Thus, because of this narrow therapeutic-toxic index, monotherapy for gram-negative bacillary meningitis with aminoglycosides is not recommended. Aminoglycosides are not metabolized and, after parenteral administration, are excreted unchanged in the kidney by glomerular filtration, with approximately 5 percent of excreted drug reabsorbed in the proximal tubular cells. Minimal amounts are excreted in saliva and feces. Urine concentrations exceed those in plasma by 25 to 100 times.

After intramuscular injection, aminoglycosides are absorbed completely, and peak serum concentrations are achieved within

90 minutes, except in some disease states that interfere with tissue perfusion, such as hypotension. When administered intravenously, the infusion should be given slowly over the course of 30 to 60 minutes to avoid the development of potential adverse effects. Peak serum concentrations are achieved within 30 to 60 minutes after infusion. Because of the polar nature of these drugs, absorption after oral administration is insignificant and inadequate to treat systemic infections, but aminoglycosides can accumulate in the presence of renal failure and result in concentrations sufficient to cause toxicity. Although aminoglycosides are nonirritating when they are instilled into pleural or peritoneal spaces, their absorption is rapid and can result in significant toxicity. In contrast, instillation into the lateral ventricles or irrigation of the bladder has not been associated with significant systemic absorption. When compared with parenteral administration, aerosol administration of aminoglycoside results in higher concentrations in bronchial secretions and less toxicity.[228] Indiscriminate use of topical aminoglycosides in the form of ointments, creams, or ophthalmic solutions may lead to the rapid development of aminoglycoside resistance, especially in immunocompromised patients.

Antimicrobial dosing in newborns and young infants differs from that in older children and adults because of developmental changes in renal function and increased total-body water composition. Neonates and young infants have a larger volume of distribution and a reduced glomerular filtration rate; these differences are more pronounced in very-low-birth-weight premature neonates.[225] Therefore, twice-daily regimens have been the standard of care in newborn infants for years. Once-daily gentamicin dosing (4 mg/kg) in term neonates has been studied extensively and is used routinely in many nurseries; no differences in effectiveness or toxicity have been observed.[274] The aminoglycoside dosing schedule currently approved for use in older infants and children is a three-times-daily regimen. Because aminoglycosides demonstrate concentration-dependent bactericidal activity, higher peak serum concentrations result in more extensive and more rapid killing.[274] Aminoglycosides also demonstrate a post-antibiotic effect against susceptible aerobic gram-negative bacilli in vitro and in vivo.[282] Furthermore, host leukocytes display enhanced phagocytosis of aminoglycoside-exposed bacteria in vitro, referred to as post-antibiotic leukocyte enhancement.[93] The higher the peak serum concentration, the longer is the duration of the post-antibiotic effect. In the presence of normal renal function, the serum concentration is low or undetectable before the administration of the next dose (trough value), but antibacterial activity persists because of the post-antibiotic effect. These characteristics favor single-daily dosing. Numerous studies in adults have shown that once-daily dosing of aminoglycosides (amikacin, gentamicin, tobramycin, or netilmicin) is either equivalent or superior to multiple-daily dosing with regard to clinical and bacteriologic effectiveness, nephrotoxicity, ototoxicity, mortality, and cost-effectiveness.[274] The Hartford nomogram originally described by investigators from Hartford, Connecticut, commonly is used to guide extended-interval aminoglycoside dosing for adults. In adults, a single serum concentration of gentamicin or tobramycin is obtained 8 to 12 hours after the start of the infusion of the first dose and plotted on the nomogram to determine the appropriate dosing interval.[99] Similar nomograms for children have not been validated. Nonetheless, published guidelines support 7.5 mg/kg per dose of gentamicin for infants and children up to 10 years of age; for adolescents and adults, 6 to 7 mg/kg per dose is appropriate.[69,274] Extended-interval aminoglycoside dosing is contraindicated if the patient's volume status is altered (third spacing, thermal injury > 20 percent total-body surface area), optimal dosing regimen is predefined (cystic fibrosis, endocarditis), renal function is impaired, or immune status is compromised.

INDICATIONS FOR USE

The major use of aminoglycosides in children (usually in combination with other antibacterial agents) is for serious infections caused by gram-negative enteric bacilli, including neonatal sepsis, sepsis in a child with malignant disease or an immunologic defect, abdominal and systemic infections associated with spillage of fecal contents into the peritoneum, endocarditis caused by certain susceptible bacteria, and complicated urinary tract infections. Because gentamicin is the least expensive aminoglycoside and the one with which physicians have the most experience, it often is considered the first-line agent for empiric treatment of suspected aerobic gram-negative bacillary infections in institutions with minimal background resistance. Combinations of an aminoglycoside and a cell wall–active antibiotic have been used for synergistic bactericidal activity. Gentamicin or streptomycin, in combination with penicillin G, ampicillin, or vancomycin, is recommended for the treatment of endocarditis caused by susceptible enterococci, viridans streptococci, or *Streptococcus bovis*. Administration of gentamicin for 3 to 5 days, in combination with an antistaphylococcal agent, should be considered for the treatment of staphylococcal endocarditis.[17] An aminoglycoside combined with an acylureidopenicillin, ceftazidime, cefepime, or a carbapenem is recommended for serious infections caused by *P. aeruginosa*. Amikacin often is used for empiric treatment of nosocomial aerobic, gram-negative, bacillary infections in patients in institutions having significant resistance to gentamicin and tobramycin. Streptomycin is indicated for use alone or in combination with other antibiotics for the treatment of tularemia and plague and in combination with other agents for the treatment of tuberculosis, brucellosis, and enterococcal endocarditis. For such purposes, gentamicin could be a more suitable agent for some of these conditions, such as brucellosis, tularemia, and plague. Paromomycin is too toxic for parenteral use, but when administered orally, it has been useful in the treatment of asymptomatic intestinal amebiasis, *Dientamoeba fragilis* infection, and *Giardia lamblia* infection during pregnancy.

ADVERSE EFFECTS

Although aminoglycosides have intrinsic toxicity, allergic reactions are uncommon. All aminoglycosides can injure the proximal renal tubules, the cochlea, the vestibular apparatus, or a combination thereof and can cause neuromuscular blockade, but the risk varies with each agent. Because aminoglycosides do not induce a significant inflammatory response, pain at intramuscular injection sites and phlebitis at intravenous infusion sites are unusual. Hypersensitivity reactions and drug fever are rare events.

Many theories have been postulated regarding the mechanism of nephrotoxicity,[188] including inhibition of lysosomal phospholipases within the proximal renal tubules. Clinical findings include a mild, nonoliguric decrease in the glomerular filtration rate that typically is reversible. When compared with traditional dosing, once-daily dosing causes similar or lower rates of nephrotoxicity.[69,274] In humans, nephrotoxicity has been associated with prolonged duration of therapy at high dosages, previous aminoglycoside therapy, administration of drugs to critically ill patients with intravascular volume depletion or hyponatremia and to those with impaired kidney function, and concomitant administration of other potentially nephrotoxic agents such as amphotericin B and loop diuretics.[78]

The mechanism by which aminoglycosides cause vestibular and cochlear ototoxicity has not been elucidated fully. Cochlear damage is manifested as tinnitus or high-frequency hearing loss, whereas vestibular toxicity is associated with vertigo, nystagmus, and ataxia.[12] Damage can be unilateral or bilateral. Occasionally,

the ototoxicity is reversible, but permanent damage occurs commonly. Mild cochlear damage may not be recognized because the high-frequency hearing range is affected first. Conventional audiograms that do not test high-frequency ranges may not detect cochlear injury. Delayed onset of high-frequency hearing loss has been observed in humans. Factors that increase the risk for development of aminoglycoside ototoxicity in humans include impaired renal function and prolonged duration of treatment. Although elevated serum peak and trough concentrations are thought to contribute to ototoxicity, a specific threshold for peak or trough concentrations has not been established. Neuromuscular blockade can occur after rapid intravenous infusion, after extensive peritoneal irrigation, or during routine parenteral aminoglycoside administration in patients with underlying conditions that affect the neuromuscular junction, such as myasthenia gravis and botulism, or during concomitant administration of agents that act on the neuromuscular junction, such as succinylcholine. Hypomagnesemia in the neonate may predispose to hearing loss.

To avoid toxicity and ensure therapeutic values, concentrations of aminoglycosides in serum should be monitored in all patients who have impaired renal function or who receive other nephrotoxic medications or prolonged treatment; such monitoring is particularly relevant to preterm, low-birth-weight infants. Monitoring also should be considered in obese and undernourished children, children with severe burns, and those with chronic disease, for which the volume of distribution of the drug can be altered (e.g., cystic fibrosis).

MACROLIDES

Erythromycin, isolated from *Streptomyces erythreus* found in a soil sample in the Philippines, was the first macrolide and was available for use in 1952. Many natural and semisynthetic erythromycin derivatives have been developed since then, three of which are approved for use in pediatrics: erythromycin, clarithromycin, and azithromycin. Macrolide antibiotics consist of a large lactone ring attached by a glycosidic bond to one or more amino or neutral sugar moieties. Erythromycin and clarithromycin have 14-membered lactone rings, whereas azithromycin, an azalide antibiotic that is grouped with the macrolides, has a tertiary amino group inserted in its 15-membered ring. In addition to similarities in chemical structure, these macrolides have similar antibacterial spectra, mechanisms of action, and mechanisms of resistance, but they differ in their pharmacokinetic characteristics.

MECHANISMS OF ACTION

Macrolide antibiotics reversibly bind to the 50S ribosomal subunit and inhibit protein synthesis. Initial studies suggested that the antibacterial activity of erythromycin usually was bacteriostatic, but against some actively growing, susceptible bacteria, large concentrations of erythromycin were bactericidal.[117] Clarithromycin and azithromycin usually are bacteriostatic, but they are bactericidal against *S. pyogenes*, *S. pneumoniae*, and *H. influenzae*.[285] Macrolides cause dissociation of peptidyl-tRNAs from bacterial ribosomes that results in accumulation of intracellular peptidyl-tRNA and depletion of the free tRNA pool, with toxic effects for bacteria.[263] The specific target appears to be the 23S ribosomal RNA. Macrolides block the entrance to the tunnel in the 50S ribosomal subunit through which many, if not all, nascent peptide chains exit the ribosome. By blocking the exit tunnel, macrolides induce premature dissociation of peptidyl-tRNAs from the ribosome just after initiation of protein synthesis. Erythromycin interferes with binding to the 50S ribosome by chloramphenicol and clindamycin, thus suggesting common or overlapping binding sites for these agents.

MECHANISMS OF RESISTANCE

Many gram-negative bacteria are inherently resistant to macrolides because of the relative impermeability of their outer membrane. Other bacteria can acquire resistance by production of enzymes that modify and inactivate the macrolide, active efflux of the antibiotic, and alteration of their ribosomal targets.[165] Two mechanisms by which bacteria can alter their ribosomes and acquire macrolide resistance have been identified. High-level resistance because of an altered protein component in domain V of the 50S ribosomal subunit has occurred after a one-step chromosomal mutation. Such resistance has been demonstrated in *M. avium*, *Helicobacter pylori*, *Treponema pallidum*, and *Propionibacterium* spp. Plasmid-mediated macrolide, lincosamide, streptogramin B (MLS_B) resistance occurs when a single adenine residue within a conserved region of domain V of the nascent 23S rRNA component of the 50S ribosomal subunit is methylated. Such methylation results in an altered common target that confers cross-resistance to macrolides (erythromycin and clarithromycin), azalides (azithromycin), lincosamides (clindamycin), and streptogramin B (quinupristin-dalfopristin).[165] The production of methylases is encoded by a class of genes referred to as *erm* (*e*rythromycin *r*ibosome *m*ethylation), nearly 40 of which have been characterized in a wide range of microorganisms, including gram-positive species, spirochetes, and anaerobes. Twenty-one classes of *erm* genes with less than 80 percent sequence homology have been designated unique letters. Pathogenic bacteria comprise one of four major classes: bacteria in classes *erm*(A) and *erm*(C) typically are in methicillin-resistant or methicillin-susceptible strains of staphylococci, respectively; members of class *erm*(B) are mostly in streptococci and enterococci; and class *erm*(F) usually is associated with anaerobic pathogens.[236] These genes are exchanged easily among different strains by plasmids, transposons, or bacterial conjugation. Resistance mediated by *erm* can be constitutive or inducible. When bacteria with inducible MLS_B resistance are exposed to a macrolide inducer (erythromycin, clarithromycin, or azithromycin), inactive methylase mRNA undergoes rearrangements that permit ribosomes to translate the methylase coding sequence. The diversity of inducible macrolide-resistant genotypes results in complex phenotypes, particularly among streptococci and enterococci with *erm*(B) genes, whereas constitutive production of a methylase generally confers a predictable high-level cross-resistance to all MLS_B antibiotics. Staphylococci that are resistant in vitro to 14- and 15-membered ring macrolides but susceptible to 16-membered ring macrolides, clindamycin, and streptogramins B should routinely undergo further testing with a double-disk diffusion test to screen for an erythromycin-induced, D-shaped zone of bacterial inhibition around a clindamycin disk. A positive D-test indicates that the *Staphylococcus* isolate has inducible MLS_B resistance and that use of clindamycin can result in clinical treatment failure, presumably related to selection of constitutive mutants in patients with severe infections and large bacterial inocula.[165] Similar inducible resistance phenotypes may be observed among various strains of streptococci for which the use of clindamycin should be discouraged.

Macrolide resistance associated with active efflux is caused by two classes of pumps: a plasmid-mediated, adenosine triphosphate (ATP)–binding cassette (ABC) transporter encoded by the *msr*(A) gene and found in *Staphylococcus* spp., and a protein of the major facilitator superfamily (MFS) found in streptococci and enterococci and encoded by the *mef*(A) gene. The MsrA pump confers a macrolide and streptogramin B (MS_B) pattern of resistance that can be distinguished from the MLS_B-inducible pheno-

type by the double-disk diffusion D-test. The Mef(A) pump affects only 14- and 15-membered ring macrolides (M phenotype).[165] Less commonly, bacteria produce enzymes that inactivate macrolides, including acetyltransferases, esterases, phosphotransferases, or glycosylases found in some strains of Enterobacteriaceae, but macrolides are not used to target these pathogens. Therefore, this mechanism of low-level resistance is not clinically important.[236] A causal association between erythromycin resistance and antibiotic use, probably mediated by selection pressure, was suggested by a nationwide epidemiologic study in Finland in which reduction of the use of erythromycin over the course of time was associated with a steady decline in erythromycin resistance among *S. pyogenes* isolates from throat swabs and pus specimens.[254] Conversely, increased consumption of macrolides in Spain since 1995 was related to an increase in macrolide resistance.[111]

IN VITRO ACTIVITY

Erythromycin is effective in vitro against a diverse group of microorganisms, including *Bacteroides* spp., *Bordetella pertussis*, *Chlamydophila* spp., *Corynebacterium diphtheriae*, *H. pylori*, the bacterium of legionnaires' disease *(Legionella pneumophila)*, mycoplasmas *(Mycoplasma pneumoniae* and *Ureaplasma urealyticum)*, spirochetes *(T. pallidum)*, and anaerobic and aerobic gram-positive cocci *(S. pneumoniae*, *S. pyogenes*, and penicillinase-producing and non–penicillinase-producing strains of oxacillin-susceptible *S. aureus)*. Erythromycin is active against *Campylobacter jejuni*, *N. meningitidis*, *N. gonorrhoeae*, and, to a lesser extent, *H. influenzae*. Macrolide resistance among diverse pathogens is increasing worldwide, and the clinical consequences are topics of active research.[103,133,159]

The newer macrolides have spectra of activity similar to that of erythromycin. When compared with erythromycin, clarithromycin has equivalent or greater activity against *M. catarrhalis*, *H. influenzae*, *M. pneumoniae*, *Chlamydia pneumoniae*, *Chlamydia trachomatis*, *L. pneumophila*, *U. urealyticum*, and *N. gonorrhoeae.* Clarithromycin is two to four times more active against most erythromycin-susceptible streptococci and staphylococci. However, pneumococci that are resistant to erythromycin (≤48% in certain regions[157]) also are resistant to clarithromycin and azithromycin; rates of macrolide resistance among pneumococci tend to correlate with rates of penicillin resistance.[285] Clarithromycin also has activity against organisms that are resistant to erythromycin, including *H. pylori*, *Toxoplasma gondii*, *Mycobacterium leprae*, MAC, and *Mycobacterium chelonae*.

When compared with erythromycin, azithromycin has less activity against gram-positive bacteria, including *S. pneumoniae*, but has better activity against gram-negative bacteria, including *Vibrio cholerae* and some Enterobacteriaceae such as *Shigella* spp. The in vitro activity of azithromycin against *M. pneumoniae*, *C. pneumoniae*, and *L. pneumophila* is similar to that of erythromycin and clarithromycin. Azithromycin is more active than is erythromycin against *C. trachomatis* and *U. urealyticum* and two- to eightfold more active than is erythromycin or clarithromycin against *M. catarrhalis*, *H. influenzae*,[18,186] *N. gonorrhoeae*, and *H. ducreyi.* Azithromycin has activity against *T. gondii* and, although less than that of clarithromycin, against MAC organisms. Azithromycin and clarithromycin are highly effective in vitro against *Borrelia burgdorferi*, *Bartonella henselae*, and *Bartonella quintana.*

PHARMACOKINETICS

The macrolides differ in pharmacokinetic properties (Table 248–8). Clarithromycin[116] and azithromycin[109] are gastric acid-stable and relatively well absorbed from the gastrointestinal tract, whereas erythromycin is acid-labile, and absorption varies with the oral preparation. Macrolides undergo metabolism by the hepatic microsomal cytochrome P-450 system. Erythromycin and clarithromycin, but not azithromycin, are inhibitors of the cytochrome P-450 enzyme system (CYP3A and CYP1A subclasses) and can interact with other drugs that are metabolized by this system.[216] Most of the metabolites are inactive, with the exception of 14-(*R*)-hydroxyclarithromycin, an active metabolite that can act additively or synergistically with clarithromycin. The lipophilic macrolides are distributed well in tissues and fluids, except CSF. High intracellular concentrations are achieved, but with the exception of azithromycin, the macrolides rapidly diffuse out of cells when extracellular concentrations are low. Clarithromycin and azithromycin are transported actively into leukocytes and macrophages. High concentrations of clarithromycin are present in the nasal mucosa, tonsils, and pulmonary epithelial lining fluid and alveolar cells.[68] Tissue concentrations of clarithromycin and azithromycin exceed those found in plasma by 2 to 20 times and 10 to 100 times, respectively. The favorable cellular penetration probably contributes to the efficacy of clarithromycin and azithromycin in the treatment of intracellular pathogens. Furthermore, sustained intracellular concentrations of azithromycin facilitate short-course therapy for pharyngitis and acute otitis media and single-dose therapy for chlamydial sexually transmitted diseases.[109] Isolated cases of intravascular bacterial infections developing during macrolide therapy for focal infections have been reported and evoke concern that, despite elevated tissue concentrations, low serum concentrations may not treat systemic infections consistently.[159] Erythromycin and azithromycin are eliminated primarily by biliary excretion, whereas clarithromycin is excreted predominantly by the kidneys. A reduction in the dosage of clarithromycin may be required in patients with moderate to severe renal insufficiency. Because of their longer serum half-life, azithromycin and clarithromycin can be administered in a once- and twice-daily regimen, respectively, as compared with the three- or four-times-daily dosing necessary for erythromycin.

Because erythromycin base is unstable at the low pH of the stomach, better-absorbed products were prepared by the addition of protective enteric coating or by alteration of the chemical structure through the formation of salts and esters. Salt and ester derivatives include ethylsuccinate or propionate (esters), stearate

TABLE 248–8 Pharmacokinetics of Macrolide Antibiotics

	Erythromycin	Clarithromycin*	Azithromycin†
Bioavailability (%)	30-65‡	55	37
Protein binding (%)	70-90	65-75	7-50§
Half-life (hr)	1.4-2	Nonlinear‖	11-14 (48-96)
C_{max} (μg/mL)¶	0.8-3‡	3-7 (1-2)	0.4 (0.25)
Elimination route			
Biliary excretion	Majority	Minimal	>50%
Renal excretion (%)	2-15	20-40 (10-15)	4.5

**Values in parentheses are for the active metabolite 14-OH-clarithromycin.*
†After one 500-mg dose; values in parentheses are at steady state after 500 mg × 1 day, then 250 mg/day (oral dosage forms).
‡Varies with the oral preparation and the presence of food.
§Protein binding varies with serum concentration.
‖Varies with dosing; 250 mg twice a day: clarithromycin = 3 to 4 hours, 14-OH-C = 5 to 6 hours; 500 mg twice a day; clarithromycin = 5 to 7 hours, 14-OH-C = 7 hours.
¶C_{max} = peak serum concentration.
Modified from USP DI: Information for the Health Care Professional. Vol. 1. Thomson MICROMEDEX, 2006.

(a salt), and estolate (salt of an ester). Estolate provides the highest concentration of antimicrobial activity in serum, but controversy continues about which preparation provides the most biologically active drug at the site of infection. Because the base is the active component, all erythromycin preparations must be hydrolyzed to the base after absorption. Formulations of erythromycin base include tablets, delayed-release tablets, and capsules. Erythromycin estolate is marketed in capsule form, suspension, and tablets. Erythromycin ethylsuccinate is available in suspension, tablets, and chewable tablets. Erythromycin stearate is only in tablet form in the United States. Parenteral preparations of erythromycin include the lactobionate and gluceptate derivatives. Intramuscular administration of these forms is painful and should be avoided. Clarithromycin can be taken without regard to food, whereas azithromycin should be taken at least 1 hour before or 2 hours after a meal. Clarithromycin is available in suspension (125 and 250 mg/5 mL) and in tablet (250- and 500-mg) forms. Azithromycin is manufactured in 250-mg capsules and as a suspension (100 and 200 mg/5 mL). Azithromycin for intravenous injection has not been approved for use in children younger than the age of 16 years, although pharmacokinetic data are available.[135]

INDICATIONS FOR USE

Erythromycin is approved for the treatment of chlamydial conjunctivitis, pneumonia and urethritis, mycoplasmal and *Legionella* pneumonia, group A streptococcal sinusitis and pharyngitis, mild pneumococcal pneumonia, uncomplicated skin and soft tissue infections caused by susceptible organisms, diphtheria, pertussis, erythrasma, listeriosis, and nongonococcal urethritis and as second-line therapy for gonococcal urethritis. It also is approved for use as a preoperative bowel preparation, for penicillin-allergic persons as prophylaxis for bacterial endocarditis and rheumatic fever, and for the treatment of syphilis in nonpregnant individuals. In the context of increasing prevalence of erythromycin resistance among common pathogens, newer macrolides or other classes of antibacterial agents may be more effective for certain infections.

Clarithromycin is approved for treatment of the following: bacterial exacerbations of bronchitis; streptococcal pharyngitis; mycoplasmal, chlamydial, and pneumococcal community-acquired pneumonia; acute maxillary sinusitis; and uncomplicated skin and soft tissue infections caused by susceptible bacteria. However, increasing rates of clarithromycin resistance among common pathogens of the respiratory tract and skin structures have been associated with treatment failure.[98,133] Clarithromycin and amoxicillin or metronidazole in combination with bismuth compounds, H_2-receptor antagonists, or proton pump inhibitors are recommended for the treatment of *H. pylori*–associated peptic ulcer disease. Clarithromycin also is approved for prophylaxis of disseminated MAC infections in children and adults with advanced human immunodeficiency virus (HIV) infection. Furthermore, clarithromycin, in combination with other antimycobacterials, is an effective therapeutic agent for disseminated MAC infections, but safety has not been established for this indication in children younger than the age of 20 months. The safety and efficacy of clarithromycin have not been established for infants younger than 6 months.

In adults, azithromycin is approved for treatment of the following: bacterial exacerbations of bronchitis; chlamydial and gonococcal cervicitis and urethritis; chancroid; streptococcal tonsillitis and pharyngitis; uncomplicated skin and soft tissue infections caused by susceptible bacteria; and acute otitis media and pneumonia caused by *H. influenzae*, *M. catarrhalis*, and *S. pneumoniae*. It also is approved in adults for prophylaxis of disseminated MAC infection in patients with advanced HIV infection. Pediatric indications include the following: group A streptococcal pharyngitis; acute otitis media caused by *H. influenzae*, *M. catarrhalis*, and *S. pneumoniae*; and mild community-acquired pneumonia caused by *S. pneumoniae*, *H. influenzae*, *M. pneumoniae*, or *C. pneumoniae*.

Five-day regimens have been approved for the treatment of pharyngitis (12 mg/kg/day) and otitis media (single dose of 10 mg/kg followed by 5 mg/kg/day). A randomized clinical trial comparing azithromycin with high-dose (90/6.4 mg/kg/day) amoxicillin-clavulanate for the treatment of acute otitis media revealed that the β-lactam–containing regimen achieved superior bacteriologic and clinical outcomes for all bacterial pathogens including penicillin-resistant *S. pneumoniae* and *H. influenzae*.[122] Consequently, consensus guidelines do not recommend azithromycin or clarithromycin for empiric treatment of acute otitis media unless the patient has a type 1 hypersensitivity reaction to penicillins.[9] Shorter courses of azithromycin for uncomplicated group A streptococcal pharyngitis (20 mg/kg/day for 3 days)[65] or acute otitis media (single dose of 30 mg/kg)[11] have been found to be equally effective as standard therapy but have not been approved by the FDA. A clinical study suggested that a 5-day course of azithromycin for localized cervical lymphadenopathy caused by *B. henselae* (cat-scratch disease) is beneficial.[23] The CDC recommends azithromycin for the treatment of chlamydial genital infections, nongonococcal urethritis, and chancroid (a single 20-mg/kg dose; maximal dose, 1 g)[278] and for prophylaxis of disseminated MAC infection in HIV-infected children.[145] Data supporting the use of azithromycin for treatment of ophthalmia neonatorum caused by *C. trachomatis* are limited to one small study in which a single oral dose of 20 mg/kg/day for 3 days appeared effective.[278]

Although the safety and efficacy of azithromycin and clarithromycin have not been determined for infants younger than 6 months old (or for pregnant or lactating women), these antimicrobials are recommended as first-line agents for treatment and postexposure prophylaxis of pertussis in individuals older than the age of 1 month, based on their in vitro effectiveness, safety and efficacy in older children, and more convenient dosing schedule. Duration of therapy or prophylaxis for pertussis is 14 days for erythromycin (40 to 50 mg/kg/day in four divided doses), 7 days for clarithromycin (15 mg/kg/day in two divided doses), and 5 days for azithromycin (10 mg/kg as single daily doses for infants <6 months of age; 10 mg/kg as a single dose on day 1, then 5 mg/kg/day on days 2 to 5 for infants and children ≥6 months of age). Azithromycin is the preferred macrolide for postexposure prophylaxis and treatment of pertussis in neonates because it has fewer adverse effects compared with erythromycin and no association with infantile hypertrophic pyloric stenosis.[269] A multicenter study established that children with cystic fibrosis chronically infected with *P. aeruginosa* had significantly improved lung function and clinical end-points associated with azithromycin treatment three times per week.[245] This effect may not persist. Studies in endemic regions showed that a single dose of azithromycin is as effective as is standard therapy with erythromycin for treatment of cholera in children.[153] A 5-day course of azithromycin (20 mg/kg/day; maximum dose, 1 g) is an effective treatment for uncomplicated typhoid fever in children and adolescents.[100] One to six doses of azithromycin for trachoma have been shown to be equivalent to prolonged topical treatment with oxytetracycline-polymyxin ointment.[77]

ADVERSE EFFECTS

The macrolides usually are well tolerated and relatively safe. The most common adverse effect is gastrointestinal disturbance, which can occur with the administration of any macrolide but is associated most commonly with the use of erythromycin. It is a

dose-related phenomenon. Because it acts as a motilin receptor agonist, gastrointestinal symptoms (nausea, vomiting, diarrhea, flatulence, and abdominal cramps) can occur with orally or parenterally administered erythromycin. Enteric coating of erythromycin does not decrease the incidence. Rapid intravenous infusions of erythromycin can result in thrombophlebitis. Cholestatic hepatitis is an unusual, but serious, macrolide toxicity. It occurs more commonly in adults and possibly in pregnant women and is associated most frequently with the estolate preparation.[45] The onset typically begins approximately 16 days after initiation of therapy and is manifested as fever, pruritus, jaundice, elevated liver function tests, and, occasionally, rash, leukocytosis, and eosinophilia. Signs and symptoms resolve after discontinuation of the macrolide but recur with subsequent therapy. Transient hearing loss has been described after the administration of large dosages of erythromycin lactobionate. *Torsades de pointes* is an uncommon reaction to intravenous infusions of erythromycin. A sevenfold increase in the rate of infantile hypertrophic pyloric stenosis was temporally associated with and probably causally related to the prophylactic use of erythromycin in neonates during a pertussis outbreak in a community hospital.[126] Gastrointestinal disturbances occur less frequently with clarithromycin and azithromycin, principally because lower dosages are required for effective therapy.

A common and potentially serious toxicity is that of drug interactions.[216] Macrolides are metabolized by hepatic microsomal cytochrome P-450 enzymes. Drug interactions can occur during concomitant therapy with two or more drugs that undergo hepatic microsomal P-450 metabolism. One proposal for this drug interaction suggests that the macrolide is *N*-demethylated to a nitrosoalkane that interacts with and inactivates the microsomal enzyme. Toxicity can occur because of interference with metabolism and consequent accumulation of the second drug. The ability to inactivate the enzyme varies with each macrolide; erythromycin is a more potent inhibitor than is clarithromycin. Azithromycin has not been associated yet with nitrosoalkane formation and resulting drug interactions, but caution should be exercised with concomitant administration of warfarin, digitoxin, and antacids. Drugs with which macrolides can interact include astemizole, benzodiazepines, buspirone, carbamazepine, cimetidine, cisapride, cyclosporine, digoxin, methylprednisolone, rifampin, tacrolimus, terfenadine, theophylline, triazolam, verapamil, warfarin, zidovudine, and certain protease inhibitors, among others.[216]

MISCELLANEOUS ANTIBIOTICS

CHLORAMPHENICOL

Chloramphenicol, originally derived from *Streptomyces venezuelae* obtained from soil near Caracas, Venezuela, in 1947, now is prepared synthetically. It is a chemically unique agent that contains an aromatic nitro group, an *N*-dichloroacetyl substituent, and two chiral centers. The availability of less toxic and equally or more effective agents has limited the usefulness of chloramphenicol in the United States.

Mechanisms of Action

Chloramphenicol reversibly binds to the 50S subunit of 70S bacterial ribosomes and thereby inhibits protein synthesis. The mechanism of action involves suppression of peptidyltransferase activity, with a resultant inability to form peptide bonds and elongate the peptide chain.[253,281] Because the ribosomal binding sites of chloramphenicol overlap with those of macrolides, clindamycin, and linezolid, concomitant use of these antibiotics can result in antagonism and should be avoided. Chloramphenicol usually is bacteriostatic, but it can be bactericidal when high concentrations are achieved against highly susceptible organisms such as meningococci and *H. influenzae*.

Mechanisms of Resistance

The most common mechanism of acquired resistance is plasmid-mediated production of chloramphenicol acetyltransferases (CATs), which acetylate chloramphenicol and render it unable to bind to the ribosomal target. Numerous *cat* genes encode classical (type A) or novel (type B) CATs and may be associated with multidrug-resistance phenotypes. This resistance has been documented in many different genera of bacteria, including *H. influenzae*, members of the Enterobacteriaceae, *Neisseria*, streptococci, and *S. aureus*. Less commonly, multiple specific or multidrug transporter mechanisms (efflux pumps) and chromosomal or plasmid-mediated alterations in permeability have resulted in chloramphenicol resistance in a wide range of bacteria including *B. cepacia*, *E. coli*, *H. influenzae*, *P. aeruginosa*, and *S. typhi* (in the absence of OmpF protein). More recently described alterations in bacterial 23S rRNA have been associated with chloramphenicol resistance by inducing frameshift mutations and readthrough of stop codons.[267] In addition, isolated cases of resistance in *Bacillus subtilis* because of altered ribosomes and in anaerobes because of inactivation of chloramphenicol by nitroreduction have been reported.[253]

In Vitro Activity

Chloramphenicol has broad-spectrum activity against aerobic and anaerobic gram-positive and gram-negative bacteria, chlamydiae, mycoplasmas, spirochetes, and rickettsiae.[233] It is bactericidal against susceptible strains of *H. influenzae*, *N. meningitidis*, and penicillin-susceptible *S. pneumoniae*, whereas it is bacteriostatic against most other susceptible microorganisms.[227] Frequently susceptible aerobic gram-positive cocci include groups A and B beta-hemolytic streptococci, viridans streptococci, and penicillin-susceptible pneumococci. Because penicillin-resistant pneumococci may be tolerant to chloramphenicol in vitro, meaning that concentrations of drug necessary to kill these pneumococci are approximately 30-fold higher than necessary to inhibit the organism, verifying chloramphenicol susceptibility by MBC testing is prudent, especially when treating meningitis.[101] Usually, chloramphenicol is active against oxacillin-susceptible *S. aureus*, but susceptibility patterns vary with the use of chloramphenicol, and more suitable alternatives for therapy exist. Susceptible gram-positive bacilli include *Bacillus* spp., *L. monocytogenes*, *C. diphtheriae*, *Clostridium* spp., and *Eubacterium*. Most *N. meningitidis* and *N. gonorrhoeae* organisms are susceptible.[143] However, several high-level chloramphenicol-resistant strains of meningococcus belonging to serogroup B were isolated from the CSF of children in Vietnam.[102] The susceptibility of Enterobacteriaceae, including that for *Salmonella* and *Shigella* spp., is variable. Other gram-negative bacilli frequently susceptible to chloramphenicol include *B. pertussis*, *B. cepacia*, *Brucella* spp., *C. jejuni*, *F. tularensis*, *H. influenzae*, *Pasteurella multocida*, *Pseudomonas pseudomallei*, *V. cholerae*, and *Y. pestis*. Virtually all obligate anaerobes are susceptible.

Pharmacokinetics

After oral administration, absorption of chloramphenicol is rapid and complete. Bioavailability is approximately 80 percent after oral administration, but it is only 70 percent after an intravenous dose because approximately 30 percent of the parenterally administered dose is excreted in urine before hydrolysis of the succinate ester to the active form. Intramuscular injection results in peak

serum concentrations comparable to those achieved after intravenous infusion. Because of its lipid solubility, chloramphenicol diffuses rapidly and widely into tissues and fluids.[233] The highest concentrations are achieved in the liver and kidneys, with high concentrations present in urine, and therapeutic concentrations are achieved in aqueous and vitreous humor. CSF concentrations range from 21 to 50 percent and from 45 to 89 percent of serum values in the presence of uninflamed and inflamed meninges, respectively. Brain tissue concentrations exceed those in plasma. Chloramphenicol also is distributed into pleural, ascitic, and synovial fluids, as well as saliva and breast milk. Protein binding ranges from 32 percent in premature newborns to 50 to 60 percent in adults. Chloramphenicol palmitate and chloramphenicol sodium succinate are esterified prodrugs of chloramphenicol. Orally administered chloramphenicol palmitate is hydrolyzed to active drug by pancreatic esterases in the small intestine before absorption. After intravenous infusion, chloramphenicol sodium succinate is hydrolyzed rapidly to active drug in the kidneys, liver, and lungs. Ninety percent of active chloramphenicol is conjugated to the inactive glucuronide primarily by the liver. Immature metabolic function of the liver in the fetus and newborn results in inadequate conjugation of chloramphenicol, with subsequent accumulation of toxic concentrations of active drug. Peak serum concentrations in children after a dose of 25 mg/kg range from 19 to 28 μg/mL, whereas in adults receiving doses of 12.5 mg/kg, peak serum values of 11 to 18 μg/mL can be achieved. Although metabolized to inactive metabolites by the liver, chloramphenicol is excreted by the kidneys: 5 to 10 percent as active drug and 80 percent as inactive metabolites. The elimination half-life is significantly longer but variable, and serum concentrations are unpredictable in neonates.[233] Dosage adjustments should be considered in persons with severe hepatic insufficiency or combined hepatic and renal insufficiency and in patients receiving drugs that compete for hepatic P-450 cytochrome oxidases, such as phenytoin, phenobarbital, and rifampin. Chloramphenicol is not removed by peritoneal dialysis or hemodialysis, but charcoal hemoperfusion may lower serum concentrations.

Formulations of chloramphenicol available include chloramphenicol palmitate in an oral suspension of 150 mg base/5 mL, 250-mg capsules of chloramphenicol base, and chloramphenicol sodium succinate for parenteral use. Other formulations include 1 percent cream for topical use, 0.5 percent otic solution, 0.5 percent ophthalmic solution, and 1 percent ophthalmic ointment.

Indications for Use

Because of its low therapeutic-toxic index, use of chloramphenicol should be reserved for serious infections for which less toxic agents are ineffective or contraindicated. In some developed countries, chloramphenicol has been replaced by third-generation cephalosporins for the treatment of bacterial meningitis and by clindamycin or metronidazole for the treatment of anaerobic infections. Ceftriaxone is a safe and effective alternative to (1) chloramphenicol for the treatment of acute typhoid fever[194] and (2) long-acting oily chloramphenicol for epidemic meningococcal meningitis in sub-Saharan Africa.[203] Infections for which chloramphenicol may be indicated include the following: pneumococcal, meningococcal, and *H. influenzae* meningitis in β-lactam–allergic individuals; brain abscesses caused by susceptible anaerobic bacteria resistant to other agents; acute typhoid fever; and rickettsial infections (typhus, Q fever, Rocky Mountain spotted fever). Doxycycline is preferred for treatment of rickettsial infections in children, even those younger than 9 years of age. Chloramphenicol is not indicated for the treatment of trivial infections, prophylaxis of infections, or treatment of typhoid carrier states.

Adverse Effects

Hematologic adverse events associated with the use of chloramphenicol include hemolytic anemia in patients with the Mediterranean type of glucose-6-phosphate dehydrogenase deficiency, reversible bone marrow suppression, and aplastic anemia. Reversible bone marrow suppression is a dose-related phenomenon. It usually occurs when serum concentrations exceed 25 μg/mL, as can be seen when administering large dosages, during prolonged therapy, or in patients with impaired liver function. Although mammalian cells contain 80S ribosomes, rather than the 70S ribosomes found in prokaryotes, mitochondria possess 70S ribosomes. A proposed mechanism of myelosuppression involves inhibition of mitochondrial protein synthesis in host bone marrow stem cells.[253] Dose-related bone marrow suppression is manifested by peripheral anemia with or without reticulocytopenia, leukopenia, and thrombocytopenia and bone marrow findings of increased cellularity, cytoplasmic vacuolization, and maturation arrest of erythroid and myeloid precursors. In contrast, aplastic anemia is a rare, often fatal idiosyncratic reaction that is unrelated to the dosage, duration, or route of therapy. The pathogenesis is understood less well but possibly is related to DNA damage from toxic metabolites of chloramphenicol produced by nitroreduction.[281] The incidence ranges from 1 in 25,000 to 40,000 courses. Onset can begin during therapy but typically occurs weeks to months or rarely years after therapy has been discontinued. Manifestations include peripheral pancytopenia and hypoplastic or aplastic marrow.

Gray syndrome or gray baby syndrome is a rare, but serious and potentially fatal adverse event that usually occurs in newborns but has been described in older children and adults with hepatic insufficiency. Most often, this syndrome occurs when serum chloramphenicol concentrations exceed 40 μg/mL, and it is thought to be a result of inhibition of mitochondrial electron transport in liver, skeletal muscle, and myocardium. The onset typically begins 2 to 9 days after initiating therapy. Manifestations include hypothermia, tachypnea, blue-gray skin color (cyanosis), abdominal distention, emesis, unresponsiveness, and refractory metabolic acidosis that can progress to vasomotor collapse and death within 2 days.

Uncommon side effects include the following: hypersensitivity reactions such as drug fever, rash, urticaria, anaphylaxis, and a Herxheimer-like reaction during therapy for syphilis, typhoid fever, and brucellosis; gastrointestinal symptoms, including nausea, emesis, diarrhea, and an unpleasant taste; and neurologic symptoms such as peripheral neuritis, headache, mental confusion, and optic neuritis, the last of which may not be entirely reversible.

Chloramphenicol can inhibit the metabolism of other drugs metabolized by the hepatic microsomal cytochrome P-450 system and thus result in the accumulation of alfentanil, barbiturates, cyclophosphamide, phenytoin, antidiabetic sulfonylureas (more common with chlorpropamide and tolbutamide than with glyburide and glipizide), and warfarin during concomitant therapy.[5] Because some agents such as rifampin, phenobarbital, and phenytoin are potent inducers of hepatic microsomal enzymes, when they are given concomitantly with chloramphenicol, metabolism is enhanced, and serum concentrations of active chloramphenicol are reduced. Other drug interactions include a reduction in the effectiveness of estrogen-containing oral contraceptives when used concurrently with chloramphenicol and a delay in the response to vitamin B_{12}, folic acid, and iron. A mild disulfiram-like reaction can occur when alcohol is ingested during chloramphenicol therapy. Both cimetidine and chloramphenicol have rare associations with aplastic anemia; a few reports of concomitant use resulting in aplastic anemia have been documented, thus suggesting a potential for additive or synergistic risk. Concomitant acetaminophen therapy has been the subject of controversy;

some studies suggest that co-administration can prolong the elimination half-life of chloramphenicol, but other reports suggest no effect or enhanced metabolism of chloramphenicol. Lincosamides and macrolides can have an antagonistic effect when they are administered concurrently with chloramphenicol because of competition for ribosomal binding sites. Chloramphenicol is physically incompatible in solution with many drugs, including tetracyclines and vancomycin.

Because of considerable variability in serum chloramphenicol concentrations, the narrow therapeutic-toxic index, and the potential for drug interactions, monitoring serum concentrations of chloramphenicol and peripheral blood counts during therapy is prudent, particularly for neonates.

COLISTIN (COLISTIMETHATE SODIUM; POLYMYXIN E)

Colistin is a cationic, cyclic polypeptide antibiotic that is structurally and pharmacologically related to polymyxin B. Colistin is less active in vitro and less toxic than is polymyxin B. Colistin was discovered in 1949 and is obtained from cultures of *Bacillus polymyxa* subspecies *colistinus*. The polymyxins have been used extensively for decades in topical ophthalmic and otic solutions. However, because of reports of common and serious nephrotoxicity and neurotoxicity, parenteral use of these drugs was abandoned in the early 1980s, except for treatment of multidrug-resistant, gram-negative bacterial infections in children and adults with cystic fibrosis. The emergence of gram-negative bacteria resistant to most classes of antibiotics and the lack of effective new antimicrobial agents led to the reconsideration of colistin as a valuable therapeutic option. Studies of patients who received intravenous polymyxins for the treatment of serious multidrug-resistant *P. aeruginosa* and *A. baumannii* infections, including pneumonia, bacteremia, and urinary tract infections, led to the conclusion that these antibiotics have acceptable effectiveness and considerably less toxicity than was reported previously.[149]

Mechanisms of Action

Colistimethate sodium is inactive until it is hydrolyzed to sulfomethylated derivatives and colistin, which acts as a cationic detergent with both lipophilic and lipophobic components. It damages the cytoplasmic membrane by displacing calcium and magnesium and binding to anionic lipopolysaccharide molecules and leads to permeability changes in the cell membrane, leakage of osmotically active intracellular metabolites and nucleosides, and cell death. Furthermore, colistin binds and neutralizes the lipid A portion of lipopolysaccharide in vitro; however, the clinical implications of this anti-endotoxin activity are unknown.[88]

Mechanisms of Resistance

Gram-negative bacteria can develop resistance to colistin by means of inherited or acquired mechanisms. Inherited mutations occur infrequently and are not influenced by exposure to colistin. Acquired resistance rarely may be induced by prolonged exposure to the drug. Studies of polymyxin-resistant *P. aeruginosa* have suggested that the development of resistance arises from modifications of the cell membrane, such as reductions in lipopolysaccharide, certain proteins or lipids, or magnesium or calcium. Currently, no evidence of efflux pump or enzyme-mediated resistance exists. Complete cross-resistance occurs between colistin and polymyxin B, but not other antimicrobial agents.

In Vitro Activity

Colistin is highly active against many strains of aerobic gram-negative bacteria, including *Acinetobacter*, *Citrobacter*, *E. coli*, *Enterobacter*, *H. influenzae*, *Klebsiella*, *Morganella morganii*, *P. aeruginosa*, *Salmonella*, *Shigella*, and variable numbers of *Stenotrophomonas* strains. However, the antibiotic is inactive against most strains of *Brucella*, *B. cepacia*, *Edwardsiella*, *Proteus*, *Providencia*, *Serratia*, gram-negative cocci, gram-positive bacteria, and anaerobic bacteria. The Clinical Laboratory Standards Institute defined bacterial resistance to colistin as an MIC of 4 μg/mL or greater. However, the sulfate form of colistin, which usually is used for in vitro testing, often is four- to eightfold more active than is the sulfomethyl form, which is used in the parenteral formulation. Limited in vitro and clinical data suggest that synergism exists with colistin and other antimicrobial agents such as aminoglycosides, antipseudomonal β-lactams, fluoroquinolones, rifampin, and TMP-SMX.[88,149]

Pharmacokinetics

The pharmacologic evaluation of the polymyxins was performed decades ago. Consequently, pharmacokinetic and pharmacodynamic information is limited. Colistin is not absorbed from the gastrointestinal tract and must be given parenterally. The drug is distributed widely in the body, except for bones and synovial, pleural, and pericardial fluids. In a reported case of multidrug-resistant *A. baumannii* meningitis in an adolescent, parenterally administered colistin achieved a concentration in the CSF that was 25 percent of the serum concentration, and it cured the infection.[137] More than 50 percent of the agent binds to serum proteins. Colistimethate sodium and its metabolites are excreted mainly by the kidneys through glomerular filtration. Therefore, the dosage and frequency of administration should be decreased in children with renal impairment. Colistin is rapidly bactericidal against susceptible bacteria in a concentration-dependent manner, and it has a post-antibiotic effect.[88,149] Considering these pharmacologic parameters, there may be a theoretical benefit of administering colistin less frequently with higher doses, but no clinical data validate this hypothesis.

Indications for Use

Colistin has been administered intravenously to infants and children of all ages for the treatment of acute or chronic infections caused by susceptible gram-negative organisms, particularly multidrug-resistant strains of *Pseudomonas* and *Acinetobacter* in patients with cystic fibrosis or complicated health care–associated infections. Colistin should be reserved for use when other more effective and less toxic antimicrobial agents are contraindicated or ineffective. The dosage of colistimethate sodium is expressed in terms of colistin. The usual parenteral dosage for adults and children with normal renal function is 2.5 to 5 mg/kg (75,000 to 150,000 IU/kg)/day divided into two to four doses, depending on the severity of infection (maximum, 300 mg/day). Intramuscular administration is not recommended because it causes severe pain at the injection site. Experience with the use of colistimethate sodium administered by nebulization for multidrug-resistant, gram-negative respiratory infections in patients with cystic fibrosis is extensive. Successful use of colistimethate sodium by the intraventricular route also has been reported. Colistin sulfate in combination with neomycin sulfate and hydrocortisone acetate is available in the United States for otic use. Colistin sulfate for oral administration no longer is available in the United States. Polymyxin B sulfate is available for topical use.[88]

Adverse Effects

Nephrotoxicity and neurotoxicity are the most serious adverse effects and occur most frequently when the drug is administered in higher than recommended doses, in patients with renal insufficiency, or in combination with other nephrotoxic agents.

Progressive azotemia or acute tubular necrosis may occur, but renal dysfunction usually is reversible when the antibiotic is discontinued. Renal function should be monitored closely during therapy, and colistin should be discontinued immediately if urine output diminishes or concentrations of blood urea nitrogen or creatinine increase. Transient effects on the nervous system, including dizziness, weakness, paresthesia, numbness, vertigo, ataxia, blurred vision, and slurred speech may occur, but these symptoms are reversible and may be alleviated by reducing the dosage of colistin. Coma, psychosis, seizures, and noncompetitive neuromuscular blockade causing apnea also have been reported and warrant prompt discontinuation of the drug.

FLUOROQUINOLONES

Fluoroquinolone antibiotics are derivatives of nalidixic acid. When compared with nalidixic acid, fluoroquinolones have the following: (1) a broader spectrum of activity that includes aerobic gram-negative enteric organisms, particularly Enterobacteriaceae, *Haemophilus* spp., *Neisseria* spp., *M. catarrhalis*, nonenteric gram-negative bacilli such as *P. aeruginosa*, oxacillin-susceptible staphylococci, some streptococci, bacteria associated with atypical pneumonia, certain genital pathogens, and certain strains of mycobacteria; (2) better penetration into tissues; (3) good intracellular penetration; and (4) rapid bactericidal activity.

Mechanisms of Action

Fluoroquinolones are the only class of antibacterial agents in clinical use that directly inhibit DNA synthesis. They bind to bacterial DNA gyrase and topoisomerase IV. Inhibition of bacterial gyrase causes relaxation of the supercoiled DNA that leads to termination of chromosomal replication and interference with cell division and gene expression. Inhibition of the activity of topoisomerase IV leads to separation of two united DNA molecules and subsequent interference with cellular replication.

Mechanisms of Resistance

A growing concern is that widespread use of fluoroquinolones among adult populations has been associated with resistance rates, especially among Enterobacteriaceae, that exceed 50 percent in some regions. Mechanisms of resistance include chromosomal mutations in genes that encode fluoroquinolone target enzymes (DNA gyrase and topoisomerase IV), efflux pumps, or porin (outer-membrane diffusion) channels. Newly identified plasmid-mediated resistance determinants in strains of *K. pneumoniae*, *E. coli*, *Enterobacter*, and other enteric bacteria include the following: (1) Qnr, a protein that protects DNA gyrase from quinolone action and is associated with multidrug resistance; and (2) a variant of the aminoglycoside acetyltransferase found in gram-negative bacteria that is capable of inactivating ciprofloxacin.[170,238] Fluoroquinolone-resistant strains of *N. gonorrhoeae* are common findings in many parts of the world and are spreading in the United States, especially in California and Hawaii. The CDC no longer recommends fluoroquinolones for treatment of gonorrhoea in the United States.[278] Other bacteria with increasing rates of resistance include *S. pneumoniae* (≈3% to ciprofloxacin with a breakpoint ≥ 4 μg/mL versus no resistance to levofloxacin as of 2004 among invasive pediatric isolates in the United States[90]) and intestinal pathogens such as *C. jejuni*, *Shigella* spp., *Salmonella* spp., certain strains of *K. pneumoniae*, *E. coli*, *P. aeruginosa*, among others.[7]

In Vitro Activity

Levofloxacin and moxifloxacin have greater potency than does ciprofloxacin against gram-positive cocci, and moxifloxacin has enhanced activity against anaerobic bacteria, although in some series, approximately 50 percent of invasive *Bacteroides* spp. are resistant to moxifloxacin.[108] Ciprofloxacin remains the most potent marketed fluoroquinolone against gram-negative bacteria, particularly against some strains of *P. aeruginosa*, *Providencia* spp., *Proteus* spp., and *S. marcescens*. In vitro studies of newer and investigational fluoroquinolones against penicillin- and cephalosporin-resistant pneumococci indicate excellent activity, whereas activity of ciprofloxacin, norfloxacin, and ofloxacin is limited. Fluoroquinolone use for MRSA and oxacillin-resistant, coagulase-negative staphylococci is limited because resistant mutants can be selected relatively easily, thus leading to treatment failure and relapse. Constitutive high-level resistance exists among MRSA isolates from patients with CA-MRSA infections in some areas of the United States. Although MICs tend to be lower for newer (e.g., moxifloxacin, gatifloxacin) as compared with older (e.g., ciprofloxacin, levofloxacin) fluoroquinolones, genes conferring fluoroquinolone resistance in *S. aureus* confer resistance to the entire class of agents.[110] Activity against enterococci is marginal or absent. Fluoroquinolones are effective against atypical pneumonia pathogens (*Chlamydophila pneumoniae*, *L. pneumophila*, *M. pneumoniae*) and certain genital pathogens (*C. trachomatis*, *U. urealyticum*, *Mycoplasma hominis*). Ciprofloxacin, levofloxacin, and moxifloxacin are active in vitro against certain strains of *M. tuberculosis*, *Mycobacterium fortuitum*, *Mycobacterium kansasii*, and *M. chelonae* and are less active against MAC. No quinolone antibiotic has been shown to be active against *T. pallidum*.

Pharmacokinetics

The fluoroquinolones generally have excellent bioavailability that is not substantially affected by food, with values of 70 percent for ciprofloxacin, 85 percent for moxifloxacin, and greater than 95 percent for ofloxacin and levofloxacin. These drugs have large volumes of distribution, and they concentrate in the kidneys and in urine (except moxifloxacin, which undergoes hepatic metabolism and biliary excretion). This class of agents has favorable pharmacokinetic properties in children, and their efficacy correlates best with the 24-hour AUC/MIC ratio because of a combination of concentration-dependent killing and post-antibiotic effect. These properties suggest that large doses given at relatively infrequent intervals would be most effective.[248] However, based on pharmacokinetic studies, doses for patients with cystic fibrosis should be higher and more frequent than for patients without cystic fibrosis, and the dosage should be decreased in proportion to increasing body weight. Fluoroquinolones can interact with a variety of other drugs including caffeine, cyclosporine, dideoxyinosine, nonsteroidal anti-inflammatory drugs, theophylline, and other agents that prolong the QT interval. Co-administration with antacids, ferrous sulfate, and multivitamins containing zinc can reduce oral bioavailability.

Indications for Use

Fluoroquinolones have been used extensively in adults for the treatment of skin and soft tissue, skeletal, and intra-abdominal infections, as well as infections of the urinogenital, gastrointestinal, and respiratory tracts. Fluoroquinolones commonly used in adults include the second-generation agents, ciprofloxacin and levofloxacin, and the fourth-generation agent, moxifloxacin. Other agents with limited indications include enoxacin, ofloxacin, lomefloxacin, norfloxacin (second-generation), and gemifloxacin (third-generation). Four agents have been withdrawn from the market in the United States: gatifloxacin, grepafloxacin (causes cardiac toxicity), sparfloxacin, and trovafloxacin (causes hepatotoxicity). Ciprofloxacin, levofloxacin, and moxifloxacin are FDA approved for the treatment of uncomplicated skin infections caused by susceptible organisms in adults. However, none of the

fluoroquinolones is FDA approved for treatment of MRSA infections.

Use in pediatric patients has been limited because of the potential for inducing cartilage damage that leads to arthropathy, as demonstrated in juvenile animal studies. However, no convincing evidence exists that quinolone-induced arthropathy occurs in humans. Reversible arthralgia and tendinopathy have a temporal association with fluoroquinolone use. Fluoroquinolones are not approved for use in children and adolescents younger than 18 years, except in the following situations: (1) complicated urinary tract infections and pyelonephritis attributable to *E. coli* in children 1 year old and older (approved by the FDA in 2004) and (2) exposure to aerosolized *B. anthracis* to prevent onset or progression of disease. However, these drugs have been used in certain clinical settings, such as cystic fibrosis, primarily because they are the only oral antibiotics with activity against *P. aeruginosa*. Furthermore, ciprofloxacin is the only fluoroquinolone available in a suspension formulation (250 mg and 500 mg [base]/5 mL). In 2002 alone, approximately 500,000 prescriptions for fluoroquinolones were written in the United States for individuals younger than 18 years old.[7] These agents, particularly ciprofloxacin, have been used to treat the following: exacerbations of chronic pseudomonal pulmonary infections in patients with cystic fibrosis; complicated urinary tract infections caused by multidrug-resistant bacteria; chronic suppurative otitis media associated with *P. aeruginosa*; osteochondritis attributable to *P. aeruginosa*; shigellosis, *C. jejuni*, and other bacterial causes of enteritis; multidrug-resistant typhoid fever; cholera; infections in neutropenic patients with cancer; in combination with other agents to treat multidrug-resistant mycobacterial disease; and multidrug-resistant gram-negative bacillary septicemia or meningitis (no controlled trials in children with meningitis have been reported using currently licensed agents).[7,54,243,249] Fluoroquinolones also effectively eradicate nasopharyngeal carriage of susceptible meningococci. One study showed that children with acute otitis media and otorrhea through tympanostomy tubes had better clinical outcomes with fewer adverse effects when they were treated with ciprofloxacin-dexamethasone otic suspension compared with high-dose amoxicillin-clavulanic acid suspension.[85] Although gatifloxacin is not approved for use in children and currently is not available in the United States, noncontrolled studies confirmed that it is safe and effective for treatment of recurrent acute otitis media and acute otitis media when therapy with standard antimicrobials has failed.[223]

The current recommendation is that parenteral and oral fluoroquinolones should be reserved for situations in which no effective, safe, and nonrestricted alternative antimicrobial agents are available to treat complicated or multidrug-resistant bacterial infections. Appropriate uses should be limited to the following clinical situations: exposure to aerosolized *B. anthracis*; urinary tract infections caused by *P. aeruginosa* or other multidrug-resistant, gram-negative bacteria; chronic suppurative otitis media or malignant otitis externa caused by *P. aeruginosa*; acute or chronic osteomyelitis or osteochondritis caused by *P. aeruginosa*; exacerbations of pulmonary disease in patients with cystic fibrosis who have *P. aeruginosa* colonization and for whom oral therapy is appropriate; susceptible mycobacterial infections as part of a combination regimen; gram-negative bacterial infections in immunocompromised hosts in whom oral therapy is appropriate or resistance to alternative agents is present; gastrointestinal infections caused by multidrug-resistant *Shigella* spp., *Salmonella* spp., *V. cholera*, or *C. jejuni*; and proven bacterial septicemia or meningitis caused by multidrug-resistant pathogens that are susceptible only to fluoroquinolones, that have failed to respond to approved agents, or that require use of a fluoroquinolone because of life-threatening hypersensitivity to alternative agents.[7]

Adverse Effects

Adverse events associated with fluoroquinolone therapy occur in 3 to 17 percent of adult and pediatric patients and cause discontinuation of treatment in 1 to 2 percent. The most common reactions are gastrointestinal, minor CNS disorders, and allergic rashes.[248] Other infrequent adverse reactions include photosensitivity, disorders of glucose homeostasis, prolongation of the QT interval with rare cases of torsade de pointes particularly among patients with long QT syndrome, leukopenia, and anaphylactoid reactions. Reversible tendinitis and tendon rupture occur uncommonly in adults.

LINCOSAMIDES

Lincosamide antibiotics consist of an amino acid linked to an amino sugar. Lincomycin, elaborated by *Streptomyces lincolnensis* variety *lincolnensis*, originally isolated from a soil sample near Lincoln, Nebraska, was the first lincosamide available for use. Clindamycin, a semisynthetic derivative of lincomycin produced by the substitution of a chlorine atom for a hydroxyl group at position 7, was available for use in the early 1970s. Because clindamycin has increased antibacterial activity and better oral absorption than does lincomycin, lincomycin rarely is used.

Mechanisms of Action

Lincosamides bind to the 50S subunit of susceptible bacterial ribosomes close to the peptidyl transferase center and thereby inhibit protein synthesis. Data indicate that clindamycin causes dissociation of peptidyl-tRNAs from the ribosome, depletion of free tRNA pools, and prevention of the egress of nascent peptides through a tunnel in the 50S subunit.[263] Because the ribosomal binding sites for lincosamides overlap with those of chloramphenicol and erythromycin, concurrent use of these agents can result in antagonism and should be avoided. Lincosamides usually are bacteriostatic but can be bactericidal against highly susceptible microorganisms in the presence of high lincosamide concentrations.

Mechanisms of Resistance

Some bacteria, including members of the Enterobacteriaceae and *Pseudomonas* and *Acinetobacter* spp., are inherently resistant to lincosamides, most likely because of relative impermeability of the outer membrane of the cell wall. Acquired resistance can develop as a result of altered ribosomal targets and, less commonly, lincosamide inactivation, whereas resistance as a result of reduced lincosamide uptake has not been described. Two mechanisms by which bacteria can alter their ribosomes and acquire lincosamide resistance have been identified. High-level resistance because of an altered protein component of the 50S ribosomal subunit after a one-step chromosomal mutation confers resistance to erythromycin and often to the lincosamides. Plasmid-mediated MLS_B resistance occurs when adenine residues on the 23S RNA component of the 50S ribosomal subunit are methylated, and an altered target is created that confers cross-resistance to macrolides, lincosamides, and streptogramin B. The production of ribosomal methylases is encoded by the *erm* class of genes.[236] This resistance can be constitutive or inducible and can be seen in staphylococci, streptococci, and *Bacteroides* spp. Erythromycin is the most potent inducer of MLS_B resistance in staphylococci, whereas any macrolide, lincosamide, or streptogramin B can be an inducer in streptococci. Some strains of *B. fragilis* can have inducible MLS_B resistance that is not detected easily by disk agar diffusion susceptibility testing[259] but

is recognizable because of frequently concurrent high-level erythromycin resistance. An MS pattern of resistance that is plasmid-mediated results in macrolide resistance as a result of active ATP-dependent efflux pumps encoded by the *mef*(A) and *msr*(A) genes. Isolates with these resistance genotypes are resistant to 14- and 15-membered ring macrolides and streptogramin B but not to clindamycin.[180] Rarely, staphylococci produce a plasmid-mediated, nonconjugative nucleotidyltransferase that inactivates lincosamides and results in high-level resistance to lincomycin and tolerance to clindamycin (MBC/MIC ratio >32).

In Vitro Activity

Both lincosamides are effective in vitro against gram-positive cocci, whereas clindamycin also is active against a wide range of anaerobic bacteria. Clindamycin is many times more active than is lincomycin and is as active as or slightly more active than is erythromycin against staphylococci, pneumococci, group A streptococci, and viridans streptococci. Unlike erythromycin, the lincosamides do not have clinically significant activity against *H. influenzae*, *M. pneumoniae*, or *Neisseria* spp. Clindamycin is active against many gram-positive cocci, including penicillinase-producing and non–penicillinase-producing staphylococci and groups A, B, C, and G beta-hemolytic streptococci and pneumococci, but it is not active against enterococci or most health care–associated methicillin-resistant staphylococci. Rates of in vitro inducible *erm*-mediated MLS_B resistance among CA-MRSA isolates vary widely, ranging from 4.5 percent in Houston[148] to 94 percent in Chicago.[97] Presence of this type of resistance can be detected in erythromycin-resistant strains of *S. aureus* with the use of the double-disk diffusion assay (D-test), in which clindamycin- and erythromycin-impregnated disks are placed 15 to 20 mm apart on Mueller-Hinton agar that has been inoculated with a standardized (0.5 McFarland) suspension of *S. aureus*. The finding of a D-shaped blunting of the circular zone of inhibition around the clindamycin disk on the side closest to the erythromycin disk indicates the presence of in vitro–inducible MLS_B resistance. These isolates have a high rate of mutation to constitutive clindamycin resistance, a trait that would confer a selective advantage during clindamycin therapy. The use of clindamycin for D-test–positive *S. aureus* infections has been reported to result in treatment failure in a few patients. Therefore, alternative effective antimicrobial agents should be sought, or, if empiric clindamycin therapy has been initiated, response to therapy should be carefully monitored.[110,180] Erythromycin and lincosamide resistance in pneumococci and group A streptococci has been increasing. Surveillance studies revealed rates of clindamycin resistance among multidrug-resistant pneumococci to range from 12 to 19 percent in the United States and Europe, respectively.[138] Most facultative aerobic gram-negative bacilli, with the exception of *Campylobacter* spp. (including *C. jejuni* and *C. fetus*) and *H. pylori*, are inherently resistant to clindamycin. Anaerobes frequently susceptible to clindamycin include the following: the anaerobic gram-negative bacilli *Bacteroides*, *Fusobacterium*, *Prevotella*, and *Porphyromonas* spp.; the non–spore-forming gram-positive bacilli *Propionibacterium*, *Eubacterium*, and actinomyces; the anaerobic gram-positive cocci *Peptococcus*, *Peptostreptococcus*, and microaerophilic streptococci; and many *Clostridium* organisms, excluding *C. difficile*, *Clostridium sporogenes*, and *Clostridium tertium*. Clindamycin resistance does occur, especially in *Fusobacterium varium*, the non-*fragilis Bacteroides* group, an increasing proportion of *B. fragilis* isolates (≤≈30% in some series),[4] and as many as 20 percent of anaerobic gram-positive cocci. When combined with other agents, clindamycin is active against certain other pathogens such as *Babesia*, *Plasmodium*, *Pneumocystis jiroveci*, and *T. gondii*.

Pharmacokinetics

Oral absorption of lincosamides occurs rapidly. The presence of food delays but does not decrease the absorption of clindamycin, but it does reduce the absorption of lincomycin. Concomitant administration of kaolin- or attapulgite-containing antidiarrheal agents can decrease absorption; therefore, these agents should not be administered within 2 hours before or 3 to 4 hours after oral lincosamides are given. Lincosamides are distributed rapidly and widely to most tissues and fluids, including saliva, sputum, respiratory tissue, pleural fluid, soft tissues, bones and joints, brain, prostate, semen, appendix, and peritoneal fluid.[202,217] Lincosamides are transported actively into macrophages and polymorphonuclear leukocytes, and high concentrations are achieved in bile, urine, and bone. Penetration into CSF is limited, unless inflammation is present. In experimental pneumococcal meningitis, concentrations of clindamycin in CSF were approximately 10 percent of the corresponding serum values. Lincosamides are highly protein-bound, with values ranging from 70 to 75 percent for lincomycin and 92 to 94 percent for clindamycin. The inactive palmitate and phosphate esters are hydrolyzed in the liver to clindamycin, the active agent. Clindamycin undergoes hepatic biotransformation to active and inactive metabolites. Ten percent of absorbed lincomycin is excreted unchanged in urine, 3 percent is excreted unchanged in feces, and the remainder is excreted as inactive metabolites, primarily in the biliary system. Elimination is delayed in the presence of severe hepatic insufficiency alone or severe concurrent renal and hepatic impairment, and, therefore, adjustments in dosages may need to be made. Formulations of lincosamides available for use include lincomycin hydrochloride in 250- and 500-mg capsules and in solution for parenteral use. Clindamycin phosphate, a water-soluble ester of clindamycin and phosphoric acid, is available for parenteral use. Oral formulations include clindamycin palmitate hydrochloride granules reconstituted to 75 mg base/5 mL and clindamycin hydrochloride capsules in 75, 150, and 300 mg of the base compound. Clindamycin also is available as a topical solution, gel, lotion, foam, pad, and vaginal cream and suppository.

Indications for Use

Lincomycin can be used for the treatment of serious infections caused by susceptible strains of staphylococci, pneumococci, other streptococci, or anaerobic organisms, but it rarely is used in pediatric patients. Clindamycin is effective against and has been approved for treatment of the following: susceptible strains of staphylococcal bone and joint infections[91]; anaerobic pelvic infections, including pelvic inflammatory disease, nongonococcal tubo-ovarian abscess, and postsurgical vaginal cuff infections; anaerobic intra-abdominal infections, including peritonitis and abscesses; pneumonitis, empyema, and lung abscesses caused by anaerobes and as a second-line agent for infections caused by susceptible strains of pneumococci and staphylococci; anaerobic septicemia; and skin and soft tissue infections caused by anaerobes, susceptible strains of staphylococci, and streptococci. Clinical prediction rules relating to resistance among community-acquired, community-onset health care–associated and traditional nosocomial staphylococcal isolates are becoming less reliable because certain strains with distinctive genetic determinants of virulence (e.g., *pvl* genes) and antibiotic resistance (e.g., SSC*mec*) are tending to predominate.[128,195] Prevalences of constitutive and inducible MLS_B resistance in community-associated *S. aureus* infections generally are increasing in the United States, although rates vary geographically and temporally as a function of predominant circulating clones.[59] Based on current experience, clindamycin should not be used for empiric therapy of suspected *S. aureus* infections if the local clindamycin resistance rate exceeds 10 to 15 percent. Conversely, clindamycin

is effective in treating serious infections caused by clindamycin-susceptible (D-test–negative) CA-MRSA infections, including bacteremia, osteomyelitis, suppurative arthritis, pleural empyema, and skin and soft tissue infections.[146,180] Clindamycin also is effective and has been approved as a topical agent for acne vulgaris. Unconfirmed retrospective data suggest that clindamycin in combination with a β-lactam antibiotic, with surgery if indicated, may be the most effective treatment of invasive *S. pyogenes* infections.[283] Other infections for which clindamycin may be effective but has not been approved for therapy include acute otitis media caused by penicillin-resistant pneumococci that has failed to respond to other antibacterial agents,[9] chronic suppurative otitis media or chronic sinusitis in which anaerobes may play a role, chronic pharyngeal carriers of group A streptococci,[260] odontogenic infections, toxoplasmosis of the CNS (in combination with pyrimethamine), uncomplicated falciparum malaria[161] or babesiosis (in combination with quinine), and mild to moderate *P. jiroveci* pneumonia in patients with acquired immunodeficiency syndrome (AIDS) (in combination with primaquine).[28] Because of poor penetration into CSF, this drug is not approved for the treatment of meningitis. Clindamycin is a third-line agent for endocarditis prophylaxis for upper respiratory tract, dental, or oral procedures in persons allergic to or intolerant of amoxicillin and erythromycin. Oral and intravenous formulations of clindamycin are approved for use in infants and children of all ages. The bitter taste of the oral suspension may limit compliance.

Adverse Effects

The most frequent side effects include generalized morbilliform-like rash and mild, self-limited diarrhea occurring in as many as 10 percent and in 2 to 20 percent of patients, respectively. Other gastrointestinal disturbances include anorexia, nausea, vomiting, flatulence, abdominal pain, and a metallic taste. Pseudomembranous colitis, a serious and sometimes fatal illness, is the adverse event posing the most concern. Most antibiotics have been associated with pseudomembranous colitis, but those most frequently implicated include ampicillin, lincosamides, and cephalosporins. The incidence of lincosamide-associated pseudomembranous colitis varies from 0.1 to 10 percent. Antibiotic-associated pseudomembranous colitis is caused by overgrowth of toxin-producing strains of *C. difficile*; at least two extracellular toxins are elaborated: toxin A, a potent enterotoxin; and toxin B, a cytotoxin.[46] The risk of developing disease is increased in elderly patients and those with chronic, debilitating conditions. Pseudomembranous colitis is unrelated to the total antibiotic dosage, duration of therapy, route of administration, or underlying disease. The onset most frequently occurs between days 4 and 9 of therapy, but signs and symptoms develop in one third of patients 2 to 10 weeks after discontinuation of the antibiotic. More than 80 percent of patients have fever, leukocytosis, crampy abdominal pain, and watery diarrhea, and 5 to 10 percent have bloody diarrhea. Sigmoidoscopic findings include plaquelike lesions on colonic or rectal mucosa consisting of polymorphonuclear leukocytes, chronic inflammatory cells, fibrin, and epithelial debris. Treatment includes prompt discontinuation of the antibiotic, avoidance of antiperistaltic agents, and oral administration of metronidazole. However, several factors that limit the effectiveness of metronidazole include occasional resistance of *C. difficile*, absence of convenient oral preparations for children, and complete absorption of the drug in the upper gastrointestinal tract so that bactericidal levels are achieved erratically in the lower gastrointestinal tract. Although this indication in children has not been approved by the FDA, metronidazole is considered the drug of choice. Use of a nonabsorbable oral vancomycin formulation is potentially effective. However, because of its expense, bad taste, associated relapse rate of 20 percent, and potential to induce resistance in enterococci, vancomycin should be reserved for children who fail to respond to metronidazole therapy. Potential alternative therapies for multiple recurrences of pseudomembranous colitis include probiotics, certain strains of yeast, solution of fresh stool from healthy donors, or cholestyramine.[46] An approved agent for traveler's diarrhea, rifaximin, has been shown to be effective in a few patients with recurrent or persistent *C. difficile* infection.

Other less common adverse events include the following: hypersensitivity reactions such as urticarial rash, drug fever, and eosinophilia; transient neutropenia, agranulocytosis, or thrombocytopenia; and a mild, reversible elevation in hepatic transaminases. Caution must be exercised when administering lincosamides to newborns; fatal gasping syndromes have been described that possibly are related to the preservative benzyl alcohol.

Drug interactions include incompatibility with many agents in solution, including ampicillin, barbiturates, calcium gluconate, phenytoin, and magnesium sulfate, as well as interaction with hydrocarbon-containing inhalational anesthetics. Lincosamides are weak neuromuscular blockers,[256] but they can enhance neuromuscular blockade when they are administered concurrently with neuromuscular blockers. Chloramphenicol and macrolides can have an antagonistic effect when they are administered concurrently with lincosamides because of competition for ribosomal binding sites.

LINEZOLID

Linezolid is the first of a new class of oxazolidinone antibiotics that was approved for use in children by the FDA in 2002. Linezolid is a totally synthetic compound with favorable activity against multidrug-resistant, gram-positive bacteria.[82]

Mechanisms of Action

Linezolid inhibits an early stage of ribosomal protein synthesis by binding to a unique site, domain V on the bacterial 23S ribosomal RNA of the 50S subunit, and prevents formation of a functional 70S initiation complex. Inhibition of this process interferes with the bacterial translation process. Furthermore, linezolid causes ribosomes to shift reading frames and to read through stop codons, thereby affecting translational fidelity.[267]

Mechanisms of Resistance

Rarely, mutations conferring resistance have been reported in the peptidyl transferase region of the 23S rRNA genes. Cross-resistance between linezolid and chloramphenicol is caused by a single nucleotide mutation in the peptidyl transferase center.[267] Cross-resistance between linezolid and other classes of antibiotics that target the ribosome such as aminoglycosides, macrolides, lincosamides, and streptogramins rarely occurs because these agents target a distinct mechanism of protein elongation. Resistance to linezolid has been reported in as many as 4 percent of clinical isolates of enterococci, particularly *E. faecium*, *S. aureus*, and coagulase-negative staphylococci.[86] Predisposing factors include indwelling devices, undrained abscesses, and prolonged administration of linezolid, particularly if subtherapeutic antibiotic concentrations are achieved.[237]

In Vitro Activity

Time-kill studies demonstrated that linezolid is bacteriostatic against enterococci and staphylococci and bactericidal against most strains of streptococci. Linezolid is active against MRSA, coagulase-negative staphylococci, and vancomycin-resistant enterococci. Furthermore, linezolid has broad activity against

Nocardia and rapidly growing mycobacteria, as well as slowly growing tuberculous and nontuberculous mycobacteria.[47]

Pharmacokinetics

Dosage adjustment is not necessary when switching from intravenous to oral formulations because the oral bioavailability is approximately 100 percent. Absorption is not significantly affected by food. The antibiotic is distributed to well-perfused tissues and has a relatively low protein binding (31%) that contributes to the high degree of penetration into bone, muscle, fat, alveolar cells, lung extracellular lining fluid, and CSF. Linezolid is metabolized primarily by oxidation and has no interaction with the cytochrome P-450 enzymes. The elimination half-life in children is less than 5 hours and is shorter than in adults. The disposition of linezolid varies as a function of age with faster clearance and greater volume of distribution in children younger than 11 years of age. The AUC values for children are lower than those for adults, whereas preterm neonates (gestational age <34 weeks) within the first week of life have larger AUC values than do many full-term neonates and older children. The pharmacokinetics of linezolid in adolescents 12 years of age and older is not significantly different from that of adults. Dosage adjustment is not necessary for patients with renal insufficiency or mild to moderate hepatic insufficiency.[144]

Indications for Use

Safety and effectiveness of linezolid have been established for pediatric patients from birth through 17 years of age. Intravenous and oral linezolid is indicated for treatment of children with (1) uncomplicated and complicated skin and skin structure infections caused by oxacillin-susceptible or oxacillin-resistant *S. aureus*, *S. pyogenes*, or *S. agalactiae*; (2) community- and health care–associated pneumonia caused by penicillin-susceptible strains of *S. pneumoniae* or oxacillin-susceptible or oxacillin-resistant *S. aureus*; and (3) infections caused by vancomycin-resistant enterococci.[147] The recommended dosage for newborns (≥34 weeks' postconceptual age) and children up to the age of 11 years is 10 mg/kg, given intravenously or orally every 8 hours for 10 to 14 days for pneumonia and complicated skin and skin structure infections and for 14 to 28 days for pneumonia or bacteremia caused by vancomycin-resistant enterococci. The 10-mg/kg dose is administered every 12 hours for 5- to 11-year-old children with uncomplicated skin and soft tissue infections and for premature neonates (gestational age < 34 weeks) within the first week of life. The dose for adolescents older than 11 years and adults is 600 mg every 12 hours, with a 1200-mg daily limit. Linezolid offers a safe therapeutic alternative to vancomycin for multidrug-resistant, gram-positive bacteria and has the additional advantage of being available in an oral liquid preparation (100 mg/5 mL), which facilitates sequential intravenous-oral therapy.[81,136,144,147] Safety and efficacy of therapy for longer than 28 days have not been evaluated in controlled clinical trials. Currently, no indication exists for treatment of mycobacterial infections, although linezolid has been used successfully for certain nontuberculous infections.

Adverse Effects

Data from multiple trials indicate that linezolid is safe and well tolerated, with adverse events similar to those of comparative drugs. The most common drug-related adverse events are diarrhea, nausea, vomiting, rash, and headache.[244] However, dose- and duration-dependent reversible myelosuppression, including mild to moderate anemia and thrombocytopenia and rarely neutropenia, have been reported in children treated with linezolid.[187,244] Therefore, weekly monitoring of complete blood counts is recommended in patients who receive linezolid for longer than 2 weeks, have preexisting myelosuppression, or receive other myelosuppressive antibiotics. Other rare adverse effects that warrant prompt evaluation include peripheral and optic neuropathy, lactic acidosis, and the serotonin syndrome in patients receiving concomitant selective serotonin reuptake inhibitors. The oral suspension formulation contains phenylalanine, which is contraindicated in children with phenylketonuria.

RIFAMYCINS

The rifamycins are a group of complex macrocyclic antibiotics that were isolated from *Streptomyces mediterranei* in the early 1960s. Rifampin (rifampicin) and rifabutin are structurally related, semisynthetic broad-spectrum antibiotics derived from rifamycin B and S, respectively. These agents avidly bind to the β subunit of the DNA-dependent RNA polymerase, prevent attachment of the enzyme to DNA, and thus block initiation of RNA transcription and protein synthesis. Rifabutin was approved in 1992 for prevention of disseminated MAC disease in adults with advanced HIV infection. Single-point mutations that determine resistance develop rapidly when rifamycins are used alone. Therefore, these antibiotics should be administered in combination with other antimicrobial agents when indicated. Rifaximin also is a semisynthetic derivative of rifamycin that was approved by the FDA in 2004 for the treatment of individuals 12 years of age or older who have nondysenteric and afebrile traveler's diarrhea caused by noninvasive strains of *E. coli*.[3]

In Vitro Activity

Rifampin usually is bactericidal, but it may be bacteriostatic, depending on the organism and drug concentration. Rifamycins have excellent in vitro activity against the following: many gram-positive cocci, including many methicillin-resistant staphylococci and penicillin-nonsusceptible *S. pneumoniae* (enterococci are notable exceptions); some gram-negative organisms, including meningococci, gonococci, and *H. influenzae*; *Legionella*; *C. trachomatis*; *T. gondii*; *M. tuberculosis*; and *M. leprae*. Rifampin is active against *M. kansasii*, but not against most other species of atypical mycobacteria. In contrast, rifabutin is active against most atypical mycobacteria, except *M. chelonae*. Rifabutin also is more active than is rifampin against rifampin-susceptible strains of *M. tuberculosis* and is active against approximately one third of rifampin-resistant strains.[174,193] Resistance to rifampin is mediated by point mutations in the *rpoB* gene that encodes the β subunit of the DNA-dependent RNA polymerase as well as alterations in membrane permeability. Surveillance data documented that 3 percent of meningococcal isolates in the United States are resistant to rifampin.[149] Rifaximin has activity against most enteric bacterial pathogens including *C. difficile*, *C. jejuni*, *E. coli*, *H. pylori*, *Salmonella* spp., *Shigella* spp., *V. cholera*, and *Yersinia enterocolitica*.[3]

Pharmacokinetics

Rifampin and rifabutin are highly lipid-soluble and highly protein-bound (72% to 89%) molecules. Consequently, they are well absorbed from the gastrointestinal tract and have excellent intracellular penetration. The drugs reach therapeutic concentrations in the CSF (≈10% to 20% of simultaneous serum concentrations), particularly in the presence of inflammation of the meninges. They are metabolized primarily by the liver through deacetylation, and 30 to 40 percent of the drug is excreted by the biliary system. In the presence of hepatic dysfunction, dosage adjustments are necessary, whereas no alteration in dosage is required for children with renal dysfunction. In adults, rifabutin

has a significantly longer mean terminal half-life than does rifampin (45 versus 2 to 5 hours).[230] Rifampin, however, has a very long post-antibiotic effect. Rifaximin is poorly absorbed, with a bioavailability of less than 0.4 percent. Therefore, dosage adjustment of rifaximin for hepatic or renal dysfunction is not necessary, and the agent is unlikely to interact with medications metabolized by the cytochrome P-450 system.

Indications for Use

The only situation in which rifampin should be considered for use as a single antibacterial agent is for prophylaxis of infection, because resistance to rifampin develops rapidly when this agent is used alone for therapeutic purposes. Prophylaxis with rifampin monotherapy is indicated to eradicate nasopharyngeal colonization in close contacts of individuals with infections caused by *H. influenzae* type b[112,197] and *N. meningitidis*,[26] as well as index cases with these infections, unless they were treated with third-generation cephalosporins.

The use of rifampin, combined with two or three other bactericidal antituberculosis agents for the treatment of active tuberculosis, as well as its combined use with clofazimine and dapsone for the treatment of leprosy, is considered an essential component of effective regimens. The effect of adding rifampin to certain antibiotics for treatment of other infections often is unpredictable and may result in synergistic, additive, indifferent, or antagonistic activity. In some patients, rifampin can be considered part of a combination regimen for the treatment of the following: (1) meningitis caused by penicillin- and cephalosporin-resistant *S. pneumoniae* in combination with a third-generation cephalosporin and vancomycin; (2) shunt- or catheter-associated staphylococcal infections, often with removal of the foreign material; (3) *S. aureus* implant-related orthopedic infections in combination with an effective agent; (4) non–life-threatening susceptible MRSA skin and skin structure infections in combination with TMP-SMX with the theoretical benefit of eradicating MRSA carriage, considering that rifampin achieves high concentrations on mucosal surfaces; (5) prosthetic valve endocarditis caused by staphylococci; (6) complicated or severe *B. henselae* infection (cat-scratch disease); (7) brucellosis in combination with TMP-SMX, a tetracycline, or an aminoglycoside[176]; (8) MAC-associated disseminated disease or lymphadenitis, if not completely resectable, in combination with ethambutol and azithromycin or clarithromycin; (9) *L. pneumophila* pneumonia in combination with a macrolide if response to macrolides alone is poor; and (10) multidrug-resistant *S. maltophilia* in combination with colistin (colistimethate sodium; polymyxin E) based on favorable in vitro data.[149,174] Furthermore, rifampin- and minocycline-impregnated central venous catheters have been used successfully in adults to prevent catheter colonization and bloodstream infection with gram-positive and gram-negative pathogens, as well as with *Candida* spp. The American Academy of Pediatrics recommends selective use of these catheters in infants weighing more than 3 kg and children if the local catheter-related bloodstream infection rate exceeds 3.3 per 1000 catheter days and if the standard protective procedures already have been implemented.[75,214] In the last circumstance, surveillance for resistance is important. Many physicians use rifampin combined with antistaphylococcal agents for treatment of disseminated MRSA infection without evidence from controlled studies of its effectiveness.

Rifabutin is effective for the treatment of most mycobacterial species, *H. pylori*, and *T. gondii* in adults. Limited data are available for the treatment of MAC infections in children with HIV infection. Clinical studies in adults with advanced HIV infection indicate that administration of daily clarithromycin or weekly azithromycin is superior to rifabutin alone for prophylaxis of MAC infection.[193] The safety and efficacy of rifabutin for prophylaxis of MAC infection in children have not been established.[145]

Rifampin is available in capsule (150- and 300-mg) and intravenous formulations. Rifabutin is available only in capsule (150-mg) form. A suspension can be compounded by the pharmacy, or the capsule contents can be mixed with thick, sweet food for infants and young children. Absorption of rifampin is optimal if administered 1 hour before or 2 hours after a meal, whereas a high-fat meal decreases the rate, but not the total amount, of absorption of rifabutin. The use of fixed-dose combinations of rifampin, isoniazid, and pyrazinamide is not recommended for children. Although the safety and efficacy of rifapentine, a rifamycin antibiotic similar in structure and activity to rifampin and rifabutin, have not been established for children younger than 12 years old, a pharmacokinetic study indicated that dosages in children need to be greater than those for adults to achieve comparable systemic exposure.[29] The approved dosage of rifaximin for the treatment of traveler's diarrhea in adolescents and adults is 200 mg three times per day for 3 days. A dosage of 400 mg twice per day for 3 days apparently is equally effective and may be better tolerated by patients. Rifaximin should not be used to treat systemic infections or mucosally invasive enteric infections. Rifaximin is not indicated currently for the treatment of dysenteric diarrhea secondary to *Shigella*, *Salmonella*, or *Campylobacter* spp. infection or for the treatment of complicated diarrhea with fever, systemic toxicity, or bloody stools. The role of this agent for prophylaxis of traveler's diarrhea is being studied.[3]

Adverse Effects

Adverse events associated with rifampin include gastrointestinal disorders, rash, hepatotoxicity, hypersensitivity, and a flulike syndrome. Adverse reactions to rifabutin include gastrointestinal disorders, rash, leukopenia, neutropenia, and, rarely, uveitis, although safety data in children are limited. The parenteral form of rifampin is associated with thrombophlebitis. Routine determination of serum aminotransferase concentrations is not recommended for children receiving brief or prolonged courses of therapy, except for those with clinical evidence of hepatitis or severe or disseminated tuberculosis. In these situations, at least monthly measurements should be performed. In addition, patients should be warned that rifamycins discolor urine, tears, sweat, and feces and permanently stain soft contact lenses. Because rifamycins induce hepatic cytochrome P-450 enzyme activity, they may interact with other medications administered concurrently and cause decreased concentrations of some antiretroviral agents, particularly protease inhibitors, azole antifungal agents, barbiturates, oral contraceptives, clarithromycin, cyclosporine, digoxin, sulfonylureas, thyroxine, and warfarin (Coumadin). Conversely, rifamycin serum drug concentrations may increase if it is administered with other drugs that inhibit hepatic enzymes, and the dose of rifamycin antibiotic may need to be reduced by as much as 50 percent. Rifabutin induces hepatic enzymes to a lesser extent than does rifampin and therefore has less potential for drug interactions.[174,193] Rifaximin is well tolerated and has an excellent safety profile.

SULFONAMIDES

Sulfachrysoidine (Prontosil), discovered in the 1930s, was the first sulfonamide developed. Sulfonamides are broad-spectrum antimicrobial agents derived from sulfanilamide (*para*-aminobenzene sulfonamide) and are structural analogues of *para*-aminobenzoic acid (PABA); they compete with PABA and result in interference with nucleotide synthesis. Sulfanilamide was manipulated to form other compounds with expanded antimicrobial activity and reduced toxicity. Sulfonamides are

distributed widely in fluids and tissues. The preparations currently available have greater solubility than earlier compounds and are less likely to cause crystalluria. Sulfonamides available for single-agent use include sulfacytine (Renoquid), sulfadiazine, sulfamethizole (Thiosulfil Forte), SMX (Gantanol), and sulfisoxazole (Gantrisin). Sulfonamide combinations, including TMP-SMX and erythromycin ethylsuccinate-sulfisoxazole acetyl (EES-SSX), are used frequently in pediatrics.

Trimethoprim-Sulfamethoxazole

TMP is a diaminopyrimidine antibiotic available for single-agent use. Combinations of TMP and sulfonamides were used in the late 1960s because of presumed synergistic antibacterial activity, although clinical evidence of synergy is equivocal. Because SMX has rates of absorption and elimination similar to those of TMP, it was the sulfonamide selected for combination. Iclaprim is a novel diaminopyrimidine currently in clinical development with a broad spectrum of activity that includes TMP-resistant strains of *S. aureus* (including MRSA and VRSA), *S. pneumoniae* (including penicillin-nonsusceptible strains), *P. jiroveci*, *Neisseria*, *M. catarrhalis*, and *C. pneumoniae*.[119]

MECHANISMS OF ACTION

SMX competitively inhibits dihydropteroate synthetase, the bacterial enzyme that assimilates PABA into dihydrofolic acid; such inhibition results in a reduction in dihydrofolic acid synthesis and therefore a reduction in the amount of tetrahydrofolic acid, a cofactor for nucleotide synthesis.[121] Only bacteria that must synthesize folic acid are potentially susceptible. SMX is bacteriostatic when used alone and can be inhibited by PABA and its derivatives (procaine and tetracaine). TMP reversibly binds and inhibits dihydrofolate reductase, an enzyme that reduces dihydrofolic acid to tetrahydrofolic acid; this activity results in diminished amounts of folic acid, an essential cofactor in nucleic acid production.[50] TMP is bacteriostatic when used alone, but in combination with SMX against susceptible bacteria, bactericidal activity can be achieved by blockade of sequential steps in folic acid metabolism. The effect of sulfonamide on bacteria is circumvented in mammals, which obtain folate from food sources. The reaction inhibited by TMP is similar in bacteria and mammals but differs quantitatively in the extent of binding of the drug to the enzyme; mammalian dihydrofolate reductase is 60,000 times less sensitive to TMP than is the enzyme in susceptible bacteria.

MECHANISMS OF RESISTANCE

Bacteria can be inherently resistant to either agent or can acquire resistance to TMP, SMX, or both agents. Resistance to SMX and other sulfonamides is associated with hyperproduction of PABA, as demonstrated in strains of *Neisseria* and staphylococci, or is caused by an altered dihydropteroate synthetase enzyme with lower affinity for sulfonamides, as found in *E. coli*, *N. meningitidis*, and *S. pneumoniae*.[265] Chromosomally mediated resistance to TMP on the basis of mutations in the *dhfr* gene has been observed in strains of *S. pneumoniae*, *H. influenzae*, *E. coli*, *Pediococcus*, *B. fragilis*, and *Nocardia* and *Clostridium* spp. Furthermore, *P. aeruginosa*, *K. pneumoniae*, and *S. marcescens* are inherently resistant to TMP because of cell wall impermeability. Acquired resistance to TMP can be plasmid-mediated or chromosomally mediated and occurs in members of the Enterobacteriaceae and in staphylococci, and streptococci. Mechanisms of acquired TMP resistance include cell wall impermeability, thymine auxotrophy, resistant dihydrofolate reductase, and overproduction of dihydrofolate reductase, the most common being plasmid-mediated production of dihydrofolate reductases, which are encoded by at least 20 genes. Resistance to sulfonamides is encoded by the *dhps* family of genes that include the *fol*P, and *sul*I and *sul*II genes. The *sul*I gene usually is linked to other transferable resistance genes, often in the transposons that belong to the Tn*21* family. Despite the reduced use of sulfonamides, the genetic determinants for sulfonamide resistance probably persist because of the efficient integron transfer mechanisms.[130] Bacterial resistance to both TMP and SMX can develop as a result of altered cell wall permeability or alternative metabolic pathways (e.g., thymine auxotrophy), whereby they obtain thymine or thymidine from the environment.[265]

IN VITRO ACTIVITY

TMP-SMX has activity against a broad spectrum of gram-positive cocci and gram-negative enteric pathogens. TMP is more active than is the sulfonamide, but the mixture is significantly more effective than is either drug alone. Synergism is more likely to occur when the bacteria are susceptible to both drugs, but it can occur even when the bacteria are resistant to only one agent. Bacteria with potential susceptibility to the combination include *Aeromonas hydrophila*, *Brucella*, *B. cepacia*, *Campylobacter*, *E. coli*, *H. influenzae*, *M. catarrhalis*, some environmental mycobacteria, *N. gonorrhoeae*, *Nocardia*, *P. jiroveci*, *Proteus*, *Salmonella*, *S. marcescens*, *Shigella*, oxacillin-susceptible *S. aureus*, *S. maltophilia* (usually with a β-lactam–lactamase inhibitor combination), penicillin-susceptible *S. pneumoniae*, and *Y. enterocolitica*. However, high levels of resistance to TMP-SMX among strains of penicillin-nonsusceptible *S. pneumoniae*, methicillin-resistant staphylococci, *M. catarrhalis*, *H. influenzae*, *Neisseria*, *E. coli*, *Klebsiella*, *Salmonella*, *Shigella*, *Campylobacter*, *P. aeruginosa*, *Acinetobacter*, *Nocardia*, *Actinomyces*, mycobacteria, *Bacteroides*, *Clostridium*, and *C. pneumonia* limit the usefulness of this antibacterial agent.[130]

PHARMACOKINETICS

Optimal synergistic activity occurs when a 1:20 ratio of TMP and SMX serum concentrations is attained, as can be achieved after the administration of a fixed 1:5 ratio of TMP to SMX. Both agents are absorbed rapidly and fairly well when administered alone and in combination. Both penetrate most body fluids and tissues, although TMP frequently penetrates extravascular tissues to a greater degree than does SMX. Both agents cross the placenta, are excreted in breast milk, and diffuse into pleural, peritoneal, and synovial fluid and CSF. Protein binding varies from 40 to 60 percent for TMP and from 60 to 70 percent for SMX. Both drugs are metabolized in the liver to inactive metabolites. The primary route of elimination is by the kidneys, with small amounts excreted in bile and feces. Dosage adjustments are required for renal impairment.

Available preparations include an oral suspension containing 40 mg of TMP and 200 mg of SMX per 5 mL, tablets containing 80 mg of TMP and 400 mg of SMX, double-strength tablets consisting of 160 mg of TMP and 800 mg of SMX, and a parenteral solution.

INDICATIONS FOR USE

TMP-SMX is approved for the treatment of acute exacerbations of chronic bronchitis in adults, enterocolitis caused by susceptible *Shigella* organisms, acute otitis media caused by susceptible strains of *H. influenzae* or pneumococcus, *P. jiroveci* pneumonia, traveler's diarrhea caused by *Shigella* and enterotoxigenic *E. coli*, and acute or chronic urinary tract infections. Although the combination is not approved for use and not usually prescribed as a first-line antibiotic, TMP-SMX has been effective therapy for typhoid fever, brucellosis, nocardiosis, sinusitis, biliary tract infections, and bone and joint infections caused by susceptible organisms.

Because early studies did not evaluate the effect of prolonged or recurrent therapy on somatic growth or bone marrow function in children, TMP-SMX was not approved for prophylaxis or prolonged treatment of otitis media. It is not recommended for treatment of group A streptococcal tonsillopharyngitis because it does not eradicate the organism or reliably prevent the nonsuppurative sequelae. TMP-SMX is not recommended as first-line monotherapy for community-acquired pneumonia, acute otitis media, or skin infections caused by *S. pyogenes* because of frequent resistance among the etiologic pathogens and absence of efficacy data. TMP-SMX is not FDA approved for the treatment of any form of staphylococcal infection. However, the medical literature contains reports of the successful use of TMP-SMX alone or in combination with one or more agents such as rifampin in the treatment of *S. aureus* infections, including MRSA.[110] TMP-SMX is recommended as an alternative agent to macrolides for treatment of pertussis in individuals 2 months of age or older.[269] TMP-SMX also is an important prophylactic agent that is indicated to prevent bacterial, *P. jiroveci*, and other infections in the following clinical settings: immunosuppressed oncology or post-transplant patients, individuals with HIV who meet clinical and hematologic criteria or certain infants who are exposed in utero to HIV, patients with urinary tract infections who are at risk for having recurrent disease, and patients with chronic granulomatous disease or other congenital immunodeficiency syndromes.

ADVERSE EFFECTS

Most of the side effects occurring during administration of TMP-SMX are caused by the sulfonamide. Gastrointestinal disturbances and hypersensitivity reactions are the adverse events most commonly observed. Anorexia, nausea, vomiting, diarrhea, drug eruption, and photosensitivity reactions can occur in 1 to 4 percent of patients. Hypersensitivity reactions that develop less frequently include erythema nodosum, erythema multiforme (including Stevens-Johnson syndrome), urticaria, anaphylaxis, and thyroid damage. Drug-induced hepatitis has been described but is an unusual event. CNS side effects include vertigo, ataxia, headache, and aseptic meningitis. TMP-SMX can affect renal function when it is administered to persons with underlying renal disease, but the side effect usually is reversible after discontinuation of therapy. Crystalluria occurred more commonly with earlier preparations because of low solubility. SMX has a greater tendency to cause crystalluria than do other sulfonamides that are currently available because of slower absorption and excretion, but with adequate fluid intake, alkalinization of the urine usually is unnecessary. Interstitial nephritis and tubular necrosis seldom are associated with the use of TMP-SMX. Blood dyscrasias can be a limiting factor to the administration of TMP-SMX. Acute hemolytic anemia has been described after the use of TMP-SMX in patients with glucose-6-phosphate dehydrogenase deficiency. Although they are uncommon in patients with normal hematopoietic systems, aplastic anemia, agranulocytosis, leukopenia, and thrombocytopenia can occur. Prolonged use can result in megaloblastic anemia because of impaired folate utilization. Administration of sulfonamide can trigger an acute attack of porphyria. Sulfonamides can displace bilirubin from albumin-binding sites. In neonates, especially premature infants, the activity of sulfonamides can be increased as a result of reduced conjugation by the immature liver. Because of the increased risk of development of kernicterus from sulfonamide displacement of bilirubin, the use of sulfonamides during the last month of pregnancy and in the first 2 months of life is discouraged.

Drug-drug interactions with TMP-SMX are numerous. Sulfonamides can displace other drugs from albumin-binding sites and thereby result in increased effective activity of the second drug, as can be seen with the concurrent administration of methotrexate, phenytoin, sulfonylurea hypoglycemic agents, thiazide diuretics, and warfarin. Drugs that, when co-administered, can displace sulfonamides from binding sites and lead to increased effective sulfonamide activity include indomethacin, probenecid, and salicylates. Agents that reduce the effect of sulfonamides include methenamine, which results in insoluble urinary precipitates of the sulfonamide, and derivatives of PABA. Sulfonamides are physically incompatible with many drugs, among them aminoglycosides, chloramphenicol, insulin, lincomycin, methicillin, tetracycline, and vancomycin.

Erythromycin Ethylsuccinate–Sulfisoxazole Acetyl

The combination of EES and SSX in a fixed ratio expands the spectrum of antibacterial activity. The mechanism of action, mechanisms of resistance, pharmacokinetics, and adverse events for EES-SSX are the same as those for each individual drug. Because EES-SSX is effective in vitro against common pathogens causing otitis media in children, it was approved for the treatment of acute otitis media.[38] However, it is not effective against many strains of pneumococci and no longer is recommended for empiric therapy of acute otitis media.[9] Although it is not approved for use, it can be effective therapy for sinusitis caused by *H. influenzae*, susceptible pneumococci, and *M. catarrhalis.* Albeit uncommon, its principal use today in pediatrics is for the treatment of acute otitis media caused by susceptible pathogens in patients with β-lactam hypersensitivity.

TETRACYCLINES

Chlortetracycline, also known as Aureomycin, was the first natural tetracycline discovered when isolated from *Streptomyces aureofaciens* in 1948.[92] Many tetracyclines have been developed since then. Those currently marketed include the natural agents tetracycline, oxytetracycline, and demeclocycline and the semisynthetic agents doxycycline and minocycline. Their basic structure consists of a hydronaphthacene nucleus with four fused rings. They differ from each other biochemically by substituent variations at carbons 5, 6, or 7. Their mechanisms of action and mechanisms of resistance, as well as their spectra of activity, are similar, but the analogues differ in the degree of activity and in pharmacokinetic properties. Tigecycline is a novel intravenously administered glycylcycline that is related structurally to minocycline. It is approved for use in adults with complicated skin and skin structure infections (including MRSA) and complicated intra-abdominal infections. It is bacteriostatic, with improved activity against multidrug-resistant bacteria, and it has no cross-resistance with other classes of antimicrobial agents.

Mechanisms of Action

Tetracyclines passively diffuse through outer-membrane porins and then traverse the cytoplasmic membrane by energy-dependent active transport. Tetracyclines are actively concentrated by most bacterial cells but not by mammalian cells, a characteristic that explains the selective action of these drugs against bacteria. Tetracyclines are bacteriostatic agents that reversibly bind to the 30S subunit of 70S bacterial ribosomes and inhibit protein synthesis. Because attachment of aminoacyl-tRNA to the ribosome acceptor site is prevented, the bacteria are unable to add amino acids to the growing peptide chain.

Mechanisms of Resistance

In many bacteria, including members of the Enterobacteriaceae, *P. aeruginosa*, staphylococci, streptococci, and *Bacteroides*, resistance to tetracyclines has developed. Resistance in most bacteria results from the acquisition of new genes often associated with

mobile elements such as plasmids or transposons, but it can also be chromosomally mediated. The genes encoding for acquired resistance are called *tet* (tetracycline)–resistance or *otr* (oxytetracycline)–resistance determinants. Resistance to one tetracycline usually implies resistance to all; however, many tetracycline-resistant bacteria are susceptible to doxycycline, minocycline, or both. At least 38 acquired tetracycline- and oxytetracycline-resistance genes have been identified that mediate (1) energy-dependent efflux of tetracycline (the most common type of resistance mechanism), (2) protection of the ribosomes from the action of tetracycline, or (3) enzymatic inactivation of tetracycline.[235] *Tet* genes that encode membrane efflux proteins are different in gram-positive and gram-negative bacteria. Efflux has been found in a wide range of bacteria including *Acinetobacter*, *Aeromonas*, *Chlamydia*, the Enterobacteriaceae, *Haemophilus*, *Moraxella*, *Neisseria*, *Pseudomonas*, staphylococci, *Stenotrophomonas*, *V. cholerae*, and others. Less commonly, ribosomal protection by a soluble ribosomal protection protein encoded by different *tet* and *otr* genes has been found in a wide variety of aerobic and anaerobic gram-positive and gram-negative bacteria. The precise mechanism by which ribosomal protection protein mediates resistance to tetracyclines is being investigated. Researchers have hypothesized that these protection determinants produce allosteric disruption of the primary tetracycline binding site that causes the tetracycline molecules to be released from the ribosome. Enzymatic inactivation of tetracycline, encoded for by other *tet* genes, has been demonstrated in in vitro studies of *Bacteroides*, and *E. coli*, but the clinical significance has not been established.[235] Susceptibility of staphylococci to this class of agents typically is tested using tetracycline. However, this approach may overestimate the prevalence of resistance to doxycycline and minocycline. Although the prevalence of tetracycline resistance remains relatively low among CA-MRSA isolates, resistance in some strains has been associated with *tet*K, a finding indicating that doxycycline and minocycline may be effective treatment options. However, because data are sparse on clinical outcomes associated with the use of minocycline or doxycycline to treat infections caused by tetracycline-resistant *S. aureus* strains, these agents are not recommended.[110]

In Vitro Activity

Tetracyclines are broad-spectrum antibiotics with activity against aerobic and anaerobic gram-positive and gram-negative bacteria, chlamydiae, mycoplasmas, rickettsiae, and spirochetes. Activity against gram-negative bacteria has been limited by the emergence of tetracycline-resistant strains. Most *P. aeruginosa* and many *Shigella* and *Salmonella* organisms are resistant. Penicillin-susceptible strains of *N. gonorrhoeae* and *N. meningitidis* usually are susceptible to tetracyclines, but penicillin-resistant strains of *N. gonorrhoeae* are not. Gram-negative organisms with continued susceptibility to tetracyclines include *A. hydrophila*, *Brucella*, *Campylobacter*, some *Haemophilus* organisms, *Helicobacter*, *P. multocida*, *Plesiomonas shigelloides*, and *Vibrio.* Tetracyclines are active against some gram-positive bacilli, including *Actinomyces israelii*, *B. anthracis*, many clostridia, *Listeria*, and *Nocardia.* Tetracyclines have excellent activity against *C. pneumoniae*, *M. pneumoniae*, and rickettsiae. Other organisms for which tetracyclines have activity include *Mycobacterium marinum* and *B. burgdorferi.* The more lipophilic agents doxycycline and minocycline generally are more active than the others. Minocycline has excellent activity against susceptible staphylococci, and both doxycycline and minocycline are more active than is tetracycline against *S. aureus* and some streptococci, but they have no activity against enterococci and group B streptococci. Because of extensive plasmid-mediated resistance in pneumococci, therapy with tetracycline should be avoided. Doxycycline and minocycline are more active in vitro than are the other agents against anaerobic bacteria, but alternative antimicrobials are preferred for anaerobic infections because more than 50 percent of *B. fragilis* isolates are resistant, and susceptibility among other anaerobes is highly variable.[234]

Pharmacokinetics

The five tetracycline compounds available for systemic use can be classified by duration of activity: tetracycline and oxytetracycline are short-acting agents, demeclocycline is intermediate-acting, and doxycycline and minocycline are long-acting agents (Table 248–9). Oral administration is the preferred route because thrombophlebitis is associated with intravenous infusion, and pain is associated with intramuscular injections. Oral absorption ranges from 58 percent for the short-acting agents to 100 percent for the long-acting ones. The presence of food decreases the absorption of demeclocycline, oxytetracycline, and tetracycline. Because tetracyclines form insoluble complexes in the gut with aluminum, calcium, iron, magnesium, zinc, and other bivalent and trivalent cations, co-administration with milk and other dairy products, antacids, calcium or iron supplements, cathartics, and other agents can reduce absorption and should be avoided. Differences in lipid solubility affect penetration of tissues by the various tetracyclines. All agents readily penetrate many tissues and fluids, among which are pleural, ascitic, and synovial fluids, sinus secretions, sputum, bone, teeth, and breast milk. Tetracyclines can cross the placenta. The highest concentrations are achieved in bile and can exceed serum concentrations by 5 to 20 times. Therapeutic concentrations of doxycycline can be achieved in tonsillar and pulmonary tissues, the eye, and the prostate, and high concentrations are achieved in myometrial and endometrial tissue and the kidney.[246] Minocycline penetrates into sputum,

TABLE 248–9 Pharmacokinetics of Tetracyclines

Drug	Oral Absorption (%)	Protein Blinding (%)	Primary Route of Excretion	Approximate Half-Life* (hr)
Short Acting				
Oxytetracycline	58	35	Renal	6-10
Tetracycline	75-77	65	Renal	6-11
Intermediate Acting				
Demeclocycline	66	91	Renal	10-17
Long Acting				
Doxycycline	90-100	93	Biliary	12-22
Minocycline	90-100	76	Biliary	11-23

**Normal renal function.*
Modified from USP DI: Information for the Health Care Professional. Vol. 1. Thomson MICROMEDEX, 2006.

saliva, and tears, as well as into cells of the vestibular apparatus.[139] Minocycline is biotransformed to inactive metabolites by the liver; the other tetracyclines are not metabolized, although whether doxycycline is metabolized is not clear. Oxytetracycline, demeclocycline, and tetracycline are eliminated unchanged in urine. Approximately 10 percent of minocycline is excreted unchanged in urine, 20 to 35 percent is excreted unchanged in feces, and the remainder is excreted as inactive metabolites in urine and feces. Thirty to 40 percent of doxycycline is excreted in urine; the remainder is excreted by the biliary tract and by diffusion through the intestinal wall, where some of it is chelated and prevented from reabsorption and enterohepatic cycling. Dosing of short- and intermediate-acting tetracyclines must be adjusted for renal failure.

Systemic formulations available in the United States include tablets, capsules, delayed-release capsules, and suspensions for many of the tetracyclines, as well as parenteral preparations of doxycycline, minocycline, and oxytetracycline. Ophthalmic and topical preparations also are available.

Indications for Use

With few exceptions, tetracyclines no longer are the drugs of choice for many infections because of the availability of cephalosporins and semisynthetic penicillins with equivalent or greater activity and less frequent side effects. As a result of the potential for dental toxicity, tetracyclines are not recommended for use in children younger than 9 years old, except for specific infections for which alternative therapy is potentially more toxic, such as chloramphenicol for Rocky Mountain spotted fever and ehrlichiosis.[1] Approved indications in the United States include treatment of actinomycosis, anthrax, brucellosis, inclusion conjunctivitis, psittacosis, Q fever, rickettsialpox, Rocky Mountain spotted fever, typhus, relapsing fever, syphilis, trachoma, yaws, and Vincent's necrotizing gingivostomatitis caused by *Fusobacterium*, as well as infections caused by *Bacteroides* spp., *Bartonella bacilliformis*, *C. fetus*, *F. tularensis*, *M. pneumoniae*, *V. cholerae*, *Y. pestis*, and others. Because of its excellent tissue penetration and spectrum of activity, doxycycline is the preferred tetracycline for treatment of atypical pneumonia caused by *M. pneumoniae* and *C. pneumoniae*, intra-abdominal or pelvic infections, and several sexually transmitted diseases, including granuloma inguinale, chlamydial infections, *U. urealyticum* infection, nongonococcal urethritis, pelvic inflammatory disease (plus cefoxitin, ampicillin-sulbactam or single dose ceftriaxone with or without metronidazole), acute epididymitis (plus ceftriaxone), proctitis (plus ceftriaxone), and prostatitis.[278] Doxycycline is an alternative drug for (nonpregnant) penicillin-allergic patients with primary, secondary, or late latent syphilis. Although minocycline is effective in eradicating nasopharyngeal carriage of meningococcus, its potential for vestibular toxicity precludes its use for this purpose. For prevention of Lyme disease after a recognized tick bite, a single dose of doxycycline may be offered to adults (200-mg dose) and to children older than 8 years of age (4 mg/kg, up to a maximum dose of 200 mg), provided the following criteria are met: (1) the attached tick can be identified reliably as an adult or nymphal *Ixodes scapularis* tick that is estimated to have been attached for more than 36 hours on the basis of the degree of engorgement of the tick, (2) prophylaxis can be started within 72 hours of tick removal, and (3) the local rate of infection of these ticks with *B. burgdorferi* is more than 20 percent. Doxycycline (4 mg/kg/day in two divided doses, up to 100 mg per dose) is indicated in children older than 8 years of age and adults for treatment of erythema migrans, isolated cranial nerve palsy, uncomplicated cardiac disease, and uncomplicated arthritis.[279] Topical tetracyclines have been used extensively for the treatment of acne vulgaris and other dermatologic illnesses.[129]

Adverse Effects

Gastrointestinal disturbances are the most common side effects associated with tetracycline and include anorexia, nausea, emesis, flatulence, and diarrhea. These symptoms occur less frequently with minocycline. Esophageal ulceration has been associated with the oral administration of tetracyclines.[95] All tetracyclines except doxycycline can cause a negative nitrogen balance, with an elevation in blood urea nitrogen that usually is not significant except in the presence of underlying renal insufficiency. Demeclocycline can cause nephrogenic diabetes insipidus and, for this reason, has been used for treatment of the syndrome of inappropriate antidiuretic hormone secretion. Rarely, tetracyclines have been associated with hepatic injury, as manifested by elevated transaminases and diffuse vacuolar fatty metamorphosis on biopsy, with or without pancreatitis. The effect is dose-related, and the risk is increased with pregnancy, malnutrition, and preexisting renal or hepatic disease and in patients receiving other hepatotoxic agents. The use of tetracyclines in children younger than 9 years of age is relatively contraindicated because tetracyclines chelate with calcium and can be deposited in developing bones and teeth and lead to a transient decrease in bone growth, permanent tooth discoloration, and enamel hypoplasia. The risk that these side effects will occur with one course at an appropriate dosage is low; the degree of tooth discoloration is associated with the total dosage administered.[113] Minocycline has been associated with reversible vestibular toxicity. All tetracyclines have been noted to cause pseudotumor cerebri, a benign elevation in intracranial pressure that is reversible after discontinuation of the drug. Some data suggest that an increased risk may exist with the concurrent use of tetracycline and isotretinoin. Tetracyclines have been associated with an exaggerated sunburn reaction after sun exposure. This risk occurs most frequently with demeclocycline and rarely with minocycline. Hypersensitivity reactions occur infrequently and include morbilliform rash, urticaria, exfoliative dermatitis, and, rarely, anaphylaxis. Hyperpigmentation of the mucosal membranes, skin, and nails has been reported with the use of tetracycline, particularly minocycline.

Drug-drug interactions are numerous. A decrease in absorption of tetracyclines can occur with the co-administration of oral antacids, bismuth subsalicylate, iron, kaolin or pectin, zinc sulfate, and other divalent or trivalent cations that chelate the antibiotic. A reduced effect of tetracyclines because of increased metabolism is associated with the concomitant use of doxycycline with barbiturates, carbamazepine, phenytoin, rifampin, and alcohol in heavy drinkers. Co-administration with tetracyclines can increase the effect of oral anticoagulants, digoxin, lithium, and theophylline and can decrease the effect of oral contraceptive agents and oral iron. Tetracyclines can inhibit the in vitro bactericidal activity of penicillins and aminoglycosides. Other drug-drug interactions include benign intracranial hypertension with the concomitant administration of vitamin A, severe nephrotoxicity with the co-administration of methoxyflurane and tetracycline, and localized hemosiderosis with amitriptyline and minocycline.

SELECTED ASPECTS OF THE ADMINISTRATION OF ANTIMICROBIAL AGENTS

DOSAGE SCHEDULES FOR INFANTS AND CHILDREN

Dosage schedules of antimicrobial agents commercially available in the United States for infants (beyond the newborn period) and children are listed in Table 248–10. The list is subdivided into

TABLE 248–10 Daily Dosage Schedules for Antimicrobial Agents in Pediatric Patients Beyond the Newborn Period

Agent, Generic (Trade name)	Route	Mild to Moderate Infections*	Severe Infections*
Penicillin G, crystalline (numerous)	IV, IM	100,000-250,000 U ÷ into 4 doses	250,000-450,000 U ÷ into 6 doses
Penicillin G, procaine (numerous)	IM	50,000 U once daily (congenital syphilis); 300,000 (<60 lb) or 600,000-1,000,000 U (≥60 lb) ÷ into 1 or 2 doses for Group A streptococcal infections	Inappropriate
Penicillin G, benzathine (Bicillin)	IM	300,000-600,000 U (<60 lb)† or 900,000 U (≥60 lb)† for Group A streptococcal infections; 50,000 U/kg (up to 2,400,000 U)† for syphilis	Inappropriate
Penicillin V, phenoxymethyl penicillin (numerous)	PO	25-50 mg ÷ into 3-4 doses	Inappropriate
Penicillinase-resistant penicillins			
Cloxacillin (Cloxapen)	PO	50-100 mg ÷ into 4 doses	Inappropriate
Dicloxacillin (Dynapen, Dycill, Pathocil)	PO	25-50 mg ÷ into 4 doses	Inappropriate
Methicillin (Staphcillin)	IV, IM	100-200 mg ÷ into 4 doses	200-300 mg ÷ into 4-6 doses
Nafcillin (Nafcil, Unipen, Nallpen)	IV, IM	50-100 mg ÷ into 4 doses	100-200 mg ÷ into 4-6 doses
Oxacillin (Prostaphlin, Bactocill)	IV, IM	100-150 mg ÷ into 4 doses	150-200 mg ÷ into 4-6 doses
Aminopenicillins			
Amoxicillin (numerous)	PO	25-90 mg ÷ into 2-3 doses	Inappropriate
Amoxicillin + clavulanate (Augmentin)	PO	45-90 mg ÷ into 2-3 doses	Inappropriate
Ampicillin (numerous)	IV, IM	100-200 mg ÷ into 4 doses	200-400 mg ÷ into 4-6 doses
	PO	50-100 mg ÷ into 4 doses	Inappropriate
Ampicillin + sulbactam (Unasny)	IV	100-200 mg of ampicillin ÷ into 4 doses	200-400 mg of ampicillin ÷ into 4-6 doses
Extended-spectrum penicillins			
Mezlocillin (Mezlin)	IV, IM	Inappropriate	200-300 mg ÷ into 4-6 doses
Piperacillin (Pipracil)	IV, IM	Inappropriate	200-300 mg ÷ into 4-6 doses
Piperacillin + tazobactam (Zosyn)	IV	Inappropriate	240-300 mg of piperacillin ÷ into 3-4 doses
Ticarcillin (Ticar)	IV, IM	100-200 mg ÷ into 4 doses	200-300 mg ÷ into 4-6 doses
Ticarcillin + clavulanate (Timentin)	IV	Inappropriate	200-300 mg ÷ into 4-6 doses
Monobactams			
Aztreonam (Azactam)	IV, IM	90 mg ÷ into 3 doses	120 mg ÷ into 4 doses
Cephalosporins			
Cefadroxil (Duricef)	PO	30 mg ÷ into 2 doses	Inappropriate
Cefazolin (Ancef, Kefzol)	IV, IM	50 mg ÷ into 3 doses	50-100 mg ÷ into 3-4 doses
Cephalexin (Keflex, Keftab)	PO	25-100 mg ÷ into 4 doses	Inappropriate
Cefaclor (Ceclor)	PO	20-40 mg ÷ into 2-3 doses	Inappropriate
Cefoxitin (Mefoxin)	IV	Inappropriate	80-160 mg ÷ into 4-6 doses
Cefprozil (Cefzil)	PO	15-30 mg ÷ into 2 doses	Inappropriate
Ceftibuten (Cedax)	PO	9 mg once daily	Inappropriate
Cefuroxime (Kefurox, Zinacef)	IV, IM	Inappropriate	100-150 mg ÷ into 3-4 doses
Cefuroxime axetil (Ceftin)	PO	20-30 mg ÷ into 2 doses	Inappropriate
Loracarbef (Lorabid)	PO	15-30 mg ÷ into 2 doses	Inappropriate
Cefdinir (Omnicef)	PO	14 mg ÷ into 1-2 doses	Inappropriate
Cefditoren (Spectracef)	PO	200-400 mg twice daily†§	Inappropriate
Cefixime (Suprax)	PO	8 mg ÷ into 1-2 doses	Inappropriate
Cefotaxime (Claforan)	IV, IM	Inappropriate	100-180 mg ÷ into 4-6 doses
Cefpodoxime proxetil (Vantin)	PO	10 mg ÷ into 2 doses	Inappropriate
Ceftazidime (Fortaz, Tazicef, Tazidime, Ceptaz)	IV, IM	Inappropriate	100-150 mg ÷ into 3 doses
Ceftriaxone (Rocephin)	IV, IM	Inappropriate	50-100 mg ÷ into 1-2 doses
Cefepime (Maxipime)	IV, IM	Inappropriate	100-150 mg ÷ into 2-3 doses
Carbapenems			
Ertapenem (Invanz)	IV, IM	Inappropriate	30 mg ÷ into 2 doses
Imipenem-cilastatin (Primaxin)	IV, IM	Inappropriate	60-100 mg ÷ into 4 doses
Meropenem (Merrem)	IV	Inappropriate	60-120 mg ÷ into 3 doses
Macrolides			
Azithromycin (Zithromax)	PO	10 mg on day 1, then 5 mg thereafter; 12 mg for pharyngitis	Inappropriate
Clarithromycin (Biaxin)	PO	15 mg ÷ into 2 doses	Inappropriate
Erythromycin base (numerous)	PO	30-50 mg ÷ into 4 doses	Inappropriate
Erythromycin estolate (llosone)	PO	30-50 mg ÷ into 4 doses	Inappropriate
Erythromycin ethylsuccinate (E.E.S., EryPed, Erythro)	PO	30-50 mg ÷ into 4 doses	Inappropriate

TABLE 248–10 Daily Dosage Schedules for Antimicrobial Agents in Pediatric Patients Beyond the Newborn Period—cont'd

Agent, Generic (Trade name)	Route	Mild to Moderate Infections*	Severe Infections*
Erythromycin gluceptate (llotycin)	IV	Inappropriate	20-50 mg ÷ into 4 doses
Erythromycin lactobionate (Erythrocin)	IV	Inappropriate	20-50 mg ÷ into 4 doses
Erythromycin Stearate (Erythrocin, Erythrocot, My-E)	PO	30-50 mg ÷ into 4 doses	Inappropriate
Lincosamides			
Clindamycin (Cleocin)	IV, IM	Inappropriate	20-40 mg ÷ into 3-4 doses
	PO	8-20 mg ÷ into 3-4 doses	Inappropriate
Vancomycin (Vancocin)	IV	Inappropriate	40-60 mg ÷ into 4 doses
Aminoglycosides			
Amikacin (Amikin)	IV, IM	Inappropriate	15-22.5 mg ÷ into 3 doses
Gentamicin (Garamycin)	IV, IM	Inappropriate	5-7.5 mg ÷ into 3 doses‖
Kanamycin (Kantrex)	IV, IM	Inappropriate	15-30 mg ÷ into 3 doses
Netilmicin (Netromycin)	IV, IM	Inappropriate	5-7.5 mg ÷ into 3 doses
Paramomycin (numerous)	PO	25-35 mg ÷ into 3 doses	Inappropriate
Streptomycin (numerous)	IM	Inappropriate	20-40 mg ÷ into 1-2 doses
Tobramycin (Nebcin)	IV, IM	Inappropriate	6-7.5 mg ÷ into 3-4 doses
Tetracyclines			
Doxycycline (numerous)	PO	2.2-4.4 mg ÷ into 1-2 doses	Inappropriate
Tetracycline (numerous)	PO	25-50 mg ÷ into 4 doses	Inappropriate
Chloramphenicol (Chloromycetin)	IV	Inappropriate	50-100 mg ÷ into 4 doses
	PO	Inappropriate	50-100 mg ÷ into 4 doses
Sulfonamides			
Erythromycin ethylsuccinate-sulfisoxazole (Pediazole, Eryzole)	PO	50 mg erythro/150 mg sulfa ÷ into 4 doses	Inappropriate
Sulfadiazine (numerous)	PO	100-150 mg ÷ into 4 doses	Inappropriate
Sulfisoxazole (Gantrisin)	PO	150 mg ÷ into 4 doses	Inappropriate
Trimethoprim-sulfamethoxazole (Bactrim, Septra, Sulfatrim, Cotrim)	PO	8-12 mg trimeth/40-60 mg sulfa ÷ into 2 doses	Inappropriate
	IV	Inappropriate	10-20 mg trimeth/50-100 mg sulfa ÷ into 4 doses
Fluoroquinolones			
Ciprofloxacin (Cipro)	PO	20-30 mg ÷ into 2 doses	Inappropriate
	IV	Inappropriate	20-30 mg ÷ into 2 doses
Rifampin (Rifadin, Rimactane)	PO	10-20 mg ÷ into 1-2 doses	20 mg ÷ into 2 doses
	IV	Inappropriate	20 mg ÷ into 2 doses
Metronidazole (Flagyl)	PO	15-50 mg ÷ into 3-4 doses	Inappropriate
	IV	Inappropriate	30 mg ÷ into 4 doses
Linezolid (Zyvox)	PO	Inappropriate	20-30 mg ÷ into 2 or 3 doses
	IV	Inappropriate	20-30 mg ÷ into 2 or 3 doses
Quinupristin-Dalfopristine (Synercid)	IV	Inappropriate	22.5 mg ÷ 3 doses
Colistimethate sodium (Colistin, Coly-Mycin M)	IV, IM	Inappropriate	2.5-5 mg ÷ into 2-4 doses

**Total daily dosage (per kg). For larger children, maximal dosages may apply.*
†*Total dose.*
‡*No longer available in the United States.*
§*Not approved for children younger than 12 years of age.*
‖*A dose of 4 mg/kg once daily is used commonly in neonates. A dose of 6-7.5 mg/kg once daily in older children is investigational.*
IM, intramuscularly; IV, intravenously; PO, orally; SC, subcutaneously.

dosage schedules for mild to moderate and for severe disease. Oral regimens are used for mild to moderate infections caused by susceptible organisms in areas that are well vascularized and in which adequate concentrations of drug are achieved at the site of infection. Parenteral administration should be considered for severe infections, especially those caused by less susceptible organisms that produce disease in areas in which diffusion of drug is limited.

DOSAGE SCHEDULES FOR NEWBORN INFANTS

The clinical pharmacology of antimicrobial agents administered to newborn infants is unique and cannot be extrapolated from data derived from older children or adults. The physiologic and metabolic processes that affect the distribution, metabolism, and excretion of drugs undergo rapid changes during the child's first few weeks of life. The increased efficiency of kidney function after the infant's first 7 days requires an increase in dosage and a decrease in the interval between doses of penicillins and aminoglycosides for maintaining therapeutic concentrations of drug in blood and tissues. Thus, different dosage schedules are provided for the first week of life and for the subsequent weeks of the neonatal period (Tables 248–11 and 248–12). With survival of very-low-birth-weight, premature infants, more data are needed on the use of antimicrobial agents in these infants with immature metabolic and physiologic mechanisms.[225] Dosages of antibiotics in these preterm infants, especially those weighing less than

TABLE 248–11 Dosage Schedules for Antimicrobial Agents Used in Neonates

		Dosage (mg/kg) and Interval of Administration				
		Weight < 1200 g	Weight 1200-2000 g		Weight > 2000 g	
Antibiotic	**Route**	**Age 0-4 wk**	**Age 0-7 Days**	**Age > 7 Days**	**Age 0-7 Days**	**Age > Days**
Penicillin G, crystalline (U)	IV	25,000-50,000 q12h	25,000-50,000 q12h	25,000-50,000 q8h	25,000-50,000 q8h	25,000-50,000 q6h
Penicillin G, procaine (U)	IM		50,000 q24h	50,000 q24h	50,000 q24h	50,000 q24h
Penicillin G, benzathine (U)	IM		50,000 once	50,000 once	50,000 once	50,000 once
Penicillinase-resistant penicillins						
Oxacillin	IV, IM	25 q12h	25-50 q12h	25-50 q8h	25-50 q8h	25-50 q6h
Nafcillin	IV, IM	25 q12h	25-50 q12h	25-50 q8h	25-50 q8h	25-50 q6h
Broad-spectrum penicillins						
Ampicillin	IV, IM					
Meningitis		50 q12h	50 q12h	50 q8h	50 q8h	50 q6h
Other infections		25 q12h	25 q12h	25 q8h	25 q8h	25 q6h
Ticarcillin	IV, IM	75 q12h	75 q12h	75 q8h	75 q8h	75 q6h
Meropenem	IV	20 q12	20 q12	20 q12	20 q12	20 q12 or q8
Cephalosporins						
Cefazolin	IV, IM	20 q12h	20 q12h	20 q12h	20 q12h	20 q8h
Cefotaxime	IV, IM	50 q12h	50 q12h	50 q8h	50 q12h	50 q6h or q8h
Ceftriaxone	IV, IM	50 q24h	50 q24h	50 q24h	50 q24h	75 q24h
Ceftazidime	IV, IM	30 q12h	30 q12h	30 q12h	30 q12h	30 q12h
Cefepime	IV, IM	30 q12	30 q12	30 q12	30 q12	30 q12 to 50 q8
Clindamycin	IV, IM	5 q12h	5 q12h	5 q8h	5 q8h	5 q6h
Erythromycin	PO	10 q12h	10 q12h	10 q8h	10 q12h	10 q6h or q8h

IM, intramuscularly; IV, intravenously; PO, orally.

Modified from Sáez-Llorens, X., and McCracken, G.H., Jr.: Clinical pharmacology of antibacterial agents. In *Remington, J.S., and Klein, J.O. (eds.): Infectious Diseases of the Fetus and Newborn Infant. 4th ed. Philadelphia, W.B. Saunders, 1995, p. 1325.*

TABLE 248–12 Dosage Schedule for Antibiotics Based on Postconceptual Age*

		Dosage (mg/kg) and Interval of Administration: Gestational Age plus Weeks of Life			
Antibiotic	**Route**	**≤26**	**27-34**	**35-41**	**≥42**
Amikacin	IV, IM	7.5 q24h	7.5 q18h	7.5 q12h	7.5 q8h
Gentamicin	IV, IM	2.5 q24h	2.5 q18h	2.5 q12h†	2.5 q8h†
Tobramycin	IV, IM	2.5 q24h	2.5 q18h	2.5 q12h	2.5 q8h
Vancomycin	IV	10-15 q24h	10-15 q18h‡	10-15 q12h‡	10-15 q8h‡ or q6h

**Dosages should be adjusted according to serum drug concentrations.*

†Single daily dosing is used commonly in neonates: 3-3.5 mg/kg (<35 wk) or 4 mg/kg (≥35 wk).

‡At 28 days of life, vancomycin is administered at 20 mg/kg per dose; the interval remains the same.

IM, intramuscularly; IV, intravenously.

1000 g, are uncertain for many agents, and measurement of serum concentrations should be considered for drugs such as vancomycin and the aminoglycosides.

SHOULD DOSAGES BE DETERMINED BY WEIGHT OR BY SURFACE AREA?

In most standard pediatric texts and in the package inserts prepared by manufacturers, dosages of antibiotics for children are based on body weight. Body surface area correlates more closely with extracellular fluid volume. Some investigators suggest that more predictable serum concentrations can be achieved by using calculations of dosages based on surface area than by using those based on weight.[120] This method may be more reliable for drugs that are distributed in extracellular fluid, such as aminoglycosides, especially when they are prescribed for obese or malnourished children. Currently, however, the convenience of calculating dosage on the basis of weight appears to be the more important consideration.

USE OF ORAL PREPARATIONS FOR SERIOUS INFECTIONS

Oral preparations of antimicrobial agents vary in their degree of absorption from individual to individual and within an individual, depending on the illness being treated and the formulation used. Because higher and more consistent serum concentrations of drug are achieved after parenteral administration, parenteral routes are preferable for serious infections. Sequential parenteral-oral antimicrobial therapy may be an option in patients with uncomplicated pneumonia, pyelonephritis, and intra-abdominal, skin and soft tissue, and suppurative skeletal infections.[185] Results of studies of orally administered antibiotics in children with skeletal infections indicate that this mode of administration can be used successfully for a portion of the therapeutic course.[205,264]

Specific guidelines for oral treatment of serious infections are recommended: (1) the patient should be able to swallow and retain the medication; (2) the dosage should be sufficiently large to provide adequate bactericidal concentrations of drug at the site of infection for at least 50 percent of the dosing interval for β-

lactams; and (3) when possible, it is advisable to have the hospital laboratory determine serum antimicrobial concentrations. When this is not possible, careful follow-up is mandatory, and measurements of inflammatory indices, such as the erythrocyte sedimentation rate and C-reactive protein, are very useful in determining resolution of the infection.

Oral therapy can be considered for patients with osteomyelitis and suppurative arthritis only after an initial period of parenteral therapy (≥5 to 7 days), after results are available from cultures and susceptibility tests, and after the patient shows definite signs of resolution of inflammation. Oral therapy should be initiated before discharge from the hospital to ascertain compliance, determine serum antimicrobial concentrations when available, and observe for significant side effects that would preclude use of the oral antibiotic.

FOOD INTERFERES WITH THE ABSORPTION OF SOME ORAL ANTIBIOTICS

The absorption of some oral antimicrobial agents is decreased significantly when the drug is taken with food or near mealtime. These drugs include unbuffered penicillin G, penicillinase-resistant penicillins (nafcillin, oxacillin, cloxacillin, and dicloxacillin), ampicillin, and lincomycin. Dairy products and other foods or medications containing calcium or magnesium salts interfere with the absorption of tetracyclines. Absorption of penicillin V, buffered penicillin G, amoxicillin, cephalexin, cefaclor, chloramphenicol, erythromycin, and clindamycin is affected only slightly by food. When absorption is affected by the concurrent ingestion of food, antibiotics should be taken 1 or more hours before or 2 or more hours after meals. A four-times-daily dosage schedule, rarely used for common infections, can be arranged for the drug to be given on arising, 1 hour before lunch and supper, and at bedtime. Most orally administered antibiotics can be administered twice or three times daily, a schedule that is accommodated easily by most parents.

INTRAVENOUS VERSUS INTRAMUSCULAR ADMINISTRATION

Although a brief period occurs when the serum antimicrobial concentration is higher after intravenous administration of an antimicrobial agent than after intramuscular administration, no therapeutic advantage of intravenous as opposed to intramuscular administration has been demonstrated. Intravenous administration should be used if the patient is in shock or is suffering from a bleeding diathesis. When prolonged parenteral therapy is anticipated, the pain of injection and the small muscle mass of infants and young children preclude the intramuscular route and render intravenous therapy preferable. The physician must be alert for thrombophlebitis, which can result from prolonged intravenous administration, and for sterile abscesses, which can develop after intramuscular administration.

Chloramphenicol, erythromycin, linezolid, tetracyclines, and vancomycin should be administered intravenously rather than intramuscularly. Chloramphenicol was thought to be absorbed poorly from intramuscular sites, although more recent data suggested that such is not the case. Intramuscular injection of parenteral tetracyclines and erythromycin causes local irritation and pain, and intramuscular injection of vancomycin causes tissue necrosis. Care should be given to the administration of intramuscular injections.[25,175] Sites that minimize the risk of local neural, vascular, or tissue injury should be selected. The preferred site varies with the age of the child: the upper anterolateral aspect of the thigh in infants, the ventrogluteal area in children older than 2 years of age, and the deltoid area for older children. Inadvertent intra-arterial injection of benzathine penicillin G can cause tissue damage.

"PUSH" VERSUS "STEADY" OR "CONTINUOUS DRIP" INTRAVENOUS ADMINISTRATION

Antimicrobial agents can be administered intravenously by the "push" method, in which case the drug is infused in 5 to 15 minutes; by "steady drip" in 1 to 2 hours; or by "continuous drip," whereby the drug is given throughout the period of administration. The push method results in high antibacterial activity in serum for short periods, whereas the steady and continuous drip methods produce lower but more sustained activity. The risk of development of adverse effects influences whether an antimicrobial agent should be administered by push or by steady drip. Pharmacodynamic studies suggest optimization of bactericidal activity when aminoglycosides are given by push once daily because these agents' activity is concentration-dependent. By contrast, β-lactams are given preferably in several doses or by a continuous drip to maintain concentrations of drug at the infection site that exceed the MIC of the pathogen for 50 percent or longer of the dosing interval (time-dependent pharmacodynamic principle). Rapid administration (<5 minutes) of large intravenous doses of penicillin should be avoided because of possible adverse CNS effects. Aminoglycosides given by the intravenous route should be infused in 20 to 60 minutes to obtain optimal peak concentrations. Antimicrobial activity, especially for penicillins, can deteriorate if drugs are kept in solution at room temperature for prolonged periods, as may occur with use of the continuous drip method. Fresh solutions of penicillins should be administered every 6 to 8 hours when the continuous drip method is used.

DIFFUSION OF ANTIMICROBIAL AGENTS ACROSS BIOLOGIC MEMBRANES

Diffusion of any drug across a biologic membrane depends on the molecular size of the drug, the degree of protein binding (only the unbound portion of the drug crosses), the degree of ionization at physiologic pH (only the un-ionized portion is available for equilibration), and solubility in lipids. Thus, the lipid solubility of the un-ionized and unbound fraction of an antimicrobial agent determines the capability of the drug to diffuse to the site of infection. Antibiotics usually are not distributed evenly throughout the body.[209,210]

Diffusion of antimicrobial agents from blood into a joint space, pleural and pericardial fluid, and middle ear fluid is relatively unimpeded, and high concentrations of many drugs are achieved in these sites after systemic administration. More than 60 percent of the peak serum concentration of various penicillins and cephalosporins is present in the inflamed joint space.[205] Loculations of fluid in the presence of fibrous adhesions may limit the passage of antimicrobial agents into infected areas.

Diffusion of antibiotics from blood into CSF or into the aqueous humor of the eye is limited. Drugs that are highly soluble in lipids, un-ionized, and minimally bound to proteins (e.g., chloramphenicol, isoniazid, rifampin, sulfonamides) pass into CSF in high concentrations, even in the absence of inflammation, whereas drugs such as the macrolides diffuse into CSF little, if at all. Fluoroquinolones pass readily into the CSF space but do not have an indication for use for meningitis in children. Penicillins, cephalosporins, and aminoglycosides pass more effectively into CSF when the membrane is inflamed; variable, but often low, concentrations of drug in CSF can be present even in the early stages of meningitis. The β-lactams are pumped actively out of

the CSF space by the choroid plexus, a process that is inhibited partially by inflammation.

DURATION OF THERAPY

Physicians must rely on empirically derived schedules of therapy for rapid and complete resolution of disease and minimal risk in terms of clinical or microbiologic failure or drug toxicity. Numerous studies evaluating the duration of therapy have been performed for streptococcal pharyngitis. The results are consistent in suggesting that the following are appropriate: 10 days of oral therapy with penicillins, cephalosporins, or macrolides; 5 days of azithromycin; or a single intramuscular dose of benzathine penicillin G. Opinions vary and data are conflicting regarding the duration of treatment for diseases such as osteomyelitis, suppurative arthritis, and infections of the urinary tract. Radetsky wrote an enlightening history of the recommendations for the duration of treatment in bacterial meningitis and pointed out: "Even in the absence of specific data certain numbers have an unaccountable power to satisfy and reassure .. 7, 10, 14 and 21 days have consistently appeared. Even in the trials performed at the dawn of the antimicrobial era, these numbers were chosen."[226]

DOSAGE SCHEDULES IN CHILDREN WITH RENAL OR HEPATIC INSUFFICIENCY

The kidneys are the major organs of excretion for most antimicrobial agents, including penicillins, cephalosporins, aminoglycosides, and tetracyclines (with the exception of doxycycline). Because impaired excretion can result in high and possibly toxic serum and tissue antimicrobial concentrations, alterations in dosage schedules should be considered in children with diminished renal function. Antibiotics that require careful dosage adjustment for renal impairment include aminoglycosides, ciprofloxacin, imipenem-cilastatin, meropenem, piperacillin, tetracyclines, ticarcillin, TMP-SMX, and vancomycin. Agents requiring dosage adjustments only when renal failure is severe include most penicillins, cephalosporins, and clindamycin. Drugs that are eliminated by nonrenal mechanisms and therefore do not require adjustment of the dosage schedule for renal impairment include chloramphenicol, cloxacillin, dicloxacillin, doxycycline, erythromycin (including the newer macrolides), metronidazole, linezolid, nafcillin, oxacillin, and rifampin.[277]

Dosage schedules for patients with renal insufficiency can be altered by administering the usual dosage for the initial dose and increasing the interval between doses or decreasing individual doses (or both, in the case of renal shutdown). Although numerous guidelines have been developed to assist the physician, these formulas have been generated from studies of adults with renal impairment, and pediatricians must be cautious in adapting the formulas for use in infants and young children.[277] Serum antimicrobial concentrations should be monitored when aminoglycosides, vancomycin, and other drugs of potential toxicity are administered to children with renal insufficiency. Serum specimens are obtained at the time of the anticipated peak and trough concentrations on the first day and repeated on subsequent days to ensure a safe and effective dosage schedule.

Hepatic disorders can alter plasma protein binding, tissue binding, hepatic metabolism, and the distribution of antimicrobials that are metabolized or excreted by the liver.[273] Few data exist regarding adjustment of dosage schedules for antibiotics that are metabolized by the liver in patients with hepatic insufficiency.[164] It would be prudent to avoid the use of tetracyclines and to exercise caution when prescribing chloramphenicol, clindamycin, metronidazole, macrolides, rifampin, and penicillinase-resistant penicillins to patients with underlying hepatic disease.

TOPICAL USE OF ANTIMICROBIAL AGENTS

Topical antimicrobial agents are used for a variety of indications: bacitracin or polymyxin ointments are available (in many cases without prescription) for first aid of minor cuts, abrasions, and burns; tetracycline, erythromycin, and clindamycin have been used for the treatment of pustular acne; and metronidazole is approved for the topical treatment of inflammatory lesions and erythema associated with rosacea. Erythromycin, chloramphenicol, sulfonamide, gentamicin, tobramycin, tetracycline, and a combination of TMP and polymyxin B ointment or drops are used for the treatment of conjunctivitis, styes, and other minor infections of the eye. Silver nitrate drops or either erythromycin or tetracycline ointment is used for the prevention of gonococcal ophthalmia in newborn infants. A controlled trial involving newborn infants in Africa demonstrated equivalent or superior efficacy of a 2.5 percent ophthalmic solution of povidone-iodine in comparison with topical silver nitrate or erythromycin for prophylaxis against ophthalmia neonatorum caused by *C. trachomatis*, *N. gonorrhoeae*, staphylococci, or gram-negative bacteria.[132] Ofloxacin drops (Floxin Otic) are approved for the treatment of otitis externa, chronic suppurative otitis media, and acute otitis media in children with tympanostomy tubes.[31] Ciprofloxacin and dexamethasone otic solution (Ciprodex) is approved for children 6 months of age and older who have tympanostomy tubes, to treat acute otitis media caused by *S. aureus*, *S. pneumoniae*, *H. influenzae*, *M. catarrhalis*, and *P. aeruginosa*, as well as acute otitis externa caused by *S. aureus* and *P. aeruginosa*. Mupirocin is effective in vitro against most *S. aureus* (including methicillin-resistant strains) and group A streptococci and is approved for the treatment of impetigo. At present, approximately 10 percent of CA-MRSA strains are resistant to mupirocin; in some areas, the rate is as high as 30 percent. Mupirocin applied to the anterior nares may be of value in eradicating nasal carriage of methicillin-resistant staphylococci. However, the few well-designed controlled studies that exist do not support the routine use of mupirocin, except possibly for patients undergoing dialysis, because prolonged use of mupirocin has been associated with resistance to this agent, and recolonization is a common occurrence.[60] Most antibiotics used topically, such as bacitracin, neomycin, and polymyxin B, are of limited use as systemic agents. Retapamulin ointment (Altabax), a pleuromutilin antibacterial, was approved by the FDA in 2007 for topical treatment of impetigo due to *S. aureus* (methicillin-susceptible isolates only) or *S. pyogenes* in patients aged 9 months or older. The dosage is twice daily applications for 5 days.

Absorption after application to the conjunctivae or large areas of denuded skin can be significant, but application to normal skin does not result in detectable concentrations of antimicrobial activity in blood or urine. Sensitization does not appear to be an important problem with most topical antibiotics, although some patients with chronic dermatoses may react to certain agents such as neomycin. Antimicrobial agents of value for systemic use should not be applied extensively to the surface of the body or used routinely in closed units (e.g., burn units) because of the risk of inducing resistance.

The Committee on Drugs of the American Academy of Pediatrics concluded that topical antimicrobial agents may prevent infection after minor cuts, abrasions, and burns, but in most instances, gentle cleansing of minor wounds and burns is sufficient antisepsis.[6] Systemic antibiotics, rather than topical drugs, are recommended for chronic pyoderma, including impetigo, especially when more than several lesions are present.

Intermittent administration of inhaled tobramycin (twice daily for 4 weeks followed by 4 drug-free weeks) is indicated for the management of children and adults with cystic fibrosis and *P. aeruginosa* infection. Clinical data from a large, randomized, placebo-controlled trial indicated that tobramycin treatment

improves pulmonary function, decreases the density of *P. aeruginosa* in sputum, reduces the risk of hospitalization, and is well tolerated. The diminished microbial reduction during the third cycle of treatment was not explained by the development of resistance to tobramycin.[229]

The use of antimicrobial-coated central venous catheters is not accepted practice in pediatrics, but limited evidence supports its potential usefulness, particularly if rates of catheter-related bloodstream infections exceed 3.3 per 1000 catheter days and if standard protective procedures already have been implemented.[214] Rates of bacterial colonization and bloodstream infections associated with the use of central venous catheters in high-risk adult patients were found to be lower in patients whose catheters were impregnated with minocycline and rifampin than in those impregnated with chlorhexidine and silver sulfadiazine.[75]

CURRENT USE OF ANTIMICROBIAL AGENTS FOR PROPHYLAXIS

Chemoprophylaxis refers to the use of drugs to prevent infection. *Antimicrobial treatment* refers to the use of drugs after infection has taken place or when early signs of infectious disease are present or infection is suspected. The use of antimicrobial agents for prophylaxis has proved to be of value in many circumstances (Table 248–13) and currently is considered to be of probable value or is investigational for the prevention of infections in many other situations. Prophylaxis is of greatest value when the following criteria are met: use of a single drug with a narrow spectrum of activity, use of a drug with limited side effects or toxicity, and prevention of colonization by an organism of known susceptibility and one that is unlikely to become resistant during the period of drug use.

USE OF ANTIMICROBIAL AGENTS FOR CHILDREN IN SCHOOL OR GROUP DAYCARE

Infants and children usually return to their school or daycare during a course of antimicrobial therapy. Because of problems with the administration of drugs outside the home, physicians should prescribe medications that are given infrequently, are relatively stable at ambient temperatures, and need only simple directions. Drugs that are administered in once- or twice-daily schedules are preferred. Chewable tablets, when available, may be of value in reducing the need for the school or daycare provider to measure specific amounts of liquid suspension and to refrigerate suspensions. Single-dose regimens, such as intramuscular benzathine penicillin G for group A streptococcal infections, may be advantageous. Guidelines for administration of medications in school have been published by the Committee of School Health of the American Academy of Pediatrics and should be useful to the physician for prescribing drugs to children who attend school.[8]

RESTRICTION ON USE OF ANTIMICROBIAL AGENTS FOR INFANTS AND CHILDREN

Many antimicrobial agents are approved for use in adults but have not been approved by the FDA for use in infants and children. The reasons for lack of approval include the following: drugs with insufficient experience in children, such as tigecycline (Tygacil); agents with real or suspected toxicity in children (e.g., damage to articular cartilage in juvenile animals associated with administration of the fluoroquinolones) that should be used only when necessary; and antibiotics for which the manufacturers have chosen not to submit data on use in children to the FDA, such as metronidazole (Flagyl), piperacillin (Pipracil), and cefotetan (Cefotan). These last three agents appear to be safe and effective in infants and children as a result of use by pediatricians, but no well-controlled studies of their efficacy or safety in this age group have been performed. Although a drug that has been approved for adults may be used in children at the discretion of the physician, the prudent physician chooses to use such a drug only when it is uniquely appropriate for the infectious illness and records the basis for choice of the unapproved drug.

Four antibiotics were approved for use in adults and have potential usefulness in the management of some pediatric patients with infections caused by multidrug-resistant pathogens. Daptomycin (Cubicin) is a cyclic lipopeptide that was approved by the FDA in 2003 for intravenous treatment of adults with compli-

TABLE 248–13 Antimicrobial Prophylaxis in Children

Prevention of Infection in Certain Patients	Antimicrobial Agent
Group A streptococcal infection in patients with a history of rheumatic fever	Benzathine penicillin G IM, penicillin V PO
Bacterial endocarditis in patients at risk during surgical procedures:	
Dental procedures, surgery on the upper respiratory tract	Amoxicillin PO; clindamycin, cephalexin, cefadroxil, azithromycin, or clarithromycin in penicillin-allergic patients
Gastrointestinal or genitourinary tract surgery or instrumentation	Amoxicillin PO, ampicillin IV, ampicillin plus gentamicin, or vancomycin plus gentamicin (for penicillin-allergic patients)
Neonatal sepsis caused by group B *Streptococcus*	Penicillin (preferred) or ampicillin IM or IV (intrapartum)
Gonococcal ophthalmia in newborn infants	Silver nitrate or erythromycin ophthalmic ointment
Meningococcal disease in contacts	Rifampin, ceftriaxone
Haemophilus influenzae type b disease in contacts	Rifampin
Recurrent episodes of acute otitis media	Amoxicillin, sulfisoxazole
Postoperative infections	Penicillinase-resistant penicillins or first- or second-generation cephalosporins
Tuberculosis infections in close contacts	Isoniazid
Recurrent urinary tract infections	Trimethoprim-sulfamethoxazole, nitrofurantoin
Sepsis in patients with functional asplenia	Penicillin, amoxicillin, trimethoprim-sulfamethoxazole

IM, Intramuscularly; IV, intravenously; PO, orally.

cated skin and skin structure infections caused by gram-positive infections, including MRSA, in a single daily dose of 4 mg/kg. Daptomycin was approved in 2006 for adults with methicillin-susceptible or methicillin-resistant bacteremia or right-sided endocarditis at a dose of 6 mg/kg daily for a minimum of 2 to 6 weeks. It has been shown to be inferior to comparator agents for community-acquired pneumonia, likely resulting from inactivation of daptomycin in the presence of pulmonary surfactant.[241] Although experience with daptomycin is meager in pediatrics, the drug does appear to be effective in the setting of persistent bacteremia in children with disseminated MRSA infection.

Quinupristin-dalfopristin (Synercid), a semisynthetic streptogramin, has synergistic activity against the same organisms as does linezolid and has been used extensively in adults in Europe. The dosage for children is 7.5 mg/kg, given intravenously three times daily. The drug has been well tolerated by children; diarrhea is the most common adverse effect. Telithromycin (Ketek) was approved in 2004 for use in adults. It is a semisynthetic ketolide antibacterial that differs chemically from the macrolide group by lack of α-L-cladinose at position 3 of the erythronolide A ring. Telithromycin is indicated for enteral treatment of adults with mild-to-moderate community-acquired pneumonia caused by common respiratory tract pathogens, including multidrug-resistant *S. pneumoniae* and atypical bacteria. In 2007, the FDA removed two previously approved indications, namely, acute exacerbations of chronic bronchitis and acute bacterial sinusitis because telithromycin has been associated with severe liver toxicity in adults. Its use is contraindicated in patients with myasthenia gravis. Safety and efficacy have not been established in children.

Tigecycline (Tygacil) is a glycylcycline related to minocycline that was approved by the FDA in 2005 for intravenous use in the treatment of adults with complicated intra-abdominal infections and complicated skin and skin structure infections caused by aerobic and anaerobic gram-positive and gram-negative bacteria, including MRSA.

HOME INTRAVENOUS ANTIBIOTIC THERAPY

Home intravenous antibiotic therapy is now available in most communities and permits discharge from the hospital earlier than in the past. The safety, effectiveness, and cost-efficiency of such a program have been proved, and these factors are of particular value for children who require 4 to 6 weeks of therapy for osteomyelitis or suppurative arthritis or who have chronic disease that can be managed in the home, such as cystic fibrosis or malignant disease. In many cases, home care enables the patient to resume normal activities, including return to school. The following are factors that are necessary before consideration of home care:

1. Availability of a team that includes the physician, the pharmacist, a vendor who will supply the drug and supplies, and an intravenous specialty nurse
2. A disease that is stable and requires only continued antimicrobial therapy
3. Unavailability of a suitable oral antibacterial agent (see the earlier section "Use of Oral Preparations for Serious Infections") and availability of a stable parenteral antibiotic with low toxicity that the patient can tolerate (as demonstrated in the hospital) and preferably with a long half-life to allow infrequent dosing
4. A member of the household who is able to administer the antibiotic and provide aseptic care of the venous access device
5. Appropriate follow-up that can be maintained for monitoring safety and effectiveness

If problems with venous access arise and ceftriaxone is appropriate therapy, the drug can be administered successfully at home by the intramuscular route once a day by a nurse.[104,240]

DRUG-DRUG INTERACTIONS

Drug-drug interactions can lead to therapeutic failure because of lack of effective activity of one or both drugs or serious adverse events resulting from toxic serum concentrations of one or both drugs.[105] Most children do not require daily medications for chronic diseases; thus, drug-drug interactions occur less commonly in pediatric than in geriatric patients, but the potential for interactions exists and must be considered when prescribing antibiotics. Because drug-drug interactions are not limited to prescription medications, inquiry into the use of over-the-counter medications should be made. Mechanisms for drug-drug interactions are classified as follows: physiochemical, whereby one drug is physically incompatible in solution with another; pharmacokinetic, whereby one drug interferes with the absorption, distribution, metabolism, or excretion of the other; and pharmacodynamic, whereby one drug affects the activity of a second drug. Examples of each mechanism include inactivation of aminoglycosides by extended-spectrum penicillins, decreased absorption of tetracyclines with co-administration of antacids, antagonism of sulfonamide activity by procaine as a result of competition for PABA-binding sites, and interference with the cytochrome P-450 oxidase enzyme system as observed with rifampin and macrolide drugs or with the azole antifungal agents and cyclosporine.

SUMMARY AND CONCLUSIONS

A summary of the information contained in this chapter is as questions that the physician must consider for appropriate use of antimicrobial agents in children.

1. Before the drug is administered:
   a. Have appropriate cultures been obtained for a specific microbiologic diagnosis?
   b. Has the patient received this drug or related compounds previously? If so, did the patient tolerate the drug? Were there any signs of toxicity or sensitization?
   c. Does the patient have a condition that requires exclusion of some drugs? For example, children with glucose-6-phosphate dehydrogenase deficiency may have induced hemolysis when a sulfonamide, nitrofurantoin, or primaquine is administered.
2. Factors to be considered when writing orders for the administration of antimicrobial agents in a hospital:
   a. If the drug is given by mouth, will co-administration with food interfere with absorption, or is diarrhea a risk?
   b. If a parenteral route is used, should the drug be administered by the intravenous or the intramuscular route?
   c. If the drug is administered by the intravenous route, is push, steady drip, or continuous drip preferred?
   d. Should the drug be instilled directly at the site of infection?
   e. Will the drug diffuse to the site of infection?
   f. Should incision and drainage of the infected area be performed before or after beginning therapy? Incision and drainage should be considered whenever a significant collection of pus is present. If a drainage procedure is performed, material should be obtained for culture and susceptibility testing.
   g. Does the patient have renal or hepatic insufficiency that requires alteration of the dosage schedule?
   h. Are any special precautions required for household contacts? Prophylaxis may be warranted in special circumstances of infection occurring in the household, daycare center, or nursery school.

3. Use of antimicrobial agents in children who are treated as outpatients:

a. Have the names and functions of the drugs been communicated to the patient and the parent? Do any of the drugs prescribed interact with each other?

b. Is the dosage schedule simple and satisfactory for the family circumstances (e.g., the child's school schedule, the schedule of the working parents)?

c. Does the child have an adequate supply of the drug until it can be purchased? If not, the use of starter packages is of value. Administration of the first dose in the clinic is advantageous because it provides knowledge of acceptance and tolerability of the drug by the child.

d. Are parents given instructions for reporting the clinical course by telephone? Is an appointment made for the next visit?

e. Does the patient or parent know how to assess adequacy of response to the drug? Does the parent know how to take the child's temperature?

f. Is the total amount of drug prescribed adequate for the course? Will refills of the prescription be needed?

g. Is the drug provided in a convenient dosage form? Will the package be provided with an adequate means of measuring the drug? Does the agent require refrigeration?

h. Has the patient or parent been informed of signs of side effects or toxicity?

i. Are generic equivalents of the drug adequate?

j. Will the patient be able to pay for the drug if it is purchased elsewhere (away from the clinic)? If applicable, will a third party pay for this prescription? (In some states, prescriptions by brand name may not be filled because reimbursement by the third-party payer, such as Medicaid, is insufficient.)

4. After the patient's course:

a. How long should the patient take the drug?

b. When should the initial choice of antimicrobial agents be reconsidered? When the results of cultures and appropriate susceptibility tests are available, should the initial choice be reevaluated and altered, if necessary?

c. What studies should be performed to monitor the safety and adequacy of the regimen? Hematologic indices must be measured during the administration of certain antibiotics to detect any adverse reaction. Vigilance is important to detect symptoms or signs attributable to adverse reactions.

d. Are repeat cultures necessary? In certain cases, the most appropriate criterion of efficacy is evaluation of the results of cultures.

e. What clinical and laboratory signs of efficacy should be monitored? Signs may differ for different diseases and various drugs but should be considered by the physician when the course of therapy is designed.

5. What factors should be considered if the patient fails to respond to the antimicrobial agent? If the patient does not respond appropriately to the course of therapy, various factors must be considered, including those related to the disease, host, drug, or organism (Table 248–14).

6. What steps can be taken to prevent re-infection? For example, in certain circumstances prophylactic antibiotics, immunizations, or surgical procedures may be warranted.

TABLE 248–14 Factors Contributing to Antimicrobial Failure

Host Related
Foreign body present
Anatomic defect
Defect in immune response to infection
Poor absorption of enteral antibiotic
Disease Related
Antibiotic inappropriate for the disease
Ancillary therapy not instituted (e.g., surgical drainage)
Sequestered focus of infection (undetected or inaccessible)
Organism Related
Acquired resistance to an antimicrobial agent
Superinfection with resistant bacteria
Drug Related
Inadequate adherence
Improper dosage schedule—route, dose, or duration
Inadequate diffusion to the site of infection
Drug-drug interactions—antibiotic inactivation or antagonism
Deterioration of drug during storage

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CHAPTER 249

Antimicrobial Prophylaxis

Gary D. Overturf

Antimicrobial prophylaxis is the provision of an antimicrobial agent or agents with the intent of preventing an infection. Prevention always is preferred, provided the means are available and the risk-benefit and cost-benefit ratios are acceptable. This chapter focuses on the prevention of morbidity and mortality from bacterial infections through the prophylactic and often empiric use of antimicrobial agents.

GENERAL PRINCIPLES OF PROPHYLAXIS

Several factors that influence the efficacy of prophylaxis are related to the potential pathogen, the prophylactic agent, the host, and the disease to be prevented (Table 249–1). Failure to consider all these factors will lead to ineffective prophylaxis, overuse of antimicrobial agents, promotion of resistant microorganisms, economic waste, and risk for development of toxicity or side effects.

TABLE 249–1 Factors Influencing Effective Prophylaxis

Single versus multiple potential pathogens
Time of exposure to the pathogen
Source of pathogens
Severity of the disease to be prevented
Targeted organs that could become infected
Spectrum of activity of the antimicrobial agent
Pharmacokinetics and the pharmacodynamics of the selected agent
Duration of chemoprophylaxis
Cost, toxicity, side effects, and acceptability of the agent
Likelihood and consequences of emerging resistance

THE BACTERIAL PATHOGEN

Prophylaxis is theoretically more effective when a single pathogen is targeted. In general, the greater the number of targeted pathogens, the less effective, the more toxic, and the more expensive the regimen becomes. Ideally, prophylaxis should be administered at the time of exposure to the potential pathogen. If exposure is prolonged or continuous, prophylaxis becomes less effective and less desirable. Bacteria that are not endogenous to the host (i.e., not part of the host's normal flora) generally are targeted more effectively if the exposure is known and identified.

THE DISEASE

The severity of the disease to be prevented is a major consideration. Potentially fatal infections (e.g., meningococcemia) or infections that result in high morbidity (e.g., endocarditis) are justifiably targeted. Prophylaxis usually is not required for minor illnesses (e.g., cuts, abrasions). The site of infection also is important. Adequate concentrations of antimicrobials are achieved

readily in organs that are highly vascular and have no barriers, whereas infections in restricted anatomic compartments (e.g., middle ear) or those that involve prosthetic materials may require special considerations.

THE ANTIMICROBIAL AGENT

The most desirable prophylactic agent is narrow spectrum, inexpensive, easily administered, and well tolerated and has minimal side effects. The less frequently an agent is given, the more reliable the adherence (compliance) of the patient.[64] When prophylaxis can be achieved effectively with a single administration of the antimicrobial agent, prophylaxis is likely to be ideal.

PROPHYLAXIS IN NEWBORN INFANTS

OPHTHALMIA NEONATORUM

The prophylaxis of ophthalmia neonatorum also is covered in the chapters on ocular infections and perinatal bacterial diseases (see Chapters 68 and 78). Prophylaxis targets *Neisseria gonorrhoeae* and *Chlamydia trachomatis.* Ideally, prophylaxis should be directed at infants who are exposed to these two pathogens; however, identification of this group with certainty is impossible. Routine prophylaxis has been discontinued in some countries (United Kingdom, Sweden)[28]; however, it is required in the United States.

Topical 1 percent silver nitrate solutions are available in single-dose ampules or single-dose tubes of an ophthalmic ointment containing 0.5 percent erythromycin or 1 percent tetracycline. All agents are effective and recommended for prophylaxis of gonococcal ophthalmia neonatorum.[58] Silver nitrate has been used for prophylaxis for more than 100 years. Because silver nitrate frequently causes chemical conjunctivitis, its use has been challenged, and alternative regimens have been sought. Erythromycin and tetracycline ophthalmic ointments appear to be as effective as silver nitrate solution for routine prophylaxis of gonococcal ophthalmia[76,120]; however, silver nitrate probably is the most effective agent against penicillinase-producing *N. gonorrhoeae.* The effectiveness of erythromycin or tetracycline in the prevention of ophthalmia caused by penicillinase-producing *N. gonorrhoea*e has not been established. No topical regimen has proven efficacy against *Chlamydia* conjunctivitis or prevention of invasive infections of the neonate, such as lower respiratory infection.[12,29,59] Furthermore, topical regimens do not eliminate *C. trachomatis* from the nasopharynx and do not prevent pneumonia.

Prophylaxis should be administered as soon as possible after birth. Each eyelid should be wiped gently with sterile cotton before local prophylaxis is administered. Care must be exercised to ensure that the solution or ointment is in the conjunctival sac and that it is not flushed from the eye after instillation.

GROUP B STREPTOCOCCAL INFECTIONS

Prophylaxis is aimed at prevention of early-onset neonatal group B streptococcal infections.[2,3,13,93,100,102] No recommendations for prophylaxis against late-onset infections exist. Prophylaxis of group B streptococcal infections also is covered in greater detail in Chapter 94.

In the past, several regimens have been used in attempts to reduce vertical transmission of group B streptococcus. Multiple studies involving the prepartum use of oral antimicrobial agents to eradicate group B streptococcus colonization of mothers (antepartum chemoprophylaxis) have not been successful, even when sexual partners were treated concurrently.[93] Prophylaxis of newborn infants with penicillin G or ampicillin soon after birth (postnatal chemoprophylaxis) is ineffective in preventing early-onset group B streptococcal disease, primarily because in most patients, infection occurs in utero and the infants are asymptomatic at or within a few hours after birth.

Current recommendations focus on treatment of all colonized women, who are tested universally at 35 to 37 weeks.[25] Previously, treatment of women in special-risk categories or the use of selective intrapartum maternal chemoprophylaxis was recommended.[1-3] The American Academy of Pediatrics as well as the current guidelines of the Centers for Disease Control and Prevention recommend that lower vaginal and anorectal (single swab) specimens for culture be obtained at 35 to 37 weeks' gestation, placed into selective broth medium, transported, and subcultured onto solid media.[3,25] Currently, both hybridization assays and DNA amplification techniques for detection of group B streptococci are available and serve as the only acceptable alternatives to culture.[10,63] Rapid amplification methods are available for testing women at the time of entry into labor and thus provide opportunities for treatment of women who may not have been tested at 35 to 37 weeks' gestation. Women who have no prenatal group B streptococcal culture results available and who begin labor with an identified risk factor (Table 249–2) thus may be tested for group B streptococcus by rapid tests or by culture. Maternal group B streptococcus carriers identified prepartum or those without culture or identification at the time of delivery with rapid testing but with one or more risk factors should be given intrapartum intravenous ampicillin (2 g initially, then 1 to 2 g every 4 to 6 hours) or penicillin G (5 million U every 6 hours) until delivery.[25] Penicillin-allergic women may be given clindamycin or erythromycin intravenously. Previous delivery of an infant with invasive group B streptococcal disease warrants intrapartum maternal chemoprophylaxis for each subsequent pregnancy, regardless of maternal colonization.

Intrapartum antibiotic prophylaxis prevents substantial numbers of but not all cases of early-onset neonatal group B streptococcal infection and will decrease the incidence of maternal group B streptococcal postpartum endometritis.[79,88] Since the institution of intrapartum antimicrobial prophylaxis, the rate of invasive group B streptococcal disease has declined approximately 65 percent. Administration of intravenous ampicillin reduces the risk for development of early-onset group B streptococcal infection by 36 percent in infants born to women with premature rupture of membranes.[9] Treatment of women with chorioamnionitis with ampicillin and gentamicin during labor reduces the

TABLE 249–2 Risk Factors for Early-Onset Group B Streptococcal Infection

Maternal Risk Factors
Premature onset of labor of <37 weeks' gestation
Premature rupture of membranes at <37 weeks' gestation
Rupture of membranes (>18 hr) at any gestation
Maternal fever during labor
Multiple births
High GBS genital inoculum
GBS bacteriuria
Low type-specific GBS capsular polysaccharide antibody
Maternal age <20 yr
Black race
Diabetes mellitus
Infant Risk Factors
Low birth weight
Prematurity

GBS, group B streptococcus.

likelihood for development of group B streptococcal infection by 86 percent. In some reports, the decrease in the rate of group B streptococcal disease was associated with an increased rate of disease caused by gram-negative pathogens. Although many obstetric care providers take some measures to prevent the development of group B streptococcal disease, reported practices often are inconsistent with the existing recommendations.[65] In the past, the most frequent deviation from accepted recommendations is timing of the antepartum screening cultures.[79]

Management of infants whose mothers received intrapartum chemoprophylaxis remains empiric and should be based on clinical manifestations and gestational age. If indicated, appropriate cultures should be performed and antimicrobial therapy initiated, pending culture results.[2]

NECROTIZING ENTEROCOLITIS

Neonatal necrotizing enterocolitis (NEC) is a multifactorial disease, with bowel wall necrosis of variable length and depth being the characteristic feature. Factors including ischemic stress, disruption of the bowel mucosal barrier, and resultant bacterial proliferation and invasion of the intestinal wall are part of the pathogenesis of NEC. Therefore, suppression of gastrointestinal flora with nonabsorbable oral antimicrobials has been used in an effort to prevent the development of NEC in premature infants. Administration of oral kanamycin or gentamicin prophylactically in the first few hours of life generated contradictory data. Furthermore, selective overgrowth of resistant organisms in the bowel and significant systemic absorption of aminoglycosides from the injured mucosa are potential risk factors. Currently, oral aminoglycosides are not recommended for the prophylaxis of NEC.

One report[92] suggests that oral vancomycin given for 48 hours before the introduction of oral feeding may be beneficial in preventing NEC. These observations have not been confirmed, and such prophylaxis is not practiced routinely or recommended.

Oral probiotics have been suggested to alter the bowel flora and to reduce the incidence or severity of NEC. Infants fed breast milk and products including *Lactobacillus acidophilus* and *Bifidobacterium infantis* had a reduced incidence and severity of NEC compared with infants fed breast milk alone.[81]

INTRAVASCULAR CATHETER INSERTION

Infection with coagulase-negative staphylococci, primarily *Staphylococcus epidermidis*, is likely to occur in premature infants or infants who have indwelling vascular catheters. Low-grade sepsis is the most common clinical manifestation; however, meningitis, endocarditis, omphalitis, cellulitis, and other focal infections may occur. Two randomized trials of low-dose vancomycin added to total parenteral nutrition fluids (25 μg of vancomycin per milliliter of fluid) suggested that such a prophylactic regimen significantly reduces coagulase-negative staphylococcal infections in small premature infants in neonatal intensive care units.[68,111] Widespread use of this regimen will not be recommended until more data are collected and the issue of emergence of vancomycin-resistant organisms is addressed adequately.[7] Currently, the American Academy of Pediatrics discourages the use of routine prophylaxis for infants of very low birth weight or the prevention of infection or colonization of indwelling central or peripheral intravascular catheters with either systemic or antibiotic lock administration techniques.[2]

Continuous administration of fluconazole has been studied in high-risk, low-birth-weight infants who are at risk for development of systemic infections caused by *Candida* spp. However, the American Academy of Pediatrics currently does not list the use of continuous fluconazole as an accepted procedure.[2]

DISEASE-TARGETED PROPHYLAXIS

RHEUMATIC FEVER

Group A streptococcal infections of the pharynx are the precipitating cause of rheumatic fever. Appropriate antibiotic treatment of streptococcal pharyngitis prevents the development of acute rheumatic fever in most cases.[38] Because at least one third of episodes of acute rheumatic fever result from inapparent streptococcal infections[32] and some symptomatic patients do not seek medical care, not all instances of rheumatic fever are preventable. Prevention of first attacks (primary prevention) is accomplished by proper identification, adequate antibiotic treatment, and eradication of this streptococcal infection. An individual who has suffered an attack of rheumatic fever is at very high risk for recurrence after subsequent group A streptococcal pharyngitis and needs continuous chemoprophylaxis to prevent such recurrence (secondary prevention).[34] The prophylaxis of rheumatic fever also is discussed in Chapter 35.

Primary Prevention

The primary treatment of episodic recurrent group A streptococcal infections is important in the prevention of rheumatic fevers and recurrent episodes. The diagnosis and treatment of group A streptococcal infections are discussed in Chapter 93, and the prevention of rheumatic fever and its prophylaxis are discussed in Chapter 35. The acceptable regimens for treatment of acute group A streptococcal infections (i.e., primary prevention) are provided in Table 249–3. Even when treatment is started as long as 9 days after the onset of acute illness, penicillin effectively prevents primary attacks of rheumatic fever.[24] Therefore, a brief delay (24 to 48 hours) for processing of the throat culture before initiation of antibiotic therapy does not increase the risk of development of rheumatic fever.

Intramuscular benzathine penicillin G is preferred to oral penicillin, particularly for patients who are unlikely to complete a 10-day course of oral therapy and patients with a personal or family history of rheumatic fever, rheumatic heart disease, or other factors that place them at substantial risk for development of rheumatic fever.[34]

Secondary Prevention

Continuous antibiotic administration is indicated in patients at risk for developing or recurrent disease after having infection with group A streptococcus or for those children with congenital or acquired cardiac disease who are at risk for development of endocarditis. Both the American Academy of Pediatrics and the American College of Cardiology provide recommendations for prophylaxis.

An individual in whom streptococcal pharyngitis develops after a previous attack of rheumatic fever is at high risk for having a recurrent attack of rheumatic fever, even after those infections that are asymptomatic or in those symptomatic infections that are treated optimally. Therefore, prevention of recurrent rheumatic fever requires continuous antimicrobial prophylaxis rather than recognition and treatment of acute episodes of streptococcal pharyngitis.[34] Continuous prophylaxis is recommended for patients with a well-documented history of rheumatic fever (including cases manifested solely by Sydenham chorea) and for those with definite evidence of rheumatic heart disease. Such prophylaxis should be initiated as soon as acute rheumatic fever or rheumatic heart disease is diagnosed. A full therapeutic course

TABLE 249–3 Prevention of Rheumatic Fever

From Dajani, A., Taubert, K., Ferrieri, P., et al.: Treatment of acute streptococcal pharyngitis and prevention of rheumatic fever: A statement for health professionals. Pediatrics 96:758-764, 1995. Copyright American Academy of Pediatrics 1995.

**Clarithromycin for 10 days or azithromycin for 5 days in recommended doses is also effective.*

of penicillin or other effective regimen should be given first to patients with acute rheumatic fever to eradicate residual group A streptococcus, even if a throat culture is negative at that time. Streptococcal infections occurring in family members of rheumatic patients should be treated promptly.

An injection of 1,200,000 U of benzathine penicillin G every 4 weeks is the recommended regimen for secondary prevention in most circumstances in the United States (see Table 249–3). In countries where the incidence of rheumatic fever is particularly high, in special circumstances, or in certain high-risk individuals, such as patients with residual rheumatic carditis, administration of benzathine penicillin G every 3 weeks is justified and recommended.[82,83] Long-acting penicillin is of particular value in patients with a high risk of having a recurrence of rheumatic fever, especially those with rheumatic heart disease, in whom recurrence is very serious. The advantages of giving benzathine penicillin G must be weighed against the inconvenience to the patient and the pain of injection, which causes some individuals to discontinue prophylaxis.

Successful oral prophylaxis (penicillin V or sulfadiazine, see Table 249–3) depends primarily on the patient's adherence to prescribed regimens.[33] Patients need to be given careful and repeated instructions about the importance of prophylaxis. Most failures of prophylaxis occur in nonadherent patients. Even with optimal adherence of the patient, the risk of having a recurrence is higher in individuals receiving oral prophylaxis than in those receiving intramuscular benzathine penicillin G.[47] Oral agents are more appropriate for patients at lower risk for having rheumatic recurrence. Accordingly, some physicians elect to switch therapy to oral prophylaxis when patients have reached late adolescence or young adulthood and have remained free of rheumatic attacks for at least 5 years.

Although sulfonamides are not effective in the eradication of group A streptococcus, they do prevent development of infection. Sulfonamide prophylaxis is contraindicated in late pregnancy because of transplacental passage of such drugs and potential competition with bilirubin for albumin-binding sites.

For patients who are allergic to penicillin and sulfisoxazole, erythromycin is recommended. No data have been published about the use of other penicillins, macrolides, or cephalosporins for the secondary prevention of rheumatic fever, but the American Academy of Pediatrics has stated that clarithromycin for 10 days or azithromycin for 5 days is adequate for secondary prevention.

The appropriate duration of prophylaxis must be determined for each situation.[34] Patients who have had rheumatic carditis are at relatively high risk for having recurrence of carditis and are likely to sustain increasingly severe cardiac involvement with each recurrence. Therefore, patients who have had rheumatic carditis should receive long-term antibiotic prophylaxis, perhaps for life. Prophylaxis should continue, even after valve surgery, including prosthetic valve replacement. Patients who have had rheumatic fever without rheumatic carditis are at considerably less risk of having cardiac involvement with a recurrence. Therefore, a physician may consider discontinuation of prophylaxis in these individuals after several years.[11] In general, prophylaxis should continue until 5 years has elapsed since the last rheumatic fever attack or the age of 21 years, whichever is longer.[34]

The decision to discontinue prophylaxis or to reinstate it should be made after discussion with the patient about the potential risks and benefits and careful consideration of various epidemiologic risk factors.[34] The risk of having a recurrence increases with multiple previous attacks, whereas the risk decreases as the interval since the most recent attack lengthens. In addition, the likelihood of acquiring a streptococcal upper respiratory tract infection is an important consideration. Individuals with increased exposure to streptococcal infections include children and adolescents, parents of young children, teachers, physicians, nurses and allied health personnel in contact with children, military recruits, and others living in crowded situations. A higher risk for recurrence has been demonstrated in economically disadvantaged populations.

BACTERIAL ENDOCARDITIS

In 2007,[117a] recommendations for antimicrobial prophylaxis were changed significantly from previous guidelines in 1997.[35] The rationale for these changes included three central concepts. First, endocarditis is much more likely to result from frequent exposure to random bacteremias associated with daily activities than from bacteremia caused by a dental, gastrointestinal tract, or genitourinary procedure. Second, prophylaxis may prevent an exceedingly small number of (if any) cases of endocarditis in individuals who undergo any procedure, and the risk of adverse reactions to antibiotics probably exceeds the benefit in any form of prophylactic treatment. Third, maintenance of optimal oral health and hygiene may reduce the incidence of bacteremia from daily activ-

ities and is more important than prophylactic antibiotics for a dental procedure to reduce the risk of endocarditis.

Therefore, recommendations for the use of antibiotic prophylaxis after 2007 were limited to the highest risk cardiac conditions. Cardiac conditions associated with the highest risk are listed in Table 249–4. Prophylaxis is no longer provide to all subjects who have the highest lifetime risk of endocarditis. For patients with high-risk conditions listed in Table 249–4, all dental procedures that involve manipulation of gingival tissues or the periapical region of teeth or perforation of the oral mucosa are reasonable for administration of dental prophylaxis. Events and procedures such as routine anesthetic injections through noninfected tissues, taking dental radiographs, placement or removal of prothodontic or orthodontic appliances, adjustment of orthodontic appliances, placement of orthodontic brackets, shedding of deciduous teeth, and bleeding from trauma to the lips or oral mucosa do not require prophylaxis. The recommendations for regimens for oral and parenteral antibiotics listed in Table 294–5 are for all dental procedures for which dental prophylaxis is reasonable for persons with high-risk conditions.

Recommendations for antimicrobial prophylaxis for respiratory procedures are only for procedures that involve the incision or incisional biopsy of the respiratory mucosa, such as tonsillectomy and adenoidectomy. Therefore, prophylaxis is not recommended for broncoscopy unless an incision of the respiratory tract will be made. An antimicrobial regimen that includes coverage of viridans group streptococci[35] can be selected from Table 249–5.

When using prophylaxis for high-risk procedures of the gastrointestinal or genitourinary tract, consideration of the possible involvement of enterococcal organisms must be considered as well as the inclusion of mixed infections with aerobic and anaerobic gram-negative and gram-positive organisms.[22,43] However, only enterococci are frequent causes of endocarditis. In the revised recommendations of 2007, the administration of prophylactic antibiotics solely to prevent endocarditis is not recommended for patients who undergo esophagogastroduodenosomy or colonoscopy, which is in contrast to previous recommendations made in 1997. For patients with infections of the gastrointestinal or genitourinary tract who may have intermittent or sustained enterococcal bacteremia or for those who receive antibiotic therapy to prevent wound infection or sepsis associated with a gastrointestinal or genitourinary tract procedure, it is reasonable to include an agent active against enterococci, such as penicillin, ampicillin, piperacillin, or vancomycin. Similarly for patients with high-risk conditions listed in Table 294–4 antibiotic therapy to eradicate the infection from the urine prior to surgery is reasonable for those who are scheduled for an elective cystoscopy or other urinary tract manipulation who have an enterococcal urinary tract infection or colonization. Amoxicillin or ampicillin is the preferred agent for enterococci, but vancomycin may be used in those intolerant of β-lactam antibiotics.

Incision of surgically scrubbed skin without an underlying or adjacent infection is not likely to cause bacteremia, and prophylaxis is not recommended. In contrast, procedures on infected skin, skin structure, or musculoskeletal tissues are likely to cause endocarditis. Therefore, for children with high-risk cardiac conditions, a therapeutic regimen administered for treatment of the infection should contain an agent active against staphylococci and β-hemolytic streptococci, such as an antistaphylococcal penicillin or cephalosporin. Vancomycin or clindamycin may be administered to children unable to tolerate β-lactam antibiotics or those who are known or suspected to be infected with a methicillin-resistant strain of staphylococcus (MRSA).

The 2007 changes in recommendations for prophylaxis for endocarditis represent substantial changes to all the recommendations before 1997. Therefore, a summary of those recommendations is listed in Table 249–6. In addition, the recommendations to withhold prophylaxis to subjects with a number of conditions have not changed, and those conditions or situations are listed in Table 249–7.

TABLE 249–4 Cardiac Conditions Associated with the Highest Risk of Adverse Outcomes from Endocarditis for Which Prophylaxis with Dental Procedures is Reasonable

Prosthetic cardiac valve or prosthetic material used for cardiac valve repair
A history of previous infectious endocarditis
Congenital heart disease • Unrepaired cyanotic CHD, including palliative shunts and conduits • Completely repaired congenital heart defect with prosthetic material or device inserted by surgery or catheter intervention during the first 6 months after the procedure • Repaired CHD with residual defects at the site or adjacent to the site of a prosthetic patch or prosthetic device (which inhibit endotheliazation)
Cardiac transplantation recipients who develop valvulopathy

CHD, congenital heart disease.

RECURRENT OTITIS MEDIA

Acute otitis media is one of the most common infections in infants and children and has a tendency to recur, particularly during the first few years of life. In addition to tympanostomy tube placement and adenoidectomy, antimicrobial prophylaxis is

TABLE 249–5 Prophylactic Antimicrobial Regimens for Dental Procedures

		Regimen, Single Dose 30–60 Minutes before Procedure	
Situation	**Agent**	**Adults**	**Children**
Oral	Amoxicillin	2 g	50 mg/kg
Unable to take oral medications	Ampicillin	2 g IV or IM	50 mg/kg IV or IM
	OR		
	Cefazolin or ceftriaxone	1 g IV or IM	50 mg/kg IV or IM
Allergic to penicillins or ampicillin—oral	Cephalexin	2 g	50 mg/kg
	OR		
	Clindamycin	600 mg	20 mg/kg
	OR		
	Azithromycin or clarithromycin	500 mg	15 mg/kg
Allergic to penicillins or ampicillin and unable to take oral medications	Cefazolin or ceftriaxone	1 g IV or IM	50 mg/kg IV or IM
	OR		
	Clindamycin	600 mg IV or IM	20 mg/kg IV or IM

TABLE 249–6 Summary of Changes and New Recommendations for Antibiotic Prophylaxis for Bacterial Endocarditis

Concluded that bacteremia resulting from daily activities is much more likely to cause endocarditis than bacteremia associated with a dental procedure.
Only an extremely small number of cases of endocarditis might be prevented by antibiotic prophylaxis even if prophylaxis were 100% effective.
Prophylaxis is now recommended only for high-risk procedures (see Table 294–4), and prophylaxis is no longer recommended for any other form of congenital heart disease except for those listed in Table 294–5.
Prophylaxis is reasonable only for dental procedures that involve the manipulation of the gingival tissues or periapical region of teeth or perforation of the oral mucosa, and only for those with very high-risk conditions.
Antibiotic prophylaxis is reasonable for procedures on respiratory tract or infected skin or skin structures or musculoskeletal tissues only for patients with underlying cardiac conditions with the highest risk of adverse outcomes from endocarditis.
Antibiotic prophylaxis solely to prevent endocarditis is no longer recommended for genitourinary or gastrointestinal tract procedures.
Conditions previously listed as not requiring prophylaxis (1997) continue to not require prophylaxis and now include vaginal delivery, hysterectomy, and tattooing, although body piercing for patients with high-risk conditions for endocarditis should not be performed.

TABLE 249–7 Conditions for Which Antiobiotics Should Not Be Used Solely for Endocarditis Prophylaxis

Cardiac Conditions
Isolated secundum atrial septal defects
Surgical repair of atrial septal defect, ventricular septal defect, or patent ductus arteriosus (without residual and beyond 6 months of age)
Previous coronary artery bypass graft surgery
Mitral valve prolapse without valvular regurgitation
Physiologic, functional, or innocent heart murmurs
Previous Kawasaki disease without valvular dysfunction
Previous rheumatic fever with valvular dysfunction
Cardiac pacemakers (intravascular and epicardial and implanted defibrillators)
Respiratory Tract
Endotracheal intubation
Bronchoscopy with a flexible bronchoscope, with or without biopsy
Tympanostomy tube insertion
Gastrointestinal Tract
Endoscopy with or without gastrointestinal biopsy
Genitourinary Tract
Vaginal hysterectomy
Vaginal delivery
Caesarean section
In uninfected tissue:
Urethral catheterization
Uterine dilation and curettage
Therapeutic abortion
Sterilization procudures
Insertion or removal of intrauterine devices
Other
Cardiac catheterization, including balloon angioplasty
Implanted cardiac pacemakers, implanted defibrillators, and coronary stents
Incision or biopsy of surgically scrubbed skin
Circumcision

one of the options recommended for the management of recurrent otitis media.[23,42,99] Chemoprophylaxis of otitis media is discussed also in Chapter 19.

Antimicrobial prophylaxis currently is recommended for a child who has had three or more episodes of acute otitis media in 6 months or four episodes within a year, with the last episode occurring during the previous 6 months.[53,95] Patients who are most likely to benefit from prophylaxis include those younger than 2 years, those in out-of-home childcare, and Native American children.[72,95] Prophylaxis is directed against the most common potential pathogens that cause otitis media: *Streptococcus pneumoniae*, *Moraxella catarrhalis*, and nontypeable *Haemophilus influenzae*. Amoxicillin, at a dose of 20 mg/kg, or sulfisoxazole, at a dose of 50 mg/kg, may be given orally each evening for a period of 3 to 6 months or during the winter months. Although many other antimicrobial agents are used for the treatment of otitis media, only amoxicillin and sulfisoxazole currently are recommended as prophylactic agents because only these two agents have undergone critical analysis in prospective trials. Antimicrobial prophylaxis must be used with great caution and balanced against the potential for increasing the emergence of resistant organisms, particularly *S. pneumoniae*, and the possible occurrence of drug-associated toxicity (e.g., neutropenia, rash, or other). Other measures that may decrease the incidence of recurrent acute otitis media include eliminating smoking in the home, reducing daycare attendance, eliminating pacifiers, and administering influenza and the heptavalent conjugated pneumococcal vaccines. If these measures do not prevent recurrent infections, referral to an otolaryngologist is recommended for evaluation and possible tympanostomy tube placement or adenoidectomy, or both procedures.

RECURRENT URINARY TRACT INFECTION

Urinary tract infection (UTI) occurs in approximately 5 percent of girls and 1 to 2 percent of boys.[123] Recurrent UTIs are noted in roughly 30 to 50 percent of children with UTIs, with most recurrences taking place within 3 months after the initial episode. Eighty percent of recurrences are new infections caused by different colonic bacterial species that have become resistant to recently administered antibiotics. The recurrence rate is not altered by extending the duration of treatment.

Renal parenchymal infections and renal scarring are well-recognized complications of UTIs in children.[62,87,107] Parenchymal scarring is found in 10 to 15 percent of children with UTIs,[110,123] hypertension will develop in an estimated 10 percent of children with this complication, and renal insufficiency may develop in a smaller number.[66] Vesicoureteral reflux is noted in 30 to 50 percent of children with UTIs,[87] the frequency being related directly to the number of UTI episodes and inversely to age. Children with reflux have a much higher incidence (30% to 60%) of pyelonephritic scarring than do children without reflux. More than 90 percent of children with renal parenchymal scarring have had vesicoureteral reflux and a history of UTI.[110,123]

Children who have three or more UTIs in a 12-month period may benefit from suppressive antibiotic therapy for as long as 6 months to allow repair of intrinsic bladder defense mechanisms.[123] In children with anatomic defects or reflux, suppressive therapy may be needed for as long as the underlying defect exists.

Appropriate prophylactic agents should result in low serum but high urinary levels of the medication, have minimal effect on fecal flora, be well tolerated, and be inexpensive.[86] Methenamine mandelate (75 mg/kg divided every 12 hours) is a suitable agent for prophylaxis because it releases formaldehyde in an acid medium. A pH of 5.5 or lower must be maintained in the urine to obtain optimal results. Ascorbic acid or other acidifying agents should be used to achieve the desired urine acidity. Other useful agents for prophylaxis in children with normal renal function are trimethoprim-sulfamethoxazole (TMP-SMX), nitrofurantoin, and nalidixic acid.[17,60,66,87,105] TMP-SMX can be given at 2 mg of TMP and 10 mg of SMX per kilogram in a single daily dose or

at 5 mg of TMP and 25 mg of SMX per kilogram twice a week. TMP has the additional unique characteristic of diffusing into vaginal and urethral fluids, thereby decreasing bacterial colonization with members of the Enterobacteriaceae and diminishing ascending re-infection.[60,112] Nitrofurantoin is recommended at 1 to 2 mg/kg, taken each night. It has been used effectively as prophylaxis for recurrent UTIs in infants and children. Pulmonary, neurologic, and hepatic adverse effects have been reported but are rare occurrences.[87] Nalidixic acid (not recommended for children) is administered at 30 mg/kg divided every 12 hours. It is a bactericidal agent for most of the common gram-negative uropathogens. More recently, various cephalosporins and amoxicillin–clavulanic acid have been used as prophylactic agents with good results.[86] Prophylactic agents are best administered as a single dose at bedtime.

Prophylaxis for UTIs may reduce the incidence of UTIs by up to 50 percent in children with nocturnal continence. No studies of prophylaxis in children without nocturnal continence have shown efficacy, and prophylactic regimens employing broad-spectrum antibiotics (e.g., amoxicillin, cephalexin) are associated with the emergence of recurrent infections caused by antibiotic-resistant organisms such as *Pseudomonas aeruginosa*.

POSTEXPOSURE PROPHYLAXIS

Prophylaxis targeted against specific organisms after an individual is exposed is discussed in this section.

PERTUSSIS

Prompt administration of erythromycin or other approved macrolide drug to those in close contact with a case of pertussis is effective in limiting secondary transmission. Close contacts are household members, attendees of childcare facilities, and other individuals who are in contact with the index case for 4 hours or more a day. Chemoprophylaxis is recommended irrespective of age or vaccination status because immunity after receiving pertussis immunization is not absolute and may not prevent development of infection.[2] The recommended dose of erythromycin is 40 to 50 mg/kg/day (maximum, 2 g/day) to be given orally in four divided doses for 14 days. Both clarithromycin, 15 mg/kg, up to a maximum of 1.0 g, divided twice daily for 7 days, and azithromycin at standard doses (10 mg/kg the first day, up to a maximum of 500 mg, followed by 5 mg/kg, up to a maximum of 250 mg, each of the days 2 through 4) have been shown to be as effective as erythromycin and are much better tolerated than is erythromycin [26] Individuals who are allergic to erythromycin or macrolides or those who cannot tolerate their side effects may be given TMP-SMX, although the efficacy of this regimen has not been documented. The dose is 8 mg/kg/day (TMP) and 40 mg/kg/day (SMX) orally in two divided doses for 14 days.[26]

Persons who have been in contact with an infected individual should be monitored closely for respiratory symptoms for 2 weeks after the last contact with the index case. The risk of contracting pertussis in adults providing medical care to children should be recognized. Symptoms may be mild and not readily recognized as pertussis; however, such individuals can transmit the infection.

MENINGOCOCCAL INFECTIONS

Close contacts of patients with invasive disease caused by *Neisseria meningitidis* (meningococcemia, meningitis, or both) are at higher risk for acquisition of infection than is the general population. Secondary cases and outbreaks may occur in households, childcare centers, nursery schools, colleges, and military camps.[2] The attack rate for household contacts is 0.3 to 1.0 percent (300 to 1000 times the rate in the general population). Spread from patients to medical care providers occurs infrequently unless intimate contact (e.g., mouth-to-mouth resuscitation, intubation, suctioning) occurs. Respiratory tract cultures are not recommended and are not of value in deciding who should receive prophylaxis.[2]

Chemoprophylaxis should be administered as soon as possible, preferably within 24 hours of identification of the index case.[2] Treatment with penicillin G, ampicillin, or sulfonamides for meningococcal disease does not eradicate nasopharyngeal carriage of *N. meningitidis* reliably, whereas treatment with extended-spectrum cephalosporins (e.g., ceftriaxone and cefotaxime) does eliminate carriage. Therefore, antimicrobial chemoprophylaxis should be administered to the index patient before discharge from the hospital if the patient has been treated with the first three antibiotics.

The antibiotic of choice in most instances is rifampin. The recommended regimen is 10 mg/kg (maximum, 600 mg) every 12 hours for a total of four doses in 2 days. A liquid preparation can be formulated, or the powder can be mixed with applesauce or a similar vehicle. The rifampin prophylaxis regimen recommended for *Haemophilus influenzae* type b disease (see later) also is effective for meningococcal prophylaxis.

Rifampin prophylaxis has several shortcomings.[103] It fails to eradicate *N. meningitidis* in 10 to 20 percent of pharyngeal carriers.[90] It is not recommended for pregnant women. Side effects occur frequently and include headache, dizziness, gastrointestinal symptoms, discoloration of body secretions (saliva, tears, urine), staining of contact lenses, and hepatotoxicity. Finally, several studies have documented the emergence of resistant meningococcal strains after the administration of rifampin prophylaxis.[103]

If the meningococcal isolate is known to be susceptible to sulfonamides, sulfisoxazole may be recommended, but currently it is difficult to obtain. The dose is 500 mg/day for infants, 500 mg every 12 hours for children 1 to 12 years of age, and 1 g every 12 hours for children older than 12 years and adults. The duration of prophylaxis is 2 days.

During an outbreak, a single intramuscular injection of ceftriaxone was significantly more effective than rifampin in eradicating meningococci at 1 week (97% versus 75%) and at 2 weeks (97% versus 81%) after prophylaxis.[103] Ceftriaxone administered as a single intramuscular dose (125 mg for children younger than 15 years and 250 mg for adults) now is recommended as an acceptable alternative for prophylaxis. Ceftriaxone has the advantages of ease of administration, possibly greater efficacy, and safety in pregnancy. For high-risk contacts 18 years of age or older, a single 500-mg oral dose of ciprofloxacin is a third option for meningococcal prophylaxis.

HAEMOPHILUS INFLUENZAE TYPE b INFECTIONS

The risk for development of secondary invasive disease with *H. influenzae* type b is age dependent.[6] The risk incidence of disease has declined dramatically with the routine use of *Haemophilus* conjugate vaccines. However, in the era when *H. influenzae* type b infections occurred frequently, household contacts younger than 1 year had the highest risk (6%) for acquisition of secondary illness; the risk in children 4 years or younger also was high (2.1%). Children older than 6 years and adults are at little or no risk. The risk for children attending childcare centers may be increased but appears to be less than that for household contacts.[19,48,85,91,94] Exposed hospital personnel do not require antimicrobial prophylaxis. Data on the risk of spread with invasive disease due to other *Haemophilus* serotypes, such as a or f, are unknown, and currently prophylaxis is not recommended for serotypes other than type b.

In addition to protecting vaccinated children against invasive disease, conjugate vaccines appear to decrease pharyngeal colonization, which further reduces *H. influenzae* type b transmission to unvaccinated children. Prophylaxis currently is recommended

for all household contacts, regardless of age, if at least one of the contacts is younger than 4 years and not immunized completely.[2] Complete immunization is defined as having received a conjugate vaccine: (1) at least one dose at 15 months or older, (2) two doses between 12 and 14 months of age, or (3) two or more doses before 12 months of age with a booster at 12 months or older.

Prophylaxis for nursery and daycare center contacts is less well defined, and definitive recommendations are lacking. In general, prophylaxis is recommended for childcare centers with the same regimen as that recommended for households if (1) the center is attended by unvaccinated or incompletely vaccinated children younger than 2 years where contact is 25 hours per week or more or (2) two or more cases of invasive *H. influenzae* type b disease occur among attendees within 60 days and unvaccinated or incompletely vaccinated children attend the facility.[2] In facilities where all contacts are older than 2 years, prophylaxis need not be given, regardless of vaccination status.

Rifampin in a single dose of 20 mg/kg/day (maximum, 600 mg) for 4 days effectively eliminates oropharyngeal carriage of *H. influenzae* type b in 95 percent of treated individuals.[2] This regimen has been shown to be effective in preventing secondary cases of invasive *H. influenzae* type b disease in household members, daycare settings, and classroom contacts.[6,19] Prophylaxis should be initiated as soon as possible because most secondary cases occur during the first week after identification of the index case.[6] The index case also should receive rifampin prophylaxis, usually initiated during hospitalization and just before discharge.

If prophylaxis is given to limit secondary spread to a cohort (household or daycare), children vaccinated with any *H. influenzae* type b vaccine and unvaccinated susceptible children should receive prophylaxis.[2] Prophylaxis is not recommended for pregnant women.

TUBERCULOSIS

The three goals of preventive therapy for tuberculosis are (1) to prevent asymptomatic (latent) infection from progressing to clinical (active) disease, (2) to prevent recurrence of past disease, and (3) to prevent initial infection in individuals who have negative tuberculin skin test results. The first two goals are covered in detail elsewhere (see Chapter 107); prevention of initial infection is addressed herein. Chemoprophylaxis is given in an attempt to prevent the establishment of infection, and the recipient is protected only as long as antituberculous therapy is continued.

In the United States, isoniazid administered for 9 months is the preferred drug for children for chemoprophylaxis against *Mycobacterium tuberculosis.* The recommended dose is 10 to 15 mg/kg/day (maximum, 300 mg/day) to be given as a single dose. However, alternative regimens, including a 6-month regimen or daily or weekly isoniazid, are recommended for children who cannot comply with a 9-month regimen. For both the 9- and 6-month regimens, twice-weekly, directly observed therapy regimens are equal in efficacy to the daily regimens. Similarly, a daily or biweekly regimen of rifampin-pyrazinamide given for 2 weeks or rifampin given daily is recommended for children exposed to individuals with isoniazid-resistant isolates.[27]

Persons exposed to an infectious case of tuberculosis should undergo tuberculin skin testing, have a chest radiograph, and receive isoniazid prophylaxis or another appropriate and approved regimen.[2] If the tuberculin test result is negative and the chest radiograph is normal and the individual is not anergic, isoniazid or another appropriate regimen should be administered for 12 weeks and contact with the index case should be broken. Isoniazid may be discontinued if the result of a repeated skin test after 12 weeks of prophylaxis remains negative. If the skin test result becomes positive, isoniazid is continued for a total of 9 months. Candidates for prophylaxis include persons with impaired immunity; household contacts, particularly children younger than 4 years; recent contacts, especially human immunodeficiency virus–positive contacts; and persons known to be anergic from populations with a high prevalence of tuberculosis.

Management of a newborn infant whose mother or other household contact has tuberculosis should be based on individual considerations.

HOST-TARGETED PROPHYLAXIS

HUMAN AND ANIMAL BITES

Human and animal bites are relatively common occurrences. According to the Centers for Disease Control and Prevention, more than 1 million animal bites that occur each year require medical attention.[116] Human bites accounted for 1 in 600 pediatric emergency visits and dog bites for 1 percent of all such visits.[104] Dog bites account for 80 to 90 percent of animal bites that require medical care.[18] The organisms most frequently isolated in human bites are *S. aureus*, gamma-hemolytic streptococci, *Bacteroides* spp., *Eikenella corrodens*, and *Fusobacterium* spp.[114] In animal bites, *Pasteurella multocida*, *S. aureus*, and anaerobic cocci are the main pathogens.[113]

Data on the use of prophylactic antimicrobial agents after bites are sparse, and the role of prophylaxis in patients who seek medical care early for bite wounds is uncertain.[5,40,104,109] However, because these wounds usually are contaminated with potential pathogens, the administration of prophylaxis should be considered for patients who have the following risk factors: delay of 18 hours or more between the time of injury and the time of initial physician assessment, facial and hand bites, deep puncture wounds, bites that are difficult to irrigate and cleanse adequately before repair, delay in primary closure of wounds, and wounds in immunocompromised individuals.[5,18,39,84,104,124] For many of these indications, the use of antibiotics is in effect early treatment rather than prophylaxis because contamination of the wound occurs at the time of the bite.

Because most human and animal bites result in polymicrobial aerobic and anaerobic infections, prophylaxis should target these organisms. For initial prophylaxis, amoxicillin–clavulanic acid (30 to 50 mg/kg/day) probably is optimal therapy.[14,18,46,109] Prophylaxis is recommended for 3 to 5 days. Combination therapy with penicillin and cephalexin or dicloxacillin has been suggested by some authorities. Although they are only moderately active against *P. multocida*, erythromycin (30 to 50 mg/kg/day) and standard doses of clarithromycin or azithromycin are accepted alternatives in penicillin-allergic children. Some experts recommend clindamycin (30 mg/kg/day divided into three doses) as an alternative in children allergic to penicillins or cephalosporins.

ASPLENIA

The spleen constitutes approximately 25 percent of the lymphoid mass. It filters blood at a rate of 150 mL/min and plays an important role in the primary defense against bacteria that gain access to the circulation.[115] The spleen has an active role in phagocytosis, is a major source of T lymphocytes, and produces IgM antibodies, complement, opsonins, and tuftsin (a phagocytosis-promoting tetrapeptide).

Asplenia may be congenital or acquired. Splenectomy is performed frequently and is done for a variety of indications. Overwhelming and often fatal septicemia and meningitis occur with increased frequency in asplenic individuals.[122] The frequency of sepsis is 60 times greater in children who undergo splenectomy than in normal children. The risk of sepsis or severe outcome from sepsis is greater in children who lose splenic function by disease or trauma before 2 years of age or in those who have

congenital absence of the spleen. Numerous congenital cardiac conditions carry high risk for development of congenital asplenia. Fatality from sepsis in splenectomized individuals is 200 times more common than that in the normal population.[115] The risk for development of sepsis is greatest in patients who have undergone splenectomies for underlying immunologic or reticuloendothelial disorders, and the risk is lowest in children after splenectomy for trauma.[73] In all categories, the risk is highest in young infants and children, but it extends to teenagers and adults as well. The period of heightened susceptibility to infection is the initial 1 to 2 years after splenectomy; however, fulminant infection has been reported as long as 25 years after splenectomy.

S. pneumoniae is the most common cause of septicemia in splenectomized individuals. Despite prompt diagnosis and treatment, pneumococcal septicemia is associated with a fatality rate as high as 50 percent. Overall, 80 percent of post-splenectomy infections are caused by bacteria with capsular polysaccharides, particularly *S. pneumoniae* and *H. influenzae*.[108,115] Although *N. meningitidis* also has a polysaccharide capsule, lack of terminal components of complement constitute the greatest risk for development of infection, and whether the incidence of sepsis is higher in persons with asplenia is unclear. However, because overwhelming meningococcal sepsis has been reported in patients with asplenia, it is reasonable to include these patients at risk.

To reduce the likelihood of serious infections after splenectomy, several measures are advisable. Splenectomy should be performed only when it is absolutely indicated. If possible, the best approach is to delay the surgical intervention until the child is 5 or 6 years of age. For children with congenital high-risk conditions, pneumococcal polysaccharide protein conjugate vaccines should be provided in the recommended schedules. Although multivalent pneumococcal polysaccharide vaccine provides incomplete protection for patients undergoing splenectomy, especially infants and young children, it should be administered to all patients who are older than 2 years, ideally 2 weeks before the splenectomy is performed.[111] Vaccination against *H. influenzae* type b and *N. meningitidis* types A, C, Y, and W-135 with the appropriate polysaccharide or polysaccharide-protein conjugate vaccine recommended for use in children should be provided.

For antibiotic prophylaxis, penicillin is the agent of choice. Penicillin V given twice daily (125 mg twice daily for children younger than 5 years; 250 mg twice daily for children older than 5 years) significantly decreases the frequency of invasive pneumococcal infection. Erythromycin and TMP-SMX are alternative options in patients with documented hypersensitivity to penicillin. The duration of prophylactic coverage remains controversial; current practice is to provide penicillin prophylaxis indefinitely in immunocompromised patients.[108]

HEMOGLOBINOPATHIES

Functional asplenia is the primary reason for susceptibility to pneumococcal infection in children with sickle-cell anemia. Serum immunoglobulins are normal or increased in these children; however, they have a dysfunctional alternative complement pathway and decreased opsonic activity (which is mediated by both the alternative and the classic components) against *S. pneumoniae*. Leukocyte function also is defective in patients with sickle-cell anemia; intracellular production of hydrogen peroxide, respiratory stimulation, and hexose monophosphate shunt activity are inadequate during phagocytosis. In contrast, leukocytes from splenectomized patients without sickle-cell anemia exhibit normal phagocytic function accompanied by adequate metabolic stimulation. Immunologic dysfunction occurs less rapidly and less commonly in children with hemoglobin C sickle-cell anemia and hemoglobin C beta-thalassemia.[77]

Patients with sickle-cell disease are at risk for development of overwhelming infection (septicemia and meningitis) by encapsulated bacteria, including *S. pneumoniae*, *H. influenzae* type b, and, rarely, *N. meningitidis*. *S. pneumoniae* is the most important and frequent cause of septicemia and meningitis in these patients.[118] The risk is particularly high in children younger than 3 years.[51] A trend toward increased frequency of invasive disease in the first 2 to 5 years after splenectomy also has been noted. Unlike very young children, school-age children appear to be less vulnerable to pneumococcal invasive infection, even though they remain functionally asplenic.[52,118,121]

The efficacy of penicillin prophylaxis in preventing pneumococcal infection in infants and young children with sickle-cell disease has been well documented in several reports.[44,51,52,96,121] The recommended dose of penicillin V is 125 mg twice daily in children younger than 3 years and 250 mg twice daily in children 3 years or older. Some physicians recommend use of amoxicillin (20 mg/kg/day) or TMP-SMX (4 mg TMP plus 20 mg SMX per kilogram daily) in children younger than 5 years to include coverage against *Haemophilus* organisms, which are less likely to be a concern in patients who are immunized adequately.

Because overwhelming infection can occur in infants as young as 3 months, detection of sickle-cell anemia should be accomplished in the neonatal period. Babies in whom sickle-cell anemia is diagnosed should start a prophylactic antibiotic regimen no later than 3 to 4 months of age.[52] The optimal duration of prophylaxis is not defined clearly, and the age at which prophylactic penicillin can be discontinued safely is determined arbitrarily. A concern is that penicillin prophylaxis may decrease the development of natural immunity against pneumococcal infection in children receiving prophylaxis, thereby rendering them more susceptible to development of infection after prophylaxis is discontinued.[20] Another concern is the accelerated development of penicillin-resistant strains of *S. pneumoniae*.[45,118] A multicenter study by the Prophylactic Penicillin Study II group suggests that in children with sickle-cell anemia who have not had a previous severe pneumococcal infection or surgical splenectomy and are receiving comprehensive care, prophylaxis may be stopped safely at 5 years of age.[45] Continuous prophylaxis has limitations, and serious overwhelming infection can occur while patients are receiving prophylaxis. Patients or parents (or both) should be aware that any febrile illness is potentially serious, and immediate medical attention should be sought.[31,96]

CEREBROSPINAL FLUID LEAKAGE

The value of antibiotic prophylaxis in patients with cerebrospinal fluid leakage has not been proved.[49,89] In the absence of meningeal inflammation, many antibiotics do not penetrate the blood-brain barrier and do not attain adequate levels in cerebrospinal fluid. Antibiotic prophylaxis often fails and frequently alters the normal flora of the respiratory tract, thereby resulting in colonization with resistant bacteria. Prophylaxis may be considered for a short duration while surgical repair is being planned.[89]

SURGICAL PROPHYLAXIS

GENERAL SURGICAL PROCEDURES

Postoperative infection always has been a feared complication of surgical procedures. Skin incision, organ manipulation, and surgical trauma increase the likelihood for development of local infection. Surgical procedures traditionally are classified as clean, clean-contaminated, and contaminated (Table 249–8). Prophylactic antibiotics are effective in reducing postoperative infections after contaminated and clean-contaminated surgical procedures, whereas their efficacy is more controversial for clean surgical procedures. Clean surgical procedures generally carry a risk for development of postoperative wound infection that is less than 5 percent and, in many hospitals, less than 1 percent.

TABLE 249–8 Surgical Procedures and Probable Pathogens

Surgical Category	Most Likely Pathogens
Clean	
Neurosurgical	CNS, *Staphylococcus aureus*
Cardiovascular	CNS, *S. aureus*
Orthopedic	CNS, *S. aureus*
Clean-Contaminated	
Burn	Group A streptococci, *S. aureus*, GNB
Gastrointestinal	GNB, anaerobes, enterococci
Urogenital	GNB, enterococci
Respiratory	Alpha-hemolytic streptococci, anaerobes
Contaminated	
Ruptured viscera	GNB, anaerobes, enterococci
Traumatic wounds	*S. aureus*, group A streptococci, clostridia

CNS, coagulase-negative staphylococci; GNB, gram-negative bacilli.

The critical period for development of infection is short, and optimal prophylaxis should be restricted to the perioperative period. Antibiotic prophylaxis of less than 24 hours' duration is effective both clinically and experimentally. Administration of antibiotics should be started at the time of induction of anesthesia or immediately before the surgical incision is made and discontinued within 24 hours.

Addressing all situations of surgical prophylaxis is beyond the scope of this chapter, and the reader is referred to several publications and reviews on the subject.[36,61,67,69,97,117] Nationwide standards and goals for surgical prophylaxis have been established.[15,16] The consensus of these statements is that effective surgical prophylaxis must include the first dose of antimicrobial agents within 60 minutes before the surgical procedure and that dosing should be discontinued within 24 hours after the end of surgery. These documents and others provide the routine basis and evidence base for all current guidelines on surgical prophylaxis, including the recommended regimens and dosing for cardiothoracic, vascular, and abdominal or colonic surgery as well as hip or knee arthroplasty, vaginal or abdominal hysterectomy, and neurosurgical procedures.[4]

NEUROSURGICAL PROCEDURES

The use of prophylactic antibiotics for clean neurosurgical procedures remains controversial.[50,56,89,99] However, because of the suggested benefit of prophylactic antibiotics in uncontrolled trials involving a large number of patients, of whom adults were the majority, and in view of the scarcity of definitive studies, the literature supports the use of a short-course prophylactic regimen.[21,41,54,98,119] Prophylaxis for clean neurosurgical procedures is particularly valuable for high-risk groups (e.g., patients undergoing operative procedures in excess of 4 hours, operations in which craniotomies are performed, or patients with major underlying disease).[37,106]

Placement of cerebrospinal fluid shunts is one of the most common neurosurgical procedures in pediatric patients. An estimated 10,000 new shunt insertions and 6000 revisions are performed annually in the United States. The frequency of shunt infection varies from 1.5 to 39 percent (average, 10% to 15%).[74,101] The major route of infection is colonization of the device or the operative wounds during placement.[54] Retrograde spread from the distal end of the catheter or hematogenous seeding accounts for some instances of infection.

Most infections are noted within 15 days to 2 months of shunt placement.[78,101] Commensal skin flora are the predominant pathogens. Coagulase-negative staphylococci are the most common pathogens and account for approximately 70 percent of shunt infections. *S. aureus* is less common. Gram-negative bacilli are the least common and often are the result of retrograde infection from the peritoneum.[74,101]

The role of prophylactic antibiotics for placement of cerebrospinal fluid shunts has been controversial.[55] A meta-analysis of 1359 patients in 12 randomized, controlled trials indicated that short-term perioperative antimicrobial prophylaxis at the time of placement of a cerebrospinal fluid shunt significantly decreases the risk for subsequent development of device-related infection.[78] Various antimicrobial regimens, including antistaphylococcal penicillins, cephalosporins, TMP-SMX, vancomycin, gentamicin, and combinations, were used in these trials. The choice of an appropriate prophylactic regimen in a particular setting should be based on the local epidemiology of suspected pathogens, local patterns of antimicrobial susceptibility, cost, and expected toxicity. The duration of perioperative prophylaxis should not exceed 48 hours.[71,78] A longer duration of prophylaxis increases cost and the risk for development of adverse reactions and promotes alteration of the normal flora and the emergence of resistant bacteria.

CARDIOVASCULAR SURGERY

Infectious complications of cardiovascular surgery can be very serious and life-threatening, and antimicrobial prophylaxis is used commonly in most medical centers.[70,75] Most available data are based on reports from adult patients,[30,57] with little specific information available on prophylactic antibiotic use in pediatric patients. The goal of prophylactic therapy is prevention of wound infection, mediastinitis, and endocarditis. A survey of 43 North American academic centers with pediatric cardiovascular surgery programs indicated that all centers use prophylactic antibiotics for all operative procedures.[80] Monotherapy prophylaxis was used by 91 percent of respondents and consisted almost exclusively of a first- or second-generation cephalosporin. In 95 percent of centers, prophylaxis was started just before surgery or intraoperatively. Prophylaxis was continued for 48 hours or less in most (68%) instances. Prophylactic antibiotics often were continued while thoracostomy tubes, mediastinal tubes, or transthoracic vascular catheters were in place but usually not for endotracheal tubes, arterial or percutaneous central venous catheters, or temporary pacing wires.

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CHAPTER 250

Outpatient Intravenous Antimicrobial Therapy for Serious Infections

John S. Bradley

Outpatient parenteral antimicrobial therapy (OPAT) is the means by which children can receive the benefits of parenteral (intravenous or intramuscular) therapy in an outpatient setting, usually the home, avoiding hospitalization with the associated exposure to nosocomial pathogens, inpatient costs, and psychological stress for child and family. The widespread acceptance of OPAT by physicians, families, and insurers has led to a tremendous increase in the outpatient treatment of serious infectious diseases.[3,13,19,21,22,29,32,36,37] The standard of care provided to children through OPAT should equal or surpass that provided to hospitalized children. National guidelines for OPAT in adults and children have been published by the Infectious Diseases Society of America and define both benefits and risks.[46] This chapter explains the basic concepts behind OPAT for children. The OPAT team, which includes the physician, OPAT coordinator, nurse, pharmacist, parent or caregiver, and third-party payer, needs to work closely to ensure a successful outcome for the child. Defined patient care outcomes in OPAT for adults and children currently are being addressed by a joint task force of the Infectious Diseases Society of America and the American Medical Association.

EVALUATING A CHILD AND PARENTS FOR OUTPATIENT PARENTERAL ANTIMICROBIAL THERAPY

Broad criteria are used to determine the medical feasibility of using OPAT (Table 250–1). The most important aspect in the

assessment of a newborn, infant, or child for OPAT is a determination that ongoing parenteral therapy is required to treat the infection appropriately and that continuous skilled nursing observation and care are not required for management. Development of complications of the infection in the child should be considered highly unlikely by the time OPAT is undertaken; unskilled clinical observations by the parents or caregivers should be considered adequate for care. No additional significant risk should be placed on the child's recovery by enrollment in an OPAT program. Whenever possible, oral antibiotic therapy should be used instead of parenteral therapy; recent options for oral therapy include fluoroquinolones (ciprofloxacin, levofloxacin) and oxazolidinones (linezolid).

A child usually is prescribed an OPAT regimen from either a hospital setting, after inpatient intravenous therapy, or an outpatient setting such as a clinic or emergency department. For a child being discharged from the hospital, an unequivocal response to parenteral anti-infective therapy should be demonstrated. Most often, appropriate cultures will have directed the use of anti-infective therapy active against the isolated pathogens. For children with no positive cultures, the signs and symptoms of the infection should be resolving with empiric therapy, as demonstrated by decreasing fever and improving function. The child's infection should be judged by the clinician to be unlikely to progress or to result in complications. No further surgical or medical interventions should be anticipated during the remainder of the parenteral antimicrobial therapy course.

The child's clinical course during receipt of OPAT needs to be monitored to be certain that the anticipated response continues to occur. An outpatient visit usually is scheduled for the day after discharge.

Before a child is enrolled in OPAT, an assessment of the parents or caregivers also is required (see Table 250–1). Because the parents are responsible for the nursing care of their child in the home, they must be willing and capable participants. Although most parents are very interested and highly motivated to return home with their child, not all are willing to perform or are capable of performing the required nursing functions. Although most parents are capable of providing limited medical assessment of their children, some are not. In addition, the home environment should have the resources required for the proposed medical care. For example, intravenous therapy administered to a child through a tunneled, central catheter requires local sterile conditions for anti-infective drug infusions and dressing changes, which will require the availability of a clean room with running water, and a refrigerator if storage of antimicrobials and diluents is necessary. Less stringent criteria clearly would apply for a child visiting the outpatient clinic daily to receive intramuscular antibiotic injections. Communication between the parents and medical personnel is essential. For parents or caregivers, telephone access to nurses and clinicians is critical. In addition, the parents should be able to bring their child back to the hospital or clinic for reevaluation if complications arise either from the infection or from therapy. Hence, access to adequate transportation within a medically appropriate time frame is required. Families with no available transportation, families who live in rural areas located several hours' travel from a medical facility, and weather conditions that may severely limit travel all should be considered before OPAT is started. In addition to observing their child and communicating information to medical providers, some parents may be asked to infuse anti-infective agents into their children. Doing so requires an additional level of skill in the parent or caregiver and further assessment of parental competence by the OPAT nursing and medical personnel.

TABLE 250–1 Medical and Social Criteria for OPAT

Medical Criteria for OPAT
Medical diagnosis of infection with a suspected or documented pathogen
Clinical response to anti-infective therapy (if therapy is started in the hospital)
No assessed need for urgent medical, surgical, or laboratory interventions to achieve a clinical response to anti-infective therapy
No significant risk of complications of the infection
No requirement for continuous skilled nursing care
Social Criteria for OPAT
Parents interested and motivated
Parents capable of assessing the child for complications of infection and therapy
24-hour telephone access to the OPAT team
Transportation for timely return to the clinic or hospital, if needed
Home environment acceptable and resources available
Parents documented to be capable of administering parenteral anti-infective agents, if required, before OPAT begins

OPAT, outpatient parenteral antimicrobial therapy.

THE OUTPATIENT PARENTERAL ANTIMICROBIAL THERAPY PROGRAM

The roles of physicians, OPAT coordinators, nurses, pharmacists, and parents or patients have been defined previously (Table 250–2).[46] Once the clinician considers that the child's condition is sufficiently stable to pursue outpatient therapy from a hospi-

TABLE 250–2 Roles of Physician, OPAT Coordinator, Nurse, Pharmacist, and Parent

Role	Physician*	OPAT Coordinator*	Pediatric Nurse*	Pediatric Pharmacist*	Parent or Patient
Assess medical stability for home therapy	+	+	–	–	–
Assess family for capability for OPAT	+	+	+	–	–
Create and implement OPAT treatment plan	+	+	+	+	–
Order nursing visits	+				
Order clinic visits, laboratory tests, imaging examinations	+				
Educate parents about infection and complications	+	+	+	–	–
Educate parents about therapy and complications	+	+	+	+	–
Manage treatment	+	+	+	–	+
Monitor IV catheter status	+	–	+	–	+
Monitor clinical status	+	–	+	–	+
Monitor antimicrobial therapy	+	–	+	+	+
Prepare antimicrobial therapy	–	–	+	+	+
Availability for complications	+	+	+	+	+
Outcome assessment	+	+	+	+	+

**Certification or experience in pediatrics.*
OPAT, outpatient parenteral antimicrobial therapy.

tal ward, a clinic, or an emergency department setting, the coordinator of the outpatient treatment program is notified. This person is capable of assessing the feasibility of implementing OPAT for that particular child and family. The OPAT coordinator interviews parents and medical staff to determine the parents' willingness and their abilities and to assess the home environment. After the clinician prescribes the anti-infective agents, required nursing visits, ongoing laboratory and imaging tests, and follow-up clinic visits, the OPAT coordinator puts together the resources required for successful therapy. With knowledge of the parents' abilities and resources, the medication and equipment requirements, and the home nursing visits required, the coordinator makes the final determination about the feasibility of the program. The coordinator also determines who is required to pay the costs of OPAT among the family, the third-party payer (private or government) if one exists, and the hospital. Authorization from third-party payers may be required before OPAT can begin; many insurance companies require a much greater financial contribution by families for the cost of care once a child is discharged. An example of an OPAT physician's order form that includes catheter management and medication orders, laboratory and imaging test orders, and clinic visits is reproduced in Figure 250–1. The coordinator of the OPAT program, whether it is clinic or hospital based, should ensure that open lines of communication exist among the clinician, the home visiting nurse, the pharmacist, and the parents. A defined, written plan for medical and nursing care should be established. All members of the OPAT team should be aware of their responsibilities before outpatient therapy is implemented.

The frequency of the required home pediatric nursing visits needs to be individualized for each child and depends on many factors: the clinical course while the child is receiving therapy, the seriousness of the infection, the ability of the parents to effectively examine the child, and the risk for development of complications that can be related to either the infection or therapy. For most children, the frequency of home nursing visits will be greatest at the beginning of OPAT, often once daily. After treatment at home has been delivered as anticipated and recovery from infection is proceeding as expected, visits may be decreased to two or three times each week, particularly if the parents have demonstrated competency in providing nursing care, including managing an intravenous catheter, changing wound dressings, and providing clinical assessment. The home visiting nurse also may have the opportunity to establish ongoing assessment of the parents' ability to administer anti-infective agents in the home. Physical assessment of neonates receiving OPAT may be particularly difficult for parents to determine, and daily visits throughout the entire course of therapy by skilled pediatric home visiting nurses may be required. Daily visits also are important for children with less stable infections, such as central nervous system infections, because subtleties in the neurologic examination findings may not be appreciated by parents or caregivers and may provide clues to impending problems. Nursing aspects of pediatric OPAT have been outlined by successful programs.[22,29,44]

Pediatric competency in nursing is not standardized. Each state government in the United States licenses nurses on the basis of criteria specific for that state. However, just as for inpatient pediatric care, pediatric experience is an important qualification for the home visiting nurse. Unfortunately, home nursing agencies providing care to children are not required to have expertise in pediatric nursing. Therefore, considerable discrepancy exists in pediatric competency among nursing agencies. Given the importance of pediatric expertise in evaluation and management of infected children in the home, the home nursing agency contracted to provide care is expected to have personnel with training and experience equivalent to that of nurses providing care in a pediatric inpatient setting. The home nursing agency should be able to document proficiency in delivering care to newborns, infants, and children to the clinician and the OPAT coordinator.

In many states, only licensed nurses (RN, LVN) may infuse anti-infective agents. However, most states have no rules prohibiting parents from administering drugs. Accordingly, parents may be expected to infuse medications to their children if licensed nurses are not deemed by the home care agency and the physician to be necessary to provide this function. The physician should be aware of the qualifications of the personnel responsible for the intravenous infusion of anti-infective agents.

The home visiting nurse should communicate with the physician about the status of the child as determined by the physician before OPAT is started. Analogous to inpatient care, the nurses and physicians are expected to be on-call 24 hours each day.

Similarly, the pharmacist providing anti-infective therapy for OPAT should be familiar with the dosages and side effects of therapy provided to all pediatric age groups, from newborns to adolescents. Dosing errors in infants and children are not uncommon occurrences, given the broad range of doses administered.[26]

The parents should have written instructions for the tasks that they are required to perform in the home, including information on sterile technique, administration of antimicrobials, flushing of catheters, and dressing changes. Their proficiency in management of the catheter and infusion of anti-infective agents should be demonstrated before the child is discharged from the hospital, emergency department, or clinic. Telephone numbers should be provided for the 24-hour on-call nursing and physician personnel. A list of complications of the medications and procedures and information on clinical assessment of the child's infection should be provided, along with a set of parameters requiring immediate notification of medical personnel. Plans for disposal of needles, dressings, and other medical waste also should be made before the child is sent home.

Visits to clinicians (primary care providers, surgeons, subspecialists) are scheduled as necessary according to the clinical status of the child and the degree of expertise required by the examiner. If the child is stable in an outpatient setting, once-weekly visits to a physician usually are sufficient unless the home visiting nurse or parent observes problems requiring more immediate attention. For any high-risk, relatively unstable child, daily physician visits may be important, particularly if skilled pediatric home nursing is not available.

Although abuse of intravenous access seldom occurs, for an adolescent who may have a history of alcohol or drug dependence, the clinician should be particularly cautious in approving OPAT. For younger children with indwelling catheters, Münchausen syndrome by proxy may be a concern for infants and children who do not recover clinically as anticipated.

INFECTIONS SUITABLE FOR OUTPATIENT PARENTERAL ANTIMICROBIAL THERAPY

Virtually any infection can be treated by OPAT at some point in the course of therapy by following the criteria listed in Table 250–1. For each child, a thorough assessment of the risks for development of complications of the infection must be made before outpatient therapy is considered. A partial list of infections treated with OPAT is given in Table 250–3; most of the studies are not prospective, randomized investigations comparing children receiving therapy in the hospital with those receiving therapy outside the hospital. The variety of infections that have been treated with OPAT at some point include bloodstream infections, such as bacteremia, catheter sepsis, and endocarditis; central nervous system infections, including meningitis, epidural abscess, subdural empyema, and brain abscess; osteomyelitis and septic arthritis; urinary tract infections; upper respiratory tract infections, including severe acute otitis media, chronic suppura-

Children's

Children's Hospital - San Diego
3020 Children's Way
San Diego, California 92123-4282

Home Care Order Sheet for Infectious Diseases

Patient: _____

MR# _____

Account # _____

1. DISCHARGE DIAGNOSIS

2. NURSING REQUIREMENTS

a. Frequency of visits:

b. Complications to watch for (include with assessment at each visit):

c. Other nursing requirements

3. MEDICATIONS/ANTIBIOTICS (type, dose, dosing interval, duration):

a.

b.

c.

d. NS FLUSH _____ cc q _____ hrs e. Heparin flush _____ u/cc _____ cc ____ q hrs

4. LABORATORY TESTING:

☐ Via Venipuncture or ☐ Venous Access Device ☐ Other

a. Antibiotic levels

b. CBC

c. ESR, CRP

d. Chem 20

e. Cultures

5. OUTPATIENT VISITS (frequency while on therapy):

a. Infectious Disease Clinic (specify attending physician)

b. Other specialists

c. Primary care physician

6. OTHER TESTS TO BE SCHEDULED (imaging, etc.):

7. DC IV AND DC FROM HOMECARE WHEN THERAPY COMPLETE:

☐ YES ☐ RE-EVAL

8. ATTENDING PHYSICIAN

Dr. NOTIFIED OF PLAN BY

Signature _____ Date _____

NON STOCK / RBF 1898 (06/96) ORIGINAL - Chart CANARY - Home Care Agency PINK - Infectious Diseases GOLD - Primary Care Physician

Figure 250–1 An example of a physician's order sheet for outpatient intravenous antimicrobial therapy (OPAT) for serious infections at Rady Children's Hospital San Diego, San Diego, California.

TABLE 250–3 Infections Treated with OPAT

Infection Treated	Reference	Design*
Collections of various infections	4, 6, 7, 15, 44	III
Appendicitis	3, 12, 42, 49	II
Catheter infection	27	III
Chronic suppurative otitis media	10	II
Cystic fibrosis	53	I
	8	II
	14, 20, 25, 29, 40, 43	III
Fever in immunocompromised children	32, 38, 51	I
	9, 41	II
	22, 50	III
Meningitis	1, 2, 11	III
Mastoiditis	34	II
Neonatal infections	47	II
	5, 11	III
Osteoarticular infections	30	III
Urinary tract infection	13	II
	33	III
Peritonitis, peritoneal catheter related	28	III

**I, randomized, prospective, comparative trial; II, prospective evaluation; III, retrospectively reviewed clinical experience.*
OPAT, outpatient parenteral antimicrobial therapy.

tive otitis, and mastoiditis; lower respiratory tract infections; fever with or without neutropenia in low-risk immunocompromised children; neonatal bacterial sepsis; intra-abdominal infections; and postoperative wound infections.

DELIVERY OF ANTIMICROBIAL THERAPY

SELECTION OF AN ANTIMICROBIAL AGENT

Selection of antimicrobial therapy for OPAT is similar to selection of therapy for any child in that the primary goal is to achieve clinical and microbiologic cure with the most efficacious, least toxic, and most cost-effective agents. Selection of an agent that is more convenient to administer but that may not be as effective is not appropriate. However, of the agents that demonstrate equivalent activity against the child's pathogen, the preferred agent is the one that is given least frequently, is nontoxic, and requires the least frequent monitoring for adverse events. Preferred agents for the most common community-acquired pathogens are given in Table 250–4. Almost any pathogen, community acquired or nosocomial, bacterial, fungal, or viral, can be treated in the home if the medical and social criteria for OPAT can be met.

In general, β-lactam agents (penicillins, cephalosporins, carbapenems) require less frequent monitoring than do aminoglycosides (gentamicin, tobramycin, amikacin), glycopeptides (vancomycin or teicoplanin), or oxazolidinones (linezolid). Agents that can be given intravenously during the course of a short period (15 minutes), such as β-lactam agents, are preferred to those that require up to an hour with each infusion (aminoglycosides and glycopeptides). Agents that can be given intramuscularly if intravenous access is temporarily lost are preferred to those that may be given only intravenously. Not only are these parameters designed to provide the optimal clinical outcome with minimal toxicity, they also are designed to facilitate therapy in the home. Dosing more frequently than every 8 hours poses a formidable challenge to many families, particularly those without extensive support. Administration of two or three antibiotics that each require dosing three or four times each day is virtually impossible for most families to accomplish. These parameters are designed to maximize the potential for parents to be able to provide care at home with minimal risk. Although more frequent nursing visits may provide relief to parents, the charges for home nursing visits to administer therapy and to provide nursing care (e.g., wound dressing changes) are substantial; the charge for three nursing visits each day approaches the cost of a day in the hospital for a stable child, and most agencies do not have sufficient staff to provide more than one visit each day to a family. Depending on the agent used, monitoring for antimicrobial toxicity in the outpatient setting should occur with the same frequency as that in the inpatient setting. Monitoring of renal function and serum antibiotic concentrations is important for children being treated with aminoglycosides or vancomycin. For children receiving long-term β-lactam therapy, periodic monitoring of the peripheral white blood cell count and renal and hepatic function every 2 to 4 weeks may detect the uncommon but well-described antibiotic-mediated toxicities of this class of antibiotics before any clinical manifestations occur.

DELIVERY OF ANTIMICROBIAL AGENTS

OPAT can be delivered by intramuscular or intravenous injection. In general, the need for more than two or three injections necessitates intravenous therapy. However, once-daily intramuscular therapy lasting 1 week or longer has not been associated with short-term complications in anecdotal reports.[5,6] Short polyethylene catheters normally used for inpatient intravenous therapy may be used for short-term therapy lasting less than 5 to 7 days. On occasion, intravenous access will be lost and the child will need to be reevaluated for ongoing parenteral therapy; if clinical improvement exceeds expectations, an early switch to oral convalescent therapy may be possible. If not, intravenous access may need to be reestablished. It may be performed in the home but more often will require the child and parent to return to the clinic or hospital. An intramuscular injection or injections may be given temporarily in the home, clinic, or emergency department until the intravenous catheter can be replaced.

For therapy lasting longer than 5 to 7 days, central catheters are preferred. Peripherally inserted silicone rubber elastomer (Silastic) central catheters (PICC lines) are practical and cost-effective and have become the intravenous access of choice for extended therapy.[45] These catheters may be inserted through many different peripheral sites (usually the antecubital vein) by physicians or nurses with training in the placement of central catheters. Sedation usually is required for younger infants and children. Alternatively, more traditional subcutaneously tunneled central catheters with multiple ports (e.g., Hickman, Groshong) may be placed by a surgeon in the operating room with the child under general anesthesia. These tunneled catheters, which also may be used for blood sampling, are preferred for children requiring several weeks of therapy with agents that require periodic blood tests for assessment of organ function and serum antibiotic concentrations. Each type of central catheter requires sterile techniques for accessing the catheter and close monitoring of the catheter exit site during administration of therapy. The home visiting nurse agency should have written protocols for the care and use of central catheters that are consistent with standards set by the Joint Commission on Accreditation of Healthcare Organizations (JCAHO) for both inpatients and outpatients.[23]

Anti-infective agents may be infused directly into the catheter by the caregiver by way of an antibiotic-containing syringe simply attached to the catheter hub ("IV push"), followed by flushing of the catheter with saline or a heparin-containing solution. Alternatively, particularly for agents that cannot be infused quickly, an intravenous drip system must be used, or the agent must be infused by a pump. Pumps come in three basic designs: those with

TABLE 250–4 Selection of Antimicrobial Agents for Common Pathogens in Community-Acquired Infections

Pathogen	Antimicrobial Agent	Dosing Frequency (hr)	Toxicity
Gram-Positive Bacteria			
Streptococcus pneumoniae	Penicillin G*	6	
	Ampicillin*	6-8	
	Cefuroxime*	8	
	Cefotaxime*	6-8	
	Ceftriaxone*†	24	
	Linezolid*	8-12	Neutropenia, thrombocytopenia
	Vancomycin (for penicillin and cephalosporin nonsusceptible strains)‡	8-12	Renal toxicity, ototoxicity
Staphylococcus aureus	Oxacillin*	6-8	
	Cefazolin†	8-12	
	Clindamycin*	8	Colitis
	Linezolid*	8-12	Neutropenia, thrombocytopenia
	Vancomycin‡	8-12	Renal toxicity, ototoxicity
Streptococcus pyogenes	See *S. pneumoniae*		
Enterococcus	Ampicillin plus gentamicin*†	Ampicillin: 6-8 Gentamicin: 8	Renal toxicity, ototoxicity (gentamicin)
	Vancomycin plus gentamicin*‡	Vancomycin: 8-12 Gentamicin: 8	Renal toxicity, ototoxicity (both gentamicin and vancomycin)
Gram-Negative Bacteria			
Haemophilus influenzae	Ampicillin*	6-8	
	Cefuroxime	8	
	Cefotaxime	8	
	Ceftriaxone†	24	
Escherichia coli	Ceftriaxone†	24	
	Ampicillin*	6-8	
	Cefotaxime‡	6-8	
	Gentamicin‡ or tobramycin	8-24	Renal toxicity, ototoxicity
	Amikacin‡	12-24	Renal toxicity, ototoxicity
Enterobacter, *Serratia*, *Citrobacter* (pathogens with inducible AmpC β-lactamases)	Cefepime†	8-12	
	Ertapenem†	12-24	
	Meropenem	8	
	Imipenem-cilastatin	6-8	
	Cefotaxime* or ceftriaxone plus an aminoglycoside‡ (gentamicin, tobramycin, amikacin)	Cefotaxime: 6-8 Ceftriaxone: 24 Gentamicin and tobramycin: 8-24 Amikacin: 24	Renal toxicity, ototoxicity (aminoglycoside)
Pseudomonas aeruginosa	Meropenem†	8	
	Cefepime	8	
	Ceftazidime* plus an aminoglycoside‡	Ceftazidime: 8 Aminoglycoside: see above	Renal toxicity, ototoxicity (aminoglycoside)
	Imipenem-cilastatin	6-8	Central nervous system irritability in children with underlying central nervous system inflammation
	Ticarcillin-clavulanate* plus an aminoglycoside‡	Ticarcillin-clavulanate: 6 Aminoglycoside: see above	Renal toxicity, ototoxicity(aminoglycoside)
	Piperacillin-tazobactam* plus an aminoglycoside‡	Piperacillin-tazobactam: 6 Aminoglycoside: see above	Renal toxicity (aminoglycoside)
	Ciprofloxacin§	8-12	Possible arthropathy
Neisseria meningitidis	Ceftriaxone†	24	
	Penicillin G	6	
Fungal Pathogens			
Candida	Fluconazole IV†	24	
	Amphotericin B (lipid preparations are better tolerated)	24, or every other day; may return to infusion center for each injection to manage side effects	Fever, chills, anemia, hypokalemia, decreased glomerular function
	Caspofungin or micafungin	24	
Viral Pathogens			
Herpes simplex virus	Acyclovir	8	Neutropenia, renal toxicity
Cytomegalovirus	Ganciclovir	12-24	Neutropenia

**If organisms are documented to be susceptible.*
†Preferred agents based on dosing frequency and side effects.
‡Requires monitoring of renal function and serum concentrations.
§Not indicated for therapy unless no other therapy options exist.

no electronic or moving parts; syringe pumps, in which the syringe containing the anti-infective agent is placed in a motorized pump designed to administer the dose during the course of a certain period; and electronic programmable pumps, which may have a variety of pump mechanisms and are programmed to control infusions of one or more agents administered during the course of different periods.

The least expensive pumps are those with no moving parts. One type of pump requires injection of the antibiotic solution, under some pressure, into a thick elastomeric "balloon." The pressure used to inject the antibiotic into the balloon then pushes the antibiotic through an infusion rate–limiting valve into the intravenous line and subsequently into the child. Other types of pumps are designed to place the antibiotic solution into a bag that then is placed into a device in which spring-loaded plates press on the solution contained in the bag, again pushing the antibiotic into the child through an infusion rate–limiting valve. These pumps generally use disposable infusion containers and are adequate for treatment with a single antibiotic administered up to a few times each day.

New tools for OPAT are becoming available each year. Resource guides to the equipment used for OPAT are available on the Internet.[35] Unfortunately for children, most of the equipment and resources available are designed for adults.

OUTCOME ANALYSIS

The anticipated outcome in the treatment of an infection is clinical and microbiologic cure with no complications of therapy. Although the psychosocial benefits of OPAT for children from a secure home environment were the original impetus for the establishment of pediatric programs, the economic benefits of outpatient therapy have been easier to document for both adults and children.[3,17,18,25,31,48,52-54] The cost of treatment is substantially less for outpatient therapy when neither hospital facilities nor 24-hour skilled nursing care is necessary. With shorter periods of hospitalization, the risk of acquiring a nosocomial viral or bacterial infection should diminish and further decrease the overall cost of care.

However, complications may occur in children receiving OPAT, just as they occur in inpatients.[16] The infection being treated may not be under control or may relapse while the child is being monitored by parents or home nursing personnel. Errors in antibiotic dosing and administration may occur,[26] particularly if parents are given the responsibility of preparing and administering antibiotics in the home. Complications of anti-infective therapy may increase when the number of anti-infective agents and doses administered to a child increases. Catheter- and pump-related complications may arise during therapy.[39] Parents may not be compliant with clinic visits and may not be in the home when the home nursing agency has scheduled a visit. Family members other than those trained to care for the child may provide care without the knowledge of the home nursing agency or physician. The clinician may need to rehospitalize the child if doing so is required to complete parenteral therapy safely.

JCAHO publishes standards[24] and reviews home care outcomes by evaluating specified parameters, including unscheduled inpatient admissions, early discontinuation of parenteral therapy, interruptions in parenteral therapy, catheter-related infections, and adverse drug reactions. Until recently, data on outcomes from various home nursing agencies were not made available to contracting physicians, hospitals, or payers. Outcome data now are required to be collected by home health organizations, which eventually will help standardize the assessment of OPAT programs. The physician or OPAT coordinator should request outcomes data from any home nursing agency proposing to care for a child by OPAT.

SUMMARY

Successful use of OPAT requires integrated delivery of care for children who are assessed to be at low risk for development of complications from their infections and therapy. Parents fulfill the role of the skilled pediatric nurse in the hospital and should be capable of providing a focused, limited assessment of their child and communicating any changes in the child's medical condition to the appropriate medical personnel. The anti-infective agents, infusion equipment, and medical follow-up all should be designed for the outpatient setting compatible with the training and resources available to parents or caregivers in the home. Outcomes of pediatric OPAT should be equivalent to or better than those achieved in an inpatient setting.

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CHAPTER 251

Antiviral Agents

Gail J. Demmler-Harrison

HISTORY AND BACKGROUND

The age of modern antiviral therapy began in the early 1950s, when methisazone, a derivative of the thiosemicarbazones (early antituberculosis compounds), was found to also have activity against vaccinia and variola viruses.[26,27,184,371,453,479] In 1959, the first antiherpes compound, idoxuridine, was synthesized; in 1962, it was approved for the topical treatment of herpetic keratitis.[244,358] Shortly thereafter, in 1964, trifluridine also was used to treat herpetic keratitis.[245,246] Also in 1964, the first description of vidarabine as an antiviral agent active against herpes simplex virus (HSV) was published, and amantadine was shown to have activity against influenza virus.[104,396] Two years later, amantadine was approved first for the prophylaxis and subsequently for the treatment of influenza A virus infection.[481] Shortly thereafter, in 1972, vidarabine was approved for the treatment of herpes encephalitis.[471] The year 1972 also marked the first description of ribavirin as a broad-spectrum antiviral agent, with activity against both DNA and RNA viruses, and in 1985, the aerosolized form of the drug was approved for the treatment of respiratory syncytial virus (RSV) bronchiolitis.[107,405] In 1977, acyclovir was reported to be a potent and selective inhibitor of the replication of both HSV and varicella-zoster virus (VZV) and boasted the added advantage of a favorable safety profile.[106,127,392] Subsequently, several structural analogues of acyclovir led to the development of antiviral agents with expanded antiviral spectra, such as ganciclovir, and agents with more favorable bioavailability, such as with the prodrugs valacyclovir and valganciclovir.[97,297] In the 1990s, the introduction of cidofovir, a broad-spectrum, long-lasting antiviral agent, and the neuraminidase inhibitors zanamivir and oseltamivir, which treat both influenza A and influenza B viruses, added new dimensions to antiviral therapy.[94,274,418] The research now being conducted no doubt will expand our antiviral armamentarium to combat viruses that may be used as biologic weapons as well as to prevent the emergence of resistant viruses and spawn creative strategies such as multidrug therapy and targeted delivery systems.[162,194]

Even though diseases caused by viruses (Table 251–1) now may be treated with a variety of antiviral agents, the close relationship between the viral replicative cycle and its host-cell metabolism unfortunately has caused the development of safe and effective antiviral agents to lag behind the development of other antimicrobials, such as antibiotics and antifungals. Clinically successful antiviral agents target and inhibit virus-specific functions while keeping cellular toxicity to a minimum. In addition, as a further testament to the codependency between

TABLE 251–1 Virus-Associated Diseases That May Be Treated with Antiviral Agents That Are Currently Available or Under Clinical Development

DNA Viruses
Herpes simplex virus
- Gingivostomatitis
- Keratoconjunctivitis
- Eczema herpeticum
- Whitlow
- Genital ulcers
- Esophagitis
- Hepatitis
- Encephalitis
- Aseptic meningitis
- Neonatal disease

Varicella-zoster virus
- Chickenpox
- Zoster
- Acute retinal necrosis
- Pneumonitis
- Hepatitis
- Cerebral vasculitis and stroke

Cytomegalovirus
- Retinitis
- Pneumonitis
- Esophagitis and colitis
- Fever and leukopenia syndrome
- Congenital disease

Epstein-Barr virus
- Mononucleosis syndrome
- Post-transplantation lymphoproliferative disease

Herpes B virus
- Encephalitis

Adenoviruses
- Disseminated disease
- Hemorrhagic cystitis
- Pneumonitis
- Colitis
- Conjunctivitis

Hepatitis B virus
- Acute and chronic hepatitis

Variola virus
- Smallpox

Vaccinia virus
- Vaccine-associated complications

Papillomaviruses
- Cutaneous and genital warts
- Laryngeal papillomatosis

Polyomaviruses (BK, JC, SV40)
- Hemorrhagic cystitis
- Progressive multifocal encephalopathy

RNA Viruses
Influenza viruses
- Influenza syndrome and complications

Parainfluenza viruses
- Laryngotracheobronchitis
- Pneumonitis

Respiratory syncytial virus
- Bronchiolitis
- Pneumonitis

Measles virus
- Measles syndrome
- Encephalitis
- Pneumonitis

Enteroviruses
- Aseptic meningitis and meningoencephalitis
- Myocarditis
- Neonatal disease

Arenavirus
- Lassa fever

Hepatitis C virus
- Acute and chronic hepatitis

viruses and host cells, some antiviral agents actually require cellular metabolism for antiviral activity, such as the terminal phosphorylation of acyclovir monophosphate to the active triphosphate form.

Antiviral agents can be categorized as virucidals, antiviral chemotherapeutic agents or drugs, and immunomodulators. Virucidal agents inactivate the virus on contact and include detergents, solvents, and ultraviolet light. These agents are not useful for treating human viral disease because healthy tissue also is destroyed. They may, however, be used to inactivate viruses on the surface of the skin or inanimate objects. Antiviral treatments that physically destroy both virus and the tissues infected or transformed by them include cryotherapy, laser therapy, and podophyllin and are used primarily to treat recalcitrant or life-threatening warts on mucocutaneous or laryngeotracheal tissue. These agents are discussed elsewhere. The host immune response also is important and in many cases essential for recovery from viral disease or even maintenance of a latent or inactivated state of the virus. Therefore, successful antiviral treatment may necessitate relief from immunosuppression when it is feasible, such as for patients with Epstein-Barr virus (EBV)–induced lymphoproliferative disease who are undergoing cancer chemotherapy or organ transplantation. It also may include the use of biologic response modifiers, or immunomodulators, that manipulate the immune system to enhance its ability to contain viral infection. Examples of immune modulators include immune globulin and monoclonal antibody preparations, cytokines such as interferons, and even novel approaches such as virus-specific cytotoxic T-cell lines designed to reconstitute host immunity.[15,24] Finally, updated knowledge about specific antiretroviral therapy is reviewed in Chapter 204.

Antiviral chemotherapeutic agents usually inhibit virus-specific events (Fig. 251–1), such as adsorption or attachment to the host cell (pleconaril), penetration and uncoating of the viral genome (amantadine), viral gene expression and nucleic acid synthesis (acyclovir, ganciclovir), and even viral assembly of intact, infectious viral particles (interferons). Therefore, antiviral agents exhibit their "antiviral effect" primarily while viral replication is active at the host-cell level. If the antiviral compound is withdrawn or discontinued, viral replication resumes. Furthermore, the currently available antiviral agents do not appear to eliminate viruses that are latent or in other dormant or nonreplicative states. The goal of antiviral therapy, for the most part, is to inhibit active viral replication to such a degree that the host immune response is able to contain or in some instances even eliminate the infection.

Antiviral agents usually have a narrow range of activity that can be predicted by their molecular mechanism of action. For example, rimantadine and amantadine have high activity against the RNA-containing influenza A virus, very limited activity against influenza B virus, and virtually no activity against the DNA herpesviruses,[94,104] whereas acyclovir, a deoxyguanosine analogue that requires monophosphorylation by the viral enzyme thymidine kinase (TK) for activation, has significant activity against DNA viruses (HSV), which carry TK, but no activity against RNA viruses such as influenza virus. Viruses infecting a host also may become resistant to a specific agent to which they originally were susceptible, usually by induced or selected mutations. Resistance is therefore most likely to occur in viruses that infect the host with a high viral load and that have a high intrinsic viral mutation rate as well as hosts who are exposed to selective drug pressure during chronic, low-dose, or repeated treatment with an antiviral agent.[213] Both resistant and sensitive viruses are capable of causing serious disease, especially in an immunocompromised host. Currently, most antiviral agents are administered alone. However, in the future, combination antiviral therapy, now a routine therapy in modern antiretroviral regimens, may someday be evaluated in clinical trials.[189,194] Such a strategy may

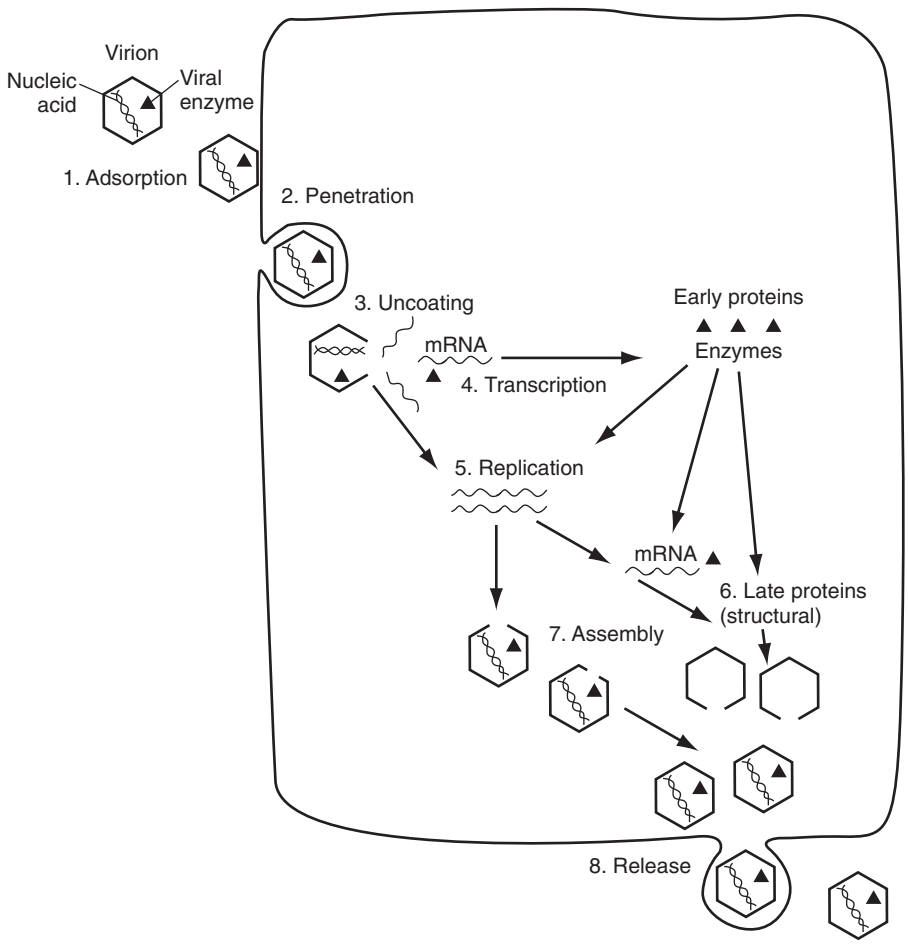


Figure 251–1 Basic steps in intracellular (DNA) replication. *1*, Adsorption of virion to the cell membrane. *2*, Penetration. *3*, Uncoating, removal of the protein coat. *4*, Early gene expression, transcription of viral proteins. *5*, Replication, synthesis of DNA strands. *6*, Late gene expression, transcription of messenger RNA, and translation of late protein synthesis. 7, Maturation and assembly of virions. *8*, Release.

increase antiviral effectiveness, prevent the emergence of drug resistance, and allow the administration of lower, less toxic dosages.

ANTIVIRAL AGENTS ACTIVE AGAINST RNA VIRUSES (Table 251–2)

AMANTADINE AND RIMANTADINE

Spectrum of Activity

Amantadine (1-adamantanamine hydrochloride) and rimantadine (α-methyl-1-adamantane methylamine hydrochloride) are tricyclic amines with specific activity against influenza A viruses (Fig. 251–2). Mean inhibitory concentrations of 0.1 to 0.4 μg/mL for amantadine have been reported, and rimantadine is 4 to 10 times more active than amantadine against influenza A virus.[56,192] Amantadine also has in vitro activity against rubella virus, but efficacy was not confirmed in animal models.[341,342,432] Much higher concentrations (10 to 50 μg/mL) appear to inhibit influenza B virus and parainfluenza viruses, but these high concentrations cannot be achieved safely in humans.[432] These agents also have activity against hepatitis C virus (HCV).[415]

Mechanism of Action and Resistance

The mechanism of action of both amantadine and rimantadine against influenza A virus appears to be primarily inhibition of the ion channel function of the M2 protein in the membrane of the virus, possibly affecting two stages of viral replication, uncoating and, to a lesser extent, assembly.[94,188] Amantadine and rimantadine resistance occurs when mutations cause an amino acid change in one of four critical sites (amino acids 26, 27, 30, or 31) in the transmembrane channel or domain of the M2 viral protein.[262] Such mutations occur frequently and rapidly during therapy because approximately 30 percent of adults and children who receive amantadine or rimantadine therapy for at least 5 days shed resistant influenza virus.[190,193] These resistant strains are genetically stable, may be transmitted to close contacts, and can produce disease.[31,190,489] Immunocompromised patients may shed drug-resistant influenza viruses for prolonged periods, and resistant strains can persist even after drug administration has been discontinued.[262] Resistant strains of influenza A subtype H3N2 have now produced community outbreaks and epidemics at this time.[31,190,202a,489] However, debate has begun among experts concerning whether patients in whom a resistant strain is identified should be isolated somehow until viral shedding has ceased.[180] Strains of influenza A virus resistant to amantadine also are resistant to rimantadine, and vice versa, but they are usually susceptible to ribavirin and neuraminidase inhibitors.

Pharmacokinetics

Amantadine is absorbed rapidly and nearly completely after oral administration, with peak plasma concentrations of 0.5 to 0.8 μg/mL attained within 2 hours of administration.[39,198] It is excreted almost unchanged in urine. Nasal and salivary secretions also contain high levels of amantadine, and the drug is present in cerebrospinal fluid (CSF) as well, at approximately half the plasma

TABLE 251–2 Clinical Indications and Usual Treatment Dosages for Antiviral Agents Active Against RNA Viruses

Agent	Indication	Dosage
Amantadine	Influenza A	2.2-4.4 mg/kg/dose PO bid (max, 100-200 mg daily)
Rimantadine	Influenza A	2.5 mg/kg/dose PO bid (max, 150 mg daily)
Oseltamivir	Influenza A and B	2 mg/kg/dose PO bid (max, 150 mg daily)
Zanamivir	Influenza A and B	5 mg/dose inhaled bid
Ribavirin	Respiratory syncytial virus	6 g/300 mL inhaled daily in an 18-hr period *or* 2-6 g/100 mL inhaled in a 2-hr period tid
	Hepatitis C	600 mg PO bid (with interferon-α)

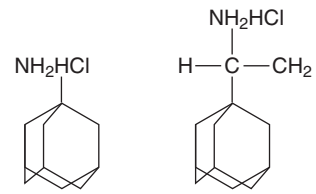


Figure 251–2 Structure of amantadine and rimantadine.

level. Dose reductions, adjusted according to creatinine clearance, are required in patients with renal insufficiency and renal failure.[222,423,481] Rimantadine, on the other hand, is more slowly absorbed than amantadine, with peak plasma levels of 0.4 to 0.6 μg/mL achieved within 2 to 4 hours of administration.[9,198,330] Rimantadine is also present in high concentration in nasal secretions. It is excreted in urine, but only after extensive metabolism by the liver. Dose reduction is therefore recommended in patients with renal or hepatic disease.[63,474] Hemodialysis does not appear to clear either drug, so supplemental doses usually are not recommended after dialysis.[423] When they are administered to chronically ill, institutionalized elderly patients, dosages also may need to be adjusted.[322,347] In addition, aerosolized forms of amantadine and rimantadine have been evaluated in human volunteers.[196,202]

Clinical Indications, Dosage, and Adverse Effects

CLINICAL INDICATIONS

Amantadine and rimantadine are equally effective for the treatment of disease caused by influenza A virus.[94,262,475] If they are administered within the first 48 hours of symptoms, both agents significantly reduce the signs and symptoms of influenza in both adults and children by significantly shortening the duration of fever and respiratory and systemic symptoms by 1 day.[94,109,287,454,475,484] The duration and amount of viral shedding from the nasopharyngeal tract also are decreased, and otologic complications may be reduced.[118] Both agents are effective for prophylaxis against influenza A virus infection and disease, with approximately 50 percent overall effectiveness in preventing infection with the virus and approximately 60 to 70 percent overall effectiveness (up to 90% in some studies) in preventing clinical disease and, in individuals with breakthrough clinical disease, in reducing the severity of the disease.* The spontaneous emergence of resistant strains during rimantadine or amantadine therapy and subsequent transmission of these resistant strains may lessen the effectiveness of prophylaxis in some families and nursing homes.[190,303] Amantadine and rimantadine are both available as tablets (100 mg) and syrup (50 mg/5 mL) for oral administration.

DOSAGE

The usual dose of amantadine for children is 4.4 to 8.8 mg/kg/day divided twice daily (many experts recommend 5 mg/kg/day), with a maximal recommended daily dose of 200 mg. The usual dose of rimantadine for children is 5 mg/kg/day divided twice daily, with a maximal recommended daily dose of 150 mg.[9,180,330,474] Older children and adolescents may take the usual adult dose for each drug, which is 200 mg/day given once daily or, for better tolerance, divided into two 100-mg doses daily.[9] Elderly patients should receive 100 mg daily or less if renal insufficiency is present. To be effective, these agents should be given as soon as possible after the onset of symptoms.[94,95] The duration of treatment of established influenza should be 3 to 7 days, or at least 2 days after the cessation of systemic signs and symptoms.[94] Prevention of influenza is accomplished best through annual vaccination; however, rimantadine and amantadine may be used to prevent or to lessen influenza symptoms in both adults and children.[83,94,96] Prophylaxis should be administered during the period of risk, whether exposure is in the family, community, or nosocomial setting. In general, prophylaxis should continue for at least 10 days after a known exposure occurs in an unprotected individual. For patients who receive the combination of inactivated vaccine and oral prophylaxis, rimantadine or amantadine should be continued for at least 2 to 4 weeks after vaccination, when maximal antibody response from the vaccine is expected to occur. For patients who are at high risk but did not receive the vaccine, oral prophylaxis should be continued for as long as the period of risk is anticipated and may be extended to the end of the community's influenza season. Doses of both drugs should be adjusted for patients with renal disease, and doses of rimantadine should be adjusted for patients with hepatic disease.[63,222,423,474] Prolonged treatment may be associated with side effects, especially in the elderly.[347] If side effects occur, the daily dose may be reduced to 100 mg and still be effective in most cases. The emergence of resistant strains during the period of drug administration may lessen the effectiveness of prophylaxis in some patients.[190,303] Combination with aerosolized ribavirin may enhance the antiviral effect of amantadine or rimantadine against influenza A virus.[189,194]

ADVERSE EFFECTS

The adverse effects of both amantadine and rimantadine include central nervous system (CNS) effects such as anxiety, depression, insomnia, dizziness, and impaired concentration; in children, these side effects appear to be rare, but they may be manifested as behavioral changes.[83,94,116,421] Rimantadine reportedly has fewer CNS side effects than amantadine does.[94,116,197,318] Hallucinations,

*See references 94, 110, 116, 156, 231, 232, 323, 338, 380, 467, 490.

tremors, and seizures also may occur, especially in patients with renal failure who have high plasma levels of amantadine. In addition, rare cases of coma and fatal cardiac dysrhythmia have been reported.[352,388] A prudent procedure is to administer amantadine and rimantadine with caution to individuals being treated for seizures or neuropsychiatric disorders or to patients also receiving other medications that affect the nervous system, such as antihistamines.[318] Furthermore, the institutionalized elderly may have increased plasma levels and experience side effects more often than younger patients do.[347] On occasion, gastrointestinal symptoms such as nausea and vomiting may accompany the oral administration of these agents in all age groups. Most reported adverse effects of these two agents have been reversible, with significant long-term complications being exceedingly rare. Of note, amantadine also is used to treat parkinsonism and drug-induced extrapyramidal reactions.[398]

NEURAMINIDASE INHIBITORS (ZANAMIVIR AND OSELTAMIVIR)

Spectrum of Activity

Zanamivir, (5-[acetylamino]-4-[(aminoiminomethyl)-amino]-2,6-anhydro-3,4,5-trideoxy-D-glycero-D-galacto-non-2-enoic acid) and oseltamivir, ([3*R*,4*R*,5*S*]-4-acetylamino-5-amino-3[1-ethylpropoxy]-1-cyclohexene-1-carboxylic acid ethyl ester phosphate [1 : 1]), are both analogues of *N*-acetylneuraminic acid, the cell surface receptor for influenza viruses, and were designed to inhibit the viral neuraminidase of influenza viruses (Fig. 251–3). Both agents are potent and specific inhibitors of the neuraminidases of influenza A and B viruses, with 90 percent of the strains inhibited at concentrations of 0.05 to 100 μmol/L.[254,456] The compounds also are capable of inhibiting neuraminidases from other pathogens and mammalian cells, but only at extremely high concentrations.

Mechanism of Action and Resistance

Neuraminidase permits influenza virus to penetrate through the mucoproteins present in respiratory secretions to the surfaces of cells, and inhibition of this enzyme prevents viral access and infection of cells. Neuraminidase also destroys receptors recognized by viral hemagglutinin and is therefore necessary for optimal release of influenza virus particles from infected cells. Inhibition prevents cell-to-cell spread of virus within the respiratory tract and lessens the intensity of the initial infection.[87,94,254,332,456] Mutations that induce amino acid changes in viral neuraminidase alter enzyme stability or activity, mutations in hemagglutinin produce a reduced affinity for cell receptors, and mutations in both produce resistant influenza viruses.[94,172-175] Cross-resistance to both zanamivir and oseltamivir also can occur, especially if the mutation is in the hemagglutinin portion.[313] Resistance has been documented not only by in vitro passage of the virus in increasing concentrations of the agents but also in patients receiving these agents. Emergence of influenza A H1N1 subtypes resistant to oseltamivir is also a concern, and clinicians should monitor antiviral resistance trends (www.cdc.gov/flu/).[202a] Clinical trials of oral oseltamivir for the treatment of naturally occurring influenza detected resistance in 1 to 2 percent of post-treatment isolates from adults and adolescents and in 5 to 8 percent of isolates from younger children.[94,174,222,486] Zanamivir resistance has been documented in one immunocompromised child receiving the drug for 2 weeks, but not in large, randomized trials of healthy individuals.[172,195,199,203,320] Preliminary evidence suggested mutant resistant strains may have reduced infectivity or virulence in comparison to susceptible strains,[442] but some resistant strains have caused outbreaks and epidemics.[202a]

Pharmacokinetics

Zanamivir is administered topically as an inhaled dry powder and is deposited in the oropharynx and, to a lesser extent, in the tracheobronchial tree and lungs.[94] Approximately 4 to 17 percent of inhaled drug is absorbed systemically, and levels of 17 to 142 ng/mL can be detected in serum 1 to 2 hours after administration of a typical inhaled dose of 10 mg to adults.[64] Serum levels in pediatric patients who received an inhaled dose were extremely low (<10 ng/mL) or undetectable, which may be related to the ability of the patient to cooperate with the inhaled drug delivery system. The oral bioavailability of zanamivir is extremely low, with less than 5 percent of the administered dose absorbed, and therefore it is not administered in this form. Intravenous and intramuscular preparations of zanamivir also have been tested in clinical trials, but this form is not available clinically at this time.[62,64,153,202a] The drug is excreted unchanged in urine.

Oseltamivir, in contrast, has good oral bioavailability. The prodrug compound oseltamivir phosphate is metabolized by hepatic esterases to the parent active compound oseltamivir carboxylate.[94] Approximately 75 to 80 percent of the administered dose is absorbed, and peak plasma concentrations are reached within 3 to 4 hours but may be delayed, but not reduced, if the drug is given with food. A typical 75-mg dose will provide plasma concentrations of 0.3 to 0.5 μg/mL. Both the prodrug and the active form are eliminated unchanged through the kidneys, and dosages should be adjusted for patients with renal insufficiency or renal failure. Whether the drug dosage should be adjusted for patients with hepatic disease or liver failure is unclear from the available evidence. Information from a small number of pediatric patients suggests that the drug may be eliminated faster in younger children. Elderly, geriatric patients, however, appear to metabolize the drug similar to young adults.

Clinical Indications, Dosage, and Adverse Effects

CLINICAL INDICATIONS

Zanamivir and oseltamivir have been shown to be effective in treating both influenza A and influenza B infection.[303a] However,

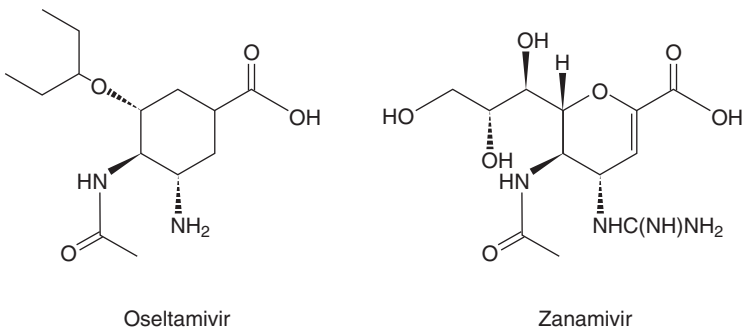


Figure 251–3 Structure of the neuraminidase inhibitors oseltamivir and zanamivir.

susceptibility of type B and HSNI and H1N1 subtypes of type A influenza may be reduced for oseltaminivir.[439a] When they are administered within the first 2 days of illness, they provide relief from symptoms up to 1.5 days earlier than placebo does and reduce the quantity and duration of viral shedding.* In some studies, the frequency of secondary complications such as sinusitis, bronchitis, and otitis media with effusion (but not pneumonia) also was reduced.[94,461] Both antiviral agents are effective for the prophylaxis of influenza A and influenza B infection in both adults and children in family, community, and nursing home situations. They appear to be 30 to 50 percent effective in preventing viral infection and 64 to 84 percent effective in preventing disease.[94,195,201,305,324,461] However, up to 89 percent efficacy in preventing disease in families has been reported, and in elderly patients in nursing homes, 92 percent of cases of influenza may be prevented if the agents are administered to those who also have received influenza vaccine.[94,195,305]

DOSAGE

Zanamivir is administered by oral inhalation with an inhalation device provided with the product. Each inhalation dose is 5 mg, and two inhalations twice daily for 5 days is the usual recommended dosage for all age groups. Use of this drug and device is dependent on the understanding and cooperation of the patient; therefore, it is not indicated for use in children younger than 7 years and may be of limited use in older children and adults who are unable to cooperate with the delivery system. No reduction in dosage is needed for patients with impaired renal function because very little of the inhaled drug is absorbed systemically. Oseltamivir is administered orally and is available in 75-mg capsules and a 12-mg/mL suspension. The usual adult dosage is 75 mg twice daily for treatment and once or twice daily for as long as 6 weeks for prophylaxis. The pediatric dose is 2 mg/kg per dose given twice daily (4 mg/kg/day divided twice daily).[94]

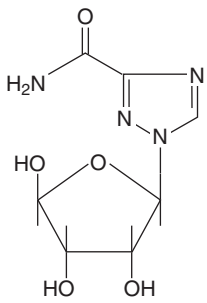
ADVERSE EFFECTS

Both neuraminidase inhibitors are generally well tolerated, with very few if any serious adverse effects observed in clinical trials. Zanamivir inhalation, however, may produce local irritation or bronchospasm in some patients, and oseltamivir has been associated with nausea and vomiting, which may be lessened by administration of the drug with food.[94]

RIBAVIRIN

Spectrum of Activity

Ribavirin (1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamide) is a synthetic guanosine analogue with broad-spectrum antiviral activity[412] (Fig. 251–4). It inhibits the in vitro replication of a wide variety of RNA viruses, including orthomyxoviruses such as influenza A and B viruses; paramyxoviruses, such as RSV, parainfluenza viruses, and measles virus; arenaviruses, such as Lassa fever virus; bunyaviruses that cause viral encephalitis; togaviruses, such as HCV; enteroviruses, such as polioviruses and coxsackie B virus; and retroviruses, including human immunodeficiency virus (HIV) type 1.[136,225,252,275,277,340,405] Also inhibited by ribavirin are many DNA viruses, such as adenoviruses, hepatitis A virus, cytomegalovirus (CMV); and poxviruses, such as vaccinia virus.[117] It inhibits most orthomyxoviruses and paramyxoviruses at concentrations of 3 to 10 μg/mL and Lassa fever virus at 4 to 40 μmol/L.[224] Because most of its clinical use has been for the treatment of RNA viruses, especially RSV, ribavirin is included in this section of the chapter.



Ribavirin

Figure 251–4 Structure of ribavirin.

Mechanism of Action and Resistance

Ribavirin most likely exerts it antiviral effects by altering the cellular nucleotide pools and by interfering with viral mRNA formation.[55,136,434] The compound is phosphorylated by host-cell enzymes to monophosphate, diphosphate, and triphosphate active forms, each with specific antiviral actions.[410] For example, ribavirin monophosphate competitively interferes with the synthesis of guanosine triphosphate and, subsequently, with viral nucleic acid synthesis. Ribavirin triphosphate inhibits the RNA polymerase of influenza virus and also interferes with the capping of its viral mRNA.[136,252,340,480] In addition, the diphosphate and triphosphate forms of ribavirin appear to inhibit the reverse transcriptase activity of HIV-1.[140] The mechanism of action of ribavirin against HCV and other viruses, however, is not well understood. Ribavirin also exerts an effect on the host immune system by inhibiting mast cell secretory responses and diminishing IgE responses.[298] Antiviral resistance is not well characterized and appears to be a rare occurrence. No ribavirin-resistant RSV isolates have been identified during clinical trials, and information is scant regarding the resistance of other viruses to ribavirin.[182,293,393]

Pharmacokinetics

Ribavirin can be administered topically by aerosol, orally, and intravenously. Aerosol administration of ribavirin with a small-particle aerosol generator delivers very high concentrations (>1000 μg/mL) of the drug to the respiratory tract.[264] The aerosolized drug also is absorbed systemically, and peak plasma levels after 8 hours of continuous aerosol administration range from 0.5 to 2.2 μg/mL; after 20 hours, they reach 0.8 to 3.3 μg/mL. After oral administration, bioavailability is 33 to 45 percent, and peak plasma levels of 1.3 to 3.2 μg/mL have been observed 1 to 2 hours after administration. Intravenous administration of ribavirin produces 10-fold higher plasma levels in less than 1 hour than after the administration of equivalent doses by different routes.[345] The higher doses used to treat Lassa fever produce plasma levels of up to 24 μg/mL. Ribavirin also crosses the blood-brain barrier and is present in CSF at approximately 70 percent of the plasma level.[91,223] The drug is metabolized by the liver, and approximately 40 percent of the dose is excreted by the kidneys.[345] The triphosphate form also concentrates in erythrocytes at an erythrocyte-to-plasma ratio of 40 : 1 and is slowly eliminated with a half-life of 40 days or longer. Hemodialysis and hemofiltration do not remove significant amounts of ribavirin from the body.[267]

*See references 61, 94, 199, 262, 305, 320, 325, 355, 450, 473.

Clinical Indications, Dosage, and Adverse Effects

CLINICAL INDICATIONS

Ribavirin aerosol is licensed for the treatment of RSV bronchiolitis and pneumonia in children.[179] Treatment shortens the duration of viral shedding and improves the fever, respiratory rate, arterial oxygen saturation, and lower respiratory tract signs on physical examination in severely ill patients who receive the antiviral agent early in the course of their illness.*

No clear benefit has been observed in patients with mild disease or in those who receive treatment late in the course of the disease. Studies of infants receiving mechanical ventilation have reported conflicting results in clinical benefit.[310,321,413] However, despite numerous clinical studies, indications for treatment remain controversial because efficacy has not been documented convincingly in all studies and the benefits observed generally have been mild and difficult to quantitate objectively.[179] Nonetheless, because antiviral treatment clearly appeared to provide benefit to some children, in 1996, the American Academy of Pediatrics recommended that ribavirin be considered for the treatment of hospitalized infants and children with severe lower respiratory tract infection caused by RSV, and these recommendations remain in effect.

Patients thought to potentially benefit the most from treatment are those younger than 6 weeks; those with chronic illnesses, such as congenital heart disease and chronic lung disease; premature infants; immunosuppressed children; and those with neurologic or neuromuscular disorders. Treatment with ribavirin also appears to decrease nasopharyngeal secretion of RSV-specific IgE and IgA responses.[378] Long-term follow-up of treated patients suggests no adverse effects but possible improvement in pulmonary function.[206,238,290] Treatment may benefit immunocompromised children who have serious or prolonged RSV infection, such as those with congenital severe combined immunodeficiency.[308] Aerosolized or intravenous ribavirin, especially combined with intravenous immune globulin or hyperimmune globulin, also may be of benefit to bone marrow transplant recipients with RSV lower tract disease.[290,292,470] Anecdotal reports suggest that early administration of ribavirin to immunocompromised patients during upper respiratory tract disease may be more efficacious than supportive care alone in preventing progression to lower tract disease or pneumonia.[132,308] Laboratory confirmation of RSV infection by viral culture or rapid diagnostic tests that detect viral antigen in respiratory secretions is recommended for all patients before treatment with ribavirin is initiated or continued beyond 12 to 24 hours. Ribavirin also is licensed in capsule form for the treatment of chronic hepatitis caused by HCV, and in combination with interferon-α, it produces a sustained decrease in HCV RNA levels, improvement in systemic symptoms such as fatigue, reduction in transaminases, and improvement in liver histologic features. These clinical benefits have been observed during initial treatment in previously untreated patients, in individuals initially unresponsive to interferon alone, and in patients treated for relapse.[38,41,105,114,152,221,307,365] Treatment with high doses of orally or intravenously administered ribavirin significantly reduces mortality rates in patients with Lassa fever.[237,306] Ribavirin also appears to benefit patients suffering from other hemorrhagic fever syndromes, including Argentine, Sabia, Bolivian, and Crimean-Congo hemorrhagic fevers, as well as those who have hemorrhagic fever with renal failure syndrome.[134,143,226,227,253] In addition, ribavirin may benefit patients with viral encephalitis, but clinical trials have not been performed.[65] Oral ribavirin also may be of benefit prophylactically in individuals who are exposed to hemorrhagic fever syndromes, including Lassa fever.[220] Treatment of hantavirus pulmonary syndrome with intravenously administered ribavirin is being evaluated in clinical trials. Ribavirin also may benefit individuals with influenza A or B virus infection. In one randomized study of young adults suffering from influenza, aerosol treatment significantly reduced fever, systemic signs and symptoms, and virus titer in respiratory secretions.[412] Another study showed clinical benefit with high-dose oral ribavirin, but other studies have not confirmed a measurable clinical benefit or virologic response consistently with aerosolized or oral ribavirin.[34,263,412,431] Intravenous ribavirin also has been used anecdotally to treat serious, life-threatening influenza.[200,361] Early treatment with aerosolized or intravenous ribavirin in bone marrow and stem cell transplant recipients infected with parainfluenza virus may help prevent progression to lower tract disease and pneumonia, but controlled trials documenting efficacy and effect on overall mortality rates have not been published.[128,200,468] Ribavirin also has been used to treat measles pneumonia in immunocompetent and immunocompromised patients, with clinical benefit reported in some.[146,176,242]

Clinical benefit, but not cure, also has been documented in case reports of children with subacute sclerosing panencephalitis caused by measles virus.[223,224,329,443] In these reports, repeated courses of large doses of ribavirin administered intravenously, combined with interferon-α, provided serum and CSF levels that exceeded the minimal inhibitory concentrations needed to inhibit the virus in vitro and improved or stabilized seizures and neurologic status in treated patients.[223] Case reports and small series using ribavirin to treat serious adenoviral disease, including pneumonia and disseminated adenoviral disease, have been published, but no controlled trials documenting clinical benefit have been published.[22,46,69,160,302,327] Despite documented antiretroviral activity, treatment of HIV-infected patients with oral ribavirin has not shown clinical, virologic, or immunologic benefits consistently.[91,140,239]

DOSAGE

A number of different dosages and routes of administration have been used to administer ribavirin to a variety of patients experiencing various infections. In normal and immunocompromised patients of all ages, the usual dosage of ribavirin, when it is administered topically by aerosol to the respiratory tract for treatment of influenza and lower respiratory tract disease caused by RSV, parainfluenza virus, and measles virus, is 6 g of drug reconstituted in a final volume of 300 mL of sterile water (final concentration, 20 mg/mL); a small-particle aerosol generator (SPAG-2 unit) is used to administer the drug continuously during a period of 12 to 18 hours for 3 to 7 days.* The drug may be delivered to an infant oxygen hood or tent, by facemask, or through pressure- or volume-cycled ventilators, provided ventilatory pressure is monitored and the device is checked for precipitation, which may cause ventilator dysfunction.[151,339]

Intermittent high-dose therapy, with drug administered at 60 mg/mL for 2 hours three times daily for 5 days, also appears to be effective and may be better tolerated in some older patients.[131] Longer courses of aerosolized therapy for up to 14 days have been used in severely immunocompromised patients with prolonged viral shedding and severe disease.[285] Oral doses of up to 600 mg twice daily for 24 to 48 weeks, usually combined with interferon-α, are used to treat HCV in adults.[38,41,105,114,221,307,365] High doses of oral ribavirin also appear to be effective in treating influenza in normal hosts.[432] Even higher doses of oral ribavirin (2-g loading dose, followed by 2 g/day divided every 8 hours for 10 days)

*See references 171, 181, 206, 238, 310, 321, 359, 413, 441, 469.

*See references 34, 128, 171, 181, 182, 242, 263, 285, 302, 310, 321, 327, 412, 413, 441, 470.

have been used for the treatment of Lassa fever and other hemorrhagic fever syndromes in adults.[134,143,220,227,237,253,306] The intravenous dose of ribavirin used to treat Lassa fever is a 2-g loading dose (25 to 33 mg/kg estimated pediatric dose), followed by 1-g doses (16 mg/kg estimated pediatric dose) every 6 hours for 4 days and then 500-mg doses (8 mg/kg estimated pediatric dose) every 8 hours for 3 days to complete a 7- to 10-day course of treatment.[237,306] This intravenous dosage regimen also has been used to treat measles pneumonia, severe influenza with complications, pneumonia caused by parainfluenza virus in immunocompromised patients, and disseminated adenovirus disease.[146,176,292,361] Continuous intravenous infusions of ribavirin have been used in patients with severe disease caused by influenza and parainfluenza viruses.[200] Doses used to treat subacute sclerosing panencephalitis in children have been considerably higher and administered for longer periods.[223,329] In one report, pediatric patients received interferon-α combined with 10 mg/kg of ribavirin administered intravenously every 8 hours daily for 7 days. Escalating doses of 20 and then 30 mg/kg given every 8 hours daily for 7 days at 7-day intervals then were administered.[223] The highest dose tolerated was given for 7 days, repeated in 7-day intervals, and administered for a period of 6 months.

ADVERSE EFFECTS

Adverse effects of aerosolized ribavirin are unusual but include local irritation causing facial rash and conjunctivitis. Abnormalities in pulmonary function, including worsening of respiratory distress and bronchospasm, also have been reported.[165,182,441] In addition, one case of water intoxication associated with aerosolized ribavirin administration has been reported.[447] Older children, adolescents, and adults may be noncompliant with treatment or may even have anxiety attacks or experience other forms of psychological stress as they are being confined for continuous treatment for a 12- to 18-hour period. In these cases, intermittent, high-dose regimens may be better tolerated and might enhance compliance of the patient.[131] Precipitation of the drug in ventilator tubing systems and on filtering devices may cause ventilator dysfunction and respiratory distress or failure in mechanically ventilated patients, especially those receiving high-dose intermittent administration, but such events may be minimized by frequent monitoring of ventilator pressure and changing of the filters.[151,310,321,339] Adverse hematologic effects such as anemia have not been documented consistently in patients who received aerosolized ribavirin. The environmental exposure to aerosolized ribavirin by health care workers providing direct care to a virus-infected patient may, in some instances, cause headache, conjunctivitis, contact lens damage, watery eyes, and bronchospasm in those with underlying reactive airway disease.[49,165,372,404] These potential adverse effects are probably more likely to be seen in instances in which ribavirin is administered by hood, tent, or facemask than when it is administered to mechanically ventilated patients.[50] Environmental exposure of health care workers and family members may be reduced by providing routine patient care during periods when the aerosol generator has been turned off, by turning off the aerosol generator during periods of more urgent care, and by using protective equipment such as masks and goggles. Dose-related, reversible, usually mild anemia occurs frequently during and after long-term oral therapy and intravenous administration of ribavirin.[221,239] Rarely, severe anemia may occur and necessitate dose reduction or cessation of therapy. Reversible increases in serum bilirubin, serum iron, and uric acid concentrations also have been documented during oral therapy. On rare occasion, intravenous administration of ribavirin has produced chills and rigors.[142,165] Ribavirin has shown teratogenic, mutagenic, and embryotoxic effects in preclinical animal models.[211] Therefore, investigators recommend that exposure of pregnant women to ribavirin, either therapeutically or through environmental exposure, be minimized or prevented whenever possible or practical.

PLECONARIL

Spectrum of Activity

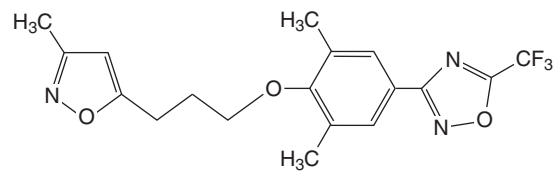
Pleconaril (3-[3,5-dimethyl-4[[3-(3-methyl-5-isoxazolyl)propyl]oly]phenyl]-5-(trifluoromethyl)-1,2,4-oxadiazole) is an antiviral agent with significant and specific activity against picornaviruses, both enteroviruses and rhinoviruses (Fig. 251–5). In vitro, pleconaril inhibits the replication of most serotypes of enteroviruses at concentrations between 0.01 and 0.1 μg/mL.[351] Echovirus 11, a commonly isolated enterovirus in the United States, appears to be the most sensitive enterovirus tested to date (inhibited at a mean concentration of 0.006 μg/mL). Most rhinovirus serotypes are inhibited at slightly higher concentrations of 0.21 to 0.78 mg/mL but still within ranges safely achievable in patients.

Mechanism of Action and Resistance

Pleconaril binds efficiently to a specific hydrophobic pocket within the viral capsid of picornaviruses and exhibits an antiviral effect by interfering with uncoating, attachment, and cell-to-cell transmission of infectious viral particles.[379] The drug induces a more rigid structure within the viral capsid, which then interferes with viral uncoating and release of viral RNA.[148,309,350,351,373,379] Pleconaril also appears to change the conformation of the canyon floor of the virus capsid when it integrates with the underlying pocket, thereby interfering with attachment of the virus to host-cell receptors.[350,379] Resistance of picornaviruses to pleconaril has not been reported.

Pharmacokinetics

The pharmacokinetics of pleconaril varies with the age of the group tested. After oral administration of single doses of 200 to 400 mg to adults, peak plasma concentrations of 1.1 to 2.4 μg/mL are achieved in 1.5 to 5 hours, with an average elimination half-life of 25 hours.[2] Repeated dosing for a 7-day period increases plasma concentrations to 1.33 to 3.4 μg/mL. When it is taken with food, especially a meal high in fat, absorption of pleconaril is enhanced significantly.[1,248] Single oral doses of 5 mg/kg in children provide plasma concentrations of approximately 1.3 μg/mL with a more rapid elimination half-life of approximately 6 hours.[247] Neonates also absorb pleconaril well, but they do not exhibit dose-proportionate pharmacokinetics as adults do.[248] The drug also has a large volume of distribution and concentrates in tissues such as the liver, brain, and nasal epithelium, where viral replication and virus-induced disease are likely to occur.[248] Pleconaril appears to be excreted in feces and urine.



Pleconaril

Figure 251–5 Structure of pleconaril.

Clinical Indications, Dosage, and Adverse Effects

CLINICAL INDICATIONS

Pleconaril currently is not licensed for therapeutic use. Pleconaril has shown efficacy in early clinical trials against picornavirus infections in adults, children, and neonates. In pretreated adults experimentally infected with coxsackie A21 virus, pleconaril significantly reduced viral titers as well as the clinical parameters of fever, nasal mucus production, and systemic symptoms compared with placebo.[191,379,394] In another trial, pleconaril resolved the symptoms of illness 2 days earlier than placebo did, and patients who received pleconaril did not require as much concomitant medication for symptomatic relief as did those who received placebo.[379] In two placebo-controlled trials in adults with aseptic meningitis caused by enterovirus, pleconaril treatment reduced the duration of headache and other symptoms of meningitis by 2 to 3 days and allowed treated patients to return to work and routine daily activities 2 days earlier than patients who received placebo.[379,394] In a placebo-controlled trial in pediatric patients with aseptic meningitis caused by enterovirus, pleconaril significantly improved illness scores and reduced the duration of headache in older children, but clinical efficacy was difficult to assess in infants and younger children.[2a] Viral shedding from the throat also was reduced in the treatment group.[389] In addition to treatment for the "common cold" and for "viral meningitis," another potential use for pleconaril is for the treatment of serious, persistent, or life-threatening illness caused by enteroviruses. More than 90 patients with unusual or life-threatening infections with enterovirus have received pleconaril on a compassionate-use basis.[148] These patients included immunocompromised patients with chronic meningitis, neonates with disseminated disease, patients with myocarditis,[374] and individuals with poliomyelitis associated with vaccine or wild-type poliovirus. In all groups, most of the patients showed clinical and virologic responses.

DOSAGE

Pleconaril is manufactured as a hard gelatin capsule and an oral solution. The capsule contains 200 mg of drug, and doses used in adults and older children have been 200 to 400 mg every 8 hours for 7 days (21 doses). An oral solution also has been prepared for pediatric use. It contains 40 mg/mL of pleconaril, medium-chain triglycerides, two surfactants (Tween 80 and Arlacel), 1.5% saccharin, and cherry-peppermint flavoring. Doses of 5 to 7.5 mg/kg of the oral solution administered every 8 to 12 hours for 7 days (14 to 21 doses) have been used in clinical trials of young children, infants, and neonates.[379] Oral pleconaril is generally well tolerated by all age groups.

ADVERSE EFFECTS

Adverse effects associated with pleconaril treatment include nausea, diarrhea, stomach upset or discomfort, and headache. Serious toxicities have not been documented to date, and preclinical studies did not show teratogenicity or fetal toxicity.

ANTIVIRAL AGENTS ACTIVE AGAINST DNA VIRUSES (Table 251–3)

ACYCLOVIR

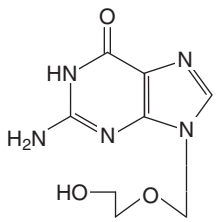
Spectrum of Activity

Acyclovir, 9-[(2-hydroxyethoxy)methyl]-9H-guanine (also known as acycloguanosine), is an analogue of the nucleoside deoxyguanosine that has selective activity against the herpes family of viruses[101,127] (Fig. 251–6). It has the most potent antiviral activity against HSV type 1 and inhibits the virus at concentrations of

TABLE 251–3 Clinical Indications and Usual Treatment Dosages for Antiviral Agents Active Against DNA Viruses

Agent	Indication	Dosage
Acyclovir	HSV, mucocutaneous	5% ointment 6× daily 15 mg/kg/dose PO 5× daily (max, 200 mg/dose) 5-10 mg/kg/dose IV q8h
	HSV, encephalitis	10-20 mg/kg/dose IV q8h
	HSV, neonatal	20 mg/kg/dose IV q8h
	VZV, chickenpox and zoster	20 mg/kg/dose (max, 800 mg) PO q6h 10-20 mg/kg/dose (500 mg/m^2/dose) IV q8h
Valacyclovir	HSV	500-1000 mg/dose PO bid*
	VZV, zoster	1000 mg/dose PO bid*
Penciclovir	HSV, mucocutaneous	1% cream q2h
Famciclovir	HSV, mucocutaneous	125-500 mg PO bid*
	VZV, zoster	500 mg PO tid*
Ganciclovir	CMV	5-6 mg/kg/dose IV q12h—induction 5 mg/kg/dose qd 5 days/wk *or* 1000 mg PO tid* *or* 500 mg PO 6× daily*—maintenance
Valganciclovir	CMV	900 mg PO bid*—induction 900 mg PO qd*—maintenance
Foscarnet	CMV	60 mg/kg/dose IV q8h—induction 90-120 mg/kg/dose daily—maintenance
Cidofovir	CMV	5 mg/kg/dose IV weekly *or* 1 mg/kg/dose IV 3×/wk—induction 5 mg/kg q2wk—maintenance

**Only adult dosages are available at this time.*
CMV, cytomegalovirus; HSV, herpes simplex virus; VZV, varicella-zoster virus.



Acyclovir

Figure 251–6 Structure of acyclovir.

0.02 to 0.9 μg/mL.[459,472] Inhibitory concentrations of HSV type 2 and VZV are also high, at 0.3 to 2.2 μg/mL and 0.8 to 4.0 μg/mL, respectively.[37,392,459,472] In addition, EBV replication is inhibited by acyclovir at a mean concentration of 1.6 μg/mL, but the drug has no effect on cells latently infected with EBV.[85,286] CMV also is inhibited by very high concentrations of acyclovir (2 to 57 μg/mL).[37] Acyclovir has in vitro activity against simian herpesvirus B and hepatitis B virus (HBV).[33,219,459]

Mechanism of Action and Resistance

Acyclovir is phosphorylated to acyclovir monophosphate by viral TK. Cellular enzymes then convert the monophosphate form to acyclovir triphosphate, the active form of the drug.[472] Acyclovir triphosphate is present in HSV-infected cells at 40 to 100 times the concentration found in uninfected cells, and it competitively inhibits viral DNA polymerase in infected cells. Acyclovir triphosphate also is incorporated into viral DNA and, because it lacks the 3′-hydroxyl group, acts as a chain terminator during viral DNA synthesis. The DNA polymerase enzymes of the various herpesviruses differ in their degree of inhibition by acyclovir triphosphate, and for EBV and CMV, which do not contain viral TK, this inhibition accounts for most of the antiviral effect of the drug.[111,154] Host cellular growth also can be inhibited by acyclovir, but only at levels thousands of times higher than those that inhibit viral replication. This relatively selective mechanism of antiviral action provides a uniquely high therapeutic index.

Three basic mechanisms of resistance have been identified for herpesviruses to become resistant to acyclovir: deficient TK activity caused by absent or low production of viral TK, altered TK activity caused by abnormal substrate specificity so that the enzyme is present and able to phosphorylate but just does not phosphorylate acyclovir, and altered DNA polymerase.[70] Most clinical isolates of HSV that are resistant to acyclovir have deficient TK activity, and most resistant VZV isolates have altered TK activity or altered DNA polymerase.[346] The prevalence of acyclovir-resistant HSV isolates in normal hosts is less than 1 percent, but it can be as high as 10 to 20 percent in immunocompromised hosts receiving acyclovir therapy for 2 weeks or longer.[79,100,133,159,208] Resistant strains of VZV, on the other hand, are unusual.[86] Resistant HSV and VZV isolates retain virulence and can cause disease, especially extensive mucocutaneous lesions in immunocompromised hosts, but invasive diseases such as keratitis, uveitis, meningoencephalitis, and pneumonia also have occurred with resistant strains of HSV.[79,133,159,208,233,417] Immunocompromised hosts may shed resistant strains of HSV for prolonged periods and may experience recurrent disease with either sensitive or resistant strains. Such mutants are also cross-resistant to other antiviral agents that require viral TK for phosphorylation and activation (e.g., penciclovir and ganciclovir), but they are inhibited by antiviral agents that have different mechanisms of action (e.g., foscarnet, cidofovir, and trifluridine).

Pharmacokinetics

Acyclovir is available for topical, oral, and intravenous administration. Systemic absorption of acyclovir after topical application to intact skin is minimal (<0.01 μg/mL), and in one study in which acyclovir ointment was applied to zoster lesions in immunocompromised patients, plasma concentrations of less than 0.1 to 0.78 μg/mL were observed, thus suggesting some degree of percutaneous absorption.[93,459] The bioavailability of oral acyclovir is low (15-21%), with peak plasma concentrations of 0.4 to 0.8 μg/mL observed after the intake of one 200-mg capsule and concentrations of up to 1.6 μg/mL observed after an 800-mg dose.[40] When an equivalent dose in liquid suspension is administered to children, slightly lower peak plasma levels of 1.0 μg/mL were observed.[438] Intravenous infusion of 5 mg/kg for 1 hour provides peak plasma levels of 9.8 μg/mL, whereas plasma levels of 20.7 μg/mL are produced after a 10-mg/kg infusion. Most of the administered dose of acyclovir is excreted unchanged in urine.[459] The elimination half-life of acyclovir is 2.5 to 3 hours in adult patients with normal renal function, slightly longer (3.8 hours) in neonates, and up to 20 hours in anuric patients.[40,150,212] Therefore, dose adjustment, according to creatinine clearance, is needed in patients with renal insufficiency or renal failure.[278] Probenecid administration decreases renal clearance and prolongs the half-life of acyclovir. Acyclovir is distributed widely into a variety of body fluids, including saliva (13% of plasma levels), vaginal secretions (15-170% of plasma levels), zoster vesicular fluid (90-100% of plasma levels), aqueous humor (37% of plasma levels), breast milk (>300% of plasma levels), amniotic fluid and placenta (>200% of plasma levels), and CSF (50% of plasma levels).[150,229,259,279,316] More than half the drug is removed by hemodialysis but very little after peritoneal dialysis.[268,278]

Clinical Indications, Dosage, and Adverse Effects

CLINICAL INDICATIONS

Topical acyclovir may decrease healing time and viral shedding slightly in mucocutaneous lesions of patients experiencing primary infection with HSV.[93] Little if any clinical benefit has been documented in patients with recurrent herpes simplex lesions; however, some patients report a soothing sensation after application of ointment to the lesions. Topical therapy also may produce a mild clinical benefit in immunocompromised patients with zoster lesions.[459] Oral acyclovir significantly reduces viral shedding, clinical symptoms, and time until lesion healing in normal and immunocompromised patients with a variety of mucocutaneous lesions (e.g., orolabial, pharyngeal, genital, rectal, gingivostomatitis, whitlow, skin) associated with primary HSV infection.[8,375,472] It also has been used to treat eye disease, including dendritic corneal ulcers.[90,205]

Oral acyclovir provides benefit to normal and immunocompromised children, adolescents, and adults with primary infection with VZV (varicella or chickenpox). Treatment reduces the duration of fever by 24 hours, the mean number of skin lesions, and the time to total crusting of lesions by 2 days. Postexposure prophylaxis with oral acyclovir also may reduce the risk of acquiring varicella by close and household contacts of patients with varicella. Administration of oral acyclovir during pregnancy is likely to clinically benefit a mother with varicella, but whether it reduces the risk of congenital or neonatal disease is not clear at this time. Recurrent HSV disease also is improved with oral acyclovir therapy. When it is administered during the prodrome or at the very first sign of lesions, treatment reduces viral shedding and time to lesion healing by 1.5 to 2 days.[366,460] Long-term, continuous suppressive therapy with oral acyclovir may be of benefit in some patients with frequent episodes of recurrent mucocutaneous disease, but asymptomatic shedding and person-to-person

transmission still may occur.[166,376] Patients with recurrent skin disease, whitlow, or erythema multiforme may benefit from long-term suppressive therapy with oral acyclovir. Oral acyclovir suppression also reduces recurrences of genital herpes simplex during the last trimester of pregnancy and decreases the need for cesarean section delivery.[53,397,399-401] Short-term prophylaxis administered during a period of risk for recurrence (sunlight exposure, for example) may reduce the clinical recurrence of mucocutaneous disease. Oral acyclovir reduces virus shedding, time to skin lesion healing, and the duration of zoster-associated pain.[29,84] Treatment combined with steroids also appears to decrease the frequency and severity of postherpetic neuralgia in older patients and the elderly, but specific trials conducted in children have not been performed because children seldom experience postherpetic neuralgia. Oral acyclovir also may reduce recurrences of HSV and VZV in bone marrow and solid organ transplant recipients. Its effect on CMV and EBV disease in these patients, however, is minimal, if any. Continuous suppressive therapy with oral acyclovir reduces cutaneous recurrences after neonatal infection when it is administered after a 14- to 21-day course of intravenous therapy has been completed.[258] Whether long-term oral suppressive therapy in neonates also prevents CNS recurrence is unclear at this time. Bell palsy associated with HSV infection may be treated with a combination of acyclovir and prednisone.[4] EBV-associated diseases have been treated with acyclovir, with variable clinical benefit.[397,439] Oral hairy leukoplakia, however, appears to respond to oral acyclovir treatment.[368] Treatment of patients with infectious mononucleosis reduces viral shedding but does not alter the disease course.[451] No clinical benefit has been documented when acyclovir was used to treat chronic hepatitis B.[33]

Intravenous acyclovir is used to treat all severe or life-threatening diseases caused by HSV and VZV, including encephalitis, hepatitis, neonatal disease, acute retinal necrosis syndrome, mucocutaneous disease, and zoster, with or without visceral dissemination, in both normal and immunocompromised patients.[177,256,261,270,344,458,483] Pediatric patients suffering from severe herpetic gingivostomatitis may benefit as well.[8] Early clinical trials also showed efficacy in the treatment of primary genital HSV infection.[92] In addition, normal patients with complications of varicella, including pneumonitis, encephalitis, and hepatitis, should receive intravenous acyclovir.[177] Other diseases associated with VZV in which acyclovir treatment is beneficial include zoster ophthalmicus with complications such as keratitis, anterior uveitis, and contralateral hemiplegia as well as acute retinal necrosis syndrome.[344,417] Intravenous acyclovir is also highly effective in reducing the incidence of disease associated with HSV and VZV in marrow and solid organ transplant recipients. Moreover, it may help reduce serious CMV disease in selected marrow and solid organ transplant recipients, although it clearly is not as effective as ganciclovir or other antiviral agents with specific activity against CMV.[18,19,43,317,455] Treatment of human herpes infection with simian virus B requires high doses of intravenous acyclovir administered for a prolonged period, followed by suppressive therapy.[219] Despite in vitro activity, clinical benefit has not been documented in patients with hepatitis B.[33]

DOSAGE

Topical acyclovir as a 5 percent ointment can be applied to local lesions every 3 to 4 hours five to six times daily for 7 days.[93] If the disease is unresponsive or severe, systemic therapy with oral or intravenous acyclovir should be initiated.

Oral acyclovir is formulated as 200-mg capsules, 800-mg tablets, and a 200-mg/5 mL banana-flavored suspension. The usual dose used to treat mucocutaneous lesions caused by primary infection with HSV in adults and adolescents is 200 mg administered five times daily for 10 days. The usual pediatric oral dose is 15 mg/kg per dose (maximum, 200 mg per dose) administered five times daily for 10 days. Recurrent HSV disease in adults and adolescents may be treated with a variety of different regimens: 800 mg three times daily for 2 days, 400 mg three times daily for 5 days, or 200 mg five times daily for 5 days.[244,245,460] Suppressive doses of 400 mg administered twice daily, often for many years, seem to be safe and effective.[166] This dose, administered to pregnant women with recent primary or recurrent genital herpes, also appears to decrease recurrences at or near delivery and diminish the need for cesarean section delivery.[53,259,401] After intravenous therapy has been completed, the usual dose for neonatal suppressive therapy is 300 mg/m^2 per dose administered three times daily for 6 months.[258] Because neonatal prophylaxis decreases but does not eliminate the risk for CNS recurrence and because the emergence of resistant strains has been documented, all early cutaneous recurrences that occur despite compliance with oral prophylaxis, as well as other signs and symptoms suggestive of recurrent visceral or CNS disease, should be evaluated carefully in young infants, and the need for systemic therapy should be considered.[258] Oral acyclovir must be administered in higher doses to treat varicella and other VZV-associated diseases.[472] Adults and adolescents require 800-mg doses administered five times daily for 7 to 10 days or until lesions have crusted over.[23,462] The pediatric dose of acyclovir to treat varicella or chickenpox is 20 mg/kg (up to 800 mg maximum) per dose (80 mg/kg/day) administered four times daily for 5 days or until lesions have crusted.[7,124]

The usual dose for intravenous administration of acyclovir to treat serious disease associated with HSV is 5 to 10 mg/kg per dose administered as a 1-hour infusion every 8 hours.[357,459,472] Treatment for a duration of 7 days is usually sufficient for mucocutaneous disease, but it should be continued until all lesions are healed. Herpes encephalitis should be treated for at least 10 to 14 days or longer, depending on clinical response and clearance of HSV DNA from the CSF.[255,256,271,471] The usual recommended dose to treat neonatal HSV disease with disseminated visceral involvement or encephalitis is a high-dose regimen of 20 mg/kg per dose administered every 8 hours (60 mg/kg/day) for at least 21 days or until HSV DNA has cleared from the CSF, whichever is longer.[255,256] Some experts use a slightly lower dose (15 mg/kg per dose or 45 mg/kg/day) and a slightly shorter duration of therapy (14 days) for neonates with proven skin, eye, or mouth disease without clinical and laboratory evidence of visceral or CNS involvement. Clinical studies have documented that these higher doses are safe in neonates, but it has not been established clearly that they have superior efficacy over lower doses. Treatment of VZV-associated disease requires higher doses of acyclovir: 500 mg/m^2 per dose (10 to 20 mg/kg per dose) administered every 8 hours (total, 1500 mg/m^2/day) for at least 5 to 7 days or until all lesions have been crusted over for 24 to 48 hours.[459,472] In all clinical indications, the dosage of intravenously administered acyclovir should be adjusted according to creatinine clearance in patients with renal insufficiency and renal failure.

ADVERSE EFFECTS

Adverse effects of acyclovir are unusual.[250] Topical application of the ointment may cause pain or local irritation, usually caused by the polyethylene glycol base.[93,459] Handwashing must be performed after each local application to lesions to avoid autoinoculation or person-to-person transmission. Oral acyclovir may cause nausea and diarrhea, rash, or headache.[459] Long-term oral acyclovir appears to be safe in adults but may be associated with neutropenia in 46 percent of infants who receive suppression after treatment of neonatal HSV infection.[258,319] Intravenous acyclovir is generally well tolerated. However, extravasation of the drug (pH 9 to 11) can cause inflammation, ulceration, and necrosis of surrounding tissue.[250,440] Phlebitis also can develop.

Neurotoxicity occurs rarely, usually in patients with renal insufficiency who have high serum concentrations.[28,139,178,457] Neurotoxicity associated with acyclovir is characterized by lethargy, confusion, tremor, and rarely seizures and coma. Renal tubular damage, crystalline nephropathy, or interstitial nephritis may occur in approximately 5 percent of patients.[360,390] These renal complications are more likely to develop in patients who are not adequately hydrated or in whom the drug has been infused at a rate faster than the recommended infusion time of 1 hour. No excess frequency of adverse events has been reported to date in pregnant women who receive acyclovir or in their fetuses or newborns.[53,67,259,397,401,483] Therefore, on the basis of available information, acyclovir seems to be safe when it is administered during pregnancy, especially during the last trimester, and does not appear to have any significant adverse effects.

VALACYCLOVIR

Spectrum of Activity

Valacyclovir (L-valine, 2-[(2-amino-1,6-dihydro-6-oxo-9H-purin-9-yl)methoxy]ethyl ester monohydrochloride) (also known as valacyclovir hydrochloride) is the hydrochloride salt of the L-valine ester of acyclovir.[30] It has essentially the same selective spectrum of antiviral activity against the herpesviruses as acyclovir does.[35]

Mechanism of Action and Resistance

Valacyclovir is converted rapidly to acyclovir and therefore has the same mechanisms of action and resistance as acyclovir does.[35] Viral isolates that are resistant to acyclovir will be resistant to valacyclovir.

Pharmacokinetics

The pharmacokinetics of valacyclovir has not been studied in the pediatric population, but information gained from adults can be applied, in many ways, to pediatric patients. After oral administration, valacyclovir is absorbed rapidly from the gastrointestinal tract. It is metabolized by first pass through the intestinal tract and also by the liver into acyclovir and L-valine.[35] It has the advantage over acyclovir of having 54 percent bioavailability (versus 20% for acyclovir), which is not altered by food.[422] A single dose of 1 g of valacyclovir administered to an adult produces plasma levels of 0.4 to 0.8 μg/mL. The half-life is 2.5 to 3 hours, similar to that of acyclovir, and is prolonged in patients with renal insufficiency or renal failure.[464,466] The drug is removed by hemodialysis. The rate but not the extent of conversion of valacyclovir to acyclovir is prolonged in patients with liver disease.

Clinical Indications, Dosage, and Adverse Effects

CLINICAL INDICATIONS

Valacyclovir has been reported to be effective in treatment of primary and recurrent mucocutaneous disease caused by HSV-1, HSV-2, and VZV.[35,36,42,284,367,429] Randomized clinical trials conducted in adult patients showed valacyclovir to be superior to placebo and comparable to acyclovir in reducing the number and duration of lesions, pain, and time to healing in patients with primary and recurrent genital herpes and zoster.[42,367,429] It is also comparable to acyclovir for suppression of genital herpes recurrences. Furthermore, in a study of 27 CMV-seropositive heart transplant recipients, valacyclovir appeared to be equal or superior to oral acyclovir in preventing both laboratory and clinical parameters of CMV reactivation.[126] Because valacyclovir is converted to acyclovir, it is likely to be effective for all the clinical indications for which acyclovir has been shown to be effective.

DOSAGE

Valacyclovir is available only in 500-mg caplets; therefore, a pediatric liquid formulation is not available at this time. The usual dose is 1 g twice daily for 10 days for primary genital herpes and 500 mg twice daily for 3 to 5 days for recurrent disease.[35,42,284,429] A daily dose of 500 mg has been used safely for longer than a year to suppress recurrences of HSV disease.[298] The dose to treat VZV-associated disease is higher than that for HSV-associated disease. A dose of 1 g three times a day for at least 7 days is usually necessary to treat zoster, and therapy should be continued until the lesions have dried.[36] The dose of valacyclovir should be adjusted according to creatinine clearance in patients with renal insufficiency or renal failure.

ADVERSE EFFECTS

The adverse effects of oral valacyclovir are similar to those of oral acyclovir and include gastrointestinal disturbances such as nausea, vomiting, and diarrhea.[35] Headache and behavioral changes also have been observed. Overdosage may produce precipitation of acyclovir in the renal tubules as well as CNS toxicity.

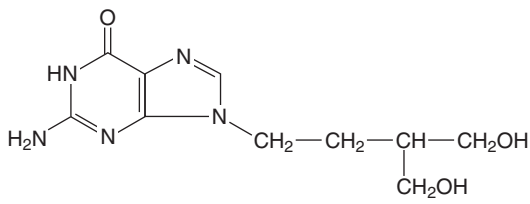
PENCICLOVIR

Spectrum of Activity

Penciclovir (9-[4-hydroxy-3-hydroxymethylbut-1-yl]guanine) is an acyclic guanosine analogue with a selective spectrum of activity against the herpesviruses similar to that of acyclovir[48] (Fig. 251–7). This compound inhibits HSV-1 at very low concentrations (0.2 to 0.6 μg/mL) and HSV-2 at slightly higher concentrations (0.3 to 2.4 μg/mL).[16,48,167,465] At even higher concentrations (0.9 to 4.0 μg/mL), it inhibits VZV.[16,17] The compound has very little activity against CMV, with concentrations greater than 50 μg/mL required to inhibit CMV. However, penciclovir does have activity against hepatitis B virus.[89,266,403]

Mechanism of Action and Resistance

Like acyclovir, penciclovir is preferentially phosphorylated by viral TK in virus-infected cells to penciclovir monophosphate and by cellular enzymes to penciclovir triphosphate, which is the active form of the drug.[125] Penciclovir triphosphate competitively inhibits viral DNA polymerase. It is approximately 100 times less potent than acyclovir triphosphate in inhibiting viral DNA polymerase, but an antiviral effect is achieved because it is present in high concentrations for a prolonged period (7 to 20 hours) in virus-infected cells. However, unlike acyclovir, penciclovir is not a chain terminator during DNA synthesis. Penciclovir triphos-



Penciclovir

Figure 251–7 Structure of penciclovir.

phate also inhibits the DNA polymerase of HBV. Emergence of HSV strains resistant to penciclovir has been minimal.[282] Similar to HSV isolates resistant to acyclovir, isolates resistant to penciclovir may have viral TK gene mutations that produce mutants with deficient or altered TK activity, or they may have mutations in DNA polymerase genes.[74] Strains of acyclovir-resistant HSV that are TK-deficient mutants also will be resistant to penciclovir, but strains of acyclovir-resistant HSV that are TK altered may be susceptible to penciclovir.[49,348]

Pharmacokinetics

Penciclovir is poorly absorbed after oral administration.[147] Adults who received a 10-mg/kg intravenous infusion of penciclovir had plasma levels of 12 μg/mL, but no pediatric information is available. Penciclovir 1 percent cream is not absorbed systemically. The systemic pharmacokinetics of penciclovir is discussed under famciclovir.

Clinical Indications, Dosage, and Adverse Effects

Penciclovir is available in the United States as a 1 percent cream in a propylene glycol base and is licensed for the treatment of herpes labialis lesions on the lips and face. Maximal clinical benefit occurs when the cream is applied to the lesions early, at the first clinical sign of disease, every 2 hours while awake for 3 to 5 days.[428] Application of penciclovir cream may be associated with local skin reactions, most likely caused by the propylene glycol base. An intravenous form of penciclovir has been tested in Europe, but it is not available in the United States. Penciclovir is not absorbed orally, but it is available as the oral prodrug famciclovir, which is converted rapidly to penciclovir. Dosages and systemic adverse effects of penciclovir are discussed under famciclovir.

FAMCICLOVIR

Spectrum of Activity

Famciclovir, 2-[(2-amino-9H-purin-9-yl)]-1,3-propanedial diacetate, is the prodrug of the antiviral agent penciclovir, which has selective activity against HSV-1, HSV-2, VZV, and HBV.

Mechanism of Action and Resistance

Famciclovir undergoes rapid transformation to penciclovir, which is monophosphorylated by viral TK to penciclovir monophosphate; the monophosphate, in turn, is converted by cellular enzymes to penciclovir triphosphate, the active form of the antiviral compound. Penciclovir then competitively inhibits viral DNA polymerase and, thereby, viral replication. The prolonged intracellular half-life observed with penciclovir also is observed with famciclovir. Penciclovir-resistant strains of HSV and VZV occur when mutations produce deficient or altered TK or altered DNA polymerase. Most TK-deficient acyclovir-resistant strains of HSV are also resistant to penciclovir.

Pharmacokinetics

Famciclovir is the diacetyl-6-deoxy analogue of the antiviral compound penciclovir. After oral administration, famciclovir is absorbed rapidly from the gastrointestinal tract with excellent (77%) bioavailability, and then it is metabolized in the liver by deacetylation and oxidation to form penciclovir. Single oral doses of 250 and 500 mg of famciclovir administered to adults produce peak plasma penciclovir levels of 1.6 to 1.9 μg/mL and 2.7 to 4.0 μg/mL, respectively. Administration of famciclovir with food reduces peak plasma concentrations by slowing the absorption time but does not alter the overall bioavailability of the drug. The half-life for penciclovir is 2 to 3 hours, after which it is excreted by filtration and active tubular secretion by the kidneys. Nonrenal clearance by fecal excretion also occurs for as much as one third of the oral dose. Excretion of penciclovir is decreased in patients with renal insufficiency and renal failure, and in patients with liver disease, peak plasma levels may be reduced.[44] Penciclovir is removed from the body by hemodialysis. After oral administration of famciclovir to rats, penciclovir is concentrated in breast milk; however, no studies have been performed in lactating mothers to confirm such concentration in humans.

Clinical Indications, Dosage, and Adverse Effects

CLINICAL INDICATIONS

In adult clinical trials, oral famciclovir was as effective as oral acyclovir and superior to placebo in treatment of recurrent mucocutaneous infections with HSV and VZV in both normal and immunocompromised patients.[82,299,381,452] When given early in the course of illness, time to lesion crusting and healing, duration of viral shedding, and length of acute pain all were shortened by treatment with famciclovir. In some studies, the duration of postherpetic neuralgia in elderly patients with zoster also was shortened.[452] Although to date it has not been studied specifically in clinical trials of patients with primary HSV and VZV infection, famciclovir probably also will provide clinical benefit in these conditions.[291] In addition, famciclovir is effective as suppressive therapy to reduce HSV recurrences and viral shedding.[113,315] Famciclovir also has been used to treat chronic HBV infection and for prophylaxis against recurrent HBV infection in liver transplant recipients.[183,299,406] No clinical trials in pediatric patients have been conducted.

DOSAGE

Famciclovir is available in 125-, 250-, and 500-mg tablets. An oral suspension for pediatric use is not available. The usual recommended oral dose of famciclovir to treat primary and recurrent mucocutaneous HSV disease is 125 mg twice daily for 5 days.[381] Dosages of suppressive therapy are slightly higher, 250 mg twice daily for as long as 1 year. Of note is that clinical trials showed that single daily doses of 250 mg were not as effective as the twice-daily regimen in suppressing recurrences. For immunocompromised patients, especially those infected with HIV, even higher doses (500 mg twice daily for 7 days) may be necessary.[391] Treatment of VZV-associated disease such as zoster also requires higher doses, 500 mg twice or three times daily for 10 days.[452] All dosage regimens should be reduced, according to creatinine clearance, in patients with renal insufficiency or renal failure.

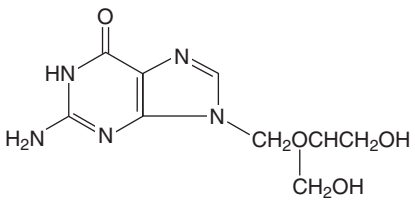
ADVERSE EFFECTS

Adverse effects of treatment with oral famciclovir are unusual and include gastrointestinal disturbances, such as nausea and diarrhea; rash; CNS complaints, such as confusion, hallucinations, and disorientation; neutropenia; and elevated liver transaminases.[82,103,386]

GANCICLOVIR

Spectrum of Activity

Ganciclovir (9-(1,3-dihydroxy-2-propoxymethyl)guanine) (also known as DHPG) is an analogue of deoxyguanosine, similar to



Ganciclovir

Figure 251–8 Structure of ganciclovir.

acyclovir, yet different in that it has an additional hydroxymethyl group on the acyclic side chain[138,141,354,409] (Fig. 251–8). This antiviral compound has selective activity against the herpesviruses, with uniquely potent antiviral activity against CMV. Ganciclovir inhibits HSV-1 and HSV-2 at 0.05 to 0.6 μg/mL, VZV at 0.4 to 10 μg/mL, CMV at 0.02 to 3.4 μg/mL, and EBV at 1.5 μg/mL. Human herpesvirus types 6 (HHV-6), 7 (HHV-7), and 8 (HHV-8) also may be inhibited by ganciclovir.[249] It has in vitro activity against adenoviruses and HBV as well.[138,164,327,446] High concentrations (30 to >700 μg/mL) of ganciclovir will inhibit the growth of most uninfected mammalian cells; bone marrow–derived cells, however, appear to be uniquely sensitive and can be inhibited at much lower concentrations (<0.7 μg/mL).

Mechanism of Action and Resistance

Ganciclovir is monophosphorylated in infected cells by virus-induced enzymes: TK in HSV-infected cells and protein kinases that are encoded by the UL97 phosphotransferase gene in CMV-infected cells.[37,138,288,409] Cellular enzymes complete the phosphorylation to ganciclovir triphosphate, the active form of the compound, which is concentrated in infected cells. Ganciclovir triphosphate competitively inhibits the incorporation of deoxyguanosine triphosphate into viral DNA, where it slows and stops viral DNA chain elongation and produces short, non-infectious viral DNA fragments.[185] Ganciclovir also preferentially inhibits viral DNA polymerase.[294] Ganciclovir resistance has been detected in 8 to 38 percent of immunocompromised adult and pediatric patients who have received prolonged administration of ganciclovir, and such resistance is an important clinical problem.[76,121,163,430]

Patients may be infected with single or multiple strains of CMV, with both drug-sensitive and drug-resistant strains mixed in the population.[135] Resistant strains may be induced or infect a patient primarily, emerge quickly after only weeks of therapy, or evolve more slowly and emerge sequentially after several months of antiviral prophylaxis or therapy.[77,135,230,377,478] These resistant mutants also retain virulence and are capable of producing serious and progressive disease.[45,135,230,420,448] Ganciclovir resistance in CMV strains occurs by at least two mechanisms: (1) point mutations or deletions in the UL97 gene that reduce intracellular phosphorylation of ganciclovir and (2) point mutations in the viral DNA polymerase UL54 (pol) gene that alter the function of the polymerase.[76,294,414,430] Most strains of CMV that are resistant to ganciclovir have UL97 mutations (most commonly at codons 460, 520, 594, and 595) and reduced phosphorylation, but they remain susceptible to foscarnet and cidofovir.[75,76,430] However, CMV strains that are resistant to ganciclovir because of a mutation in the UL54 DNA polymerase gene also may be resistant to foscarnet or cidofovir, or to both.[294,387] Moreover, multiple highly resistant strains of CMV may occur if both UL97 phosphotransferase and UL54 DNA polymerase gene mutations are present.[77,414] Resistance conferred by UL54 mutations usually emerges after UL97 mutations have occurred.[163,414] Ganciclovir resistance in HSV occurs in TK-deficient, acyclovir-resistant strains of the virus.

Pharmacokinetics

Intravenous administration of a 5-mg/kg dose of ganciclovir in adults produces peak plasma levels of 8 to 11 μg/mL.[138,144] Similar pharmacokinetics in 10 pediatric patients aged 9 months to 12 years has been observed.[155] A study of the pharmacokinetics of ganciclovir in 27 neonates aged 2 to 49 days who were administered intravenous ganciclovir for treatment of congenital CMV disease showed a dose of 6 mg/kg to be the most appropriate dose for that age group.[449,488] After intravenous administration, ganciclovir is distributed in CSF at 24 to 70 percent of plasma levels, and 38 percent of plasma levels enter brain tissue.[82] Drug levels in aqueous, vitreous, and subretinal fluid in the eye are comparable to serum levels, and even higher levels can be achieved with intravitreal implants.[13,144,269,301,328] Ganciclovir also accumulates in breast milk in animal models.[5] The plasma half-life is 2 to 4 hours in adults with normal renal function and longer than 24 hours in patients with renal insufficiency and renal failure.[419] The drug is eliminated by renal excretion, and dose reduction is required for patients with impaired creatinine clearance. Hydration will enhance elimination of the drug, and hemodialysis removes 60 percent of ganciclovir in plasma.[419] Ganciclovir also can be administered orally. Its oral bioavailability is rather poor: 5 percent under fasting conditions and 6 to 9 percent if it is administered with food.[10,280] Oral doses of 1000 mg administered to adults every 8 hours produce plasma levels of 0.9 to 1.2 μg/mL.[10,424] A recently licensed valine ester prodrug of ganciclovir, valganciclovir, has high oral bioavailability.[300]

Clinical Indications, Dosage, and Adverse Effects

CLINICAL INDICATIONS

Ganciclovir is used for the treatment of established CMV disease, for early or preemptive therapy in immunocompromised patients with virologic markers of active infection that are predictive of CMV disease, and for prophylaxis of high-risk patients such as those who are CMV seropositive or who have received transplants from CMV-seropositive donors. Ganciclovir is licensed for treatment and chronic suppression of sight-threatening CMV retinitis in immunocompromised patients and for prevention of CMV disease in transplant recipients. Clinical trials have demonstrated efficacy in these conditions.* Treatment with ganciclovir also is beneficial in immunocompromised individuals with other forms of invasive CMV disease, including pneumonia, colitis, esophagitis, myocarditis, encephalitis, persistent fever and leukopenia syndrome, and viral sepsis syndrome.† Treatment of established CMV pneumonia in bone marrow and stem cell transplant recipients is difficult, and the disease may not respond to ganciclovir treatment, with or without immune modulators or globulins. Most clinical trials have been performed in adults, but children and infants with CMV disease benefit from treatment as well. Ganciclovir treatment also has been evaluated in newborns congenitally infected with CMV with CNS involvement.[257,449,488] In addition, ganciclovir is used for preemptive therapy for patients (primarily recipients of solid organ, marrow, and stem cell transplants) with virologic markers that are predictive of serious CMV disease.[52,169,207,426] Prophylaxis with ganciclovir to prevent CMV infection and disease in immunocompromised patients, including solid organ and bone marrow transplant recipients and patients with acquired immunodeficiency

*See references 52, 99, 123, 168, 169, 207, 218, 314, 395, 476.
†See references 60, 80, 88, 115, 119, 129, 363, 364, 424, 426, 427.

syndrome (AIDS), also has been beneficial in most groups studied.[52,123,168,426,476,477] Prophylaxis reduces the risk for the acquisition of serious CMV disease or, in some groups, prolongs the incubation period, but it does not eliminate the risk.[123,168,395,476] Ganciclovir prophylaxis is also effective in preventing HSV infection in immunocompromised patients.[168] Ganciclovir appears to decrease HBV DNA levels and to improve hepatic enzymes in patients with post-transplantation infection or reactivation with HBV.[164] In addition, ganciclovir has been used to treat patients with serious disease associated with adenovirus infection, but clinical trials proving efficacy have not been performed.[446]

DOSAGE

Ganciclovir is supplied as a solution for intravenous infusion and as 250- and 500-mg capsules for oral administration. The intravenous infusions are delivered best in a concentration of 10 mg/mL or less and administered during a 1-hour period. Intravitreal implants are also available and are designed to slowly release relatively large doses of ganciclovir locally into the eye during a period of many months. Ganciclovir therapy for serious CMV disease usually is administered in two phases, induction and maintenance. The recommended dose for induction therapy for serious CMV disease in adults and children with normal renal function is 5 mg/kg per dose administered intravenously every 12 hours for 2 to 3 weeks. Successful induction therapy is accompanied by clinical and virologic response. Maintenance therapy at doses of 5 mg/kg/dose administered intravenously every 12 to 24 hours should be continued in severely immunocompromised patients who are at risk for relapse. Selected patients may receive oral ganciclovir for maintenance therapy. The usual recommended dose for adults is 1000 mg three times daily or, alternatively, 500 mg administered six times daily. The pediatric dose for maintenance oral therapy has not been established. The duration of maintenance therapy should be individualized for each patient, but such therapy usually lasts through the period of greatest risk, such as rejection or immune suppression, or may be lifelong, as in patients with AIDS. Some patients who experience CMV disease, such as solid organ transplant recipients with minimal immune suppression and minimal or no rejection, will respond dramatically to induction therapy and will not require long-term maintenance therapy. A dose of 6 mg/kg administered every 12 hours for 6 weeks has been used to treat newborns with congenital CMV disease.[257,276,488] This dose appears to be adequate for term newborns and premature newborns as young as 32 weeks' gestation, provided renal function seems to be normal for age. The dose for extremely premature newborns is not known and should be individualized according to the clinical judgment of the infectious diseases specialist. Preemptive therapy, 5 mg/kg per dose administered every 12 hours, is initiated when virologic markers for active, invasive infection, such as positive cultures for CMV from bronchoalveolar lavage samples or the presence of CMV DNAemia or antigenemia, are identified by routine virologic surveillance.[395,425] After an induction period of 7 to 14 days, maintenance therapy usually is continued for 100 to 120 days after transplantation or longer if the patient remains at high risk for relapse of CMV disease. Prophylaxis with ganciclovir is administered immediately before transplantation and for a defined period after transplantation in patients who are at high risk, such as those who are CMV seropositive before transplantation and those who receive marrow or solid organ transplants from a CMV-seropositive donor.[51,123,157,168,395,426,476,477] Patients who do not respond to induction therapy or who relapse or progress during maintenance therapy with ganciclovir may have a resistant strain of CMV. The addition of another antiviral with different mechanisms of action, such as foscarnet or cidofovir, may be beneficial. If clinical or virologic responses still are not maintained, the possibility of a multiply resistant CMV strain should be considered. Patients with disseminated adenovirus disease have received doses of 5 mg/kg every 12 hours for 14 or more days, but clinical benefit has not been proved.[327]

ADVERSE EFFECTS

Adverse effects of ganciclovir can be both local and systemic. Local reactions such as phlebitis, irritation, blistering, or ulceration at or around the infusion site can occur and usually are attributed to the alkaline pH (pH 11) of the intravenous solution. Local reactions can be minimized by paying careful attention to the infusion site or by administering the antiviral agent through a central venous catheter. The most common systemic toxicity associated with ganciclovir administration is dose-dependent, reversible neutropenia, which occurs in a third to a half of patients (adults, children, and newborns) who receive this antiviral for longer than 2 weeks.[99,138] Thrombocytopenia also can occur, most often in patients with AIDS who are receiving other antiviral agents, including antiretrovirals.[216] If the neutropenia is severe (absolute neutrophil count <500/mm^3), ganciclovir administration should be halted temporarily until the neutrophil count recovers. Ganciclovir then may be readministered, if it is still clinically indicated, at the same or half the original dose while the patient is carefully monitored for recurrence of the neutropenia. Some experts have used recombinant granulocyte-macrophage colony-stimulating factor successfully to treat ganciclovir-induced neutropenia.[187] Anemia associated with ganciclovir administration is an unusual occurrence. Other adverse effects associated with ganciclovir include CNS disturbances, such as headache, behavioral changes, psychosis, seizures, and coma; mild nephrotoxicity with azotemia; liver dysfunction with elevated transaminases; and rash. Ganciclovir also is mutagenic, carcinogenic, and immunosuppressive. In addition, preclinical animal studies showed reproductive toxicity, with teratogenicity, embryotoxicity, and testicular atrophy.[138] Long-term studies in children who received ganciclovir as newborns are being conducted to determine the long-term effects of ganciclovir administration, if any. Also, caretakers who prepare and administer ganciclovir to patients should take precautions to minimize direct exposure to the antiviral agent.

VALGANCICLOVIR

Spectrum of Activity

Valganciclovir (L-valine, 2-[(2-amino-1,6-dihydro-6-oxy-9H-purin-9-yl)methoxy]-3-hydroxypropyl ester) is a prodrug of ganciclovir and therefore has the same selective spectrum of activity as ganciclovir against herpesviruses, especially CMV, as well as limited activity against adenoviruses and HBV.

Mechanism of Action and Resistance

Valganciclovir is the L-valyl ester (prodrug) of ganciclovir.[300] It is metabolized rapidly in the body to ganciclovir. Ganciclovir then is monophosphorylated by viral protein kinase (coded for by UL97 genes) in CMV-infected cells and further phosphorylated to the active form ganciclovir triphosphate by cellular kinases. Viral DNA synthesis is inhibited by ganciclovir triphosphate (see ganciclovir for details). Isolates of CMV become resistant to valganciclovir by mutations in UL97, the viral kinase gene, or in UL54, the viral DNA polymerase gene. Mutations in UL97 confer resistance to ganciclovir and therefore to valganciclovir, whereas mutations in UL54 confer double or triple resistance to ganciclovir, foscarnet, and cidofovir. Mutations in both genes can produce highly resistant strains of CMV.

Pharmacokinetics

Valganciclovir is well absorbed after oral administration and is rapidly hydrolyzed in the intestine and liver to ganciclovir.[349] The bioavailability of valganciclovir is high, approximately 60 percent (versus 6% to 9% for ganciclovir), and an oral dose of 900 mg administered to adults produces ganciclovir blood levels equivalent to a 5-mg/kg dose administered intravenously.[54,241,300] Absorption is enhanced significantly with the ingestion of food, so physicians recommend that valganciclovir be taken with food or meals. The drug is excreted by the kidneys, and renal insufficiency produces prolonged excretion and a longer half-life (see ganciclovir). Oral valganciclovir solution administered to neonates and infants up to 6 weeks of age, at a dosage of 16 mg/kg/dose, is 41% bioavailable and provides plasma concentrations comparable to intravenously administered ganciclovir.[259a]

Clinical Indications, Dosage, and Adverse Effects

Valganciclovir is available in 450-mg tablets, and the usual adult dose is 900 mg (two 450-mg tablets) twice daily for 14 days (induction therapy), followed by a maintenance dose of 900 mg administered once daily.[300] Special oral solutions are still investigational.[259a] Valganciclovir is licensed for induction and maintenance treatment of CMV retinitis in immunocompromised patients, but clinical trials are likely to show that valganciclovir is beneficial to a variety of patients with a number of different CMV infections.[300] A liquid, pediatric formulation is not available at this time. As with ganciclovir, if a patient treated with valganciclovir experiences progression of disease or recurrence during maintenance therapy, a resistant strain of CMV should be considered a possibility. Adverse effects associated with the oral administration of valganciclovir are similar to those with oral ganciclovir and include diarrhea and dose-dependent, reversible neutropenia.[300]

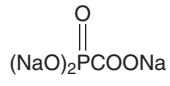
FOSCARNET

Spectrum of Activity

Foscarnet (phosphonoformic acid or trisodium phosphonoformate hexahydrate) is a pyrophosphate analogue that selectively inhibits herpesviruses[337] (Fig. 251–9). It also has activity against HIV and HBV.[20,137] At concentrations of 100 to 300 µmol/L, CMV is inhibited; whereas slightly lower concentrations (80 to 200 µmol/L) inhibit HSV types 1 and 2, VZV, EBV, and HHV-8.[337] Concentrations between 20 and 200 µmol/L appear to inhibit HBV. Foscarnet also inhibits most but not all acyclovir-resistant HSV and VZV strains and most but not all ganciclovir-resistant CMV strains.[149,296,382] Combinations of ganciclovir and foscarnet are synergistic against CMV, and combinations of zidovudine and foscarnet appear to be synergistic against HIV.[137,296] At high concentrations (500 to 1000 µmol/L), foscarnet inhibits cellular DNA synthesis in uninfected cells.[78]

Mechanism of Action and Resistance

Foscarnet is not a nucleoside analogue, and it does not require phosphorylation or any other form of intracellular metabolism to be activated. Rather, it is a pyrophosphate analogue that directly inhibits viral and cellular DNA polymerase.[98,112] Selective viral inhibition is accomplished by noncompetitive and reversible blocking of the pyrophosphate binding site of the viral polymerase, in much lower concentrations than it inhibits cellular DNA polymerases. Because foscarnet does not require phosphorylation by viral TK or other kinases, it inhibits TK-deficient and altered strains of HSV and VZV that are resistant to acyclovir as well as UL97 phosphotransferase mutants of CMV that are resistant to ganciclovir. However, strains of herpesviruses of all types that are resistant to acyclovir or ganciclovir by mutation of the viral DNA polymerase gene are also resistant to foscarnet.[21,384]



Foscarnet

Figure 251–9 Structure of foscarnet.

Pharmacokinetics

Intravenous administration of 60 mg of foscarnet every 8 hours to adults produces plasma levels of 450 to 575 µmol/L; 90 mg administered every 12 hours produces plasma levels of 420 to 746 µmol/L.[112,382] CSF levels are usually approximately 60 percent of plasma levels, and vitreous concentrations in the eye are the same or slightly higher than plasma levels.[204] Most (80%) of the dose of foscarnet is eliminated unmetabolized from the body through the kidneys, and plasma clearance decreases if renal function is impaired. The remaining 20 percent appears to be deposited in teeth and bone, where it accumulates and remains for months. The drug is removed by hemodialysis, but not appreciably by peritoneal dialysis.[6,295] Oral foscarnet has poor bioavailability (less than 10%) and causes diarrhea, and it is unlikely to be available for patient use.[336] Pharmacokinetic data in infants and children have not been published. However, preclinical studies showed that deposition of foscarnet in teeth and bones is greater in younger than in older animals.

Clinical Indications, Dosage, and Adverse Effects

CLINICAL INDICATIONS

Foscarnet is licensed for both induction and maintenance treatment of CMV retinitis in immunocompromised patients and for treatment of mucocutaneous disease caused by acyclovir-resistant HSV.* Foscarnet also may be of benefit in patients with zoster caused by acyclovir-resistant VZV.[382] Because the combination of foscarnet and ganciclovir appears to be synergistic in vitro, immunocompromised patients who experience progression or relapse of CMV disease while receiving therapy with one or the other antiviral agent may benefit from combination therapy in some instances.[122,149,234] Foscarnet also may be used for the treatment or prophylaxis of serious or life-threatening CMV disease when ganciclovir is contraindicated or otherwise deemed clinically undesirable because of its myelosuppressive effects.[369,482] The antiretroviral properties of foscarnet, when it is combined with other antiviral agents, also may be beneficial in patients with HIV infection or AIDS.[32,236,343]

DOSAGE

Treatment with foscarnet usually is divided into two phases, induction and maintenance. The usual dosage of foscarnet for induction therapy is 60 mg/kg per dose administered intravenously every 8 hours for 3 weeks; maintenance therapy is 90 to 120 mg/kg/day administered indefinitely or through the period of risk.[243] The dose of foscarnet should be given slowly, during the course of 2 hours (or no faster than 1 mg/kg/min), to reduce renal toxicity. Creatinine clearance should be used to adjust

*See references 112, 122, 234, 281, 295, 343, 384, 435, 436, 463.

dosage regimens in patients with renal insufficiency or renal failure. Published experience on the use of foscarnet in pediatric patients is limited, but undoubtedly, certain pediatric patients benefit from receiving foscarnet therapy.[463] In these cases, the same per-kilogram dosage regimens can be used for most patients.

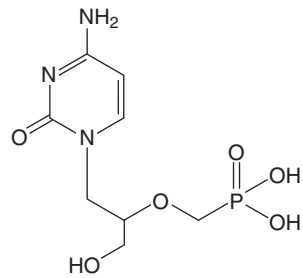
ADVERSE EFFECTS

Foscarnet is associated with serious adverse effects and should be used only after thorough consideration of the risks and benefits involved.[78,112] Renal toxicity with azotemia, proteinuria, crystalluria, renal tubular acidosis or necrosis, and interstitial nephritis can occur in as many as a third of patients who receive foscarnet.[110,240] Renal toxicity generally occurs after the first week of therapy and usually is reversible. The risk for the development of renal toxicity is increased if the drug is given by rapid infusion or administered in high doses, if the patient is dehydrated, or if other nephrotoxic drugs are administered concomitantly with foscarnet.[333] Hydration, including saline loading, and administration of each dose during the course of at least 2 hours appear to reduce the risk for development of renal toxicity. Foscarnet binds divalent metal ions such as calcium in the body, and metabolic abnormalities, including hypocalcemia and hypercalcemia (total or ionized), hypophosphatemia and hyperphosphatemia, hypomagnesemia, and hypokalemia, may occur in approximately a third of patients who receive foscarnet.[235] Symptoms of these acute metabolic abnormalities include perioral tingling, numbness or paresthesias of the limbs, and, if severe, seizures, tetany, and cardiac dysrhythmias. Administration of the dose during the course of at least 2 hours also reduces the risk for development of metabolic abnormalities. Foscarnet also can be deposited and concentrate in bone, with as yet unclear long-term consequences.[112] CNS side effects also occur in approximately a fourth of patients and include headache, tremor, seizures, and behavioral changes. Abnormal liver function test results have been noted, and high urinary concentrations of foscarnet may produce painful genital ulcerations and rash in some patients.[417] Preclinical studies showed foscarnet to be mutagenic and associated with anomalies of skeletal development in young animals.[109]

CIDOFOVIR

Spectrum of Activity

Cidofovir (*S*)-1-3-hydroxy-2-[phosphonomethoxypropyl])cytosine dihydrate (also known as HPMPC) is an acyclic phosphonate nucleotide analogue of deoxycytidine monophosphate with broad-spectrum in vitro antiviral activity against all DNA viruses, including herpesviruses (HSV types 1 and 2; CMV, EBV, and VZV; and HHV types 6, 7, and 8), adenoviruses, polyomaviruses (JC and BK viruses), papillomaviruses, and poxviruses (vaccinia, variola or smallpox, cowpox, monkeypox, camelpox, and molluscum contagiosum and orf viruses) (Fig. 251–10).* Cidofovir exhibits its most specific and potent antiviral activity against CMV (minimal inhibitory concentration, 0.25 μmol/L).[71,72,120,130] The compound also is very active against VZV (0.79 μmol/L), HSV-1 (12.7 μmol/L), and acyclovir-resistant, TK-deficient HSV-1 strains (6.24 μmol/L).[214,312,418] Cidofovir inhibits HSV-2 at concentrations of 31.7 μmol/L, adenoviruses at 10.8 μmol/L, and vaccinia virus at 12.7 μmol/L.[108,170,411] Strains of CMV UL97 mutants that are resistant to ganciclovir are inhibited by cidofovir, and cidofovir in combination with ganciclovir or foscarnet shows synergistic inhibition of CMV in vitro.[383]



Cidofovir

Figure 251–10 Structure of cidofovir.

Mechanisms of Action and Resistance

Cidofovir inhibits the replication of CMV and other viruses by selective inhibition of viral DNA polymerase.[72,214,312] The compound is phosphorylated by cellular enzymes to the active form cidofovir diphosphate. Cidofovir diphosphate inhibits both viral and cellular DNA polymerase; however, because the concentration necessary to inhibit cellular DNA synthesis is hundreds of times higher than that needed to inhibit viral DNA synthesis, cidofovir appears to selectively inhibit viral DNA synthesis at concentrations safely administered to humans. Cidofovir diphosphate has a long intracellular half-life that provides prolonged and persistent antiviral activity and allows infrequent dosing regimens in humans.[145,215,418] Because the compound does not require TK for initial phosphorylation, it is active against TK-deficient and TK-altered acyclovir-resistant HSV strains.[312] Resistance to cidofovir is unusual but can occur by mutations in viral DNA polymerase genes (codons 375 to 540 and possibly 978 to 988), most likely in patients who have received prolonged or repeated periods of treatment with ganciclovir or foscarnet. Strains of CMV that are resistant to cidofovir are also usually resistant to ganciclovir, and occasionally, triple mutants resistant to ganciclovir, foscarnet, and cidofovir occur.[120,445]

Pharmacokinetics

After an intravenous infusion of 5 mg/kg of cidofovir, peak plasma levels range from 11.6 to 26.1 μg/mL, with the latter occurring after administration of probenicid.[102,383,445] The plasma half-life is 2 to 3 hours, but the intracellular half-life is very prolonged (between 17 and 65 hours).[418] CSF penetration by cidofovir is not well studied, but in at least one patient, the drug did not appear to cross the blood-brain barrier in detectable amounts. After topical administration to the eye or intact skin, systemic absorption is low, with peak plasma levels usually less than 0.5 μg/mL.[273,274] However, patients with abraded or denuded skin may have significant absorption.[36] The systemic absorption that occurs after intralesional or subcutaneous administration is not well characterized at this time. Intravitreal administration produces sustained antiviral effects in animal models.[145] Cidofovir is not well absorbed orally, with less than 5 percent bioavailability. However, bioavailable alkoxyalkyl esters of cidofovir are in development and may lead to an oral compound in the near future.[251] Aerosolized cidofovir also is being studied in animal models and appears to deliver high concentrations of antiviral to the lungs.[51] Cidofovir is eliminated through the kidney by glomerular filtration and active tubular secretion, and more than 90 percent of the original dose can be recovered unchanged in urine.

*See references 11, 71, 72, 108, 120, 130, 161, 170, 214, 312, 334, 335, 383, 407, 408, 411, 418, 453.

Clinical Indications, Dosage, and Adverse Effects

CLINICAL INDICATIONS

Cidofovir is licensed for induction and maintenance treatment of CMV retinitis in immunocompromised adults with AIDS.[383] Clinical trials have shown that cidofovir significantly delays progression of CMV retinitis in previously untreated patients as well as in those who previously failed or were intolerant of foscarnet or ganciclovir therapy.[273,274,356,437] Cidofovir also has been shown to be effective for the treatment of CMV infection and disease in marrow and stem cell transplant recipients. In addition, it has been used, in selected patients, for preemptive treatment of CMV infection after marrow and stem cell transplantation.[68,289,353] One study also showed that cidofovir helped prevent post-transplantation CMV-associated atherosclerosis in rats.[57] Patients infected with CMV strains resistant to ganciclovir or foscarnet, or both, may benefit from receiving cidofovir treatment.[120] Cidofovir is used by clinicians to treat immunocompromised children with serious CMV disease despite the lack of published experience in children.[59,66] Cidofovir has broad-spectrum antiviral activity, and published case reports show that it has been used to treat patients with serious infections caused by a wide variety of DNA viruses. For example, patients with acyclovir- and foscarnet-resistant HSV infection have been treated successfully with topical and systemic cidofovir.[58,265,272,274] It also may be effective in treatment of selected patients with acyclovir-resistant VZV infections.[444] Moreover, one report showed that treatment with cidofovir and anti-CD20 monoclonal antibody was associated with remission and regression of post-transplantation EBV-associated lymphoproliferative disease.[186] The use of cidofovir to treat patients with AIDS and HHV-8 viremia and disease also has been reported.[47,304] Human papillomavirus–induced epithelial cell proliferation may be responsive to treatment with topical and intralesional administration of cidofovir. Several small, uncontrolled case series have described the successful use of cidofovir to treat juvenile laryngeal papillomatosis, hypopharyngeal and esophageal papillomatous lesions, anogenital condylomas, and cervical intraepithelial neoplasia.[14,73,416,433,485] Furthermore, intravenous and intralesional therapy with cidofovir has been used to treat individual patients, including children, with refractory disseminated respiratory papillomatosis of the lung.[14,296a] Human BK polyomavirus–associated acute hemorrhagic cystitis in immunocompromised patients has been treated with cidofovir, with varying results.[25,137a] Case reports and small clinical trials evaluating cidofovir treatment in patients with progressive multifocal leukoencephalopathy who have AIDS or other immunocompromising conditions have not shown consistent benefit in survival or sustained improvement in neurologic status.[12,81,158,228,326,362,385,402] Successful cidofovir treatment of adenovirus disease in marrow and stem cell transplant recipients has been published in reports of case series, with the best results noted in patients in whom the disease was localized and treatment was initiated early.[46,160,217,283,370] Controlled clinical trials evaluating eyedrops containing an investigational topical solution of cidofovir to treat patients with acute keratoconjunctivitis caused by adenovirus have not shown consistent benefit in the doses used.[209,210] Poxviruses are inhibited by cidofovir in concentrations safely achievable in humans, and at least one case report of successful treatment of orf (ecthyma contagiosum) has been published.[161] In addition, in vitro and animal model data suggest that cidofovir may be effective in the treatment and short-term, postexposure prophylaxis of smallpox and other related poxvirus infections in humans as well as in the treatment of complications that may occur after inoculation with smallpox (vaccinia-like) vaccine.[11,71,72,108,120,383,411] However, no clinical trials in humans to evaluate the efficacy of cidofovir for the treatment of poxvirus infections have been published.

DOSAGE

The usually recommended dose for induction therapy with cidofovir is 5 mg/kg given as an intravenous infusion during the course of 1 hour administered once weekly for 2 consecutive weeks. Some clinicians suggest a reduced dosage regimen of 1 mg/kg administered three times weekly to reduce the risk of renal toxicity.[217] Maintenance therapy usually is administered as 1-hour infusions of 5 mg/kg once every 2 weeks to complete a total of at least five doses. These doses should be decreased to 1 to 3 mg/kg if renal insufficiency is present and discontinued if significant elevation of serum creatinine concentration or proteinuria occurs. Probenecid must be administered orally with each dose of cidofovir, 3 hours before and then 2 and 8 hours after completion of the intravenous infusion. Prehydration with normal saline before each infusion also is recommended. Investigational topical preparations of eyedrops containing 0.2 to 1 percent cidofovir and creams containing 1 percent cidofovir are being investigated.[209,210] The usual concentration used for intralesional injection is 2.5 mg/mL.[433]

ADVERSE EFFECTS

Cidofovir is nephrotoxic and produces clinically apparent proximal tubular dysfunction, including Fanconi syndrome and acute renal failure, in as many as half the patients who receive the drug.[311,383] Early laboratory signs of renal toxicity include proteinuria, glycosuria, azotemia, and metabolic acidosis. Therefore, patients receiving cidofovir should have a urinalysis and serum tests for renal function performed before taking each dose. The risk for development of renal toxicity can be reduced but not eliminated by probenecid and saline prehydration. Moreover, renal toxicity is more likely to occur if other nephrotoxic agents, such as aminoglycosides, amphotericin B, or foscarnet, are administered concurrently with cidofovir.[487] In clinical trials of patients with AIDS, administration of cidofovir also was associated with neutropenia. Ocular toxicity, including ocular hypotony (decreased intraocular pressure) and anterior uveitis and iritis, also has been reported in patients receiving cidofovir therapy.[3] Therefore, frequent monitoring by an ophthalmologist, including measurement of intraocular pressure, is recommended for patients receiving cidofovir therapy. Intravitreal administration of cidofovir has produced uveitis, vitreitis, reduced intraocular pressure, and loss of vision.[260,331] Local or topical treatment with cidofovir may produce local reactions in the skin.

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CHAPTER 252

Antifungal Agents

Andreas H. Groll ❁ Thomas J. Walsh

Invasive fungal infections are important causes of morbidity and mortality in children with severe underlying illnesses. These infections remain difficult to diagnose and can be rapidly fatal. As a consequence, early and aggressive antifungal chemotherapy is pivotal for successful management and survival. For a long time, options for antifungal chemotherapy were limited to amphotericin B deoxycholate with or without the addition of flucytosine. The 1990s, however, witnessed major progress through the introduction of fluconazole and itraconazole and the development of less toxic formulations of amphotericin B. More recent advances include the advent of novel, potent, and broad-spectrum antifungal triazoles and the clinical development of echinocandins, an entirely new class of antifungal agents that target the fungal cell wall (Fig. 252–1). This chapter is devoted to the clinical pharmacology of systemic antifungal agents; emphasis is placed on pharmacokinetics, dosing, and safety in pediatric age groups.

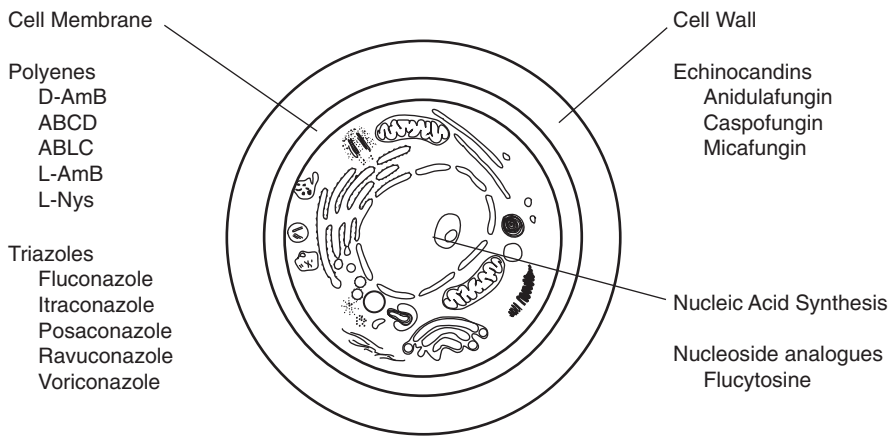


Figure 252–1 Cellular targets of approved and investigational antifungal agents for treatment of invasive mycoses at the beginning of the 21st century. *(Modified from Groll, A. H., Piscitelli, S. C., and Walsh, T. J.: Antifungal pharmacodynamics: Concentration-effect relationships in vitro and in vivo. Pharmacotherapy 21[Suppl. 8]:133-148, 2001.)*

AGENTS FOR TREATMENT OF INVASIVE MYCOSES

POLYENE ANTIBIOTICS

Amphotericin B Deoxycholate

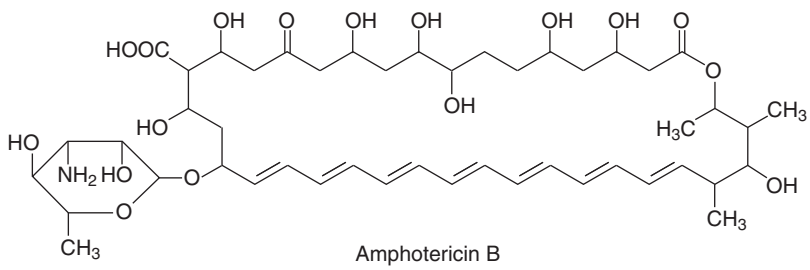
Despite an expanded antifungal armamentarium, amphotericin B deoxycholate still has a role in the treatment of selected invasive fungal infections. First isolated in the 1950s as a natural product of a soil actinomycete,[161] amphotericin B belongs to a family of approximately 200 polyene macrolide antibiotics and consists of 7 conjugated double bonds, an internal ester, a free carboxyl group, and a glycoside side chain with a primary amino group (Fig. 252–2). The compound is amphoteric, not orally or intramuscularly absorbed, and virtually insoluble in water. For parenteral use, amphotericin B has been solubilized with deoxycholate as micellar suspension, and this formulation has been available for more than 50 years.[38]

MECHANISM OF ACTION. Amphotericin B, similar to other polyenes, acts primarily by binding to ergosterol, the principal sterol in the cell membrane of most fungi. This interaction with ergosterol results in the formation of ion channels, loss of protons and monovalent cations, depolarization, and concentration-dependent cell death. Although with less avidity, the compound also binds to cholesterol, the main sterol of mammalian cell membranes; this action accounts for most of the toxicities associated with this drug. A second mechanism of action of amphotericin B may involve oxidative damage of the cell through a cascade of oxidative reactions linked to its own oxidation, with formation of free radicals or an increase in membrane permeability. In addition to its antifungal activity, amphotericin B has stimulatory effects on phagocytic cells that also are related to oxidation-dependent events.[63,185]

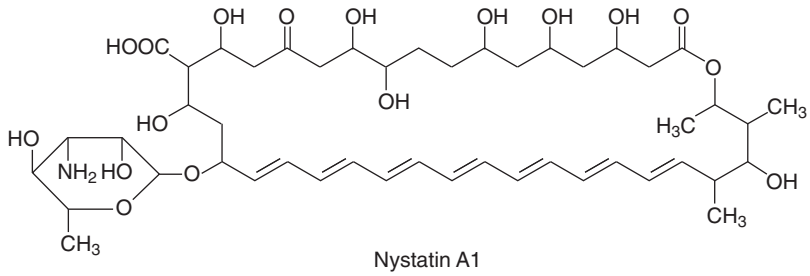
ANTIFUNGAL ACTIVITY. Amphotericin B has a broad spectrum of antifungal activity that includes most fungi pathogenic in humans. This characteristic maintains amphotericin B as the gold standard in the development of other antifungal agents. True microbiologic resistance to antifungal polyenes has been associated with qualitative or quantitative differences in the sterol composition of the cell membrane, but this resistance may also be related to increased catalase activity with decreased susceptibility to oxidative damage.[185] Resistance to amphotericin B remains rare in *Candida* spp. other than *Candida lusitaniae*, although the compound appears somewhat less active against *Candida guilliermondii*, *Candida parapsilosis*, and *Candida tropicalis*.[471,486,514] *Aspergillus* spp. and other opportunistic molds, but not the dimorphic molds, tend to have more variable susceptibility to amphotericin B; *Aspergillus terreus*[223,442] and some of the emerging pathogens such as *Trichosporon asahii*,[11,491,492] *Fusarium* spp.,[61,380] *Scedosporium apiospermum*,[453,494] *Scedosporium prolificans*,[46,286] and certain dematiaceous fungi[171] may be completely resistant to amphotericin B at concentrations achievable in patients by maximum tolerated dosages. Acquisition of secondary resistance is an uncommon occurrence and has not been a clinical problem.[184]

PHARMACODYNAMICS. In time-kill studies, amphotericin B displays concentration-dependent fungicidal activity against susceptible *Candida albicans*, *Cryptococcus neoformans*, and *Aspergillus fumigatus*.[245,246,375] In addition to its concentration-dependent fungicidal dynamics, a prolonged post-antifungal effect (PAFE) of amphotericin B of up to 12 hours' duration has been demonstrated in *C. albicans* and *C. neoformans*.[130,461] Studies in laboratory animals support the concentration-dependent kill kinetics of amphotericin B in vitro. In neutropenic pharmacokinetic/pharmacodynamic mouse models of disseminated candidiasis and pulmonary aspergillosis, peak plasma concentration (C_{max})/minimal inhibitory concentration (MIC) was the parameter that provided the best correlation with outcome as measured by the residual organismal burden in tissue.[22,512] These laboratory findings indicate that large doses will be most effective and that achievement of optimal peak concentrations is important. Therefore, the dosage of amphotericin B should not be uncritically reduced, and infusion for durations longer than that recommended by the manufacturer should be avoided.

PHARMACOKINETICS. After intravenous administration, amphotericin B rapidly dissociates from its vehicle and becomes highly protein-bound before distributing into tissues.[86] The dis-



Amphotericin B



Nystatin A1

Figure 252–2 Structural formulas of antifungal polyenes: amphotericin B and nystatin A1.

position of the compound follows a three-compartment model, with rapid initial clearance from plasma followed by a biphasic pattern of elimination with a beta half-life of 24 to 48 hours and a prolonged terminal (gamma) half-life of 15 days or more.[28] Tissue levels of amphotericin B in laboratory animals are highest in liver, spleen, bone marrow, kidney, and lung; concentrations in body fluids other than plasma are generally low.[225,270] However, despite mostly undetectable concentrations in the cerebrospinal fluid (CSF) and comparatively low concentrations in brain tissue across all species, amphotericin B is effective in the treatment of fungal infections of the central nervous system (CNS). Although no metabolites of amphotericin B have been identified, only small quantities of parent compound are excreted into urine and bile, a finding suggesting that tissue accumulation accounts for most of the disposition of drug.[39,40,98,385] Accordingly, adjustment of dose is not necessary in patients with unrelated renal or hepatic dysfunction. Because of the high protein binding of amphotericin B, hemodialysis usually does not affect plasma concentrations.[99]

Reported pharmacokinetic data in pediatric age groups are characterized by a high interindividual variability, which may be related to differences in underlying diseases and modes of administration (Table 252–1).[31,44,253,327,437] However, infants and children appear to clear the drug from plasma more rapidly than do adults, as indicated by a significant negative correlation between age and clearance in two separate studies.[44,253] Because distribution into tissues appears to be the main route of clearance from plasma, the faster clearance in individuals of younger age may be explained by the larger relative volume of parenchymatous organs in comparison with adults.[316] Whether the enhanced clearance from the bloodstream has implications for dosing remains unknown. Currently, dosage recommendations for all pediatric age groups do not differ from those in adult patients.

ADVERSE EFFECTS. Infusion-related reactions and nephrotoxicity are major problems associated with the use of conventional amphotericin B and often limit successful therapy. Infusion-related reactions (fever, rigors, chills, myalgias, arthralgias, nausea, vomiting, and headaches) are thought to be mediated by the release of cytokines from monocytes in response to the drug.[24] These reactions can be noted in as many as 73 percent of patients prospectively monitored at the bedside.[485] In a more recent prospective interventional study of pediatric patients with cancer, investigators observed fever or rigors, or both, associated with the infusion of conventional amphotericin B in 19 of 78 treatment courses (24%).[327] However, these characteristic adverse effects of amphotericin B are observed only rarely in the neonatal setting.[242] In clinical practice, infusion-related reactions associated with amphotericin B therapy may be blunted by slowing the infusion rate but often require premedication with acetaminophen (10 to 15 mg/kg), hydrocortisone (0.5 to 1.0 mg/kg), or meperidine (0.2 to 0.5 mg/kg).[486] Less common acute adverse effects are hypotension, hypertension, flushing, and vestibular disturbances; bronchospasm and true anaphylaxis are rare occurrences.[184] Cardiac arrhythmias and cardiac arrest resulting from acute potassium release may occur with rapid infusion (<60 minutes), especially if the patient has preexisting hyperkalemia or renal impairment.[73,166,313]

The hallmarks of amphotericin B–associated nephrotoxicity are azotemia, wasting of potassium and magnesium; tubular acidosis and impaired urinary concentration ability rarely are of clinical significance.[163,411] Relevant electrolyte wasting occurs in approximately 12 percent of prospectively monitored patients.[485] Hypokalemia can be quite refractory to replacement until hypomagnesemia is corrected.[411] Azotemia is a common occurrence: In a large prospective clinical trial, the baseline serum creatinine rose by more than 100 percent in 34 percent of 344 unstratified pediatric and adult patients receiving conventional amphotericin B for empiric therapy of fever and neutropenia.[485] Azotemia can be exacerbated by concomitant nephrotoxic agents, in particular by cyclosporine and tacrolimus: in a more recent clinical trial in persistently febrile neutropenic patients, renal toxicity occurred in 67 percent of patients receiving these drugs as compared with 31 percent in patients not receiving them concurrently with amphotericin B.[509] Data from another clinical trial suggested a somewhat lower rate of azotemia in children as compared with adults,[370] but this observation does not appear to be consistent.[509] A frequency of amphotericin B–associated azotemia of only 2 percent was reported for pediatric patients with cancer who were receiving the drug at 1 mg/kg/day for empiric antifungal therapy.[327] In contemporary series reporting safety data of conventional amphotericin B (0.5 to 1.0 mg/kg) in premature neonates, the incidence of azotemia ranged from zero to 15 percent,[72,159,242,373] a finding indicating that the compound is much better tolerated in this setting than reported early during its use.[30]

Renal toxicity associated with the use of conventional amphotericin B has the potential to lead to renal failure and dialysis,[515] but azotemia often stabilizes with therapy and usually is reversible after discontinuation of the drug.[486] Avoiding concomitant nephrotoxic agents, appropriate hydration and normal saline loading (10 to 15 mL NaCl/kg/day)[25,201] may greatly lessen the likelihood and severity of azotemia associated with amphotericin B therapy.

TABLE 252–1 Pharmacokinetic Parameters of Amphotericin B Deoxycholate in Pediatric Patients*

Population/Reference	Dosage (mg/kg)	C_{max} (μg/mL)	$AUC_{0-\infty}$ (μg/mL/hr)	Vd_{ss} (L/kg)	Cl (L/hr/kg)	$t_{1/2}$ (hr)
Preterm neonates[437] (*n* = 5, 0.5-7.5 mo)	1.0/md	0.96	N/A	4.1	0.122	39
Preterm neonates[31] (*n* = 13, 0.06-1.8 mo)	0.5/md	0.96	N/A	1.5	0.036	14.8
Infants/children[253] (*n* = 13, 0.08-18 yr)	0.5/sd	1.5	N/A	0.37	0.026	9.9
Infants/children[44] (*n* = 12, 0.3-14 yr)	0.68/md	2.9	N/A	0.76	0.027	18.1
Infants/children[327] (*n* = 20, 2.2-14.3 yr)	0.98/sd	2.43	22.0	0.92	0.039	15.1
Children/adults[9] (*n* = 20, 4-66 yr)	1.0/md	2.9	36	1.1	0.028	39

**All values are given as means.*

$AUC_{0-\infty}$, area under the concentration versus time curve from time zero to infinity; Cl, plasma clearance; C_{max}, peak plasma concentration; md, multiple-dose data; N/A, not assessed; sd, single-dose data; $t_{1/2}$, elimination half-life; Vd_{ss}, apparent volume of distribution at steady state.

Other, potentially relevant adverse effects of amphotericin B include demyelinizing encephalopathy in bone marrow transplant recipients conditioned with total body irradiation and/or receiving cyclosporine and concomitant high-dose amphotericin B therapy,[319] and normocytic, normochromic anemia associated with low erythropoietin levels after long-term administration.[99] Amphotericin B deoxycholate is topically irritating; therefore, a central line should be used for infusion, and local instillation of amphotericin B should be considered only in conjunction with expert consultation.

THERAPEUTIC MONITORING. Historically, 1-hour postinfusion plasma concentrations of twice the MIC of the fungal isolate have been proposed as targets for treatment of yeast infections.[123] However, monitoring of amphotericin B concentrations in plasma or CSF appears to be of little value because relationships between plasma and tissue concentrations and clinical efficacy or toxicity have not been adequately characterized.[184]

The toxicity of amphotericin B and the practice of normal saline loading implicate close monitoring of related laboratory parameters. The drug must not be infused in less than 60 minutes and only under particularly careful cardiac monitoring in newborns and in patients with hyperkalemia and renal impairment, circumstances in which arrhythmias resulting from acute potassium release have been observed.[73,179]

DRUG INTERACTIONS. Drug-drug interactions caused by shared metabolic pathways are unknown for amphotericin B. Hypokalemia may be aggravated by corticosteroids, and, in turn, can potentiate digoxin toxicity, cause rhabdomyolysis, and enhance the effects of nonpolarizing muscle relaxants. Similarly, hypomagnesemia may become especially profound in patients with cancer and platinum-associated nephropathy. Impairment of glomerular filtration by amphotericin B may enhance plasma levels and, thereby, toxicity of many renally cleared drugs, including aminoglycosides, vancomycin, fluorocytosine, and cyclosporine.[184] Finally, the simultaneous infusion of granulocytes has been associated with acute pulmonary reactions[521] and therefore should be avoided.

INDICATIONS. With the advent of newer antifungal agents and after the completion of pivotal phase III clinical trials, few indications are left for antifungal treatment of opportunistic mycoses with conventional amphotericin B deoxycholate. These indications include candidemia and acute tissue invasive candidiasis, particularly in neonates, and induction therapy for cryptococcal meningitis; however, in the absence of adequate clinical trials, amphotericin B deoxycholate is still a valid option for treatment of severe forms of the endemic mycoses (Tables 252–2 to 252–4). Depending on both the type of infection and the host, the recommended daily dosage ranges from 0.7 to 1.0 mg/kg/day administered over 2 to 4 hours as tolerated.[184]

For empiric antifungal therapy in the persistently febrile neutropenic host, the historical standard dosage is 0.5 to 0.6 mg/kg/day.[126,366] Efficacy of prophylactic intravenous amphotericin B in the setting of anticancer therapy has not been documented,[186] and a large, randomized, multicenter study failed to show any preventive benefit of aerosolized amphotericin B in neutropenic patients at high risk for developing invasive mold infections.[420] However, aerosolized amphotericin B deoxycholate may have a preventive role in patients who have undergone lung transplantation.[120]

On principle, treatment should be started at the full target dose, with careful bedside monitoring during the first hour of infusion to allow for prompt intervention for infusion-related reactions.[486] With the exception of uncomplicated candidemia and induction therapy of cryptococcal meningoencephalitis, the duration of treatment is ill defined for most infections. Prolonged and individualized therapy often is required until complete resolution of the individual disease process, including the use of lipid formulations of amphotericin B, triazoles, and echinocandins for salvage or consolidation therapy.

TABLE 252–2 Medical Management of Invasive Infections by Opportunistic Yeast

Fungal Disease	Management
Esophageal candidiasis	Fluconazole (8-12 mg/kg QD; max, 800 mg QD PO/IV) Itraconazole (2.5 mg/kg BID PO) Voriconazole (4 mg/kg BID PO/IV)* Amphotericin B deoxycholate (0.5-1.0 mg/kg QD) Echinocandin lipopeptides‡
Uncomplicated candidemia or invasive candidiasis	Amphotericin B deoxycholate (0.6-1.0 mg/kg QD IV) Fluconazole (8-12 mg/kg QD; max, 800 mg QD IV) Fluconazole (16 mg/kg QD) plus amphotericin B deoxycholate (0.7 mg/kg QD for day 1 to 5 IV) Voriconazole (4 mg/kg BID IV; day 1:12 mg/kg IV)* Liposomal amphotericin B (3 mg/kg QD IV) Echinocandin lipopeptides‡
Second-line therapy for • Refractory infections • Limiting toxicity	Liposomal amphotericin B (3-5 mg/kg QD IV) Amphotericin B Lipid Complex (5 mg/kg QD IV) Voriconazole (4 mg/kg BID IV; day 1:12 mg/kg)* Amphotericin B deoxycholate (0.7-1.0 mg/kg QD) plus flucytosine† (100 mg/kg/day in 3 to 4 dosages) Echinocandin lipopeptides‡
Cerebral cryptococcosis	Amphotericin B deoxycholate (0.7 mg/kg QD IV) plus flucytosine† (100 mg/kg/d in 3-4 dosages) for ≥2 weeks (induction), followed by fluconazole (8-12 mg/kg QD PO) (consolidation/maintenance) "Second line" for intolerance of amphotericin B deoxycholate: Liposomal amphotericin B (5 mg/kg QD IV); in case of polyen intolerance: fluconazole (8-12 mg/kg QD) plus flucytosine†
Extracerebral manifestations	Amphotericin B deoxycholate (0.7-1.0 mg/kg QD IV) Fluconazole (8-12 mg/kg QD) Amphotericin B deoxycholate (0.7 mg/kg QD) plus flucytosine‡ (100 mg/kg/d in 3-4 dosages)

**IV dosage for patients > 11 years; IV dosage for children from 2 to 11 years: 7 mg/kg QD without loading dose. PO dosage from 2 to 18 years: 200 mg BID.*
†Monitoring of plasma concentrations recommended (>40 bis < 100 μg/mL).
‡For details of pediatric approval status of the three compounds please see text.

TABLE 252–3 Medical Management of Invasive Infections by Opportunistic Molds

Fungal Disease	Management
Invasive Aspergillosis	
First-line therapy	Voriconazole (4 mg/kg IV bid, day 1: 12 mg/kg)*
	Liposomal amphotericin B (3 mg/kg qd IV)†
Second-line options for	Liposomal amphotericin B (≥5 mg/kg qd IV)
Refractory infections	Amphotericin B lipid complex (5 mg/kg qd IV)
Limiting toxicity	Caspofungin (50 mg qd IV, day 1: 70 mg/m^2)‡
	Voriconazole (4 mg/kg IV bid, day 1: 12 mg/kg)*
	Posaconazole (400 mg bid or 200 mg qid PO)§
Therapy for immediately life-threatening infections	Liposomal amphotericin B (5 mg/kg qd IV) plus caspofungin (50 mg qd IV, day 1: 70 mg)‡
	Voriconazole (4 mg/kg IV qd, day 1: 12 mg/kg)† plus caspofungin (50 mg/m^2 qd IV, day 1: 70 mg/m^2)‡
Consolidation therapy	Voriconazole (200 mg PO bid)*
	Itraconazole (2.5 mg/kg bid PO)¶
	Posaconazole (400 mg bid or 200 mg qid PO)§
Non-*Aspergillus* hyalohyphomycetes	Voriconazole (4 mg/kg IV bid, day 1: 12 mg/kg)*
	Liposomal amphotericin B (≥5 mg/kg IV qd)
	Amphotericin B lipid complex (5 mg/kg IV qd)
	Posaconazole (400 mg bid or 200 mg qid PO)§
Zygomycetes infections	Liposomal amphotericin B (≥5 mg/kg IV qd)
	Amphotericin B lipid complex (≥5 mg/kg IV qd)
	Posaconazole (400 mg bid or 200 mg qid PO) for second-line therapy only§
Dematiaceous molds	Voriconazole (4 mg/kg bid IV, day 1: 12 mg/kg)*
	Liposomal amphotericin B (5 mg/kg qd IV)
	Amphotericin B lipid complex (5 mg/kg qd IV)
	Posaconazole (400 mg bid or 200 mg qid PO)§
	Itraconazole (2.5 mg bid PO)¶

**IV dosage for patients >11 years; IV dosage for children from 2 to 11 years: 7 mg/kg qd without loading dose. PO dosages from 2 years onward: 200 mg bid.*
†Based on a recently presented clinical trial.[92]
‡Children and adolescents aged 3 months to 17 years; max, dose: 70 mg. For adult dosage, please see text.
§Not approved in pediatric patients; 800 mg/day safely given to children >12 years of age.
¶Proposed pediatric dosage; monitoring of trough concentrations recommended (target: >0.5 μg/mL).

Amphotericin B Lipid Formulations

During the late 1990s, three novel formulations of amphotericin B were approved in the United States and most of Europe: AmB colloidal dispersion (ABCD, Amphocil, or Amphotec), AmB lipid complex (ABLC or Abelcet), and a small unilamellar vesicle (SUV) liposomal formulation (L-AmB, AmBisome). Because of their reduced nephrotoxicity in comparison with deoxycholate amphotericin B, these compounds allow for the safe delivery of higher dosages of the parent. However, data from animal models also suggest that higher dosages are required for equivalent antifungal efficacy.[207,520]

PHYSICOCHEMICAL PROPERTIES AND PHARMACOKINETICS. The carriers of the lipid formulations are composed of biodegradable, amphiphilic bilayered membranes in which the hydrophilic heads of the lipid molecules face outward to shield the hydrophobic tails. The membranes may form either spherical vesicles called liposomes or bilayered complexes or dispersions with no specific vesicular structure. Incorporated into these water-soluble carriers, amphotericin B becomes soluble in plasma and available for distribution. Each of the lipid formulations of amphotericin B possesses distinct physicochemical and pharmacokinetic properties. All three, however, preferentially distribute to organs of the mononuclear phagocytic system (MPS) and functionally spare the kidney. Although the micellar dispersion of ABCD behaves kinetically very similarly to amphotericin B deoxycholate, the small unilamellar liposomal preparation has a prolonged circulation time in plasma, achieves strikingly high C_{max} and area under the concentration versus time curve (AUC) values, and is only slowly taken up by the MPS. In contrast, the large, ribbon-like aggregates of ABLC are efficiently opsonized by plasma proteins and rapidly taken up by the MPS, thus resulting in lower peak plasma and AUC values (Table 252–5).[182,207] Whether and how the distinct physicochemical and pharmacokinetic features of each formulation translate into different pharmacodynamic properties in vivo are largely unknown. However, experimental head-to-head comparisons of all four formulations of amphotericin B against defined invasive mycoses suggest that important differences exist in antifungal efficacy depending on the agent, dose, type, and site of infection.[88,176,340]

SAFETY AND ANTIFUNGAL EFFICACY. Safety and antifungal efficacy of ABCD, ABLC, and L-AmB were demonstrated in an array of phase II and III clinical trials in immunocompromised, mostly adult patients with a wide spectrum of underlying disorders.[92,386,394,488,509] The overall response rates in these trials ranged from 53 to 84 percent in patients with invasive candidiasis and 34 to 59 percent, respectively, in patients with presumed or documented invasive aspergillosis.[182,207] A few randomized, controlled trials were completed in which one of the newer formulations was compared with amphotericin B deoxycholate. These studies consistently showed at least equivalent therapeutic efficacy and reduced nephrotoxicity of the investigated lipid formulation. Infusion-related side effects of fever, chills, and rigor appeared to be less frequent with L-AmB only.[16,485,508,509] Several individual cases of substernal chest discomfort, respiratory distress, and sharp flank pain were noted during infusion of L-AmB,[230,389] and in comparative studies, hypoxic episodes associated with fever and chills occurred more frequently in ABCD recipients than in recipients of the deoxycholate formulation.[62,508] Mild increases in serum bilirubin and alkaline phosphatase have been observed with all three formulations, as well as mild increases in serum transaminases with L-AmB. However, no case of fatal liver disease has occurred.[182,207,520]

EXPERIENCE IN PEDIATRIC PATIENTS. Considerable numbers of pediatric patients have been treated with ABCD,

ABLC, or L-AmB on protocols in the clinical trials cited earlier. Separately published pediatric data are discussed in the following subsections.

ABCD. ABCD is a complex of amphotericin B and sodium cholesteryl sulfate in an approximate 1:1 molar ratio that forms disklike colloidal structures on dissolution.[182] Population-based, multiple-dose pharmacokinetic studies with ABCD in bone marrow transplant recipients with systemic fungal infections included the compartmental analysis of five children younger than 13 years of age who received the compound at 7.0 and 7.5 mg/kg/day. Estimated pharmacokinetic parameters in these children were not significantly different from those obtained in a dose-matched cohort of adult patients: under conditions of steady state, the mean AUC from 0 to 24 hours ($AUC_{0\text{-}24}$) was 7.10 μg/mL/hour (normalized to a 1-mg/kg/day dose), the mean volume of distribution was 4.57 L/kg, and the mean total clearance was 0.144 L/hour/kg.[9]

A double-blind, randomized trial comparing ABCD (4 mg/kg/day) with D-AmB (0.8 mg/kg/day) for empiric antifungal therapy of febrile neutropenic patients separately reported safety data from 46 children (≥2 to <16 years of age) either randomized to ABCD (n = 25) or D-AmB (n = 21). ABCD was overall significantly less nephrotoxic than was D-AmB, and no differences in adverse events and efficacy were reported as compared with the (much larger) adult study population.[508] An additional 70 children (0 to 15 years; mean, 8.8 years) with presumed or proven invasive fungal infections refractory to or intolerant of amphotericin B were treated on five different open-label studies of ABCD. The dosages ranged from 0.8 to 7.5 mg/kg (mean, 4.5 mg/kg), administered for a mean of 30 days (range, 1 to 192). Although 67 percent of patients reported infusion-related reactions, nephrotoxicity, defined as an increase in serum creatinine to two times or greater the baseline value, was reported in only 12 percent. Other unexpected toxicities were not observed.[406]

The published experience in the neonatal setting is limited to 16 very-low-birth-weight infants (779 ± 170 g; 25 ± 2 weeks) with invasive candidiasis and a serum creatinine concentration of 1.2 mg/dL or greater.[278] Infants received 3 mg/kg ABCD on day 1, followed by 5 mg/kg/day thereafter; a second agent was permitted for candidemia that persisted for 7 or more days. Thirteen of 14 evaluable patients cleared the organism after therapy with ABCD alone (n = 8) or in combination with another agent (n = 5), and overall survival was 75 percent. ABCD was well tolerated without infusion-related reactions, increases in serum creatinine, hepatotoxicity, or hepatotoxicity.

These data overall indicate no fundamental differences in disposition, safety, and antifungal efficacy of ABCD in comparison with adult populations. The U.S. Food and Drug Administration (FDA)–approved indication is treatment of probable or proven invasive aspergillosis refractory to or intolerant of ampho-

TABLE 252–4 Medical Management of Invasive Infections by Endemic Molds

Fungal Disease	Management
Histoplasmosis	Liposomal amphotericin B (3 mg/kg qd IV) Amphotericin B deoxycholate (0.7 mg/kg qd IV) Itraconazole*† (2.5 mg/kg bid PO) Fluconazole (8-12 mg/kg qd PO/IV)
Coccidioidomycosis	Amphotericin B deoxycholate (0.5-1.0 mg/kg qd IV) Fluconazole‡ (8-12 mg/kg qd PO/IV) Itraconazole*† (2.5 mg/kg bid) Posaconazole (400 mg bid or 200 mg qid PO)§
Blastomycosis	Amphotericin B deoxycholate (0.5-1.0 mg/kg qd IV) Itraconazole*† (2.5 mg/kg bid)
Paracoccidioidomycosis	Amphotericin B deoxycholate (0.5-1.0 mg/kg qd IV) Itraconazole*† (2.5 mg/kg bid) (B-III)
Penicilliosis	Amphotericin B deoxycholate (0.5-1.0 mg/kg qd IV) Itraconazole*† (2.5 mg/kg bid) (A-II)
Sporotrichosis	Amphotericin B deoxycholate (0.5-1.0 mg/kg qd) Itraconazole*† (2.5 mg/kg bid) Fluconazole (8-12 mg/kg qd PO/IV) Terbinafine (lymphocutaneous disease only)

**Clinically stable patients with mild to moderate disease outside and no central nervous system involvement, or as consolidation or maintenance therapy. Dosages refer to the cyclodextrin solution.*

†Monitoring of trough plasma concentrations is recommended (target: >0.5 μg/mL). Intravenous therapy: 200 mg bid for 2 days, followed by 200 mg qd for patients >18 years of age.

‡Agent of first choice in (1) consolidation therapy of meningeal coccidioidomycosis; (2) Coccidioides *meningitis; (3) coccidioidomycosis in stable patients with mild to moderate disease or as consolidation or maintenance therapy.*

§Second-line therapy; not approved in pediatric patients; 800 mg/day safely given to children >12 years of age.

TABLE 252–5 Physicochemical Properties and Multiple-Dose Pharmacokinetic Parameters of the Four Currently Marketed Amphotericin B Formulations*

	D-AmB	ABCD	ABLC	L-AmB
Lipids (molar ratio)	Deoxycholate	Cholesteryl sulfate	DMPC/DMPG (7:3)	HPC/CHOL/DSPG (2:1:0.8)
Mol% AmB	34%	50%	50%	10%
Lipid configuration	Micelles	Micelles membrane-like	SUVs	
Diameter (μm)	0.05	0.12-0.14	1.6-11	0.08
Dosage (mg AmB/kg)	1	5	5	5
C_{max} (μg/mL)	2.9	3.1	1.7	58
$AUC_{0\text{-}\infty}$ (μg/mL/hr)	36	43	14	713
Vd_{ss} (L/kg)	1.1	4.3	131	0.22
Cl (L/hr/kg)	0.028	0.117	0.476	0.017

**Data represent mean values, stem from adult patients and were obtained after different rates of infusion.*

$AUC_{0\text{-}\infty}$, area under the concentration versus time curve from time zero to infinity; CHOL, cholesterol; Cl, plasma clearance; Cmax, peak plasma concentration; DMPC, dimiristoyl phosphatidylcholine; DMPG, dimiristoyl phosphatidylglycerol; DSPG, disteaoryl phosphatidylglycerol; HPC, hydrogenated phosphatidylcholine; SUV, small unilamellar vesicles; Vd_{ss}, apparent volume of distribution at steady state.

Modified from Groll, A. H., Muller, F. M., Piscitelli, S. C., and Walsh, T. J.: Lipid formulations of amphotericin B: Clinical perspectives for the management of invasive fungal infections in children with cancer. Klin. Padiatr. 210:*264-273, 1998.*

tericin B deoxycholate, and the approved dosage is 3 to 4 mg/kg/day, administered over the course of 2 hours.

ABLC. ABLC is composed of dimyristoyl phosphatidylcholine/dimyristoyl phosphatidylglycerol (DMPC/DMPG) in a 1:1 molar ratio of lipid to amphotericin B and forms large, ribbon-like structures. The pharmacokinetic properties of ABLC were studied in whole blood in three pediatric patients with cancer who received the compound at 2.5 mg/kg over the course of 6 weeks for hepatosplenic candidiasis.[498] Steady state was achieved by day 7 of therapy; following the final dose, the mean $AUC_{0\text{-}24}$ was 11.9 ± 2.6 μg/mL/hour, the mean C_{max} was 1.69 ± 0.75, and clearance was 0.218 L/kg/hour. In the six patients evaluable for safety assessment, mean serum creatinine levels were stable at the end of therapy and at 1-month follow-up, and no increase in hepatic transaminases occurred. Five of the patients had infusion-related reactions to the first dose, which was prospectively monitored without prior premedication; however, infusion-related adverse reactions were well controlled thereafter by conventional premedications. All evaluable patients responded to therapy.

Safety and antifungal efficacy of ABLC were studied in 111 treatment episodes in pediatric patients (21 days to 16 years of age) refractory of or intolerant to conventional antifungal agents through an open-label, emergency-use protocol in the United States.[496] ABLC was administered at a mean daily dosage of 4.85 mg/kg (range, 1.1 to 9.5 mg/kg/day) for a mean duration of 38.9 days (range, 1 to 198 days). The mean serum creatinine for the entire study population did not significantly change between baseline (1.23 ± 0.11 mg/dL) and cessation of ABLC therapy (1.32 ± 0.12 mg/dL) during 6 weeks. No significant differences were observed between baseline and end-of-therapy levels of serum potassium, magnesium, hepatic transaminases, alkaline phosphatase, and hemoglobin. However, an increase in the mean total bilirubin (3.66 ± 0.73 to 5.13 ± 1.09 mg/dL) occurred at the end of therapy ($p = .054$). In 7 patients (6%), ABLC therapy was discontinued because of one or more adverse effects, and in 6 patients (5%), ABLC was discontinued because of progression of disease. Among 54 cases fulfilling criteria for evaluation of antifungal efficacy, a complete or partial therapeutic response was obtained in 38 patients (70%).

The safety and efficacy of ABLC also were assessed in 548 children and adolescents who were enrolled in the Collaborative Exchange of Antifungal Research (CLEAR) registry of the manufacturer between 1996 and 2000. Most patients were either intolerant of or refractory to conventional antifungal therapy. Response data were evaluable for 255 of the 285 patients with documented single or multiple pathogens. A complete (cured) or partial (improved) response was achieved in 54.9 percent of patients. No significant difference was noted between the rates of new hemodialysis versus baseline hemodialysis. Elevations in serum creatinine of greater than 1.5 times baseline and greater than 2.5 times baseline values were seen in 24.8 and 8.8 percent of all patients, respectively. The overall response rate and safety profile in pediatric patients were consistent with earlier reported findings of smaller trials.[513]

Eleven infants aged 6 months and younger with candidemia were enrolled on the U.S. open-label, emergency-use protocol[496] and received 5 to 41 daily doses of ABLC; these infants were between 3 and 13 weeks of age and weighed between 0.8 and 5 kg. Seven of the 11 patients maintained a stable mean serum creatinine; in 4 patients, a rise in serum creatinine was observed, but in each case, the increase was less than 40 percent of the baseline value. No differences were observed between baseline and end-of-therapy mean bilirubin levels. Among the 8 evaluable infants, a complete response was observed in 6 (75%).[496]

A population pharmacokinetic study in 28 mostly immature neonates with invasive *Candida* infections demonstrated that the disposition of ABLC in neonates is similar to that observed in other age groups: weight was the only factor that influenced clearance. Based on the results of this study and a cure rate of greater than 80 percent, a dosage of 2.5 to 5.0 mg/kg is recommended for treatment of neonatal candidiasis.[522]

The current data suggest no fundamental differences in disposition, safety, and antifungal efficacy of ABLC as compared with adults. The FDA-approved indication is treatment of invasive fungal infections refractory to or intolerant of amphotericin B deoxycholate, and the approved dosage is 5 mg/kg/day administered over the course of 2 hours.

L-AmB. L-AmB consists of small, unilamellar spherical vesicles (true liposomes) composed of hydrogenated soy phosphatidylcholine and distearoyl phosphatidylglycerol stabilized by cholesterol and combined with amphotericin B in a 2:0.8:1:0.4 molar ratio.[173] The pharmacokinetic properties of L-AmB in pediatric patients beyond the neonatal period were investigated in a formal phase II, dose-escalation trial investigating dosages of 2.5, 5.0, and 7.5 mg/kg in immunocompromised patients, as well as by using a population-based approach. The results of these studies indicate that the disposition of L-AmB in pediatric patients is not substantially different from that in adults and that weight is a covariate that determines clearance and volume of distribution.[211,424] A pharmacokinetic pilot study was conducted in 12 children at risk for developing invasive fungal infections who received once-weekly, high-dose L-AmB (10 mg/kg over 2 hours) as prophylaxis. L-AmB was well tolerated and showed measurable amphotericin B plasma concentrations 7 days after the dose, a finding suggesting that once-weekly administration may provide useful protection against fungal infections.[303]

Many pediatric patients have been enrolled on clinical trials with L-AmB but have not been reported separately.[307,485] Two hundred four children (mean age, 7 years) with neutropenia and fever of unknown origin were randomized in an open-label, multicenter trial to receive conventional amphotericin B deoxycholate at 1 mg/kg/day ($n = 63$), L-AmB at 1 mg/kg/day ($n = 70$), or L-AmB at 3 mg/kg/day ($n = 71$) for empiric antifungal therapy.[370] Twenty-nine percent of patients treated with L-AmB 1, 39 percent of patients treated with L-AmB 3, and 54 percent of patients treated with AMBD experienced adverse effects ($p = .01$); nephrotoxicity, defined as 100 percent or more increase in serum creatinine from baseline, was noted in 8, 11, and 21 percent, respectively (NS). Hypokalemia (<2.5 mmol/L) occurred in 10, 11, and 26 percent of patients, respectively ($p = .02$); increases in serum transaminase levels (≥110 U/L) occurred in 17, 23, and 17 percent, respectively (NS); and increases in serum bilirubin (≥35 μmol/L) occurred in 11, 12, and 10 percent of patients, respectively. Efficacy assessment by intent-to-treat analysis indicated successful therapy in 51 percent of children treated with AMBD and 64 or 63 percent in children treated with L-AmB at either 1 or 3 mg/kg/day ($p = .22$), respectively. L-AmB at either 1 or 3 mg/kg/day was significantly safer and at least equivalent to AMBD with regard to resolution of fever of unknown origin. L-AmB was well tolerated and effective in cohorts of immunocompromised children requiring antifungal therapy for proven or suspected infections, including patients with bone marrow transplants for primary immunodeficiencies[352] and patients with cancer.[387] A phase IV analysis of 141 courses of L-AmB administered for a mean of 17 days' duration at a mean maximum dosage of 2.5 mg/kg for various indications to pediatric patients with cancer and hematopoietic stem cell transplantation (HSCT) revealed a low rate of adverse events (4%), necessitating discontinuation. Whereas mean aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, and bilirubin values were slightly higher at the end of treatment ($p < .01$), bilirubin and creatinine values were not different from baseline. L-AmB had acceptable safety and tolerance and displayed efficacy in prevention and treatment of invasive fungal infections.[250]

L-AmB (2.5 to 7 mg/kg/day) was evaluated prospectively in 24 very-low-birth-weight infants (mean birth weight, 847 ± 244 g; mean gestational age, 26 weeks) with systemic candidiasis. In 13 infants, previous antifungal therapy with amphotericin B (with or without 5-flucytosine) had failed. *Candida* spp. were isolated from the blood in all 25 episodes and from skin abscesses and urine in 4 infants each, respectively. The mean duration of therapy was 21 days; the cumulative L-AmB dose was 94 mg/kg. Fungal eradication was achieved in 92 percent of the episodes; 20 (83%) infants were considered clinically cured at the end of treatment. No major adverse effects were recorded. One infant developed increased bilirubin and hepatic transaminase levels during therapy. Four (17%) infants died; in 2 of them (8%), the cause of death was attributed directly to systemic candidiasis.[233] In a second study undertaken by the same investigators, high-dose (5 to 7 mg/kg/day) L-AmB was evaluated prospectively in 41 episodes of systemic candidiasis occurring in 37 neonates (36 of the 37 were premature infants with very low birth weights). *Candida* spp. were isolated from blood in all patients and from urine, skin abscesses, and peritoneal fluid in 6, 5, and 1 neonates, respectively. Twenty-eight, 5, and 8 infants received 7, 6 to 6.5, and 5 mg/kg/day, respectively. Median duration of therapy was 18 days; median cumulative dose was 94 mg/kg. Fungal eradication was achieved in 39 of 41 (95%) episodes. One patient died of systemic candidiasis on day 12 of therapy. High-dose L-AmB was effective and safe in the treatment of neonatal candidiasis. Fungal eradication was achieved more rapidly in patients treated early with high doses and in patients who received high-dose L-AmB as first-line therapy.[232]

Current data indicate no substantial differences in pharmacokinetics and pharmacodynamics of L-AmB between pediatric and adult patients. The FDA-approved dosages are 3 mg/kg/day (empiric antifungal therapy in febrile neutropenic patients), 3 to 5 mg/kg/day (therapy of invasive infections intolerant or refractory to amphotericin B deoxycholate), and 6 mg/kg/day for cryptococcal meningitis, administered over the course of 2 hours.

INDICATIONS. The lipid formulations of AmB represent an important therapeutic advance in the management of invasive opportunistic fungal infections in immunocompromised patients. All three compounds have less renal toxicity than does conventional amphotericin B as defined by development of azotemia; distal tubular toxicity also may be somewhat reduced. Infusion-related reactions of fever, chills, and rigor appear to occur substantially less frequently only with L-AmB, and no new toxicities have been noted. The available pharmacokinetic and safety data from children so far indicate no fundamental differences from data obtained in the adult population.

Therapeutically, the lipid formulations are at least as effective as is conventional amphotericin B for treatment of most opportunistic human mycoses, and these formulations can be effective if conventional amphotericin B has failed. They may be indicated when toxicity prohibits the administration of effective dosages of AMBD and when standard therapies fail to induce a therapeutic response against an organism susceptible to amphotericin B. The experience with life-threatening endemic mycoses, however, is limited. The lipid formulations currently are approved for the treatment of patients with invasive mycoses refractory of or intolerant to AMBD, and, limited to L-AmB, for empiric therapy of persistently neutropenic patients (see Tables 252–2 to 252–4).

FLUCYTOSINE

Flucytosine (5-fluorocytosine) is a low-molecular-weight, water-soluble, synthetic fluorinated pyrimidine analogue (Fig. 252–3). It is taken up into the fungal cell by the fungus-specific enzyme cytosine permease and is converted in the cytoplasm by cytosine deaminase to 5-fluorouracil, a potent antimetabolite that causes RNA miscoding and inhibits DNA synthesis (Fig. 252–4).[119] Flucytosine is relatively nontoxic to mammalian cells because of the absence or very low level of activity of cytosine deaminase. In the United States, flucytosine is available only as oral formulation; an intravenous formulation is available outside the United States in select countries.

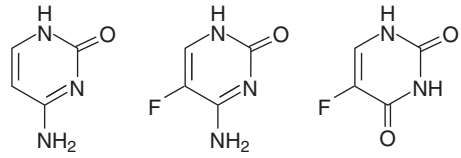


Figure 252–3 Structural formulas of cytosine, flucytosine, and fluorouracil.

ANTIFUNGAL ACTIVITY. The antifungal activity of flucytosine in vitro is essentially limited to *Candida* spp., *C. neoformans*, *Cryptococcus gattii*, *Saccharomyces cerevisiae*, *Rhodotorula* spp. and some dematiaceous molds.[114,140,209,360,362,456] Flucytosine generally is thought to have no or weak activity against *Aspergillus* spp. and other hyaline molds,[153,295] although this lack of activity in vitro may be pH-dependent[444] and does not correlate with the documented efficacy in animal models.[446] Notably, whereas *Candida krusei* appears to be less susceptible to flucytosine, the compound is highly active against *Candida glabrata*.[119,140,360] Synergistic or additive effects in combination with amphotericin B have been observed against *Candida*[302,314] and *Aspergillus* spp.[445] and in combination with amphotericin B, fluconazole, voriconazole, and posaconazole against *C. neoformans*.[34,194,302,332,422] Combination with echinocandins was additive or indifferent in vitro against *Candida* spp.[235]

Two mechanisms of resistance have been reported: (1) mutations in enzymes necessary for cellular uptake and transport of flucytosine or its metabolism and (2) increased synthesis of pyrimidine that competes with the fluorinated antimetabolites of the compound.[117,477] In pretreatment isolates, intrinsically resistant strains have been found in 3 to 8 percent of *C. albicans*, in 0 to 8 percent of non-*albicans Candida* spp., and in 2 percent or less of *C. neoformans* isolates.[301,360] In an analysis of 8803 clinical isolates of *Candida* spp. (18 species) obtained from more than 200 medical centers worldwide between 1992 and 2001 that used the broth microdilution test according to National Committee on Clinical Laboratory Standards guidelines, primary resistance to flucytosine was an uncommon occurrence among *Candida* spp. (95% sensitive, 2% intermediate, and 3% resistant), with the exception of *C. krusei* (5% sensitive, 67% intermediate, and 28% resistant).[360] Development of resistance to flucytosine can be observed during treatment,[140] and it is thought to be caused predominantly by selection of resistant clones.[477] As a consequence, flucytosine is rarely given alone but instead is given in combination with amphotericin B or fluconazole.

PHARMACODYNAMICS. Time-kill assays against *Candida* spp. and *C. neoformans* demonstrated a predominantly concentration-independent fungistatic (99% reduction in colony-forming units) activity of flucytosine at concentrations exceeding the MIC of the investigated isolates.[275,467] A prolonged PAFE against these organisms was noted consistently that was dependent on concentration and duration of exposure and ranged from 0.8 to 10 hours.[413,526] In addition, synergistic PAFEs were demonstrated with flucytosine in combination with fluconazole and amphotericin B, respectively, against *C. albicans*.[309,413,414] Investigation of pharmacokinetic and pharmacodynamic relationships in a neutropenic mouse

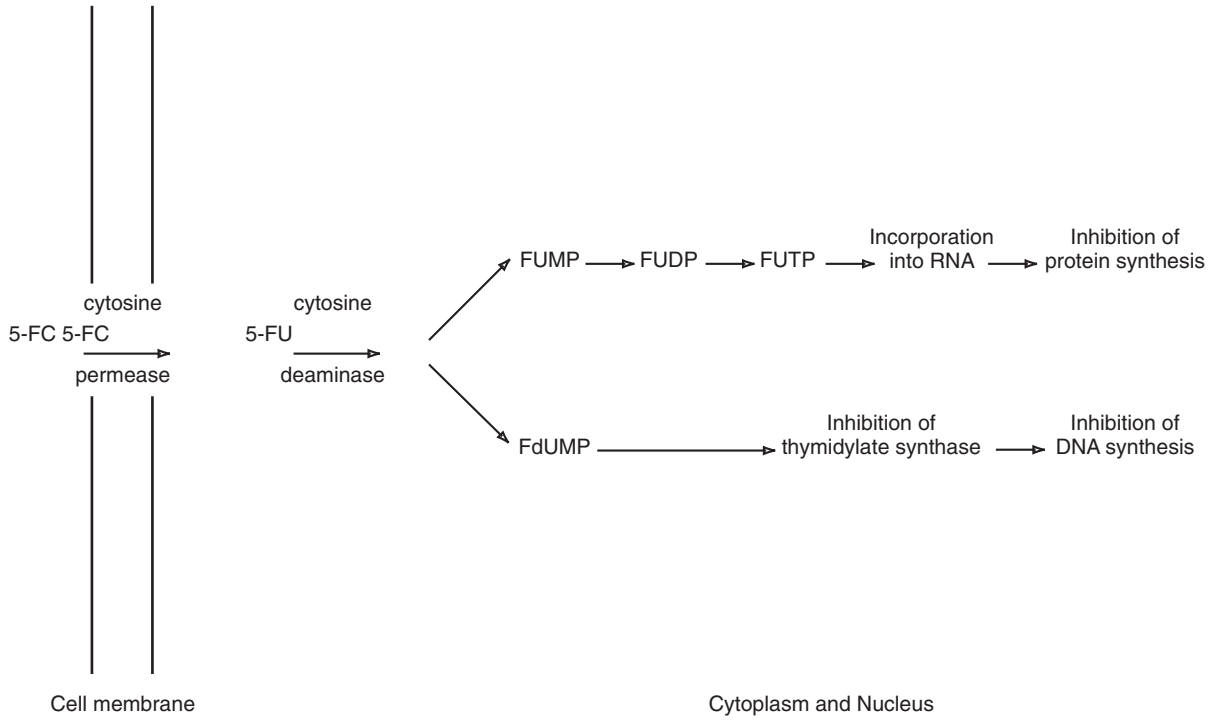


Figure 252–4 Schematic of intracellular pathways and mechanism of action of flucytosine. 5-FC, flucytosine; FdUMP, 5-fluorodeoxyuridine monophosphate; 5-FU, 5-fluorouracil; FUDP, 5-fluoridine diphosphate; FUMP, 5-fluoridine monophosphate; FUTP, 5-fluoridine triphosphate. *(Modified from Groll, A. H., Piscitelli, S. C., and Walsh, T. J.: Antifungal pharmacodynamics: Concentration-effect relationships in vitro and in vivo. Pharmacotherapy 21[Suppl. 8]:133-148, 2001; and Vermes, A., Guchelaar, H. J., and Dankert, J.: Flucytosine: A review of its pharmacology, clinical indications, pharmacokinetics, toxicity and drug interactions. J. Antimicrob. Chemother. 46:171-179, 2000.)*

model of disseminated candidiasis that used the residual fungal burden in kidney tissue as the end-point of antifungal efficacy revealed that both the time above the MIC and the AUC/MIC ratio were important in predicting efficacy; the peak level/MIC ratio was the least important parameter. Maximal efficacy was observed when levels exceeded the MIC for only 20 to 25 percent of the 24-hour dosing interval.[18] In experiments that used a pharmacodynamic model of disseminated candidiasis and bridging to humans by population pharmacokinetics and Monte Carlo simulation, an in vivo drug exposure breakpoint for flucytosine was apparent when serum levels were greater than the MIC for 45 percent of the dosing interval. The Monte Carlo simulations suggested that, using a human dose of 100 mg/kg/day in four divided doses, flucytosine resistance was defined at an MIC of 32 mg/L. Target attainment rates after administration of 25, 50, and 100 mg/kg/day were similar.[213] These data collectively suggest that lower dosages or less frequent dosing may yield identical antifungal efficacy while further reducing potential toxicities of flucytosine that are thought to be concentration-dependent.[140]

PHARMACOKINETICS. Flucytosine is absorbed readily from the gastrointestinal tract, and oral bioavailability exceeds 80 percent (Table 252–6). C_{max} occurs 1 to 2 hours after administration. As a water-soluble compound, flucytosine has negligible protein binding (4%) and is widely distributed in the body, with a volume of distribution that approximates that of total body water. Mean CSF concentrations usually are 65 to 90 percent of simultaneous plasma concentrations. The drug penetrates well into peritoneal fluid, inflamed joints, and other fluid compartments, including the eye.[99,184]

In humans, less than 1 percent of a given dose of flucytosine is thought to undergo hepatic metabolism. In addition to 5-fluorouracil, several other metabolites of yet unclear toxic potential have been identified.[289] Some evidence suggests that bacteria of the gastrointestinal flora deaminate flucytosine to resorbable 5-fluorouracil,[196,289] a finding that may account for some of the toxicities observed after oral administration of the drug. Approximately 95 percent of a given dose of flucytosine is excreted into the urine in unchanged, active form by simple glomerular filtration, and the plasma elimination half-life is 3 to 6 hours in adult patients with normal renal function.[184] Because the compound's elimination parallels the glomerular filtration rate, adjustment of the dosage is necessary in patients with impaired renal function.[99] In patients undergoing hemodialysis, a dose of 37.5 mg/kg is recommended following dialysis,[51] and in those undergoing hemofiltration, the dosage needs to be adjusted to the individual filtration rate.[222,268] In peritoneal dialysis, the compound can be administered systemically or intraperitoneally.[308] Although the data are limited, impaired liver function does not appear to alter the disposition of flucytosine.[99]

In infants and children, the pharmacokinetic properties of flucytosine have not been systematically characterized thus

TABLE 252–6 Pharmacokinetics of Flucytosine in Adults

Parameter or Characteristic	Value
Oral bioavailability	≥80%
C_{max}	50-120 μg/mL*
T_{max}	1-2 hr
Protein binding	4%
Vd_{ss}	0.6-0.7 L/kg
$t_{1/2}$beta	3-6 hr
Clearance	≥95% renal
Unchanged drug in urine	≥95%
Relative cerebrospinal fluid levels	65%-90%

**At steady state in patients with cryptococcal meningitis receiving 4 × 2 g/day PO.*
C_{max}, peak plasma concentration; T_{max}, time until occurrence of peak plasma concentration; Vd_{ss}, volume of distribution at steady state.
Modified from Groll, A. H., Piscitelli, S. C., and Walsh, T. J.: Antifungal pharmacodynamics: Concentration-effect relationships in vitro and in vivo. Pharmacotherapy 21(Suppl. 8):133-148, 2001.

far. However, because of very similar physicochemical and pharmacokinetic properties, developmental changes in disposition similar to those found with fluconazole can be anticipated. Indeed, a similarly marked interindividual variability in clearance and volume of distribution has been reported in neonates[31] that renders uniform dosing recommendations in this population impossible.

A starting dosage for both adults and children of 100 mg/kg daily divided into three or four doses is recommended currently. Monitoring of plasma concentrations is essential to adjust dosage to changing renal function and to avoid toxicity. After oral administration, near peak levels 2 hours after dosing overlap with trough levels as patients reach steady state, and thus these levels are sufficient for therapeutic monitoring.[140] In practice, plasma levels between 40 (trough) and less than 100 μg/mL (peak) correlate with antifungal efficacy and seldom are associated with hematologic adverse effects.[140,478] However, retrospective evaluations in the United Kingdom revealed that only a fraction of plasma levels ordered for therapeutic monitoring were in this concentration range.[353,435]

ADVERSE EFFECTS. Common adverse effects associated with flucytosine, effects that occur in 5 to 6 percent of patients, include gastrointestinal intolerance and reversible elevations of hepatic transaminases and alkaline phosphatase. Rarer side effects are skin rashes, blood eosinophilia, and crystalluria.[184]

Hematologic adverse effects have been reported in overall 6 percent of patients receiving oral flucytosine and may include neutropenia, thrombocytopenia, or pancytopenia. Although these effects usually are reversible after discontinuation of the drug or dosage reduction, fatal outcomes have been reported.[140,184] Some of the adverse effects of flucytosine may result either from conversion of the compound to 5-fluorouracil by the gastrointestinal bacterial flora[196,289] or from toxic effects of endogenously produced metabolites. Notably, hematologic adverse effects occur less frequently if plasma levels of flucytosine do not exceed 100 μg/mL.[140,278] However, this relationship is not absolute, and hematologic toxicity may occur at levels much lower than threshold.

DRUG INTERACTIONS. Orally administered, nonresorbable antibiotics and administration of aluminum/magnesium hydroxide–based antacids may delay but do not impair absorption of the compound from the gastrointestinal tract.[184] Flucytosine undergoes only minor hepatic metabolism, and it is not known to interfere with the cytochrome P-450 enzyme system. However, any drug that can cause a reduction in the glomerular filtration rate may lead to increased flucytosine serum levels and thereby has the potential to enhance flucytosine-associated toxicity. This phenomenon almost invariably is encountered with concomitant administration of amphotericin B, but it can occur similarly with numerous antimicrobial agents, anticancer drugs, and cyclosporine, to name only the most common examples.[99] The anticancer drug cytosine arabinoside competitively inhibits the action of flucytosine, and these drugs should not be given concomitantly.[77]

CLINICAL INDICATIONS. Because of the propensity of susceptible organisms to develop resistance in vitro,[367] flucytosine traditionally is not administered as a single agent. Ample laboratory and clinical experience exists regarding the combination of conventional amphotericin B and flucytosine (see Table 252–2).[119,184,477] Randomized clinical trials established the use of amphotericin B in combination with flucytosine as standard for induction therapy of cryptococcal meningitis in non–human immunodeficiency virus (HIV)-infected and HIV-infected patients.[43,468] Indeed, a small but well-designed trial demonstrated that the clearance of cryptococci from the CSF was significantly faster with amphotericin B in combination with flucytosine than with amphotericin B alone, amphotericin B in combination with fluconazole, or triple therapy, a finding confirming that the combination is the most rapidly fungicidal regimen.[66]

Although no comparative trials have been performed, the cumulative clinical experience supports the combination of amphotericin B with flucytosine for the treatment of *Candida* infections involving deep tissues, particularly in critically ill patients and when non-*albicans Candida* spp. are involved.[140,215,296,448] This includes *Candida* meningitis, endophthalmitis, endocarditis, vasculitis, and peritonitis, as well as osteoarticular, renal, and disseminated candidiasis.[184,486] Susceptibility of *Aspergillus* spp. to flucytosine in vitro is controversial,[153,295,444] and high-dose amphotericin B in combination with flucytosine compared with high-dose amphotericin B alone in the treatment of invasive aspergillosis has not been investigated. Although the combination has been employed in cases of successful treatment of invasive aspergillosis,[69,106,236] its role remains unclear.

Investigators also have begun to explore combinations of flucytosine with fluconazole.[184] The combination of flucytosine with fluconazole has been studied in a prospective study in 32 patients with acquired immunodeficiency syndrome (AIDS) and cryptococcal meningitis. Clinical and microbiologic responses were superior to those reported with either amphotericin B or fluconazole alone but not as favorable as those documented for the combination of amphotericin B and flucytosine.[267] Thus, flucytosine in combination with fluconazole may be used for cryptococcal meningitis, when treatment with conventional or liposomal amphotericin B is not feasible. In addition, this combination also may be useful as second-line therapy for individual patients with invasive *Candida* infections involving aqueous body compartments.

Flucytosine has demonstrated impressive therapeutic efficacy against chromoblastomycosis[281] and significant activity in murine models of phaeohyphomycosis.[52,116] Given the high case-fatality rate of invasive phaeohyphomycoses in humans, further investigation of flucytosine in combination with other antifungal compounds against these infections appears warranted.

ANTIFUNGAL TRIAZOLES

The antifungal azoles are a class of synthetic compounds that have one or more azole rings and—attached to one of the nitrogen atoms—a more or less complex side chain. Whereas the imidazoles have two, the triazoles have three nitrogen atoms in the five-member ring. The triazole ring confers improved resistance to metabolic degradation, greater target specificity, and an expanded spectrum of activity.[167,168] The imidazoles miconazole and ketoconazole (Fig. 252–5) were the first azole compounds developed for systemic treatment of human mycoses. Severe toxicities associated with the drug carrier (miconazole) and erratic absorption and significant interference with the human cytochrome P-450 system (ketoconazole), however, have limited their clinical usefulness.[184] The triazoles fluconazole and itraconazole (Fig. 252–6), in contrast, have become extremely useful components of the antifungal armamentarium. Overall, they are well tolerated and possess a broad spectrum of activity. Whereas fluconazole and itraconazole have been available since the 1990s, a new generation of antifungal triazoles has entered clinical practice more recently; these so-called second-generation triazoles include voriconazole and posaconazole (Fig. 252–7).

MECHANISM OF ACTION. The antifungal azoles, as a class, target ergosterol biosynthesis by inhibiting the fungal cytochrome P-450–dependent enzyme lanosterol 14α-demethylase. This inhibition interrupts the conversion of lanosterol to ergosterol and thus leads to accumulation of aberrant 14α-methylsterols and depletion of ergosterol in the fungal cell mem-

brane (Fig. 252–8). These effects alter cell membrane properties and function and, depending on organism and compound, may lead to cell death or inhibition of cell growth and replication. In addition, the azoles also inhibit cytochrome P-450–dependent enzymes of the fungal respiration chain, but the contribution of this action to their overall activity is unclear. Interaction with structurally similar mammalian cytochrome P-450–dependent enzyme systems is responsible for most toxicities and drug interactions of this class of compounds.[184,470]

ANTIFUNGAL ACTIVITY. Fluconazole and itraconazole are active principally against dermatophytes, *Candida* spp., *C. neoformans*, *C. gattii*, *T. asahii*, and other uncommon yeast organisms, as well as against dimorphic fungi such as *Histoplasma capsulatum*, *Coccidioides immitis*, *Blastomyces dermatitidis*, *Paracoccidioides brasiliensis*, and *Sporothrix schenkii*.[167,168,456] These drugs have less activity against *C. glabrata* and none against *C. krusei*.[184,383] Clinically useful activity against *Aspergillus* spp. and dematiaceous molds is restricted to itraconazole, and both itraconazole and fluconazole are considered inactive against *Fusarium* spp. and the Zygomycetes.[184] The second-generation triazoles voriconazole and posaconazole have enhanced target activity and are active against a wide spectrum of clinically important fungi, including *Candida* spp., *T. asahii*, *C. neoformans*, *Aspergillus* spp., *Fusarium* spp. and other hyaline molds, as well as dematiaceous and dimorphic molds.[5,81,84,87,184] In contrast to fluconazole and itraconazole, both agents are active against *C. glabrata* and *C. krusei*; posaconazole also is active against Zygomycetes, a feature that distinguishes it from all current azole compounds.[172]

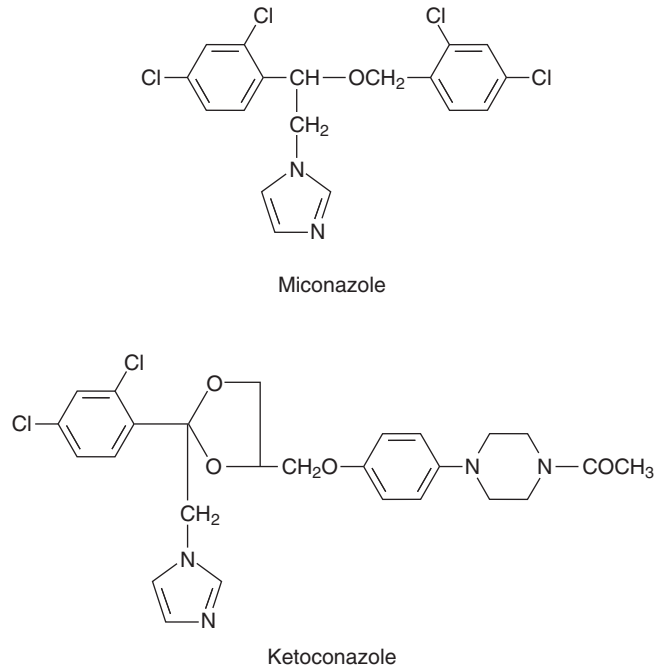


Figure 252–5 Structural formulas of systemic antifungal imidazoles: miconazole and ketoconazole.

RESISTANCE. Selection and nosocomial spread of azole-resistant *Candida* spp. have become matters of concern in hospitalized or persistently immunocompromised patients. Several mechanisms of resistance have been identified and include, but are not limited to, molecular alterations at the target binding site, increased target expression, and induction of cellular efflux pumps.[407,510] In contrast to pathogenic bacteria, genetic exchange mechanisms are largely unknown in fungi. Exposure-induced cumulative molecular events that lead to stable azole resistance have been reported; however, in the clinical setting, resistance is encountered most commonly in the form of a primarily resistant species or through selection of resistant subclones during exposure to azoles.[383,510]

Acquisition of microbiologic and clinical azole resistance was reported first in patients with chronic mucocutaneous candidiasis who were receiving long-term therapy with ketoconazole.[216] In the 1990s, before the advent of highly active antiretroviral therapy (HAART), azole-resistant oropharyngeal and esophageal candidiasis became a major clinical conundrum in patients with advanced HIV infection.[377,393] Emergence of *C. glabrata* and *C. krusei* infections in association with fluconazole prophylaxis has been observed in several bone marrow transplant centers[226,300,516,517] and in large cancer centers.[252] However, a large prospective series from Seattle showed an altogether low incidence of breakthrough candidemia (4.6%; in two thirds caused by *C. glabrata* or *C. krusei*)

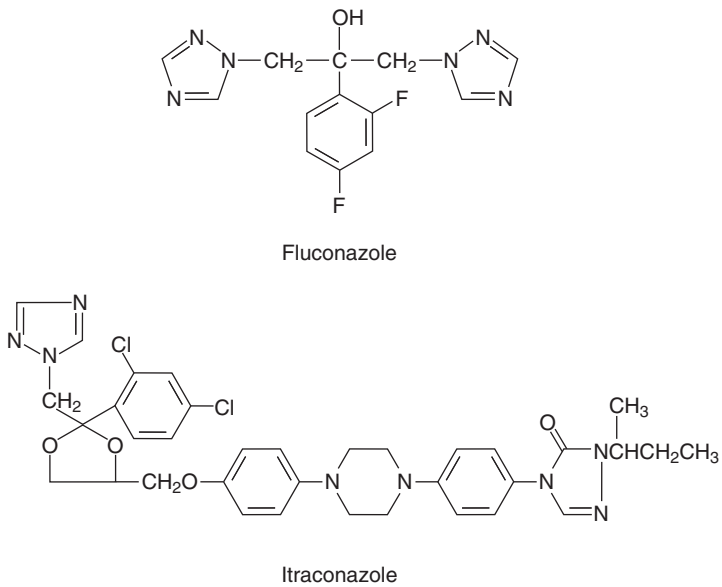
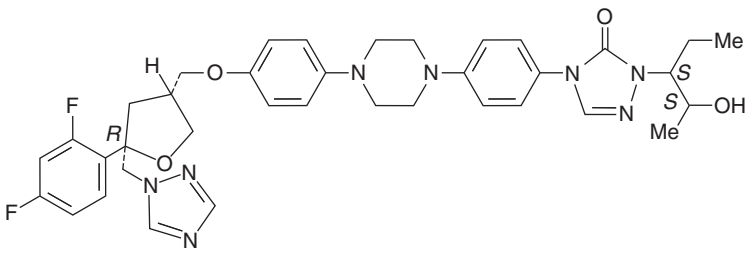
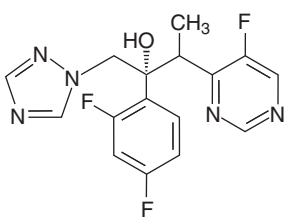


Figure 252–6 Structural formulas of first generation systemic antifungal triazoles: fluconazole and itraconazole.

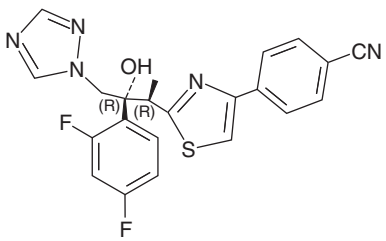


Posaconazole (SCH 56592)

Figure 252–7 Structural formulas of second generation systemic antifungal triazoles: posaconazole, ravuconazole, and voriconazole.



Voriconazole (UK 109496)



Ravuconazole (BMS 207147)

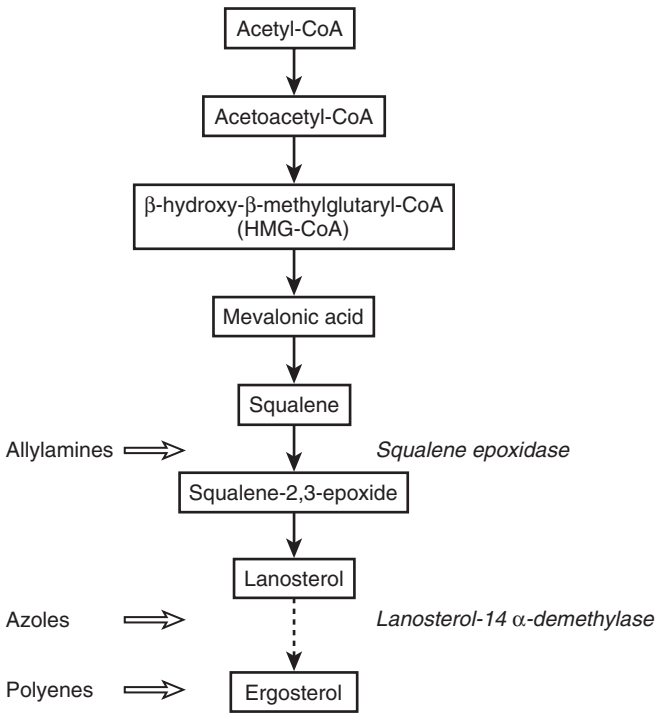


Figure 252–8 Ergosterol biosynthesis and targets of current antifungal agents. CoA, coenzyme A. *(Modified from Groll, A. H., and Walsh, T. J.: Uncommon opportunistic fungi: New nosocomial threats. Clin. Microbiol. Infect. 7[Suppl. 2]:8-24, 2001.)*

and a low attributable mortality (20%) in patients receiving fluconazole prophylaxis despite frequent colonization with fluconazole-resistant *Candida* spp.[300] Although cross-resistance of *Candida* spp. to antifungal azoles is a common occurrence,[36,162,320,355] it is not obligate: for example, patients with microbiologic and clinical fluconazole-resistant mucosal candidiasis may respond to itraconazole or second-generation triazoles.[200,400] Nonetheless, patients who have been exposed to azoles and who have breakthrough candidemia or an azole-resistant *Candida* isolate have a high likelihood of cross-resistance, and treatment with alternative agents is advised.[288,347] Acquired resistance to azoles was documented in a few patients with *C. neoformans* meningitis who were receiving maintenance therapy, but little is known about the frequency and mechanisms of secondary azole resistance and cross-resistance in filamentous fungi.[80,184,312]

Fluconazole

Fluconazole is a synthetic, low-molecular-weight, water-soluble *bis*-triazole (see Fig. 252–6). In comparison with ketoconazole, the compound has similar potency at the target enzyme but is much more specific and therefore is better tolerated. Fluconazole is active against *Candida* spp., *C. neoformans*, *C. gattii*, *T. asahii*, and endemic dimorphic fungi, but not against *Aspergillus* spp. and other hyaline or dematiaceous molds. Fluconazole has intermediate activity against *C. glabrata* and is inactive against *C. krusei*.[160,168,361,456]

PHARMACODYNAMICS. Fluconazole generally is considered to be a fungistatic agent.[185] Time-kill assays performed over incubation periods of 24 to 48 hours in susceptible *Candida* spp. and *C. neoformans* showed fungistatic activity of fluconazole with variable concentration-related growth effects.[70,71,112,245,246] However, time-kill studies that used extended periods of incubation up to 14 days and nonproliferating growth conditions demonstrated direct fungicidal activity of fluconazole against *C. albicans*.[434] These observations raise the possibility that fluconazole may ultimately be able to eliminate *Candida* spp. without help from host defenses. In serum-free growth media, fluconazole displays no measurable PAFE against *C. albicans* and *C. neoformans*, but concentration-dependent PAFEs of 1 to 3.6 hours were observed in the presence of fresh serum. Finally, pretreatment of *C. albicans* with fluconazole increased its vulnerability to killing by polymorphonuclear leukocytes.[130,310]

In vivo pharmacodynamic studies of fluconazole in murine models of disseminated *C. albicans* candidiasis using the fungal burden in kidney tissue as an end-point for antifungal efficacy collectively suggested that the AUC/MIC ratio is the pharmacodynamic parameter that best predicts antifungal efficacy of fluconazole[17,284,436] and that dosing regimens that produce prolonged sub-MIC concentrations are associated with development of

resistance.[19] Pharmacodynamic studies of fluconazole in patients with fungal infections have not been presented to date. The dose-independent pharmacokinetics and the available experimental and clinical data are in support of once-daily dosing regimens. Current susceptibility breakpoints and dosing recommendations for fluconazole against *Candida* spp. have been derived from MIC and outcome information of patients with mostly superficial *Candida* infections.[382] The feasibility of this approach is supported not only by the pharmacodynamic models discussed earlier and several animal studies that have demonstrated a correlation between MIC and antifungal efficacy[13,33,390,487] but also by an analysis of 1295 patient-episode-isolate events from 12 published clinical studies that demonstrated success rates of 85 percent for those episodes in which the fluconazole MIC was 8 μg/mL or less, 67 percent for those episodes in which the MIC was 16 to 32 μg/mL, and 42 percent for those episodes with resistant (MIC = 64 μg/mL) isolates.[359] However, in view of the availability of alternative agents, treatment of serious invasive mycoses caused by organisms in the susceptible-dose dependent MIC range by dose escalation remains controversial.

PHARMACOKINETICS. Fluconazole has favorable pharmacokinetic properties. It is available for oral and parenteral use, and its disposition is independent of route and formulation. Fluconazole exhibits linear plasma pharmacokinetics that fit best into a two-compartment open model.[65]

Independent of food or intragastric pH, the oral bioavailability of fluconazole is greater than 90 percent. C_{max} occurs 1 to 2 hours after ingestion. Multiple dosing increases C_{max} approximately 2.5-fold. Steady state generally is reached within 4 to 7 days after once-daily dosing, but it can be achieved rapidly by doubling the dose on the first day.[160,168] Because of the free solubility in water of this drug, protein binding is low. Fluconazole distributes well into virtually all tissue sites and body fluids. The ratio of CSF and serum concentrations ranges from 0.5 to 0.9 during the dosing interval, and penetration into brain tissue and the different compartments of the eye is excellent. Fluconazole is relatively stable to metabolic conversion; more than 90 percent of a dose is excreted by the kidney, with approximately 80 percent recovered as unchanged, active drug and 11 percent recovered as inactive metabolites.[65,160,168] Because excretion of fluconazole parallels the glomerular filtration rate, the dosage must be adjusted in patients with renal failure. A 50 percent reduction is required in patients with a creatinine clearance of 50 mL/minute or less, and a 75 percent reduction is required in patients with a creatinine clearance of less than 21 mL/minute; the initial loading dose need not be adjusted.[48,168] Fluconazole is dialyzable; in patients undergoing hemodialysis, 100 percent of the target dose is given after each dialysis session. In continuous venovenous hemofiltration and hemodiafiltration, dosing at the higher end of the dosing range (i.e., 800 mg/day in adults) is suggested. A dose of 150 mg in a single 2-L dialysate bag has been used for continuous ambulatory peritoneal dialysis.[147,160,321,333,418,453] Hepatic insufficiency per se does not require adjustments of dosing, but careful monitoring of additional hepatic toxicity is required.[395]

The plasma pharmacokinetic properties of fluconazole in pediatric age groups reflect developmental changes in the volume of distribution and clearance that are characteristic for a highly water-soluble drug with minor metabolism and predominantly renal elimination (Table 252–7). Except for premature neonates, in whom clearance initially is decreased, pediatric patients tend to have an increased weight-normalized clearance rate from plasma that leads to a shorter half-life in comparison with that in adults.[64,262,272,412,423,451] As a consequence, dosages at the high end of the recommended dosage range are necessary for the treatment of invasive mycoses in children. Because exposure over the course of time appears to be the most predictive pharmacodynamic parameter,[17,284] fractionating the dose is not required in infants and children, despite the shorter half-life in these age groups.

TABLE 252–7 Pharmacokinetic Parameters of Fluconazole in Pediatric Patients*

Age Group	Vd_{ss} (L/kg)	Cl (L/hr/kg)	$t_{1/2}$beta (hr)
Preterm <1500 g			
Day 1	1.18	0.010	88
Day 6	1.84	0.019	67
Day 12	2.25	0.031	55
Term neonates	1.43	0.036	28
Infants >1-6 mo	1.02	0.037	19
Children, 5-15 yr	0.84	0.031	18
Adult volunteers	0.65	0.015	30

**Data represent mean values.*
Cl, total plasma clearance; $t_{1/2}$beta, elimination half-life; Vd_{ss}, apparent volume of distribution at steady state.
Data from references 64, 262, 272, 323, 412, and 423.

ADVERSE EFFECTS. In adults, fluconazole has been administered safely over prolonged periods of time at dosages of up to 1200 mg/kg/day; dose-escalation to 1600 mg/day resulted mainly in increased hepatotoxicity, and dose-limiting neurotoxicity was observed at 2000 mg/day.[14] Compiled data from adult patients who received the drug at dosages of 100 to 400 mg/day over the course of at least 7 days indicate an overall incidence of possibly related adverse effects of 16 percent; significant adverse effects or laboratory abnormalities leading to the discontinuation of the drug were noted in overall 2.8 percent.[168] Nausea, vomiting, and other gastrointestinal symptoms are seen in fewer than 5 percent, skin rashes and headaches in fewer than 2 percent, and usually reversible, asymptomatic hepatic transaminase elevations were reported in as many as 7 percent of adult patients.[89,168]

In pediatric patients of all age groups, at dosages up to 12 mg/kg/day, fluconazole generally is well tolerated. The most common reported side effects in pediatric patients include gastrointestinal disturbances (8%), increases in hepatic transaminases (5%), and skin reactions (1%); toxicity-related discontinuation of therapy with fluconazole occurs in approximately 3 percent of patients.[355] Severe side effects, including severe hepatotoxicity and exfoliative skin reactions, have been reported anecdotally in association with fluconazole therapy; fluconazole does not appear to affect the synthesis of human steroid hormones at the dosages currently used.[89,178]

DRUG INTERACTIONS. Fluconazole undergoes minimal cytochrome P-450–mediated metabolism; it inhibits CYP3A4 and several other cytochrome P-450 isoforms in vitro and interacts with enzymes involved in glucuronidation, thus leading to certain significant drug-drug interactions.[89,174,188,365] Most important, concurrent therapy with cisapride and newer antihistamines results in inhibition of the metabolic pathways of these drugs and potentially serious cardiac arrhythmias and is therefore strictly contraindicated.[184] By similar mechanisms, fluconazole can precipitate phenytoin toxicity,[311] may lead to increased plasma concentrations of cyclosporine and tacrolimus,[271,344] and may potentiate the effects of warfarin, sulfonylurea drugs, rifabutin (in particular short-acting benzodiazepines, carbamazepine), and nifedipine.[271,324,339] At a small magnitude, fluconazole can decrease the plasma clearance of theophylline and zidovudine.[251,404] Fluconazole also may interfere with the plasma clearance of cyclophosphamide, a widely used anticancer agent that must be metabolized by cytochrome P-450 enzymes to produce alkylating species. This interaction may potentially result in a relevant reduction in the therapeutic efficacy of cyclophosphamide.[525]

Further potential interactions with anticancer drugs have been observed with busulfan[68] and all-*trans*-retinoic acid.[419]

Conversely, drugs notorious for hepatic enzyme induction may lead to decreased fluconazole levels and therapeutic failure as the ultimate consequence.[89,168] In addition, the potential for added hepatotoxicity must be monitored when fluconazole is given in combination with these compounds.[168,324]

The clinical indications for fluconazole are summarized in Tables 252–2, 252–4, 252–15, and 252–16. Fluconazole is highly effective against superficial infections caused by dermatophytes and *Pityrosporum* spp.,[143,192] and it has excellent activity in the treatment of mucosal candidiasis, including vaginal, oropharyngeal, esophageal, and chronic mucocutaneous candidiasis.[136,183,205,294]

Several controlled studies including both neutropenic and non-neutropenic adult patients indicated that intravenous fluconazole (400 to 800 mg/day) is as effective as is D-AmB (0.5 to 1.0 mg/kg/day) against candidemia and other forms of invasive candidiasis, but it is better tolerated.[11,15,363,381] Fluconazole thus can be used for invasive *Candida* infections caused by susceptible organisms in patients who are in stable condition.[350,383] In patients who have received antifungal azoles for prophylaxis, the role of fluconazole as a therapeutic agent is very limited; breakthrough infections in this setting are likely to be caused by fluconazole-resistant *Candida* spp.,[252,300] and alternative agents are recommended.

Fluconazole is a useful agent for treatment of invasive *Candida* infections in the neonatal setting: in 6 published series including 10 or more patients with proven invasive *Candida* infections, treatment with fluconazole at a daily dosage of mostly 5 to 6 mg/kg was successful in 83 to 97 percent, and crude mortality ranged from 10 to 33 percent. In none of the altogether 125 patients was fluconazole discontinued because of toxicity.[50,60,121,122,133,221,482] The recommended dosage range for pediatric patients of all age groups is 6 to 12 mg/kg/day. However, in view of the faster clearance rate, the larger volume of distribution, and the safety profile of fluconazole, 12 mg/kg/day may be the more appropriate dosage for treatment of serious infections in term neonates, infants, and children. A dosing regimen of 6 to 12 mg/kg once every 72 hours has been advocated during the first week of life in preterm neonates who weigh less than 1500 g, based on an initially decreased clearance of fluconazole.[412] However, this dosage regimen has not been validated in the therapeutic setting; given the extreme variability in extravascular water content and renal function in may of these children, predictably effective and safe treatment with fluconazole may not be possible in the early postnatal period.

Fluconazole has been useful in the treatment of focal *Candida* urinary tract infections and uncomplicated funguria, and it has been used successfully in *Candida* peritonitis, endocarditis, osteomyelitis, meningitis, and endophthalmitis.[184,274] Further potential indications for fluconazole beyond the treatment of acute invasive *Candida* infections include consolidation therapy for chronic disseminated candidiasis[10,239] and cryptococcal meningitis.[399,468] High-dose fluconazole is an option for systemic infections caused by the yeast *T. asahii* in non-neutropenic hosts.[12,171] Fluconazole is the current drug of choice for treatment of coccidioidal meningitis,[148,405] and it has proven effectiveness in nonmeningeal coccidioidal infections.[79,178] Fluconazole appears comparatively less active than is itraconazole in the treatment of paracoccidioidomycosis, blastomycosis, histoplasmosis, and sporotrichosis.[113,193,240,241,348,349,506]

Fluconazole can prevent mucosal candidiasis in patients with HIV infection or cancer,[177,334,368] and it has proven efficacy in preventing invasive *Candida* infections in high-risk patients with acute leukemia or with bone marrow or liver transplants.[165,391,519] Fluconazole, given at 400 mg once daily from the start of the conditioning regimen until day 75, can reduce the frequency of invasive *Candida* infections and lower mortality at day 110 following allogeneic bone marrow transplantation,[432] and it also may provide persistent protection against invasive candidiasis and *Candida*-related death. Fluconazole can decrease the frequency of severe, gut-related graft-versus-host disease (GVHD), and it has an independent overall survival benefit of 17 percent at 8 years after the end of treatment.[299] Fluconazole also has been shown to reduce the incidence of *Candida* infections in low-birth-weight infants.[49,197,237,238,293,462] Thus, fluconazole prophylaxis is a valid option for centers with a high frequency (>10%) of invasive *Candida* infections in premature infants of less than 1000 g birth weight or in the setting of a nosocomial outbreak by a fluconazole-susceptible *Candida* spp. Fluconazole is approved by the FDA for treatment of vaginal, oropharyngeal, and esophageal candidiasis, invasive *Candida* infections, and cryptococcal meningitis, as well as for prophylaxis of candidiasis in patients undergoing bone marrow transplantation.

Itraconazole

Itraconazole is a high-molecular-weight, highly lipophilic *bis*-triazole (see Fig. 252–6). Structurally closely related to ketoconazole, it has a broader spectrum of antifungal activity that includes dermatophytes, *Candida* spp. (*C. krusei* excluded), *C. neoformans* and *C. gattii*, *Aspergillus* spp., various dematiaceous molds, and the dimorphic fungi. Itraconazole binds more avidly to its fungal target than does ketoconazole, but only weakly to human cytochrome P-450, thus leading to comparatively fewer mechanism-associated adverse effects.[167]

PHARMACODYNAMICS. In vitro, itraconazole exerts species- and strain-dependent fungistatic or fungicidal pharmacodynamics. Time-kill experiments in serum-free and serum-containing media demonstrated the concentration-independent, fungistatic activity of itraconazole against *Candida* spp. and *C. neoformans*.[70,71,146,526] Against *Aspergillus* spp., however, itraconazole displayed time- and concentration-dependent fungicidal activity with 87 to greater than 97 percent killing within 24 hours of exposure to the drug.[290] Persistent effects have not been reported thus far.

The principal feasibility of a correlation between in vitro susceptibility and outcome was demonstrated in mice with experimental disseminated aspergillosis.[108] Relationships between drug concentrations and antifungal efficacy of itraconazole were assessed in a model of invasive pulmonary aspergillosis in methylprednisolone/cyclosporine-immunosuppressed rabbits. In this model, an inhibitory sigmoid maximum effect model predicted a significant pharmacodynamic relationship ($r = .87$, $p < .001$) between itraconazole concentrations in plasma and antifungal efficacy as a function of the burden of *A. fumigatus* in lung tissue.[45]

In patients, however, the main rationale for monitoring plasma levels has been the erratic oral bioavailability of itraconazole, particularly in neutropenic patients. Historically, the target plasma level for itraconazole has been estimated at 0.25 μg/mL (high-performance liquid chromatography [HPLC]) at trough, based on the concentration that inhibits 90 percent of a large set of clinical isolates.[58,101] More recently, the predictive value of threshold concentrations of prophylactic itraconazole was analyzed in a large cohort of patients undergoing intensive chemotherapy for acute leukemia. The median itraconazole trough concentration after the first week of prophylaxis was significantly lower in patients who developed invasive fungal infections (0.46 versus 0.82 μg/mL; $p = .008$). Multivariate logistic regression analysis demonstrated a significant ($p = .028$) statistical association of trough concentrations less than 0.5 μg/mL with the occurrence of invasive fungal infections. A threshold of 0.25 μg/mL did not influence the occurrence of invasive mycoses in univariate and multivariate analyses.[157]

In a phase I/II clinical trial of oral cyclodextrin-itraconazole in HIV-infected children with oropharyngeal candidiasis, the relationships between pharmacodynamic parameters and therapeutic response as assessed by standardized scoring of mucosal disease after 14 days of therapy fitted to inhibitory maximum effect pharmacodynamic models. Best fits were observed for area under the curve (AUC), AUC/MIC, C_{max}, and C_{max}/MIC ($r = .483$ to $.595$; $p < .01$). In this study, no significant correlations were found between the MIC values of itraconazole for the fungal isolates at baseline and the therapeutic response.[179]

PHARMACOKINETICS. Itraconazole is available as capsules, as oral solution in hydroxypropyl-β-cyclodextrin (HP-β-CD), and as parenteral solution that also uses HP-β-CD as solubilizer. Absorption of itraconazole from the capsule form is dependent on a low intragastric pH and is compromised in the fasting state, and it becomes erratic in patients with granulocytopenic cancer or hypochlorhydria.[89,178,184] Absorption can be somewhat improved when the capsules are taken with food or an acidic cola beverage.[100,178] The oral solution of itraconazole in HP-β-CD, however, leads to an improved oral bioavailability of the parent compound that is further enhanced in the fasting state.[36,384]

After oral administration, C_{max} is measured within 1 to 4 hours; following once-daily dosing, steady state is achieved after 7 to 14 days.[167,178] Steady state can be reached more rapidly by doubling the dose during the first 2 to 3 days. In adult patients with cancer who were receiving the standard regimen of 2.5 mg/kg of HP-β-CD itraconazole twice daily, mean trough levels were 0.8 μg/mL under conditions of steady state[369]; systemic absorption of the carrier was negligible.[101] After administration of intravenous HP-β-CD itraconazole, drug and carrier rapidly dissociated and followed their own disposition. After administration of 200 mg twice daily for 2 days followed by 200 mg daily for 5 days to patients with hematologic malignancies, mean trough levels 24 hours after the last dose were 0.53 μg/mL.[57] The carrier HP-β-CD is not significantly metabolized, and virtually 100 percent is eliminated from plasma within 24 hours in unchanged form through glomerular filtration.[527]

Itraconazole exhibits dose-dependent pharmacokinetics with hyperproportional increases in the AUC with increasing and split dosages.[167,179,369] Itraconazole is highly (95%) protein-bound; only 0.2 percent circulates as free drug, whereas the remainder is bound to blood cells.[206] The compound is distributed extensively throughout the body. Although concentrations in nonproteinaceous body fluids are negligible, tissue concentrations in many organs, including the brain, exceed corresponding plasma levels by 2 to 10 times.[100,206]

Itraconazole is metabolized extensively in the liver by numerous pathways to more than 30 metabolites and is excreted in metabolized form into bile and urine. The major metabolite, hydroxyitraconazole, possesses antifungal activity similar to that of itraconazole. It is eliminated more rapidly, but its plasma concentrations at steady state are 1.5 to 2 times higher than those of the parent compound.[100,178] As a consequence, plasma concentrations of itraconazole measured by bioassay are approximately 3.5 times higher than those determined by HPLC.[500] The elimination from plasma follows a biphasic pattern. In healthy adult volunteers, the elimination half-life of the compound is 20 to 24 hours after single dosing and 35 to 40 hours under terms of steady state, a finding reflecting saturable excretion mechanisms.[195] The dosage of oral itraconazole does not need to be adjusted in patients with renal insufficiency or dialysis. Because the elimination of HP-β-CD parallels the glomerular filtration rate, intravenous itraconazole is contraindicated in patients with a creatinine clearance of less than 30 mL/minute. In patients undergoing hemodialysis, administration just before dialysis appears to produce adequate systemic exposure while allowing dialysis clearance of the carrier.[312] No data are available for continuous hemofiltration methods. In patients with severe hepatic insufficiency, the elimination half-life of itraconazole can be prolonged, and additional hepatic toxicity or possible drug interactions should be monitored carefully.

Data on the plasma pharmacokinetics of itraconazole in pediatric patients are limited to the oral HP-β-CD solution (Table 252–8).[111,179,415] In 26 infants and children aged 6 months to 12 years with cancer ($n = 20$) or liver transplantation who received

TABLE 252–8 Pharmacokinetics of Itraconazole and Hydroxy-Itraconazole after Administration of Hydroxypropyl-beta-Cyclodextrin Oral Solution to Immunocompromised Infants and Children*

	Children with Cancer/ Liver Transplant† ($n = 8$, 0.5-2 yr) 5.0 mg/kg qd × 14 days	Children with Cancer† ($n = 7$, 2-5 yr) 5.0 mg/kg qd × 14 days	Children with Cancer† ($n = 11$, 6-12 yr) 5.0 mg/kg qd × 14 days	Children with Cancer‡ ($n = 9$, 2-5 yr) 2.5 mg/kg bid × 14 days	Children with Cancer‡ ($n = 6$, 6-12 yr) 2.5 mg/kg bid × 14 days
Itraconazole					
C_{max} (μg/mL)	0.571 ± 0.416	0.534 ± 0.431	0.631 ± 0.358	1.024 ± 0.351	1.524 ± 0.770
T_{max} (hr)	1.9 ± 0.1	2.9 ± 2.5	3.1 ± 2.1	N/A	N/A
C_{min} (μg/mL)	0.159 ± 0.218	0.179 ± 0.100	0.233 ± 0.14	0.711 ± 0.251	1.072 ± 0.408
$AUC_{0-\infty}$ (μg/mL/hr)	6.930 ± 5.83	7.33 ± 5.42	8.77 ± 5.05	N/A	N/A
$t_{1/2}$beta (hr)	47.4 ± 55.0	30.6 ± 25.3	28.3 ± 9.6	N/A	N/A
Accumulation factor	6.2 ± 5.0	3.3 ± 3.0	8.6 ± 7.4	N/A	N/A
OH-Itraconazole					
C_{max} (μg/mL)	0.690 ± 0.445	0.687 ± 0.419	0.699 ± 0.234	1.358 ± 0.373	2.180 ± 0.753
T_{max} (hr)	4.4 ± 2.3	4.8 ± 2.7	10.8 ± 14.3	N/A	N/A
C_{min} (μg/mL)				1.272 ± 0.322	1.964 ± 0.562
AUC_{0-24} (μg/mL/hr)	13.20 ± 11.40	13.4 ± 9.1	13.45 ± 7.19	N/A	N/A
$t_{1/2}$beta (hr)	18.0 ± 18.1	17.1 ± 14.5	17.9 ± 8.7	N/A	N/A
Accumulation factor	11.4 ± 16.0	2.3 ± 1.9	6.4 ± 5.6	N/A	N/A

**Pharmacokinetic parameters were obtained after daily dosing over 14 days. All values represent mean values ± SD.*

†Data from de Repentigny, L., Ratelle, J., Leclerc, J. M., et al.: Repeated-dose pharmacokinetics of an oral solution of itraconazole in infants and children. Antimicrob. Agents Chemother. 42:404-408, 1998.

‡Data from Schmitt, C., Perel, Y., Harousseau, J., et al.: Pharmacokinetics of itraconazole oral solution in neutropenic children during long-term prophylaxis. Antimicrob. Agents Chemother. 45:1561-1564, 2001.

Accumulation factor; AUC_{0-24} day 14/$AUC_{0-\infty}$ day 1; $AUC_{0-\infty}$, area under the concentration versus time curve from zero to infinity; C_{max}, peak plasma levels; C_{min}, minimum plasma levels; N/A, not assessed; $t_{1/2}$beta, elimination half-life; T_{max}, time until occurrence of peak plasma concentration.

the compound at 5 mg/kg once daily, plasma concentrations were substantially lower than those reported in adult patients with cancer, particularly in children younger than 2 years of age.[111,369] In contrast, in a pharmacokinetic study in 16 neutropenic children (1.7 to 14.3 years of age) who received cyclodextrin itraconazole for antifungal prophylaxis in a split dosing regimen of 2.5 mg/kg twice daily, peak and trough levels of itraconazole were substantially higher; nonetheless, a similar trend toward lower plasma concentrations occurred in the group of children 5 years of age and younger.[415] Finally, in a cohort of 26 HIV-infected children and adolescents (1.25 to 18 years), cyclodextrin itraconazole was safe and effective for treatment of oropharyngeal candidiasis at dosages of 2.5 mg once a day or 2.5 mg twice daily given for at least 14 days.[179] Many (77%) of the patients were receiving concomitant therapy with protease inhibitors or clarithromycin, drugs that are strong inhibitors of the CYP3A4-dependent metabolism of itraconazole.[365] Peak and trough levels measured after administration of the split-dosage regimen were similar to those observed in patients with cancer who were receiving the same dosage regimen.[415]

Despite the tremendous interpatient variability that results from both variable absorption and hepatic metabolism, the pharmacokinetic properties of itraconazole in pediatric patients appear not to be fundamentally different from those of adults. Although oral cyclodextrin itraconazole is not approved for pediatric age groups, a starting dosage of 2.5 mg/kg twice daily can be advocated, based on the available pharmacokinetic data.[111,179,415] Data on the use of intravenous itraconazole in pediatric patients are currently lacking; the dosage regimen used in the published adult studies is 200 mg twice daily for 2 days, followed by 200 mg once a day for a maximum of 12 days.[57,75] Because some evidence suggests that a relationship exists between trough concentrations and antifungal efficacy,[157] trough levels should be monitored, and dosing should be adjusted to maintain plasma concentrations of the parent itraconazole higher than 0.5 μg/mL. Only anecdotal reports have been published on the use of itraconazole in the neonatal setting.

ADVERSE EFFECTS. Itraconazole usually is well tolerated, with a pattern similar to that of fluconazole and a frequency of adverse effects approximately identical to that seen with fluconazole.[89] In 189 patients treated for systemic fungal infections at dosages of 50 to 400 mg/day for a median of 5 months, the rate of possibly or definitely related adverse effects was 39 percent.[460] Most of the observed reactions were transient and included nausea and vomiting (<10%), hypertriglyceridemia (9%), hypokalemia (6%), elevated hepatic transaminases (5%), rash or pruritus (2%), headaches or dizziness (<2%), and pedal edema (1%). Four percent of patients discontinued itraconazole treatment because of adverse effects. Gastrointestinal intolerance appears to be the dose-limiting toxicity of the oral cyclodextrin formulation. In a comparative study in adult patients with acute leukemia, 46 percent of patients receiving a daily dose of 800 mg stopped treatment early because of severe nausea and vomiting. Crossover to the identical dose of the capsule formulation was well tolerated by all patients; patients receiving 400 mg/day of the solution had no gastrointestinal adverse effects.[156] Only a few cases of more severe hepatic injury or hepatitis have been described.[269] Itraconazole can have negative inotropic effects; because of a low but possible risk of cardiac toxicity, itraconazole should not be administered to patients with ventricular dysfunction.[6]

Cyclodextrin itraconazole solution was safe and well tolerated for at least 14 days in reported phase I/II pharmacokinetic studies in immunocompromised pediatric patients.[111,179,415] Vomiting (12%), abnormal liver function tests (5%), and abdominal pain (3%) were the most common adverse effects considered definitely or possibly related to cyclodextrin itraconazole solution in an open study in 103 neutropenic pediatric patients with cancer who received the drug at 5 mg/kg daily or 2.5 mg/kg twice daily for antifungal prophylaxis for a median duration of 37 days; 18 percent of patients withdrew from the study because of adverse events.[138] In another report on pediatric patients with cancer who were receiving prophylactic oral itraconazole, adverse effects that led to the cessation of the itraconazole prophylaxis occurred in 11 percent of all 44 courses.[430]

DRUG INTERACTIONS. In comparison with fluconazole, both the propensity to and the extent of drug-drug interactions are greater.[175,178] Itraconazole is a substrate of CYP3A4, but it also interacts with the heme moiety of CYP3A, thus resulting in noncompetitive inhibition of oxidative metabolism of many CYP3A substrates. An interaction also can result from inhibition of P-glycoprotein–mediated efflux; P-glycoprotein is extensively co-localized and exhibits overlapping substrate specificity with CYP3A.[174,188] Inhibition of hepatic cytochrome P-450 enzyme systems may lead to increased and potentially toxic concentrations of co-administered drugs. Most important, the co-administration of cisapride, pimozide, terfenadine, astemizole, oral midazolam, quinidine, dofetilide, triazolam, and levacetylmethadol with itraconazole can lead to serious cardiac arrhythmias and is thus strictly contraindicated.[212,365] Similarly contraindicated is the co-administration of cholesterol-lowering agents such as lovastatin and simvastatin, which are associated with rhabdomyolysis,[331] and that of ergot alkaloids metabolized by CYP3A4, which may result in ergotism. Potentially toxic levels of the co-administered drug also can be reached when itraconazole is given along with phenytoin, carbamazepine, benzodiazepines, cyclosporine, tacrolimus, sirolimus, methylprednisolone, budesonide, atorvastatin, cerivastatin, digoxin, warfarin, sulfonylurea compounds, rifampin, rifabutin, ritonavir, indinavir, haloperidol, clarithromycin, verapamil, felodipine, busulfan, and vinca alcaloids.[55,257,339,365,403,472] Increased metabolism of itraconazole resulting in decreased plasma levels can be induced by rifampin, rifabutin, isoniazid, carbamazepine, phenobarbital, and phenytoin.[178,365,459] As a consequence, patients who receive itraconazole along with one of the listed drugs should be followed closely, and plasma concentrations of ideally both compounds as well as hepatic function should be monitored carefully.

CLINICAL INDICATIONS. The clinical indications for itraconazole are summarized in Tables 252–2, 252–3, 252–4, 252–15 and 252–16. Itraconazole is a useful agent for dermatophytic infections, pityriasis versicolor,[4,142,167,191] and vaginal candidiasis.[438] It is effective in treating patients with oropharyngeal and esophageal candidiasis, including adult and pediatric patients who have developed resistance to fluconazole.[1,111,179,400] The clinical efficacy of itraconazole in candidemia and other deeply invasive *Candida* infections has not been evaluated systematically. Although the experience with itraconazole in the primary treatment of cryptococcal meningitis is scant, itraconazole has been used with success for long-term treatment of cryptococcal meningitis in patients with HIV infection.[399,468]

Itraconazole may be a second-line option for treatment of invasive *Aspergillus* infections, in particular as maintenance or consolidation therapy in non-neutropenic patients. Two separate uncontrolled studies investigated oral itraconazole for treatment of proven or probable invasive aspergillosis; the results suggest a response rate comparable to that of conventional amphotericin B.[110,439] The published experience with the intravenous formulation for this indication is limited.[75] Beyond invasive aspergillosis, itraconazole may be useful in the management of infections by certain dematiaceous molds.[171,418] However, the compound has no well-documented clinical efficacy against zygomycosis and fusariosis.

Itraconazole is the current treatment of choice for lymphocutaneous sporotrichosis[240,379] and non–life-threatening, nonmenin-

geal paracoccidioidomycosis, blastomycosis, and histoplasmosis in non-immunocompromised patients.[82,115,325,329,450,507] It also has established efficacy in both induction and maintenance therapy of mild to moderate, nonmeningeal histoplasmosis in HIV-infected patients.[504,505,507] Although earlier uncontrolled clinical trials suggested a somewhat inferior efficacy against nonmeningeal and meningeal coccidioidomycosis in comparison with fluconazole,[169,457,458] a more recent randomized, double-blind comparative study in patients with progressive, nonmeningeal coccidioidomycosis showed a trend toward slightly greater efficacy when both drugs were given at a daily dosage of 400 mg.[149] However, amphotericin B remains the treatment of choice for most immunocompromised patients and for those with life-threatening forms of endemic mycoses.[184]

Prophylactic itraconazole may reduce the incidence of proven or suspected invasive fungal infections in patients with hematologic malignancies[306] and those who have undergone HSCT.[297,518] Efficacy in the prevention of invasive aspergillosis is supported by a large meta-analysis,[158] but not by a randomized, comparative trial. Finally, itraconazole was at least as effective as is conventional amphotericin B and was superior with respect to its safety profile when it was investigated as empiric antifungal therapy in persistently neutropenic patients with cancer.[59]

Posaconazole

Posaconazole (see Fig. 252–7) is a novel lipophilic antifungal triazole with potent and broad-spectrum activity against opportunistic, endemic, and dermatophytic fungi in vitro. This activity extends to organisms that often are refractory to existing triazoles or amphotericin B or to echinocandins such as *C. glabrata*, *C. krusei*, *A. terreus*, and *Fusarium* spp. Posaconazole also possesses activity against Zygomycetes both in vitro and in vivo, a feature that distinguishes it from all other available azoles.[172,402,452]

PHARMACODYNAMICS. Posaconazole is considered fungistatic against *Candida* and fungicidal against *Aspergillus* spp.[172] In time-kill assays, posaconazole showed concentration- and time-dependent fungicidal activity against *A. fumigatus*.[180,291,292] In vivo, in a neutropenic kidney target murine model of *C. albicans* infection, the AUC/MIC ratio was the parameter that was most predictive of antifungal efficacy.[20]

PHARMACOKINETICS. Posaconazole is available as oral suspension only and achieves optimal exposure when it is administered in two to four divided doses given with food or a nutritional supplement. The compound has dose-proportional pharmacokinetics in the 50- and 800-mg dose range, with saturation of absorption occurring at doses higher than 800 mg; after repeat dosing, steady state is achieved after 7 to 10 days with a 6- to 8-fold accumulation of plasma concentrations.[95] Posaconazole has a large volume of distribution in the order of 5 L/kg and a prolonged elimination half-life of approximately 20 hours. It is not significantly metabolized through the cytochrome P-450 enzyme system but is primarily excreted in unchanged form in the feces. The drug is inhibitory against cytochrome P3A4 but has no effects on 1A2, 2C8, 2C9, 2D6, and 2E1 isoenzymes, and therefore, a limited spectrum of drug-drug interactions can be expected (Table 252–9).[172,260,503] Posaconazole is a substrate and inhibitor of P-glycoprotein. However, investigation of the relationship between MDR1 mRNA expression and posaconazole exposure showed no correlation. Thus, the moderate variability in the compound's pharmacokinetics is unlikely to be caused by interindividual differences in P-glycoprotein expression.[96]

ADVERSE EFFECTS. The overall safety of posaconazole was assessed in two open-label clinical trials of more than 400 patients with invasive fungal infections who received posaconazole suspension (800 mg/day in divided doses). Treatment-related adverse events occurred in 38 percent of patients (164/428); the most common were nausea (8%), vomiting (6%), headache (5%), abdominal pain (4%), and diarrhea (4%). Treatment-related abnormal liver function test results were observed in as many as 3 percent of patients. No clinically significant differences occurred in mean QTc interval change from baseline. Serious adverse events considered possibly or probably related to posaconazole occurred in 35 (8%) patients. The most common severe adverse events were altered drug level, increased hepatic enzymes, nausea, rash, and vomiting (1% each). No significant trends related to age, sex, or race were observed, and no unique treatment-related adverse events were identified in patients during long-term exposure (>6 months) compared with those identified during shorter-duration therapy.[354,373] In two large, prospective, randomized comparative clinical trials investigating posaconazole for prevention of invasive fungal infections in high-risk patients with leukemia or HSCT, posaconazole was well tolerated, and the rate of study drug discontinuations in posaconazole-treated subjects was not different from that in subjects in the control cohort receiving fluconazole or itraconazole.[93,463]

TABLE 252–9 Principal Pharmacokinetic Properties of New Antifungal Triazoles, Posaconazole and Voriconazole

	Posaconazole	Voriconazole
Formulation	PO (IV)	PO/IV
Dose linearity	Yes	No
Oral bioavailability (%)	>50	>90
Protein binding (%)	>95	58
Volume of distribution (L/kg)	>5	2
Elimination half-life (hr)	25	6
Substrate/inhibitor of CYP450	3A4	3A4, 2C9, 2C19
Elimination through		
Feces (%/% metabolites)	77/-	<20/?
Urine (%/% metabolites)	14/14	80/78

Data from references 95, 96, 175, 260, 371, and 503.

INTERACTIONS. Posaconazole is not significantly metabolized through the cytochrome P-450 enzyme system, and cytochrome P-450–mediated drug-drug interactions will have limited potential to affect posaconazole pharmacokinetics.[260] Posaconazole is inhibitory against cytochrome P3A4, but it has no effect on 1A2, 2C8, 2C9, 2D6, or 2E1 isoenzymes.[503] The drug-drug interaction potential of posaconazole was investigated in seven open-label, crossover drug interaction studies. No adjustments of doses are needed when posaconazole is co-administered with glipizide, zidovudine, and lamivudine, whereas dosages of ritonavir and indinavir may need to be lowered. Monitoring of cyclosporine and tacrolimus blood concentrations is mandatory, and adjustments of doses should be made accordingly. Because of a relevant decrease in posaconazole concentrations, concomitant use with rifabutin, phenytoin, or cimetidine should be avoided. As with other azoles, caution is advised when posaconazole is co-administered with CYP3A4 substrates that have the potential to prolong the QTc interval.[408,409]

CLINICAL EFFICACY. Posaconazole has been targeted for prevention and treatment of serious infections caused by opportunistic and endemic fungal organisms. Posaconazole has demonstrated strong antifungal efficacy in phase II clinical trials in immunocompromised patients with primary or refractory oropharyngeal and esophageal candidiasis.[431,474] In a randomized, comparative phase III trial, posaconazole (100 mg daily; day 1: 200 mg) was as effective as was fluconazole (100 mg daily; day 1: 200 mg) in the primary treatment of HIV-associated

oropharyngeal candidiasis: 155 of 169 (91.7%) patients receiving posaconazole compared with 148 of 160 (92.5%) of patients receiving fluconazole achieved a complete clinical response (cure). The relapse rate at day 42 was greater in fluconazole recipients than in posaconazole-treated subjects (38.2% versus 31.5%; $p = .038$).[475] Posaconazole (800 mg in divided doses) also was investigated as salvage therapy in a large phase II study including 330 patients with invasive fungal infections intolerant to or refractory to standard therapies and a contemporaneous external control of 279 patients.[372] Most patients (86%) were refractory to previous therapy. Successful outcomes at the end of treatment in the posaconazole cohort and in the contemporaneous external control cohort were 42 versus 26 percent in aspergillosis (107 and 86 patients; separately published[495]), 39 versus 50 percent in fusariosis (18 versus 4 patients), 56 versus 50 percent in zygomycoses (11 versus 8 patients), 69 versus 43 percent (16 versus 7 patients) in coccidioidomycosis, 52 versus 53 percent in candidiasis (23 versus 30 patients), 48 versus 58 percent in cryptococcosis (31 versus 64 patients), 81 versus 0 percent in chromoblastomycosis (11 versus 2 patients), and 64 versus 60 percent in other invasive fungal infections (30 versus 20 patients). In a subset analysis of 39 patients with mostly refractory proven or probable CNS infections, posaconazole proved efficacious against cryptococcal meningitis (success rate in 29 patients, 48%) and against infections caused by opportunistic or endemic molds (5 of 10 patients, 50%).[364] These data are in agreement with the compound's extended spectrum of activity in vitro and demonstrate efficacy for treatment of refractory invasive opportunistic and endemic mycoses. A retrospective analysis of the manufacturer's compassionate-use program including 91 patients with proven or probable zygomycosis refractory or intolerant to prior antifungal therapy revealed a 60 percent success rate (complete and partial responses) at 12 weeks after initiation of therapy, thus providing first evidence for the clinical utility of posaconazole as second-line or consolidation therapy of zygomycosis.[465]

Two pivotal preventive randomized phase III studies in high-risk patients with HSCT and GVHD[463] and acute leukemias[93] have been completed. In the first study, patients received either posaconazole 200 mg three times a day or fluconazole 400 mg daily, respectively, with the start of immunosuppression for a total of 16 weeks. Treatment with posaconazole led to a decreased incidence of invasive fungal infections at 16 weeks (5% versus 9%; $p = .07$), with a statistically significant decrease in invasive *Aspergillus* infections (2% versus 7%; $p = .006$). Seven days after end of treatment, fewer patients had invasive fungal disease (2% versus 8%; $p = .004$), and fewer patients had invasive aspergillosis (1% versus 6%; $p = .001$). No differences in overall mortality rates occurred at 12 weeks.[463] In the second study, patients received either posaconazole 200 mg three times a day and either fluconazole 400 mg daily or itraconazole 200 mg twice a day, respectively. Treatment was started with each cycle following a drop of the absolute neutrophil count (ANC) to 500 μL or less for up to 12 weeks. Significantly fewer patients enrolled in the posaconazole arm of the trial developed an invasive fungal infection at day 7 after the end of treatment as compared with the comparator arm (2% versus 8%; $p < .01$); most important, treatment with posaconazole resulted in a significant decrease in the rate of invasive aspergillosis (1% versus 7%; $p < .001$). At day +100 after randomization, the rate of invasive fungal infections was 5 and 11 percent ($p < .01$), respectively, and patients treated with posaconazole had a significantly improved survival probability ($p = .035$).[93] These two important studies demonstrate the preventive efficacy of posaconazole, in particular against invasive *Aspergillus* infections in high-risk patients, and a statistically significant survival benefit in patients with acute myeloblastic leukemia/myelodysplastic syndrome who are undergoing remission induction chemotherapy.

APPROVAL STATUS AND DOSING. In the United States, posaconazole is approved for treatment of oropharyngeal candidiasis, including disease refractory to itraconazole or fluconazole, and for prophylaxis of invasive *Candida* and *Aspergillus* infections in high-risk patients 13 years of age and older with HSCT and GVHD or those with hematologic malignancies and prolonged neutropenia. In the European Union, the compound also is approved for treatment of aspergillosis, fusariosis, chromoblastomycosis, and coccidioidomycosis refractory to or intolerant of standard therapies in subjects 18 years of age and older (see Tables 252–3, 252–4, and 252–16).

The recommended dose for primary treatment of oropharyngeal candidiasis is 100 mg/day (day 1: 100 mg twice daily) and 400 mg twice a day for refractory disease; for prophylaxis of invasive *Candida* and *Aspergillus* infections, the recommended dose is 200 mg three times a day. The dose for salvage treatment is 400 mg twice a day given with food; for patients not tolerating solid food, a dose of 200 mg four times a day is recommended, preferentially together with a nutritional supplement.

Current data indicate no need for adjustments to dosage based on differences in age, gender, race,[410] or renal or hepatic function.[94,97] The pharmacokinetic properties of posaconazole in pediatric patients (<18 years of age) have not been studied. Limited data obtained in 12 pediatric subjects 8 years of age and older indicated no fundamental differences in trough plasma concentrations as compared with adults.[261] Salvage treatment with posaconazole resulted in successful outcomes in 5 of 11 pediatric subjects (8 to 17 years of age), a result that resembles the outcome in the adult population.[54]

Voriconazole

Voriconazole (see Fig. 252–7) is a synthetic antifungal triazole with activity against a wide spectrum of clinically important yeasts and molds, including *Candida* spp., *C. neoformans*, *Aspergillus*, and other hyaline molds, dematiaceous molds as well as dimorphic molds, both in vitro and in animal models. A notable exemption are the Zygomycetes, against which voriconazole is intrinsically inactive.[175,228,229,317]

PHARMACODYNAMICS. Against *Candida* spp. and *C. neoformans*, voriconazole exhibited non-concentration against *A. fumigatus*, and displayed time-dependent fungicidal activity.[244,290] A concentration-dependent PAFE of 0.2 to 4.1 hours has been observed in the presence of serum against *C. albicans*.[150] In a murine kidney target model of disseminated candidiasis, the AUC/MIC ratio was the pharmacodynamic parameter that correlated best with efficacy. Using 10 *C. albicans* isolates of different voriconazole susceptibilities, the free drug AUC/MIC ratios were similar to all the organisms studied and similar to those observed for other azoles.[21] Because voriconazole has nonlinear pharmacokinetics and underlies considerable interindividual variability in metabolism, assessment of concentration-effect relationships continues to be a challenge.[185]

PHARMACOKINETICS. Voriconazole is available in oral and intravenous formulations; oral bioavailability exceeds 90 percent in the fasted state. In adults, the compound has nonlinear pharmacokinetics. Plasma protein binding is 58 percent, and the mean volume of distribution accounts for 2 L/kg. Tissue and CSF levels exceed those of trough plasma levels severalfold. The plasma half-life is 6 hours, with elimination occurring primarily by oxidative hepatic metabolism to at least eight metabolites that are eliminated through the urine; less than 2 percent of a dose of voriconazole is excreted unchanged in urine. The major isoenzyme involved in voriconazole metabolism is CYP2C19, but CYP2C9 and CYP3A4 also contribute (see Table 252–9). Wide between-subject variability exists in the

disposition of voriconazole that is related to genetic CYP2C19 polymorphism.[175,371]

SAFETY. Voriconazole has an acceptable safety profile. The accrued clinical data indicate that side effects include four distinct clinical categories: transient liver enzyme abnormalities (10% to 20%), skin reactions (<10%), hallucinations or confusion (<10%), and transient, dose-related visual disturbances (altered or enhanced perception of light, blurred vision; 25% to 45%).[175] With regard to the visual disturbances, no morphologic correlation was noted in animal models, and the underlying mechanism remains to be elucidated.[229] Drug-related adverse effects requiring the discontinuation of voriconazole were infrequent in comparative clinical trials and ranged from 2 to 13 percent.[8,203,493]

Serious hepatic reactions including hepatic failure have been observed during treatment with voriconazole; therefore, evaluation of liver function tests before and during treatment with voriconazole is advised. Azole therapy has been associated with QTc prolongation; for this reason, voriconazole should be administered with caution to patients with proarrhythmic conditions. Voriconazole is teratogenic in animals and may cause fetal harm when it is administered to a pregnant woman.[175]

INTERACTIONS. Voriconazole is both substrate and inhibitor of CYP2C19, CYP2C9, and CYP3A4, and therefore, numerous clinically relevant and potentially hazardous drug-drug interactions need to be considered.[175] Voriconazole significantly increases exposure to cyclosporin, tacrolimus, benzodiazepines, methadone, vinca alkaloids, the statins, omeprazole, warfarin, sulfonylurea drugs, phenytoin, protease inhibitors other than indinavir, and non-nucleoside reverse transcriptase inhibitors, thus requiring dosage adjustment or monitoring. Voriconazole exposure is significantly decreased by phenytoin, ritonavir, efavirenz, rifabutin, carbamazepine, rifampin, and phenobarbital. Concurrent use of the last three enzyme inducers with voriconazole is contraindicated (risk of subtherapeutic levels of voriconazole), as is the concurrent use of terfenadine, astemizole, cisapride, quinidine, pimozide (risk of QTc prolongation resulting from increased exposure to these agents), ergotamine (risk of ergotism resulting from increased exposure), and sirolimus (increased exposure). Dosage adjustments of voriconazole are necessary when the drug must be used concurrently with phenytoin or rifabutin.[175,229,247]

CLINICAL EFFICACY. Voriconazole has demonstrated excellent clinical efficacy in phase II and III clinical trials in patients with oropharyngeal candidiasis and esophageal candidiasis.[8,200] In salvage studies in patients with fungal infections refractory or intolerant to treatment, complete and partial responses were seen in 43 and 48 percent of patients with invasive aspergillosis,[109,356,490] in 45 to 52 percent of patients with invasive candidiasis,[356] in 30 to 63 percent of patients with scedosporiosis,[356,490] in 45 percent of patients with fusariosis,[356] and in 38 percent of patients with cryptococcosis.[356]

A large, multinational, randomized phase III clinical trial of voriconazole and conventional amphotericin B followed by other licensed antifungal therapeutic agents for primary therapy of invasive aspergillosis revealed superior outcomes in voriconazole-treated patients[203]: At week 12, successful outcomes were noted in 52.8 percent of the patients in the voriconazole group and in 31.6 percent of those in the amphotericin B group (absolute difference, 21.2 percentage points; 95% confidence interval (CI), 10.4 to 32.9). The survival rate at 12 weeks was 70.8 percent in the voriconazole group and 57.9 percent in the amphotericin B group (hazard ratio, 0.59; 95% CI, 0.40 to 0.88). Voriconazole-treated patients had significantly fewer severe drug-related adverse events. A randomized comparative study of voriconazole versus conventional amphotericin B followed by fluconazole for treatment of candidemia in non-neutropenic patients showed similar response rates and end-of-treatment and similar survival rates at 3 months.[263] Twelve weeks after the end of treatment, successful outcomes were observed in 41 percent of patients in both treatment groups (95% CI for difference, –10.6% to 10.6%). Voriconazole cleared blood cultures as quickly as did amphotericin B/fluconazole, and significantly fewer serious adverse events and cases of renal toxicity occurred than in the group treated with amphotericin B/fluconazole. In a large, international collaborative study of voriconazole versus liposomal amphotericin B for empiric therapy, voriconazole did not meet the prespecified statistical end-point for non-inferiority in a composite end-point but was associated with significantly fewer breakthrough invasive fungal infections, particularly those caused by invasive aspergillosis.[493] Finally, several reports also suggested the potential usefulness of voriconazole for treatment of infections by unusual hyaline and dematiaceous fungi,[356] as well as for treatment of cerebral mold infections.[421]

APPROVAL STATUS AND DOSING. Voriconazole is approved for treatment of the following: invasive aspergillosis, fusariosis, and scedosporiosis; esophageal candidiasis; and primary treatment of candidemia and certain forms of invasive candidiasis in non-neutropenic patients (see Table 252–2 and 252–3). The recommended intravenous dosages for patients 12 years and older are 6 mg/kg twice a day on day 1, followed by 4 mg/kg twice a day. Oral doses in adults are 400 mg twice a day on day 1 (<40 kg: 200 mg twice daily), followed by 200 mg twice a day (<40 kg: 100 mg twice daily). In patients with renal insufficiency, no adjustment of dosage is needed for the oral formulation; because of the renal clearance of the intravenous carrier, patients with a creatinine clearance of less than 50 mL/minute should receive voriconazole by the oral route. In patients with mild to moderate hepatic function abnormalities, half of the daily maintenance dosage is recommended after the initial loading dose. Recommendations for severe liver failure are lacking.[175,229]

Pediatric patients 2 to 12 years have a higher capacity for elimination of voriconazole per kilogram of body weight than do adult healthy volunteers; the result is lower, potentially nontherapeutic exposure at similar dosages (Table 252–10).[489] An intraindividual dosage escalation study exploring pharmacokinetics and safety of higher dosage regimens of voriconazole in this patient population has been completed; based on the population-based

TABLE 252–10 Simulated Plasma Concentrations of Voriconazole following Multiple Doses of 3 and 4 mg/kg in Pediatric versus Adult Patients

	Value*			
	Pediatric Patients 2-11 yr		Adult Patients	
Parameter	3 mg/kg	4 mg/kg	3 mg/kg	4 mg/kg
AUC_{tau} (ng/hr/mL)	10,670	14,227	13,855	38,605
$C_{average}$ (ng/mL)	889	1,186	1,155	3,217

**Data are reported as medians following 6 mg/kg every 12 hours on day 1 and maintenance dose 3.*

Whereas in pediatric patients, an increase in dosage by a factor of 1.3 leads to proportional increase in the mean average plasma concentration ($C_{average}$) and in the mean area under the concentration versus time curve (AUC_{tau}), adult patients display a 2.8-fold, hyperproportional increase in exposure, indicating nonlinear disposition. As a consequence, pediatric patients given 4 mg/kg do not achieve the same exposure as adults given 4 mg/kg, the dosage that has led to the approval of voriconazole for first-line treatment of invasive aspergillosis.

Modified from Walsh, T. J., Karlsson, M. O., Driscoll, T., et al.: Pharmacokinetics and safety of intravenous voriconazole in children after single- or multiple-dose administration. Antimicrob. Agents Chemother. 48*:2166-2172, 2004.*

analysis of the data set of that study, an intravenous dosage of 7 mg/kg twice a day and an oral dose of 200 mg twice a day (oral suspension) without loading doses is currently suggested and approved by the European Medicine Agency for children between the ages of 2 and 12 years.[484] Voriconazole has been administered safely and with success to children younger than 12 years of age who had no therapeutic alternative. Of 58 immunocompromised children with proven or probable invasive fungal infection refractory to or intolerant of conventional antifungal therapy, 26 patients (45%) had a complete or partial response. Four patients (7%) discontinued therapy because of intolerance. A total of 23 patients had voriconazole-related adverse events, most commonly elevation in hepatic transaminases or bilirubin ($n = 8$), skin rash ($n = 8$), abnormal vision ($n = 3$), and photosensitivity reactions ($n = 3$).[490] The safety and tolerance of voriconazole were further analyzed in a retrospective cohort study of 37 immunocompromised children and adolescents who required therapy for various indications. Voriconazole was administered intravenously or orally at dosages ranging from 2 to 8 mg/kg twice daily for a mean duration of 174 days (range, 5 to 998 days). Grade I or II adverse events were observed in 19 patients (51%); the most frequent events included transient increases in hepatic transaminases (19) and transient visual disturbances (5). Four patients (10%) experienced grade III/IV adverse events, and three (8%) permanently discontinued therapy. Voriconazole showed promising efficacy as a preventive and therapeutic modality, although these outcomes were not primary end-points of the analysis.[249] Voriconazole also has been used with success and acceptable tolerance in many patients with cystic fibrosis and allergic bronchopulmonary aspergillosis.[208]

ECHINOCANDIN LIPOPEPTIDES

The echinocandins are a novel class of semisynthetic amphiphilic lipopeptides that are composed of a cyclic hexapeptide core linked to a variably configured lipid side chain. The first compound of this class undergoing preclinical evaluation was cilofungin (LY 121019), a semisynthetic echinocandin B derivative with activity limited to *Candida* spp. However, clinical development was abandoned in early stages because of concerns regarding toxicity associated with the intravenous polyethylene glycol formulation vehicle.[184] Since the 1990s, a second generation of semisynthetic echinocandins with extended antifungal spectrum against *Candida* and *Aspergillus* spp., a favorable safety profile and pharmacokinetic characteristics, has been developed: anidulafungin (Eraxis), caspofungin (Cancidas), and micafungin (Mycamine) (Fig. 252–9). The data accumulated thus far indicate that these agents are not fundamentally different with respect to spectrum, pharmacokinetics, safety, and antifungal efficacy.

MECHANISM OF ACTION. The echinocandins act by noncompetitive inhibition of the synthesis of 1,3-β-D-glucan, a polysaccharide in the cell wall of many pathogenic fungi (Fig. 252–10). Together with chitin, the ropelike glucan fibrils are responsible for the cell wall's strength and shape. They are important in maintaining the osmotic integrity of the fungal cell and play a key role in cell division and cell growth.[104,105,199] The proposed molecular target of the echinocandins, glucan synthase, is a heteromeric enzyme complex composed of at least one large integral membrane protein encoded by the *FKS* gene that binds the substrate (uridine diphosphate glucose), and one small regulatory subunit, Rho1p, a guanosine triphosphate–binding protein; additional, yet unidentified, components also may be involved.[264,449]

ANTIFUNGAL ACTIVITY. All three compounds have potent and broad-spectrum fungicidal in vitro activity against *Candida* spp. and potent inhibitory activity against *Aspergillus* spp.; their antifungal efficacy against these organisms in vivo has been demonstrated in various animal models. The current echinocandins have variable activity against dematiaceous and endemic mold and are considered inactive against most Hyalohyphomycetes, Zygomycetes, *C. neoformans*, and *T. asahii*. All echinocandins have demonstrated preventive and therapeutic activity in animal models of *Pneumocystis carinii* (now *Pneumocystis jiroveci*) pneumonitis.[145,147,473]

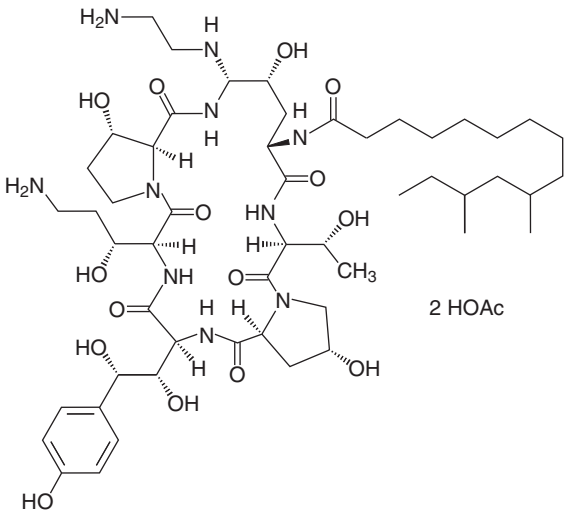
As expected from their mechanism of action, the echinocandins show no cross-resistance to amphotericin B and fluconazole-resistant *Candida* isolates. Resistance to echinocandins in otherwise susceptible fungal yeast species is rare; most mutations conferring resistance have been mapped to the *FKS* gene.[264,351] Little is known thus far about potential mechanisms of echinocandin resistance among *Aspergillus* spp.[152]

PHARMACODYNAMICS. The echinocandins demonstrate a species-dependent mode of antifungal activity. Whole-cell in vitro assays reveal fungicidal activity against most *Candida* spp. but not against *Aspergillus* spp.[37,443] Microscopical examination of drug-exposed *A. fumigatus* shows a dose-dependent formation of microcolonies with progressively truncated, swollen hyphal elements that appear to be cell wall deficient but are able to regain their cell walls on subculture in the absence of drug.[118,265,336,378] These observations demonstrate differences in functional target sensitivity that are not fully understood. Time-kill studies in *Candida* spp. demonstrated predominantly concentration-dependent fungicidal activity and rate-of-kill at concentrations greater than the MIC for all three compounds.[129,131,357] In addition, PAFEs between 5 and 12 hours at concentrations greater than the MIC have been demonstrated.[130,292]

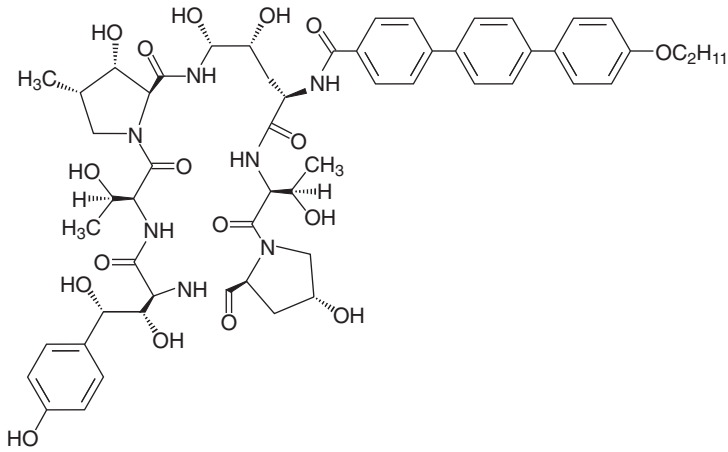
In persistently neutropenic rabbit models, anidulafungin showed highly predictable concentration-effect relationships in experimental disseminated candidiasis; however, no concentration-effect relationships were observed in experimental pulmonary aspergillosis, despite full exploration of the dosage range.[181] Pharmacodynamic studies in murine kidney target models of disseminated candidiasis showed prolonged in vivo antifungal effects and indicated that the AUC/MIC ratio is the pharmacodynamic parameter that predicts caspofungin efficacy.[283] In a murine model of invasive pulmonary aspergillosis, the C_{max}/MEC ratio appeared to be the parameter most closely associated with efficacy.[511]

PHARMACOKINETICS. At present, all current echinocandins are available only for intravenous administration. They exhibit dose-proportional plasma pharmacokinetics with a triexponential elimination pattern. Their beta half-life is between 10 and 15 hours, thus allowing for once-daily dosing without major accumulation after multiple dosing. All echinocandins are highly (>95%) protein-bound and distribute into all major organ sites including the brain; however, concentrations in uninfected CSF are low. The echinocandins are chemically degraded or metabolized by the liver and slowly excreted into urine and feces; only small fractions (<2%) of a dose are excreted into urine in unchanged form (Table 252–11).[145,147,473] The current echinocandins are not dialyzable, and no adjustment in dose is required for patients with renal insufficiency or renal failure. Similarly, no adjustment in dose is necessary for patients with mild to moderate (all) or severe (anidulafungin) hepatic failure. Whether the minor differences in individual pharmacokinetic parameters such as AUC, peak plasma levels, volume of distribution, and clearance of the echinocandins are of clinical significance remains to be elucidated.

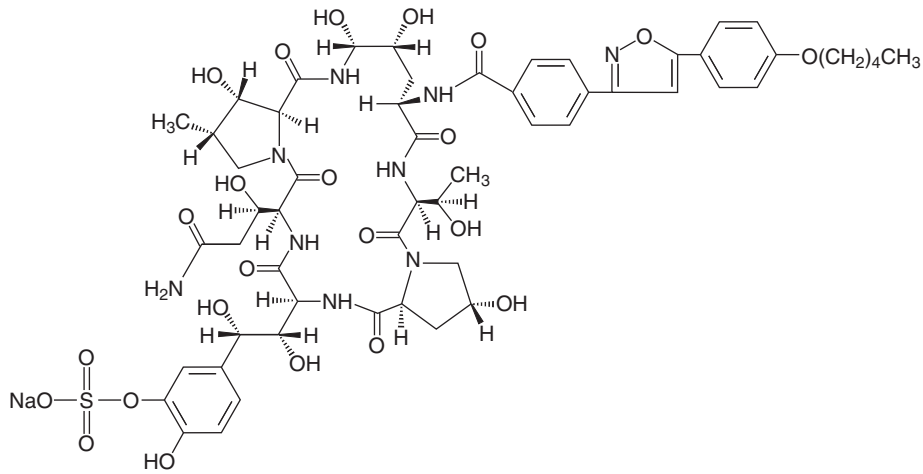
ADVERSE EFFECTS. At current dosages, anidulafungin, caspofungin, and micafungin generally are well tolerated. Fewer than 10 percent of patients enrolled on the various randomized



Caspofungin (MK0991)



Anidulafungin (LY303366)



Micafungin (FK463)

Figure 252–9 Structural formulas of echinocandin lipopeptides: anidulafungin, caspofungin, and micafungin.

comparative clinical trials discontinued echinocandin therapy because of drug-related adverse events.[103,259,315,376,466,497] Increased liver transaminases, gastrointestinal symptoms, skin rash, and headache probably or definitely associated with echinocandin treatment have been reported to occur at a frequency of less than 5 percent each. Like other basic polypeptides, the echinocandins have the theoretical potential to cause release of histamine. Symptoms such as rash, facial swelling, pruritus, or sensation of warmth and anaphylactic reactions have been reported in isolated cases.[103,259,315,376,466,497] Because of transient elevations of hepatic transaminases in interaction studies in healthy volunteers,[170] the concomitant use of caspofungin and cyclosporine currently is not recommended; clinical experience, however, indicates that both drugs can be given safely under careful monitoring.[173,298,454]

DRUG INTERACTIONS. In vitro studies showed that anidulafungin does not significantly inhibit the activities of clinically important human cytochrome P-450 isoforms at clinically relevant concentrations and that it is not an inhibitor of P-glycoprotein. Interaction studies demonstrated that no adjustment of dosage of either drug is warranted when anidulafungin is co-administered with cyclosporine, tacrolimus, voriconazole, liposomal amphotericin B, or rifampin.[76]

Caspofungin is not a substrate of P-glycoprotein and is a poor substrate and a weak inhibitor of cytochrome P-450 enzymes.[29,523] Clinical studies showed that the pharmacokinetic properties of caspofungin are not altered by itraconazole, amphotericin B, mycophenolate, nelfinavir, or tacrolimus. Caspofungin has no effect on the pharmacokinetics of itraconazole, amphotericin B, or the active metabolite of mycophenolate.[76,346,440] Caspofungin can reduce the AUC of tacrolimus by approximately 20 percent, but it has no effect on cyclosporine levels. Inducers of drug clearance or mixed inducer/inhibitors, namely efavirenz, nelfinavir, nevirapine, phenytoin, rifampin, dexamethasone, and carbamazepine, may reduce caspofungin concentrations, and dose adjustment to a daily dose of 70 mg of caspofungin should be considered.[76,170]

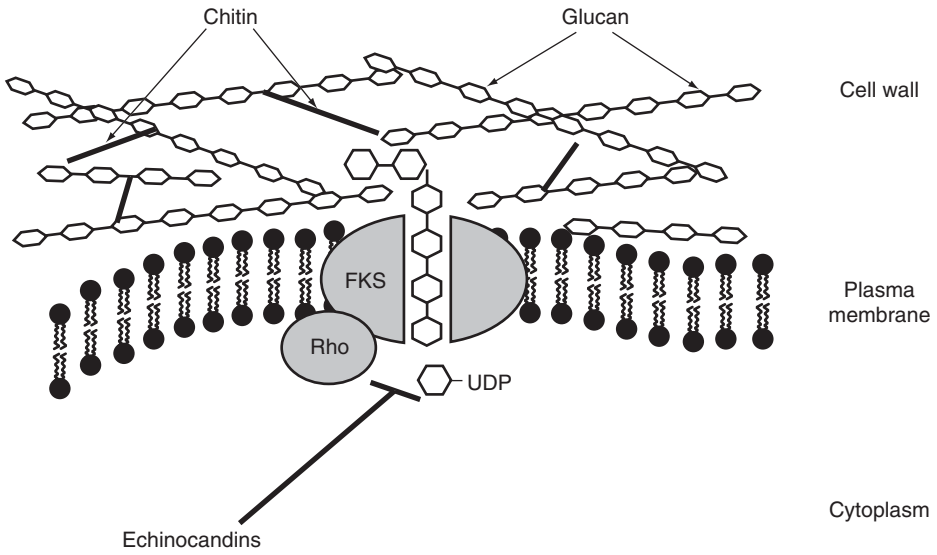


Figure 252–10 Schematic of the proposed mechanism of action of echinocandin lipopeptides. Echinocandins inhibit the synthesis of cell wall 1,3-β-glucan at the level of the cell membrane. Fks is the proposed catalytic subunit, and Rho the proposed regulatory subunit of the glucan synthase complex. *(Modified from Kurtz, M. B., and Douglas, C. M.: Lipopeptide inhibitors of fungal glucan synthase. J. Med. Vet. Mycol. 35:79-86, 1997.)*

TABLE 252–11 Principal Pharmacokinetic Properties of the Echinocandin Lipopeptides Caspofungin, Anidulafungin, and Micafungin

	Caspofungin	Anidulafungin	Micafungin
Formulation	IV	IV	IV
Dose linearity	Yes	Yes	Yes
Oral bioavailability (%)	N/A	N/A	N/A
Protein binding (%)	97	84	99
Volume of distribution (L/kg)	N/A	0.7-0.9	0.24
Elimination half-life (hr)	8-10	24	15
Substrate/inhibitor of CYP450	N/A	N/A	N/A
Routes of elimination	Degradation/metabolization, urine > feces	Degradation only, feces	Metabolization, feces > urine

N/A, not applicable.
Data from references 145, 147, and 473.

Micafungin is a weak inhibitor of CYP3A in vitro, but it is neither a P-glycoprotein substrate nor inhibitor in vitro. In clinical studies, micafungin had no effect on mycophenolate mofetil, cyclosporine, tacrolimus, prednisolone, and fluconazole pharmacokinetics, but it increased the AUC of sirolimus and nifedipine by approximately 20 percent. Patients receiving sirolimus or nifedipine in combination with micafungin should be monitored for sirolimus or nifedipine toxicity, and sirolimus or nifedipine dosage should be reduced if necessary.[187,322]

Caspofungin

CLINICAL EFFICACY. The clinical efficacy of caspofungin against *Candida* spp. was demonstrated first in phase II and III studies in immunocompromised patients with esophageal candidiasis.[23,480,481] A multicenter, randomized, double-blind phase III clinical trial investigated the efficacy of caspofungin for primary treatment of invasive *Candida* infections in 224 mostly non-neutropenic patients with amphotericin B deoxycholate (0.6 to 1.0 mg/kg) as comparator agent. Among patients receiving at least one dose of the study drug, 73 percent of patients in the caspofungin cohort and 61.7 percent of patients in the amphotericin B deoxycholate cohort had therapeutic success at the end of intravenous therapy. Among patients who received five or more doses, the response rates were 80.7 and 64.9 percent, respectively. No difference occurred in relapse or survival, but caspofungin was better tolerated.[315] A multicenter phase II salvage trial of caspofungin was completed in 83 patients with definite or probable invasive aspergillosis refractory to or intolerant of standard therapies. As determined by an independent expert panel, a complete or partial response was observed in 45 percent of patients receiving at least one dose of caspofungin; in patients receiving the drug for more than 7 days, the response rate was 56 percent.[287] Finally, in a large, randomized, double-blind clinical trial including 1095 patients, caspofungin was as effective as was liposomal amphotericin B for empiric antifungal therapy in persistently febrile granulocytopenic patients, but it was better tolerated. The proportion of patients who survived at least 7 days after therapy was greater in the caspofungin group (92.6% versus 89.2%).[497]

APPROVAL STATUS AND DOSING. Caspofungin is licensed in both the United States and Europe in patients 3 months of age or older with candidemia and certain forms of invasive *Candida* infections in non-neutropenic patients, as second line therapy for invasive aspergillosis, and for empirical antifungal therapy in granulocytopenic patients. In the United States, caspofungin also is approved for treatment of esophageal candidiasis (see Tables 252–2, 252–3 and 252–16).[76] The adult dose regimen is 50 mg daily with a single 70-mg loading dose on day 1, administered over 1 hour. No dosage adjustment is required in patients with renal insufficiency and mild hepatic insufficiency. In patients with moderate hepatic insufficiency (Child-Pugh category B), a maintenance dose of 35 mg/day is recommended after the loading dose of 70 mg. No recommendations exist for patients with severe hepatic insufficiency (Child-Pugh category C).[186]

In children and adolescents 2 to 17 years of age, the pharmacokinetics and safety of caspofungin was investigated using either a weight-based or a body surface area regimen. Although a maintenance dosage of 1 mg/kg/day achieved suboptimal exposure,

TABLE 252–12 Single-Dose Caspofungin Pharmacokinetics in Pediatric versus Adult Patients*

Dosage	Children (2-11 yr) 1 mg/kg	Children (2-11 yr) 50 mg/m^2	Adolescents (12-17 yr) 50 mg/m^2	Adults 50 mg
$AUC_{0\text{-}24\ hr}$ (μg/hr/mL)	41.5	96.4	77.6	70.6
C1 (μg/mL)	6.59	13.9	7.67	7.67
C24 (μg/mL)	0.45	1.09	1.35	1.35
$t_{1/2}$beta (hr)	7.2	7.6	11.7	11.7
Cl (mL/min/m^2)	8.57	7.78	6.07	6.07

**Least square means are reported for AUC, C1 (peak plasma concentration [C_{max}]), and C24 (minimum plasma concentration [C_{min}]), and harmonic means for $t_{1/2}$beta. Data were obtained in groups of 6 to 10. Data in pediatric patients were compared with those obtained in 32 adult patients with mucosal candidiasis. In comparison with adult values, a 1-mg/kg dosage does not achieve the target trough concentration of caspofungin of 1 μg/mL and leads to a lower exposure as measured by the $AUC_{0\text{-}24\ hr}$; the dosage regimen of 50 mg/m^2 leads to similar or slightly higher trough concentrations and similar or slightly higher exposure ($AUC_{0\text{-}24\ hr}$) and has been selected for the further development program in pediatric patients.*

AUC, area under the concentration versus time curve from 0 to 24 hours; Cl, clearance; $t_{1/2}$beta, elimination half-life.

Modified from Walsh, T. J., Adamson, P. C., Seibel, N. L., et al.: Pharmacokinetics, safety, and tolerability of caspofungin in children and adolescents. Antimicrob. Agents Chemother. 49:4536-4545, 2005.

dosing with 50 mg/m^2/day provided similar or slightly higher exposure relative to adults (Table 252–12).[483] As a consequence, in conjunction with pharmacokinetic data obtained in small children,[328a] the dosage of 50 mg/m^2/day (day 1: 70 mg/m^2/day; maximum daily dose; 70 mg) has been selected for pediatric patients between 3 months and 17 years of age. In neonates up to 3 months of postnatal age, limited pharmacokinetic data suggest a dosage of 25 mg/m^2/day.[338,403a]

Caspofungin appears to be well-tolerated in pediatric patients. In the above-mentioned phase I/II dose-finding study, none of the patients developed a serious drug-related adverse event or discontinued because of toxicity.[483] In a phase III estimation trial with safety as the primary objective, 82 patients between the ages of 2 and 17 years with persistent fever and neutropenia were randomly assigned to receive caspofungin or liposomal amphotericin B (3 mg/kg daily) in a 2:1 ratio. Rates of drug-related adverse events were similar (clinical 48.2% vs. 46.2%; laboratory 10.7% vs. 19.2%); serious drug-related adverse events occurred in 1.8% of caspofungin-treated and in 11.5% of amphotericin B-treated patients. Overall success rates were not different. The interim analysis of the first 28 patients enrolled on a phase II trial in patients with invasive infections revealed no serious drug-related adverse events and an overall response rate of 75%.[224] Similarly favorable safety experiences have been reported in immunocompromised pediatric patients who received the compound for various indications, mostly in combination with other antifungal agents,[141,170] and in neonates with refractory invasive candidiasis.[326,337,338]

Anidulafungin

CLINICAL EFFICACY. The clinical efficacy of anidulafungin against *Candida* spp. was demonstrated in phase II or phase III studies in immunocompromised patients with esophageal candidiasis and candidemia. Anidulafungin had equivalent efficacy to fluconazole in esophageal candidiasis in a randomized, double-blind, international multicenter study; success was documented in 242 of 249 evaluable anidulafungin-treated patients (97.2%) and in 252 of 255 fluconazole-treated patients (98.8%). Adverse events leading to discontinuation were reported in 29 anidulafungin-treated patients (10%) versus 23 fluconazole-treated patients (8%).[258] In a noncomparative dose-ranging study in 123 patients with invasive candidiasis randomized to one of three intravenous regimens, 50, 75, or 100 mg once daily continued for 2 weeks beyond resolution or improvement of signs and symptoms, success rates at end of therapy were 84, 90, and 89 percent in the 50-, 75-, and 100-mg groups, respectively.[259] This study was followed by a randomized, double-blind phase III study that compared anidulafungin (100 mg once daily) with fluconazole (400 mg once daily) in a total of 245 mostly non-neutropenic patients with invasive candidiasis.[376] The preliminarily presented data indicate that more patients receiving anidulafungin had clinical and microbiologic success at the end of intravenous therapy (75.6% versus 60.2%); similar superiority was found at the 2- and 6-week follow-ups after end of all therapy (64.6% versus 49.2% and 55.9% versus 44.1%, respectively). Survival at end of therapy was higher in the anidulafungin-treated group (74% versus 69%).

APPROVAL STATUS AND DOSING. Anidulafungin is licensed in both the United States and Europe for patients 18 years of age or older for candidemia and certain forms of invasive *Candida* infections in non-neutropenic patients. In the United States, the compound is also licensed for esophageal candidiasis (see Table 252–2).[128] The recommended dose regimen consists of 100 mg (day 1: 200 mg) for invasive candidiasis and 50 mg QD (day 1: 100 mg) for esophageal candidiasis, administered at a rate of 1.1 mg/minute or higher. No dosage adjustment is needed in subjects with renal impairment, those undergoing hemodialysis, and patients with hepatic impairment (Child-Pugh class A, B and C).[128,475]

A pediatric phase I/II multicenter study of the pharmacokinetics and safety of anidulafungin has been completed in 19 granulocytopenic children with cancer. Patients were divided into two age cohorts (2-11 and 12-17 years) and were enrolled into sequential groups to receive 0.75 or 1.5 mg/kg/day[41] (Table 252–13). No drug-related serious adverse events were recorded. Pharmacokinetic parameters were similar across age groups and dosage cohorts and similar relative to adult subjects. Following single and multiple daily doses of 0.75 mg/kg and 1.5 mg/kg, plasma concentration data corresponded to those in adults following a daily 50 and 100 mg dose, respectively.[41] The pediatric development program of the compound is in advanced stages.

Micafungin

CLINICAL EFFICACY. Micafungin was studied in open-label dose-ranging trials of endoscopically proven esophageal candidiasis in patients with HIV infection.[358,441] A double-blind comparative study investigating 50, 100, and 150 mg/day versus fluconazole 200 mg/day for HIV-associated esophageal candidiasis showed similar endoscopic cure rates and safety profiles for micafungin at doses of 100 and 150 mg/day and fluconazole.[103] A further randomized, double-blind comparative trial in 523 patients 16 years old and older with esophageal candidiasis investigated micafungin (150 mg/day) versus fluconazole (200 mg/day).[102] For the primary end-point of endoscopic cure, treatment difference was –0.3 percent (micafungin, 87.7%; fluconazole, 88.0%). A large, phase III, 1:1 randomized, double-blind non-inferiority trial was completed that compared micafungin (100 mg

TABLE 252–13 Single-Dose Anidulafungin Pharmacokinetics in Pediatric versus Adult Patients*

	Pediatric Patients (2-17 yr)		Adults	
Dosage	**0.75 mg/kg**	**1.5 mg/kg**	**50 mg**	**100 mg**
C_{max} (μg/mL)	4.02	6.09	2.51	3.82
$AUC_{0\text{-}24\ hr}$ (μg/hr/mL)	48.0	89.7	53.3	104.8
$t_{1/2}$beta (hr)	20.8	19.5	39.3†	42.3†
Cl (L/hr/kg)	0.0175	0.0191	N/A	N/A
Vd_{ss} (L/kg)	0.45	0.49	0.72	0.78

**Pharmacokinetic parameters are expressed as mean values. Data were obtained in groups of 6 pediatric patients with compromised immunity and neutropenia per age group and dosage level and were compared with those obtained in 26 adult healthy volunteers.*

†$t_{1/2}$gamma.

AUC, area under the concentration versus time curve from 0 to 24 hours; Cl, clearance; C_{max}, peak plasma concentration; N/A, not available; $t_{1/2}$beta, elimination half-life; Vd_{ss}, volume of distribution at steady state.

Data from Benjamin, D. K., Jr., Driscoll, T., Seibel, N. L., et al.: Safety and pharmacokinetics of intravenous anidulafungin in children with neutropenia at high risk for invasive fungal infections. Antimicrob. Agents Chemother. 50:632-638, 2006; and Vasquez, J. A., and Sobel, J. D.: Anidulafungin: A novel echinocandin. Clin. Infect. Dis. 43:215-222, 2006.

TABLE 252–14 Single-Dose Micafungin Pharmacokinetics in Pediatric versus Adult Patients*

	Pediatric Patients (2-17 yr)			Adults	
Dosage	**1 mg/kg**	**2 mg/kg**	**4 mg**	**50 mg**	**100 mg**
C_{max} (μg/mL)	10.8	15.3	30.3	3.6	7.1
$AUC_{0\text{-}24\ hr}$ (μg/hr/mL)	40.3	83.0	191.4	33.9	59.9
$t_{1/2}$beta (hr)	12.5	13.2	11.6	12.5	13.0
Cl (L/hr/kg)	0.021	0.020	0.017	0.017*	0.018*
Vd_{ss} (L/kg)	0.33	0.31	0.28	0.31*	0.32*

**Pharmacokinetic parameters are expressed as mean values. Data were obtained in groups of 7 to 15 pediatric patients with compromised immunity and neutropenia per age group and dosage level and were compared with those obtained in cohorts of 8 to 9 adult patients with hematopoietic stem cell transplantation.*

†Weight normalization calculated by assuming an average body weight of 70 kg.

AUC, area under the concentration versus time curve from 0 to 24 hours; Cl, clearance; C_{max}, peak plasma concentration; $t_{1/2}$beta, elimination half-life; Vd_{ss}, volume of distribution at steady state.

Modified from Seibel, N. L., Schwartz, C., Arrieta, A., et al.: Safety, tolerability, and pharmacokinetics of Micafungin (FK463) in febrile neutropenic pediatric patients. Antimicrob. Agents Chemother. 49:3317-3324, 2005; and Groll, A. H., Stergiopoulou, T., Roilides, E., and Walsh, T. J.: Micafungin: Pharmacology, experimental therapeutics and clinical applications. Expert Opin. Investig. Drugs 14:489-509, 2005.

once daily) and liposomal amphotericin B (3 mg/kg once daily) for first-line therapy of invasive *Candida* infections in a total of 531 adult patients. The overall success rate in both treatment arms of the trial was similar (89.6% versus 89.5%). No difference in survival occurred. Predefined safety parameters showed micafungin to have advantages over liposomal amphotericin B in renal function.[394] In another large, phase III, randomized clinical trial of a total of 593 patients with invasive candidiasis, micafungin at 100 mg/day, micafungin at 150 mg/day, and caspofungin at 50 mg/day (day 1: 70 mg) were equivalent in terms of efficacy, adverse events, and mortality.[124] A multinational, noncomparative open-label clinical trial investigated micafungin for proven or probable invasive aspergillosis alone or in combination with another systemic antifungal agent. A favorable response rate at the end of therapy was seen in 35.6 percent (80/225) of patients. Of those treated with only micafungin, favorable responses were seen in 6 of 12 (50%) of the primary and 9 of 22 (40.9%) of the salvage therapy group, with corresponding numbers in the combination treatment groups of 5 of 17 (29.4%) and 60 of 174 (34.5%) of the primary and salvage treatment groups, respectively.[106] Finally, micafungin (50 mg/day; 1 mg/kg for patients <50 kg) versus fluconazole (400 mg/day; 8 mg/kg for patients <50 kg) was investigated for prophylaxis of invasive fungal infections in 882 patients undergoing HSCT. Prophylaxis was given from the start of the conditioning regimen until 5 days after engraftment. The overall success rate was significantly higher for patients randomized to receive micafungin (80.0% versus 73.5%; $p = .03$). Drug-related adverse events were comparable.[466]

APPROVAL STATUS AND DOSING. In the United States, micafungin is licensed in adults for prevention of *Candida* infections in patients undergoing hematopoietic stem cell transplantation (HSCT), for treatment of candidemia and defined forms of invasive candidiasis, and for treatment of esophageal candidiasis (see Table 252–2). In Europe, the compound is licensed for use in neonates, children, and adults for treatment of invasive candidiasis and as prophylaxis of *Candida* infections in patients with allogeneic HSCT and those with prolonged neutropenia; it is also licensed for treatment of esophageal candidiasis in individuals 16 years and older. The recommended dosage is 100 mg/day for invasive candidiasis (≤40 kg body weight: 2 mg/kg), 150 mg/day for esophageal candidiasis (≤40 kg: 3 mg/kg), and 50 mg/day (≤40 kg: 1 mg/kg) for the preventive indication. Renal dysfunction, dialysis, or mild-to-moderate hepatic dysfunction (Child-Pugh class A and B) does not alter the pharmacokinetics of micafungin.[198,322]

The pharmacokinetics and safety of micafungin have been studied in 70 febrile granulocytopenic children aged 2 to 17 years. Micafungin was well tolerated at dosages of 0.5 to 3.0 mg/kg/day; pharmacokinetics were linear and similar to those observed in adults[424] (Table 252–14). A phase I trial investigated the safety and pharmacokinetics of micafungin (0.75 mg/kg, 1.5 mg/kg, and 3.0 mg/kg) in 18 premature infants weighing more than 1000 g. Micafungin pharmacokinetics were linear, but clearance rates were more rapid compared with those of older children and adults. No serious drug-related adverse events were observed.[204] A noncomparative phase II study investigated the activity of micafungin alone or in combination with other agents in 58 pediatric patients with mostly refractory invasive aspergillosis. Most patients ($n = 56$) received micafungin in combination with another agent, primarily liposomal amphotericin B ($n = 47$). The mean daily dose was 2.0 ± 1.2 mg/kg/day, and the mean duration of dosing was 67 ± 85 days. Overall response was 26/58 (45%). Thirteen patients (22%) discontinued micafungin, primarily because of a progression of underlying disease or invasive aspergillosis.[137] Micafungin was further investigated in the pediatric subpopulation of a double-blind, phase III trial in patients with invasive candidiasis or candidemia. Patients were randomized to receive intravenous micafungin (2 mg/kg/day) or liposomal amphotericin B (3 mg/kg/day) for a minimum of 14 days. There was no difference in the success rates (69.2% vs. 74.1%). The incidence of serious adverse events (3.8% vs. 9.3%) and the rate of patients discontinuing therapy because of an adverse event (3.8% vs. 16.7%) were lower in micafungin-treated patients.[26,371a] In a pooled analysis of adverse events data from six clinical trials that included close to 300 patients, 26.7 percent of patients had a treatment-related adverse event and 2.4 percent had a treatment-related adverse event that led to treatment discontinuation. No trends were seen with respect to dose or duration of treatment, and the types and rates of events wre similar to those observed in adults.[23a]

In Europe, the Summary of Product Characteristics for micafungin recommends careful monitoring of liver function and early discontinuation in the presence of significant and persistent elevation of ALT/AST values. It also recommends that micafungin treatment be conducted on a careful risk-benefit assessment,

particularly in patients with liver diseases or concomitant hepatotoxic-genotoxic therapies.[27a] This recommendation is based on preclinical data from a high-dose, long-term exposure model in rats treated with micafungin for either 3 or 6 months (equivalent to 12.5% or 25% of the total life span of the rat, respectively). The observed development of foci of altered hepatocytes and liver tumors in this species was dependent on both dose and duration of micafungin treatment. The human relevance of this finding is presently not known.

AGENTS FOR SYSTEMIC TREATMENT OF MYCOSES OF THE SKIN AND ITS APPENDAGES

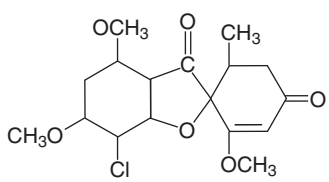
GRISEOFULVIN

Griseofulvin (Fig. 252–11) was isolated originally in 1939 as a natural product of *Penicillium griseofulvum*.[346] However, only in the late 1950s was it was reported to be effective as an antifungal agent.[154] Griseofulvin has been used since extensively for the systemic treatment of superficial dermatophyte infections in both adults and children.

MECHANISM OF ACTION. Griseofulvin interferes with fungal microtubule formation. It disrupts the cell's mitotic spindle formation and thus arrests the metaphase of cell division.[189] Several additional mechanisms of action have been proposed, including inhibition of nucleic acid synthesis, interference with the synthesis of cell wall chitin, and anti-inflammatory properties.[192] Griseofulvin is deposited in keratin precursor cells and produces an unfavorable environment for fungal invasion; infected skin, hair, and nails are replaced with tissue not infected by the dermatophyte.

ANTIFUNGAL ACTIVITY. As evident from its mechanism of action, griseofulvin is a fungistatic compound. It is active against *Trichophyton*, *Microsporon*, and *Epidermophyton* spp. The drug has no activity against yeastlike organisms such as *Candida* spp., *Pityrosporum* spp., and *C. neoformans*. It also is inactive against opportunistic hyaline and dematiaceous molds and the dimorphic (endemic) molds.[142]

PHARMACOKINETICS. Griseofulvin is commercially available for oral administration only as griseofulvin microsize and griseofulvin ultramicrosize. Microsize griseofulvin contains predominantly particles in the order of 4 μm in diameter, and ultramicrosize griseofulvin contains predominantly particles of less than 1 μm in diameter. Griseofulvin is weakly water soluble and poorly absorbed from the gastrointestinal tract. In comparison with nonmicronized drug preparations, micronized and ultramicronized formulations display enhanced absorption, particularly when polyethylene glycol is used as dispersion carrier in the ultramicronized formulations. Oral bioavailability of the micronized formulation is variable and ranges from 25 to 70 percent; ultramicronized griseofulvin, in contrast, is almost completely absorbed.[53,192]



Griseofulvin

Figure 252–11 Structural formula of griseofulvin.

After oral administration, C_{max} occurs approximately 4 hours after dosing. Griseofulvin distributes to keratin precursor cells and is concentrated in skin, hair, nails, liver, adipose tissue, and skeletal muscles. In skin, over time, a concentration gradient is established, with the highest concentrations found in the outermost stratum corneum.[127,427] Non–protein-bound drug is carried in the extracellular fluid, in sweat, and through transepidermal fluid loss; in addition, reversible protein binding and high lipid solubility cause griseofulvin to partition into the stratum corneum, where its concentrations exceed that of serum. Within 48 to 72 hours after discontinuation of the drug, plasma concentrations of griseofulvin are markedly reduced, and the compound no longer is detectable in the stratum corneum.[53,192]

Griseofulvin is oxidatively demethylated and conjugated with glucuronic acid primarily in the liver; its major metabolite, 6-desmethylgriseofulvin, is microbiologically inactive.[276] The elimination of the compound from plasma is bi-exponential, with a terminal elimination half-life of 9 to 21 hours.[392] Approximately one third of a single dose of micronized griseofulvin is excreted in feces, and 50 percent is excreted in urine within 5 days. In urine, the drug is excreted mainly as free and glucuronized 6-desmethylgriseofulvin. Unchanged drug in the urine accounts for less than 1 percent of the dose.[276,277]

Separate pharmacokinetic data for pediatric patients have not been published; however, the compound is approved for children older than 2 years of age. The recommended pediatric dosage of microsize griseofulvin is 10 to 20 mg/kg/day (maximum, 1 g) administered in two divided doses; the recommended pediatric dosage of ultramicronized griseofulvin is 5 to 10 mg/kg/day (maximum, 750 mg), also given in two divided doses.[125,143]

ADVERSE EFFECTS. Griseofulvin has an acceptable safety profile. More common adverse effects include headaches and a variety of gastrointestinal symptoms. Griseofulvin can cause photosensitivity and can exacerbate lupus and porphyria. Cases of erythema multiforme–like reactions, toxic epidermal necrolysis, and a reaction resembling serum sickness have been reported. Proteinuria, nephrosis, hepatotoxicity, leukopenia, and menstrual irregularities have been reported rarely in association with griseofulvin therapy. Griseofulvin has been noted to produce estrogen-like effects in children and reversible diminution of hearing.[53,142,192] Griseofulvin is contraindicated in patients with porphyria or hepatocellular failure. The compound has been shown to be teratogenic in animals and should not be administered to pregnant women. Griseofulvin also has mutagenic and carcinogenic potential; the significance of these observations for humans, however, is unclear.[142]

DRUG INTERACTIONS. Griseofulvin has been noted to enhance the clearance of oral contraceptives, cyclosporine, theophylline, aspirin, and warfarin. Concurrent use of phenobarbital may lead to decreased levels of griseofulvin. Finally, concurrent ingestion of alcohol may lead to a disulfiram-like reaction.[142]

INDICATIONS. Griseofulvin remains an important agent for the treatment of tinea capitis and refractory tinea corporis (Table 252–15).[135] For tinea capitis, 6 to 8 weeks of treatment usually are required.[125,217] The usual duration of therapy for refractory tinea corporis is 4 weeks.[125,144] Nail infections, which are rare occurrences in the pediatric population, usually fail to respond to therapy with griseofulvin and are better treated with itraconazole or terbinafine. Because griseofulvin is not effective against other fungal infections (Table 252–16), the infecting organism always should be identified as a dermatophyte before therapy is initiated. In vitro resistance of dermatophytes to griseofulvin has been reported and may be the cause of therapeutic failure.[27]

TABLE 252–15 Medical Management of Superficial Infections by Dermatophytes

Fungal Disease	Management
Tinea capitis	Griseofulvin (micronized: 5-10 mg/kg bid; ultramicronized: 2.5-5 mg/kg bid) for a total of 6 to 8 wk
	Fluconazole (6 mg/kg qd) for 4 wk
	Itraconazole (2.5 mg/kg bid) for 4 wk*
	Terbinafine (<20 kg: 62.5 mg qd; 20-40 mg: 125 mg qd; >40 mg: 250 mg qd) for 4 wk*
Tinea unguium	Itraconazole (2.5 mg/kg bid 1 wk/mo) for 3 to 4 mo*
	Terbinafine (<20 kg: 62.5 mg qd; 20-40 mg: 125 mg qd; >40 mg: 250 mg qd) for 6 wk (fingernail) or 12 wk (toenail)*
Tinea corporis	Topical antifungal azoles:
Tinea facialis	Miconazole, clotrimazole, econazole,
Tinea pedis	ketoconazole, sulconazole, oxiconazole bid for 2-4 wk
	Topical allyl/benzylamines, thiocarbamates:
	Terbinafine, naftifine, butenafine, tolnaftate qd/bid for 2-4 wk
	Other topical agents:
	Ciclopirox olamine bid for 2 to 4 wk
	Refractory infections/immunocompromised patients:
	Griseofulvin (micronized: 5-10 mg/kg bid; ultramicronized: 2.5-5 mg/kg bid) for 2-4 wk
	Fluconazole (3-6 mg/kg qd) for 2-4 wk
	Itraconazole (2.5 mg/kg bid) for 2-4 wk*
	Terbinafine (<20 kg: 62.5 mg qd; 20-40 mg: 125 mg qd; >40 mg: 250 mg qd) for 2-4 wk*

**Not approved by the U.S. Food and Drug Administration in individuals <18 years of age.*

Data from Friedlander, S. F., and Suarez, S.: Pediatric antifungal therapy. Dermatol. Clin. 16:527-537, 1998; and Howard, R. M., and Frieden, I. J.: Dermatophyte infections in children. Adv. Pediatr. Infect. Dis. 14:73-107, 1999.

TABLE 252–16 Medical Management of Superficial Infections by Yeastlike Fungi

Fungal Disease	Management
Candida dermatitis	Topical antifungal azoles:
Tinea versicolor	Miconazole, sulconazole, econazole, oxiconazole, clotrimazole bid for 2-4 wk
	Topical polyenes:
	Amphotericin B or nystatin bid/qid for 2-4 wk
	Other topical agents:
	Ciclopirox olamine bid for 2-4 wk
	Refractory infections/immunocompromised patients:
	Fluconazole (3-6 mg/kg qd) for 2-4 wk
	Itraconazole (2.5 mg/kg bid) for 2-4 wk*
Oropharyngeal candidiasis	Topical polyenes:
	Nystatin (200-600.000 U) or amphotericin B (100 mg) four times daily for ≥2 wk
	Topical antifungal azoles:
	Clotrimazole lozenges (10 mg) five times daily for ≥2 wk
	Refractory infections/immunocompromised patients:
	Fluconazole (3-6 mg/kg qd) for ≥2 wk
	Itraconazole (2.5 mg/kg bid) for ≥2 wk*
	Voriconazole (200 mg bid PO)
	Posaconazole (100-400 mg bid PO)*
	Amphotericin B deoxycholate (0.5-1 mg/kg qd)
	Echinocandin lipopeptides
Vulvovaginal candidiasis	Topical antifungal azoles:
	Miconazole, clotrimazole, butoconazole, terconazole, tioconazole qhs for ≤7 days
	Topical polyenes:
	Nystatin qhs for 14 days
	Systemic antifungal azoles:
	Fluconazole 150 mg PO × 1day/50 mg PO × 3days
	Refractory infections/immunocompromised patients:
	Fluconazole (3-6 mg/kg qd) for ≥2 wk
	Itraconazole (2.5 mg/kg bid) for ≥2 wk*
	Amphotericin B deoxycholate (0.5-1 mg/kg qd)

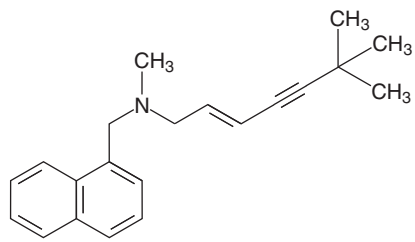
**Not approved by the U.S. Food and Drug Administration in individuals of <18 years of age.*

Data from Bennett, J. E.: Antifungal agents. In *Mandell, G. L., Bennett, J. E., and Dolin, R. (eds.): Principles and Practice of Infectious Diseases. 4th ed. New York, Churchill Livingstone, 1995, pp. 401-410; and Walsh, T. J., Gonzalez, C., Lyman, C. A., et al.: Invasive fungal infections in children: Recent advances in diagnosis and treatment. Adv. Pediatr. Infect. Dis. 11:187-290, 1996.*

TERBINAFINE

The synthetic allylamine terbinafine (Fig. 252–12) is a relatively novel antifungal agent that is useful for topical and systemic (oral) treatment of superficial infections of the skin and its appendages by dermatophytes and yeasts, as well as possibly for treatment of cutaneous sporotrichosis. Terbinafine acts by inhibiting the biosynthesis of fungal ergosterol at the level of squalene epoxidase (see Fig. 252–8), thus leading to depletion of ergosterol and accumulation of toxic squalenes in the fungal cell membrane.[32]

ANTIFUNGAL ACTIVITY. Terbinafine has exceptionally potent and fungicidal in vitro activity against dermatophytes.[134] It also is highly active against *Aspergillus* spp., certain Hyalohyphomycetes, dematiaceous and dimorphic fungi, and *P. jiroveci* (formerly *P. carinii*). Its in vitro activity against yeasts appears more variable.[151,184,227,397] Comparative studies have indicated that terbinafine may be more active against *Aspergillus* spp. than is itraconazole[426] or amphotericin B,[416] more active than is itraconazole against *S. schenkii*,[248] and less or comparably active than are the azoles against yeasts.[426] Synergy with triazoles against *C. albicans*,[139,501] *C. glabrata*,[35] *C. neoformans*,[139] *A. fumigatus*,[318,396] non-*Aspergillus* Hyalohyphomycetes[304,305,342,397] and Zygomycetes[164] has been reported. Although more variable, synergy with amphotericin B also has been demonstrated for filamentous and yeastlike fungi.[397] Terbinafine resistance in clinical dermatophyte isolates is a rare occurrence; only two cases have been documented in which the resistance mechanism was identified as a single amino acid substitution in squalene epoxidase.[343]



Terbinafine

Figure 252–12 Structural formula of terbinafine.

PHARMACOKINETICS. The pharmacokinetic properties of terbinafine in adults are well characterized.[32] Independent of food, oral bioavailability is 70 to 80 percent. Over the adult

dosage range of 125 to 750 mg once daily, terbinafine displays linear plasma pharmacokinetics. C_{max} of 0.5 to 2.7 μg/mL is measured within 2 hours,[254] and steady state is reached after 10 to 14 days after only twofold accumulation.[32] As a lipophilic drug, terbinafine is strongly bound to plasma proteins. The compound is distributed extensively to tissues and accumulates throughout adipose tissues, dermis, epidermis, and nail. It exhibits a triphasic distribution pattern in plasma, with a terminal half-life up to 3 weeks; microbiologically active concentrations can be measured in plasma for weeks to months after the last dose, a finding consistent with a slow redistribution from peripheral tissue and adipose tissue sites.[32,254,328]

Terbinafine undergoes extensive and complex hepatic biotransformation that involves at least seven cytochrome P-450 enzymes.[479] Fifteen metabolites have been identified, mainly in urine; none has been shown to be mycologically active.[255] Studies employing radiolabeled drug demonstrated that urinary excretion accounts for more than 70 percent and fecal elimination for 10 percent of radioactivity; the extent of enterohepatic recycling is unknown.[219,220] As a consequence of the compound's extensive hepatic metabolism and urinary excretion, caution is warranted in treating patients with severe hepatic and renal impairment.[32]

Several studies were conducted to evaluate the safety, pharmacokinetics, and antifungal efficacy in the pediatric population.[2,67,144,220,231,256,279,330] The pharmacokinetic properties of terbinafine and five known major metabolites in plasma and urine were investigated carefully after single and repeated oral administration of 125 mg/day to 12 pediatric patients for up to 56 days (mean age, 8 years; age range, 5 to 11 years; weight range, 17 to 34 kg) (Table 252–17). No differences were found regarding the metabolism of terbinafine in comparison with healthy adults. Steady state was reached at least on day 21, and no further accumulation occurred between days 21 and 56.[219,330] Comparison of the kinetic parameters of terbinafine after single administration of 125 mg showed comparable C_{max} and T_{max} (time until occurrence of C_{max}) values, and a 40 percent higher AUC; when dose was calculated as milligrams per kilogram or milligrams per square meter, children showed a lower AUC (range, –29% to –45%) as compared with adults, a finding indicating a higher, weight-normalized volume of distribution into lipophilic tissue. Children had shorter beta-phase elimination half-lives, but the gamma-phase, terminal half-life determined after multiple dosing during washout was similar to that in adults. Thus, in children weighing 17 to 34 kg, a dose of 125 mg terbinafine yields pharmacokinetic properties similar to those in adults without drug accumulation, and use of a calculation of milligrams per kilogram or milligrams per square meter would lead to lower drug levels than those recorded in adults.[2,220,231,330] Exploration of lower doses (62.5 mg/day) in eight children weighing 19 to 35 kg revealed an approximate reduction in trough level of 50 percent, a finding indicating linearity of plasma pharmacokinetics in children.[231]

Based on the experience with dosages of 10 mg/kg and less in adults and the described pharmacokinetic profile of the compound in children, a dose of 250 mg/day has been proposed for children weighing more than 40 kg, a dose of 125 mg/day for children weighing 20 to 40 kg, and 62.5 mg/day for children weighing less than 20 kg.[231]

TABLE 252–17 Pharmacokinetic Parameters of Terbinafine in Children after a Single Dose of 125 mg Compared with Similar Parameters in Healthy Adults*

	Children with Tinea Capitis (n = 12)	Healthy Adults (n = 16)	Statistical Comparison
Age (yr)	8 ± 2 (5-11)	26 ± 4 (21-34)	—
Weight (kg)	26 ± 5 (17-34)	64 ± 6 (54-80)	—
C_{max} (μg/mL)	0.706 ± 0.277 (0.333-1.212)	0.565 ± 0.329 (0.196-1.172)	NS
T_{max} (hr)	2.1 ± 1.1 (1.0-4.0)	5 ± 0.7 (0.7-2.5)	NS
$AUC_{0-\infty}$ (μg/mL/hr)	2.967 ± 0.965 (1.474-4.841)	2.135 ± 1.131 (0.758-4.435)	$p < .05$
$t_{1/2}$beta (hr)	14.7 ± 4.3 (10-26)	27 ± 12 (12-58)	$p < .001$

**Mean values ± SD (range).*

$AUC_{0-\infty}$, area under the concentration versus time curve from zero to infinity; C_{max}, peak plasma concentration; NS, not significant; $t_{1/2}$beta, elimination half-life; T_{max}, time until occurrence of peak plasma concentration.

Modified from Jones, T. C.: Overview of the use of terbinafine (Lamisil) in children. Br. J. Dermatol. 132:683-689, 1995.

ADVERSE EFFECTS. In adults, terbinafine usually is well tolerated at doses of up to 500 mg/day, and it has a relatively low incidence of adverse effects. The primary adverse effects associated with terbinafine include gastrointestinal upsets and skin reactions in 2 to 7 percent of patients. Terbinafine can cause hepatitis and liver failure. Potentially severe hepatotoxicity is estimated to occur in 1 of 120,00 patients, and asymptomatic rises in liver enzyme activities are likely to occur at a frequency of 1 per 200. Although hepatotoxicity may occur in patients with or without chronic or active liver disease, the drug should not be administered in patients with an underlying liver problem, and liver function tests should be obtained before terbinafine is prescribed. Less common significant adverse effects have included reversible loss of taste, severe skin eruptions including generalized erythematous pustulosis and drug-induced lupus erythematosus, Stevens-Johnson syndrome, and blood dyscrasias.[3] No evidence indicates that these idiosyncratic effects are increasing in incidence with the increasing use of terbinafine.[345]

Several clinical studies documented the safety of terbinafine in pediatric patients.[67,74,144,145,231,256,279,330,429] Terbinafine, administered for a median duration of 4 weeks (range, 1 to 28 weeks) was safe in children between 2 and 17 years of age who received the drug for various dermatophyte and yeast infections of the skin: of a total of 196 patients enrolled in 6 studies, 22 adverse events were observed in 15 patients. Adverse events probably associated with the use of terbinafine occurred in 6 of these patients (3%), but in none of these patients did terbinafine therapy have to be discontinued.[231] Terbinafine was similarly well tolerated in two double-blind, randomized trials in children with tinea capitis.[144,279]

DRUG INTERACTIONS. Multiple cytochrome P-450 enzymes are involved in the metabolism of terbinafine. However, with the possible exception of CYP2D6 substrates, in vitro studies revealed little or no effect on the metabolism of many characteristic CYP substrates.[479] Inhibition of CYP2D6-mediated metabolism may be relevant with the concomitant use of tricyclic antidepressants, beta blockers, selective serotonin reuptake inhibitors, and type B monoamine oxidase inhibitors.

In clinical interaction studies, no pharmacokinetic or pharmacodynamic interactions were observed with the concomitant administration of terfenadine,[388] midazolam,[7] and alfentanil.[401] Terbinafine can (1) reduce the clearance of theophylline[455]; (2) increase levels of nortryptiline,[469] amitryptiline,[78] desipramine,[285] imipramine,[447] and paroxetine[524]; (3) increase or reduce warfarin exposure[190,501]; and (4) reduce the trough cyclosporine concentration in patients with transplants.[280] The metabolism of terbinafine may be decreased by cimetidine and increased by rifampin.[184]

CLINICAL INDICATIONS. Terbinafine is approved by the FDA in subjects older than18 years of age for the treatment of

onychomycosis of toenails and fingernails caused by dermatophytes. It may be indicated for treatment of other superficial infections of the skin and its appendages caused by dermatophytes,[3,32,143] for cutaneous and lymphocutaneous sporotrichosis[83,218] subcutaneous phaeohyphomycosis[56,374] (see Tables 252–4 and 252–15), and, investigationally, for seborrhoic dermatitis.[476] Recommended durations of treatment for tinea capitis, tinea corporis and pedis, fingernail onychomycosis, and toenail onychomycosis in adults are 4, 2, 6, and 12 weeks, respectively.[231]

Several studies investigated the safety and clinical efficacy of terbinafine in the pediatric population.[67,74,144,145,231,256,279,330,429] The overall mycologic and clinical efficacy for 152 children aged 2 to 17 years with various dermatophyte and yeast infections of the skin exceeded 95 percent.[231] In a prospective, randomized clinical trial in 210 children comparing treatment with 4 weeks of terbinafine versus 8 weeks of griseofulvin for *Trichophyton* tinea capitis, both regimens showed overall similar efficacy.[145] In a subsequent study of the duration of treatment in 176 patients, both 2 and 4 weeks of treatment were clinically superior to a 1-week regimen and had similar treatment efficacy, a finding suggesting that a 2-week course may be sufficient for patients with *Trichophyton* tinea capitis.[144] A similar study performed in 134 patients with *Microsporon* tinea capitis investigating 6, 8, 10, or 12 weeks of therapy demonstrated that 6 weeks of therapy were equivalent to griseofulvin.[279] Finally, a meta-analysis of 6 randomized, comparative clinical trials indicated that a 2- to 4-week course of terbinafine was at least as effective as a 6- to 8-week course of griseofulvin for the treatment of tinea capitis caused by *Trichophyton* spp.[135]

The broad-spectrum fungicidal in vitro activity, systemic availability, and lack of significant side effects suggested potential usefulness of terbinafine against deep-seated fungal infections. However, terbinafine was ineffective in animal models of pulmonary and disseminated aspergillosis,[243,417] systemic sporotrichosis,[234] cerebral phaeohyphomycosis,[116] disseminated candidiasis, and pulmonary cryptococcosis,[396] findings explained by nonsaturable protein-binding kinetics.[417] Against experimental pulmonary pneumocystosis, terbinafine was active in some,[90,91] but not all,[499] models.

TOPICAL ANTIFUNGAL AGENTS

Apart from fungal keratitis, the use of topical antifungal agents is confined to superficial infections of the skin and mucosal surfaces. The decision to treat superficial infections of the skin and mucosal surfaces with a topical or systemic agent depends mainly on the site and extent of the infection. Immunocompromised children, however, usually require systemic therapies, as do patients with tinea capitis and onychomycosis (see Tables 252–15 and 252–16).[125]

TOPICAL THERAPEUTICS FOR SUPERFICIAL SKIN INFECTIONS

Dermatophytosis is caused by the filamentous fungi *Microsporon* spp., *Trichophyton* spp., and *Epidermophyton floccosum*. Many different agents and formulations are available for topical treatment of dermatophytic skin infections (tinea corporis, facialis, or pedis), including allylamines and azoles. Agents for treatment of *Candida* dermatitis and tinea (pityriasis) versicolor (caused by *Malassezia furfur* or *Malassezia pachydermatidis*) include various topical azoles and topical polyenes. Most topical agents are applied twice daily well beyond the clinical resolution of the infection. A detailed review of the pharmacologic properties of these topical drugs and of the treatment of cutaneous mycoses is beyond the scope of this chapter and can be found elsewhere.[125,143,217]

TOPICAL THERAPEUTICS FOR MUCOSAL CANDIDIASIS

Agents for the topical treatment of vulvovaginal candidiasis include a large variety of antifungal azoles and the polyene nystatin.[42,433] Azole agents may be absorbed to a minor extent and potentially can interfere with the metabolism of concomitant drugs. For example, potentiation of the anticoagulatory effects of acenocoumarol was noted after vaginal administration of miconazole capsules to two postmenopausal patients[266] and after oral administration of miconazole gel in three elderly patients with oral candidiasis.[341]

Antifungal azoles such as clotrimazole and miconazole and antifungal polyenes such as amphotericin B and nystatin are effective in the treatment of oropharyngeal candidiasis. Many clinical trials evaluated the usefulness of these agents for prevention of fungal infections in immunocompromised patients with cancer or HSCT. Although most agents have documented efficacy in the prevention of oropharyngeal candidiasis, they are not effective in preventing invasive mycoses and in improving infection-related and overall mortality in this setting.[186,214,282,464]

FUTURE DIRECTIONS

Pediatric age groups display important differences in host biology, predisposing conditions, epidemiology, and presentation of fungal infections relative to the adult population. Since the late 1990s, major advances have been made in the field of medical mycology. Most importantly, an array of new antifungal agents has entered the clinical arena. Although the final pediatric approval of several of these agents remains to be established, the development of pediatric therapies is moving forward at a steady pace. Invasive fungal infections will remain important causes of morbidity and mortality in immunocompromised pediatric patients. The availability of alternative therapeutic options is an important advance; at the same time, however, antifungal therapy has become increasingly complex. In addition to information on prior antifungal therapies, microbiologic data, existing comorbidities, and concurrent medications, detailed knowledge of the available antifungal armamentarium and contemporary clinical trials are needed more than ever in the management of the individual patient.[85]

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CHAPTER 253

Drugs for Parasitic Infections

The Medical Letter (Peter J. Hotez)

With increasing travel, immigration, and use of immunosuppressive drugs and the spread of acquired immunodeficiency syndrome (AIDS), physicians anywhere may see infections caused by parasites. The table below lists first-choice and alternative drugs for most parasitic infections. A later table summarizes the known prenatal risks of antiparasitic drugs. The brand names and manufacturers of the drugs are listed at the end of the chapter. The table was modified from *The Medical Letter*, Vol. 5 (Suppl), last modified August 2008.

Infection		Drug	Adult Dosage	Pediatric Dosage
ACANTHAMOEBA keratitis				
Drug of choice:		See footnote 1		
AMEBIASIS *(Entamoeba histolytica)*				
Asymptomatic				
Drug of choice:		Iodoquinol[2]	650 mg PO tid × 20 d	30-40 mg/kg/d (max. 2 g) PO in 3 doses × 20 d
	OR	Paromomycin[3]	23-35 mg/kg/d PO in 3 doses × 7 d	25-35 mg/kg/d PO in 3 doses × 7 d
	OR	Diloxanide furoate[4]*	500 mg PO tid × 10 d	20 mg/kg/d PO in 3 doses × 10 d
Mild to moderate intestinal disease				
Drug of choice:[5]		Metronidazole	500-750 mg PO tid × 7-10 d	35-50 mg/kg/d PO in 3 doses × 7-10 d
	OR	Tinidazole[6]	2 g once PO daily × 3 d	≥3 yr: 50 mg/kg/d (max. 2 g) PO in 1 dose × 3 d
		either followed by		
		Iodoquinol[2]	650 mg PO tid × 20 d	30-40 mg/kg/d (max. 2 g) PO in 3 doses × 20 d
	OR	Paromomycin[3]	25-35 mg/kg/d PO in 3 doses × 7 d	25-35 mg/kg/d PO in 3 doses × 7 d
Severe intestinal and extraintestinal disease				
Drug of choice:		Metronidazole	750 mg PO tid × 7-10 d	35-50 mg/kg/d PO in 3 doses × 7-10 d
	OR	Tinidazole[6]	2 g once PO daily × 5 d	≥3 yr: 50 mg/kg/d (max. 2 g) PO in 1 dose × 3 d
		either followed by		
		Iodoquinol[2]	650 mg PO tid × 20 d	30-40 mg/kg/d (max. 2 g) PO in 3 doses × 20 d
	OR	Paromomycin[3]	25-35 mg/kg/d PO in 3 doses × 7 d	25-35 mg/kg/d PO in 3 doses × 7 d
AMEBIC MENINGOENCEPHALITIS, primary and granulomatous				
Naegleria				
Drug of choice:		Amphotericin B[7,8]	1.5 mg/kg/d IV in 2 doses × 3 d, then 1 mg/kg/d × 6 d plus 1.5 mg/d intrathecally × 2 d, then 1 mg/d every other day × 8 d	1.5 mg/kg/d IV in 2 doses × 3 d, then 1 mg/kg/d × 6 d plus 1.5 mg/d intrathecally × 2 d, then 1 mg/d every other day × 8 d
Acanthamoeba				
Durg of choice:		See footnote 9		

* *Availability problems. See table on page 3309.*

1. *Topical 0.02% chlorhexidine and polyhexamethylene biguanide (PHMB, 0.02%, either alone or in combination, have been used successfully in a large number of patients. Treatment with either chlorhexidine or PHMB is often combined with propamidine isethionate* (Brolene) *or hexamidine* (Desmodine). *None of these drugs is commercially available or approved for use in the United States, but they can be obtained from compounding pharmacies (see footnote 2). Leiter's Park Avenue Pharmacy, San Jose, CA (800-292-6773); www.leiterrx.com) is a compounding pharmacy that specializes in ophthalmic drugs. Propamidine is available over the counter in the United Kingdom and Australia. Hexamidine is available in France. The combination of chlorhexidine, natamycin (pimaricin), and débridement also has been successful (K Kitagawa et al, Jpn J Ophthalmol 2003; 47:616). Débridement is most useful during the stage of corneal epithellial infection. Most cysts are resistant to neomycin; its use is no longer recommended. Azole antifungal drugs (ketoconazole, itraconazole) have been used as oral or topical adjuncts (FL Shuster and GS Visvesvara, Drug Resist Update 2004; 7:41). Use of corticosteroids is controversial (K Hammersmith, Curr Opinions Ophthal 2006; 17:327; ST Awwad et al, Eye Contact Lens 2007; 33:1).*
2. *Iodoquinol should be taken after meals.*
3. *Paromomycin should be taken with a meal.*
4. *Not available commercially. It may be obtained through compounding pharmacies such as Panorama Compounding Pharmacy, 6744 Balboa Blvd, Van Nuys, CA 91406 (800-247-9767) or Medical Center Pharmacy, New Haven, CT (203-688-6816). Other compounding pharmacies may be found through the National Association of Compounding Pharmacies (800-687-7850) or the Professional Compounding Centers of America (800-331-2498, www.pccarx.com).*
5. *Nitazoxanide may be effective against a variety of protozoan and helminth infections (DA Bobak, Curr Infect Dis Rep 2006; 8:91; E Diaz et al, Am J Trop Med Hyg 2003; 68:384). It was effective against mild to moderate amebiasis, 500 mg bid × 3 d, in a recent study (JF Rossignol et al, Trans R Soc Trop Med Hyg 2007 101:1025). It is FDA-approved only for treatment of diarrhea caused by* Giardia or Cryptosporidium *(Med Lett Drugs Ther 2003; 45:29). Nitazoxanide is available in 500-mg tables and an oral suspension; it should be taken with food.*
6. *A nitroimidazole similar to metronidazole, tinidazole appears to be as effective as metronidazote and better tolerated (Med Lett Durgs Ther 2004; 46:70). It should be taken with food to minimize GI adverse effects. For children and patients unable to take tablets, a pharmacist can crush the tablets and mix them with cherry syrup (*Humco, *and others). The syrup suspension is good for 7 days at room temperature and must be shaken before use (HB Fung and TL Doan et al, Clin Ther 2005; 27:1859). Ornidazole, a similar drug, is also used outside the United States.*
7. *Not FDA-approved for this indication.*
8. *Although a* Naegleria fowleri *infection was treated successfully in a 9-year-old girl with combination of amphotericin B and miconazole both intravenous and intrathecal, plus oral rifampin (JS Seidel et al, N. Engl. J. Med. 1982; 306:346). Amphotericin B and miconazole appear to have a synergistic effect, but* Medical Letter *consultants believe the rigampin probably had no additional effect (GS Visvesvara et al, FEMS Immunol Med Microbiol 2007; 50:1). Parenteral miconazole is no longer available in the United States. Azithromycin has been used successfully in combination therapy to treat* Balmuthia *infection, but was changed to clarithromycin because of toxicity concerns and for better penetration into the cerebrospinal fluid. In vitro, azithromycin is more active than clarithromycin against* Naegleria, *so may be a choice combined with amphotericin B for treatment of* Naegleria *(TR Deetz et al, Clin Infect Dis 2003; 37:1304; FL Schuster and GS Visvesvara, Drug Resistance Updates 2004; 7:41). Combinations of amphotericin B, ornidazote and rifampin (R Jain et al, Neurol Indian 2002; 50:470) and amphotericin B fluconazole and rifampin have also been used (J Vargas-Zepeda et al, Arch Med Research 2005; 36:83). Case reports of other successful therapy have been published (FL Schuster and GS Visvesvara, Int J Parasitol 2004; 34:1001).*

Infection	Drug	Adult Dosage	Pediatric Dosage
AMEBIC MENINGOENCEPHALITIS (continued)			
Balamuthia mandrillaris			
Drug of choice:	See footnote 10		
Sappinia diploidea			
Drug of choice:	See footnote 11		
ANCYLOSTOMA caninum (Eosinophilic enterocolotis)			
Drug of choice:	Albendazole[7,12]	400 mg PO once	400 mg PO once
OR	Mebendazole	100 mg PO bid × 3 d	100 mg PO bid × 3 d
OR	Pyrantel pamoate[7,13]*	11 mg/kg (max. 1 g) PO × 3 d	11 mg/kg (max. 1 g) PO × 3 d
OR	Endoscopic removal		
Ancylostoma duodenale, see HOOKWORM			
ANGIOSTRONGYLIASIS *(Angiostrongylus cantonensis, Angiostrongylus costaricensis)*			
Drug of choice:	See footnote 14		
ANISAKIASIS (*Anisakis spp*)			
Treatment of choice:[15]	Surgical or endoscopic removal		
ASCARIASIS (*Ascaris lumbricoides,* roundworm)			
Drug of choice:[5]	Albendazole[7,12]	400 mg PO once	400 mg PO once
OR	Mebendazole	100 mg bid PO × 3 d or 500 mg once	100 mg PO bid × 3 d or 500 mg once
OR	Ivermectin[7,16]	150-200 µg/kg PO once	150-200 µg/kg PO once
BABESIOSIS *(Babesia microti)*			
Drug of choice:[17]	Clindamycin[7,18]	1.2 g bid IV or 600 mg tid PO × 7-10 d	20-40 mg/kg/d PO in 3 doses × 7-10 d
	plus quinine[7,19]	650 mg PO tid × 7-10 d	30 mg/kg/d PO in 3 doses × 7-10 d
OR	Atovaquone[7,20]	750 mg PO bid × 7-10 d	40 mg/kg/d PO in 2 doses × 7-10 d
	plus azithromycin[7]	600 mg PO daily × 7-10 d	12 mg/kg/d PO × 7-10 d
Balamuthia mandrillaris, see AMEBIC MENINGOENCEPHALITIS, PRIMARY			
BALANTIDIASIS *(Balantidium coli)*			
Drug of choice:	Tetracycline[7,21]	500 mg PO qid × 10 d	400 mg/k/d (max. 2 g) PO in 4 doses × 10 d
Alternative:	Metronidazole[7]	700 mg PO tid × 5 d	35-50 mg/kg/d PO in 3 doses × 5 d
OR	Iodoquinol[2,7]	650 mg PO tid × 20 d	30-40 mg/k/d (max. 2 g) PO in 3 doses × 20 d

9. Several patients with granulomatous amebic encephalitis (GAE) have been successfully treated with combinations of pentamidine, sulfadiazine, flucytosine, and either fluconazole or itraconazole (GS Visvesvara et al, FEMS Immunol Med Microbiol 2007; 50:1). GAE in an AIDS patient was treated successfully with sulfadiazine, pyrimethamine, and fluconazole combined with surgical resection of the CNS lesion (M Seijo Martinez et al, J Clin Microbiol 2000; 38:3892). Chronic Acanthamoeba *meningitis was successfully treated in two children with a combination of oral trimethoprim/sulfamethoxazole, rifampin, and ketoconazole (T Singhal et al, Pediatr Infect Dis J 2001; 20:623). Disseminated cutaneous infection in an immunocompromised patient was treated successfully with IV pentamidine, topical chlorhexidine, and 2% ketoconazole cream, followed by oral itraconazole (CA Slater et al, N Engl J Med 1994; 331:85) and with voriconazole and amphotericin B lipid complex (R Walia et al, Transplant Infect Dis 2007;9:51). Other reports of successful therapy have been described (FL Schuster and GS Visvesvara, Drug Resistance Updates 2004; 7:41). Susceptibility testing of* Acanthamoeba *isolates has shown differences in drug sensitivity between species and even among strains of a single species; antimicrobial susceptibility testing is advisable (FL Schuster and GS Visvesvara, Int J Parasitiol 2004;34:1001).*

10. B. mandrillaris *is a free-living ameba that causes subacute to fatal granulomatous amebic encephalitis (GAE) and cutaneous disease. Two cases of* Balamuthia *encephalitis have been successfully treated with flucytosine, pentamidine, fluconazole and sulfadiazine plus either azithromycin or clarithromycin (phenothiazines were also used) combined with surgical resection of the CNS lesion (TR Deetz et al, Clin Infect Dis 2003; 37:1304). Another case was successfully treated following open biopsy with pentamidine, fluconazole, sulfadiazine, and clarithromycin (S Jung et al, Arch Pathol Lab Med 2004; 128:466).*

11. A free-living ameba once thought not to be pathogenic to humans. S. diploidea *has been successfully treated with azithromycin, pentamidine, itraconazole, and flucytosine combined with surgical resection of the CNS lesion (BB Gelman et al, J Neuropathol Exp Neurol 2003; 62:990).*

12. Albendazole must be taken with food; a fatty meal increases oral bioavailability.

13. Pyrantel pamoate suspension can be mixed with milk or fruit juice.

14. A. cantonensis *causes predominantly neurotropic disease.* A. costaricensis *causes gastrointestinal disease. Most patients infected with either species have a self-limited course and recover completely. Analgesics, corticosteroids and careful removal of CSF at frequent intervals can relieve symptoms from increased intracranial pressure (V Lo Re III and SJ Gluckman, Am J Med 2003; 114:217). Treatment for* A. cantonensis *is controversial and varies across endemic areas. No antihelminthic drug is proven to be effective and some patients have worsened with therapy (TJ Slom et al, N Engl J Med 2002; 346:668). Mebendazole and a corticosteroid, however, appear to shorten the course of infection (H-C Tsai et al, Am J Med 2001; 111:109; V Chotmongkol et al, Am J Trop Med Hyg 2006; 74:1122). Albendazole has also relieved symptoms of angiostrongyliasis (XG Chen et al, Emerg Infect Dis 2005; 11:1645).*

15. A Repiso Ortega et al, Gastroenterol Hepatol 2003; 26:341. Successful treatment of Anisakiasis *with albendazole 400 mg PO bid × 3-5 d has been reported, but the diagnosis was presumptive (DA Moore et al, Lancet 2002; 360:54; E Pacios et al, Clin Infect Dis 2005; 41:1825).*

16. Safety of ivermectin in young children (<15 kg) and pregnant women remains to be established. Ivermectin should be taken on an empty stomach with water.

17. Exchange transfusion has been used in severely ill patients and those with high (>10%) parasitemia (VI Powell and K Grima, Transfus Med Rev 2002; 16:239). In patients who were not severely ill, combination therapy with atovaquone and azithromycin was as effective as clindamycin and quinine and may have been better tolerated (PJ Krause et al, N Engl J Med 2000; 343:1454). Longer treatment courses may be needed in immunosuppressed patients and those with asplenia. Patients are commonly co-infected with Lyme disease (Med Lett Drugs Ther 2007; 49:49; AC Steere et al, Clin Infect Dis 2003; 36:1078).

18. Oral clindamycin should be taken with a full glass of water to minimize esophageal ulceration.

19. Quinine should be taken with or after a meal to decrease gastrointestinal adverse effects.

20. Atovaquone is available in an oral suspension that should be taken with a meal to increase absorption.

21. Use of tetracyclines is contraindicated in pregnancy and in children <8 years old. Tetracycline should be taken 1 hour before or 2 hours after meals and/or dairy products.

Infection	Drug	Adult Dosage	Pediatric Dosage
BAYLISASCARIASIS *(Baylisascaris procyonis)*			
Drug of choice:	See footnote 22		
BLASTOCYSTIS *hominis* infection			
Drug of choice:	See footnote 23		
CAPILLARIASIS *(Capillaria philippinensis)*			
Drug of choice:	Metronidazole[7]	200 mg PO bid × 20 d	200 mg PO bid × 20 d
Alternative:	Albendazole[7,12]	400 mg PO daily × 10 d	400 mg PO daily × 10 d
Chagas disease, see TRYPANOSOMIASIS			
Clonorchis sinensis, see FLUKE infection			
CRYPTOSPORIDIOSIS *(Cryptosporidium)*			
Non-HIV infected			
Drug of choice:	Nitazoxanide[5]	500 mg PO bid × 3 d	1-3 yr: 100 mg PO bid × 3 d 4-11 yr: 200 mg PO bid × 3 d >12 yr: 500 mg PO q12h × 3 d
HIV infected			
Drug of choice:	See footnote 24		
CUTANEOUS LARVA MIGRANS (creeping eruption, dog and cat hookworm)			
Drug of choice:[25]	Albendazole[7,12]	400 mg PO daily × 3 d	400 mg PO daily × 3 d
OR	Ivermectin[7,16]	200 μg/kg PO daily × 1-2 d	200 μg/kg PO daily × 1-2 d
CYCLOSPORIASIS *(Cyclospora cayetanensis)*			
Drug of choice:[26]	Trimethoprim/sulfamethoxazole[7]	TMP 160 mg/SMX 800 mg (1 DS tab) PO bid × 7-10 d	TMP 5 mg/kg/SMX 25 mg/kg/d PO in 2 doses × 7-10 d
CYSTICERCOSIS, see TAPEWORM infection			
DIENTAMOEBA fragilis infection[27]			
Drug of choice:	Iodoquinol[2,7]	650 mg PO tid × 20 d	30-40 mg/kg/d (max. 2 g) PO in 3 doses × 20 d
OR	Paromomycin[3,7]	25-35 mg/kg/d PO in 3 doses × 7 d	25-35 mg/kg/d PO in 3 doses × 7 d
OR	Tetracycline[7,21]	500 mg PO qid × 10 d	40 mg/kg/d (max. 2 g) PO in 4 doses × 10 d
OR	Metronidazole[7]	500-750 mg PO tid × 10 d	35-50 mg/kg/d PO in 3 doses × 10 d
Diphyllobothrium latum, see TAPEWORM infection			
DRACUNCULUS medinensis (guinea worm) infection			
Drug of choice:	See footnote 28		
Echinococcus, see TAPEWORM infection			
Entamoeba histolytica, see AMEBIASIS			
ENTEROBIUS *vermicularis* (pinworm) infection			
Drug of choice:[29]	Mebendazole	100 mg PO once; repeat in 2 wk	100 mg PO once; repeat in 2 wk
OR	Pyrantel pamoate[13]*	11 mg/kg base PO once (max. 1 g); repeat in 2 wk	11 mg/kg base PO once (max. 1 g); repeat in 2 wk
OR	Albendazole[7,12]	400 mg PO once; repeat in 2 wk	400 mg PO once; repeat in 2 wk
Fasciola hepatica, see FLUKE infection			
FILARIASIS[30]			
Wuchereria bancrofti, Brugia malayi, Brugia timori			
Drug of choice:[31]	Diethylcarbamazine*	6 mg/kg/d PO in 3 doses × 12d[32,33]	6 mg/kg/d PO in 3 doses × 12d[32,33]

22. No drug has been demonstrated to be effective. Albendazole 25 mg/kg/d PO × 20 d started as soon as possible (up to 3 d after possible infection) might prevent clinical disease and is recommended for children with known exposure (ingestion of raccoon stool or contaminated soil) (WJ Murray and KR Kazacos, Clin Infect Dis 2004; 39:1484). Mebendazole, levamisole, or ivermectin could be tried if albendazole is not available. Steroid therapy may be helpful, especially in eye and CNS infections (PJ Gavin et al, Clin Microbiol Rev 2005; 18:703). Ocular baylisascariasis has been treated successfully using laser photocoagulation therapy to destroy the intraretinal larvae (CA Garcia et al, Eye 2004; 18:624).

23. Clinical significance of these organisms is controversial; metronidazole 750 mg PO tid × 10 d, iodoquinol 650 mg PO tid × 20 d, or trimethoprim/sulfamethoxazole 1 DS tab PO bid × 7 d have been reported to be effective (DJ Stenzel and PFL Borenam, Clin Microbiol Rev 1996; 9:563; UZ Ok et al, Am J Gastroenterol 1999; 94:3245). Metronidazole resistance may be common in some areas (K Haresh et al, Trop Med Int Health 1999; 4:274). Nitazoxanide has been effective in clearing organism and improving symptoms (E Diaz et al, Am J Trop Med Hyg 2003; 68:384; JF Rossignol, Clin Gastroenterol Hepatol 2005; 18:703).

24. No drug has proven efficacy against cryptosporidiosis in advanced AIDS (I Abubakar et al, Cochrane Database Syst Rev 2007; 1:CD004932). Treatment with HAART is the mainstay of therapy. Nitazoxanide (JF Rossignol, Aliment Pharmacol Ther 2006; 24:807), paromomycin (P Maggi et al, Clin Infect Dis 2000; 33:1609), or a combination of paromomycin and azithromycin (NH Smith et al, J Infect Dis 1998; 178:900) may be tried to decrease diarrhea and recalcitrant malabsorption of antimicrobial drugs, which can occur with chronic cryptosporidiosis.

25. G Albanese et al, Int J Dermatol 2001; 40:67; D Malvy et al, J Travel Med 2006; 13:244.

26. HIV-infected patients may need higher dosage and long-term maintenance. Successful use of nitazoxanide (see also footnote 5) has been reported in one patient with sulfa allergy (SM Zimmer et al, Clin Infect Dis 2007; 44:466).

27. A Norberg et al, Clin Microbiol Infect 2003; 9:65; O Vandenberg et al, Int J Infect Dis 2006; 10:255.

28. No drug is curative against Dracunculus. *A program for monitoring local sources of drinking water to eliminate transmission has dramatically decreased the number of cases worldwide (M Barry, N Engl J Med 2007; 356:2561). The treatment of choice is slow extraction of worm combined with would care and pain management (C Greenaway, Can Med Assoc J 2004; 170:495).*

29. Since family members are usually infected, treatment of the entire household is recommended.

30. Antihistamines or corticosteroids may be required to decrease allergic reactions to components of disintegrating microfilariae that result from treatment, especially in infection caused by Loa loa. *Endosymbiotic* Wolbachia *bacteria may have a role in filarial development and host response, and may represent a potential target for therapy. Addition of doxycycline 100 or 200 mg/d PO × 6-8 wk in lymphatic filariasis and onchocerciasis has resulted in substantial loss of* Wolbachia *and decrease in both micro- and macrofilariae (MJ Taylor et al, Lancet 2005; 365:2116; AY Debrah et al, PLOS Pathog 2006; e92:0829); but use of tetracyclines is contraindicated in pregnancy and in children <8 yr old.*

Infection	Drug	Adult Dosage	Pediatric Dosage
Loa loa			
Drug of choice:[34]	Diethylcarbamazine*	6 mg/kg/d PO in 3 doses × 12d[32,33]	6 mg/kg/d PO in 3 doses × 12d[32,33]
FILARIASIS[30]			
Mansonella ozzardi			
Drug of choice:	See footnote 35		
Mansonella perstans			
Drug of choice:	Albendazole[7,12]	400 mg PO bid × 10 d	400 mg PO bid × 10 d
OR	Mebendazole[7]	100 mg PO bid × 30 d	100 mg PO bid × 30 d
Mansonella streptocerca			
Drug of choice:[36]	Diethylcarbamazine*	6 mg/kg/d PO × 12 d[33]	6 mg/kg/d PO × 12 d[33]
OR	Ivermectin[7,16]	150 mcg/kg PO once	150 mcg/kg PO once
Tropical Pulmonary Eosinophilia (TPE)[37]			
Drug of choice:	Diethylcarbamazine*	6 mg/kg/d in 3 doses × 12-21 d[33]	6 mg/kg/d in 3 doses × 12-21 d[33]
Onchocerca volvulus (River blindness)			
Drug of choice:	Ivermectin[16,38]	150 mcg/kg PO once, repeated every 6-12 mo until asymptomatic	150 mcg/kg PO once, repeated every 6-12 mo until asymptomatic
FLUKE, hermaphroditic, infection			
Clonorchis sinensis (Chinese liver fluke)			
Drug of choice:	Praziquantel[39]	75 mg/kg/d in 3 doses × 2 d	75 mg/kg/d in 3 doses × 2 d
OR	Albendazole[7,12]	10 mg/kg/d PO × 7 d	10 mg/kg/d PO × 7 d
Fasciola hepatica (sheep liver fluke)			
Drug of choice:[40]	Triclabendazole*	10 mg/kg/d PO once or twice[41]	10 mg/kg/d PO once or twice[41]
Alternative:	Bithionol*	30-50 mg/kg on alternate days × 10-15 doses	30-50 mg/kg on alternate days × 10-15 doses
OR	Nitazoxanide[5,7]	500 mg PO bid × 7 d	1-3 yr: 100 mg PO q12h × 7 d 4-11 yr: 200 mg PO q12h × 7 d >12 yr: 500 mg PO q12h × 7 d
Fasciolopsis buski, Heterophyes heterophyes, Metagonimus yokogawai (intestinal flukes)			
Drug of choice:	Praziquantel[7,39]	75 mg/kg/d PO in 3 doses × 1 d	75 mg/kg/d PO in 3 doses × 1 d
Metorchis conjunctus (North American liver fluke)			
Drug of choice:	Praziquantel[7,39]	75 mg/kg/d PO in 3 doses × 1 d	75 mg/kg/d PO in 3 doses × 1 d
Nanophyetus salmincola			
Drug of choice:	Praziquantel[7,39]	60 mg/kg/d PO in 3 doses × 1 d	60 mg/kg/d PO in 3 doses × 1 d
Opisthorchis viverrini (Southeast Asian liver fluke)			
Drug of choice:	Praziquantel[39]	75 mg/kg/d PO in 3 doses × 2 d	75 mg/kg/d PO in 3 doses × 2 d
Paragonimus westermani (lung fluke)			
Drug of choice:	Praziquantel[7,39]	75 mg/kg/d PO in 3 doses × 2 d	75 mg/kg/d PO in 3 doses × 2 d
Alternative:[42]	Bithionol*	30-50 mg/kg on alternate days × 10-15 doses	30-50 mg/kg on alternate days × 10-15 doses

31. Most symptoms are caused by adult worm. A single-dose combination of albendazole (400 mg PO) with either ivermectin (200 mcg/kg PO) or diethylcarbamazine (6 mg/kg PO) is effective for reduction or suppression of W. bancrofti *microfilaria, but the albendazole/ivermectin combination does not kill all the adult worms (D Addiss et al, Cochrane Database Syst Rev 2004; CD003753).*

32. For patients with microfilaria in the blood, Medical Letter consultants start with a lower dosage and scale up: d1: 50 mg; d2: 50 mg tid; d3: 100 mg tid; d4-14: 6 mg/kg in 3 doses (for Loa Loa *d4-14: 9 mg/kg in 3 doses). Multidose regimens have been shown to provide more rapid reduction in microfilaria than single-dose diethylcarbamazine, but microfilaria levels are similar 6-12 months after treatment (LD Andrade et al, Trans R Soc Trop Med Hyg 1995; 89:319; PE Simonsen et al, Am J Trop Med Hyg 1995; 53:267). A single dose of 6 mg/kg is used in endemic areas for mass treatment (J Figueredo-Silva et al, Trans R Soc Trop Med Hyg 1996; 90:192; J Noroes et al, Trans R Soc Trop Med Hyg 1997; 91:78).*

33. Diethylcarbamazine should not be used for treatment of Onchocerca volvulus *due to the risk of increased ocular side effects, including blindness associated with rapid killing of the worms. It should be used cautiously in geographic regions where* O. volvulus *coexists with other filariae. Diethylcarbamazine is contraindicated during pregnancy. See also footnote 38.*

34. In heavy infections with Loa loa, *rapid killing of microfilariae can provoke encephalopathy. Apheresis has been reported to be effective in lowering microfilarial counts in patients heavily infected with* Loa loa *(EA Ottesen, Infect Dis Clin North Am 1993; 7:619). Albendazole may be useful for treatment of loiasis when diethylcarbamazine is ineffective or cannot be used, but repeated courses may be necessary (AD Klion et al, Clin Infect Dis 1999; 29:680; TE Tabi et al, Am J Trop Med Hyg 2004; 71:211). Ivermectin has also been used to reduce microfilaremia, but albendazole is preferred because of its slower onset of action and lower risk of precipitating encephalopathy (AD Klion et al, J Infect Dis 1993; 168:202; M Kombila et al, Am J Trop Med Hyg 1998; 58:458). Diethylcarbamazine, 300 mg PO once/wk, has been recommended for prevention of loiasis (TB Nutman et al, N Engl J Med 1988; 319:752).*

35. Diethylcarbamazine has no effect. A single dose of ivermectin 200 mcg/kg PO reduces microfilaria densities and provides both short- and long-term reductions in M. ozzardi *microfilaremia (AA Gonzalez et al, W Indian Med J 1999; 48:231).*

36. Diethylcarbamazine is potentially curative due to activity against both adult worms and microfilariae. Ivermectin is active only against microfilariae.

37. AK Boggild et al, Clin Infect Dis 2004; 39:1123. Relapses occur and can be treated with a repeated course of diethylcarbamazine.

38. Diethylcarbamazine should not be used for treatment of this disease because rapid killing of the worms can lead to blindness. Periodic treatment with ivermectin (every 3-12 months), 150 mcg/kg PO, can prevent blindness due to ocular onchocerciasis (DN Udall, Clin Infect Dis 2007; 44:53). Skin reactions after ivermectin treatment are often reported in persons with high microfilarial skin densities. Ivermectin has been inadvertently given to pregnant women during mass treatment programs; the rates of congenital abnormalities were similar in treated and untreated women. Because of the high risk of blindness from onchocerciasis, the use of ivermectin after the first trimester is considered acceptable according to the WHO. Doxycycline (100 mg/day PO for 6 weeks), followed by a single 150 μg/kg PO dose of ivermectin, resulted in up to 19 months of amicrofilaridermia and 100% elimination of Wolbachia *species (A Hoerauf et al, Lancet 2001; 357:1415).*

39. Praziquantel should be taken with liquids during a meal.

40. Unlike infections with other flukes, Fasciola hepatica *infections may not respond to praziquantel. Triclabendazole (*Egaten*; Novartis) appears to be safe and effective, but data are limited (DY Aksoy et al, Clin Microbiol Infect 2005; 11:859). It is available from Victoria Pharmacy, Zurich, Switzerland (www.pharmaworld.com; 41-1-211-24-32) and should be given with food for better absorption. Nitazoxanide also appears to have efficacy in treating fasciolosis in adults and in children (L Favennec et al, Aliment Pharmacol Ther 2003; 17:265; JF Rossignol et al, Trans R Soc Trop Med Hyg 1998; 92:103; SM Kabil et al, Curr Ther Res 2000; 61:339).*

41. J Keiser et al, Expert Opin Investig Drugs 2005; 14:1513.

42. Triclabendazole may be effective in a dosage of 5 mg/kg PO once/d × 3 d or 10 mg/kg PO bid × 1 d (M Calvopiña et al, Trans R Soc Trop Med Hyg 1998; 92:566). See footnote 40 for availability.

Infection		Drug	Adult Dosage	Pediatric Dosage
GIARDIASIS *(Giardia duodenalis)*				
Drug of choice:		Metronidazole[7]	250 mg PO tid × 5-7 d	15 mg/kg/d PO in 3 doses × 5-7 d
	OR	Tinidazole[6]	2 g PO once	50 mg/kg PO once (max 2 g)
	OR	Nitazoxanide[5]	500 mg PO tid × 3 d	1-3 yr: 100 mg PO q12h × 3 d 4-11 yr: 200 mg PO q12h × 3 d >12 yr: 500 mg PO q12h × 3 d
Alternative:[43]		Paromomycin[3,7,44]	25-35 mg/kg/d PO in 3 doses × 5-10 d	25-35 mg/kg/d PO in 3 doses × 5-10 d
	OR	Furazolidone*	100 mg PO qid × 7-10 d	6 mg/kg/d PO in 4 doses × 7-10 d
	OR	Quinacrine[4,45]*	100 mg PO tid × 5 d	2 mg/kg/d PO in 3 doses × 5 d (max 300 mg/d)
GNATHOSTOMIASIS *(Gnathostoma spinigerum)*[46]				
Treatment of choice:		Albendazole[7,12]	400 mg PO bid × 21 d	400 mg PO bid × 21 d
	OR	Ivermectin[7,16] either	200 mcg/kg/d PO × 2 d	200 mcg/kg/d PO × 2 d
	±	Surgical removal		
GONGYLONEMIASIS *(Gongylonema* sp*)*[47]				
Treatment of choice:		Surgical removal		
	OR	Albendazole[7,12]	400 mg/d PO × 3 d	400 mg/d PO × 3 d
HOOKWORM infection *(Ancylostoma duodenale, Necator americanus)*				
Drug of choice:		Albendazole[7,12]	400 mg PO once	400 mg PO once
	OR	Mebendazole	100 mg PO bid × 3 d or 500 mg once	100 mg PO bid × 3 d or 500 mg once
	OR	Pyrantel pamoate[7,13]*	11 mg/kg (max 1 g) PO × 3 d	11 mg/kg (max 1 g) PO × 3 d
Hydatid cyst, see TAPEWORM infection				
Hymenolepis nana, see TAPEWORM infection				
ISOSPORIASIS *(Isospora belli)*				
Drug of choice:[48]		Trimethoprim-sulfamethoxazole[7]	TMP 160 mg/SMX 800 mg (1 DS tab) PO bid × 10 d	TMP 5 mg/kg/d/SMX 25 mg/kg/d PO in 2 doses × 10 d
LEISHMANIA				
Visceral[49,50]				
Drug of choice:		Liposomal amphotericin B[51]	3 mg/kg/d IV d 1-5, 14 and 21[52]	3 mg/kg/d IV d 1-5, 14, and 21[52]
	OR	Sodium stibogluconate*	20 mg Sb/kg/d IV or IM × 28 d	20 mg Sb/kg/d IV or IM × 28 d
	OR	Miltefosine[53]*	2.5 mg/kg/d PO (max 150 mg/d) × 28 d	2.5 mg/kg/d PO (max 150 mg/d) × 28 d

43. Another alternative is albendazole 400 mg/d PO × 5 d in adults and 10 mg/kg/d PO × 5 d in children (K Yereli et al, Clin Microbiol Infect 2004;10:527; O Karabay et al, World J Gastroenterol 2004; 10:1215). Combination treatment with standard doses of metronidazole and quinacrine × 3 wk has been effective for a small number of refractory infections (TE Nash et al, Clin Infect Dis 2001; 33:22). In one study, nitazoxanide was used successfully in high doses to treat a case of Giardia *resistant to metronidazole and albendazole (P Abboud et al, Clin Infect Dis 2001; 32:1792).*

44. Poorly absorbed; may be useful for treatment of giardiasis in pregnancy.

45. Quinacrine should be taken with liquids after a meal.

46. P Nontasut et al, Southeast Asian J Trop Med Pub Health 2005; 36:650; M de Gorgolas et al, J Travel Med 2003; 10:358. All patients should be treated with medication whether surgery is attempted or not.

47. ME Wilson et al, Clin Infect Dis 2001; 32:1378; G Molavi et al, J Helminth 2006; 80:425.

48. Usually a self-limited illness in immunocompetent patients. Immunosuppressed patients may need higher doses, longer duration (TMP/SMX qid × 10 d, followed by bid × 3 wk) and long-term maintenance. In sulfonamide-sensitive patients, pyrimethamine 50-75 mg daily in divided doses (plus leucovorin 10-25 mg/d) has been effective.

49. To maximize effectiveness and minimize toxicity, the choice of drug, dosage, and duration of therapy should be individualized based on the region of disease acquisition, a likely infecting species, and host factors such as immune status (BL Herwaldt, Lancet 1999; 354:1191). Some of the listed drugs and regimens are effective only against certain Leishmania *species/strains and only in certain areas of the world (J Arevalo et al, Clin Infect Dis 2007; 195:1846). Medical Letter consultants recommend consultation with physicians experienced in management of this disease.*

50. Visceral infection is most commonly due to the Old World species L. donovani *(kala-azar) and* L. infantum *and the New World species* L. chagasi.

*51. Liposomal amphotericin B (*AmBisome*) is the only lipid formulation of amphotericin B FDA-approved for treatmetn of visceral leishmania, largely based on clinical trials in patients infected with* L. infantum *(A Meyerhoff, Clin Infect Dis 1999; 28:42). Two other amphotericin B lipid formulations, amphotericin B lipid complex (*Abelcet*) and amphotericin B cholesteryl sulfate (*Amphotec*) have been used, but are considered investigational for this condition and may not be as effective (C Bern et al, Clin Infect Dis 2006; 43:917).*

52. The FDA-approved dosage regimen for immunocompromised patients (e.g., HIV infected) is 4 mg/kg/d IV on days 1-5, 10, 17, 24, 31 and 38. The relapse rate is high; maintenance therapy (secondary prevention) may be indicated, but there is no consensus as to dosage or duration.

53. Effective for both antimony-sensitive and -resistant L. donovani *(Indian); miltefosine (*Impavido*) is manufactured in 10- or 50-mg capsules by Zentaris (Frankfurt, Germany at info@zentaris.com) and is available through consultation with the CDC. The drug is contraindicated in pregnancy; a negative pregnancy test before drug initiation and effective contraception during and for 2 months after treatment is recommended (H Murray et al, Lancet 2005; 366:1561). In a placebo-controlled trial in patients ≥12 years old, oral miltefosine 2.5 mg/kg/d × 28 d was also effective for treatment of cutaneous leishmaniasis due to* L. (V.) panamensis *in Colombia, but not* L. (V.) braziliensis *or* L. mexicana *in Guatemala (J Soto et al, Clin Infect Dis 2004; 38:1266). "Motion sickness," nausea, headache, and increased creatinine are the most frequent adverse effects (J Soto and P Soto, Expert Rev Anti Infect Ther 2006; 4:177).*

Infection		Drug	Adult Dosage	Pediatric Dosage
Alternative:		Meglumine antimonate*	20 mg Sb/kg/d IV or IM × 28 d	20 mg Sb/kg/d IV or IM × 28 d
	OR	Amphotericin B[7]	1 mg/kg IV daily × 15-20 d or every second day for up to 8 wk	1 mg/kg IV daily × 15-20 d or every second day for up to 8 wk
	OR	Paromomycin[7,13,54]*	15 mg/kg/d IM × 21 d	15 mg/kg/d IM × 21 d
Cutaneous[49,55]				
Drug of choice:		Sodium stibogluconate*	20 mg Sb/kg/d IV or IM × 20 d	20 mg Sb/kg/d IV or IM × 20 d
	OR	Meglumine antimonate*	20 mg Sb/kg/d IV or IM × 20 d	20 mg Sb/kg/d IV or IM × 20 d
	OR	Miltefosine[53]*	2.5 mg/kg/d PO (max 150 mg/d) × 28 d	2.5 mg/kg/d PO (max 150 mg/d) × 28 d
Alternative:[56]		Paromomycin[7,13,54]*	Topically 2×/d × 10-20 d	Topically 2×/d × 10-20 d
	OR	Pentamidine[7]	2-3 mg/kg/IV or IM daily or every second day × 4-7 doses[57]	2-3 mg/kg/IV or IM daily or every second day × 4-7 doses[57]
Mucosal[49,58]				
Drug of choice:		Sodium stibogluconate*	20 mg Sb/kg/d IV or IM × 28 d	20 mg Sb/kg/d IV or IM × 28 d
	OR	Meglumine antimonate*	20 mg Sb/kg/d IV or IM × 28 d	20 mg Sb/kg/d IV or IM × 28 d
	OR	Amphotericin B[7]	0.5-1 mg/kg IV daily or every second day for up to 8 wk	0.5-1 mg/kg IV daily or every second day for up to 8 wk
	OR	Miltefosine[53]*	2.5 mg/kg/d PO (max 150 mg/d) × 28 d	2.5 mg/kg/d PO (max 150 mg/d) × 28 d
LICE infestation *(Pediculus humanus, P. capitis, Phthirus pubis)*[59]				
Drug of choice:		0.5% Malathion[60]	Topically	Topically
	OR	1% Permethrin[61]	Topically	Topically
Alternative:		Pyrethrins with piperonyl butoxide[61]	Topically	Topically
	OR	Ivermectin[7,16,62]	200 µg/kg PO	≥15 kg: 200 µg/kg PO

54. *Paromomycin IM has been effective against leishmania in India; it has not yet been tested in South America or the Mediterranean and there are insufficient data to support its use in pregnancy (S Sundar et al, N Engl J Med 2007; 356:2371). Topical paromomycin should be used only in geographic regions where cutaneous leishmaniasis species have low potential for mucosal spread. A formulation of 15% paromomycin/12% methylbenzethonium chloride* (Leshcutan*) in soft white paraffin for topical use has been reported to be partially effective against cutaneous leishmaniasis due to* L. major *in Israel and* L. mexicana *and* L. *(V.)* braziliensis *in Guatemala, where mucosal spread is very rare (BA Arana et al, Am J Trop Med Hyg 2001; 65:466). The methylbenzethonium is irritating to the skin; lesions may worsen before they improve.*

55. *Cutaneous infection is most commonly due to the Old World species* L. major *and* L. tropica *and the New World species* L. mexicana, L. (Viannia) braziliensis, *and others.*

56. *Although azole drugs (fluconazole, ketoconazole, itraconazole) have been used to treat cutaneous disease, they are not reliably effective and have no efficacy against mucosal disease (AJ Magill, Infect Dis Clin North Am 2005; 19:241). For treatment of* L. major *cutaneous lesions, a study in Saudi Arabia found that cral fluconazole, 200 mg once/d × 6 wk appeared to speed healing (AA Alrajhi et al, N Engl J Med 2002; 346:891). Thermotherapy may be an option for cutaneous* L. tropica *infection (R Reithinger et al, Clin Infect Dis 2005; 40: 1148). A device that generates focused and controlled heating of the skin has been approved by the FDA for this indication (*ThermoMed-*ThermoSurgery Technologies Inc., Phoenix, AZ, 602-264-7300; www.thermosurgery.com).*

57. *At this dosage pentamidine has been effective in Colombia predominantly against* L. (V.) panamensis *(J Soto-Mancipe et al, Clin Infect Dis 1993; 16: 417; J. Soto et al, Am J Trop Med Hyg 1994; 50:107). Activity against other species is not well established.*

58. *Mucosal infection is most commonly due to the New World species* L. (V.) braziliensis, L. (V.) panamensis, or L. (V.) guyanensis.

59. *Pediculocides should not be used for infestations of the eyelashes. Such infestations are treated with petrolatum ointment applied 2-4 daily/d × 8-10 d. Oral TMP/SMX has also been used (TL Meinking and D Taplin, Curr Probl Dermatol 1996; 24:157). For pubic lice, treat with 5% permethrin or ivermectin as for scabies. TMP/SMX has also been effective when used together with permethrin for head lice (RB Hipolito et al, Pediatrics 2001; 107:E30).*

60. *Malathion is both ovicidal and pediculocidal; 2 applications at least 7 days apart are generally necessary to kill all lice and nits.*

61. *Permethrin and pyrethrin are pediculocidal; retreatment in 7-10 d is needed to eradicate the infestation. Some lice are resistant to pyrethrins and permethrin (TL Meinking et al, Arch Dermatol 2002; 138:220).*

62. *Ivermectin is pediculocidal, but more than one dose is generally necessary to eradicate the infestation (KN Jones and JC English 3rd, Clin Infect Dis 2003; 36:1355). The number of doses and interval between doses has not been established, but in one study of body lice, 3 doses administered at 7-day intervals were effective (C Fouault et al, J Infect Dis 2006; 193:474).*

63. *Chloroquine-resistant* P. falciparum *occurs in all malarious areas except Central America (including Panama north and west of the Canal Zone), Mexico, Haiti, the Dominican Republic, Paraguay, northern Argentina, North and South Korea, Georgia, Armenia, most of rural China, and some countries in the Middle East (chloroquine resistance has been reported in Yemen, Oman, Saudi Arabia, and Iran). For treatment of multiple-drug-resistant* P. falciparum *in Southeast Asia, especially Thailand, where mefloquine resistance is frequent, atovaquone/proguanil, quinine plus either doxycycline or clindamycin, or artemether/lumefantrine may be used.*

64. P. vivax *with decreased susceptibility to chloroquine is a significant problem in Papua New Guinea and Indonesia. There are also a few reports of resistance from Myanmar, India, the Solomon Islands, Vanuatu, Guyana, Brazil, Colombia, and Peru (JK Baird et al, Curr Infect Dis Rep 2007; 9:39).*

65. *Chloroquine-resistant* P. malariae *has been reported from Sumatra (JD Maguire et al, Lancet 2002; 360:58).*

Infection		Drug	Adult Dosage	Pediatric Dosage
Loa loa, see FILARIASIS				
MALARIA, Treatment of *(Plasmodium falciparum,*[63] *P. vivax,*[64] *P. ovale, and P. malariae*[65]*)*				
ORAL[66]				
P. falciparum or unidentified species acquired in areas of chloroquine-resistant *P. falciparum*[63]				
Drug of choice:[67]		Atovaquone/ proguanil[68]	2 adult tabs bid[69] or 4 adult tabs once/d × 3 d	<5 kg: not indicated 5-8 kg: 2 peds tabs once/d × 3 d 9-10 kg: 3 peds tabs once/d × 3 d 11-20 kg: 1 adult tab once/d × 3 d 21-30 kg: 2 adult tabs once/d × 3 d 31-40 kg: 3 adult tabs once/d × 3 d >40 kg: 4 adult tabs once/d × 3 d
	OR	Quinine sulfate **plus**	650 mg q8h × **or** 7 d[70]	30 mg/kg/d in 3 doses × 3 **or** 7 d[70]
		doxycycline[7,21,71] **or plus**	100 mg bid × 7 d	4 mg/kg/d in 2 doses × 7 d
		tetracycline[7,21] **or plus**	250 mg qid × 7 d	6.25 mg/kg/d in 4 doses × 7 d
		clindamycin[7,18,72]	20 mg/kg/d in 3 doses × 7 d[73]	20 mg/kg/d in 3 doses × 7 d
Alternative:[67]		Mefloquine[74,75]	750 mg followed 12 hr later by 500 mg	15 mg followed 12 hr later by 100 mg
	OR	Artemether/ lumefantrine[76,77]*	6 doses over 3 d (4 tabs/dose at 0, 8, 24, 36, 48, and 60 hours)	6 doses over 3 d at same intervals as adults; <15 kg: 1 tab/dose 15-25 kg: 2 tabs/dose 25-35 kg: 3 tabs/dose >35 kg: 4 tabs/dose
	OR	Artesunate[76]* **plus** see footnote 78	4 mg/kg/d × 3 d	4 mg/kg/d × 3 d
P. vivax acquired in areas of chloroquine-resistant *P. vivax*[64]				
Drug of choice:[67]		Mefloquine[74]	750 mg PO followed 12 hr later by 500 mg	15 mg/kg PO followed 12 hr later by 10 mg/kg
	OR	Atovaquone/ proguanil[68]	2 adult tabs bid[69] or 4 adult tabs once/d × 3 d	<5 kg: not indicated 5-8 kg: 2 peds tabs once/d × 3 d 9-10 kg: 3 peds tabs once/d × 3 d 11-20 kg: 1 adult tab once/d × 3 d 21-30 kg: 2 adult tabs once/d × 3 d 31-40 kg: 3 adult tabs once/d × 3 d >40 kg: 4 adult tabs once/d × 3 d

66. *Uncomplicated or mild malaria may be treated with oral drugs. Severe malaria (e.g., impaired consciousness, parasitemia >5%, shock) should be treated with parenteral drugs (KS Griffin et al, JAMA 2007; 297:2264).*

67. *Primaquine is given for prevention of relapse after infection with* P. vivax *or* P. ovale. *Some experts also prescribe primaquine phosphate 30 mg base/d (0.6 mg base/kg/d for children) for 14 d after departure from areas where these species are endemic (Presumptive Anti-Relapse Therapy [PART]), "terminal prophylaxis"). Because this is not always effective as prophylaxis (E Schwartz et al, N Engl J Med 2003; 349:1510), others prefer to rely on surveillance to detect cases when they occur, particularly when exposure was limited or doubtful. See also footnote 79.*

68. *Atovaquone/proguanil is available as a fixed-dose combination tablet: adult tablets (*Malarone*; 250 mg atovaquone/100 mg proguanil) and pediatric tablets (*Malarone Pediatric*; 62.5 mg atovaquone/25 mg proguanil). To enhance absorption and reduce nausea and vomiting, it should be taken with food or a milky drink. Safety in pregnancy is unknown; outcomes were normal in 24 women treated with the combination in the 2nd and 3rd trimester (R McGready et al, Eur J Clin Pharmacol 2003; 59:545). The drug should not be given to patients with severe renal impairment (creatinine clearancxe <30 mL/min). There have been isolated case reports of resistance in* P. falciparum *in Africa, but Medical Letter consultants do not believe there is a high risk for acquisition of* Malarone*-resistant disease (E Schwartz et al, Clin Infect Dis 2003; 37:450; A Farnert et al, BMJ 2003; 326:628; S Kuhn et al, Am J Trop Med Hyg 2005; 72:407; CT Happi et al, Malaria Journal 2006; 5:82).*

69. *Although approved for once-daily dosing, Medical Letter consultants usually divide the dose in two to decrease nausea and vomiting.*

70. *Available in the United States in a 324-mg capsule; 2 capsules suffice for adult dosage. In Southeast Asia, relative resistance to quinine has increased and treatment should be continued for 7 d. Quinine should be taken with or after meals to decrease gastrointestinal adverse effects.*

71. *Doxycycline should be taken with adequate water to avoid esophageal irritation. It can be taken with food to minimize gastrointestinal adverse effects.*

72. *For use in pregnancy and in children <8 yr.*

73. *B Lell and PG Kremsner, Antimicrob Agents Chemother 2002; 46:2315; M Ramharter et al, Clin Infect Dis 2005; 40:1777.*

74. *At this dosage, adverse effects include nausea, vomiting, diarrhea and dizziness. Disturbed sense of balance, toxic psychosis, and seizures can also occur. Mefloquine should not be used for treatment of malaria in pregnancy unless there is no other treatment option because of increased risk for stillbirth (F Nosten et al, Clin Infect Dis 1999; 28:808). It should be avoided for treatment of malaria in persons with active depression or with a history of psychosis or seizures and should be used with caution in persons with any psychiatric illness. Mefloquine can be given to patients taking β-blockers if they do not have an underlying arrhythmia; it should not be used in patients with conduction abnormalities. Mefloquines should not be given together with quinine or quinidine, and caution is required in using quinine or quinidine to treat patients with malaria who have taken mefloquine for prophylaxis. Mefloquine should not be taken on an empty stomach; it should be taken with at least 8 oz of water.*

75. P. falciparum *with resistance to mefloquine is a significant problem in the malarious areas of Thailand and in areas of Myanmar and Cambodia that border on Thailand. It has also been reported on the borders between Myanmar and China, Laos and Myanmar, and in Southern Vietnam. In the United States, a 250-mg tablet of mefloquine contains 228 mg mefloquine base. Outside the United States, each 275-mg tablet contains 250 mg base.*

76. *The artemisinin derivatives, artemether and artesunate, are both frequently used globally in combination regimens to treat malaria. Both are available in oral, parenteral and rectal formulations, but manufacturing standards are not consistent (HA Karunajeewa et al, JAMA 2007; 297:2381; EA Ashley and NJ White, Curr Opin Infect Dis 2005; 18:531). In the United States, only the IV formulation of artesunate is available; it can be obtained through the CDC under an IND for patients with severe disease who do not have timely access, cannot tolerate, or fail to respond to IV quinidine (www.cdc.gov/malaria/features/artesunate_now_available.htm). To avoid development of resistance, monotherapy should be avoided (PE Duffy and CH Sibley, Lancet 2005; 366:1908). In animal studies artemisinins have been embryotoxic and caused a low incidence of teratogenicity; no adverse pregnancy outcome has been observed in limited studies in humans (S Dellicour et al, Malaria J 2007; 6:15).*

77. *Artemether/lumefantrine is available as a fixed-dose combination tablet (*Coartem *in countries with endemic malaria,* Riamet *in Europe and countries without endemic malaria); each tablet contains 20 mg artemether and 120 mg lumefantrine (M van Vugt et al, Am J Trop Med Hyg 1999; 60:936). It is contraindicated during the first trimester of pregnancy; safety during the second and third trimester is not known. The tablets should be taken with food. Artemether/lumefantrine should not be used in patients with cardiac arrhythmias, bradycardia, severe cardiac disease or QT prolongation. Concomitant use of drugs that prolong the QT interval or are metabolized by CYP2D6 is contraindicated.*

78. *Adults treated with artesunate should also receive oral treatment doses of either atovaquone/proguanil, doxycycline, clindamycin or mefloquine; children should take either atovaquone/proguanil, clindamycin, or mefloquine (F Nosten et al, Lancet 2000; 356:297; M van Vugt, Clin Infect Dis 2002; 35:1498; F Smithuis et al, Trans R Soc Trop Med Hyg 2004; 98:182). If artesunate is given IV, oral medication should be started when the patient is able to tolerate it (SEAQUAMAT group, Lancet 2005; 366:717).*

Infection		Drug	Adult Dosage	Pediatric Dosage
		either followed by		
		primaquine phosphate[79]	30 mg base/d PO × 14 d	0.6 mg/kg/d PO × 14 d
Alternative:[67]		Chloroquine phosphate[80]	25 mg base/kg PO in 3 doses over 48 hr[81]	25 mg base/kg PO in 3 doses over 48 hr[81]
	OR	Quinine sulfate **plus**	650 mg PO q8h × 3-7d[70]	30 mg/kg/d PO in 3 doses × 3-7 d[70]
		doxycycline[7,21,71]	100 mg PO bid × 7 d	4 mg/kg/d PO in 2 doses × 7 d
		either followed by		
		primaquine phosphate[79]	30 mg base/d PO × 14 d	0.6 mg/kg/d PO × 14 d
All *Plasmodium* species except chloroquine-resistant *P. falciparum*[63] and chloroquine-resistant *P. vivax*[64]				
Drug of choice:[67]		Chloroquine phosphate[80]	1 g (600 mg base) PO, then 500 mg (300 mg base) 6 hr later, then 500 mg (300 mg base) at 24 and 48 hr[81]	10 mg base/kg (max 600 mg base) PO, then 5 mg base/kg 6 hr later, then 5 mg base/kg at 24 and 48 hr[81]
PARENTERAL[66]				
All *Plasmodium* species (Chloroquine-sensitive and resistant)				
Drug of choice:[67,82]		Quinidine gluconate[83]	10 mg/kg IV loading dose (max 600 mg) in normal saline over 1-2 hr, followed by continuous infusion of 0.02 mg/kg/min until PO therapy can be started	10 mg/kg IV loading dose (max 600 mg) in normal saline over 1-2 hr, followed by continuous infusion of 0.02 mg/kg/min until PO therapy can be started
	OR	Quinidine dihydro-chloride[83]*	20 mg/kg IV loading dose in 5% dextrose over 4 hr, followed by 10 mg/kg over 2-4 hr q8h (max 1800 mg/d) until PO therapy can be started	20 mg/kg IV loading dose in 5% dextrose over 4 hr, followed by 10 mg/kg over 2-4 hr q8h (max 1800 mg/d) until PO therapy can be started
	OR	Artesunate[76]*	2.4 mg/kg/dose IV × 3d at 0, 12, 24, and 48 hr	2.4 mg/kg/dose IV × 3d at 0, 12, 24, and 48 hr
		plus see footnote 78		
MALARIA, Prevention of[84]				
All *Plasmodium* species in chloroquine-sensitive areas[63,64,65]				
Drug of choice:[67,85]		Chloroquine phosphate[80,86]	500 mg (300 mg base) PO once/wk[87]	5 mg/kg base PO once/wk, up to adult dose of 300 mg base[87]
All *Plasmodium* species in chloroquine-resistant areas[63,64,65]				
Drug of choice:[67]		Atovaquone/ proguanil[68]	1 adult tab/d[88]	5-8 kg: $^1/_2$ peds tabs/d[68,88] 9-10 kg: $^3/_4$ peds tab/d[68,88] 11-20 kg: 1 peds tab/d[68,88] 21-30 kg: 2 peds tab/d[68,88] 31-40 kg: 3 peds tab/d[68,88] >40 kg: 1 adult tabs/d[68,88]
	OR	Doxycycline[7,21,71]	100 mg PO daily[89]	2 mg/kg/d PO, up to 100 mg/d[89]

79. Primaquine phosphate can cause hemolytic anemia, especially in patients whose red cells are deficient in G-6-PD. This deficiency is most common in African, Asian, and Mediterranean peoples. Patients should be screened for G-6-PD deficiency before treatment. Primaquine should not be used during pregnancy. It should be taken with food to minimize nausea and abdominal pain. Primaquine-tolerant P. vivax *can be found globally. Relapses of primaquine-resistant strains may be retreated with 30 mg (base) × 28 d.*

80. Chloroquine should be taken with food to decrease gastrointestinal adverse effects. If chloroquine phosphate is not available, hydroxychloroquine sulfate is as effective; 400 mg of hydroxychloroquine sulfate is equivalent to 500 mg of chloroquine phosphate.

81. Chloroquine combined with primaquine was effective in 85% of patients with P. vivax *resistant to chloroquine and could be a reasonable choice in areas where other alternatives are not available (JK Baird et al, J Infect Dis 1995; 171:1678).*

82. Exchange transfusion is controversial, but has been helpful for some patients with high-density (>10%) parasitemia, altered mental status, pulmonary edema, or renal complications (VI Powell and K Grima, Transfus Med Rev 2002; 16:239; MS Riddle et al, Clin Infect Dis 2002; 34:1192).

83. Continuous EKG, blood pressure, and glucose monitoring are recommended, especially in pregnant women and young children. For problems with quinidine availability, call the manufacturer (Eli Lilly, 800-821-0538) or the CDC Malaria Hotline (770-488-7788). Quinidine may have greater antimalarial activity than quinine. The loading dose should be decreased or omitted in patients who have received quinine or mefloquine. If more than 48 hours of parenteral treatment is required, the quinine or quinidine dose should be reduced by 30-50%.

84. No drug guarantees protection against malaria. Travelers should be advised to seek medical attention if fever develops after they return. Insect repellents, insecticide-impregnated bed nets and proper clothing are important adjuncts for malaria prophylaxis (Med Lett Drugs Ther 2005; 47: 100). Malaria in pregnancy is particularly serious for both mother and fetus; prophylaxis is indicated if exposure cannot be avoided.

85. Alternatives for patients who are unable to take choloroquine include atovaquone/proguanil, mefloquine, doxycycline, or primaquine dosed as for chloroquine-resistant areas.

86. Has been used extensively and safely for prophylaxis in pregnancy.

87. Beginning 1-2 wk before travel and continuing weekly for the duration of stay and for 4 wks after leaving.

88. Beginning 1-2 d before travel and continuing for the duration of stay and for 1 wk after leaving. In one study of malaria prophylaxis, atovaquone/proguanil was better tolerated than mefloquine in nonimmune travelers (D Overbosch et al, Clin Infect Dis 2001; 33:1015). The protective efficacy of Malarone *against* P. vivax *is variable ranging from 84% in Indonesian New Guinea (J Ling et al, Clin Infect Dis 2002; 35:825) to 100% in Colombia (J Soto et al, AM J Trop Med Hyg 2006; 75:430). Some Medical Letter consultants prefer alternate drugs if traveling to areas where* P. vivax *predominates.*

89. Beginning 1-2 d before travel and continuing for the duration of stay and for 4 wk after leaving. Use of tetracyclines is contraindicated in pregnancy and in children <8 years old. Doxycycline can cause gastrointestinal disturbances, vaginal moniliasis, and photosensitivity reactions.

Infection		Drug	Adult Dosage	Pediatric Dosage
	OR	Mefloquine[74,75,90]	250 mg PO once/wk[91]	5-10 kg: 1/8 tab once/wk[91] 11-20 kg: 1/4 tab once/wk[91] 21-30 kg: 1/2 tab once/wk[91] 31-45 kg: 3/4 tab once/wk[91] >45 kg: 1 tab once/wk[91]
Alternative:[92]		Primaquine[7,79] phosphate	30 mg base/d PO daily[93]	0.6 mg/kg base PO daily[93]
MALARIA, Prevention of Relapses: *P. vivax* and *P. ovale*[67]				
Drug of choice:		Primaquine phosphate[79]	30 mg base/d PO × 14 d	0.6 mg base/kg/d PO × 14 d
MALARIA, Self-Presumptive Treatment[94]				
Drug of Choice:		Atovaquone/proguanil[7,68]	4 adult tabs once/d × 3 d[69]	<5 kg: not indicated 5-8 kg: 2 peds tabs once/d × 3 d 9-10 kg: 3 peds tabs once/d × 3 d 11-20 kg: 1 adult tab once/d × 3 d 21-30 kg: 2 adult tabs once/d × 3 d 31-40 kg: 3 adult tabs once/d × 3 d >40 kg: 4 adult tabs once/d × 3 d[69]
	OR	Quinine sulfate **plus**	650 mg PO q8h × 3 or 7 d[70]	30 mg/kg/d PO in 3 doses × 3 or 7 d[70]
		doxycycline[7,21,71]	100 mg PO bid × 7 d	4 mg/kg/d PO in 2 doses × 7 d
	OR	Artesunate[76]* **plus** see footnote 78	4 mg/kg/d PO × 3 d	4 mg/kg/d PO × 3 d
MICROSPORIDIOSIS				
Ocular *(Encephalitozoon hellem, E. cuniculi, Vittaforma corneae [Nosema corneum])*				
Drug of choice:		Albendazole[7,12] **plus** fumagillin[95]*	400 mg PO bid	
Intestinal *(E. bieneusi, E. [Septata] intestinalis)*				
E. bieneusi				
Drug of choice:		Fumagillin[96]*	20 mg PO tid × 14 d	
E. intestinalis				
Drug of choice:		Albendazole[7,12]	400 mg PO bid × 21 d	
Disseminated (*E. hellem*, *E, cuniculi*, *E. intestinalis*, *Pleistophora* sp, *Trachipleistophora* sp, and *Brachiola vesicularum*)				
Drug of choice:[97]		Albendazole[7,12]*	400 mg PO bid	
Mites, see SCABIES				
MONILIFORMIS *moniliformis* infection				
Drug of choice:		Pyrantel pamoate[7,13]*	11 mg/kg PO once, repeat twice, 2 wk apart	11 mg/kg PO once, repeat twice, 2 wk apart

90. Mefloquine has not been approved for use during pregnancy. However, it has been reported to be safe for prophylactic use during the second and third trimester of pregnancy and possibly during early pregnancy as well (CDC Health Information for International Travel, 2008, page 228; BL Smoak et al, J Infect Dis 1997; 176:831). For pediatric doses <1/2 tablet, it is advisable to have a pharmacist crush the tablet, estimate doses by weighing, and package them in gelatin capsules. There is no data for use in children <5 kg, but based on dosages in other weight groups, a dose of 5 mg/kg can be used. Not recommended for use in travelers with active depression or with a history of psychosis or seizures and should be used with caution in persons with psychiatric illness. Mefloquine can be given to patients taking β-blockers if they do not have an underlying arrhythmia; it should not be used in patients with conduction abnormalities.

91. Beginning 1-2 wk before travel and continuing weekly for the duration of stay and for 4 wk after leaving. Most adverse events occur within 3 doses. Some Medical Letter consultants favor starting mefloquine 3 weeks prior to travel and monitoring the patient for adverse events, this allows time to change to an alternative regimen if mefloquine is not tolerated.

92. The combination of weekly chloroquine (300 mg base) and daily proguanil (200 mg) is recommended by the World Health Organization (www.WHO.int) for use in selected areas; this combination is no longer recommended by the CDC. Proguanil (Paludrine; AstraZeneca, United Kingdom) is not available alone in the United States but is widely available in Canada and Europe. Prophylaxis is recommended during exposure and for 4 weeks afterwards. Proguanil has been used in pregnancy without evidence of toxicity (PA Phillips-Howard and D Wood, Drug Saf 1996; 14:131).

93. Studies have shown that daily primaquine beginning 1 d before departure and continued until 3-7 d after leaving the malarious area provides effective prophylaxis against chloroquine-resistant P. falciparum *(JK Baird et al, Clin Infect Dis 2003; 37:1659). Some studies have shown less efficacy against* P. vivax. *Nausea and abdominal pain can be diminished by taking with food.*

94. A traveler can be given a course of medication for presumptive self-treatment of febrile illness. The drug given for self-treatment should be different from that used for prophylaxis. This approach should be used only in very rare circumstances when a traveler would not be able to get medical care promptly.

95. CM Chan et al, Ophthalmology 2003; 110:1420. Ocular lesions due to E. hellem *in HIV-infected patients have responded to fumagillin eyedrops prepared from* Fumidil-B *(bicyclohexyl ammonium fumagillin) used to control a microsporidial desease of honey bees (MJ Garvey et al, Ann Pharmacother 1995; 29:872), available from Leiter's Park Avenue Pharmacy (see footnote 1). For lesions due to* V. corneae, *topical therapy is generally not effective and keratoplasty may be required (RM Davis et al, Ophthalmology 1990; 97:953).*

*96. Oral fumagillin (*Flisint-*Sanofi-Aventis, France) has been effective in treating* E. bieneusi *(J-M Molina et al, N Engl J Med 2002; 346:1963), but has been associated with thrombocytopenia and neutropenia. Highly active antiretroviral therapy (HAART) may lead to microbiologic and clinical response in HIV-infected patients with microsporidial diarrhea. Octreotide (*Sandostatin*) has provided symptomatic relief in some patients with large-volume diarrhea.*

97. J-M Molina et al, J Infect Dis 1995; 171:245. There is no established treatment for Pleistophora. *For disseminated disease due to* Trachipleistophora *or* Brachiola, *itraconazole 400 mg PO once/d plus albendazole may also be tried (CM Coyle et al, N Engl J Med 2004; 351:42).*

98. Albendazole or pyrantel pamoate may be effective (JB Ziem et al, Ann Trop Med Parasitol 2004; 98:385).

Infection		Drug	Adult Dosage	Pediatric Dosage
***Naegleria* species,** see AMEBIC MENINGOENCEPHALITIS, PRIMARY				
***Nacator americanus*,** see HOOKWORM infection				
OESOPHAGOSTOMUM bifurcum				
Drug of choice:		See footnote 98		
***Onchocerca volvulus*,** see FILARIASIS				
***Opisthorchis viverrini*,** see FLUKE infection				
***Paragonimus westermani*,** see FLUKE infection				
***Pediculus capitis, humanus, Phthirus pubis*,** see LICE				
Pinworm, see ENTEROBIUS				
PNEUMOCYSTIS jiroveci (formerly *carinii*) pneumonia (PCP)[99]				
Drug of choice:		Trimethoprim/ sulfamethoxazole	TMP 15 mg/SMX 75 mg/kg/d, PO or IV in 3 or 4 doses × 21 d	TMP 15 mg/SMX 75 mg/kg/d, PO or IV in 3 or 4 doses × 21 d
Alternative:		Primaquine[7,79]	30 mg base PO daily × 21 d	0.3 mg/kg base PO daily × 21 d
		plus clindamycin[7,18]	600 mg IV q6h × 21 d, or 300-450 mg PO q6h × 21 d	15-25 mg IV q6h × 21 d, or 10 mg/kg PO q6h × 21 d
	OR	Trimethoprim[7]	5 mg/kg PO tid × 21 d	5 mg/kg PO tid × 21 d
		plus dapsone[7]	100 mg daily × 21 d	2 mg/kg/d PO × 21 d
	OR	Pentamidine	3-4 mg/kg IV daily × 21 d	3-4 mg/kg IV daily × 21 d
	OR	Atovaquone	750 mg PO bid × 21 d	1-3 mo: 30 mg/kg/d PO × 21 d 4-24 mo: 45 mg/kg/d PO × 21 d >24 mo: 30 mg/d PO × 21 d
Primary and secondary prophylaxis[100]				
Drug of choice:		Trimethoprim/ sulfamethoxazole	1 tab (single or double strength) daily or 1 DS tab PO 3d/wk	TMP 150 mg/SMX 750 mg/m^2/d PO in 2 doses 3d/wk
Alternative:		Dapsone[7]	50 mg PO bid or 100 mg PO daily	2 mg/kg/d (max 100 mg) PO or 4 mg/kg (max 200 mg) PO each wk
	OR	Dapsone[7]	50 mg PO daily or 200 mg PO each wk	
		plus pyrimethamine[101]	50 mg PO or 75 mg PO each wk	
	OR	Pentamidine	300 mg aerosol inhaled monthly via Respirgard II nebulizer	≥5 yr: 300 mg inhaled monthly via Respirgard II nebulizer
	OR	Atovaquone[7,20]	1500 mg PO daily	1-3 mo: 30 mg/kg/d PO 4-24 mo: 45 mg/kg/d PO >24 mo: 30 mg/kg/d PO
River Blindness, see FILARIASIS				
Roundworm, see ASCARIASIS				
***Sappinia diploidea*,** See AMEBIC MENINGOENCEPHALITIS, PRIMARY				
SCABIES *(Sarcopted scabiei)*				
Drug of choice:		5% Permethrin	Topically once[102]	Topically once[102]
Alternative:[103]		Ivermectin[7,16,104]	200 mcg/kg PO once[102]	200 mcg/kg PO once[102]
		10% Crotamiton	Topically once/d × 2	Topically once/d PO × 2
SCHISTOSOMIASIS *(Bilharziasis)*				
S. haematobium				
Drug of choice:		Praziquantel[39]	40 mg/kg/d PO in 2 doses × 1 d	40 mg/kg/d PO in 2 doses × 1 d
S. japonicum				
Drug of choice:		Praziquantel[39]	60 mg/kg/d PO in 3 doses × 1 d	60 mg/kg/d PO in 3 doses × 1 d
S. mansoni				
Drug of choice:		Praziquantel[39]	40 mg/kg/d PO in 2 doses × 1 d	40 mg/kg/d PO in 2 doses × 1 d
Alternaticve:		Oxamniquine[105]*	15 mg/kg PO once[106]	20 mg/kg/d PO in 2 doses × 1 d[106]
S. mekongi				
Drug of choice:		Praziquantel[39]	60 mg/kg/d PO in 3 doses × 1 d	60 mg/kg/d PO in 3 doses × 1 d

99. *Pneumocystis has been reclassified as a fungus. In severe disease with room air P_{O_2} ≤ 70 mm Hg or Aa gradient ≥35 mm Hg, prednisone should also be used (S Gagnon et al, N Engl J Med 1990; 323:1444; E Caumes et al, Clin Infect Dis 1994; 18:319).*
100. *Primary/secondary prophylaxis in patients with HIV can be discontinued after CD4 count increases to >200 × 10^6/L for >3 mo.*
101. *Plus leucovorin 25 mg with each dose of pyrimethamine. Pyrimethamine should be taken with food to minimize gastrointestinal adverse effects.*
102. *Treatment may need to be repeated in 10-14 days. A second ivermectin dose taken 2 weeks later increases the cure rate to 95%, which is equivalent to that of 5% permethrin (V Usha et al, J Am Acad Dermatol 2000; 42:236; O Chosidow, N Engl J Med 2006; 354:1718; J Heukelbach and H Feldmeier, Lancet 2006; 367:1767).*
103. *Lindane (γ-benzene hexachloride) should be reserved for treatment of patients who fail to respond to other drugs. The FDA has recommended it not be used for immunocompromised patients, young children, the elderly, pregnant and breast-feeding women, and patients weighing <50 kg.*
104. *Ivermectin, either alone or in combination with a topical scabicide, is the drug of choice for crusted scabies in immunocompromised patients (P del Giudice, Curr Opin Infect Dis 2004; 15:123).*
105. *Oxamniquine, which is not available in the United States, is generally not as effective as praziquantel. It has been useful, however, in some areas in which praziquantel is less effective (ML Ferrari et al, Bull World Health Organ 2003; 81:190; A Harder, Parasitol Res 2002; 88:395). Oxamniquine is contraindicated in pregnancy. It should be taken after food.*
106. *In East Africa, the dose should be increased to 30 mg/kg, and in Egypt and South Africa to 30 mg/kg/d × 2 d. Some experts recommend 40-60 mg/kg over 2-3 d in all of Africa (KC Shekhar, Drugs 1991; 42:379).*

Infection	Drug	Adult Dosage	Pediatric Dosage
Sleeping sickness, see TRYPANOSOMIASIS			
STRONGYLOIDIASIS *(Strongyloides stercoralis)*			
Drug of choice:[107]	Ivermectin[16]	200 mcg/kg/d PO × 2 d	200 mcg/kg/d PO × 2 d
Alternative:	Albendazole[7,12]	400 mg PO bid × 7 d	400 mg PO bid × 7 d
TAPEWORM infection			
—**Adult** (intestinal stage)			
Diphyllobothrium latum **(fish),** ***Taenia saginata*** **(beef),** ***Taenia solium*** **(pork),** ***Dipylidium caninum*** **(dog)**			
Drug of choice:[107]	Praziquantel[7,39]	5-10 mg/kg PO once	5-10 mg/kg PO once
Alternative:	Niclosamide[108]*	2 g PO once	50 mg/kg PO once
Hymenolepis nana **(dwarf tapeworm)**			
Drug of choice:	Praziquantel[7,39]	25 mg/kg PO once	25 mg/kg PO once
Alternative:	Nitazoxanide[5,7]	500 mg PO once/d or bid × 3 d[109]	1-3 yr: 100 mg PO bid × 3 d[109] 4-11 yr: 200 mg PO bid × 3 d[109]
—**Larval** (tissue stage)			
Echinococcus granulosus (hydatid cyst)			
Drug of choice:[110]	Albendazole[12]	400 mg PO bid × 1-6 mo	15 mg/kg/d (max 800 mg) × 1-6 mo
Echinococcus multilocularis			
Treatment of choice:	See footnote 111		
Taenia solium *(Cysticercosis)*			
Treatment of choice:	See footnote 112		
Alternative:	Albendazole[12]	400 mg PO bid × 8-30 d; can be repeated as necessary	15 mg/kg/d (max 800 mg) PO in 2 doses × 8-30 d; can be repeated as necessary
OR	Praziquantel[7,39]	100 mg/kg/d PO in 3 doses × 1 day then 50 mg/kg/d in 3 doses × 29 days	100 mg/kg/d PO in 3 doses × 1 day then 50 mg/kg/d in 3 doses × 29 days
Toxocariasis, see VISCERAL LARVA MIGRANS			
TOXOPLASMOSIS *(Toxoplasma gondii)*			
Drug of choice:[113]	Pyrimethamine[114]	25-100 mg/d PO × 3-4 wk	2 mg/kg/d PO × 2 d, then 1 mg/kg/d (max 25 mg/d) × 4 wk[115]
	plus		
	sulfadiazine[116]	1-1.5 g PO qid × 3-4 wk	100-200 mg/kg/d PO × 3-4 wk
TRICHINELLOSIS *(Trichinella spiralis)*			
Drug of choice:	Steroids for severe symptoms		
	plus		
	Albendazole[7,12]	400 mg PO bid × 8-14 d	400 mg PO bid × 8-14 d
Alternative:	Mebendazole[7]	200-400 mg PO tid × 3 d , then 400-500 mg tid × 10 d	200-400 mg PO tid × 3 d , then 400-500 mg tid × 10 d
TRICHOMONIASIS *(Trichomonas vaginalis)*			
Drug of choice:[117]	Metronidazole	2 g PO once or 500 mg bid × 7 d	15 mg/kg/d PO in 3 doses × 7 d
OR	Tinidazole[6]	2 g PO once	50 mg/kg once (max 2 g)

107. In immunocompromised patients or disseminated disease, it may be necessary to prolong or repeat therapy, or to use other agents. Veterinary parenteral and enema formulations of ivermectin have been used in severely ill patients with hyperinfection who were unable to take or reliably absorb oral medications (J Orem et al, Clin Infect Dis 2003; 37:152; PE Tarr Am J Trop Med Hyg 2003; 68:453; FM Marty et al, Clin Infect Dis 2005; 41:e5). In disseminated strongyloidiasis, combination therapy with albendazole and ivermectin has been suggested (S Lim et al, Can Med Assoc J 2004; 171:479).

108. Niclosamide must be chewed thoroughly before swallowing and washed down with water.

109. JO Juan et al, Trans R Soc Trop Med Hyg 2002; 96:193; JC Chero et al, Trans R Soc Trop Med Hyg 2007; 101:203; E Diaz et al, Am J Trop Med Hyg 2003; 68:384.

110. Patients may benefit from surgical resection or percutaneous drainage of cysts. Praziquantel is useful preoperatively or in case of spillage of cyst contents during surgery. Percutaneous aspiration-injection-reaspiration (PAIR) with ultrasound guidance plus albendazole therapy has been effective for management of hepatic hydatid cyst disease (RA Smego, Jr., et al, Clin Infect Dis 2003; 37:1073; S Nepalia et al, J Assoc Physicians India 2006; 54:458; E Zerem and R Jusufovic Surg Endosc 2006; 20:1543).

111. Surgical excision is the only reliable means of cure. Reports have suggested that in nonresectable cases use of albendazole (400 mg bid) can stabilize and sometimes cure infection (P Craig, Curr Opin Infect Dis 2003; 16:437; O Lidove et al, Am J Med 2005; 118:195).

112. Initial therapy for patients with inflamed parenchymal cysticercosis should focus on symptomatic treatment with anti-seizure medication (LS Yancey et al, Curr Infect Dis Rep 2005; 7:39; AH del Brutto et al, Ann Intern Med 2006; 145:43). Patients with live parenchymal cysts who have seizures should be treated with albendazole together with steroids (dexamethasone 6 mg/d or prednisone 40-60 mg/d) and an anti-seizure medication (HH Garcia et al, N Engl J Med 2004; 350:249). Patients with subarachnoid cysts or giant cysts in the fissures should be treated for at least 30 d (JV Proaño et al, N Engl J Med 2001; 345:879). Surgical intervention (especially neuroendoscopic removal) or CSF diversion followed by albendazole and steroids is indicated for obstructive hydocephalus. Arachnoiditis, vasculitis, or cerebral edema is treated with prednisone 60 mg/d or dexamethasone 4-6 mg/d together with albendazole or praziquantel (AC White, Jr., Annu Rev Med 2000; 51:187). Any cysticercocidal drug may cause irreparable damage when used to treat ocular or spinal cysts, even when corticosteroids are used. An ophthalmic exam should always precede treatment to rule out intraocular cysts.

Infection	Drug	Adult Dosage	Pediatric Dosage
TRICHOSTRONGYLUS infection			
Drug of choice:	Pyrantel pamoate[7,13]*	11 mg/kg base PO once (max. 1 g)	11 mg/kg PO once (max 1 g)
Alternative:	Mebendazole[7]	100 mg PO bid × 3 d	100 mg PO bid × 3 d
OR	Albendazole[7,12]	400 mg PO once	400 mg PO once
TRICHURIASIS (*Trichuris trichiura*, whipworm)			
Drug of choice:	Mebendazole	100 mg PO bid × 3 d or 500 mg once	100 mg PO bid × 3 d or 500 mg once
Alternative:	Albendazole[7,12]	400 mg PO × 3 d	400 mg PO × 3 d
OR	Ivermectin[7,16]	200 mcg/kg PO daily × 3 d	200 mcg/kg/d PO × 3 d
TRYPANOSOMIASIS[118]			
T. cruzi (American trypanosomiasis, Chagas disease)			
Drug of choice:	Nifurtimox*	8-10 mg/kg/d PO in 3-4 doses × 90-120 d	1-10 yr: 15-20 mg/kg/d PO in 4 doses × 90-120 d 11-16 yr: 12.5-15 mg/kg/d in 4 doses × 90-120 d
OR	Benznidazole[119]*	5-7 mg/kg/d PO in 2 doses × 30-90 d	≤12 yr: 10 mg/kg/d PO in 2 doses × 30-90 d >12 yr: 5-7 mg/kg/d in 2 doses × 30-90 d
T. brucei gambiense (West African trypanosomiasis, sleeping sickness)			
Hemolymphatic stage			
Drug of choice:[120]	Pentamidine[7]	4 mg/kg/d IM × 7 d	4 mg/kg/d IM × 7 d
Alternative:	Suramin*	100-200 mg (test dose) IV, then 1 g IV on days 1, 3, 7, 14, and 21	20 mg/kg on d 1, 3, 7, 14, and 21
Late disease with CNS involvement			
Drug of Choice:	Eflornithine[121]*	400 mg/kg/d IV in 4 doses × 14 d	400 mg/kg/d IV in 4 doses × 14 d
OR	Melarsoprol[122]	2.2 mg/kg/d IV × 10 d	2.2 mg/kg/d IV × 10 d
T. b. rhodesiense (East African trypanosomiasis, sleeping sickness)			
Hemolymphatic stage			
Drug of choice:	Suramin*	100-200 mg (test dose) IV, then 1 g IV on days 1, 3, 7, 14, and 21	20 mg/kg on d 1, 3, 7, 14, and 21
Late disease with CNS involvement			
Drug of choice:	Melarsoprol[122]	2-3.6 mg/kg/d IV × 3 d; after 7 d 3.6 mg/kg/d × 3 d; repeat again after 7 d	2-3.6 mg/kg/d × 3 d; after 7 d 3.6 mg/kg/d × 3 d; repeat again after 7 d
VISCERAL LARVA MIGRANS[123] *(Toxocariasis)*			
Drug of choice:	Albendazole[7,12]	400 mg PO bid × 5 d	400 mg PO bid × 5 d
OR	Mebendazole[7]	100-200 mg PO bid × 5 d	100-200 mg PO bid × 5 d
Whipworm, see TRICHURIASIS			
Wuchereria bancrofti, see FILARIASIS			

113. To treat CNS toxoplasmosis in HIV-infected patients, some clinicians have used pyrimethamine 50-100 mg/d (after a loading dose of 200 mg) with sulfadiazine and, when sulfonamide sensitivity developed, have given clindamycin 1.8-2.4 g/d in divided doses instead of the sulfonamide. Treatment is usually given for at least 4-6 weeks. Atovaquone (1500 mg PO bid) plus pyrimethamine (200 mg loading dose, followed by 75 mg/d PO) for 6 weeks appears to be an effective alternative in sulfa-intolerant patients (K Chirgwin et al, Clin Infect Dis 2002; 34:1243). Atovaquone must be taken with a meal to enhance absorption. Treatment is followed by chronic suppression with lower dosage regimens of the same drugs. For primary prophylaxis in HIV patients with <100 × 16^6/L CD4 cells, either trimethoprim-sulfamethoxazole, pyrimethamine with dapsone, or atovaquone with or without pyrimethamine can be used. Primary or secondary prophylaxis may be discontinued when the CD4 count increases to >200 × 10^6/L for >3 mo (MMWR Morb Mortal Wkly Rep 2004; 53 [RR15]:1). In ocular toxoplasmosis with macular involvement, corticosteroids are recommended in addition to antiparasitic therapy for an anti-inflammatory effect. In one randomized single-blind study, trimethoprim/sulfamethoxazole was reported to be as effective as pyrimethamine/sulfadiazine for treatment of ocular toxoplasmosis (M Soheilian et al, Ophthalmology 2005; 112:1876). Women who develop toxoplasmosis during the first trimester of pregnancy should be treated with spiramycin (3-4 g/d). After the first trimester, if there is no documented transmission to the fetus, spiramycin can be continued until term. If transmission has occurred in utero, therapy with pyrimethamine and sulfadiazine should be started (JG Montoya and O Liesenfeld, Lancet 2004; 363:1965). Pyrimethamine is a potential teratogen and should be used only after the first trimester.

114. Plus leucovorin 10-25 mg with each dose of pyrimethamine. Pyrimethamine should be taken with food to minimize gastrointestinal adverse effects.

115. Congenitally infected newborns should be treated with pyrimethamine every 2 or 3 days and a sulfonamide daily for about one year (JS Remington and G Desmonts in JS Remington and JO Klein, eds, Infectious Disease of the Fetus and Newborn Infant, *6th ed, Philadelphia: Saunders, 2006, page 1038).*

116. Sulfadiazine should be taken on an empty stomach with adequate water.

117. Sexual partners should be treated simultaneously with same dosage. Metronidazole-resistant strains have been reported and can be treated with higher doses of metronidazole (2-4 g/d × 7-14 d) or with tinidazole (MMWR Morb Mortal Wkly Rep 2006; 55 [RR11]:1).

118. MP Barrett et al, Lancet 2003; 362:1469. Treatment of chronic or indeterminate Chagas disease with benznidazole has been associated with reduced progression and increased negative seroconversion (R Viotti et al, Ann Intern Med 2006; 144:724).

119. Benznidazole should be taken with meals to minimize gastrointestinal adverse effects. It is contraindicated during pregnancy.

120. Pentamidine and suramin have equal efficacy, but pentamidine is better tolerated.

121. Eflornithine is highly effective in T.b. gambiense, *but not in* T.b. rhodesiense *infections. In one study of treatment of CNS disease due to* T.b. gambiense, *there were fewer serious complications with eflornithine than with melarsoprol (F Chappuis et al, Clin Infect Dis 2005;41:748). Eflornithine is available in limited supply only from the WHO. It is contraindicated during pregnancy.*

122. E Schmid et al, J Infect Dis 2005; 191:1922. Corticosteroids have been used to prevent arsenical encephalopathy (J Pepin et al, Trans R Soc Trop Med Hyg 1995; 89:92). Up to 20% of patients with T.b.gambiense *fail to respond to melarsoprol (MP Barrett, Lancet 1999; 353:1113). In one study, a combination of low-dose melarsoprol (1.2 mg/kg/d IV) and nifurtimox (7.5 mg/kg PO bid) × 10 d was more effective than standard-dose melarsoprol alone (S Bisser et al, J Infect Dis 2007; 195:322).*

123. Optimum duration of therapy is not known; some Medical Letter consultants would treat ×20 d. For severe symptoms or eye involvement, corticosteroids can be used in addition (D Despommier, Clin Microbiol Rev 2003; 16:265).

Drug	Toxicity in Pregnancy	Recommendations
Albendazole (*Albenza*)	Teratogenic and embryotoxic in animals	Caution*
Amphotericin B (*Fungizone*, and others)	None known	Caution*
Amphotericin B liposomal (*AmBisome*)	None known	Caution*
Artemether/lumefantrine *(Coartem, Riamet)*[1]	Embryocidal and teratogenic in animals	Caution*
Artesunate[1]	Embryocidal and teratogenic in animals	Caution*
Atovaquone (*Mepron*)	Maternal and fetal toxicity in animals	Caution*
Atovaquone/proguanil (*Malarone*)[2]	Maternal and fetal toxicity in animals	Caution*
Azithromycin (*Zithromax*, and others)	None known	Probably safe
Benznidazole (*Rochagan*)	Unknown	Contraindicated
Chloroquine (*Aralen*, and others)	None known with doses recommended for malaria prophylaxis	Probably safe in low doses
Clarithromycin (*Biaxin*, and others)	Teratogenic in animals	Contraindicated
Clindamycin (*Cleocin*, and others)	None known	Caution*
Crotamiton (*Eurax*)	Unknown	Caution*
Dapsone	None known; carcinogenic in rats and mice; hemolytic reactions in neonates	Caution*, especially at term
Diethylcarbamazine (DEC; *Hetrazan*)	Not known; abortifacient in one study in rabbits	Contraindicated
Diloxanide *(Furamide)*	Safety not established	Caution*
Doxycycline (*Vibramycin*, and others)	Tooth discoloration and dysplasia, inhibition of bone growth is fetus; hepatic toxicity and azotemia with IV use in pregnant patients with decreased renal function or with overdosage	Contraindicated
Eflornithine (*Ornidyl*)	Embryocidal in animals	Contraindicated
Fluconazole (*Diflucan*, and others)	Teratogenic	Contraindicated for high dose; caution* for single dose
Flucytosine (*Ancoban*)	Teratogenic in rats	Contraindicated
Furazolidone (*Furoxone*)	None known; carcinogenic in rodents; hemolysis with G-6-PD deficiency in newborn	Caution*; contraindicated at term
Hydroxychloroquine (*Plaquenil*)	None known with doses recommended for malaria prophylaxis	Probably safe in low doses
Itraconazole (*Sporanox*, and others)	Teratogenic and embryotoxic in rats	Caution*
Iodoquinol (*Yodoxin*, and others)	Unknown	Caution*
Ivermectin (*Stromectol*)	Teratogenic in animals	Contraindicated
Ketoconazole (*Nizoral*, and others)	Teratogenic and embryotoxic in rats	Contraindicated; topical probably safe
Lindane	Absorbed from the skin; potential CNS toxicity in fetus	Contraindicated
Malathion, topical (*Ovide*)	None known	Probably safe
Mebendazole (*Vermox*)	Teratogenic and embryotoxic in rats	Caution*
Mefloquine (*Lariam*)[3]	Teratogenic in animals	Caution*
Meglumine *(Glucantine)*	Not Known	Caution*
Metronidazole (*Flagyl*, and others)	None known—carcinogenic in rats and mice	Caution*
Miconazole (*Monistat i.v.*)	None known	Caution*
Miltefosine (*Impavido*)	Teratogenic in rats and induces abortions in animals	Contraindicated; effective contraception must be used for 2 months after the last dose
Niclosamide (*Niclocide*)	Not absorbed; no known toxicity in fetus	Probably safe
Nitazoxanide (*Alinia*)	None known	Caution*
Oxamniquine (*Vansil*)	Embryocidal in animals	Contraindicated
Paromomycin (*Humatin*)	Poorly absorbed; toxicity in fetus unknown	Oral capsules probably safe
Pentamidine (*Pentam 300*, *NebuPent*, and others)	Safety not established	Caution*
Permethrin (*Nix*, and others)	Poorly absorbed; no known toxicity in fetus	Probably safe
Praziquantel (*Biltricide*)	Not known	Probably safe
Primaquine	Hemolysis in G-6-PD deficiency	Contraindicated
Pyrantel pamoate (*Antiminth*, and others)	Absorbed in small amounts; no known toxicity in fetus	Probably safe
Pyrethrins and piperonyl butoxide (*RID*, and others)	Poorly absorbed; no known toxicity in fetus	Probably safe
Pyrimethamine (*Daraprim*)[4]	Teratogenic in animals	Caution*; contraindicated during 1st trimester
Quinacrine (*Atabrine*)	Safety not established	Caution*
Quinidine	Large doses can cause abortion	Probably safe
Quinine (*Qualaquin*)	Large doses can cause absortion; auditory nerve hypoplasia, deafness in fetus; visual changes, limb anomalies, visceral defects also reported	Caution*
Sodium stibogluconate (*Pentostam*)	Not known	Caution*
Sulfonamides	Teratogenic in some animal studies; hemolysis in newborn with G-6-PD deficiency; increased risk of kernicterus in newborn	Caution*; contraindicated at term
Suramin sodium (*Germanin*)	Teratogenic in mice	Caution*
Tetracycline (*Sumycin*, and others)	Tooth discoloration and dysplasia, inhibition of bone growth in fetus; hepatic toxicity and azotemia with IV use in pregnant patients with decreased renal function or with overdosage	Contraindicated
Tinidazole (*Tindamax*)	Increased fetal mortality in rats	Caution*
Trimethoprim (*Proloprim*, and others)	Folate antagonism; teratogenic in rats	Caution*
Trimethoprim-sulfamethoxazole (*Bactrim*, and others)	Same as sulfonamides and trimethoprim	Caution*; contraindicated at term

**Use only for strong clinical indication in absence of suitable alternative.*

1. See also footnote 76 on page 3315.

2. See also footnote 68 on page 3315.

3. See also footnotes 74 on page 3315 and 90 on page 3317.

4. See also footnote 113 on page 3320.

Manufacturers of Drugs Used to Treat Parasitic Infections

albendazole—*Albenza* (GlaxoSmithKline)
Albenza (GlaxoSmithKline)—albendazole
Alinia (Romark)—nitazoxanide
AmBisome (Gilead)—amphotericin B, liposomal
amphotericin B—*Fungizone* (Apothecon), others
amphotericin B, liposomal—*AmBisome* (Gilead)
Ancobon (Valeant)—flucytosine
§ *Antiminth* (Pfizer)—pyrantel pamoate
• *Aralen* (Sanofi)—chloroquine HCl and chloroquine phosphate
§ artemether—*Artenam* (Arenco, Belgium)
§ artemether/lumefantrine—*Coartem*, *Riamet* (Novartis)
§ *Artenam* (Arenco, Belgium)—artemether
§ artesunate—(Guilin No. 1 Factory, People's Republic of China)
atovaquone—*Mepron* (GlaxoSmithKline)
atovaquone/proguanil—*Malarone* (GlaxoSmithKline)
azithromycin—*Zithromax* (Pfizer), others
• *Bactrim* (Roche)—TMP/Sulfa
§ benznidazole—*Rochagan* (Brazil)
• *Biaxin* (Abbott)—clarithromycin
§ *Biltricide* (Bayer)—praziquantel
† bithionol—*Bitin* (Tanabe, Japan)
† *Bitin* (Tanabe, Japan)—bithionol
§ *Brolene* (Aventis, Canada)—propamidine isethionate chloroquine HCl and chloroquine phosphate—*Aralen* (Sanofi), others
clarithromycin—*Biaxin* (Abbott), others
• *Cleocin* (Pfizer)—clindamycin
clindamycin—*Cleocin* (Pfizer), others
Coartem (Novartis)—artemether/lumefantrine
crotamiton—*Eurax* (Westwood-Squibb)
dapsone—(Jacobus)
§ *Daraprim* (GlaxoSmithKline)—pyrimethamine USP
† diethylcarbamazine citrate (DEC)—*Hetrazan*
• *Diflucan* (Pfizer)—fluconazole
§ diloxanide furoate—*Furamide* (Boots, United Kingdom)
doxycycline—*Vibramycin* (Pfizer), others
eflornithine (difluoromethylornithine, DFMO)—*Ornidyl* (Aventis)
§ *Egaten* (Novartis)—triclabendazole
Elimite (Allergan)—permethrin
Ergamisol (Janssen)—levamisole
Eurax (Westwood-Squibb)—crotamiton
• *Flagyl* (Pfizer)—metronidazole
§ *Flisint* (Sanofi-Aventis, France)—fumagillin
fluconazole—*Diflucan* (Pfizer), others
flucytosine—*Ancobon* (Valeant)
§ fumagillin—*Flisint* (Sanofi-Aventis, France)
• *Fungizone* (Apothecon)—amphotericin
§ *Furamide* (Boots, United Kingdom)—diloxanide furoate
§ furazolidone—*Furozone* (Roberts)
§ *Furozone* (Roberts)—furazlidone
† *Germanin* (Bayer, Germany)—suramin sodium
§ *Glucantime* (Aventis, France)—meglumine antimonate
† *Hetrazan*—diethylcarbamazine citrate (DEC)
Humatin (Monarch)—paromomycin
§ *Impavido* (Zentaris, Germany)—miltefosine
iodoquinol—*Yodoxin* (Glenwood), others
itraconazole—*Sporanox* (Janssen-Ortho), others
ivermectin—*Stromectol* (Merck)
ketoconazole—*Nizoral* (Janssen), others
† *Lampit* (Bayer, Germany)—nifurtimox
Lariam (Roche)—mefloquine
§ *Leshcutan* (Teva, Israel)—topical paromomycin
levamisole—*Ergamisol* (Janssen)
lumefantrine/artemether—*Coartem*, *Riamet* (Novartis)
Malarone (GlaxoSmithKline)—atovaquone/proguanil
malathion—*Ovide* (Medicis)
mebendazole—*Vermox* (McNeil), others
mefloquine—*Lariam* (Roche)
§ meglumine antimonate—*Glucantime* (Aventis, France)
† melarsoprol—*Mel-B*
† *Mel-B*—melarsoprol
Mepron (GlaxoSmithKline)—atovaquone
metronidazole—*Flagyl* (Pfizer), others
§ miconazole—*Monistat i.v.*
§ miltefosine—*Impavido* (Zentaris, Germany)
§ *Monistat i.v.*—miconazole
NebuPent (Fujisawa)—pentamidine isethionate
Neutrexin (US Bioscience)—trimetrexate
§ niclosamide—*Yomesan* (Bayer, Germany)
† nifurtimox—*Lampit* (Bayer, Germany)
nitazoxanide—*Alinia* (Romark)
• *Nizoral* (Janssen)—ketoconazole
Nix (GlaxoSmithKline)—permethrin
§ ornidazole—*Tiberal* (Roche, France)
Ornidyl (Aventis)—eflornithine (difluoromethylornithine, DFMO)
Ovide (Medicis)—malathion
§ oxamniquine—*Vansil* (Pfizer)
§ *Paludrine* (AstraZeneca, United Kingdom)—proguanil
paromomycin—*Humatin* (Monarch); *Leshcutan* (Teva, Israel; (topical formulation not available in US)
Pentam 300 (Fujisawa)—pentamidine isethionate
pentamidine isethionate—*Pentam 300* (Fujisawa), *NebuPent* (Fujisawa)
† *Pentostam* (GlaxoSmithKline, United Kingdom)—sodium stibogluconate
permethrin—*Nix* (GlaxoSmithKline), *Elimite* (Allergan)
§ praziquantel—*Biltricide* (Bayer)
primaquine phosphate USP
§ proguanil—*Paludrine* (AstraZeneca, United Kingdom)
proguanil/atovaquone—*Malarone* (GlaxoSmithKline)
§ propamidine isethionate—*Brolene* (Aventis, Canada)
§ pyrantel pamoate—*Antiminth* (Pfizer)
pyrethrins and piperonyl butoxide—*RID* (Pfizer), others
§ pyrimethamine USP—*Daraprim* (GlaxoSmithKline)
Qualaquin—quinine sulfate (Mutual Pharmaceutical Co/AR Scientific)
* quinidine gluconate (Eli Lilly)
§ quinine dihydrochloride
quinine sulfate—*Qualaquin* (Mutual Pharmaceutical Co/AR Scientific)
Riamet (Novartis)—artemether/lumefantrine
• *RID* (Pfizer)—pyrethrins and piperonyl butoxide
• *Rifadin* (Aventis)—rifampin
rifampin—*Rifadin* (Aventis), others
§ *Rochagan* (Brazil)—benznidazole
* *Rovamycine* (Aventis)—spiramycin
† sodium stibogluconate—*Pentostam* (GlaxoSmithKline, United Kingdom)
* spiramycin—*Rovamycine* (Aventis)
• *Sporanox* (Janssen-Ortho)—itraconazole
Stromectol (Merck)—ivermectin
sulfadiazine—(Eon)
† suramin sodium—*Germanin* (Bayer, Germany)
§ *Tiberal* (Roche, France)—ornidazole
Tindamax (Mission)—tinidazole
tinidazole—*Tindamax* (Mission)
TMP/Sulfa—*Bactrim* (Roche), others
§ triclabendazole—*Egaten* (Novartis)
trimetrexate—*Neutrexin* (US Bioscience)
§ *Vansil* (Pfizer)—oxamniquine
• *Vermox* (McNeil)—mebendazole
• *Vibramycin* (Pfizer)—doxycycline
• *Yodoxin* (Glenwood)—iodoquinol
§ *Yomesan* (Bayer, Germany)—niclosamide
• *Zithromax* (Pfizer)—azithromycin

**Available in the United States only from the manufacturer.*
§ *Not available in the United States; may be available through a compounding pharmacy (see footnote 4).*
† *Available from the CDC Drug Service, Centers for Disease Control and Prevention, Atlanta, Georgia 30333; 404-639-3670 (evenings, weekends, or holidays: 770-488-7100).*
• *Also available generically.*

CHAPTER 254

Immunomodulating Agents

Timothy R. La Pine ✪ Harry R. Hill

Considerable clinical interest and basic science research into the functional mechanisms of the immune response and identification of the specific biologic factors that modulate this response have been generated lately. This research has established that a critical and delicate balance in the regulation of both cellular and humoral function is essential for complete immunologic response to invasive pathogens and that alteration of this regulation may have potential clinical significance. Attempts to augment immune function in the challenged host or during specific immunodeficiency states have focused on numerous modifiers of immune biologic response. These efforts, in combination with advances made in hybridoma and recombinant DNA technology, have resulted in numerous clinical trials conducted to explore the therapeutic utility of immunomodulating agents in the treatment of specific human disease states. I review the biologic agents used to manipulate immune regulation for the prevention and management of infectious diseases in infants and children.

MONOCLONAL ANTIBODIES

The use of serum antibody therapy, in the late 1800s, was among the first clinical attempts to modulate the immune response in the treatment of human sepsis. By the middle 1930s, serum-based therapy was the standard of care for many infectious illnesses, particularly pneumonia. Controlled trials during that time demonstrated that the administration of type-specific pneumococcal serum reduced the mortality rate by 50 percent in patients with pneumococcal pneumonia.[43,44,73] With the introduction of antimicrobial pharmacologic agents in the 1940s, the use of serum antibody therapy for sepsis became less popular. The development of hybridoma technology by Kohler and Milstein[137] in 1975, however, provided the means to generate virtually unlimited amounts of monoclonal antibodies for potential clinical use. This technology in combination with the advances in recombinant DNA technology has allowed researchers to generate highly specific human monoclonal antibodies and to humanize murine monoclonal antibodies.[31,264] Monoclonal antibodies now are considered attractive molecules to be used potentially as antimicrobial agents and immunomodulators, to deliver pharmacologic substances to sites of inflammation, or even to target certain cancerous tissues. This advance has led to the development of monoclonal antibody–based therapies to treat sepsis, septic shock, and a number of inflammatory diseases in infants, children, and adults.

A monoclonal antibody could, in theory, be generated that would alter the clinical course of any infectious or inflammatory disease state. This hypothesis has fostered numerous studies evaluating the use of monoclonal antibodies in a variety of clinical diseases, particularly those related to alloimmune and autoimmune phenomena.[53,64,99,165,207] The use of monoclonal antibodies to alter the pathogenesis of sepsis and septic shock also has received considerable experimental and clinical attention. These efforts have focused on two phases in the development of the septic shock syndrome: (1) to block bacteria and their components that induce shock and (2) to modify the release and action of the proinflammatory mediators that lead to septic shock.

Monoclonal antibody preparations to block bacteria or their components in the development of septic shock were initially employed in early studies using type-specific antisera against *Streptococcus pneumoniae*, *Neisseria meningitidis*, and *Haemophilus influenzae*.[43] Attempts using monoclonal antibody therapy to alter the pathogenesis of bacterial sepsis leading to septic shock have focused on group B streptococcus (GBS) and *Escherichia coli*. These two bacteria are significant causes of neonatal morbidity and mortality. Infants usually acquire these bacterial infections from exposure in the birth canal; however, only a small number of the exposed infants actually develop bacterial sepsis. Factors predisposing infants to acquisition of infection with these organisms include prematurity, prolonged rupture of membranes, and maternal sepsis. Neonatal infections occur more commonly with bacteria that possess specific capsular polysaccharides (i.e., the type III polysaccharide of GBS or the K-1 capsule of *E. coli*). Bacteria bearing these capsules are able to avoid opsonization and subsequent phagocytic killing by polymorphonuclear leukocytes (PMNs). Newborns with deficiencies of type-specific antibodies to these bacterial capsular antigens due to prematurity, which contributes to decreased maternal transplacental antibody transport, or inadequate maternal stores are predisposed to acquisition of GBS and *E. coli* infections.[111] These observations led investigators to hypothesize that passive antibody administration may be beneficial in preventing or reducing neonatal morbidity and mortality observed with these bacterial infections.

Rebecca Lancefield in 1933 originally demonstrated a protective efficacy for GBS antibody therapy using rabbit antisera. She established that this protective efficacy is type specific and classified three types of GBS strains. Antisera raised against type II or type III GBS did not protect against infection with type I bacteria. These experiments also suggested that antibodies to group B non–type-specific determinants (i.e., expressed on all GBS bacteria) are not protective.[82,150,151] Further experiments by Lancefield and coworkers with type I GBS demonstrated that antibodies to both carbohydrate and protein capsular components can be protective.[149,151] The first human studies to suggest that the administration of antibody to the infecting strain of bacteria could improve survival rates from early-onset GBS in infants occurred in the middle 1970s.[229-232] In these studies, fresh whole blood either containing or lacking opsonic antibody was administered to infants with early-onset GBS disease. All nine infants who demonstrated a rise in opsonic antibody after transfusion survived. In contrast, three of the six infants (50%) who received blood lacking antibody to their infection strain died.[228] Subsequent studies have suggested that intravenous immune globulins (IVIG) could offer some protection against GBS infections in experimental animal models.[76,116] Because of their lack of specificity and decreased opsonic activity, however, polyclonal-type IVIG antibody preparations have had only variable and limited ability to alter the course and outcome of GBS or other bacterial infections in clinical trials.[106]

The development of monoclonal antibodies to GBS type III occurred in the early 1980s.[229-232] The GBS type III–specific antibodies, which were of the IgM class, protected rats against intraperitoneal infection with homologous type group B streptococci. Survival rates were 95 to 100 percent for rats protected by monoclonal antibody compared with 17 percent for unprotected rats. Protection was afforded even when therapy was delayed up to 24 hours after inoculation. Antibody administration resulted in the rapid accumulation of PMNs at the site of infection and prevented the depletion of bone marrow granulocyte stores commonly seen in animal models and human neonates with GBS

infection.[231] Monoclonal IgG and IgA antibody preparations also have been generated to type III GBS.[21,190,229] A protective effect in animal models of GBS infection for all three immunoglobulin isotypes has been established.[230] In GBS infections, the ability of an antibody isotype preparation to activate the complement system is related directly to its protective and opsonic activity.[232] IgM is much more active in triggering complement than are monoclonal IgG and IgA preparations,[133,232] which may explain why monoclonal IgM is quantitatively more effective against GBS infections.[110,113,232] Although human monoclonal IgM antibodies to GBS are available and have demonstrated effectiveness in reducing mortality in animal models of GBS sepsis, they have yet to be tried clinically to prevent or to attenuate GBS sepsis and septic shock in human neonates.[210,232]

Concomitant with the studies of monoclonal antibody preparations for use in experimental models of GBS infections were similar studies using monoclonal preparations in *E. coli* sepsis. *E. coli* is an antigenically complex, gram-negative bacterium having more than 150 somatic (O) and 100 capsular (K) antigens. Several factors associated with *E. coli* have been implicated as contributing to the virulence of these organisms. Among these, the K-1 capsular polysaccharide and lipopolysaccharide (LPS) have received the most attention.[26] The K-1 capsular polysaccharide on *E. coli* as a contributing factor to the virulence of this organism was suggested first by Robbins and colleagues in 1974.[214] Subsequent studies established that *E. coli* strains that possess the K-1 capsular polysaccharide are resistant to opsonization through the alternative complement pathway.[28,237] Several investigators have shown that antibody preparations to the K-1 capsular polysaccharide are opsonic and protective against lethal *E. coli* bacteremia in animals.[27] Although protection has been demonstrated primarily with IgM antibody, polyclonal hyperimmune IgG has shown some protection in animal models of lethal *E. coli* sepsis.[209] Because the serum from newborn infants is deficient in opsonic activity for *E. coli*, some investigators suggested that the administration of antibody against *E. coli* K-1 would enhance neonatal resistance to infections with this organism.[29,52] Investigators since have developed murine and human monoclonal IgM antibodies to *E. coli* K-1 and demonstrated that these antibodies are opsonic and protective against lethal *E. coli* infections in animal models.[209] Human clinical trials using monoclonal antibodies to *E. coli* K-1 have not been initiated to my knowledge.

Staphylococci are a major cause of acquired infections in prematurely born infants. Antibiotic resistance among staphylococcal species in these immunocompromised patients is of significant concern. A human chimeric monoclonal antibody has been generated that is opsonic for *Staphylococcus epidermidis* and *Staphylococcus aureus.* In animal models of *S. epidermidis* and *S. aureus* sepsis, this monoclonal antibody enhanced bacterial clearance and significantly improved survival against both staphylococcal species. Clinical trials with this monoclonal antibody are currently under way to determine the clinical usefulness of monoclonal antibody preparations in preventing nosocomial staphylococcal infections in high-risk preterm neonates.[77,254]

During the past several decades, the importance of pneumococcal vaccination for individuals at risk for acquiring infection has been underscored by the emergence of antibiotic resistance among pneumococcal strains and the increased prevalence of invasive pneumococcal disease in immunocompromised patients. Unfortunately, pure pneumococcal capsular polysaccharide vaccines are poorly immunogenic in many immunodeficient patients who are at risk for acquiring infection. This concern has led to the development of monoclonal antibodies against type-specific pneumococcal infections. A human monoclonal IgM antibody has been generated and shown to provide type-specific protection against lethal pneumococcal infections in mice, even in the presence of complement deficiency.[267] Thus, type-specific monoclonal antibody preparations likely could provide protection against pneumococcal infection in high-risk immunocompromised humans.

The use of monoclonal antibodies as a single-agent therapy to treat or to prevent bacterial sepsis has been limited to experimental animal models, primarily because of the narrow spectrum of monoclonal antibody therapy and the exceedingly large number of patients required to demonstrate the efficacy of human monoclonal antibody therapy in clinical trials. The future clinical use of monoclonal antibodies directed at bacteria or their components in the treatment of sepsis requires several clinical considerations. First, because antibodies are more effective in preventing an infection than in treating an established infection, monoclonal antibody therapy is most useful when it is administered early in the course of a disease. Second, the successful implementation of monoclonal antibody therapy directed at a specific pathogen requires refinements in diagnostic laboratory methodology to hasten identification of pathogens, leading to early treatment. Third, monoclonal antibody therapy is pathogen specific, which is problematic in dealing with unknown infections early in their course and with mixed infections with multiple organisms or serotypes. For pathogens that are antigenically variable, use of monoclonal antibody cocktail preparations that combine monoclonal antibodies to common antigenic serotypes may be beneficial. Antibody cocktails also may be designed to include monoclonal antibodies of different isotypes (IgM, IgG, IgA) to further enhance their effectiveness. Fourth, combination therapy with monoclonal antibodies and antimicrobial chemotherapy agents may be an alternative means to treat bacteria and their components in the development of septic shock. Such therapies could potentially reduce the amount or duration of antibiotic treatment needed and reduce the number of antibiotic-resistant pathogens. Combination therapy also may be clinically beneficial in cases in which the antimicrobial agents are toxic themselves. Fifth, monoclonal antibodies may elicit self-neutralizing antibodies. Thus, the administration of a monoclonal antibody could promote the production of antibody against the monoclonal antibody itself, which would diminish the clinical response and potentially cause severe anaphylaxis in cases of antibody retreatment. Likewise, widespread use of monoclonal antibody therapy could select for antibody-resistant pathogens or other serotypes or even entirely different infecting organisms.[148]

Researchers have examined the possible blockade of the proinflammatory cascade that accompanies severe infections.[1,13,86,146] The experimental attempts using monoclonal antibodies to modify the release and action of the proinflammatory mediators that lead to septic shock have focused on the proinflammatory effects of the cytokine family, endotoxin, and the functions of neutrophils and complement.

THE CYTOKINES

LYMPHOKINES AND MONOKINES

The cytokines are a family of small soluble protein molecules responsible for cell-to-cell communication. They are produced by several cell types and play crucial roles in many biologic processes, including growth, inflammation, immunity, and hematopoiesis. During infection, genes for nearly all the cytokines are expressed. The biologic activity of the prototype cytokines, the lymphokines and monokines, include interleukins (ILs) and tumor necrosis factor–α (TNF-α); these molecules, along with the granulocyte colony-stimulating factors, have received considerable attention as potential immunomodulatory agents. In response to pathogen invasion, these cytokines perform a complex series of interactions to initiate a cascade of biologic events resulting in the propagation and subsequent regulation of the inflammatory response, leading to pathogen alienation while maintaining

host preservation. Thus, the cytokine family, through a complex web of interactions, functions to initiate and then both to up-regulate and down-regulate the inflammatory response. On the basis of their roles of either up-regulating or down-regulating immune responsiveness, the cytokines generally and historically have been classified as either proinflammatory or anti-inflammatory molecules. Although many cytokines have the potential to perform dual functions, either directly or indirectly, their proinflammatory or anti-inflammatory properties are of considerable basic science and clinical therapeutic interest.[25,45,62,80,146,154,164,202]

Some prominent proinflammatory cytokines and their cellular sources are listed in Table 254–1. TNF-α and IL-1 generally are considered to be prominent early proinflammatory mediators. They induce gene expression of other proinflammatory cytokines, including IL-6 and IL-8, leading to neutrophil activation, recruitment, and degranulation. TNF-α and IL-1 also activate a secondary cascade of inflammatory mediators, including arachidonic acid–derived prostaglandin I_2, thromboxane A_2, prostaglandin E_2, platelet-activating factor, and the complement system. The colony-stimulating factors and IL-3 also are proinflammatory cytokines that induce bone marrow stem cell production of granulocytes and monocytes in addition to activating neutrophils and inducing production of IL-1 and TNF-α.[25,45,62,80,146,154,164]

Prominent among the anti-inflammatory cytokines (Table 254–2) are IL-4, IL-10, IL-13, and transforming growth factor–β. These cytokines may block endotoxin induction of IL-1 and TNF-α and suppress lymphocyte and monocyte function. In addition, IL-1 receptor antagonist blocks the proinflammatory action of IL-1 by binding its receptor.[25,45,62,80,146,154,164]

Considerable experimental and clinical interest has focused on the proinflammatory cytokines TNF-α and IL-1 as important early mediators in the pathogenesis of sepsis and septic shock syndrome. The basis for the potential therapeutic utility of these two cytokines in human disease states involves two clinically distinct hypotheses: (1) excessive cytokine production results in host immune injury, leading to severe shock; and (2) deficient cytokine production renders a host susceptible to infection by invasive pathogens.[146]

The hypothesis that cytokine overproduction can lead to severe lethal shock is demonstrated in several animal models and is suggested in patients with overwhelming sepsis. The outer membranes of gram-negative bacteria contain lipopolysaccha-

TABLE 254–1 Proinflammatory Cytokines

Cytokine	Function	Predominant Cell Source
Tumor necrosis factor–α	Stimulates interleukin-6 and colony-stimulating factors Depresses erythropoiesis, stimulates interleukin-8 and interleukin-9 Promotes tumor necrosis and endotoxic shock	Monocytes and macrophages
Interleukin-1	Stimulates proliferation and differentiation of T and B lymphocytes Stimulates T lymphocytes to produce interleukin-2 Promotes colony-stimulating factor, interleukin-8 and interleukin-9 production, and endotoxic shock	Macrophages, astrocytes, monocytes, fibroblasts, keratinocytes, B cells, corneal epithelium, and other cell types
Interleukin-2	Stimulates growth of T lymphocytes Stimulates B-lymphocyte and monocyte differentiation Increases cytotoxicity of T lymphocytes and natural killer cells	Activated T lymphocytes
Interleukin-3	Multipotential hematopoietic cell growth factor Stimulates early B and T lymphocytes Mast cell growth factor	Activated T lymphocytes, natural killer cells
Interleukin-5	Stimulates eosinophil formation and differentiation Augments T lymphocyte cytotoxicity and proliferation of B lymphocytes	T lymphocytes, mast cells
Interleukin-7	Supports growth of pre-B lymphocytes Stimulates T lymphocytes	B lymphocytes, bone marrow fibroblasts, monocytes
Interleukin-8	Stimulates neutrophil, monocyte, and lymphocyte activation chemotaxis	Monocytes
Interleukin-9	Stimulates neutrophil, monocyte, and lymphocyte activation chemotaxis Stimulates erythroid progenitors, helper T-lymphocyte growth factor	T lymphocytes
Interleukin-11	T lymphocyte–dependent stimulator of B lymphocytes	Bone marrow fibroblasts
Interleukin-12	Stimulates helper T-lymphocyte differentiation and interleukin-2 production Stimulates interferon-γ production Increases cytotoxicity of natural killer cells	T and B lymphocytes, lymphoblastoid cells
Interleukin-14	Stimulates proliferation of activated B lymphocytes Inhibits immunoglobulin secretion from B lymphocytes	T lymphocytes
Interleukin-15	Stimulates T-lymphocyte function and proliferation Enhances natural killer cell function	Monocytes and macrophages
Interleukin-16	Promotes migration of T lymphocytes	T lymphocytes
Interleukin-17	Stimulates interleukin-6 and interleukin-8 production	T lymphocytes
Interleukin-18	Stimulates interferon-γ and tumor necrosis factor–α production	Macrophages, mononuclear cells, and dendritic cells
Granulocyte colony-stimulating factor	Stimulates neutrophil colony formation	Monocytes and fibroblasts
Granulocyte-macrophage colony-stimulating factor	Stimulates granulocyte and monocyte formation Induces tumor necrosis factor	T lymphocytes, natural killer cells, endothelial cells, fibroblasts, and keratinocytes
Macrophage colony-stimulating factor	Activates monocytes and granulocytes Stimulates macrophage colony formation Induces interleukin-1 and tumor necrosis factor	Fibroblasts, monocytes, and endothelial cells

TABLE 254–2 Anti-inflammatory Cytokines

Cytokine	Function	Predominant Cell Source
Interleukin-4	Stimulates proliferation of T and B lymphocytes and megakaryocytes A growth factor for mast cells and erythroid precursors	T lymphocytes
Interleukin-6	Blocks endotoxin induction of interleukin-1 and tumor necrosis factor B and T lymphocyte–stimulating activity	Monocytes, T and B lymphocytes, fibroblasts, epithelial and endothelial cells
Interleukin-10	Blocks production of interleukin-1 and tumor necrosis factor Inhibits primary allogeneic T-lymphocyte responses Inhibits interleukin-2, interleukin-8, and granulocyte-macrophage colony-stimulating factor	T and B lymphocytes, macrophages and monocytes
Interleukin-13	Blocks production of interleukin-1, tumor necrosis factor, and interleukin-8 Suppresses nitric oxide formation	T lymphocytes
Interleukin-1 receptor antagonist	Binds interleukin-1 receptors, blocking interleukin-1 effects	Monocytes and macrophages
Transforming growth factor–β	Reduces endotoxin-induced interleukin-1 and tumor necrosis factor production	Monocytes and macrophages

rides or endotoxin, which induce the early proinflammatory cytokines TNF-α and IL-1. Although these cytokines may protect the host from infection, if they are expressed in excessive amounts, their effects can result in multiple organ failure and death. During severe sepsis, the levels of both TNF-α and IL-1 increase proportionately with the degree of hypotension and organ failure. The combination of these two cytokines can result in synergism over their individual effects by severalfold, leading to lethal septic shock syndrome.[35,36] In animal models of shock and gram-negative sepsis, TNF-α levels rise rapidly after injection of bacteria or endotoxin, reaching peak concentrations at 60 to 90 minutes, whereas IL-1 levels rise more slowly, peaking at 180 minutes. A similar time course response has been observed in human subjects injected with endotoxin.[36,184] Children with septicemia and purpura fulminans and children with meningococcal disease demonstrated an association between morbidity and mortality and high serum levels of TNF-α and IL-1.[87,192] These studies and others implicate TNF-α and IL-1 as prominent modulators in the development of the septic shock syndrome and suggest that a potential therapeutic benefit may be obtained by inhibiting the production of these cytokines and reducing their proinflammatory effects.

Experimental attempts to attenuate the excessive proinflammatory cytokine activity of TNF-α and IL-1 have focused on (1) inhibiting release of endotoxin, (2) blocking endotoxin–target cell binding and preventing the transmembrane signaling mechanisms leading to production of TNF-α and IL-1, (3) controlling the synthesis of TNF-α and IL-1 by inhibiting or suppressing specific cytokine gene transcription and translation, (4) inhibiting release of TNF-α and IL-1, (5) administering TNF-α and IL-1 neutralizing antibodies and soluble receptors, (6) producing and administering TNF-α and IL-1 receptor antagonists that block specific cytokine binding to target cell receptors, and (7) blocking TNF-α or IL-1 intracellular transmembrane signaling mechanisms and preventing their action on target cells.[154]

The use of monoclonal anti-endotoxin antibodies to inhibit the binding of endotoxin to its target cells has received considerable attention. Numerous in vitro and in vivo animal experiments have suggested that blocking of endotoxin leads to improved survival by inhibiting proinflammatory cytokine production and expression.[193] The initial clinical studies in patients with gram-negative bacteremia treated with immunoglobulin preparations directed against endotoxin demonstrated a significant reduction in mortality rates.[14,222] Further studies suggested a reduction of septic shock in similarly treated high-risk surgical patients.[14] These observations led to the development of several clinical trials using human monoclonal anti-endotoxin antibodies. HA-1A is a human monoclonal antibody against the lipid A moiety of bacterial endotoxin. The mechanism of HA-1A action is to block endotoxin triggering of the intracellular events leading to proinflammatory cytokine synthesis. In placebo-controlled clinical trials of HA-1A, either HA-1A or placebo was infused during the course of 20 minutes to patients with severe sepsis. These patients also received cardiopulmonary support and antibacterial therapy. The etiologic agents of sepsis included *E. coli*, *Pseudomonas*, and *Klebsiella* and *Enterobacter* spp. The authors reported that HA-1A significantly reduced the incidence of mortality in adults with septic shock and gram-negative bacteremia. The mortality rate for those patients who were in severe shock before receiving HA-1A was reduced by 42 percent.[268] Although these early clinical studies using HA-1A were encouraging, a protective role for anti-endotoxin antibodies has not been established in subsequent clinical trials.[168] Anti-endotoxin therapy may be more effective if it is given earlier during the sepsis syndrome before the development of shock. Because bacterial lysis caused by antibiotics is an ongoing process and results in further release of endotoxin, multiple dosing of anti-endotoxin antibodies also may prove beneficial in treating sepsis. The future clinical use of anti-endotoxin antibodies may be in combination with other immunomodulation therapies, directed at simultaneously blocking several steps in both the propagation and action of proinflammatory cytokines.

Studies of cytokine inhibition have focused on controlling TNF-α and IL-1 with anti–TNF-α antibodies directed at these specific cytokines and their receptors. Control of proinflammatory cytokine synthesis is specific for each individual cytokine and requires an understanding of the unique temporal relationships these molecules have during the propagation of inflammatory responses. A critical aspect of TNF-α and IL-1 gene expression in a variety of cell types has been the reported exquisite sensitivity these cytokines have to bacterial endotoxin.[45,62,146,154] Human blood monocytes synthesize TNF-α and IL-1 in the presence of endotoxin. In the absence of endotoxin, however, gene expression occurs, but protein translation does not take place.[223] Thus, these cells may be viewed as being primed for bacterial endotoxin exposure. TNF-α and IL-1 transcription is suppressed by the anti-inflammatory cytokines IL-10, IL-13, and transforming growth factor–β, but the clinical therapeutic benefits these cytokines have in treating septic shock remain to be defined.[47,103,186,224] Agents blocking the lipoxygenase pathway of arachidonate metabolism also have been implicated in the reduction of TNF-α and IL-1 synthesis, and corticosteroids have been shown to sup-

press both TNF-α and IL-1 transcription and synthesis, but only when they have been administered before transcription has been initiated.[78,221] The use of corticosteroids in infants and children with bacterial meningitis has demonstrated that treatment with a combination of dexamethasone and antibiotics results in lower cerebrospinal fluid levels of TNF-α than occurs with treatment with placebo and antibiotics.[192] In addition, patients treated with corticosteroids had fewer neurologic symptoms.[132] Subsequent multicenter trials, however, have failed to establish a protective effect of corticosteroid use in the treatment of children with meningitis.[249] One possible explanation for this discrepancy may be that corticosteroids almost exclusively suppress endotoxin-induced, proinflammatory cytokine gene transcription but have little or no effect on proinflammatory cytokine translation.[62,233] Thus, some investigators have suggested that the early administration of corticosteroids, before transcription has been initiated, would block cytokine synthesis. Timing of corticosteroid administration, therefore, may account for some of the clinical variability seen with its use in children with meningitis. Clinical trials are focusing on TNF-α and IL-1 synthesis with the administration of corticosteroids either before or while antibiotics are administered. Similarly, the temporal use of antibiotics, corticosteroids, and anti-endotoxin therapy may result in an additive therapeutic benefit.

Neutralizing monoclonal antibodies against murine or human TNF-α have been shown to decrease mortality rates in several experimental animal models of sepsis.[18,176,243] Studies with soluble TNF-α receptors or their immunoadhesin constructs also have demonstrated an immunoprotective effect of TNF-α blockade in animal models of endotoxemia or bacteremia.[17] Although anti–TNF-α antibodies are being used with caution in humans, anti–TNF-α treatment in a limited number of patients with established septic shock resulted in increased vascular hemodynamics and left ventricular stroke volume.[97] The in vitro use of free soluble TNF-α receptors to bind TNF-α results in a 10- to 50-fold increase in binding affinity over that observed with anti–TNF-α monoclonal antibodies.[98] Results from phase II clinical trials using soluble TNF-α receptors, however, have not shown improvement in survival rates; moreover, administration of high doses actually increased the incidence of mortality.[98,219]

Certain naturally occurring substances inhibit IL-1 synthesis and action, but they also have effects on many of the other cytokines. Specific inhibitors of IL-1, however, have been identified. Most prominent of these is the IL-1 receptor inhibitor that competes with the binding of IL-1 to its cell surface receptor. Recombinant IL-1 receptor antagonist (IL-1ra) that blocks IL-1 effects has been studied in various animal models.[4,200] For instance, IL-1ra prevents death from endotoxic shock in rabbits.[200] The therapeutic use of IL-1ra in treating septic shock in early phase II human clinical trials showed improved survival rates at 28 days.[79] The subsequent randomized phase III trials, however, failed to show improvement in patients with severe shock.[78] Further clinical studies are ongoing. IL-1ra also has been used in treatment trials of patients with acute myelogenous leukemia. The uncontrolled production of IL-1 by leukemic blasts has been proposed to result in the continued proliferation of these cells and the development of acute myelogenous leukemia. Studies have shown that IL-1ra blocks the spontaneous proliferation and production of granulocyte-macrophage colony-stimulating factor (GM-CSF), IL-1, and IL-6 in the peripheral blood and bone marrow cells of these patients.[211] The potential clinical use of IL-1ra is being investigated in patients with psoriasis, rheumatoid arthritis, and myelogenous leukemia.[100] Similarly, combination therapy with the simultaneous blockade of both TNF-α and IL-1 is being investigated in endotoxin models of shock and a number of clinical disease states.[217]

Another approach to attenuate excessive proinflammatory cytokine responses is the use of pharmacologic agents. Salyer and colleagues[220] demonstrated that pentoxifylline, a methylxanthine derivative that blocks TNF-α transcription and production, could override some of the effects of TNF-α on PMNs. In their study, the profound decrease in human PMN chemotactic ability caused by excessive TNF-α was restored to normal by treatment with pentoxifylline. The specific mechanisms of pentoxifylline's effect are not known, but it has been shown to restore PMN membrane fluidity inhibited by TNF-α that is critical for cell movement.[197] Furthermore, pentoxifylline can block PMN adhesion to endothelium and result in decreased PMN respiratory burst activity, which is thought to be responsible for the improved survival rates seen in pentoxifylline-treated animal models of endotoxin-induced septic shock.[201] Some of the clinical trials using pentoxifylline in surgical patients with early evidence of systemic inflammation have suggested a cardioprotective effect, although other studies have been less convincing.[71,236] Pentoxifylline also has been evaluated in preterm infants with sepsis.[156] A total of 78 infants with documented sepsis were randomly assigned to receive pentoxifylline (5 mg/kg/hr for 6 hours on 6 successive days) or placebo. Pentoxifylline-treated infants had significantly lower levels of TNF-α but not of IL-1. Only 1 of 40 infants in the pentoxifylline-treated group died, compared with 6 of 38 in the control group ($p < .05$). Amrinone, a phosphodiesterase inhibitor, also has been shown to reduce TNF-α levels in LPS-challenged mice.[88] Thus, these and other pharmacologic agents may be useful in preventing or reducing some of the adverse and even fatal effects that are mediated by proinflammatory cytokines.

IVIG may have a potential inhibitory effect on proinflammatory cytokine activity. Patients in the active phase of Kawasaki disease have increased levels of TNF-α and IL-1. These cytokines have been postulated to stimulate local inflammatory responses by regulating leukocyte adherence and activation, leading to the vascular damage that is a critical clinical aspect of this disease. Another suggestion is that the effects of IVIG therapy in Kawasaki disease, and perhaps in other diseases, may be results of the attenuating production of the proinflammatory cytokines TNF-α and IL-1.[6,10,110] Animal models of LPS-induced TNF-α and IL-1 synthesis demonstrate a suppression of mononuclear cell synthesis of these proinflammatory cytokines when they are treated with IVIG.[12] The peripheral blood mononuclear cell production of TNF-α and IL-1, however, in patients with Kawasaki disease receiving IVIG therapy showed decreased synthesis of IL-1 but not of TNF-α.[157,158] The role of IVIG as an immunomodulator has been suggested in an ever growing number of clinical disease states because of its broad immunoregulatory potential.[10,107] Its specific role in arresting proinflammatory cytokine activity, either directly or indirectly, warrants further clinical investigation.

The hypothesis that diminished levels of cytokines may render a host susceptible to development of infection was introduced by Weatherstone and Rich.[252] They suggested that the increased susceptibility to infection observed in premature neonates may be secondary to deficient proinflammatory cytokine production. In their study, they measured cord blood monocyte secretion of TNF-α and IL-1 with and without stimulation of LPS. IL-1 activity by LPS-stimulated preterm monocytes did not differ from that observed by LPS-stimulated adult monocytes. TNF-α activity, however, in the LPS-stimulated monocytes from preterm neonates was significantly lower than that in both stimulated and unstimulated adult monocytes. Thus, they concluded that diminished production of TNF-α may predispose the preterm infant to acquisition of infection. This finding has not been supported by the studies of infected animals, which show markedly elevated TNF-α levels, and subsequent studies in preterm infants have not established a deficiency in TNF-α.[259] Williams and colleagues[259] and Peat and coworkers[203] have, in contrast, shown that mononuclear cells from term newborns produced enhanced levels of TNF-α in response to group B streptococci or endo-

toxin. Ongoing studies are defining the role of cytokine production in neonatal and other infections.

The proinflammatory cytokine IL-2 acts on activated T cells and to some extent on B cells and natural killer cells and causes them to proliferate or differentiate. IL-2 is synthesized by both T cells and natural killer cells.[185,250] Decreased IL-2 production or IL-2 receptor expression has been noted in a number of clinical disease states, most notably in cases of severe combined immunodeficiency disease. Lesser degrees of abnormalities may occur in acquired immunodeficiency syndrome (AIDS), type 1 diabetes mellitus, systemic lupus erythematosus, and hypogammaglobulinemia.[146,154,164] The most intriguing potential for IL-2 in the treatment of disease involves its use in tumor therapy.[216] IL-2 currently is approved for the treatment of metastatic renal carcinoma. Use of IL-2–activated natural killer cells results in a decrease in tumor burden in approximately 20 percent of patients, although serious side effects occur. In addition to its potential role as an antitumor agent, IL-2 shares many of the same effects as interferon-γ (IFN-γ) and may, someday, function as a therapeutic agent in infection, autoimmunity, and immunodeficiency.[154,216]

Both IL-8 and IL-9 have been investigated in various human disease states, primarily because of their powerful role in stimulating neutrophil function, including activation, adhesion, and chemotaxis. Patients with cystic fibrosis, bronchiectasis, and chronic bronchitis have demonstrated elevated levels of IL-8 in their sputum.[212] The sputum from these patients is highly chemotactic to neutrophils, but with treatment with monoclonal antibodies to IL-8, this chemotactic effect was inhibited. Aerosolized IL-8 inhibitors have been used in patients with cystic fibrosis and have resulted in decreased inflammation in these patients.[178,212] IL-8 inhibitors are being evaluated in infants with bronchopulmonary dysplasia. Researchers have speculated that the persistently elevated levels of IL-8 in the tracheal fluid of ventilated preterm infants lead to neutrophil accumulation and the development of pulmonary fibrosis, which may be reduced by IL-8 inhibitors.[129,138] Similar studies are being conducted with IL-9 inhibitors. Thus, these cytokines may play an important role in the acute inflammatory response leading to chronic disease states, and blocking of this response may be of therapeutic benefit. The proinflammatory IL-5 has not been implicated in a specific human disease state, but its strong B-cell proliferative effects suggest that it has a possible role in the pathogenesis of immunodeficiency, and its potent effects on eosinophil production, activation, and migration implicate its action in allergic responses.[33]

The proinflammatory activities of IL-12 and IL-18 are of current immunologic interest as well. IL-12 is an integral immune regulator that is produced primarily by macrophages and dendritic cells.[198] IL-12 has been shown to induce the production of IFN-γ by T cells and NK cells.[171,172,198] IL-18 is another recently described cytokine with many proinflammatory functions.[180] IL-18 was defined initially as IFN-γ–inducing factor.[37,61,155] IL-12 and IL-18, both alone and in synergy, regulate IFN-γ production in response to infection with intracellular parasites and bacteria and in certain autoimmune diseases.* The regulation of these cytokines may be therapeutically beneficial in certain disease states.[66,93] The other proinflammatory cytokines also are in various developmental stages of experimental investigation. As we learn more about their specific actions, we will better be able to determine their use as potential immunomodulating agents.

The anti-inflammatory cytokines have limited clinical use. IL-4 can block both IL-1 and TNF-α transcription.[103,224] IL-4 has been shown to inhibit human neutrophil adhesion to human endothelial cells while enhancing the adhesion of eosinophils.[189] These effects have not been implicated in specific human diseases, but an IL-4 action is suggested in allergic responses. Although IL-6 has both anti-inflammatory and proinflammatory effects, the levels of this cytokine were found to correlate with mortality rates in children with gram-negative and gram-positive sepsis, suggesting that monitoring of IL-6 levels may be of prognostic value.[246] Similarly, IL-10 has been shown to block production of IL-1, IL-6, IL-8, IL-12, and TNF-α as well as GM-CSF in animal models.[54,173] It also has effects on mast cells, T cells, and natural killer cells, and it inhibits primary allogeneic T-cell responses. Thus, IL-10 may have a potential role in treating acute and chronic inflammation and may be effective in suppressing transplant rejection.[15,54] Studies examining the safety and immunomodulatory effects of the intravenous injection of IL-10 in humans demonstrate that it is well tolerated and results in decreased production of both TNF-α and IL-1.[25] Additional clinical studies are being conducted on the potential immunoregulatory effects of the anti-inflammatory cytokines, which may indicate their therapeutic use in human disease states.

*References 61, 66, 93, 120, 131, 175, 199, 234, 238, 247, 253, 265.

COLONY-STIMULATING FACTORS

Colony-stimulating factors are involved principally in the production of neutrophils and monocytes. They were discovered because of their ability to stimulate the formation of colonies of granulocytes and monocyte-macrophages in cultured bone marrow cells and were named according to the primary cell colony type that they elicited. Granulocyte-macrophage colony-stimulating factor (GM-CSF) induces peripheral blood macrophages and granulocytes. It also has other pleiotropic effects, including stimulation of precursors of megakaryocytes, mast cells, and eosinophils. In addition, GM-CSF has effects on neutrophil migration and phagocytosis. Granulocyte colony-stimulating factor (G-CSF) induces peripheral blood granulocytes. Its actions are on both the production and function of neutrophils, including migration, phagocytosis, and superoxide generation. Macrophage colony-stimulating factor (M-CSF) induces peripheral mononuclear phagocytes. IL-3 increases mast cell populations as well as the induction of granulocytes, macrophages, eosinophils, and megakaryocytes.[63,96,154,155,163,183]

Clinical studies with GM-CSF and G-CSF as adjuvant therapy have been performed in individuals with distinct lymphopoietic disorders (i.e., congenital agranulocytosis, cyclic neutropenia, Shwachman-Diamond syndrome) or those due to the consequences of cytotoxic chemotherapy, AIDS, and aplastic anemia. The colony-stimulating factors can stimulate granulocyte and monocyte populations in these individuals, but the response is restricted to the number of available stem cells. Colony-stimulating factor treatment can partially or completely reverse congenital neutropenia and has shown great promise in regeneration of lymphopoietic cells after administration of cytotoxic chemotherapy and high-dose chemotherapy followed by autologous bone marrow transplantation. Recombinant human GM-CSF (rhGM-CSF) also has been shown to be beneficial in patients with aplastic anemia.[9,244] Hammond and colleagues[101] demonstrated a dramatic increase in neutrophil counts in children with cyclic neutropenia treated with recombinant human G-CSF (rhG-CSF). Cyclic neutropenia is a rare disorder characterized by regular 21-day cyclic fluctuations in the number of blood neutrophils, monocytes, eosinophils, lymphocytes, platelets, and reticulocytes. Although the exact mechanism of this disorder is not known, it is attributed to a regulatory abnormality affecting proliferation of stem cells. These infants have recurrent aphthous stomatitis, pharyngitis, lymphadenopathy, fever, and numerous infections during the periods of neutropenia. The length of cycling in treated infants decreased from 21 days to 1 day, and the neutrophil turnover rate increased nearly fourfold, signifi-

cantly reducing the frequency of infection. Recombinant G-CSF also has shown promise for infants and children with neutrophil production disorders. Infants with congenital agranulocytosis, Kostmann syndrome, and Shwachman-Diamond syndrome, disorders characterized by severe, persistent absolute neutropenia, show a dramatic increase in neutrophil count after treatment with rhG-CSF.[24] In addition, rhG-CSF and rhGM-CSF have been used in neutropenic patients with AIDS. Despite the concern that the effects of GM-CSF to activate macrophages may in turn promote replication of human immunodeficiency virus (HIV), current studies are defining the role of rhGM-CSF and rhG-CSF in patients with AIDS.[95,163]

Thus, colony-stimulating factors have emerged as significant modulators of human immune function and lymphopoiesis. Because the colony-stimulating factors can functionally activate mature granulocytes and monocytes, considerable attention has focused on their future role in treating individuals at risk for acquiring infection. English and colleagues[65] reported decreased GM-CSF production by neonatal T cells. Because a major factor contributing to the increased susceptibility of human neonates to severe infections is their inability to produce adequate numbers of neutrophils in response to bacterial infections, these neonates may benefit from colony-stimulating factor treatment during severe infection.[20] Similar treatment in immunologically stressed burn and trauma patients also may prove beneficial.[56,196]

INTERFERONS

INTERFERONS α AND β

The interferons are glycoproteins that were discovered because of their antiviral properties. They are known to possess antitumor and immunomodulatory activities in addition to their antiviral effects.[11] The interferons have been classified into three major groups: α, β, and γ. IFN-α and IFN-β were previously known as type I interferon and have similar protein structures and bind the same receptor. IFN-γ, formerly type II interferon, has a much different structure and its own receptor.[8] IFN-α is produced by leukocytes. The earliest demonstration of its clinical usefulness was in the treatment of AIDS-related Kaposi sarcoma. In these initial studies, a Kaposi sarcoma tumor response occurred in 30 to 50 percent of the patients treated with recombinant IFN-α.[94,142,187,191] Researchers have speculated that IFN-α exerts its antitumor effect by activating cytotoxic T cells. Since then, placebo-controlled clinical trials have shown that IFN-α also may have significant antiretroviral effect in HIV-infected patients.[141,152] Clinical trials have been conducted to determine the effect of early treatment with IFN-α in reducing progression of HIV disease and to determine the therapeutic effect of IFN-α in combination with other drugs in the treatment of HIV infection and HIV-related diseases.[140,140a]

IFN-α also has been used in various parts of the world for the treatment of chronic myeloid leukemia, hairy-cell leukemia, basal cell carcinoma, multiple myeloma, hepatitis B and C, and condylomata acuminata. In chronic myeloid leukemia, early treatment with IFN-α has been reported to elicit complete hematologic remission in more than 70 percent of the patients treated. Many of these patients had total elimination of the Philadelphia chromosome, which is a hallmark of the disease.[239,240,241] Complete to 80 percent remissions also have been reported in the treatment of hairy-cell leukemia.[208] Intralesional injection of IFN-α into basal cell carcinoma of the skin resulted in 81 percent tumor remission as determined by biopsy.[50,125] Despite the variability of the outcomes among reports on the use of IFN-α in the treatment of multiple myeloma, it has been approved as a therapy for these patients in a number of European countries.[174]

IFN-α also is used for the treatment of hepatitis B and C.[155] Clinical responses have been reported to be of long duration, often with complete loss of both hepatitis B surface antigen and evidence of viral replication. In chronic hepatitis C, complete responses to therapy determined by the decline of serum aspartate aminotransferase to normal levels have been observed in 50 to 70 percent of the patients treated. Although serum aspartate aminotransferase levels in half of the patients who had improved returned to pretreatment levels within 6 to 12 months after discontinuation of therapy, nearly 20 percent achieved sustained remission.[57,60,119,139,204] Studies also have shown that IFN-α is useful in treating genital warts, condylomata acuminata caused by papillomaviruses.[32] Intralesional injections of IFN-α completely eliminated warts in more than 50 percent of the patients treated. IFN-α therapy also is suggested to reduce the number of lesions in juvenile laryngeal papillomatosis after systemic use.[169] Thus, IFN-α has therapeutic potential as both an antitumor and an antiviral agent.

IFN-β also has received attention as an antiviral and antitumor agent. Its clinical usefulness as a single-agent therapy is suggested in relapsing multiple sclerosis. The mechanism of IFN-β therapeutic action in multiple sclerosis is unknown.[126] Ongoing studies are investigating IFN-β therapy in combination with IFN-α in the treatment of various malignant neoplasms.

INTERFERON γ

IFN-γ is produced by $CD4^+$ and $CD8^+$ cells as well as by natural killer cells.[108] Investigators have reported that circulating mononuclear cells and T lymphocytes from neonates are markedly deficient in their ability to produce IFN-γ in response to a variety of stimuli, compared with adult cells.[162,226,227,260] Studies using recombinant IFN-γ have shown that preincubation of neonatal neutrophils, which are deficient in their chemotactic ability, with recombinant IFN-γ enhances their chemotactic response to a level equal to that of adult neutrophils.[108] Other studies have shown that neonatal mixed mononuclear cells are deficient in the production of the IFN-γ–stimulating cytokines IL-12 and IL-18 in response to GBS.[112,114,130] In vitro treatment with recombinant IL-12 and IL-18 can correct this defect in IFN-γ production in response to GBS. These findings suggest that a potential role exists for the regulation of IFN-γ in neonatal host defense.

Job syndrome was first described by Davis, Schaller, and Wedgwood[58] in 1965 in two patients with recurrent staphylococcal abscesses. Patients with this syndrome often develop chronic sinopulmonary infections and mucocutaneous *Candida* infections. Hill and coworkers[115] observed that the patients with Job syndrome also have a profound defect in neutrophil chemotactic responsiveness along with extreme hyperimmunoglobulin E. This defect in neutrophil chemotaxis is intermittent and occurs predominantly when the patient is symptomatic.[55,109,115] Because production of IFN-γ by mononuclear leukocytes in patients with hyperimmunoglobulin E is markedly deficient or absent, in vitro studies were conducted to determine the effect of recombinant IFN-γ on the chemotactic responsiveness of neutrophils from patients with this syndrome. After pretreatment with IFN-γ, the chemotactic response of the neutrophils from patients with Job syndrome increased significantly, with an average enhancement of 300 percent above baseline to levels not significantly different from those of matched healthy controls.[128] In four patients with Job syndrome, preliminary trials of IFN-γ therapy with hyperimmunoglobulin E suggested clinical benefit in three, with a significant decrease in eczema as well as in pulmonary symptoms and secretions.

Patients with chronic granulomatous disease (CGD) have an inherited deficiency in the proteins required for nicotinamide

adenine dinucleotide phosphate oxidase activity. Phagocytes with this enzymatic defect are able to engulf bacteria but cannot generate the respiratory burst necessary to kill the organisms. Consequently, patients with CGD suffer severe chronic, recurrent life-threatening infections. The usefulness of treating CGD patients with IFN-γ was suggested by studies showing that this lymphokine can stimulate the respiratory burst of normal phagocytes. Results of studies by Ezekowitz and colleagues[69] and Sechler and coworkers[225] showed that when macrophages from patients with CGD were treated with IFN-γ in vitro, a respiratory burst occurred and superoxide anion was generated. Sechler and colleagues[225] further demonstrated a partial correction in neutrophils and monocytes from patients with CGD after subcutaneous treatment with recombinant IFN-γ. These initial results suggested that when it is administered in vivo to patients with CGD, recombinant IFN-γ could partially correct the defective ability of phagocytes to kill bacteria. Ezekowitz and coworkers[70] extended these findings in a double-blind placebo-controlled trial. They showed that recombinant IFN-γ significantly decreased the relative risk for development of serious infection in patients with CGD, but these studies failed to demonstrate an effect of IFN-γ on respiratory burst activity. This cytokine most likely affects the arginine–nitric oxide pathway in neutrophils and macrophages. Patients who received IFN-γ had a 70 percent reduction in the risk for development of serious infection compared with controls. Overall, IFN-γ decreases the risk for development of infection and the duration of hospitalization in patients with CGD. When it is administered with prophylactic antibiotics, an additive effect occurs, resulting in a nearly 20 percent increase in the infection-free rate of patients with CGD compared with IFN-γ alone.[70] IFN-γ was licensed by the Food and Drug Administration in December 1990 for the treatment of patients with CGD. The authors of the collaborative studies recommended its use with the addition of prophylactic antibiotics for treatment of patients diagnosed with CGD.[84]

TOLL-LIKE RECEPTORS

Toll-like receptors (TLRs) are pathogen pattern-recognition receptors central to innate immune responses.[127] TLRs are a family of type 1 integral membrane glycoproteins that detect pathogens and initiate cytokine production by activation of NF-κB. The ligands recognized by TLRs consist of a wide range of evolutionarily conserved motifs, such as LPS, single- and double-stranded RNA, and zymosan. Collectively, these motifs are called pathogen-associated molecular patterns (PAMPs). TLR recognition of PAMPs contributes to the immediate response and broad specificity of innate immune recognition toward bacterial, viral, fungal, and parasitic pathogens. Twelve TLRs have been described in mammals, with at least 10 known in humans.[2,3,6]

The role of TLRs as immunomodulating agents is of contemporary interest. Amlie-Lefond and colleagues[5] described the potential use of TLR agonists as protective agents against biothreat pathogens. TLRs 1 to 10 in humans act to augment the immune response. TLR agonists can bolster this response and potentially offer protection against specific infections or diseases. Stimulation of TLR-7 and TLR-8 has shown antiviral and antineoplastic properties in humans. A drug known to do this, imiquimod (Aldara), has been shown to be beneficial against human papillomavirus, molluscum contagiosum, genital warts, and basal cell carcinoma.[5,51,242]

Immunomodulatory oligonucleotides stimulate Toll-like receptors and induce cytokine production. Immunomodulatory oligonucleotides offer potential clinical use for treatment of a number of inflammatory diseases, including severe acute respiratory syndrome (SARS), for which clinical trials are under way.[5] Similar use of immunomodulatory oligonucleotides in patients with renal carcinomas and HIV infection also is under investigation. Recent evidence suggests that TLR-3 activation can enhance innate immune response and enhances production of IFN-α.[143] These features render TLR-3 agonists attractive agents in the treatment of HIV-related infections and infections in certain immunocompromised patients.

TLR expression in human neonates is currently being defined.[218] TLR-2, TLR-4, and TLR-8 functions have been described in neonates. Researchers have speculated that deficiency of TLR activation may contribute to the neonate's increased susceptibility to infection.[248] The results of TLR expression in neonates have varied. In general, similar expression of TLRs 1 to 4 occurs in newborn infants and adults. Low-birthweight or premature infants, however, demonstrate significantly decreased expression of TLR-4.[81,242] Most studies describe decreased cytokine production to TLR agonists in neonates compared with adults. Caron and colleagues[147] reported significantly lower LPS stimulation of TLR-4 in neonates compared with adults and speculated that this decreased stimulation may contribute to the neonate's increased susceptibility to *E. coli* and other gram-negative infections.[147] Similarly, Levy and colleagues[161] demonstrated that TLR-8 agonists are beneficial in activating co-stimulatory responses in neonatal antigen-presenting cells and suggested that this type of agent may be used adjuvantly to enhance the neonatal immune response.

NEUTROPHILS AND COMPLEMENT

Recruitment of neutrophils from the bloodstream to extravascular sites of inflammation is a critical event in host defense against bacterial infection and in the repair of tissue damage. Under certain circumstances, accumulation of neutrophils may contribute to vascular and tissue injury. Thus, the regulatory mechanisms involved in neutrophil activation, recruitment, and subsequent degranulation are of potential clinical significance. Neutrophil adherence to and migration through capillary endothelium is a critical early event in the acute inflammatory response. The adhesive interactions between leukocytes and endothelial cell surfaces are regulated by two novel families of glycoproteins: the integrins and the selectins. The β_2 integrins are membrane-bound glycoprotein receptors found on the surface of PMNs. The β_2 integrins CD11 and CD18 are required for adherence of PMNs to endothelial cell surfaces. The selectins also are membrane-bound glycoproteins that mediate neutrophil adhesion to endothelial cells. They include L-selectin, which is found on the surface of PMNs, and P-selectin and E-selectin, which are expressed on the surface of activated endothelial cells.[19,42,235,255,270]

The interaction between the β_2 integrins and the selectins serves to regulate PMN responses during inflammation. In general, the selectins P and E on the activated endothelial cell surface and L-selectin on the PMN cell surface function to facilitate PMN rolling and tethering to activated capillary endothelium. Once this tethering has occurred and the PMN itself is activated, the β_2 integrin CD11/CD18 receptors on the PMN form a tight adhesion with the endothelial cell surface, which facilitates PMN attachment and polarization, leading to migration.[19,42,235,270] Congenital β_2 integrin CD11/CD18 deficiency states have been described (leukocyte adhesion deficiency type I). These patients have profound PMN adhesion and motility defects and recurrent life-threatening infections along with delayed separation of the umbilical cord and juvenile periodontitis.[68,83] A second type, leukocyte adhesion deficiency type II, has been described as being due to a deficiency of sialyl $Lewis^X$, the PMN ligand for E-selectin on endothelial cells.[205] Researchers also have shown that the tethering of PMNs to P-selectin on activated endothelial cells is critical for PMN priming by platelet-

activating factor and that monoclonal antibodies to P-selectin can block this response.[166] Monoclonal antibodies to P-selectin have been used in animal models of ischemia and reperfusion injury and have been shown to reduce significantly the severe edema and endothelial cell injury observed after reperfusion.[261] Similarly, monoclonal antibodies to P-selectin have resulted in significant endothelial cell preservation in animal models of lung injury and cardiac ischemia.[190,256] Monoclonal antibodies to E-selectin and L-selectin also are being tested in animal models.[179] With the rapid advancements occurring in identification of new molecules that influence endothelial cell–leukocyte interactions, we will gain greater understanding of the complexity of cellular communication during inflammation.

BACTERICIDAL/PERMEABILITY-INCREASING FACTOR AND DEFENSINS

In addition to their respiratory burst activity in antimicrobial defense, human neutrophils have been shown to contain a variety of granule-associated antibacterial proteins and peptides.[213] These include bactericidal/permeability-increasing protein (BPI) and the defensin family of peptides. BPI is a 55-kd receptor present in neutrophil granules, which contain two domains. One of the domains binds with LPS to increase membrane permeability and the lysis of gram-negative bacteria, whereas the other promotes the opsonization of gram-negative bacteria.[117] Neutrophil BPI functions best in inflamed tissues, where it acts in concert with defensins and the membrane attack complex of complement to cause cell lysis.

Meningococcemia is a severe gram-negative infection that occurs predominantly in infants and young adults. Mortality rates of meningococcemia range from 10 to 20 percent.[8,30,105,257,263] Endotoxin levels may be profoundly elevated and correlate with the severity of the illness.[30] Recombinant BPI (rBPI) has been used as an adjunct to antimicrobial therapy in children with severe meningococcal sepsis[159]; 14 of 193 patients with severe meningococcal disease who received rBPI (2 mg/kg during 30 minutes) died, compared with 20 of 203 control infants, an insignificant difference. Among the surviving treated patients, however, a modest improvement was noted in long-term functional outcome, suggesting a possible beneficial effect in decreasing complications associated with septic shock.[89,159] Neonates have been shown to have reduced release and activity of BPI, which may contribute to their enhanced susceptibility to gram-negative bacterial infections.[160]

The defensins are strongly cationic, single-chain peptides contained in neutrophil primary, or azurophilic, granules with molecular weights between 3 and 4.5 kd. Defensins compose 50 percent of the protein content of the neutrophil primary granules.[85,266] These peptides possess broad antimicrobial activity against gram-positive and gram-negative bacteria, fungi, mycobacteria, and some viruses. The defensins create voltage-sensitive pores in microbial membranes, resulting in cell lysis. They are divided into α and β defensins. Humans have six human α defensins (HAD 1-6) and two human β defensins (HBD-1 and HBD-2).

The α defensins, HAD 1-6, are made by neutrophils and compose 30 to 50 percent of the primary granule content.[104] Defensins appear at the site of inflammation. They are present after neutrophil degranulation induced by LPS, IL-8, C5a, and other stimuli. They also are found on the epithelial surfaces of the bronchi and in bronchial lavage fluid of patients with various types of inflammatory lung disease. The antimicrobial activity of the defensins is inhibited by high salt content, which may be clinically important in the immune response of patients with cystic fibrosis.[91]

The β defensins are produced by epithelial cells of the respiratory and gastrointestinal tracts.[22,227] HBD-1 is expressed constitutively by epithelial cells in the bronchi and the intestine, whereas HBD-2 synthesis is up-regulated by inflammatory stimuli, including LPS, TNF-α, bacterial infection, and injury. Thus, HBD-1 acts to kill organisms in the absence of inflammation, whereas HBD-2 acts primarily as part of the inflammatory process.

In inflammatory lung disease, such as chronic bronchitis and chronic obstructive lung disease, both α defensins HAD 1-6 and β defensin HBD-2 may be increased significantly, perhaps contributing to airway inflammation.[266] There are no reported clinical disorders of defensin production. Potential roles for the defensins as immunoregulatory-antimicrobial agents are being investigated.

Endotoxin and other proinflammatory mediators activate the complement system and the chemotactic properties of C5a, which recruits neutrophils to sites of infection. Interruption of this process at various levels may be possible with use of monoclonal antibody strategies that counteract the effects of the complement leading to septic shock. Although monoclonal antibodies directed at the specific components of the complement cascade have not been tested in human clinical trials, the use of monoclonal antibodies to inhibit C5a in primates challenged with *E. coli* improved the survival rate and reduced the incidence of adult respiratory distress syndrome.[7,23]

PLATELET-ACTIVATING FACTOR

Platelet-activating factor (PAF) is a potent phospholipid inflammatory mediator with many biologic effects. Its synthesis is regulated by phospholipase A_2, an enzyme associated with the arachidonic acid pathway. PAF has a very short half-life in vivo because of its rapid degradation by PAF acetylhydrolase. PAF is synthesized by many cell types, including macrophages, neutrophils, platelets, eosinophils, endothelial cells, and hepatocytes.[258] Intravenous infusion of PAF into animals results in pulmonary hypertension, bronchoconstriction, neutropenia, thrombocytopenia, and ischemic bowel necrosis.[16,46,102] Production of PAF is stimulated in numerous clinical disease states, including hypoxia and ischemia, and after administration of biologic agents such as LPS, GM-CSF, TNF-α, IL-1, bradykinin, and thrombin.[72,144,182,206,216,262] Corticosteroids decrease levels of PAF by the induction of its natural inhibitor, PAF acetylhydrolase.[92] PAF has been shown to stimulate the production of many other mediators of inflammation, including TNF-α, complement breakdown products, oxygen radicals, catecholamines, prostaglandins, thromboxane, and the leukotrienes.[74,123,144,215,245,269] It also activates endothelial cells and neutrophils and monocytes, leading to their adherence and migration.[181] Thus, PAF is a ubiquitous phospholipid mediator with many biologic effects and interactions within the inflammatory cascade.

The regulation of PAF has been studied in numerous potential clinical disease states, including sepsis and septic shock.[121] In the clinical phase III trials in septic patients and patients in septic shock who received a PAF antagonist, a significant reduction in the mortality rate was noted.[59] The role of PAF, however, in the pathogenesis of necrotizing enterocolitis (NEC) has received considerable recent attention.[39,40] NEC often is a fatal gastrointestinal disease that predominantly affects premature infants. Exogenous administration of PAF into rat mesenteric circulation causes ischemic bowel necrosis and disease similar to that seen in neonatal NEC.[122] Endotoxin-induced intestinal injury is associated with increased PAF levels, and the infusion of high doses of endotoxin into animals produces a similar pathologic model of NEC, which can be prevented by administration of dexamethasone, PAF acetylhydrolase, or PAF receptor antagonists.[38,124] These animal studies suggest a link between PAF and its regulation and the development of NEC and implicate PAF as a poten-

tial endogenous inflammatory mediator in the pathogenesis of neonatal NEC.

Evidence in humans supports an association between PAF and human neonatal NEC. PAF levels are higher in infants with NEC compared with controls, and PAF acetylhydrolase activity is lower in infants with NEC.[41] PAF acetylhydrolase is suppressed with prematurity.[37] Because enteral feedings are necessary for the development of NEC, PAF levels were measured in feeding premature infants. In these studies, feedings alone increased circulating PAF levels but not PAF acetylhydrolase, and infants fed human breast milk had lower PAF levels and a lower incidence of NEC, suggesting a protective effect of human milk through PAF regulation.[170] Human milk is known to have a number of factors protective against infectious disease, including PAF acetylhydrolase.[34,90] Because PAF acetylhydrolase activity is present in human milk and absent in formulas, the suggestion has been made that the protective activity observed in human milk against the development of NEC may result from blocking of PAF-related inflammatory responses.[34] The modulation of the many interactions of PAF within the inflammatory cascade may have future clinical potential in regulating neonatal NEC and other inflammatory disease states.

NITRIC OXIDE

Nitric oxide is a membrane-permeable gas that functions in the regulation of vascular tone and in the inhibition of platelet aggregation and leukocyte adhesion. In addition, nitric oxide has been shown to have antitumor as well as antimicrobial activity. Under normal conditions, nitric oxide synthase induces endothelial cell production of nitric oxide. The signal transduction pathway for nitric oxide is linked to pathways involving vasodilation. Some evidence indicates that the L-arginine–nitric oxide pathway is activated in sepsis, in which the effects of nitric oxide on the vasculature are associated with the severe vascular failure observed during septic shock.[48,136,188,194,195] Thus, inhibition of production of nitric oxide has been proposed as a novel approach for the treatment of the severe hypotension associated with septic shock.[251]

The increased production of nitric oxide observed during septic shock may have several harmful effects. Nitric oxide may be largely responsible for sepsis-induced hypotension. In vitro studies implicate nitric oxide in sepsis-induced myocardial depression, although nitric oxide synthase inhibitors have not been shown to prevent endotoxin-induced myocardial depression in vivo. Nitric oxide also has direct cytotoxic effects, and its overproduction in septic shock can lead to tissue injury and organ failure.[49,75,134] In addition, in vitro experiments suggest that nitric oxide may enhance the release of proinflammatory cytokines during septic shock.[67,194] Production of nitric oxide may, however, have some beneficial effects during septic shock. It is implicated in maintaining visceral and other microvasculature blood flow, both as a counterregulatory mechanism to the vasoconstrictive mediators released during sepsis and by its ability to block platelet adhesion, reducing potential microvasculature stasis and thrombosis.[135,177] In addition, high levels of nitric oxide have antimicrobial activity and enhance LPS-induced production of cytokine,[145] although whether these levels of nitric oxide reflect actual physiologic states has yet to be determined.

Because hypotension during sepsis is an important predictor of organ injury and death, use of nitric oxide synthase inhibitors may improve survival in severe septic shock by increasing mean arterial pressure. Nitric oxide synthase inhibitors have been shown to restore vascular responsiveness to catecholamines in animal models of endotoxin-induced septic shock.[118] In addition, nitric oxide synthase inhibition has been shown to normalize mean arterial pressure in anesthetized animals challenged with endotoxin or TNF-α without causing hypertension.[134] These considerations have led to the use of nitric oxide synthase inhibitors to treat hypotension in patients with sepsis and in those receiving cytokine therapy for cancer.[133,167] Although these agents can alter mean arterial pressure, beneficial effects on clinical outcomes, including survival, are only suggested in human clinical trials.[194] Studies of endotoxin-challenged rats showed that partial nitric oxide synthase inhibition improved survival, whereas complete inhibition of nitric oxide production clearly is harmful, suggesting a beneficial effect with selective partial nitric oxide inhibition. Studies of nitric oxide inhibition that is more selective are being explored, and in the future such therapy may have clinical utility in the treatment of infectious disease states.

CONCLUSION

The initial attempts to augment immune function in the treatment of sepsis consisted of serum antibody therapy. The development of and refinements in hybridoma and recombinant technologies have provided the means to generate highly specific monoclonal antibodies. Although monoclonal antibodies directed toward bacteria and their components currently are not used during a course of sepsis, their future may be in combination with antimicrobial pharmacologic agents to treat infections with certain pathogens. The recent advances in basic science and clinical research also have demonstrated that the cytokine family plays many crucial roles in the pathogenesis of septic shock. The interaction among the proinflammatory cytokines initiates the development of a cascade of biologic events leading to the propagation and regulation of inflammation. Although the proinflammatory cytokines are effective in augmenting immune responses, their overexpression can lead to severe septic shock. The use of neutralizing monoclonal antibodies to endotoxin, anti–TNF-α antibodies, and soluble TNF-α and IL-1 receptors as well as IL-1 receptor antagonists in human clinical trials of septic shock, however, has shown limited therapeutic potential. The future clinical use of cytokine inhibition may include a combination of a number of both recombinant and pharmacologic agents temporally administered to regulate multiple proinflammatory cytokine–mediated steps in the development of septic shock. The hematopoietic growth factors have demonstrated considerable clinical effect, especially in individuals with distinct lymphopoietic disorders and in patients receiving immunosuppressive chemotherapy. The interferons α, β, and γ have received considerable attention during the past decade as potential immunomodulators. IFN-α has shown broad clinical application as an antitumor as well as an antiviral agent; IFN-β is being used with some success in patients with relapsing multiple sclerosis. The stimulatory effect of IFN-γ on human neutrophils is demonstrated in infants and children with specific neutrophil disorders and in neonatal sepsis. Toll-like receptor agonists are emerging as attractive therapeutic agents in a number of infectious diseases. Investigations defining the actions of the integrins and the selectins, bactericidal/permeability-increasing factor and defensins, and platelet-activating factor and nitric oxide may provide novel future clinical therapeutic approaches to attenuate acute inflammatory responses. As we learn more about the complexity of intracellular and extracellular interactions and the delicate balance these molecules have in regulating immune responses, we will be better able to implement their clinical use in regulating infectious disease states in infants and children.

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CHAPTER 255

Active Immunizing Agents

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Prevention of infectious diseases in children by immunization is one of the outstanding accomplishments of medical science. Children enjoy better health today because of effective immunization programs, which in many countries have markedly diminished the morbidity and mortality of once common contagious diseases. The striking decline in the United States in vaccine-preventable childhood diseases is demonstrated in Table 255–1. Immunization programs have led to the global eradication of smallpox, elimination of measles and poliomyelitis in regions of the world, and substantial reductions in the morbidity and mortality attributed to diphtheria, tetanus, and pertussis.[156] The World Health Organization (WHO) estimated that 2 million child deaths were prevented by vaccinations in 2003.

To achieve this progress in child health, scientific technology and medical practice have combined efforts to (1) understand the biology of causal infectious agents, (2) purify these agents and in some cases their components, (3) develop and test safe and effective vaccines, (4) manufacture and administer these vaccines to appropriate segments of the population, (5) develop appropriate indications and implement schedules for immunizations, and (6) identify necessary contraindications.

Infectious diseases can be prevented through immunization by the following means: (1) stimulating an active immunologic defense (e.g., from humoral antibody) through the administration of antigens, usually before natural exposure to an infectious agent (i.e., active immunization), or (2) temporarily supplying preformed human or animal antibody to persons before or soon after exposure to certain infectious agents (i.e., passive immunization). Active immunizations, including the currently available vaccines, are discussed in this chapter. Major vaccines (i.e., those discussed in this chapter) and their composition and routes of administration are listed in Table 255–2.

ACTIVE IMMUNOPROPHYLAXIS: CONSIDERATIONS AND RECOMMENDATIONS

VACCINES

An ideal immunizing agent should include the following characteristics: (1) the agent should be easy to produce in well-standardized preparations that are readily quantifiable and stable in immunobiologic potency, (2) it should be easy to administer, (3) it should not produce disease in the recipient or susceptible contacts, (4) it should induce long-lasting (ideally permanent) immunity that is measurable by available and inexpensive techniques, (5) it should be free of contaminating and potentially toxic substances, and (6) adverse reactions should be minimal and minor in consequence. All these objectives rarely, if ever, are met with the currently available vaccines because they are neither completely safe nor completely effective. Partial immunity or undesirable side effects or reactions, or both, including rare severe reactions, can occur. Nonetheless, vaccines in current use are highly effective and very safe.

All active immunizing agents (vaccines) contain one or more antigens that stimulate a protective immunologic response. Some are live attenuated viruses or bacteria; other vaccines consist of killed microorganisms or contain inactivated components such as exotoxins (i.e., toxoids). In some vaccines, the antigen is a highly defined, single constituent, such as *Haemophilus influenzae* type b (Hib) polysaccharide, whereas in others, the antigen component is less well defined (e.g., live viruses or whole-cell pertussis vaccines composed of killed *Bordetella pertussis* organisms). Immunizing agents are administered in suspending fluids such as sterile water, saline solution, or complex tissue culture fluid that can contain proteins or other constituents derived from the medium from which the vaccine was produced (e.g., serum proteins, egg antigens, or other tissue culture–derived antigens). With some vaccines, certain preservatives, stabilizers, or antibiotics are added; in some cases, these additives can result in hypersensitivity reactions. To enhance immunogenicity, particularly for vaccines containing inactivated microorganisms or their extracted components, adjuvants such as aluminum compounds may be added.

Immunization Schedules

The age and timing of immunization are critical for the success of vaccination. The schedule by which a vaccine is provided is based on multiple factors, including the epidemiology of naturally occurring disease, the age-specific risk of complications caused by the natural disease, the anticipated immunologic response of the host to the antigens, the duration of immunity

TABLE 255–1 Reduction in Morbidity of Some Vaccine-Preventable Diseases in the United States

	Maximum Cases (Yr)	2004	Percent Decrease (%)
Diphtheria	206,939 (1921)	0	100
Pertussis	265,269 (1934)	25,827	90
Tetanus	1,314 (1922-6)	34	97
Poliomyelitis, paralytic	21,269 (1952)	0	100
Measles	894,134 (1941)	37	>99
Mumps	152,209 (1968)	258	>99
Rubella	57,686 (1969)	10	>99
Congenital rubella syndrome	20,000 (1964-5)	0	100
Haemophilus influenzae type b	20,000 (before 1987)	19	>99
Invasive pneumococcal disease (<5 yr)	15,933 (2000)	1,162	93
Hepatitis B	21,102 (1990)	6,212	71
Varicella*	158,364 (1992)	32,931	79

**Data from <40 states.*

Adapted from Jajosky, R. A., Hall, P. A., Adams, D. A., et al.: Summary of notifiable diseases: United States, 2004. M. M. W. R. Morb. Mortal. Wkly. Rep. 53:1, 2006.

TABLE 255–2 Available Vaccines in the United States for Use in Children and Their Routes of Administration*

Vaccine	Type	Recommended Route
BCG	Live bacteria	ID (preferred) or SC
DTaP	Toxoids and inactivated bacterial components	IM
DTaP, hepatitis B, and IPV (combination)	See DTaP, hepatitis B, and IPV	IM
DTaP, Hib conjugate, and IPV (combination)	See DTaP, Hib, and IPV	IM
DTaP and IPV (combination)	See DTaP and IV	IM
Hepatitis A	Inactivated virus	IM
Hepatitis B	Inactivated viral antigen yeast-derived recombinant	IM
Hepatitis A and hepatitis B (combination)	See hepatitis A and hepatitis B	IM
Hib conjugates	Polysaccharide-protein conjugate	IM
Hib conjugate and DTaP (combination)	See Hib and DTaP	IM
Hib conjugate and hepatitis B (combination)	See Hib and hepatitis B	IM
Influenza	Inactivated viral components	IM
Influenza	Live attenuated virus	Intranasal
Japanese encephalitis	Inactivated virus	SC
Measles	Live attenuated virus	SC
Measles-rubella	Live attenuated viruses	SC
Meningococcal	Polysaccharide	SC
Meningococcal conjugate	Polysaccharide-protein conjugate	IM
MMR	Live attenuated viruses	SC
MMRV	Live attenuated viruses	SC
Mumps	Live attenuated virus	SC
Pneumococcal	Polysaccharide	IM or SC
Pneumococcal conjugate	Polysaccharide-protein conjugate	IM
Poliovirus (IPV)	Inactivated viruses	SC
Rabies	Inactivated virus	IM
Rotavirus	Live attenuated viruses	oral
Rubella	Live attenuated virus	SC
Td and DT (adsorbed)	Toxoids	IM
Tdap	Toxoids and inactivated bacterial components	IM
Typhoid		
Parenteral	Capsular polysaccharide	IM
Oral	Live attenuated bacteria	Oral
Varicella	Live attenuated virus	SC
Yellow fever	Live attenuated virus	SC

**Only major childhood vaccines and selective others are included.*

BCG, bacille Calmette-Guérin (tuberculosis); DT, diphtheria-tetanus toxoids (for children <7 yr); DTaP, diphtheria-tetanus toxoids–acellular pertussis vaccine (for children <7 yr); Hib, Haemophilus influenzae type b conjugate vaccine; ID, intradermal; IM, intramuscular; IPV, inactivated poliovirus vaccine; MMR, live measles-mumps-rubella viruses vaccine; MMRV, live measles-mumps-rubella-varicella viruses vaccine; SC, subcutaneous; Td, tetanus-diphtheria toxoid (for children ≥7 yr and adults); Tdap, diphtheria–reduced tetanus toxoids–acellular pertussis vaccine (for children ≥10 yr and adults).

that can be induced, and, often, the recommended ages for routine health care visits. In general, vaccines are recommended at the youngest age at which significant risk for the natural disease and its complications exist and at which a protective immunologic response to the vaccine will occur. An example is measles vaccine, which in the United States is recommended routinely at 12 to 15 months of age because many children have residual, transplacentally acquired maternal measles serum antibody in the first year of life that will interfere with the antibody response. However, during measles outbreaks in preschool children, measles vaccination is recommended for infants as young as 6 months because the risk of complications with measles is high in children younger than 1 year.[14,25,111] These infants should be vaccinated again at 12 to 15 months of age. Similarly, in countries where measles causes significant morbidity and mortality in infants younger than 9 months, the Global Advisory Group of the WHO's Expanded Programme on Immunization (EPI) has recommended giving measles vaccine to infants as young as 6 months.[250]

The recommended doses of vaccine are determined by the number necessary to achieve a uniform and predictable immunologic response and to sustain protection. Some immunizing agents require the administration of more than one dose for development of an adequate antibody response and require a booster dose to maintain protection; examples are pertussis, diphtheria, and tetanus vaccines. Intervals between doses are based on the kinetics of primary and secondary antibody responses.

Route of Administration

An example of the effect of the route of administration on immunologic response is provided by poliomyelitis vaccines. Inactivated poliovirus vaccines given intramuscularly induce systemic immunity through serum antibody production; however, they do not consistently evoke local secretory immunoglobulin A (IgA) antibodies in the intestinal tract and thereby effectively prevent subsequent transmission of wild-type virus. Because live attenuated oral polio vaccine (OPV) induces optimal intestinal as well as systemic antibody, it was the preferred vaccine for routine immunization of children in the United States against poliomyelitis for 3 decades and remains the recommended vaccine by the WHO for global eradication.[503] Vaccines containing adjuvants must be injected deep into the muscle mass because if they are administered subcutaneously or intradermally, they can cause local irritation, inflammation, granuloma formation, or necrosis.[6,308]

Injectable vaccines should be administered in areas unlikely to cause local neural, vascular, or tissue injury (Table 255–3).[6,308] Although the upper, outer quadrant of the buttocks has been used as a frequent site of immunization, this area ordinarily should not be used because the gluteal region consists mostly of fat in young children and because of potential injury to the sciatic nerve. Ideally, intramuscular injections should be given in the anterolateral aspect of the upper part of the thigh or the deltoid muscle of the upper part of the arm. The anterolateral aspect of the thigh

TABLE 255–3 Site and Needle Length by Age for Intramuscular Immunization

Age Group	Needle Length, inches (mm)	Suggested Injection Site
Infants		
Preterm newborn	5/8(16)	Anterolateral thigh muscle
Term newborn	5/8(16)	Anterolateral thigh muscle
Infant, age 2-12 mo	1 (25)	Anterolateral thigh muscle
Toddlers and Children	5/8-1 (16-25) 1-1 1/4 (25-32)	Deltoid muscle of the arm Anterolateral thigh muscle
Adolescents and Young Adults		
Female and male, weight <60 kg	5/8-1 (16-25)	Deltoid muscle of the arm
Female, weight 60-90 kg or Male, weight 60-118 kg	1 (25)	Deltoid muscle of the arm
Female, weight >90 kg or Male, weight >118 kg	1 1/2 (38)	Deltoid muscle of the arm

Adapted from Pickering, L. K., Baker, C. J., Long, S. S., et al. (eds.): Red Book: 2006 Report of the Committee on Infectious Diseases. 27th ed. Elk Grove Village, IL, American Academy of Pediatrics, 2006.

is preferred for infants because of its muscle mass relative to other sites. For older children, the deltoid muscle usually is sufficiently large for intramuscular injection. The incidence of significant pain in 18-month-old children injected intramuscularly in the thigh is greater than that after deltoid injections and can result in transient limping.[284] The deltoid generally is the preferred site for intramuscular administration of vaccines in children 18 months and older, although some physicians prefer the anterolateral aspect of the thigh for toddlers.[6,308] Subcutaneous inoculations also usually should be given in the thigh of infants and the deltoid area of older children. Intradermal vaccines generally should be administered on the volar aspect of the forearm.

Recommended routes for administration of vaccines are provided in their package inserts and are summarized in recommendations for immunizations by the Committee on Infectious Diseases of the American Academy of Pediatrics (AAP)[6] and the Advisory Committee on Immunization Practices (ACIP) of the Centers for Disease Control and Prevention (CDC).[308]

Vaccine Dose

The recommended dose of each immunizing agent is derived from theoretical considerations and vaccine trials. Because inactivated immunizing agents cannot replicate in the host, these vaccines must contain an adequate antigenic mass to stimulate the desired immunologic response. Long-lasting immunity with such vaccines requires repeated doses. Exceeding the recommended dose can be hazardous because of excessive local or systemic concentrations of immunizing agents, whereas administration of doses smaller than those recommended may result in inadequate response and protection.[6,308]

Lapsed Immunizations

In general, intervals between multiple doses of an antigen longer than those recommended do not affect the antibody responses achieved, provided the immunization series is completed. Thus, restarting the series after interruption of the vaccine schedule or giving additional doses is not necessary.

TABLE 255–4 Guidelines for Spacing the Administration of Live and Inactivated Antigens

Antigen Combination	Recommended Minimum Interval between Doses
≥2 inactivated*	None; may be administered simultaneously or at any interval between doses
Inactivated and live	None; may be administered simultaneously or at any interval between doses
≥2 live parenteral†	28-day minimum interval if not administered simultaneously

**If simultaneous administration of diphtheria–reduced tetanus toxoids–acellular pertussis vaccine (Tdap) and meningococcal polysaccharide–protein conjugate vaccine (MCV4) is not feasible (a vaccine is not available), the vaccines should be separated by at least 28 days.*

†Some live oral vaccines (e.g., Ty21a typhoid vaccine, oral poliovirus vaccine, rotavirus vaccine) can be administered simultaneously or at any interval before or after inactivated or live parenteral vaccines.

Simultaneous Administration of Multiple Vaccines

Because most vaccines can be given simultaneously without impairment of effectiveness or safety, multiple vaccines are given to children concurrently.[301] Simultaneous administration of vaccines is particularly important for inadequately immunized children whose return for further immunization is doubtful or for patients with imminent travel plans. An inactivated vaccine and a live virus vaccine can be administered simultaneously at different sites without interference with the immune response. Exceptions are yellow fever and cholera vaccines because if they are administered simultaneously, antibody responses are diminished. If possible, administration of these vaccines should be separated by at least an interval of 3 weeks.[308]

In the case of live virus vaccines, the immune response to one live virus vaccine can be impaired if it is given within 4 weeks of another.[131,308,392] Thus, parenteral live virus vaccines not administered on the same day should be given at least 4 weeks apart. This consideration is the basis of the recommended minimal interval of 1 month (defined as 4 weeks) between doses of measles vaccine, such as would be the case for a previously unimmunized person who is entering college. Guidelines for spacing live and killed antigen vaccines are given in Table 255–4.

Record Keeping, Patient Information, Informed Consent, and Reporting

Accurate record keeping by physicians is required, and parents (or patients) should keep up-to-date immunization records for their children. The 1986 National Childhood Vaccine Injury Act (NCVIA) requires that for routinely recommended childhood vaccines, health care providers record in the child's permanent medical record the date of administration of the vaccine, manufacturer, lot number, and the name of the health care provider administering the vaccines.[6]

As a general principle, all children and their parents or caregivers should be informed about the benefits and risks of any vaccines to be administered. For vaccines currently specified in the NCVIA, Vaccine Information Statements (VISs) have been prepared by the CDC and must be used by vaccine administrators.

Informed consent should be obtained before the administration of vaccines. Some physicians and other health care providers may choose to obtain the parent's signature, but current law does not require written consent. An appropriate alternative to written consent is to note in the patient's record that the VISs have been provided and discussed with the parent, patient, or legal guardian.[6]

TABLE 255–5 Reportable Events Following Immunization, as Required by the National Childhood Vaccine Injury Act*

Vaccine/Toxoid	Adverse Event	For Reporting
Tetanus toxoid–containing vaccines (e.g., DTaP, Tdap, DTP-Hib, DT, Td, or TT)	Anaphylaxis or anaphylactic shock	0-4 hr
	Branchial neuritis	2-28 days
	Any acute complication or sequela (including death) of above events	Not applicable
Pertussis antigen–containing vaccines (e.g., DTaP, Tdap, DTP, P, DTP-Hib)	Anaphylaxis or anaphylactic shock	0-4 hr
	Encephalopathy (or encephalitis)	0-72 hr
	Any acute complication sequela (including death) of above events	Not applicable
Measles, mumps, and rubella virus–containing vaccines in any combination (e.g., MMR, MR, M, R)	Anaphylaxis or anaphylactic shock	0-4 hr
	Encephalopathy (or encephalitis)	5-15 days
	Any acute complication or sequela (including death) of above events	Not applicable
Measles virus–containing vaccines (e.g., MMR, MR, M)	Thrombocytopenic purpura	7-30 days
	Vaccine-strain measles viral infection in an immunodeficient recipient	0-6 mo
	Any acute complication or sequela (including death) of above events	Not applicable
Rubella virus–containing vaccines (e.g., MMR, MR, R)	Chronic arthritis	7-42 days
	Any acute complication or sequela (including death) of above events	Not applicable
Live poliovirus–containing vaccines (OPV)	Paralytic polio	
	In a non-immunodeficient recipient	0-30 days
	In an immunodeficient recipient	0-6 mo
	In a vaccine-associated community case	Not applicable
	Vaccine-strain polio infection	
	In a non-immunodeficient recipient	0-30 days
	In an immunodeficient recipient	0-6 mo
	In a vaccine-associated community case	Not applicable
	Any acute complication or sequela (including death) of above events	Not applicable
Inactivated poliovirus–containing vaccines (e.g., IPV)	Anaphylaxis or anaphylactic shock	0-4 hr
	Any acute complication or sequela (including death) of above events	Not applicable
Hepatitis B antigen–containing vaccines	Anaphylaxis or anaphylactic shock	0-4 hr
	Any acute complication or sequela (including death) of above events	Not applicable
Varicella	No condition specified	Not applicable
Rotavirus vaccine	No condition specified	Not applicable
Hib conjugate vaccines	No condition specified	Not applicable
Pneumococcal conjugate vaccines	No condition specified	Not applicable
Any new vaccine recommended by the CDC for routine administration to children, after publication by the Secretary of a notice of coverage†	No condition specified	Not applicable

**Effective date: November 10, 2008. For updates to the table, see www.hrsa.gov/vaccinecompensation/table.htm.*

†As of December 1, 2004, hepatitis A virus (HAV) vaccines have been added to the VIT under this category. As of July 1, 2005, trivalent influenza vaccines (TIV, LAIV) have been added to the VIT under this category. (Influenza vaccines routinely administered in the United States are trivalent.) As of February 1, 2007, meningococcal (conjugate and polysaccharide) and human papillomavirus (HPV) vaccines have been added to the table under this category. See "News" on the VICP Web site for more information.

The Reportable Events Table (RET) reflects what is reportable by law (42 USC 300aa-25) to the Vaccine Adverse Event Reporting System (VAERS). In addition, individuals are encouraged to report any clinically significant or unexpected events (even if you are not certain the vaccine caused the event) for any vaccine, whether or not it is listed on the RET. Manufacturers are also required by regulation (21 CFR 600.80) to report to the VAERS program all adverse events made known to them for any vaccine.

CDC, Centers for Disease Control and Prevention; DT, diphtheria-tetanus; DTaP, diphtheria-tetanus-acellular pertussis; DTP, diphtheria-tetanus-pertussis; Hib, Haemophilus influenzae *type b; M, measles, MMR, measles-mumps-rubella; MR, measles-rubella; OPV, oral poliovirus; P, pertussis; R, rubella; Td, tetanus-diphtheria toxoid; Tdap, diphtheria–reduced tetanus toxoids–acellular pertussis; TT, tetanus toxoid.*

To increase knowledge about adverse reactions, all temporally associated events severe enough to require the patient to seek medical attention should be reported to the Vaccine Adverse Events Reporting System (VAERS). Health care providers who administer vaccines are required in the United States to report to the VAERS specific adverse events in recipients of the vaccines covered by the act (Table 255–5). This system for reporting adverse events associated with vaccination was established by the U.S. Department of Health and Human Services to foster recognition of vaccine-related reactions and further study, as indicated, to establish possible causation. VAERS forms can be obtained by calling 800-822-7967 or by logging onto their Web site: http://www.vaers.org.

The decrease in the occurrence of vaccine-preventable infectious diseases has resulted in a greater number of adverse events temporally related to immunization than cases of disease. Although in some cases, such as vaccine-associated paralytic poliomyelitis (VAPP), vaccine has been established to be the cause, in other circumstances, such as brain damage alleged to be attributed to whole-cell pertussis vaccine, causation by vaccine has not been proved.[27]

Increased public visibility of vaccine reactions contributed to a marked increase in vaccine litigation in the 1980s as compensation was sought through the judicial system by those alleged to have suffered serious vaccine-related sequelae. A marked increase in manufacturers' actual and anticipated liability costs and subsequent escalating increases in the price of vaccines occurred concomitantly. These and other developments, such as threats to the vaccine supply, concerns by parents about vaccine safety, and recognition of the benefits derived from improved coordination and planning of vaccine programs, led to passage of the 1986 NCVIA and the National Vaccine Injury Compensation Program, a no-fault system to compensate victims of certain presumed vaccine-related events.

The Department of Health and Human Services administers the compensation program. Decisions on compensation are made by the U.S. Court of Federal Claims and are based on the Vaccine Injury Table, which was revised since passage of the original

legislation in response to new findings and analysis by the several Institute of Medicine (IOM) committees. Compensation for injuries occurring after the program's effective date of October 1, 1988, is provided by excise taxes on each vaccine. The program has been successful in reducing vaccine-related litigation, stabilizing vaccine prices, and creating a favorable environment for the introduction of new vaccines.[216]

Vaccine Recommendations and Schedules

In developing recommendations for immunization, multiple factors are considered and include the vaccine's characteristics, scientific knowledge about the principles of immunization, assessment of the benefits of the vaccine, the risk of development of the disease and its complications, vaccine costs, and the risk of having adverse reactions. Changes in relative benefits and risks necessitate continued review of recommendations. In the United States, recommendations for immunization of infants and children are made by two different committees, the ACIP of the CDC and the AAP Committee on Infectious Diseases. These committees work closely together, and, in most circumstances, their recommendations are similar. These two committees and the American Academy of Family Practice (AAFP) issue a single vaccine schedule each year. The 2009 schedule for routine administration of childhood vaccines is given in Figures 255–1 and 255–2.

A major change in 1996 was the establishment of a routine pre-adolescent immunization visit at 11 to 12 years of age.[101] The dose of adult tetanus-diphtheria-acellular pertussis (Tdap) vaccine should be given at that time, as should a dose of the meningococcal conjugate vaccine and the first dose of human papillomavirus (HPV) vaccine.[38] In addition, at this pre-adolescent visit, children not previously vaccinated with hepatitis B, varicella, or the second dose of measles-containing vaccine (or any combination of these vaccines) should be given the necessary immunizations and scheduled for future visits to receive any vaccines not administered during this visit.

Other countries have similar national mechanisms for formulating immunization schedules and recommendations that are based on the local epidemiology of diseases and available vaccines. In developing countries, practices are guided by the WHO EPI.[502] Current recommendations are listed in Table 255–6.

As new vaccines and scientific knowledge become available, vaccine recommendations and schedules are modified and changed. Examples of changes in the last decade include the use of pneumococcal conjugate vaccine beginning at 2 months of age, universal toddler hepatitis A immunization, recommendations for administration of a second dose of varicella vaccine, and the introduction of acellular pertussis vaccines for adolescents and adults.

Implementation of Vaccine Programs

In addition to the availability of safe and effective vaccines and appropriate schedules for their use, effective means of implementation and delivery are necessary for the success of vaccine programs. In the United States, high rates of immunization in school-age children have been achieved, in part because of public health programs for vaccine administration, government support for vaccine purchase, and state laws requiring immunization for school entry. In contrast to rates of approximately 95 percent or higher in school-age children, however, immunization rates in infants and young children in the 1980s were significantly lower.[511] In a survey of 21 primarily urban areas throughout the United States, 11 to 58 percent (median, 44%) of children who entered school in 1991 and 1992 were fully vaccinated by their second birthday. Failure to immunize young children was a major factor in the outbreaks of measles in major urban areas in the United States in 1989 to 1991.[452]

This epidemic and the recognition of low immunization rates prompted a national campaign to achieve the U.S. Public Health Service's goal of a 90 percent vaccine coverage rate in children by the time they were 2 years of age. Initiatives have included improved access to vaccines, education of health care providers in the community, and the development of standards for pediatric immunization practice.[369] These standards have been endorsed by the AAP, AAFP, and other major professional organizations and serve as guidelines to be followed for improving the delivery of vaccines. They include evaluation of the immunization status of patients at all medical visits, the use of valid contraindications, simultaneous administration of all indicated vaccines, and routine audit of the immunization status of patients by providers (Table 255–7). These and other initiatives have resulted in increasing immunization rates of young children. According to the National Immunization Survey, coverage rates in children 19 to 35 months of age in 2005 were 83 percent for completion of the four doses of tetanus-diphtheria-pertussis/diphtheria-tetanus toxoid-acellular pertussis/diphtheria-tetanus (DTP/DTaP/DT), three of poliovirus vaccine, and one dose of measles-mumps-rubella (MMR) vaccine.[152] Vaccination coverage rates in the United States for preschool-age children consistently have been greater than 75 percent since 1995.

Vaccine Contraindications, Precautions, and Use in Special Circumstances

Recommendations for the use of specific vaccines include contraindications and use in special circumstances, such as immunocompromised patients (from underlying disease or therapy, such as high-dose steroids) and pregnancy.[14,111] Established, generic contraindications are moderate or severe illness, a previous anaphylactic reaction to the specific vaccine, and a severe hypersensitivity reaction, such as anaphylaxis, to a vaccine constituent.

The decision to defer immunization in a febrile child should be based on the physician's assessment of the severity of the illness rather than the degree of fever. Children with minor illness and low-grade fever generally should be vaccinated, especially if a child is unlikely to return promptly for the deferred immunization.

Administration of live virus vaccines such as varicella and MMR vaccines generally is contraindicated in patients with altered immunity. However, the morbidity and mortality rates of measles and the lack of complications from vaccination of children infected with human immunodeficiency virus (HIV) have led to recommendations that these children, unless significantly immunocompromised, receive the MMR vaccine.[39,102,484]

Because of a theoretical risk to the developing fetus, administration of live virus vaccines in most cases is not recommended for pregnant women.[12,308] However, inadvertent administration of vaccine is not necessarily a reason for termination of the pregnancy, and some live virus vaccines, such as those for yellow fever and poliomyelitis, can be given safely to pregnant women. Inactivated bacterial and viral vaccines such as tetanus toxoids, hepatitis B, and influenza vaccine, which are composed of antigenic components or killed organisms, can and should be given during pregnancy if indicated.

In some recipients, vaccines can cause severe reactions, which may be a contraindication or precaution to subsequent administration of the specific vaccine. An example is a child in whom a fever of 40.5° C or higher develops after receiving DTaP vaccine, for whom administration of further doses of pertussis-containing vaccine is not indicated in most cases. This recommendation is based on the unproven but reasonable presumption that children who experience adverse reactions after receiving

Recommended immunization schedule for persons aged 0 through 6 Years — United States, 2009 (for those who fall behind or start late, see the catch-up schedule [Table])

Vaccine ▼ Age ▶	Birth	1 month	2 months	4 months	6 months	12 months	15 months	18 months	19–23 months	2–3 years	4–6 years
Hepatitis B[1]	HepB	HepB		see footnote 1	HepB						
Rotavirus[2]			RV	RV	RV[2]						
Diphtheria,Tetanus,Pertussis[3]			DTaP	DTaP	DTaP	see footnote 3	DTaP				DTaP
Haemophilus influenzae type b[4]			Hib	Hib	Hib[4]	Hib					
Pneumococcal[5]			PCV	PCV	PCV	PCV				PPSV	
Inactivated Poliovirus			IPV	IPV	IPV						IPV
Influenza[6]					Influenza (Yearly)						
Measles, Mumps, Rubella[7]						MMR		see footnote 7			MMR
Varicella[8]						Varicella		see footnote 8			Varicella
Hepatitis A[9]						HepA (2 doses)				HepA Series	
Meningococcal[10]										MCV	

Range of recommended ages

Certain high-risk groups

This schedule indicates the recommended ages for routine administration of currently licensed vaccines, as of December 17, 2008, for children aged 0 through 6 years. Any dose not administered at the recommended age should be administered at a subsequent visit, when indicated and feasible. Licensed combination vaccines may be used whenever any component of the combination is indicated and other components are not contraindicated and if approved by the Food and Drug Administration for that dose of the series. Providers should consult the relevant Advisory Committee on Immunization Practices statement for detailed recommendations, including high-risk conditions: http://www.cdc.gov/vaccines/pubs/acip-list.htm. Clinically significant adverse events that follow immunization should be reported to the Vaccine Adverse Event Reporting System (VAERS). Guidance about how to obtain and complete a VAERS form is available at http://www.vaers.hhs.gov or by telephone, 800-822-7967.

1.Hepatitis B vaccine (HepB). ***(Minimum age: birth)***

At birth:

- Administer monovalent HepB to all newborns before hospital discharge.
- If mother is hepatitis B surface antigen (HBsAg)-positive, administer HepB and 0.5 mL of hepatitis B immune globulin (HBIG) within 12 hours of birth.
- If mother's HBsAg status is unknown, administer HepB within 12 hours of birth. Determine the HBsAg status as soon as possible and, if HBsAg-positive, administer HBIG (no later than age 1 week).

After the birth dose:

- The HepB series should be completed with either monovalent HepB or a combination vaccine containing HepB. The second dose should be administered at age 1 or 2 months. The final dose should be administered no earlier than age 24 weeks.
- Infants born to HBsAg-positive mothers should be tested for HBsAg and antibody to HBsAg (anti-HBs) after completion of at least 3 doses of the HepB series, at age 9 through 18 months (generally at the next well-child visit).

4-month dose:

- Administration of 4 doses of HepB to infants is permissible when combination vaccines containing HepB are administered after the birth dose.

2. Rotavirus vaccine (RV). ***(Minimum age: 6 weeks)***

- Administer the first dose at age 6 through 14 weeks (maximum age: 14 weeks 6 days). Vaccination should not be initiated for infants aged 15 weeks or older (i.e., 15 weeks 0 days or older).
- Administer the final dose in the series by age 8 months 0 days.
- If Rotarix® is administered at ages 2 and 4 months, a dose at 6 months is not indicated.

3. Diphtheria and tetanus toxoids and acellular pertussis vaccine (DTaP). ***(Minimum age: 6 weeks)***

- The fourth dose may be administered as early as age 12 months, provided at least 6 months have elapsed since the third dose.
- Administer the final dose in the series at age 4 through 6 years.

4. *Haemophilus influenzae* type b conjugate vaccine (Hib). ***(Minimum age: 6 weeks)***

- If PRP-OMP (PedvaxHIB® or Comvax® [HepB-Hib]) is administered at ages 2 and 4 months, a dose at age 6 months is not indicated.
- TriHiBit®(DTaP/Hib) should not be used for doses at ages 2, 4, or 6 months but can be used as the final dose in children aged 12 months or older.

5. Pneumococcal vaccine. ***(Minimum age: 6 weeks for pneumococcal conjugate vaccine [PCV]; 2 years for pneumococcal polysaccharide vaccine [PPSV])***

- PCV is recommended for all children aged younger than 5 years. Administer 1 dose of PCV to all healthy children aged 24 through 59 months who are not completely vaccinated for their age.
- Administer PPSV to children aged 2 years or older with certain underlying medical conditions (see *MMWR* 2000;49[No. RR-9]), including a cochlear implant.

6. Influenza vaccine. ***(Minimum age: 6 months for trivalent inactivated influenza vaccine [TIV]; 2 years for live, attenuated influenza vaccine [LAIV])***

- Administer annually to children aged 6 months through 18 years.
- For healthy nonpregnant persons (i.e., those who do not have underlying medical conditions that predispose them to influenza complications) aged 2 through 49 years, either LAIV or TIV may be used.
- Children receiving TIV should receive 0.25 mL if aged 6 through 35 months or 0.5 mL if aged 3 years or older.
- Administer 2 doses (separated by at least 4 weeks) to children aged younger than 9 years who are receiving influenza vaccine for the first time or who were vaccinated for the first time during the previous influenza season but only received 1 dose.

7. Measles, mumps, and rubella vaccine (MMR). ***(Minimum age: 12 months)***

- Administer the second dose at age 4 through 6 years. However, the second dose may be administered before age 4, provided at least 28 days have elapsed since the first dose.

8. Varicella vaccine. ***(Minimum age: 12 months)***

- Administer the second dose at age 4 through 6 years. However, the second dose may be administered before age 4, provided at least 3 months have elapsed since the first dose.
- For children aged 12 months through 12 years the minimum interval between doses is 3 months. However, if the second dose was administered at least 28 days after the first dose, it can be accepted as valid.

9. Hepatitis A vaccine (HepA). ***(Minimum age: 12 months)***

- Administer to all children aged 1 year (i.e., aged 12 through 23 months). Administer 2 doses at least 6 months apart.
- Children not fully vaccinated by age 2 years can be vaccinated at subsequent visits.
- HepA also is recommended for children older than 1 year who live in areas where vaccination programs target older children or who are at increased risk of infection. See *MMWR* 2006;55(No. RR-7).

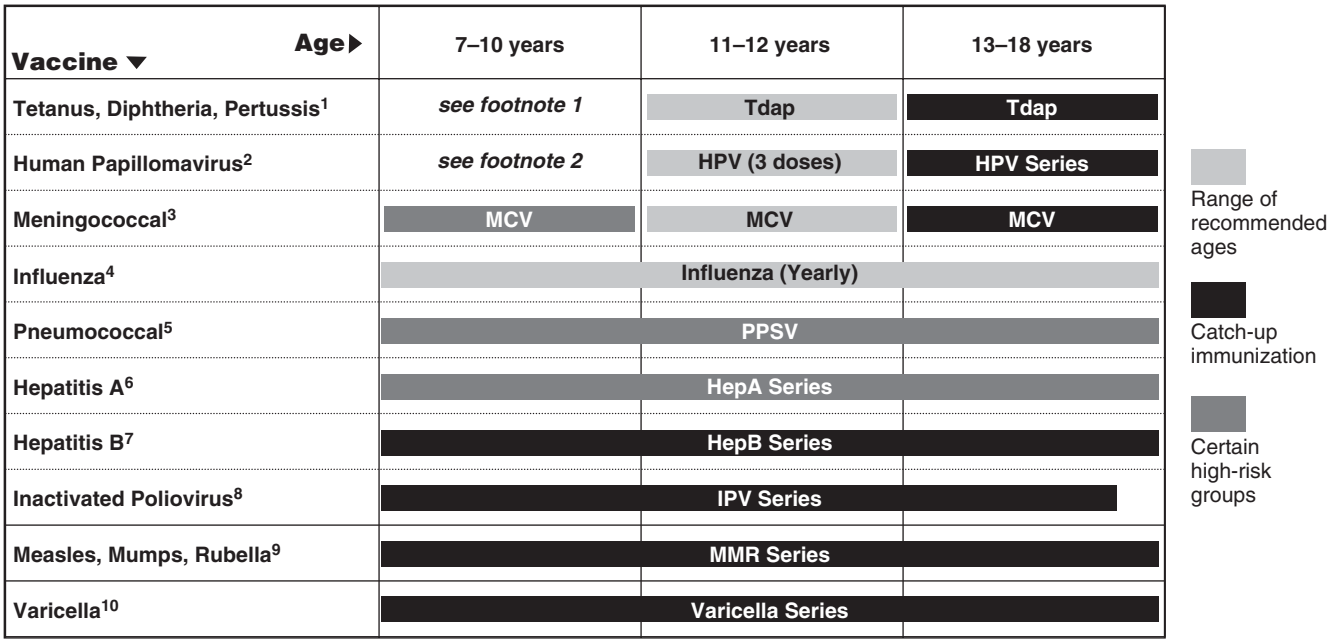
10. Meningococcal vaccine ***(Minimum age: 2 years for meningococcal conjugate vaccine [MCV] and for meningococcal polysaccharide vaccine [MPSV])***

- Administer MCV to children aged 2 through 10 years with terminal complement component deficiency, anatomic or functional asplenia, and certain other high-risk groups. See *MMWR* 2005;54(No. RR-7).
- Persons who received MPSV 3 or more years previously and who remain at increased risk for meningococcal disease should be revaccinated with MCV.

The Recommended Immunization Schedules for Persons Aged 0 though 18 Years are approved by the Advisory Committee on Immunization Practices (http://www.cdc.gov/vaccines/recs/acip), the American Academy of Pediatrics (http://www.aap.org), and the American Academy of Family Physicians (http://www.aafp.org).

Figure 255–1 Recommended immunization schedule for persons aged 0 to 18 years: United States. (*From Centers for Disease Control and Prevention: Immunization Schedules. Available at cdc.gov/vaccines/recs./schedules/default.htm*)

Recommended immunization schedule for persons aged 7 through 18 years — United States, 2009 (for those who fall behind or start late, see the schedule below and the catch-up schedule [Table])



This schedule indicates the recommended ages for routine administration of currently licensed vaccines, as of December 17, 2008, for children aged 7 through 18 years. Any dose not administered at the recommended age should be administered at a subsequent visit, when indicated and feasible. Licensed combination vaccines may be used whenever any component of the combination is indicated and other components are not contraindicated and if approved by the Food and Drug Administration for that dose of the series. Providers should consult the relevant Advisory Committee on Immunization Practices statement for detailed recommendations, including high risk conditions: http://www.cdc.gov/vaccines/pubs/acip-list.htm. Clinically significant adverse events that follow immunization should be reported to the Vaccine Adverse Event Reporting System (VAERS). Guidance about how to obtain and complete a VAERS form is available at http://www.vaers.hhs.gov or by telephone, 800-822-7967.

1.Tetanus and diphtheria toxoids and acellular pertussis vaccine (Tdap). ***(Minimum age: 10 years for BOOSTRIX® and 11 years for ADACEL®)***
- Administer at age 11 or 12 years for those who have completed the recommended childhood DTP/DTaP vaccination series and have not received a tetanus and diphtheria toxoid (Td) booster dose.
- Persons aged 13 through 18 years who have not received Tdap should receive a dose.
- A 5-year interval from the last Td dose is encouraged when Tdap is used as a booster dose; however, a shorter interval may be used if pertussis immunity is needed.

2. Human papillomavirus vaccine (HPV). ***(Minimum age: 9 years)***
- Administer the first dose to females at age 11 or 12 years.
- Administer the second dose 2 months after the first dose and the third dose 6 months after the first dose (at least 24 weeks after the first dose).
- Administer the series to females at age 13 through 18 years if not previously vaccinated.

3. Meningococcal conjugate vaccine (MCV).
- Administer at age 11 or 12 years, at age 13 through 18 years if not previously vaccinated.
- Administer to previously unvaccinated college freshmen living in a dormitory.
- MCV is recommended for children aged 2 through 10 years with terminal complement component deficiency, anatomic or functional asplenia, and certain other groups at high risk. See *MMWR* 2005;54(No. RR-7).
- Persons who received MPSV 5 or more years previously and remain at increased risk for meningococcal disease should be vaccinated with MCV.

4. Influenza vaccine.
- Administer annually to children aged 6 months through 18 years.
- For healthy nonpregnant persons (i.e., those who do not have underlying medical conditions that predispose them to influenza complications) aged 2 through 49 years, either LAIV or TIV may be used.
- Administer 2 doses (separated by at least 4 weeks) to children aged younger than 9 years who are receiving influenza vaccine for the first time or who were vaccinated for the first time during the previous influenza season but only received 1 dose.

5. Pneumococcal polysaccharide vaccine (PPSV).
- Administer to children with certain underlying medical conditions (see *MMWR* 1997;46[No. RR-8]), including a cochlear implant. A single revaccination should be administered to children with functional or anatomic asplenia or other immunocompromising condition after 5 years.

6. Hepatitis A vaccine (HepA).
- Administer 2 doses at least 6 months apart.
- HepA is recommended for children older than 1 year who live in areas where vaccination programs target older children or who are at increased risk of infection. See *MMWR* 2006;55(No. RR-7).

7. Hepatitis B vaccine (HepB).
- Administer the 3-dose series to those not previously vaccinated.
- A 2-dose series (separated by at least 4 months) of adult formulation Recombivax HB® is licensed for children aged 11 through 15 years.

8. Inactivated poliovirus vaccine (IPV).
- For children who received an all-IPV or all-oral poliovirus (OPV) series, a fourth dose is not necessary if the third dose was administered at age 4 years or older.
- If both OPV and IPV were administered as part of a series, a total of 4 doses should be administered, regardless of the child's current age.

9.Measles, mumps, and rubella vaccine (MMR).
- If not previously vaccinated, administer 2 doses or the second dose for those who have received only 1 dose, with at least 28 days between doses.

10.Varicella vaccine.
- For persons aged 7 through 18 years without evidence of immunity (see *MMWR* 2007;56[No. RR-4]), administer 2 doses if not previously vaccinated or the second dose if they have received only 1 dose.
- For persons aged 7 through 12 years, the minimum interval between doses is 3 months. However, if the second dose was administered at least 28 days after the first dose, it can be accepted as valid.
- For persons aged 13 years and older, the minimum interval between doses is 28 days.

The Recommended Immunization Schedules for Persons Aged 0 though 18 Years are approved by the Advisory Committee on Immunization Practices (http://www.cdc.gov/vaccines/recs/acip), the American Academy of Pediatrics (http://www.aap.org), and the American Academy of Family Physicians (http://www.aafp.org).

Figure 255–1 *Continued*

Catch-up immunization schedule for persons aged 4 months through 18 years who start late or who are more than 1 month behind — United States, 2009

The table below provides catch-up schedules and minimum intervals between doses for children whose vaccinations have been delayed. A vaccine series does not need to be restarted, regardless of the time that has elapsed between doses. Use the section appropriate for the child's age.

CATCH-UP SCHEDULE FOR PERSONS AGED 4 MONTHS THROUGH 6 YEARS					
Vaccine	**Minimum Age for Dose 1**	**Minimum Interval Between Doses**			
		Dose 1 to Dose 2	**Dose 2 to Dose 3**	**Dose 3 to Dose 4**	**Dose 4 to Dose 5**
Hepatitis B[1]	Birth	4 weeks	8 weeks (and at least 16 weeks after the first dose)		
Rotavirus[2]	6 wks	4 weeks	4 weeks[2]		
Diphtheria,Tetanus,Pertussis[3]	6 wks	4 weeks	4 weeks	6 months	6 months[3]
Haemophilus influenzae type b[4]	6 wks	4 weeks if first dose administered at younger than age 12 months; 8 weeks (as final dose) if first dose administered at age 12–14 months; No further doses needed if first dose administered at age 15 months or older	4 weeks[4] if current age is younger than 12 months; 8 weeks (as final dose)[4] if current age is 12 months or older and second dose administered at younger than age 15 months; No further doses needed if previous dose administered at age 15 months or older	8 weeks (as final dose)[4] This dose only necessary for children aged 12 months through 59 months who received 3 doses before age 12 months	
Pneumococcal[5]	6 wks	4 weeks if first dose administered at younger than age 12 months; 8 weeks (as final dose for healthy children) if first dose administered at age 12 months or older or current age 24 through 59 months; No further dose needed for healthy children if first dose administered at age 24 months or older	4 weeks if current age is younger than 12 months; 8 weeks (as final dose for healthy children) if current age is 12 months or older; No further dose needed for healthy children if previous dose administered at age 24 months or older	8 weeks (as final dose) This dose only necessary for children aged 12 months through 59 months who received 3 doses before age 12 months or for high-risk children who received 3 doses at any age	
Inactivated Poliovirus[6]	6 wks	4 weeks	4 weeks	4 weeks[6]	
Measles, Mumps, Rubella[7]	12 mos	4 weeks			
Varicella[8]	12 mos	3 months			
Hepatitis A[9]	12 mos	6 months			
CATCH-UP SCHEDULE FOR PERSONS AGED 7 THROUGH 18 YEARS					
Tetanus, Diphtheria/ Tetanus,Diphtheria, Pertussis[10]	7 yrs[10]	4 weeks	4 weeks if first dose administered at younger than age 12 months; 6 months if first dose administered at age 12 months or older	6 months if first dose administered at younger than age 12 months	
Human Papillomavirus[11]	9 yrs	Routine dosing intervals are recommended[11]			
Hepatitis A[9]	12 mos	6 months			
Hepatitis B[1]	Birth	4 weeks	8 weeks (and at least 16 weeks after first dose)		
Inactivated Poliovirus[6]	6 wks	4 weeks	4 weeks	4 weeks[6]	
Measles, Mumps, Rubella[7]	12 mos	4 weeks			
Varicella[8]	12 mos	3 months if the person is younger than age 13 years; 4 weeks if the person is aged 13 years or older			

1. Hepatitis B vaccine (HepB).
- Administer the 3-dose series to those not previously vaccinated.
- A 2-dose series (separated by at least 4 months) of adult formulation Recombivax HB® is licensed for children aged 11 through 15 years.

2. Rotavirus vaccine (RV).
- The maximum age for the first dose is 14 weeks 6 days. Vaccination should not be initiated for infants aged 15 weeks or older (i.e., 15 weeks 0 days or older).
- Administer the final dose in the series by age 8 months 0 days.
- If Rotarix® was administered for the first and second doses, a third dose is not indicated.

3. Diphtheria and tetanus toxoids and acellular pertussis vaccine (DTaP).
- The fifth dose is not necessary if the fourth dose was administered at age 4 years or older.

4. *Haemophilus influenzae* type b conjugate vaccine (Hib).
- Hib vaccine is not generally recommended for persons aged 5 years or older. No efficacy data are available on which to base a recommendation concerning use of Hib vaccine for older children and adults. However, studies suggest good immunogenicity in persons who have sickle cell disease, leukemia, or HIV infection, or who have had a splenectomy; administering 1 dose of Hib vaccine to these persons is not contraindicated.
- If the first 2 doses were PRP-OMP (PedvaxHIB® or Comvax®), and administered at age 11 months or younger, the third (and final) dose should be administered at age 12 through 15 months and at least 8 weeks after the second dose.
- If the first dose was administered at age 7 through 11 months, administer 2 doses separated by 4 weeks and a final dose at age 12 through 15 months.

5. Pneumococcal vaccine.
- Administer 1 dose of pneumococcal conjugate vaccine (PCV) to all healthy children aged 24 through 59 months who have not received at least 1 dose of PCV on or after age 12 months.
- For children aged 24 through 59 months with underlying medical conditions, administer 1 dose of PCV if 3 doses were received previously or administer 2 doses of PCV at least 8 weeks apart if fewer than 3 doses were received previously.
- Administer pneumococcal polysaccharide vaccine (PPSV) to children aged 2 years or older with certain underlying medical conditions (see *MMWR* 2000;49 [No. RR-9]), including a cochlear implant, at least 8 weeks after the last dose of PCV.

6. Inactived poliovirus vaccine (IPV).
- For children who received an all-IPV or all-oral poliovirus (OPV) series, a fourth dose is not necessary if the third dose was administered at age 4 years or older.
- If both OPV and IPV were administered as part of a series, a total of 4 doses should be administered, regardless of the child's current age.

7. Measles, mumps, and rubella vaccine (MMR).
- Administer the second dose at age 4 through 6 years. However, the second dose may be administered before age 4, provided at least 28 days have elapsed since the first dose.
- If not previously vaccinated, administer 2 doses with at least 28 days between doses.

8. Varicella vaccine.
- Administer the second dose at age 4 through 6 years. However, the second dose may be administered before age 4, provided at least 3 months have elapsed since the first dose.
- For persons aged 12 months through 12 years, the minimum interval between doses is 3 months. However, if the second dose was administered at least 28 days after the first dose, it can be accepted as valid.
- For persons aged 13 years and older, the minimum interval between doses is 28 days.

9. Hepatitis A vaccine (HepA).
- HepA is recommended for children older than 1 year who live in areas where vaccination programs target older children or who are at increased risk of infection. See *MMWR* 2006;55(No. RR-7).

10. Tetanus and diphtheria toxoids vaccine (Td) and tetanus and diphtheria toxoids and acellular pertussis vaccine (Tdap).
- Doses of Dtap are counted as part of the Td/Tdap series.
- Tdap should be substituted for a single dose of Td in the catch-up series or as a booster for children aged 10 through 18 years; use Td for other doses.

11. Human papillomavirus vaccine (HPV).
- Administer the series to females at age 13 through 18 years if not previously vaccinated.
- Use recommended routine dosing intervals for series catch-up (i.e., the second and third doses should be administered at 2 and 6 months after the first dose). However, the minimum interval between the first and second doses is 4 weeks. The minimum interval between the second and third doses is 12 weeks, and the third dose should be given at least 24 weeks after the first dose.

Information about reporting reactions after immunization is available online at http://www.vaers.hhs.gov or by telephone, 800-822-7967. Suspected cases of vaccine-preventable diseases should be reported to the state or local health department. Additional information, including precautions and contraindications for immunization, is available from the National Center for Immunization and Respiratory Diseases at http://www.cdc.gov/vaccines or telephone, 800-CDC-INFO (800-232-4636).

Figure 255–2 Catch-up immunization schedule for persons aged 4 months to 18 years who start late or who are more than 1 month behind. *(From Centers for Disease Control and Prevention: Immunization Schedules. Available at cdc.gov/vaccines/recs./schedules/default.htm)*

TABLE 255–6 Schedule of the Expanded Programm on Immunization of the World Health Organization*

Age	Vaccines	Hepatitis B†	
		Schedule A or	Schedule B
Birth	BCG, OPV	HB-1	
6 wk	DTP, OPV	HB-2	HB-1
10 wk	DTP, OPV		HB-2
14 wk	DTP, OPV	HB-3	HB-3
9 mo	Measles, yellow fever‡		

**Modifications may be made by the ministries of health in individual countries on the basis of local conditions.*
†Schedule A is recommended in countries where perinatal transmission of HBV is important (e.g., Southeast Asia) and B in countries where perinatal transmission is less important (e.g., Sub-Saharan Africa).
‡In countries where yellow fever poses a risk.
BCG, bacille Calmette-Guérin; DTP, diphtheria, tetanus, and pertussis; HB, hepatitis B; OPV, oral poliovirus.

TABLE 255–7 Standards for Child and Adolescent Immunization Practices*

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Please refer to the printed publication.

From National Vaccine Advisory Committee: Standards for child and adolescent immunization practices. Pediatrics 112:*958, 2003.*

pertussis immunization are at risk of having similar reactions of equal or greater magnitude on subsequent immunization.[165]

Anaphylactic reactions caused by allergenic components of a vaccine, such as gelatin or egg protein (in vaccine prepared in embryonated chicken eggs), have occurred in rare cases. Vaccines posing a potential risk for egg-sensitive persons include those against measles, mumps, inactivated influenza, and yellow fever. Before administering vaccines to persons with possible hypersensitivity to vaccine constituents, physicians should review current recommendations for these vaccines. In other circumstances, specific immunizations may be contraindicated because of previous reactions and the child's past history, such as with DTaP (e.g., evolving neurologic disorders) and MMR (e.g., immune thrombocytopenia occurring in temporal association with vaccination).[308]

Misconceptions

Appropriate and safe use of vaccines requires knowledge of the patient's relevant medical history, adverse reactions associated with previous receipt of vaccines, and specific indications and contraindications. Without this information, vaccines may be administered inadvertently or not given in circumstances in which immunization is indicated, thereby resulting in missed opportunities for receiving the recommended immunization and susceptibility of the child to a preventable disease. Examples of common misconceptions concerning contraindication to vaccines are given in Table 255–8.

International Travel

Foreign travel often is an indication for giving vaccines not routinely administered to children.[12] The risk of exposure to certain vaccine-preventable diseases may be increased relative to that in the United States, and travelers may be exposed to infections that are uncommon or do not occur in the United States. Examples include vaccines against hepatitis A, typhoid fever, yellow fever, and Japanese encephalitis (JE) (Table 255–9), depending on the location and circumstances of the person's visit. Some countries may require yellow fever vaccination for entry. The second dose of measles vaccine should be given to children and adolescents who have received only one dose (provided 4 weeks or more have elapsed since administration of the first dose), irrespective of age, because the risk of exposure to cases of measles may be substantial in some foreign countries. In addition, children and adolescents should have received all vaccines routinely recommended for their age. Information on vaccine requirements for international travel to different countries is provided in a publication by the CDC entitled *Health Information for International Travel*,[143] which is revised semiannually and can be obtained from the Superintendent of Documents, U.S. Government Printing Office, Washington, D.C., 20402-9235. Information also is available from the CDC International Travelers Hotline by calling 404-332-4559 or on the Internet at the CDC Web site.

Vaccine Safety

Immunizations are among the most cost-effective and widely used public health interventions. Public health recommendations for vaccine programs and practices represent a dynamic balancing of risks and benefits. Vaccine safety or monitoring of risk is necessary to weigh this balance accurately and adjust vaccination policies accordingly.

No vaccine is perfectly safe or effective. As the incidence of vaccine-preventable diseases is reduced, public concern refocuses from the risk of contracting disease to the health risks associated with vaccines. A higher standard of safety generally is expected of vaccines than of other medical interventions because, in con-

TABLE 255–8 Misconceptions Concerning Vaccine Contraindications

- Mild acute illness with low-grade fever or mild diarrheal illness in an otherwise well child
- Current antimicrobial therapy or the convalescent phase of illness
- Reaction to previous vaccine dose that involved only soreness, redness, or swelling in the immediate vicinity of the vaccination site or temperature of less than 105° F (40.5° C)
- Prematurity. The appropriate age for initiating most immunization in the prematurely born infant is the usual recommended chronologic age. Vaccine doses should not be reduced for preterm infants.
- Pregnancy of mother or other household contact. Vaccine viruses in MMR vaccine are not transmitted by vaccine recipients. Although varicella vaccine and influenza vaccine virus has been transmitted by a healthy vaccine recipient to contacts, the frequency is rare, only mild or asymptomatic infection has been reported, and use of vaccine is not contraindicated by pregnancy of either the child's mother or other household contacts.
- Recent exposure to an infectious disease
- Breast-feeding. The only vaccine virus that has been isolated from breast milk is rubella vaccine virus. No evidence indicates that breast milk from women immunized against rubella is harmful to infants.
- A history of nonspecific allergies or relatives with allergies
- Allergies to penicillin or any other antibiotic, except anaphylactic reactions to neomycin or streptomycin. These reactions occur rarely, if ever. None of the vaccines licensed in the United States contain penicillin.
- Allergies to duck meat or duck feathers. No vaccine available in the United States is produced in substrates containing duck antigens.
- Family history of convulsions in persons considered for pertussis or measles vaccination
- Family history of sudden infant death syndrome in children considered for DTaP vaccination
- Family history of an adverse event, unrelated to immunosuppression after vaccination
- Malnutrition

DTaP, diphtheria-tetanus-acellular pertussis; MMR, measles-mumps-rubella.
Adapted from Pickering, L. K., Baker, C. J., Long, S. S., et al. (eds.): Red Book: 2006 Report of the Committee on Infectious Diseases. 27th ed. Elk Grove Village, IL, American Academy of Pediatrics, 2006.

TABLE 255–9 Recommended Immunizations for Travelers to Developing Countries

Review and complete age-appropriate childhood schedule.
- DTaP, poliovirus, pneumococcal, and *Haemophilus influenzae* type b vaccines may be given at 4-week intervals if necessary to complete the recommended schedule before departure.
- Measles: Two additional doses given if younger than 12 months of age at first dose
- Varicella
- Hepatitis B*

Vaccines against the following diseases should be considered depending on the geographic area and circumstances of the visit.
- Hepatitis A†
- Japanese encephalitis‡
- Meningococcal disease§
- Rabies¶
- Typhoid fever¶
- Yellow fever**

**If insufficient time to complete 6-month primary series, accelerated series can be given.*
†Indicated for travelers to areas with intermediate or high endemic rates of HAV infection.
‡For regions with endemic infection. For high-risk activities in areas experiencing outbreaks, vaccine is recommended, even for brief travel.
§Recommended for regions of Africa with endemic infection and during local epidemics and required for travel to Saudi Arabia for the Hajj.
¶Indicated for people with high risk of animal exposure (especially to dogs) and for travelers to countries with endemic infection.
¶Indicated for travelers who will consume food and liquids in areas of poor sanitation.
***For regions with endemic infection.*
DTaP, diphtheria-tetanus-acellular pertussis.
Adapted from Pickering, L. K., Baker, C. J., Long, S. S., et al. (eds.): Red Book: 2006 Report of the Committee on Infectious Diseases. 27th ed. Elk Grove Village, IL, American Academy of Pediatrics, 2006; and Centers for Disease Control and Prevention: Health Information for International Travel, 2005-2006: International Travel with Infants and Young Children. Available at http://www2.ncid.cdc.gov/travel/yb/utils/ybGet.asp?section=&obj=child-vax.htm&cssNav=browseoyb.

trast to most pharmaceutical products that are administered to ill persons for curative purposes, vaccines generally are given to healthy persons to prevent the acquisition of disease. Public tolerance of adverse reactions related to products given to healthy persons, especially healthy infants, is substantially lower than that to products administered to persons who are already ill. This lower tolerance of risk for vaccines necessitates investigating possible causes of rare adverse events after administration of vaccinations more aggressively than would be acceptable for other pharmaceutical products. Because vaccination is such a common event, any health problem that occurs after immunization may be attributed to the vaccine. Health effects reported as being associated with vaccines may be true adverse reactions or may be associated with vaccination only by coincidence. Because a temporal relationship alone does not necessarily indicate causation, cause-and-effect relationships often are impossible to establish. Epidemiologic and related studies must be performed to ascertain the incidence and nature of adverse reactions to vaccines, and such studies are important in ensuring a scientific rationale for recommendations for vaccine use and optimal public and professional acceptance of vaccines.

The topic of vaccine safety became prominent during the mid-1970s with increases in the number of lawsuits filed on behalf of patients presumably injured by the DTP vaccine.[223] Legal decisions were made and damages awarded despite the lack of scientific evidence to support claims of vaccine injury.[223] As a result of the liability, prices soared, and several manufacturers halted production. A shortage of vaccines resulted, and public health officials became concerned about the return of epidemic diseases. To reduce liability and respond to public health concerns, Congress passed the NCVIA in 1986.

The NCVIA mandates that all health care providers report certain adverse events that occur after the administration of vaccination with routinely recommended childhood vaccines. As a result, VAERS was established by the U.S. Food and Drug Administration (FDA) and the CDC in 1990. VAERS provides a mechanism for the collection and analysis of adverse events associated with vaccines currently licensed in the United States. Adverse events are defined as health effects occurring after immunization that may or may not be related to the vaccine. VAERS data are monitored continually to detect previously unknown adverse events or increases in known adverse events.[164]

The gaps that exist in the scientific knowledge of rare vaccine adverse events prompted the CDC to develop the Vaccine Safety Datalink (VSD) project.[162] This project involved forming partnerships with four large health maintenance organizations to monitor vaccine safety continually. VSD is an example of a large, linked database and includes information on more than 6 million people. The VSD project allows for planned vaccine safety studies, as well as timely investigations of hypotheses.

In the late 1990s, reports in the lay press questioned the safety of routine immunizations and alarmed parents with unsupported accounts of the dangers of vaccines. One major national television network broadcast a feature piece that linked diabetes to childhood immunizations. In the United Kingdom, a report linked receipt of measles vaccine to the development of autism, and in France, hepatitis vaccine was reported to cause multiple sclerosis (MS).

VACCINATION AND DIABETES

Classen and Classen[169] suggested that certain vaccines, if given at birth, may decrease the occurrence of type 1 diabetes, whereas if initial vaccination is performed after children are 2 months of age, the occurrence of diabetes increases. Other researchers have not found an increased risk for diabetes associated with vaccination.[299] The Classen theory is based on results from experiments in laboratory animals, as well as comparisons of rates of diabetes among countries with different immunization schedules.[169] Applying findings from laboratory animals to humans is fraught with uncertainties. Comparison of diabetes rates among countries with different vaccination policies also provides weak evidence because many factors, including vaccination schedules, may differ by country. In addition, other factors, including genetic predisposition and numerous possible environmental exposures unrelated to the administration of vaccines, may influence the development of diabetes.[298]

The most rigorous epidemiologic study of infant vaccinations and type 1 diabetes to date found that measles vaccine was associated with a decreased risk and no association was found with bacille Calmette-Guérin (BCG), smallpox, tetanus, pertussis, rubella, or mumps vaccine.[66] In a large clinical trial of Hib vaccine conducted in Finland, no statistically significant association was found between the receipt of Hib vaccine and the development of type 1 diabetes during 10 years of follow-up.[293] The IOM Immunization Safety Committee reviewed the clinical and epidemiologic literature in 2002 and concluded that multiple immunizations do not lead to a risk for development of type 1 diabetes.[279]

MEASLES VACCINE AND AUTISM

The causes of autism are unknown. Most experts agree, however, that autism is a condition that begins before birth.[410] Genetic factors seem to play a major role in the development of autism. Autism usually is diagnosed in children when they are 18 to 30 months old, shortly after they have received many of the recommended vaccinations. Because of this coincidence in timing, some parents of children with autism believe that an immunization may have caused their child's condition.

A 1998 report published in *The Lancet* of 12 patients who had inflammatory bowel disease and autism raised the hypothesis that a link could exist between the MMR vaccine and autism.[479] The authors of the report speculated that MMR vaccine was the possible cause of bowel problems, with the resultant malabsorption of essential vitamins and nutrients leading to autism.

Epidemiologic studies in both the United States and Great Britain do not support a causal association between MMR vaccine and inflammatory bowel disease,[198,363] nor do epidemiologic studies support a causal association between MMR vaccine (and other measles-containing vaccines) and autism.[201,202] Expert panels convened by both the IOM and the AAP examined the available data on autism and MMR vaccine. Both panels concluded that the available evidence does not support the hypothesis that MMR vaccine causes autism or associated disorders or inflammatory bowel disease.[252,281]

HEPATITIS B VACCINE AND MULTIPLE SCLEROSIS

Reports of MS developing after receipt of hepatitis B vaccine led to the concern that immunization with hepatitis B vaccine may precipitate the onset of MS or lead to relapses. Since licensure, the safety of the hepatitis B vaccine has continued to be monitored. The several studies conducted did not find a scientific association between hepatitis B vaccination and the development of severe neurologic adverse events such as optic neuritis and Guillain-Barré syndrome (GBS). Two large epidemiologic studies examined the risk of an association between vaccines and MS. The first, a case-control study in a large cohort of nurses, demonstrated no significant association between hepatitis B vaccination and development of MS.[47] A French study of patients with MS found that vaccination did not appear to increase the short-term risk of relapse.[185]

The Immunization Safety Review Committee of the IOM reviewed all available data and issued a report in 2004. The committee concluded that the evidence did not support a causal relationship between hepatitis B vaccine administered to adults and onset or relapse of MS.[279]

THIMEROSAL-CONTAINING VACCINES AND NEURODEVELOPMENTAL DISORDERS

Thimerosal is a mercury-containing preservative that has been used as an additive to biologics and vaccines since the 1930s because it is effective in preventing bacterial and fungal contamination, particularly in multidose containers. The FDA Modernization Act of 1997 required a review and assessment of the risk of all mercury-containing pharmaceuticals. Assessment of vaccines led to the recognition that, during the first 6 months of life, some children could be exposed to a cumulative quantity of mercury that exceeds one of the federal guidelines on methylmercury. However, a significant safety margin is incorporated into all the acceptable mercury exposure limits, and no data determined or evidence was found that any harm was caused by the level of exposure with the existing immunization schedules. In addition, the risk posed by exposure to thimerosal, which contains ethylmercury, not methylmercury, on which the federal guidelines were based, is unknown. However, to avoid any potential risk, the Public Health Service, the AAP, and vaccine manufacturers agreed in July 1999 that thimerosal-containing vaccines should be removed as soon as possible. As of March 2001, thimerosal no longer was used as a preservative in any of the pediatric vaccines given to children 6 years old or younger in the United States.[125]

A possible association between thimerosal-containing vaccines and increasing diagnoses of autism and autistic spectrum disorders has been investigated in studies in the United States and elsewhere. The Immunization Safety Review Committee of the IOM reviewed all available data and, in October of 2001, issued a report stating that the evidence is inadequate to accept or reject a causal relationship between exposure to thimerosal-containing vaccines and the neurodevelopmental disorders of autism, attention-deficit/hyperactivity disorder (ADHD), and speech and language delay. In addition, the report concluded that the hypothesis that exposure to thimerosal could be associated with the development of neurodevelopmental disorders, although not established, is biologically plausible.[282] The IOM committee recommended that thimerosal-free DTaP, hepatitis B, and Hib vaccines be used for infants, children, and pregnant women, provided an adequate supply is available.[282]

Since the IOM report, five large studies have compared the risk of autism in children who received vaccines containing thimerosal with that in children who received vaccines without thimerosal.[42,222,263,275,469] The studies found the incidence of autism to be the same in both groups.

Reference Sources

Several comprehensive sources of information about pediatric vaccines are available. The AAP publishes *The Red Book: Report of the Committee on Infectious Diseases* every 3 years. The next edition will be published in 2009. In the interval between editions, the AAP publishes recommendations in its newsletter *AAP News* and subsequently in *Pediatrics*. The ACIP issues vaccine recommendations and relevant information in *Morbidity and Mortality*

Weekly Report. Manufacturers provide product information for each vaccine in the FDA-approved package inserts.

VACCINES RECOMMENDED FOR ROUTINE ADMINISTRATION

DIPHTHERIA TOXOID

The introduction of diphtheria toxoid–containing vaccine in the 1940s led to a dramatic reduction in the incidence of diphtheria in the United States. From 1980 through 2004, 57 cases of diphtheria were reported in the United States, an average of 2 to 3 per year.[307] Only 5 cases have been reported since 2000. However, diphtheria is still a potentially significant public health problem. Serologic surveys in the United States and England have suggested that many adults are not immune.[186,307,335] Although a study published in 1996 demonstrated that most adults in the United States do have protective concentrations of serum antitoxin,[245] more recent data obtained from a national population based serosurvey published in 2002 revealed that the prevalence of immunity to diphtheria, as determined by the level of diphtheria antitoxin, progressively decreased from 91 percent among children 6 to 11 years of age to approximately 30 percent in those 60 to 69 years of age.[350] Of the reported cases with known patient age since 1980, 58 percent were in persons 20 years of age or older and 44 percent of cases were among persons 40 years of age or older. Most cases have occurred in unimmunized or inadequately immunized persons. The current age distribution of cases corroborates the finding of inadequate levels of circulating antitoxin in many adults. In addition, adequate immunization has not eliminated the potential for transmission of *Corynebacterium diphtheriae* completely because immunization does not prevent carriage of *C. diphtheriae* in the nasopharynx or on the skin.[71,307]

As a result of inadequate immunity in adults as well as in infants and children, an epidemic of diphtheria occurred in the 1990s throughout the former Soviet Union including Russia, Ukraine, and the central Asian republics, with case-fatality rates ranging from 3 to 23 percent.[224,255] In addition, diphtheria continues to be a significant cause of morbidity and mortality in developing countries,[224] and, therefore, it remains a source of possible exposure during travel to endemic countries. Because humans are the only known reservoir for *C. diphtheriae*, universal immunization with a diphtheria toxoid–containing vaccine is the only effective control measure.

Preparations

Diphtheria toxoid is produced by growing toxigenic *C. diphtheriae* in liquid medium. The filtrate is incubated with formaldehyde to convert toxin to toxoid and then is adsorbed onto an aluminum salt adjuvant.

Diphtheria toxoid is available in combination with DTaP for routine immunization of infants and children younger than 7 years of age and for adolescents and adults aged 11 to 64 years as a single booster (Tdap). Two brands of Tdap are available: Boostrix (approved for children 10 to 18 years of age) and Adacel (approved for persons 11 to 64 years of age). DTaP and Tdap vaccines do not contain thimerosal as a preservative. Diphtheria toxoid also is available as a DTaP-inactivated poliovirus (IPV)-hepatitis B combination, a DTaP-inactivated poliovirus (IPV)-*Haemophilus influenzae* type b conjugate combination, and in combination with tetanus toxoid (DT and Td) alone for use when pertussis vaccination is contraindicated. *H. influenzae*, pneumococcal, and meningococcal conjugate vaccines containing diphtheria toxoid or CRM_{197} protein, a nontoxic variant of diphtheria toxin, are not substitutes for diphtheria toxoid immunization.[8]

Pediatric formulations (DT and DTaP) contain a similar amount of tetanus toxoid as adult Td, but they contain three to four times as much diphtheria toxoid. The concentration of diphtheria toxoid (D) for children younger than 7 years of age per 0.5 mL intramuscular dose of DTaP or DT vaccine is 6.7 to 25 limit of flocculation units (Lf), depending on the vaccine manufacturer. Vaccines approved for children 7 years or older and adults (Tdap and Td) contain only a fraction of the diphtheria toxoid (d, 2 to 2.5 Lf) because of adverse reactions related to dose and age.[8,71,307]

Immunogenicity

After a primary series of three properly spaced diphtheria toxoid doses in adults or four doses in infants, a protective level of antitoxin (defined as > 0.1 international unit [IU] of antitoxin/mL) is reached in more than 95 percent of vaccinees. Diphtheria toxoid has been estimated to have a clinical efficacy of 97 percent. Immune response to diphtheria toxoid was measured following administration of each adult Tdap vaccine and compared with that elaborated after Td vaccine. Seroprotective anti-diphtheria levels, defined as a titer of 0.1 or more IU per mL, and booster response rates to diphtheria were determined to be non-inferior following administration of Tdap as compared with Td vaccination.[307]

Adverse Events

Other than local reactions of pain and swelling at the site of vaccine injection, immunization does not cause significant adverse events. These local reactions have been attributed to hypersensitivity reactions in response to the pertussis component and are not a contraindication for administration of further vaccination if otherwise indicated.

Regarding adverse reactions reported following administration of Tdap, vaccination with Boostrix was associated with a statistically higher rate of moderate to severe headache compared with Td, and Adacel was associated with higher rates of mild injection site pain and low-grade fever compared with Td vaccine. No serious adverse events have been reported for Boostrix. There have been two reports of serious adverse events, both characterized as neuropathic reactions, in adults, possibly related to having received Adacel (none reported in adolescents), and in both cases symptoms resolved completely within several days.[71]

Indications

Primary immunization consists of five doses of diphtheria toxoid, provided as DTaP or as DT if pertussis is contraindicated.[8,71,307] The first three doses are administered routinely to children who are 2, 4, and 6 months of age. A fourth dose is administered 6 to 12 months after the third dose, between 12 to 18 months of age, to maintain adequate antibody concentrations for the ensuing preschool years. For those not immunized in infancy, the initial dose is followed by two doses given 2 and 8 to 14 months later. A single booster dose given when the child is 4 to 6 years of age, before school entry, is indicated unless the preceding dose was given after the fourth birthday. Interruption of the recommended schedule or delay in administering subsequent doses during primary immunization does not reduce immunity or necessitate restarting the series.

In 2005, the FDA licensed two vaccines containing tetanus toxoid, reduced diphtheria toxoid, and acellular pertussis antigens (Tdap) for routine use in adolescents and adults. Tdap is approved as a one-time only substitute for the next scheduled or recommended Td booster in those individuals who previously completed their primary vaccination series with DTP or DTaP. The preferred age for Tdap vaccination is 11 to 12 years. Adolescents

aged 11 to 18 years who completed their primary series with DTP or DTaP and received a Td booster at least 5 years previously are encouraged to receive Tdap. Although an interval of 5 years between Td and Tdap is recommended to reduce the likelihood of adverse reactions, periods shorter than 5 years between doses can be used when the patient has an increased risk of exposure to or complications from pertussis. Every 10 years thereafter, tetanus booster should be provided by Td. Adolescents between the ages of 11 and 18 years who have never been vaccinated against tetanus, diphtheria, or pertussis initially should receive Tdap followed by Td for the subsequent two doses at 4 weeks or more and 6 to 12 months following. No pertussis-containing vaccine has been approved for children between the ages of 7 and 10 years. For children in this age group who have not been vaccinated previously, three doses of Td are recommended following the schedule for those aged 11 to 18 years. A booster with Tdap is recommended when these children become adolescents.

After exposure to a case of strongly suspected or proven diphtheria, asymptomatic previously immunized persons should receive a booster of an age-appropriate diphtheria-containing vaccine if at least 5 years have transpired since their last receipt of a diphtheria toxoid–containing vaccine.[8] If not previously immunized, carriers should receive vaccine as soon as identified and should complete the entire series. If a carrier has been immunized and a period of 5 years or more has transpired since they received their most recent booster, a booster containing diphtheria (DTaP, Tdap, DT, or Td) should be administered. Patients recovering from diphtheria infection should be immunized because infection does not confer immunity.

Precautions and Contraindications

The only contraindication to diphtheria toxoid is a history of severe hypersensitivity developing after receipt of a previous vaccine dose. Vaccination with either diphtheria or tetanus toxoid is not known to be associated with an increased risk of having convulsions. Local reactions alone do not preclude continued use.

HAEMOPHILUS INFLUENZAE TYPE B VACCINE

Before the introduction of routine infant and childhood vaccination against Hib, this pathogen was the major cause of invasive bacterial infections in young children in the United States. It was the most common cause of bacterial meningitis and epiglottitis and a significant cause of septic arthritis, occult febrile bacteremia, and pneumonia in children younger than 5 years, in whom it caused an estimated 12,000 cases of meningitis and 8000 additional cases of invasive Hib disease annually.[175] The cumulative risk of Hib disease was approximately 1 in every 200 U.S. children in the first 5 years of life, with the peak incidence of Hib meningitis occurring in infants between 6 and 12 months of age. In high-risk populations, such as Native Americans, rates of disease in the absence of immunizations were higher, and a greater proportion of cases of meningitis occurred in the first year of life than in non–high-risk populations.[175,265,491]

Meningitis and pneumonia caused by Hib remain significant causes of morbidity and mortality, particularly among children younger than the age of 5 years, in many parts of the developing world. As of November of 2006, the WHO estimated that at least 3 million cases of serious disease and approximately 386,000 deaths were attributable to Hib annually. Hib remains the most common cause of non-epidemic bacterial meningitis in unvaccinated children younger than 1 year of age. Mortality rates for Hib meningitis treated with appropriate antibiotics range up to 20 percent, and 30 to 40 percent of survivors suffer severe neurologic sequelae.[506]

Because most cases of Hib disease occur in infancy, vaccines that induce protection before the age of 6 months are necessary for effective control of Hib disease. The Hib polysaccharide capsule is the major virulence factor, and antibodies directed against the polysaccharide antigens are protective. Capsular type b is responsible for more than 90 percent of systemic *H. influenzae* infections. However, purified polysaccharide vaccines induce primarily a T-lymphocyte–independent IgM antibody response that is poorly immunogenic, particularly in children younger than 18 months who lack immunologic memory. Covalent linkage of the purified capsular polysaccharide, polyribosylribitol phosphate (PRP), to a protein carrier creates a conjugate glycoprotein that is T-lymphocyte dependent and elicits protective antibody in infants and young children and significantly greater concentrations of circulating anti-PRP at all ages than does the unconjugated polysaccharide.[320,376] T-cell–dependent antigens involve helper T-lymphocyte activation of a B-cell humoral antibody response. T-cell–dependent antigens also are able to prime for a booster response.

The introduction of Hib conjugate vaccines in the United States, first in children at least 18 months of age in 1987 and for routine infant immunization in 1991, decreased the incidence of meningitis and bacteremia caused by Hib dramatically. As of 2000, the incidence of invasive Hib disease in the United States had decreased by 99 percent since the pre-vaccine era, with an incidence rate of less than 1 case per 100,000 children younger than 5 years old.[137] The remarkably rapid reduction in incidence of disease was partly the result of the ability of the vaccine to reduce asymptomatic nasopharyngeal carriage of the organism, an action that had the indirect effect of reducing exposure and infection in those not immunized (herd immunity).[49,260]

Preparations

Three single-antigen Hib conjugate vaccine products and three combination vaccine products that contain Hib conjugate are available in the United States. All Hib vaccines contain PRP, the organism's polysaccharide capsular antigen. The three currently licensed single-antigen Hib conjugate vaccines, HbOC (HibTITER), PRP-OMP (PedvaxHIB), and PRP-T (ActHIB), are approved for use in early infancy (Table 255–10). Of these prod-

TABLE 255–10 *Haemophilus influenzae* Type b Conjugate Vaccines

Manufacturer	Abbreviation (Trade Name)	Carrier Protein
Wyeth-Lederle Vaccines	HbOC (HibTITER)	CRM_{197} (a nontoxic mutant diphtheria toxin)
Merck & Co.	PRP-OMP*(PedvaxHIB)	OMP (an outer-membrane protein complex of *Neisseria meningitidis*)
Sanofi Pasteur	PRP-T† (ActHIB)	Tetanus toxoid

**PRP-OMP is also available as a combination vaccine with hepatitis B (COMVAX).*
†PRP-T may be reconstituted with diphtheria-tetanus-acellular pertussis (DTaP) (Tripedia, manufactured by Sanofi Pasteur) to form TriHIBit. TriHIBit is approved only for the fourth dose of the DTaP and Haemophilus influenzae *type b (Hib) series. Other licensed formulations of DTaP have not been approved by the Food and Drug Administration for reconstitution and may not be used for this purpose.*
Adapted from Pickering, L. K., Baker, C. J., Long, S. S., et al. (eds.): Red Book: 2006 Report of the Committee on Infectious Diseases. 27th ed. Elk Grove Village, IL, American Academy of Pediatrics, 2006.

ucts, only PRP-T can be mixed with DTaP for the fourth dose. Each conjugate vaccine is chemically and immunologically unique. All three are composed of the type b PRP antigen conjugated to a protein and differ in the protein carrier, size of the saccharide component, and the chemical linkage. The carrier proteins for HbOC, PRP-OMP, and PRP-T are a nontoxic mutant diphtheria toxin, an outer membrane protein from *Neisseria meningitidis*, and tetanus toxoid, respectively. Since July of 2000, the entire Hib vaccine supply for the United States has been thimerosol-free. The dose of each Hib conjugate vaccine is 0.5 mL, given intramuscularly.

Three licensed combination vaccines containing Hib are as follows: (1) DTaP/PRP-T (TriHIBit), which, as noted earlier is the combination of Hib with DTaP approved for the fourth dose only; and (2) PRP-OMP/Hep B (Comvax), the bivalent Hib and recombinant hepatitis B virus (HBV) vaccine approved for use in children aged 6 weeks to 15 months who were born to hepatitis B surface antigen (HBsAg)–negative mothers, and (3) DTaP/IPV/PRP-T(Pentocel), which is approved for immunization at 2, 4, 6, and 15 to 18 months of age.

Immunogenicity and Efficacy

Placebo-controlled field trials of Hib conjugate vaccines in infants in the United States demonstrated nearly 100 percent protection and provided the basis for the initial approval of these vaccines for use in this country. In a study of HbOC in northern California, vaccine efficacy was 100 percent for infants receiving the three-dose schedule at 2, 4, and 6 months of age.[62] In a Navajo population of infants at high risk of acquiring Hib disease who were vaccinated at 2 and 4 months of age with either PRP-OMP or placebo, vaccine efficacy was 100 percent at 1 year of age and 93 percent in total.[422] Licensure of PRP-T was based on immunogenicity in a three-dose schedule that was comparable to that of the other two products. In addition, an efficacy trial in Great Britain and the lack of cases in trials indicate an efficacy of PRP-T comparable to that of HbOC and PRP-OMP.[67,422,462] Although the data are limited, some evidence suggests that administration of the first dose of Hib before the child reaches the age of 6 weeks may result in immunologic tolerance to the Hib antigen and reduce the immune response to subsequent doses.[351]

Adverse Events

Hib vaccines are well tolerated. Local reactions occur in approximately 25 percent of recipients but typically are mild and last less than 24 hours.[200,491] Systemic reactions, such as fever and irritability, are infrequent occurrences. When conjugate vaccines are administered concurrently with DTaP vaccine, the incidence of systemic reactions is similar to that observed when only DTaP is given.[200]

Indications

Routine vaccination against Hib disease is recommended for all children beginning at approximately 2 months of age.[9,89,94] Four vaccines, HbOC, PRP-T, PRP-OMP and DTaP/IPV/PRP-T, are licensed in the United States for use in infants. One of these products, PRP-T, can be given with DTaP, mixed in the same syringe for the fourth dose only.

Two or three doses, depending on the product, given at 2-month intervals (optimal) are indicated by the time that the child is 6 months of age. The recommended age to initiate the primary series is 2 months, with a minimum age of 6 weeks. The minimum interval between doses is 4 weeks, with at least 8 weeks separating the last dose in the primary series and the booster. Excellent immune responses have been achieved when vaccines from different manufacturers have been interchanged in the primary series.[41,60,241] HbOC, PRP-T, and PRP-OMP are considered interchangeable for primary as well as booster vaccinations. If more than a single brand of vaccine is used, the child should receive a three-dose primary series. A final dose of any product, irrespective of the previous vaccines received, is acceptable when the child is 12 to 15 months of age for completion of the Hib immunization schedule. When feasible, the conjugate vaccine product used for the first dose should be used for subsequent doses in children younger than 12 months.

For children in whom Hib immunization has not been initiated by the time that they reach 7 months of age, the recommended schedules differ according to the child's age and the choice of conjugate vaccine.[29,89] Previously unimmunized children between 15 and 59 months should be immunized with at least a single dose of any licensed conjugate Hib vaccine. For previously unimmunized children 5 years or older, immunization is indicated only if they have an underlying condition predisposing to Hib disease, such as asplenia, immunoglobin deficiency, or stem cell transplant, or are immunosuppressed because of chemotherapy or HIV infection. More than one dose may be recommended for this population.[9]

HEPATITIS A VACCINE

The occurrence of hepatitis A is highest in developing countries and reflects the primary route of transmission, fecal-oral, person-to-person spread. In the United States, before a vaccine became available, hepatitis A was a common occurrence and caused substantial morbidity with significant associated costs.[103,304] In 1995, the FDA licensed hepatitis A vaccine, and during the decade that followed the rate of hepatitis A infection in the United States dramatically declined, reaching an all time low of 5683 cases in 2004.[219]

In the pre-vaccine era, the incidence of hepatitis A varied considerably in different populations.[219,445] A shift occurred in the epidemiology of hepatitis A in the United States following the introduction of hepatitis A vaccine. Disease in the United States most commonly occurred in children 5 to 14 years of age.[219] After vaccines were licensed, rates of infection among children declined more rapidly than did rates among adults, thus resulting in similar rates among all age groups. Historically, rates of infection were highest among Alaskan Natives and American Indians, and most cases consistently occurred in a small number of states and counties in the western and southwestern regions of the country. During the decade after vaccine was licensed, age-specific, racial, and geographic disparities narrowed considerably.[219]

Individuals at risk for acquiring hepatitis A infections include close contacts of persons infected with hepatitis A virus (HAV), travelers to developing countries, those who engage in homosexual and bisexual activity, and injecting drug users. However, in approximately 50 percent of reported cases, no risk factor is identified.[10] These infections likely are attributable to fecal-oral spread from asymptomatic contacts. Because children frequently have asymptomatic infections and may shed virus for prolonged periods, they play an important role in transmission of HAV. Children and infants shed virus for longer periods than adults do, as long as several months after the onset of clinical illness. In one study involving adults without an identified source of infection, 52 percent of their households included a child younger than 6 years.[219] Therefore, it has long been recognized that the control and ultimate elimination of hepatitis A by active immunization would be best achieved through universal childhood immunization.[464] However, it was not until 2005 that hepatitis A vaccine was licensed for use in children from 12 to 23 months of age.

Before then, it had been restricted to children older than 2 years old and consequently could not be incorporated readily into routine infant vaccinations. In May of 2006, the ACIP recommended including hepatitis A vaccine in the routine infant immunization schedule.[219]

Before vaccine was available, community-wide outbreaks, recurring every 3 to 10 years in high-risk communities, accounted for much of the occurrence of hepatitis A infection and disease. Outbreaks among children attending daycare and daycare staff are common occurrences and have been associated with community outbreaks.[249] However, the prevalence of hepatitis A infection in daycare center staff and in children and adolescents who previously attended daycare is not increased, a finding suggesting that infections within daycare settings most commonly reflect transmission within the community that extends to these settings.[249] Transmission of HAV also can occur in institutions for the developmentally disabled and in neonatal intensive care units. Transmissions from hospitalized patients to health care professionals have been reported.[10] In addition to these examples of direct person-to-person transmission, infection can be acquired by the ingestion of contaminated food or water.

Preparations

Both inactivated and attenuated HAV vaccines have been developed.[189] However, only inactivated vaccines are licensed in the United States. Inactivated HAV vaccine is prepared by methods similar to those used for inactivated poliomyelitis vaccine. Virus is propagated in human diploid fibroblast cell cultures, formalin inactivated, and adsorbed to aluminum hydroxide adjuvant.[26,219] Two such products are licensed in the United States: Havrix (SmithKline Beecham Biologicals) and Vaqta (Merck & Co.). Both Vaqta and Havrix have two formulations, an adult and a pediatric product with different antigen content (Table 255–11). The pediatric formulation is indicated for persons aged 12 months to 18 years of age. The vaccines can be used interchangeably.[75] Limited data indicate that hepatitis A vaccine may be administered simultaneously with other vaccinations.[10]

Immunogenicity and Efficacy

Inactivated viral vaccine is highly immunogenic. After receiving a single dose, 95 percent of children and nearly all adults seroconvert within 1 month.[26,219] After receipt of a second dose in children, seroconversion approximates 100 percent. Hepatitis A vaccine is immunogenic in children younger than 2 years old who do not have passively acquired maternal antibody.[393,457] In two large clinical trials of inactivated hepatitis A vaccine in children older than 2 years, protective efficacy was greater than 90 percent.[277,493] In a double-blind, placebo-controlled, randomized study in Thailand involving approximately 34,000 vaccinees, the protective efficacy against clinical hepatitis A was 94 percent after administration of two doses given 1 month apart; it was 100 percent after subsequent administration of a 12-month booster dose.[277]

Vaccination also has been demonstrated to be effective in controlling outbreaks in communities with high rates of disease.[219] For example, in a New York State community in which HAV is highly endemic in children, a single dose of vaccine was 100 percent effective beginning 3 weeks after immunization in preventing symptomatic disease.[493] Moreover, observations from countries where routine hepatitis A vaccination of infants or children has been implemented suggest a strong herd immunity effect.[193,203] The duration of protection after vaccination is likely to be prolonged. Protective antibody levels of anti-HAV were observed in 99 percent of children evaluated 5 to 6 years after receiving Vaqta.[492] Kinetic models of antibody decline indicate that protective levels of antibody could be present for more than 25 years in adults and up to 20 years in children.[463,465] No data are available currently to determine whether and when children will need a hepatitis A booster vaccine. In an ongoing study designed to address the need for a booster dose, no cases of hepatitis A among children 9 years after initial vaccination have been reported.[494]

Reduced vaccine immunogenicity has been observed in infants with passively acquired anti–hepatitis A antibody who are administered hepatitis A vaccines.[190,321,325] Studies demonstrated that, despite the presence of lower antibody levels in infants born to anti–hepatitis A–positive mothers, most infants with passively acquired antibody had an anamnestic response to a booster dose 1 to 6 years later.[190,218,291] In most infants, maternally acquired anti–hepatitis A antibody declines to undetectable levels by the time the child reaches 12 months of age.[324] Hepatitis A vaccine is highly immunogenic for all infants when administered when they are older than 1 year of age, irrespective of maternal antibody status.[55,190]

Concurrent administration of immune globulin and vaccine inhibits the peak serum antibody concentration achieved but not the rate of seroconversion.[477] Because antibody levels are much higher the protective concentration, this inhibition is not considered clinically significant and supports passive-active immunoprophylaxis when indicated.

Adverse Events

Except for rare reports of anaphylaxis and anaphylactoid reaction in adults in Europe and Asia, serious reactions to inactivated HAV vaccine have not been reported.[218] Pain, tenderness, and infection at the injection site can occur.[277]

TABLE 255–11 Recommended Doses and Schedules for Inactivated Hepatitis A Vaccines

Age (yr)	Vaccine*	Hepatitis A Antigen Dose	Volume per Dose (mL)	No. of Doses	Schedule
1-18	Havrix	720 ELU	0.5	2	Initial and 6-12 mo later
1-18	Vaqta	25 U†	0.5	2	Initial and 6-18 mo later
≥19	Havrix	1440 ELU	1.0	2	Initial and 6-12 mo later
≥19	Vaqta	50 U†	1.0	2	Initial and 6-18 mo later
≥18	Twinrix‡	720 ELU	1.0	3	Initial and 1 and 6 mo later

**Havrix and Twinrix are manufactured by GlaxoSmithKline Biologicals; Vaqta is manufactured and distributed by Merck & Co.*

†Antigen units (each unit is equivalent to ≈1 μg of viral protein).

‡A combination of hepatitis B (Engerix-B, 20 μg) and hepatitis A (Havrix, 720 ELU) vaccine (Twinrix) is licensed for use in people 18 years of age and older in a three-dose schedule. Havrix 360 ELU in single-dose vials is licensed in the United States but no longer is available.

ELU, enzyme-linked immunosorbent assay units.

Adapted from Pickering, L. K., Baker, C. J., Long, S. S., et al. (eds.): Red Book: 2006 Report of the Committee on Infectious Diseases. 27th ed. Elk Grove Village, IL, American Academy of Pediatrics, 2006.

Indications

In May of 2006, the ACIP recommended that hepatitis A vaccine be integrated as part of the childhood vaccination schedule.[10,26,218] All children should receive hepatitis A vaccine routinely according to the licensed two-dose schedule, with the initial dose administered at age 1 year (12 to 23 months) and the second dose 6 to 12 months later. Catch-up vaccination of unvaccinated children 2 to 18 years of age also can be considered. In addition, hepatitis A vaccination is recommended routinely for the following persons who are at increased risk of acquiring infection:

- All susceptible persons traveling to or working in countries that have a high or intermediate hepatitis A endemicity; these persons should be vaccinated or receive immune globulin before departure
- Persons who receive clotting factor concentrates, especially solvent detergent–treated preparations
- Sexually active homosexual and bisexual males
- Illicit drug users
- Persons working with infected primates or with HAV in a laboratory
- Persons with chronic liver disease; routine vaccination is recommended because these persons may be at increased risk for developing fulminate hepatitis if they become infected with HAV

Although the ACIP guidelines do not recommend routine vaccination of food handlers, consideration may be given to vaccination of these workers in areas where state and local health authorities or private employers determine that vaccination is cost-effective.

Effectiveness of vaccination has not been demonstrated in localized outbreaks occurring in institutions for the developmentally disabled, daycare centers, schools, and prisons; administration of intramuscular immune globulin currently is recommended for close contacts of infected persons in these circumstances. At present, HAV vaccine is not indicated routinely for daycare attendees and staff.

Precautions and Contraindications

Hepatitis A vaccine should not be administered to persons with a hypersensitivity reaction to any of the vaccine components, such as alum or, in the case of Havrix, phenoxyethanol.[10,26,219] Safety data in pregnant women are not available, but the risk is considered to be low or nonexistent because the vaccine contains inactivated, purified viral proteins.

HEPATITIS B VACCINE

HBV infection is a leading cause of acute hepatitis and a major public health problem of global importance. Its incidence is especially high in many Asian and African countries. Individuals with chronic infection are at risk for the development of chronic hepatitis, cirrhosis, and primary hepatocellular carcinoma. Rates of new infection are highest among adults, but chronic infection is more likely to occur in individuals infected as infants or children. These persons not only are at increased risk for chronic and malignant liver disease but also, as chronic carriers, serve as the reservoir for transmission of HBV.

The initial strategies for prevention of hepatitis B through vaccination reflect the varying epidemiology of HBV infection in different areas of the world.[90] In the United States, for example, infection is of comparatively low endemicity and occurs primarily in adolescents and adults. The risk of acquiring infection, however, is much greater in certain populations. Examples include those born and living in areas or among groups in which HBV is highly endemic and those with lifestyles predisposing to the acquisition of HBV, such as male homosexual activity, intravenous drug abuse, and promiscuous heterosexual activity.[3] In contrast, in geographic areas in which HBV infection is highly endemic, infection usually is acquired at birth or during childhood, a pattern resulting in the recommendation for universal vaccination of infants.

In 1991, the CDC initiated a comprehensive hepatitis B vaccination strategy to eliminate transmission of HBV in the United States.[90] Critical elements of this strategy included preventing perinatal transmission of HBV by identifying and providing immunoprophylaxis to infants of HBsAg-positive mothers and universal hepatitis B vaccination of infants to interrupt transmission and prevent future infection. The advantages of this approach included the existence of effective programs of routine childhood immunization, protection without any need to identify specific risk factors, and protection before significant exposure occurred. In addition, the positive effects of universal infant immunization had been observed in Taiwan, where the strategy of universal infant immunization already was being used. In Taiwan, the overall prevalence rate of HBV for children aged 1 to 10 years decreased from 9.8 percent in 1984 to 1.3 percent in 1994.[161]

In 1994, the ACIP expanded the recommendations to include previously unvaccinated children, 11 to 12 years old.[100] In October 1997, these recommendations were expanded still further to include all unvaccinated children 0 to 18 years old and made hepatitis B vaccine available through the Vaccines for Children (VFC) program for persons 0 to 18 years of age who are eligible for this program.[117] The goal of the 1997 recommendations was to increase access to hepatitis B vaccine by encouraging the vaccination of previously unvaccinated children and adolescents 0 to 18 years old whenever they are seen for routine medical visits.[107] This expansion of the recommended age group for receiving vaccination and for VFC eligibility simplifies previous recommendations and the eligibility criteria for receiving VFC vaccine. The other ACIP priorities for giving hepatitis B vaccination to children remained unchanged and included the following groups: all infants; children in populations at high risk for acquiring HBV infection, such as Alaska Natives, Pacific Islanders, and children who reside in households of first-generation immigrants from countries where HBV infection is moderately or highly endemic; previously unvaccinated children 11 to 12 years old; and older adolescents and adults in defined at-risk groups.[90,100]

Between 1990 and 2004, the incidence of HBV infection in the United States declined by 75 percent. The decline was greatest among children and adolescents. Coincident with the decline in HBV incidence, vaccine coverage for children aged 19 to 35 months increased from 16 to 92 percent from 1991 to 2004.[144] Among adolescents 13 to 15 years old, vaccine coverage increased from zero to 74 percent between 1993 and 2004 (CDC, unpublished data). Clearly, since its inception in 1991, many aspects of the national immunization strategy to eliminate HBV transmission have been implemented with great success, especially the routine vaccination of all infants. Nonetheless, many challenges remain.

Despite the overall decline in hepatitis B incidence and the recommendations for routine vaccination of infants and children, increasing proportions of new HBV infections in the United States occur among adolescents and adults who have defined risk factors, including persons with multiple sex partners (more than one partner during the preceding 6 months), men who have sex with men, and injecting drug users.[332] The primary means of preventing these infections is to identify settings such as correctional facilities, sexually transmitted disease clinics, and drug treatment centers where adolescents and adults with high-risk drug and sexual practices can be routinely accessed and vaccinated. In correctional facilities where previously unvaccinated

inmates are offered HBV vaccination, 60 to 80 percent of inmates offered accepted vaccination.[489]

Even though universal screening of all pregnant women has been widely adopted across the United States, fewer than half of all HBsAg-positive pregnant women are identified through prenatal screening.[215] These women with unknown HBsAg status are not screened consistently, even when they are hospitalized during labor and delivery; consequently, their infants do not receive appropriate post-exposure prophylaxis.[453] Furthermore, the birth dose of HBV vaccine, which could serve as a safety net for infants born to mothers for whom testing either was not performed or not performed correctly, was administered to only 45 percent of infants born in the United States in 2004, less than the rate of 54 percent seen before July of 1999, when recommendations were made to suspend the birth dose of hepatitis B vaccine temporarily until a thimerosal-free vaccine became available.[331]

To enhance existing strategies aimed at prevention of perinatal HBV transmission, the ACIP recommended in December of 2005 that all delivery hospitals institute specific policies and procedures to improve identification of infants born to HBsAg-positive mothers and mothers with unknown HBsAg status. These recommendations were made to ensure administration of appropriate post-exposure prophylaxis and a birth dose of HBV vaccine to all medically stable infants. This statement also updated recommendations to improve vaccination coverage of adolescents and children by implementing immunization record reviews for all children 11 to12 years old and children younger than 19 years of age who were born in countries with high or intermediate HBV endemicity and vaccinating all unvaccinated adolescents in settings that provide health care services to this age group.[343]

Preparations

Hepatitis B vaccines consisting of inactivated purified HBsAg derived from chronic hepatitis B plasma were introduced first in the early 1980s. In the late 1980s, two recombinant vaccines (Recombivax HB, Merck & Co.; and Engerix-B, SmithKline Beecham Biologicals) were licensed in the United States and currently are available in both single-antigen and combined formulations. Only the recombinant vaccines are available in the United States, but plasma-derived vaccines are widely used in other areas of the world. The recombinant vaccines contain 10 to 40 μg/mL of HBsAg protein. Pediatric formulations contain no thimerosal or only trace amounts. Vaccine is administered intramuscularly in the anterolateral thigh or deltoid area, depending on the age and size of the recipient. Administration in the buttocks or intradermally has been associated with decreased immunogenicity and is not recommended at any age.

Immunogenicity and Efficacy

The recommended series of three doses of vaccine induces a protective antibody response in more than 95 percent of infants, children, adolescents, and adults younger than 40 years.[11,343] In field trials, efficacy has been 80 to 95 percent and generally correlates with immunogenicity. Protection against disease is virtually 100 percent for persons who develop adequate serum antibody concentrations (anti-HBs ≥10 mIU/mL) after receiving vaccination. Adults older than 40 years of age and immunosuppressed persons are less likely to develop protective anti-HBs concentrations.

Hepatitis B vaccine is highly immunogenic. Therefore, post-vaccination serologic testing is not indicated, with the exception of infants born to HBsAg-positive women, persons with ongoing occupational exposure to blood, and persons with immunosuppressive conditions. Active immunization in combination with passive immunoprophylaxis with hepatitis B immune globulin (HBIG) administered within 12 to 24 hours after birth to infants born to chronically infected mothers is more than 90 percent effective in preventing transmission of HBV to the infant. Active post-exposure vaccination without HBIG administered soon after birth has been shown to be effective in preventing perinatal transmission and is used in areas where the use of HBIG is impractical.[240]

Vaccine-induced protection against symptomatic infection in a normal host is prolonged and correlates with immunologic memory, which has been demonstrated in immunized children and adults for at least 12 years after vaccination. Children immunized at birth are protected for at least 10 years. Thus, a need for routine booster doses has not been demonstrated.

Vaccine failures related to HBV variants with mutations in the S gene leading to conformational changes in the HBsAg protein, the major target for neutralizing anti-HBs antibody, have occurred in fully vaccinated children and perinatally exposed infants who received appropriate active and passive post-exposure prophylaxis and in many cases had protective anti-HBs antibody levels.[273] Although no evidence to date establishes that these escape mutations pose a threat to the effectiveness of current hepatitis B vaccine programs, future surveillance to detect emergence of HBV variants in immunized populations is warranted.[343,514]

Adverse Events

Other than soreness at the injection site, reactions to hepatitis B vaccine rarely occur. Post-vaccination surveillance performed after licensure of the plasma-derived vaccine indicated a possible association between GBS and receipt of the first vaccine dose, but no evidence indicates an association of GBS with recombinant vaccine.[343,344] Anaphylaxis has been estimated to occur in 1 in 600,000 doses distributed.[343,344] Several nonfatal cases have been reported in children.

Indications

Routine immunization with hepatitis B vaccine is recommended for all infants in the United States and should be completed by 6 to 18 months of age for all infants born to HBsAg-negative mothers.[11,336] Delivery hospitals should develop policies that ensure administration of a birth dose for all infants weighing 2000 g or more at birth unless a physician's order to defer immunization is in place and the serologic status of the mother is in the infant's medical record. Administering the first dose of hepatitis B vaccine soon after birth should minimize the risk for development of infection because of errors in maternal HBsAg testing or reporting or from exposure to persons with chronic HBV infection in the household and can increase the likelihood of completing the vaccine series. For infants weighing less than 2 kg who are born to HBsAg-negative mothers, initiation of vaccine should begin starting at 1 month of chronologic age. Administration of the second dose of vaccine is recommended 1 to 2 months after administration of the first dose, followed by a third dose when the infant is 6 to 18 months of age.

Only single-antigen hepatitis B vaccine can be used for doses given to infants between birth and 6 weeks of age. Single-antigen or combination vaccine may be used to complete the series; four doses of vaccine may be administered if a birth dose is given and a combination vaccine containing a hepatitis B component is used to complete the series.

Routine screening of all pregnant women for HBsAg is recommended because of the necessity for administering a birth dose of vaccine and HBIG.[11,343] Infants born to HBsAg-positive mothers, including pre-term and low-birth-weight infants, should be given HBIG (0.5 mL) within 12 hours of birth at a separate injection site, as well as the birth dose of HBV vaccine.[11,343] If a

pre-term infant weighing less than 2000 g is born to an HBsAg-positive mother, the birth dose of hepatitis B vaccine should not be counted toward completion of the vaccine series, and three additional doses of hepatitis B vaccine should be administered beginning when the child is 1 month of age. In addition to receiving HBIG and the hepatitis B vaccine series, infants of HBsAg-positive mothers should be tested for HBsAg and antibody to HBsAg (anti-HBs) at 9 to 15 months of age to identify those with chronic HBV infection or those who may require re-vaccination.[343]

A woman whose HBsAg status is unknown at delivery should undergo blood testing as soon as possible to determine her HBsAg status. The infant should receive the first dose of hepatitis B vaccine within 12 hours. If the woman is found to be HBsAg-positive, her infant should receive HBIG as soon as possible within 7 days. In populations where HBsAg testing of pregnant women is not feasible, all infants should receive hepatitis B vaccine at birth and 2 months and complete the series by the time they reach 6 months of age.

Hepatitis B vaccination is recommended for all children and adolescents younger than 19 years old in the United States. Children who have not been immunized previously may begin the vaccine series during any visit. All children aged 11 to 12 years old should have a review of their immunization records and receive the complete hepatitis B vaccine series if they have not been vaccinated previously. All children and adolescents younger than 19 years old who were born in Asia, the Pacific Islands, Africa, or other intermediate- or high-endemic countries or have at least one parent who was born in one of these areas should have a review of their immunization records and complete the hepatitis B vaccine series if they were not vaccinated previously.[343]

Vaccination also is recommended for those with one or more of the following risk factors for acquiring HBV infection:

- Sexually active heterosexual adolescents and adults who have a recently acquired sexually transmitted disease, are identified as prostitutes, or have had one or more sex partners in the previous 6 months
- Sexually active men who have sex with men
- Household contacts or sexual partners of HBsAg-positive persons
- Injecting drug users
- Persons at occupational risk of acquiring infection through exposure to blood or blood-contaminated body fluids, such as health care workers and public safety workers
- Residents and staff of institutions for the developmentally disabled
- Patients undergoing hemodialysis; vaccination of those with early renal failure is encouraged before they require hemodialysis
- Patients who receive clotting factor concentrates
- Members of households with international adoptees who are HBsAg-positive
- Travelers, especially children, to areas with high and intermediate rates of infection with HBV who have close contact with the local population or are likely to have contact with blood, such as in a medical setting, or sexual contact with residents
- Inmates in long-term correctional facilities

In addition to active and passive immunoprophylaxis of infants born to HBsAg-positive mothers, post-exposure prophylaxis is recommended in the following circumstances:

- Sexual partner of an HBsAg-positive person. A single dose of HBIG within 14 days of the last sexual contact and initiation of the three-dose hepatitis B vaccination is recommended for susceptible persons.
- Household exposure of an unvaccinated infant younger than 12 months old to a primary caregiver who has acute hepatitis B. Infants in this circumstance should receive HBIG, in addition to being vaccinated.
- Accidental percutaneous or per mucosal exposure of a susceptible person to HBsAg-positive blood. This indication is exemplified most by a needle-stick or other accident involving blood in a hospital. Another example is an injury to a susceptible person caused by the bite of an HBsAg-positive child. For this indication, HBIG as well as vaccine is given. Recommendations in these circumstances are complex and based on the availability of the blood source for HBsAg testing and the hepatitis B vaccination status of the exposed person.

Booster doses are not recommended except in the case of patients undergoing hemodialysis and possibly other immunocompromised patients, in whom annual antibody testing should assess the need. An additional dose is indicated for those whose serum anti-HBsAg concentration is less than 10 mIU/mL.

The current recommended doses and schedule for the use of hepatitis B vaccines licensed in the United States in infants and other age groups are shown in Table 255–12 (see elsewhere for more information[11,343]). For completing the hepatitis B vaccine series and achieving complete vaccination for hepatitis B, the two licensed hepatitis B vaccines are interchangeable when administered in doses recommended by the manufacturers.[11,343] In September of 1999, the FDA approved an optional two-dose schedule of Recombivax HB for vaccination of adolescents 11 to 15 years of age. The ACIP recommended that this schedule be included in the VFC program in February 2000.[120] With the two-dose schedule, the adult dose of Recombivax HB is administered to adolescents 11 to 15 years old, with the second dose given 4 to 6 months after the first dose. In immunogenicity studies in adolescents 11 to 15 years of age, antibody concentrations and seroprotection rates were similar with the two-dose schedule and the currently licensed three-dose schedule. Follow-up data collected during the course of 2 years indicate that the rate of decline in concentration of antibody for the two-dose schedule was similar to that for the three-dose schedule. No data are available to assess long-term protection or immune memory after vaccination with the two-dose schedule, and whether booster doses of vaccine will be required is not known. Children and adolescents who have begun vaccination with a dose of the pediatric formulation should

TABLE 255–12 Recommended Doses and Schedules of Hepatitis B Virus Vaccines in the United States

Group	Recombivax HB Dose µg (mL)	Engerix-B Dose µg (mL)
Infants of HBsAg-negative mothers, children, and adolescents <20 yr	5 (0.5)	10 (0.5)
Infants of HBsAg-positive mothers (HBIG also recommended)	5 (0.5)	10 (0.5)
Adolescents 11-15 yr	10 (0.5)*	N/A
Adults (≥20 yr)	10 (1.0)	20 (1.0)
Patients undergoing dialysis and other immunocompromised persons	5 (0.5)	10 (0.5)
<20 yr†	40 (1.0)‡	40 (2.0)§
≥20 yr		

**Adult formulation administered on a two-dose schedule.*
†Higher doses may be more immunogenic, but no specific recommendations have been made.
‡Special formulation for patients administered for patients undergoing dialysis.
§Two 1.0-mL doses administered at one site in a four-dose schedule at 0, 1, 2, and 6 months.
HBIG, hepatitis B immune globulin; HBsAg, hepatitis B surface antigen.
Data from references 11, 343, and 344.

complete the three-dose series with this dose. Similarly, if the formulation administered to an adolescent at the start of a series is not known, the series should be completed with the three-dose schedule.

In December of 2006, the ACIP approved recommendations for hepatitis vaccine use in adults.[344] In settings in which high proportions of adults have risks for acquiring HBV infection, the ACIP recommends universal hepatitis B vaccination for all unvaccinated adults. In other primary care and specialty medical settings in which adults at risk for developing HBV infection receive care, the ACIP recommends that health care providers should inform all patients about the health benefits of vaccination, including risks for acquiring HBV infection, and persons for whom vaccination is recommended, and should vaccinate adults who report having risks for developing HBV infection and any adults requesting protection from HBV infection. To promote vaccination in all settings, health care providers should implement standing orders to identify adults recommended for hepatitis B vaccination and administer vaccination as part of routine clinical services, not require acknowledgment of an HBV infection risk factor for adults to receive vaccine, and use available reimbursement mechanisms to remove financial barriers to receiving hepatitis B vaccination.

Contraindications

The only contraindication to receiving HBV vaccination is a history of anaphylaxis to a previous dose of vaccine. Although data on the safety of HBV vaccines are not available for pregnant women, these vaccines contain only HBsAg and not live virus and should not be deleterious to the developing fetus. Because HBV infection during pregnancy can result in transmission to the newborn, susceptible women at increased risk of acquiring infection should be vaccinated during pregnancy. Inadvertent vaccination of HBsAg-positive persons has no deleterious effects.

HUMAN PAPILLOMAVIRUS VACCINE

Genital HPV infection is thought to be the most common sexually transmitted viral infection, accounting for 6.2 million new cases annually in the United States.[490] The estimated prevalence of HPV infection ranges from 20 to 40 percent among sexually active 20-year-old women.[330] HPV typically infects girls and women soon after sexual exposure.[180,497] Median time from first intercourse to first detection of HPV is only 3 months.[180] The lifetime risk for women to acquire one or more genital HPV infections exceeds 75 percent.[79,330]

More than 40 HPV types can infect the genital tract and are classified on the basis of their association with cervical cancer.[366] The "low risk," non-oncogenic HPV types (e.g., types 6 and 11) are associated with anogenital warts, mild cervical dysplasia, and recurrent respiratory papillomatosis. The "high-risk" oncogenic HPV types are causally linked to several human cancers in both women and men, including cancers of the cervix, vulva, vagina, anus, and penis and a subset of head and neck cancers.[366] Infection with HPV causes virtually all cases of cervical cancer, the second most common cause of death from cancer among women globally, surpassed only by breast cancer.[480] The development of cervical cancer often occurs decades after initial infection. HPV types 16 and 18 account for 70 percent of cervical cancers.[68] Other types account for the remaining 30 percent of cervical cancers, and these types vary in their distribution globally.

Although the incidence of HPV is high, most infections clear without intervention. Seventy percent of new infections clear within 1 year, and 91 percent clear within 2 years.[174] Persistent infection with high-risk HPV types, defined as detection of the same viral type at two or more visits 6 months apart, is the most important risk factor for development of cervical cancer precursor lesions. Studies have demonstrated that persistent infection with a high-risk HPV type is associated with a greater than 10-fold risk of high-grade cervical cancer precursors.[269,358]

A first generation of prophylactic HPV subunit vaccines has been developed.[306] These vaccines are expected to have a significant impact on the incidence of HPV-related cervical and other anogenital cancers in women. Incidence of recurrent respiratory papillomatosis also is likely to decrease as a result of widespread vaccination.

Preparations

In 2006, the FDA licensed the first HPV vaccine for use in girls and women in the United States. This quadrivalent vaccine (GARDASIL, Merck & Co.) contains HPV types 6, 11, 16, and 18 virus-like particles (VLPs) and protects against four HPV types, which together cause 70 percent of cervical cancers (HPV types 18 and 16) and 90 percent of genital warts (HPV types 6 and 11).

The quadrivalent HPV vaccine is prepared from the highly purified VLPs of the major capsid (L1) protein of HPV types 6, 11, 16, and 18. The L1 proteins are produced by *Saccharomyces cerevisiae* and self-assemble into VLPs. The VLPs mimic the HPV virus, but they contain no viral DNA. Each 0.5-mL dose contains 20 μg HPV 6 L1 protein, 40 μg HPV 11 L1 protein, 40 μg HPV 16 L1 protein, and 20 μg HPV 18 L1 protein. The quadrivalent vaccine uses alum as an adjuvant and does not contain thimerosal or antibiotics. The vaccine should be stored at 2° C to 8° C (36° F to 46° F) and not frozen.

Primary vaccination with quadrivalent HPV vaccine consists of three 0.5-mL doses administered intramuscularly. The second and third doses should be administered 2 and 6 months after administration of the first dose.

Immunogenicity and Efficacy

Early data from randomized controlled trials have shown consistently that prophylactic HPV VLP vaccines are immunogenic and efficacious in preventing infection and lesions caused by the targeted HPV types.[306,330,352,444] Vaccine efficacy seems to be mediated by a type-specific humoral immune response. Evaluations of the quadrivalent vaccine have demonstrated that vaccinated individuals develop high antibody titers to the respective HPV types that exceed the antibody titers seen with natural HPV infection.[444] However, the level of antibody that confers a protective response is unknown.[444] For the quadrivalent HPV VLP vaccine, an antibody response significantly greater than placebo was sustained for at least 3 years without booster doses.[352,474] Whereas vaccine efficacy studies have been done only in adolescents ages 13 years and older, immunogenicity bridging studies have been performed in children as young as 9 years of age. Antibody titers to HPV types 6, 11, 16, and 18 were found to be higher in younger adolescents than in young adults.[352] From these data, investigators inferred that protection exists against cervical cancer, cancer precursor lesions, and genital warts, even for young adolescent girls who receive the quadrivalent vaccine.

The results from four randomized controlled trials repeatedly demonstrate that a regimen of three intramuscular injections of HPV VLP vaccine provides high-level protection from infection and lesions caused by targeted HPV types.[306,330] Efficacy from a combined analysis of quadrivalent HPV vaccine efficacy from both phase II and phase III clinical trials is 100 percent against precancerous lesions (carcinoma in situ II/III or adenoma in situ) caused by HPV type 16 or 18, and 99 percent against development of genital warts.[352] Whether HPV vaccines provide long-

term protection and whether booster doses will be necessary is not known. The quadrivalent HPV vaccine has not been found to have efficacy against existing disease or infection caused by HPV.

Adverse Events

HPV vaccines appear to be generally safe and well tolerated. The quadrivalent HPV vaccine has been associated with minimal adverse reactions.[206,352] The most common are mild fever (<38° C) and local injection site reactions such as pain, redness, pruritus, or swelling. Additional data on vaccine safety, including data on pregnancy and fetal and infant outcomes, are being collected in ongoing trials and will continue to be collected in post-licensure studies.

Indications

In March of 2007, the ACIP issued recommendations for the use of the quadrivalent HPV vaccine.[158] Routine vaccination with three doses of vaccine is recommended for girls 11 to 12 years of age. The vaccination series can be started in girls as young as 9 years of age, with physician and parental discretion. Catch-up vaccination is recommended for girls and women 13 to 26 years old who have not been vaccinated previously or who have not completed the full vaccination series. Ideally, vaccination should be administered before potential exposure to HPV through sexual contact occurs.

Vaccine is administered in a three-dose schedule, and the second and third doses should be administered 2 and 6 months after the first dose. Immunogenicity data allow for some degree of flexibility in the dosing schedule, often an important consideration in providing multidose vaccines to adolescents for whom contact with health providers can be sporadic. For the quadrivalent vaccine, the minimum interval from dose 1 to dose 2 is 4 weeks. The minimum interval from dose 2 to dose 3 should not be less than 12 weeks.

Quadrivalent HPV vaccine can be administered at the same visit when other age-appropriate vaccines, such as Tdap, Td, and MCV4 (meningococcal) are provided. At present, recommendations for cervical cancer screening have not changed for girls and women who receive quadrivalent HPV vaccine.

Quadrivalent HPV vaccine can be given to girls and women who have an equivocal or abnormal Papanicolaou (Pap) test, a positive hybrid capture-II high risk test, or genital warts. Vaccine recipients should be advised that data from clinical trials do not indicate the vaccine will have any therapeutic affect on existing Pap abnormalities, HPV infection, or genital warts. However, vaccination of these girls and women would provide protection against infection with vaccine HPV types not already acquired.

Lactating women can receive quadrivalent HPV vaccine. Girls and women who are immunocompromised from either disease or medication can receive quadrivalent HPV vaccine. However, the immune response to vaccination and vaccine effectiveness may be less than in girls and women who are immunocompetent. Quadrivalent vaccine falls into pregnancy category B and is not recommended for use in pregnancy.[352] If an adolescent becomes pregnant during vaccination, subsequent doses should be deferred until after parturition. The vaccine has not been associated causally with adverse outcomes of pregnancy or adverse events to the developing fetus. However, data on vaccination during pregnancy are limited at this time.

Contraindications

Quadrivalent HPV vaccine is contraindicated for people with a history of immediate hypersensitivity to yeast or to any vaccine component.

INFLUENZA VACCINE

Influenza virus infection continues to cause significant morbidity and mortality despite the availability of effective vaccines and antiviral therapy for prevention and treatment of influenza. In the United States, epidemics of influenza typically occur during the winter months and have been associated with an average of approximately 36,000 deaths per year in the United States.[454] Influenza viruses cause disease among all age groups.[234,235,362] Rates of infection are highest among children, but rates of serious illness and death are highest among persons aged 65 years and older, children aged 2 years and younger, and persons of any age who have medical conditions that place them at increased risk for developing complications from influenza.[50,51,233,362] The impact of influenza on both normal children and those with underlying high-risk conditions is appreciable. Attack rates in normal children have been estimated at 10 to 40 percent each year, and approximately 1 percent of these influenza infections result in hospitalization.[236,397]

A major difficulty in the development and provision of satisfactory immunizing agents for the prevention of influenzal disease is the antigenic variation in these viruses. Periodic minor antigenic changes in influenza A or B virus are the major factors in the continuing occurrence of yearly influenzal disease. Although outbreaks generally are limited in magnitude, the resulting morbidity and mortality remain discouragingly high. Major antigenic changes in influenza A virus, as occurred in 1957 to 1958 (Asian strain) and again in 1968 to 1969 (Hong Kong strain), account for the pandemic spread of disease associated with greater overall morbidity and mortality, especially in high-risk populations.

Preparations

Two types of influenza vaccines are licensed in the United States for use in children, inactivated trivalent influenza vaccine (TIV) and live attenuated influenza vaccine (LAIV) (Table 255–13).[13,440] These multivalent vaccines contain three virus strains (usually two type A and one type B), with the composition changed periodically in anticipation of the prevalent influenza strains expected to circulate in the United States in the following winter. Both vaccines are prepared from virus grown in the allantoic sac of the chick embryo.

Inactivated TIV distributed in the United States consists of either subvirion vaccine, prepared by disrupting the lipid-containing membrane of the virus, or purified surface-antigen vaccine. TIV is administered intramuscularly into the anterolateral thigh of infants and young children and into the deltoid muscle of older children and adults. Children younger than 9 years of age who are being immunized against influenza for the first time should receive two doses of TIV given 4 weeks apart, before the start of the influenza season.

Concerns about thimerosal have prompted some parents to reconsider influenza immunization. However, the benefits of protecting children against the known risks of influenza far outweigh the theoretical risks associated with the small amounts of thimerosal in some currently available forms of influenza vaccine. In addition, certain types of TIV can be obtained thimerosal-free and include single-dose Fluzone (Sanofi Pasteur) and Fluvirin (Novartis Vaccines).

Of the four inactivated influenza vaccines currently licensed in the United States (see Table 255–13), only two are approved for persons younger than 18 years of age. One influenza vaccine, Fluzone, is approved for use in children, beginning at 6 months of age. The other influenza vaccine, Fluvirin, is approved in the United States only for persons 4 years of age or older because its efficacy in younger persons has not been demonstrated.

LAIV is cold-adapted, developed by passaging the viruses at successively lower temperatures in tissue culture, so that replica-

TABLE 255–13 Licensed Influenza Vaccines for Different Age Groups: United States, 2006 to 2007 Season

Vaccine	Trade Name	Manufacturer	Dose/Presentation	Thimerosal Mercury Content (μg Hg/0.5-mL Dose)	Age Group
Inactivated					
TIV	Fluzone	Sanofi Pasteur	0.25-mL prefilled syringe	0	6-35 mo
			0.5-mL prefilled syringe	0	≥36 mo
			0.5-mL vial	0	≥36 mo
			5.0-mL multidose vial	25	≥6 mo
TIV	Fluvirin	Novartis (formerly Chiron)	0.5-mL prefilled syringe	<1.0	≥4 yr
			5.0-mL multidose vial	24.5	≥4 yr
TIV	Fluarix	GlaxoSmithKline	0.5-mL prefilled syringe	<1.25	≥18 yr
TIV	Flulaval	GlaxoSmithKline	10-mL multidose vial	25	≥18 yr
Live Attenuated					
LAIV	FluMist	MedImmune	0.2-mL sprayer	0	2-49 yr

LAIV, live attenuated influenza vaccine; TIV, trivalent influenza vaccine.
Adapted from Centers for Disease Control and Prevention: Prevention and control of influenza: Recommendations of the Advisory Committee on Immunization Practices (ACIP). M. M. W. R. Recomm. Rep. 55:1-42, 2006.

tion occurs only in the upper respiratory tract.[57,58,173,400] LAIV is administered intranasally. The cold-adapted formulation that is licensed in the United States must be stored at 2° to 8° C (35-46° F). When the vaccine is warmed to room temperature for intended use, it must be used within 30 minutes.

LAIV is licensed by the FDA for healthy individuals aged 5 through 49 years. Children younger than 9 years of age being immunized against influenza for the first time should receive two doses of LAIV given 6 weeks apart, before the start of the influenza season. Studies are ongoing to assess safety and efficacy in children between 6 months and 5 years of age.

Immunogenicity and Efficacy

High post-vaccination hemagglutination inhibition antibody titers develop in the majority of vaccinated children and young adults.[315,370,379] These antibodies are protective against illness caused by strains that are antigenically similar to those strains of the same type or subtype included in the vaccine.[268,370,379,401]

Children younger than 9 years of age who have not been immunized previously against influenza require two doses of TIV or LAIV vaccine administered at least 1 month apart to produce a satisfactory antibody response. Children previously primed with a related strain of influenza by infection or immunization mount a brisk antibody response to one dose of the vaccine. In previously unimmunized populations, such as children younger than 9 years old, a single dose of split-product vaccine may be significantly less immunogenic than is a single dose of whole-virus preparation, and two doses may be required to achieve a satisfactory serum antibody response.[440] Children younger than 9 years of age who received two doses of either TIV or LAIV vaccine in a previous year need only one dose in subsequent years. Children 9 years of age or older require only one dose, regardless of their influenza immunization history.

Variable immunogenicity of influenza vaccine has been reported in immunocompromised individuals, including those with malignant disease. Successful immunologic responses in these populations are most likely to occur when immunized individuals have been primed previously by exposure to antigenically similar influenza strains.[243,316] The optimal time to immunize children with malignant diseases who still must undergo chemotherapy is 3 to 4 weeks after chemotherapy has been discontinued and the peripheral granulocyte and lymphocyte counts are greater than 1000/mm.[6,13]

Corticosteroids administered for brief periods or every other day have only a minimal effect on the antibody response to influenza vaccine. Prolonged administration of high doses of corticosteroids (i.e., a dose equivalent to either 2 mg/kg or greater or a total of 20 mg/day of prednisone) may impair the antibody response. Influenza immunization can be deferred temporarily during the time of receipt of high-dose corticosteroids, provided deferral does not compromise the likelihood of administering immunization before the start of the influenza season.[13]

The effectiveness of inactivated influenza vaccine depends primarily on the age and immunocompetence of the vaccine recipient, the degree of similarity between the viruses in the vaccine and those in circulation, and the outcome being measured. Vaccine efficacy and effectiveness studies may have various end-points, including the prevention of medically attended acute respiratory illness (MAARI), prevention of culture-positive influenza virus illness, prevention of influenza or pneumonia-associated hospitalizations or deaths, seroconversion to vaccine serotypes, or prevention of seroconversion to circulating influenza virus subtypes.

Protection against virologically confirmed influenza illness after immunization with TIV in healthy children older than 2 years of age usually is 70 to 80 percent, with a range of 50 to 95 percent depending on the closeness of vaccine strain match to the circulating wild strain.[440] Efficacy of TIV in children 6 to 23 months of age is lower than in older children, although data are limited. Efficacy of LAIV was 86 to 96 percent against virologically confirmed influenza A (H3N2) virus infection in a large pre-licensure pediatric trial during 1 year. The effectiveness of influenza immunization on acute respiratory tract illness is less evident in pediatric than in adult populations because of the frequency of upper respiratory tract infections and influenza-like illness caused by other viral agents in young children. The protection afforded by influenza vaccine is transient, and yearly immunization is necessary irrespective of whether significant antigenic changes have occurred in a prevailing influenza strain.

Vaccine effectiveness is lower among previously unvaccinated children younger than 9 years of age if they have received only

one dose of influenza vaccine, compared with children who have received two doses. A retrospective study among approximately 5000 children aged 6 to 23 months conducted during a year with a suboptimal vaccine match indicated vaccine effectiveness of 49 percent against medically attended, clinically diagnosed pneumonia or influenza among children who had received two doses of influenza vaccine. No effectiveness was demonstrated among children who had received only one dose of influenza vaccine, a finding illustrating the importance of administering two doses of vaccine to previously unvaccinated children younger than 9 years old.[406] Similar results were observed in a case-control study of children aged 6 to 59 months with laboratory confirmed influenza.[434] A study assessing protective antibody responses after administration of one and two doses of vaccine among vaccine-naïve children aged 5 to 8 years also demonstrated the importance of compliance with the two-dose recommendation.[371]

Two studies documented that TIV vaccine decreases the incidence of influenza-associated otitis media among young children by approximately 30 percent,[171,261] but a third study determined that vaccination did not reduce the burden of acute otitis media (AOM).[270]

Adverse Events

TIV is an inactivated vaccine that contains killed viruses and therefore cannot produce signs or symptoms of influenza caused by active virus infection. The most common symptoms associated with TIV administration are soreness at the injection site and fever. Fever, usually occurring 6 to 24 hours after immunization, affects approximately 10 to 35 percent of children younger than 2 years old. Mild systemic symptoms, such as nausea, lethargy, headache, muscle aches, and chills, also can occur after receipt of TIV.

LAIV generally is well tolerated and may produce mild signs or symptoms related to influenza virus infection that include headache and runny nose or nasal congestion in vaccinees. Transmission of LAIV strains to unimmunized contacts has been documented only once in pre-licensure studies. The proposed explanation for the uncommon occurrence of transmission is that the vaccine virus is shed for a shorter duration and in a much smaller quantity than are wild-type strains.

Indications

Annual immunization with TIV is recommended for the following groups[13,181,440]:

- Healthy children 6 through 18 years of age
- High-risk children 6 months and older AND adolescents with underlying medical conditions, including the following:
  - Asthma or other chronic pulmonary diseases, such as cystic fibrosis
  - Hemodynamically significant cardiac disease
  - Immunosuppressive disorders or therapy
  - HIV infection
  - Sickle cell anemia and other hemoglobinopathies
  - Diseases requiring long-term salicylate therapy, such as rheumatoid arthritis or Kawasaki disease
  - Chronic renal dysfunction
  - Chronic metabolic disease, such as diabetes mellitus
  - Any condition that can compromise respiratory function or handling of secretions or can increase the risk of aspiration, such as cognitive dysfunction, spinal cord injuries, seizure disorders, or other neuromuscular disorders
  - Any girl or woman who will be pregnant during influenza season

To prevent additional cases of influenza and transmission from these cases to at-risk individuals, influenza immunization with TIV or LAIV is recommended for the following persons, unless contraindicated:

- Healthy household contacts and out-of-home caregivers of either high-risk children and adolescents or children younger than 5 years old; immunization of close contacts of children younger than 6 months old is especially important because influenza vaccine is not licensed for use in these infants
- Healthy contacts and caregivers of other children or adults at high risk of developing complications of influenza infection
- Close contacts of immunosuppressed people
- Health care professionals or volunteers in hospitals or medical offices

Previously unimmunized children aged 6 months to younger than 9 years of age should receive two doses of influenza vaccine before the onset of influenza season. Available data suggest that children younger than 9 years who did not receive the second dose of influenza vaccine in the initial year that influenza vaccine was given may not be adequately protected the next influenza season with only one dose. In this group, levels of protection can be suboptimal, especially if the antigenic specificity of the predominant strains has changed from the previous year. Thus, the AAP recommends that two doses be given to these children the following influenza season.[181] This recommendation applies only to the influenza season that follows the first year that a child younger than 9 years old receives influenza vaccine.

Precautions and Contraindications

Current influenza vaccines contain egg proteins and on rare occasion may induce immediate allergic reactions, including anaphylaxis. Skin testing has been used for children with severe anaphylactic reactions to eggs who are to receive influenza vaccine, but these children generally should not receive influenza vaccine because of the risk of reaction, the probable need for yearly immunization, and the availability of chemoprophylaxis against influenza infection. Less severe or local manifestations of allergy to egg or to feathers are not contraindications to administering influenza vaccine and do not warrant performing vaccine skin testing.[13]

Pregnancy is not a contraindication to influenza vaccine administration, and vaccination is advised for pregnant girls and women who have an underlying high-risk condition.[440]

Persons with acute febrile illness usually should not be vaccinated until their symptoms have abated. However, minor illnesses with or without fever do not contraindicate the use of influenza vaccine, particularly in children with mild upper respiratory tract infection or allergic rhinitis.[440]

People should not receive LAIV if they are taking salicylates, have a known or suspected immune deficiency, have a history of GBS, have a history of anaphylactic reaction to egg protein, or have reactive airway disease or other conditions traditionally considered high risk for severe influenza (chronic pulmonary disorders or cardiac disorders, pregnancy, chronic metabolic disease, renal dysfunction, hemoglobinopathies, or immunosuppressive therapy).

Precaution also should be taken when considering LAIV administration to persons with minor acute illness, such as a mild upper respiratory tract infection with or without fever. Although the vaccine most likely can be given in this case, LAIV should be deferred temporarily until the congestion-inducing illness is resolved if nasal congestion will impede the delivery of the vaccine to the nasopharyngeal mucosa.

LAIV or TIV can be used to prevent influenza in those who are in close contact with most immunosuppressed individuals. People who are in contact with severely immunosuppressed individuals, such as those receiving care in a protective environment after undergoing hematopoietic stem cell transplantation, should NOT receive LAIV. For such individuals, TIV is recommended.

MEASLES VACCINE

Since the introduction of both an inactivated and a live virus, attenuated measles vaccine (Edmonston B strain) in the United States in 1963, the reported incidence of measles has decreased by more than 99 percent. Although the incidence of measles has declined in all age groups, the decline has been greatest in children aged 5 to 14 years.

Measles was targeted for elimination in the United States by 1982. Efforts to eliminate measles were not successful as a result of two factors. The first was vaccine failure. In the late 1980s, outbreaks occurred in older children in schools in which immunization rates usually were greater than 95 percent.[247,340,372] Attack rates were 1 to 5 percent because of the accumulation of measles-susceptible individuals from vaccine failure. The recurrent measles outbreaks among vaccinated school-age children in the mid-1980s prompted both the ACIP and the AAP in 1989 to recommend that all children be given two doses of measles-containing vaccine, preferably as MMR.[25,84] Although administration of the second dose originally was recommended either at entry to primary school (ACIP) or middle/secondary school (AAP), the ACIP, the AAP, and the AAFP now recommend that a child be given the second dose at age 4 to 6 years of age rather than delaying it until the child is 11 to 12 years old.[111,337,339,342,345,381,481] The major benefit of administering the second dose is a reduction in the proportion of persons who remain susceptible because of primary vaccine failure. Waning immunity is not a major cause of vaccine failure and has little influence on transmission of measles, and re-vaccination of children who have low concentrations of measles antibody produces only a transient rise in antibody concentration.[337,339,342,345,381,481]

The second factor leading to outbreaks of measles was the failure to implement current immunization strategies, especially in the inner cities, where a high proportion of preschool-age children had not been vaccinated. From 1989 through 1991, the proportion of unvaccinated persons with measles increased, as reflected by outbreaks among unvaccinated inner-city preschool-age children. Multiple barriers to providing timely immunization to these children were identified during investigation of the measles resurgence that occurred between 1989 and 1991. Reported cases of measles declined rapidly after the 1989 to 1991 resurgence because of intensive efforts to vaccinate preschool-age children. Measles vaccination levels among 2-year-old children increased from 70 percent in 1990 to 91 percent in 1997. Since 1993, fewer than 500 cases of measles have been reported annually, and fewer than 200 cases per year have been reported since 1997. A record low annual total of 37 cases was reported in 2004.[287]

Available epidemiologic and virologic data indicate that transmission of measles in the United States has been interrupted. Most cases now are imported from other countries or linked to imported cases. Most imported cases originate in Asia and Europe and occur both among U.S. citizens traveling abroad and persons visiting the United States from other countries. Since 1993, the largest outbreaks of measles in the United States have occurred in populations that refuse vaccination for religious or personal belief reasons. Most outbreaks have involved limited spread from measles imported from outside the United States. During 2005, the CDC reported 66 confirmed cases of measles, 34 of which were from a single outbreak in Indiana associated with infection in a traveler returning to the United States.[151]

Since the mid-1990s, no age group has predominated among reported cases of measles. Relative to earlier decades, increased proportions of cases now occur among adults. Persons aged 20 years and older accounted for only about 3 percent of cases in 1983, whereas in 2001 this age group accounted for 48 percent of all reported cases.

The recommended age for receiving routine vaccination with measles vaccine has been lowered in the past decade from 15 months to 12 to 15 months of age.[135] The decision to lower the age for receiving routine primary vaccination was based on the observation that most children are susceptible to measles by the time that they reach 12 months of age because of waning transplacental immunity.[334,338] Most mothers now have vaccine-induced immunity rather than immunity conferred by infection with wild virus. Antibody concentrations induced by measles vaccination generally are lower than those induced by natural measles. Therefore, measles-specific antibodies acquired transplacentally are lower in infants of vaccinated mothers, and these infants therefore are susceptible at an earlier age.

Preparations

The live measles virus vaccine (Moraten strain) available in the United States is prepared in chick fibroblast cell culture. Each dose of vaccine contains neomycin, sorbitol, and hydrolyzed gelatin as a stabilizer. Preparations include a monovalent (measles only) vaccine and two combinations, measles-rubella, MMR, and measles-mumps-rubella-varicella (MMRV). MMRV is approved for children 12 months through 12 years of age. MMRV should not be administered to persons 13 years of age or older. The ACIP and AAP do not express a preference for use of combination MMRV over separate administration of MMR and varicella vaccines. In all situations in which measles vaccine is to be used, MMR or MMRV should be given if the recipient is likely to be susceptible to contracting rubella, mumps, or varicella.[14,111]

Inadequate protection against measles can result from the administration of improperly stored vaccine. Before reconstitution, measles vaccine must be stored at a temperature between 2° C and 8° C (35.6° F to 46.4° F) or colder and must be protected from light, which may inactivate the virus. Reconstituted vaccine should be stored in a refrigerator and discarded if not used within 8 hours.

Immunogenicity and Efficacy

Immunization produces a mild or inapparent, noncommunicable infection. Measles antibodies develop in approximately 95 percent of children vaccinated at 12 months of age and in 98 percent of children vaccinated at 15 months of age.[338] Studies indicate that serologic evidence of measles immunity develops in more than 99 percent of persons who receive two doses of measles vaccine, separated by at least 1 month, on or after their first birthday.[166,178] Although vaccine-induced antibody titers are lower than those after natural disease, persistence of protective titers for as long as 16 years after administration of vaccine has been demonstrated.[311,339] Most vaccinated persons who appear to lose antibody have an anamnestic response after re-vaccination, thus indicating that they most likely are still immune.[377] A small percentage of vaccinated individuals may lose protection after several years as a result of secondary vaccine failure.[345,513]

Adverse Events

Vaccine-associated symptoms, consisting of fever higher than 39.4° C (102.9° F) occurring 5 to 10 days after immunization or transient rash, develop in 5 to 18 percent of recipients.[319,388] Serious complications related to vaccine use occur far less frequently than after natural measles.[385]

Thrombocytopenia occurs at a rate of 1 case for every 30,000 to 40,000 doses distributed. Based on data from Sweden and Finland, the IOM concluded that a causal association exists between MMR and thrombocytopenia.[280] The decrease in platelet count presumably is caused by the measles component and usually is not clinically apparent. However, thrombocytopenic purpura occurring after vaccination has been reported.

Central nervous system disease, specifically encephalitis or encephalopathy, is reported at a rate of less than 1 case per 1 million doses of vaccine administered. Because the incidence of encephalitis or encephalopathy after the administration of measles vaccination to healthy children is lower than the observed incidence of encephalitis of unknown origin, some or most of the reported severe neurologic disorders may be only temporally, rather than causally, related to measles immunization. The risk of subacute sclerosing panencephalitis (SSPE) in vaccinated children is extremely low and is estimated to be approximately one twelfth the risk of SSPE after a case of natural measles (0.7 SSPE cases per million vaccine doses versus 8.5 cases per million natural measles infections).[64,81,280] Whether measles vaccine causes SSPE is unclear. Some cases of SSPE occur in children with no history of having measles or measles vaccination.[14]

Reactions to measles vaccine are not age-related and occur only in susceptible vaccinees. After re-vaccination, reactions should be expected only in those who failed to respond to the first immunization.

To date, no convincing evidence establishes that any vaccine causes autism or autistic spectrum disorder. Concern has been raised about a possible relation between MMR vaccine and autism by some parents of children with autism. Symptoms of autism often are noticed by parents during the second year of life and may manifest after administration of MMR by weeks or months. Two independent nongovernmental groups, the IOM and the AAP, reviewed the evidence regarding a potential link between autism and MMR vaccine.[252,281,283] Both groups independently concluded that available evidence does not support an association and that the United States should continue its current MMR vaccination policy.

Indications

Unless otherwise contraindicated, measles vaccine is indicated for persons susceptible to measles.[14,111] The recommended age for receiving the first dose of measles vaccine is 12 to 15 months. In high-risk areas, such as those with recurrent measles transmission, the initial dose should be administered when the child reaches 12 months of age. The second dose is given routinely at 4 to 6 years and no later than 11 to 12 years of age. Both doses of measles vaccine preferably should be given as MMR or MMRV. The minimal interval between the two doses is 4 weeks.

Adults born before 1957 generally may be considered immune to measles because of previous natural infection. Those born after 1956 and in whom immunoprophylaxis is indicated should receive two doses of vaccine.

During outbreaks, when the likelihood of exposure to measles is high, measles vaccine should be given to infants as young as 6 months. Seroconversion rates to vaccine are significantly less in children vaccinated before reaching 1 year of age than those in older children. Children immunized before their first birthday then should be re-vaccinated with MMR at 12 to 15 months of age, with a third dose given according to local policy.

Measles remains endemic in many areas of the world. Although vaccination against measles is not a requirement for entry into any country, susceptible children, adolescents, and adults born after 1956 should be offered measles vaccination (usually as MMR) before embarking on international travel. Infants 6 months or older who are traveling to areas where measles is endemic or epidemic should be vaccinated before departure and re-vaccinated at 12 to 15 months. Vaccination of infants younger than 6 months is not necessary because most young infants are protected by maternally derived antibodies.

Exposure of susceptible individuals to measles is not a contraindication to administering vaccination; vaccine given within 72 hours of exposure may provide protection. If exposure does not result in infection, immunization will protect against future infection.

Precautions and Contraindications

Immunocompromised patients with conditions such as lymphoreticular or other generalized malignant disease and primary or secondary immunodeficiency states should not be given live virus, attenuated measles vaccine.[14,111] After cessation of their chemotherapy, these individuals generally should not receive measles vaccine for at least 3 months. However, because the intensity and type of immunosuppressive therapy, radiation therapy, underlying disease, and other factors determine when immunologic responsiveness will be restored, arriving at a definitive recommendation for an interval after cessation of immunosuppressive therapy when measles vaccine can be safely and effectively administered often is not possible.

An exception to the contraindication of administering measles vaccine to immunocompromised patients is asymptomatic HIV-infected patients, for whom measles vaccination, given as MMR at 12 to 15 months of age, is recommended. The need to protect HIV-infected persons who are at increased risk for having severe complications if infected with measles has been balanced against the risk of having adverse reactions. Measles vaccine is not recommended for HIV-infected persons with evidence of severe immunosuppression. A case of progressive measles pneumonitis occurred in a person with acquired immunodeficiency syndrome (AIDS) and severe immunosuppression to whom MMR vaccine was administered,[132] and morbidity related to measles vaccination has been reported in persons with severe immunosuppression unrelated to HIV infection.[21,276,283,285] In addition, the antibody response to measles vaccine in severely immunocompromised HIV-infected persons is diminished.[45,56,346,357,359] In the United States, the incidence of measles currently is very low. Among HIV-infected persons who do not have evidence of severe immunosuppression, no serious or unusual adverse events have been reported after receiving measles vaccination.[349,375,382,442] Therefore, MMR vaccination is recommended for all asymptomatic HIV-infected persons who do not have evidence of severe immunosuppression and for whom measles vaccination would otherwise be indicated. MMR vaccination also should be considered for all symptomatic HIV-infected persons who do not have evidence of severe immunosuppression. Testing asymptomatic persons for HIV is not necessary before administering MMR.[39]

Systemically absorbed corticosteroids can suppress the immune system of an otherwise healthy person. However, neither the minimal dose nor the duration of therapy sufficient to cause immune suppression is well defined. Although the immunosuppressive effects of steroid treatment vary, many clinicians consider that a steroid dose equivalent to or greater than a prednisone dose of 2 mg/kg of body weight per day or a total of 20 mg/day is sufficiently immunosuppressive to raise concern about the

safety of administering live virus vaccines. Persons who have received systemic corticosteroids in doses of 2 mg/kg of body weight or 20 mg daily or on alternate days for an interval of 14 days or longer should avoid receiving vaccination with MMR and its component vaccines for at least 1 month after cessation of steroid therapy.[111] Persons who have received prolonged or extensive topical, aerosolized, or other local corticosteroid therapy that causes clinical or laboratory evidence of systemic immunosuppression also should avoid receiving vaccination with MMR for at least 1 month after cessation of therapy.[111]

The live attenuated measles virus vaccine used for immunization is not communicable. Therefore, contacts of immunocompromised patients should be vaccinated to prevent the spread of natural measles to such patients.

Although no direct evidence has demonstrated that measles vaccine is harmful to a pregnant woman or her fetus, the vaccine should not be administered to women known to be pregnant or who are considering becoming pregnant, because of the theoretical risk of fetal infection associated with a live virus vaccine. Women vaccinated with MMR should avoid conception for 28 days after being vaccinated.[130]

Because measles vaccination may diminish cutaneous manifestations of cell-mediated immunity temporarily, a tuberculin test performed several days to 6 weeks after receiving immunization can yield a false-negative result. Although natural measles infection can exacerbate tuberculosis, no evidence indicates that measles vaccination is associated with such an effect. Therefore, tuberculin skin testing is not a prerequisite for administering measles immunization. If a tuberculin test is indicated, it should be performed on the day of immunization or postponed for 4 to 6 weeks because measles vaccination may suppress tuberculin reactivity temporarily.

In persons allergic to eggs, the risk of having serious allergic reactions such as anaphylaxis after receiving MMR vaccine is extremely low, and skin testing with vaccine is not predictive of allergic reaction to vaccination.[14,288,296] Therefore, obtaining a skin test is not required before administering MMR to persons who are allergic to eggs. Similarly, the administration of gradually increasing doses of vaccine is not required.[111] Data indicate that most anaphylactic reactions to measles- and mumps-containing vaccines are not associated with hypersensitivity to egg antigens but to other components of the vaccines such as the gelatin stabilizer.[242,262,295,318]

Children with a previous history of thrombocytopenic purpura or thrombocytopenia may be at risk for developing clinically significant thrombocytopenia after receiving immunization with MMR.[54,280] The decision to vaccinate should be based on the benefits of immunity to measles, mumps, and rubella and the risk of re-occurrence or exacerbation of the thrombocytopenia after receiving vaccination or from natural infection with measles or rubella. For children in whom thrombocytopenia develops in the month after receiving a dose of measles-containing vaccine, withholding the second dose of measles vaccine is prudent if the incidence of measles remains low.

Receipt of antibody-containing blood products (whole blood, plasma, or parenteral immunoglobulin) may interfere with seroconversion to measles vaccine. High doses of immunoglobulin preparations can inhibit the immune response to measles vaccine for 3 or more months, depending on the dosage.[435] The length of time that such passively acquired antibody persists depends on the concentration and quantity of the blood product received (Table 255–14).[14,308]

As with any condition that induces fever during the second year of life, children predisposed to having febrile seizures may

TABLE 255–14 Suggested Intervals between Immune Globulin Administration and Measles or Varicella Vaccination

Product/Indication	Route	Dose		Interval (mo)*
		U or mL	mg/kg	
RSV monoclonal antibody (Synagis)	IM		15	None
Tetanus (as TIG)	IM	250 U	10	3
Hepatitis A prophylaxis (as IG)				
Contact prophylaxis	IM	0.02 mL/kg	3.3	3
International travel	IM	0.06 mL/kg	10	3
Hepatitis B prophylaxis (as HBIG)	IM	0.06 mL/kg	10	3
Rabies prophylaxis (as RIG)	IM	20 IU/kg	22	4
Varicella prophylaxis (as VariZIG)	IM	125 U/10 kg (maximum, 625 U)	20-40	5
Measles prophylaxis (as IG)				
Standard	IM	0.25 mL/kg	40	5
Immunocompromised contact	IM	0.50 mL/kg	80	6
Blood Transfusion				
Washed RBCs	IV	10 mL/kg	Negligible	None
RBCs, adenine-saline added	IV	10 mL/kg	10	3
Packed RBCs	IV	10 mL/kg	20-60	5
Whole blood	IV	10 mL/kg	80-100	6
Plasma/platelet products	IV	10 mL/kg	160	7
CMV IVIG	IV	3 mL/kg	150	6
IVIG				
Replacement therapy for immune deficiencies	IV		400	8
ITP	IV		400	8
ITP	IV		1000	10
ITP or Kawasaki disease	IV		1600-2000	11

**These intervals should provide sufficient time for decreases in passive antibodies in all children to allow for an adequate response to measles or varicella vaccine. Physicians should not assume that children are fully protected against measles during these intervals. Additional doses of immune globulin or measles vaccine may be indicated after exposure to measles.*

CMV, cytomegalovirus; HBIG, hepatitis B immune globulin; IG, immune globulin; IM, intramuscular; ITP, immune thrombocytopenic purpura; IV, intravenous; IVIG, intravenous immune globulin; RIG, rabies immune globulin; RBCs, red blood cells; RSV, respiratory syncytial virus; TIG, tetanus immune globulin; VariZIG, varicella-zoster immune globulin.

experience seizures after receiving measles vaccination. Most convulsions that develop after receiving measles immunization are simple febrile seizures and occur in children without known risk factors. Febrile seizures that occur after the administration of vaccinations do not increase the risk for the subsequent development of epilepsy or other neurologic disorders.[52] An increased risk of having seizures after receiving measles vaccination may occur in children with a previous history of convulsions or those with a history of convulsions in first-degree family members.[473] Although the exact risk cannot be determined, it appears to be low. The recommendation to immunize children with a personal history of seizures or those with a history of seizures in first-degree family members is based on factors indicating that the benefits greatly outweigh the risks. Prophylactic use of anticonvulsants usually is not feasible because therapeutic concentrations of many currently prescribed anticonvulsants are not achieved for some time after the initiation of therapy.

MENINGOCOCCAL VACCINE

N. meningitidis became a leading cause of bacterial meningitis in the United States after dramatic reductions in the incidence of *Streptococcus pneumoniae* and Hib infections occurred with the introduction of conjugate vaccines.[425,496] The disease is transmitted by person-to-person spread through close contact. Meningococcal disease is fatal in 10 to 14 percent of cases. Of patients who recover, 11 to 19 percent have permanent hearing loss, mental retardation, loss of limbs, or other serious sequelae.

Each year, an estimated 1400 to 2800 cases of meningococcal disease occur in the United States. Although rates of disease are highest among children aged younger than 2 years old, 62 percent of meningococcal disease in the United States occurs among persons aged 11 years and older.[61] Serogroups B, C, and more recently Y are the major causes of meningococcal disease in the United States, and each serogroup is responsible for approximately one third of cases. The proportion of cases caused by each serogroup varies by age group. Among infants aged younger than 1 year, more than 50 percent of cases are caused by serogroup B, for which no vaccine is licensed or available in the United States.[413] Of all cases of meningococcal disease among persons aged 11 years and older, 75 percent are caused by serogroups (C, Y, or W-135), which are included in vaccines available in the United States.

An increased incidence of meningococcal disease in adolescents and young adults was noted in the United States in the mid-1990s.[258,414] A prospective surveillance study of meningococcal disease among college students by the CDC and the American College Health Association suggested that the incidence is similar to that of the general population of 18- to 22-year-olds but that dormitory residents, especially freshmen, are at increased risk.[73] In a Maryland study, the incidence of disease was significantly higher in on-campus residents than off-campus residents.[257] In 2000, ACIP and the AAP concluded that college students, especially those living in dormitories, are at moderately increased risk for acquiring meningococcal disease compared with other persons their age[119] and recommended that (1) college students and their parents be informed by health care providers of the risks of acquiring meningococcal disease and of the potential benefits of vaccination, (2) college and university health services facilitate implementation of educational programs about meningococcal disease and the availability of vaccination services, and (3) meningococcal vaccine be made available to those persons requesting vaccination. As of November of 2004, a total of 31 states had adopted legislation requiring colleges to provide information on risks of acquiring meningococcal disease either to matriculating students or to students residing on campus, and 10 states had mandated vaccination for certain students, unless a vaccination waiver is provided.

Since the early 1990s, outbreaks of meningococcal disease have occurred with increasing frequency in the United States. From July 1994 to June 2002, a total of 76 outbreaks were identified.[72] Serogroup C accounted for 63 percent of the outbreaks, 25 percent were serogroup B, and 12 percent were serogroup Y. These outbreaks accounted for fewer than 2 percent of the total number of cases of meningococcal disease in the United States during this period. One third of the outbreaks were community-based and the remainder organization-based, occurring in colleges, primary and secondary schools, and nursing homes.

In other parts of the world, the number of cases is much higher.[146] In the sub-Saharan African "meningitis belt," which extends from Mali to Ethiopia, peaks of serogroup A meningococcal disease occur regularly during the dry season. In addition, major epidemics occur every 8 to 12 years. The last epidemic during 1996 to 1997 resulted in 213,658 cases, with 21,830 deaths. Meningococcal disease continues to cause epidemics outside the meningitis belt, including epidemics in the Great Lakes of Africa. Serogroup W-135 also emerged as an epidemic strain causing disease in Saudi Arabia in association with the Hajj pilgrimage in 2000, in Burkina Faso in 2002, and in several African countries in 2003 and 2004.

Preparations

Two meningococcal vaccines are licensed in the United States for use in children and adults against serotypes A, C, Y, and W-135. The tetravalent meningococcal polysaccharide vaccine (MPSV4) was licensed in 1981 for use in children 2 years of age and older and has been recommended for use only for people at increased risk of acquiring meningococcal disease. Each vaccine dose contains 50 mg of each of the four purified bacterial capsular polysaccharides.[123] MPSV4 is available both as a single dose that is thimerosal preservative-free and in 10-dose vials that contain 25 μg of thimerosal/0.5 mL; 50-dose vials no longer are available. The MPSV4 is administered subcutaneously as a single 0.5-mL dose.

The second vaccine, a tetravalent meningococcal polysaccharide–protein conjugate vaccine (MCV4), is licensed for use among persons aged 2 to 55 years.[61] Each dose of vaccine contains 4 μg each of the four capsular polysaccharides conjugated to 48 μg of diphtheria toxoid. MCV4 is available as a single dose that is thimerosal preservative-free. The vaccine is administered intramuscularly as a single 0.5-mL dose.

No vaccine is available in the United States for prevention of serogroup B meningococcal disease because unconjugated group B polysaccharide is poorly immunogenic in humans.

Immunogenicity and Efficacy

POLYSACCHARIDE VACCINE

Protective antibody concentrations are achieved within 10 to 14 days of administration of MPSV4. Both vaccines elicit protective levels of bactericidal antibody to all four serogroups in more than 97 percent of recipients as measured at 28 days after vaccination. The antibody responses to each of the four polysaccharides in the polysaccharide vaccine are serogroup-specific and independent. Group A polysaccharide induces antibody in some children as young as 3 months, although a response comparable to that in adults is not achieved until the child is 4 to 5 years of age.[390] The serum antibody response to serogroup C is age-dependent, with a poor response in children younger than 2 years.[238] Serum concentrations of antibodies against group A and C polysaccharides decrease markedly during the first 3 years after receipt of a single dose of vaccine. The decrease in antibody occurs more rapidly in infants and young children than in adults.[294,510]

Field trials of A and C meningococcal vaccines in Europe and Africa demonstrated efficacy rates against serogroup A of 85 to 95 percent 1 year after vaccination.[390,478] After 3 years, efficacy rates were 67 percent in older children but only 10 percent in children younger than 4 years old at the time of immunization with serogroup A vaccine.[403] In an epidemic, serogroup C vaccine demonstrated clinical efficacy rates similar to those of the serogroup A vaccine.[451]

Serogroup Y and W135 antigens are immunogenic and safe in children more than 2 years of age. However, clinical efficacy has not been demonstrated as yet for these preparations.[4,44,475] Persons with deficiencies of the terminal components of serum complement and those with anatomic or functional asplenia have antibody responses to quadrivalent meningococcal vaccines consistent with protection.[416,419] However, the clinical efficacy of vaccination has not been evaluated in these persons.

CONJUGATE VACCINE

Protective antibody concentrations are achieved within 8 days after administration of MCV4.[61] In studies conducted among persons aged 11 to 55 years that compared the immunogenicity of conjugate vaccine with that of the polysaccharide vaccine at 28 days after vaccination, the percentage of subjects achieving at least a fourfold increase in bactericidal titer for each serogroup was higher in the MPSV4 group than in the MCV4 group for persons older than 18 years of age; nonetheless, the criteria for demonstrating immunologic non-inferiority to MPSV4 were still achieved. The percentage of subjects with at least a fourfold rise was highest for serogroup W-135 and lowest for serogroup Y. The percentage of subjects achieving a protective level of bactericidal antibody was greater than 97 percent for all serogroups in both MCV4 and MPSV4 groups.

Response to re-vaccination with MCV4 was assessed by administering MCV4 to subjects previously vaccinated with MPSV4 or MCV4 and to vaccine-naïve control subjects. All subjects in all three groups achieved protective bactericidal antibody titers at both 8 and 28 days after receiving MCV4. Subjects initially primed with MCV4 achieved higher bactericidal antibody concentrations than those of naïve control subjects for all serogroups except A. In contrast, bactericidal antibody titers of those primed with MPSV4 were lower than those of vaccine-naïve control subjects on both days 8 and 28 for all serogroups.

Little information is available to determine the need for or timing of re-immunization when the risk of acquiring disease continues or recurs. In children aged 11 years and older and in adults, concentrations of antibodies against serogroups A, C, Y, and W-135 3 years after a single dose of MCV4 are equal to or greater than those of people given MPSV4, and the duration of protection after the conjugate vaccine is expected to exceed 5 years. Studies to determine duration of protection and need for re-immunization with MCV4 are under way.

MPSV4 is to be administered subcutaneously, whereas MCV4 is to be administered intramuscularly. More than 100 persons have inadvertently received the MCV4 vaccine by the subcutaneous route. For a subset of these individuals, CDC determined that, although the serologic responses were lower after MCV4 was administered subcutaneously compared with intramuscularly, the proportions of individuals who achieved antibody levels thought to be protective were similar. Therefore, the CDC did not recommend that those who had received MCV4 needed to be re-immunized.[150]

Adverse Events

MPSV4 has been used extensively in mass immunization programs as well as in the military and among international travelers. Adverse reactions to MPSV4 generally are mild; the most frequent reactions are pain and redness at the injection site that last for 1 or 2 days.[48]

Estimates of the incidence of such local reactions have varied, ranging from 4 to 56 percent. Transient fever occurs in as many as 5 percent of vaccine recipients in some studies but is less common in older children and adults.

Among adolescents 11 to 18 years of age, safety of administering MCV4 and MPSV4 was assessed in two randomized, controlled trials. Common adverse reactions after MPSV4 and MCV4 immunization include localized pain, headache, and fatigue, all of which are mild and last for 1 to 2 days. Pain, induration, swelling, and redness at the injection site are slightly greater after administration of MCV4 compared with MPSV4. The frequency of local adverse reactions reported after MCV4 administration was similar to that reported after Td administration, which, like MCV4, is given intramuscularly. Fever is reported by 2 to 5 percent of adolescents who receive either MPSV4 or MCV4.[61]

GBS was reported in 17 persons, including 15 adolescents aged 11 to 19 years, who received MCV4 between July and September of 2006.[150] This CDC study could not determine with certainty whether MCV4 increases the risk for development of GBS. Because invasive meningococcal disease causes significant morbidity and mortality, the ACIP and AAP continue to recommend administration of MCV4. However, given the temporal association, the recommendation is that MCV4 should not be given to adolescents or adults with a history of GBS. Cases of GBS or other clinically significant adverse events after administration of MCV4 should be reported to the CDC (www.vaers.hhs.gov).

Indications

Routine childhood immunization with MPSV4 is not recommended because the infection rate in the general population is low, response is poor in young children, immunity is relatively short- lived, and the response to subsequent vaccine doses is impaired for some serogroups.[15,35,61] Immunization with MCV4 is recommended for children aged 2 to 10 years in high-risk groups, including those with functional or anatomic asplenia, terminal complement component, or properdin deficiencies, and children aged 2 to 10 years who travel to or reside in areas where *N. meningitidis* is hyperendemic or epidemic.

If MCV4 is unavailable, MPSV4 is an acceptable alternative for persons at elevated risk. MPSV4 is not recommended and should not be administered routinely for adolescents aged 11 to12 years or for adolescents entering high school. Adolescents in these age groups are recommended to receive only MCV4.

Routine vaccination of young adolescents (persons aged 11 to 12 years) with MCV4 is recommended at the preadolescent health care visit (at age 11 to 12 years). Students entering college who plan to live in dormitories should be immunized with MCV4 routinely. People at increased risk of acquiring meningococcal disease should be immunized with MCV4 if they are at least 2 years of age. These people include those who have terminal complement or properdin deficiencies, have anatomic or functional asplenia, or travel to or reside in countries where *N. meningitidis* is hyperendemic or epidemic. Because people with HIV infection are likely to be at higher risk of acquiring meningococcal disease, although not to the extent that they are at risk of acquiring invasive *S. pneumoniae* infection, they may elect to be immunized with MCV4 if they are at least 2 years of age. People who wish to decrease their risk of contracting meningococcal disease may elect to receive MCV4 if they are 2 years of age or older. Immunization with MCV4 may be indicated for adolescents previously immunized with MPSV4. These people should

be considered for re-immunization 3 to 5 years after receiving MPSV4 if they remain at increased risk of contracting meningococcal disease. The same recommendation applies to entering college students previously immunized with MPSV4 and for people at high risk of developing an infection.

For control of meningococcal outbreaks caused by vaccine-preventable serogroups, the preferred vaccine in adults and children older than 2 years is MCV4, but MPSV4 is acceptable.

Precautions and Contraindications

Because of theoretical considerations, meningococcal polysaccharide vaccines should not be administered to pregnant women unless the risk of acquiring disease is substantial. However, evaluation of pregnant women immunized during an epidemic in Brazil demonstrated no adverse effects.[348]

Immunization with MCV4 is contraindicated among people known to have hypersensitivity to any component of the vaccine, including diphtheria toxoid, and to dry, natural rubber latex, which is used in the vial stopper. Because of the temporal association between MCV4 and GBS, MCV4 should not be given to adolescents or adults with a history of GBS who are not in a high-risk group for developing invasive meningococcal disease.

MUMPS VACCINE

Live virus mumps vaccine became available in the United States in 1967 and was recommended for routine use in 1977. After vaccine licensure, the incidence of reported mumps cases decreased rapidly. A relative resurgence of mumps occurred in 1986 and 1987. In 1989, the ACIP and AAP implemented a two-dose combined MMR schedule given at 4 to 6 or at 11 to 12 years of age. After implementation of the two-dose MMR vaccination requirement, the incidence of mumps disease decreased and reached a record low of 258 cases in 2004.[132]

Before vaccine licensure in 1967 and during the early years of vaccine use, most reported cases occurred in the 5- to 9-year age group; 90 percent of cases occurred among children 15 years of age and younger. In the late 1980s, a shift toward older children occurred. Since 1990, persons age 15 years and older have accounted for 30 to 40 percent of cases per year.

Between January and October of 2006, more than 5700 confirmed or probable mumps cases were reported, predominantly among patients aged 18 to 24 years, many of whom were college students. Twelve midwestern states were most affected, but sporadic cases were documented in many other states, mostly related to travel from primary outbreak areas. Nearly half of all cases occurred in Iowa, where the resurgence began.[149] The outbreak underscored limitations in the 1998 recommendations relating to prevention of mumps transmission in health care and other settings with high risk for mumps transmission. After reviewing data from this outbreak and previous evidence on mumps vaccine effectiveness and transmission, the ACIP issued updated recommendations for mumps vaccination.[153]

Preparations

The live attenuated mumps virus vaccine (Jeryl Lynn strain) in current use in the United States is prepared in chick embryo cell culture and is available individually (monovalent, mumps only) and in combination as mumps-rubella vaccine, MMR, and MMRV. The AAP and ACIP recommend that combined MMR vaccine be used when any of the individual components is indicated and MMRV is recommended if the vaccinee is 12 months through 12 years of age. Use of single-antigen mumps vaccine is not recommended.

Each dose of vaccine contains neomycin, sorbitol, and hydrolyzed gelatin as stabilizers. Before reconstitution, mumps vaccine must be stored at 2 to 8° C (35.6° F to 46.4° F) or colder and protected from light to avoid inactivation. After reconstitution, the vaccine should be used within 8 hours or discarded.

Immunogenicity and Efficacy

The vaccine induces an asymptomatic, noncommunicable infection. More than 97 percent of susceptible recipients develop protective antibody titers, albeit lower than those after natural infection.[488] Post-licensure studies in the United States demonstrated that one dose of mumps vaccine was 78 to 91 percent effective in preventing clinical mumps with parotitis.[395] Studies of vaccine effectiveness during outbreaks suggested substantially higher levels of protection with a second dose of MMR.[70,256,389]

The effectiveness of the mumps component of MMR vaccine was estimated during the large outbreak in the United Kingdom in 2004 to 2005. Vaccine effectiveness was 88 percent for one dose and 95 percent for two doses. The effectiveness of one dose declined from 96 percent in 2-year-olds to 66 percent in 11- to 12-year-olds, and the effectiveness of two doses declined from 99 percent in 5- to 6-year-olds to 86 percent in 11- to 12-year-olds.[177]

The duration of vaccine immunity is unknown, but serologic data showed persistence of antibody for more than 30 years.[486] However, data from more recent studies suggested that waning immunity may contribute to mumps outbreaks in older vaccinated populations.[177]

Adverse Events

The use of mumps vaccine is associated with very few side effects. Parotitis and fever have been reported rarely. Hypersensitivity reactions, including rash, pruritus, and purpura, have been associated temporally with vaccination, but they are transient and generally mild. Administration of MMR is not harmful if it is given to an individual already immune to one or more of the viruses.[16,111]

The frequency of reported central nervous system dysfunction after vaccination is not greater than the observed background rate in unimmunized persons.[111] The IOM concluded that evidence is inadequate to establish a causal relationship between the Jeryl Lynn strain of mumps vaccine used in the United States and aseptic meningitis, encephalitis, or sensorineural deafness.[280]

Indications

Routine active immunization as MMR in children 12 to 15 months of age is recommended.[16,111,149] Most children will receive a second dose of mumps vaccine in childhood as a result of the recommendation for routine measles re-vaccination with MMR. Susceptible older children, adolescents, and adults also should be vaccinated against mumps.

Evidence of immunity through documentation of vaccination is now defined as one dose of live mumps vaccine for preschool-age children and adults not at high risk for exposure and infection and two doses of live mumps vaccine for school-age children (i.e., grades kindergarten to12) and adults at high risk for exposure and development of infection (i.e., health care workers, international travelers, and students at post–high school education institutions). Additional recommendations for outbreak control include administering a second dose of MMR for preschool children and adults not at high risk for exposure and acquisition of infection if these persons are part of a group that is experiencing an outbreak.[149] To ensure high levels of immunity, especially among groups at high risk for exposure and development of infection,

every opportunity should be used to provide the first or second dose of MMR vaccine to those without adequate evidence of immunity (e.g., documentation of vaccination).

Mumps remains endemic throughout most of the world. Although vaccination against mumps is not a requirement for entry into any country, susceptible children, adolescents, and adults born after 1956 should be offered mumps vaccination, usually as MMR, before engaging in international travel.

Mumps vaccine is of no proven value in the prevention of disease in susceptible individuals after exposure to mumps, probably because the time required to develop protective antibody titers after immunization exceeds the incubation period of clinical mumps. However, if the exposure does not result in infection, the vaccine confers subsequent immunity.

Precautions and Contraindications

Among persons who are allergic to eggs, the risk of having serious allergic reactions such as anaphylaxis after receiving MMR is extremely low, and skin testing with vaccine is not predictive of allergic reaction to vaccination [16,288,296] Therefore, performing skin testing is not required before administering MMR to persons who are allergic to eggs. Similarly, the administration of gradually increasing doses of vaccine is not required.[111] Data indicate that most anaphylactic reactions to measles- and mumps-containing vaccines are not associated with hypersensitivity to egg antigens but rather with hypersensitivity to other components of the vaccines, such as the gelatin stabilizer.[242,262,295,318]

Because of the theoretical risk of fetal damage, mumps vaccine should not be administered to women known to be pregnant or who are considering becoming pregnant. Women vaccinated with MMR should avoid conception for 28 days after vaccination.[130]

Lymphoreticular or other generalized malignancy and primary or secondary immunodeficiency states represent contraindications to the use of mumps vaccine. Exceptions are children with HIV infection who are immunized against measles with MMR (see "Measles Vaccine"). Because infection after vaccination is noncommunicable, susceptible close contacts of immunosuppressed patients should be vaccinated to avoid exposure to mumps in such patients.

After cessation of immunosuppressive therapy, live virus mumps vaccine generally is withheld for at least 3 months. Because the intensity and type of immunosuppressive therapy, radiation therapy, underlying disease, and other factors determine when immunologic responsiveness will be restored, making a definitive recommendation for an interval after cessation of immunosuppressive therapy when mumps vaccine can be safely and effectively administered often is not possible.

The effect of immune globulin preparations on the response to mumps vaccine is unknown. High doses of immune globulin preparations can inhibit the immune response to measles vaccine for 3 or more months, depending on the dosage.[341] If mumps vaccine is given as the MMR vaccine, the recommendations for measles vaccine should be followed (see Table 255–14).

Administration of mumps vaccine should be avoided if the individual is receiving immunosuppressive dosages of systemic corticosteroids. The effects of corticosteroids vary, but many clinicians consider that a dose equivalent to either 2 mg/kg of body weight or 20 mg/day of prednisone is sufficiently immunosuppressive to raise concern about the safety of vaccination with live virus vaccines.

PERTUSSIS VACCINE

Pertussis (whooping cough), caused by the fastidious, gram-negative, pleomorphic bacillus *B. pertussis*, continues to produce significant morbidity and mortality worldwide among young children.[365] In the absence of vaccination, the WHO estimated that approximately 1 million deaths would have occurred from the disease and its complications. In the United States, the number of cases has been reduced by approximately 95 percent during the vaccine era. Despite an effective vaccine, however, pertussis continues to occur in the United States in all age groups. Since the historic low point achieved in the 1980s, the incidence of pertussis has increased steadily. Surveillance data collected by the CDC's National Immunization Program for the periods from 1994 to 1996 and 1997 to 2000 demonstrated that the incidence of pertussis increased 60 percent in adolescents and adults and 11 percent in infants younger than 6 months.[136] Data from the National Notifiable Disease Surveillance System reveal an escalation in the increase in all age groups between 2001 and 2004.[71] Incidence rates among the pediatric population peak in infants younger than 6 months of age and again during adolescence between 11 and 18 years of age.[36] The increase in the number of cases among young infants suggests that a true increase in pertussis circulation has occurred. The number of cases in children old enough to receive vaccine has remained stable. The likely reason that pertussis remains endemic in the United States despite high vaccination rates is that neither infection nor immunization provides life-long immunity. Humans are the only known reservoir for pertussis; immunity wanes after 5 to 10 years, thus maintaining a population of susceptible hosts for the continued circulation of bacteria.

The experiences of countries where rates of pertussis vaccination have markedly declined provide strong support for continuing routine immunization of infants and young children.[165] In the United Kingdom, as a result of adverse publicity about pertussis vaccination, a decrease in immunization rates in 2-year-old children from 77 percent in 1974 to 30 percent in 1978 was followed by an epidemic of 102,500 cases of pertussis. A similar experience occurred in Japan, which reported 13,105 cases and 41 deaths in 1979 after routine immunization had been suspended temporarily in 1975.

In the United States during the 1980s, publicity about alleged serious reactions to pertussis vaccine generated public controversy about the risk of receiving pertussis vaccine that resulted in costly litigation and escalating vaccine costs and also jeopardized vaccine supply and development.[364,391] The experience in countries such as England and Japan, the severity of pertussis in young infants, and the usually benign or self-limited sequelae of pertussis vaccination clearly justify continuing routine childhood immunization. Several risk-to-benefit analyses provided additional evidence in support of vaccination.[165,267]

Effective primary preventive programs necessitate immunizing young infants, usually beginning at 2 months of age, because the morbidity and mortality of pertussis are greatest in infants, especially those younger than 6 months.[99,165] Approximately 26 percent of reported cases in the United States occur in infants younger than 6 months. During the period from 1997 to 2000, the case-fatality rate for infants younger than 6 months was 0.8 percent; 63 percent of these infants were hospitalized with frequent complications including pneumonia (11.8%), seizures (1.4%), and encephalopathy (0.2%).[136] From 2000 to 2004, 100 pertussis-related deaths were reported; 90 percent were among infants younger than 4 months of age and 76 percent occurred in infants younger than 2 months of age.[71] Maintaining high rates of immunization in children beyond infancy, with booster immunization for adolescents and adults, may reduce the risk of infection in infants by decreasing the incidence of infection in older family members and the resultant transmission of *B. pertussis* within the household. Vaccination, including boosters, is essential because the disease is highly infectious and transmission often occurs before adults seek medical care.

Preparations

Whole-cell pertussis vaccine was introduced first in the United States in the 1920s. Not until the 1940s to 1950s, however, did pediatric whole-cell pertussis vaccine became routinely recommended for children. This vaccine, unavailable in the United States since 2002, consisted of a suspension of inactivated *B. pertussis* combined with diphtheria and tetanus toxoids (DTP).[71] To reduce the incidence of local and systemic reactions caused by whole-cell vaccines, less reactogenic pediatric acellular vaccines composed of one or more purified components of *B. pertussis* combined with diphtheria and tetanus toxoids (DTaP) were developed. Initially licensed in 1991 for use as the fourth or fifth dose in the series, DTaP was licensed in 1997 for all five doses in the series. Numerous acellular vaccines formulated from the different components have been tested in children. All currently U.S. licensed vaccines contain detoxified/inactivated pertussis toxin (i.e., pertussis toxoid) and filamentous hemagglutinin (FHA).[199] In addition, most vaccines include one or both of the following *B. pertussis* antigens: pertactin (PRN, a 69-kd outer-membrane protein), and fimbriae proteins (FIM, agglutinogens). The various vaccines differ in the amount of each component. No pertussis-only vaccine is available.

From 2001 until 2008, numerous changes were made to the list of licensed acellular pertussis–containing vaccines available in the United States. Three acellular vaccines currently are available for use in the primary vaccination series for children younger than 7 years of age (Table 255–15). Tripedia (Sanofi Pasteur) and Infanrix (GlaxoSmithKline Biologicals) are licensed for the five-dose DTaP vaccination series, and Daptacel (Sanofi Pasteur) is licensed for the first four doses of DTaP vaccine only. Pediarix (GSK), which combines DTaP with IPV and HBV vaccines, was licensed in 2002 for the first three doses in the DTaP series for use in children 6 weeks to 6 years old and born to mothers known to be HBsAg-negative. This vaccine is not approved for the first HBV vaccine, for the fourth or fifth DTaP dose, or for infants born to women known to be HBsAg-positive or whose hepatitis B status is unknown. TriHIBit (Sanofi Pasteur), the combination of Tripedia and ActHIB (Hib conjugate vaccine), was licensed by the FDA in 2001 for use as the fourth booster dose given when the infant is 15 to 18 months of age and not for use as part of the initial three-dose series. Pentacel (Sanofi Pasteur), which combines DTaP with IPV and Hib (PRP-T) vaccines, was licensed in 2008 for the first four doses in the DTaP series in children 6 weeks to 6 years of age. Kinrix (GSK), a combination of DTaP with IPV, was also licensed in 2008 for use for the fifth dose of DTaP in children 4 to 6 years of age.

For enhanced control of pertussis, routine re-vaccination of adolescents and adults with an acellular pertussis vaccine is recommended now to minimize the morbidity associated with infection in these age groups and to reduce the reservoir of infection. In 2005, the FDA licensed two Tdap vaccines (see Table 255–15); Boostrix (GSK) is approved for adolescents aged 10 to 18 years, and Adacel (Sanofi Pasteur) is approved for adolescents and adults aged 11 to 64 years. Both Tdap vaccines contain the same pertussis antigen components as are in the pediatric vaccines (some in reduced quantities). The tetanus and diphtheria components are the same as for the current adult formations of Td, with reduced diphtheria content as recommended for use in persons 7 years of age and older.

The ACIP recommends that, whenever feasible, the same DTaP vaccine product be used for all doses of the vaccination series. If the vaccine provider does not know or does not have available the type of DTaP previously administered, any of the available licensed DTaP vaccines may be used to complete the vaccination series.[126]

Immunogenicity

The Multicenter Acellular Pertussis Trial evaluated the immunogenicity and safety of 13 different acellular pertussis–containing vaccines as compared with whole-cell DTP administered to infants at 2, 4, and 6 months of age in the United States.[199] Serologic correlates of immunity to diphtheria and tetanus toxoids, defined as antibody levels 0.1 IU/mL or greater, were achieved after vaccination with Tripedia and Infanrix and were comparable with those achieved with DTP vaccination. Pertussis immunity, determined by at least a fourfold rise in antibody titer to pertussis toxin and FHA, was equal to or greater than with DTP, following immunization with either of the two DTaP vaccines noted earlier.[211]

Efficacy

For both whole-cell and acellular vaccines, serologic correlates of immunogenicity have not been established for assessing efficacy. As a result, field and other epidemiologic studies must be performed to demonstrate efficacy. Studies in the United States of household contacts exposed to pertussis indicate that the efficacy of whole-cell vaccine was 80 percent or greater.[99,165,376] Studies reporting lower rates of vaccine efficacy often reflect the use of different criteria for the diagnosis of pertussis and lesser effectiveness of the vaccine in protecting against mild infection than against severe disease.[217] Vaccine-induced immunity persists for at least 3 years and subsequently diminishes with time. Pertussis in individuals previously vaccinated is less severe and is associated with fewer complications than in unvaccinated persons.

In studies of the efficacy of eight acellular pertussis vaccines in infants, rates of prevention of pertussis ranged from 58 to 93 percent.[396] Comparing efficacy among the different products, however, often is not possible because of differences in study design, vaccine schedule (specifically, the number of doses and age of administration), case definitions of pertussis, and other confounding variables. In general, these acellular vaccines appear to be similar in efficacy to most whole-cell vaccines. Whereas in two large trials in Sweden and Italy several acellular vaccines

TABLE 255–15 Acellular Pertussis Vaccines Licensed for Use in the United States

Trade Name	Vaccine Manufacturer	No. of Pertussis Antigens	Antigenic Content	Dose Series Approved
DTaP Vaccines for Children <7 yr of Age				
Tripedia	Sanofi Pasteur	2	PT, FHA	5
Infanrix	GlaxoSmithKline Biologicals	3	PT, FHA, PE	5
DAPTACEL	Sanofi Pasteur	5	PT, FHA, PE, 2 types of FIM	4
Tdap Vaccines for Adolescents				
BOOSTRIX	GlaxoSmithKline Biologicals	3	PT, FHA, PE	1
ADACEL	Sanofi Pasteur	5	PT, FHA, PE, 2 types of FIM	1

DTaP, diphtheria-tetanus-acellular pertussis; FHA, filamentous hemagglutinin; FIM, fimbriae; PE, pertactin; PT, pertussis toxin; Tdap, tetanus-diphtheria-acellular pertussis.

demonstrated substantially greater efficacy than noted in the one U.S. whole-cell vaccine previously approved, other whole-cell vaccines studied appeared to be slightly more effective than were acellular vaccines in other trials.[210] In addition, the vaccines in these Swedish and Italian trials were given in a three-dose schedule, in contrast to the four-dose primary schedule for vaccination of young children in the United States.

Adverse Events

Local and febrile reactions to whole-cell vaccines occur in more than half of DTP recipients.[176] These manifestations usually develop within the first 24 hours and are brief in duration. More serious reactions to whole-cell vaccines are uncommon. Such reactions include prolonged crying for 3 hours or longer occurring within the first 48 hours of receiving vaccination (1% to 3% of DTP recipients) and a temperature of 40.5° C or greater (≥104.8° F) within 48 hours (0.3%); a hypotonic-hyporesponsive episode (HHE) described as collapse or shocklike state within 48 hours and seizure within 3 days of vaccination each was estimated to occur once per 1750 doses.[176] Episodes of inconsolable crying, high fever, and HHE reactions following receipt of DTP vaccine resolved without sequelae. Most post-DTP seizures occurring within 48 hours of receipt of vaccine are brief, self-limited, and generalized and occur in association with fever. These seizures have not been demonstrated to result in the subsequent development of epilepsy or other neurologic sequelae. Predisposing factors include an underlying convulsive disorder, a personal history of previous convulsion, and a family history of convulsions.[106]

The incidence of local and febrile reactions after the administration of acellular vaccines is significantly lower.[199,396] Comparison of rates of adverse events with different acellular vaccines compared with whole-cell vaccines demonstrated similar safety profiles for each of these vaccines.[168,424] The most common adverse events include injection site reactions (erythema, induration, tenderness) and the following mild system symptoms: slight to moderate fever, drowsiness, irritability, and loss of appetite. Rates of local reactions increase with each subsequent dose of DTaP vaccine.[394,455] Booster doses of acellular pertussis vaccine may be associated with extensive local swelling (i.e., limb swelling), especially with vaccines having a high diphtheria content.[404] The pathogenesis of this reaction is not understood but spontaneously resolves without sequelae and is not associated with an increased risk of similar adverse events occurring after receipt of the fifth dose.[17] Severe reactions to acellular vaccines such as prolonged crying for 3 hours or longer, temperatures of 40.5° C or greater (≥104.8° F), HHEs, and seizures are rare occurrences.[168,199,239,247,396,424,456,458] As with local and febrile reactions, these occurrences with acellular pertussis vaccination are significantly less frequent than those that occur after administration of whole-cell vaccination.

SERIOUS NEUROLOGIC ILLNESS

The National Childhood Encephalopathy Study (NCES), a large case-control study from Great Britain that was published in 1985, estimated that the occurrence of acute neurologic illness resulting in hospitalization was 1 in 140,000 DTP vaccinations.[355] In a 10-year follow-up study published in 1993, neurologic sequelae were found to be common occurrences, but no more so than in children with unrelated, acute neurologic illness in infancy,[354] and reviews of the data have disputed the conclusion that pertussis vaccine can cause neurologic sequelae.[27,106,447] More recent data from Canada evaluating more than 12,000 pediatric hospitalizations for serious neurologic illness between 1993 and 2002 found no association after administration of more than 6.5 million doses between development of encephalopathy and receipt of DTaP vaccination.[17] Taken together, the data do not support a role for whole-cell pertussis vaccine in causing brain damage.[88]

Indications

Vaccination against pertussis with DTaP is recommended routinely for children at 2, 4, and 6 months of age, followed by a fourth dose at 12 to 18 months of age and a fifth dose at 4 to 6 years of age.[17,36,71] Immunization can be started when the child is as young as 6 weeks of age if pertussis is prevalent in the community. The interval between administrations of the three doses of the initial series can be as short as 4 weeks. The AAP and ACIP recommend exclusive use of acellular pertussis vaccines for all doses of the pertussis vaccine series.[17,36,71] DTP is not an acceptable alternative because of its higher rates of local reactions, fever, and other common systemic reactions. However, in many countries, including several in Europe as well as in developing countries, whole-cell vaccine remains the recommended product.

A single dose of Tdap is now recommended for adolescents aged 11 to 18 years; the preferred age is 11 to 12 years, for those who have completed their primary DTP/DTaP series and have not yet received the adult tetanus and diphtheria toxoids (Td) booster.[17,36,71] Adults aged 19 to 64 years who have not received Tdap previously should receive a single dose of Tdap in place of the Td booster if 10 years or more have transpired since they last received a tetanus-containing vaccine. Intervals as short as 2 years have been shown to be safe,[17,36,71] and intervals of 2 years or less may be indicated in certain high-risk situations (e.g., adults who are likely to have close contact with an infant younger than 12 months, exposed health care personnel). Women should receive Tdap before becoming pregnant. Women who have not been immunized previously with Tdap should receive Tdap in the immediate postpartum period. Every 10 years thereafter, booster vaccination should be provided by Td (or TT).

Adolescents between the ages of 11 and 18 years who have never been vaccinated against tetanus, diphtheria, or pertussis should initially receive a single dose of Tdap followed by Td for the subsequent two doses at 4 weeks or more and 6 to 12 months later. No pertussis-containing vaccine is approved for children between the ages of 7 and 10 years. For these children who have not previously been vaccinated, three doses of Td are recommended at 0 (initial dose), 4 weeks or more, and 6 to 12 months. The third dose may be delayed until the child is old enough to receive Tdap; otherwise, a booster with Tdap is recommended in adolescence.

As further protection against transmission of pertussis to infants who are at greatest risk of pertussis-related morbidity or mortality compared with other age groups, the ACIP has recommended the use of Tdap vaccine in pregnant girls and women between the ages of 11 and 64 years, including those breast-feeding who have previously not received Tdap vaccine.[17,36,71,307]

Contraindications and Precautions

The contraindications and precautions for administering pertussis vaccine are based on adverse reactions associated with whole-cell vaccine. Although reactions occurring after the administration of DTaP are much less common than are those associated with DTP, at present, the contraindications and precautions for DTaP are the same.[17,36,71] Adverse events temporally related to pertussis immunization that contraindicate further administration of DTaP are as follows:

- An immediate anaphylactic reaction. Subsequent immunization with any of the three components of the vaccine should be avoided.

- Encephalopathy occurring within 7 days. Encephalopathy in this context is defined as a severe, acute, central nervous system disorder unexplained by another cause and may be manifested by major alterations in consciousness or by generalized or focal seizures that persist for more than a few hours without recovery within 24 hours. Additional doses of DT (or Td) vaccine should be substituted for any pertussis-containing vaccine.

Post-vaccination reactions constituting precautions are as follows:

- A convulsion, with or without fever, occurring within 3 days of receiving DTP or DTaP vaccination
- Persistent, severe, inconsolable screaming or crying for 3 or more hours within 48 hours of vaccination
- HHE occurring within 48 hours of vaccination
- Temperature of 40.5° C or of 104.8° F or greater that is unexplained by another cause and occurs within 48 hours of vaccination

With these adverse events occurring in temporal association with DTaP vaccination, the decision to administer additional doses of pertussis vaccine should be considered carefully. In circumstances such as a pertussis outbreak in which the potential benefits of pertussis immunization outweigh the possible risks, vaccination is indicated, particularly because these events have not been proved to cause permanent sequelae. In addition, the risk of these reactions occurring after receipt of DTaP is substantially lower than after receipt of DTP.

In children with an evolving neurologic disorder, pertussis immunization should be deferred until the nature and cause of the disorder have been established. A personal history of having a previous convulsion unrelated to DTaP vaccination or a family history of convulsions (in the absence of a possible evolving neurologic disorder) is not a contraindication.

PNEUMOCOCCAL VACCINE

S. pneumoniae is a leading bacterial pathogen, especially among young children, elderly persons, and persons with predisposing conditions. In children, it is the most common cause of otitis media, occult bacteremia, and bacterial pneumonia requiring hospitalization. After the widespread introduction of conjugate Hib vaccination and subsequent marked decline in occurrence of Hib meningitis, *S. pneumoniae* became a leading cause of bacterial meningitis in children in the United States. In some populations, such as Native Alaskans, the incidence of bacteremia is markedly higher than that reported in other geographic areas of the United States.[197] Groups considered at high risk for invasive pneumococcal disease include children with sickle-cell disease, asplenia, Hodgkin disease, congenital humoral immunodeficiency, HIV infection, and nephrotic syndrome, as well as recipients of organ transplants. Children who have received cochlear implants are at particular risk for developing meningitis.[139] Other chronic diseases associated with an increased risk for development of severe pneumococcal disease include chronic cardiovascular and pulmonary diseases, diabetes mellitus, and renal failure. The role of these chronic diseases in predisposing individuals to the development of pneumococcal infection, however, has been demonstrated primarily in adults. Mortality rates are highest in those who have bacteremia or meningitis, elderly persons, and patients with impaired humoral immunity or certain chronic diseases.

The purified polysaccharide vaccine has been effective in reducing severe disease in the adult population,[76] but it has had little impact in young children because the vaccine is not immunogenic in children younger than 2 years old.[18] In addition, the polysaccharide vaccine has not been effective in preventing otitis media caused by *S. pneumoniae*.[18]

Several factors have made the development of new preventive strategies for pneumococcal disease a high priority.[184] Morbidity and mortality rates of pneumococcal infection appear to be particularly high in developing countries. The increasing incidence of antimicrobial-resistant pneumococci further underscores the need for developing effective pneumococcal vaccines for young children.[69] Resistance of *S. pneumoniae* to multiple antibiotics has increased rapidly in the United States and even more rapidly in other parts of the world.[292] Children younger than 2 years of age have the highest rate of invasive pneumococcal infection but do not develop an effective antibody response to polysaccharide vaccine. In addition, children 2 to 5 years of age may have relatively poor responses to serotypes 6B, 14, 19F, and 23F, common causes of pediatric infections and the most prevalent penicillin-resistant serotypes.

These factors prompted the development of conjugated polysaccharide–protein vaccines, one of which has been licensed in the United States. These vaccines are similar in design to the licensed Hib conjugate vaccines. Because of the large number of serotypes of *S. pneumoniae* that cause disease, development of these conjugate pneumococcal vaccines has been more difficult than has the development of similar vaccines for Hib. Each pneumococcal antigen must be coupled to a protein carrier, and the vaccine must be prepared to ensure that antigen is sufficient to induce an immune response but insufficient to elicit an adverse reaction.

One potential problem with conjugate pneumococcal vaccines is the need to immunize against many different serotypes of pneumococci. Because of local reactions to the protein component, conjugate vaccines that contain more than 12 serotypes may be difficult to produce. As a result, different formulations of conjugate pneumococcal vaccine may be developed that would contain different serotypes targeted for a specific group of patients. Vaccine containing types 4, 6B, 9V, 14, 18C, 19F, and 23F (PCV7) would be necessary for prevention of otitis media in the United States, whereas types 1, 2, and 5 would need to be added to prevent pneumonia in developing countries. In addition, the use of conjugate vaccines against limited serotypes may lead to the emergence of pneumococcal serotypes that are currently less common and require adjustment of a vaccine's composition.[441]

Routine infant immunization using these conjugate vaccines has led to significant reductions in the instances of *S. pneumoniae* disease. Since the widespread introduction of the seven-valent pneumococcal conjugate vaccine in infants in 2000, rates of invasive pneumococcal disease have decreased markedly in the United States. The incidence of all invasive pneumococcal infections has decreased by 80 percent for children younger than 2 years of age and by up to 90 percent for infections caused by vaccine and vaccine-related serotypes.[18,496] An increase in non-vaccine serotype disease has occurred, but it is small compared with the overall reductions in vaccine-type disease.[77,142]

A decline in the incidence of vaccine-type disease in unvaccinated children and adults has occurred since the introduction of routine vaccination for infants in the United States.[142] In addition, rates of invasive pneumococcal disease in infants younger than 2 months of age have decreased significantly, thus providing evidence that vaccinating children aged 2 to 23 months has led to changes in pneumococcal carriage in infants too young to receive PCV7.[398] Herd immunity is estimated to prevent twice as many cases as do the direct effects of vaccination alone.[142] Although the exact mechanism of herd immunity is uncertain, one hypothesis is that vaccinated children are less likely to have nasal carriage of pneumococcus and hence less pneumococcal transmission to their contacts.[192] Use of conjugate vaccines has been demonstrated to reduce nasopharyngeal carriage of vaccine

serotypes.[194,195] Pneumococcal conjugate vaccination also has led to increased nasopharyngeal colonization of children with non-vaccine serotypes.[229,353] Ongoing surveillance of invasive pneumococcal disease is needed to determine whether non-vaccine serotypes will predominate.

Preparations

Two pneumococcal vaccines are available for use in children in the United States. The first is a heptavalent vaccine (PCV7) containing the capsular polysaccharides from serotypes 4, 6B, 9V, 14, 18C, 19F, and 23F conjugated to mutant diphtheria toxin (CRM_{197}). This vaccine (Prevnar, Wyeth Vaccines) was licensed in the United States in 2000. Serotypes included in PCV7 and potentially cross-reactive serotypes (i.e., 6A, 9A, 9L, 18B, and 18F) accounted for 86 percent of cases of bacteremia, 83 percent of cases of meningitis, and 65 percent of AOM cases occurring in children younger than 6 years in the United States during the period 1978 to 1994.[122] The second vaccine (PPV23 [Pneumovax]) is composed of purified, capsular polysaccharide antigens of 23 pneumococcal serotypes. Although 90 different serotypes have been identified, vaccine serotypes in the 23-valent vaccine are responsible for 85 to 90 percent of adult infections and nearly 100 percent of invasive disease and 85 percent of otitis media cases in children.[85,230] Each vaccine is recommended in a dose of 0.5 mL to be given intramuscularly.

Immunogenicity and Efficacy

The immunogenicity of the conjugate polysaccharide vaccine appears to be determined by the pneumococcal polysaccharide serotype, rather than by the carrier protein. Some serotypes (14, 18C, and 19F) are excellent immunogens in that they elicit antibody protection after a single dose, whereas others (6B and 23F) require three doses of vaccine.[213] Conjugate vaccine elicits immunologic memory.[213] The antibody concentrations achieved after the initial series of three doses usually are sustained for only a few months and then decline to nearly pre-immunization levels. A dose of pneumococcal vaccine, either polysaccharide or conjugate, given in the second year of life elicits an amnestic-type response.

The licensed heptavalent polysaccharide conjugate vaccine was studied in a large, prospective, placebo-controlled efficacy trial in northern California involving 38,000 children. The vaccine was 89 percent effective in preventing invasive disease caused by any pneumococcal serotype and 97 percent effective against disease caused by the seven vaccine serotypes.[63] For non-invasive disease, a decrease of 7 percent in the number of cases of otitis media and 23 percent in doctor visits for recurrent otitis (six or more visits per year) occurred in vaccinated children. The study also demonstrated an 11 percent decrease in the number of clinical cases of pneumonia and efficacy of 26 percent against a first episode of radiograph-confirmed pneumonia in vaccines.[254,432]

The ability of the heptavalent vaccine to protect children against AOM also was evaluated in an efficacy trial conducted in Finland.[214] For prevention of AOM caused by pneumococci of any serotype, efficacy was estimated to be 34 percent, whereas efficacy against AOM irrespective of etiology was 6 percent. Efficacy against AOM caused by vaccine-related serotypes was 57 percent, but an increase of 33 percent in the rate of AOM episodes caused by non-vaccine serotypes occurred in the group receiving the heptavalent vaccine in comparison with controls. However, in spite of the increase in disease caused by non-vaccine serotypes, the net effect on pneumococcal AOM was a reduction of 34 percent.

Randomized controlled trials conducted in African children using a nine-valent pneumococcal conjugate vaccine demonstrated efficacy in the prevention of radiologically confirmed pneumonia and vaccine-type invasive pneumococcal disease.[188,303] The vaccine was efficacious in preventing vaccine-type invasive disease in HIV-infected children.[303] One of the trials demonstrated a reduction in all-cause mortality among vaccine recipients.[188] These data have presented a compelling case for extending the benefits of pneumococcal conjugate vaccination to developing countries.

Vaccination with purified polysaccharide vaccine results in serologic type-specific antibody in most healthy adults and older children. Immunocompromised patients may respond less well. In children younger than 2 years old, antibody response is poor to most serotypes, including those most likely to cause infection, such as types 6A and 14.[85,108,230] Patients with AIDS have impaired antibody responses to vaccination, but asymptomatic HIV-infected adults do respond.[85,108]

Vaccine efficacy in preventing serious pneumococcal infection has been demonstrated for the purified polysaccharide vaccine primarily in immunocompetent adults, including elderly persons and those with chronic diseases such as chronic pulmonary and cardiac disorders and diabetes mellitus, which predispose these patients to development of pneumococcal infections. Efficacy against vaccine serotypes ranges from 61 to 75 percent in adults.[76,429] Investigations in adults in whom vaccine protection against pneumococcal infection has been substantially less have been criticized for methodologic problems.[85,108] Efficacy in the limited studies of children has been consistent with that in adults. In children with sickle-cell disease or anatomic asplenia, an octavalent vaccine was highly effective in preventing bacteremic infection.[40]

Adverse Events

Pneumococcal conjugate vaccines appear to be safe. The reactions most commonly reported have been local reactions at the injection site, but they occur at a lower frequency than do local reactions with other childhood vaccines such as DTP.[63]

Local reactions at the injection site, such as erythema and pain, are reported in approximately 50 percent of recipients of the purified polysaccharide vaccine.[18] However, more severe local and systemic reactions, such as fever and myalgia, are rare events that occur in less than 1 percent of vaccine recipients. Severe systemic reactions such as anaphylaxis rarely have been reported.[498] In adults who were re-vaccinated within 1 to 2 years in early studies, local reactions occurred more commonly than did those after initial immunization.[85,108] However, subsequent investigations, including studies in children, indicated no increase in the incidence or severity of local or systemic reactions on re-vaccination after longer intervals.[305,405]

Indications

Recommendations by the AAP and the ACIP for the use of PCV7 have been issued.[33,122] The AAP and ACIP recommend universal use of PCV7 in children 23 months and younger. For children in whom pneumococcal immunization is initiated before they reach 7 months of age, four doses of PCV7 are recommended at 2, 4, 6, and 12 to 15 months of age. For children beginning PCV7 immunization between 7 months and 23 months of age, the recommended schedule differs according to the child's age (see elsewhere for further information[18,33,122]). In addition, two doses of PCV7 are recommended for children 24 to 59 months of age who are at high risk of acquiring invasive pneumococcal infection and have not been immunized previously with PCV7. These children also should receive the 23-valent polysaccharide vaccine (PPV23) to expand serotype coverage.

Routine immunization of children 24 months or older who are at low and moderate risk is not recommended by the AAP at this time.[33] The ACIP recommends that PCV7 be considered for all children 24 to 59 months of age, with priority given to the

following: those aged 24 to 35 months; persons of Alaskan Native, American Indian, or African American descent; and those who attend group daycare centers.[122] Children 24 to 59 months old at high risk who have not previously received PCV7 but who have already received PPV23 should be vaccinated with two doses of PCV7 given 2 or more months apart.[122] Current data do not support a recommendation to replace the PPV23 with PCV7 vaccine for older children and adults.[122]

The purified polysaccharide vaccine is recommended by the AAP[33] for children 5 years or older who have one or more of the following risk factors:

- Sickle-cell disease
- Functional or anatomic asplenia
- Nephrotic syndrome or chronic renal failure
- Immunosuppression, such as from chemotherapy, organ transplantation, or malignant disease
- Cerebrospinal fluid leak
- HIV infection, symptomatic or asymptomatic
- Cochlear implants[139]

For previously vaccinated children 10 years old or younger who are at high risk of acquiring severe pneumococcal infection, re-vaccination with purified polysaccharide vaccine after 3 to 5 years is recommended.[33,122] Such children include those who have functional (e.g., sickle-cell disease) or anatomic asplenia, children and adolescents with HIV infection, and those who have a rapid antibody decline (e.g., nephrotic syndrome, renal failure, or organ transplantation).[231] Re-vaccination should be considered for high-risk older children and adults who were vaccinated 6 years or more previously.

Contraindications

No contraindications to initial vaccination exist.[33,122] The safety of pneumococcal vaccine in pregnant women has not been evaluated, but adverse consequences to the fetus have not been observed in newborns whose mothers were vaccinated inadvertently during pregnancy. Ideally, women at high risk of acquiring pneumococcal disease should be vaccinated before pregnancy. In persons who have had a severe reaction, such as anaphylaxis or a localized, severe hypersensitivity response, re-vaccination should be avoided.

POLIOMYELITIS VACCINE

The widespread implementation of poliovirus vaccine programs resulted in a dramatic reduction in the incidence of paralytic poliomyelitis throughout the world. In contrast to the pre-vaccine era, when more than 18,000 cases of paralytic disease occurred in the United States annually, the last known case in this country caused by indigenous wild-type virus occurred in 1979.[448] Other than rare imported cases, the only cases of paralytic poliomyelitis in the United States since then have been vaccine-related. The effectiveness of polio vaccination has led to major and successful initiatives by the WHO for global eradication of poliovirus infection. Currently, all nations in the Western Hemisphere, the Pacific region (including China), and Europe are free of poliomyelitis.[95,147,407] The number of polio-endemic countries decreased from 125 in 1988 to 6 (Afghanistan, Egypt, India, Niger, Nigeria, and Pakistan) in 2003.[147,504] However, from 2002 to 2005, a total of 21 previously polio-free countries were affected by importations of wild poliovirus type 1 from the 6 nations where wild poliovirus was endemic. Most of these importations were from northern Nigeria, where endemic disease increased dramatically after an 11-month suspension in immunization activities.[148] During that period, 4 countries, Indonesia, Somalia, Sudan, and Yemen, had outbreaks of more than 100 polio cases. Approximately 60 percent of all cases reported globally in 2005 occurred in previously polio-free countries. By the end of 2005, wild poliovirus transmission in all 21 countries except Somalia had been interrupted or substantially curtailed. The hope is, that with a sustained effort, complete global eradication of wild-type polio can be achieved.

The elimination of poliovirus infection has been achieved primarily through the use of OPV. This product had been the vaccine of choice for children in the United States since the early 1960s because it induced optimal intestinal immunity, was painless to administer, and secondarily immunized some contacts by fecal-oral spread of the vaccine virus and thus contributed to the immunity of the population.[80] Because 8 to 10 cases of vaccine-associated paralytic polio (VAPP) occurred annually and the risk of exposure to wild-type poliovirus had been markedly reduced or eliminated in the United States, expanded use of IPV was recommended by the CDC and the AAP beginning in 1997.[28] In 1999, as a result of progress in the global eradication of poliomyelitis, the need for further reduction in the risk for acquiring VAPP, and the acceptance of IPV by parents and physicians,[110] IPV was recommended for the first two doses of poliovirus vaccine for routine childhood vaccination.[31,116] To eliminate the risk of VAPP completely, in January of 2000 an all-IPV schedule was recommended for routine childhood vaccination in the United States.[32,116,124]

Since 2000, outbreaks of paralytic poliomyelitis caused by virulent polioviruses that are derived from OPV vaccine strains have occurred among underimmunized children living in certain economically deprived regions. The low immunization rates in these areas have permitted these viruses, called vaccine-derived polioviruses (VDPVs), to circulate for long periods of time and, by continuous mutation, to acquire biologic properties that are indistinguishable from naturally occurring wild polioviruses. Outbreaks associated with circulating VDPVs have occurred in Hispaniola (2000 to 2001), the Philippines (2001), and Madagascar (2001 to 2002).[121,127,138,297] Retrospective studies also detected the circulation of endemic VDPV in Egypt (1988 to 1993) and the likely localized spread of OPV-derived virus in Belarus (1965 to 1966).[128] Gaps in OPV coverage and the previous eradication of the corresponding serotype of indigenous wild poliovirus were the critical risk factors for all VDPV outbreaks.[298] The VDPV outbreaks were stopped by mass immunization campaigns using OPV. The potential risk of emergence of VDPV has increased dramatically in recent years as wild poliovirus circulation has ceased in most of the world. The risk appears to be highest for the type 2 OPV strain because of its greater tendency to spread to contacts. The emergence of VDPVs underscores the critical importance of eliminating the last pockets of wild poliovirus circulation, maintaining universally high levels of polio vaccine coverage, stopping OPV use as soon as it is safely possible to do so, and continuing sensitive poliovirus surveillance into the foreseeable future. Particular attention must be given to areas where the risks for wild poliovirus circulation have been highest and where the highest rates of polio vaccine coverage must be maintained to suppress VDPV emergence.

Preparations

Trivalent IPV is the only vaccine available for routine infant and childhood immunization in the United States. IPV is prepared by inactivation of naturally occurring polioviruses by treatment with dilute formalin.[85,116] Four trivalent IPV vaccines are now available in the United States: IPOL (Aventis Pasteur), Pediarix (in combination with DTaP vaccine and HBV vaccine, GlaxoSmithKline), Pentacel (in combination with DTaP vaccine and Hib vaccine, Sanofi Pasteur), and Kinrix (in combination with DTaP vaccine, GSK). These vaccines are produced in monkey kidney (Vero) cells, and each contains 40, 8, and 32 D antigen units of poliovirus types 1, 2, and 3, respectively. OPV is no longer available in the United States.

The trivalent OPV formulations available globally contain approximately $10^{6.5}$ 50 percent tissue culture infectious doses ($TCID_{50}$), $10^{5.5}$ $TCID_{50}$, and $10^{6.2}$ $TCID_{50}$ of poliovirus types 1, 2, and 3, respectively. The unequal contribution of each type to the trivalent preparation represents a "balanced" formulation designed to account for the more efficient replication of type 2 OPV virus in the gastrointestinal tract; if type 2 were given in equal concentrations with types 1 and 3, it would interfere with the replication of the two latter types. The live oral OPV strains originally were attenuated by passage in monkeys and in monkey kidney cell culture.

Trivalent IPV is now the preferred vaccine for other developed countries. However, because OPV maintains certain advantages for children in developing countries, including cost, ease of administration, and transmission of vaccine virus to unimmunized contacts, it is the vaccine currently recommended by the WHO EPI.[503] The trivalent form of OPV has been the mainstay of EPI and the WHO Polio Eradication Program for many years. Monovalent OPV vaccines have been introduced for outbreak control in certain settings.

Immunogenicity and Efficacy

Neutralizing antibodies are detectable to all three poliovirus types in 99 percent of recipients of IPV after two doses and in 100 percent after the third dose.[347,438] IPV-immunized children experience a large boost in antibody titer after receiving the third dose.[407] Detectable antibody persists at protective levels for at least 5 years, although geometric mean titers decline considerably.[449] However, few data are available regarding vaccine efficacy of the currently licensed "enhanced-potency" IPV formulations. A case-control study in Senegal indicated lower than expected protection rates of 36 and 89 percent for recipients of one and two doses, respectively.[408] However, because most IPV recipients experience a substantial boost in antibody titer after receiving a third dose, efficacy after three IPV doses likely would be quite high.

An optimal immune response to trivalent OPV requires multiple doses. In developed countries, three doses given at least 2 months apart are sufficient, with antibody prevalence to all three types being approximately 96 percent after the third dose.[347] Detectable serum antibody to all three types persists in 84 to 98 percent of vaccinees 5 years after receiving primary immunization.[310]

OPV efficacy was directly evaluated during a type 1 poliovirus outbreak in Taiwan in the early 1980s. During this outbreak, vaccine efficacy was estimated to be 82, 96, and 98 percent for one, two, and three or more doses, respectively.[299] In tropical countries, the series of OPV at 6, 10, and 14 weeks of age recommended by the WHO EPI fails to produce active immunity in a significant proportion of infants. Low seroconversion rates have been documented in many locations, averaging 73, 90, and 70 percent for types 1, 2, and 3, respectively.[386] Diarrheal disease at the time of immunization is a major factor.[368] The impact of diarrhea on seroconversion persists despite the administration of three or four OPV doses.

The monovalent OPV vaccines have higher type-specific seroconversion rates than does the trivalent vaccine and thus may be more effective when used in response to an outbreak caused by one poliovirus type.[78]

Adverse Events

No serious adverse events have been associated with use of the currently available IPV vaccine.[19,32,116] Because IPV vaccine contains trace amounts of streptomycin, neomycin, and polymyxin B, allergic reactions are possible in recipients with hypersensitivity to one or more of these antibiotics.

The OPV vaccine can cause VAPP, the overall risk of which is approximately 1 case per 2.4 million doses distributed. The rate after the first dose is approximately 1 case per 750,000 doses, including vaccine recipient and contact cases.

Indications

The polio vaccination series in the United States consists of four doses of IPV administered subcutaneously or intramuscularly.[19,32,116] The first and second doses are administered at 2 and 4 months of age, respectively. The third dose is usually given at 6 to 18 months of age, and a fourth dose is given routinely at 4 to 6 years of age, before school entry. Administration of a fourth dose is not necessary if the third dose was given on or after the child's fourth birthday. IPV is a component of Pediarix. Pentacel, and Kinrix. Pentacel may be used in children from 6 months to 18 months of age, and Kinrix may be used for children 4 to 6 years of age.

The ACIP and AAP continue to support the global eradication initiative and use of OPV as the vaccine of choice to eradicate wild-type polio in endemic areas and during epidemics. In the immunization schedule of the EPI of the WHO, doses of OPV are recommended at birth and when the child is 6 weeks, 10 weeks, and 14 weeks of age.[6] In geographic areas with endemic polio, a dose may be given when the newborn is discharged from the hospital. Supplementary doses often are given during mass community programs in these areas. Breast-feeding does not interfere with successful immunization with OPV.

Routine poliovirus vaccination is not necessary in adults residing in the United States. Such individuals are at minimal risk for exposure, and most are adequately protected because of vaccination during childhood. However, vaccination is recommended for individuals who have increased risk for exposure, including persons traveling to countries where poliomyelitis is epidemic or endemic, members of communities of specific population groups experiencing wild-type poliovirus disease, health care workers in close contact with patients who may be excreting wild-type poliovirus, and laboratory workers in contact with specimens that may contain wild-type poliovirus. Previously immunized adults who are at increased risk of exposure to poliomyelitis, such as those traveling to countries where poliomyelitis is still endemic, should receive a single dose of IPV. Available data do not indicate the need for more than a single lifetime booster dose of IPV. Adults who are unvaccinated or whose vaccination status is not documented should receive a primary vaccination series with IPV. This consists of two doses of IPV at 4- to 8-week intervals and a third dose 6 to 12 months after the second dose.

Precautions and Contraindications

IPV is contraindicated in persons who have experienced an anaphylactic reaction after receiving a previous dose of IPV or an anaphylactic reaction to one of the antibiotics in the vaccine preparation (i.e., streptomycin, polymyxin B, or neomycin).[19,32,116]

Poliomyelitis vaccination generally is contraindicated in pregnant women because of the theoretical risk of harm to the fetus. However, no deleterious effects from IPV administered during pregnancy have been demonstrated, and if immediate protection against poliomyelitis is needed, IPV may be given.

ROTAVIRUS VACCINE

Rotavirus, a major cause of childhood morbidity and mortality, causes an annual 440,000 deaths or approximately 5 percent of all childhood deaths worldwide. Rotavirus is also a major cause of acute gastroenteritis in the United States. In their first 5 years

of life, 4 out of 5 children in the United States will develop rotavirus gastroenteritis,[246,412,467] 1 in 7 will require a clinic or emergency department visit, 1 in 70 will be hospitalized, and 1 in 200,000 will die of this disease.[232,459]

Rotavirus gastroenteritis is preventable with live oral rotavirus vaccines. In 1998, the only approved rotavirus vaccine, a tetravalent rhesus-based rotavirus vaccine (RRV-TV, Rotashield, Wyeth Laboratories), was withdrawn from the market in the United States because of an association with an increased incidence of intussusception among vaccinated infants.[367] In 2006, a bovine-based pentavalent rotavirus vaccine (RotaTeq, Merck & Co.) was licensed by the FDA for use in infants in the United States. Both the ACIP of the CDC and the AAP have recommended universal vaccination of U.S. infants against rotavirus.[37,383] In 2008 a live attenuated human rotavirus vaccine (Rotarix, GlaxoSmithKline) was licensed in the United States.

Preparations

The licensed pentavalent rotavirus vaccine is an oral vaccine that contains five live reassortant rotaviruses. The rotavirus parent strains of the reassortants were isolated from human and bovine hosts. Four reassortant rotaviruses express one of the outer capsid proteins (G1, G2, G3, or G4) from the human rotavirus parent strain and the attachment protein (P7[5]) from the bovine rotavirus parent strain. The fifth reassortant virus expresses the attachment protein (P1A[8]) from the human rotavirus parent strain and the outer capsid protein G6 from the bovine rotavirus parent strain. The reassortants are propagated in Vero cells using standard tissue culture techniques.

Pentavalent rotavirus vaccine is provided in a squeezable plastic dosing tube with a twist-off cap designed to allow for the vaccine to be administered directly to infants by mouth. Each tube contains a single 2-mL dose of the vaccine as a liquid buffered-stabilized solution is stored at refrigerator temperatures (2° C to 8° C) for 24 months. Pentavalent rotavirus vaccine should be administered as soon as possible after being removed from refrigeration. The human rotavirus vaccine is a G1 P1A[8] virus attenuated by passage in cell culture. The lyphilized vaccine is stored at 2° to 8° C and must be reconstituted with a supplied diluent prior to administration.

Immunogenicity and Efficacy

The immune correlates of protection from rotavirus infection and disease are not fully understood. In a large phase III clinical trial of pentavalent rotavirus vaccine, an increase in titer of rotavirus group-specific serum IgA antibodies was used as one of the measures of the immunogenicity. Serum samples were obtained from a subset of study participants before they were immunized and approximately 2 weeks after they received the third dose; seroconversion was defined as a threefold or greater increase in antibody titer from baseline. Seroconversion rates for IgA antibody to rotavirus were 95 percent among vaccine recipients versus 14 percent in recipients of the placebo.[471]

The efficacy of pentavalent rotavirus vaccine was evaluated in two phase III trials.[65,471] In these trials, the efficacy of pentavalent rotavirus vaccine after completion of a three-dose regimen against rotavirus gastroenteritis of any severity was 74 percent and against severe rotavirus gastroenteritis was 98 percent. Efficacy was observed against all G1 to G4 and G9 serotypes, but relatively few non-G1 rotavirus cases were reported. Pentavalent rotavirus vaccine reduced the incidence of office visits by 86 percent, emergency department visits by 94 percent, and hospitalizations for rotavirus gastroenteritis by 96 percent. The efficacy of pentavalent rotavirus vaccine in the second rotavirus season after immunization was 63 percent against rotavirus gastroenteritis of any severity and 88 percent against severe rotavirus gastroenteritis. Neither breast-feeding nor concurrent administration of other childhood vaccines appears to diminish the efficacy of a three-dose series of pentavalent rotavirus vaccine.

Adverse Events

Safety with respect to intussusception was evaluated in 71,725 subjects enrolled in phase III efficacy trials.[65,471] For the prespecified 42-day post-immunization end-point, six cases of intussusception were observed in the pentavalent rotavirus vaccine group versus five cases of intussusception in the placebo group (multiplicity-adjusted relative risk, 1.6). The data did not suggest an increased risk of development of intussusception relative to placebo. Among vaccine recipients, no confirmed cases of intussusception occurred within the 42-day period after administration of the first dose, which was the period of highest risk for the previously licensed RRV-TV vaccine. In addition, no evidence of clustering of cases of intussusception was observed within a 7- or 14-day window after immunization for any dose. For the 1-year follow-up period after administration of the first dose, 13 cases of intussusception were observed in the pentavalent rotavirus vaccine group versus 15 cases of intussusception in the placebo group (multiplicity-adjusted relative risk, 0.9).

Among pentavalent rotavirus vaccine and placebo recipients, the incidence of serious adverse events, including deaths, was similar. In the 7-day period after immunization, vaccinees had a small but significantly greater rate of diarrhea, with an excess of 1 percent after dose 1 (10% versus 9%, respectively), 3 percent after dose 2 (9% versus 6%, respectively), and 3 percent after any dose (18% versus 15%, respectively). Similarly, vaccinees had a small but significantly greater rate of vomiting, with an excess of 2 percent after dose 1 (7% versus 5%, respectively) and 2 percent after any dose (12% versus 10%, respectively). The incidence of fever and irritability during the 7-day period after receipt of any vaccine dose was similar among pentavalent rotavirus vaccine and placebo recipients. Among preterm infants given pentavalent rotavirus vaccine and placebo, the incidence of serious adverse events (5.5% versus 5.8%, respectively) was similar.

Vaccine virus was shed in 8.9 percent subjects after receiving dose 1, in no subjects after dose 2, and in 0.3 percent of subjects after dose 3. Shedding was observed as early as 1 day and as late as 15 days after a dose. The potential for transmission of vaccine virus was not assessed through epidemiologic studies.

Indications

Infants should receive three doses of pentavalent rotavirus vaccine administered orally at 2, 4, and 6 months of age (http://www.cdc.gov/vaccines/recs/provisional/downloads/roto-7-1-08-508.pdf) or 2 doses of the human rotavirus vaccine at 2 and 4 months of age.[37,383] The first dose should be administered when the child is between 6 and 14 weeks of age (i.e., on or before 14 weeks, 0 days of age). Subsequent doses should be administered at a minimum of 4-week intervals, and all doses of vaccine should be administered by the time the child is 32 weeks of age (i.e., on or before 32 weeks, 0 days).

Immunization should not be initiated for infants older than 14 weeks because of insufficient data on safety of the first dose of rotavirus vaccine in older infants. Vaccine should not be administered after 8 months of age because of insufficient data on the safety and efficacy of rotavirus vaccine in infants after this age. For infants in whom the first dose of rotavirus vaccine is inadvertently administered off-label at 15 weeks or older, the rest of the rotavirus immunization series should be completed as per the schedule defined earlier, because timing of the first dose should not affect the safety and efficacy of the second and third doses.

Infants documented to have had rotavirus gastroenteritis before receiving the full course of rotavirus immunizations should

still start or complete the three-dose schedule because the initial infection frequently provides only partial immunity.

Infants who are being breast-fed can receive rotavirus vaccine. Like other childhood vaccines, rotavirus vaccine can be administered to infants with transient, mild illnesses, with or without low-grade fever.[308] Rotavirus vaccine can be administered together with DTaP, Hib, IPV, hepatitis B, and pneumococcal conjugate vaccines.

The AAP and ACIP support immunization of preterm infants under the following conditions: the infant is at least 6 weeks of age, the infant is clinically stable, and the first dose of vaccine is given at the time of discharge or after the infant has been discharged from the hospital nursery.

Infants living in households with persons who have or are suspected of having an immunodeficiency disorder or impaired immune status or with a pregnant woman can be immunized. To minimize potential virus transmission, all members of the household should employ measures such as good handwashing after contact with the feces of the immunized infant (e.g., after changing a diaper) for at least 1 week after the first dose of rotavirus vaccine has been given.

An infant who regurgitates, spits out, or vomits during or after receiving a dose of rotavirus vaccine should not have that dose readministered. The infant can receive the remaining recommended doses of rotavirus vaccine at appropriate intervals. If a recently immunized child is hospitalized for any reason, no precautions other than standard precautions need be taken to prevent the spread of vaccine virus in the hospital setting.

Contraindications and Precautions

Rotavirus vaccine should not be administered to infants who have severe hypersensitivity to any component of the vaccine or those individuals who have experienced a serious allergic reaction to a previous dose of rotavirus vaccine.[37,383] The human rotavirus vaccine oral applicator contains latex rubber. Pentavalent rotavirus vaccine may be preferred for children at high risk for acquiring latex sensitization.

Practitioners should consider the potential risks and benefits of administering rotavirus vaccine to infants with known or suspected altered immunocompetence. Children and adults who are immunocompromised because of congenital immunodeficiency, bone marrow transplantation, or solid organ transplantation sometimes experience severe, prolonged, and even fatal rotavirus gastroenteritis. However, no safety or efficacy data are available for the administration of rotavirus vaccine to infants who are potentially immunocompromised.

Rotavirus vaccine should not be administered to infants with acute, moderate to severe gastroenteritis until the condition improves. Rotavirus vaccine has not been studied among infants with concurrent acute gastroenteritis, among whom its immunogenicity and efficacy theoretically can be compromised. Infants with moderate to severe illness should be immunized as soon as they have recovered from the acute phase of the illness.

Infants with preexisting chronic gastrointestinal conditions and who are not undergoing immunosuppressive therapy should benefit from rotavirus vaccine immunization, and the benefits outweigh the theoretical risks. However, the safety and efficacy of rotavirus vaccine have not been established for infants with these preexisting conditions (e.g., congenital malabsorption syndromes, Hirschsprung disease, short-gut syndrome, or persistent vomiting of unknown cause).

After administration of a previously licensed rotavirus vaccine (RRV-TV), an increased risk of intussusception was observed. Available pre-licensure data from a large trial of 70,000 infants show no evidence of an association between intussusception and pentavalent rotavirus vaccine. However, additional post-licensure surveillance data are required to confirm that the vaccine is not associated with intussusception at a lower rate than what would have been detected in pre-licensure trials. In addition, some data suggest that infants with a history of intussusception may be at higher risk of having a repeat episode than are other infants. Therefore, until post-licensure data on safety of rotavirus vaccine are available, the risks and benefits of immunization should be considered when immunizing infants with a previous episode of intussusception.

RUBELLA VACCINE

Rubella is a viral disease that usually is manifested as a mild febrile rash illness in adults and children; however, 20 to 50 percent of infected persons are asymptomatic. Rubella can have severe adverse effects on the fetuses of pregnant women who contract the disease during the first trimester of pregnancy; it causes a wide range of congenital defects known as congenital rubella syndrome (CRS). The primary objective of the rubella vaccination program is to prevent intrauterine rubella infection. The primary strategies for rubella control in the United States are universal childhood vaccination, prenatal screening of pregnant women for rubella immunity, and vaccination of rubella-susceptible women post partum.

In the pre-vaccine era, epidemics of rubella occurred every 6 to 9 years, with the last major epidemic in the United States taking place in 1964 to 1965. The incidence of reported cases of rubella fell sharply after routine rubella immunization of young children was initiated in the United States in 1969. From the estimated 2 million cases per year in the pre-vaccine era, fewer than 1000 cases were reported in 1983. The incidence of rubella continued to fall during the 1980s and 1990s, although clusters of disease occurred among groups of susceptible individuals, including people with religious or philosophic exemptions to immunization. Although rubella had been a disease of childhood, the proportion of remaining cases among people 20 years of age and older increased to 79 percent in 1998.[476] Sustained implementation of the rubella vaccination program resulted in a marked decrease in incidence among all age groups. Since the mid-1990s, most reported cases of rubella have occurred among foreign-born young adults (particularly from Latin America) who were born in countries without routine rubella immunization programs.[196,402]

In October of 2004, 35 years after initiation of the rubella immunization program, an independent panel of international experts was convened by the CDC to assess progress toward elimination of rubella and CRS. Based on data showing fewer than 25 cases of rubella reported each year since 2001, at least 95 percent vaccination coverage among school-age children, an estimated 91 percent population immunity, adequate surveillance to detect rubella outbreaks, and a pattern of virus genotypes consistent with virus originating in other parts of the world, panel members concluded unanimously that rubella no longer is endemic in the United States.[140]

Rubella continues to be endemic in many parts of the world. Internationally imported rubella cases may give rise to indigenous transmission. The United States will need to continue its vigilance against rubella and CRS by the following means: (1) maintaining high vaccination rates among children; (2) ensuring vaccination among women of child-bearing age, especially women born outside the United States; (3) continuing surveillance of both rubella and CRS; and (4) responding rapidly to any outbreak.[140]

Preparations

Since 1979, RA 27/3 (rubella abortus, 27th specimen/third extract) vaccine, prepared in human diploid tissue culture, has

been the only vaccine available in the United States; it replaced the earlier HPV-77 and Cendehill vaccines. RA 27/3 induces higher antibody titers and more closely parallels the immune response after natural infection than did previous vaccines.[320,378] In addition to MMR vaccine, monovalent rubella and measles-rubella and MMRV vaccines are available. MMR and MMRV vaccines generally are used for routine infant immunization programs. Rubella vaccine should be kept at 2° C to 8° C (35.6° F to 46.4° F) or colder during storage and should be protected from light to avoid inactivation of the virus. Once reconstituted, the vaccine should be used within 8 hours.

Immunogenicity and Efficacy

At least 98 percent of susceptible vaccinees aged 12 months or older develop antibody titers that are protective.[356] Vaccine-induced rubella antibodies have persisted in more than 90 percent of vaccinees aged 16 years old after receiving the RA 27/3 vaccine.[167] Lifelong protection against clinical re-infection, asymptomatic viremia, or both, usually results from a single dose of vaccine given early in childhood.

In some cases, vaccinees exposed to natural rubella developed a rise in antibody titer unassociated with clinical symptoms. Re-infection is associated only rarely with viremia. Significant pharyngeal shedding also is observed infrequently. Person-to-person transmission, however, has not been reported. Re-infection caused by wild-type rubella virus also may be observed in individuals with previous natural rubella. The risk of CRS developing from rubella re-infection during pregnancy is extremely low.[409]

Adverse Events

Rubella vaccines generally are well tolerated. The most frequent complaints after vaccination are fever, lymphadenopathy, or rash, which occur in 5 to 15 percent of children 5 to 12 days after receiving vaccination.[20] Transient peripheral neuritis (paresthesia and pain in the arms and legs) has been observed uncommonly, primarily in older age groups.[423]

Approximately 3 percent of children have transient joint manifestations, including arthralgia and, less commonly, arthritis 1 to 3 weeks after being immunized. Although 25 percent of women report having joint pain after being vaccinated, arthritis with objective clinical findings lasting less than 10 days occurs in 13 to 15 percent. Cases of persistent or recurrent joint symptoms have been reported but are rare events. In 1992, the IOM reviewed the existing data on rubella and adverse joint events and concluded that the evidence available was consistent with a causal relationship between rubella vaccination and chronic arthritis in women, although data on current vaccine strains are limited.[272] The incidence of joint manifestations after immunization is lower than that after natural infection at the corresponding age.

Rubella re-vaccination is well tolerated, even among college-age and older vaccinees, and is associated with a much lower incidence of adverse reactions than is primary rubella immunization of young adult populations. Reported rates of joint-related complaints of 4 to 18 percent after re-vaccination are lower than those reported after primary vaccination.[163,426]

Indications

Live virus rubella vaccine generally is recommended for all children aged 12 months or older; it is given as MMR or MMRV when children are 12 to 15 months of age.[20,111,129] A second dose of rubella vaccine administered as MMR or MMRV is given at the time they enter school, usually 4 to 6 years of age, according to recommendations for routine measles immunization. The vaccine should be provided to previously unimmunized preschool-age children or older schoolchildren despite a history of having clinical rubella, unless serologic tests confirm immunity.

Emphasis should be placed on the immunization of the postpubertal male and female population, especially college students and those in the military. Rubella vaccine also should be administered to adolescent girls and women of child-bearing age who lack a history of previous vaccination. Other opportunities for immunization include premarital screening, routine gynecologic examinations, visits for newborn infants and well-child care, or other medical visits. The immediate postpartum period also is an excellent time for giving immunizations. Rubella vaccine may be given after administration of anti-Rho(D) immune globulin, but serologic testing to determine whether seroconversion has occurred should be performed at least 8 weeks after vaccination. When practical, potential vaccinees may be screened for susceptibility. However, vaccination of girls and women of child-bearing age is justifiable, and may be preferable, without previous serologic testing in women not known to be pregnant.

Adults in the United States who were born in countries where rubella vaccination was not offered are at higher risk for contracting rubella and having infants with CRS. Health care practitioners who treat foreign-born adults should document the rubella immunity of these patients with a written record of rubella-containing vaccine or by serologic testing. Susceptible adults, especially women of child-bearing age, should be vaccinated. During rubella outbreaks, all susceptible persons who have no contraindications to rubella vaccine should be identified and vaccinated.

Precautions and Contraindications

Specific contraindications to administration of live rubella vaccine include the following: (1) pregnancy; (2) severe febrile illness; (3) known history of anaphylactic reaction to rubella vaccine, gelatin, or neomycin, which are contained in the vaccine; and (4) immunodeficiency conditions (i.e., malignancy, primary immunodeficiency disease, immunosuppressive or corticosteroid therapy, and radiation therapy).[20,111,129]

Persons with mild immunosuppression, such as those with asymptomatic HIV infection or those taking short-term or low-dose corticosteroids, may be vaccinated.

Postpubertal women of child-bearing age who are known to be pregnant or who are attempting to become pregnant should not be vaccinated. Vaccinated women should be counseled about the need to avoid pregnancy for 28 days after receiving vaccination.[130] Although pregnancy is a contraindication to administering rubella vaccination, the maximal theoretical risk to the fetus is estimated to be 1.6 percent. From 1979 until 1989, the CDC registered 321 susceptible women who inadvertently had received RA 27/3 rubella vaccine within 3 months before or after conception and carried their pregnancies to term. None of their infants had defects compatible with CRS, although 2 percent had serologic evidence of intrauterine infection.[86] Because rubella virus has been isolated from the products of conception of women vaccinated during pregnancy, continued caution with respect to vaccination during pregnancy is advised. However, the evidence available indicates that rubella vaccination inadvertently given during pregnancy ordinarily does not represent a reason to consider interruption of pregnancy.

Although vaccine virus may be isolated from the pharynx, vaccinees do not transmit rubella to others, except in the case of a vaccinated breast-feeding mother. In this situation, the infant may be infected through breast milk and a mild rash illness may develop, but serious adverse effects have not been noted. Infants infected through breast-feeding respond normally to rubella vaccination at 15 months of age. Breast-feeding is not a contraindication to receiving rubella vaccination.

Concern about potential transmission of disease from immunized children to susceptible contacts, including pregnant women,

has not been supported by studies of susceptible household contacts. Therefore, susceptible children whose household contacts are pregnant may be vaccinated.

Persons with a history of thrombocytopenia may experience thrombocytopenia after receipt of MMR vaccine. The decision to vaccinate should depend on the benefits of immunity versus the risk for recurrence or exacerbation of thrombocytopenia, either after vaccination or during natural infection with measles or rubella.

Rubella vaccine should not be given during an interval beginning 2 weeks before and extending 3 months after the administration of immune globulin or blood transfusion. Because rubella vaccine usually is given as MMR or MMRV, and evidence suggests that high doses of immune globulin preparations can inhibit the immune response to measles vaccine for 3 or more months, depending on the dosage, rubella vaccination with MMR or MMRV necessitates deferral for longer periods (see "Measles Vaccine").[20,111,435]

TETANUS TOXOID

The efficacy of active immunization against tetanus was demonstrated most dramatically in military personnel during World War II, when tetanus toxoid virtually eliminated tetanus in injured soldiers.[327] Since the 1940s, routine immunization of civilians in this country with tetanus toxoid has been successful in nearly eliminating tetanus. In almost all cases, disease has been reported in unimmunized or inadequately immunized individuals.[87] The primary three-dose series of a tetanus toxoid-containing vaccine confers protective immunity for 10 years or longer.[71,307]

Without a tetanus booster, immunity wanes over time. The potential for occurrence of tetanus is indicated by the significant number of adults in the United States who lack protective concentrations of serum antibody.[226] Although the incidence of disease is exceptionally low (a total of 624 cases of tetanus were reported in the United States between 1990 and 2004), the case-fatality rate was 18 percent.[384,443]

Neonatal tetanus also has been nearly eliminated in the United States. However it is a leading cause of morbidity in newborns in developing countries. As a result, global elimination of neonatal tetanus remains a goal of the WHO.[96]

Preparations

Tetanus toxoid is prepared by formaldehyde treatment of *Clostridium tetani* toxin.[71,307] It has been prepared in both fluid and aluminum salt-adsorbed preparations, but in the United States, only the latter is available. Fluid toxoid preparations result in a significantly shorter duration of immunity than that induced by aluminum-adsorbed antigens; therefore, adsorbed antigens are recommended.

Tetanus toxoid is available in combination with diphtheria toxoid and acellular pertussis vaccine (DTaP) for routine administration to infants and children younger than 7 years of age and for adolescents and adults aged 11 to 64 years old as a single booster (Tdap). For all persons in whom pertussis vaccine is contraindicated, tetanus toxoid is combined with diphtheria toxoid as either DT or Td (see "Diphtheria Toxoid"). The first two Tdap vaccines approved for use in adolescents and adults in the United States, Boostrix (GlaxoSmithKline Biologicals) and Adacel (Sanofi Pasteur), were licensed in 2005. Preparations of DT, Td, and Tdap are identical in the amounts of tetanus toxoid they contain, but they differ in the quantity of diphtheria toxoid. The dose of Tdap is the same as that of DT or Td, 0.5 mL administered intramuscularly.

Immunogenicity and Efficacy

Adequate primary immunization provides sufficient protective titers of antitoxin for at least 10 years and ensures prompt, anamnestic responses to subsequent booster injections. Immune response to tetanus toxoid was measured following administration of each adult Tdap vaccine and compared with that elaborated after Td vaccine. Seroprotective anti-tetanus antibody concentrations, defined as a titer 0.1 IU/mL or greater, and booster response rates to tetanus were determined to be noninferior after vaccination with Tdap as compared with Td vaccination.[71,307]

Adverse Events

Local reactions of pain, swelling, and induration can occur, but these reactions in children usually are attributable to the pertussis component contained in the vaccine rather than to response to tetanus toxoid. Hypersensitivity reactions can occur in adolescents and adults but very rarely are severe. Neurologic reactions occurring after the administration of tetanus toxoid are rare events. Such reactions include brachial neuritis and GBS.

Regarding adverse reactions reported following Tdap, vaccination with Boostrix was associated with a statistically higher rate of moderate to severe headache compared with Td, and Adacel was associated with higher rates of mild injection site pain and low-grade fever compared with Td vaccine. No serious adverse events have been reported for Boostrix. Two cases of serious adverse advents, both characterized as neuropathic reactions, in adults possibly related to having received Adacel (none reported in adolescents), have been reported, and in both cases symptoms resolved completely within several days.[71,307]

Indications

PRE-EXPOSURE

For primary immunization, doses of tetanus toxoid, provided as DTaP, should be administered when the child is 2, 4, and 6 months of age. [22,36,71] A fourth dose is given 6 to 12 months after the third dose (i.e., at 12 to 18 months of age) to maintain adequate serum antibody concentrations for the ensuing preschool years. For children younger than 7 years of age not immunized in infancy, DTaP is given at 0 (initial dose), 2, 4, and 10 to 16 months later, followed by a single booster dose at age 4 to 6 years, just before school entry.

In 2005, the FDA licensed two Tdap vaccines for routine use in adolescents and adults, Adacel (Sanofi Pasteur, approved for persons 11 to 64 years of age) and Boostrix (GlaxoSmithKline Biologicals, approved for adolescents 10 to 18 years of age). These vaccines are approved for one-time use only in those individuals who have previously completed their primary vaccination series with DTP or DTaP as a substitute for their next Td booster. The preferred age for Tdap vaccination is 11 to 12 years. Catch-up vaccination with Tdap is encouraged for adolescents 13 to 18 years old who completed their primary series with DTP or DTaP and received a Td booster at least 5 years previously. Although an interval of 5 years between Td and Tdap is recommended to reduce the likelihood of adverse reactions, periods shorter than 5 years can be used when increased risks of exposure to or complications from pertussis exist. Every 10 years thereafter, tetanus booster should be provided by Td. Adolescents between the ages of 11 and 18 years who have never been vaccinated against tetanus, diphtheria, or pertussis should receive Tdap initially, followed by Td for the subsequent two doses 4 or more weeks, and 6 to 12 months later. For children between the ages of 7 and 10 years who have not been vaccinated previously, three doses of Td are recommended at 0 (initial dose), 4 or more

weeks, and 6 to 12 months. The third dose may be delayed until the child is old enough to receive Tdap; otherwise, a booster with Tdap is recommended when these children become adolescents. Interruption of the recommended schedule or delay in administering subsequent doses during primary immunization does not reduce immunity.

ANTEPARTUM

In areas of the world where the risk of acquiring neonatal tetanus is significant, previously unimmunized, pregnant women should receive two antepartum doses of a tetanus toxoid-containing vaccine, properly spaced, and should complete the three-dose series subsequently.[13,307] Women immunized more than 10 years previously should receive a booster dose.

The ACIP and the AAP recommend for administration a single dose of Tdap vaccine in pregnancy to women between the ages of 11 and 64 years, including those breast-feeding who have not received Tdap vaccine previously.[22,36,307] If vaccination is not provided before delivery, women should receive a single dose of Tdap in the immediate postpartum period, preferably before being discharged from their hospital or birthing center.

POST-EXPOSURE: WOUND MANAGEMENT

The potential need for immunoprophylaxis is an integral aspect of wound management at the time of trauma or injury. The recommended use of tetanus toxoid in addition to tetanus immune globulin at the time of injury is given in Table 255–16. To ensure adequate immunity, children and adults receiving tetanus toxoid for wound management should be given age-appropriate preparations of vaccines containing diphtheria and pertussis, unless contraindicated, as well as tetanus toxoid.[22,36,307] Specific recommendations depend on the individual's immunization status, the nature of the wound, and the duration of time between when the injury occurred and evaluation and treatment were undertaken. After prophylaxis is provided, primary immunization should be completed subsequently in those lacking the recommended number of doses. This conservative approach to the frequent administration of booster doses of tetanus toxoid in wound management for previously immunized persons is supported by the prolonged immunity from tetanus vaccination and the increased incidence of hypersensitivity reactions associated with receipt of frequent booster injections.[387] Patients convalescing from tetanus infection should complete active immunization because infection often does not confer immunity.

TABLE 255–16 Recommended Tetanus Prophylaxis in Wound Management

History of Tetanus Toxoid (Number of Doses)	Clean, Minor Wounds		All Other Wounds*	
	Td or Tdap†	TIG	Td or Tdap†	TIG
<3 or unknown	Yes	No	Yes	Yes
≥3	No‡	No	No§	No

**Such as, but not limited to, the following: wounds contaminated with dirt, feces, soil, and saliva; puncture wounds; avulsions; and wounds resulting from missiles, crushing, burns, and frostbite.*

†For children younger than 7 years of age; diphtheria-tetanus, acellular pertussis (DTaP) or diphtheria-tetanus (DT) (depending on vaccine status of patient) is preferred to tetanus toxoid (TT) alone. Tdap is preferred to Td for adolescents who have never received Tdap. Td is preferred to TT for adolescents who received Tdap previously or when Tdap is not available.

‡Yes, if more than 10 years since last tetanus-containing vaccine dose.

§Yes, if more than 5 years since last tetanus-containing vaccine dose. More frequent boosters are not needed and can accentuate side effects.

Td, tetanus-diphtheria; Tdap, tetanus-diphtheria-acellular pertussis; TIG, tetanus immune globulin.

Precautions and Contraindications

A history of having an immediate, severe hypersensitivity reaction to tetanus toxoid-containing preparations that is severe or anaphylactic in type is a contraindication to receiving further vaccination.[22,36,71,307] Persons who experience Arthus-type hypersensitivity reactions after receiving tetanus toxoid usually have high serum tetanus antitoxin concentrations and should not be given doses of Td more frequently than every 10 years, even if they have a tetanus-prone wound. If an anaphylactic reaction to a previous dose of tetanus toxoid is suspected, intradermal skin testing may be helpful in determining whether to discontinue tetanus toxoid vaccination.[286] Because tetanus toxoid administration has been associated with recurrence of GBS in rare cases,[278] the decision to give additional doses in persons with a previous history of this syndrome within 6 weeks after receipt of tetanus toxoid should be based on consideration of the benefit of revaccination and the comparative risk of having a recurrence of GBS.[106] No physician-diagnosed cases of anaphylaxis or Arthus reactions or GBS in any adolescent or adult following either Tdap vaccine have been reported.

VARICELLA VACCINE

In the pre-vaccine era, varicella was endemic in the United States, and virtually all persons acquired varicella by the time they reached adulthood. Varicella infection was responsible for an estimated 4 million cases, 11,000 hospitalizations, and 100 deaths each year in the United States.[115] Approximately 90 percent of cases occurred in children, with the highest incidence in children 1 to 6 years old.

Since the introduction of varicella vaccine in 1995, the incidence of varicella has decreased as vaccination coverage has increased, and the number of hospitalizations and deaths from varicella has declined more than 90 percent.[144,427] Active surveillance for varicella has been conducted at sites in Pennsylvania, Texas, and California since 1995 in a CDC-sponsored study.[427] From 1995 to 2000, vaccine coverage in 1- to 2-year-old children rose to approximately 80 percent, whereas overall cases of varicella declined 70 to 80 percent. The greatest decline was in children 1 to 4 years of age, but the number of cases also declined in all other age groups, including infants younger than 1 year old and adults, thus suggesting herd immunity. Decreasing rates of varicella also have been associated with increasing use of varicella vaccine in a daycare center population.[170] Varicella vaccine coverage among 19- to 35-month-old children was estimated by the National Immunization Survey to be 88 percent in 2005.[152]

Despite the high one-dose vaccination coverage and the success of the vaccination program in reducing varicella morbidity and the mortality rate, varicella surveillance indicates that the number of reported varicella cases appears to have plateaued. Increasing proportions of cases represent breakthrough infection (chickenpox occurring in a previously vaccinated person). In 2001 to 2005, outbreaks were reported in schools with 96 to 100 percent varicella vaccination coverage.[208,328,460] These outbreaks had many similarities: all occurred in elementary schools; vaccine effectiveness was within the expected range of 72 to 85 percent; the highest attack rates occurred among the younger students; each outbreak lasted approximately 2 months; and persons with breakthrough infection transmitted the virus, although the breakthrough disease was mild. Overall attack rates among vaccinated children were 11 to 17 percent, with attack rates in some classrooms as high as 40 percent. These data indicate that even in settings where almost everyone was vaccinated and vaccine performed as expected, varicella outbreaks could not be prevented with the current one-dose vaccination policy. Although varicella typically was mild, the outbreaks lasted for several months and

were challenging and costly for health departments to control.[155] These observations led to the recommendation in 2006 for a second routine dose of varicella vaccine.

In June of 2005, the ACIP provisionally recommended a second dose of varicella vaccine in outbreak settings for persons who have had only one dose of varicella vaccine and no disease history (provided an appropriate interval has elapsed since the first dose).[154] On the basis of a 10-year follow-up pre-licensure study of the vaccine, a two-dose vaccination regimen has been determined to be more effective than a one-dose regimen.[313] In a 2006 position statement, the Council of State and Territorial Epidemiologists supported a routine two-dose varicella vaccination policy to improve varicella control and outbreak prevention. In June of 2006, ACIP approved a routine two-dose varicella vaccination policy for children (first dose at 12 to 15 months, second dose at 4 to 6 years) and catch-up vaccinations for children, adolescents, and adults who previously had received only one dose.[154] Establishing a routine two-dose vaccination regimen may make the two-dose outbreak response for susceptible populations more feasible to implement.

Preparations

Three varicella-containing vaccines are now approved for use in the United States: varicella vaccine (Varivax), combination MMRV vaccine (ProQuad), and herpes zoster vaccine (Zostavax). Varicella vaccine was licensed in the United States in 1995. It is a preparation of the Oka strain of varicella-zoster virus (VZV) obtained from the vesicle fluid of a healthy child with varicella that has been attenuated by serial propagation in human embryo lung fibroblasts, guinea pig embryonic cells, and human diploid cell cultures. The vaccine contains trace amounts of neomycin, fetal bovine serum, sucrose, residual components of human diploid (MRC-5) cells, and gelatin. The vaccine does not contain preservatives.

Varicella vaccine is lyophilized and stored frozen at –15° C or colder until reconstituted. Any freezer that reliably maintains an average temperature of –15° C and has a separate sealed freezer door is acceptable for storing vaccine. The vaccine also may be stored at refrigerator temperature (2° C to 8° C) for as long as 72 hours before reconstitution. Vaccine stored at 2° C to 8° C that is not used within 72 hours should be discarded. Reconstituted vaccine should be stored at room temperature and discarded if it is not used within 30 minutes.

In September of 2005, a combined live attenuated MMRV vaccine was licensed for use in persons aged 12 months through 12 years.[145] The attenuated measles, mumps, and rubella vaccine viruses in MMRV are identical and of equal titer to those in the MMR vaccine. The titer of Oka/Merck VZV is higher in MMRV vaccine than in single-antigen varicella vaccine. Each 0.5-mL dose contains a small quantity of hydrolyzed gelatin, human albumin, residual components of MRC-5 cells, neomycin, bovine calf serum, and other buffer and media ingredients. Unlike single-antigen varicella vaccine, MMRV vaccine cannot be stored at refrigerator temperature. MMRV vaccine must be stored frozen at an average temperature of –15° C or lower for up to 18 months. Once reconstituted, the vaccine should be used immediately to minimize loss of potency and should be discarded if it is not used within 30 minutes. The diluent should be stored separately at room temperature or in the refrigerator. MMRV vaccine contains no preservative. In May of 2006, the FDA approved herpes zoster vaccine for use in persons 60 years of age and older. No indications for use of this vaccine in children exist.

Immunogenicity and Efficacy

Varicella vaccine is highly immunogenic in susceptible children. Seroconversion has occurred in more than 96 percent of children aged 12 months to 12 years after one dose of vaccine.[495] Preexisting antibody, if present at 12 months of age, does not appear to interfere with antibody response. As with other viral vaccines, the antibody response after immunization is lower than that from natural disease. Adolescents and adults have age-related decreases in the ability to develop a primary response to varicella virus.[228] Seroconversion rates of 78 to 82 percent after one dose and 99 percent after two doses have been reported in those older than 12 years.[228,495]

In ongoing studies in the United States and Japan, serum antibodies to varicella have been detected for as long as 10 to 20 years after immunization in more than 95 percent of immunized children.[46,290] Antibody concentrations have persisted for at least 1 year in 97 percent of adults and adolescents who were administered two doses of vaccine 4 to 8 weeks apart.[228] Cell-mediated immunity to VZV has been detected in 87 percent of children and 94 percent of adults 5 years after vaccination.[512]

MMRV vaccine was licensed on the basis of equivalence of immunogenicity of the antigenic components, rather than clinical efficacy. Clinical studies involving healthy children aged 12 to 23 months indicated that those who received a single dose of MMRV vaccine developed levels of antibody to measles, mumps, rubella, and varicella similar to those of children who received MMR and varicella vaccines concomitantly at separate injection sites.[312]

Varicella vaccine has been demonstrated to be highly effective in preventing varicella in children and in reducing the severity of infection if they do become infected. In pre-licensure clinical trials, vaccine was 70 to 90 percent effective in preventing varicella and more than 95 percent effective in preventing severe disease.[256,314,373,487] Several post-licensure studies have shown similar results, with vaccine effectiveness ranging from 83 to 100 percent in preventing varicella and 87 to 100 percent in preventing severe disease.[172,285,466] In follow-up studies, chickenpox has developed in approximately 0.2 to 2.3 percent of vaccinated children per year after exposure to wild-type varicella virus, a rate that does not seem to increase with length of time after immunization.[472] These vaccine failure cases are mild, with fewer skin lesions, lower rates of fever, and faster recovery, and they are less contagious than are moderate to severe cases of varicella.[59,428,472,483] These infections have been termed *breakthrough varicella*.

Although findings of some studies have suggested otherwise, most investigations have not identified time since vaccination as a risk factor for breakthrough varicella. Some, but not all, investigations have identified the presence of asthma, use of steroids, and vaccination at younger than 15 months of age as risk factors for breakthrough varicella.[208,225,285,460,470] Breakthrough varicella infection could be a result of several factors, including interference of vaccine virus replication by circulating antibody, impotent vaccine resulting from storage or handling errors, or inaccurate record keeping. Interference from live viral vaccine administered before varicella vaccine could also reduce vaccine effectiveness. A study of 115,000 children in two health maintenance organizations during 1995 to 1999 found that children who received varicella vaccine less than 30 days after MMR vaccination had a 2.5-fold increased risk of breakthrough varicella compared with those who received varicella vaccine before, simultaneously with, or more than 30 days after receiving MMR.[470] Inactivated vaccines (DTaP, Hib, IPV, and hepatitis B) and OPV did not increase the risk of breakthrough varicella if they were administered less than 30 days before administration of varicella vaccine.

In adults and adolescents who have seroconverted, varicella vaccine provides protective efficacy rates of approximately 70 percent after household exposure. In the remaining 30 percent, attenuated disease with fewer skin lesions and little or no systemic toxicity develops, as in children.[228]

Adverse Events

Varicella vaccine produces relatively few adverse reactions.[430,499] The most common adverse reactions that occur after receipt of varicella vaccine are local reactions, such as pain, soreness, erythema, and swelling. Based on information from the manufacturer's clinical trials of varicella vaccine, local reactions after receiving the first dose are reported by 19 percent of children and by 24 percent of adolescents and adults.[430,499] These local adverse reactions generally are mild and self-limited.

A varicella-like rash at the site of injection is reported by 3 percent of children and by 1 percent of adolescents and adults following receipt of the second dose. In both circumstances, a median of two lesions has been present. These lesions generally occur within 2 weeks and usually are maculopapular rather than vesicular.[430,499]

In post-licensure studies, the adverse event most frequently reported is a mild vesicular rash that occurs in approximately 5 percent of vaccinees.[115,227] Most of these generalized rashes occur within 3 weeks, and most are maculopapular. In one study, vesicular rashes that occurred within 2 weeks of vaccination were more likely to be caused by wild-type varicella, whereas rashes that occurred more than 2 weeks after vaccination were more likely to be caused by the Oka vaccine strain.[430]

Systemic reactions are not common. Fever within 42 days of vaccination is reported by 15 percent of children and 10 percent of adolescents and adults. Most of these episodes of fever have been attributed to concurrent illness rather than to the vaccine.

Clinical trials of MMRV that compared events that occurred within 42 days of receiving either MMRV or MMR and varicella vaccine separately in different anatomic sites found the frequencies of local reactions and generalized varicella-like rash similar to those described for varicella vaccine.[312] A temperature of 102° F or higher within 42 days of vaccination was more common in the MMRV group (22%) than in the group that received MMR and varicella vaccine at different sites (15%). A measles-like rash also occurred more frequently in MMRV recipients (3%) than in the group receiving separate injections (2%). Both fever and measles-like rash usually occurred 5 to 12 days after receipt of vaccination. In a postlicensure study, an increased risk of seizure was seen in children 12 to 23 months of age receiving MMRV vaccine when compared with those who received MMR and varicella vaccines given separately in the 7- to 10-day post-vaccination period.[159a]

Varicella vaccine is a live virus vaccine and may result in a latent infection, similar to that caused by wild-type varicella virus. Consequently, zoster caused by the vaccine virus has been reported, mostly among vaccinated children. Based on reports to VAERS, the rate of herpes zoster after varicella vaccination is 2.6 per 100,000 vaccine doses distributed.[115] The incidence of herpes zoster after natural varicella infection in healthy persons younger than 20 years is 68 per 100,000 person-years[244] and, for all ages, 215 per 100,000 person-years.[204] However, these rates should be compared cautiously because the latter rates are based on populations monitored for longer periods than the vaccinees were. Cases of herpes zoster have been confirmed by polymerase chain reaction (PCR) to be caused by both vaccine virus and wild-type virus, a finding thus suggesting that some herpes zoster cases in vaccinees may result from antecedent natural varicella infection.[115,253] Most cases of herpes zoster that occur after administration of vaccine have been mild and have not been associated with complications such as postherpetic neuralgia.

Transmission of the vaccine virus occurs rarely and most often from immunocompromised vaccinees. Of the 15 million doses of varicella vaccine distributed, on only 3 occasions has transmission from immunocompetent persons been documented by PCR analysis.[317,421] All these cases resulted in mild disease without complications. In one case, a child 12 months old transmitted the vaccine virus to his pregnant mother.[326,421] The mother elected to terminate the pregnancy, but fetal tissue tested by PCR was negative for varicella vaccine virus. The other two documented cases involved transmission from healthy children aged 1 year to a healthy sibling aged 4 1/2 months and to a healthy father.[115] Transmission also has occurred from a person with herpes zoster caused by vaccine strain virus.[74] Transmission has not been documented in the absence of a vesicular rash after vaccination. No evidence indicates reversion to virulence of the vaccine strain during transmission; siblings of leukemic vaccine recipients who acquired vaccine virus had mild rash in 75 percent of cases and symptomless seroconversion in 25 percent.[43]

Indications

Varicella vaccine is licensed for use in individuals aged 12 months or older who have not had varicella.[24,34,115,159] The initial dose of varicella vaccine is recommended at 12 to 15 months of age. In June of 2006, the ACIP voted to recommend a routine second dose of varicella vaccine.[159] The second dose should be administered at 4 through 6 years of age, at the same visit during which the second dose of MMR vaccine is given. The second dose may be administered earlier than 4 through 6 years of age if at least 3 months have elapsed following the first dose (i.e., the minimum interval between doses of varicella vaccine for children <13 years is 3 months). However, if the second dose is administered at least 28 days following the first dose, the second dose does not need to be repeated.

A second dose of varicella vaccine also is recommended for persons older than 4 through 6 years of age who have received only one dose. Varicella vaccine doses administered to persons younger than 13 years should be separated by at least 3 months.

Varicella vaccine also is recommended for all children without evidence of varicella immunity by their 13th birthday. Children who have not been vaccinated previously and who do not have a reliable history of chickenpox are considered susceptible. Efforts should be made to ensure varicella immunity by age 13 years, because after this age varicella disease is more severe and complications are more frequent.

All varicella-containing vaccines should be administered by the subcutaneous route. Varicella vaccine has been shown to be safe and effective in healthy children when administered at the same time as MMR vaccine at separate sites and with separate syringes. If varicella and MMR vaccines are not administered at the same visit, they should be separated by at least 28 days. Varicella vaccine also may be administered simultaneously (but at separate sites with separate syringes) with all other childhood vaccines. The ACIP strongly recommends that varicella vaccine be administered simultaneously with all other vaccines recommended at 12 through 15 months of age.

Children with a clinician-diagnosed or verified history of typical chickenpox can be assumed to be immune to varicella. Serologic testing of such children before vaccination is not warranted because most children between 12 months and 12 years of age without a clinical history of chickenpox are not immune. Prior history of chickenpox is not a contraindication to receiving varicella vaccination.

Varicella vaccine should be administered to all adolescents and adults 13 years of age and older who do not have evidence of varicella immunity. Persons aged 13 years and older should receive two doses of varicella vaccine with administration of the doses separated by at least 4 weeks. If a lapse of more than 4 weeks occurs after the first dose, the second dose may be administered at any time without repeating the first dose. Assessment of varicella immunity in all adolescents and adults and vaccination of those who lack evidence of varicella immunity is desirable to

protect these individuals from the higher risk of developing complications from acquired varicella. Vaccination may be offered at the time of routine health care visits. However, specific assessment efforts should be focused on adolescents and adults who are at highest risk of exposure and those most likely to transmit varicella to others.

The ACIP recommends that all health care personnel be immune to varicella. In health care settings, serologic screening of personnel who are uncertain of their varicella history, or who claim not to have had the disease, is likely to be cost-effective. Testing for varicella immunity after administration of two doses of vaccine is not necessary because 99 percent of persons are seropositive after the second dose. Moreover, available commercial assays are not sufficiently sensitive to detect antibody after receipt of vaccination in all instances.

Seroconversion does not always result in full protection against disease, although no data regarding correlates of protection are available for adults. If a vaccinated health care provider is exposed to VZV, the employee should be monitored daily (screen for fever, skin lesions, and systemic symptoms) from day 10 to day 21 after exposure through the employee health or infection control program to determine clinical status. Persons with varicella may be infectious starting 2 days before onset of rash. In addition, the health care worker should be instructed to report fever, headache, or other constitutional symptoms and any skin lesions (which may be atypical) immediately. The person should be placed on sick leave immediately if symptoms occur. The risk of transmission of vaccine virus from a vaccinated person to a susceptible contact appears to be very low, and the benefits of vaccinating susceptible health care providers clearly outweigh this potential risk. Transmission of vaccine virus appears to occur primarily if and when the vaccine develops a vaccine-associated rash. As a safeguard, institutions may wish to consider protocols for personnel who develop a rash following receipt of vaccination (e.g., avoidance of contact with persons at high risk of developing serious complications, such as immunosuppressed persons who do not have evidence of varicella immunity).

MMRV vaccine is indicated for vaccination against measles, mumps, rubella, and varicella in children 12 months through 12 years of age.[145] Persons 13 years of age and older should not receive MMRV. When used, MMRV vaccine should be administered on or after the first birthday, preferably as soon as the child becomes eligible for vaccination. MMRV may be used for both the first and second doses of MMR and varicella in children younger than 13 years. The minimum interval between doses of MMRV is 3 months. However, if the second dose is administered at least 28 days after the first dose, the second dose does not need to be repeated.

POST-EXPOSURE PROPHYLAXIS

Data from the United States and Japan in a variety of settings indicate that varicella vaccine is 70 to 100 percent effective in preventing illness or modifying the severity of illness if it is used within 3 days, and possibly up to 5 days, after exposure.[420,482] The ACIP recommends the vaccine for use in persons who do not have evidence of varicella immunity following exposure to varicella. If exposure to varicella does not cause infection, post-exposure vaccination should induce protection against subsequent exposure. If the exposure results in infection, no evidence indicates that administration of varicella vaccine during the incubation period or prodromal stage of illness increases the risk of vaccine-associated adverse reactions. Although post-exposure use of varicella vaccine has potential applications in hospital settings, pre-exposure vaccination of all health care workers without evidence of varicella immunity is the recommended and preferred method for preventing varicella in health care settings.

Varicella outbreaks in some settings (e.g., childcare facilities and schools) can persist up to 6 months. Varicella vaccine has been used successfully to control these outbreaks. The ACIP recommends a second dose of varicella vaccine for outbreak control. During a varicella outbreak, persons who have received one dose of varicella vaccine should receive a second dose, provided the appropriate vaccination interval has elapsed since the first dose (3 months for persons 12 months to 12 years old and at ≥4 weeks for persons ≥13 years of age).

In 2006, the ACIP approved a revised definition for evidence of immunity to varicella.[159] Revised criteria for evidence of immunity to varicella includes any of the following:

- Documentation of age-appropriate vaccination
  - Preschool-age children 12 months of age and older: one dose
  - School-age children, adolescents, and adults: two doses
  - Laboratory evidence of immunity or laboratory confirmation of disease
  - Born in the United States before 1980
- Diagnosis of varicella or verification of history of varicella disease by a health care provider
- History of herpes zoster based on health care provider diagnosis.

Varicella vaccine is not licensed for use in persons who have blood dyscrasias, leukemia, lymphoma of any type, or other malignant neoplasms affecting the bone marrow or lymphatic systems. According to current ACIP and AAP recommendations, varicella vaccine should not be administered to persons who have cellular immunodeficiencies, but persons with impaired humoral immunity may be vaccinated.[115]

HIV-infected children 12 months of age and older who are in CDC clinical class N, A, or B with $CD4^+$ T-lymphocyte counts of 15 percent or greater and without evidence of varicella immunity should receive two doses of single-antigen varicella vaccine at a minimum interval of 3 months. Varicella vaccine was recommended previously for asymptomatic or mildly symptomatic HIV-infected children (CDC clinical class N and A) with age-specific $CD4^+$ T-lymphocyte counts 25 percent or greater (http://www.cdc.gov/mmwr/PDF/rr/rr4806.pdf).[115] Because data are not available on safety, immunogenicity, or efficacy of MMRV vaccine in HIV-infected children, MMRV vaccine should not be administered as a substitute for the component vaccines when vaccinating HIV-infected children.

Women should be assessed prenatally for evidence of varicella immunity. On completion or termination of their pregnancies, women who do not have evidence of varicella immunity should receive the first dose of varicella vaccine before discharge from the health care facility. The second dose should be administered 4 to 8 weeks later (at the postpartum or other health care visit). To ensure administration of varicella vaccine, standing orders are recommended for health care settings where completion or termination of pregnancy occurs.

Precautions and Contraindications

Varicella-containing vaccines are contraindicated in the following situations: (1) pregnancy, (2) severe febrile illness, (3) known history of anaphylactic reaction to vaccine components, and (4) immunodeficiency states (malignancy, primary immunodeficiency disease, immunosuppressive or corticosteroid therapy, and radiation therapy).[24,34,104,115]

Immunocompromised patients with conditions such as lymphoreticular or other generalized malignancy and primary or secondary immunodeficiency conditions should not be given live attenuated varicella vaccine. Exceptions as previously noted include (1) persons with impaired humoral immunity, (2) asymp-

tomatic or mildly symptomatic HIV-infected children, and (3) susceptible children with acute lymphocytic leukemia in continuous remission for at least 1 year and with a lymphocyte count greater than 700/μL (0.7×10^9/L) and a platelet count greater than 100×10^3/μL (100×10^9/L). With appropriate monitoring, these children have been immunized safely as part of a research protocol.[92,104]

Administration of varicella vaccine should be avoided if the individual is receiving immunosuppressive doses of systemic corticosteroids. The effects of corticosteroids vary, but many clinicians consider a dose equivalent to either 2 mg/kg body weight or 20 mg/day of prednisone to be sufficiently immunosuppressive to raise concern about the safety of vaccination with live virus vaccines.

After cessation of immunosuppressive therapy, varicella vaccine generally is withheld for at least 1 month. Because the intensity and type of immunosuppressive therapy, radiation therapy, underlying disease, and other factors determine when immunologic responsiveness will be restored, making a definitive recommendation for an interval after cessation of immunosuppressive therapy when varicella vaccine can be safely and effectively administered often is not possible.

Transmission of the live attenuated varicella vaccine virus used for immunization has been documented rarely. Therefore, contacts of immunocompromised patients should be vaccinated to prevent the spread of natural varicella to such patients. Vaccinees in whom a rash develops in the month after immunization should avoid direct contact with immunocompromised, susceptible individuals for the duration of the rash.

Receipt of antibody-containing blood products (whole blood, plasma, or parenteral immunoglobulin) may interfere with seroconversion to varicella vaccine. The length of time that such passively acquired antibody persists depends on the concentration and quantity of the blood product received (see Table 255–11).[308]

Although no direct evidence demonstrates that varicella vaccine is harmful to a pregnant female or her fetus, the vaccine should not be administered to women known to be pregnant or considering becoming pregnant within the month because of the theoretical risk of fetal infection associated with a live virus vaccine. Vaccinated women should avoid conception for 1 month after receiving vaccination. The manufacturer, in collaboration with the CDC, has established a Varicella Vaccination in Pregnancy Registry to monitor the maternal-fetal outcomes of pregnant women inadvertently given varicella vaccine. The telephone number for the registry is 800-986-8999.

Varicella vaccine may be considered for a nursing mother. Although most live virus vaccines are not secreted in breast milk, whether varicella vaccine virus is excreted in human milk and, if so, whether the infant can be infected are unknown.

Reye syndrome has occurred in children infected with varicella who receive salicylates. Whether varicella vaccine may induce Reye syndrome is not known, but the vaccine manufacturer recommends that salicylates not be given within at least 6 weeks after administration of varicella vaccine.

DISEASES FOR WHICH COMBINATION VACCINES ARE AVAILABLE

Increasing numbers of new and improved vaccines are being introduced. Incorporation of these vaccines into already complex childhood immunization schedules poses a challenge. In the 2009 Recommended Immunization Schedule in the United States, a minimum of 22 separate injections are needed to immunize a child from birth to 2 years of age.[38,157] At some visits, the administration of three to five separate injections can be indicated.

Combination vaccines represent one solution to the problem of increased numbers of injections. These vaccines incorporate, into a single product, antigens that prevent several diseases. Combinations licensed in recent years in the United States are shown in Table 255–17.

The ACIP, AAP, and AAFP have indicated a preference for the use of licensed combination vaccines over separate injections of the equivalent component vaccines, except in the case of MMRV.[30,113] Separate vaccines should not be combined into the same syringe for administration together unless such mixing is indicated on the package insert approved by the FDA. The safety, immunogenicity, and efficacy of unlicensed combinations are unknown.

Mixing antigens in the same syringe can result in an increase or, more commonly, a decrease in the immunogenicity of one or more components of the combination vaccine. An antigen may have chemical incompatibility with other antigens or may cause interference with the immune response to those antigens when incorporated into a single vaccine. The unpredictability of the effect of combining different products in the same syringe can be the result of interference, antigenic competition, or carrier protein interference. Interference can result from chemical incompatibility among the vaccine antigens and the stabilizers, adjuvants, or preservatives. For example, thimerosal, a preservative used in DTP, a vaccine no longer used in the United States, decreases the potency of poliovirus antigens in IPV. Formulations of DTP/IPV used in Europe and Canada avoid interference by using a different preservative.

Antigenic competition occurs when one or more vaccine strains in a combination vaccine replicate more efficiently than the other strains do. For example, the concentration of the varicella component in MMRV is higher than in single-component varicella vaccine because measles replication interfered with that of varicella.[145] Carrier protein interference was identified when multiple polysaccharide–protein conjugate vaccines using the same carrier protein were administered simultaneously with large doses of the carrier protein antigen. An example would be the simultaneous administration of a tetravalent pneumococcal vaccine conjugated with tetanus toxoid and a DTP-IPV-PRP-T vaccine.[191] This problem should be preventable by using different carrier proteins in new combination conjugate vaccines.

Clinical studies in infants have demonstrated that using some combination vaccine products containing Hib vaccine may induce a suboptimal immune response to the Hib vaccine component. Because of the potential for a suboptimal immune response to

TABLE 255–17 Combination Vaccines Licensed in the United States since 1995

Vaccine	Trade Name	Manufacturer	Date of First Licensure
DTaP-IPV-Hib conjugate (PRP-T)	Pentacel	Sanofi Pasteur	2008
DTaP-IPV	Kinrix	GlaxoSmithKline	2008
Measles-mumps-rubella-varicella	ProQuad	Merck	2005
DTaP-IPV-hepatitis B	Pediarix	GlaxoSmithKline	2002
Hepatitis A-hepatitis B	Twinrix	GlaxoSmithKline	2001
Hib conjugate (PRP-T)–DTaP	TriHIBit	Aventis Pasteur	1996
Hib conjugate (PRP-OMP)–hepatitis B	COMVAX	Merck	1996

DTaP, diphtheria-tetanus-acellular pertussis; Hib, Haemophilus influenzae *type b*, *IPV, inactivated poliovirus vaccine.*

the Hib component,[209] the currently licensed DTaP/Hib combination product should not be used for primary vaccination in infants aged 2, 4, or 6 months.[112]

In general, vaccines from different manufacturers that protect against the same disease are interchangeable. Data are not available on the interchangeability of DTaP vaccines. Vaccines from the same manufacturer should be used throughout a series whenever feasible. Licensure of combination vaccines usually is based on studies indicating that the product's immunogenicity (or efficacy) and safety are similar to those of monovalent products licensed previously. A combination vaccine may be used interchangeably with monovalent formulations and other combination products with similar component antigens produced by the same manufacturer.

VACCINES WITH SELECTIVE INDICATIONS FOR CHILDREN AND ADOLESCENTS

BACILLE CALMETTE-GUÉRIN VACCINE

Effective control of tuberculosis in the United States has been achieved by the early identification and treatment of cases, followed by surveillance of household and other close contacts and institution of appropriate preventive measures for those at high risk for development of disease. In the United States, the mainstay of preventive therapy is isoniazid chemoprophylaxis, which is used in asymptomatically infected persons to prevent the progression of infection to disease. In selected instances, however, the potential for acquiring disease, poor compliance in contacts instructed to take chemoprophylaxis, or failure of chemoprophylaxis may justify the use of immunoprophylaxis.[105] Elsewhere in the world, BCG vaccine is used in more than 100 countries and is recommended routinely at birth by the WHO (see Table 255–6).[501] Of primary concern to pediatricians is the risk to an infant born to a tuberculous mother or living within a household with other identified tuberculous individuals.[23]

Preparations

BCG is a live attenuated strain derived from *Mycobacterium bovis*. All currently available BCG vaccines are derived from the original strain at the Pasteur Institute in Paris, but they have been propagated by different methods in many laboratories and therefore vary in their immunogenic and reactogenic properties. BCG vaccines manufactured by Organon Teknika Corporation (Durham, NC) and Connaught Laboratories (Willowdale, Ontario, Canada) are licensed in the United States. Comparative evaluations of these and other BCG vaccines have not been performed. BCG is administered either percutaneously or intradermally. BCG preparations instilled in treatment of bladder cancer are not intended to be used as vaccines.[105]

Immunogenicity and Efficacy

BCG is used primarily in young infants in an attempt to prevent disseminated and other life-threatening manifestations of *Mycobacterium tuberculosis* disease. However, BCG does not prevent infection with *M. tuberculosis*. The efficacy of different BCG vaccines seems to be highly variable. Two meta-analyses of published clinical trials and case-control studies concerning the efficacy of BCG vaccines concluded that BCG has relatively high protective efficacy (≈80%) against meningeal and miliary tuberculosis in children.[179,411] The protective efficacy against pulmonary tuberculosis, however, differed significantly among the studies, thus precluding a specific conclusion. Protection afforded by BCG in one meta-analysis was estimated to be 50 percent.

Adverse Events

BCG vaccination usually results in scarring at the site of injection. BCG preparations have been associated uncommonly (1% to 2% of vaccinations) with local adverse reactions such as subcutaneous abscess and lymphadenopathy, which are not generally serious. Osteitis affecting the epiphyses of long bones is a rare complication that develops in one per million vaccinees and may occur as long as several years after receiving BCG immunization. The rate may be higher in newborns. Disseminated fatal disease occurs rarely (0.1 to 1 case per million vaccinees), primarily in persons with severely impaired immune systems.[105,309,329,446] Antituberculosis therapy, except for pyrazinamide, is recommended to treat osteitis and disseminated disease caused by BCG. Some experts also recommend treatment of chronic suppurative lymphadenitis caused by BCG. Persons with complications caused by BCG should be referred, if possible, to a tuberculosis expert for management.

Indications

In the United States, administration of BCG should be considered only in limited and select circumstances, such as unavoidable risk of exposure to *M. tuberculosis* and failure or unfeasibility of other methods of control of tuberculosis. The ACIP and the AAP have published recommendations for the use of BCG to control tuberculosis in children.[23,105]

Healthy infants from birth to 2 months of age may be given BCG without tuberculin skin testing; thereafter, BCG is given only to children with a negative tuberculin skin test. In infants and children, BCG immunization should be considered for those who are not infected with HIV in the following circumstances:

- The child is exposed continually to a person or persons with contagious pulmonary tuberculosis resistant to isoniazid and rifampin and the child cannot be removed from this exposure.
- The child is exposed continually to a person or persons with untreated or ineffectively treated contagious pulmonary tuberculosis, and the child cannot be removed from such exposure or given antituberculosis therapy.

Careful assessment of the potential risks and benefits of BCG vaccine and consultation with personnel in local area control programs for tuberculosis are strongly recommended before the use of BCG. When BCG vaccine is given, care should be taken to observe the precautions and directions for administration on the product label.

Skin Test Reactivity

Recipients of BCG should have repeat tuberculin skin tests 2 to 3 months after immunization to establish that tuberculin cellular reactivity has developed. Failure to react dictates the need for repeat BCG vaccination followed by repeat tuberculin testing.[105] The tuberculin reaction to the BCG vaccine available in the United States generally results in 7 to 15 mm of induration after vaccination and diminishes gradually during subsequent years; without re-vaccination or repeated exposure to *M. tuberculosis*, reactivity usually disappears within 10 years.[105] The size of the area of induration may be correlated with the number of doses of BCG.[276] However, tuberculin skin test sensitivity does not correlate with BCG efficacy.[259] In BCG recipients, differentiating between a tuberculin reaction representing acquired tuberculous infection and persisting post-vaccination reactivity is difficult. Because the degree and duration of protection against tuberculous disease afforded by BCG are uncertain, a positive

tuberculin reaction always must be suspected to be indicative of disease.

Precautions and Contraindications

BCG vaccine should not be administered to individuals with burns, skin infections, and primary or secondary immunodeficiencies, including HIV infection. The use of BCG also is contraindicated in persons receiving immunosuppressive medications, including high-dose corticosteroids.

In the United States, where the risk of acquiring tuberculosis is low, BCG vaccine should not be administered to children with known or suspected asymptomatic HIV infection.[105,374] However, in populations where the risk of contracting tuberculosis is high, the WHO has recommended that asymptomatic HIV-infected children receive BCG vaccine at birth or shortly thereafter.[501,505] Although no harmful effects of BCG vaccine on the fetus have been documented, women should avoid receiving vaccination during pregnancy.

CHOLERA VACCINE

Fewer than 300 cases of cholera have been recognized in the United States since the late 1980s.[98] Most cases have occurred in travelers to cholera-affected areas or persons who have eaten contaminated food brought or imported from these areas.[98] Although cholera remains a significant public health concern in African, South American, and Asian countries, even in these countries the risk to U.S. travelers is low. Persons following the usual tourist itinerary who use standard accommodations in countries reporting cholera are at virtually no risk of acquiring infection.[146]

Preparations

As of August 2000, the only cholera vaccine approved for use in the United States was no longer being manufactured or sold.[146] Two oral vaccines have been licensed for commercial use in other countries. One vaccine consists of killed whole-cell *Vibrio cholerae* O1 with purified recombinant B-subunit of cholera toxoid (WC/rBS; Dukoral; SBL Vaccin AB, Stockholm). The other vaccine, an attenuated live oral genetically modified *V. cholerae* O1 strain (CVD 103-HgR; Orochol; Berna Biotech, Bern; known as Mutachol in Canada), is not currently being produced.[266]

Immunogenicity and Efficacy

WC/rBS vaccine confers protection specific to *V. cholerae* serogroup O1.[266] Immunization does not protect against *V. cholerae* serogroup O139 or other species of *Vibrio*. Field trials in Bangladesh, Peru, and Sweden have shown that the WC/rBS vaccine confers 85 to 90 percent protection for 6 months in all age groups after administration of two doses, 1 week apart. In Bangladesh, protection declined rapidly after 6 months in young children, but it was still approximately 60 percent in older children and adults after 2 years.

Adverse Events

More than 5 million doses of WC/rBS vaccine have been supplied worldwide.[266] According to manufacturer information from clinical trials and post-marketing surveillance, mild gastrointestinal symptoms (abdominal pain, cramping, diarrhea, nausea) are most commonly reported, occurring at a frequency of 0.1 to 1 percent. Serious adverse events, including a flulike syndrome, rash, arthralgia, and paraesthesias, are rare, occurring in less than 1 in 10,000 doses distributed.

Indications

Routine immunization is not recommended for travelers.[146,333] Oral cholera vaccines may be considered for those at higher than average risk, such as health professionals in endemic areas, aid workers in refugee camps, and those traveling to remote areas where cholera epidemics are occurring and access to medical care is limited. At present, no country requires proof of cholera immunization as a condition for entry, and the WHO recommends against such a requirement. Some local authorities, however, may require immunization (to determine local requirements, travelers may consult the embassies of the countries to which they will be traveling).

Only limited data are available on the safety and immunogenicity of the vaccine in children aged 1 to 2 years, and protective efficacy has not been studied. Therefore, WC/rBS vaccine is not recommended to be used in children younger than 2 years of age.

Precautions and Contraindications

Oral cholera vaccine should not be administered to persons with a hypersensitivity reaction to any of the vaccine components, Administration of oral cholera vaccine should be postponed for subjects suffering from acute gastrointestinal illness or acute febrile illness.

JAPANESE ENCEPHALITIS VIRUS VACCINE

JE virus, the most important cause of epidemic arboviral encephalitis in Asia, has a wide clinical spectrum, ranging from asymptomatic infection to permanent neurologic sequelae, and a high case-fatality rate of 30 to 70 percent.[361,461] An inactivated mouse brain–derived vaccine has controlled JE virus infection successfully among human populations in Japan, Korea, and Taiwan since 1968.[380] Two other JE virus vaccines have been used widely, primarily in China: an inactivated primary hamster kidney cell–derived vaccine and a live attenuated vaccine.[336] Only the inactivated mouse brain–derived vaccine is available internationally for travelers from non-endemic countries. This vaccine is licensed in the United States for use in persons living in or traveling to Asia.

Preparations

The JE vaccine licensed in the United States is a formalin-inactivated vaccine prepared by purifying JE virus from the brains of mice inoculated intracerebrally with JE virus.[93] Although the vaccine undergoes two major purification steps, including ultracentrifugation, complete removal of mouse proteins is not possible. Each 1-mL dose of vaccine contains less than 50 ng of mouse serum proteins and no detectable murine myelin basic protein.[93] The vaccine contains gelatin from bovine and porcine sources, formaldehyde, and thimerosal as a preservative. This vaccine has limited availability in the United States. A new inactivated cell culture–derived vaccine has been developed but is not expected to be licensed and available in the United States until 2009.

Immunogenicity and Efficacy

Immunogenicity studies in the United States indicate that three doses are needed to provide protective concentrations of serum neutralizing antibody in greater than 80 percent of vaccinees.[399] Protective concentrations have been defined by animal challenge experiments.[380] The longevity of neutralizing antibody after the primary vaccination series is not known. In one Japanese study, protective antibody titers persisted for 3 years after the administration of a booster dose.[300]

A field trial of the currently licensed JE vaccine conducted in Thai children demonstrated an efficacy of 91 percent when compared with placebo.[271] The efficacy for a single year of a prototype of the currently licensed vaccine, field-tested in Taiwanese children, was 80 percent.[274]

Adverse Reactions

JE vaccination is associated with a moderate frequency of local and mild systemic side effects. Local reactions occur in approximately 20 percent of vaccines, and approximately 10 percent have reported systemic side effects such as fever, headache, malaise, or rash.[93]

Neurologic adverse reactions, including acute disseminated encephalomyelitis, also have been reported. In Denmark, acute disseminated encephalomyelitis has been estimated to occur in 1 in 50,000 to 75,000 vaccinees. However, a review of post-marketing data in the United States from 1993 to 1999 found no serious neurologic events occurring after receipt of JE immunization.[450]

Hypersensitivity reactions have been reported. Urticaria or angioedema of the extremities, face, and oropharynx, especially the lips, characterizes these reactions. They occur a median of 12 hours after administration of the first dose of vaccine. The interval between administration of a second dose and onset of symptoms generally is longer, with a median of 3 days and possibly as long as 2 weeks. Reactions have occurred after the administration of a second or third dose when the preceding doses did not cause symptoms. Hypersensitivity reaction rates are similar after the administration of both first and second doses—a rate of approximately 15 to 62 per 10,000 immunizations in U.S. citizens. The vaccine component responsible for these adverse events has not been identified.[93]

Indications

JE vaccine is recommended for persons older than 3 years of age who will be residing in areas where JE is endemic or epidemic.[93] No data are available on vaccine safety and efficacy in infants. The risk for acquiring JE varies highly within endemic regions. Therefore, the incidence of JE in the area of residence, conditions of housing, the nature of activities, and the possibility of unexpected travel to high-risk areas are factors that should be considered in the decision to vaccinate.

JE vaccine is *not* recommended for all travelers to Asia. The vaccine should be offered to persons spending a month or longer in endemic areas during the transmission season, especially if travel will include rural areas. *Health Information for International Travel*, updated regularly by the CDC, provides a useful table that lists affected areas by country and notes the transmission season.[146]

The decision to use JE vaccine should balance the risks for exposure to the virus and the development of illness, the availability and acceptability of mosquito repellents and other alternative protective measures, and the side effects of vaccination.

The recommended primary immunization series is three doses administered on days 0, 7, and 30. An abbreviated schedule of days 0, 7, and 14 can be used when a longer schedule is impractical because of time constraints. Two doses administered 1 week apart will confer short-term immunity in 80 percent of vaccinees. However, this schedule should be used only under unusual circumstances. The last dose should be administered at least 10 days before travel commences to ensure an adequate immune response and access to care if a delayed, adverse reaction occurs.[93] No data are available regarding vaccine safety and efficacy in infants.[7]

The duration of protection is unknown, and definitive recommendations cannot be given on the timing of booster doses. Booster doses may be administered after 2 years.

Precautions and Contraindications

Because generalized urticaria and angioedema can occur within minutes to as long as 2 weeks after administration of vaccination, epinephrine, other medications, and equipment to treat anaphylaxis should be available. Vaccinees should be observed for 30 minutes after receiving vaccination and should be warned about the possibility of delayed development of urticaria and angioedema, which can occur as long as 2 weeks after being vaccinated. Vaccinees should be advised to remain in areas with ready access to medical care for 10 days after receiving a dose of JE vaccine.

Hypersensitivity to proteins of rodent or neural origin, to thimerosal, or to a previous dose of JE vaccine is a contraindication to receiving vaccination.

A study in U.S. military personnel found an association between reactions to JE vaccine and a past history of having urticaria. A history of urticaria should be considered when weighing the risks and benefits of vaccination.

No specific information is available on the safety of JE vaccine in pregnancy. Limited data suggest that the vaccine can be given to patients with altered immune status. Little information is available on the effect of concurrent administration of other vaccines on the safety and immunogenicity of JE vaccine.

RABIES VACCINE

Rabies is a viral zoonosis transmitted in saliva and other tissue of infected mammals. The virus enters the central nervous system of the host and causes an acute, progressive encephalomyelitis that almost always is fatal. Post-exposure prophylaxis is possible because of the long incubation period, usually weeks to months, of this infection.

Wild animals are the most important source of infection for both humans and domestic animals. Rabid, insectivorous bats carrying variants of the rabies virus have been responsible for nearly all human cases of rabies in the continental United States in recent years. Transmission has been shown to occur from even minor and unrecognized bites from infected bats. Of the 36 human cases of rabies diagnosed in the United States between 1980 and 1997, 21 (58%) were associated with bat variants.[114] Wild carnivores, especially skunks, foxes, coyotes, and raccoons, are the terrestrial animals most often infected with rabies. Wildlife rabies occurs throughout the continental United States; only Hawaii remains consistently rabies-free. Domestic animals including dogs, cats, and ferrets also may be infected, with three times as many reports of rabies annually in cats compared with dogs.[141] The likelihood of human exposure to rabies from a domestic animal in the United States is a rare occurrence, a result of effective national rabies control programs. In most other countries (e.g., Asia, Africa, and Latin America), dogs remain the most important potential source of infection. Therefore, international travelers to areas where canine rabies is still endemic have an increased risk of exposure to rabies. Twelve of the 36 human rabies deaths reported to the CDC from 1980 through 1997 appear to have been related to rabid animals outside the United States.[109,373]

Preparations

Three rabies vaccines are licensed commercially for pre-exposure and post-exposure prophylaxis in the United States: human diploid cell vaccine (HDCV), rabies vaccine adsorbed (RVA), and purified chicken embryo cell (PCEC), but only HDCV and PCEC are being produced. The HDCV and PCEC vaccines are licensed for intramuscular administration. The HDCV product for intradermal use is no longer available. HDCV (Imovax Rabies, Sanofi Pasteur) is derived from the Pitman-Moore strain grown

in human diploid cell culture. PCEC (RabAvert, Chiron Corporation-Novartis) is prepared from the fixed rabies virus strain Flury LEP grown in primary culture of chicken fibroblasts.[205] The HDCV and PCEC vaccines each contain the WHO recommended standard of at least 2.5 IU of rabies virus antigen per 1.0-mL intramuscular dose. Although not licensed for use in the United States, two intradermal rabies vaccines are recommended by the WHO and used in countries where the two tissue culture vaccines (HDCV and PCEC) are prohibitively expensive.[5]

Immunogenicity and Efficacy

Viral neutralizing antibodies (VNAs) are produced within 7 to 10 days, and protective immunity usually persists for 2 years or longer. Although a definitive protective titer has not been identified, the WHO[507] and the CDC adhere to working guidelines for an acceptable response to immunization.[82] The CDC currently specifies complete viral neutralization at a 1:5 or greater titer by the rapid fluorescent-focus inhibition test as acceptable; the WHO specifies 0.5 IU/mL or more as acceptable.

No randomized, placebo-controlled, human trials have documented the efficacy of vaccine for pre-exposure or post-exposure prophylaxis. However, substantial field experience, as well as direct evidence from controlled animal studies, demonstrates a protective effect. The paucity of human cases attests to the efficacy of post-exposure prophylaxis with the currently recommended vaccine and immunoglobulin preparations. To date, rabies has not been reported in the United States in any patient who received the currently recommended post-exposure measures. Cases of human rabies occurring after post-exposure administration of prophylaxis have resulted from failure to adhere to established guidelines, such as those of the CDC or the WHO.[91,207,220,431,485]

Adverse Events

Reactions occurring after administration of HDCV or PCEC vaccine are less serious and less common than those associated with the previously available vaccines.[114] Local reactions at the injection site occur in 30 to 74 percent of injections, and mild systemic reactions such as fever, headache, nausea, abdominal pain, muscle aches, and dizziness occur in 5 to 40 percent of vaccine recipients. In one report, approximately 6 percent of persons had an immune complex–like reaction 2 to 21 days after receipt of the booster dose of HDCV.[83] This systemic hypersensitivity reaction occurred less frequently in persons receiving primary vaccination. The reactions have been associated with the presence of beta-propiolactone–altered human albumin in HDCV and the development of IgE antibodies to this allergen.[221]

Indications and Precautions

When used as indicated, both types of rabies vaccine are considered equally safe and effective for both pre-exposure and post-exposure prophylaxis (see the ACIP recommendations[114]). Usually, an immunization series is initiated and completed with one vaccine product. No clinical studies have been conducted that documented a change in efficacy or the frequency of adverse reactions when the series is completed with a second vaccine product. Ideally, an immunization series should be completed with the same product unless serious allergic reactions occur. Corticosteroids, immunosuppressive conditions, and concurrent administration of antimalarials can interfere with the adequate development of an active immune response. Post-vaccination titers should be checked in these circumstances.

For adults, rabies vaccination always should be administered in the deltoid area. For children, the anterolateral aspect of the thigh also is acceptable. The gluteal area never should be used for HDCV or PCEC injections because administration of HDCV in this area results in lower neutralizing antibody titers and decreased immunogenicity.[220]

POST-EXPOSURE PROPHYLAXIS

The essential components of rabies post-exposure prophylaxis are wound treatment and, for previously unvaccinated persons, concurrent administration of human rabies immune globulin (HRIG) and vaccine (Table 255–18).[114] The combination of active and passive immunization is indicated for the treatment of

TABLE 255–18 Rabies Post-exposure Prophylaxis for Individuals not Previously Immunized

Animal Type	Evaluation and Disposition of the Animal	Post-exposure Prophylaxis Recommendations
Wild		
Skunk Fox Raccoon Most other carnivores Bat	Regard as rabid unless animal proven negative by laboratory tests*	Consider immediate vaccination
Domestic		
Dog	Healthy and available for 10 days observation	Persons should not begin prophylaxis unless animal develops clinical signs of rabies†
Cat	Rabid or suspected rabid	Immediately vaccinate
Ferret	Escaped (unknown)	Consult public health officials
Other		
Livestock	Consider individually	Consult public health officials.
Small rodents Large rodents (woodchucks and beavers) Lagomorphs (rabbits and hares) Other mammals		Bites of squirrels, hamsters, guinea pigs, gerbils, chipmunks, rats, mice, other small rodents, rabbits, and hares almost never require antirabies prophylaxis

**The animal should be euthanized and tested as soon as possible. Holding for observation is not recommended. Discontinue vaccine if immunofluorescence test results of the animal are negative.*

†During the 10-day observation period, begin post-exposure prophylaxis at the first sign of rabies in a dog, cat, or ferret that has bitten someone. If the animal exhibits clinical signs of rabies, it should be euthanized immediately and tested.

Adapted from Centers for Disease Control and Prevention: Human rabies prevention: United States, 1999. Recommendations of the Advisory Committee on Immunization Practices (ACIP). M. M. W. R. Morb. Mortal. Wkly. Rep. 48:*1*, *1999.*

all bite and all non-bite exposures inflicted by animals suspected or proven to be rabid. Recommendations for the management of persons with possible exposure to rabies include giving meticulous attention to thorough cleansing of the wound with soap and water. The decision to give rabies immunoprophylaxis depends on the circumstances precipitating the exposure, the species and condition of the animal inflicting the wound, and the prevalence of rabies in local animal populations. Bite and non-bite exposures, including scratches, abrasions, open wounds, or mucous membranes contaminated with saliva, are considered significant. Because the need for preventive measures is based on these specific circumstances, the local department of health should be consulted promptly concerning the necessity for initiating post-exposure prophylaxis.

When possible, the brains of wild animals (skunks, foxes, coyotes, raccoons, and bats), stray dogs or cats, or symptomatic animals implicated in an exposure should be examined in certified laboratories for evidence of rabies. Immunization always should be initiated promptly and discontinued only if laboratory results are negative. Individuals exposed to healthy dogs or cats that are available for observation do not require immediate prophylactic treatment. Implicated healthy domestic dogs or cats should be quarantined and observed for at least 10 days. If symptoms develop that suggest rabies, the exposed individual should begin post-exposure prophylaxis, and the brain of the animal should be examined. An unknown or unavailable animal must be regarded as potentially rabid.

Studies conducted in the United States by the CDC have documented that a regimen of one dose of HRIG and five doses of rabies vaccine (1.0 mL for each dose) given on days 0, 3, 7, 14 and 28 was safe and induced an excellent antibody response in all recipients.[114] If anatomically feasible, the full dose of HRIG (20 IU/kg body weight) should be infiltrated thoroughly in the area around and into the wounds. Any remaining volume should be injected intramuscularly at a site distant from that of vaccine administration. The dose of HRIG should not exceed that recommended and should not be given beyond the seventh day because HRIG can partially suppress active antibody production.

Persons previously fully vaccinated do not require HRIG and should receive only vaccine (two doses 3 days apart) because an amnestic response will occur after the administration of a booster regardless of the antibody titer.[114] Serum for antibody testing should be obtained from persons whose prophylaxis history or immune status is uncertain, and the course of post-exposure active and passive immunoprophylaxis as described for nonimmune individuals should be initiated immediately. If serologic testing demonstrates adequate anti-rabies antibody, post-exposure prophylaxis may be discontinued.

Once initiated, rabies prophylaxis should not be interrupted or discontinued because of local or mild systemic adverse reactions to rabies vaccine. Usually, such reactions can be successfully managed with anti-inflammatory and antipyretic agents such as ibuprofen or acetaminophen. When a person with a history of having had serious hypersensitivity to rabies vaccine must be re-vaccinated, antihistamines can be administered. Epinephrine should be readily available to counteract any anaphylactic reactions, and the person should be observed carefully immediately after receiving vaccination.

PRE-EXPOSURE PROPHYLAXIS

Active immunization should be considered for high-risk groups (i.e., veterinarians, animal handlers and control officers, selected laboratory workers, persons visiting countries where rabies is hyperendemic, and persons whose pursuits may involve frequent contact with rabid animals, such as spelunkers). Persons whose risk of exposure is less but whose access to immediate competent medical care is restricted also should be considered for pre-exposure prophylaxis. The primary series consists of three doses (1.0 mL per dose) administered on days 0, 7, and 21 or 28, given intramuscularly in the deltoid area. This three-dose series provides long-term protective immunity, and routine serologic testing for rabies antibody following primary immunization is not necessary. For those individuals who may be immunosuppressed, measurement of anti-rabies antibodies should be performed. In the case of continued or frequent exposure, serum samples should be tested every 2 years and a booster dose of rabies vaccine provided when the antibody titer falls below the minimum accepted level.[114]

Precautions and Contraindications

Because of the potential consequences of inadequately treated rabies exposure and because no indication exists that fetal abnormalities have been associated with rabies vaccination, pregnancy is not considered a contraindication to post-exposure prophylaxis.[114] If the risk of exposure to rabies is substantial, pre-exposure prophylaxis may also be indicated during pregnancy.

Persons who have a history of serious hypersensitivity to rabies vaccine should be re-vaccinated with caution. Although serious systemic, anaphylactic, or neuroparalytic reactions are rare events during and after the administration of rabies vaccines, such reactions pose a serious dilemma for the patient and the attending physician. A patient's risk of acquiring rabies must be carefully considered before deciding to discontinue vaccination. Advice and assistance on the management of serious adverse reactions for persons receiving rabies vaccines may be sought from the state health department or the CDC.

TYPHOID VACCINE

Typhoid fever, an acute, life-threatening febrile illness caused by the bacterium *Salmonella enterica* serovar *typhi* (*S. typhi*), remains a serious public health problem throughout the developing world. Although disease prevalence varies greatly depending on the specific population studied, current global estimates suggest that 21.6 million cases of typhoid and 200,000 deaths occur annually.[187] In the United States and much of the developed world, typhoid fever has virtually disappeared.[187] Approximately 400 cases of typhoid fever, mostly among travelers, are reported to the CDC each year. Most reported cases in the United States are acquired during travel to developing countries.[97] Therefore, the primary indication for typhoid vaccination in the United States is international travel to an endemic area. In developing countries without safe water and sanitation, mass immunization is a potentially effective strategy to limit both the severity and impact of typhoid fever.[2]

The changing epidemiology of typhoid fever underscores the importance of vaccination in both international travelers and high-risk populations in endemic regions. Current studies report that the highest incidence rate is now found in children younger than 5 years of age and results in more severe disease than previously.[436,439] *S. typhi* is increasingly resistant to ampicillin, chloramphenicol, and co-trimoxazole, and quinolone resistance is now being reported.[418,433] Case-fatality rates, which had decreased from 10 to 1 percent with appropriate antibiotic therapy,[508] could rise again without safe, effective, and affordable vaccination strategies.

Preparations

The three types of typhoid vaccines are as follows: inactivated, whole-cell vaccines; live attenuated bacterial vaccines; and subunit vaccines.[146] The parenteral, heat-phenol-inactivated, whole-cell

TABLE 255–19 Dosage and Schedule for Typhoid Fever Vaccination

Vaccination	Age (yr)	Dose/Route of Administration	No. of Doses	Dosing Interval	Boosting Interval
Oral Live Attenuated TY21a Vaccine					
Primary series	≥6	1 capsule*/oral	4	48 hr	Not applicable
Booster	≥6	1 capsule*/oral	4	48 hr	Every 5 yr
Vi Capsular Polysaccharide Vaccine					
Primary series	≥2	0.50 mL/IM	1	Not applicable	Not applicable
Booster	≥2	0.50 mL/IM	1	Not applicable	Every 2 yr

**Administer with cool liquid no warmer than 37° C (98.6° F).*

Adapted from Centers for Disease Control and Prevention: Health Information for International Travel 2005-2006. Atlanta, U.S. Department of Health and Human Services, Public Health Service, Centers for Disease Control and Prevention, 2005.

vaccine widely used for many years was highly immunogenic but also was highly reactogenic and no longer is available in the United States. It is the only vaccine currently approved for use in children as young as 6 months and is still available in several developing countries because of its affordability, although manufacturing of this vaccine may not be up to international standards.

Two vaccines currently are licensed for use in the United States[57,212]: an oral live, attenuated vaccine (TY21a, Vivotif Berna) and a purified Vi (virulence) capsular polysaccharide of *S. typhi* vaccine (ViCPS) (Typhim Vi, Aventis Pasteur) for intramuscular use. Table 255–19 provides information on vaccine dosage and administration. The time required for primary vaccination differs for the two vaccines, as do the lower age limits for use in children.

Ty21a (Vivotif, BernaBiotech) is an oral, live attenuated vaccine consisting of a stable mutant, Ty21a, developed by chemical mutagenesis of a pathogenic *S. typhi* strain. This vaccine was licensed in the United States in 1989 for use in adults and children 6 years of age and older and is available as an enteric-coated capsule. The four-dose primary vaccine series (in the United States and Canada, three-dose series in other parts of the world) is administered as one capsule every other day, taken 1 hour before a meal, for a total of four capsules. The capsules should be kept refrigerated (not frozen), and all four doses must be taken to achieve maximum efficacy. Each capsule should be taken with cool liquid no warmer than 37° C (98.6° F), approximately 1 hour before a meal. This regimen should be completed 1 week before potential exposure. The vaccine manufacturer recommends that Ty21a not be administered to infants or children younger than 6 years of age.

A parenteral subunit vaccine, ViCPS (Typhim Vi, Sanofi Pasteur), was licensed in 1994 for use in adults and children as young as 2 years of age. Primary vaccination with ViCPS consists of one 0.5-mL (25 μg) dose administered intramuscularly and should be given at least 2 weeks before potential exposure. The manufacturer does not recommend the vaccine for children younger than 2 years of age.

In circumstances of continued or repeated exposure to *S.* serotype *typhi*, booster doses are recommended to maintain immunity after primary immunization. The optimal booster schedule for either vaccine has not been determined. Current recommendations for re-vaccination with either vaccine are provided in Table 255–19. No data have been reported concerning the use of one vaccine as a booster after primary immunization with the other.

Efficacy

Although field trials have demonstrated the efficacy of each vaccine, no comparative studies have been performed.[97] For the heat-phenol-inactivated, whole-cell vaccine, efficacy ranged from 51 to 88 percent and lasted for as long as 7 seven years. The oral, live attenuated Ty21a vaccine stimulates humoral (both serum IgG and mucosal IgA antibodies) as well as cell-mediated immune responses. In trials of the Ty21a vaccine, efficacy ranged from 42 to 96 percent after administration of the initial series of three doses, with the lower efficacies seen in trials from areas with highly endemic disease.[322,437] The optimum schedule for booster immunizations has not been determined for this vaccine. Protective immunity has been shown to persist for at least 7 years in field trials in endemic countries where herd immunity may have an effect.[248] Re-vaccination is recommended with the same four-capsule regimen every 5 years for as long as continued exposure is likely to occur.

Immunization with ViCPS results in seroconversion, defined as a fourfold rise in serum anti-Vi antibody, in 80 percent of vaccinees within 2 weeks in endemic as well as non-endemic areas.[248,264,302] Because the vaccine is administered parenterally, mucosal immunity does not develop. The efficacy of ViCPS vaccine in clinical trials was 72 percent at 17 months[1] and 64 percent at 21 months[302] after administration of a single dose. Polysaccharide vaccines do not stimulate T cells and cannot establish immunologic memory. Additional doses of ViCPS therefore do not elicit a booster effect. Re-vaccination with a single dose is recommended every 2 years for people who remain at risk.

None of the typhoid vaccines approach 100 percent efficacy, and a large inoculum of *S. typhi* can overcome vaccine-induced immunity. Vaccines do not substitute for proper hygiene and appropriate food handling practices.

Adverse Events

Reactions to the oral Ty21a vaccine generally are mild and consist of transient gastrointestinal upset, fever, headache, and occasionally rash; these reactions occur in less than 5 percent of recipients.[97] Adverse systemic effects are reported in less than 1 percent of vaccines, and neither the development of bacteremia nor person-to-person transmission has been reported.[248]

Reactions to the ViCPS vaccine also occur infrequently, reported in approximately 7 percent of recipients.[97] These reactions include tenderness at the injection site, erythema, and induration, fever, and, very rarely, rashes; systemic complaints occur in less than 2 percent.[248] In contrast, reactions to the inactivated, whole-cell vaccine occur more commonly and are more severe; they include fever in as many as 24 percent of recipients, headache, and severe local pain or swelling in as many as 35 percent of vaccinees. Thirteen to 24 percent of vaccinees subsequently have missed school or work.[323]

Indications

Typhoid vaccination in the United States is recommended only for the following groups[21,97,146,251]:

- Travelers to areas where typhoid fever is endemic and in whom a risk of exposure is recognized; risk of exposure is greatest with travel to the Indian subcontinent, Latin America, Asia, the Middle East, and Africa (see www.cdc.gov/travel)
- Persons with intimate exposure to a documented *S. typhi* carrier, such as occurs with continuing household contact
- Laboratory workers who have frequent contact with *S. typhi* and people living in areas outside the United States with endemic typhoid infection

Vaccination is not recommended for persons attending summer camp or for those in areas of natural disaster or for control of common-source outbreaks. Doses and schedules for the different typhoid vaccines are given in recommendations of the CDC.[97,146]

Contraindications

Ty21a is a live attenuated vaccine and should not be given to immunocompromised patients, including those receiving high doses of corticosteroids and persons with HIV infection.[97,146] Ty21a vaccine also should not be administered during a gastrointestinal illness or concurrent with certain antibiotics. The 2006 *Red Book* recommends avoiding antimicrobial therapy for at least 24 hours before the first dose of oral typhoid vaccine through 7 days after the last dose.[21] Reports are conflicting regarding the use of the anti-malarial agents atovaquone and proguanil; however, chloroquine and mefloquine do not appear to interfere with the immune response to oral Ty21a. Neither of the available typhoid vaccines should be given to anyone with a history of having severe local or systemic reactions after receiving a previous dose of vaccine.

Ty21a is not approved for children younger than 6 years of age, and ViCPS is not approved for children younger than the age of 2 years. The whole-cell, inactivated vaccine available outside of the United States is approved for use in for children younger than the age of 2 years and older than 6 months of age.

Information is not available on the safety of these vaccines when they are used during pregnancy. It is prudent on theoretical grounds to avoid vaccinating pregnant women.

YELLOW FEVER VACCINE

Yellow fever occurs only in sub-Saharan Africa and tropical South America, where it causes an estimated 200,000 cases annually.[360] As a result, yellow fever is an important vaccine-preventable disease among travelers to areas where yellow fever occurs. From 1996 to 2002, five cases of yellow fever among unvaccinated travelers from the United States and Europe to areas where yellow fever is endemic were fatal.[118,134,341] The risk to unvaccinated travelers of acquiring yellow fever probably is increasing because potential zones of transmission of yellow fever are expanding to include urban areas with large populations of susceptible humans and abundant competent mosquito vectors. Vaccination is the most effective preventive measure against yellow fever, a disease that has no specific treatment and may cause death in 20 percent of patients.[360]

Preparations

Yellow fever vaccines are derived from the original 17D yellow fever vaccine strain. The live attenuated 17D-204 and 17DD yellow fever strains are the yellow fever vaccines most commonly used.[360] The 17D-204 yellow fever vaccine, which is prepared in chick embryos, is licensed in the United States.[160] Primary immunization consists of a single, subcutaneous injection of reconstituted, freeze-dried vaccine for both adults and children.

Immunogenicity and Efficacy

Seroconversion rates of 93 percent have been documented in young children receiving yellow fever vaccine.[509] Immunity acquired from immunization with the 17D strain virus has been demonstrated to persist for more than 10 years.[160,415,500] Revaccination is required no more frequently than every 10 years.[415]

Adverse Events

The 17D-204 and 17DD yellow fever vaccines are among the safest and most effective viral vaccines.[360] Since 1965, approximately 8 million doses of 17D-derived yellow fever vaccine have been administered to U.S. travelers, and approximately 300 million doses have been administered to persons in areas where yellow fever is endemic. Although 2 to 5 percent of persons who receive vaccine report headaches, myalgia, and low-grade fever 5 to 10 days after receiving vaccination, less than 1 percent will report curtailing their usual activities.

Serious adverse events associated with yellow fever vaccine rarely occur. Immediate hypersensitivity reactions, characterized by rash, urticaria, or asthma, or a combination of these, are uncommon (incidence <1 case per 131,000 vaccinees). Unrecognized allergy to eggs or chicken or to the hydrolyzed gelatin used to stabilize the vaccine may be responsible for hypersensitivity reactions.

Historically, yellow fever vaccine–associated adverse events were seen primarily among infants and presented as encephalitis. Since 1992, five cases of encephalitis among adult recipients of yellow fever vaccine have been reported to the U.S. VAERS.[146] In addition, 10 cases of autoimmune neurologic disease, including patients with GBS and acute disseminated encephalomyelitis, have been reported to VAERS. All patients with yellow fever vaccine–associated neurologic disease had an onset of illness 4 to 23 days after receiving vaccination. All cases were in first-time vaccine recipients. The risk for vaccine-associated neurologic disease does not appear to be limited to infants, and crude estimates in the United States of the reported frequency range from 4 to 6 cases per 1,000,000 doses distributed.

A serious adverse reaction syndrome has been described among recipients of yellow fever vaccines produced by several different manufacturers. This syndrome previously was reported as febrile multiple organ system failure and now is called *yellow fever vaccine–associated viscerotropic disease*. Since 1996, 9 cases of yellow fever vaccine–associated viscerotropic disease, a disease clinically and pathologically resembling naturally acquired yellow fever, have been reported in the United States; an additional 17 cases had been identified worldwide as of October of 2004.[133,468] All U.S. patients required intensive care after experiencing fever, hypotension, respiratory failure, elevated hepatocellular enzymes, hyperbilirubinemia, lymphocytopenia, and thrombocytopenia; 8 of the 9 also had renal failure that required hemodialysis. Six of the U.S. cases were fatal. In several cases for which tissue samples were available, immunohistochemistry demonstrated viral dissemination throughout the body, including liver, lung, spleen, lymph node, brain, and smooth muscle; however, in many cases, tissue samples were not available for histopathologic review or detection of virus. All cases reported thus far have occurred in primary vaccinees.

Yellow fever vaccines must be considered as a possible, but rare, cause of yellow fever vaccine–associated viscerotropic disease that is similar to fulminant yellow fever caused by wild-type yellow fever virus. Accurately measuring the incidence of vaccine-associated viscerotropic disease is currently precluded by lack of adequate prospective data; however, crude estimates in the United

States of the reported frequency range from 3 to 5 cases per 1,000,000 doses distributed. This frequency appears to be higher for persons older than 60 years of age, as high as 19 cases per million doses distributed.

Indications

Yellow fever vaccine is recommended for persons 9 months of age or older traveling to or residing in areas where yellow fever is endemic.[160] Because of the increased risk for development of neurologic complications, infants aged 4 to 8 months should be considered for vaccination only when travel to high-risk areas is required and high-level protection against mosquito exposure is not feasible. Vaccination for international travel is required. To obtain an international certificate of vaccination, a yellow fever vaccine approved by the WHO and administered at a designated yellow fever vaccine center is required. Yellow fever vaccine centers in the United States can be identified by contacting state or local health departments. The International Health Regulations require re-vaccination at intervals of 10 years. Re-vaccination may boost antibody titers; however, evidence from several studies suggests that yellow fever immunity persists for at least 30 to 35 years and probably for life.[160]

Precautions and Contraindications

The risk for having adverse reactions appears to be age-related.[160] Infants younger than 6 months should not receive yellow fever vaccine because of the increased risk of vaccine-associated neurotropic disease developing in this age group.[7] Immunization should be delayed until an infant is at least 9 months of age. In unusual circumstances, physicians considering vaccinating infants younger than 9 months of age should contact the Division of Vector-Borne Infectious Diseases (970-221-6400) or the Division of Global Migration and Quarantine (404-498-1600) at the CDC for advice.

No adverse effects of yellow fever vaccine on the developing fetus have been demonstrated. Vaccine administration to pregnant women, however, generally is not indicated because the vaccine is a live virus. Pregnant women should be considered for vaccination only when travel to high-risk areas is required and protection against mosquito exposure is not feasible.

Whether this vaccine is excreted in breast milk is not known. No reports exist of adverse events or transmission of the vaccine viruses from nursing mother to infant. As a precautionary measure, vaccination of nursing mothers should be avoided because of the theoretical risk of the transmission of virus to the breast-fed infant. When travel of nursing mothers to high-risk yellow fever endemic areas cannot be avoided or postponed, these women may be vaccinated.

Yellow fever vaccine poses a theoretical risk to patients with altered immunity as a result of underlying disease or immunosuppressive therapy. These patients should not be vaccinated. If travel to an epidemic or endemic area is necessary, the patient should be instructed in ways to avoid mosquitoes and given a vaccine waiver letter.

A history of thymus disease has been identified as a contraindication to receiving yellow fever vaccine. Four of the 26 vaccine recipients with yellow fever vaccine–associated viscerotropic disease worldwide have had a history of diseases involving the thymus, all of which are extremely rare, a finding suggesting that compromised thymic function may be another independent risk factor for viscerotropic disease.[53] Health care providers should ask about a history of thymus disorder when they are screening a patient before administering yellow fever vaccine. For persons with such a history, alternative means of prevention should be recommended, if travel plans cannot be altered to avoid yellow fever-endemic areas.

Persons with a history of systemic anaphylaxis to eggs should not be vaccinated because the vaccines contain egg proteins and on rare occasion may induce immediate allergic reactions. Less severe or local manifestations of allergy to eggs or to feathers are not contraindications to yellow fever vaccine administration and do not warrant performing vaccine skin testing.[6] If international quarantine regulations are the only reason to immunize a patient known to be hypersensitive to eggs, attempts should be made to obtain a waiver. If immunization of an individual with a questionable history of egg hypersensitivity is considered essential because of a high risk of exposure, an intradermal skin test may be given as directed in the vaccine package insert.[160]

VACCINES RELATED TO BIOTERRORISM

Two vaccines, anthrax and smallpox, are potentially available to protect children against bioterrorist attacks with *Bacillus anthracis* and variola. Neither vaccine is available commercially at this time and does not have indications for children and adolescents in the absence of a bioterrorist attack.[417] The vaccines are reviewed in the relevant disease-specific chapters.

INVESTIGATIONAL VACCINES

Routine immunizations for children have virtually eliminated many infectious diseases from the United States. These successes have encouraged research to develop vaccines to prevent other serious viral and bacterial diseases affecting children. A 1985 report by the IOM of the National Academy of Sciences reviewed the benefits that would be associated with the development and use of new and improved vaccines in the United States.[182] The report listed 14 diseases for which vaccines were desirable. A 1999 study by the IOM noted that considerable progress had been made since the 1985 study.[183] Seven of 14 vaccines listed in the 1985 study as domestic priorities for development are now licensed. They include acellular pertussis vaccine, LAIV, and vaccines against hepatitis A and B, Hib, varicella, and rotavirus.

The 1999 IOM report used a new quantitative model to compare the cost and health benefits of developing candidate vaccines.[183] This model can be used to evaluate the potential impact of a new vaccine on public health. In the 1999 report, the model was used to evaluate diseases for which candidate vaccines were being developed. The report divided 26 candidate vaccines into 4 groups, from most to least favorable for development. The 4 vaccines in the top tier include a cytomegalovirus vaccine given to adolescents, a universal influenza vaccine, a group B streptococcus vaccine for high-risk adults and pregnant women, and an *S. pneumoniae* vaccine for infants and seniors. Other diseases for which vaccines would be desirable included *Chlamydia trachomatis*, enterotoxigenic *Escherichia coli*, Epstein-Barr virus, *Helicobacter pylori*, hepatitis C virus, herpes simplex virus, HPV, *M. tuberculosis*, *Neisseria gonorrhoeae*, respiratory syncytial virus, parainfluenza virus, *Shigella*, and groups A and B streptococcus.

Two vaccines listed in the 1999 study are now licensed, pneumococcal conjugate vaccine for infants and HPV vaccine. Candidate vaccines are in human trials for many of the other pathogens listed in the 1999 IOM report.

Advances in biotechnology, increased understanding of the virulence factors of infectious agents, and knowledge of the host immune response have led to new approaches for vaccine development.[212] The three general categories of approaches include the following: live vaccines; killed, inactivated, or subunit vaccines; and, most recently, DNA-based vaccines. In addition, newer technologies such as adjuvants or delivery systems and vectors can be applied to improve immunogenicity. Vaccines are

being developed by the application of these newer technologies to numerous infectious agents for which vaccines are not currently available, especially those pathogens listed in the 1999 IOM report.

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CHAPTER 256

Passive Immunization

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GENERAL PRINCIPLES OF PASSIVE IMMUNITY

DEFINITION

Passive immunization is the administration of antibodies from an immune subject to provide immediate protection against a microbial agent, toxic substance, or cell. In general, passive immunization is used to provide temporary immunity in an unimmunized subject exposed to an infectious disease when active immunization is unavailable (e.g., respiratory syncytial virus infection), is contraindicated (e.g., varicella in an immunocompromised child), or has not been given before exposure (e.g., tetanus, rabies).

Passive immunization also is used in the management of certain disorders associated with toxins (e.g., diphtheria), in certain bites (e.g., snake and spider), in drug overdose (e.g., digoxin), as a specific (e.g., Rh_0[D] immune globulin) or nonspecific (e.g., antithymocyte globulin) immunosuppressant, and in the treatment of certain infectious diseases.

Several types of preparations are used in passive immunization (Table 256–1):

1. standard human immune serum globulin (HISG) for general use, which is available in three forms: immune globulin (IG) for intramuscular use (IGIM), intravenous use (IGIV), and subcutaneous use (IGSC);
2. special high-titer IGs with a known antibody content for specific illnesses;
3. animal serums and antitoxins;
4. monoclonal antibodies (Tables 256–1, 256-2).

These preparations are listed in Table 256–1. Most of the licensed special IGs are for intramuscular use only. Plasma, serum, and even breast milk also can be used in passive immunization.

Passive immunization is not always effective; the duration is short and variable (1-6 weeks), and undesirable reactions may occur, especially if the antibody is of nonhuman origin. High-titer special IGs and IGIVs are identical to regular IGs and IGIVs, except that they are derived from patients hyperimmunized or convalescing from a specific infection or selected from donors with high titers to a specific antigen; they are useful in several disorders in which regular IG and IGIV are of little or no value.

ANIMAL SERUMS AND ANTITOXINS

Animal serums and antitoxins are derived from the serum of immunized animals, usually horses (equine). Because these sera are foreign proteins, they carry a significant risk of sensitization. Thus, they should be administered only when specifically indicated, after sensitivity tests, and by a physician prepared to deal with a hypersensitivity reaction.

A careful history must be taken before an animal serum is injected. Inquiry must be made about asthma, hay fever, urticaria, and previous injections of animal serum. Patients with a history of asthma, allergic rhinitis, or other allergic symptoms on exposure to horses may be dangerously sensitive to the corresponding serum and should be given only with the utmost caution.

SENSITIVITY TESTS FOR ANIMAL SERUM

A scratch, prick, or puncture skin test, followed by an intradermal skin test, always should be performed before any injection of animal serum, regardless of whether the patient has had the serum previously. A scratch, prick, or puncture test is performed by applying a drop of a 1:100 dilution of the serum in saline to the site of a superficial scratch, prick, or puncture on the volar aspect of the forearm and observing it for 20 minutes. A positive control (histamine phosphate, 0.1%) and negative control (saline) also should be applied. A positive reaction consists of erythema or wheal formation 3 mm greater than the control. (Note: previous use of antihistamines may render results of these tests negative.)

If the scratch, prick, or puncture test result is negative, an intradermal test is performed by injecting 0.02 mL of a 1:1000 saline dilution; again, positive (histamine phosphate, 0.1%) and negative control tests should be performed. The reaction is read after 10 to 30 minutes and is positive if a wheal appears that is 3 mm greater than the negative control. If the test result is negative, it should be repeated with 0.02 mL of a 1:100 dilution. For persons with negative histories of animal allergy and no previous exposure to animal sera, the 1:100 dilution may be used initially if the scratch, prick, or puncture test result is negative.

Although intradermal skin tests have resulted in fatalities, scratch, prick, or puncture tests have not but can still be associated with immediate reactions. Therefore, a skin test never should be performed (nor a serum injected) unless a syringe containing 1 mL of 1:1000 epinephrine is within immediate reach.

Skin tests can indicate the probability of sensitivity. However, a negative skin test result is not an absolute guarantee of the absence of sensitivity. Therefore, either a specific history of allergy or a positive skin test reaction with horse serum is sufficient reason for special caution. A positive history of sensitivity to horse dander is an indication of the need for extreme caution.

ADMINISTRATION OF ANIMAL SERUM

If the history and sensitivity test reactions are negative, the indicated dose of serum may be given intramuscularly, with epinephrine at hand. The patient should be watched closely for an hour for adverse reactions.

Intravenous injection may be indicated if a high concentration of circulating antibody is required rapidly, as in severe tetanus or diphtheria. The manufacturer's instructions should be consulted. If they are unavailable, a preliminary dose of 0.5 mL of serum should be diluted in 10 mL of either physiologic saline or 5 percent glucose solution. This preparation should be given intravenously during the course of 5 minutes, and the patient should be watched for 30 minutes for reactions. If no reaction occurs, the remainder of the serum, diluted 1:20, may be given at a rate not to exceed 1 mL/min.

TABLE 256–1 Antibody Preparations Available for Passive Immunity in the United States

Product	Abbreviations and Brand Names	Principal Use
Standard human immune serum globulins for intramuscular or subcutaneous use		
Immune globulin, intravenous	IGIV, IVIG	Treatment of antibody deficiency, immune thrombocytopenic purpura, Kawasaki disease, other immunoregulatory and inflammatory diseases
Immune globulin, intramuscular	IGIM, IG, ISG	Treatment of antibody deficiency; prevention of measles, hepatitis A
Immune globulin, subcutaneous	IGSC	Treatment of antibody deficiency
Special human immune globulins for intramuscular or subcutaneous use		
Hepatitis B immune globulin	HBIG	Prevention of hepatitis B
Varicella-zoster immune globulin	VZIG	Prevention or modification of chickenpox
Rabies immune globulin	RIG	Prevention of rabies
Tetanus immune globulin	TIG	Prevention or treatment of tetanus
Vaccinia immune globulin	VIG, VIGIM	Prevention or treatment of vaccinia, prevention of smallpox
Rh_0(D) immune globulin	RhoGAM	Prevention of Rh hemolytic disease
Special human immune globulins for intravenous use		
Cytomegalovirus immune globulin	CMV-IGIV, CMVIG, CytoGam	Prevention or treatment of cytomegalovirus infection
Hepatitis B immune globulin, intravenous	HBIGIV, HepaGam B	Prevention of hepatitis B (including liver transplantation)
Vaccinia immune globulin	VIGIV	Prevention or treatment of vaccinia, prevention of smallpox
Rh_0(D) immune globulin, intravenous	WinRho SDF	Treatment of immune thrombocytopenic purpura
Botulinum immune globulin	BIG, BabyBIG	Treatment of newborn botulism
Animal serums and globulins		
Tetanus antitoxin (equine)	TAT	Prevention or treatment of tetanus (when TIG is unavailable)
Diphtheria antitoxin (equine)	DAT	Treatment of diphtheria
Botulinum antitoxins (equine)		Treatment of botulism
Latrodectus mactans antivenin (equine)		Treatment of black widow spider bites
Crotalidae polyvalent antivenin (equine)		Treatment of most snake bites
Crotalidae polyvalent immune Fab (ovine)		Treatment of most snake bites
Micrurus fulvius antivenin (equine)		Treatment of coral snake bites
Digoxin immune Fab fragments (ovine)†	Digibind, DigiFab	Treatment of digoxin or digitoxin overdose
Lymphocyte/thymocyte immune globulin (equine)	Equine ATG, Atgam	Immunosuppression
Lymphocyte/thymocyte immune globulin (rabbit)	Rabbit ATG, Thymoglobulin	Immunosuppression
Monoclonal antibodies		
Muromonab (anti-CD3)	OKT3	Immunosuppression
Daclizumab (anti-CD25)	Zenapax	Immunosuppression
Basiliximab (anti-CD25)	Simulect	Immunosuppression
Infliximab (anti–TNF-α)	Remicade	Treatment of inflammatory bowel disease, rheumatoid arthritis
Trastuzumab (anti–HER-2)	Herceptin	Treatment of breast cancer
Rituximab (anti-CD20)	Rituxan	Treatment of B-cell lymphoma, autoimmune diseases
Tositumomab (anti-CD20*)	Bexxar	Treatment of refractory B-cell lymphoma
Omalizumab (anti-IgE)	Xolair	Prevention and treatment of asthma, allergic disorders
Efalizumab (anti-CD11α)	Raptiva	Treatment of psoriasis
Adalimumab (anti–TNF-α)	Humira	Treatment of rheumatoid arthritis, inflammatory bowel disease
Bevacizumab (anti-VEGFR)	Avastin	Treatment of metastatic colon cancer
Cetuximab (anti-EGFR)	Erbitux	Treatment of metastatic colon cancer
Natalizumab (anti–α_4 integrin)	Tysabri	Treatment of multiple sclerosis
Ranibizumab (anti-VEGFR†)	Lucentis	Treatment of macular degeneration
Gemtuzumab (anti-CD33‡)	Mylotarg	Treatment of acute myelocytic leukemia
Alemtuzumab (anti-CD52)	Campath	Treatment of chronic lymphocytic leukemia
Abciximab (anti-GPIIb/IIIa†)	ReoPro	Prevention of thrombosis
Palivizumab (anti-RSV)	Synagis	Prevention of respiratory syncytial virus infection
Ibritumomab (anti-CD20§)	Zevalin	Treatment of refractory B-cell lymphoma

Monoclonal antibody characteristics:
**Radiolabeled with iodine 131.*
†Fab fragment.
‡Conjugated with calicheamicin.
§Radiolabeled with yttrium 90.
EGFR, epithelial growth factor receptor; GP, glycoprotein; HER-2, human epithelial growth factor receptor 2; RSV, respiratory syncytial virus; TNF, tumor necrosis factor; VEGFR, vascular endothelial growth factor receptor.

If the skin test reaction is positive or a history of allergy to animal serum is present and the need for the serum is unquestioned (and epinephrine is at hand), a procedure commonly called desensitization can be undertaken, but any significant desensitization is unlikely to occur. This procedure merely results in establishing temporary tolerance to the serum. Desensitization should be performed by trained personnel with the necessary emergency equipment and drugs immediately available.

Desensitization consists of periodic (at 15-minute intervals) injections or infusions of progressively larger doses of the serum,

TABLE 256–2 USAD Nomenclature for Monoclonal Antibodies*

The suffix -mab is used for all monoclonal antibodies and fragments.	
The following letters preceding -mab identify the product source:	
u	human
e	hamster
o	mouse
i	primate
a	rat
xi	chimeric
zu	humanized
The general disease state subclass for which the monoclonal antibody is used is encoded by the syllables preceding the above:	
Disease or target class	
-vir	viral
-bac	bacterial
-lim	immune
-les	infectious lesions
-circ	cardiovascular
Tumors	
-col	colon
-mel	melanoma
-mar	mammary
-got	testis
-gov	ovary
-pr(o)	prostate
-tum	miscellaneous

**United States Adopted Name.*

starting at a very low dose, until tolerance is achieved. Schedules for intravenous and intradermal, subcutaneous, or intramuscular desensitization are given in the *2006 Red Book*.[21] Administration of sera after desensitization has been achieved must be continuous; protection from desensitization is lost rapidly.

HYPERSENSITIVITY REACTIONS TO ANIMAL SERUM

Hypersensitivity reactions to animal serum may be of four general types:

1. anaphylactic reactions consisting of urticaria or other rashes; respiratory distress with wheezing, dyspnea, cough, hoarseness, or stridor; cardiovascular reactions including tachycardia, hypotension, arrhythmia, cyanosis, shock, and unconsciousness; and gastrointestinal reactions, such as cramps, diarrhea, and vomiting, all occurring seconds to minutes after an injection has been administered;
2. acute febrile reactions consisting of moderate or severe hyperpyrexia within 2 hours after an injection has been given;
3. serum sickness reactions consisting of urticaria, arthritis, adenopathy, and fever occurring hours to days after an injection has been administered, depending on the dose and the presence or degree of previous sensitization (serum sickness occurs within hours or a few days after the second injection and within 7 to 12 days after the first injection); and
4. delayed reactions of varying nature, including peripheral neuritis, nephritis, Guillain-Barré syndrome, and myocarditis.

TREATMENT OF HYPERSENSITIVITY REACTIONS TO ANIMAL SERUM

For anaphylactic reactions, 1:1000 epinephrine at a dose of 0.01 mL/kg (0.5 mL maximum dose) is given subcutaneously or intramuscularly immediately. If improvement is not achieved immediately, 1:10,000 epinephrine at a dose of 0.01 mL/kg is given intravenously. This dose can be repeated every 10 minutes for up to three doses. The 1-mL 1:1000 epinephrine vials must be diluted 1:10 in physiologic saline (now 1:10,000) and injected slowly at a dose of 0.01 mL/kg. (A 1:10,000 dilution in a 10-mL vial also is available.)

Administration of epinephrine may be repeated in 5 to 15 minutes if the response is not satisfactory. Vasopressors and positive-pressure oxygen are helpful. For severe urticaria or edema, particularly edema of the larynx, intramuscular injection of antihistamines and corticosteroids is indicated. Administration of serum therapy (if necessary) should be resumed 6 to 8 hours later or after all visible signs of reaction have subsided.

Mild febrile reactions (temperatures of less than 39° C [102.8° F]) are treated with aspirin, ibuprofen, or acetaminophen. Severe febrile reactions can cause convulsions and death and should be treated rigorously with sponge baths or other cooling means to reduce the temperature promptly. Serum sickness and serum neuritis generally are treated with corticosteroids.

HUMAN IMMUNE SERUM GLOBULIN

Human immune serum globulin (HISG, or gamma globulin) is available in three forms for general use: intramuscular (IGIM), intravenous (IGIV, IVIG), and subcutaneous (IGSC). IGIM is used primarily for the prevention of certain infectious disorders, as outlined in Table 256–1, and less commonly for the treatment of antibody immunodeficiencies; IGIV is used in the treatment of primary and secondary antibody deficiencies, many immunoregulatory disorders (e.g., immune thrombocytopenic purpura, Kawasaki disease), and neurologic disorders (e.g., Guillain-Barré syndrome, peripheral neuritis). IGSC is used exclusively for treatment of antibody deficiency.

Several high-titer IGIMs and IGIVs are available for use in certain illnesses as listed in Table 256–1.

INTRAMUSCULAR IMMUNE GLOBULIN

Pharmacology

Intramuscular immune globulin (IGIM, IMIG) is prepared from pooled human serum by the Cohn alcohol fractionation procedure (thus deriving its alternative name of Cohn fraction II). This procedure and viral inactivation steps remove most other serum proteins, hepatitis viruses, and human immunodeficiency viruses (HIV-1 and HIV-2), thereby providing a safe product for intramuscular injection. It is reconstituted as a sterile approximate 16 percent solution (160 mg/mL) with or without preservative. It contains a wide spectrum of antibodies to viral and bacterial antigens.

IGIM is greater than 95 percent immunoglobulin G (IgG), but trace quantities of IgM and IgA and other serum proteins are present. The IgM and IgA are therapeutically insignificant because of their rapid half-lives (approximately 7 days) and their low concentrations in IG. IG contains all IgG allotypes (Gm and Km types).

IGIM is approved only for intramuscular or subcutaneous use, and intravenous injection of IG generally is contraindicated. IG aggregates in vitro to high-molecular-weight complexes (9.5S to 40S) that are strongly anticomplementary. These aggregates probably are responsible for the occasional systemic reactions to IGIM. The incidence of these reactions is increased if the patient has received IGIM previously or if it is inadvertently given intravenously. Agammaglobulinemic boys with affected male relatives (suggesting X-linked inheritance) may have a lower incidence of

reactions.[395] Small intradermal injections of IGIM are not of value (except as a placebo), and they are contraindicated.

Intramuscular Immune Globulin in Antibody Immunodeficiency

IGIM is used infrequently now for IgG replacement therapy in immunodeficiency. The usual dosage is 100 mg/kg per month, about equivalent to 0.7 mL/kg per month of the 16 percent (160 mg/mL) product. Two or three such injections are given at the onset of therapy as a loading dose, often during the course of a 3- to 5-day period. The maximal maintenance dosage should not exceed 20 or 30 mL per week.

Few studies on optimal dosage are available; however, the Medical Research Council Working Party[395] found that 25 mg/kg per week (100 mg/kg per month) was therapeutically equivalent to 50 mg/kg per week but that 10 mg/kg per week was inadequate. Use of IGIM by this route has been supplanted largely by IGIV administration.

IGIM should be given at multiple sites to avoid giving more than 5 mL at any one site (10 mL in a large adult). The buttocks are the preferred site, but the anterior of the thighs also can be used. Tenderness, sterile abscesses, fibrosis, and sciatic nerve injury may result from these injections. The danger of sciatic nerve injury is especially great in a small malnourished infant with inadequate muscle and fat in the gluteal regions. Large doses of IGIM should not be given to patients with severe thrombocytopenia because of the risk for development of hematoma and infection.

The injections are given initially at monthly intervals. If the patient continues to have infection or if a characteristic symptom (e.g., cough, conjunctivitis, diarrhea, arthralgia, or purulent nasal discharge) recurs at the end of the injection period, the interval between doses is decreased to 3 or 2 weeks. Older patients often report that they can tell when their IgG level is low and they need another injection. During acute infections, IgG catabolism increases, so extra injections of IGIM often are given.

Because high serum levels of IgG cannot be maintained, serial immunoglobulin assays are unnecessary in assessing the effectiveness of treatment. The maximal increase in serum IgG level after a standard IGIM injection will vary from patient to patient and from dose to dose because of different rates of absorption, local proteolysis at the injection site, and distribution within tissues.

An intramuscular injection of 100 mg/kg of IGIM usually raises the IgG serum level by 100 mg/dL after 2 to 4 days.[565] Thus, a recent IGIM injection usually does not obscure the diagnosis of hypogammaglobulinemia.

Intramuscular Immune Globulin and Special Intramuscular Immune Globulins for Prevention of Infectious Diseases

IGIM is recommended for prophylaxis of hepatitis A and measles. The recommended doses are found in the sections on these disorders. Special high-titer IGIMs are available for several disorders (see Table 256–1), such as hepatitis B and varicella. These products are identical to IGIM except for the high titers of specific antibodies. These products also are discussed in the sections on these disorders.

Adverse Effects of Intramuscular Immune Globulin

Although IGIM is one of the safest biologic products available, rare anaphylactic reactions to intramuscular injections have been reported, particularly in patients requiring repeated injections.[166] The Medical Research Council Working Party[395] noted such reactions in 33 of 175 patients (19%) treated during a 10-year period. In all, 85 reactions occurred after approximately 40,000 injections; in eight patients, the injections were stopped as a result of these adverse effects, and one death was recorded. Such reactions occurred at any stage of treatment and were unrelated to any particular lot number of IGIM or its anticomplementary activity. Symptoms include anxiety, nausea, vomiting, malaise, flushing, facial swelling, cyanosis, and loss of consciousness. Immediate treatment with epinephrine and antihistamines is indicated.

Persons who experience such reactions should be evaluated before receiving a repeated injection. Skin testing should be performed with several lots of IGIM.[166] A skin test result that is positive for an old but not a new lot of IG may indicate a particular idiosyncratic reaction to a particular lot. Under these circumstances, incremental doses of IG from a new lot are recommended. In other patients, IgE antibodies to IgG develop and result in positive immediate skin test reactions to all IGIM lots. In many other patients, no cause of the reactions can be found. Some of these patients will tolerate gradually increasing doses of IG, particularly if they are premedicated with a nonsteroidal anti-inflammatory agent, diphenhydramine, or corticosteroids. Finally, in a few patients, antibodies have developed to the IgA present in minute quantities in IG; these IgA antibodies can be detected by serologic means in several laboratories.[623] This topic is discussed further under adverse effects of IGIV.

Administration of exogenous gamma globulin may inhibit the endogenous synthesis of gamma globulin. In a few patients given IGIM or IGIV from early infancy, we have noted depressed IgG levels that return to normal when the injections are stopped. Amer and colleagues[11] reported decreased IgG levels in premature infants given monthly IGIM injections since birth.

IGIM injections or infusions may inhibit antibody responses to live virus vaccines such as measles or varicella. Siber and associates[536] recommend an interval of 3 months between IVIG or IGIM therapy and administration of live virus vaccines after IG doses of less than 40 mg/kg, an interval of 6 months after doses of 40 to 80 mg/kg, an interval of 8 months after doses of 80 to 400 mg/kg, and an interval of 12 months after large doses (1 to 2 g/kg).

Late side effects after IGIM injections are uncommon occurrences; however, fibrosis of the buttocks or localized subcutaneous atrophy may develop at the site of repeated injections in some patients. Repeated injections of IGIM may result in high levels of mercury from the thimerosal preservative. Although symptoms of acrodynia (mercury toxicity) developed in one patient as a result of such therapy,[382] most remain asymptomatic.

INTRAVENOUS IMMUNE GLOBULIN

Intravenous immune globulin (IGIV, IVIG) is further-treated Cohn fractionated human IG that has been rendered free of complexes and is thus safe for intravenous infusion. These products can be given in large quantities for antibody deficiencies[99]; for several autoimmune, inflammatory and neurologic disorders[49,162]; and for prevention of transplant rejection.[289,290]

Pharmacology

The first IGIV produced in the United States in 1981 was Gamimune (Cutter Laboratories), a reduced and alkylated 5 percent solution containing 10 percent maltose.[30] The second IVIG produced was prepared by acidification and treatment with pepsin; the lyophilized powder could be reconstituted as a 3, 6, or 12 percent solution (Sandoglobulin, Sandoz Pharmaceuticals). Since then, several other IGIVs have been introduced by different manufacturers.[443]

Several methods of treating Cohn fraction II, including treatment with proteolytic enzymes, ultracentrifugation, chromatog-

raphy, reduction of sulfhydryl bonds by alkylation, and incubation at low pH, have been used to eliminate high-molecular-weight complexes. Solvent and detergent treatment, pasteurization, and addition of the fatty acid caprylate are used to ensure that viral inactivation occurs.[251] Various stabilizers, such as maltose, albumin, or sucrose, then are added. These variations in manufacturing techniques result in unique characteristics among the different IG products.[443]

Although these products vary somewhat from brand to brand and batch to batch,[545,565] they are for the most part therapeutically equivalent and usually are selected on the basis of cost and convenience. Minor IgA and IgG subclass differences exist.[32,545] Antibody titers to specific pathogens also may vary from lot to lot as well as among different IGIVs.[187] Products differ as to their IgA content, which can be a consideration for patients with anti-IgA antibodies.[32] Premixed liquids have the advantage of convenience because the reconstitution step is not required; however, most solutions must be kept refrigerated.

All IGIV products currently available have all IgG subclasses, adequate serum half-lives (15 to 25 days), wide spectrum of antibody activity, and minimal anticomplementary activity, and they are free of bacterial and viral contamination.[269] Indeed, individual donors are checked for viral pathogens, and the final product is checked for sterility. The ability of the fractionation process to remove viral and prion particles is validated by spiking test pools with model pathogens and subjecting them to the same fractionation process used by the plasma pools for human use. Some of them are 5 to 10 percent solutions; others are lyophilized powders that are reconstituted as 3 to 12 percent solutions.[443,565]

IGIV has several advantages over intramuscular IG, including the following: larger quantities of IgG can be given, high levels of serum IgG can be achieved rapidly, painful intramuscular injections are avoided, tissue pooling and local proteolysis are avoided, and home administration and self-administration are easier to perform.

Administration of Intravenous Immune Globulin

Administration of IGIV requires venous access, sometimes a problem in small children or obese subjects. It also requires close monitoring during the infusion, which usually takes 2 to 5 hours.[565] The initial rate is 0.01 mL/kg per minute, and if no side effects occur, it can be doubled at 15- to 30-minute intervals to a maximal rate of 0.08 mL/kg per minute. Rates above 5 mL/kg per hour (0.08 mL/kg per minute) are not recommended. Vital signs should be monitored frequently, and trained personnel should be available to treat adverse reactions. Adverse effects tend to be associated with rapid rates of infusion in patients with concurrent acute infections, previously untreated patients, or significant time elapse between infusions (>6-week intervals).

IGIV is contraindicated in patients who have had an anaphylactic reaction to IGIV or other blood products. It should be administered with great caution to patients who have IgG subclass deficiencies along with IgA deficiency or anti-IgA antibodies (or both).[146]

In responsible, older patients receiving infusions without adverse effects, infusion by home self-administration can be accomplished with considerable cost savings.[38,324,553] However, in most cases, IGIV infusions are performed in the clinic setting or by a home infusion service.

A few investigators have given high concentrations (9% to 12% solutions) infused rapidly during a period of 20 to 40 minutes; this rapid rate can be tolerated by some patients.[516] However, it should not be done except by experienced personnel equipped to manage adverse reactions.

The brand and lot number should be recorded, as should the dose, premedication, and any side reactions. Cold solutions should be warmed to room temperature before administration.

Premedication, such as acetaminophen or diphenhydramine, often is given 30 minutes before infusions are started, particularly for the first infusion or for patients who have had prior reactions. Intravenous hydrocortisone (6 mg/kg in children, 100-200 mg in adults) can be used for prevention of anticipated severe reactions. Oral prednisone can also be used.

Side Effects of Intravenous Immune Globulins

Certain brands of IVIG may be more reactogenic; in one study involving Kawasaki disease, the two IGIVs used were equivalent therapeutically, but one had a 12-fold (2% versus 25%) increase in side effects.[495]

Immediate reactions, such as headaches, shaking chills, nausea and vomiting, or myalgia and arthralgia that occur during or shortly after the infusions, are common occurrences.[158,565,566] They may occur in as many as 10 percent of recipients, especially with a first infusion or in individuals with prior reactions.[567] They can be treated by slowing or stopping the infusions and giving nonsteroidal anti-inflammatory drugs, diphenhydramine (1 mg/kg), or hydrocortisone or other steroids. On occasion, switching to a different product (generally one available as a solution) may alleviate the reactions.

SEVERE IMMEDIATE REACTIONS. Anaphylaxis, shortness of breath, hypotension and hypertension, and other such immediate reactions are uncommon events. Because they rarely involve IgE antibodies, they are termed anaphylactoid reactions, and they may be associated with cytokine release, complement activation, or presence of vasoactive substance in the preparations.

IMMEDIATE REACTIONS IN IgA-DEFICIENT PATIENTS. An occasional IgA-deficient patient on exposure to blood, plasma, or any form of IG may develop IgE anti-IgA antibodies, which may cause a reaction to the small amount of IgA present in IG.[101] IgG anti-IgA in high titers may cause a complement-mediated anaphylactic reaction.[623] These reactions are extremely rare, however, and testing patients for IgA deficiency before administration of IGIV is unnecessary. Further, patients with profound antibody deficiency do not develop anti-IgA antibodies. For the sensitized patient, use of a low-content IgA product or of premedication is indicated. Although IgA deficiency is rare, patients with this condition should not be given IGIV unless it is absolutely indicated. However, we do not recommend testing for IgA deficiency before administration of IGIV.

ASEPTIC MENINGITIS. Headache is a common occurrence during or after IGIV infusions. Yet some patients develop severe headaches 6 to 24 hours after the infusion, sometimes associated with photophobia, stiff neck, and nausea.[304,615] Several of these patients have had spinal taps that show cerebrospinal fluid pleocytosis but negative viral and bacterial cultures. The symptoms usually resolve after 96 hours. This complication may recur despite changing brands. Most occur when IGIV is used in large doses (e.g., 2 g/kg) for neurologic diseases. Individuals with a past history of migraine may be more susceptible to aseptic meningitis.[523] Such patients may tolerate slow infusions of subcutaneous IG.[482,562] Headaches and aseptic meningitis often can be avoided by premedication with steroids or with antimigraine medications.

RENAL COMPLICATIONS. Renal failure, usually temporary, has been reported with IGIV, particularly among subjects with a history of renal disease.[482,567] When such a problem is anticipated, IGIV should be given slowly at no more than 0.5 g/kg per day.[567]

THROMBOTIC COMPLICATIONS. Several thrombotic events, including stroke, myocardial infarction, transient ischemic events, and central vein thrombosis, have occurred after IGIV infusions.[567] These events may be associated with high doses and rapid infusion, particularly among patients with preexisting cardiovascular illness, obesity, or immobility.[567,651]

RARE SIDE EFFECTS. Transient neutropenia, hemolytic anemia, acidosis, and symptoms of hyperviscosity occur rarely.[92,567]

Transmission of Pathogens, Including Hepatitis C, by Intravenous Immune Globulin

Because IGIV is derived from human donors, a remote chance of transmission of viral or other pathogens exists. Tests of donors and the final products are performed, and newer testing for prions is in place but is not of proven reliability. No instance of HIV infection or Jakob-Creutzfeldt or new-variant (mad cow) Jakob-Creutzfeldt disease has been linked to the use of IGIV. Nonetheless, some patients with immunodeficiency receiving long-term IGIV therapy have developed progressive central nervous system problems.[666]

In the early 1990s, hepatitis C was transmitted by experimental IGIV lots,[354,430] some European preparations,[68,69,352,642] and some commercially available U.S. lots.[119,517] Until the report of an outbreak of hepatitis C in 1994,[119] no U.S. cases of hepatitis associated with a commercially available IVIG had been reported. This transmission occurred shortly after hepatitis C–seropositive donors were excluded from the donor pools used in the manufacture of IGIV.

A plausible explanation for the lack of transmission before this Food and Drug Administration policy was instituted is that the hepatitis C antibodies neutralized trace amounts of hepatitis C virus not eliminated during the fractionation. As of October 1994, 137 suspected cases were reported, 88 of which were confirmed.[119,517] Of the 88 patients, 51 (58%) had primary immunodeficiencies, and 63 percent eventually became symptomatic.

Bjoro and associates[69] reported that immunocompromised patients had a severe and rapidly progressive course of hepatitis C infection and that responses to interferon were poor. Razvi and colleagues[483] have not confirmed this finding in U.S. patients.

Newer manufacturing processes and more rigorous testing seemingly have eliminated the risk for occurrence of hepatitis C transmission.

Intravenous Immune Globulin and Intramuscular Immune Globulin Inhibition of Vaccine Antibody Responses

All IGs contain antibody to measles and other viral and bacterial pathogens and thus may inhibit antibody responses to these vaccines. In general, for IGIM or IGIV in doses less than 100 mg/kg, a period of 3 months should elapse before vaccines are given. For doses between 100 and 400 mg/kg, a period of 6 months should elapse; and for doses of 1000 to 2000 mg/kg, a period of 11 months should elapse. The pediatric *Red Book* gives detailed recommendations.[21]

Intravenous Immune Globulin in Primary Immunodeficiencies

Administration of IGIV is indicated for patients with profound primary antibody deficiency (quantitative and qualitative), for patients with combined immunodeficiencies, and for those with secondary immunodeficiency along with significant antibody deficiency (Table 256–3). Regular infusions of IGIV can keep patients with primary antibody immunodeficiencies free of infections for long periods or lessen the severity and frequency of chronic infections.

TABLE 256–3 Some Immunodeficiencies in Which Human Immune Globulin May Be Beneficial

Antibody deficiencies
Congenital agammaglobulinemias
Common variable immunodeficiency
Immunodeficiencies with hyper-IgM
Transient hypogammaglobulinemia of infancy (sometimes)
IgG subclass deficiency ± IgA deficiency (sometimes)
Antibody deficiency with normal immunoglobulins
Combined deficiencies
Severe combined immunodeficiencies (all types)
Wiskott-Aldrich syndrome
Ataxia-telangiectasia
Short-limbed dwarfism
X-linked lymphoproliferative syndrome
Secondary immunodeficiencies
Malignant neoplasms with antibody deficiencies; multiple myeloma, chronic lymphocytic leukemia, other cancers
Protein-losing enteropathy with hypogammaglobulinemia
Nephrotic syndrome with hypogammaglobulinemia
Pediatric acquired immunodeficiency syndrome
Intensive care patients: trauma, surgery, or shock
Post-transplantation
Burns
Prematurity

Patients with hereditary agammaglobulinemias, common variable immunodeficiency, and immunodeficiencies with hyper-IgM clearly benefit from replacement therapy. In combined antibody and cellular defects and in secondary antibody immunodeficiencies, administration of IVIG serves as an important ancillary treatment, but it does not correct the associated T-cell defect or underlying cause of the secondary immunodeficiency.

The recommended dose of IGIV is 400 to 600 mg/kg, usually given every 3 to 4 weeks.[443,565] It should be given every 3 weeks if symptoms develop late in the period between monthly infusions. In general, the trough IgG level should be maintained at approximately 400 mg/dL higher than the pretreatment level, which will normalize the IgG level in most patients (e.g., a level >600-1000 mg/dL).

Certain patients who experience continued infections may receive even higher doses (i.e., 600-800 mg/kg every 3-4 weeks).[165,297] Patients receiving these larger doses may have less frequent sinopulmonary infections, improved pulmonary function, and decreased number of days of illness and hospitalization compared with when they received lower doses.[565] Some investigators recommend giving even higher doses (i.e., keeping trough levels above 800 mg/dL) in an effort to prevent pulmonary complications.[165,297]

Some patients with severe disease do not respond to higher doses or more frequent infusions because of permanent tissue damage or deep-seated chronic infection.

IGIV also is used in patients with antibody deficiency but with normal or nearly normal immunoglobulin levels; often, these patients need higher or more frequent doses of IGIV because their IgG catabolism is increased as a result of their high serum IgG levels. On occasion, IGIV is indicated in infants with transient hypogammaglobulinemia and persistent infection.

IGIV also has been used in patients with IgG subclass deficiencies, but controlled studies demonstrating efficacy are lacking.[67,323,539]

Special Uses of Intravenous Immune Globulin in Antibody Deficiencies

A syndrome of polymyositis or chronic encephalitis, or both, caused by persistent enteroviral infection in patients with agammaglobulinemia has been treated successfully with very high doses of IGIV containing specific antibody to the virus.[394,476] However, some patients do not respond.[144]

Very high dose IGIV (up to 2 g/kg per day) is used occasionally in patients with primary immunodeficiency who develop parvovirus B19 infection, autoimmune disease such as immune thrombocytopenic purpura, or persistent respiratory viral infection.[565]

SUBCUTANEOUS HUMAN IMMUNE GLOBULIN

An alternative to intramuscular injection of IG or infusion of IGIV is slow subcutaneous infusion of IGIM or IGIV.[3,64,65,195] This route is used extensively in Europe, with a 16 percent preservative-free IG. A recently licensed preservative-free 16 percent product (Vivaglobin, CSL) was licensed in 2006 in the United States for subcutaneous use.[432] However, 10 to 12 percent solutions for IGIV also can be used safely.[562]

These products usually are infused into the abdominal wall or thigh with a battery-operated portable infusion pump.[65,195] The usual dose is 100 to 150 mg/kg per week, the same monthly dose as recommended for IGIV, given at a rate of 0.05 to 0.20 mL/kg per hour. Larger doses and more rapid infusions can be accomplished by using multiple injection sites. If a patient is beginning IGSC therapy and an immediate therapeutic level is sought, loading doses of IGIV at double the monthly maintenance dose can be given 1 week before the start of IGSC. Peak serum IgG levels after IGSC occur 48 to 96 hours after the infusion.

Subcutaneous IG infusions, which can be self-administered, are well tolerated, safe, and preferred by some patients because the side reactions are considerably less.[65,195,236] Indeed, premedication rarely is needed. Thus, they are more suitable than is IGIV for home self-administration.[194,195]

Stiehm and coworkers[562] used this route successfully in patients with poor intravenous access, aseptic meningitis, anaphylactic reactions after receiving IGIV infusions, or rapid gastrointestinal protein loss; they used a 10 percent IGIV solution. Subcutaneous IG has been used safely in IgA-deficient adults with frequent respiratory infections,[236] during pregnancy,[63] and in children.[3]

Rapid administration of subcutaneous infusions of IG has been achieved by using two pumps at four infusion sites and increasing the rate to 20 mL/hr per pump, thereby allowing the entire infusion to be completed in little more than an hour.[195]

IMMUNE GLOBULIN ADMINISTRATION BY OTHER ROUTES (ORAL, INTRATHECAL, AEROSOL, LOCAL)

Multiple types of antibody have been given by unusual routes with varying degrees of success. None except intravitreous injection of monoclonal antibody is standard care.

Oral Immunoglobulin

Breast milk is the ultimate oral passive immune agent with preventive benefit for newborns. Oral IG administered to provide antimicrobial activity to the gastrointestinal tract mimics the action of antibody-rich colostrum and breast milk. In humans, little or no ingested IG is absorbed intact into the systemic circulation.[29] Some oral IG traverses the entire gastrointestinal tract undigested, particularly in premature infants.[74] Oral IG may neutralize microorganisms, inhibit colonization, and prevent microbial attachment to the gastrointestinal mucosa.

ROTAVIRUS INFECTION. Barnes and colleagues[53] fed human IG or placebo for 7 days to premature infants in a nursery in which rotavirus was endemic. Rotavirus-associated diarrhea developed in 6 of 11 babies given placebo and in 1 of 14 given oral IG.

Losonsky and coworkers[363] administered oral human IG to two children with severe combined immunodeficiency and chronic rotavirus infection and demonstrated a decrease in the amount of free rotavirus excretion and survival of the IG through the gastrointestinal tract. Kanfer and associates[301] used oral human IG to treat rotavirus infection that occurred after bone marrow transplantation.

Bovine hyperimmune colostral immunoglobulin enriched in rotavirus antibodies (from immunized cows) also has been used in the prevention of rotavirus infection. Davidson and colleagues[148] prevented rotavirus infection in 55 children admitted to an Australian hospital by administering a 10-day course of bovine colostrum; 9 of 65 control children became infected.

Turner and Kelsey[603] added at least 360 mL of cow colostrum to the formula of 31 term infants aged 3 to 7 months for an average of 101 days (range, 16 to 202 days). Rotavirus infections occurred in 11 (35%) of the colostrum-fed group and 14 (42%) of the control group, an insignificant difference, but symptomatic infection occurred in 1 (3%) of the infants receiving colostrum and 6 (18%) of the controls.

This product also has been used in two studies of the treatment of rotavirus diarrhea in Bangladesh.[403,514] In both studies, less diarrhea, fewer days of illness, less stool output, and more rapid clearance of rotavirus from the stool were noted. Guarino and coworkers[229] used oral human IG (300 mg/kg) as a single dose and observed more rapid recovery from rotavirus diarrhea.

In sum, oral IG is a promising but unproven agent for the prevention and treatment of rotavirus infection.

NECROTIZING ENTEROCOLITIS. Eibl and colleagues[164] were able to prevent necrotizing enterocolitis in all 90 infants given oral IG rich in serum IgA. Six cases occurred in 91 control infants. Rubaltelli and colleagues[497] in Italy achieved similar results with oral monomeric IgG.

CRYPTOSPORIDIAL INFECTION. Bovine colostrum was used successfully to treat cryptosporidial diarrhea in HIV infection.[530]

Borowitz and Saulsbury[82] used oral IG successfully to treat cryptosporidial infection in a child with acute leukemia.

OTHER DIARRHEAS. Oral human IG has been used in the post–bone marrow transplantation period to prevent the development of viral gastroenteritis[301] and to treat nonspecific diarrhea in immunodeficient subjects.[397] Commercial products from bovine colostrum are available in Sweden and India.[188,461]

Considerable experimental study in veterinarian medicine is available on other oral immunoglobulins.

Aerosolized and Intratracheal Immunoglobulin

Aerosolized and intratracheal immunoglobulin has been used with encouraging results in experimental models of pneumonia caused by respiratory syncytial virus (RSV), pneumococci, staphylococci, *Yersinia pestis*, and parainfluenza and influenza viruses.[217,257,446,479-481,489] Aerosolized monoclonal antibodies also are being studied for specific infections, bioterrorist toxins (e.g., anthrax, ricin), and lung immunomodulation.[174,347,471,619]

Rimensberger and colleagues[489] conducted a placebo-controlled trial of aerosolized human IG (not RSV-IVIG) in the

treatment of RSV infection. No significant benefit was found, but the treatment was well tolerated.

Heikkinen and coworkers[248] used an IgA-enriched human IG as a nasal spray twice daily for 8 weeks to reduce respiratory infections in children between 1 and 4 years of age attending daycare. A 42 percent reduction was achieved in the 19 children receiving the IG versus the 20 children in the placebo group. The authors suggested that this treatment also may decrease the incidence of otitis media.

Intrathecal Immunoglobulin

Human IG has been used intrathecally for the treatment of viral encephalomyelitis in patients with antibody deficiency.[172] Monoclonal antibodies occasionally are given intrathecally in cancer therapy.[559] The use of antitoxin intrathecally in tetanus is discussed in the section on tetanus.

Other Routes

Local application of antibody has been used in the prevention or treatment of surgical or traumatic wounds.[311,470] Injections of the monoclonal antibody bevacizumab (Lucentis) into the vitrea to treat macular degeneration are under way.[141] Inner ear injection of infliximab, a tumor necrosis factor monoclonal antibody, has been used in autoimmune neurosensory hearing loss.[613]

Intra-articular IG was of no clinical benefit to patients with rheumatoid arthritis.[43] We have used IG in the conjunctiva of a child with ligneous conjunctivitis, without benefit.

IMMUNE GLOBULIN IN SECONDARY IMMUNODEFICIENCIES

Many chronically ill patients have a primary illness that results in immunodepression and increased susceptibility to infection. These secondary immunodeficiencies are considerably more common than is primary immunodeficiency and are particularly common developments in hospitalized patients. Although most such patients have cellular (T-cell) deficiencies, a number have antibody deficiencies either isolated or combined with other immune defects. These patients may have low immunoglobulin levels, poor antibody responses to antigenic challenge, and low levels of natural antibodies that may result from loss of immunoglobulin, loss of immune cells, or the toxic effect of therapy or infection on the immune system. Table 256–3 lists diseases and conditions in which secondary antibody immunodeficiency can occur.

Laboratory criteria that support the use of IGIV include significant hypogammaglobulinemia (serum IgG < 200 mg/dL or total immunoglobulin [IgG + IgM + IgA] < 400 mg/dL), absent or unexpectedly low titers of natural antibodies, absent or poor response to antigenic challenge (e.g., tetanus, pneumococcal vaccines), and lack of an antibody response to the infecting organism.[566] Some of the more common secondary immunodeficiencies with antibody defects are discussed.

HEMATOLOGIC AND ONCOLOGIC DISEASES

Antibody deficiencies can occur with multiple myeloma, chronic lymphocytic leukemia, lymphoma, and advanced cancer, aggravated by the immunosuppressive agents used in their therapy. A double-blind multicenter study concluded that the prophylactic infusion of 400 mg/kg of IGIV every 3 weeks reduced the incidence of bacterial infections in patients with chronic lymphocytic leukemia.[138] The treatment group had fewer infections with *Streptococcus pneumoniae* and *Haemophilus influenzae*, but no difference was noted in infections caused by other gram-negative bacteria, fungi, or viruses.

This beneficial effect was confirmed in a subsequent study,[192] and although concern has been raised about its cost-effectiveness,[630] IGIV also has been shown to reduce the incidence of infections in patients with multiple myeloma[124] and in those receiving chemotherapy for lung cancer.[519]

PROTEIN-LOSING STATES: ENTEROPATHY, NEPHROTIC SYNDROME, PLASTIC BRONCHITIS

In some pediatric patients, antibody deficiency results from massive proteinuria (nephrosis), diarrhea (protein-losing enteropathy), or loss into the lung (plastic bronchitis) associated with accelerated IgG loss. Most of these patients have minimal trouble with recurrent infection, probably because antibody synthesis is intact and most likely accelerated; however, if the loss of IgG greatly exceeds synthetic capacity, symptomatic severe hypogammaglobulinemia may result. IGIV infusions can be used diagnostically in such cases; a large intravenous infusion, followed by serial measurements of serum IgG levels, can document an accelerated IgG half-life (i.e., <10 days).

These patients are candidates for IGIV therapy if they have recurrent infections or very low IgG levels (e.g., <200 mg/dL). Administration of large and repeated doses is necessary. On occasion, antibody infusions will help control the severe diarrhea of protein-losing enteropathy.[109] Subcutaneous IG has been used in this situation because its delayed release into the circulation may result in higher long-term IgG levels than can be achieved with an equivalent IGIV monthly dose.[562]

INTENSIVE CARE PATIENTS: TRAUMA, SURGERY, SEPTIC SHOCK

Patients undergoing severe stress associated with trauma or extensive surgery have profound exposure and susceptibility to infection and a spectrum of immune deficiencies, including cutaneous anergy, leukocyte dysfunction, hypogammaglobulinemia, and transiently impaired antibody synthesis.[213,414,644] Bowel stasis and hypotension may promote gram-negative sepsis or endotoxemia, or both, along with the development of severe and often irreversible shock.

Early studies by Ziegler and associates[665] and Baumgartner and colleagues[57] suggested that when antisera to a mutant J5 *Escherichia coli* endotoxin with anti–lipid A activity is used in bacteremic or surgical intensive care unit (ICU) patients, the incidence and severity of severe shock could be reduced. However, Calandra and colleagues,[106] using a human IGIV to J5 *E. coli* in 71 patients with gram-negative infections and shock, could not confirm their results. They gave a single infusion of 200 mg/kg; a control group received a similar dose of regular IGIV. No differences were found in mortality rates, onset of time to shock, and complications.

Just and coworkers[294] administered regular IGIV and antibiotics to 50 patients in the ICU thought to have infection and compared their outcomes with those of 54 control patients who received antibiotics alone. Although no difference in survival occurred, they noted a trend indicating that the IGIV-antibiotic group had a shortened stay in the ICU, a shorter period in which respirator therapy was required, and improved renal function as well as a favorable effect on infection (i.e., infections were a less likely cause of death in these patients).

Rodriquez and colleagues[491] gave an IgM-enriched IGIV or albumin to 56 patients with sepsis undergoing abdominal surgery and found a reduction of sepsis from 55 to 25 percent, a significant difference. Another multicenter study of 352 postsurgical

patients confirmed the observation that standard IGIV (400 mg/kg at weekly intervals) reduced the incidence of infections and shortened the stay in the ICU compared with patients treated with placebo or hyperimmune core-lipopolysaccharide IG.[272]

Werdan[634] has reviewed the use of IGIV in sepsis and suggests that certain specific subgroups may benefit (e.g., postoperative sepsis, septic shock with endotoxinemia, and sepsis with neutropenia). A single-institution study from Egypt suggested that IGIV was of benefit in children younger than 2 years in the ICU who had sepsis.[167]

Studies of IGIV in trauma patients requiring surgery[152,157,209] and in patients with head trauma[644] have shown questionable efficacy.

Monoclonal antibodies to endotoxin have been tested in clinical trials of patients in septic shock, but none was of proven efficacy.[418,635,664] Monoclonal antibody to tumor necrosis factor–α also has shown no efficacy in the treatment of adults with septic shock.[2,136] Monoclonal antibodies to interleukin-6 or interleukin-1 receptor likewise have not been shown to be effective in the treatment of shock.[196]

In summary, no compelling data suggest that antibody, either polyclonal or monoclonal, is of benefit in an acutely ill patient with shock.

PREMATURITY

All premature infants have low levels of maternally derived IgG at birth, and levels approaching 100 mg/dL will develop in the first months of life in most.[48] These IgG levels may be further depressed by pulmonary disease (with transudation into the lungs), fever and stress (with increased IgG catabolism), and multiple blood sampling.[500] In addition, their sluggish antibody responses, their concurrent IgM and IgA deficiency, and their immature complement, phagocytic, and T-cell systems render low-birth-weight infants extraordinarily susceptible to development of infection.[356]

Attempts to decrease the incidence of infections in premature infants by using periodic IG injections date to the 1960s. Four controlled studies enrolling a total of 363 infants used IG doses of 80 to 240 mg/kg per month during their stay in the nursery (two studies), for 4 months, and for 1 year.[11,137,154,557] In two studies, a slight decrease in the number and severity of infections was noted, but no difference in survival. These mixed results suggest that IG at the doses used was not of prophylactic value in these patients.

In the last 2 decades, the increasing rate of survival of very tiny premature infants and the availability of IGIV have reawakened interest in the use of antibody to prevent infections in premature infants. In general, IGIV is well tolerated, with minimal adverse effects, in doses of up to 1 g/kg per day.

An initial double-blind clinical trial by Baker and colleagues[44] suggested that IGIV at a dose of 0.5 g/kg at frequent intervals in the first weeks of life significantly reduced the frequency of sepsis, particularly that caused by *Staphylococus epidermidis.* Subsequent studies, however, have not shown a clear benefit.

Meta-analyses of prospective, randomized, placebo-controlled prevention studies (representing 5000 infants) were performed by Jenson and Pollock[282,283] and Ohlsson and Lacy.[438] These studies differed in entry criteria, dose, and brand of IGIV, but most infants were less than 2000 g, were given IGIV therapy within the first week of life, and received doses of at least 400 mg/kg per month; culture-proven sepsis was used as an end-point. Overall, there was a slight reduction (3%) in the incidence of sepsis but no difference in mortality rates, length of stay, or other complications of prematurity, such as necrotizing enterocolitis, bronchopulmonary dysplasia, and intraventricular hemorrhage.

By contrast, IGIV has been of significant benefit in the meta-analysis of six controlled studies for the treatment of neonatal sepsis. For infants with proven sepsis, IGIV reduced the mortality in the control group from 20 percent (27 deaths among 133 infants) to 11 percent (14 deaths among 129 infants; $p = .04$).[439] Among infants with suspected sepsis, there was suggestive evidence of benefit ($p = .05$).

Shamin and coworkers[526] gave sufficient IGIV for 6 months to 15 of 30 matched premature infants with severe bronchopulmonary dysplasia to maintain their IgG levels above 400 mg/dL, whereas the untreated infants had levels below 200 mg/dL. The number of infections, notably pneumonia, was significantly reduced in the IGIV group (5 episodes of pneumonia and 4 other infections) in comparison with the control group (15 episodes of pneumonia and 12 other infections).

Thus, the evidence to date still supports the 1990 National Institutes of Health consensus statement that IGIV should not be given routinely to infants of low birth weight[425] but that it may be of value in selected premature infants at high risk for acquiring infection.

IGIV is sometimes of benefit in premature infants with a history of serious infections and underlying lung disease; most such infants will have hypogammaglobulinemia and should be regarded as having transient hypogammaglobulinemia of infancy. Such infants also need RSV prophylaxis with palivizumab.

Hyperimmune IGIV or monoclonal antibodies enriched in antibodies against specific microbial antigens (e.g., *Staphylococcus aureus*) may be of benefit to certain high-risk newborn and premature infants. Two large multicenter double-blind clinical trials of *S. aureus* hyperimmune human immunoglobulins were completed in infants of very low birth weight. One used IG from donors immunized with a staphylococcal vaccine against serotypes 5 and 8 (termed Altastaph).[61,377] The other used IG from donors with a high titer to C1fA, a staphylococcal fibrinogen-binding protein (termed Veronate).[72,110,150,305] Neither trial showed a significantly decreased severity or incidence of staphylococcal infections.

TRANSPLANTATION

Conditioning regimens to eliminate or to reduce the host's hematopoietic and immune systems before transplantation (bone marrow and solid organ) render these patients extremely susceptible to infection, particularly cytomegalovirus (CMV) infections.[604] Thus, IGIV and CMV-IGIV have been used to prevent infection as well as to modify graft-versus-host disease and to prevent solid organ rejection.

Early studies of IGIV therapy after allogeneic bone marrow transplantation were modestly successful in preventing infections, decreasing graft-versus-host reaction, and increasing survival rates.[56,139,218,577-579] This effect was less dramatic when CMV infection was controlled by ganciclovir and screening of blood products.[231] Two more multicenter studies showed only a modest or no effect on modifying acute graft-versus-host reactions or mortality rates.[140,646] In single-center studies, CMV-IGIV was of no benefit in CMV-positive lung transplant recipients[332] or liver transplant recipients.[222]

IGIV is of benefit in adult kidney transplant recipients with high titers of HLA antibodies. They have a shorter time to transplantation, reduced HLA antibody titers, and improved allograft survival rates.[289,290]

BURNS

Bacterial sepsis, particularly *Pseudomonas* spp. and *E. coli* sepsis, is the leading cause of death in the 300,000 patients hospitalized

annually in the United States for burns.[413,414] The protein loss in these patients induces hypogammaglobulinemia in proportion to the severity of the burn. High-dose IGIV prolongs survival in experimentally burned mice infected with *Pseudomonas* spp., and preliminary studies of hyperimmune IG and plasma in human burn patients were encouraging, but proof of efficacy is lacking[310,472,574] (see later section on burn infections).

HUMAN IMMUNODEFICIENCY VIRUS INFECTION

The rationale for use of IGIV in patients with advanced HIV disease is their increased susceptibility to common bacterial and viral infections, poor primary antibody responses to vaccine antigens despite hypergammaglobulinemia, and, in young children, a limited antibody spectrum to common bacterial pathogens. Although the central immune defect in acquired immunodeficiency syndrome (AIDS) is a loss of helper T-cell ($CD4^+$) number and function, the polyclonal B-cell activation and defective helper T-cell function result in a significant B-cell deficiency.[66,431]

Thus, in children with AIDS, bacterial infections occur more commonly than do opportunistic infections. Immune-mediated thrombocytopenic purpura and viral diseases (e.g., RSV and parvovirus and other disorders amenable to IGIV prophylaxis or therapy) also may develop in patients with AIDS.

After preliminary uncontrolled studies suggested a benefit of IGIV in children with advanced HIV infection,[107,537] two large multicenter controlled studies undertaken by the National Institutes of Health–supported Pediatric AIDS Clinical Trial Group determined the efficacy of IGIV in decreasing infections and improving survival in AIDS patients.[419,555]

In the first study, 372 children received 400 mg/kg of IGIV every 4 weeks or an albumin control for 2 years.[419] Thirty percent of all children in the IGIV-treated group had serious infections compared with 42 percent in the placebo group. Children who were less ill (those with $CD4^+$ lymphocyte counts >200/mL) benefited in particular from IGIV. Children in this category had fewer serious infections and were hospitalized less frequently than were those in the placebo group, and their $CD4^+$ counts dropped less rapidly than did those of the placebo group.[405] The mortality in both groups, however, was identical. Children with $CD4^+$ counts less than 200 cells/mL (i.e., those with severely impaired immunity) who were given IGIV had no significant decrease in infections, days of hospitalization, or mortality compared with their counterparts in the placebo group.

The authors concluded that IGIV significantly reduced the risk for development of serious infection in some children with symptomatic HIV infection, primarily those with $CD4^+$ counts between 200 and 500 cells/mL. In a follow-up study, the placebo group was allowed to cross over to receive IGIV, with a drop in the rate of serious infections and hospitalizations.[408]

In a second trial of IGIV in which all the children received zidovudine, a significant decrease in serious bacterial infections was found again, but this benefit was limited to children who did not receive trimethoprim-sulfamethoxazole prophylaxis for *Pneumocystis jiroveci* pneumonia.[555] A consensus of an HIV Working Group was that HIV-infected children with significant hypogammaglobulinemia or documented poor antibody formation may be candidates for receiving IGIV therapy.[652] IGIV also may be of benefit to HIV-infected children who have recurrent infections not controlled by antibiotics or chronic nonspecific diarrhea with failure to thrive.

IGIV also prevents serious infections in adults with advanced HIV infection.[317]

In addition to preventing serious bacterial infections, IGIV given to children with HIV infection reduced the number of nonserious bacterial infections (by 60% for ear infections, 13% for skin infections, and 10% for other upper respiratory infections) and viral infections by approximately a third in both trials.[404,406-408,555]

Regular IGIV infusion also improved left ventricular function in HIV-infected children with dilated cardiomyopathy.[361] It is also effective in treating certain other complications of HIV infection, including thrombocytopenia,[105,343] red cell aplasia after erythrovirus (parvovirus B19) infection,[325] Guillain-Barré syndrome,[206] and certain drug reactions.[184] IGIV, even in high doses, provides no antiviral activity to HIV.[443]

INTRAVENOUS IMMUNE GLOBULIN IN IMMUNOREGULATORY AND NEUROLOGIC DISORDERS

High-dose IGIV has immunosuppressive and anti-inflammatory effects that render it a valuable agent in the treatment of several autoimmune, inflammatory, and neurologic disorders (Table 256–4).[162,443] A consensus committee has evaluated these uses in terms of likely efficacy.[443]

High-dose IGIV (1 to 2 g/kg) may work by one or more of the following mechanisms:[49,162,540,589]

1. IGIV may decrease the high levels of a pathogenic antibody (such as may be present in myasthenia gravis or idiopathic thrombocytopenic purpura) by accelerating IgG catabolism. IGIV increases IgG levels acutely and increases its catabolism, thus decreasing pathogenic antibodies present in high concentrations. It also saturates the FcRn receptor, thus inhibiting the recirculation of IgG molecules.[658] IGIV also inhibits antibody synthesis by having a direct effect on proliferating B cells, possibly through activation of the FcRII receptor.[510]
2. IGIV contains anti-idiotypic antibodies that can combine with autoimmune antibodies and remove them rapidly from the circulation, which may be a mechanism by which IGIV reduces anti–human leukocyte antigen (HLA) antibodies in transplant recipients.
3. IGIV binds to Fc receptors on reticuloendothelial cells and causes an Fc receptor blockade, thus reducing the destruction of antibody-coated cells in the spleen or liver. It is a mechanism for the rapid response to IGIV noted in patients with immune thrombocytopenic purpura. Dimeric IGIV may be particularly effective in causing FcR blockage.
4. IGIV combines with other surface receptors to inhibit cellular activation; for example, in toxic epidermal necrolysis, IGIV prevents Fas-mediated cell death of keratinocytes by blocking the Fas receptor.[617]
5. IGIV may promote solubilization and clearance of pathologic immune complexes, such as may be present in lupus nephritis.
6. IGIV down-regulates T-cell immune activation by decreasing inflammatory cytokine release or action. In Kawasaki disease, an immediate damping of the cytokine "storm" occurs with this illness.
7. IGIV antibodies neutralize bacterial superantigens and prevent them from activating T cells. It is the probable mechanism for its beneficial effect in toxic shock syndrome.[556,589]
8. IGIV combines with complement components to prevent complement-mediated tissue injury. An example is inhibition of complement-mediated myocyte damage in dermatomyositis.
9. IGIV neutralizes virus or bacterial antigens or toxins that may trigger or cause the disease. An example may be the neutralization of bacterial products in hemolytic-uremic syndrome.
10. IGIV contains substances such as soluble HLA antigens and soluble cytokine receptors that may inhibit immune responses.

TABLE 256–4 Likelihood of Benefit of Intravenous Immune Globulin (IGIV) in Autoimmune, Inflammatory, and Neurologic Diseases

Proven benefit*
Kawasaki disease
Graves ophthalmopathy
Immune thrombocytopenic purpura (e.g., idiopathic)
Dermatomyositis
Post–bone marrow transplantation
Neurologic diseases
  Guillain-Barré syndrome
  Chronic inflammatory demyelinating polyneuropathy
  Multifocal motor neuropathy
  Demyelinating neuropathy with IgM gammopathy

Probable benefit†
Toxic shock syndrome
Post-infectious thrombocytopenic purpura
Neonatal isoimmune or autoimmune thrombocytopenic purpura
Neonatal isoimmune or autoimmune hemolytic anemia
Autoimmune or isoimmune neutropenia
Autoimmune hemolytic anemia
Toxic epidermal necrolysis
Prevention of antibody-mediated kidney transplant rejection
Autoimmune uveitis
Neurologic diseases
  Polymyositis
  Lambert-Eaton myasthenia syndrome
  Myasthenia gravis
  Stiff-man syndrome
  Relapsing-remitting multiple sclerosis

Possible benefit‡
Anticardiolipin antibody syndrome
Coagulopathy with factor VIII inhibitor
Bullous pemphigoid and other blistering skin diseases
Churg-Strauss vasculitis
Vasculitis with antineutrophil cytoplasmic antibodies
Other vasculitides
Stevens-Johnson syndrome
Severe rheumatoid arthritis
Systemic lupus erythematosus
Autoimmune diabetes mellitus
Post-transfusion purpura
Prevention of allograft rejection in high-risk patients with solid organ transplants
Recurrent spontaneous abortion
Cardiomyopathy
Severe persistent high-dose steroid-dependent asthma
Chronic urticaria
Autoimmune liver disease
Delayed pressure urticaria
Neurologic diseases
  Multiple sclerosis
  Rasmussen syndrome
  Intractable childhood epilepsy
  Demyelinating neuropathy with anti-GAM antibodies
  Acute disseminated encephalomyelitis
  Cerebral infarction with antiphospholipid antibodies
  HTLV-1–associated myelopathy
  Lumbosacral or brachial plexitis
  Paraproteinemic neuropathy
  Opsoclonus-myoclonus
  Postinfectious cerebellar ataxia
  Acute idiopathic dysautonomia

Unproven or no benefit§
Non–steroid-dependent asthma
Atopic dermatitis
Epidermolysis bullosa acquisita
Hemolytic-uremic syndrome
Thrombotic thrombocytopenic purpura
Pure red cell or white cell aplasia
Acquired von Willebrand disease
Chronic fatigue syndrome
Infantile autism
Acute myocarditis
Chronic graft-versus-host disease
Inflammatory bowel disease
Neurologic diseases
  Inclusion body myositis
  Amyotrophic lateral sclerosis
  Paraneoplastic cerebellar degeneration, sensory neuropathy, encephalitis syndrome (POEMS)
  Sensory neuropathy
  Pediatric autoimmune neuropsychiatric disorders associated with streptococcal infections (PANDAS)
  Alzheimer's disease

Levels of benefits:
**Controlled studies demonstrate efficacy.*
†Several case reports or uncontrolled series are convincing.
‡Preliminary studies are encouraging but incomplete.
§Preliminary studies are limited, equivocal, or negative.
HTLV, human T-lymphotropic virus; POEMS, polyneuropathy, organomegaly, endocrinopathy, M protein, skin changes.

The illnesses for which IGIV may be of benefit often are of unknown cause, refractory to standard treatment, somewhat responsive to steroids, and associated with local or generalized immune activation with fever, inflammatory cells, and cytokine release.[435] Large doses of IGIV (e.g., usually 1 to 2 g/kg/day) are necessary and may need to be repeated at weekly intervals. IGIV preparations may not always be equivalent in action; Tsai and associates[602] found that certain brands of IGIV were less effective in Kawasaki disease than were others.

If a favorable response does not occur immediately or within a few weeks, it probably will not occur later. Proof of efficacy requires a double-blind study (e.g., as conducted for Kawasaki disease) or dramatic reversal of progressive severe disease (e.g., as noted in some cases of toxic shock syndrome). The use of IGIV in Kawasaki disease is covered in another chapter.

MONOCLONAL ANTIBODIES

Monoclonal antibodies specific for a single antigenic epitope have been available for laboratory use for several decades and are used increasingly as therapeutic agents. The first monoclonal antibody, licensed in 1984, was a murine antilymphocyte antibody (anti-CD3 [OKT3]) used for immunosuppression in transplant recipients. Since then, 18 other monoclonal antibodies have been licensed for treatment, and many others are being studied in the United States and abroad (see Table 256–1).[104]

NOMENCLATURE AND STRUCTURE

The generic names of therapeutic monoclonal antibodies have been codified, as summarized in Table 256–2.[104] All end in *mab* or *nab*. Preceding this ending is a one- or two-letter prefix that denotes the animal from which it has been produced (e.g., *o* for mouse, as in murom*o*nab; *u* for human, as in adalim*u*mab).

Two types of monoclonal antibodies contain both human and murine components. Chimeric monoclonal antibodies, designated *xi*, contain the Fab portion of a murine IG and the Fc portion of human IG, usually an IgG1 molecule (e.g., ritu*xi*mab). Humanized monoclonal antibodies, designated *zu*, have only the antibody-combining site of a murine IG (termed the complemen-

tary determinant region), and the rest of the molecule is a human IgG1 molecule (e.g., omali*zu*mab). Chimeric and humanized antibodies are less antigenic and have a longer half-life than do mouse monoclonal antibodies and thus usually are preferred.

One completely human monoclonal antibody is derived from isolated and recombined human L and H chains (adalimumab).

The final prefix before the source prefix designates the disease for which the antibody is used (e.g., *vir* for viral disease, *lim* for immune modulation, *tu* for cancer). Examples include pali*vi*zumab, oma*li*zumab, trans*tu*zumab. Sometimes the last consonant is dropped for the sake of pronunciation. The beginning of the generic name is left to the manufacturer, as is, of course, the trade name.

FRAGMENTED AND CONJUGATED MONOCLONAL ANTIBODIES

Two of the licensed monoclonal antibodies are Fab fragments of the whole molecule. Fragmented molecules retain their antibody activity, but their survival and therapeutic duration are markedly shortened. They include abciximab (ReoPro) directed against platelet glycoprotein IIb/IIIa for prevention of thrombosis and ranibizumab (Lucentis) directed against vascular endothelial growth factor for intravitreous injection into the eye for macular degeneration. It is derived from bevacizumab (Avastin) used for treatment of metastatic cancers.

Three of the licensed monoclonal antibodies are combined with other molecules to increase their therapeutic effectiveness. Two of these are anti-CD20 (B-cell) antibodies similar to rituximab that are radiotagged and used for rituximab-refractory B-cell lymphomas. Ibritumomab (Zevulin) is conjugated to yttrium 90, and tositumomab (Bexxar) is conjugated to iodine 131. Thus, these monoclonal antibodies deliver radiation only to cells carrying the CD20 antigen.

A third tagged antibody is gemtuzumab ozogamicin (Mylotarg) used in the treatment of relapsed acute myelocytic leukemia. It is conjugated with the potent antitumor antibiotic calicheamicin to increase its concentration on myeloid cells expressing CD33.

Uses of Monoclonal Antibodies

Therapeutic monoclonal antibodies developed to date are licensed for graft rejection, cancer, autoimmunity (e.g., rheumatoid arthritis, inflammatory bowel disease, immune cytopenias), thrombosis, infectious disease, multiple sclerosis, asthma and allergy, and macular degeneration. Like many new drugs, monoclonal antibodies also are used for disorders other than those for which they are licensed.

Only one of these antibodies, palivizumab (Synagis), directed against RSV, is used in infectious diseases for the prevention of RSV infection in high-risk infants (see section on RSV).[23] However, monoclonal antibodies to microbial antigens and inflammatory mediators will play an important role in tomorrow's battle with infectious pathogens. For example, monoclonal antibodies to anthrax toxin, staphylococcal antigens, and HIV antigens and co-receptors are being studied in clinical trials.

PASSIVE IMMUNITY IN BACTERIAL DISEASES

Antibodies have been used for a century for the prevention and treatment of infectious diseases (Table 256–5). In bacterial disease, antibodies neutralize toxins, facilitate opsonization, and, with complement, promote bacteriolysis; in viral disease, antibodies block viral entry into uninfected cells, promote antibody-directed cell-mediated cytotoxicity by natural killer cells, and neutralize virus alone or with the participation of complement.

Before the use of antibiotics, antibodies were the only specific agents for the treatment of certain infections. Although this role has been supplanted largely by antibiotics, antibody still has a crucial role in the treatment of certain infectious diseases (see Table 256–5). The remainder of this chapter discusses the roles of antibody in prevention and treatment of bacterial and viral diseases. Other reviews are available.[112,113,351]

ANTHRAX

Anthrax is a rare but serious infectious disease, predominantly of ruminant animals, caused by an aerobic gram-positive rod, *Bacillus anthracis*.[132,367,368] Humans can be infected through the skin (cutaneous anthrax), by ingestion (gastrointestinal anthrax), or by inhaling the spores (inhalational anthrax).[368] Inhalational anthrax results from prolonged exposure to animal hides or carcasses and infected soil or, rarely, by deliberate spore exposure in the bioterrorism setting, as occurred among U.S. postal workers in 2001.[284,367]

After inhalation, the spores are ingested by alveolar phagocytic cells and transported to regional nodes, where they germinate and release exotoxins (lethal toxin and edema toxins), which damage cell membranes, increase capillary permeability, initiate pulmonic changes, and ultimately lead to shock and cardiovascular collapse.

A vaccine is available for individuals at high risk for exposure and the military. It is effective against cutaneous anthrax but of unproven efficacy against inhalational anthrax.[83] Others are in development.[85]

Before the antibiotic era and as early as 1903, anthrax antitoxin (usually equine) was used in therapy for anthrax.[211,322] An antitoxin would be of value in a bioterrorism attack, both before exposure and after exposure. The Centers for Disease Control and Prevention (CDC) and the National Institutes of Health are collecting plasma units from military recruits and volunteers immunized with the anthrax vaccine[171] to develop a human high-titer IGIV from these donors.[133]

Several monoclonal antibodies against anthrax antigens have been produced.[31,88,126,266,373,384,409,576,626] The best studied is a human monoclonal antibody 5H3 (ABthrax).[126] ABthrax was safe and well tolerated either intravenously or intramuscularly in human volunteers and provided good antibody titers with a half-life of 15 to 19 days.[576] The U.S. government plans to buy 20,000 treatment doses of this monoclonal antibody for the Strategic National Stockpile, contingent on approval by the Food and Drug Administration.[254]

Recommendation

No current commercial antitoxin is available in the United States for prophylaxis or treatment. The only available source is the limited supply of immune plasma stockpiled by the U.S. army. This supply would be used in conjunction with antibiotics and with vaccine, the latter to provide immunity after metabolism of the antibody.

BOTULISM, BOTULINUM ANTITOXIN, AND BOTULISM IMMUNE GLOBULIN

Botulism, a severe paralytic poisoning, results from ingestion or absorption of neurotoxins or spores from *Clostridium botulinum*. Several clinical variants are recognized: food poisoning from ingestion of contaminated canned food, wound botulism from a

TABLE 256–5 Summary of the Efficacy of Antibody in the Prevention and Treatment of Infectious Diseases

Infection	Prophylaxis	Treatment
Bacterial infections		
Respiratory infections (streptococcal, *Streptococcus pneumoniae*, *Neisseria meningitidis*, *Haemophilus influenzae*)	Proven (NR)*	Proven (NR)*
Diphtheria	Probable benefit (NR)	Proven
Pertussis	Unproven	Unproven
Tetanus	Proven	Proven
Other clostridial infections		
C. botulinum	Proven	Proven
Infantile botulism	Unproven	Proven
C. difficile	Unproven	Possible benefit
Staphylococcal infections		
Toxic shock syndrome	Unproven	Probable benefit
Refractory infection	Unproven	Possible benefit (NR)
S. epidermidis in newborns	Possible benefit	Unproven
Toxic shock syndrome	Unproven	Probable benefit
Newborn sepsis	Possible benefit (NR)	Probable benefit
Shock, intensive care, and trauma	Unproven	Possible benefit (NR)
Pseudomonas infections		
Cystic fibrosis	Unproven	No benefit
Burns	Unproven	No benefit
Viral infections		
Hepatitis A	Proven	No benefit
Hepatitis B	Proven	No benefit
Hepatitis C	Unproven	No benefit
Human immunodeficiency virus infection	Unproven	Unproven
Respiratory syncytial virus infection	Proven	Unproven
Herpesvirus infections		
Cytomegalovirus	Proven	Possible benefit
Epstein-Barr virus	Unproven	Unproven
Herpes simplex virus	Possible benefit (NR)	Unproven
Varicella-zoster virus	Proven	Unproven
Parvovirus infection	Probable benefit (NR)	Proven
Enterovirus infection in newborns	Unproven	Possible benefit
Enteroviral central nervous system infection	Proven	Proven
Ebola	Unproven	Unproven
Rabies	Proven	No benefit
Measles	Proven	No benefit
Rubella	Unproven	No benefit
Mumps	Unproven	No benefit
Tick-borne encephalitis	Possible benefit	No benefit
Vaccinia	Proven	Proven
Variola (smallpox)	Proven	Unproven

**Except for immunodeficient patients.*
NR, not recommended.
Modified from Keller, M.A., and Stiehm, E. R.: Passive immunity in prevention and treatment of infectious diseases. Clin. Microbiol. Rev. 13*:602-614, 2000.*

contaminated local infection, inhalational botulism among individuals working with the toxin or in bioterrorism,[34] infant botulism from ingestion of *C. botulinum* spores, and adult-type infant botulism in adults with preexisting gastrointestinal disease. In the last two types, ingested spores multiply in the gastrointestinal tract and elaborate toxin, and the absorbed toxin results in a paralytic disorder.[62,123]

There are reports of botulism as a result of the cosmetic use of an unapproved botulinum toxin A.[128,554]

Seven immunologic types of *C. botulinum*, designated A through G, have been identified, each elaborating an immunologically distinct toxin. Almost all human botulism has resulted from the ingestion of toxins A, B, and E, the last usually associated with fish and marine mammal products.[326,327,527]

Japanese studies, reviewed by Dolman and IIda,[155] indicate that antitoxin therapy is effective for type E botulism. The mortality rate associated with type E botulism was 49 percent in 135 untreated cases and 3.5 percent in 85 antitoxin-treated cases.

Tacket and associates[585] noted that 46 patients who had type A food-borne botulism and who received trivalent antitoxin had a lower mortality rate (27%) than did 13 patients who did not receive antitoxin (46% mortality). Early antitoxin administration resulted in a shorter clinical course.

The presence of toxin in the blood long after the appearance of clinical symptoms or the ingestion of toxin gives theoretical support to the use of antitoxin to prevent further binding of toxin to tissue. Some antitoxin remains in the circulation for more than 30 days, thus indicating that a single initial dose is adequate therapy.

Equine antitoxins to types A, B, and E are the primary antitoxins available in the United States. The decision to use antitoxin is complicated by its unknown efficacy and its side effects, but approximately 80 percent of patients with ingestion-type botulism receive antitoxin.[527]

Recommendations

All forms of botulism, except for infant botulism, are treated with bivalent (types A and B) and type E antitoxin as soon as possible, after testing for sensitivity to horse serum.[14,21,123,527] They are

available in the United States from the CDC in Atlanta, Georgia (404-639-3670 or 2888). One vial of bivalent antitoxin contains 7500 IU of type A and 5500 IU of type B antitoxin, and one vial of type E antitoxin contains 5000 IU. They usually are given intravenously. Both vials are given unless the toxin type is known; additional doses usually are unnecessary, but with severe wound infections, higher doses may be needed, as guided by antibody titers.

Antitoxin can be given prophylactically to individuals known to have ingested contaminated food. The risk for development of serum reactions (about 10%) must be weighed against the risk of contracting the disease. An investigational equine botulism heptavalent IG fragment $F(ab')_2$ also is being studied for treatment.[255]

A 5-year, placebo-controlled, double-blind trial of human botulinum intravenous immune globulin (BIG) for infant botulism showed a significant reduction in hospital days, mechanical ventilation, tube feedings, and days of hospitalization in the BIG recipients, with considerable cost savings.[35] BIG should be started immediately after the illness is suspected. The dose is 50 mg/kg. Each vial contains 200 mg and is diluted to a 50 mg/mL 5 percent solution. One treatment costs about $45,000.

OTHER CLOSTRIDIAL INFECTIONS

CLOSTRIDIUM DIFFICILE INFECTIONS

Clostridium difficile is a ubiquitous spore-forming anaerobe often involved in antibiotic-associated diarrhea and pseudomembranous colitis.[14] The severity of the disease can range from an asymptomatic carrier state to fulminant colitis with toxic megacolon. Toxic strains of *C. difficile* release two antigenically distinct toxins, both of which have potent cytotoxic and inflammatory properties.[135] Infection generally leads to an antibody response to the toxins, and most individuals older than 2 years have such antibodies; thus, *C. difficile* antibodies are present in IGIV preparations.[507]

High levels of IgG antibodies acquired after colonization are associated with the asymptomatic carrier state; individuals who have persistent diarrhea have significantly lower serum IgG but not IgA to toxin A.[345] Several patients have been identified with relapsing symptomatic disease who have low IgG antibody levels to toxin A.[244,353] Some of these patients had low levels of IgG[353] or IgG1 subclass deficiency[244] and were treated successfully with IGIV.

Recommendations

Patients with refractory *C. difficile* infections (with or without a global antibody deficiency) can be treated with 300 to 500 mg/kg of IGIV every 1 to 3 weeks.[292,393,637,638] Such therapy has been shown to increase antitoxin levels, to control diarrhea, and to prevent relapses during the treatment period.[353,507] However, controlled trials have not been conducted, and thus IGIV treatment is unproved.[286,292]

Studies with an oral anti–*C. difficile* bovine IG for both treatment and prevention were initiated several years ago.[629]

GAS GANGRENE (*CLOSTRIDIUM PERFRINGENS*)

Treatment of gas gangrene with equine antitoxin to *Clostridium perfringens* was of unproven efficacy and no longer is available. Food poisoning caused by *C. perfringens* is self-limited and is not treated with antitoxin.

TETANUS (*CLOSTRIDIUM TETANI*)

See the later section on tetanus, tetanus antitoxin, and tetanus immune globulin.

DIPHTHERIA AND DIPHTHERIA ANTITOXIN

Diphtheria is caused by toxigenic strains of *Corynebacterium diphtheriae* and occasionally *Corynebacterium ulcerans*. Diphtheria is exceedingly uncommon now because of widespread immunization, but a massive epidemic in the countries of the former Soviet Union occurred in the 1990s, with many fatalities.[296,620]

Many of the adverse consequences of diphtheria result from the toxin elaborated and absorbed from the diphtheritic membrane. This toxin not only has a local effect of perpetuating membrane formation but also is distributed through the blood to the heart, nervous system, kidney, and other organs. The larger the membrane, the more toxin elaborated; in addition, more toxin is elaborated from a membrane involving the pharynx and tonsils than from one involving the larynx and trachea.

Diphtheria toxin is present in three forms: (1) circulating and unbound, (2) loosely bound to tissues, and (3) firmly bound to tissues. Antitoxin neutralizes circulating toxin and competes with and partially neutralizes loosely bound toxin but has no effect on tissue-bound toxin. Thus, optimal passive immunity must be initiated at an early stage of the disease by the intravenous route so that toxin can be intercepted before it becomes tissue bound.

In the 1890s, diphtheria antitoxin was used successfully to prevent spread to newly admitted pediatric patients and nurses.[173a]

Antitoxin of animal origin remains the mainstay of treatment, as it was in the pre-antibiotic era. Diphtheria was the first illness in which antiserum was used as standard therapy. Emil von Behring was awarded the first Nobel Prize in Medicine (in 1901) for this achievement. Fibiger[183] proved the efficacy of diphtheria antitoxin in 1898 when he showed that 5 of 204 patients given horse antitoxin died, whereas 14 of 201 not given antiserum died. Paschlau[458] demonstrated the importance of early administration of antitoxin in 1949; the fatality rate of 197 cases treated within 48 hours was 1.96 percent versus 8.9 percent when treatment was delayed until the fourth or fifth day. Other authors have noted similar findings.[592,593]

Tasman and associates[592,593] emphasized the importance of intravenous administration of antitoxin because rapid achievement of high blood levels results in rapid neutralization of antitoxin and the appearance, within 30 minutes, of antitoxin in saliva. They showed that the mortality rate and the severity of the myocarditis and neuritis in experimental diphtheria in guinea pigs could be reduced by giving antitoxin intravenously rather than intramuscularly.

McCloskey and Smilack[386] determined the antitoxin content of standard human IG. None of the lots tested contained diphtheria antitoxin in sufficient titer to allow its use for antitoxin therapy. They suggested that an IVIG with higher titer antitoxin be developed to eliminate the risk of giving horse serum intravenously. Indeed, such a product is produced and used in the Ukraine.[178]

Recommendations

Diphtheria antitoxin of equine origin is indicated for all suspected or proven cases of diphtheria.[15] It is available in vials containing 20,000 U from the CDC. Before administration, skin tests must be performed to determine sensitivity. If the patient has a previous history of serum reactions or these test results are

positive, a schedule of desensitization as outlined earlier must be followed.

The amount of antitoxin given depends on the location and the extensiveness of the membrane, the degree of systemic toxicity, and the duration of illness. The preferred route is intravenous, although intramuscular administration has been used historically in milder cases.

In all cases, diphtheria antitoxin should be given promptly rather than be delayed while awaiting bacterial confirmation of the diagnosis.[15]

In cutaneous diphtheria, antitoxin is of uncertain value. When used, the dose is 20,000 to 40,000 U to prevent toxic sequelae. In pharyngeal or laryngeal disease, 20,000 to 40,000 U is given; in nasopharyngeal disease, 40,000 to 60,000 U is given; and in extensive disease with neck edema or disease of more than 3 days' duration, 80,000 to 120,000 U is given.[15] Although antimicrobial therapy is a valuable aid in the treatment of diphtheria, it is not a substitute for antitoxin therapy.

Routine use of antitoxin in an asymptomatic, exposed susceptible patient is not recommended. With heavy exposure or an extremely susceptible host, antitoxin, 5000 to 10,000 U intramuscularly, can be given in addition to antibiotics and diphtheria immunization. Proof of efficacy is lacking, however.

Human IGIV has variable amounts of diphtheria antitoxin, so it cannot be used as a replacement for animal antitoxin.[15]

PERTUSSIS

Pertussis antiserum was used in the 1930s for the treatment of pertussis.[84] Human pertussis IG was developed in the 1960s but was shown to have no additive benefit to antibiotics in the treatment of pertussis[45,410] and no longer is available commercially.

Granström and coworkers[219] used an experimental human hyperimmune serum from subjects immunized with a two-component acellular vaccine to treat 33 children, and an equal number received an albumin placebo. Both groups received the same antibiotic and supportive treatment. The treated children had decreased coughing and whooping, particularly if treatment was started early.

Ichimaru and colleagues[268] successfully used a high-titer human IGIV preparation to treat a severely ill 1-year-old child.

Bruss and associates[97] studied a 4 percent high-titer human pertussis IG in 26 children with pertussis and found that the product was safe at three dose levels (250, 750, and 1500 mg/kg) and provided good serum pertussis IgG levels with a half-life of 38 days. Little antibody appeared in nasal secretions. Efficacy was not evaluated in this study, but the product was effective in the treatment of aerosol-induced pertussis in mice.[96]

A subsequent randomized placebo-controlled trial was begun that enrolled 25 infants; it was terminated because of unavailability of study product. Preliminary analysis did not suggest a clinical benefit in symptoms such as cough, apnea, and oxygen desaturation compared with the placebo.[239]

Recommendation

No agent is available for passive immunity for pertussis.

RESPIRATORY AND OTHER BACTERIAL INFECTIONS

Respiratory tract infections caused by group A streptococci, *S. pneumoniae*, *H. influenzae* type b, and, to a lesser extent, *Neisseria meningitidis*, *Chlamydia pneumoniae*, and *Mycoplasma pneumoniae* are well recognized as occurring more frequently in patients with primary antibody deficiencies, and these infections can be reduced markedly by the regular administration of IGIV or IGIM.[351,433] Furthermore, specific animal antisera to some of these organisms were used in the early 1930s for the treatment of severe infections (e.g., meningitis), even after the introduction of sulfonamides.[10] Efficacy varied but was clearly better than no treatment at all, and a combination of sulfonamides and antibody seemed to be synergistic.[10]

Small doses of IGIM (100 mg/kg per month) did not prevent or improve the course of respiratory infections in normal children.[54,186] However, Nydahl-Persson and colleagues[429] gave 24 children with repeated bacterial respiratory infection (pneumonia or otitis) either trimethoprim-sulfamethoxazole or IGIV, 400 mg/kg per month; both agents were effective in reducing the number of infections in comparison with a control group.

Santoshan and associates[513] administered a human intramuscular IGIM prepared from the sera of donors immunized with pneumococcal, meningococcal, and *H. influenzae* type b polysaccharide vaccines (termed bacterial polysaccharide immune globulin [BPIG]) to Apache Indian infants living on reservations in Arizona. The 222 infants in the study group received 80 mg/kg of BPIG at 2, 6, and 10 months of age, and the 218 infants in the control group received saline injections at the same ages. During the study period, seven cases of invasive *H. influenzae* type b disease and four cases of invasive pneumococcal disease occurred in the control group compared with one and two cases, respectively, in the BPIG-treated group, a significant difference ($p < .05$).

OTITIS

Immunologically normal otitis-prone children seem to derive little preventive or therapeutic benefit from low-dose IG given intramuscularly (100 mg/kg per month)[291] or intravenously (200 mg/kg per month).[299]

In patients with primary immunodeficiency, the frequency and severity of otitis are diminished dramatically by the use of IG in large doses. In children with secondary antibody deficiency associated with HIV infection, IGIV in large doses (400 mg/kg per month) reduced the frequency of otitis by 60 percent.[407,408] Patients with subtle immunologic abnormalities such as IgG subclass deficiencies, partial IgA deficiency, and polysaccharide antibody deficiencies may have recurrent otitis that can be reduced with a large dose of IGIV (e.g., 400 mg/kg per month).[299,323,429,534,539]

BPIG also was shown to reduce the number of episodes of pneumococcal otitis media in high-risk Native American infants.[534] It did not, however, decrease the total number of episodes of otitis media. Large doses of RSV-IVIG (750 mg/kg per month) reduced the frequency of non-RSV otitis in young infants.[541] This product may have high antibody titers to bacteria and other viruses.[169]

Ishizaka and coworkers[275] successfully treated seven children with recurrent pneumococcal otitis with IGIV.

These studies indicate that low-dose IGIM is ineffective in the prevention or treatment of otitis, but high doses of IGIV (e.g., >400 mg/kg per month) may reduce the frequency and severity of otitis in immunodeficient and otitis-prone normal children, probably by reducing both virally and bacterially mediated disease.[243] Englund and Glezen[169] suggested that passive immunization transplacentally from a recently immunized pregnant mother with *H. influenzae* or pneumococcal vaccine also should be considered.

IGIV should not be used routinely for otitis-prone normal children but in extreme cases can be considered after failure of prophylactic antibiotics and pneumococcal immunization.

SINUSITIS

Sinusitis is a common occurrence in immunodeficient patients and is difficult to prevent or to eradicate, even with optimal treatment. Mofenson and colleagues[407] found that neither IGIV, 400 mg/kg per month, or three times weekly sulfonamide prophylaxis prevented sinusitis from developing in children with HIV infection.

In patients with no or subtle immune defects (e.g., IgG subclass deficiencies, impaired polysaccharide antibody deficiencies), high-dose IGIV sometimes may decrease the frequency and severity of sinusitis.[86,127,478,539]

LOWER RESPIRATORY TRACT INFECTIONS

Although high-dose IGIV is of proven benefit in preventing pneumonia in immunodeficient subjects,[103] no evidence has established that it will prevent pneumonia in normal subjects. Anecdotal cases of IGIV used as adjunctive therapy for refractory viral pneumonia have been reported.[505,569]

CYSTIC FIBROSIS

Winnie and associates[645] suggested that IGIV may improve pulmonary function in pulmonary exacerbations of cystic fibrosis. All subjects received antibiotics, were older than 12 years, and had no long-term benefit from the IGIV. Van Wye and coworkers[614] had similar results with the use of hyperimmune *Pseudomonas* IVIG in patients with cystic fibrosis.

A controlled trial of *Pseudomonas* hyperimmune IGIV in 116 patients with cystic fibrosis was discontinued because a 6-month interim analysis showed no reduction in acute pulmonary exacerbations.[474]

Balfour-Lynn and colleagues[47] suggested that high-dose IGIV was of short-term benefit, both in improving pulmonary function and in decreasing the need for steroids, in selected patients with cystic fibrosis and respiratory obstruction receiving inhaled and oral steroids.

BURN INFECTIONS

Kefalides and associates[310] reduced the mortality rate of severely burned children by administering plasma (1 mL/kg for each 1% of surface area burned) or IGIM (1 mL/kg on days 1, 3, and 5) from 40 to 20 percent. They concluded that solutions containing antibodies (plasma or IG) were more effective in reducing the complications of infections than were other colloids. However, Stone and colleagues[574] could not achieve any clinical benefit from IGIM therapy (0.4 mL/kg every third day until skin coverage) in 60 burned subjects versus 40 controls.

Convalescent plasma, special IG with high antibody titer to *Pseudomonas*, and *Pseudomonas* vaccines also have been used in burn patients in an attempt to reduce infections, but without proof of efficacy.[143,156,288,416,533]

An observational study suggested that the combined use of IGIV and polymyxin B reduced the number of septic episodes and shortened the length of hospital stay in severely burned children.[372]

GRAM-NEGATIVE INFECTIONS

The most extensive use of IG for infections in adults has involved trauma, shock, and postoperative patients thought to have gram-negative infections, as reviewed earlier under secondary immunodeficiency.[57,106,152,209,294,664,665] A meta-analysis involving 492 patients receiving polyclonal IGIV for sepsis and septic shock suggested a benefit, particularly among adults.[9] Monoclonal antibodies to endotoxin or cytokines were not of benefit. We conclude that this is a promising yet unproven use for IGIV.

NEWBORN SEPSIS

As noted earlier, newborns and premature infants in particular are highly susceptible to bacterial sepsis and its sequelae. In addition to antibiotics, leukocytes, granulocyte colony-stimulating factor, and IGIV have been used as adjunctive therapy. As discussed earlier under secondary immunodeficiencies, numerous studies on the prevention and treatment of newborn sepsis are available and suggest only slight benefit in prevention but significant benefit in treatment of established sepsis.

Thus, IGIV is recommended for all septic premature infants not responding well to conventional therapy. It may be particularly valuable for neutropenic septic infants because it may help mobilize leukocytes from the storage pool. We recommend a dose of 500 mg/kg daily for 4 days and repeated as necessary.

STAPHYLOCOCCAL INFECTIONS

Staphylococcal infections are ubiquitous and of varying severity. They include superficial skin infections, acute infections such as abscesses and wound infections, deep-seated indolent cellulitis, and three toxin-mediated diseases: scalded skin syndrome, toxic shock, and acute food poisoning. Antibiotics usually are effective in controlling the infections, but in some instances, the organism is antibiotic resistant or the disease is rapidly progressive. IGIV may be of adjunctive benefit in some of these situations.[311,624]

STAPHYLOCOCCAL TOXIC SHOCK SYNDROME

Patients with staphylococcal toxic shock syndrome, often associated with tampon use by menstruating women, have a rapid onset of fever, shock, macular desquamating rash, and multisystem organ failure.[27,398] The pathogenesis of the disorder results from infection with a strain that releases toxic shock syndrome toxin (TSST-1).[27] Approximately 20 percent of all staphylococcal isolates carry the gene for this toxin.[364] TSST-1 is a potent superantigen that directly activates the 5 percent of T cells that have a Vβ2 T-cell receptor[364]; such activation results in the rapid release of multiple cytokines and a clinical picture of rapidly progressive illness.

IGIV contains neutralizing antitoxins to the staphylococcal (and streptococcal) superantigens[589] and has been used successfully both in animal models of toxic shock syndrome[27] and in several patients.[55,435,455,518] Although no controlled clinical trials have been performed for staphylococcal toxic shock syndrome, most authorities recommend large IVIG doses, at least 400 mg/kg, in addition to antibiotic treatment and circulatory support.[27] IGIV also down-regulates cytokine synthesis and action and inhibits immune activation, thereby providing additional benefits.[27]

Higuchi and coauthors[256] reported a large family that had recurrent episodes of staphylococcal toxic shock syndrome associated with normal immunoglobulin levels but low serum antibody titers to staphylococcal superantigens; two boys in the family achieved successful prophylaxis with regular IGIV infusions.

NEONATAL STAPHYLOCOCCAL INFECTIONS

A second situation in which antibody may be of value is neonatal staphylococcal infection. Coagulase-negative staphylococcal infection, such as with *S. epidermidis*, is the most common cause of sepsis in premature infants and is aggravated in part by the use of catheters and parenteral lipid infusions.[44,187]

One controlled study showed that IGIV was of value in decreasing the incidence of but not eliminating infections,[44] but this benefit has not been found in all trials of IGIV in newborns.

This variability may be explained by differing amounts of staphylococcal antibodies present in different IGIV brands and lots, particularly opsonic antibodies, which have been shown in animal models to be the most important correlate of clinical protection.[187] Because antibody variability exists in lots of IGIV made from pools of thousands of donors, it is not surprising that Krediet and colleagues[331] could not reliably increase the opsonic titers of premature infants by administering single-donor fresh-frozen plasma. However, polyclonal hyperimmune staphylococcal human IGIVs also have not been of benefit (see IGIV in secondary immunodeficiencies). Monoclonal antistaphylococcal antibodies are under study.

REFRACTORY STAPHYLOCOCCAL INFECTIONS

A final use of IG is for the treatment of antibiotic-resistant chronic staphylococcal infection, along with intravenous antibiotics. Waisbren[624] treated 16 such patients with an antibiotic–gamma globulin combination, with recovery noted in 13 of them. Indeed, hyperimmune plasma and immunoglobulin from subjects immunized with staphylococcal toxoids are widely used in Russia with apparent success.[311]

We (E. R. S.) successfully treated a woman with antibiotic-resistant dissecting cellulitis of the scalp with a combination of high-dose IVIG and antibiotics. Animal studies support such a combined approach.[187a]

Recommendations

Large doses of IGIV are indicated for the treatment of staphylococcal toxic shock syndrome.[27] The recommended regimen is a single dose of 1 to 2 g/kg or 400 mg/kg daily for 5 days. A repeated dose may be necessary because of rapid clearance of IGIV in this illness.[27]

The value of IGIV in refractory chronic staphylococcal infections is unproven, although anecdotal reports of efficacy exist.[624]

IGIV is not recommended routinely for the prevention of infection in premature newborn infants. As noted earlier, IGIV may be of adjunctive benefit in the treatment of neonates with sepsis, particularly those with neutropenia.[283] We recommend 500 mg/kg on 4 consecutive days if the patient has not responded to optimal antimicrobial and supportive management. This regimen may be repeated as needed.

STREPTOCOCCAL INFECTIONS

Circulating antibody may play a role in the prevention and treatment of group A streptococcal infection.[302] Newborns rarely develop invasive streptococcal illness, in part because of protective transplacental antibodies.[302] Equine antitoxin was used with some success in the treatment of erysipelas and scarlet fever in the 1920s and 1930s.[366,582] A preventive streptococcal vaccine to the M component has been proposed but as yet is unavailable.

INVASIVE GROUP A INFECTIONS

Invasive group A streptococcal infections, including septicemia, necrotizing fasciitis or myositis, and toxic shock syndrome, are of increasing severity and frequency.[13] Streptococcal pyrogenic exotoxins, including types A, B, and C, and mitogenic factor elaborated by certain strains of streptococci may be responsible for these serious complications of infection. Streptococcal pyrogenic exotoxins are potent superantigens that activate certain T lymphocytes directly and lead to the massive production of multiple cytokines, with resultant shock, fever, and organ failure. IGIV contains neutralizing antibodies to these antigens,[426,427] but at varying titers from batch to batch.[520]

Invasive soft tissue infections with group A streptococcus may benefit from high-dose IGIV.[145,207,426] By contrast to these reports, Mehta and colleagues[396] could not document a benefit for IGIV in patients admitted to the ICU with invasive group A infections from any cause.

STREPTOCOCCAL TOXIC SHOCK SYNDROME

Many case reports have attested to the value of IGIV in streptococcal toxic shock syndrome since the report by Barry and coauthors in 1992.[55,348,465] Kaul and colleagues[306] reported a study of 21 consecutive patients at several Canadian medical centers in 1994 and 1995 treated with IGIV and compared them with 32 similar patients not given IGIV at the same centers for the 3 preceding years. Both groups received appropriate antibiotics and were similar with regard to demographics, severity score, and timing of intervention. The median IGIV dose was 2 g/kg. The survival rate for the IGIV-treated group was significantly greater at 7 and 30 days (90% and 67%, respectively) than that in the untreated controls (50% and 34%, respectively; $p = .02$), and the days of hospitalization were insignificantly shortened (29 versus 39). Serum from the IGIV-treated patients caused a marked inhibition of lymphocyte mitogenic activity to their own bacterial isolates after a single IGIV dose.

PANDAS SYNDROME

Swedo and coauthors[581] reported a syndrome of tics or obsessive-compulsive behavior, or both, occurring in prepubertal children soon after the onset of a streptococcal infection. They termed this disorder pediatric autoimmune neuropsychiatric disorder associated with streptococcal disease (PANDAS syndrome). They reported suggestive benefit of IGIV.[466]

Recommendations

IGIV is recommended for moderate and severe cases of streptococcal toxic shock syndrome, even if the organism has not been identified. The recommended dose is 1 to 2 g/kg given in a single dose or in divided doses of 400 mg/kg. Repeated administration of IGIV may be necessary after a few days because of rapid IGIV catabolism.

IGIV is probably of benefit in severe streptococcal soft tissue infections.

IGIV is of unproven benefit in PANDAS syndrome.[443]

TETANUS, TETANUS ANTITOXIN, AND TETANUS IMMUNE GLOBULIN

Antitoxin for the treatment of tetanus was introduced into medicine by Behring and Kitasato[460] in 1890; large doses (50 to 100 mL) of serum from horses immunized with tetanus toxin

were used. The dose was increased gradually to 300 to 500 mL, equivalent to 300 to 500 U of antitoxin. As the means to increase the production and concentration of antitoxin developed and a high mortality rate persisted, the dosage of antitoxin was increased until doses as high as 200,000 U, repeated at weekly intervals, were recommended.[460] Despite such heroic therapy, no solid proof of efficacy was obtained.

However, in 1960, Brown and colleagues,[89] using sequential analysis, found that the mortality rate was 49 percent in 41 patients with tetanus who received 200,000 U of antitoxin, a statistically significant difference that established therapeutic efficacy. Extensive controlled studies established that mortality rates did not improve when doses of 100,000 to 500,000 U were used.[365,606-608]

Similarly, Patel and associates[460] could find no difference in the mortality rates of patients with tetanus treated with antitoxin doses of 5000 to 60,000 U. Adequate blood levels of antitoxin were noted in all cases, even in fatal cases, with a dose of 10,000 U. In mild cases, no antitoxin was necessary. They and others have reported considerable differences in mortality rates, ranging from 0 to 98 percent; the rate depends primarily on the severity of illness rather than on the dose of antitoxin.

Athavale[40] established that antitoxin was of benefit in tetanus neonatorum and in tetanus in children up to 12 years of age. Antitoxin affected the mortality rate in mild and moderate cases but not in severe ones. A dose of 10,000 U was as effective as one of 30,000 U.

The mechanism of action of antitoxin is to neutralize toxin before it is transported to the nervous system through the circulation. Antitoxin also can neutralize toxin locally and prevent its systemic absorption. Thus, antitoxin can be given locally, at the site of production of toxin (e.g., at the site of a wound), intravenously (in severe cases), or intramuscularly (in less severe cases).

In 1962, an estimated 750,000 annual doses of equine tetanus antitoxin were needed in the United Kingdom, which is equivalent to more than a million doses in the United States.[499] Serum sickness occurs in 6 to 14 percent and fatal anaphylaxis in 1 of every 100,000 injections. Thus, hyperimmune human tetanus immune globulin (TIG), first available in the early 1960s, gradually has replaced equine tetanus antitoxin.

Rubbo and Suri[498] and Rubinstein[499] showed that TIG given intramuscularly (5 to 10 U/kg) provides adequate circulating antitoxin levels and is maintained in the circulation for considerably longer than is equine tetanus antitoxin.

The efficacy of TIG is equivalent to that of equine tetanus antitoxin. McCracken and associates[388] compared the results of 550 U of TIG with 10,000 U of tetanus antitoxin in the treatment of tetanus neonatorum. Among the 65 infants in each treatment group, no difference was noted in severity, length of hospitalization, need for sedation or gavage feeding, or mortality rate. Blake and colleagues[70] analyzed 545 tetanus cases reported to the CDC from 1965 to 1971 and could find no difference in the outcome of patients treated with equine tetanus antitoxin versus TIG.

Gupta and associates[235] gave TIG intrathecally to alternate patients with early tetanus. Among 49 patients given intrathecal TIG (250 U), three got worse and one died; among 48 patients given intramuscular TIG (1000 U), 15 got worse and 10 died. No side effects occurred. Herrero and coworkers[253] could not prove a benefit of administering intrathecal antitoxin for tetanus neonatorum. Although an early meta-analysis of intrathecal therapy cast doubt on its efficacy,[4] studies from India[197] and Brazil[401] and a larger meta-analysis[295] suggested beneficial effect of intrathecal antibody.

TIG can be given along with tetanus toxoid (10 Lf U) for passive-active immunization. A dose of 250 U of TIG given intramuscularly at a site different from that of the toxoid does not interfere with the active antibody response.[387]

Lee and Lederman[350] measured the tetanus antitoxin titers in 29 lots of IGIV and noted considerable variability, although all lots had titers greater than 4 U/mL (mean, 21 U/mL). All lots would provide sufficient anti–tetanus toxin antibody if used at doses of 100 mg/kg as an alternative to TIG or tetanus antitoxin.

Recommendations

PROPHYLAXIS

If a non-immunized person sustains a serious injury or a bite, 250 to 500 U of TIG should be given intramuscularly as soon as possible.[26] The larger dose is used in the event of an extensive wound or delay in treatment. TIG is available for intramuscular administration in individual vials containing 250 U. Alum-precipitated toxoid to initiate active immunity is given at a different site with a separate syringe.

Human IGIV also can be used if TIG is not available, at a dose of 200 to 400 mg/kg.[26]

If TIG or IGIV is unavailable, 3000 to 5000 U of tetanus antitoxin (equine) is administered (after screening and testing for serum sensitivity have been performed).[26] Equine antitoxin for human use is available in several countries where TIG is unavailable. Equine tetanus antitoxin is available for veterinary use in vials containing 1500 or 20,000 U.

TREATMENT

In addition to administration of antibiotics and management of the wound, TIG should be given, but the optimal dose has not been established. At least 500 U is recommended, but doses as high as 3000 to 6000 U have been used. Part of the TIG is infiltrated near the wound, and the remainder is administered intramuscularly.[26] If the wound is extensive, TIG can be diluted with saline to infiltrate the entire area. TIG also is indicated for the treatment of tetanus neonatorum. Recent meta-analyses suggest that intrathecal use is of benefit in both adults and infants.[295]

If TIG is unavailable, equine tetanus antitoxin should be given in a single dose of 100,000 U, with 50,000 U given intramuscularly and 50,000 intravenously (after appropriate testing for sensitivity). On recovery, the patient should undergo primary immunization.

In tetanus neonatorum, McCracken and associates[388] found that 500 U of TIG given intramuscularly and 10,000 U of equine antitoxin were equally efficacious. Intrathecal TIG has been recommended[235,295,401] but is not always of benefit.[60,253] However, TIG is not licensed for this use.

PASSIVE IMMUNITY IN VIRAL INFECTION

ENTEROVIRUSES

POLIOVIRUS

Before the development of poliomyelitis vaccines in the mid-1950s, IGIM was used extensively for the prevention of poliomyelitis. Bodian[75,76] showed that Red Cross IGIM had neutralizing antibody to all three strains of poliovirus in approximately equal titer and that rhesus monkeys given intramuscular poliovirus could be protected against disease by the subcutaneous administration of IGIM.

Bloxsom,[73] in an uncontrolled study performed during a 1948 Texas epidemic, gave 841 contacts an average dose of 2 mL of IGIM and noted only four cases at 1, 2, 3, and 42 days after administration of the IGIM injection. His interpretation was that IGIM had been given too late to prevent the first three cases and that protection had worn off in the fourth case.

A committee on immunization of the National Foundation for Infantile Paralysis recommended in March 1951 that a controlled study be conducted on the efficacy of IGIM for the prevention of poliomyelitis during epidemics. Hammon and associates[242] subsequently undertook a massive field study in communities in three states during poliomyelitis epidemics.

Fifty-five thousand children aged 1 to 11 years received either IGIM (average dose, 0.14 mL/lb) or gelatin in a double-blind fashion. During the first week after injection was administered, 12 cases occurred in the IGIM recipients and 16 cases in the gelatin recipients. In the two groups, 3 and 23 cases occurred in the second week and 6 and 38 cases occurred in the third to fifth weeks, respectively. When protection was incomplete, decreased severity was noted. Protection waned by 6 weeks and disappeared by 8 weeks. These clinical results were confirmed by isolation of the virus or a rise in antibody titer in affected patients.

IGIM was an inefficient method of poliomyelitis prophylaxis in that it prevented only one case for every 500 to 2000 injections, and then only for a brief time. Its chief value was in close family contacts of affected children and in aborting severe local epidemics.

Recommendations

The use of IGIM rarely is indicated for the prevention of poliomyelitis. An exposed unimmunized subject can be given 0.31 mg/kg of IGIM. An unimmunized patient who is traveling to an endemic or epidemic area and who cannot have vaccine can be given this dose of IGIM for temporary protection.

An immunodeficient patient inadvertently exposed to live attenuated polio vaccine who is excreting poliovirus in stool may be a candidate for receiving IGIV and oral IG in an effort to rid the gastrointestinal tract of the virus.

OTHER ENTEROVIRUSES

Enteroviruses, particularly echoviruses and coxsackieviruses, can cause severe disease in neonates[274] and in immunodeficient patients, particularly those with X-linked agammaglobulinemia. IGIM and IGIV have been used in both groups for the prevention and treatment of these infections.

MENINGOENCEPHALITIS. Chronic enteroviral meningoencephalitis with severe neurologic findings may develop in agammaglobulinemic patients; the incidence has decreased since routine treatment with IGIV has been instituted for these patients. IGIV does not prevent all cases, possibly because of differing titers of antibodies to different enteroviral serotypes. McKinney and coworkers[391] summarized the results of treatment of 42 patients with chronic enterovirus meningoencephalitis: 10 received IGIM or plasma, but only 1 survived, a patient who received high-titer human serum; 10 received IGIV, and 7 survived; and 12 received both intraventricular IGIV and systemic IGIV, and 6 improved and 10 survived.

Misbah and colleagues[402] reviewed the cases of 15 patients with chronic enteroviral meningoencephalitis not included in the McKinney report, some of whom, however, had been described by others.[161,328] Of 5 patients treated with IGIV, 3 survived, and of 10 treated with IGIV and intraventricular or intrathecal IGIV (only 1 patient received intrathecal, not intraventricular, IGIV), 5 survived. Mellouli and colleagues[399] successfully treated an agammaglobulinemic boy who developed echovirus meningoencephalitis while receiving IGIV with intraventricular IG through an Omaya reservoir in addition to aggressive intravenous therapy.

Quartier and associates[476] reported complete clinical and virologic remission, as determined by both culture and polymerase chain reaction (PCR) assay, in one patient treated with IGIV and a brief course of pleconaril; remission was maintained for 37 months by keeping the serum IgG trough level at 800 mg/dL. A second patient was treated with IGIV and pleconaril, but intraventricular IG was added when cerebrospinal fluid pleocytosis persisted after 11 months of therapy. Complete clinical and virologic remission resulted and persisted at the 20-month follow-up. This study indicated that cerebrospinal fluid PCR assay can be used, in addition to viral culture, as a guide to successful therapy.

NEONATAL ENTEROVIRAL INFECTION. Severe and sometimes fatal disseminated enterovirus infection can develop in neonates.[477] Case reports have suggested benefit with the use of IGIV.[274,287,610] Kimura and coworkers[318] treated coxsackievirus B3 infection in four term infants with IGIV; the three who were treated early survived, but the infant treated 6 days after the onset of infection died. Abzug and associates[5] treated nine infants with echovirus and coxsackievirus B infection with IGIV but without apparent benefit; however, three of five infants who received high-titer IGIV had a shortened period of viremia. Rentz and colleagues[488] suggested that the maternal plasma might be used in lieu of IGIV as a source of high-titer antibody in newborns with a severe enteroviral infection. In sum, IGIV is of uncertain value, particularly if its titer against the particular virus is not known.

Nagington and colleagues[415] used IGIM successfully in an echovirus 11 outbreak in a special care nursery, but Kinney and coworkers[319] could not demonstrate a benefit of using IGIM in a similar nursery with the same virus. Pasic and associates[459] administered IGIV at 400 mg/kg prophylactically (neutralization titer, 1:32) during a nursery echovirus 6 outbreak and thought that the severity of illness was decreased, although viral transmission continued.

Recommendations

IGIV may be used for critically ill neonates with disseminated enterovirus infection, although its benefit is unproven. In an outbreak situation with a known serotype and the availability of IGIV with significant titer to this serotype, IGIV should be considered. Multiple doses of 500 mg/kg as well as single doses of 750 to 1000 mg/kg have been used.[5,287,318,610]

For chronic enteroviral meningoencephalitis, McKinney and colleagues[391] recommend that sufficient IGIV be administered to maintain an IgG serum trough level of 900 to 1000 mg/dL. Even higher doses may be needed if the IGIV titer for the infecting virus is low. Quartier and coworkers[476] used 500 mg/kg of IGIV every 24 to 48 hours for 2 weeks, followed by 500 mg/kg two to three times per week for 6 weeks and then gradual reductions, but with maintenance of the trough IgG level at greater than 800 mg/dL.

Administration of IGIV by intraventricular catheter should be considered for patients who do not improve despite receiving aggressive therapy with IGIV. IGIV for intraventricular administration should have a neutral pH, be given slowly, and be at room temperature.[391] Dwyer and Erlendsson[161] used 6 percent IGIV intraventricularly starting at doses of 120 mg/day and increasing to 600 mg/day during the first week of treatment, followed by doses of 300 mg/day for 1 to 4 weeks. Quartier and

colleagues[476] administered 300 mg of IGIV daily for 15 days and then 300 mg three times a week to a patient whose intravenous IGIV therapy had failed. The duration and frequency of both intraventricular and intravenous IGIV must be individualized and determined by clinical and virologic response and normalization of cerebrospinal fluid and ventricular fluid. An IGIV with a high titer to the infecting serotype should be used for both systemic and intraventricular therapy.

HEPATITIS A

The widest use for human IGIM has been for the prevention of hepatitis A, but this indication has been decreased by the widespread use of hepatitis A vaccine.[16] The efficacy of IGIM was demonstrated in 1945 by Stokes and Neefe[570] in aborting an epidemic in a children's summer camp, by Havens and Paul[245] in controlling an institutional epidemic, and by Gellis and associates[198] in preventing hepatitis A in the Mediterranean theater of operations at the close of World War II. The administration of combined IGIM and hepatitis A vaccine and scrupulous cleanliness can be used to interrupt the intestinal-oral circuit of transmission to abort an incipient epidemic.

IGIM is efficacious in preventing hepatitis A if it is given within 2 weeks of the last exposure.[16] Protection persists for 6 to 8 weeks. Stokes and associates[571] noted that a single small dose of IGIM (0.02 mL/kg) provided a degree of protection for up to 9 months in individuals residing at an institution in which hepatitis A was endemic.

The effectiveness of IGIM in hepatitis A varies from 80 to 95 percent, depending on how soon it is administered after exposure and the severity of the exposure.[627] IGIM suppresses clinical manifestations of the disease, but anicteric hepatitis is not prevented, and the ratio of anicteric to icteric hepatitis may be as high as 12:1.[337] Because the period of protection exceeds the expected duration of the IGIM, the concept of passive-active immunity has emerged; as a result of continuous exposure, a mild illness ensues and in turn confers long-lasting immunity.[337,571,627]

In initial studies, Stokes and Neefe[570] used an IGIM dose of 0.15 mL/lb. Other early workers used doses of 0.06 to 0.12 mL/lb.[198,245] Stokes and associates[571] in 1951 showed that doses as low as 0.01 mL/lb were effective in limiting spread but not in totally preventing hepatitis. Hsia and colleagues[263] in 1954 also noted that a dose of 0.01 mL/lb was effective in preventing hepatitis among family contacts. However, Ward and associates[628] in 1958 were able to reduce the incidence of hepatitis in institutionalized patients from 19.5 to 7.4 cases per 1000 with 0.01 mL/kg and to 1.7 cases per 1000 with 0.06 mL/kg. The larger dose may be particularly important in adults because they are subject to more severe disease.

The use of serologic tests for hepatitis A provides a way to determine the immunity of a subject, the presence of inapparent infection, the titer of hepatitis A virus (HAV) in lots of IGIM, and the validity of the passive-active immunity concept.[570,571] In other earlier studies, Krugman[338] showed that an IGIM preparation with a titer of 1:3200 by an immune adherence test was effective in neutralizing the infectivity of MS-1 serum, a substance known to contain HAV. Among seronegative children who received the IGIM–hepatitis A mixture, six remained seronegative and two became seropositive; one became ill. By contrast, hepatitis developed in 8 of 14 children who received MS-1 serum without IG.

Most current lots of IGIM have anti-HAV antibodies when they are assayed by a competitive-inhibition radioimmunoassay. Titers greater than 1:100 are protective.[546]

RECOMMENDATIONS

HOUSEHOLD AND SEXUAL CONTACTS. Individuals, either adults or children, with a known intimate exposure to hepatitis A, such as a household or sexual contact, should be given a single IGIM dose of 0.02 mL/kg as soon as possible after being exposed.[16] Hepatitis A vaccine also can be initiated. Serologic testing for hepatitis A is unnecessary and may delay the administration of IGIM. The use of IGIM longer than 2 weeks after exposure has occurred is not indicated.[16]

SCHOOL EXPOSURE. IGIM usually is not necessary for children and their teachers exposed to a single case of hepatitis A in the classroom of a day school. However, if several children are infected or if transmission in the school is documented, IGIM (0.02 mL/kg) and immunization are indicated for the students and teachers. New students and employees should be given IGIM and vaccine for 6 weeks after the last case is identified.[16] IGIM prophylaxis is recommended for children and staff exposed at a boarding school or in a school for retarded children, where opportunities for transmission by the fecal-oral route are increased.

INSTITUTIONAL OUTBREAKS. Hepatitis A outbreaks in institutions such as boarding schools, daycare centers, facilities for the mentally retarded, and prisons require aggressive action. Other cohorts, employees, and adult members of the households of infants who wear diapers and who attend these daycare facilities should be treated immediately with 0.02 mL/kg of IGIM. If recognition of an outbreak is delayed 3 weeks or more after onset of the index case or if spread to other cohorts, staff, or household contacts appears to be occurring, all personnel (staff and children) should be given IGIM. If an outbreak of hepatitis A is traced to a food handler, IGIM should be given to close contacts and other restaurant employees.[111]

HOSPITAL AND CLINIC EXPOSURE. Administration of IGIM to unimmunized hospital employees caring for patients with hepatitis A or to patients usually is not recommended unless evidence of an outbreak among patients or between patients and staff exists.[16]

COMMON-SOURCE EXPOSURE. These cases generally are identified too long after the exposure occurs for IGIM to be effective. If a person has been exposed within the previous 2 weeks, IGIM can be given (0.02 mL/kg).[16]

COMMUNITY OUTBREAKS. Unless a source of the infection is identified, mass use of IGIM is ineffective and not recommended because it will not interfere with transmission.[39] Immunization is recommended.

FOREIGN TRAVEL. Individuals going to developing countries should receive vaccine 1 month before departure. If the departure date is less than 1 month away, 0.02 mL/kg of IGIM and vaccine can be used.[16] The IGIM will provide immediate protection and not interfere with efficacy of the vaccine. If exposure to hepatitis A will continue beyond 3 months, the IGIM dose should be 0.06 mL/kg and repeated every 5 months if exposure to hepatitis A continues and the patient cannot be immunized.

PRIMATE EXPOSURE. Certain subhuman primates such as chimpanzees may carry HAV. Animal handlers should observe scrupulous hygiene and be given hepatitis A vaccine. If bitten and unimmunized, they should receive 0.02 mL/kg of IGIM and vaccine.

NEEDLE EXPOSURE. IGIM is indicated for susceptible persons accidentally inoculated with blood or serum from a patient with hepatitis A. The recommended dose is 0.02 mL/kg; pregnancy is not a contraindication to the administration of IGIM.

NEWBORN INFANTS OF INFECTED MOTHERS. If the mother becomes symptomatic with acute hepatitis A between 2 weeks before and 1 week after delivery, the infant can be given 0.02 mL/kg of IGIM. Efficacy has not been established, however.

HEPATITIS B AND HEPATITIS B IMMUNE GLOBULIN

Hepatitis B virus causes a wide spectrum of illness ranging from asymptomatic seroconversion to fulminant hepatitis. Although most normal subjects recover completely, the carrier state occurs commonly in exposed immunocompromised subjects, such as newborns, patients taking immunosuppressive medications, and patients with primary or secondary immunodeficiencies.

Transmission occurs after exposure to blood or other body fluids, through inoculation or sex, and by close personal contact such as may occur in daycare centers. The main route of transmission used to be by blood transfusion, but with donor testing, this route no longer is common in developed countries. An important route of transmission is from the mother to her newborn infant, who is likely to remain chronically infected for a lifetime. This route usually can be prevented by active and passive immunization.

IMMUNE GLOBULIN IN HEPATITIS B

The initial study of IGIM in hepatitis B was conducted in 1945 by Grossman and associates,[228] who treated alternate battle casualties given whole blood or plasma with two 10-mL injections of IGIM 1 month apart. The incidence of icteric hepatitis was 1.3 percent in 384 IGIM-treated patients and 9.9 percent in 384 control patients, a highly significant difference that suggested a beneficial effect of IGIM.

In 1947, Duncan and associates[160] reported the results from a similar study, although they gave only one 10-mL injection; hepatitis occurred in 1.2 percent of 2406 patients in the IGIM-treated group and in 0.9 percent of patients in the control group, an insignificant difference. However, the mean incubation period was significantly prolonged in the IGIM-treated group (to 103 days) compared with the control group (87 days).

Holland and associates[260] could not alter the incidence or severity of post-transfusion hepatitis in 84 open heart surgery patients given two 10-mL doses of IGIM 1 month apart in comparison to 83 non–IGIM-treated controls. These findings were confirmed in a large cooperative study of 5189 transfused cardiovascular patients given 10 mL of IGIM during the first, fourth, and seventh postoperative weeks.[216] Redeker and associates[484] could not demonstrate that IGIM protected spouses of individuals with type B hepatitis. Similarly, Kuhns and colleagues[340] could not reduce the incidence of post-transfusion hepatitis B with 20 mL of IGIM. Both these latter studies used IGIM with low titers of antibody to hepatitis B surface antigen (anti-HBs).

Several factors probably are responsible for the variation in effectiveness of IGIM in hepatitis B. One is the variable degree of exposure to hepatitis B virus, which can be massive (as with a blood transfusion) or minimal (e.g., a household contact). A second factor is the variable levels of anti-HBs antibody in different lots of IGIM. Because IGIM does not always contain high titers of anti-HBs, it is not recommended for hepatitis B prophylaxis.

HEPATITIS B IMMUNE GLOBULIN

Soon after hepatitis B surface antigen (HBsAg) and its antibody (anti-HBs) were identified, researchers realized that measurement of the antibody content of IG would permit selection of lots (or donors) with high titers of anti-HBs; such selection results in IG lots with anti-HBs titers of at least 1:100,000 by radioimmunoassay. This product, hepatitis B immune globulin (HBIG), has been licensed since 1978 for prevention of hepatitis B.[17,120,122]

Krugman and associates[333,335,337] in 1971 evaluated high-titer HBIG in institutionalized children injected with the infective serum MS-2. Hepatitis developed in all 11 children exposed to MS-2 serum; two became icteric, and five remained HBsAg carriers after 320 days. Among five children given MS-2 serum and standard IG, hepatitis developed in three, two were icteric, but none became a carrier. Of 10 children exposed to MS-2 serum and HBIG, six were completely protected, one had a transient infection, and three developed classic hepatitis. They concluded that HBIG was 70 percent effective under these circumstances. Their later studies confirmed that HBIG could reduce the incidence, severity, and carrier rate of HBsAg significantly after prenatal exposure to hepatitis B virus.[338]

Szmuness and associates[583] tested the efficacy of HBIG versus IGIM by giving either standard IGIM or HBIG to institutionalized children at admission and at 4-month intervals for 1.5 to 2 years and compared the incidence of hepatitis with that in untreated subjects. Both globulin-treated groups had a lower attack rate (11% versus 25% in the untreated subjects) and a lower incidence of persistent antigenemia (none in the globulin-treated group versus 13.5% in the untreated group). Thus, both IGIM and HBIG were effective in preventing or modifying nonparenterally transmitted hepatitis B in an endemic setting.

Of note is that anti-HBs developed in 55 percent of the patients treated with standard IGIM, whereas antibody developed in only 23 percent of the patients treated with HBIG, thus suggesting that passive-active immunity occurred more frequently in the group that received standard IGIM than in the group that received HBIG.

Seeff and associates[522] gave either HBIG or standard IGIM to 302 individuals definitively exposed orally or parenterally to material that was infectious for hepatitis B. The incidence of both clinical and subclinical hepatitis during the first 6 months was 0.7 percent in the HBIG-treated group and 6.1 percent in the IGIM-treated group. At 6 months, 32 percent of the IGIM recipients and 6 percent of the HBIG recipients had antibody, a finding indicative of minimal passive-active immunity in the HBIG-treated group.

Grady[215] reported similar results with HBIG after accidental exposure. The incidence of hepatitis at 6 months was 7 percent (of 251 patients) with standard IGIM, 5 percent (of 208 patients) with intermediate-titer HBIG, and 2 percent (of 253 patients) with high-titer HBIG. This protection waned after 6 months, and differences in the groups became less apparent after 9 months, possibly because of reexposure, delayed onset of infection, or failure of passive-active immunity

PREVENTION OF VERTICAL TRANSMISSION

Beasley and associates[58,59] studied the efficacy of HBIG in preventing perinatal transmission of the hepatitis B virus carrier state from a mother to her newborn infant. HBIG or placebo was

given at birth to the infants of hepatitis B early antigen (HBeAg)–positive, HBsAg-positive carrier mothers, and the infants were monitored for at least 15 months. Of 61 placebo recipients, 92 percent became carriers; of 67 infants who received 1.0 mL of HBIG at birth, 54 percent became carriers; and of 57 infants who received 0.5 mL of HBIG at birth and at 3 and 6 months, 26 percent became carriers. Passive-active immunization, indicated by the presence of anti-HBs, occurred in 27 percent of the single-dose group and in 61 percent in the three-dose group.

On the basis of these and other studies[285,487] suggesting that multiple HBIG doses were more effective in interrupting vertical transmission of the HBsAg carrier state than was a single HBIG dose, advisory committees in the early 1980s recommended that all infants of HBsAg-positive mothers be given HBIG (0.5 mL) immediately after birth and again at 3 and 6 months.[120,122] However, many of these infants became infected sometime after their last HBIG dose (i.e., in the second or third year of life), thus indicating a need for more durable active immunity.

Wong and associates[650] studied the efficacy of hepatitis B vaccine given in conjunction with HBIG in the prevention of vertical transmission of the carrier state from mother to infant. They gave hepatitis B vaccine (36 infants), hepatitis B vaccine plus one dose of HBIG (35 infants), hepatitis B vaccine plus seven monthly HBIG doses (35 infants), or placebo (35 infants) to infants of HBsAg-positive mothers. In all vaccine groups, development of a persistent carrier state was reduced significantly in comparison to the placebo group (21% with hepatitis B vaccine alone, 2.9% with hepatitis B vaccine plus one dose of HBIG, and 6.8% with hepatitis B vaccine plus seven doses of HBIG versus 73.2% for placebo groups). That anti-HBs developed in all infants of the treatment groups indicated that HBIG did not interfere with active immunization.

This and other studies[131,300,586] indicate that HBIG given at the same time as hepatitis B vaccine provides optimal passive-active immunity for long-lasting prevention of the carrier state, and this dose is the current recommendation. Studies in adults also confirm that HBIG given before or simultaneously with the first dose of hepatitis B vaccine does not interfere with the antibody response to hepatitis B vaccine.[584,659] This approach will not prevent every case of vertical transmission because some infants may acquire the infection in utero.

HBIG is not of value in the treatment of either acute[6] or chronic[486] hepatitis B infection.

HEPATITIS B IMMUNE GLOBULIN IN LIVER TRANSPLANTATION

Liver transplantation in a patient infected with hepatitis B is associated with a high rate of its recurrence in the new liver (about 50% in 3 years) and subsequent mortality. The HBIG doses needed to prevent re-infection are very high, and thus HBIG often is given intravenously.[595] One brand of HBIG (Nabi-HB) is formulated as a preservative-free 5 percent solution rather than the usual 16 percent formulation; it has been tested but not licensed for intravenous use.[596] A second 5 percent formulation (HepaGam B, Cangene) was licensed in 2007 for intravenous administration. Despite their lower protein content, these 5 percent products provide levels of HBs antibody equivalent to or higher than those of the 16 percent formulations.

The 5 percent HBIG given intravenously or by the usual intramuscular route will reduce the rate of recurrence of hepatitis B.[508] In a retrospective analysis of 359 such transplants, hepatitis B recurrence was 74 ± 6 percent in 67 patients given no HBIG, 74 ± 5 percent in 83 patients given HBIG for 2 months, and 36 ± 4 percent in the 209 patients given HBIG for 6 months or longer.

Grazi and coworkers[221] reduced significantly the recurrence rate in HBsAg-positive, hepatitis B virus DNA–negative cirrhotic individuals undergoing liver transplantation with the use of HBIG for 1 year after transplantation; recurrence was noted in 8 of 10 controls (80%) and 4 of 25 HBIG-treated patients (16%). Several other reports are available.[595,596]

In addition to HBIG therapy, pre-transplantation and post-transplantation antiviral drugs such as lamivudine are used. When administered in the immediate pre-transplantation period to patients with a high viral burden, these drugs enhance the effectiveness of HBIG because less virus must be neutralized.[594] Long-term maintenance of antivirals with discontinuation of HBIG after several months may be feasible.[649]

Preliminary studies of monoclonal antibodies to hepatitis B antigens are under way.[663] They would be particularly useful for liver transplantation because of the large antibody doses required.

Recommendations

PROPHYLAXIS

HBIG is recommended after a person's parenteral or mucous membrane (oral, sexual, ophthalmic) has contact with blood or body fluids from individuals with hepatitis B or with HBsAg-positive materials (e.g., blood, plasma) and for neonates born to HBsAg-positive mothers (Table 256–6).[17] It is available in 0.5-mL prefilled syringes and 1- and 5-mL vials. Two products are 5 percent product, whereas others are the usual 16 percent product characteristic of most special IGIMs for specific infectious diseases. Both contain equivalent amounts of anti-HBs antibodies per milliliter. The older 5 percent product (Nabi-HB) has been used intravenously after liver transplantation, but it is an off-label use.

Exposure to Blood That Contains (or May Contain) Hepatitis B Surface Antigen

No prospective studies have tested the efficacy of a combination of HBIG and hepatitis B vaccine in preventing hepatitis B after accidental exposure, including exposure by the percutaneous, ocular, and mucous membrane routes, as well as by human bites that penetrate the skin. Because health care workers at risk for incurring such accidents are candidates for receiving hepatitis B vaccine and because the combination of HBIG and hepatitis B vaccine is more effective than HBIG alone in perinatal exposure, this combination also is recommended after accidental exposure.

If the exposed patient is unimmunized and the blood or secretions come from an individual known to be HBsAg positive or if the infection status of the source is unknown but the source is high risk, immediate prophylaxis is indicated. A single dose of HBIG (0.06 mL/kg or 5 mL for adults) should be given intramuscularly as soon as possible, preferably within 24 hours of exposure.[17] Hepatitis B vaccine should be given simultaneously at a different site and repeated after 1 and 6 months (see Table 256–6). After massive exposure (i.e., by blood transfusion), much larger doses of HBIG probably are indicated.

If the exposed patient has been immunized but the serologic response is not known, anti-HBs titers should be determined and the individual managed according to the results; if nonresponsive (anti-HBs <10 mIU/mL by radioimmunoassay), HBIG and a full series of vaccine should be given. In patients with a contraindication to vaccine, two doses of HBIG (0.06 mL/kg) should be used, the second administered 1 month after the first. Two doses of HBIG also should be given to an exposed health care worker who is known to have no response to two series of hepatitis B vaccine.[17] Current recommendations from the CDC[115b] for nonoccupational exposures are to administer vaccine alone to previously immunized individuals as a booster dose without the need to document serologic response if the exposed person has written

TABLE 256–6 Hepatitis B Post-exposure Recommendations for Hepatitis B Immune Globulin (HBIG) and Hepatitis B Vaccine

	HBIG		Hepatitis B Vaccine	
Type of Exposure	**Dose**	**Recommended Timing**	**Dose**	**Recommended Timing**
Perinatal				
Infant of HBsAg-positive mother	0.5 mL IM	Within 12 hr of birth	0.5 mL	First dose within 12 hr, repeat 2 times
Infant of mother whose HBsAg status is unknown	0.5 mL IM	Within 7 days of birth if mother is found to be HBsAg positive	0.5 mL	First dose within 12 hr, repeat 2 times
Premature infant (<2000 g) whose mother's HBsAg status is unknown	0.5 mL IM	Within 12 hr of birth (unless maternal HBsAg test result is negative by this time)	0.5 mL	First dose within 12 hr, repeat 3 times
Mucous membrane or percutaneous with known non-occupational exposure				
Nonvaccinated	0.06 mL/kg IM	Immediately	0.5 mL*	First dose immediately, repeat 2 times
Previously vaccinated, known responder	None	None	None	
Previously vaccinated, unknown response	0.06 mL/kg IM if HBs	Test patient for antiHBs; omit HBIG† if > 10 mIU/mL	0.5	Give booster†
Previously vaccinated, known nonresponder	0.06 mL/kg IM	Immediately, may repeat in 1 month if < 10 mIU/mL‡	0.5 mL*	Give booster immediately, repeat 2 times if necessary
Sexual—known exposure				
Nonvaccinated	0.06 mL/kg IM	Immediately, but no later than 14 days	0.5 mL*	First dose immediately, repeat 2 times
Vaccinated†	None	None	0.5 mL	Give booster if unknown response

**Dose for individuals younger than 20 years. The adult vaccine dose is 1.0 mL. The dose for immunosuppressed patients and patients undergoing dialysis varies with the vaccine used. Vaccine doses given are for Recombivax HB or Engerix-B.*

†Recent CDC recommendations[115b] for exposure (sexual, sexual assault, percutaneous, or mucosal) from a source with unknown hepatitis B status are to forgo vaccine and HBIG if the exposed patient is already immunized and has written documentation of complete vaccine series and did not receive serologic testing for response. Individual assessments of potential risk must be made. See text.

‡See text for discussion of persons who have not responded to two vaccine series.

HBsAg, hepatitis B surface antigen

documentation of a completed hepatitis B vaccine series. The *Red Book*[17] also agrees with this booster vaccine approach. We recommend a more individualized approach for assessing risks of the addition of HBIG.

Perinatal Exposure

Among mothers who are HBsAg positive and HBeAg positive, 85 percent of their untreated infants will become infected and be chronic carriers, and chronic hepatitis, cirrhosis, or hepatic cancer will develop in some of them. If the mother is HBsAg positive only, the risk of her offspring's becoming a carrier is less but is still significant. Accordingly, these infants should receive HBIG prophylaxis.

INFANTS BORN TO MOTHERS WHO ARE HBsAg POSITIVE. For optimal passive-active immunity, HBIG (0.5 mL) is given to the newborn at birth (preferably in the delivery room but within 12 hours at the latest). Hepatitis B vaccine (at a dose half that of the adult dose) is begun simultaneously and repeated at 1 and 6 months (see Table 256–6). This combination is only approximately 90 percent effective in preventing the carrier state because intrauterine infection will not be prevented.

TERM INFANTS BORN TO MOTHERS NOT TESTED FOR HBsAg. If the HBsAg status of the mother is unknown, hepatitis B vaccine should be given to the term infant within 12 hours, and HBIG should be administered as soon as the mother is shown to be a carrier. Its effectiveness is diminished markedly if administration is delayed beyond 48 hours after birth but is given up to 7 days after birth.[17]

The mother should be tested immediately; if she is HBsAg negative, vaccine is continued as recommended for other infants. If the mother is found to be HBsAg positive later, HBIG and hepatitis B vaccine should be given to her infant, even if a significant delay has occurred. After the final dose, the infant can be tested for HBsAg and anti-HBs at 12 to 15 months to determine the success of the HBIG and vaccine regimen. If HBsAg is present, the infant is a carrier; if anti-HBs is present, the child was successfully immunized. Administration of HBIG at birth should not interfere with polio or diphtheria-pertussis-tetanus (DPT) vaccines starting at 2 months of age.

PREMATURE INFANTS. Premature infants with birth weights less than 2000 g who are born to women not tested for HBsAg should be given 0.5 mL HBIG within 12 hours of birth unless the mother's HBsAg test results can be available within 12 hours and are negative. Immunization should be started immediately and repeated for a total of four doses (rather than the usual three doses) because of the poorer response of preterm infants to the vaccine.[17]

Sexual Exposure to Hepatitis B or a Carrier of Hepatitis B

Sexual exposure (including rape) to an individual who has hepatitis B or is a carrier is an indication for administration of HBIG (0.06 mL/kg intramuscularly, 5 mL maximum) and initiation of hepatitis B vaccination. HBIG should be given as soon as possible but not after 14 days post-exposure. If only HBIG is given, a second dose of HBIG is recommended after 30 days. The CDC[115b] also recommends booster vaccine alone in this situation if written documentation is available for a completed hepatitis B vaccine series.

Possible Exposure

After possible exposure (percutaneous, ingestion, sexual) to an unidentified person or body fluid in which the HBsAg status is

unknown, the decision to treat with HBIG must be made individually on the basis of the likelihood that the source is HBsAg positive and the seriousness of the exposure. Hepatitis B vaccine should be initiated immediately if the exposed person is not immunized. Current *Red Book* recommendations and CDC recommendations[17] suggest that with an unknown source, vaccine alone is acceptable for an unvaccinated exposed patient and no treatment is required if the exposed person has written documentation of having previous immunization. We recommend a more individual approach to assess risks.

Ideally, the source subject should be tested for HBsAg positivity; if the results are available within 7 days for a percutaneous exposure and 14 days for a sexual exposure,[17] HBIG (0.06 mL/kg) can be given immediately and again at 1 month if the source is HBsAg positive and the patient did not or could not receive vaccine. When the source subject cannot be tested or when the source is likely to be HBsAg positive, HBIG is administered immediately.

If the exposed individual is a high-risk patient (e.g., immunodeficient, immunosuppressed, institutionalized, or undergoing hemodialysis) or is in an environment or health care unit for which past environmental control measures have been ineffective, HBIG should be given in addition to hepatitis B vaccine.

HBIG is not indicated on a routine basis after administration of blood transfusions. School or hospital exposure or usual household exposure is not an indication for HBIG, but household exposure is an indication for vaccine for unimmunized exposed persons.[17]

HEPATITIS B IMMUNE GLOBULIN IN LIVER TRANSPLANTATION

For patients who are HBsAg positive at the time they undergo liver transplantation or in the rare situation in which the donor of the liver is HBsAg positive, administration of HBIG is recommended in the preoperative period and after transplantation to prevent recurrence in the transplanted liver.[221,412,508,509,594-596]

The exact dose, frequency, route, and duration of HBIG in this situation have not been established, but the dose is large and adds significantly to the cost of the transplant procedure.

The typical regimen is to use very high HBIG doses (i.e., 10,000 units) before transplantation and immediately after transplantation, along with antiviral medications.[594] The HBIG is continued for several months, the dose determined by monitoring of the hepatitis B viral burden by PCR. After several months, the HBIG may be reduced or discontinued, but the antivirals are continued.[649]

HEPATITIS C

Early studies suggested that lots of polyvalent IGIM or HBIG not screened for hepatitis C antibodies provided some protection against the acquisition of non-A, non-B hepatitis (presumably hepatitis C) after heart surgery or hemodialysis.[321,512,543]

Two studies provide some evidence for a prophylactic effect of IGIM. Piazza and colleagues[468] gave polyvalent IGIM (from pools containing antibodies to hepatitis C virus [HCV]) or placebo monthly for 4 to 20 months to the seronegative sexual partners of 884 subjects who were seropositive for HCV. One of the 450 (representing 560 subject-years) in the IGIM group became infected versus 6 of the 449 (500 subject-years) in the placebo group ($p = .03$; relative risk, 10.7). The authors concluded that sexual transmission of hepatitis C occurs and that IG has a protective effect.

Féray and associates[181] reviewed the records of 218 patients with hepatitis C co-infection undergoing liver transplantation who received an HBIG product that contained antibody to HCV. The incidence of HCV viremia 1 year after transplantation was significantly lower (25 of 46 [54%]) in patients receiving HBIG than in those not receiving it (162 of 172 [94%]; $p < .001$). They also reviewed 210 transplanted HCV-seronegative patients and found that hepatitis C developed within 1 year in 18 of 68 patients (26%) who received the HBIG product containing anti-HCV antibody, compared with 40 of 86 patients (47%) who did not receive HBIG, a significant difference ($p < .001$) suggesting a preventive effect of hepatitis C antibodies.

Also supporting this concept was the observation that HCV was transmitted by pooled human immune globulin in the early 1990s, shortly after HCV antibody–positive donors were excluded from the donor pool.[119,517] These antibodies presumably neutralized the small amounts of HCV present in the unscreened lots. Indeed, Yu and colleagues[657] demonstrated that HCV antibodies in unscreened immune globulin lots containing HCV antibodies prevented low-dose infection in chimpanzees. Screened IGIM lots after 1991 lacking HCV antibodies did not prevent infection.

Because HBIG can prevent recurrence of HBIG among patients with hepatitis B undergoing liver transplantation,[221,509] researchers have postulated that hepatitis C antibodies might prevent recurrence in hepatitis C–positive liver transplant recipients.

EXPERIMENTAL HEPATITIS C IMMUNE GLOBULIN

This product (HCV-IGIV) is derived from human plasma units rejected for HBIG or IVIG manufacture because of the presence of hepatitis C antibodies: it does not contain hepatitis C as tested by PCR.

Krawczynski and colleagues[330] showed that HCV-IGIV delayed but did not prevent experimental hepatitis C infection in chimpanzees compared with regular IVIG without hepatitis C antibodies.

Willems and colleagues[641] gave HCV-IGIV to 26 hepatitis C–positive liver transplant recipients during the ahepatic and postoperative periods, without decreasing the rate of recurrence of hepatitis C. Davis and colleagues[149] reported a multicenter double-blind study of HCV-IGIV (Civacir, Nabi) in hepatitis C–positive liver transplant recipients. No effect occurred on HCV RNA levels, but some decrease in liver enzyme activities compared with the control patients was noted.

Thus, the use of antibody to prevent development of hepatitis C after liver transplantation has not been successful, possibly because of the inadequacy of the doses used or the lack of critical antibodies in the preparations. Studies using monoclonal antibodies, alone or in combination with other monoclonals or polyclonals, are in progress.[81,308,447]

Recommendation

No passive immune product is available for hepatitis C prophylaxis.

HERPESVIRUSES

The common herpesviruses that cause human infection are cytomegalovirus, Epstein-Barr virus, herpes simplex virus types 1 and 2, varicella-zoster virus, and human herpesviruses 6, 7, and 8. Although these DNA viruses produce latent infection that can reactivate, particularly in immunodeficient patients, the major uses of IGs have been in the prevention of primary varicella-zoster virus infection and prevention and treatment of cytomegalovirus infection in transplant recipients.

CYTOMEGALOVIRUS

Use of Cytomegalovirus Intravenous Immune Globulin or Intravenous Immune Globulin in Transplantation

Human antibodies to cytomegalovirus (CMV) in the form of CMV-IGIV (CMVIG) or IGIV have been used for more than a decade to prevent development of CMV infection in bone marrow and solid organ transplant recipients. CMVIG is prepared from the plasma of donors with high anti-CMV titers, but regular IGIV also contains anti-CMV antibodies at lower titer. Early studies used CMVIG or IGIV alone, but later studies have combined IG with antiviral agents.

Early studies in renal and liver transplant recipients showed the efficacy of CMVIG or IGIV.[100,175,189,548] Two meta-analyses demonstrated decreased CMV mortality rates with the use of CMVIG or IGIV in bone marrow and solid organ transplant recipients.[210,648] Others also have shown a similar prophylactic benefit of IGIV or CMVIG.[56,231,400,535] Ruutu and coworkers[504] showed no prophylactic benefit of CMVIG in CMV-seronegative bone marrow transplant recipients.

Several factors that have reduced the need for CMVIG or IGIV in transplant recipients include the realization of a markedly reduced risk for development of CMV if both the donor and recipient are CMV antibody negative (D–/R–), the use of CMV-seronegative or filtered blood products, the early detection of CMV by PCR, and the use of ganciclovir-valganciclovir prophylaxis or early treatment.[240]

Snydman and colleagues,[549] in an open label, multicenter study in liver transplantation, found that the combination of CMVIG and ganciclovir was superior to different immunosuppressive regimens in historical controls. Campbell and colleagues[108] recommended universal prophylaxis for the patients at highest risk, children receiving liver transplants (D+/R–), but the role of immunoglobulin was uncertain.

In heart and lung transplants, the use of CMVIG for CMV infection prophylaxis has been recommended by some groups if either the donor or the recipient is CMV seropositive. Weill and associates[631] showed that combined ganciclovir and CMVIG reduced the incidence of CMV infection in high-risk lung transplant recipients compared with historical controls. Ruttmann and colleagues[503] showed, in a retrospective study, decreased morbidity due to CMV infection in lung transplant recipients given both CMVIG and ganciclovir compared with the use of ganciclovir alone.

Zamora and associates[662] recommended intravenous ganciclovir and CMVIG for all lung transplant recipients if either the donor or recipient is CMV seropositive; and in the situation of D+/R–, they gave seven infusions of CMVIG in the first 90 days after transplantation. Other investigators[80,609] and consensus statements[147,473] permit the use of combined therapy with CMVIG in high-risk heart-lung transplant recipients and other solid organ recipients, despite the absence of definitive controlled studies.

Use of Cytomegalovirus Intravenous Immune Globulin in Perinatal Cytomegalovirus Infection

Another use of CMVIG may be the treatment of in utero CMV infection.[421] Two infusions of CMVIG were given intraperitoneally at 28 and 29 weeks' gestation to a CMV-infected fetus. The therapeutic benefit was unclear because the infant had intracranial calcifications at 2 weeks of age but was free of neurologic symptoms at 1 year of age.

Nigro and associates[423] treated a pregnant woman with primary CMV infection and in utero infection of one twin fetus with intravenous CMVIG at 30 weeks' gestation. CMVIG also was injected into the amniotic sac. The affected twin had better growth, decreased placental thickening, and lessened cord edema. Although born with CMV infection and hepatosplenomegaly, the child was normal at the age of 2 years.

Subsequently, Nigro and colleagues[422] gave 31 pregnant women with primary CMV infection at least one dose of CMVIG (200 mg/kg) during pregnancy. There was ultrasonic evidence of CMV infection in 15 fetuses. Nine women received additional CMVIG into their amniotic sac or umbilical cord. Only one woman gave birth to an infant with CMV infection, compared with 7 of 14 women who did not receive CMVIG during pregnancy. All of these women had in utero infection confirmed by CMV-positive (culture or PCR) amniotic fluid.

In a second group of 37 pregnant women with primary CMV infection (but without confirmatory amniocentesis) who were given 100 mg/kg of CMVIG monthly until delivery, none had an infant with CMV infection compared with three infants delivered to 47 untreated pregnant women.[423] Although these data are encouraging, the women in the study were not randomized, nor were the results statistically significant.

Nigro and colleagues[424] used CMVIG successfully to treat chronic cervical CMV infection associated with either recurrent abortion or infertility in three women.

Recommendations

Patients undergoing allogeneic human stem cell transplantation who are at risk for acquiring CMV disease (e.g., a CMV-seropositive recipient or a CMV-seronegative recipient and a CMV-seropositive donor) should receive either prophylactic antiviral therapy or preemptive antiviral therapy after early detection of infection by CMV PCR. CMVIG no longer is recommended for prevention of CMV disease.[77]

For solid organ recipients, the highest risk is a CMV-seropositive donor and a CMV-seronegative recipient, in which case antiviral prophylaxis is used. Antiviral prophylaxis rather than CMVIG or IGIV is used currently in kidney and liver transplantation.[281,525] In high-risk situations such as heart-lung transplants with either the donor or the recipient CMV seropositive, CMVIG is added at some centers to antiviral therapy, despite lack of proof by multicenter, randomized studies.[80,473,609]

CMVIG or IGIV in combination with antiviral agents is used to treat severe CMV disease, such as pneumonia, in transplant recipients.[193,448,462,485,661] For CMVIG, 400 mg/kg on days 1, 2, and 7 and 200 mg/kg on days 14 and 21 have been used in addition to antiviral therapy,[485] although others have used a lower dose (100 mg/kg every other day for 2 weeks).[199] IGIV, 500 mg/kg every other day for 10 to 59 days, followed by 500 mg/kg once or twice weekly for an extended period, also has been reported.[448] Garcia-Gallo and colleagues[193] have reported the use of CMVIG as an adjunct to antiviral therapy to prevent development of pneumonitis in lung transplant recipients with invasive CMV disease and as preemptive therapy (with ganciclovir) in post-transplant recipients with persistent CMV viral load.

CMVIG for in utero CMV infection is not recommended routinely but may be of some value in selected situations with confirmed ultrasonic evidence of fetal infection.

EPSTEIN-BARR VIRUS

Epstein-Barr virus (EBV), the etiologic agent of infectious mononucleosis, causes severe infection in immunocompromised patients and males with X-linked lymphoproliferative syndrome. IGIV has been given to EBV-seronegative males with X-linked lymphoproliferative syndrome in an attempt to prevent EBV infection, but EBV infection developed in some of them while they were taking IGIV.[185]

Transplant recipients with EBV-induced post-transplantation lymphoproliferative syndrome or hepatitis have been treated successfully with a combination of IGIV or CMVIG, antiviral therapy, and interferon-α.[151,434,587] A similar approach was not successful in X-linked lymphoproliferative syndrome.[441] CMVIG was used in one case because it contains higher titers of anti-EBV antibody than does IGIV.[151] One case of fulminant EBV hepatitis was treated successfully with liver transplantation, antiviral therapy, and CMVIG.[180]

A recent multicenter trial indicated that CMVIG given to liver transplant recipients did not prevent post-transplantation lymphoproliferative disease or other EBV disease.[222] However, the EBV serology was unavailable in 50 of the 82 donors, hampering interpretation of the results.

Ohta and colleagues[440] reported marked improvement of a patient with chronic EBV hepatitis following kidney transplantation by treatment with the anti-CD20 monoclonal antibody rituximab after failure of reduction of immunosuppression. There are other reports of the use of rituximab in the treatment of EBV-induced post-transplantation lymphoproliferative disease in stem cell[177,532] or solid organ transplants.[252,590,655]

Rituximab has also been used in preemptive therapy in stem cell transplantation based on EBV viral load.[134,612] Boesch and colleagues[78] reported the successful use of rituximab to treat an adolescent without immunodeficiency who developed EBV-associated lymphoproliferative disease that involved massive submucosal infiltration of B cells in the lingual tonsils, trachea, and bronchi and resultant near-complete airway obstruction unresponsive to steroids.

Recommendations

Current approaches to EBV-induced post-transplantation lymphoproliferative disease include reduction of immunosuppression and consideration of preemptive therapy or treatment with rituximab,[551] although antiviral medication, anticytokine therapy, chemotherapy, and immunotherapy all have been used. In a recent literature review, Giulino and colleagues[206a] found evidence of a better response to rituximab in children compared with adults and suggested that rituximab may be appropriate as first-line therapy for post-transplantation lymphoproliferative disease in children. The role of IGIV or CMVIG is not clearly defined.

HERPES SIMPLEX INFECTIONS

Antibody has a preventive effect in herpes simplex virus (HSV) infection in the newborn period. Mothers with a reactivated herpes infection during delivery are 10-fold less likely to transmit HSV to their newborn infants during vaginal delivery than are mothers with primary HSV infection at delivery, presumably because of the transplacental transfer of anti-herpes antibody.[18] IGIV containing HSV antibodies has not been used in the prevention of neonatal herpes infection, although Whitley[636] has proposed that HSV monoclonal antibody or hyperimmune IGIV be evaluated for treatment of disseminated neonatal disease.

Masci and colleagues[381] compared monthly IGIV(400 mg/kg) with intermittent acyclovir treatment (800 mg twice daily, 1 week each month) in patients with recurrent genital HSV infection for 6 months and noted fewer recurrences in the IGIV-treated group.

Recommendation

IGIV is not recommended for the prevention or treatment of HSV infections.

VARICELLA-ZOSTER VIRUS AND VARICELLA-ZOSTER IMMUNE GLOBULIN

Immune Globulin in Varicella

After success was achieved with IGIM in the prevention of measles and hepatitis, IGIM was evaluated for the prevention of varicella. Although Funkhouser[191] in 1948 showed some beneficial effects of standard IGIM in the prevention of varicella in an uncontrolled study, Greenberg[225] and Schaeffer and Toomey[515] were unable to prevent chickenpox in exposed children with IGIM doses of 2.5 to 20 mL.

Others have reported anecdotal evidence for the efficacy of IGIM in large doses during the early stages of chickenpox and herpes zoster. These claims included prompt relief of pain in zoster[633] and rapid resolution of skin lesions.[349,490] However, even high-titer IG (zoster immune globulin) does not prevent dissemination of herpes zoster.[560]

Ross[496] in 1962 gave 242 children IGIM in doses of 0.1 to 0.6 mL/lb within 3 days of exposure to chickenpox; 209 similarly exposed, uninjected children were used as controls. The attack rate was the same (97%) in both groups, thus indicating that IGIM does not prevent varicella under these conditions. However, with doses of IGIM above 0.2 mL/lb, the severity of the disease was reduced, as indicated by a decreased number of pox and reduced temperature. Children receiving the largest dose of IGIM (0.6 mL/lb) had maximal temperatures of 38.9° C (102° F) versus 41.1° C (106° F) for controls and 40 pox versus 207 for controls. Other investigators also have reported similar but uncontrolled observations that IG modifies the severity of chickenpox.[273,601]

Varicella-Zoster Immune Globulin in Varicella

The prophylactic value of large IG doses in decreasing the incidence and severity of varicella led to a trial of high-titer plasma or IG preparations to prevent varicella.[46] These preparations include zoster immune globulin (ZIG) and plasma (ZIP) from convalescing zoster patients and varicella-zoster immune globulin (VZIG) prepared from high-titer plasma from normal adults.

Brunell and associates[95] in 1969 selected convalescing patients with zoster whose complement-fixing antibody titers were 1:256 or greater and prepared ZIG from their plasma; this material had titers considerably higher than standard IG did. Exposed children from six families in which chickenpox was occurring were given ZIG or IG at doses of 2 mL. In none of six children receiving ZIG did chickenpox develop, whereas it developed in all six children given IG. No antibody developed in the ZIG-treated group, thus indicating that the disease was prevented.

Because this dose did not prevent varicella in leukemic children or other high-risk patients, a larger dose (5 mL) was used in a later study to successfully modify or to prevent varicella in eight of nine high-risk children.[93,94] Severe varicella developed in one child given a less potent preparation of ZIG. These observations were confirmed in two later studies. Judelsohn and associates[293] gave ZIG to 56 exposed high-risk children; mild varicella occurred in 7 patients and was prevented in the others, most of whom were susceptible as determined by absence of serum antibody. Gershon and colleagues[200] gave ZIG to 15 seronegative high-risk exposed children; varicella was severe in 1, mild in 9, and subclinical in 5. Subclinical infection was determined by the acquisition of membrane antibody, as detected by fluorescent microscopy.

Orenstein and associates[445] studied 553 exposed, high-risk patients who received ZIG of two different titers (1:1280 versus 1:2560 or greater). They found that the clinical attack rate after receipt of ZIG correlated with the type of exposure (varicella

developed in 36% with household exposure, 7.7% with hospital exposure, and 0% with school exposure), the rise in antibody titer (45% of patients without a fourfold titer increase became ill compared with 22% of patients with a fourfold or greater rise in titer), and the titer of the administered ZIG (significantly more complications and deaths occurred in recipients of the lower titer ZIG).

Because of the limited supply of ZIG and ZIP, VZIG from normal adults became the commercially available product in the United States. Zaia and associates[660] compared the efficacy of ZIG and VZIG in immunocompromised children exposed to varicella. Varicella attack rates and the clinical severity in recipients of VZIG and ZIG did not differ significantly. A higher incidence of subclinical infection was indicated by the rise in antibody titer in ZIG recipients (31.3%) versus that in VZIG recipients (16%). A larger dose of VZIG (2.5 mL/10 kg versus 1.25 mL/10 kg) reduced the frequency of subclinical infection (from 20% to 4.3%). Varicella developed in several high-risk patients with demonstrable serum antibody at exposure, thus indicating that history-negative, seropositive patients are at risk for development of clinical varicella-zoster infection and should be given VZIG regardless of antibody titer.

In 2006, VZIG from the Massachusetts Pubic Health Laboratories became unavailable, replaced by a new investigational VZIG product, VariZIG, available through FFF Enterprises (800-843-7477).[116] IGIV and acyclovir prophylaxis are reasonable alternatives if VariZIG is not available.[28]

Intravenous Immune Globulin in Varicella Prophylaxis

Paryani and colleagues[456,457] in 1984 observed that IGIV at doses of 200 to 300 mg/kg resulted in serum varicella-zoster virus antibody titers comparable to those in patients receiving VZIG at standard doses. Subsequently, Chen and Liang[125] demonstrated that varicella was prevented by a single dose of IGIV at 200 mg/kg in five children with leukemia.

Immunocompromised patients who are receiving IGIV at 400 mg/kg are thought to be protected for 3 weeks after infusion and do not require additional VZIG on exposure.[28] However, Ferdman and Church[182] reported that varicella occurred in two HIV-infected children receiving 500 mg/kg of IGIV after exposure to varicella 7 and 11 days after infusion. Both had mild disease and responded well to acyclovir. They suggest that for patients with profound immunodeficiency, VZIG can be given in addition to IGIV. Kavaliotis and associates[307] found that an IGIV dose of 1 g/kg given within 6 to 24 hours of exposure was 90 percent effective in preventing varicella in an oncology unit, with no additional advantage noted with administration of VZIG.

Tokat and colleagues[598] described a 32-year-old man with severe varicella pneumonia that progressed to acute respiratory distress syndrome. IGIV (400 mg/kg for 5 days) was given with a successful outcome.

Recommendations

The decision to administer VZIG or IGIV or acyclovir to prevent chickenpox is based on the patient's susceptibility, the type of exposure, and the patient's immune competence.

DETERMINATION OF SUSCEPTIBILITY

With the exception of bone marrow transplant recipients, most immunocompromised individuals with previous varicella infection are considered immune; however, their immune status should be confirmed with varicella antibody titers. Healthy adults reared in the United States are considered immune even without a history of varicella, zoster, or vaccination, but immunocompromised children and adults without a history of varicella are considered susceptible because of their higher risk for development of severe infection. Bone marrow transplant recipients are considered susceptible regardless of the varicella history of the donor or recipient. If varicella or herpes zoster develops after transplantation, the patient then is considered to be immune.[116,232]

An immunocompromised, unimmunized child without a history of chickenpox is considered susceptible even if antibody titers are present because receipt of blood products containing immunoglobulin may result in transient seropositivity. Alternatively, such patients who received VZIG may have had asymptomatic varicella with the subsequent development of varicella antibodies, yet they may not be protected on re-exposure.[28] The value of antibody titer to determine susceptibility in these patients is controversial. However, oncology patients who have been vaccinated for varicella before illness and have been seropositive for varicella antibodies before receipt of blood products are considered immune and do not routinely need VZIG. The severity of immune compromise is also a factor, and bone marrow transplant recipients are considered susceptible despite prior history of immunization or disease.

VZIG is not recommended for immunodeficient patients who have been vaccinated in the past, provided they previously were shown to have varicella antibody, even if they are seronegative at the time of exposure. These individuals are expected to have mild disease.[116] However, verifying varicella immunity with antibody before forgoing VZIG administration in this situation seems to be a reasonable approach.

TYPE OF EXPOSURE

Individuals residing in the same household as a patient with varicella, persons who have had face-to-face contact with a patient considered infectious, and patients sharing the same hospital room are considered to have significant exposure. Because the duration of exposure that results in transmission is not known, each exposure must be evaluated individually. Contact with a varicella vaccine recipient with a varicella rash must be considered a significant exposure for an immunodeficient patient because the vaccinee may have wild-type virus infection; vaccine strain virus rarely has been transmitted.[232]

CANDIDATES FOR VARICELLA-ZOSTER IMMUNE GLOBULIN OR INTRAVENOUS IMMUNE GLOBULIN (Table 256–7)

Normal Adults, Children, and Adolescents

Normal children, adolescents, and adults exposed to chickenpox or zoster usually are not candidates for receiving VZIG or IGIV. They may be given chickenpox vaccine if they are older than 12 months. VZIG is not necessary for normal term infants exposed

TABLE 256–7 Candidates for Varicella-Zoster Immune Globulin (VZIG) After Significant Exposure

Immunocompromised children without a history of varicella and without documented response to immunization*
Susceptible, pregnant women
Newborn infants whose mother had an onset of varicella within 5 days before delivery or within 48 hours after delivery
All hospitalized premature infants born at less than 28 weeks of gestation or less than 1000 g at birth, regardless of maternal history
Hospitalized premature infants of more than 28 weeks' gestation whose mother has no history of chickenpox or who is seronegative; may consider its use for other premature infants. See text.

**Severity of immune compromise is a factor in the decision to give VZIG despite immunization and disease history. See text.*

to varicella after 48 hours of life, but it is considered optional for infants younger than 1 week.[202]

Immunocompromised Children and Adults

The principal use of VZIG is to prevent chickenpox in susceptible immunocompromised children exposed to zoster or chickenpox (see Table 256–7). These children include those with primary immunodeficiency and those with secondary immunodeficiency, including HIV infection and neoplastic disease, and those receiving immunosuppressive therapy (e.g., systemic steroids, chemotherapy).[37,437,445]

Term and Premature Newborns

Newborns who acquire varicella after birth are not at high risk for development of severe disease and are not candidates for receiving VZIG, with the possible exception of those whose mothers have severe skin disease.[28] Newborn infants born to mothers in whom varicella developed within 5 days before delivery or within 2 days after delivery are at high risk for having severe varicella because they have not received transplacental protective antibodies.[116] Others, questioning the validity of these limits, have treated a small number of infants born to mothers in a window ranging from 7 days before delivery to 5 days after delivery with IGIV (500 mg/kg) and a prophylactic acyclovir regimen.[265]

Exposed hospitalized premature infants born at less than 28 weeks' gestation or less than 1000 g should receive VZIG. These infants are susceptible, regardless of the maternal history of varicella, because of incomplete transplacental transfer of maternal antibody. Hospitalized premature infants with significant exposure who were born at more than 28 weeks' gestation and whose mothers do not have a history of varicella or are seronegative also are considered candidates for receiving VZIG because transplacental antibody would not be present.[28,116]

Gold and colleagues[212] showed that gestational age of more than 28 weeks and birth weight above 1000 g do not always accurately predict the presence of maternal varicella antibody. Ogilvie[437] suggested that infants born before 30 weeks' gestation be given VZIG and that some infants born after 28 weeks will lose their maternal antibody by 60 days of age; she recommends antibody testing on exposure if it can be performed quickly to assess the need for VZIG. Linder and colleagues[360] also noted that only 24% of prematures born at 29 to 35 weeks had positive FAMA (fluorescent antibody to membrane antigen) titers at 2 months of age.

Gold and associates[212] recommended the routine use of VZIG in the neonatal ICU if the FAMA assay is not available to determine susceptibility. Previous administration of blood products in the neonatal ICU may render these results uninterpretable.

Pregnant Women

Susceptible pregnant women are candidates for receiving VZIG because of their increased risk for development of severe varicella pneumonia.[547]

DOSAGE

VariZIG brand of VZIG is supplied in lyophilized vials of 125 U (reconstituted to a 5% solution for intramuscular use). The dose of VZIG is 125 U/10 kg intramuscularly. The minimal dose for infants is 125 U (one vial), and the maximal dose is 625 U for adults (five vials).[28,117]

Although VZIG should be given to susceptible candidates as soon as possible after exposure for maximal protection, it can be given up to 96 hours after exposure.[112] A second dose is not needed for subsequent exposure unless it occurs beyond 3 weeks after administration of the VZIG dose.[28] IGIV (400 mg/kg)[116] is an acceptable alternative if the child has a bleeding diathesis and cannot receive intramuscular injections or if VZIG is not available.

VZIG is not recommended for the treatment of chickenpox, zoster, disseminated varicella, or post-herpetic neuralgia; zoster immune globulin did not prevent dissemination of zoster in immunocompromised patients.[560]

HUMAN IMMUNODEFICIENCY VIRUS INFECTION

As noted earlier in the chapter, regular infusions of IGIV have been used to prevent concomitant infection in patients with HIV infection, notably children, who have compromised immune systems with antibody deficiencies. Attempts to use HIV-specific antibody to prevent HIV infection or to provide an antiviral effect have met with very limited success.

These studies have used human immune plasma or hyperimmune HIV immunoglobulin (HIVIG) from asymptomatic HIV-seropositive patients, porcine antisera, or monoclonal antibodies.[564] In two of three controlled clinical trials of immune plasma in adult patients with AIDS, a modest clinical benefit was achieved as judged by decreased opportunistic infections, a slight increase in $CD4^+$ cell counts, and improved survival; however, no striking decreases in viral burden were noted.[277,355,621]

In a double-blind, placebo-controlled multicenter study, HIV-seropositive pregnant women receiving zidovudine were given HIVIG monthly during the last trimester of pregnancy, and their newborns were given one HIVIG dose at birth; no effect on the rate of maternal-fetal HIV transmission versus that in a similar group given regular IGIV was found.[563] The rate of transmission in both groups was unexpectedly low (less than 5%), so the study was discontinued after 800 patients had been enrolled because the study was not statistically powered to detect a slight reduction in transmission rates.

Another study[561] examined the antiviral effect of large doses of HIVIG in 30 children with moderately advanced HIV infection who were receiving stable antiviral treatment and showed a measurable viral burden. No striking beneficial effect was observed as indicated by plasma HIV RNA levels, cellular viral culture titers, or immunologic assays.

Despite these failures, efforts to develop antibody preparations that will neutralize the virus continue; such preparations would be particularly valuable after needle-stick, perinatal, or sexual exposure.[564] Under study are monoclonal antibodies to neutralizing regions of the virus, antibodies to co-receptors that may interfere with HIV attachment, or combinations of antibodies with different specificities, including combinations of HIVIG with monoclonal antibodies.[33,102,342,358,359,452] An effective neutralizing antibody also would provide valuable information toward an effective vaccine.

Recommendations

IGIV is used in immunosuppressed patients with HIV infection to prevent bacterial and viral infections and to treat certain complications (e.g., thrombocytopenia, anemia of parvovirus infection) but has no direct antiviral activity. HIVIG or plasma derived from infected patients has little or no antiviral activity or clinical benefit for HIV-infected adults or children, nor has HIVIG given during pregnancy decreased maternal-infant transmission.

Monoclonal antibodies directed against the virus or its cellular receptors show considerable promise but are not yet available.

MEASLES

The first successful prophylaxis of measles with convalescent serum was reported by Cenci[114] in 1907. Convalescent serum was

used in the United States first in 1916 by Park, Freeman, and Zingher, who gave either 4 or 8 mL of serum to 41 recently exposed children at New York Metropolitan Hospital.[453,667] Measles did not develop in any of the 20 children who received the 8-mL dose and developed in only three of the 20 children who received the 4-mL dose. Park and Freeman[453] in 1926 found that 6 to 10 mL of convalescent serum was 92 percent efficacious in preventing measles in recently exposed individuals, a finding confirmed and extended by Stillerman and associates.[568] Placental extracts containing serum antibodies also were used in the prevention and modification of measles.[390]

Stokes and colleagues[573] and Ordman and associates[444] both confirmed in 1944 that immunoglobulin given immediately after exposure could prevent or modify measles and given in the early stages of clinical illness could lessen its severity. Greenberg and coworkers[224] in 1955 noted a lower incidence of measles encephalitis in IGIM-modified measles.

A major use of IGIM in the 1960s was to diminish the side effects of the Edmonston strain of measles vaccine. Krugman and associates[336] in 1962 noted that the simultaneous administration of 0.02 mL/lb of IG at the time of Edmonston measles vaccination reduced the incidence of high fever from 40 to 14 percent and the incidence of rash from 10 to 2 percent. The mean titer of measles antibody achieved was somewhat reduced by the IGIM, and a slight decrease in the rate of seroconversion was noted; nonetheless, the vaccine-IGIM combination was 95 percent effective.[572] The current measles vaccine does not require the need for concomitant IGIM injections.

Audet and colleagues[41] report that the measles neutralizing antibody titer in recent IGIV lots is decreasing because of the lower titer of vaccine-induced antibodies compared with natural infection among donors. Although not tested, IGIM probably has lower titers of measles antibodies, so that the recommended prophylactic doses may be inadequate.

Recommendations

IGIM or IGIV can be given to susceptible contacts within 6 days of exposure to prevent or to modify measles. Infants younger than 1 year, immunocompromised patients, and pregnant women are at risk for development of severe disease and should receive immunoprophylaxis.[19] Because immunodeficient patients with HIV infection may contract measles after exposure despite receiving immunization, they should receive passive immunoprophylaxis.[201] IGIM also can be given to close family contacts. Measles vaccine has some efficacy if it is given within 72 hours of exposure and is preferred for the management of outbreaks in healthy children older than 1 year.[19]

The standard dose of IGIM is 0.25 mL/kg for healthy patients and 0.5 mL/kg for immunocompromised subjects. The maximal dose is 15 mL. IGIV at comparable doses also can be used, but one should realize that the titers in both products may be diminished in recent years.[41]

Immunodeficient children receiving regular IGIV treatments at doses of at least 100 mg/kg are protected for 3 weeks after its administration. Additional IGIV can be considered if the exposure occurs more than 2 weeks after the IGIV dose.[19]

Administration of IGIM to a healthy infant precludes the use of measles vaccine for 5 months, and after the administration of large doses of IGIV, longer intervals are needed (e.g., 8 to 11 months).[19]

MUMPS

Mumps immune globulin was ineffective as postexposure prophylaxis, and this product no longer is manufactured in the United States.[20]

PARVOVIRUS

Human parvovirus B19, also termed erythrovirus, the cause of the benign childhood exanthem termed fifth disease (slapped-cheek syndrome), also may cause polyarthropathy, transient aplastic crises in patients with hemolytic anemia, chronic red cell aplasia in immunocompromised patients, and hemophagocytic syndrome.[91,271,656] Patients susceptible to chronic parvovirus infection include those with congenital[591] or acquired immunodeficiencies,[130,325] those receiving immunosuppressive therapy,[656] and organ transplant recipients.[303,378] In utero parvovirus infection can result in hydrops fetalis.[600] The use of parvovirus DNA PCR has facilitated establishment of the diagnosis in immunodeficient patients unable to make antibodies or in patients who have passively acquired parvovirus antibodies.

Parvovirus B19 has tropism for marrow erythroid progenitor cells because these cells express the blood group P antigen, the cellular receptor for the virus. Most adults have encountered and made antibodies to parvovirus, and thus IGIV is an excellent source of antiparvovirus antibodies.[521] IGIV has been used successfully to treat parvovirus-induced red cell aplasia.[344] Patients with AIDS are particularly susceptible to this disease. Successfully treated patients have decreased viremia, reticulocytosis, and resolution of the anemia within 2 weeks. Sometimes relapse or persistent infection occurs, requiring maintenance IGIV therapy, particularly in patients with HIV infection and low $CD4^+$ cell counts.[325]

IGIV has been used successfully to treat parvovirus B19 anemia resulting from chemotherapy,[392] rituximab therapy,[528] or both.[276,552] However, IGIV was not of value in four children with persistent parvovirus infection undergoing chemotherapy for acute lymphocytic leukemia.[176] IGIV also has been used successfully for treatment of parvovirus B19 anemia in renal transplant recipients.[129,379,449,550]

Viguier and coauthors[618] administered 1 g/kg of IGIV for 2 days to treat successfully a 33-year-old woman with parvovirus B19–associated polyarteritis nodosa and fever, palpable purpura, intense myalgia, paresthesias, and polyarthritis of the hand joints. IGIV has been used with success in parvovirus B19–associated chronic fatigue syndrome in three adults[315] and one teenage boy.[389]

IGIV has been used as a supplement to transfusions in severe in utero parvovirus infection with hydrops fetalis. Selbing and coworkers[524] described a 24-week pregnant women with severe preeclampsia whose fetus had ascites and pericardial effusion; she was given 25 g of IGIV with resolution of the ascites, effusion, and anemia. Matsuda and colleagues[383] treated a parvovirus-infected hydropic fetus with two injections of immunoglobulin into the fetal peritoneum with resolution of the anemia and the hydrops.

Rugolotto[501] treated an anemic newborn secondary to intrauterine parvovirus infection with IGIV (1 g/kg every 3 weeks for 8 months). Heegaard and coauthors[247] reported similar success of IGIV, in addition to in utero transfusions. Earlier attempts to treat older infants with persistent parvovirus-induced congenital anemia with IGIV were unsuccessful.[90]

Human neutralizing monoclonal antibodies have been generated from infected individuals[204] for possible future therapeutic use.

Recommendations

Red cell aplasia caused by parvovirus infection in immunodeficient patients, including those with HIV infection, should be treated with IGIV, 400 mg/kg per day for 5 days or 1 g/kg per day for 2 days.[91,325,411] If relapse is likely to occur because of the patient's severe immunocompromised status, IGIV at 400 mg/kg can be given every 4 weeks.

Neonates with persistent congenital anemia caused by parvovirus B19 should receive 400 mg/kg of IGIV for 5 days, with additional doses given if the anemia does not resolve.

Pregnant women carrying fetuses with in utero hydrops fetalis caused by parvovirus B19 infection also may be candidates for IGIV therapy.

RABIES AND RABIES IMMUNE GLOBULIN

Rabies is the ideal disease for passive immunization because the exact moment, the exact source, and the exact location of exposure usually are known. Furthermore, the long incubation period and the fact that the virus remains localized to the wound for several days enhance the effectiveness of passive immunization. Although a rabies serum was prepared initially in 1889 by Babes and Lepp,[42] experiments of Habel[238] in mice, guinea pigs, and monkeys involving the use of rabbit hyperimmune serum demonstrated that antibody worked by two mechanisms: (1) neutralizing the virus while it is still in tissues and (2) retarding the spread of virus within the nervous system, thereby prolonging the incubation period and permitting active immunity by vaccine to become established.

On the basis of these studies, a World Health Organization Expert Committee in 1950 recommended that a field trial of the efficacy of hyperimmune rabies serum in conjunction with vaccine be conducted.[173] It was undertaken in Iran because multiple bites by a single rabid wolf coming into isolated villages were not uncommon occurrences, and this severe exposure had an associated 40 to 50 percent mortality. In 1954, a single rabid wolf bit 27 individuals, 17 of whom were bitten on the head. These 17 were divided into three groups: five received vaccine alone, seven received vaccine and one dose of antirabies serum, and five received vaccine and two doses of antirabies serum.[50] Three of five persons treated with vaccine alone died of rabies, one of seven in the one-dose antiserum group died, and none of five in the two-dose antiserum group died.

Antibody studies conducted on these patients indicated that a single or a double dose of antiserum, followed by 14 to 21 daily doses of vaccine, results in significant levels of circulating antibody for as long as 50 days.[237] The antibody found early is supplied passively; after the tenth day, the antibody present is a result of the vaccine. Thus, optimal treatment requires both passive and active immunization.

Before 1971, the only available antiserum was of equine origin. It is still the only product available in some countries. Since 1971, human rabies immune globulin (RIG) has been available in the United States and many other countries and is preferred because of the lessened risk of serum reactions.[538] In addition, human antibody has a half-life in the circulation twice that of equine antibody, with the result that higher levels of passive antibody are maintained. However, the antibody response to the vaccine given concomitantly is suppressed more effectively.[362]

A case of fatal rabies was reported in a 19-year-old man bitten on the finger by a rabid mongoose despite the recommended postexposure prophylaxis (RIG and five doses of human diploid cell rabies vaccine).[531] Possible reasons for failure included (1) inadequacy of the recommended dose of RIG; (2) vaccine injection into the gluteal region, where more fat is found than in the recommended deltoid region; and (3) decreased antibody response to the vaccine as a result of a possible immunodeficiency state.

Prevention of rabies consists of three essential components: thorough washing of the wound with a 20 percent soap solution followed by irrigation with povidone-iodine,[71,502] passive immunization with rabies antibody, and active immunization. Although most cases of failure are caused by lack of adherence to these three strategies,[469] isolated cases of rabies occurring despite appropriate management have been reported.

Wilde and associates[640] reported failure of prophylaxis in five Thai children who received multiple bites on the face and head; they suggested that failure to infiltrate all wounds with RIG and surgical closure before the wound was infiltrated might have been factors. Hemachudha and coauthors[249] reported failure of prophylaxis in a child with minor scratches on the nose and in a woman with a deep wound on the cheek; they suggested that direct inoculation of virus into nerve endings may have occurred.

Goudsmit and associates[214] found that a human monoclonal antibody combination was equivalent to RIG when it was used with vaccine in a hamster model of rabies.

Recommendations

Human RIG or animal antiserum is recommended in nonimmunized individuals for all bites by animals in which rabies cannot be ruled out and for nonbite exposure to animals proved to be or suspected of being rabid.[22,121] Such treatment should be given as early as possible after exposure and followed by vaccine administration. Two human RIG products are available in the United States, BayRab and Imogam Rabies-HT; both are prepared from the plasma of hyperimmunized donors. The recommended dose of 20 IU/kg of RIG must not be exceeded because higher doses can suppress the vaccine response.

If RIG is not available, the dose of equine RIG is 40 IU/kg, and desensitization may be required. We now recognize[267] that the entire RIG dose should be infiltrated around the wounds. If this is not anatomically feasible, any remaining RIG should be given intramuscularly.[22]

Wilde and colleagues,[640] recognizing the difficulty in infiltrating multiple wounds with a small volume of RIG, suggested dilution of the RIG. The World Health Organization[653] recommends dilution of the RIG twofold to threefold in saline to ensure an adequate volume for infiltration. RIG should not be frozen, and if it is frozen, it should not be used.

Although administration of one of the three vaccines licensed in the United States should be started at the same time as RIG, the vaccine and RIG should not be administered in the same syringe or with the same syringe or needle. Only two of the licensed vaccines are currently produced, human diploid cell rabies vaccine and purified chicken embryo cell vaccine. Vaccine should be given in the deltoid muscle or, for an infant, in the anterolateral aspect of the thigh and at an anatomic site removed from the injury or any injection sites of RIG.

If RIG is not available immediately, immunization should be started and RIG given if it is available within 7 days.[22,121] If vaccine is not available immediately, RIG should be administered and the vaccine given as soon as possible. If both RIG and vaccine are delayed, both should be given whenever available, regardless of the interval between exposure and treatment.[22,121]

RIG is not recommended for patients who previously have received a full postexposure vaccine course of human diploid cell rabies vaccine, rabies virus adsorbed, or purified chicken embryo cell vaccine; who have received a three-dose pre-exposure intramuscular series of these vaccines; who have received a three-dose pre-exposure intradermal human diploid cell rabies vaccine used in the United States; or who have been immunized with any vaccine and have a documented rabies titer.[22,121]

Postexposure prophylaxis of immunodeficient patients is problematic. Jaijaroensup and coauthors[280] reported poor or nondetectable neutralizing antibody in five HIV-infected patients who received intradermal vaccine and proposed that higher doses of vaccine or additional boosters be considered. Serologic failure of both pre-exposure and postexposure rabies immunizations in children with HIV infection[450,597] has been reported.

Thus, immunodeficient patients should undergo serologic testing to document seroconversion after completion of the

vaccine. An adult patient with lymphoma[246] was attacked by a rabid jackal and received RIG and rabies vaccine while chemotherapy was delayed. When antibody titer was low after the series of vaccines, a second course of vaccine and RIG was administered. The patient survived both the lymphoma and rabies exposure.

RIG is not indicated for the treatment of rabies. Hemachudha and colleagues[250] described a patient with rabies who developed total limb and facial paralysis after receiving RIG.

RESPIRATORY SYNCYTIAL VIRUS

Acute RSV infection of the respiratory tract is the most common cause of hospitalization of infants and young children and thus is a significant public health expense. For high-risk patients such as premature infants and infants with chronic lung disease, RSV infection can be severe and life-threatening. The observation that passively transferred maternal antibody provided some protection from RSV infection[436] led to the development of passive immunity products to prevent and to modify the severity of infection. No effective vaccine exists.

RESPIRATORY SYNCYTIAL VIRUS–INTRAVENOUS IMMUNE GLOBULIN

The first product used was RSV-IGIV (RespiGam), a 5 percent polyclonal human intravenous IG prepared from healthy donors with high RSV-neutralizing antibody titers. RSV-IGIV was licensed in 1996 after studies in high-risk infants demonstrated its efficacy. Groothuis and colleagues[226] conducted a prospective, blinded, randomized trial at five centers and showed that 58 premature infants who received RSV-IGIV at 750 mg/kg per month during the winter months had a significantly lower incidence of RSV lower respiratory tract disease than that observed in 58 premature control infants who did not receive RSV-IGIV (6.9% versus 24.1%; $p = .01$). The RSV-IGIV–treated patients also had a lower incidence of moderate to severe RSV lower respiratory tract disease ($p = .006$), fewer days of hospitalization ($p = .020$), and fewer days in the ICU ($p = .05$).

Another large RSV-IGIV multicenter, double-blind, placebo-controlled study[475] of 510 infants with either prematurity or bronchopulmonary dysplasia also demonstrated decreased number of hospitalizations (13.5% versus 8.0%; $p = .047$), decreased total number of RSV hospital days ($p = .045$), and decreased days with oxygen use ($p = .007$). RSV-IGIV also decreased days of hospitalization for any respiratory illness by 38 percent and total respiratory illness hospital days by 46 percent, presumably because of antibodies to other respiratory pathogens.

An added benefit of RSV-IGIV prophylaxis was a decreased incidence of otitis media (number of episodes, 0.15 per patient versus 0.78 per control; $p = .003$).[243,541]

In 1997, the American Academy of Pediatrics recommended RSV-IGIV for high-risk children with prematurity or bronchopulmonary dysplasia[12] but not for infants with cyanotic congenital heart disease[227,542] because of the risk for development of serious side effects. RSV-IGIV no longer is available.

PALIVIZUMAB

In 1996, palivizumab (Synagis), a humanized IgG1 monoclonal antibody against the F (fusion) glycoprotein of RSV,[208,575] entered clinical trials. Palivizumab neutralizes both type A and type B RSV and is 50 to 100 times more potent than RSV-IGIV.[511] A multicenter, randomized, double-blind, placebo-controlled study of 1502 high-risk infants (premature infants and infants younger than 2 years with bronchopulmonary dysplasia) demonstrated the efficacy of 15 mg/kg of palivizumab given intramuscularly every 30 days.[270] It resulted in significantly decreased number of RSV hospitalizations (55%; $p < .001$), RSV hospital days ($p < .001$), and days of oxygen therapy ($p < .001$).

Palivizumab was licensed in 1998, the first monoclonal antibody approved for the prevention of an infectious disease. Postlicensure studies also have supported its effectiveness.[463,494,544] Although debate continues about the cost-effectiveness of palivizumab in some countries,[309,442,580] palivizumab prophylaxis for high-risk infants is now the standard of practice in the United States.[23]

In 2003, Feltes and colleagues[179] reported the results of an international, randomized, double-blind, placebo-controlled trial of palivizumab prophylaxis in 1287 children with congenital heart disease. This study showed a 45 percent reduction in RSV hospitalizations, a 56 percent reduction in total number of hospital days, and a 73 percent reduction in total number of hospital days with oxygen with the use of palivizumab. European cardiologists and the American Academy of Pediatrics recommend use of palivizumab for infants with hemodynamically significant heart disease (uncorrected, partially corrected, or with residual disease), pulmonary hypertension or chronic lung disease, and hemodynamically significant dilated or hypertrophic cardiomyopathy causing cyanosis or requiring cardiac medication.[23,599]

The use of palivizumab for more than one season has been a concern because of the theoretical risk of side effects resulting from the development of an antibody to the murine component.[346,428] Null and colleagues[428] found that only one child of 55 receiving palivizumab for a second season developed an anti-palivizumab titer of greater than 1:40. This child had a titer of 1:160 on day 30 that fell to 1:10 on day 120 (30 days after the fourth injection), suggesting a nonspecific reactivity. No associated adverse events occurred, and the palivizumab trough concentrations were as expected.

Palivizumab or other RSV antibodies are not indicated for the treatment of RSV infection. Saez-Llorens and colleagues[506] found in a small pharmacokinetic study that although palivizumab was safe, it was of no clinical benefit for previously healthy children with acute RSV infection. Banna and colleagues[51] successfully used palivizumab and steroid therapy to treat an adult woman with RSV-related pneumonia after stem cell transplantation. An experimental RSV monoclonal antibody (Medi-493), given intravenously, was not of benefit for intubated patients with RSV infection and respiratory failure.[376] Studies of high-titer monoclonal antibodies may yield different results.[190]

PASSIVE IMMUNITY IN RESPIRATORY SYNCYTIAL VIRUS TREATMENT

Although a single dose of 1500 mg/kg of RSV-IGIV was of no benefit in normal and high-risk children with RSV infection,[492,493] DeVincenzo and associates[153] gave a single dose of RSV-IGIV (1500 mg/kg) to 11 bone marrow transplant recipients after the onset of RSV infection. All but one patient also received ribavirin. Only one patient died in this group, which compares favorably with historical controls given ribavirin therapy alone.

Ghosh and coworkers[203] gave IGIV (500 mg/kg every other day) and aerosolized ribavirin to 14 adult bone marrow transplant recipients with RSV upper respiratory tract infection to prevent pneumonia and death. Ten patients resolved their infection, pneumonia developed in four, and two died. Aerosolized human IGIV was of no clinical benefit in the treatment of patients with RSV infection.[489]

Recommendations

Palivizumab is indicated for prevention of RSV infection during the RSV infection season for high-risk premature infants or infants with chronic lung disease.[23] The period of prophylaxis should be individualized, depending on the duration of the local RSV infection season.

CANDIDATES FOR PROPHYLAXIS[23]

1. Infants younger than 24 months with chronic lung disease of prematurity requiring medical treatment in the past 6 months at the start of the RSV infection season. Two seasons of treatment may be required for some infants who require ongoing medical treatment.
2. Premature infants without chronic lung disease who were born before 32 weeks' gestation. Infants born at 28 weeks or earlier may benefit from 12 months of prophylaxis, whereas infants born at 29 to 32 weeks' gestation may benefit most from prophylaxis for 6 months,[23] although decisions should be individualized.
3. Premature infants of 32 to 35 weeks' gestation who have two or more high-risk factors and are younger than 6 months at the start of the RSV infection season. Such factors may include severe neuromuscular disorders, group daycare, school-age siblings, environmental air pollutants, and congenital abnormalities of the airways.[511]
4. Children younger than 24 months with hemodynamically significant heart disease. These criteria include children requiring medication for congestive heart failure, children with moderate to severe pulmonary hypertension, and children with cyanotic heart disease. Children with hemodynamically insignificant heart disease are not candidates for palivizumab. This category includes atrial septal defect, small ventricular septal defect, pulmonic stenosis, mild coarctation of the aorta, and patent ductus arteriosus.

   Children who have had surgical correction and do not require medications for congestive heart failure also are not candidates for prophylaxis. After cardiopulmonary bypass, a postoperative dose of palivizumab can be given to maintain protection because decreased levels of antibody have resulted from cardiopulmonary bypass.[179]
5. Infants who are severely immunocompromised can be considered for prophylaxis, although there are no studies on which to base a recommendation.

POSSIBLE INDICATIONS

1. Palivizumab in addition to implementation of strict isolation practices may be of value in aborting a nosocomial outbreak of RSV infection in the premature nursery.[1,142] However, current dosing recommendations for outpatients may not be adequate for some hospitalized premature infants.[654]
2. Patients with cystic fibrosis who are younger than 2 years also may be candidates for RSV prophylaxis, but no data exist on which to base a recommendation.
3. Older high-risk infants who received palivizumab the previous season but remain unwell, are immunocompromised, or have recently undergone a procedure such as transplantation or operation may benefit from a second season of palivizumab. They include immunodeficient infants receiving IGIV because IGIV has only low titers of RSV antibodies.

DOSAGE AND ADMINISTRATION

Prophylaxis is given monthly from just before the start of the local RSV infection season until the end of the season. Usually, five injections are given from November to March, but starting earlier or extending into April may be required. Thus, it is important to be aware of the local outbreak data. If an infant develops RSV infection while receiving prophylaxis, palivizumab should be continued because more than one RSV strain could infect the patient in a single season.[23]

The dose of palivizumab is 15 mg/kg per month given intramuscularly. It is supplied in 50- and 100-mg vials.

As discussed before, palivizumab is not indicated for the treatment of RSV infections.

ROTAVIRUS

Several reviews have summarized the role of oral IG in the prevention and treatment of rotavirus infection.[79,148,241,632] Its effectiveness varies, in part because of differences in antibody titer in the various products.[53,163,229,230,258,403,514] Human IG has been used to prevent rotavirus infection; Barnes and colleagues[53] gave human gamma globulin orally to low-birth-weight infants in a nursery in which rotavirus was endemic. Patients given human IG had milder disease than placebo recipients did.

Human IG has been infused into the duodenum[230] to treat two children with prolonged rotavirus infection. One patient responded rapidly, but the other had a prolonged course. Losonsky and coworkers[363] gave human IG to three immunodeficient patients and demonstrated that it survived passage of the gastrointestinal tract in an immunologically active form.

Pooled colostrum concentrate from hyperimmunized pregnant cows with high titers to several rotavirus serotypes[98] has been used in several studies. In a controlled study, bovine immune colostrum[603] did not prevent development of symptomatic rotavirus infection in infants, but treated patients had a decrease in the duration of diarrhea. Immune bovine colostrum also was used prophylactically for 2 weeks in 10 infants in a baby care center, with evidence of a protective effect.[163]

A double-blind, placebo-controlled trial of oral lyophilized rotavirus bovine colostrum in Bangladesh showed that a 4-day course decreased diarrhea, the need for oral rehydration, and viral shedding.[514] Mitra and associates[403] also showed that cow colostrum[603] significantly shortened the duration of diarrhea and decreased stool output. Hilpert and colleagues[258] demonstrated that rotavirus colostrum decreased virus excretion in infants with rotavirus diarrhea.

A lactobacillus engineered to express a neutralizing antibody fragment of a llama immunoglobulin (lactobodies) was given orally to experimental animals with rotavirus diarrhea and markedly shortened disease duration, severity, and viral load.[451,611]

A recently licensed vaccine for rotavirus seems safe and effective.[24]

Recommendation

Because rotavirus infection usually is self-limited, no commercial passive antibody product has been developed, although it would be of use in developing countries. The neutralizing titer of human IG probably is not high enough to be consistently beneficial. Oral vaccines may decrease the need for antibody-based therapies.

RUBELLA

IGIM rarely is used for prevention of rubella because of its uneven efficacy. Early studies suggested some prophylactic benefit. Korns[329] in 1952 showed that one lot of IGIM at a dose of 0.1 mL/lb partially protected mentally handicapped institutionalized subjects against development of epidemic rubella. Rubella developed in 9 of 45 IGIM-injected subjects versus 35 of 60 noninjected controls, a significant difference. However,

another IGIM lot was ineffective, and a third was only slightly effective, suggesting that the titers of rubella antibodies varied significantly.

Studies in epidemic situations have shown that 5 mL of IGIM can prevent development of clinical rubella,[220] but Brody and coworkers[87] showed that an IGIM dose of 0.55 mg/kg resulted in a decreased clinical attack rate but also a high incidence of subclinical infection. Military recruits were protected by a 15-mL dose of IGIM administered before exposure.[262]

Extensive studies on IGIM prophylaxis of rubella conducted by Green, Krugman, and their associates[223,334] did not demonstrate a protective effect of 0.12 to 0.2 mL of IGIM per pound. Lundström and coworkers[369] gave 251 exposed pregnant Swedish women 4 mL of convalescent rubella IGIM and 28 exposed pregnant women 24 mL of standard IGIM. Rubella developed in 6 of the 251 (2.4%); three of these six women aborted, and one had an infant with probable congenital rubella. None of the 28 women given 24 mL of standard IGIM contracted rubella. Their subsequent study demonstrated that convalescent rubella IGIM given to pregnant women with clinically manifested rubella did not protect against congenital rubella or lessen the probability of fetal damage.[370]

Recommendations

IGIM prophylaxis is not indicated for children and nonpregnant adults exposed to an infected contact because of the mildness of the infection.

IGIM is not recommended for most exposed pregnant women because the clinical syndrome may be masked and congenital rubella not prevented. For exposed pregnant women who will not consider abortion if rubella develops, a large dose of IGIM may be of some value. A dose of 0.55 mL/kg of IGIM given intramuscularly is the recommended dose.[25] However, mothers who have received such prophylaxis after exposure have delivered infants with congenital rubella.[25]

VACCINIA, VARIOLA, AND VACCINIA IMMUNE GLOBULIN

Vaccinia virus is closely related antigenically to smallpox (variola) virus, and thus immunity to vaccinia virus through vaccination prevents smallpox.[420] Although smallpox has been eradicated from the world since 1977, the virus nonetheless exists in a few research laboratories and is a potential weapon in biologic warfare.

Vaccination is still given to members of the military, to some health care workers, and to laboratory workers who handle cultures or animals contaminated with vaccinia virus, some recombinant vaccinia viruses, and other orthopoxviruses (e.g., monkeypox or cowpox). Passive immunization occasionally is necessary after laboratory accidents, inadvertent vaccination of high-risk individuals, and exposure of high-risk individuals to a recently vaccinated individual.

Passive immunization against variola was known as early as 1895, when Hlava and Honl[259] showed that schoolchildren could be protected from variola by the injection of 3 to 10 mL of serum from a cowpox-immune calf. At the same time, protective human antibodies developed after vaccination, and passive transmission of these antibodies from mothers to infants was demonstrated.[558] Thereafter, effective prevention of smallpox by well-organized mass vaccination campaigns diminished interest in passive immunization.

Janeway, quoted by Enders,[168] found neutralizing vaccinia antibody in IG in 1944, and Verlinde and Spaander[616] in 1949 found high titers of such antibodies in convalescent IG from recently vaccinated individuals. Gispen and associates[205] in 1956 developed a human vaccinia immune globulin (VIG) that did not interfere with active immunity, and they proposed its use with vaccine as prophylaxis against vaccinia encephalitis. Human VIG is derived from the plasma of vaccinated donors.[118]

The value of human VIG in treating smallpox or disseminated vaccinia is based on the presence of viremia, which leads to secondary dissemination. Administration of neutralizing antibody will prevent or limit the spread of infection and thus modify clinical expression of the disease.

Studies on the efficacy of VIG in treating smallpox and vaccinia complications were initiated by Kempe and colleagues in 1955.[52,312] They used a VIG that was prepared from recently vaccinated donors with a neutralizing titer of 1:256 to 1:512, in contrast to titers of 1:16 or 1:32 in standard IGIM. The households of new admissions to the Madras (India) Smallpox Hospital were visited, and alternate family contacts received VIG (1.0 g in adults, 0.5 g in children).[312] After 25 days, eight cases of smallpox developed in 75 contacts not given VIG, and two cases developed in 56 contacts given VIG, a significant difference. A similar, more extensive study disclosed 21 cases of smallpox (four severe) among 379 contacts serving as controls and five cases of smallpox (none severe) among 326 contacts given VIG.[313]

Kempe[314] also reported the results of 300 cases of smallpox vaccination (vaccinia) complications treated with VIGIM, including 62 cases of generalized vaccinia, 132 cases of eczema vaccinatum, 23 cases of vaccinia necrosum, 12 cases of vaccinia encephalitis, and 28 cases of autoinoculation. In addition, VIGIM was given prophylactically to 44 eczematoid children requiring smallpox vaccine (0.6 to 1.2 mL/kg). VIGIM did not affect the course of vaccinia encephalitis. Twenty-seven of 28 patients with autoinoculation who received VIGIM did well. Nine deaths occurred in the 132 patients with eczema vaccinatum given VIGIM; this 7 percent mortality compares favorably with the usual mortality of 30 to 40 percent with supportive care only. All 62 patients with generalized vaccinia who were given VIGIM did well, although four children required a second course.

Seven of the 23 patients with vaccinia necrosum who received VIGIM died (30%); however, this disease generally is fatal, and immune defects were present in most of these patients.[52] These results strongly supported the efficacy of VIGIM and subsequently were confirmed by studies in Sweden[371] and the United Kingdom.[529]

In 1997, Kesson and coauthors[316] reported a severely immunocompromised patient with progressive vaccinia who had inadvertently received a vaccinia melanoma oncolysate vaccination but was successfully treated with VIGIM and ribavirin. Nanning[416a] studied the effect of VIGIM on postvaccinia encephalitis. He gave a placebo or 2 mL of VIG to Dutch military recruits at the time of primary vaccination; three cases of encephalitis occurred in 43,630 vaccinated recruits given VIG, compared with 13 cases in 53,044 recruits in the control group. This 77 percent reduction is statistically significant.

INTRAMUSCULAR VACCINIA IMMUNE GLOBULIN AND INTRAVENOUS VACCINIA IMMUNE GLOBULIN

Two types of VIG are now available, VIGIM (for intramuscular use) and VIGIV (for intravenous use). VIGIM was approved in the United States in 2005 for specific indications: treatment of infections that involve accidental implantation in the eye (except for isolated keratitis), mouth, or other hazardous areas; eczema vaccinatum; progressive vaccinia; severe generalized vaccinia; and vaccinia in patients with burns, impetigo, varicella-zoster, poison ivy, or severe eczema.

Both licensed VIG preparations are prepared from the plasma of vaccinated donors; one is produced by DynPort Vaccine Company LLC, Frederick, Maryland, and the other is produced by Cangene, Mississauga, Ontario, Canada[647] (http://www.fda.gov).

VIGIV used at a dose of 100 mg/kg results in a more rapid and higher antibody peak than does an injection of VIGIM.[261] The first civilian treated with VIGIV was a woman with vaccinia blepharoconjunctivitis; she also received topical antiviral medication.[264] Another laboratory worker with ocular vaccinia also was treated with a single dose of VIGIV.[357]

Recommendations

Complications of vaccination are the usual indications for VIG. They include accidental or intentional vaccination of a patient with a contraindication to the vaccine, autoinoculation of the eye, eczema vaccinatum, severe generalized vaccinia, and vaccinia necrosum.

The initial dose for VIGIM is 0.6 mL/kg. In adults, the dose must be divided and administered during the course of 24 to 36 hours. Repeated doses can be given at 2- to 3-day intervals for vaccine complications until recovery begins, as evidenced by no new lesions. VIG should be given as soon as possible after diagnosis is established.[7,115]

For unimmunized patients exposed to smallpox, VIGIM in combination with vaccine can be given. A dose of 0.6 mL/kg should be given as soon as possible, preferably within 1 to 3 days.[7,385,605] Current recommendations from the U.S. Army Medical Research Institute of Infectious Diseases suggest that VIGIM may be of value in postexposure prophylaxis of smallpox when it is given within the first week after exposure and concurrently with vaccination. Vaccination alone is recommended for those without contraindications. Both vaccine and VIGIM should be given if more than 1 week has elapsed since exposure occurred.

The current supplies of VIGIM would not permit its routine use in combination with vaccine for bioterrorism exposure,[115] but if additional supplies become available, a VIGIM dose of 0.3 mL/kg in combination with vaccine could be used. No data are available on the use of VIGIV for a bioterrorism exposure or its combination with vaccination.

Recommendations for VIGIM for vaccinia complications are summarized on the CDC bioterrorism Web site (http://www.bt.cdc.gov/agent/smallpox/vaccination/vig.asp). Cidofovir also has been used.[84a,84b] VIGIM is not indicated for established smallpox, postvaccinial encephalitis, or hypersensitivity and toxic rashes that occur after vaccination. However, the lack of efficacy of VIGIM in the past does not exclude a potential beneficial effect for VIGIV. The pathogenesis of vaccinial encephalitis is poorly understood and may involve both infection and immune response,[261a] and thus VIGIV may be beneficial.

Inadvertent vaccination of a pregnant woman occasionally results in fetal complications, with an estimate of one case of fetal vaccinia for 10,000 to 100,000 vaccinated pregnant women. Although VIGIV has not been licensed for this purpose, it can be offered. The VIGIM is now considered an investigational preparation because of a change in color with age; further, VIGIM contains a mercury preservative, so it should not be used in pregnant women. Some investigators suggest use of VIGIV if it is given within 10 days of vaccination of a pregnant woman.[115,115a,417]

In ocular vaccinia, VIGIM is contraindicated for isolated vaccinial keratitis because it may increase corneal scarring,[357] as suggested by animal studies. If additional indications for VIGIM exist, such as conjunctivitis, blepharitis, and eczema vaccinatum, the presence of keratitis is not a contraindication for use of VIGIM. Indeed, scarring can occur from vaccinial keratitis in the absence of VIGIM treatment. Topical medications also are recommended.[464]

REGIONAL VIRUSES

ARGENTINE HEMORRHAGIC FEVER

Junin virus, the etiologic agent of Argentine hemorrhagic fever, causes a febrile illness with high mortality rates from vascular or neurologic complications.[467] Patients may either improve or have severe hemorrhagic or neurologic manifestations (or both) within the first 2 weeks of illness.[374] Maiztegui and colleagues[374] found that immune plasma given before the ninth day of illness to 91 patients reduced the mortality rate to 1.1 percent compared with a mortality rate of 16.5 percent in the 97 patients given normal plasma ($p < .01$). Ten of the patients who received immune plasma relapsed with fever and cerebellar signs after several weeks, as did eight others who were not part of the study. The neurologic relapse was self-limited in all but one patient, who died.

Difficulty in obtaining immune plasma, possible neurologic complications of plasma treatment, and lack of efficacy late in the course of the disease have led to the use of ribavirin, despite its limitation late in the disease.[170] An effective, live attenuated viral vaccine has now been developed.[375]

EBOLA INFECTION

Ebola virus, a filovirus, causes severe and often fatal hemorrhagic fever; it has no effective treatment. Kudoyarova-Zubavichene and coworkers[339] reviewed the use of hyperimmune goat or equine serum in the prevention and treatment of Ebola infection. Goat hyperimmune serum protects guinea pigs from experimental infection if it is given less than 24 hours before exposure and provides some benefit if it is given within 72 hours after exposure. This product was used for emergency prophylaxis in four patients exposed by laboratory accidents. Mild infection developed in one definitely exposed patient, but Ebola disease did not develop in the other three patients with questionable exposure. All four patients also received recombinant human interferon-α–2. Equine serum has protected baboons from low-dose virus challenge but not cynomolgus monkeys from a high-dose virus challenge.[278] Equine serum given to monkeys on the day of infection and 5 days later did not prevent death.[279]

Gupta and coauthors[234] reported that a murine polyclonal immune serum protected 100 percent of normal mice and mice with severe combined immunodeficiency after a lethal challenge. Wilson and colleagues[643] were able to protect mice with monoclonal antibodies to Ebola glycoprotein for up to 2 days after infection. Parren and associates[454] were able to protect guinea pigs by giving a human neutralizing antibody up to 1 hour after infection with Ebola virus. Protection of mice by neutralizing monoclonal antibody before and up to 2 days after virus was also shown.[588] Thus, antibody may be a useful agent in the prevention of Ebola disease. Other human monoclonal antibodies have been developed.[380]

Recommendation

The use of antibody in prevention and treatment of Ebola infection is unproved, but monoclonal antibodies are being developed. Animal studies support a role for antibody in the prevention of Ebola infection.

TICK-BORNE ENCEPHALITIS

Tick-borne encephalitis is caused by a flavivirus that is endemic in Russia and eastern and central Europe. Several neurologic syndromes are associated with this infection: febrile headache, aseptic meningitis, meningoencephalitis, meningomyeloencephalitis, and postencephalitic syndrome.[159] Meningoencephalitis is the most severe form and can result in death or permanent paresis. Effective vaccines are available[159,233] and have been successful in dramatically lowering the incidence of this disease.[341]

Passive immunization with a hyperimmune human globulin has been used for postexposure prophylaxis in endemic countries, but it may result in antibody-dependent enhancement of infection.[320,625] Arras and colleagues[36] question these case reports compared with the large number of doses of passive antibody given. Günther and Hagland[233] contend that hyperimmune globulin is not indicated for children younger than 14 years because of concerns that antibody may worsen the disease. The lack of efficacy trials also has led to questioning of the use of this product.[8]

However, von Hedenström and colleagues[622] have recommended its use for postexposure prophylaxis in addition to immunization. In a review of 656 patients with tick-borne encephalitis in southern Germany, Kaiser[298] concluded that postexposure prophylaxis with hyperimmunoglobulin is less effective than is vaccine and should be used only when necessary. Vaccine is the best method for disease prevention.

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SECTION XXV

OTHER PREVENTIVE CONSIDERATIONS

CHAPTER 257

PUBLIC HEALTH ASPECTS OF INFECTIOUS DISEASE CONTROL

Laurene Mascola ❂ David E. Dassey

The dramatic achievements of public health in the 20th century have improved our quality of life: an increase in life expectancy, a worldwide reduction in infant and child mortality rates, and the elimination or reduction in incidence of many communicable diseases. Public health is the science of protecting and improving the health of communities through education, promotion of healthy lifestyles, and research for prevention of disease and injury.[175] Its core functions can be summarized as assessment, assurance, and development of policies. Public health involves the application of many different disciplines, including biology, sociology, mathematics, anthropology, public policy, medicine, education, psychology, computer science, business, engineering, and more.[13] Its scope covers many human health areas, including birth defects, disabilities, communicable diseases, emergency preparedness and response, environmental health, genetics and genomics, health promotion, injury and violence, travelers' health, vaccines and immunizations, and safety and health in the workplace.

For the benefit of this textbook, this chapter focuses on communicable disease control, although the very same principles can be applied to many other health problems. Key tools for control of public health problems fall into two categories: recognition-evaluation and intervention. Foremost among the tools for recognition and evaluation is surveillance, which can be defined as the systematic collection of information about the occurrence of disease and risk behaviors in a community. When an unusual cluster of disease is recognized through surveillance or serendipity, an investigation is indicated. This investigation includes a description of the disease or problem, determination of the pattern of its occurrence in the community, formulation of hypotheses of the cause of the unusual frequency, testing of the hypotheses, and framing of control methods. Of assistance in both surveillance and epidemic investigation is the recognition of common patterns of disease spread either within groups of people or from the environment to people.

Many tools are available for communicable disease intervention. Two important categories are not considered in this chapter because they are addressed at length elsewhere in this book; one is immunization, and the other is treatment of individual patients. Public health tools that are discussed here include isolation, quarantine, chemoprophylaxis, water and food sanitation (including the special situation of international travel), and vector control.

Other public health tools are not discussed here but are as relevant to the control of infectious diseases. They include but are not limited to short- or long-term modifications to human behaviors that affect exposure to infectious diseases such as sexual behavior, food preparation methods, and intravenous drug use practices. In addition, certain control measures, such as vaccination and chemoprophylaxis or chemotherapy, can be applied to animals to decrease the risk of their spreading diseases to humans.[184] And last, control measures that can be applied to the environment, such as proper disposal of waste and appropriate design and proper maintenance of buildings and other equipment, can reduce opportunities for transmission of diseases. An example of such a control measure is proper maintenance of spas or cooling towers to prevent transmission of *Legionella*.

SURVEILLANCE

Public health surveillance is the ongoing, systematic collection, analysis, interpretation, and dissemination of data about a health-related event for use in public health action to reduce morbidity and mortality rates and to improve health. Data disseminated by a public health surveillance system can be used for immediate public health action, program planning and evaluation, and formulation of research hypotheses.[83] Surveillance involves the collection, collation, and analysis of data and feedback of information. When it is used properly, this information helps in directing disease control activities and evaluating their impact. In the primary collection of information, three major factors must be defined. The first is the population to be surveyed, which may be circumscribed geographically (e.g., the inhabitants of the region surrounding the New York City World Trade Center towers) or restricted to a certain age group (e.g., infants, for the surveillance of birth defects, or child-bearing women, for surveillance of postpartum infections) or to a certain institution (e.g., a school, a hospital, the U.S. military). Second, the data to be monitored can be as crude as the number of cases of disease or deaths from a disease. Additional information that could be collected and be useful epidemiologically includes the characteristics of ill persons with regard to age, sex, place of residence, and occupation or detailed characteristics of the organisms involved, such as antibiotic sensitivity, serogroup, nucleic acid molecular profile, or site of the body from which an isolate was recovered. The third and most important element of the reporting system is the primary reporting source. Unless a report of disease is initiated by a physician, infection control professional, or laboratory, no information is generated. Incentives to the primary reporting source to maintain good reporting—such as ongoing feedback—are essential for maintaining a useful surveillance system. Increased use of automated reporting systems using electronic medical records and laboratory results has the potential for improving completeness and timeliness of surveillance data.[67]

In each state, reporting of specified infectious diseases is required by law. However, state laws vary with regard to the persons who are responsible for reporting, the diseases or conditions that are reportable, and the circumstances under which

reporting is to occur.[178,274] Specific information generally is available on request from state or local health agencies.

Collection of surveillance information on the occurrence of disease is pointless unless the data are used to initiate or to modify control actions. The first step is collation and analysis, which may be done at the level of the local health department, the state health department, or the federal government. If this step is performed in a timely fashion, problems can be recognized early and a response initiated.

After this information is collated and analyzed, the results are fed back to the primary reporting source and to others interested in the data. Such feedback serves to maintain interest in the reporting system, thereby maintains the quality of data obtained, and continuously updates persons at each level of the surveillance system about the changing patterns of occurrence of disease and new strategies for disease prevention and control.

One vehicle for the feedback of national surveillance information is the *Morbidity and Mortality Weekly Report* (*http://www.cdc.gov/mmwr/*), which is published by the Centers for Disease Control and Prevention (CDC). Also published by the CDC are periodic summaries of surveillance activities relating to specific diseases. Regular summaries of surveillance activities also are published by the World Health Organization (*http://www.who.int/wer/en/*) and by many state and local health departments.

Identification of a disease problem in the course of surveillance should prompt an attempt to control the problem. Control may involve the initiation of an outbreak investigation if surveillance data suggest an unusual frequency of occurrence of a disease or the adjustment in a public health program if, for example, surveillance data suggest that vaccine is not being given to a particular age group of susceptible people.

OUTBREAK INVESTIGATION

When faced with a possible outbreak, a useful approach is for the investigator to address the inquiry systematically.

1. *Verify the diagnosis.* Review of available clinical and laboratory information and examination of a few patients will permit the creation of a list of possible diagnoses and will suggest additional diagnostic specimens that may be needed to secure the diagnosis. The investigation may proceed without a microbiologic diagnosis if a clearly defined clinical syndrome can be identified. With or without microbiologic confirmation, a standardized case definition is needed.

2. *Confirm the existence of an epidemic.* Through the use of an objective and standardized case definition, the baseline incidence of the illness (number of cases per period of time) before the apparent occurrence of an epidemic should be determined by review of appropriate microbiologic, serologic, or clinical records. Armed with these data, the investigator can decide whether recent events indicate an unusual clustering of illness (i.e., whether an outbreak or epidemic [a large outbreak] exists).

3. *Describe the time, place, and person*. Graphic display of the distribution of cases in time not only confirms the existence of an epidemic but also indicates whether the outbreak is waxing or waning. It may permit judgment about whether the outbreak is spread from person to person or from a common source of exposure and an estimate of the incubation period. Comparison of the characteristics of case patients with regard to age, sex, geographic location, exposure risks, and other relevant factors with those of persons who reasonably might be considered to have been at risk (exposed) but who did not become ill forms the basis for making epidemiologic hypotheses. Determining the attack rate (number ill/[number ill + number well]) is a useful method of describing the risk for development of illness in various groups.

4. *Frame an epidemiologic hypothesis.* By taking the diagnosis (presumed or confirmed) and the pattern of occurrence of disease into account, the investigator may hypothesize one or several possible common sources of infection and one or more modes of spread (food or water borne, airborne, vector borne, or by a particular therapeutic or diagnostic procedure, and from a single point source or an ongoing source of exposure). Alternatively, the hypothesis may be of person-to-person spread. Any thought of an intentional release of a pathogen should be shared immediately with public health authorities

5. *Identify the control group or groups.* Central to the epidemiologic process is identification of a group of persons in whom illness might have developed but did not.[110] The activities and exposures that set well persons apart from sick persons often point to the source of the outbreak. For gastroenteritis occurring after a church supper, one control group might be those who went to the supper but did not get sick, and another control group might be residents of the same community who did not attend the supper. For histoplasmosis in schoolchildren occurring after a school clean-up program, controls might be the well children.

6. *Test the hypothesis statistically.* The attack rates of illness among persons exposed or not exposed to a hypothesized source then are compared by using the number of cases and controls in each group. A true common source should be associated with a higher attack rate in those exposed to it than in those not so exposed. Statistical tests are useful for assessing the significance of differences observed in attack rates of those exposed and not exposed.

7. *Explain the outliers.* Any case that occurs in a person apparently not exposed to an implicated common source needs to be explained. As an example, if vanilla ice cream is implicated as the common source in a food-borne outbreak and ill persons are found who ate only chocolate ice cream, one might hypothesize reasonably that cross-contamination of the ice cream scoop has occurred. Faulty memory of exposure to a common source and the simultaneous occurrence of endemic cases unrelated to the outbreak may complicate this stage of the investigation.

8. *Confirm the hypothesis microbiologically.* Cultures of the implicated common-source material may permit recovery of the infectious agent. When a microorganism is isolated, typing methods may be used to support the epidemiologic hypothesis by demonstrating that the same strain was responsible for disease in all affected patients and can be recovered from the common source. Traditional methods for typing microorganisms—serotyping, biotyping, antimicrobial sensitivity testing, and immunoblotting—remain very useful. In recent years, these techniques have been supplemented and, in some settings, replaced by nucleic acid–based typing methods, such as plasmid profile analysis, pulsed-field gel electrophoresis of chromosomal DNA, and polymerase chain reaction assay to identify genome segments.[220,315] However, culturing of the agent from a particular site without any epidemiologic implication of that site as a source of infection provides little or no information needed to direct control measures.

9. *Implement control measures.* Knowledge of the agent, source, and mode of spread permits drafting of control measures and their implementation. Without careful attention to this step, the investigation is an academic exercise only.

10. *Measure the impact of control measures.* This step doubles as the ultimate test of the epidemiologic hypothesis and as an assessment of the design and implementation of the control measures. Surveillance for the occurrence of disease in groups shown to be at high risk is the most common technique used for this measurement, and a decrease in the attack rate to zero (no new cases of disease) or to the baseline incidence of disease is the measure of success.

Several exercises illustrating these principles, including communicable and noncommunicable disease outbreaks, can be found at the CDC's training site for epidemic intelligence service officers (*http://www.cdc.gov/eis/casestudies/casestudies.htm*).

COMMON PATTERNS OF DISEASE SPREAD

DISEASE SPREAD FROM ANIMALS TO PEOPLE

Many infectious diseases are transmitted from other animals to humans (zoonoses). Of all emerging diseases in the 20th and 21st centuries, more than 75 percent are zoonotic in origin.[138] From the United States perspective alone, emerging zoonotic diseases that have caused human infection or concern include West Nile virus, anthrax (weaponized to cause intentional harm by contaminating some mail), severe acute respiratory syndrome (SARS) pandemic caused by a newly identified coronavirus,[279] monkeypox,[285] bovine spongiform encephalopathy,[41] and H5N1 avian influenza.[229] Physicians who routinely inquire about animal contacts as part of the clinical and epidemiologic history of an ill patient frequently find valuable clues to an obscure diagnosis.[1] Table 257–1 lists infectious disease agents for which some suggestive or definitive evidence exists that the disease can be spread from animal to human, either directly or by a vector.[1,21,161,189,303] A distinctive disease name associated with the organism also is listed, as are the various animal groups that have been suggested or shown to act as sources of transmission to humans. The frequency with which an animal group serves as a source of infection for humans and the frequency with which a vector is involved are indicated in a semiquantitative fashion.

Perhaps the most recent disease recognized to be spread from animals to humans by mosquitoes in the United States is West Nile virus. West Nile virus was introduced first in the northeastern part of the United States in the summer and fall of 1999. By 2005, West Nile virus activity detected in mosquitoes, people, dead birds, captive sentinel animals, and other sources had been reported from 596 counties in 42 states.[84] From a global standpoint, a devastating zoonotic disease emerged in November 2002 in China; called SARS, it was identified as being caused by a new human coronavirus and was thought to be transmitted from animals and the markets that trade in live game animals to humans.[253] In 1997, the highly pathogenic avian influenza strain H5N1 emerged and infected 18 humans in Hong Kong; it continues to infect humans, felids, pigs, and other mammals, leaving the world in anticipation of a possible next influenza pandemic.[204] In recent years, infections with zoonotic diseases such as rabies, trypanosomiasis (Chagas disease), West Nile virus, and human lymphocytic choriomeningitis have caused infections in organ recipients, escalating the damage caused by zoonotic diseases to a new paradigm.[43,80,196]

Omitted in Table 257–1 are many species that may be infected with the organism but do not appear to act as a source of human infection. Also omitted are certain rare or accidental infectious diseases. The complex life cycles of some parasites are difficult to display in tabular form. Details of the clinical manifestations, differential diagnoses, and treatments of the zoonoses are found in the appropriate chapters elsewhere in this book. For a complete description of the circumstances under which each agent is spread from animal to person, consult a textbook of zoonoses.[1,22] Excluded from Table 257–1 are several interesting groups of infectious diseases in which human beings and animals appear to interact but in which infection does not spread from animals to people. The first group includes organisms that infect both animals and humans from a common source in nature. Such organisms include *Burkholderia pseudomallei*, *Blastomyces dermatitidis*, and *Aspergillus* spp., which can infect a wide variety of animal species, including humans, from soil or other inanimate sources.

A few diseases are spread from humans to animals. Perhaps the most prominent example is *Herpesvirus hominis*, which can cause fatal encephalitis in nonhuman primates when it is spread from humans.[172] Tuberculosis also can spread from humans to nonhuman primates, and even elephants, as well as the reverse. *Cryptococcus neoformans* and *Histoplasma capsulatum* live in animal feces but do not infect animals. From the fecal material, these organisms may spread to infect humans. Some organisms can be transmitted by fomites of animal origin. They act as mechanical carriers, and the process does not involve infection of the animals. Examples include *H. capsulatum*, which has been observed to be spread by chicken feathers, and *Coccidioides immitis*, which can be spread in bales of wool contaminated with fungus-laden dust.

Another group of diseases are spread from person to person through an insect vector, either typically or on occasion. Filariasis is a prime example of a disease in which vector-borne person-to-person spread is the rule. Typhoid and yaws have been shown to be spread by vectors on occasion. Evidence that *Mycobacterium leprae* can be recovered from mosquitoes and sandflies[292] that have fed on patients with lepromatous leprosy exists, as does speculation that such mosquitoes or sandflies may serve as vectors of transmission.

DISEASE SPREAD FROM PERSON TO PERSON

As members of groups including the family, school, camp, daycare center, and religious organizations, children are at risk of acquiring or spreading infectious agents that are transmitted from person to person. Knowledge of the typical patterns of spread can be useful in planning surveillance or in designing control measures. For example, recognition that diarrhea commonly results from person-to-person spread in daycare centers and that it can indicate inadequate hygienic practices may justify specific surveillance for diarrhea in that setting. Documentation of secondary cases in various categories of contacts of cases of meningococcal disease permits focusing the delivery of chemoprophylaxis on just the high-risk groups.

In this section, spread in the family and schools is discussed, but several groups of interest are only briefly mentioned. The important areas of transmission of disease within daycare centers and within hospitals are discussed in separate chapters in more depth. Maternal transmission of specific infections to the fetus or neonate also is reviewed elsewhere in this book. Spread of enteric diseases within institutions for the mentally challenged is recognized widely as an important problem, but it does not impinge directly on the practice of most pediatricians and is not discussed in this chapter.

Infection caused by the human immunodeficiency virus (HIV), which leads to the acquired immunodeficiency syndrome (AIDS), is one of the conditions for which public health surveillance is most critical. The CDC has reported that in 2003, more than 1 million persons in the United States were estimated to be living with HIV infection.[116] During 2001 to 2004, HIV infection was diagnosed in an estimated 157,253 persons in 33 states that have been conducting name-based HIV/AIDS surveillance for at least 4 years.[116] Another CDC report, which collects data from those 33 states, Guam, and the Virgin Islands, showed that whereas HIV infection reporting decreased year to year from 2001 to 2003, it increased 1 percent from the end of 2003 (38,188 cases) through the end of 2004 (38,730 cases).[42] From 2001 to 2004, the estimated number of HIV/AIDS cases by year of diagnosis decreased among children younger than 13 years (124 cases to 48 cases), remained stable in those aged 13 to 14 years (at 60 cases), and increased in those aged 15 to 19 years (291 cases to 326). Survival after a diagnosis of AIDS was established was

Text continued on p. 3460

TABLE 257–1 Infectious Disease Spread from Animals to Humans (with or without Vectors)

		Animal Source*																
Agent	**Disease**	**Vector-borne†**	**Dogs**	**Cats**	**Cattle**	**Horses**	**Swine**	**Sheep, goats**	**Domestic fowl**	**Pigeons**	**Parrots, parakeets**	**Mice, rats**	**Rabbits**	**Fish**	**Shellfish**	**Turtles**	**Nonhuman primates**	**Other**
Bacterial diseases																		
Spirochetes and curved species bacteria																		
Borrelia burgdorferi	Lyme disease	++	1	1	1	1		?			1	2	?					Deer 2 Birds 1 Other rodents 2 Other carnivores?
Borrelia species	Relapsing fever	++			?	?						2					?	Opossums? Armadillos? Squirrels 2 Small rodents 2 Skunks? Weasels?
Borrelia lonestari	STARI	++																White-tailed deer?
Leptospira interrogans	Leptospirosis		2	1	2	1	2	1				2	?					Skunks 1 Squirrels 1 Raccoons 1 Wildlife 2
Spirillum minus	Rat-bite fever											2						Carnivores 1 Other rodents?
Gram-negative rods																		
Aeromonas species															2			
Bartonella henselae/ Bartonella numerous other species	Cat-scratch disease			2														
Burkholderia mallei	Glanders		?			2		?										Mules 2 Donkeys 2 Wolves?
Bordetella bronchiseptica			2	1								?	2					Guinea pigs 2
Brucella abortus	Brucellosis				2													Elk 1 Bison 1 Llamas 1 Rodents 1 Wild boars 1
Brucella canis	Brucellosis		2															
Brucella melitensis	Brucellosis							2										
Brucella suis	Brucellosis				2		2											
Campylobacter species	Campylobacteriosis		1	1	2		1	1	2			1						Rodents 1 Birds 1
Capnocytophaga canimorsus			2	1														Coyotes? Bears? Tigers?

Escherichia coli O157:H7, other serotypes	Enterohemorrhagic gastroenteritis		1		2		1	1	2										Deer 2 Wildlife 1
Francisella tularensis	Tularemia	+	1	1	1		1	1				2	2						Muskrats 2 Beavers 1 Other rodents 1 Squirrels 1 Hamsters 2
Helicobacter canis			?	?															
Helicobacter felis			1	1															
Helicobacter heilmannii			1	1															
Pasteurella haemolytica																			Deer 1
Pasteurella multocida			2	2	2	1	2	1	1				1						Opossums 1 Guinea pigs 1 Birds 1 Wild and zoo animals 1
Pasteurella pneumotropica			2	2															
Salmonella species	Salmonellosis		1	1	1	1	1	1	2	1	1	1	1	1	1	2	2		Many mammals 2 Reptiles 2
Shigella species	Shigellosis																2		
Streptobacillus moniliformis	Rat-bite fever											2							
Vibrio parahaemolyticus															2				
Vibrio vulnificus															2				
Yersinia enterocolitica	Yersiniosis		1	1	1	1	2	1											
Yersinia pestis	Plague	++	1	2								2	1						Ground squirrels 2 Prairie dogs 2 Wild carnivores 1 Tree squirrels 1 Other rodents 1
Yersinia *pseudotuberculosis*			1	1			1		2			1	2						Canaries 1 Hamsters? Guinea pigs? Rodents 2 Raccoons 1
Gram-positive cocci																			
Group A streptococci				1	?												?		Ducks?
Group B streptococci				?															

TABLE 257–1 Infectious Disease Spread from Animals to Humans (with or without Vectors)—cont'd

		Animal Source*																
Agent	**Disease**	**Vector-borne†**	**Dogs**	**Cats**	**Cattle**	**Horses**	**Swine**	**Sheep, goats**	**Domestic fowl**	**Pigeons**	**Parrots, parakeets**	**Mice, rats**	**Rabbits**	**Fish**	**Shellfish**	**Turtles**	**Nonhuman primates**	**Other**
Group C streptococci *(Streptococcus equi)*					1	2												
Group S streptococci *(Streptococcus suis)*							2											
Streptococcus iniae														2				
Staphylococcus aureus			1				?					?						
Staphylococcus aureus, methicillin resistant			1															
Staphylococcus intermedius			1															
Gram-positive rods																		
Bacillus anthracis	Anthrax		1	1	2	2	1	2										Wildlife 1
Clostridium difficile			?			?							?					Hamsters?
Clostridium tetani	Tetanus				?	?												
Corynebacterium (Rhodococcus) equi					?	1	?	?										
Corynebacterium pseudotuberculosis					?	?		1										
Corynebacterium ulcerans					1	1												
Dermatophilus congolensis	Dermatophilosis				?	?		?										
Erysipelothrix rhusiopathiae (insidiosa)			1		1	1	2	1	2			?	2					Seals 1 Wild boars 1
Listeria monocytogenes	Listeriosis				1		1	1	1			1	1			1		Whales 1 Chinchilla 1
Mycobacterium avium							?											Birds?
Mycobacterium bovis			1	1	2													
Mycobacterium leprae	Leprosy	?																Armadillos?
Mycobacterium marinum														2				Dolphins 1 Poikilotherms 1
Mycobacterium tuberculosis	Tuberculosis		1	1	1			1									1	Elephants 1

Chlamydiae															
Chlamydophila psittaci	Psittacosis	?						2	2	1					Other birds 2 Muskrats?
Rickettsiae															
Anaplasma phagocytophilum human granulocytic ehrlichiosis [HGE]	Anaplasmosis (previously HGE)	++	2	?			1			1					Deer? Other rodents 1
Coxiella burnetii	Q fever	+	1	1	2		2				1				Small mammals 2 Birds?
Ehrlichia chaffeensis	Human monocytic ehrlichiosis (HME)	++	?				?								Deer?
Rickettsia akari	Rickettsialpox	++									2				
Rickettsia australis	Queensland tick typhus	++									2				Small marsupials 2
Rickettsia conorii	Fercel boutonneuse	++	2								2				Rodents 1
Rickettsia felis	Muline typhus	++		1							2				Opossums 2
Rickettsia prowazekii	Louse-borne typhus	++			?	?	?	?							Flying squirrels?
Rickettsia rickettsii	Rocky Mountain spotted fever	++	2	2							2	2			Other rodents 2 Birds?
Rickettsia sibirica	North Asian tick typhus	++									2				Birds? Other rodents 1
Rickettsia tsutsugamushi	Scrub typhus	++									2				Birds? Marsupials 1
Rickettsia typhi	Murine typhus	++		1							2				Rodents 1 Opossums 2
Fungal diseases															
Microsporum canis	Ringworm		2	2		1	1	1			1	1		1	Zoo animals 1
Microsporum equinum	Ringworm					2									
Microsporum gallinae	Ringworm								1						
Microsporum nanum	Ringworm						2								
Pneumocystis carinii	*P. carinii* pneumonia		?								?				
Trichophyton equinum	Ringworm				1	2									
Trichophyton mentagrophytes var. *erinacei*	Ringworm														Hedgehogs 2
Trichophyton mentagrophytes var. *mentagrophytes*	Ringworm	1	2				1				2	1		1	Guinea pigs 1 Hamsters 1 Chinchillas 1
Trichophyton mentagrophytes var. *quinckeanum*	Ringworm					2					2				
Trichophyton verrucosum	Ringworm				2	1		1							

TABLE 257–1 Infectious Disease Spread from Animals to Humans (with or without Vectors)—cont'd

		Animal Source*																
Agent	**Disease**	**Vector-borne†**	**Dogs**	**Cats**	**Cattle**	**Horses**	**Swine**	**Sheep, goats**	**Domestic fowl**	**Pigeons**	**Parrots, parakeets**	**Mice, rats**	**Rabbits**	**Fish**	**Shellfish**	**Turtles**	**Nonhuman primates**	**Other**
Parasitic diseases																		
Helminths																		
Angiostrongylus species												2		2	2			Amphibians 2 Reptiles 2
Capillaria hepatica		?										2						
Capillaria philippinensis														2				Piscivorous birds?
Dioctophyma renale	Dioctophymiasis		?	?										2				Mink and other fish-eating mammals 2
Trichinella spiralis	Trichinosis	?	?				2											Bears 1 Walruses 1 Whales 1 Raccoons 1
Filaria																		
Brugia malayi	Filariasis	++	?	2													2	Other carnivores 2 Wild felids 1
Dirofilaria immitis	Heartworm	++	2															
Dirofilaria tenuis		++																Raccoons 2
Dirofilaria repens		++	2	2														
Nematodes																		
Ancylostoma braziliense	Cutaneous larva migrans		2	2														
Ancylostoma caninum	Cutaneous larva migrans		2															
Ancylostoma ceylonicum	Cutaneous larva migrans		2	2														
Angiostrongylus cantonensis	Eosinophilic meningitis											2			1			
Anisakis species													2	1				Marine mammals 2
Ascaris lumbricoides							?											
Ascaris suum							?											
Baylisascaris procyonis																		Raccoons 2
Gnathostoma spinigerum	Visceral and cutaneous larva migrans		2	2			2		1					1				Frogs 1 Weasels 2 Snakes 1
Strongyloides stercoralis	Strongyloidiasis		2	2													1	
Trichostrongylus species					2	2		2										Donkeys 2 Herbivores 2

Toxocara canis	Visceral larva migrans		2													Other canids 2
Toxocara cati	Visceral larva migrans			2												
Uncinaria stenocephala	Cutaneous larva migrans		2	2												Foxes 1
Trematodes																
Clinostomum complanatum													2			
Clonorchis sinensis	Oriental liver fluke		2	2			1			1	2	2				Fish-eating mammals 1
Dicrocoelium dendriticum					?											Ants 2
Dicrocoelium hospes												2				Ants 2
Echinostoma species	Echinostomiasis		1	1			1		?	1	2					Ducks and geese 1
Fasciola gigantica	Liver fluke				2	2		2				2				
Fasciola hepatica	Liver fluke				2			2				2				
Fasciolopsis buski	Liver fluke						2					2				
Gastrodiscoides (Amphistomum) hominis							2			?				?		
Haplorchis species											2	2				Numerous birds 1 Mammals 1
Heterophyes species	Heterophyiasis		1	1							2	2				Other birds 1 Mammals 1
Metagonimus species	Metagonimiasis											2	2			Numerous birds 2 Mammals 2
Opisthorchis felineus				2							2	2				Numerous birds 1 Reptiles 1
Paragonimus species	Paragonimiasis		?	?			1					2				African civet? Other mammals?
Schistosoma haematobium	Schistosomiasis						?			?		2		?		
Schistosoma japonicum	Schistosomiasis		1	1	1	1	1	1		1		2				
Schistosoma mansoni	Schistosomiasis				?					?						Baboons 1
Cestodes																
Bertiella species	Tapeworm	++												1		
Dipylidium caninum	Tapeworm	++	2	2												
Diphyllobothrium latum	Fish tapeworm		1	1			?				2					
Echinococcus granulosus	Hydatid disease		2		1		1	2								Other carnivores 2
Echinococcus multilocularis	Hydatid disease		2	2						1						Foxes 2 Other canids 1
Hymenolepis diminuta	Tapeworm	++								2						
Hymenolepis nana	Tapeworm	?								2						Hamsters?

TABLE 257–1 Infectious Disease Spread from Animals to Humans (with or without Vectors)—cont'd

		Animal Source*																
Agent	**Disease**	**Vector-borne†**	**Dogs**	**Cats**	**Cattle**	**Horses**	**Swine**	**Sheep, goats**	**Domestic fowl**	**Pigeons**	**Parrots, parakeets**	**Mice, rats**	**Rabbits**	**Fish**	**Shellfish**	**Turtles**	**Nonhuman primates**	**Other**
Mesocestoides species	Tapeworm	?	?									1						Snakes 1 Birds 1 Other amphibians 1
Spirometra species	Sparganosis		?	?			?											Frogs 2 Copepods 1 Snakes 1 Hyenas 1
Taenia saginata	Taeniasis				2													
Taenia solium	Taeniasis Cysticercosis						2											
Protozoa																		
Babesia divergens	Babesiosis	++			2													
Babesia microti	Babesiosis	++										2						Deer 2
Balantidium coli	Balantidiasis						2					1	1				1	
Cryptosporidium species	Cryptosporidiosis		?	?	2	?	?	?	?		?	?	?	?			?	Deer?
Encephalitozoon cuniculi	Microsporidiosis		1									1	1					
Entamoeba histolytica	Amebiasis		?															
Enterocytozoon bieneusi	Microsporidiosis			1	1		1					?					1	Mammals 2
Giardia intestinalis	Giardiasis		1	1	1		1	1				1					1	Beavers 1 Muskrats 1
Leishmania aethiopica	Oriental sore	++																Hyrax 2
Leishmania brasiliensis	Espundia, uta	++	1									2						Other rodents 2 Sloths? Anteaters?
Leishmania chagasi	Kala-azar	++	2									2						Foxes 2 Other rodents 2
Leishmania donovani	Kala-azar	++	2	?	?	?		?				2						Jackals 2 Foxes 2
Leishmania major	Oriental sore	++																Gerbils 2 Other rodents 2
Leishmania mexicana	Espundia, uta	++										2						Small rodents 2
Leishmania tropica	Oriental sore	++	2									2						Gerbils 2 Other rodents 2

Plasmodium knowlesi	Malaria	++												2	
Plasmodium simium	Malaria	++												?	
Sarcocystis species					1			?							
Toxoplasma gondii	Toxoplasmosis			2	1	1	1	1	?		?				
Trypanosoma cruzi	Chagas disease	++	2	2								2			Raccoons 2 Guinea pigs 2 Opossums? Armadillos? Numerous small mammals 2
Trypanosoma brucei gambiense	Gambian sleeping sickness	++	?				?	?							
Trypanosoma brucei rhodesiense	Rhodesian sleeping sickness	++			2			1							Bushbuck 2 Wild animals 1 Domestic animals 1
Viral diseases															
Parapoxvirus species	Bovine papular stomatitis				2										
Coronavirus SARS	SARS (severe acute respiratory syndrome)		1												Bats 2 Wild animals 1
Cowpox	Cowpox				2										
Ebola	Hemorrhagic fever (HF)													1	Bats 1
Foot-and-mouth disease					1		1	1							Wild animals 1
Hantaan, other hantavirus	HF with renal syndrome										2				Shrews 1 Voles 1 Other rodents 1
Hepatitis A	Hepatitis												1	1	
Hendra virus	Pulminary edema, meningitis					1									Bats 2
Cercopithecine herpesvirus 1	B virus encephalitis													2	
Influenza A/swine	Swine influenza						2								
Influenza A/avian	Avian influenza			1					1						Tigers? Other birds 2
Junin virus	Argentine HF										2				
Lassa virus	HF										2				
Lymphocytic choriomeningitis	Meningitis										2				Hamsters 2

TABLE 257–1 Infectious Disease Spread from Animals to Humans (with or without Vectors)—cont'd

		Animal Source*																
Agent	**Disease**	**Vector-borne†**	**Dogs**	**Cats**	**Cattle**	**Horses**	**Swine**	**Sheep, goats**	**Domestic fowl**	**Pigeons**	**Parrots, parakeets**	**Mice, rats**	**Rabbits**	**Fish**	**Shellfish**	**Turtles**	**Nonhuman primates**	**Other**
Machupo	Bolivian HF											2						
Marburg	HF																2	Bats 2
Menangle virus	Flu-like illness						1											
Monkeypox	Pox																2	Gambian rats 1 Prairie dogs 2
Nipah virus	Encephalitis						2											Bats 1
Newcastle	Conjunetivitis								2									
Orf	Pox							2										
Pseudocowpox	Milker's nodule				2													
Rabies			2	1	1	1	1	1										Wolves 2 Bats 2 Foxes 1 Skunks 2
Sabia virus	Venezuelan HF											2						
Sin Nombre	Hantavirus pulmonary syndrome											2						
Vesicular stomatitis		+			?													
Arboviruses																		
California encephalitis		++											?					Squirrels? Bats?
Chikungunya		++															2	
Colorado tick fever		++										1						Deer mice 1 Ground squirrels 2 Porcupines?
Crimean hemorrhagic fever (CHF)	HF	++			2	1		1				1	1					Other birds 2 Hedgehogs 1 Ostriches 1
Dengue		++															?	
Eastern equine encephalitis		++				1			1									Other birds 2
Group C viruses	Fevers	++										2					1	Other birds?
Japanese encephalitis		++	?	?		?	2											Other birds 2

Kemerovo		++		2	?					2				Other birds?
Kyasanur Forest disease	HF	++		?						2			2	Porcupines 2
La Crosse		++									1			Chipmunks 2 Foxes 2 Squirrels 2
Louping ill		++					2			?				Deer? Other birds?
Murray Valley encephalitis		++												Other birds?
Nairobi sheep disease		++					2							
Omsk HF		++		?						?				Muskrats?
Oropouche	Oropouche fever	++											?	Other birds? Sloths?
Powassan		++								?	?			Birds 2 Squirrels? Woodchucks?
Rift Valley fever		++		2			2			?				
Ross River	Epidemic polyarthritis	++	?			?	?							Kangaroos?
St. Louis encephalitis		++						?	?					Other birds 2
Tick-borne encephalitis		++		2			2			2				Other birds 2
Venezuelan equine encephalitis		++	1		2					2				Bats 1
Western equine encephalitis		++			1			1						Other birds 2
Wesselsbron		+	?	2			2							
West Nile		++	?						?					Other birds 2
Yellow fever		++											2	
Prion disease														
Bovine spongiform encephalopathy	Variant Creutzfeldt-Jakob			2			2							
Chronic wasting disease														Elk? Deer?

**Animal species that seem to be sources, either directly or through a vector, or microbial agents that infect humans: 1, rare source of human infection; 2, typical or common source of human infection; ?, speculative source of human infection.*
†Disease acquired through bites from, contact with, or ingestion of insects or arachnids: ++, disease typically vector borne; +, disease occasionally vector borne; ?, speculation that disease occasionally may be vector borne.
HF, Hemorrhagic fever; HGE, Human granulocytic ehrlichiosis.

greatest among children with perinatally acquired HIV infection compared with other age groups. Maternal transmission and person-to-person sexual and blood-borne spread of HIV infection are at the top of the list of public health concerns and are covered extensively in Chapter 204.

THE FAMILY

Spread of disease agents in the family can be considered in two stages: introduction of the agent into the family, usually by a single member ("index case" or "index carrier"), and spread to other family members. Spread to other members can be described in terms of rapidity and intensity, the latter commonly measured by the secondary infection rate (or secondary attack rate), which is defined as the proportion of the contacts of the index carrier or index case who become infected (or ill) in a given interval after exposure. The secondary infection rate varies from agent to agent and may be affected by the age and sex of the introducer; the size of the family; crowding; the age of household contacts; previous immunity; and interventions attempted, including vaccination, isolation, treatment, and chemoprophylaxis. The ratio of the secondary attack rate to the incidence of disease in the community is a measure of the importance of the family as a focus of disease.[131]

Of the bacteria spread by the respiratory route, pneumococci, group A streptococci, meningococci, and *Haemophilus influenzae* historically have been studied most completely. In a 1950s study of English families of five persons each (mother, father, and three children, the youngest of whom was younger than 5 years), type-specific introduction of pneumococci was attributed most frequently to the middle child.[31] The oldest and middle children were the most efficient spreaders of pneumococci to other family members. Among household contacts, children were more likely than were their parents to become infected. The overall secondary infection rate was 9 percent.

Whereas older studies supported the idea that parents do not obtain disease from their children, keeping the theory of "an immunologic barrier between adults and children" intact, others have demonstrated an extremely high rate of transmission of penicillin-resistant *Streptococcus pneumoniae* between parents and children.[170,186] The recent routine administration of a seven-valent pneumococcal conjugate vaccine (PCV7) to young infants has altered the epidemiology of pneumococcal colonization, invasive infection, and transmission not only in vaccinated children but in nonimmunized persons as well, similar to what has been observed with the conjugate vaccine for *H. influenzae* type b.[203] Conjugate vaccines interrupt the transmission of vaccine-type strains among vaccinees and their household members.[139] Several reports have documented the reduction of incidence of pneumococcal nasopharyngeal carriage of vaccine serotypes.[308] One study among vaccinated South African children showed a reduction of nasal carriage of pneumococcal vaccine serotypes from 36 percent in control children to 18 percent in vaccine recipients. Another study among vaccinated Israeli toddlers also showed a 50 percent reduction in new colonization by vaccine serotypes compared with controls. The decrease in nasopharyngeal colonization by vaccine serotypes resulting from conjugate vaccine in children has conferred benefits to their nonimmunized contacts through herd immunity. The incidence of invasive pneumococcal disease among adults aged 50 years or older caused by the seven conjugate serotypes in the United States declined 55 percent (95% confidence interval, –58% to –51%) from 1998-1999 to 2002-2003.[205] Pneumococcal carriage also was altered in adults as demonstrated by a study in which adults who lived in a household in which at least one child had been vaccinated appropriately with PCV7 had a lower likelihood of having PCV7-type pneumococci carriage than did adults living in a household in which no child had been vaccinated (adjusted odds ratio, 0.49; 95% confidence interval, 0.28-0.83).[149] In addition, the vaccine interrupted the transmission of resistant pneumococci from children to adults, which was not associated with an increased use of that vaccine. Kyaw and colleagues[194] showed that among all age groups between 1999 and 2004, the estimated number of cases caused by pneumococcal strains with reduced susceptibility to penicillin or multiple antibiotics fell by half after the introduction of the conjugate vaccine.

One interesting association, needing validation, recently showed that *S. pneumoniae* carriage, specifically of vaccine-type strains, was negatively associated with *Staphylococcus aureus* carriage in children, suggesting that *S. pneumoniae* carriage protects again *S. aureus* carriage in children.[269] Therefore, if *S. pneumoniae* carriage decreases in children because of vaccination efforts and causes increasing *S. aureus* carriage, this change could have important implications, especially in light of the recent worldwide emergence of methicillin-resistant *S. aureus* (MRSA) disease among children. Prior incidence of *S. aureus* infections in children is unknown, although nasal carriage of the organism in all age groups is estimated to be 32.4 percent, and carriage with the organism has long been known to be one of the most strongly associated risk factors for subsequent infection.[191] Fortunately, MRSA carriage prevalence estimates were found to be much lower, at 0.8 percent, and unusual in healthy persons, with age older than 60 years and being female found to be risk factors. However, family outbreaks of invasive community-associated MRSA infections have occurred, even though intrafamilial spread of invasive disease remains a relatively rarely reported phenomenon.[182,183] A study that evaluated 249 MRSA skin and soft tissue infections from 11 university-affiliated emergency departments in August 2004 found that 18 percent reported close contact with a person who had a similar infection.[236] The epidemiology of this disease is still being elucidated, and no current recommendations exist for screening or treatment of carriers of this organism.

Children with group A streptococcal infection in members of families in Cleveland, Ohio, were even more important than were children with pneumococcal infection as introducers and recipients of intrafamilial transmission and carriage; the organism may persist for many months, resulting in multiple cases of disease in a family widely separated in time.[179] A more recent reference also corroborates the fact that children are most likely to introduce group A streptococci into the home and spread disease and that children with sore throats are likely reservoirs of group A streptococci for adults who develop invasive disease.[118] Crowding, as measured by the number of rooms in the house, also influences the development of invasive group A streptococcal infection, although familial transmission of invasive group A streptococcal infection is a rare occurrence. Only a few studies have described intrafamilial transmission of invasive group A streptococcal infections.[132] However, a consensus working group concluded that no definite recommendations should be made regarding chemoprophylaxis to household contacts of persons with invasive group A streptococcal infection.[305]

The pattern of spread of meningococcal infection has been reported to be strikingly different. An article in 1971 stated that the most common introducers of meningococci into families are men, followed distantly and in decreasing order of frequency by women, girls, and boys.[143] Once meningococcal bacteria are introduced, all family members may become infected, although studies of carriers in household transmission of meningococcal disease in Brazil suggest that spread may proceed from an adult to older children and only thereafter to infants.[237] A 1993 study by Tappero and colleagues[304] showed in Los Angeles County, California, that 45 percent of persons with sporadic meningococcal disease had household contact with an adult man recently released from or employed by the county's men's jail system, establishing a new risk factor for acquisition of meningococcal

disease. The recently approved quadrivalent meningococcal conjugate vaccine (MCV4), licensed in January 2004 for use in persons aged 11 to 55 years, also may exert substantial herd immunity effects through reductions in acquisition of nasopharyngeal carriage for this disease as well.[150] Before the development of this vaccine, carriage of meningococci could persist for months, although half of the secondary cases of meningococcal disease occurred within 5 days of the index case.[143,237]

Children younger than 5 years have the greatest risk of acquiring serious disease from *H. influenzae* type b infection. Before vaccination was routine, overall carriage rates were less than 5 percent, although high rates of colonization had been reported in settings such as daycare, where young children are in close contact, and in families with a child who had invasive *H. influenzae* type b disease.[230] Among unvaccinated contacts of persons with *H. influenzae* type b disease, children younger than 5 years have the highest carriage rates (30-40%), with rates dropping progressively with increasing age.[17] However, the epidemiology of *H. influenzae* type b disease has been changed markedly by the use of vaccines; the annual incidence of disease in children younger than 5 years fell more than 99 percent (from 100/100,000 to 0.3/100,000) from 1990 to 2000 after the introduction of conjugate vaccines.[2,68] Unlike earlier polysaccharide vaccines, conjugate vaccines appear to prevent colonization as well as disease; one study using a matched case-control analysis showed that conjugate vaccines had an 81 percent efficacy in decreasing colonization.[238] Before this vaccine as well, mostly because of nasopharyngeal carriage of the index cases, secondary cases of *H. influenzae* type b disease occurred within 5 days of the index case.[129,142]

An ever-present respiratory bacterial pathogen, *Bordetella pertussis*, spreads easily within families. In a study of 21 families in Finland, 83 percent of the family members of primary cases showed serologic evidence of secondary infection.[228] Secondary attack rates for symptomatic disease were lower in older children and adults. Children 2 to 15 years of age were more likely to introduce pertussis into the family than were younger children or family members older than 15 years. However, adults may be of increasing importance as reservoirs of *B. pertussis*, leading to infection in infants.[208,223] A German study of 122 households with an index case of pertussis showed that adult index cases were as likely to spread pertussis within the family as were index cases who were children. Fourteen (78%) of 18 adults and 74 (71%) of 104 children spread pertussis to at least one other family member.[321] Outbreaks have been reported in which an adult or adolescent with persistent bronchitis acted as a source of pertussis for children and other adults.[86,193] In the United States during 2002 to 2003, of the pertussis cases reported to the CDC for which the age of the person was known, 23 percent occurred in persons 20 years or older, up from 20 percent during the prior 3 years.[85] The annual incidence of pertussis among persons aged 10 to 19 years also increased from 5.5 per 100,000 to 10.9 in 2003.

Both tuberculosis and leprosy usually are acquired by children from adults who introduce the mycobacteria into the household.[122] These introducers typically shed large numbers of bacteria from the respiratory tract (especially from the nose in lepromatous leprosy) for months or years. Secondary attack rates are similar for the two diseases and are highest for tuberculosis in children younger than 5 years.[267] For leprosy, children of all ages beyond the neonatal period are at significant risk.

Introducers who are ill tend to be more efficient spreaders of bacteria in the respiratory tract. In the Cleveland study,[179] spread of group A streptococci was 2.7 times as frequent when the introducer was symptomatic as when the introducer was asymptomatic. Spread of group A streptococci was observed in Egyptian families particularly frequently if the person who introduced the strain into the household was ill enough to seek medical care or if the strain persisted in the respiratory tract of the introducer for 3 months or more.[111] Nasal shedders of bacterial pathogens have been shown to be more efficient spreaders, and nasal shedding commonly is found early in illness.[221] In 14 (56%) of the 25 episodes of transfer of pneumococci observed in Virginia families, the spreader experienced sneezing, rhinorrhea, nasal congestion, or cough during the 2-week period in which transfer occurred, whereas such symptoms were present in only 159 (37%) of the other 423 2-week periods of observation.[145] Again, this finding may relate to higher rates of recovery of pneumococci from the nose during symptoms of upper respiratory tract infection.

Crowding can be an important risk factor for spread of disease. Crowding has been observed inconsistently to affect spread of pneumococci. English families living in only two rooms had a secondary infection rate of 16.0 percent, whereas those living in four or more rooms had an 11.2 percent secondary infection rate.[31] Floor space was not shown to affect spread in families in Syracuse, New York.[107] Severe overcrowding, inadequate ventilation, and altered host susceptibility were important factors in an outbreak of pneumococcal disease in a large urban jail.[168] Limited ventilation of houses seems to promote the spread of meningococci in the meningitis belt in Africa.[137] In a population-based case-control study, household crowding was shown to be a risk factor for *H. influenzae* disease.[91] Ill children were 2.6 times more likely to live in households containing one or more persons per room than were well children in control families. Crowding also was found to be a risk factor for pediatric invasive group A streptococcal disease.[118] The magnitude of risk associated with MRSA infection or colonization increased as the extent of crowding or closeness to a teammate with an illness increased, as evidenced in outbreaks of MRSA among athletic teams and in jails.[53,241]

The changing epidemiology of measles and mumps demonstrates the impact of vaccines on the transmission of viral diseases spread by the respiratory route. The age at which a person is most at risk for acquiring one of these diseases is determined largely by the communicability of the virus and the completeness of immunity. Long before vaccines were available for measles, varicella, and mumps, Hope-Simpson[169] calculated that disease developed in 75 percent of susceptible family contacts exposed to a case of measles, whereas the corresponding figures for varicella and mumps were 61 percent and 31 percent, respectively. In that study, the mean ages at which infection occurred were 5.6, 6.7, and 11.5 years for measles, varicella, and mumps, respectively, typical of ages obtained in the pre-vaccine era. Spread of measles before the introduction of vaccine occurred primarily in younger school-age children (5 to 9 years of age) within the community, with secondary spread to siblings occurring within the family. From 1960 to 1964, 53 percent of all measles cases occurred in children 5 to 9 years of age and 37 percent in children younger than 5 years.[126] However, new cases are now most likely to occur in older school-age children and college students, whereas preschoolers, especially those too young to receive vaccine, constitute a continuing pool of susceptible persons.[219] In 2004, of 37 cases, 49 percent were in children younger than 5 years, 19 percent were in children 5 to 19 years of age and persons aged 20 to 34 years, and 14 percent were in infants younger than 12 months.[52] A comparable change has been seen in the epidemiology of mumps, with a decreasing proportion of cases in younger school-age children and an increasing proportion in older children, adolescents, and young adults.[92,310] The "new" epidemiology of mumps disease was confirmed in a recent outbreak of mumps in 11 states in the United States from January 1 to May 2, 2006, which resulted in 2597 cases.[87] The median age of case patients was 21 years, and the outbreak started among college students.

Another childhood viral disease, varicella, has undergone significant changes in its epidemiology. Varicella vaccine has been

shown to be more than 85 percent effective in preventing varicella in clinical practice,[311] resulting in a dramatic decrease in varicella disease (between 71% and 84%) as measured in three varicella disease active surveillance sites across all ages groups, along with an observed 66 percent national decrease in varicella-associated deaths.[242,286] However, as the vaccine is not 100 percent effective in preventing every case of varicella, mild cases of varicella continued to occur in vaccinated persons (known as breakthrough disease). These vaccinated cases are half as contagious as are unvaccinated cases to household members unless they have 50 lesions or more.[287]

For respiratory syncytial virus, secondary attack rates of infants have been estimated at 45 percent, higher than the corresponding rate of 27 percent for all family contacts.[147] The infant's older sibling is the most frequent introducer of respiratory syncytial virus into the family. In a recent report among infants in a community study from Guinea-Bissau, researchers found that mothers with recent exposure to respiratory syncytial virus were two times more likely to infect boy children (prevalence ratio = 2.04 [1.18-3.53]) than girls.[299] These findings await corroboration.

In the Cleveland families study of the 1960s, common respiratory diseases (including the common cold, rhinitis, laryngitis, and bronchitis) occurred more often in young school-age children than in preschool children and more frequently in siblings of school-age children than in preschool children without such siblings.[103] Secondary attack rates of common respiratory diseases averaged 25 percent, were highest in children younger than 5 years (37-49%), and were similar in preschool children with or without siblings in school.

The epidemiology of some acute respiratory diseases also may be changing. Transmission varies both with levels of immunity and with opportunities for exposure of susceptible persons. A comparison of the Cleveland family study data with data from a more recent longitudinal study of children in daycare suggested that out-of-home childcare may be lowering the age of first exposure to and infection with acute respiratory pathogens.[102] Young children in daycare are more likely than children in home care to have more than six upper respiratory tract infections per year and more than 60 days of illness per year.[314] In a study of lower respiratory tract infections in children younger than 2 years, children hospitalized with lower respiratory tract infection were three times more likely to have received care in a daycare center attended by six or more children.[11] A Japanese study evaluated the carriage rates of respiratory bacterial pathogens in children attending daycare centers and found high colonization rates by respiratory bacterial pathogens (especially by antibiotic-resistant strains), suggesting horizontal spread among children in daycare centers. More than 50% of children carried more than one pathogen.[222] Not surprisingly, crowding in the child's home also was observed to be an independent factor that increased the risk of acquiring respiratory disease.

In the Seattle Virus Watch study of family spread of influenza virus in 1972, school-age children were the most likely family members to acquire community-acquired infection and then to introduce the infection into the household; they also were most at risk for spread from other family members (57-63% secondary attack rate).[124] However, secondary attack rates among all household members were highest (70-75%) when the introducer was younger than 5 years. For infants in the first year of life, the risk of acquiring influenza infection is related to the number of siblings in the household.[140] Studies using laboratory confirmation of transmission of influenza confirm the importance of disease transmission from children to their parents (attack rate, 13.6%) and their siblings (attack rate, 19.9%) and support the recent 2008 recommendations of the Advisory Committee on Immunization Practices (ACIP) of the CDC to expand the routine age of annual influenza vaccination to children aged 6 months to 18 years.[263] Much attention has been placed recently on the role of children in the spread of influenza viruses to family members and the community, especially with respect to pandemic influenza planning.

Parvovirus B19 infection appears to be transmitted effectively within families, with a secondary attack rate of 50 percent among close contacts; the risk of transmission seems to decrease with advancing age.[73,187] The risk for pregnant women to acquire acute parvovirus B19 infection increases with an increasing number of children in the household. Having children 6 to 7 years old resulted in the highest risk for acquiring acute infection in these mothers.[193] Preschool-age children, especially those in daycare, may introduce cytomegalovirus infection into the family, infect seronegative mothers-to-be, and thereby pose a risk to the fetus if the woman with cytomegalovirus infection is pregnant.[250] The risk of transmission of cytomegalovirus from child to mother is greatest if the child is younger than 20 months.[3]

Several novel respiratory viruses recently have been associated with acute respiratory infection in humans. A new disease caused by a human coronavirus, SARS, was introduced into the world in the fall of 2002. Between November 2002 and July 2003, more than 8000 cases, with almost 800 deaths, occurred in 32 countries. Children had less severe morbidity and lower mortality rates compared with adults. The virus was thought to be transmitted by the respiratory route, mostly through large droplets.[201,319] Household transmission from a known SARS case occurred at a secondary average attack rate of 14.9 percent, although mostly among adults in one Hong Kong study.[197] Another newly studied respiratory virus, human metapneumovirus, is thought to play an important role in infections in young children, having a spectrum of disease similar to that of respiratory syncytial virus.[320] Information on the epidemiology of this RNA virus in families is still being evaluated. Two other paramyxoviruses that cause encephalitis and respiratory tract disease in humans, Hendra and Nipah viruses, have been identified recently in Australia and Malaysia. Neither of these viruses has caused pediatric infections to date.[319]

The potential spread of other strange and newly emerging or potentially emerging diseases within families was illustrated recently during an outbreak of monkeypox (a zoonotic DNA double-stranded orthopoxvirus) in the Midwestern United States in June 2003.[285] Transmission occurs primarily by large droplets or direct contact, although the disease is much less transmissible than is smallpox. In this small outbreak of 37 human infections, one cluster of three cases occurred in the same family, mostly through person-to-person transmission.

The spread of some enteric diseases within the family also has been studied. In two community outbreaks of shigellosis, Weissman and associates[317] found that in 86 percent of families, the bacterium was introduced by a child younger than 10 years. The secondary attack rate averaged 31 percent and was highest in children younger than 5 years. The secondary attack rate was significantly higher in families with an initial case in a preschool-age child than in families with an initial case in an older person. Spread within the family did not correlate with family size or household crowding but appeared to be highly dependent on whether an ill preschool-age child was in the family.

After a food-borne outbreak of Norwalk virus (now referred to as norovirus) gastroenteritis associated with a school cafeteria occurred, secondary transmission was reported in 44 percent of the households with a primary case. In households with a primary case, the risk for occurrence of secondary illness was twice as high among preschool-age children (70% attack rate) as among adults (31%); other investigators have found lower attack rates in households, ranging from 15 to 26 percent.[101,160,167] de Wit and colleagues[101] showed that a risk factor for development of norovirus

gastroenteritis was having a household gastroenteritis contact, with that risk being slightly higher if the household contact was a child rather than an adult. Another outbreak in 2004 among 191 children who acquired norovirus after playing in a recreational water fountain found that in 111 families in which information was complete, the secondary attack rate for illness was 15 percent and occurred mostly among parents (74%) of the affected schoolchildren.[167] As expected, risk for spread of illness into households was higher if the primary case was a child.[141]

Poliovirus seems to spread more readily than does *Shigella* in families. Gelfand and associates[134] studied household contacts of children found to have wild poliovirus infection. Among household contacts, evidence of recent infection, usually no later than 1 month after that in the index child, was found in 73 percent of adults, 96 percent of older siblings, and 84 percent of younger siblings. By studying the spread of live poliomyelitis vaccine virus strains in families, they found a secondary infection rate among susceptible persons of 53 percent in a lower economic group and 9 percent in a higher economic group.[135] Secondary infection rates were higher with type 3 poliovirus (77%) than with type 1 (47%) or type 2 (36%). Virus appeared to spread more readily from those with pharyngeal excretion and less readily from adults than from children.

In the older study of Cleveland families, generically termed "infectious gastroenteritis" was introduced most commonly by children younger than 6 years.[103] The secondary attack rate averaged 11 percent and was highest in children younger than 8 years. The secondary attack rate was related directly to the number of major gastrointestinal symptoms (vomiting, diarrhea, and abdominal pain) suffered by the index case: 10 percent for contacts of those with one symptom, 16 percent for contacts of those with two, and 32 percent for contacts of those with all three.

Helicobacter pylori infections cluster within families, and the family is the most important source for transmission.[108] Families of children with *H. pylori* infection identified in the gastric antrum after endoscopy were studied for the presence of *H. pylori* antibody. Seventy-four percent of the parents of infected children were seropositive, compared with 24 percent of the parents of uninfected children; 82 percent of the siblings of infected children also were positive. In addition, in a study conducted among 241 sibling and nonsibling children aged 2 to 18 years, compared with children with no infected siblings or nonsiblings and adjusting for age, the odds of acquiring *H. pylori* infection were 2.3, 3.2, and 9.4 for children residing with at least one infected nonsibling, one infected sibling, or at least one infected sibling and one nonsibling, respectively. This study thereby implicates intersibling transmission as a route of *H. pylori* infection in childhood as well.[133] Another study also corroborated that having an infected mother or at least one infected sibling was a risk factor, whereas the influence of infected fathers was nonsignificant.[185] A study of 215 adults in England found an association between seropositivity for *H. pylori* antibodies and a history of crowded living conditions in childhood.[226] However, another study did not find crowding to be a risk factor, nor was the size of the family.[185]

In a community-wide study of rotavirus transmission, children younger than 2 years were most likely to introduce the infection into the family.[188] In persons living in households with a child younger than 2 years, the risk of becoming infected was 2.9 (for adults) to 3.5 times (for children 10 to 17 years of age) higher than that for persons of comparable age in other families. A similarly high risk of household transmission (21%) was found if a young child had rotavirus in the home.[101] Young children attending daycare centers also are likely to introduce infections associated with parasitic enteropathogens into their families. A study in Houston, Texas, found a 17 percent secondary attack rate for giardiasis in families with a child who became infected in daycare facilities.[256] At least one secondary case of giardiasis was identified in 47 percent of all households with a *Giardia*-infected child during an outbreak at a Washington, DC, daycare center.[258] During an outbreak of diarrheal disease at a daycare center in Oklahoma, stool samples were positive for *Cryptosporidium* in 23 percent of the household contacts of children with confirmed *Cryptosporidium* infection.[156] *Cryptosporidium parvum* has family transmission and infectivity rates as high as those of *Shigella*, which has added concern in countries where the prevalence of family HIV seropositivity is high and in whom the parasite can cause more severe morbidity.[240]

Young children may acquire asymptomatic hepatitis A infection in daycare centers and transmit this infection to adult household contacts, who may become quite ill.[312] In a population with a low prevalence of hepatitis A, adults who lived in the same household with children of primary school age were at increased risk of acquiring the infection during community outbreaks, with the risk increasing as the number of primary school–age pupils sharing the household increased.[273] Households with a primary school–age child had better than a threefold higher risk of having hepatitis in an unmatched analysis. The epidemiology of this disease will change as well with the recent ACIP recommendation for routine vaccination of all children aged 1 year to 18 years with hepatitis A vaccine.[65]

International adoptees may introduce infectious diseases that are endemic in the child's country of origin into families that might otherwise not be at risk. In one study, parents of hepatitis B surface antigen (HBsAg)–seropositive adopted children were five times more likely to have serologic evidence of hepatitis B virus infection than were parents of HBsAg-seronegative adoptees.[130] Children from various countries also have been shown to have tuberculosis, cytomegalovirus infection, congenital syphilis, and infections with enteric pathogens when they join their adopted families.[171] Careful screening has been recommended for such children to identify and to treat these infections and thereby to prevent transmission.[231]

SCHOOLS

A well-known fact is that infectious disease agents are transmitted easily among children in daycare centers. Lu and colleagues[211] showed that risks of acquiring diarrheal disease and upper respiratory tract infection in children who attended daycare centers are greater than those in home-schooled children; the difference was most significant for children younger than 1.5 years. Interventions that have proved valuable for reducing the incidence of infections within childcare centers include the following: formal written policies for infection control within the childcare center, formal education of staff in childcare centers concerning infection control practices, practice of good hand hygiene by both staff and children, appropriate cleaning of contaminated surfaces, separation of food preparation and diaper changing areas, exclusion of certain ill children, cohorting of ill children when exclusion is not possible, adequate age-appropriate immunization of childcare attendees and staff, and optimal ratios of children to staff.[29]

Evans and Maguire[117] summarized 95 reported outbreaks of infectious intestinal disease in schools and nurseries in England and Wales between 1992 and 1994 (7% of 1280 reported outbreaks). The most common pathogens were *Salmonella*, *Shigella sonnei*, and noroviruses. The mode of transmission was mainly from person to person in 55 outbreaks and mainly food-borne in 30. The mean attack rate was 30 percent, and median duration was 10 days; the attack rate and duration varied with the pathogen involved. Forty-five (1.5%) of the 3118 people reported ill were admitted to the hospital. The authors concluded that outbreaks

in schools and nurseries are common occurrences, attack rates are high, and such outbreaks often are prolonged. Effective infection control policies and appropriate training of staff are needed.

A CDC study of meningococcal disease in schools between January 1989 and June 1994 identified 22 clusters in 15 states.[327] Three quarters of the school clusters occurred in secondary schools. The estimated incidence of secondary meningococcal disease among schoolchildren aged 5 to 18 years was 2.5 per 100,000 population, a relative risk of 2.3. More than 70 percent of secondary cases occurred within 2 weeks of the index case. Surveillance in preschool and school settings in England and Wales during the course of a 6-year period identified 114 clusters of meningococcal disease; 20 clusters were reported in preschool settings, 43 in primary, 46 in secondary, and 5 in independent schools.[100] The relative risk for development of more cases in the 4 weeks after a single case occurred compared with the background rate was raised in all settings, ranging from a relative risk of 27.6 in preschool settings to a relative risk of 3.6 in secondary schools. Most (68%) secondary cases occurred within 7 days of the first case. Although risk for occurrence of more cases of meningococcal disease in schools was higher, especially in preschool settings, whether widespread use of antibiotics after single cases reduces risk of more cases could not be determined. College students, particularly freshmen living in dormitories, have an increased risk of acquiring meningococcal disease, and the recommendation of the American Academy of Pediatrics is that these students and their parents be informed of this increased risk and that vaccination with the quadrivalent conjugate meningococcal vaccine be offered.[16,254]

Attack rates of shigellosis during a group of outbreaks in elementary schools ranged from 0.4 to 1.1 percent; the higher values occurred in schools with larger numbers of children younger than 8 years.[307] Enforced handwashing and disinfection of items potentially contaminated with feces have been associated with control of a shigellosis outbreak in an elementary school.[136]

Typically, spread in schools is less than that in families. In a measles outbreak that involved children in 20 of 26 classrooms in one school, the attack rate in unimmunized pupils with no history of measles was only 16 percent.[97] Landrigan[195] observed in a community outbreak that measles spread most quickly in situations in which unvaccinated children were brought together for the first time; in urban areas, such spread occurred in daycare centers and nursery schools, whereas in rural areas, where children seldom attended daycare centers, it occurred in elementary schools. Measles spread rapidly in a school with children ranging in age from 5 to 14 years due to a high personal belief exemption policy.[63] Although it no longer is endemic in the United States, measles continues to be imported. An outbreak in a boarding school started from a student who returned from the Middle East and caused 11 confirmed cases, including 9 students in the school. High coverage with two doses of measles-containing vaccine among students was effective in limiting the extent of the outbreak.[51] Health care providers should maintain a high index of suspicion for measles, especially in those who have traveled abroad recently, and recommendations for two doses of measles-containing vaccine in all school-age children should be followed.

Schools may serve to spread infection from family to family. In outbreaks of measles, mumps, and pertussis, children infected within classroom settings appear to have spread disease to younger siblings at home. These outbreaks have occurred in school settings in which a high proportion of students have been immunized previously.[32,219,233] In outbreaks of measles and mumps, failure of the vaccine to evoke a protective immunologic response (primary vaccine failure) has been a more significant factor resulting in susceptibility to infection than has waning immunity from previous successful vaccination (secondary vaccine failure).[105,112] Failure of primary coverage due to shortage of measles-mumps-rubella (MMR) vaccine resulted in a large cohort of United Kingdom children in the mid-1990s receiving only MR, thus remaining susceptible to mumps; a large outbreak among young adults 18 to 25 years old occurred in 2004 to 2005 as a result.[57] A large outbreak in the United States in 2006, however, was due to a combination of primary and secondary vaccine failure.[88] Pertussis outbreaks are being recognized increasingly in schools, with a substantial proportion of cases occurring among older children and adolescents, with subsequent spread to the community.[61,74] Waning immunity has been shown to be a particular problem leading to susceptibility to pertussis in adolescents and adults[157]; the American Academy of Pediatrics Committee on Infectious Diseases now recommends that adolescents 11 to 18 years of age receive a booster dose of acellular pertussis vaccine.[6,33]

Miller and associates[232] described a diphtheria outbreak in an elementary school in which 34 percent of all students were found to be infected and clinical diphtheria developed in 30 percent of the unimmunized students. The reason for the intense spread was not found.

The absence of tuberculosis infection and disease among children is a key indicator of a community's success in interrupting the transmission of tuberculosis.[26] The resurgence of tuberculosis in the United States in 1985 to 1992 included a reversal of the long-term decline in the incidence of tuberculosis among children, which indicated a failure of the public health system to prevent transmission of the disease. A study of 165 children with tuberculosis in California in 1994 found that for 59 (37%), an adult source-case was identified.[209] Factors that contributed to transmission to children included delayed reporting, delayed initiation of contact investigations, and poor management of adult source-cases. Improvements in contact investigations might have prevented 17 (10%) of those cases.

Although most pediatric tuberculosis infection is caused by adults with active disease, outbreaks of tuberculosis in children have been reported[180,297]; in most cases, the outbreaks occurred in schools. Widespread tuberculin skin test (Mantoux) screening of school-age children has been shown not to be cost-effective[234]; screening is recommended only for groups of children considered to be at high risk for development of infection.[30,252] Recent review articles highlight the importance of caring for the child's disease in the context of the family and community.[120,215,216]

Settings that result in a greater level of person-to-person contact than is found in typical classroom settings, such as participation in school sports[79,235] or attendance at a boarding school (or summer camp), also can result in more efficient transmission of disease.[34,283] Outbreaks of community-associated MRSA have occurred among football teams at both the high-school and college levels.[241,271]

The number of varicella outbreaks in childcare settings and schools has declined since initiation of varicella immunization in the United States in 1995, but problems remain.[69] One-dose varicella vaccination does not provide sufficient herd immunity levels to prevent outbreaks in school settings, where exposure can be intense. Furthermore, transmission of varicella among vaccinated children is effective, and the difficulty in establishing the diagnosis of mild cases in vaccinated persons hinders early implementation of control measures.[210] Vaccine failure is associated with administration to children younger than 19 months, administration to children with eczema, and a longer time since immunization.[151,199] Furthermore, as one dose of varicella vaccine was deemed insufficient to prevent school outbreaks and to allow a further decrease in related morbidity and mortality,[210] the ACIP recommended in June 2006 a two-dose varicella vaccination for children and a second-dose, catch-up program for children, adults, and adolescents who previously had only one dose.[324]

FOOD- AND WATER-BORNE ILLNESSES

According to a 1999 estimate, food-related disease develops in an estimated 76 million people each year in the United States, thus rendering food safety one of the most important objectives of public health officials.[225] The most common clinical manifestations of food-borne illness are gastrointestinal. The CDC periodically publishes a summary of food-borne illnesses in the United States based on active surveillance by the CDC (FoodNet data)[62] as well as incidence summaries of reported cases and outbreaks of several food-borne diseases.[77] Physicians must work in conjunction with public health officials by confirming diagnoses and promptly reporting findings or suspicions so that food-borne outbreaks can be recognized in a timely fashion to control and to prevent these illnesses most effectively.[45] Proper food preparation, whether it is done commercially or in the home, is critical for community health. Outbreaks have resulted from cross-contamination, improper cooking, and inherently risky raw foodstuffs including unpasteurized dairy products[99,213,313,318]; on occasion, the etiologic agent cannot be identified.[295] Poor personal hygiene may lead to environmental contamination of hands or food, with disastrous consequences.[96] Drinking water, taken for granted in some parts of the world, continues to spread diseases such as norovirus gastroenteritis, giardiasis, cryptosporidiosis, cholera, hepatitis A, and typhoid fever.[227,243,280] Water-borne infections from municipal drinking water are rare events when water companies are well managed and the treatment process includes filtration as well as disinfection.[326] Municipal water systems that draw supplies from open sources such as rivers and lakes are prone to contamination,[28] and ground water sources may become contaminated and cause illness.[90,296]

BIOTERRORISM AND DISASTER RESPONSE

Unfortunately, an important responsibility of public health officials is to help prepare the nation and pediatricians for the possibility of biologic or chemical terrorism and natural disasters. Children have special vulnerabilities to biologic terrorism and chemical agents because of their unique physiology and the limited availability of age-appropriate and weight-appropriate antidotes and treatment.[217,251] In addition, clinicians' lack of familiarity with presenting clinical syndromes from either biologic or chemical exposures can delay establishment of the appropriate diagnosis and initiation of treatment.[94,251] In April 2000, the CDC issued a strategic plan for preparedness and response to biologic and chemical terrorism.[40] Eighteen months later, in October 2001, the first case of intentional exposure to *Bacillus anthracis* was reported in Palm Beach County, Florida. Eventually, 23 cases of anthrax were identified as a result of intentional exposure, predominantly through envelopes containing or contaminated with highly purified anthrax spores. Eleven cases were inhalational and 12 cutaneous (one was pediatric); one cutaneous case was laboratory acquired. Four individuals died.[79,181] Public health officials had to address the public's fear and hysteria after the initial cases were reported. Guidelines for the proper handling of mail and recommendations for rational use of antibiotics, including prophylaxis, were developed quickly and disseminated.[82] Subsequently, autonomous detection systems were developed in postal facilities to detect aerosolized *B. anthracis*.[72] Special recommendations for anthrax prophylaxis were required for children and breast-feeding mothers because ciprofloxacin was the agent of choice for adults and could not be used in these groups.[81,217] Many more cases of anthrax probably were prevented, in part as a result of the rapid response and recommendations of public health officials to this bioterrorist attack. However, certain barriers need to be addressed to fully provide protection for children against biologic and chemical terrorism agents. The Strategic National Stockpile, which is a national repository of antimicrobial agents, chemical antidotes, antitoxins, and other life-support medications and equipment, can stockpile only items licensed by the U.S. Food and Drug Administration (FDA) and only for their FDA-approved indications.[218] Often FDA indications for antimicrobial and other therapeutic agents for children after release of biologic terrorist agents are lacking, causing the stockpile to be deficient in certain therapeutic agents for children.

The CDC also identified smallpox virus as a likely bioterrorism agent and developed guidelines for preparations in case of a threat related to smallpox.[325] On June 21, 2002, the ACIP of the CDC made the following recommendations with respect to smallpox vaccination:

- Under current circumstances, with no confirmed smallpox and the risk of an attack assessed as low, vaccination of the general population is not recommended, as the potential benefits of vaccination do not outweigh the risks of vaccine complications.
- Smallpox vaccination is recommended for persons predesignated by the appropriate bioterrorism and public health authorities to conduct investigation and follow-up of initial smallpox cases that would necessitate direct patient contact.
- Smallpox vaccination is recommended for health care personnel at risk for exposure to the initial smallpox cases in facilities that are pre-designated to receive these patients.

Updated smallpox recommendations are summarized in a recent article.[71] Undoubtedly, as the situation changes and perhaps improved vaccines against smallpox virus and other biologic agents are developed, the CDC and other public health agencies will issue updated recommendations and guidelines for combating and preventing infections related to bioterrorism.

Terrorism preparedness is a unique and, it is to be hoped, rare component of general emergency and disaster preparedness. Since December of 2004 through 2006, several natural disasters of significant proportions have struck the world, causing extreme destruction, loss of lives and property, and potential exposure to infectious diseases.[206] These have included tsunamis in Sri Lanka, Thailand, and Indonesia (2004); earthquakes in Sumatra, Pakistan, and India (2005); powerful hurricanes, Katrina and Rita, in the United States (2005); and volcanic eruptions in El Salvador (2005) and Indonesia (2006). During these disasters, infections can result from compromised personal hygiene; wounds and injuries; contaminated food and water, such as diarrheal disease from viruses (e.g., rotavirus and hepatitis A), parasites, and bacteria (cholera, shigellosis, leptospirosis, typhoid, *Vibrio cholerae* and *Vibrio vulnificus*); and vector-borne diseases. The vector-borne diseases may vary, depending on the location of the disaster, but may consist of West Nile virus, rabies, dengue, and malaria.[206] After hurricanes Rita and Katrina, illnesses caused by *V. vulnificus*, *Vibrio parahaemolyticus*, and nontoxigenic and toxigenic *V. cholerae* were reported.[114,301] These findings underscore the importance of prompt recognition and appropriate management of these often rare diseases by pediatricians during and after disaster situations.

CONTROL METHODS

ISOLATION

Indications for isolation of patients with infectious diseases have changed greatly in this century with increasing knowledge of modes of spread and with antimicrobial therapy, which rapidly renders patients with many diseases noninfectious. Isolation of patients with diphtheria was shown to be of value in Providence,

Rhode Island, in 1904 to 1913 and probably remains so today. The attack rate among family contacts of diphtheria patients treated at home was 6.5 percent, whereas that among contacts of patients treated in the hospital was 4.3 percent; after adjustment for the ages of contacts in the two groups, hospitalization was associated with a 43 percent decrease in cases among household contacts.[106] Heymann's *Control of Communicable Diseases Manual* still suggests strict isolation for pharyngeal diphtheria and contact isolation for cutaneous diphtheria until cultures are negative after cessation of antibiotherapy. Specific therapy has made such institutional isolation unnecessary for some diseases.[161]

Isolation of patients with bacilliferous leprosy, which once was a central part of leprosy control, no longer is necessary because dapsone and rifampin quickly kill most *M. leprae*, thereby rapidly preventing further risk of transmission.

Indications for and techniques of isolating patients in hospitals to prevent nosocomial transmission are discussed elsewhere in this book. An extensive, practical discussion of disease control strategies, including isolation, is readily available in *Control of Communicable Diseases Manual*.[161] The *Red Book Report of the Committee on Infectious Diseases* of the American Academy of Pediatrics also is an important resource for information about isolation recommendations.[255] In most instances, the need for isolation of children at home is occasioned by bacterial respiratory disease, diarrhea, acute viral diseases of childhood, conjunctivitis, contagious skin diseases, and arthropod-borne diseases.

For bacterial respiratory diseases for which therapy rapidly and consistently renders a patient noninfectious, isolation of infected children may be as brief as 1 or 2 days (as for group A streptococcal pharyngitis or meningococcal infections). Isolation of patients known to have pertussis still is recommended for at least 5 days after the initiation of erythromycin or other therapy. For patients with infection or colonization by vancomycin-resistant enterococcus, standard and contact precautions remain in force until culture results from multiple sites (stool, wound, perineal area) are negative on three separate series of cultures obtained at least 1 week apart.[70]

An attempt should be made to limit contamination with the feces of children with diarrhea. Careful handwashing is of great importance, both for the children and for those who take care of them. Clothing and linen contaminated with excreta should be laundered. Adults who are infected with and may be excreting *Salmonella*, *Shigella*, *Yersinia enterocolitica*, hepatitis A virus, or other enterically transmitted pathogens should be excluded from preparing food or providing direct care to young children. (See Chapter 258 for recommendations for excluding children from daycare.)

Indications for isolation of patients with acute viral diseases of childhood depend on the severity of the illness and the degree of contagiousness. No value has been established for isolation for the common acute viral respiratory diseases. For rubella, isolation is recommended only from women in early pregnancy. Currently, children with SARS would be strictly isolated in the hospital even if they are not seriously ill. Isolation of patients with poliomyelitis within the home is of little value because spread occurs most commonly during the prodrome. The same holds true for measles, which spreads efficiently, although the period of communicability can extend until 4 days after the onset of rash, and children with measles should be kept home from school at least that long. Attempts are made to isolate children with varicella and mumps from school for 7 and 9 days, respectively, after the onset of typical illness; these diseases spread less efficiently than measles does.[169] Isolation of children with influenza would also be difficult because most cases are infectious before they are symptomatic.[63] Children with conjunctivitis caused by transmissible agents should be excluded from school during the acute phase of illness. This form of isolation may help limit the spread of *H. influenzae* biogroup *aegyptius*, pneumococci, picornavirus, adenovirus, and *Chlamydia trachomatis*.

Children with contagious skin diseases should avoid having skin contact with other children outside their family. Those with molluscum contagiosum, herpes simplex, impetigo, or MRSA skin infections should avoid engaging in wrestling or other contact sports.[19] Children with MRSA skin infections are becoming increasingly prevalent; in addition to single-case occurrences, these children are also involved in outbreaks on athletic teams, in daycare centers, and in other situations where crowding and skin-to-skin contact occur.[183] Children with scabies should be excluded from school until the day after treatment. Children with tinea corporis should avoid gymnasia, swimming pools, and contact sports.

Children in the early phases of arthropod-borne diseases, when the infecting organism still may be in the blood, should avoid being bitten by the vector. The wide variety of such illnesses includes arboviral diseases, bartonellosis, leishmaniasis, microfilarial infections, malaria, and African trypanosomiasis.

QUARANTINE

In addition to containing the spread of contagious illnesses through isolation, quarantine is another less commonly used control measure. The history and practice of quarantine began in Italy to control plague as early as the 14th century by requiring ships arriving in Venice from infected ports to anchor for 40 days before landing.[39] The Italian word *quaranta*, meaning forty, turned into the commonly used word *quarantine*. Although the term *isolation* refers to separation of people known to have an illness, the strategy of *quarantine* refers to keeping people who have been exposed to an illness but who may or may not be infected away from the general population.[75] Health departments in the past and recent present have used quarantine infrequently for people exposed to illnesses for which they refuse prophylactic treatment (such as vaccine-preventable diseases) or for diseases of historical or medical importance, such as plague, diphtheria, cholera, smallpox, and yellow fever.[206,260] However, not until the arrival in 2002 of SARS, an untreatable infectious viral disease spread mostly by droplets, was this control measure used with regularity. Its effectiveness in decreasing the spread of SARS was aided by the long incubation period of the disease and the fact that the peak of viral shedding was during the second week of illness.[161,177] Contacts of patients suspected of having SARS were placed in quarantine for 10 days.[161] The viral hemorrhagic fever agents (Ebola, Marburg) recently were added as quarantinable diseases along with pandemic influenza. With the possibility of a pandemic influenza strain imminent, the strategy of quarantine as an early containment measure once again has become prominent in some disease control plans.[60]

CHEMOPROPHYLAXIS

The word *chemoprophylaxis* is used in this section to describe the use of antimicrobial agents to prevent acquisition of infection before exposure (as with malaria), to treat asymptomatic infection and thereby to prevent disease (as with isoniazid treatment of tuberculin skin test–positive children), to treat contacts of a proven case before presentation of signs or symptoms in the contacts (as with pertussis), and to treat disease and thereby to prevent its complications (as with primary prevention of acute rheumatic fever). In each situation, the primary purpose is to prevent a disease by administration of an antimicrobial agent that affects the causative microorganism.

The decision to initiate chemoprophylaxis and the choice and dosage of agents require careful consideration of both the child's

clinical status and the epidemiologic factors that may place the child at risk for acquiring the disease. Local public health departments should be notified of exposure to reportable communicable diseases; they may need to conduct an investigation to ensure that all exposed persons have been identified and, when necessary, treated or placed under observation. In addition, they often can provide useful additional information on recommended treatment regimens.

A summary of several diseases for which chemoprophylaxis may be administered to children is given in Table 257–2. Some special situations, such as chemoprophylaxis for immunodeficient children and perioperative prophylaxis to prevent epidemics, are omitted from the discussion. For more detailed discussion of antimicrobial drug use in prevention of disease, one should consult the regularly updated *Red Book* of the American Academy of Pediatrics.[7]

Acute rheumatic fever that occurs after streptococcal pharyngitis can be prevented in 90 percent of patients by administration of repository penicillin.[98,275] Secondary prophylaxis for prevention of bacterial endocarditis after acute rheumatic fever also can be up to 90 percent effective and depends largely on the faithfulness with which an effective drug is taken. For acute glomerulonephritis, one study has suggested that antibiotics will prevent the development of acute glomerulonephritis after group A streptococcal pharyngitis,[265] but the apparent reduction in incidence was not statistically significant. No evidence indicates that administration of antibiotics will prevent the development of acute glomerulonephritis after streptococcal skin infection.[316] Studies to determine the effectiveness of chemoprophylaxis and optimal drug regimens are still needed.[300]

Invasive group A streptococcal infection also is associated with necrotizing fasciitis and streptococcal toxic shock syndrome. For household contacts of index patients, routine screening for and chemoprophylaxis against group A streptococcal infection are not recommended. Providers and public health officials may choose to offer chemoprophylaxis to household contacts who are at an increased risk of contracting sporadic disease or dying of group A streptococcal infection.[153,262]

Cholera typically is not spread from person to person in households. However, in developing countries, if the household secondary attack rate is known to be high, family contacts have been treated prophylactically with tetracycline or doxycycline to prevent illness.[302] Mass treatment of the community never is warranted; improvement of food and water hygiene and attention to rehydration are more important.[277,278] At present, nearly all strains of *V. cholerae* in Bangladesh are resistant to tetracycline, trimethoprim-sulfamethoxazole, and erythromycin. Although the strains are still sensitive to ciprofloxacin, the minimal inhibitory concentration is increasing, and azithromycin and ciprofloxacin are being administered in their place. Patients with cholera recover even without an antibiotic if hydration is maintained, but antibiotics should be used for severely and moderately dehydrated patients to reduce excess losses of fluids and electrolytes and to minimize the supply and logistical requirements for caring for a large patient population. Vaccines are poorly immunogenic, especially in children, and provide modest protection at best.[163]

Prevention of secondary cases of sporadic invasive meningococcal disease depends on rapid identification of close contacts and administration of an effective antibiotic. The risk for spread of meningococcal disease among household contacts of a patient can be reduced by an estimated 89 percent if they take antibiotics known to eradicate meningococcal carriage. In the United States, the primary means for prevention of sporadic meningococcal disease is antimicrobial chemoprophylaxis of close contacts of infected persons.[64] Close contacts include household members, daycare center contacts, and anyone directly exposed to the patient's oral secretions (e.g., through kissing, mouth-to-mouth resuscitation, endotracheal intubation, or sharing of cigarettes or pipes). The attack rate for household contacts exposed to patients who have sporadic meningococcal disease is an estimated four cases per 1000 persons exposed, which is 500 to 800 times greater than for the total population. Because the rate of secondary disease for close contacts is highest during the first few days after onset of disease in the index patient, antimicrobial chemoprophylaxis should be administered as soon as possible (ideally within 24 hours after identification of the index case patient). Conversely, chemoprophylaxis administered later than 14 days after onset of illness in the index patient is probably of limited or no value. Oropharyngeal or nasopharyngeal cultures are not helpful in determining the need for chemoprophylaxis and may unnecessarily delay institution of this preventive measure. Internationally, no uniform recommendations have been established for chemoprophylaxis,[264] and jurisdictions vary with respect to their recommendations for prophylaxis, in the absence of an outbreak, of other types of close contacts and even for the index case. Rapid initiation of a chemoprophylaxis program after two cases of meningococcal disease were identified in a school potentially would have prevented 50 percent of subsequent cases in the clusters described.[327] A European study examined the efficacy of chemoprophylaxis after single cases of meningococcal disease in daycare or nursery settings, comparing countries recommending chemoprophylaxis only to close contacts and those recommending chemoprophylaxis for all children in the same nursery. Results suggested that possible benefit was achieved from mass prophylaxis, although the difference was not statistically significant.[27]

Rifampin, ciprofloxacin, and ceftriaxone are 90 to 95 percent effective in reducing nasopharyngeal carriage of *Neisseria meningitidis*, and all are acceptable alternatives for chemoprophylaxis. However, rifampin is associated with adverse side effects and is not recommended during pregnancy.[281] Systemic antimicrobial therapy for meningococcal disease with agents other than ceftriaxone or other third-generation cephalosporins may not reliably eradicate nasopharyngeal carriage of *N. meningitidis*. If other agents have been used for treatment, the index patient should receive a chemoprophylactic antibiotic for eradication of nasopharyngeal carriage before being discharged from the hospital. Given that the widespread use of rifampin in an outbreak setting might lead to the circulation of isolates resistant to rifampin, use of ciprofloxacin or ceftriaxone should be considered. More trials comparing the effectiveness of ceftriaxone, ciprofloxacin, and rifampin for eradication of *N. meningitidis* could provide important insights.[128]

To prevent gonococcal ophthalmia neonatorum, a prophylactic agent should be instilled into the eyes of all newborn infants; this procedure is required by law in most states. All of the prophylactic regimens listed here prevent gonococcal ophthalmia. However, the efficacy of these preparations in preventing chlamydial ophthalmia is less clear, and they do not eliminate nasopharyngeal colonization by *C. trachomatis*.[76] Ocular prophylaxis is warranted because it can prevent sight-threatening gonococcal ophthalmia and because it is safe, easy to administer, and inexpensive. Recommended regimens include erythromycin (0.5%) ophthalmic ointment in a single application or tetracycline ophthalmic ointment (1%) in a single application. One of these recommended preparations should be instilled into both eyes of every neonate as soon as possible after delivery. If prophylaxis is delayed (i.e., not administered in the delivery room), a monitoring system should be established to ensure that all infants receive prophylaxis. All infants should be administered ocular prophylaxis, regardless of whether they are delivered vaginally or by cesarean section. Single-use tubes or ampules are preferable to multiple-use tubes. Bacitracin is not effective. Use of povidone-iodine has not been studied adequately. The best method for preventing neonatal gonococcal and chlamydial disease is the diagnosis and treatment of gonococcal and chlamydial infections in pregnant women; not all women, however, receive prenatal

TABLE 257–2 Chemoprophylaxis against Communicable Diseases in Children

Disease	Target Group	Value of Chemoprophylaxis	Recommended Drug	Vaccine for Target Group	References
Acute glomerulonephritis	Those with group A streptococcal infection in nephritis outbreaks	Proposed	Benzathine penicillin G IM Penicillin V PO	No	265, 316
Acute rheumatic fever	Those with group A streptococcal infection other than impetigo	Shown	Benzathine penicillin G IM Penicillin V PO Erythromycin PO	No	98, 275, 300
	Those with previous acute rheumatic fever	Shown	Benzathine penicillin G IM Penicillin V PO Sulfadiazine or sulfisoxazole PO Erythromycin PO		
Anthrax	Those exposed in an intentional release	Proposed	Doxycycline PO Ciprofloxacin PO Amoxicillin PO	Yes	49, 176
Chlamydia	Infant born to mother with genitoanal chlamydial infection	Shown	Erythromycin PO Topical antibiotic therapy alone is inadequate and is unnecessary if systemic treatment is administered.	No	76
	Sexual contacts of confirmed cases	Shown	Erythromycin PO Azithromycin PO Doxycycline PO		
Cholera	Close contacts of case	Rarely indicated	Tetracycline PO Doxycycline PO Trimethoprim-sulfamethoxazole PO Furazolidone PO Erythromycin PO	Yes (limited value)	163, 277, 278
Diphtheria	Close contacts	Shown	Erythromycin PO Benzathine penicillin G IM	Yes	119, 224, 244
Gonorrhea	Infant born to mother with gonorrhea	Shown	Ceftriaxone IM Topical antibiotic therapy alone is inadequate and is unnecessary if systemic treatment is administered.	No	76
	Sexual contacts of confirmed cases	Shown	Ceftriaxone IM Cefixime PO Ciprofloxacin PO Ofloxacin PO Levofloxacin PO In a single dose plus treatment for chlamydia if chlamydial infection is not ruled out		
Group A streptococcus	Household contacts	Unknown		No	153
Haemophilus influenzae type b	Index case All household contacts when at least 1 contact younger than 48 months is partially immunized or unimmunized	Shown	Rifampin (contacts) Cefotaxime or ceftriaxone (index case)	Yes	9, 23, 95, 154
Influenza	Close contacts High-risk patients Unvaccinated patients	Shown	Amantadine PO Oseltamivir PO Rimantadine PO Zanamivir inhaled	Yes	4, 152
Leprosy	Household contacts	Shown	Dapsone Rifampicin	No	15, 289
Malaria	Travelers to malarious areas	Shown	Specifics are highly detailed. Consult an authority.	No	14, 50, 125, 144
Meningococcal disease	Close contacts of index case who shared oral secretions	Shown	Rifampin PO Ceftriaxone IM Ciprofloxacin PO	Serogroups A, C, Y, W135	27, 64, 100, 128, 264, 281, 327
	Index case		Same		
Ophthalmia neonatorum (chlamydia and gonorrhea)	Neonate at birth	Shown	Tetracycline ointment Erythromycin ointment	No	76

TABLE 257–2 Chemoprophylaxis against Communicable Diseases in Children—cont'd

Disease	Target Group	Value of Chemoprophylaxis	Recommended Drug	Vaccine for Target Group	References
Pertussis	Close contacts, irrespective of vaccination status	Proposed	Erythromycin PO Azithromycin PO Clarithromycin PO Trimethoprim-sulfamethoxazole PO	Yes	5, 104, 148, 291, 298
Plague	Contacts of pneumonic case Those exposed in an intentional release	Proposed	Ciprofloxacin PO Doxycycline PO Amoxicillin PO	Yes	174
Syphilis	Infant born to mother with syphilis	Shown	Consult an expert. See CDC recommendations.	No	76
	Sexual contact of confirmed case	Shown	Benzathine penicillin G IM		
Tuberculosis	Close contact of a case, Mantoux nonsignificant	Shown	Isoniazid PO Rifampin PO	Bacille Calmette-Guérin (BCG) (limited indications in United States)	44, 78, 290
	Mantoux significant	Shown	Same		
Yaws	Close contact	Shown	Benzathine penicillin G IM Penicillin V PO	No	12, 284

care. For sexually transmitted gonorrhea, partners of case patients should be treated with a drug or drugs that will eradicate the organism at all anatomic sites of exposure, not waiting for test results. Concurrent treatment of chlamydial infection generally also is warranted.[76]

Because the number of tuberculosis cases has dropped in the United States and the disease has became concentrated among persons at high risk in particular subpopulations, consequently, most U.S. children have negligible risk for acquiring latent tuberculosis infection (LTBI).[44] Among children at low risk, most positive tuberculin skin test results are false-positives caused by nonspecific reactivity or exposure to nontuberculous mycobacteria in the environment. False-positive results lead to unnecessary health care expenditures and anxiety for the child, family, school, and health care workers. Thus, whereas the testing of children with an expected high prevalence of LTBI is desirable, mass testing of children with a low prevalence of LTBI is counterproductive and should not be undertaken. The optimal approach is to perform tuberculin skin testing only of those children with specific risk factors for acquiring LTBI, thereby diminishing the number of false-positive results. Factors that have correlated highly with risk for LTBI among children in more than one study include the following: previous positive tuberculin skin test result; birth in a foreign country with high prevalence; nontourist travel to a high-prevalence country for longer than 1 week; contact with a person with tuberculosis; and presence in the household of another person with LTBI. Isoniazid prophylaxis can prevent development of infection in uninfected tuberculin-negative contacts of tuberculosis cases.[290] Isoniazid prophylaxis also has been shown to decrease the incidence of disease in tuberculin-positive children. In certain treatment programs for LTBI among children in the United States, the completion rate associated with 6 to 9 months of self-supervised isoniazid therapy is only 30 to 50 percent. Because LTBI among young children might progress rapidly to tuberculosis disease, daily observed therapy is recommended. The only recommended regimen for treatment of LTBI in HIV-uninfected children is a 9-month course of isoniazid as self-administered daily therapy or by daily observed therapy twice weekly. Routine monitoring of serum liver enzyme concentrations is not necessary but should be considered in children at risk for development of hepatic disease.[78] Children with LTBI, who are most likely to benefit from daily observed therapy because of their high risk for rapid progression of infection to disease, include contacts of persons with recently diagnosed cases of pulmonary tuberculosis, infants and young children, and children with immunologic deficiencies, especially HIV infection.

Chemoprophylaxis of leprosy has been shown in a meta-analysis to be an effective way to reduce the incidence of leprosy, particularly in household contacts.[289] Rifampicin has been the drug tested most often[15]; the role of chemoprophylaxis needs to be re-examined with use of newer drugs, given the continuing case detection rates globally.

Prophylaxis of contacts to pertussis cases has been questioned in recent analyses, although past studies have demonstrated benefits.[104,148] Administration of erythromycin prophylaxis to contacts of pertussis cases during a community outbreak was associated with a decreased likelihood of occurrence of secondary cases, and delays in beginning erythromycin treatment of primary cases and prophylaxis of contacts were associated with increased secondary spread.[291] Similarly, in a study of a pertussis outbreak in an institutionalized population, erythromycin was shown to be effective in reducing the attack rate and severity of disease among exposed persons.[298] Twelve trials with 1720 participants, including 10 trials investigating treatment regimens and two investigating prophylaxis regimens, were summarized in a meta-analysis.[5] Results showed that short-term antibiotics (azithromycin for 3 days, clarithromycin for 7 days, or erythromycin estolate for 7 days) were equally effective with long-term antibiotic treatment (erythromycin estolate or erythromycin for 14 days) in the microbiologic eradication of *B. pertussis* from the nasopharynx. Side effects were fewer with short-term therapy. No differences were noted in clinical improvement or microbiologic relapse between short- and long-term treatment regimens. Contact prophylaxis (of contacts older than 6 months) with antibiotics did not significantly improve clinical symptoms or decrease the number of cases that developed culture-positive *B. pertussis*. The authors con-

cluded that antibiotics are effective in eliminating *B. pertussis* from patients with the disease, rendering them noninfectious, but do not alter the subsequent clinical course of the illness. Effective regimens for both treatment and chemoprophylaxis include 3 days of azithromycin, 7 days of clarithromycin, 7 or 14 days of erythromycin estolate, and 14 days of erythromycin ethylsuccinate. Considering microbiologic clearance and side effects, 3 days of azithromycin and 7 days of clarithromycin are the best regimens. Seven days of trimethoprim-sulfamethoxazole also appeared to be effective for the eradication of *B. pertussis* from the nasopharynx and may serve as an alternative antibiotic treatment for patients who cannot tolerate a macrolide. Evidence was insufficient to determine the benefit of prophylactic treatment of pertussis contacts.

Erythromycin and benzathine penicillin G have been shown to be effective in eliminating pharyngeal carriage of *Corynebacterium diphtheriae* in 92 and 84 percent, respectively, of those treated.[224] The effectiveness of these drugs as chemoprophylaxis against diphtheria has not been studied. However, the high incidence of diphtheria in nonimmune household contacts of cases,[106] the side effects of diphtheria antitoxin, and the delay associated with and the possible false negativity of cultures are points in favor of giving chemoprophylaxis. Because the vaccine is directed against the toxin, immunized persons still may be carriers and become infected; thus, chemoprophylaxis should be administered to all close contacts after nasopharyngeal cultures have been obtained, regardless of immunization status. All close contacts should have samples cultured and be monitored by a public health representative for 7 days.[119] Patients with positive cultures should complete a full course of antimicrobial treatment. Previously immunized contacts should receive a booster dose of vaccine. Active immunization should be initiated for those who have not been immunized fully in the past.[244]

Individuals with primary or secondary pneumonic plague caused by infection with *Yersinia pestis* are potential sources of person-to-person spread, as would be people exposed through the criminal release of plague bacteria by aerosol. Recommendations for the use of antibiotics after exposure to a plague weapon are conditioned by the lack of published trials in treating plague in humans, the limited number of studies in animals, and the possible requirement to treat large numbers of persons. A number of possible therapeutic regimens for treatment of plague have yet to be studied adequately or submitted for approval to the FDA. A working group offered consensus recommendations based on the best available evidence.[174] It recommends that the tetracycline class of antibiotics be used to treat pneumonic plague if aminoglycoside therapy cannot be administered, which might be the case in a mass exposure scenario when parenteral therapy is either unavailable or impractical. Doxycycline would be considered pharmacologically superior to other antibiotics in the tetracycline class for this indication because it is well absorbed without food interactions, is well distributed with good tissue penetration, and has a long half-life. The fluoroquinolone family of antimicrobials has demonstrated efficacy in animal studies against plague. In studies of mice with experimentally induced pneumonic plague, ciprofloxacin has been demonstrated to be at least as efficacious as aminoglycosides and tetracyclines. In vitro studies also suggest equivalent or greater activity of ciprofloxacin, levofloxacin, and ofloxacin against *Y. pestis* compared with aminoglycosides or tetracyclines. However, no trials of the use of fluoroquinolones in human plague have been performed, and they are not FDA approved for this indication. Thus, in a setting requiring mass postexposure prophylaxis, the drugs of choice are doxycycline, ciprofloxacin, and chloramphenicol; the same holds for pregnant women. However, physicians have recommended that ciprofloxacin and other fluoroquinolones not be used in children younger than 16 to 18 years because of a link to permanent arthropathy in adolescent animals and transient arthropathy in a small number of children, and chloramphenicol should not be given to children younger than 2 years. No comparative studies assessing efficacy or safety of alternative treatment strategies for plague in children have or can be performed. Given these considerations, the working group recommends that children in the contained casualty setting receive streptomycin or gentamicin. In a mass casualty setting or for postexposure prophylaxis, doxycycline is recommended.[174]

Mass campaigns of treatment of patients with yaws and their community contacts have been successful in markedly decreasing the prevalence of the disease, although transmission has not been stopped completely.[12,284]

Prevention of malaria generally is discussed in terms of prophylaxis among nonimmune travelers visiting regions of the world where malaria remains endemic. New concepts of mass treatment in high-risk regions also are being explored by some researchers.[144] For short-term prevention, chloroquine is effective prophylaxis against malarial parasitemia caused by *Plasmodium vivax*, *Plasmodium ovale*, *Plasmodium malariae*, and chloroquine-sensitive strains of *Plasmodium falciparum;* however, chloroquine resistance of *P. falciparum* has spread to most areas of the world with malaria.[50] Thus, persons anticipating travel to areas with chloroquine-resistant *P. falciparum* must weigh carefully the risks of acquiring disease and the problems associated with prophylaxis for small children and pregnant women.[14,125] Health care providers needing assistance with diagnosis or management of suspected cases of malaria should call the CDC Malaria Hotline, 770-488-7788 (Monday to Friday, 8 AM to 4:30 PM, Eastern time); for emergency consultation after hours, call 770-488-7100 and request to speak with a CDC Malaria Branch clinician. Detailed recommendations for prevention of malaria also are available on the Internet at *http://www.cdc.gov/travel/diseases.htm#malaria*.

P. vivax and *P. ovale* have an extra-erythrocytic (hepatic) form, and thus relapse may occur after chloroquine therapy has been stopped. Primaquine, taken as terminal prophylaxis after leaving the malarious area, decreases the risk of having relapses; however, it may cause severe hemolysis in persons with glucose-6-phosphate dehydrogenase deficiency. The decision to give terminal prophylaxis with primaquine depends on individual factors, including the degree of the traveler's risk of acquiring *Plasmodium* with an extra-erythrocytic form and the potential for having adverse reactions; primaquine generally is indicated for persons who have had prolonged exposure in endemic areas.

Four antiviral drugs are available for treatment and prophylaxis of influenza; FDA approval differs by strain of influenza (type A or type B) and by the patient's age. Surveillance of circulating strains of influenza A viruses found increasing amantadine- and rimantadine-resistant viruses after 2005. Therefore, those antivirals should not be used as first-line drugs for either treatment or prophylaxis of seasonal influenza. Zanamivir and oseltamivir are chemically-related antiviral drugs known as neuraminidase inhibitors that have activity against influenza A and B viruses alike. Both zanamivir and oseltamivir were first approved in 1999 for treatment of uncomplicated influenza virus infections; shortly thereafter resistant strains of influenza began to be detected. Neuraminidase inhibitor resistance remained low in the United States through the 2006-2007 influenza season. In 2007-2008, increased resistance to oseltamivir was reported among A (H1N1) viruses in many countries.[323a] In the United States, approximately 10 percent of influenza A (H1N1) viruses, no A (H3N2) viruses, and no influenza B viruses were resistant to oseltamivir during the 2007-2008 influenza season. No viruses resistant to zanamivir were identified.[47a] Zanamivir is now approved for chemoprophylaxis of children 5 years of age or older and for treatment of influenza among children 7 years of age or

older; it is administered by inhaler.[63a] Oseltamivir is approved for treatment and chemoprophylaxis among persons 1 year of age or older. Recommended treatment and chemoprophylaxis dosages of oseltamivir for children vary by the weight of the child. Consult with the Centers for Disease Control and Prevention for oseltamivir and zanamivir dosages and current information about antiviral resistance. One study determined the efficacy of postexposure prophylaxis and treatment of ill index cases with oseltamivir in an attempt to prevent influenza transmission in households randomized to receive treatment (5 days) if illness developed or postexposure prophylaxis for 10 days.[152] Postexposure prophylaxis provided a protective efficacy of 59 percent for households against proven influenza and 68 percent for individual contacts, compared with treatment of index cases alone. Postexposure prophylaxis of household contacts of those with influenza reduces the secondary spread of influenza in families when the initial household case is treated. This study included 129 exposed children aged 1 to 12 years, for whom postexposure prophylaxis protection was slightly lower at 55 percent.

The use of antiviral drugs for treatment and chemoprophylaxis of influenza is a key component of influenza outbreak control in institutions. In addition to antiviral medications, other outbreak-control measures include instituting droplet precautions and establishing cohorts of patients with confirmed or suspected influenza, reoffering influenza vaccinations to unvaccinated staff and patients, restricting staff movement between wards or buildings, and restricting contact between ill staff or visitors and patients. The majority of published reports concerning use of antiviral agents to control influenza outbreaks in institutions are based on studies of influenza A outbreaks among nursing home populations that received amantadine or rimantadine. Less information is available concerning use of neuraminidase inhibitors in influenza A or B institutional outbreaks. When confirmed or suspected outbreaks of influenza occur in institutions that house persons at high risk, chemoprophylaxis should be started as early as possible to reduce the spread of the virus. In these situations, having preapproved orders from physicians or plans to obtain orders for antiviral medications on short notice can substantially expedite administration of antiviral medications. When outbreaks occur in institutions, chemoprophylaxis should be administered to all residents, regardless of whether they received influenza vaccinations during the previous fall, and should continue for a minimum of 2 weeks. If surveillance indicates that new cases continue to occur, chemoprophylaxis should be continued until approximately 1 week after the end of the outbreak. The dosage for each resident should be determined individually. Chemoprophylaxis also can be offered to unvaccinated staff members who provide care to persons at high risk. Chemoprophylaxis should be considered for all employees, regardless of their vaccination status, if the outbreak is suspected to be caused by a strain of influenza virus that is not well matched to the present vaccine.

Guidelines for which populations would require postexposure prophylaxis to prevent inhalational anthrax after the release of a *B. anthracis* aerosol as a biologic weapon will need to be developed by public health officials, depending on epidemiologic circumstances. These decisions would require estimates of the timing, location, and conditions of the exposure. Ongoing case monitoring would be needed to define the high-risk groups, to direct follow-up, and to guide the addition or deletion of groups requiring postexposure prophylaxis. Ciprofloxacin, doxycycline, and penicillin G procaine are approved by the FDA for postexposure prophylaxis of inhalational anthrax. Therefore, for postexposure prophylaxis, the same antibiotic regimen as that recommended for treatment should be used for prevention; prophylaxis should be continued for at least 60 days after exposure. As noted previously, ciprofloxacin and other fluoroquinolones should not be used in children younger than 16 to 18 years because of a link to permanent arthropathy in adolescent animals and transient arthropathy in a small number of children. However, as with mass prophylaxis for plague, balancing these risks against the risks of anthrax infections caused by an engineered antibiotic-resistant strain, an expert panel recommends that ciprofloxacin be used as a component of combination therapy for children with inhalational anthrax. For postexposure prophylaxis or after a mass casualty attack, monotherapy with fluoroquinolones is recommended by the working group.[49] The American Academy of Pediatrics has recommended that doxycycline not be used in children younger than 9 years because the drug has resulted in retarded skeletal growth in infants and discolored teeth in infants and children.[5a] However, the serious risk of acquisition of infection after an anthrax attack supports the consensus recommendation that doxycycline, instead of ciprofloxacin, be used in children if antibiotic susceptibility testing, exhaustion of drug supplies, or adverse reactions preclude use of ciprofloxacin. According to the CDC recommendations for the bioterrorist attacks in 2001, in which *B. anthracis* was susceptible to penicillin, amoxicillin was a suitable alternative for postexposure prophylaxis in infants and children.[81,176] In a contained casualty setting, the working group recommends that children with inhalational anthrax receive intravenous antibiotics. In a mass casualty setting and as postexposure prophylaxis, the working group recommends that children receive oral antibiotics. The United States anthrax vaccine is licensed for use only in persons aged 18 to 65 years because studies to date have been conducted exclusively in this group. No data exist for children, but on the basis of experience with other inactivated vaccines, the vaccine probably would be safe and effective.

Since the introduction of a conjugate vaccine against *H. influenzae* type b in the United States and other countries, rates of invasive disease have plummeted to almost undetectable levels.[23] Developing countries with effective vaccine programs have experienced similar success.[95] Prophylaxis is indicated for household and close contacts to a case of *H. influenzae* type b infection when an unimmunized or partially immunized child younger than 4 years has been exposed.[9] Replacement of type b strains with other invasive serotypes has been documented[154]; however, no proven benefit has been shown for antibiotic prophylaxis for strains other than type b.

DRINKING AND RECREATIONAL WATER

Provision of a safe water supply is a major public health function that factors in preventing water-borne communicable diseases as well as exposure to chemicals such as heavy metals and pesticides. Purification of water occurs both naturally and through human intervention. Natural methods include evaporation and condensation, filtration through the earth, and a series of processes acting during the flow of a stream or on standing: aeration, light-accelerated plant growth, gravity, and oxidation and reduction of organic material by bacteria.[245] Municipal water treatment programs use a variety of measures, including protection of the watershed, chemical disinfection, and filtration of surface water supplies such as lakes and rivers, to ensure the safety of community water supplies.[326] Failure in one of these systems can result in widespread outbreak of water-borne disease.[198] Although many such outbreaks involve a few dozen to a few hundred persons, more than 400,000 cases of illness may have resulted from an outbreak of cryptosporidiosis in Milwaukee, Wisconsin, in 1993.[212] The outbreak was associated with a marked increase in turbidity of the water supply and failure of coagulation and filtration at the water treatment plant to remove oocysts of *Cryptosporidium* from the treated water. An outbreak of toxoplasmosis occurred in Greater Victoria, British Columbia, Canada, in 1995,

resulting in at least 100 cases, including children and 38 pregnant or postpartum women (nearly 1% of all pregnant women)[28]; unfiltered surface water contaminated by rain run-off was blamed for the contamination. Since 1971, the CDC and the Environmental Protection Agency have maintained a voluntary surveillance system for the reporting of water-borne disease outbreaks.[24] The factors apparently resulting in these outbreaks include treatment deficiencies, use of untreated ground water, deficiencies in the distribution system, and use of untreated surface water. An increasingly important problem is the occurrence of outbreaks of water-borne disease associated with recreational water use, as for swimming or wading,[38,214,259] hot tubs and spas,[192,268] water parks,[59] and decorative fountains.[164] Diseases transmitted in this manner include shigellosis, cryptosporidiosis, norovirus gastroenteritis, viral conjunctivitis, giardiasis, legionellosis, enterohemorrhagic *E. coli* O157:H7 gastroenteritis, and *Pseudomonas* dermatitis.[326] Although cryptosporidiosis and giardiasis cases can occur sporadically, outbreaks of each disease are well documented. During the years 1991 to 2000, *Cryptosporidium* was identified as a causal agent of 38 percent (40 of 106) of reported cases of recreational water–associated outbreaks of gastroenteritis of known and suspected infectious etiology.[165] During the same period, *Giardia* was identified as a causal agent of 9.4 percent (10 of 106) of reported cases of recreational water–associated and 16 percent (21 of 130) of reported drinking water–associated outbreaks of gastroenteritis of known or suspected infectious etiology.[166] Deaths may occur, especially with cases of primary amebic meningoencephalitis caused by *Naegleria* infection in persons who recently have been swimming.[66]

MILK SANITATION

Milk has been demonstrated as the vehicle of transmission in outbreaks of brucellosis, tuberculosis, and diphtheria as well as in outbreaks of diseases caused by group A streptococci, *Salmonella*, *Shigella*, *Campylobacter*, *Listeria*, *Y. enterocolitica*, and staphylococcal toxin.[261] Although the quality of milk has improved steadily in the United States[246]—largely through mechanization and sanitation of milking operations, pasteurization, and cold storage—the use of raw milk as a "health food" continues to account for outbreaks of disease, particularly outbreaks caused by *Salmonella* and *Campylobacter*.[55,202] Children sometimes drink raw milk while visiting dairy farms on school field trips or other youth activities; a retrospective survey of state health departments identified 20 outbreaks involving a total of 458 cases of *Campylobacter* enteritis occurring as a result of such activities during the period 1981 to 1990.[322]

The quality of milk is monitored by a standard bacterial plate count, the phosphatase test (an assay for prior pasteurization), and measurement of the density of coliform organisms.[127] Nonetheless, pasteurized milk has been associated with outbreaks of enteric disease and listeriosis.[123,207] Both post-pasteurization contamination at the dairy[276] or in transit[158] and intrinsic bovine contamination with survival of organisms despite adequate pasteurization[248] have been suggested as possible mechanisms of transmission. The incidence of outbreaks of milk-borne disease has decreased greatly in the past 40 years in the United States. From 1993 to 1997, fewer than three milk-borne outbreaks per year were reported for the entire country.[247]

FOOD SANITATION

Foods other than milk have been shown to be the vehicle of transmission in numerous outbreaks caused by a wide range of bacteria, viruses (hepatitis A, noroviruses), and parasites, in addition to many naturally occurring toxins and man-made chemicals. Several reports of illness associated with tortillas have been published.[54] The incubation period is short (<24 hours), and the dominant symptoms are headache and nausea. These characteristics suggest an as yet unidentified preformed toxin or chemical agent. Rarely, the contamination is done with criminal intent.[36,309] Major public health programs in the United States involving inspection of meat and poultry, shellfish sanitation, and inspection of public eating and drinking places have had a significant impact, notably in reduction of the incidence of tuberculosis and brucellosis in cattle and limitation of spread of typhoid, hepatitis A, and vibriosis by contaminated shellfish. Significant problem areas remain, however, such as in schools. From 1973 through 1997, states and local health departments reported 604 outbreaks of food-borne disease in schools, with a median number of 25 outbreaks annually (range, 9 to 44). In 60 percent of the outbreaks, a cause was not determined; in 45 percent, a specific food vehicle of transmission was not determined. *Salmonella* was the pathogen most commonly identified, accounting for 36 percent of outbreak reports with a known etiology. The most commonly reported food preparation practices that contributed to these school-related outbreaks were improper food storage and holding temperatures and food contaminated by a food handler.[99]

Inherent contamination of meat, poultry, and eggs with *Salmonella*, *Yersinia*, Shiga toxin–producing *E. coli*, *Listeria*, and other pathogens and deficiencies in food storage and preparation both in eating establishments and in the home still contribute to outbreaks and sporadic cases.[20,200,282,293] Several outbreaks of *E. coli* O157:H7 infection and hemolytic-uremic syndrome have been related to apple juice,[93,162] lettuce,[266] spinach,[58] and other food items. In addition, new problems can result from changes in the food industry and in patterns of food consumption.[155,249] Since the 1970s, several large outbreaks of salmonellosis have been associated with the widespread distribution of fresh produce, a previously rare vehicle of *Salmonella* transmission.[56] These outbreaks have occurred after notable increases during the past 2 decades in the per-capita consumption of fresh fruits and vegetables in the United States.[288] Larger, centralized production and processing facilities with extensive distribution networks may increase the number of persons affected when commercial products do become contaminated. In a global economy, the importation of food from resource-poor countries is another source of contaminated food. For example, multistate outbreaks have been linked to the importation of foods, such as cyclosporiasis associated with raspberries from Guatemala[159] and strawberries from Mexico contaminated with hepatitis A virus.[173] Adequate cooking of foods, proper canning techniques (both commercial and home), and refrigeration are major contributors to control of outbreaks of food-borne disease. In approximately half of the outbreaks of food-borne disease, one or more food preparation practices are reported to be contributing factors; improper storage or holding temperature is reported in approximately two thirds of these outbreaks, and poor personal hygiene of the food handler in approximately two fifths.[247]

Although the number of outbreaks reported to public health authorities represents only a small fraction of those that occur, these reports can provide important insight into the epidemiology of food-borne disease. From 2000 to 2004, the number of outbreaks and the number of cases reported annually remained relatively constant (Table 257–3). The specific cause is confirmed in approximately 30 percent of all outbreaks, and of these, bacterial pathogens, particularly *Salmonella*, account for the majority. Other infectious etiologic agents include *Clostridium botulinum*, *Clostridium perfringens*, *Campylobacter*, *Shigella*, *Vibrio cholerae*, hepatitis A virus, *Giardia*, and *Trichinella spiralis*. From 2000 to 2004, *Salmonella enteritidis* remained the most commonly isolated cause of outbreaks of food-borne disease.

TABLE 257–3 Food-Borne Disease Outbreaks and Cases Reported in the United States, 2000-2004, by Etiology

	2000*	2001†	2002‡	2003§	2004¶
Total number of outbreaks/cases reported	1417/26,043	1238/25,035	1332/24,971	1072/22,791	1319/28,229
Total number of reported outbreaks/cases with a confirmed cause	448/14,112	445/13,945	494/16,117	409/15,569	519/16,372
Percent of all reported outbreaks with a confirmed cause	32	37	37	38	39
Number of outbreaks/cases caused by bacterial diseases	226/6528	235/7062	226/8356	196/8047	208/5269
Number of outbreaks/cases caused by *Salmonella*	112/2591	111/3205	109/4368	108/3963	121/2609
Percent of all bacterial outbreaks caused by *Salmonella*	50	47	48	55	58
Number of outbreaks/cases caused by chemical agents	37/185	52/223	46/272	54/415	47/153
Number of outbreaks/cases caused by viral diseases	176/7208	156/6451	205/6611	149/6505	251/9994
Number of outbreaks/cases caused by parasitic diseases	6/169	5/90	5/88	3/155	8/230

*http://www.cdc.gov/foodborneoutbreaks/us_outb/fbo2000/fbofinal2000.pdf.
†http://www.cdc.gov/foodborneoutbreaks/us_outb/fbo2001/2001linelists.pdf.
‡http://www.cdc.gov/foodborneoutbreaks/us_outb/fbo2002/2002linelist.pdf.
§http://www.cdc.gov/foodborneoutbreaks/us_outb/fbo2003/2003LineList.pdf.
¶http://www.cdc.gov/foodborneoutbreaks/us_outb/fbo2004/Outbreak_Linelist_Final_2004.pdf.

HEALTH INFORMATION FOR INTERNATIONAL TRAVEL

International travel, whether by child or adult, necessitates preparation beforehand to obtain the proper immunizations and contingency plans for the possibility of encountering unsanitary food or water during the travel.[18,121,239] Illnesses acquired abroad may not be manifested until return home, with consequent diagnostic difficulty.

Needs for active and passive immunization are covered elsewhere in this book and are summarized fully in two continuously updated CDC publications to which the reader is referred: *Health Information for International Travel*[47] (which can be purchased online at *http://wwwn.cdc.gov/travel/contentYellowBook.aspx* or from the Superintendent of Documents, U.S. Government Printing Office, Washington, DC 20402) and the *Morbidity and Mortality Weekly Report* (available at *http://www.cdc.gov/mmwr/*). The CDC travel Web site also is very helpful as a source of up-to-date information for travelers (*http://wwwn.cdc.gov/travel/default.aspx*).

Safe water may be found in many hotels in large cities throughout the world, but only water from adequately chlorinated sources can be considered truly safe. Where chlorinated water is not available, canned or bottled carbonated beverages and beverages made from boiled water (as well as beer or wine) may be safe; however, transmission of cholera by uncarbonated bottled mineral water has been described.[25] Ice made from untreated water may contaminate an otherwise safe beverage, either directly or by leaving contaminated water on the outside of the container. Although heat treatment by boiling vigorously for at least 1 minute is the most reliable method to render questionable water potable, chemical treatments also can be used.[46] Cloudy water should be strained through a clean cloth into a container to remove any sediment or floating matter before treatment with heat or iodine. Although chlorine has been used for chemical disinfection, its germicidal activity varies greatly with pH, temperature, and the organic content of the water to be purified, and therefore it is less reliable than iodine. Detailed information and illustrations can be found at the Web site of the U.S. Environmental Protection Agency.[113] Such information is important for coping with domestic natural disasters as well as foreign travel. More specific information on how to keep water safe is as follows:

Heat

1. Bring the water to a vigorous boil for 1 minute. Allow to cool at room temperature—do not add ice. At very high altitudes, for an extra margin of safety, boil for several minutes or use chemical disinfection.

2. Add a pinch of salt to each quart or pour the water from one clean container to another several times to aerate and improve the taste.

Chemicals

1. Tincture of iodine. Follow the manufacturer's directions. Let stand for at least 30 minutes.

2. Tetraglycine hydroperiodide tablets (can be purchased at pharmacies and sporting goods stores). The manufacturer's instructions should be followed. If the water is cloudy, the number of tablets should be doubled; if the water is extremely cold, an attempt should be made to warm the water, and the recommended contact time should be increased to achieve reliable disinfection.

3. Household bleach will kill some but not all types of disease-causing organisms that may be in water. If the water is cloudy, filter it through clean cloths or allow it to settle and draw off the clear water for disinfection. Add $^1/_8$ teaspoon (or 8 drops) of regular, unscented, liquid household bleach for each gallon of water, stir it well, and let it stand for 30 minutes before use. Store disinfected water in clean containers with covers.

Food should be selected carefully. In areas of the world where hygiene and sanitation are poor, the traveler should avoid intake of salads, uncooked vegetables, unpasteurized milk, and milk products such as cheese and should eat only fruits and vegetables that can be peeled by the traveler or have been cooked and are still hot.

Children younger than 2 years have a high risk of acquiring traveler's diarrhea.[257] Immediate medical attention should be sought for an infant or child with blood or mucus in the stool, fever with rigors, or persistent vomiting or diarrhea with dehydration. Because infants and small children are especially at risk for becoming dehydrated, parents need to be aware of the signs of dehydration and be prepared to use oral rehydration solutions (ORS) as a preventive measure while medical attention is being obtained. Replacement of fluid losses may be achieved best by the use of an ORS containing appropriate concentrations of electrolytes and glucose. Recent efforts to improve the efficacy of ORS have focused on solutions of reduced osmolarity (e.g., sodium ranges of 60 to 75 mEq/L and glucose ranges of 75 to 90 mmol/L). These solutions generally preserve the 1:1 molar ratio of sodium to glucose that is critical for efficient co-transport of sodium but present a lower osmolar load to the intestinal tract than does the original WHO ORS.[109] In a meta-analysis that evaluated the effects of reduced-osmolarity ORS in 15 randomized trials of nearly 2400 children, a reduced-osmolarity ORS was associated with less frequent use of unscheduled intravenous fluids and less vomiting. In addition, a statistically significant reduction in stool output was observed. The incidence of hyponatremia was not significantly elevated among children who received reduced-osmolarity ORS in these trials.[146]

Although prophylaxis with several different agents, including trimethoprim-sulfamethoxazole, doxycycline, fluoroquinolone antibiotics, and bismuth subsalicylate, may prevent some cases of traveler's diarrhea in adults, no drug has been shown to be both safe and effective for this purpose in children. When diarrhea is severe or associated with fever or bloody stools, self-treatment with a 3-day course of ciprofloxacin, levofloxacin, norfloxacin, or ofloxacin usually is recommended. One- and 2-day courses also may be effective.[294] Azithromycin is an alternative for travelers to areas with fluoroquinolone-resistant *Campylobacter*, such as Thailand, and for pregnant women, children, and those who do not respond to a fluoroquinolone within 48 hours. Rifaximin, a nonabsorbed oral antibiotic derived from rifampin, has been approved by the FDA for treatment of traveler's diarrhea caused by noninvasive strains of *E. coli* in patients 12 years of age or older. It has been available in Europe since 1987.[115,270] Iodoquinol is especially dangerous because of its association with subacute myelo-optic neuropathy. Administration of bismuth subsalicylate, as found in Pepto-Bismol or other generic products, may lead to salicylate toxicity.

VECTOR CONTROL

Techniques of environmental control of vectors responsible for the transmission of disease have included eradication of habitats for mosquito larvae, construction of rat-proof houses, and elimination of rodent habitats.[323] In some instances, dam construction, large-scale irrigation projects, and deforestation have led to increases in the density of vectors responsible for the trans-mission of schistosomiasis, onchocerciasis, and mosquito-borne diseases.

Chemical control of vectors, which seemed so attractive a few years ago, has been complicated by environmental contamination by undegraded chemicals and by the appearance of vectors resistant to the organochlorine insecticides (DDT, dieldrin), organophosphorus insecticides (malathion, fenitrothion), and carbonates (propoxur, carbaryl). The persistence of DDT and dieldrin in the environment has resulted in their being banned in some countries and their use being severely restricted in others. Ultra-low-volume spraying, as with malathion in the control of malaria, has permitted the use of minimal amounts of insecticide while still effecting a decrease in the incidence of disease over a considerable area.[190]

The rising incidence of tick-borne diseases, including ehrlichiosis and Lyme disease in certain parts of the United States, has led to an increased interest in methods for the control of ticks and their animal hosts. Area-wide spraying of pesticides in residential areas may reduce tick populations but also may pose health or environmental risks if these chemicals are applied improperly. Personal protection includes avoidance of vector-infested areas, wearing of protective clothing, application of insect repellents to skin (e.g., *N, N*-diethyl-*m*-toluamide, DEET) or to clothing (permethrin), and frequent inspection for and prompt removal of attached ticks. These individual preventive measures can be practiced in any circumstance where exposure to infected ticks may occur.[10]

The rising incidence of West Nile virus in the United States since its first detection in New York City in 1999 has led to new advances in repellant choices, even though repellants containing DEET or picaridin, when used as labeled, are still the most effective defenses.[272,306] Repellants containing oil of lemon eucalyptus provide protection similar to that of low concentrations of DEET. Two percent soybean oil also can be used for short (approximately $2^1/_2$ hours) periods of exposure.[8,48]

Experimental work with genetic and biologic control of vectors with insect growth regulators,[37] as with the use of larvivorous fish, chemosterilized male mosquitoes, and parasites that attack the vector, has had limited success.[10,35]

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CHAPTER 258

Infections in Daycare Environments

Ellen R. Wald

The ever-increasing number of young women in the work force has contributed to the large number of infants and toddlers receiving out-of-home care. Current estimates are that approximately 75 percent of the mothers of children younger than 5 years work outside the home and that 13 million children receive some form of child daycare. About half of these children receive care in their own homes provided by parents or relatives. Twenty percent receive care in family daycare, and 28 percent are cared for in daycare centers. As children grow older, families increasingly rely on center care and decrease the use of parental care.[24]

The term *daycare* is used to describe a variety of different options for the supervision of children in the absence of their parents. Types of daycare include (1) large daycare centers housed in nonresidential settings that provide care for at least seven children in one location (included here are situations in which the parent's employer is the sponsor of the daycare center, frequently at the site of employment, and also preschool and nursery schools in which childcare is provided in a structured, primarily educational setting); (2) daycare homes in which care is provided in a residential setting for six or fewer children; (3) before- and after-school care designed to bridge the gap between organized schools (e.g., preschool or public or private school) and parental departure for or return from work; and (4) cooperatives in which childcare is rendered by parents who alternate in caring for their own children as well as other enrolled children.

The quality of care provided to children attending various daycare facilities varies widely. Although daycare centers are licensed in most states, only 23 percent of children in out-of-home care are enrolled in these facilities.[205] State regulations vary from state to state; some are quite lenient in setting suggested ratios for staff to child, whereas others have no regulations governing group size. Even if impeccable regulations and well-trained caregivers were available, the regulations would not protect the vast majority of children who are in unlicensed facilities.

Many factors contribute to the transmission of infectious agents in the daycare setting (Table 258–1), the most important of which are host factors relating to the immunologic susceptibility of young children. Once infection is established, it is transmitted easily because of the natural tendency for intimacy in an age group that has not established acceptable toileting practices and is ignorant of basic hygienic practices. Respiratory and gastrointestinal pathogens frequently contaminate toys.

Caregivers may have received insufficient training in infection control practices. A lack of policy regarding immunization and health care screening of employees may contribute to the spread of infection. In addition, overcrowding, understaffing, and poorly designed physical environments foster transmission of infectious agents in daycare facilities. A lack of or inadequate number of handwashing facilities for both children and providers creates an almost insurmountable barrier to infection control.

TABLE 258–1 Risk Factors for Infectious Disease in Daycare

Children
Immunologic susceptibility to infectious agents
Lack of toilet training
Natural tendency to intimacy
Frequent oral contact with environment
Lack of awareness and practice of good hygiene
Caregivers
Insufficient training in infection control
Lack of policy regarding immunization
Inadequate screening for infectious diseases
Environmental and Economic Problems
Inappropriate staff-child ratios
Overcrowding
Failure to separate age groups
Poorly designed physical layout
Inadequate or poorly placed sinks
Failure to separate toilet areas from areas of food preparation
Parental pressure to admit sick children to daycare

Major risk factors for spread of infection are related to the age of the participants, the number of children, and the ratio of staff to children. The last is determined in part by the age of the children and the experience of the daycare provider. In overcrowded situations with inadequate staff, infection is spread easily because of inadequate washing of hands and attention to other facets of infection control.

Factors identified as particularly important in the spread of gastrointestinal organisms are large numbers of diaper-aged children in centers where staff members who diaper children are also responsible for food preparation.[15,77] Separating diaper-aged children from those who are older is important to limit spread of enteric illness. If meals are prepared in the daycare setting, separating this activity from toilet areas is essential. If food is brought from home, it should be transported properly and stored to avoid spoilage. Lack of attention to this issue may result in additional illness. Daycare centers that operate for profit also are at higher risk for having infectious disease outbreaks, probably as a result of lower staff-to-children ratios.[119] Finally, parents may not withdraw ill children from attendance at daycare voluntarily because of the expense and inconvenience of creating alternate childcare arrangements.

MODES OF TRANSMISSION OF INFECTIOUS DISEASES IN DAYCARE

The most important pathogens and their modes of transmission of infection in daycare are shown in Table 258–2. Respiratory infections are by far the most common causes of illness in infants, toddlers, and preschoolers, regardless of whether these children attend daycare.

RESPIRATORY

For some microbiologic species causing infection, the mode of transmission is the airborne route. The organism is aerosolized and remains in the air, like cigarette smoke. Direct contact with the infected individual is not necessary for spread of the infection. Illnesses known to be transmitted by this means include measles, varicella, and tuberculosis.

Most commonly, respiratory organisms are spread by the production of droplets laden with infective particles. These droplets may be transmitted directly from mucosa-to-mucosa when close physical contact occurs. More often, droplets land on nonporous surfaces (e.g., cribs, tables, chairs) or on clothes and paper (fomites) and remain infective for minutes to hours. Hand contact with contaminated surfaces and fomites can result in infection if the hands touch the nasal or conjunctival mucosa.[80] Agents that can be transmitted by droplet spread (mucosa to mucosa), from finger to mucosa, or by fomites include most respiratory viruses (respiratory syncytial virus [RSV], rhinovirus, human metapneumovirus, coronavirus, human herpes virus 6 [HHV-6], influenza virus, parainfluenza virus, adenovirus, parvovirus B19, measles virus, mumps virus, rubella virus, and varicella-zoster virus), *Haemophilus influenzae* type b (Hib), *Streptococcus pneumoniae*, *Neisseria meningitidis*, and *Streptococcus pyogenes*.[64,65,68,76,79,80] Finger-to-mucosa spread of respiratory pathogens is the most important and common mechanism for transmission of viral and bacterial infections.[87] Consequently, handwashing is an essential element of preventing spread of infection.

GASTROINTESTINAL

Spread of gastrointestinal organisms is by fecal-oral transmission. The number of organisms required to produce infection will determine whether infection occurs by person-to-person spread or whether a food or fluid intermediary is required. For example, rotavirus, *Giardia lamblia*, and *Shigella* spp. are transmitted readily in very small numbers of organisms on the hands (without obvious gross contamination) after person-to-person contact or by touching infected surfaces. In contrast, *Salmonella*, rarely a cause of diarrheal outbreaks in the daycare setting, requires large numbers of organisms to produce infection. Accordingly, an intermediary step of food or beverage contamination is required to allow organisms to replicate up to the necessary inocula.

Numerous studies have demonstrated fecal organisms on daycare center environmental surfaces with which infants and toddlers have had contact, as well as on the hands of care providers.[54,107,206,213] Contamination of the environment is highest when the daycare children are younger than 3 years old. This age predilection correlates with the number of children still wearing diapers.[207] Important pathogens, including fecal bacteria, rotavirus, hepatitis A virus (HAV), and *G. lamblia* cysts, are able to survive on environmental surfaces for periods ranging from hours to weeks.[35,114]

TABLE 258–2 Pathogens and Modes of Transmission of Infection in Daycare

Mode of Transmission	Bacteria	Viruses	Parasites
Respiratory	*Haemophilus influenzae* type b *Streptococcus pneumoniae* *Neisseria meningitidis* *Streptococcus pyogenes* *Bordetella pertussis* *Mycobacterium tuberculosis* *Kingella kingae*	Adenovirus Coronavirus Human metapneumovirus Influenza A and B Measles Mumps Parainfluenza Parvovirus B19 Respiratory syncytial Rhinovirus Rubella Varicella	
Fecal-oral	*Campylobacter* organisms *Clostridium difficile* *Escherichia coli* O157:H7 *Salmonella* organisms *Shigella* organisms	Enteroviruses Hepatitis A Adenovirus Astrovirus Calicivirus Enterovirus Rotavirus Astrovirus	*Cryptosporidium parvum* *Enterobius vermicularis* *Giardia lamblia*
Person-to-person by skin contact	*Streptococcus pyogenes* *Staphylococcus aureus*	Herpes simplex	*Pediculus capitis* *Sarcoptes scabei* *Trichophyton* species *Microsporum* species
Contact with blood, urine, or saliva		Cytomegalovirus Hepatitis B Herpes simplex Human immunodeficiency virus	

Adapted from American Academy of Pediatrics: Children in out-of-home child care. In *Pickering, L. (ed): 2006 Red Book: Report of the Committee on Infectious Diseases. 27th ed. Elk Grove Village, IL, American Academy of Pediatrics, 2006, p. 132.*

SKIN

Bacterial, viral, and parasitic infections of the skin can be transmitted by person-to-person spread by direct contact. Bacterial pathogens such as *S. pyogenes* and *Staphylococcus aureus* usually are not primarily invasive unless a break in the integument occurs, such as after minor trauma (e.g., insect bites). Herpes simplex virus may be transmitted from skin or mucosa to skin by direct contact, again only if the skin is broken. Infestations such as scabies and lice are transmitted by person-to-person spread by mobile parasites. The superficial dermatophytes responsible for tinea infections (*Trichophyton*, *Microsporum*, and *Epidermophyton*) are transmitted by person-to-person spread or by contact with infected fomites such as combs, hairbrushes, and hats.

BLOOD, URINE, AND SALIVA

Hepatitis B virus (HBV), hepatitis C virus (HCV), human immunodeficiency virus (HIV), and cytomegalovirus (CMV) can be transmitted by blood and sexual activity (presumably resulting in microtrauma, thereby leading to blood exposure). Transmission of HCV by sexual activity is an uncommon occurrence, however. Although both HBV and HIV can be demonstrated in urine and saliva, exchange of these body fluids is very unlikely to transmit infection. At daycare centers, transmission of CMV probably occurs between attendees by contamination of toys with saliva. Mothers and care providers can become infected with CMV by the finger-to-mucosa route after contamination of hands by urine or saliva.

PATTERNS OF INFECTION IN DAYCARE

Infections in children in daycare occur in several different patterns (Table 258–3).[73] Most of the increased infectious disease burden found in daycare centers is experienced by the children themselves. They have an excess of respiratory and gastrointestinal infections when compared with children who are in home care.[138,139,211] Certain specific infections, such as those caused by Hib, rotavirus, and RSV, are shouldered almost exclusively by the children. However, adult personnel working as providers occasionally experience some of the same respiratory and gastrointestinal infections as the children. Examples of infections for which daycare attendees and staff share an increased burden include shigellosis, giardiasis, and invasive meningococcal disease.

TABLE 258–3 Patterns of Occurrence of Infections Experienced in Daycare

Patterns of Occurrence	Examples
Clinical manifestation of infection primarily in children	Respiratory syncytial virus, rotavirus infections
Infection affecting children, daycare staff, and close family members	Shigellosis, giardiasis, *Neisseria meningitidis* infection
Inapparent infection in children with clinically important infection in adult contacts	Hepatitis A
Inapparent or mild infection in children and adults but with possibly serious consequences for fetus in pregnant contact	Cytomegalovirus infection, rubella, parvovirus B19 infection, coxsackievirus A16 infection

Adapted from Goodman, R. A., Osterholm, M. T., Granoff, D. M., et al.: Infectious diseases and child day care. Pediatrics 74:134-139, 1984. Copyright 1984, American Academy of Pediatrics.

Several infections barely recognizable in a daycare child (because these infections are mild or asymptomatic) cause significant repercussions when spread to adults, whether parents or daycare providers. The most notable of these diseases is hepatitis A, which in adults tends to be an illness of moderate severity and often leads to extended absences from work.

Finally, with some infections, clinical manifestations in adults are mild, but the infection may have potential effects on the fetus if the childcare provider or mother of a daycare attendee is pregnant. CMV and rubella virus are associated with severe birth defects if infection occurs in the first trimester. Infection with parvovirus B19 during pregnancy poses a risk for the development of nonimmune hydrops in the fetus.

INFECTIOUS AGENTS IN DAYCARE

INFECTIONS SPREAD BY THE RESPIRATORY ROUTE

Viral Upper Respiratory Infections

Respiratory viruses (rhinovirus, coronavirus, RSV, human metapneumovirus, parainfluenza virus, influenza virus, adenoviruses, HHV-6, and Epstein-Barr virus) are the most common causes of infection in preschool-age children.[17,50,65,216] The range of clinical manifestations includes asymptomatic infection, simple upper respiratory infection (rhinitis), acute otitis media, pharyngitis, croup, tracheitis, bronchiolitis, and pneumonitis. Disease may be mild or severe and involve a single level or more than one level of the respiratory tree. Children may experience multiple infections with each agent because of antigenic diversity within virus subtypes (e.g., influenza A virus), existence of multiple subtypes (e.g., rhinoviruses, coronaviruses), and failure of immunity to develop after a single exposure (e.g., RSV). Viruses are shed from the site of infection (conjunctiva, nose, throat), even before clinical symptoms develop, thereby rendering control of the spread of these infections in daycare difficult. Documentation that children in daycare (family or large daycare centers) experience more respiratory infections than those noted in children in home care is ample.[51,60,93,121-123,138,139,188,190,211] The risk of acquiring infection for children in group or family care is intermediate, between that for large daycare centers and home care.[211]

The most common bacterial complication of viral upper respiratory infection is acute otitis media. The peak age incidence of otitis media is between 6 and 18 months, similar to that of viral upper respiratory tract infections. Most children have had at least one episode of acute otitis media by the time they reach their second birthday, and approximately one third will have had at least three episodes by that time.[199] Not surprisingly, because the frequency of episodes of viral respiratory infections is increased in children attending daycare, these children experience a notable increase in the frequency of episodes of otitis media.[165,188,190,192,211] Two reviews strongly supported the notion that attendance at daycare is a major risk factor for acquiring acute otitis media (Table 258–4).[171,204] Wald and colleagues[211] provided data indicating that the risk of hospitalization for performance of myringotomy with tube placement was highest for children in large daycare environments. Similarly, the risk of contracting recurrent acute otitis media and persistent middle ear effusion also is higher for children in daycare than for those who receive care at home.[43,58,189,202] Researchers do not recommend that children with mild respiratory infections be excluded from daycare or be separated from the group of well children because this strategy has not been shown to achieve an overall reduction in infections for children in daycare facilities.

TABLE 258–4 Comparisons of Annualized Rates of Acute Respiratory Tract Infections, Otitis Media, and Antibiotic Treatment among Children in Various Modes of Childcare

Study	Length of Time Observed	Annual Rate of Respiratory Infection (per Child-Year)	Annual Rate of Otitis Media (per Child-Year)	Annual Rate of Antibiotic Treatment (per Child-Year)
Wald et al.[211]*				
DCC (n = 33)	12-18 mo	6.3		
DCH (n = 23)	12-18 mo	5.1		
HC (n = 97)	12-18 mo	3.9		
Strangert[192]*†				
DCC (n = 108)	8 mo	7.5	1.2	1.9
DCH (n = 42)	8 mo	7.5	1.3	1.6
HC (n = 57)	8 mo	3.0	0.5	0.8
Stahlberg[188‡]				
DCC (n = 23)	8 wk	13.8	2.3	4.8
DCH (n = 23)	8 wk	12.0	0.9	2.8
HC (n = 23)	8 wk	9.9	1.1	1.4
Reves and Jones[165§]				
DCC (n = 42)	8 wk	8.1	6.0	9.1
DCH (n = 72)	8 wk	2.6	1.6	2.7
HC (n = 156)	8 wk	3.0	1.6	2.5

**Telephone interview every 2 weeks.*
†Active surveillance in DCC; telephone call every 4 months for children in DCH and HC.
‡Daily diary in all groups.
§Health maintenance organization abstraction covering an 8-week period for each child.
DCC, daycare center; DCH, daycare home; HC, home care.
Modified from Reves, R. R., and Jones, J. A.: Antibiotic use and resistance patterns in day care centers. Semin. Pediatr. Infect. Dis. 1:*212-221, 1990.*

TABLE 258–5 Excretion of Cytomegalovirus in Urine from Children in Daycare

Study	No. of Daycare Centers Studied	Age of Children Studied (mo)	No. of Children Studied	No. (%) with CMV in Urine
Strangert[191,192]	1	21-30	10	7 (70)
	13	6-36	40	9 (23)
Strom[193]	1	24-36	18	13 (72)
Pass et al.[150]	1	3-12	11	1 (9)
		13-24	18	15 (83)
		25-36	16	10 (63)
		36-60	25	10 (40)
Pass et al.[150]	1	0-60	103	59 (57)
Hutto et al.[95]	3	<12	10	0 (0)
		12-24	38	14 (37)
		25-36	35	27 (77)
		38-48+	143	36 (25)
Jones et al.[104]	11	<12	33	8 (21)
		12-24	51	15 (29)
		25-48	52	9 (17)
Adler[1]	1	0-24	31	8 (25)
		24-60	34	7 (20)
Murph et al.[134]	1	0-9	8	2 (25)
		12-24	12	8 (67)
		25-36	14	4 (29)
		37-48+	39	3 (8)

CMV, cytomegalovirus.

Systemic Viral Infections

CYTOMEGALOVIRUS

CMV is a common cause of infection in preschool-age children. Most often, the infection is completely asymptomatic.[94] Rarely, the child may experience a febrile illness with lymphadenopathy and hepatosplenomegaly. Sources of CMV in the daycare setting are infants and children who have been infected by their mothers by vertical transmission in utero (1% to 2%), perinatally (2% to 3%), or post partum through breast-feeding (≈6%). Strangert[190] reported the isolation of CMV from 7 of 10 children between 21 and 30 months of age in one daycare center. Strom[193] found that 13 of 18 (72%) children between 24 and 36 months of age in a single nursery excreted CMV. Children younger than 3 years of age who acquire CMV after birth excrete CMV in urine and saliva for 6 to 42 months (mean, 18 months).[5] Other investigators demonstrated that peak rates of infection occur in children between 1 and 3 years of age, when viral excretion may be documented in as many as 70 percent of children in daycare, as shown in Table 258–5.[1,95,104,134,150,152]

Infection in preschool-age children leads to viral shedding documented by positive CMV cultures obtained from swabs of the throat (thereby contaminating saliva) and urine. Transmission probably occurs through fomites (toys and blankets) contaminated with saliva, rather than by respiratory droplets.[186] Shedding of CMV is as chronic among infected toddlers in daycare centers as it is in children with perinatal or congenital infection.[5,151,152]

CMV is a problem if acquisition of infection occurs during early pregnancy in daycare providers or mothers of children attending daycare.[1-4] Such acquisition may lead to clinically evident congenital CMV infection (microcephaly, hepatosplenomegaly, chorioretinitis, psychomotor retardation, and deafness) in approximately 5 percent of infected children. Another 10 to 15 percent of infants experience occult, but potentially damaging, infection that results in milder degrees of hearing loss and learning disabilities.[187]

PARVOVIRUS B19

Parvovirus B19, the etiologic agent of erythema infectiosum, or fifth disease, is spread by the respiratory route. Viral replication, viremia, and nasopharyngeal shedding occur approximately 1 week before the development of clinical symptoms. This benign disease of childhood (in a normal host) may occur in preschool- and school-age children. The clinical illness is characterized by an erythematous rash on the face that gives a "slapped-cheek" appearance.[113] Patients usually do not have fever or other constitutional symptoms. The rash progresses after the first day and spreads as a maculopapular eruption beginning on the proximal ends of the extremities and extending to the distal portions and trunk. After several days, these lesions develop into a lacy reticular pattern on the proximal parts of the extremities and then fade. Unrecognized in immunocompetent hosts is an infection of the erythrocyte precursor that leads to transient red blood cell aplasia.

In adults, parvovirus B19 infection frequently causes arthralgia and arthritis. Its clinical importance in the context of daycare infections is transmission from an infected preschool-age child to a pregnant mother or daycare provider.[69] In a large outbreak of erythema infectiosum in Connecticut, 19 percent of susceptible adult school personnel became infected; the highest rates of seroconversion were observed in personnel in contact with large numbers of young children. Infection of the fetus may lead to nonimmune hydrops secondary to infection of erythrocyte progenitor cells. Estimates of fetal loss when a pregnant woman of unknown antibody status is exposed to parvovirus are 2.5 percent after household exposure and 1.5 percent after occupational exposure in a school or daycare facility.[13]

COXSACKIEVIRUS A16

Epidemics of infection with coxsackievirus A16 cause hand, foot, and mouth disease, a clinical syndrome characterized by a vesicular exanthem on the distal parts of the extremities and mild stomatitis. Two outbreaks have been reported in children attending daycare.[56,57] The disease is mild in children but has a high attack rate. Its importance relates to the possibility of infection developing during pregnancy, which may lead to spontaneous abortion.[57,143]

Local Bacterial Infections

STREPTOCOCCUS PYOGENES OR GROUP A STREPTOCOCCI

Group A streptococci (GAS) are a frequent cause of respiratory and skin infections. The most common expression of infection with GAS is the development of pharyngitis and fever in an elementary school-age child. Usually, the throat infection is accompanied by tender anterior cervical nodes. In classic infection, none of the usual signs of viral upper respiratory disease—coryza, cough, and conjunctivitis—are present. Illness peaks in the late winter and spring. In contrast, streptococcal infection of a preschool-age child or child in daycare takes the form of a protracted upper respiratory infection. Specifically, these patients have low-grade fever, persistent nasal discharge, anorexia, and cervical adenopathy.

Although exposure of daycare children to an index case of streptococcal pharyngitis can be assumed to result in secondary cases, relatively few epidemics of GAS infection have been reported in daycare facilities.[55,91,184] An outbreak occurred in a daycare center in which preschool children shared facilities with kindergarten children in an after-school program.[184] During a 3-month period, 47 percent of the daycare population had positive cultures for GAS or positive results on a rapid antigen-detection test. Outbreaks of scarlet fever and perianal cellulitis each have been reported from daycare centers.[133] A cluster of 12 cases of perianal cellulitis, characterized by a well-demarcated rash around the anus with itching, rectal pain, blood-streaked stools, and occasionally a purulent discharge, was reported from a kindergarten setting in Denmark.[154] Children with GAS infections of the throat or skin should be excluded from daycare for 24 hours after the initiation of appropriate antibiotic treatment.

Invasive Bacterial Diseases

HAEMOPHILUS INFLUENZAE TYPE b

Hib used to be the major bacterial pathogen of childhood and was responsible for meningitis, epiglottitis, pneumonia, facial cellulitis, and septic arthritis. These infections occurred in children between 2 months and 5 years of age, with a peak occurring in those between 6 and 18 months of age. The organism colonizes the nasopharynx before becoming bloodborne; hematogenous dissemination results in a distant focus of infection. Infected children usually have a several-day history of mild upper respiratory symptoms followed by an abrupt onset of high fever and localized symptoms indicating the site of infection (e.g., irritability or seizures in central nervous system infection, cutaneous findings in cellulitis). Colonized individuals who lack anticapsular antibody are susceptible to the development of invasive disease.[75]

In the 1980s, considerable debate ensued regarding whether children in daycare had a higher risk for having primary episodes of infection with Hib than did those in home care. The risk that primary disease will develop in an individual child is defined as the risk of Hib disease in that child in the absence of known contact with another case in the previous 60 days. Many Hib infections have occurred in children attending daycare centers,[34,61,71,98,136,163,196] with estimates of 28 to 40 percent in several studies.[98,163] However, the presence and magnitude of the increased risk have shown significant variation that was dependent on the age of the child, the type and size of the daycare facility, and the geographic location of the facility. In general, studies have found that the risk of contracting primary Hib infection is highest in younger children (≤23 months of age) during their first month of enrollment in daycare and in children attending a larger daycare center, as opposed to a daycare home.[98,163]

An additional concern was whether the risk of development of secondary cases of Hib infection in the daycare setting was significantly elevated. Although several studies substantiated an increased risk for secondary cases of invasive Hib infection in daycare attendees exposed to another attendee infected with Hib,[14,60,124] others did not.[135,147] Based on available data,[14,60,124,135,147] the risk that secondary disease will develop in daycare classroom contacts of a child with invasive Hib probably was higher than

that in children of similar age in the general population; however, the magnitude was not well defined and depended on the characteristics and geographic location of the daycare center. Management of daycare attendees younger than 2 years who were exposed to an individual with invasive Hib infection was controversial.[45]

The availability and universal use (in infancy) of effective vaccines for the prevention of Hib infection have changed the epidemiology of infections caused by this pathogen dramatically.[181] Schulte and coworkers[176] reviewed the pattern of invasive Hib infection in New York State. These investigators demonstrated a dramatic decline in the incidence of Hib disease in children younger than 5 years old, both those in daycare and those in home care. The decrease in cases of infection attributable to Hib occurred in children attending daycare centers before it was observed in non-daycare attendees, perhaps because of the requirement for Hib vaccine before enrollment in daycare. Rarely, cases are reported among fully vaccinated children in a daycare center.[129] The virtual disappearance of this once common and serious problem and the high immunization rates with Hib vaccine obviate a lengthy discussion regarding the need for rifampin prophylaxis after exposure to cases of invasive disease. In the rare instance of a case of invasive Hib disease occurring in a family daycare center housing other susceptible children (<4 years of age), all daycare contacts (adults and children) should receive rifampin, 20 mg/kg/day once daily for 4 days, to a maximum dose of 600 mg.

NEISSERIA MENINGITIDIS

N. meningitidis is a gram-negative, polysaccharide-encapsulated diplococcus found colonizing the nasopharynx. It is the second leading cause of meningitis in the United States. Other manifestations of infection include pericarditis, sepsis, and pneumonia. Similar to that in patients experiencing infection with Hib, the attack rate is highest in children 2 months to 5 years of age, with a peak in children between 6 and 12 months of age. The pathogenesis is hematogenous dissemination from the nasopharynx as a site of colonization. Household contacts exposed to patients with meningococcal disease have a significantly higher risk of acquiring that disease than does the general population.[46,130,132]

Secondary spread of infection with *N. meningitidis* has been recognized as a potential risk, especially in situations of crowding such as in military barracks and college dormitories. In addition, reports suggest that the risk of acquiring secondary disease caused by *N. meningitidis* is increased in the daycare setting.[44,101,117,142,172] In a Belgian report,[49] exposure in a daycare nursery during a prolonged meningococcal epidemic conferred a risk for infection that was 76 times greater than that in children of similar age who received care at home. Prompt institution of rifampin prophylaxis is the recommended strategy for management of all intimate child contacts of a person with invasive *N. meningitidis* disease, to prevent development of secondary or associated illness. A single oral dose of ciprofloxacin (500 mg) or an intramuscular dose of ceftriaxone (250 mg) is an alternative for adult contacts. Physicians do not recommend that throat cultures be performed to identify those who require prophylaxis. The cultures may be insensitive, and waiting for results will delay the administration of appropriate management.

STREPTOCOCCUS PNEUMONIAE

Pneumococci are the most common cause of bacterial infections of the upper and lower respiratory tracts, including otitis media, sinusitis, and pneumonia.[177] As respiratory pathogens, they also cause other upper respiratory tract infections, such as conjunctivitis, and important systemic illnesses, including occult bacteremia, bacteremic periorbital cellulitis, and meningitis. Multiple antibiotic–resistant *S. pneumoniae*, a problem that began in the 1960s, is now widespread in South Africa, in large parts of Europe, and, since 1990, in the United States.

Although *S. pneumoniae* has been the most common cause of otitis media and otitis media is the most common infection caused by *S. pneumoniae*, the rarity of performing tympanocentesis (despite the frequency of otitis media in children in daycare) explains the delayed appreciation that *S. pneumoniae* is spread easily in the daycare setting. The ease of transmission of penicillin-resistant *S. pneumoniae* (tracked by antibiotic resistance patterns, capsular types, and genetic patterns) in the daycare setting now has been demonstrated amply.[52,86,160,161] In addition, although the increased risk of the acquisition by children of secondary cases of pneumococcal disease in daycare versus home care is unknown, several outbreaks of invasive *S. pneumoniae* disease in daycare centers have brought greater attention to this issue.[38,161] A study of children in an Ohio daycare center[164] showed that prophylactic doses of antibiotics and frequent use of antibiotics were risk factors for nasopharyngeal carriage of antibiotic-resistant *S. pneumoniae*. A cluster of cases of invasive pneumococcal disease occurring in young children in childcare[31] involved a 12F serotype of pneumococcus that was penicillin-sensitive; three of six children in the daycare facility experienced bacteremic infections. In a more recent outbreak of disease, three cases of meningitis caused by multidrug-resistant serotype 14 *S. pneumoniae* occurred at a daycare center over a period of 5 days.[38]

Several reports claim that rifampin is ineffective or only partially effective in eradicating carriage of *S. pneumoniae*.[161,164] However, this finding may be explained by inadequacy of the dose of rifampin,[161] lack of compliance with drug administration, or inappropriate timing for test-of-cure cultures.

A pneumococcal conjugate vaccine containing seven common serotypes of *S. pneumoniae* (PCV7) was licensed in March of 2000. The vaccine is administered to children at 2, 4, and 6 months of age and again when they are between 12 and 15 months old. In a large field trial conducted at the Kaiser Permanente Vaccine Study Center, the vaccine performed very well. The trial achieved a 93 percent reduction in invasive disease (meningitis and bacteremia), a 73 percent reduction in consolidative pneumonia, and a 7 percent reduction in episodes of otitis media.[21] Universal use of the vaccine has reduced the most serious manifestations of infection with *S. pneumoniae* substantially.[9] In addition, use of PCV7 and other pneumococcal conjugate vaccines has been shown to reduce nasopharyngeal colonization with the serotypes of *S. pneumoniae* contained in the vaccine, including the carriage of antibiotic-resistant *S. pneumoniae*.[41,42] This secondary effect of the conjugate vaccines has resulted in a decreased number of instances of colonization and disease among the contacts of vaccinees.[72]

KINGELLA KINGAE

Kingella kingae is a fastidious gram-negative coccobacillus that colonizes the oropharynx and is becoming increasingly recognized as an important cause of skeletal infections in young children (<2 years of age). An outbreak of skeletal infections in a childcare center in Minnesota involving two confirmed and one possible case of osteomyelitis and septic arthritis in the same toddler classroom was described.[109] In addition to causing osteomyelitis and septic arthritis, which are the major manifestations of disease, *K. kingae* also causes bacteremia and endocarditis.[219]

MYCOBACTERIUM TUBERCULOSIS

The number of reported cases of tuberculosis has been on the decline in the United States since 1992 and includes a decrease in the number of infected children. In general, children with tuberculosis are not infectious. Rather, an adult in the environ-

ment invariably has active disease. Three clusters of cases of tuberculosis in children have been traced to attendance at daycare.[106,118,140] Screening of all daycare personnel, both staff and volunteers, with tuberculin skin testing is essential to eliminate this problem. One case of tuberculosis in a 9-year-old boy resulted in positive tuberculin skin tests in 79 percent of his classroom contacts and 16 percent of his daycare contacts.[40]

INFECTIONS OF THE GASTROINTESTINAL TRACT

Giardia lamblia

G. lamblia is one of the most common causes of diarrhea in the daycare setting.[20,162,178] Infection can be caused by the ingestion of as few as 10 cysts. Transmission most commonly results from person-to-person spread, although waterborne outbreaks have been documented.[108] Parents of daycare attendees are at risk for acquiring infection.[157] Demonstration of *G. lamblia* cysts on environmental surfaces indicates additional potential for transmission.[35] Infection with this parasite causes infestation of the duodenum and proximal jejunum and results in asymptomatic carriage more often than clinical disease.[97] After an incubation period of approximately 2 weeks, symptomatic patients experience diarrhea, intermittent abdominal pain, anorexia, and flatulence. The most notable feature of the infection is its tendency to become protracted, thereby leading to weight loss, failure to thrive, and anemia. The diagnosis is made by recovery of the organism from stool or occasionally by examination of duodenal aspirates or intestinal biopsy specimens.[102,200] After resolution of the symptoms, either by virtue of treatment or by spontaneous cure, patients may continue to shed cysts for a very long time.[97] Treatment is not recommended for asymptomatic individuals shedding *G. lamblia* cysts. For symptomatic individuals, treatment may be undertaken with metronidazole, furazolidone, nitazoxanide, or quinacrine. Relapses occur in approximately 15 percent of patients.

Cryptosporidium

Cryptosporidium is a cause of severe diarrhea in immunocompromised hosts, but infection usually results in self-limited illness in immunocompetent children and adults.[37] The parasite may be spread by person-to-person spread or through food or water. The usual clinical symptoms are watery diarrhea and low-grade fever with abdominal pain and weight loss. Vomiting occurs in approximately 30 percent of patients.

Cryptosporidium, like *G. lamblia*, has a very low infectious dose for humans. Ninety percent of infant nonhuman primates will become infected with 10 to 50 oocysts.[131] Spread of infection is facilitated in daycare centers because oocysts shed in the feces of infected persons are highly resistant to common disinfectants. Many outbreaks of diarrhea caused by *Cryptosporidium* have been reported from daycare centers.[7,8,36,39,85,137,198] Asymptomatic children and adults shed oocysts for weeks after infection.[34]

The diagnosis is made by examination of stool for oocysts with special stains. Recommended treatment is a 3-day course of nitazoxanide. Exclusion from daycare is recommended until the patient is asymptomatic.

Shigella

Infection with *Shigella* organisms causes illness of variable severity but easy transmissibility. Accordingly, *Shigella* is one of the most common causes of diarrhea outbreaks in the daycare population,[25,27,68,155] and *Shigella* infection was among the first enteric diseases recognized to be spread in daycare centers. Infection can be caused by as few as 10 to 100 organisms. Although a low inoculum is sufficient to cause infection, the organism does not survive well outside the human host. Person-to-person transmission is considered more important than environmental contamination. Although infection with *Shigella* may be caused by one of four species (*Shigella sonnei*, *Shigella flexneri*, *Shigella dysenteriae*, and *Shigella boydii*), *S. sonnei* and *S. flexneri* are the most common.

Shigellosis is primarily a disease of young children. In outbreaks, younger children have the highest attack rate and are the most effective transmitters of infection.[201] Daycare personnel and family contacts also experience high attack rates.[90,197] After an incubation period of several days, fever and watery diarrhea, followed by crampy abdominal pain, tenesmus, and mucoid bloody stools, occur in patients with classic disease. In many cases, the illness is mild and self-limited and is indistinguishable from other causes of gastroenteritis. The presence of fecal leukocytes on examination of stool provides supportive information; stool culture is diagnostic.

Treatment of shigellosis with an appropriate antimicrobial effectively terminates the illness. Antimicrobial treatment decreases the duration of symptoms and fecal shedding. Untreated persons continue to excrete organisms for several weeks. Susceptibility varies both within and outside the United States, and, therefore, testing of stool isolates is indicated.

Campylobacter jejuni

Campylobacter jejuni is the most common cause of bacterial diarrhea in children in the United States, but it is less likely than *Shigella* to cause outbreaks of diarrheal disease in daycare centers.[99,115,149] Most often, a high inoculum of *Campylobacter* is required to produce infection, thereby necessitating a food or water vehicle. Less often, transmission can occur by person-to-person spread of smaller numbers of organisms. The clinical findings are variable, but the usual onset includes fever, abdominal pain, and diarrhea. Many cases resemble classic shigellosis. Watery diarrhea and low-grade fever with only modest constitutional signs of illness also may be observed. The illness usually lasts between 3 and 7 days. The diagnosis can be made by culture of stool; examination of stool by darkfield microscopy or direct smear may be very informative if performed by an experienced observer.

Treatment with erythromycin and azithromycin is effective in terminating excretion of the infective organism, which may be important in controlling epidemics or outbreaks. The impact of macrolides on the clinical course of disease is variable.

Salmonella

Salmonella is the most common cause of bacterial diarrhea in many parts of the United States, but it is an uncommon cause of gastroenteritis outbreaks in daycare centers.[120] Because the organism is widespread in nature and has 2200 different serotypes, it has been very difficult to control. Infection usually occurs after the ingestion of contaminated food or beverages. Person-to-person spread seldom occurs, except in infancy, when the infective dose is low.[217]

The most common expression of infection with *Salmonella* is uncomplicated gastroenteritis. The illness begins approximately 12 to 36 hours after exposure and often is characterized initially by vomiting and subsequently by diarrhea. Abdominal pain and fever are frequent accompaniments; occasionally, stools contain mucus and blood. Although most cases are self-limited, *Salmonella* infection can be distinguished from other causes of gastroenteritis by its occasionally protracted course. The diagnosis is made by culture of stool. In general, antimicrobials are not recommended unless bacteremia is documented or the episode becomes protracted and severe. Management of special

hosts, such as neonates and immunocompromised patients, is controversial. Although some authors recommend antimicrobial therapy for these groups, few data support this recommendation. Exclusion from daycare is recommended until the diarrhea resolves. Once stools are normal, little reason exists to restrict attendance. Obtaining stool cultures from daycare contacts or the index case in convalescence is not necessary.[201] A very high rate of asymptomatic colonization with *Salmonella* of multiple serotypes has been documented in daycare centers in Mexico.[141]

Clostridium difficile

Clostridium difficile is the classic cause of pseudomembranous colitis in patients who have received or are receiving antimicrobial agents, and it rarely causes disease unassociated with antimicrobial use. The organism, a gram-positive, spore-forming rod, is distributed widely in soil and in the gastrointestinal tract of humans. It frequently is found colonizing asymptomatic newborns. Disease is a consequence of the elaboration of toxin or toxins by vegetative organisms. Hospital environments and daycare facilities are reservoirs for the organism.[110,111] During outbreaks of *C. difficile* diarrhea in daycare centers, environmental contamination is increased, as is recovery of *C. difficile* from the hands of children and staff.[111]

Clinical symptoms include fever, diarrhea, and abdominal cramps. Stools may contain blood and mucus. The illness varies in severity from mild to life-threatening. The diagnosis of *C. difficile* gastroenteritis is made by recovery of the toxin from a stool sample. Treatment includes cessation of antibiotics and supportive care. Specific antimicrobial therapy with oral vancomycin or metronidazole may be necessary to achieve clinical cure. Metronidazole is favored in an era of concern regarding the development of vancomycin-resistant enterococci after the use of oral vancomycin. Children and personnel should be excluded from daycare until they are asymptomatic.

Escherichia coli

Several outbreaks of diarrhea caused by various *Escherichia coli* strains have been associated with significant morbidity and mortality in the childcare setting. In 1986, an outbreak of diarrhea caused by *E. coli* O157:H7 was reported from a daycare attended by 107 children.[185] Thirty-four percent of attendees became ill, with a significant increase in risk for younger children. Approximately one third of the children with diarrhea had bloody stools, and hemolytic-uremic syndrome developed in three children. Although infection with *E. coli* O157:H7 usually occurs after the ingestion of contaminated beef, person-to-person spread of infection most likely occurred in this epidemic. The diarrheal illness also was documented in family members of ill children. Subsequent epidemiologic studies in Minnesota confirmed the mode of transmission for *E. coli* O157:H7 to be person-to-person in the daycare setting.[18] Accordingly, symptomatic children should be excluded from daycare until stools are formed and culture-negative for *E. coli* O157:H7. Unfortunately, fecal shedding of *E. coli* O157:H7 may be quite prolonged in young children.[195]

E. coli O157:H7 is recovered from stool samples after being plated on MacConkey-sorbitol agar. Sorbitol-negative colonies of *E. coli* are picked and screened with O157 antisera by tube agglutination. Several sensitive, specific, and rapid immunologic assays for detection of Shiga toxin are available commercially. Antibiotic treatment is not recommended for persons with diarrhea caused by *E. coli* O157:H7.[218] Hemolytic-uremic syndrome develops in approximately 5 to 10 percent of children infected with *E. coli* O157:H7.

Outbreaks of diarrhea caused by enteropathogenic *E. coli* in the childcare setting have been characterized by chronic, often relapsing diarrhea in infants and toddlers.[23,153] The diarrheal illness has had a high attack rate (56% to 90%) and has led to prolonged hospitalization in 8 to 30 percent of affected children.

Rotavirus

Rotavirus is the most common etiologic agent of gastroenteritis in infants and children and the leading cause of hospitalization for gastroenteritis in industrialized countries, including the United States.[22,89,125] It is an important cause of diarrhea in developing countries as well and contributes substantially to the worldwide mortality figures for gastroenteritis. It is the pathogen most commonly present in daycare settings.[47]

Rotavirus frequently is recovered from asymptomatic hosts, especially neonates and infants.[30] It is shed in large numbers in the stool of symptomatic patients, thereby contributing to the high prevalence of the virus on environmental surfaces during outbreaks.[215] Viral shedding occurs both before and after symptoms have appeared.[156] Only a low inoculum is needed to cause infection.

Most illnesses caused by rotavirus occur in the winter in temperate climates, but they occur year-round in tropical areas. The peak attack rate is in the 6- to 24-month-old age group. After a brief incubation period of 2 to 3 days, patients may have prodromal respiratory symptoms (coryza and cough) and then varying combinations of vomiting, diarrhea, and low-grade fever that last 3 to 5 days. The range of severity is broad, and dehydration occasionally may be profound. Spread of rotavirus infection is extremely common in hospitals.[169] Not surprisingly, transmission within a daycare center is very rapid, and many outbreaks have been reported in this setting.[16,145,146,156,168] In prospective studies of diarrheal illness in children attending daycare, rotavirus was implicated in 6 to 24 percent of cases of gastroenteritis,[15,88,194] as well as in 20 to 40 percent of outbreaks.[16] Daycare workers and family contacts are at risk for acquiring rotavirus infection.

Rotavirus cannot be cultivated easily in tissue culture in a viral diagnostic laboratory. The diagnosis is made with an antigen-detection method on a stool specimen. Treatment is supportive, and exclusion from daycare is recommended until the diarrhea resolves.

In early 2006, a bovine rotavirus-based pentavalent rotavirus vaccine (Rota-Teq) was licensed for use in U.S. infants. Three doses of the vaccine are given orally at 2, 4, and 6 months of age.[12] In 2008, a monovalent attenuated human rotavirus (Rota Rix) was licensed for a two-dose schedule given orally at 2 and 4 months. Both have been shown to be effective against rotavirus of any severity.

Norovirus

Noroviruses are nonenveloped RNA viruses that have a worldwide distribution and are a major cause of sporadic and epidemic gastroenteritis. Transmission is by person-to-person spread through the fecal-oral route or through contaminated food or water. Outbreaks frequently are observed in closed populations, including childcare centers and on cruise ships. The attack rate usually is high. Two reports highlighted the transmissibility of this agent in the childcare setting.[74,96]

Astroviruses

In addition to rotavirus and noroviruses, astroviruses are common causes of diarrheal outbreaks in daycare[6,183] and other closed populations. Children younger than age 4 years of age are infected most commonly, and the illness has a winter predominance similar to that caused by rotaviruses. Transmission usually is by person-to-person spread by the fecal-oral route. This illness is indistinguishable from the other viral causes of gastroenteritis.

Hepatitis A Virus

HAV, an enterovirus, is the most common cause of acute hepatitis in children. As with other enteric pathogens, this organism is transmitted by the fecal-oral route. Manifestations of infection vary remarkably according to age. In young children (<6 years), infection with HAV may be entirely asymptomatic or associated with relatively mild and nonspecific symptoms such as low-grade fever, anorexia, nausea, vomiting, and diarrhea in 30 percent of patients.[11] Jaundice, a more specific marker of liver disease, occurs in fewer than 10 percent of children younger than 6 years.[70] In contrast, adults with hepatitis A often are icteric in conjunction with other gastrointestinal symptoms. Infection, when symptomatic, usually lasts several weeks but occasionally can become protracted.

HAV is shed in high density in the stool of infected persons from 2 weeks before until 1 week after the onset of clinical symptoms. Transmission is primarily by person-to-person spread, but fomites may play an important role because the organism can persist and remain infective in the dried state for months.[126] The diagnosis usually is made by detecting serologic evidence of marker antibodies against HAV (immunoglobulin M anti-HAV). Treatment is symptomatic. Prevention of illness in contacts can be accomplished with intramuscular immune serum globulin and, more recently, with hepatitis A vaccine.[214] A study conducted among children residing in a Hasidic Jewish community in New York State demonstrated the efficacy of inactivated hepatitis A vaccine in preventing hepatitis A during 7 months of follow-up after vaccination.[148]

HAV can be spread easily in daycare centers with diaper-aged children because of the facility of person-to-person transmission.[78] Spread of infection is barely noticeable until symptomatic HAV infection develops in an adult contact (usually a parent or daycare worker). Other contacts, including siblings, extended family members, and babysitters, also are affected frequently. Cases of hepatitis A in communities have been associated with direct and indirect contact with child daycare settings.[209] The risk of a hepatitis outbreak occurring in a daycare center has been shown to be related directly to the number and age of children in attendance and the hours that the center is open. Larger centers with longer hours and diaper-aged children have the highest rates of infection and greatest incidence of spread to the community.[77] Hepatitis A vaccine is now recommended for all children at 1 year of age (i.e., 12 to 23 months).[11] Integration of this vaccine into the routine childhood immunization schedule will reduce the risk of transmission of HAV in daycare dramatically.

INFECTIONS SPREAD BY SKIN CONTACT

Group A Streptococci

GAS have become a less common cause of impetigo and pyoderma in children during the last decade, with a concomitant rise in the number of cases caused by *S. aureus.* Nonetheless, these organisms still can cause superficial infection of traumatized skin (insect bites, scratches), cellulitis, and erysipelas. The latter infections can result in an abrupt onset of fever and dramatic cutaneous erythema and tenderness often accompanied by regional adenopathy. An outbreak of perianal *S. pyogenes* infection in a daycare center has been reported. Typical signs and symptoms include perianal inflammation, itching, rectal pain, and blood-streaked stool. Four documented cases in three children and two presumed cases were treated without obtaining specific cultures.[175] The diagnosis of streptococcal skin infection can be made by careful performance of wound or surface cultures (obtained after carefully cleansing the periwound area) or examination of tissue aspirates. Spread of typical impetigo occurs commonly within families and presumably also would occur within daycare centers. Children should be excluded from daycare until they have completed 24 hours of appropriate antimicrobial therapy (of a 10-day course).

Scabies

Scabies is an infection of the skin caused by infestation with the female mite *Sarcoptes scabiei.* Transmitted by person-to-person spread from an infested individual, the mite buries itself beneath the stratum corneum and burrows along for its 30-day life span while laying two to three eggs per day. The larval and nymphal mites scatter after hatching to embed themselves in skin at distant sites. Some 3 to 6 weeks later, a pruritic eruption (worse at night) develops and leads to excoriation, bleeding, and crusting. The distribution of lesions varies with age. In adults and older children, the eruption, which consists of papules, vesicles, and nodules, occurs commonly in the interdigital spaces of the hands, on the extensor surface of the elbows, and around the umbilicus, waist, axillary lines, and genital area. In infants and young children, vesicular and eczematous lesions are found on the hands and feet, as well as on the face and head.

The diagnosis is made by scraping the lesions and demonstrating the mite, ova, or mite feces. Treatment of the index case should be undertaken with a scabicide, preferably 5 percent permethrin, applied to the entire body. Alternative, less desirable drugs are lindane and crotamiton. Asymptomatic contacts should be treated simultaneously because they may be infected unknowingly and may be capable of transmitting the mite during this asymptomatic period. Clothing and bed linen should be washed, dry cleaned, or stored for a week to ensure that they are not infectious.

Although scabies is spread primarily by intimate personal contact, skin-to-skin transmission can result from prolonged casual contact, as occurs in institutional settings, nursing homes, and daycare centers.[174] Mites can survive on inanimate surfaces for 2 to 3 days, a period that permits transmission by fomites such as clothes, bed linen, and furniture. An outbreak of scabies was reported in a hospital-affiliated daycare facility.[174] Elimination of the problem required a coordinated effort with simultaneous treatment of all potentially infected individuals. An infected child should be excluded from daycare for 24 hours after treatment is undertaken. More commonly, transmission occurs within the family setting.

Head Lice

Head lice infestation occurs commonly in daycare and school-age children as a consequence of infection with *Pediculus capitis.* The insect, a hemophagocytic ectoparasite, obtains nourishment by sucking capillary blood from the scalp. Female lice attach egg cases (nits) to the hair shafts at or very near the scalp. The eggs hatch 8 to 11 days later, and the louse nymphs are released.

The diagnosis is made when the symptoms of scalp pruritus, excoriation, pyoderma, and regional lymphadenopathy cause the caregiver to inspect the scalp closely. Nits are observed readily, although live lice may be difficult to see, especially in light infestations. Transmission is by direct contact with or spread by fomites of live lice. Spread by fomites is facilitated with common storage of hats and coats.

Treatment is with permethrin shampoos to eradicate the lice. Nits can be removed mechanically with spiral combs after preparation of the hair with vinegar soaks or a commercially prepared rinse. All clothing and infested bedding can be disinfected by machine washing or drying (at temperatures of ≥128.3°F [53.5°C]), dry cleaning, or storage in plastic bags for approximately 10 days.

INFECTIONS SPREAD BY CONTACT WITH BLOOD, URINE, AND SALIVA

Hepatitis B Virus

HBV is a DNA-containing virus that causes infections with a wide range of clinical manifestations from asymptomatic seroconversion to fatal hepatitis. Infection is more likely to be asymptomatic in children than in adults.[128] Common symptoms include fever, fatigue, anorexia, malaise, and jaundice. Other gastrointestinal symptoms, such as nausea, vomiting, and diarrhea, may be prominent. Joint symptoms (arthritis and arthralgias) and cutaneous lesions (papular acrodermatitis) may be noted early in the course of the illness.

The most common modes of transmission of HBV in adults are contact with blood and sexual activity. Young children usually acquire infection with HBV by vertical transmission from their mother at delivery. The maternal infection may be acute or chronic. Infection acquired vertically by an infant usually is asymptomatic, but it leads to chronic carriage of hepatitis B surface antigen in most cases. With increasing immigration and adoption of infants from HBV-endemic areas, more HBV-carrier children will be identified.[83]

Transmission of HBV within the daycare setting has been documented twice in the United States[48,179] and once in Australia.[127] In one U.S. case, the probable source was a bite by a child who was a carrier of HBV.[179] In the other, a daycare worker with chapped hands was exposed to the blood of a child who was an HBV carrier.[48] The case in Australia involved an aggressive 21-month-old child with weeping dermatitis and a history of biting other children. Three other investigations failed to demonstrate transmission in daycare facilities despite long-term contact, including one situation with a high potential for blood exposure.[48,179,180] The most recent surveillance activity showed only 1 of 496 HBV-infected children in daycare to be without a family member as a potential source.[63] In Japan, where the background prevalence of hepatitis B surface antigen carriage is higher than that in the United States, data suggested that transmission of HBV most probably occurs among children in nursery schools.[84] Implementation of the current recommendation to screen all parturients for HBV and to undertake universal immunization against hepatitis B in infancy is the most effective way to prevent the spread of this infection. The National Immunization Survey provides vaccination coverage estimates for children aged 19 to 35 months of age. The last published results for the estimated vaccination with at least three doses of hepatitis B vaccine in 2004 was 92.4 percent.[28] Because of the low risk of transmission within the daycare setting, the American Academy of Pediatrics (AAP), the American Public Health Association, and the Centers for Disease Control and Prevention (CDC) do not recommend exclusion of HBV-infected children from daycare or HBV screening of children as a criterion for entry.[11]

Cytomegalovirus

CMV can be transmitted by blood, urine, and saliva, as described earlier.

Human Immunodeficiency Virus

To date, HIV infection has not been reported to be transmitted in a daycare center. In light of its rare horizontal transmission to nonsexual contacts within households with an infected member, HIV would not be expected to spread in daycare.[66,103] The risk of transmission of HIV is less than 1 in 500 exposures, even when direct inoculation with HIV-infected blood has occurred by needle-stick injury to a health care worker. The risk of exposure other than by direct inoculation is far less than 1 in 500. The risk in daycare is even lower. Potential high-risk situations may involve HIV-positive children who are persistent biters or who have extensive weeping skin lesions.

One example of possible transmission of HIV between siblings was reported from Germany.[210] In this case, a bite may have been the source, although complete information on other interactions between the brothers was not available. Other reports indicated a lack of transmission to 35 individuals bitten or scratched by a person infected with HIV.[53,182,203] In addition, a study of family members of children infected with HIV reported no seroconversion to HIV in nine contacts bitten by HIV-infected children and seven uninfected children who bit children infected with HIV.[170] These studies support the lack of evidence for transmission of HIV in the daycare setting.

VACCINE-PREVENTABLE DISEASES

DIPHTHERIA

Diphtheria is now a very rare disease in the United States; fewer than five cases a year are observed. The infection can cause nasal symptoms, membranous pharyngitis, obstructive laryngotracheitis, or skin manifestations. The prominent symptoms are usually a severe sore throat and croup accompanied by toxemia.

Corynebacterium diphtheriae is spread by droplets from people with infection or who are carriers after intimate contact. Individuals are susceptible if they have not been immunized or have been immunized only partially. Treatment is with antitoxin and antibiotics (erythromycin or penicillin). Prophylaxis can be accomplished with erythromycin or penicillin if an individual is found to be a carrier. Immunization with a diphtheria toxoid–containing vaccine is effective in preventing disease and spread of infection.

INFLUENZA

Influenza is a common cause of upper respiratory infection in preschool-age children. The severity of infection is variable, from mild nasal congestion to a severe sepsis syndrome. Influenza is transmitted by person-to-person spread through droplets. Secondary transmission to household and daycare contacts is a very common occurrence. Enthusiasm for annual universal immunization of children has escalated in the last few years and now is recommended for all children 6 through 59 months of age. In addition, immunization is recommended for household contacts and out-of-home caregivers of children 0 to 59 months of age.

VARICELLA

Varicella, or chickenpox, is a common, highly contagious infection of childhood that affects more than 80 percent of the population by the time they reach 10 years of age. The infection is spread easily by the airborne route and by respiratory droplets. It is characterized by a pruritic, generalized vesicular rash that occurs in one to six crops, each crop separated by 24 to 36 hours. Fever usually is mild. The most common complication is secondary bacterial pyoderma. Varicella-zoster virus remains latent in the body after primary infection but can be reactivated as herpes zoster or "shingles," in which case it is manifested as a vesicular eruption involving one to three sensory dermatomes. If infection is acquired in early pregnancy, varicella embryopathy (limb bands or amputation) has been noted in a small fraction of children.

Rates of varicella peak during the preschool and kindergarten years (3 to 6 years), and only 20 percent of children remain susceptible to chickenpox after reaching 8 years of age. Data on the

relative risk of varicella development in children in daycare are minimal.[59,105] However, outbreaks of varicella in daycare centers are known to occur commonly, and the prevalence of varicella in children who attend daycare appears to be higher than that documented for the general population.[62] Children with varicella are excluded from daycare but may return 6 days after the onset of rash or when all their lesions are crusted.

In 1995, varicella vaccine became available for universal use in children between 12 and 15 months of age. The availability of this vaccine altered the epidemiology of varicella in the United States dramatically. The vaccine has been demonstrated to be highly effective during most outbreaks of varicella in daycare[33,67,100] and in clinical practice.[208] The general effectiveness of the vaccine is 83 to 86 percent against all forms of the disease and 97 to 100 percent against moderate to severe disease. The varicella vaccine is available in combination with measles, mumps, and rubella vaccines (as MMRV) and also as a monovalent vaccine. The Advisory Committee on Immunization Practices made a provisional recommendation for a universal routine two-dose varicella vaccination, with a first dose given when the child is 14 to 15 months of age and a second dose at 4 to 6 years of age.[29a]

MEASLES

Measles (rubeola) is a highly contagious respiratory infection. After many years of decline, it again reached epidemic proportions in impoverished and medically underserved inner-city areas in the United States between 1989 and 1992. Although susceptibility to measles virus infection occurs when maternal antibody wanes, the peak age group for measles in the pre-immunization era was children between 5 and 9 years old. More recently, outbreaks have involved preschool-age children, thus setting the stage for outbreaks in daycare facilities.

Measles virus is spread by droplets, hand transmission, and the airborne route. The illness usually is moderate to severe; high fever and prominent respiratory symptoms (cough and coryza) and conjunctivitis are present for several days before onset of the rash. Diarrhea occasionally may be a prominent feature. After the rash erupts, the fever and respiratory symptoms persist for several more days. Pneumonia and encephalitis are complications of measles virus infection; each occurs at an incidence of 1 per 1000 cases.

Measles is prevented effectively by immunization. Current recommendations are to immunize twice: once when the child is between 12 and 15 months of age and again at 4 to 6 years of age.[11] In epidemic situations, primary immunization may be given before a child is 12 months of age. In such cases, another vaccination is given when the child is between 12 and 15 months of age, and the first is not counted toward the two required for full protection.

When measles is diagnosed in a child attending daycare after several days of illness, intramuscular immune serum globulin is the most effective way to prevent secondary cases in susceptible contacts. When there has been a single recent exposure to a case of measles, administration of MMR vaccine within 3 days of exposure should be effective in preventing natural infection.

RUBELLA

Rubella, another exanthematous disease of childhood, is much milder than is rubeola but attacks a similar age group. As with measles, until recently rubella had been nearly eradicated by routine universal immunization with MMR vaccine.

Clinically, rubella is characterized by mild fever, lymphadenopathy (postauricular and occipital), and rash. The illness is difficult to distinguish from other viral exanthems. Infection may be completely asymptomatic. Children with documented rubella should be excluded from daycare until 5 days after the onset of illness.

If rubella is contracted during the first or early second trimester of pregnancy, a severe fetal infection resulting in microcephaly, deafness, congenital heart defects, eye disorders, and psychomotor retardation may result. Acquisition of infection during pregnancy may be a potential hazard for daycare personnel or mothers of children who attend daycare if an outbreak of rubella occurs in the daycare setting. In a large outbreak of rubella in Nebraska, 14 children (9 of whom were <12 months old) and 2 parents acquired their infection in a daycare center.[44]

The incidence of congenital rubella syndrome can be minimized by appropriate immunization of preschoolers and personnel. No specific recommendations exist for care after exposure of a susceptible pregnant adult.

PERTUSSIS

Pertussis, or whooping cough, is a highly communicable respiratory disease caused by *Bordetella pertussis*. The attack rate and severity of disease are highest in the first year of life. The illness classically is divided into three phases: catarrhal, paroxysmal, and convalescent. In the first stage, the child has no fever but has symptoms, primarily rhinorrhea and a cough. When the nasal symptoms resolve, however, the cough becomes and remains very prominent for many weeks. The cough is characterized by paroxysms that are followed by an inspiratory effort (whoop) or leave the child exhausted and occasionally apneic. The convalescent stage is the many weeks necessary for complete recovery. Treatment with erythromycin is effective in decreasing the shedding of organisms, but it does not alter the course of the disease.

Complete immunization with a five-dose series of acellular pertussis vaccine is effective in preventing most cases of pertussis, although illness does occur in partially and occasionally in fully immunized children.[32] After one has been exposed to a case, erythromycin, clarithromycin, or azithromycin provides effective prophylaxis, despite the negligible clinical effect of these drugs after the paroxysmal stage begins. Prophylaxis is recommended for exposure in daycare. If appropriate, booster doses of acellular diphtheria-tetanus-pertussis vaccine should be given to exposed children in a daycare or household setting. A child with pertussis should be excluded from daycare until 5 days of an appropriate course of a macrolide (azethromycin or erythromycin) has been received.[32] Daycare workers are an appropriate target group for receipt of a tetanus toxoid, reduced diphtheria toxoid, and acellular pertussis vaccine licensed as a single booster vaccine for persons 11 to 64 years of age.[29]

POLIOMYELITIS

Poliovirus is an enterovirus that now rarely causes disease in the United States. Although most infections with poliovirus are subclinical, in the pre-immunization era, polio was the major cause of acquired paralytic disease, especially in older children and young adults. In some developing countries, it remains a major health problem.

Poliomyelitis is prevented effectively by use of either the live oral poliovirus vaccine or the inactivated, enhanced-potency parenteral vaccine (IPV).[159] The Advisory Committee on Immunization Practices of the CDC and the AAP recommend a four-dose all-IPV vaccine schedule for routine immunization of all infants and children in the United States.

MUMPS

Mumps is a relatively benign infection of childhood that often is asymptomatic. The most prominent clinical feature is parotitis. Occasionally, a clinically significant central nervous system infection causes unilateral sensorineural deafness. This infection had been dramatically reduced by widespread use of the MMR vaccine until 2005 to 2006, when a large outbreak was documented in the Midwest of the United States. Children with mumps should be excluded from the daycare setting until the parotid swelling subsides or, if the swelling is prolonged, until 9 days after the onset of swelling.

ROTAVIRUS

Rotavirus is now a vaccine-preventable infection that is transmitted by person-to-person spread through the fecal-oral route. Availability of two different oral vaccines to be administered in infancy has dramatically altered the epidemiology of this infection.

HEPATITIS B

HBV is a vaccine-preventable infection that is spread by contact with blood, urine, and saliva, as described earlier.

MANAGEMENT OF INFECTIONS

EXCLUSION POLICY

The AAP and the American Public Health Association have reached a consensus regarding exclusion policies for children attending daycare.[10] The recommendations reflect the understanding that when children have moderate to severe illnesses, they should not be allowed to participate in usual activities or may require more individualized care than available and that the spread of certain communicable diseases within the daycare center will be reduced by the exclusion of people with infections. Accordingly, children known to have highly infectious illnesses should not be allowed to attend daycare until treatment is initiated (e.g., head lice or GAS infections) or the symptoms have resolved (e.g., diarrhea caused by *Shigella*, rotavirus, or *Giardia*) or until transmissibility has waned, as in pertussis, varicella, measles, and mumps. In addition, children should be excluded if the contagiousness of their illness is uncertain (e.g., if a child has a high fever and a rash). A complete list of recommendations for exclusion is presented in Table 258–6.

Important conditions that do not necessarily require exclusion from daycare include (1) asymptomatic excretion of an enteropathogen, (2) nonpurulent conjunctivitis, (3) a rash without a fever or behavioral change, (4) CMV infection, (5) the carrier state of HBV infection, (6) HIV infection, and (7) parvovirus B19 infection in an immunocompetent host. Any exceptions to this statement are found in the individual discussions of the infectious agents. For many infections, the highest risk of transmission of disease occurs before the appearance of recognizable symptoms. Once illness occurs, other children already have been exposed, and exclusion is a less effective strategy. For this reason, exclusion has not been shown to be successful in reducing the frequency of viral upper respiratory tract infections.

TABLE 258–6 Recommendations for Exclusion from Daycare

Symptoms
Illness that prevents the child from comfortably participating in program activities
Illness that results in a greater need for care than the staff can provide without compromising the health and safety of other children
Any of the following conditions in a child: fever, lethargy, irritability, persistent crying, difficulty breathing, or other manifestations of possible severe illness
Diarrhea or stools that contain blood or mucus
Escherichia coli O157:H7 or *Shigella* infection, until the diarrhea resolves and two stool cultures are negative for these organisms
Vomiting two or more times during the previous 24 hours, unless the vomiting is determined to result from a noncommunicable condition and the child is not in danger of dehydration
Mouth sores associated with drooling, unless the child's physician or local health department states that the child is noninfectious
Rash with fever or behavior change, until a physician has determined that the illness is not a communicable disease
Specific Diseases
Purulent conjunctivitis (defined as pink or red conjunctiva with white yellow eye discharge, often with matted eyelids after sleep and eye pain or redness of the eyelids or skin surrounding the eye) until examined by a physician and approved for re-admission, with treatment
Tuberculosis, until the child's physician or local health department authority states that the child is noninfectious
Impetigo, until 24 hours after treatment has been initiated
Streptococcal pharyngitis, until 24 hours after treatment has been initiated
Head lice (pediculosis), until after the first treatment
Scabies, until after treatment has been given
Varicella, until all lesions have dried and crusted (usually 6 days)
Pertussis, until 5 days of appropriate antibiotic therapy has been completed
Mumps, until 9 days after the onset of parotid gland swelling
Measles, until 4 days after the onset of rash
Hepatitis A, until 1 week after the onset of illness or jaundice (if symptoms are mild)

Adapted with permission of the American Academy of Pediatrics: Children in out-of-home child care. In *Pickering, L. (ed.): 2000 Red Book: Report of the Committee on Infectious Diseases. 25th ed. Elk Grove Village, IL, American Academy of Pediatrics, 2000, p. 108.*

PROPHYLAXIS OF INFECTION

Prophylaxis is a strategy that may be helpful in the management of some infections that occur in daycare centers. For example, if a child has had invasive disease caused by *N. meningitidis*, rifampin prophylaxis for all daycare contacts may prevent secondary or associated cases. If a child has pertussis, exposed children may be protected by a booster immunization, if appropriate, and administration of erythromycin or azithromycin for prophylaxis. Intramuscular immune serum globulin may be used to protect susceptible contacts after exposure to measles.

VACCINATION

Vaccination can be used as a strategy to prevent infection during epidemics in a community. For example, immunization with MMR vaccine is successful in terminating epidemics of measles in elementary or high schools. Varicella vaccine also can be used throughout a community and up to 3 days after exposure to curtail the spread of chickenpox. Hepatitis A vaccine is an appropriate intervention for controlling an outbreak of hepatitis A in daycare centers and other settings.[92]

HBV rarely has been reported to be transmitted in a daycare setting in the United States. Hepatitis B vaccine, now recommended for universal use in infants, protects against transmission of infection if a carrier of HBV is identified.

PREVENTION OF INFECTIONS

VACCINATION

Currently, the 14 vaccine-preventable diseases for a preschool child are diphtheria, tetanus, pertussis, polio, influenza, measles, mumps, rubella, varicella, rotavirus, Hib infection, *S. pneumoniae* infection, hepatitis A, and hepatitis B. The timely and appropriate use of immunizations will eliminate or dramatically reduce these problems within the daycare setting. Studies have shown that children who attend registered daycare facilities are more likely to be up to date in their immunizations than are children cared for at home.[88] Laws requiring age-appropriate vaccination of children attending licensed child daycare programs exist in almost all states.[26]

TABLE 258–7 Immunizations for Daycare Employees

Vaccine	Personnel	Schedule
Diphtheria, tetanus	All	Every 10 yr
Pertussis	All	Once
Measles-mumps-rubella	All	If born after 1955, evidence of previous infection or 2 doses at least 1 mo apart
Varicella	Nonimmune	Two doses 1 mo apart
Polio	All	Primary immunization with inactivated poliovirus vaccine if needed; consider booster if previously immunized
Influenza A/B	All	Annually
Hepatitis B	Advised	0, 1, and 6 mo
Hepatitis A	All	Two doses 1 mo apart

EDUCATION

An integral part of the control of infection within a daycare center is education of staff and families. The staff must understand the general principles of infection transmission and control. Education before job placement and frequent inservice seminars reinforce the importance of some basic techniques, especially handwashing. Supervision is essential to ensure compliance with policies.[112] Two studies showed a modest benefit of increased attention to handwashing in preventing respiratory infections in children younger than 2 years old and in preventing episodes of diarrhea in children older than 2 years.[166,167] A possible role for alcohol-based hand gels is being explored as a strategy to reduce transmission of respiratory and gastrointestinal illnesses in the home.[116,173] Applicability to the daycare environment also has been evaluated as part of several multipronged interventional programs.[81]

Parents should be educated regarding recognition of illness, especially illnesses for which the child receives the best care at home. The rationale and importance of compliance with daycare center rules should be emphasized. The childcare program should inform parents of the need to share information about communicable illnesses in the child or a family member.

WRITTEN POLICIES

Each daycare facility should have written policies for managing child and employee illness.[10] It should have written procedures for handwashing, personal hygiene policies, environmental sanitation policies and procedures, and policies for filing and updating immunization records. Employees should be screened for tuberculosis by skin testing when hired and annually if the skin test initially is negative. If the skin test result is positive, a chest radiograph should be obtained. Other recommendations regarding immunization of daycare employees are shown in Table 258–7. Screening daycare attendees or employees for HIV, HBV, or CMV is not necessary.

PHYSICAL PLANT CHARACTERISTICS

In the planning of daycare facilities, areas for infants and toddlers should be separated from those for older children. Because fecal contamination is related strongly and inversely to age, having physical premises large enough to separate children younger than 3 years of age from older children is important. If such separation cannot be achieved, an age restriction should be placed on admission. The kitchen and food storage areas should be separated from the toilet space. Because contamination of hands is the most critical factor in transmission of infection, handwashing facilities must be available to staff and children. Such facilities are especially important in the diaper-changing and food preparation areas. The handwashing facility preferably should be pedal-operated and in easy reach of soap and towel dispensers.

When construction materials are selected, the choice should be based on durability and ease of cleaning. Diaper-changing areas should be made of materials that can be cleaned easily and are light in color so that soilage can be detected. A pedal-operated, closed receptacle is ideal for disposal of soiled diapers. Only paper diapers should be used. The toddler area should be equipped with training toilets and junior-sized toilets. The use of potty chairs should be discouraged. If they are used, they should be emptied into the toilet, cleaned in a utility sink, and disinfected after each use.[10] These areas must be cleaned frequently.

HYGIENIC STANDARDS/INTERVENTIONS

The key factor in prevention of disease in the daycare setting is maintenance of optimal hygienic standards based on recognized mechanisms of transmission of infection. Handwashing is considered the single most important preventive measure,[19,73,112,157] in recognition of the finding that both respiratory and enteric pathogens are spread by contaminated hands.

Haskins published a review article in 2000 summarizing the published reports of interventional studies designed to reduce the frequency of common respiratory and gastrointestinal infections.[81] Although he concluded that studies indicate that multidimensional interventional programs can reduce infection rates, he acknowledged a wide variety of methodologic problems that could confound interpretation of the data. Similarly, the same set of methodologic concerns can be applied to two studies assessing the effect of probiotic infant formula on infections in childcare centers.[82,212] The differences in reported outcomes may be more a function of study design than of actual effectiveness.

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CHAPTER 259

Human Bites

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Human bites have been recorded since the biblical era and currently are a common cause of serious medical and surgical diseases. They are the third most frequent type of bite after dog and cat bites. Reports of secondary infection occurring after a human bite in children have been noted in the United States since at least 1910.[42] Approximately 30 to 50 percent of human bites are accidental and result from injuries that are self-inflicted (such as bitten lips and nail biting) or from injuries suffered while playing sports or games, during falls, during dental therapy, and during treatment of seizures. They also may be intentional, with 50 to 70 percent resulting from aggressive behavior. Fighting is the primary cause of human bites, but aggressive bites also may occur during play (especially in the childcare setting). In addition, bite wounds may be incurred in the course of restraining impaired patients, as a consequence of criminal or police activities, or from child abuse and child battering.[25] Sexual bites or "love nips" account for 5 to 20 percent of adult human bite wounds and may be either intentional or accidental and occur in children of all ages.[1,64,78,79]

INCIDENCE AND EPIDEMIOLOGY

In 1977 the New York City Department of Health altered its reporting system to include human bites. From that year's data of 892 reports, Marr and colleagues[51] noted an incidence of 10.7 human bites per 100,000 population per year, with a range of 0.9 to 60.9 per 100,000 population for different geographic areas. Although a bias was suggested that median income, population density, and younger age might have been factors in this geographic variance, it could not be substantiated. Stockheim and colleagues[70] noted 734 human bite incidents, of which 84 percent were deliberate, in New York City schools for the 1999 and 2000 school years. There was a 2.8:1 male-to-female ratio, with 0.07 percent of both general education and special education students initiating bite incidents. In 2001 to 2002, the Edinburgh Adult Accident and Emergency Department reported an annual incidence of 45.1 human bites per 100,000 catchment population.[75] Merchant and colleagues[54] noted that human bites accounted for approximately 0.4 percent of all pediatric emergency department visits. Human bites have their peak incidence in the spring and early summer for both children[4,48] and adults[51] and on Saturdays, especially for the 15- to 30-year-old age group. Alcohol and drug abuse may play roles in some of these instances. For children[14,48] and adults,[51] bites occur more commonly in males than in females (1.35-1.5:1), except for the 15- to 20-year-old and the 55- to 60-year-old age groups, in which females are bitten more frequently; human bites occur most often in men 20 to 25 years of age. Most bites in teenagers are associated with aggressive behavior. This pattern was documented in 1936 by Welch,[77] who reported that teenagers accounted for 5 of 13 human bite cases, some of which resulted in amputations. Farmer and Mann[20] noted that 12 percent of their patients with bites at a large urban hospital were younger than 20 years. Bites to the lip occurred most often in women (78% in women versus 22% in men) and to the lower lip (65%) more often than the upper lip (35%).[73] Baker and Moore[4] reported that of 322 children who suffered human bites, 21 percent were younger than 5 years, 21 percent were 5 to 10 years of age, and 58 percent were older than 10 years. When corrected for gender, 64 percent of the girls and 39 percent of the boys were 12 years of age or older.

Biting is a common occurrence in the childcare center setting, for which it is the third most frequently reported injury. The incidence peaks in midmorning and during the early school year

in September. Toddlers from 13 to 30 months of age are bitten more often than are infants and other preschoolers,[69] and males are bitten more frequently than are females.[24] Bites account for 3 and 6 percent of reported injuries in boys and girls, respectively,[22] and may be self-inflicted in many cases.[14] Other researchers have noted that approximately 50 percent of all children enrolled in daycare suffer bite wounds, with a rate of 3.9 bites per child per year in general, but 9.4 bites per male child per year for full-time enrollees.[24,67] Garrard and colleagues[24] reported that 104 of 224 children (46%) experienced 347 bites in a single year, and Solomons and Elardo[67] noted that 66 of 133 children (50%) experienced 224 bites in a 42-month period. Fortunately, most of these bites are minor and do not break the skin. A higher proportion of bites in younger children (such as preschoolers) are to the face, whereas most bites in adolescents are on the upper extremities and hands.

Paronychial infections are not actual bites, but when infection occurs, it is related to contamination by oral flora when children bite or suck their fingers. Brook[11] noted children from 2 to 9 years of age (mean age, 5 years 8 months) who were treated at a children's hospital for paronychial infection and reported the bacteriologic and clinical findings of these infections.

Bites as harbingers or signs of child abuse are noted more commonly in the 0- to 4-year-old age group[60]; the age of the abusing parents was younger than 20 years for mothers and younger than 22 years for fathers. An attempt should be made to identify the biter and measure distances between circular bite marks to ascertain the spread. In addition, biting children may have learned this behavior from abusive adults and may themselves be victims of human bites.

BACTERIOLOGY

The bacteriology of these wounds, including occlusional bites, clenched-fist injuries, and paronychia, reflects the human oral flora of the biter and has been the focus of various studies.[8,12,27,32,34,36,71] Human saliva and dental plaque can contain more than 42 different species of bacteria in concentrations of 10^8 colony-forming units per milliliter. Table 259–1 lists common human bite wound isolates and their relative frequency of isolation. Alpha-hemolytic streptococci, especially *Streptococcus anginosus*, are the most frequent isolates. Other common bacterial isolates include *Streptococcus pyogenes*, *Staphylococcus aureus*, *Haemophilus* spp., *Eikenella corrodens*, and oral anaerobes, especially *Fusobacterium*, *Prevotella*, *Veillonella*, and *Peptostreptococcus* spp. Cultures of virtually all infected bite wounds grow bacterial pathogens; wound cultures of patients who initially are seen less than 8 hours after incurring an injury, before the development of clinical infection, yield potential bacterial pathogens in 85 percent of cases.[26,32] The bacteriology of early-treated, colonized wounds is remarkably similar to the bacteria isolated from wounds evaluated later with established infection. The average bite wound yields between 3.4 and 5.4 bacterial isolates per wound, including 1.7 to 2.4 aerobes and 1.7 to 3 anaerobes.[12,32,71] Anaerobes were recognized as important pathogens and markers for serious bite infections, especially bite infections involving the hand, as early as 1936.[5,10,30,57,58,77] Anaerobes are isolated from more than 50 percent of human bite wounds, almost always in mixed culture with aerobes, and they are associated more often with more serious infections, amputation, and the presence of abscesses.[30,32,71] Many of the anaerobes isolated from human bite wounds, especially *Prevotella* and *Porphyromonas* spp., are β-lactamase producers.[12,36,71]

E. corrodens, a capnophilic, gram-negative rod that is part of the normal human oral flora, has been recognized as an important pathogen in approximately 20 percent of clenched-fist injuries.[27,34] It has an unusual antimicrobial susceptibility pattern in that it is susceptible to penicillin but resistant to first-generation cephalosporins, β-lactamase-stable penicillins (such as oxacillin), and erythromycin.[28,33] When unrecognized or treated with the incorrect antibiotic, *E. corrodens* has been associated with therapeutic failure.[27] Usually isolated in mixed culture, it may be missed by

TABLE 259–1 Approximate Prevalence and Bacteriology of Isolates from Human Bite Wound Infections

Isolate	Prevalence (%)	Present in OB	Present in CFI
Aerobes			
Streptococci			
Alpha-hemolytic	28-90	+	+
Streptococcus anginosus	52	+	+
Beta-hemolytic			
Group A	14-26	+	+
Other	12	+	+
Gamma-hemolytic	3-33	+	–
Enterococci	6-11	–	+
Staphylococcus aureus	13-55	+	+
Coagulase-negative staphylococci	6-53	+	+
Haemophilus influenzae	2-6	+	+
Haemophilus species (other)	11-20	+	+
Eikenella corrodens	10-30	+	+
Micrococcus species	3-5	–	+
Moraxella species	3	+	–
Neisseria species	11-15	+	+
Corynebacterium species	28-41	+	+
Acinetobacter calcoaceticus	2-6	–	+
Campylobacter species	16	+	+
Escherichia coli	6	+	–
Klebsiella pneumoniae	3-6	+	+
Enterobacter species	3-4	+	–
Nocardia species	3	–	+
Anaerobes			
Acidaminococcus species	2	+	+
Actinomyces species	4-8	+	+
Bacteroides ovatus	6	–	+
Bacteroides ureolyticus	3-11	+	–
Bacteroides species (unspeciated)	12-33	+	+
Bifidobacterium species	11	–	+
Clostridium perfringens	4	+	–
Prevotella (Bacteroides) melaninogenica	15-22	+	+
Prevotella (Bacteroides) intermedia	11-26	+	+
Prevotella (Bacteroides) oralis	6-17	+	+
Prevotella buccae	15	+	+
Prevotella disiens	4	–	+
Prevotella loescheii	3	+	+
Porphyromonas species	2	+	+
Eubacterium species	3-11	+	+
Fusobacterium nucleatum	12-33	+	+
Fusobacterium necrophorum	4-6	–	+
Fusobacterium species (other)	17	–	+
Peptostreptococcus anaerobius	3	+	–
Peptostreptococcus asaccharolyticus	22	+	+
Peptostreptococcus intermedius	3	+	–
Peptostreptococcus (Finegoldia) magnus	2-17	+	+
Peptostreptococcus micros	12-20	+	+
Gemella (Peptostreptococcus) morbillorum	3	+	–
Peptostreptococcus prevotii	3	+	–
Peptostreptococcus species (other)	3-22	+	+
Veillonella parvula	9-11	+	+
Veillonella species (other)	6-18	+	+
Spirochetes	5-30	+	+

CFI, clenched-fist injuries; OB, occlusional bite wounds.
+, present; –, absent.
Based on a compilation of data from references 6, 12, 35, 56, and 71.

microbiologists because of its slow growth characteristics and overgrowth of other bacterial colonies. *E. corrodens* produces a small colony that "pits" or "corrodes" the agar surface and has a light yellow pigment and an odor like that of hypochlorite bleach.

The bacteriology of paronychial infections is similar to that of other human bite infections, except that aerobes and anaerobes each have been isolated in pure culture in 27 percent of cases.[11] Brook[11] found a total of 3.6 isolates per specimen: 1.4 aerobes, 2 anaerobes, and 0.2 *Candida albicans;* β-lactamase–producing organisms, including isolates of *Prevotella melaninogenica* and *Prevotella oralis*, were found in 45 percent of the wounds.

In addition to oral bacterial infection, human bites have been associated with viral infections such as those caused by herpes simplex virus,[23,52] cytomegalovirus,[52] hepatitis B virus,[13,16,41] hepatitis C virus,[19] and possibly human immunodeficiency virus (HIV),[1,3,7,17,43,52,61,63,66,72] as well as syphilis,[21] tuberculosis,[26] actinomycosis,[9] and tetanus.[55,62]

CLINICAL MANIFESTATIONS

Human bites may be categorized into three groups: (1) bites resulting in paronychial infection, (2) occlusional bites, and (3) clenched-fist injuries. Patients in the latter two categories may be seen early (<8 hours after injury) for wound care or tetanus boosters or late (>8 hours after injury), usually because of established infection or infectious complications. All share the predominance of oral aerobic and anaerobic bacteria as primary etiologic pathogens. Noninfectious complications may include injury to tendons and nerves or fractures. Potential complications of human bites are noted in Table 259–2. Table 259–3 lists some of the diseases acquired as a result of human bites. The incidence of infection developing after human bites is estimated to be 10 to 30 percent. Because of the typically superficial nature of human bite wound injuries in children, the infection rate in children is estimated to be approximately 10 percent.

TABLE 259–2 Potential Complications of Human Bite Wounds

Abscess
Cellulitis
Compartment syndrome
Fracture
Necrotizing fasciitis
Nerve severance/injury
Osteomyelitis
Scarlet fever
Sepsis
Septic arthritis
Tendon severance/injury
Tenosynovitis
Toxic shock syndrome

TABLE 259–3 Diseases Transmitted by Human Bite Wounds

Actinomycosis
Cytomegalovirus infection
Hepatitis B virus infection
Hepatitis C virus infection
Herpesvirus infection
HIV infection
Invasive group A streptococcal infection
Syphilis
Tetanus
Toxic shock syndrome
Tuberculosis
Whitlow

BITES RESULTING IN PARONYCHIAL INFECTION

Paronychia is an infection (inflammation) of the structures of the distal phalanx, either those surrounding the nail or the bone itself. Most are caused by finger sucking in younger children but may be caused by accidental biting. The area is red, tender, and swollen and may have some underlying purulence.

OCCLUSIONAL BITES

Occlusional bites occur when the teeth actually contact any part of the human anatomy. Human bite wounds occur most often on the upper extremity (18-71%), the head and neck (4-33%), the thorax and abdomen (6-25%), the breasts or genitals (3-25%), and the lower extremity (3-11%).[4,24,32,51,74] Vale and Noguchi[74] reported the anatomic distribution of bite marks in 67 forensically evaluated cases, including 13 (19%) cases in children younger than 15 years old. They noted more than one bite mark in 40 percent of victims and that female victims were bitten most frequently on the breasts, arms, and legs, whereas bites to males more often were on the upper extremities and shoulders. Marr and colleagues[51] did not differentiate the location of bites by sex of the victim but noted that 15 percent were to the head and neck, 12 percent were to the thorax and abdomen, 61 percent were to the upper extremities, 4 percent were to the lower extremities, and 9 percent were to unknown locations. Similarly, MacBean and colleagues[48] reported that 20 percent of human bites were to the face and head; 58 percent were to the hand, wrists, and arms; 5 percent were multiple injuries; and 9 percent were to "missing/other body regions." Our experience with teenagers and adults is similar to those cited,[48,51] perhaps because of a selection bias involving patients who seek medical care or come to the attention of infectious disease consultants. When the hand is involved, wounds tend to occur most frequently on the terminal phalanx of the middle (long) finger of the dominant hand.[8,18,32] Bites to the upper extremities occur most frequently in toddlers (66%), infants (71%), and preschoolers (46%)[24] and in children overall (42%).[4]

Most wounds are minor and require routine care with cleansing and bandaging. The infection rate after occlusional bite wounds has been estimated to be 10 to 30 percent, 87 percent of which may require hospitalization.[4,46] Wounds to the hand and with any edema or crush injury and those that involve a bone or a joint have a greater potential to become infected. Infections, when they occur, usually are manifested as cellulitis. If the child is treated early,[18,45] these injuries rarely result in serious complications. Patients in whom treatment is delayed (>12 hours after injury) probably have a preselection bias because they usually seek attention for an already established infection. These infections generally spread proximally and not distally and in less than 5 percent of cases have associated fever, lymphangitis, or lymphadenopathy. A malodorous discharge or abscess may be present if anaerobes are involved.

Complications may be limited to skin defects (when skin avulsion has occurred), but septic arthritis and osteomyelitis may develop if the joint or bone is involved.[37] In immunocompromised hosts, such as those with hematologic malignancy or neutropenia, sepsis may occur. Amputation may be necessary as a result of the initial bite in approximately 2 to 5 percent of cases or as a result of serious chronic infection in 0 to 18 percent of cases.[5,18,45,47,53,56,77] Tendon injury, primary nerve injury and tenosynovitis, compartment syndrome, and resultant secondary nerve damage may occur.[59]

CLENCHED-FIST INJURIES

Clenched-fist injuries, or "fight bites," are the most serious of human bite wounds and occur when the closed fist of one person strikes another in the teeth and a break in the skin occurs.[38] The most common cause is a fight, although cases can occur during contact sports or boxing if gloves are not worn. The metacarpophalangeal joint (knuckle) of the middle (long) finger of the dominant hand is involved most frequently.[27,34,65] The break in the skin may be only 2 to 5 mm in length but often results in penetration into the joint or even the bone. Patients and physicians may underestimate the potentially serious nature of these wounds. During the initial contact, bacteria penetrate the knuckle area and, on relaxation of the hand, are carried by the tendons further back into the potential spaces of the hand. Infection may spread laterally, between the collateral and accessory ligaments, or dorsally into the thin-walled bursa overlying the metacarpal head or into the palmar deep spaces of the hand. The typical patient avoids seeking attention because of the circumstances surrounding the injury and often wakes up 6 to 8 hours later with a painful, throbbing, swollen, infected hand and then seeks medical attention. Often, the patient has a purulent exudate emanating from the injury, which may be foul-smelling if *P. melaninogenica* or other anaerobes are present, and the patient comes to the physician with the hand elevated to diminish the pain. The swelling usually spreads proximally and not distally from the site of injury.

The physician should take samples for both aerobic and anaerobic cultures and secure the assistance of a surgeon experienced in hand cases. Chuinard and D'Ambrosia[15] and others[49,50,60] have outlined the surgical management of these wounds; such management includes a determination, under a bloodless field, of whether penetration of the joint capsule has occurred or whether only localized cellulitis is present. Complications occur frequently, and in our experience, these patients have a 50 percent chance of developing septic arthritis, osteomyelitis, or both. The range of motion of the hand usually is limited by swelling and edema but also may be limited as a result of tendon or nerve injury or severance. Permanent limitation of range of motion and joint stiffness are frequent results of osteomyelitis in the affected joints. Osteomyelitis, which usually involves the small bones of the hand, often is manifested as continued pain, swelling, and erythema with or without drainage at 10 days to 3 weeks after the injury has occurred.[20] Osteomyelitis and septic arthritis caused by *E. corrodens*, often in association with alpha-hemolytic streptococci, frequently are insidious and persistent and may lead to amputation, especially if treated with the wrong antibiotics.[27] Other complications include fracture, collar-button abscess, and muscle atrophy.

POTENTIAL FOR TRANSMISSION OF THE HUMAN IMMUNODEFICIENCY VIRUS

Reports[3,43,66,72] have noted the transmission of HIV from biter to victim. HIV is isolated uncommonly from the saliva of infected persons, and when recovered, it has been in low number. Consequently, the possibility of HIV transmission occurring is considered unlikely, though negligible.[7,17,61,63] Rickman and Richman[63] reviewed instances of reported HIV transmission via human bites and concluded that "no well documented case of HIV transmission through bites exists"; the risk of HIV transmission occurring is possible biologically but appears to be unlikely. A review and commentary of professional sports-related injuries also noted the unlikely possibility of HIV being transmitted via sporting events and outlined similar commonsense control measures.[52] Most schools allow preschool-aged children with HIV to attend child-care centers unless a significant, real risk exists in an individual's behavior or hygienic habits.[17]

TREATMENT

The basic elements of management are outlined in Table 259–4. They include obtaining a complete history of the circumstances of the injury, with an attempt to identify the biter. If the biter is identified, some questions about the presence of herpes and other potentially orally transmitted viral diseases should be asked. Awareness of the potential for child abuse or battering must be considered, and a search should be performed for other associated injuries. The examination must include an evaluation of range of motion if a hand or joint is involved; determination of the integrity of tendons, nerves, and the vascular supply; and a diagram of the location of the bite marks. Because most victims have multiple wounds, a thorough search of the entire body should be made and the wounds should be measured. Special attention must be paid to any wound close to bones or joints, especially when it involves the hands, and the possibility of bone or joint penetration should be considered. Radiographic studies of the hand should be obtained for all clenched-fist injuries or in any situation in which suspicion of fracture or the potential for osteomyelitis exists. Magnification radiography may be helpful in identifying bone penetration. If tetanus immunization is not current, a toxoid booster should be given. If the patient has no record of immunization, tetanus immunoglobulin and tetanus toxoid should be administered.

The wound should be cleansed with normal saline, and any foreign body or debris and necrotic tissue should be removed. Cautious débridement is indicated for some wounds, with care being taken to not create a potential skin defect. The wound then is irrigated, with a syringe and needle/catheter tip used as a

TABLE 259–4 Management Procedures for Human Bite Wounds

Obtain history from patient
Situation leading to injury
Place of occurrence
Patient allergies
Other medications (potential interactions)
Perform evaluation of patient
Nerve function
Tendon function
Vascular integrity
Range of motion
Potential bone and joint involvement
Diagram or photograph wound
Mark leading edge of cellulitis
Culture wound (if infected)
Irrigate wound
Débride wound cautiously
Drain abscess
Administer antimicrobial agents
Prophylactic therapy, 3 to 5 days
Longer duration for established infection
Elevate injured area
Immobilize wound area (3 days for hands)
Have the patient exercise the injured area (if previously immobilized)
Close the wound
Primary for face and head
Delayed for early wounds
Secondary intent for infected wounds
Administer tetanus toxoid
Obtain radiograph (if indicated)
Submit health department report (if required)

high-pressure jet to diminish the bacterial inoculum. Aerobic and anaerobic cultures should be obtained, and Gram stain performed if infection is present. In cases of clenched-fist injury, a hand surgeon should examine the patient to determine whether the joint capsule or bone has been compromised.[15] With many hand infections, especially after exploration of clenched-fist injuries, immobilization in a plaster splint is recommended. Elevation of a swollen or inflamed part is crucial to healing. The elevation should be maintained above the level of the heart. Promises made by patients or family to "keep the hand up" should not be accepted unless a properly fitting sling is issued or made from a scarf; in addition, a tubular stockinette and an intravenous pole may be used. Legs and hands also can be elevated with pillows. The use and value of topical agents have not been studied, although most patients have applied them before seeking medical attention.[32]

Approximately 14 to 32 percent of victims of occlusional bite wounds will require hospitalization,[54,71] compared with approximately 55 percent of those with clenched-fist injuries.[71]

Antimicrobial agents should be selected empirically according to the most likely pathogens and their usual susceptibility patterns to antimicrobial agents.[29,35,39,40] If cultures are performed, therapy should be adjusted according to the organisms isolated and their specific susceptibility to antimicrobial agents. The activity of antibiotics commonly used against the usual human bite wound pathogens is outlined in Table 259–5. Common pathogens that need to be considered in the selection of antimicrobial therapy include streptococci, *S. aureus*, *Haemophilus* spp., *E. corrodens*, and β-lactamase–producing oral anaerobes. The oral agent of choice is amoxicillin-clavulanic acid; for penicillin-allergic children, a combination such as trimethoprim-sulfamethoxazole plus clindamycin can be substituted. Many other combination regimens, including cefuroxime plus clindamycin or metronidazole, may be used. The intravenous agents of choice are ampicillin-sulbactam and ticarcillin-clavulanate.[2] For penicillin-allergic children, combination therapy with an extended-spectrum cephalosporin or trimethoprim-sulfamethoxazole plus clindamycin should be administered.[2] Additional potentially useful intravenous agents include cefoxitin, imipenem, or ertapenem and combinations of such agents as cefuroxime or cefotaxime plus metronidazole. First-generation cephalosporins are of limited utility because of poor activity against *E. corrodens* and some anaerobes[28,33] and should not be used as empirical monotherapy. Erythromycin also has poor activity against *E. corrodens* and *Fusobacterium nucleatum*,[35] and its use as monotherapy can lead to therapeutic failure. Newer macrolides, including azithromycin, show improved activity when compared with erythromycin against the spectrum of bite wound pathogens, but *Haemophilus* spp., and *F. nucleatum* remains relatively resistant.[35]

The quinolones, particularly moxifloxacin, exhibit broad activity against the pathogens implicated in bite wounds.[71] The risk-to-benefit ratio should be considered before they are administered because the quinolones are not approved for use in children younger than 18 years old. Linezolid, an oxazolidinone, appears to be more active than are the macrolides against the gram-positive organisms and fusobacteria isolated from human bite wounds, but some aerobic gram-negative organisms are at the susceptibility breakpoint.[31] Once patient-specific cultures return, antimicrobial therapy should be adjusted in accordance with the patient's individual isolates and susceptibility pattern. The duration of antimicrobial therapy is determined by the type and severity of infection. Prophylactic antimicrobial agents typically are given for 3 to 5 days, whereas the course for established infection usually is longer, such as 10 to 14 days for cellulitis and 4 to 6 weeks for septic arthritis and osteomyelitis. If any question exists about the prompt filling of a prescription because of financial or other concerns, a dose of intramuscular or intravenous antibiotics should be administered and hospitalization considered.

Patients' wounds should be re-examined within 24 to 48 hours. If outpatient therapy is initiated and the cellulitis advances, hospitalization is indicated. Table 259–6 lists the reasons for hospitalization of patients with human bite wounds. Such indications include patient noncompliance and virtually all clenched-fist injuries.

Infected wounds should not be closed. However, the value and risks associated with primary closure in patients who are initially seen less than 8 hours after injury and without any symptoms or signs of established infection have not been studied in a prospective or randomized manner. Exceptions are wounds to the face and neck and losses of the lip, for which early primary closure has been successful.[73,76] However, the information about those wounds probably is not applicable to bite wounds to the hands or other parts of the body for several reasons: (1) head and face wounds are débrided and copiously irrigated with as much as a liter of normal saline, which diminishes the bacterial inoculum; (2) most surgeons give a course of 5 or more days of antibiotics; (3) the blood supply to the head and face area is superior to that of most other anatomic areas; and (4) these areas are rarely dependent, and, therefore, edema and swelling resolve more rapidly or do not develop. The use of primary wound closure in early-treated, uninfected wounds remains at the physician's dis-

TABLE 259–5 Comparative In Vitro Antimicrobial Activity of Selected Oral Antimicrobial Agents Against Common Human Bite Wound Pathogens

	*Staphylococcus aureus**	*Eikenella corrodens*	Streptococci	*Haemophilus*	Anaerobes
Amoxicillin	–	+	+	v	v
Amoxicillin-clavulanic acid	v	+	+	+	+
Cephalexin	v	–	+	–	–
Cefaclor	v	–	+	+	–
Cefuroxime	v	v	+	+	–
Cefprozil	v	–	+	+	–
Dicloxacillin	v	–	+	–	–
Erythromycin	v	–	v	–	–
Azithromycin	v	v	+	–	v
Clarithromycin	v	v	+	–	–
Trimethoprim-sulfamethoxazole	+	+	+	+	–
Chloramphenicol	v	+	v	+	+
Moxifloxacin	+	+	+	+	+
Clindamycin	+	–	+	–	+

**Community-acquired methicillin-resistant S. aureus (CA-MRSA) has emerged as a significant problem in some regions.*
Note: Because of contraindications in pediatric patients, tetracyclines and fluoroquinolones are not included.
+, active; –, poorly active or inactive; v, variable.

TABLE 259–6 Indications for Hospitalization of a Victim of a Human Bite Injury

Clenched-fist injury
Immunocompromised host
Asplenia (diagnostic or traumatic)
Cirrhosis
Leukemia
Lupus/steroids
Mastectomy (radical or modified radical)
Crush injury
Edema
Preexisting in the injured area or developed during therapy
Cirrhosis
Mastectomy
Malnutrition
Congestive heart failure
Fever (>100.5° F)
Lymphadenopathy
Patient noncompliance
Failure to take medication
Failure to elevate the injured area
Osteomyelitis
Septic arthritis
Progression of infection despite outpatient therapy

cretion. Wounds to the hands should be observed and left open for either delayed primary or secondary closure.

In general, hyperbaric oxygen therapy for human bite wounds remains of unproven benefit. Lehman and colleagues[44] prospectively studied the use of a portable hyperbaric oxygen chamber in 16 of 43 patients admitted to the hospital for human bite infections of the hand, almost all caused by clenched-fist injuries. They found that no benefit was obtained for mild or moderate infections, but the duration of the hospital stay was shortened (4.7 versus 11.2 days) in patients with severe infections. Although the authors thought that return of function was more rapid in the hyperbaric group, limitations in follow-up precluded evaluation and the institution of an early, aggressive exercise program may have been an important factor.

PREVENTION

Mast and colleagues[52] reviewed both risk and prevention strategies for the transmission of blood-borne pathogens during sports. Prevention strategies include appropriate infection control measures in the sports setting and education of young athletes, as well as their coaches and trainers. Bites may occur in the daycare setting, even "when caregivers are vigilant." The use of "disciplinary techniques," including a developmentally appropriate curriculum ("busy, happy children are less likely to get into serious mischief"), avoidance of too much open space in classrooms, and discipline that pays attention to redirected positive behavior, has been advocated.[67,68] More attention should be given to the victim than to the aggressor; teachers should work with the biter on behavior modification with reinforcement, extinction, and punishment strategies, which should be limited to "time-out" procedures.

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CHAPTER 260

Animal Bites

Morven S. Edwards

> Many children delight in teasing dogs, and without caution go too near them, by which they get miserably torn and mangled. . . . What these boys had been doing to enrage the dog we cannot tell, but suspect they had been tormenting him in some way, thinking that as he was chained he could not injure them. But they were mistaken in this, and one of them is likely to be bitten very severely.[5]
>
> *Author unknown, 1830*

HISTORICAL ASPECTS

The consequences to children of bites resulting from provoking dogs, as noted in the opening quotation, were of concern in the 19th century just as they are today.[23] In early reports, infected bite wounds were noted to contain fusiform bacilli and spirochetal organisms.[80] We now appreciate that a vast number of aerobic and anaerobic organisms comprising the normal flora of the biting animal's mouth are potential bite-wound pathogens.

The importance of débridement and drainage in treatment of infected bite wounds was understood in the pre-antibiotic era, but these infections carried a high morbidity rate.[78] In a report from 1936, amputation was required in one third of cases in which treatment was delayed for 24 hours or longer.[98] Adjuncts to cleansing, such as electrocauterization and even radiation therapy, were used in efforts to prevent or treat infection, but not until the introduction of penicillin was the outcome of bite-wound infections improved over that achieved by symptomatic therapy alone.[78]

EPIDEMIOLOGY

More than 108 million cats and dogs are kept as pets in the United States.[43] The estimated annual incidence is 1 to 2 million

dog bites, 400,000 cat bites, and 45,000 snake bites.[42,96] The direct medical cost annually for dog bites alone is $165 million.[81] Rabbits, skunks, squirrels, horses, rats, hogs, and monkeys cause at least 1 percent of bite injuries. Unprovoked pet ferret attacks have caused severe facial injuries.[74] Taken together, however, bites from nondomestic animals, generally thought to pose a higher risk for transmission of rabies, constitute less than 1 percent of reported bite wounds. The right arm is bitten most frequently, presumably because of defensive attempts by victims using their dominant arm. At least three quarters of bites are located on extremities. Facial bites comprise only 10 percent of the total, but two thirds of them are sustained by children younger than 10 years of age.

Children are the most common victims of animal bites. From one half to three fourths of dog bites are reported in persons younger than 20 years of age. The peak incidence is in children 5 to 14 years of age. Many bites do not require a visit to the physician and are not reported. The number of children with bites reported exceeds those of all reportable childhood diseases.[14] Up to 1 percent of all pediatric emergency department visits during the summer months are for treatment of animal bites.[27,54] Most bites occur in late afternoon and early evening hours. Boys sustain dog bites twice as often as do girls, but girls are bitten more frequently by cats.[66]

Large dogs account for most animal bites and are implicated most frequently in bites with a fatal outcome. Ten to 20 fatal human attacks occur yearly in the United States. At least 25 breeds of dogs were implicated in 238 fatalities from dog bites during the past 25 years, but pit bull–type dogs and rottweilers were involved in more than half the deaths.[87] These animals can exert a biting force of 1500 psi, several times that of a German shepherd. The severity of wounds inflicted by pit bull breeds also is due to their tendency to inflict multiple bites and to bite and grind their molars into tissue. Ninety-four percent of injuries caused by pit bulls in one study were the consequence of unprovoked attacks.[8] In most instances (75%), the dog's owner is known by the victim, although only a small percentage of bites are caused by a family-owned dog. Stray dogs account for 10 percent of bites inflicted. When the circumstances are known, most mammalian bites are provoked, although the victim may not have agitated the animal intentionally.[19,91]

Infection is a common complication of animal bites. Between 3 and 20 percent of dog bites and 20 to 50 percent of cat bites for which medical care is sought become infected.[43,96] With the exception of monkey bites, which have a high infection rate, infection seldom develops after bites by other mammals. Factors influencing the risk for development of infection include the patient's age, type and location of the wound, and length of time that transpires between the bite and initiation of treatment. Wounds of the hand are more likely to become infected than are those of the arm, leg, or face.[18] Puncture wounds more often become infected than do lacerations, superficial wounds, or wounds with skin and soft tissue defects.[1] Infection is likely to develop when wounds are repaired surgically or when care is delayed more than 24 hours after injury.

MICROBIOLOGY

The mammalian mouth has been viewed as a microbial incubator supporting the growth of some 200 species of facultative organisms and obligate anaerobes.[33] The oral flora of the animal, rather than skin flora of the victim, is the source of most isolates from bite wounds,[66] but both can be sources for infection. Infections usually are polymicrobial and contain mixed aerobic-anaerobic isolates. Table 260–1 enumerates bacterial isolates from the wounds of 50 patients with dog bites and 57 patients with cat bites that were infected at the time of arrival at an emergency department for care.[93] A median of five bacterial isolates were found per culture. Slightly more than half the wounds yielded both aerobes and anaerobes, and anaerobes alone were isolated from slightly more than a third of the wounds. *Pasteurella* spp. were the isolates most frequently found in dog and cat bites (50% and 75%, respectively), with *Pasteurella canis* being isolated most commonly from dog bites and *Pasteurella multocida* subspecies *multocida* and *septica* the most common isolates from cat bites. *Pasteurella* carrier rates are as high as 66 percent for dogs and 90 percent for cats, so one is not surprised that this organism is associated so commonly with bite-wound infections. Infection has occurred as a consequence of bites by large cats, including lions,[17] cougars,[57] and tigers,[21] as well as by house cats. The type of wound commonly inflicted (i.e., puncture by cats versus laceration by dogs) might explain the species-specific disparity in infection rates.

TABLE 260–1 Bacteria Isolated from 50 Infected Dog Bites and 57 Infected Cat Bites

	Number of Patients (%)	
Bacteria	**Dog Bite**	**Cat Bite**
Aerobes		
Pasteurella	25 (50)	43 (75)
Streptococci	23 (46)	26 (46)
Staphylococcus aureus	10 (20)	2 (4)
Other staphylococci	13 (23)	18 (31)
Neisseria	8 (16)	11 (19)
Corynebacterium	6 (12)	16 (28)
EF-4b	5 (10)	9 (16)
Moraxella	5 (10)	20 (35)
Enterococcus	5 (10)	7 (12)
Bacillus	4 (8)	6 (11)
Pseudomonas	3 (6)	3 (5)
Actinomyces	3 (6)	2 (4)
Brevibacterium	3 (6)	2 (4)
Weeksella	2 (4)	4 (7)
Eikenella corrodens	1 (2)	1 (2)
Capnocytophaga	1 (2)	4 (7)
Acinetobacter	0	4 (7)
Other	19 (38)	19 (33)
Anaerobes		
Fusobacterium	16 (32)	19 (33)
Bacteroides	15 (30)	16 (28)
Porphyromonas	14 (28)	17 (30)
Prevotella	14 (28)	11 (19)
Propionibacterium	10 (20)	10 (18)
Peptostreptococcus	8 (16)	3 (5)
Eubacterium	2 (4)	1 (2)
Others	1 (2)	5 (9)

Modified from Talan, D. A., Citron, D. M., Abrahamian, F. M., et al.: Bacteriologic analysis of infected dog and cat bites. N. Engl. J. Med. 340:*85-92, 1999.*

Other aerobic agents commonly isolated from infected dog or cat bites include streptococci, staphylococci, *Moraxella*, *Neisseria*, and corynebacteria. Although streptococci often are beta-hemolytic, *Streptococcus pyogenes* was isolated from only 12 percent of dog bites and none of the infected cat bites in one large series.[93] *Staphylococcus aureus* was isolated frequently from infected dog bites but infrequently from cat bite wounds. Species such as *Capnocytophaga canimorsus* and *Neisseria weaveri*, previously classified under the Centers for Disease Control and Prevention alpha-numeric system, are notable because infections in association with these species have occurred in splenectomized persons or those with immunocompromising conditions.[4,61]

Animal bites are implicated as a vehicle for transmission of a number of systemic infectious diseases caused by viruses, fungi, and mycobacteria, in addition to bacteria (Table 260–2).[33] For

TABLE 260–2 Some Systemic Infections Transmissible by Animal Bites

Infection	Representative References
Viral	
B-virus	73
Hemorrhagic fever	29
Monkeypox	71
Rabies	30
Bacterial	
Brucellosis	82
Cat-scratch disease	40
Leptospirosis	63
Plague	38
Rat-bite fever*	101
Tularemia	20
Mycobacterial	
Mycobacterium marinum	37
Mycobacterium fortuitum	7, 60
Fungal	
Blastomycosis	41
Sporotrichosis	89

**Both Haverhill fever caused by* Streptobacillus moniliformis *and sodoku caused by* Spirillum minus.

some of these infections, the lists of animals that may transmit the infection are extensive. For example, tularemia can be transmitted by the bite of a wild boar, coyote, hog, lamb, muskrat, opossum, raccoon, rat, skunk, squirrel, snapping turtle, or weasel.[6,20,64]

CLINICAL MANIFESTATIONS

Signs of infection after animal bites develop within hours to several days after injury. Infection is the usual reason that patients seek medical attention more than 12 hours after injury, whereas those seen earlier are more concerned with prophylaxis or surgical repair. Findings suggestive of wound infection include localized swelling, erythema, and pain with or without serosanguineous or purulent drainage (Fig. 260–1). The clinical findings vary with the infecting organism, site of injury, and type of bite.

The characteristic findings of *P. multocida* infection, which include intense pain, swelling, and erythema, develop rapidly, often within hours after injury. Cellulitis is usually evident within 24 to 36 hours after the bite; occasionally the manifestation is delayed for 3 to 5 days. Despite these intense local symptoms, patients generally are afebrile, and less than 20 percent have lymphangitis and regional adenitis. Patients with wound infection caused by staphylococci or streptococci usually experience less intense pain, have a delay between injury and the onset of symptoms of days rather than hours, and may have a more diffuse, less fiery cellulitis. Gas in tissues of the forearm clinically suggestive of clostridial gas gangrene has occurred in infections caused by *Streptococcus anginosus* and *Streptococcus mutans* after horse bite lacerations.[68] Wound infection clinically resembling that caused by *P. multocida* from which the related but more unusual gram-negative rod *Actinobacillus lignieresii* was isolated has been reported in a child who sustained a facial bite by a horse.[26]

"Seal finger" deserves mention because failure to initiate appropriate therapy can result in permanent sequelae. The etiologic agent is unknown; a possible role is suggested for *Mycoplasma*.[9] Infection can result from contact through a laceration from the skin of a seal or from a seal tooth- or claw-associated puncture wound.[67] The incubation period averages 4 to 8 days, and the onset is characterized by severe pain and often massive swelling and erythema. In some cases, regional adenopathy and ascending lymphangitis occur. A predilection for involvement of the joint closest to the inoculation site has been noted. Once the diagnosis is established, treatment should be initiated with a tetracycline, the drug class of choice.

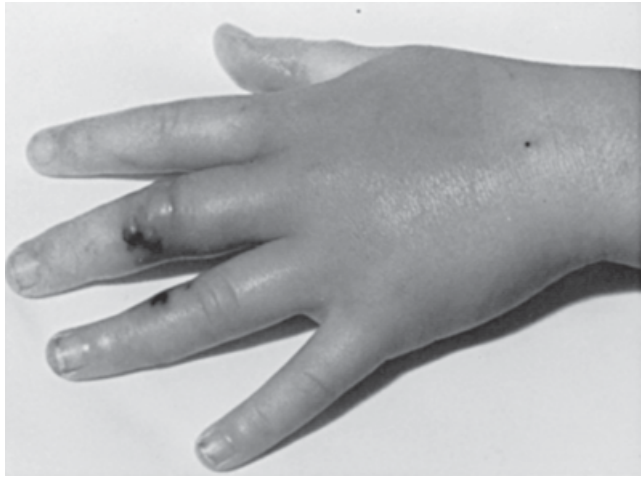

Figure 260–1 Dog bite wound–associated tenosynovitis in a 4-year-old girl. Group D *Streptococcus* was isolated from the wound culture.

In patients with systemic infections transmitted by animal bites, the incubation periods and clinical manifestations vary with the causative agent. For example, streptobacillary rat-bite fever occurs after an incubation period of less than 1 week, whereas spirillary rat-bite fever, or sodoku, has a 2-week asymptomatic interval after the bite. However, rat-bite fevers caused by *Spirillum minor* and *Streptobacillus moniliformis* can occur together. For most of the infections listed in Table 260–2, the bite wound serves as the site of inoculation and has healed completely during the incubation period. For example, fatal encephalitis has resulted from the bite or scratch of a monkey that is actively shedding B virus *(Herpesvirus simiae)*. Institution of acyclovir treatment intravenously at the time of injury may abort progression of the disease.[11,73] The symptoms heralding the onset of systemic infections caused by animal bites do not depend on the mode of transmission and are discussed in their respective chapters. A high index of suspicion can be required to trace the infection to the animal source. For example, most cases of human plague in the United States result from bites by infected fleas, but contact with a domestic cat infected with *Yersinia pestis* can be the source of infection.[38] With tularemia, an ulcerative[81] or pustular[34] lesion develops at the bite site 4 to 7 days after injury and is associated with fever, chills, and painful regional adenopathy. In a case of *Mycobacterium marinum* infection that developed after a dolphin bite, one of several discrete fluctuant masses containing the isolate developed in an area just proximal to the original wound.[37] With cat-scratch disease caused by *Bartonella henselae*, a papule or pustule may be present at the original bite site when systemic signs develop.

The jaws and teeth of dogs are likely to produce multiple puncture wounds, as well as jagged lacerations with devitalized tissue. These lesions can be associated with depressed skull fractures, sometimes in more than one cranial region.[100] Puncture wounds, particularly those inflicted by cat bites, often are deceptively innocuous. Inoculation of organisms deep into poorly vascularized areas, such as tendon sheaths, fascia, joints, and bones, is likely to result in an infected wound.

Some of the complications resulting from direct extension or generalized spread of infection caused by animal bites are summarized in Table 260–3. Tenosynovitis caused by *P. multocida* can be apparent within hours or can present days to weeks after

TABLE 260–3 Some Infectious Complications of Animal Bites

Complication	Representative References
Direct Extension	
Arthritis	17
Brain abscess	2, 12, 58
Endophthalmitis	103
Orbital cellulitis	28
Osteomyelitis	17, 34, 55, 69
Synovitis	7
Tendonitis, tenosynovitis	34, 51
Generalized	
Endocarditis	13, 88, 99
Generalized Shwartzman reaction	70
Meningitis ± sepsis	10, 17, 83
Mycotic aneurysm	50
Pneumonia	52
Sepsis ± coagulopathy	35, 56
Sepsis, infected knee joint prostheses	72
Sepsis, postsplenectomy	61

injury, when persistence of swelling, tenderness or pain, and a mass overlying the involved tendon sheath suggest the diagnosis. *Pasteurella* osteomyelitis that develops after a cat bite is characterized by pain, swelling, and tenderness over the involved bone.[55] With chronic infection, a draining sinus or a persistently draining wound can be the presenting signs. When the periosteum has been entered, osteomyelitis can develop despite early administration of local and antibiotic treatment. Feline incisors are more likely to penetrate the periosteum than are canine incisors, but osteomyelitis can occur as a consequence of dog bites as well.[55]

Bites to the cranium occur with relative frequency in small children because their heads are at the level of the mouth of medium- to large-sized dogs. Complications of perforating cranial bites in children include compound depressed skull fractures, dural lacerations, and extensive intracerebral injuries, which can prove fatal.[17,100] Brain abscess and meningitis can occur as complications of these injuries (see Table 260–3).

Generalized or systemic complications from animal bites occur usually, but not exclusively, in hosts with altered immune status. Bacteremia and fatal endocarditis caused by this organism have occurred in immunocompetent patients. Common underlying findings in infections associated with *Capnocytophaga canimorsus* are splenectomy, alcoholism, and chronic lung disease.[79] Disseminated intravascular coagulation, hypotension, cutaneous gangrene, and renal failure have occurred in patients with leukemia or lymphoma and in association with steroid therapy. Symptoms ensue 1 day to 2 weeks after dog or, occasionally, cat bites occur; the overall mortality rate from *C. canimorsus* septicemia exceeds 20 percent.[25] Although removal of bilaterally affected knee joint prostheses was required to achieve cure of infection caused by *P. multocida* and *Pseudomonas aeruginosa* in one report,[72] cure also has been achieved with antibiotics and drainage with the prosthesis remaining in situ.

DIAGNOSIS AND TREATMENT

The most important aid in establishing the diagnosis of infection after animal bite wounds is the proper use of wound cultures. A culture need not be obtained from children evaluated in the immediate postinjury period (the first 8 hours) unless the bite is located on the face or hand or signs of infection are present. Isolates from an uninfected but contaminated wound reflect the normal flora of the biting animal and do not predict the future development of infection. Routine determination of the microorganisms colonizing bites on the face or hands is useful because of the potentially devastating consequences of infection at these locations. After the wound has been cleansed, culture should be performed routinely when evaluation is at an interval exceeding 8 hours from the time of injury, except for wounds evaluated more than 24 hours after injury and with no signs of infection.

When material is obtained for wound culture, the microbiology laboratory should be informed that the source of the specimen is an animal bite wound. This information should optimize accuracy because *P. multocida* can be mistaken morphologically for *Neisseria* or *Haemophilus influenzae*. Appropriate media must be used for the isolation of anaerobes, and gram-negative rods should be considered pathogens. A blood culture should be obtained for children with a temperature greater than 38.9°C [102°F] rectally) or if systemic toxicity is evident, although associated bacteremia occurs rarely.[34] Radiographic evaluation of the bite-injured area is indicated when the periosteum has been penetrated. A computed tomographic or magnetic resonance imaging study may be an aid to detecting periosteal defects, particularly in children with cranial bite wounds. For patients in whom wound infection has extended locally, sutures, if present, should be removed and material from the involved tissue compartment harvested for study. Material should be aspirated from areas of cellulitis or drained from areas of frank abscess formation. If osteomyelitis is suspected, a bone biopsy specimen should be submitted for Gram stain, culture, and histopathologic evaluation. If the course of the wound infection is indolent, acid-fast stains and mycobacterial cultures should be performed. Serum for serologic testing should be obtained from patients with symptoms suggesting cat-scratch disease, tularemia, syphilis, or blastomycosis. The details of the indicated diagnostic evaluation for systemic infections transmissible by animal bite (see Table 260–3) are specified in the appropriate chapters.

The first step in care of the wound is to cleanse it and the surrounding area. Visible dirt should be sponged away gently to avoid further damage to traumatized tissue. The wound should be irrigated copiously with normal saline. Cleansing by high-pressure syringe irrigation with a 20-mL syringe and 19-gauge needle is effective.[95] This method of irrigation decreased the incidence of infection associated with wounds from dog bites by fivefold, from 69 to 12 percent. Puncture wounds should be cleansed but not irrigated because irrigation may damage tissues further.

Devitalized tissues should be cautiously débrided. The issue of closure for bite wounds is controversial because of concern that sutured wounds have an increased rate of infection. No difference in the infection rate in 57 patients assessed prospectively was noted by one investigator, with the exception of those suffering hand bites, which were more likely to become infected when sutured.[18] In another prospective study, primary closure in patients presenting less than 8 hours after injury was associated with only slightly higher development of wound infection than that occurring after nonbite wound lacerations.[22] After appropriate cleansing and débridement have been achieved, most nonpuncture animal bite wounds except those involving the hand can be treated by primary closure without substantially increasing the risk of development of infection.[39,65]

Definitive data on which to base the use of antibiotic prophylaxis after animal bites are lacking. Smith and colleagues[90] provide a summary of prospective clinical trials that have addressed the issue. One investigator found that oxacillin did not reduce the incidence of infection after dog bite wounds[31] but did after cat bites.[32] Another study found a trend toward a reduced rate of infection, particularly for hand wounds, for patients receiving penicillin prophylaxis after incurring dog bites.[18] Significantly lower infection rates were observed for prophylaxis of wounds in patients presenting at 9 to 24 hours after injury but not for those presenting less than 9 hours later in another study.[15] Prophylactic

dicloxacillin, cephalexin, or erythromycin was not beneficial therapy for low-risk dog bite wounds in another prospective study because infection rates for wounds in both the antibiotic and control groups did not exceed 5 percent.[27]

A meta-analysis of eight randomized trials totaling 783 patients with dog bite wounds found that prophylactic antibiotics did reduce the incidence of infection.[24] The estimated cumulative incidence of infection was 16 percent in controls, and the relative risk was 0.56 (95% confidence interval 0.38-0.82) in patients given antibiotics. Treatment of approximately 14 patients was required to prevent one infection. Thus, one view is that prophylaxis has a role, but it should be used selectively.[19] Alternatively, some researchers consider antibiotic use therapeutic rather than prophylactic in this setting and suggest that antibiotic therapy be given in all cases of dog bites except those initially evaluated more than 24 hours after injury with no clinical signs of infection.[99]

Until additional data are available, a reasonable approach appears to be administration of prophylactic antibiotics in the following circumstances: (1) dog bites more than 8 hours old; (2) moderate to severe dog bites less than 8 hours old, especially if edema or crush injury is present; (3) puncture wounds, particularly if bone or joint penetration may have occurred; (4) facial wounds; (5) all hand bites; (6) wounds in the genital area; and (7) wounds in immunocompromised persons.[43,66,95,97]

Empiric treatment should be directed toward the most common infecting organisms: *P. multocida*, staphylococci, streptococci, and anaerobes.[66] Amoxicillin-clavulanate is active against almost all species of bacteria found in bite wounds and is the drug of choice for empiric oral treatment.[3,16,44] The recommended dosage is 40 mg/kg/day administered at 8-hour intervals. Erythromycin is only moderately active against *P. multocida*.[66,92] A recommended oral regimen for penicillin-allergic children is trimethoprim-sulfamethoxazole (10 mg/kg/day of the trimethoprim component) plus clindamycin (30 mg/kg/day).[3] Numerous other combination regimens such as cefuroxime axetil (30 mg/kg/day) plus clindamycin or metronidazole (30 mg/kg/day) can be used. For hospitalized patients requiring treatment, ampicillin-sulbactam (200 mg/kg/day) and ticarcillin-clavulanate (200 mg/kg/day) are the drugs of choice.[3] Imipenem (80 mg/kg/day) is an alternative choice. Consideration should be given, in the era of methicillin-resistant *S. aureus*, to empiric addition of clindamycin or vancomycin to the regimen in children with serious bite injuries, pending culture results. For penicillin-allergic children, combination therapy with an extended-spectrum cephalosporin or trimethoprim-sulfamethoxazole plus clindamycin should be administered.[3]

Newer macrolides, including azithromycin, show improved activity (versus erythromycin) against the organisms causing animal bite wound infections, but some strains of staphylococci and fusobacteria are resistant.[47] Azithromycin is more active than is clarithromycin against all *Pasteurella* spp.[45] To date, clinical trials documenting its efficacy are not available, and the use of regimens for which clinical experience exists is advisable. Similarly, quinolones and, in particular, gatifloxacin and moxifloxacin exhibit broad activity against the spectrum of pathogens isolated from bite wounds, with the exception of fusobacteria.[48,49]

Penicillin is the drug of choice for *P. multocida* infection. This organism has a median minimal inhibitory concentration to penicillin G of 0.1 to 0.8 μg/mL.[34] Other antibiotics with in vitro activity are ampicillin, tetracycline, chloramphenicol, cephalothin,[92] trimethoprim-sulfamethoxazole, and third-generation cephalosporins.[34] Cefuroxime is potentially useful and more active than is cephalexin.[44] Antibiotics with poor activity against *P. multocida* include the penicillinase-resistant penicillins, clindamycin, and aminoglycosides.[92]

As a general guideline, a suggested duration of therapy, assuming that proper drainage has been established, is 10 days for cellulitis or localized abscess, 2 to 3 weeks for tenosynovitis, and 3 to 4 weeks for osteomyelitis. When improvement is evident, treatment can be completed orally. At least 4 weeks of intravenous therapy should be administered to patients with endocarditis. Although the number of patients is too small to establish the required dosage with certainty, ampicillin (400 mg/kg/day) has been used successfully for the treatment of *P. multocida* meningitis.[10,51a] Antimicrobial therapy for wound infections should be adjusted according to the susceptibility of organisms isolated from the wound cultures.

In every child sustaining a bite wound, immunization status should be determined to assess the need for tetanus prophylaxis and whether rabies prophylaxis should be undertaken (see the appropriate chapters).

REPTILE BITES

Approximately 8000 people in the United States are bitten yearly by poisonous snakes. Approximately half occur in people younger than 20 years old. The curiosity of children can contribute to this exposure because many are bitten while handling poisonous snakes.[75] Between 9 and 15 persons die of snake bites each year.[59]

Venomous snakes are divided into four families, two of which, the Crotalidae (rattlesnakes, copperheads, and water moccasins) and the Elapidae (coral snakes), are found in the United States. Snake venoms are among the most complex of proteins. The local effects of the venom of pit vipers (Crotalidae), which is rich in proteolytic activity, include swelling, pain, edema, ecchymosis, and tissue necrosis with the formation of bullae.[85] Systemic effects result from increased blood vessel permeability and hemolysis and may include hematuria, hematemesis, and disseminated intravascular coagulopathy.[85] The venom of the eastern coral snake causes minimal local tissue destruction. It is a neurotoxin that produces paresthesia of the involved extremity, followed by involvement of the cranial nerves and bulbar paralysis.

Since the preantibiotic era, researchers have recognized that the oral flora of snakes' mouths frequently consists of multiple organisms, particularly gram-negative bacilli, staphylococci, and anaerobes.[76,102] Goldstein and associates[46] isolated 58 aerobic and 28 anaerobic organisms after culturing the venom from 15 rattlesnakes. The aerobes most commonly isolated were *P. aeruginosa*, *Proteus* spp., and *Staphylococcus epidermidis*. Among the anaerobes, *Clostridium* spp. were the isolates found most frequently; *Bacteroides fragilis* also was recovered. Defecation of prey during ingestion contributes to this preponderance of gastrointestinal flora. When the fangs are cleansed carefully before collection, the venom itself is sterile, thus indicating that bacterial isolates potentially contaminating snake bite wounds are a reflection of the oral flora.

In persons sustaining alligator bite wounds, infection is caused most commonly by *Aeromonas hydrophila*. A report of mixed infection with *A. hydrophila*, *Enterobacter agglomerans*, and *Citrobacter diversus* and the frequency with which *Proteus vulgaris* and *Pseudomonas* spp. are isolated from alligator mouths suggest that treatment of alligator bites should be directed at gram-negative species.[36] *Vibrio* infection should be suspected in victims of shark bites, as well as all wounds exposed to salt water.[77] *Aeromonas* spp. and other gram-negative bacilli, as well as *S. aureus* and anaerobes, all can infect shark bites.[84]

The exotic pet industry is growing in the United States, and the common green iguana is a popular pet. Few cases of infections from iguana bites have been reported because these creatures are generally nonaggressive, but *Serratia marcescens* cellulitis has been observed.[53]

The role of empiric antibiotics after reptile wounds is undefined, as is the incidence of infection and subsequent

complications. The inflammatory changes of envenomation can be difficult to differentiate from those of infection. At least one instance of osteomyelitis as a complication of an infected snake bite has been documented.[86] Some experts suggest using a broad-spectrum antibiotic for injuries with severe tissue involvement but not for bites with minor or minimal envenomation.[86] Others suggest that prophylactic antibiotics are not required in snake bite victims.[62,94] Amoxicillin-clavulanate is an appropriate oral antimicrobial and ampicillin-sulbactam plus gentamicin is an appropriate parenteral regimen for empiric treatment of reptile bite wounds.[3] The oral alternatives for penicillin-allergic children are the same as those given for other animal bite wounds; one parenteral regimen for a penicillin-allergic child is clindamycin plus gentamicin. Treatment should be guided by the results of Gram stain and susceptibility testing.

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CHAPTER 261

Bioterrorism

Robert J. Leggiadro

The intentional delivery of *Bacillus anthracis* spores through mailed letters or packages established the clinical reality of bioterrorism in the United States in the autumn of 2001.[7,32] An understanding of the epidemiology, clinical manifestations, and management of the more credible biologic agents is critical to limiting morbidity and mortality from a bioterrorism attack.[14,26,29,38]

Implementation of an effective response to deliberate release of biologic agents by terrorists requires detection and reporting of cases as soon as possible.[5,10] Prompt recognition of unusual clinical syndromes and increases above seasonal levels in the incidence of common syndromes or deaths from infectious agents is critical to an effective response.[5,21,29,36]

HISTORY

The concept of biologic warfare or terrorism is not a new one. In 1346 the Tatar force attacking Kaffa (also, Caffa) (now Feodossia, Ukraine) was struck by a plague epidemic.[11] To take advantage of this development, the Tatars catapulted the corpses of their plague victims into Kaffa. An outbreak of plague did occur in the city, representing one of the first biologic attacks recorded.[51]

Smallpox first was used as a biologic weapon during the French and Indian War of 1754 to 1767.[11] Apparently, British troops deliberately infected Native Americans with smallpox by giving them blankets from infected patients.

A secret branch of the Japanese army, Unit 731, reportedly caused outbreaks of plague by dropping plague-infected fleas over populated areas of China in World War II.[28] Bomb experiments of weaponized anthrax spores were conducted by the Allies on uninhabited Gruinard Island near the coast of Scotland and resulted in heavy contamination in 1942.[45] Viable anthrax spores persisted until the island was decontaminated with formaldehyde and seawater in 1986.

In response to a perceived German biologic warfare threat, the United States began research into the offensive use of biologic agents in 1943 at Camp Detrick (renamed Fort Detrick in 1956) in Frederick, Md.[19] President Nixon stopped all offensive biologic and toxin weapon research and production by executive order in 1969. Included among the agents destroyed as a result

of this action were *B. anthracis*, botulinum toxin, *Francisella tularensis*, *Coxiella burnetii*, Venezuelan equine encephalitis virus, *Brucella suis*, and staphylococcal enterotoxin B. Begun in 1953, the U.S. defensive program at Fort Detrick continues today as the U.S. Army Medical Research Institute of Infectious Diseases (USAMRIID).

The Biological and Toxin Weapons Convention was ratified in 1972 and went into effect in 1975.[52] It prohibits the development, production, stockpiling, or transfer of biologic weapons agents (microbial pathogens and toxins) for other than peaceful purposes and any devices used to deliver these agents. One hundred forty-three states are parties to the convention, with an additional 18 signatories. Unfortunately, the treaty was not accompanied by effective provisions for verification.

Although the Soviet Union signed the convention at its inception in 1972, it formed and funded an organization known most recently as Biopreparat (Chief Directorate for Biological Preparations) 2 years later.[16] This organization was designed to carry out offensive biologic weapons research, development, and production concealed behind legal and civil biotechnology research. Its capacity for production of biologic weapons included plague, tularemia, glanders, brucellosis, anthrax, smallpox, and Venezuelan equine encephalomyelitis.[1]

An incident at a military microbiology facility in Sverdlovsk (now Yekaterinburg) in the former Soviet Union in 1979 proved to be a grim warning of the dangers of biologic weapons science.[40] An accidental aerosolized release of anthrax spores resulted in at least 79 cases of anthrax, including 68 deaths. This largest reported outbreak of inhalational anthrax occurred in people living or working within a 4-km zone downwind of the facility.

Iraq's biologic warfare program commenced in earnest in 1985 after initial explorations in the late 1970s.[54] By the end of the Persian Gulf War in 1991, Iraqi scientists had investigated the biologic warfare potential of five bacterial strains, one fungal strain, five viruses, and four toxins. The United Nations Special Commission suspects that from 1991 to 1995, Iraq actively preserved biologic weapons capability, including botulinum toxin, anthrax, and *Clostridium perfringens* spores.[16]

The Japanese cult Aum Shinrikyo made several unsuccessful attempts at disseminating anthrax from rooftops and trucks in central Tokyo in the early 1990s before successfully releasing sarin nerve gas into Tokyo's subways and killing a dozen people in 1995.[53] A large community outbreak of salmonellosis was caused by the intentional contamination of restaurant salad bars for political reasons by members of a religious commune in The Dalles, Oregon, in 1984.[49]

EPIDEMIOLOGY

Potential biologic weapons share several characteristics. Ease of acquisition and production is a primary consideration. Other ideal properties include the potential to be aerosolized (particle size, 1-10 μm) and dispersed over a wide geographic area, as well as resistance to sunlight, desiccation, and heat. The potential to cause lethal or debilitating disease and person-to-person transmission are important features, as is lack of effective therapy or prophylaxis.[22,26,38]

Any small or large outbreak of disease merits evaluation as a potential biologic event.[41] Unusually high rates of disease (e.g., a cluster of life-threatening pneumonia cases in previously healthy adults), as well as unusual clinical syndromes, should signal a warning. Once definition of the cause and rate of attack have been determined, an epidemic curve based on the number of cases during a specific time can be calculated. The epidemic curve in a biologic event triggered by a point-source exposure most likely would be compressed, with a peak occurring in a matter of hours or days. The occurrence of a second curve peak would be possible with contagious agents as a result of person-to-person transmission. The steep epidemic curve expected in a bioterrorism attack is similar to what would be seen with other point-source exposures such as food-borne outbreaks.

Several epidemiologic clues may be helpful in determining whether further investigation into an outbreak as a potential biologic attack is warranted. A large epidemic, especially in a discrete population; more severe disease than expected for a given pathogen; and a disease unusual for a given geographic area (e.g., pulmonic tularemia in an urban setting) are major indicators. Multiple simultaneous epidemics of different diseases, outbreaks with both human and zoonotic consequences, and unusual strains or susceptibility profiles are additional helpful parameters. Variable attack rates as a function of agent release relative to the interior or exterior of a building also would be useful.[41] Although most bioterrorism attacks will be covert,[5,10] intelligence revealing plans for an attack, terrorist claims of deliberate release, or direct physical evidence of an attack obviously would point to a biologic event.

The emergence of mosquito-borne West Nile virus encephalitis in New York City in the summer of 1999 is an example of a naturally occurring outbreak that had elements of a potential bioterrorist attack.[5,21,47] This outbreak represented a disease occurring in an unusual (previously nonendemic) area, as well as one with zoonotic (birds) in addition to human consequences. It marked the first documented appearance of West Nile virus in the Western Hemisphere and the first arboviral outbreak in New York City since the yellow fever epidemics of the 19th century.[21] A large avian die-off, affecting primarily crows, preceded the outbreak in humans by at least several weeks.

CRITICAL BIOLOGIC AGENTS

In addition to anthrax, critical biologic agents include brucellosis, plague, Q fever, and tularemia; smallpox, viral encephalitis, and viral hemorrhagic fever; and illnesses related to botulinum and staphylococcal enterotoxin B toxins (Table 261–1).[5,33,36] The five more credible biologic agents are discussed further.

ANTHRAX

B. anthracis is a large sporulatory, gram-positive rod with three distinct life cycles featuring multiplication of spores in soil, animal (herbivore) infection, and human infection.[34] Anthrax continues to occur in developing countries where the organism is highly endemic and the use of animal anthrax vaccine is not comprehensive (e.g., Iran, Iraq, Turkey, Pakistan, and sub-Saharan Africa). Human cases may be classified as either agricul-

TABLE 261–1 Critical Biologic Agents

Bacteria
Anthrax
Plague
Q fever
Tularemia
Brucellosis
Viruses
Smallpox
Viral encephalitis
Viral hemorrhagic fevers
Toxins
Botulinum toxin
Staphylococcal enterotoxin B

tural or industrial. Herders, butchers, and slaughterhouse workers in direct contact with infected animals are susceptible to acquiring agricultural infection, and workers in mills that process animal hair and those handling bone meal may acquire industrial infection.[43]

The three forms of human anthrax are cutaneous, inhalational, and gastrointestinal. The most common form is cutaneous, which is acquired through contact with an infected animal or animal products. The much less common inhalational form results from the deposition of spores in the lungs, and gastrointestinal anthrax occurs after the ingestion of infected meat. Because human-to-human transmission of anthrax has not been reported, standard precautions are recommended for hospitalized patients with all forms of anthrax infection.[30,48]

In the United States, 224 cases of cutaneous anthrax were reported between 1944 and 1994.[30] Most cases in recent decades were a result of exposure to wool or animal hair.[43]

The clinical features and course of the first 10 confirmed cases of inhalational anthrax associated with bioterrorism in the United States in the fall of 2001 have been reported.[32] Epidemiologic investigation indicated that the outbreak was a result of intentional delivery of *B. anthracis* spores through mailed letters or packages. The median incubation period was 4 days and ranged from 4 to 6 days. Several clinical features of these patients were not emphasized in earlier reports of inhalational anthrax, a previously rare disease. Drenching sweating, nausea, and vomiting were common manifestations of the initial phase of illness in this outbreak. Pleural effusions were a remarkably consistent clinical feature. No predominant underlying diseases or conditions were noted.

None of the 10 patients had an initially normal chest radiograph. In addition to characteristic mediastinal widening (Fig. 261–1), paratracheal or hilar fullness, pleural effusions, and parenchymal infiltrates were noted. Computed tomography of the chest was more sensitive than was chest radiography in revealing mediastinal lymphadenopathy, and an elevation in the proportion of neutrophils or band forms represented an early diagnostic clue.

Inhalational anthrax previously was reported to be a biphasic illness with influenza-like symptoms such as fever, cough, malaise, fatigue, and chest discomfort in the first phase, followed briefly by 1 to 2 days of improvement before development of the acute phase 2 to 5 days later.[13] However, this brief period of improvement between the initial and fulminant phases of illness was not observed in the first intentional outbreak associated with mail.[27]

The 55 percent survival rate in these patients was higher than previously reported (<15%). Limited data on treatment of survivors suggest that early treatment with a fluoroquinolone and at least one other active drug (e.g., rifampin, clindamycin, or vancomycin) may improve survival.[7,48]

Nasal congestion, rhinorrhea, and sore throat, infrequently seen in this series, might help distinguish influenza-like illness from inhalational anthrax.[15] Newer diagnostic methods for *B. anthracis* infection include polymerase chain reaction (PCR), immunohistochemistry, and sensitive serologic tests. Recommendations for antibiotic treatment of inhalation anthrax include use of either ciprofloxacin or doxycycline, plus one or two additional antibiotics known to be active in vitro against *B. anthracis.* Additional antibiotics include clindamycin, vancomycin, imipenem, meropenem, chloramphenicol, penicillin, ampicillin, rifampin, and clarithromycin. Corticosteroids should be considered for meningitis or significant mediastinal edema.[3,27]

Cutaneous anthrax is characterized by a skin lesion evolving from a papule, through a vesicular stage, to a depressed black eschar, often surrounded by significant edema and erythema.[18,46,48] The lesion, which may mimic a spider bite, is usually painless and located on exposed parts of the body (i.e., face, neck, and arms). The incubation period ranges from 1 to 12 days but commonly is less than 7 days. With effective antimicrobial therapy, fatalities are rare occurrences (<1%). Cutaneous anthrax occurred in a 7-month-old infant who was exposed at his mother's workplace as a result of the Fall 2001 attack. This infant displayed severe microangiopathic hemolytic anemia with renal involvement, coagulopathy, and hyponatremia, unusual findings with cutaneous anthrax.[23]

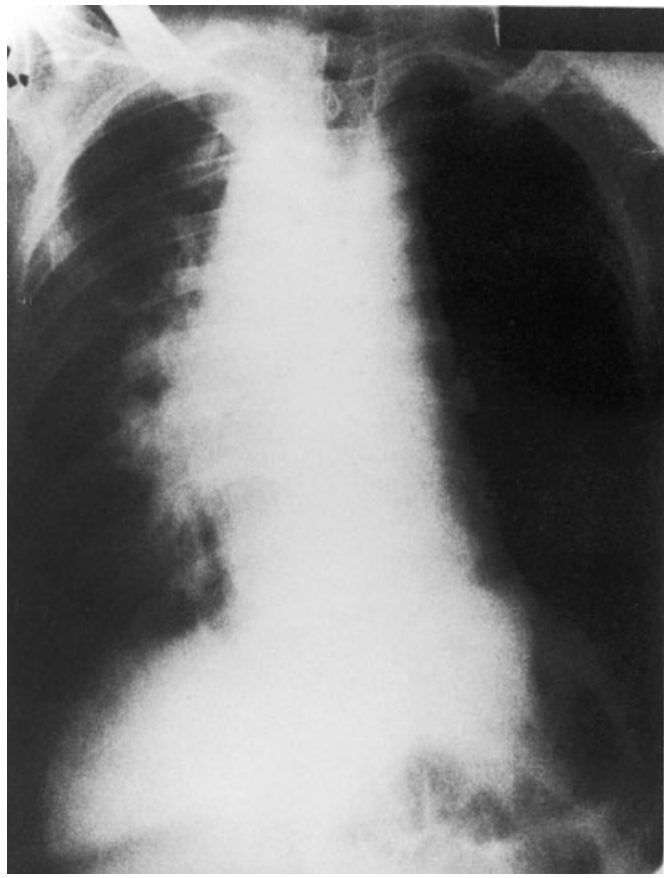

Figure 261–1 Chest radiograph showing a widened mediastinum secondary to hemorrhagic mediastinitis in a patient with a fatal case of inhalation anthrax. *(From LaForce, F. M., Bumford, F. H., Feeley, J. C., et al.: Epidemiologic study of a fatal case of inhalation anthrax. Arch. Environ. Health* 18*:798-805, 1969. Reprinted with permission of Helen Dwight Reid Educational Foundation. Published by Heldref Publications, 1319 Eighteenth St., NW, Washington, DC 20036-1802. Copyright 2000.)*

The organism grows readily on sheep blood agar and forms rough, gray-white colonies 4 to 5 mm in size with characteristic comma-shaped or "comet tail" protrusions.[34] *B. anthracis* is differentiated from other *Bacillus* spp. by an absence of the following: hemolysis, motility, growth on phenylethyl alcohol blood agar, gelatin hydrolysis, and salicin fermentation. Biosafety level 2 conditions for safe specimen processing in the microbiology laboratory and prompt confirmation of suspected isolates at the Centers for Disease Control and Prevention (CDC) or the USAMRIID in Fort Detrick are warranted.[30,43]

Postexposure vaccination with an inactivated, cell-free anthrax vaccine may be indicated along with ciprofloxacin, doxycycline, or amoxicillin chemoprophylaxis after a proven biologic event has occurred.[48,50] Recommendations for postexposure prophylaxis (and prolonged therapy following initial therapy for inhalational or cutaneous anthrax) include three options: (1) a 60-day course

of antibiotics followed by careful clinical observation, (2) extension of antibiotic therapy to 100 days, or (3) extension of antibiotic therapy to 100 days combined with administration of anthrax vaccine in three doses at 2-week intervals.[3,27] Pre-exposure vaccination may be indicated for the military and other select populations or for groups for which a calculable risk can be assessed.

SMALLPOX

After a worldwide eradication program, the last known endemic case of smallpox occurred in Somalia in 1977, and the World Health Organization declared smallpox eradicated in 1980.[24] No animal reservoir exists. Current recognized stocks of variola virus are authorized only at the CDC in Atlanta and a Russian state laboratory in Koltsovo. However, additional variola isolates, either long held unreported or acquired through security breaches, also may exist. Because vaccination against smallpox ceased in the United States in 1972, virtually the entire population now would be considered susceptible because immunity wanes over the course of time. Release of an aerosol would be the most likely route of transmission during an act of bioterrorism.

Smallpox is transmitted by respiratory secretions and requires close person-to-person contact. The incubation period generally is 12 to 14 days, with a range of 7 to 17 days. The prodromal illness of classic variola major features an acute onset of malaise, fever, rigors, vomiting, headache, and backache. Two to 3 days later, a discrete rash appears on the face, hands, forearms, and mucous membranes; it spreads to the legs and then centrally to the trunk during the second week of illness. Lesions progress from macules to papules to pustular vesicles during the course of 1 to 2 days. Umbilicate scabs form 8 to 14 days after onset and leave depressions and depigmented scars.[25]

In contrast to varicella (chickenpox), the rash of smallpox is centrifugal, with a concentration of lesions on the face and extremities, including the palms and soles, versus the trunk in varicella (Fig. 261–2). Smallpox lesions also are synchronous in stage of development, whereas the lesions of chickenpox appear in crops every few days, which results in lesions at very different stages of maturation in different areas of the skin. Any confirmed case of smallpox represents an international emergency and must be reported to national authorities through local and state health departments.[24]

Historically, the mortality rate associated with smallpox was 30 percent for unvaccinated contacts, and currently no antiviral therapy of proven efficacy has been developed. Supplies of vaccinia vaccine and vaccinia immune globulin are available only through the CDC.[24] Postexposure vaccination and strict quarantine are indicated for all household and other face-to-face contacts of suspected smallpox cases.[24]

In a limited outbreak with few cases, hospitalized patients should receive care in negative-pressure rooms with high-efficiency particulate air filtration. In addition, precautions using gloves, gowns, and masks are indicated. Home isolation and care are appropriate for most patients in larger outbreaks.[24]

Between January 24 and October 31, 2003, 37,901 volunteers in 55 jurisdictions received 38,885 smallpox vaccinations through the U.S. Department of Health and Human Services program, with a take rate of 92 percent.[9] The Vaccine Adverse Event Reporting System (VAERS) received 822 adverse event reports related to these vaccines, an overall reporting rate of 217 per 10,000 vaccinees. Seven hundred twenty-two nonserious reports to VAERS included multiple signs and symptoms of mild systemic and self-limited local reactions, including fever, rash, pain, and headache.[9]

No cases of preventable life-threatening adverse reactions, such as eczema vaccinatum, progressive vaccinia, or fetal vaccinia, were reported. No cases of vaccinia contact transmission occurred. No vaccinee or contact of this program received vaccinia immune globulin. Rigorous smallpox vaccine safety screening, educational programs, and older vaccinees may have contributed to low rates of preventable life-threatening adverse reactions.[9]

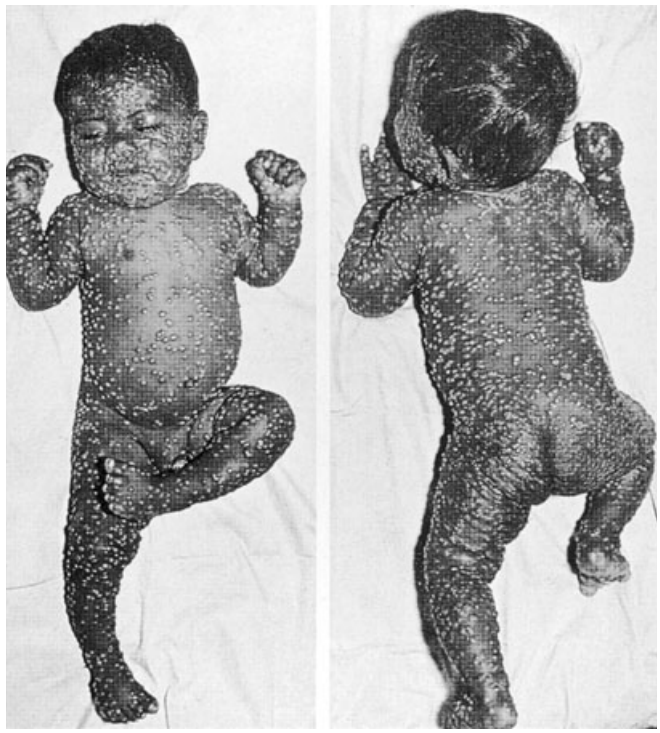

Figure 261–2 The lesions of smallpox are at the same stage of development on each area of the body, are deeply embedded in the skin, and are more densely concentrated on the face and extremities. *(From Henderson, D. A.: Smallpox: Clinical and epidemiologic features. Emerg. Infect. Dis. 5:537-539, 1999.)*

One-hundred adverse events were designated as serious, resulting in 85 hospitalizations, two permanent disabilities, 10 life-threatening illnesses, and three deaths. Among the serious adverse events, 21 cases were classified as myocarditis and/or pericarditis and 10 as ischemic cardiac events that were not anticipated on historical data. Two cases of generalized vaccinia and one case of postvaccinial encephalitis also were detected.[9]

PLAGUE

Plague, a zoonotic illness caused by the gram-negative bacillus *Yersinia pestis*, is primarily a disease of rodents, with transmission occurring through infected fleas. Human disease is acquired through rodent flea vectors, as well as respiratory droplets from animals to humans and humans to humans. Transmission of plague to humans in the United States primarily is via the bites of fleas from infected rodents. From 1970 to 1995, 341 cases of human plague were reported in the United States, most commonly from Arizona, California, Colorado, and New Mexico.[44] Indications of a deliberate release of plague bacilli would include the occurrence of cases in locations not known to have enzootic infection, in persons without known risk factors, and in the absence of previous rodent deaths.[28]

The three clinical forms of human plague are bubonic, primary septicemic, and pneumonic. Bubonic plague, characterized by the development of an acute regional lymphadenopathy, is the most

frequent clinical form and accounts for 80 to 90 percent of U.S. cases.[44] However, the pneumonic form would be the most likely manifestation as a result of release of an aerosol during a biologic attack.[22,28] This clinical form is the least common, but it has the highest mortality rate; it is almost always fatal if antibiotics are not begun within 24 hours of the onset of symptoms. Septicemic plague without obvious lymphadenopathy may be more difficult to diagnose than is bubonic plague because of nonspecific manifestations (i.e., fever, chills, abdominal pain, nausea, vomiting, diarrhea, tachycardia, tachypnea, and hypotension).[28] Delay in establishing the diagnosis and initiating appropriate therapy may lead to death.[28,37]

The incubation period for primary pneumonic plague is 1 to 3 days. Fever, chills, headache, and rapidly progressive weakness are characteristic of all clinical forms of plague. Cough, dyspnea, and hemoptysis are distinctive of primary pneumonic plague. The sudden appearance of a large number of previously healthy patients with fever, cough, shortness of breath, chest pain, and a fulminant course leading to death should suggest the possibility of pneumonic plague or inhalational anthrax immediately. The presence of hemoptysis would strongly suggest plague.[4,28]

Y. pestis may be identified in clinical specimens by Gram, Wright-Giemsa, Wayson, and immunofluorescence staining methods, in addition to standard bacterial culture. Appropriate clinical specimens include lymph node aspirates and blood, as well as tracheal washes or sputum smears if pneumonic plague is suspected. Tests that would be used to confirm a suspected diagnosis, including antigen detection, immunoglobulin M (IgM) enzyme immunoassay, and PCR assay, are available only through state health departments, the CDC, and military laboratories.[28]

Effective therapy is available in the form of streptomycin, gentamicin, chloramphenicol, doxycycline, and ciprofloxacin.[28] Parenteral aminoglycoside therapy is recommended in a contained casualty setting (modest number of patients requiring treatment); oral therapy is recommended in a mass casualty scenario.[28] The potential benefits of doxycycline and ciprofloxacin in the treatment of pneumonic plague infection in children substantially outweigh the risks.[10,28] An inactivated, whole-cell *Y. pestis* vaccine was discontinued by its manufacturers in 1999 and no longer is available.[28]

In addition to standard precautions, droplet precautions are indicated for all patients with suspected plague until pneumonia is excluded and appropriate therapy has been initiated. Droplet precautions should be continued in patients with confirmed pneumonic plague for 48 hours after the initiation of appropriate therapy.[28] Only standard precautions are recommended for bubonic plague.

TULAREMIA

The etiologic agent of this zoonotic illness is *F. tularensis*, a gram-negative coccobacillus. The disease may be acquired from ticks and deer flies, contact with animals such as rabbits and rodents, ingestion of contaminated water, or inhalation of aerosols. In a bioterrorist event, inhalation of an aerosol would be the most likely route of infection.[4,17] Human-to-human transmission of tularemia has never been reported. The annual incidence of tularemia in the United States is less than 200 cases; all suspected or confirmed cases must be reported to health authorities.

Clinical forms of the disease include ulceroglandular, glandular, oculoglandular, oropharyngeal, pneumonic, and typhoidal, the type reflecting the organism's portal of entry.[20,31] Either pneumonic alone or typhoidal, with or without a pneumonic component, would be the most likely clinical manifestation of tularemia as a result of aerosol release during a biologic attack.[4,17] The incubation period for tularemia is 3 to 6 days, with a range 1 to 21 days. Typhoidal tularemia may manifest as fever of unknown origin. Standard precautions are indicated for hospitalized patients with all forms of tularemia.[17]

The diagnosis is established most often by serologic testing, and isolation of *F. tularensis* from clinical specimens requires cysteine-enriched media or inoculation of laboratory mice. In addition to a need for special media, the laboratory should always be informed when tularemia is suspected because of the potential hazard to laboratory personnel. Suspected isolates should be confirmed by the CDC or USAMRIID through local or state health departments.[17]

Effective therapeutic agents include streptomycin, gentamicin, tetracycline, ciprofloxacin, and chloramphenicol; post-exposure prophylaxis with doxycycline or ciprofloxacin may be considered.[17] The benefits of tetracycline or ciprofloxacin therapy may outweigh the risks for children younger than 8 years in select clinical situations, including tularemia.[17,35] A live attenuated vaccine for pre-exposure use is available through the USAMRIID.[12]

BOTULISM

Seven distinct but related neurotoxins, A through G, are produced by different strains of *Clostridium botulinum*, an anaerobic gram-positive rod. The most common types in U.S. food-borne outbreaks are A, B, and E; outbreaks with unusual botulinum toxin types (i.e., C, D, F, and G, or E not acquired from an aquatic food) would suggest deliberate release.[2] Classic neuroparalytic disease is acquired through the ingestion of preformed neurotoxin. Other forms include localized infection (wound botulism) and *C. botulinum* intestinal colonization in infants with in vivo toxin production (infant botulism). Botulism in the United States is seen most often in small clusters or single cases associated with home-canned foods. Although airborne transmission of botulinum neurotoxin does not occur naturally, aerosolization of preformed toxin would be the most likely route of transmission in a bioterrorism event.[2,22] Sabotage of food supplies is also possible. Botulism is not transmitted from human to human; standard precautions are recommended for hospitalized patients.

The incubation period for food-borne botulism generally is 12 to 36 hours (range, 6 hours to 8 days). The clinical manifestations of disease acquired by inhalation would be the same as those for food-borne botulism. Early manifestations include blurred vision, diplopia, and dry mouth. Patients are afebrile with a clear sensorium.

Later clinical features indicative of more severe disease include dysphonia; dysarthria; dysphagia; ptosis; and symmetric, descending, progressive muscular weakness with respiratory failure.[2] Clinical suspicion is critical because a recognized source of exposure may be absent in a biologic attack. Botulism is a reportable disease.

A toxin neutralization bioassay in mice is used to identify botulinum toxin in serum, stool, or food. *C. botulinum* also may be cultured from stool and food. Electromyography can be helpful diagnostically. Botulinum antitoxin of equine origin, available from the CDC and state or municipal health departments, should be administered as soon as possible to patients symptomatic with botulism after testing for hypersensitivity to equine sera.[2,19] A pentavalent toxoid of *C. botulinum* toxin types A, B, C, D, and E is available as a vaccine under investigational drug status through the CDC or Department of Defense.[2,12]

VIRAL HEMORRHAGIC FEVER

The term viral hemorrhagic fever (VHF) refers to a clinical illness associated with fever and a bleeding diathesis caused by a

virus belonging to one of four distinct families: Filoviridae (e.g., Ebola, Marburg), Arenaviridae (e.g., Lassa fever), Bunyaviridae (e.g., Rift Valley fever), and Flaviviridae (e.g., yellow fever). VHF agents are RNA viruses normally transmitted to humans from animal reservoirs or arthropod vectors, although the natural reservoirs and vectors of the Ebola and Marburg agents are unknown. Most of these agents are considered serious, potential biologic threats because of their potential to be aerosolized and their high morbidity and/or mortality rates. Clinical features vary with the specific virus, but all are capable of causing fever, myalgia, prostration, petechiae, hemorrhage, and shock.[6]

Treatment generally is supportive, although ribavirin has some in vitro and in vivo activity against arenaviruses and bunyaviruses, but not against filoviruses or flaviviruses. VHF-specific barrier precautions, as well as airborne precautions, are recommended for any patient with suspected or documented VHF. Effective prophylaxis following exposure to an VHF agent is not available.[6]

PREPAREDNESS AND RESPONSE

Being prepared for a bioterrorist attack or any other large-scale infectious disease outbreak relies on similar critical elements.[21,29,39,42,47] Clinician awareness and education are paramount. Because practicing physicians most likely will be the first group to encounter diseases caused by biologic weapons, they must be familiar with the signs and symptoms of the more credible biologic agents (e.g., smallpox, anthrax).[29,39,42] Recognition of an unusual case or cluster of illnesses should prompt a report to the local public health authorities.[8,21] Improved communication between human and animal health authorities is warranted because many potential bioterrorist agents, such as anthrax, brucellosis, Q fever, plague, and tularemia, are zoonotic diseases.[21] Knowledge of the processes by which the hospital laboratory, the local or state health department, or the CDC performs diagnostic studies to implicate or exclude biologic agents also is important.[5,21,29] Local, state, and federal public health agencies must coordinate plans for dealing with a large-scale outbreak caused by a biologic event.[5,8,21,47] They should address rapid investigation of the outbreak, public education, mass distribution of antibiotics and vaccines, the capacity to care for mass casualties, and proper, expeditious treatment of the dead.[5,21,29,47]

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CHAPTER 262

Bacterial Laboratory Diagnosis

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The plethora of bacterial pathogens causing infections in children poses multiple challenges for the pediatrician. Overlapping or similar clinical presentations may result in the simultaneous consideration of established and emerging pathogens. Fortunately for pediatric medicine, the continuous development and refinement of molecular strategies has complemented ongoing improvements in routine approaches for the laboratory diagnosis of bacterial infections.

Bacteria are unicellular prokaryotic organisms that may cause infections as single pathogens or disease-causing strains. At the same time, commensal bacteria comprise 90 percent of the cellular content of human beings, with an estimated 10^{14} bacteria compared with estimates of 10^{13} human cells.[7] The gene content of the intestinal microbiota is estimated to be 100-fold greater in magnitude than the content of the human genome. The challenge for diagnostic microbiology laboratories often becomes a quest to distinguish potential pathogens from commensal bacteria not involved in disease. Such pathogens may belong to a specific clonal or genetic lineage with well-characterized toxins or virulence factors. Additionally, polymicrobial infections may be caused by two or more pathogens, resulting in mixed infections. In the current era of metagenomics, scientists are beginning to understand disorders of microbial ecology and how changes in complex bacterial communities may affect human health.

Bacteria are defined by several unifying features, including the ability to proliferate as single-celled organisms lacking a nuclear membrane or cellular organelles. Their ability to proliferate as free-living cells enables the diagnostic laboratory to employ routine culture-based approaches for pathogen isolation. In addition to culture on plated (solid) or liquid media, pathogens or toxins may be detected directly by protein (antigen) immunoassays or nucleic acid (DNA or RNA) diagnostics. Adaptive immune responses to specific pathogens may be exploited for pathogen detection by antibody immunoassays, and such approaches may offer diagnostic solutions when pathogens cannot be cultured readily in the laboratory.

The sections in this chapter will be grouped by the basic category of infection. The central issues of specimen collection, transport, and reporting will be considered within the context of each type of infection. More detailed diagnostic strategies are described in other chapters of this edition and in the context of specific pediatric infections. Rather than provide a detailed listing of tests and methods, this chapter will highlight basic laboratory strategies and important diagnostic principles important for each category of pediatric infections (Table 262–1).

RESPIRATORY TRACT INFECTIONS

Bacterial pathogens represent important causes of pediatric upper and lower respiratory tract infections. Other pathogens such as respiratory viruses or fungi may cause respiratory tract infections and mimic the clinical presentations of bacterial infections. Furthermore, children may be co-infected with viruses and bacteria so that even if a predominant bacterial pathogen is identified, a viral pathogen also may need to be considered. Respiratory tract infections caused by different pathogens may have overlapping features and may not be clinically distinguishable with respect to the etiologic agent.

BACTERIAL CULTURES OF THE UPPER RESPIRATORY TRACT

The diagnoses of upper and lower respiratory tract infections require sampling of different sites and by different methods. Upper respiratory tract specimens include throat or nasopharyngeal swabs, sputa, or tracheal aspirates and are considered nonsterile. Lower respiratory tract specimens include bronchoscope-assisted collections of fluids or tissues of the lower respiratory tract and are considered relatively sterile samples. The distinctions between sterile and nonsterile specimens are important considerations in the laboratory because potential pathogens must be distinguished from the commensal microbiota of the respiratory tract.

The throat and nasopharynx may serve as important reservoirs and primary sites for upper respiratory tract infections. Oropharyngeal or throat swabs from children may be processed for antigen immunoassays, throat cultures, or nucleic acid amplifica-

TABLE 262–1 Basic Laboratory Strategies for Diagnosis of Bacterial Infections

Site of Infection	Rapid Assessment	Routine/Special Tests
Central nervous system	CSF Gram stain	CSF culture DNA amplification
Gastrointestinal tract		
Gastric	*Helicobacter pylori* serology Urea breath testing	Tissue urease testing Histology with special stains
Intestinal	Stool microscopy (O&P) Stool antigen testing	Stool culture
Genitourinary tract	Urinalysis Urine Gram stain	Urine culture DNA amplification
Peripheral blood	Leukocyte count/differential Serology	Blood culture
Respiratory tract	Direct Gram stain Urine antigen testing	Respiratory culture DNA amplification
Wounds and superficial sites	Direct Gram stain	Tissue/aspirate culture

CSF, cerebrospinal fluid; O&P, ova and parasites.

tion. Bacterial cultures are recommended as a standard approach in the laboratory to evaluate patients with suspected streptococcal pharyngitis.[29,54] Swabs made of synthetic fibers such as Dacron are preferable for collection and are used to obtain samples from the tonsillar areas and posterior oropharynx. Plated media with blood agar, including media selective for streptococci, are used to isolate *Streptococcus pyogenes* or other β-hemolytic streptococci from throat swabs. Non–serogroup A β-hemolytic streptococci, especially groups C and G, may cause pharyngitis and may be isolated from throat cultures. *Arcanobacterium hemolyticum*, a gram-positive pathogen that may cause pharyngitis in adolescents, can be isolated from throat cultures after prolonged incubation (>72 hours) and has colonies that produce small zones of β-hemolysis.[2,41] Nasopharyngeal cultures and sinus aspirates may be useful for the evaluation of children with acute otitis media and sinusitis.[13,88] Although nasopharyngeal or sinus aspirate cultures are not performed routinely, pathogens such as *Streptococcus pneumoniae*, *Haemophilus influenzae*, or *Moraxella catarrhalis* may be isolated on plated media with blood or chocolate agar and may facilitate establishing the diagnoses in children with protracted illnesses.[13,24] Collection of sinus aspirates may require sinus endoscopy by pediatric otorhinolaryngologists. Bacterial cultures of sinus aspirates should include both routine (aerobic) and anaerobic cultures with blood agar and media capable of isolating fastidious pathogens such as *Haemophilus* spp.

BACTERIAL CULTURES OF THE LOWER RESPIRATORY TRACT

The diagnosis of pneumonia can be challenging, even with the proper clinical specimens for laboratory evaluation. In a recent report, evidence of a potential etiologic agent varied tremendously (24 to 85% of cases) among children being evaluated for pneumonia, depending on the numbers and types of tests performed.[82] A pathogen was identified in 79 percent of hospitalized children (age range, 2 months to 17 years) with radiographically confirmed lower respiratory tract infections.[57] Sputum Gram stain and cultures are recommended as standard approaches for the evaluation of bacterial pneumonia or of infections of the lower respiratory tract, especially in older children and adolescents. In young children, sputum samples may be difficult to obtain, and bronchoscope-assisted collection of lower respiratory tract specimens may be required. Sputum and bronchoscopic specimens are evaluated routinely by light microscopy and Gram stain in order to visualize polymorphonuclear infiltration and predominant microbial morphologies. Careful Gram stain evaluation continues to be a useful tool for routine evaluation of patients suspected of having acute bacterial pneumonia. The presence of abundant neutrophils and a single predominant microbial morphotype provides strong support for the diagnosis of acute bacterial pneumonia.[61,98] Neutrophils may be sparse in children with neutropenia, so the immune status of patients should be considered.

Sputum and tracheal aspirate cultures are plated on different solid media including blood agar, chocolate agar, and MacConkey agar for a minimum of 48 hours. Primary bacterial etiologies of acute bacterial pneumonia such as *S. pneumoniae*, *Staphylococcus aureus*, and *S. pyogenes* are isolated routinely by sputum culture on plated blood agar. In a recent study of radiographically confirmed pneumonia in children, 60 percent of cases included a bacterial pathogen and 73 percent of cases with a bacterial pathogen included *S. pneumoniae* as the primary agent.[57] Up to 23 percent of children in this study yielded evidence of viral and bacterial co-infections in the context of pneumonia. Gram-positive pathogens can be diagnosed by morphologic and limited biochemical testing. The successful isolation of *S. pneumoniae* from sputum or bronchoscopic cultures continues to be the top priority for the laboratory evaluation of bacterial pneumonia in children. Colonies of *S. pneumoniae* may be visible as alpha-hemolytic colonies on blood agar, requiring 24 to 48 hours for successful culture. Optochin susceptibility and Gram stain morphology are key tests for identification of pneumococcal isolates.[16,63]

Different media formulations may be important for the isolation of different respiratory pathogens. Chocolate agar may be important for the isolation of fastidious pathogens such as non-typeable *H. influenzae*, whereas other *Haemophilus* spp. may be identified subsequently with additional agar-based nutrient testing and limited biochemical evaluation. Gram-negative pathogens such as *Klebsiella pneumoniae* or *Pseudomonas aeruginosa* may be cultivated on MacConkey agar. Cases of aspiration pneumonia in pediatrics may necessitate the addition of anaerobic cultures with plated blood agar and selective media for organisms such as *Bacteroides* or *Fusobacterium* spp.[12] Tracheal aspirates may facilitate establishing the diagnosis of aspiration pneumonia, ventilator-associated pneumonia, and tracheitis. Like sputum cultures, tracheal aspirate cultures are considered nonsterile, and differential or selective media are helpful. Children, especially younger pediatric patients, may not produce sufficient or adequate sputum for evaluation in the laboratory. If sputum is difficult to obtain, tracheal aspirates or induced sputum specimens may be collected. Bronchoscope-assisted collection, such as bronchial wash or bronchoalveolar lavage (BAL), may be necessary for adequate sampling and assessment. Such specimens should be evaluated by direct Gram stain and usually lack confounding features of the upper respiratory tract such as epithelial cell shedding. Bronchial wash and BAL cultures also should be plated on different media such as blood and MacConkey agar in addition to thioglycollate broth for the isolation of gram-positive and gram-negative bacterial pathogens. Unusual bacterial respiratory pathogens such as *Legionella* spp. must be cultured on specialized media such as buffered charcoal yeast extract (BCYE) agar.[93]

Children with cystic fibrosis and associated respiratory tract infections pose special challenges for the diagnostic microbiology laboratory.[58] *S. aureus* is an important cause of respiratory tract infections in cystic fibrosis. Although *S. aureus* isolates usually are relatively easy to grow and identify in the laboratory, small-colony variants with different colonial features can be identified in up to 50 percent of patients with cystic fibrosis.[39,40] The small-colony variants are nonhemolytic, nonpigmented, and slow-growing, rendering them difficult to identify as *S. aureus*. Media such as mannitol salt agar can effectively support *S. aureus* auxotrophs while minimizing overgrowth for successful isolation. *P. aeruginosa* also represents a primary respiratory pathogen of special significance in this population. *P. aeruginosa* can be observed by sputum Gram stain and cultured on blood agar during routine examination of respiratory cultures. *P. aeruginosa* isolates that are mucoid in appearance in routine cultures may be associated with virulent features.[50,62] Although these organisms usually can be identified by routine biochemical approaches, isolates from patients with cystic fibrosis may be difficult to identify by conventional methods. In these cases, DNA sequencing may be helpful for identification purposes. In addition, organisms of the *Burkholderia cepacia* complex may contribute to the respiratory pathology of cystic fibrosis, and nine different species comprise the *B. cepacia* complex.[48] One species in particular, *Burkholderia cenocepacia*, has been associated with most cases of cepacia syndrome, an overwhelming systemic infection that may cause rapid demise and preempt pulmonary transplantation.[52] The specialized media required to culture these organisms are additional formulations that complement the routinely used media for respiratory cultures. Although *B. cepacia* complex organisms can be cultured and identified by biochemical testing as members of the complex, speciation requires DNA sequencing of informative target genes, which typically is performed by reference laboratories. The identification of particular species by

molecular methods may affect decisions regarding lung transplantation and appropriate patient management.[4,58]

ANTIGEN AND ANTIBODY IMMUNOASSAYS

Direct immunoassays for antigens or antibodies specific to individual pathogens may facilitate establishing the diagnoses of atypical pneumonia, bacterial pneumonia, pertussis, and legionellosis. Such immunoassays may be used to detect pathogens directly in respiratory tract specimens, serum, or urine. The most common application of direct antigen detection in pediatrics is rapid immunoassay-based screening for *S. pyogenes.* Point-of-care tests include optical and membrane immunoassays for detection of antigens specific for *S. pyogenes* (group A Streptococcus).[46,96] Immunoassay-based screening of *S. pyogenes* should be supplemented with throat cultures, and direct nucleic acid amplification represents an alternative for diagnosis of streptococcal pharyngitis.[17,35,73]

Antigen immunoassays that rely on immunofluorescence may be helpful for establishing the diagnosis of legionellosis and pertussis in respiratory tract specimens, although these tests have diminished sensitivities compared with direct DNA detection.[26,49,75] Immunoassays may detect circulating IgM or IgG antibodies specific for *Mycoplasma pneumoniae* or *Chlamydia pneumoniae* and provide a minimally invasive diagnostic modality. However, serologic tests usually are supplanted by direct DNA amplification due to superior sensitivity and specificity.

Alternative methods of diagnosing respiratory infections include urinary antigen testing for *Legionella pneumophila* or *S. pneumoniae*.[3,38] Urinary antigen testing for *L. pneumophila* provides excellent sensitivity and is a useful adjunctive test, especially when delays in specimen transport and culture processing are encountered. If the diagnosis is considered after initiation of broad-spectrum therapy, it may be more convenient to obtain a urine specimen because antigen excretion may occur for several weeks.[45,85] In the case of *S. pneumoniae*, urinary antigen testing has been controversial, but this test may be useful in cases for which transport of the specimen is delayed and cultures may be nonviable.

MOLECULAR DIAGNOSTICS—NUCLEIC ACID AMPLIFICATION AND PROBES

A patient with a chronic, nonproductive cough with or without fever may highlight the need for evaluation of atypical respiratory pathogens. These organisms include *Bordetella pertussis* and other *Bordetella* spp., *C. pneumoniae*, and *M. pneumoniae*. Nasopharyngeal aspirates or Dacron swabs should be sampled for the diagnosis of *Bordetella* pathogens, including *Bordetella parapertussis*, *B. pertussis*, and *Bordetella holmesii*.[95] Infections caused by these organisms cause similar symptoms, although *B. pertussis* is more widely prevalent and causes more serious infections. When considering respiratory infections by *Bordetella* spp., most laboratories process nasopharyngeal aspirates or swab specimens for direct DNA amplification. Immunoassays and culture also may be performed for *Bordetella* spp. using direct immunofluorescence or specialized media, respectively, but sensitivities are reduced compared with DNA amplification.[30,32,55] Pathogens associated with atypical pneumonia such as *C. pneumoniae* and *M. pneumoniae* are difficult to culture in most laboratory settings. Therefore, throat swabs or sputum specimens from patients suspected of having atypical pneumonia should be directed for testing by serology or direct DNA amplification.[28,60] A significant proportion of children up to 15 years of age with *C. pneumoniae* or *M. pneumoniae* infections will either present with pneumonia or subsequently progress to pneumonia if untreated.[33,74,99] The diagnosis of *M. pneumoniae* as a cause of atypical pneumonia requires IgM serologic testing or molecular methods for direct detection. The utility of polymerase chain reaction (PCR) has been demonstrated for the detection of *M. pneumoniae* DNA in throat swabs, sputa, and lower respiratory specimens.[14,21,56,59] DNA probe-based hybridization and real-time PCR have been used successfully for detection and diagnosis of streptococcal pharyngitis using throat swabs.[17,90]

GASTROINTESTINAL TRACT INFECTIONS

Infections of the gastrointestinal (GI) tract may occur in different regions such as the stomach, small intestine, or colon, and direct sampling of intestinal contents may or may not be required. Bacterial cultures of intestinal contents remain important for the diagnostic evaluation of infectious gastroenteritis or colitis, but other approaches such as breath testing and direct antigen detection may be important for assessment of different bacterial pathogens. An obvious challenge with diagnostic approaches for GI tract infections is the need to distinguish pathogens from commensal bacteria.

GASTRIC INFECTIONS

In the stomach, *Helicobacter pylori* and a restricted set of other *Helicobacter* spp. may cause chronic gastritis and predispose patients to an increased lifetime risk of developing peptic ulcer disease and gastric cancer.[10,92] Nonendoscopic approaches are attractive for diagnostic evaluation of children because pediatric patients usually present with dyspepsia and evidence of chronic or erosive gastritis without evidence of peptic ulcer disease. Diagnostic methods include the collection of peripheral blood for assessment of serum IgG antibodies to *H. pylori.*[92] Detection of *H. pylori* may be performed conveniently in children without endoscopy or culture by fecal antigen testing or the urea breath test.[64,92] If biopsy sampling is required, *H. pylori* may be visualized by special stains in addition to the typical pattern of mixed inflammation seen on hematoxylin and eosin (H&E) stained biopsy specimens. Additionally, rapid tissue-based urea testing may be performed directly with biopsy samples by different colorimetric tests.[64] The detection of other *Helicobacter* spp. such as "Candidatus Helicobacter heilmannii" may require visualization with special stains following esophagogastroduodenoscopic evaluation of gastric biopsy specimens.[83]

INTESTINAL INFECTIONS AND STOOL CULTURES

Acute bacterial gastroenteritis usually is caused by various gram-negative enteric pathogens, and direct visualization of stool specimens by Gram stain rarely is beneficial in evaluating patients with infectious gastroenteritis. Routine stool cultures should be performed with at least two selective and differential media formulations (e.g., MacConkey agar, xylose-lysine-deoxycholate agar, Hektoen enteric agar) in order to distinguish candidate enteric pathogens from the commensal microbiota. The inoculated formulations are incubated at 37°C for 24 to 48 hours. Likely enteric pathogens such as *Salmonella* spp. and *Shigella* spp. can be distinguished by their lactose-negative phenotype on differential media, followed by comprehensive biochemical panels for confirmation of genus and species identifications. As a rule, gram-negative enteric bacterial pathogens share close phylogenetic relationships, and as such, species-level identification necessitates comprehensive biochemical panels that may be

performed via automated (e.g., Vitek, bioMérieux; MicroScan, Dade Behring) or manual approaches (e.g., API, bioMérieux) in the laboratory.

Additional pathogens such as enterohemorrhagic *Escherichia coli* (EHEC) and *Campylobacter jejuni* may require different approaches for detection. EHEC may be cultured on MacConkey-sorbitol agar medium, but occasionally, EHEC isolates may be able to ferment sorbitol. In addition to plate-based screening for the lack of sorbitol-fermenting capacity, direct toxin immunoassays or real-time PCR may be used to test EHEC organisms. Importantly, many EHEC isolates belong to serotypes other than O157:H7, limiting the value of serotyping in the laboratory. Microaerobic bacterial species such as *C. jejuni* or *Campylobacter coli* may cause acute bacterial gastroenteritis and require specialized media and atmospheric conditions for culture.[44] Most diagnostic microbiology laboratories currently include microaerobic cultures for *Campylobacter* spp. as part of routine stool culture procedures. *C. jejuni* and *C. coli* are thermophilic campylobacters that can be cultured readily at 42° C in the laboratory and identified by microscopic morphology and biochemical testing. Hippurate hydrolysis is the key laboratory feature for identification of *C. jejuni*, although rare hippuricase-negative mutants have been isolated.[42,76] *Campylobacter fetus* tends to be associated with invasive infections and usually is isolated from surgical and blood cultures, instead of stool cultures, at 37° C.[9,78,100] *Yersinia enterocolitica* and *Yersinia pseudotuberculosis* can cause clinical symptoms mimicking appendicitis[25] and may contaminate blood products due to their ability to thrive at lower temperatures.[11,71] If suspected, laboratory evaluation of *Yersinia* spp. usually must be requested in addition to routine stool cultures because *Yersinia* spp. require different culture conditions, such as incubation at room temperature on MacConkey or cefsulodin-irgasan-novobiocin (CIN) agar.

DIRECT ANTIGEN DETECTION

Clostridium difficile is a primary cause of disorders of intestinal microbial ecology such as antibiotic-associated colitis and antibiotic-associated diarrhea. This bacterial pathogen includes two toxin genes, *tcdA* and *tcdB*, in a mosaic genome[80] that encode proteins with well-defined enteropathogenic and cytopathic effects.[80,94] Direct toxin detection is the most commonly used approach for the diagnosis of toxigenic *C. difficile* infection (see Chapter 51**)** because *C. difficile* isolates lacking toxin production (non-toxigenic *C. difficile*) are not associated with disease. Briefly, commercial stool immunoassays consist of antibodies that detect the presence of toxins, TcdA or TcdB, directly in stool specimens. Stool toxin immunoassays for *C. difficile* detection are relatively insensitive (30-60%) when compared with next-generation molecular methods and may require repeated testing.[1,84] Anaerobic culture with specialized media such as cycloserine-cefoxitin-fructose agar (CCFA) can be followed by direct toxin detection in cultured organisms to improve diagnostic sensitivity.[69] However, because of the labor-intensive nature of bacterial culture-based strategies and mammalian cell culture methods for cytotoxicity studies, direct toxin detection by various commercial antigen immunoassays provides an attractive strategy for most laboratories. DNA amplification for direct toxin gene detection in fecal specimens is a new option for diagnostic laboratories, and toxin gene testing may eventually supplant immunoassays for toxin detection.[6,91] In addition to their usefulness in detecting *C. difficile*-associated disease, direct antigen tests for *Campylobacter* spp. and EHEC provide alternative diagnostic modalities if stool samples are collected in physicians' offices at remote clinic locations and preserving the organism's viability in clinical specimens poses a challenge.[23,67]

BACTEREMIA AND SEPSIS

The annual incidence of sepsis and number of sepsis-related deaths in the United States have steadily increased from 83 cases per 100,000 population in 1979 to 240 cases per 100,000 population in 2000.[53] Shifts in the etiologic patterns of sepsis also have been noted in recent years. Since approximately 1990, gram-positive pathogens have surpassed gram-negative pathogens as primary causes of sepsis. However, in early-onset sepsis in very-low-birth-weight infants, gram-negative pathogens, especially *E. coli*, are predominant causes of sepsis in the era of maternal prophylaxis targeting *Streptococcus agalactiae*.[86] Fortunately, most pathogens associated with sepsis can be cultured routinely using automated blood culture systems.

PERIPHERAL BLOOD CULTURES

The diagnosis of bacteremia requires the growth of pathogenic bacteria from peripheral blood, and continuously monitoring, automated detection systems are used routinely for this purpose. Blood samples from infected individuals typically contain low levels of organisms (1-10 organisms per mL in adults) so that incubation with continuously monitoring systems usually requires a minimum of 8 to 12 hours of inoculation before detection of growth in a blood culture bottle. Infants and young children typically contain higher concentrations of organisms in peripheral blood in cases of bacteremia and sepsis (10-100 organisms/mL), so smaller blood volumes are sufficient for detection. Blood culture bottles have been adapted for pediatric patients, with smaller blood volumes required for continuously monitoring systems. Weight-based, graduated volumes of peripheral blood collection enhanced efforts to successfully isolate pathogens in immunocompromised children[27] and may have more general implications for pediatrics. Pediatric collection systems may not be sufficient for older children and adolescents, and older patients may need sampling of larger peripheral blood volumes combined with adult blood culture bottles.

Automated blood culture systems yield positive signals usually within 24 hours of inoculation, and signal-positive samples must be processed for rapid Gram-stain assessment and subculturing onto blood agar media. In the absence of a signal, blood culture bottles typically are incubated for a minimum of 5 days. With the currently sophisticated liquid medium formulations, incubations rarely need to be extended beyond 7 to 10 days. In one comprehensive study of blood culture samples spanning a 16-month period, HACEK microorganisms were detected in only 16 of 15,826 (0.1%) samples, and all isolates were detected within 3 days of incubation.[70] Aerobic blood culture bottles usually are used in pediatric practice because of the relative paucity of systemic anaerobic infections in children. In specific cases, anaerobic bottles may be added for comprehensive evaluation. Direct Gram-stain evaluation facilitates patient management by providing microscopic morphologies at the time of subculture from a positive, blood culture. Occasionally, organisms are not visualized by Gram stain despite a positive signal; accordingly, supplemental staining by acridine orange or species-specific nucleic acid probes may be performed.

Positive blood cultures are transferred to media containing blood agar and alternative formulations for fastidious organisms, depending on the Gram-stain morphology. Following successful subculture on plated media (usually 24-48 hours after a positive signal is generated in an automated blood culture system), microscopic evaluation by Gram stain and biochemical testing are performed routinely to facilitate species-level identification. Depending on the species identification, organisms can be con-

sidered likely pathogens or likely contaminants. Time to detection of peripheral blood cultures also may be helpful, as coagulase-negative staphylococci associated with true pediatric infections were isolated 11 hours postinoculation versus isolates from chronically ill patients with contamination (mean time to positivity equaled 24 hours).[31] Several reports have documented the primary pathogens to consider in peripheral blood cultures, and organisms, which represent likely contaminants.[97] In neonates, predominant pathogens include *E. coli* and *S. agalactiae* (group B streptococcus). Gram-positive pathogens include *S. aureus*, coagulase-negative staphylococci, *S. pneumoniae*, *Listeria monocytogenes*, and *E. faecium*. Predominant gram-negative pathogens include *E. coli* and other enteric organisms, *P. aeruginosa*, and *Kingella kingae*. These pathogens can be isolated using routine culture methods that include chocolate agar.

CENTRAL NERVOUS SYSTEM INFECTIONS

In children, acute bacterial meningitis and meningoencephalitis represent the most common central nervous system (CNS) infections caused by bacterial pathogens. The most likely causes of acute bacterial meningitis in children depend on the age groups and include pathogens that are culturable using routine methods in the microbiology laboratory. In neonates, *S. agalactiae*, *L. monocytogenes*, *S. pneumoniae*, and *E. coli* are the pathogens found most commonly. In infants and children younger than 2 years of age, *S. pneumoniae*, *Neisseria meningitidis*, and *S. agalactiae* were the most commonly identified pathogens, whereas *N. meningitidis* and *S. pneumoniae* represent the most likely pathogens in older children.[79] Childhood vaccination strategies have diminished the risk of meningitis due to *H. influenzae* serotype b infection in North America.[15]

DIRECT MICROSCOPIC EXAMINATION OF CEREBROSPINAL FLUID

If acute bacterial meningitis is suspected, cerebrospinal fluid (CSF) specimens should be submitted routinely for Gram stain and culture. CSF is usually collected in sterile tubes as sequential volumes for testing in different areas of the clinical laboratory. CSF should be collected in the initial fraction for the microbiology laboratory and transported rapidly to minimize contamination risks while preserving pathogen viability.[20] Immediate Gram-stain evaluation is useful, but its sensitivity is limited by the requirement for 10^5 organisms/mL for visualization. Pathogens such as *L. monocytogenes* are often difficult to visualize directly because the organisms are often present in lower concentrations. Gram-stain evaluation may "facilitate the rapid" assessment of the presence of bacterial pathogens, as well as provide microscopic morphologic information (e.g., gram-negative cocci versus gram-positive cocci) in order to guide patient management. Direct microscopic evaluation of CSF includes Wright-Giemsa stains for leukocytes so that neutrophils or lymphocytes can be distinguished and quantified. CSF specimens may also demonstrate elevated total protein and reduced glucose levels in cases of acute bacterial meningitis.[22] Elevated CSF lactate concentrations also may be consistent with bacterial meningitis and active inflammation.[47,51]

BACTERIAL CULTURES OF CEREBROSPINAL FLUID

Regardless of the Gram stain results, CSF specimens are plated routinely on media containing blood and chocolate agar and are incubated for 48 to 72 hours at 37° C. Most bacterial pathogens of the CNS may be isolated successfully on blood agar, although chocolate agar improves the yield and reduces time to positive culture for pathogens such as *N. meningitidis* and *S. pneumoniae*. *N. meningitidis* may be identified readily by microscopic morphology and limited biochemical testing (e.g., oxidase testing, sugar fermentation).[72] *S. pneumoniae* and *S. agalactiae* usually can be identified by microscopic morphology, limited biochemical testing, and Lancefield group determination. If gram-negative enteric rods are visualized by CSF Gram stain, culture on MacConkey agar may be indicated in order to provide a differential medium to facilitate isolation of pathogens such as *E. coli*. Prior antimicrobial treatment may preclude performing bacterial culture, even if antimicrobial agents were administered only several hours before CSF collection.

Liquid bacteriologic media such as thioglycollate broth also are used routinely to maximize opportunities for successful culture of bacterial pathogens. Children undergoing neurosurgical interventions, including placement of prosthetic devices such as ventricular shunts or drains, may be at risk for acquiring CNS infections caused by *S. aureus* or coagulase-negative staphylococci, as well as the primary bacterial pathogens that cause CNS infections.[43,81,89] CSF samples obtained from CSF shunts may yield clinically significant cultures of coagulase-negative staphylococci in thioglycollate cultures.

DIRECT ANTIGEN AND NUCLEIC ACID DETECTION

Direct bacterial antigen testing of CSF rarely is useful for establishing the diagnosis of bacterial meningitis and is not recommended routinely.[34,65,68] Direct Gram-stain evaluation of CSF enables the laboratory to detect organisms not evaluated by antigen testing and matches or exceeds the sensitivity of bacterial antigen detection. Direct DNA amplification may improve the diagnostic accuracy for bacterial pathogens such as *N. meningitidis*. More than 4000 CSF specimens were screened in one study describing real-time PCR for detection of bacterial pathogens in CSF[19]; the improvement in the meningococcal detection rate was 2.9 percent. That is, 87 additional cases of meningococcal meningitis were identified by real-time PCR and missed entirely by culture. *M. pneumoniae* may cause meningoencephalitis or transverse myelitis in children, and DNA amplification of *M. pneumoniae* in CSF facilitated establishing the diagnoses of *Mycoplasma* infections in these patients.[8]

GENITOURINARY TRACT INFECTIONS

The principal bacterial pathogens that cause urinary tract infections in children include gram-negative enteric bacteria such as *E. coli* and *Proteus* spp., and other pathogens such as *Enterococcus* spp., *P. aeruginosa*, and *S. aureus*.[101] *E. coli* represents the most common cause of nongonococcal urethrocystitis, and yeasts including *Candida* spp. may be isolated as clinically significant pathogens in bacterial urine cultures.

BACTERIAL URINE CULTURES

Pediatric urinary tract infections (UTIs) usually are diagnosed by semiquantitative evaluation of urine cultures obtained by various collection methods.[101] Urine samples usually are obtained as voided urine or via urinary catheters, although suprapubic needle aspiration in infants also may be performed. In young children, urinary catheters may be required in order to obtain adequate urine specimens. Likely bacterial pathogens can be cultured on plated media with standardized volumes using inoculating loops in the laboratory. Plated media including blood agar and MacConkey agar typically are used for urine cultures, and plates

are incubated for 24 to 48 hours at 37° C. Likely pathogens are defined as predominant pathogens that exceed 100,000 organisms per mL in clean-catch voided urine specimens. In "sterile" catheter specimens from young children (especially younger than 2 years of age), lower cutoff values of 10,000 to 50,000 organisms/mL may be clinically significant. Importantly, 65 percent of cultures with colony counts between 10,000 and 49,000 colony-forming units/mL yielded mixed cultures with evidence of contamination.[36] If one or two predominant pathogens are found at concentrations exceeding those of commensal bacteria by 10-fold or more, they may be clinically significant and should be reported by the laboratory. The urinary Gram stain may represent a useful test for rapid screening of young, febrile children with suspected UTIs.[101]

The relative abundance of the likely pathogens in comparison with the commensal microbiota, in addition to the estimated concentrations of organisms by routine culture, is an important consideration for laboratories. Organisms such as corynebacteria, lactobacilli, coagulase-negative staphylococci, and gamma- or non-hemolytic streptococci may be considered as likely commensal bacteria. Laboratories should consider likely commensal organisms of males and females in different age groups. Yeasts may be part of the commensal genitourinary microbiota in adolescent females, and *Gardnerella vaginalis*, although usually part of the commensal microbiota, may cause infections if isolated as predominant pathogens from female patients. Fungi, especially yeasts, are a prominent cause of nosocomial urinary tract infections in children and are isolated in routine urine cultures.

DIRECT ANTIGEN OR NUCLEIC ACID DETECTION

Especially in adolescent females, sexually transmitted pathogens such as *Chlamydia trachomatis* and *Neisseria gonorrhoeae* often require molecular diagnostics for detection because of the relative difficulty and inadequate sensitivity of culture or antigen testing.[66] Endocervical swabs or urine specimens may be submitted for DNA amplification or hybridization for either *C. trachomatis* or *N. gonorrhoeae*. Sexually active adolescents have a high incidence of *C. trachomatis* and *N. gonorrhoeae* infections in addition to pathogen-associated pelvic inflammatory disease.[87] Nucleic acid amplification methods facilitate widespread screening of sexually active adolescents via self-collected urine and vaginal samples. Direct antigen detection for either pathogen also may be helpful, but nucleic acid detection methods have largely supplanted antigen detection by virtue of greatly improved sensitivity.[66]

MISCELLANEOUS AND SUPERFICIAL SITE INFECTIONS

Miscellaneous cultures may be processed for evaluation of tissue abscesses, infected wounds, ocular and ear specimens, and superficial or skin infections. Surgical site infections may include wound infections and abdominal abscesses, as well as complications associated with pediatric liver transplantation, including surgical leaks at sites of anastomosis.[37] Tissue specimens and aspirates of superficial sites generally are recommended, and swabs of superficial sites are discouraged for routine microbiologic evaluation. Direct Gram stain may be helpful for rapidly assessing optimal culture strategies and for focused consideration of potential pathogens. Bacterial cultures from such miscellaneous sites are plated on solid media, including blood agar, chocolate agar, and MacConkey agar. Thioglycollate broth also may be useful as a liquid medium for supplemental cultures of sterile specimens such as tissue or aspirates. Evaluating for bartonellosis is difficult because *Bartonella henselae* is extremely difficult to culture. The diagnosis usually is accomplished through serologic testing and histologic assessment of lymph node tissue using special silver impregnation stains to highlight microorganisms. However, DNA amplification from tissue provides a rapid and sensitive diagnostic modality in suspected cases, especially when organisms are visible.[5,18,77]

SUMMARY

Pediatric infections present a multitude of challenges for the diagnostic microbiology laboratory. Point-of-care methods such as direct antigen testing for *S. pyogenes* in the context of throat infections continue to make a major impact in routine diagnostics. Expanded proliferation of point-of-care tests, including direct antigen and nucleic acid detection, will increase the role of pediatric office-based testing for screening of children. The laboratory continues to evolve, with a multipronged approach to patient diagnosis and disease monitoring. Rapid assessment methods using microscopy and qualitative antigen or antibody detection continue to contribute to evaluation of sterile body fluids and disease screening in the laboratory. The ability to grow organisms by routine culture methods remains important for in-depth studies of pathogens in the laboratory, including antimicrobial susceptibility testing. Molecular testing is eroding the role of biochemical testing and cumbersome phenotypic approaches to pathogen identification. Nucleic acid amplification, sequencing, and array-based diagnostics will expand the contributions of molecular testing to the diagnosis and monitoring of pediatric infections in the future.

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CHAPTER 263

Fungal Laboratory Analysis: Specimen Collection, Direct Detection, and Culture

David A. Bruckner

Fungi were regarded as insignificant causes of infection historically. The most important factor responsible for the increase in the number of fungal infections has been an alteration in the immune response of humans. Organ transplant recipients, particularly during the post-transplant period, and patients with malignant neoplasms and debilitating immunologic or metabolic disorders such as systemic lupus erythematosus, diabetes mellitus, alcohol, or intravenous drug abuse are particularly susceptible to fungal infections. Opportunistic fungi of low or limited virulence are detected with increasing frequency in subcutaneous or disseminated infections.

SPECIMEN COLLECTION

When an infectious process is suspected, the most appropriate techniques directed toward the detection of the etiologic agent must be ordered by the physician. Knowledge of the pathophysiology of the disease process is important in determining the optimal time for specimen collection. These collections include specimens for culture, direct detection of the agent using antigen tests, nucleic acid detection, stains, or antibody methods.[1,3,5,7,8] All specimens should be considered hazardous, and universal precaution must be used for all specimen-collection procedures. A sufficient quantity of a specimen must be obtained for the techniques required to detect the fungal agent directly, for culture, for antigen detection, or for serology. Procedures used for specimen collection for culture of bacteria also can be used for culture of fungi (Table 263–1). Microbiologists and pathologists are available to guide physicians in selecting the most appropriate specimen for culture and other tests to achieve rapid detection of maximum recovery of the microorganism causing the infection. Most clinical laboratories have established guidelines for specimen collection with minimal volumes of specimen to be collected. In addition, specimens should be collected in sterile containers before the initiation of therapy to ensure that optimal recovery of organisms is achieved. An important factor is that adequate specimen volumes, which include up to 5 mL of cerebrospinal fluid (CSF) and 200 mL urine for fungal culture, be submitted to the laboratory. Specimens collected for 24 hours,

TABLE 263–1 Specimen Collection Guidelines

Specimen	Recommendations	Transport Device/Comments
Abscess/wounds	Collect as per bacterial culture.	Collect in sterile container with as much material as possible. If swab used, use exclusively for mycology. RT
Blood	Collect as per bacterial culture.	BacT/Alert, BACTEC, ESP, or Septi-Chek (biphasic) bottles. Isolator (lysis-centrifugation). RT, never refrigerate
Bone marrow	Collect as per bacterial culture	Collect in sterile container, Isolator, or heparin (green top) tube. If clotted may have to homogenize or cut into pieces to place on mycology media. RT
CSF	Collect as per bacterial culture. Fluid volumes greater than 1 mL should be centrifuged or filtered.	Collect in sterile container. RT, never refrigerate
Eye	Corneal scrapings should be inoculated directly onto mycology media. Vitreous fluid should be collected by needle aspiration.	Direct inoculation onto mycology media. Vitreous fluid can be placed in a sterile container or the syringe with needle removed can be sent to the laboratory. RT
Fluids other than CSF and urine	Collect as per bacterial culture. Fluid volumes greater than 1 mL should be centrifuged or filtered.	Collect in sterile container. RT
Hair	Can use Wood lamp to help select infected hairs. Also scrape border of infected area if lesions are present.	Place hairs in sterile container. RT
Nails	Swab surface with 70% alcohol. Collect softened material from beneath nail bed.	Use sterile container or directly inoculate mycology media. RT
Prostatic secretions	Empty bladder before giving prostate massage. For detection of *Blastomyces* and less commonly *Coccidioides*, *Cryptococcus*, and *Histoplasma*.	Place in sterile container or directly inoculate mycology plates. RT
Respiratory	Collect as per bacterial culture using same rejection criteria for sputum. Early morning sputum is the preferred specimen.	Use sterile container. RT
Skin	Disinfect surface area with 70% alcohol to remove bacterial contaminants. Scrape skin surface at the edge of the lesion.	Use sterile container or directly inoculate mycology media. RT
Tissue/biopsy	Collect specimen per surgical technique.	Sterile container to which a small amount of sterile saline may be added to keep tissue moist. RT
Urine	Collect as per bacterial culture. Early morning specimen is preferred. Use midstream collection technique.	Use sterile wide-mouthed container. RT
Vaginal	Collect specimen as per bacterial culture. Primarily for *Candida* infections.	Swab transport device. RT

CSF, cerebrospinal fluid; RT, room temperature.

such as urine and sputa, are unsuitable for fungal culture because of bacterial overgrowth. Swab specimens are considered inadequate for the recovery of fungi, particularly when multiple culture tests (bacteriology, mycology, mycobacteriology, and virology) from the same swab specimen are requested. In many instances, surgeons send tissue for a histologic diagnosis and neglect to submit a request for culture. If an infection caused by a dimorphic fungi such as *Coccioides immitis* is suspected, the physician should notify the laboratory because of hazards associated with culturing this organism. A poorly collected specimen may result in the failure to recover the organism, or it may lead to therapy directed at a contaminating or commensal organism.

Specimens should be transported to the laboratory as soon as possible under optimal conditions, which usually are advised in the laboratory specimen collection manual that should be available to all physicians. Specimen transport and storage at room temperature are recommended. Ideally, specimens should be received in the laboratory within 2 hours of being collected. If a delay occurs in setting up cultures, the specimen should be held at room temperature or 4°C to prevent bacterial overgrowth. Unacceptable specimens that should not be processed include specimens in containers that leak, specimens received many days postcollection, and specimens held under inappropriate temperature conditions. Some fungal isolates such as *Cryptococcus neoformans* and *Histoplasma capsulatum* do not survive well at refrigerated or freezing temperatures.

DIRECT DETECTION

In the laboratory, direct wet mounts or smears can be prepared and a portion of the specimen can be used for culture (Table 263–2). Blood, CSF, and urine also can be used for the detection of antigens and nucleic acids by enzyme immunoassay, latex agglutination, polymerase chain reaction (PCR), and radioimmunoassay.[2,5,6] These assays have proven to be useful for the detection of infections caused by *Aspergillus*, *C. neoformans*, and *H. capsulatum*.[2] Although PCR techniques may be used for the direct detection of fungal infections, they are used in research or have been validated in specific laboratories for clinical use but are not readily available in most clinical laboratories.[5] Direct microscopic examination, including frozen sections of tissue to provide an immediate presumptive diagnosis, should be performed on most specimens. A definitive diagnosis may be made when specific fungal elements pathognomonic for fungal infections are detected in direct specimen stains. Examples include *Blastomyces dermatitidis*, *C. neoformans*, *Coccioides immitis*, and *Pneumocystis jiroveci*. Detection of wide and narrow, wavy or ribbon-like, nonseptate hyphae is consistent with a Zygomycetes infection. Direct specimen examinations always must be followed with culture because the direct examination can never be used to rule out infection, and recovery of the organism may be necessary for speciation.

TABLE 263–2 Methods for Detection of Fungi in Human Specimens

Stain/Method	Use	Comments
Aspergillus galactomannan	To detect galactomannan in serum	An EIA test used as an adjunct to culture for the detection of *Aspergillus* in high-risk patients. Test has low sensitivity and specificity.
Acid-fast, modified	To detect *Nocardia*	Most *Nocardia* will stain pink.
Calcofluor white	Stains chitin and cellulose of fungal cell wall	Rapid detection of fungi and *Pneumocystis.* Staining time is 1 minute; requires fluorescent microscope. Organisms fluoresce blue-white. May be used with Evans blue to suppress background fluorescence.
Cryptococcal antigen test (EIA)	Detects antigen in CSF and blood	A reliable and sensitive method for the detection of cryptococcal antigen. Can be used to monitor therapy.
Giemsa	Stains most bacteria and fungi	Used for the detection of *Histoplasma* and *Penicillium marneffei* in blood and bone marrow. *Pneumocystis* trophozoites and intracystic sporozoites can be detected. Organisms stain blue-purple.
Gomori methenamine silver (GMS)	Stains most organisms in tissue	Fungi including *Pneumocystis* cysts stain gray-black. Difficult to control staining reaction and may be difficult to interpret due to background staining.
Gram stain	Used primarily for detection of bacteria	Yeast cells and pseudohyphae stain gram-positive; *Cryptococcus* stains gram-positive surrounded by a capsular halo; hyphae will stain gram-negative.
Hematoxylin and eosin	Used to demonstrate patient's tissue reactions	Fungi stain violet to blue-purple. Can see fungal pigments.
Histoplasma urine antigen (EIA)	To identify infections of *H. capsulatum*	More sensitive and faster than culture. Test could cross-react with *Coccidioides immitis*.
India ink	To detect *Cryptococcus* in CSF specimens	To help detect capsule of *Cryptococcus neoformans*. Not very sensitive, and capsule may be difficult to detect.
Papanicolaou	Used to detect the presence of malignant cells	Fungal elements stain pink.
Periodic acid–Schiff (PAS)	Stains glycogen, mucin, mucoproteins, and glycoprotein	A mucin stain used to stain the capsular mucopolysaccharide of fungal organisms. Capsule will have a pink-magenta or purple color.
Pneumocystis DFA	Detects *Pneumocystis* cysts and trophozoites	Rapid staining method specific for *Pneumocystis* in respiratory specimens.
PCR	Can be used for direct detection of fungal pathogens	Not commercially available but has been developed in specific diagnostic laboratories for the detection of selected fungal pathogens.
Potassium hydroxide	To detect yeast and hyphal elements	Used as a clearing agent to make fungi more visible in tissue, hair, nails, and body fluids. Digests proteinaceous material of host cells, leaving fungi intact.
Wright	Stains most bacteria and fungi	Used for the detection of *Histoplasma* and *Penicillium marneffei* in blood and bone marrow. *Pneumocystis* trophozoites and intracystic sporozoites can be detected. Organisms stain blue-purple.

CSF, cerebrospinal fluid; DFA, direct fluorescent antibody; EIA, enzyme immunoassay; PCR, polymerase chain reaction.

Serology may be used to diagnose fungal infections either because other direct detection methods were negative or the organism failed to grow in culture. In general, serology is not as sensitive as are other direct methods such as antigen detection, particularly in immunocompromised patients. Serology has been useful in the diagnosis of *C. immitis* and *H. capsulatum* infections and can be used to follow successful therapy in coccioidiomycosis.[6] Serologic methods used include complement fixation, enzyme-immunoassay, immunodiffusion, and latex agglutination.

PRIMARY ISOLATION MEDIA

The most reliable and sensitive means of diagnosing a fungal infection is isolation of the organism on culture media.[1,3,7,8] A variety of media are commercially available for primary isolation of fungal organisms (Table 263–3). The medium chosen should support the growth of fungal isolates while inhibiting bacterial organisms from specimens collected from nonsterile sites. Traditional antibiotics used include chloramphenicol, gentamicin, penicillin, and streptomycin. Antibacterial agents can inhibit actinomycetes; therefore, media without antibiotics should be chosen if this agent is suspected, or a combination of media with and without antibiotics could be used.

Media may use cycloheximide, a protein synthesis inhibitor to suppress the growth of saprophytic fungi. It also inhibits *Aspergillus*, *Candida*, *Cryptococcus*, *Trichophyton*, and most Zygomycetes. Cycloheximide can be used in combination with various antibacterial agents listed earlier.

Chromogenic media are useful for the recovery and identification of *Candida* spp.[4] Dermatophyte test medium was designed specifically for the culture and isolation of dermatophytes. Other media used can be those containing caffeic acid for the detection of *Cryptococcus neoformans* by its phenol oxidase activity or media overlaid with olive oil (long-chain fatty acid source) for the recovery of *Malassezia furfur*.

When processing tissue, avoid the use of a mortar and pestle or tissue grinder. Hyphal elements, particularly aseptate hyphae, are easily destroyed in this process, rendering culture recovery impossible. Recommendations are to mince the tissue with a scissors or scalpel blade into 1-mm cubes and place these fragments onto the agar surface.

Nonselective media will permit the recovery of rapidly growing yeast and fungi, as well as fastidious, slow-growing fungi. Selective media will enhance the recovery of slower-growing dimorphic fungi while inhibiting the faster-growing yeast and fungi that may contaminate the medium

TABLE 263–3 Primary Isolation Media

Media	Supplements	Use
CHROMagar	Chromogenic substrates and antibiotics	Selective and differential medium for recovery of clinically important yeasts. Assimilation of chromogenic substrates and colony color development allow presumptive identification of common *Candida* species.
Dermatophyte test medium	Chloramphenicol, cycloheximide, gentamicin	Selective and differential medium for recovery of dermatophytes *(Epidermophyton*, *Microsporum*, *Trichophyton)*. Medium turns from yellow to red with growth of dermatophytes.
Inhibitory mold agar (IMA)	Chloramphenicol; chloramphenicol may be added	Selective and enriched medium used to isolate cycloheximide-sensitive fungi *(Candida*, *Cryptococcus*, *Histoplasma*, *Zygomycetes*, some *Aspergillus)* and inhibit bacterial growth.
Mycosel	Chloramphenicol and cycloheximide	Selective medium for isolation of dimorphic fungi and dermatophytes. Chloramphenicol inhibits bacterial growth and cycloheximide inhibits rapidly growing molds and yeasts.
Potato flake agar	Can be made selective by addition of chloramphenicol and cycloheximide	Useful for recovery of dermatophytes.
Sabouraud brain heart infusion agar (SABHI)	Used with (selective) or without antibiotics. Antibiotics include chloramphenicol, cycloheximide, penicillin and streptomycin. Could add sheep blood for recovery of *Histoplasma*	General purpose medium for growth of dimorphic and other fungi.
Sabouraud dextrose agar (SDA)	None	General purpose medium supporting growth of aerobic actinomycetes and all fungi.
	With olive oil on surface	Selective for cultivation of *Malassezia furfur*.
Yeast extract phosphate medium	Ammonium hydroxide and chloramphenicol	Selective medium promotes recovery of slow-growing dimorphic fungi. Chloramphenicol inhibits bacterial growth and ammonium hydroxide inhibits growth of other fungi.

and render isolation of the slower growing organisms impossible.

In general, a battery of media, including media containing sheep blood (BHI and Sabouraud's brain heart infusion agar [SABHI]), is used for the recovery of fungal isolates. Although sheep blood inhibits conidiation, it does improve the recovery of fungal isolates. Agar plates or screw-capped tubes can be used for isolating fungal isolates. Most mycologists prefer agar plates because of the ease of handling the cultures, and the larger surface area provides for better isolation of colonies. Plates usually are sealed with air-permeable tape or shrink-wrap to prevent inadvertent opening of the plate and also to slow down or retard dehydration of the media because many plates are held for 4 weeks in the laboratory before the specimen is signed out as negative. Examination of culture plates or agar tubes should be performed in a certified biologic safety cabinet. Most fungi will grow on routine bacteriology plates; however, the incubation time usually is short and may not allow sufficient time for the fungi to grow and be visually noticed on the plates.

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CHAPTER 264

VIRAL LABORATORY DIAGNOSIS

Marjorie J. Miller

In the past, clinical and virologic services were perceived as too impractical and nonproductive to warrant implementation in the hospital laboratory. Cell culture propagation and maintenance, a requirement for isolation of virus, was considered complex, slow, expensive, and beyond the scope of the typical laboratory. Availability of commercial reagents and products also was a constraining factor. Serology, one of the earliest methods used for viral diagnosis, was limited by the delay in timely reporting of results, rendering it a more useful tool for epidemiologic rather than clinical purposes. In addition, few treatments were available for viral infections, further diminishing interest in and emphasis on the importance of clinical and laboratory diagnosis of viral infections. This circular cause-and-effect resulted in reduced and inappropriate utilization of such services when available and

TABLE 264–1 Why Clinical Virologic Laboratory Services Should Be Available on a Routine Basis

Knowledge of etiology in the clinical setting tunes the physician's interest and leads to better care.
Knowledge of viral infection reduces inappropriate antibiotic administration.
Knowledge of viral etiology reduces the cost and discomfort of unnecessary diagnostic procedures and lengthy hospital stays.
Virologic diagnosis is critical for the use of presently available antiviral drugs. As new drugs are developed, such diagnosis becomes even more important.
Wider use of viral diagnostic services leads to a better understanding of disease processes: viral-bacterial interactions and the relationship of viruses to "noninfectious diseases" such as myocardial infarction and pancreatitis.
Success and failure of viral vaccines are monitored more accurately.
Better patient awareness results from specific viral study. Patients like to know their illness, and such knowledge can be useful historically when future medical illnesses occur.
More accurate prognosis of disease outcome is possible when the specific etiologic agent is known.
The routine availability and use of viral diagnostic services make the physician more aware of priorities in medical research. The morbidity and mortality associated with specific viral agents, such as respiratory syncytial virus, should lead to interest in the development of new vaccines.

offered little reason to expand the availability of such services elsewhere.

Fortunately, technologic developments have allowed virologic diagnostic services to be integrated into many hospital laboratories.[293] Advances include commercial availability of (1) traditional and modified cell lines and media; (2) monoclonal antibodies for the detection of viral antigens directly in patient specimens and identification of culture isolates; (3) rapid (<30 minutes) virus antigen detection kits; (4) traditional molecular assays; and, more recently, (5) real-time molecular assays. Many manufacturers offer laboratory educational and technical support to assist in the validation and implementation of these assays. These technologic advances enable laboratories to provide effective diagnostic services; turnaround time to results and charges are similar to those for other areas of the microbiology laboratory.[125,126,201,240]

Presently, clinical laboratories have a variety of options when offering virologic services. Services may range from referral to a reference laboratory to limited detection of viral antigens (e.g., respiratory syncytial virus, influenza, rotavirus) or expansion to include culture and molecular assays. Whatever the choice, diagnosis is the foundation of all medical care. Specific reasons why clinical virologic laboratory services should be routinely available are listed in Table 264–1.

This chapter presents general aspects of diagnosis of viral infections, including specimen selection, collection, and transport; conventional and modified culture for virus isolation and identification; and rapid detection of viral antigens and nucleic acids directly in specimens. Specific aspects of diagnosis are presented in the respective chapters covering the particular viral agents.

SPECIMEN COLLECTION AND TRANSPORT

Laboratory diagnosis of viral infections depends primarily on the collection of appropriate specimens, in sufficient volume, at the correct time during the course of the illness using optimal transport and storage conditions. Improvements in the sensitivity and accuracy of laboratory assays will be rendered insignificant if the input sample is compromised.

SPECIMEN COLLECTION SITES

The selection of appropriate sites for collection of specimens as early in the acute phase of the illness as possible is critical to the recovery of viruses. A guide to recommended collection sites for specific viral syndromes is presented in Table 264–2. In general, the extent of the diagnostic investigation should be dictated by the characteristics of the illness being studied. For example, in common respiratory illnesses such as pharyngitis or croup, collection of a single specimen from the throat usually is all that is necessary. In other situations in which an illness is severe or unique, collection of specimens from multiple sites is important. In addition, in unusual cases, serum should be obtained for frozen storage in case subsequent serologic studies are required.

In unusual and severe illnesses, invasive procedures (e.g., brain, cardiac, liver, lung biopsies; needle aspiration of body fluids) frequently must be performed to obtain material for laboratory study. Many medical specialists argue against using these invasive procedures because little can be done to treat viral illnesses. However, in our experience, the knowledge gained from a positive viral identification justifies some risk incurred in collecting specimens. At present, antiviral drugs are similar to antibacterial and antifungal agents in that they generally can be used effectively only after definitive identification of the etiologic agent. Demonstration of a viral etiologic agent in diseases such as encephalitis, pneumonia, or cardiac disease can prevent the unnecessary administration of antibiotics and steroids. In many cases, overutilization of services, patient trauma, and overall cost can be reduced when a viral etiologic agent is confirmed. Finally, the prognosis of a particular illness is more accurate when the specific etiologic agent is known.

COLLECTION OF SPECIMENS

Specimens for virus culture or direct examination generally are obtained as they are for other microbiologic studies. The primary purpose of a transport medium is to provide a protective protein, neutral pH, and antibiotics for control of microbial contamination and, most importantly, for prevention of desiccation. Many viral transport and storage media are available commercially or are prepared readily in the laboratory; their utility has been reviewed.[16,50,147] Convenient and practical collection devices, such as the Culturette (Becton Dickinson, Cockeysville, Md) or the Virocult (Medical Wire and Equipment Co., Victory Gardens, NY), consist of a swab, usually Dacron or rayon, on a plastic or aluminum shaft accompanied by a self-contained transport medium (Stuart or Amies). These devices are routinely available in most hospitals for bacteriologic culture. Calcium alginate swabs, which are toxic to herpes simplex virus (HSV), and wooden shafts, which may be toxic to viruses as well as the cell culture system itself, should not be used. Saline or holding media that contain serum also should be avoided. Useful liquid transport media (2-mL aliquots in screw-capped vials) consist of tryptose phosphate broth with 0.5 percent bovine albumin; Hanks balanced salt solution with 5 percent gelatin or 10 percent bovine albumin; or buffered sucrose phosphate (0.2 mol/L, 2-SP),[131,147] which has been used as a combined transport medium for viral, chlamydial, and mycoplasmal culture requests[291] and is appropriate for long-term frozen storage of specimens and isolates.[131]

Some of these transport media also have been evaluated and found acceptable for use in rapid methods (e.g., enzyme-linked immunosorbent assay [ELISA][227,328] and polymerase chain reaction [PCR]).[207]

TABLE 264–2 Specimen Collection Guide for the Diagnosis of Viral Infections Based on the Viral Syndrome and Etiologic Agent Suspected

Main Location or Category of illness	Clinical Diagnosis	Specimen Collection Source		Other Sources	Etiologic Agent Suspected*
		Most Practical	*Most Definitive*		
Upper respiratory tract	Common cold, nasopharyngitis	Nasopharynx/ nose	Nasopharynx/ nose	Nasopharyngeal/ nasal wash, stool blood	Rhinoviruses, coronaviruses, parainfluenza viruses, respiratory syncytial virus enteroviruses, adenoviruses
	Pharyngitis	Throat	Throat	Stool, blood	Adenoviruses, enteroviruses, Epstein-Barr virus, influenza viruses, parainfluenza viruses
	Herpangina, other enanthems	Throat	Lesions	Stool, blood	Enteroviruses, herpes simplex virus
	Laryngitis, laryngotracheitis	Throat	Larynx or trachea	Nasopharyngeal/ nasal wash	Parainfluenza viruses, influenza viruses
	Parotitis, other salivary gland enlargement	Throat	Stensen duct	Urine, blood, cerebrospinal fluid	Mumps virus, enteroviruses
Lower respiratory tract	Bronchitis, bronchiolitis, pneumonia	Throat	Bronchoalveolar lavage, bronchial washing biopsy	Stool, blood, sputum, nasopharyngeal/ nasal wash	Respiratory syncytial virus, human metapneumovirus, parainfluenza viruses, adenoviruses, influenza viruses, cytomegalovirus
	Pleurodynia	Throat	Throat	Stool	Enteroviruses
	Pleural effusion	Pleural fluid	Pleural fluid	Throat, stool, blood	Enteroviruses, adenoviruses
Heart	Myocarditis, pericarditis, conduction defects	Throat	Pericardial fluid, biopsy	Stool, blood, urine	Enteroviruses, influenza viruses, adenoviruses
Central nervous system	Meningitis	Throat	Cerebrospinal fluid	Stool, blood, urine	Enteroviruses, mumps virus, arboviruses, herpes simplex virus type 2, lymphocytic choriomeningitis virus
	Encephalitis	Throat	Brain biopsy	Cerebrospinal fluid, stool, blood, urine	Arboviruses, mumps virus, enteroviruses, herpes simplex virus type 1, influenza viruses
	Guillain-Barré syndrome, cerebellar ataxia, transverse myelitis, poliomyelitis	Throat	Throat	Stool, blood, cerebrospinal fluid	Influenza viruses, arboviruses, enteroviruses, Epstein-Barr virus
Genital tract	Orchitis, epididymitis	Throat	Testicular biopsy	Stool, blood, urine	Mumps, enteroviruses, lymphocytic choriomeningitis virus
	Herpes genitalis	Lesions	Lesions	Vagina, cervix, urethra	Herpes simplex virus
Urinary tract	Cytomegalovirus infection	Urine	Urine	Throat, blood	Cytomegalovirus
	Hematuria and/or pyuria	Throat, urine	Urine	Blood, stool	Arboviruses, enteroviruses, mumps virus, adenoviruses
Gastrointestinal tract	Nausea and/or vomiting	Throat	Throat	Stool, blood	Enteroviruses, influenza viruses, noroviruses
	Diarrhea	Stool	Stool	Throat	Rotaviruses, enteroviruses, adenoviruses, noroviruses
	Abdominal pain	Throat	Throat	Stool, blood	Enteroviruses, adenoviruses
	Acute abdomen mesenteric adenitis	Throat	Mesenteric lymph node biopsy, peritoneal fluid	Stool, blood	Enteroviruses, adenoviruses
	Hepatitis	Throat	Liver biopsy	Stool, blood	Hepatitis A, B, C, E, and G viruses, Epstein-Barr virus, adenoviruses, enteroviruses, cytomegalovirus
	Pancreatitis	Throat	Duodenal fluid	Stool, blood	Enteroviruses
	Reye syndrome	Throat	Liver biopsy	Blood	Influenza viruses, varicella virus
Reticuloendothelial system	Hepatosplenomegaly	Throat	Blood, liver biopsy	Stool, urine	Adenoviruses, enteroviruses, Epstein-Barr virus, cytomegalovirus
	Generalized lymphadenopathy	Throat	Blood, lymph node biopsy	Stool, urine	Adenoviruses, enteroviruses, Epstein-Barr virus, cytomegalovirus
	Immunodeficiency	Blood	Blood	Lymph nodes	HIV
Bone or joints	Osteomyelitis	Bone	Bone	Throat, blood, urine, skin lesion	Rubella virus, vaccinia virus
	Arthritis	Joint fluid	Joint fluid	Throat, blood, urine	Rubella virus, arboviruses

TABLE 264–2 Specimen Collection Guide for the Diagnosis of Viral Infections Based on the Viral Syndrome and Etiologic Agent Suspected—cont'd

Main Location or Category of illness	Clinical Diagnosis	Specimen Collection Source		Other Sources	Etiologic Agent Suspected*
		Most Practical	*Most Definitive*		
Muscle	Myositis	Throat	Muscle biopsy	Stool, blood	Influenza virus, enteroviruses
Skin	Exanthematous disease	Throat	Vascular fluid, skin biopsy	Stool, blood, urine, eye	Measles virus, rubella virus, varicella virus, enteroviruses, herpes simplex virus
Eye	Conjunctivitis, including pharyngoconjunctival fever	Conjunctiva	Conjunctiva	Throat, stool	Adenoviruses, enteroviruses
	Keratoconjunctivitis	Conjunctiva, cornea	Conjunctiva, cornea	Throat, stool	Adenoviruses, herpes simplex virus, varicella virus
	Retinitis	Vitreous aqueous fluid	Vitreous aqueous fluid		Cytomegalovirus, herpes simplex virus, varicella virus
Fever	Nonspecific febrile illness (human-to-human transmission)	Throat	Blood	Stool, urine, cerebrospinal fluid	Enteroviruses, influenza viruses, adenoviruses, cytomegalovirus
	Nonspecific febrile illness (arthropod vector)	Blood	Blood	Throat, urine, cerebrospinal fluid	Arboviruses
	Fever of unknown origin	Blood	Blood	Urine, stool, throat	Hepatitis A, B, C, E, and G viruses, cytomegalovirus, herpes simplex virus, Epstein-Barr virus, adenoviruses
Congenital infection	Rubella virus, cytomegalovirus infections	Throat	Blood	Urine, nasopharynx, biopsy material, cerebrospinal fluid	Rubella virus, cytomegalovirus
	HIV infection	Blood	Blood	Lymph node	HIV
Perinatal and neonatal infections	Herpes simplex virus, cytomegalovirus, enterovirus infections	Throat	Blood	Urine, nasopharynx, stool, skin lesions, cerebrospinal fluid	Enteroviruses, respiratory syncytial virus, influenza viruses, herpes simplex virus, cytomegalovirus

**This listing includes only the more commonly associated agents: It is likely that with more general use of viral diagnostic services, new virus-disease associations will be made.*
HIV, human immunodeficiency virus.

Throat

Specimens from the throat should be obtained with a swab in a manner similar to that used for bacterial culture. The posterior pharyngeal wall and tonsillar surfaces, any inflamed or erythematous areas, and any visible lesions are swabbed firmly without contact with the tongue and anterior oral cavity.

Nose and Nasopharynx

Specimens should be collected with nasopharyngeal swabs that have thin, flexible wire shafts by inserting the swab into the nasopharynx and rotating it to obtain the maximal number of ciliated, columnar epithelial cells and then placing the swab in transport medium. Alternatively, a flexible nasal probe with a cupped tip may be used (Rhinoprobe, Rhinotechnics, San Diego, Calif).[144,332] Nasopharyngeal cultures can be obtained from infants by the wash technique described by Hall and Douglas.[119] In this method, a small amount of sterile phosphate-buffered saline (3-7 mL) is squeezed into the nose with a nasal bulb aspirator (1 oz, tapered) and then immediately withdrawn and placed in a sterile, screw-capped container. Alternatively, nasopharyngeal aspirates may be obtained with a mucus collection device. An appropriately sized catheter is inserted nasally into the posterior of the nasopharynx; intermittent suction is applied as the catheter is withdrawn. Aspirate is washed through the tubing with 5 to 8 mL of transport medium or sterile phosphate-buffered saline. Washes and aspirates (versus swabs) are preferred for direct antigen detection because more epithelial cells are obtained with this method.[4]

Other Respiratory Specimens (Sputum, Tracheal Aspirates, Bronchial Washings, Bronchoalveolar Lavage)

Collection depends on the volume obtained. Volumes of 0.5 mL or larger should be placed in a sterile container and sealed tightly. If the volume is less than 0.5 mL, the specimen is placed in 2 mL of transport medium.

Eye

Exudate or pus should be removed first with a sterile swab. Conjunctival specimens may be obtained by pressing a swab premoistened with sterile saline firmly against the inflamed areas. The swab is returned to the self-contained transport device, or the tip is broken off into 2 mL of transport medium. Corneal scrapings should be obtained by an ophthalmologist or other trained person and placed in transport medium immediately.

Body Fluids Other than Blood

Body fluids such as urine (clean voided, 10-20 mL), cerebrospinal fluid (CSF) (2-5 mL), pleural effusion, and peritoneal, pericardial, or joint fluid should be collected under sterile conditions and placed in securely sealed, sterile containers. For small volumes (<0.5 mL), the specimen may be placed in transport medium.

Lesions

For lesions, fresh vesicles should be selected because recovery of virus from older lesions decreases significantly. Gently swab the area with sterile saline. Rupture the vesicle and collect both fluid and cells from the base of the lesion. Material obtained from several lesions may be pooled. Desiccation must not be allowed to occur; swabs should be submitted moistened with their self-contained transport medium or, if that is unavailable, placed in 2 mL of transport medium. Specimens may be collected with a swab or aspirated with a 26-gauge needle attached to a tuberculin syringe. Aspirated fluid should be rinsed into 1 to 2 mL of transport medium.

For direct antigen detection by immunofluorescence (IF) (direct fluorescent antibody [DFA]), the vesicle is ruptured as previously described and epithelial cells are collected by firmly swabbing the base of the lesion. Cells are transferred to a clean glass slide by firmly rolling the swab back and forth over a 5- to 10-mm area (dime size); for differentiation of HSV types 1 and 2 (HSV-1 and HSV-2) and varicella-zoster virus (VZV), three areas should be prepared.

Stool and Rectal Specimens

Because of initial studies with polioviruses and other enteroviruses, culture of fecal material has been overemphasized in virology. Both enteroviruses and adenoviruses can be recovered readily from stool, but because these agents are carried in the lower gastrointestinal tract for considerable periods of time after acute infection, use of this source for establishing the diagnosis of specific illnesses is limited. A specimen of fecal material is practical only when the specific primary diagnosis is diarrhea or when concomitant serologic study with paired sera can be performed.

For recovery of enteroviruses, fresh stool specimens are better than are rectal swabs; 2 to 5 g (2-3 tsp) of formed or liquid specimen should be transferred to a sterile, leak-proof container. However, from a practical point of view, a rectal swab is the simplest method of specimen collection. A rectal swab can be obtained immediately, whereas collection of a stool specimen usually entails considerable delay. Of importance is that the swab contain visible stool; the swab should be inserted 3 to 5 cm into the rectum, rolled against the mucosa, and then transported in a Culturette or similar device.

Blood

Some viruses can be recovered from serum or red blood cells (RBCs), but in general, leukocytes are a better source of virus. Fresh blood (optimally 5 mL) is collected in a suitable anticoagulant (heparin, ethylenediaminetetraacetic acid [EDTA], acid citrate dextrose) and transported to the laboratory for processing by density gradient centrifugation[132] or other methods to enrich for leukocytes. EDTA is the anticoagulant of choice because it is an acceptable transport for culture, as well as for molecular methods such as hybridization and PCR.

Bone Marrow

Approximately 2 mL should be aspirated into a tube or syringe containing heparin anticoagulant, mixed thoroughly to prevent clotting, and transported without further additives or diluents.

Biopsy Specimens

For biopsy specimens, fresh tissue obtained from the affected site should be placed in 2 mL of transport medium to prevent desiccation.

Autopsy Specimens

Most postmortem specimens are almost useless for viral cultivation because of the manner in which autopsies usually are performed. If labile agents are to be recovered, the autopsy should be performed within 4 hours of death. All tissues (1-2.5-cm cubes) for study should be obtained aseptically, and individual tissue should be collected with sterile instruments and placed in separate sterile containers. Because the usual autopsy routine entails fixation of specimens, of vital importance is that specimens for viral isolation not be placed in containers with fixatives such as formalin or other preservatives.

TRANSPORT TO THE LABORATORY

The method of handling specimens from the time of collection to laboratory processing is critical for preservation of virus infectivity and subsequent recovery in culture. In general, the less time that transpires between collection and inoculation into cell culture, the greater the chance of recovering the virus. Specimens should not be frozen or exposed to temperatures higher than 22° C. For short-term storage (<5 days) of most viruses, the specimen should be held at 4° C until it can be processed in the laboratory. For transport to the laboratory, this temperature can be achieved with the use of cold packs or simply by placing one ice cube next to the specimen container within an aluminum foil wrapping.

Although the degree of sensitivity varies, all viruses are inactivated by ultraviolet light. Therefore, shielding specimens from sunlight is important and can be accomplished by using opaque transport boxes or wrapping individual specimens in aluminum foil or opaque paper.

When considerable delay between collection and culture will elapse (transport to other laboratories, holiday schedules, and so on), special preparation is necessary. Such preparation needs to be individualized and based on the most likely viral cause of a particular illness. For example, when an enteroviral etiologic agent is suspected, freezing the specimen (–70° C) and shipping it with dry ice are satisfactory. In contrast, a urine specimen from a patient with possible cytomegalovirus (CMV) infection should not be frozen. It should be shipped under wet ice at 4° C because CMV in urine is stable for several days at this temperature. In addition, media that protect viruses against the deleterious effects of temperature fluctuations, particularly freeze-thawing, are available for transport as well as for long-term storage.[131,147] For specific characteristics of individual viruses, see Chapters 164-204 and the general references at the end of this chapter.[180,216,278,293]

LABORATORY DIAGNOSIS OF VIRAL INFECTIONS

Previously, the perception that isolation of virus was difficult, nonproductive, and too slow to be of clinical utility, combined with the lack of consistently available reliable reagents, discouraged the use of diagnostic virology services. Since the mid-1980s, a dramatic increase has occurred in the quality and availability of diagnostic reagents, primarily monoclonal antibodies, which has significantly improved the turnaround time of reporting virology results. Direct detection of viral antigens in clinical specimens such as the herpes group viruses (HSV, VZV, CMV); respiratory syncytial virus (RSV); influenza viruses; adenoviruses; and rotaviruses, usually by IF; an immunoperoxidase (IP) test; or ELISA has become routine and has made obtaining same-day results a reality. In addition, detection of viral antigens in cell culture by the application of IF/IP staining or ELISA, before the appear-

ance of cytopathic effect (CPE), has greatly reduced the time needed to report a positive result. More recently, molecular techniques for the direct detection and quantitation of viral nucleic acids in specimens[69,142,237] have begun the transition process from research tool to routine use in the clinical laboratory as commercial reagents become readily available, methods are simplified and standardized, and some assays are manufactured in kit format. Methods for the laboratory diagnosis of specific viral infections are presented in Chapters 164-204, and detailed methods are provided in the general references at the end of this chapter.[142,180,216,257,278,293] Table 264–3 summarizes specific methods for the laboratory identification of viruses, in addition to indications for serologic study of selected viruses.

VIRUS ISOLATION

Despite technologic advances and innovations, culture remains the cornerstone of the diagnostic virology laboratory. Culture still is among the most sensitive of diagnostic methods because theoretically, a single infectious virus can be detected. Unlike rapid methods, which are limited to the detection of a specific viral antigen or nucleic acid, culture is open-ended and permits the detection of unexpected viruses, new viruses, or multiple viruses within the same specimen. In addition, a broad range of specimens can be evaluated, whereas rapid methods usually are approved or licensed for use with specimens collected from specific sites. Viruses that can be isolated and identified readily include adenoviruses, HSV, VZV, CMV, enteroviruses, rhinoviruses, influenza and parainfluenza viruses, RSV, rubella virus, mumps virus, and measles virus. The number and type of isolates encountered depend on the patient population, season, and type of specimens submitted. For optimal laboratory diagnosis, indicating the virus suspected on the requisition is important because some viruses require specific cell lines, procedures for identification, or both. Cultures are maintained for different time frames before finalizing a report of negative results, depending on the virus being sought; for example, HSV cultures usually are observed for 5 to 7 days, CMV for 1 month, and all others for 2 to 4 weeks. Improvements and modifications in culture methods have resulted in more rapid identification of isolates, with greater than 70 percent being reported within 5 days.[67,137]

Traditional Culture

Although animals such as suckling mice and chicken embryos originally were the only means of isolating viruses, most laboratories now use cell culture exclusively. A variety of cell lines capable of supporting the growth of a broad spectrum of viruses can be obtained fresh weekly from commercial sources. After inoculation of the specimen, cultures are observed at regular intervals for evidence of viral infection characterized by the appearance of CPE or the ability to hemadsorb or hemagglutinate RBCs. Adenoviruses, CMV, HSV, VZV, rhinoviruses, and enteroviruses can be identified and reported by their characteristic CPE. The mean time between inoculation of a specimen and the appearance of CPE usually is 1 week or less for most of this group, except CMV and some adenoviruses, which may require longer incubation. Cultures containing HSV and enteroviruses often are positive within 1 to 2 and 3 to 5 days, respectively. In the event of atypical or questionable CPE, IF with monoclonal antibodies can be used to identify CMV, VZV, HSV-1, HSV-2, adenoviruses, enteroviruses, and some select coxsackieviruses and echoviruses in infected cell cultures. Although it rarely is performed, the neutralization test is used to definitively identify adenoviruses and enteroviruses if serotyping is requested for epidemiologic or other reasons.[142,278]

Respiratory viruses, including parainfluenza and influenza viruses, which may or may not produce CPE, generally can be detected within 5 to 7 days of inoculation of the specimen with the traditional hemadsorption method, in which a suspension of guinea pig RBCs is added to the cell culture. The RBCs are observed to adhere to tissue culture cells infected with these viruses, usually 3 to 5 days after the inoculation of specimens for most isolates.[213] An adaptation of this method uses cell culture medium and guinea pig RBCs in a microtiter plate format to detect viral hemagglutinins in suspension. The results are similar to those observed with hemadsorption, but the adapted method is more rapid and simple to perform when screening numerous specimens.[151] RSV is identified presumptively, usually within 3 to 5 days of inoculation of the specimen, by its typical CPE (syncytium formation) and inability to hemadsorb/hemagglutinate guinea pig RBCs. This group of viruses is identified definitively by IF with monoclonal antibodies.[142,278]

Finally, the traditional method for isolation and identification of rubella virus uses primary African green monkey kidney cells in conjunction with the interference assay. After specified intervals following inoculation have transpired, cultures are challenged with a second virus, usually echovirus 11, incubated for an additional 3 to 4 days, and examined for CPE. The presence of rubella virus interferes with growth of the challenge virus, and no CPE is observed; on the other hand, if the challenge virus grows and produces CPE, the specimen is considered negative for rubella virus. Final identification of the isolate requires neutralization of the interference with specific rubella antibody.[216,278]

Serologic diagnosis is recommended for viruses that are difficult or impossible to isolate in commonly available cell cultures or that require special techniques for detection of their presence. For instance, in most cases of arbovirus infection, the study of paired sera for antibody development is the most rewarding for diagnosis. Human immunodeficiency virus (HIV) infection generally is identified best by the demonstration of serum antibody or antigen with the use of ELISA. Measles virus is relatively difficult to grow, and identification can take considerable time; when measles is suspected, the diagnosis is confirmed most easily by serologic study. Paired sera collected 1 week apart usually reveal a fourfold antibody titer rise. Alternatively, demonstration of specific IgM antibody allows the diagnosis to be established with the use of a single serum sample. Similarly, serology is useful for establishing the diagnosis of mumps virus. Although mumps virus can be isolated in the same cell lines used for other paramyxoviruses and detected by hemadsorption/hemagglutination as previously described, such requests are rare. Rubella virus requires 2 or more weeks for isolation and specific identification. Because of this delay, rubella virus infection, other than that acquired congenitally, is confirmed best by serologic study. In all instances of suspected congenital rubella, isolation of the virus should be attempted.

Modified Culture

A variety of physical and chemical methods have been evaluated for their ability to enhance the rapid detection of virus in culture.[137] These methods include the effect of low- and high-speed rolling of cultures, centrifugation, chemicals such as dimethyl sulfoxide, hormones such as dexamethasone, and enzymes such as trypsin on the isolation of viruses. Early detection of viral antigens in culture by IF and IP, before the appearance of CPE, also has been evaluated.[89,208,241,263] A technique that entails the physical method of centrifugation with early antigen detection in cell culture and is called variously the shell vial assay (SVA), the spin-amplified technique, or culture-amplified antigen detection is used routinely in most laboratories to decrease the

TABLE 264–3 Common Laboratory Methods for Virus Isolation, Identification, and Direct Detection and Most Useful Methods for Diagnosis of Specific Viral Illnesses

Virus	Culture/Direct Detection	Identification of Isolates	Serology	Most Useful Method for Diagnosis of Specific Viral Illness
Adenoviruses	Primary HEK, A549, WI-38, HEp-2, HeLa, KB cell, R-Mix, R-Mix Too culture/FA, ELISA	Group identified by characteristic CPE and/or FA; type identified by neutralization with specific antiserum	CF on paired sera, ELISA HAI	Virus isolation
Arboviruses of North America	Suckling mouse intracerebral inoculation, vero, BHK-21, LLC-MK2 cell culture	Neutralization with specific antiserum	CF on paired sera, HAI, indirect FA, IgM antibody capture ELISA	CF on paired sera
Coronaviruses	Human embryo tracheal organ culture, HEK PCR	Neutralization with specific antiserum	Neutralization, CF, HAI on paired sera, ELISA, indirect FA	No practical method presently available
Rhinoviruses	WI-38, MRC-5 cell culture	Characteristic CPE: stability on exposure to lipid solvents and inactivation at pH 3	Neutralization on paired sera	Virus isolation
Enteroviruses	MK, WI-38 cell culture, suckling mouse inoculation (intracerebral and intraperitoneal)/PCR	Group identified by characteristic CPE in cell culture or illness or pathology in mice; type identified by neutralization with specific antiserum; FA for some serotypes	Neutralization, CF, HAI on paired sera	Virus isolation
Cytomegalovirus	WI-38, MRC-5, foreskin cell culture/FA, IP, PCR, hybrid capture	Characteristic CPE; FA/IP for definitive identification	CF on paired sera, ELISA, LA, indirect FA	Virus isolation; PCR
Epstein-Barr virus (EBV)	Practical method not available/PCR	Practical method not available	Indirect FA against viral capsid antigen on paired sera; indirect FA against early antigen in single serum	Infectious mononucleosis rapid slide tests; rarely, EBV-specific FA test; PCR
Herpes simplex virus	WI-38, MRC-5, primary RK cell culture/FA, IP, ELISA, PCR	Characteristic CPE; FA, IP, ELISA for definitive identification	CF on paired sera, ELISA, indirect FA, ACIF	Virus isolation; antigen detection by FA, ELISA; PCR
HIV	ELISA, PCR, bDNA	ELISA, Western blot	ELISA, Western blot, indirect FA	ELISA
Human metapneumovirus	LLC-MK2, Vero, A549, HEp-2, R-Mix, R-Mix Too cell culture PCR, FA, ELISA	FA	ELISA	PCR; virus isolation
Influenza viruses	MK, LLC-MK2, MDCK, R-Mix Too cell culture and chicken embryo (amniotic sac and aliantoic cavity) inoculation/FA, ELISA	HA or hemadsorption of guinea pig or chicken erythrocytes and inhibition with specific antiserum for type/strain identification; FA for identification of type	CF, HAI, ELISA on paired sera	Virus isolation; antigen detection by FA, ELISA
Measles virus	Primary MK, HEK, R-Mix, R-Mix Too cell culture/FA	Hemadsorption and HA of monkey erythrocytes and inhibition with specific antiserum or identification by FA	CF, HAI on paired sera identification of measles-specific IgM antibody by ELISA, indirect FA	Identification of measles-specific IgM antibody by ELISA, Indirect FA or CF/HAI antibody titer rise
Parainfluenza viruses, mumps virus	Primary MK, LLC-MK2, HEK, R-Mix, R-Mix Too cell culture/FA	Hemadsorption and HA of guinea pig erythrocytes; specific identification by FA	CF, HAI on paired sera, ELISA, indirect FA	Virus isolation; antigen detection by FA
Respiratory syncytial virus	HEp-2, WI-38, R-Mix, R-Mix Too cell culture/FA, ELISA	Characteristic CPE in absence of hemadsorption; specific identification by FA	CF on paired sera, ELISA, indirect FA	Detection of viral antigen by FA/ELISA: virus isolation
Rabies virus (animal infections)	Demonstration of Negri bodies by microscopic examination of brain or demonstration of viral antigen by FA, mouse inoculation	Demonstration of Negri bodies or antigen by FA	Neutralization, ELISA	Antigen detection by FA

TABLE 264–3 Common Laboratory Methods for Virus Isolation, Identification, and Direct Detection and Most Useful Methods for Diagnosis of Specific Viral Illnesses—cont'd

Virus	Culture/Direct Detection	Identification of Isolates	Serology	Most Useful Method for Diagnosis of Specific Viral Illness
Rubella virus	African green monkey kidney cell culture	Interference of enteroviral CPE; neutralization with specific antiserum	HAI on paired sera; identification of rubella-specific IgM antibody by ELISA, indirect FA	Identification of rubella-specific IgM antibody by ELISA, indirect FA or HAI antibody titer rise
Poxviruses	MK, WI-38 cell culture/FA, EM	Characteristic CPE; FA or appearance on EM	Not useful for diagnosis	Antigen detection by FA, EM
Varicella-zoster virus	WI-38, MRC-5 cell culture/FA, PCR	Characteristic CPE: FA for definitive identification	CF on paired sera, EIA, LA, IHA, IAHA, ACIF, FAMA	Detection of viral antigen by FA; virus isolation
Hepatitis A virus	Demonstration of antigen by ELISA, IEM, RIA	Practical method not available	ELISA, RIA, HA	Identification of HAV-specific IgM by ELISA, RIA
Hepatitis B virus	Demonstration of antigen by ELISA, RIA, HA, demonstration of DNA by hybridization, PCR	Practical method not available	ELISA, RIA, HA	Demonstration of antigen, nucleic acid
Hepatitis C virus	Demonstration of antigen by IEM, RIA	Practical method not available	ELISA, RIBA	ELISA, RIBA, determination of viral RNA
Notovirus	Determination of antigen by IEM, RIA	IEM, RIA	ELISA, RIA-BL; serology not used routinely	Demonstration of antigen
Rotavirus	Demonstration of antigen by ELISA, LA, EM	EM, ELISA	ELISA; serology not used routinely	Demonstration of antigen
Adenovirus 40/41	Demonstration of antigens by ELISA	ELISA	CF, ELISA on paired sera; serology not used routinely	Demonstration of antigen

For details, see Chapters 164-204.

ACIF, anticomplement immunofluorescence; A549, human lung carcinoma: bDNA, branched-chain DNA; BHK-21, continuous babv hamster kidney cell line; CF, complement fixation; CPE, cytopathic effect; ELISA, enzyme-linked immunosorbent assay; EM, electron microscopy; FA, immunofluorescent antibody; FAMA, fluorescent antibody to membrane antigen; HA, hemagglutination; HAI, hemagglutination inhibition; HEK, human embryonic kidney; HEp-2, human laryngeal carcinoma cells; IAHA, immune adherence hemagglutination assay; IEM, immune electron microscopy; IHA, indirect hemagglutination; IP, immunoperoxidase test; KB, oral cavity carcinoma; LA, latex agglutination; LLC-MK2 continuous line of Rhesus MK; MDKC Madin-Darby canine kidney; MK, monkey kidney; PCR, polymerase chain reaction; RIA, radioimmunoassay; RIA-BL, radioimmunoassay blocking test; RIBA, recombinant immunoblot assay; RK, rabbit kidney; R-Mix, A549 and mink lung mixture; R-Mix Too, A549 and MDCK mixture; Vero, continuous line of African green monkey kidney; WI-38, MRC-5, human total diploid lung fibroblasts.

time to detection of a positive culture. Cell lines are propagated on 12-mm round coverslips in 1-dram shell vials. An aliquot of specimen is inoculated into the vial, centrifuged at 700 × *g* for 1 hour, and incubated 1 to 3 days, depending on the virus being sought. Antigen typically is detected by IF or occasionally by IP staining with monoclonal antibodies directed against a specific virus.

SVA was described initially and evaluated extensively for HSV[88,108,188,239,244,267] and CMV,[90,102,106,107,179,233,234,249] and its use since then has been extended to the rapid identification of VZV,[103,275] adenovirus,[10,78,191,314] RSV,[26,290] influenza virus,[84,210,222,299] parainfluenza virus,[274] measles virus,[211] and human metapneumovirus[172,235] antigens in culture. Pooled antibodies directed against seven commonly isolated respiratory viruses (adenovirus; influenza viruses A and B; parainfluenza virus types 1, 2, and 3; and RSV) also have been evaluated.[197,229,248] In addition, SVA has been applied to the detection of enteroviruses[160,183] and human herpesvirus type 6.[205] Recently, the application of mixed cell lines in tube and shell vial culture has resulted in several improvements in isolation of virus. Combination cell lines in a single tube or shell vial broaden the range of culturable viruses, reduce the requirement for multiple single cell lines, and decrease the time to detection of a positive result, with sensitivity similar to that of conventional culture. Mixed cell lines are useful for rapid isolation of respiratory viruses,[13,92,135,349] human metapneumovirus,[152,284] enteroviruses,[12,95,136] and herpes group viruses.[134] The sensitivity of mixed cell versus conventional single-cell lines for virus detection ranges from 92 to 100 percent for respiratory viruses[13,92,135,349] to 85 to 97 percent for enteroviruses,[95,136] with time to detection of a positive result reduced from an average of 5 to 10 days to 1 to 2 days for respiratory viruses and 1 to 3 days for enteroviruses.

Although it is rapid, sensitive, and specific, SVA is an adjunct to and not a substitute for traditional culture. When compared with traditional culture, the sensitivity of SVA averages approximately 85 percent (range, 70-100%) and depends on numerous variables, including the cell line,[359] the age of cells,[90] the concentration of virus,[359] the type of specimen,[179,233] the number of vials inoculated,[233] and the type of reagents used.[82,108,208,239,267] Table 264–4 summarizes the typical time to detection of a positive result in traditional culture and SVA, the sensitivity of SVA versus culture, and the turnaround time for each.

Other culture modifications include hybridization[82,94] and ELISA[10,64,204,329,336] for rapid detection of viruses. A modification of cell culture for the rapid detection of HSV is the enzyme-linked virus-inducible system (ELVIS).[295,296] This system consists of a mixture of modified baby hamster kidney cells and MRC-5 cells. The modified baby hamster kidney cells contain an HSV-

TABLE 264–4 Detection of Virus in Traditional Culture and Shell Vial Assay

Virus	Traditional Culture		Shell Vial Assay	
	Usual Day Positive, Range	*Negative Turnaround Time*	*Day Stained (Range)*	*% Sensitivity vs. Culture*
Adenovirus	4-10	2-3 wk	2 (1-3)	92-100
CMV	7-14	1 mo	2 (1-3)	75-100
Enterovirus	1-7	2-3 wk	3	85-100
HSV	1-3	5-7 days	1	70-100
HHV-6	NA	2-3 wk	3 (2-3)	86
hMPV	≥14	3-4 wk	1 (1-2)	92-100
Influenza	3-7	2-3 wk	2 (1-3)	92-100
Parainfluenza	3-7	2-3 wk	2 (1-3)	92-100
RSV	3-7	2-3 wk	2 (1-3)	92-100
VZV	5-10	2-3 wk	3 (2-5)	80-100

CMV, cytomegalovirus; HHV-6, human herpes virus type 6; hMPV, human metapneumovirus; HSV, herpes simplex virus; RSV, respiratory syncytial virus; VZV, varicella-zoster virus.

inducible promoter and an *Escherichia coli LacZ* reporter gene that produces β-galactosidase only when cells are infected with HSV. After the addition of a substrate for the β-galactosidase, infected cells stain blue, whereas uninfected cells remain colorless. ELVIS appears to be comparable with culture for the detection of HSV-positive specimens (95-100% sensitivity) in a similar time frame, 1.4 to 1.7 days.[42,194] This system may have future application in antiviral susceptibility testing.[280]

DIRECT DETECTION

Cytology

Historically, rapid detection of viral infection relied on light microscopy and evaluation of tissues and exfoliated cells for viral inclusions or other CPEs. Cytologic identification of virus is most useful in illnesses with vesicular exanthems such as HSV or VZV infection. A scraping of the base of an HSV or VZV lesion (Tzanck smear) reveals multinucleated giant and balloon cells when stained with hematoxylin and eosin, Wright, or Giemsa stain. In contrast, vesicular lesions caused by enteroviral infections do not contain giant or balloon cells, and allergy-related lesions contain eosinophils. Cytologic study of urine in congenital CMV infection reveals cells with the characteristic "owl's eye" intranuclear inclusions in 25 to 50 percent of cases, and nasal or pharyngeal smears in measles may reveal typical giant cells. These types of stains no longer are performed routinely in most diagnostic virology laboratories because they have been replaced by IF, which is more sensitive and specific.[45,46] Whereas the Tzanck smear cannot differentiate HSV from VZV and the presence of multinucleated giant cells is necessary to make the diagnosis, IF can identify the causative agent specifically, and the presence of viral antigen in balloon or giant cells is diagnostic.

Antigen Detection

Antigen-detection methods are available for most commonly isolated viruses. Advantages of these methods include speed; the use of standardized reagents and kits; the ability to detect nonviable, nonculturable, or difficult-to-grow viruses; and the ability to detect antigen when culture may be negative late in the course of infection. Generally, for viruses that can be cultured, these methods are not as sensitive as is optimized culture; thus, culture backup is recommended. These tests also are approved for specific-specimen types. DFA, indirect immunofluorescent assay (IFA), and ELISA are used most commonly for antigen detection in the clinical laboratory.

Immunofluorescence

Antigen detection by IF requires collection of the appropriate cell types (i.e., those in which the virus propagates) in adequate numbers for evaluation. In some cases, the specimen is placed on the slide directly (e.g., HSV or VZV detection), but usually the sample is transported to the laboratory for processing and slide preparation (e.g., for respiratory virus detection).

DFA methods use a virus-specific monoclonal antibody labeled with fluorescein dye to detect antigen and usually require approximately 30 to 45 minutes to complete. If processing of the specimen is required and slides are prepared in the laboratory, additional time is necessary. For IFA, unlabeled monoclonal antibody is added to the sample and incubated for 30 minutes, followed by a wash and the addition of fluorescein-labeled antimouse immunoglobulin. After an additional 30 minutes of incubation has transpired and a wash step has been performed, the slide can be examined for typical fluorescence (for excellent photographic examples, see Rossier and colleagues[257]). IFA can be completed in 2 to 3 hours, depending on specimen-processing requirements and the number of viral antigens being sought. The advantage of using DFA or IFA is the ability to determine specimen adequacy and perform small-batch and demand testing. A high-quality microscope and skilled, experienced personnel are required for optimal preparation and evaluation of the specimen.

The first DFA reagent available for use in the diagnostic laboratory was for the detection of HSV[109] in genital, dermal, oral, and anal lesions. The test has not been validated for the detection of HSV in CSF, asymptomatic genital specimens, tracheal aspirates, or bronchoalveolar lavage (BAL) specimens from immunocompromised patients. The sensitivity of DFA versus culture for HSV detection ranges from 72 to 92 percent[167,243] and, like culture, depends on the stage of the lesion.[45,46] The ability to detect HSV by DFA or culture decreases with the stage and age of the lesion. DFA or culture detected HSV in 96.7 percent of vesicles, 79.2 percent of pustules, 44.7 percent of ulcers, and 16.7 percent of crusts; HSV was detected in 82 percent of lesions less than 24 hours old, 77 percent of lesions 25 to 72 hours old, 50 percent of lesions 73 to 121 hours old, and 15 percent of lesions older than 122 hours.[45]

DFA is an excellent method for detecting VZV in lesion material, and because the virus is labile, the sensitivity of DFA is superior to that of culture.[41,68,103,275,279] In a comparison of DFA

and culture for the detection of VZV in skin lesions, the sensitivity and negative predictive values were 97.5 and 96.8 percent, respectively, for DFA and 49.4 and 60.4 percent, respectively, for culture. Because of the sensitivity of DFA, culture backup is not necessary.

Rapid detection of CMV antigen in lung tissue or BAL specimens[73,118,192,331,340] and in liver tissue[232] for the diagnosis of CMV pneumonia or hepatitis also has been evaluated. The sensitivity is less than that of culture or SVA and ranges from 56 to 100 percent; results were best when a mixture of antibodies directed against early and late viral antigens was used.[331] Sensitivity may be reduced further because BAL specimens virtually have replaced lung biopsy for the diagnosis of CMV pneumonia. A comparison of the cellular portion, supernate, and whole BAL for isolation of CMV in culture suggested that in most BAL specimens, CMV is associated with the cell-free rather than the cellular component.[40]

The antigenemia assay[325,326] is a sensitive, specific, and rapid method for detecting CMV viremia. Antigenemia has been used for establishing the diagnosis of CMV infection early[76,77,173]; for monitoring patients at high risk for acquiring CMV disease, including bone marrow,[19] heart transplant,[101] renal transplant,[324] and liver transplant patients,[322] as well as those with acquired immunodeficiency syndrome (AIDS)[198]; and for monitoring the treatment of severe disease after organ transplantation has been performed.[101,323] Clinically, it has been useful in detecting infection before the onset of symptoms, and through quantitation of viral load, it has enabled differentiation of disease from asymptomatic infection.[89,170,311] The assay consists of separation of leukocytes from whole blood, spotting or cytocentrifuging of cells onto microscope slides, fixation and permeabilization, staining with monoclonal antibodies, and detection by fluorescein- or peroxidase-labeled secondary antibodies. Antigen-positive leukocytes are quantitated. Once the assay was optimized,[20,99,100] standardized IFA[173] and IP[76,77] kits became available commercially. Antigenemia is more sensitive than is traditional culture or SVA for detecting CMV viremia. Antigenemia results were positive in 91 percent (90 of 99) of confirmed active CMV infections as compared with 34 percent (34 of 99) for culture[173]; another study yielded similar antigenemia results: 91 percent sensitivity versus 66 and 57 percent sensitivity for culture and SVA, respectively.[77] Disadvantages of the assay are that it is labor-intensive, time-consuming, and subjective; the leukocyte suspension must be adjusted to 10^6/mL, and the specimen must be processed quickly because storage for longer than 6 hours results in inaccurate quantitation of positive cells.[20,170]

Finally, DFA and IFA are useful for the rapid detection of common respiratory virus antigens[200,202,251,292,301,333,335,346] in nasopharyngeal washes, aspirates, and suctions. DFA for RSV has been evaluated extensively,[35,154,200,212,251,301,312] and its sensitivity in comparison to that of optimized culture ranges from 80 to 97 percent. As is the case for VZV, RSV is relatively labile, and antigen detection actually may be more sensitive than is culture; thus, culture backup usually is not required. However, culture still may be advisable because 5 to 25 percent of specimens submitted for establishing the diagnosis of RSV contain other viruses.[18] The sensitivity of DFA and IFA for detection of influenza virus A and B, parainfluenza virus 1, 2, and 3, and adenovirus antigens varies when compared with that of culture (ranging from 29-100%[10,139,171,197,200,292,301]), so culture must be performed for optimal diagnosis.

Enzyme-Linked Immunosorbent Assay

Standardized, well-characterized commercial kits for the detection of viral antigen by ELISA are available for HSV, RSV, influenza virus A, rotavirus, adenovirus, and hepatitis B virus (HBV). Intracellular and extracellular antigen can be detected, the method is simple technically, and suboptimal specimen handling still may yield positive results. ELISAs come in two basic formats, either a classic microtiter/tube system or the more recently developed self-contained membrane assays. The microtiter/tube format uses antibodies lining the surface of the solid phase to capture viral antigen, which in turn is reacted with an enzyme-labeled secondary antibody, followed by detection with the addition of substrate and color development. Results are read spectrophotometrically and thus are objective, thereby eliminating some of the difficulties inherent in microscopic techniques, which require technical skill and expertise for evaluation of specimens. These tests are relatively inexpensive, can be automated, are suitable for processing large numbers of specimens, and generally can be completed within 2 to 4 hours. Specimens normally are batched and cannot be tested on demand as previously described for IF. In addition, the adequacy of the specimen cannot be ascertained. In contrast, membrane assays use the membrane as the solid support to retain viral antigen. Enzyme-labeled antibodies are added to react with trapped antigen, and antigen-antibody complexes are detected via a chromogenic substrate. The results are read visually. Although they are rapid (15-20 minutes) and simple to perform, these tests are more expensive, require more hands-on time, and do not result in objective end-points. False-positive results and interpretation can be a problem.

Numerous studies have evaluated the performance of ELISA for detection of HSV in genital, dermal, oral, and ocular specimens.[44,54,105,111,149,227,283,328,330] Herpchek (DuPont Pharmaceuticals, Wilmington, Del) has been investigated most frequently and, when compared with culture, yields sensitivities and specificities ranging from 72 to 99 percent and 92 to 100 percent, respectively (the average sensitivity is approximately 90%). An automated system, VIDAS (bioMerieux, Hazelwood, Mo), also has shown promise, with a sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of 92, 89, 83, and 95 percent, respectively.[149] Results obtained with another assay (Ortho ELISA) yielded an unacceptably low sensitivity of 35 percent, but the specificity, PPV, and NPV were 100, 100, and 85 percent, respectively,[111] thus illustrating the substantial differences among assays and the need for evaluation and validation by the laboratory. Membrane assays are less sensitive than is microwell ELISA, with a reported sensitivity, specificity, PPV, and NPV of 73, 99, 97, and 84 percent, respectively, and, therefore, they are used infrequently.[66,360]

Detection of RSV antigen in nasopharyngeal aspirates, washes, suctions, and swabs, like HSV, also has been evaluated extensively in standard[91,120,150,175,312,321,332] and membrane ELISA formats. The reported sensitivity, specificity, PPV, and NPV for standard ELISA were 71 to 91, 87 to 100, 95, and 94 percent, respectively, and for membrane enzyme immunoassay (EIA), 57 to 94, 73 to 100, 38 to 100, and 75 to 97 percent, respectively. When the results of TestPack RSV (Abbott Laboratories, Abbott Park, Ill) and Directigen RSV (Becton Dickinson) membrane EIAs were compared, TestPack appeared to be more sensitive and specific for the detection of RSV antigen; the mean sensitivity, specificity, PPV, and NPV for TestPack and Directigen were 87, 93, 89, and 92 percent and 76, 81, 63, and 89 percent, respectively. In many of these studies, antibody-blocking assays confirmed the specificity of ELISA. Other RSV rapid membrane assays include Directigen EZ RSV (Becton Dickinson), NOW RSV (Binax), RSV OIA (Biostar), XPECT RSV (Remel), QuickVue RSV (Quidel), and RSV ImmunoCard STAT (Meridian). Reported sensitivity, specificity, PPV, and NPV are similar to those mentioned earlier.[5,25,228] The recent introduction of antiviral therapy for influenza A and B has increased the demand for rapid diagnosis, thus prompting the development of several new assays. Table 264–5 summarizes the features of these assays. The assays are rapid and require less than 30 minutes to perform.

TABLE 264–5 Summary of Rapid Influenza Assays

Rapid Test (Manufacturer)	Virus Detected	Acceptable Specimens	Assay Time	Sensitivity	References
Directigen Flu A (Becton Dickinson)	A	NP wash/aspirate; NP and throat swabs	15-20 min	64-90%	148, 169, 334
Directigen Flu A + B (Becton Dickinson)	A and B	Same as Flu A and lower nasal swab, BAL	15-20 min	47-86%	264, 289, 303, 339
Directigen EZ Flu A + B (Becton Dickinson)	A and B	Same as Flu A + B	15-20 min	39%	339
Flu OIA (Biostar)	A/B*	Nasal aspirate, NP and throat swabs, sputum	15-20 min	54-88%	23, 48, 128
Flu OIA A-B (Biostar)	A and B	Same as Flu OIA	15-20 min	54-88%	48, 128
NOW Influenza A (Binax)	A	Nasal wash/aspirate, NP swab	15-20 min	53-59%	289
NOW Influenza B (Binax)	B	Same as NOW Flu A	15-20 min	53-59%	289
NOW Influenza A + B (Binax)	A and B	Same as NOW Flu A	15-20 min	47-88%	24, 289, 339
QuickVue Influenza (Quidel)	A/B	Nasal wash/aspirate, NP swab	10-15 min	73-94%	11, 264
QuickVue Influenza A + B (Quidel)	A and B	Same as QuickVue Flu	10-15 min	69-85%	3
XPECT Flu A + B (Remel)	A and B	Nasal wash, NP and throat swabs	15-30 min	94%	33
ZstatFlu (Zyme Tx)	A/B	Throat swab	20-30 min	41-87%	121, 226

**A/B does not differentiate A from B.*
BAL, bronchoalveolar lavage; NP, nasopharyngeal.

Some assays detect influenza A or B, some detect both but do not differentiate between them, and some simultaneously detect A and B and differentiate between them. Various specimen types have been validated and approved for use with these rapid assays, but accuracy may vary, even when acceptable specimens (e.g., throat swab versus NP swab versus NP aspirate) are used. In general, these rapid assays are expensive, are best suited to small-batch testing, require subjective interpretation, and are less sensitive than is culture or rapid IF detection. Sensitivity is variable (median 70-75%) and highly dependent on the type and quality of the specimen; median specificities are 90 to 95 percent.[23,48,148,169,181,265,303,334] Directigen Flu A and culture were compared for the detection of influenza A in nasopharyngeal washes, throat gargles, nasopharyngeal swabs, and throat swabs obtained from experimentally infected volunteers. Culture yielded 3.6, 1.2, 1.8, and 0.6 log of virus from these sources, respectively. The sensitivity of Directigen Flu A versus culture was 75, 22, and 64 percent for nasopharyngeal washes, throat gargles, and combined throat and nasopharyngeal swabs, respectively.[153] For optimal diagnosis, these devices should not be used when disease prevalence is low (at a 1-2% prevalence, the predictive value of a positive result is <15%) or in the off-season, adequate specimen collection must be emphasized, and negative results should be backed up by culture or IF testing.[115]

For the diagnosis of rotavirus and adenovirus 40/41 infection, ELISA is the only rapid test available in the clinical laboratory. Rotavirus antigen detection by standard ELISA has been studied thoroughly.[51,56,163,313] Many of the assays have excellent sensitivity (93-100%) and specificity (98-100%) when compared with that of electron microscopy (EM), although the best results were obtained with monoclonal (versus polyclonal) antibody-based kits (see elsewhere[57,196] for a review of available tests). Some assays can be read either visually or spectrophotometrically, so instrumentation is not always necessary. Several membrane EIAs also are available for rotavirus antigen detection (TestPack, Abbott Laboratories; ImmunoCard, Meridian Diagnostics, Cincinnati, Ohio) and, as is typical of this format, are somewhat less sensitive than is standard ELISA, with approximately 2 to 4×10^7 virions/mL required for detection of a positive specimen. The reported sensitivity and specificity versus EM are 94 to 100 percent and 90 to 100 percent, respectively.[27,37,57] An excellent ELISA for detection of adenovirus 40/41 in stool specimens is available and yields a reported sensitivity, specificity, PPV, and NPV of 96, 96, 95, and 96 percent, respectively.[127,146,347]

Latex Agglutination

Latex agglutination (LA) has been used primarily to diagnose rotavirus infection,[56,196,230,268,269] although assays also are available for detection of adenovirus[114] and HSV[300] antigens. LA is the least technically demanding of the tests described thus far, is rapid (2-5 minutes for rotavirus, 25 minutes for HSV), and is adequate for testing specimens collected early in the course of infection, but it is not as sensitive as is standard ELISA, with 10^7 virions/mL required for detection of a positive specimen. The sensitivity, specificity, PPV, and NPV for detection of rotavirus antigen by LA ranged from 70 to 93, 80 to 100, 76 to 100, and 85 to 95 percent, respectively (see elsewhere[56,196] for review); those for HSV were 73, 89, 89, and 72 percent, respectively.[300] The sensitivity and specificity of LA versus EM for detection of adenovirus were 95 and 100 percent, respectively.[114]

Nucleic Acid Detection

Molecular methods for the detection, identification, and characterization of microorganisms are being adapted increasingly for use in the clinical laboratory.[161,236-238,242,309,310,341,343,344] The two broad categories for detecting nucleic acids are direct detection via labeled DNA or RNA probes and hybridization or amplification of selected nucleic acid targets followed by detection of the amplified product. Direct hybridization formats include solid-phase (slot/spot/dot blot, microwell/bead capture, Southern/Northern blot), solution-phase, and in situ hybridization.[74,161,237,310,344] Nucleic acid amplification techniques are characterized as target (PCR), probe (ligase chain reaction), or signal (branched-chain DNA [bDNA]) amplification types.[74,237,309,344] Some of this methodology is moving from the research to the clinical laboratory for the following reasons: (1) replacement of radiolabels with enzyme, chemiluminescent, or affinity labels (e.g., biotin); (2) replacement of more cumbersome, labor-intensive detection methods with simplified, objective, ELISA-like formats; (3) simplified specimen preparation methods for releasing and exposing target nucleic acid; (4) the availability of standardized, optimized, and quality-controlled reagents and kits; and (5) the publication of guidelines and recommendations for the use of molecular testing in infectious disease diagnosis.[74] A few of these techniques are described in the following section with respect to their application for the diagnosis and monitoring of certain viral infections. Table 264–6 lists various methods and

TABLE 264–6 Comparison of Analytic Sensitivities

Detection Method	Approximate Detection Limit of Assay (Virions or Copies/mL)
Culture	1-10
Antigen	
IF-, IP-stained cells	1-10
Microwell ELISA	10^3-10^6
Membrane ELISA	10^7
Latex agglutination	10^7
Electron microscopy	10^6-10^7
Nucleic acid	
Radiolabeled oligonucleotide probes	10^6
Radiolabeled full-length probes	10^4
Enzyme-labeled probes	10^4
Chemiluminescent probes	10^4
Compound or branched probes	50-10^3
Nucleic acid amplification	≤10-10^3

ELISA, enzyme-linked immunosorbent assay; IF, immunofluorescence; IP, immunoperoxidase.

their relative analytic sensitivity for the detection of viruses, viral antigen, or viral nucleic acid.

IN SITU HYBRIDIZATION

In situ hybridization is a type of solid-phase hybridization assay in which whole cells or tissue sections fixed to microscope slides are taken through the hybridization process within the morphologic context of infected tissue. Cell suspensions and fresh-frozen and formalin-fixed, paraffin-embedded tissue sections have been used as the starting material for hybridization. The target nucleic acid is made accessible for hybridization via proteases and heat to increase availability of the target while preserving cellular architecture and morphology through the use of fixatives such as formalin, paraformaldehyde, or glutaraldehyde.[237] Detection systems include radiolabeled, as well as enzyme-, biotin-, and digoxigenin-labeled, probes. Some commercial kits are available, but methods often are optimized individually, thereby leading to variations in sensitivity among laboratories. In situ hybridization has been applied to the study and diagnosis of numerous infections,[237,316,318] particularly CMV viremia,[53,298] pneumonia,[104,217,340] hepatitis,[79,232] and HSV infection.[93,174,252,302] In some cases, culture,[104] immunocytochemistry,[302] and histopathology[79] had equivalent or superior sensitivity in comparison with hybridization. Although in situ hybridization probably is one of the first molecular techniques applied to the diagnosis of viral infections, lack of sensitivity, cost, and complexity have precluded its widespread use in diagnostic virology laboratories. In addition, the technique is related more closely to routine histologic staining and immunocytochemistry and, thus, is better suited to other areas of the pathology laboratory.[317,318]

SIGNAL AMPLIFICATION

In signal amplification, target nucleic acid is hybridized with complementary DNA or RNA probes, captured onto a solid surface such as a microtiter well or test tube, and, in an assay similar to ELISA, detected after the addition of enzyme-labeled probes and substrate. The attachment of multiple enzyme-labeled probes to each captured target molecule allows for amplification of the signal rather than the target nucleic acid itself. Thus, the signal generated is approximately proportional to the quantity of target nucleic acid in the sample, which is determined from a standard curve. Two signal amplification systems that have become commercially available are bDNA assays (VERSANT [formerly Quantiplex], Siemens-Bayer, Tarrytown, NY), and Hybrid Capture System (HCS) (Digene Corp., Gaithersburg, Md).

bDNA

Quantiplex assays have been developed for the detection of HBV,[36,124,357] hepatitis C virus (HCV),[58,186,225] and HIV[60,253] viremia. Virus is concentrated from plasma or serum; the target nucleic acid is released by disruption, hybridized with target and capture probes, and incubated overnight. The captured complex is hybridized with bDNA, incubated and washed, hybridized with alkaline phosphatase–labeled probes, and detected via a chemiluminescent substrate.[58] As many as 3000 alkaline phosphatase–labeled probe molecules can be incorporated onto each target molecule, thereby amplifying the signal and increasing the sensitivity of detection.[237] Because the signal is proportional to the quantity of nucleic acid in the specimen, the assay can be used to quantitate viral burden. Although the analytic sensitivity of bDNA is less than that obtained with PCR (10^1 to 10^5 copies/mL versus 10^1 to 10^2 copies/mL, respectively), bDNA's ability to quantitate viral burden by using a relatively simple and reproducible method has rendered it useful for establishing the diagnoses and prognoses of HBV,[34,36,124,133,357,353] HCV,[58,133,225] and HIV[14,138,176,177,273,288] infections, as well as for monitoring the response to antiviral therapy.

HYBRID CAPTURE SYSTEM

HCS assays have been developed for the detection and monitoring of human papilloma virus, HBV,[9,34,1130] HSV,[185] and CMV[14,138,140,176,187,199,209,273,288] infections. Briefly, specimens are treated to release viral DNA, target DNA combines with specific RNA probes to create RNA/DNA hybrids, hybrids are captured onto a test tube or microwell coated with capture antibody specific for the RNA/DNA hybrids, and captured hybrids are detected with multiple antihybrid antibodies conjugated to alkaline phosphatase. The bound alkaline phosphatase is detected with a chemiluminescent substrate. Similar to the case with bDNA, as many as 3000 alkaline phosphatase molecules can bind to each captured hybrid, and the signal generated is proportional to the quantity of nucleic acid in the original sample.

HCS has been evaluated for the detection and quantitation of CMV viremia; correlation to symptomatic infection; and response to therapy in renal,[14,138,176,177,273,288] liver,[176,177,187] heart,[176,177] and bone marrow transplant[176] and HIV-positive patients.[140,199,209] HCS was more sensitive than is traditional tube culture, SVA, or both, and it was useful for the early detection of CMV infections. Higher DNA concentrations correlated with clinically significant disease or progression, and quantitative DNA was useful in monitoring antiviral therapy. As with all quantitative methods, the threshold levels that predict disease must be determined in various patient populations. The lower limit of detection is 600 copies/mL, levels similar to those detected by PCR.

TARGET AMPLIFICATION BY POLYMERASE CHAIN REACTION

Of the molecular techniques developed recently, probably none has had a greater impact on basic and clinical research than has PCR. PCR has been applied to the detection and study of numerous, if not most, bacteria, fungi, parasites, and viruses. PCR is a target amplification technique in which a specific nucleic acid sequence is replicated by repeated cycles of denaturation, annealing, and extension directed by an oligonucleotide primer pair that defines the region of interest.[237,344] Target nucleic acid is produced in sufficient quantities to render it detectable by a variety of methods. The most practical and adaptable detection methods use an ELISA-like format with 96-well microtiter

plates. These methods entail the use of biotinylated primers in the amplification step, which are incorporated into the PCR product and subsequently detected via avidin-biotin interaction. In one scheme (Roche Diagnostic Systems, Indianapolis, Ind), the biotinylated PCR product is captured through hybridization with a specific probe attached to the microwell and detected with avidin–horseradish peroxidase conjugate. This system also features enzymatic prevention of carryover contamination.[156,165,186,225,253,260,281,337,354] Another system (Digene Corp.) uses solution hybridization with specific RNA probes to form RNA/DNA hybrids that are captured onto streptavidin-coated microwells. Bound PCR product then is detected by the addition of alkaline phosphatase–conjugated antibodies directed against RNA/DNA hybrids. This universal product detection system has been used to detect amplified CMV,[83,166] HBV,[320] and HIV[182] and can be adapted for the detection of numerous targets with the use of specific primer/probe sets. Issues that surround the implementation of PCR diagnostics include the production of diagnostic kits, the availability of standardized kits approved by the Food and Drug Administration (FDA), guidelines for use and interpretation, standardization and quality control of test procedures and specimen preparation, qualitative versus quantitative PCR, validation of test protocols, participation in proficiency surveys such as the College of American Pathologists survey, and re-evaluation of the gold standard. Although PCR is a powerful and sensitive technique, as with all other diagnostic tests, interpretation of results must integrate the clinical features, patient history, supporting laboratory data, and treatment records. PCR is a rapid, sensitive, and specific tool that is clinically useful for establishing the diagnosis of particular viral infections in select situations. PCR is useful (1) when culture is too insensitive, lengthy, expensive, difficult, impractical, or unavailable; (2) for confirmation of infection; (3) for early detection of infection; (4) for detection late in disease; (5) for resolution of indeterminate serology results; (6) to monitor the status of disease; and (7) to monitor response to therapy (Table 264–7). Use of PCR for diagnosing certain viral infections is addressed in the following section.

PCR has been used to detect HSV DNA in a variety of clinical specimens, including CSF and brain biopsy material,* skin lesions,[218,219] genital lesions,[43,61,123] and ocular samples.[143,351] The application of PCR to CSF specimens for diagnosing HSV encephalitis (HSE) has received a great deal of emphasis. Brain biopsy remains the gold standard for diagnosis. However, it rarely is performed, and patients are treated empirically. Traditional laboratory methods, including culture, antigen detection, intrathecal antibody production, CSF and serum antibodies, and the serum/CSF antibody ratio, lack sensitivity or specificity or are too slow; they are useful primarily for retrospective diagnosis. Numerous reports have documented the utility of CSF PCR for diagnosing HSE in a timely manner, with sensitivity and specificity greater than 95 and 100 percent, respectively.* Most of these studies have used some combination of traditional methods along with clinical observation and radiodiagnostic studies for case definition. Lakeman and Whitley[168] compared CSF PCR and brain biopsy in 101 patients, 54 with biopsy-proven HSE and 47 who were biopsy culture-negative. The sensitivity and specificity of CSF PCR were 98 percent (53 of 54) and 94 percent (44 of 47), respectively. The PCR-positive, biopsy-negative specimens remained PCR-positive in repeat assays with two different primer pairs and were considered true-positive; culture failures were perhaps due to sampling problems. The PCR-negative, biopsy-positive sample remained PCR-negative after DNA extraction and was not inhibitory to PCR. Patients also were monitored after receiving acyclovir treatment; 100, 98, 47, and 21 percent of CSF specimens were PCR-positive at times 0, 0 to 7, 8 to 14, and greater than 15 days, respectively, after brain biopsy was performed and therapy was initiated. The authors concluded that PCR should be the standard for establishing the diagnosis of HSE because its sensitivity and specificity were adequate for establishing the diagnosis of focal biopsy-proven HSE; its usefulness for defining the spectrum of HSV infection of the central nervous system (CNS) continues to be investigated. Detection of HSV DNA in skin and genital lesions has shown that PCR is more sensitive than culture, is able to identify asymptomatic HSV, detects the presence of HSV for a longer period of time (in older lesions and even fixed tissue), and has greater diagnostic value than culture and clinical observations for assessing genital HSV and treatment efficacy in patients with recurrent genital HSV.[43,61,123,218,219] PCR also is useful for diagnosing ocular infections caused by specific viruses or as supporting information in the clinical diagnosis of specific ocular disease syndromes.[143,351]

PCR has been used to diagnose cutaneous,[62,157,164,218,219] ocular,[222,351] CNS,[247,286] and congenital VZV infection[141] and as a tool for studying the natural history of primary and reactivated disease.[62,157,164] The principal clinical use of PCR in VZV disease is for the diagnosis of VZV CNS infection because isolation of virus rarely is successful and intrathecal antibodies cannot be used as an early diagnostic tool.[247,286]

TABLE 264–7 Clinical Use of Polymerase Chain Reaction for the Diagnosis of Selected Viral Infections

Virus	Clinical Utility
CMV	Diagnosis of CNS infection, diagnosis of neonatal/congenital infection, diagnosis of ocular infections, diagnosis and monitoring of active infection/viral load in immunocompromised hosts
EBV	Diagnosis of active EBV infection, diagnosis of CNS infection, diagnosis and monitoring of EBV-related lymphoproliferative disorders in liver and bone marrow transplants undergoing suppression
Enteroviruses	Rapid diagnosis of CNS infection
HBV	Early detection of infection, confirmation of infectivity, monitoring of viral load and therapeutic efficacy in chronic hepatitis
HCV	Detection before seroconversion, resolution of indeterminate or ambiguous serology, monitoring of viral load and therapeutic efficacy in chronic infection
HHV-6	Confirmation of infection
HIV	Detection before seroconversion, resolution of indeterminate immunoblot results, evaluation of suspected prenatal and intrapartum HIV infection, monitoring of viral load to evaluate therapeutic efficacy and disease progression
HSV	Diagnosis of HSV encephalitis, confirmation of neonatal HSV infection, detection of virus in late lesions, diagnosis of ocular infections
HTLV-I/II	Detection of HTLV-I in adult T-cell leukemia and lymphoma and tropical spastic paraparesis/HTLV-I–associated myelopathy
Parvovirus B19	Detection of infection, early detection before seroconversion
VZV	Diagnosis of CNS infection, detection of virus in late or atypical lesions, diagnosis of ocular infections

CMV, cytomegalovirus; CNS, central nervous systems; EBV, Epstein-Barr virus; HBV, hepatitis B virus; HCV, hepatitis C virus; HHV-6, human herpesvirus type 6; HIV, human immunodeficiency virus; HSV, herpes simplex virus; HTLV-I/II, human T-cell lymphotropic virus types I and II; VZV, varicella-zoster virus.

*See references 6, 59, 116, 158, 168, 245, 246, 262, 276, 315, 319, 342.

Significant morbidity and mortality are associated with CMV infection, primarily in organ transplant recipients and patients with AIDS, and surveillance and monitoring for infection or disease by culture or antigenemia are required. Increasingly, PCR is being used to detect CMV DNA in blood,* CSF,[2,8,39,113,345] urine,[55,207] and the same spectrum of specimens submitted for culture.[207] Whole blood, peripheral blood leukocytes, serum, and plasma have been evaluated extensively for detection of infection, association with clinical disease, and therapeutic monitoring. Although PCR was positive earlier, remained positive longer, and was more sensitive than culture or antigenemia for the detection of CMV infection in whole blood or peripheral blood leukocytes, in the absence of a positive culture or antigenemia, PCR was not always associated with clinical symptoms and did not necessarily predict or correlate with the appearance of clinical disease, although it did predict a risk of relapse.[71,101,145,361] Detection of CMV DNA in serum or plasma, however, correlated with viremia, clinical disease, or both in patients with CMV infection,[28,294,337] including congenital CMV infection.[220] Detection of mRNA in peripheral blood leukocytes by reverse transcriptase PCR also has been used to assess active productive CMV infection and to identify patients at risk for symptomatic infection.[17,112] Detection of CMV viremia by PCR likewise is useful for monitoring antiviral therapy, both for the early initiation of treatment and as a predictor of treatment efficacy.[70,101,337,361] In addition, commercial kits (Roche Diagnostic Systems) are being evaluated for the qualitative and quantitative detection of CMV DNA from plasma.[21,29,287,337] PCR also is useful for diagnosing CMV CNS infection, primarily in patients with AIDS,[8,39,113,345] with reported sensitivity ranging from 79 to 100 percent.[8]

Conventional diagnostic methods generally are not useful in the evaluation of Epstein-Barr virus (EBV)-related disorders in immunosuppressed patients. Culture is not practical or useful, and serology is difficult to interpret in the setting of immunosuppression. PCR offers the possibility of rapid detection of EBV in a variety of clinical specimens and allows a semiquantitative or quantitative estimate of viral load. PCR has been used for establishing the diagnosis of AIDS-related CNS lymphoma,[7] EBV-related lymphoproliferative disorders,[49,159,193,221,297,307] and other EBV-associated diseases (e.g., infectious mononucleosis, fatal infectious mononucleosis, chronic active EBV infection, and EBV-associated hemophagocytic syndromes) in which increased DNA concentrations are associated with more severe clinical categories.[350] PCR is particularly useful for the early identification and diagnosis of lymphoproliferative disorders in pediatric patients after they have undergone liver transplantation, for monitoring EBV levels so that immunosuppression can be adjusted, and as a prognostic marker.[49,159,193,221,297,307]

PCR is useful for the early detection of HIV infection, resolution of indeterminate Western blot results, evaluation of suspected prenatal and intrapartum infection, and monitoring of the viral load for prognosis and evaluation of therapeutic efficacy.[129,156,165,203,253,254] Qualitative[156,165] (for detection of proviral DNA in peripheral blood leukocytes) and quantitative[72,75,215,253,266] (for detection of viral RNA in plasma) kits are available commercially (Roche Diagnostic Systems), and the quantitative assay has FDA approval. Quantitative PCR is useful for correlating RNA levels to disease stage, predicting clinical outcome, and monitoring response to therapy.[129,203] However, guidelines for the optimal use of PCR and other quantitative assays (bDNA, nucleic acid sequence–based amplification [NASBA]) still are evolving.[266] In a comparison of PCR, NASBA, and bDNA,[205] no significant differences in sensitivity were found for baseline measurement of HIV-1 RNA levels in the three assays. In addition, changes in RNA levels in response to therapy were comparable. The lower limit of detection for PCR, NASBA, and bDNA was 200, 4000, and 10,000 copies/mL, respectively; the turnaround time was 6 hours, 5 hours, and 1.5 days, respectively. Improvements in PCR and bDNA assays have lowered the limits of detection to 50 copies/mL.[15,72,75,215] Commercial kits (Roche Diagnostic Systems) also are available for the detection of HCV RNA in plasma.[186,225,260,281,358] Detection of HCV RNA is useful for establishing the diagnosis of acute hepatitis before seroconversion, for detection of chronic HCV in seronegative patients, for resolution of indeterminate serologic test results, and to monitor patients receiving therapy. PCR was more sensitive than was bDNA,[186,225] with approximately 11 percent of true-positives undetected by bDNA.[225] The analytic sensitivity of PCR and bDNA was 400 and 3.5×10^5 copies/mL, respectively.[225] Modifications to the VERSANT HCV version 3.0 bDNA assay have lowered the detection limit to 617 IU/mL, with a linear detection range of 617 to 7,700,000 IU/mL.[30] The unit of measure for HCV PCR assays has been converted from copies per milliliter to international units per milliliter, with lower limits of detection of 600 and 50 IU/mL for the quantitative and qualitative assays, respectively.[178] The qualitative assay is the first HCV assay to receive FDA approval.

With regard to enteroviruses, PCR has been used to diagnose enteroviral CNS[32,255,256,258,259,270,277,354] and neonatal infections[1,52,256] and to identify vaccine[352] and wild-type[38] poliovirus infections. Major emphasis, however, has been placed on developing a rapid, sensitive method for diagnosing aseptic meningitis because up to 25 to 35 percent of specimens from patients with characteristic enterovirus infection are negative and, when positive, require an average of 4 to 8 days for detection in culture.[258,270,354] CSF evaluation for pleocytosis is not always reliable because specimens with no pleocytosis also may be culture- or PCR-positive.[255,270] PCR for the diagnosing of enteroviral CNS infections has a sensitivity, specificity, PPV, and NPV of 95 to 100, 97 to 100, approximately 98, and 98 percent, respectively.[255,256,258,259,270,277] Commercial kits also have become available and yield rapid (<6 hours), sensitive, and specific results.[31,259,270,305,354,355] In addition, rapid diagnosis by PCR will have an effect on patient management with regard to number of days of hospitalization, antibiotic treatment, and, therefore, overall cost.[122,250,255,277]

Increased use of PCR for the diagnosis of disease has stimulated several recent developments, including automation of nucleic acid extraction, amplification, detection, and reporting and interpretation of results. Automation of specimen processing significantly improves the PCR process by rapidly and efficiently providing standardized, reproducible nucleic acids for amplification, comparable with those obtained with more labor-intensive manual methods.[81,215] Roche has developed numerous qualitative and quantitative PCR assays for the COBAS, an instrument that automates PCR amplification and detection and yields results similar to those of manual methods. Assays that have been developed for the COBAS include HCV,[63,98,356] HBV,[155,224] CMV,[21,29,287] and HIV.[215] In addition to increased automation is a trend to obtain more rapid, clinically relevant results through the use of real-time PCR. Amplification and detection are integrated in real-time PCR and occur simultaneously, in contrast to conventional PCR, in which these processes are separate. Turnaround time for conventional PCR ranges from 4 to 48 hours, depending on the detection system, whereas real-time PCR can be completed in 20 minutes to 2 hours, depending on the system and instrumentation used. Potential contamination is reduced in real-time PCR because amplification and detection occur in a closed system. For quantitative assays, real-time PCR also offers linearity over a broader dynamic range (5-6 logs) than possible with conventional PCR (2-3 logs). Real-time amplification methods, instrumentation, assay development, validation, and implementation in the clinical microbiology laboratory have been

*See references 28, 70, 71, 97, 101, 112, 145, 207, 220, 285, 294, 337, 361.

reviewed.[87,117,338] Although the majority of real-time amplification methods are home-brew, standardized and optimized commercial reagents are increasingly available, rendering validating and incorporating new molecular assays easier for diagnostic laboratories. Real-time PCR has been used for the qualitative and quantitative detection of CMV,[96,223,271,272,306] HSV,[80,86] VZV,[85] EBV,[159,221,297] HBV,[184,231] influenza A and B,[22,190,308,327] RSV,[22,110,190,214,308] parainfluenza viruses,[308] and human metapneumovirus[47,189] from a variety of specimens. See Espy and colleagues[87] for a complete review.

SUMMARY

Methods for the laboratory diagnosis of viral infections, including culture, SVA, antigen detection, and molecular diagnostic methods, have been reviewed. The use of these tests in some combination is most practical because a single test may not yield a diagnosis. For many viruses, culture has been the gold standard or reference method for evaluation of the new, rapid tests. However, culture is neither 100 percent sensitive nor always diagnostic of symptomatic infection. With the newer methods, particularly molecular diagnostic methods, the gold standard is changing, and evaluation of new techniques must be compared against a spectrum of laboratory and clinical data. Standards for performance and guidelines for the use and interpretation of molecular techniques are becoming available. Quantitative (versus qualitative) viral results may be useful for interpreting tests, particularly with regard to viruses causing latent infection or for monitoring therapy or disease progression. Finally, interpretation of any result requires integration of the clinical history, laboratory data, treatment records, and observation of trends over time.

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CHAPTER 265

Parasitic Laboratory Diagnosis

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Parasitic infections usually are associated with tropical areas; however, many infections (*Dientamoeba fragilis*, *Entamoeba histolytica*, *Enterobius vermicularis*, *Giardia lamblia*, and *Toxoplasma gondii*) have worldwide distribution. With the ability of travelers to move freely throughout the world in a short time span, clinical laboratories must be able to recommend specimen collection and detection/identification methods for all types of parasitic infections. It is helpful if the physician informs the laboratory of the parasitic infection suspected or travel history of the patient because some organisms are detected more easily using specific methods (*Giardia* and *Cryptosporidium*—enzyme immunoassay [EIA] or direct fluorescent antibody [DFA] techniques) or alternative methods (microfilaria—Nuclepore filtration or Knott concentration procedure).[5,7,9] The life cycle of the parasite and the time of infection are important factors in determining the type of specimens to be collected and the techniques used to detect the infection (Table 265–1). Multiple specimens might have to be examined before the etiologic agent can be detected (e.g., colon aspirates or biopsy tissue for *Entamoeba histolytica*, blood for malaria). All specimens must be considered infectious and

TABLE 265–1 Diagnostic Stages and Anatomic Sites of Parasites

Protozoa	Blood	CNS	Eye	GI	G/U	Li/Sp	Lung	Lymph node	Muscle	Skin	Other
Acanthamoeba spp.		t*	c/t							c/t	
Babesia spp.	**RBC**										Serology
Balantidium coli				**c/t**							
Cryptosporidium spp.				**oocyst**							
Cyclospora cayetanensis				**oocyst**							
Dientamoeba fragilis				**t**							
Entamoeba histolytica		t		**c/t**		t	t				Serology
Giardia lamblia				**c/t**							
Isospora belli				**oocyst**							
Leishmania donovani	A**(WBC)**					**A**					Serology
Leishmania spp.										**A**	Serology
Naegleria spp.		**t**								c/t	
Plasmodium spp.	**RBC**										Serology
Toxoplasma gondii	t(WBC)	c	c			c/t	c/t	c/t	c		Serology
Trichomonas vaginalis					t						
Trypanosoma cruzi	T(plasma)								**A**		Serology
Trypanosoma gambiense	**T(plasma)**	**T**									
Trypanosoma rhodesiense	**T(plasma)**	**T**									
Helminths											
Ancylostoma duodenale											
Ascaris lumbricoides				**a/e**			l				
Brugia malayi	**mf**							a			
Clonorchis sinensis				**e**		a					
Diphyllobothrium latum				**a/e**							
Dipylidium caninum				**a/e**							
Echinococcus spp.		l				l	l				Serology
Enterobius vermicularis				**a/e**							
Fasciola hepatica				**e**		a					
Fasciolopsis buski				**a/e**							
Heterophyes spp.				**a/e**							
Hymenolepis spp.				**a/e**							
Loa loa	**mf**		mf								
Metagonimus spp.				**a/e**							
Necator americanus				**a/e**							
Onchocerca volvulus			mf							**a/mf**	
Opisthorchis spp.				**e**		a					
Paragonimus spp.				e			**a/e**				
Schistosoma spp.	a			**e**	**e**						Serology
Strongyloides stercoralis				**a/l**							Serology
Taenia spp.		l	l	**a/e**						l	
Trichinella spiralis				a/l					**a**		Serology
Trichuris trichiura				**a/e**							
Wuchereria bancrofti	**mf**							a			

**Bold type, most frequently detected at that source.*

a, adult; CNS, central nervous system; c, cyst; GI, gastrointestinal tract; GU, genitourinary tract; l, larvae; Li/Sp, liver/spleen; mf, microfilaria; RBC, red blood cell; WBC, white blood cell.

must be handled with universal safety precautions using proper protective equipment.

The diagnosis of parasitic infections is dependent on macroscopic and microscopic examination of blood, feces, respiratory, tissue, and urine specimens. Specimens must be collected using methods that allow for the preservation of parasites when they are transported to the laboratory. Most stool specimens are collected at home or in a physician's office that may be a considerable distance from the laboratory performing the stool examination. Trophozoites disintegrate rapidly and do not form cysts after passage; therefore, stool specimens should be collected in containers from stool collection kits containing preservatives. The preservatives fix the specimens so that the trophozoites can be recognized easily on permanent stained slides and prevent the continued development of larvae and eggs.[3,5,9]

If questions of when and how to collect specimens to maximize the recovery of parasites occur, one's laboratory should be consulted about the collection methods of choice.

STOOL SPECIMENS FOR DETECTION OF INTESTINAL PARASITES

Liquid or semisolid stool specimens should be examined within 30 to 60 minutes, respectively, from the time of collection for protozoan trophozoites. Because most clinical laboratories cannot perform the examination in this time frame, stool specimens should be placed in preservative immediately after passage (Table 265–2). Most stool collection kits contain a combination of vials such as sodium acetate–acetic acid–formalin (SAF) and polyvinyl alcohol (PVA).[3,5,9] Specimens should be collected before radiologic examination with barium is performed, which may prevent the detection of intestinal parasites for up to 10 days after administration. Other substances or medications that also can interfere with the detection of intestinal parasites for a prolonged period of time include antibiotics (aminoglycosides, metronidazole, and tetracycline), antimalarial agents, bismuth, mineral oil, and nonabsorbable antidiarrheal agents. The specimen should be collected in a clean, wide-mouthed container and must not be contaminated with toilet water or urine. Once collected, the specimen should be transferred to a container with preservative. After being mixed with the preservative, the specimen can be stored at room temperature until it is transported to the laboratory.

Physicians recommend that three specimens be collected on alternate days within 10 days to rule out most intestinal infections. The probability of detecting stool parasites with a single stool specimen is as low as 50 percent. Multiple specimens collected from the same patient on the same day should not be accepted. Many intestinal protozoa such as *Giardia* are shed in a cyclic pattern, and the concentration of trophozoites and cysts in the stool can vary considerably from day to day.

OTHER SPECIMENS FOR DETECTION OF INTESTINAL PARASITES

Sigmoidoscopy samples may be submitted for the detection of *E. histolytica*. No fewer than six samples (slides) should be submitted

TABLE 265–2 Common Intestinal Tract Specimen Preservatives

Stool Preservative	Concentrate	Permanent Stain	Immunoassay	Advantages	Disadvantages
Formalin	Yes	No	Yes	Easy to prepare and has a long shelf life; preserves helminth eggs and larvae and protozoan cysts well; can be used with modified acid-fast and trichome stains	Does not preserve trophozoite morphology adequately; not suitable for permanent stains; specimen does not adhere to slide; cannot be used with all immunoassays
MIF*	Yes	No	ND	Has a long shelf life; fixes and stains parasite forms simultaneously	Two solutions must be mixed immediately before use; trophozoite morphology is poorly preserved; not suitable for trichrome stains; iodine interferes with immunoassays
PVA	Yes	Yes	No	Excellent preservation of trophozoite morphology; suitable for PCR assays	Contains Hg making disposal quite costly; morphology of larvae and eggs makes identification difficult; unsuitable for modified stains; PVA interferes with immunoassays
PVA, modified	Yes	Yes	No	Does not contain Hg; disposal is less expensive	Cu or Zn (substitute for Hg) does not preserve trophozoite and cyst morphology, making identification difficult; PVA interferes with immunoassays
SAF	Yes	Yes	Yes	Does not contain Hg; disposal is less expensive; has a long shelf life; permanent stain of choice is iron hematoxylin; preserves helminth eggs and larvae and protozoan cysts well; can be used with most immunoassays and special stains	Must use albumin-coated slides for permanent stains
Schaudinn	No	Yes	No	Excellent preservation of trophozoite morphology	Contains Hg, making disposal quite costly; not recommended for concentrates and has poor adhesive properties for liquid and mucoid specimens

MIF, merthiolate-iodine-formalin; PCR, polymerase chain reaction; PVA, polyvinyl alcohol; SAF, sodium acetate–acetic acid–formalin.

to the laboratory. Aspirates or scrapings can be collected and sent to the laboratory for direct wet mount observations, immunoassay, or polymerase chain reaction (PCR) (not commercially available). Preservatives such as Schaudinn fluid or PVA can be used to fix the samples to microscopic slides. Biopsy specimens should be submitted to the pathology laboratory for histologic preparation.

Duodenal aspirates and biopsies can be collected for the detection of *Cryptosporidium*, *Giardia*, and *Strongyloides*. Collection techniques similar to those mentioned for the detection of *E. histolytica* can be used. In addition, duodenal contents may be sampled using the EnteroTest (HEDECO, Mountain View, Calif). The nylon string can be sent directly to the laboratory for processing and microscopic examination for parasitic organisms.

E. vermicularis infections are detected best using the cellulose tape method. Specimens should be collected in the early morning before bathing or having a bowel movement. The adhesive side of the cellulose tape is pressed against the anal folds and after completion of sampling, the tape is placed with the adhesive side down onto a microscope slide. *Enterobius* eggs and adults may be trapped on the tape (commercial collection kits are available).[3] Multiple specimens (four to six) may be collected on consecutive days to rule out the presence of infection. Eggs from infections with *Taenia* adult worms also may be detected using this method.

STOOL PROCESSING AND EXAMINATION FOR PARASITES

All specimens should be examined using a concentration technique and performing a permanently stained slide (Fig. 265–1). If fresh, unpreserved specimens are received, a direct wet mount should be performed for the detection of motile protozoan trophozoites and protozoan cysts. Helminth eggs and larvae also may be seen. If necessary, a few drops of saline may be used to emulsify the stool and a drop placed on a microscopic slide and covered with a 22 × 22-mm coverslip. The wet mount should be examined using 100× and 400× magnification. A drop of iodine solution may be added to highlight parasite internal structures; however, parasite motility will be lost. A concentrate and a permanent stained slide also should be prepared from this fresh specimen.

Common concentration techniques include the flotation method using zinc sulfate ($ZnSO_4$) and sedimentation method. Specimens fixed in formalin, PVA, or SAF can be used. The flotation method gives a cleaner preparation to observe cysts and eggs, but the high specific gravity tends to distort cysts and eggs, rendering identification difficult. Heavier eggs (*Ascaris* or operculated eggs) will not float and will be found in the sediment; therefore, one should examine both top and bottom layers for a complete examination. The sedimentation method is used most widely in laboratories. Parasites and fecal debris are pelleted to the bottom of the tube by centrifugation. After the supernatant is discarded, the sediment is examined for parasites at 100× and 400× magnification.

Permanent stained slides should be prepared on every stool specimen that is examined for ova and parasites.[3,5,9] The stained slide is used primarily to detect and identify protozoan, trophozoites, and cysts. Gomori trichrome (Wheatley modification) and iron hematoxylin stains frequently are used in laboratories. Both stains have advantages and disadvantages that should be weighed, as should the expertise of laboratory personnel, before selecting the stain of choice for the laboratory.

Certain parasites are not detected easily by wet mount or routine permanent stained slides stained with Gomori trichrome (Wheatley modification) and iron hematoxylin. They include *Cryptosporidium* spp., *Cyclospora cayetanensis*, *Isospora belli*, and microsporidia. The oocysts of *Cryptosporidium*, *Cyclospora*, and *Isospora* can be detected easily by the modified acid-fast stain. Microsporidia can be detected using a modified trichrome stain.[6,9]

Culture of fresh stool or contents of the large intestine have been used to detect *E. histolytica;* however, this methodology is not practical for most clinical laboratories.

EIA kits are commercially available for the detection of *E. histolytica/Entamoeba dispar* group, *G. lamblia*, and *Cryptosporidium*. For *E. histolytica/E. dispar* group, only fresh or frozen stool can be used in the assay. Formalin interferes with reagents to detect

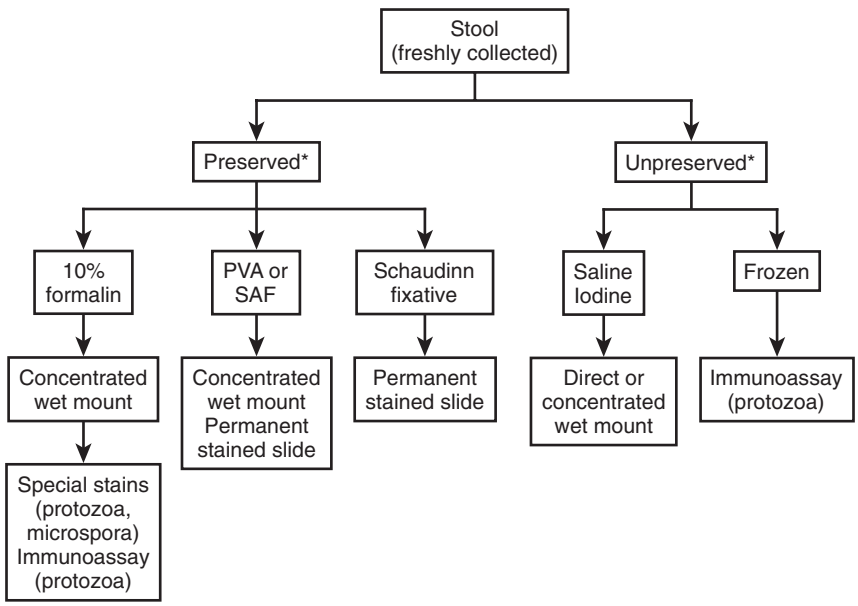

Figure 265–1 Parasite stool examination. *Worms (cestodes, nematodes, trematodes)—fix in 70% ethanol. PVA, polyvinyl alcohol; SAF, sodium acetate–acetic acid–formalin.

E. histolytica and cannot be used. EIA kits are available that will discriminate between *E. histolytica* and *E. dispar*, using fresh or frozen stool. EIA kits for the detection of *G. lamblia* and *Cryptosporidium* can be used with fresh, frozen or formalin fixed stool. Direct fluorescent antibody tests have been developed to detect *G. lamblia* and *Cryptosporidium*. Depending on the manufacturer, either fresh or formalinized specimens can be used. PCR also has been used to detect *E. histolytica*, *G. lamblia*, and *Cryptosporidium*. PCR test kits are not commercially available, whereas DFA and EIA kits are readily available. The DFA and EIA kits have excellent sensitivity and specificity compared to microscopy. The disadvantage of using DFA or EIA kits is that they detect only one or two pathogens at one time and other pathogens might be missed. Additional parasite examinations would have to be performed to detect other parasitic pathogens.

Detection of *Strongyloides stercoralis* is enhanced by culturing a small amount of fresh stool on an agar plate and looking for larval tracks across the plate as evidenced by lines of bacterial growth.[4] Other methods include the Baermann concentrate, Harada-Mori, or Petri dish filter paper slant.[3,5,9] It is possible that hookworms eggs could hatch and the larvae would have to be differentiated from *Strongyloides* larvae.

BLOOD PARASITES

Numerous important parasites can be detected in blood, although most laboratories will see these organisms only during proficiency testing (Fig. 265–2). These parasites include *Babesia*, *Leishmania donovani*, microfilaria, *Plasmodium*, and *Trypanosoma*. They may be found in macrophages, plasma, or red blood cells depending on the parasite. Maintaining proficiency to speciate *Plasmodium* or to recognize the amastigote stages of *Leishmania* is difficult for many laboratorians because these infections are seen so infrequently in the United States. A very important factor is that the physician inform the laboratory about the travel and medication history of the patient. If the patient took partial prophylaxis for malaria, a longer time should be spent examining the blood film due to low parasitemias. Similarly, differences in periodicity of microfilaria in the blood exist, and the timing of collection of specimens becomes more important. Alternative concentration techniques not normally used in most laboratories, such as Nuclepore filtration or Knott concentration procedure, can be used to detect microfilaria in low numbers in the blood, and Nuclepore filtration also may be used with urine.[3,5,9] Without the knowledge of what infection the physician may be considering, these concentration procedures are not routinely performed on blood specimens and the diagnosis of infections could be delayed or missed. Requests for the diagnosis of malaria must be considered to be STAT requests due to the morbidity and mortality mostly associated with *Plasmodium falciparum* infections.

Although some parasites (trypanosomes and microfilaria) can be detected by their motility in whole blood, identification is dependent on morphology as seen with a permanent stain. The blood film stain of choice is Giemsa, although Wright stain can also be used, or Delafield hematoxylin stain can be used, for the microfilarial sheath. Because Giemsa stains may not differentiate the microfilaria nuclei adequately, Delafield hematoxylin stain may be necessary to speciate the microfilaria.

Although blood films can be prepared in clinics or at the bedside (finger or ear lobe sticks), the recommendation is that blood be collected in EDTA (lavender top) so that multiple slides (thick and thin blood films) may be prepared in the laboratory and concentration methods be used. EDTA-collected blood provides the best overall morphology of parasites, and the tubes should be filled to the proper level to reduce distortion of the blood cells and organisms by the anticoagulant. Heparin tubes (green top) can also be used; however, these blood-filled tubes cannot be used for PCR testing. The blood should be examined for parasites within 4 to 6 hours of being collected.

Blood should be drawn immediately when a parasitemia is suspected. For malaria, blood should be drawn every 6 to 8 hours for 36 hours to rule out infection. Pediatric patients may be drawn at 10-hour intervals. A set of one negative blood films does not rule out the presence of malaria. When parasitemias are low, concentration procedures other than thick films, serology, or PCR may be the more sensitive method to detect *Babesia*, Chagas' disease, malaria, or microfilaria. Serology has not been shown to be an effective method to detect active infections of African trypanosomiasis in nonendemic areas.

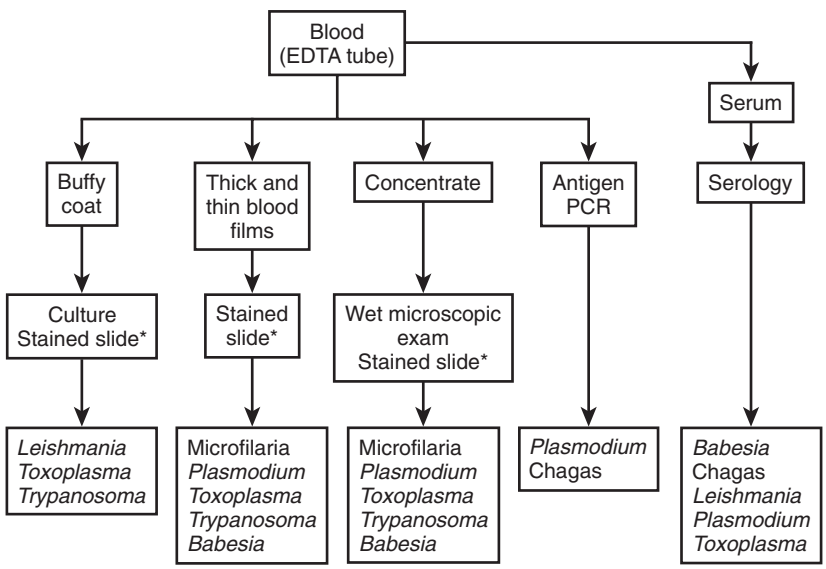

Figure 265–2 Blood parasite examination. *Preferred stain is Giemsa; however, Wright stain is also acceptable. PCR, polymerase chain reaction.

BLOOD PROCESSING AND EXAMINATION FOR PARASITES

If the physician does not indicate the specific blood parasite suspected, fresh blood should be used as a wet mount to look for the presence of microfilaria or trypanosomes by their characteristic shape and motility. If these parasites are detected, a permanent stained slide will have to be performed to identify the organisms to species. When thick and thin blood films are examined for the presence of parasites, no fewer than 300 microscopic oil fields should be examined before finalizing a result for the presence of *Babesia* or malaria. One may use lower magnification when looking for the presence of trypanosomes or microfilaria, but the entire film should be observed before finalizing a result. Thin films are used for the detection of blood parasites, enumeration of infected cells, and also to determine the species of parasite. Once the films are air dried, the film can be fixed with 100 percent methanol and stained with Giemsa stain or Wright stain without the methanol fixation process. If *Babesia* or malarial parasites (*P. falciparum* and *Plasmodium vivax*) are present, one should quantitate the number of infected red blood cells. This quantification can be expressed as the number of infected cells/100 RBC observed in the blood film. Serial specimens collected at various times during therapy can be used to monitor the success or failure of therapy. Continued presence of parasites in blood films without a marked decrease in parasitemia within the first 24 hours is an indication of therapeutic failure.

Thick films are prepared with one or two drops of blood that should be stirred in a small circle for 10 to 20 seconds at various intervals as it dries on the slide to prevent the blood from sloughing from the slide during the staining process. The thicker the film, the more likely the blood will slough. Detecting and identifying the presence of parasites on thick films is extremely difficult, particularly with inexperienced laboratory personnel. Once the thick film has dried, the blood film is laked with water or buffer before staining if Wright stain is used, or the films can be placed directly into Giemsa stain without prior laking. Laking disrupts the red cell membrane, allowing the hemoglobin to be removed and leaving parasites, white blood cells, and platelets intact. The search for microfilaria can be performed at a lower magnification, whereas the search for *Babesia*, malaria, and trypanosomes must be done with oil magnification looking at 200 to 300 oil microscopic fields.

Concentration techniques also can be used to detect *Babesia*, *Leishmania*, malaria, microfilaria, and trypanosomes. Microhematocrit capillary tubes can be centrifuged, and the parasitized red blood cells and trypanosomes can be detected near the buffy coat interface. The tube can be scored and snapped, and the buffy coat can be used to prepare a thin film. Alternatively, the tube can be observed under a microscope at the buffy coat interface for the presence of motile trypanosomes.

Microfilaria can be concentrated using the Knott procedure or Nuclepore membrane filtration technique. Normally, a 5-μm filter is used, but if *Mansonella perstans* microfilaria is suspected, a 3-μm filter is recommended. Most laboratories would not be familiar with or normally use concentration techniques unless specifically asked to do so.

Antigen tests for malaria have been developed and approved for clinical use in the United States.

UROGENITAL SPECIMENS

Trichomonas vaginalis is the most common pathogen; however, schistosome eggs, microfilaria, and microsporidium also may be recovered from urogenital specimens. Many *T. vaginalis* infections are diagnosed on clinical signs and symptoms with a specimen being collected. The detection of *T. vaginalis* usually is based on direct observation in wet mounts prepared from vaginal and urethral discharges, prostatic secretions, and urine sediment. Urine specimens should be first-voided urine in the morning. Specimens collected for the detection of *T. vaginalis* should not be refrigerated because *T. vaginalis* is susceptible to cold temperatures. Specimens can be diluted with a drop of saline and observed under low power in reduced illumination for the presence of motile flagellates. Stained slides, such as Giemsa or PAP smears, also can be used, but the majority of the infections with *T. vaginalis* are identified by the direct wet mount.

Culture can be used and is recommended by the Centers for Disease Control and Prevention (CDC) for patients suspected of harboring *T. vaginalis* infections, but no organisms were detected by the direct wet mount method.[1] Specimens can be collected with a brush, swab, or sponge. Specimens should be inoculated as soon as possible into the media. Cultures showing no organisms after incubation for 4 days can be reported as negative. Commercially available DFA, EIA, and DNA probe kits have been approved by the FDA for the detection of *T. vaginalis*.[2,3,5,9]

The Knott concentration procedure and the Nuclepore membrane filtration technique have been used for the detection of microfilaria in the urine. Microfilaria most frequently detected using these methods are *Wuchereria bancrofti* and *Onchocerca volvulus*.

Schistosoma hematobium eggs found in the urine can be concentrated by sedimentation, centrifugation, or the Nuclepore membrane filtration procedure. The preferred specimen is urine collected at midday, when the patient is most active and most likely to have eggs in the urine.

OTHER SPECIMENS

Other important pathogenic parasites, such as ocular infections with *Acanthamoeba* and *Toxoplasma gondii*, can be detected in other anatomic sites and are addressed in specific chapters.

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CHAPTER 266

Epidemiology and Biostatistics

Eugene D. Shapiro

Clinicians care for individual patients, each of whom has certain unique problems. Clinicians base their decisions on a panoply of factors that include features of the acute and chronic medical problems of the patients, as well as features of both their personalities (e.g., How likely are they to comply with a certain therapeutic regimen?) and their private lives (e.g., Can they afford a certain medication? Are they planning to travel out of the country in the next few days?).

In contrast, epidemiologists study groups of people. They draw conclusions from studies of large numbers of patients and usually base conclusions on probabilities and biostatistical analyses of average (mean) outcomes.

Fortunately, these two approaches—the individualistic approach of the clinician and the probabilistic approach of the classic epidemiologist—are not incompatible. Indeed, each can illuminate the other. So it is that a chapter on epidemiology and biostatistics is included in a clinical textbook of pediatric infectious diseases. The goal of this chapter is not to make the reader an expert in these fields—courses and textbooks are designed for that purpose.[6-7,9-12,20-22] Instead, the goal is to summarize how selected key aspects of epidemiology and biostatistics can be applied to understand studies that will improve our ability to evaluate and treat children with infectious diseases. Chapter 7 addresses some epidemiologic issues that are specific to infectious diseases, whereas this chapter addresses more general issues related to designs of epidemiologic studies, as well as statistical analyses of such studies.

EPIDEMIOLOGY

DESIGN OF STUDIES

Overview

Epidemiologic studies generally are designed to be either descriptive or analytic. In *descriptive* studies, the goal is to describe a population (e.g., the clinical manifestations and prognosis of children with tuberculosis). In *analytic* studies, the goal is to assess associations between two or more variables (e.g., whether children who receive an experimental vaccine are less likely to develop the infection that the vaccine is intended to prevent than are controls). Indeed, analytic studies usually are designed to assess whether a causal association exists between the variables (e.g., Is a vaccine efficacious? Do children who attend daycare centers have a higher risk of acquiring infections caused by certain bacteria or viruses?). Of course, categorizing a study so easily may not be possible. In a study that is primarily descriptive, causal associations within subgroups may be assessed. For instance, in a study of the clinical epidemiology of tuberculosis in children, the investigators may compare the mortality rates of younger children with those of older children or the mortality rates of children with tuberculous meningitis with those of children who have infection at other sites.

Most studies, whether descriptive or analytic, seek to reach conclusions about the particular group that is being assessed (e.g., children with tuberculosis). However, because studying all persons with the condition of interest is not possible, investigators inevitably study a *sample* of persons and hope that the conclusions drawn from studying the sample apply to the entire population of interest (the *target population*). The *validity* of a study refers to the extent to which the results are true (accurate). The extent to which the conclusions drawn from the study sample (e.g., children with tuberculosis at Bellevue Hospital in New York City) are valid for the target population (e.g., all children with tuberculosis in New York City) is a measure of the study's *internal validity*. The extent to which the conclusions drawn from the study sample are valid for a less restrictively defined, larger population (e.g., all children with tuberculosis in the United States) is a measure of the study's *external validity*, which also is called its *generalizability*. To ensure that a study is both valid and generalizable, investigators must take steps to protect against a variety of potential biases (discussed later) that may distort the results of a study.

Elements of an Analytic Study

Epidemiologists refer to the major elements of an analytic study as the *exposure* and the *outcome*. The *exposure* is the factor that the investigator hypothesizes is related causally to the outcome of interest. In this context, the term *exposure* is not used in the classic sense of potential contact with an infectious agent; rather, it is any factor (e.g., living at a certain altitude, race, receipt of either a vaccine or a medication, smoking cigarettes) that may be associated causally with the outcome. The *outcome* is the effect (e.g., disease caused by a particular bacterium) that putatively is related causally to the exposure. The putative association may be one in which the exposure either causes or prevents the outcome of interest.

Before the specific type of study is defined, several additional elements of a study must be considered. One is how the *sample* for the study is selected. In general, investigators select a sample of the population either on the basis of exposure (e.g., patients who either received or did not receive a vaccine or who either attend or do not attend group daycare) or on outcome (e.g., patients who either had or did not have pneumococcal bacteremia or patients who either died or survived). Another key element is the *timing* of the study in relation to the timing of exposure and outcome. In studies in which the timing is *historical*, both the exposure and the outcome occurred before the study was initiated (e.g., a case-control study of a vaccine's efficacy in which cases with disease are identified from historical logbooks and antecedent receipt of the vaccine is determined from medical records). Timing may be *concurrent*—that is, both the exposure and the outcome occur after the study is initiated (e.g., a randomized clinical trial of the efficacy of a new antibiotic). Timing also may be *mixed*, a mixture of historical and concurrent (e.g., a study of the current IQ of children who previously had aseptic meningitis).

A final important element of a study is its *direction*. Direction is used to describe the order in which outcome and exposure are assessed. It may be done in a *forward* direction, from exposure to outcome (e.g., in a clinical trial of a vaccine's efficacy, the exposure [receipt or nonreceipt of the experimental vaccine] occurs first, and the occurrence of the outcome [the infection that the vaccine is designed to prevent] is determined subsequently). Alternatively, a study's direction may be *backward*, from outcome to exposure (e.g., in a case-control study, the outcome [e.g.,

pneumococcal bacteremia] is determined first, and exposure [e.g., previous receipt of pneumococcal vaccine] is determined subsequently). Alternatively, exposure and outcome may be determined simultaneously (e.g., a survey in which determination of whether subjects have sickle-cell disease and whether they are taking penicillin occurs at the same time). A study's direction can have important implications for inferences about causal associations; for example, even though a strong statistical association may exist between sickle-cell disease and the use of penicillin, concluding that the penicillin caused the sickle-cell disease would be erroneous. Although such an inference obviously is not plausible in this instance, the dangers of making erroneous inferences about causality are very real when exposures and outcomes that are less well understood are studied.

Although the terms *prospective* and *retrospective* are used widely, substantial variability exists in how they are applied. The term *prospective* is used to describe studies in which selection of the sample is based on the exposure, in which the timing is concurrent, or in which the direction of the study is forward (from exposure to outcome). The term *retrospective* is used to describe studies in which selection of the sample is based on the outcome, in which the timing is historical, or in which the direction of the study is backward (from outcome to exposure). Referring to the specific elements of the study is preferable to using the terms *prospective* and *retrospective*, which are applied so imprecisely.

Types of Studies

Epidemiologic studies may be classified as either experimental or observational. In *experimental studies*, the exposure is assigned by the investigators (e.g., in a clinical trial of an experimental vaccine, the investigator assigns subjects to receive either the experimental vaccine or a placebo). In *observational studies*, the exposure occurs naturally (e.g., rainfall in a study that assesses the effect of the amount of rainfall on rates of mosquito-borne infections), is selected by the subjects or their parents (e.g., attendance at group daycare in a study that assesses the frequency of infectious illnesses in children who attend and others who do not attend group daycare), or is assigned in the course of regular medical care (e.g., receipt of an approved vaccine in a case-control study of the effectiveness of the vaccine). Observational studies also are called *surveys*.

EXPERIMENTAL STUDIES

The paradigm for an experimental study is the *randomized clinical trial*, which is a special type of a *longitudinal cohort study* in which the exposure is assigned randomly to the subjects by the investigators. In randomized trials, the direction of the study always is forward, selection (or categorization) of subjects always is based on exposure (i.e., exposure is determined at the time of randomization), and timing always is concurrent (both exposure and outcome are determined during the real period of the study). Randomized trials are the *sine qua non* for evaluating the effect of new agents designed either to prevent (e.g., vaccines, prophylactic drugs) or treat (e.g., antimicrobials) diseases. By randomly allocating subjects to either receive or not receive the agent that is being tested, potential bias is minimized. Theoretically, if the size of the sample is adequate, the only difference between the groups is whether they received the experimental agent. Consequently, if the study is conducted properly, inferring that statistically significant differences in outcomes between the groups were related causally to the experimental agent is reasonable. For this reason, in most instances, the efficacy of new therapeutic agents or new vaccines must be demonstrated in clinical trials before the U.S. Food and Drug Administration (FDA) will approve them (although in some instances new products are approved if criteria for safety and for some surrogate end-point, such as a serologic correlate of immunity, are met).

TABLE 266–1 Randomized Clinical Trials

Advantages
Gold standard for scientific validity
Randomization ensures unbiased allocation of the exposure (e.g., a new drug or an experimental vaccine)
Blinding ensures unbiased assessment of outcomes
Disadvantages
Poor statistical power for rare diseases
Requires large samples
Requires longitudinal follow-up
Logistically complex and expensive
Impaired generalizability when the study population differs from the ultimate target population
Ethical issues
Requires informed consent
Difficult to use to evaluate approved (presumably efficacious) products

The advantages and disadvantages of randomized trials are summarized in Table 266–1. Although clinical trials are the gold standard for investigators who wish to design a scientifically valid study, they do have numerous limitations. A major problem is that clinical trials usually are expensive. Subjects need to be selected, enrolled, and monitored longitudinally to detect the outcomes. When the outcome is a disease that is relatively rare (e.g., pneumococcal bacteremia), large samples are necessary to provide adequate statistical power. Because sponsors of clinical trials usually want to test a new drug or an experimental vaccine under conditions that will maximize the chance that it will be found to be efficacious, patients with comorbid conditions (e.g., sickle-cell disease, asplenia, metastatic cancer) that might affect their response to the new agent often are excluded from the study. If the subjects who are excluded are an important part of the target population for the new agent (e.g., the patients with comorbid conditions are likely to be at high risk for serious complications of the disease and thus potentially could benefit greatly from an effective new intervention), the generalizability of the results of the clinical trial may be impaired.

Most randomized clinical trials are conducted in a double-blind manner; that is, neither the investigators nor the subjects know whether they received the experimental intervention (e.g., a new drug) or the comparison agent (e.g., either a placebo or an agent used in the standard manner). Double-blinding helps ensure lack of bias in ascertainment of the outcome because neither the subject nor the investigator can be influenced either to (or not to) seek medical care or undergo diagnostic tests on the basis of which of the interventions was received.

Randomized clinical trials may pose difficult ethical problems because the new (and potentially efficacious) agent is not given to the controls. By the time that clinical trials are begun, usually some evidence suggests that the agent is efficacious (e.g., preliminary studies showing that a new vaccine induces antibodies). Accordingly, some patients and patient advocates might suggest that withholding a potentially efficacious therapeutic agent or a vaccine from persons at risk is not ethical; consequently, it may be difficult to have persons agree to be potential controls in studies of a promising new therapy.

Problems also may arise when approval of a product for the target population is based on studies that are conducted in a different population. For example, polyvalent pneumococcal polysaccharide vaccine was approved in the United States for the elderly and for adults with chronic conditions, such as chronic obstructive pulmonary disease and congestive heart failure, that

put them at increased risk of acquiring serious pneumococcal infections. However, the data on which approval was based were derived from clinical trials conducted among young gold miners in South Africa who were at risk largely not because of their age or underlying illnesses but because of the conditions in which they lived and worked. Reports of vaccine failure and poor antibody response to the vaccine in the target population in the United States led to questions about the vaccine's efficacy.[5] However, once the vaccine was approved, conducting a randomized clinical trial in the target population was difficult ethically because it would mean withholding, on a random basis, an approved (and presumably efficacious) vaccine from patients at risk. Consequently, all but one of the postlicensure studies of this vaccine's efficacy were observational studies.

Conducting an experimental study in which the exposure is not assigned randomly is possible. For example, one might conduct an experimental cohort study in which volunteers receive a certain intervention (e.g., a new drug) while controls receive no intervention. Both groups would be monitored forward in time while undergoing surveillance for the outcome event. Although such a study incorporates some of the features of a randomized clinical trial, because the intervention is not assigned randomly, such studies are subject to significant biases.

OBSERVATIONAL STUDIES

Observational Cohort Studies

The direction of cohort studies always is forward, and the selection (or categorization) of subjects always is based on exposure; however, the timing may be concurrent, historical, or mixed. Thus, a cohort study may identify a cohort of subjects at a point (or at several points) in time in the recent or remote past, categorize them regarding their status with respect to the exposure (e.g., receipt of either a vaccine or a drug), and then monitor them forward in time for the occurrence of the outcome event (e.g., an infection) until a certain point in time, which could be in the past, present, or future. As in a clinical trial, subjects must not have the outcome at the time of enrollment in the study.

Cohort studies share many of the disadvantages of randomized clinical trials (see Table 266–1) but have the additional disadvantage that because they are not experimental, they are subject to many potential biases. On the other hand, cohort studies have many practical advantages (e.g., the timing can be historical, so conducting a 30-year follow-up study in just months is possible), and because they are observational studies, they usually have fewer potential ethical problems than does a randomized trial. In addition, for rare exposures, a critical factor is to be able to base selection of subjects on exposure.

Case-Control Studies

In both clinical trials and observational cohort studies, the selection of subjects is based on their exposures, and they are monitored in a forward direction until the outcome is determined. In *case-control studies*, the process is reversed. Subjects are selected on the basis of outcomes. The cases have the outcome, usually a disease; the controls do not have the outcome. The direction of the study is backward—the previous exposure is ascertained after the subjects are selected. The timing of case-control studies usually is historical, but it may be mixed; the timing of the exposure always is historical, but the timing of the outcome may be either historical or concurrent. For example, an investigator may conduct a case-control study of a vaccine's effectiveness in which persons who are infected (cases) are identified concurrently through active surveillance of a microbiology laboratory. Because case-control studies are nonexperimental and the exposure (and, sometimes, the outcome) occurred in the past, the potential for bias is great, both in selection of the sample and in ascertainment of both the exposure and the outcome. The advantages and disadvantages of case-control studies are shown in Table 266–2. Recently, use of "sham" outcomes and/or exposures in addition to the true outcomes or exposures of interest has been suggested as a means of assessing the internal validity of case-control studies.[24]

TABLE 266–2 Case-Control Studies

Advantages
Statistically powerful method to assess outcomes that are rare or delayed
Logistically easier and more efficient than large experimental or observational cohort studies
No longitudinal follow-up, so it can be completed relatively quickly and inexpensively
Ethically acceptable because it is an observational study
Disadvantage
Subject to many potential biases

Cross-Sectional Studies

Unlike cohort studies (both experimental and observational), in which subjects are monitored forward from exposure to outcome, and case-control studies, in which subjects are monitored backward from outcome to exposure, in cross-sectional studies, outcome and exposure are determined at the same time. In cohort studies, causal inference is made from cause to effect, whereas in case-control studies, causal inference is made from effect to cause. In cross-sectional studies, determining whether the exposure preceded the outcome may not be possible. Consequently, making valid causal inferences from a cross-sectional study also may not be possible.

Selection of subjects for a cross-sectional study may be based on outcome, exposure, or neither. However, because cross-sectional studies include only persons with prevalent outcomes, they are particularly problematic for studies of infectious diseases because patients whose illnesses (outcomes) either resolved or resulted in death before the study is conducted are not counted as having the outcome. Consequently, cross-sectional studies are more suitable to the study of chronic conditions and rarely are used in studies of infectious diseases.

ANALYSIS OF EPIDEMIOLOGIC STUDIES

The results of epidemiologic studies with dichotomous exposures and outcomes often are displayed in a 2×2 *contingency table*. However, the way that the results are analyzed statistically depends on the type of study.

Cohort Studies

Analysis of longitudinal cohort studies (both experimental trials and observational studies) is shown in Table 266–3. The measure of association between the exposure and the outcome is the *relative risk* (sometimes called the *risk ratio)*, which is an expression of the *magnitude* of this association in the study sample and represents an *estimate* of the association in the population from which the study sample was drawn. A relative risk of 1 indicates that no association exists between the exposure and the outcome, a relative risk greater than 1 indicates that the exposure is associated with an increased risk of the outcome, and a relative risk less than 1 indicates that the exposure is associated with a decreased risk of the outcome. The *attributable risk* (the risk of the outcome that is attributable to the exposure) is calculated as the risk in

TABLE 266–3 Analysis of Experimental or Observational Cohort Studies

	Outcome Present	Outcome Absent	Total
Exposed	a	b	a + b
Unexposed	c	d	c + d
Total		a + c	b + d

Risk in exposed subjects: a/(a + b).
Risk in unexposed subjects: c/(c + d).
Relative risk: (a/a + b) ÷ (c/c + d).
Attributable risk: (a/a + b) − (c/c + d).

exposed subjects minus the risk in unexposed subjects. Of course, testing the statistical significance of any association is necessary because the relative risk could be greater than 1 or less than 1 by chance (see later sections on stochastic statistics and confidence intervals).

These analyses assume that no attrition exists among the subjects in the study. However, because of death, migration out of the study area, and loss to follow-up, as well as variation in the time of enrollment (enrollment in either a clinical trial or an observational cohort study may occur over a prolonged period or, indeed, throughout a study), variation virtually always exists among subjects in the duration of time that they are at risk for the outcome. If the average time at risk among the exposed and the unexposed subjects is equal, the analysis shown in Table 266–3 is likely to be valid. However, if a great deal of irregular attrition or new enrollment occurs during the study, the denominator is expressed better as person-time at risk (e.g., person-months, person-years) rather than as the number of persons in the group. When this method is used, the rate then is called the *incidence density rate* and the index of comparison is called the *incidence density ratio*.

In studies of infectious diseases, the outcome event often is the occurrence of an infection. Frequently, persons who become infected recover and may remain at risk for the outcome event again. Nonetheless, a subject usually should be censored (i.e., removed from the study) once the outcome event occurs. The fact that the outcome occurred may indicate that the subject has an increased risk for the outcome, independent of the exposure that is being assessed. For example, consider a study of the protective efficacy of a conjugate pneumococcal vaccine in which one of the subjects (whether the subject is a vaccinee or a control does not matter) experiences three or four episodes of invasive infection caused by pneumococci (perhaps because the subject has a previously unrecognized underlying condition, such as acquired immunodeficiency syndrome [AIDS] or an immunoglobulin deficiency). The estimate of the vaccine's efficacy would be distorted substantially if each of the outcome events were counted. Certainly, in a primary analysis, only the initial event should be counted. Another reason to censor the subject is that in some instances, the outcome event may be fatal. If some subjects die of the outcome (and, therefore, must be censored), not censoring subjects with the outcome who survive is, in effect, incorporating an assessment of the exposure's effect on prognosis and not just on the risk of the outcome event.

Using a different method to adjust for unequal durations of follow-up in a study may be necessary, particularly when a long latent period exists between exposure and outcome (e.g., a condition that often is met when the study involves the effect of a potential carcinogen). In such instances, simply to sum the total person-times of exposure may be misleading because the outcome is more likely to occur many years after exposure. For example, although the total person-time at risk would be the same, the risk of an outcome developing clearly may be substantially different for 500 persons, each of whom is monitored for 2 years after exposure, than it may be for 40 persons, each of whom is monitored for 25 years. In such situations, the analyses must be adjusted for variation in the length of follow-up, which can be done with the use of *survival analysis* (also known as *life-table analysis*). The two basic methods of survival analysis, the *actuarial* method and the *Kaplan-Meier (product-limit)* method, are described in detail elsewhere.[14]

TABLE 266–4 Analysis of Case-Control Studies

	Cases (Outcome Present)	Controls (Outcome Present)
Exposed	a	b
Unexposed	c	d

Proportion of exposed cases: a/(a + c).
Proportion of exposed controls: b/(b + d).
Odds of exposure among cases: a/c.
Odds of exposure among controls: b/d.
Odds ratio: (a/c) ÷ (b/d) or (ad) ÷ (bc).

Case-Control and Cross-Sectional Studies

Analysis of a case-control study (with dichotomous outcomes and exposures) is shown in Table 266–4. Because selection of subjects is based on their outcomes, one cannot calculate the risk of development of the outcome, as one would in a longitudinal study (such as a clinical trial). Instead, one calculates the proportion of each group (cases and controls) that is exposed. The measure of association in a case-control study is the *odds ratio*. The *odds* of some occurrence means the probability that it will occur divided by the probability that it will not occur. In a case-control study, we are interested in the odds of exposure. The odds ratio is the ratio of the odds of exposure among cases (a/c) divided by the odds of exposure among controls (b/d). For rare events, the odds ratio closely approximates the relative risk of exposure that would be found in a longitudinal study of the same association. Use of a method known as *risk set sampling* allows odds ratios from case-control studies to approximate a risk ratio even if the outcome is not rare.[1] Cross-sectional studies are analyzed either like a case-control study (if the selection of subjects was based on their outcomes) or like a cohort study (if the selection of subjects was based on their exposures).

Bias

Bias occurs when the estimate of the association between the exposure and the outcome in the sample differs systematically from the true value in the population. Bias in the estimate of the association is different from bias in the measurement of individual variables. The latter bias (which may occur, for example, if an instrument such as a thermometer is calibrated incorrectly) may or may not affect the estimate of association in an epidemiologic study. Biased measurements usually affect the exposed and the unexposed groups equally. By contrast, *analytic bias* is the effect of *differential error (nonrandom error)* on the assessment of the relationship between exposure and outcome. Analytic bias can occur because of *information bias* (as a result of differential error in ascertainment of either the exposure or the outcome), *sample distortion bias* (because the joint distribution of the exposure and the outcome in the sample chosen for the study is not representative of the distribution of these factors in the target population), and *confounding bias* (because the joint distribution of one or more variables that independently are related both to the exposure and to the outcome is unequal in the groups that are being compared). Ensuring that bias does not affect a study is critical to its validity. Furthermore, although using statistical methods to adjust for certain sources of bias (particularly for confounding bias) in analysis of the results of a study sometimes is possible, adjustment for bias after the fact may be impossible.

An example of information bias is *detection bias*, which occurs when there is differential detection of the outcome in the exposed

and the unexposed groups. For example, numerous studies in which clinical scales to assess whether children with fever have a serious illness have been developed.[15] Often, however, the "serious illness" may include certain abnormal laboratory test results (such as an abnormal radiograph of the chest or a low concentration of sodium in serum). Because children with high scores on these scales were more likely to undergo diagnostic tests, detection bias may have occurred because similar abnormalities (such as a "silent" pneumonia or a mildly depressed concentration of sodium in serum) might go undetected in the "unexposed" group (children with lower scores on the clinical scale), a much smaller proportion of whom underwent a diagnostic test to detect a possible outcome. The best way to avoid having information bias is to use standardized, consistent methods to ascertain both the exposure and the outcome and to blind subjects and investigators to ensure that no differential ascertainment of either the outcome (in longitudinal studies) or the exposure (in case-control studies) occurs.

Sample distortion bias occurs when the sample in a study is not representative of the target population. However, a nonrepresentative sample does not lead to bias necessarily; bias in an assessment of the association between exposure and outcome occurs only if differential distribution of the exposure (or differential risk of the outcome) occurs in the subjects who are selected for the study versus the overall target population. This distribution can be the result of *selection bias*. For example, consider a case-control study of the effectiveness of an approved vaccine in which the control subjects (the uninfected patients) were chosen from private practices in affluent suburbs, whereas the case subjects (the infected patients) were any patients in whom a serious infection developed and who were hospitalized. The association between antecedent vaccination and infection probably is biased because the controls are not a representative sample of the population from which the cases emerged, and the proportion of them likely to have been vaccinated is higher than in the group of all uninfected persons in the population. Another possible source of sample distortion bias is differential loss to follow-up in a longitudinal study. The potential for sample distortion bias can be minimized by maximizing the probability that a representative sample will be selected, ideally by random sampling (or some other kind of unbiased sampling), and by minimizing loss to follow-up in longitudinal studies.

Confounding bias occurs when the association between exposure and outcome is distorted by a variable (a *confounder*) that is distributed unequally between the groups, is associated independently with both the exposure and the outcome, and is not in the causal pathway from exposure to outcome. One example of confounding bias is *susceptibility bias*, which occurs when the risk of the subjects (to development of the outcome) differs in the exposed and unexposed groups, independent of the exposure. For example, imagine a longitudinal study of the protective efficacy of a vaccine against *Haemophilus influenzae* type b in infants. Ensuring that an equal proportion of subjects in the vaccinated and the unvaccinated groups attended group daycare is important because attendance at group daycare is associated with an increased risk of developing infection with *H. influenzae* type b. If a substantially higher proportion of vaccinees than of controls attended group daycare, their attendance might confound assessment of the vaccine's efficacy and result in a biased (lower) estimate of the effect of the vaccine. By contrast, if a disproportionate number of controls attended group daycare, the estimate of the vaccine's efficacy might be biased in the opposite direction (it would be erroneously high because controls would have a disproportionately higher risk of acquiring infection with *H. influenzae* type b than vaccinees would).

In the previous example, adjusting for the effect of the confounder might be possible by either stratification or multivariable analysis (see later). However, identifying confounders may not always be possible. Random allocation of subjects probably is the best strategy to protect against confounding, especially potential confounders that cannot be identified.

STATISTICS

SUMMARY STATISTICS

Perhaps the most basic use of statistical analysis is to summarize data. This type of statistical analysis often is called either *summary* or *descriptive* statistics. The specific summary measures that are used depend, in part, on whether the variables being summarized are *continuous* (variables with equal distance between intervals) or *categorical* (variables with two or more discrete categories). Age, weight, height, temperature, concentration of creatinine in serum, and the number of hours spent in group daycare all are examples of continuous variables (sometimes called *dimensional* or *quantitative* variables). Race, sex, country of residence, and whether a patient is being ventilated artificially are examples of categorical variables (sometimes called *discrete* variables). Of course, continuous variables can be analyzed categorically (e.g., age can be divided into either a *dichotomous* variable [<45 years or = 45 years] or a *polychotomous* variable [20-44 years, 45-59 years, or = 60 years]).

Continuous Variables

A *frequency distribution* is classification of the values of a sample of continuous variables into successive categories (e.g., for a sample of different ages, one might place each value into different 5-year [0-4 years, 5-9 years, etc.], 3-year [0-2 years, 3-5 years, etc.], or 1-year [0 years, 1 year, 2 years, 3 years, etc.] categories) and expression of the frequency of values within each category.

A sample of values of a continuous variable also can be summarized mathematically by describing its central tendency, shape, and spread. The *central tendency* can be expressed as a *mean* (or *average*), a *median* (the middle value of the sample), or a *mode* (the most frequent value in the sample). The mean value is calculated by summing all the individual values in the sample and dividing by the number of individual values. Thus, for a sample that is composed of subjects aged 1, 1, 2, 2, 2, 3, 3, 4, 10, and 12 years, the mean age is 4 years (40/10), the median age (the age for which half the subjects are older than and half are younger than its value—if the number of subjects is even, the median is the mean of the two middle values) is 2.5 years [(2 + 3)/2], and the mode is 2 years. These summary measures provide only a limited view of the data. For example, the mean of a distribution can be shifted in the direction of extreme outlying values; in the example, the mean is 4 years even though 70 percent of the subjects are younger than 4 years. Likewise, from the median value alone, one would not know whether outlying values existed. Consequently, describing the spread of a distribution is important.

The *spread* of a sample may be expressed by its *range* and by its *standard deviation*. The range of a sample is the interval between the highest and the lowest value in its distribution. In the example, the range is from 1 to 12 years. Another useful way of mathematically summarizing the spread of a distribution is to express the interval between each individual value and the mean value of the sample. To summarize these values, one cannot simply sum all the differences because the sum always equals zero (the sum of the positive differences equals the sum of the negative differences). To avoid this problem, the standard deviation of a sample is used. This parameter is calculated by taking the square root of the *variance* of the sample. The variance is the sum of the squares of the differences from the mean of each individual value, divided by the number of *degrees of freedom* of the sample (which is equal to the number of values in the sample minus one, or $N - 1$). The

sum of the squares is divided by the number of degrees of freedom of the sample ($N - 1$), rather than by N (the actual number in the sample), because this calculation is thought to represent more accurately the true variance of the population from which a sample is taken.

To calculate the standard deviation of the sample in the example, one must determine the variance, which is the sum of the squares of the difference of each individual value from the mean of the sample:

$$(2[4 - 1]^2 + 3[4 - 2]^2 + 2[4 - 3]^2 + [4 - 4]^2 + [4 - 10]^2 + [4 - 12]^2) \div (10 - 1)$$

which equals 132/9, or 14.6667 years. The standard deviation simply is the square root of the variance, which is 3.8297 years.

In the medical literature, investigators often use the *standard error of the mean* (which is the square root of the variance divided by the square root of the number in the sample) to express the spread of the frequency distribution of a sample. However, the standard error actually is designed to be a measure of the standard deviation of the means of repeated samples from a single source population. Because the standard error always is smaller than the standard deviation of a single sample, it gives the erroneous impression that the spread of a sample is smaller than it actually is. A large sample with a large spread (and a large standard deviation) may have a small standard error. The standard error should not be used to describe the spread of a single sample.

The general shape of the frequency distribution can be inferred from the parameters given earlier. The characteristics of the most important distribution in statistics, the familiar bell-shaped curve of the normal distribution, are shown in Figure 266–1 and are discussed in a later section about diagnostic tests. The peak value of any distribution is the mode. A distribution may be bimodal, trimodal, and so on if it has two or more modes. A frequency distribution may be asymmetric, or *skewed*. If the mean is greater than the median (as in the example), the distribution is skewed to the right; conversely, if the mean is less than the median, the distribution is skewed to the left.

An investigator may want to summarize the relationship between two different continuous variables, which can be done with the use of linear regression, in which a straight line is constructed to represent the relationship between a continuous *dependent variable* (y) and a continuous *independent variable* (x). An equation ($y = a + bx$) can be created that represents the best "fit" to describe the relationship between the actual values of y and x, in which a (the *intercept*) is the value of y when x equals zero and b (the *regression coefficient*) is the slope of the line. Such an equation allows us to summarize the relationship between these variables and to extrapolate the value of each variable for conditions that may not have been observed in the sample. For example, one can calculate the incremental value of y for each interval change in the value of x.

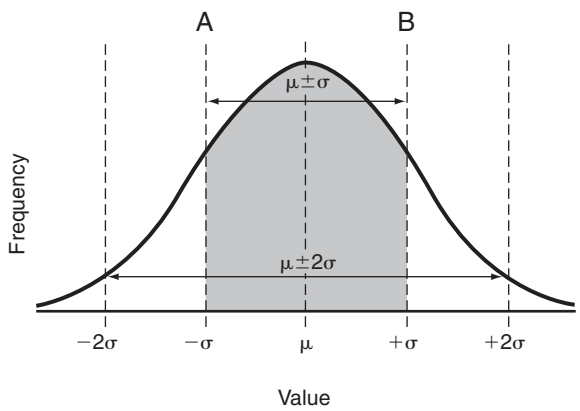

Figure 266–1 Theoretic normal (gaussian) distribution showing where 1 and 2 standard deviations (SD) above and below the mean would fall. The Greek letter mu (μ) stands for the mean in the theoretic distribution, and the Greek letter sigma (σ) stands for the standard deviation in the theoretic population. In this figure, the area under the curve represents all the observations in the distribution. One standard deviation above and below the mean, shown in dark gray and represented by the distance from point A to point B, is equivalent to 68 percent of the area under the curve, and therefore 68 percent of the observations in a normal distribution fall within this range. Two standard deviations above and below the mean, represented by the areas shown in dark and light gray, are equivalent to 95.4 percent of the area of the curve or 95.4 percent of the observations in a normal distribution. *(From Jekel, J. F., Elmore, J. G., and Katz, D. L.: Epidemiology, Biostatistics, and Preventive Medicine. Philadelphia, W. B. Saunders, 1996, p. 118.)*

Categoric Variables

The measure generally used to summarize categoric data is called a *rate* or a *proportion.* Rates consist of a numerator and a denominator. The numerator represents the actual number of persons in the sample who have the characteristic of interest (e.g., those with fever, those with tuberculosis, those who received a vaccine), and the denominator is the total number of persons in the sample. The persons in the numerator must be included in the group that the denominator represents, and the persons in the denominator must be able to have the characteristic that the numerator represents. For example, if one were interested in the rate of testicular cancer in the population, women should not be included in the denominator because they cannot have testicular cancer. Some experts believe that rates, by definition, should include a time factor (e.g., incident cases per person-months or per year). However, the term is used widely to refer to a proportion of a sample, as described earlier.

One of the most important rates that is calculated in epidemiologic studies is the *incidence rate*, which is the number of persons in whom a certain outcome develops divided by the number of persons in whom the outcome could have developed during a specified period. For example, a comparison may be made of the incidence rates of an infection over time in two groups in which the interventions given to prevent an infection differed (e.g., vaccine, placebo).

Another important rate, the *prevalence rate*, is the number of persons in whom a certain outcome develops divided by the total number of persons in the group at a specific point in time. In calculating the incidence rate, persons who at *zero time* (before the study begins) already have the outcome (e.g., prevalent cases) are excluded from both the numerator and the denominator. At time zero, the numerator of the incidence rate is zero. Thus, if one were interested in the incidence rate of lung cancer, persons who had lung cancer at the beginning of the study period would be excluded. The prevalence rate, by contrast, is measured at one point in time, and the numerator includes all persons with the outcome at that time.

Certain features of many infectious diseases affect both the prevalence rate and the incidence rate as measures of the occurrence of disease. The prevalence rate of any condition is related to its chronicity and the mortality rate associated with it; conditions that are common and chronic but are not associated necessarily with immediate mortality (e.g., atherosclerotic heart disease) have a relatively high prevalence rate. On the other hand, the prevalence rates are relatively low for conditions that either have a low mortality rate and resolve completely (such as many infectious illnesses) or have a high short-term mortality rate. To get a reliable estimate of the importance of such conditions, one must determine their incidence rates.

STOCHASTIC STATISTICS

Statistical Significance

Stochastic statistics deals with probability. Most readers are familiar with the use of "*p* values" to assess the *statistical significance* of a finding from a study. Usually, the threshold used to separate "significant" from "nonsignificant" results is a probability (*p* value) of less than 0.05, which means that (arbitrarily, by convention) if a result is likely to be observed by chance less than 1 in 20 times, the finding is considered to be "statistically significant." It must be emphasized, however, that statistical significance and clinical significance are very different. A statistically significant result means that the result is considered unlikely to be attributable to chance alone. As we shall see, the *p* value is related not only to the magnitude of the difference between the things that are being compared (e.g., a characteristic of either the exposed and the unexposed groups in a longitudinal study or the cases and the controls in a case-control study) but also to the size of the sample. Consequently, differences that are clinically unimportant (e.g., a difference in mean weight of 0.2 kg between two groups, a difference of 4% in the distribution of whites between two groups) can be statistically significant if the size of the sample is large. Of importance is to remember that a statistically significant finding may be of no clinical significance.

By the same token, the "magic" *p* value of 0.05 itself may be misleading because it is an arbitrary threshold; in fact, usually little difference exists in the results associated with a *p* value of 0.04 and one associated with a *p* value of 0.06. If the size of a sample is small, a large, clinically important difference may not be statistically significant. One should not automatically dismiss the importance of a finding for which the *p* value is 0.05 or higher.

Hypothesis Testing

In most epidemiologic studies, stochastic tests are used to check the validity of a hypothesis. By convention, one tests the validity of the *null hypothesis* that no difference exists in either the mean value of the characteristic (for continuous variables) or the frequency distribution of the characteristic (for categorical values) between the groups being compared. In essence, one is testing the probability that the two samples being compared (exposed subjects versus unexposed subjects or cases versus controls) emerged from the same population—that is, whether a difference between the groups could have been the result of chance if the two samples were selected randomly from the same population or whether the difference is so great that it probably (<1 chance in 20, or a *p* value of <0.05) did not occur by chance; that is, the samples probably actually represent a true difference in the populations of either exposed subjects versus unexposed subjects or cases versus controls.

Hypotheses can be either *bidirectional (nondirectional)* or *unidirectional (directional)*. A nondirectional hypothesis asks whether an association exists between exposed and unexposed subjects, but it does not assume in which direction the association will occur. For example, one might ask whether an association exists between the height of fever and the outcomes of children with typhoid fever without specifying whether the association would be in the direction of better or worse outcomes (perhaps high fever indicates a more severe infection, or perhaps it indicates a more vigorous immune response). In such instances, the associated *p* value should be interpreted in a bidirectional, or *two-tailed*, manner because the distribution of the test statistic contains two "tails," one at either end of the curve; the *p* value represents the sum of the area under the two tails of the curve. In effect, one is specifying that for a result to be statistically significant, the probability of the result being in a positive direction is less than 2.5 percent and the probability of the result being in a negative direction is less than 2.5 percent. Most tables of *p* values provide results that are to be interpreted in a two-tailed manner (because this is the most conservative approach to testing a hypothesis), so the *p* value for a given test statistic can be read directly from the table.

In some instances, however, one might have good reason to suspect that an exposure is associated with an outcome in a unidirectional manner (e.g., that possession of a handgun is associated with an increased risk of sustaining a gunshot wound). If, in advance, the investigator decides to test this hypothesis in a unidirectional manner (the null hypothesis for which would be that ownership of a gun is not associated with an increased risk of incurring a gunshot wound), interpreting the results of a test statistic in a unidirectional, or *one-tailed*, manner might be appropriate. To derive a one-tailed *p* value, the two-tailed value is divided by 2. Thus, if the two-tailed *p* value is 0.06, the one-tailed value for the same result is 0.03. Clearly, obtaining statistically significant results by testing hypotheses in a unidirectional manner is easier. However, of importance is to specify before the study is conducted the *a priori* plan for how the results of statistical tests will be interpreted. Furthermore, although many hypotheses might seem to lend themselves to unidirectional analyses (e.g., one might think that an experimental vaccine that has been shown to be immunogenic could only be beneficial), in fact, unidirectional analyses are used only rarely. First, the possibility that an exposure may have an effect opposite to that expected usually cannot be ruled out in advance. For example, researchers found that persons who were immunized with a vaccine composed of inactivated respiratory syncytial virus had an increased risk for developing symptomatic infection with the virus. In addition, because two-tailed interpretation of data is more conservative, many authorities (such as editors of journals and such licensing agencies as the FDA) demand two-tailed analyses.

Type I Error, Type II Error, and Statistical Power

Two types of errors can be made when testing the validity of a null hypothesis. The first occurs when the null hypothesis is true but is rejected erroneously, which leads to an erroneous conclusion that a difference exists between the exposed and unexposed subjects; this kind of mistake is termed a *type I error*; or *alpha error*. If a test statistic with a *p* value of less than 0.05 is accepted as statistically significant (and the null hypothesis is rejected on that basis), approximately 5 percent of the time, a type I error is made because if no difference actually exists between the groups, that result will occur by chance 1 in 20 times. By contrast, if the *p* value is less than 0.001, a type I error will occur less than 1 in 1000 times if the null hypothesis is rejected.

The other kind of error, *type II error*; or *beta error*; occurs when the null hypothesis erroneously is not rejected even though a difference between the two groups truly exists. If the *p* value associated with the result is 0.05 or greater, the probability that the null hypothesis is false is not sufficiently low to reject it (even though the probability that the observed result could have occurred by chance may be only 6%).

Type I and type II errors are mutually exclusive. If one rejects the null hypothesis, one risks committing a type I error, the probability of which is equal to the *p* value. In such instances, a type II error cannot occur. If one accepts (i.e., fails to reject) the null hypothesis, one may commit a type II error (in which case a type I error cannot occur). The probability of committing a type II error is not related directly to the observed *p* value. Instead, one can estimate the probability of committing a type II error (beta error) before the study begins on the basis of an assumed (clinically important) degree of association between the exposure and the outcome. Certain assumptions also must be made about the magnitude of the effect among the exposed subjects and the

frequency of the outcome in the unexposed subjects. One is calculating a unidirectional probability that, by chance, the study will fail to detect an association equal to or greater than the magnitude that is specified for what is, in effect, an *alternative hypothesis* (i.e., that an association truly exists between the exposure and the outcome). The *statistical power* of a study (the unidirectional probability that the null hypothesis will be rejected given a specified degree of type I error—usually <5%) is equal to 1 minus beta.

In designing a study, adequate statistical power is critical to ensure that a true association between the exposure and the outcome is not missed erroneously. The probability of a type II error depends on the size of the sample, the degree of variance within the sample, and the magnitude of the association that one wants to be able to detect: the smaller the sample, the larger the variance, and the smaller the magnitude of the association that one wants to detect, the larger the beta error (type II error) and the lower the statistical power of the study to detect an association. Of these factors, the investigator usually has control over only the size of the sample (because in the population, the variance generally is a relatively fixed attribute of the characteristic being measured and the magnitude of association that is chosen usually is the minimal association that is clinically meaningful; for example, to design a study of an experimental vaccine so that it could detect as little as 30 percent efficacy would not make sense because such a product would not be approved in most instances unless the magnitude of its effect was much greater).

Exactly what constitutes adequate power is debatable. Because enrolling a large number of subjects in a study often is expensive and time-consuming, frequently the power that a study is designed to have depends on both the financial resources and the time available to conduct the study. A statistical power of 80 percent often is chosen as a reasonable compromise between ensuring that the study has a sufficient number of subjects to answer the research hypothesis and the realities of logistic and financial exigencies. On the other hand, if a pharmaceutic company has invested many years and millions of dollars to develop a new drug or a new vaccine, it may demand that a pivotal clinical trial of the product have at least 90 percent power to detect a clinically meaningful effect in an attempt to ensure that the product will be approved if it is efficacious.

Multiple Comparisons

Another more subtle problem in interpreting results arises when more than one hypothesis is assessed in a single study. If multiple tests of statistical significance are performed in a study, the probability that by chance alone the p value associated with any one variable will be less than 0.05 is substantially greater than 5 percent. In fact, the probability that by chance at least one test will be associated with a p value less than 0.05 is equal to $1 - (0.95)^k$, with k equal to the number of independent tests of statistical significance that are performed. Thus, if 10 different hypotheses are tested, a 40 percent probability exists that at least one of them will be statistically significant (i.e., that at least one of the tests will have a p value of <0.05). Of course, this matter violates the arbitrary rule that we consider a finding to be statistically significant only if it is likely to have occurred by chance less than 5 percent of the time. If multiple different associations in a study have p values less than 0.05, determining which occurred by chance and which truly are statistically significant is difficult.

On the other hand, studies often are expensive, time-consuming, and logistically difficult to conduct. Therefore, sometimes a reasonable approach is to try to increase efficiency by assessing more than one hypothesis in a single study. To address this problem, one can specify in advance the *primary hypothesis* and the *secondary* and *tertiary hypotheses.* One then might give most credence to the statistical test of the primary hypothesis. Another approach is to lower the threshold used to define statistical significance by dividing 0.05 by the number of different tests that are performed. Thus, if five independent hypotheses are tested, any one would have to be associated with a p value of less than 0.01 before the null hypothesis would be rejected. However, this approach might be too stringent because the different hypotheses that are being tested often are not truly independent, and the probability that two or more associations will be statistically significant may be greater than the product of their individual probabilities. For example, one might assess whether infection is associated both with diarrhea and with vomiting; if it is associated with one of these symptoms, usually a higher than chance probability exists that it also is associated with the other. At the least, an investigator should acknowledge this potential problem and its consequences before drawing conclusions from the study.

Tests of Statistical Significance

Going into detail about the many different stochastic tests that are available is beyond the scope of this chapter. However, several general comments, as well as descriptions of a few commonly used tests, are in order.

Tests of statistical significance are either parametric or nonparametric. The test statistic of a *parametric* test is based on the assumption that the frequency distribution of the characteristic being assessed in the source population follows certain parameters. For example, for certain tests, one assumes that the characteristic in the population has a normal distribution. If the assumption is violated (e.g., the characteristic is not distributed normally), the statistical test will not be valid. In such instances, an appropriate approach might be to use a test based on a different, skewed distribution, such as the *Poisson distribution* for rare events, which might reflect more accurately the distribution of the characteristic in the population.

Alternative approaches to assess the statistical significance of differences between groups, if the distribution of the characteristic in the population is known to be highly skewed, include transforming the data so that they are normalized, usually by converting the values to their logarithmic equivalent, or using a *nonparametric* test.[25] Nonparametric tests, such as the Mann-Whitney U test and the Wilcoxon rank sum test, do not depend directly on the numeric values of the data; instead, the individual values are ranked in order of their values, and the statistical analyses are based on the relative ranks of the values in the different groups rather than on an assumed distribution in the population or in the sample.

For large samples, parametric tests usually are valid and often are easier to calculate than are nonparametric tests. For smaller samples and for samples with highly skewed distributions, nonparametric tests or tests that use log-transformed data may be preferable.

Continuous Variables

To test the statistical significance of differences between groups in continuous variables (such as degree of fever, IQ, age, or a score on a standardized questionnaire), the mean values of the different groups can be compared.[26] The tests of statistical significance use the magnitude of the difference of the means and the variance in the sample to assess the probability that the difference could occur by chance. If more than two groups are being compared for a single variable, the procedure is called *one-way analysis of variance.* The null hypothesis is that the mean values from each group are the same (i.e., that the different groups are random samples from the same population). The total variance in all the subjects is separated into the amount that is attributable to differences between the groups of subjects (the *intergroup variance*) and the amount that is attributable to differences among the

subjects within each group (the *intragroup variance*). The greater the quotient of the intergroup variance divided by the intragroup variance (the *F ratio*), the more likely that differences between the groups are statistically significant (i.e., that they are not due to chance variation). The statistical significance of the F ratio can be determined from the *F test* table of *p* values. When this type of test is performed to assess the statistical significance of the simultaneous effects of two factors (by stratifying into additional groups), the test is called *two-way analysis of variance*.

The familiar t *test* is just a special form of one-way analysis of variance in which the means of just two groups are compared. A *one-sample* t *test* assesses whether the mean value of the study sample is statistically significantly different from the mean value in the source population. It assumes that the mean value in the source population is known and is calculated by dividing the difference of the mean of the sample minus the mean of the population by the quotient of the variance of the sample divided by the square root of the number in the sample.

A more common use of the t *test* is to assess the statistical significance of the difference between the mean values of either a characteristic or an outcome of subjects in two different samples (e.g., of the exposed and the unexposed groups in a study). The null hypothesis of this *two-sample* t *test* is that the mean values of the two samples are not statistically significantly different (i.e., that they could be random samples from the same population). The *t* test statistic is equal to the difference in the mean values of the two samples divided by the square root of the pooled variance of the samples (the standard error of the difference of the means of the two groups). The *p* value of the result (*t*) is read from the table of the *t* distribution, with the degrees of freedom equal to the sum of the number of subjects in each sample minus 2.

Categoric Values

The chi-square statistic (χ^2) commonly is used to assess the statistical significance of differences in categorical values between groups.[6] Most often, proportions of two different groups (e.g., the proportions of the exposed and of the unexposed groups in which the outcome developed) are compared. Typically, the data are displayed in a *2 × 2 contingency table* (Table 266–5). The null hypothesis is that no association exists between the exposure and the outcome (or whatever the rows and columns represent)—that is, that the two groups could be random samples from the same population. This hypothesis is tested statistically by comparing the number of subjects who would be expected to be in a given cell by chance with the actual frequency (the observed frequency). The expected frequency can be calculated from the total number of subjects in the rows and the columns (the marginal totals) and is equal to the product of the number of subjects in the row times the number of subjects in the column divided by the total number of subjects in the sample.

The value of χ^2 (with N equal to the total number of subjects) is shown in Table 266–5. The statistical significance of χ^2 can be determined by reading the *p* values from a table of the χ^2 distribution. The larger the value of χ^2, the less likely that the observed proportions could have occurred by chance. Unfortunately, assuming that the probability that the observed proportions of a random sample of subjects will follow the χ^2 distribution is not necessarily accurate if the expected frequency of any cell is small. Consequently, many experts use a *continuity correction* (sometimes called the *Yates correction*) to adjust for the deviation from the smooth, continuous theoretic distribution of χ^2 when the discrete values of small numbers of expected subjects disrupt the validity of the assumed distribution. The equation for χ^2 with the continuity correction is

$$\chi_c^2 = [(ad - bc) - (N/2)]^2 N \div [(a + b)\,(a + c)\,(b + d)\,(c + d)]$$

If the expected frequency in any cell is very small (often defined as <5), the χ^2 test, even with the continuity correction, is not a valid estimate of the probability that the observed values could have occurred by chance. In such instances, the *Fisher exact test*, which is based on a hypergeometric distribution, should be used to assess the statistical significance of differences in categoric values.

TABLE 266–5 2 × 2 Contingency Table

	Outcome Present	Outcome Absent	Total
Exposed	a	b	a + b
Unexposed	c	d	c + d
Total	a + c	b + d	N

$\chi^2 = [(ad - bc)^2 N] \div [(a + b)(a + c)(b + d)(c + d)]$

Confidence Intervals

Although the validity of a null hypothesis typically is tested by stochastic tests and the use of *p* values, construction of a *confidence interval* (usually a 95% confidence interval [CI]) has the advantage of providing information about both the precision of the estimate of the association (the narrower the confidence interval, the more precise the estimate) and its statistical significance.[4] CIs have become standard components of reports of the results of epidemiologic studies.

Analyses of studies are concerned with both estimation (of the "true" value of the association between the exposure and the outcome in the population) and hypothesis testing (i.e., what probability that any association that is observed could have occurred by chance alone). A sample of the population is enrolled in any study. The association observed in the study is hoped to be an accurate estimate of the true value of the association in the entire population. However, if an unbiased study were repeated many times, by chance alone the results would vary (because of random sampling error). A CI is a range of values, based on the estimate and the spread of the data from a single study, within which the true value of the association in the population is likely to lie with a specified probability (i.e., 95% of the time for a 95% CI, 99% of the time for a 99% CI). CIs provide information about both the magnitude of associations (so that the clinical significance of the estimate can be assessed) and their statistical significance. If the CI is calculated for a single outcome and if the 95 percent CI for the difference between two groups does not include zero, the outcome is statistically significant (i.e., <5% probability that the null hypothesis of no difference existing between the groups is true). If the CI is for a risk ratio or an odds ratio, the association will be statistically significant if the 95 percent CI for the association does not include 1 (i.e., <5% probability that the null hypothesis that no association exists is true). CIs can be calculated with both continuous and categoric data, as well as for estimates of a single proportion and for differences (or associations) between two groups.

Adjustment for Potential Confounding Variables

The effect of confounding variables on the associations between exposure and outcome may be controlled in either the design or the analysis of a study. Random allocation of subjects to the exposed and the unexposed groups is a common strategy to avoid the effect of confounding in longitudinal studies. Although randomization actually ensures only lack of bias if a confounding variable is distributed unevenly between the groups (because an uneven distribution is as likely to favor the exposed group as the

unexposed group), randomization is likely to be effective in preventing confounding if the size of the sample is large (which renders substantial inequalities between groups in the distribution of an independent variable unlikely to occur). Nonetheless, an investigator must check to ensure that confounding does not occur, even in a randomized clinical trial.

Another way to ensure that a potential confounding variable does not affect the results of a study is to *match* on that variable. Subjects in the exposed and the unexposed groups (or, in a case-control study, the cases and the controls) can be matched on certain variables that are known to be associated independently with both the exposure and the outcome. Such matching can be achieved either by matching subjects individually or by *frequency matching* (i.e., ensuring that the overall frequency distribution of the potential confounder is the same among the cases and the controls). Matching can ensure that the matched variable (e.g., race, age) does not affect the observed association between exposure and outcome.

One disadvantage of matching is that the effect of the matched variable on the association between exposure and outcome cannot be assessed as it might be if either stratification or multivariable analysis were used to control for confounding. If matching is used in the design of the study, analyzing the data with special tests for matched designs is necessary. For example, in a matched-pairs case-control study, only discordant pairs (matched pairs in which the cases and controls differ in their exposure status) provide information. Calculation of the matched odds ratio and the associated test of statistical significance (*McNemar* χ^2) is shown in Table 266–6.

Adjusting for the effect of potential confounders in the analyses also is possible. One way to accomplish this adjustment is with *stratification*, which is performed by dividing the data into different groups, or *strata*, according to the potential confounder. For example, a study may indicate that children whose parents both work were three times more likely to become febrile during the course of a study than were children whose parents both do not work. We may suspect, however, that it is not having both parents at work, per se, that results in this increased risk of fever; rather, another factor probably is related independently to the risk for fever and to the probability that both parents work. Attendance at group daycare is such a factor. If we stratified the results of the study by whether the children attended group daycare, we most likely would find that a disproportionate number of children whose parents both work also attended group daycare. When the risk ratios in the two different strata are combined (by using the Mantel-Haenszel technique), we probably would find that the apparent association between having both parents work and the risk of fever disappears when the effect of attendance of group daycare is controlled by stratification.

Advantages of stratification are that it can be understood easily and it can be performed without changing the design of the study after it is completed. One disadvantage is that it requires being able to identify and to measure accurately the potential confounders (which is not necessary if one allocates subjects to different groups randomly). In addition, if several different potential confounders exist, performing the calculations may be difficult and time-consuming, and the sizes of the individual strata may be so small that the ability to make statistical inferences is poor.

Finally, one can adjust for confounding with the use of one of many multivariable statistical techniques, such as *multiple linear regression* (if both the dependent and the independent variables are continuous) or *logistic regression* (if the dependent variable is dichotomous and the independent variables are either categoric or continuous). Providing a detailed description of multivariable techniques is beyond the scope of this chapter. However, in general, these techniques involve creating a mathematic model that fits the data and then using that model to analyze various associations.[2-3,6,22] Although multivariable techniques are a powerful tool for both assessing the effects of and controlling for potential confounding variables, they depend on mathematic assumptions that may not always be valid and use techniques that are not intuitively obvious.

TABLE 266–6 Analysis of a Matched Case-Control Study

	Cases	
Controls	**Exposed**	**Unexposed**
Exposed	a	b
Unexposed	c	d

Matched odds ratio: b/c.
McNemar χ^2*:* $(b - c)^2/(b + c)$.
McNemar χ^2 *(with continuity correction):* $(b - c - 1)^2/(b + c)$.

META-ANALYSIS

The term *meta-analysis* refers to a number of different types of analysis. The common feature of all meta-analyses is that they summarize the results of two or more different studies. The term often is used to refer to a quantitative technique in which the results of different studies are combined after they are weighted in the calculations according to the sizes of their samples and the variances of their results. The term also sometimes refers to a qualitative analysis of a group of studies, including critical analyses that rate the methodological soundness of different studies but do not combine their results quantitatively. For example, one might review a group of studies that reached different conclusions about a topic. If the methodologically rigorous studies had similar results that differed from those of the weaker studies, one might conclude that the results of the more rigorous studies were more likely to be valid.

The concept of producing a single, statistically valid result by combining the results of different, often contradictory, studies has great appeal. However, quantitative meta-analysis has many shortcomings. Perhaps chief among them is that no distinction is made between studies that are sound methodologically and those that are not. Thus, the results of a meta-analysis may be dominated by a large, but methodologically questionable, study, whereas a superbly performed, but smaller study may fail to override the impact of the larger study in the meta-analysis. Another major problem is the selection of studies to include in a meta-analysis. Should only published, peer-reviewed studies be included? Clearly a bias exists that studies with positive results are more likely to be published than studies with negative results? Imagine that many small studies with poor statistical power are conducted and submitted to journals for publication. If the study found no association with the putative risk factor, it likely would not be published, on the grounds that the statistical power of the study is poor (i.e., the risk of making a type 2 error and erroneously concluding there is no association when such an association truly exists is great). On the other hand, if the study did find a statistically significant association with the risk factor, it might be published and could be included in a meta-analysis. If many such small studies that found an association were published, but even more small studies that did not find an association were not published, this kind of *publication bias* could lead to erroneous conclusions in a meta-analysis. Should the analysis be limited to only those studies that meet certain basic criteria for methodological rigor (and who should decide on the criteria and whether they are met), or should only randomized clinical trials be included? What formula should be used to weight the different studies? Is combining the results of methodologically different

studies valid? Results of meta-analyses should be interpreted with great circumspection.[27]

DIAGNOSTIC TESTS

Although clinicians typically order diagnostic tests many times each day, misinterpretation and misuse of diagnostic tests are extremely common occurrences.[19] The widespread practice of interpreting continuous values (e.g., the total white blood cell count) in a dichotomous manner (so that the result is either "normal" or "abnormal") and failure to appreciate fully the difference between the accuracy of a diagnostic test (i.e., its sensitivity and specificity) and its predictive value are major factors in the misuse of diagnostic tests.

WHAT IS NORMAL?

In most instances the "cutoff" for an abnormal test of a continuous variable, such as the total white blood cell count or the concentration of antibodies against an infectious agent, is based on the distribution of the results of the test in the population. One usually assumes that the distribution of most such results in the population will follow a gaussian (or "normal") distribution, which is illustrated by the bell-shaped curve in Figure 266–1. Gauss and other statisticians used the term *normal* to refer to the shape of this curve. However, in medicine, the term *normal* long has been used to mean something very different—the dichotomy between a state of health *(normal)* and one of disease *(abnormal)*. Unfortunately, the different meanings of these terms often have been used interchangeably, which has added to confusion in the interpretation of diagnostic tests.

In a normal distribution, the mode (the most frequent value) is the mean value, and 68.3 percent, 95.4 percent, and 99.7 percent of the values lie within (plus or minus) 1, 2, and 3 standard deviations (SD) from the mean value, respectively. The cutoff for an abnormal test result often is defined arbitrarily as any value that is more than 2 SD from the mean of a normally distributed population; thus, 5 percent (actually 4.6%) of the population would be categorized as abnormal. Half the people with abnormal results will be 2 SD below the mean, and half will be 2 SD above the mean. Thus, 2.5 percent (actually 2.3%) of the population will have abnormally high values. In some instances, 3 SD (or some other arbitrary parameter) is used to determine the cutoff. However, of importance is to realize that these definitions of normal and abnormal are merely statistical models that should not necessarily be translated to mean that 2.5 percent of the population is "diseased." As noted earlier, with this kind of statistical logic, the diagnosis of disease may be related to the number of tests performed because if multiple tests are conducted, the likelihood that the result of any one will be abnormal is increased. If 15 independent tests are performed on a patient, the probability that any one of the test results will be abnormal by chance is 54 percent.

Other statistical models might be more appropriate for assessing a diagnostic test. For example, interpreting diagnostic tests would be relatively easy if two separate, nonoverlapping normal distributions of diseased and disease-free patients existed. Unfortunately, although this model may apply for some rare conditions (such as genetic disorders in which affected persons are missing an enzyme that metabolizes certain substances, which leads to uniquely high concentrations of the substance in persons with the disorder), the model rarely is applicable. A model that is more appropriate for most infectious illnesses is shown in Figure 266–2. In this model (the example of which uses levels of serum calcium), the diseased and nondiseased populations have separate, partially overlapping distributions. The specificity of a diagnostic test is related directly to the degree to which the two distributions overlap.

ACCURACY OF A DIAGNOSTIC TEST

Two important characteristics of a diagnostic test—its *reproducibility* and its *validity*—are the key components of its accuracy. The reproducibility (sometimes called *reliability* or *precision)* of a test simply is the degree to which retesting yields the same result. A test that provides results that are not reproducible is of little diagnostic value. The validity of the results of a diagnostic test may be divided into two components: *sensitivity* and *specificity* (Table 266–7). The *sensitivity* of a test is the proportion of persons

TABLE 266–7 Statistical Indices of a Diagnostic Test

Result of Test	Disease	No Disease
Positive	a	b
Negative	c	d
True positives: a	Sensitivity = a/(a + c)	
False positives: b	Specificity = d/(b + d)	
True negatives: d	Positive predictive value = a/(a + b)	
False negatives: c	Negative predictive value = d/(c + d)	

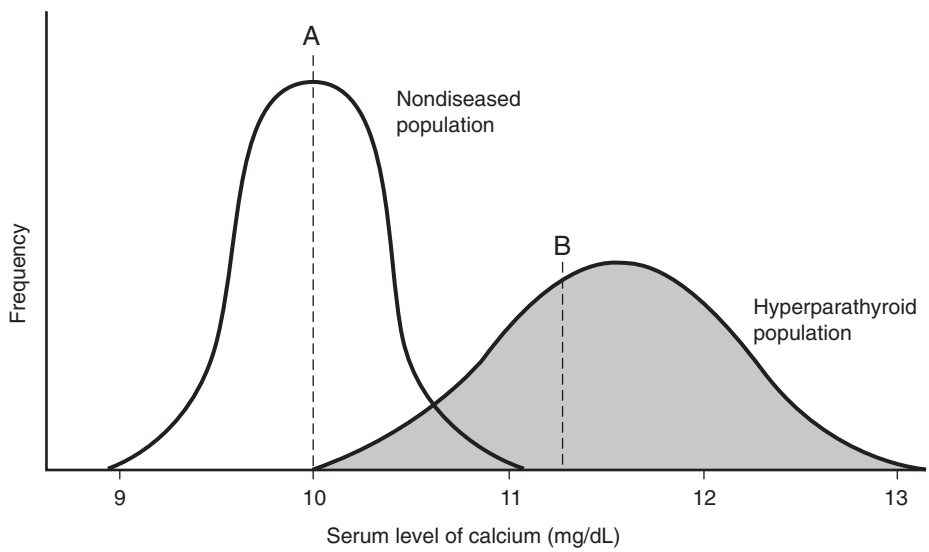

Figure 266–2 Overlap in values of randomly performed tests in a population in which most of the people are healthy (curve on the *left*) but some of the people are diseased (curve on the *right*). A person with a level of calcium below point A would be unlikely to have hyperparathyroidism. A person with a level of calcium above point B would be likely to have an abnormality in calcium metabolism, possibly hyperparathyroidism. A person with a level of calcium between point A and point B may or may` not have an abnormality in calcium metabolism. (*Note:* The normal range of calcium depends on the method used in a specific laboratory. In some laboratories, the range is from 8.5 to 10.5 mg/dL. In others, as in this illustration, it is from 9 to 11 mg/dL.) *(From Jekel, J. F., Elmore, J. G., and Katz, D. L.: Epidemiology, Biostatistics, and Preventive Medicine. Philadelphia, W. B. Saunders, 1996, p. 88.)*

with disease who are identified accurately by the test as having disease (true positives/[true positives + false negatives]). The *specificity* of a test is the proportion of persons without disease who are identified accurately by the test as not having disease (true negatives/[true negatives + false positives]). The ideal test has both a specificity and a sensitivity of 1. Of course, no diagnostic tests are that accurate. Indeed, a tradeoff exists between sensitivity and specificity—usually, the more sensitive a test is, the less specific it is, and vice versa.

Although the sensitivity and specificity of a given test often are considered to be absolute characteristics of a test, in fact, these characteristics do depend on the types of patients included in the studies in which the indices of the test were developed. Indeed, tests that appear to be both highly sensitive and highly specific in preliminary studies commonly prove to be much less so when used in actual clinical practice because the preliminary studies that establish a test's sensitivity and specificity frequently are affected by problems of spectrum and bias.[18] If the patients enrolled in studies used to assess the test (both those with disease and those without disease) have a relatively narrow spectrum of clinical manifestations, both the sensitivity and the specificity of a diagnostic test may appear to be better than they actually are. For example, if one were to assess the sensitivity of a differential heterophile test to diagnose infection with Epstein-Barr virus (EBV), its sensitivity would appear to be far better if all the subjects infected with EBV were teenagers with severe pharyngitis (a high proportion of whom will have a positive heterophile test result) than if it included subjects with a broader spectrum of manifestations of EBV infection (such as toddlers with symptoms of an infection of the upper respiratory tract, who are much less likely to have a positive heterophile test result when infected with EBV). Likewise, the specificity of a diagnostic test may appear to be better when only asymptomatic, healthy persons are used as nondiseased subjects instead of including patients with other illnesses that produce symptoms similar to those of the target illness. The effects of spectrum of the illness on sensitivities and specificities of a diagnostic test also have been reported for dipstick urinalyses as a diagnostic test for urinary tract infections.[13]

In addition, of importance is to realize that the cutoffs for classifying results as either normal or abnormal usually are somewhat arbitrary and may depend on how the test is used, as well as the nature of the disease that one is trying to detect. If a diagnostic test is being used to screen a population for a relatively mild disorder with a low prevalence, one would want to use a cutoff that results in high specificity to avoid having a large number of false-positive results, even at the expense of lower sensitivity. On the other hand, if the diagnostic test is for a serious disorder or is used not for screening but for diagnosis in a targeted population with at least a moderate prevalence of the disorder (e.g., a white blood cell count in cerebrospinal fluid to detect possible bacterial meningitis in febrile children), one might want to err on the side of overdiagnosis and, therefore, use a cutoff with a high sensitivity at the expense of lower specificity.

PREDICTIVE VALUE OF A DIAGNOSTIC TEST

Sensitivity and specificity are important characteristics of a diagnostic test, but to calculate them, one must know whether the patients do or do not have the disease; clearly, if a physician already has this knowledge, the test does not need to be performed in the first place. From the perspective of a clinician who is caring for an individual patient, the key characteristic of a diagnostic test is its *predictive value* (see Table 266–7). That is, if a test result is positive, what is the probability that the patient has the disease *(positive predictive value)*? Conversely, if a test result is negative, what is the probability that a patient does not have the disease *(negative predictive value)*? However, unlike sensitivity and specificity, the predictive value of a test depends critically on the prevalence of the disease among the subjects who are tested.

This concern is illustrated by the example in Table 266–8. In example *a*, the prevalence of the disease in the sample is 1 percent and the positive predictive value of a positive test result is only 8.8 percent. By contrast, as shown in examples *b* and *c*, when the prevalence of disease in the sample rises to 10 percent and to 50 percent, respectively, the positive predictive value of a positive test result rises to 51.4 percent and to 90.5 percent, respectively, even though the sensitivity and the specificity of the test are constant. By the same token, as the prevalence of disease in the sample that is tested rises, the predictive value of a negative test result falls, although the negative predictive value remains reasonably good until the prevalence of disease becomes high.

Of importance for clinicians is to be aware of these epidemiologic truths when ordering diagnostic tests for patients. Sometimes a physician orders tests to "rule out" the possibility that a patient's symptoms are caused by a disease that, based on the patient's history, physical examination, and previous laboratory tests, is unlikely to be the cause of the problem (i.e., the "prior probability" that the patient has the disease is low). Unless the specificity of such tests approaches 100 percent (which is unlikely for most serologic tests), in such situations the great majority of positive test results will be falsely positive; the physician then will be in the unfortunate position of ignoring the result, ordering

TABLE 266–8 Predictive Value of a Diagnostic Test with 95% Sensitivity and 90% Specificity with Different Prevalence of Disease in the Sample

	Test	Disease	No Disease	Total
a*	Positive	95	990	1,085
	Negative	5	8910	8,915
	Total	100	9900	10,000
	Positive predictive value = 8.8%	Negative predictive value = 99.9%		
b†	Positive	950	900	1,850
	Negative	50	8100	8,150
	Total	1000	9000	10,000
	Positive predictive value = 51.4%	Negative predictive value = 99.4%		
c‡	Positive	4750	500	5,250
	Negative	250	4500	4,750
	Total	5000	5000	10,000
	Positive predictive value = 90.5%	Negative predictive value = 94.7%		

**Prevalence of disease: 1%.*
†Prevalence of disease: 10%.
‡Prevalence of disease: 50%.

additional diagnostic tests, or treating the patient for a disease that probably is not the cause of the symptoms.[23] Consequently, clinicians should be selective in ordering diagnostic tests.

ASSESSMENT OF THE PROTECTIVE EFFICACY OF A VACCINE (OR OF ANY INTERVENTION)

This section will discuss assessment of the protective efficacy of a vaccine, but the same methods (with different outcomes specified, of course) can be applied to any intervention such as a drug or physical therapy. The protective efficacy (PE) of a vaccine may be assessed with either experimental or observational studies. The classic way to assess a vaccine's efficacy is in an experimental, randomized, double-blind clinical trial. The major observational designs available are cohort studies and case-control studies—although a large variety of hybrid designs such as household exposure studies and indirect cohort studies are available—but complete descriptions of them are beyond the scope of this chapter.[16,17] Table 266–9 illustrates calculation of the PE of a pneumococcal vaccine with data from clinical trials, cohort studies, and case-control studies.

CLINICAL TRIALS

In a randomized clinical trial, subjects at risk of acquiring the infection in question would be assigned randomly either to receive the vaccine (vaccinees) or not to receive the vaccine (controls). Subsequently, all subjects would be observed for occurrence of the infection during an appropriate period of follow-up. At the conclusion of the study, the *protective efficacy* of the vaccine, an index devised by Greenwood and Yule[8] to indicate the proportionate reduction in the frequency of disease attributable to the vaccine, would be calculated. PE is defined as follows:

$$PE = (\text{Risk of infection in controls} - \text{Risk of infection in vacciness}) \div \text{Risk of infection in controls}$$

A PE of 100 percent indicates complete protection against infection, a PE of 0 percent indicates no protection, and negative values indicate that a greater risk of acquiring infection existed among vaccinees than among controls. By rearranging terms, PE equals 1 minus the risk of infection in vaccinees divided by the risk of infection in controls. Because the ratio in this equation is the relative risk of infection developing in vaccinees versus controls, PE is, by definition, equal to 1 minus the relative risk.

TABLE 266–9 Calculation of Protective Efficacy (PE) of Pneumococcal Vaccine

Clinical Trial or Cohort Study	Pneumococcal Infection	No Pneumococcal Infection	Total
Vaccinated	a	b	a + b
Not vaccinated	c	d	c + d

$PE = [(c/c + d) - (a/(a + b)] \div (c/c + d) = 1 - [(a/(a + b) \div (c/c + d)]$*

	Pneumococcal	No Pneumococcal Infection
Case-Control Study	Infection (Cases)	(Controls)
Vaccinated	a	b
Not vaccinated	c	d

$PE = 1 - [(ad) \div (bc)]$†

**The expression $[(a/(a + b) \div (c/c + d)]$ also is known as the relative risk of infection in vaccinated versus unvaccinated persons.*

†The expression $[(ad) \div (bc)]$ is the odds ratio relating vaccination to infection. In a case-control study, the odds ratio approximates the relative risk if the outcome is rare.

OBSERVATIONAL COHORT STUDIES

In a cohort study, subjects at risk would be selected on the basis of whether they had been vaccinated or had been left unvaccinated during routine clinical care. The vaccinated and unvaccinated groups then would be monitored longitudinally to assess frequencies of infection. The analysis of a vaccine's PE for cohort studies is similar to that for clinical trials, but because the study is nonexperimental, the results are more subject to bias than are those of a well-conducted, randomized clinical trial.

CASE-CONTROL STUDIES

In a case-control study, patients with antecedent conditions that place them at high risk for pneumococcal infection would be eligible to be subjects. Patients with serious pneumococcal infection would become subjects in the case group. The control group would consist of subjects with similar high-risk conditions but without pneumococcal infection. The two groups then would be compared for the frequency of antecedent vaccination with pneumococcal vaccine.

In a case-control study, the strength of the relationship between vaccination and subsequent infection is measured by an odds ratio (the ratio of the odds of vaccination in the case subjects to the odds of vaccination in the controls). Because the odds ratio in a case-control study closely approximates the relative risk of pneumococcal infection in a longitudinal study and because PE is defined as 1 minus the relative risk, the value (1 – odds ratio) closely approximates the PE that would be calculated from a longitudinal study.

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